Tuberculosis Drug Discovery

Application of computational approaches in a unique industry/academic collaboration

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Tuberculosis Drug Accelerator

Panorama Global

Prologue

"Jack of all trades, master of none" – Robert Greene on Shakespeare

"Drug discovery is a team sport. It requires collaboration, creativity, and perseverance" – David Julius (2021 Nobel Laureate)

"If you don't have results tell a good story" – Jim Bobbitt (Uconn Chemistry)

"Scientists tend to come in two stripes: those who have tremendous appetite and aptitude for the details, and those who illuminate the big picture"

– Carolyn Porco (regarding Carl Sagan)

"This porridge is just right" – Goldilocks

Outline

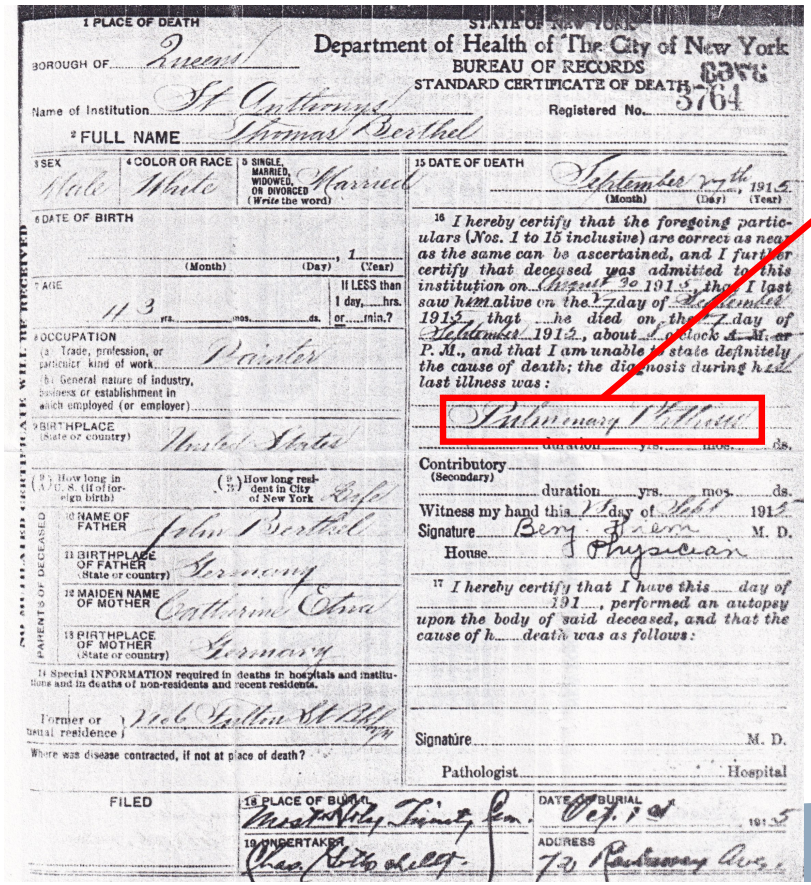
Tuberculosis Drug Discovery

Application of computational approaches in a unique industry/academic collaboration

- Tuberculosis (TB) and *Mycobacterium Tuberculosis (Mtb)*
- Tuberculosis Drug Accelerator (TBDA)
- Drug Discovery Process
- TBDA drug design Team (TddT)
- Example (Rv1297, Rho)

Tuberculosis Drug Discovery

Personal connection



Department of Health of The City of New York
BUREAU OF RECORDS
STANDARD CERTIFICATE OF DEATH
Registered No. 3764

Name of Institution: St Catherines
2 FULL NAME: Thomas Berthel
3 SEX: Male
4 COLOR OR RACE: White
5 SINGLE, MARRIED, WIDOWED, OR DIVORCED (Write the word): Married
6 DATE OF BIRTH: (Month) (Day) (Year): 1
7 AGE: 11 3 yrs. mos. ds. If LESS than 1 day, hrs. or min.?
8 OCCUPATION: (a) Trade, profession, or particular kind of work: Painter
(b) General nature of industry, business or establishment in which employed (or employer):
9 BIRTHPLACE (State or country): United States
(a) How long in U. S. (If of foreign birth):
(b) How long resident in City of New York: Born
10 NAME OF FATHER: John Berthel
11 BIRTHPLACE OF FATHER (State or country): Germany
12 MAIDEN NAME OF MOTHER: Catharine Graf
13 BIRTHPLACE OF MOTHER (State or country): Germany
14 Special INFORMATION required in deaths in hospitals and institutions and in deaths of non-residents and recent residents.
Former or usual residence: 216 Sutton St Bklyn
Where was disease contracted, if not at place of death?
15 DATE OF DEATH: September 27th 1915 (Month) (Day) (Year)
16 I hereby certify that the foregoing particulars (Nos. 1 to 15 inclusive) are correct as near as the same can be ascertained, and I further certify that deceased was admitted to this institution on August 30 1915, that I last saw him alive on the 7 day of September 1915, that he died on the 7 day of September 1915, about 1 o'clock A. M. or P. M., and that I am unable to state definitely the cause of death; the diagnosis during his last illness was:
Pulmonary Phthisis
duration yrs. mos. ds.
Contributory (Secondary)
duration yrs. mos. ds.
Witness my hand this 11 day of Oct 1915
Signature Benj Boem M. D.
House Physician
17 I hereby certify that I have this day of 191, performed an autopsy upon the body of said deceased, and that the cause of h death was as follows:
Signature M. D.
Pathologist Hospital
FILED
18 PLACE OF BURIAL: Mount Olivet Trinity Ave.
DATE OF BURIAL: Oct 1st 1915
19 UNDERTAKER: Chas. Ritchey
ADDRESS: 72 Rockaway Ave.

Pulmonary Phthisis

Phthisis Pulmonalis

[Form A.—Jan. 1, 1880.] DEPARTMENT OF HEALTH OF THE CITY OF BROOKLYN 13102
CERTIFICATE OF DEATH.
1.—Full Name. John Berthe
2.—Age. 60 years, 6 months, days.
3.—Sex, Male, Female. 4.—White, Colored.
5.—Single, Married, Widow, Widower.
6.—Birthplace, Germany 7.—Occupation, Watchmaker
8.—If of foreign birth, how long in the U. S. 25 years. 9.—How long resident in City, 25 years.
10.—Father's Birthplace, Germany 11.—Mother's Birthplace, Germany
12.—Place of Death, No. Liberty Ave near E. Jersey St Brooklyn, Ward 24th
13.—Number of Families in House, 4 14.—On what Floor, 1st
15.—I HEREBY CERTIFY that I attended the deceased from August 7th 1890, to August 16th 1890, that I last saw h alive on the 16th day of August 1890; that he died on the 21st day of August 1890, about 8 o'clock A. M. or P. M., of the following disease, as I verily believe, and that the following was the
I. Phthisis Pulmonalis — Time from attack till Death, Several months
II. Asthenia
This Certificate delivered to Carl Beikle at 8 A. M. August 21st 1890.
Signed J. A. Winter M. D. Medical Attendant. No. 181 Meserole St Street or Avenue. Address.
See other side for explanations and directions.

Tuberculosis

By any other name…

- Phthisis Pulmonalis
- Pulmonary Phthisis
- Consumption
- Scrofula
- White plague

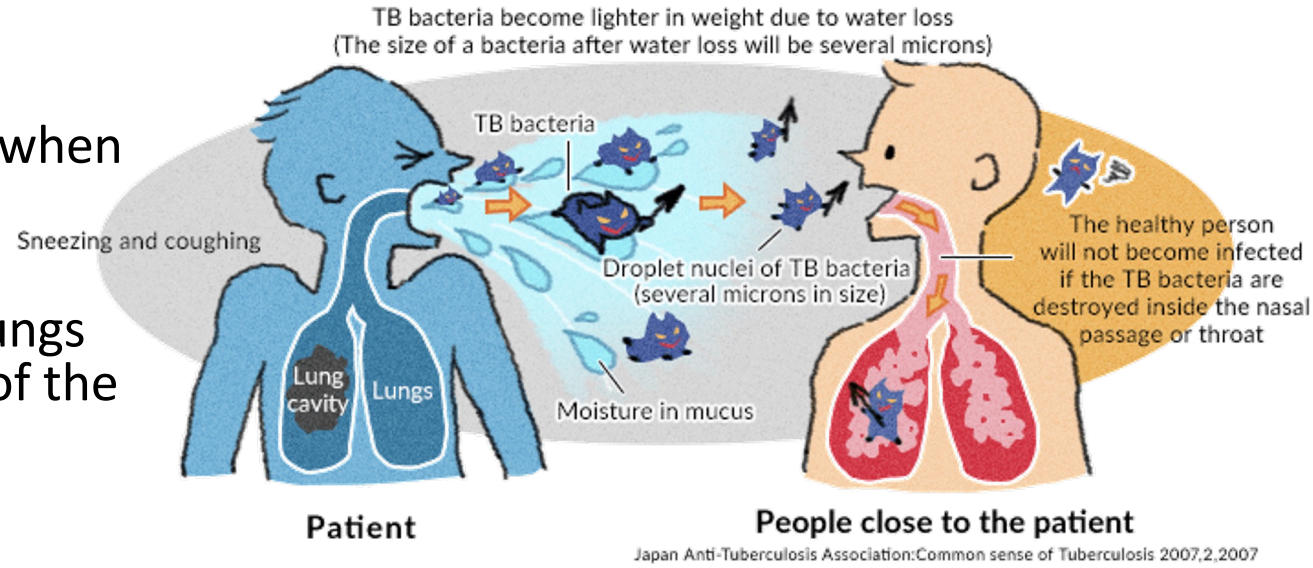
- Tuberculosis
- TB

Tuberculosis Drug Accelerator

Tuberculosis

What is TB?

- An infectious disease
- Spreads through the air when infected people cough, sneeze or spit[1]
- Most often affects the lungs but can attack any part of the body such as the kidney, spine, and brain[2]
- Caused by a bacterium called *Mycobacterium tuberculosis (Mtb)*



1. World Health Organization
2. Centers for Disease Control and Prevention

Tuberculosis

Mycobacterium tuberculosis

- The organism is an acid-fast, aerobic bacillus with a high cell wall content of high-molecular-weight lipids (mycolic acid)
- "Waxy" cell wall allows bacterium to avoid destruction by immune cells
- Ability to modulate metabolism allows for persistence in host tissues for decades (granulomas)
- Evidence Mtb has been a human pathogen for thousands of years

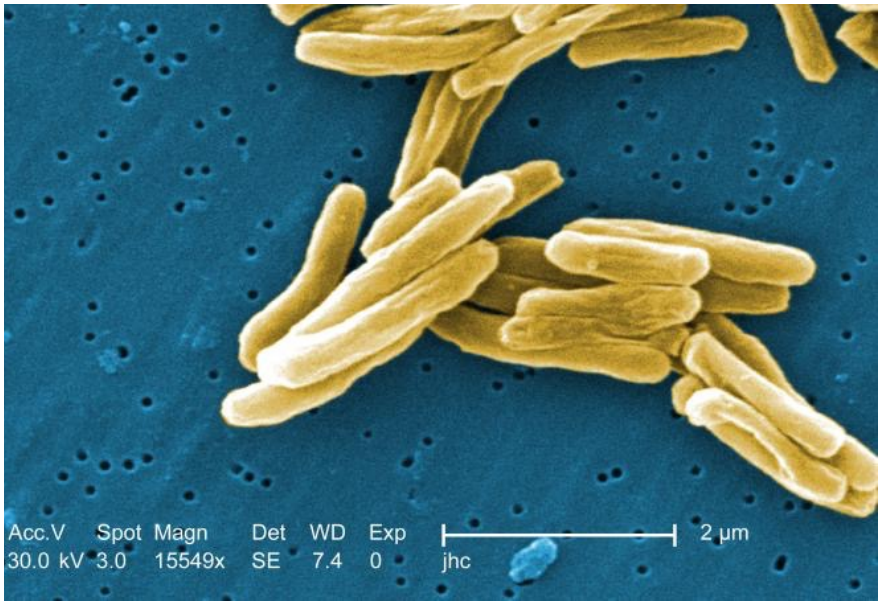
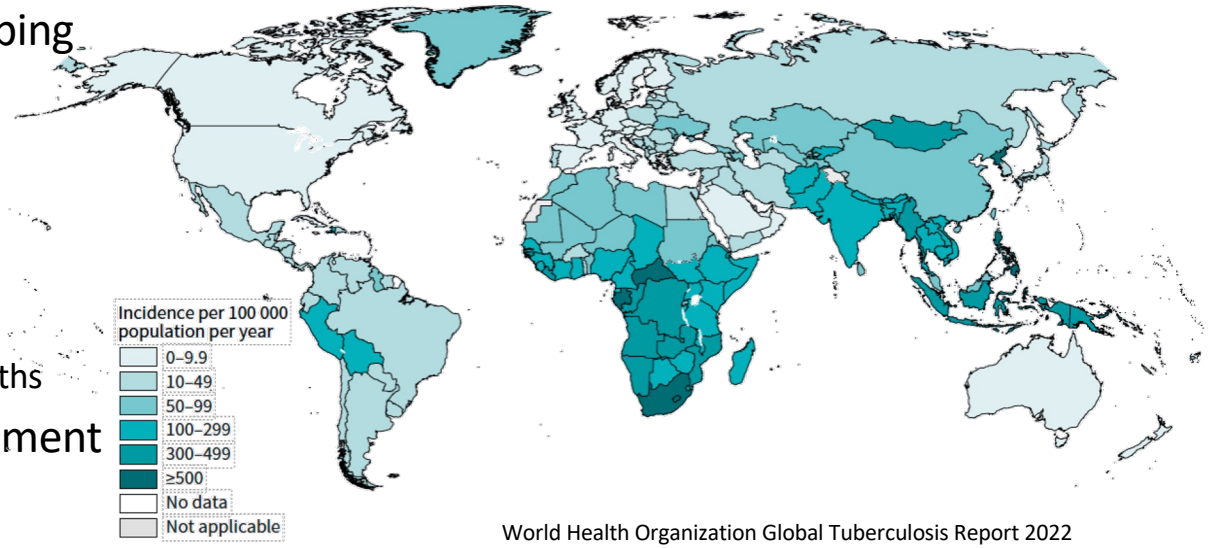


Photo: Janice Haney Carr, 2006 (CDC)

Tuberculosis

Global burden

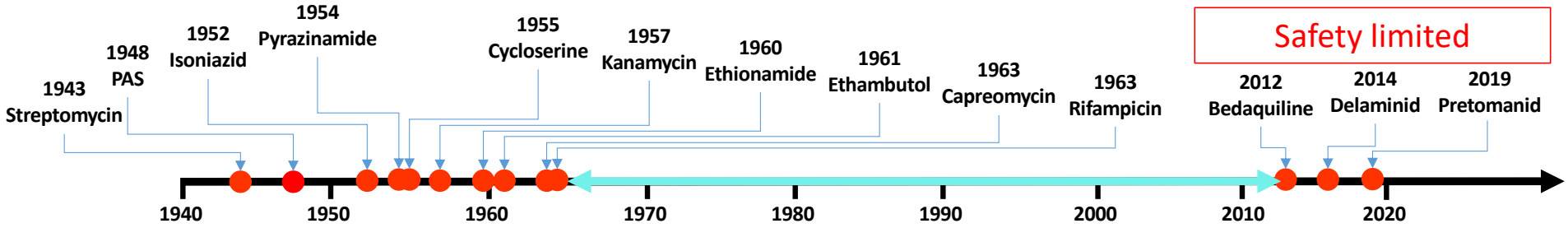
- Until COVID-19, TB was the world's **leading infectious killers**
- Disproportionately affects developing countries, in 2021:
  - 1.4 M deaths
  - 10.6 M new infections
  - 187 K multidrug-resistant cases
- First-line therapies for TB
  - Antiquated and inadequate
  - Standard of Care (SOC) 4 drugs/6 months
- Regimen contributes to high treatment default rates
  - Increased transmission
  - Drug resistance



World Health Organization Global Tuberculosis Report 2022

Tuberculosis

TB Drug discovery



adapted from TB Alliance graphic

- Limited investment in biology and drug discovery for ~50 years
- Lack of understanding of how to improve therapy
- Few well validated targets
- Poor assays to screen for drugs
- Safety of newer drugs limit use
- Resistance to only true sterilizing and treatment shortening agent (Rifampicin)
- Limited Candidates

Tuberculosis Drug Accelerator

What is the TBDA[1]?

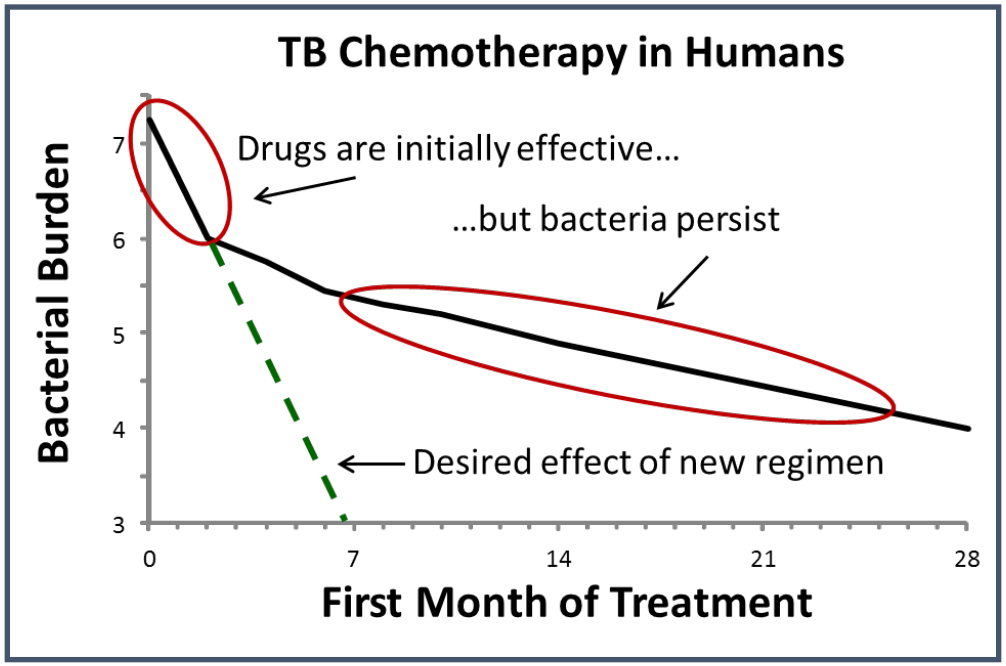
- The TBDA is a groundbreaking partnership between:
  - Pharmaceutical companies
  - Major Universities
  - Biotechs
  - Research Institutes
  - National Institutes
  - Nonprofit PDP
- With participation from:
  - Bill and Melinda Gates Foundation
- Managed through:
  - The CEO roundtable at Panorama Global

1. learn more at www.tbdrugaccelerator.org

Tuberculosis Drug Accelerator

Focus, Strategy and Goals

- Current TB regimens drive down bacterial levels quickly, but require months of treatment to rid the body of all TB
- The only way to overcome this persistence is through a shorter more effective regimen
- GOAL-To generate 1-2 mechanistically distinct TB drug candidates per year sufficient to advance at least one universal drug regimen to a 1-month clinical proof of concept
- Need to create a balanced portfolio
  - Novel mechanisms
  - Sequestered sites (granulomas, cavities)
  - Tolerant sub-populations
  - Safety
  - Resistance



Tuberculosis Drug Accelerator

"quasi-biotech"

Biotech/Pharma like

- Discovery, preclinical and early clinical capabilities
- Multiple projects at multiple centers covering different modes of action
- Funding and portfolio management oversight

Unique

- Comprises normally competitive organizations that share information and resources at an unprecedented level
- Investigating a single disease from many, many angles
- Output has global access requirements

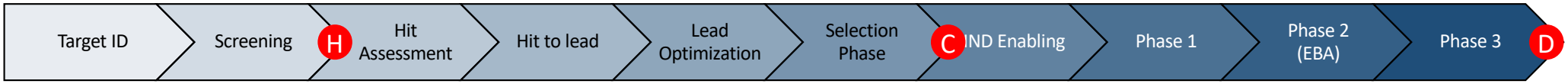
Tuberculosis Drug Accelerator

Drug Design Team (TddT)

- Comprises scientists from TBDA organizations and affiliates
- Medicinal Chemistry, Drug design, Molecular Modelling, Computer science, Structural biology, Chem and Bioinformatics expertise
- **Objective-** To identify starting points for discovery projects through computational methods
- Methodology
  - Virtual screening (docking) utilizing public domain software
  - Texas A&M HPRC

Tuberculosis Drug Accelerator

Drug Discovery and Development Progression



- The TBDA is focused on preclinical discovery
  - Goal- Clinical candidates
  - Paradigms
    - Screening: Target, Phenotypic, DNA encoded, and Virtual
    - Rational: Design and information based
- The TddT is focused on early preclinical discovery
  - Goal- Hit series for further evaluation
  - Methodology- Virtual screening, rational design

Tuberculosis Drug Accelerator

General considerations for target selection

- **Biology-** impact on bacteria
  - **Essentiality[1-3]-** Is the gene required for survival and growth
  - **Vulnerability[4]-** Magnitude of gene inhibition as it relates to bacterial fitness
  - **Validation-** Are there compounds that show bactericidal/bacteriostatic activity *in vitro*, *in vivo* and/or in the clinic
- **Chemistry-** Is it druggable?
  - **Assayable-** Can you produce protein, create an assay and screen compounds?
  - **Structure/function-** Is there structural data that suggests you can inhibit function with a small molecule?
  - **Safety-** Is it different enough to human orthologs that you can achieve selectivity and thereby avoid side effects (toxicity)?

1. DeJesus, M. A.; Gerrick, E. R.; Xu, W.; Park, S. W.; Long, J. E.; Boutte, C. C.; Rubin, E. J.; Schnappinger, D.; Ehrt, S.; Fortune, S. M.; Sassetti, C. M.; Ioerger, T. R. Comprehensive Essentiality Analysis of the Mycobacterium Tuberculosis Genome via Saturating Transposon Mutagenesis. *mBio* **2017**, *8* (1), e02133-16
2. Griffin, J. E.; Gawronski, J. D.; DeJesus, M. A.; Ioerger, T. R.; Akerley, B. J.; Sassetti, C. M. High-Resolution Phenotypic Profiling Defines Genes Essential for Mycobacterial Growth and Cholesterol Catabolism. *PLOS Pathogens* **2011**, *7* (9), e1002251.
3. Sassetti, C. M.; Boyd, D. H.; Rubin, E. J. Genes Required for Mycobacterial Growth Defined by High Density Mutagenesis. *Molecular Microbiology* **2003**, *48* (1), 77–84
4. Bosch, B.; DeJesus, M. A.; Poulton, N. C.; Zhang, W.; Engelhart, C. A.; Zaveri, A.; Lavalette, S.; Ruecker, N.; Trujillo, C.; Wallach, J. B.; Li, S.; Ehrt, S.; Chait, B. T.; Schnappinger, D.; Rock, J. M. Genome-Wide Gene Expression Tuning Reveals Diverse Vulnerabilities of M. Tuberculosis. *Cell* **2021**, *184* (17), 4579-4592.e24.

Tuberculosis Drug Accelerator

Druggability as it applies to virtual screening

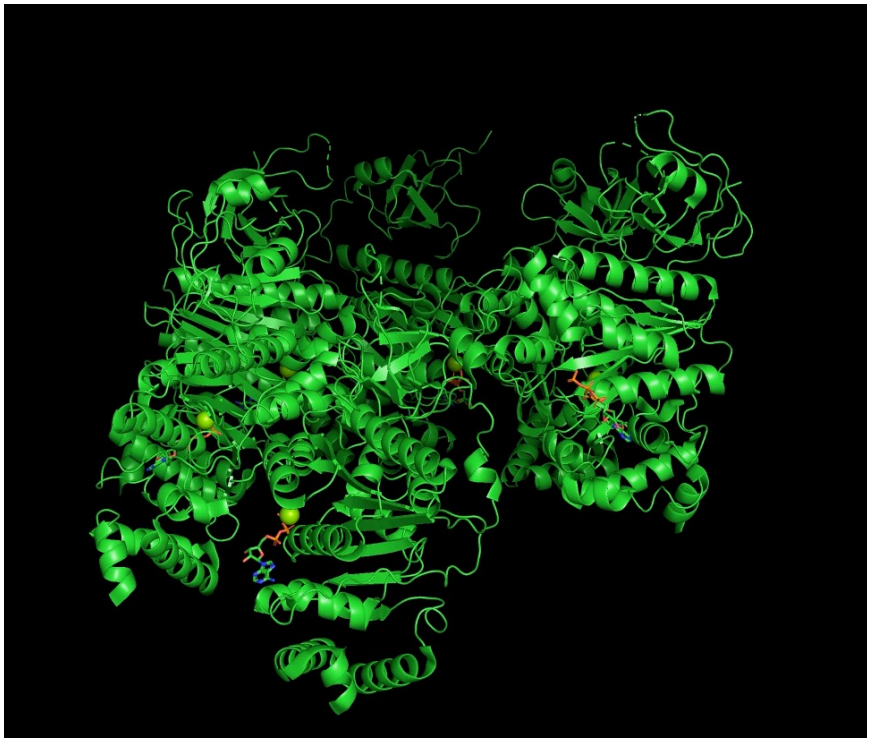
- **Structure**
  - Xray Crystallography
  - Cryogenic electron microscopy
  - Nuclear Magnetic Resonance
- **Features**
  - Resolution (Å, 10^{-10} m)
  - Well defined binding pocket(s)
    - Active site
    - Allosteric site
  - Bound ligand
  - Composition (Electrostatics, hydrophobicity)
- **Computational Tools**
  - **PockDrug**[1] (University of Paris)-calculated descriptor-based analysis of protein structure
  - **Pocketome**[2] (UCSD, RAS)- Bioinformatic "encyclopedia" classification based on known druggable binding sites
  - **Datawarrior**[3] (Actelion)- Fragment based calculation of druglikeness of known protein ligands.
- **Bioinformatics**
  - Ortholog identification (human, bacterial)
  - Degree of homology (overall and active site)

1. Hussein, H. A.; Borrel, A.; Geneix, C.; Petitjean, M.; Regad, L.; Camproux, A.-C. PockDrug-Server: A New Web Server for Predicting Pocket Druggability on Holo and Apo Proteins. Nucleic Acids Research 2015, 43 (W1), W436–W442.
2. Kufareva, I.; Ilatovskiy, A. V.; Abagyan, R. Pocketome: An Encyclopedia of Small-Molecule Binding Sites in 4D. Nucleic Acids Res 2012, 40 (Database issue), D535–D540.
3. Sander, T.; Freyss, J.; Von Korff, M.; Rufener, C. DataWarrior: An Open-Source Program For Chemistry Aware Data Visualization And Analysis. J. Chem. Inf. Model. 2015, 55 (2), 460–473.

Rho

Basic information

- Rv1297- Transcription termination factor
- Transcription- Process in which information in DNA is copied to mRNA
- Enzyme
  - Clamps on to RNA
  - “Molecular machine” that unwinds RNA
- Validation
  - Essential
  - Highly vulnerable
  - Known drugs in other bacterial species
  - No human ortholog
- Considered a high priority target



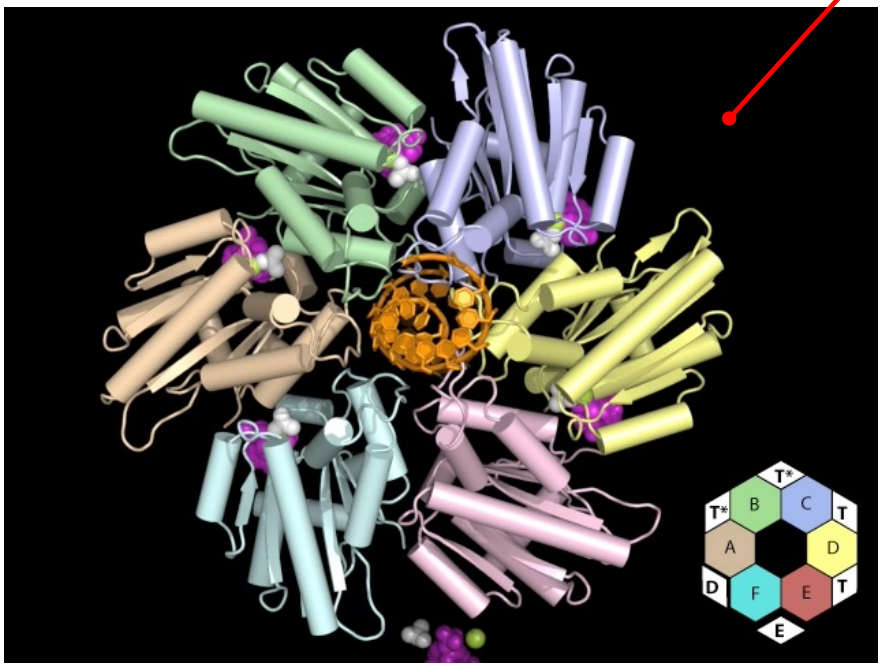
1. Bossi, L.; Figueroa-Bossi, N.; Bouloc, P.; Boudvillain, M. Regulatory Interplay between Small RNAs and Transcription Termination Factor Rho. *Biochimica et Biophysica Acta (BBA) - Gene Regulatory Mechanisms* **2020**, *1863* (7), 194546.
2. Saridakis, E.; Vishwakarma, R.; Lai-Kee-Him, J.; Martin, K.; Simon, I.; Cohen-Gonsaud, M.; Coste, F.; Bron, P.; Margeat, E.; Boudvillain, M. Cryo-EM Structure of Transcription Termination Factor Rho from Mycobacterium Tuberculosis Reveals Bicyclomycin Resistance Mechanism. *Commun Biol* **2022**, *5* (1), 1–9.

Rho

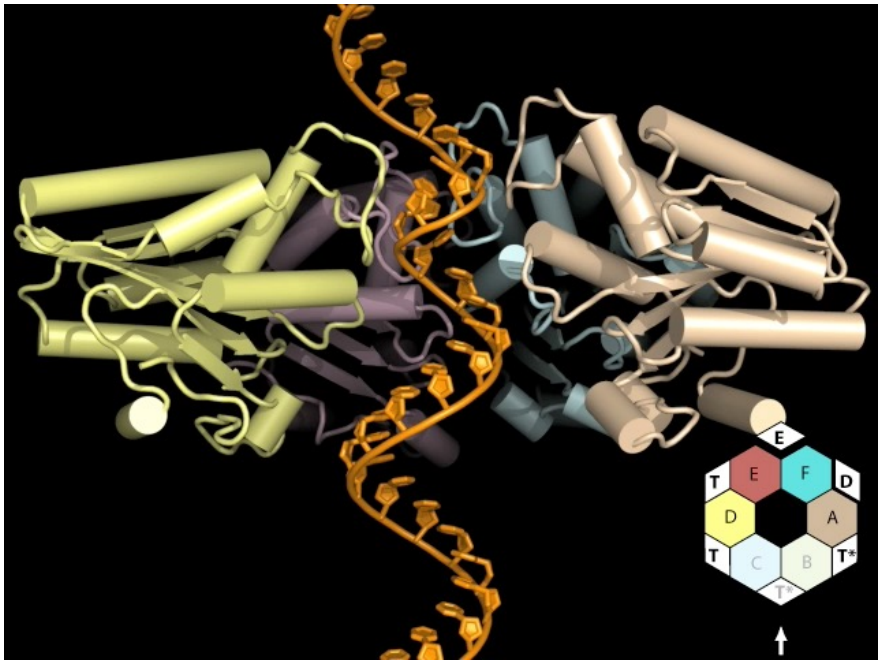
E. Coli ortholog in action[1]

ATP->ADP

Top view



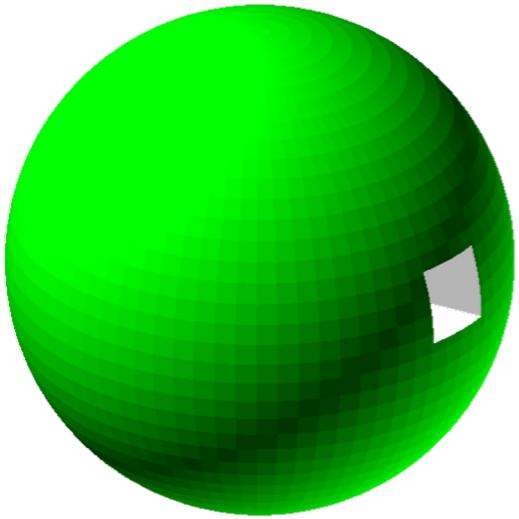
side view



1. Movies taken from Thomsen, N. D.; Berger, J. M. Running in Reverse: The Structural Basis for Translocation Polarity in Hexameric Helicases. *Cell* **2009**, *139* (3), 523–534 who used programs from the Yale Morph Server

Rho

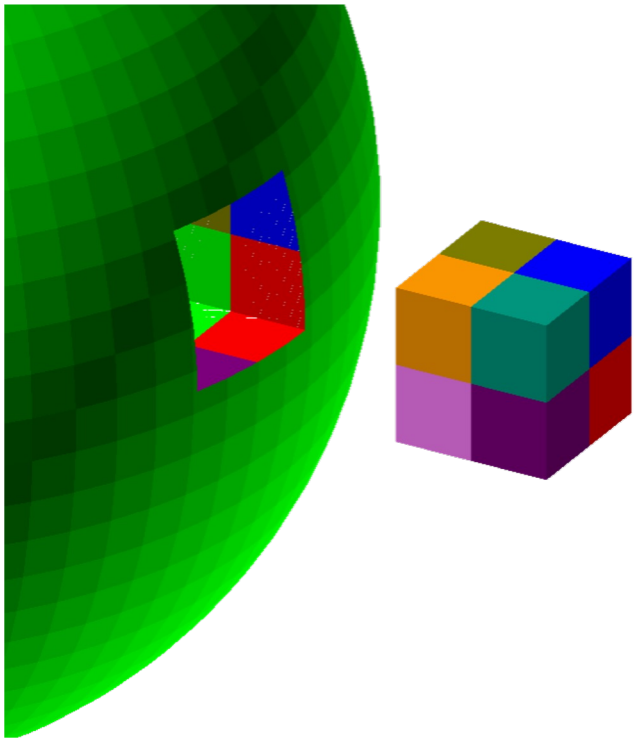
Enzyme inhibition



- Molecular Recognition
  - Size and Shape complementarity

Rho

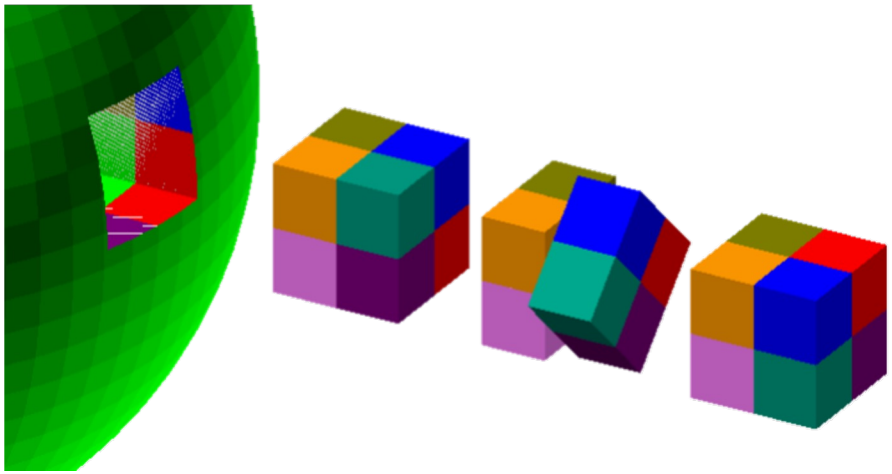
Enzyme inhibition



- Molecular Recognition
  - Size and Shape complementarity
  - Chemical complementarity
    - hydrogen bonding
    - Van der Waals interactions
    - Electrostatic interactions
    - Hydrophobic interactions

Rho

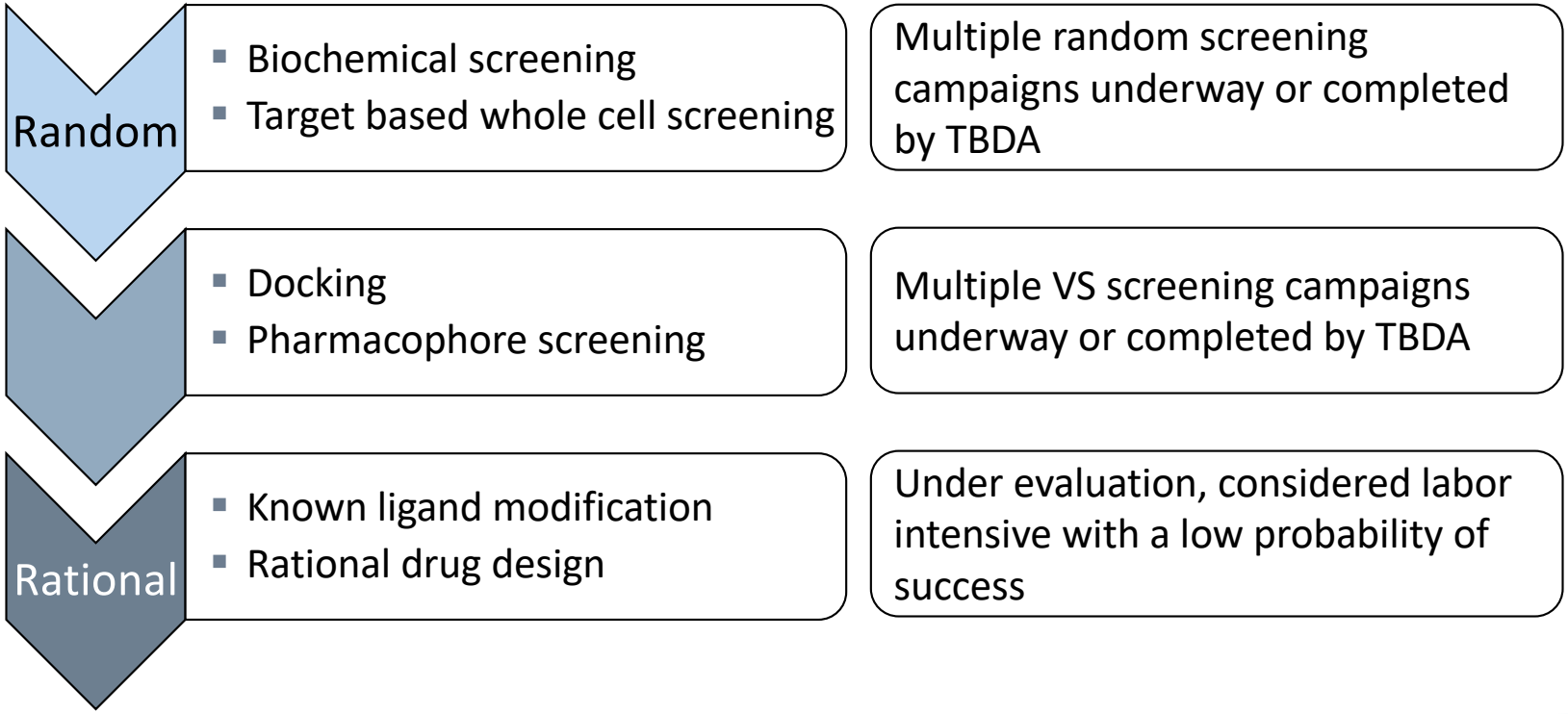
Enzyme inhibition



- Molecular Recognition
  - Size and Shape complementarity
  - Chemical complementarity
    - hydrogen bonding
    - Van der Waals interactions
    - Electrostatic interactions
    - Hydrophobic interactions.
  - Correct ligand conformation
- A good inhibitor has:
  - **High affinity-** usually translates to greater inhibition
  - **High Specificity-** usually translates to lower side effects/adverse events

Rho

TBDA Discovery approaches



Rho

Virtual Screening: Docking

- Method of assessing, *in silico*, whether ligands can bind to a protein
- Utilizes complex scoring functions to calculate binding affinity
- Has numerous assumptions and caveats:
  - Crystal structure vs. solution structure, homology models
  - Ligand conformations
  - Protein flexibility
  - Weighting of electrostatics, hydrogen bonding, hydrophobic interactions
  - Binding may not result in inhibition
- Must be confirmed *in vitro* with a binding or functional assay

Rho

Virtual Screening: Docking

- Requires
  - Protein structure of suitable quality
  - Ligand database(s) of accessible compounds (own or buy)
- Software
  - Several commercial vendors
  - **Autodock Vina-** open-source molecular docking program (Scripps)[1-2]
- Hardware
  - Desktop PC (few ligands)
  - HPRC (100,000 to millions of ligands)

1. Trott, O.; Olson, A. J. AutoDock Vina: Improving the Speed and Accuracy of Docking with a New Scoring Function, Efficient Optimization, and Multithreading. *J. Comp. Chem.* **2010**, *31* (2), 455–461.
2. Eberhardt, J.; Santos-Martins, D.; Tillack, A. F.; Forli, S. AutoDock Vina 1.2.0: New Docking Methods, Expanded Force Field, and Python Bindings. *J. Chem. Inf. Model.* **2021**, *61* (8), 3891–3898

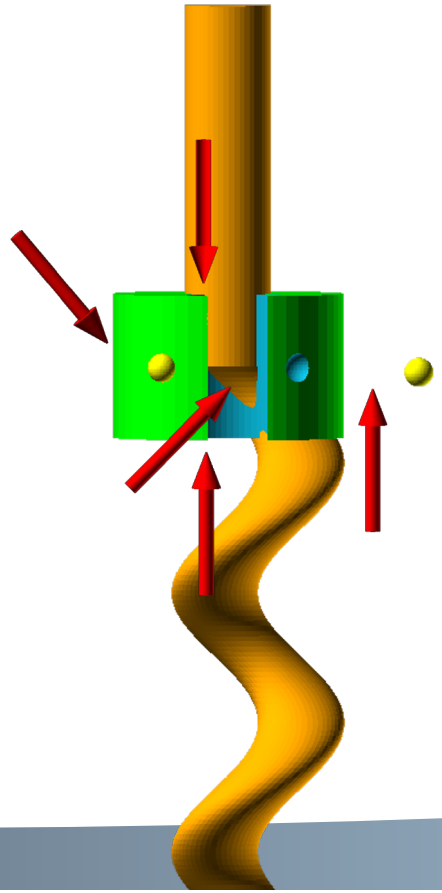
Rho

Virtual Screening: Docking

- Protein preparation
  - Determine protein search area (active or allosteric site)
  - Describe ”bounding box” (Bigger- more hits, more time/mol)
  - Determine flexibility of key sidechains (More flexible sidechains, more hits more time/mol)
  - Determine exhaustiveness (Higher- more attempts to dock, more hits, more time/mol)
- Ligand database preparation
  - Prefilter (drug likeness, size, complexity)
  - Calculate conformations

Rho

Enzyme inhibition: How to prevent function?



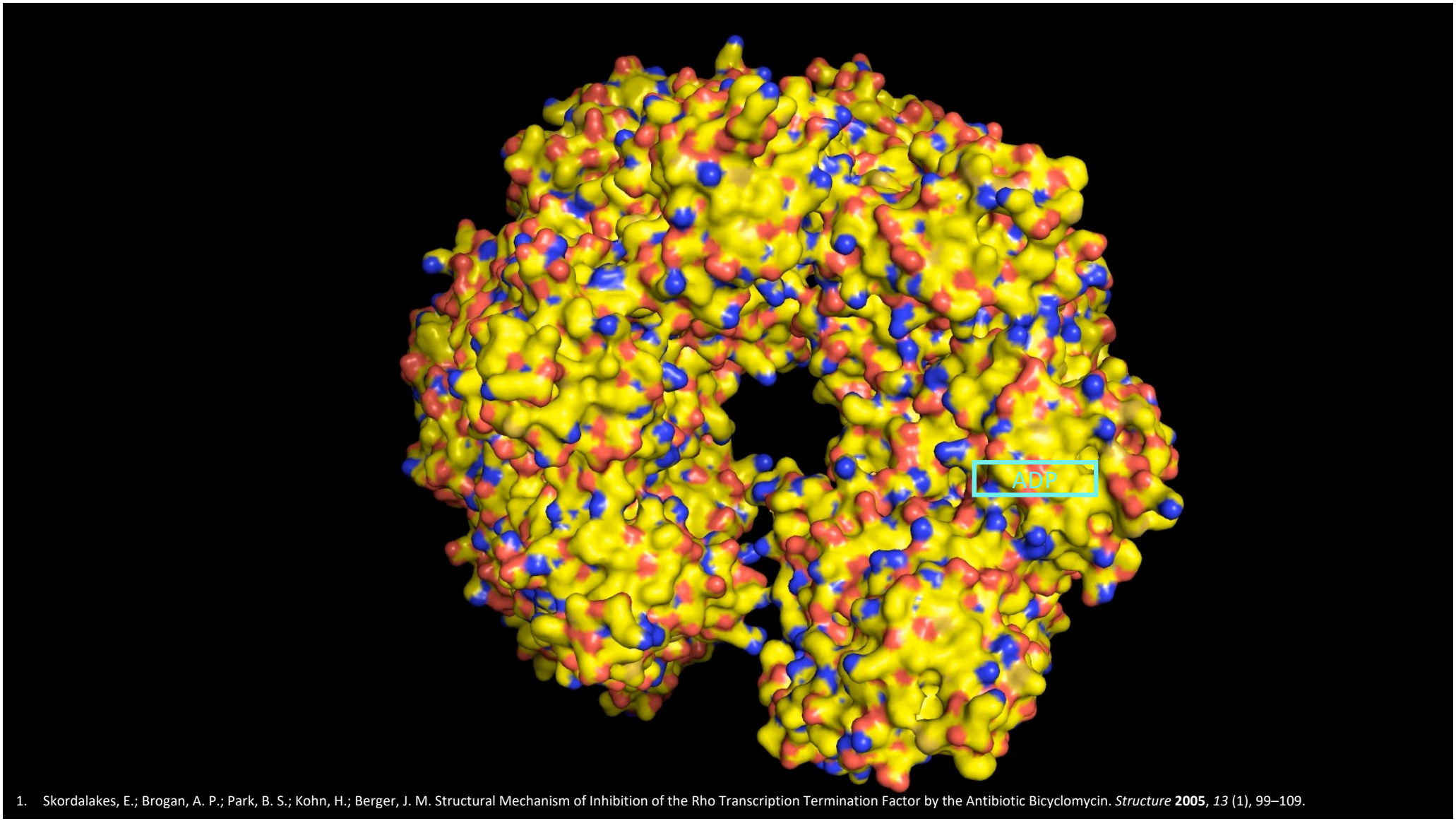
- Active Site
  - Interfere with unwinding mechanism
  - Interfere with power source (ATP->ADP)
- Allosteric site
  - Identify remote site that through small molecule binding function is blocked
    - hinge or pivot
    - entrance/exit of RNA

Rho

Virtual Screening

- Protein Structure
  - Mtb Rho structure unavailable
  - E. Coli ortholog structures available (PDB 1XPO[1], 5JJI[2])

1. Skordalakes, E.; Brogan, A. P.; Park, B. S.; Kohn, H.; Berger, J. M. Structural Mechanism of Inhibition of the Rho Transcription Termination Factor by the Antibiotic Bicyclomycin. *Structure* **2005**, *13* (1), 99–109.
2. Thomsen, N. D.; Lawson, M. R.; Witkowsky, L. B.; Qu, S.; Berger, J. M. Molecular Mechanisms of Substrate-Controlled Ring Dynamics and Substepping in a Nucleic Acid-Dependent Hexameric Motor. *Proceedings of the National Academy of Sciences* **2016**, *113* (48), E7691–E7700.



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Rho
Virtual Screening

- Protein Structure
  - Mtb Rho structure unavailable
  - E. Coli ortholog structures available (PDB 1XPO[1], 5JJI[2])
    - ATP active site identified
    - *Allosteric site identified as well*
  - Build homology model
- Ligand Database(s)

1. Skordalakes, E.; Brogan, A. P.; Park, B. S.; Kohn, H.; Berger, J. M. Structural Mechanism of Inhibition of the Rho Transcription Termination Factor by the Antibiotic Bicyclomycin. *Structure* **2005**, *13* (1), 99–109.
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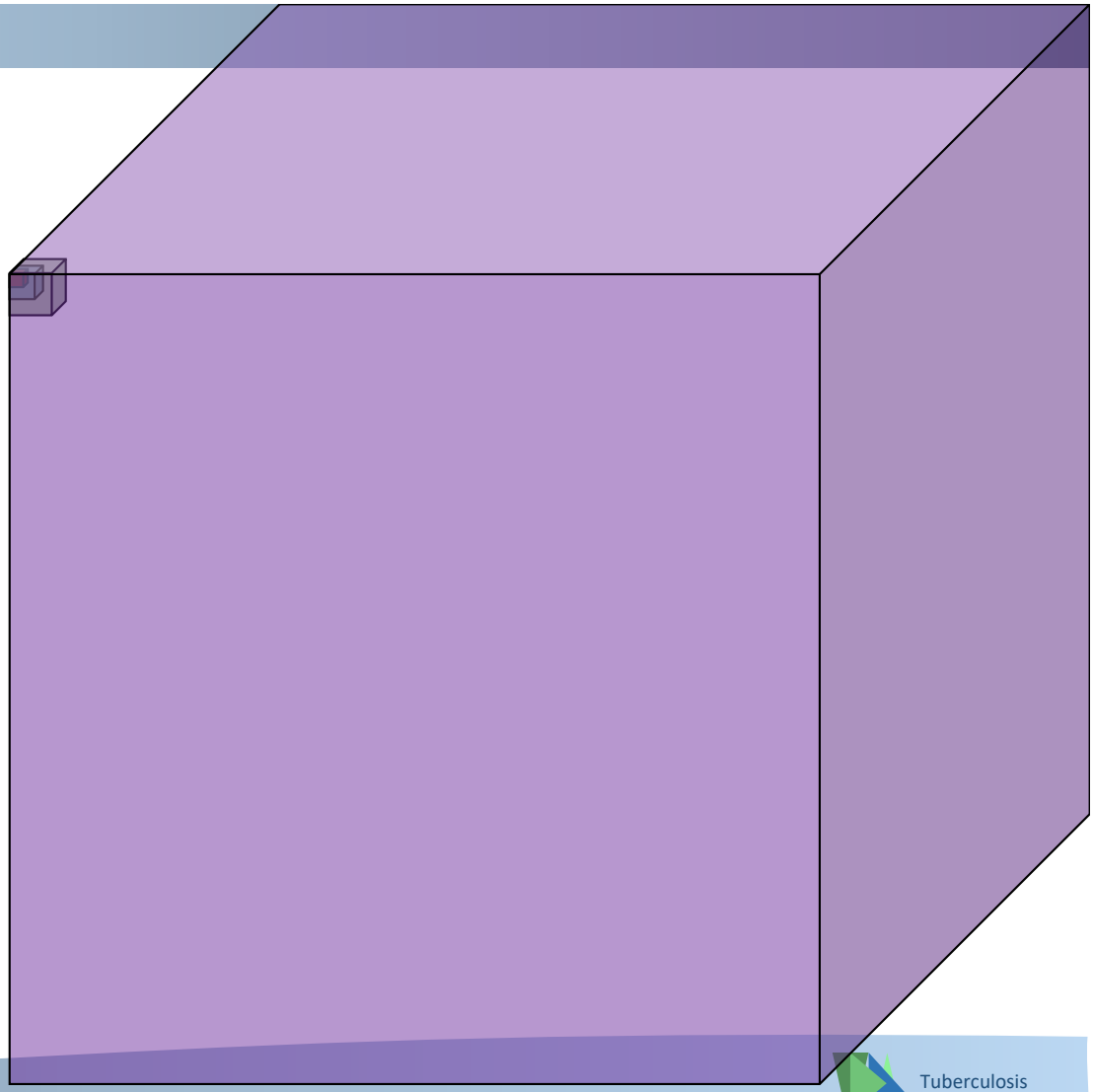
Rho

Virtual screen

Ligand database and “chemical space” considerations

- Virtual vs Real
- “Real” Sources
  - Texas A&M ~140K
  - Pharma libraries 1-2M
  - Commercial 5M
  - Enamine “real” 35B
- Druglike?
- Singletons vs. analogs



Tuberculosis
Drug Accelerator

Rho

Virtual Screening

Library size trade-offs

- Bigger may mean more diversity
  - Better chance of finding a hit
- Bigger may mean more analogs
  - Easier hit assessment and validation
  - Early Structure Activity and Property Relationships (SAR/SPR)
- Bigger definitely means more time!
  - Computational
  - Work-up
  - Actual assays

Rho

Virtual Screening

- Full Pharma collections are large (500K to 2-3 Million)
- Created over time
  - Internal projects (final products, byproducts, intermediates)
  - Commercial sources
  - Focused efforts (Parallel synthesis, natural products)
  - Company mergers/acquisitions
- More drug like?
- Not just singletons (100s to 1000s of potential analogs)
- Not disclosed- Highly proprietary, closely guarded

Rho

Virtual Screening

- TBDA has pharma partners, but access to collections blinded
  - OK for “wet” screening
  - Useless for virtual screening
- “Patent Space[1]” libraries
  - SureChEMBL Open patent database (EMBL-EBI, Wellcome Trust, NIH)
  - United States Patent and Trade Office
  - Company history (**GSK-** Glaxo Wellcome, Burroughs Wellcome, SmithKline Beecham, SmithKline French, etc.)
  - Filtered by drug likeness criteria (eliminate proteins, reactive intermediates, reagents)
  - 250-500K

1. Mark Gardner, AMG consultants Ltd

Rho

Virtual Screening

- Patent space is a “Best Guess” as to pharma collection content
  - Over estimation (not every patented compound in collection)
  - Under estimation (not every compound in series makes patent)
  - Clearly missing purchased and purpose made compounds
- Virtual screening hits
  - Request to pharma (1-2K compounds)
  - Return to screeners
    - 500-1000 compounds
    - Blinded (activity attached to compound proprietary)
  - Selective disclosure of validated hits
    - Requires bacteriostatic or bactericidal activity due to target inhibition
    - Requires Pharma legal (encumbrance)

Rho

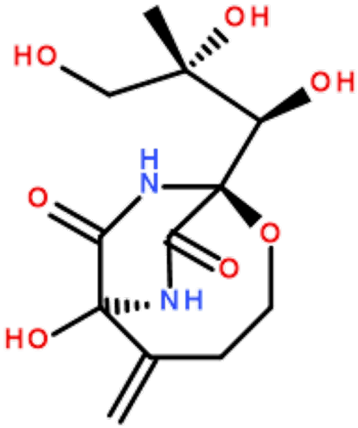
Virtual screen

- Protein Structure
  - Mtb Rho structure unavailable
  - E. Coli ortholog structures available (PDB 1XPO[1], 5JJI[2])
    - ATP active site identified
    - *Allosteric site identified as well*
  - Build homology model
- Ligand Database(s)
  - TAMU, commercial and “patent space”
- Campaigns
  - 15 to date using 4 models and 6 libraries

1. Skordalakes, E.; Brogan, A. P.; Park, B. S.; Kohn, H.; Berger, J. M. Structural Mechanism of Inhibition of the Rho Transcription Termination Factor by the Antibiotic Bicyclomycin. *Structure* **2005**, *13* (1), 99–109.
2. Thomsen, N. D.; Lawson, M. R.; Witkowsky, L. B.; Qu, S.; Berger, J. M. Molecular Mechanisms of Substrate-Controlled Ring Dynamics and Substepping in a Nucleic Acid-Dependent Hexameric Motor. *Proceedings of the National Academy of Sciences* **2016**, *113* (48), E7691–E7700.

Rho

Bicyclomycin

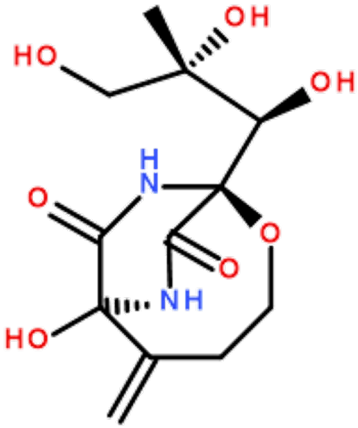


- Isolated in 1972 from *Streptomyces sapporonesis* and *Streptomyces aizumenses*[1-2]
- Broad spectrum antibiotic against Gram-negative and some Gram-positive bacteria
- 2,3-diketopiperazine natural product
- Once sold under the trade name Bicozamycin (Fujisawa) in Japan, it showed some clinical utility as an antidiarrheal in humans, calves and pigs[3]

1. Miyoshi, T.; Miyairi, N.; Aoki, H.; Kohsaka, M.; Sakai H-I.; Imanaka, H. Bicyclomycin, A New Antibiotic I. Taxonomy, Isolation and Characterization. J. Antibiotics 1972, 25(10), 569-575.
2. Miyamura, S.; Ogasawara, N.; Otsuka, H.; Niwayama, S.; Tanaka, H.; Take, T.; Uchiyama, T.; Ochiai, H.; Abe, K.; Koizumi, K.; Asao, K. ;Matsuki, K.; Hoshino, T. Antibiotic No. 5879, a new water-soluble antibiotic against gram-negative bacteria. J. Antibiotics 1972, 25(10), 610–612.
3. Kohn, H.; Widger, W. The molecular basis for the mode of action of bicyclomycin. Current Drug Targets. Infectious Disorders. 2005, 5(3), 273–295

Rho

Bicyclomycin

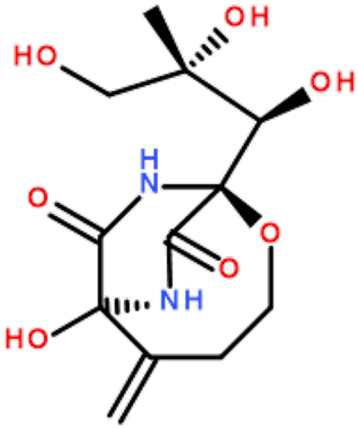


- Inhibitor of bacterial Rho[1-3]
- Crystallography
  - E. Coli Rho structures available (1XPO)[2]
  - Bicyclomycin binding site elucidated
- Not active against Mtb Rho, not active against Mtb *in vitro*
- Why?

1. Zwiefka, A.; Kohn, H.; Widger, W. R. Transcription Termination Factor Rho: The Site of Bicyclomycin Inhibition in Escherichia Coli. *Biochemistry* **1993**, *32* (14), 3564–3570
2. Skordalakes, E.; Brogan, A. P.; Park, B. S.; Kohn, H.; Berger, J. M. Structural Mechanism of Inhibition of the Rho Transcription Termination Factor by the Antibiotic Bicyclomycin. Structure 2005, 13 (1), 99–109.
3. Cardinale, C. J.; Washburn, R. S.; Tadigotla, V. R.; Brown, L. M.; Gottesman, M. E.; Nudler, E. Termination Factor Rho and Its Cofactors NusA and NusG Silence Foreign DNA in E. Coli. Science 2008, 320 (5878), 935–938.

Rho

Bicyclomycin



Why doesn't Bicyclomycin work against Mtb?

- E. Coli and Mtb sequence homology 56%
- Homology in bicyclomycin binding site even higher
  - Key difference: Leucine 320 in E. Coli is Methionine in Mtb
  - Leucine to Methionine considered a conservative change

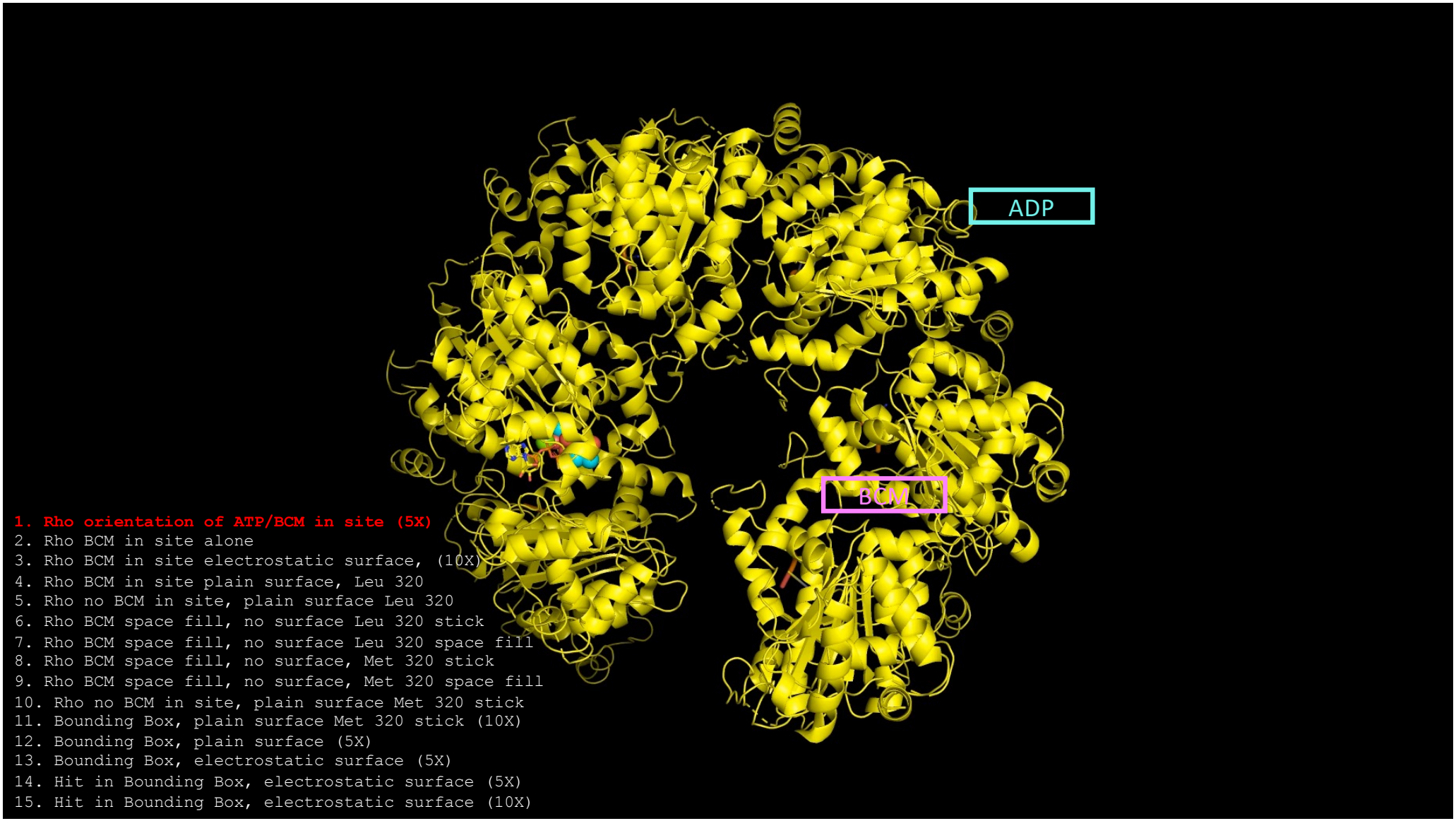
Theory- Bicyclomycin fit is so perfect, small change enough to disrupt

- Modify bicyclomycin?
- Find something like bicyclomycin

Rho

Bicyclomycin

- Modify bicyclomycin?
  - Identify part(s) that don't fit- easy
  - Identify analogs that might fit- easy to identify, hard to obtain
  - Synthesize analogs that might fit- very hard
- Find something like bicyclomycin?
  - Virtually screen- easy
  - Design *de novo*- very hard


ADP
BCM
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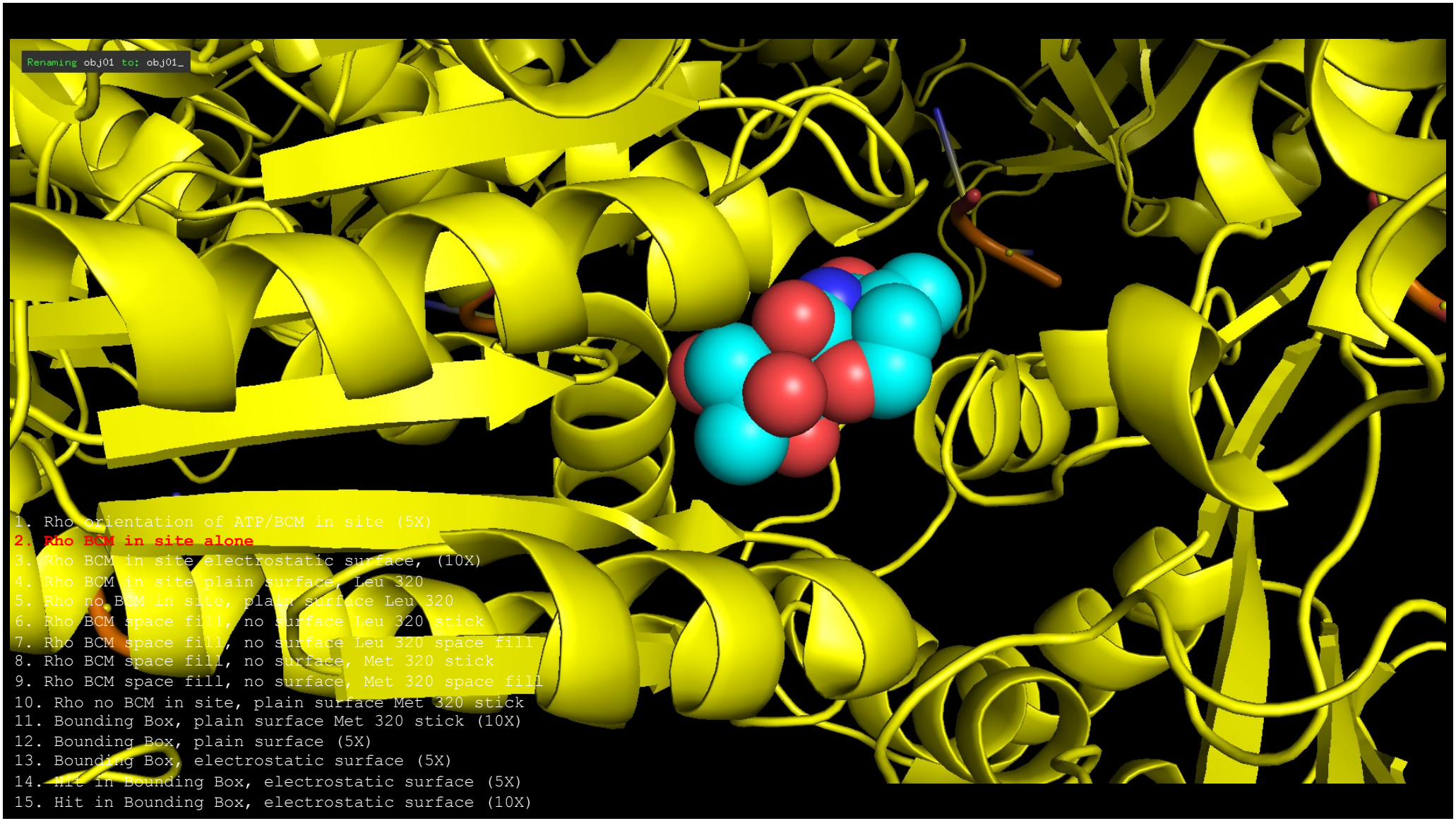
2. Rho BCM in site alone
3. Rho BCM in site electrostatic surface, (10X)
4. Rho BCM in site plain surface, Leu 320
5. Rho no BCM in site, plain surface Leu 320
6. Rho BCM space fill, no surface Leu 320 stick
7. Rho BCM space fill, no surface Leu 320 space fill
8. Rho BCM space fill, no surface, Met 320 stick
9. Rho BCM space fill, no surface, Met 320 space fill
10. Rho no BCM in site, plain surface Met 320 stick
11. Bounding Box, plain surface Met 320 stick (10X)
12. Bounding Box, plain surface (5X)
13. Bounding Box, electrostatic surface (5X)
14. Hit in Bounding Box, electrostatic surface (5X)
15. Hit in Bounding Box, electrostatic surface (10X)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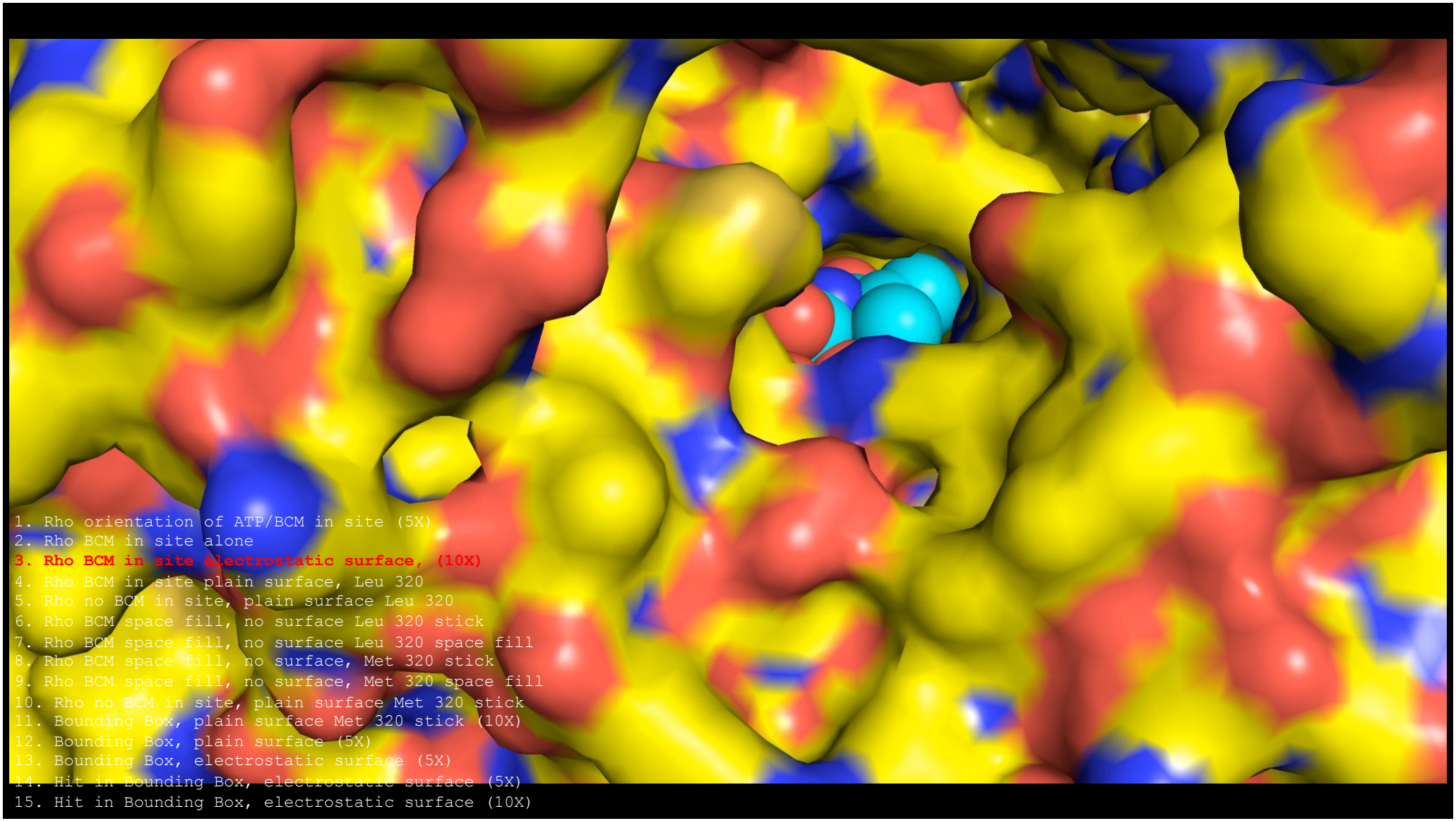
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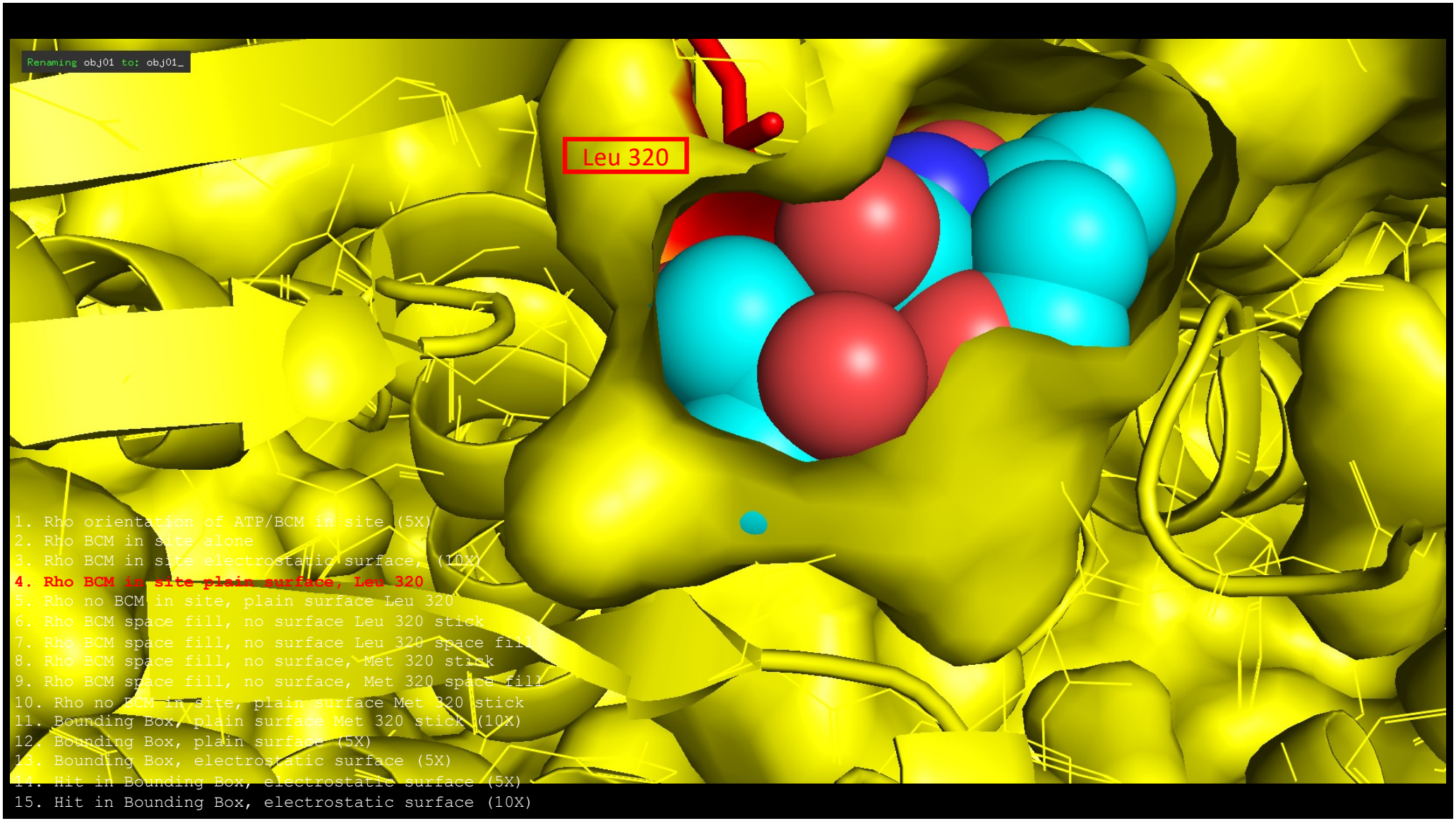
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Leu 320
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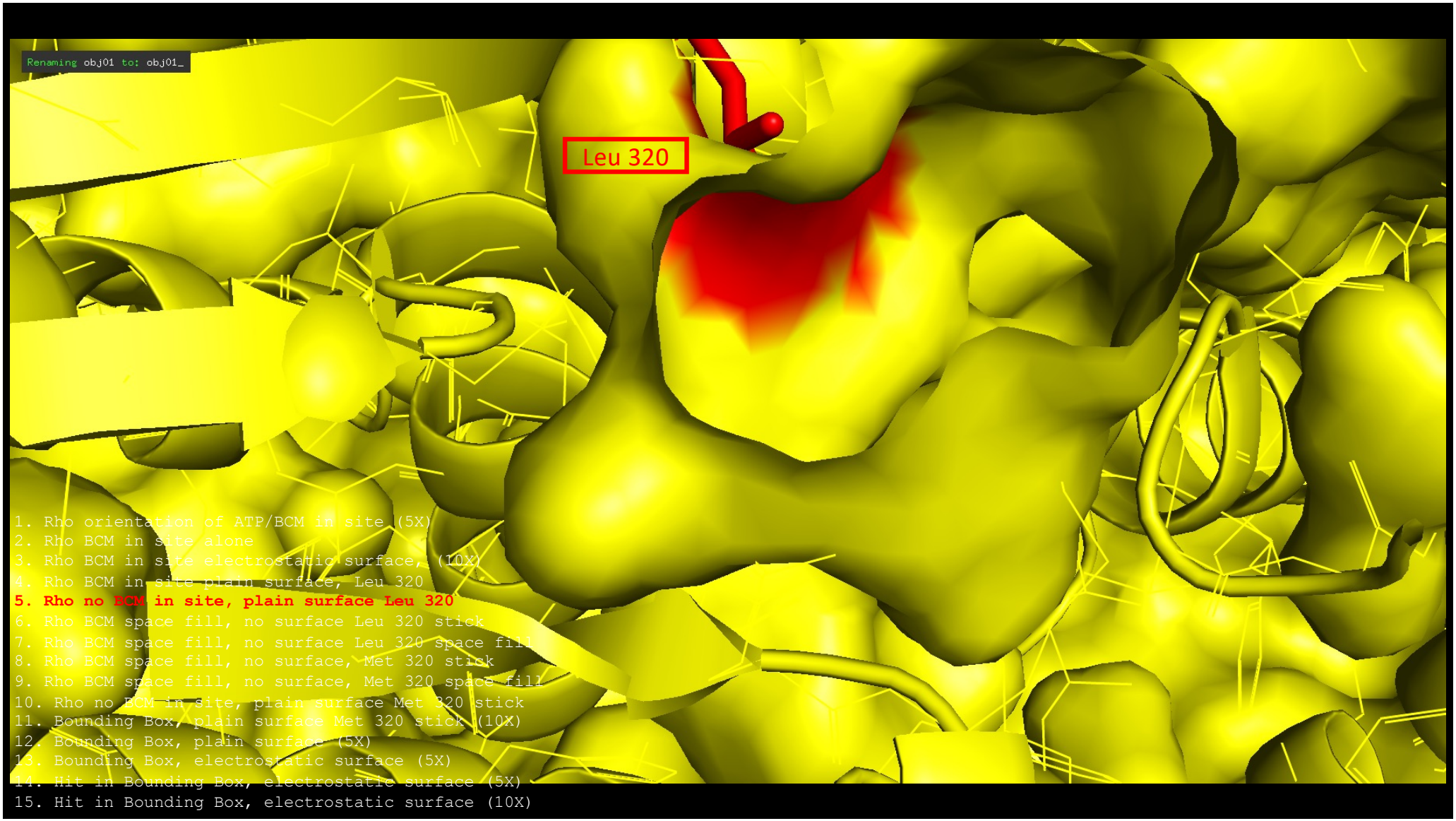
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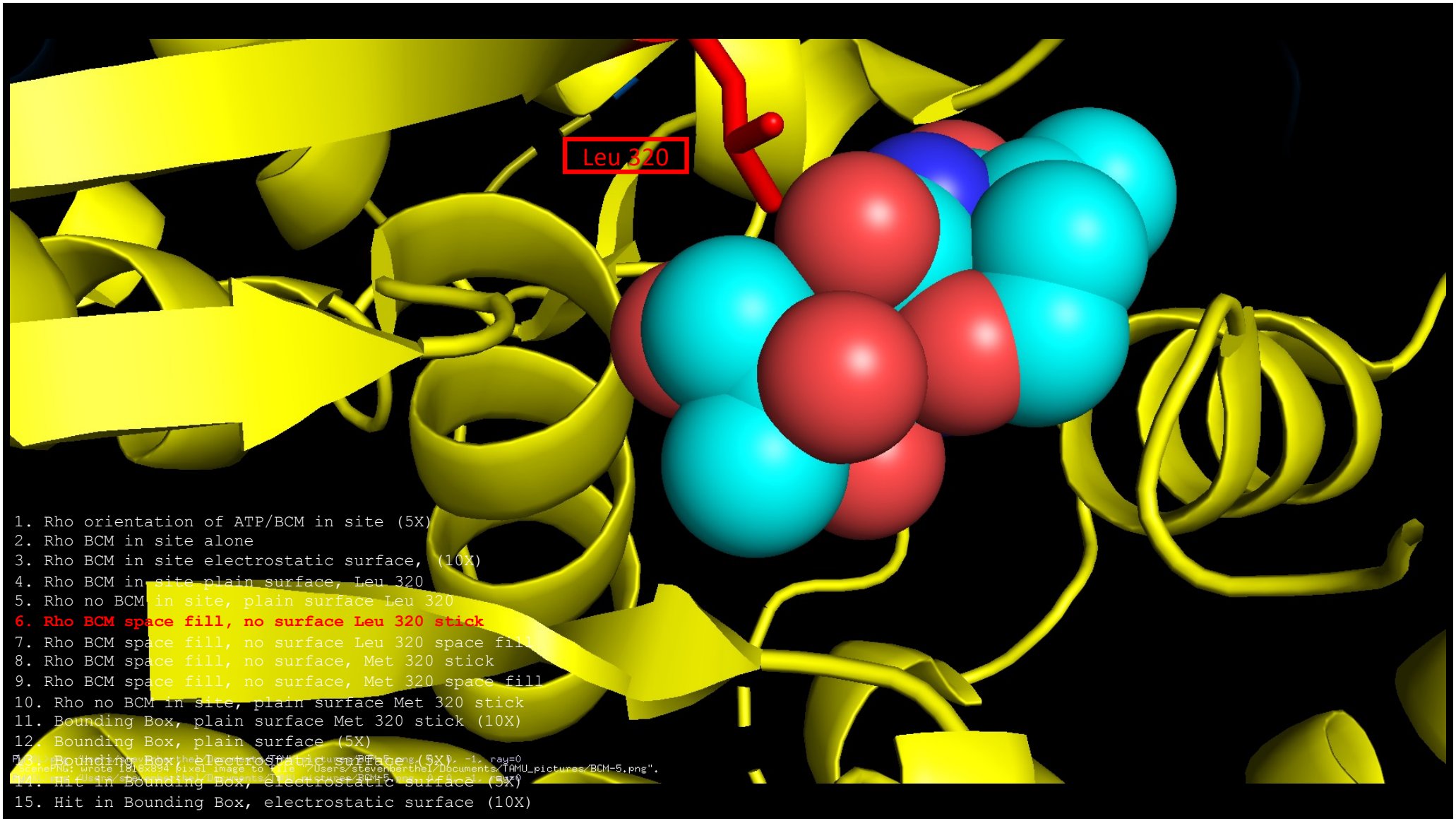
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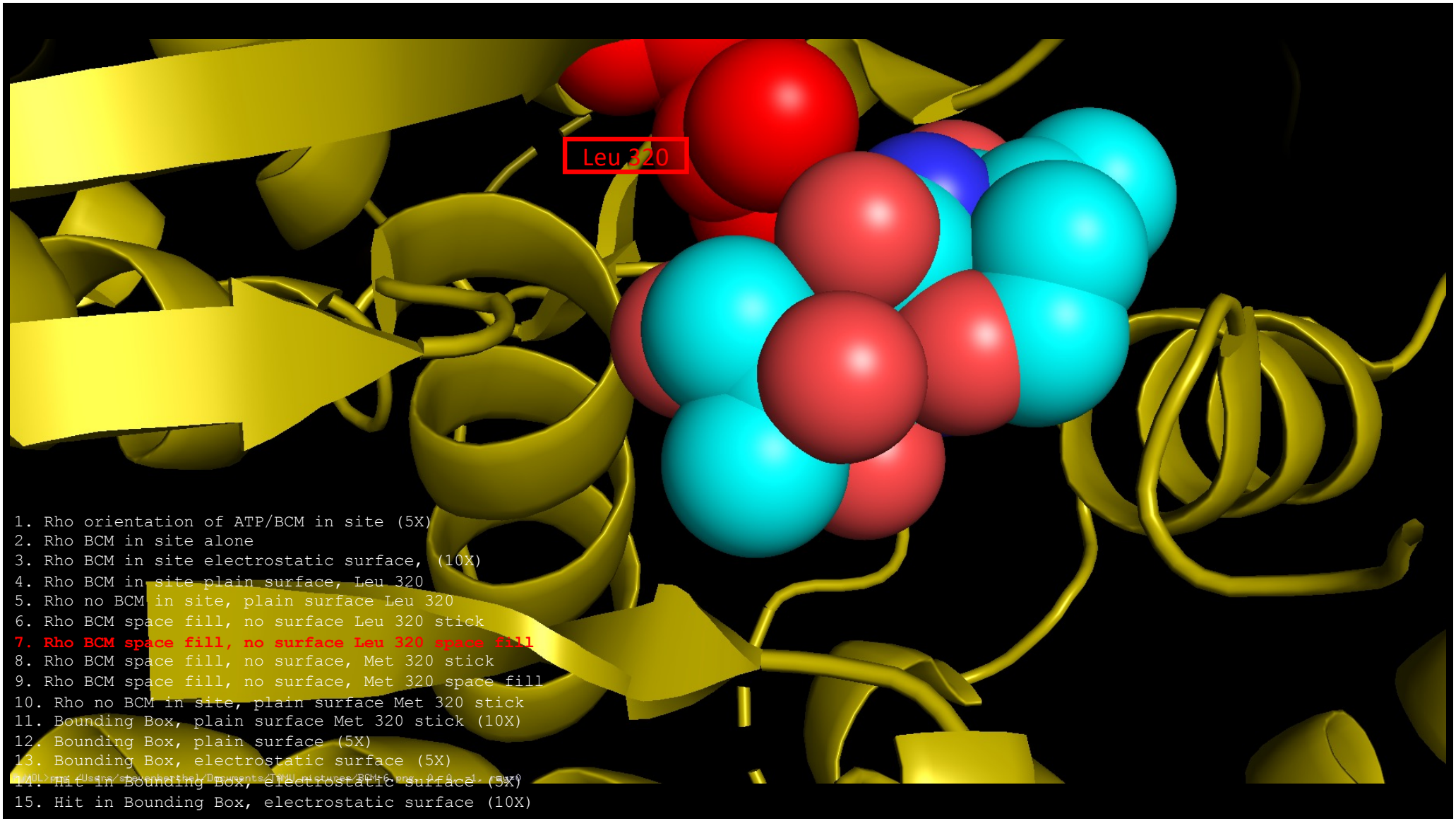
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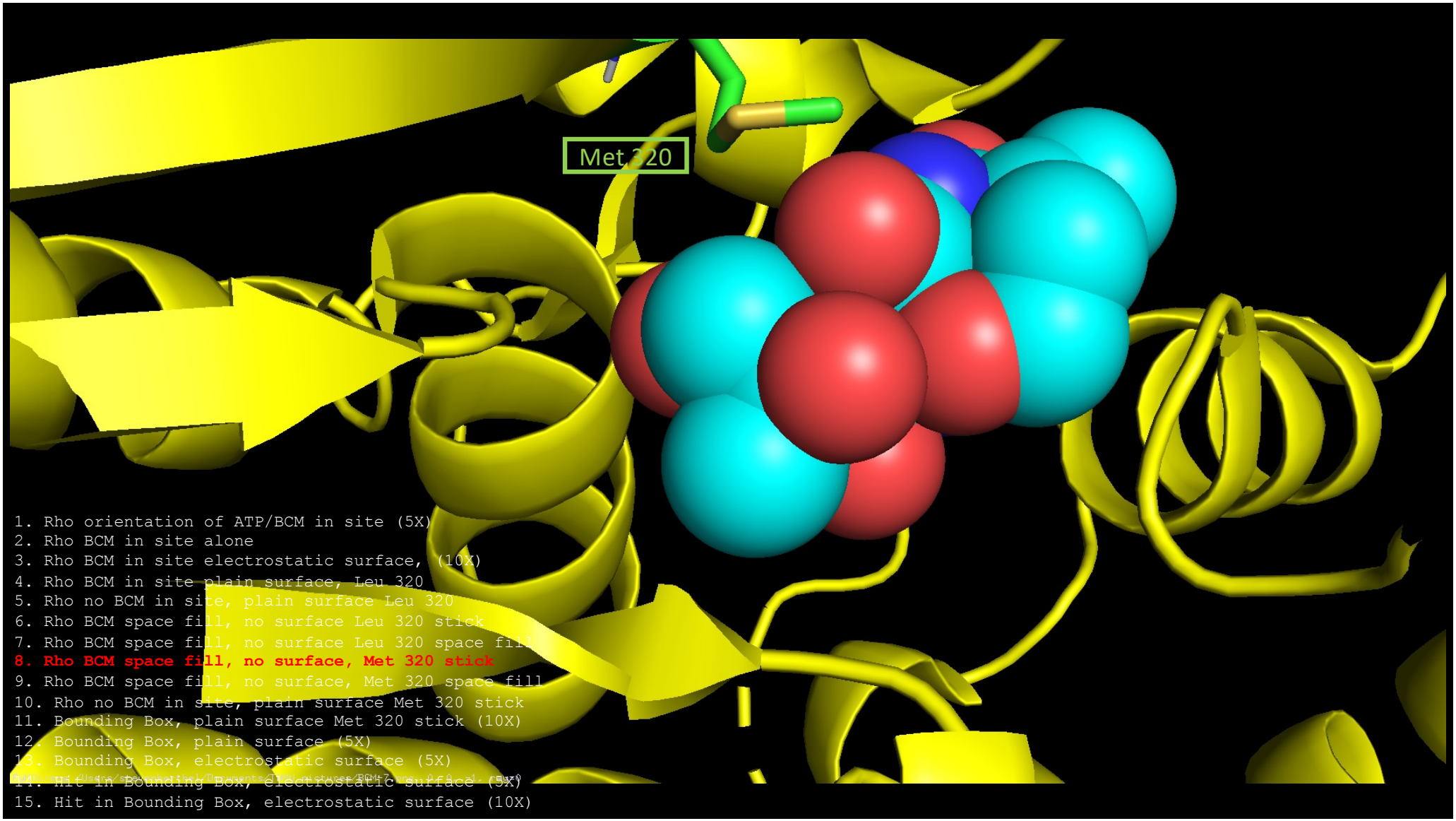
Met 320
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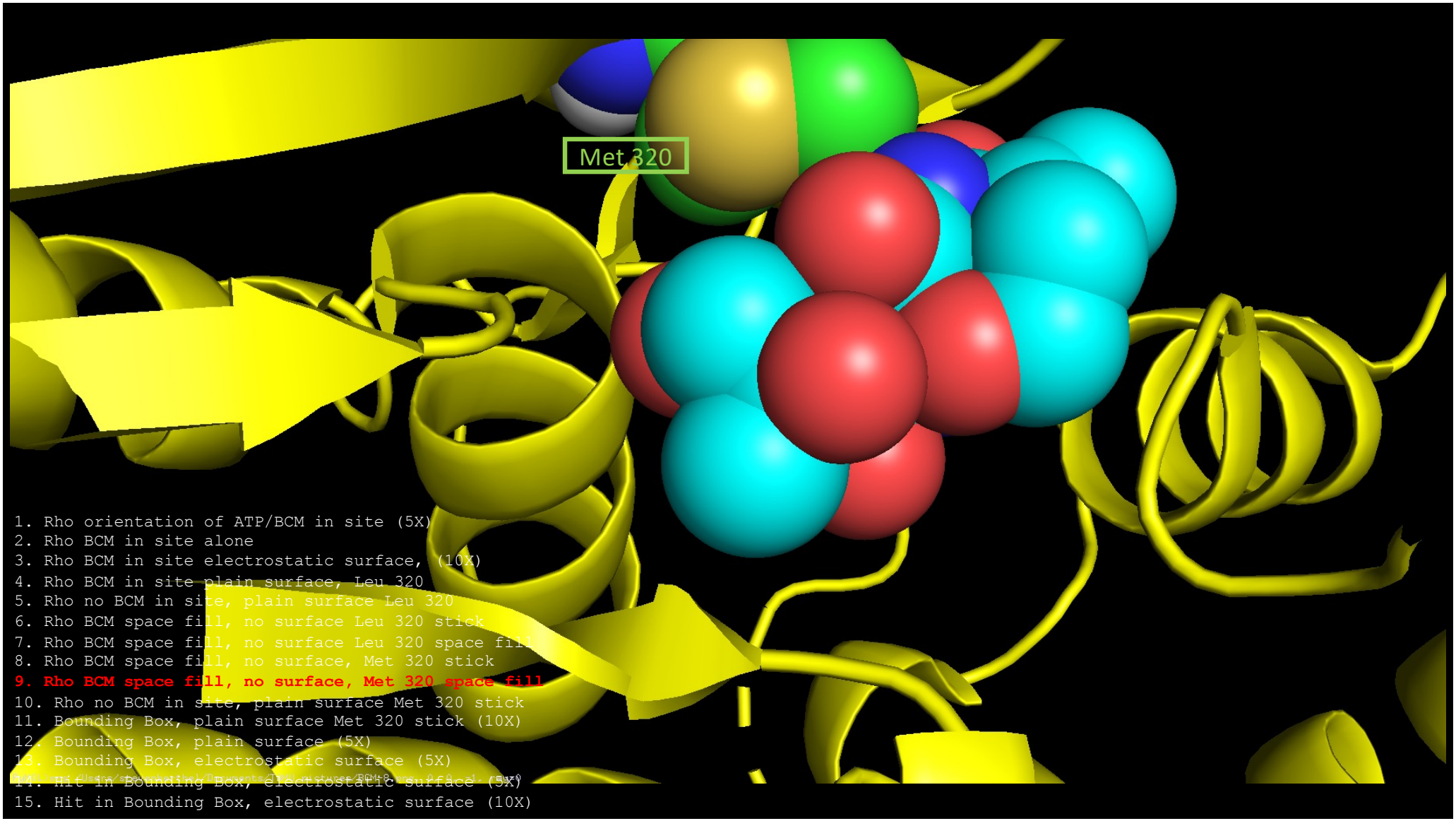
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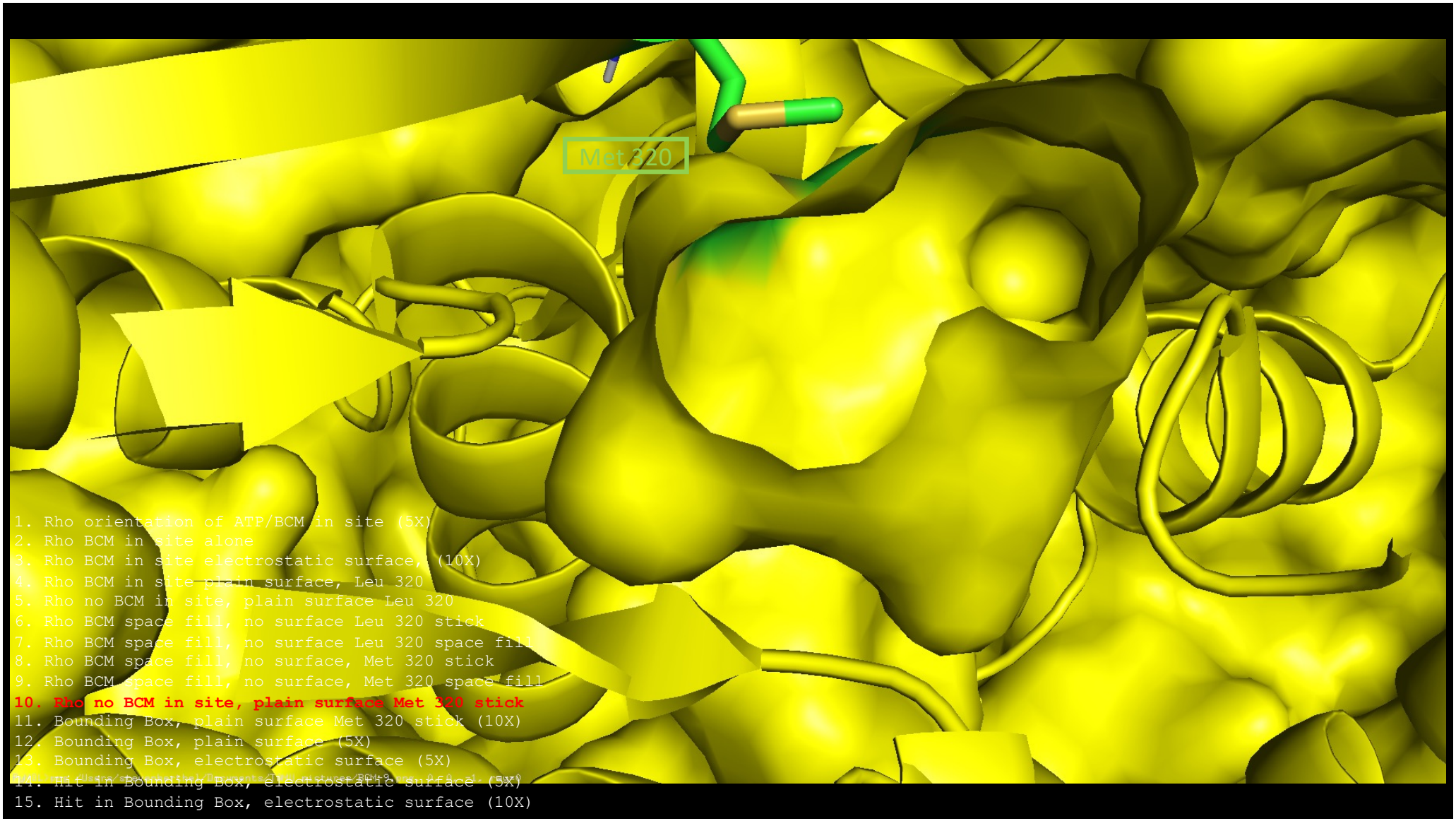
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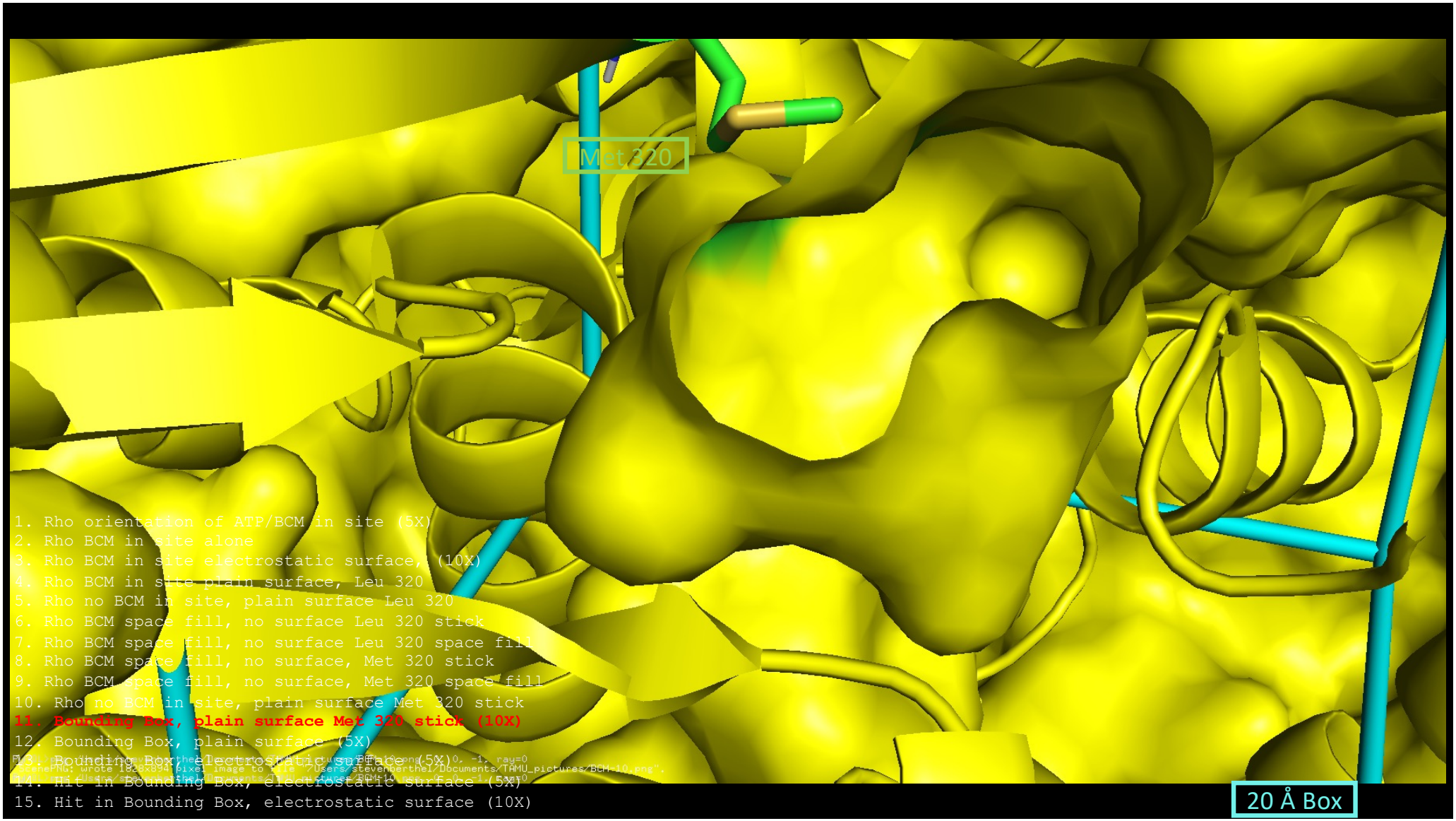
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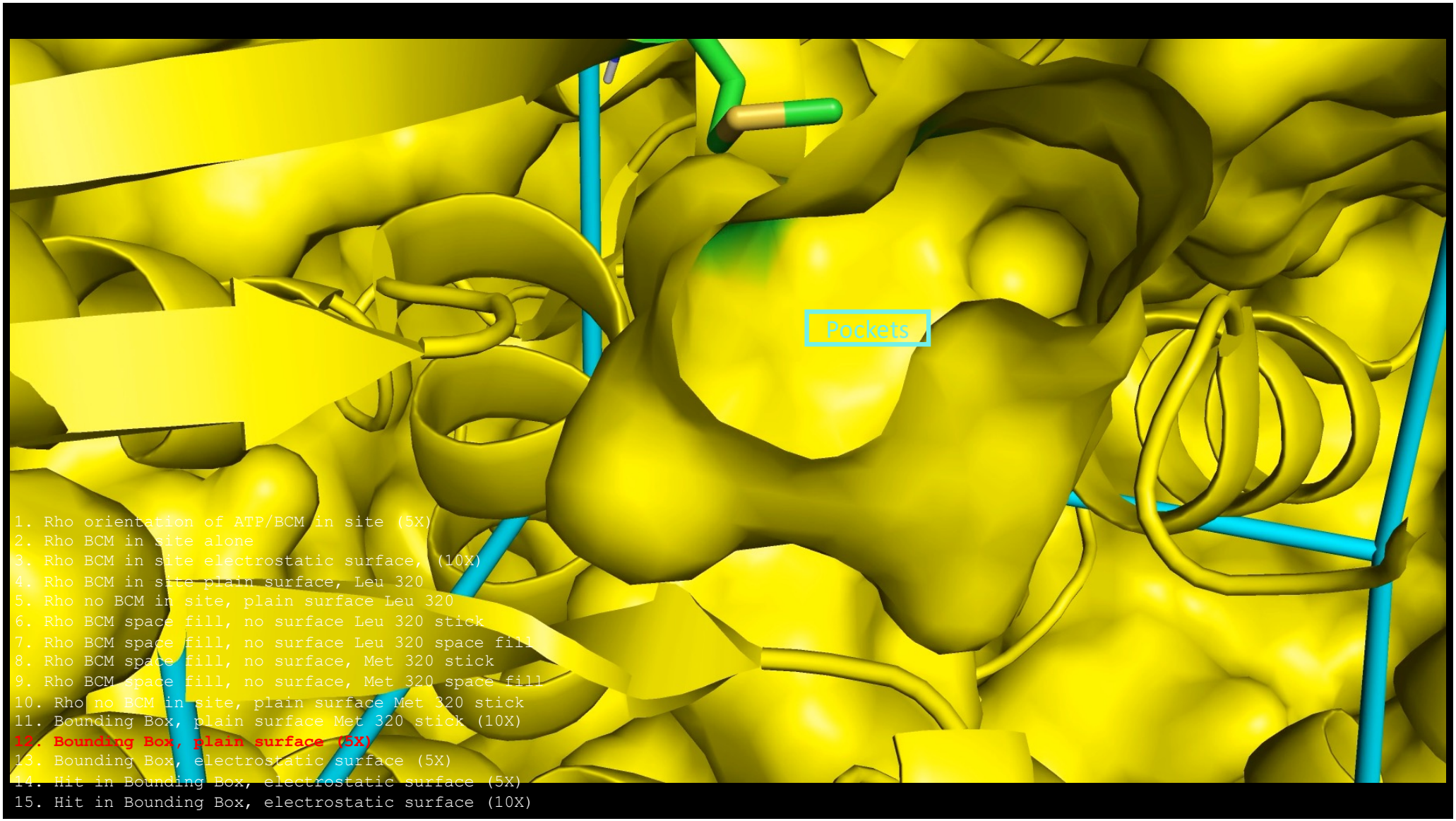
20 Å Box


Pockets
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5. Rho no BCM in site, plain surface Leu 320
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13. Bounding Box, electrostatic surface (5X)
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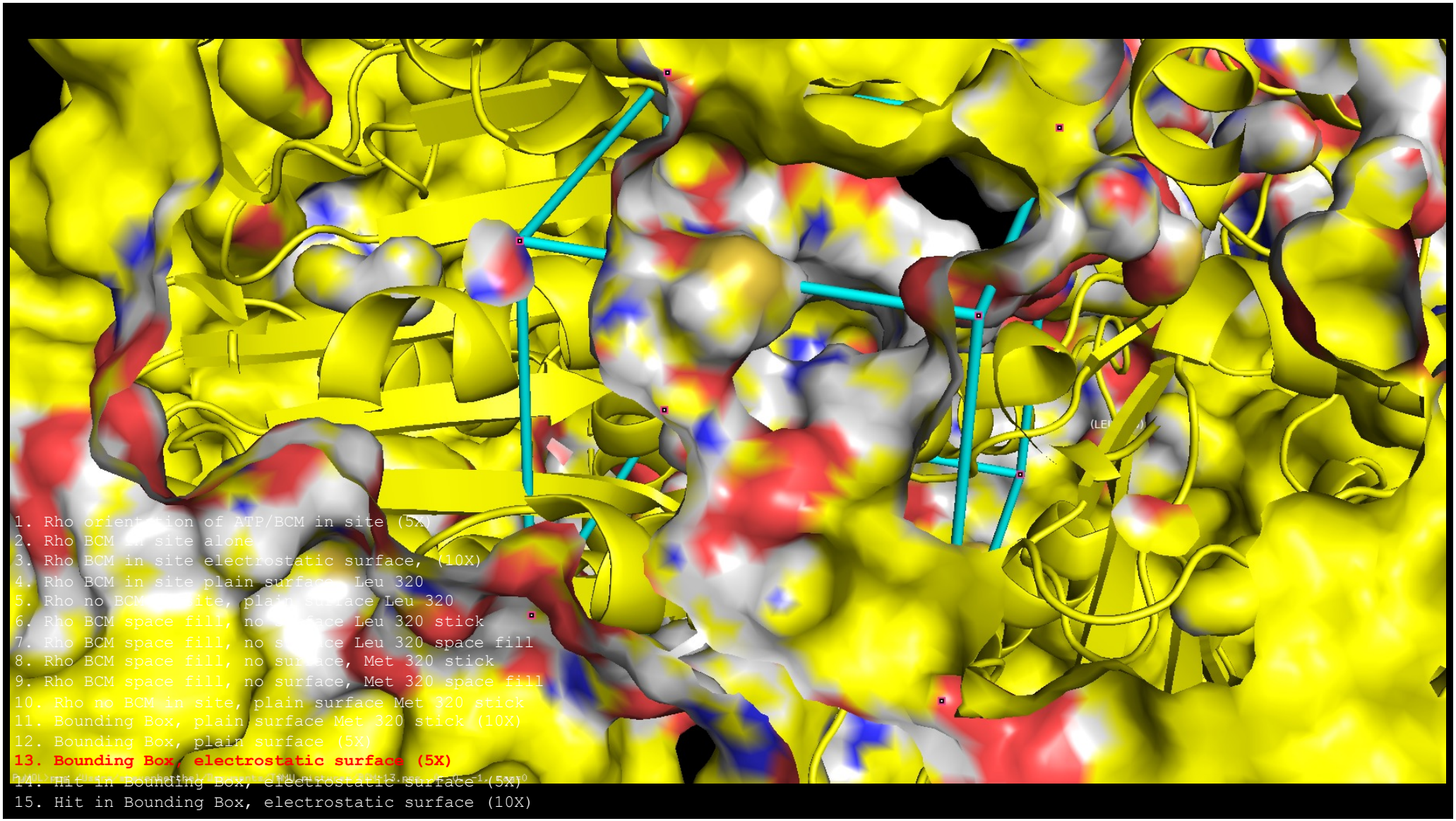
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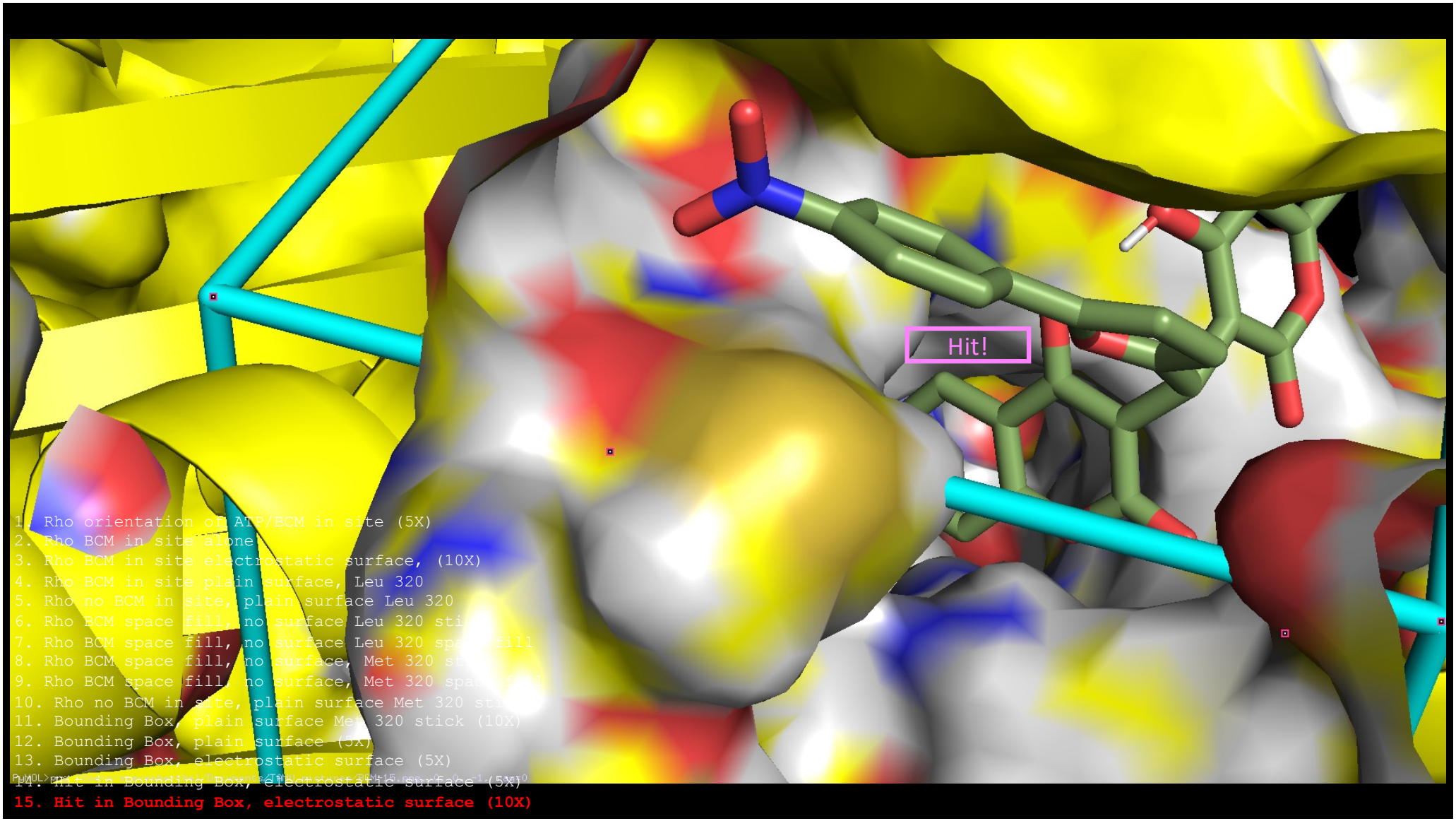
Hit!
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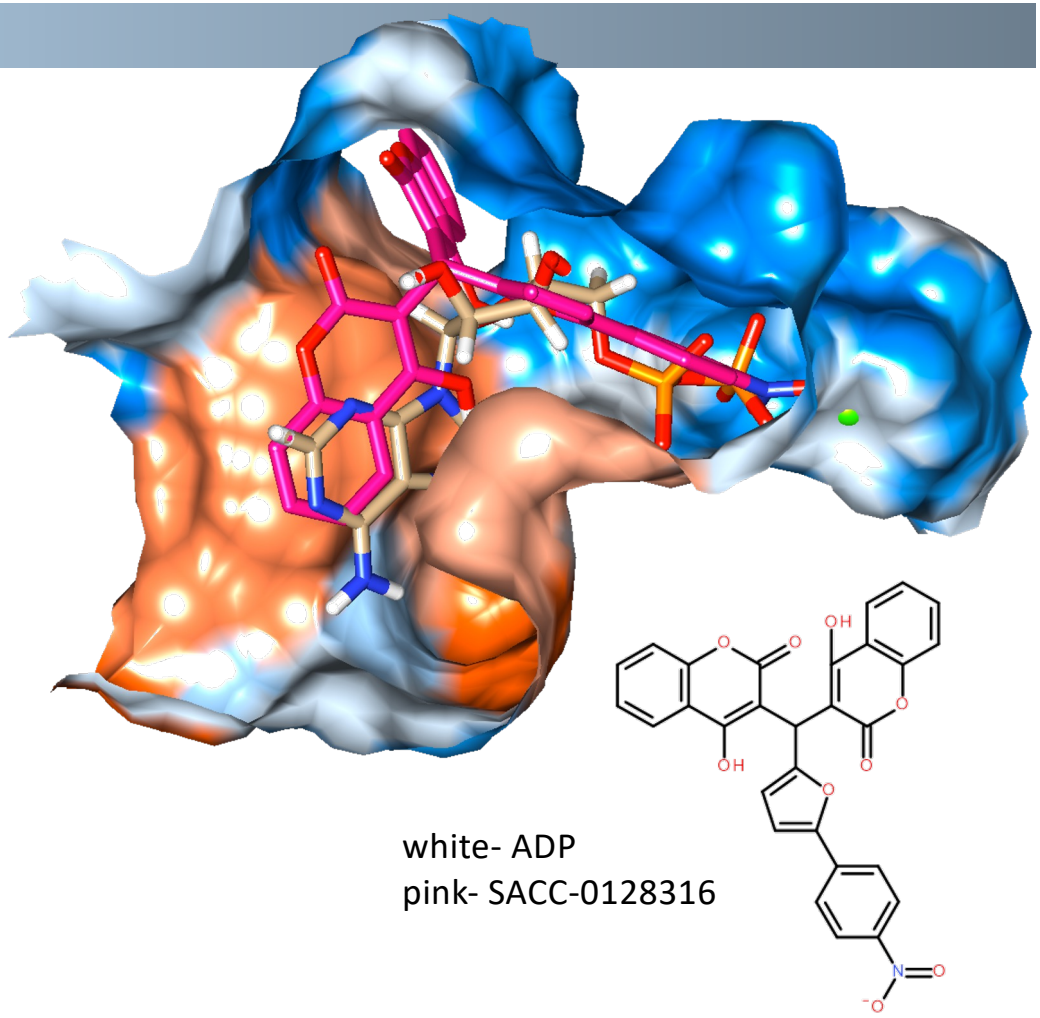
8. Rho BCM space fill, no surface, Met 320 st
9. Rho BCM space fill, no surface, Met 320 spa fill
10. Rho no BCM in site, plain surface Met 320 st
11. Bounding Box, plain surface Met 320 stick (10X)
12. Bounding Box, plain surface (5X)
13. Bounding Box, electrostatic surface (5X)
14. Hit in Bounding Box, electrostatic surface (5X)
15. Hit in Bounding Box, electrostatic surface (10X)

Rho

SACC-0128316

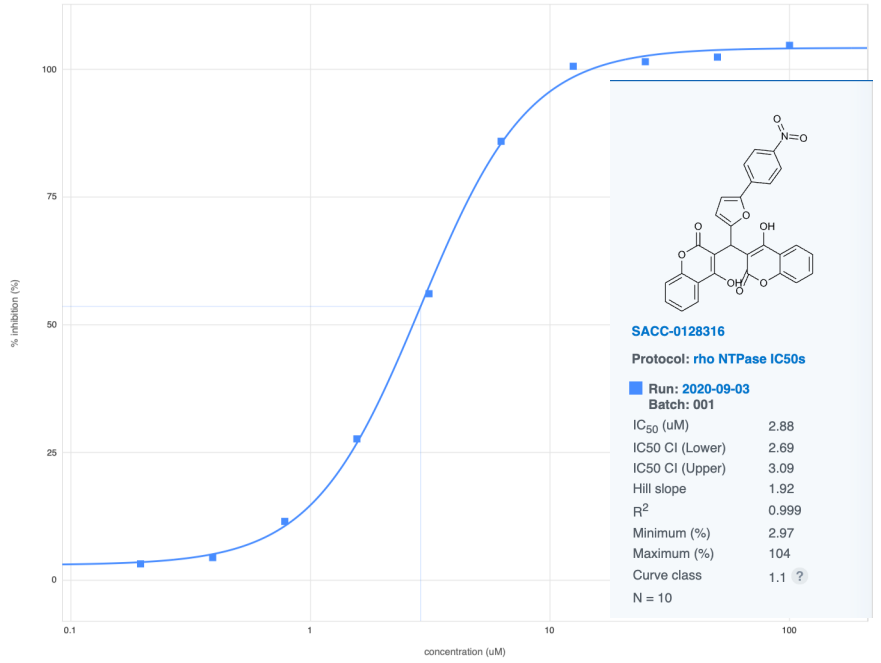
- Top 10 hit
- GSK (Zagreb) patent WO2006111858
  - Prophylaxis and treatment of asthma and other inflammatory diseases
  - ~1000 analogs exemplified
- 1 step, 1 pot synthesis



Rho

SACC-0128316

- Inhibits Rho *in vitro*
  - Rho NTPase assay IC_{50} 1.7-2.88 µM[1-2]
  - Rho Helicase assay 78% rate reduction (w and w/o ATP)[3]
- Active against Mtb
  - WCA (Luciferase) EC_{50} 15.2 µM[3]
  - WCA (Resuazurin) EC_{50} 18.6 µM[3]
- Suitable for Hit Assessment



1. Qingan Sun, Texas A&M Sacchettini lab
2. Aashish Srivastava, Texas A&M Sacchettini lab
3. Kuo-Sen Hwang, CepterBio
4. Katherine Bonilla , Texas A&M Sacchettini lab

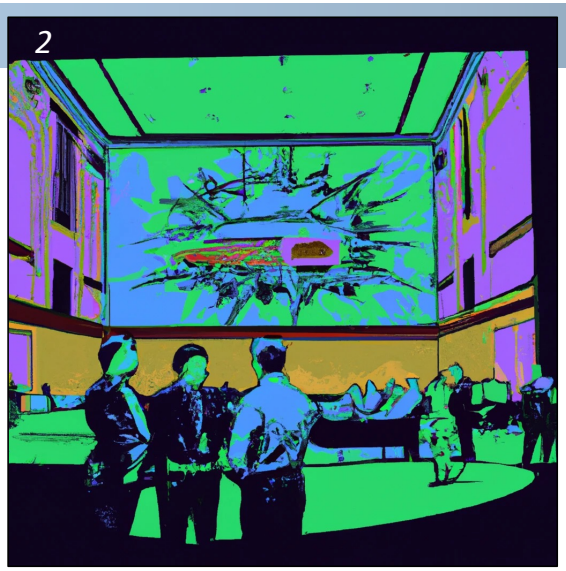
Rho

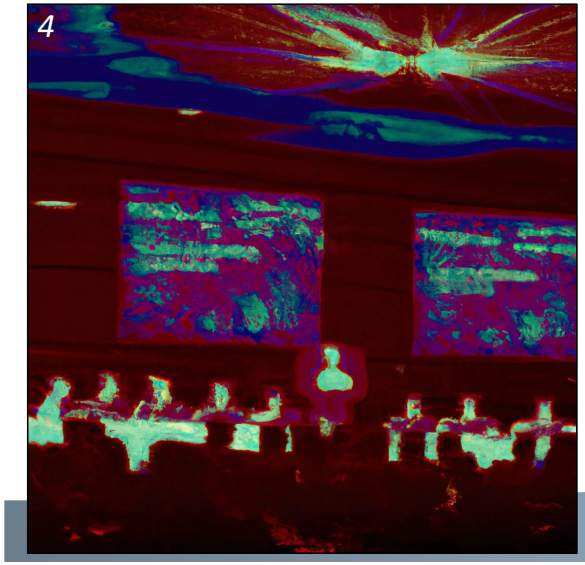
SACC-0128316

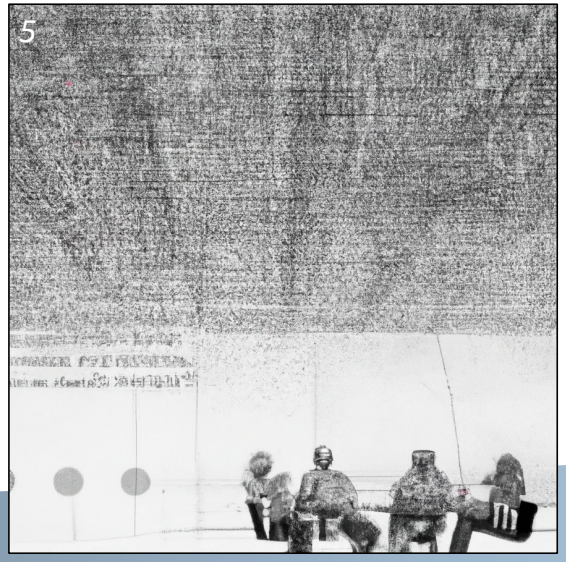
- ~400 analogs prepared (A-E)
- Structure Activity Relationship (SAR)
  - <2 orders of magnitude
  - none < 1 µM
- Series assessment
  - Pros:
    - Activity against enzyme and cells
    - Calorimetry shift (binding)
  - Cons:
    - Flat SAR
    - No structure to date
    - Detergent effect (aggregator?)
- Series currently on hold
- New VS campaigns with large libraries planned

Acknowledgements

- **Alexander Alex** (AMG, formerly Pfizer)
- **Greg Durst** (Lgenia, formerly Lilly)
- **Mark Gardner** (AMG, formerly Pfizer)
- **Tom Ioerger** (Texas A&M)
- **Inna Krieger** (Texas A&M)
- **Jiankun Lyu** (Rockefeller University)
- **Lisa Perez** (Texas A&M)
- **Jim Sacchettini** (Texas A&M)
- **Dirk Schnappinger** (Weill Cornell Medicine)
- ***Yvonne Martin*** *(AbbVie, retired)*
- ***Caroline Low*** *(AMG, currently DeepMind)*
- **Elliott Nickbarg** (Merck)
- **Rob Bates** (GSK)
- **Mike Schrimpf** (AbbVie)
- **Cory Reidl** (AbbVie)
- **Eric Rubin** (Harvard)
- **Phil Hipskind** (Lgenia, formerly Lilly)
- **Ying Yuan** (Global Health Drug Discovery Institute)
- Bill and Melinda Gates Foundation
- Panorama Global
- TBDA Member organizations and scientists
- Texas A&M







ChatGPT and DALL-E*

Prompts: Famous Texas painters, Conference, Supercomputing, Texas

in the style of:
(1) Everett Spruce
(2) Porfirio Salinas
(3) Jose Arpa
(4) Ben Culwell
(5) Dawson Dawson-Watson

*OpenAI

Tuberculosis Drug Accelerator