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Thermodynamic dissection of an affinity matured antibody and implications for library design

Sriram Jakkaraju, Robert James Hoey IV and **James R Horn**
Northern Illinois University, USA

A frequent goal of library-based antibody engineering is affinity maturation. Due to the complex nature of protein-protein interactions, little is known regarding the thermodynamic origins of enhanced affinity for such engineered proteins. Consequently, there is interest to understand the common energetic trends that drive affinity maturation. The resulting knowledge can be used to more efficiently direct library design and *in vitro* selection strategies. Here we explore the thermodynamic origins of an *in vitro* affinity matured anti-RNase A VHH Antibody (VHH-mat) that possesses an approximate 100-fold increase in affinity when compared to the parent antibody, VHH-wt. ΔΔΔG values of nine relevant interface residues were calculated from alanine scanning mutagenesis, which revealed four of nine residues were critical for VHH-mat's increased in affinity. Thermodynamic binding and stability studies were conducted on individual, double and triple mutants as well as isolated CDR1 or CDR3 loop variants, where wild-type residues were replaced with the VHH-mat residues. These studies revealed the enhanced binding energy (~3 kcal/mol) of VHH-mat was primarily through non-additive contributions (~70% non-additive vs. ~30% additive). Interestingly, non-additive contributions correlated with the addition (positive) or removal (negative) of likely salt-bridge interactions between CDR3 residues. When comparing inter-CDR loop interactions (CDR1 and CDR3), only additive contributions were observed. The observed thermodynamic signature of VHH-mat's enhanced binding appears to directly follow the nature in which CDR1 and CDR3 were initially randomized individually during affinity maturation before combining together, thus greatly diminishing chances for inter-CDR loop non-additive contributions. These findings reveal the important role that localized, intramolecular interactions may have on enhanced affinity. Furthermore, the detailed dissection of the binding thermodynamics may help shape library design with the goal of developing affinity matured antibodies.

Biography

Sriram Jakkaraju is currently pursuing PhD in Hornlab at Northern Illinois Univeristy. His PhD dissertation focuses on understanding thermodynamic orgins of enhanced affinity and its implementations on library based protien engineering.

sriram.niu@gmail.com

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