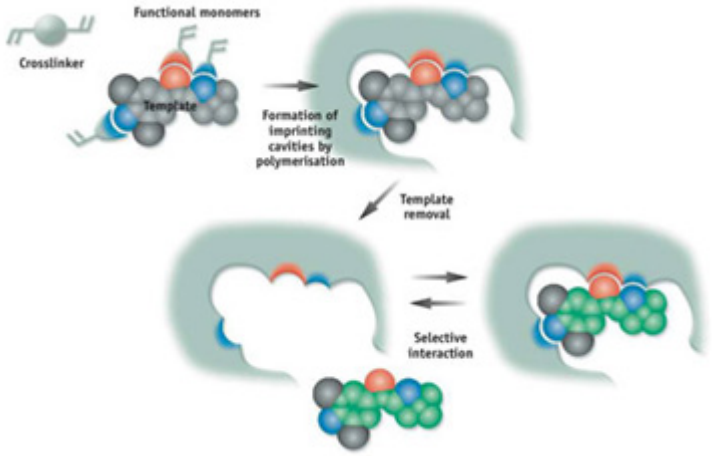
Molecularly Imprinted Polymers (MIPs)

MIPs are engineered cross-linked polymers that can exhibit high affinity and selectivity towards a single compound or they can be designed to exhibit 'Class Selectivity' for a family of related compounds. MIPs are able to bind analytes even when these are present in complex matrices (e.g. plasma, urine, muscle tissue, food matrices, environmental samples, process solutions etc). An important strength of MIPs is that they are able to bind to trace levels of the target analyte, in the presence of large excess of other compounds that have similar physico-chemical properties.



How do they behave?

Binding

Unlike most separation particles that exhibit only non-selective interactions, MIP particles have a selective synthetic recognition site (or imprint), which is sterically and chemically complementary to a particular analyte or class of analytes. The interactions mimic antibody or receptor binding and are stronger than interactions obtained with conventional separation materials. A particularly interesting characteristic of MIPs is their ability to achieve high recoveries at low analyte concentrations (see Table 1 below where extraction data for the AFFINILUTE MIP - Clenbuterol are taken from -Shimelis, O., Aurand, C., and Trinh, A., Reporter 25.2). This may be attributed to the spectrum of affinity sites within a given MIP polymer where typically there is a low concentration of high affinity sites.

	% Recovery of Clenbuterol from Urine	
Spike Level (ng/mL)	AFFINILUTE™ MIP – Clenbuterol	Mixed Mode Polymer (market leader)
0.1	99%	8%
0.5>	75%	66%
1.0	75%	69%

For further information on affinity distributions in MIPs, see Stanley et al (2003) Langmuir, 19, 772-778

Economics and Stability

MIPs are economical and fast to produce and are robust and stable under storage. They can be

used at elevated temperatures, in organic solvents and at extreme pH values. They also display a higher sample load capacity for small molecules than is typical for immuno-affinity based sorbents. This results in higher recoveries for analytical applications and suitability of using the sorbents for semi-preparative or preparative scale separations.

Material Design

In trace analysis applications, the level of compounds that may leach out from the MIP are extremely low, due to efficient washing procedures and do not interfere with the quantification of the target analyte. Template molecules used are usually analogs that differ sufficiently from the target analytes to avoid co-elution problems. Typically, any leachable compound from the MIP preparation is removed (around 99 %) and is normally not detected during analysis.
Biotage has extensive experience in designing the polymer components, that include specialized monomers and optimized templates which may be isosteric or isoelectronic analogues of the desired analytes. Our ‘Rule of six’ for analytical applications has been developed after many years of experience (see below).

The Analyte

MIPs can be routinely prepared for a variety of low-molecular weight compounds, with molecular weights up to about 3000 Da. Larger target analytes can also be targeted, such as peptides, carbohydrates and proteins by making use of accessible epitopes.

The MIP ‘Rule of Six’

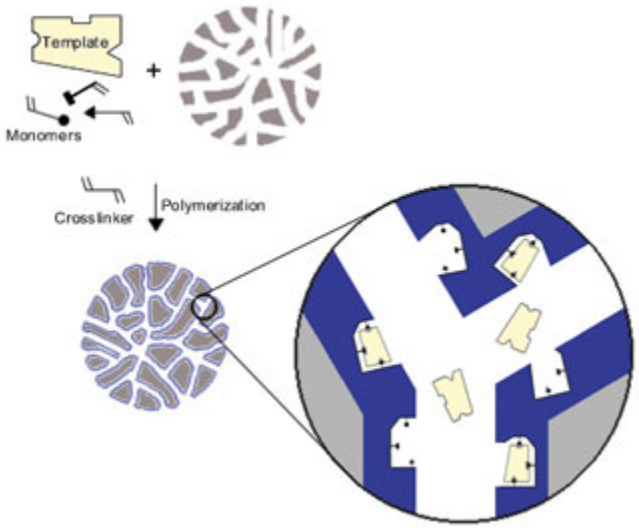
- Never use the analyte as a template unless there is absolutely no alternative
- Make rational choices about which regions of an analyte are likely to command the best types of interaction in a low dielectric medium (organic solvent) and then incorporate these elements in an analog of the analyte molecule
- Select monomers that are likely to form strong interactions in the chosen solvent (e.g., Brönsted acids or bases/H-donors or acceptors/nonpolar groups, etc.) - this will increase capacity and influence homogeneity of the binding cavities
- Choose templates and monomers that will be soluble in the porogenic solvent to be used in the polymerization - this may seem obvious but it sometimes requires carrying out solubility tests
- Ensure as far as possible that the template-monomer mixture is stable and does not undergo side reactions under the polymerization conditions
- Consider the nature of the matrix from which the analyte will eventually be extracted when selecting the cross-linking monomer - a range of di- or tri-unsaturated cross-linking monomers (e.g., vinylic, acrylic, methacrylic, acrylamide, etc.) with varying chemistries are available to create the porous organic network material.

Methodology

High Performance Molecular Imprinting

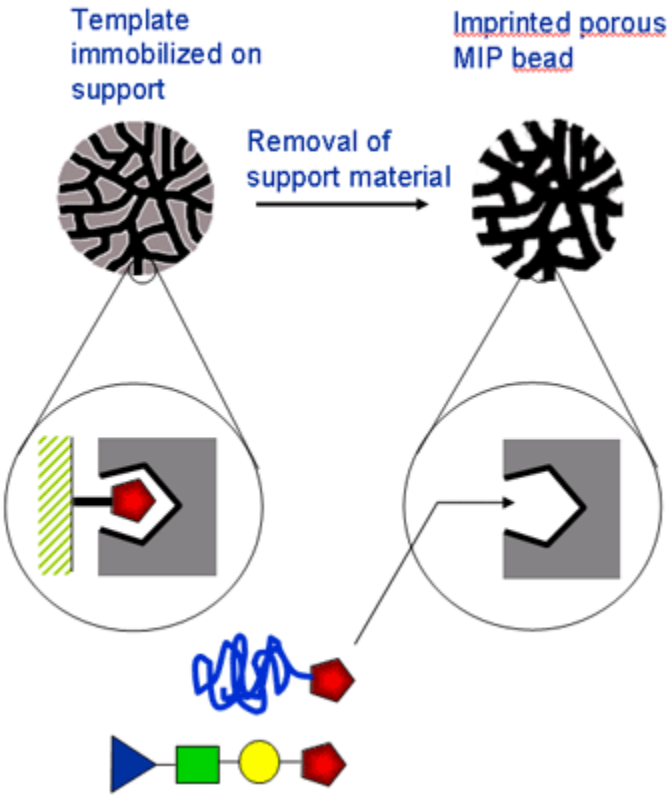
Thin-layer MIPs give higher surface areas and improved mass transfer.

With its new proprietary technology known as ‘Grafting’, in which MIPs are directed to the surface of porous silica particles, Biotage can produce solid phase materials that have large surface areas (up to 1000m2 per gram) and improved mass transfer properties. This is particularly important where separation of similar molecules (eg enantiomers) is required or fast kinetic resolution is desirable.

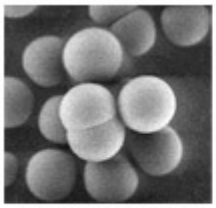


Surface Imprinted MIPs are suitable for larger molecules

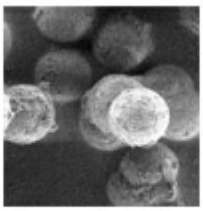
Biotage holds patents in the area of hierarchical imprinting, where the template is immobilized onto a support material during polymerization, whereafter the support is removed. The thereby polymers obtained now have binding sites that are highly accessible to molecules that are much larger than the imprinted template. This is particularly relevant to epitope imprinting of proteins and similar applications. As depicted in the figure, a fragment of the target is sufficient to form a specific cavity into which the whole molecule (eg. protein or peptide) can than bind.



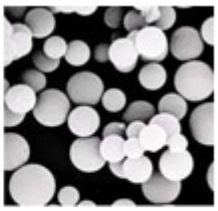
Grafting



Micrograph of naked silica particles



Micrograph of silica particles coated with a grafted MIP



SEM picture of imprinted porous MIP beads

Applications

Analytical scale

Solid-phase extraction (SPE) for trace analysis from complex matrices.
AFFINILUTE MIP products, developed and produced by Biotage are currently available for specific analysis of chloramphenicol, tobacco specific nitrosamine derivatives, NNAL, triazines, clenbuterol, ß-agonists and ß-blockers. These products serve control, analytical or contract research laboratories operating in the following sectors:

- Pharma
- Food & beverage
- Forensic
- Doping control
- Health
- Veterinary
- Environmental

Process Scale

Biotage's process materials can be engineered for a large variety of molecules including:

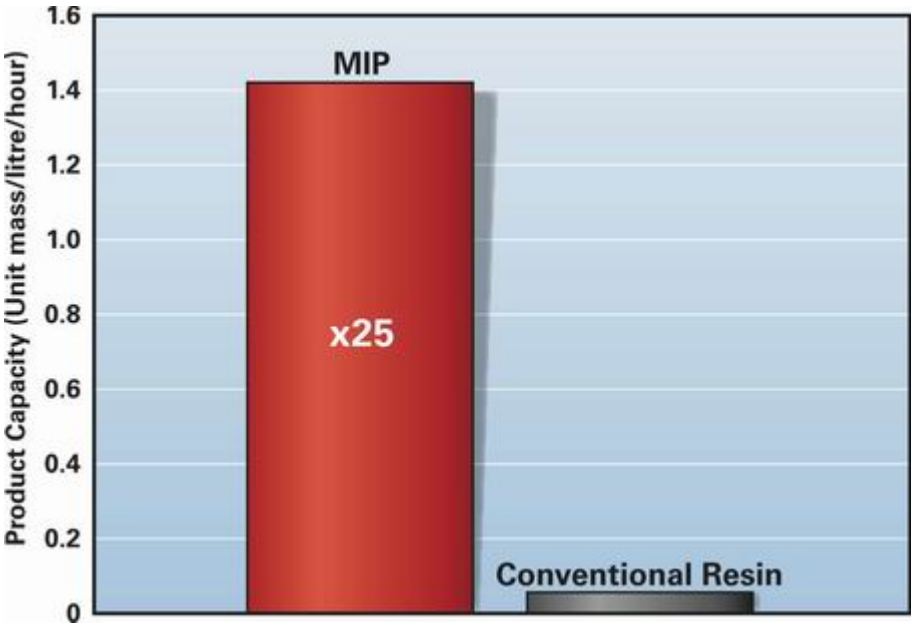
- Small molecules
- Chiral compounds
- Proteins (via epitope imprinting)
- Peptides
- Carbohydrates

Selectivity towards one specific molecule or a class of related molecules can be designed into the MIP phase.
Example of application areas:

- Pharmaceuticals
- Foods
- Chemicals
- Consumer products
- Environmental clean-up

Biotage process materials introduce selectivity into the separation/extraction process. It is possible to extract unwanted chemical compounds from complex mixtures, leading to a significantly increased productivity by decreasing the number of purification cycles which in turn leads to a more cost efficient process.

Increased Productivity by Molecularly Imprinted Polymers



The diagram shows a 25 times higher product capacity in unit mass/liter per hour using a MIP phase compared with a conventional resin.

ExploraSep is particularly advantageous in the intermediate and/or polishing steps of the purification cycle, where resolution is an important factor. As the diagram shows, MIPs can dramatically increase the productivity of a process due to their selectivity compared with conventional resins.