Molecularly imprinted polymers for the selective recognition of annexin I

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Specific molecular association is a fundamental biological process. One of the most promising approaches to produce artificial systems that mimic natural recognition is molecular imprinting [1,2]. This technology is based on the synthesis of a polymeric network in the presence of the molecule to be recognized. After polymerization, the template is removed, leaving cavities that are complementary in shape, size and chemical functionalities to the target molecule, thus allowing specific recognition. Molecular imprinted polymers (MIPs) have been successfully used for the recognition of small molecules. By contrast, imprinting of macromolecules is affected by several problems. For instance, large target molecules such as proteins are not able to freely diffuse in the polymeric network to reach the binding sites [3]. However, if the imprinted cavities are present only on the surface of the polymer, this complication is circumvented, and highly cross-linked polymers can be employed. The rigidity of these scaffolds increases the selectivity of the binding sites toward the imprinted protein.
In this study, we developed surface MIPs able to recognize annexin I, a peripheral membrane protein of about 39 KDa, which is a marker for tumours since it is exposed only on the outer surface of cancer cells. The protein was successfully expressed in *Escherichia coli* cells and purified, and was labelled with rhodamine B to allow protein quantification through fluorescence spectroscopy. Surface imprinting was realized by using a glass microscope slide as a solid support. The target protein was absorbed on a coverslip, which was then placed on the microscope slide covered with a solution of the polymerization components (monomer, crosslinker and photoinitiator). After polymerization, the coverslip was gently removed, resulting in a glass-supported polymeric film. Multiple washing steps with high ionic strength solutions and detergents achieved removal of the target protein. To determine the general applicability of this approach, MIPs were realized also with ovalbumin, a protein of size and physico-chemical properties similar to those of annexin I.
To analyse the effect of imprinting, we realized a non-imprinted sample (NIP, non imprinted polymer), polymerized in the absence of the protein. Binding of the target molecule to both polymers (MIP and NIP) was determined by measuring the fluorescence intensity of rhodamine. Although some aspecific adsorption of the protein to the NIPs was observed, MIPs systematically displayed a 2-3 fold higher affinity (Figure 1). Similarly, binding of the target protein was significantly higher than association of other proteins (Figure 2), indicating a good selectivity of the system toward its template.
These preliminary findings, which need to be confirmed, indicate that the MIPs developed in the present study are a good starting point for the realization of sensors able to specifically recognize proteins.

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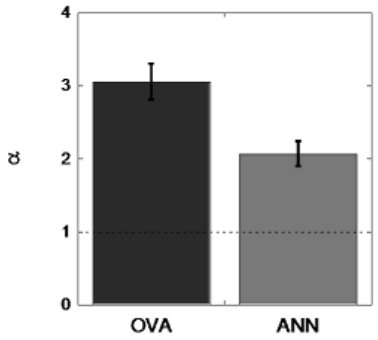

Figure 1. Imprinting factor α (ratio of the protein amounts bound to MIP and NIP) for a MIP imprinted with ovalbumin or annexin I.

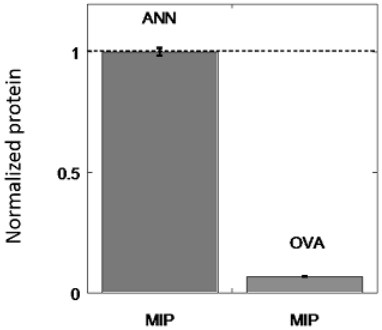

Figure 2. Normalized amount of annexin I and ovalbumin bound to the polymer imprinted with annexin I.