

# Imprinted nanomaterials: a new class of synthetic receptors

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**Abstract** The molecular imprinting approach provides a unique opportunity for the creation of three-dimensional cavities with tailored recognition properties. Over the last decade this field has expanded considerably, across a variety of disciplines, leading to novel approaches and many potential applications. Progress in the field of materials science has led to significant breakthroughs and the application of the imprinting approach to novel polymeric formats offers new insights and attractive methods for the preparation of synthetic receptors. In particular, nanomaterials have received considerable attention in the developing field of nanotechnology. With a large number of recent developments in the field of molecular imprinting available, this article is focused on a selection of new systems, in particular the different formats of nanomaterials, such as nanogels, nanofibres, nanowires and nanotubes.

**Keywords** Molecular imprinting ·  
Molecularly imprinted polymers · Synthetic receptors ·  
Sensing · Catalytic nanomaterials · Nanogels

## Introduction

Molecular recognition plays a very important role in biological activity: enzymes, antibodies and receptors are typical examples. The high specificity and/or catalytic activity of these macromolecules are generated by three-

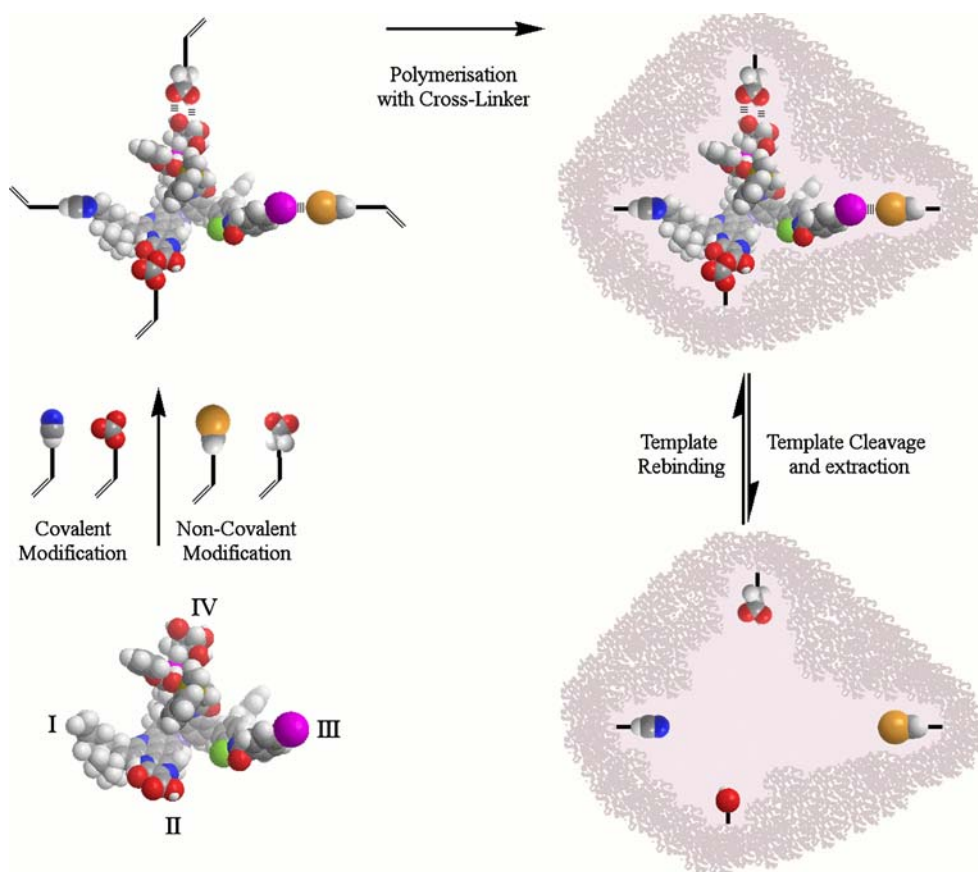
dimensional structures in which multiple interactions involving covalent or non-covalent bonds can be formed with the target molecule. The recognition specificity is an important parameter that affects the applications of these macromolecules and considerable effort has been spent on understanding the mechanism that controls recognition with the view to generating artificial macrostructures, for a variety of applications related to binding and/or catalysis. An interesting approach for the development of synthetic receptors is molecular imprinting and its application to polymeric materials.

Molecular imprinting is a process where functional and cross-linking monomers are copolymerized in the presence of the target analyte (the imprint molecule), which acts as a molecular template [1]. The functional monomers initially form a complex with the imprint molecule, and following polymerization, the specific functional groups are held in position by the highly cross-linked polymeric structure. Upon removal of the imprint molecule, a cavity, or recognition site, is created which is complementary to the original template used. A molecular memory is therefore created in the polymeric matrix, which can recognize and rebind the analyte with a high degree of specificity. The interactions between the template and the functional monomers can be covalent, non-covalent or combinations of both, where the choice is largely dependent on the chemical structure of both the template and the monomers, and the intended application (Fig. 1).

The first example of molecular imprinting in synthetic organic polymers was reported in 1972 by Wulff and Sarhan [2] and described the preparation of imprinted polymers for the separation of racemic mixtures using the covalent approach, where the template–monomer complex is formed via a reversible covalent bond. Research in the field accelerated when Arshady and Mosbach [3] introduced the non-

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**Fig. 1** The imprinting process, showing some of the interactions used in creating affinity in the binding site for the template. *I* reversible covalent interaction; *II* semicovalent method; *III* electrostatic interaction with an oppositely charged monomer; *IV* non-covalent hydrogen bonding



covalent strategy, where non-covalent self-assembly of the template with functional monomers is the key step prior to polymerization. This is currently the most widely used approach to generate imprinted binding sites. Advantageously, molecularly imprinted polymers (MIPs) are often characterized by long-term stability as well as the ability to perform well under harsh conditions, such as high temperature, extreme pH values and in the presence of organic solvents.

MIPs have attracted much interest in recent decades and have found applicability in a number of research areas, such as stationary phases for chromatographic separations and affinity solid-phase extraction, catalysis, controlled drug release, sensor technology and as antibody replacements in diagnostics. The final application of a target-imprinted polymer plays a key role in determining the type of polymeric matrix that is required and “bulk” polymerization has certainly been the most widely used technique. However, this material must be ground mechanically, resulting in a large size distribution of irregularly shaped particles. Significant advances over the last decade in the field of materials science have allowed the development of methods for the preparation of a variety of polymeric formats that are compatible with molecular imprinting technology with the aim of developing a variety of novel synthetic receptors. To exploit the full potential of

molecular imprinting as a technique, substantial effort has been devoted to progress from the microscale to the nanoscale. This advancement has offered considerable advantages, such as greater surface-to-volume ratio, accessibility to the maximum number of recognition sites, lower diffusion times to facilitate greater uptake and release of the template and overall improved efficiency. In addition to these, there are also advantages related to the distinct differences in properties (optical, electrical, mechanical, etc.) demonstrated by nanomaterials when compared with their macroscopic counterparts. In response to this, a new generation of molecularly imprinted synthetic receptors has arisen over the past decade that display physical properties which are often closer to those demonstrated by enzymes and antibodies, such as physical size, solubility, flexibility and recognition site accessibility. Here we highlight some interesting examples of imprinting with nanomaterials, without covering the extensive literature available on the topic outside the remit of this article.

### Nanoparticles

A number of different approaches have been reported for the preparation of imprinted nanoparticles, based mainly on

emulsion and precipitation polymerization methods; however, these approaches are rather broad and a number of variations have been described for each. For example when considering emulsion polymerization, Pérez et al. [4, 5] reported a successful approach for the preparation of cholesterol-imprinted core-shell nanoparticles. Initially, non-imprinted core particles of 30–40 nm in diameter were prepared. These core particles were subsequently used in a second-stage polymerization where the imprinted shell was produced using a covalent imprinting approach. Cholesterol-specific recognition sites were prepared in this way, following hydrolytic cleavage and removal of the template from the shell of the nanoparticle. The successful preparation of imprinted core-shell nanoparticles by a non-covalent imprinting approach was subsequently also reported [6, 7].

Miniemulsion and microemulsion polymerization have also been utilized for the preparation of imprinted nanoparticles, the latter often using a template-directed method of imprinting. In this strategy the target molecule is used as the head of a surfactant and is therefore directed to the inner surface of the micelle, thus leaving an imprinted recognition site at the surface of the nanoparticle. Markowitz et al. [8, 9] used this type of method for imprinting of an  $\alpha$ -chymotrypsin transition state analogue at the surface of silica nanoparticles.

Precipitation polymerization is a method in which the polymeric reaction begins in a homogenous phase, where monomers, cross-linkers and initiators are in a dilute solution of porogenic solvent. As the polymerization proceeds, the expanding polymer becomes insoluble and aggregates into particles, which are stabilized against coagulation either sterically or by their rigid cross-linked surfaces. Ye et al. [10] reported the first synthesis of imprinted nanoparticles using the precipitation polymerization technique. They demonstrated that both hydrophobic and hydrophilic template molecules could be successfully imprinted using this method. A variety of imprinted nanoparticles have subsequently been prepared using this method, with potential applications in research areas such as sensing [11], capillary electrochromatography [12] and drug delivery [13].

## Microgels/nanogels

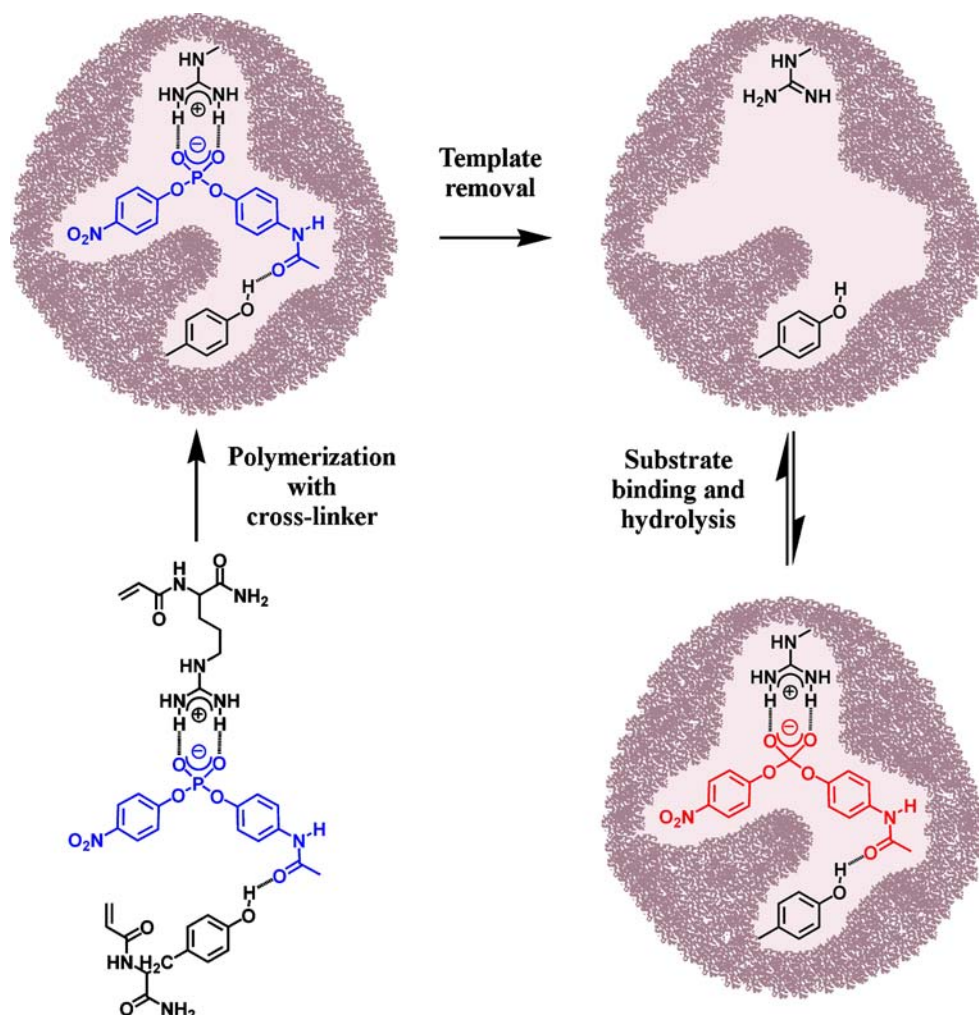
The precipitation polymerization method can be optimized to produce microgels/nanogels in the size range of 10–300 nm. A characteristic of these materials is that they produce low-viscosity colloidal solutions after preparation in a suitable solvent that never reaches the point of precipitation. The nature of microgels/nanogels makes them an attractive material for the development of synthetic receptors with enzymelike activity. Initial research by Biffis

et al. [14] demonstrated the feasibility of applying the imprinting approach to microgels, which were subsequently shown to recognize and to selectively bind sugar molecules. Subsequently, Maddock et al. [15, 16] reported the first soluble imprinted microgels that demonstrated hydrolytic catalytic activity (Fig. 2). In this work the authors imprinted a phosphate transition state analogue, using polymerizable amino acids as functional monomers, in order to mimic the catalytic mechanism of hydrolytic antibodies and hydrolase enzymes with carbonate substrates. The imprinted microgels were found to display rate accelerations of about an order of magnitude over the uncatalysed reaction. Following this, Wulff et al. [17] reported the preparation of hydrolytically active nanogels with an average of one active site per macromolecule. Here they demonstrated the use of a novel postdilution method for the preparation of more highly cross-linked particles, thus aiding the imprinting process.

More recently Carboni et al. [18] applied this strategy (i.e. imprinting in nanogels) to a more energetically demanding carbon-carbon bond forming reaction, and reported the first example of molecularly imprinted nanogels with aldolase type I activity. In this work a covalent imprinting approach was used to obtain a nanogel preparation capable of catalysing the cross-aldol reaction. A polymerizable proline derivative was used as a functional monomer, to mimic the enamine-based mechanism of aldolase type I enzymes, and coupled reversibly to a diketone template, designed to mimic the intermediate of the reaction (Fig. 3). Nanogels were prepared with an average particle size of 20 nm and kinetic characterization was performed using very low catalytic loads, following estimation of active-site concentrations. The advantage of solubility and flexibility, previously unattainable with monolithic polymers, allowed the demonstration of the highest catalytic rate enhancements for a carbon-carbon bond forming reaction achieved to date using imprinted polymers. In a comparison of the catalytic constants ( $k_{\text{cat}}$ ) obtained for the imprinted and non-imprinted nanogels, a ratio of 18.8 was evident, which is indicative of good imprinting efficiency.

In addition to being used as catalysts, microgels/nanogels have been shown to act as proficient receptors for use in immunoassay technology by Hunt et al. [19]. They reported the first pseudo-immunoassay, using a fluorescent probe, that did not necessitate separation of the 2,4-dichlorophenoxyacetic acid imprinted polymer from the analyte solution. As it is important in such an assay that fluorescence is distinguished from scattering, it was advantageous to use imprinted microgels, as these do not sediment easily and can be obtained with an average diameter (180 nm) considerably lower than the fluorescence excitation wavelength (323 nm).

**Fig. 2** Non-covalent imprinting of a phosphate transition state analogue using polymerizable arginine and tyrosine as functional monomers [15]



### Nanowires, nanotubes and nanofibres

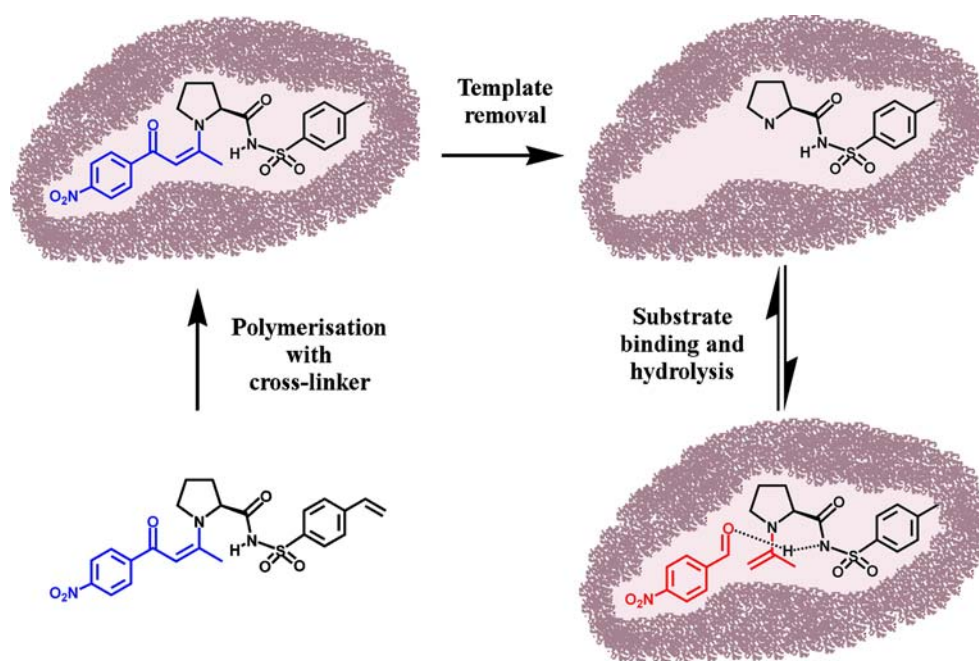
While considerable effort was devoted to the development of polymerization methods to obtain a variety of imprinted nanoparticles, a number of research groups have focused on the application of the molecular imprinting approach to other types of nanomaterials, such as nanowires, nanotubes and nanofibres, to extend the range of possible applications using the imprinting approach. The group led by Wang was the first to successfully prepare nanowires by a nano-moulding process, using the molecular imprinting approach for the creation of molecular recognition sites at the surface of the nanowires [20]. In this approach a nanoporous alumina membrane was used, where the inner walls of the pores were functionalized with the template. The nanopores were subsequently filled with the monomer mixture and polymerized, followed by chemical dissolution of the alumina membrane, leaving behind polypyrrole nanowires with glutamic acid binding sites situated at the surface.

The same protocol has also been used for the surface imprinting of a variety of proteins, including albumin,

haemoglobin and cytochrome *c* [21]. The isolated nanowires showed significant evidence of imprinting efficiency with a sevenfold difference between imprinted and non-imprinted nanowires, and this result was complemented by a larger binding capacity, resulting from the high surface area of the nanowires and the complete removal of the template molecule. Xie et al. [22] described the preparation of TNT surface-imprinted nanowires where alumina membranes with pore diameters of 70 nm were used (Fig. 4).

In this work the authors also reported the synthesis of imprinted nanotubes. These are effectively hollow nanowires that display a larger surface-to-volume ratio and may be prepared following small alterations to the nanowire polymerization procedure. Aside from their use as an effective recognition element, their successful implementation for the chemical separation of a number of hormones has also been reported [23]. Li et al. [21] even demonstrated that magnetic properties could be imparted to this type of nanostructured material, with potential applications in drug delivery, as biochemical sensors and for trace enrichment of specific analytes.

**Fig. 3** Covalent imprinting of the diketone template using a polymerizable proline derivative as a functional monomer [17]



Using a very different approach, Ye and colleagues worked intensively on the application of imprinting to nanofibres and not only were they able to demonstrate the incorporation of imprinted nanoparticles into electrospun nanofibres [24–26] but they were also able to achieve the generation of artificial molecular recognition sites in the nanofibre itself [27]. The nanofibres were prepared from a solution of poly(ethylene terephthalate) and polyallylamine in the presence of a template molecule, 2,4-dichlorophenoxyacetic acid. These materials have shown good imprinting efficiencies and provide further evidence of the flexibility of the imprinting approach. Initial reports have also demonstrated their potential applications as recognition materials both for solid-phase extraction [25] and as sensing elements [26]. In the latter case, imprinted nanoparticles were incorporated into the nanofibre, where proximity scintillation was used as the

transduction method, permitting direct read-out of a binding event and avoiding the requirement of a separation step.

### Single-site macromolecules

One could argue that the preparation of imprinted polymeric structures such as nanowires and nanotubes produced using macroscopic template material may be categorized as employing the “top-down” approach. Using a similar train of thought, one could describe the preparation of a single macromolecule, with a single recognition site, using molecular self-assembly as employing the “bottom-up” approach. This idea was previously described by Zimmerman and Lemcoff [28] and was initially realized with the first “homogeneous nanoscale imprinting system”, described by Ishi-i et al. [29], wherein a saccharide template was covalently bound by two boronic

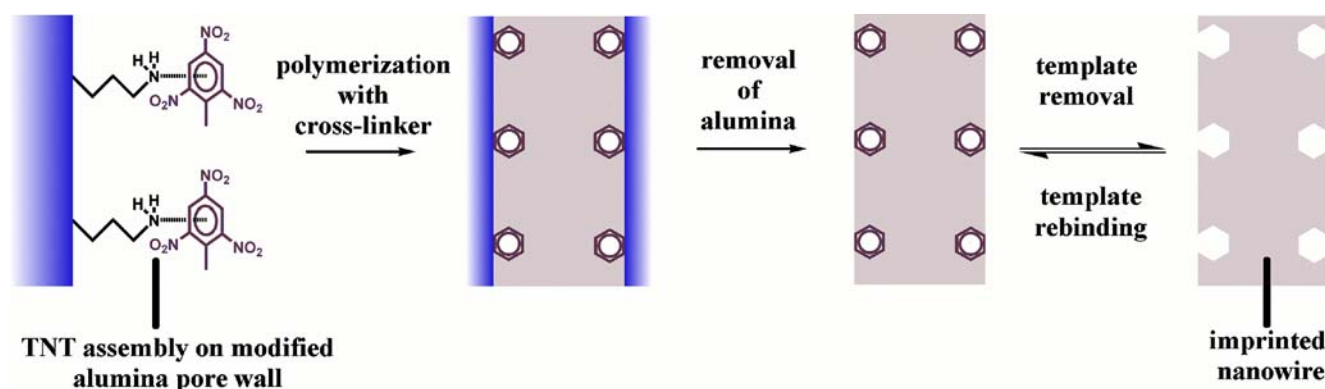


**Fig. 4** Non-covalent imprinting of TNT at the surface of acrylate-based nanowires. (Adapted from [22])

acid groups and bound onto the surface of [60] fullerene. This strategy was used to produce one single binding site per molecule and avoid the creation of numerous binding sites with different recognition properties. Although the selectivity in this system was not extraordinary, it may be emphasized that this was the first instance in which molecular imprinting was applied to a system with a soluble nano-sized matrix in homogeneous solution.

Zimmerman et al. [30] more recently used a different strategy, wherein a single porphyrin template was dynamically imprinted into a single macromolecule (dendrimer), in order to prepare molecularly homogeneous hosts, each with a single recognition site. In this work the authors used the covalent approach, where the porphyrin acted as the core and was covalently bound through ester linkages to eight third-generation dendrons to form the dendrimer (Fig. 5).

The porphyrin was cleaved by hydrolysis, following the cross-linking of homoallyl end groups at the exterior of the dendrimer by Grubbs ring-closing metathesis. They also showed that the imprinted dendrimer is selective, binding porphyrins with at least four hydrogen bond donor/acceptor sites in toluene. The imprinted cavity is also size-selective and although the template itself is too large to bind, porphyrins of smaller physical size and appropriate functionality complex strongly.

More recently Beil and Zimmerman [31] reported the preparation of an imprinted dendrimer with a less symmetrical template molecule with a view to producing an amine-selective sensor. Here, very high affinity binding was detected, from a three-point interaction, which included two hydrogen bonds and one covalent linkage to a reporter group that transduced binding by colour change. This monomolecular imprinting approach results in properties

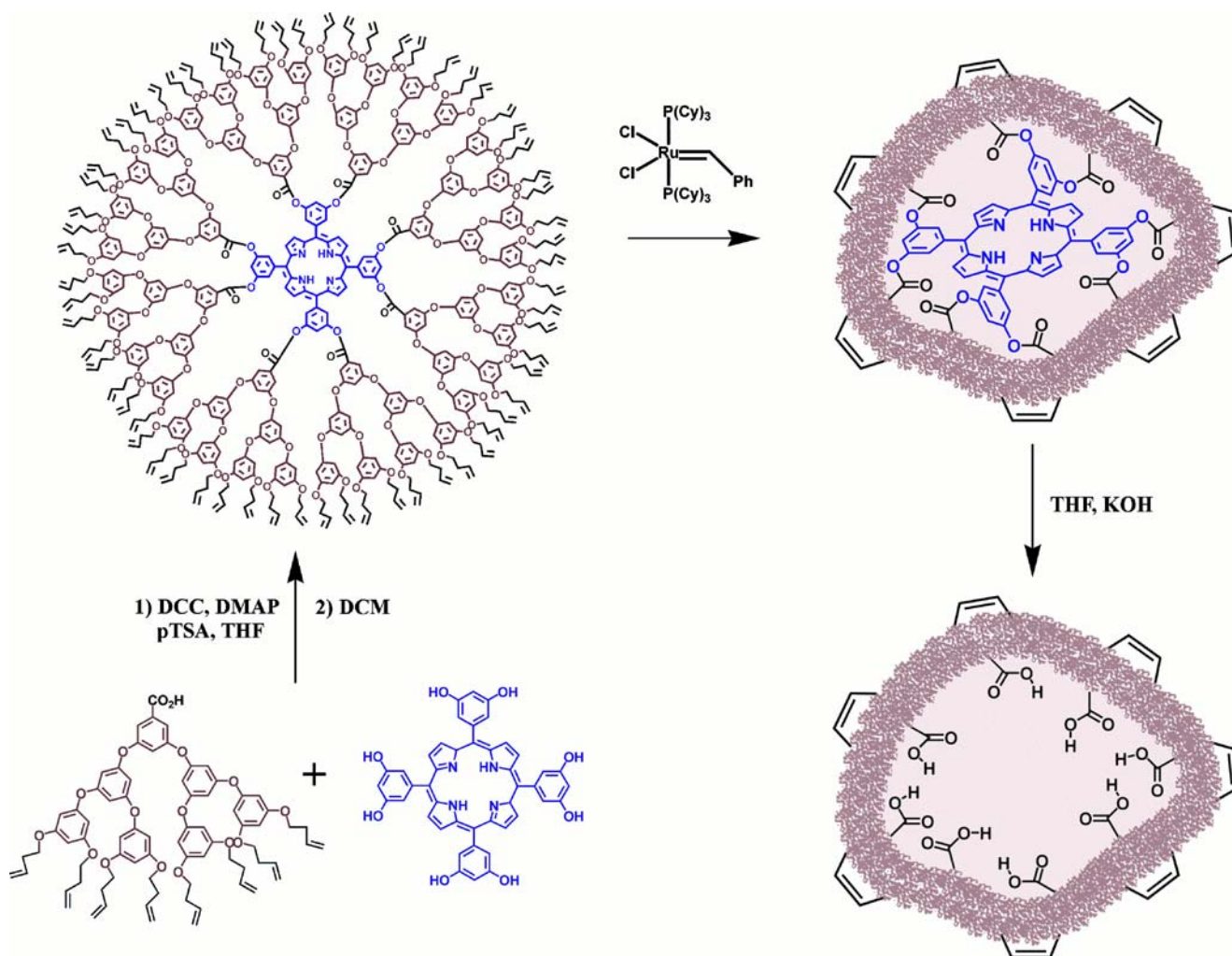


**Fig. 5** A monomolecular covalent imprinting strategy with dendrimers for the recognition of porphyrins. DCC *N,N*-dicyclohexylcarbodiimide, DMAP 4-dimethylaminopyridine, pTSA *p*-toluenesulfonic acid, THF tetrahydrofuran, DCM dichloromethane. (Adapted from [28])

difficult to achieve by conventional polymerization methods, such as high-efficiency imprinting, quantitative removal of the template, solubility of the imprinted material in common organic solvents, and possibility of separation of imperfectly assembled binding sites. However, limitations of this approach are the need for multistep synthesis of the dendrimers, the high dilution conditions needed for the ring-closing metathesis reaction and the incompatibility of many templates with this imprinting approach.

### Imprinting with diverse nanomaterials

As previously stated, when progressing from the macro-level/microlevel to the nanoscale, materials not only demonstrate advantages such as greater surface area and recognition site accessibility, but also start exhibiting new properties. A number of research groups have taken advantage of these altered properties, an example of which involved the incorporation of quantum dots (semiconductor nanoparticles that exhibit remarkable photoluminescence behaviour) into MIPs, as reported by Lin et al. [32, 33]. The authors demonstrated that binding to recognition sites in the vicinity of the fluorescent nanoparticles caused quenching of the photoluminescence, presumably owing to the fluorescence resonance energy transfer between quantum dots and template molecules.

More recently, Willner and co-workers employed gold nanoparticles for the electrochemical analysis of TNT, where an imprinting strategy was used for the enhancement of detection limits. In this case functionalized gold nanoparticle electrodes were electropolymerized and utilized as the sensitive electrochemical recognition layer. In the future, the use of such metallic or semiconductor nanoparticles in conjunction with molecular imprinting may well be of importance in the design and development of enhanced optical and electrochemical sensing methods

### Outlook

Nanomaterials are one of the most important targets of the developing field of nanotechnology. The advanced scientific knowledge acquired on the synthesis and physical-chemical characteristics of these materials has led to a considerable increase in the number of applications and commercial exploitations. The application of the imprinting technology to nanomaterials has provided a new insight offering the possibility to create tailored cavities with specific recognition properties.

Over the last decade, considerable effort has been devoted to the development of novel synthetic strategies leading to imprinted materials in a variety of formats. The

main focus has been applied to the development of various approaches for the production of imprinted nanoparticles and the reproducibility and efficiency related to the polymerization methods used. Although there have been significant advances in this area, widespread commercialization of such materials has yet to be realized. On the other hand, constant developments in the field of nanomaterials has led to various promising polymeric formats, such as soluble cross-linked imprinted dendrimers, microgels and nanogels, which more closely mimic the physical characteristics of enzymes and antibodies. Further formats such as nanofibres, nanowires and nanotubes have arisen and have been successfully used in conjunction with imprinting technology. These materials exploit the advantages of high surface-to-volume ratios and often also surface imprinting techniques that allow easy access of target molecules to recognition sites. The results in this field are the outcome of the increasing interest in the molecular imprinting approach by both academics and industrial enterprises and are expected to lead to significant applications.

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