Parsing for Differences between Lck and Src Members

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Abstract

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There are hundreds of kinases that have been identified and grouped within eukaryotic organisms and one specific family of these kinases are known as Src Family Kinases (SFKs). A unique trait about SFKs is the large similarity amongst them yet they exhibit non-redundancy in their specialized roles – and one of these members, Lck, is specifically involved with immune response involving T-cell receptors. Understanding what differentiates Lck from the rest of the SFKs would provide insight into how SFKs serve specialized roles and potentially be used towards immunological applications.

Lck belongs to a family of tyrosine kinases known as SRC family kinases (SFKs) that also consist of other members such as Src, Fyn, Yes, Fgr, Lyn, Hck and Blk. These SFK members all share conserved structure of a unique domain, an SH4 domain, an SH3 domain, an SH2 domain, their kinase domain, and a C-terminus tail segment. Unlike other enzymes that exhibit distinct conformational states when active or inactive, SFKs can exist in different degrees due to various regulatory mechanisms understood to be present. Asides the conformational shifts in how an SKFs domains are packed together in a globally closed state or spread out in their globally opened state, there are a few other factors that affects their activity state (Fang et al., 2020). Of particular importance for Lck are tyrosine residues at position 394, located in the activation loop of the kinase domain, and position 505, located in the C-terminus tail segment (Ahler et al., 2019, Gervais et al., 1993). It's been shown that when Tyr394 is phosphorylated, but not Tyr505, the globally open state or activated Lck is stabilized. Whereas on the opposite end of the spectrum, the inactivated state has the Tyr505 position phosphorylated and interacting with SH2 domain to stabilize the inactive form. Lck is a key protein that can be found within lymphocytes, such as T cells, and is understood to be involved with T-cell receptor signaling (TCR) (Bozso et al., 2020). Contrary to their shared similarities in structure and regulation mechanisms, Lck has the distinct role in TCR that cannot be fulfilled by another SFK. Further understanding what key differences may exist in regulation or structural components of Lck versus the rest of the SFKs could provide insight into how the SFKs may differ from one another and serve specific functions, despite the large similarities among them.

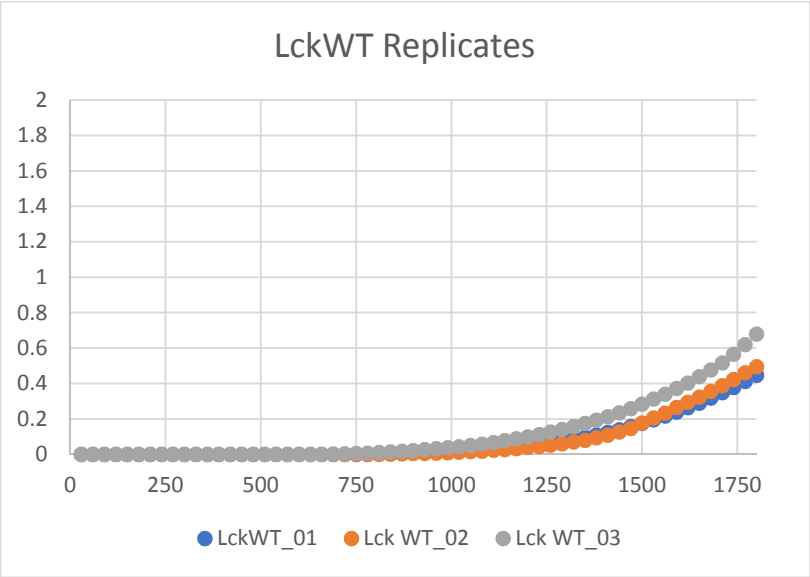
Using previous data obtained through DMS experiments and from the Maly lab, where there were clusters of Lck residues that exhibited change in kinase activity, whereas the corresponding residue changes in Src did not exhibit the same effects. The following mutants listed in table 1 were created to validate the expected increases or decreases in activity. To confirm the expected results, the Lck constructs were transformed into yeast and biological triplicates were utilized for a growth assay. The individual yeast triplicates were periodically measured for OD measurements to calculate growth rate per constructs and growth curves were plotted as shown in figure 1. Adjacent to looking at these residues identified that are expected to have unique significance to Lck in contrast to Src, there was also a broader comparison between Lck's peptide sequence and the remaining SFK members. Using this comparison to identify other potential residues that may be conserved among SFKs or within sub-groups are shown in table 2. Taking that comparison and mapping onto the Lck kinase domain, the different residues where at least one comparison is shown to be either an activating or de-activating mutation, based off Lck DMS.

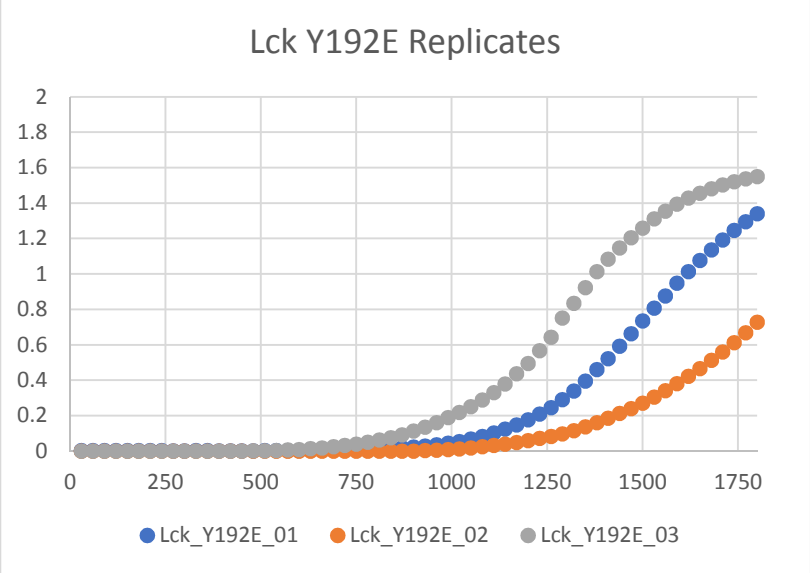
For future steps to take with these Lck constructs, would be to validate the results from the growth assay through western blot analysis. To further prove that the changes in kinase activity are in fact occurring due to activated Lck by probing for levels of phosphotyrosine, with an emphasis for an increase in Tyr394 levels – which should correspond to more activated kinases. Another point that could be useful to follow-up, may be to take some other residues identified from the comparison between Lck and SKFs and perform similar experiments to quantify growth rates and phosphotyrosine levels, if they accurately reflect the expected kinase activity effect.

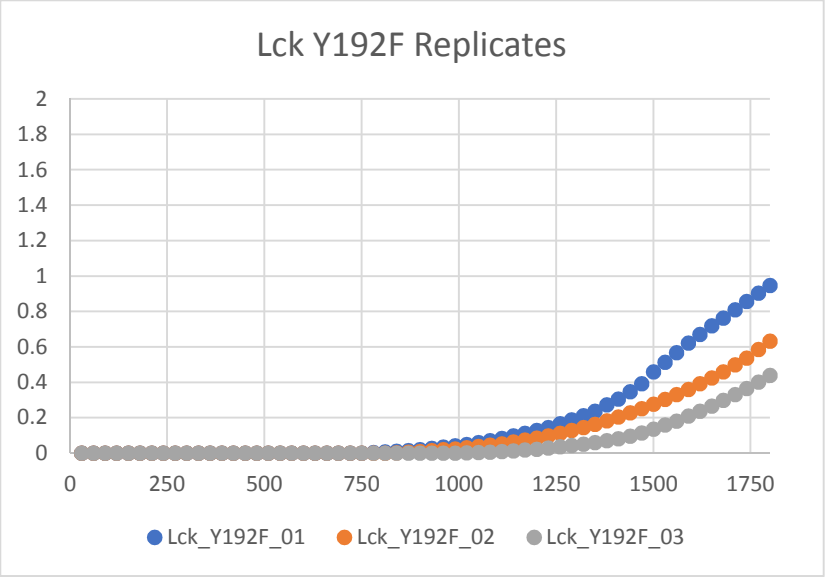
Table 1. Lck mutants/strains tested & their activity scores & growth rates

Lck Construct	**Activity Score**	**Growth Rate (per hr)**
Y192E	-	0.342
K273R	-	0.164
p415_Empty	-	0.156
Y192F	-	0.278
Lck_Wt	-	0.250
D496M	1.19	0.220
T316I	-	0.082
R302S	1.94	0.176
K379V	2.24	0.150
E454W	2.07	0.087
Y304E	2.57	0.124

Figure 1. Lck triplicate growth curves & table with growth rates



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1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
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0
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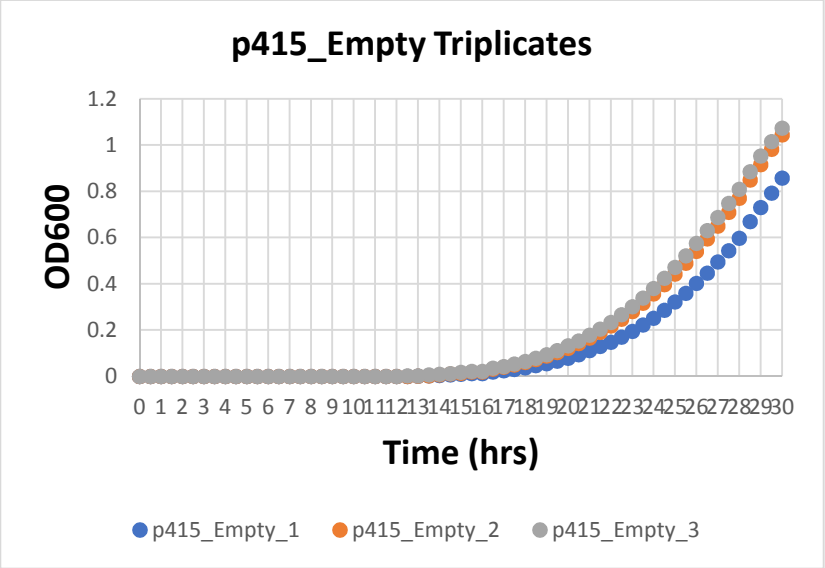
500
750
1000
1250
1500
1750
Lck_Y192F_01
Lck_Y192F_02
Lck_Y192F_03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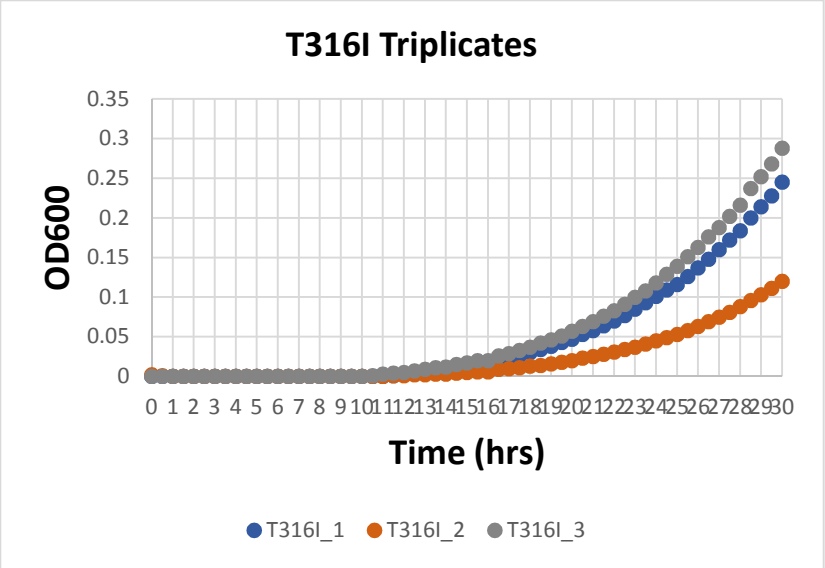
1
0.8
0.6
0.4
0.2
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Time (hrs)
p415_Empty_1
p415_Empty_2
p415_Empty_3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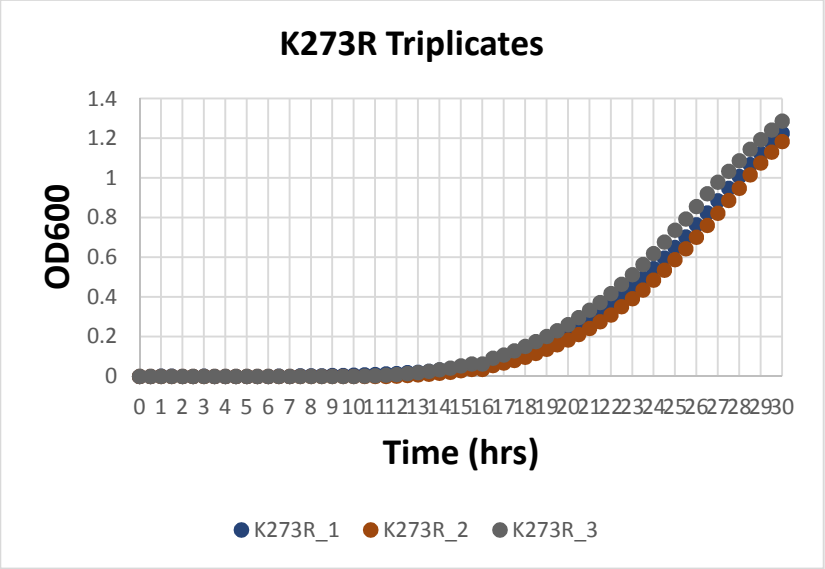
0.25
0.2
0.15
0.1
0.05
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Time (hrs)
T316I_1
T316I_2
T316I_3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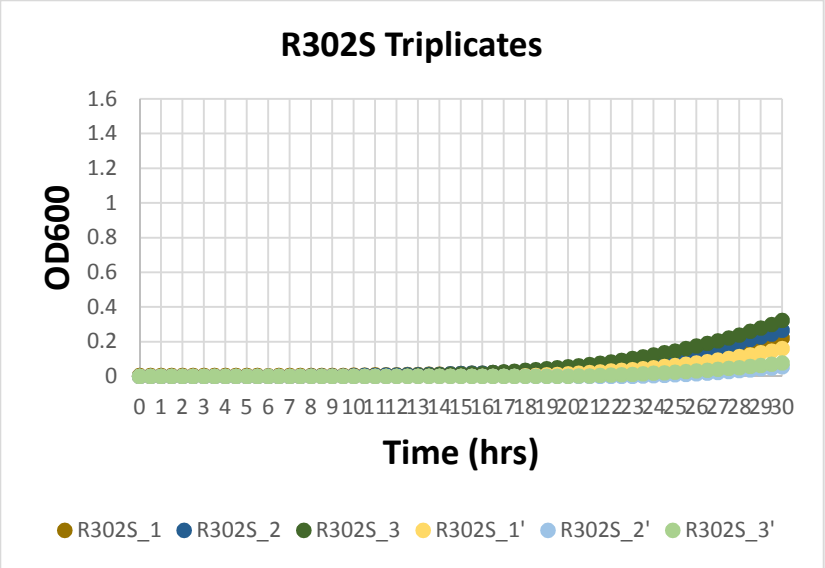
0.8
0.6
0.4
0.2
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Time (hrs)
K273R_1
K273R_2
K273R_3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1.4
1.2
1
0.8
0.6
0.4
0.2
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Time (hrs)
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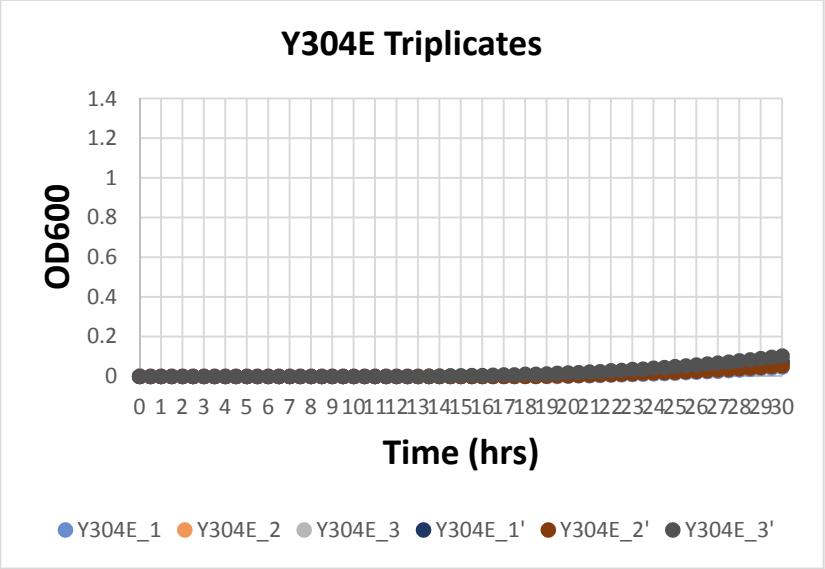
R302S_2
R302S_3
R302S_1'
R302S_2'
R302S_3'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OD600
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1.2
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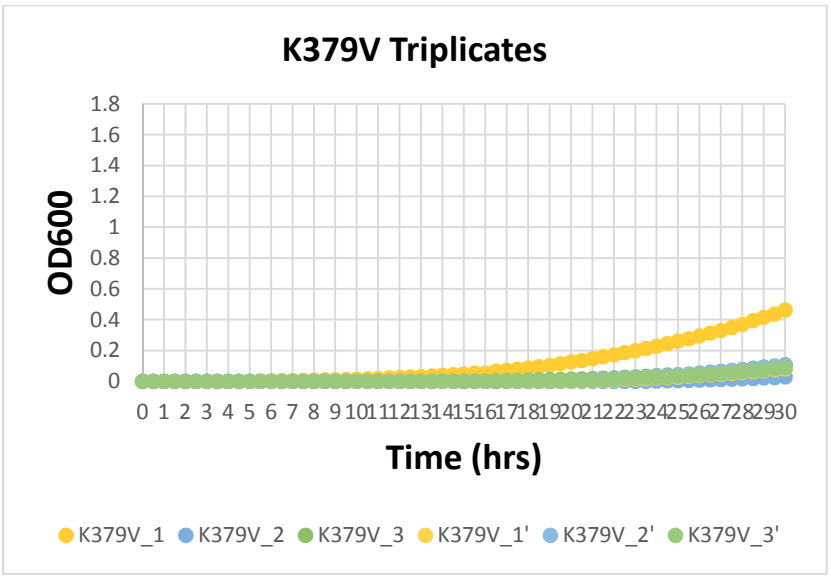
0.8
0.6
0.4
0.2
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Time (hrs)
Y304E_1
Y304E_2
Y304E_3
Y304E_1'
Y304E_2'
Y304E_3'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1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Time (hrs)
K379V_1
K379V_2
K379V_3
K379V_1'
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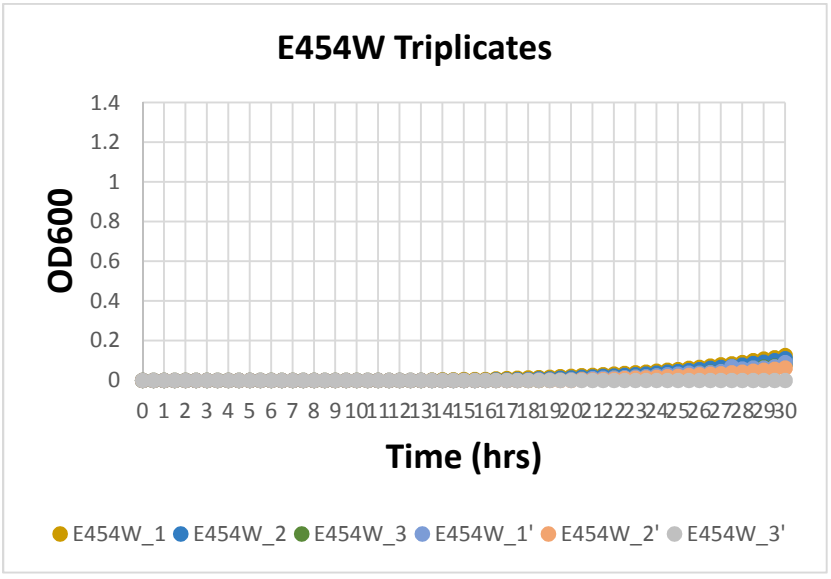
K379V_3'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1.2
1
0.8
0.6
0.4
0.2
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Time (hrs)
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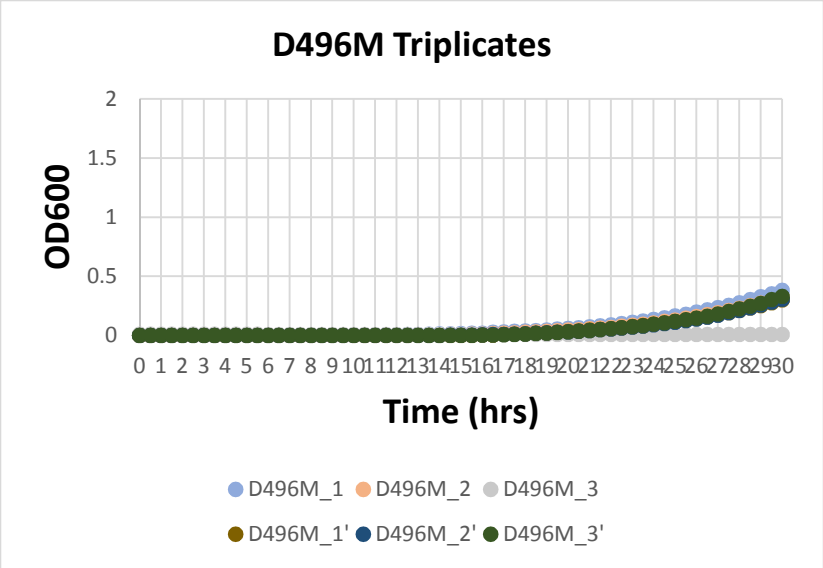
E454W_2
E454W_3
E454W_1'
E454W_2'
E454W_3'


D496M Triplicates
OD600
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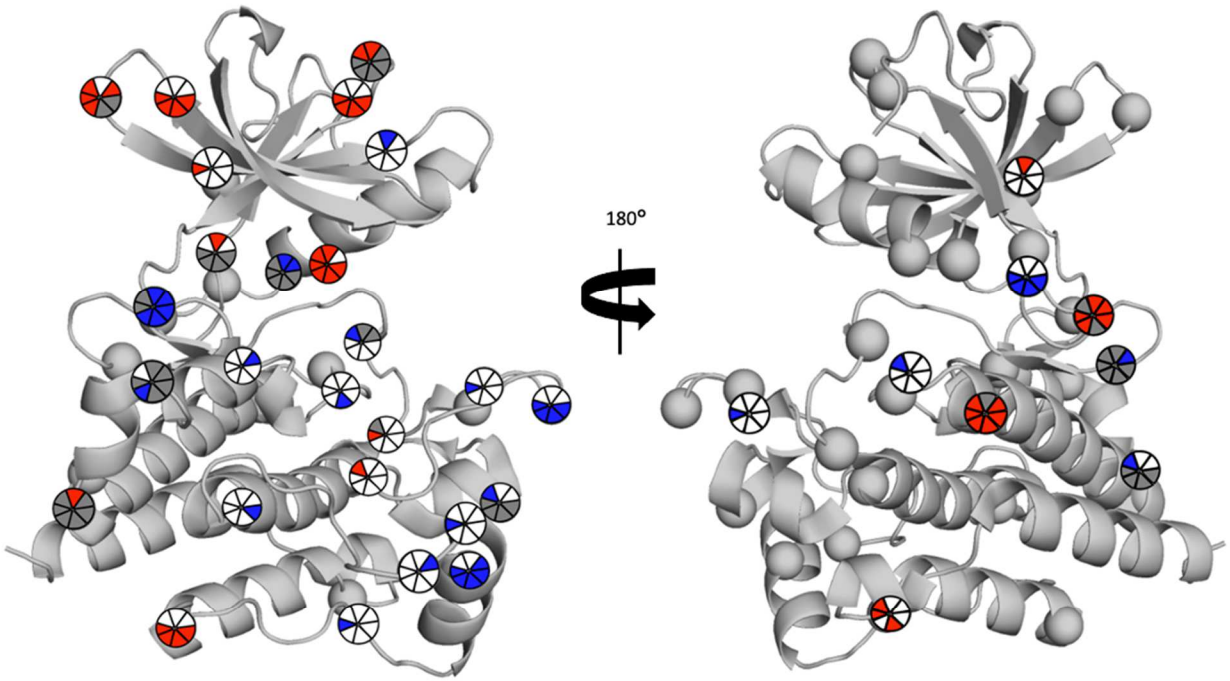
1.5
1
0.5
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Time (hrs)
D496M_1
D496M_2
D496M_3
D496M_1'
D496M_2'
D496M_3'

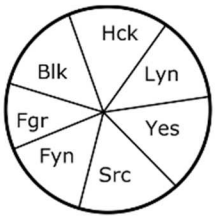
Table 2. Lck vs SFKs (specifically kinase domain only)

Lck No.	Blk	Hck	Lyn	Fgr	Fyn	Src	Yes
261	0.000	0.000	0.000	1.013	0.000	0.000	0.000
264	0.000	0.000	0.000	1.749	1.749	1.749	1.749
267	0.776	0.000	0.692	0.692	0.776	0.266	0.266
275	0.000	-0.632	0.000	0.000	0.000	0.000	0.000
291	0.540	0.540	0.000	0.540	1.211	0.540	1.211
294	0.017	-0.539	-0.539	0.157	-0.087	-0.087	-0.087
296	0.000	0.000	0.000	-1.227	-1.262	-1.227	-1.227
298	-0.119	0.769	0.769	0.769	0.769	-0.119	0.769
302	0.000	0.880	0.000	1.435	1.435	1.435	0.194
304	0.000	0.604	0.000	0.000	0.000	0.000	0.000
308	0.000	0.000	0.000	0.747	0.747	0.747	0.747
309	0.572	0.572	0.017	0.017	0.017	0.017	0.017
321	-0.327	-0.522	-0.522	-0.039	-0.522	-0.522	-0.522
330	0.000	-0.217	-0.217	-0.240	-0.503	-0.300	-0.439
337	-0.221	0.580	-0.010	0.084	0.404	0.084	0.404
340	-0.734	0.000	0.000	-0.415	-0.339	-0.415	-0.415
357	0.584	0.136	0.584	0.584	0.584	0.584	0.584
360	-0.556	0.000	0.000	0.000	0.000	0.000	0.000
361	0.000	0.000	0.000	0.000	0.000	-0.849	0.000
370	0.000	0.000	-0.648	0.000	0.000	0.000	0.000
377	-0.129	0.160	-0.734	0.471	-0.445	0.160	0.160
388	-0.516	-0.325	-0.325	0.000	0.000	0.000	0.000
392	-0.030	0.000	0.000	0.827	0.000	0.000	0.000
395	0.000	0.000	0.000	-0.515	0.000	0.000	0.000
396	0.000	0.000	0.000	-1.279	0.000	0.000	0.000
398	0.000	0.000	0.000	-0.819	-0.819	-0.819	-0.819
413	-0.584	0.000	0.000	0.208	0.208	0.208	0.208
421	0.577	0.000	0.000	0.000	0.000	0.000	0.000
430	0.000	0.000	0.000	0.000	0.000	0.000	-1.505
444	0.000	0.000	-1.470	0.000	0.000	0.000	0.000
446	0.000	0.000	0.000	-0.928	0.000	0.000	0.000
450	0.000	0.000	-0.769	-0.499	-0.499	-0.499	-0.499
458	0.000	0.000	0.000	-0.824	0.000	0.000	0.000
468	0.000	0.000	0.000	0.636	0.636	0.636	0.636
478	0.574	0.000	0.000	0.574	0.000	0.574	0.000

Lck No.	Blk	Hck	Lyn	Fgr	Fyn	Src	Yes
261	Identical	Identical	Identical	M261L	Identical	Identical	Identical
264	Identical	Identical	Identical	Y264W	Y264W	Y264W	Y264W
267	H267N	Identical	H267S	H267S	H267N	H267T	H267T
275	Identical	L275M	Identical	Identical	Identical	Identical	Identical
291	L291V	L291V	Identical	L291V	L291I	L291V	L291I
294	Q294A	Q294T	Q294T	Q294L	Q294K	Q294K	Q294K
296	Identical	Identical	Identical	Q296R	Q296K	Q296R	Q296R
298	Q298E	Q298D	Q298D	Q298D	Q298D	Q298E	Q298D
302	Identical	R302K	Identical	R302Q	R302Q	R302Q	R302P
304	Identical	Y304H	Identical	Identical	Identical	Identical	Identical
308	Identical	Identical	Identical	T308S	T308S	T308S	T308S
309	Q309K	Q309K	Q309E	Q309E	Q309E	Q309E	Q309E
321	N321R	N321K	N321K	N321H	N321K	N321K	N321K
330	Identical	T330S	T330S	T330N	T330D	T330G	T330E
337	T337S	T337P	T337L	T337R	T337K	T337R	T337K
340	K340R	Identical	Identical	K340Q	K340N	K340Q	K340Q
357	E357R	E357Q	E357R	E357R	E357R	E357R	E357R
360	Y360S	Identical	Identical	Identical	Identical	Identical	Identical
361	Identical	Identical	Identical	Identical	Identical	I361V	Identical
370	Identical	Identical	I370V	Identical	Identical	Identical	Identical
377	S377C	S377V	S377M	S377A	S377I	S377V	S377V
388	L388I	L388V	L388V	Identical	Identical	Identical	Identical
392	N392S	Identical	Identical	N392D	Identical	Identical	Identical
395	Identical	Identical	Identical	T395N	Identical	Identical	Identical
396	Identical	Identical	Identical	A396P	Identical	Identical	Identical
398	Identical	Identical	Identical	E398Q	E398Q	E398Q	E398Q
413	N413H	Identical	Identical	N413L	N413L	N413L	N413L
421	S421A	Identical	Identical	Identical	Identical	Identical	Identical
430	Identical	Identical	Identical	Identical	Identical	Identical	L430Q
444	Identical	Identical	M444R	Identical	Identical	Identical	Identical
446	Identical	Identical	Identical	N446K	Identical	Identical	Identical
450	Identical	Identical	I450M	I450L	I450L	I450L	I450L
458	Identical	Identical	Identical	R458H	Identical	Identical	Identical
468	Identical	Identical	Identical	E468S	E468S	E468S	E468S
478	K478R	Identical	Identical	K478R	Identical	K478R	Identical

Figure 2. Lck mapped with table 2 comparisons





Classification	Color
Gain of Func	
Loss of Func	
Wild-type	
Identical	

Crystal Structure of Lck KD: PDB ID 3AD6

References

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