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Effect of point mutations on the mechanics of enzymes ZAHRASADAT ALAVI, PhD student — Using real time nano-rheology measurements, we have previously shown that the hydration layer of enzymes partially controls conformational dynamics. We perturbed the hydration layer of the enzyme Guanylate Kinase by adding small amounts of dimethyl sulfoxide (DMSO) and observed that the enzyme becomes more viscous. Here we explore the effect of point mutations on the mechanical response of the same molecule. We prepared point mutants where one Gly (the smallest amino-acid) is substituted at putative locations of high strain during enzymatic activity. In contrast to the surface perturbation, these internal changes of amino-acid sequence did not have a significant effect on the overall stiffness of the molecule, although in some cases the activity changes significantly. Using an enhanced detection system which takes advantage of surface plasmon resonance (SPR) we then studied the small changes in the mechanical response of the enzyme upon binding the different reactants and products (GMP, ATP, ADP and GDP for GK), for the different mutants. These small molecules typically modify the mechanical stiffness of the enzyme when binding to the active site. The goal here is to understand the significance of these changes.

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