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Electrosynthesized molecularly imprinted polymers for protein recognition

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ABSTRACT

Molecularly imprinted polymers (MIPs) for the recognition of proteins are expected to possess high affinity through the establishment of multiple interactions between the polymer matrix and the large number of functional groups of the target. However, while highly affine recognition sites need building blocks rich in complementary functionalities to their target, such units are likely to generate high levels of non-specific binding. This paradox, that nature solved by evolution for biological receptors, needs to be addressed by the implementation of new concepts in molecular imprinting of proteins. Additionally, the structural variability, large size and incompatibility with a range of monomers made the development of protein MIPs to take a slow start. While the majority of MIP preparation methods are variants of chemical polymerization, the polymerization of electroactive functional monomers emerged as a particularly advantageous approach for chemical sensing application. Electropolymerization can be performed from aqueous solutions to preserve the natural conformation of the protein templates, with high spatial resolution and electrochemical control of the polymerization process. This review compiles the latest results, identifying major trends and providing an outlook on the perspectives of electrosynthesised protein-imprinted MIPs for chemical sensing.

Contents

1. Introduction

Molecular imprinting is a universal concept to generate materials with "molecular memory" by performing a polymerization of suitable functional monomers in the presence of a target molecule acting as a template. The subsequent removal of the template creates recognition sites in the molecularly imprinted polymer (MIP) that can, further on, selectively rebind the target. Whilst nature has an arsenal of 20 amino acids, MIPs are typically prepared from one

Dedicated to the memory of Professor Marco Mascini.

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to four monomers. Nevertheless, this concept proved to be successful in preparing selective sorbents for compounds of small molecular weight (~200–1200 Da). However, one should confront several specific problems if the target is a macromolecular protein [1] as the classical bulk methodologies worked out for small molecular weight compounds generally fail to address the peculiarities of protein targets. The difficulties are largely attributed to the intrinsic properties of the proteins as detailed below.

- Due to their fragility irreversible conformational changes may occur during polymerization [2] and the rebinding of the native conformation to such imprinted sites is not favored.
- The large size of the proteins makes them difficult to remove from, or rebind to a highly cross-linked 3D polymeric network traditionally used in small molecule imprinting, i.e., during imprinting the macromolecules may become irreversibly entrapped in the polymeric material.
- The large number of potential interaction sites on the surface of proteins may lead to cross-reactivity of the imprinted polymers and nonspecific adsorption onto bulk polymeric material.

All these difficulties caused a considerable lag in the development of protein MIPs compared to small molecule imprinting. Thus while the first research paper on a protein-imprinted MIP appeared already in 1985 [3] a steady increase in the publications can only be observed since 2005 (Fig. 1). However, even in 2014 less than 1% of the papers published on MIPs involved protein targets. Additionally, the range of implemented protein targets is very narrow and rather restricted to templates having properties that facilitates the imprinting process. In this respect proteins with good conformational stability, and distinct physical-chemical properties (e.g., high isoelectric point, and glycosylation) were generally preferred. Such properties facilitate the formation of strong and/or selective interactions such as electrostatic interaction between the positively charged proteins (e.g., lysozyme [4], avidin [5]) and negatively charged polymers as well as between glycan moieties and aminophenyl boronic acid (APBA) monomers [66]. This is a strong indication that the field of macromolecular imprinting is still very much at the proof of concept level and an enhancement in selectivity and affinity is required.

Despite the difficulties, imprinting methodologies custom tailored for protein targets are worthwhile to pursue beyond the inherent importance of protein analysis for several reasons.

- The prospects of preparing "plastic antibodies" are in general better for proteins than for small molecular weight targets as proteins are expected to generate higher affinity MIPs through the establishment of multiple interactions with their large number of functional groups.
- Molecularly imprinted polymers are expected to outperform their biological counterparts in terms of robustness and shelf-life.
- MIPs are more robust to environmental conditions and cost-effective as compared to antibodies and such their application in affinity assays is resourceful.

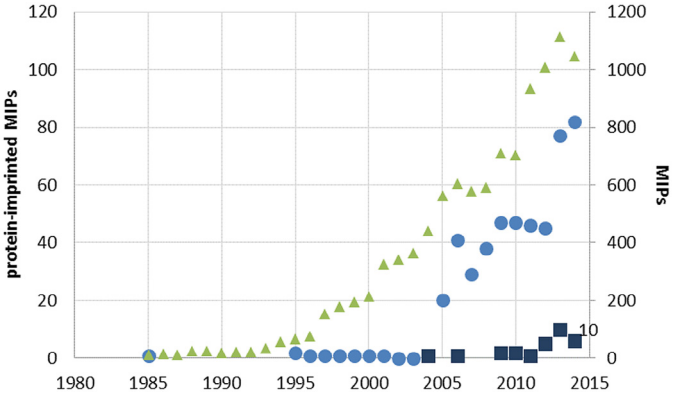

Fig. 1. Number of publications on all (circles) and electrosynthesized (squares) protein-imprinted polymers and on all MIPs (triangles, right axis), until the end of 2014 (ref. www.mipdatabase.com).

1.1. *Main concepts to overcome the difficulties of imprinting protein targets*

During the past decade different strategies have been introduced to overcome the barriers of protein imprinting and these were reviewed in several monographies [1,7–20]. To avoid denaturation of protein templates polymerization in aqueous media using water soluble monomers and initiators was an evident choice, although there have been some concerns about the deteriorating role of water on H-bonding and dipole-dipole interactions between the functional monomers and the template.

The problem of the restricted diffusion of the bulky macromolecular template in the highly cross-linked polymer network initiated fundamentally different strategies. The first approach, using *lightly cross-linked hydrogels* similar to the ones used in gel electrophoresis, was initiated by Hjertén and his coworkers [21,22]. Though not many direct evidences, the formation of highly permeable polymer structures or gels seem to not fulfil expectations with respect to the quality and stability of the imprints owing to excessive chain flexibility [20]. Another important approach is the "*epitope imprinting*", whereby a small, representative peptide sequence is used as the template instead of the native protein. This approach enables the implementation of non-aqueous polymerization media and complete template removal [23,24]. However, the rebinding of the targeted, much larger native protein may be still hindered in the MIP monolith. Therefore *surface imprinting* emerged as the main strategy for macromolecular imprinting. This approach restricts the formation of imprinted binding sites to the surface of a polymer or to a very thin polymer layer the thickness of which is comparable to the size of the protein template. In extreme cases proteins are captured by 2D monomolecular layers of suitable functional monomers anchored to an existing surface [25,26]. To maximize the binding capacity of the respective MIPs, generation of polymeric nanostructures with high area/volume ratio comes as a natural necessity to take full advantage of the surface imprinted sites. Creative methods have been devised to confine the templated sites exclusively to the polymer surface such as microcontact imprinting techniques [4,27–30] and sacrificial template support methods [5,31]. Imprinted soluble nanogels, having dimensions comparable to that of protein clusters were also shown to allow facile template exchange between the polymer and the solution [32]. The so-called solid-phase synthesis approach made feasible the reproducible, controlled and large scale production of such imprinted nanogels [33–35]. Interfacial polymerization on planar surfaces or supporting beads [36–38] can create ultra-thin polymer films that only partially bury the protein template without completely encapsulating it. Adherent MIP films can be polymerized *in situ* by free-radical polymerization for example by spin coating [39] or drop-casting the pre-polymerization mixture onto the substrate and inducing polymerization [40]. Better control over the thickness of the sensing layer can be achieved with surface confined polymerization methods, where either the initiator [41,42] or a pendant polymerizable group [43] is attached to the surface. Not only the thickness, but the area of the polymer film can be kept very small by the spatial confinement of the initiation process using light [44] or heat [45] supplied by a laser.