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# Molecularly Imprinted Polymers as Selective Receptors for Sensing Nanosized Species

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**Abstract**

Molecular imprinting into highly cross-linked polymers has attracted substantial interest for a wide range of analytes. Herein, we present two application examples of molecularly imprinted polymers (MIP) as artificial receptors for chemosensors: both analytes, namely high densitiy lipoprotein (HDL) and engineered gold nanoparticles, respectively, share their dimensions in the range of a few ten nm. Utilizing the resulting surface MIP as artificial receptors on quartz crystal microbalance (QCM) sensors leads to appreciable results. In both cases, MIP yield much higher sensor responses (up to an order of magnitude higher), than their non-imprinted counterparts. MIP sensors reveal dynamic sensor signals, for HDL in the physiologically interesting range. The two examples hence show the potential of molecular imprinting for designing receptor layers targeting analytes in the nanometer range.

**Key words:** Molecular imprinting, mass-sensitive sensors, biomimetic recognition, nanoparticle sensors, lipoprotein sensor.

## Introduction

Molecularly imprinted polymers attract increasing scientific interest and have found applications in such diverse areas as preconcentration, separation, chemical sensing, catalysis, and others [1]. The rationale behind their synthesis is outlined in Figure 1:

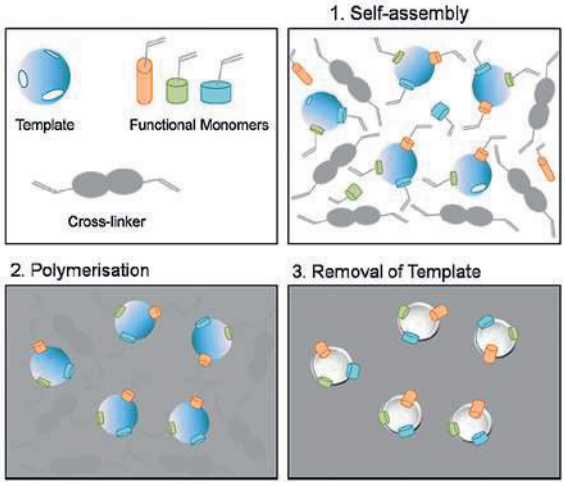


*Fig. 1. Principle of molecular imprinting. Reproduced with permission from [2]. © Elsevier B.V.*

Basically, the approach comprises polymerizing a highly cross-linked matrix in the presence of a template component. Pre-organization between this template and (functional) monomers predefines a non-covalent interaction network between functional groups of the polymer and the template. After hardening and removing said template, cavities remain in the matrix. They exactly fit the steric and functional properties of the respective template and are hence useful for re-incorporating those compounds in a selective manner.

Such recognition behavior has made MIP very interesting for the design of novel, highly selective chemical sensors, because they combine bio-analogous recognition abilities with the ruggedness and processability of man-made polymers. Hence, a wide variety of MIP sensor applications has been suggested so far, with analytes ranging from small molecules [2], to viruses [3] and bacteria [4]. Among these classes, nanometer-sized species deserve special attention, because to date hardly any rapid analysis techniques exist in this size range: Those species are too small to be accessible by light scattering techniques and usually also cannot be assessed by impedance measurements/impedance spectroscopy.

Within this paper we present two MIP strategies for such nanosized species, namely high density lipoprotein (HDL) and engineered nanoparticles. Whereas the former are potentially highly interesting for diagnostic purposes, the latter topic is of more general interest, especially in

the fields of environmental monitoring and food safety.

### High density lipoprotein MIP

HDL is one of several classes of cholesterol-based lipoproteins. Due to its involvement in atherosclerosis it serves, amongst others, as an important biomarker. It consists of a composite of cholesterol, lipids and proteins. In the blood stream, it is present as a globular structure with roughly 10 nm in radius [5]. While a sensor has already been reported for low density lipo-protein (LDL) [6] no comparable system exists for HDL. The reason most probably can be found in one of the main analytical challenges in this case: the concentration of HDL in physiological/clinical samples is usually lower (ideally >60 mg/dl, in clinical cases down to below 15 mg/dl compared to 25 to >200 mg/dl for LDL). Nonetheless, the polymers used to synthesize LDL MIP turned out suitable starting points for designing HDL MIP sensors. Again, a copolymer of methacrylic acid (MAA) and vinyl pyrrolidone (NVP) cross-linked with ethylene glycol dimethacrylate (EGDMA) as the cross linker turned out most useful. The optimized matrix brought sensitivity of respective MIP-coated quartz crystal microbalance (QCM) transducers down to the clinically relevant range. Such sensors were able to detect HDL concentrations well below 100 mg/dL. Sensor signals for samples containing 50 mg/dl and 12.5 mg/dl (see Fig. 2), respectively, are well above the noise level of the measurement, which in this case is in the range of a few Hz. The NIP shows anti-Sauerbrey behavior, which is to be observerd frequently with globular particles on flat surfaces. The beauty of the approach lies in the fact that in contrast to current clinical tests, the sensor proposed here detects HDL itself rather than assessing its amount via the HDL(C), i.e. cholesterol content. This is also interesting from the MIP point of view, because hardly any imprinting has been reported on biochemical aggregates so far.

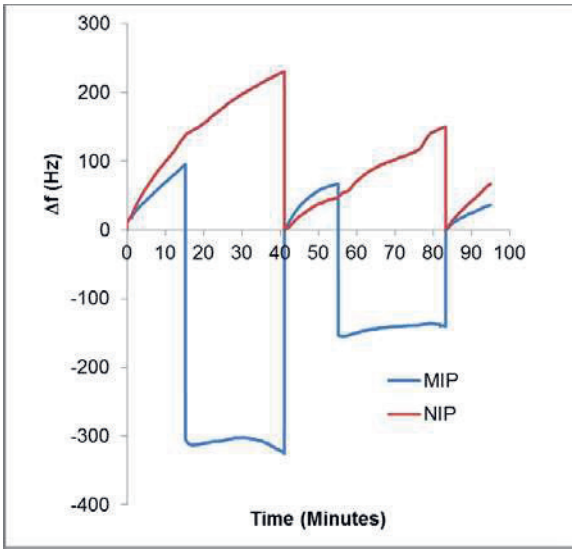


*Fig. 2. Sensor QCM sensor responses of HDL-MIP and respective NIP toward to HDL standards in PBS buffer (pH=7.4)*

### MIP for Engineered Nanoparticles

Nanoparticles are defined as objects of uniform size and shape in the region of 1 – 100 nm and have attracted steadily increasing attention during the last decade: due to their size, their physical properties (reactivity, surface energy etc.) more closely resemble those of surface atoms than those of bulk atoms. This leads to promising technological applications, among others in the fields of cosmetics/personal healthcare and food industry, respectively. However, engineered nanoparticles have come under close scrutiny as a result of this in an ever increasing number of commercial products: Even materials which are otherwise known to be harmless may pose a risk to public health when scaled down to nanometer size. This can be explained by the inherently high surface-to-volume ratio of nanoparticles and their small size which allows them to pass through cell membranes. Although this is known, only few data exist on toxicology of nanoparticles and their pharmacokinetics. Same can be said about the effects resulting from human long-term exposure to NPs This makes analyzing them a pressing issue.

In the course of the research work shown three different methods were developed and optimized for nanoparticle imprinting. These include: one-step, two-step and sedimentation imprinting. Based on affinity tests carried out on different polymer systems it was found that polyurethane yielded the most appreciable

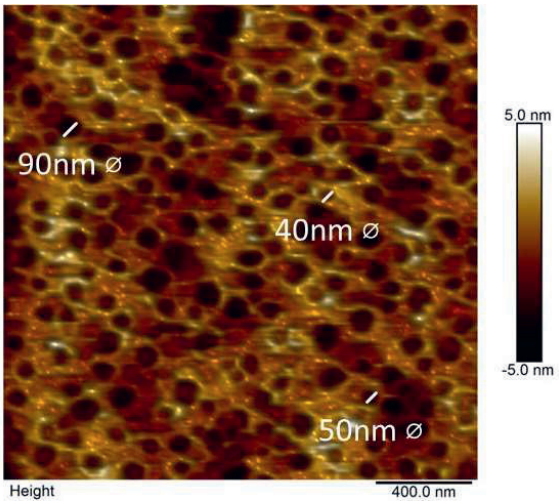


*Fig. 3. AFM image of Au NP-MIP surface*

results with sensor effects generally being reversible as well as reproducible. First results of these experiments were published for Ag nanoparticles [7]. The concept, however, is not limited to silver nanoparticles as laid out there. As can be seen in Figure 3, gold nanoparticles also lead to very appreciable imprinted cavities in the polymer surface. This is appreciable, yet not too surprising, because diameters of these engineered nanoparticles are in the same size range, as those of viruses. Actually, several MIP have already been published for those (some recent examples can e.g. be found in [3,8,9]). Figure 4 shows QCM results revealing that gold NP also lead to appreciable sensor

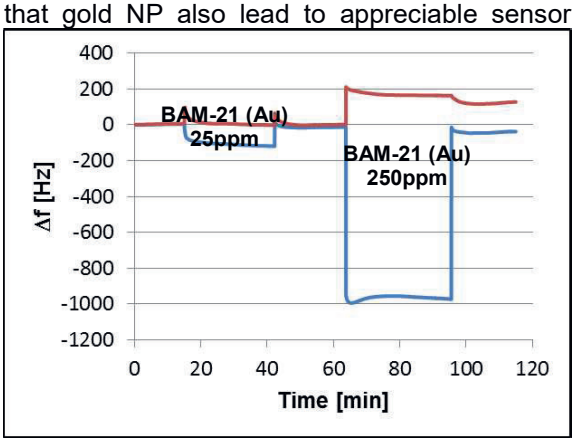


*Fig. 4. QCM sensor responses of MIP (blue) and NIP (red), respectively, toward Au NP.*

responses. Obviously, the mass sensitive signals depend on nanoparticle concentration and can be traced back to molecular imprinting: signals on the electrode coated with the non-imprinted material (non-imprinted polymer – NIP) lead to sensor responses that reach only about 25% of the corresponding MIP. Given the high density of gold ($\rho$=19.2 g/cm$^3$) these are very appreciable results. Nonetheless, a range of questions still wait to be answered, including e.g. the influence of the respective stabilizer shell on the sensor responses caused by a nanoparticle. Furthermore, selectivity of the systems also requires further experiments.

## Summary and conclusion

The two analytes presented herein are from very different origin: HDL is obviously a natural compound that is present in the human blood stream; engineered nanoparticles (PVP-stabilized gold in the concrete case) on the other hand represent fully artificial species. However, they have their physical dimensions in common (in the concrete case, both species have a radius just below 10 nm). In both cases it is possible to generate MIP, which demonstrates the generality of the synthetic approach and closes an “analytical gap” between sensors for small molecules and those aiming at detecting larger species, such as bacteria and entire cells. Despite appreciable results, several points remain a challenge, for instance the fact that surface roughness of MIP is also in the nm-range. Despite such current shortcomings, both HDL and NP sensors seem fit for use in real-life matrices. The former ones lead to appreciable sensor responses near the physiologically interesting concentration range.

## Acknowledgements

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