

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

EMBO WORKSHOP: INTEGRATING GENOMICS AND BIOPHYSICS TO COMPREHEND FUNCTIONAL GENETIC VARIATION

TURIN, ITALY

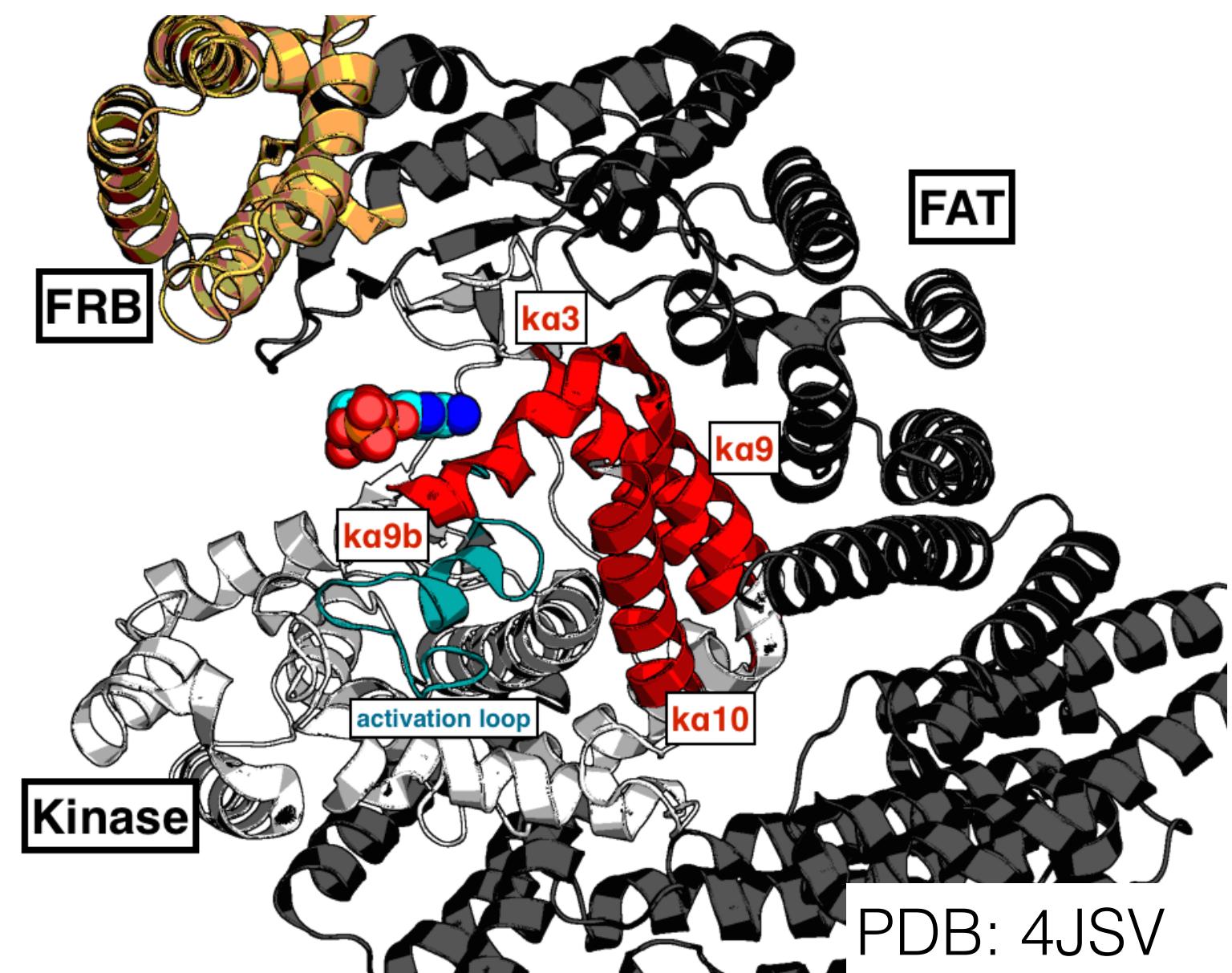
09/08/17



STEVEN ALBANESE
CHODERA LAB // MSKCC

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

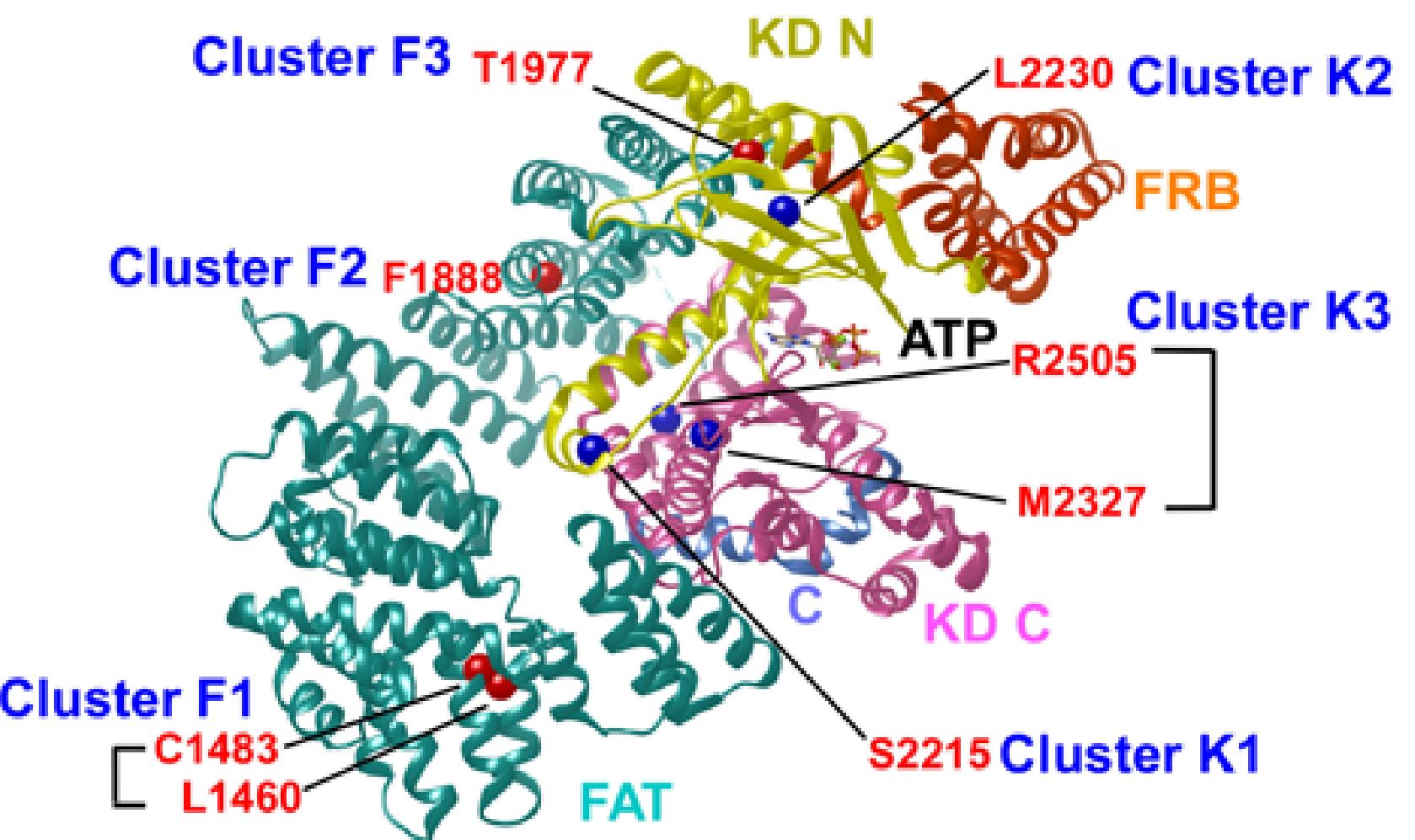
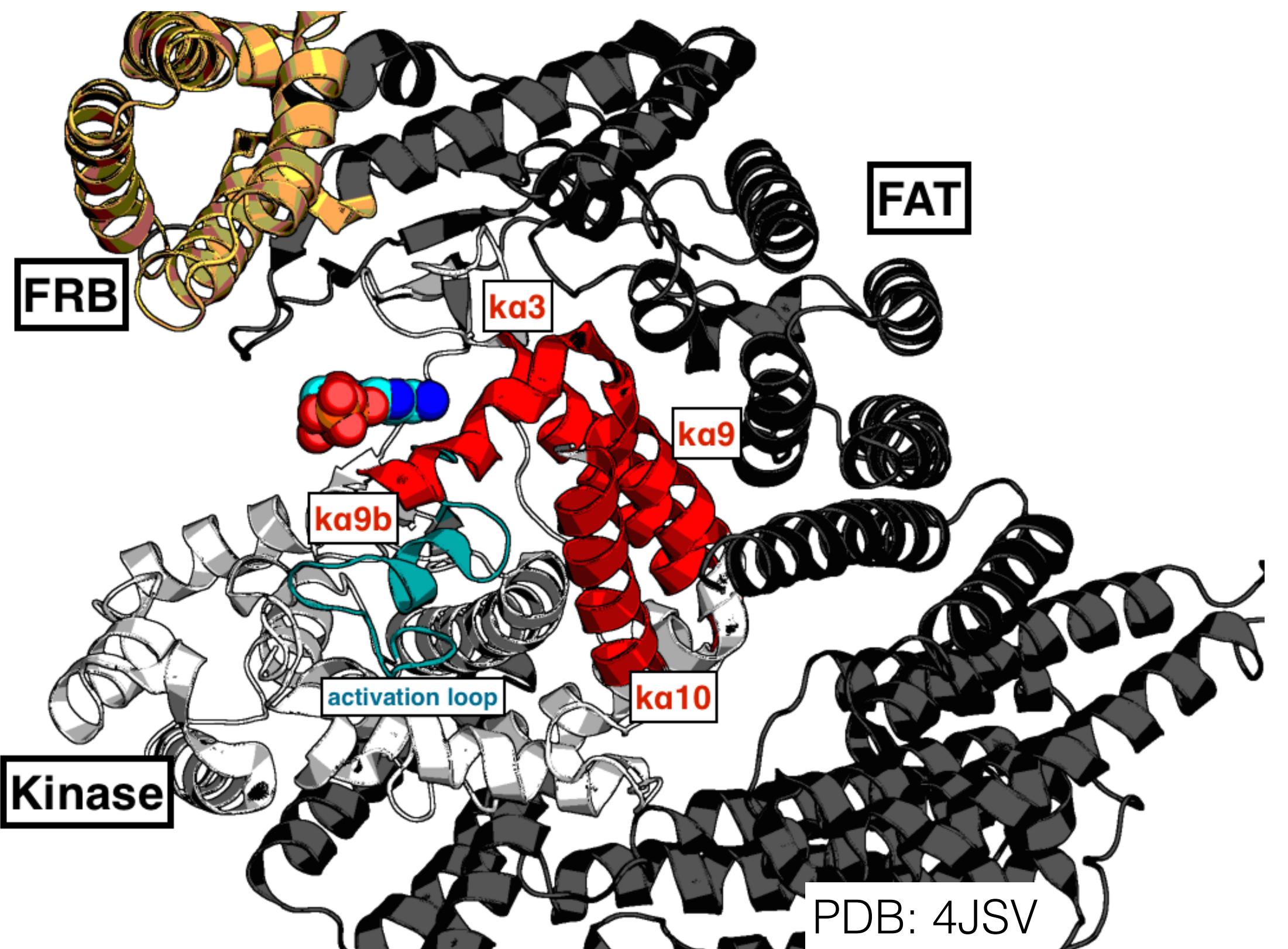
G5R R717L K860N E919V A1105P



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)

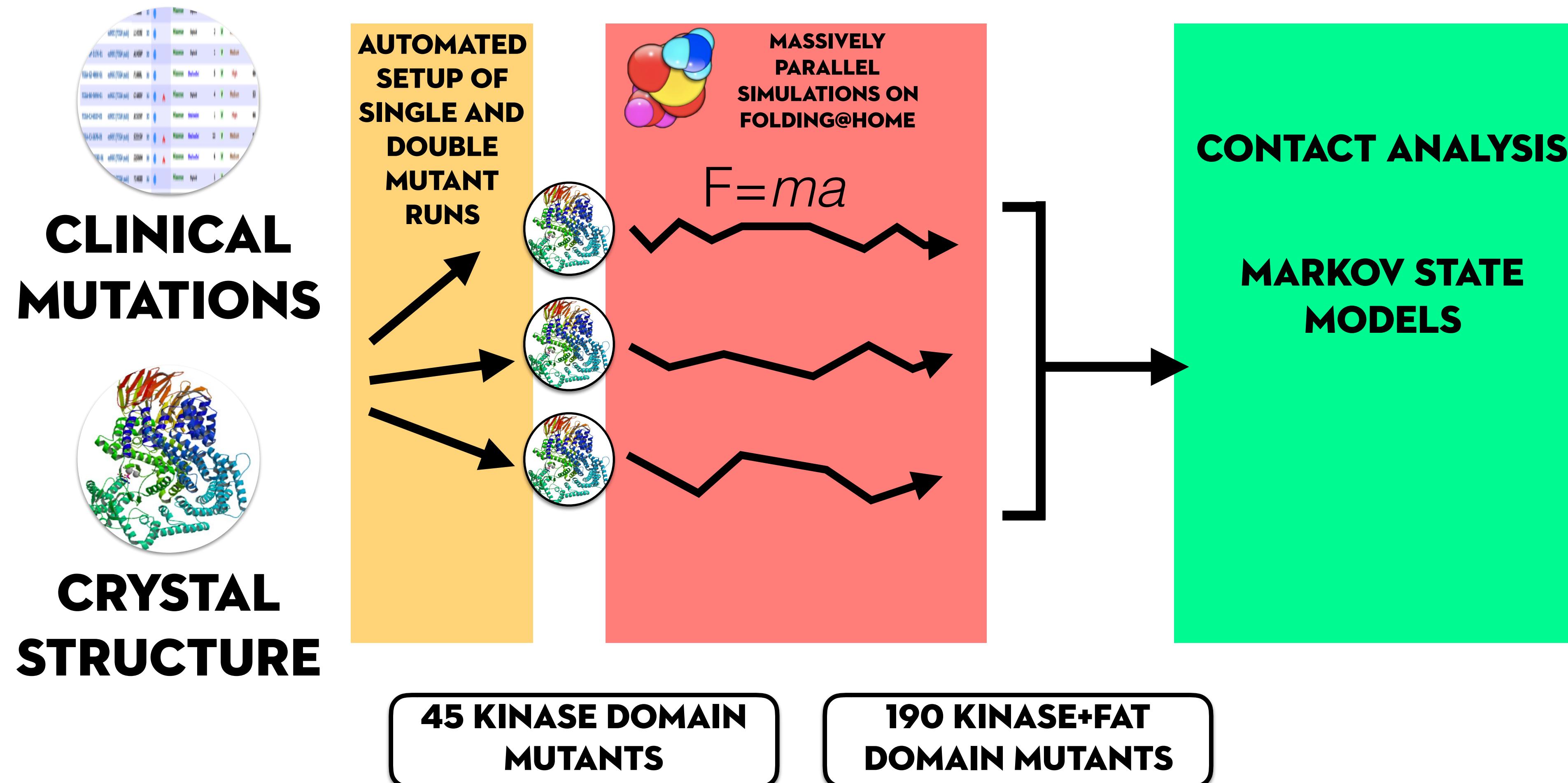
CAN WE USE PHYSICAL MODELING TO GAIN MECHANISTIC INSIGHT INTO CLINICAL MUTATIONS?



DEPTOR RAPTOR	DEPTOR RAPTOR	-	RAPTOR	RAPTOR Kinase	-	Kinase	-	DEPTOR -	Mechanism
F1	F1	F2	F3	K1	K2	K3	K3		Cluster
L1460P	C1483F	F1888L	T1977K	S2215F	L2230V	M2327I	R2505P	Mutation	
-	-	+	+	+	+	+	-	L1460P	F1
		+	+	+	+	+	-	C1483F	F1
		+	+	+	+	+	+	F1888L	F2
			+	+	+	+	+	T1977K	F3
				+	+	+	+	S2215F	K1
				+	-	-	-	M2327I	K3
					+	+	+	L2230V	K2
						-	-	R2505P	K3

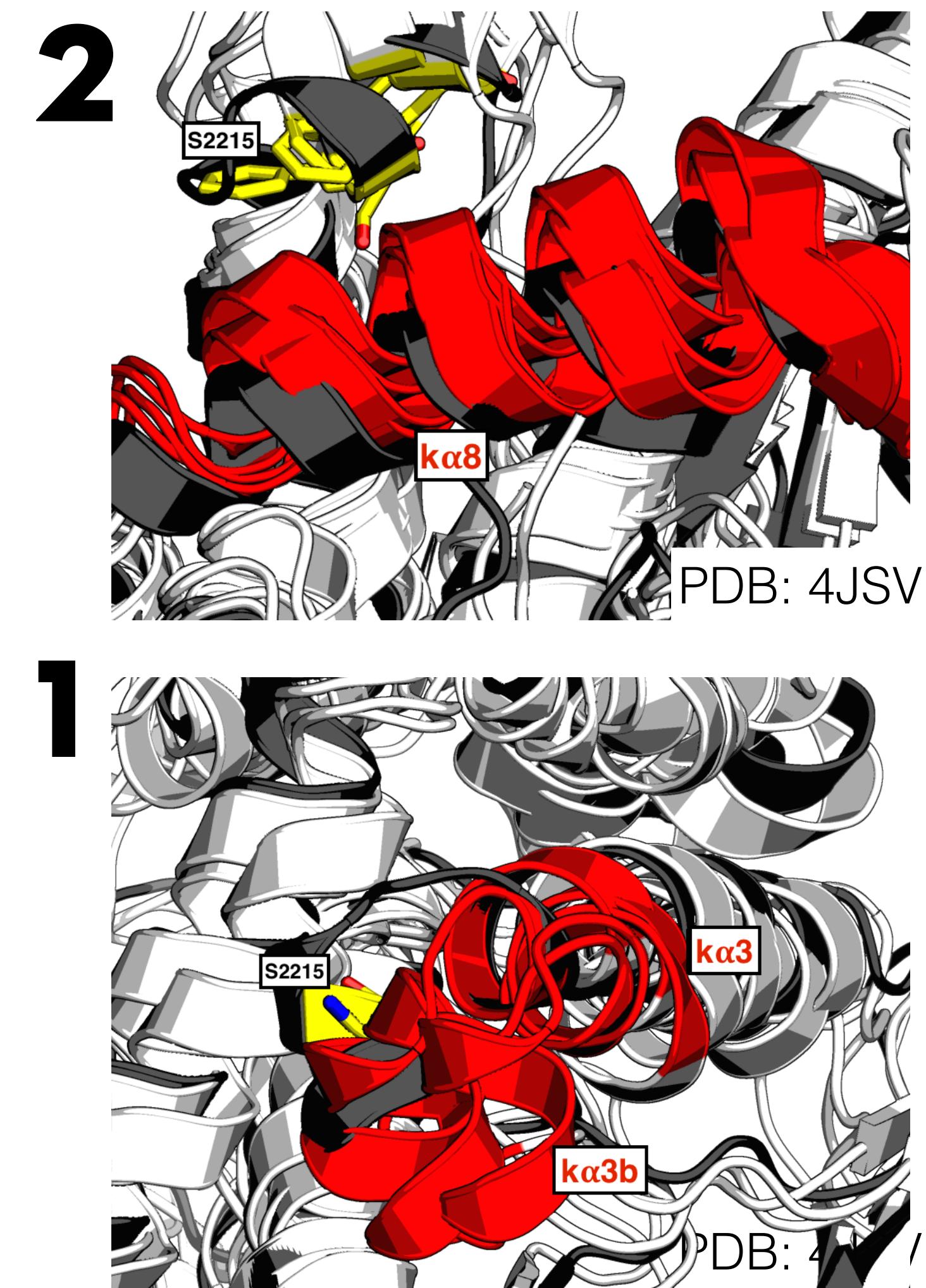
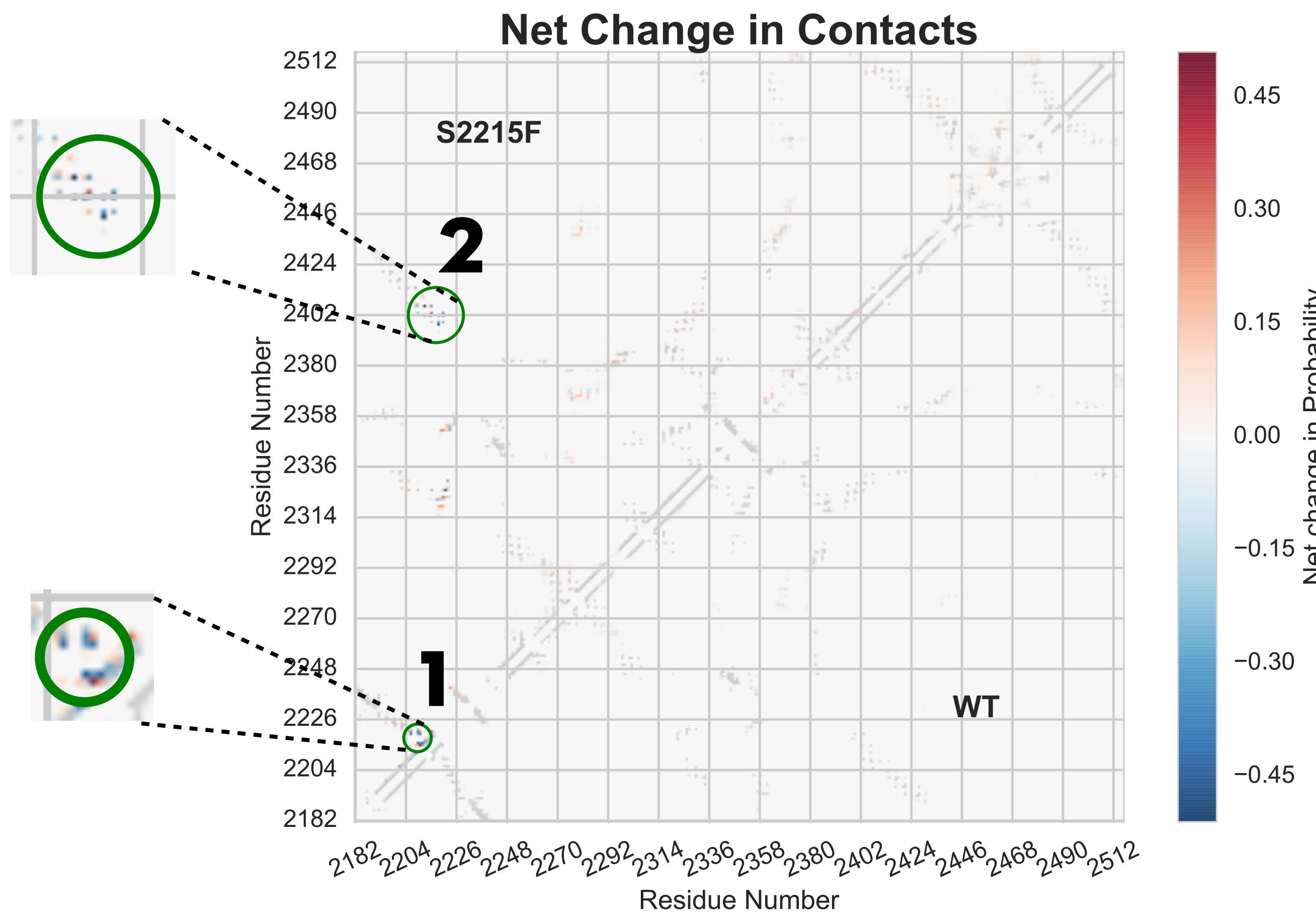
-	No synergism
+	synergism

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

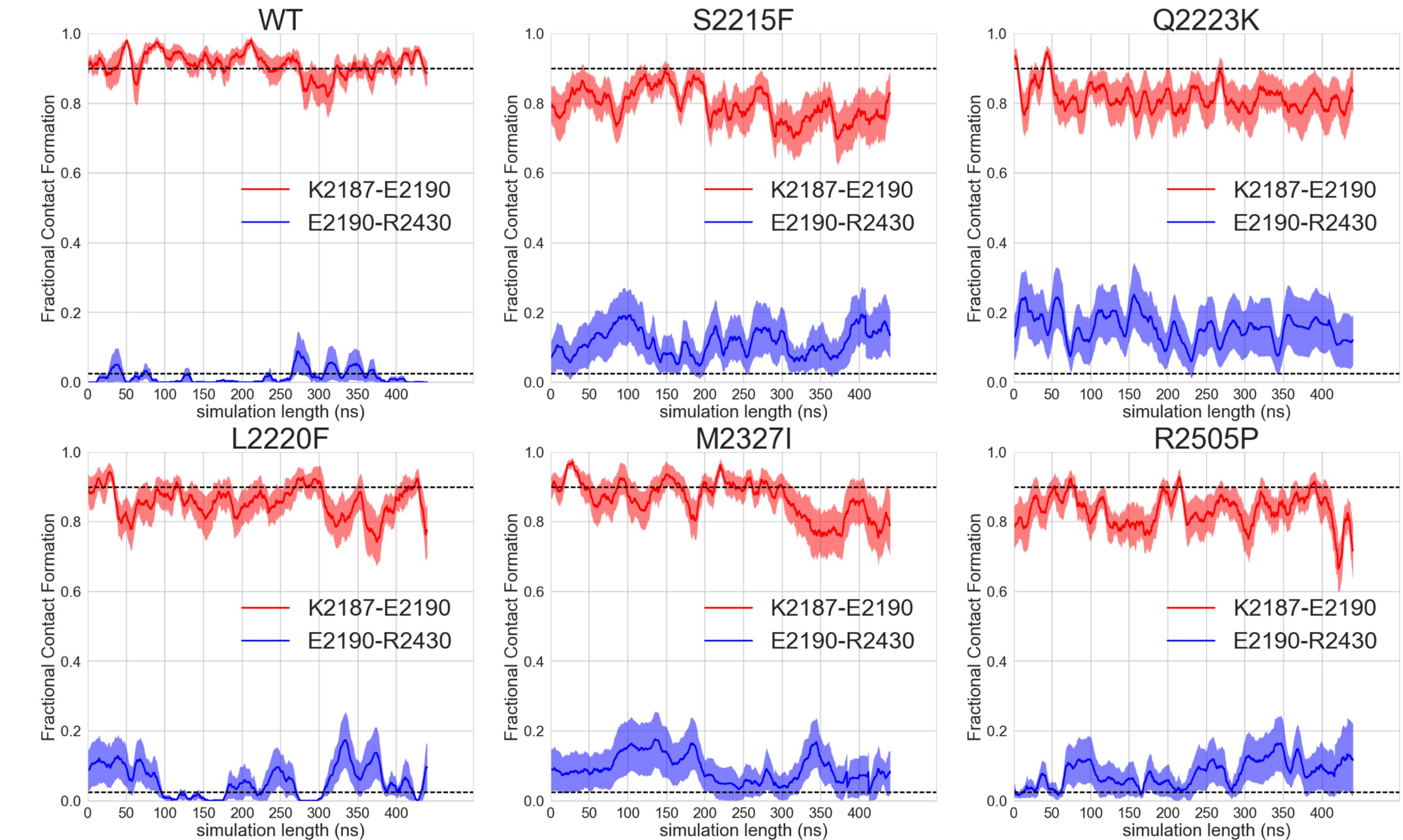
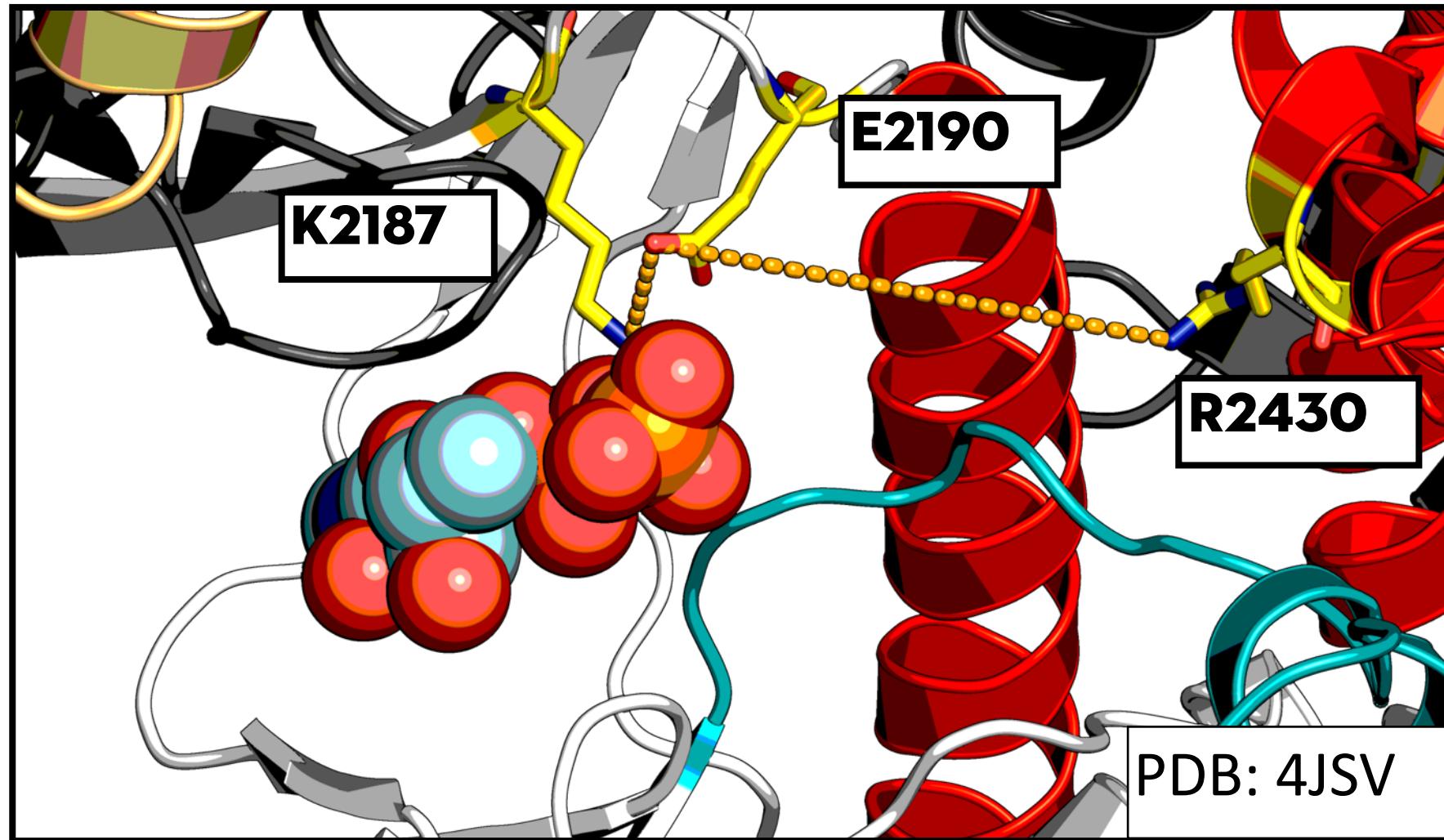


Adapted from Danny Parton

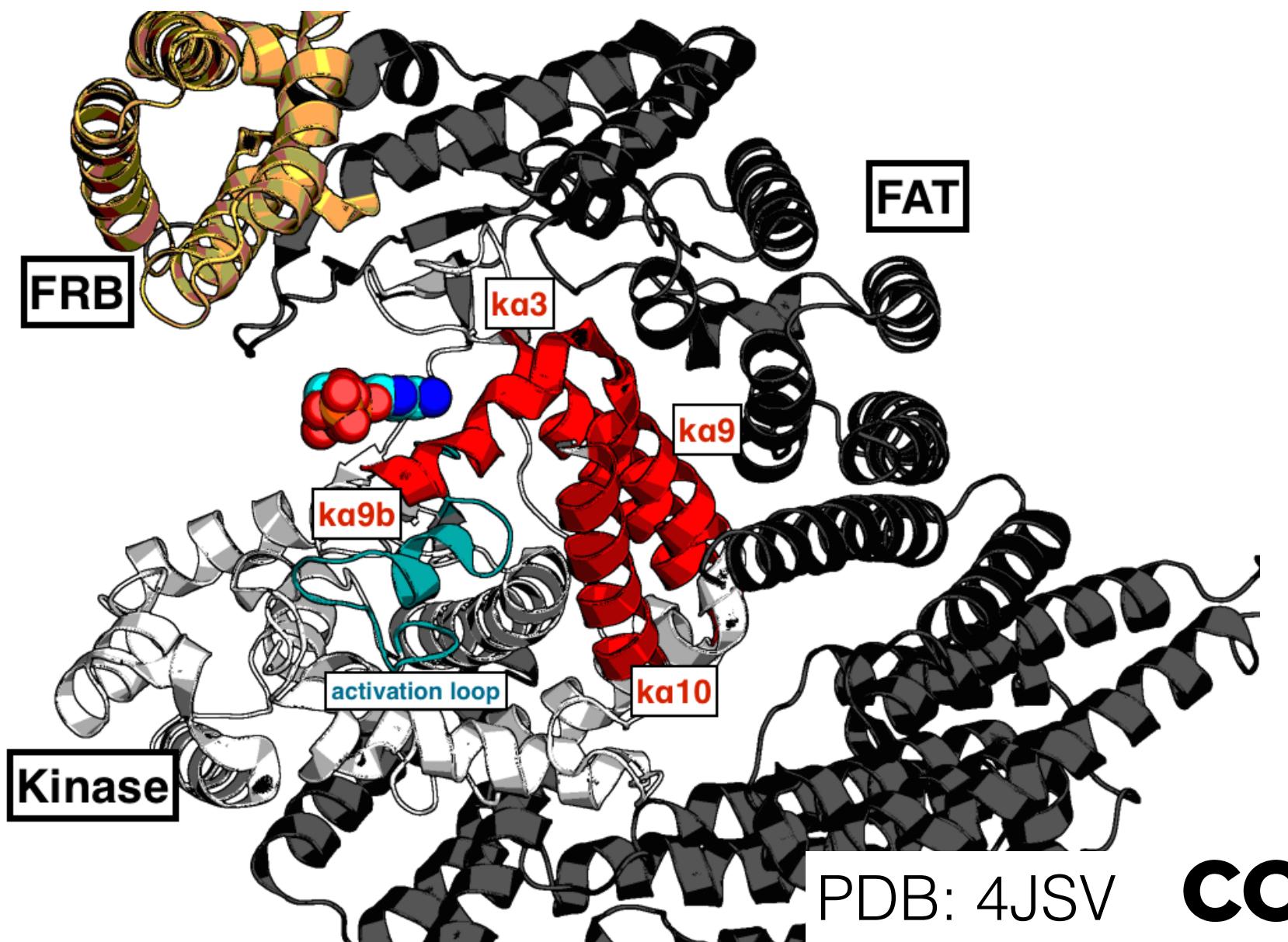
ACTIVATING MUTATION S2215F CAUSES HELICAL UNWINDING



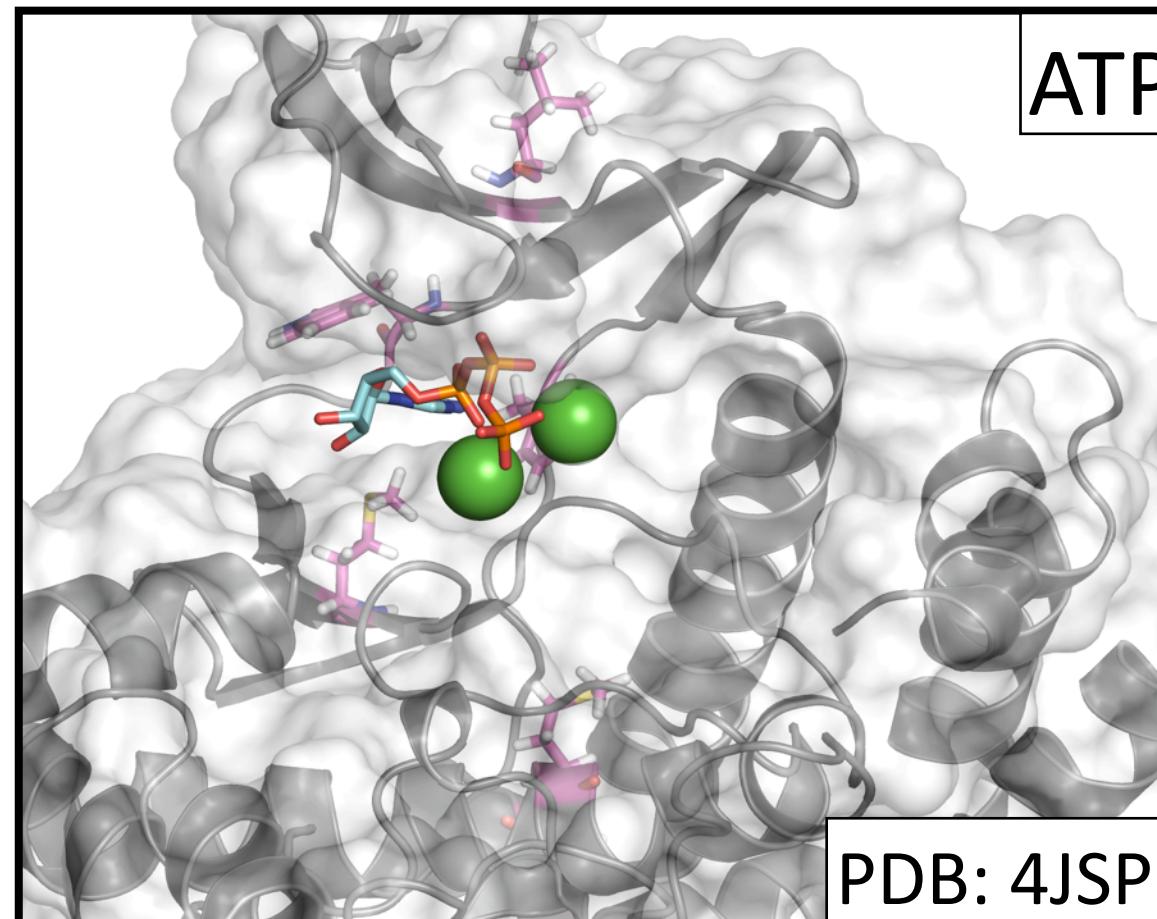
DO MUTATIONS STABILIZE KEY SALT BRIDGES REQUIRED FOR ACTIVITY?



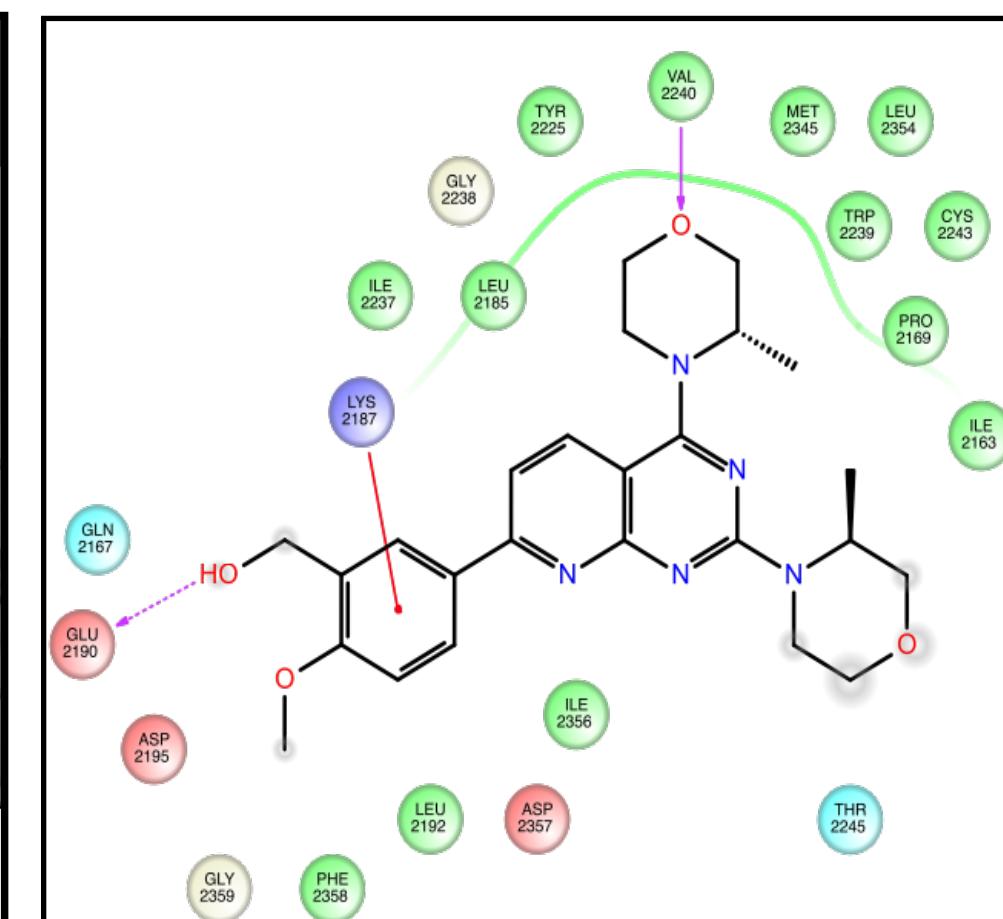
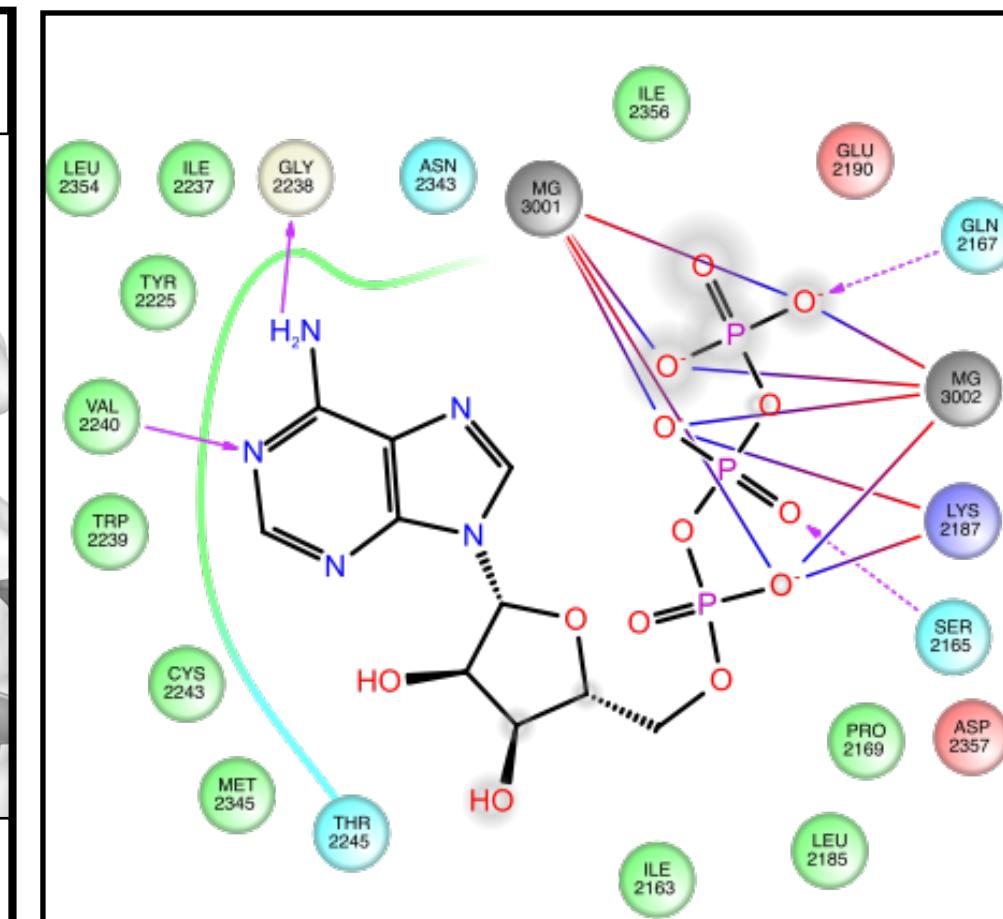
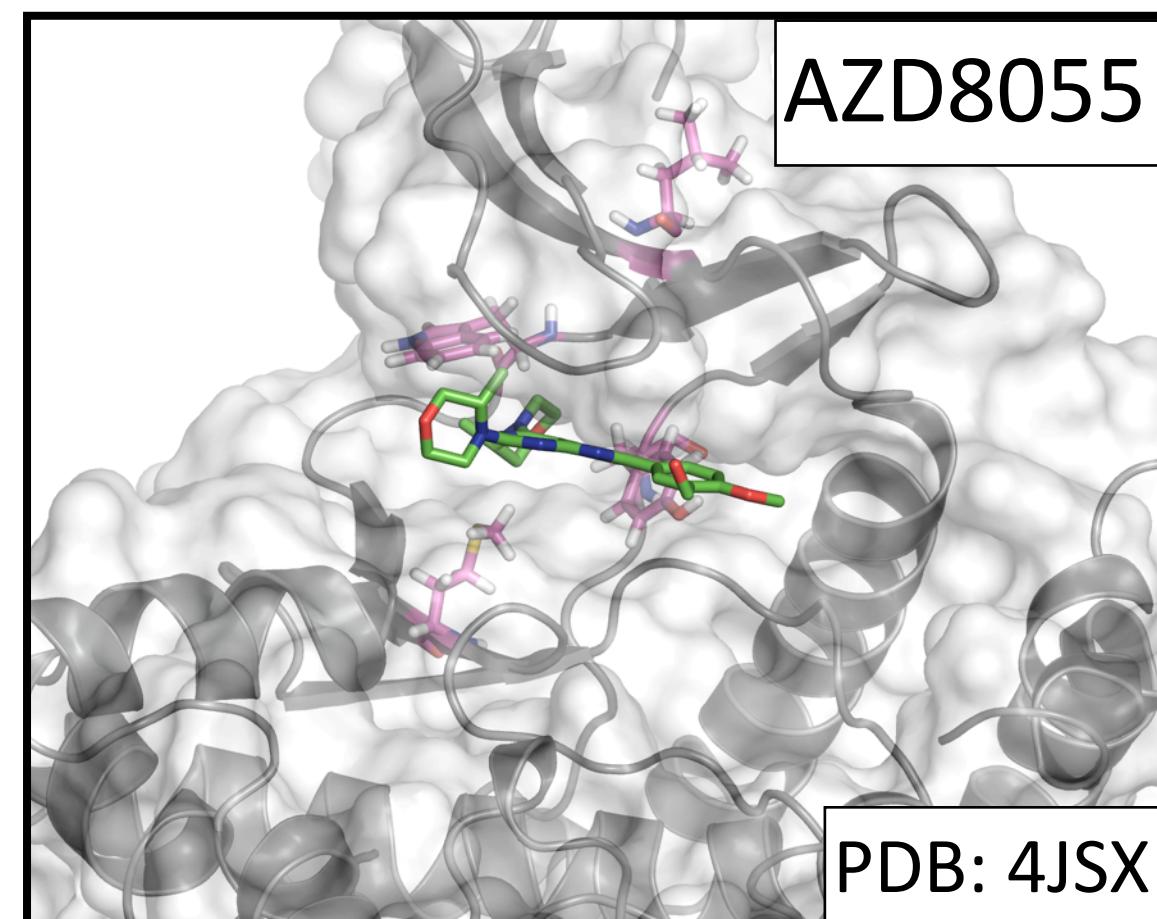
A KEY CHALLENGE: COMPUTING PHYSICAL PROPERTIES THAT CAN BE TESTED



ACTIVATE MTOR BY INCREASING ATP AFFINITY?



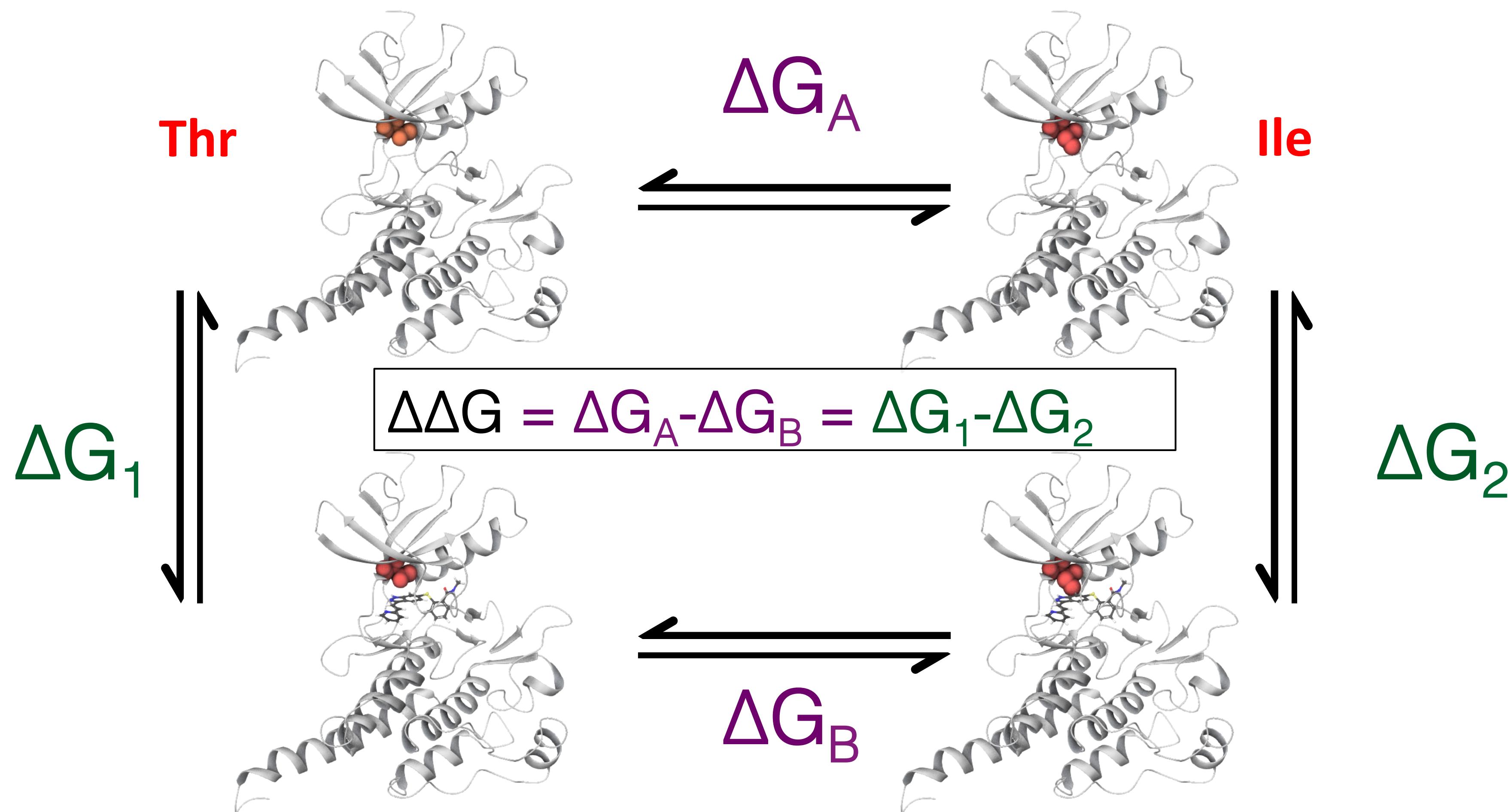
CONFER SENSITIVITY OR RESISTANCE TO AN ATP-COMPETITIVE INHIBITOR?



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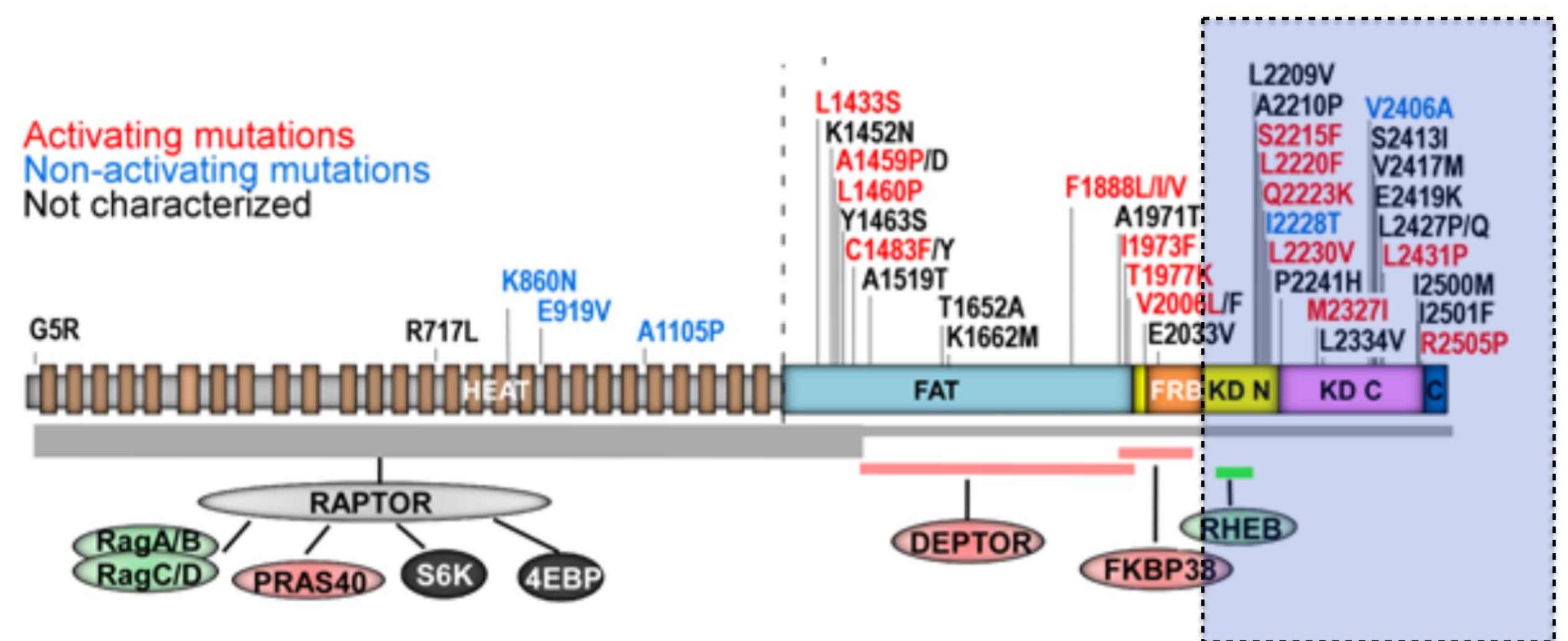
PROTEIN MUTATION FREE ENERGY PERTURBATION USES A THERMODYNAMIC CYCLE



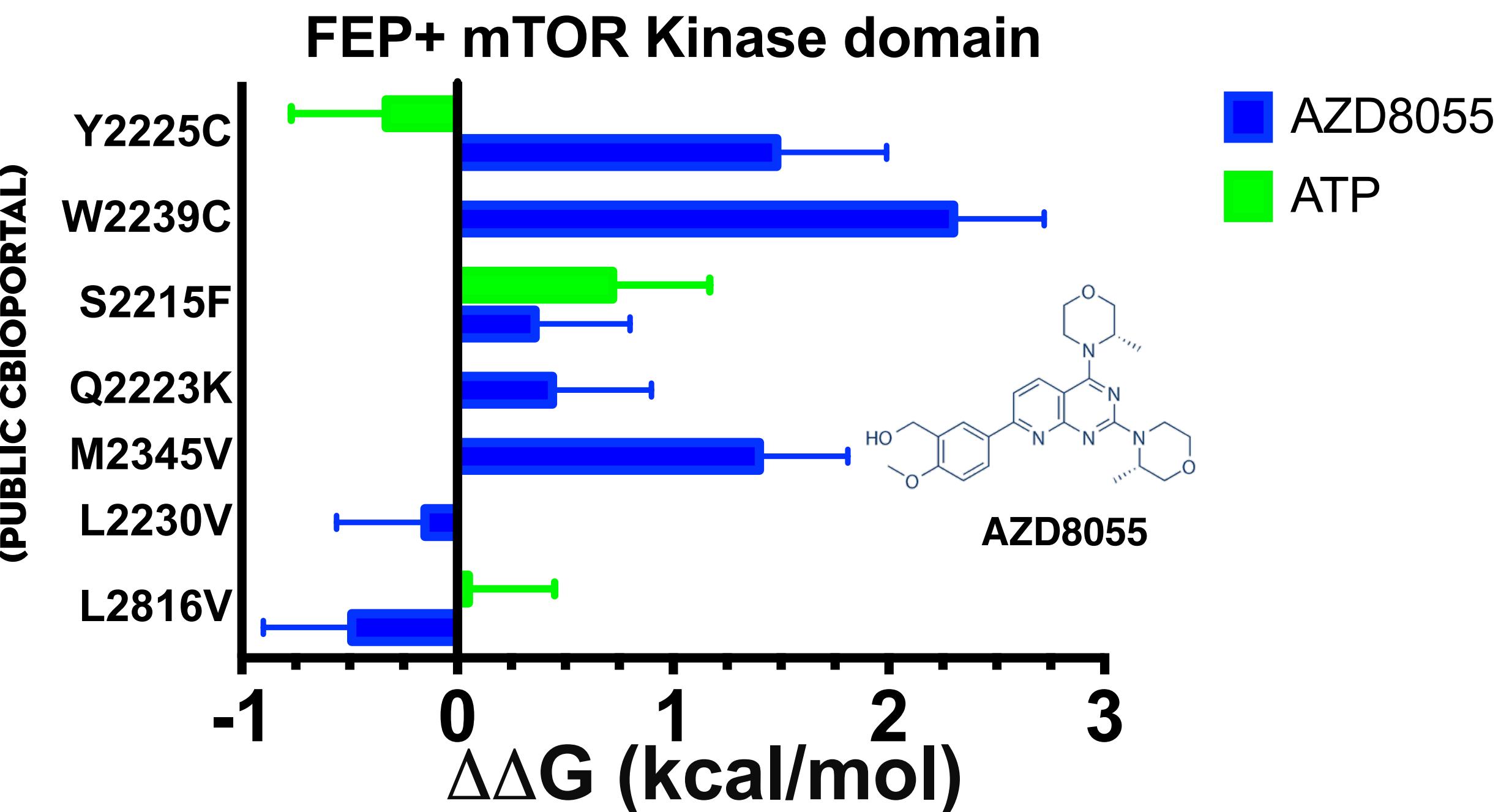
KEVIN HAUSER
SCHRÖDINGER

PRELIMINARY CALCULATIONS SUGGEST POTENTIAL RESISTANCE MUTATIONS TO AZD8055

KINASE DOMAIN HARBORS ACTIVATING MUTATIONS



CLINICAL MUTATIONS
(PUBLIC CBIOPORTAL)

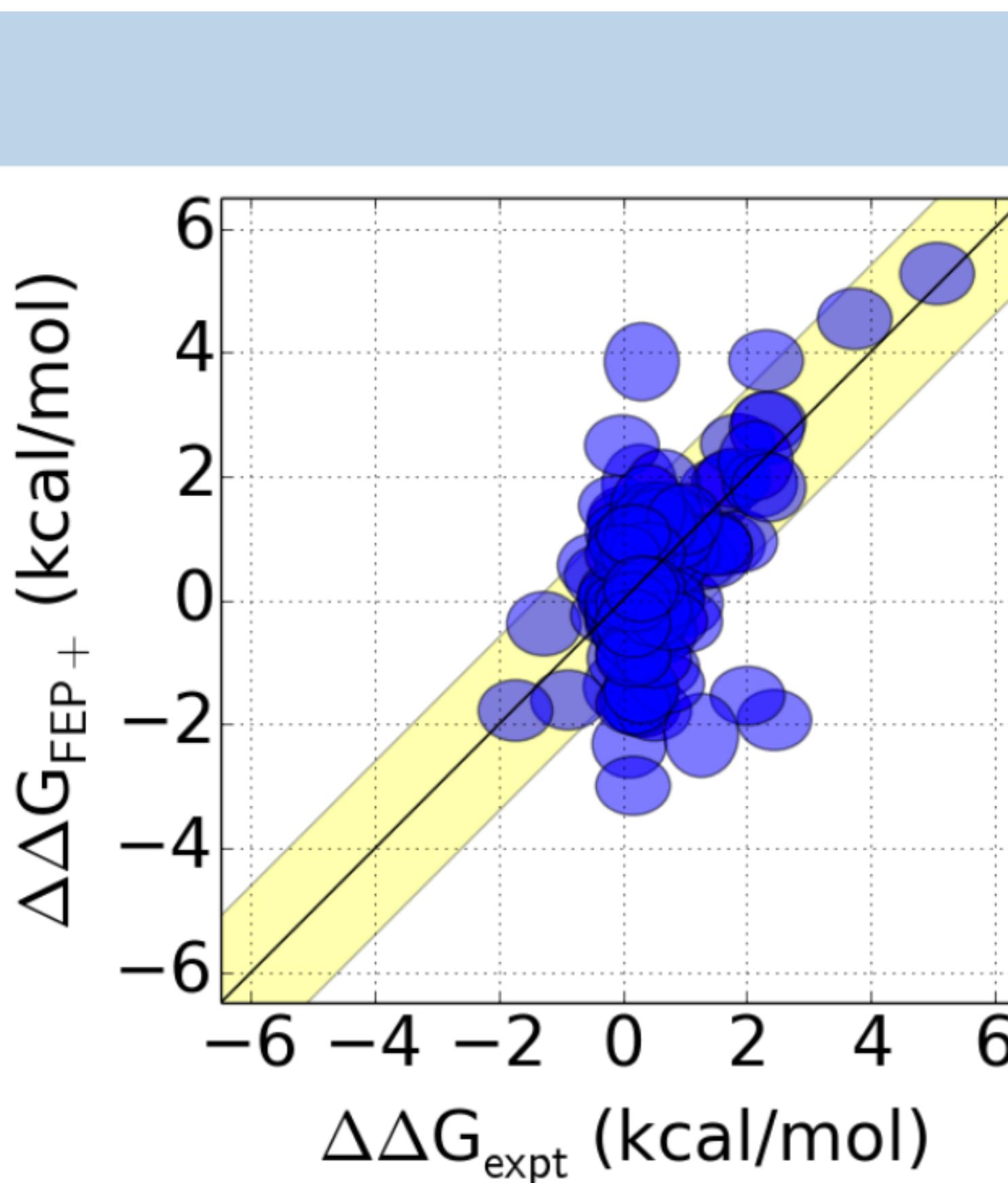


CURRENTLY WORKING TO TEST DRUG SENSITIVITY IN CANCER CELL LINES WITH JAMES HSIEH LAB @ WUSTL

A RETROSPECTIVE STUDY SUGGESTS FEP IS CAPABLE OF PREDICTING RESISTANCE MUTATIONS

FEP+

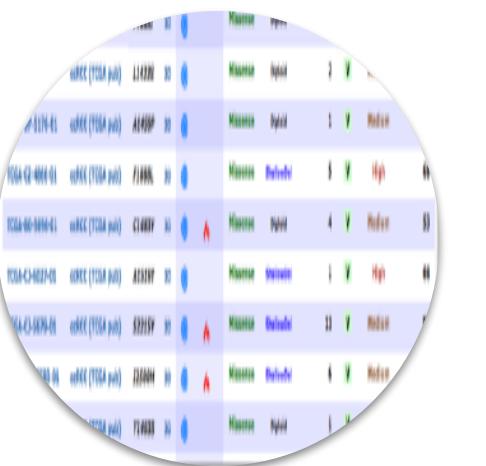
MUE (kcal/mol)	0.79 ^{0.80} _{0.79}
RMSE (kcal/mol)	1.11 ^{1.12} _{1.11}



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

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USING COMPUTATION TO GUIDE EXPERIMENT



GATHER CLINICAL
MUTATIONS

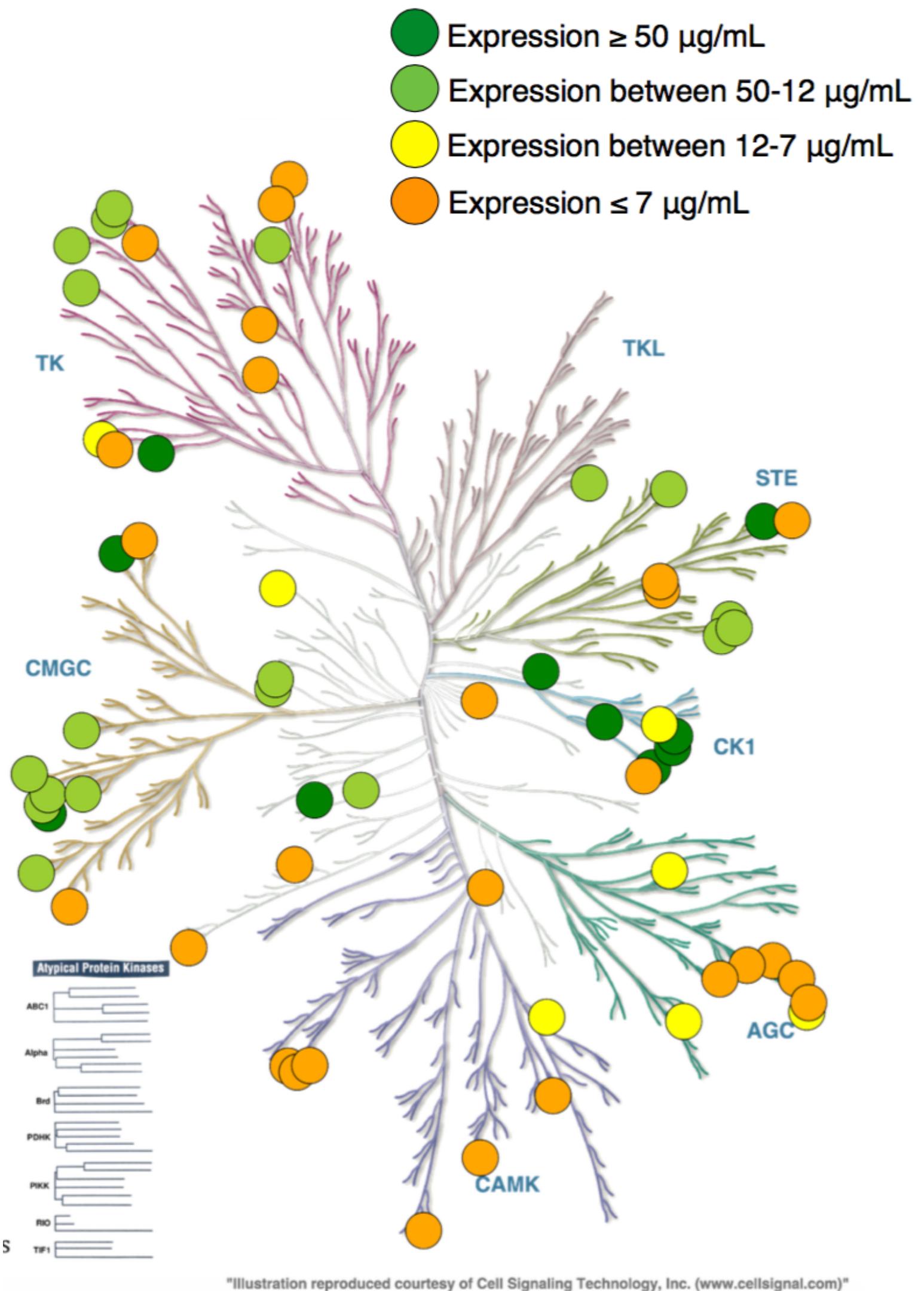


FEP CALCULATIONS
PRIORITIZE MUTANTS
TO TEST



EXPERIMENT CAN
TEST PREDICTIONS
AND IDENTIFY
OUTLIERS FOR
FOLLOW UP

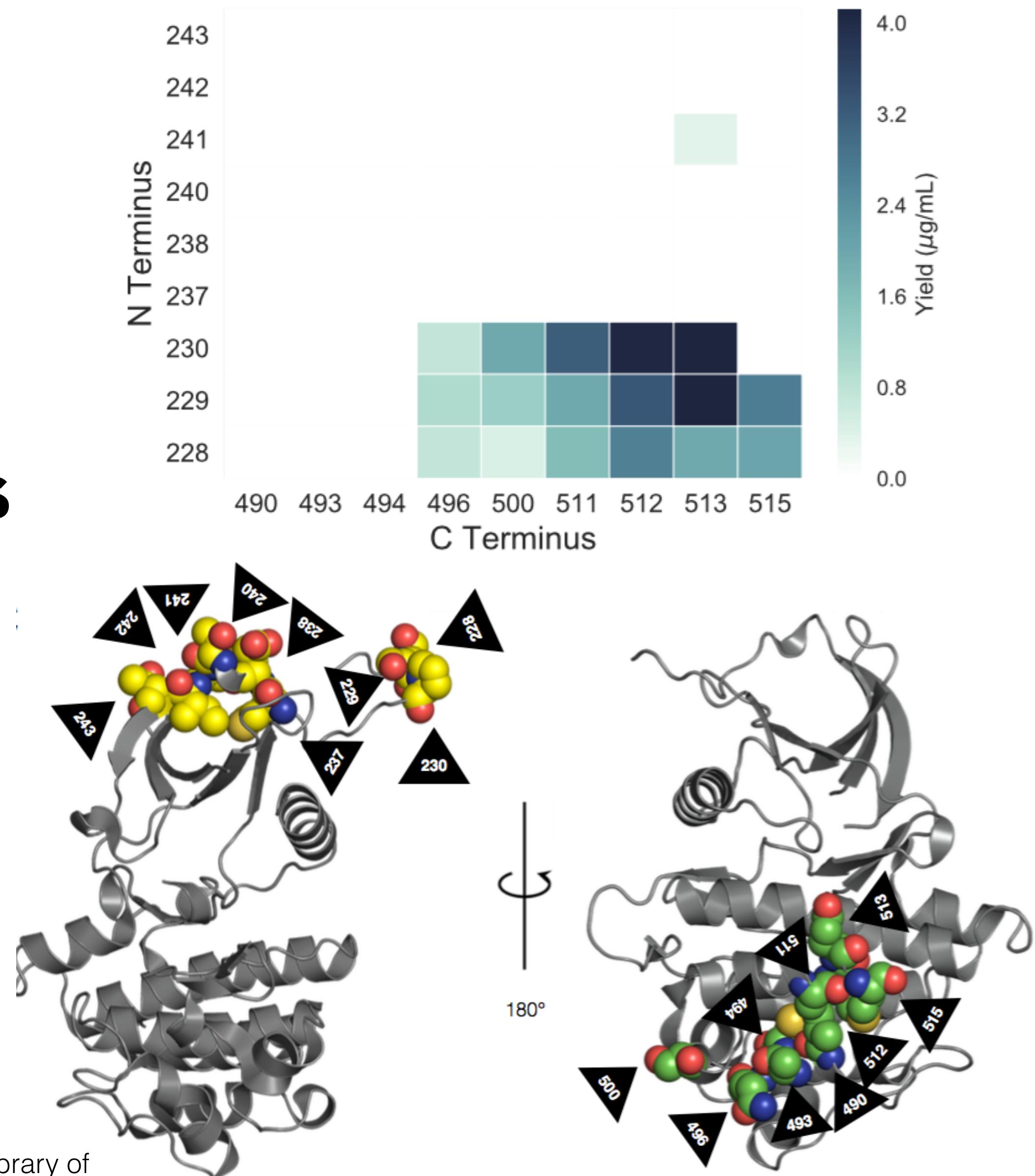
WHICH KINASES ARE EXPERIMENTALLY TRACTABLE?



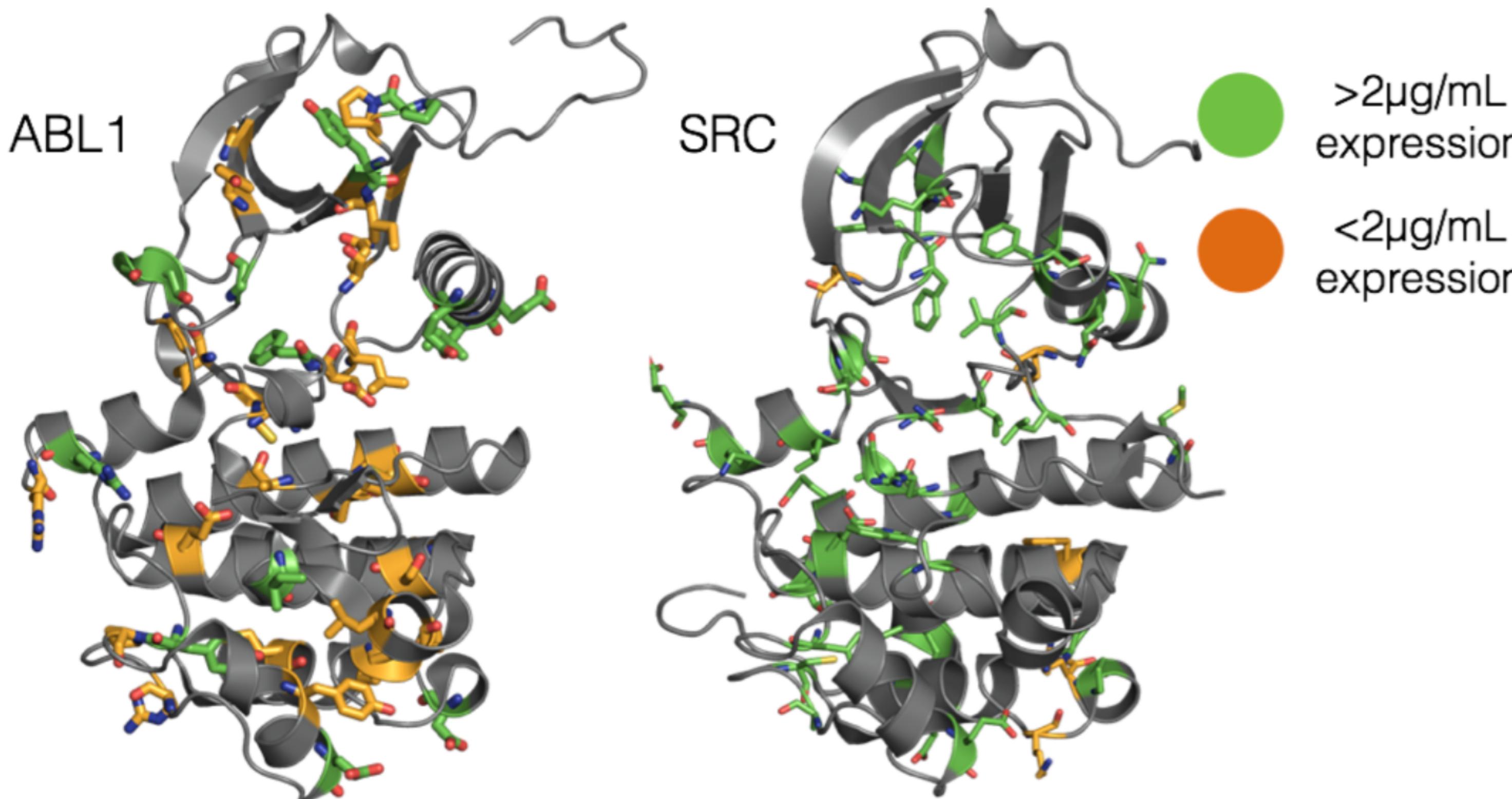
**SCREENED MULTIPLE
ABL1 CONSTRUCTS TO
FIND PROTOCOL FOR
CONSTRUCT
SELECTION**

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

***KINASE PLASMIDS
ARE AVAILABLE ON
ADDGENE**

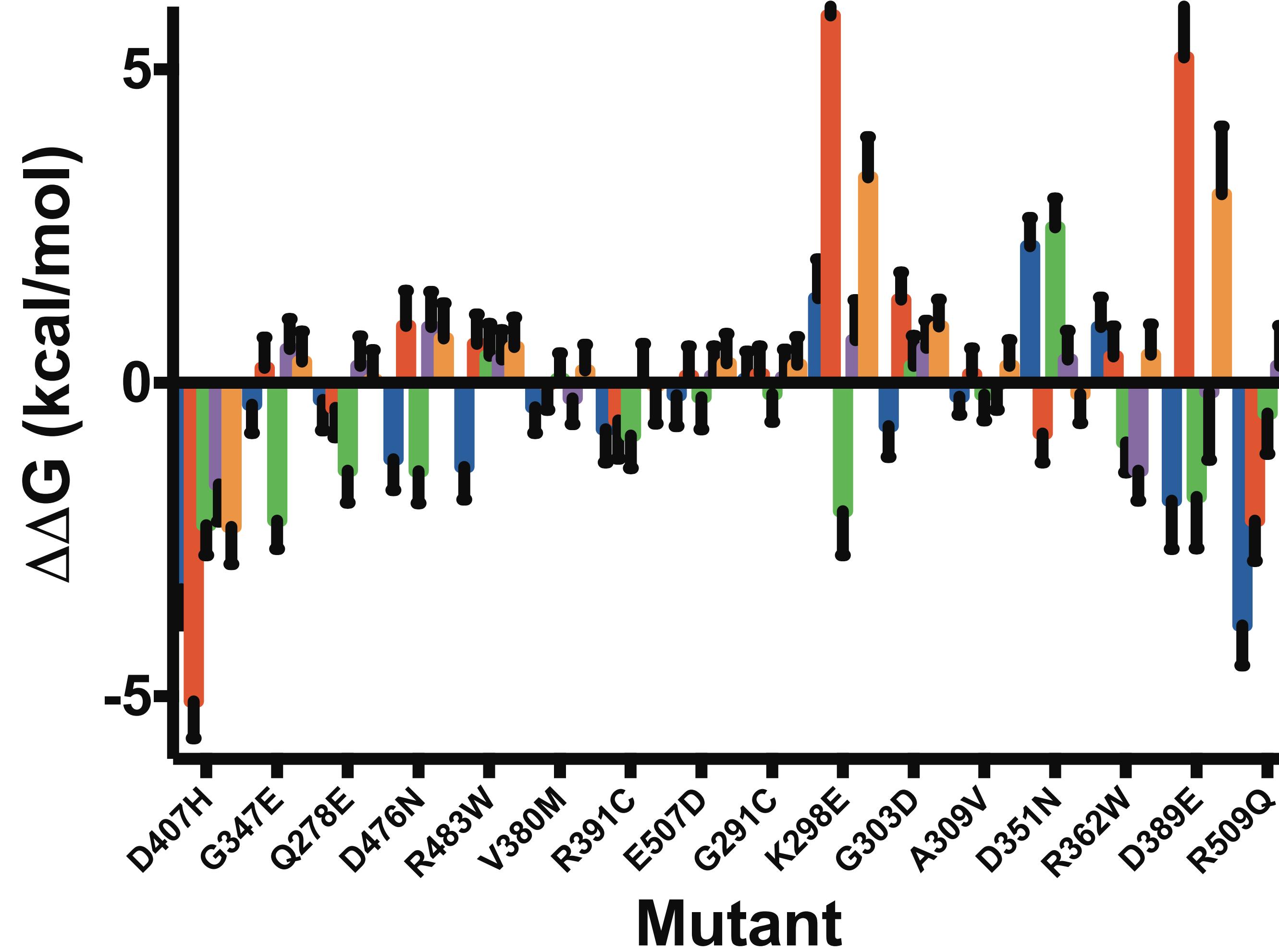


ARE THESE CONSTRUCTS SUITABLE FOR EXPRESSING CLINICAL MUTANTS?

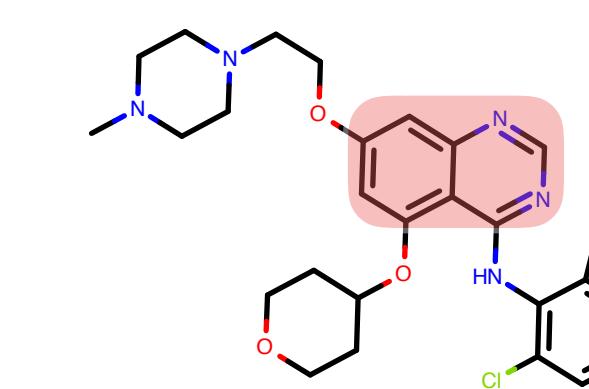
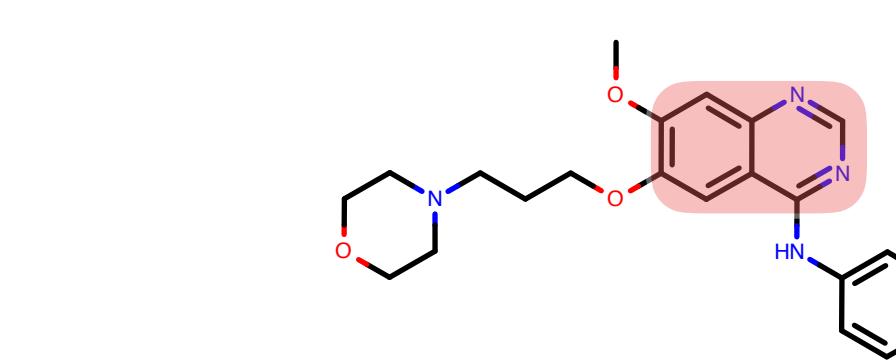
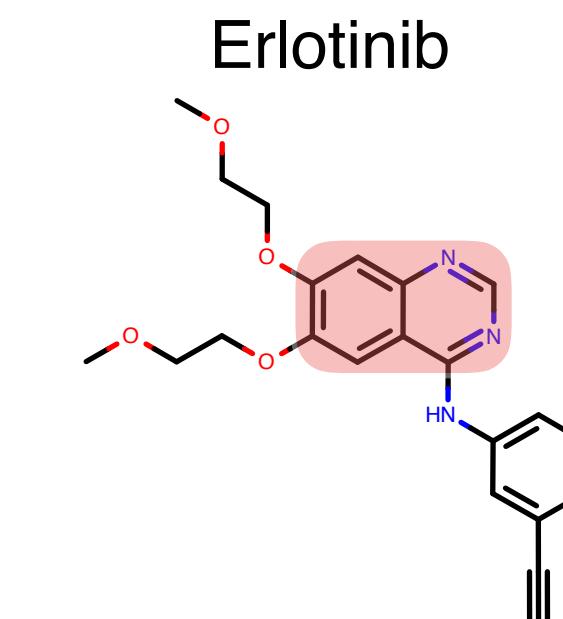
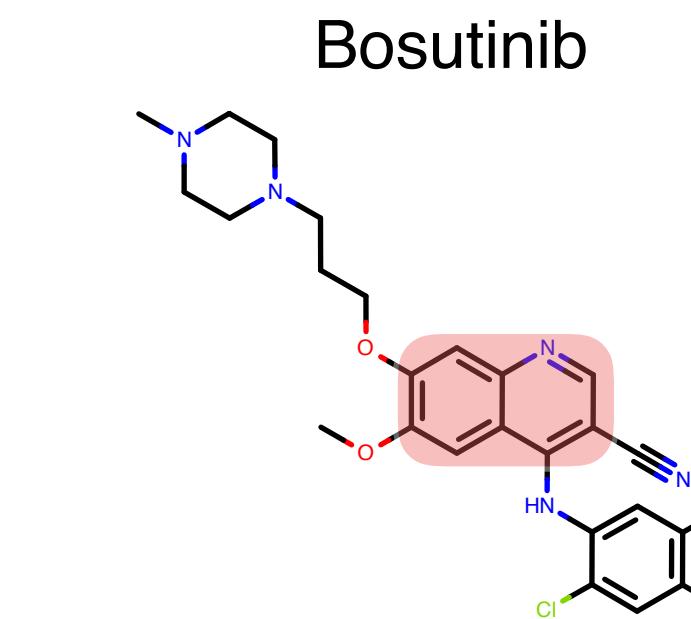


Src (254–536)	Mutation ¹	Functional Impact Score ²	yield (µg/mL)	% of WT expression
WT	-	-	35.7	-
T456S	Neutral	80.9	227	
R388G	Medium	61.5	172	
K298E	High	54.5	153	
V380M	Neutral	51.7	145	
D368N	Neutral	49.9	140	
D521N	Low	42.8	120	
R463Q	Neutral	38.4	108	
R391C	Neutral	37.5	105	
E323D	Low	37.2	104	
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

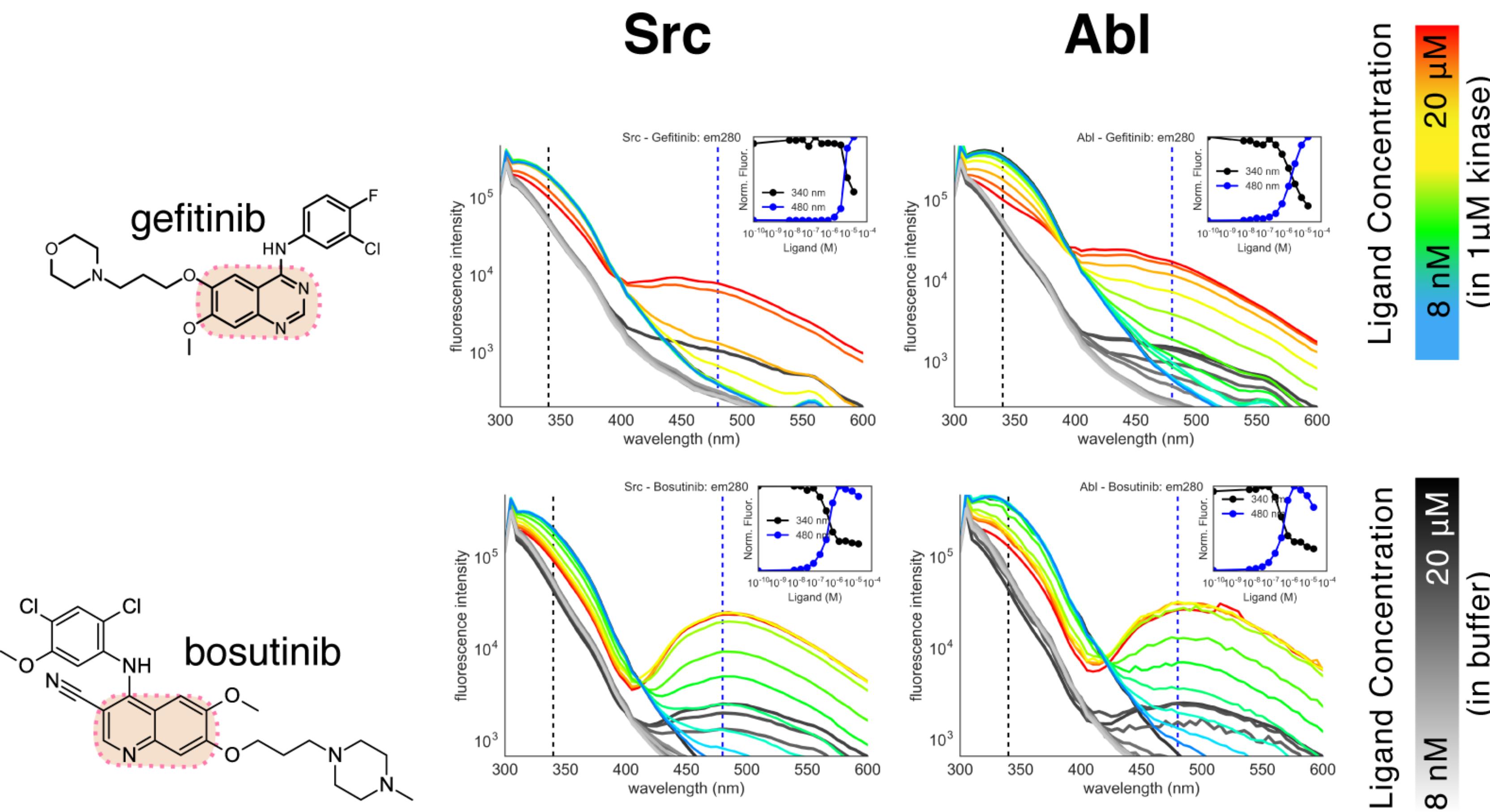


- Bosutinib
- Axitinib
- Gefitinib
- Erlotinib
- Seracatinib



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SCHRÖDINGER

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

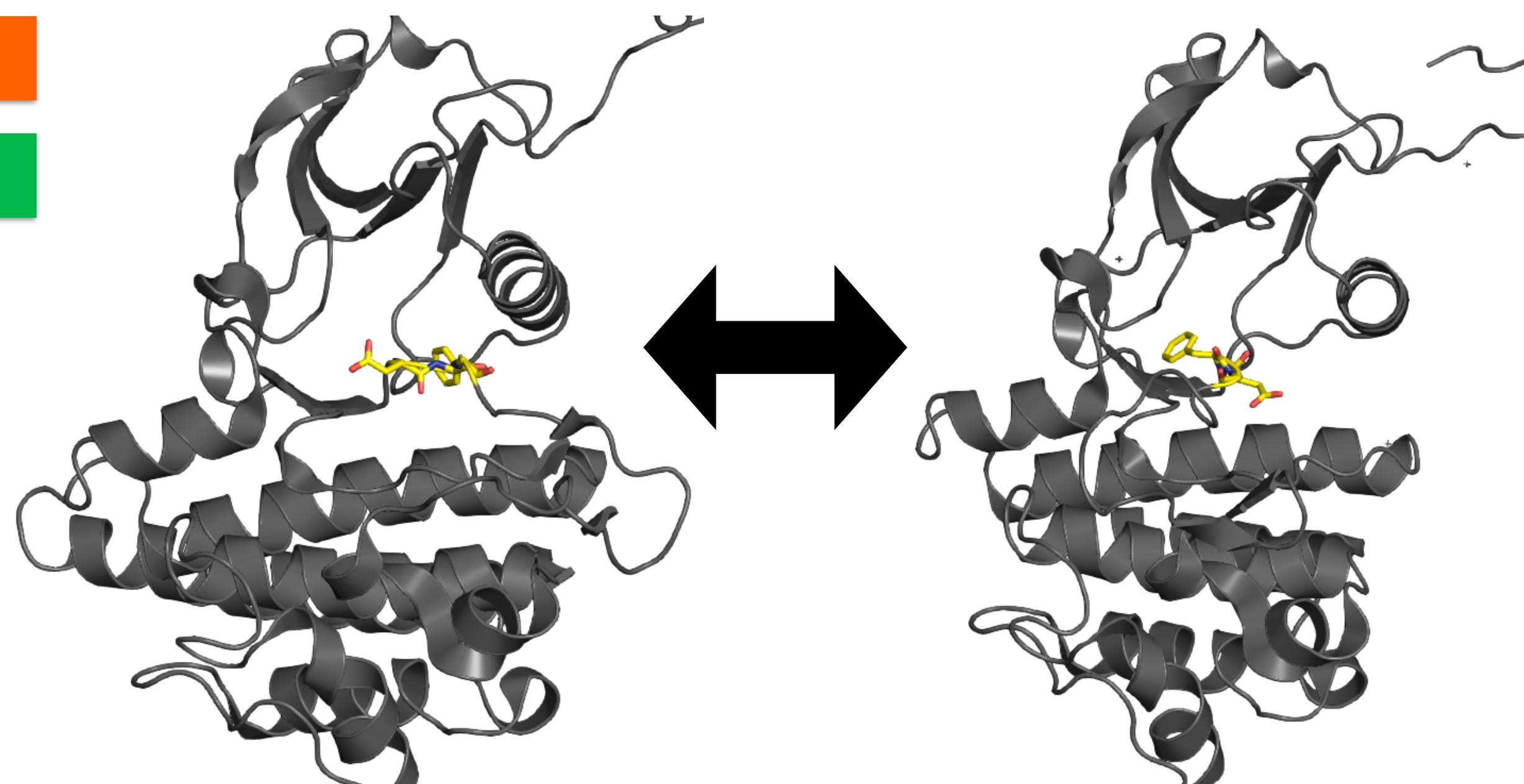


TESTING PREDICTIONS ALLOWS IDENTIFICATION OF OUTLIERS AND REFINING FUTURE PREDICTIONS

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

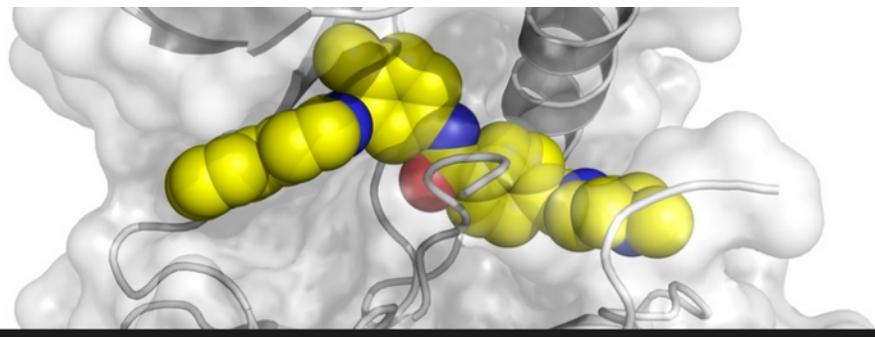


PDB: 2F4J

PDB: 1IEP

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

ACKNOWLEDGEMENTS



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