STRUCTURAL BIOLOGY

One domain, multiple conformations

Conformational transitions triggered by the binding of neurotransmitters open the gate of the ion channel domain of glutamate receptors, regulating the transmission of information through the nervous system. Using single-molecule fluorescence resonance energy transfer (smFRET), the agonist-binding domain of the GluA2 receptor, isolated in solution, is observed to interconvert among distinct large-scale conformational states.

Albert Y Lau & Benoît Roux

Cells of the nervous system communicate with each other by sending and receiving chemical messages, which take the form of neurotransmitter molecules. The major excitatory neurotransmitter is the amino acid glutamate. It transmits information by binding to ionotropic glutamate receptors, which are large protein complexes localized in the post-synaptic membrane at the junction between nerve cells. The binding of glutamate to the extracellular agonist-binding domain (ABD) induces conformational changes that ultimately lead to the opening of the transmembrane ion channel domain of the receptor. Subsequent cation flow through the channel causes an electric depolarization of the membrane that triggers the generation of a nerve impulse in the post-synaptic neuron. Proper messaging within the brain and spinal cord rely on this regulated gating of glutamate receptors. Indeed, abnormal behavior of the receptor can contribute to a number of neurological disorders, including schizophrenia and Alzheimer's disease[1]. In this issue, Landes *et al.*[2] use single-molecule FRET experiments to provide an incisive analysis of the multiple conformational states that are visited by an isolated glutamate receptor ABD in solution.

The physical phenomenon of FRET occurs when energy is transferred from an electronically excited donor chromophore to an acceptor chromophore via a nonradiative dipole-dipole interaction mechanism. Because the rate of energy transfer is greatly affected by the distance and relative orientation of the donor and acceptor, FRET is a highly sensitive technique that offers a powerful route for monitoring conformational transitions in proteins. FRET analyses of ABD molecules in bulk solution have previously been carried out to probe large-scale structural rearrangements important to receptor gating[3]. In these studies, however, the observables were limited to ensemble averages, which may obscure the fine details of macromolecular dynamics. With recent technical advances, it is now possible to go beyond ensemble averages and monitor conformational changes in individual macromolecules. By combining single-molecule FRET analysis with a wavelet-based signal processing technique for 'denoising' the data and subsequently fitting the denoised trajectories using a hidden Markov model, Landes *et al.* were able to resolve distinct conformational states visited by the ABD of the GluA2 subunit of α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors (see **Fig. 1**).

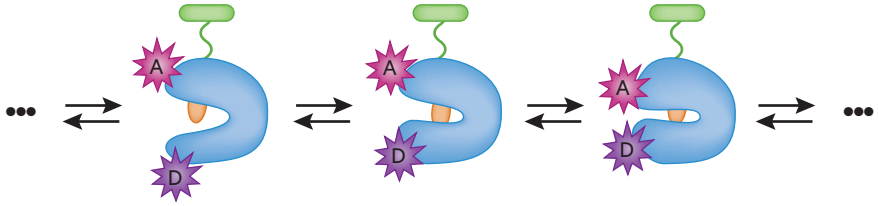

Figure 1 | FRET pairs detect large-scale conformational transitions in the isolated GluA2 glutamate receptor ABD in solution. For single-molecule FRET analyses, individual protein molecules are sparsely immobilized on a glass surface. Donor and acceptor chromophores (the FRET pair) are labeled "D" and "A," respectively. The glutamate ligand is colored orange. Glutamate binding to the ABD triggers a conformational change, activating the receptor.

The authors conclude that the glutamate-bound ABD interconverts among four conformations in a sequential manner, in striking similarity to predictions from molecular dynamics free energy simulations[4,5]. Rate constants describing transitions among these conformational states are also extracted from the data. Both single-molecule and ensemble FRET studies[2,3] and NMR studies[6–9] are consistent in concluding that the glutamate-bound ABD explores a range of conformations rather than being locked into a single one. In general, these studies demonstrate that the ABD is considerably more complex than a simple two-state 'switch' that interconverts between a ligand-bound and a ligand-free conformation.

Examination of conformational transitions for additional ABD forms, the apo ABD and the glutamate-bound T686S variant, reveal that these protein forms explore broader conformational space than the aforementioned glutamate-bound form, again in agreement with computational predictions[4,5]. The T686S mutation, which disrupts a hydrogen bond between the two structural lobes of the ABD, has been shown to decrease agonist affinity and efficacy and also to hasten recovery from desensitization[10]. Interestingly, the single-molecule FRET data suggest the ABD dynamics are best described by sequential interconversions among five conformational states.

A general limitation of FRET analysis is the inability to precisely identify the nature of the conformational transitions that give rise to changes in the distance between chromophores. For example, local side chain rearrangements can produce signals that could potentially be misinterpreted as large-scale protein motions. In the analysis of Landes *et al.*, single conformational states could potentially appear to be multiple states, owing to limitations in the hidden Markov model state-finding procedure. Nonetheless, Landes *et al.* present an exciting advance in the elucidation of GluA2 ABD conformational distributions and dynamics that are key to receptor activation[2]. We anticipate analogous single-molecule FRET studies of ABDs belonging to members of other glutamate receptor families, such as *N*-methyl-D-aspartate (NMDA) and kainate receptors. These studies would help characterize the dynamical diversity among glutamate receptors that exhibit different

functional behavior. We also look forward to the possibility of single-molecule FRET studies of full-length glutamate receptor complexes. These studies would bring further insight into the allosteric mechanism controlling receptor activation.

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Competing financial interests

The authors declare no competing financial interests.

CARBOHYDRATE DYNAMICS

Antibody glycans wiggle and jiggle

Many proteins bear sugar residues that modulate their functionality. Clues about the energy landscape and accessibility of sugar conformations in immunoglobulin G will contribute to an understanding of the mechanistic and energetic aspects of glycobiology and immunology.

Sebastian Meier & Jens Duus

Richard Feynman's famous postulate from 1963 that "everything that living things do can be understood in terms of the jiggling and wiggling of atoms" has since been tested and supported by ever-sharper experimental and computational tools. Proteins, in particular, have been found to exhibit functionally important structural fluctuations over a broad range of timescales[1]. Data on protein dynamics at unique spatial and temporal resolutions can be gathered with nuclear magnetic resonance (NMR) spectroscopy, which provides structural and dynamic information at atomic resolution in close-to-natural conditions[2]. In particular, structural rearrangements on the microsecond-to-millisecond timescale seem to be relevant for many biochemical events and have been in the spotlight of NMR-based dynamic analysis in recent years[1]. Many proteins must be covalently conjugated with asparagine-linked sugars (N-glycans) to perform their roles, and nearly two thirds of sequence entries in protein sequence data banks contain N-glycosylation consensus sequences[3]. Glycosylation modulates intermolecular recognition events as well as folding and other properties of the protein itself. Experimental probes for the structural fluctuations of sugar epitopes in glycoproteins are, however, relatively sparse. In this issue[4], Barb and Prestegard experimentally map the energy landscape and motions of an antibody N-glycan by introducing NMR approaches to dynamic structural glycobiology. The approach characterizes conformational substates of the glycoprotein carbohydrate and yields interconversion rates and populations of the substates on separable timescales, thereby showing that antibody glycans are considerably more dynamic than previously assumed.

IgG is the main serum immunoglobulin and is composed of two identical heavy chains with four domains each and two

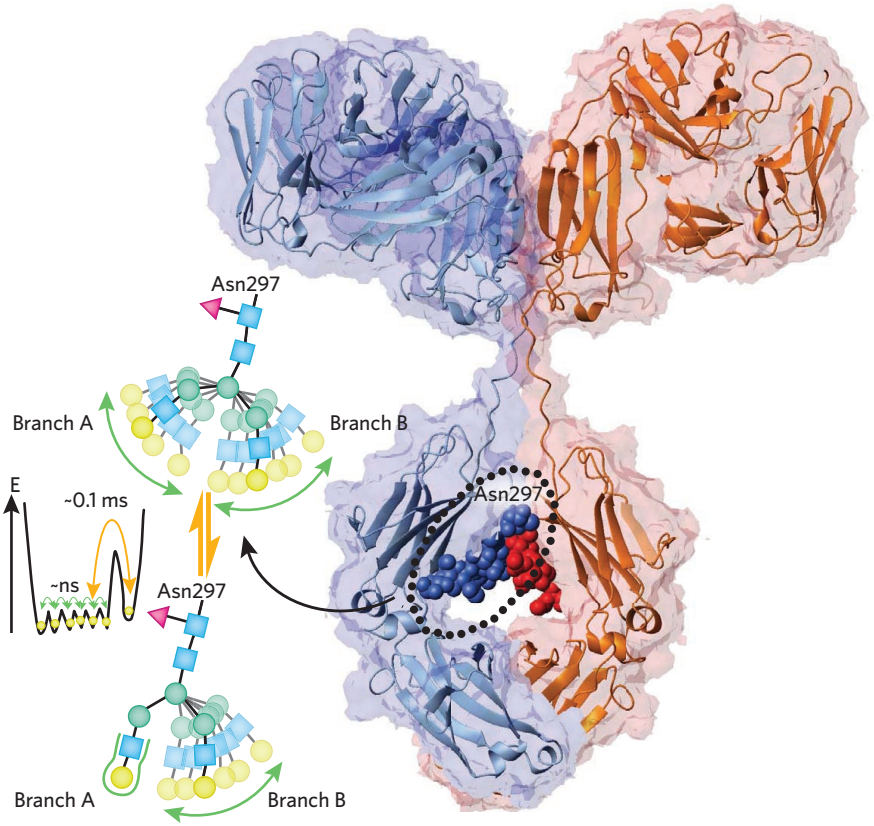

Figure 1 | Structure of IgG carrying an N-linked glycan at Asn297. The experimentally determined conformational substates of the two fluctuating glycan branches A and B are schematically depicted.