Simulating mTOR hyperactivating mutations to understand functionally significant structural rearrangements

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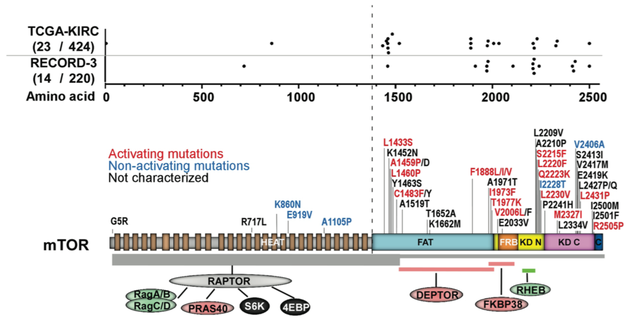
mTOR mutations are observed in RCC patients



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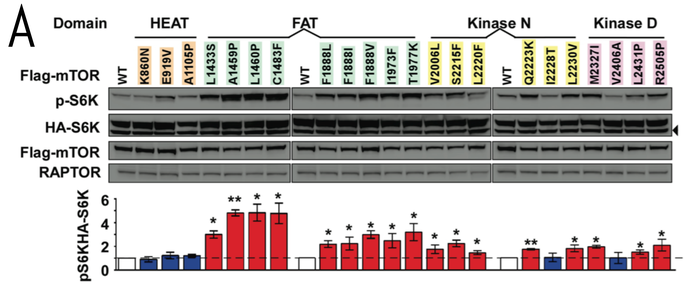
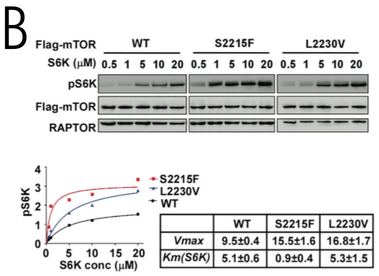
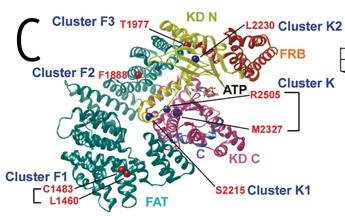
mTOR mutations are hyperactivating through multiple mechanisms





	WT	S2215F	L2230V
Vmax	9.5±0.4	15.5±1.6	16.8±1.7
Km(S6K)	5.1±0.6	0.9±0.4	5.3±1.5

(A) Mutations in the FAT and kinase domains induce higher phosphorylation of S6K. Shown is an immunoblot of whole cell lysate from 293T cells transfected with HA-tagged S6K and FLAG-tagged mTOR and densitometry of phospho-S6K vs. HA-S6K (mean ± SEM for 3 independent experiments) **(B)** In vitro kinase activity assay analyzed by immunoblot and quantitated via densitometry. **(C)** Mutations from different functional clusters exhibit complementary activation when combined. Determined on the basis of S6K phosphorylation.



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

-	No cooperativity
+	Cooperativity

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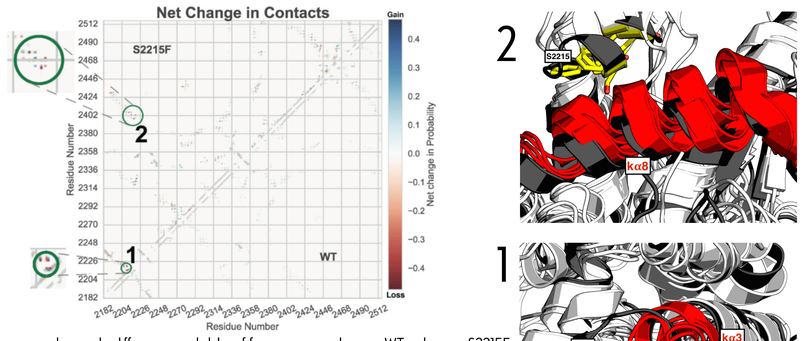
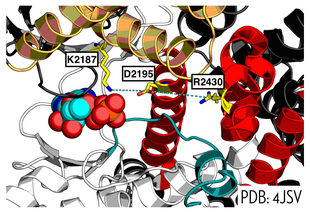
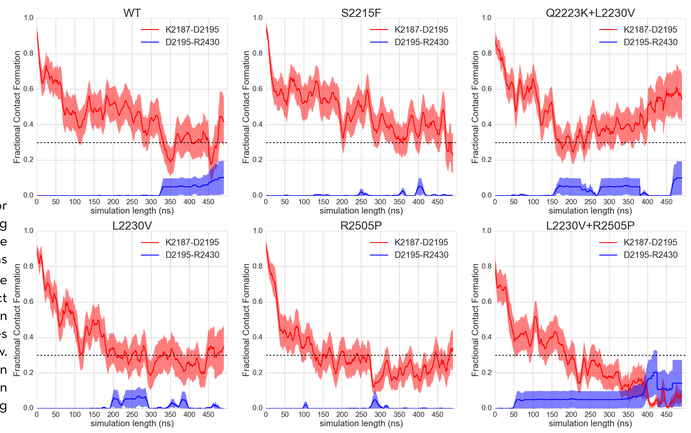
Automated detection of structural rearrangement from multiple MD simulations



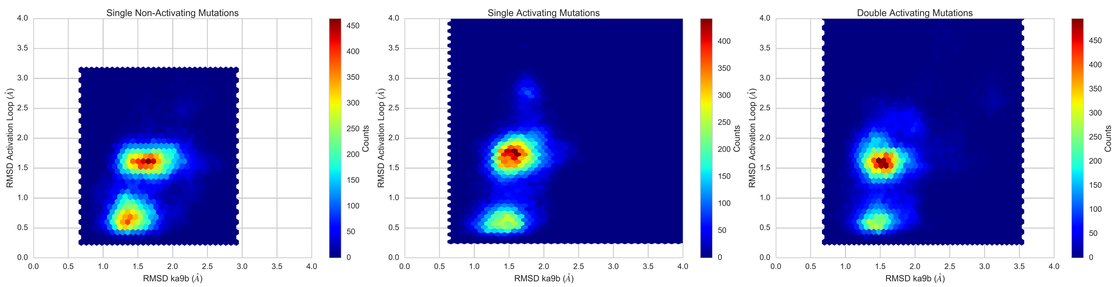
(Top) Contact map showing the difference in probability of forming a contact between WT and mutant S2215F. **(Left, upper)** Region two highlighted in contact map, showing a structural perturbation in helix kα8. Starting structure is shown in gray, the residues indicated in the contact map are shown in red and residue 2215 is shown in yellow. **(Left, lower)** Region one highlighted in contact map, showing a displacement and relaxing of helix kα3. Color is same as above. All trajectories started from PDB: 4JSV from Yang et. al, 2013

Hyperactivating mutations may perturb population of structural conformations

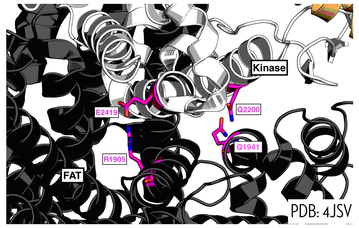




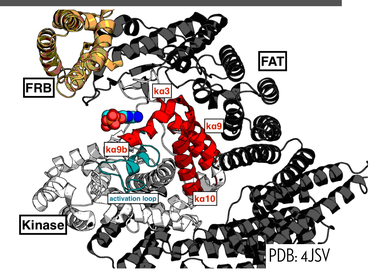
(Right) Fractional contact formation analysis for 20 500ns trajectories, analyzed in 10ns sliding window chunks. Contacts formed when the distance between the closest heavy atoms was 4Å or less. Plotted is the mean ± SEM. The dashed line represents 0.3 fractional contact formation, the proportion roughly populated in the WT simulations. **(Top)** Illustration of distances measured between residues shown in yellow. Kinase domain shown in white, FAT domain in black, FRB in gold, activation loop in teal. Shown in red are helices important in regulating substrate access to catalytic cleft.



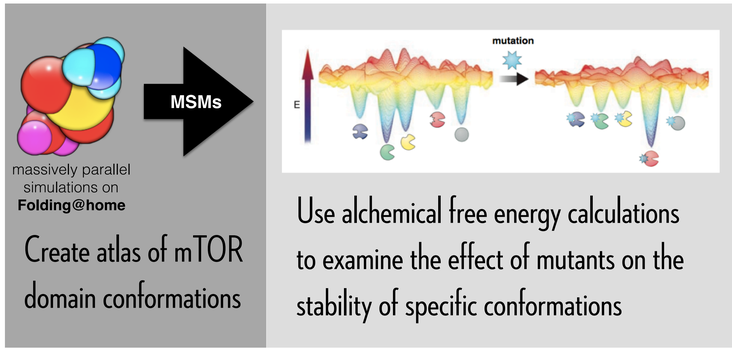
Going forward: investigating substrate access, the FAT domain and MSMs



There are a number of contacts between the FAT and kinase domains. Previous work has shown that mutations of residues involved in forming these key salt bridges activate the kinase domain. This provides a potential mechanism of activation of hyper activating mutants that are able to disrupt the formation of these salt bridges. Exploring these interactions can help understand the role of the FAT domain in regulating the kinase domain.



A proposed mechanism for hyperactivation is modulation of substrate access through changes in helix packing that centers on ka9b. The FRB domain (shown in gold) is also proposed to regulate substrate access, and the distance between this domain and the active site could provide insight into how the physical size of the substrate cleft changes in the presence of mutations.



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

References:

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