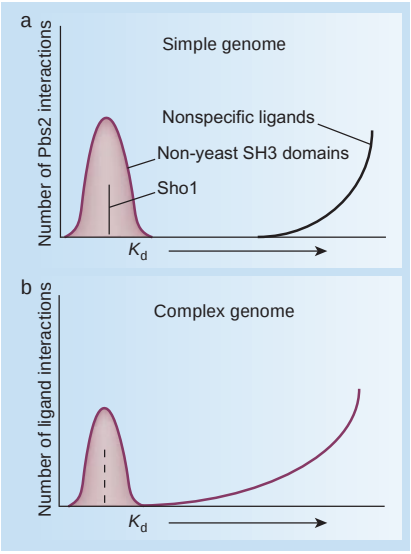

Figure 1 **How organisms achieve specificity of protein–protein interactions. A general feature is the presence of structural 'domains' in one protein and complementary 'motifs' in their binding partners (ligands).** a, **In baker's yeast, which has a relatively simple genome encoding relatively few proteins, a single motif-containing protein (here, Pbs2) binds to just one SH3-domain-containing partner (Sho1) with a reasonably low dissociation constant (K_d; that is, with high affinity). Other yeast SH3-domain proteins bind to Pbs2 with lower affinity (black curve). Zarrinpar *et al.*[2] find that this specificity results from evolutionary negative selection against nonspecific interactions. SH3 domains from other organisms are not subject to negative selection in yeast, and so bind promiscuously to Pbs2 with dissociation constants similar to that of Sho1 (purple curve).** b, **In organisms with more complex genomes, which encode many SH3 domains and many ligands that bind these domains, additional mechanisms may work to restrict a large number of potential interactions (purple curve) to a single domain–ligand pair (dashed line).**

the 12 non-yeast SH3 domains functioned well enough to allow cell growth under high salt conditions (where the HOG pathway is important). Why should this be? Zarrinpar *et al.* suggest that, through natural selection, the amino acids within and around the proline–X–X–proline motif on Pbs2 have evolved to be recognized only by the SH3 domain of Sho1, and not by any other yeast SH3 domain. No such negative selection would have occurred against the non-yeast SH3 domains. In general terms, then, an evolving system composed of intermixing parts could use negative selection to eliminate spurious interactions.

To test this model, the authors changed the Pbs2 SH3-binding motif so as to increase or decrease the strength of the Sho1–Pbs2 interaction. All changes reduced the specificity of interaction for the Sho1 SH3 domain, suggesting that the Pbs2 motif was already 'optimized' for the combination of binding strength and SH3 specificity. As a second test, Zarrinpar *et al.* used a competitive growth assay to compare yeast containing wild-type Pbs2 with yeast containing either a mutant Pbs2 that does not interact with Sho1, or a promiscuous Pbs2 mutant that interacts with both Sho1 and most other yeast SH3 domains. Both the wild-type and the promiscuous strains outgrew the 'non-interacting' strain under high salt conditions. But the wild-type and non-interacting strains outgrew the promiscuous strain under conditions that do not require the HOG pathway. The success of the non-interacting strain under these conditions supports the general model that a cell is better off with components that don't interact than with those that bind to one another indiscriminately. Promiscuous proteins in a particular cell type are selected against in order to maintain a 'self-consistent' protein-interaction network that is free of detrimental interactions.

What happens in more complex animals, in which there are greater numbers of protein–protein interactions (Fig. 1b)? Is negative selection alone sufficiently powerful to maintain interaction specificity? The data here are incomplete. Analysis of some mammalian SH3 domains by techniques such as phage display and bioinformatics suggests more promiscuous protein binding, and a lack of strong, specific motif selection[5]. But some of this apparent promiscuity is clearly overcome by temporal and spatial segregation; not all components are present at the same time and place. So negative selection such as that described by Zarrinpar *et al.* need only operate within the confines of a particular subcellular compartment or cell type in higher animals.

In addition, these organisms may have developed further mechanisms for maintaining interaction specificity. Genomic analyses of multicellular organisms suggest the evolution of more complex multidomain protein architectures[6,7], in which SH3 domains are mixed-and-matched with other modular domains. This might allow a series of weak and otherwise promiscuous individual interactions with any potential target protein to occur simultaneously, with the sum of these interactions providing much higher specificity than that possible with any single domain acting alone.

Zarrinpar and colleagues' work[2] reveals an elegant example of how biology has solved the problem of wiring dynamic systems at the molecular scale. A complete understanding of such systems, and of the mechanisms that underlie their proper function and maintenance, will greatly aid our analysis of — and interaction with — the living world. ■

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Chirality

Organic films with a twist

Michael D. Ward

Left- and right-handed helical molecules form mirror-image chiral crystals on a copper substrate. It seems that the substrate and the molecules work in concert to determine the handedness of the crystal domains.

Chirality is central to the building blocks of life, and to commercial chemical enterprises. Most amino acids, sugars and pharmaceuticals contain chiral carbon centres — a carbon atom bonded to four different substituents in a tetrahedral geometry. Such chiral molecules exist in two mirror-image forms, like left and right human hands, that are called enantiomers. Our understanding of this peculiar property can be traced back to Louis Pasteur, who discovered that 'racemic acid' (a crystalline deposit formed on wine casks during fermentation) consisted of equal amounts of left- and right-handed crystals of sodium ammonium tartrate, which were easily distinguished as mirror images under an optical microscope[1]. As they report in *Angewandte Chemie International Edition*, Fasel *et al.*[2] have exploited the atomic-level imaging capabilities of the scanning tunnelling microscope (STM) to observe chirality directly at the molecular level, in enantiomorphic two-dimensional crystals of chiral molecules on a copper surface.

In three dimensions, chiral molecules can form either racemic (heterochiral) crystals, which contain equal numbers of left- and

right-handed molecules in the same crystal, or conglomerates of separate left- or right-handed (homochiral) crystals of the pure enantiomers, like Pasteur's tartrate salts. Racemic crystals, however, tend to greatly outnumber conglomerates[3]. Despite the passage of 150 years since Pasteur's studies, the factors responsible for the transmission of chiral information between molecules during crystal formation are not fully understood. Nevertheless, theory has suggested that discrimination between hetero- and homochiral ordering becomes more likely when short-range repulsive forces (such as those experienced by molecules in close-packed organic crystals) are significant[4]. Under these conditions, the selectivity for racemates or conglomerates would be governed by molecular shape and symmetry.

How chiral structures form on surfaces, and how chiral information propagates from single molecules to supramolecular ensembles, are important questions in several technological applications, including crystal growth[5,6], the fabrication of liquid-crystal displays[7] and enantioselective catalysis[8,9]. Like many organic molecules, chiral molecules can adsorb on crystalline substrates, such as graphite or copper, to form two-dimensional crystals that are only a single molecule thick. If the substrate is electrically conductive, the adsorbed molecules can be viewed with an STM. To create an image, an ultrasharp electrified tip is scanned over the crystals and the tunnelling current measured between the tip and crystals on the conductive substrate.

The extraordinary spatial resolution and the sensitivity of the tunnelling current to the different chemical groups in a molecule means that the handedness of chiral structures can be assigned[10], as can their epitaxial alignment on the substrate[11]. Scanning tunnelling microscopy has revealed that, unlike their three-dimensional counterparts, racemic compounds often form conglomerates of two-dimensional enantiomorphic crystals when confined to a substrate surface[12,13], suggesting that confinement in two dimensions aids chiral discrimination[14]. These enantiomorphic two-dimensional crystals are discernible as mirror images[15], not unlike Pasteur's tartrates.

Ernst and co-workers previously found[16] that racemic mixtures of '[7]H' — which consists of seven benzene rings fused edge-to-edge to form a chiral helical coil — formed separate enantiomorphous domains aligned through epitaxy on a Cu(111) substrate (the Cu(111) surface is a flat plane of copper atoms in a hexagonal arrangement). Now Fasel *et al.*[2] have prepared films of the pure enantiomers, designated (M)-[7]H and (P)-[7]H, on the Cu(111) surface. STM images revealed spheres corresponding to individual molecules of [7]H organized into two-dimensional crystalline monolayers. When

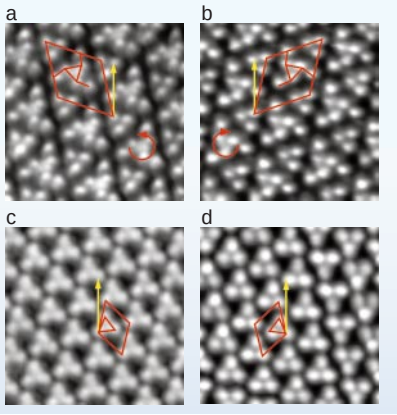

Figure 1 **Left- and right-handed forms of [7]H.** a, b, **The '6&3' structure;** c, d, **the '3-structure'. Unit cells and the arrangement of molecules within them are outlined in red; the yellow arrow indicates the same surface direction in each figure.**

95% of the surface was covered with [7]H, the molecules organized as pairs of triangular clusters, one with three molecules and the other with six (a '6&3' structure; Fig. 1a, b). Surprisingly, increasing the coverage just slightly, to 100%, produced a different film structure, consisting of three-molecule clusters (a '3-structure'; Fig. 1c, d). The chirality of these films is apparent from the STM images — the enantiomeric (M)-[7]H and (P)-[7]H lattices are mirror images aligned along opposite directions on the substrate surface. These features signify epitaxial ordering and, more importantly, they reveal that the chirality of the [7]H molecules is transmitted to the molecule–substrate interface.

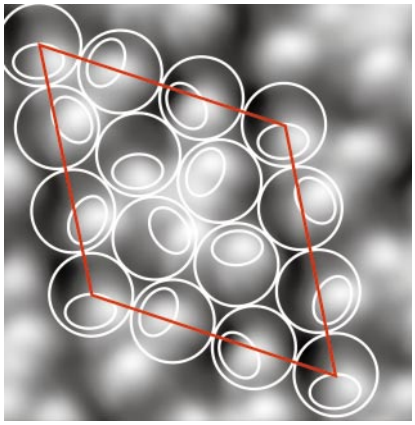

Figure 2 **Chirality established. Fasel *et al.*[2] have overlaid their STM image of (M)-[7]H molecules with a model of the structure of these 6&3 molecule clusters. Each circle represents a molecule, and the bright oval inside each sphere is the topmost part of the molecule. Tracing a path between them, each molecule is rotated through 60° with respect to the previous one, indicating that chirality is transmitted between adjacent molecules.**

Particularly striking is the tunnelling contrast observed in the 6&3 clusters. The six-membered clusters resemble a 'pinwheel', with the (M)-[7]H pointing anticlockwise and (P)-[7]H pinwheel clockwise, a direct consequence of the chirality of the helical molecules. Zooming in on the images reveals that each molecular sphere has a circular bright spot tipped to one side (Fig. 2). The authors ascribe this feature, quite reasonably, to tunnelling at the uppermost ring of the [7]H molecule, which is closest to the STM tip. Remarkably, tracing an anticlockwise circuit on the periphery of the (M)-[7]H six-membered cluster, molecule by molecule, reveals that this bright spot rotates in successive steps by 60° about the surface normal. This produces a total of six different in-plane molecular orientations. A three-molecule circuit around the three-membered cluster of a 6&3 structure or a 3-structure cluster also reveals successive 120° orientations of the bright spot.

The observation that successive 60° (or 120°) orientations are observed during these circuits, instead of random 60° orientations, can only mean that chirality is transmitted between adjacent [7]H molecules. This [7]H is not decorated with strong dipoles or hydrogen-bonding groups that could direct molecular organization. Instead, it would seem that the helical shape of [7]H prescribes a gear-like rotation of adjacent molecules in these close-packed two-dimensional crystals. Fasel *et al.*[2] have thus provided a molecular-level insight into how chirality evolves in crystals, which will undoubtedly enhance our understanding of chirality in supramolecular ensembles. ■

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