

USING PHYSICAL MODELING TO DIRECT BIOPHYSICAL EXPERIMENTS AND PREDICT THE FUNCTIONAL IMPACT OF CLINICAL CANCER MUTATIONS IN KINASES ON DRUG SUSCEPTIBILITY

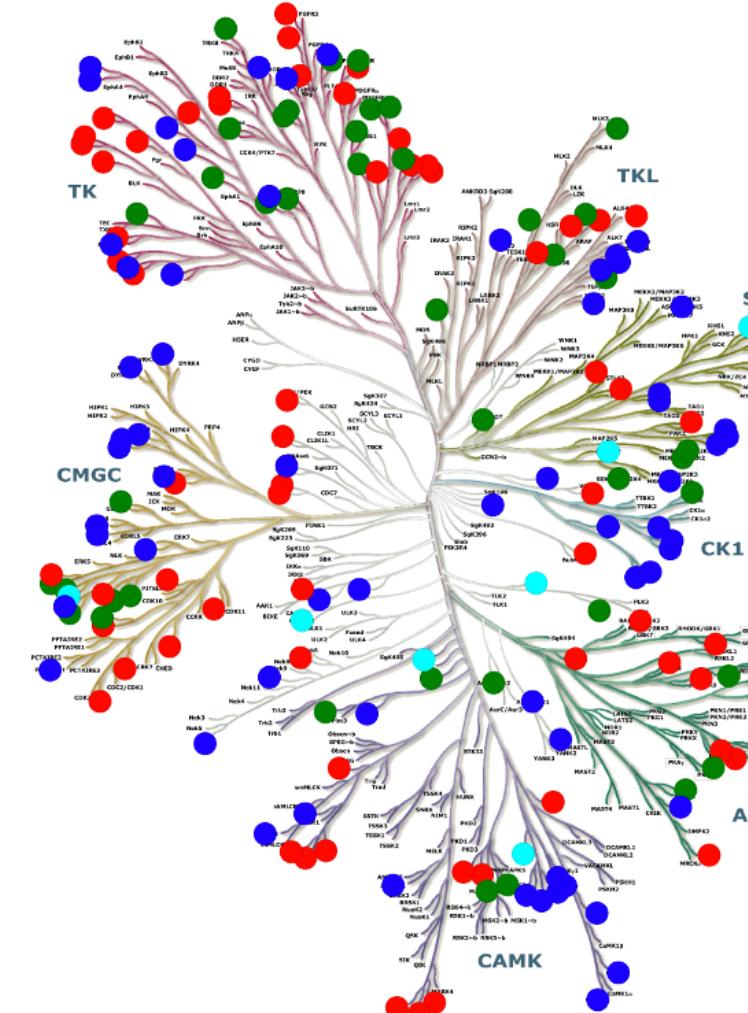
MED INTO GRAD
COLUMBIA UNIVERSITY
10/14/17



STEVEN ALBANESE
CHODERA LAB // MSKCC

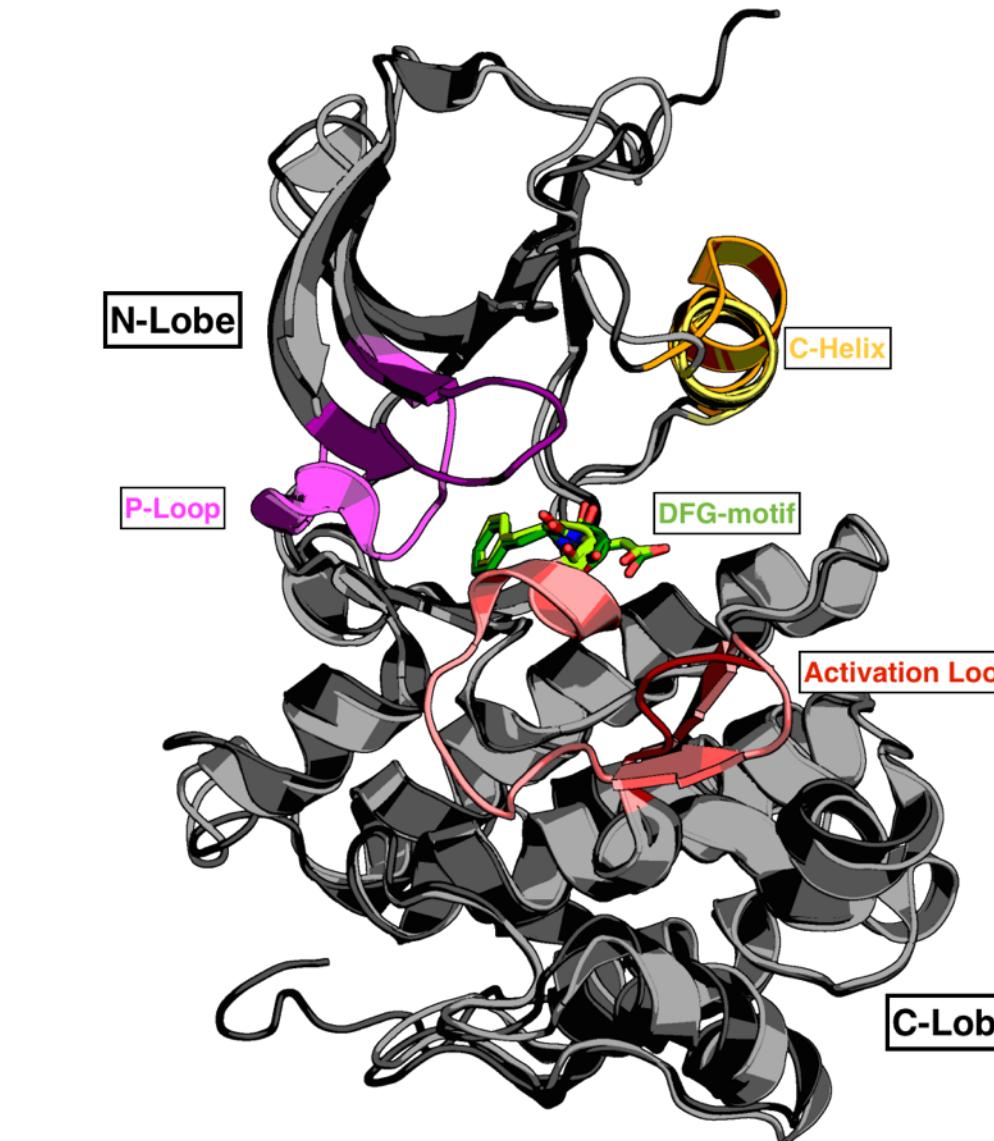
KINASES HAVE A SHARED FOLD AND ARE A COMMON THERAPEUTIC TARGET

518 human kinases...

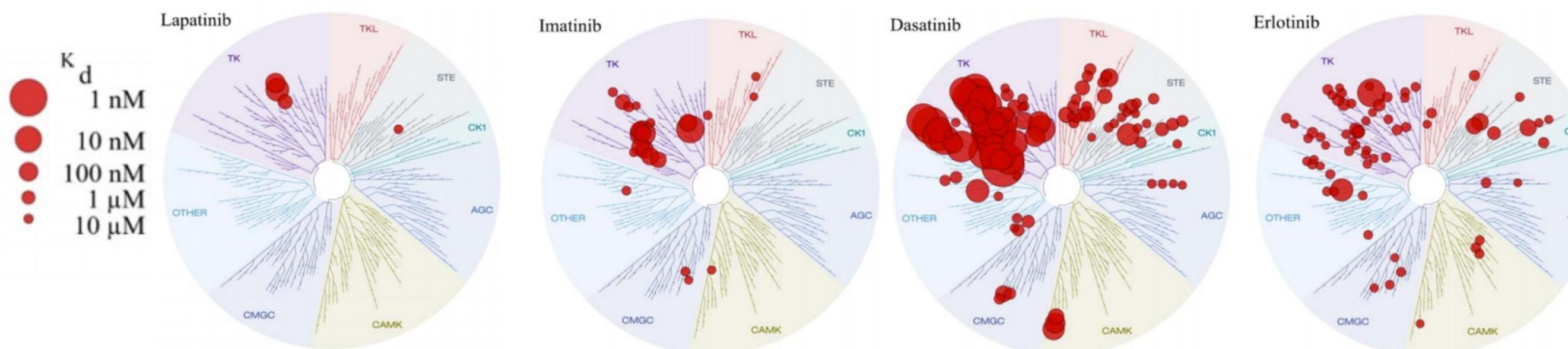


<http://www.thescg.org/scientists/resources/kinases>

...with a shared fold

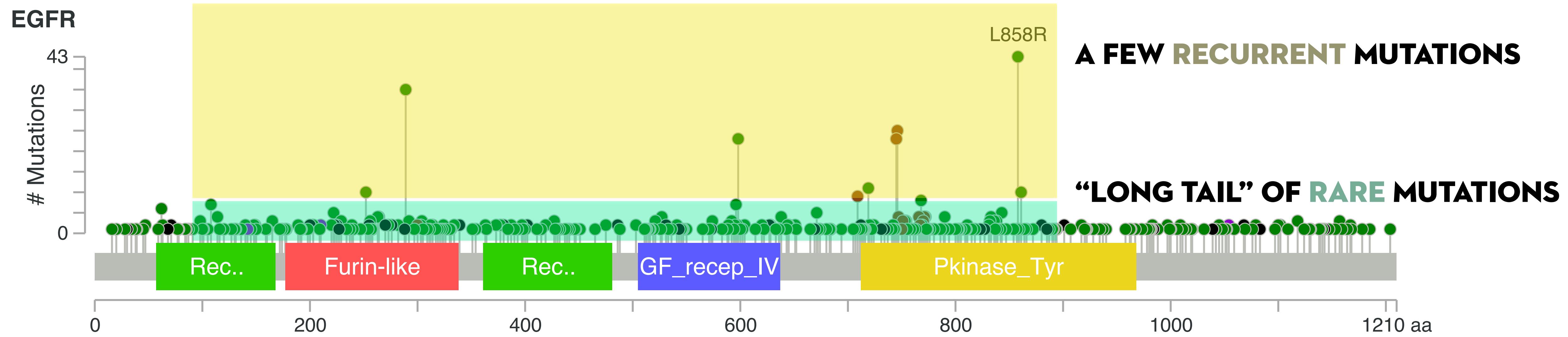


PDB: 2OIQ, 2YHH

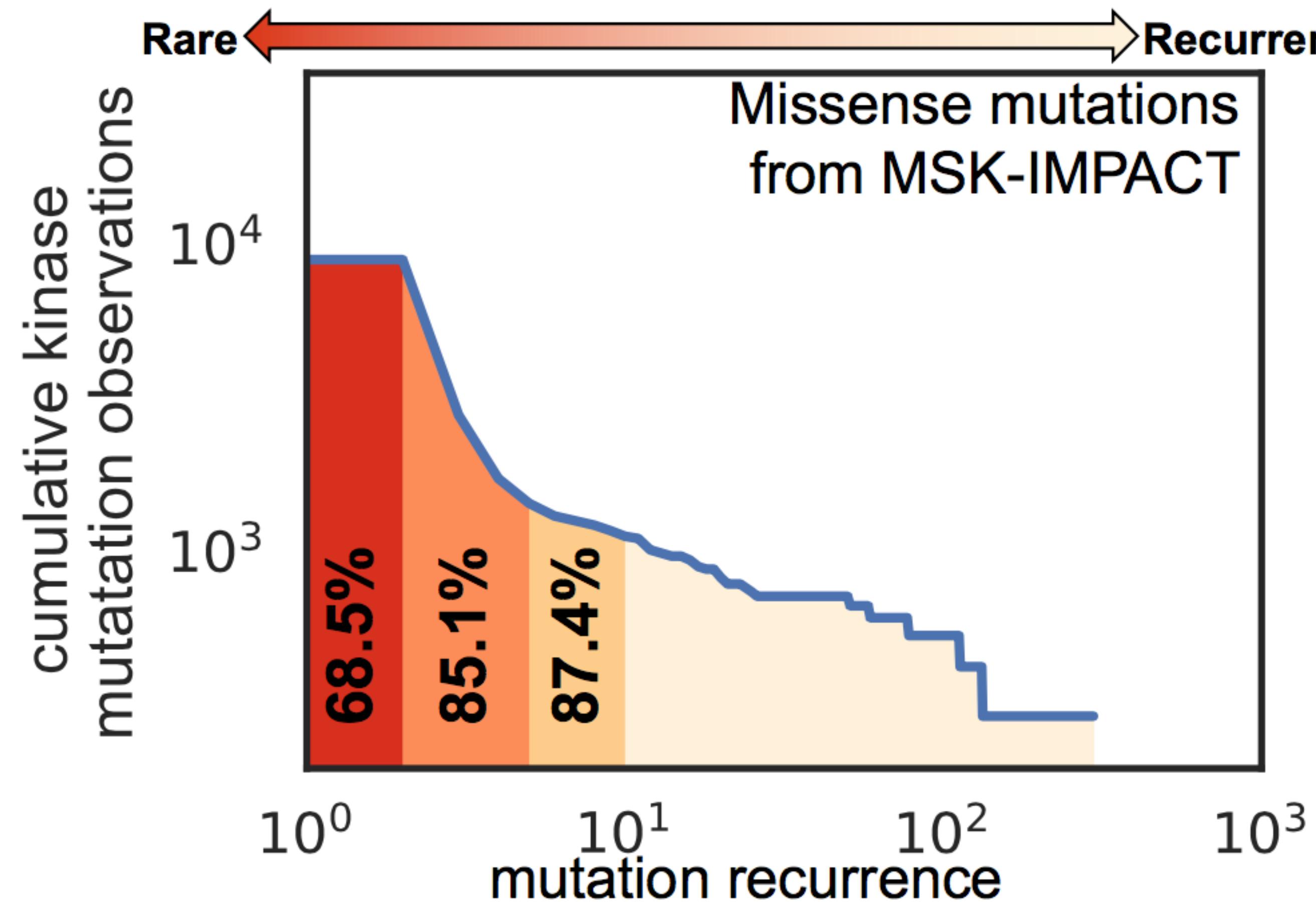


Davis et. al. Nat. Biotechnol. 29:11, 2011

LONG TAIL MUTATIONS CAN BE DIFFICULT TO ASSESS FUNCTIONALLY

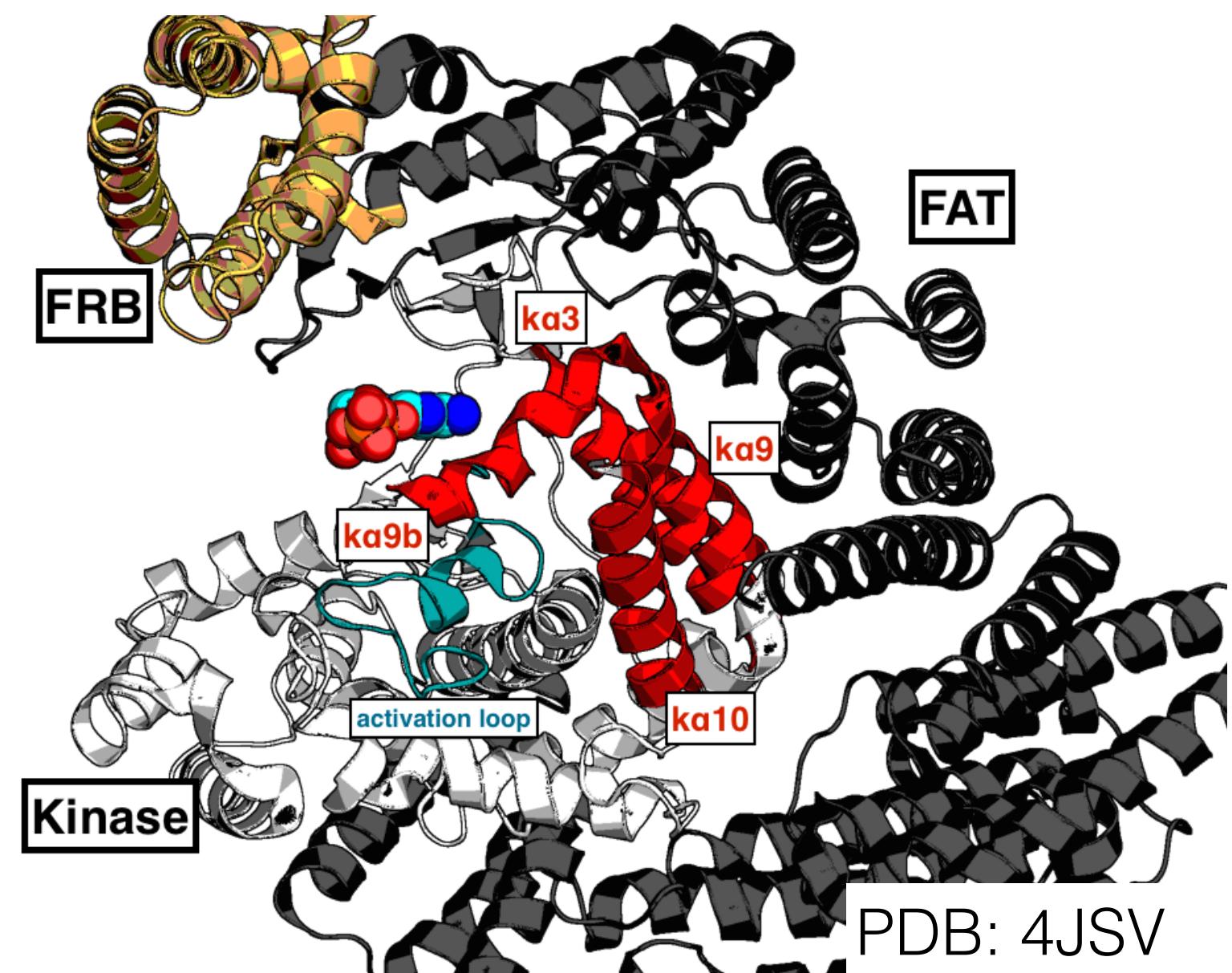


LONG TAIL MUTATIONS CAN BE DIFFICULT TO ASSESS FUNCTIONALLY



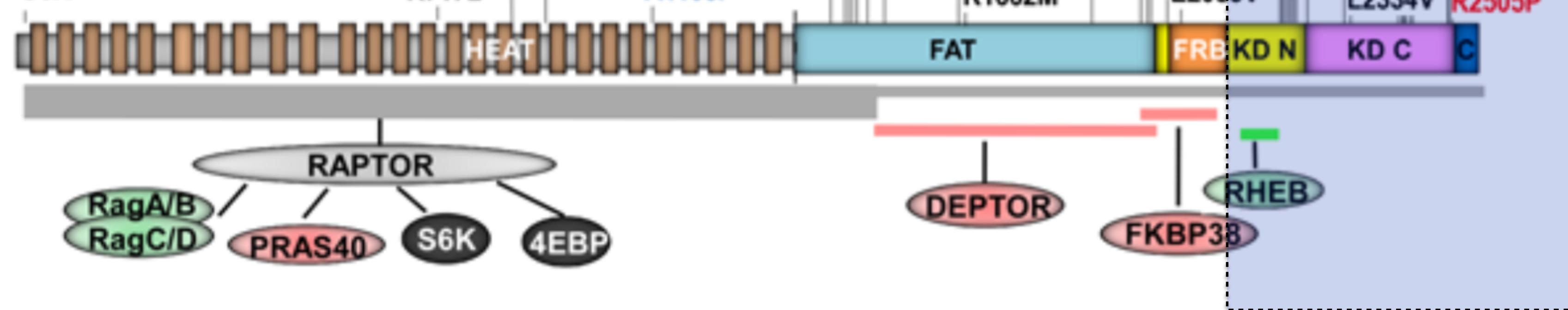
CAN WE USE PHYSICAL MODELING TO GAIN MECHANISTIC INSIGHT INTO CLINICAL MUTATIONS?

~2% OF ALL CANCERS HARBOR MTOR MUTATIONS
MANY MUTATIONS IN KINASE DOMAIN ARE ACTIVATING



Activating mutations
Non-activating mutations
Not characterized

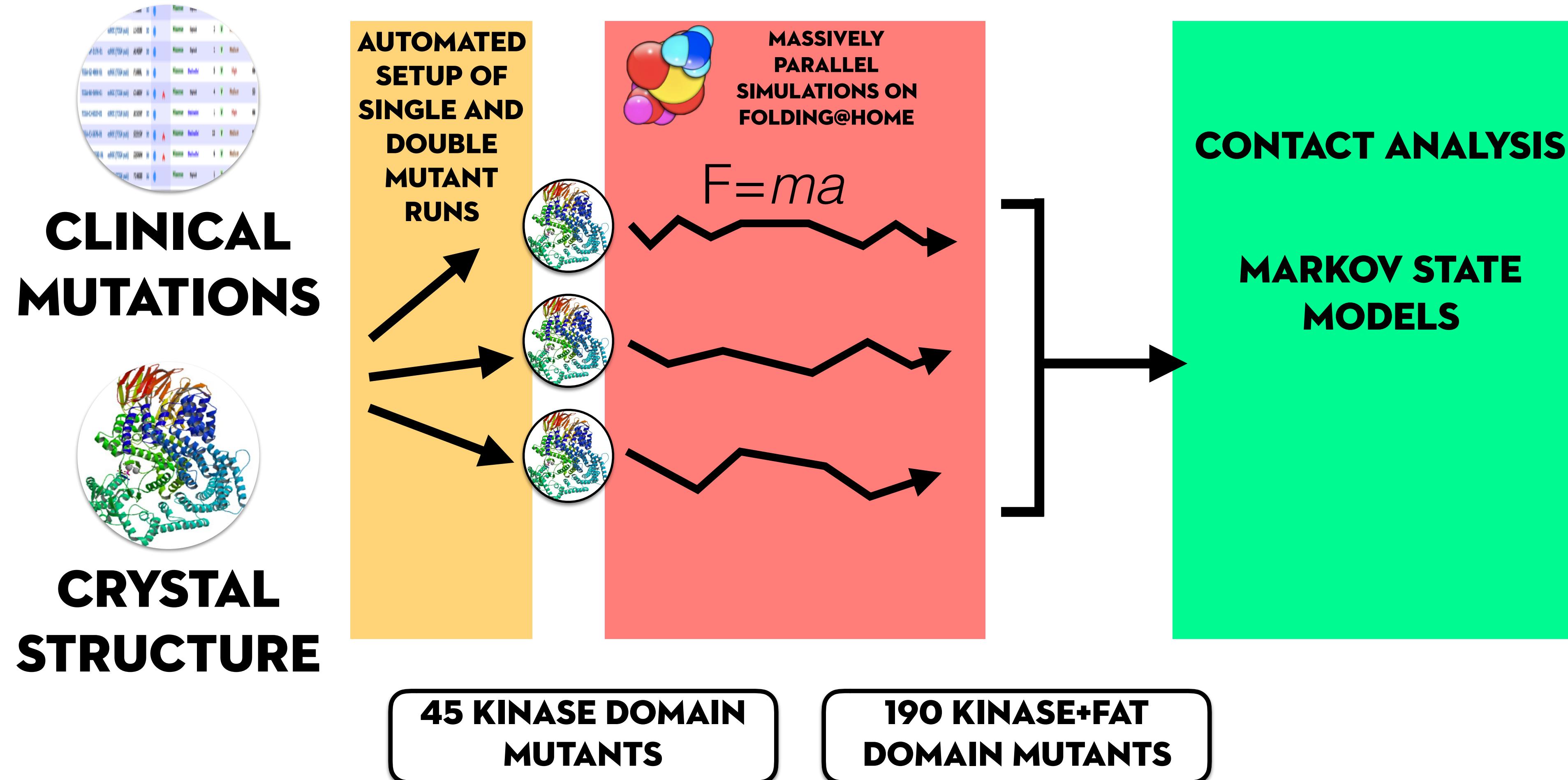
G5R R717L K860N E919V A1105P



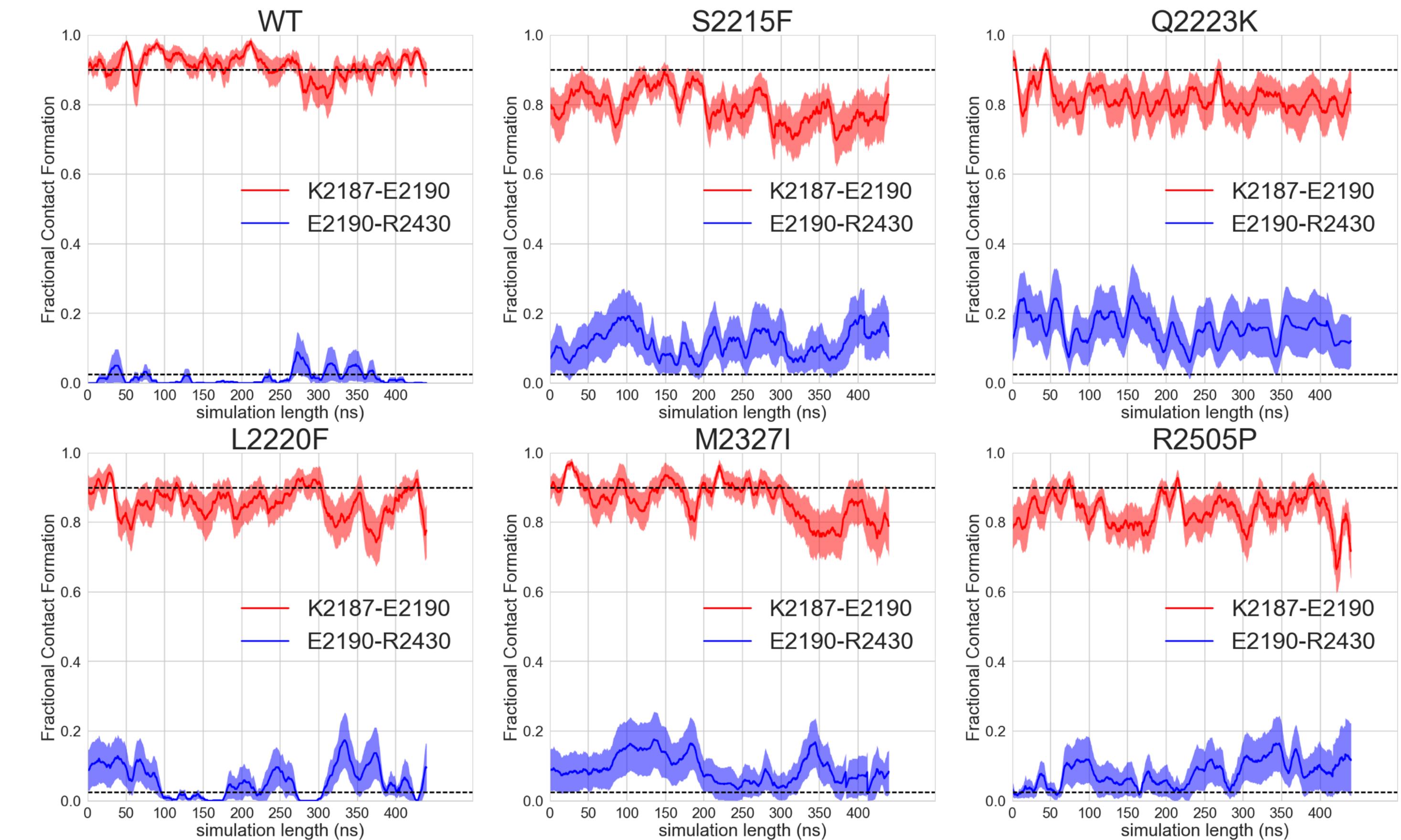
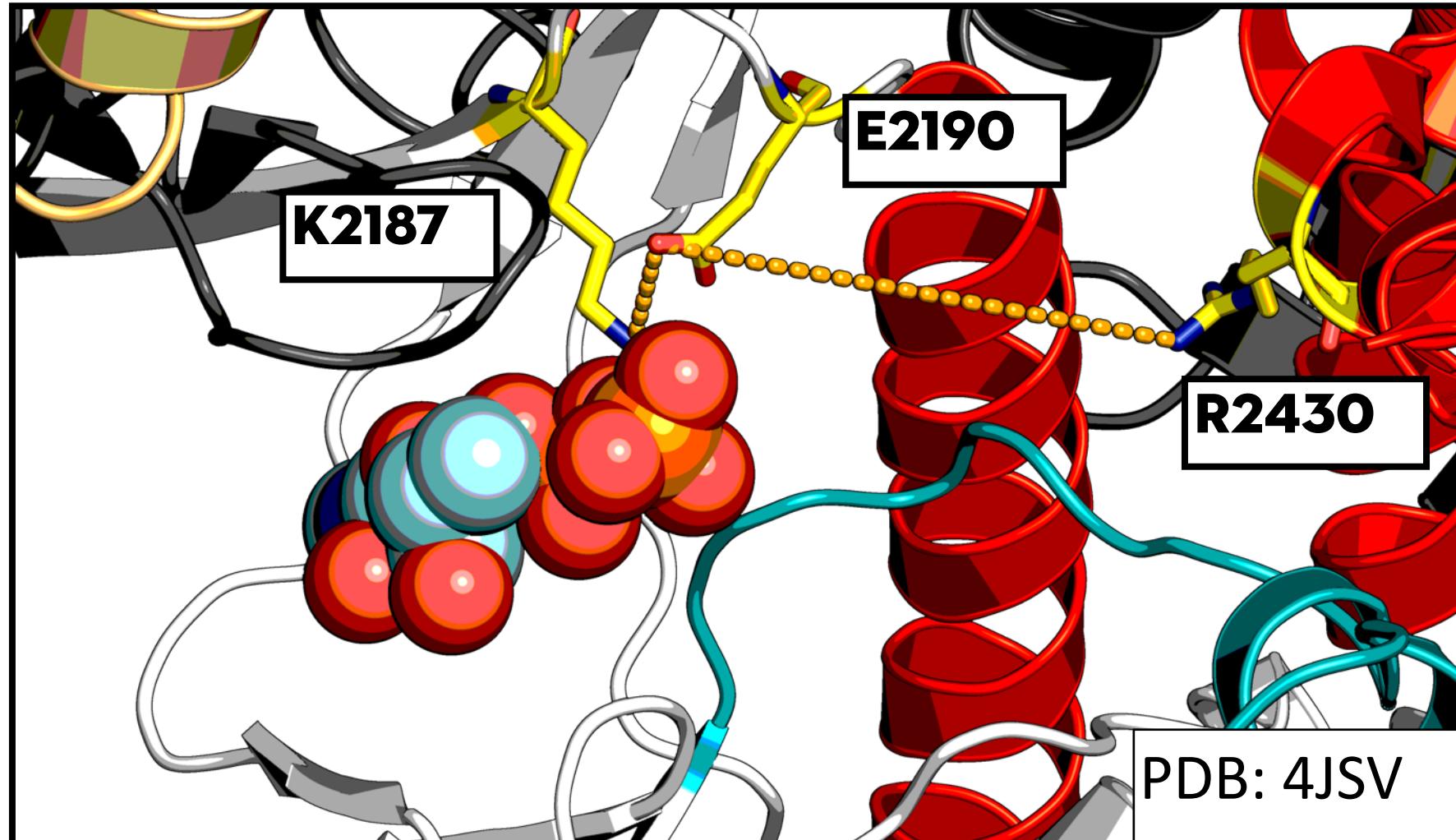
Xu, Pham, **Albanese**, Dong, Oyama, Lee, Rodrik-Outmezguine, Yao, Han, Chen, Parton, Chodera, Rosen, Cheng, and Hsieh.
Journal of Clinical Investigation, 126:3526, 2016

Collaboration with Kevin Hauser, Christopher Negron, and Robert Abel (Schrödinger); Jianing Xu and James Hsieh (WUSTL)

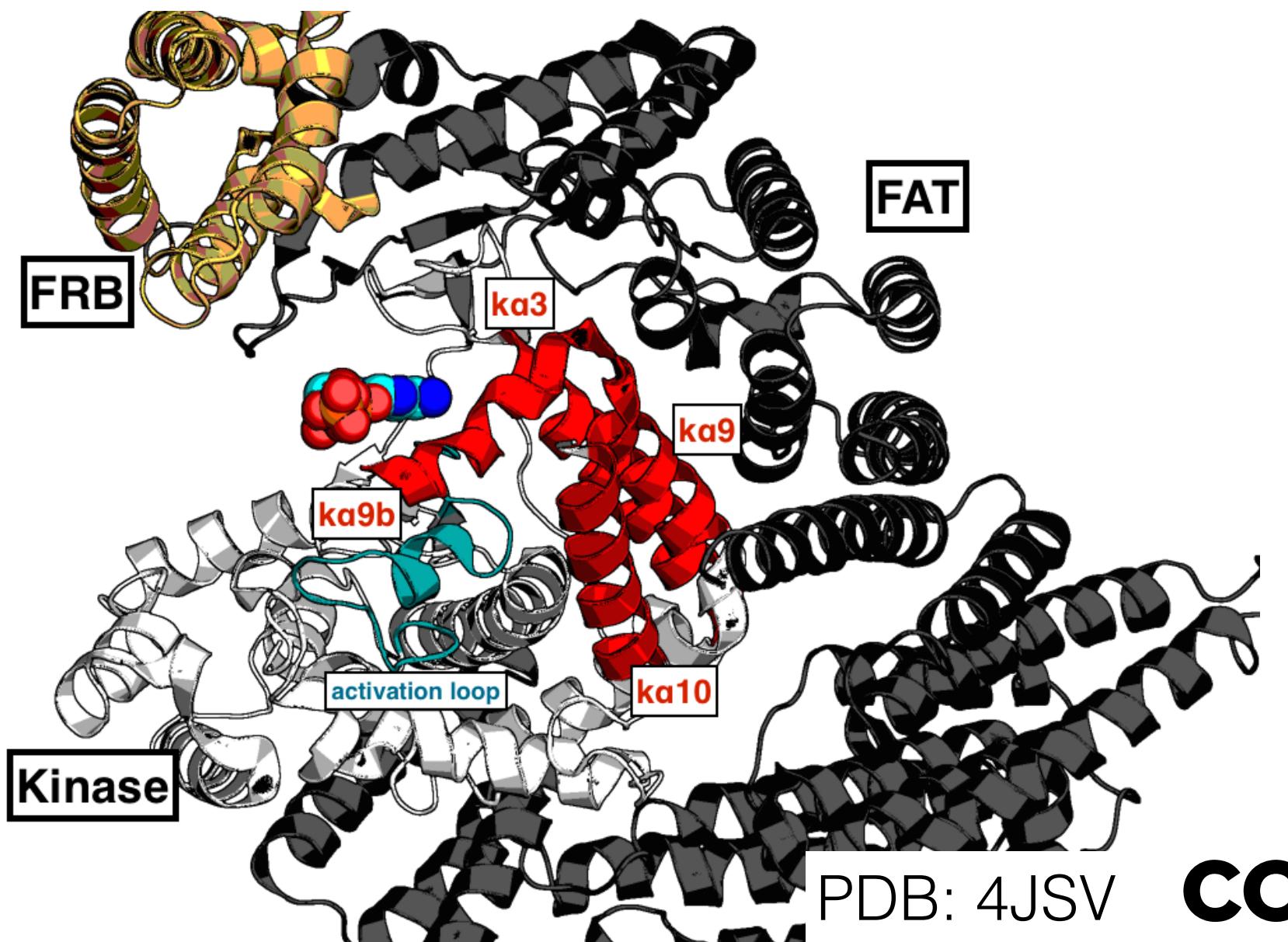
CAN WE USE PHYSICAL MODELING TO GAIN MECHANISTIC INSIGHT INTO CLINICAL MUTATIONS?



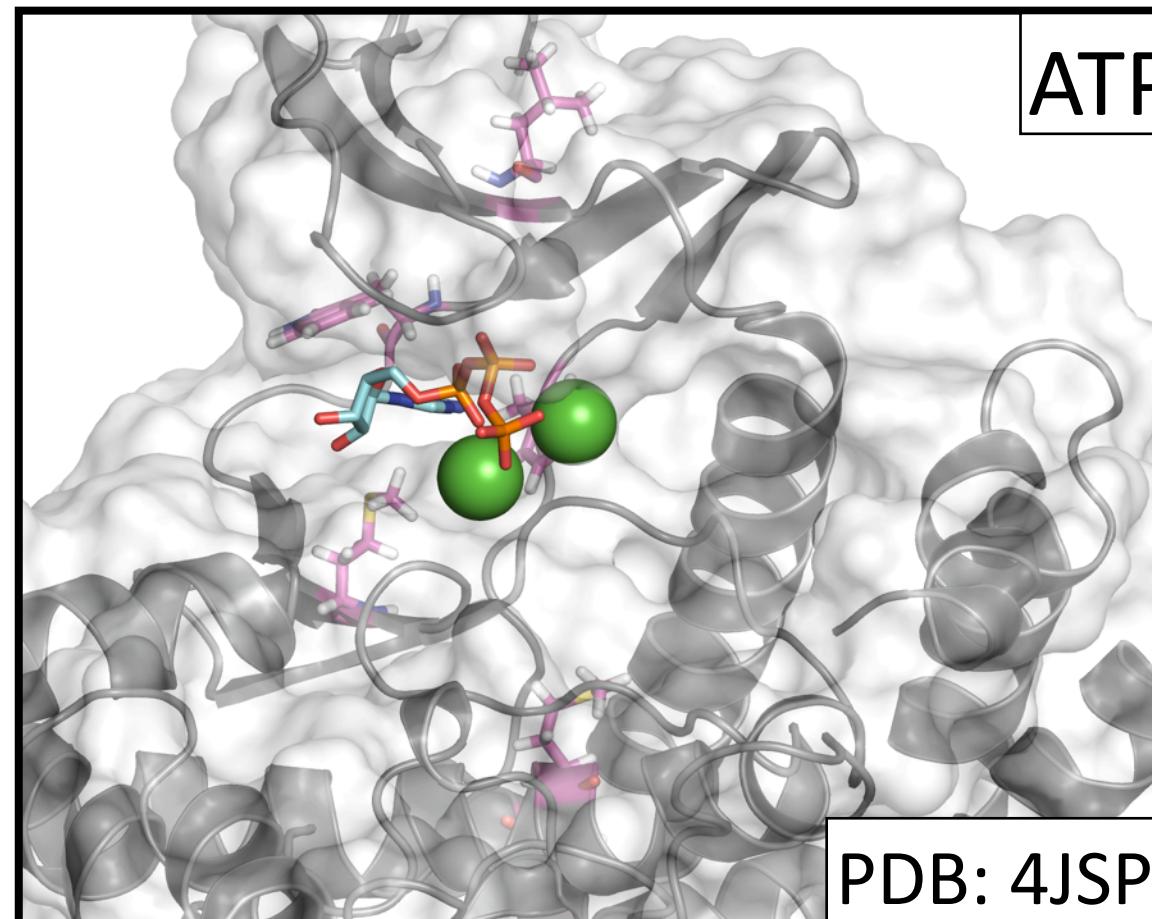
DO MUTATIONS STABILIZE KEY SALT BRIDGES REQUIRED FOR ACTIVITY?



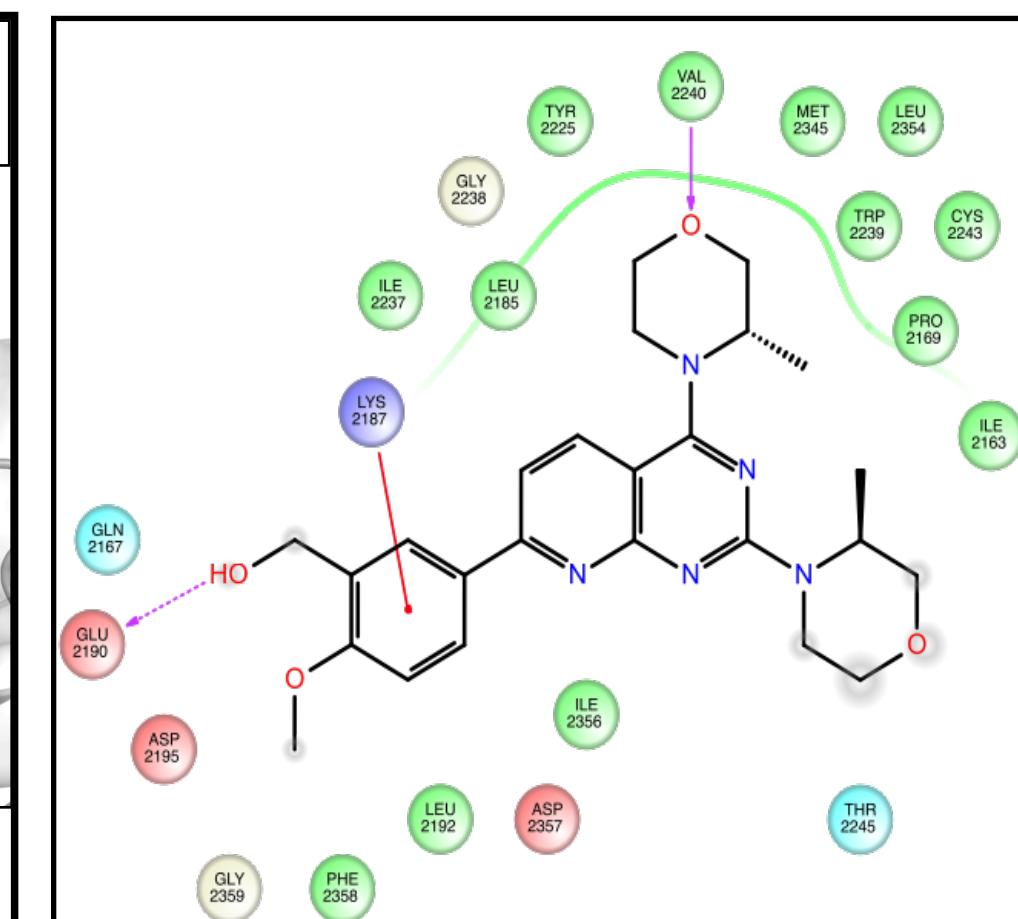
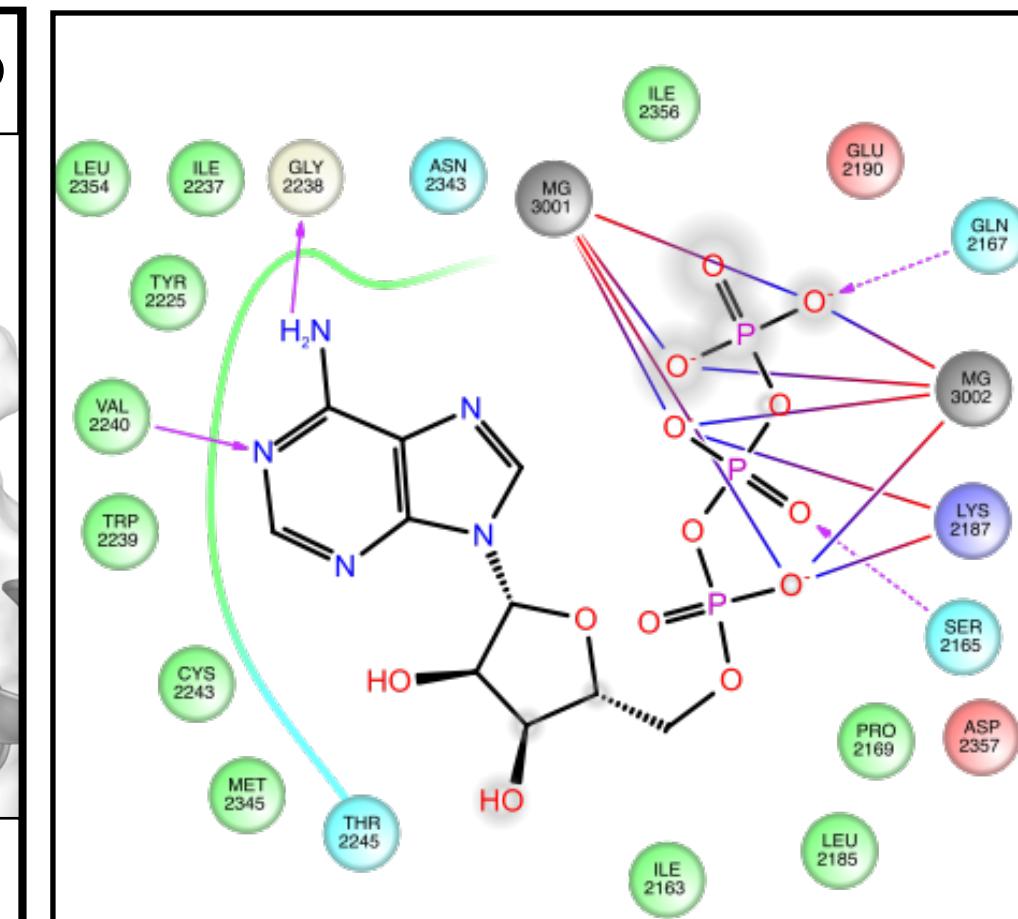
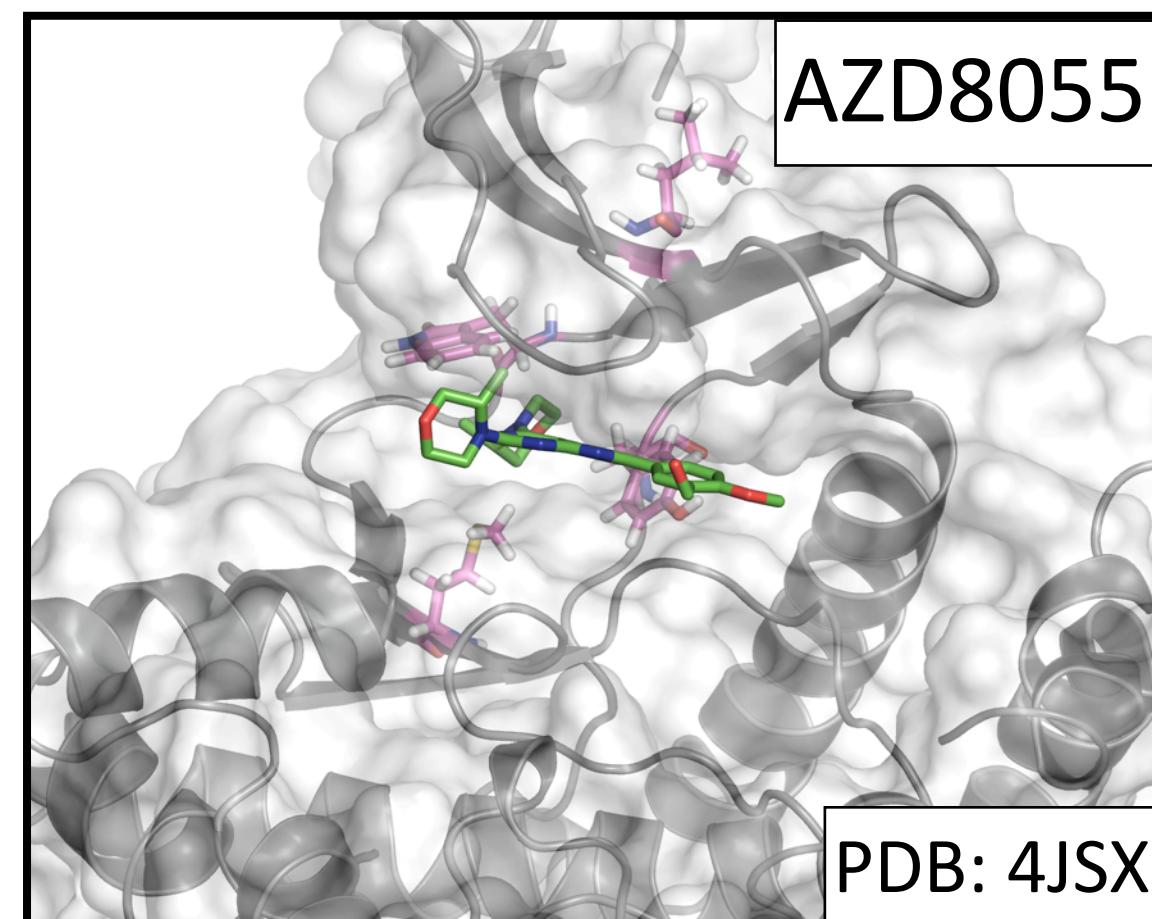
A KEY CHALLENGE: COMPUTING PHYSICAL PROPERTIES THAT CAN BE TESTED



ACTIVATE MTOR BY INCREASING ATP AFFINITY?



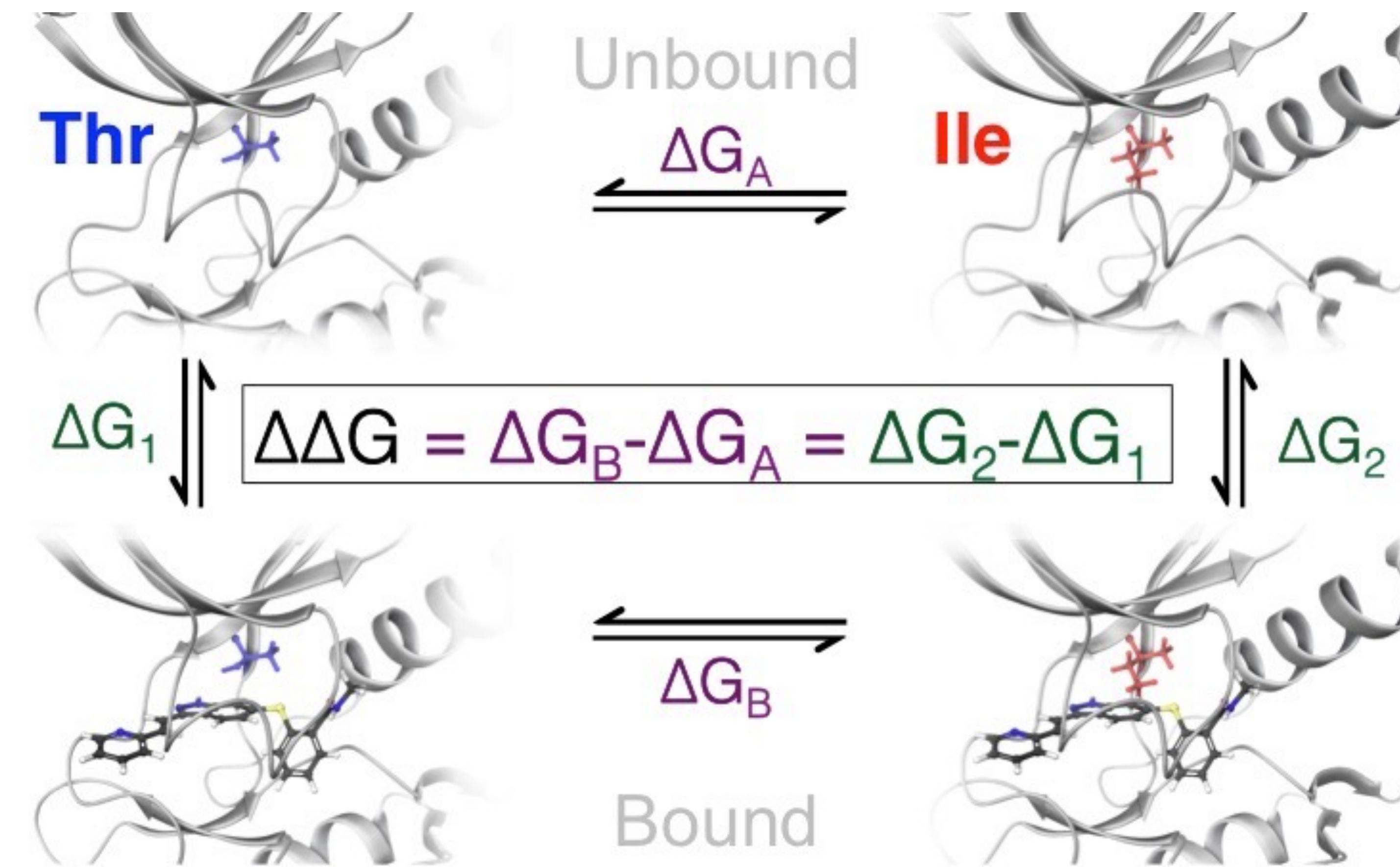
CONFER SENSITIVITY OR RESISTANCE TO AN ATP-COMPETITIVE INHIBITOR?



Xu, Pham, **Albanese**, Dong, Oyama, Lee, Rodrik-Outmezguine, Yao, Han, Chen, Parton, Chodera, Rosen, Cheng, and Hsieh.
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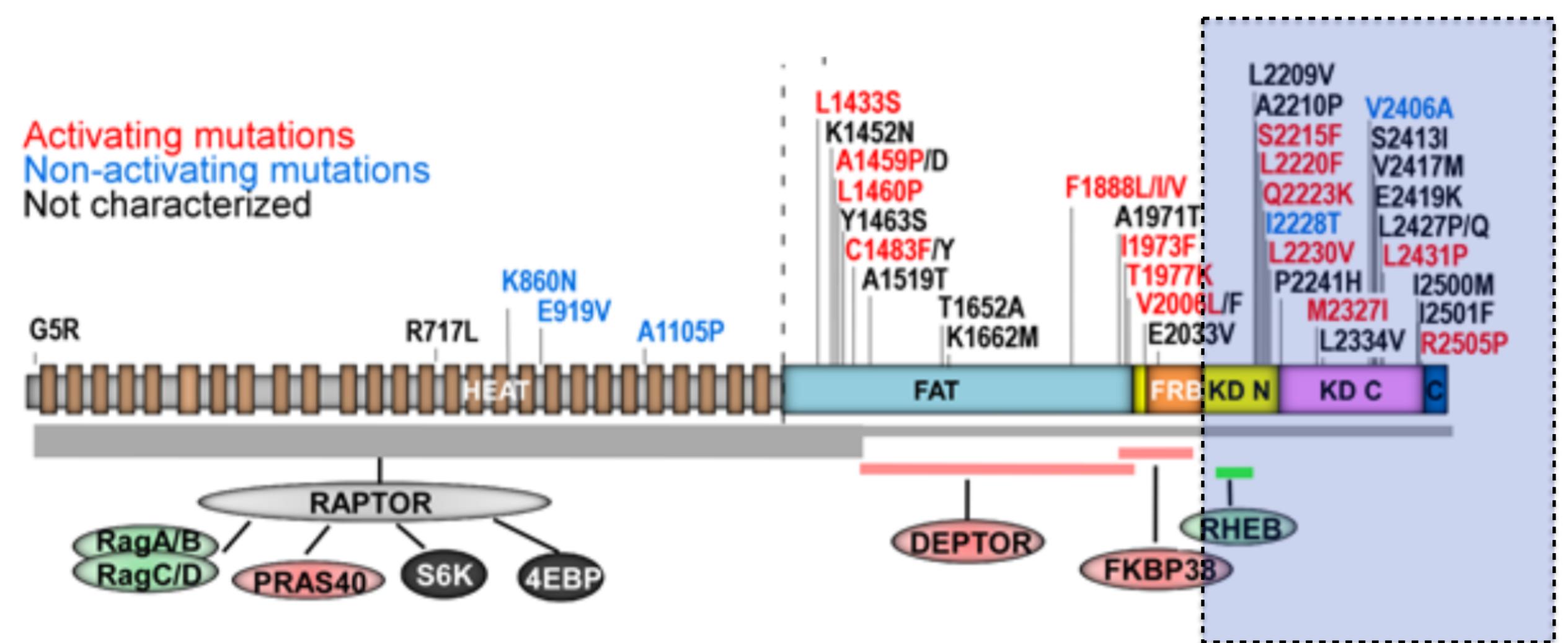
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PROTEIN MUTATION FREE ENERGY PERTURBATION USES A THERMODYNAMIC CYCLE

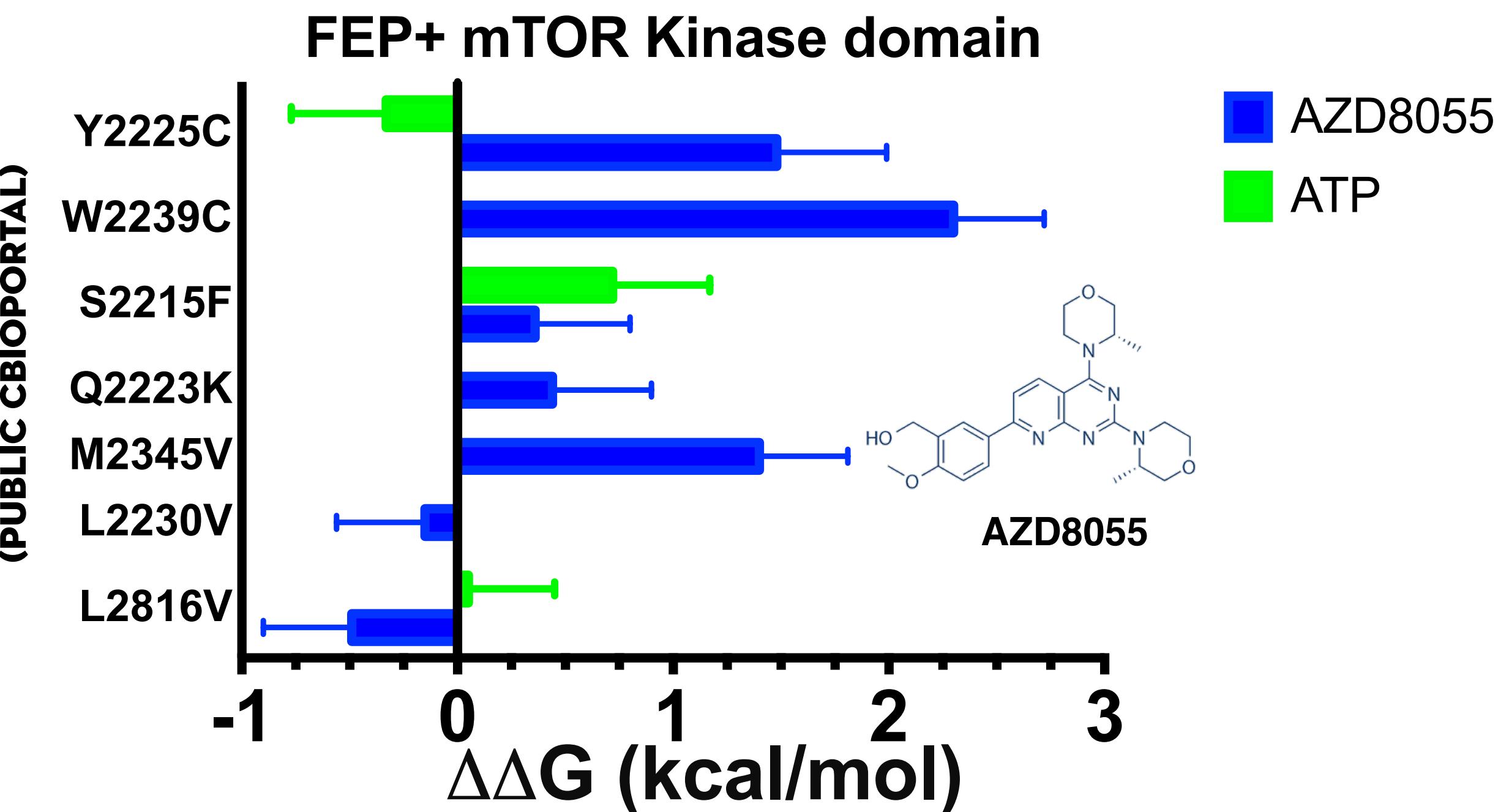


PRELIMINARY CALCULATIONS SUGGEST POTENTIAL RESISTANCE MUTATIONS TO AZD8055

KINASE DOMAIN HARBORS ACTIVATING MUTATIONS

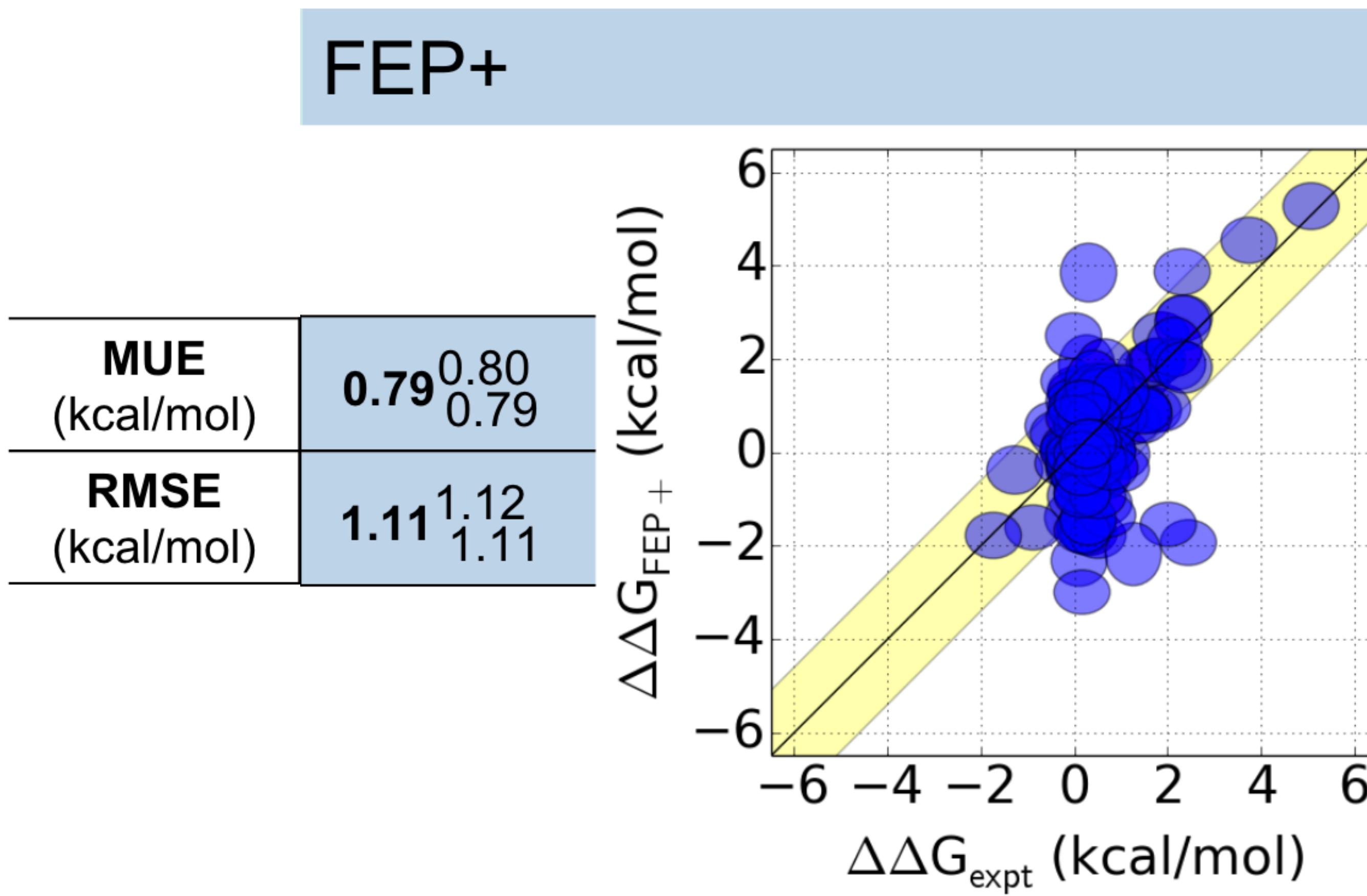


CLINICAL MUTATIONS
(PUBLIC CBIOPORTAL)



CURRENTLY WORKING TO TEST DRUG SENSITIVITY IN CANCER CELL LINES WITH JAMES HSIEH LAB @ WUSTL

A RETROSPECTIVE STUDY SUGGESTS FEP IS CAPABLE OF PREDICTING RESISTANCE MUTATIONS

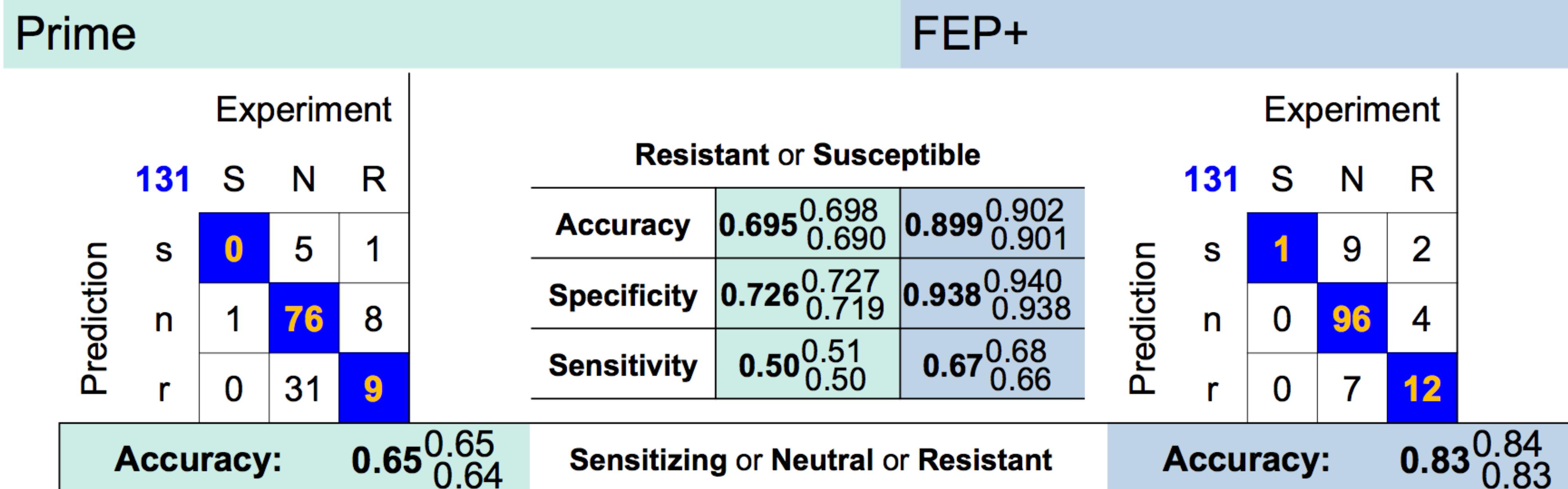


- PROTEIN MUTATION FEP+ FOR 131 MUTATION:INHIBITOR PAIRS
- COMPARES CALCULATION TO PUBLISHED IC50 DATA FOR 6 FDA APPROVED INHIBITORS



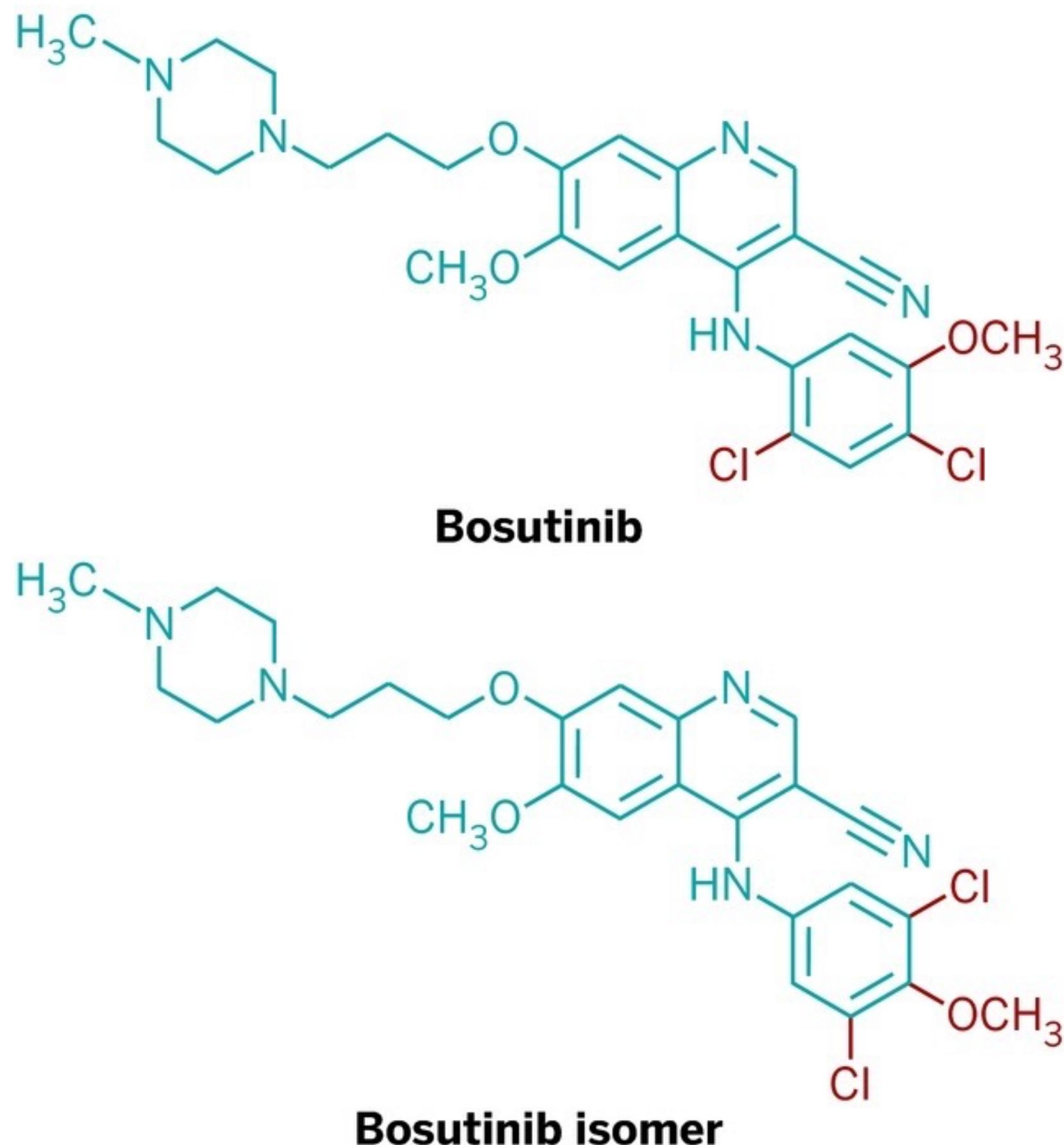
KEVIN HAUSER
SCHRÖDINGER

A RETROSPECTIVE STUDY SUGGESTS FEP IS CAPABLE OF PREDICTING RESISTANCE MUTATIONS

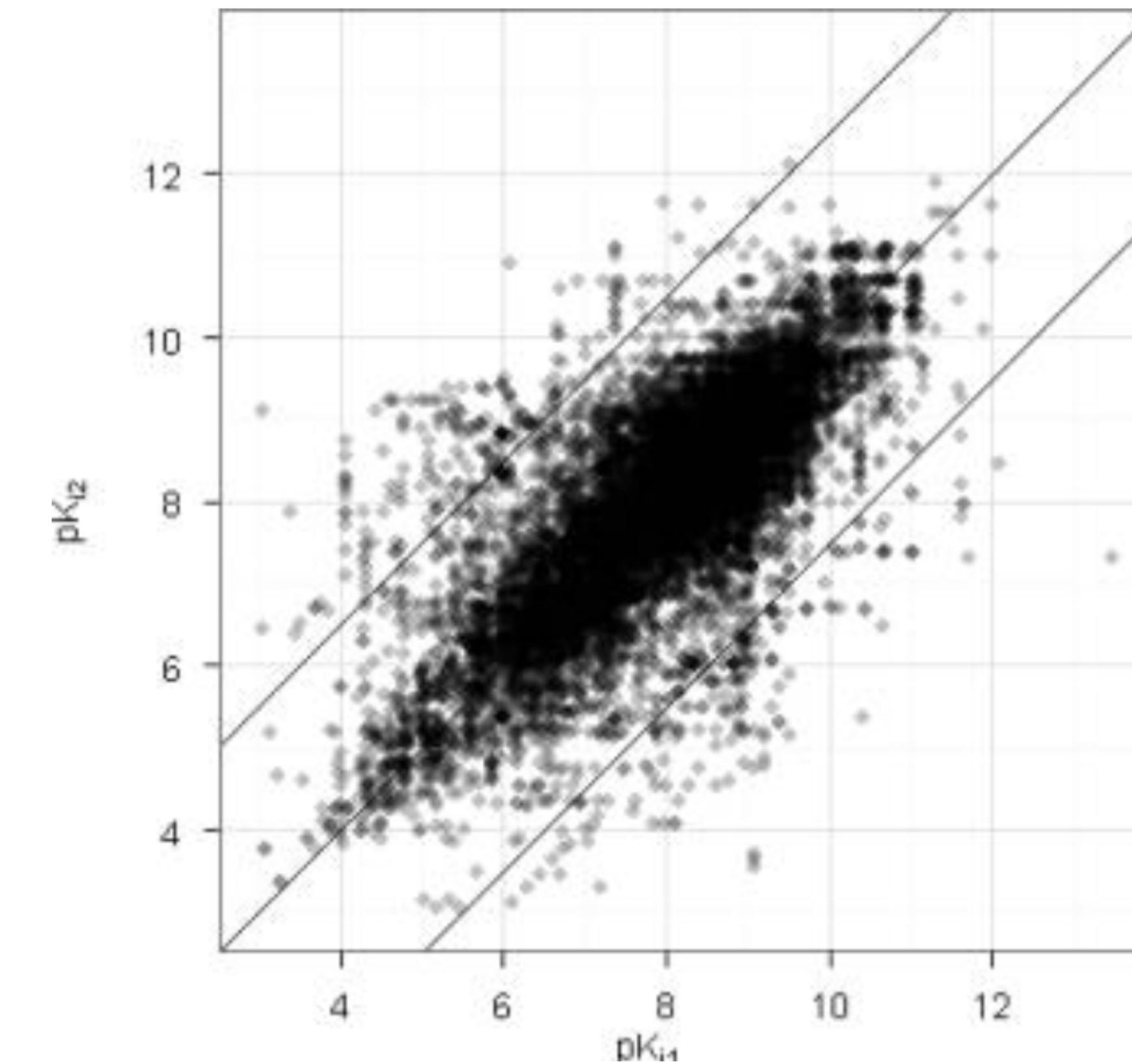


KEVIN HAUSER
SCHRÖDINGER

PUBLICLY AVAILABLE DATA CAN BE UNRELIABLE

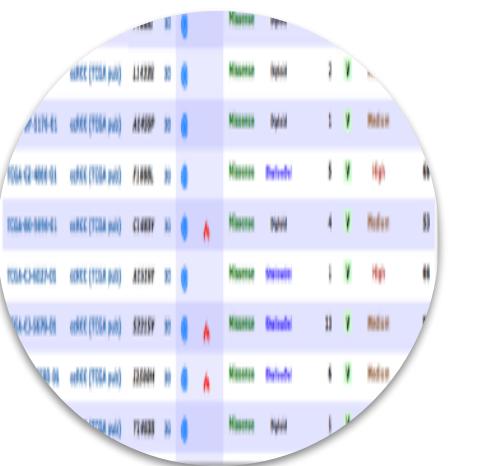


<https://cen.acs.org/articles/90/web/2012/05/Bosutinib-Buyer-Beware.html>

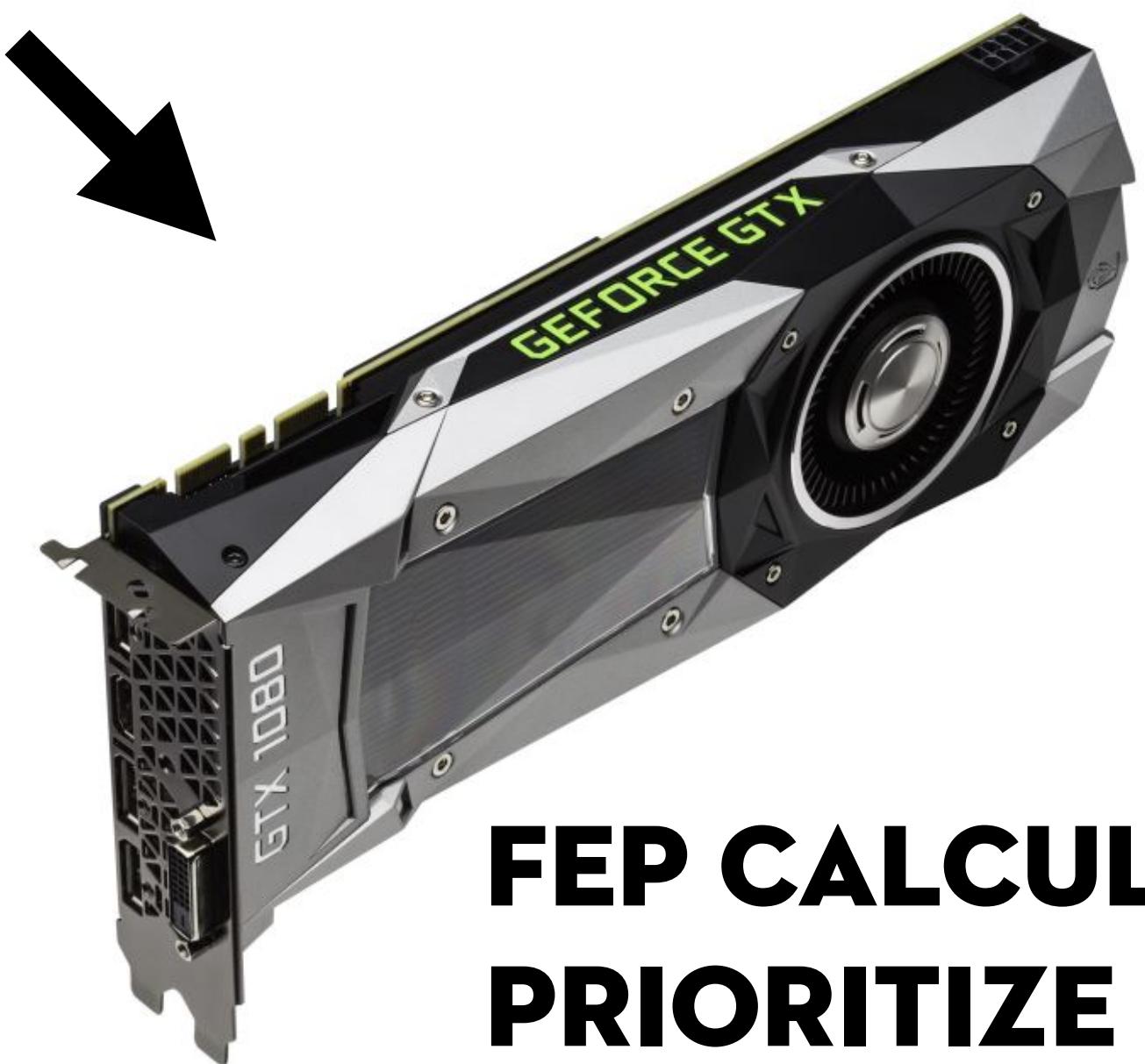


Kramer, C., Kalliokoski, T., Gedeck, P., & Vulpetti, A. (2012). The Experimental Uncertainty of Heterogeneous Public Ki Data. *Journal of Medicinal Chemistry*, 55(11), 5165–5173. <http://doi.org/10.1021/jm300131x>

USING COMPUTATION TO GUIDE EXPERIMENT



GATHER CLINICAL
MUTATIONS



FEP CALCULATIONS
PRIORITIZE MUTANTS
TO TEST



EXPERIMENT CAN
TEST PREDICTIONS
AND IDENTIFY
OUTLIERS FOR
FOLLOW UP

WHICH KINASES CAN WE EASILY EXPRESS IN BACTERIA?



FOR THE RECORD

High yield bacterial expression of active c-Abl and c-Src tyrosine kinases

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¹Howard Hughes Medical Institute, and ²Department of Molecular and Cell Biology and Department of Chemistry, University of California, Berkeley, California 94720, USA

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(RECEIVED August 3, 2005; FINAL REVISION September 2, 2005; ACCEPTED September 4, 2005)

Markus Seeliger
Stony Brook

Abstract

The Abl and Src tyrosine kinases are key signaling proteins that are of considerable interest as drug targets in cancer and many other diseases. The regulatory mechanisms that control the activity of these proteins are complex, and involve large-scale conformational changes in response to phosphorylation and other modulatory signals. The success of the Abl inhibitor imatinib in the treatment of chronic myelogenous leukemia has shown the potential of kinase inhibitors, but the rise of drug resistance in patients has also shown that drugs with alternative modes of binding to the kinase are needed. The detailed understanding of mechanisms of protein–drug interaction and drug resistance through biophysical methods demands a method for the production of active protein on the milligram scale. We have developed a bacterial expression system for the kinase domains of c-Abl and c-Src, which allows for the quick expression and purification of active wild-type and mutant kinase domains by coexpression with the YopH tyrosine phosphatase. This method makes practical the use of isotopic labeling of c-Abl and c-Src for NMR studies, and is also applicable for constructs containing the SH2 and SH3 domains of the kinases.

Keywords: Src; Abl; imatinib; tyrosine kinases; biophysical methods; bacterial expression; NMR

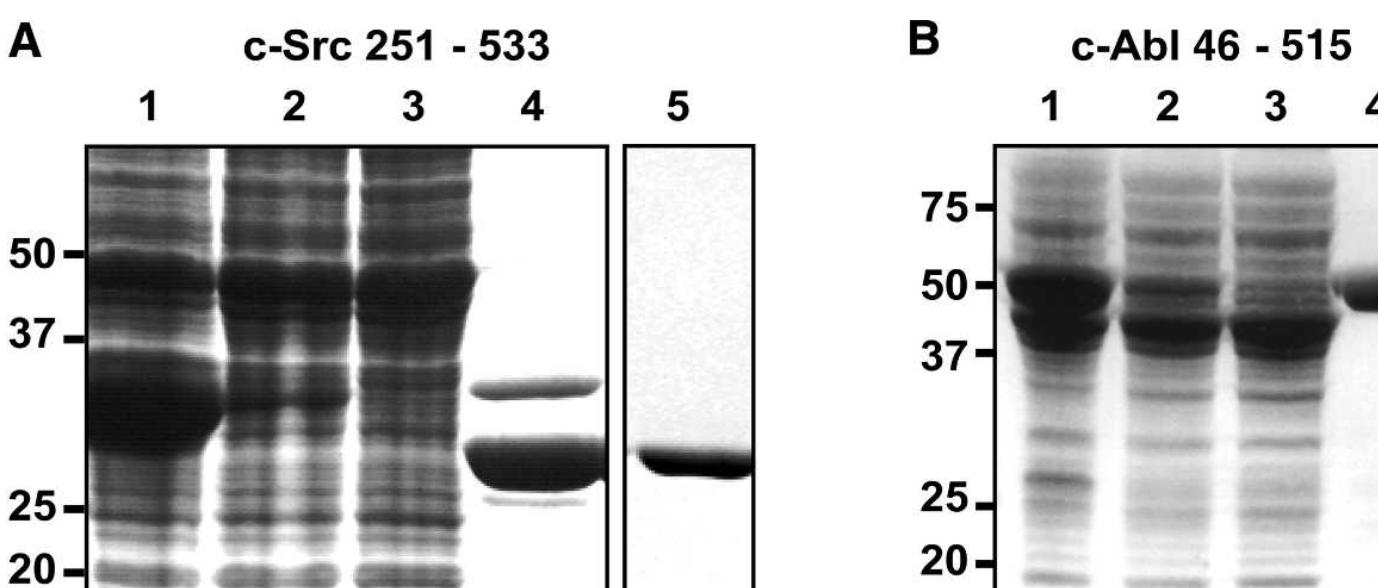
Protein tyrosine kinases play a central role in cellular signaling. The disruption of the regulatory mechanisms that control protein tyrosine kinase activity is associated with many diseases, particularly cancer. c-Src and c-Abl are closely related nonreceptor tyrosine kinases that contain SH2 and SH3 domains in addition to the catalytic tyrosine kinase domain (Thomas and Brugge 1997; Pendegast 2002). Due to the success of the Abl tyrosine kinase inhibitor imatinib (Gleevec, Glivec, STI-571, [Novartis]) in the treatment of chronic myelogenous leu-

kemia, protein kinase inhibitors have now been established as excellent therapeutic agents in the clinic (Noble et al. 2004; Krause and Van Etten 2005). The high degree of conservation in the sequences of protein kinases and the fact that most if not all kinase inhibitors are competitors of ATP, which is the common substrate of protein kinases, makes it difficult to achieve specificity for individual kinases (Capdeville et al. 2002). Considerable effort is therefore being invested in understanding the nuanced differences in conformation and dynamics that distinguish one kinase from the other. Moreover, a substantial fraction of patients undergoing treatment with imatinib develop resistance mutations in the Abl kinase domain, which render the kinase resistant to imatinib, and understanding the molecular basis of resistance is also an important issue (Shah et al. 2002; Deininger et al. 2005).

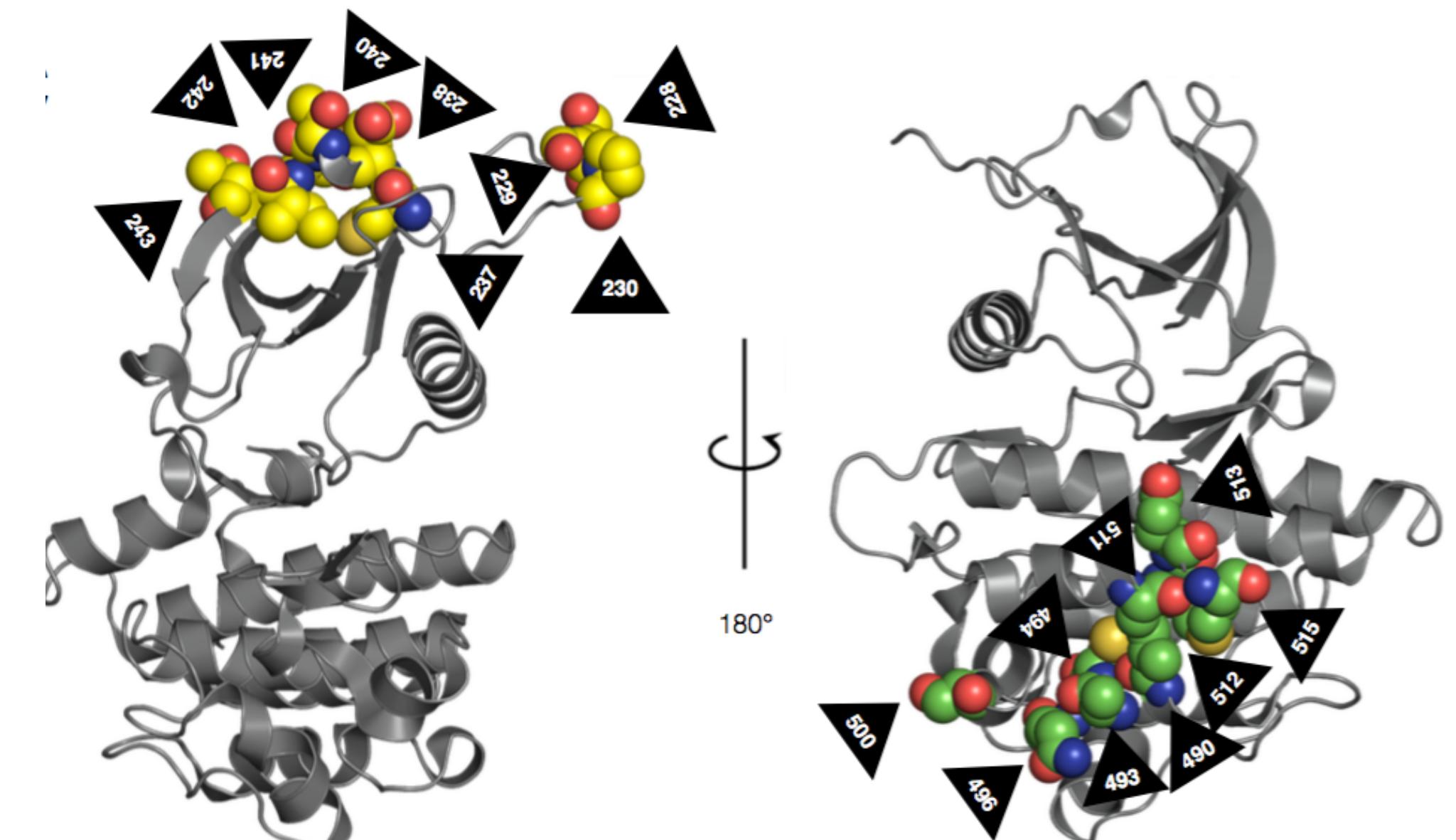
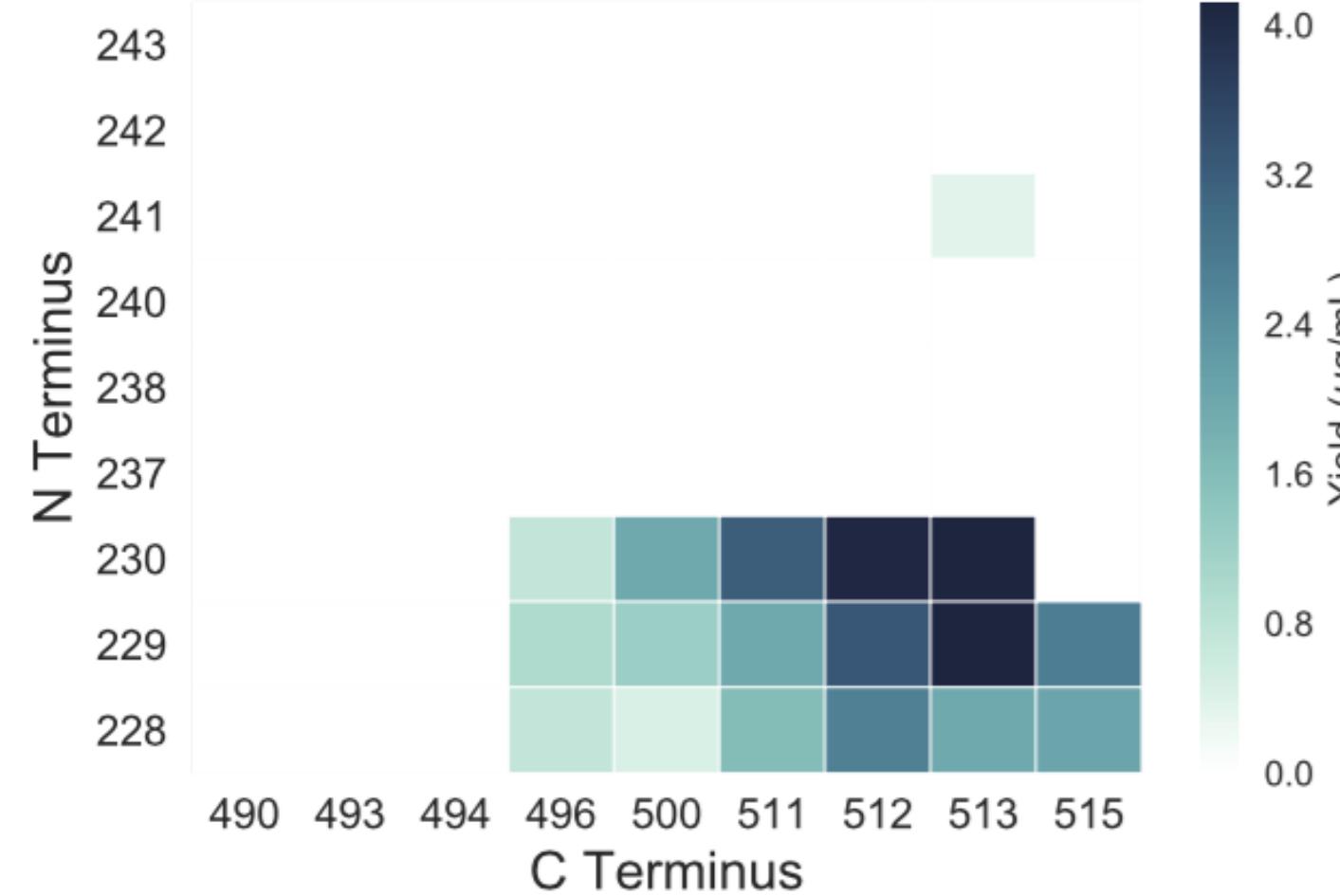
Reprint requests to: John Kuriyan, Department of Molecular and Cell Biology, 16 Barker Hall, University of California, Berkeley, CA 94720-3202, USA; e-mail: kuriyan@berkeley.edu; fax: (510) 643-2352.

Article published online ahead of print. Article and publication date are at <http://www.proteinscience.org/cgi/doi/10.1110/ps.051750905>.

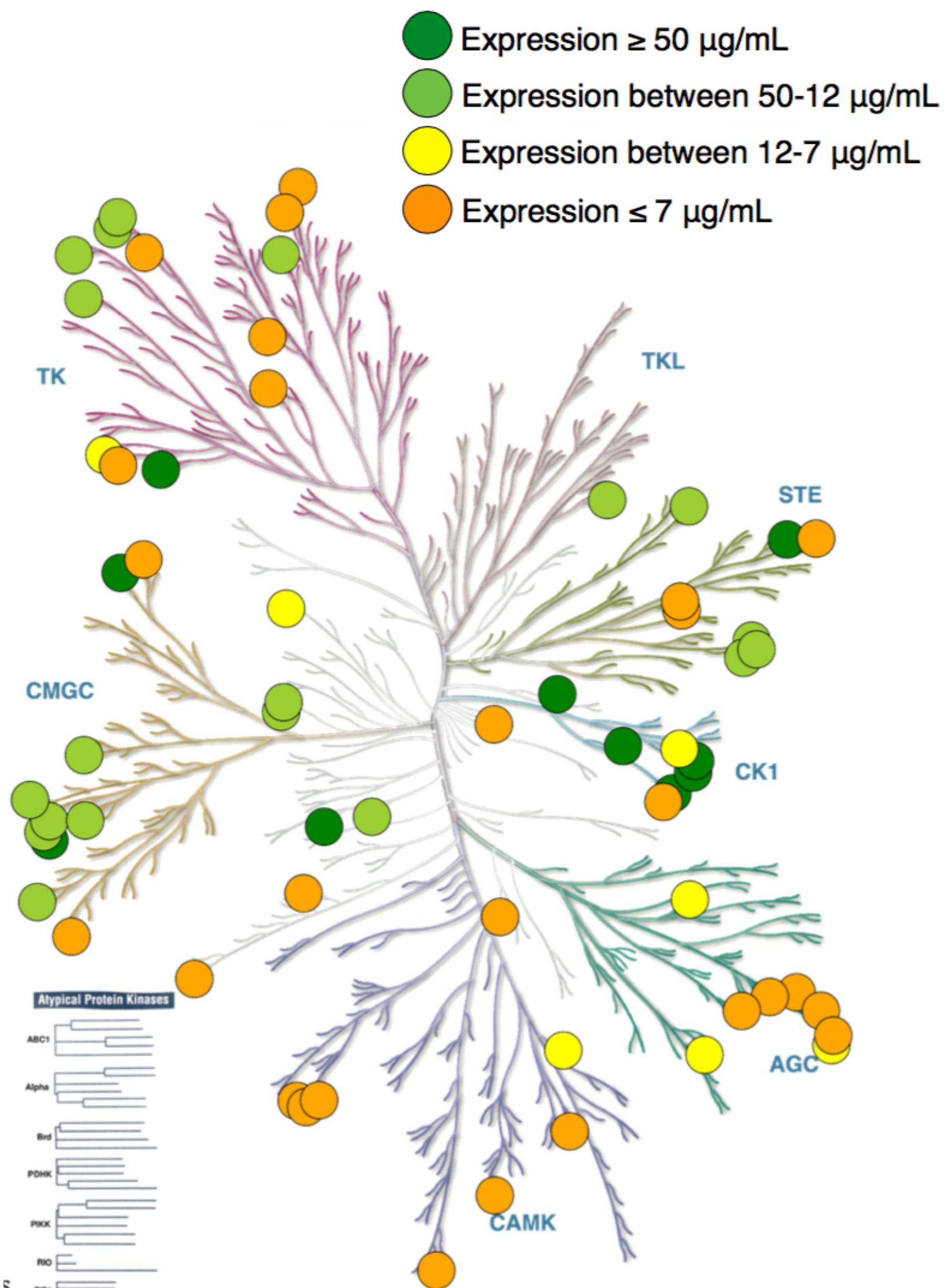
co-transform tyrosine kinases with YopH plasmid



KINASE EXPRESSION IS SENSITIVE TO CONSTRUCT BOUNDARY CHOICE



WHICH KINASES ARE EXPERIMENTALLY TRACTABLE?

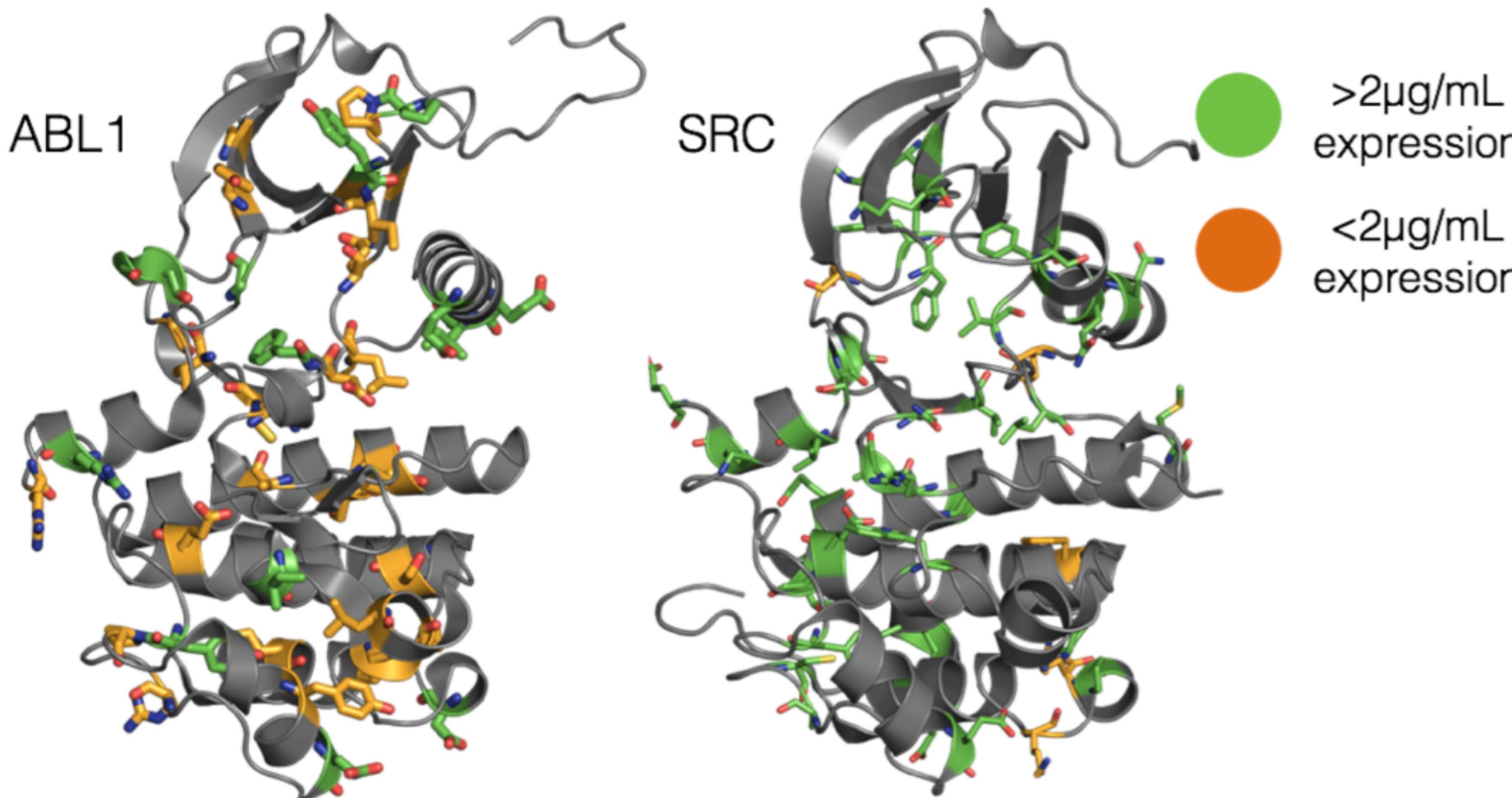


*Illustration reproduced courtesy of Cell Signaling Technology, Inc. (www.cellsignal.com)

**WE USED PREVIOUSLY REPORTED
CONSTRUCT BOUNDARIES**

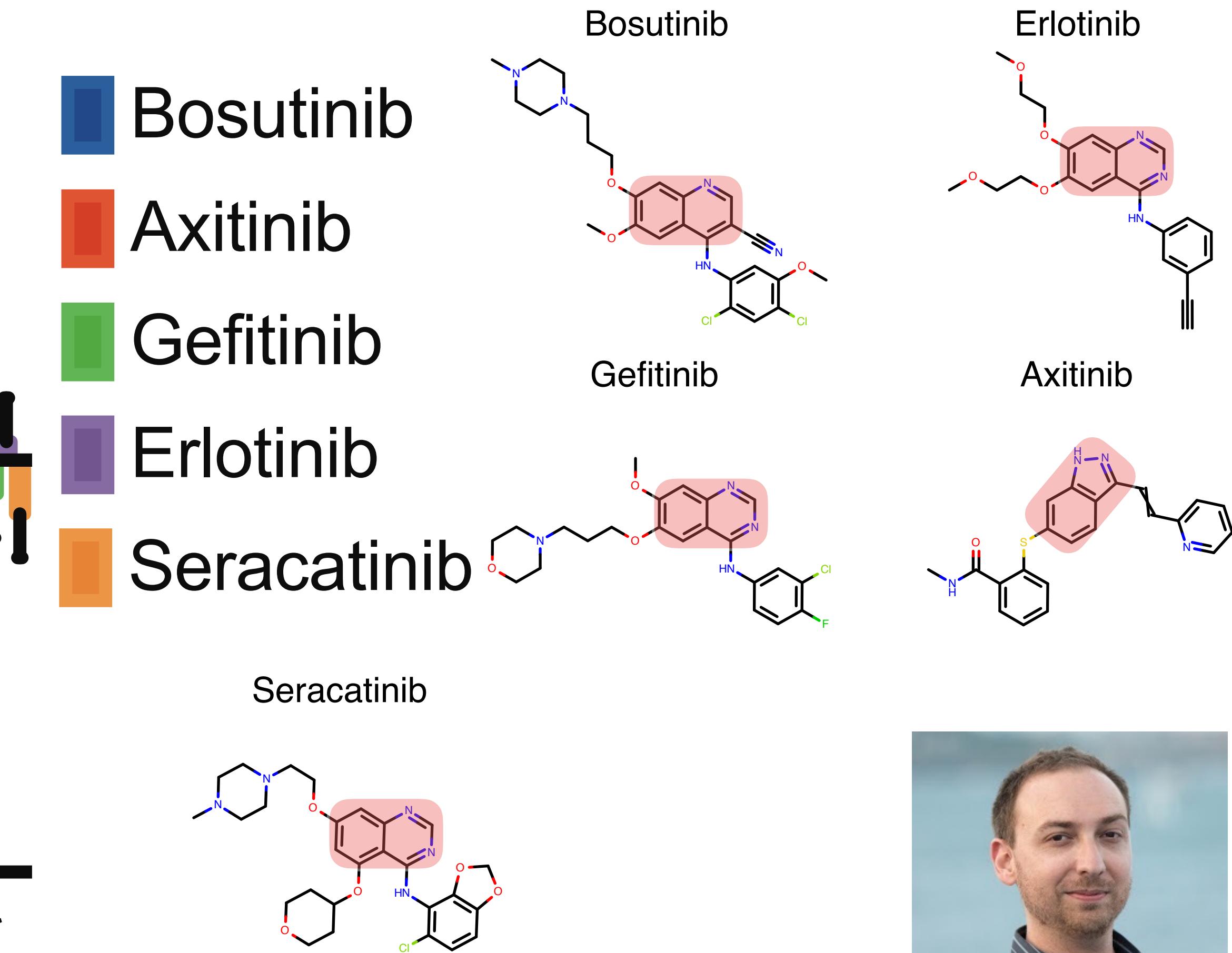
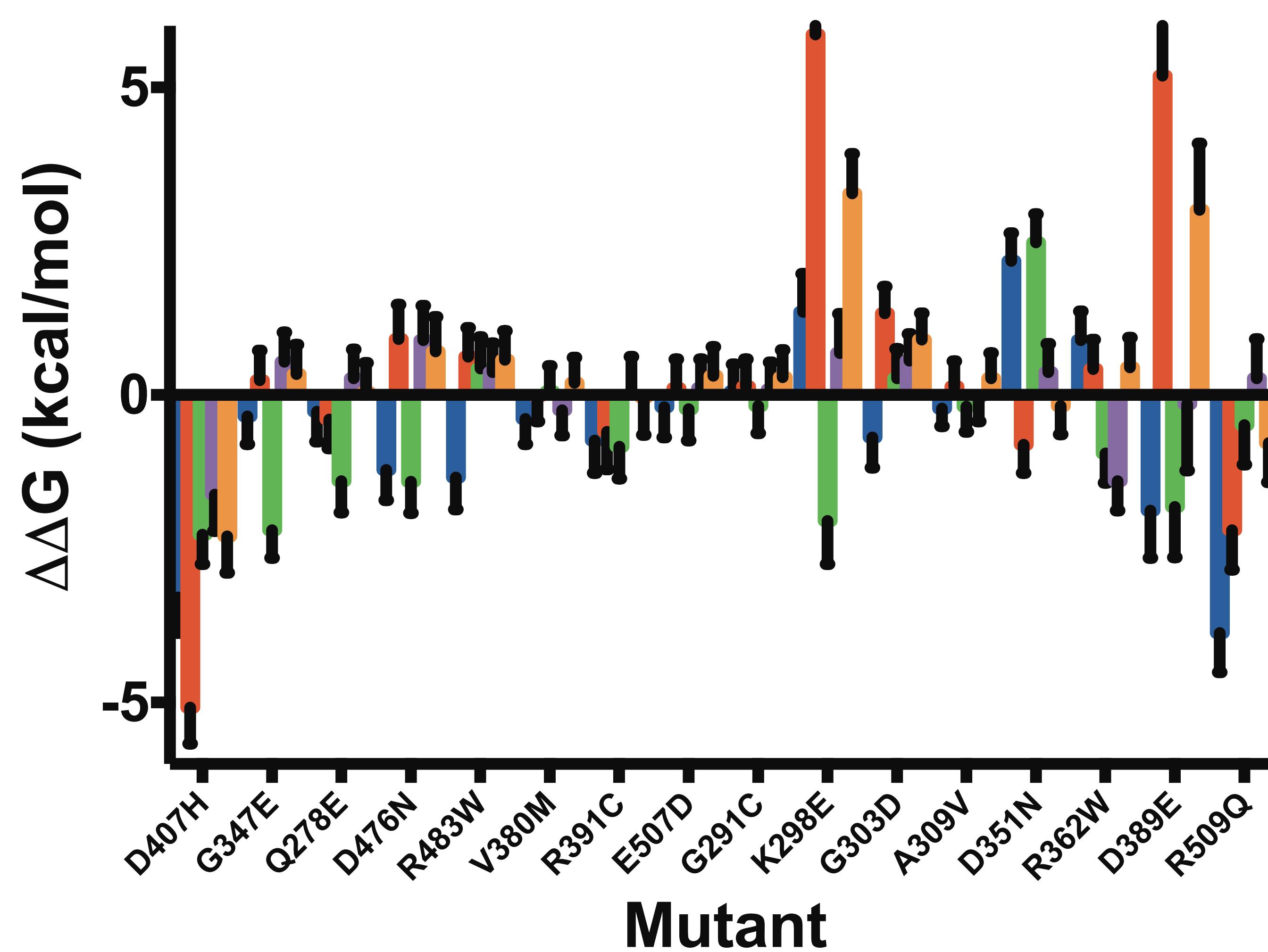
**SCREENED 96 KINASES TO IDENTIFY
KINASES EXPRESSIBLE IN E COLI**

ARE THESE CONSTRUCTS SUITABLE FOR EXPRESSING CLINICAL MUTANTS?



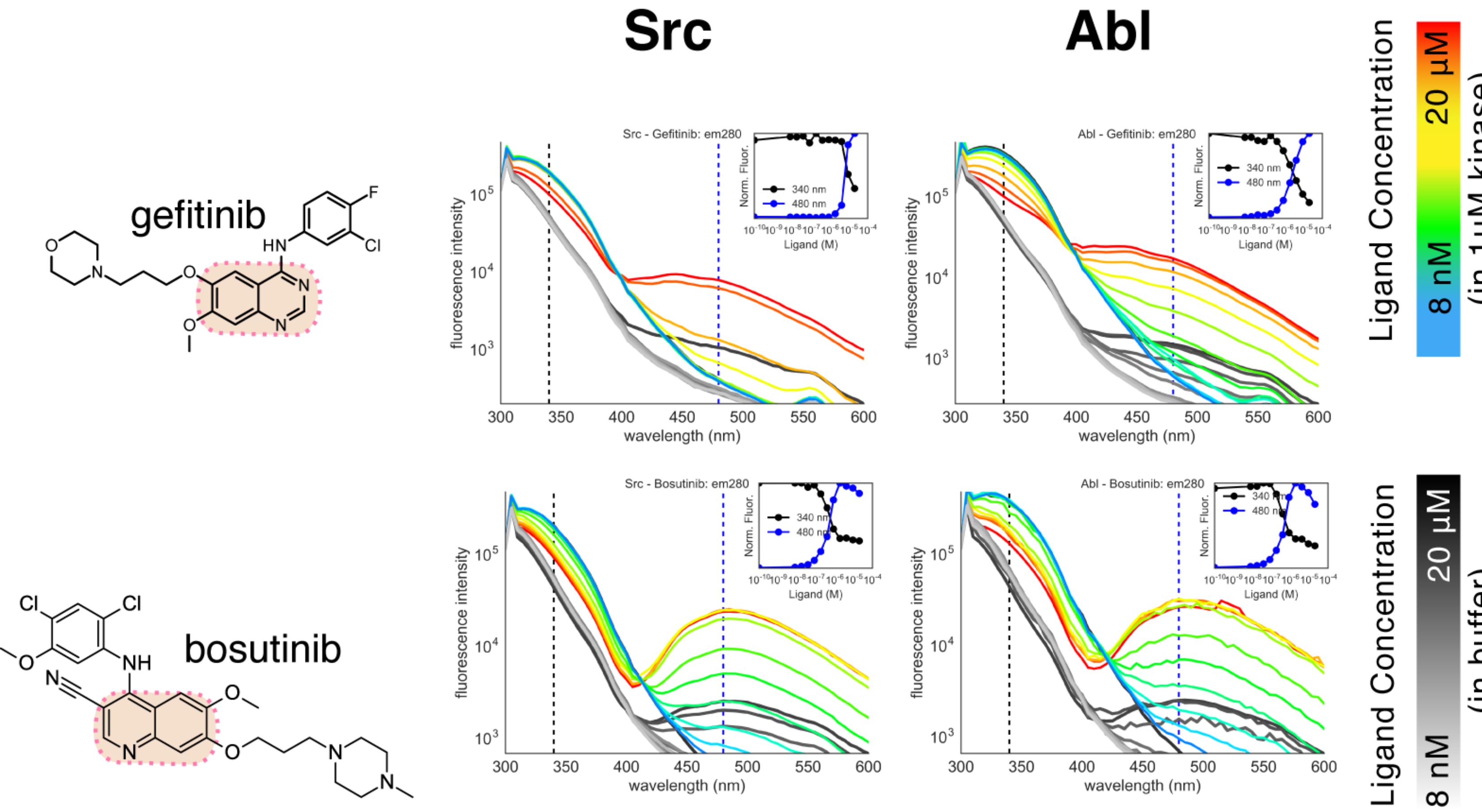
Src (254–536)	Mutation ¹	Functional Impact Score ²	yield (μ g/mL)	% of WT expression
WT	-	-	35.7	-
T456S	Neutral	80.9	227	
R388G	Medium	61.5	172	
K298E	High	54.5	153	
V380M	Neutral	51.7	145	
D368N	Neutral	49.9	140	
D521N	Low	42.8	120	
R463Q	Neutral	38.4	108	
R391C	Neutral	37.5	105	
E323D	Low	37.2	104	
A309V	Low	35.9	98	
G303D	Neutral	34.1	96	
R362Q	Neutral	33.6	94	
L361M	Medium	31.7	89	
A421V	Neutral	30.7	86	
V402L	Neutral	30.6	86	
V397M	Medium	29.8	84	
Q278E	Neutral	29.6	83	
Q312H	Low	29.5	83	
L353V	Medium	29.0	81	
L454V	Neutral	29.0	81	
P307R	Neutral	28.6	80	
V340I	Low	28.0	78	
P307S	Neutral	24.2	68	
D476N	Neutral	23.3	65	
D351N	Neutral	22.9	64	
T293A	Neutral	22.2	62	
S345C	Low	22.2	62	
P428S	Medium	22.2	62	
E507D	Neutral	20.7	58	
D389E	High	20.0	56	
R503Q	Neutral	17.3	49	
D407H	High	15.9	45	
R463L	Neutral	14.9	42	
G291C	Medium	11.9	33	
G347E	Medium	10.2	29	
R483W	High	9.8	27	
P487L	Medium	6.0	17	
R463W	Medium	5.2	15	
R362W	Low	3.9	11	
S493F	Low	3.0	8	
P491S	Low	2.2	6	

PRELIMINARY FEP CALCULATIONS REVEAL SENSITIZING AND RESISTANT SRC MUTANTS



KEVIN HAUSER
SCHRÖDINGER

A FLORESCENCE ASSAY CAN MEASURE THE BINDING AFFINITIES FOR KINASES AND THEIR MUTANTS

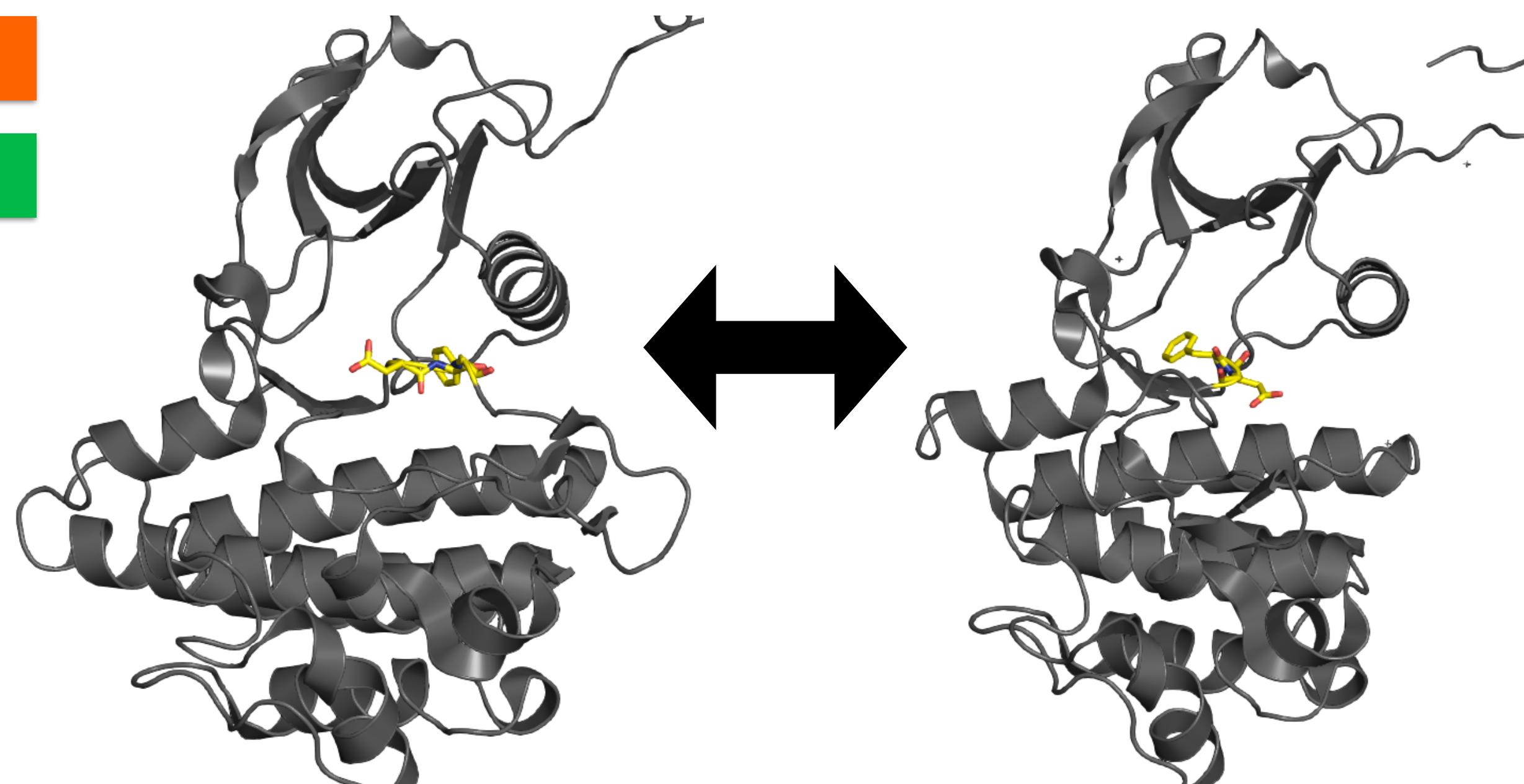


TESTING PREDICTIONS ALLOWS IDENTIFICATION OF OUTLIERS AND REFINING FUTURE PREDICTIONS

PROTONATION STATE CHANGES IN THE LIGAND OR KINASE MAY IMPACT PREDICTIONS

pdbid	inhibitor	kinase	Δ protein	Δ inhibitor	Δ protomer	
3UE4	Bosutinib	ABL	0	0.5	YES	proton gain
2GQG	Dasatinib	ABL	-0.1	0.6	YES	
4XEY	Dasatinib	ABL	0.12	0.82	YES	
2HYY	Imatinib	ABL	-0.2	-0.01	NO	proton loss
3PYY	Imatinib	ABL	-0.28	0.01	NO	
3CS9	Nilotinib	ABL	0.1	0.06	NO	
3OXZ	Ponatinib	ABL	-0.6	0.02	NO	
3IK3	Ponatinib	ABL T315I	-0.63	0.06	NO	
3AOX	Alectinib	ALK	0	0.13	NO	
4MKC	Ceritinib	ALK	0.7	0	NO	
2XP2	Crizotinib	ALK	-0.04	-0.77	YES	
4ANQ	Crizotinib	ALK G1269A	-0.1	-0.76	YES	
2YFX	Crizotinib	ALK L1196M	-0.1	-0.77	YES	
4ANS	Crizotinib	ALK L1196M/G1269A	-0.1	-0.77	YES	

LARGE CONFORMATIONAL CHANGES OR NEW BINDING MODES CAN BE IDENTIFIED WITH X-RAY CRYSTALLOGRAPHY

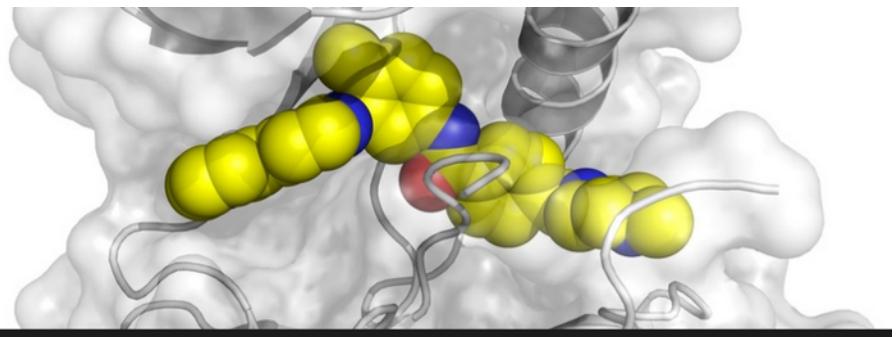


Marilyn Gunner and Salah Salah (CCNY) in collaboration with Markus Seeliger (Stony Brook) and Paul Czodrowski (Merck Serono)

PDB: 2F4J

PDB: 1IEP

ACKNOWLEDGEMENTS



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JOHN CHODERA
LEVI NADEN
PATRICK GRINAWAY
CHAYA STERN
BAS RUSTENBURG
MEHTAP ISIK
RAFAL WIEWIORA
JOSH FASS
ANDREA RIZZI



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MARILYN GUNNER
SALAH SALAH

