dynamic manner, may therefore restore normal tau glycosylation and thus prevent pathological phosphorylation.

The design of the O-GlcNAcase inhibitor Thiamet-G (**2a**), as Yuzwa *et al.*[2] have termed their agent, represents an elegant example of structure-based drug design. Stabilization of high-energy transition states that occur during the chemical conversion of substrates to products is at the heart of enzyme catalysis. Thiamet-G is a stable compound whose fused thiazoline ring system (red in **2**) geometrically mimics a transition state of the substrate-assisted enzymatic hydrolysis of protein-*O*-GlcNAc units and, in this way, effectively inhibits O-GlcNAcase function. Similar oxazoline systems that inspired the design of Thiamet-G are found in natural products with glycoside hydrolase inhibitory activities, such as allosamidin (**3**) and allosamizoline (**4**)[3], as well as in synthetic derivatives (such as **2b** and **2c**)[4]. Unlike known O-GlcNAcase inhibitors, Thiamet-G, designed by reference to X-ray crystallographic structures of hexosaminidase–inhibitor complexes, shows high selectivity for O-GlcNAcase over related hexosaminidases, especially lysosomal Hex A and Hex B. For example, replacement of simple small thiazoline 2′-substituents with the ethylamino group of Thiamet-G increases not only affinity by virtue of favorable electrostatic interactions between the inhibitor amino group and the enzyme's aspartatecatalytic residues (**Fig. 1b–d**) but also enhances selectivity through optimal occupation of the pocket that accommodates the substrate *N*-acetyl group, which is noticeably larger in O-GlcNAcase than in Hex A and Hex B (**Fig. 1e–g**). Because deficiency of Hex A and Hex B is associated with several serious lysosomal storage disorders (such as Tay-Sachs and Sandhoff diseases), inhibitor selectivity for O-GlcNAcase over lysosomal hexosaminidases is important for precisely elucidating the roles of these proteins, as well as for potential therapeutic safety in drug development.

Apart from its enzyme potency and selectivity, the notable features of Thiamet-G as a first-generation pharmacological O-GlcNAcase inhibitor are its oral bioavailability and ability to cross the blood-brain barrier. According to Yuzwa *et al.*[2] the secondary amino functional group of Thiamet-G shows a pK_a value of 8.0, which is likely to be beneficial not only for enzyme affinity but also for CNS bioavailability, as compounds that are somewhat basic under physiological conditions are more likely to be blood-brain barrier permeant[5]. In neuronal cell culture, Thiamet-G was able to reduce tau phosphorylation at pathologically relevant sites up to threefold. Immunohistochemical analysis of brain samples from animals that had received Thiamet-G by the oral administration route revealed a similar picture of specific suppression of tau phosphorylation.

Recent results suggest that direct inhibition of the kinases that are implicated in pathological tau phosphorylation may be a viable therapeutic strategy for tauopathies (reviewed in ref. 6). The results of Yuzwa *et al.* now suggest an alternative and indirect strategy. As we saw, decreased glucose availability in the brain limits O-GlcNAcylation and thus allows tau hyperphosphorylation. Elsewhere in the body, however, excess glucose can lead to an increased flux through the hexosamine biosynthetic pathway, leading to elevated *O*-GlcNAcylation of many proteins, some of which are known to contribute to metabolic disease states such as hyperglycemia, hyperinsulinemia and hyperlipidemia, as well as the adverse affects of these disorders on the heart[7]. For this reason, it might be expected that global inhibition of O-GlcNAcase could result in undesirable side effects. Although Yuzwa *et al.* do not report any gross toxicity of their O-GlcNAcase inhibitor upon *in vivo* administration, even at what seems to be a very high dose, further O-GlcNAcase target validation and Thiamet-G toxicology studies are clearly warranted. Apart from representing an interesting new drug lead, Thiamet-G will certainly be a valuable tool for the *in vivo* study of the role of O-GlcNAcase in tauopathies.

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Protein dynamics under light control

Michele Vendruscolo

A stochastic view of allostery is providing quantitative estimates of the energy made available through protein photoswitches.

Living cells constantly monitor their own state and their surroundings in order to respond effectively to changes in them. Their ability to perform these tasks relies on the presence of complex networks of interacting proteins that sense the environmental conditions and enable appropriate biochemical reactions to take place to maintain homeostasis and promote development. At the molecular level, the transfer of information is often achieved through the relay of highly specific conformational changes within and between proteins. A longstanding challenge has been to understand how much energy is required to initiate and sustain these processes. As reported in this issue, Yao, Rosen and Gardner have now been able to provide a quantitative estimate of the energy made available by a protein photosensor[1].

Key to the result obtained by Gardner and co-workers[1] has been the recognition that functionally important conformational changes of proteins are often achieved through a shift in the equilibrium populations of interconverting states. According to this view, conformational fluctuations of proteins, rather than being uniformly distributed in a random manner, are organized to prompt them to sample their active states[2,3]. This dynamical view of protein behavior, which has already generated major advances in understanding enzymatic catalysis[4,5] and allosteric activity[6], has now been applied to the study of sensory proteins.

To study the molecular basis of the photosensor activity of proteins, Gardner and co-workers[1] focused their attention on phototropins—blue-light receptor proteins that control the ability of algae and plants to carry out photosynthesis by

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regulating a variety of processes, including phototropism, chloroplast movement and stomatal opening[7]. As with other photosensory proteins, phototropins function by binding light-absorbing chromophores to convert the energy of photons into structural and dynamical changes. The absorption process itself takes place in two so-called LOV domains in the N-terminal region of the phototropin. The subsequent conformational changes are then transmitted to the kinase domain at the C-terminal region of the protein. Previous studies identified as a key step in the signaling process the partial unfolding upon light absorption of Jα, an α-helical connective element between the second light-absorbing LOV domain (LOV2) and the kinase domains[8]. The energy involved in such an event, however, was not precisely known.

By adopting a stochastic view of protein allostery, Gardner and co-workers have been able to fill this gap, and to demonstrate that the conformations dominant in the lit state of the phototropin that they studied are present also in the dark state, although with a low statistical weight[1]. They exploited the opportunities offered by NMR spectroscopy to measure, at least in favorable cases, the relative populations of states and the rates of their interconversion with high accuracy. By using the Carr-Purcell-Gill-Meiboom (CPGM) relaxation dispersion technique[9], which enables detailed information to be obtained about low populated states in equilibrium with the native state, they first estimated the free energy difference between the ground and the excited states of the LOV2-Jα complex in the dark as 2.4 kcal mol^{-1} (**Fig. 1a**). Then, by comparing the chemical shifts of the Jα peptide in isolation and in the lit state, they estimated the free energy difference between the ground and the excited states in the presence of light as –1.4 kcal mol^{-1} (**Fig. 1b**). Hence, they concluded that upon light absorption a total of 3.8 kcal mol^{-1} becomes available for transmission from the LOV2-Jα complex to the phototropin kinase. Thus, they have been able to quantify the free energy associated with this conformational change, and in the process to reveal that this particular photoswitch functions by changing the equilibrium populations of the dark and the lit states.

As 3.8 kcal mol^{-1} is almost 20-fold smaller than the energy made directly available by the absorption of a blue-light photon[1], phototropins would not seem to be very efficient as light sensor devices. However, the energy that they yield is close, for instance, to that provided through ATP hydrolysis, which is itself comparable to the overall stability of most globular proteins[10]. Thus, this amount of energy seems to be of just about the right scale to prompt functional conformational transitions in folded proteins. Therefore, rather than being directly related to the efficiency of molecular machines, the specific amount of energy generated by phototropins seems to be scaled down from the substantial energy of photons to the right level to alter specific conformational states of proteins. These results obtained by Gardner and co-workers open the possibility of generating a variety of photoswitches with a large dynamical range by altering—for example, through protein engineering[10]—the efficiency of the conversion of the energy of photons into conformational and dynamical changes of proteins.

The ability to make quantitative estimates of the energy provided in proteins through light absorption, or indeed through other sensory processes, holds the promise of providing powerful further tools to engineer protein sensors with accurately tunable responses. These advances will be firmly rooted in the view that the control of protein behavior—including their signaling activity—can be very effectively achieved through the modulation of their equilibrium structural fluctuations.

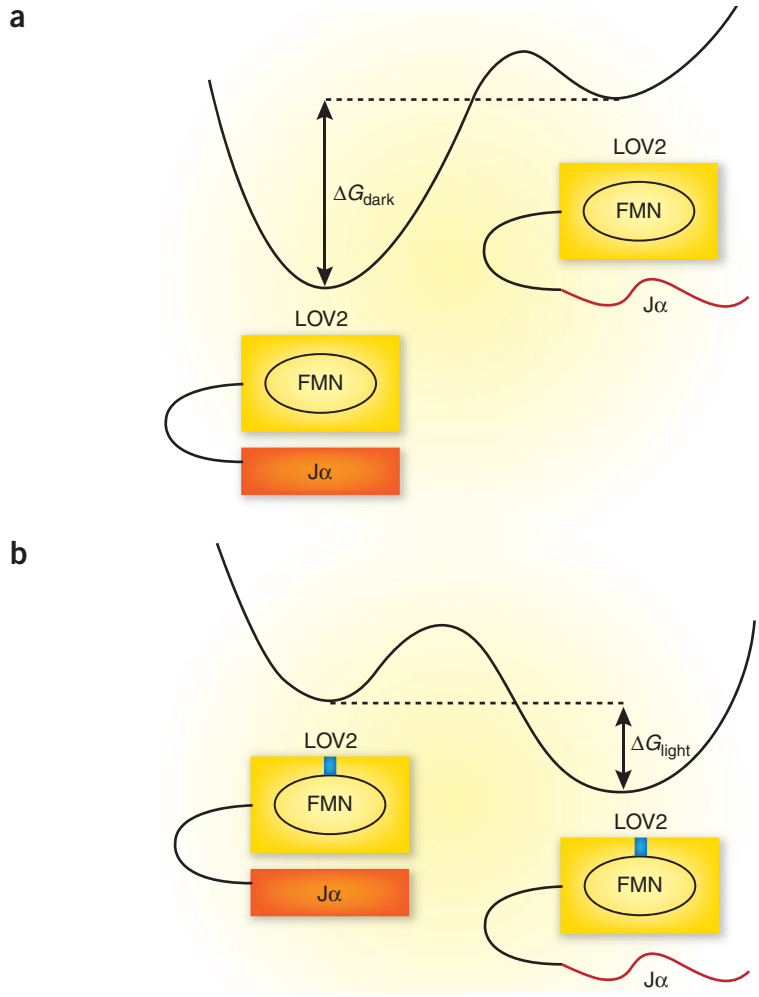

Figure 1 Equilibrium shift of a protein photoswitch. (**a**) In the absence of light, the LOV2-Jα complex fluctuates on the micro- to millisecond timescale between the dark state and a lit-like state, with a free energy difference ΔG_{dark} = 2.4 kcal mol^{-1} between them. (**b**) By contrast, in the presence of light, the covalent binding of a chromophore (flavin mononucleotide FMN) to LOV2 triggers conformational and dynamical changes leading to a reversal in the free energy difference, which becomes ΔG_{light} = –1.4 kcal mol^{-1}, between a dark-like state and the lit state. Hence, light absorption makes 3.8 kcal mol^{-1} available to the protein for initiating its kinase activity.

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