corroborated the controversial prediction.

And that was where the story ended — until now. Latos-Grażyński and colleagues[1] have synthesized a new kind of ring compound that exhibits Hückel or Möbius behaviour depending on the polarity of the surrounding solvent. Their molecule incorporates a 'porphyrin' structure (Fig. 1b). Porphyrins are aromatic molecules that are ubiquitous in nature — they form the reactive sites of haemoglobin, myoglobin and cytochrome enzymes, for example. Structures related to porphyrins are also found in chlorophyll and vitamin B_{12}.

Latos-Grażyński and colleagues[1] have inserted two benzene rings into the porphyrin core (Fig. 1b), so increasing the number of mobile electrons from 18 (a $4n+2$ system) to 28 (a $4n$ system). Their nuclear magnetic resonance (NMR) experiments show that the compound is antiaromatic in nonpolar solvents, as expected from the Hückel rule. But in polar solvents, the antiaromaticity is lost. This change in electronic behaviour can be attributed to a molecular conformation change.

The difference in conformation seen in polar and nonpolar solvents is brought about by a small twist of one of the molecule's benzene rings, which acts like a topology switch (Fig. 2). If this benzene ring is perpendicular to the other one on the opposite side of the porphyrin, the molecule adopts a twisted, one-sided Möbius topology. But if the benzene rings are parallel to each other, the structure is untwisted and two-sided. The authors[1] did not observe aromaticity when the molecule was in the Möbius conformation, however, probably because the large amount of twist prevents efficient overlap of the electrons' orbitals.

This is the first example of a molecule with reversible topology that switches from an antiaromatic to a non-aromatic (or weakly aromatic) state. Naturally occurring porphyrin-containing compounds bind to metal cations to perform various functions, such as inducing redox reactions, transferring methyl (CH_3) groups between molecules or storing oxygen. Artificial porphyrins that include a topology switch might extend the list of applications for these molecules.

When the first Möbius annulene was prepared in 2003, it was predicted that additional molecules would be synthesized that would have stronger Möbius aromaticity[7]. We are still waiting for an example. But Latos-Grażyński and colleagues' work suggests that such a compound will probably be found among the extended porphyrins[8]. ■

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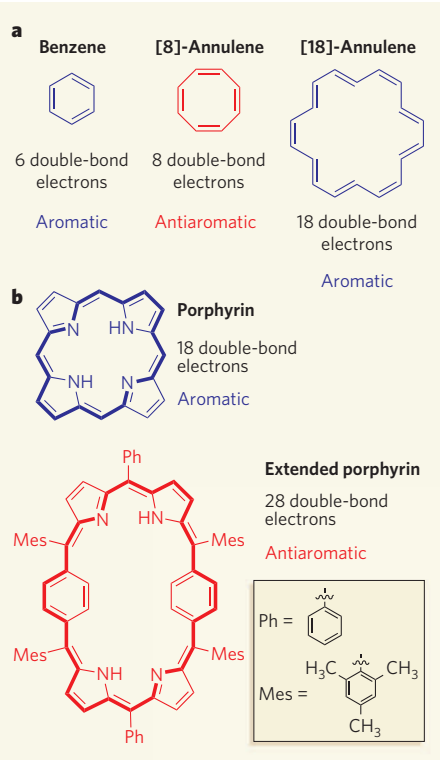

Figure 1 | Annulenes and aromaticity. **a**, Annulenes are cyclic hydrocarbons that contain alternating single and double bonds, as shown in these examples. Annulenes with $4n+2$ double-bond electrons (where $n \geq 0$) are stable, and are described as aromatic compounds. Annulenes with $4n$ double-bond electrons are unstable, and are described as antiaromatic compounds. **b**, The porphyrin structure is aromatic. Latos-Grażyński and colleagues[1] have made an extended porphyrin that is antiaromatic in nonpolar solvents. Only the bonds shown in bold affect the aromatic state of the molecule. The three-dimensional structure of the extended porphyrin is shown in Figure 2.

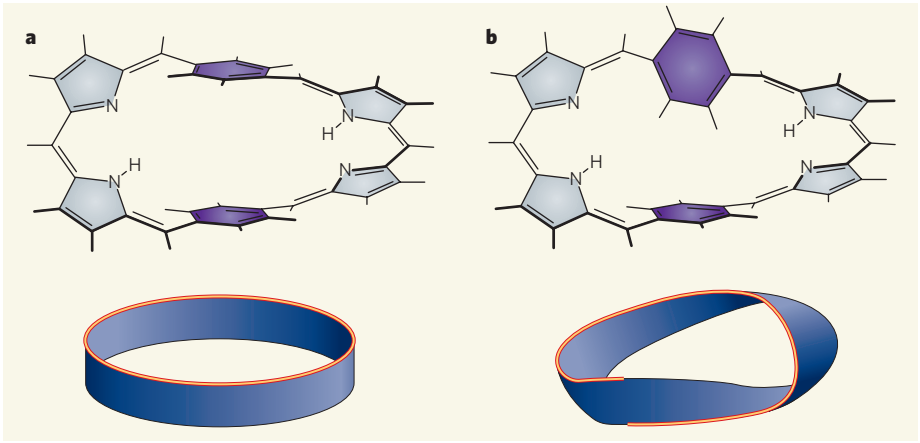

Figure 2 | A molecular topological switch. Latos-Grażyński and colleagues[1] have made a compound that is antiaromatic in nonpolar solvents, but not in polar solvents. **a**, In nonpolar solvents, the two benzene rings (purple) in the molecule are parallel, and the molecule is a two-sided, non-twisted band. **b**, In polar solvents, the upper benzene ring twists by 90°, so that the molecule becomes a one-sided, Möbius structure. This conformational change alters the aromaticity of the molecule.

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VISION

Dynamic platforms

Roger C. Hardie

Scaffolding proteins are so named because they function as platforms for the assembly of molecular signalling complexes. But at least one such protein is more than a passive bystander and has its own signalling role.

Living cells are dynamic machines. They contain thousands of different proteins that participate in a maze of signalling pathways, all of which must be accurately organized in space and time for efficient cellular functioning. To achieve this level of organization, one strategy that cells use involves scaffolding proteins, which assemble proteins of a particular signalling pathway into multimolecular complexes, thereby promoting signalling specificity and speed while avoiding spurious crosstalk with other pathways[1]. Scaffolding proteins are often regarded as passive binding platforms. But in a paper published in *Cell*, Mishra *et al.*[2] show that INAD, a scaffolding protein essential for phototransduction in flies, undergoes

a light-induced conformational change, indicating that it has a direct and dynamic role in signalling.

Many scaffolding proteins contain one or more PDZ structural domains, which are modules of about 90 amino acids that bind to the carboxyl termini of specific protein partners[2]. PDZ-domain proteins have been implicated in almost all aspects of cell biology as structural binding platforms, but there are also reports[3] indicating that they have more direct signalling roles. Mishra *et al.* provide fascinating structural insight into such a role by solving the crystal structure of INAD — one of the best-characterized PDZ-domain scaffolding proteins[4–8] — in dynamically interchangeable states.

In the fruitfly *Drosophila*, INAD underpins the phototransduction cascade, a canonical, G-protein-coupled signalling pathway that can generate electrical responses within 20 milliseconds of the absorption of a single photon[7,8]. Crucial to the speed and amplification of this signalling cascade is the localization of its components within an array of about 30,000 tiny, finger-like membrane extensions, known as microvilli, that form the light-absorbing organelle in the photoreceptor cells.

Correct localization of the components of the phototransduction pathway depends on INAD, which has five interlinked PDZ domains that bind to different cascade components[4–8] (Fig. 1a). The core components of the resulting signalling complex include the enzyme required for excitation (PLC), the ion channel that mediates the electrical response to light (TRP) and a regulatory enzyme (PKC). PKC phosphorylates TRP and INAD, and possibly other proteins, and is required for the deactivation of the response to light[6–8].

Using X-ray crystallography, Mishra *et al.*[2] have solved the structure of INAD's fifth PDZ domain (PDZ5) at 2 Å resolution. The ligand-binding site in PDZ domains is a groove between an α-helix and a β-sheet, which is evolutionarily conserved in all 15 previously solved PDZ-domain protein structures[9]. Unexpectedly, the authors found that, owing to the formation of a disulphide bond, this ligand-binding groove becomes severely distorted in PDZ5 (Fig. 1b). In crystals grown under reducing conditions, however, they found that the disulphide 'bridge' was broken, and that PDZ5 had its expected topology.

The researchers then showed that, when flies are kept in darkness, PDZ5 is in its reduced form, but, after light stimulation, the disulphide bridge forms transiently, resulting in a conformational switch to the oxidized form. This 'redox switch' failed to operate in mutant flies lacking PKC, indicating that phosphorylation by this enzyme might trigger INAD's conformational change.

What is the functional significance of the redox switch in INAD's structure? Mishra *et al.* mutated one of the two cysteines in INAD that form the disulphide bridge (*Inad*^{C645S}), thereby

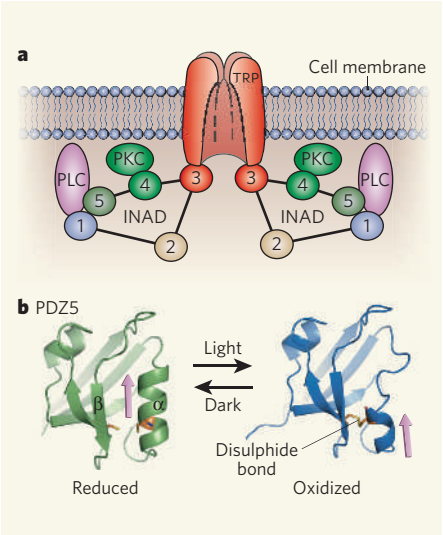

Figure 1 | Phototransduction in *Drosophila* and the INAD complex. a, The five PDZ domains of INAD (1–5) assemble components of the phototransduction cascade, including PLC, the TRP channel and PKC, into a signalling complex at the cell membrane. **b**, Mishra *et al.*[2] report that, in response to light, the PDZ5 domain of INAD undergoes a conformational change. In the dark, PDZ5 is in its canonical, reduced form, in which a groove between an α-helix and a β-sheet serves as a ligand-binding site. After stimulation with light, the PDZ5 domain undergoes a conformational change to an oxidized state, whereby the formation of a disulphide bond between two cysteine residues results in the unravelling of the α-helix and the distortion of the ligand-binding groove. Following this conformational switch, the ligand (arrowed) — putatively part of the PLC enzyme — can no longer bind. (Adapted from ref. 2.)

effectively locking PDZ5 in the reduced state with its ligand-binding groove available. When tested with dim light, the photoreceptor cells of *Inad*^{C645S}-mutant flies showed normal responses. But, at higher light intensities, light responses deactivated slowly. The authors underlined the biological importance of the redox switch by showing that *Inad*^{C645S} mutants have dramatic defects in visually mediated escape behaviour.

On the basis of these observations, Mishra *et al.* suggest a model according to which phosphorylation of INAD by PKC facilitates the transition of PDZ5 to the oxidized state. This results in the dissociation of the ligand — possibly PLC — from PDZ5, thereby inactivating and/or making PLC temporarily unavailable for renewed activation. But in *Inad*^{C645S}-mutant flies PLC would remain bound and could potentially be rapidly re-activated following the absorption of a second photon in the same microvillus, explaining the deactivation defect at high light levels.

This model is supported by earlier work[10] showing that PKC is required for rapid, calcium-dependent termination of PLC activity. As PLC is normally inactivated within milliseconds of Ca^{2+} influx into the cell, but can be reactivated after a refractory period of about 100 milliseconds (ref. 7), this model implies that the reversible conformational switch in PDZ5 must be remarkably fast.

New advances always raise as many questions as they answer, and the study of Mishra *et al.* is no exception. Although earlier studies[5] concluded that PLC binds to PDZ5, Mishra *et al.* cite their own unpublished data questioning such an interaction. So, depending on whether PLC really is the ligand that binds to the PDZ5 domain of INAD, Mishra *et al.* suggest that their model may need to be refined.

The authors find that, although PDZ5 is stable in the oxidized state, when grown as crystals, the pre-stimulation, 'ground' state *in vivo* is the reduced form. How is the reduced state stabilized? Are there factors in the microvilli that create an unusually reducing environment, or do other (allosteric) factors favour the reduced state in the intact protein complex?

In addition, how does the activation of the phototransduction cascade facilitate the conformational switch? The requirement for PKC suggests phosphorylation of INAD may trigger the switch. But INAD is not the only PKC target. Light stimulation also rapidly initiates dramatic changes in the local (microvillar) environment. These include Ca^{2+} influx through TRP channels, which leads to exceptionally high Ca^{2+} concentrations, and huge changes in the levels of the PLC substrate $PtdInsP_2$ and its metabolites DAG and $InsP_3$. Whether light exposure also induces changes in the redox state within the microvilli is not known.

Finally, how common is this new mechanism? The specific cysteine residues involved in the formation of the disulphide bridge seem to be unique to the PDZ5 domain of INAD in flies that belong to the Brachycera suborder of the order Diptera. This suggests that the redox switch may be a specific adaptation for the rapidly signalling visual systems of these organisms. However, Mishra and colleagues' graphic demonstration[2] of a dynamic conformational switch and its functional significance will surely motivate the search for similar dynamic behaviour among other members of the large and ancient family of PDZ-domain proteins. ■

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