mass transfer and other issues that have limited the use of SPR in protein-DNA interactions under physiologically relevant conditions. We found that distamycin binds to all three site-specific reaction rates of the ternary complex.

Based on preliminary computational studies, we hypothesize that the alanine editing domain of ProRS undergoes a conformational change to facilitate YbaK binding. In addition, the CCA-3' end of the tRNA must also be involved in significant conformational changes, translocating between the synthetic active site, the ProRS editing domain active site located 35 Å away, and YbaK. To probe these protein and RNA domain movements, we devised a fluorescence resonance energy transfer (FRET)-based approach. To date, using ensemble time-resolved FRET, we have measured an ~20 Å conformational change in the ProRS editing domain upon YbaK binding, confirming our hypothesis of a large conformational change to accommodate YbaK. Moreover, the distance between tRNA and YbaK differs by ~15 Å in the presence and absence of ProRS, further verifying a conformational change. Current studies are aimed at obtaining additional distance constraints between components of the ternary complex.

1310-Pos Board B202 Proteins Searching for their Target on DNA by One-Dimensional Diffusion: Overcoming the “Speed-Stability” Paradox Shi Yu, Shihui Wang, Ronald G. Larson. University of Michigan, Ann Arbor, MI, USA.

The sequence dependence of DNA-protein interactions that allows proteins to find the correct reaction site also slows down the 1D diffusion of the protein along the DNA molecule, leading to the so-called “speed-stability paradox,” wherein fast diffusion along the DNA molecule is seemingly incompatible with stable targeting of the reaction site. Here, we develop diffusion-reaction models that use discrete and continuous Gaussian random 1D diffusion landscapes with or without a high-energy cut-off, and two-state models with a transition to and from a “searching” mode in which the protein diffuses rapidly without recognizing the target. We show the conditions under which such considerations lead to a predicted speed-up of the targeting process, and under which the presence of a “searching” mode in a two-state is nearly equivalent to the existence of a high-energy cut-off in a one-state model. We also determine the conditions under which the search is either diffusion-limited or reaction-limited, and develop quantitative expressions for the rate of successful targeting as a function of the site-specific reaction rate, the roughness of the DNA-protein interaction potential, and the presence of a “searching” mode. In general, we find that a rough landscape is compatible with a fast search if the highest energy barriers can be avoided by “hopping” or by the protein transitioning to a lower-energy “searching” mode. We validate these predictions with the results of Brownian dynamics, kinetic Metropolis, and Kinetic Monte Carlo simulations of the diffusion and targeting process, and apply these concepts to the case of 77 RNA polymerase searching for its target site on T7 DNA.


Many vital biological processes rely on the fast searching of DNA by proteins with an average scanning rate of about 10 microseconds per base pair. The underlying mechanism involves the ability of the protein to first bind nonspecifically and then to move along DNA with “facilitated diffusion” involving four processes: sliding, hopping, jumping, and intersegmental transfer. In order to fully explain sliding dynamics of proteins along DNA, an atomic representation of the system is needed. Studying the dynamics of the nonspecific binding and facilitated search mechanisms on DNA strands in atomic detail will not only provide an understanding of how one of the most important cellular regulatory