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MOLECULARLY IMPRINTED NANOPARTICLES COUPLED TO MALDI-TOF MASS SPECTROMETRY FOR TARGETED PROTEIN ANALYSIS

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Molecularly imprinted nanoparticles (MIP NPs) are tailor made recognition materials prepared by template assisted synthesis, targeting small molecules but also peptides and proteins. MIP NPs demonstrated to possess size, affinity and selectivity on the par of natural antibodies, but the robustness and processability typical of polymeric materials. Aiming at developing a flexible analytical platform performing high sensitivity and high selectivity measurements, suitable for a simultaneous multi-biomarker determination, a library of peptide- and protein-imprinted MIP NPs was prepared and coupled to a well-established high sensitive detection method: matrix assisted laser desorption ionization mass spectrometry (MALDI-MS). The analytical performance and the extent of the applicability of the MIP NPs/MALDI-MS were studied by challenging the system with selected biomarkers of clinical interest, all found in serum at concentrations spanning from the nano- to pico-molar, but bearing different characteristics in size, folding and complexity. The selected biomarkers were: the random coiled 11mer peptide of the cardiac troponin I, marker of cardiac failure, the tightly folded 25 residues oligopeptide hepcidin and the folded whole

protein transferrin (77 kDa), both markers of iron metabolism. The MIP NPs compositions were rationally defined. Poly-acrylamido-based MIP NPs with average sizes of 30-60 nm were obtained by precipitation-polymerization. Isothermal titration calorimetry measurements demonstrated for exquisite template selectivity and allowed to estimate nanomolar dissociation constants. In view of developing a rapid semi-quantitative protocol for the analysis of targeted protein biomarkers, model and serum samples were incubated with the MIP NPs (5-30 min), briefly rinsed and spotted on the MALDI target plate, covered with organic matrix and mass analyzed. Femtomoles of the target analytes, both small peptides and whole proteins, both from model solutions and serum samples, were detected from the MALDI-TOF-MS analyses. Results proved the hyphenation MIP NPs/MALDI-TOF-MS as a rapid and highly sensitive method; it was demonstrated that the MIP NPs are suitable "baits" for the general development of flexible platforms for the targeted proteomics analysis. Foreseen applications of the MIP NPs/MALDI-TOF-MS platform will be monitoring patients by single analysis on targeted multi-protein markers.

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