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UNIVERSITY OF MICHIGAN

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"Population Shifts from Allosteric Coupling of RNA and Tryptophan in the Gene-Regulating Ring-Shaped Protein TRAP"

ABSTRACT

Heterotropic allostery pervades macromolecular function, providing a means for regulating molecular properties. The undecameric (11-mer) protein TRAP from Bacillus spp. participates in a feedback regulatory mechanism in which excess free tryptophan (Trp) activates TRAP to bind a specific mRNA sequence, resulting in attenuated expression of the trp operon. Thus, Trp and RNA are heterotropically coupled through their mutual interaction with TRAP.

Understanding the mechanism by which the Trp ligand regulates the RNA binding activity of TRAP requires quantifying the structural and thermodynamic coupling between the bound Trp ligands (up to 11), and between the Trp ligands and RNA at the microscopic level. However, this goal is complicated because allosteric effects can distort the proportionality between populations and traditional experimental observables like NMR chemical shifts (Fig 1, left). On the other hand, the mass shift in native mass spectrometry (nMS) depends only on the mass and number of bound ligands, making it ideal for monitoring populations of liganded states.

We test the suitability of a nearest neighbor (NN) statistical thermodynamic model to describe calorimetric data of Trp binding to TRAP rings, and obtain microscopic thermodynamic parameters.1 Population distributions predicted from this model are observed directly by high-resolution nMS (Figure 1, center). We also use nMS to quantify how heterotropic coupling of RNA to Trp causes redistribution of Trp ligands to TRAP rings with bound RNA. These findings illustrate how allostery is achieved by population shifts, and demonstrate the utility of nMS for describing complex allosteric behavior of regulatory macromolecules.