Grand Challenges
African Drug Discovery Accelerator

Drug Discovery and Development Course

in silico approaches to drug design

Joe Eyermann

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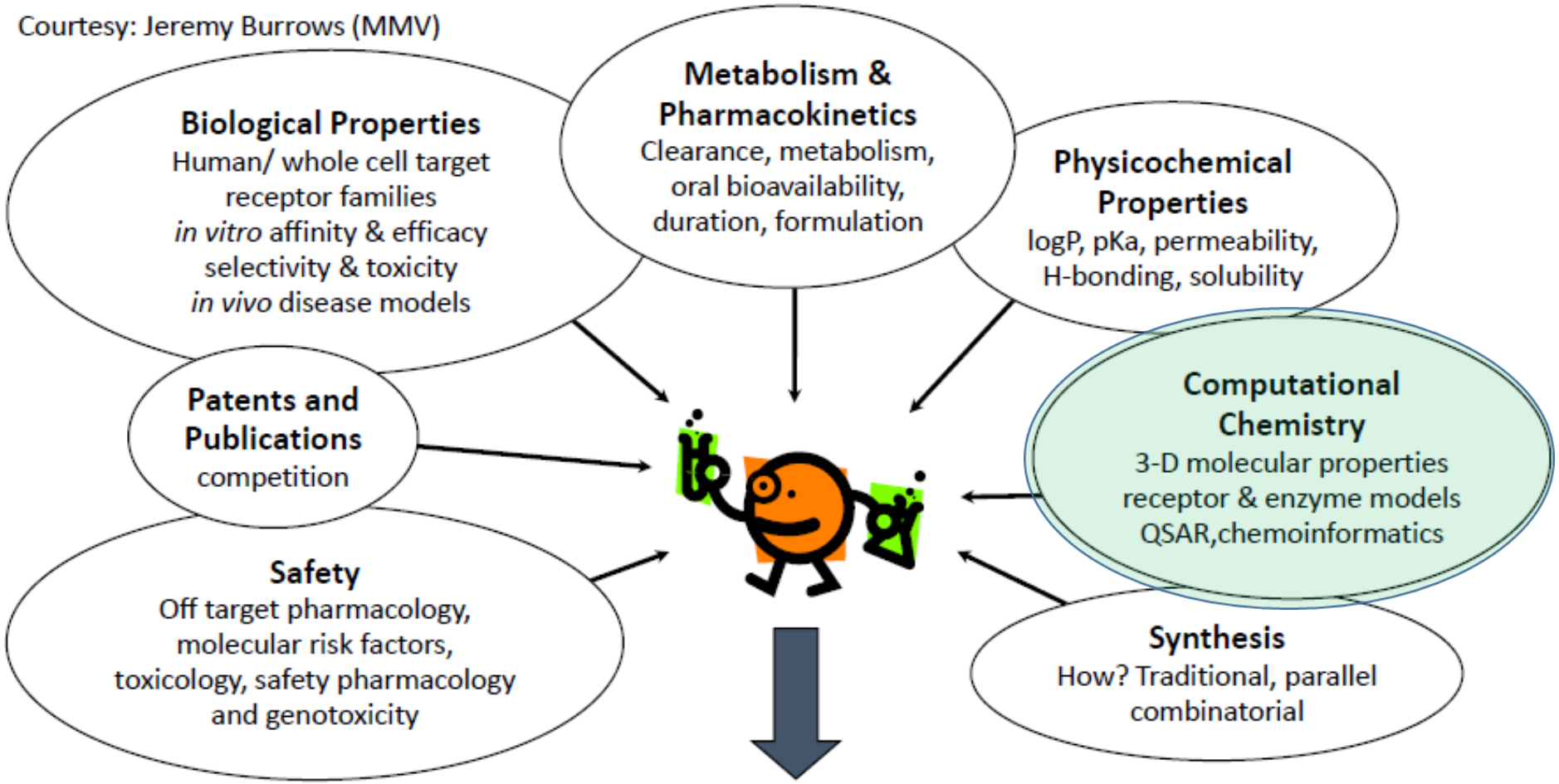
Learning Objectives

- **Overview of *in silico (CADD)* resources**
- **Integrating *in silico* methods with drug discovery data**
- **Building strong relationships with drug discovery teams**

Drug Discovery Process



Being able to use all of the above information and knowledge to design a molecule that overcomes any hurdles to become a drug

Computer-Aided Drug Design Tools

Small Molecule Tools

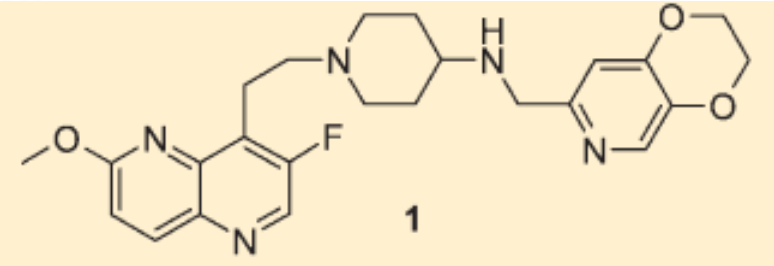
- 2D structure databases (reagents, screening compounds)
- Physical property predictions (lipophilicity, solubility, pKa, etc)
- Cambridge Crystal Structure Database
- 2D to 3D structure conversion
- Conformation analysis
- Pharmacophore model generation
- Pharmacophore searching
- Electronic structure properties (quantum mechanics)
- Metabolism and toxicity prediction
- ……

Protein Tools

- Protein Data Bank crystal structures
- Genome databases
- Homology modelling
- Protein-ligand docking and scoring
- Active-site ‘hotspot’ analysis
- Molecular dynamics
- …..

As much or more about thinking than calculations!!!

Ligand-Based Scaffold Hopping



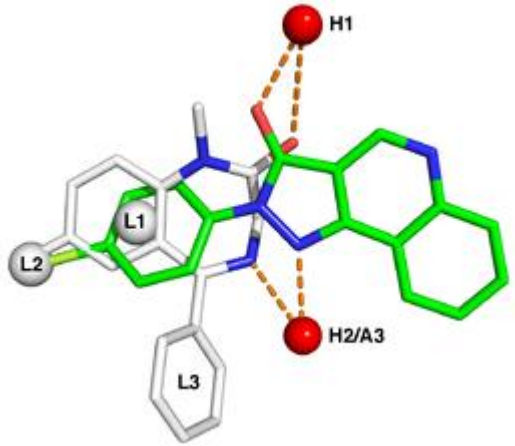
- **Phenotypic hit from whole cell antibacterial screen**
- **Topoisomerase inhibitors in patents**
- **Attractive series with novel mechanism**
  - Potent broad spectrum antibacterial activity
  - Excellent drug-like properties
  - No cross resistance with clinical agents (e.g. fluoroquinolones)
- **Challenges**
  - Significant cardiovascular risk
    - Original lead from sodium channel blocker program (GSK)
- **No protein-ligand x-ray structure available**

Pharmacophore Models

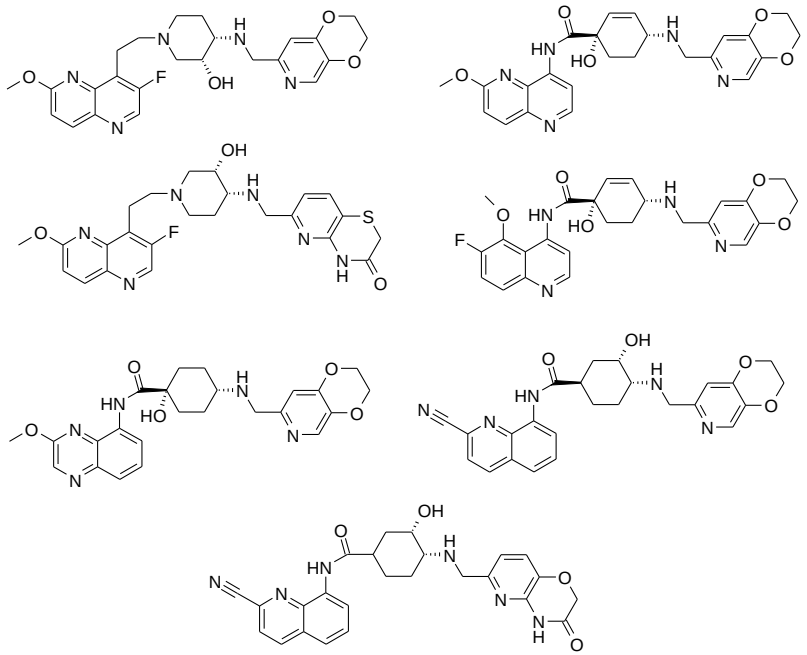
An ensemble of steric and electronic features necessary to ensure the optimal interactions with a specific biological target and to trigger (or block) its biological response *(and the 3D relationship between those features)*

Pharmacophore features can include:

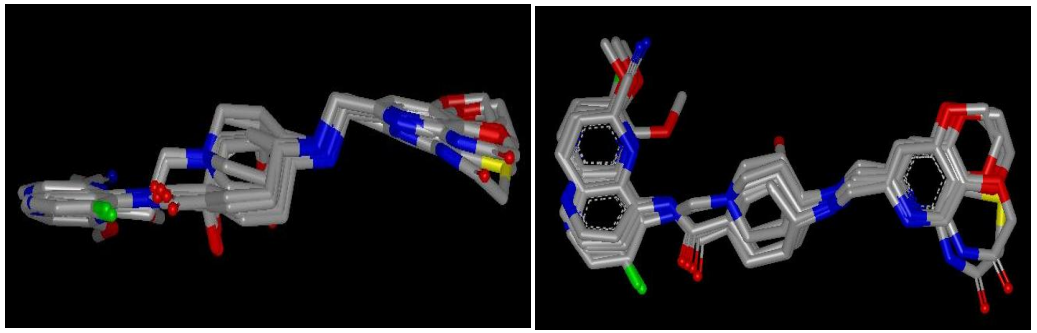
- Hydrogen-bond donors and acceptors
- Aromatic rings
- Positive or negative charges
- Hydrophobic groups



Pharmacophore Model Based on Patents



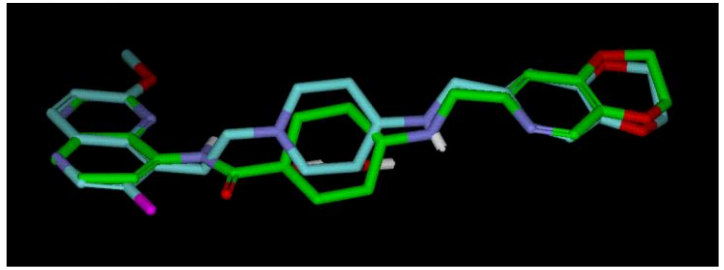
- Select conformationally constrained analogs
- Overlap ring centers and H-bond donors/acceptors using QXP software
- Use MM and QM to confirm low-energy conformers



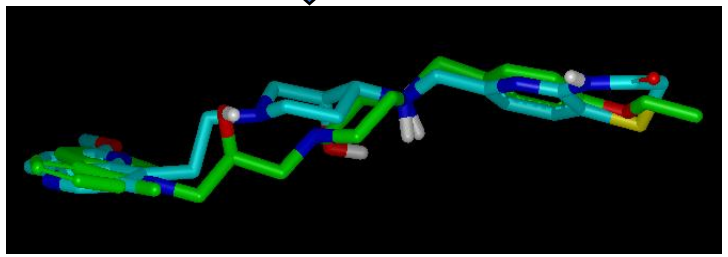
Side View | Top View

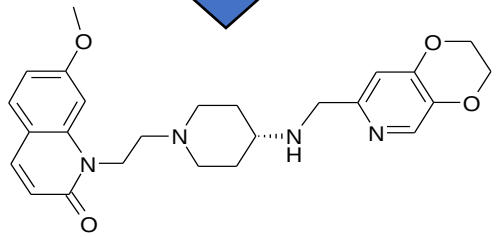
QXP: McMartin and Bohacek, *J. Comput. Aided Mol . Des.*, **1997,** *4*, 333.

3D Search Generates Novel Target Idea

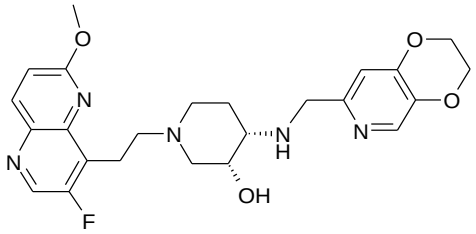


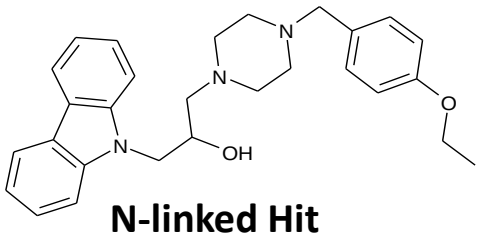
3D Pharmacophore Search of
AstraZeneca Compound Collection





LHS Linker RHS



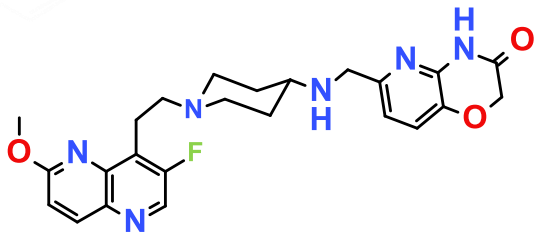


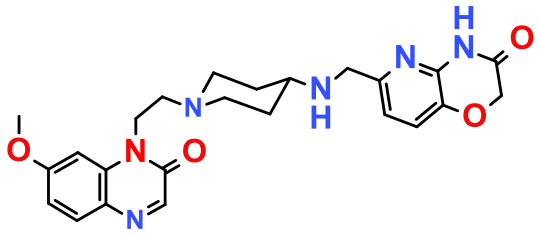
N-linked Hit
No MIC or IC50
N-linked not covered in patents

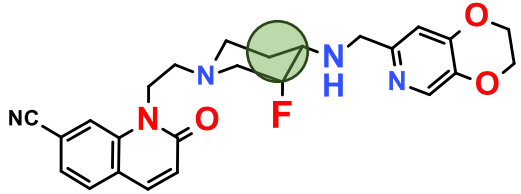
Extensive medicinal chemistry efforts explored variations in the LHS, linker and RHS groups – guided by the pharmacophore model

Reck, et. al., *J. Med. Chem.* **2011**, *54*, 7834

AstraZeneca's Topoisomerase II Inhibitors





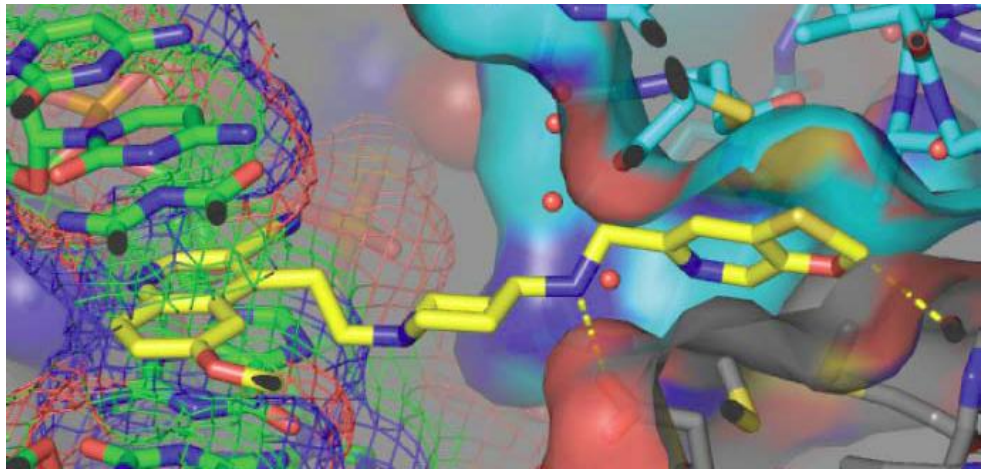


GSK, C-linked	AZD5206, N-linked	AZD9742
MIC Sau 516 = 0.03 uM	0.06 uM	0.14 uM
hERG = 4.4 uM	**35 uM**	**233 uM**
fu (human) = 6 %	**26%**	**25%**
Log D = 1.8	**0.6**	**0.96**
pKa	**8.5**	**7.03**

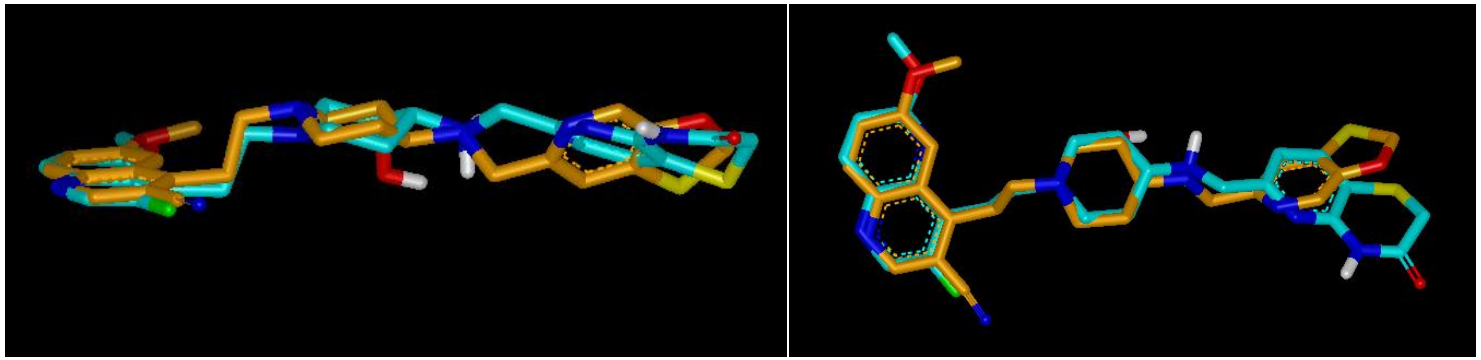
- **pKa reduction (>1 log unit)**
- **hERG improvement over GSK (>50-fold)**
- **Protein binding significantly improved**

Reck, et. al., *J. Med. Chem.* **2012**, *55*, 6916

Pharmacophore Model *vs* X-ray Structure

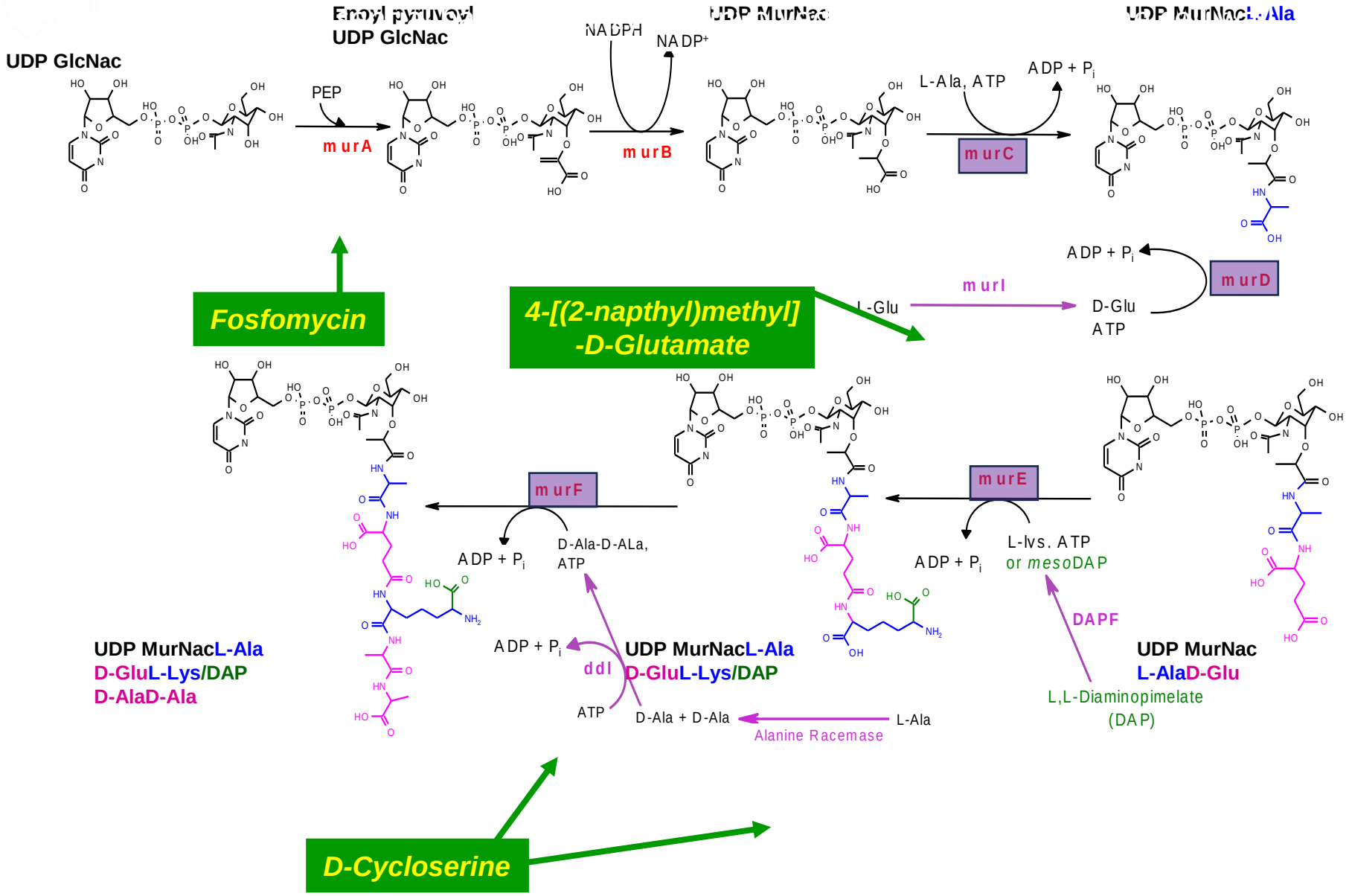


Bax, et. al*, Nature* **2010**, *466,* 935-940.

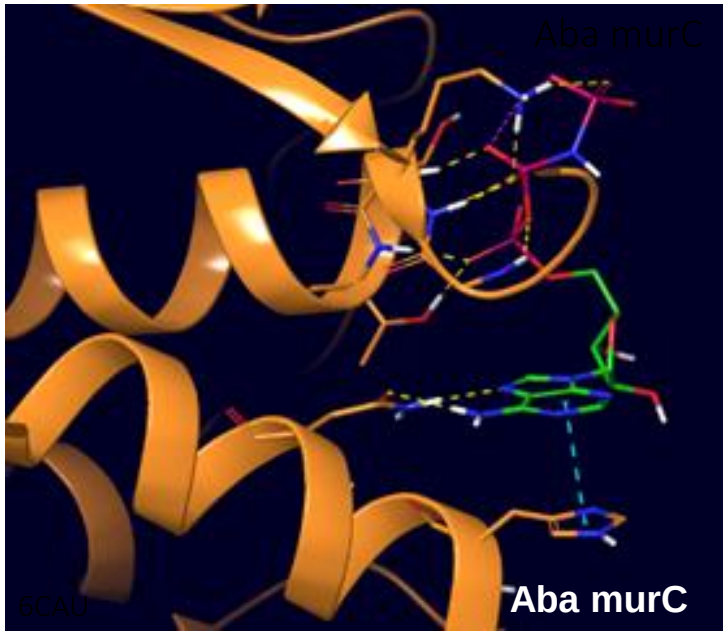


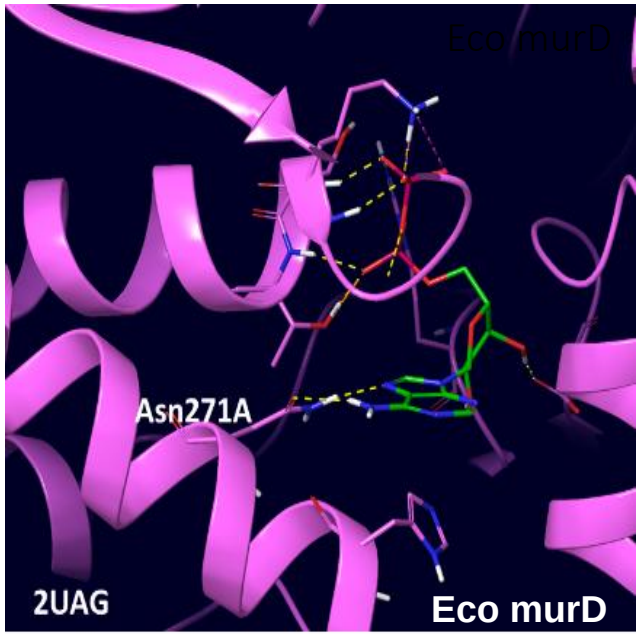
Overlay of a NBTI inhibitor from pharmacophore modeling (cyan carbons) versus NBTI inhibitor conformation (gold carbons) observed bound to GyrA from the reported crystal structure

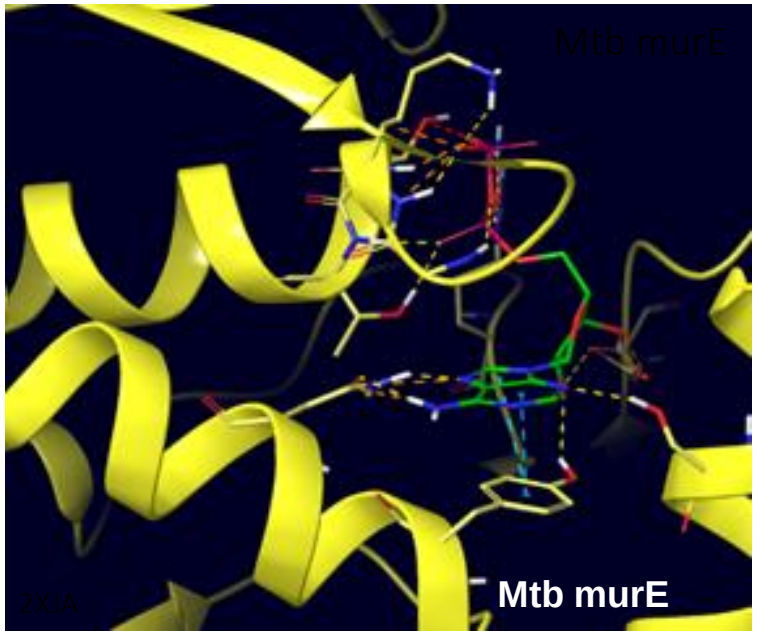
Mur Ligases as Antibacterial Targets

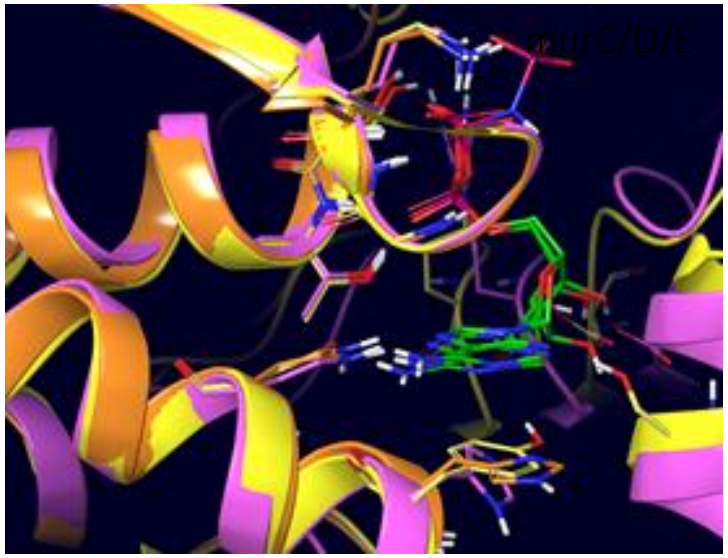


Mur Ligase ATP Binding Sites are Well Conserved









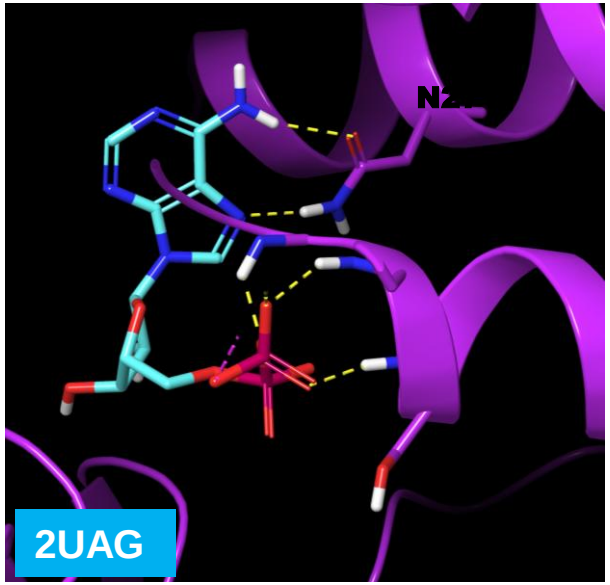
%Idn/%Sim	**Eco murC**	**Pae murC**	**Aba murC**	**Eco murD**	Eco murE	Mtb murE
Eco murC	*					
Pae murC	100/100	*				
Aba murC	100/100	100/100	*			
Eco murD	86/100	83/100	83/100	*		
Eco murE	44/56	67/67	86/100	83/100	*	
Mtb murE	70/80	71/86	75/88	67/89	70/90	*

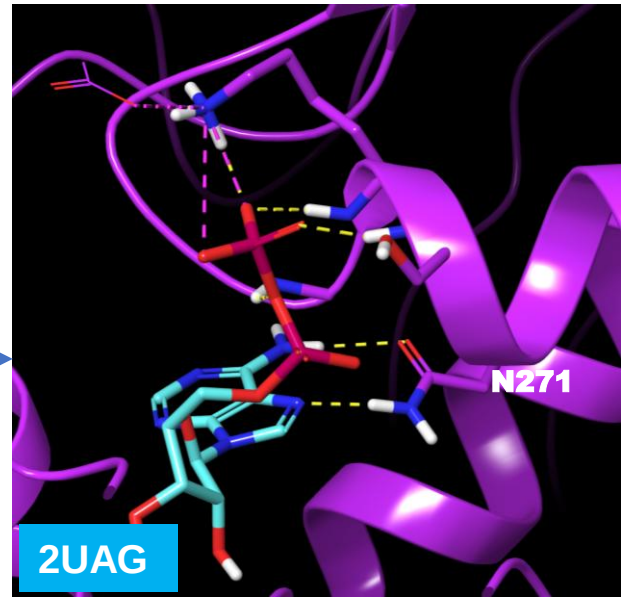
Percent identity and similarity of mur ligase ADP binding sites

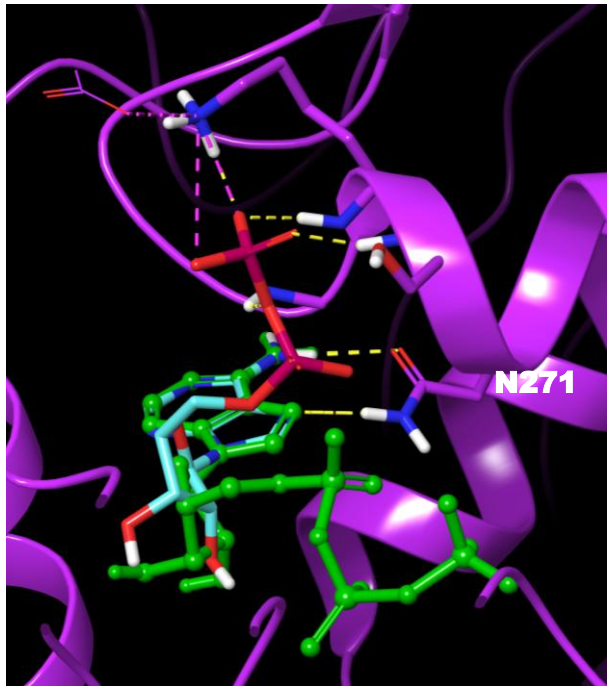
Pursue a multitargeting approach to limit resistance

Human Kinase vs Bacterial Mur Ligase ATP Binding Sites

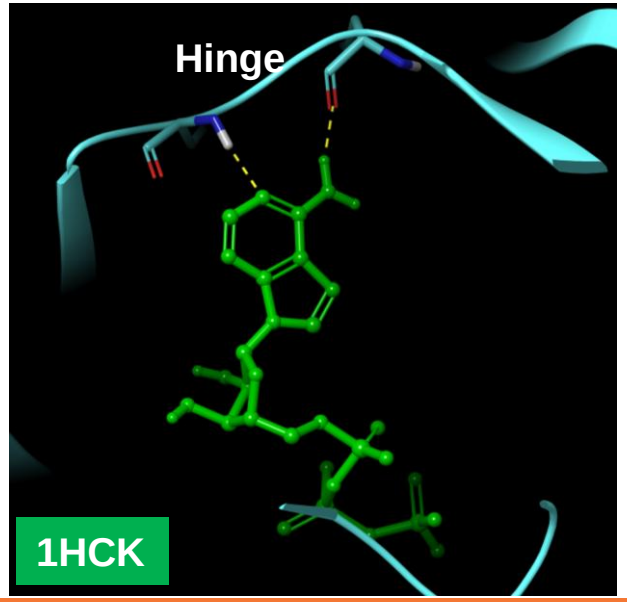
E. coli **murD Ligase**

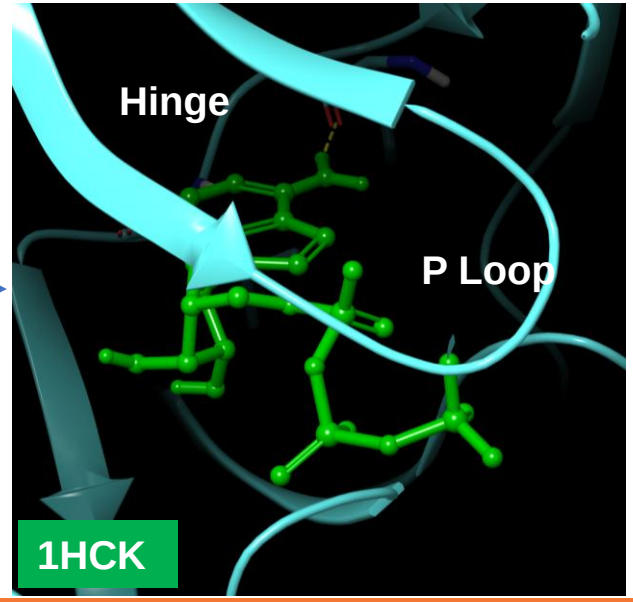






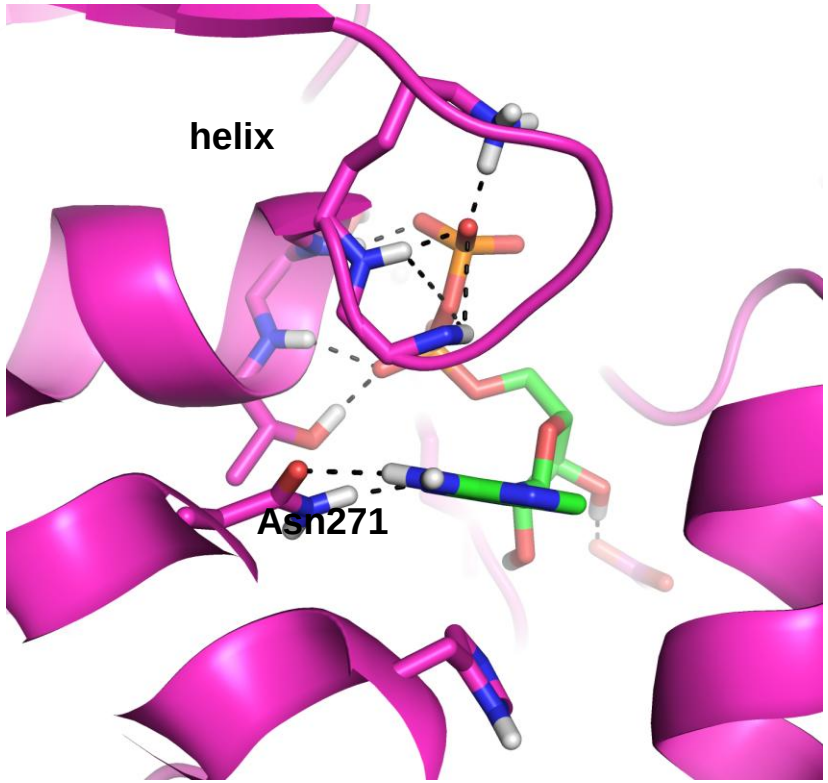
Human CDK2 Kinase

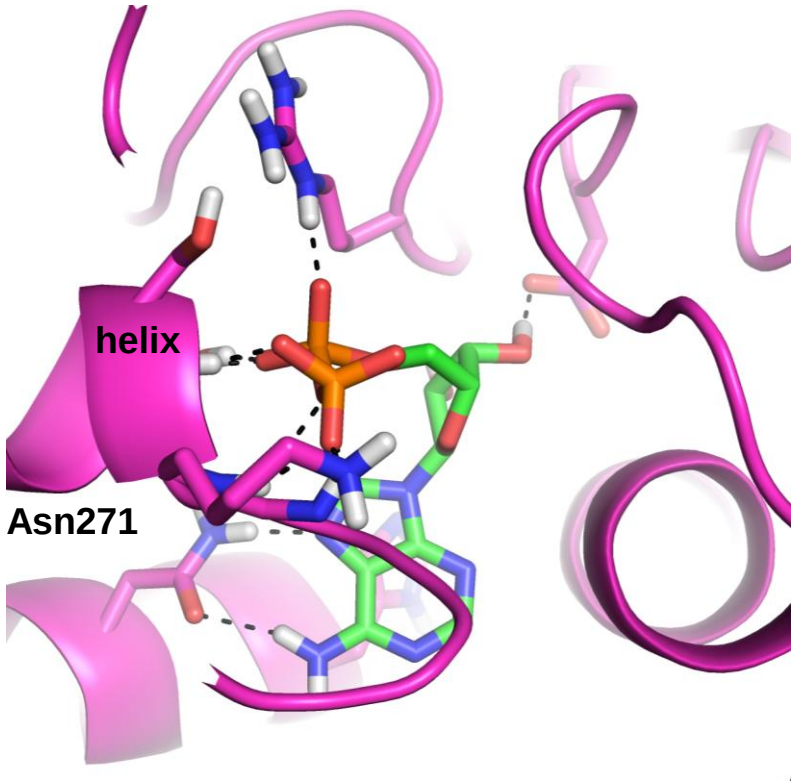




murD ADP forms H-bonds to end of murD helix – significant difference to ATP interaction with catalytic lysine in human kinases

Analyze Active Site Hotspots: *E. coli* murD with ADP

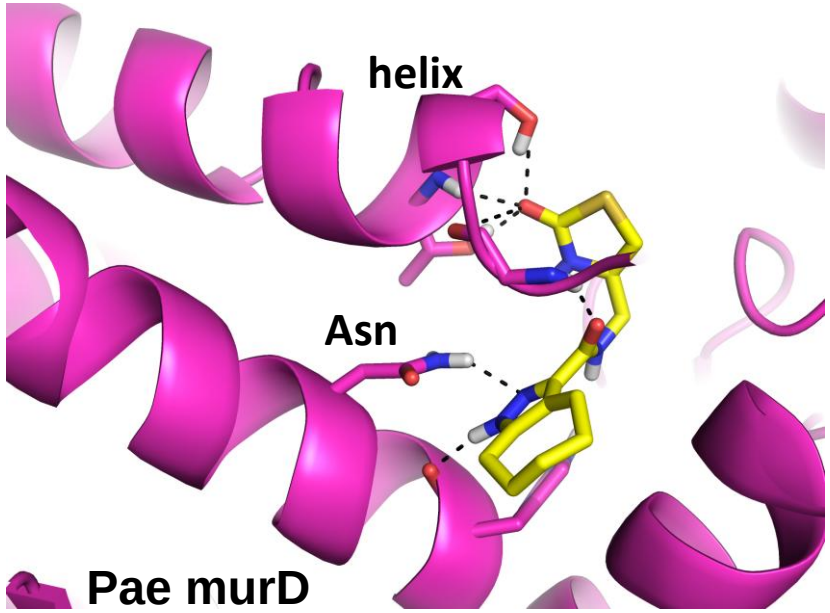


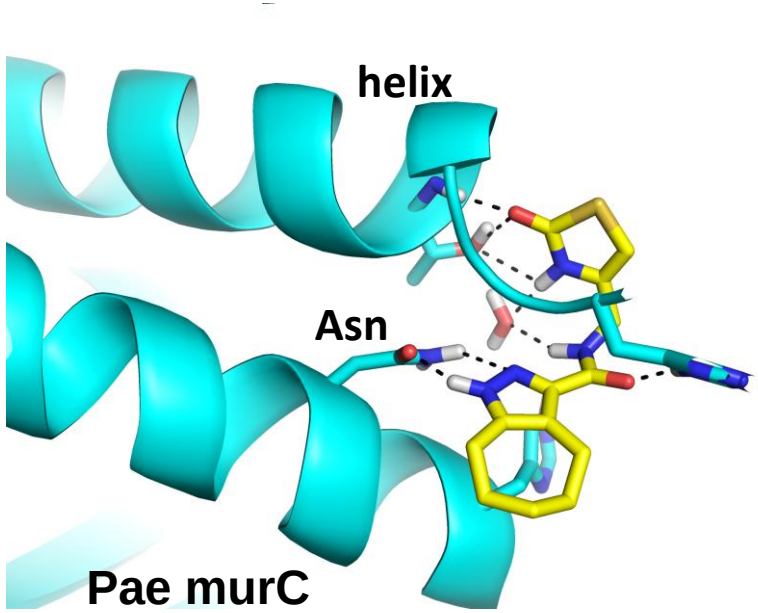


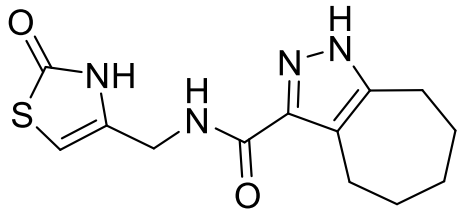
- Adenine ring of ADP forms two H-bonds to Asn271
- β-phosphate forms multiple H-bonds to murD helix
- Helix terminus provides multiple H-bond donors to interact with ligand H-bond acceptors
- Acceptors in murD inhibitors improves chances of inhibitor permeability against Gram- bacteria

Virtual Screen

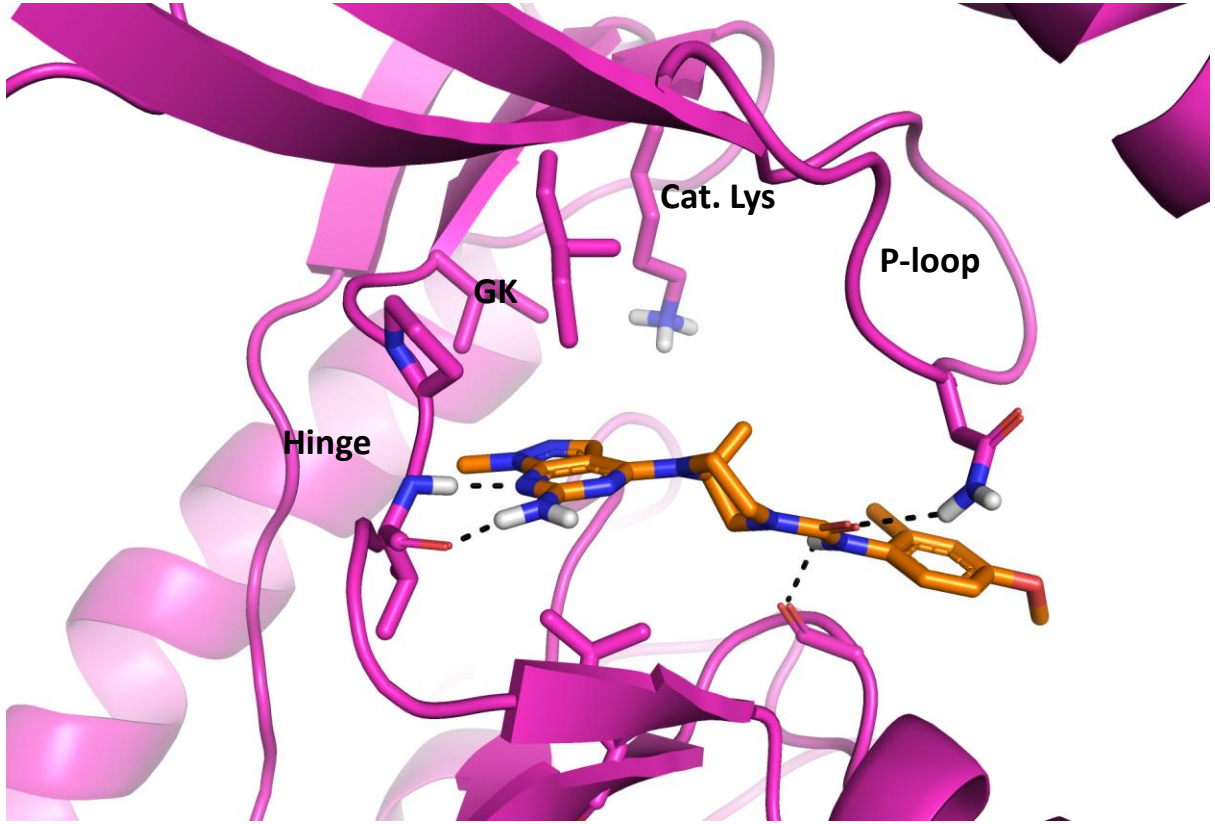
- **Virtual screen of 40,000 compound Enamine kinase focused library**
  Virtual screen against *S. alg*, MurD, *E. coli* murD and *E. coli* MurE
  Selected compounds binding to Asn sidechain and H-bond donors interacting helix terminus







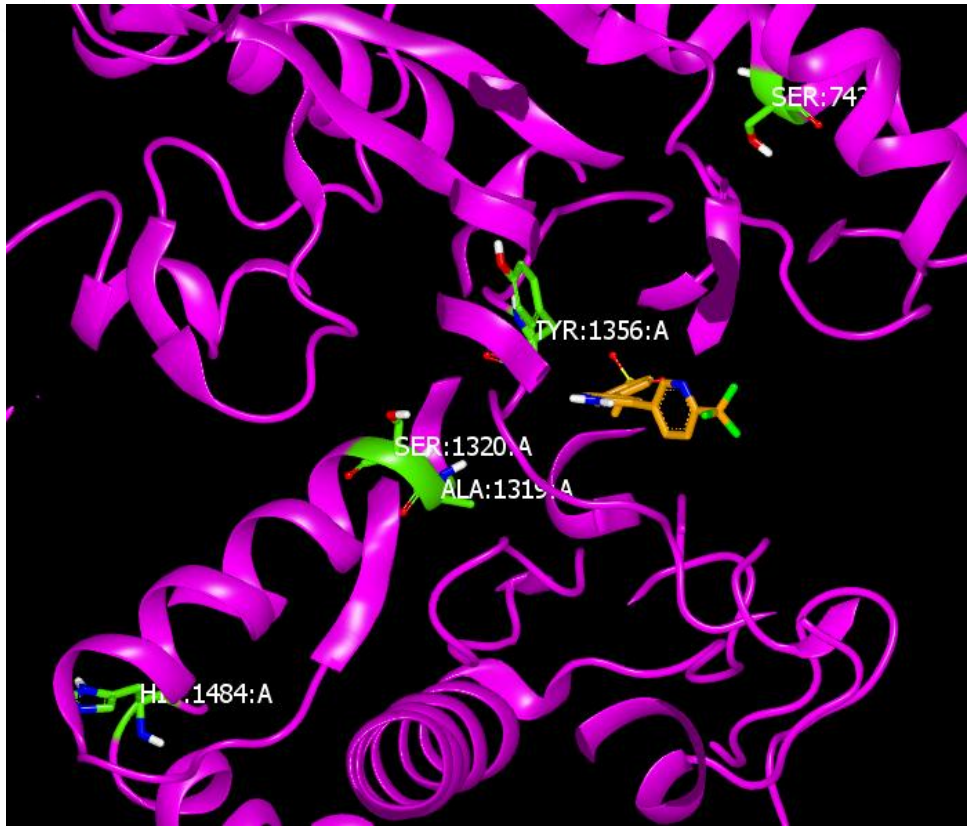
Human PI4Kb x-ray Structure



Crystal structure (PDB: 6GL3) of *Hs PI4K3b* with inhibitor illustrating key interaction in the ATP binding site.

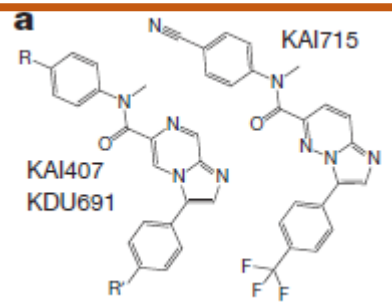
Hs PI4Kb vs PfPI4K sequence
Identity 36%
Similarity 61%

Mutations Observed for Pf PI4K inhibitors

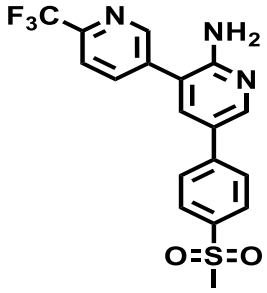


PI4K Mutations

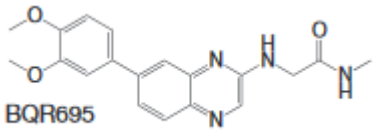
H3D ‘048:	S743T, A1319V
Quinoxaline (BQR695):	Y1356F(**14X**)
Imidazopyridine:	S1320L (6X), H1484Y(2)



Imidazopyrazines



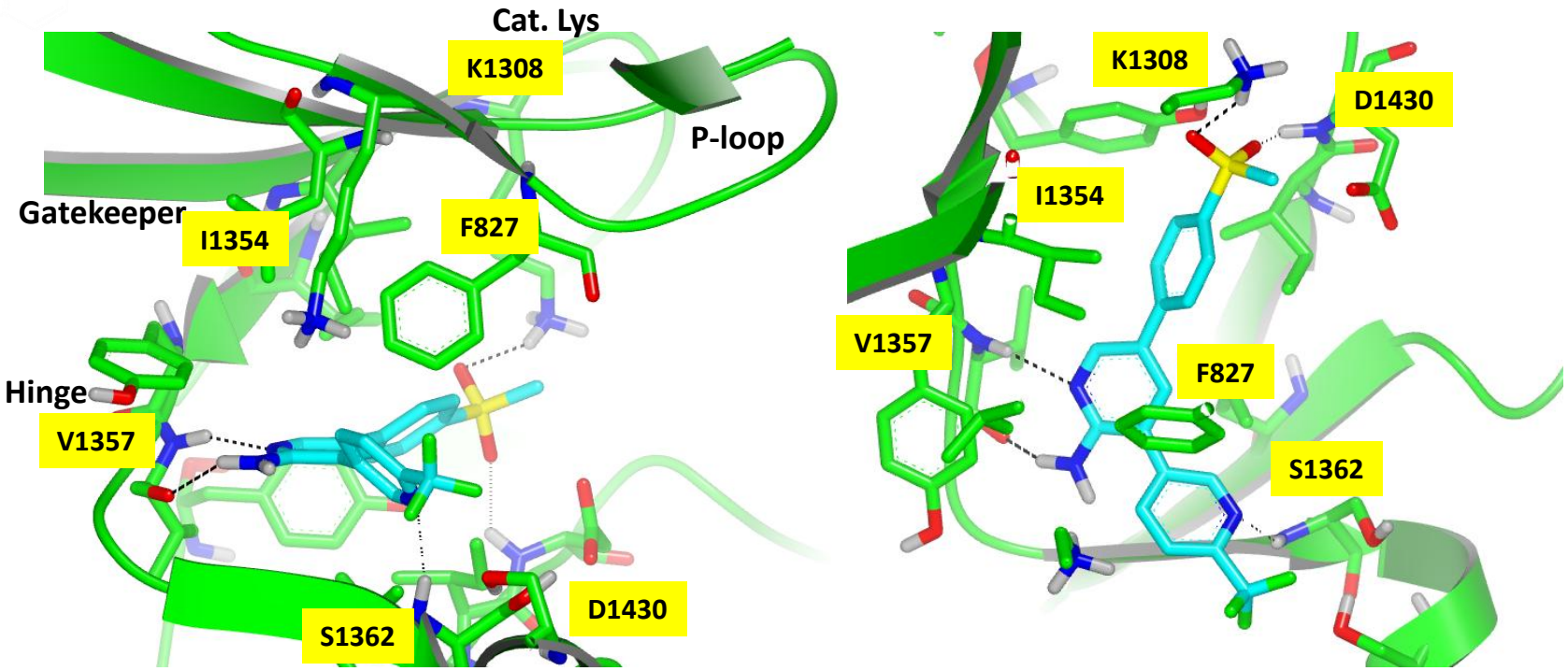
H3D MMV ‘048

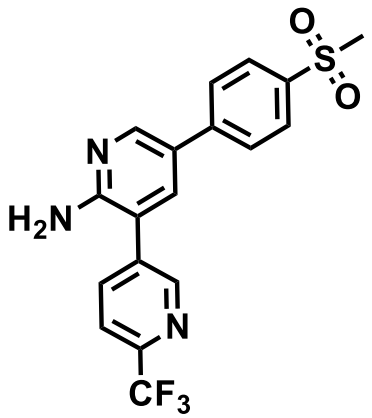


Quinoxaline

H3D PI4K inhibitor IC50 is 10X more potent than PfNF54 IC50 (cell activity)
PI4K mutation to H3D PI4K inhibitor occur distal to binding site (‘second layer’ and beyond)
Mutations to H3D PI4K inhibitor cause only 2-3 fold shift in Pf IC50
Use mutation data and human PI4K x-ray structure to build Pf PI4K homology model

MMV0048 model in Pf PI4K Homology Model





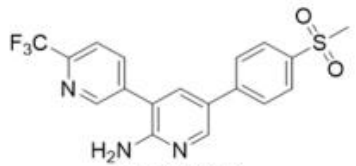
PvPI4K IC_{50} = 3.4 nM

PfPI4K Inhibitor MMV0048 Kinase Selectivity

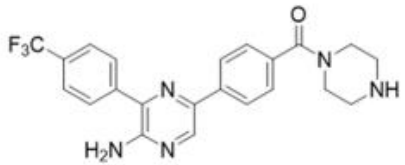
Structural Basis for Inhibitor Potency and Selectivity of *Plasmodium falciparum* Phosphatidylinositol 4-Kinase Inhibitors

Stephen Fienberg, Charles J. Eyermann, Lauren B. Arendse, Gregory S. Basarab, Jacob A. McPhail, John E. Burke, and Kelly Chibale*

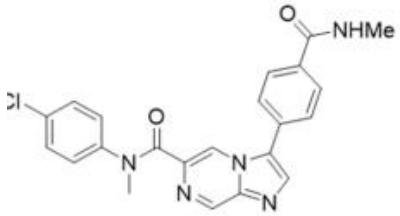
ACS Infect. Dis. 2020, 6, 3048−3063



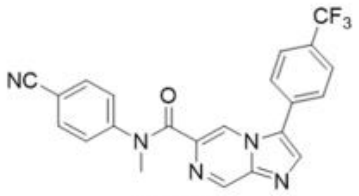
MMV390048
*Pv*PI4K IC_{50} - 3.4 nM



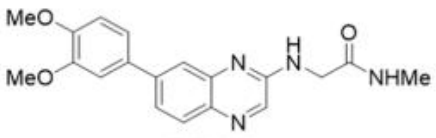
1
*Pv*PI4K IC_{50} - 23 nM



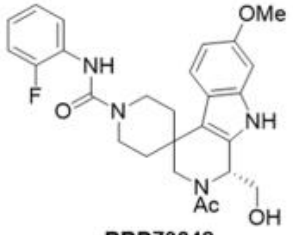
KDU691
*Pv*PI4K IC_{50} - 1.5 nM



KAI407
*Pv*PI4K IC_{50} - 120 nM

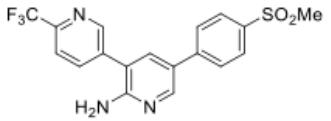


BQR695
*Pv*PI4K IC_{50} - 3.5 nM

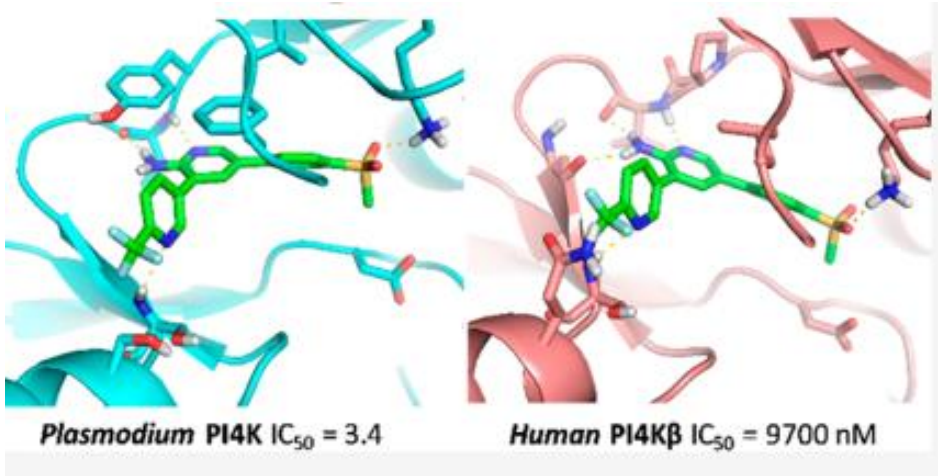


BRD73842
*Pv*PI4K IC_{50} - 21 nM

Table 8. Comparison of Available Inhibition Data of *Pv*PI4K and Human PI4K*β*

Compound	PvPI4K pIC_{50}	HuPI4Kβ pIC_{50}	HuPI4Kβ IC_{50}/*Pv*PI4K IC_{50}
	8.47[a]	5.01[c*]	2852

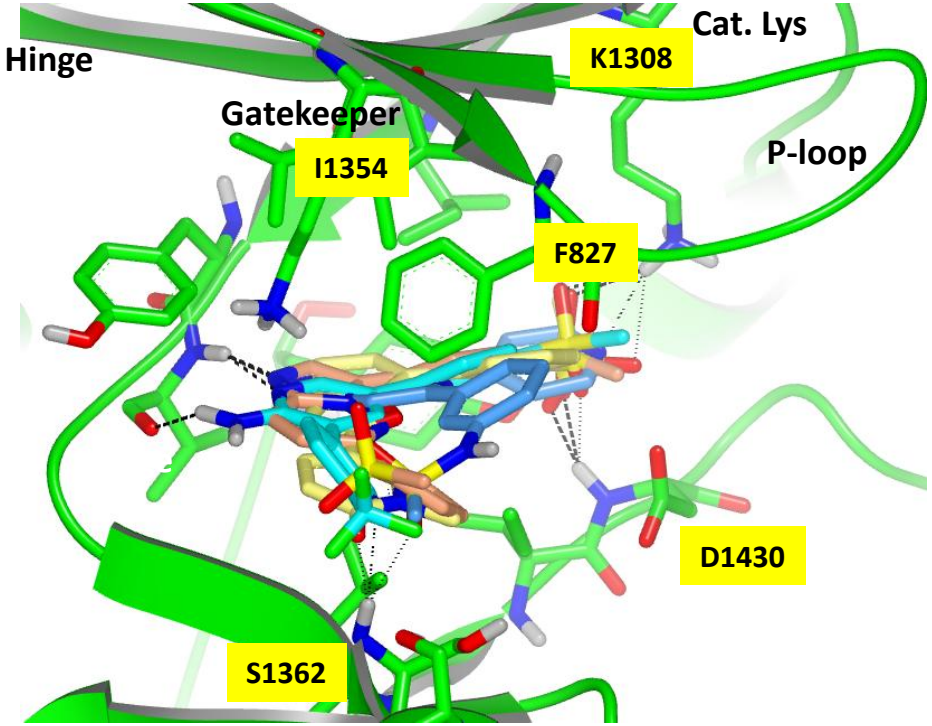
MMV390048

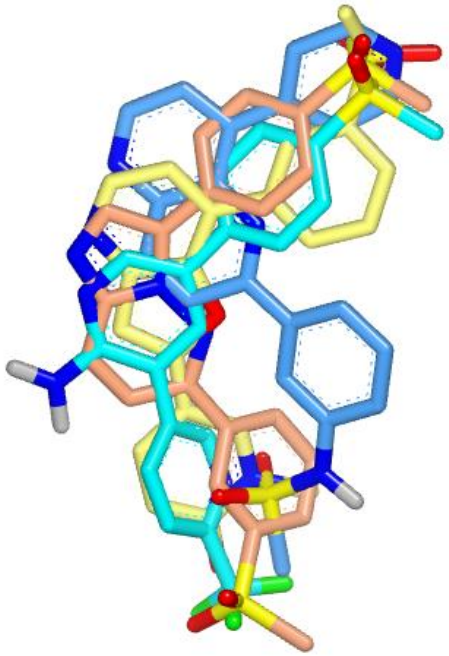


Also examined MMV0048 *Hs*PI3K isoform selectivity

Pf PI4K Inhibitor Models – Scaffold Hopping Opportunity

Use models and structure activity relationships for scaffold hopping to identify next generation Pf PI4K inhibitors





in silico resources

- **PubMed Central: https://pmc.ncbi.nlm.nih.gov/:**
- **ChEMBL: https://www.ebi.ac.uk/chembl:**
- **SciFinder: https://www.cas.org/solutions/cas-scifinder-discovery-platform/cas-scifinder:**
- **BLAST: https://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGETYPE=BLASTHome**
- **Plasmodium Database PlasmoDB: https://plasmodb.org/plasmo/app**
- **Trypanosome Database TriTrpDB: https://tritrypdb.org/tritrypdb/app**
- **DataWarrior: https://openmolecules.org/datawarrior/**
- **WebCSD: https://www.ccdc.cam.ac.uk/solutions/software/webcsd/**
- **Protein Structure Data Bank: https://www.rcsb.org/**
- **KLIFS: https://klifs.net/index.php**
- **AlphaFold: https://alphafold.ebi.ac.uk/**
- **MetaSite: https://www.moldiscovery.com/software/metasite/**
- **pyMol (free): https://anaconda.org/conda-forge/pymol-open-source**
- **Schrodinger: https://www.schrodinger.com/**
- **MOE: https://www.chemcomp.com/en/index.htm**
- **Openeye: https://www.eyesopen.com/**
- **Compound vendors: (e.g. SelleckChem, Enamine, Chembridge, eMolecules, etc)**

Integrating CADD into Drug Discovery

- **Drug discovery (hunting) is a TEAM sport!**
- **CADD and computational chemists can help with offense and defense**
- **Deep dive on all data – both internal project data and external data**
- **Thinking about the data is as important as the calculations**
- **Important to benchmark/validate CADD methods for your project**
- **Still challenge to predict binding affinities *a priori***

Grand Challenges
African Drug Discovery Accelerator

Phenotypic or Target-Based Projects?

Which approach provides the best opportunity to integrate CADD and data to deliver a drug candidate?

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