Negative Cooperativity in Cytosolic Taurocyamine Kinase from *Arenicola brasiliensis* and its Implication in the Evolution of the Phosphagen Kinase Family

Mingyuan "Merlin" Li*, Mark J. Snider*, Dean Fraga*

*Biochemistry and Molecular Biology, College of Wooster, OH

Background & Significance

- Phosphagen kinases (PK) are highly conserved enzymes that catalyze the reversible phosphoryl transfer from ATP to guanidino-containing substrate
- The diverse structural and functional properties within the PK family make it an excellent model for protein evolution, although the evolutionary relationship remains largely unknown.

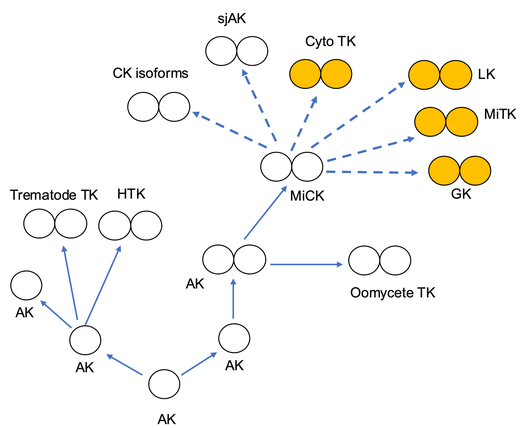

Figure 1: Proposed pathways for PK evolution. An unknown ancestral AK diverges two separate AK branches, one of which undergoes dimerization and then gave rise to various PKs, including annelid specific PKs (Yellowed circles). Dash lines illustrate the exact evolutionary pathway in this group is highly debatable.

- Negative cooperativity was seen in some dimeric PKs with a proposed CK ancestor but not all.

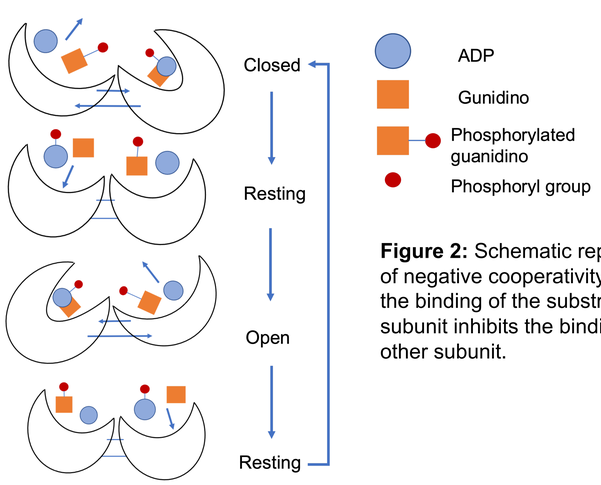

Figure 2: Schematic representation of negative cooperativity in which the binding of the substrates by one subunit inhibits the binding of the other subunit.

Results

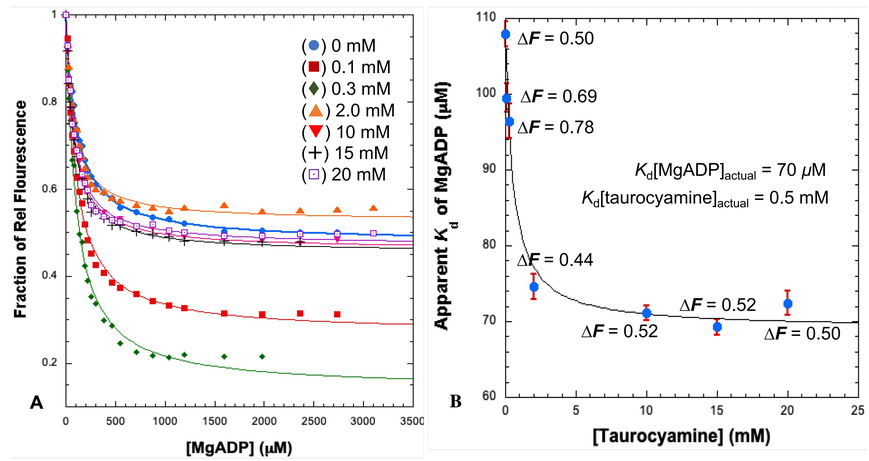

Figure 3: Effects of different concentrations of taurocyamine on the (**A**) ΔF and (**B**) the apparent K_d for MgADP binding to CytoTK. Apparent Kd[MgADP] decreases whereas ΔF first increases and then decreases, which suggests although the saturating presence of taurocyamine favors the formation of TSAC, it reduces the amount of binding, providing an indirect evidence for negative cooperativity between subunits.

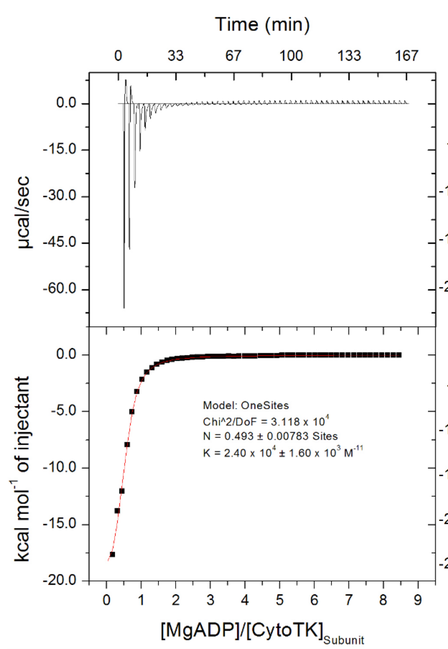

Figure 4: 1st ITC experiment fits a one-site model, providing the number of binding sites per dimer = 0.99 ± 0.02, overall K_d = 42 (± 3) μM, indicating extreme negative cooperativity between the subunits in CytoTK

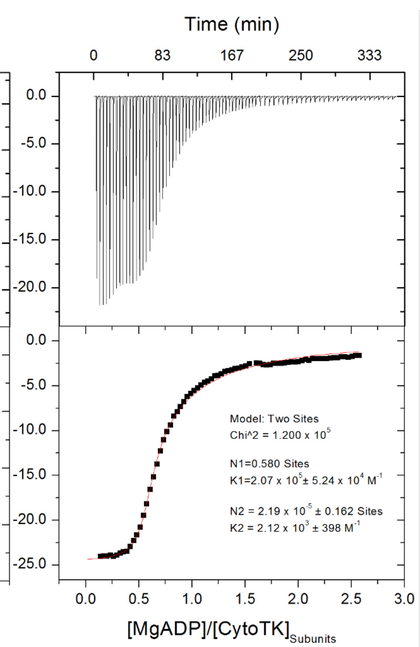

Figure 5: 2nd ITC experiment fits a two-sites binding model (Origin, v7), which provides K_{d1} = 5.2 (± 1.3) μM, K_{d2} = 470 (±110) μM, suggesting negative cooperativity, consistent with the 1st ITC experiment.

Possible Evolutionary Models

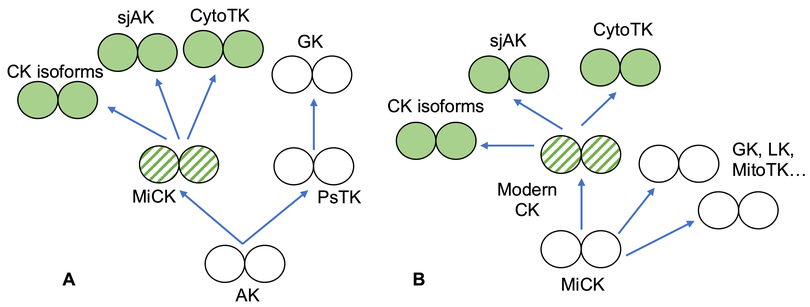

Figure 6: Two possible trajectories of annelid PKs and CK isoforms evolution. Shaded green circles represent PKs whose negative cooperativity was confirmed experimentally. Pattern shaded green circles represent a possible PK that might have negative cooperativity

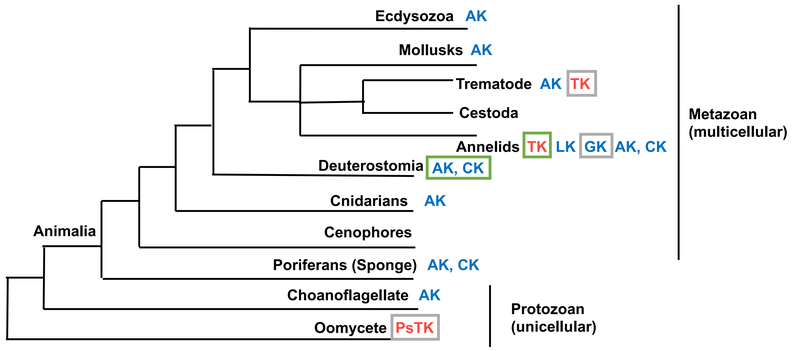

Figure 7: Phylogeny of major protozoan and metazoan species and distribution of PKs. TKs were labeled as red. PKs with known negative cooperativity are circled green, and those without negative cooperativity are circled grey.

Future Directions

- Given that *A. brasiliensis* has a second, mitochondrial TK (**MiTK**), it will be interesting to determine whether the **MiTK also displays cooperativity**.
- What are the possible **biological significance** for negative cooperativity in the PK family?
- Use **molecular dynamic simulation** to predict whether newly found PK species have negative cooperativity.

Key References

- Wu, X. et al. Structural basis for a reciprocating mechanism of negative cooperativity in dimeric phosphagen kinase activity. The FASEB Journal 24, 242–252 (2010).
- Borders, C. L., Snider, M. J., Wolfenden, R. & Edmiston, P. L. Determination of the Affinity of Each Component of a Composite Quaternary Transition-State Analogue Complex of Creatine Kinase. 6.
- Palmer, A., Begres, B. N., Van Houten, J. M., Snider, M. J. & Fraga, D. Characterization of a putative oomycete taurocyamine kinase: Implications for the evolution of the phosphagen kinase family. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 166, 173–181 (2013)
- Suzuki, T. et al. Evolution of the diverse array of phosphagen systems present in annelids. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 152, 60–66 (2009).