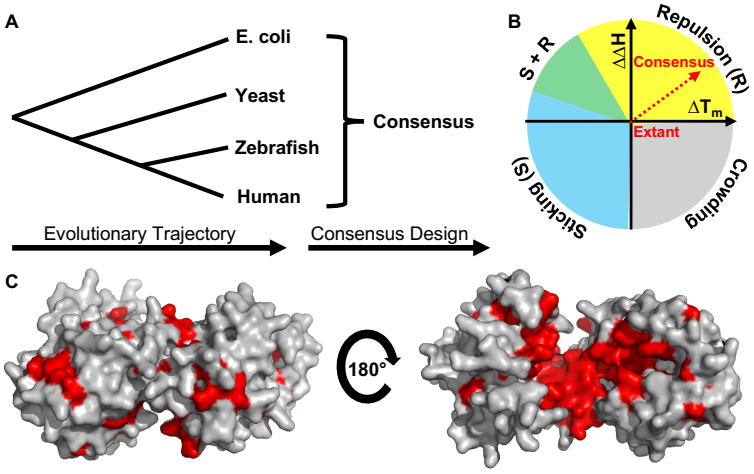
**BIOPHYSICAL TRAINING GRANT | RESEARCH IN PROGRESS TALKS**

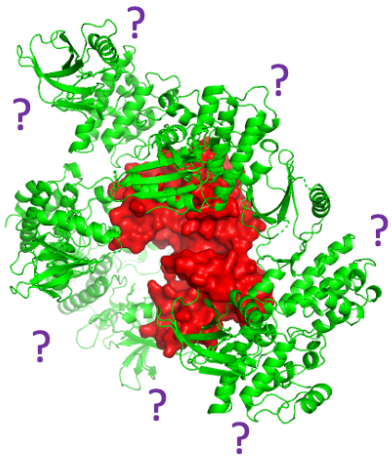
# Eddie Knab




## *Stability vs. environmental sensitivity: The importance of protein surface charge adaptations*

AND

# Kimberly Vish



## *Understanding the enzymatic regulation of an understudied MAPK cascade*

Friday, March 15, 2024 at 12 pm
BASS 305
*Coffee and snacks will be provided*

**Eddie Knab** is a fourth-year graduate student in Caitlin Davis' lab. Eddie's current research involves protein folding in cells. He likes microscopes, camping, and hiking. His favorite place is Hershey Park in Pennsylvania.

**Abstract:** Although biomolecules evolved to function in the cell, most biochemical and biophysical studies have been carried out in dilute aqueous solution in vitro. Such studies neglect differences in the local environments of cells that can impact protein stability and function. Here we have experimentally tested the role of the local environment on the folding of FRET-labeled phosphoglycerate kinase (PGK) in vitro, E. coli, and in U-2 OS cancer cells. To investigate cellular pressures on protein sequence evolution, we compared a global consensus PGK, made with all extant sequences of PGK, with eight extant sequences. Temperature-dependent fluorescence microscopy was used to monitor thermodynamics and kinetics via the single-cell FRET signal. Strikingly, we found the folding of PGK-FRET to be dependent on cell type. Analysis of extant homologs of PGK revealed that sequence differences are predominantly located at surface residues. Further analysis of the surface properties of consensus sequences generated from orders of bacteria, yeast, archaea, Actinopterygii and Mammalia revealed sequence differences are consistent across the taxonomic orders of the extant sequences tested. Therefore, it is likely that these surface properties were evolved to assist proteins in functioning in disparate environments. We find that PGK stability is strongly correlated with more negative net surface charge that leads to repulsive interactions, whereas environmental sensitivity is correlated with high positive surface charge that promotes sticking in the cytosol. We conclude that residues at the surface positions are not weakly conserved because they are less important for stability in the cell than the highly conserved interior residues, but rather that their lack of conservation results from unique selective pressures imposed by the intracellular environment. Taken together, our results demonstrate that different cellular environments contribute to different protein sequences, stabilities, and kinetic phenotypes.

**Kimberly Vish** is a fifth year MBB student in Titus Boggon's lab. She spent her early PhD years working on understanding the role of phosphorylated binding partners in p120RasGAP function and has since switched to understanding the regulation of MAPK pathways. She obtained her B.S. from Siena College where she worked in Jesse Karr's lab comparing the enzyme kinetics of horseradish peroxidase and myoglobin.

**Abstract:** Mitogen Activated Protein Kinase (MAPK) cascades are important regulators in transducing extracellular signals into the cell interior to elicit a cellular response. While some of these cascades are very well studied, including ERK1/2, p38, and JNK, others, particularly ERK5, are less well studied. ERK5 signaling has been shown to be vital for vascular development and abnormalities in ERK5 signaling are associated with numerous vascular diseases. Here I assess signaling in the ERK5 cascade using biophysical and biochemical techniques.