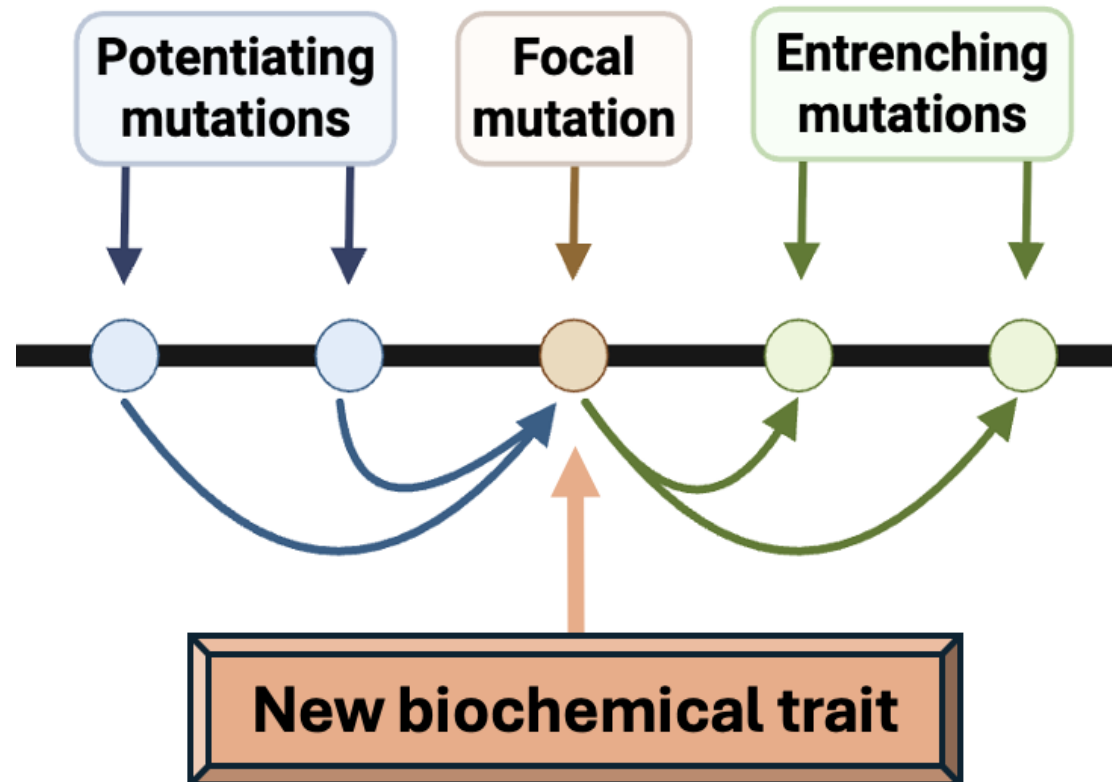


**Investigating *Stichopus japonicus*
Arginine Kinase (SjAK) to Understand
the Evolution of Negative Cooperativity
in Phosphagen Kinases**

Abigail R. Williams

Protein evolution allows for the development of complex traits



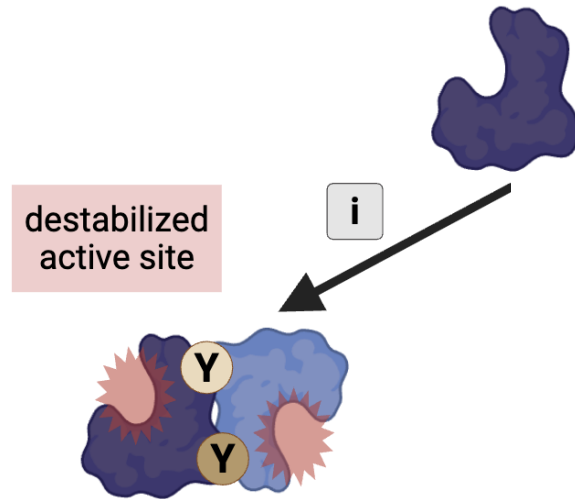
Shah, P., McCandlish, D. M., & Plotkin, J. B. (2015). Contingency and entrenchment in protein evolution under purifying selection. *Proceedings of the National Academy of Sciences of the United States of America*, 112(25), E3226–E3235. <https://doi.org/10.1073/pnas.1412933112>.

'Neutral' mutations lay the groundwork for new biochemical traits



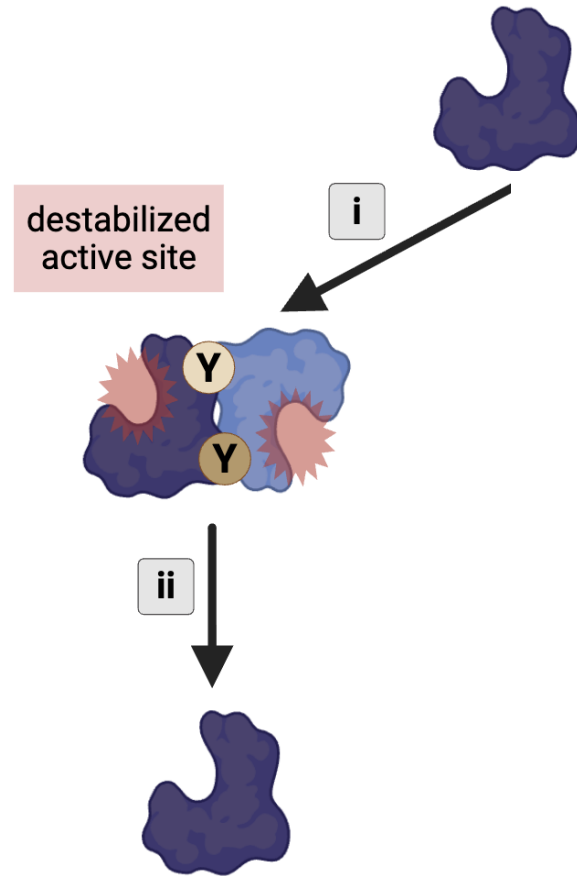
- X** potentiating mutation
- Y** focal mutation
- Z** entrenching mutation

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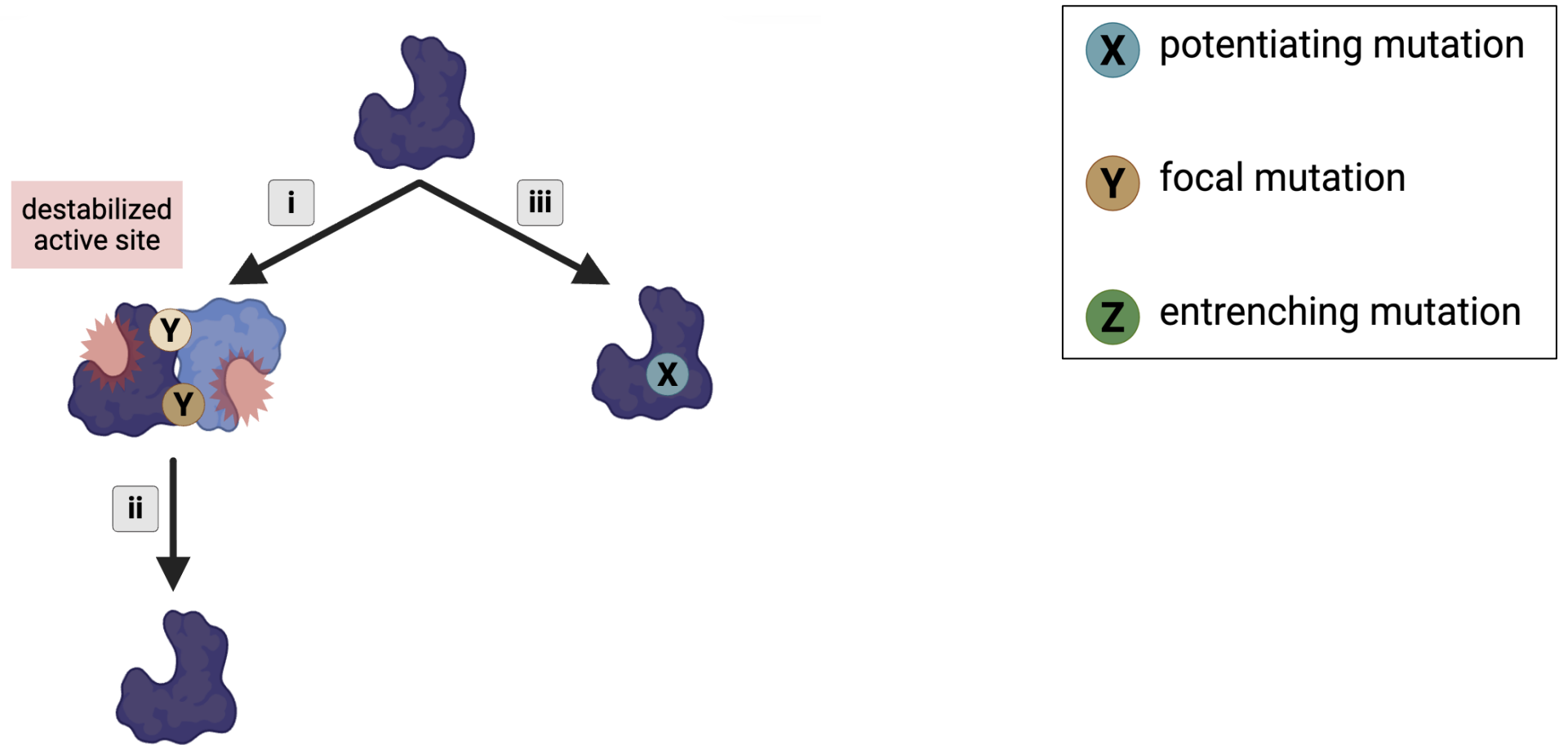
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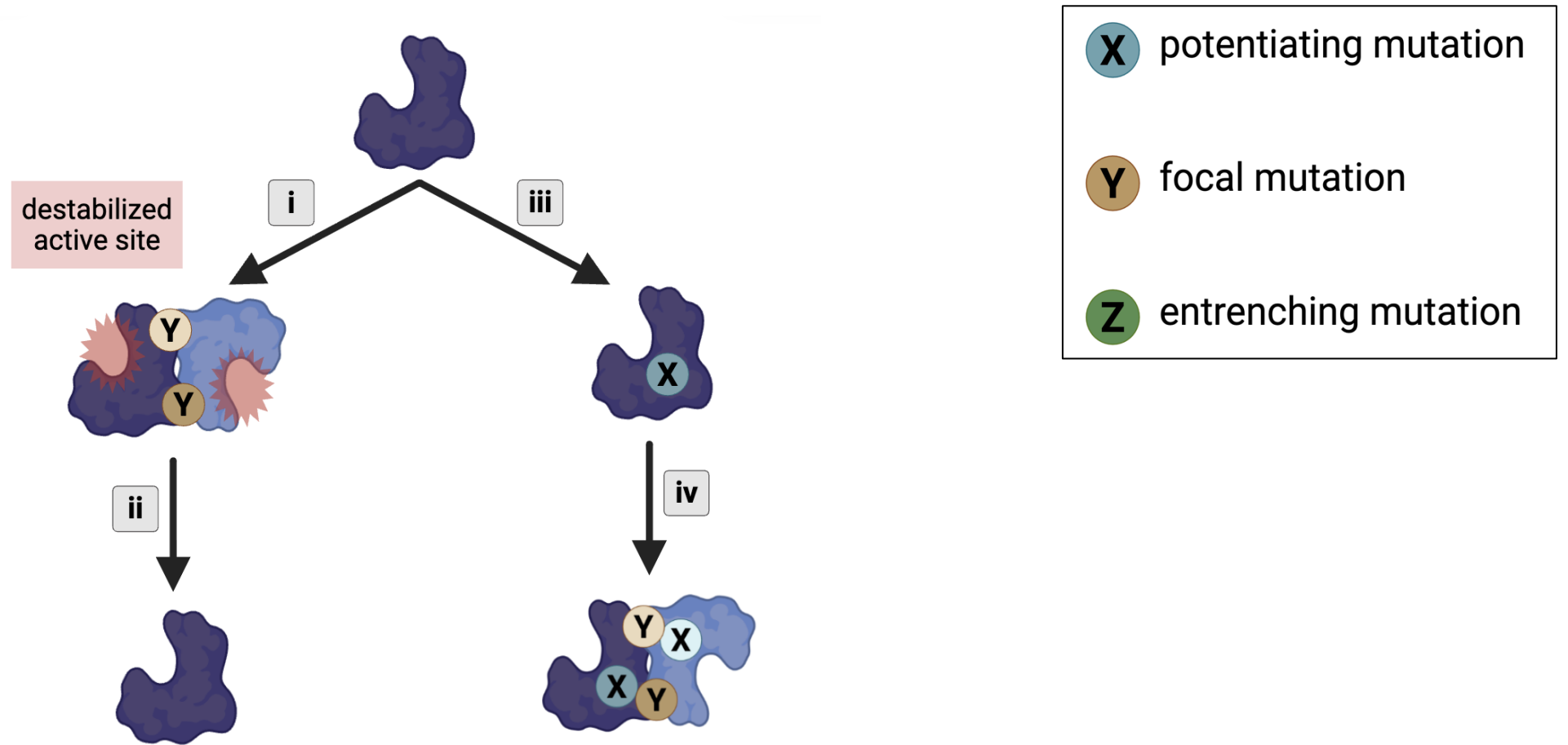


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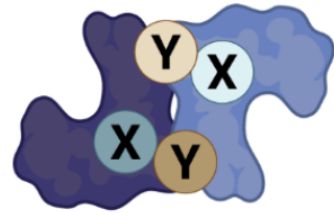
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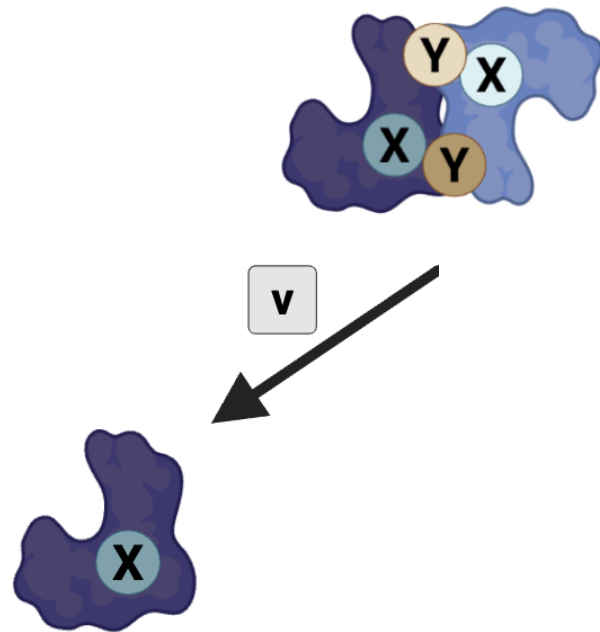


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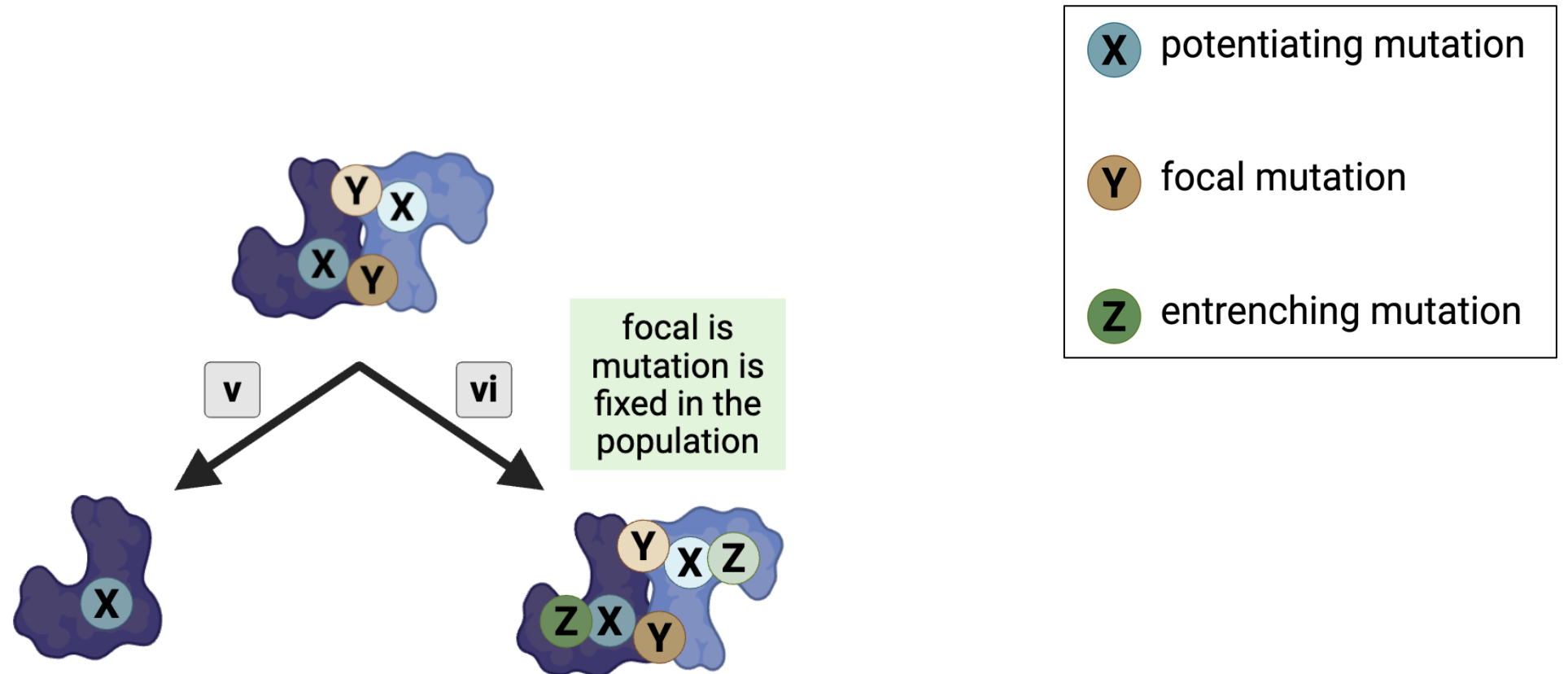
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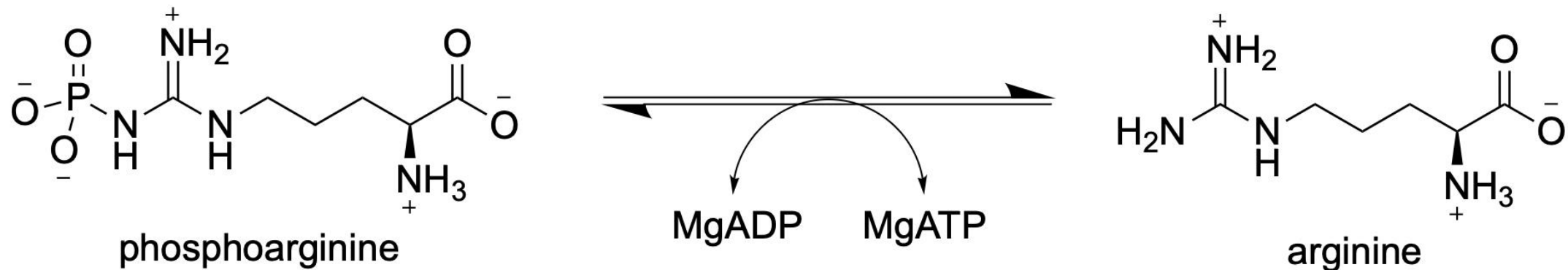


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'Neutral' mutations lay the groundwork for new biochemical traits

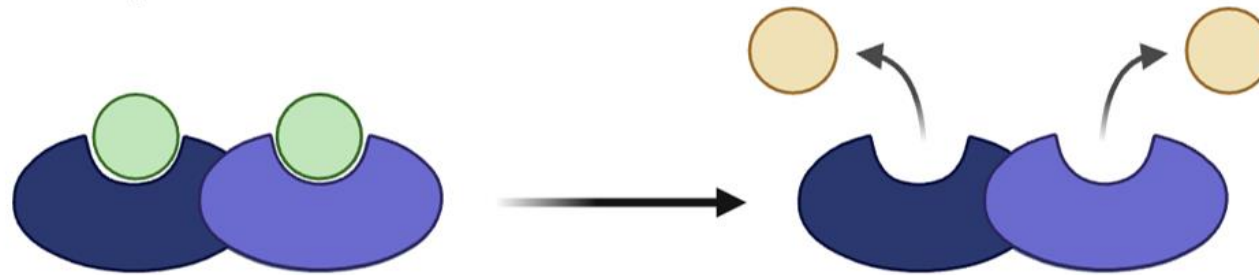


Phosphagen kinases (PKs) can be used as a model system to investigate protein evolution

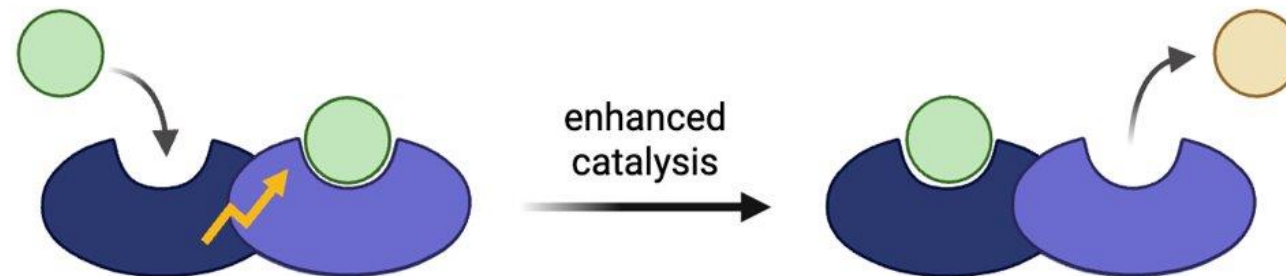


Cooperativity is a complex trait that can provide an evolutionary and biochemical advantage

Non-cooperative:



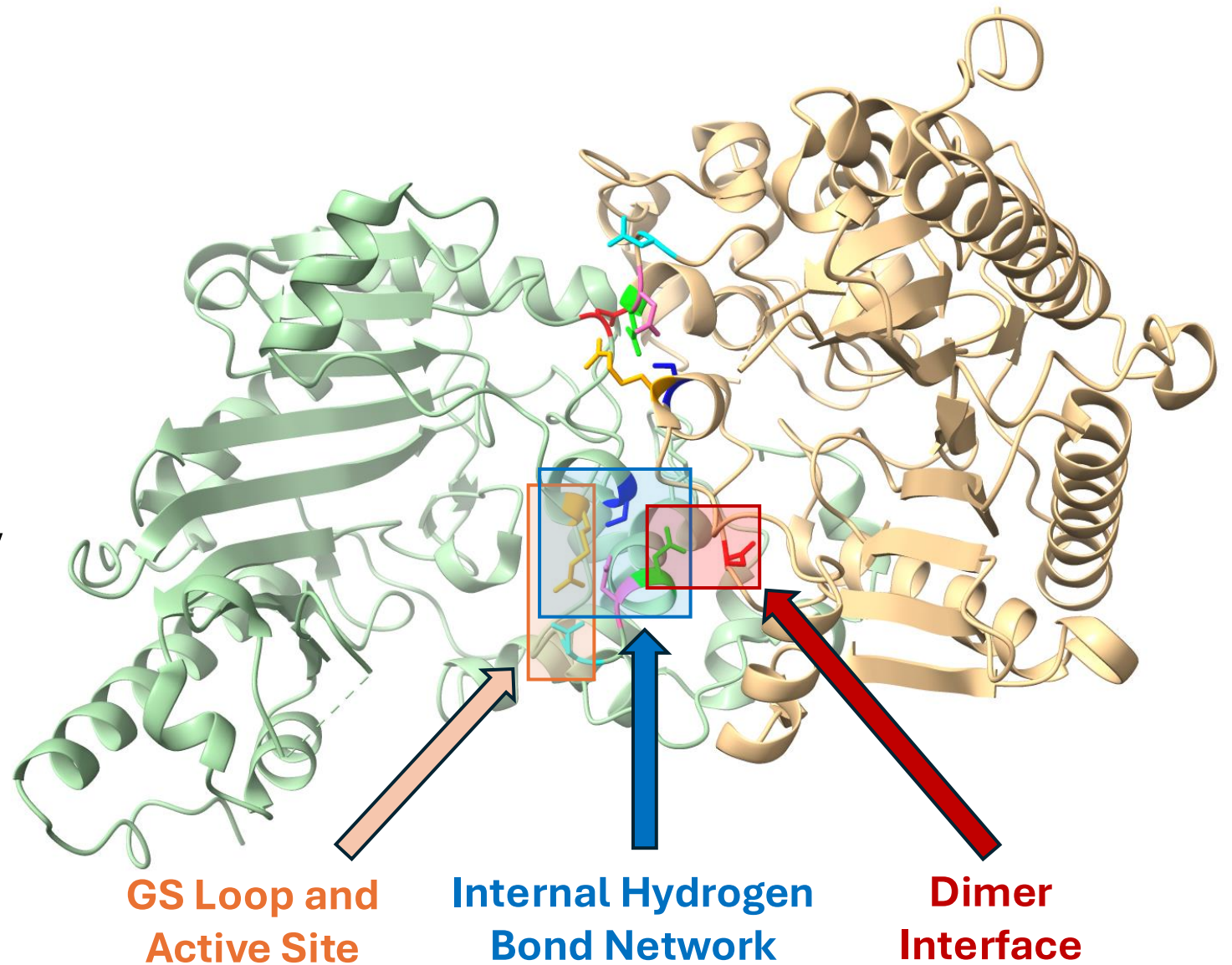
Cooperative:



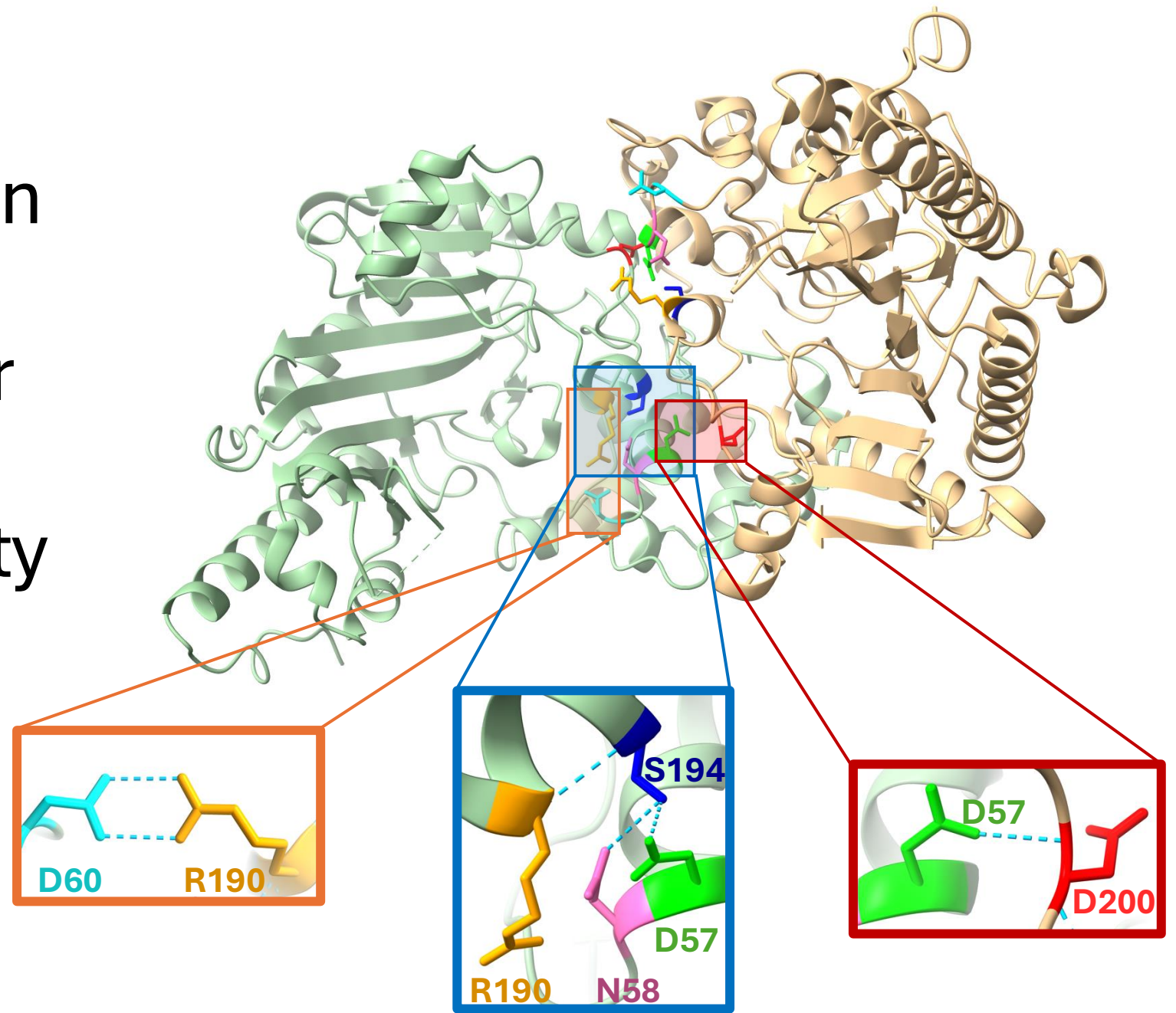
Chen, K.-W., Sun, T.-Y., & Wu, Y.-D. (2023). New Insights into the Cooperativity and Dynamics of Dimeric Enzymes. *Chemical Reviews*, 123(16), 9940–9981. <https://doi.org/10.1021/acs.chemrev.3c00042>.

Wu, X., Ye, S., Guo, S., Yan, W., Bartlam, M., & Rao, Z. (2010). Structural basis for a reciprocating mechanism of negative cooperativity in dimeric phosphagen kinase activity. *The FASEB Journal*, 24(1), 242–252. <https://doi.org/10.1096/fj.09-140194>.

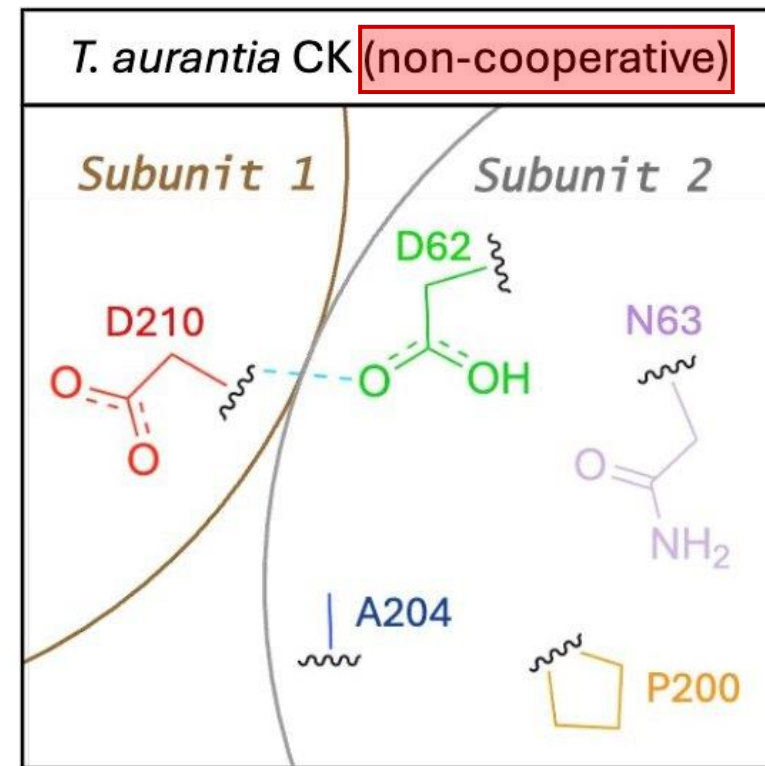
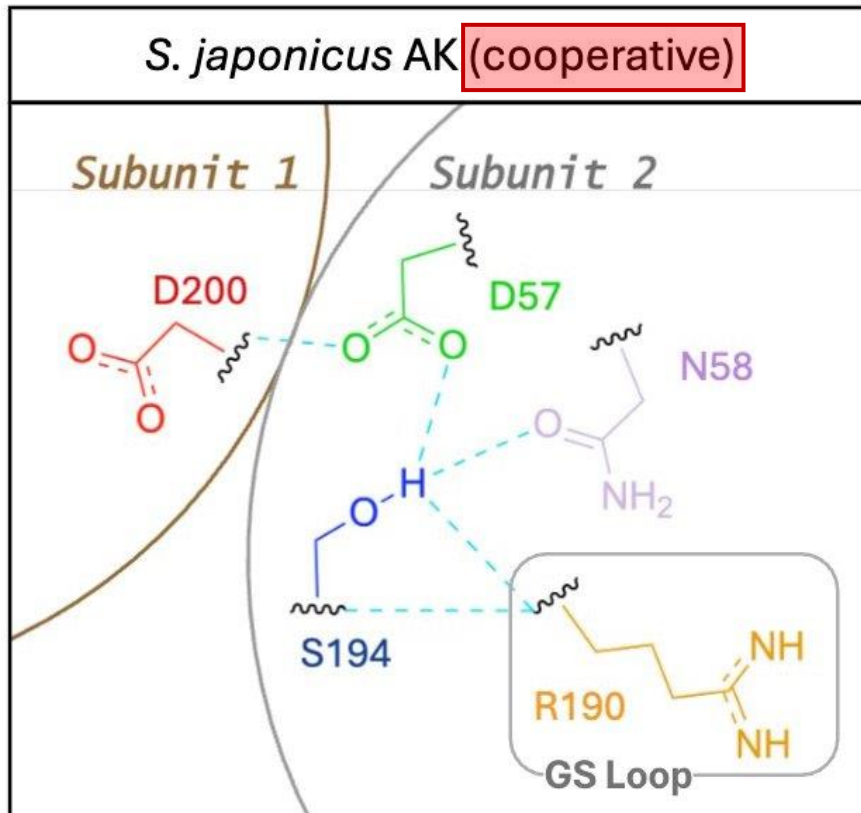
In cooperative PKs
an internal hydrogen
bond network
connects the dimer
interface to the
guanidine specificity
(GS) loop



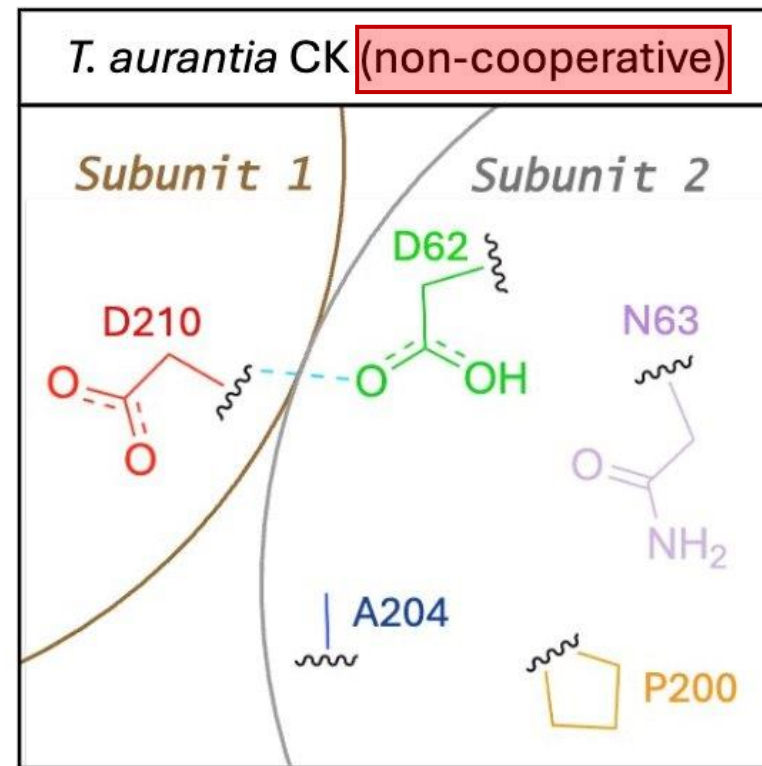
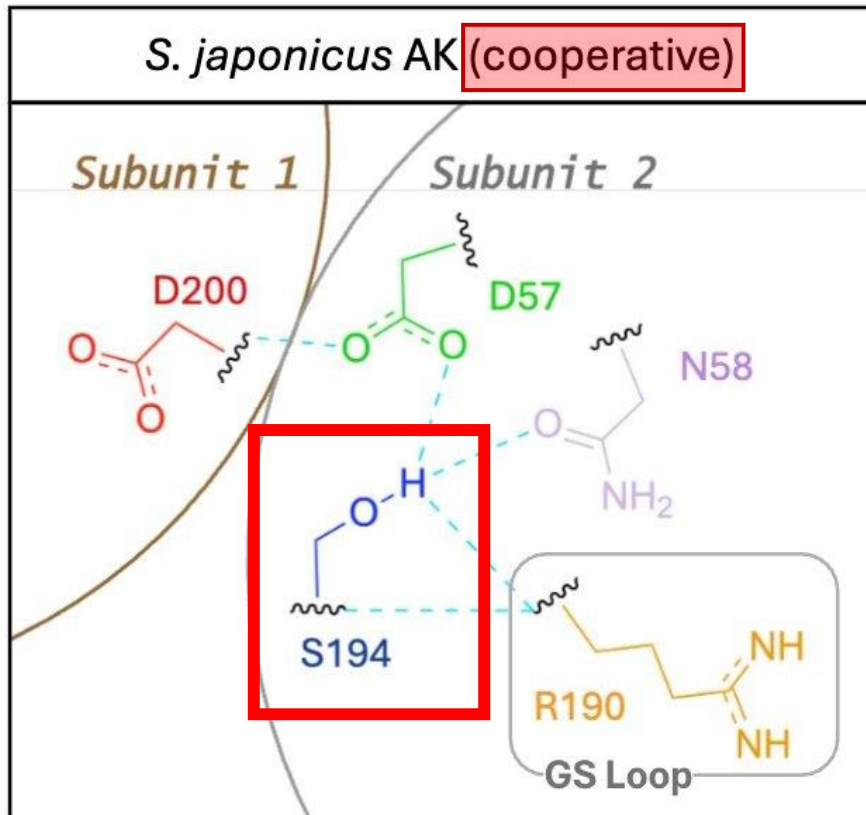
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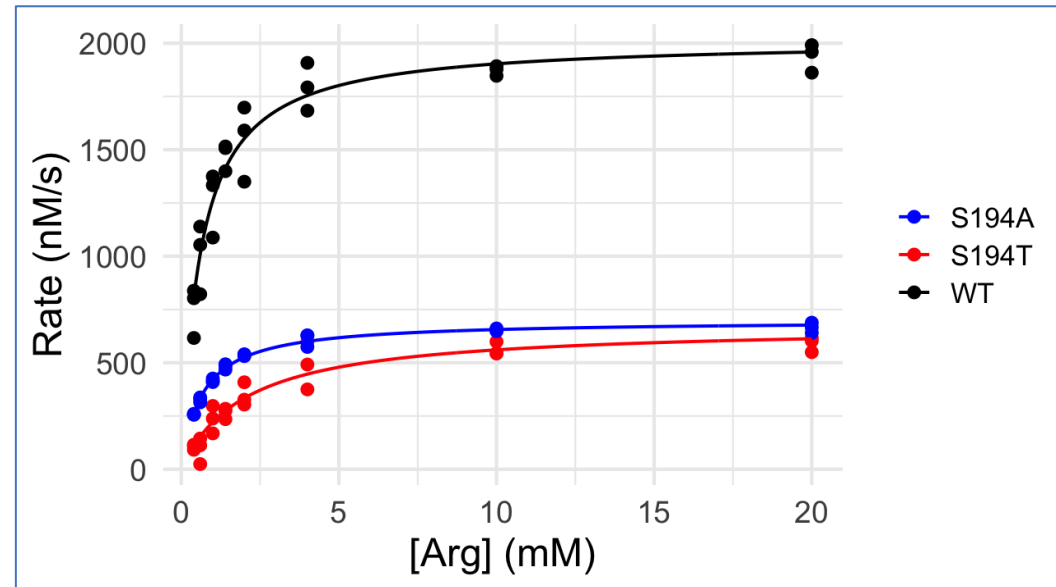
Do the residues within the internal hydrogen bond network serve as a series of potentiating mutations that allow for the evolution of PK cooperativity?



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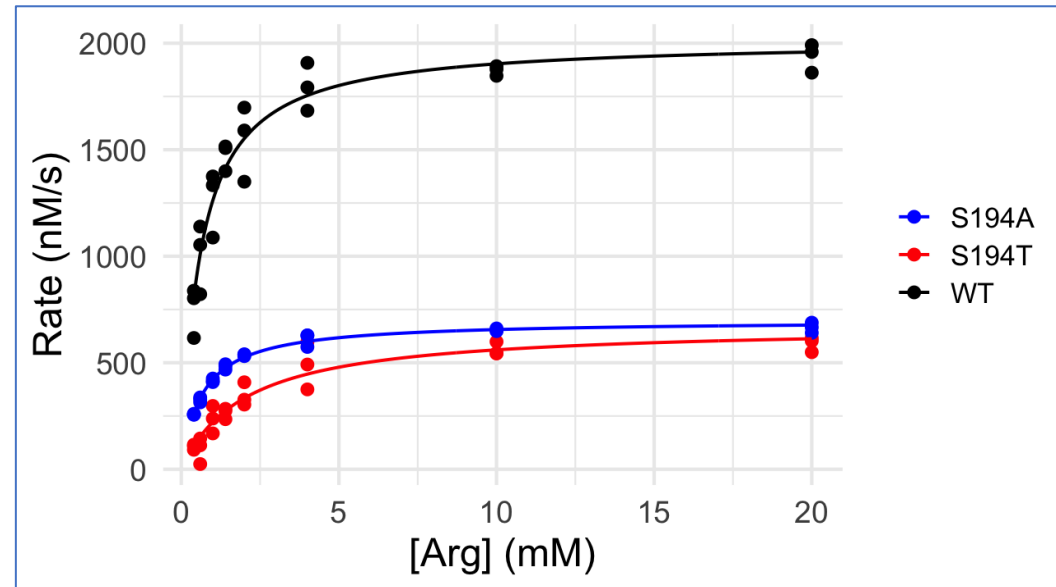


Substituting internal residue S194 affects kinetic parameters



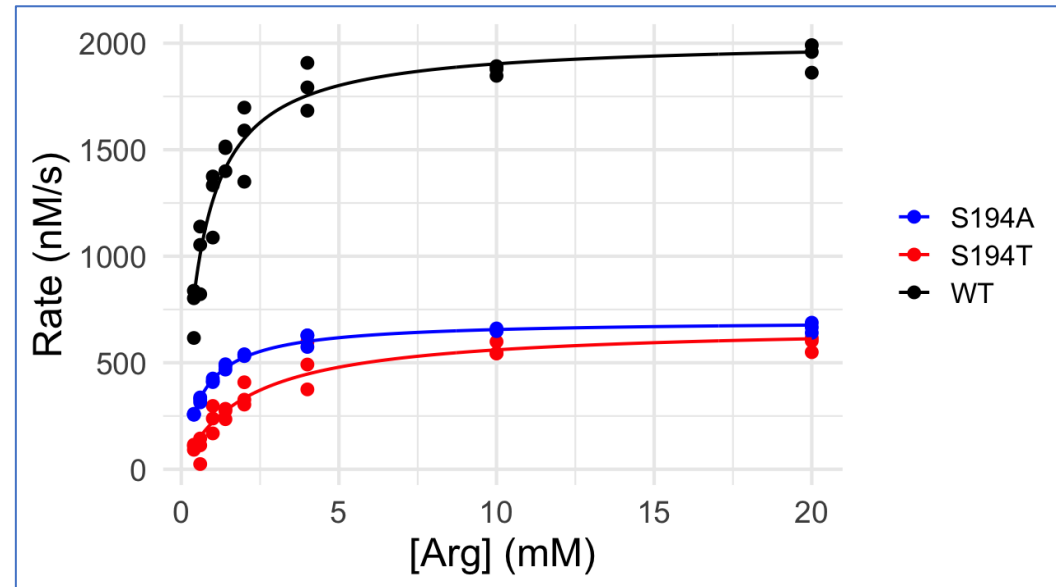
Enzyme	Quaternary Structure	K_{MArg} (mM)	k_{cat} (s ⁻¹)	k_{cat}/K_{MArg} (mM ⁻¹ s ⁻¹)
SjAK WT	Dimer	0.6 ± 0.1	202 ± 2	337 ± 77
SjAK S194A	Dimer	0.66 ± 0.02	70 ± 0.6	106 ± 3
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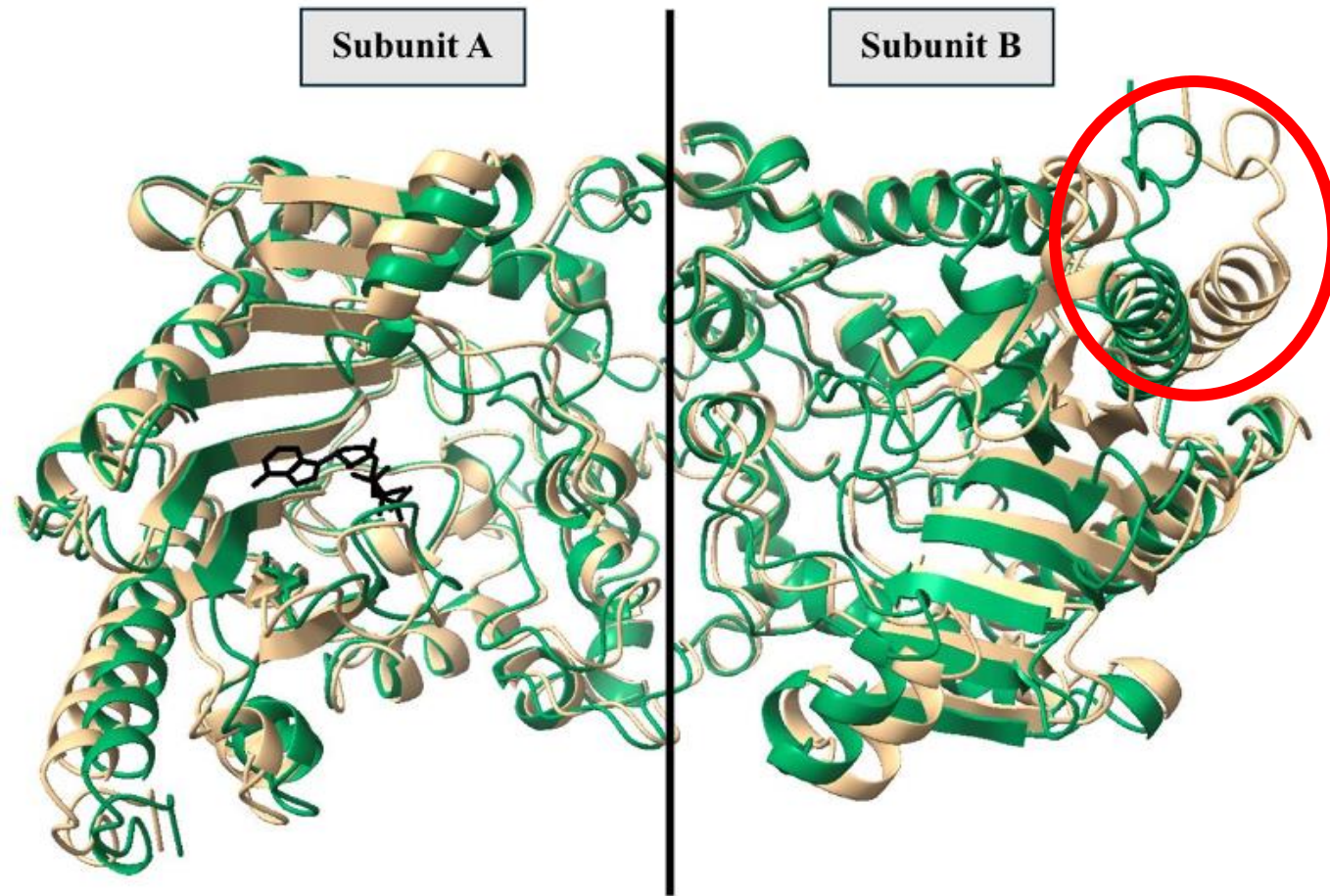


Enzyme	Quaternary Structure	K_{MArg} (mM)	k_{cat} (s^{-1})	k_{cat}/K_{MArg} ($mM^{-1}s^{-1}$)
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SjAK WT undergoes conformational changes upon ATP binding

Tan:
unbound

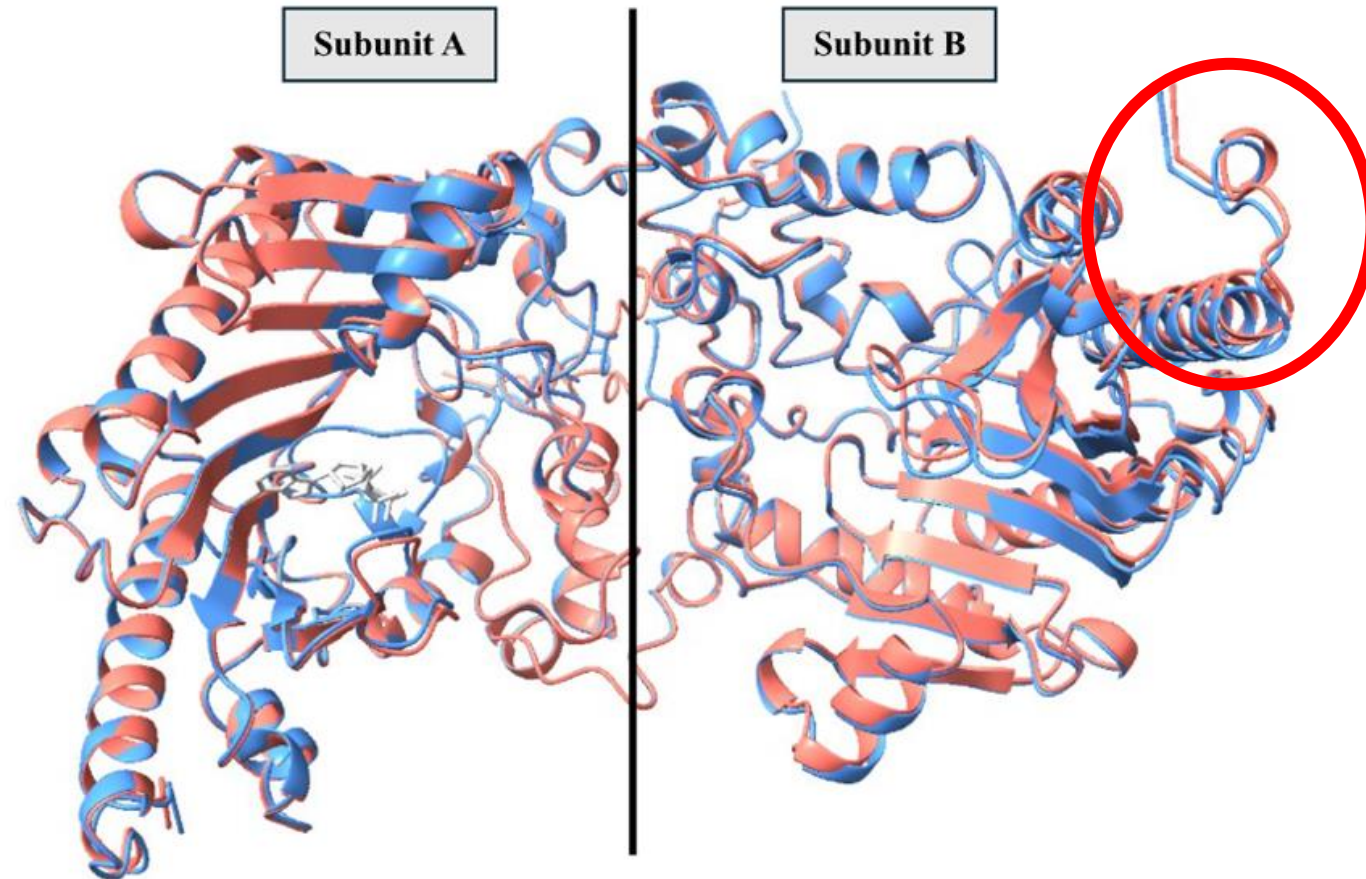
Green:
ATP bound



SjAK S194T does not undergo the same conformational changes as WT upon ATP binding

Pink:
unbound

Blue:
ATP bound

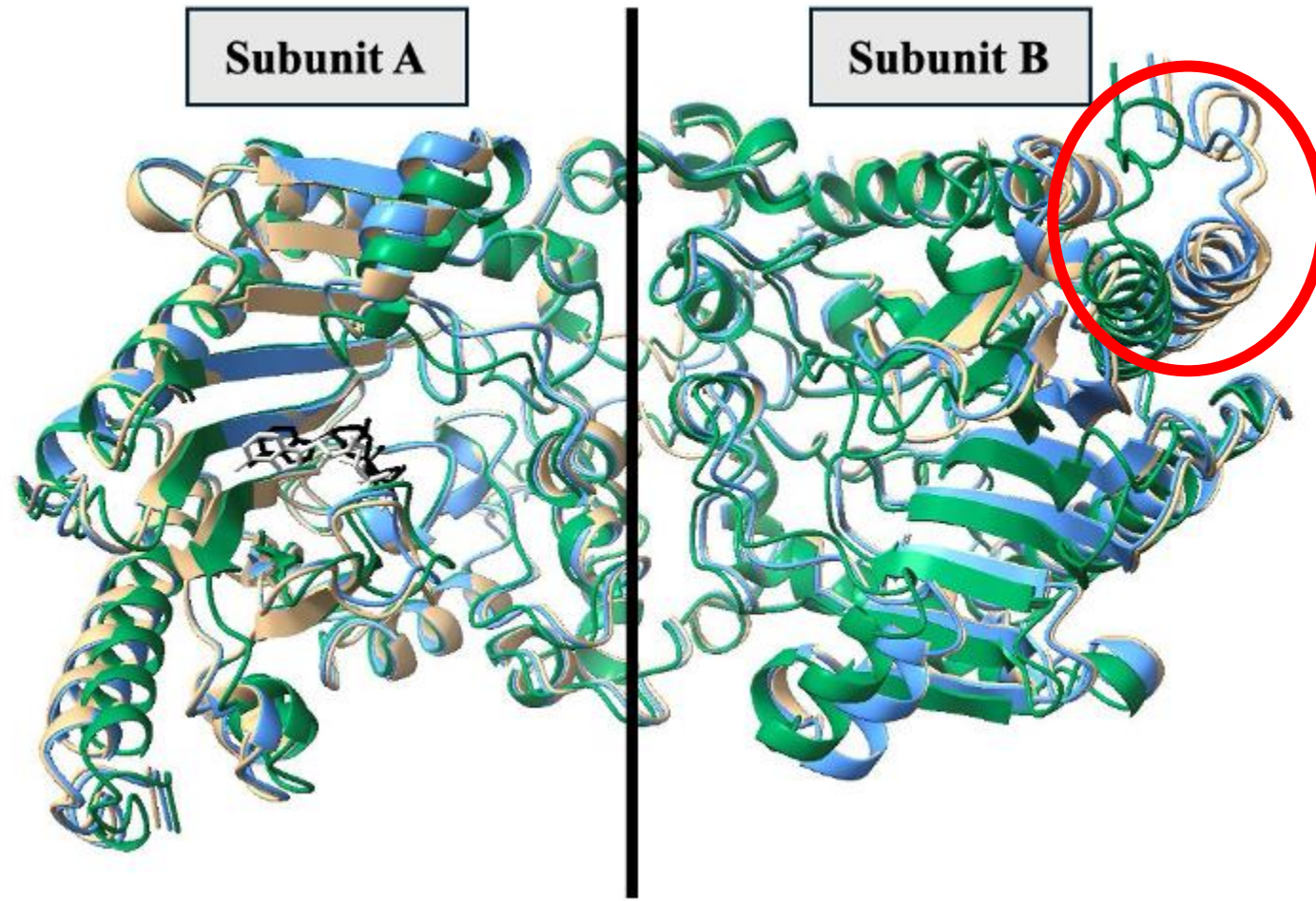


ATP bound S194T is more similar to unbound WT than ATP bound WT

Tan:
unbound WT

Green:
ATP bound WT

Blue:
ATP bound S194T



Conclusions

- S194T had 3-fold increased K_M suggesting decreased substrate affinity

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- S194T may not undergo the proper conformational changes upon ATP binding

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- S194T had 3-fold increased K_M suggesting decreased substrate affinity
- S194T may not undergo the proper conformational changes upon ATP binding
- S194 may serve as a potentiating mutation that helps stabilize the ATP bound form of SjAK

Future Directions

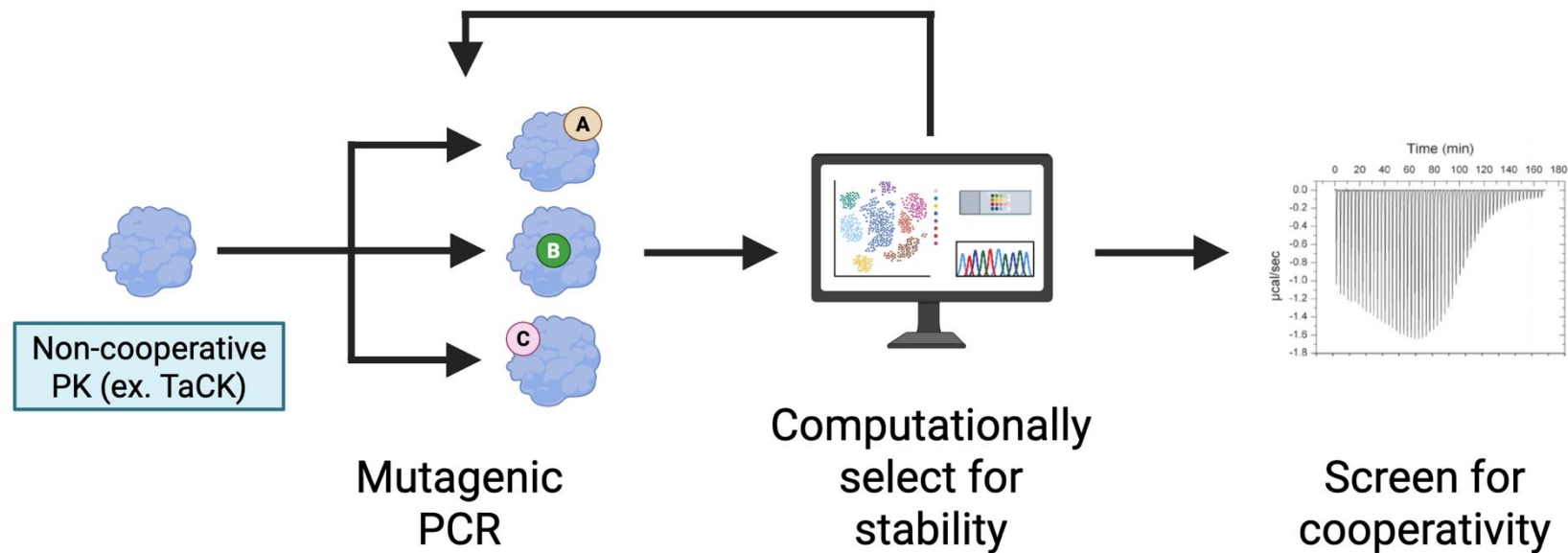
- Determine whether S194A and S194T are cooperative

Future Directions

- Determine whether S194A and S194T are cooperative
- Mutate other residues in the internal hydrogen bond network

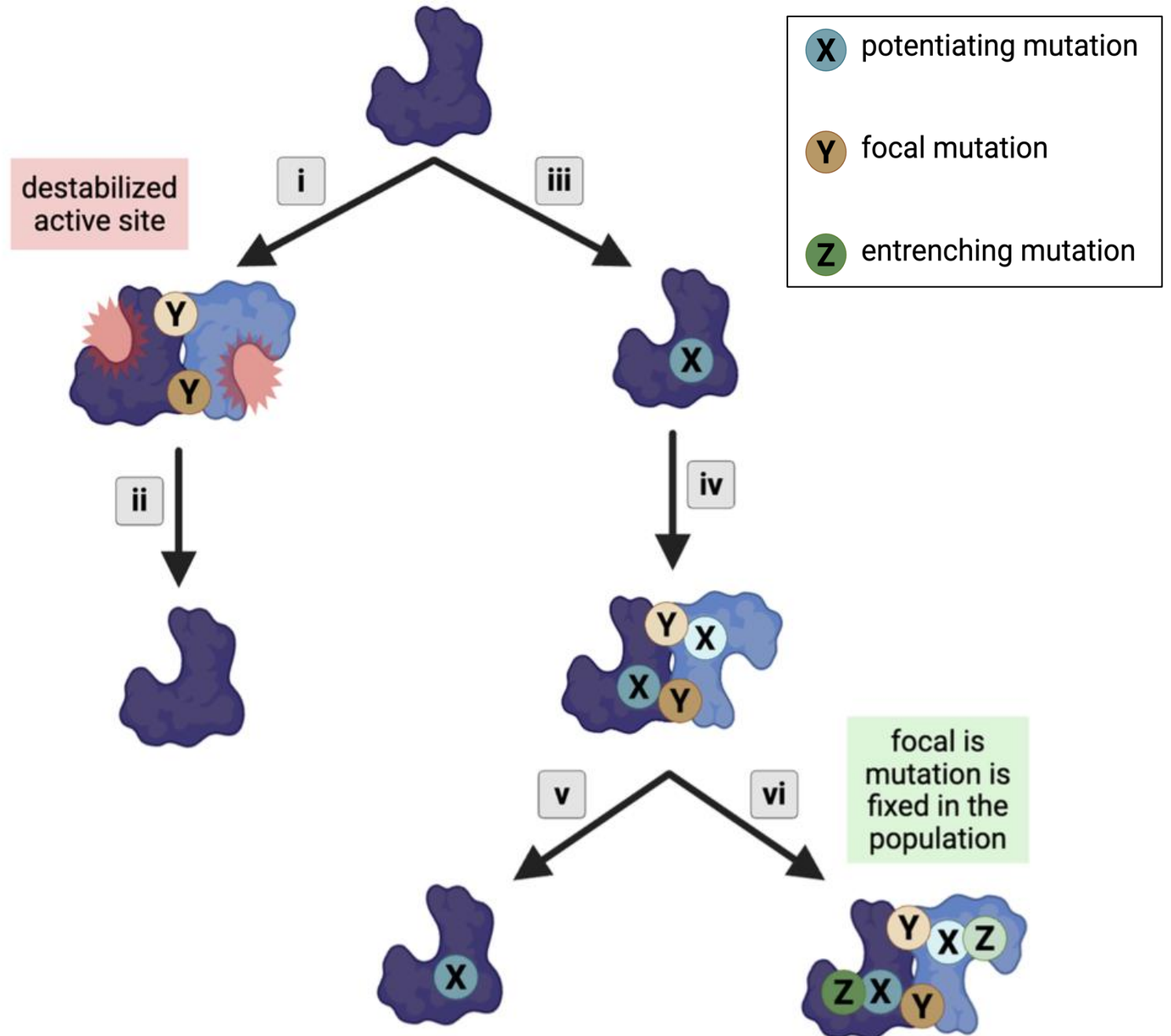
Future Directions

- Use directed evolution as less biased approach to investigate PK evolution

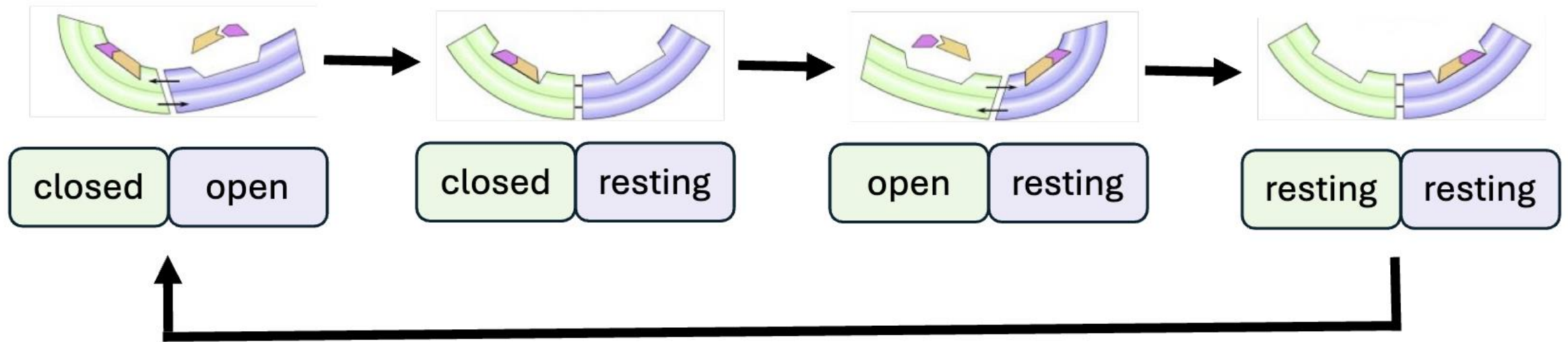


Thank you for listening!

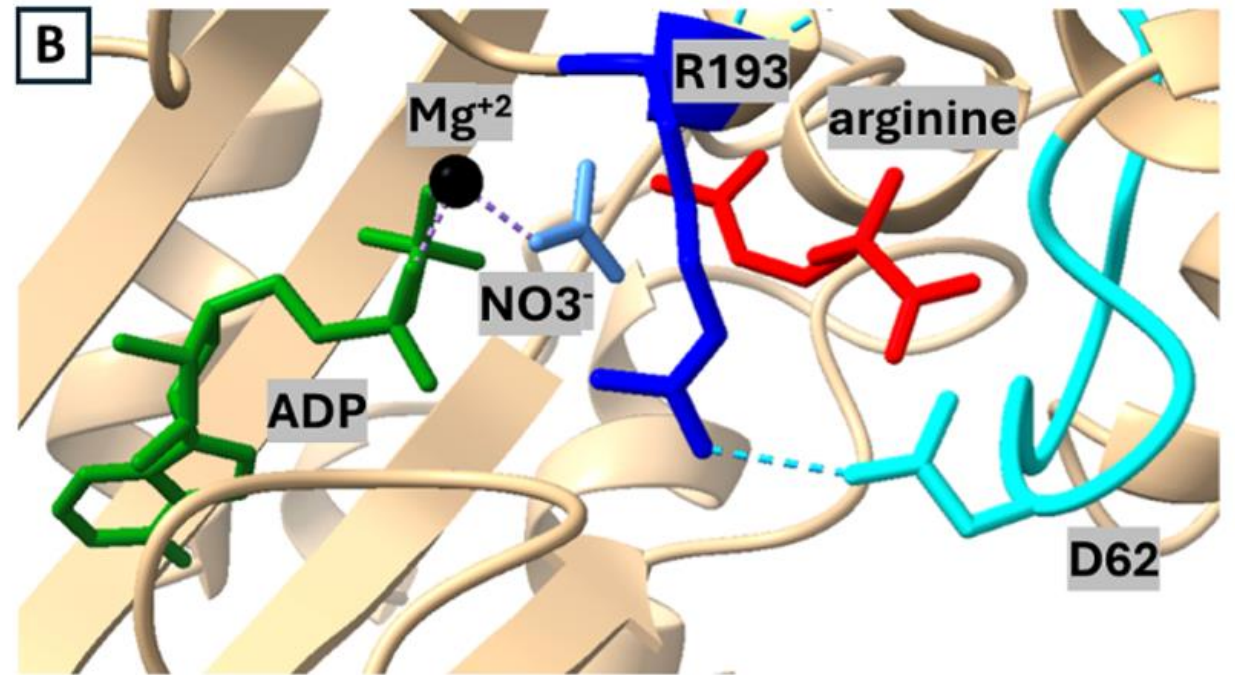
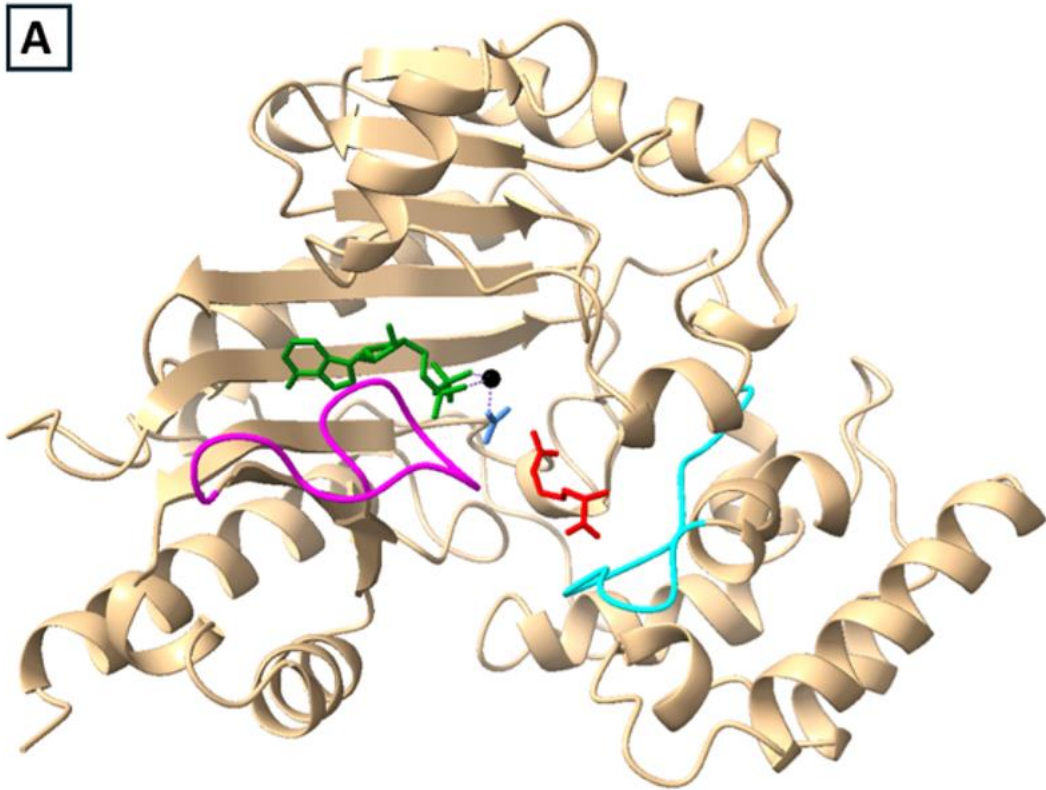
'Neutral'
mutations lay
the groundwork
for new
biochemical
traits



Structural analysis reveals that cooperative PKs utilize a reciprocating mechanism



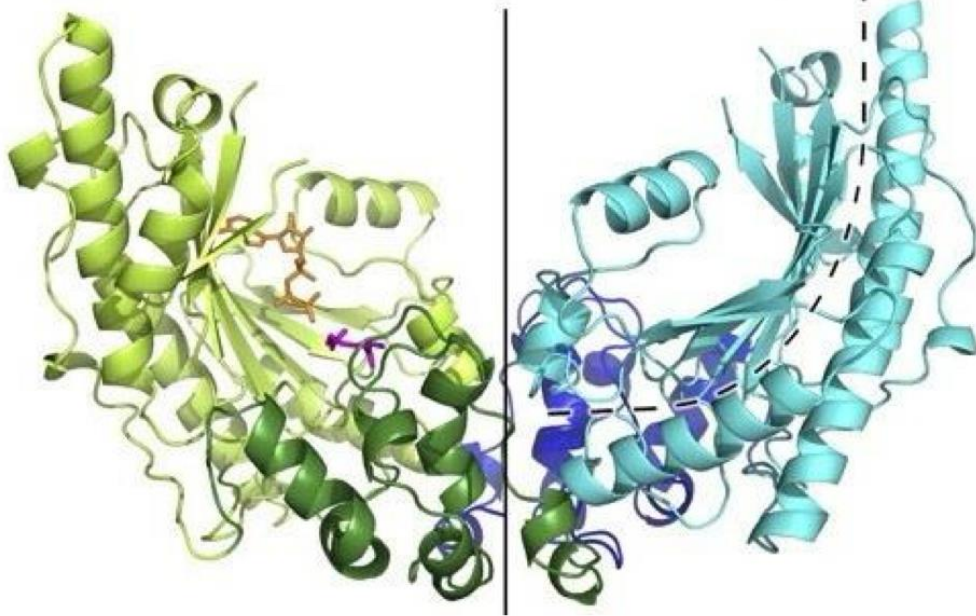
Transition state analogue complex of HcAK



Stichopus japonicus AK exhibits extreme negative cooperativity

TSAC

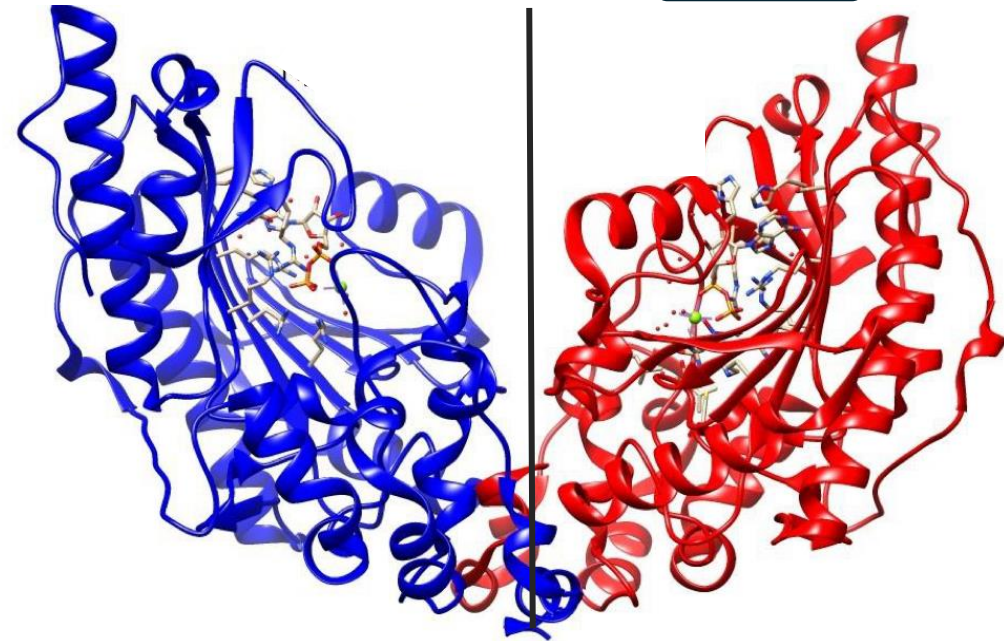
No substrate



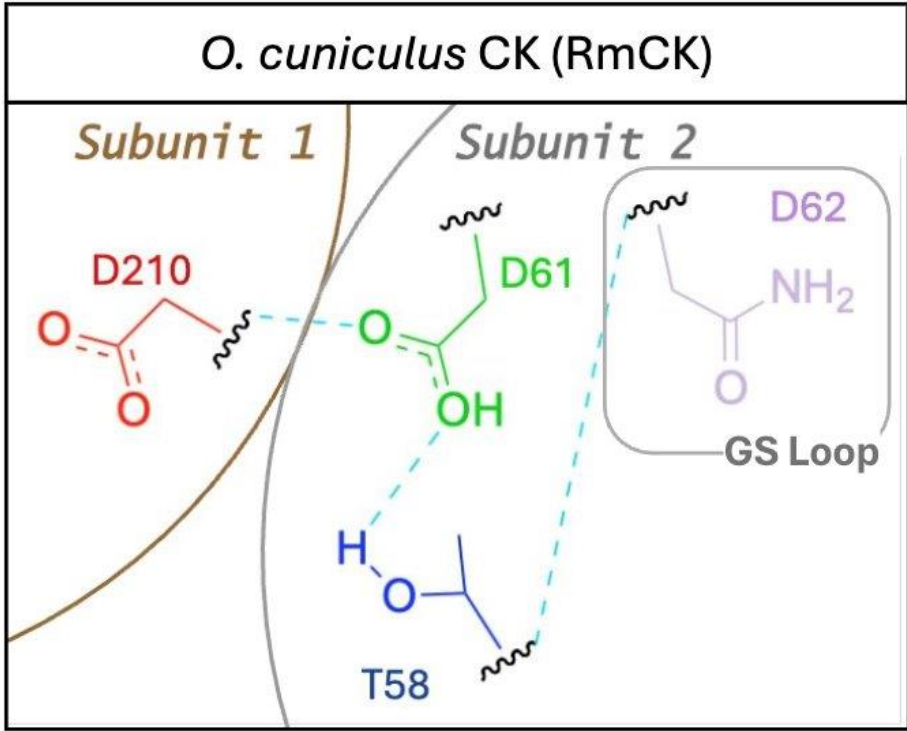
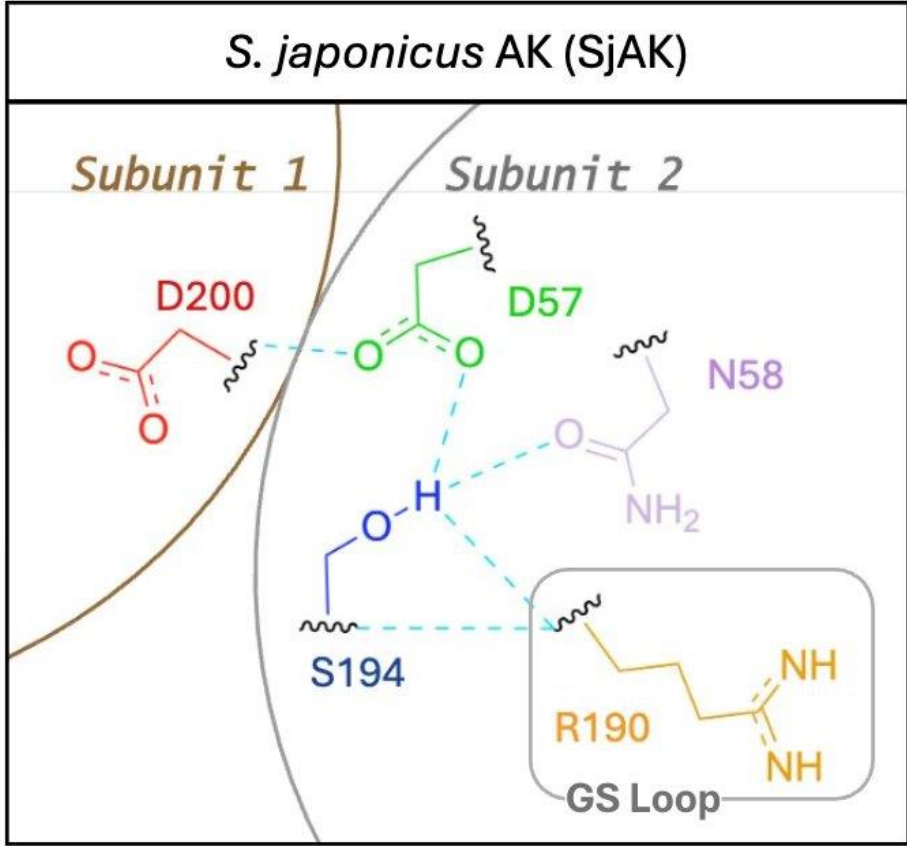
Stichopus japonicus AK (SjAK)

MgADP

TSAC



Oryctolagus cuniculus CK (RmCK)



Because S194 is not conserved, this could mean that there is a network of pre-existing potentiating mutations that make it easy for a focal mutation for cooperativity to evolve

S194

D200

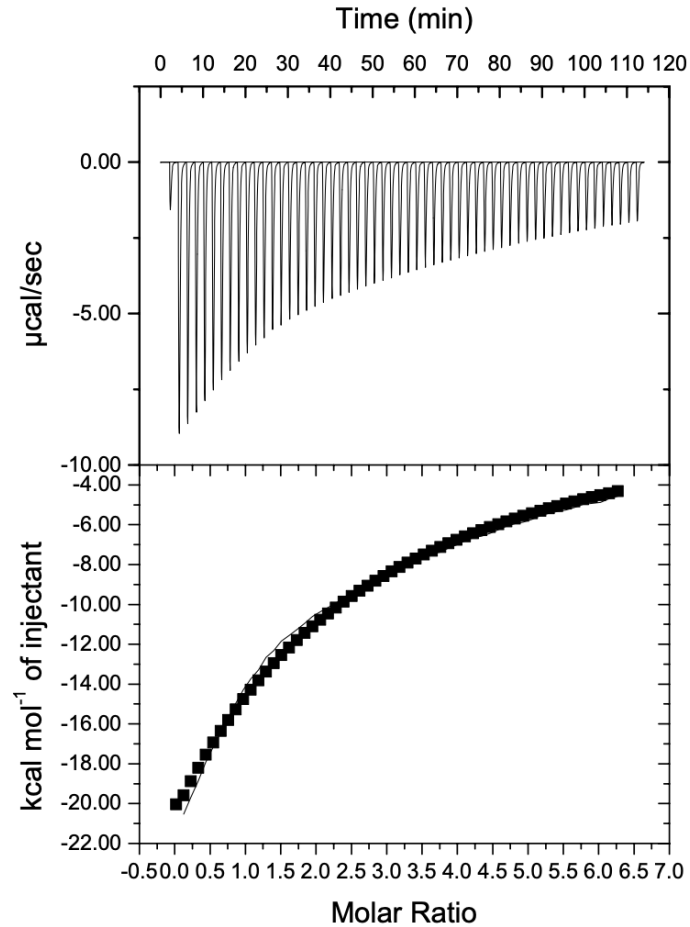
SjAK-coop-Apost	170	DEKTQQQLIDDHFLFDRPVS-RHFT	S	GGMARD	D	FPDGRGIW	208
MbAK-coop-Monos	181	SEADRKQLVADHFLFKKGDR--FLQ	S	AGANRD	D	WPESRGIF	218
RmCK-coop-Oryct	180	TEQEQQQLIDDHFLFDKPVS-PLLLASGMARD			D	WPDARGIW	218
TcCK-coop-Torpe	180	SDAEQQQLIDDHFLFDKPIS-PLLLASGMARD			D	WPDGRGIW	218
BtCK-coop-Bos t	180	TEQEQQQLIDDHFLFDKPVS-PLLLASGMARD			D	WPDARGIW	218
HsCK-coop?-Homo	180	TEKEQQQLIDDHFLFDKPVS-PLLLASGMARD			D	WPDARGIW	218
AbMiTK-coop-Are	209	TDKEQQQLIDDHFLFQKPTG-ALLLASGMARD			D	WPDARGIW	247
HcAK	174	SKATQQQLIDDHFLFKEGDR--FLQTANACRY			D	WPTGRGIF	211
TaCK-nc-Tethy a	180	TDAEQQLIDDHFLFDKPVS-PLLLASNWARD			D	WPDARGIW	218
AbCK-nc-Aphroca	172	TDQEQLINDHFLFDKPVS-PLLLASGMARD			D	WPDARGIW	210
PsTK-nc-Phytoph	230	TKEEQQLIDDHFLFQKPGGGTLLTNAGAARD			D	WPDGRGIF	269
NGK-nc-Namalyca	175	NEKDQEQLIEDHFLFEKPTG-ALLTSGCARD			D	WPDGRGIW	213

SjAK-coop-Apost	21	---HKSLLSKYLTADMYAKLRDVATPSGYTLDRAIQNGVD	57
MbAK-coop-Monos	30	SS--HSLLKKHLTKDAYLQCETRVTPSGFTLQHAIQSGVD	67
RmCK-coop-Oryct	26	---HNNHMAKVLTLPDLYKKLRDKETPSGFTLDDVIQTGVD	62
TcCK-coop-Torpe	26	---HNNHMAKALTLDIYKKLRDKETPSGFTLDDIIQTGVD	62
BtCK-coop-Bos t	26	---HNNHMAKALTLEIYKKLRDKETPSGFTLDDVIQTGVD	62
HsCK-coop?-Homo	26	---HNNHMAKVLTLELYKKLRDKETPSGFTVDDVIQTGVD	62
AbMiTK-coop-Are	56	---HYNMLKDHLTPGIYARLRDKATPSGFTFDQAIQCGVD	92
HcAK	20	ASDCKSLLKKHLTKDVFDSEIKNKKTGMGATLLDVIQSGVE	59
TaCK-nc-Tethy a	24	GE-YNNWMAKCLTEEIYAKLRDTPTASGFTLDQCIQTGVD	62
AbCK-nc-Aphroca	18	---HNNWMSRCLTPDLYRQLQGVKTDTGFTLDEAIQTGVD	54
PsTK-nc-Phytoph	81	-K-HKSLMAKHLTPEIYDKLKDRKTASGYTIDQAIQTGID	118
NGK-nc-Namalyca	21	---HNNVMASQLTYELYEKYWDKVTPNGVTFDKCIQTGVD	57

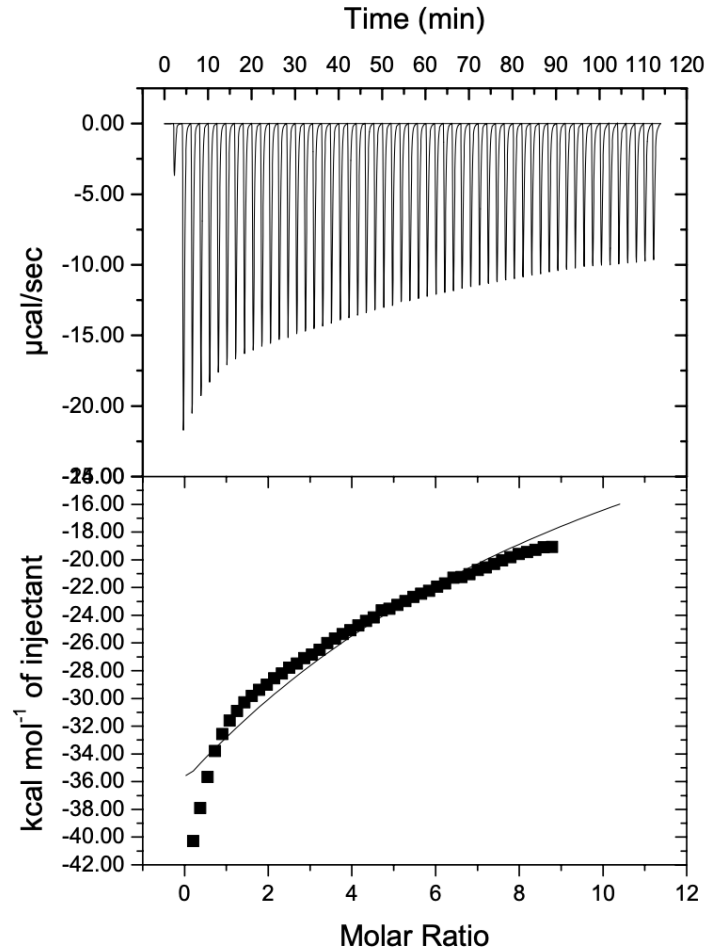
N58

SjAK-coop-Apost	58	NPD-----FHLGLLAGDEETYTVFADLFDPVIEEYHNGFK	92
MbAK-coop-Monos	68	NQD-----SGVGLYAGDEESYTVFAPVFDVIEDYHNGYK	102
RmCK-coop-Oryct	63	NPGHPFI-MTVGCVAGDEESYTVFKDLFDPIIQDRHGGFK	101
TcCK-coop-Torpe	63	NPGHPFI-MTVGCVAGDEECYEVFKDLFDPVIEDRHGGYK	101
BtCK-coop-Bos t	63	NPGHPFI-MTVGCVAGDEESYTVFKDLFDPIIQDRHGGFK	101
HsCK-coop?-Homo	63	NPGHPFI-MTVGCVAGDEESYEVEFKELFDPIISDRHGGYK	101
AbMiTK-coop-Are	93	NPGYPHT-KTVGLVAGDEESYEVEFAELFDKIIDDKHGGFK	131
HcAK	60	NLD-----SGVGIYAPDAESYRTFGPLFDPIIDDYHGGFK	94
TaCK-nc-Tethy a	63	NPGHPFI-MTVGCVAGDEESYETFADLLDKVIDKRHNGYG	101
AbCK-nc-Aphroca	55	NPGHPFI-MTVGVVAGDEDYKAFAPFLDDVIEKRHGGYS	93
PsTK-nc-Phytoph	119	TPH-----LGVGVVAGDEESYHTFKDLMDPVIEGWHG-YK	152
NGK-nc-Namalyca	58	NPGNKFYGKKTGCVFGDEYSYECYKEFFDKCIEEIH-HFK	96

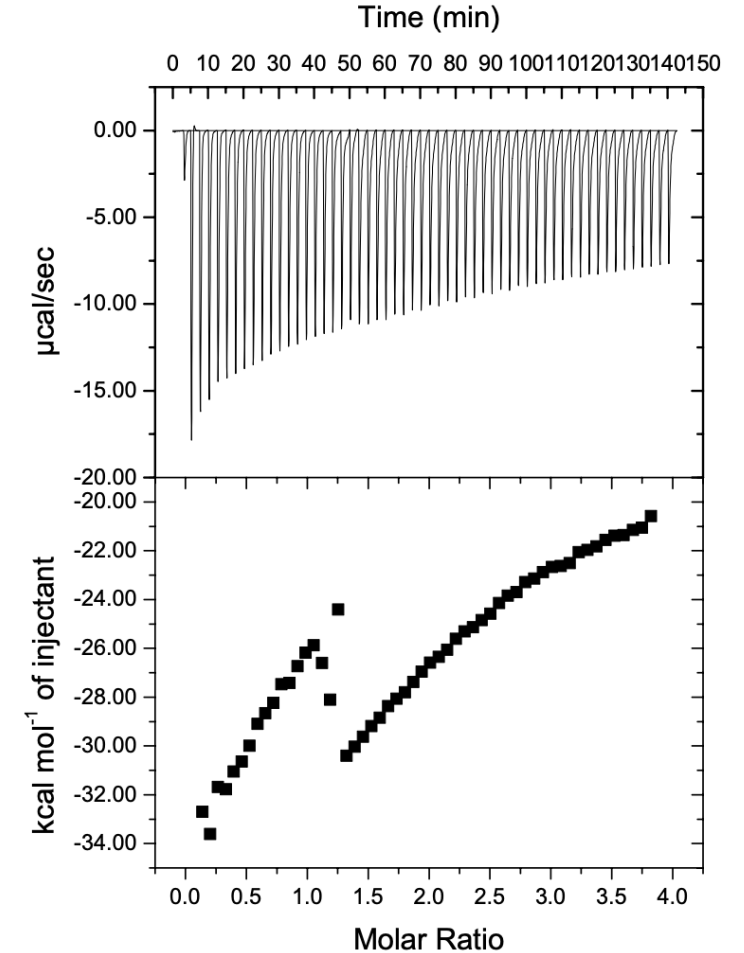
ITC cooperativity assays were inconclusive



SjAK WT

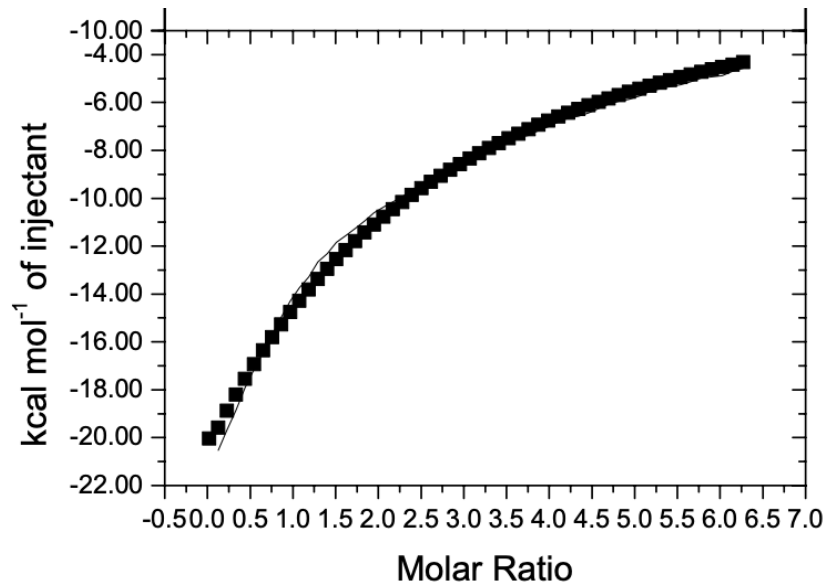


SjAK S194A

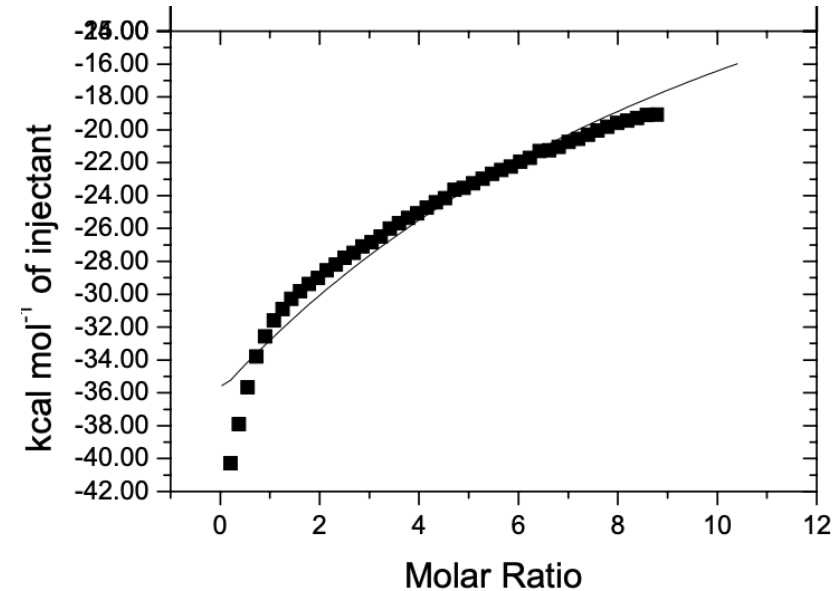


SjAK S194T

ITC cooperativity assays were inconclusive



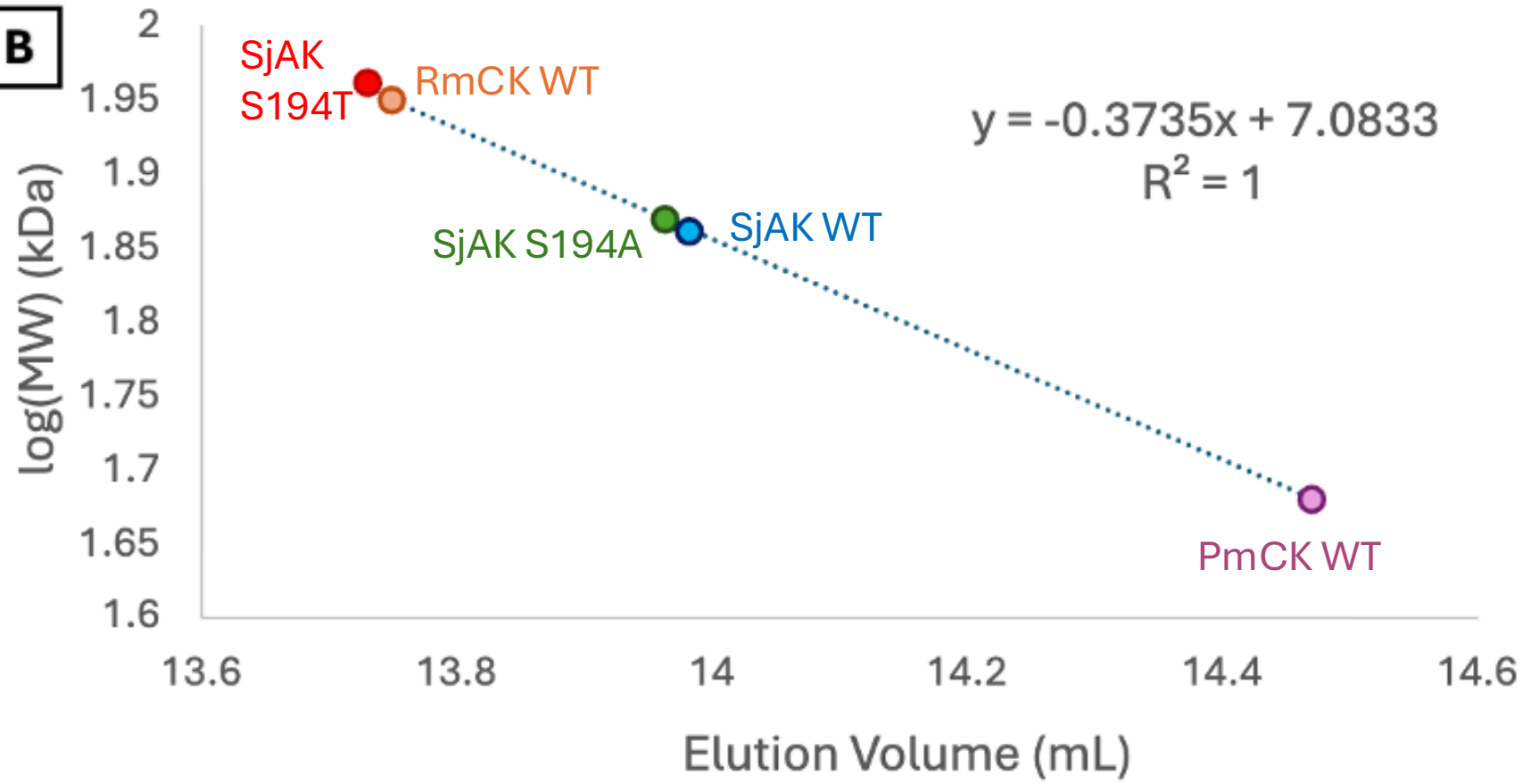
SjAK WT





SjAK S194A

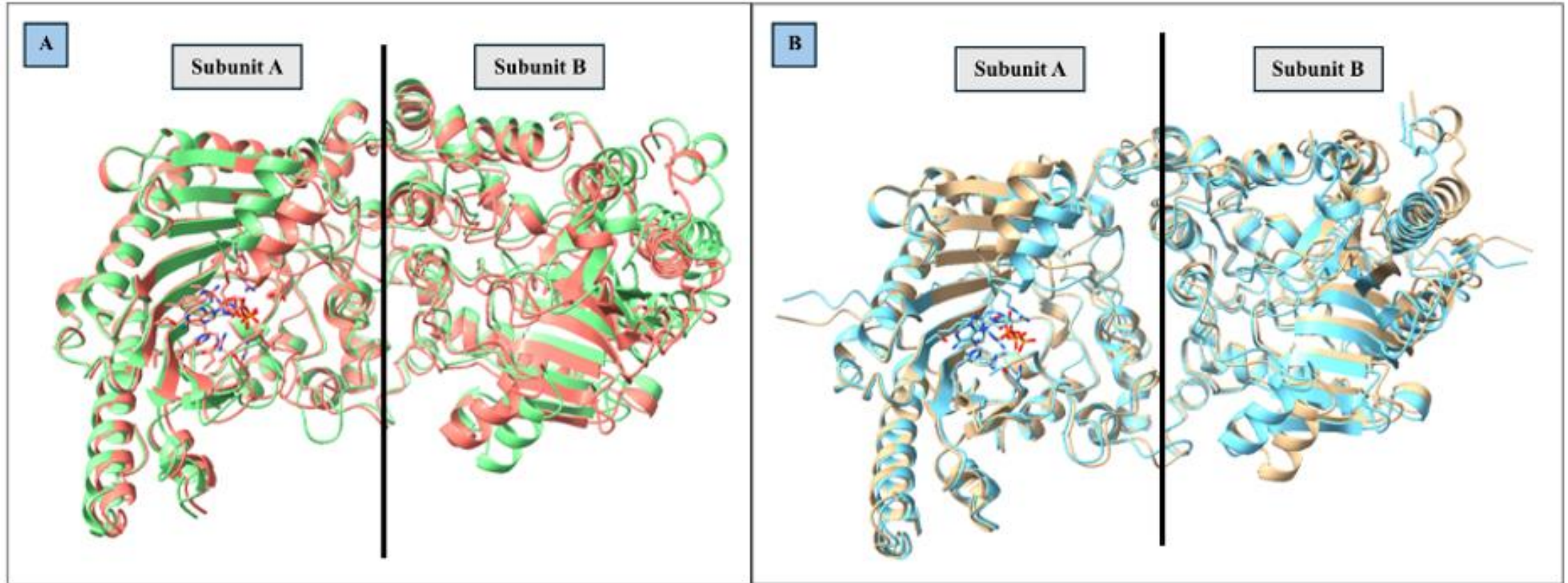
Enzyme	N (sites)	K (M ⁻¹)	H (cal/mol)
SjAK WT	$3.72\text{E-}5 \pm 0.590$	$2.05\text{E}3 \pm 278$	$-3.626\text{E}9 \pm 5.749\text{E}13$
SjAK S194A	0.00114 ± 25.2	464 ± 726	$-4.01\text{E}6$

B

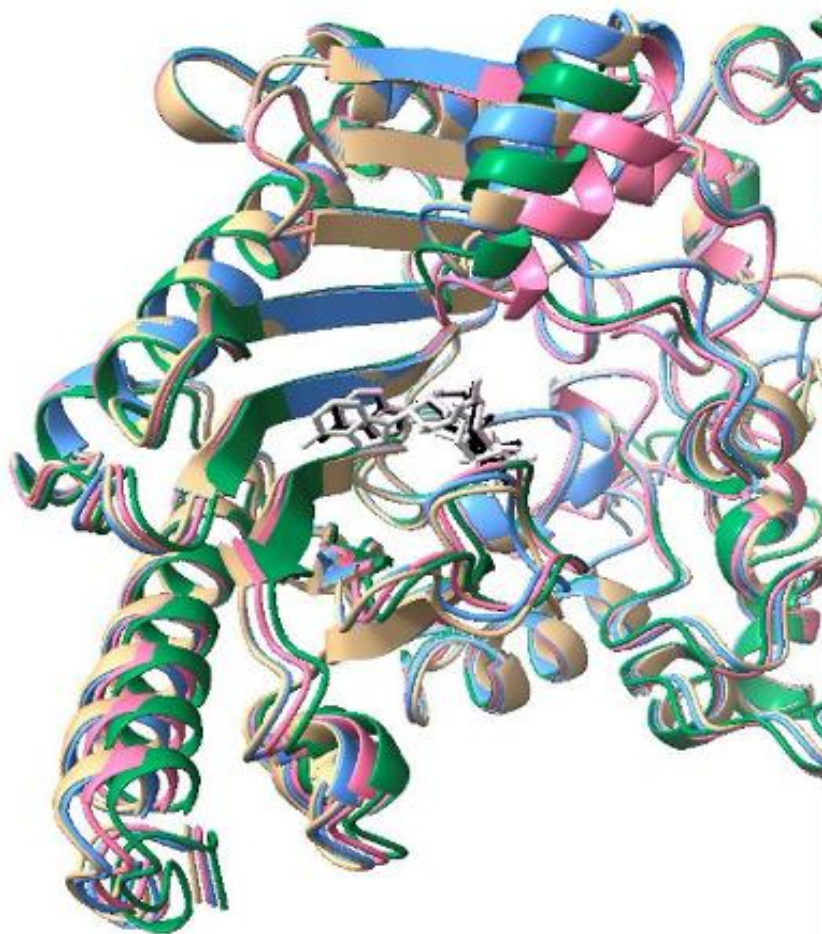


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S194A	Dimer	0.66 ± 0.02	70 ± 0.6	106 ± 3
S194T	Dimer	2.1 ± 0.6	68 ± 4	33 ± 10
L65G		ND	0.002 ± 0.0001	
N56-G57-V58 Deletion		ND	Inactive	
D63-G-F64 Insertion		1.2 ± 0.5	0.03 ± 0.01	

RmCK (A) and TaCK (B) also undergo conformational changes upon ATP binding



Subunit A



Subunit B

