

Molecular Imprinting

**Principles and Applications of Micro-and
Nanostructured Polymers**

edited by Lei Ye



Molecular Imprinting

Principles and Applications of Micro-and
Nanostructured Polymers

edited by Lei Ye



Published by

Pan Stanford Publishing Pte. Ltd.
Penthouse Level, Suntec Tower 3
8 Temasek Boulevard
Singapore 038988

Email: editorial@panstanford.com

Web: www.panstanford.com

British Library Cataloguing-in-Publication Data

A catalogue record for this book is available from the British Library.

Molecular Imprinting: Principles and Applications of Micro- and Nanostructured Polymers

Copyright © 2013 by Pan Stanford Publishing Pte. Ltd.

All rights reserved. This book, or parts thereof, may not be reproduced in any form or by any means, electronic or mechanical, including photocopying, recording or any information storage and retrieval system now known or to be invented, without written permission from the publisher.

For photocopying of material in this volume, please pay a copying fee through the Copyright Clearance Center, Inc., 222 Rosewood Drive, Danvers, MA 01923, USA. In this case permission to photocopy is not required from the publisher.

ISBN 978-981-4310-99-4 (Hardcover)

ISBN 978-981-4364-87-4 (eBook)

Printed in the USA

Molecular Imprinting

The background of the cover is a textured, light gray surface. It features several white, stylized molecular structures, including a large, complex one on the right side and a smaller, simpler one on the left. At the bottom, there is a dense field of overlapping, semi-transparent circles in various shades of gray, creating a bokeh-like effect.

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

<i>Preface</i>	xi
----------------	----

1. New Frontiers in Molecular Imprinting: From Micro- to Nanofabrication	1
---	----------

Lei Ye

1.1 Introduction	1
1.2 The Emergence of Micro- and Nanosized MIP Materials	4
1.3 Combination of Imprinted Polymer with Nanomaterials	8
1.4 From Bottom-Up Synthesis to Top-Down Fabrication	12
1.5 Conclusion	18

2. Synthetic Chemistry in Molecular Imprinting	25
---	-----------

Börje Sellergren and Andrew J. Hall

2.1 Introduction	25
2.2 Molecular Imprinting and Host-Guest Chemistry	26
2.3 Host Monomers in Non-Covalent Imprinting	32
2.3.1 Hydrogen-Bonding Host Monomers	32
2.3.1.1 Donor-acceptor monomers	32
2.3.1.2 Donor-donor monomers	38
2.3.1.3 Donor-acceptor-donor monomers	53
2.3.2 Hosts Monomers Designed for Imprinting in Water	58
2.4 Template Design	62
2.5 Conclusion	65

3. Rational Molecularly Imprinted Polymer Design: Theoretical and Computational Strategies	71
---	-----------

Ian A. Nicholls, Håkan S. Andersson, Kerstin Golker, Henning Henschel, Björn C. G. Karlsson, Gustaf D. Olsson, Annika M. Rosengren, Siamak Shoravi, Jesper G. Wiklander, and Susanne Wikman

3.1 Introduction	71
------------------	----

3.2	Electronic Structure Methods	72
3.3	Molecular Dynamics Simulations	78
3.4	Multivariate Statistical Analyses	86
3.5	Summary and Future Perspectives	91
4.	Application of Controlled/"Living" Radical Polymerization Techniques in Molecular Imprinting	105
	<i>Huiqi Zhang</i>	
4.1	Introduction	105
4.2	Application of CRP Techniques in Molecular Imprinting	114
4.2.1	Brief Introduction of CRP Techniques	114
4.2.1.1	Advantages of CRP over conventional free radical polymerization	114
4.2.1.2	Typical CRPs	116
4.2.1.3	Synthesis of cross-linked polymers with homogeneous network structures via CRPs	121
4.2.2	Controlled Preparation of MIPs via Various CRPs	124
4.2.2.1	Application of iniferter-induced "living" radical polymerization	124
4.2.2.2	Application of ATRP	130
4.2.2.3	Application of RAFT polymerization	139
4.2.2.4	Application of NMP	144
4.3	Summary and Outlook	144
5.	Molecularly Imprinted Nanoparticles	161
	<i>Zhiyong Chen and Lei Ye</i>	
5.1	Introduction	161
5.2	Synthesis of Molecularly Imprinted Nanoparticles	163
5.2.1	Precipitation Polymerization	164
5.2.2	Solution Polymerization	166
5.2.3	Mini-Emulsion Polymerization	168
5.2.4	Micro-Emulsion Polymerization	169
5.2.5	Nonaqueous Emulsion Polymerization	169

5.2.6	Core-Shell Nanoparticles	170
5.2.6.1	Core-shell nanoparticles by emulsion polymerization	170
5.2.6.2	Two-step precipitation polymerization	172
5.2.6.3	Grafting approaches	173
5.2.6.4	Sol-gel process	175
5.2.6.5	Surface deposition	176
5.2.7	Hyper-branched Polymers and Dendrimers	177
5.3	Applications of MIP Nanoparticles	177
5.3.1	Separation	177
5.3.2	Binding Assays	180
5.3.3	Chemical Sensing	182
5.3.4	Catalysis	183
5.3.5	Controlled Release and Drug Delivery	186
5.4	Conclusions and Perspectives	187
6.	Molecularly Imprinted Nano- and Microstructures by Electrospinning	197
	<i>Ioannis S. Chronakis and Lei Ye</i>	
6.1	Introduction	197
6.2	Electrospinning Process	199
6.3	Electrospinning Processing Parameters: Control of Fiber Morphology	201
6.3.1	Solution Properties	201
6.3.2	Process Conditions	202
6.3.3	Ambient Conditions	202
6.4	Advantages of Molecularly Imprinted Nano- and Microstructures	203
6.5	Generation of Artificial Molecular Recognition Sites in Nano- and Microfibers	204
6.6	Surface Protein Molecular Imprinting Employing Polymer Brushes	207
6.7	Thin Films of MIP Grafted on Nano- and Microfiber by Surface-Initiated Polymerization	208
6.7.1	2D Molecularly Imprinted Surfaces	210
6.7.2	3D Molecularly Imprinted Microfibrous Structures	210

6.8	Electrospun Nanofibers with Encapsulated MIP Nanoparticles	212
6.8.1	Effect of Particle Size	213
6.9	Conclusion	217
7.	Molecular Monolayers, Thin Films, and Membranes	221
	<i>Oliver Brüggemann and Wolfgang Fürst</i>	
7.1	Introduction	221
7.2	Molecularly Imprinted Molecular Monolayers	222
7.2.1	Monolayers on Electrodes	222
7.2.2	Sensors for Biomolecules	229
7.2.3	Piezoelectric Sensors	232
7.3	Molecularly Imprinted Thin Films	236
7.3.1	Molecularly Imprinted Thin Films Based on Polymers	236
7.3.1.1	MIP thin films obtained by spin coating	236
7.3.1.2	MIP thin films obtained by drop coating	238
7.3.1.3	MIP thin films obtained by spray coating	240
7.3.1.4	MIP thin films obtained by grafting	241
7.3.1.5	MIP thin films obtained by other coating methods	242
7.3.1.6	Polymeric MITF via electropolymerization	243
7.3.2	Molecularly Imprinted Thin Films Obtained by a Sol–Gel Process	246
7.4	Molecularly Imprinted Membranes	250
7.4.1	Molecularly Imprinted Membranes Based on Polymers	251
7.4.1.1	In situ preparation of MIPs as coatings on a supporting membrane	251
7.4.1.2	MIP membranes obtained by phase inversion	253
7.4.1.3	MIP membranes obtained by solvent evaporation	258

7.4.1.4 Hybrids of membrane matrix and pre-synthesized MIPs	259
7.4.2 Molecularly Imprinted Membranes Fabricated with Inorganic Compounds	261
7.5 Conclusion and Perspectives	264
<i>Index</i>	273

Chapter 1

New Frontiers in Molecular Imprinting: From Micro- to Nanofabrication

Lei Ye

*Division of Pure and Applied Biochemistry, Lund University,
Box 124, 221 00 Lund, Sweden*

Lei.Ye@tbiokem.lth.se

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

Molecular Imprinting: Principles and Applications of Micro- and Nanostructured Polymers

Edited by Lei Ye

Copyright © 2013 Pan Stanford Publishing Pte. Ltd.

ISBN 978-981-4310-99-4 (Hardcover), 978-981-4364-87-4 (eBook)

www.panstanford.com

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.

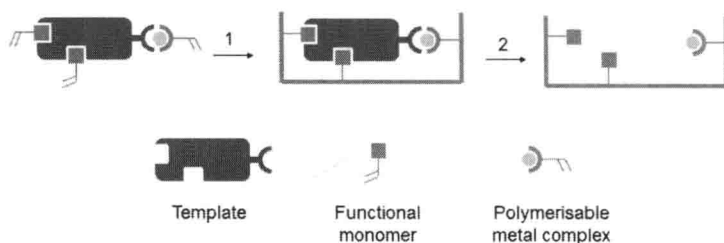


Figure 1.1 Schematic of molecular imprinting. Self-assembled template-monomer complex (top, left) is stabilized by co-polymerization with excess cross-linking monomer (step 1). After removal of the molecular template, a well-defined recognition site (molecular “cavity”) is obtained in the polymer matrix (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.

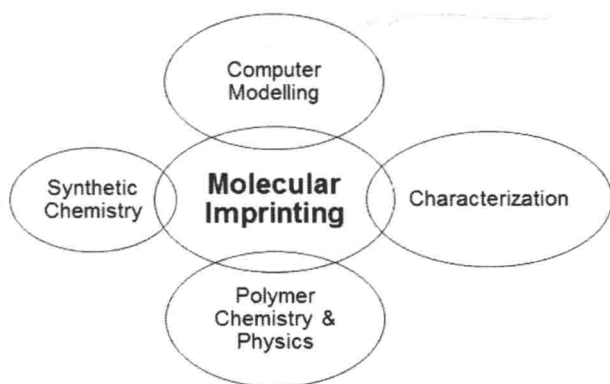


Figure 1.2 The interdisciplinary nature of molecular imprinting. Technological development of the field is also driven by practical applications and new micro- and nanofabrication techniques.

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].

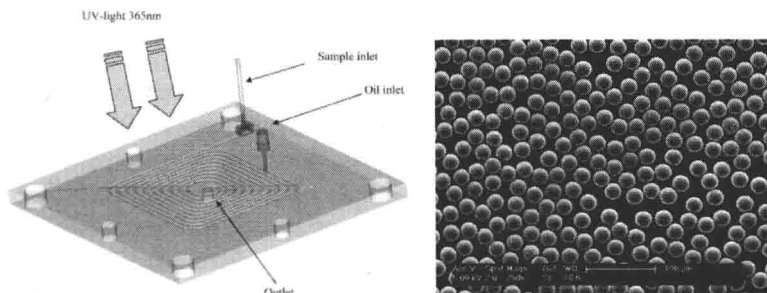


Figure 1.3 Preparation of MIP beads using suspension polymerization in a spiral microfluidic reactor. Propranolol was used as the template, and mineral oil was used as the continuous phase. Reproduced with permission from *Lab Chip* 2006, 6, 296. Copyright 2006 The Royal Society of Chemistry.

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).

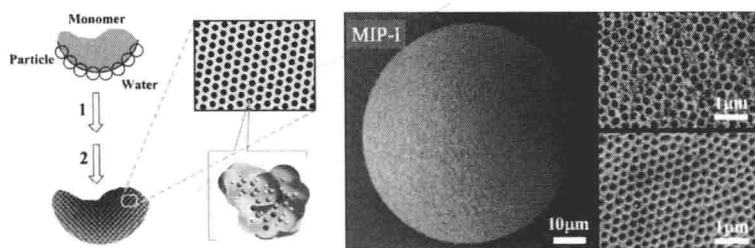


Figure 1.4 Left: interfacial molecular imprinting in nanoparticle-stabilized emulsions. Step 1: Pickering emulsion polymerization, where the silica nanoparticles are modified with a molecular template; Step 2: removal of silica and template. Right: SEM image of single MIP bead and the nanoindentations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

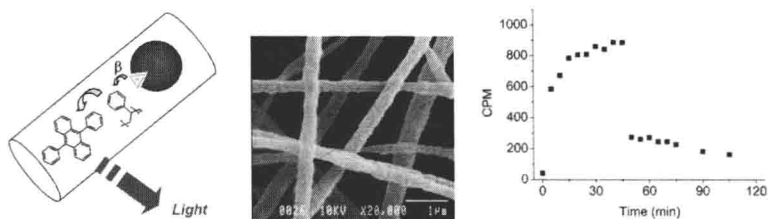


Figure 1.5 Composite electrospun nanofiber containing propranolol-imprinted nanoparticles allowed real-time monitoring of molecular association and dissociation events. The small nanoparticles allowed the molecular binding sites to be located in close proximity of the remaining fluorescence relay components embedded in the nanofiber. Binding of tritium-labeled propranolol to the imprinted site triggered 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