Creating a good impression

By the imprinting of a molecular memory in their core, dendrimers can be tailored to bind to defined molecular targets in a selective and reversible fashion.

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Like a hand in a glove, specialized structures such as antibodies, hormone receptors, and enzymes fit perfectly with their natural targets. Such macromolecules are, therefore, invaluable in biotechnology, medicine, and analytic chemistry. However, although "nature's own," such structures are far from perfect "tools"—they are unstable out of their native environment and often low in abundance, and a natural receptor for the particular molecule of interest may not exist. Researchers have long dreamed of building such structures *de novo*: creating tailor-made receptors for the desired molecular target in bulk. One surprisingly simple way of generating artificial macromolecular receptors is through the molecular imprinting of synthetic polymers. Now, in a recent publication in *Nature*[1], Zimmermann and colleagues have made an exciting new contribution to this field: they created a molecular impression in the core of a dendrimer, obtaining a soluble artificial receptor the size of a small protein.

In molecular imprinting, a target molecule (or a derivative thereof) acts as the template around which interacting and crosslinking monomers are arranged and co-polymerized to form a cast-like shell (Fig. 1). Initially, the monomers form a complex with the template through covalent or noncovalent interactions. After polymerization and removal of the template, binding sites complementary to the target molecule in size, shape, and position of functional groups are exposed and their confirmation is preserved by the crosslinked structure. In essence, a molecular memory is imprinted on the polymer, which is now capable of selectively rebinding the target. Thus, molecularly imprinted polymers (MIPs) possess two of the most important features of biological receptors—the ability to recognize and bind specific target molecules.

Nevertheless, MIPs do differ from biological receptors: MIPs are large, rigid, and insoluble macromolecules, whereas their natural counterparts are smaller, flexible, and in most instances soluble. Depending on their size, MIPs can bear thousands or millions of binding sites per molecule, whereas biological receptors have a few binding sites, or just one. Moreover, the population of binding sites in MIPs, especially those of noncovalently imprinted polymers, is heterogeneous because of the influence of the equilibria that govern the template–monomer complex formation, and the dynamic of the growing polymer chains before co-polymerization. Although not always problematic, these characteristics can prevent MIPs from being substituted for natural receptors in certain applications.

Now, Zimmermann and colleagues describe a system that avoids some of these potential shortcomings[1]. Their strategy is to make the molecular imprint inside dendrimers—macromolecules of highly regular structure consisting of a polyfunctional central core covalently linked to layers of repeating units (so-called generations). In this study, the template molecule and core used was the porphyrine derivative tetrakis-meso(3,5-dihydroxyphenyl)-porphyrine, to which eight third-generation dendrons were covalently attached through ester links. The outer layer of the dendrons was composed of homoallyl end groups, and thanks to these extremities, the outer "shell" of the dendrimer could be crosslinked or "polymerized" intramolecularly. Finally, hydrolytic removal of the porphyrine template liberated the binding sites, which then contained eight precisely positioned carboxyl groups. This imprinted dendrimer was shown to selectively rebind structural analogs of the template, for example the isomeric compound tetrakis-meso(2,6-dihydroxyphenyl)-porphyrine, although for spatial reasons not the template itself.

Have Zimmermann and colleagues made an imprinter's dream come true? At first glance, the answer seems to be yes: the researchers produced a low-molecular-mass MIP (~10^4 Da), that is soluble in common organic solvents and has only one well-defined, and apparently readily accessible, binding site for the template per MIP molecule. The team postulates that binding of the target by the MIP involves a dynamic "breathing process," indicating that the MIP has some flexibility in its structure. Indeed, accessibility is an advantage of these imprinted dendrimers, allowing an easy and complete removal of the template that is not always possible with traditional MIPs because of their highly crosslinked and compact structure.

First employed by Wulff[2], covalently imprinted complexes are more stable and structurally better defined than noncovalent MIPs. The resulting material therefore contains a population of binding sites with a more or less homogeneous affinity for their targets. Zimmermann and colleagues took covalently generated MIPs one step further—producing MIPs with just one binding site per molecule. The originality of their system lies in how they generated the imprinted molecules: they presynthesized all parts of the molecules, assembled them with the help of the template, and stabilized the whole by crosslinking the outer shell. Although they relied on covalent bonding during the imprinting process, rebinding of the target molecule by the MIP occurs through noncovalent interactions. Whitcombe and colleagues used a similar strategy to imprint a peptide using monomers covalently linked to the target molecule via short sacrificial spacers[3]. This creates space in

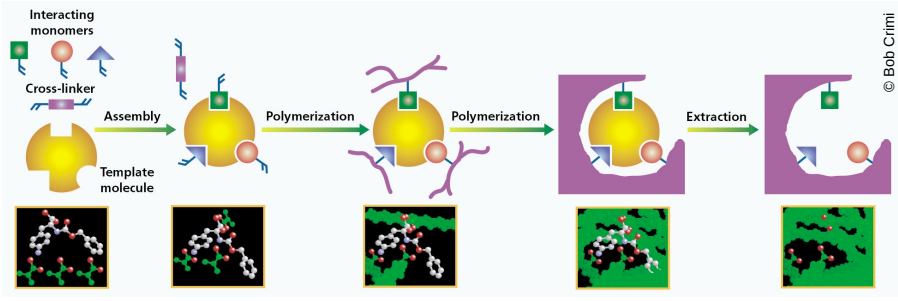

Figure 1. Creating a molecular imprint using a synthetic polymer. The interacting monomers, crosslinker, and template molecule are mixed together (left). The interacting monomers assemble into a complex with the template molecule (here through noncovalent interactions). Polymerization is initiated, and the interacting monomers co-polymerize with the crosslinker, forming soluble polymer chains. As polymerization proceeds, an insoluble, highly crosslinked polymeric network is formed around the template. Removal of the template then liberates complementary binding sites. Inset panel, the different steps of the process are illustrated for the amino-acid derivative Cbz-*p*-aminophenylalanine as the template and methacrylic acid as the interacting monomer.

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the binding site that, after the template is removed, allows accommodation of the original target molecule though noncovalent interactions, resulting in a stable imprinting complex and fast rebinding of the target.

However, the imprinting of polymers using covalent complexes—including this new dendrimer system—involves a substantial amount of time-consuming organic chemistry, and is limited to targets to which monomers can be readily attached. By comparison, noncovalent imprinting, a concept pioneered by Mosbach[4], seems much more straightforward: mix together target and suitable monomers, initiate polymerization, and let the MIP grow itself. This often works, but the noncovalent imprinting approach is not as easy as it sounds. A closer look at the recent literature suggests that a substantial amount of work is devoted to improving noncovalently imprinted polymers. For example, new monomers are being designed that can form stronger noncovalent interactions with targets. Other researchers propose to rely on combinatorial methods[5] or molecular modeling[6] to find the optimal combination of monomers for noncovalent imprinting of a given target. Wulff and coworkers have recently attempted to obtain MIPs of the same size as proteins, by synthesizing imprinted microgels with molecular masses of 10^4–10^5 Da (ref. 7).

Industry is currently evaluating the potential application of and commercial opportunities for MIPs, and here, proof of principle is not the only criteria for future investment. Companies need to investigate the selectivity of MIPs for their targets, and their compatibility with the environment in which they are to be used, including biological fluids and tissues. Criteria such as the ready integration of molecular imprinting within existing industrial fabrication processes, yields, cost, and the competitiveness of MIPs with existing affinity materials also need to be examined.

The day when MIPs were only used for the separation of isomers is long past. After Mosbach *et al.*[8] described the use of MIPs as antibody mimics in immunoassays, the number of publications in the area rose exponentially. Today, the main opportunities for the technology are in analytical chemistry[9], but interest is growing in the biomedical field for the use of MIPs, for example, in drug discovery or as therapeutics themselves. For example, Mosbach's group has recently described synthesizing new enzyme inhibitors by assembling and interconnecting different building blocks at the binding sites of a MIP imprinted with a known inhibitor of the enzyme[10].

For many of potential MIP applications, it is highly desirable, if not essential, for the MIPs to have well-defined binding sites. Zimmermann and colleagues' imprinted dendrimers could be used in many of the traditional applications of MIPs, either in soluble form, immobilized to a support, or perhaps tagged with a marker. Several outstanding questions about this technology remain to be answered: is the new method flexible enough to be used with different kinds of templates, including those that are non-symmetrical? Can they be made water soluble? Can stable noncovalent complexes be imprinted in the same manner? Whatever the answers, one thing is for sure: these imprinted dendrimers are an impressive piece of supramolecular chemistry.

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