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Towards Mass-Sensitive Assay Formats for Medical Drugs Using MIP Nanobodies

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Summary:
Solid-phase synthesis of molecularly imprinted polymers yields nanoparticles that inherently may replace antibodies in bioassays. Herein, we demonstrate the approach for mass-sensitive detection of two medical drugs, namely salbutamol and vancomycin. While both yield appreciable sensor responses on quartz crystal microbalances – for salbutamol including a competitive assay, the responses to vancomycin are limited due to template size (and, thus, fewer binding events). One way to overcome this is to link the MIP nanoparticles to heavier particles to increase the response per binding event.

Keywords: mass-sensitive sensing, molecularly imprinted polymers, nanoparticles, solid-phase synthesis, proteins, peptides

Background, Motivation an Objective

The last three decades have seen substantial progress in molecularly imprinted polymers (MIP), which – among others – have also firmly established themselves for designing chemical sensors [1]. Even though straightforward to synthesize and cost-effective, especially compared to natural antibodies, MIP come with certain limitations not least regarding batch-to-batch reproducibility [2]. This has so far prevented them from delivering on the promise of replacing natural systems in sensing formats and assays. The groups of S. Piletsky [3] and K. Haupt [4] have proposed a solid-phase approach to synthesize MIP nanoparticles (nanoMIPs) from immobilized template species, which display high binding site homogeneity and affinities comparable to those of natural antibodies.

Experimental Background

The research presented focuses on the development of nanoMIP-based gravimetric assay systems for the detection of medically relevant compounds. Combining nanoMIPs with highly sensitive quartz crystal microbalance (QCM) transducers allows for the design of reliable, robust, yet inexpensive sensing devices that can be used without the need for expensive laboratory equipment and trained personnel.

Via solid phase synthesis, nanoMIPs for the detection of salbutamol (SAL), a beta-agonist commonly used for asthma treatment and as leanness-enhancing agent in the meat industry as well as the antibiotic vancomycin were obtained and tested. The process involves immobilizing the respective template on silica gel by usual APTES modification followed by EDC/NHS-catayzed coupling. Consecutive washing steps with cold and hot water, respectively, allows for selectively enriching high-affinity particles for the respective analyte.

Furthermore, MIP nanobodies were conjugated to heavier inorganic nanoparticles to increase the QCM signal, owing to the higher mass increase per binding event.

Results

Binding affinity of the nanoMIPs was studied on dual channel QCM chips with one analyte-functionalized sensor surface, while the second measurement electrode was used for selectivity investigations.

As shown in Figure 1, injecting SAL-imprinted nanoMIPs results in strong, concentration-dependent frequency shifts for the template-

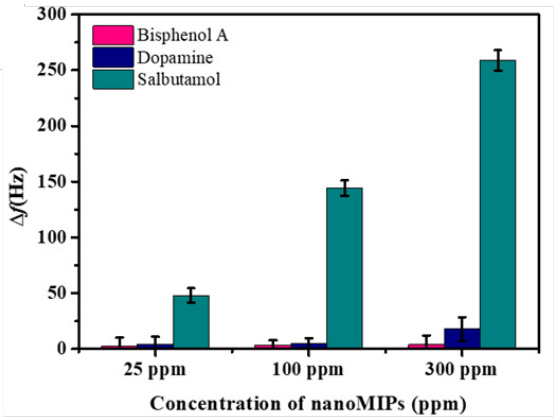

Fig. 1. Frequency response of QCMs modified with either SAL, bisphenol A or dopamine upon injection of varying concentrations of SAL-imprinted nanoMIPs.

functionalized electrode. Only negligible binding is visible on sensor surfaces modified with dopamine and bisphenol A, respectively.

Overall, this suggests appreciable affinity and selectivity of the SAL-MIPs. To establish a competitive assay, nanoMIPs at a fixed concentration of 300 ppm were mixed with varying concentrations of the analyte. As shown in Figure 2, one can observe an inverse relationship between SAL concentration and frequency shift, yielding a limit of detection of 2.85 ppm and a dynamic range of 2.5-50 ppm SAL in solution.

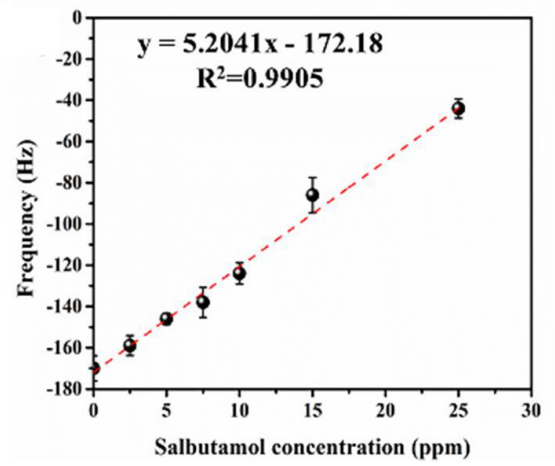

Fig. 2. Calibration curve for the competitive SAL-assay using mixtures of 300 ppm SAL-imprinted nanoMIPs and varying SAL-concentrations.

While for VM-imprinted MIPs, good selectivity compared to human serum albumin (HAS) is observed, the frequency response was significantly lower. This might be attributed to the size difference between the two analytes. With a sixfold molecular weight compared to SAL, the number of VM molecules that can be immobilized onto the sensor surface serving as nano-MIP binding site is significantly reduced. Thus, a concentration of 100 ppm of VM-imprinted nanoMIPs yields relatively low frequency shifts of -24 ± 4 Hz. To improve the limit of detection, the MIP particles were coupled to aminated TiO_2 nanoparticles via carbodiimide crosslinking. TiO_2 surface modification and coupling protocol were optimized towards minimal cluster formation. As shown in figure 3, first binding experiments on VM functionalized QCMs suggest a strong signal enhancing effect of the coupled MNPs with sensitivities in the sub-ppm region, proving that conjugation is a promising method for improving the obtainable limit of detection.

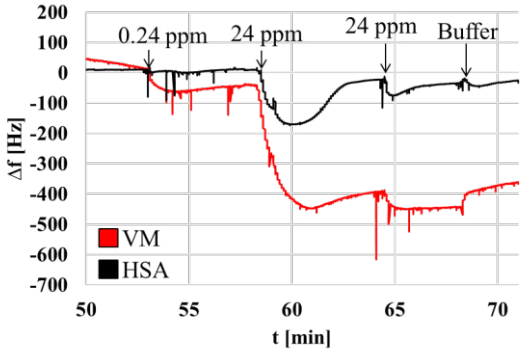

Fig. 3. QCM measurement with VM (red) and HSA (black) functionalized channel using increasing concentrations of VM-imprinted nanoMIPs conjugated to TiO_2 NPs.

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