



INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

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SHORT ABSTRACT

Protein-RNA recognition plays major role in biology, viz., charging tRNA by synthetase, structural stability of ribosome, stop-codon recognition by translation factors in the ribosome, viral-RNA recognition by innate immune proteins, etc. This thesis discusses the application of classical molecular dynamics free energy calculations to understand protein-RNA recognition events involved in gene expression and viral-RNA recognition by host protein. Energetics of discrimination (i.e., free energy differences between correct and incorrect protein-RNA complex) is linked to molecular recognition. Using X-ray structures of protein-RNA complexes as a template, we performed molecular dynamics simulations, and using appropriate thermodynamic cycle, we computed the strength of discrimination (cognate from non-cognate) employing popular statistical methods (e.g., Thermodynamic integration, Free energy perturbation etc.). We have shown that reliable free energy estimates are possible the energetics of cognate versus non-cognate interactions could be linked to 3D-structures. In this thesis, protein-RNA interactions were studied for three cases: (1) Alanine tRNA recognition by alanine-tRNA synthetase (AlaRS), (2) Stop-codon recognition by eukaryotic release factor 1 (eRF1), and (3) Viral RNA recognition by retinoic inducible gene I (RIG-I).

First case discusses the principle of alanine tRNA selection by alanine tRNA synthetase based on the critical G3•U70 base pair. Presence of a single G3•U70 mismatch in the acceptor stem of tRNA^{Ala} is essential for aminoacylation with alanine by alanyl-tRNA synthetase (AlaRS) in archaea, bacteria, and eukarya. In this chapter, we discussed quantitative estimation of relative binding free energies associated

with AlaRS binding to 3•70 mutant tRNA^{Ala} with respect wild-type tRNA^{Ala}/ G3•U70. Based on our computed relative binding free energetics, we have proposed a simple three-state kinetic scheme for aminoacylation which could explain the experimentally measured kinetics. The quantitative estimation of tRNA^{Ala} selectivity by AlaRS offers a simple view of how accuracy in the aminoacylation process is achieved, thereby establishing the link between 3D structures, thermodynamics, and experimentally measured kinetics.

In second case, energetics of stop codon (UAA, UGA, and UAG) recognition by eukaryotic release factor 1 (eRF1) have been discussed. Results suggest that eRF1 imposes a very high energetic penalty for binding to sense codon (CAA, UGG) programmed mRNA in the ribosomal A-site. The discriminatory power of eRF1 (favouring stop-codons relative to sense codons) is larger than its bacterial analog (RF1 and RF2). eRF1 ensures high selectivity by (a) introducing strain in the mRNA, (b) disrupting protein-mRNA interactions, and (c) placing sense-codons in the dry desolvated pocket with unsatisfied h-bonds in the near-cognate complexes. This chapter provides a clue to how eRF1 selectivity between the stop and sense codons could control error during translation termination.

In third case, viral RNA recognition by RIG-I has been reported. RIG-I protein recognizes viral RNAs and initiates an antiviral response, but unresponsive to similar host RNA's. Viral RNA's are usually double-stranded bearing 5'-tri/diphosphates (5'-ppp/pp-dsRNA), differing from host RNA's (bearing 5'-monophosphate/hydroxyl: 5'-P/OH-dsRNA). Experiments confirmed that RIG-I binds to viral RNA's with a very high affinity relative to host RNA's. Interestingly, 5'-p-dsRNA binds to RIG-I with surprisingly low affinity, and binding affinity is much weaker than 5'-OH-dsRNA. The mechanism of RNA discrimination by RIG-I is unclear and poorly understood. This chapter consists of two parts. The first part discusses viral (5'-ppp/pp-dsRNA) and host (5'-p) dsRNA discrimination by RIG-I. The second part discusses RIG-I discrimination between host RNA's (5'-p-dsRNA vs. 5'-OH-dsRNA). Based on our calculations, we proposed a possible mechanism of RNA selection by RIG-I. We propose that dsRNA binding to RIG-I requires Mg²⁺ dissociation from its 5' terminus dsRNA. The free energy cost associated with Mg²⁺ dissociation from RIG-I could tune the energetics and results in (1) weak discrimination by RIG-I between 5'-ppp vs. 5'-pp dsRNA (2) preferential binding of 5'-OH-dsRNA over 5'-p-dsRNA to RIG-I.