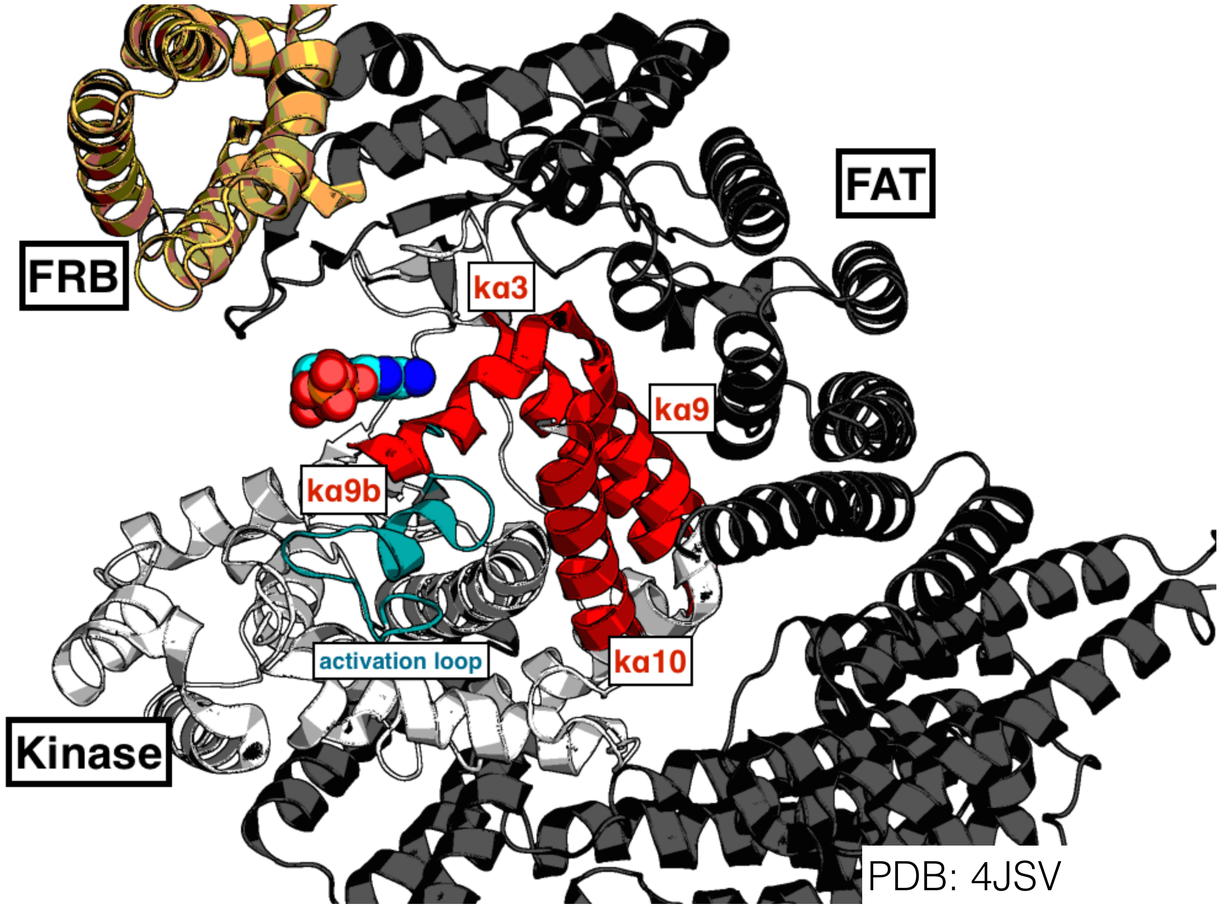
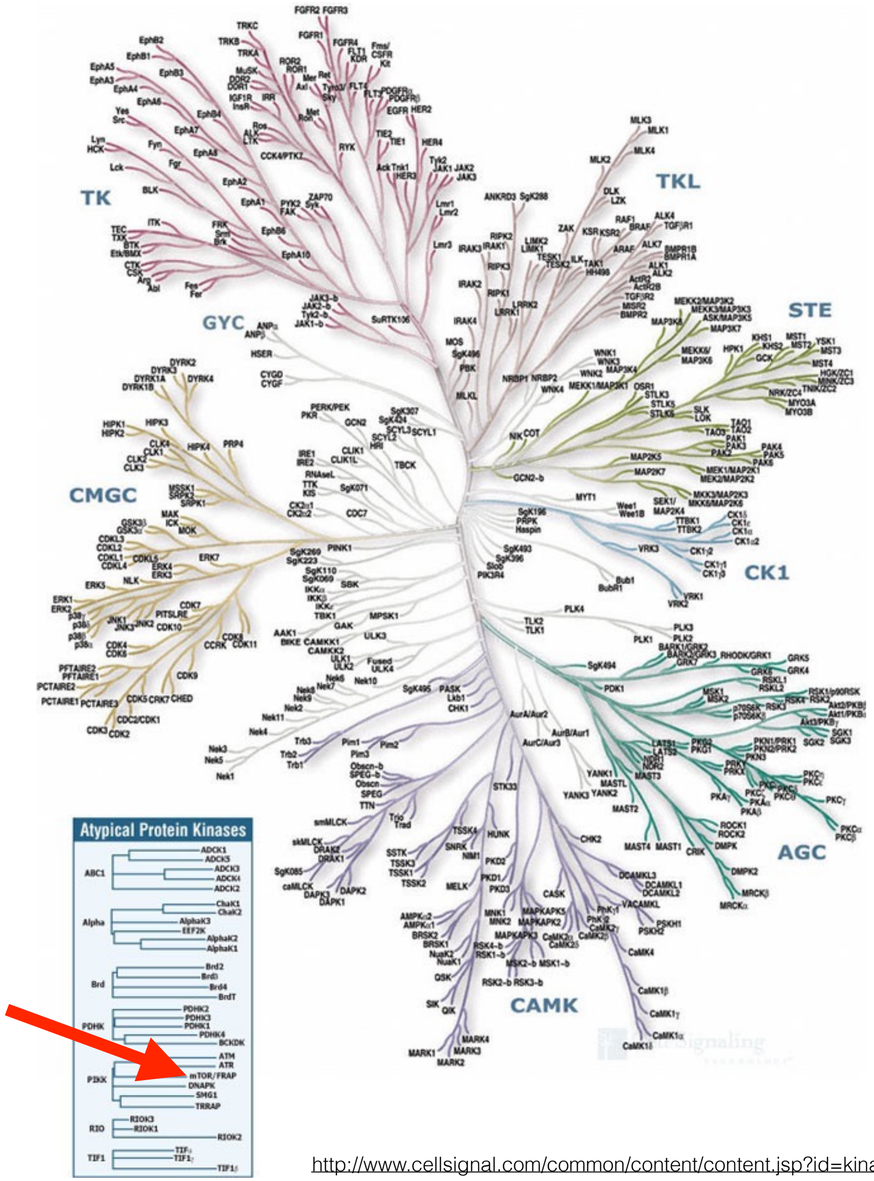
SIMULATING MTOR HYPERACTIVATING MUTATIONS TO UNDERSTAND FUNCTIONALLY SIGNIFICANT STRUCTURAL REARRANGEMENTS

LAB MEETING
JULY 18, 2016

STEVEN ALBANESE
CHODERA LAB // MSKCC

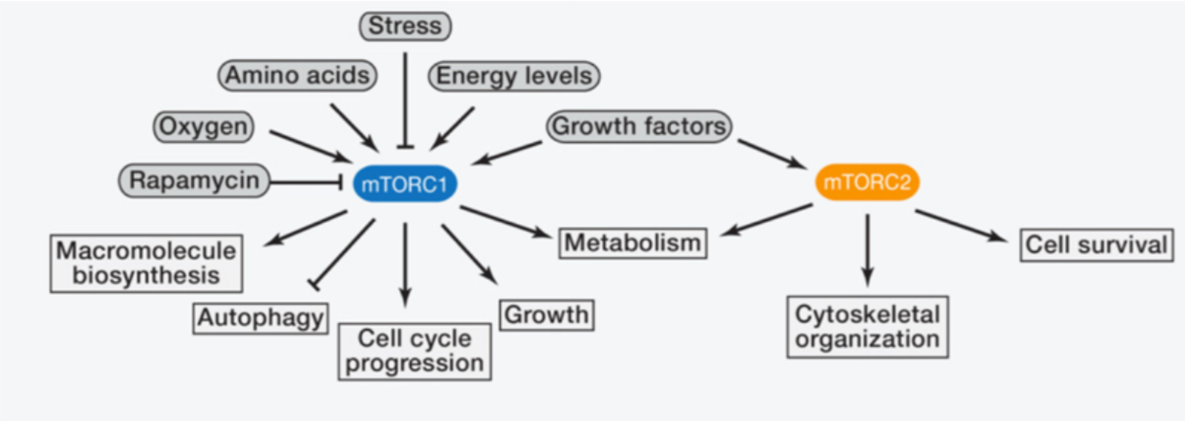
MTOR IS AN ATYPICAL SERINE/THREONINE KINASE

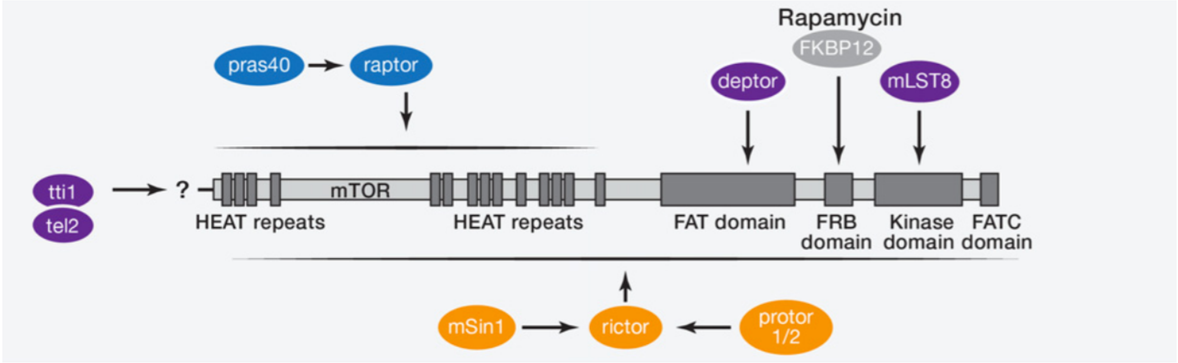




http://www.cellsignal.com/common/content/content.jsp?id=kinases-human-protein

MTOR FORMS THE CATALYTIC CORE OF TWO COMPLEXES

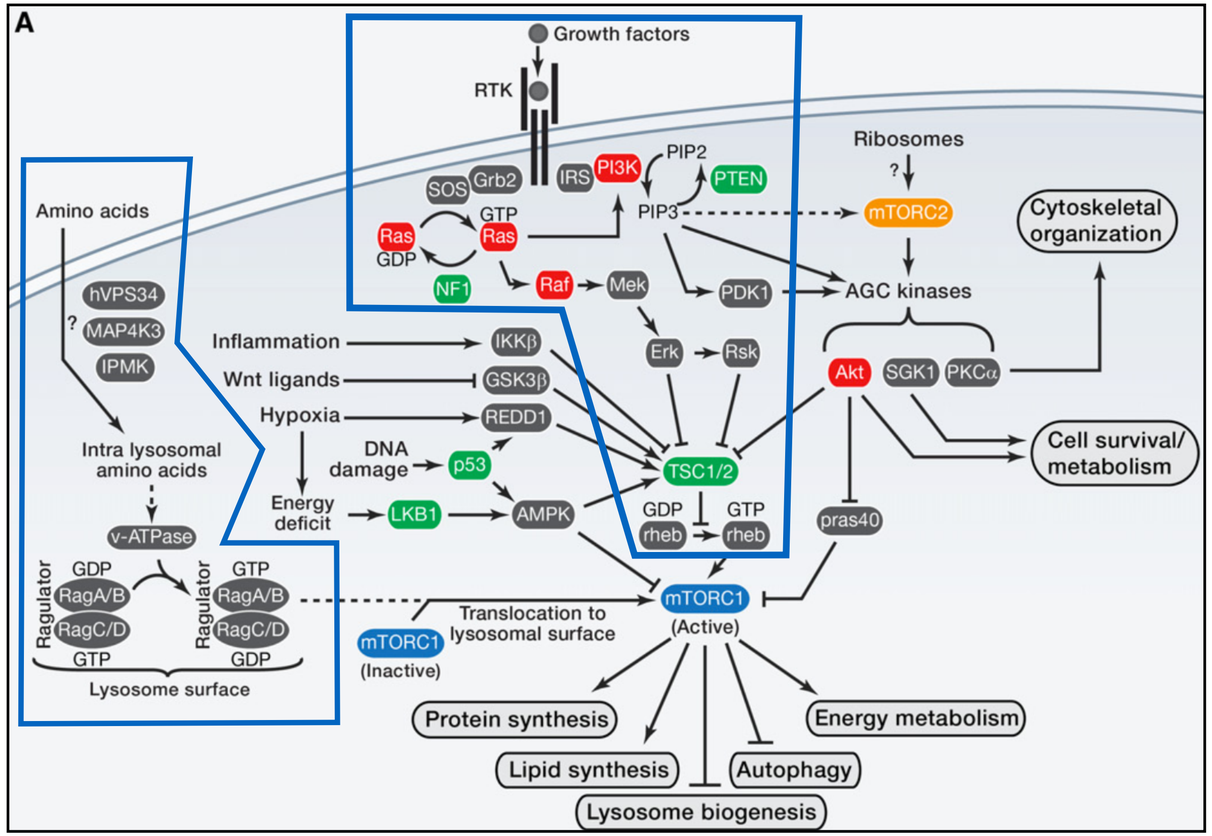




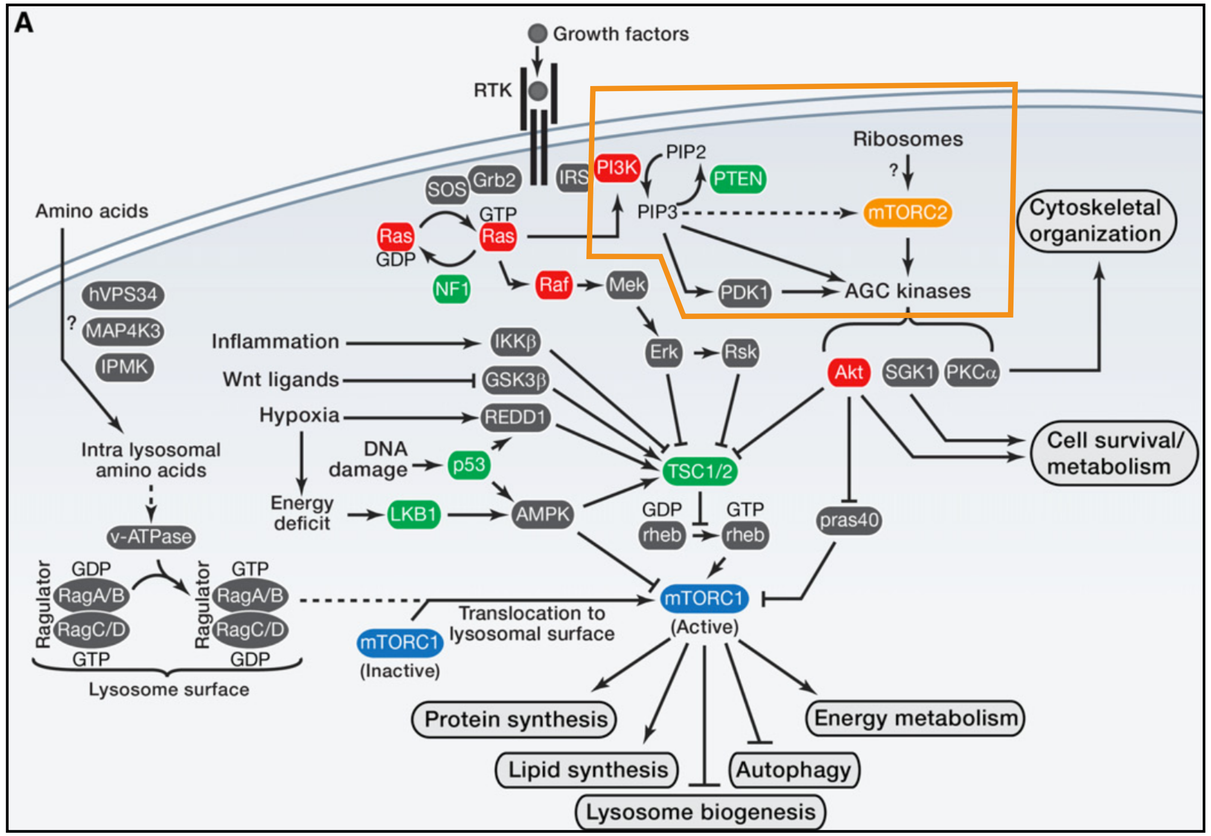
Laplante, M. & Sabatini, D. M. mTOR signaling in growth control and disease. Cell 149, 274–293 (2012).

MTOR FORMS THE CATALYTIC CORE OF TWO COMPLEXES

MTORC1



MTORC2



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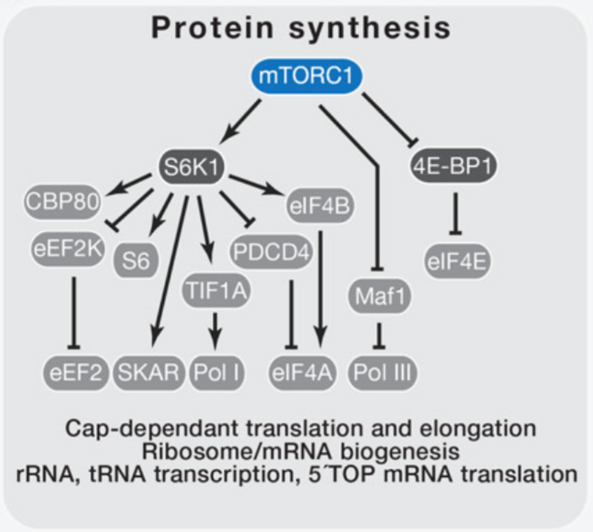
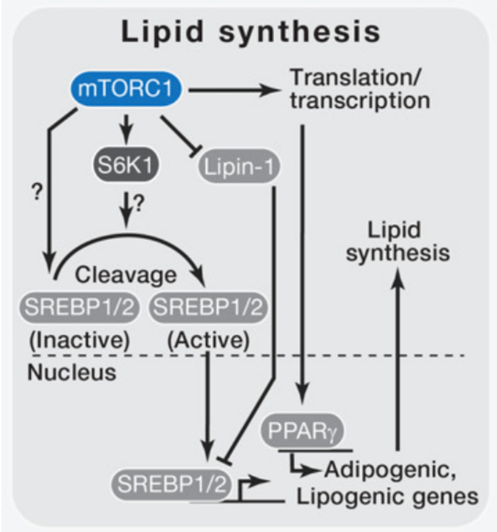
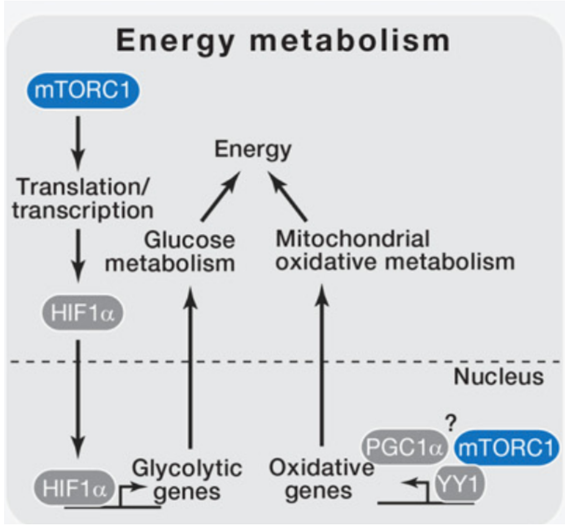
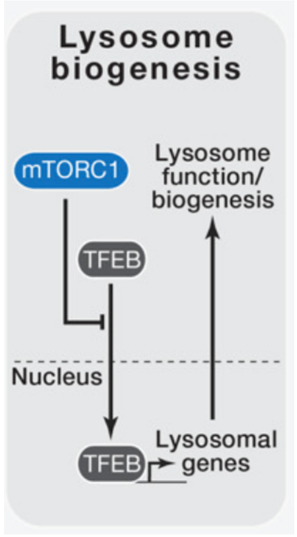
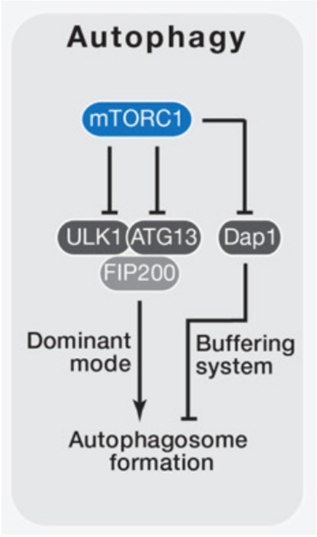
MTORC1 CONTROLS A NUMBER OF IMPORTANT PROCESSES











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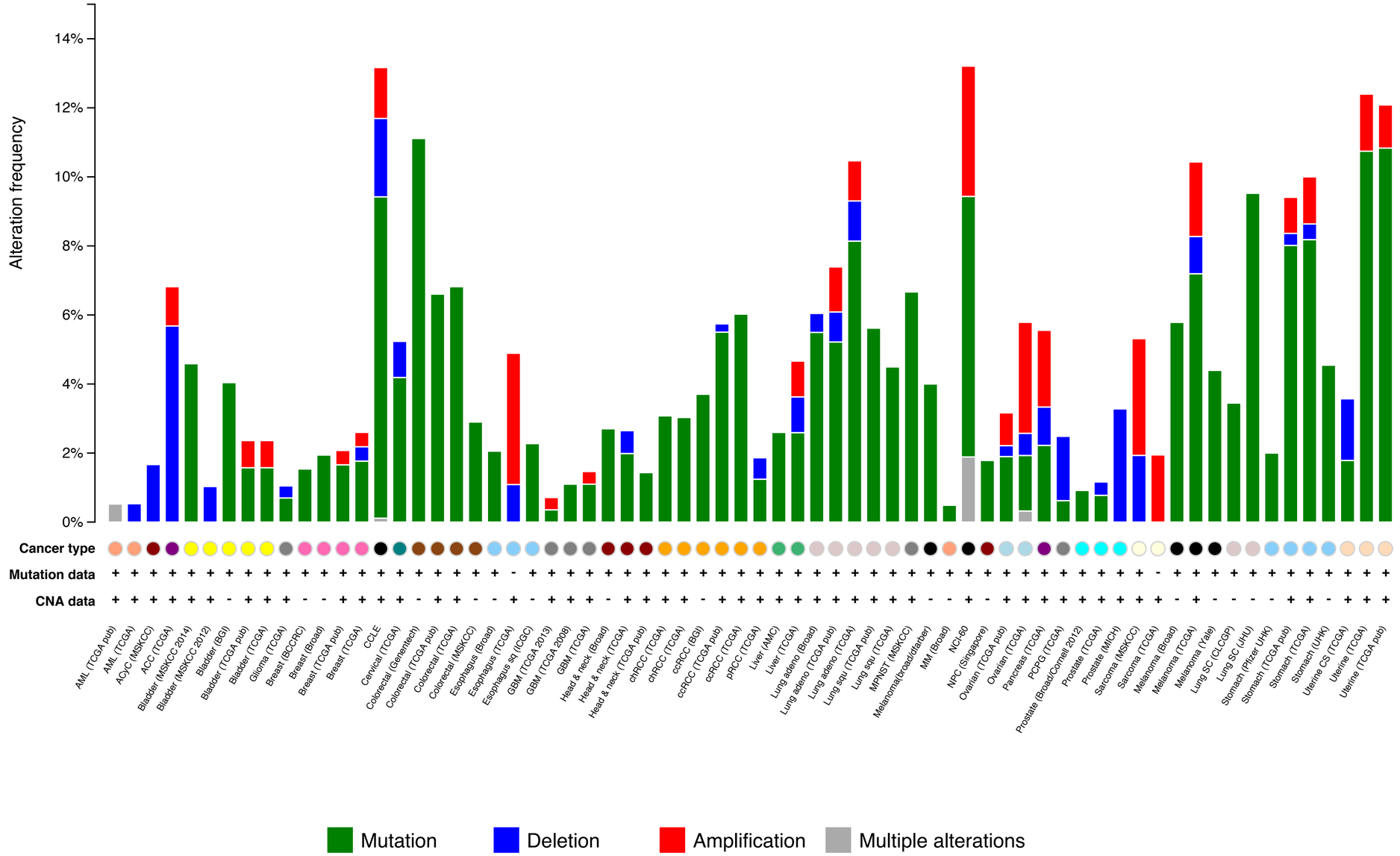
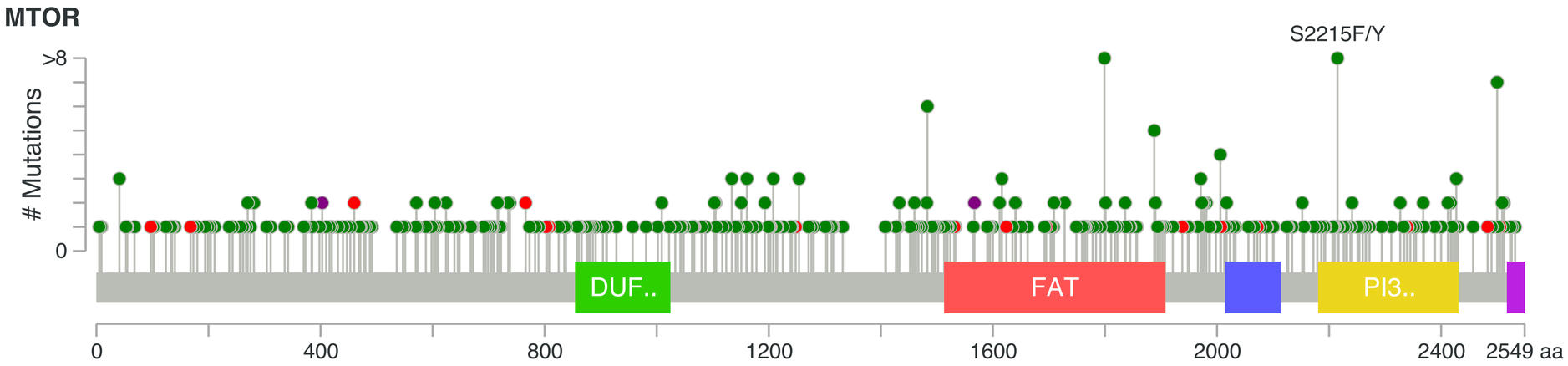
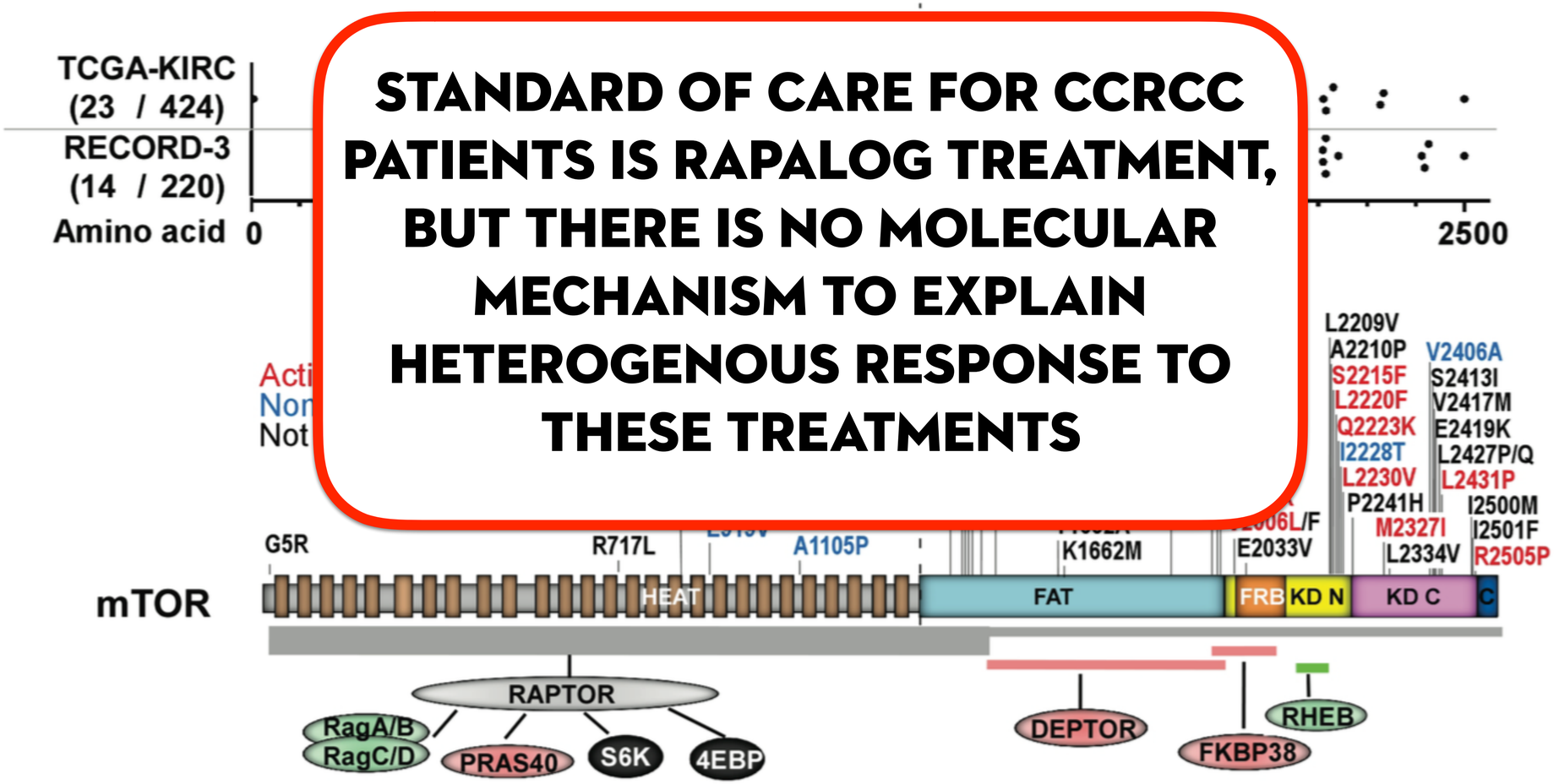
MTOR MUTATIONS ACROSS CANCER TYPES



MTOR MUTATIONS OCCUR THROUGHOUT THE PROTEIN



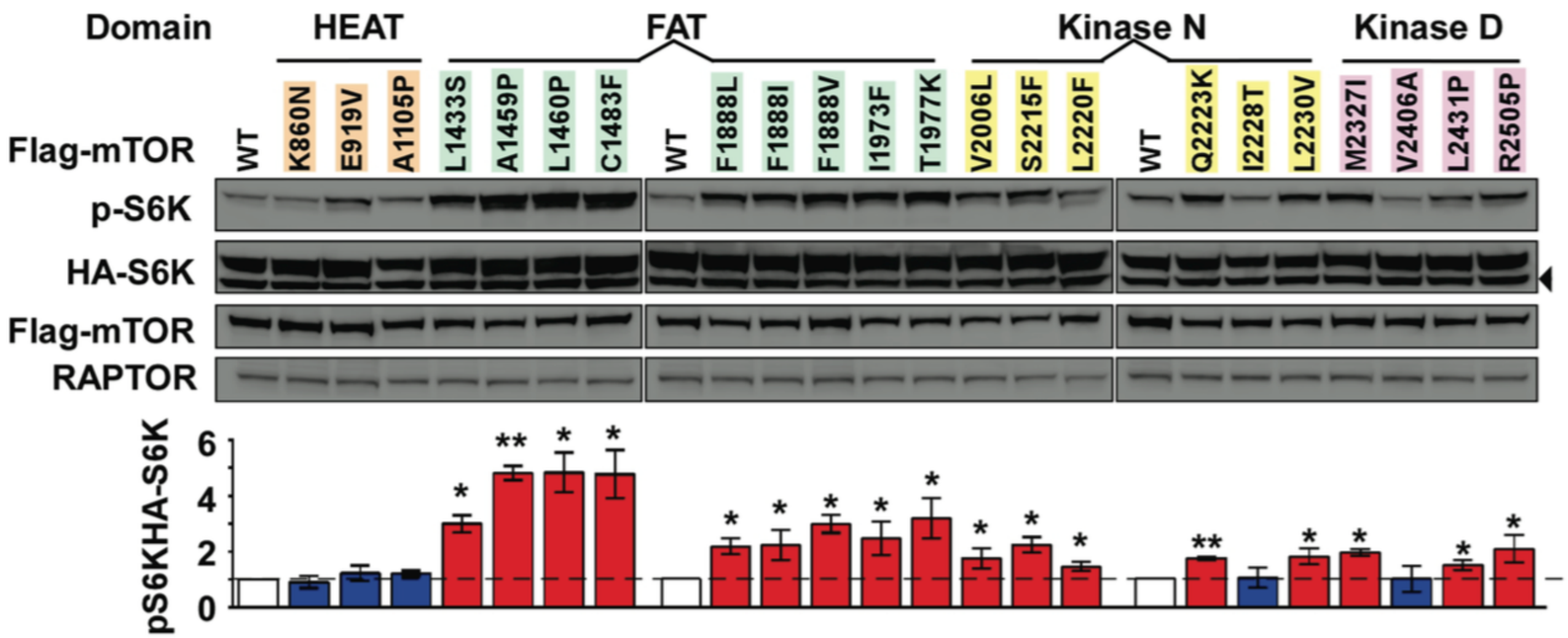
MTOR MUTATIONS ARE PREVALENT IN CLEAR CELL RENAL CELL CARCINOMA (CCRCC)



STANDARD OF CARE FOR CCRCC PATIENTS IS RAPALOG TREATMENT, BUT THERE IS NO MOLECULAR MECHANISM TO EXPLAIN HETEROGENOUS RESPONSE TO THESE TREATMENTS

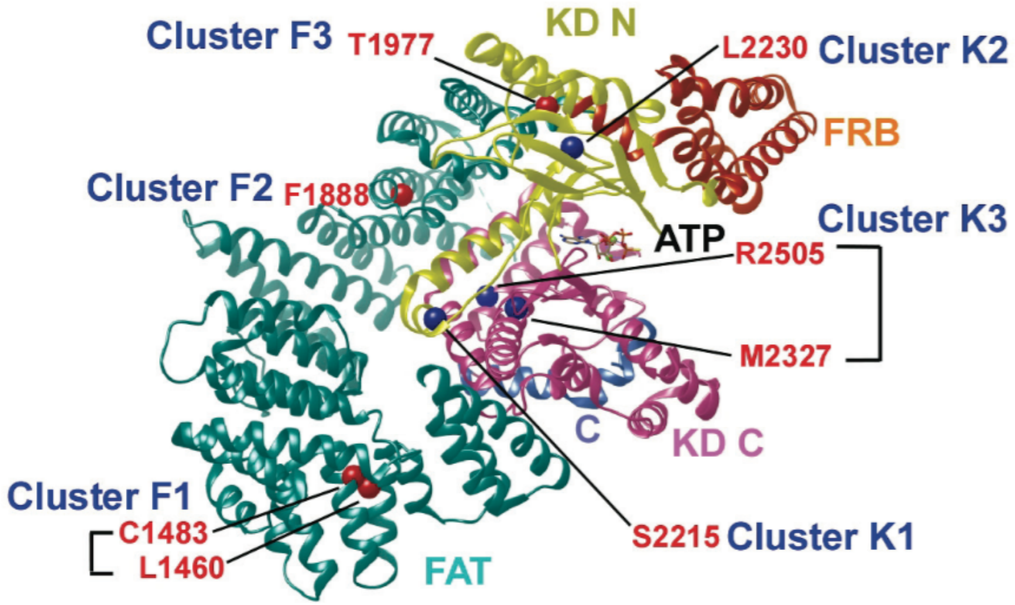
Xu *et al.*, in press

MTOR MISSENSE MUTATIONS ARE HYPER ACTIVATING



Xu *et al.*, in press

SOME DOUBLE MUTATION COMBINATIONS ARE COOPERATIVE



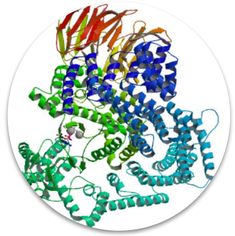
F1	F1	F2	F3	K1	K2	K3	K3	Cluster	
L1460P	C1483F	F1888L	T1977K	S2215F	L2230V	M2327I	R2505P	Mutation	
	-	+	+	+	+	+	-	L1460P	F1
		+	+	+	+	+	-	C1483F	F1
			+	+	+	+	+	F1888L	F2
				+	+	+	+	T1977K	F3
					+	-	-	S2215F	K1
						+	+	L2230V	K2
							-	M2327I	K3
								R2505P	K3

-	No cooperativity
+	Cooperativity

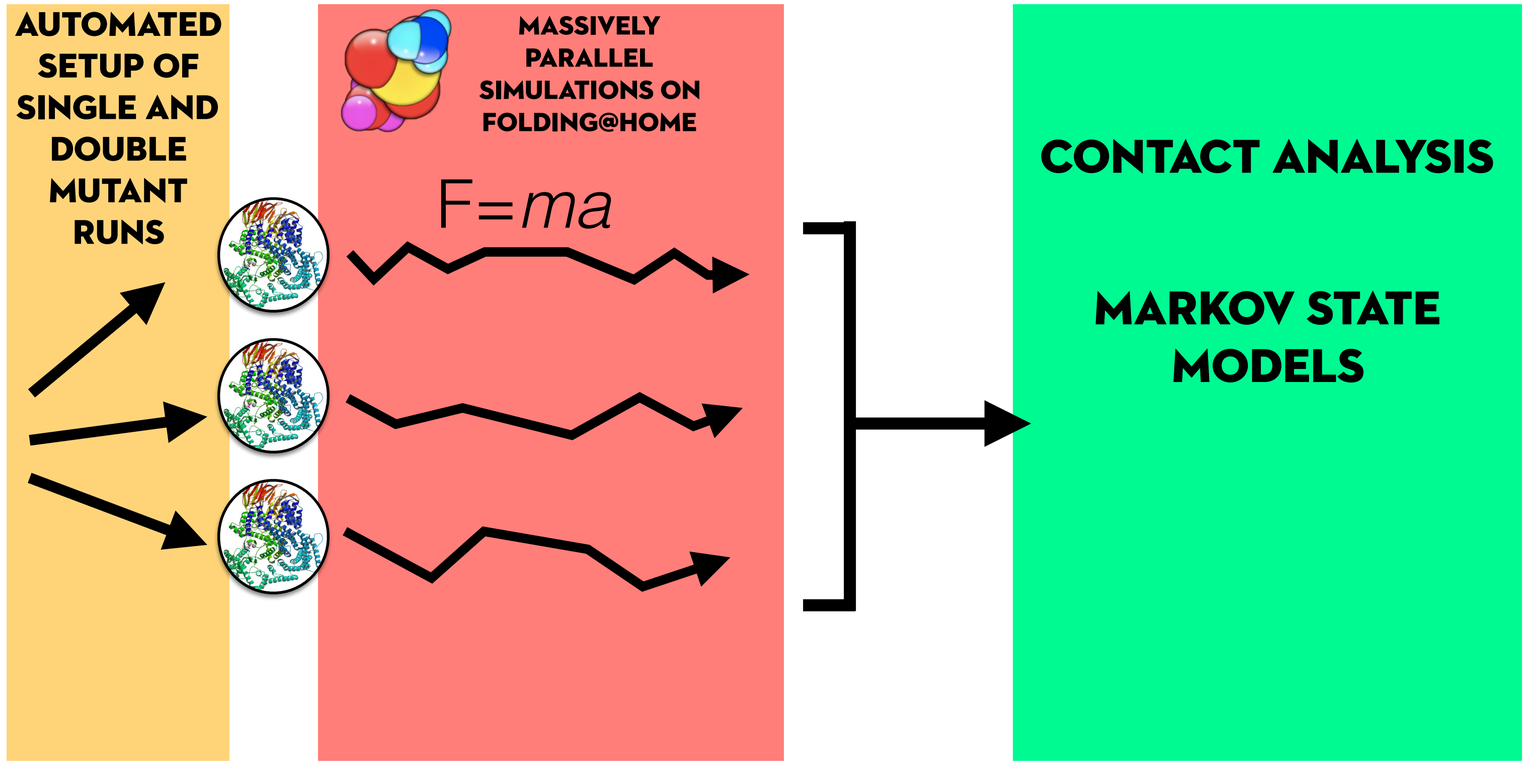
Xu *et al.*, in press

CAN WE DISSECT DIFFERENT MECHANISMS OF ACTIVATION?

CLINICAL MUTATIONS



CRYSTAL STRUCTURE

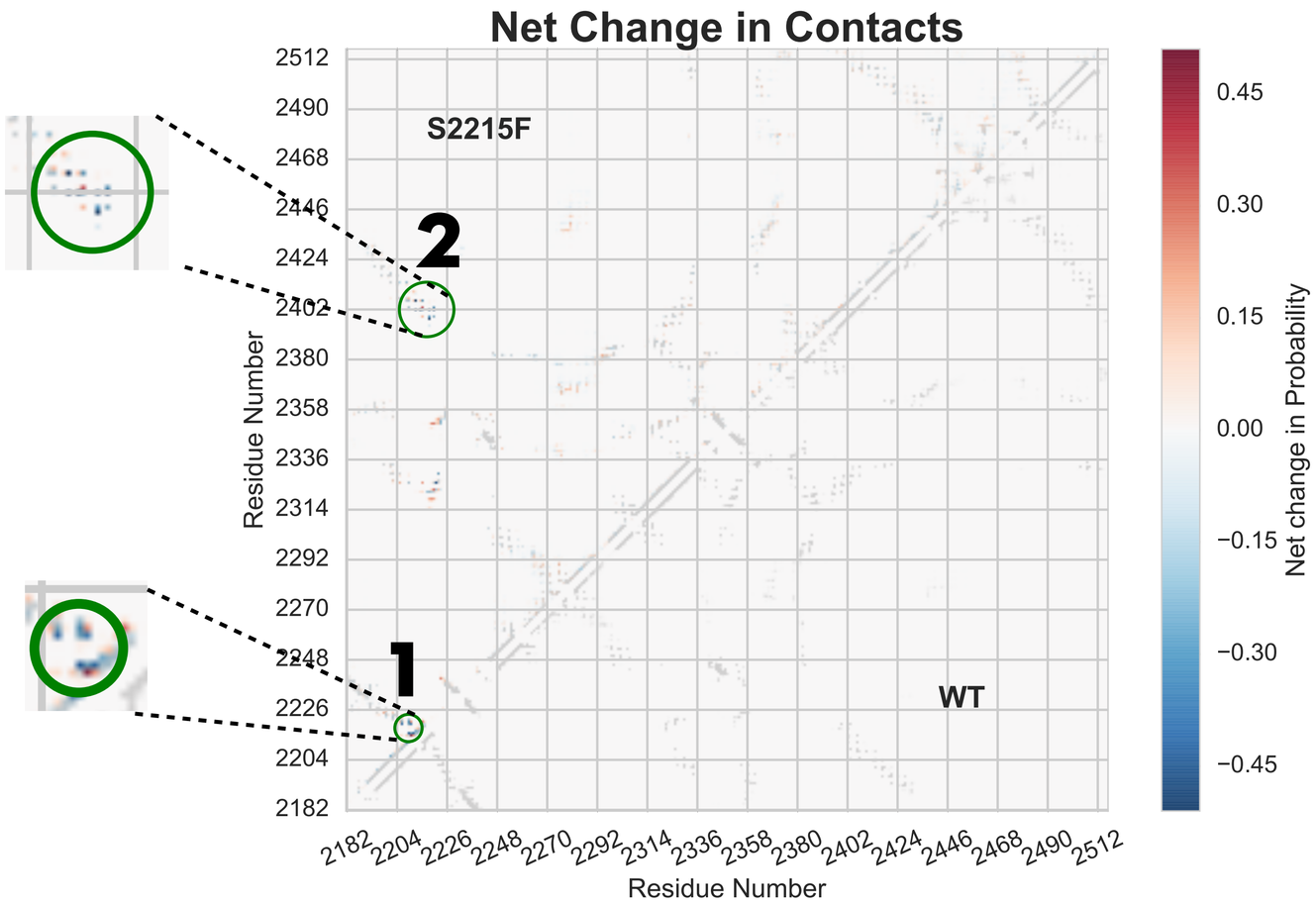


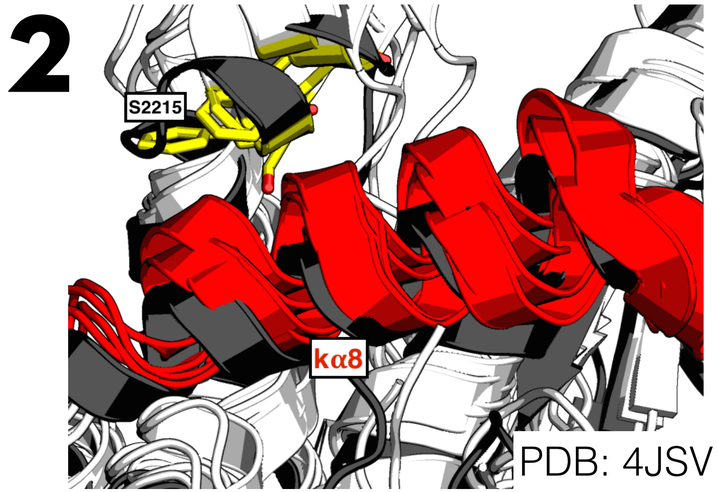
45 KINASE DOMAIN MUTANTS

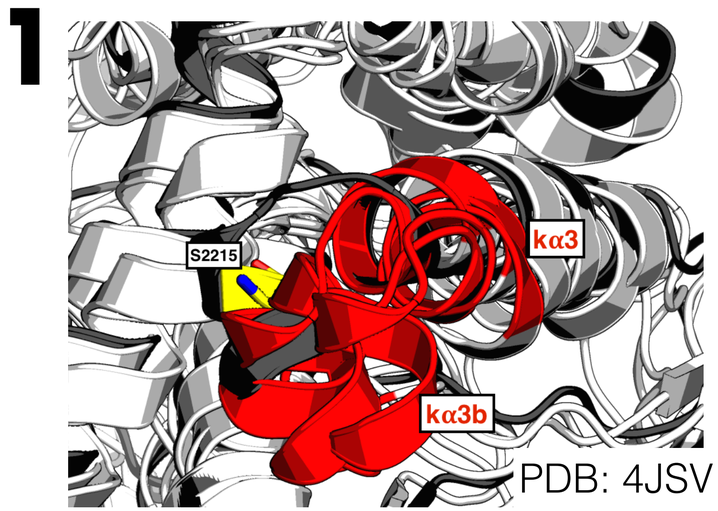
190 KINASE+FAT DOMAIN MUTANTS

Adapted from Danny Parton

CAN WE DETECT LOCAL STRUCTURAL CHANGES IN THE MUTANTS?

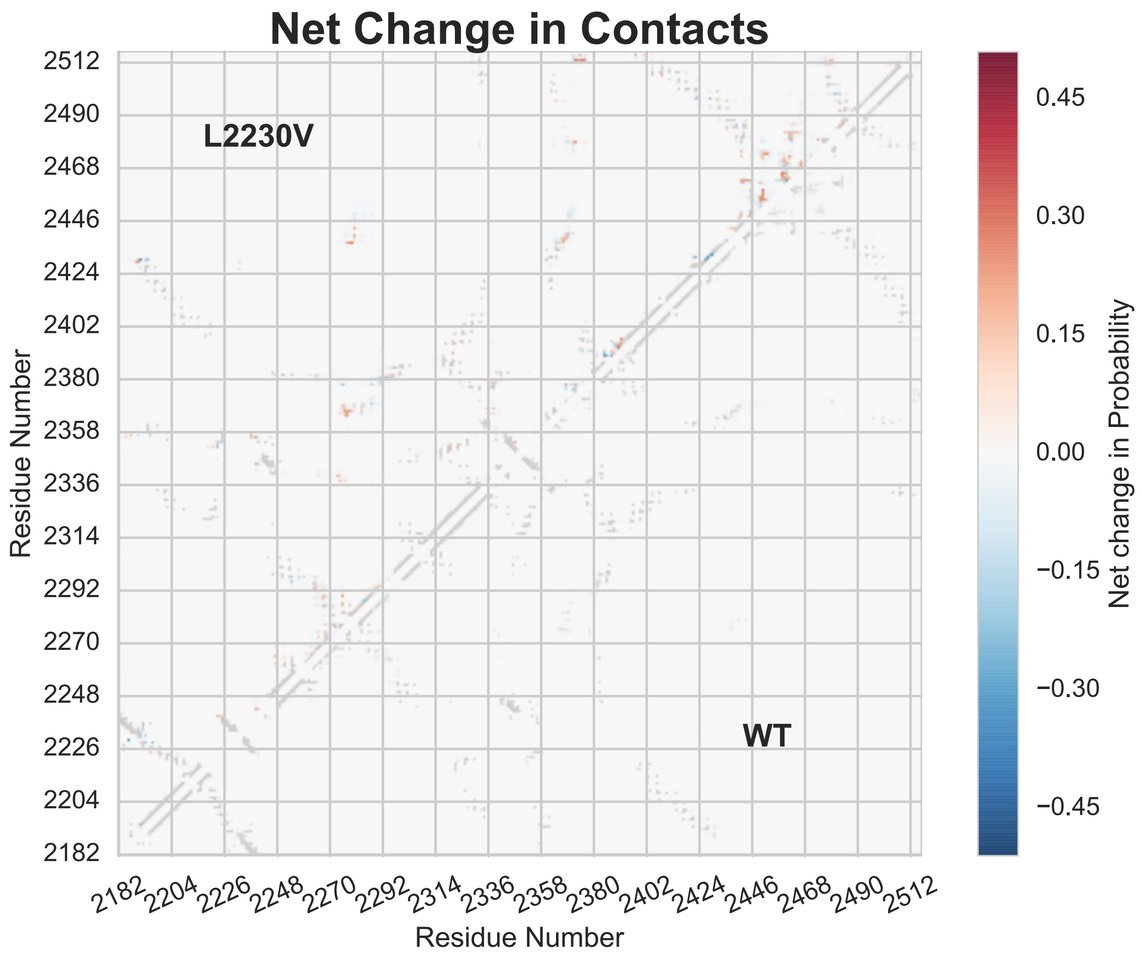


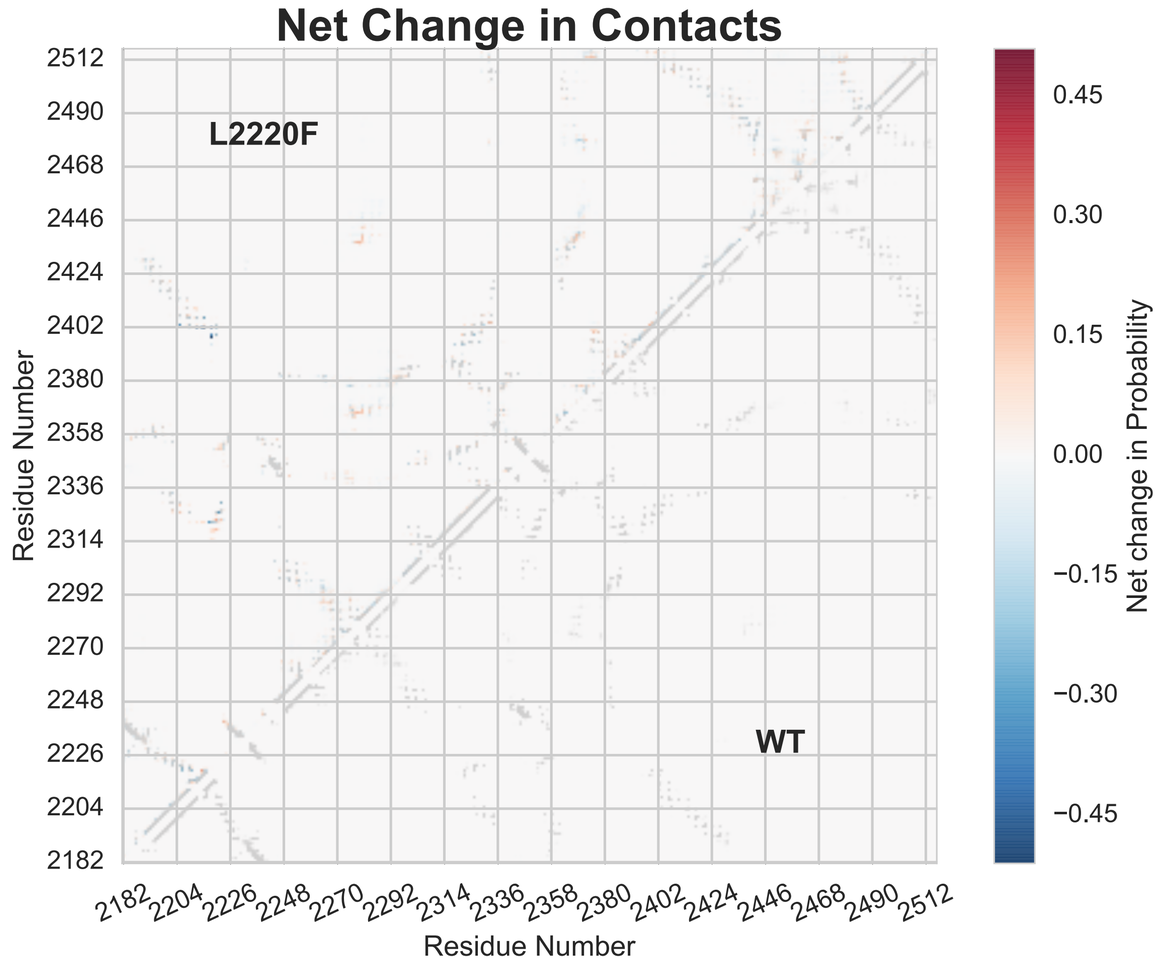




Xu *et al.*, in press

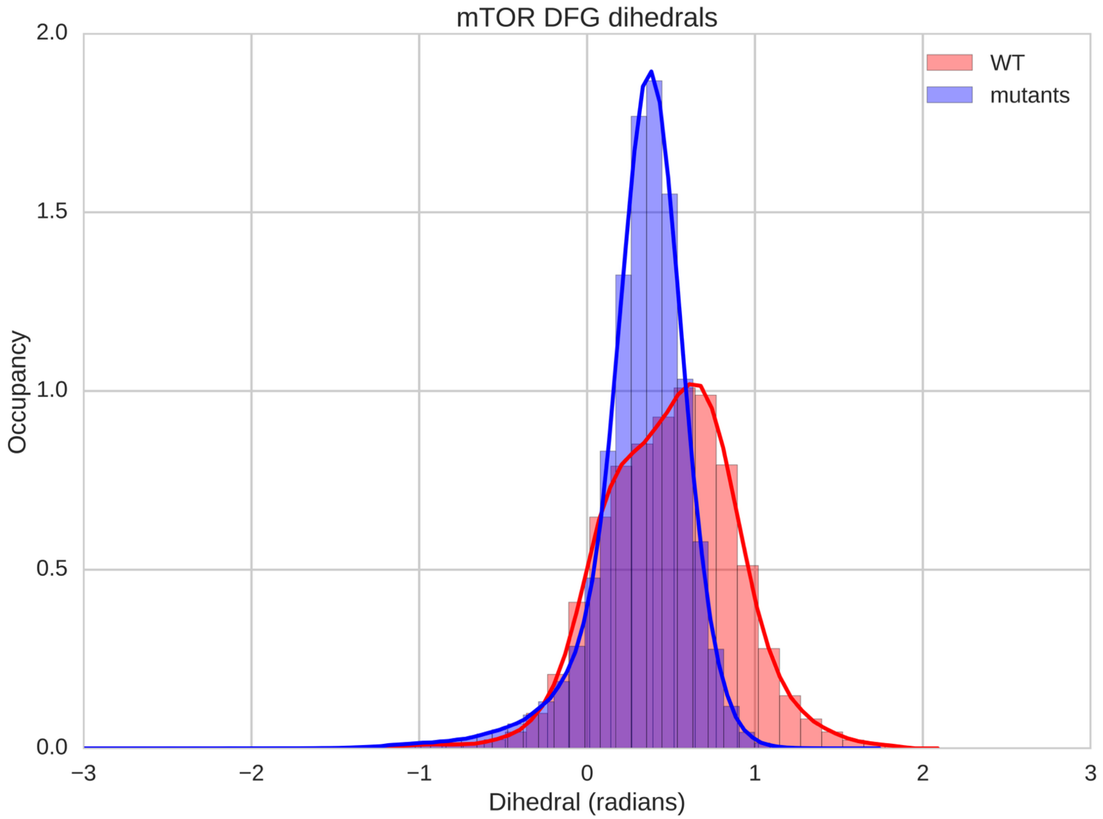
CAN WE DETECT LOCAL STRUCTURAL CHANGES IN THE MUTANTS?



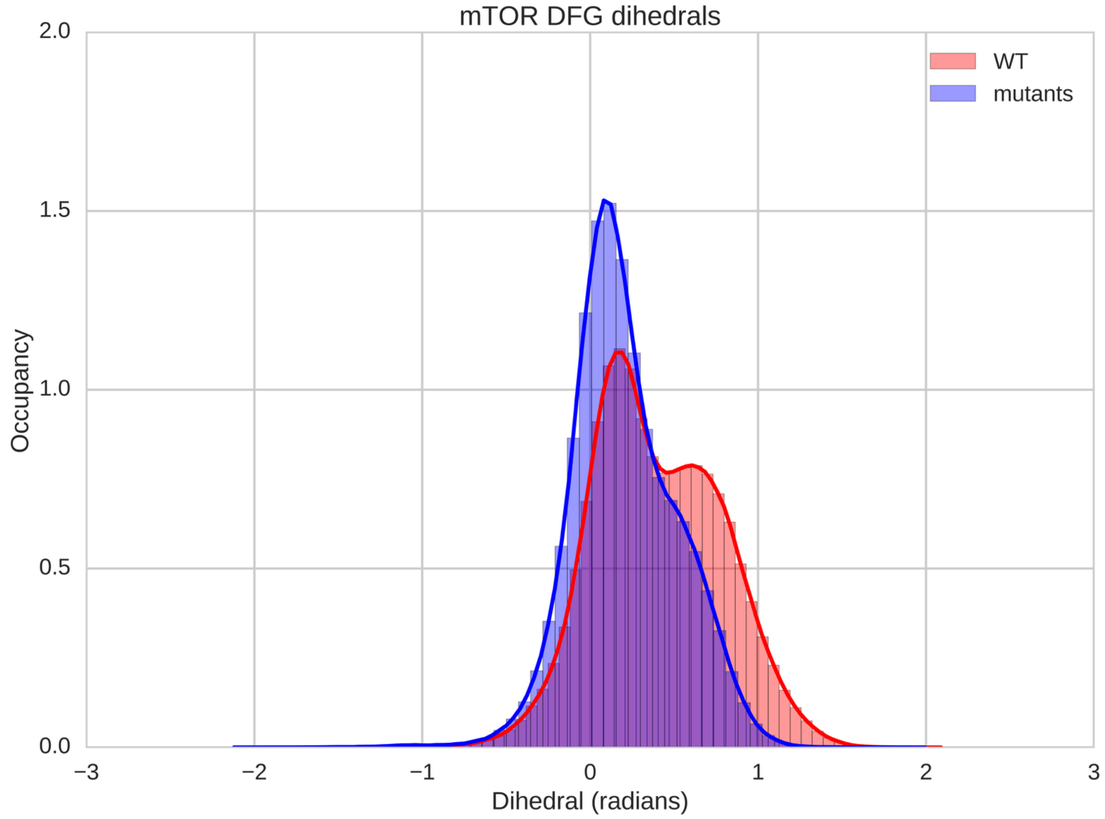


DO MUTANTS AFFECT DFG-IN VS. OUT CONFORMATION?

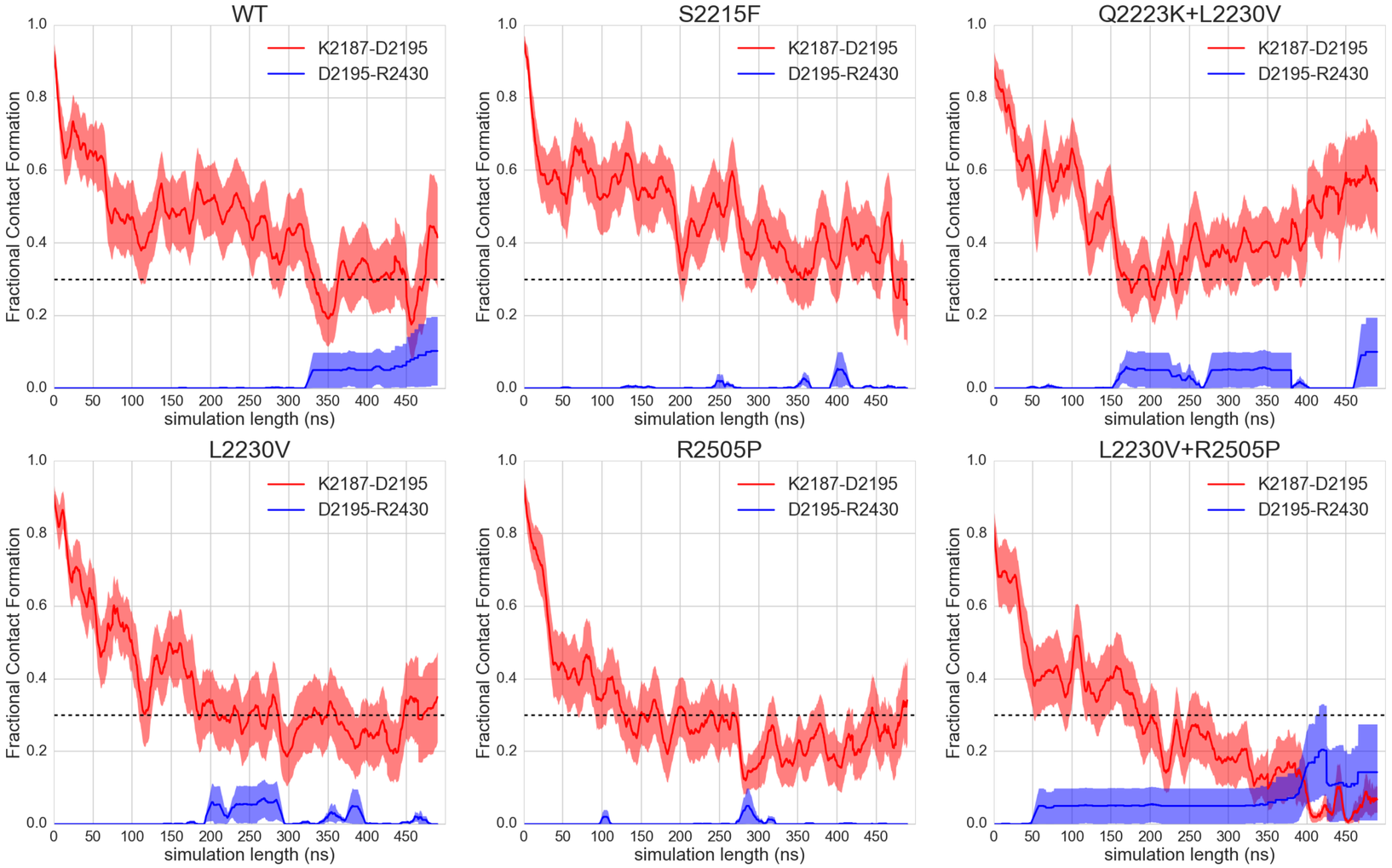
KINASE DOMAIN

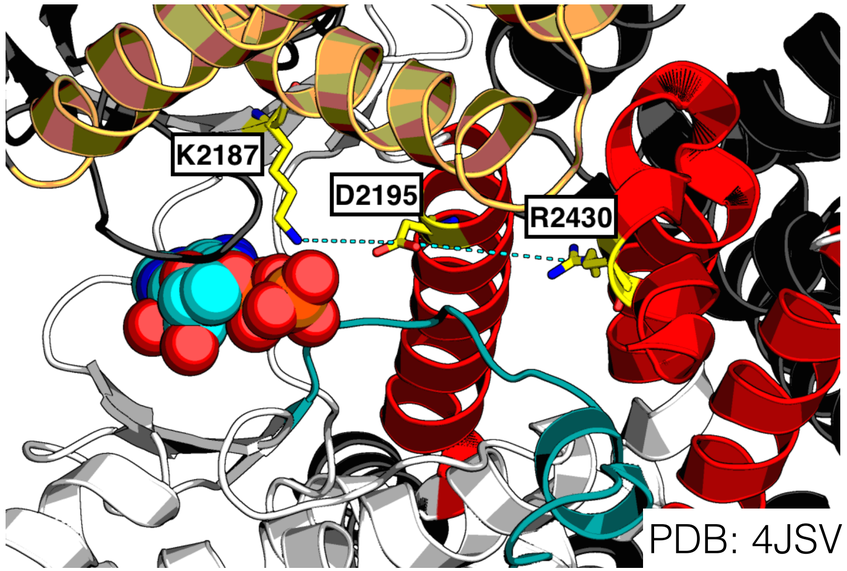


KINASE+FAT DOMAIN



HYPERACTIVATING MUTATIONS MAY PERTURB POPULATION OF STRUCTURAL CONFORMATIONS





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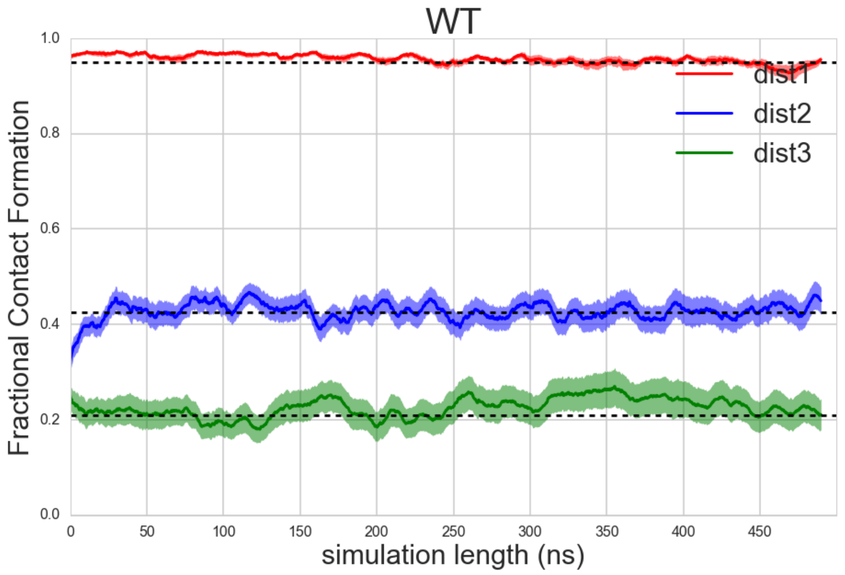
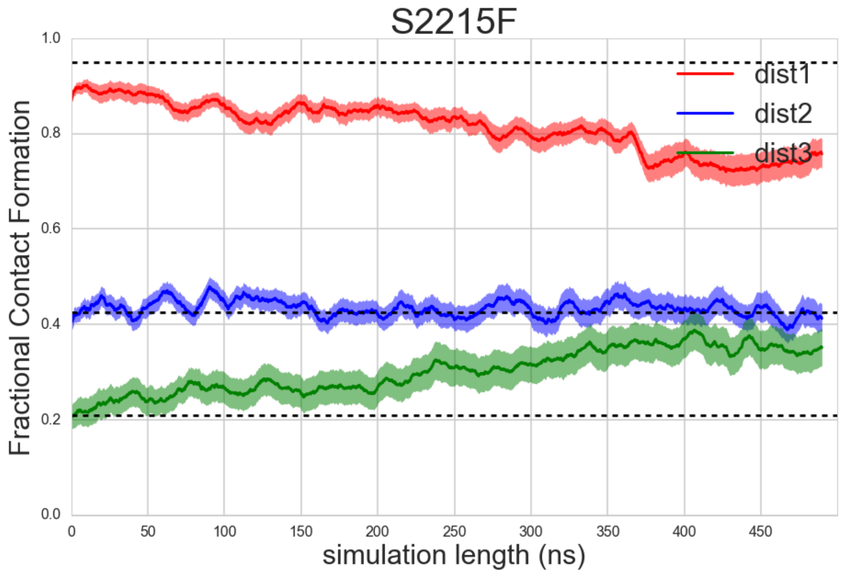
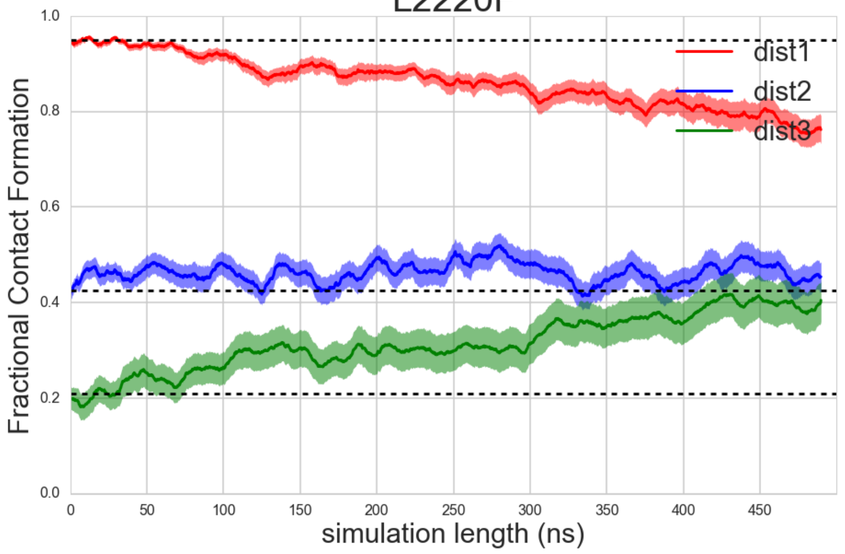
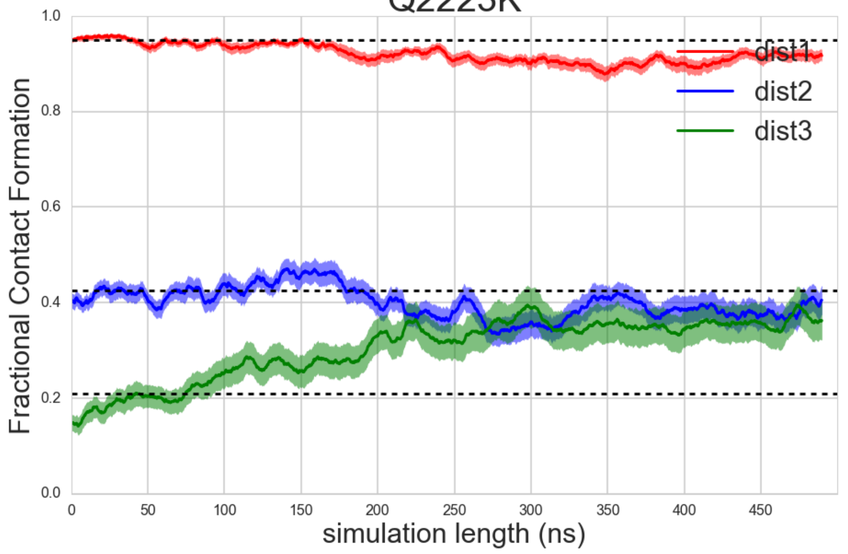
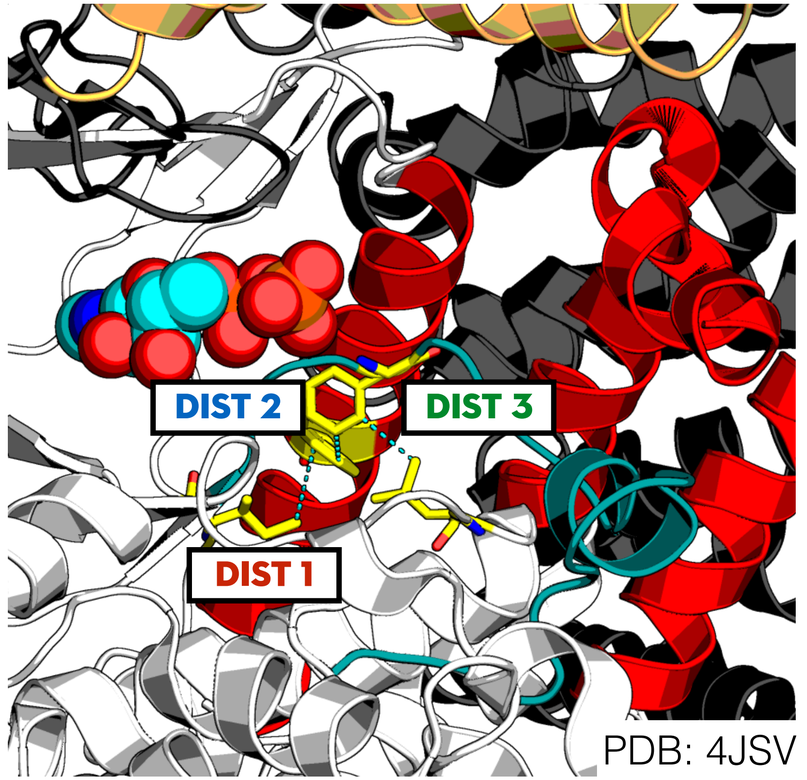


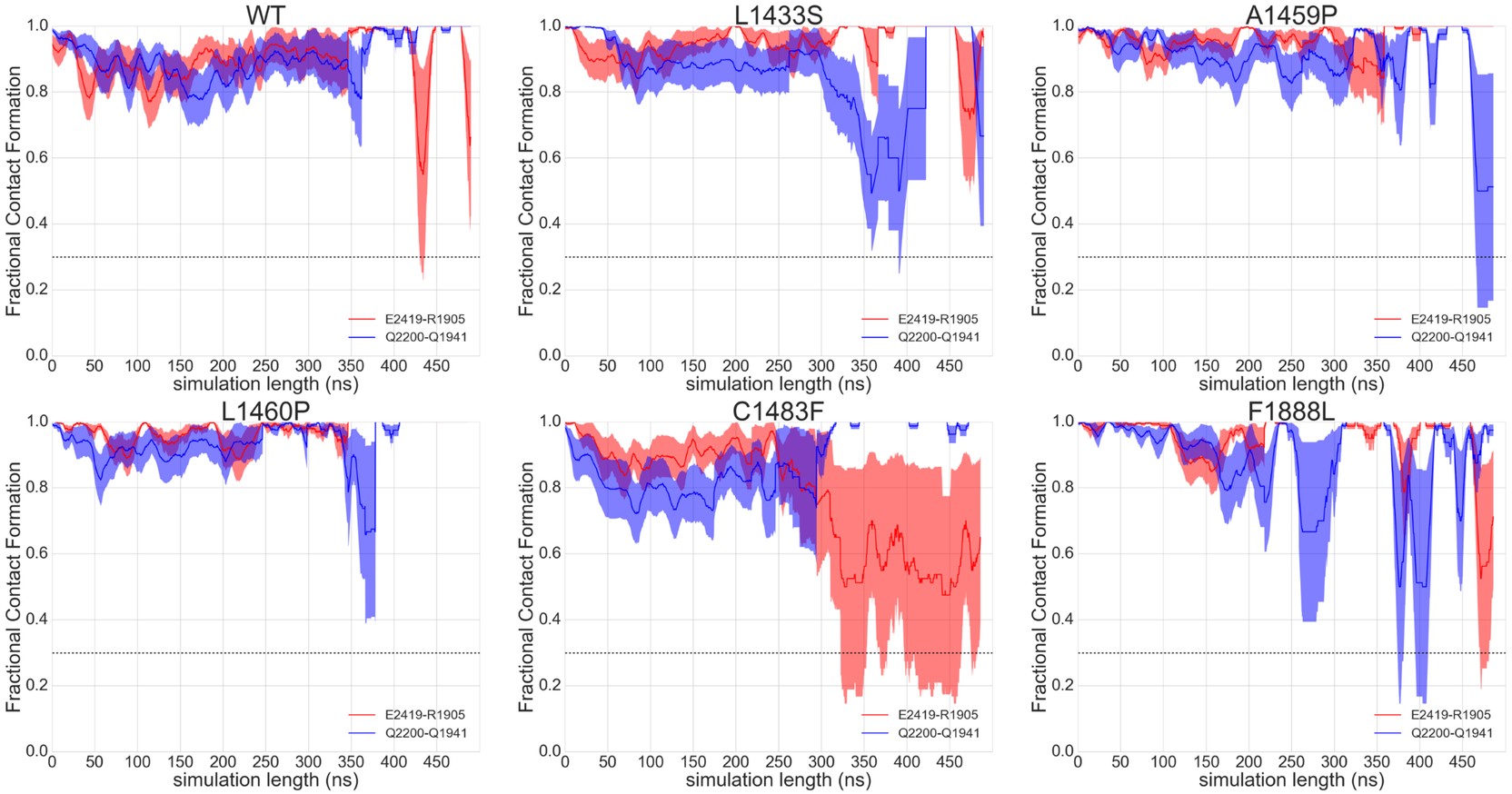


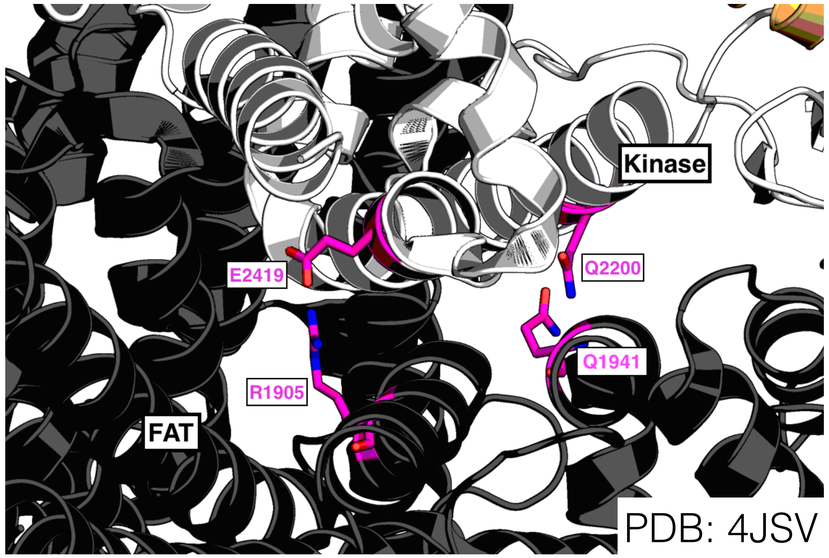




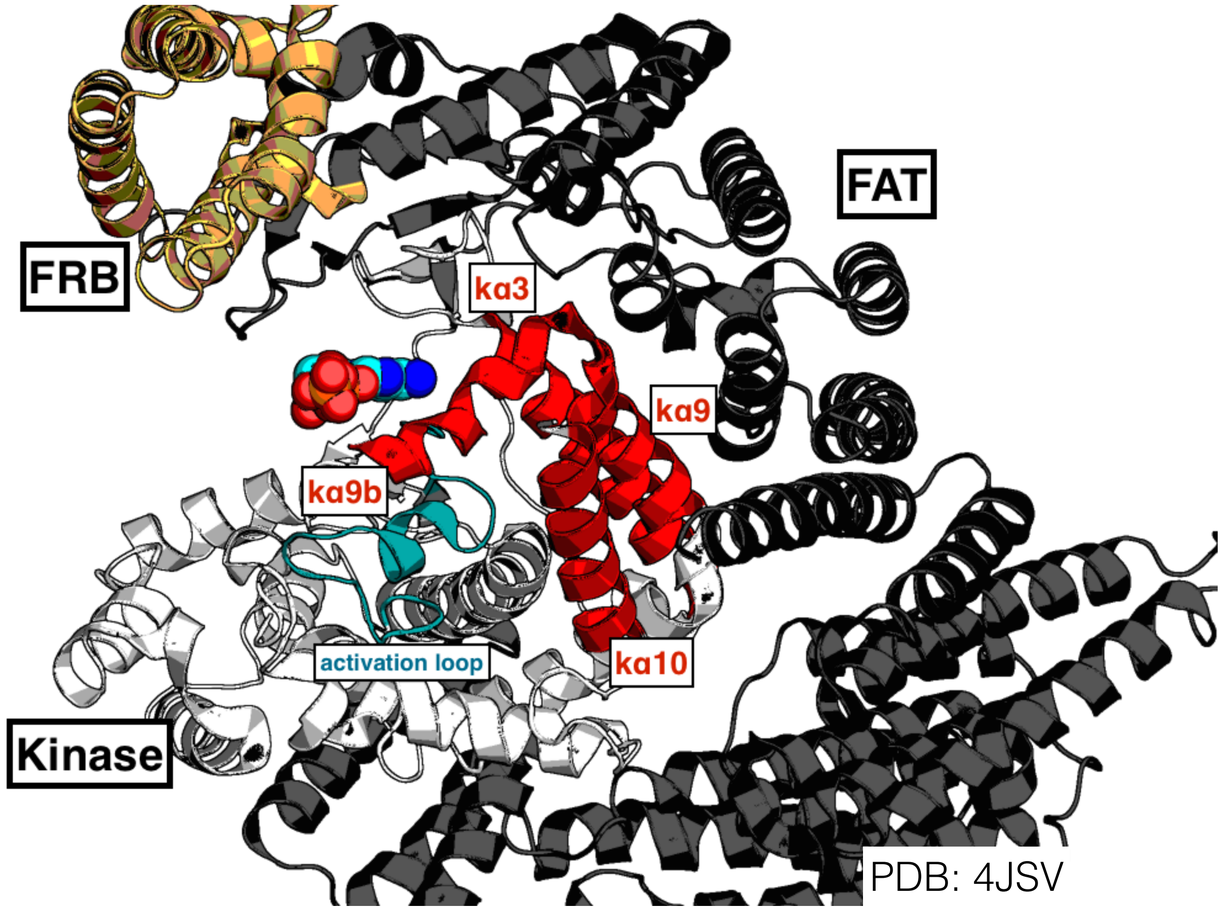


HYPERACTIVATING MUTATIONS MAY PERTURB POPULATION OF STRUCTURAL CONFORMATIONS





FUTURE DIRECTIONS: WHAT OTHER COORDINATES CAN WE LOOK AT TO DISSECT MECHANISMS OF ACTIVATION?



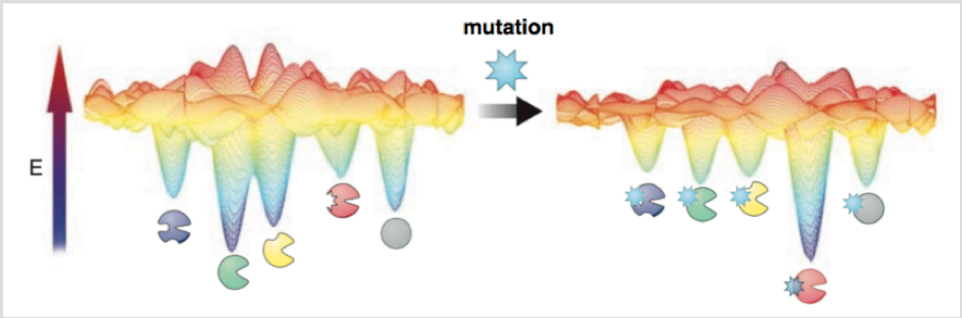
LOOK AT HYPOTHESES FOR REGULATION: SUBSTRATE ACCESS AND HELIX PACKING (SHOWN IN RED)

FUTURE DIRECTIONS: BUILD AN MSM OF WT MTOR FOR BOTH KINASE DOMAIN AND KINASE+FAT DOMAIN

MSMs

MASSIVELY PARALLEL SIMULATIONS ON FOLDING@HOME

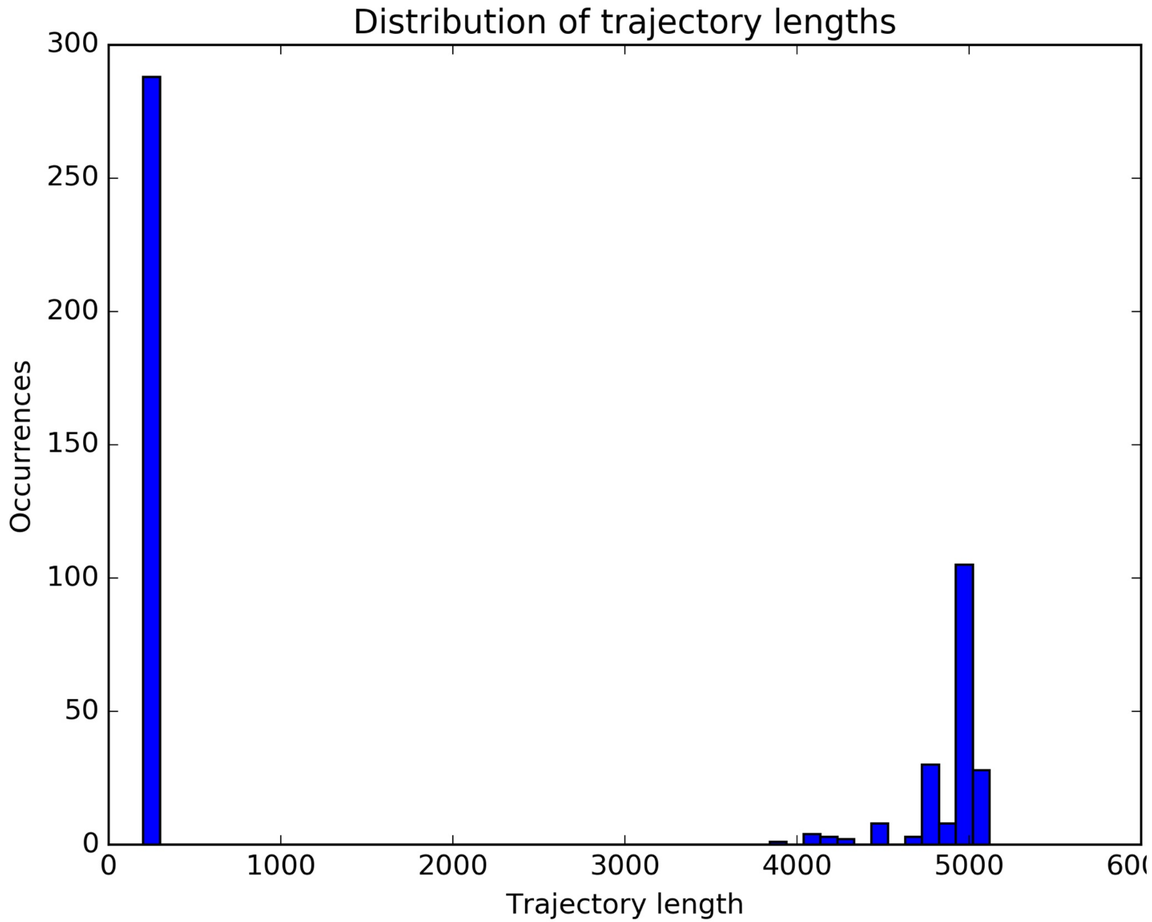
CREATE ATLAS OF MTOR DOMAIN CONFORMATIONS

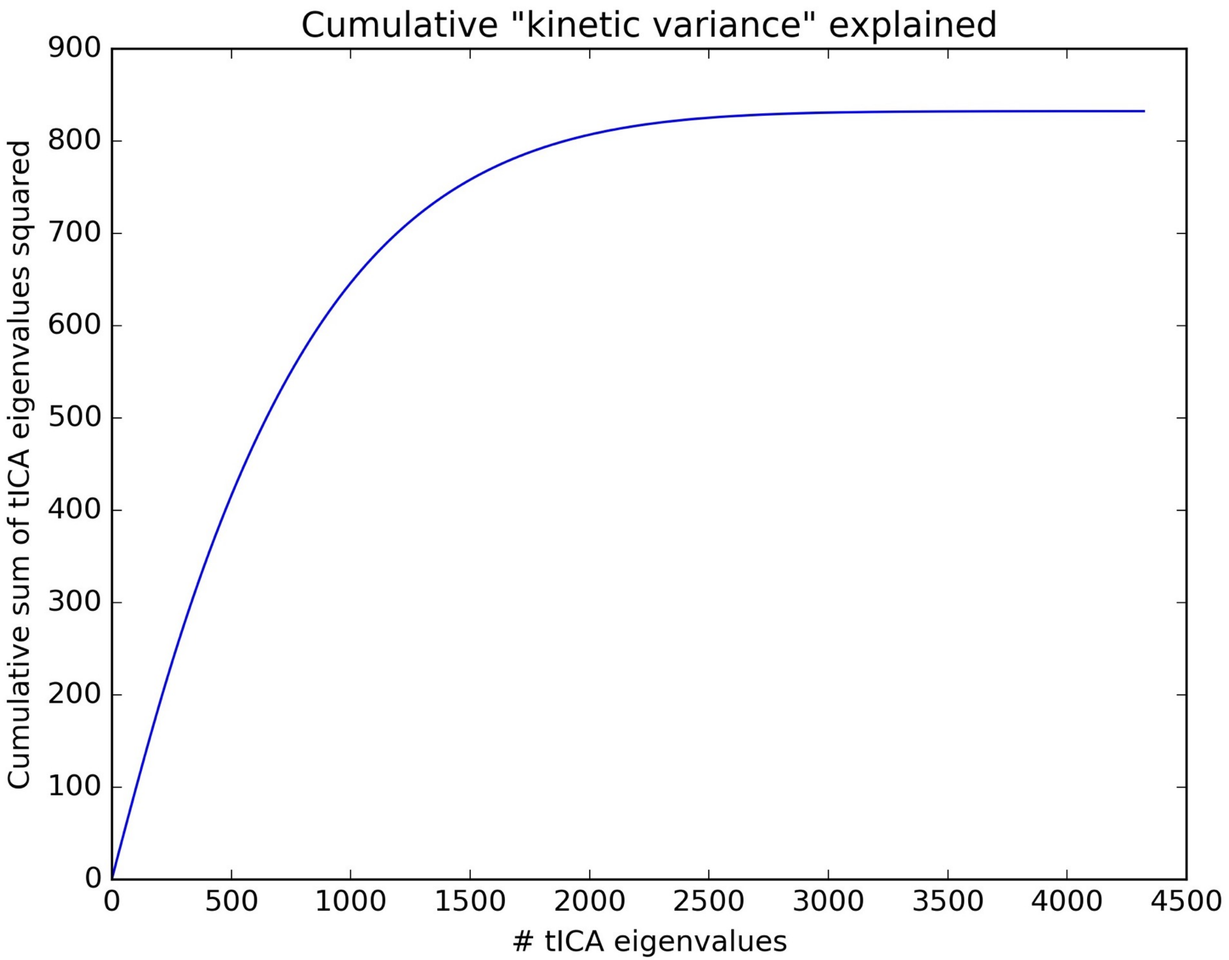


USE ALCHEMICAL FREE ENERGY CALCULATIONS TO EXAMINE THE EFFECT OF MUTANTS ON THE STABILITY OF SPECIFIC CONFORMATIONS

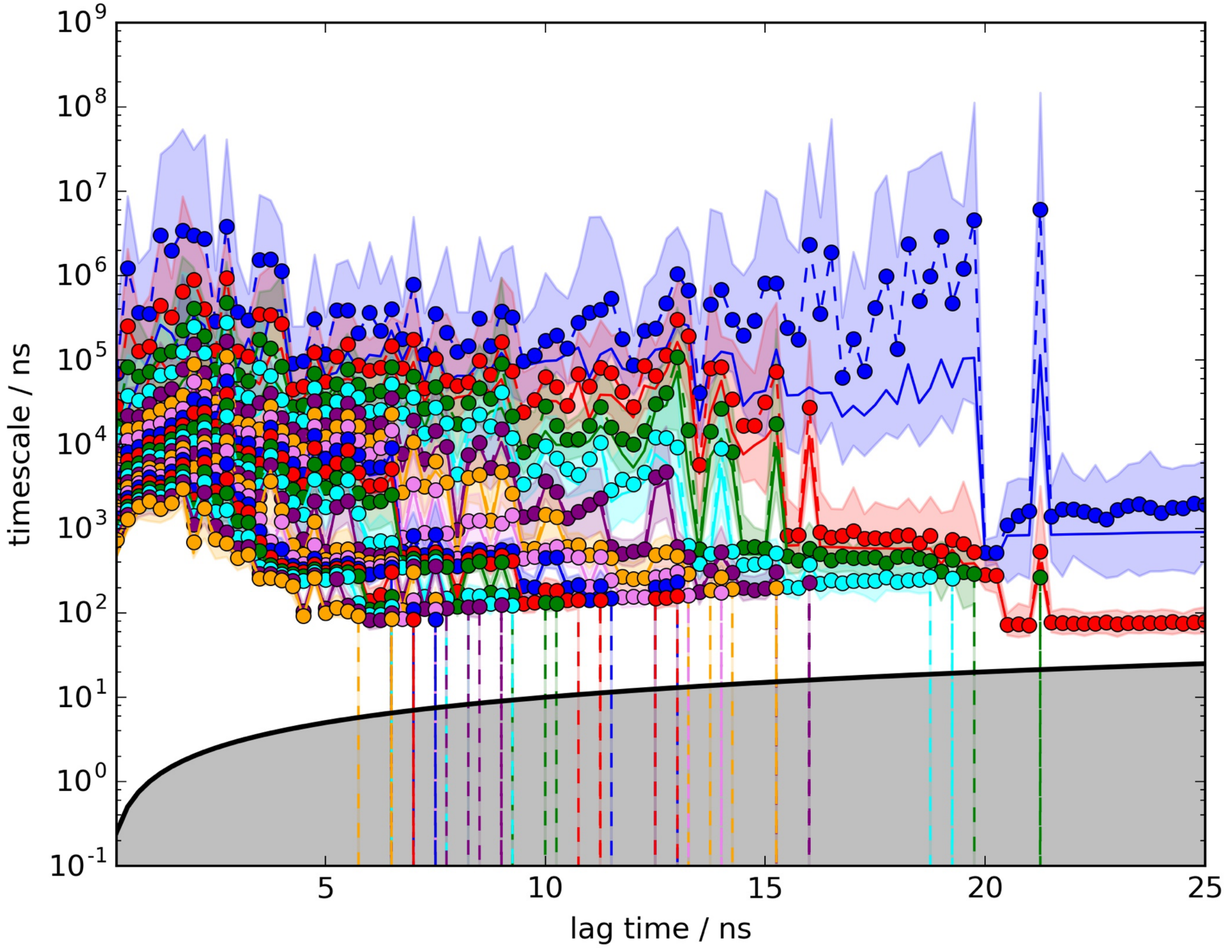
Lee and Craik, 2009

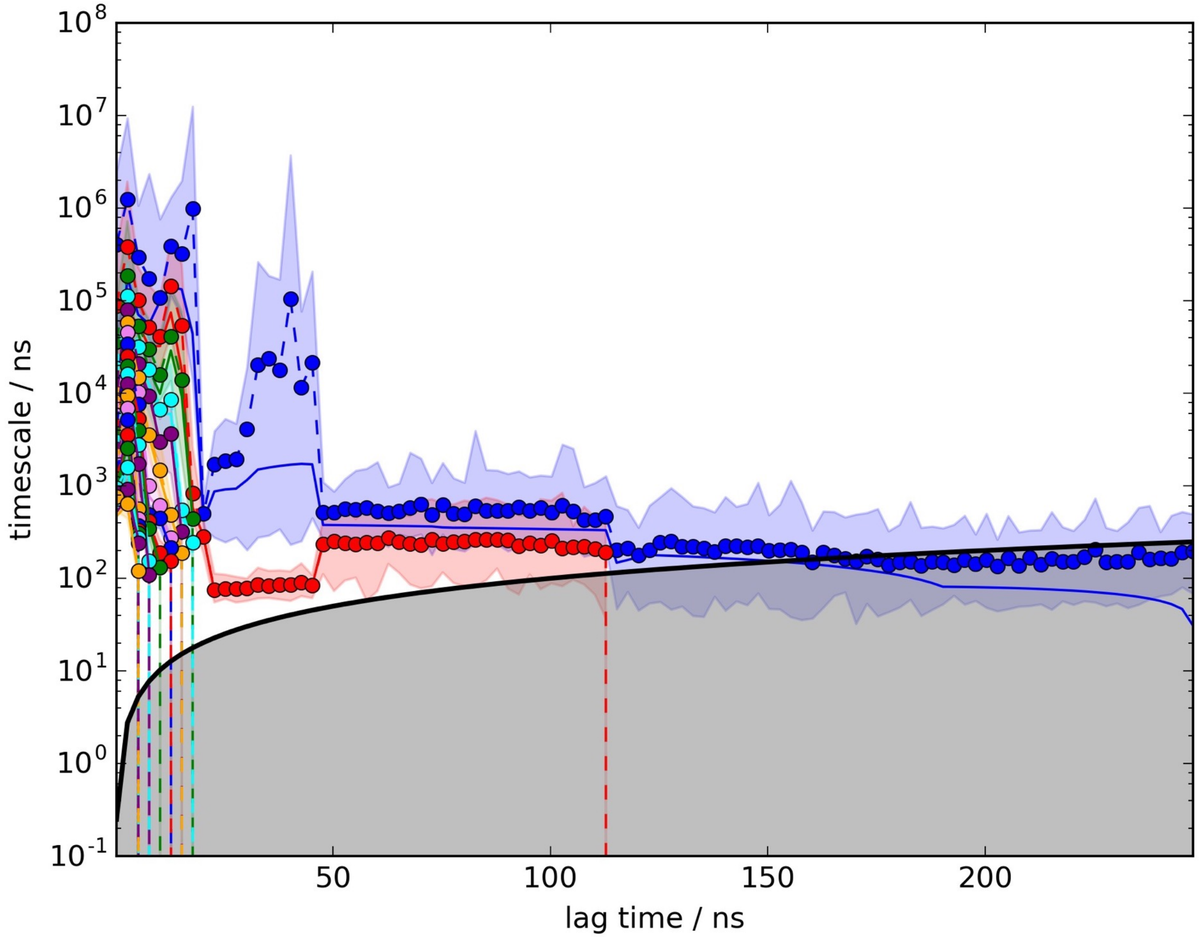
FIRST PASS MTOR KINASE DOMAIN MSM





FIRST PASS MTOR KINASE DOMAIN MSM





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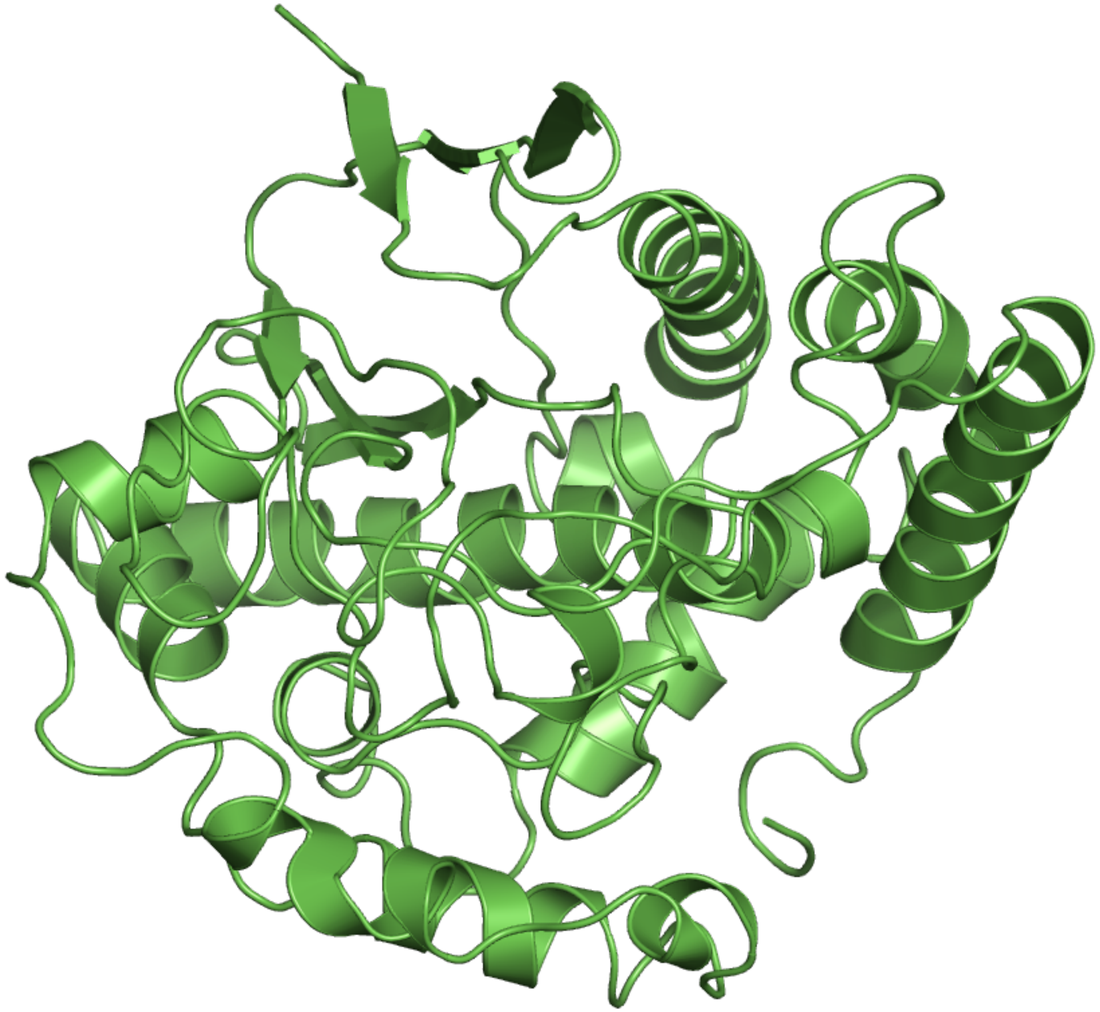
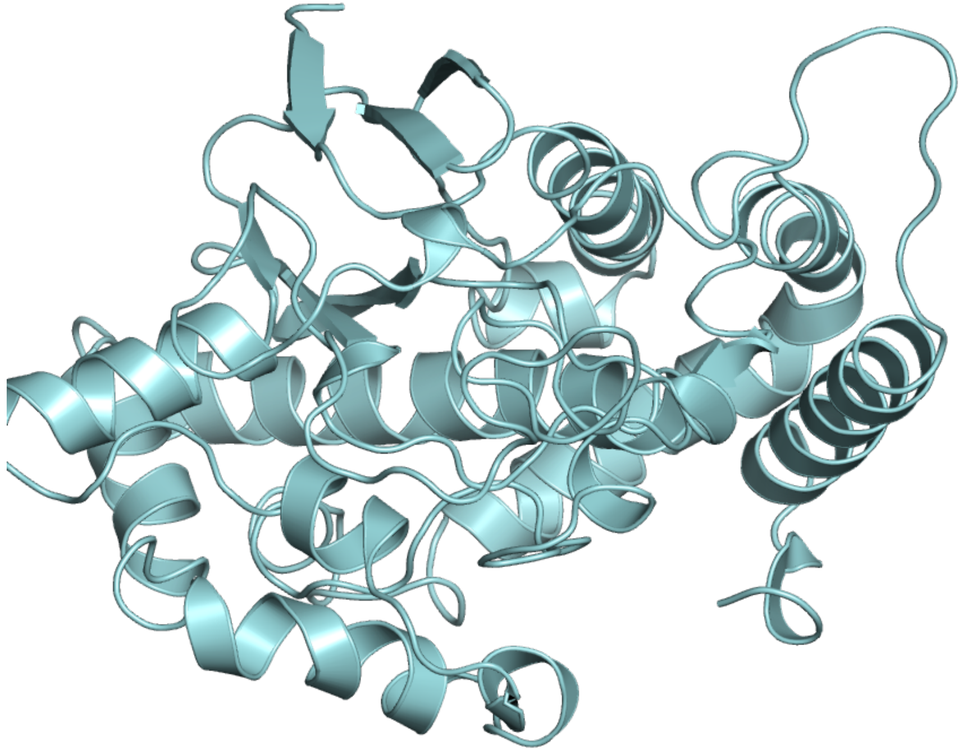
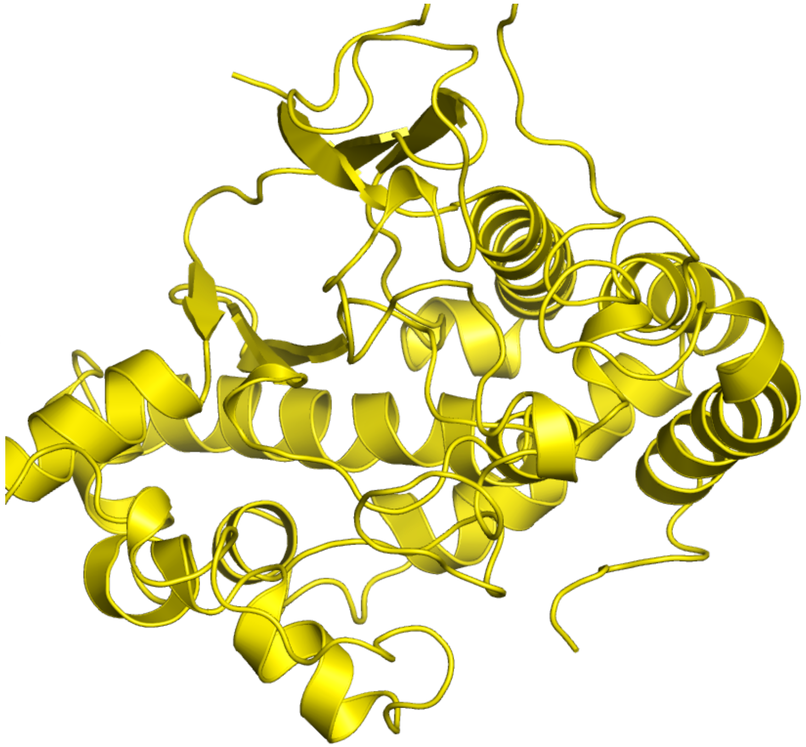
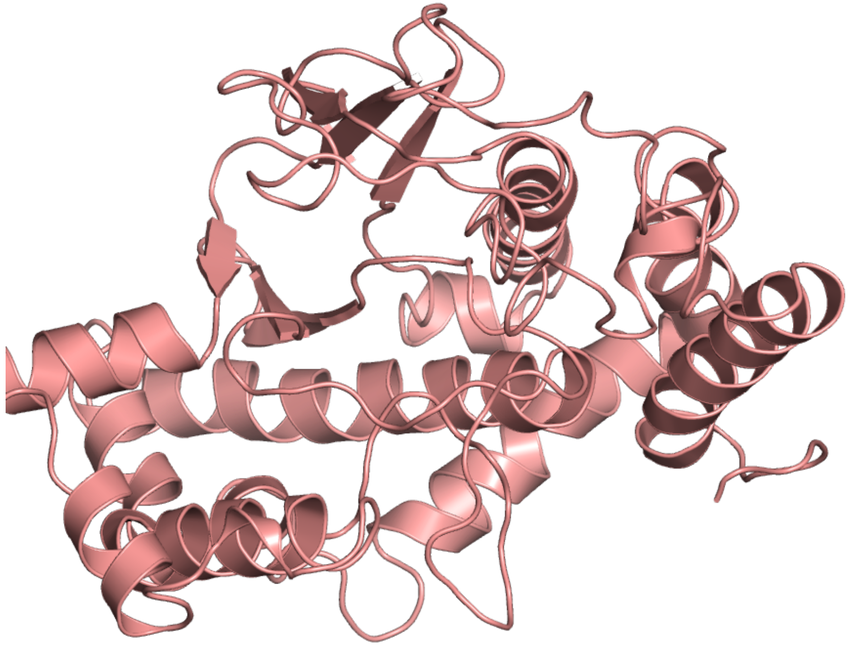


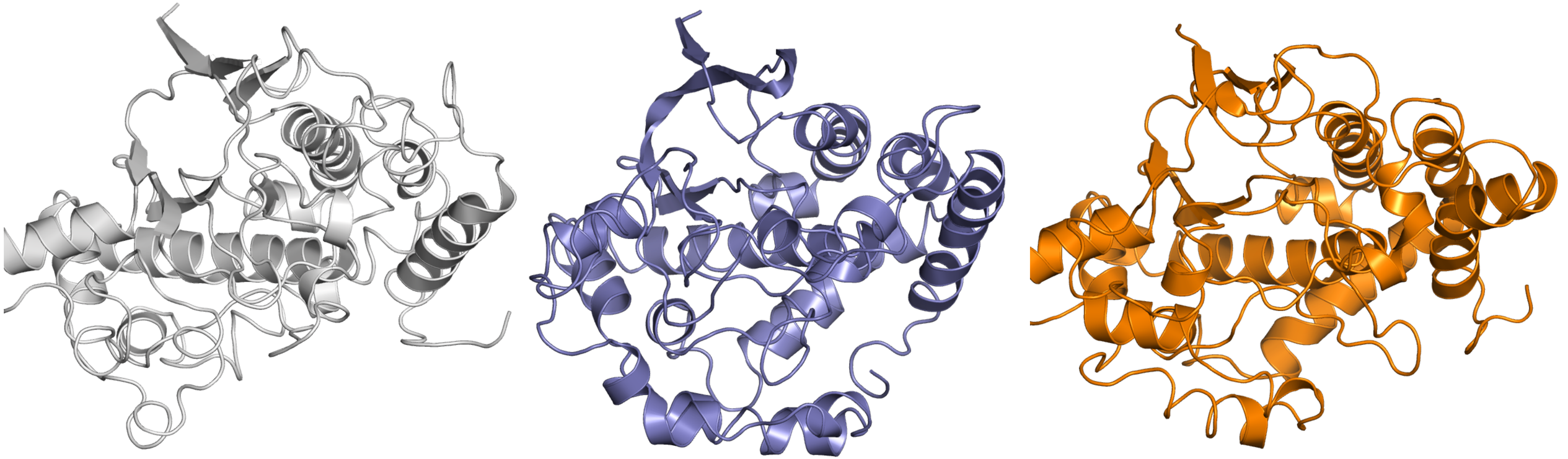


FIRST PASS MTOR KINASE DOMAIN MSM





FIRST PASS MTOR KINASE DOMAIN MSM



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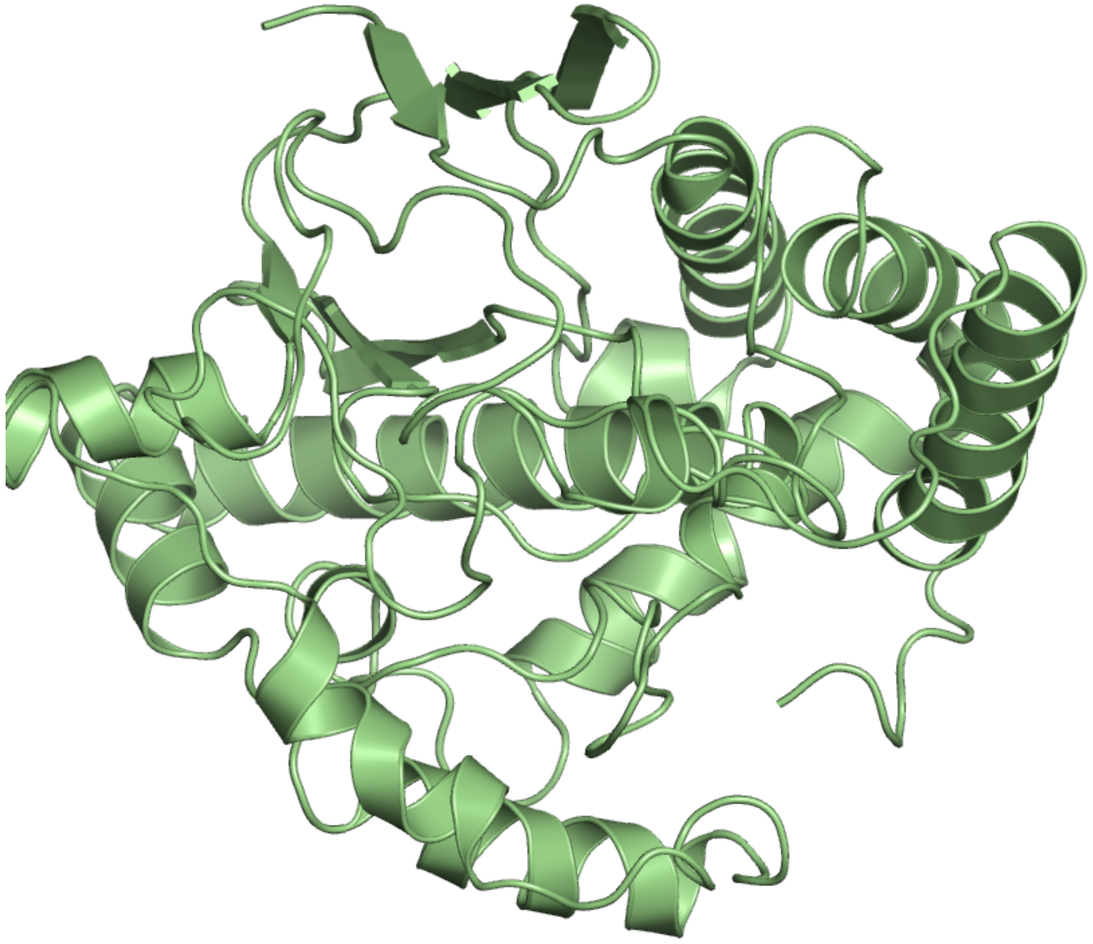
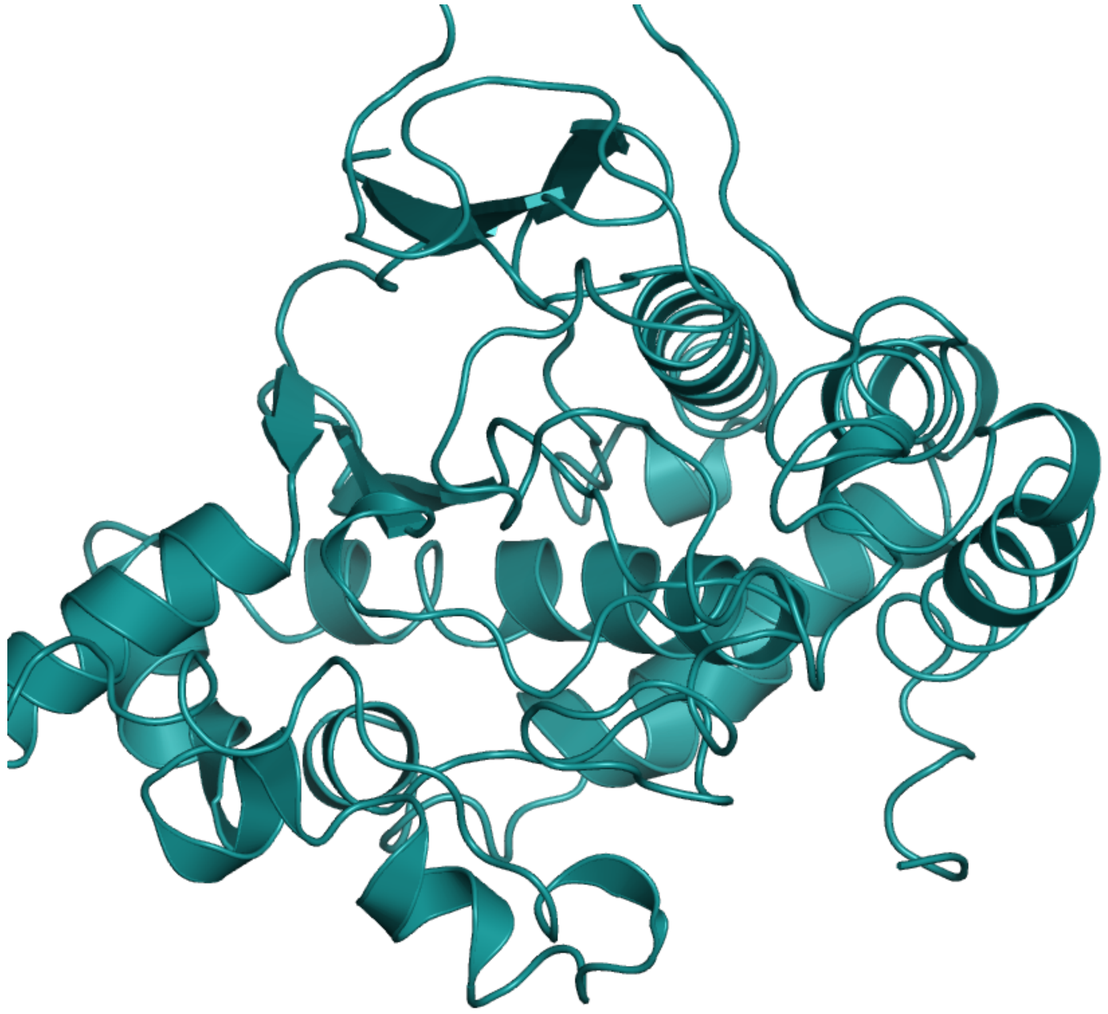
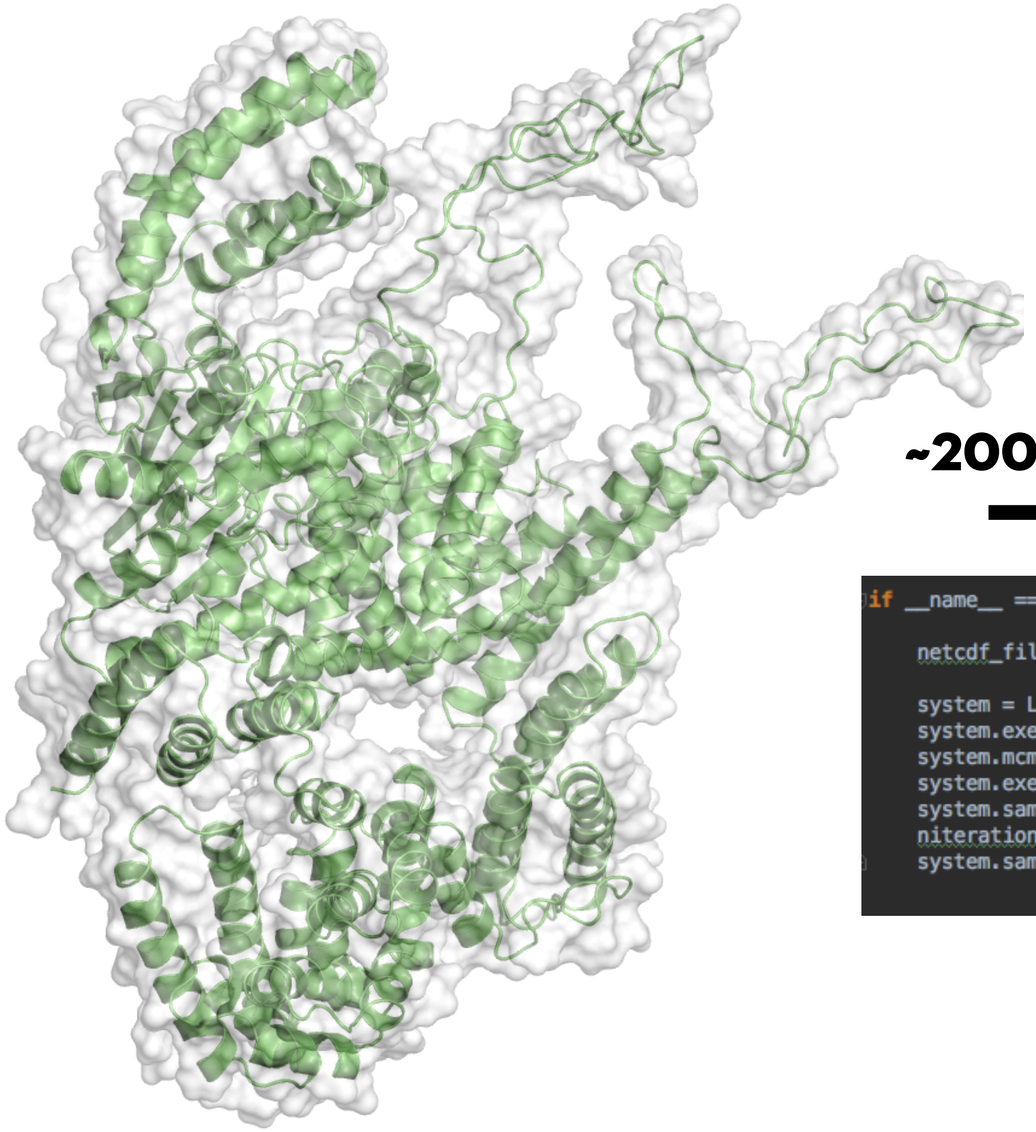




CAN WE CHOOSE BETTER STARTING CONFIGURATIONS FOR LARGE LOOPS?



~2000 ITERATIONS OF SAMS

```
if __name__ == '__main__':

    netcdf_filename = 'output.nc'

    system = LoopSoftening(netcdf_filename=netcdf_filename)
    system.exen_sampler.update_scheme = 'global-jump'
    system.mcmc_sampler.nsteps = 5000
    system.exen_sampler.locality = 10
    system.sams_sampler.update_method = 'optimal'
    niterations = 10000
    system.sams_sampler.run(niterations)
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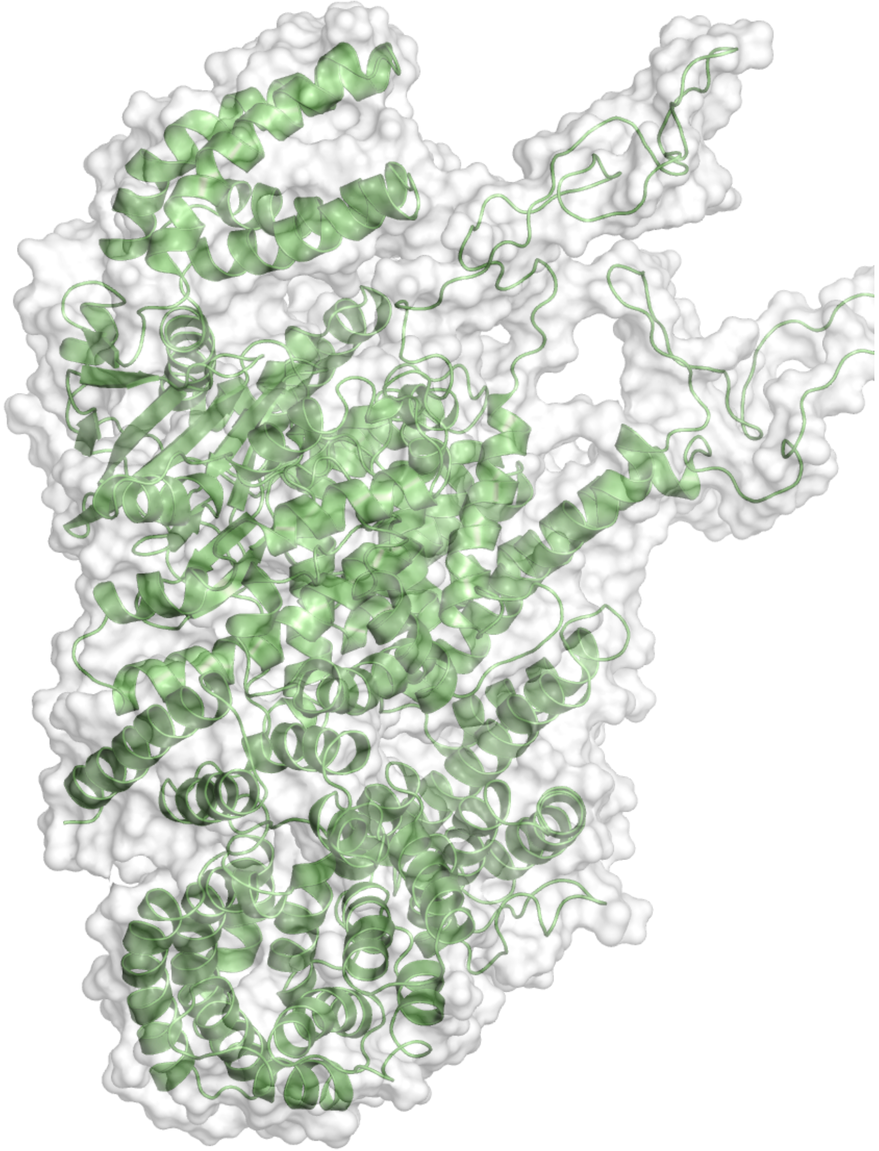
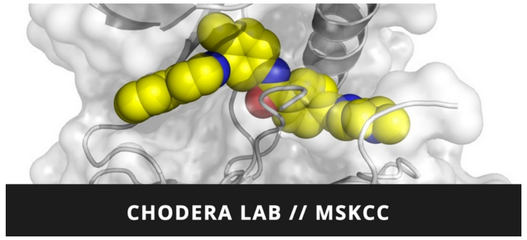


FUTURE DIRECTIONS

- IMPORTANT TO FIND GOOD PARAMETERS TO IDENTIFY ACTIVE CONFORMATIONS OF MOTOR
  - LOOKING AT PIKK-FAMILY OF KINASES MIGHT BE A GOOD PLACE TO START
- NEED TO BUILD A BETTER MSM FOR THE KINASE DOMAIN ALONE- WILL BE AIDED BY HAVING ALL OF MY DATA PRESENT
- NEED TO BUILD AN MSM+FAT DOMAIN MSM AND INVESTIGATE GOOD WAYS TO COMPARE MSMS FOR DIFFERENT CONSTRUCTS
- WORK ON CONVINCING SOMEONE TO ASSAY KINASE ACTIVITY FOR KINASE DOMAIN ALONE AND COMPARE TO KINASE+FAT CONSTRUCT

ACKNOWLEDGEMENTS



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