

Molecularly Imprinted Polymers

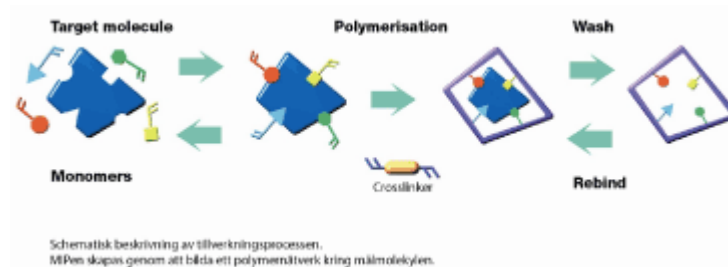
Molecularly Imprinted Polymers (MIPs) are used to specifically bind and enrich medium and small sized molecules. They can be used to detect a wide variety of compounds and most molecules having molecular weights below 5 kDa are suitable for imprinting. One can either design a specific MIP for each molecule of interest, or one can design a single imprinted polymer for a whole family of compounds.

At present MIPs are used mainly for purification and/or enrichment of target compounds in chromatographic columns. When integrated into a sensing system they can be used as chemically specific substrates to enhance sensitivity and specificity of detection by selective binding of dedicated targets. Few forthcoming applications of MIPs in sensorics include detection of pharmaceutical waste, of toxins, drugs, volatile mould products, etc., in water food or agriculture.

When the target molecule is absorbed by the molecularly imprinted polymer network and binds to the tailor made hollow spaces in the polymer, two properties of the polymer become often altered: its charge state and its volume. At Imego we evaluate different generic detection techniques for monitoring of the binding of different targets where the transduction aims at detecting either changes of volume or of the charge state of the imprinted polymer. Imego's strategy is to evaluate and optimize several detection schemes using model imprint systems. The goal is to identify the optimal detection technique using model analyte and built a demonstrator instrument able to perform outside the laboratory and then demonstrate the performance of this system for targets of commercial interest or of environmental importance.

Imego works closely together with a research group at Lund University with many years of experience and expertise in Molecularly Imprinted Polymer production. Researchers develop the MIP chemistry and produce MIPs either as nanoparticles or as thin films on sensor substrates provided and/or developed by Imego. Imego's expertise lies in the development of an appropriate sensoric system, with MIPs used as the absorbing material.

Principle of production



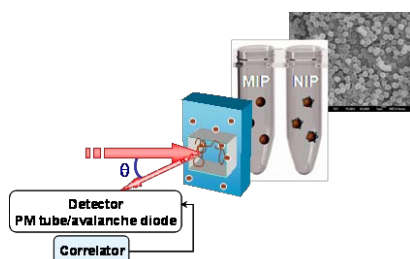
To achieve optimal molecular specificity the polymer has to contain target-specific monomers and have a well defined degree of cross linking. As rule-of-the-thumb the monomers are chosen to promote binding to at least two, preferably three, different functionalities on the target, depicted as blue object in the figure above. Too few binding sites on the target will decrease the specificity of the resulting imprint while too many bindings between the target and the imprinting site will inhibit target removal. Usually the monomer-target binding is non-covalent. It is determined mainly by electrostatic interaction and to some extent by hydrogen bonding and the hydrophobic interactions, respectively.

The degree of cross-linking determines two important parameters. First it governs the penetration speed of the target into an imprinted film or (nano- micro-) particles, i.e., the target diffusion constant. Secondly it influences the shape of the target specific cavity and the variation of the cavity geometry and its local surrounding. Both lead to spread of the target-imprint binding energies. This spread manifests itself via specificity variations – some sites perform better than other sites, i.e., the frequency of false molecule binding to a particular site varies.

At first the molecule of interest is mixed with functional monomers and is allowed to bind to different monomers (depicted by different colors in the figure above). A light or temperature sensitive cross-linking molecule is then added to a suspension. The illumination or heating of the mixture initiates polymerisation during which the polymer network is built up which locks the structure around the target molecule. The target molecules are then washed away, leaving hollow spaces in a sponge like polymeric network. The hollow spaces are able to encase and rebind the target molecule if encountered with for example a water sample containing the molecule. The rebinding has a specificity connected to both the geometrical structure of the hollow site and the chemically functional groups within the site.

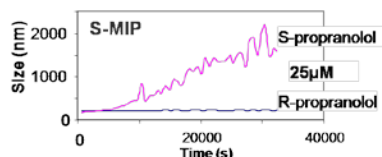
Sensing agglomeration of imprinted nanoparticles

The imprint-target binding is often electrostatic. When it occurs the charge of the imprinted polymer changes. Therefore the charge state of imprinted nanoparticles in colloidal suspensions changes which may lead to aggregation. Aggregation of imprinted polymer nanoparticles upon target binding can be detected using dynamic light scattering. The main advantage to use nanoparticles is the simplicity of the detection scheme. One mixes two suspensions: the one containing imprinted nanoparticles with the suspension to be investigated and monitors the progress of agglomeration, as in the figure below.



Figurtext *Dynamic light scattering has been used as a proof-of-the-principle for the possibility to detect agglomeration of imprinted nanospheres. If requested we shall adapt the laboratory instrumentation for the in-field use. This will significantly reduce the instrumentation costs.*

To ensure that the binding is due to a targeted molecule one determines the degree of non-specific binding by performing similar measurement but using the non-imprinted nanoparticles. An example of the results is shown in the figure below where the polymer nanoparticles imprinted against propranolol of left-hand-symmetry (S-propranolol) were used to distinguish between two chirally different propranolol species. The propranolol of right-hand-symmetry (R-propranolol) was used to show how specific this particular imprint is.



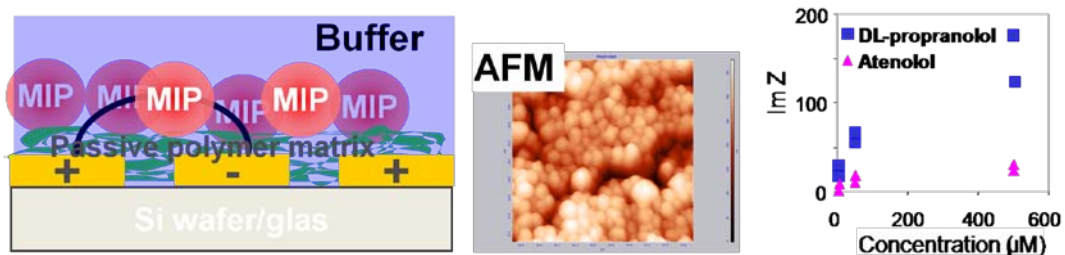
Figurtext *Aggregation of imprinted nanoparticles upon target adsorption. The nanoparticles imprinted against S-propranolol did not adsorb R-propranolol. The stereoselectivity can be used to monitor quality of stereo-separation during screening of drug candidates or it can be used to monitor presence of compounds of toxic chirality in foodstuff.*

We develop measurements of dynamic light scattering from the quantum dots surrounded by the imprinted shells (patent pending) using fluorescence correlation spectroscopy. Quantum dots will allow us to separate signal from the imprinted nanoparticles from the other particles in a strongly turbid suspension like water waste samples.

Measurement of the complex impedance of imprinted polymer films

Another detection scheme that probes changes of the charge state of imprinted polymer upon target binding involves measurements of the changes of complex impedance of the imprinted films that are anchored to (micro) electrodes.

The principle behind the method is schematically illustrated in the figure below where we assumed that imprinted nanoparticles constitute the imprinted film. The idea is to measure changes of the complex impedance of the film. The imaginary part of complex impedance is related to the capacitance of the film. The capacitance, in turn changes when the total charge accumulated in the film changes.

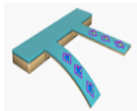


Figurtext *The schematic illustration of a film of imprinted nanoparticles (LHS) deposited onto the home-made interdigitated microelectrodes developed at Imego AB and AFM image of similar film. The RHS shows the changes of imaginary part of electrode impedance collected at 10kHz. Note large change of the signal with increasing propranolol concentrations and virtually no change in the signal upon exposure of the imprinted film to atenolol.*

The method is very sensitive but requires that the contribution from the film itself is singled out of the total signal that includes also the (parasitic) signal due to the buffer.

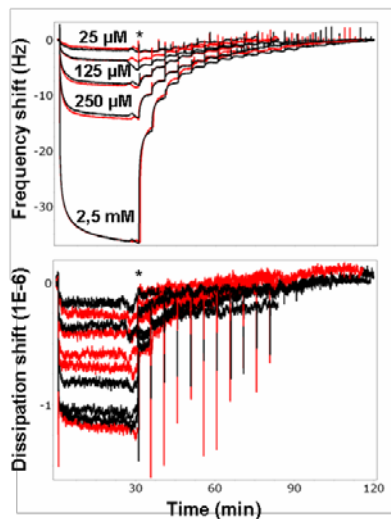
QCM-D measurements of the volume changes of imprinted films

It is well known that some MIPs tend to expand or shrink upon target absorption and their surface energy changes. These changes can be monitored using micro-cantilevers or gravimetrically using microbalances.



The cantilever with the deposited imprinted film will bend in proportion to the adsorbed analyte when the volume of the film changes. Changes in the beam curvature can be detected either optically or using strain gauges and can be related to the target uptake and the concentration of the target in the sample solution.

Figure below shows the results obtained using QCM-D (Quartz Crystal Microbalance with Dissipation) device to monitor volume changes of the film of nanoparticles deposited onto the electrode of the transducer.



Figurtext Apparent mass increase and the dissipation decrease measured using the QCM-D instrument from Q-Sense AB. The polymer film was imprinted against propranolol. Exposure to the buffer containing the target (propranolol) resulted in rapid adsorption of the molecule and stiffening of the film. Exchange of the buffer to an empty one resulted in full desorption of the target (emptying of the imprinted film. This sequence was repeated at least 10 times.