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Study of Atypical Alarmone Synthesis in *Clostridioides difficile*

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STUDY OF ATYPICAL ALARMONE SYNTHESIS IN CLOSTRIDIOIDES DIFFICILE

by

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ABSTRACT

STUDY OF ATYPICAL ALARMONE SYNTHESIS IN *CLOSTRIDIOIDES DIFFICILE*

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Clostridioides difficile is a Gram-positive anaerobic bacterium that causes infections in humans that costs healthcare systems billions per year. *C. difficile* infection has high rates of recurrence due to multiple antibiotic resistance. When bacteria are in stressful environments, they produce hyperphosphorylated guanosine ribonucleotide signaling molecules called alarmones. The accumulation of alarmones activates the stringent response (SR), in which bacterial cells induce transcription of stress survival genes to delay growth and replication. The *C. difficile* SR is regulated by enzymatic activity of a bifunctional synthetase/hydrolase, RelA-SpoT homolog (RSH), and a monofunctional small alarmone synthetase (RelQ). Additionally, the SR is potentially regulated by a third putative synthetase (RelC) which has an uncharacterized domain. Unlike other characterized SR-utilizing bacteria, *C. difficile* exclusively produces a single triphosphate alarmone through atypical mechanisms despite high active site sequence homology. The first goal of this project was *in silico* mutational analysis of RSH and RelQ to highlight important residues potentially responsible for the unique alarmone metabolism and the second goal was to predict the functionality of RelC. Together, these aims provide the theoretical framework to identify the structural basis of unusual SR activity in *C. difficile*.

This thesis is dedicated to everyone who helped at every step of the way.

ACKNOWLEDGMENTS

I could not have completed this without the help and support of my family and loved ones. I am indebted to them for their countless sacrifices in ensuring I could perform to the best of my abilities. Savannah led the way and provided more help than can be recognized. Eoghan made sure everything was as logical as possible. Dad, Juliana, and Amelia made sure to help in every way possible. Mom, Jim, Walter, and Emmett gave much of their own time for support.

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NOMENCLATURE

CDI	*Clostridioides difficile* infection
FMT	Fecal microbiota transplantation
TcdA	*Clostridioides difficile* glucosylating toxin A
TcdB	*Clostridioides difficile* glucosylating toxin B
CDT	*Clostridioides difficile* transferase
SR	Stringent response
pppGpp	Guanosine pentaphosphate
ppGpp	Guanosine tetraphosphate
pGpp	Guanosine triphosphate
ATP	Adenosine triphosphate
GTP	Guanosine triphosphate
GDP	Guanosine diphosphate
GMP	Guanosine monophosphate
RSH	RelA-SpoT homolog
TLC	Thin layer chromatography
GDPβS	Non-hydrolyzable GDP analog
GXP	GTP, GDP, or GMP
PDB	Protein Data Bank
MSA	Multiple sequence alignment
3D	Three-dimensional
SOE	Splice overlap extension

PCR	Polymerase chain reaction
RMSD	Root Mean Square Deviation
KEGG	Kyoto Encyclopedia of Genes and Genomes
NTD	Beginning terminal domain of a protein that contains the free amine group
CTD	End terminal domain of a protein that contains the free carboxylic group
NIH	National Institutes of Health
BLAST	Basic Local Alignment Search Tool
CO_2	Carbon dioxide
H_2	Hydrogen
N_2	Nitrogen
BHIS	Brain heart infusion-supplemented growth medium
mL	Milliliter
mc	Microcentrifuge
°C	Degrees Celsius
x g	Times Earth's gravitational force
NEB	New England Biolabs
TAE	Tris base, acetic acid, and ethylenediaminetetraacetic acid
µL	Microliter
µg	Microgram
IPTG	Isopropyl-ß-D-thiogalactopyranoside
$MgCl_2$	Magnesium chloride
SDS	Sodium Dodecyl Sulfate
PAGE	Polyacrylamide Gel Electrophoresis

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CHAPTER I

INTRODUCTION

Epidemiology

Clostridioides difficile, formerly known as *Clostridium difficile* and *Bacillus difficilis*, is a Gram-positive, flagellated, obligate anaerobic bacterium that is the leading cause of nosocomial infections in global healthcare systems (1–5). The reason *C. difficile* is called a Gram positive bacterium because it has a thick cell wall that binds the purple 'Gram' stain; this wall protects the cell from environmental stresses such as antibiotics, ultraviolet light, peroxides, bleach, extreme pH, and heat (6–9). Obligate anaerobes do not have mechanisms to protect themselves from oxidative damage and can only live in environments with no diatomic oxygen (10). *C. difficile* infection (CDI) can lead to stomach pain, nausea, fever, inflammation of the colon, profuse diarrhea, and death (11–15). People at high risk for acquiring a CDI are those most likely to be found in a healthcare setting: the elderly, the immunocompromised, and people treated with broad-spectrum antibiotics (9, 16, 17). For those groups, the rates of infection are increased from the elimination of the best protection against a CDI—their natural gut bacteria. Through the combination of decreased nutrient competition from commensal gut bacteria and germination by bile acids in the digestive tract, metabolically dormant *C. difficile* endospores (spores) can establish a niche in the anoxic environment in the colon and germinate into toxin producing vegetative cells (11–15, 18–23). The exact cascade(s) of signaling events required to become the type of cell solely responsible for toxin production are unknown (24).

Every year, *C. difficile* spores are ingested and cause approximately 500,000 new cases of CDI with 100,000 recurrent infections and 30,000 deaths, which contribute to approximately 2.4 million days of inpatient hospitalizations in the United States healthcare system (18, 25–27). *C. difficile* not only infects those with decreased gut bacteria but also people not in the high risk category, people with healthy/typical gut microbiomes, who account for about a third of acquired CDIs (25, 28). *C. difficile* is a pathogen that has the potential of infecting anyone. The rate of healthy community acquired CDI is increasing (28–31). The high hospitalization rates, increasing community infections, and high recurrence of CDIs lead to an annual financial burden of $5.4-6.3 billion for management and treatment of *C. difficile* for the U.S. healthcare system alone (25, 26). Other global healthcare systems are experiencing similar issues and all are noticing specific *C. difficile* strains developing antibiotic resistance (28). The use of broad-spectrum antibiotics such as clindamycin, cephalosporins, and fluoroquinolones are most associated with wiping out the natural gut bacteria and increasing the risk of acquiring a CDI (7, 32). Furthermore, treating a CDI with those broad-spectrum antibiotics allows *C. difficile* cells to adapt and form spores with increased antibiotic resistance traits (28). There are more specific medications used to treat CDIs–metronidazole vancomycin, and fidaxomicin,–but they are losing clinical efficacy and the latter two are often not prescribed due to cost (7, 33).

Treatment Options

There are three non-pharmaceutical options for the treatment of CDIs, although all have downsides. These treatments include bezlotoxumab infusion, fecal microbiota transplantation (FMT), and purposeful infection of non-toxigenic *C. difficile* (34). *C. difficile* can produce three toxic enzymatic proteins that are lethal to host cells. The toxins produced by vegetative *C.*

difficile cells consist of two large glucosylating toxins and a third binary toxin that together prevent the host from maintaining or developing epithelial cellular cytoskeletons within the colon, which results in cellular death (35, 36). The two glucosylating toxins—toxin *C. difficile* A (TcdA) and toxin *C. difficile* B (TcdB)—inhibit major biochemical cascades by glucosylating small GTPases in host epithelial cells that regulate actin polymerization, dysregulating cytoskeletal formation and maintenance. The third toxin, *C. difficile* transferase (CDT), is a binary toxin that causes immense dysregulation in host cells through covalent modification of essential intracellular regulators (34, 36). The uptake of those toxins by host cells results in the degradation of the colonic lining, inflammatory responses, major tissue damage, and the deadly symptoms associated with a CDI (11–15, 35–38). Bezlotoxumab, a monoclonal antibody, combats CDIs by preventing the secreted bacterial toxin TcdB from binding to host cells, thus preventing activation of biochemical cascades responsible for cellular damage (34). The unintended consequence of FMT and colonization of non-toxigenic *C. difficile* is that there remains a non-zero probability of transmitting other pathogenic microorganisms that could cause immense dismay within the enigmatic natural gut microbiome; ultimately, the efficacy and exact mechanisms that influence successful elimination of *C. difficile* through those three treatment options remain unknown (39). There is an urgent need for the development of new therapies against CDI that are more accessible and consistent than these treatments.

Surviving Stressful Conditions

If the environment of *C. difficile* becomes unfavorable, either due to attack by the innate immune system, antibiotic exposure, or nutrient limitation, growth is halted, and survival mechanisms are activated. One conserved bacterial survival mechanism that is found throughout

all *C. difficile* strains is the stringent response (SR). When stressed, *C. difficile* produces intracellular signal molecules—called alarmones—that activate survival pathways to reduce growth and cellular replication (7, 13, 15, 40, 41). The SR is a conserved response that has been identified as a survival mechanism in nearly all bacteria. The activation of the SR allows bacteria to survive stressful environmental conditions by activating, through mechanisms that vary between species, intracellular pathways that prevent DNA replication, limit protein synthesis, slow metabolic rates, promote biofilm formation, and increase transcription of survival genes such as nutrient transporters, efflux pumps to remove antibiotics, genes that promoted biofilm formation, and virulence factors (1, 9, 40–42). The alarmones that trigger those mechanism are hyperphosphorylated ribonucleotide signaling molecules: guanosine pentaphosphate (pppGpp), guanosine tetraphosphate (ppGpp), and guanosine triphosphate (pGpp) (1). The p's to the left of the guanosine (G) represent the phosphates attached to the 5' hydroxyl group of the ribose sugar, while the p's to the right of the G represent the phosphates attached to the 3' hydroxyl group of the guanosine nucleotide. It is the 3' phosphates that differentiate alarmones from canonical ribonucleotides, which are only phosphorylated at the 5' site. During stressful conditions, alarmone synthetase enzymes transfer a pyrophosphate, two phosphates bonded by a phosphoanhydride bond, from an adenosine triphosphate (ATP) donor to the 3' end of guanosine triphosphate (GTP), guanosine diphosphate (GDP), or guanosine monophosphate (GMP) to produce pppGpp, ppGpp, or pGpp, respectively (Figure 1). *C. difficile* encodes two conserved synthetases: the difunctional synthetase/hydrolase RelA-SpoT homolog, RSH, and the monofunctional small alarmone synthetase, RelQ (1, 9, 43).

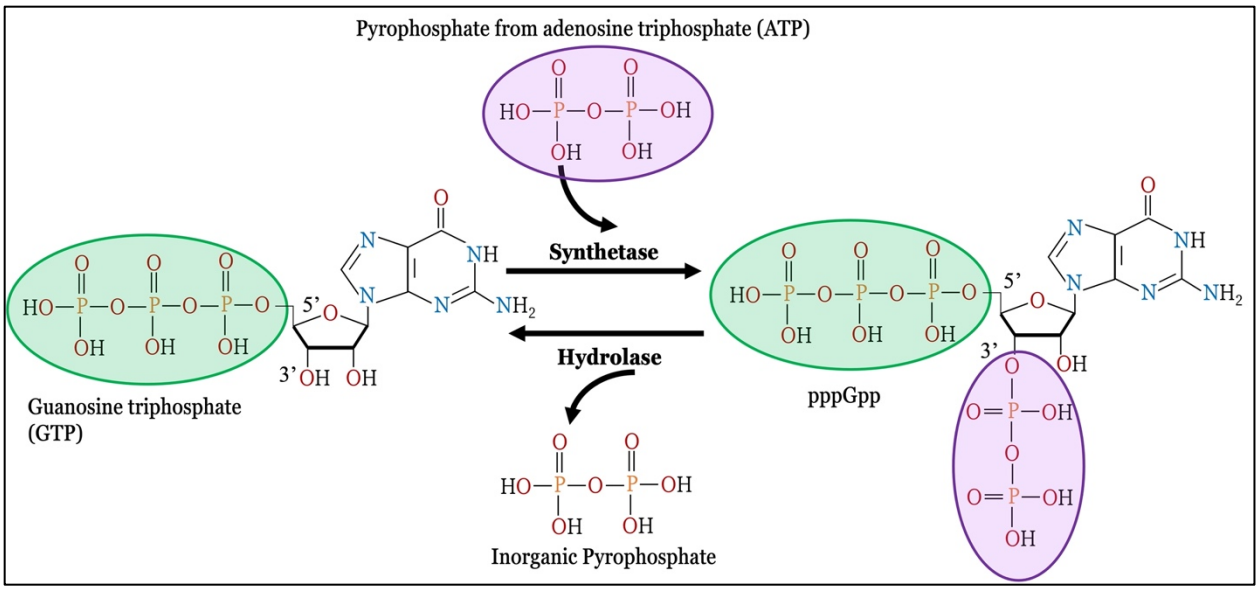


Figure 1. Typical alarmone synthesis and degradation. When conditions are stressful, bacteria transfer a pyrophosphate (purple) from ATP to the 3' hydroxyl of a guanosine recipient. The number of 5' phosphates (green) on the guanosine substrate typcially determine the identity of the alarmone product. Shown is a triphosphate group on a GTP substrate and pppGpp product. The reaction is reversible by the removal of the 3' pyrophosphate. Adapted from Hauryliuk V. et al. (42).

When the environment becomes favorable, alarmones are deconstructed by a hydrolysis reaction (performed by the RSH enzyme or a small alarmone hydrolase), which releases an inorganic pyrophosphate and the original guanosine nucleotide (Figure 1) (42). The size of alarmones produced in typical synthesis is usually dependent on the number of phosphates 'n'originally attached to the guanosine nucleotide; the alarmone product has 'n' phosphates plus two. Thus, use of a GDP precursor yields ppGpp and use of GTP yields pppGpp. However, *C. difficile* has a unique alarmone production mechanism in that both RSH and RelQ exclusively produce the smallest alarmone, pGpp, but cannot utilize GMP as a precursor (Figure 2A,C) (1, 9). Both clostridial enzymes must hydrolyze a 5'β phosphate bond on the guanosine substrate, leaving a 5' monophosphate and converting all alarmone products to pGpp, and are unable to utilize a non-cleavable GDP analog (Figure 2D, E) (9). A control enzyme from *B. subtilis* can utilize GMP and non-hydrolyzable GDP analogs (Figure 2B,F) (9). While there are some

NUDIX hydrolases that trim pppGpp and ppGpp to pGpp, there are no other documented synthetases that affect the 5' phosphate groups while transferring a pyrophosphate to the 3' hydroxyl (44). It is not known how it benefitted *C. difficile* to evolve a non-conserved alarmone synthetase mechanism requiring a guanosine phosphate hydrolysis.

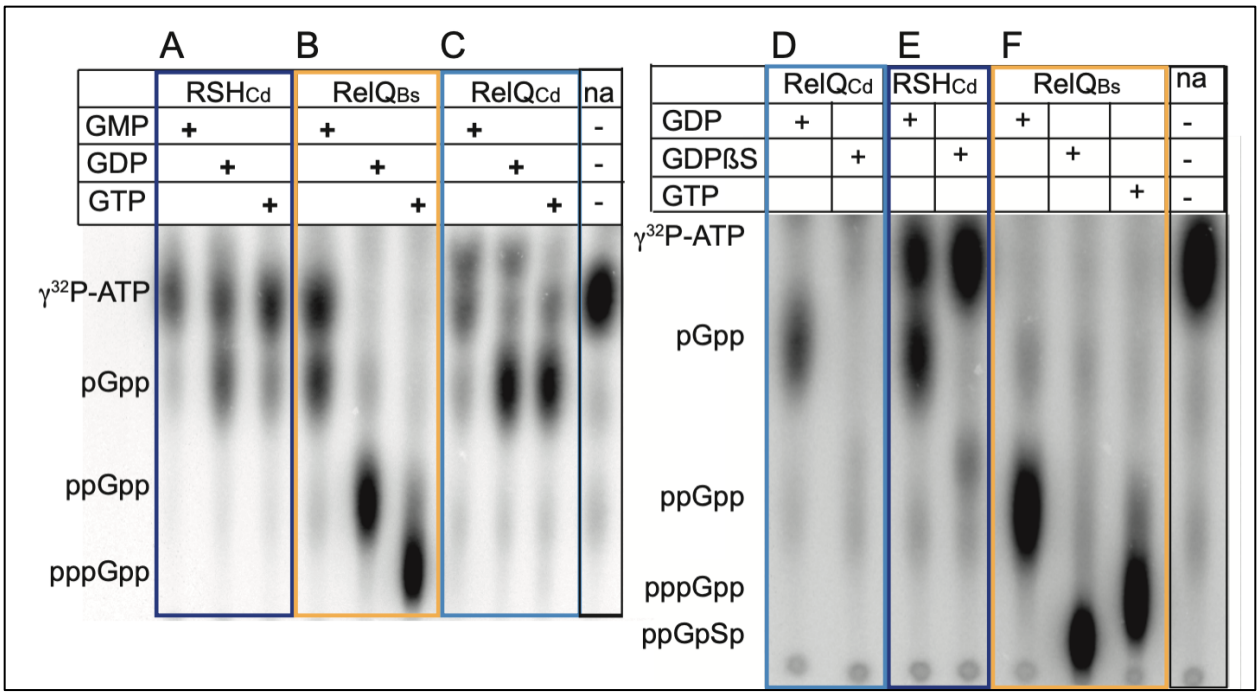
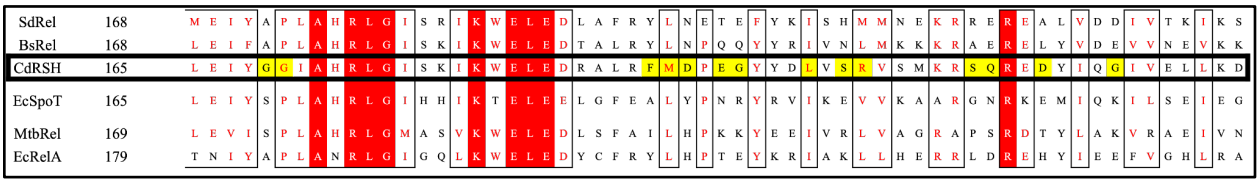


Figure 2. Unique clostridial alarmone synthesis. The transfer of radioactive phosphate from γ^{32}P-ATP to GXP substrates to form radiolabeled alarmones can be visualized through thin layer chromatography (TLC). (A) *C. difficile* RSH cannot make alarmones from GMP and only uses trace amounts of GTP, but readily makes an alarmone product from GDP which is the same apparently size and charge as pGpp, not ppGpp. (B) The well-characterized small alarmone synthetase RelQ from *B. subtilis* uses GMP, GDP, or GTP to make three differently-sized alarmones. (C) The RelQ homolog from *C. difficile* utilizes eithor GDP or GTP but only synthesizes a single size of alarmone, which exhibits the same motiity as pGpp. (D, E) Clostridial RelQ can synthesize alarmone products from GDP but not the non-hydrolyzable GDP analog GDPßS. (F) *B. subtilis* RelQ can utilize GDPßS. Reproduced from Poudel A. et al. (9).

The two clostridial enzymes, RSH and RelQ, both have high sequence homology with other bacterial SR enzymes that make larger alarmones, and well-conserved catalytic regions, but they behave differently within *C. difficile* (Figure 3). The primary sequences of these enzymes offer

no clue as to why or how they perform the guanosine phosphate bond hydrolysis. In addition, much of what is known about alarmone regulation is from research in model organisms such as *Escherichia coli* and *Bacillus subtilis,* and many of the receptors that respond to alarmones in other species don't have homologs in *C. difficile,* so it is unclear what the triphosphate alarmone does in the cell once synthesized. It is has been demonstrated that alarmone production occurs and affects antibiotic survival and biofilm formation in this organism, but the evolutionary rationale and mechanism(s) behind the atypical alarmone synthesis are unknown (1, 44). Understanding why *C. difficile* only produces the triphosphate alarmone is a possible avenue in developing a SR-inhibitor specific to CDI which might not affect canonical alarmone synthesis in beneficial gut bacteria.

A.



B.

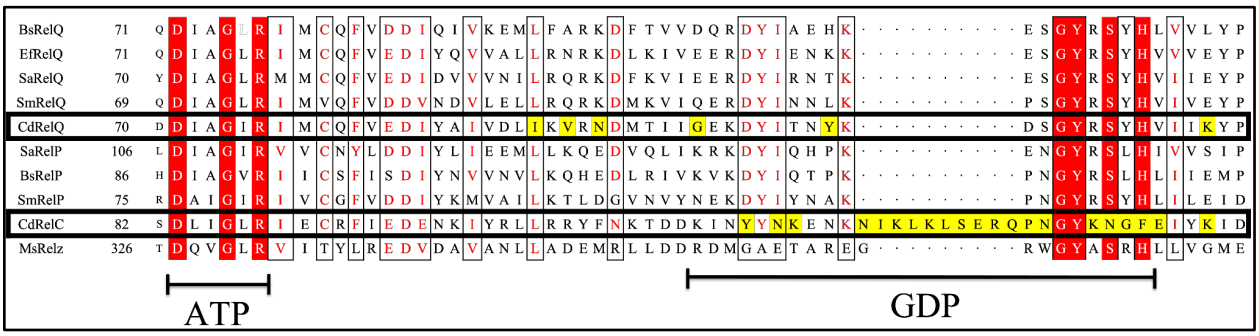


Figure 3. *C. difficile* enzyme divergence. *C. difficile* (Cd) RSH, RelQ, and RelC sequences (black outline) were compared to the active sites of Steptococcus dysgalactae (Sd), Bacillus subtilis (Bs), Escherichia coli (Ec), Mycobacterium tuberculosis (Mtb), Enterococcus faecalis (Ef), Staphyloccocus aureus (Sa), Streptococcus mutans (Sm), and Mycobacterium smegmatis (Ms) of (A.) RSH enzymes and (B.) SAS synthetases. How *C. difficile* differs from the consensus sequences across other bacterial species are highlighted in yellow. Red columns with white

lettering are highly conserved residues in the active site. Red letters are moderately conserved residues. Vertical boxes were used for sequence alignments. Bold horizontal boxes are *C. difficile* enzymes. The sections labeld ATP and GDP are where those compounds bind to the active site in. Reproduced from Poudel A. et al (9).

RelC

The clostridial genome contains a third gene with a putative synthetase domain, although it lacks some conserved active site residues and has an insertion in the predicted GXP-binding region (Figure 3). This putative synthetase domain is at the N-terminus of the protein, which has a C-terminus domain of unknown function. This gene has homologs, but only in Firmicutes bacteria, while RSH and RelQ are more evolutionarily widespread. Alarmone synthetases are typically not part of multidomain proteins outside of difunctional synthetase/hydrolases like RSH, although there is one reported case where a synthetase domain is bound to another domain other than the normal hydrolase domain. RelZ, from *Mycobacterium smegmatis*, contains an RNase H domain in tandem with a (p)ppGpp synthetase domain (45). No alarmone synthetase activity has been demonstrated for an enzyme from this family, which we have named RelC for 'Rel homolog from Clostridioides.' It is possible that it is an alarmone-binding effector or that it is a nucleotide hydrolase that does not product alarmones. Whatever its role, its narrow distribution in Firmicutes species make it a potential target for developing a narrow-spectrum anti-clostridial drug if it does something important that can be inhibited.

Utilizing AlphaFold for *in silico* Predictions

RSH and RelQ are both members of highly conserved families, and high-resolution crystal structures exist for *Bacillus subtilis* RelQ (Protein Data Bank code: 5DEC), *Bacillus subtilis* RSH (PDB Entry: 6YXA), and *Streptococcus equisimilis* RSH (PDB Entry: 1VJ7) (46–48). There are several non-conserved residues near the active sites in each protein (Figure 3),

although it is unclear which one(s) are important for 5' guanosine phosphate bond hydrolysis. Mutational analysis of individual and combined point mutants will be necessary to define this, but there are so many potential mutants that this would be a very extensive set of experiments. Similarly, the RelC active site exhibits such poor conservation (Figure 3) that it is unclear whether it would be worth expressing it to test for alarmone synthesis. Computation analysis of these proteins can be used to guide experimental design. Because RSH and RelQ have functionality unique to *C. difficile* and RelC is only found in Firmicutes, all these proteins represent potential opportunities to interfere with *C. difficile* viability in a very specific way and are therefore worth further study.

AlphaFold first gathers multiple sequence alignments (MSA) with different regions weighted by importance. Then the software utilizes a Evoformer module to extract information about interrelationships between protein sequences and template structures. The structure module has the protein act as a residue gas that is placed into different locations to generate a three-dimensional (3D) structure. This structure is then followed by local refinement to provide the final prediction. We have predicted the structures of *C. difficile* RSH and RelQ to identify which residues to prioritize for mutational analysis and analyzed structural predictions of RelC to assess its potential as an alarmone synthetase. This project lays the groundwork for determining the structural basis for atypical alarmone synthesis in *C. difficile*.

CHAPTER II

COMPUTATIONAL ANALYSIS OF CLOSTRIDIAL RSH AND RELQ

Introducing the Stress Survival Mechanism

The stringent response has been confirmed to have different roles in different organisms, and to contribute to antibiotic resistance in *C. difficile* (1). Since the SR is a conserved response that has been identified as a survival mechanism in nearly all bacteria, targeting this mechanism for drug design was originally thought of as a bad idea, as targeting a mechanism that is found in nearly all bacteria would create yet another broad-spectrum treatment that damages the native gut bacteria and promotes the conditions for *C. difficile* to thrive. However, the atypical alarmone synthesis mentioned above makes researching this pathway an exciting and unique way to specifically target *C. difficile* cells. Exploring the unique alarmone synthesis that triggers the SR in *C. difficile* may provide the foundation for an exploitable drug target that would not create the issues that arise from broad-spectrum antibiotics in CDIs. There are several non-conserved residues in both RSH and RelQ (Figure 3), so it will be important to generate RSH and RelQ point mutants to understand their role in unique *C. difficile* alarmone synthesis. We have identified several non-conserved residues near the active sites RSH and RelQ (summarized in Table 3 and Table 4, respectively). Some of these amino acid changes are conservative such as aspartic acid to glutamic acid and glycine to alanine. However, some of these changes are drastic changes that could have profound change on structure and function at that site function such as glycine to proline. It is possible that mutating some or all these *C. difficile* residues to the consensus residues could affect substrate specificity and processing, allowing the *C. difficile*

enzymes to produce ppGpp and pppGpp. Such mutagenesis could be performed by splice overlap extension polymerase chain reaction (SOE PCR).

***C. difficile* RSH Amino Acid Residue**	***C. difficile* RSH residue Position**	**Consensus RSH Amino Acid Residue**	**Consequence**
G	169	A	Size: Small → Big
G	170	P	Size: Small → Big
M	192	L	Size: Big → Small
D	193	N	Polarity: Acidic → Polar
G	196	E	Size & Polarity: Small & Polar → Big & Acidic
G	196	K	Size & Polarity: Small & Polar → Big & Basic
D	213	A	Size & Polarity: Big & Acidic → Small & Nonpolar
D	213	H	Size & Polarity: Small & Acidic → Big & Basic
G	217	D	Size & Polarity: Small & Polar → Big & Acidic

Table 1. RSH Point Mutations and their Consequences. Change in *C. difficile* RSH amino acid size and polarity. Consensus residues as described in Figure 3.

C. difficile RelQ Amino Acid Residue	*C. difficile* RelQ residue Position	Consensus RelQ Amino Acid Residue	Consequence
I	92	L	Size: Small → Big
V	94	N	Size and Polarity: Small & Nonpolar → Big & Polar
V	94	K	Size and Polarity: Small & Nonpolar → Big & Basic
N	96	K	Polarity: Polar → Basic
N	96	E	Polarity: Polar → Acidic
G	102	D	Size and Polarity: Small & Polar → Big & Acidic
G	102	K	Size and Polarity: Small & Polar → Big & Basic
Y	110	H	Size and Polarity: Small & Polar → Big & Basic
Y	110	P	Size and Polarity: Big & Polar → Small & Nonpolar
K	123	E	Polarity: Basic → Acidic

Table 2. RelQ Point Mutations and their Consequences. Change in *C. difficile* RelQ amino acid size and polarity. Consensus residues as described in Figure 3.

Using AlphaFold to Analyze RelQ and RSH

To determine which residues to prioritize, we performed predictive structural analysis of *C. difficile* RSH and RelQ. Google's DeepMind program, AlphaFold 3.0, was utilized to predict these structures, which we compared to high-resolution crystal structures of homologs with canonical synthetic activity (3' pyrophosphotransfer with no 5' phosphorolysis). We then assessed the structural effects of creating single- and double-point mutations in the clostridial sequences *in silico*.

Crystal structures of *Bacillus subtilis* RelQ (PDB Entry: 5DEC), *Bacillus subtilis* RSH (PDB Entry: 6YXA), and *Streptococcus equisimilis* RSH (PDB Entry: 1VJ7) were used to validate AlphaFold structural prediction (46–48). The primary amino acid sequences were entered into

AlphaFold 3.0 along with the combination ATP, magnesium, and either GDP or GTP (Appendix code). The outputs were saved as .cif files and loaded into PyMOL (The PyMOL Molecular Graphics System, Version 2.5.7 Schrödinger, LLC.). Each prediction was loaded together with the .pdb file of its experimentally obtained X-ray crystal structure. The two models were aligned using PyMOL align command. Then selections of the amino acid chains were made using the selection command to be compared for RMSD calculations (Appendix code). For comparison of X-ray crystallography structures, the residue numbers were manually adjusted to match one another. Then the RMSD script (Appendix code) was loaded onto PyMOL to calculate the RMSD per residue, which generated a tgt_gzt object and displayed in cartoon-sausage mode with a color ramp that showed the relative deviations. The RMSD values were automatically stored and saved under a pdb file, rmsdBFactor_XXXX.pdb where the B-factor contained the RMSD of the Cα atoms. Finally, the RMSD script saved a csv file for easy access to the RMSD per residue values.

The sequences of *C. difficile* RSH (CD630_27440) and RelQ (CD630_03450) were obtained from the Kyoto Encyclopedia of Genes and Genomes (KEGG) and entered AlphaFold 3 to generate .cif files as described above (49). For *in silico* mutational analysis, residues were manually altered, and the predictions were repeated.

AlphaFold predictions of clostridial enzymes were aligned with the AlphaFold models of the homologous structures and the average RMSD and the RMSD per residue were calculated as described above.

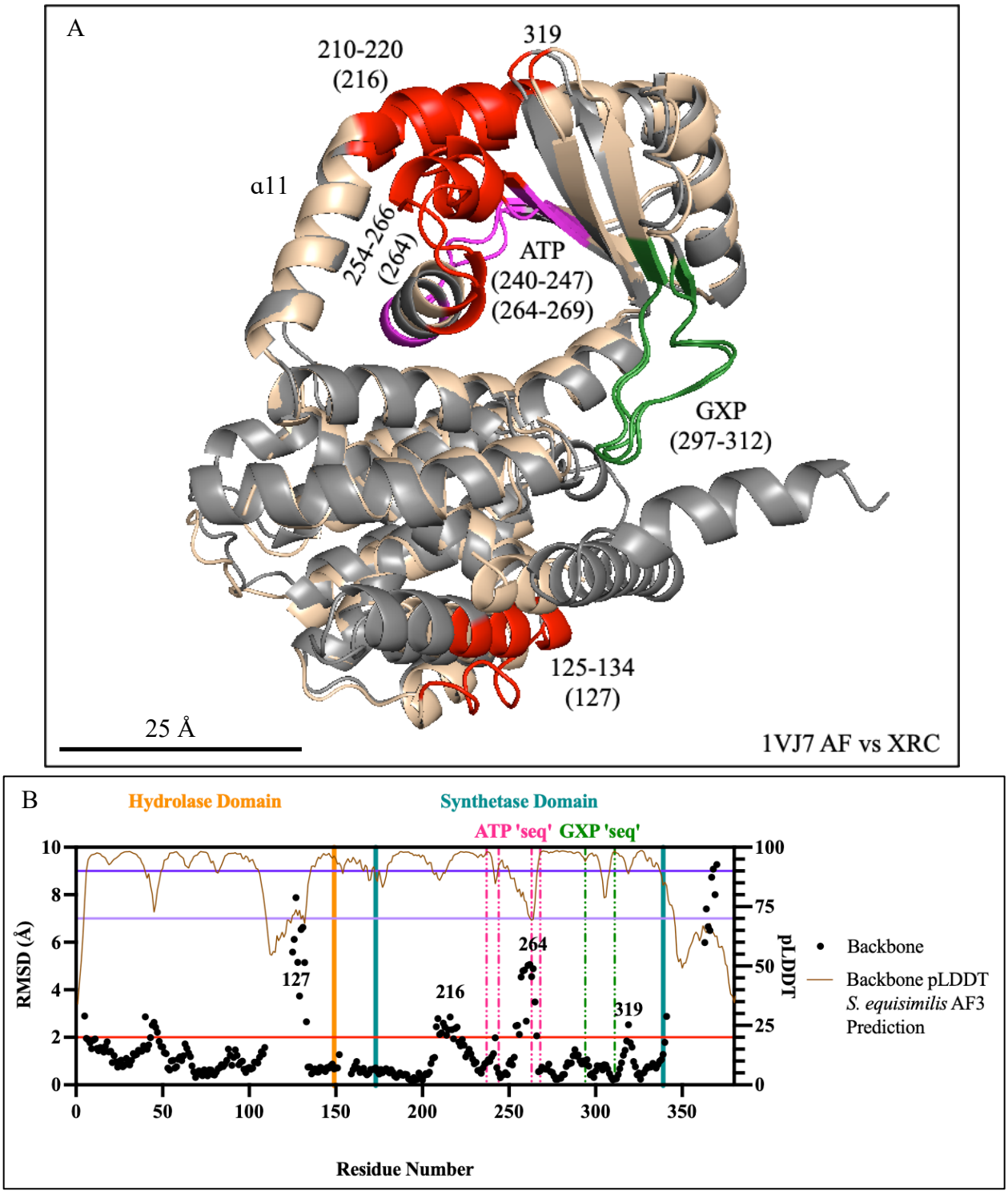


Figure 4. Validating AlphaFold 3 predictions with *S. equisimilis* RSH. (A) *S. equisimilis* RSH AlphaFold (AF) (gray) vs *S. equisimilis* RSH (PDB code 1VJ7) X-ray crystallography (SRC) (tan) structure. The conserved ATP binding region (magenta), conserved GDP/GTP (GXP) binding region (green), and hot spot (red) are shown in their respective colors on each structure. The hot spots are locations on the AlphaFold model with an RMSD above 2 Å compared to the crystal structure. When aligning the two structures together, there was an overall RMSD of 1.059 Å

(2098 to 2098 atoms). (B) RMSD plot. Black dots are the average RMSD of the atoms (nitrogen, alpha carbon, carbonyl carbon, and carbonyl oxygen) that are a part of the backbone at each residue. The red line at an RMSD of 2 Å that is the cutoff for amino acids that are considered hotspots. The magenta (ATP residues 240-247 GRPKHIYS and 264-269 DLIAIR) and lime green (GXP residues 297-312 KDYIAAPKANGYQIH) dash/dot lines are the conserved residues that make up binding regions of the indicated nucleotide. The light and dark purple lines are at a pLDDT value of 70 and 90, respectively, to show the predicted confidence levels of 'normal confidence' and 'very confident.' pLDDT values, per residue, are traced with a mocha colored line.

Root Mean Squared Deviation (RMSD) quantifies how far apart two entities differ from one another and is measured in Ångström (Å), which is 10^{-10} meters. Less than or equal to 2 Å is considered 'fairly good' alignment (50). pLDDT is a measure of confidence of predicted structure, which is a per-residue measure of local confidence automatically calculated by AlphaFold. pLDDT values are scaled from zero to one hundred. The closer a pLDDT value is to one hundred, the more confident and accurate the prediction. pLDDT is computed over all pairs of atoms in the reference structure at a distance closer than a predefined threshold inclusion radius (Ro). A distance is considered preserved in the model (M) if it is, within a certain tolerance threshold 0.5 Å, 1 Å, 2 Å and 4 Å and any value above 90 is very confident (51, 52).

To use AlphaFold for novel studies in *C. difficile*, the software must be validated as being able to correctly predict proteins involved in alarmone synthesis. To begin, homologous enzymes with available X-ray crystallography structures were selected for their primary structure similarity (Figure 3) to *C. difficile*. *Streptococcus equisimilis* RSH (PDB code 1VJ7), which has a resolution of 2.10 Å, was selected as a model for RSH and *Bacillus subtilis* RelQ (PDB code 5DEC), with a resolution of 2.00 Å, was selected as a model for RelQ (47, 48). The primary sequence of *S. equisimilis* RSH was run through the AlphaFold software and was overlaid with the experimentally determined X-ray crystallography structure. There are gaps within the RMSD plot due to the X-ray crystallography structure having floppy regions and areas of low resolution that manifested as gaps in the structure, leaving no residues at those positions with which to

compare the AlphaFold model. AlphaFold also struggled to produce reproducible structures–as measured by pLDDT values–at those floppy loop regions of the protein.

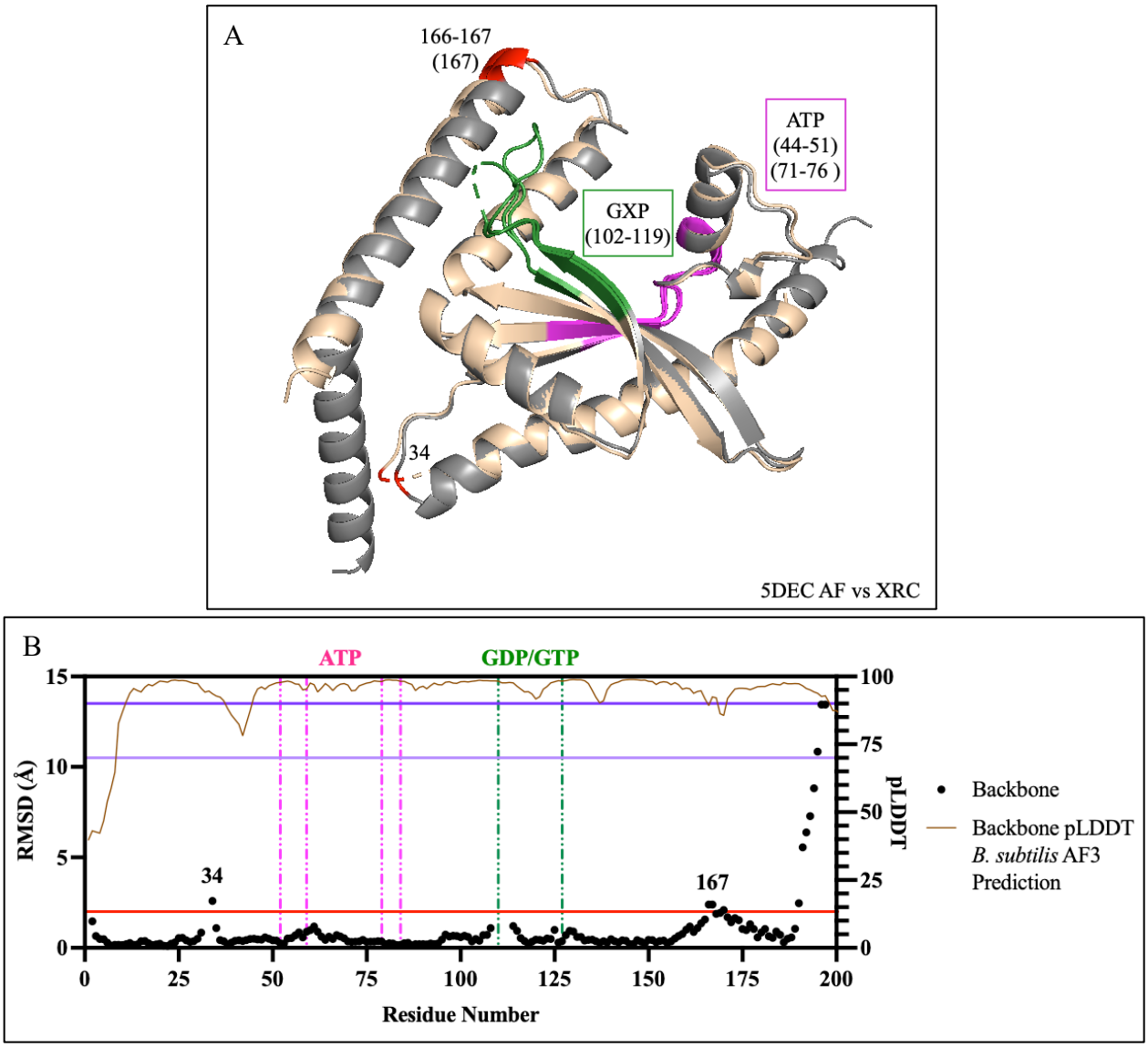


Figure 5. Validating AlphaFold predictions with *B. subtilis* RelQ. (A) *B. subtilis* RelQ AF (gray) vs *B. subtilis* RelQ (PDB code 5DEC) X-ray crystallography (tan) structure. The conserved ATP binding region (magenta), conserved GXP binding region (green), and hot spot (red) are shown in their respective colors on each structure. The hot spots are anywhere on the protein with an RMSD above 2 Å. When aligning the two structures together, there was an overall RMSD of 0.558 Å (1190 to 1190 atoms). The missing RMSD within the conserved GXP binding site is at within the loop that connects to the beginning of the beta sheet. (B) RMSD plot. Black dots are the average RMSD of the atoms (nitrogen, alpha carbon, carbonyl carbon, and carbonyl oxygen) that are a part of the backbone. The red line at an RMSD of 2 Å that is the cutoff for amino acids that are considered hotspots. The magenta (ATP residues 44-51 GRVKPVAS and 71-76 DIAGLR) and lime green (GXP residues 102-119 DQRDYIAEHKESGYRSYH) dash/dot lines are the conserved residues that make up binding regions of the

indicated nucleotide. The light and dark purple lines are at a pLDDT value of 70 and 90, respectively, to show the predicted confidence levels of ‘normal confidence’ and ‘very confident.’ pLDDT values per residue are traced with a mocha colored line.

The comparison of the X-ray crystallography structure and AlphaFold model of *B. subtilis* RelQ had even lower RMSD spikes and higher pLDDT values than that of *S. equisimilis* RSH. Since the AlphaFold structures were comparable to true structures and had no unresolved residues, the AlphaFold models were used as the basis of comparison for the clostridial enzymes. *S. equisimilis* RSH was compared to *C. difficile* RSH (Figure 6).

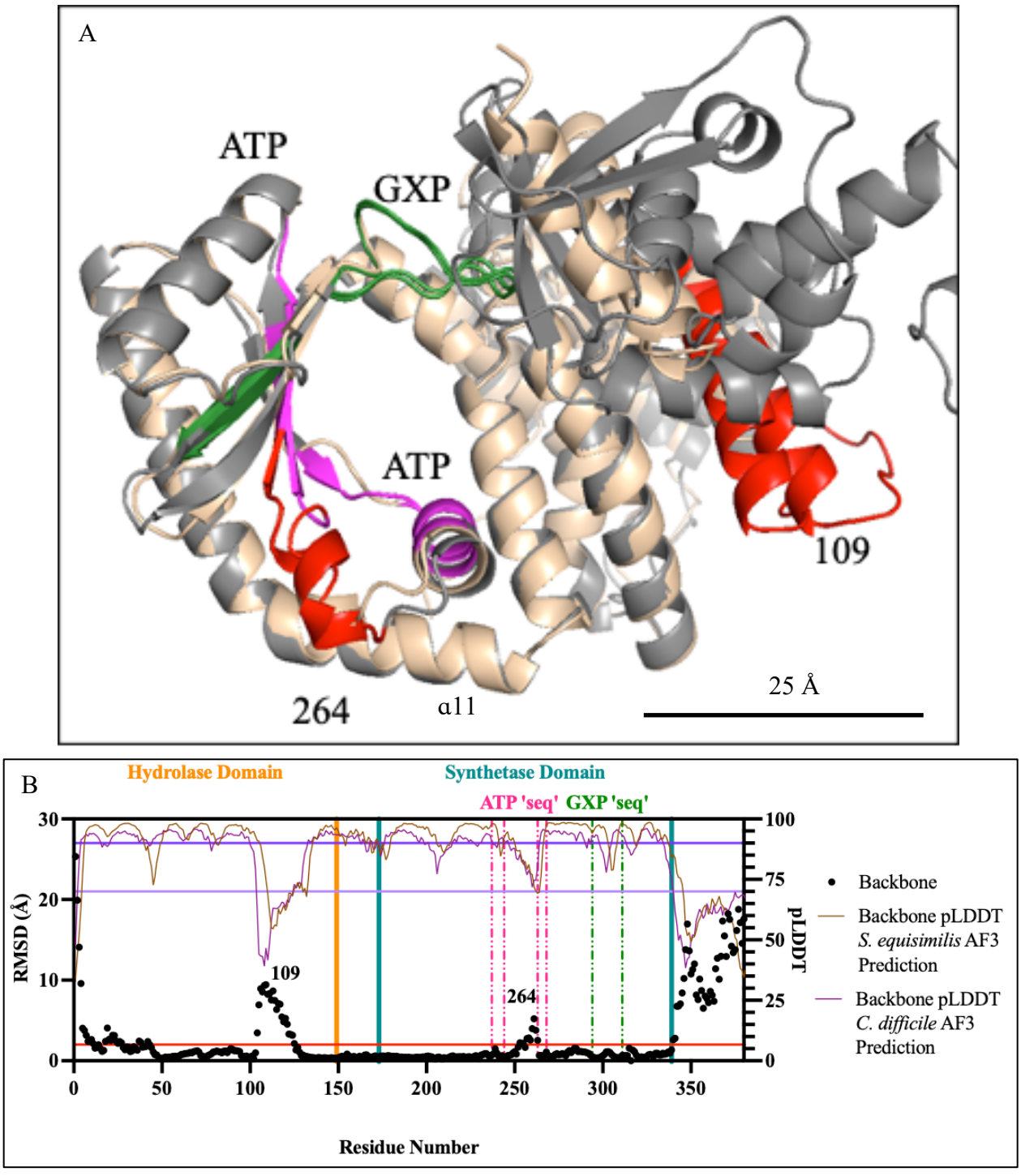


Figure 6. Comparing *S. equisimilis* RSH against *C. difficile* AlphaFold. (A) *S. equisimilis* RSH AlphaFold (gray) vs *C. difficile* RSH AlphaFold (tan) structure. Conserved ATP binding region (magenta), conserved GXP binding region (green), and hot spot (red) are shown in their respective colors. The hot spots are anywhere on the protein with an RMSD above 2 Å. When aligning the two structures together, there was an overall RMSD of 0.836 Å (1913 to 1913 atoms). (B) RMSD plot. Black dots are the average RMSD of the atoms (nitrogen, alpha carbon, carbonyl

carbon, and carbonyl oxygen) that are a part of the backbone. The red line at an RMSD of 2 Å that is the cutoff for amino acids that are considered hotspots. The magenta and lime green dash/dot lines are the conserved binding regions of the indicated nucleotide. The light and dark purple lines are at a pLDDT value of 70 and 90, respectively, to show the predicted confidence levels of 'normal confidence' and 'very confident.' pLDDT values, per residue, are traced with a mocha colored line for *S. equisimilis* and a salmon color for *C. difficile.*

RMSD spikes are observed around residues 109 and 264 when comparing the AlphaFold structure of *S. equisimilis* RSH to that of *C. difficile* RSH (Figure 6), which resembles the RMSD spikes of the Xray structure of *S. equisimilis* RSH to the predicted AlphaFold structure of *S. equisimilis* RSH (Figure 4). Figure 6a shows the actual structures overlaid on top of one another which shows the cause of those spikes for similar reasons as to the spikes seen in comparing the same protein (Figure 4 and Figure 5). All other spots within the protein are nearly identical with RMSD values well below the 2 Å cutoff. When the two RelQ models were compared, similar results were acquired. The majority of *C. difficile* RelQ had nearly identical coordinates to the the *B. subtilis* RelQ with a few exceptions. These exceptions for *B. subtilis* RelQ vs *C. difficile* RelQ are different than the comparison of the *B. subtilis* RelQ AlphaFold model versus experimentally determined *B. subtilis* RelQ. The RMSD jumps are caused by loops found at different locations. In *B. subtilis* RelQ versus *B. subtilis* RelQ (Figure 5) the jumps were at residues 34 and 167 while in *B. subtilis* RelQ vs *C. difficile* RelQ, the RMSD jumps were found within residues 64 and 131 (Figure 7) which were also found within loops.

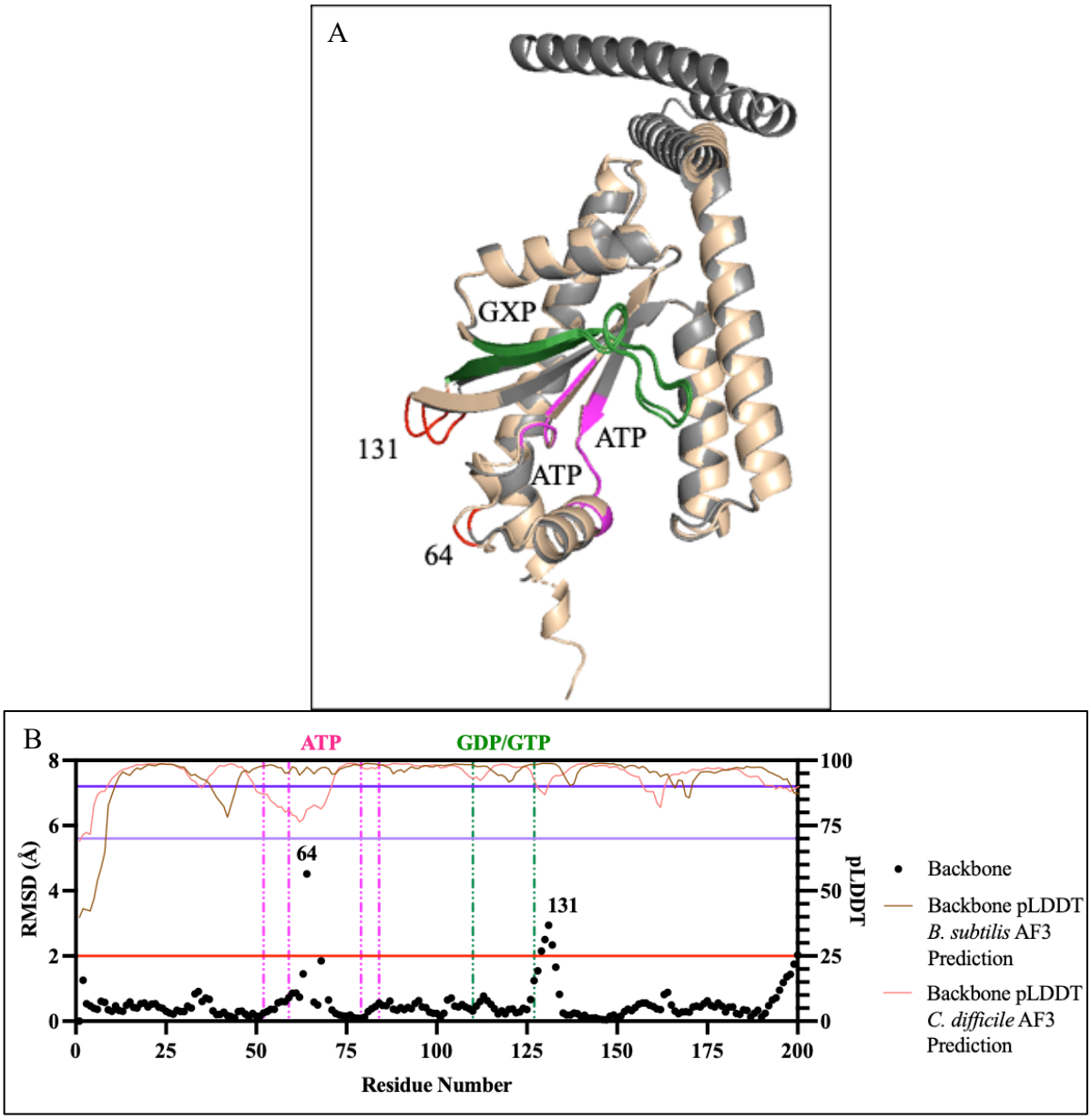


Figure 7. Comparing the RelQ of *B. subtilis* to *C. difficile*. (A) *C. difficile* RelQ AF (gray) vs *B. subtilis* RelQ AlphaFold prediction (tan) structure. Conserved ATP binding region (magenta), conserved GXP binding region (green), and hot spot (red) are shown in their respective colors. The hot spots are anywhere on the protein with an RMSD above 2 Å. When aligning the two structures together, there was an overall RMSD of 0.435 Å (1183 to 1183 atoms). The missing RMSD within the conserved GXP binding site is at within the loop that connects to the beginning of the beta sheet. (B) RMSD plot. Black dots are the average RMSD of the atoms (nitrogen, alpha carbon, carbonyl carbon, and carbonyl oxygen) that are a part of the backbone. The red line at an RMSD of 2 Å that is the cutoff for amino acids that are considered hotspots. The magenta and lime green dash/dot lines are the conserved binding regions of the indicated nucleotide. The light and dark purple lines are at a pLDDT value of 70 and 90, respectively, to show the predicted confidence levels of 'normal confidence' and 'very confident.' pLDDT values, per residue, are traced with a mocha colored line for *B. subtilis* and a salmon colored line for *C. difficile.*

Once AlphaFold was validated and baseline structural models for the clostridial enzymes were established, *in silico* point mutations were compared to the wild-type models. Since structure determines function, any notable differences in RMSD plots of the point mutations may provide insight as to the residues responsible for the unique alarmone synthesis of *C. difficile*. The divergent clostridial residues (Figure 3) were replaced with conserved homologous sequences in the sequences entered into AlphaFold. For RSH, nine single point mutations selected (Table 1). For RelQ, ten point mutations were selected for *in silico* analysis (Table 2). These initial 19 point mutations were run through the AlphaFold software to predict their actual consequences to overall structure. These point mutations were originally tested using AlphaFold 2 (Figure 8), but the comparisons were repeated in AlphaFold 3 after the ability to model non-covalently bound ligands was added.

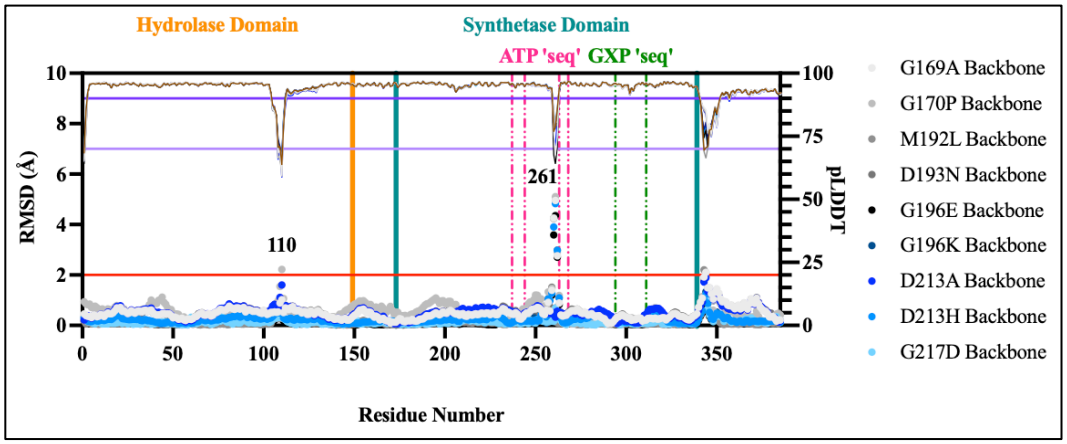


Figure 8. *C. difficile* RSH Single SOE RMSD AlphaFold 2.0. Figure 8b Overlay of the RMSD traces of all AlphaFold 2.0 RSH single mutants. Conserved ATP binding region (magenta), conserved GXP binding region (green), and hot spot (red) are shown in their respective colors. The hot spots are anywhere on the protein with an RMSD above 2 Å. Black dots are the average RMSD of the atoms (nitrogen, alpha carbon, carbonyl carbon, and carbonyl oxygen) that are a part of the backbone. The red line at an RMSD of 2 Å that is the cutoff for amino acids that are considered hotspots. The magenta and lime green dash/dot lines are the conserved binding regions of the indicated nucleotide. The light and dark purple lines are at a pLDDT value of 70 and 90, respectively, to show the predicted confidence levels of 'normal confidence' and 'very confident.' pLDDT values, per residue, are traced with a mocha (wildtype prediction) line trace and the same color as gradient in the legend for the mutations.

The AlphaFold 2 and AlphaFold 3 results differed. Of the *C. difficile* RSH single mutants, all except G196K, D213A, and G217D had an RMSD spike at residue 261 (Figure 8). This spike was eliminated in the AlphaFold 3 predictions of the same mutations (Figure 9a). The pLDDT values of AlphaFold 2 mutant predictions are much lower than the pLDDT values of the exact same single point mutations for the AlphaFold 3 predictions. The *in silico* single point mutations of RSH had notable RMSD jumps. When the mutations were combined with ATP, GDP/GTP, and magnesium the spike at residue 261 did not return but a new RMSD spike formed at residue 207 (Figure 9b and Figure 9c). For this RMSD jump, the mutated residues responsible were G170P, G196K, D213A, and D213H for ATP, GDP, and magnesium. There were more residues responsible for the jump when modeled with ATP, GTP, and magnesium; these residues were G169A, G170P, D193N, G196E, G196K, D213A, D213H, and D217D. Therefore, the only single point mutation to not cause any RMSD spike at residue 207 was M192L.

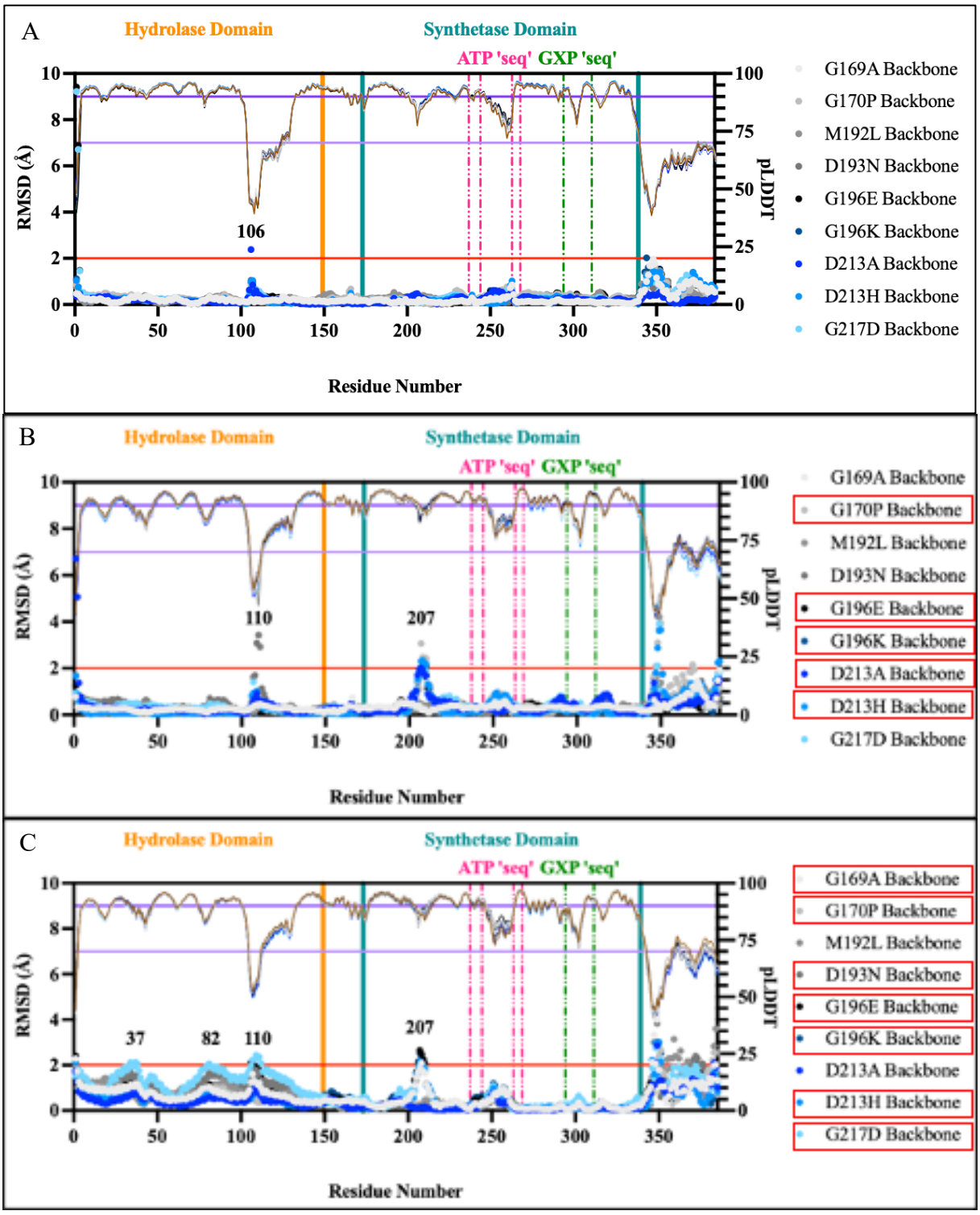


Figure 9. *C. difficile* RSH Single SOE RMSD AlphaFold 3.0. (A) Overlay of the RMSD traces of all AlphaFold 3 WT RSH point mutants compared with wild-type RSH. (B) Overlay of the RMSD traces of all AlphaFold 3 RSH single mutants compared with RSH in which ATP, GDP, and Mg are modeled into the active site. (C) Overlay of the RMSD traces of all AlphaFold 3 ATP, GTP, and Mg RSH single mutants. Conserved ATP binding region (magenta), conserved GXP binding region (green), and hot spots (red) are shown in their respective colors. The hot

spots are anywhere on the protein with an RMSD above 2 Å. Black dots are the average RMSD of the atoms (nitrogen, alpha carbon, carbonyl carbon, and carbonyl oxygen) that are a part of the backbone. The red line at an RMSD of 2 Å that is the cutoff for amino acids that are considered hotspots. The magenta and lime green dash/dot lines are the conserved binding regions of the indicated nucleotide. The light and dark purple lines are at a pLDDT value of 70 and 90, respectively, to show the predicted confidence levels of 'normal confidence' and 'very confident.' pLDDT values, per residue, are traced with a mocha (wildtype prediction) line trace and the same color as gradient in the legend for the mutations.

Residue 207 is the first instance where there is an RMSD jump between wild-type and mutant in a location that is not a loop and has a high pLDDT score. Also, it is the first time of only some mutations causing the change in structure and other mutations have no impact on overall structure. For RelQ, all ten selected point mutations had no impact on overall structure (Figure 10). Those predictions had very high confidence, meaning those residues by themselves probably do not contribute to the atypical alarmone synthesis observed within *C. difficile.*

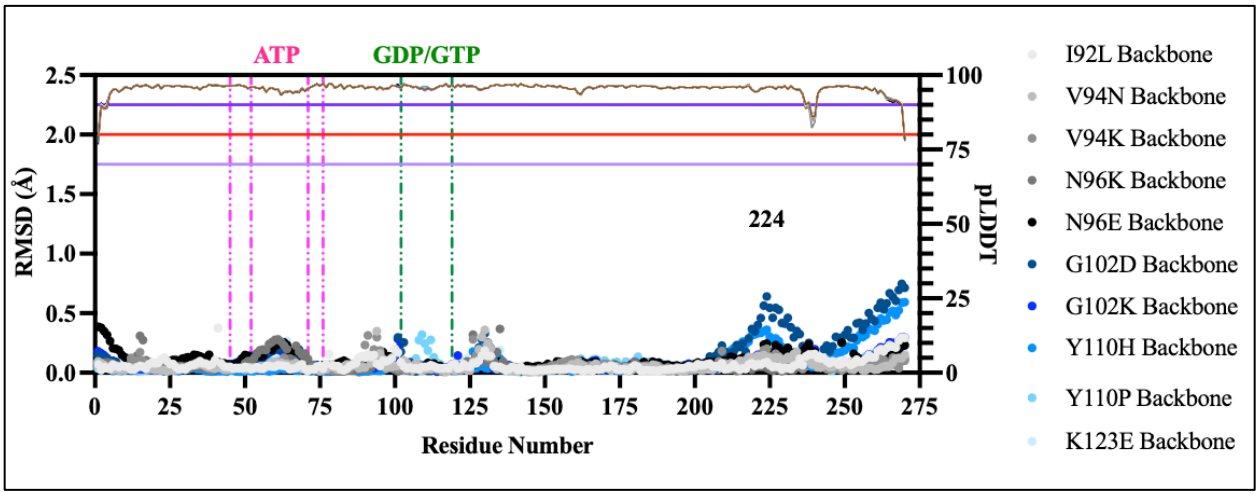


Figure 10. *C. difficile* RelQ Single SOE RMSD AlphaFold 2.0. Overlay of the RMSD traces of all AlphaFold 2 RelQ single mutants. Conserved ATP binding region (magenta), conserved GXP binding region (green), and hot spots (red) are shown in their respective colors. The hot spots are anywhere on the protein with an RMSD above 2 Å. Black dots are the average RMSD of the atoms (nitrogen, alpha carbon, carbonyl carbon, and carbonyl oxygen) that are a part of the backbone. The red line at an RMSD of 2 Å that is the cutoff for amino acids that are considered hotspots. The magenta and lime green dash/dot lines are the conserved binding regions of the indicated nucleotide. The light and dark purple lines are at a pLDDT value of 70 and 90, respectively, to show the predicted confidence levels of 'normal confidence' and 'very confident.' pLDDT values, per residue, are traced with a mocha (wildtype prediction) line trace and the same color as gradient in the legend for the mutations.

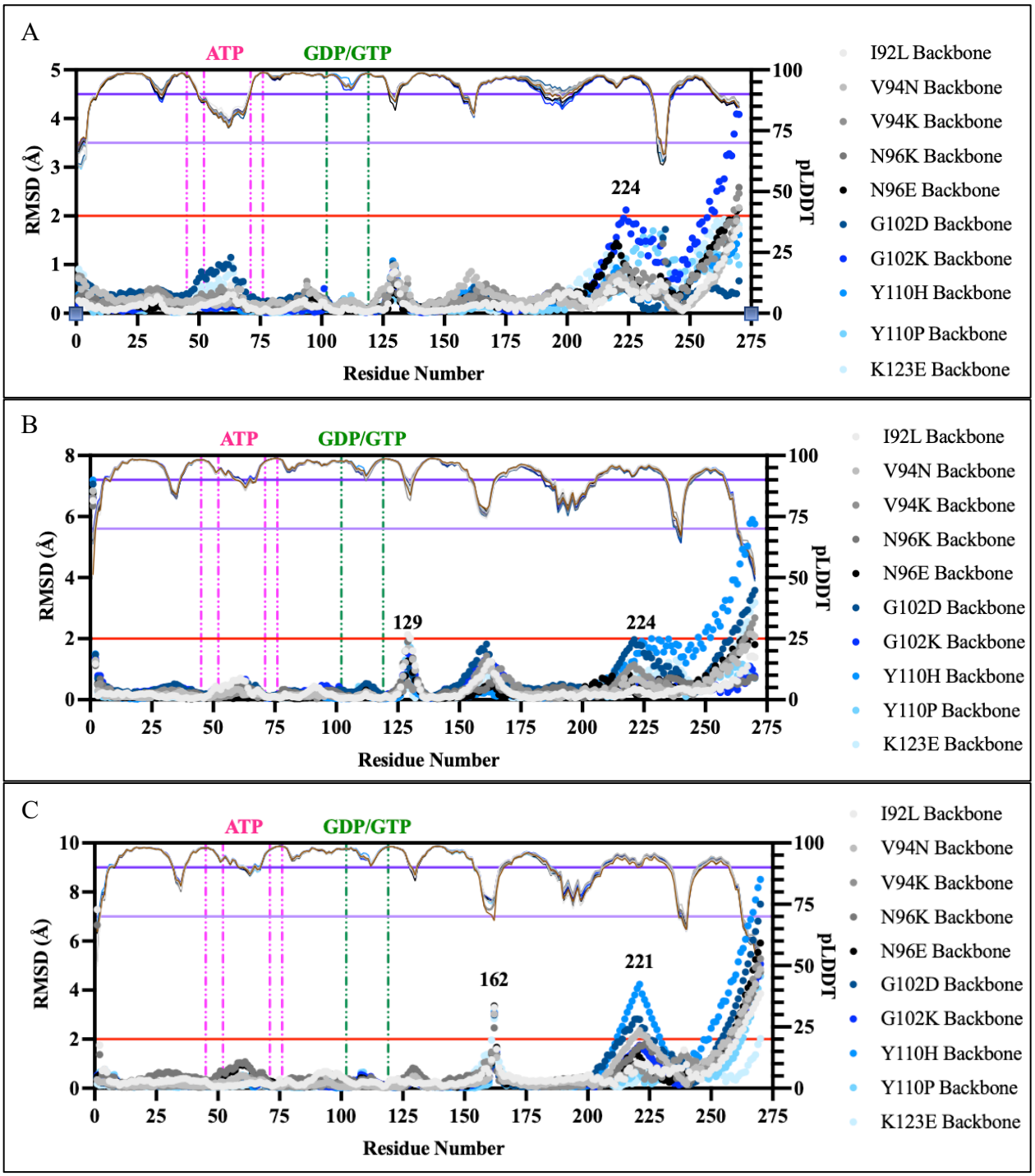


Figure 11. *C. difficile* RelQ Single SOE RMSD AlphaFold 3.0. (A) Overlay of the RMSD traces of all AlphaFold 3 WT RelQ single mutants. (B) Overlay of the RMSD traces of all AlphaFold 3 ATP, GDP, and Mg RelQ single mutants. (C) Overlay of the RMSD traces of all AlphaFold 3 ATP, GTP, and Mg RelQ single mutants. Conserved ATP binding region (magenta), conserved GXP binding region (green), and hot spots (red) are shown in their respective colors. The hot spots are anywhere on the protein with an RMSD above 2 Å. Black dots are the average

RMSD of the atoms (nitrogen, alpha carbon, carbonyl carbon, and carbonyl oxygen) that are a part of the backbone. The red line at an RMSD of 2 Å that is the cutoff for amino acids that are considered hotspots. The magenta and lime green dash/dot lines are the conserved binding regions of the indicated nucleotide. The light and dark purple lines are at a pLDDT value of 70 and 90, respectively, to show the predicted confidence levels of 'normal confidence' and 'very confident.' pLDDT values, per residue, are traced with a mocha (wildtype prediction) line trace and the same color as gradient in the legend for the mutations.

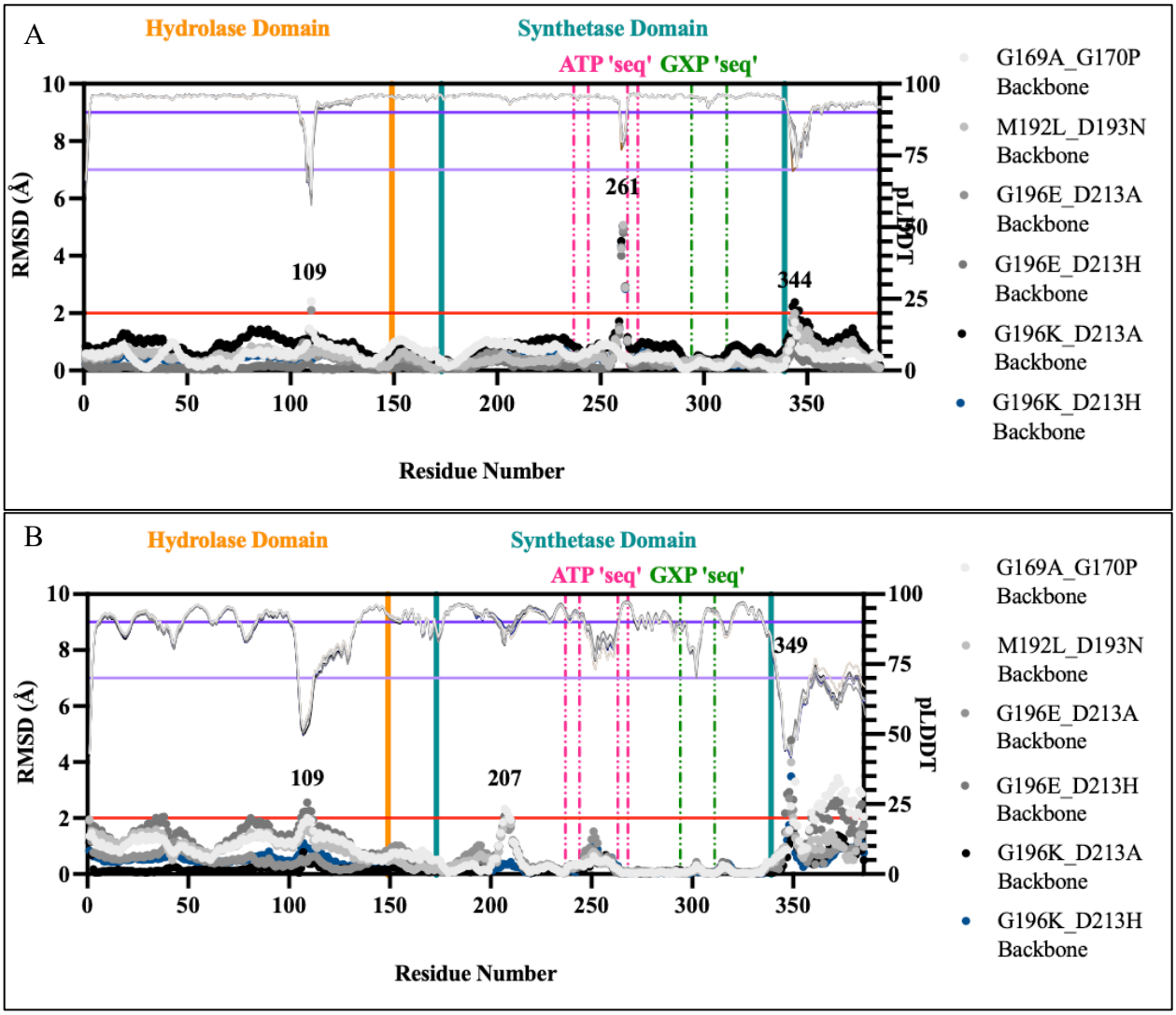


Figure 12. Double Point Mutations *C. difficile* RSH Predictions. Testing all mutations against (A) wildtype AlphaFold 2 *C. difficile* and (B) wildtype AlphaFold 3 *C. difficile*. Conserved ATP binding region (magenta), conserved GXP binding region (green), and hot spot (red) are shown in their respective colors. The hot spots are anywhere on the protein with an RMSD above 2 Å. Black dots are the average RMSD of the atoms (nitrogen, alpha carbon, carbonyl carbon, and carbonyl oxygen) that are a part of the backbone. The red line at an RMSD of 2 Å that is the cutoff for amino acids that are considered hotspots. The magenta and lime green dash/dot lines are the conserved binding regions of the indicated nucleotide. The light and dark purple lines are at a pLDDT value of 70 and 90, respectively, to show the predicted confidence levels of 'normal confidence' and 'very confident.' pLDDT

values, per residue, are traced with a mocha (wildtype prediction) line trace and the same color as gradient in the legend for the mutations.

All the point mutations were combined and visualized for their effects on overall structure. These results were compared against *C. difficile* and *S. equisimilis* to see if normal structure, a structure that matches an enzyme that produces all alarmones, returned. The combination of: G169A, G170P, M192L, D193N, G196E, and D213A; G169A, G170P, M192L, D193N, G196K, and D213H created no spike in residue 207. However, the combination of: G169A, G170P, M192L, D193N, G196E, and D213H; G169A, G170P, M192L, D193N, G196K, and D213A created the same observed 3 Å spike within the alpha helix at residue 207.

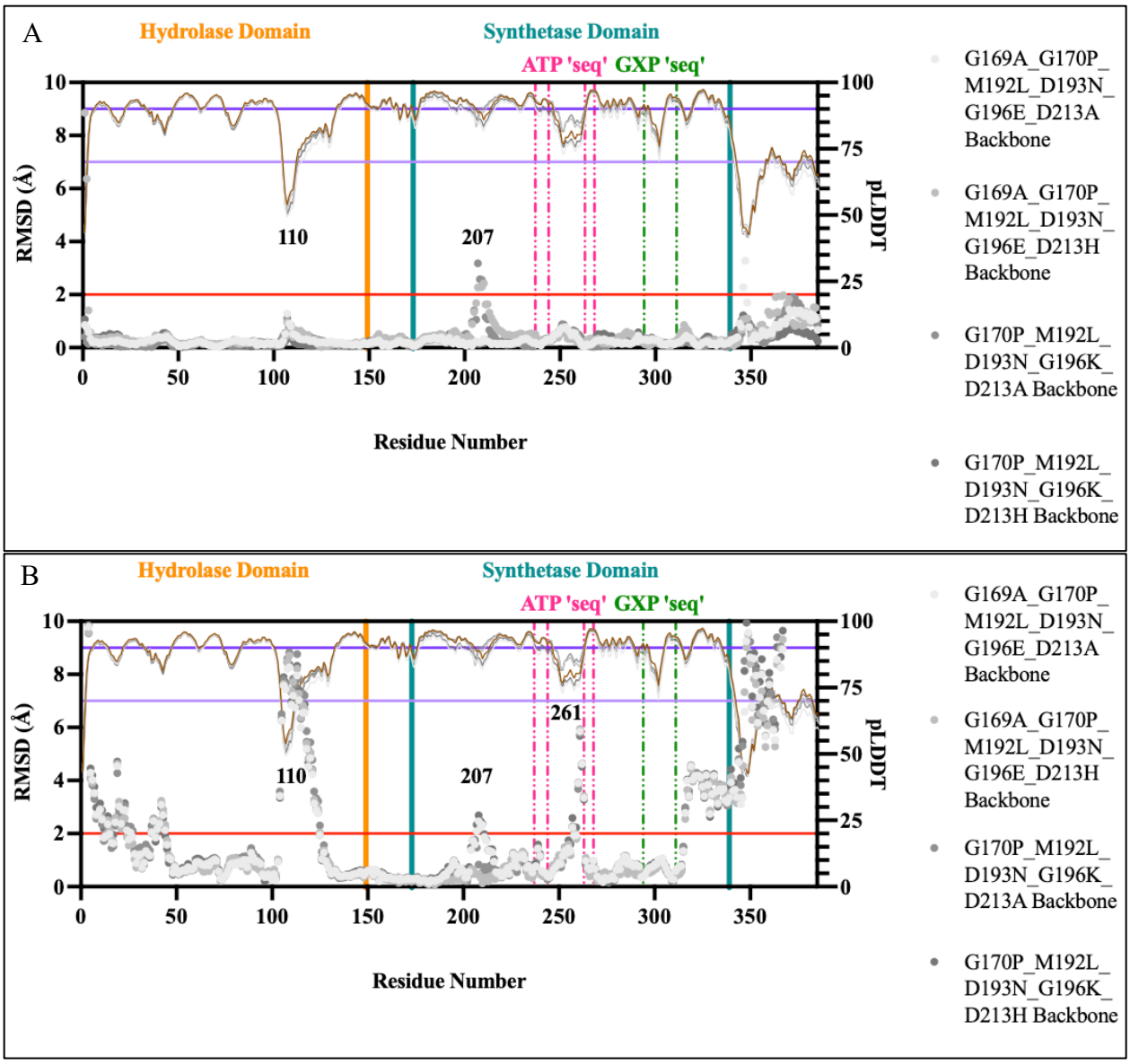


Figure 13 Multiple AlphFold SOE. Testing all mutations against (A) wildtype AlphaFold *C. difficile* and (B) wildtype AlphaFold *S. equisimilis*. These mutations were modeled with ATP, GDP, and magnesium. Conserved ATP binding region (magenta), conserved GXP binding region (green), and hot spot (red) are shown in their respective colors. The hot spots are anywhere on the protein with an RMSD above 2 Å. Black dots are the average RMSD of the atoms (nitrogen, alpha carbon, carbonyl carbon, and carbonyl oxygen) that are a part of the backbone. The red line at an RMSD of 2 Å that is the cutoff for amino acids that are considered hotspots. The magenta and lime green dash/dot lines are the conserved binding regions of the indicated nucleotide. The light and dark purple lines are at a pLDDT value of 70 and 90, respectively, to show the predicted confidence levels of 'normal confidence' and 'very confident.' pLDDT values, per residue, are traced with a mocha (wildtype prediction) line trace and the same color as gradient in the legend for the mutations.

The RMSD measurements only assessed the effects of mutations on the residues of the protein. To see if the mutations caused any changes in ligand positioning or orientation, the

distance between the phosphate groups, GXP 3’ hydroxyl, and catalytic magnesium ions were measured using PyMOL and compared between the wild-type and mutant structures.

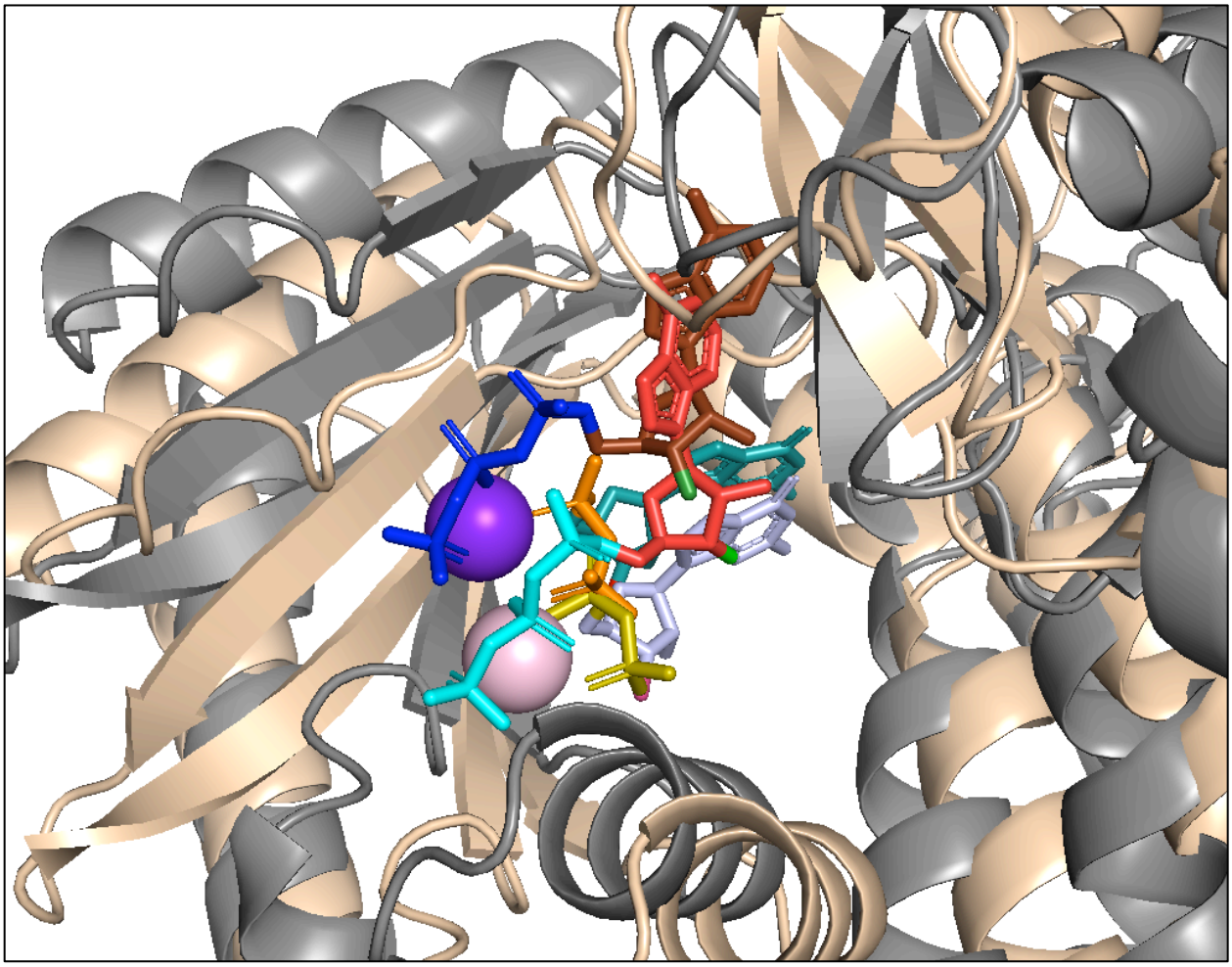


Figure 14. Determining Predicted Distance of Ligands. Wildtype (WT) *C. difficile* RSH (tan) vs mutated G196E *C. difficile* RSH (gray). (A) *S. equisimilis* RSH AlphaFold (gray) vs *C. difficile* RSH AlphaFold (tan) structure. The various colors of the ligands are: deepteal, Mutated GDP; chocolate, Mutated ATP; lightblue, WT GDP; tv_red, WT ATP; purpleblue, Mutated Mg; lightpink, WT Mg; red, Mutated GDP 3’ OH; orange, Mutated Phosphate Groups; warmpink, WT GDP 3’ OH; olive, WT GDP Phosphate groups; cyan, WT ATP Phosphate Groups; blue, Mutated ATP Phosphate Groups; forest, Mutated ATP 3’ OH; green, WT 3’ OH.

Each atom of the ligands and coordination ions were compared against wildtype structure and the distance was measured using the get_distance command in PyMOL (Figure 15).

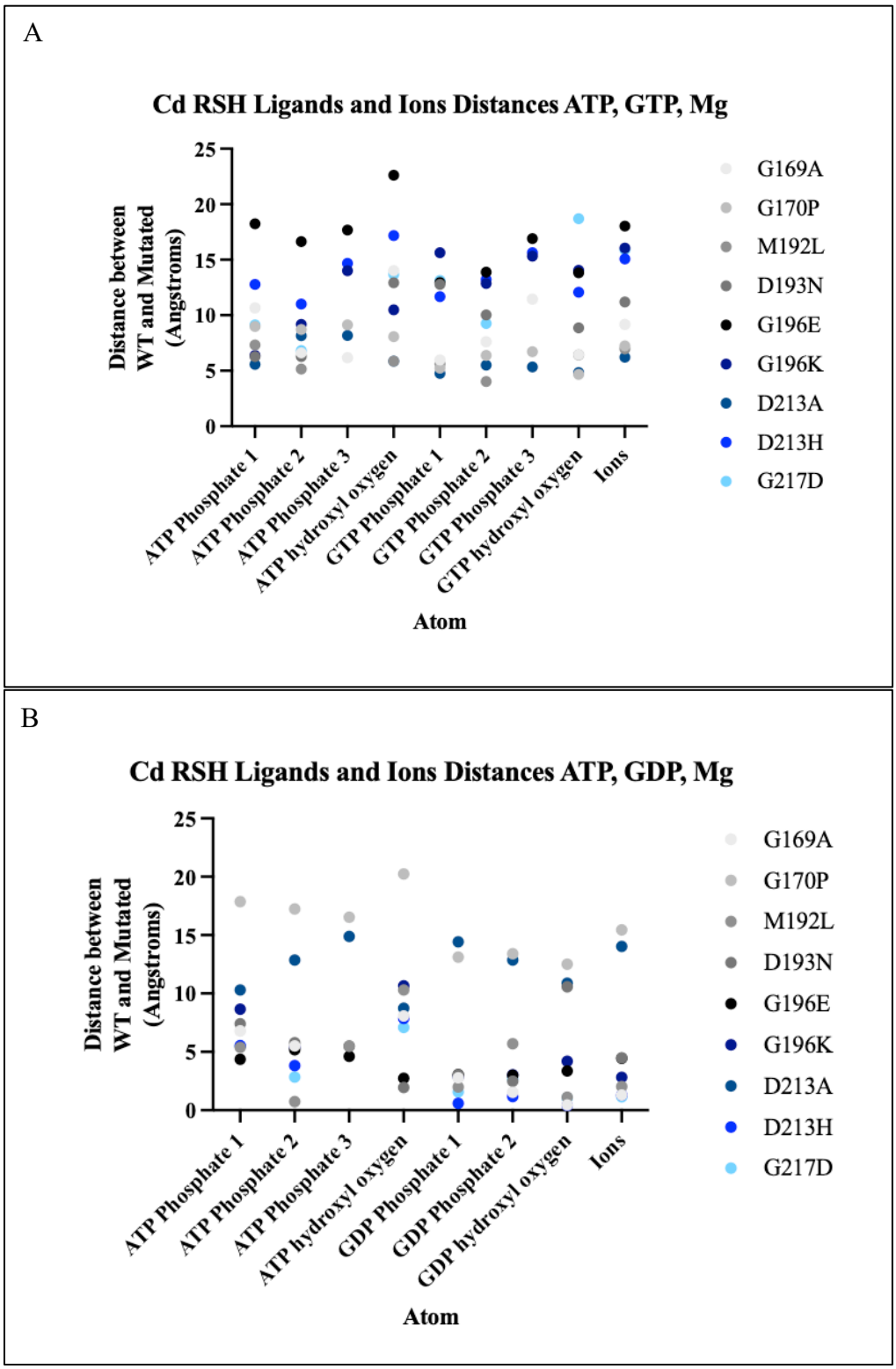


Figure 15. Measured Differences between ligands and ions. (A). Measured difference (Å) of specified atoms between wildtype and mutations containing GTP. (B). Measured difference (Å) of specified atoms between wildtype and mutations containing GDP.

There were more residues responsible for the jump when modeled with ATP, GTP, and magnesium; G169A, G170P, D193N, G196E, G196K, D213A, D213H, and D217D. Therefore, the only single point mutation to not cause any RMSD spike at residue 207 was M192L. However, for the multiple mutations done (Figure 13) the combination of: G196E and D213A; G196K and D213H created no spike in residue 207. However, the combination of: G196E and D213H; G196K and D213A created the same observed 3 Å spike within the alpha helix at residue 207. The largest differences in ligand atom distances that were calculated came from G170P, G196K, and D193N.

Discussion of *In silico* Mutagenesis

These predictive structural analyses of *C. difficile* RSH and RelQ were used to assess the structural effects of creating single, double, and multiple point mutations in the clostridial sequences *in silico*. Predictions of *B. subtilis* RelQ and Se RSH overlay well with the actual crystal structures. There are gaps, caused by low resolution, in the X-ray crystallography structures, which cause similar gaps in the RMSD plots (Figure 4 and Figure 5). The AlphaFold predictions were able to fill in those gaps, which is the reason all mutant predictions were compared to homolog structures. Apart from loops, *C. difficle* RSH and RelQ have similar overlays of structure calculated by the RMSD to homologs (Figure 6 and Figure 7). If the overall movement of those loop regions could be averaged, then the RMSD spikes would be eliminated if the true structures are meant to be identical. The problem is that the predictions and X-ray structures is that they are pictures of a constantly moving protein. All the RelQ RMSD predictions were near zero, meaning that the selected residues are predicted to not have a role in

overall structure of the protein. Therefore, those *in silico* mutations do not provide insight into the structural basis of *C. difficile* unique alarmone synthesis. However, RSH had some exciting results. The hotspot created at residue 207 in Figure 9 was unexpected as it has a relatively high pLDDT value above 80, was caused by mutations upstream and downstream of it, and is close to a metabolically active site. While this position is a conserved residue among RSH enzymes, the fact that multiple distant point mutations all had predicted allosteric effects at the same position suggests that this position is very responsive to its surroundings. This lysine is adjacent to an arginine, which are both conserved residues involved in coordinating the GXP substrate. Indeed, the mutations which shift RSH residue 207 also affect the positioning of the nucleotide substrates in the AlphaFold 3 models, which could contribute to the different ways the 5' phosphates are processed by *C. difficile* RSH and canonical enzymes. The largest differences in ligand atom distances that were calculated were for G170P, G196K, and D193N. These phosphate groups were above 10 Å away from each other meaning the predicted structure had modified their position in space to a location unsuitable for alarmone production. These computational studies have allowed us to hypothesize about which mutations will be significant and prioritize our future mutational experiments accordingly rather than guess, perform the experiment, and check fewer positions.

Chapter III

EXPLORING THE PUTATIVE SYNTHETASE RELC

Introducing RelC as a Potential Novel Alarmone Synthetase

The Kyoto Encyclopedia of Genes and Genomes (KEGG) predicts a third member of the RelA/SpoT family in the *C. difficile* genome. This gene contains an N-terminal region identified as a putative guanosine pyrophosphotransferase and a C-terminal region with no characterized homologs. When this sequence is submitted to the NIH BLAST database, it has exactly one hundred homologs which are all found in Firmicutes species (Figure 16) (53–60). By contrast, BLAST searches of *C. difficile* RSH and RelQ have broader matches. We have named this gene *relC*, for potential Rel from Clostridia.

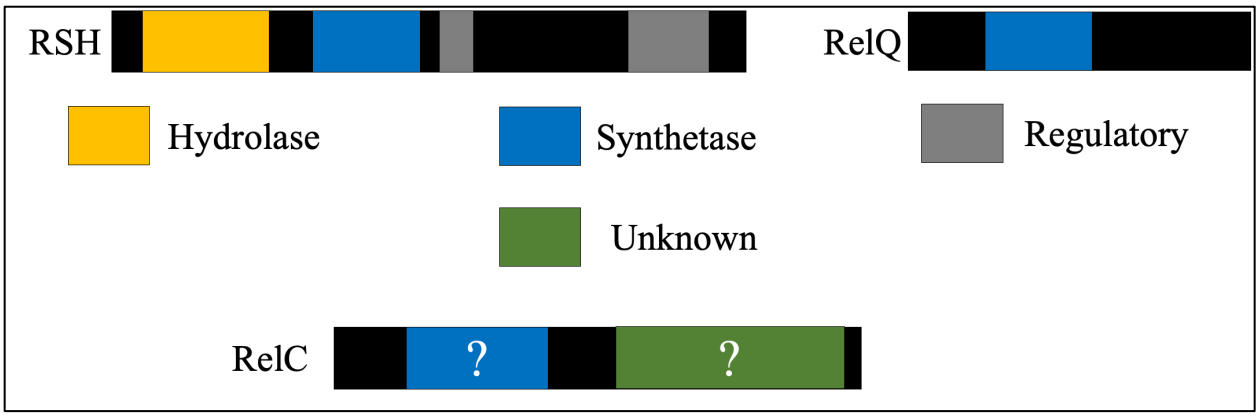


Figure 16. Domains of Alarmone Enzymes. RSH and RelQ are already characterized alarmone enzymes with synthetase domains, but there is a third putative enzyme that has similarities to the synthetase domain. Using the NIH's protein BLAST tool, the sequence of RelC (KEGG entry: CD630_17080) has only been characterized in firmicutes bacteria. This signifies the exciting path of developing the genetic tools to study the novel protein for future studies of drug development specific to *C. difficile.*

Because RelC lacks several catalytically important alarmone synthetase domain residues and contains an inserted sequence in the middle of the predicted GTP binding region (Figure 3), we suspected that it would not be an active alarmone synthetase but sought to predict its sequence to determine whether to express it for characterization.

Utilizing AlphaFold for RelC Synthetase Function

To assess whether it would be worthwhile to express RelC and test it as an alarmone synthetase, we performed AlphaFold structural modeling of this protein (Figure 17) and compared its putative alarmone synthetase domain to the confirmed pGpp synthetase *C. difficile* RelQ (Figure 18).

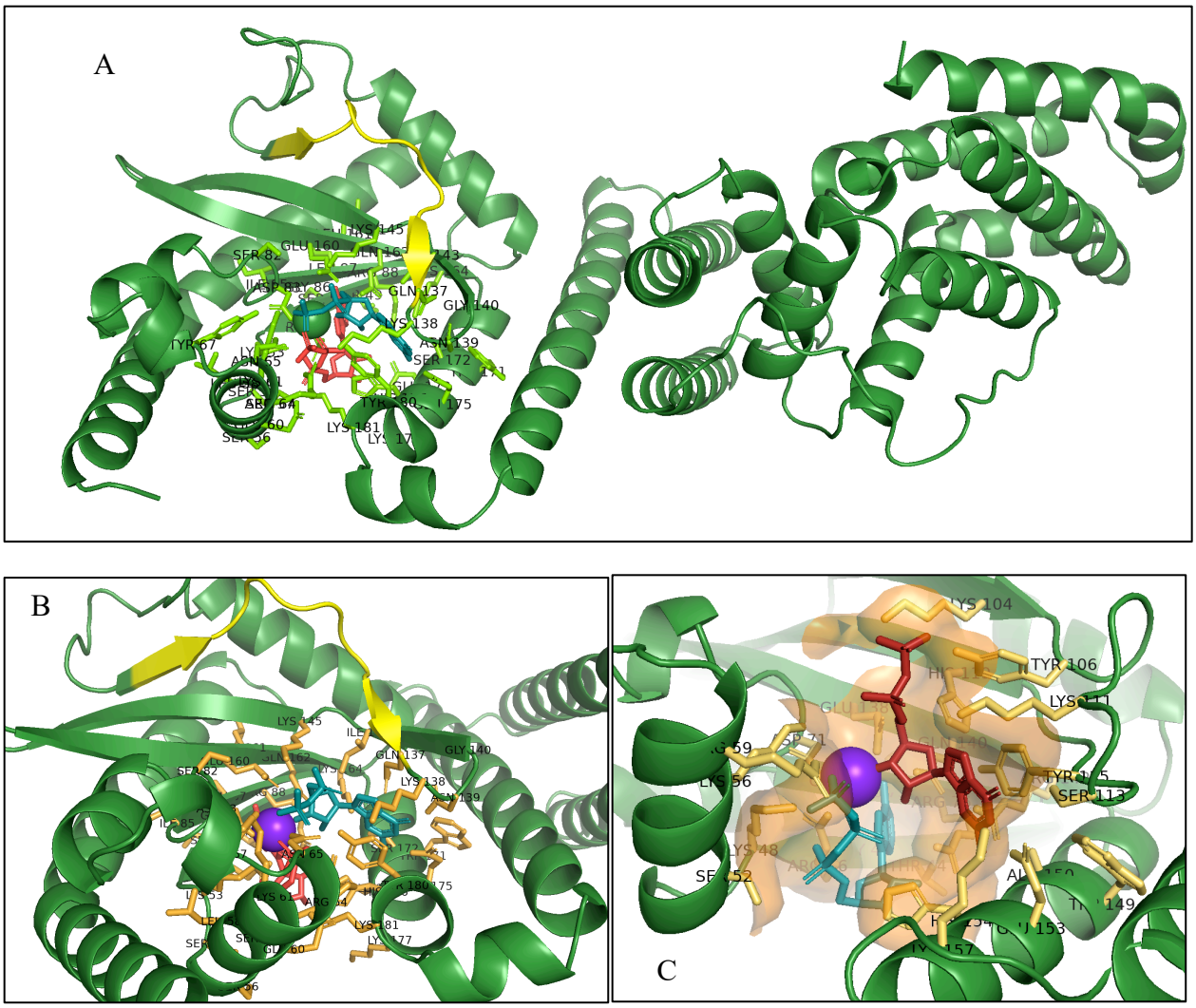


Figure 17 Modeling RelC with Ligands. (A) full length RelC. (B) Zoom on NTD with ATP, GDP, and magnisium (C) ATP (red), GTP (teal), and magnesium (purple). The inserted loop has no impact on the active site as previously predicted through primary structure overlay.

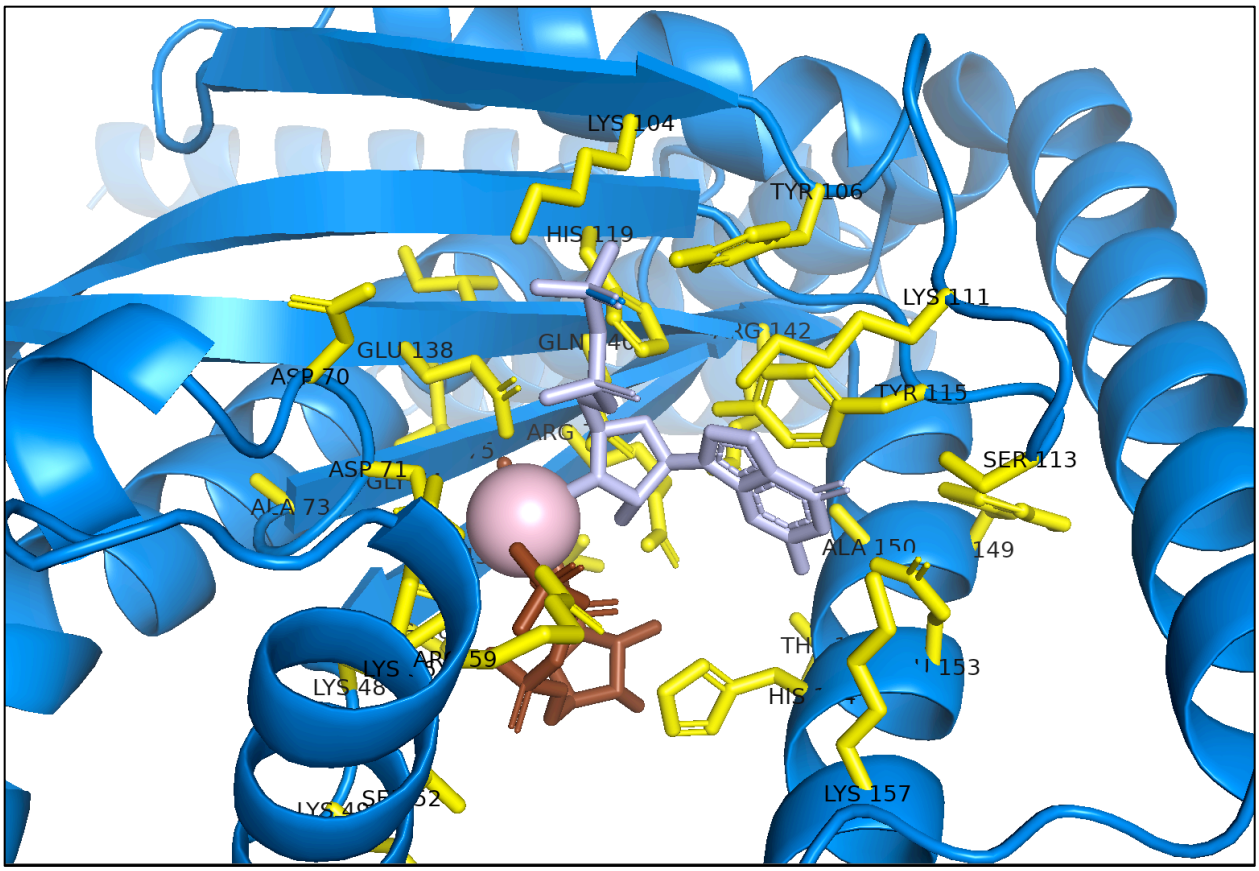


Figure 18. RelQ with Ligands. RelQ (blue) modeled with ATP (chocolate), GDP (light blue), and magnesium (ligth pink). Side chains that make up the binding pocket are colored yellow and are labeled.

Because the potential active site of the predicted RelC synthetase domain exhibited more structural similarity to RelQ than expected, and because the insertional loop in the putative GXP-binding picket (Figure 3) did not occlude potential GXP binding as we had predicted (Figure 19), we attempted to express RelC for functional analysis. We designed primers that would amplify the *relC* gene, adding a C-terminal hexahistidine tag and restriction enzyme cut sites, and amplified the gene from *C. difficile strain* R20291 genomic DNA. Because the *relC* is located in a highly repetitive, AT-rich region of the *C. difficile* genome, amplification was challenging, and we had to test multiple polymerases and experimental protocols before amplifying a piece of DNA the predicted size of the *relC* gene (Figure 21).

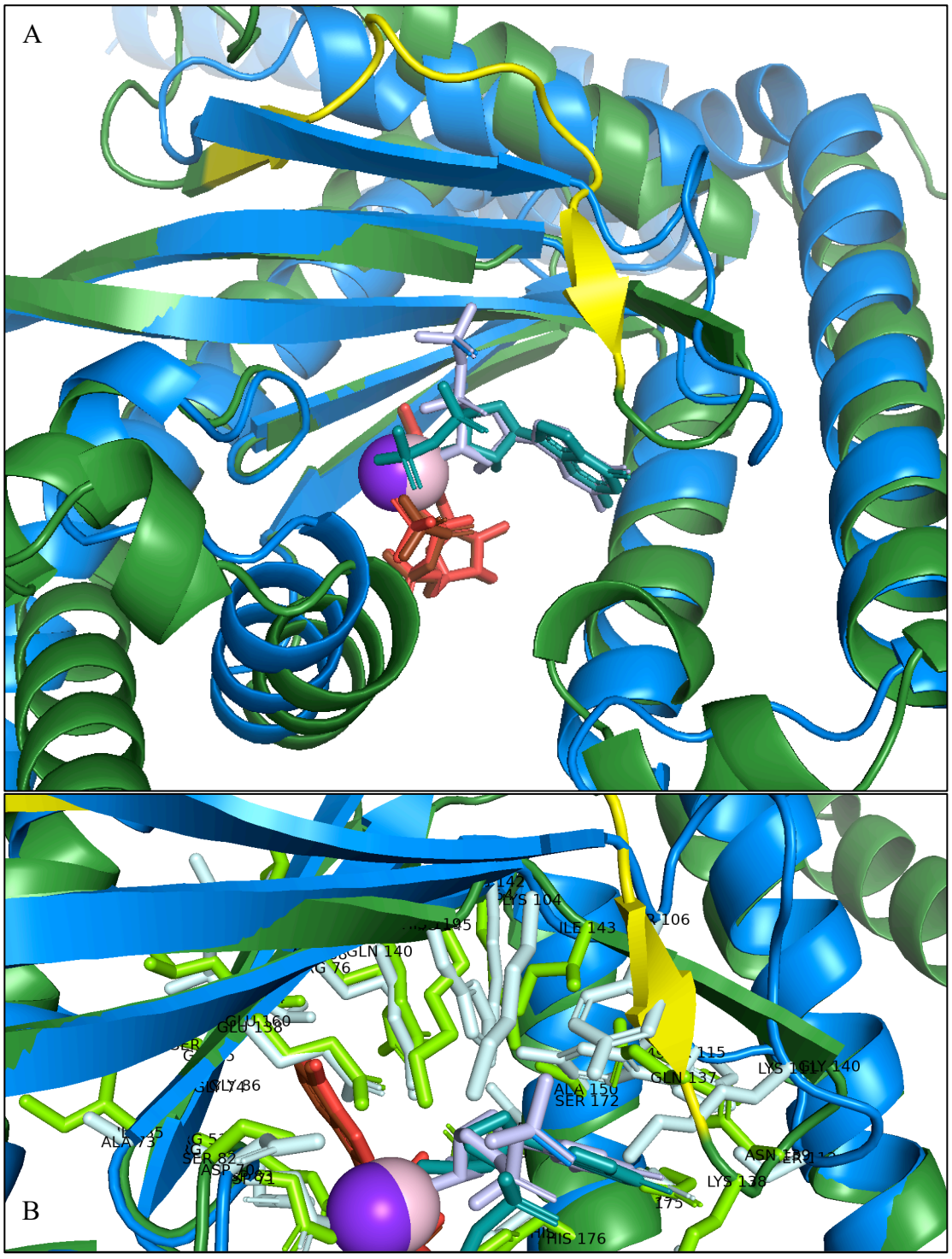


Figure 19. NTD RelC vs RelQ. (A). NTD RelC (green) vs RelQ (blue). ATP (red), GDP (teal), and magnesium (purple). RMSD plot. Inserted loop (yellow). (B). NTD RelC (green) with labeled sidechains (bright green) vs RelQ (blue) with labeled side chains (light blue). ATP (red), GDP (teal), and magnesium (purple). RMSD plot. Inserted loop (yellow).

To visualize if *C. difficile* RelC is capable of oligomerizing, it was overlayed with the tetramer structure of *B. subtilis* (Figure 20).

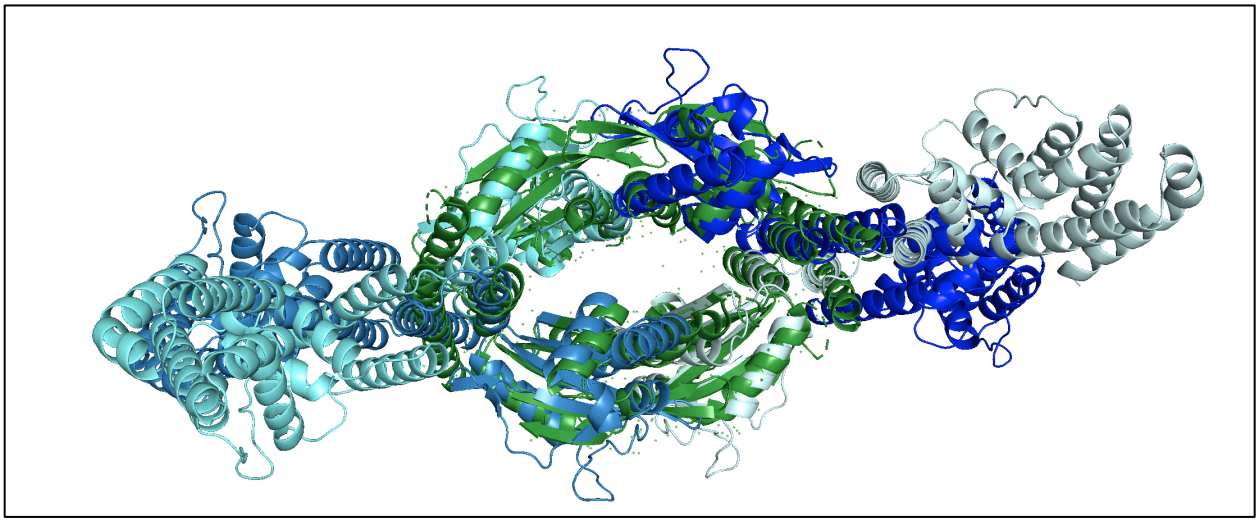


Figure 20. Potential RelC Tetramer. Green *Bacillus subtilis* RelQ tetramer. Shades of blue *C. difficile* RelC.

We did amplify a single product with reasonably high yield and purity (Table 4), so we performed restriction enzyme digests and ligated the amplified sequence into the pMMBneo vector for protein expression in E. coli. After digestion, another gel was run with all the digested relC product and 2.5 µL pMMBneo (Figure 23). The bands seen in Figure 23, rows 3 and 4, were cut out and gel purified. Additionally, for Figure 22, since no pMMBneo band was observed in row 8, another gel check was performed with 2.5 µL from a different miniprep sample of a DH5α colony containing the pMMBneo vector and its concentration was measured using a BioDrop DUO spectrophotometer (Table 5).

Preliminary Experiments in Determining RelC Function

The sequences of *C. difficile* RelC (CD630_17080) were obtained from the Kyoto Encyclopedia of Genes and Genomes and pasted into AlphaFold 3.0 to generate. cif files as described above (49). The N-terminus domain of the resulting model was aligned with the AlphaFold prediction for *C. difficile* RelQ (KEGG Entry: CD630_03450).

RelC-HIS_{CTD} cloning: Amplification

C. difficile 630Δ*erm* was grown at 37 ºC in a Coy anaerobic chamber (Coy Laboratory) with an atmosphere of 10.0 % CO_2, 5.0 % H_2, and 85.0 % N_2 on a brain heart infusion-supplemented (BHIS, VWR) medium agar plate. A single colony was picked from the plate and placed into a glass test tube containing liquid BHIS medium and grown overnight. The next day, 1 mL of liquid culture was placed into a 1.5 mL microcentrifuge (mc) tube, which was taken out of the glove box and immediately sterilized with SporGon, 70% ethanol, and 10% bleach. The cells were boiled in a 95 °C dry bath incubator for 10 minutes and spun for 2 minutes at 8,000 x g. The supernatant was removed and the DNA within the pellet was extracted using the GeneJET Plasmid Miniprep Kit (Thermo Fisher Scientific) according to manufacturer instructions. The primers used for amplification are listed in Table 3. The primers were designed to introduce a C-terminal hexahistidine tag between the last functional codon and the stop codon. PCR amplification with these primers was performed with Phusion HS II DNA Polymerase (New England Biolabs) and Q5 DNA Polymerase (NEB) and tested in the presence and absence of 10 µM supplemental magnesium chloride. An initial PCR gradient was run ranging from 75-57 °C that tested two high-fidelity DNA polymerases, Q5 and Phusion, with and without supplemented magnesium chloride ($MgCl_2$) for 10 µL total volume reactions. PCR was performed with the following conditions: the initial denaturation was set at 98 °C for 2 minutes; subsequent

denaturation at 98 °C for 30 seconds; annealing of primers was performed at a temperature gradient ranging from 75 °C to 57 °C for 30 seconds; and an extension phase at 72 °C for 2 minutes. Denaturation, annealing, and extension were repeated for 34 additional cycles with the 35th cycle having a final extension at 72 °C for 5 minutes and held at 4 °C.

RelC-HIS_{CTD} Cloning

Products were assessed by visualization on a 1% agarose gel. A gel electrophoresis apparatus (Bio-Rad) was used to determine the success of the reaction, which required setting up an agarose gel made from the combination of the following: 1% agarose powder; 1X Tris base, acetic acid, and ethylenediaminetetraacetic acid (TAE) buffer (Invitrogen); and 0.01% SYBR™ Safe (Thermo Fisher Scientific). 5 µL of SimplyLoad™ Tandem DNA Ladder (Lonza) was run alongside 5 µL of each PCR sample that contained 1 µL of 6X Loading Dye (Lonza) and any remaining PCR product was stored at -20 °C. After running the gel at 120 volts for 15 minutes, it was imaged using a SYNGENE U:GENIUS^3 ultra-violet gel imaging system. The remaining PCR product was taken out from the -20 °C freezer and any sample with a band (Figure 21) was combined into one 1.5 mL mc tube and the DNA concentration was determined using a BioDrop spectrophotometer (BioDrop Ltd.) (Table 4).

RelC-HIS_{CTD} Digestion and Ligation

Amplified sequence was digested with KpnI-HF® (New England Biolabs) and 1 µL PstI-HF® (New England Biolabs). Plasmid was digested in 1X rCutSmart™ Buffer (New England Biolabs) at 37 °C for 10 minutes. After the incubation, both samples were placed into a 1% agarose TAE gel using the same procedure outlined above for the confirmation of digestion success. The *relC* sequence that was digested with KpnI and PstI restriction enzymes was cut out of the gel, using a razor blade, and placed into a pre-weighed mc tube. This DNA product was

then purified in a similar fashion to the Thermoscientific GeneJet miniprep process of *C. difficile* cells. Then 50 ng of the digested sequence was quickly at room temperature for 10 minutes ligated into a *E coli* compatible vector, pMMBneo, in a 1:3 vector to insert ratio within a 20 µL T4 DNA ligase reaction (NEB #M0202S). The vector contains a promoter which is induced by a signal molecule, isopropyl-ß-D-thiogalactopyranoside (IPTG) a nonhydrolyzable lactate analog that mimic lactose but cannot be metabolized. After ligation, the plasmid with the *relC* insert was transformed into chemically competent DH5α *E. coli* (NEB). This strain was grown to exponential phase in media containing 50 µg/mL kanamycin and the plasmid was purified from a 5 mL cell culture. This plasmid was transformed into chemically competent *E. coli* BL21 (NEB). DH5α and BL21 strains containing the plasmid were stored in a 20% glycerol stock at -80°C.

Primer Name	**Sequence**	**Sequence Introduces**
Forward RelC	CA**ggtacc**TTGGAGAATACTTACTTATGATAGGAA	KpnI site (bold)
Reverse RelC	CC**ctgcag**TTAgtgatggtgatggtgatgCTCAAAGATTTGAATAATC	PstI site (bold) His-tag underlined

Table 3 relC Amplification Primer Sequences. Designed by Dr. E.B. Purcell.

RelC Protein Expression

For to express the RelC protein using *E. coli*. An inoculation loop was used to scrap some transformed cells, stored in a 20% glycerol LB media treated with 50 µg/mL kanamycin antibiotic at -20°C, and placed into fresh 50 mL of liquid LB media treated with 50 µg/mL kanamycin. pMMBneo vector contains kanamycin resistance. The cells were grown overnight and diluted in a 1:20 ratio into fresh kanamycin 50 µg/mL liquid LB broth until the cells reached

log phase. To induce the *E. coli* to make RelC, varying IPTG concentrations were added to the to troubleshoot effective induction concentrations: 37°C incubation for 3 hours 0 mM IPTG, 37°C incubation for 3 hours 1 mM IPTG, 37°C incubation for 3 hours 5 mM IPTG, 4°C incubation for 24 hours 0 mM IPTG, 4°C incubation for 24 hours 1 mM IPTG, 4°C incubation for 24 hours 5 mM IPTG, ~22°C incubation for 8 hours 0 mM IPTG, ~22°C incubation for 8 hours 1 mM IPTG, and ~22°C incubation for 8 hours 5 mM IPTG.

SDS-PAGE Preparation

Acrylamide 10% polyacrylamide gel. Tris-HCl buffer (pH 8.8) was added to establish the appropriate running environment. Polymerization of both gel solutions was initiated by the addition of ammonium persulfate (APS) and N,N,N',N'-Tetramethylethylenediamine (TEMED). APS serves as a free radical initiator, while TEMED accelerates the polymerization process. The solution within the 15 mL conical tube was then inverted 3-6 times to ensure proper mixing. A lower concentration of acrylamide (4%) was used in this layer to create a zone where proteins can concentrate before entering the running gel. The electrophoresis chamber was assembled with cleaned glass plates and spacers. The running gel solution was carefully poured between the plates, leaving space at the top for the stacking gel. An overlay of 70% ethanol was added to prevent dehydration during polymerization and create a solid line separating stacking from running gel. After the running gel solidified (approximately 5 minutes), the isopropanol layer was discarded using a chem wipe. The stacking gel solution was then pipetted on top of the running gel, and a well-forming comb was inserted to create sample loading wells. The entire assembly was left to polymerize completely. While the gels were solidifying, protein samples are prepared for loading. A 5x sample buffer containing sodium dodecyl sulfate (SDS), and DTT, and tracking dye was mixed with 20 µL protein samples. SDS disrupts protein structure and

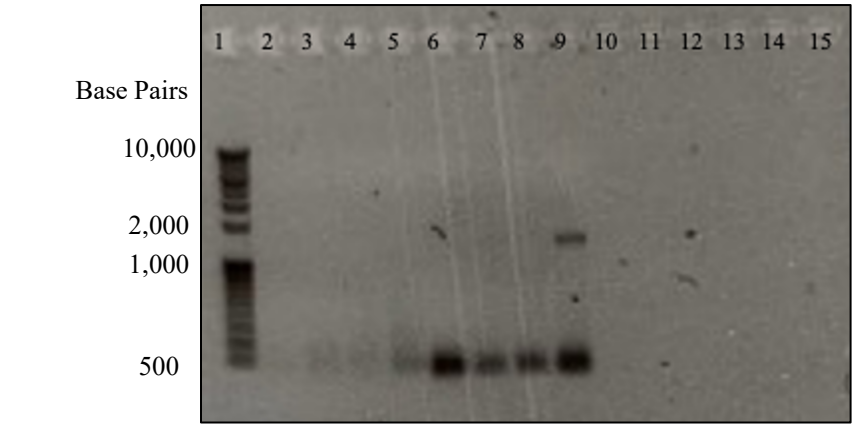
imparts a uniform negative charge to each protein, allowing separation based solely on size. The reducing agent, DTT, breaks disulfide bonds within proteins, ensuring complete linearization. The tracking dye allows for visualization of protein migration during electrophoresis. The protein-sample-buffer mixture was heated to facilitate protein denaturation and then placed on ice until loading. Finally, the electrophoresis chamber was filled with running buffer (Tris-glycine-SDS solution) to establish electrical continuity. The polymerized gel cassette was placed within the BioRad chamber. Prepared protein samples, along with a reference protein ladder, were carefully loaded into the wells using micropipettes. The power supply was connected, and electrophoresis was run at a constant 200 voltage for 40 minutes. After, the SDS-PAGE was taken out from the electrophoresis chamber, stained with commassie brilliant blue for 1 hour on a rocking table set at half speed, destained using a reagent alcohol/acetic acid solution for 8-12 hours and imaged on a table light.

Western Blot

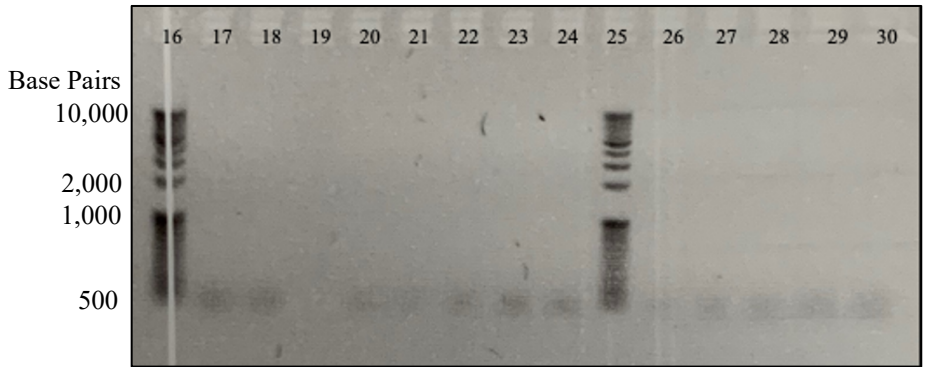
A 10% running gel solution and a 4% stacking gel solution was used to separate proteins based off their size following the SDS-PAGE protocol above. After the 40 minutes of applied 200 volts, The protein-laden gel was equilibrated in transfer buffer then sandwiched onto a nitrocellulose membrane. Following transfer, the membrane was blocked with a milk solution to prevent non-specific antibody binding. The anti-histidine primary antibody (Invitrogen Thermo Scientific) , designed to recognize the His-tag, was incubated with the nitrocellulose membrane. This antibody specifically binds only to proteins harboring the His-tag, ensuring detection of RelC. Next, the unbound primary antibody was thoroughly washed away using a PBS solution in a rocker for 10 minutes in 3 separate washes. A secondary antibody was then incubated with the

nitrocellulose membrane. This secondary antibody recognized the Fc region of the anti-His primary antibody, acting as a signal amplifier and was visualized with an iPhone camera.

A.



B.



C.

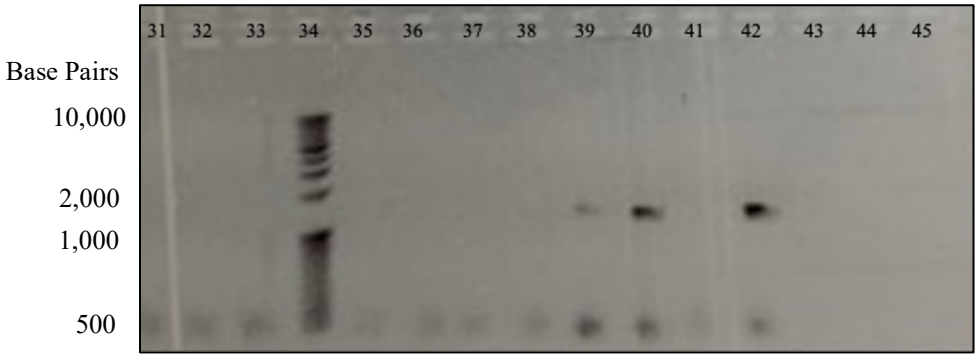


Figure 21. Images of Gel Electrophoresis Confirming PCR. Gel ran at 120V for 25 minutes. (1) 5 µL Tandem DNA Ladder. (2)-(9) 1µL 6X Loading Dye with 5 µL DNA template in Phusion buffer with temperature gradient from 75-57 °C. (10)-(15) blank. (16) 5 µL Tandem DNA ladder. (17)-(24) 1µL 6X Loading Dye with 5 µL DNA

template with Q5 buffer buffer temperature gradient from 75-57 °C. (25) 5 µL Tandem DNA Ladder. (26)-(33) 1µL 6X Loading Dye with 5 µL Q5 with additional 10 mM MgCl2 in temperature gradient from 75-57 °C. (34) 5 µL Tandem DNA Ladder. (35)-(38) 5 µL DNA template with Phusion buffer with additional 10 mM MgCl2 in temperature gradient from 75-57 °C. (43)-(45) Blank.

Sample	Run	A230 (A)	A260 (A)	A280 (A)	A320 (A)	$\frac{A260}{A230}$	$\frac{A260}{A280}$	Concentration (ng/µL)
RelC PCR column purified	1	0.063	0.030	0.025	0.017	0.285	1.615	13.12
	2	0.063	0.029	0.024	0.016	0.274	1.640	12.81

Table 4. DNA Concentrations and Purities. Values recorded by a BioDrop DUO spectrophotometer. Average of the two concentrations is 12.97 ng/µL.

After gel purification, all digested *relC* was lost as multiple BioDrop replicates read zero concentration, data not shown. Thus, another colony PCR was performed in a 20 µL total reaction of Phusion with supplemented $MgCl_2$ at an annealing temperature of 57°C. However, no bands were seen; data not shown. Thus, another gradient was performed with a total 20 µL reaction at 58.1°C and 55.8°C annealing temperatures using both high-fidelity DNA polymerases and 2.5 µL of the sample was used in another gel (Figure 23). For Figure 23, only one band was seen in row 15 that had supplemented $MgCl_2$ and an annealing temperature of 55.8°C. That sample was used as a template for further amplification and 2.5 µL of each PCR was used in a gel check, and every trial produced a band (Figure 23).

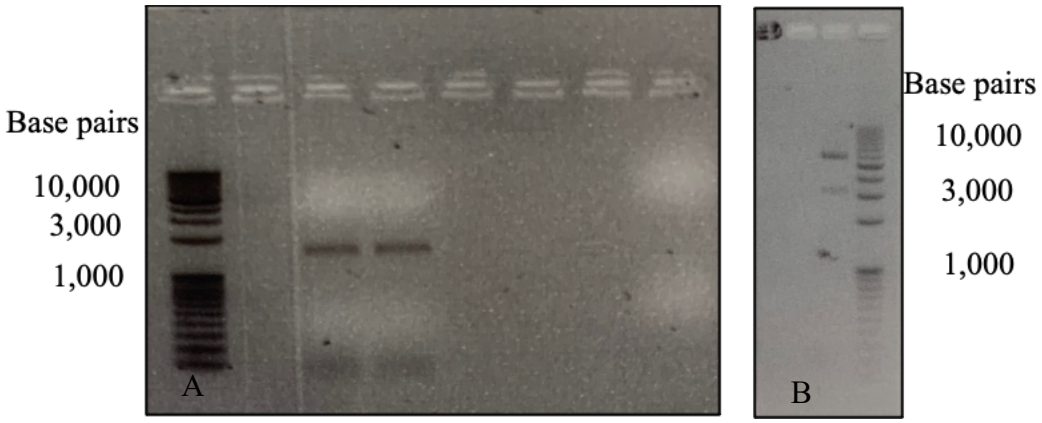


Figure 22. *relC* and pMMBneo Gel Electrophoresis Purification. Gel ran at 120 V for 20 minutes. (1) 5 µL Tandem DNA Ladder. (2) Blank. (3) and (4) the entire digested PCR amplified *relC* from lanes 9, 39, 40, and 42 from Figure 21. (5)-(7) Blank. (8) Digested pMMBneo. B) Gel Check of pMMBneo. Gel ran at 120 V for 30 minutes. (1) Blank (2) 2.5 µL DNA extracted from DH5α cells containing pMMBneo (Table 4) (3) 5 µL Tandem DNA ladder.

Sample	Run	A230 (A)	A260 (A)	A280 (A)	A320 (A)	$\frac{A260}{A230}$	$\frac{A260}{A280}$	Concentration (ng/µL)
pMMBneo Extraction for Figure 22	1	-0.036	0.046	0.013	-0.014	-2.757	2.214	60.18
	2	-0.032	0.051	0.017	-0.012	-3.161	2.170	63.05

Table 5. *relC*, Post-PCR, and Pre-Gel Purification and Isolation of pMMBneo. Average of the two pMMBneo concentrations was 61.62 ng/µL.

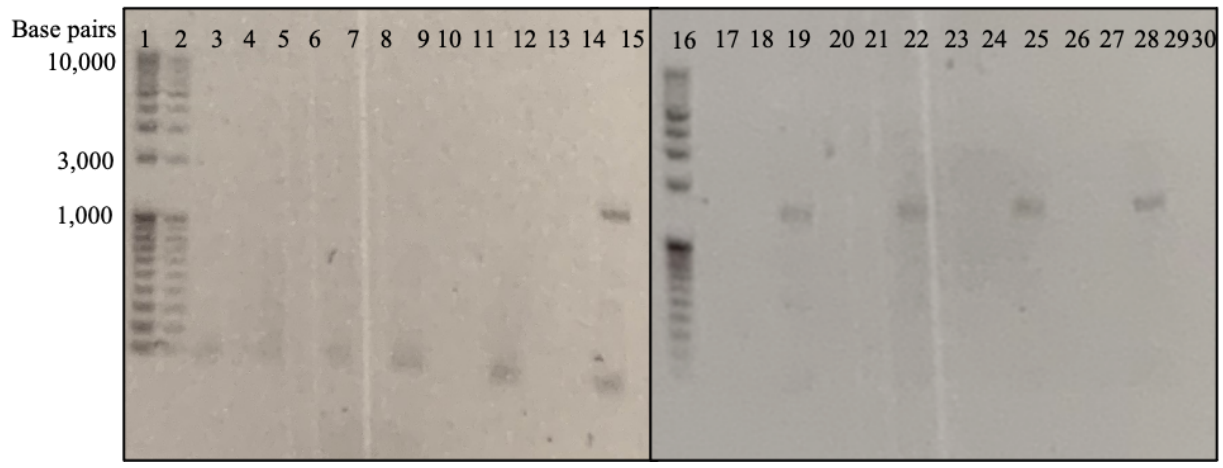


Figure 23. *relC* Amplification. Colony PCR gradient. Gel ran at 120 V for 30 minutes. (1) 5 µL Tandem DNA Ladder (TDL). All subsequent samples mixed with 0.5 µL 6x Loading Dye. (2) cracked well wall with some TDL from well 1. (3) 2.5 µL PCR sample ran with Phusion at 58.1°C annealing temperature. (4) Blank. (5) Phusion at 58.1°C annealing temperature supplemented with $MgCl_2$. (6) Blank (7) Q5 at 58.1°C annealing temperature (8) Blank. (9) Q5 at 55.8°C annealing temperature (10-11) Blank (12) Q5 at 58.1°C annealing temperature with supplemented $MgCl_2$. (13-14) Blank. (15) Q5 at 55.8°C annealing temperature with supplemented $MgCl_2$. B) Further amplification of *relC.* The previous round of PCR was used as the DNA template. Gel ran at 120 V for 25 minutes. (16) 5 µL Tandem DNA Ladder. (17-18, 20-21, 23-24, 26-27, 29-30) Blank. (19, 22, 25, 28) 2.5 µL Q5 at 55.8°C annealing temperature with supplemented $MgCl_2$.

After successful transformation of *relC* into pMMBneo, the cells were induced, as described above, and the SDS-PAGE and western blot were run and imaged (Figure 24). These were done to visualize any RelC production by *E. coli*.

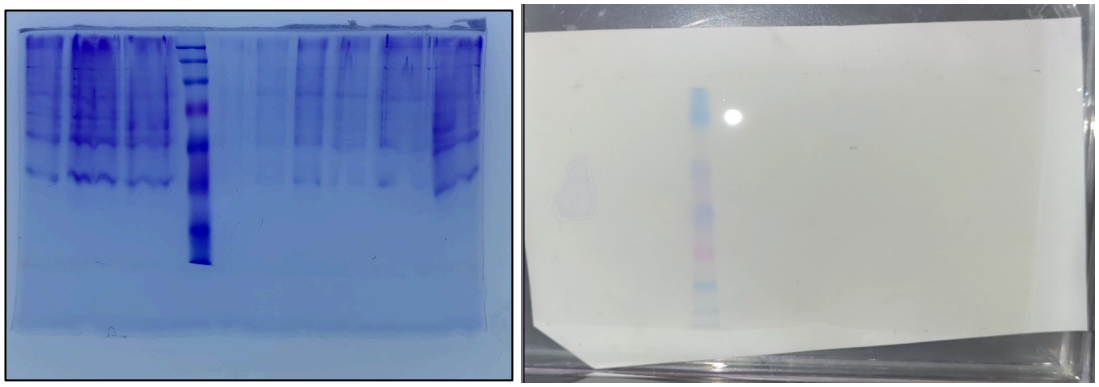


Figure 24. SDS-PAGE and Western Blot. For RelC. (1) 37°C incubation for 3 hours 0 mM IPTG (2) 37°C incubation for 3 hours 1 mM IPTG. (3) 37°C incubation for 3 hours 5 mM IPTG. (4) Molecular weight ladder. (5) 4°C incubation for 24 hours 0 mM IPTG. (6) 4°C incubation for 24 hours 1 mM IPTG. (7) 4°C incubation for 24 hours 5 mM IPTG. (8) ~22°C incubation for 8 hours 0 mM IPTG. (9) ~22°C incubation for 8 hours 1 mM IPTG. (10) ~22°C incubation for 8 hours 5 mM IPTG.

Discussion of the Potentially Novel Synthetase

RelC is a novel *C. difficile* protein with no available information on its function or interactions. Whether or not it is an alarmone synthetase, its narrow distribution in Firmicutes species (Figure 16) could provide a target for drug development that would specifically target *C. difficile*. It is a worthwhile target of study, but it was unclear whether we could hypothesize that it is an alarmone synthetase. It has the most homology to alarmone synthetases but has substantial sequence divergence (Figure 3). However, the predicted structure of RelQ is more conserved compared to *C. difficile* RelC than expected, and we have concluded that it is likely capable of binding ATP and GXP simultaneously, so we intend to assess its functionality as an alarmone synthetase. Even if it is not a synthetase, we will assess its capacity to hydrolyze both ATP and GDP/GTP. There is also a possibility that it is a degenerate synthetase domain that has been evolutionarily repurposed as an alarmone binding effector. This is very commonly found among the GGDEF domains that either synthesize or bind the GTP-derived signal molecule

cyclic di-GMP, but has never been reported for alarmone synthetases (61, 62). The ability of purified RelC to bind as well as synthesize pppGpp, ppGpp, and pGpp must be assessed.

The *rel C* sequence was difficult to amplify with a C-terminal histidine tag and the resulting *E. coli* expression strain yielded no protein, so future work will create an expression construct with an N-terminal affinity tag.

CHAPTER V

CONCLUSIONS

This work has provided a foundation for future research in learning more about characteristics unique to *C. difficile* as potential targets for drug therapies. *C. difficile* is hard to work with is and is the reason that at every name change, 'difficile' remained in the name. Anyone who works with this pathogen will become proficient at any experimental method from the number of failed replicates caused by the difficult nature of *C. difficile*. The goals of this project were to create the genetic tools for studying the novel RelC protein and do the bioinformatic work needed to create RSH and RelQ point mutants to compare their *in vitro* function to wild type RSH and RelQ. These *in silico* mutational analyses has accelerated these studies from being guess and check to hypothesis driven. We now know which mutations lead to slight conformational change that could result in enzyme function. Future studies can utilize these results to preform mutagenesis and characterize protein function. If normal alarmone production returns, then additional studies can be conducted to target those enzymatically important residues/domains.

Further work is needed to determine the function of the RelC protein with the use of transformed BL21 *E. coli* cells that can be plated, expressed, and purified for protein characterization. This will confirm whether RelC has a role in *C. difficile* atypical alarmone synthesis or not. For the other part of the project, once 2-3 residues are identified as important to uniqueness in alarmone synthesis, a screening drug library can be performed to see if any drug will affect *C. difficile* enzyme. With the control of those studies being the point mutants

responsible for creating typical alarmone synthesis. The designed drug may not be an antibiotic, it could just be an alarmone inhibitor that can be given with antibiotics. Ultimately, research of atypical alarmone synthesis of *C. difficile* opens avenues for CDI treatments that would reduce the billion-dollar burden on global healthcare systems caused by recurrent infections and save thousands of people from untimely deaths around the world.

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APPENDIX A

DESIGNED FORWARD SOE PRIMERS

RSH Forward SOE Primers	Mutation
GAAATTTATG**c**TGGTATAGCTCATAGATTAGG	G169A
GAAATTTATGGT**cc**TATAGCTCATAGATTAGGAATTTC	G170P
ATTAAGATTT**c**TGGACCCAGAAG	M194L
ATTAAGATTTATG**a**ACCCAGAAGGG	D195N
ATTAAGATTTATGGACCCAGAAG**a**GTATTATGATTTAG	G198E
ATTAAGATTTATGGACCCAGAA**aa**GTATTATGATTTAG	G198K
CAAAGAGAGG**c**TTATATTCAAGGAATTG	D215A
CAAAGAGAG**c**ATTATATTCAAGGAATTGTTG	D215H
CAAAGAGAGGATTATATTCAAG**ac**ATTGTTGAAC	G219D
RelQ Forward SOE Primers	**Mutation**
TATTATAGTCATATCATTTCTTACTTTTA**g**CAAATCAAC	I92L
TATTATAGTCATATCATTTCT**gtt**TTTTATCAAATCAAC	V94N
TATTATAGTCATATCATTTCT**ctt**TTTTATCAAATCAAC	V94K
TATTATAGTCATATC**c**TTTCTTACTTTTATCAAATC	N96K
TATTATAGTCATATC**ctc**TCTTACTTTTATCAAATC	N96E
AATCTTTTTC**gt**CTATTATAGTCATATCATTTCTTAC	G102D
AATCTTTTTC**ctt**TATTATAGTCATATCATTTCTTACTTTTATC	G102K
CACTATCTTTGT**g**ATTTGTAATATAATCTTTTTC	Y110H
CACTATCTTT**cgg**ATTTGTAATATAATCTTTTTC	Y110P

Table 6. Designed Forward SOE Primers. The highlighted yellow letters are mutations that will be introduced using SOE. The numbers are the location of the residue in the amino acid sequence. The letter before the number is the *C. difficile* residue. The letter after the number is the new amino acid that the nucleic acid change, in yellow, will cause.

APPENDIX B

DESIGNED REVERSE SOE PRIMERS

RSH Reverse SOE Primers	**Mutation**
AAGTGTCTCTTTTGCTTTATACTTTGCTTTTTCTGG	G169A and G170P
GCTCTGTCTTCTAGTTCCCAC	M194L, D195N, G198E, and G198K
ACTTCTCTTCATTGATACTCTACTAACTAAATCATAATACCC	D215A, D215H, and G219D
RelQ Reverse SOE Primers	**Mutation**
GGAGAAAAAGATTATATTACAAATTACAAAGATAGTGG	I92L, V94N, V94K, N96K, and N96E
ATATTACAAATTACAAAGATAGTGGTTATAGAAGCTATC	G102D and G102K
GTTATAGAAGCTATCATGTTATAATAAAATACCCTATAAAC	Y110H and Y110P
AAACTCCATTGCGGGTTC	K123E

Table 7. Designed Reverse SOE Primers. The letter before the number is the *C. difficile* residue. The letter after the number is the mutated amino acid that matches homologus enzyme residues.

APPENDIX C

P-31 NMR OF NUCLEOTIDES AND ALARMONES

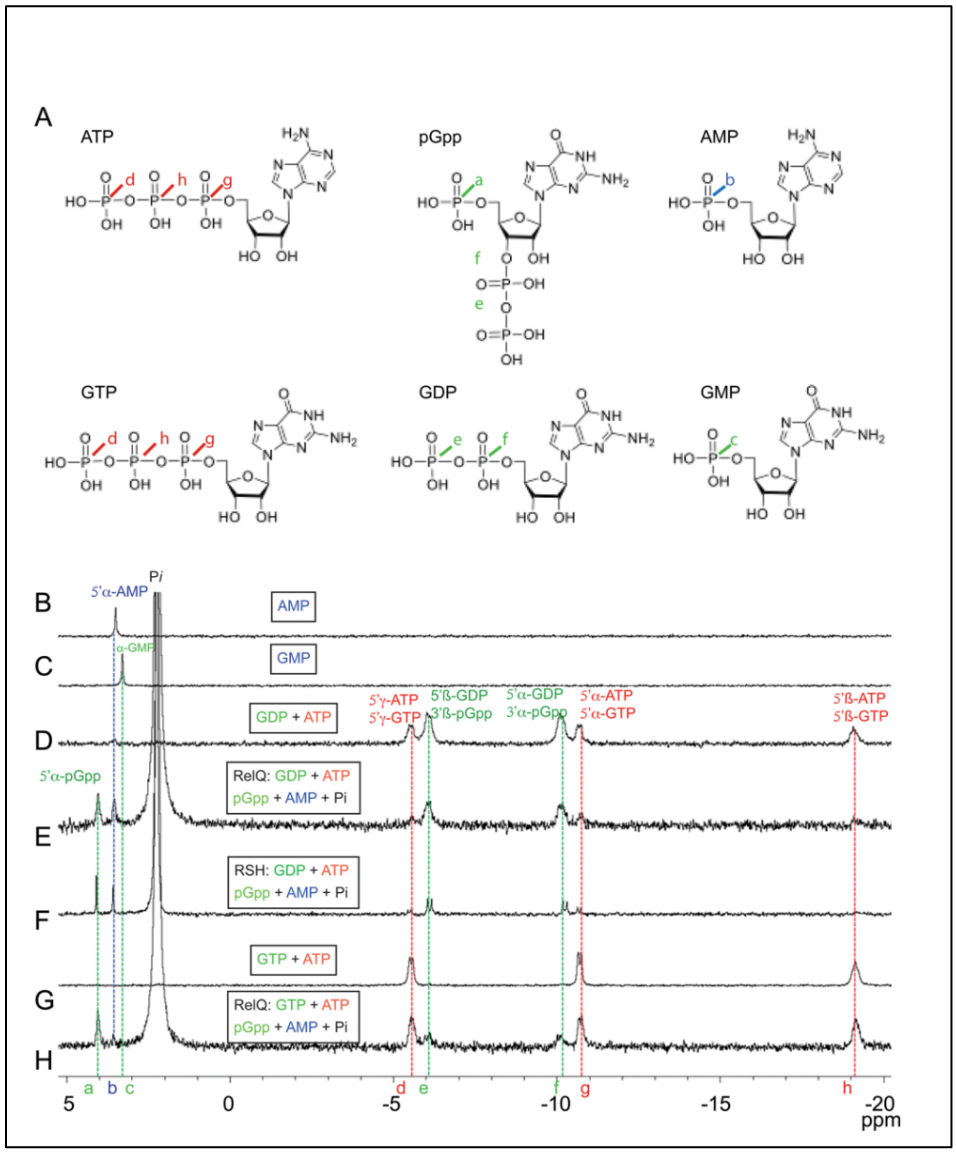

Figure 25. P-31 NMR of Nucleotides and Alarmones. Cleavage of ATP and pyrophosphate transfer. (A) Structures of guanosine nucleotides and resulting alarmones. (B) AMP standard. (C) GMP standard. (D) GDP and ATP standards. (E) GDP and ATP incubated with *C. difficile* RelQ. (F) GDP and ATP incubated with *C. difficile* RSH (G) GTP and ATP standards. (H) GTP and ATP incubated with *C. difficile* RelQ. Reproduced from Poudel A. et al. (2022) (9).

APPENDIX D

PYMOL CODE FOR ALPHAFOLD VISUALIZATION

The following code was used in the PyMOL command window to create all visualizations:

```
RelQ  X-ray WT B. subtilis vs AF3 WT B. subtilis RelQ
01
set seq_view, 1


select Xray, 5dec
select AF3, fold_Bs_RelQ_5DEC_AF3_model_0

alter Xray, resi=str(int(resi)-1)
sort
alter AF3, resi=str(int(resi)-8)
sort


align Xray, AF3


zoom Xray

deselect
.
.
.
rmsdCA Xray, AF3

bg_color white
color wheat, Xray
color gray50, AF3

select RelQ_E1_ATP_Binds, resi 44-51
select RelQ_E2_ATP_Binds, resi 71-76
select wide_E1_and_E2, resi 44-76
select RelQ_E1_and_E2_ATP_Binds, wide_E1_and_E2 and (not resi 52-70)
delete wide_E1_and_E2
color magenta, RelQ_E1_and_E2_ATP_Binds
select RelQ_GDP_GTP_Binds, resi 102-119
color forest, RelQ_GDP_GTP_Binds

select wideHotSpot, resi 34-167
select hotSpot, wideHotSpot and (not resi 35-165)
color red, hotSpot

RelQ AF3 X-ray WT B. subtilis vs AF3 WT C. difficile
01
```

```
set seq_view, 1

select Bs, fold_Bs_RelQ_5DEC_AF3_model_0
select Cd, fold_Cd_RelQ_AF3_model_0

alter Bs, resi=str(int(resi)-8)
sort
alter Cd, resi=str(int(resi)-1)
sort

select CdAfter, Cd and (not resi 0-63)
alter CdAfter, resi=str(int(resi)+2)
sort

select BsAfter, Bs and (not resi 0-68)
alter BsAfter, resi=str(int(resi)+1)
sort

align Bs, Cd

zoom Bs

deselect
.
.
.
rmsdCA Bs, Cd

bg_color white
color wheat, Bs
color gray50, Cd

select BsHotSpot1, Bs and resi 64
color red, BsHotSpot1
deselect

select CdHotSpot1, Cd and resi 63
color red, CdHotSpot1
deselect

select HotSpot2, resi 129-132
color red, HotSpot2
deselect


select BsRelQ_E1_ATP_Binds, Bs and resi 52-59
select BsRelQ_E2_ATP_Binds, Bs and resi 79-84
select BsRelQ_E1_and_E2, Bs and resi 52-84
select BsRelQ_E1_and_E2_ATP_Binds, BsWide_E1_and_E2 and (not resi 60-78)
delete BsRelQ_E1_and_E2
color magenta, BsRelQ_E1_and_E2_ATP_Binds
select BsRelQ_GDP_GTP_Binds, Bs and resi 110-127
color forest, BsRelQ_GDP_GTP_Binds


select CdRelQ_E1_ATP_Binds, Cd and resi 45-52
```

select CdRelQ_E2_ATP_Binds, Cd and resi 71-76
select CdWide_E1_and_E2, Cd and resi 45-76
select CdRelQ_E1_and_E2_ATP_Binds, CdWide_E1_and_E2 and (not resi 53-70)
delete CdWide_E1_and_E2
color magenta, CdRelQ_E1_and_E2_ATP_Binds
select CdRelQ_GDP_GTP_Binds, resi 102-119
color forest, CdRelQ_GDP_GTP_Binds

deselect

RelQ WT *C. difficile*
01

set seq_view, 1

select OG, fold_relq_af3_model_0
select I92L, fold_01_cd_relq_i92l_model_0

align I92L, OG

deselect
.
.
.
rmsdCA OG, I92L

02

set seq_view, 1

select OG, fold_relq_af3_model_0
select V94N, fold_02_cd_relq_v94n_model_0

align V94N, OG

deselect
.
.
.
rmsdCA OG, V94N

03

set seq_view, 1

select OG, fold_relq_af3_model_0
select V94K, fold_03_cd_relq_v94k_model_0

align V94K, OG

deselect
.
.
.
rmsdCA OG, V94K

04

set seq_view, 1

select OG, fold_relq_af3_model_0
select N96K, fold_04_cd_relq_n96k_model_0

align N96K, OG

deselect
.
.
.
rmsdCA OG, N96K

05

set seq_view, 1

select OG, fold_relq_af3_model_0
select N96E, fold_05_cd_relq_n96e_model_0

align N96E, OG

deselect
.
.
.
rmsdCA OG, N96E

06

set seq_view, 1

select OG, fold_relq_af3_model_0
select G102D, fold_06_cd_relq_g102d_model_0

```
align G102D, OG

deselect
.
.
.
rmsdCA OG, G102D
```

07

```
set seq_view, 1

select OG, fold_relq_af3_model_0
select G102K, fold_07_cd_relq_g102k_model_0

align G102K, OG

deselect
.
.
.
rmsdCA OG, G102K
```

08

```
set seq_view, 1

select OG, fold_relq_af3_model_0
select Y110H, fold_08_cd_relq_y110h_model_0

align Y110H, OG

deselect
.
.
.
rmsdCA OG, Y110H
```

09

```
set seq_view, 1

select OG, fold_relq_af3_model_0
select Y110P, fold_09_cd_relq_y110p_model_0
```

align Y110P, OG

deselect
.
.
.
rmsdCA OG, Y110P

10

set seq_view, 1

select OG, fold_relq_af3_model_0
select K123E, fold_10_cd_relq_k123e_model_0

align K123E, OG

deselect
.
.
.
rmsdCA OG, K123E

RelQ Single SOE *C. difficile* ATP, GDP, and Mg
01

set seq_view, 1

select OG, fold_relq_af3_gdp_mg_atp_model_0
select I92L, fold_01_cd_relq_i92l_gdp_mg_atp_model_0

align I92L, OG

deselect
.
.
.
rmsdCA OG, I92L

02

set seq_view, 1

select OG, fold_relq_af3_gdp_mg_atp_model_0
select V94N, fold_02_cd_relq_v94n_gdp_mg_atp_model_0

align V94N, OG

deselect

.
.
.
rmsdCA OG, V94N

03

set seq_view, 1

select OG, fold_relq_af3_gdp_mg_atp_model_0
select V94K, fold_03_cd_relq_v94k_gdp_mg_atp_model_0

align V94K, OG

deselect
.
.
.
rmsdCA OG, V94K

04

set seq_view, 1

select OG, fold_relq_af3_gdp_mg_atp_model_0
select N96K, fold_04_cd_relq_n96k_gdp_mg_atp_model_0

align N96K, OG

deselect
.
.
.
rmsdCA OG, N96K

05

set seq_view, 1

select OG, fold_relq_af3_gdp_mg_atp_model_0
select N96E, fold_05_cd_relq_n96e_gdp_mg_atp_model_0

align N96E, OG

deselect
.

.
.
rmsdCA OG, N96E

06

set seq_view, 1

select OG, fold_relq_af3_gdp_mg_atp_model_0
select G102D, fold_06_cd_relq_g102d_gdp_mg_atp_model_0

align G102D, OG

deselect
.
.
.
rmsdCA OG, G102D

07

set seq_view, 1

select OG, fold_relq_af3_gdp_mg_atp_model_0
select G102K, fold_07_cd_relq_g102k_gdp_mg_atp_model_0

align G102K, OG

deselect
.
.
.
rmsdCA OG, G102K

08

set seq_view, 1

select OG, fold_relq_af3_gdp_mg_atp_model_0
select Y110H, fold_08_cd_relq_y110h_gdp_mg_atp_model_0

align Y110H, OG

deselect
.

.
.
rmsdCA OG, Y110H

09

set seq_view, 1

select OG, fold_relq_af3_gdp_mg_atp_model_0
select Y110P, fold_09_cd_relq_y110p_gdp_mg_atp_model_0

align Y110P, OG

deselect
.
.
.
rmsdCA OG, Y110P

10

set seq_view, 1

select OG, fold_relq_af3_gdp_mg_atp_model_0
select K123E, fold_10_cd_relq_k123e_gdp_mg_atp_model_0

align K123E, OG

deselect
.
.
.
rmsdCA OG, K123E

select ATP_GTP_Mg, org. ino.
select prot_ATP_GTP_Mg, polymer
set surface_carve_cutoff, 4.5
set surface_carve_selection, ATP_GTP_Mg
set surface_carve_normal_cutoff, -0.1
show surface, prot_ATP_GTP_Mg within 8 of ATP_GTP_Mg
set two_sided_lighting
set transparency, 0.5
orient ATP_GTP_Mg
zoom ATP_GTP_Mg, 2
select close_ATP_GTP_Mg, br. polymer near_to 5 of ATP_GTP_Mg
show sticks, close & sc. + close & n. CA + close & r. PRO & n. N
set surface_color, orange
distance hbond_ATP_GTP_Mg, ATP_GTP_Mg, prot, mode=2

```
color magenta, hbond


RelQ ATP GTP Mg (C. difficile)
set seq_view, 1
bg_color white

select RelQ_E1_ATP_Binds, resi 45-52
select RelQ_E2_ATP_Binds, resi 71-76
select wide_E1_and_E2, resi 45-76
select RelQ_E1_and_E2_ATP_Binds, wide_E1_and_E2 and (not resi 53-70)
delete wide_E1_and_E2
color red, RelQ_E1_and_E2_ATP_Binds
select RelQ_GDP_GTP_Binds, resi 102-119
color blue, RelQ_GDP_GTP_Binds

select seleATP, bm. first org.
select seleGTP, bm. last org.
select seleMg, bm. first ino.

select seleATP_GTP_Mg, org. ino.
select prot_ATP_GTP_Mg, polymer
set surface_carve_cutoff, 4.5
set surface_carve_selection, seleATP_GTP_Mg
set surface_carve_normal_cutoff, -0.1
set two_sided_lighting
set transparency, 0.5

select close_ATP_GTP_Mg, br. polymer near_to 5 of seleATP_GTP_Mg
create bindingPocket_Sticks, close & sc. + close & n. CA + close & r. PRO & n. N
hide everything, bindingPocket_Sticks
show sticks, bindingPocket_Sticks
label n. CA and bindingPocket_Sticks, "%s %s" % (resn, resi)
color yelloworange, bindingPocket_Sticks

create bindingPocket_Surface, prot_ATP_GTP_Mg within 8 of seleATP_GTP_Mg, 0, 1
hide everything, bindingPocket_Surface
show surface, bindingPocket_Surface
set surface_color, orange
set transparency, 0.5

distance hbond_ATP, seleATP, prot_ATP_GTP_Mg, mode=2
distance hbond_GTP, seleGTP, prot_ATP_GTP_Mg, mode=2
color magenta, hbond_ATP
color magenta, hbond_GTP

create ATP, seleATP
create GTP, seleGTP
create Mg, seleMg
create ATP_GTP_Mg, seleATP_GTP_Mg

select close_GTP, br. polymer near_to 5 of seleGTP
select close_ATP, br. polymer near_to 5 of seleATP

color yelloworange, bindingPocket_Sticks
```

```
color forest, prot_ATP_GTP_Mg
color firebrick, seleATP
color deepteal, seleGTP
color purpleblue, seleMg
color firebrick, ATP
color deepteal, GTP
color purpleblue, Mg

deselect

orient ATP_GTP_Mg
zoom ATP_GTP_Mg, 5
```

```
select GTP, first org.
select prot_GTP, polymer
set surface_carve_cutoff, 4.5
set surface_carve_selection, GTP
set surface_carve_normal_cutoff, -0.1
show surface, prot_GTP within 8 of GTP
set two_sided_lighting
set transparency, 0.5
orient GTP
zoom GTP, 2
select close_GTP, br. polymer near_to 5 of GTP
show sticks, close & sc. + close & n. CA + close & r. PRO & n. N
set surface_color, orange
distance hbond_ATP_GTP_Mg, ATP_GTP_Mg, prot, mode=2
color magenta, hbond
```

RelQ and RSH ATP GTP Mg (*C. difficile*)

```
set seq_view, 1

select RelQ, fold_relq_af3_gtp_mg_atp_model_0
select RSH, fold_cd_rsh_synthetase_atp_mg_gtp_resi173_339_model_0

align RelQ, RSH

alter RelQ, resi=str(int(resi)-37)
alter RSH,  resi=str(int(resi)-58)
```

RSH (*C. difficile*) WT vs. Single SOE 01

```
set seq_view, 1
```

```
select OG, fold_rsh_af3_model_0
select G169A, fold_01_cd_rsh_g169a_model_0

select bifunctionalDomain, OG and (not resi 386-735)

align G169A, bifunctionalDomain

deselect
.
.
.
rmsdCA OG, G169A
```

02

```
set seq_view, 1

select OG, fold_rsh_af3_model_0
select G170P, fold_02_cd_rsh_g170p_model_0

select bifunctionalDomain, OG and (not resi 386-735)

align G170P, bifunctionalDomain

deselect
.
.
.
rmsdCA OG, G170P
```

03

```
set seq_view, 1

select OG, fold_rsh_af3_model_0
select M192L, fold_03_cd_rsh_m192l_model_0

select bifunctionalDomain, OG and (not resi 386-735)

align M192L, bifunctionalDomain

deselect
.
.
.
rmsdCA OG, M192L
```

04

set seq_view, 1

select OG, fold_rsh_af3_model_0
select D193N, fold_04_cd_rsh_d193n_model_0

select bifunctionalDomain, OG and (not resi 386-735)

align D193N, bifunctionalDomain

deselect
.
.
.
rmsdCA OG, D193N

05

set seq_view, 1

select OG, fold_rsh_af3_model_0
select G196E, fold_05_cd_rsh_g196e_model_0

select bifunctionalDomain, OG and (not resi 386-735)

align G196E, bifunctionalDomain

deselect
.
.
.
rmsdCA OG, G196E

06

set seq_view, 1

select OG, fold_rsh_af3_model_0
select G196K, fold_06_cd_rsh_g196k_model_0

select bifunctionalDomain, OG and (not resi 386-735)

align G196K, bifunctionalDomain

deselect
.
.
.
rmsdCA OG, G196K

07

set seq_view, 1

select OG, fold_rsh_af3_model_0
select D213A, fold_07_cd_rsh_d213a_model_0

select bifunctionalDomain, OG and (not resi 386-735)

align D213A, bifunctionalDomain

deselect
.
.
.
rmsdCA OG, D213A

08

set seq_view, 1

select OG, fold_rsh_af3_model_0
select D213H, fold_08_cd_rsh_d213h_model_0

select bifunctionalDomain, OG and (not resi 386-735)

align D213H, bifunctionalDomain

deselect
.
.
.
rmsdCA OG, D213H

09

set seq_view, 1

select OG, fold_rsh_af3_model_0
select G217D, fold_09_cd_rsh_g217d_model_0

select bifunctionalDomain, OG and (not resi 386-735)

align G217D, bifunctionalDomain

deselect
.
.
.
rmsdCA OG, G217D

RSH WT S. equisimilis vs. WT Se RSH 1VJ7 AF3

01

```
set seq_view, 1

select Se, 1vj7_A_Se
select AF3_Se, fold_Se_RSH_1VJ7_AF3_model_0

align AF3_Se, Se

zoom Se

deselect
.

.
.
rmsdCA Se, AF3_Se

RSH X-ray WT S. equisimilis 1VJ7 vs. AF3 WT Se RSH 1VJ7

set seq_view, 1

select Xray, 1vj7_A_Se
select AF3, fold_Se_RSH_1VJ7_AF3_model_0

align Xray, AF3

zoom Xray

deselect
.
.
.
rmsdCA Xray, AF3

bg_color white
color wheat, Xray
color gray50, AF3

select RSH_E1_ATP_Binds, resi 240-247
select RSH_E2_ATP_Binds, resi 264-269
select wide_E1_and_E2, resi 240-269
select RSH_E1_and_E2_ATP_Binds, wide_E1_and_E2 and (not resi 248-263)
delete wide_E1_and_E2
color magenta, RSH_E1_and_E2_ATP_Binds
select RSH_GDP_GTP_Binds, resi 297-312
color forest, RSH_GDP_GTP_Binds

select wideHotSpot, resi 125-319
select wideHotSpot2, wideHotSpot and (not resi 267-318)
select wideHotSpot3, wideHotSpot2 and (not resi 221-253)
select hotSpot, wideHotSpot3 and (not resi 134-209)
color red, hotSpot
deselect
RSH X-ray WT S. equisimilis 1VJ7 vs. AF3 WT Cd RSH
```

```
set seq_view, 1

select Se, fold_Se_RSH_1VJ7_AF3_model_0
select Cd, fold_Cd_RSH_AF3_model_0

alter Se, resi=str(int(resi)-4)
sort
alter Cd, resi=str(int(resi)-1)
sort

select CdAfter, Cd and (not resi 0-315)
alter CdAfter, resi=str(int(resi)-1)
sort

align Se, Cd

zoom Se

deselect
.
.
.
rmsdCA Se, Cd

bg_color white
color wheat, Se
color gray50, Cd

select SeHotSpot1, Se and resi 108-129
select SeHotSpot2, Se and resi 257-267
color red, SeHotSpot1
color red, SeHotSpot2
deselect

select CdHotSpot1, Cd and resi 105-126
select CdHotSpot2, Cd and resi 254-264
color red, CdHotSpot1
color red, CdHotSpot2
deselect

select SeRSH_E1_ATP_Binds, Se and resi 240-247
select SeRSH_E2_ATP_Binds, Se and resi 264-269
select SeWide_E1_and_E2, Se and resi 240-269
select SeRSH_E1_and_E2_ATP_Binds, SeWide_E1_and_E2 and (not resi 248-263)
delete Sewide_E1_and_E2
color magenta, SeRSH_E1_and_E2_ATP_Binds
select SeRSH_GDP_GTP_Binds, Se and resi 297-312
color forest, SeRSH_GDP_GTP_Binds

select CdRSH_E1_ATP_Binds, Cd and resi 237-244
select CdRSH_E2_ATP_Binds, Cd and resi 261-266
select Cdwide_E1_and_E2, Cd and resi 237-266
select CdRSH_E1_and_E2_ATP_Binds, Cdwide_E1_and_E2 and (not resi 245-260)
delete Cdwide_E1_and_E2
color magenta, CdRSH_E1_and_E2_ATP_Binds
select CdRSH_GDP_GTP_Binds, Cd and resi 294-311
```

```
color forest, CdRSH_GDP_GTP_Binds

deselect
RSH X-ray WT S. equisimilis 1VJ7 vs. AF3 WT Se RSH 1VJ7

set seq_view, 1

select Xray, 1vj7_A_Se
select AF3, fold_Se_RSH_1VJ7_AF3_model_0

align Xray, AF3

zoom Xray

deselect
.
.
.
rmsdCA Xray, AF3

bg_color white
color wheat, Xray
color gray50, AF3

select RSH_E1_ATP_Binds, resi 240-247
select RSH_E2_ATP_Binds, resi 264-269
select wide_E1_and_E2, resi 240-269
select RSH_E1_and_E2_ATP_Binds, wide_E1_and_E2 and (not resi 248-263)
delete wide_E1_and_E2
color magenta, RSH_E1_and_E2_ATP_Binds
select RSH_GDP_GTP_Binds, resi 297-312
color forest, RSH_GDP_GTP_Binds

select wideHotSpot, resi 125-319
select wideHotSpot2, wideHotSpot and (not resi 267-318)
select wideHotSpot3, wideHotSpot2 and (not resi 221-253)
select hotSpot, wideHotSpot3 and (not resi 134-209)
color red, hotSpot
deselect
RSH WT S. equisimilis vs. WT Se RSH 1VJ7 AF2 GDP and Mg

set seq_view, 1

select Se, fold_Se_RSH_1VJ7_AF3_model_0
select AF, fold_Cd_RSH_AF3_model_0

alter Se, resi=str(int(resi)-4)
sort
alter Cd, resi=str(int(resi)-1)
sort

select CdAfter, Cd and (not resi 0-315)
alter CdAfter, resi=str(int(resi)-1)
sort

align Se, Cd
```

zoom Se

deselect
.
.
.
rmsdCA Se, Cd

bg_color white
color wheat, Se
color gray50, Cd

select SeHotSpot1, Se and resi 108-129
select SeHotSpot2, Se and resi 257-267
color red, SeHotSpot1
color red, SeHotSpot2
deselect

select CdHotSpot1, Cd and resi 105-126
select CdHotSpot2, Cd and resi 254-264
color red, CdHotSpot1
color red, CdHotSpot2
deselect

select SeRSH_E1_ATP_Binds, Se and resi 240-247
select SeRSH_E2_ATP_Binds, Se and resi 264-269
select SeWide_E1_and_E2, Se and resi 240-269
select SeRSH_E1_and_E2_ATP_Binds, SeWide_E1_and_E2 and (not resi 248-263)
delete Sewide_E1_and_E2
color magenta, SeRSH_E1_and_E2_ATP_Binds
select SeRSH_GDP_GTP_Binds, Se and resi 297-312
color forest, SeRSH_GDP_GTP_Binds

select CdRSH_E1_ATP_Binds, Cd and resi 237-244
select CdRSH_E2_ATP_Binds, Cd and resi 261-266
select Cdwide_E1_and_E2, Cd and resi 237-266
select CdRSH_E1_and_E2_ATP_Binds, Cdwide_E1_and_E2 and (not resi 245-260)
delete Cdwide_E1_and_E2
color magenta, CdRSH_E1_and_E2_ATP_Binds
select CdRSH_GDP_GTP_Binds, Cd and resi 294-311
color forest, CdRSH_GDP_GTP_Binds

deselect
RSH WT S. equisimilis vs. WT Se RSH 1VJ7 AF2

01

set seq_view, 1

select Se, 1vj7_A_Se
select AF2_Se, ranked_0_Se_RSH_AlphaFold

align AF2_Se, Se

zoom Se

deselect
.

.
.
rmsdCA Se, AF2_Se

set seq_view, 1

select Se, 1vj7_A_Se
select AF2_Se, ranked_0_Se_RSH_AlphaFold
select afterATP_AF2_Se, AF2_Se and (not resi 1-237)
select afterATP_Se, Se and (not resi 5-237)
align afterATP_AF2_Se, afterATP_Se

zoom Se

deselect
.

.
.
rmsdCA afterATP_Se, afterATP_AF2_Se
RSH WT S. equisimilis vs. Cd single SOE AF2

01

set seq_view, 1

select Se, 1vj7_A_Se
select G169A, ranked_0_Cd_RSH_G169A

select bifunctionalDomain, G169A and (not resi 386-735)

alter Se, resi=str(int(resi)-4)
sort
alter G169A, resi=str(int(resi)-1)
sort

align bifunctionalDomain, Se

zoom Se

deselect
.

.
.
rmsdCA Se, G169A

02

set seq_view, 1

select Se, 1vj7_A_Se
select G170P, ranked_0_Cd_RSH_G170P

alter Se, resi=str(int(resi)-4)
sort
alter G170P, resi=str(int(resi)-1)
sort

align G170P, Se

zoom Se

deselect
.

.
.
rmsdCA Se, G170P

03

set seq_view, 1

select Se, 1vj7_A_Se
select M192L, ranked_0_Cd_RSH_M192L

alter Se, resi=str(int(resi)-4)
sort
alter M192L, resi=str(int(resi)-1)
sort

align M192L, Se

zoom Se

deselect
.

.
.
rmsdCA Se, M192L

04

set seq_view, 1

select Se, 1vj7_A_Se

```
select D193N, ranked_0_Cd_RSH_D193N

alter Se, resi=str(int(resi)-4)
sort
alter D193N, resi=str(int(resi)-1)
sort

align D193N, Se

zoom Se

deselect
.

.
.
rmsdCA Se, D193N
```

05

```
set seq_view, 1

select Se, 1vj7_A_Se
select G196E, ranked_0_Cd_RSH_G196E

alter Se, resi=str(int(resi)-4)
sort
alter G196E, resi=str(int(resi)-1)
sort

align G196E, Se

zoom Se

deselect
.

.
.
rmsdCA Se, G196E
```

06

```
set seq_view, 1

select Se, 1vj7_A_Se
select G196K, ranked_0_Cd_RSH_G196K

alter Se, resi=str(int(resi)-4)
sort
alter G196K, resi=str(int(resi)-1)
sort
```

```
align G196K, Se

zoom Se

deselect
.

.
.
rmsdCA Se, G196K
```

07

```
set seq_view, 1

select Se, 1vj7_A_Se
select D213A, ranked_0_Cd_RSH_D213A

alter Se, resi=str(int(resi)-4)
sort
alter D213A, resi=str(int(resi)-1)
sort

align D213A, Se

zoom Se

deselect
.

.
.
rmsdCA Se, D213A
```

08

```
set seq_view, 1

select Se, 1vj7_A_Se
select D213H, ranked_0_Cd_RSH_D213H

alter Se, resi=str(int(resi)-4)
sort
alter D213H, resi=str(int(resi)-1)
sort

align D213H, Se

zoom Se

deselect
.
```

.
.
rmsdCA Se, D213H

09

set seq_view, 1

select Se, 1vj7_A_Se
select G217D, ranked_0_Cd_RSH_G217D

alter Se, resi=str(int(resi)-4)
sort
alter G217D, resi=str(int(resi)-1)
sort

align G217D, Se

zoom Se

deselect
.

.
.
rmsdCA Se, G217D

RSH WT S. equisimilis vs. Cd double SOE AF3
01

set seq_view, 1

select Se, 1vj7_A_Se
select G169A_G170P, fold_01_rsh_doublesoe_g169a_g170p_gtp_mg_atp_model_0

alter Se, resi=str(int(resi)-4)
sort
alter G169A_G170P, resi=str(int(resi)-1)
sort

align G169A_G170P, Se

zoom Se

deselect
.

.
.
rmsdCA Se, G169A_G170P

02

```
set seq_view, 1

select Se, 1vj7_A_Se
select M192L_D193N, fold_02_rsh_doublesoe_M192L_D193N_gtp_mg_atp_model_0

alter Se, resi=str(int(resi)-4)
sort
alter M192L_D193N, resi=str(int(resi)-1)
sort

align M192L_D193N, Se

zoom Se

deselect
.

.
.
rmsdCA Se, M192L_D193N
```

03

```
set seq_view, 1

select Se, 1vj7_A_Se
select G196E_D213A, fold_03_rsh_doublesoe_G196E_D213A_gtp_mg_atp_model_0

alter Se, resi=str(int(resi)-4)
sort
alter G196E_D213A, resi=str(int(resi)-1)
sort

align G196E_D213A, Se

zoom Se

deselect
.

.
.
rmsdCA Se, G196E_D213A
```

04

```
set seq_view, 1

select Se, 1vj7_A_Se
select G196E_D213H, fold_04_rsh_doublesoe_G196E_D213H_gtp_mg_atp_model_0
```

```
alter Se, resi=str(int(resi)-4)
sort
alter G196E_D213H, resi=str(int(resi)-1)
sort

align G196E_D213H, Se

zoom Se

deselect
.

.
.
rmsdCA Se, G196E_D213H
```

05

```
set seq_view, 1

select Se, 1vj7_A_Se
select G196K_D213A, fold_05_rsh_doublesoe_G196K_D213A_gtp_mg_atp_model_0

alter Se, resi=str(int(resi)-4)
sort
alter G196K_D213A, resi=str(int(resi)-1)
sort

align G196K_D213A, Se

zoom Se

deselect
.

.
.
rmsdCA Se, G196K_D213A
```

06

```
set seq_view, 1

select Se, 1vj7_A_Se
select G196K_D213H, fold_06_rsh_doublesoe_G196K_D213H_gtp_mg_atp_model_0

alter Se, resi=str(int(resi)-4)
sort
alter G196K_D213H, resi=str(int(resi)-1)
sort

align G196K_D213H, Se
```

zoom Se

deselect
.

.
.
rmsdCA Se, G196K_D213H

RSH X-ray WT B. subtilis vs. AF3 Bs RSH WT AF3
01

set seq_view, 1

select Bs, 6yxa
select RSH, fold_Bs_RSH_6YXA_AF3_model_0

select bifunctionalDomain, RSH and (not resi 386-735)

align Bs, bifunctionalDomain

zoom Bs

deselect
.

.
.
rmsdCA Bs, RSH
RSH X-ray WT B. subtilis vs. Cd RSH WT AF3
01

set seq_view, 1

select Bs, 6yxa
select RSH, fold_Cd_RSH_AF3_model_0

select bifunctionalDomain, RSH and (not resi 386-735)

alter Bs, resi=str(int(resi)-4)
sort
alter RSH, resi=str(int(resi)-1)
sort

align Bs, bifunctionalDomain

zoom Bs

deselect
.

.

.
rmsdCA Bs, RSH

RSH WT B. subtilis vs. Cd RSH WT AF2
01

set seq_view, 1

select Bs, 6yxa
select RSH, ranked_0_Cd_RSH_Original

select bifunctionalDomain, RSH and (not resi 386-735)

alter Bs, resi=str(int(resi)-4)
sort
alter RSH, resi=str(int(resi)-1)
sort

align Bs, bifunctionalDomain

zoom Bs

deselect
.

.
.
rmsdCA Bs, RSH
RSH WT B. subtilis vs. Cd Single SOE
01

set seq_view, 1

select Bs, 6yxa
select G169A, ranked_0_Cd_RSH_G169A

select bifunctionalDomain, G169A and (not resi 386-735)

alter Bs, resi=str(int(resi)-4)
sort
alter G169A, resi=str(int(resi)-1)
sort

align bifunctionalDomain, Bs

zoom Bs

deselect
.

.
.
rmsdCA Bs, G169A

02

```
set seq_view, 1

select Bs, 6yxa
select G170P, ranked_0_Cd_RSH_G170P

select bifunctionalDomain, G170P and (not resi 386-735)

alter Bs, resi=str(int(resi)-4)
sort
alter G170P, resi=str(int(resi)-1)
sort

align bifunctionalDomain, Bs

zoom Bs

deselect
.

.
.
rmsdCA Bs, G170P
```

03

```
set seq_view, 1

select Bs, 6yxa
select M192L, ranked_0_Cd_RSH_M192L

select bifunctionalDomain, M192L and (not resi 386-735)

alter Bs, resi=str(int(resi)-4)
sort
alter M192L, resi=str(int(resi)-1)
sort

align bifunctionalDomain, Bs

zoom Bs

deselect
.

.
.
rmsdCA Bs, M192L
```

04

set seq_view, 1

select Bs, 6yxa
select D193N, ranked_0_Cd_RSH_D193N

select bifunctionalDomain, D193N and (not resi 386-735)

alter Bs, resi=str(int(resi)-4)
sort
alter D193N, resi=str(int(resi)-1)
sort

align bifunctionalDomain, Bs

zoom Bs

deselect
.

.
.
rmsdCA Bs, D193N

05

set seq_view, 1

select Bs, 6yxa
select G196E, ranked_0_Cd_RSH_G196E

select bifunctionalDomain, G196E and (not resi 386-735)

alter Bs, resi=str(int(resi)-4)
sort
alter G196E, resi=str(int(resi)-1)
sort

align bifunctionalDomain, Bs

zoom Bs

deselect
.

.
.
rmsdCA Bs, G196E

06

```
set seq_view, 1

select Bs, 6yxa
select G196K, ranked_0_Cd_RSH_G196K

select bifunctionalDomain, G196K and (not resi 386-735)

alter Bs, resi=str(int(resi)-4)
sort
alter G196K, resi=str(int(resi)-1)
sort

align bifunctionalDomain, Bs

zoom Bs

deselect
.

.
.
rmsdCA Bs, G196K
```

07

```
set seq_view, 1

select Bs, 6yxa
select D213A, ranked_0_Cd_RSH_D213A

select bifunctionalDomain, D213A and (not resi 386-735)

alter Bs, resi=str(int(resi)-4)
sort
alter D213A, resi=str(int(resi)-1)
sort

align bifunctionalDomain, Bs

zoom Bs

deselect
.

.
.
rmsdCA Bs, D213A
```

08

```
set seq_view, 1

select Bs, 6yxa
```

select D213H, ranked_0_Cd_RSH_D213H

select bifunctionalDomain, D213H and (not resi 386-735)

alter Bs, resi=str(int(resi)-4)
sort
alter D213H, resi=str(int(resi)-1)
sort

align bifunctionalDomain, Bs

zoom Bs

deselect
.

.
.
rmsdCA Bs, D213H

09

set seq_view, 1

select Bs, 6yxa
select G217D, ranked_0_Cd_RSH_G217D

select bifunctionalDomain, G217D and (not resi 386-735)

alter Bs, resi=str(int(resi)-4)
sort
alter G217D, resi=str(int(resi)-1)
sort

align bifunctionalDomain, Bs

zoom Bs

deselect
.

.
.
rmsdCA Bs, G217D

RSH ATP (*C. difficile*)
01

set seq_view, 1

select OG, fold_cd_rsh_af3_atp_model_0
select G169A, fold_01_rsh_g169a_atp_model_0

select bifunctionalDomain, OG and (not resi 386-735)

align G169A, bifunctionalDomain

deselect
.
.
.
rmsdCA OG, G169A

02

set seq_view, 1

select OG, fold_cd_rsh_af3_atp_model_0
select G170P, fold_02_rsh_g170p_atp_model_0

select bifunctionalDomain, OG and (not resi 386-735)

align G170P, bifunctionalDomain

deselect
.
.
.
rmsdCA OG, G170P

03

set seq_view, 1

select OG, fold_cd_rsh_af3_atp_model_0
select M192L, fold_03_rsh_m192l_atp_model_0

select bifunctionalDomain, OG and (not resi 386-735)

align M192L, bifunctionalDomain

deselect
.
.
.
rmsdCA OG, M192L

04

set seq_view, 1

select OG, fold_cd_rsh_af3_atp_model_0
select D193N, fold_04_rsh_d193n_atp_model_0

select bifunctionalDomain, OG and (not resi 386-735)

```
align D193N, bifunctionalDomain

deselect
.
.
.
rmsdCA OG, D193N


05

set seq_view, 1

select OG, fold_cd_rsh_af3_atp_model_0
select G196E, fold_05_rsh_g196e_atp_model_0

select bifunctionalDomain, OG and (not resi 386-735)

align G196E, bifunctionalDomain

deselect
.
.
.
rmsdCA OG, G196E


06

set seq_view, 1

select OG, fold_cd_rsh_af3_atp_model_0
select G196K, fold_06_rsh_g196k_atp_model_0

select bifunctionalDomain, OG and (not resi 386-735)

align G196K, bifunctionalDomain

deselect
.
.
.
rmsdCA OG, G196K


07

set seq_view, 1

select OG, fold_cd_rsh_af3_atp_model_0
select D213A, fold_07_rsh_d213a_atp_model_0

select bifunctionalDomain, OG and (not resi 386-735)
```

align D213A, bifunctionalDomain

deselect
.
.
.
rmsdCA OG, D213A

08

set seq_view, 1

select OG, fold_cd_rsh_af3_atp_model_0
select D213H, fold_08_rsh_d213h_atp_model_0

select bifunctionalDomain, OG and (not resi 386-735)

align D213H, bifunctionalDomain

deselect
.
.
.
rmsdCA OG, D213H

09

set seq_view, 1

select OG, fold_cd_rsh_af3_atp_model_0
select G217D, fold_09_rsh_g217d_atp_model_0

select bifunctionalDomain, OG and (not resi 386-735)

align G217D, bifunctionalDomain

deselect
.
.
.
rmsdCA OG, G217D

RSH ATP GTP Mg (*C. difficile*)
01

set seq_view, 1

select OG, fold_cd_rsh_af3_atp_mg_gdp_model_0
select G169A, fold_01_cd_rsh_g169a_gdp_mg_atp_model_0

select bifunctionalDomain, OG and (not resi 386-735)

align G169A, bifunctionalDomain

deselect
.
.
.
rmsdCA OG, G169A

02

set seq_view, 1

select OG, fold_cd_rsh_af3_atp_mg_gdp_model_0
select G170P, fold_02_cd_rsh_g170p_gdp_mg_atp_model_0

select bifunctionalDomain, OG and (not resi 386-735)

align G170P, bifunctionalDomain

deselect
.
.
.
rmsdCA OG, G170P

03

set seq_view, 1

select OG, fold_cd_rsh_af3_atp_mg_gdp_model_0
select M192L, fold_03_cd_rsh_m192l_gdp_mg_atp_model_0

select bifunctionalDomain, OG and (not resi 386-735)

align M192L, bifunctionalDomain

deselect
.
.
.
rmsdCA OG, M192L

04

set seq_view, 1

select OG, fold_cd_rsh_af3_atp_mg_gdp_model_0
select D193N, fold_04_cd_rsh_d193n_gdp_mg_atp_model_0

select bifunctionalDomain, OG and (not resi 386-735)

align D193N, bifunctionalDomain

deselect
.
.
.
rmsdCA OG, D193N

05

set seq_view, 1

select OG, fold_cd_rsh_af3_atp_mg_gdp_model_0
select G196E, fold_05_cd_rsh_g196e_gdp_mg_atp_model_0

select bifunctionalDomain, OG and (not resi 386-735)

align G196E, bifunctionalDomain

deselect
.
.
.
rmsdCA OG, G196E

06

set seq_view, 1

select OG, fold_cd_rsh_af3_atp_mg_gdp_model_0
select G196K, fold_06_cd_rsh_g196k_gdp_mg_atp_model_0

select bifunctionalDomain, OG and (not resi 386-735)

align G196K, bifunctionalDomain

deselect
.
.
.
rmsdCA OG, G196K

07

set seq_view, 1

select OG, fold_cd_rsh_af3_atp_mg_gdp_model_0
select D213A, fold_07_cd_rsh_d213a_gdp_mg_atp_model_0

select bifunctionalDomain, OG and (not resi 386-735)

align D213A, bifunctionalDomain

deselect
.
.
.
rmsdCA OG, D213A

08

set seq_view, 1

select OG, fold_cd_rsh_af3_atp_mg_gdp_model_0
select D213H, fold_08_cd_rsh_d213h_gdp_mg_atp_model_0

select bifunctionalDomain, OG and (not resi 386-735)

align D213H, bifunctionalDomain

deselect
.
.
.
rmsdCA OG, D213H

09

set seq_view, 1

select OG, fold_cd_rsh_af3_atp_mg_gdp_model_0
select G217D, fold_09_cd_rsh_g217d_gdp_mg_atp_model_0

select bifunctionalDomain, OG and (not resi 386-735)

align G217D, bifunctionalDomain

deselect
.
.
.
rmsdCA OG, G217D

RSH *C. difficile* WT vs. ALL SOE ATP GDP Mg
01

set seq_view, 1

select OG, fold_cd_rsh_af3_atp_mg_gdp_model_0
select G169A_G170P_M192L_D193N_G196E_D213A,
fold_01_cd_rsh_g169a_g170p_m192l_d193n_g196e_d213a_atp_gdp_mg_model_4

select bifunctionalDomain, OG and (not resi 386-735)

align G169A_G170P_M192L_D193N_G196E_D213A, bifunctionalDomain

deselect
.
.
.
rmsdCA OG, G169A_G170P_M192L_D193N_G196E_D213A

01

set seq_view, 1

select OG, fold_cd_rsh_af3_atp_mg_gdp_model_0
select G169A_G170P_M192L_D193N_G196E_D213A,
fold_01_cd_rsh_g169a_g170p_m192l_d193n_g196e_d213a_atp_gdp_mg_model_0

select bifunctionalDomain, OG and (not resi 386-735)

align G169A_G170P_M192L_D193N_G196E_D213A, bifunctionalDomain

deselect
.
.
.
rmsdCA OG, G169A_G170P_M192L_D193N_G196E_D213A

02

set seq_view, 1

select OG, fold_cd_rsh_af3_atp_mg_gdp_model_0
select G169A_G170P_M192L_D193N_G196E_D213H,
fold_02_rsh_g169a_g170p_m192l_d193n_g196e_d213h_atp_gdp_mg_model_0

select bifunctionalDomain, OG and (not resi 386-735)

align G169A_G170P_M192L_D193N_G196E_D213H, bifunctionalDomain

deselect
.
.
.
rmsdCA OG, G169A_G170P_M192L_D193N_G196E_D213H

03

set seq_view, 1

select OG, fold_cd_rsh_af3_atp_mg_gdp_model_0

select G170P_M192L_D193N_G196K_D213A,
fold_03_rsh_g169a_g170p_m192l_d193n_g196k_d213a_atp_gdp_mg_model_0

select bifunctionalDomain, OG and (not resi 386-735)

align G170P_M192L_D193N_G196K_D213A, bifunctionalDomain

deselect
.
.
.
rmsdCA OG, G170P_M192L_D193N_G196K_D213A

04

set seq_view, 1

select OG, fold_cd_rsh_af3_atp_mg_gdp_model_0
select G170P_M192L_D193N_G196K_D213H,
fold_04_rsh_g169a_g170p_m192l_d193n_g196k_d213h_atp_gdp_mg_model_0

select bifunctionalDomain, OG and (not resi 386-735)

align G170P_M192L_D193N_G196K_D213H, bifunctionalDomain

deselect
.
.
.
rmsdCA OG, G170P_M192L_D193N_G196K_D213H

C. difficile All SOE RSH ATP, GDP, and Mg

set seq_view, 1
bg_color white

select OG, fold_cd_rsh_af3_atp_mg_gdp_model_0
select G169A_G170P_M192L_D193N_G196E_D213H,
fold_02_rsh_g169a_g170p_m192l_d193n_g196e_d213h_atp_gdp_mg_model_0
deselect

select bifunctionalDomain, OG and (not resi 386-735)
align G169A_G170P_M192L_D193N_G196E_D213A, bifunctionalDomain
deselect

create AllSOE, G169A_G170P_M192L_D193N_G196E_D213A
create NonSOE, OG

disable fold_cd_rsh_af3_atp_mg_gdp_model_0
disable G169A_G170P_M192L_D193N_G196E_D213A

color gray50, AllSOE

```
color wheat, NonSOE

select seleNonSOEIonsLigands, NonSOE and not polymer
deselect
create ionsNonSOELigands, NonSOE and not polymer

select seleNonSOE_ATPGXP, NonSOE and not polymer and org.
deselect
create NonSOE_ATPGXP, NonSOE and not polymer and org.

select seleNonSOE_ATP, bm. first NonSOE_ATPGXP
color tv_red, seleNonSOE_ATP
create NonSOE_ATP, bm. first NonSOE_ATPGXP
deselect
select seleNonSOE_GDP, bm. last NonSOE_ATPGXP
color deepteal, seleNonSOE_GDP
create NonSOE_GDP, bm. last NonSOE_ATPGXP
deselect

select seleAllSOEIonsLigands, AllSOE and not polymer
deselect
create ionsAllSOELigands, AllSOE and not polymer

select seleAllSOE_ATPGXP, AllSOE and not polymer and org.
deselect
create AllSOE_ATPGXP, AllSOE and not polymer and org.
deselect

select seleAllSOE_ATP, bm. first AllSOE_ATPGXP
color chocolate, seleAllSOE_ATP
create AllSOE_ATP, bm. first AllSOE_ATPGXP
deselect
select seleAllSOE_GDP, bm. last AllSOE_ATPGXP
color lightblue, seleAllSOE_GDP
create AllSOE_GDP, bm. last AllSOE_ATPGXP
deselect

disable all
enable NonSOE_ATP
enable NonSOE_GDP
enable AllSOE_ATP
enable AllSOE_GDP
enable AllSOE
enable NonSOE

set surface_carve_cutoff, 6
set surface_carve_selection, seleNonSOEIonsLigands
set surface_carve_normal_cutoff, -0.1
set two_sided_lighting
set transparency, 0.5

select close_ATP_GDP_Mg, br. NonSOE near_to 6 of seleNonSOEIonsLigands
create LabelBindingPocket_Sticks, close & sc. + close & n. CA + close & r. PRO & n. N
create bindingPocket_Sticks, LabelBindingPocket_Sticks
hide everything, LabelBindingPocket_Sticks
hide everything, bindingPocket_Sticks
```

show sticks, bindingPocket_Sticks
show sticks, LabelBindingPocket_Sticks
label n. CA and LabelBindingPocket_Sticks, "%s %s" % (resn, resi)
color yelloworange, LabelBindingPocket_Sticks
color yelloworange, bindingPocket_Sticks

disable LabelBindingPocket_Sticks

create bindingPocket_Surface, NonSOE within 6 of seleNonSOEIonsLigands, 0, 1
hide everything, bindingPocket_Surface
show surface, bindingPocket_Surface
set surface_color, orange
set transparency, 0.5

distance hbond_ATP, seleNonSOE_ATP, NonSOE, mode=2
distance hbond_GDP, seleNonSOE_GDP, NonSOE, mode=2
color magenta, hbond_ATP
color magenta, hbond_GDP

select AllSOE_hotSpot, resi 207-209 and AllSOE
color red, AllSOE_hotSpot
deselect
select NonSOE_hotSpot, resi 207-209 and NonSOE
color salmon, NonSOE_hotSpot
deselect

RSH S. equisimilis WT vs. ALL SOE ATP GDP Mg

01

set seq_view, 1

select Se, fold_Se_RSH_1VJ7_AF3_model_0
select G169A_G170P_M192L_D193N_G196E_D213A,
fold_01_cd_rsh_g169a_g170p_m192l_d193n_g196e_d213a_atp_gdp_mg_model_0

select bifunctionalDomain, G169A_G170P_M192L_D193N_G196E_D213A and (not resi 386-735)

alter Se, resi=str(int(resi)-4)
sort
alter G169A_G170P_M192L_D193N_G196E_D213A, resi=str(int(resi)-1)
sort

align Se, bifunctionalDomain

deselect
.
.
.
rmsdCA Se, G169A_G170P_M192L_D193N_G196E_D213A

02

set seq_view, 1

select Se, fold_Se_RSH_1VJ7_AF3_model_0
select G169A_G170P_M192L_D193N_G196E_D213H,
fold_02_rsh_g169a_g170p_m192l_d193n_g196e_d213h_atp_gdp_mg_model_0

select bifunctionalDomain, G169A_G170P_M192L_D193N_G196E_D213H and (not resi 386-735)

alter Se, resi=str(int(resi)-4)
sort
alter G169A_G170P_M192L_D193N_G196E_D213H, resi=str(int(resi)-1)
sort

align Se, bifunctionalDomain

deselect
.
.
.
rmsdCA Se, G169A_G170P_M192L_D193N_G196E_D213H

03

set seq_view, 1

select Se, fold_Se_RSH_1VJ7_AF3_model_0
select G170P_M192L_D193N_G196K_D213A,
fold_03_rsh_g169a_g170p_m192l_d193n_g196k_d213a_atp_gdp_mg_model_0

select bifunctionalDomain, G170P_M192L_D193N_G196K_D213A and (not resi 386-735)

alter Se, resi=str(int(resi)-4)
sort
alter G170P_M192L_D193N_G196K_D213A, resi=str(int(resi)-1)
sort

align Se, bifunctionalDomain

deselect
.
.
.
rmsdCA Se, G170P_M192L_D193N_G196K_D213A

04

set seq_view, 1

select Se, fold_Se_RSH_1VJ7_AF3_model_0
select G170P_M192L_D193N_G196K_D213H,
fold_04_rsh_g169a_g170p_m192l_d193n_g196k_d213h_atp_gdp_mg_model_0

select bifunctionalDomain, G170P_M192L_D193N_G196K_D213H and (not resi 386-735)

```
alter Se, resi=str(int(resi)-4)
sort
alter G170P_M192L_D193N_G196K_D213H, resi=str(int(resi)-1)
sort

align Se, bifunctionalDomain

deselect
.
.
.
rmsdCA Se, G170P_M192L_D193N_G196K_D213H
```

RSH WT (*C. difficile*)

```
set seq_view, 1
bg_color white

select CdRSH, fold_Cd_RSH_AF3_model_0
color forest, CdRSH

select overallCatalyticDomain, CdRSH and resi 165-224

select pointMutation169,  CdRSH and resi 169
    color yellow, pointMutation169

select pointMutation170,  CdRSH and resi 170
    color yellow, pointMutation170

select pointMutation170,  CdRSH and resi 170
    color yellow, pointMutation170

select pointMutation191,  CdRSH and resi 191
    color yellow, pointMutation191

select pointMutation192,  CdRSH and resi 192
    color yellow, pointMutation192

select pointMutation193,  CdRSH and resi 193
    color yellow, pointMutation193

select pointMutation195,  CdRSH and resi 195
    color yellow, pointMutation195

select pointMutation195,  CdRSH and resi 195
    color yellow, pointMutation195

select pointMutation196,  CdRSH and resi 196
    color yellow, pointMutation196

select pointMutation200,  CdRSH and resi 200
    color yellow, pointMutation200
```

select pointMutation202, CdRSH and resi 202
color yellow, pointMutation202

select pointMutation203, CdRSH and resi 203
color yellow, pointMutation203

select pointMutation209, CdRSH and resi 209
color yellow, pointMutation209

select pointMutation210, CdRSH and resi 210
color yellow, pointMutation210

select pointMutation213, CdRSH and resi 213
color yellow, pointMutation213

select pointMutation217, CdRSH and resi 217
color yellow, pointMutation217

select conservedResidue172, CdRSH and resi 172
color red, conservedResidue172

select conservedResidue174to176, CdRSH and resi 174-176
color red, conservedResidue174to176

select conservedResidue178, CdRSH and resi 178
color red, conservedResidue178

select conservedResidue180to182, CdRSH and resi 180-182
color red, conservedResidue180to182

select conservedResidue211, CdRSH and resi 211
color red, conservedResidue211

deselect
RSH WT ATP GDP Mg *C. difficile*

set seq_view, 1
bg_color white

select seleATP, bm. first org.
select seleGDP, bm. last org.
select seleMg, bm. first ino.

select seleATP_GDP_Mg, org. ino.
select prot_ATP_GDP_Mg, polymer
set surface_carve_cutoff, 6
set surface_carve_selection, seleATP_GDP_Mg
set surface_carve_normal_cutoff, -0.1
set two_sided_lighting
set transparency, 0.5

select close_ATP_GDP_Mg, br. polymer near_to 6 of seleATP_GDP_Mg
create LabelBindingPocket_Sticks, close & sc. + close & n. CA + close & r. PRO & n. N

```
create bindingPocket_Sticks, LabelBindingPocket_Sticks
hide everything, LabelBindingPocket_Sticks
hide everything, bindingPocket_Sticks
show sticks, bindingPocket_Sticks
show sticks, LabelBindingPocket_Sticks
label n. CA and LabelBindingPocket_Sticks, "%s %s" % (resn, resi)
color yelloworange, LabelBindingPocket_Sticks
color yelloworange, bindingPocket_Sticks


create bindingPocket_Surface, prot_ATP_GDP_Mg within 6 of seleATP_GDP_Mg, 0, 1
hide everything, bindingPocket_Surface
show surface, bindingPocket_Surface
set surface_color, orange
set transparency, 0.5

distance hbond_ATP, seleATP, prot_ATP_GDP_Mg, mode=2
distance hbond_GDP, seleGDP, prot_ATP_GDP_Mg, mode=2
color magenta, hbond_ATP
color magenta, hbond_GDP

create ATP_GDP_Mg, seleATP_GDP_Mg
create ATP, seleATP
create GDP, seleGDP
create Mg, seleMg

select close_GDP, br. polymer near_to 6 of seleGDP
select close_ATP, br. polymer near_to 6 of seleATP

color forest, prot_ATP_GDP_Mg
color firebrick, ATP
color deepteal, GDP
color purpleblue, Mg
disable ATP_GDP_Mg

select seleCd_RSH_Bifunctional_Fragment, prot_ATP_GDP_Mg and resi 1-385
select seleCd_RSH_Synthetase, prot_ATP_GDP_Mg and resi 173-339
select seleCd_RSH_Central3HelixBundle, prot_ATP_GDP_Mg and resi 132-192
select seleCd_RSH_Hydrolase, prot_ATP_GDP_Mg and resi 1-149

create Cd_RSH_Bifunctional_Fragment, seleCd_RSH_Bifunctional_Fragment
create Cd_RSH_Synthetase, seleCd_RSH_Synthetase
create Cd_RSH_Central3HelixBundle, seleCd_RSH_Central3HelixBundle
create Cd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase_NoOrg
select seleCd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase and (not org.)
select seleCd_RSH_Hydrolase_NoIno, Cd_RSH_Hydrolase_NoOrg and (not ino.)
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase_NoIno
delete seleCd_RSH_Hydrolase_NoOrg
delete Cd_RSH_Hydrolase_NoOrg
delete seleCd_RSH_Hydrolase_NoIno
select seleCd_RSH_Hydrolase, Cd_RSH_Hydrolase
deselect
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase

disable Cd_RSH_Bifunctional_Fragment
disable Cd_RSH_Synthetase
```

```
disable Cd_RSH_Central3HelixBundle
disable Cd_RSH_Hydrolase

orient ATP_GDP_Mg
zoom ATP_GDP_Mg, 5
```

RSH WT ATP GTP Mg *C. difficile*

```
set seq_view, 1
bg_color white

select seleATP, bm. first org.
select seleGTP, bm. last org.
select seleMg, bm. first ino.

select seleATP_GTP_Mg, org. ino.
select prot_ATP_GTP_Mg, polymer
set surface_carve_cutoff, 6
set surface_carve_selection, seleATP_GTP_Mg
set surface_carve_normal_cutoff, -0.1
set two_sided_lighting
set transparency, 0.5


select close_ATP_GTP_Mg, br. polymer near_to 6 of seleATP_GTP_Mg
create LabelBindingPocket_Sticks, close & sc. + close & n. CA + close & r. PRO & n. N
create bindingPocket_Sticks, LabelBindingPocket_Sticks
hide everything, LabelBindingPocket_Sticks
hide everything, bindingPocket_Sticks
show sticks, bindingPocket_Sticks
show sticks, LabelBindingPocket_Sticks
label n. CA and LabelBindingPocket_Sticks, "%s %s" % (resn, resi)
color yelloworange, LabelBindingPocket_Sticks
color yelloworange, bindingPocket_Sticks


create bindingPocket_Surface, prot_ATP_GTP_Mg within 6 of seleATP_GTP_Mg, 0, 1
hide everything, bindingPocket_Surface
show surface, bindingPocket_Surface
set surface_color, orange
set transparency, 0.5

distance hbond_ATP, seleATP, prot_ATP_GTP_Mg, mode=2
distance hbond_GTP, seleGTP, prot_ATP_GTP_Mg, mode=2
color magenta, hbond_ATP
color magenta, hbond_GTP

create ATP_GTP_Mg, seleATP_GTP_Mg
create ATP, seleATP
create GTP, seleGTP
create Mg, seleMg

select close_GTP, br. polymer near_to 6 of seleGTP
select close_ATP, br. polymer near_to 6 of seleATP
```

color forest, prot_ATP_GTP_Mg
color firebrick, ATP
color deepteal, GTP
color purpleblue, Mg
disable ATP_GTP_Mg

select seleCd_RSH_Bifunctional_Fragment, prot_ATP_GTP_Mg and resi 1-385
select seleCd_RSH_Synthetase, prot_ATP_GTP_Mg and resi 173-339
select seleCd_RSH_Central3HelixBundle, prot_ATP_GTP_Mg and resi 132-192
select seleCd_RSH_Hydrolase, prot_ATP_GTP_Mg and resi 1-149

create Cd_RSH_Bifunctional_Fragment, seleCd_RSH_Bifunctional_Fragment
create Cd_RSH_Synthetase, seleCd_RSH_Synthetase
create Cd_RSH_Central3HelixBundle, seleCd_RSH_Central3HelixBundle
create Cd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase_NoOrg
select seleCd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase and (not org.)
select seleCd_RSH_Hydrolase_NoIno, Cd_RSH_Hydrolase_NoOrg and (not ino.)
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase_NoIno
delete seleCd_RSH_Hydrolase_NoOrg
delete Cd_RSH_Hydrolase_NoOrg
delete seleCd_RSH_Hydrolase_NoIno
select seleCd_RSH_Hydrolase, Cd_RSH_Hydrolase
deselect
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase

disable Cd_RSH_Bifunctional_Fragment
disable Cd_RSH_Synthetase
disable Cd_RSH_Central3HelixBundle
disable Cd_RSH_Hydrolase

orient ATP_GTP_Mg
zoom ATP_GTP_Mg, 5
RSH SOE ATP GDP Mg *C. difficile*

set seq_view, 1
bg_color white

select seleATP, bm. first org.
select seleGDP, bm. last org.
select seleMg, bm. first ino.

select seleATP_GDP_Mg, org. ino.
select prot_ATP_GDP_Mg, polymer
set surface_carve_cutoff, 6
set surface_carve_selection, seleATP_GDP_Mg
set surface_carve_normal_cutoff, -0.1
set two_sided_lighting
set transparency, 0.5

select close_ATP_GDP_Mg, br. polymer near_to 6 of seleATP_GDP_Mg
create LabelBindingPocket_Sticks, close & sc. + close & n. CA + close & r. PRO & n. N
create bindingPocket_Sticks, LabelBindingPocket_Sticks
hide everything, LabelBindingPocket_Sticks

```
hide everything, bindingPocket_Sticks
show sticks, bindingPocket_Sticks
show sticks, LabelBindingPocket_Sticks
label n. CA and LabelBindingPocket_Sticks, "%s %s" % (resn, resi)
color yelloworange, LabelBindingPocket_Sticks
color yelloworange, bindingPocket_Sticks


create bindingPocket_Surface, prot_ATP_GDP_Mg within 6 of seleATP_GDP_Mg, 0, 1
hide everything, bindingPocket_Surface
show surface, bindingPocket_Surface
set surface_color, orange
set transparency, 0.5

distance hbond_ATP, seleATP, prot_ATP_GDP_Mg, mode=2
distance hbond_GDP, seleGDP, prot_ATP_GDP_Mg, mode=2
color magenta, hbond_ATP
color magenta, hbond_GDP

create ATP_GDP_Mg, seleATP_GDP_Mg
create ATP, seleATP
create GDP, seleGDP
create Mg, seleMg

select close_GDP, br. polymer near_to 6 of seleGDP
select close_ATP, br. polymer near_to 6 of seleATP

color forest, prot_ATP_GDP_Mg
color firebrick, ATP
color deepteal, GDP
color purpleblue, Mg
disable ATP_GDP_Mg

select seleCd_RSH_Bifunctional_Fragment, prot_ATP_GDP_Mg and resi 1-385
select seleCd_RSH_Synthetase, prot_ATP_GDP_Mg and resi 173-339
select seleCd_RSH_Central3HelixBundle, prot_ATP_GDP_Mg and resi 132-192
select seleCd_RSH_Hydrolase, prot_ATP_GDP_Mg and resi 1-149

create Cd_RSH_Bifunctional_Fragment, seleCd_RSH_Bifunctional_Fragment
create Cd_RSH_Synthetase, seleCd_RSH_Synthetase
create Cd_RSH_Central3HelixBundle, seleCd_RSH_Central3HelixBundle
create Cd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase_NoOrg
select seleCd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase and (not org.)
select seleCd_RSH_Hydrolase_NoIno, Cd_RSH_Hydrolase_NoOrg and (not ino.)
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase_NoIno
delete seleCd_RSH_Hydrolase_NoOrg
delete Cd_RSH_Hydrolase_NoOrg
delete seleCd_RSH_Hydrolase_NoIno
select seleCd_RSH_Hydrolase, Cd_RSH_Hydrolase
deselect
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase

disable Cd_RSH_Bifunctional_Fragment
disable Cd_RSH_Synthetase
disable Cd_RSH_Central3HelixBundle
disable Cd_RSH_Hydrolase
```

```
select hotSpot, prot_ATP_GDP_Mg and resi 204-210
color red, hotSpot

orient ATP_GDP_Mg
zoom ATP_GDP_Mg, 5
```

RSH G169A ATP GDP Mg *C. difficile*

```
set seq_view, 1
bg_color white

select prot_ATP_GDP_Mg, polymer
select seleATP_GDP, org. and (not prot_ATP_GDP_Mg)
create ATP_GDP, seleATP_GDP
select seleATP_atom, first org. and ATP_GDP
create ATP_atom, seleATP_atom
select seleATP,  bm. seleATP_atom

create ATP, seleATP

select seleGDP_atom, last org. and ATP_GDP
create GDP_atom, seleGDP_atom
select seleGDP,  bm. seleGDP_atom
create GDP, seleGDP
deselect

delete ATP_atom
delete GDP_ato
delete seleATP_atom
delete seleGDP_atom
delete GDP_atom
delete ATP_atom
delete seleGDP
delete seleATP

select seleATP, ATP
select seleGDP, GDP
deselect

select seleMg, bm. first ino.
create Mg, seleMg
deselect

select seleATP_GDP_Mg, org. ino. and (not prot_ATP_GDP_Mg)

set surface_carve_cutoff, 6
set surface_carve_selection, seleATP_GDP_Mg
set surface_carve_normal_cutoff, -0.1
set two_sided_lighting
set transparency, 0.5
```

```
select close_ATP_GDP_Mg, br. polymer near_to 6 of seleATP_GDP_Mg
create LabelBindingPocket_Sticks, close & sc. + close & n. CA + close & r. PRO & n. N
create bindingPocket_Sticks, LabelBindingPocket_Sticks
hide everything, LabelBindingPocket_Sticks
hide everything, bindingPocket_Sticks
show sticks, bindingPocket_Sticks
show sticks, LabelBindingPocket_Sticks
label n. CA and LabelBindingPocket_Sticks, "%s %s" % (resn, resi)
color yelloworange, LabelBindingPocket_Sticks
color yelloworange, bindingPocket_Sticks


create bindingPocket_Surface, prot_ATP_GDP_Mg within 6 of seleATP_GDP_Mg, 0, 1
hide everything, bindingPocket_Surface
show surface, bindingPocket_Surface
set surface_color, orange
set transparency, 0.5

distance hbond_ATP, seleATP, prot_ATP_GDP_Mg, mode=2
distance hbond_GDP, seleGDP, prot_ATP_GDP_Mg, mode=2
color magenta, hbond_ATP
color magenta, hbond_GDP

create ATP_GDP_Mg, seleATP_GDP_Mg
create ATP, seleATP
create GDP, seleGDP
create Mg, seleMg

select close_GDP, br. polymer near_to 6 of seleGDP
select close_ATP, br. polymer near_to 6 of seleATP

color gray50, prot_ATP_GDP_Mg
color firebrick, ATP
color deepteal, GDP
color purpleblue, Mg
disable ATP_GDP_Mg

select seleCd_RSH_Bifunctional_Fragment, prot_ATP_GDP_Mg and resi 1-385
select seleCd_RSH_Synthetase, prot_ATP_GDP_Mg and resi 173-339
select seleCd_RSH_Central3HelixBundle, prot_ATP_GDP_Mg and resi 132-192
select seleCd_RSH_Hydrolase, prot_ATP_GDP_Mg and resi 1-149

create Cd_RSH_Bifunctional_Fragment, seleCd_RSH_Bifunctional_Fragment
create Cd_RSH_Synthetase, seleCd_RSH_Synthetase
create Cd_RSH_Central3HelixBundle, seleCd_RSH_Central3HelixBundle
create Cd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase_NoOrg
select seleCd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase and (not org.)
select seleCd_RSH_Hydrolase_NoIno, Cd_RSH_Hydrolase_NoOrg and (not ino.)
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase_NoIno
delete seleCd_RSH_Hydrolase_NoOrg
delete Cd_RSH_Hydrolase_NoOrg
delete seleCd_RSH_Hydrolase_NoIno
select seleCd_RSH_Hydrolase, Cd_RSH_Hydrolase
```

```
deselect
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase

disable Cd_RSH_Bifunctional_Fragment
disable Cd_RSH_Synthetase
disable Cd_RSH_Central3HelixBundle
disable Cd_RSH_Hydrolase
disable ATP_GDP

select hotSpot, resi 204-209
color red, hotSpot
deselect

select mutation, resi 169
color yellow, mutation
deselect

orient ATP_GDP_Mg
zoom ATP_GDP_Mg, 5
```

RSH G169A ATP GTP Mg *C. difficile* (can be used for WT *C. difficile*)

```
set seq_view, 1
bg_color white

select prot_ATP_GTP_Mg, polymer
select seleATP_GTP, org. and (not prot_ATP_GTP_Mg)
create ATP_GTP, seleATP_GTP
select seleATP_atom, first org. and ATP_GTP
create ATP_atom, seleATP_atom
select seleATP,  bm. seleATP_atom

create ATP, seleATP

select seleGTP_atom, last org. and ATP_GTP
create GTP_atom, seleGTP_atom
select seleGTP,  bm. seleGTP_atom
create GTP, seleGTP
deselect

delete ATP_atom
delete GTP_ato
delete seleATP_atom
delete seleGTP_atom
delete GTP_atom
delete ATP_atom
delete seleGTP
delete seleATP

select seleATP, ATP
select seleGTP, GTP
deselect

select seleMg, bm. first ino.
create Mg, seleMg
deselect
```

```
select seleATP_GTP_Mg, org. ino. and (not prot_ATP_GTP_Mg)

set surface_carve_cutoff, 6
set surface_carve_selection, seleATP_GTP_Mg
set surface_carve_normal_cutoff, -0.1
set two_sided_lighting
set transparency, 0.5


select close_ATP_GTP_Mg, br. polymer near_to 6 of seleATP_GTP_Mg
create LabelBindingPocket_Sticks, close & sc. + close & n. CA + close & r. PRO & n. N
create bindingPocket_Sticks, LabelBindingPocket_Sticks
hide everything, LabelBindingPocket_Sticks
hide everything, bindingPocket_Sticks
show sticks, bindingPocket_Sticks
show sticks, LabelBindingPocket_Sticks
label n. CA and LabelBindingPocket_Sticks, "%s %s" % (resn, resi)
color yelloworange, LabelBindingPocket_Sticks
color yelloworange, bindingPocket_Sticks


create bindingPocket_Surface, prot_ATP_GTP_Mg within 6 of seleATP_GTP_Mg, 0, 1
hide everything, bindingPocket_Surface
show surface, bindingPocket_Surface
set surface_color, orange
set transparency, 0.5

distance hbond_ATP, seleATP, prot_ATP_GTP_Mg, mode=2
distance hbond_GTP, seleGTP, prot_ATP_GTP_Mg, mode=2
color magenta, hbond_ATP
color magenta, hbond_GTP

create ATP_GTP_Mg, seleATP_GTP_Mg
create ATP, seleATP
create GTP, seleGTP
create Mg, seleMg

select close_GTP, br. polymer near_to 6 of seleGTP
select close_ATP, br. polymer near_to 6 of seleATP

color gray50, prot_ATP_GTP_Mg
color firebrick, ATP
color deepteal, GTP
color purpleblue, Mg
disable ATP_GTP_Mg

select seleCd_RSH_Bifunctional_Fragment, prot_ATP_GTP_Mg and resi 1-385
select seleCd_RSH_Synthetase, prot_ATP_GTP_Mg and resi 173-339
select seleCd_RSH_Central3HelixBundle, prot_ATP_GTP_Mg and resi 132-192
select seleCd_RSH_Hydrolase, prot_ATP_GTP_Mg and resi 1-149

create Cd_RSH_Bifunctional_Fragment, seleCd_RSH_Bifunctional_Fragment
create Cd_RSH_Synthetase, seleCd_RSH_Synthetase
```

create Cd_RSH_Central3HelixBundle, seleCd_RSH_Central3HelixBundle
create Cd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase_NoOrg
select seleCd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase and (not org.)
select seleCd_RSH_Hydrolase_NoIno, Cd_RSH_Hydrolase_NoOrg and (not ino.)
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase_NoIno
delete seleCd_RSH_Hydrolase_NoOrg
delete Cd_RSH_Hydrolase_NoOrg
delete seleCd_RSH_Hydrolase_NoIno
select seleCd_RSH_Hydrolase, Cd_RSH_Hydrolase
deselect
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase

disable Cd_RSH_Bifunctional_Fragment
disable Cd_RSH_Synthetase
disable Cd_RSH_Central3HelixBundle
disable Cd_RSH_Hydrolase
disable ATP_GTP

select hotSpot, resi 204-209
color red, hotSpot
deselect

select mutation, resi 169
color yellow, mutation
deselect

orient ATP_GTP_Mg
zoom ATP_GTP_Mg, 5

RSH G170P ATP GTP Mg *C. difficile* (can be used for WT *C. difficile*)

set seq_view, 1
bg_color white
color gray50, fold_02_cd_rsh_g170p_atp_mg_gtp_model_0

select prot_ATP_GTP_Mg, fold_02_cd_rsh_g170p_atp_mg_gtp_model_0
select seleATP_GTP, org.
create ATP_GTP, seleATP_GTP
select seleATP_atom, first org. and ATP_GTP
create ATP_atom, seleATP_atom
select seleATP, bm. seleATP_atom

create ATP, seleATP

select seleGTP_atom, last org. and ATP_GTP
create GTP_atom, seleGTP_atom
select seleGTP, bm. seleGTP_atom
create GTP, seleGTP
deselect

delete ATP_atom
delete GTP_ato
delete seleATP_atom
delete seleGTP_atom
delete GTP_atom
delete ATP_atom

```
delete seleGTP
delete seleATP

select seleATP, ATP
select seleGTP, GTP
deselect

select seleMg, bm. first ino.
create Mg, seleMg
deselect


select seleATP_GTP_Mg, org. ino. and (not prot_ATP_GTP_Mg)

set surface_carve_cutoff, 6
set surface_carve_selection, seleATP_GTP_Mg
set surface_carve_normal_cutoff, -0.1
set two_sided_lighting
set transparency, 0.5



select close_ATP_GTP_Mg, br. fold_02_cd_rsh_g170p_atp_mg_gtp_model_0 near_to 6 of seleATP_GTP_Mg
create LabelBindingPocket_Sticks, close & sc. + close & n. CA + close & r. PRO & n. N
create bindingPocket_Sticks, LabelBindingPocket_Sticks
hide everything, LabelBindingPocket_Sticks
hide everything, bindingPocket_Sticks
show sticks, bindingPocket_Sticks
show sticks, LabelBindingPocket_Sticks
label n. CA and LabelBindingPocket_Sticks, "%s %s" % (resn, resi)
color yelloworange, LabelBindingPocket_Sticks
color yelloworange, bindingPocket_Sticks


create bindingPocket_Surface, prot_ATP_GTP_Mg within 6 of seleATP_GTP_Mg, 0, 1
hide everything, bindingPocket_Surface
show surface, bindingPocket_Surface
set surface_color, orange
set transparency, 0.5

distance hbond_ATP, seleATP, prot_ATP_GTP_Mg, mode=2
distance hbond_GTP, seleGTP, prot_ATP_GTP_Mg, mode=2
color magenta, hbond_ATP
color magenta, hbond_GTP

create ATP_GTP_Mg, seleATP_GTP_Mg
create ATP, seleATP
create GTP, seleGTP
create Mg, seleMg

select close_GTP, br. fold_02_cd_rsh_g170p_atp_mg_gtp_model_0 near_to 6 of seleGTP
select close_ATP, br. fold_02_cd_rsh_g170p_atp_mg_gtp_model_0 near_to 6 of seleATP

color firebrick, ATP
color deepteal, GTP
color purpleblue, Mg
```

```
disable ATP_GTP_Mg

select seleCd_RSH_Bifunctional_Fragment, prot_ATP_GTP_Mg and resi 1-385
select seleCd_RSH_Synthetase, prot_ATP_GTP_Mg and resi 173-339
select seleCd_RSH_Central3HelixBundle, prot_ATP_GTP_Mg and resi 132-192
select seleCd_RSH_Hydrolase, prot_ATP_GTP_Mg and resi 1-149

create Cd_RSH_Bifunctional_Fragment, seleCd_RSH_Bifunctional_Fragment
create Cd_RSH_Synthetase, seleCd_RSH_Synthetase
create Cd_RSH_Central3HelixBundle, seleCd_RSH_Central3HelixBundle
create Cd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase_NoOrg
select seleCd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase and (not org.)
select seleCd_RSH_Hydrolase_NoIno, Cd_RSH_Hydrolase_NoOrg and (not ino.)
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase_NoIno
delete seleCd_RSH_Hydrolase_NoOrg
delete Cd_RSH_Hydrolase_NoOrg
delete seleCd_RSH_Hydrolase_NoIno
select seleCd_RSH_Hydrolase, Cd_RSH_Hydrolase
deselect
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase

disable Cd_RSH_Bifunctional_Fragment
disable Cd_RSH_Synthetase
disable Cd_RSH_Central3HelixBundle
disable Cd_RSH_Hydrolase
disable ATP_GTP

select hotSpot, resi 204-209
color red, hotSpot
deselect

select mutation, resi 170
color limegreen, mutation
deselect

disable fold_02_cd_rsh_g170p_atp_mg_gtp_model_0
enable fold_02_cd_rsh_g170p_atp_mg_gtp_model_0

disable LabelBindingPocket_Sticks
disable bindingPocket_Sticks

orient ATP_GTP_Mg
zoom ATP_GTP_Mg, 5
```

RSH ATP GTP Mg G170P and WT ATP GTP Mg *C. difficile*

```
set seq_view, 1
bg_color white

select seleOG, fold_cd_rsh_af3_atp_mg_gtp_model_0
select seleG170P, fold_02_cd_rsh_g170p_atp_mg_gtp_model_0
color wheat, seleOG
color gray50, seleG170P
select bifunctionalDomain, seleOG and (not resi 386-735)
```

```
align seleG170P, bifunctionalDomain
deselect

select seleHotSpot, resi 204-209
Deselect

select seleMutation, resi 170
deselect
create HotSpot, resi 204-209
create Mutation, resi 170

select OG_HotSpot, seleOG and resi 204-209
color salmon, OG_HotSpot
deselect

select mutationHotSpot, seleG170P and resi 204-209
color red, mutationHotSpot
deselect

select OG_Mutation, seleOG and  resi 170
color paleyellow, OG_Mutation
deselect

select G170P_Mutation, seleG170P and resi 170
color limegreen, G170P_Mutation
deselect



select prot_ATP_GTP_Mg, fold_02_cd_rsh_g170p_atp_mg_gtp_model_0
select seleATP_GTP, org. and (not prot_ATP_GTP_Mg)
create ATP_GTP, seleATP_GTP
select seleATP_atom, first org. and ATP_GTP
create ATP_atom, seleATP_atom
select seleATP,  bm. seleATP_atom

create ATP, seleATP

select seleGTP_atom, last org. and ATP_GTP
create GTP_atom, seleGTP_atom
select seleGTP,  bm. seleGTP_atom
create GTP, seleGTP
deselect

delete ATP_atom
delete GTP_ato
delete seleATP_atom
delete seleGTP_atom
delete GTP_atom
delete ATP_atom
delete seleGTP
delete seleATP

select seleATP, ATP
select seleGTP, GTP
deselect
```

```
select seleMg, bm. first ino.
create Mg, seleMg
deselect


select seleATP_GTP_Mg, org. ino. and (not prot_ATP_GTP_Mg)

set surface_carve_cutoff, 6
set surface_carve_selection, seleATP_GTP_Mg
set surface_carve_normal_cutoff, -0.1
set two_sided_lighting
set transparency, 0.5


select close_ATP_GTP_Mg, br. fold_02_cd_rsh_g170p_atp_mg_gtp_model_0 near_to 6 of seleATP_GTP_Mg
create LabelBindingPocket_Sticks, close & sc. + close & n. CA + close & r. PRO & n. N
create bindingPocket_Sticks, LabelBindingPocket_Sticks
hide everything, LabelBindingPocket_Sticks
hide everything, bindingPocket_Sticks
show sticks, bindingPocket_Sticks
show sticks, LabelBindingPocket_Sticks
label n. CA and LabelBindingPocket_Sticks, "%s %s" % (resn, resi)
color yelloworange, LabelBindingPocket_Sticks
color yelloworange, bindingPocket_Sticks


create bindingPocket_Surface, prot_ATP_GTP_Mg within 6 of seleATP_GTP_Mg, 0, 1
hide everything, bindingPocket_Surface
show surface, bindingPocket_Surface
set surface_color, orange
set transparency, 0.5

distance hbond_ATP, seleATP, prot_ATP_GTP_Mg, mode=2
distance hbond_GTP, seleGTP, prot_ATP_GTP_Mg, mode=2
color magenta, hbond_ATP
color magenta, hbond_GTP

create ATP_GTP_Mg, seleATP_GTP_Mg
create ATP, seleATP
create GTP, seleGTP
create Mg, seleMg

select close_GTP, br. fold_02_cd_rsh_g170p_atp_mg_gtp_model_0 near_to 6 of seleGTP
select close_ATP, br. fold_02_cd_rsh_g170p_atp_mg_gtp_model_0 near_to 6 of seleATP

color firebrick, ATP
color deepteal, GTP
color purpleblue, Mg
disable ATP_GTP_Mg

select seleCd_RSH_Bifunctional_Fragment, prot_ATP_GTP_Mg and resi 1-385
select seleCd_RSH_Synthetase, prot_ATP_GTP_Mg and resi 173-339
select seleCd_RSH_Central3HelixBundle, prot_ATP_GTP_Mg and resi 132-192
select seleCd_RSH_Hydrolase, prot_ATP_GTP_Mg and resi 1-149
```

create Cd_RSH_Bifunctional_Fragment, seleCd_RSH_Bifunctional_Fragment
create Cd_RSH_Synthetase, seleCd_RSH_Synthetase
create Cd_RSH_Central3HelixBundle, seleCd_RSH_Central3HelixBundle
create Cd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase_NoOrg
select seleCd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase and (not org.)
select seleCd_RSH_Hydrolase_NoIno, Cd_RSH_Hydrolase_NoOrg and (not ino.)
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase_NoIno
delete seleCd_RSH_Hydrolase_NoOrg
delete Cd_RSH_Hydrolase_NoOrg
delete seleCd_RSH_Hydrolase_NoIno
select seleCd_RSH_Hydrolase, Cd_RSH_Hydrolase
deselect
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase

disable Cd_RSH_Bifunctional_Fragment
disable Cd_RSH_Synthetase
disable Cd_RSH_Central3HelixBundle
disable Cd_RSH_Hydrolase

disable ATP_GTP
disable LabelBindingPocket_Sticks
disable hbond_ATP
disable hbond_GTP
disable GTP

disable fold_cd_rsh_af3_atp_mg_gtp_model_0
disable fold_02_cd_rsh_g170p_atp_mg_gtp_model_0
enable fold_cd_rsh_af3_atp_mg_gtp_model_0
enable fold_02_cd_rsh_g170p_atp_mg_gtp_model_0

orient ATP_GTP_Mg
zoom ATP_GTP_Mg, 5

RSH d193n ATP GTP Mg *C. difficile* (can be used for WT *C. difficile*)

set seq_view, 1
bg_color white
color gray50, fold_04_cd_rsh_d193n_atp_mg_gtp_model_0

select prot_ATP_GTP_Mg, polymer
select seleATP_GTP, org. and (not prot_ATP_GTP_Mg)
create ATP_GTP, seleATP_GTP
select seleATP_atom, first org. and ATP_GTP
create ATP_atom, seleATP_atom
select seleATP, bm. seleATP_atom

create ATP, seleATP

select seleGTP_atom, last org. and ATP_GTP
create GTP_atom, seleGTP_atom
select seleGTP, bm. seleGTP_atom
create GTP, seleGTP

```
deselect

delete ATP_atom
delete GTP_ato
delete seleATP_atom
delete seleGTP_atom
delete GTP_atom
delete ATP_atom
delete seleGTP
delete seleATP

select seleATP, ATP
select seleGTP, GTP
deselect

select seleMg, bm. first ino.
create Mg, seleMg
deselect


select seleATP_GTP_Mg, org. ino. and (not prot_ATP_GTP_Mg)

set surface_carve_cutoff, 6
set surface_carve_selection, seleATP_GTP_Mg
set surface_carve_normal_cutoff, -0.1
set two_sided_lighting
set transparency, 0.5



select close_ATP_GTP_Mg, br. fold_04_cd_rsh_d193n_atp_mg_gtp_model_0 near_to 6 of seleATP_GTP_Mg
create LabelBindingPocket_Sticks, close & sc. + close & n. CA + close & r. PRO & n. N
create bindingPocket_Sticks, LabelBindingPocket_Sticks
hide everything, LabelBindingPocket_Sticks
hide everything, bindingPocket_Sticks
show sticks, bindingPocket_Sticks
show sticks, LabelBindingPocket_Sticks
label n. CA and LabelBindingPocket_Sticks, "%s %s" % (resn, resi)
color yelloworange, LabelBindingPocket_Sticks
color yelloworange, bindingPocket_Sticks


create bindingPocket_Surface, prot_ATP_GTP_Mg within 6 of seleATP_GTP_Mg, 0, 1
hide everything, bindingPocket_Surface
show surface, bindingPocket_Surface
set surface_color, orange
set transparency, 0.5

distance hbond_ATP, seleATP, prot_ATP_GTP_Mg, mode=2
distance hbond_GTP, seleGTP, prot_ATP_GTP_Mg, mode=2
color magenta, hbond_ATP
color magenta, hbond_GTP

create ATP_GTP_Mg, seleATP_GTP_Mg
create ATP, seleATP
create GTP, seleGTP
```

```
create Mg, seleMg

select close_GTP, br. fold_04_cd_rsh_d193n_atp_mg_gtp_model_0 near_to 6 of seleGTP
select close_ATP, br. fold_04_cd_rsh_d193n_atp_mg_gtp_model_0 near_to 6 of seleATP

color firebrick, ATP
color deepteal, GTP
color purpleblue, Mg
disable ATP_GTP_Mg

select seleCd_RSH_Bifunctional_Fragment, prot_ATP_GTP_Mg and resi 1-385
select seleCd_RSH_Synthetase, prot_ATP_GTP_Mg and resi 173-339
select seleCd_RSH_Central3HelixBundle, prot_ATP_GTP_Mg and resi 132-192
select seleCd_RSH_Hydrolase, prot_ATP_GTP_Mg and resi 1-149

create Cd_RSH_Bifunctional_Fragment, seleCd_RSH_Bifunctional_Fragment
create Cd_RSH_Synthetase, seleCd_RSH_Synthetase
create Cd_RSH_Central3HelixBundle, seleCd_RSH_Central3HelixBundle
create Cd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase_NoOrg
select seleCd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase and (not org.)
select seleCd_RSH_Hydrolase_NoIno, Cd_RSH_Hydrolase_NoOrg and (not ino.)
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase_NoIno
delete seleCd_RSH_Hydrolase_NoOrg
delete Cd_RSH_Hydrolase_NoOrg
delete seleCd_RSH_Hydrolase_NoIno
select seleCd_RSH_Hydrolase, Cd_RSH_Hydrolase
deselect
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase

disable Cd_RSH_Bifunctional_Fragment
disable Cd_RSH_Synthetase
disable Cd_RSH_Central3HelixBundle
disable Cd_RSH_Hydrolase
disable ATP_GTP

select hotSpot, resi 204-209
color red, hotSpot
deselect

select mutation, resi 193
color limegreen, mutation
deselect

disable fold_04_cd_rsh_d193n_atp_mg_gtp_model_0
enable fold_04_cd_rsh_d193n_atp_mg_gtp_model_0

disable LabelBindingPocket_Sticks

orient ATP_GTP_Mg
zoom ATP_GTP_Mg, 5
```

RSH ATP GTP Mg d193n and WT ATP GTP Mg *C. difficile*

```
set seq_view, 1
bg_color white

select seleOG, fold_cd_rsh_af3_atp_mg_gtp_model_0
select seled193n, fold_04_cd_rsh_d193n_atp_mg_gtp_model_0
color wheat, seleOG
color gray50, seled193n
select bifunctionalDomain, seleOG and (not resi 386-735)
align seled193n, bifunctionalDomain
deselect

select seleHotSpot, resi 204-209
Deselect

select seleMutation, resi 193
deselect
create HotSpot, resi 204-209
create Mutation, resi 193

select OG_HotSpot, seleOG and resi 204-209
color salmon, OG_HotSpot
deselect

select mutationHotSpot, seled193n and resi 204-209
color red, mutationHotSpot
deselect

select OG_Mutation, seleOG and  resi 193
color paleyellow, OG_Mutation
deselect

select d193n_Mutation, seled193n and resi 193
color limegreen, d193n_Mutation
deselect



select prot_ATP_GTP_Mg, fold_04_cd_rsh_d193n_atp_mg_gtp_model_0
select seleATP_GTP, org. and (not prot_ATP_GTP_Mg)
create ATP_GTP, seleATP_GTP
select seleATP_atom, first org. and ATP_GTP
create ATP_atom, seleATP_atom
select seleATP,  bm. seleATP_atom

create ATP, seleATP

select seleGTP_atom, last org. and ATP_GTP
create GTP_atom, seleGTP_atom
select seleGTP,  bm. seleGTP_atom
create GTP, seleGTP
deselect

delete ATP_atom
delete GTP_ato
delete seleATP_atom
```

```
delete seleGTP_atom
delete GTP_atom
delete ATP_atom
delete seleGTP
delete seleATP

select seleATP, ATP
select seleGTP, GTP
deselect

select seleMg, bm. first ino.
create Mg, seleMg
deselect


select seleATP_GTP_Mg, org. ino. and (not prot_ATP_GTP_Mg)

set surface_carve_cutoff, 6
set surface_carve_selection, seleATP_GTP_Mg
set surface_carve_normal_cutoff, -0.1
set two_sided_lighting
set transparency, 0.5


select close_ATP_GTP_Mg, br. fold_04_cd_rsh_d193n_atp_mg_gtp_model_0 near_to 6 of seleATP_GTP_Mg
create LabelBindingPocket_Sticks, close & sc. + close & n. CA + close & r. PRO & n. N
create bindingPocket_Sticks, LabelBindingPocket_Sticks
hide everything, LabelBindingPocket_Sticks
hide everything, bindingPocket_Sticks
show sticks, bindingPocket_Sticks
show sticks, LabelBindingPocket_Sticks
label n. CA and LabelBindingPocket_Sticks, "%s %s" % (resn, resi)
color yelloworange, LabelBindingPocket_Sticks
color yelloworange, bindingPocket_Sticks


create bindingPocket_Surface, prot_ATP_GTP_Mg within 6 of seleATP_GTP_Mg, 0, 1
hide everything, bindingPocket_Surface
show surface, bindingPocket_Surface
set surface_color, orange
set transparency, 0.5

distance hbond_ATP, seleATP, prot_ATP_GTP_Mg, mode=2
distance hbond_GTP, seleGTP, prot_ATP_GTP_Mg, mode=2
color magenta, hbond_ATP
color magenta, hbond_GTP

create ATP_GTP_Mg, seleATP_GTP_Mg
create ATP, seleATP
create GTP, seleGTP
create Mg, seleMg

select close_GTP, br. fold_04_cd_rsh_d193n_atp_mg_gtp_model_0 near_to 6 of seleGTP
select close_ATP, br. fold_04_cd_rsh_d193n_atp_mg_gtp_model_0 near_to 6 of seleATP
```

color firebrick, ATP
color deepteal, GTP
color purpleblue, Mg
disable ATP_GTP_Mg

select seleCd_RSH_Bifunctional_Fragment, prot_ATP_GTP_Mg and resi 1-385
select seleCd_RSH_Synthetase, prot_ATP_GTP_Mg and resi 173-339
select seleCd_RSH_Central3HelixBundle, prot_ATP_GTP_Mg and resi 132-192
select seleCd_RSH_Hydrolase, prot_ATP_GTP_Mg and resi 1-149

create Cd_RSH_Bifunctional_Fragment, seleCd_RSH_Bifunctional_Fragment
create Cd_RSH_Synthetase, seleCd_RSH_Synthetase
create Cd_RSH_Central3HelixBundle, seleCd_RSH_Central3HelixBundle
create Cd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase_NoOrg
select seleCd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase and (not org.)
select seleCd_RSH_Hydrolase_NoIno, Cd_RSH_Hydrolase_NoOrg and (not ino.)
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase_NoIno
delete seleCd_RSH_Hydrolase_NoOrg
delete Cd_RSH_Hydrolase_NoOrg
delete seleCd_RSH_Hydrolase_NoIno
select seleCd_RSH_Hydrolase, Cd_RSH_Hydrolase
deselect
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase

disable Cd_RSH_Bifunctional_Fragment
disable Cd_RSH_Synthetase
disable Cd_RSH_Central3HelixBundle
disable Cd_RSH_Hydrolase

disable ATP_GTP
disable LabelBindingPocket_Sticks
disable hbond_ATP
disable hbond_GTP
disable GTP

disable fold_cd_rsh_af3_atp_mg_gtp_model_0
disable fold_04_cd_rsh_d193n_atp_mg_gtp_model_0
enable fold_cd_rsh_af3_atp_mg_gtp_model_0
enable fold_04_cd_rsh_d193n_atp_mg_gtp_model_0

deselect

orient ATP_GTP_Mg
zoom ATP_GTP_Mg, 5

RSH G196K ATP GTP Mg *C. difficile* (can be used for WT *C. difficile*)

set seq_view, 1
bg_color white
color gray50, fold_06_cd_rsh_g196k_atp_mg_gtp_model_0

select prot_ATP_GTP_Mg, polymer
select seleATP_GTP, org. and (not prot_ATP_GTP_Mg)
create ATP_GTP, seleATP_GTP
select seleATP_atom, first org. and ATP_GTP

```
create ATP_atom, seleATP_atom
select seleATP,  bm. seleATP_atom

create ATP, seleATP

select seleGTP_atom, last org. and ATP_GTP
create GTP_atom, seleGTP_atom
select seleGTP,  bm. seleGTP_atom
create GTP, seleGTP
deselect

delete ATP_atom
delete GTP_ato
delete seleATP_atom
delete seleGTP_atom
delete GTP_atom
delete ATP_atom
delete seleGTP
delete seleATP

select seleATP, ATP
select seleGTP, GTP
deselect

select seleMg, bm. first ino.
create Mg, seleMg
deselect


select seleATP_GTP_Mg, org. ino. and (not prot_ATP_GTP_Mg)

set surface_carve_cutoff, 6
set surface_carve_selection, seleATP_GTP_Mg
set surface_carve_normal_cutoff, -0.1
set two_sided_lighting
set transparency, 0.5



select close_ATP_GTP_Mg, br. fold_06_cd_rsh_g196k_atp_mg_gtp_model_0 near_to 6 of seleATP_GTP_Mg
create LabelBindingPocket_Sticks, close & sc. + close & n. CA + close & r. PRO & n. N
create bindingPocket_Sticks, LabelBindingPocket_Sticks
hide everything, LabelBindingPocket_Sticks
hide everything, bindingPocket_Sticks
show sticks, bindingPocket_Sticks
show sticks, LabelBindingPocket_Sticks
label n. CA and LabelBindingPocket_Sticks, "%s %s" % (resn, resi)
color yelloworange, LabelBindingPocket_Sticks
color yelloworange, bindingPocket_Sticks


create bindingPocket_Surface, prot_ATP_GTP_Mg within 6 of seleATP_GTP_Mg, 0, 1
hide everything, bindingPocket_Surface
show surface, bindingPocket_Surface
set surface_color, orange
set transparency, 0.5
```

```
distance hbond_ATP, seleATP, prot_ATP_GTP_Mg, mode=2
distance hbond_GTP, seleGTP, prot_ATP_GTP_Mg, mode=2
color magenta, hbond_ATP
color magenta, hbond_GTP

create ATP_GTP_Mg, seleATP_GTP_Mg
create ATP, seleATP
create GTP, seleGTP
create Mg, seleMg

select close_GTP, br. fold_06_cd_rsh_g196k_atp_mg_gtp_model_0 near_to 6 of seleGTP
select close_ATP, br. fold_06_cd_rsh_g196k_atp_mg_gtp_model_0 near_to 6 of seleATP

color firebrick, ATP
color deepteal, GTP
color purpleblue, Mg
disable ATP_GTP_Mg

select seleCd_RSH_Bifunctional_Fragment, prot_ATP_GTP_Mg and resi 1-385
select seleCd_RSH_Synthetase, prot_ATP_GTP_Mg and resi 173-339
select seleCd_RSH_Central3HelixBundle, prot_ATP_GTP_Mg and resi 132-192
select seleCd_RSH_Hydrolase, prot_ATP_GTP_Mg and resi 1-149

create Cd_RSH_Bifunctional_Fragment, seleCd_RSH_Bifunctional_Fragment
create Cd_RSH_Synthetase, seleCd_RSH_Synthetase
create Cd_RSH_Central3HelixBundle, seleCd_RSH_Central3HelixBundle
create Cd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase_NoOrg
select seleCd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase and (not org.)
select seleCd_RSH_Hydrolase_NoIno, Cd_RSH_Hydrolase_NoOrg and (not ino.)
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase_NoIno
delete seleCd_RSH_Hydrolase_NoOrg
delete Cd_RSH_Hydrolase_NoOrg
delete seleCd_RSH_Hydrolase_NoIno
select seleCd_RSH_Hydrolase, Cd_RSH_Hydrolase
deselect
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase

disable Cd_RSH_Bifunctional_Fragment
disable Cd_RSH_Synthetase
disable Cd_RSH_Central3HelixBundle
disable Cd_RSH_Hydrolase
disable ATP_GTP

select hotSpot, resi 204-209
color red, hotSpot
deselect

select mutation, resi 196
color limegreen, mutation
deselect

disable fold_06_cd_rsh_g196k_atp_mg_gtp_model_0
enable fold_06_cd_rsh_g196k_atp_mg_gtp_model_0

disable LabelBindingPocket_Sticks
```

```
orient ATP_GTP_Mg
zoom ATP_GTP_Mg, 5
```

RSH ATP GTP Mg G196K and WT ATP GTP Mg *C. difficile*

```
set seq_view, 1
bg_color white

select seleOG, fold_cd_rsh_af3_atp_mg_gtp_model_0
select seleG196K, fold_06_cd_rsh_g196k_atp_mg_gtp_model_0
color wheat, seleOG
color gray50, seleG196K
select bifunctionalDomain, seleOG and (not resi 386-735)
align seleG196K, bifunctionalDomain
deselect

select seleHotSpot, resi 204-209
Deselect

select seleMutation, resi 196
deselect
create HotSpot, resi 204-209
create Mutation, resi 196

select OG_HotSpot, seleOG and resi 204-209
color salmon, OG_HotSpot
deselect

select mutationHotSpot, seleG196K and resi 204-209
color red, mutationHotSpot
deselect

select OG_Mutation, seleOG and  resi 196
color paleyellow, OG_Mutation
deselect

select G196K_Mutation, seleG196K and resi 196
color limegreen, G196K_Mutation
deselect


select prot_ATP_GTP_Mg, fold_06_cd_rsh_g196k_atp_mg_gtp_model_0
select seleATP_GTP, org. and (not prot_ATP_GTP_Mg)
create ATP_GTP, seleATP_GTP
select seleATP_atom, first org. and ATP_GTP
create ATP_atom, seleATP_atom
select seleATP,  bm. seleATP_atom

create ATP, seleATP

select seleGTP_atom, last org. and ATP_GTP
create GTP_atom, seleGTP_atom
```

```
select seleGTP,  bm. seleGTP_atom
create GTP, seleGTP
deselect

delete ATP_atom
delete GTP_ato
delete seleATP_atom
delete seleGTP_atom
delete GTP_atom
delete ATP_atom
delete seleGTP
delete seleATP

select seleATP, ATP
select seleGTP, GTP
deselect

select seleMg, bm. first ino.
create Mg, seleMg
deselect


select seleATP_GTP_Mg, org. ino. and (not prot_ATP_GTP_Mg)

set surface_carve_cutoff, 6
set surface_carve_selection, seleATP_GTP_Mg
set surface_carve_normal_cutoff, -0.1
set two_sided_lighting
set transparency, 0.5


select close_ATP_GTP_Mg, br. fold_06_cd_rsh_g196k_atp_mg_gtp_model_0 near_to 6 of seleATP_GTP_Mg
create LabelBindingPocket_Sticks, close & sc. + close & n. CA + close & r. PRO & n. N
create bindingPocket_Sticks, LabelBindingPocket_Sticks
hide everything, LabelBindingPocket_Sticks
hide everything, bindingPocket_Sticks
show sticks, bindingPocket_Sticks
show sticks, LabelBindingPocket_Sticks
label n. CA and LabelBindingPocket_Sticks, "%s %s" % (resn, resi)
color yelloworange, LabelBindingPocket_Sticks
color yelloworange, bindingPocket_Sticks


create bindingPocket_Surface, prot_ATP_GTP_Mg within 6 of seleATP_GTP_Mg, 0, 1
hide everything, bindingPocket_Surface
show surface, bindingPocket_Surface
set surface_color, orange
set transparency, 0.5

distance hbond_ATP, seleATP, prot_ATP_GTP_Mg, mode=2
distance hbond_GTP, seleGTP, prot_ATP_GTP_Mg, mode=2
color magenta, hbond_ATP
color magenta, hbond_GTP

create ATP_GTP_Mg, seleATP_GTP_Mg
```

create ATP, seleATP
create GTP, seleGTP
create Mg, seleMg

select close_GTP, br. fold_06_cd_rsh_g196k_atp_mg_gtp_model_0 near_to 6 of seleGTP
select close_ATP, br. fold_06_cd_rsh_g196k_atp_mg_gtp_model_0 near_to 6 of seleATP

color firebrick, ATP
color deepteal, GTP
color purpleblue, Mg
disable ATP_GTP_Mg

select seleCd_RSH_Bifunctional_Fragment, prot_ATP_GTP_Mg and resi 1-385
select seleCd_RSH_Synthetase, prot_ATP_GTP_Mg and resi 173-339
select seleCd_RSH_Central3HelixBundle, prot_ATP_GTP_Mg and resi 132-192
select seleCd_RSH_Hydrolase, prot_ATP_GTP_Mg and resi 1-149

create Cd_RSH_Bifunctional_Fragment, seleCd_RSH_Bifunctional_Fragment
create Cd_RSH_Synthetase, seleCd_RSH_Synthetase
create Cd_RSH_Central3HelixBundle, seleCd_RSH_Central3HelixBundle
create Cd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase_NoOrg
select seleCd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase and (not org.)
select seleCd_RSH_Hydrolase_NoIno, Cd_RSH_Hydrolase_NoOrg and (not ino.)
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase_NoIno
delete seleCd_RSH_Hydrolase_NoOrg
delete Cd_RSH_Hydrolase_NoOrg
delete seleCd_RSH_Hydrolase_NoIno
select seleCd_RSH_Hydrolase, Cd_RSH_Hydrolase
deselect
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase

disable Cd_RSH_Bifunctional_Fragment
disable Cd_RSH_Synthetase
disable Cd_RSH_Central3HelixBundle
disable Cd_RSH_Hydrolase

disable ATP_GTP
disable LabelBindingPocket_Sticks
disable hbond_ATP
disable hbond_GTP
disable GTP

disable fold_cd_rsh_af3_atp_mg_gtp_model_0
disable fold_06_cd_rsh_g196k_atp_mg_gtp_model_0
enable fold_cd_rsh_af3_atp_mg_gtp_model_0
enable fold_06_cd_rsh_g196k_atp_mg_gtp_model_0

deselect

orient ATP_GTP_Mg
zoom ATP_GTP_Mg, 5

RSH D213H ATP GTP Mg *C. difficile*

set seq_view, 1

```
bg_color white
color gray50, fold_08_cd_rsh_d213h_atp_mg_gtp_model_0

select prot_ATP_GTP_Mg, polymer
select seleATP_GTP, org. and (not prot_ATP_GTP_Mg)
create ATP_GTP, seleATP_GTP
select seleATP_atom, first org. and ATP_GTP
create ATP_atom, seleATP_atom
select seleATP,  bm. seleATP_atom

create ATP, seleATP

select seleGTP_atom, last org. and ATP_GTP
create GTP_atom, seleGTP_atom
select seleGTP,  bm. seleGTP_atom
create GTP, seleGTP
deselect

delete ATP_atom
delete GTP_ato
delete seleATP_atom
delete seleGTP_atom
delete GTP_atom
delete ATP_atom
delete seleGTP
delete seleATP

select seleATP, ATP
select seleGTP, GTP
deselect

select seleMg, bm. first ino.
create Mg, seleMg
deselect


select seleATP_GTP_Mg, org. ino. and (not prot_ATP_GTP_Mg)

set surface_carve_cutoff, 6
set surface_carve_selection, seleATP_GTP_Mg
set surface_carve_normal_cutoff, -0.1
set two_sided_lighting
set transparency, 0.5



select close_ATP_GTP_Mg, br. fold_08_cd_rsh_d213h_atp_mg_gtp_model_0 near_to 6 of seleATP_GTP_Mg
create LabelBindingPocket_Sticks, close & sc. + close & n. CA + close & r. PRO & n. N
create bindingPocket_Sticks, LabelBindingPocket_Sticks
hide everything, LabelBindingPocket_Sticks
hide everything, bindingPocket_Sticks
show sticks, bindingPocket_Sticks
show sticks, LabelBindingPocket_Sticks
label n. CA and LabelBindingPocket_Sticks, "%s %s" % (resn, resi)
color yelloworange, LabelBindingPocket_Sticks
color yelloworange, bindingPocket_Sticks
```

```
create bindingPocket_Surface, prot_ATP_GTP_Mg within 6 of seleATP_GTP_Mg, 0, 1
hide everything, bindingPocket_Surface
show surface, bindingPocket_Surface
set surface_color, orange
set transparency, 0.5

distance hbond_ATP, seleATP, prot_ATP_GTP_Mg, mode=2
distance hbond_GTP, seleGTP, prot_ATP_GTP_Mg, mode=2
color magenta, hbond_ATP
color magenta, hbond_GTP

create ATP_GTP_Mg, seleATP_GTP_Mg
create ATP, seleATP
create GTP, seleGTP
create Mg, seleMg

select close_GTP, br. fold_08_cd_rsh_d213h_atp_mg_gtp_model_0 near_to 6 of seleGTP
select close_ATP, br. fold_08_cd_rsh_d213h_atp_mg_gtp_model_0 near_to 6 of seleATP

color firebrick, ATP
color deepteal, GTP
color purpleblue, Mg
disable ATP_GTP_Mg

select seleCd_RSH_Bifunctional_Fragment, prot_ATP_GTP_Mg and resi 1-385
select seleCd_RSH_Synthetase, prot_ATP_GTP_Mg and resi 173-339
select seleCd_RSH_Central3HelixBundle, prot_ATP_GTP_Mg and resi 132-192
select seleCd_RSH_Hydrolase, prot_ATP_GTP_Mg and resi 1-149

create Cd_RSH_Bifunctional_Fragment, seleCd_RSH_Bifunctional_Fragment
create Cd_RSH_Synthetase, seleCd_RSH_Synthetase
create Cd_RSH_Central3HelixBundle, seleCd_RSH_Central3HelixBundle
create Cd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase_NoOrg
select seleCd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase and (not org.)
select seleCd_RSH_Hydrolase_NoIno, Cd_RSH_Hydrolase_NoOrg and (not ino.)
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase_NoIno
delete seleCd_RSH_Hydrolase_NoOrg
delete Cd_RSH_Hydrolase_NoOrg
delete seleCd_RSH_Hydrolase_NoIno
select seleCd_RSH_Hydrolase, Cd_RSH_Hydrolase
deselect
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase

disable Cd_RSH_Bifunctional_Fragment
disable Cd_RSH_Synthetase
disable Cd_RSH_Central3HelixBundle
disable Cd_RSH_Hydrolase
disable ATP_GTP

select hotSpot, resi 204-209
color red, hotSpot
deselect

select mutation, resi 213
```

color limegreen, mutation
deselect

disable fold_08_cd_rsh_d213h_atp_mg_gtp_model_0
enable fold_08_cd_rsh_d213h_atp_mg_gtp_model_0

disable LabelBindingPocket_Sticks

orient ATP_GTP_Mg
zoom ATP_GTP_Mg, 5

RSH ATP GTP Mg D213H and WT ATP GTP Mg *C. difficile*

set seq_view, 1
bg_color white

select seleOG, fold_cd_rsh_af3_atp_mg_gtp_model_0
select seleD213H, fold_08_cd_rsh_d213h_atp_mg_gtp_model_0
color wheat, seleOG
color gray50, seleD213H
select bifunctionalDomain, seleOG and (not resi 386-735)
align seleD213H, bifunctionalDomain
deselect

select seleHotSpot, resi 204-209
Deselect

select seleMutation, resi 213
deselect
create HotSpot, resi 204-209
create Mutation, resi 213

select OG_HotSpot, seleOG and resi 204-209
color salmon, OG_HotSpot
deselect

select mutationHotSpot, seleD213H and resi 204-209
color red, mutationHotSpot
deselect

select OG_Mutation, seleOG and resi 213
color paleyellow, OG_Mutation
deselect

select D213H_Mutation, seleD213H and resi 213
color limegreen, D213H_Mutation
deselect

select prot_ATP_GTP_Mg, fold_08_cd_rsh_d213h_atp_mg_gtp_model_0
select seleATP_GTP, org. and (not prot_ATP_GTP_Mg)
create ATP_GTP, seleATP_GTP
select seleATP_atom, first org. and ATP_GTP

```
create ATP_atom, seleATP_atom
select seleATP,  bm. seleATP_atom

create ATP, seleATP

select seleGTP_atom, last org. and ATP_GTP
create GTP_atom, seleGTP_atom
select seleGTP,  bm. seleGTP_atom
create GTP, seleGTP
deselect

delete ATP_atom
delete GTP_ato
delete seleATP_atom
delete seleGTP_atom
delete GTP_atom
delete ATP_atom
delete seleGTP
delete seleATP

select seleATP, ATP
select seleGTP, GTP
deselect

select seleMg, bm. first ino.
create Mg, seleMg
deselect


select seleATP_GTP_Mg, org. ino. and (not prot_ATP_GTP_Mg)

set surface_carve_cutoff, 6
set surface_carve_selection, seleATP_GTP_Mg
set surface_carve_normal_cutoff, -0.1
set two_sided_lighting
set transparency, 0.5



select close_ATP_GTP_Mg, br. fold_08_cd_rsh_d213h_atp_mg_gtp_model_0 near_to 6 of seleATP_GTP_Mg
create LabelBindingPocket_Sticks, close & sc. + close & n. CA + close & r. PRO & n. N
create bindingPocket_Sticks, LabelBindingPocket_Sticks
hide everything, LabelBindingPocket_Sticks
hide everything, bindingPocket_Sticks
show sticks, bindingPocket_Sticks
show sticks, LabelBindingPocket_Sticks
label n. CA and LabelBindingPocket_Sticks, "%s %s" % (resn, resi)
color yelloworange, LabelBindingPocket_Sticks
color yelloworange, bindingPocket_Sticks


create bindingPocket_Surface, prot_ATP_GTP_Mg within 6 of seleATP_GTP_Mg, 0, 1
hide everything, bindingPocket_Surface
show surface, bindingPocket_Surface
set surface_color, orange
set transparency, 0.5
```

```
distance hbond_ATP, seleATP, prot_ATP_GTP_Mg, mode=2
distance hbond_GTP, seleGTP, prot_ATP_GTP_Mg, mode=2
color magenta, hbond_ATP
color magenta, hbond_GTP

create ATP_GTP_Mg, seleATP_GTP_Mg
create ATP, seleATP
create GTP, seleGTP
create Mg, seleMg

select close_GTP, br. fold_08_cd_rsh_d213h_atp_mg_gtp_model_0 near_to 6 of seleGTP
select close_ATP, br. fold_08_cd_rsh_d213h_atp_mg_gtp_model_0 near_to 6 of seleATP

color firebrick, ATP
color deepteal, GTP
color purpleblue, Mg
disable ATP_GTP_Mg

select seleCd_RSH_Bifunctional_Fragment, prot_ATP_GTP_Mg and resi 1-385
select seleCd_RSH_Synthetase, prot_ATP_GTP_Mg and resi 173-339
select seleCd_RSH_Central3HelixBundle, prot_ATP_GTP_Mg and resi 132-192
select seleCd_RSH_Hydrolase, prot_ATP_GTP_Mg and resi 1-149

create Cd_RSH_Bifunctional_Fragment, seleCd_RSH_Bifunctional_Fragment
create Cd_RSH_Synthetase, seleCd_RSH_Synthetase
create Cd_RSH_Central3HelixBundle, seleCd_RSH_Central3HelixBundle
create Cd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase_NoOrg
select seleCd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase and (not org.)
select seleCd_RSH_Hydrolase_NoIno, Cd_RSH_Hydrolase_NoOrg and (not ino.)
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase_NoIno
delete seleCd_RSH_Hydrolase_NoOrg
delete Cd_RSH_Hydrolase_NoOrg
delete seleCd_RSH_Hydrolase_NoIno
select seleCd_RSH_Hydrolase, Cd_RSH_Hydrolase
deselect
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase

disable Cd_RSH_Bifunctional_Fragment
disable Cd_RSH_Synthetase
disable Cd_RSH_Central3HelixBundle
disable Cd_RSH_Hydrolase

disable ATP_GTP
disable LabelBindingPocket_Sticks
disable GTP
disable ATP


disable fold_cd_rsh_af3_atp_mg_gtp_model_0
disable fold_08_cd_rsh_d213h_atp_mg_gtp_model_0
enable fold_cd_rsh_af3_atp_mg_gtp_model_0
enable fold_08_cd_rsh_d213h_atp_mg_gtp_model_0

deselect
```

```
orient ATP_GTP_Mg
zoom ATP_GTP_Mg, 5
```

RSH G217D ATP GTP Mg *C. difficile* (can be used for WT *C. difficile*)

```
set seq_view, 1
bg_color white
color gray50, fold_09_cd_rsh_g217d_atp_mg_gtp_model_0

select prot_ATP_GTP_Mg, polymer
select seleATP_GTP, org. and (not prot_ATP_GTP_Mg)
create ATP_GTP, seleATP_GTP
select seleATP_atom, first org. and ATP_GTP
create ATP_atom, seleATP_atom
select seleATP,  bm. seleATP_atom

create ATP, seleATP

select seleGTP_atom, last org. and ATP_GTP
create GTP_atom, seleGTP_atom
select seleGTP,  bm. seleGTP_atom
create GTP, seleGTP
deselect

delete ATP_atom
delete GTP_ato
delete seleATP_atom
delete seleGTP_atom
delete GTP_atom
delete ATP_atom
delete seleGTP
delete seleATP

select seleATP, ATP
select seleGTP, GTP
deselect

select seleMg, bm. first ino.
create Mg, seleMg
deselect


select seleATP_GTP_Mg, org. ino. and (not prot_ATP_GTP_Mg)

set surface_carve_cutoff, 6
set surface_carve_selection, seleATP_GTP_Mg
set surface_carve_normal_cutoff, -0.1
set two_sided_lighting
set transparency, 0.5



select close_ATP_GTP_Mg, br. fold_09_cd_rsh_g217d_atp_mg_gtp_model_0 near_to 6 of seleATP_GTP_Mg
create LabelBindingPocket_Sticks, close & sc. + close & n. CA + close & r. PRO & n. N
create bindingPocket_Sticks, LabelBindingPocket_Sticks
hide everything, LabelBindingPocket_Sticks
```

```
hide everything, bindingPocket_Sticks
show sticks, bindingPocket_Sticks
show sticks, LabelBindingPocket_Sticks
label n. CA and LabelBindingPocket_Sticks, "%s %s" % (resn, resi)
color yelloworange, LabelBindingPocket_Sticks
color yelloworange, bindingPocket_Sticks


create bindingPocket_Surface, prot_ATP_GTP_Mg within 6 of seleATP_GTP_Mg, 0, 1
hide everything, bindingPocket_Surface
show surface, bindingPocket_Surface
set surface_color, orange
set transparency, 0.5

distance hbond_ATP, seleATP, prot_ATP_GTP_Mg, mode=2
distance hbond_GTP, seleGTP, prot_ATP_GTP_Mg, mode=2
color magenta, hbond_ATP
color magenta, hbond_GTP

create ATP_GTP_Mg, seleATP_GTP_Mg
create ATP, seleATP
create GTP, seleGTP
create Mg, seleMg

select close_GTP, br. fold_09_cd_rsh_g217d_atp_mg_gtp_model_0 near_to 6 of seleGTP
select close_ATP, br. fold_09_cd_rsh_g217d_atp_mg_gtp_model_0 near_to 6 of seleATP

color firebrick, ATP
color deepteal, GTP
color purpleblue, Mg
disable ATP_GTP_Mg

select seleCd_RSH_Bifunctional_Fragment, prot_ATP_GTP_Mg and resi 1-385
select seleCd_RSH_Synthetase, prot_ATP_GTP_Mg and resi 173-339
select seleCd_RSH_Central3HelixBundle, prot_ATP_GTP_Mg and resi 132-192
select seleCd_RSH_Hydrolase, prot_ATP_GTP_Mg and resi 1-149

create Cd_RSH_Bifunctional_Fragment, seleCd_RSH_Bifunctional_Fragment
create Cd_RSH_Synthetase, seleCd_RSH_Synthetase
create Cd_RSH_Central3HelixBundle, seleCd_RSH_Central3HelixBundle
create Cd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase_NoOrg
select seleCd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase and (not org.)
select seleCd_RSH_Hydrolase_NoIno, Cd_RSH_Hydrolase_NoOrg and (not ino.)
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase_NoIno
delete seleCd_RSH_Hydrolase_NoOrg
delete Cd_RSH_Hydrolase_NoOrg
delete seleCd_RSH_Hydrolase_NoIno
select seleCd_RSH_Hydrolase, Cd_RSH_Hydrolase
deselect
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase

disable Cd_RSH_Bifunctional_Fragment
disable Cd_RSH_Synthetase
disable Cd_RSH_Central3HelixBundle
disable Cd_RSH_Hydrolase
disable ATP_GTP
```

```
select hotSpot, resi 204-209
color red, hotSpot
deselect

select mutation, resi 217
color limegreen, mutation
deselect

disable fold_09_cd_rsh_g217d_atp_mg_gtp_model_0
enable fold_09_cd_rsh_g217d_atp_mg_gtp_model_0

disable LabelBindingPocket_Sticks

orient ATP_GTP_Mg
zoom ATP_GTP_Mg, 5
```

RSH ATP GTP Mg G217D and WT ATP GTP Mg *C. difficile*

```
set seq_view, 1
bg_color white

select seleOG, fold_cd_rsh_af3_atp_mg_gtp_model_0
select seleG217D, fold_09_cd_rsh_g217d_atp_mg_gtp_model_0
color wheat, seleOG
color gray50, seleG217D
select bifunctionalDomain, seleOG and (not resi 386-735)
align seleG217D, bifunctionalDomain
deselect

select seleHotSpot, resi 204-209
Deselect

select seleMutation, resi 217
deselect
create HotSpot, resi 204-209
create Mutation, resi 217

select OG_HotSpot, seleOG and resi 204-209
color salmon, OG_HotSpot
deselect

select mutationHotSpot, seleG217D and resi 204-209
color red, mutationHotSpot
deselect

select OG_Mutation, seleOG and  resi 217
color paleyellow, OG_Mutation
deselect

select G217D_Mutation, seleG217D and resi 217
color limegreen, G217D_Mutation
deselect
```

```
select prot_ATP_GTP_Mg, fold_09_cd_rsh_g217d_atp_mg_gtp_model_0
select seleATP_GTP, org. and (not prot_ATP_GTP_Mg)
create ATP_GTP, seleATP_GTP
select seleATP_atom, first org. and ATP_GTP
create ATP_atom, seleATP_atom
select seleATP,  bm. seleATP_atom

create ATP, seleATP

select seleGTP_atom, last org. and ATP_GTP
create GTP_atom, seleGTP_atom
select seleGTP,  bm. seleGTP_atom
create GTP, seleGTP
deselect

delete ATP_atom
delete GTP_ato
delete seleATP_atom
delete seleGTP_atom
delete GTP_atom
delete ATP_atom
delete seleGTP
delete seleATP

select seleATP, ATP
select seleGTP, GTP
deselect

select seleMg, bm. first ino.
create Mg, seleMg
deselect


select seleATP_GTP_Mg, org. ino. and (not prot_ATP_GTP_Mg)

set surface_carve_cutoff, 6
set surface_carve_selection, seleATP_GTP_Mg
set surface_carve_normal_cutoff, -0.1
set two_sided_lighting
set transparency, 0.5



select close_ATP_GTP_Mg, br. fold_09_cd_rsh_g217d_atp_mg_gtp_model_0 near_to 6 of seleATP_GTP_Mg
create LabelBindingPocket_Sticks, close & sc. + close & n. CA + close & r. PRO & n. N
create bindingPocket_Sticks, LabelBindingPocket_Sticks
hide everything, LabelBindingPocket_Sticks
hide everything, bindingPocket_Sticks
show sticks, bindingPocket_Sticks
show sticks, LabelBindingPocket_Sticks
label n. CA and LabelBindingPocket_Sticks, "%s %s" % (resn, resi)
color yelloworange, LabelBindingPocket_Sticks
color yelloworange, bindingPocket_Sticks
```

```
create bindingPocket_Surface, prot_ATP_GTP_Mg within 6 of seleATP_GTP_Mg, 0, 1
hide everything, bindingPocket_Surface
show surface, bindingPocket_Surface
set surface_color, orange
set transparency, 0.5

distance hbond_ATP, seleATP, prot_ATP_GTP_Mg, mode=2
distance hbond_GTP, seleGTP, prot_ATP_GTP_Mg, mode=2
color magenta, hbond_ATP
color magenta, hbond_GTP

create ATP_GTP_Mg, seleATP_GTP_Mg
create ATP, seleATP
create GTP, seleGTP
create Mg, seleMg

select close_GTP, br. fold_09_cd_rsh_g217d_atp_mg_gtp_model_0 near_to 6 of seleGTP
select close_ATP, br. fold_09_cd_rsh_g217d_atp_mg_gtp_model_0 near_to 6 of seleATP

color firebrick, ATP
color deepteal, GTP
color purpleblue, Mg
disable ATP_GTP_Mg

select seleCd_RSH_Bifunctional_Fragment, prot_ATP_GTP_Mg and resi 1-385
select seleCd_RSH_Synthetase, prot_ATP_GTP_Mg and resi 173-339
select seleCd_RSH_Central3HelixBundle, prot_ATP_GTP_Mg and resi 132-192
select seleCd_RSH_Hydrolase, prot_ATP_GTP_Mg and resi 1-149

create Cd_RSH_Bifunctional_Fragment, seleCd_RSH_Bifunctional_Fragment
create Cd_RSH_Synthetase, seleCd_RSH_Synthetase
create Cd_RSH_Central3HelixBundle, seleCd_RSH_Central3HelixBundle
create Cd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase_NoOrg
select seleCd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase and (not org.)
select seleCd_RSH_Hydrolase_NoIno, Cd_RSH_Hydrolase_NoOrg and (not ino.)
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase_NoIno
delete seleCd_RSH_Hydrolase_NoOrg
delete Cd_RSH_Hydrolase_NoOrg
delete seleCd_RSH_Hydrolase_NoIno
select seleCd_RSH_Hydrolase, Cd_RSH_Hydrolase
deselect
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase

disable Cd_RSH_Bifunctional_Fragment
disable Cd_RSH_Synthetase
disable Cd_RSH_Central3HelixBundle
disable Cd_RSH_Hydrolase

disable ATP_GTP
disable LabelBindingPocket_Sticks
disable GTP
disable ATP
```

```
disable fold_cd_rsh_af3_atp_mg_gtp_model_0
disable fold_09_cd_rsh_g217d_atp_mg_gtp_model_0
enable fold_cd_rsh_af3_atp_mg_gtp_model_0
enable fold_09_cd_rsh_g217d_atp_mg_gtp_model_0

deselect

orient ATP_GTP_Mg
zoom ATP_GTP_Mg, 5
```

RSH ATP GTP Mg G169A and WT ATP GTP Mg *C. difficile*

```
set seq_view, 1
bg_color white

select seleOG, fold_cd_rsh_af3_atp_mg_gtp_model_0
select seleG169A, fold_01_cd_rsh_g169a_atp_mg_gtp_model_0
color wheat, seleOG
color gray50, seleG169A
select bifunctionalDomain, seleOG and (not resi 386-735)
align seleG169A, bifunctionalDomain
deselect

select seleHotSpot, resi 204-209
Deselect

select seleMutation, resi 169
deselect
create HotSpot, resi 204-209
create Mutation, resi 169

select OG_HotSpot, seleOG and resi 204-209
color salmon, OG_HotSpot
deselect

select mutationHotSpot, seleG169A and resi 204-209
color red, mutationHotSpot
deselect

select OG_Mutation, seleOG and  resi 169
color paleyellow, OG_Mutation
deselect

select G169A_Mutation, seleG169A and resi 169
color limegreen, G169A_Mutation
deselect


select prot_ATP_GTP_Mg, polymer
select seleATP_GTP, org. and (not prot_ATP_GTP_Mg)
create ATP_GTP, seleATP_GTP
select seleATP_atom, first org. and ATP_GTP
```

```
create ATP_atom, seleATP_atom
select seleATP,  bm. seleATP_atom

create ATP, seleATP

select seleGTP_atom, last org. and ATP_GTP
create GTP_atom, seleGTP_atom
select seleGTP,  bm. seleGTP_atom
create GTP, seleGTP
deselect

delete ATP_atom
delete GTP_ato
delete seleATP_atom
delete seleGTP_atom
delete GTP_atom
delete ATP_atom
delete seleGTP
delete seleATP

select seleATP, ATP
select seleGTP, GTP
deselect

select seleMg, bm. first ino.
create Mg, seleMg
deselect


select seleATP_GTP_Mg, org. ino. and (not prot_ATP_GTP_Mg)

set surface_carve_cutoff, 6
set surface_carve_selection, seleATP_GTP_Mg
set surface_carve_normal_cutoff, -0.1
set two_sided_lighting
set transparency, 0.5



select close_ATP_GTP_Mg, br. fold_cd_rsh_af3_atp_mg_gtp_model_0 near_to 6 of seleATP_GTP_Mg
create LabelBindingPocket_Sticks, close & sc. + close & n. CA + close & r. PRO & n. N
create bindingPocket_Sticks, LabelBindingPocket_Sticks
hide everything, LabelBindingPocket_Sticks
hide everything, bindingPocket_Sticks
show sticks, bindingPocket_Sticks
show sticks, LabelBindingPocket_Sticks
label n. CA and LabelBindingPocket_Sticks, "%s %s" % (resn, resi)
color yelloworange, LabelBindingPocket_Sticks
color yelloworange, bindingPocket_Sticks


create bindingPocket_Surface, prot_ATP_GTP_Mg within 6 of seleATP_GTP_Mg, 0, 1
hide everything, bindingPocket_Surface
show surface, bindingPocket_Surface
set surface_color, orange
set transparency, 0.5
```

distance hbond_ATP, seleATP, prot_ATP_GTP_Mg, mode=2
distance hbond_GTP, seleGTP, prot_ATP_GTP_Mg, mode=2
color magenta, hbond_ATP
color magenta, hbond_GTP

create ATP_GTP_Mg, seleATP_GTP_Mg
create ATP, seleATP
create GTP, seleGTP
create Mg, seleMg

select close_GTP, br. polymer near_to 6 of seleGTP
select close_ATP, br. polymer near_to 6 of seleATP

color firebrick, ATP
color deepteal, GTP
color purpleblue, Mg
disable ATP_GTP_Mg

select seleCd_RSH_Bifunctional_Fragment, prot_ATP_GTP_Mg and resi 1-385
select seleCd_RSH_Synthetase, prot_ATP_GTP_Mg and resi 173-339
select seleCd_RSH_Central3HelixBundle, prot_ATP_GTP_Mg and resi 132-192
select seleCd_RSH_Hydrolase, prot_ATP_GTP_Mg and resi 1-149

create Cd_RSH_Bifunctional_Fragment, seleCd_RSH_Bifunctional_Fragment
create Cd_RSH_Synthetase, seleCd_RSH_Synthetase
create Cd_RSH_Central3HelixBundle, seleCd_RSH_Central3HelixBundle
create Cd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase_NoOrg
select seleCd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase and (not org.)
select seleCd_RSH_Hydrolase_NoIno, Cd_RSH_Hydrolase_NoOrg and (not ino.)
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase_NoIno
delete seleCd_RSH_Hydrolase_NoOrg
delete Cd_RSH_Hydrolase_NoOrg
delete seleCd_RSH_Hydrolase_NoIno
select seleCd_RSH_Hydrolase, Cd_RSH_Hydrolase
deselect
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase

disable Cd_RSH_Bifunctional_Fragment
disable Cd_RSH_Synthetase
disable Cd_RSH_Central3HelixBundle
disable Cd_RSH_Hydrolase
disable ATP_GTP

disable fold_cd_rsh_af3_atp_mg_gtp_model_0
disable fold_05_cd_rsh_g196e_atp_mg_gtp_model_0
enable fold_cd_rsh_af3_atp_mg_gtp_model_0
enable fold_05_cd_rsh_g196e_atp_mg_gtp_model_0

orient ATP_GTP_Mg
zoom ATP_GTP_Mg, 5

RSH G170P ATP GDP Mg *C. difficile* (can be used for WT *C. difficile*)

```
set seq_view, 1
bg_color white
color gray50, fold_02_cd_rsh_g170p_gdp_mg_atp_model_0

select prot_ATP_GDP_Mg, polymer
select seleATP_GDP, org. and (not prot_ATP_GDP_Mg)
create ATP_GDP, seleATP_GDP
select seleATP_atom, first org. and ATP_GDP
create ATP_atom, seleATP_atom
select seleATP,  bm. seleATP_atom

create ATP, seleATP

select seleGDP_atom, last org. and ATP_GDP
create GDP_atom, seleGDP_atom
select seleGDP,  bm. seleGDP_atom
create GDP, seleGDP
deselect

delete ATP_atom
delete GDP_ato
delete seleATP_atom
delete seleGDP_atom
delete GDP_atom
delete ATP_atom
delete seleGDP
delete seleATP

select seleATP, ATP
select seleGDP, GDP
deselect

select seleMg, bm. first ino.
create Mg, seleMg
deselect


select seleATP_GDP_Mg, org. ino. and (not prot_ATP_GDP_Mg)

set surface_carve_cutoff, 6
set surface_carve_selection, seleATP_GDP_Mg
set surface_carve_normal_cutoff, -0.1
set two_sided_lighting
set transparency, 0.5



select close_ATP_GDP_Mg, br. fold_02_cd_rsh_g170p_gdp_mg_atp_model_0 near_to 6 of seleATP_GDP_Mg
create LabelBindingPocket_Sticks, close & sc. + close & n. CA + close & r. PRO & n. N
create bindingPocket_Sticks, LabelBindingPocket_Sticks
hide everything, LabelBindingPocket_Sticks
hide everything, bindingPocket_Sticks
show sticks, bindingPocket_Sticks
show sticks, LabelBindingPocket_Sticks
label n. CA and LabelBindingPocket_Sticks, "%s %s" % (resn, resi)
```

```
color yelloworange, LabelBindingPocket_Sticks
color yelloworange, bindingPocket_Sticks


create bindingPocket_Surface, prot_ATP_GDP_Mg within 6 of seleATP_GDP_Mg, 0, 1
hide everything, bindingPocket_Surface
show surface, bindingPocket_Surface
set surface_color, orange
set transparency, 0.5

distance hbond_ATP, seleATP, prot_ATP_GDP_Mg, mode=2
distance hbond_GDP, seleGDP, prot_ATP_GDP_Mg, mode=2
color magenta, hbond_ATP
color magenta, hbond_GDP

create ATP_GDP_Mg, seleATP_GDP_Mg
create ATP, seleATP
create GDP, seleGDP
create Mg, seleMg

select close_GDP, br. fold_02_cd_rsh_g170p_gdp_mg_atp_model_0 near_to 6 of seleGDP
select close_ATP, br. fold_02_cd_rsh_g170p_gdp_mg_atp_model_0 near_to 6 of seleATP

color firebrick, ATP
color deepteal, GDP
color purpleblue, Mg
disable ATP_GDP_Mg

select seleCd_RSH_Bifunctional_Fragment, prot_ATP_GDP_Mg and resi 1-385
select seleCd_RSH_Synthetase, prot_ATP_GDP_Mg and resi 173-339
select seleCd_RSH_Central3HelixBundle, prot_ATP_GDP_Mg and resi 132-192
select seleCd_RSH_Hydrolase, prot_ATP_GDP_Mg and resi 1-149

create Cd_RSH_Bifunctional_Fragment, seleCd_RSH_Bifunctional_Fragment
create Cd_RSH_Synthetase, seleCd_RSH_Synthetase
create Cd_RSH_Central3HelixBundle, seleCd_RSH_Central3HelixBundle
create Cd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase_NoOrg
select seleCd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase and (not org.)
select seleCd_RSH_Hydrolase_NoIno, Cd_RSH_Hydrolase_NoOrg and (not ino.)
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase_NoIno
delete seleCd_RSH_Hydrolase_NoOrg
delete Cd_RSH_Hydrolase_NoOrg
delete seleCd_RSH_Hydrolase_NoIno
select seleCd_RSH_Hydrolase, Cd_RSH_Hydrolase
deselect
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase

disable Cd_RSH_Bifunctional_Fragment
disable Cd_RSH_Synthetase
disable Cd_RSH_Central3HelixBundle
disable Cd_RSH_Hydrolase
disable ATP_GDP

select hotSpot, resi 204-209
color red, hotSpot
deselect
```

```
select mutation, resi 170
color limegreen, mutation
deselect

disable fold_02_cd_rsh_g170p_gdp_mg_atp_model_0
enable fold_02_cd_rsh_g170p_gdp_mg_atp_model_0

disable LabelBindingPocket_Sticks

orient ATP_GDP_Mg
zoom ATP_GDP_Mg, 5
```

RSH ATP GDP Mg G170P and WT ATP GDP Mg *C. difficile*

```
set seq_view, 1
bg_color white

select seleOG, fold_cd_rsh_af3_atp_mg_gdp_model_0
select seleG170P, fold_02_cd_rsh_g170p_gdp_mg_atp_model_0
color wheat, seleOG
color gray50, seleG170P
select bifunctionalDomain, seleOG and (not resi 386-735)
align seleG170P, bifunctionalDomain
deselect

select seleHotSpot, resi 204-209
deselect

select seleMutation, resi 170
deselect
create HotSpot, resi 204-209
create Mutation, resi 170

select OG_HotSpot, seleOG and resi 204-209
color salmon, OG_HotSpot
deselect

select mutationHotSpot, seleG170P and resi 204-209
color red, mutationHotSpot
deselect

select OG_Mutation, seleOG and  resi 170
color paleyellow, OG_Mutation
deselect

select G170P_Mutation, seleG170P and resi 170
color limegreen, G170P_Mutation
deselect

select prot_ATP_GDP_Mg, fold_02_cd_rsh_g170p_gdp_mg_atp_model_0
select seleATP_GDP, org. and (not prot_ATP_GDP_Mg)
```

```
create ATP_GDP, seleATP_GDP
select seleATP_atom, first org. and ATP_GDP
create ATP_atom, seleATP_atom
select seleATP,  bm. seleATP_atom

create ATP, seleATP

select seleGDP_atom, last org. and ATP_GDP
create GDP_atom, seleGDP_atom
select seleGDP,  bm. seleGDP_atom
create GDP, seleGDP
deselect

delete ATP_atom
delete GDP_ato
delete seleATP_atom
delete seleGDP_atom
delete GDP_atom
delete ATP_atom
delete seleGDP
delete seleATP

select seleATP, ATP
select seleGDP, GDP
deselect

select seleMg, bm. first ino.
create Mg, seleMg
deselect


select seleATP_GDP_Mg, org. ino. and (not prot_ATP_GDP_Mg)

set surface_carve_cutoff, 6
set surface_carve_selection, seleATP_GDP_Mg
set surface_carve_normal_cutoff, -0.1
set two_sided_lighting
set transparency, 0.5



select close_ATP_GDP_Mg, br. fold_02_cd_rsh_g170p_gdp_mg_atp_model_0 near_to 6 of seleATP_GDP_Mg
create LabelBindingPocket_Sticks, close & sc. + close & n. CA + close & r. PRO & n. N
create bindingPocket_Sticks, LabelBindingPocket_Sticks
hide everything, LabelBindingPocket_Sticks
hide everything, bindingPocket_Sticks
show sticks, bindingPocket_Sticks
show sticks, LabelBindingPocket_Sticks
label n. CA and LabelBindingPocket_Sticks, "%s %s" % (resn, resi)
color yelloworange, LabelBindingPocket_Sticks
color yelloworange, bindingPocket_Sticks


create bindingPocket_Surface, prot_ATP_GDP_Mg within 6 of seleATP_GDP_Mg, 0, 1
hide everything, bindingPocket_Surface
show surface, bindingPocket_Surface
```

```
set surface_color, orange
set transparency, 0.5

distance hbond_ATP, seleATP, prot_ATP_GDP_Mg, mode=2
distance hbond_GDP, seleGDP, prot_ATP_GDP_Mg, mode=2
color magenta, hbond_ATP
color magenta, hbond_GDP

create ATP_GDP_Mg, seleATP_GDP_Mg
create ATP, seleATP
create GDP, seleGDP
create Mg, seleMg

select close_GDP, br. fold_02_cd_rsh_g170p_gdp_mg_atp_model_0 near_to 6 of seleGDP
select close_ATP, br. fold_02_cd_rsh_g170p_gdp_mg_atp_model_0 near_to 6 of seleATP

color firebrick, ATP
color deepteal, GDP
color purpleblue, Mg
disable ATP_GDP_Mg

select seleCd_RSH_Bifunctional_Fragment, prot_ATP_GDP_Mg and resi 1-385
select seleCd_RSH_Synthetase, prot_ATP_GDP_Mg and resi 173-339
select seleCd_RSH_Central3HelixBundle, prot_ATP_GDP_Mg and resi 132-192
select seleCd_RSH_Hydrolase, prot_ATP_GDP_Mg and resi 1-149

create Cd_RSH_Bifunctional_Fragment, seleCd_RSH_Bifunctional_Fragment
create Cd_RSH_Synthetase, seleCd_RSH_Synthetase
create Cd_RSH_Central3HelixBundle, seleCd_RSH_Central3HelixBundle
create Cd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase_NoOrg
select seleCd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase and (not org.)
select seleCd_RSH_Hydrolase_NoIno, Cd_RSH_Hydrolase_NoOrg and (not ino.)
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase_NoIno
delete seleCd_RSH_Hydrolase_NoOrg
delete Cd_RSH_Hydrolase_NoOrg
delete seleCd_RSH_Hydrolase_NoIno
select seleCd_RSH_Hydrolase, Cd_RSH_Hydrolase
deselect
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase

disable Cd_RSH_Bifunctional_Fragment
disable Cd_RSH_Synthetase
disable Cd_RSH_Central3HelixBundle
disable Cd_RSH_Hydrolase

disable ATP_GDP
disable LabelBindingPocket_Sticks
disable GDP
disable ATP


disable fold_fold_cd_rsh_af3_atp_mg_gdp_model_0
disable fold_02_cd_rsh_g170p_gdp_mg_atp_model_0
enable fold_fold_cd_rsh_af3_atp_mg_gdp_model_0
enable fold_02_cd_rsh_g170p_gdp_mg_atp_model_0
```

```
deselect

orient ATP_GDP_Mg
zoom ATP_GDP_Mg, 5

RSH G196E ATP GDP Mg C. difficile

set seq_view, 1
bg_color white
color gray50, fold_05_cd_rsh_g196e_gdp_mg_atp_model_0

select prot_ATP_GDP_Mg, polymer
select seleATP_GDP, org. and (not prot_ATP_GDP_Mg)
create ATP_GDP, seleATP_GDP
select seleATP_atom, first org. and ATP_GDP
create ATP_atom, seleATP_atom
select seleATP,  bm. seleATP_atom

create ATP, seleATP

select seleGDP_atom, last org. and ATP_GDP
create GDP_atom, seleGDP_atom
select seleGDP,  bm. seleGDP_atom
create GDP, seleGDP
deselect

delete ATP_atom
delete GDP_ato
delete seleATP_atom
delete seleGDP_atom
delete GDP_atom
delete ATP_atom
delete seleGDP
delete seleATP

select seleATP, ATP
select seleGDP, GDP
deselect

select seleMg, bm. first ino.
create Mg, seleMg
deselect


select seleATP_GDP_Mg, org. ino. and (not prot_ATP_GDP_Mg)

set surface_carve_cutoff, 6
set surface_carve_selection, seleATP_GDP_Mg
set surface_carve_normal_cutoff, -0.1
set two_sided_lighting
set transparency, 0.5


select close_ATP_GDP_Mg, br. fold_05_cd_rsh_g196e_gdp_mg_atp_model_0 near_to 6 of seleATP_GDP_Mg
create LabelBindingPocket_Sticks, close & sc. + close & n. CA + close & r. PRO & n. N
```

```
create bindingPocket_Sticks, LabelBindingPocket_Sticks
hide everything, LabelBindingPocket_Sticks
hide everything, bindingPocket_Sticks
show sticks, bindingPocket_Sticks
show sticks, LabelBindingPocket_Sticks
label n. CA and LabelBindingPocket_Sticks, "%s %s" % (resn, resi)
color yelloworange, LabelBindingPocket_Sticks
color yelloworange, bindingPocket_Sticks


create bindingPocket_Surface, prot_ATP_GDP_Mg within 6 of seleATP_GDP_Mg, 0, 1
hide everything, bindingPocket_Surface
show surface, bindingPocket_Surface
set surface_color, orange
set transparency, 0.5

distance hbond_ATP, seleATP, prot_ATP_GDP_Mg, mode=2
distance hbond_GDP, seleGDP, prot_ATP_GDP_Mg, mode=2
color magenta, hbond_ATP
color magenta, hbond_GDP

create ATP_GDP_Mg, seleATP_GDP_Mg
create ATP, seleATP
create GDP, seleGDP
create Mg, seleMg

select close_GDP, br. fold_05_cd_rsh_g196e_gdp_mg_atp_model_0 near_to 6 of seleGDP
select close_ATP, br. fold_05_cd_rsh_g196e_gdp_mg_atp_model_0 near_to 6 of seleATP

color firebrick, ATP
color deepteal, GDP
color purpleblue, Mg
disable ATP_GDP_Mg

select seleCd_RSH_Bifunctional_Fragment, prot_ATP_GDP_Mg and resi 1-385
select seleCd_RSH_Synthetase, prot_ATP_GDP_Mg and resi 173-339
select seleCd_RSH_Central3HelixBundle, prot_ATP_GDP_Mg and resi 132-192
select seleCd_RSH_Hydrolase, prot_ATP_GDP_Mg and resi 1-149

create Cd_RSH_Bifunctional_Fragment, seleCd_RSH_Bifunctional_Fragment
create Cd_RSH_Synthetase, seleCd_RSH_Synthetase
create Cd_RSH_Central3HelixBundle, seleCd_RSH_Central3HelixBundle
create Cd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase_NoOrg
select seleCd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase and (not org.)
select seleCd_RSH_Hydrolase_NoIno, Cd_RSH_Hydrolase_NoOrg and (not ino.)
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase_NoIno
delete seleCd_RSH_Hydrolase_NoOrg
delete Cd_RSH_Hydrolase_NoOrg
delete seleCd_RSH_Hydrolase_NoIno
select seleCd_RSH_Hydrolase, Cd_RSH_Hydrolase
deselect
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase

disable Cd_RSH_Bifunctional_Fragment
disable Cd_RSH_Synthetase
disable Cd_RSH_Central3HelixBundle
```

```
disable Cd_RSH_Hydrolase
disable ATP_GDP

select hotSpot, resi 204-209
color red, hotSpot
deselect

select mutation, resi 196
color limegreen, mutation
deselect

disable fold_05_cd_rsh_g196e_gdp_mg_atp_model_0
enable fold_05_cd_rsh_g196e_gdp_mg_atp_model_0

disable LabelBindingPocket_Sticks

orient ATP_GDP_Mg
zoom ATP_GDP_Mg, 5
```

RSH ATP GDP Mg G196E and WT ATP GDP Mg *C. difficile*

```
set seq_view, 1
bg_color white

select seleOG, fold_cd_rsh_af3_atp_mg_gdp_model_0
select seleG196E, fold_05_cd_rsh_g196e_gdp_mg_atp_model_0
color wheat, seleOG
color gray50, seleG196E
select bifunctionalDomain, seleOG and (not resi 386-735)
align seleG196E, bifunctionalDomain
deselect

select seleHotSpot, resi 204-209
deselect

select seleMutation, resi 196
deselect
create HotSpot, resi 204-209
create Mutation, resi 196

select OG_HotSpot, seleOG and resi 204-209
color salmon, OG_HotSpot
deselect

select mutationHotSpot, seleG196E and resi 204-209
color red, mutationHotSpot
deselect

select OG_Mutation, seleOG and  resi 196
color paleyellow, OG_Mutation
deselect

select G196E_Mutation, seleG196E and resi 196
color limegreen, G196E_Mutation
```

```
deselect


select prot_ATP_GDP_Mg, fold_05_cd_rsh_g196e_gdp_mg_atp_model_0
select seleATP_GDP, org. and (not prot_ATP_GDP_Mg)
create ATP_GDP, seleATP_GDP
select seleATP_atom, first org. and ATP_GDP
create ATP_atom, seleATP_atom
select seleATP,  bm. seleATP_atom

create ATP, seleATP

select seleGDP_atom, last org. and ATP_GDP
create GDP_atom, seleGDP_atom
select seleGDP,  bm. seleGDP_atom
create GDP, seleGDP
deselect

delete ATP_atom
delete GDP_ato
delete seleATP_atom
delete seleGDP_atom
delete GDP_atom
delete ATP_atom
delete seleGDP
delete seleATP

select seleATP, ATP
select seleGDP, GDP
deselect

select seleMg, bm. first ino.
create Mg, seleMg
deselect


select seleATP_GDP_Mg, org. ino. and (not prot_ATP_GDP_Mg)

set surface_carve_cutoff, 6
set surface_carve_selection, seleATP_GDP_Mg
set surface_carve_normal_cutoff, -0.1
set two_sided_lighting
set transparency, 0.5


select close_ATP_GDP_Mg, br. fold_05_cd_rsh_g196e_gdp_mg_atp_model_0 near_to 6 of seleATP_GDP_Mg
create LabelBindingPocket_Sticks, close & sc. + close & n. CA + close & r. PRO & n. N
create bindingPocket_Sticks, LabelBindingPocket_Sticks
hide everything, LabelBindingPocket_Sticks
hide everything, bindingPocket_Sticks
show sticks, bindingPocket_Sticks
show sticks, LabelBindingPocket_Sticks
label n. CA and LabelBindingPocket_Sticks, "%s %s" % (resn, resi)
color yelloworange, LabelBindingPocket_Sticks
```

```
color yelloworange, bindingPocket_Sticks


create bindingPocket_Surface, prot_ATP_GDP_Mg within 6 of seleATP_GDP_Mg, 0, 1
hide everything, bindingPocket_Surface
show surface, bindingPocket_Surface
set surface_color, orange
set transparency, 0.5

distance hbond_ATP, seleATP, prot_ATP_GDP_Mg, mode=2
distance hbond_GDP, seleGDP, prot_ATP_GDP_Mg, mode=2
color magenta, hbond_ATP
color magenta, hbond_GDP

create ATP_GDP_Mg, seleATP_GDP_Mg
create ATP, seleATP
create GDP, seleGDP
create Mg, seleMg

select close_GDP, br. fold_05_cd_rsh_g196e_gdp_mg_atp_model_0 near_to 6 of seleGDP
select close_ATP, br. fold_05_cd_rsh_g196e_gdp_mg_atp_model_0 near_to 6 of seleATP

color firebrick, ATP
color deepteal, GDP
color purpleblue, Mg
disable ATP_GDP_Mg

select seleCd_RSH_Bifunctional_Fragment, prot_ATP_GDP_Mg and resi 1-385
select seleCd_RSH_Synthetase, prot_ATP_GDP_Mg and resi 173-339
select seleCd_RSH_Central3HelixBundle, prot_ATP_GDP_Mg and resi 132-192
select seleCd_RSH_Hydrolase, prot_ATP_GDP_Mg and resi 1-149

create Cd_RSH_Bifunctional_Fragment, seleCd_RSH_Bifunctional_Fragment
create Cd_RSH_Synthetase, seleCd_RSH_Synthetase
create Cd_RSH_Central3HelixBundle, seleCd_RSH_Central3HelixBundle
create Cd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase_NoOrg
select seleCd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase and (not org.)
select seleCd_RSH_Hydrolase_NoIno, Cd_RSH_Hydrolase_NoOrg and (not ino.)
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase_NoIno
delete seleCd_RSH_Hydrolase_NoOrg
delete Cd_RSH_Hydrolase_NoOrg
delete seleCd_RSH_Hydrolase_NoIno
select seleCd_RSH_Hydrolase, Cd_RSH_Hydrolase
deselect
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase

disable Cd_RSH_Bifunctional_Fragment
disable Cd_RSH_Synthetase
disable Cd_RSH_Central3HelixBundle
disable Cd_RSH_Hydrolase

disable ATP_GDP
disable LabelBindingPocket_Sticks
disable GDP
disable ATP
```

```
disable fold_cd_rsh_af3_atp_mg_gdp_model_0
disable fold_05_cd_rsh_g196e_gdp_mg_atp_model_0
enable fold_cd_rsh_af3_atp_mg_gdp_model_0
enable fold_05_cd_rsh_g196e_gdp_mg_atp_model_0

deselect

orient ATP_GDP_Mg
zoom ATP_GDP_Mg, 5
```

RSH G196K ATP GDP Mg *C. difficile* (can be used for WT *C. difficile*)

```
set seq_view, 1
bg_color white
color gray50, fold_06_cd_rsh_g196k_gdp_mg_atp_model_0

select prot_ATP_GDP_Mg, polymer
select seleATP_GDP, org. and (not prot_ATP_GDP_Mg)
create ATP_GDP, seleATP_GDP
select seleATP_atom, first org. and ATP_GDP
create ATP_atom, seleATP_atom
select seleATP,  bm. seleATP_atom

create ATP, seleATP

select seleGDP_atom, last org. and ATP_GDP
create GDP_atom, seleGDP_atom
select seleGDP,  bm. seleGDP_atom
create GDP, seleGDP
deselect

delete ATP_atom
delete GDP_ato
delete seleATP_atom
delete seleGDP_atom
delete GDP_atom
delete ATP_atom
delete seleGDP
delete seleATP

select seleATP, ATP
select seleGDP, GDP
deselect

select seleMg, bm. first ino.
create Mg, seleMg
deselect


select seleATP_GDP_Mg, org. ino. and (not prot_ATP_GDP_Mg)

set surface_carve_cutoff, 6
set surface_carve_selection, seleATP_GDP_Mg
set surface_carve_normal_cutoff, -0.1
```

```
set two_sided_lighting
set transparency, 0.5


select close_ATP_GDP_Mg, br. fold_06_cd_rsh_g196k_gdp_mg_atp_model_0 near_to 6 of seleATP_GDP_Mg
create LabelBindingPocket_Sticks, close & sc. + close & n. CA + close & r. PRO & n. N
create bindingPocket_Sticks, LabelBindingPocket_Sticks
hide everything, LabelBindingPocket_Sticks
hide everything, bindingPocket_Sticks
show sticks, bindingPocket_Sticks
show sticks, LabelBindingPocket_Sticks
label n. CA and LabelBindingPocket_Sticks, "%s %s" % (resn, resi)
color yelloworange, LabelBindingPocket_Sticks
color yelloworange, bindingPocket_Sticks


create bindingPocket_Surface, prot_ATP_GDP_Mg within 6 of seleATP_GDP_Mg, 0, 1
hide everything, bindingPocket_Surface
show surface, bindingPocket_Surface
set surface_color, orange
set transparency, 0.5

distance hbond_ATP, seleATP, prot_ATP_GDP_Mg, mode=2
distance hbond_GDP, seleGDP, prot_ATP_GDP_Mg, mode=2
color magenta, hbond_ATP
color magenta, hbond_GDP

create ATP_GDP_Mg, seleATP_GDP_Mg
create ATP, seleATP
create GDP, seleGDP
create Mg, seleMg

select close_GDP, br. fold_06_cd_rsh_g196k_gdp_mg_atp_model_0 near_to 6 of seleGDP
select close_ATP, br. fold_06_cd_rsh_g196k_gdp_mg_atp_model_0 near_to 6 of seleATP

color firebrick, ATP
color deepteal, GDP
color purpleblue, Mg
disable ATP_GDP_Mg

select seleCd_RSH_Bifunctional_Fragment, prot_ATP_GDP_Mg and resi 1-385
select seleCd_RSH_Synthetase, prot_ATP_GDP_Mg and resi 173-339
select seleCd_RSH_Central3HelixBundle, prot_ATP_GDP_Mg and resi 132-192
select seleCd_RSH_Hydrolase, prot_ATP_GDP_Mg and resi 1-149

create Cd_RSH_Bifunctional_Fragment, seleCd_RSH_Bifunctional_Fragment
create Cd_RSH_Synthetase, seleCd_RSH_Synthetase
create Cd_RSH_Central3HelixBundle, seleCd_RSH_Central3HelixBundle
create Cd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase_NoOrg
select seleCd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase and (not org.)
select seleCd_RSH_Hydrolase_NoIno, Cd_RSH_Hydrolase_NoOrg and (not ino.)
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase_NoIno
delete seleCd_RSH_Hydrolase_NoOrg
delete Cd_RSH_Hydrolase_NoOrg
delete seleCd_RSH_Hydrolase_NoIno
```

```
select seleCd_RSH_Hydrolase, Cd_RSH_Hydrolase
deselect
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase

disable Cd_RSH_Bifunctional_Fragment
disable Cd_RSH_Synthetase
disable Cd_RSH_Central3HelixBundle
disable Cd_RSH_Hydrolase
disable ATP_GDP

select hotSpot, resi 204-209
color red, hotSpot
deselect

select mutation, resi 196
color limegreen, mutation
deselect

disable fold_06_cd_rsh_g196k_gdp_mg_atp_model_0
enable fold_06_cd_rsh_g196k_gdp_mg_atp_model_0

disable LabelBindingPocket_Sticks

orient ATP_GDP_Mg
zoom ATP_GDP_Mg, 5
```

RSH ATP GDP Mg G196K and WT ATP GDP Mg *C. difficile*

```
set seq_view, 1
bg_color white

select seleOG, fold_cd_rsh_af3_atp_mg_gdp_model_0
select seleG196K, fold_06_cd_rsh_g196k_gdp_mg_atp_model_0
color wheat, seleOG
color gray50, seleG196K
select bifunctionalDomain, seleOG and (not resi 386-735)
align seleG196K, bifunctionalDomain
deselect

select seleHotSpot, resi 204-209
deselect

select seleMutation, resi 196
deselect
create HotSpot, resi 204-209
create Mutation, resi 196

select OG_HotSpot, seleOG and resi 204-209
color salmon, OG_HotSpot
deselect

select mutationHotSpot, seleG196K and resi 204-209
color red, mutationHotSpot
deselect
```

```
select OG_Mutation, seleOG and  resi 196
color paleyellow, OG_Mutation
deselect

select G196K_Mutation, seleG196K and resi 196
color limegreen, G196K_Mutation
deselect


select prot_ATP_GDP_Mg, fold_06_cd_rsh_g196k_gdp_mg_atp_model_0
select seleATP_GDP, org. and (not prot_ATP_GDP_Mg)
create ATP_GDP, seleATP_GDP
select seleATP_atom, first org. and ATP_GDP
create ATP_atom, seleATP_atom
select seleATP,  bm. seleATP_atom

create ATP, seleATP

select seleGDP_atom, last org. and ATP_GDP
create GDP_atom, seleGDP_atom
select seleGDP,  bm. seleGDP_atom
create GDP, seleGDP
deselect

delete ATP_atom
delete GDP_ato
delete seleATP_atom
delete seleGDP_atom
delete GDP_atom
delete ATP_atom
delete seleGDP
delete seleATP

select seleATP, ATP
select seleGDP, GDP
deselect

select seleMg, bm. first ino.
create Mg, seleMg
deselect


select seleATP_GDP_Mg, org. ino. and (not prot_ATP_GDP_Mg)

set surface_carve_cutoff, 6
set surface_carve_selection, seleATP_GDP_Mg
set surface_carve_normal_cutoff, -0.1
set two_sided_lighting
set transparency, 0.5


select close_ATP_GDP_Mg, br. fold_06_cd_rsh_g196k_gdp_mg_atp_model_0 near_to 6 of seleATP_GDP_Mg
create LabelBindingPocket_Sticks, close & sc. + close & n. CA + close & r. PRO & n. N
```

```
create bindingPocket_Sticks, LabelBindingPocket_Sticks
hide everything, LabelBindingPocket_Sticks
hide everything, bindingPocket_Sticks
show sticks, bindingPocket_Sticks
show sticks, LabelBindingPocket_Sticks
label n. CA and LabelBindingPocket_Sticks, "%s %s" % (resn, resi)
color yelloworange, LabelBindingPocket_Sticks
color yelloworange, bindingPocket_Sticks


create bindingPocket_Surface, prot_ATP_GDP_Mg within 6 of seleATP_GDP_Mg, 0, 1
hide everything, bindingPocket_Surface
show surface, bindingPocket_Surface
set surface_color, orange
set transparency, 0.5

distance hbond_ATP, seleATP, prot_ATP_GDP_Mg, mode=2
distance hbond_GDP, seleGDP, prot_ATP_GDP_Mg, mode=2
color magenta, hbond_ATP
color magenta, hbond_GDP

create ATP_GDP_Mg, seleATP_GDP_Mg
create ATP, seleATP
create GDP, seleGDP
create Mg, seleMg

select close_GDP, br. fold_06_cd_rsh_g196k_gdp_mg_atp_model_0 near_to 6 of seleGDP
select close_ATP, br. fold_06_cd_rsh_g196k_gdp_mg_atp_model_0 near_to 6 of seleATP

color firebrick, ATP
color deepteal, GDP
color purpleblue, Mg
disable ATP_GDP_Mg

select seleCd_RSH_Bifunctional_Fragment, prot_ATP_GDP_Mg and resi 1-385
select seleCd_RSH_Synthetase, prot_ATP_GDP_Mg and resi 173-339
select seleCd_RSH_Central3HelixBundle, prot_ATP_GDP_Mg and resi 132-192
select seleCd_RSH_Hydrolase, prot_ATP_GDP_Mg and resi 1-149

create Cd_RSH_Bifunctional_Fragment, seleCd_RSH_Bifunctional_Fragment
create Cd_RSH_Synthetase, seleCd_RSH_Synthetase
create Cd_RSH_Central3HelixBundle, seleCd_RSH_Central3HelixBundle
create Cd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase_NoOrg
select seleCd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase and (not org.)
select seleCd_RSH_Hydrolase_NoIno, Cd_RSH_Hydrolase_NoOrg and (not ino.)
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase_NoIno
delete seleCd_RSH_Hydrolase_NoOrg
delete Cd_RSH_Hydrolase_NoOrg
delete seleCd_RSH_Hydrolase_NoIno
select seleCd_RSH_Hydrolase, Cd_RSH_Hydrolase
deselect
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase

disable Cd_RSH_Bifunctional_Fragment
disable Cd_RSH_Synthetase
disable Cd_RSH_Central3HelixBundle
```

```
disable Cd_RSH_Hydrolase

disable ATP_GDP
disable LabelBindingPocket_Sticks
disable GDP
disable ATP


disable fold_cd_rsh_af3_atp_mg_gdp_model_0
disable fold_06_cd_rsh_g196k_gdp_mg_atp_model_0
enable fold_cd_rsh_af3_atp_mg_gdp_model_0
enable fold_06_cd_rsh_g196k_gdp_mg_atp_model_0

deselect

orient ATP_GDP_Mg
zoom ATP_GDP_Mg, 5
```

RSH D213A ATP GDP Mg *C. difficile* (can be used for WT *C. difficile*)

```
set seq_view, 1
bg_color white
color gray50, fold_07_cd_rsh_d213a_gdp_mg_atp_model_0

select prot_ATP_GDP_Mg, polymer
select seleATP_GDP, org. and (not prot_ATP_GDP_Mg)
create ATP_GDP, seleATP_GDP
select seleATP_atom, first org. and ATP_GDP
create ATP_atom, seleATP_atom
select seleATP,  bm. seleATP_atom

create ATP, seleATP

select seleGDP_atom, last org. and ATP_GDP
create GDP_atom, seleGDP_atom
select seleGDP,  bm. seleGDP_atom
create GDP, seleGDP
deselect

delete ATP_atom
delete GDP_ato
delete seleATP_atom
delete seleGDP_atom
delete GDP_atom
delete ATP_atom
delete seleGDP
delete seleATP

select seleATP, ATP
select seleGDP, GDP
deselect

select seleMg, bm. first ino.
create Mg, seleMg
deselect
```

```
select seleATP_GDP_Mg, org. ino. and (not prot_ATP_GDP_Mg)

set surface_carve_cutoff, 6
set surface_carve_selection, seleATP_GDP_Mg
set surface_carve_normal_cutoff, -0.1
set two_sided_lighting
set transparency, 0.5


select close_ATP_GDP_Mg, br. fold_07_cd_rsh_d213a_gdp_mg_atp_model_0 near_to 6 of seleATP_GDP_Mg
create LabelBindingPocket_Sticks, close & sc. + close & n. CA + close & r. PRO & n. N
create bindingPocket_Sticks, LabelBindingPocket_Sticks
hide everything, LabelBindingPocket_Sticks
hide everything, bindingPocket_Sticks
show sticks, bindingPocket_Sticks
show sticks, LabelBindingPocket_Sticks
label n. CA and LabelBindingPocket_Sticks, "%s %s" % (resn, resi)
color yelloworange, LabelBindingPocket_Sticks
color yelloworange, bindingPocket_Sticks


create bindingPocket_Surface, prot_ATP_GDP_Mg within 6 of seleATP_GDP_Mg, 0, 1
hide everything, bindingPocket_Surface
show surface, bindingPocket_Surface
set surface_color, orange
set transparency, 0.5

distance hbond_ATP, seleATP, prot_ATP_GDP_Mg, mode=2
distance hbond_GDP, seleGDP, prot_ATP_GDP_Mg, mode=2
color magenta, hbond_ATP
color magenta, hbond_GDP

create ATP_GDP_Mg, seleATP_GDP_Mg
create ATP, seleATP
create GDP, seleGDP
create Mg, seleMg

select close_GDP, br. fold_07_cd_rsh_d213a_gdp_mg_atp_model_0 near_to 6 of seleGDP
select close_ATP, br. fold_07_cd_rsh_d213a_gdp_mg_atp_model_0 near_to 6 of seleATP

color firebrick, ATP
color deepteal, GDP
color purpleblue, Mg
disable ATP_GDP_Mg

select seleCd_RSH_Bifunctional_Fragment, prot_ATP_GDP_Mg and resi 1-385
select seleCd_RSH_Synthetase, prot_ATP_GDP_Mg and resi 173-339
select seleCd_RSH_Central3HelixBundle, prot_ATP_GDP_Mg and resi 132-192
select seleCd_RSH_Hydrolase, prot_ATP_GDP_Mg and resi 1-149

create Cd_RSH_Bifunctional_Fragment, seleCd_RSH_Bifunctional_Fragment
create Cd_RSH_Synthetase, seleCd_RSH_Synthetase
create Cd_RSH_Central3HelixBundle, seleCd_RSH_Central3HelixBundle
```

create Cd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase_NoOrg
select seleCd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase and (not org.)
select seleCd_RSH_Hydrolase_NoIno, Cd_RSH_Hydrolase_NoOrg and (not ino.)
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase_NoIno
delete seleCd_RSH_Hydrolase_NoOrg
delete Cd_RSH_Hydrolase_NoOrg
delete seleCd_RSH_Hydrolase_NoIno
select seleCd_RSH_Hydrolase, Cd_RSH_Hydrolase
deselect
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase

disable Cd_RSH_Bifunctional_Fragment
disable Cd_RSH_Synthetase
disable Cd_RSH_Central3HelixBundle
disable Cd_RSH_Hydrolase
disable ATP_GDP

select hotSpot, resi 204-209
color red, hotSpot
deselect

select mutation, resi 213
color limegreen, mutation
deselect

disable fold_07_cd_rsh_d213a_gdp_mg_atp_model_0
enable fold_07_cd_rsh_d213a_gdp_mg_atp_model_0

disable LabelBindingPocket_Sticks

orient ATP_GDP_Mg
zoom ATP_GDP_Mg, 5

RSH ATP GDP Mg D213A and WT ATP GDP Mg *C. difficile*

set seq_view, 1
bg_color white

select seleOG, fold_cd_rsh_af3_atp_mg_gdp_model_0
select seleD213A, fold_07_cd_rsh_d213a_gdp_mg_atp_model_0
color wheat, seleOG
color gray50, seleD213A
select bifunctionalDomain, seleOG and (not resi 386-735)
align seleD213A, bifunctionalDomain
deselect

select seleHotSpot, resi 204-209
deselect

select seleMutation, resi 213
deselect
create HotSpot, resi 204-209
create Mutation, resi 213

```
select OG_HotSpot, seleOG and resi 204-209
color salmon, OG_HotSpot
deselect

select mutationHotSpot, seleD213A and resi 204-209
color red, mutationHotSpot
deselect

select OG_Mutation, seleOG and  resi 213
color paleyellow, OG_Mutation
deselect

select D213A_Mutation, seleD213A and resi 213
color limegreen, D213A_Mutation
deselect



select prot_ATP_GDP_Mg, fold_07_cd_rsh_d213a_gdp_mg_atp_model_0
select seleATP_GDP, org. and (not prot_ATP_GDP_Mg)
create ATP_GDP, seleATP_GDP
select seleATP_atom, first org. and ATP_GDP
create ATP_atom, seleATP_atom
select seleATP,  bm. seleATP_atom

create ATP, seleATP

select seleGDP_atom, last org. and ATP_GDP
create GDP_atom, seleGDP_atom
select seleGDP,  bm. seleGDP_atom
create GDP, seleGDP
deselect

delete ATP_atom
delete GDP_ato
delete seleATP_atom
delete seleGDP_atom
delete GDP_atom
delete ATP_atom
delete seleGDP
delete seleATP

select seleATP, ATP
select seleGDP, GDP
deselect

select seleMg, bm. first ino.
create Mg, seleMg
deselect


select seleATP_GDP_Mg, org. ino. and (not prot_ATP_GDP_Mg)

set surface_carve_cutoff, 6
set surface_carve_selection, seleATP_GDP_Mg
set surface_carve_normal_cutoff, -0.1
```

```
set two_sided_lighting
set transparency, 0.5


select close_ATP_GDP_Mg, br. fold_07_cd_rsh_d213a_gdp_mg_atp_model_0 near_to 6 of seleATP_GDP_Mg
create LabelBindingPocket_Sticks, close & sc. + close & n. CA + close & r. PRO & n. N
create bindingPocket_Sticks, LabelBindingPocket_Sticks
hide everything, LabelBindingPocket_Sticks
hide everything, bindingPocket_Sticks
show sticks, bindingPocket_Sticks
show sticks, LabelBindingPocket_Sticks
label n. CA and LabelBindingPocket_Sticks, "%s %s" % (resn, resi)
color yelloworange, LabelBindingPocket_Sticks
color yelloworange, bindingPocket_Sticks


create bindingPocket_Surface, prot_ATP_GDP_Mg within 6 of seleATP_GDP_Mg, 0, 1
hide everything, bindingPocket_Surface
show surface, bindingPocket_Surface
set surface_color, orange
set transparency, 0.5

distance hbond_ATP, seleATP, prot_ATP_GDP_Mg, mode=2
distance hbond_GDP, seleGDP, prot_ATP_GDP_Mg, mode=2
color magenta, hbond_ATP
color magenta, hbond_GDP

create ATP_GDP_Mg, seleATP_GDP_Mg
create ATP, seleATP
create GDP, seleGDP
create Mg, seleMg

select close_GDP, br. fold_07_cd_rsh_d213a_gdp_mg_atp_model_0 near_to 6 of seleGDP
select close_ATP, br. fold_07_cd_rsh_d213a_gdp_mg_atp_model_0 near_to 6 of seleATP

color firebrick, ATP
color deepteal, GDP
color purpleblue, Mg
disable ATP_GDP_Mg

select seleCd_RSH_Bifunctional_Fragment, prot_ATP_GDP_Mg and resi 1-385
select seleCd_RSH_Synthetase, prot_ATP_GDP_Mg and resi 173-339
select seleCd_RSH_Central3HelixBundle, prot_ATP_GDP_Mg and resi 132-192
select seleCd_RSH_Hydrolase, prot_ATP_GDP_Mg and resi 1-149

create Cd_RSH_Bifunctional_Fragment, seleCd_RSH_Bifunctional_Fragment
create Cd_RSH_Synthetase, seleCd_RSH_Synthetase
create Cd_RSH_Central3HelixBundle, seleCd_RSH_Central3HelixBundle
create Cd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase_NoOrg
select seleCd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase and (not org.)
select seleCd_RSH_Hydrolase_NoIno, Cd_RSH_Hydrolase_NoOrg and (not ino.)
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase_NoIno
delete seleCd_RSH_Hydrolase_NoOrg
delete Cd_RSH_Hydrolase_NoOrg
delete seleCd_RSH_Hydrolase_NoIno
```

```
select seleCd_RSH_Hydrolase, Cd_RSH_Hydrolase
deselect
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase

disable Cd_RSH_Bifunctional_Fragment
disable Cd_RSH_Synthetase
disable Cd_RSH_Central3HelixBundle
disable Cd_RSH_Hydrolase

disable ATP_GDP
disable LabelBindingPocket_Sticks
disable GDP
disable ATP


disable fold_cd_rsh_af3_atp_mg_gdp_model_0
disable fold_07_cd_rsh_d213a_gdp_mg_atp_model_0
enable fold_cd_rsh_af3_atp_mg_gdp_model_0
enable fold_07_cd_rsh_d213a_gdp_mg_atp_model_0

deselect

orient ATP_GDP_Mg
zoom ATP_GDP_Mg, 5
```

RSH D213H ATP GDP Mg *C. difficile* (can be used for WT *C. difficile*)

```
set seq_view, 1
bg_color white
color gray50, fold_08_cd_rsh_d213h_gdp_mg_atp_model_0

select prot_ATP_GDP_Mg, polymer
select seleATP_GDP, org. and (not prot_ATP_GDP_Mg)
create ATP_GDP, seleATP_GDP
select seleATP_atom, first org. and ATP_GDP
create ATP_atom, seleATP_atom
select seleATP,  bm. seleATP_atom

create ATP, seleATP

select seleGDP_atom, last org. and ATP_GDP
create GDP_atom, seleGDP_atom
select seleGDP,  bm. seleGDP_atom
create GDP, seleGDP
deselect

delete ATP_atom
delete GDP_ato
delete seleATP_atom
delete seleGDP_atom
delete GDP_atom
delete ATP_atom
delete seleGDP
delete seleATP

select seleATP, ATP
```

```
select seleGDP, GDP
deselect

select seleMg, bm. first ino.
create Mg, seleMg
deselect


select seleATP_GDP_Mg, org. ino. and (not prot_ATP_GDP_Mg)

set surface_carve_cutoff, 6
set surface_carve_selection, seleATP_GDP_Mg
set surface_carve_normal_cutoff, -0.1
set two_sided_lighting
set transparency, 0.5


select close_ATP_GDP_Mg, br. fold_08_cd_rsh_d213h_gdp_mg_atp_model_0 near_to 6 of seleATP_GDP_Mg
create LabelBindingPocket_Sticks, close & sc. + close & n. CA + close & r. PRO & n. N
create bindingPocket_Sticks, LabelBindingPocket_Sticks
hide everything, LabelBindingPocket_Sticks
hide everything, bindingPocket_Sticks
show sticks, bindingPocket_Sticks
show sticks, LabelBindingPocket_Sticks
label n. CA and LabelBindingPocket_Sticks, "%s %s" % (resn, resi)
color yelloworange, LabelBindingPocket_Sticks
color yelloworange, bindingPocket_Sticks


create bindingPocket_Surface, prot_ATP_GDP_Mg within 6 of seleATP_GDP_Mg, 0, 1
hide everything, bindingPocket_Surface
show surface, bindingPocket_Surface
set surface_color, orange
set transparency, 0.5

distance hbond_ATP, seleATP, prot_ATP_GDP_Mg, mode=2
distance hbond_GDP, seleGDP, prot_ATP_GDP_Mg, mode=2
color magenta, hbond_ATP
color magenta, hbond_GDP

create ATP_GDP_Mg, seleATP_GDP_Mg
create ATP, seleATP
create GDP, seleGDP
create Mg, seleMg

select close_GDP, br. fold_08_cd_rsh_d213h_gdp_mg_atp_model_0 near_to 6 of seleGDP
select close_ATP, br. fold_08_cd_rsh_d213h_gdp_mg_atp_model_0 near_to 6 of seleATP

color firebrick, ATP
color deepteal, GDP
color purpleblue, Mg
disable ATP_GDP_Mg

select seleCd_RSH_Bifunctional_Fragment, prot_ATP_GDP_Mg and resi 1-385
select seleCd_RSH_Synthetase, prot_ATP_GDP_Mg and resi 173-339
```

select seleCd_RSH_Central3HelixBundle, prot_ATP_GDP_Mg and resi 132-192
select seleCd_RSH_Hydrolase, prot_ATP_GDP_Mg and resi 1-149

create Cd_RSH_Bifunctional_Fragment, seleCd_RSH_Bifunctional_Fragment
create Cd_RSH_Synthetase, seleCd_RSH_Synthetase
create Cd_RSH_Central3HelixBundle, seleCd_RSH_Central3HelixBundle
create Cd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase_NoOrg
select seleCd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase and (not org.)
select seleCd_RSH_Hydrolase_NoIno, Cd_RSH_Hydrolase_NoOrg and (not ino.)
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase_NoIno
delete seleCd_RSH_Hydrolase_NoOrg
delete Cd_RSH_Hydrolase_NoOrg
delete seleCd_RSH_Hydrolase_NoIno
select seleCd_RSH_Hydrolase, Cd_RSH_Hydrolase
deselect
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase

disable Cd_RSH_Bifunctional_Fragment
disable Cd_RSH_Synthetase
disable Cd_RSH_Central3HelixBundle
disable Cd_RSH_Hydrolase
disable ATP_GDP

select hotSpot, resi 204-209
color red, hotSpot
deselect

select mutation, resi 213
color limegreen, mutation
deselect

disable fold_08_cd_rsh_d213h_gdp_mg_atp_model_0
enable fold_08_cd_rsh_d213h_gdp_mg_atp_model_0

disable LabelBindingPocket_Sticks

orient ATP_GDP_Mg
zoom ATP_GDP_Mg, 5

RSH ATP GDP Mg D213H and WT ATP GDP Mg *C. difficile*

set seq_view, 1
bg_color white

select seleOG, fold_cd_rsh_af3_atp_mg_gdp_model_0
select seleD213H, fold_08_cd_rsh_d213h_gdp_mg_atp_model_0
color wheat, seleOG
color gray50, seleD213H
select bifunctionalDomain, seleOG and (not resi 386-735)
align seleD213H, bifunctionalDomain
deselect

select seleHotSpot, resi 204-209
deselect

```
select seleMutation, resi 213
deselect
create HotSpot, resi 204-209
create Mutation, resi 213

select OG_HotSpot, seleOG and resi 204-209
color salmon, OG_HotSpot
deselect

select mutationHotSpot, seleD213H and resi 204-209
color red, mutationHotSpot
deselect

select OG_Mutation, seleOG and  resi 213
color paleyellow, OG_Mutation
deselect

select D213H_Mutation, seleD213H and resi 213
color limegreen, D213H_Mutation
deselect


select prot_ATP_GDP_Mg, fold_08_cd_rsh_d213h_gdp_mg_atp_model_0
select seleATP_GDP, org. and (not prot_ATP_GDP_Mg)
create ATP_GDP, seleATP_GDP
select seleATP_atom, first org. and ATP_GDP
create ATP_atom, seleATP_atom
select seleATP,  bm. seleATP_atom

create ATP, seleATP

select seleGDP_atom, last org. and ATP_GDP
create GDP_atom, seleGDP_atom
select seleGDP,  bm. seleGDP_atom
create GDP, seleGDP
deselect

delete ATP_atom
delete GDP_ato
delete seleATP_atom
delete seleGDP_atom
delete GDP_atom
delete ATP_atom
delete seleGDP
delete seleATP

select seleATP, ATP
select seleGDP, GDP
deselect

select seleMg, bm. first ino.
create Mg, seleMg
deselect
```

```
select seleATP_GDP_Mg, org. ino. and (not prot_ATP_GDP_Mg)

set surface_carve_cutoff, 6
set surface_carve_selection, seleATP_GDP_Mg
set surface_carve_normal_cutoff, -0.1
set two_sided_lighting
set transparency, 0.5


select close_ATP_GDP_Mg, br. fold_08_cd_rsh_d213h_gdp_mg_atp_model_0 near_to 6 of seleATP_GDP_Mg
create LabelBindingPocket_Sticks, close & sc. + close & n. CA + close & r. PRO & n. N
create bindingPocket_Sticks, LabelBindingPocket_Sticks
hide everything, LabelBindingPocket_Sticks
hide everything, bindingPocket_Sticks
show sticks, bindingPocket_Sticks
show sticks, LabelBindingPocket_Sticks
label n. CA and LabelBindingPocket_Sticks, "%s %s" % (resn, resi)
color yelloworange, LabelBindingPocket_Sticks
color yelloworange, bindingPocket_Sticks


create bindingPocket_Surface, prot_ATP_GDP_Mg within 6 of seleATP_GDP_Mg, 0, 1
hide everything, bindingPocket_Surface
show surface, bindingPocket_Surface
set surface_color, orange
set transparency, 0.5

distance hbond_ATP, seleATP, prot_ATP_GDP_Mg, mode=2
distance hbond_GDP, seleGDP, prot_ATP_GDP_Mg, mode=2
color magenta, hbond_ATP
color magenta, hbond_GDP

create ATP_GDP_Mg, seleATP_GDP_Mg
create ATP, seleATP
create GDP, seleGDP
create Mg, seleMg

select close_GDP, br. fold_08_cd_rsh_d213h_gdp_mg_atp_model_0 near_to 6 of seleGDP
select close_ATP, br. fold_08_cd_rsh_d213h_gdp_mg_atp_model_0 near_to 6 of seleATP

color firebrick, ATP
color deepteal, GDP
color purpleblue, Mg
disable ATP_GDP_Mg

select seleCd_RSH_Bifunctional_Fragment, prot_ATP_GDP_Mg and resi 1-385
select seleCd_RSH_Synthetase, prot_ATP_GDP_Mg and resi 173-339
select seleCd_RSH_Central3HelixBundle, prot_ATP_GDP_Mg and resi 132-192
select seleCd_RSH_Hydrolase, prot_ATP_GDP_Mg and resi 1-149

create Cd_RSH_Bifunctional_Fragment, seleCd_RSH_Bifunctional_Fragment
create Cd_RSH_Synthetase, seleCd_RSH_Synthetase
create Cd_RSH_Central3HelixBundle, seleCd_RSH_Central3HelixBundle
```

create Cd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase_NoOrg
select seleCd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase and (not org.)
select seleCd_RSH_Hydrolase_NoIno, Cd_RSH_Hydrolase_NoOrg and (not ino.)
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase_NoIno
delete seleCd_RSH_Hydrolase_NoOrg
delete Cd_RSH_Hydrolase_NoOrg
delete seleCd_RSH_Hydrolase_NoIno
select seleCd_RSH_Hydrolase, Cd_RSH_Hydrolase
deselect
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase

disable Cd_RSH_Bifunctional_Fragment
disable Cd_RSH_Synthetase
disable Cd_RSH_Central3HelixBundle
disable Cd_RSH_Hydrolase

disable ATP_GDP
disable LabelBindingPocket_Sticks
disable GDP
disable ATP

disable fold_cd_rsh_af3_atp_mg_gdp_model_0
disable fold_08_cd_rsh_d213h_gdp_mg_atp_model_0
enable fold_cd_rsh_af3_atp_mg_gdp_model_0
enable fold_08_cd_rsh_d213h_gdp_mg_atp_model_0

deselect

orient ATP_GDP_Mg
zoom ATP_GDP_Mg, 5

RSH G196E ATP GTP Mg *C. difficile*

set seq_view, 1
bg_color white

select seleOG, fold_cd_rsh_af3_atp_mg_gtp_model_0
select seleG196E, fold_05_cd_rsh_g196e_atp_mg_gtp_model_0
color wheat, seleOG
color gray50, seleG196E
select bifunctionalDomain, seleOG and (not resi 386-735)
align seleG196E, bifunctionalDomain
deselect

select seleHotSpot, resi 204-209
Deselect

select seleMutation, resi 196
deselect
create HotSpot, resi 204-209
create Mutation, resi 196

select OG_HotSpot, seleOG and resi 204-209
color salmon, OG_HotSpot
deselect

```
select mutationHotSpot, seleG196E and resi 204-209
color red, mutationHotSpot
deselect

select OG_Mutation, seleOG and  resi 196
color paleyellow, OG_Mutation
deselect

select G196E_Mutation, seleG196E and resi 196
color limegreen, G196E_Mutation
deselect



select prot_ATP_GTP_Mg, polymer
select seleATP_GTP, org. and (not prot_ATP_GTP_Mg)
create ATP_GTP, seleATP_GTP
select seleATP_atom, first org. and ATP_GTP
create ATP_atom, seleATP_atom
select seleATP,  bm. seleATP_atom

create ATP, seleATP

select seleGTP_atom, last org. and ATP_GTP
create GTP_atom, seleGTP_atom
select seleGTP,  bm. seleGTP_atom
create GTP, seleGTP
deselect

delete ATP_atom
delete GTP_ato
delete seleATP_atom
delete seleGTP_atom
delete GTP_atom
delete ATP_atom
delete seleGTP
delete seleATP

select seleATP, ATP
select seleGTP, GTP
deselect

select seleMg, bm. first ino.
create Mg, seleMg
deselect


select seleATP_GTP_Mg, org. ino. and (not prot_ATP_GTP_Mg)

set surface_carve_cutoff, 6
set surface_carve_selection, seleATP_GTP_Mg
set surface_carve_normal_cutoff, -0.1
set two_sided_lighting
set transparency, 0.5
```

```
select close_ATP_GTP_Mg, br. fold_05_cd_rsh_g196e_atp_mg_gtp_model_0 near_to 6 of seleATP_GTP_Mg
create LabelBindingPocket_Sticks, close & sc. + close & n. CA + close & r. PRO & n. N
create bindingPocket_Sticks, LabelBindingPocket_Sticks
hide everything, LabelBindingPocket_Sticks
hide everything, bindingPocket_Sticks
show sticks, bindingPocket_Sticks
show sticks, LabelBindingPocket_Sticks
label n. CA and LabelBindingPocket_Sticks, "%s %s" % (resn, resi)
color yelloworange, LabelBindingPocket_Sticks
color yelloworange, bindingPocket_Sticks


create bindingPocket_Surface, prot_ATP_GTP_Mg within 6 of seleATP_GTP_Mg, 0, 1
hide everything, bindingPocket_Surface
show surface, bindingPocket_Surface
set surface_color, orange
set transparency, 0.5

distance hbond_ATP, seleATP, prot_ATP_GTP_Mg, mode=2
distance hbond_GTP, seleGTP, prot_ATP_GTP_Mg, mode=2
color magenta, hbond_ATP
color magenta, hbond_GTP

create ATP_GTP_Mg, seleATP_GTP_Mg
create ATP, seleATP
create GTP, seleGTP
create Mg, seleMg

select close_GTP, br. polymer near_to 6 of seleGTP
select close_ATP, br. polymer near_to 6 of seleATP

color firebrick, ATP
color deepteal, GTP
color purpleblue, Mg
disable ATP_GTP_Mg

select seleCd_RSH_Bifunctional_Fragment, prot_ATP_GTP_Mg and resi 1-385
select seleCd_RSH_Synthetase, prot_ATP_GTP_Mg and resi 173-339
select seleCd_RSH_Central3HelixBundle, prot_ATP_GTP_Mg and resi 132-192
select seleCd_RSH_Hydrolase, prot_ATP_GTP_Mg and resi 1-149

create Cd_RSH_Bifunctional_Fragment, seleCd_RSH_Bifunctional_Fragment
create Cd_RSH_Synthetase, seleCd_RSH_Synthetase
create Cd_RSH_Central3HelixBundle, seleCd_RSH_Central3HelixBundle
create Cd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase_NoOrg
select seleCd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase and (not org.)
select seleCd_RSH_Hydrolase_NoIno, Cd_RSH_Hydrolase_NoOrg and (not ino.)
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase_NoIno
delete seleCd_RSH_Hydrolase_NoOrg
delete Cd_RSH_Hydrolase_NoOrg
delete seleCd_RSH_Hydrolase_NoIno
select seleCd_RSH_Hydrolase, Cd_RSH_Hydrolase
deselect
```

```
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase

disable Cd_RSH_Bifunctional_Fragment
disable Cd_RSH_Synthetase
disable Cd_RSH_Central3HelixBundle
disable Cd_RSH_Hydrolase
disable ATP_GTP


disable fold_cd_rsh_af3_atp_mg_gtp_model_0
disable fold_05_cd_rsh_g196e_atp_mg_gtp_model_0
enable fold_cd_rsh_af3_atp_mg_gtp_model_0
enable fold_05_cd_rsh_g196e_atp_mg_gtp_model_0


orient ATP_GTP_Mg
zoom ATP_GTP_Mg, 5
```

RSH ATP GTP Mg G196E and WT ATP GTP Mg *C. difficile*

```
set seq_view, 1
bg_color white

select seleOG, fold_cd_rsh_af3_atp_mg_gtp_model_0
select seleG196E, fold_05_cd_rsh_g196e_atp_mg_gtp_model_0
color wheat, seleOG
color gray50, seleG196E
select bifunctionalDomain, seleOG and (not resi 386-735)
align seleG196E, bifunctionalDomain
deselect

select seleHotSpot, resi 204-209
Deselect

select seleMutation, resi 196
deselect
create HotSpot, resi 204-209
create Mutation, resi 196

select OG_HotSpot, seleOG and resi 204-209
color salmon, OG_HotSpot
deselect

select mutationHotSpot, seleG196E and resi 204-209
color red, mutationHotSpot
deselect

select OG_Mutation, seleOG and  resi 196
color paleyellow, OG_Mutation
deselect

select G196E_Mutation, seleG196E and resi 196
```

```
color limegreen, G196E_Mutation
deselect


select prot_ATP_GTP_Mg, polymer
select seleATP_GTP, org. and (not prot_ATP_GTP_Mg)
create ATP_GTP, seleATP_GTP
select seleATP_atom, first org. and ATP_GTP
create ATP_atom, seleATP_atom
select seleATP,  bm. seleATP_atom

create ATP, seleATP

select seleGTP_atom, last org. and ATP_GTP
create GTP_atom, seleGTP_atom
select seleGTP,  bm. seleGTP_atom
create GTP, seleGTP
deselect

delete ATP_atom
delete GTP_ato
delete seleATP_atom
delete seleGTP_atom
delete GTP_atom
delete ATP_atom
delete seleGTP
delete seleATP

select seleATP, ATP
select seleGTP, GTP
deselect

select seleMg, bm. first ino.
create Mg, seleMg
deselect


select seleATP_GTP_Mg, org. ino. and (not prot_ATP_GTP_Mg)

set surface_carve_cutoff, 6
set surface_carve_selection, seleATP_GTP_Mg
set surface_carve_normal_cutoff, -0.1
set two_sided_lighting
set transparency, 0.5


select close_ATP_GTP_Mg, br. fold_05_cd_rsh_g196e_atp_mg_gtp_model_0 near_to 6 of seleATP_GTP_Mg
create LabelBindingPocket_Sticks, close & sc. + close & n. CA + close & r. PRO & n. N
create bindingPocket_Sticks, LabelBindingPocket_Sticks
hide everything, LabelBindingPocket_Sticks
hide everything, bindingPocket_Sticks
show sticks, bindingPocket_Sticks
show sticks, LabelBindingPocket_Sticks
label n. CA and LabelBindingPocket_Sticks, "%s %s" % (resn, resi)
```

```
color yelloworange, LabelBindingPocket_Sticks
color yelloworange, bindingPocket_Sticks


create bindingPocket_Surface, prot_ATP_GTP_Mg within 6 of seleATP_GTP_Mg, 0, 1
hide everything, bindingPocket_Surface
show surface, bindingPocket_Surface
set surface_color, orange
set transparency, 0.5

distance hbond_ATP, seleATP, prot_ATP_GTP_Mg, mode=2
distance hbond_GTP, seleGTP, prot_ATP_GTP_Mg, mode=2
color magenta, hbond_ATP
color magenta, hbond_GTP

create ATP_GTP_Mg, seleATP_GTP_Mg
create ATP, seleATP
create GTP, seleGTP
create Mg, seleMg

select close_GTP, br. polymer near_to 6 of seleGTP
select close_ATP, br. polymer near_to 6 of seleATP

color firebrick, ATP
color deepteal, GTP
color purpleblue, Mg
disable ATP_GTP_Mg

select seleCd_RSH_Bifunctional_Fragment, prot_ATP_GTP_Mg and resi 1-385
select seleCd_RSH_Synthetase, prot_ATP_GTP_Mg and resi 173-339
select seleCd_RSH_Central3HelixBundle, prot_ATP_GTP_Mg and resi 132-192
select seleCd_RSH_Hydrolase, prot_ATP_GTP_Mg and resi 1-149

create Cd_RSH_Bifunctional_Fragment, seleCd_RSH_Bifunctional_Fragment
create Cd_RSH_Synthetase, seleCd_RSH_Synthetase
create Cd_RSH_Central3HelixBundle, seleCd_RSH_Central3HelixBundle
create Cd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase_NoOrg
select seleCd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase and (not org.)
select seleCd_RSH_Hydrolase_NoIno, Cd_RSH_Hydrolase_NoOrg and (not ino.)
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase_NoIno
delete seleCd_RSH_Hydrolase_NoOrg
delete Cd_RSH_Hydrolase_NoOrg
delete seleCd_RSH_Hydrolase_NoIno
select seleCd_RSH_Hydrolase, Cd_RSH_Hydrolase
deselect
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase

disable Cd_RSH_Bifunctional_Fragment
disable Cd_RSH_Synthetase
disable Cd_RSH_Central3HelixBundle
disable Cd_RSH_Hydrolase
disable ATP_GTP


disable fold_cd_rsh_af3_atp_mg_gtp_model_0
```

```
disable fold_05_cd_rsh_g196e_atp_mg_gtp_model_0
enable fold_cd_rsh_af3_atp_mg_gtp_model_0
enable fold_05_cd_rsh_g196e_atp_mg_gtp_model_0


orient ATP_GTP_Mg
zoom ATP_GTP_Mg, 5
```

RSH GDP Mg Xray S. equisimilis

```
set seq_view, 1
bg_color white

select seleGDP, bm. last org.
select seleMg, bm. first ino.

select seleGDP, org.
select prot_GDP, polymer
set surface_carve_cutoff, 6
set surface_carve_selection, seleGDP
set surface_carve_normal_cutoff, -0.1
set two_sided_lighting
set transparency, 0.5

deselect
hide nonbonded

select close_GDP, br. polymer near_to 6 of seleGDP
create LabelBindingPocket_Sticks, close & sc. + close & n. CA + close & r. PRO & n. N
create bindingPocket_Sticks, LabelBindingPocket_Sticks
hide everything, LabelBindingPocket_Sticks
hide everything, bindingPocket_Sticks
show sticks, bindingPocket_Sticks
show sticks, LabelBindingPocket_Sticks
label n. CA and LabelBindingPocket_Sticks, "%s %s" % (resn, resi)
color yelloworange, LabelBindingPocket_Sticks
color yelloworange, bindingPocket_Sticks


create bindingPocket_Surface, prot_GDP within 6 of seleGDP, 0, 1
hide everything, bindingPocket_Surface
show surface, bindingPocket_Surface
set surface_color, orange
set transparency, 0.5

distance hbond_GDP, seleGDP, prot_GDP, mode=2
color magenta, hbond_GDP

create GDP, seleGDP
```

```
select close_GDP, br. polymer near_to 6 of seleGDP

color forest, prot_GDP
color deepteal, GDP

select seleCd_RSH_Bifunctional_Fragment, prot_GDP and resi 1-385
select seleCd_RSH_Synthetase, prot_GDP and resi 173-339
select seleCd_RSH_Central3HelixBundle, prot_GDP and resi 132-192
select seleCd_RSH_Hydrolase, prot_GDP and resi 1-149

create Cd_RSH_Bifunctional_Fragment, seleCd_RSH_Bifunctional_Fragment
create Cd_RSH_Synthetase, seleCd_RSH_Synthetase
create Cd_RSH_Central3HelixBundle, seleCd_RSH_Central3HelixBundle
create Cd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase_NoOrg
select seleCd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase and (not org.)
select seleCd_RSH_Hydrolase_NoIno, Cd_RSH_Hydrolase_NoOrg and (not ino.)
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase_NoIno
delete seleCd_RSH_Hydrolase_NoOrg
delete Cd_RSH_Hydrolase_NoOrg
delete seleCd_RSH_Hydrolase_NoIno
select seleCd_RSH_Hydrolase, Cd_RSH_Hydrolase
deselect
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase

disable Cd_RSH_Bifunctional_Fragment
disable Cd_RSH_Synthetase
disable Cd_RSH_Central3HelixBundle
disable Cd_RSH_Hydrolase

orient GDP
zoom GDP, 5

RSH GDP Mg AF3 S. equisimilis

set seq_view, 1
bg_color white

select seleATP, bm. first org.
select seleGDP, bm. last org.
select seleMg, bm. first ino.

select seleATP_GDP_Mg, org. ino.
select prot_ATP_GDP_Mg, polymer
set surface_carve_cutoff, 6
set surface_carve_selection, seleATP_GDP_Mg
set surface_carve_normal_cutoff, -0.1
set two_sided_lighting
set transparency, 0.5



select close_ATP_GDP_Mg, br. polymer near_to 6 of seleATP_GDP_Mg
create LabelBindingPocket_Sticks, close & sc. + close & n. CA + close & r. PRO & n. N
create bindingPocket_Sticks, LabelBindingPocket_Sticks
hide everything, LabelBindingPocket_Sticks
```

```
hide everything, bindingPocket_Sticks
show sticks, bindingPocket_Sticks
show sticks, LabelBindingPocket_Sticks
label n. CA and LabelBindingPocket_Sticks, "%s %s" % (resn, resi)
color yelloworange, LabelBindingPocket_Sticks
color yelloworange, bindingPocket_Sticks


create bindingPocket_Surface, prot_ATP_GDP_Mg within 6 of seleATP_GDP_Mg, 0, 1
hide everything, bindingPocket_Surface
show surface, bindingPocket_Surface
set surface_color, orange
set transparency, 0.5

distance hbond_ATP, seleATP, prot_ATP_GDP_Mg, mode=2
distance hbond_GDP, seleGDP, prot_ATP_GDP_Mg, mode=2
color magenta, hbond_ATP
color magenta, hbond_GDP

create ATP_GDP_Mg, seleATP_GDP_Mg
create ATP, seleATP
create GDP, seleGDP
create Mg, seleMg

select close_GDP, br. polymer near_to 6 of seleGDP
select close_ATP, br. polymer near_to 6 of seleATP

color forest, prot_ATP_GDP_Mg
color firebrick, ATP
color deepteal, GDP
color purpleblue, Mg
disable ATP_GDP_Mg

select seleCd_RSH_Bifunctional_Fragment, prot_ATP_GDP_Mg and resi 1-385
select seleCd_RSH_Synthetase, prot_ATP_GDP_Mg and resi 173-339
select seleCd_RSH_Central3HelixBundle, prot_ATP_GDP_Mg and resi 132-192
select seleCd_RSH_Hydrolase, prot_ATP_GDP_Mg and resi 1-149

create Cd_RSH_Bifunctional_Fragment, seleCd_RSH_Bifunctional_Fragment
create Cd_RSH_Synthetase, seleCd_RSH_Synthetase
create Cd_RSH_Central3HelixBundle, seleCd_RSH_Central3HelixBundle
create Cd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase_NoOrg
select seleCd_RSH_Hydrolase_NoOrg, seleCd_RSH_Hydrolase and (not org.)
select seleCd_RSH_Hydrolase_NoIno, Cd_RSH_Hydrolase_NoOrg and (not ino.)
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase_NoIno
delete seleCd_RSH_Hydrolase_NoOrg
delete Cd_RSH_Hydrolase_NoOrg
delete seleCd_RSH_Hydrolase_NoIno
select seleCd_RSH_Hydrolase, Cd_RSH_Hydrolase
deselect
create Cd_RSH_Hydrolase, seleCd_RSH_Hydrolase

disable Cd_RSH_Bifunctional_Fragment
disable Cd_RSH_Synthetase
disable Cd_RSH_Central3HelixBundle
disable Cd_RSH_Hydrolase
```

```
orient ATP_GDP_Mg
zoom ATP_GDP_Mg, 5
```

RelC and RelQ both *C. difficile* ATP GDP Mg

```
set seq_view, 1
bg_color white

select seleRelC_NTD, fold_relc_af3_atp_mg_gdp_model_0 and resi 1-220

align fold_relq_af3_gdp_mg_atp_model_0, seleRelC_NTD
align fold_relq_af3_gdp_mg_atp_model_0, seleRelC_NTD

create RelC_NTD, seleRelC_NTD

select seleRelC, fold_relc_af3_atp_mg_gdp_model_0
select seleRelQ, fold_relq_af3_gdp_mg_atp_model_0

create RelC, fold_relc_af3_atp_mg_gdp_model_0
create RelQ, fold_relq_af3_gdp_mg_atp_model_0

show surface, fold_relc_af3_atp_mg_gdp_model_0
hide surface, fold_relc_af3_atp_mg_gdp_model_0

show surface, fold_relq_af3_gdp_mg_atp_model_0
hide surface, fold_relq_af3_gdp_mg_atp_model_0

color forest, RelC
color marine, RelQ
color blue, RelC_NTD

select seleRelCIonsLigands, RelC and not polymer
deselect
create ionsRelCLigands, RelC and not polymer

select seleRelC_ATPGXP, RelC and not polymer and org.
deselect
create RelC_ATPGXP, RelC and not polymer and org.

select seleRelC_Mg, RelC and not polymer and ino.
deselect
create RelC_Mg, RelC and not polymer and ino.
color purpleblue, RelC_Mg

select seleRelC_ATP, bm. first RelC_ATPGXP
color tv_red, seleRelC_ATP
create RelC_ATP, bm. first RelC_ATPGXP
deselect
select seleRelC_GDP, bm. last RelC_ATPGXP
color deepteal, seleRelC_GDP
```

```
create RelC_GDP, bm. last RelC_ATPGXP
deselect

select seleRelQIonsLigands, RelQ and not polymer
deselect
create ionsRelQLigands, RelQ and not polymer

select seleRelQ_ATPGXP, RelQ and not polymer and org.
deselect
create RelQ_ATPGXP, RelQ and not polymer and org.
deselect

select seleRelQ_Mg, RelQ and not polymer and ino.
deselect
create RelQ_Mg, RelQ and not polymer and ino.
color lightpink, RelQ_Mg

select seleRelQ_ATP, bm. first RelQ_ATPGXP
color chocolate, seleRelQ_ATP
create RelQ_ATP, bm. first RelQ_ATPGXP
deselect
select seleRelQ_GDP, bm. last RelQ_ATPGXP
color lightblue, seleRelQ_GDP
create RelQ_GDP, bm. last RelQ_ATPGXP
deselect

disable all
enable RelC_Mg
enable RelQ_Mg
enable RelC_ATP
enable RelC_GDP
enable RelQ_ATP
enable RelQ_GDP
enable RelC_NTD
enable RelQ
enable RelC
enable RelC_NTD
deselect

orient RelC
zoom RelC, 5


bg_color white
set seq_view, 1

select OG, fold_cd_rsh_af3_atp_mg_gdp_model_0
select Mutation, fold_05_cd_rsh_g196e_gdp_mg_atp_model_0

align OG, Mutation

zoom OG
deselect

delete OG
delete Mutation
```

```
select seleMutation, fold_05_cd_rsh_g196e_gdp_mg_atp_model_0
create Mutation, fold_05_cd_rsh_g196e_gdp_mg_atp_model_0
select seleOG, fold_cd_rsh_af3_atp_mg_gdp_model_0
create OG, fold_cd_rsh_af3_atp_mg_gdp_model_0

color gray50, Mutation
color wheat, OG

select seleMutationIonsLigands, seleMutation and not polymer
deselect
create ionsMutationLigands, seleMutation and not polymer

select seleMutation_ATPGXP, Mutation and not polymer and org.
deselect
create Mutation_ATPGXP, Mutation and not polymer and org.

select seleMutation_Mg, Mutation and not polymer and ino.
deselect
create Mutation_Mg, Mutation and not polymer and ino.
select seleMutation_ATP, bm. last Mutation_ATPGXP
create Mutation_ATP, bm. last Mutation_ATPGXP
deselect
select seleMutation_GDP, bm. first Mutation_ATPGXP
create Mutation_GDP, bm. first Mutation_ATPGXP
deselect

select seleOGIonsLigands, seleOG and ino. + org.
deselect
create ionsOGLigands, seleOG and ino. + org.

select seleOG_ATPGXP, seleOG and not polymer and org.
deselect
create OG_ATPGXP, seleOG and not polymer and org.
deselect

select seleOG_Mg, seleOG and not polymer and ino.
deselect
create OG_Mg, seleOG and not polymer and ino.

select seleOG_GDP, bm. last OG_ATPGXP
color lightblue, seleOG_GDP
create OG_GDP, bm. last OG_ATPGXP
deselect

select seleOG_ATP, bm. first OG_ATPGXP
color lightblue, seleOG_ATP
create OG_ATP, bm. first OG_ATPGXP
deselect

select waters, sol.
hide everything, waters

set surface_carve_cutoff, 6
set surface_carve_selection, seleOGIonsLigands
set surface_carve_normal_cutoff, -0.1
```

```
set two_sided_lighting
set transparency, 0.5

select closeOG_ATP_GDP_Mg, br. seleOG near_to 6 of seleOGIonsLigands
create Label_OGBindingPocket_Sticks, closeOG_ATP_GDP_Mg & sc. + closeOG_ATP_GDP_Mg & n. CA +
closeOG_ATP_GDP_Mg & r. PRO & n. N
create OG_BindingPocket_Sticks, Label_OGBindingPocket_Sticks
hide everything, Label_OGBindingPocket_Sticks
hide everything, OG_BindingPocket_Sticks
show sticks, OG_BindingPocket_Sticks
show sticks, Label_OGBindingPocket_Sticks
label n. CA and Label_OGBindingPocket_Sticks, "%s %s" % (resn, resi)
color yellow, Label_OGBindingPocket_Sticks
color yellow,OG_BindingPocket_Sticks

disable Label_OGBindingPocket_Sticks

create OG_BindingPocket_Surface, seleOG within 6 of seleOGIonsLigands, 0, 1
hide everything, OG_BindingPocket_Surface
show surface, OG_BindingPocket_Surface
set surface_color, blue, OG_BindingPocket_Surface
set transparency, 0.5

distance hbond_ATP, OG_ATP, seleOG, mode=2
distance hbond_GDP, OG_GDP, seleOG, mode=2
color magenta, hbond_ATP
color magenta, hbond_GDP

set surface_carve_cutoff, 6
set surface_carve_selection, seleMutationIonsLigands
set surface_carve_normal_cutoff, -0.1
set two_sided_lighting
set transparency, 0.5

select closeMutation_ATP_GDP_Mg, br. seleMutation near_to 6 of seleMutationIonsLigands
create Label_MutationBindingPocket_Sticks, closeMutation_ATP_GDP_Mg & sc. +
closeMutation_ATP_GDP_Mg & n. CA + closeMutation_ATP_GDP_Mg & r. PRO & n. N
create Mutation_BindingPocket_Sticks, Label_MutationBindingPocket_Sticks
hide everything, Label_MutationBindingPocket_Sticks
hide everything, Mutation_BindingPocket_Sticks
show sticks, Mutation_BindingPocket_Sticks
show sticks, Label_MutationBindingPocket_Sticks
label n. CA and Label_MutationBindingPocket_Sticks, "%s %s" % (resn, resi)
color brightorange, Label_MutationBindingPocket_Sticks
color brightorange,Mutation_BindingPocket_Sticks

disable Label_MutationBindingPocket_Sticks

create Mutation_BindingPocket_Surface, seleMutation within 6 of seleMutationIonsLigands, 0, 1
hide everything, Mutation_BindingPocket_Surface
show surface, Mutation_BindingPocket_Surface
set surface_color, red, Mutation_BindingPocket_Surface
set transparency, 0.5

distance hbond_ATP, Mutation_ATP, seleMutation, mode=2
distance hbond_GDP, Mutation_GDP, seleMutation, mode=2
```

color magenta, hbond_ATP
color magenta, hbond_GDP

select seleOG_GDP_Phosphates, OG_GDP and bto. elem p + OG_GDP and elem p
deselect

select seleOG_GDP_O, OG_GDP and (not bto. elem P) and elem o and don.
create OG_GDP_O, OG_GDP and (not bto. elem P) and elem o and don.
disable OG_GDP_O
select seleOG_GDP_OH, first seleOG_GDP_O
deselect
select seleMutation_GDP_O, Mutation_GDP and (not bto. elem P) and elem o and don.
create Mutation_GDP_O, Mutation_GDP and (not bto. elem P) and elem o and don.
disable Mutation_GDP_O
select seleMutation_GDP_OH, first seleMutation_GDP_O
deselect

select seleOG_ATP_O, OG_ATP and (not bto. elem P) and elem o and don.
create OG_ATP_O, OG_ATP and (not bto. elem P) and elem o and don.
disable OG_ATP_O
select seleOG_ATP_OH, first seleOG_ATP_O
deselect
select seleMutation_ATP_O, Mutation_ATP and (not bto. elem P) and elem o and don.
create Mutation_ATP_O, Mutation_ATP and (not bto. elem P) and elem o and don.
disable Mutation_ATP_O
select seleMutation_ATP_OH, first seleMutation_ATP_O
deselect

select seleMutation_PhosphateGroups, Mutation_GDP and bto. elem p + Mutation_GDP and elem p
deselect

select seleOG_GDP_Phosphates, OG_GDP and elem P and (not Mutation_GDP)
select seleOG_GDP_Phosphate1, first seleOG_GDP_Phosphates and (not Mutation_GDP)
create NotOG_GDP_Phosphate1, OG_GDP and elem P and (not seleOG_GDP_Phosphate1) and (not Mutation_GDP)
select seleNotOG_GDP_Phosphate1, OG_GDP and elem P and (not seleOG_GDP_Phosphate1) and (not Mutation_GDP)
select seleOG_GDP_Phosphate2, first seleNotOG_GDP_Phosphate1 and (not Mutation_GDP)
create OG_GDP_Phosphate2, seleOG_GDP_Phosphate2 and (not Mutation_GDP)

select seleMutation_GDP_Phosphates, Mutation_GDP and elem P and (not OG_GDP)
select seleMutation_GDP_Phosphate1, first seleMutation_GDP_Phosphates and (not OG_GDP)
create NotMutation_GDP_Phosphate1, Mutation_GDP and elem P and (not seleMutation_GDP_Phosphate1) and (not OG_GDP)
select seleNotMutation_GDP_Phosphate1, Mutation_GDP and elem P and (not seleMutation_GDP_Phosphate1) and (not OG_GDP)
select seleMutation_GDP_Phosphate2, first seleNotMutation_GDP_Phosphate1 and (not OG_GDP)
create Mutation_GDP_Phosphate2, seleMutation_GDP_Phosphate2 and (not OG_GDP)

select seleMutation_ATP_Phosphates, Mutation_ATP and elem P and (not OG_ATP)
select seleMutation_ATP_Phosphate1, first seleMutation_ATP_Phosphates and (not OG_ATP)
create NotMutation_ATP_Phosphate1, Mutation_ATP and elem P and (not seleMutation_ATP_Phosphate1) and (not OG_ATP)
select seleNotMutation_ATP_Phosphate1, Mutation_ATP and elem P and (not seleMutation_ATP_Phosphate1) and (not OG_ATP)
select seleMutation_ATP_Phosphate2, first seleNotMutation_ATP_Phosphate1 and (not OG_ATP)

```
create Mutation_ATP_Phosphate2, seleMutation_ATP_Phosphate2 and (not OG_ATP)
select seleMutation_ATP_Phosphate3, seleNotMutation_ATP_Phosphate1 and elem P and (not
seleMutation_ATP_Phosphate2) and (not OG_ATP)
select seleOG_ATP_Phosphates, OG_ATP and elem P and (not Mutation_ATP)
select seleOG_ATP_Phosphate1, first seleOG_ATP_Phosphates and (not Mutation_ATP)
create NotOG_ATP_Phosphate1, OG_ATP and elem P and (not seleOG_ATP_Phosphate1) and (not
Mutation_ATP)
create seleNotOG_ATP_Phosphate1, OG_ATP and elem P and (not seleOG_ATP_Phosphate1) and (not
Mutation_ATP)
create NotOG_ATP_Phosphate1, OG_ATP and elem P and (not seleOG_ATP_Phosphate1) and (not
Mutation_ATP)
select seleOG_ATP_Phosphate2, first seleNotOG_ATP_Phosphate1 and (not Mutation_ATP)
create OG_ATP_Phosphate2, seleOG_ATP_Phosphate2 and (not Mutation_ATP)
select seleOG_ATP_Phosphate3, seleNotOG_ATP_Phosphate1 and elem P and (not seleOG_ATP_Phosphate2) and
(not Mutation_ATP)

disable all
enable Mutation_Mg
enable OG_Mg

enable Mutation_GDP
enable OG_GDP
enable OG
enable Mutation
enable OG_ATP
enable Mutation_ATP
deselect

color deepteal, Mutation_GDP
color chocolate, Mutation_ATP
color lightblue, OG_GDP
color tv_red, OG_ATP
color purpleblue, Mutation_Mg
color lightpink, OG_Mg
color red, seleMutation_GDP_OH
color orange, seleMutation_PhosphateGroups
color warmpink, seleOG_GDP_OH
color olive, OG_GDP and bto. elem p + OG_GDP and elem p
color cyan, OG_ATP and elem p + OG_ATP and bto. seleOG_ATP_Phosphates
color blue, Mutation_ATP and elem p + Mutation_ATP and bto. seleMutation_ATP_Phosphates
color forest, seleMutation_ATP_OH
color green, seleOG_ATP_OH

orient seleMutation
zoom seleMutation, 5

get_distance seleOG_ATP_Phosphate1, seleMutation_ATP_Phosphate1
get_distance seleOG_ATP_Phosphate2, seleMutation_ATP_Phosphate2
get_distance seleOG_ATP_Phosphate3, seleMutation_ATP_Phosphate3
get_distance seleOG_ATP_OH, seleMutation_ATP_OH

get_distance seleOG_GDP_Phosphate1, seleMutation_GDP_Phosphate1
get_distance seleOG_GDP_Phosphate2, seleMutation_GDP_Phosphate2
get_distance seleOG_GDP_OH, seleMutation_GDP_OH
get_distance seleOG_Mg, seleMutation_Mg
```

```
print(">begin","ATP_Phosphates,","ATP_hydroxyl_oxygens,","GDP_Phosphates,","GDP_hydroxyl_oxygens,","ion
s,",cmd.get_distance(atom1="seleOG_ATP_Phosphate1", atom2 ="seleMutation_ATP_Phosphate1"), "," ,
cmd.get_distance(atom1 = "seleOG_ATP_Phosphate2", atom2 = "seleMutation_ATP_Phosphate2"), "," ,
cmd.get_distance(atom1 = "seleOG_ATP_Phosphate3", atom2 = "seleMutation_ATP_Phosphate3"),"," ,
cmd.get_distance(atom1 = "seleOG_ATP_OH", atom2 = "seleMutation_ATP_OH"),",",
cmd.get_distance(atom1="seleOG_GDP_Phosphate1",atom2 ="seleMutation_GDP_Phosphate1"), "," ,
cmd.get_distance(atom1 = "seleOG_GDP_Phosphate2", atom2 = "seleMutation_GDP_Phosphate2"), ",",
cmd.get_distance(atom1 = "seleOG_GDP_OH", atom2 = "seleMutation_GDP_OH"),",",
cmd.get_distance(atom1 = "seleOG_Mg", atom2 = "seleMutation_Mg"), "end")


bg_color white
set seq_view, 1

select OG, fold_cd_rsh_af3_atp_mg_gtp_model_0
select Mutation, fold_09_cd_rsh_g217d_atp_mg_gtp_model_0

align OG, Mutation

zoom OG
deselect

delete OG
delete Mutation

select seleMutation, fold_09_cd_rsh_g217d_atp_mg_gtp_model_0
create Mutation, fold_09_cd_rsh_g217d_atp_mg_gtp_model_0
select seleOG, fold_cd_rsh_af3_atp_mg_gtp_model_0
create OG, fold_cd_rsh_af3_atp_mg_gtp_model_0

color gray50, Mutation
color wheat, OG

select seleMutationIonsLigands, seleMutation and not polymer
deselect
create ionsMutationLigands, seleMutation and not polymer

select seleMutation_ATPGXP, Mutation and not polymer and org.
deselect
create Mutation_ATPGXP, Mutation and not polymer and org.

select seleMutation_Mg, Mutation and not polymer and ino.
deselect
create Mutation_Mg, Mutation and not polymer and ino.
select seleMutation_ATP, bm. first Mutation_ATPGXP
create Mutation_ATP, bm. first Mutation_ATPGXP
deselect
select seleMutation_GTP, bm. last Mutation_ATPGXP
create Mutation_GTP, bm. last Mutation_ATPGXP
deselect

select seleOGIonsLigands, seleOG and ino. + org.
deselect
create ionsOGLigands, seleOG and ino. + org.

select seleOG_ATPGXP, seleOG and not polymer and org.
```

```
deselect
create OG_ATPGXP, seleOG and not polymer and org.
deselect

select seleOG_Mg, seleOG and not polymer and ino.
deselect
create OG_Mg, seleOG and not polymer and ino.

select seleOG_GTP, bm. last OG_ATPGXP
color lightblue, seleOG_GTP
create OG_GTP, bm. last OG_ATPGXP
deselect

select seleOG_ATP, bm. first OG_ATPGXP
color lightblue, seleOG_ATP
create OG_ATP, bm. first OG_ATPGXP
deselect

select waters, sol.
hide everything, waters

set surface_carve_cutoff, 6
set surface_carve_selection, seleOGIonsLigands
set surface_carve_normal_cutoff, -0.1
set two_sided_lighting
set transparency, 0.5

select closeOG_ATP_GTP_Mg, br. seleOG near_to 6 of seleOGIonsLigands
create Label_OGBindingPocket_Sticks, closeOG_ATP_GTP_Mg & sc. + closeOG_ATP_GTP_Mg & n. CA +
closeOG_ATP_GTP_Mg & r. PRO & n. N
create OG_BindingPocket_Sticks, Label_OGBindingPocket_Sticks
hide everything, Label_OGBindingPocket_Sticks
hide everything, OG_BindingPocket_Sticks
show sticks, OG_BindingPocket_Sticks
show sticks, Label_OGBindingPocket_Sticks
label n. CA and Label_OGBindingPocket_Sticks, "%s %s" % (resn, resi)
color yellow, Label_OGBindingPocket_Sticks
color yellow,OG_BindingPocket_Sticks

disable Label_OGBindingPocket_Sticks

create OG_BindingPocket_Surface, seleOG within 6 of seleOGIonsLigands, 0, 1
hide everything, OG_BindingPocket_Surface
show surface, OG_BindingPocket_Surface
set surface_color, blue, OG_BindingPocket_Surface
set transparency, 0.5

distance hbond_ATP, OG_ATP, seleOG, mode=2
distance hbond_GTP, OG_GTP, seleOG, mode=2
color magenta, hbond_ATP
color magenta, hbond_GTP

set surface_carve_cutoff, 6
set surface_carve_selection, seleMutationIonsLigands
set surface_carve_normal_cutoff, -0.1
set two_sided_lighting
```

```
set transparency, 0.5

select closeMutation_ATP_GTP_Mg, br. seleMutation near_to 6 of seleMutationIonsLigands
create Label_MutationBindingPocket_Sticks, closeMutation_ATP_GTP_Mg & sc. + closeMutation_ATP_GTP_Mg
& n. CA + closeMutation_ATP_GTP_Mg & r. PRO & n. N
create Mutation_BindingPocket_Sticks, Label_MutationBindingPocket_Sticks
hide everything, Label_MutationBindingPocket_Sticks
hide everything, Mutation_BindingPocket_Sticks
show sticks, Mutation_BindingPocket_Sticks
show sticks, Label_MutationBindingPocket_Sticks
label n. CA and Label_MutationBindingPocket_Sticks, "%s %s" % (resn, resi)
color brightorange, Label_MutationBindingPocket_Sticks
color brightorange,Mutation_BindingPocket_Sticks

disable Label_MutationBindingPocket_Sticks

create Mutation_BindingPocket_Surface, seleMutation within 6 of seleMutationIonsLigands, 0, 1
hide everything, Mutation_BindingPocket_Surface
show surface, Mutation_BindingPocket_Surface
set surface_color, red, Mutation_BindingPocket_Surface
set transparency, 0.5

distance hbond_ATP, Mutation_ATP, seleMutation, mode=2
distance hbond_GTP, Mutation_GTP, seleMutation, mode=2
color magenta, hbond_ATP
color magenta, hbond_GTP

select seleOG_GTP_Phosphates, OG_GTP and bto. elem p + OG_GTP and elem p
deselect

select seleOG_GTP_O, OG_GTP and (not bto. elem P) and elem o and don.
create OG_GTP_O, OG_GTP and (not bto. elem P) and elem o and don.
disable OG_GTP_O
select seleOG_GTP_OH, first seleOG_GTP_O
deselect
select seleMutation_GTP_O, Mutation_GTP and (not bto. elem P) and elem o and don.
create Mutation_GTP_O, Mutation_GTP and (not bto. elem P) and elem o and don.
disable Mutation_GTP_O
select seleMutation_GTP_OH, first seleMutation_GTP_O
deselect

select seleOG_ATP_O, OG_ATP and (not bto. elem P) and elem o and don.
create OG_ATP_O, OG_ATP and (not bto. elem P) and elem o and don.
disable OG_ATP_O
select seleOG_ATP_OH, first seleOG_ATP_O
deselect
select seleMutation_ATP_O, Mutation_ATP and (not bto. elem P) and elem o and don.
create Mutation_ATP_O, Mutation_ATP and (not bto. elem P) and elem o and don.
disable Mutation_ATP_O
select seleMutation_ATP_OH, first seleMutation_ATP_O
deselect

select seleMutation_PhosphateGroups, Mutation_GTP and bto. elem p + Mutation_GTP and elem p
deselect

select seleOG_GTP_Phosphates, OG_GTP and elem P and (not Mutation_GTP)
```

select seleOG_GTP_Phosphate1, first seleOG_GTP_Phosphates and (not Mutation_GTP)
create NotOG_GTP_Phosphate1, OG_GTP and elem P and (not seleOG_GTP_Phosphate1) and (not Mutation_GTP)
select seleNotOG_GTP_Phosphate1, OG_GTP and elem P and (not seleOG_GTP_Phosphate1) and (not Mutation_GTP)
select seleOG_GTP_Phosphate2, first seleNotOG_GTP_Phosphate1 and (not Mutation_GTP)
create OG_GTP_Phosphate2, seleOG_GTP_Phosphate2 and (not Mutation_GTP)
select seleOG_GTP_Phosphate3, seleNotOG_GTP_Phosphate1 and elem P and (not seleOG_GTP_Phosphate2) and (not Mutation_GTP)

select seleMutation_GTP_Phosphates, Mutation_GTP and elem P and (not OG_GTP)
select seleMutation_GTP_Phosphate1, first seleMutation_GTP_Phosphates and (not OG_GTP)
create NotMutation_GTP_Phosphate1, Mutation_GTP and elem P and (not seleMutation_GTP_Phosphate1) and (not OG_GTP)
select seleNotMutation_GTP_Phosphate1, Mutation_GTP and elem P and (not seleMutation_GTP_Phosphate1) and (not OG_GTP)
select seleMutation_GTP_Phosphate2, first seleNotMutation_GTP_Phosphate1 and (not OG_GTP)
create Mutation_GTP_Phosphate2, seleMutation_GTP_Phosphate2 and (not OG_GTP)
select seleMutation_GTP_Phosphate3, seleNotMutation_GTP_Phosphate1 and elem P and (not seleMutation_GTP_Phosphate2) and (not OG_GTP)

select seleMutation_ATP_Phosphates, Mutation_ATP and elem P and (not OG_ATP)
select seleMutation_ATP_Phosphate1, first seleMutation_ATP_Phosphates and (not OG_ATP)
create NotMutation_ATP_Phosphate1, Mutation_ATP and elem P and (not seleMutation_ATP_Phosphate1) and (not OG_ATP)
select seleNotMutation_ATP_Phosphate1, Mutation_ATP and elem P and (not seleMutation_ATP_Phosphate1) and (not OG_ATP)
select seleMutation_ATP_Phosphate2, first seleNotMutation_ATP_Phosphate1 and (not OG_ATP)
create Mutation_ATP_Phosphate2, seleMutation_ATP_Phosphate2 and (not OG_ATP)
select seleMutation_ATP_Phosphate3, seleNotMutation_ATP_Phosphate1 and elem P and (not seleMutation_ATP_Phosphate2) and (not OG_ATP)
select seleOG_ATP_Phosphates, OG_ATP and elem P and (not Mutation_ATP)
select seleOG_ATP_Phosphate1, first seleOG_ATP_Phosphates and (not Mutation_ATP)
create NotOG_ATP_Phosphate1, OG_ATP and elem P and (not seleOG_ATP_Phosphate1) and (not Mutation_ATP)
create seleNotOG_ATP_Phosphate1, OG_ATP and elem P and (not seleOG_ATP_Phosphate1) and (not Mutation_ATP)
create NotOG_ATP_Phosphate1, OG_ATP and elem P and (not seleOG_ATP_Phosphate1) and (not Mutation_ATP)
select seleOG_ATP_Phosphate2, first seleNotOG_ATP_Phosphate1 and (not Mutation_ATP)
create OG_ATP_Phosphate2, seleOG_ATP_Phosphate2 and (not Mutation_ATP)
select seleOG_ATP_Phosphate3, seleNotOG_ATP_Phosphate1 and elem P and (not seleOG_ATP_Phosphate2) and (not Mutation_ATP)

disable all
enable Mutation_Mg
enable OG_Mg

enable Mutation_GTP
enable OG_GTP
enable OG
enable Mutation
enable OG_ATP
enable Mutation_ATP
deselect

```
color deepteal, Mutation_GTP
color chocolate, Mutation_ATP
color lightblue, OG_GTP
color tv_red, OG_ATP
color purpleblue, Mutation_Mg
color lightpink, OG_Mg
color red, seleMutation_GTP_OH
color orange, seleMutation_PhosphateGroups
color warmpink, seleOG_GTP_OH
color olive, OG_GTP and bto. elem p + OG_GTP and elem p
color cyan, OG_ATP and elem p + OG_ATP and bto. seleOG_ATP_Phosphates
color blue, Mutation_ATP and elem p + Mutation_ATP and bto. seleMutation_ATP_Phosphates
color forest, seleMutation_ATP_OH
color green, seleOG_ATP_OH

orient seleMutation
zoom seleMutation, 5

get_distance seleOG_ATP_Phosphate1, seleMutation_ATP_Phosphate1
get_distance seleOG_ATP_Phosphate2, seleMutation_ATP_Phosphate2
get_distance seleOG_ATP_Phosphate3, seleMutation_ATP_Phosphate3
get_distance seleOG_ATP_OH, seleMutation_ATP_OH

get_distance seleOG_GTP_Phosphate1, seleMutation_GTP_Phosphate1
get_distance seleOG_GTP_Phosphate2, seleMutation_GTP_Phosphate2
get_distance seleOG_GTP_Phosphate3, seleMutation_GTP_Phosphate3
get_distance seleOG_GTP_OH, seleMutation_GTP_OH
get_distance seleOG_Mg, seleMutation_Mg

print(">begin","ATP_Phosphates,","ATP_hydroxyl_oxygens,","GTP_Phosphates,","GTP_hydroxyl_oxygens,","ion
s,",cmd.get_distance(atom1="seleOG_ATP_Phosphate1", atom2 ="seleMutation_ATP_Phosphate1"), "," ,
cmd.get_distance(atom1 = "seleOG_ATP_Phosphate2", atom2 = "seleMutation_ATP_Phosphate2"), "," ,
cmd.get_distance(atom1 = "seleOG_ATP_Phosphate3", atom2 = "seleMutation_ATP_Phosphate3"),"," ,
cmd.get_distance(atom1 = "seleOG_ATP_OH", atom2 = "seleMutation_ATP_OH"),",",
cmd.get_distance(atom1="seleOG_GTP_Phosphate1",atom2 ="seleMutation_GTP_Phosphate1"), "," ,
cmd.get_distance(atom1 = "seleOG_GTP_Phosphate2", atom2 = "seleMutation_GTP_Phosphate2"), "," ,
cmd.get_distance(atom1 = "seleOG_GTP_Phosphate3", atom2 = "seleMutation_GTP_Phosphate3"),",",
cmd.get_distance(atom1 = "seleOG_GTP_OH", atom2 = "seleMutation_GTP_OH"),",",
cmd.get_distance(atom1 = "seleOG_Mg", atom2 = "seleMutation_Mg"), "end")
```

VITA

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