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Review

Single molecule insights on conformational selection and induced fit mechanism

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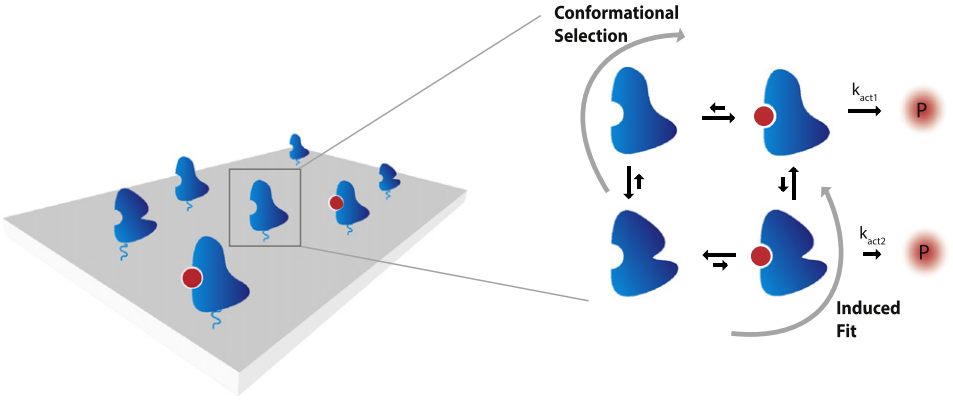
HIGHLIGHTS

- Single molecule studies decipher the mechanism of biomolecular recognition.
- Conformational selection and ligand recognition of proteins and enzymes.
- Allosteric regulation of monomeric enzymes operates via conformational selection.
- Single molecule insights for nano-medicine and drug design.
- Single molecule insights for de novo protein design with tailor made functionalities.

GRAPHICAL ABSTRACT



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ABSTRACT

Biomolecular interactions regulate a plethora of vital cellular processes, including signal transduction, metabolism, catalysis and gene regulation. Regulation is encoded in the molecular properties of the constituent proteins; distinct conformations correspond to different functional outcomes. To describe the molecular basis of this behavior, two main mechanisms have been advanced: 'induced fit' and 'conformational selection'. Our understanding of these models relies primarily on NMR, computational studies and kinetic measurements. These techniques report the average behavior of a large ensemble of unsynchronized molecules, often masking intrinsic dynamic behavior of proteins and biologically significant transient intermediates. Single molecule measurements are emerging as a powerful tool for characterizing protein function. They offer the direct observation and quantification of the activity, abundance and lifetime of multiple states and transient intermediates in the energy landscape, that are typically averaged out in non-synchronized ensemble measurements. Here we survey new insights from single molecule studies that advance our understanding of the molecular mechanisms underlying biomolecular recognition.

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1. Intro

Proteins are dynamic entities, sampling a plethora of interconverting conformations via structural fluctuations on a broad range of time scales from nanoseconds to minutes [1–3]. This fine-tuned network of motions and its remodeling by regulatory inputs drives biomolecular recognition and thus protein function and regulation. Two opposing mechanisms describing the conformational sampling underlying biomolecular recognition have been proposed: 'induced fit' and 'conformational selection' [4,5]. Induced fit posits that ligand binding remodels the protein landscape, inducing a new conformational state [6,7]. Conformational selection on the other hand proposes that this 'ligand-bound' conformation exists even before the ligand has bound, albeit as a weakly populated state [5,7]. The ligand recognizes and selectively binds this state, shifting the conformational equilibrium to make it the predominant conformation in the ensemble [8–10]. Deciphering the prevailing mechanism requires observations of weakly populated conformational states and conformational heterogeneities within ensembles of proteins.

Our understanding of these mechanisms, until recently, relied primarily on the combined results of spectrometric studies like nuclear magnetic resonance (NMR) and computational studies. NMR studies offer a wealth of information of protein conformational motions [2,3,11–13] but despite great advances [13,14] the observation of transiently formed conformational states [15,16] often remains masked due to averaging over a large ensemble of unsynchronized molecules. Computational studies on the other hand provide unprecedented insights in protein dynamics [17–23] but are limited to submillisecond time scales. The advent of single molecule techniques added new insights in the complex tapestry of protein function, the heterogeneity that is a fundamental feature of protein behavior and regulation. Despite providing limited structural information single molecule studies offered the direct observation of weakly populated states, conformational heterogeneities, transient intermediates as well as the existence of long lived stable conformational states within an ensemble of proteins with significantly different activity [24–26]. Last but not least single molecule studies offer simultaneous observation of both the conformational states of an individual protein and the ligand binding capturing and thus which of the state(s) the ligand recognizes and interacts with. Single molecule measurements thus emerge as an instrumental tool, complementing computational and spectrometric studies in attaining a comprehensive description of protein conformational and functional dynamics and in deciphering the mechanism underlying biomolecular recognition.

In this review we will firstly introduce shortly the prevailing hypothesis underlying biomolecular recognition and the limitation of current averaging techniques. We will then focus on the insights acquired by recent single molecule data in deciphering the controversy between conformational selection or induced fit as the mechanism underlying enzymatic function and regulation. Comparison of the two models has been extensively discussed earlier [5–7,10,20,27,28] and will not be discussed further here. The pivotal role of NMR [2,3,11–13] and computational studies [17–23] on the mechanism underlying biomolecular recognition has been extensively reviewed elsewhere but also by GM Clore and JA McCammon in this special issue.

2. Current models underlying biomolecular recognition and state of the art

Tightly regulated biomolecular recognition is central for controlling plethora of vital cellular processes from signaling to metabolism and gene regulation as it encompasses protein-ligand and substrate, protein–protein interactions and allostery. Comprehensive description of biomolecular recognition is critical for understanding these processes and the design of novel therapeutics for controlling them. The two prevailing hypothesis underlying allostery for oligomeric proteins proposed in the 1960s, are the MWC (Monod Wyman Changeux) model [29] and the KNF (Koshland Nemethy Filmer) model [30,31] (Fig. 1). Both models describe the allosteric effect as a binding event at one partner of the oligomeric protein causing a conformational change affecting the activity of the rest of the partners. The MWC model proposes signaling proteins to exist as oligomers that preexist in equilibrium between two conformational states. Ligand interactions would shift the conformational equilibrium leading to the allosteric activation [32,33]. The KNF model on the other hand proposes ligand-binding interactions to drive the protein towards a new conformation that is complementary to the ligand [30,31].

The modern view of molecular recognition has evolved to the "conformational selection" and the "induced fit" mechanisms extending to account for monomeric proteins and to encompass their inherent dynamics (Fig. 1C). While the term conformational selection was first used in the 1980s [34,35] it is only the last decade it has gained significant ground as the prevailing mechanism underlying biomolecular recognition [5,7,36,37] by the insightful contributions of R. Nussinov, also discussed in this special issue. Conformational selection (or different incarnations of it: population selection, population-shift, selected fit, and stabilization of conformational ensembles) has been initially rationalized to explain protein ligand recognition [36,38,39] and folding of disordered structures [37]. Later studies extended CS to explain dynamics along the reaction coordinate for a plethora of monomeric non regulated metabolic [40–44], or signaling [27], enzymes and the dynamics and mechanisms of coupled binding and folding reactions [36,45]. Recent pivotal single molecule and NMR studies showed monomeric allosterically regulated enzymes to operate via mechanism similar to conformational selection [46–48]. Although we emphasized the importance of conformational selection mechanisms, it is well understood that conformational selection and induced fit constitute the two extreme ends of the molecular recognition mechanism. In fact often both conformational selection and induced fit or induced fit alone play important roles in molecular recognition [5,7,49]. Deciphering the prevalence of any of the two mechanisms to date is often based on the quantification of the kinetic rate constants describing the thermodynamic cycle for varying ligand concentrations (see Fig. 1C) [28,49]. The critical role of the kinetics rates [39] in sorting out the prevailing mechanism is described by E Di Cera in this special issue.

The key advancement in the field of protein conformational dynamics and biomolecular recognition has come from the provision of a new