NMR Dynamics Reveals Allostery in Ubiquitination ⇔ HARD and Geometric Approximation

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Many biological processes derive from the interplay of two or more proteins. This interplay is often driven by allosteric and dynamic changes. NMR (structure and dynamics), combined with other structural (X-ray, SAXS) and biophysical techniques, is unique in the ability to dissect these interactions. However, generally, these tools provide ground-state structures, while it is often the case that low-population excited states are the keys to the binding interactions. The field of NMR dynamics is expanding, such that both a wider range of rate processes can be measured, and more complex exchange models can be analyzed (Chao & Byrd, Emerging Topics in Life Sciences (in press, 2018). We have focused on ubiquitin-conjugating enzymes (E2s) interacting with ubiquitin ligases (E3s) to create post-translational modification by ubiquitination (Mol. Cell 2009, 2013; Structure 2012; EMBO J. 2014), which is critical to protein regulation in ERAD. RING finger proteins constitute the majority of E3s and function by interacting with E2s charged with ubiquitin. How low-affinity RING:E2 interactions result in highly processive substrate ubiquitination remains largely unknown. The RING E3, gp78, represents an excellent model to study this process. gp78 includes a high-affinity secondary binding region, G2BR, for its cognate E2, Ube2g2. Structural analyses reveal two allosteric events that are critical to the recognition, ubiquitin-transfer, and release of these proteins. These processes suggest a role for conformational dynamics in this biological machine; however, recent attempts to apply CPMG techniques to similar E2 systems have proven refractory by a number of groups. We have been able to overcome these difficulties and are beginning to understand the role of conformational dynamics in the recognition of the E2 by the E3. CPMG experiments combined with molecular dynamics are revealing new insight into the interconversion between the various states of the E2 along the reaction pathway (Structure 2017). Overall, these studies reveal that gp78:Ube2g2 is a ubiquitination machine where multiple E2-binding sites coordinately facilitate processive ubiquitination. Continuation of these studies involve examination of more complete functional complexes, which places stringent demands on experimental methods. In addition to the gp78 G2BR, other secondary binding domains have been identified for Ube2g2, and we are exploring the comparative allosteric effects in these systems.

In order to explore a broader range of exchange rate processes, we have continued development of the heteronuclear adiabatic relaxation dispersion (HARD) experiments combined with the novel analysis tools of geometric approximation (Chao & Byrd: JACS 2016, JMR 2017, Emerging Topics in Life Sciences (in press, 2018). These tools indicate the processes approaching 40-60 x 10^3 sec-1 can be detected and quantified. We will describe these tools and discuss the challenges associated with analyses of more complex exchange models/processes using geometric approximation methodology.