Molecular Imprinting

Principles and Applications of Micro-and Nanostructured Polymers

edited by Lei Ye

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Preface

Specific interactions between molecules play important roles in all living organisms. One example is the very specific interaction between the antibody and the antigen in our immune systems. Many therapeutic drugs are designed to bind biological catalysts (enzymes) to alter their activity. Besides curing diseases, specific recognition materials are very useful for various technical applications, such as product purification, monitoring of toxic pollutants in food and the environment, and fast and convenient chemical analysis and diagnostics.

Out of curiosity, some 40 years ago scientists started to think about using a target molecule as a template to create its own binding pocket in cross-linked polymers. This concept has been developed over the past years to the modern era of molecular imprinting, which is now considered a powerful synthetic method for creating tailor-designed molecular recognition sites in micro- and nanostructured polymers.

Compared with antibodies and other biological recognition materials, molecularly imprinted polymers (MIPs) are easier to prepare and are much more robust. Micro- and nanostructured MIPs can be produced from relatively simple starting materials using one or two reaction steps, which is much less time-consuming and more cost-effective than other synthetic approaches.

The aim of this book is to provide an overview of the state of the art of the molecular imprinting technology, with a focus on molecularly imprinted micro- and nanostructured materials. The book is divided into seven chapters. Chapter 1 highlights the new frontiers of molecular imprinting research. Chapter 2 explains the principle of designing functional monomers based on supramolecular chemistry. Chapter 3 introduces the use of theoretical and computational approaches to improve the performance of MIPs. In Chapter 4, controlled (living) radical polymerization techniques are presented, followed by a review on how these techniques have been applied to prepare MIPs. The subject of Chapter 5 is MIP nanoparticles, where both synthetic approaches and applications of MIP nanoparticles are

discussed. Chapter 6 focuses on molecularly imprinted nanofibers and microstructures involving the electrospinning technique. Chapter 7 describes imprinted molecular monolayers and thin films and membranes, as well as their analytical applications.

As a powerful synthetic method, molecular imprinting is fascinating and continues to bring in new functional materials. I hope this book will be useful for the readers to initiate new ideas and contribute to the field.

The editor would like to thank all the authors for their excellent contributions. Thanks to Stanford Chong, Jenny Rompas, and Arvind Kanswal, without whom this book would not have been possible.

Lei Ye
Lund, Sweden
August 2013

Contents

Chapter 1

New Frontiers in Molecular Imprinting: From Micro- to Nanofabrication

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1.1 Introduction

Molecular imprinting is now a widely accepted synthetic method to prepare polymer materials bearing pre-designed molecular recognition sites. Molecularly imprinted polymers (MIPs), due to their antibody-like binding affinity and selectivity, are frequently named artificial antibodies, plastic antibodies, or enzyme mimics if the imprinted polymers are designed to catalyze specific chemical reactions. The basic concept of molecular imprinting is very straightforward and can be illustrated using a simple illustration as shown in Fig. 1.1.

Compared to biologically derived molecular recognition materials, MIPs have much higher stability and can be prepared in large quantity at much lower cost. These advantages are very

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attractive for many practical applications requiring high molecular binding selectivity, such as for affinity separations, bioanalytical assays, chemical sensors and enzyme-like catalysis. To some extent, advancement of molecular imprinting has been driven by various intended applications, as exemplified by the recent development of water-compatible MIPs that can offer satisfactory molecular recognition under aqueous conditions, which is essential for addressing many biologically active molecules.

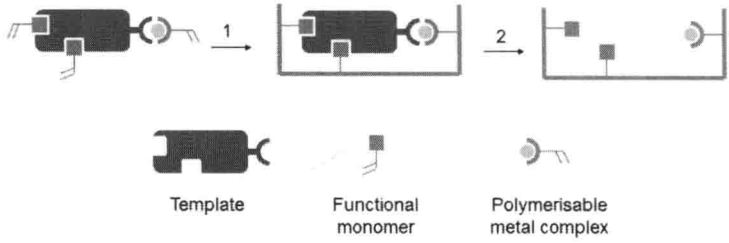

Figure 1.1 Schematic of molecular imprinting. Self-assembled template-monomer complex (top, left) is stabilized by co-polymerization with excess cross-linking monomer (step 1). After removal of the molecular template, a well-defined recognition site (molecular "cavity") is obtained in the polymer matrix (step 2). The 3D structure of an ideal recognition site is complementary to that of the template in terms of both size and interacting groups. For simplicity, the cross-linking monomer is not shown.

Molecular imprinting is an interdisciplinary field that involves synthetic chemistry, polymer chemistry and physics, analytical sciences and computational design. The links among the related disciplines can be found in Fig. 1.2. As such, progress in molecular imprinting has been dependent on advancements in all the basic disciplines involved. Recent examples include the new developments of supramolecular (host–guest) chemistry, controlled or "living" radical polymerization techniques, computer modeling, and the various micro- and nanofabrication techniques, subjects that are covered by the following chapters in this volume.

As generally accepted, the modern era of molecular imprinting can be dated back to the 1970's when Wulff et al. published the first covalent molecular imprinting research [1]. The introduction of noncovalent molecular imprinting strategy by Mosbach et al. in

the 1980's brought in significant interests in this area [2], especially after the successful demonstration that MIPs can be used as antibody substitutes in bioanalytical drug assays [3]. Because of its simplicity and the easy access to numerous commercially available functional monomers, noncovalent molecular imprinting can be carried out in most chemical and analytical laboratories and has become the method of choice in most application-oriented research projects, as well as for preparation of a handful of commercial MIP products presently on the market.

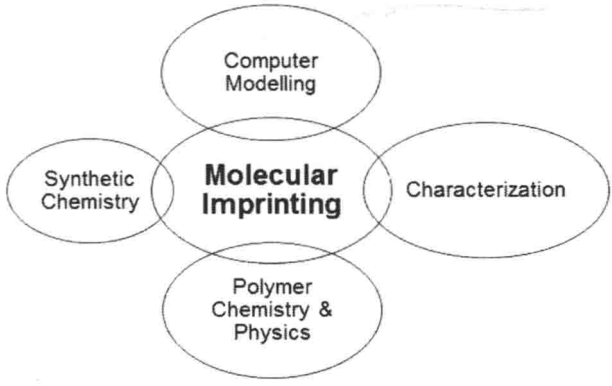

Figure 1.2 The interdisciplinary nature of molecular imprinting. Technological development of the field is also driven by practical applications and new micro- and nanofabrication techniques.

To circumvent the disadvantages of covalent molecular imprinting (slow binding kinetics) and noncovalent molecular imprinting (low imprinting fidelity), a semicovalent molecular imprinting strategy was introduced by Whitcombe et al., allowing covalently imprinted MIPs to bind target molecules through noncovalent interactions [4]. The semicovalent strategy requires careful design of the sacrificial spacer that links the template and the functional monomer, and often requires custom synthesis that can only be carried out by experienced researchers. Nevertheless, it will remain a very useful strategy to address some tricky templates that cannot be imprinted successfully using the noncovalent strategy.

From the conceptual point of view, the three molecular imprinting strategies proposed by Wulff, Mosbach and Whitcombe et al. have

remained. The major developments in the past decade are improved molecular imprinting effect through the use of new host–guest chemistry knowledge, computational modeling, new polymerization techniques, and micro- and nanofabrication methods, as well as new insights into the actual molecular imprinting process that are being revealed by new analytical and characterization techniques.

1.2 The Emergence of Micro- and Nanosized MIP Materials

Nanomaterials can have unique functions that are different from bulk materials made from the same chemical composition. Metal and semiconductor nanoparticles are good examples for their new catalytic and optical properties, and are finding increased uses in practical applications. The original initiative of developing micro- and nanostructured MIPs was to improve the uniformity of imprinted polymers to achieve better separation efficiency and to minimize batch to batch variation of the synthesized materials. Suspension polymerization has been used to synthesize MIP microspheres, both in aqueous and nonaqueous continuous phases. The use of perfluorocarbon and mineral oil as the continuous phase allowed successful imprinting of polar organic molecules that form hydrogen bond interactions with the functional monomer [5, 6], which otherwise can be easily disrupted if water is used as the continuous phase. This heterogeneous polymerization method is also easy to scale up, therefore more suitable for commercial production of MIPs. It has been demonstrated that using microfluidic reactors, very uniform MIP beads can be prepared from standard molecular imprinting recipe through suspension polymerization (Fig. 1.3) [7].

The tendency of nanoparticles to situate on water–oil interface has been utilized in the past to form stable nanoparticle-stabilized emulsions (Pickering emulsions). Pickering emulsion polymerization is similar to suspension polymerization, except that the monomer droplets are stabilized by nanoparticles instead of surfactants. The use of Pickering emulsion polymerization to synthesize MIP beads has been recently demonstrated. As reported by Shen and Ye, the new method of molecular imprinting using Pickering emulsion polymerization allowed hydrophilic MIP beads to be produced [8].

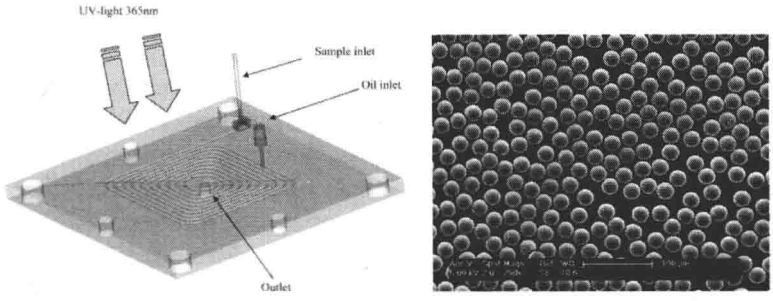

Figure 1.3 Preparation of MIP beads using suspension polymerization in a spiral microfluidic reactor. Propranolol was used as the template, and mineral oil was used as the continuous phase. Reproduced with permission from *Lab Chip* 2006, *6*, 296. Copyright 2006 The Royal Society of Chemistry.

More importantly, the nanoparticles themselves can be decorated with molecular template before the particles are used to establish the Pickering emulsion [9]. In this manner the immobilized template is located on the surface of the monomer droplets, and by interacting with the functional monomer, the template creates surface-accessible binding sites in the finished MIP beads. This surface molecular imprinting protocol was demonstrated using a segment of propranolol as template, with the obtained MIP beads being able to recognize propranolol and its structural analogs containing the same template structure (Fig. 1.4).

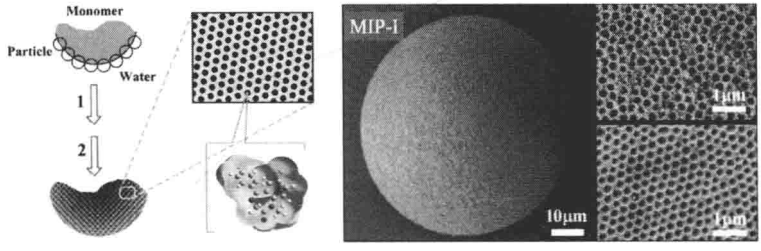

Figure 1.4 Left: interfacial molecular imprinting in nanoparticle-stabilized emulsions. Step 1: Pickering emulsion polymerization, where the silica nanoparticles are modified with a molecular template; Step 2: removal of silica and template. Right: SEM image of single MIP bead and the nanoindentations on its surface created by the nanoparticles [9].

Reducing the physical size of MIP particles can increase their effective capacity and binding kinetics due to the improved accessibility of the imprinted sites. In addition, new possibilities can be realized when moving from bulk MIPs to micro- and nanosized MIP materials. An example is the signal transduction scheme that was realized in propranolol-imprinted composite nanofibers, where the small physical size of the MIP nanoparticles allowed effective proximity scintillation to be employed for real-time monitoring of molecular association and dissociation events (Fig. 1.5) [10].

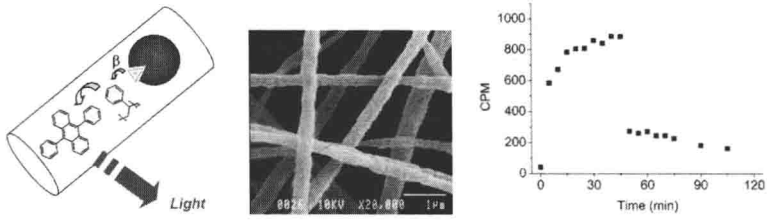

Figure 1.5 Composite electrospun nanofiber containing propranolol-imprinted nanoparticles allowed real-time monitoring of molecular association and dissociation events. The small nanoparticles allowed the molecular binding sites to be located in close proximity of the remaining fluorescence relay components embedded in the nanofiber. Binding of tritium-labeled propranolol to the imprinted site trigged fluorescence energy transfer, thereby allowing direct monitoring of the labeled propranolol. From ref. 10.

MIPs prepared by noncovalent molecular imprinting often have heterogeneous binding sites [11]. The heterogeneity of affinity distribution of noncovalent MIPs may be caused by several reasons. The major factor contributing to the site inhomogeneity is the dynamic association–dissociation of template-functional monomer complexes during the kinetically controlled free radical polymerization process [12]. Depending on the exact status of a functional monomer (template-bound or free) when it is being incorporated into cross-linked polymer chains, this functional monomer may contribute to either high or low affinity site. Although the heterogeneous affinity sites of noncovalent MIPs can be tolerated in many practical applications, several synthetic strategies have been introduced to increase the homogeneity of binding sites of MIPs [13–15]. Until now, the only monoclonal MIP material reported in the literature is based on dendrimers with cross-linked