

Introduction

Molecularly imprinted polymers (MIPs) are polymers that have been prepared by polymerizing an either pre-formed or self-assembled monomer-template complex together with a crosslinking monomer. After removal of the template molecule, a polymer with binding sites for the template is obtained (see figure 1). Although MIPs can be highly selective and comparable to antibodies,¹ the binding sites often have a degree of flexibility in their recognition properties and the MIP will not only bind the template molecule, but also other similar molecules.

This flexibility can be utilized in different ways:

- **Template analogues.** Frequently, it is found that it is not possible to completely remove the template from the MIP and that traces of template will 'bleed' from the MIP, which causes problems in analytical applications. One way to solve this problem is to use an analogue of the target molecule as the template instead of the target molecule itself. Thanks to the flexibility of the binding sites, the MIP will still bind the intended target molecule.

- **Class-selective MIPs.** A degree of flexibility in the recognition properties of the MIP can be very useful as it may lead to a MIP that shows class-selectivity. MIPs that can recognize entire classes of molecules are often more useful than MIPs that only recognize a single compound.

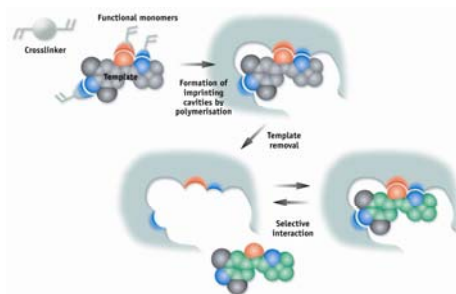


Figure 1. Overview of the preparation of a MIP.

Cross-reactivity of MIPs

The imprinted sites may sometimes also recognize molecules that differ more significantly from the template molecule used for imprinting. Two examples from the literature:

1) Haupt et al² imprinted 2,4-dichlorophenoxyacetic acid and were able to use the non-related molecule 7-carboxymethoxy-4-methylcoumarine as a fluorescent probe (Figure 2).

2) Martin et al³ imprinted the beta antagonist propranolol and found that the MIP also bound tamoxifen, a compound used in the treatment of breast cancer (Figure 3).

Even though the recognized molecule is different from the template in these two cases, they have common features (marked in yellow in figures 2 and 3). 2,4-Dichlorophenoxyacetic acid and 7-carboxymethoxy-4-methylcoumarine have large portions in common, and even though propranolol and tamoxifen differ more, both contain the O-CH₂-CH₂-NH moiety. The role of this moiety in recognition was not investigated, but it is seems likely that it was involved.

These findings suggest that MIPs should have a broader selectivity than just for the template molecule and closely related analogues, and that instead it is the placement of a few key recognition elements that matter. This is analogous to the concept of 'pharmacophores' in medicinal chemistry, where different molecules can have the same function as long as they contain the pharmacophore that is recognized by the natural receptor.

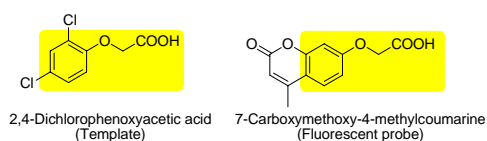


Figure 2. Template and bound molecule in the work of Haupt et al.²

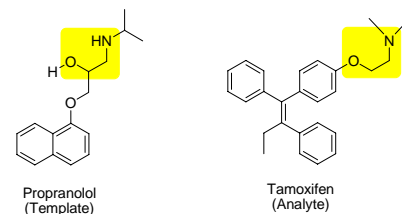


Figure 3. Template and bound molecule in the work of Martin et al.³

Exploring the cross-reactivity of MIPs through screening

We decided to start screening our existing in-house MIPs for interesting selectivity. The MIPs were categorized into sub-libraries based on the functional monomer used and were screened against analytes of commercial and scientific interest. The screening process involves passing a solution of the analyte through SPE columns on a 96-well plate and measuring the percentage of bound analyte. The results of some experiments are shown in figure 4.

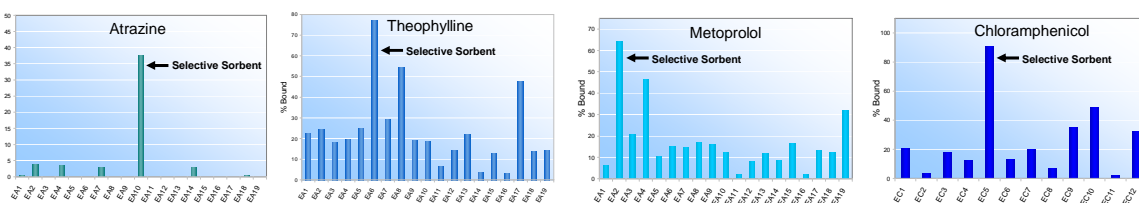


Figure 4. Results from screening experiments with atrazine, theophylline, metoprolol and chloramphenicol on MIP libraries.

In these four cases, MIPs with selectivity for the analyte were found. Atrazine was included as a control and the MIP that binds it was actually designed for this type of compound, but the other 'hits' are with MIPs that were prepared with unrelated templates. In some cases, this cross-reactivity can be explained. For example, theophylline binds to MIP EA6 which was imprinted against nicotinamide. If theophylline and nicotinamide are placed next to each other (Figure 5), one can see that the best hydrogen-bond acceptors in theophylline match the best hydrogen-bond donors in nicotinamide, and so it is not so surprising that imprinting with nicotinamide as the template leads to binding sites where also theophylline can bind. In other cases, the cross-reactivity is more difficult to explain.

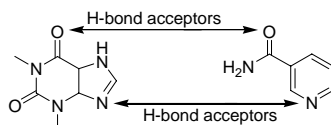


Figure 5. Comparison between theophylline (left) and nicotinamide (right).

Conclusions

MIPs exhibit a degree of cross-reactivity that can be utilized and MIPs with suitable selectivity can be found through the screening of MIP libraries against the target molecule. The 'hit' MIPs can then be used for developing an analytical method based on that MIP or as a starting point for further MIP development. As our MIP libraries continue to grow, we expect to find more and more MIPs with interesting cross-reactivity.

References

1. Vlatakis, G.; Andersson, L. I.; Müller, R.; Mosbach, K. *Nature* **1993**, *361*, 645-647.
2. Martin, P. D.; Wilson, T. D.; Wilson, I. D.; Jones, G. R. *Analyst* **2001**, *126*, 757-759.
3. Haupt, K.; Mayes, A. G.; Mosbach, K. *Anal. Chem.* **1998**, *70*, 3936-3939.

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