Comments to the Editor

Reply to: “Only Kinetics Can Prove Conformational Selection”

Because of their dynamic nature, understanding the structural properties of intrinsically disordered proteins (IDPs) is extremely challenging. In this context, we recently contributed an accurate characterization of the conformational properties of a fragment from the disordered Gab2 protein, namely, Gab2503-524, which provided key insights into its interaction with the binding partner Grb2 (1). In particular, by exploiting the information contained in NMR chemical shifts ($^1$H, $^1$HN, $^{13}$Ca, $^{13}$Cβ, $^{15}$N), we accurately quantified residual secondary structure and dynamics by using δ2D (2,3) and random coil index (4) methods, respectively, as well as refined accurate structural ensembles by means of NMR-restrained molecular-dynamics simulations (5,6). Furthermore, we assessed the effect of point mutations on the conformations of this disordered fragment by highlighting a correlation between the population of bound-like conformations in the free state of the Gab2503-524 mutants and their binding affinity with the Grb2 receptor (1). This finding unambiguously shows that some conformations explored by the IDP in isolation are both energetically and structurally similar to those observed in the bound state. It is worth noting that only the P519A variant, among those considered in our comparison, may have additional effects on the complex due to a partial loss of favorable native interactions.

We read with interest the observations by Dogan and Jemth, who point out that inferring the mechanism of binding between Gab2 and Grb2 would demand a rigorous kinetic analysis (7). The question of whether equilibrium studies are relevant for the definition of reaction mechanisms is a recurrent theme in biophysics. In this context, we note that the Monod-Wyman-Changeaux model (8) was conceived on the basis of earlier crystallographic studies of hemoglobin by Perutz et al. (9), who introduced the concept of R and T states and set the scene for understanding the allosteric transition.

We certainly agree that a kinetic analysis would be natural step toward completing the puzzle in understanding Gab2/Grb2 binding and would complement published studies on Gab2503-524 conformations (1) and the structure and thermodynamics of the Gab2/Grb2 complex (10). One possible scenario, which we suggested in our paper, is the selection of preformed conformations along the binding.

On the other hand, as suggested by Dogan and Jemth, it is indeed possible that the binding occurs via an initial docking of disordered Gab2503-524 conformations, and that the intrinsic propensity of Gab2503-524 to adopt bound-like conformations would come into play in a subsequent step, e.g., by lowering the energy barrier for a rate-limiting step and/or the energy of the protein-protein complex (7). However, this scenario implies that, upon docking with the receptor as a disordered molecule, Gab2503-524 would possess an energy landscape resembling that of its unbound state, which at this stage remains a strong assumption.

Although they involve significantly different binding mechanisms, these two scenarios share some common ground, that is, the key role played by the conformational preferences of Gab2503-524 in influencing the interaction with Grb2. Thus, although we agree with the observations by Dogan and Jemth and believe that more kinetic studies are needed in the field of IDPs, we stress the importance of new approaches that will allow for a quantitative characterization of the heterogeneous ensembles that disordered proteins explore at equilibrium, and ultimately will lead to a better understanding of the fundamental principles underlying their function, as obtained in our study (1,11,12). As new techniques become available to address the nature of IDPs, a clear picture of the distinctive properties of these molecules, which appear to be significantly different from those of purely random coil states, will begin to emerge.

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REFERENCES


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