Much binding in the lab

Andrew D. Hamilton

Application of a concept drawn from two areas of macromolecular chemistry shows how artificial binding sites that resemble those found in globular proteins can be made.

Chemists have often looked with awe at the dazzling array of biochemical functions and chemical transformations in nature. So, if nature can do it, why can't we? Part of the answer is, of course, the many millions of years over which natural selection has had time to optimize an enzyme active site or a protein–DNA interface, compared to the usual term of a research grant or the four or five years available to PhD students. Synthetic chemists are, nonetheless, on the case, and a clever way of creating molecular-recognition systems is described by Zimmerman *et al.*[1] on page 399 of this issue. They have taken an important step in establishing a family of synthetic compounds that can be easily prepared and show recognition properties that bear comparison to those of antibodies.

The exquisite recognition of an antigen by its antibody is just one instance of the remarkable chemical control achieved in biology. Regulation of gene expression by transcription factors and the seemingly effortless execution of difficult reactions by enzymes are other examples. Yet these transformations are carried out with a very limited set of building-blocks — just four nucleotide bases and 20 amino acids. Nature can fashion the correct chemical microenvironments for binding a substrate or cleaving a bond by simply folding a string of amino acids, but synthetic chemists have yet to design and create an equivalent receptor or catalyst. There are essentially no examples of synthetic molecules that bind to an antigen with the strength or selectivity of an antibody, or that split the bonds between amino acids at physiological pH and temperature like the digestive enzyme trypsin. But although chemists don't have the time that nature has had, they do have a large array of synthetic reagents and potential building-block designs for constructing functional mimics of proteins or nucleotide chains.

Using entirely synthetic components, Zimmerman *et al.*[1] have created a globular molecule containing an interior recognition site that can bind to certain modified porphyrins and differentiate between closely related analogues. Porphyrins are a family of large organic molecules that occur naturally (for instance, as a component of haemoglobin) but are commonly used in synthetic chemistry. The key element in Zimmerman and colleagues' approach is the merging of two areas of macromolecular chemistry, dendrimers and molecularly imprinted polymers.

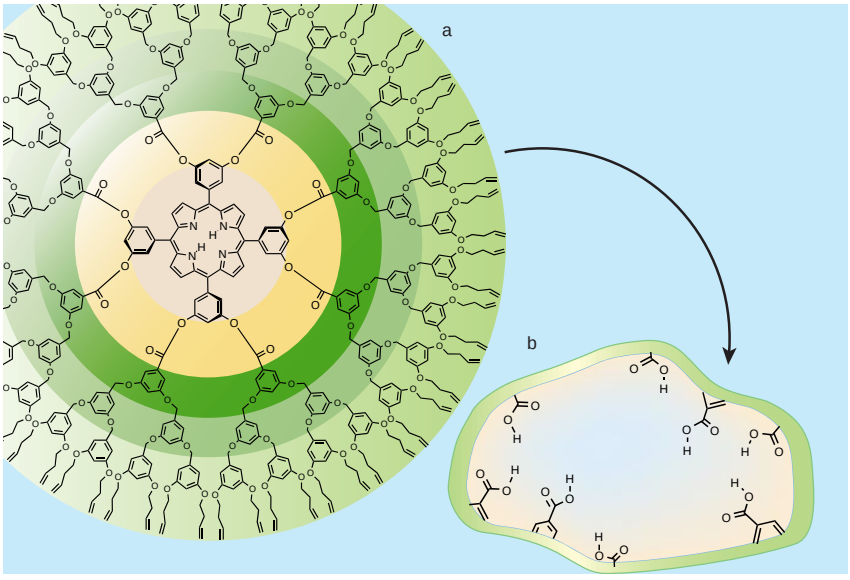

Figure 1 **The approach used by Zimmerman *et al.*[1] to create a synthetic globular structure enclosing a molecular recognition site.** a, **Dendrimer construction. The template porphyrin with its four phenyl groups (beige) is linked by ester bonds (yellow) to three generations of benzene derivatives (green). This is a two-dimensional depiction of a three-dimensional process, and with only partial representation of the benzene groups. The particular innovation described by Zimmerman *et al.* is crosslinking of terminal alkenes on the third generation, which stabilizes the structure and allows the central porphyrin to be removed by cleaving the ester bonds.** b, **The result is a globular molecule, containing a cavity of a specific size and shape, lined by eight carboxylic-acid groups, that serves as a well-defined binding pocket for other modified porphyrins. (Figure based on Fig. 1 of ref. 1.)**

Dendrimers have long been seen as a discrete branch of polymer chemistry, where the macromolecule is grown out from a central core to create a roughly spherical compound with a single molecular weight[2]. But dendrimers suffer from their design. They tend to be unduly flexible and have little interior space in which to form an active site with well-positioned binding groups. On the other hand, molecularly imprinted polymers often contain effective binding pockets within the confines of a stable polymer network. The pockets are formed by carrying out the polymerization around a molecular template which is then removed from the matrix[3]. The resulting cavity possesses the shape (and complementary chemical characteristics) of the template and can be used to bind related chemical structures. But molecularly imprinted polymers can be limited by the chemical heterogeneity of the polymer in terms of size and binding-site structure, as well as restricted solubility.

Zimmerman *et al.*[1] have designed a single molecular template from which they construct a dendrimer by attaching functional groups to create a divergent framework. The template is a porphyrin with four phenyl groups, each of which bears two hydroxyl groups (Fig. 1a). All eight hydroxyls are linked through easily cleaved ester bonds to a wedge-shaped dendrimer segment composed of three generations of benzene derivatives[4]. The third-generation component contains terminal alkenes. The result is a classical, almost spherical dendrimer with 64 alkene groups on its surface.

The significance of the alkenes is that they contain carbon–carbon double bonds, which can be broken and re-formed as new double bonds between neighbouring alkene groups. It is here that Zimmerman and colleagues' approach comes into its own. Using a catalyst — Grubbs' olefin metathesis catalyst[5] — the peripheral alkenes are crosslinked to stabilize the shape of the dendrimer that

has been formed around the porphyrin template. Analysis with nuclear magnetic resonance, mass spectrometry and chromatography confirmed that virtually every alkene had reacted with a neighbour. The whole superstructure is then sufficiently stable to allow the porphyrin template to be removed by cleaving the ester bonds. This leaves behind a cavity, lined with eight carboxylic-acid groups, within the interior of the globular molecule (Fig. 1b). The size and shape of the cavity betray its origins from the porphyrin template.

The authors show that the dendrimer host binds strongly to a test substrate — a porphyrin with four pyrimidine groups, with a total of eight basic nitrogen atoms in appropriate positions to form hydrogen bonds to the carboxylic-acid groups in the cavity. There is subtlety in the recognition, suggesting a well-defined binding pocket. The original porphyrin template with its eight hydroxyls does not itself bind because the combined sizes of a carboxylic acid and a hydroxyl group make too tight a fit.

However, the design is not perfect. There is flexibility in the dendrimer host structure that leads to intramolecular hydrogen bonds between carboxylic acids in the binding pocket. This results in an affinity that is similar for substrates irrespective of whether four or eight hydrogen bonds are formed. Also, the lack of discrimination among different isomers of a tetrapyridyl porphyrin suggests that the carboxylic-acid groups can move within the binding cavity and contact the nitrogen atoms in different positions.

Nonetheless, the overall concept outlined by Zimmerman *et al.* is a promising one. The idea of using a substrate (or at least a close analogue) as the template for the synthesis of its own host will find many future applications, for example in drug delivery and catalyst design, and in devising novel separation strategies. Nature still has an edge in terms of affinity and selectivity, but Zimmerman and colleagues' approach will now permit the construction of artificial binding sites that resemble those in globular proteins. ■

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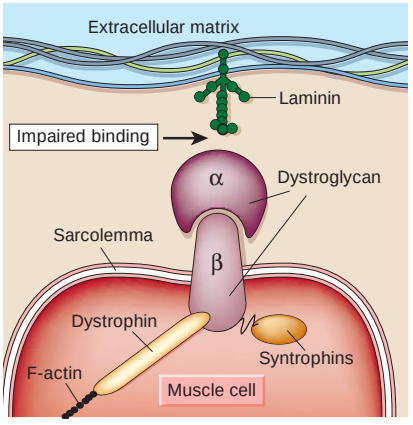

Figure 1 **Congenital muscular dystrophy and α-dystroglycan[3]. In muscle, dystrophin binds both F-actin in the cytoskeleton and the 'dystrophin glycoprotein complex', which includes dystroglycan. α-dystroglycan is a secreted component that lies outside the muscle cell. To function properly, it must be glycosylated — have sugar groups attached — and bind both β-dystroglycan in the cell's membrane, the sarcolemma, and proteins in the extracellular matrix such as laminin. Failure of glycosylation impairs binding to the extracellular matrix, destroying the muscle fibre over time.**

Neurobiology

Full circle to cobbled brain

M. Elizabeth Ross

A biochemical link between certain congenital muscular dystrophies and the associated brain malformation known as cobblestone lissencephaly has been elusive. But it looks as if that link has been found.

Muscular dystrophies are genetic diseases that cause progressive muscle weakness. The best known is that described by Duchenne, which affects boys and is evident from about five years of age, and which results from mutations in the gene encoding a protein called dystrophin. Another subclass is the congenital muscular dystrophies, where muscle weakness is apparent at birth or shortly afterwards. Two of these for which gene mutations have been found are muscle–eye–brain disease (MEB) and Fukuyama congenital muscular dystrophy (FCMD). Children carrying the faulty MEB or FCMD genes[1,2] suffer from both muscle weakness and 'cobblestone lissencephaly', in which a flaw in neuronal migration results in a brain with a bumpy, cobblestone appearance and loss of the normal folding pattern.

How the two very different muscle and brain defects arise in the same patient has not been known. Now, however, companion papers by Michele *et al.*[3] and Moore *et al.*[4] (pages 417 and 422 of this issue) describe an impressive array of data that points to a common mechanism. To function properly in muscle, dystrophin has to form complexes that include two components, α and β, of another protein, dystroglycan. Each of these has to be appropriately modified by glycosylation — the addition of sugar molecules by glycotransferase enzymes. The β-dystroglycan in the membranous sheath of a muscle cell, the sarcolemma, binds α-dystroglycan; in turn, α-dystroglycan binds to proteins such as laminin in the extracellular matrix. The two papers provide evidence that the defect underlying muscle weakness and brain abnormalities in both MEB and FCMD is disrupted glycosylation of α-dystroglycan (Figs 1 and 2).

The MEB and FCMD genes both have similarity to genes known to encode glycosyltransferases, although it was unclear which substrates of these enzymes are relevant to the congenital dystrophies. Likely candidates, however, lie in the dystrophin–dystroglycan complex. Mutations in dystroglycan have not hitherto been associated with human disease. But a 'knockout' of dystroglycan in mice proves lethal at the embryo stage. So there have been hints that other proteins cannot substitute for dystroglycan, and that even reduced dystroglycan activity might underlie the human disorders.

Michele *et al.*[3] now provide evidence that reduced (hypo) glycosylation of α-dystroglycan is involved in the two diseases, as well as in a naturally occurring mouse mutant, the myodystrophy (*myd*) mouse. Muscle biopsies from patients with MEB and FCMD revealed normal patterns of β-dystroglycan but no glycosylated α-dystroglycan. In electrophoresis, the core α-dystroglycan protein showed a shift in mobility, interpreted as a change in apparent molecular weight due to loss of sugar groups. Furthermore, the change in mobility of the α-dystroglycan component was identical for the MEB and FCMD samples, implying that the different glycosyltransferases mutated in these diseases affect the same sugar residues on α-dystroglycan.

The authors further show that the hypoglycosylated α-dystroglycan from patients was impaired in binding proteins such as laminin, agrin and neurexin — all of which are components of basement membrane, the specialized sheet of extracellular matrix that surrounds muscle and other cells. Similar biochemical abnormalities were evident in both the muscle and brain of a *myd* mouse with a mutation in a gene — the *LARGE* gene — which again is thought to encode a glycosyltransferase[5]. Finally, Michele *et al.* find that defects in neuronal migration in the *myd* mouse brain are like those seen in MEB and FCMD patients.

In sum, Michele *et al.*[3] show that muta-