Obey the Peptide Assembly Rules

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a-factor (*6*) (see the figure). Such a channel could be a resident membrane protein of the pre-autophagosomal organelle (or MVB) or be delivered to that structure from some other membrane during the maturation of the autophagosome. An analogous channel has been proposed to promote the entry of chaperone-assisted autophagy cargo into the lysosome (*19*). In this model, cargoes reside in the intermembrane space between the outer and inner membrane (exosomal membranes) of the autophagosome (or MVB). Imported cargo would then be discharged in soluble form to the cell exterior concomitant with organelle fusion at the cell surface.

Two key questions must be answered to address the alternative models for unconventional secretion. One is whether the leaderless cargoes within an autophagosome or amphisome/autolysosome are conveyed directly to the cell surface. A temporal precursor-product relationship between autophagosome-bound and secreted material has not been established. It remains possible that the effect of mutations in the autophagy-related genes (Atg) or Atg protein depletion on secretion is indirect; for example, the autophagic organelle may not carry secretory cargo but instead traffic the machinery for direct translocation of unconventional cargo from the cytosol through the plasma membrane. In the case of a direct role, there is a question about the topologic location of the leaderless cargo within the autophagosome or subsequently in the amphisome/autolysosome, which must be established by visualization or biochemical fractionation. Both tests require knowledge of the origin of the autophagosome membrane, and specifically the structure responsible for unconventional secretion.

Although a unified picture of the pathways and mechanism of unconventional secretion remain elusive, the existence of this process now seems fairly secure. In the future, the application of genetic and biochemical analysis should prove crucial in identifying the essential machineries for unconventional secretion.

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MATERIALS SCIENCE

Obey the Peptide Assembly Rules

Application of simple design rules enables controlled assembly of discrete peptide nanostructures.

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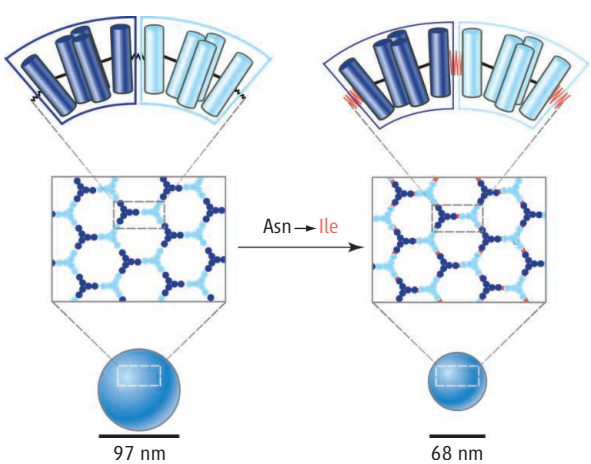
The complex properties of systems as diverse as ant colonies and stock markets emerge from the interplay of many components interacting through a small number of simple rules (*1*). Establishing the interaction rules not only helps to understand the emergence of the larger system; exploitation of the rules can provide rational control over the system itself. On page 595 of this issue, Fletcher *et al*. (*2*) describe the design of molecular components that assemble into enclosed, hollow nanostructures, the morphologies of which are defined by rules governing the interactions between the building blocks. Manipulation of these rules results in the controlled alteration of the the nanostructure's size (see the figure).

Using such rules, synthetic biology (*3*, *4*) aims to push natural biological systems in novel directions or to generate biomimetic systems with new properties. Synthetic attempts to construct biomimetic structures and systems often rely on engineering assemblies of DNA (*5*) and proteins or peptides (*6*) to adopt new structures with potentially novel functions. In living cells, proteins act as scaffolds for dynamic nanoassemblies essential for cellular structural integrity. Learning from protein noncovalent binding motifs, researchers have used short peptide structures to make self-assembled materials. The assembly properties of the peptides are governed by how their folding results in the projection of chemical functional groups into space.

Cyclic, β sheet, and α-helical peptides are among the protein-like fragments commonly used as building blocks for assembled materials ranging from gels to mesoscopic objects (*7*). Some of the first peptide nanotubes were developed in the 1990s based on the precise assembly of cyclic peptides made up of alternating D– and L–amino acids (*8*).

Fletcher *et al*. use three design rules to control the assembly of peptide-based building blocks into discrete morphologies. The building blocks consist of three "interaction helices" radiating from a central hub. The first rule involves noncovalent binding interactions designed to link the building blocks through heterodimeric coiled coils (*9*) formed by the interaction heli-



Emergent morphology. In Fletcher *et al.*'s study, discrete capsular morphologies emerge through implementation of three simple rules imposed on their peptide building blocks. Subtle manipulation of the helix-helix binding through a single amino acid change (Asn → Ile) causes a drastic change in nanostructure size.

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ces. Second, the three-fold symmetry of the building blocks is established by the central hub, which is made up of a three helix bundle to which the interaction helices are projected by means of a disulfide bond. Third, the wedge shape of the building blocks in cross section results from charge repulsion at the N-termini of the central hub and the interaction helices.

Coiled-coil binding between α-helices has been used in other assembling molecular systems. However, designs for nanoscale peptide assemblies often result in discrete structure in one or two dimensions but are polydisperse overall because they aggregate continuously in a third dimension. This lack of control over uniformity is often due to design principles that fail to take into account competing, but desired processes to limit assembly. For example, one design combines heterodimeric coiled coils with overhanging "sticky ends." These peptides assemble into extended fibers of variable length (*10*). The value of coiled-coil binding to Fletcher *et al*.'s design is underlined by the fact that a subtle single amino acid change at the molecular interface results in a shift to an alternate, but still discrete, structure (see the figure). Presumably the mutation enhances binding affinity between the building blocks, resulting in an increase in the rate of closure.

Establishing symmetry in building blocks, a strategy which is incorporated into Fletcher *et al*.'s second design rule, has previously been shown to be key for the generation of other closed, hollow nanostructures, albeit small ones, using designed proteins. For example, King *et al*. induced natively symmetric multicomponent proteins to form monodisperse, hollow protein cages by using the protein structures as hubs upon which protein-protein interactions between the hubs were grafted (*11*).

The wedge shape of Fletcher *et al*.'s building block is also an essential design rule. Not unlike the size of a lipid head group influencing the convex shape of a bilayer (*12*), or the angle of the keystone affecting the diameter of an architectural arch (*13*), the skew of the wedge shape defines the curvature of the resulting enclosed nanostructure. Equally important, the wedge shape provides a means to limit the assembly as it forms. The other two design rules allow symmetric assembly but could result in the generation of extended sheets of undefined size. Curving these sheets increases the likelihood that the high-energy edges of the sheets meet, thus generating closed and highly monodisperse structures.

The three simple design rules defining the interactions in Fletcher *et al*.'s system result in the formation of a robustly controlled morphology (see the figure). Because the system is so precisely defined from the molecular to the nanoscale level, it provides easily testable mechanistic hypotheses and predictions for the controlled manipulation of the rules to generate new morphologies (*14*). It could provide a robust model system to understand how nanoscale properties emerge from molecular components. Moreover, the structures could be used for protein delivery or as biomimetic mini-organelles to sequester and transport enzymatic activity.

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BIOCHEMISTRY

As Good as Chocolate

Structural details of how ligands bind to serotonin receptors should guide the development of pharmaceuticals with fewer side effects.

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No one could have imagined how important the 1948 discovery of the vasoconstrictor serotonin (5-hydroxytryptamine or 5-HT) would be to the field of human physiology (*1*). Elucidation of the 5-HT structure (*2*) and synthesis of the molecule with the expected biological activity (*3*) soon followed. This monoamine is a ligand for 15 receptors, and drugs that target 5-HT receptors are widely used to treat conditions including migraine headache, depression, anxiety, nausea, vomiting, and irritable bowel syndrome, reflecting the wide diversity of physiological and pathophysiological processes in which 5-HT is involved (*4*). On page 615 and 610 in this issue, Wacker *et al*. (*5*) and Wang *et al*. (*6*), respectively, report the crystal structure of human 5-HT_{2B} bound to the antimigraine agent ergotamine and compare it with the 5-HT_{1B}-ergotamine structure. Together with biochemical and computational data, these structures reveal molecular mechanisms responsible for divergent signaling patterns of ergotamine, serotonin, and the psychedelic drug lysergic acid diethylamide (LSD).

The structures were obtained by fusing either receptor to a thermally stabilized bacterial protein [apocytochrome b562RIL (BRIL)]. This approach stabilizes the receptor to promote crystallization but does not alter ligand-binding properties. The structural information, together with computational ligand-docking experiments, reveal similar binding modes for ergotamine, 5-HT, and LSD to the ligand-binding pocket formed by residues conserved in the 5-HT receptor family, thereby clarifying the family-wide agonist activity of 5-HT. However, there are some key differences between the two receptors (see the figure). In both structures, an accessory binding pocket adjacent to the binding site for the natural ligand (5-HT) can accommodate chemical groups located distal to the core indoleamine moiety in a differential manner, which possibly could control signaling. The 5-HT_{1B} receptor displays a 3 Å outward shift at the extracellular end of helix V relative to the 5-HT_{2B} receptor, resulting in a more open, extended pocket that explains receptor subtype selectivity for ligands.

5-HT receptor subtypes are classified according to their ligand-binding preferences, sequence homology, and signaling mechanisms. With the exception of the type 3 recep-

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