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Review

Designing selective sites in templated polymers utilizing coordinative bonds

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Abstract

This review gives a survey over recent achievements on the design of selective sites in templated polymers. Particularly, coordinative bonds as driving force for the interaction between a substrate and a templated polymer are discussed. Recent achievements on the selective recognition of larger molecules, such as dipeptides and disaccharides, are highlighted that promise a fast development on biomolecule templated material towards enzyme-like catalysis in the up-coming years. Additionally, the achievements on the incorporation of catalytic centers based on transition metal complexes are summarized.

Keywords: Reviews; Selective sites; Coordinative bonds; Templated polymers

Contents

1. Introduction

The selective recognition of a given target molecule by a specific receptor is an important issue in chemistry and biology. Nature elegantly solves this challenging task by providing specific protein binding sites, utilizing hydrogen-bonding, π–π stacking interactions, metal coordination and charged amino acid residues not only to discriminate between different ligands, but also to enable selective transformations on them. Towards an increased understanding of these natural systems and towards the preparation of novel systems with unknown material properties, considerable attention has been devoted to the design of new highly selective devices based on the above mentioned molecular recognition principles [1].

Although the macromolecular structures of enzymes play a special role in their performance, many synthetic receptors are still small molecules [2]. Polymeric receptors, however, offer various advantages over low molecular weight compounds due to their macromolecular nature. They exhibit high selectivity due to cooperativity of functional groups, and dynamic effects, such as the induced fit, the allosteric effect. Steric strain can be modeled into the matrix, if a recognition center with pre-arranged binding sites can be defined in a three-dimensional neighborhood [3]. Polymers are reasonably stable against heat, chemicals, and solvents, and they can easily be fabricated in a form suitable for in-

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dustrial applications. Taking advantage of this, the preparation of selective recognition sites is central to the template polymerization approach and is used to prepare material for various purposes including applications in chromatography [4–6], medical diagnostics [7,8], solid phase extraction [9], sensors [10,11] or catalysis [3,12,13].

Simple mixing of target templates with polymerizable monomers followed by a one-pot polymerization procedure is unlikely to create outstanding material properties, such as highly selective, and ideally homogeneously shaped and distributed binding sites. Instead, the rational design of selective cavities is a difficult, time consuming and sometimes even frustrating task, which requires a careful investigation of the coordination properties of the compounds associated prior to immobilization in a highly cross-linked matrix. Driving forces applied for binding between templates and functional, i.e. with the template strongly interacting, monomers include hydrogen bonds [11], covalent bonds [3] and metal coordination [13,14]. As a random immobilization of functional monomers in a polymer reduces its selectivity, interactions between the compounds associated should be as strong as possible during the polymerization step—ideally they would be covalent [15]. Systems utilizing reversible covalent bonds often exhibit slow kinetics during rebinding and require harsh conditions for template removal after polymerization [15]. Hydrogen bonds are more versatile in binding strength than covalent bonds, but less suitable and effective for pre-arranging a template and a functional monomer in aqueous solution. As water remains the preferred solvent of nature and as the templated cavities are demonstrated to memorize the solvent used during templating [16,17], the appropriate choice for the solvent applied during material preparation and recognition events is of high importance. As a consequence, aqueous solutions should be used for preparation of biomolecule templated material. Although protic solvents such as alcohols and water are compatible with free radical polymerization, these have been largely excluded from use in template polymerization due to their ability to compete with hydrogen-bonding interactions [18]. Practical matters, however, such as lacking solubility of biomolecules in organic media, make water the solvent of choice [18], but require sufficiently strong binding interactions at the same time.

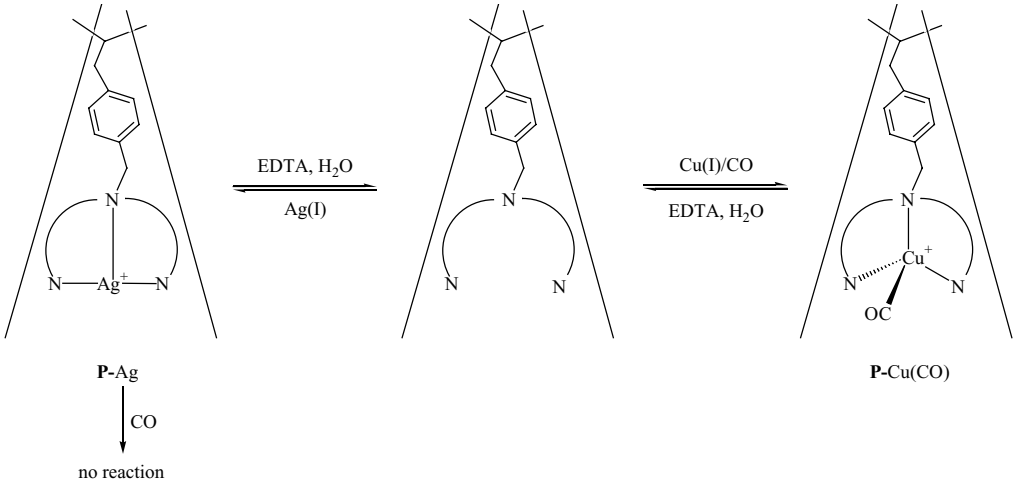
Metal coordination as binding force appears advantageous for several reasons as coordinative bonds are very flexible in strength, even in water. Additionally, metal-coordination based compounds can be thermodynamically stable and kinetically labile at the same time, which permits fast ligand exchange reactions on the metal center. Further advantages should be also emphasized: firstly, metal coordination is a spontaneous and kinetically fast process in aqueous solutions and organic solvents, which binding strengths can be adjusted as needed by choosing an appropriate coordinating metal ion; secondly, transition metal ions, such as copper(II), platinum(II) or iron(III), do not only act as binding partners but also have inherent catalytic properties, which can be utilized for a chemical transformation of a coordinated compound; thirdly, due to the unique electronic properties of transition metal ions in various oxidation states, their complexes can be characterized by diverse spectroscopic methods to investigate binding events and catalytic reactions; and lastly, ligands for transition metal complexes can be synthesized according to specific needs and used to fine-tune the selective and catalytic properties of the metal ion complex.

A key step for the preparation of molecularly imprinted polymers includes the pre-organization of the template and the functional monomer. In case of metal coordination as binding force, the functional monomer consists of a transition metal ion that is bound to a supporting ligand. Using a suitable polymerizable group of the supporting ligand, the resulting ternary template–metal ion–supporting ligand complex can be captured in a highly cross-linked matrix by radical polymerization of the solution in the presence of a large excess of cross-linking agent. The polymerization is commonly initiated thermally or by UV light in the presence of an appropriate initiator. After solidification of the polymerization solution, the template is removed during repeated washing steps, which also may result in partial removal of metal ions. Subsequent washing steps with metal salts, such as metal chlorides, are applied for metal ion reloading. If the nature of the metal ion is to be altered for the subsequent use of the material, washing steps with complexing ligands, such as EDTA, are preferred to remove *all* accessible metal ion content, prior to reloading a different one. Alternatively, repeated washing of the template free material with diluted metal ion solutions may lead to the same result. The metal ion reloaded matrix is consequently used for template rebinding experiments to estimate the fidelity of the templated sites or for the characterization of the matrix properties.

An intriguing example for the effect of metal ion exchange are Cu(I) and Ag(I) network polymers, which were used to demonstrate reversible binding of carbon monoxide, CO, to the immobilized Cu(I) metal site, while correspondingly derived Ag(I) sites did not enable rebinding of the gas. Instead CO binding is permitted after removal of Ag(I) ions and occupation of the metal site with Cu(I) ions (Scheme 1) [19].

Isolation of metal coordinating binding sites was subsequently demonstrated with oxygen binding to immobilized cobalt salen complexes, which form dimeric peroxide species in solution [20].

Applying the bait- and switch approach [21,22] to templated polymers, the binding strength of the ternary template–metal ion–supporting ligand complex can be altered for subsequent recognition experiments by the choice of the metal ion, which, consequently, also changes the correlated binding properties of the metal coordinating matrix [23,24]. Following that procedure, a high-fidelity imprint can be obtained by using strong coordination between the target template and a metal complex during material preparation, while weak binding interactions are used to explore the prepared rebinding sites afterwards [23,24]. Nevertheless, some limitations due to the nature of the metal ion



Scheme 1. Investigation of Cu(I) and Ag(I) network polymers for reversible binding of carbon monoxide, CO.

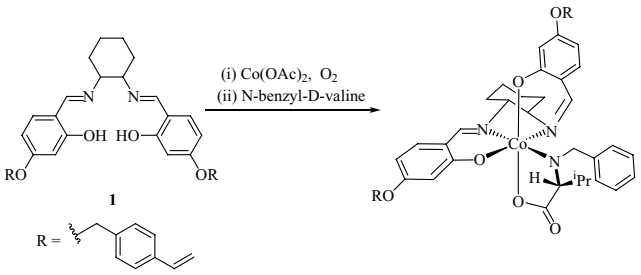
applied should be illustrated comparing titanium(IV) and cobalt(III) ions as representative examples: Ti(IV) ions coordinate strongly to oxygen donor ligands, whereas Co(III) ions also form complexes with nitrogen donors; Ti(IV) complexes strongly prefer a coordination number of 4 with a tetrahedral geometry, while cobalt(III) complexes preferably exist in coordination number 6 with octahedral arrangement of the surrounding ligands. As a consequence, the functionality of the chosen template largely dominates the choice of the metal complexes applicable for strong coordination, whereas Lewis acidity or redox capability associated with a particular metal ion might be beneficially used in subsequent recognition events or for catalytical transformation of substrates afterwards. The preferred coordination mode of the metal complexes under consideration should be carefully evaluated to ensure that the immobilized binding sites in the templated matrix are able to uptake the desired ions and, thus, gain full functionality. Nevertheless, the very different nature of metal ions in size, preferred donor ligands, coordination numbers or spatial arrangements of donor ligands enables a flexible design of the binding interactions between matrix and template.

The frequently claimed selectivity obtained for metal ions in 'metal templated' sites [25–63], however, is often in contrast to these distinct differences in coordination characteristics of metal ions as result of their diverse nature. Appropriate control experiments on the 'metal-templated' material are therefore mandatory and should ensure that the claimed 'metal ion discrimination' capability is truly related to a templating effect and not due to other, more conventional incentives for recognition, such as a preferred ion radius [64], thermodynamic parameters or a higher binding strength of a certain metal to the supporting ligand immobilized in the matrix. Comparing the metal coordination capability of the supporting ligand in solution with that found in the material certainly helps to clarify this task, while only distinct differences in the performance of the material from that of the metal complex in solution account for a template effect. As metal containing species usually exist in several equilibria in solution, an in-depth investigation should also cover the coordination mode and the stoichiometry upon complex formation between the supporting ligand and the metal ion prior to material preparation. It is commonly known that the molar ratio of metals to supporting ligands provide a sensitive tool to direct the stoichiometry of the complexes formed and may be characterized by diagrams of the distribution of species [65]. However, appropriate control experiments on the obtained material also include a comparison of the rebinding performance of metal ions, which show the same coordination capability to the supporting ligand in solution. To choose metal ions, which show distinct differences in coordination already in solution, is not well suited to verify a template effect, but rather shows the binding characteristics of the immobilized supporting ligand.

2. Recognition sites for amino acids, peptides and proteins

Most complexes derived from Co(III) are kinetically inert and therefore very stable against ligand exchange reactions. Taking advantage of this fact, complexes consisting of D-phenylalanine [66], or *N*-benzyl-D-valine, respectively [67], and the *N*,*N*′-bis(2-hydroxybenzaldehyde)cyclohexyldiimin cobalt(III) complex **1** as functional monomer were prepared from Co(II) acetate in the presence of excess oxygen (Scheme 2).

The stoichiometry and the geometry of various amino acid–**1** complexes were studied by single crystal structure X-ray analysis in the solid state [68,69]. Additionally, a UV-Vis, CD and ^{1}H NMR spectroscopic evaluation established the formation and stoichiometry of the complexes in solution prior to immobilization of the assembly in a styryl–divinylbenzyl copolymer. The D-phenylalanine–**1** complex was subsequently used for the preparation of a templated polymer [66]. After the cleavage of the template

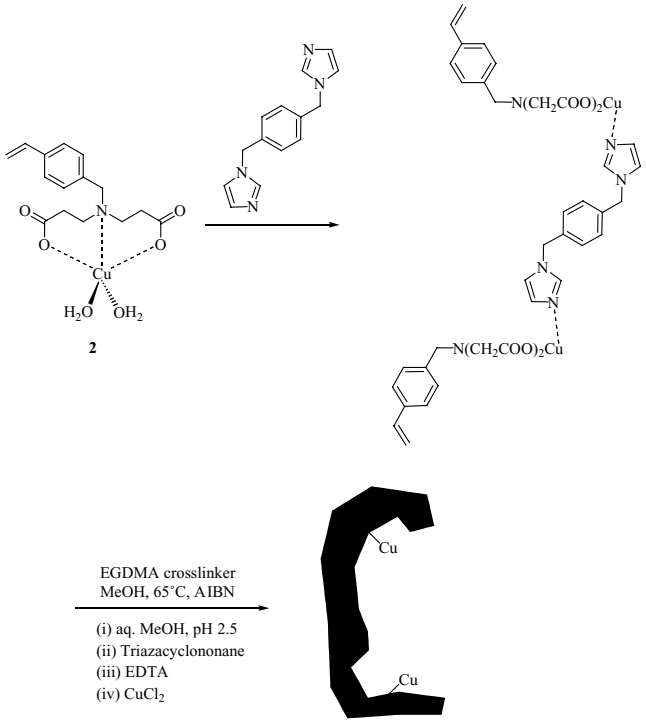


Scheme 2. Formation of a chiral complex from *N*-benzyl-D-valine and the *N*,*N*′-bis(2-hydroxybenzaldehyde)cyclohexyldiimin cobalt(III) complex **1** [67].

amino acid from the immobilized metal site, Fujii and co-workers evaluated in batch mode rebinding experiments with racemic D,L-phenylalanine the amino acid rebinding capability of the D-phenylalanine templated material with respect to a control polymer, which was prepared without template as well as in relation to the amino acid discrimination capability of the complex **1** itself. Although the functional monomer **1** shows some amino acid discrimination in solution, the chiral discrimination capability of the templated polymer is definitely larger and thus demonstrates a template effect. The control polymer is not selective at all, but coordinates a very high amount of amino acid as well [66]. The investigation was repeated for a *N*-benzyl-D-valine templated polymer with similar results [67].

For practical applications of a templated material in chromatography, however, a more flexible complex for amino acid coordination, which is not substitution-inert, is preferable to allow fast and selective ligand exchange reactions of amino acids [70]. Such a system is particularly interesting as a sensitive tool for the purification of proteins based on the selective recognition of imidazole groups of surface-exposed histidine residues, such as demonstrated by immobilized metal affinity chromatography (IMAC) [71–74] or ligand exchange based separations [75,76] (Scheme 3).

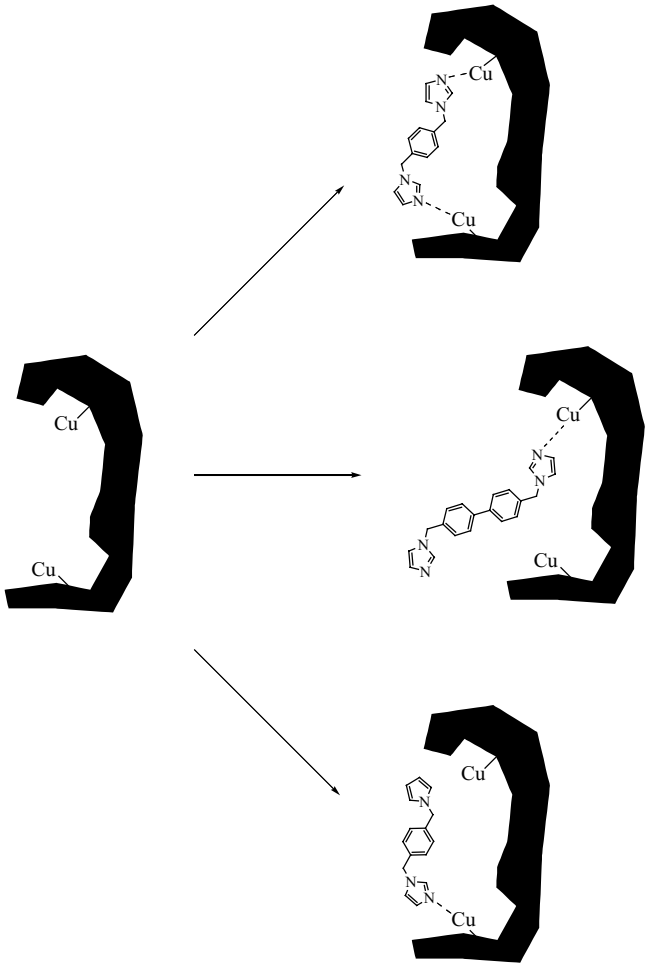
Towards this overall goal, different bis-imidazole molecules, which were inseparable on a conventional reversed-phase material, were used as small protein analogs for a model study with copper(II) iminodiacetic acid **2** as functional monomer [15,70,77–79]. Based on the stoichiometry of the compounds evaluated in solution, the interaction between the immobilized copper(II) complexes and various imidazole based substrates demonstrated the polymer's potential to distinguish bis(imidazole) substrates that differ only slightly in the spatial arrangements of the imidazole ligands (Scheme 4). The microstructure of the binding cavities and the distribution of the metal ion complexes were found to be significantly different in both templated and random polymers. The strong Cu(II)–imidazole interactions, however, desirable for creating a high-fidelity imprint, lead to excessive retention in elution chromatography [23]. Therefore, thin coatings of templated, metal complexing polymers have been grafted to activated silica



Scheme 3. Preparation of bis(imidazole) templated polymers based on copper(II) iminodiacetic acid 2 as functional monomer [15,77].

beads suitable for high-performance liquid chromatography (HPLC) [80]. By replacing the copper in the templated metal-complexing polymers with weaker binding Zn(II) ions, these ligand exchange supports can effect partial to complete chromatographic separation of their bis-imidazole templated from other similar imidazole-containing substrates. All together, these model studies revealed promising features of metal coordination for the preparation of highly specific templated polymers capable for recognition of biological molecules in recognition and separation material via the arrangements of metal-coordinating ligands on the material surfaces [15,23,70,75,77,78,80].

However, the same idea on improving the capabilities on synthetic material for protein purification by specifically placed metal complexes on an templated adsorbent was followed in simultaneous studies by the Mosbach group [81]. Taking advantage of the two surface-exposed histidine groups of the enzyme bovine pancreatic ribonuclease A (RNase A), which are capable of co-ordinating metal ions [71], chelating monomer **2** was polymerized onto methacrylate-derivatized silica particles in the presence of this enzyme. After the necessary washing steps to remove the template, the prepared adsorbent was used in subsequent rebinding experiments as stationary phase and investigated by high-performance liquid chromatography. The study revealed separation of RNase A from lysozyme, when the prepared material contained metal ions. The mechanism and the origin of the observed selectivity, however, remained unexplored.



Scheme 4. Rebinding studies with different bis(imidazole) derivatives as small protein analogues [15].

The Arnold group focused instead in more detail on the recognition of natural, underivatized aliphatic and aromatic amino acids in order to evaluate the role of the side group in imparting enantioselectivity into templated material [75]. Coordinating a chiral amino acid with an achiral monomer, such as **2**, that cannot itself distinguish between L- and D-amino acids, and subsequent immobilization of the resulting chiral amino acid–**2** complex demonstrated that enantioselectivity of the adsorbent arises form the chirality of the recognition sites created during polymerization [82]. A silica coated D-phenylalanine imprinted material allows the enantioselective resolution of a D,L-phenylalanine (**3**) racemate, but it is not capable of recognizing the small differences between **3** and tyrosine (**4**), which consequently results in resolution of a D,L-tyrosine racemate on the D-phenylalanine imprinted matrix as well (Fig. 1) [82].

The origin of enantioselectivity of a target molecule in amino acid templated polymers is discussed in view of the three-point binding model introduced by Davankov [76,83] as a requirement for chiral recognition. Enantiomers of other related amines or amino acids with smaller side groups compared to phenylalanine, such as D,L-alanine and D,L-valine racemates, also fit into binding sites that distinguish enantiomers of phenylalanine and may be retained on the material during chromatographic evaluation. However, as the number of binding interactions with the material is smaller than for the original template, the retardation is not due to the same binding mechanism and, in view of the Davankov model, not enantioselectively. This conclusion was further supported by chromatographic experiments with racemic α-methylphenethylamine **5** and α-methylhydrocinnamic acid **6**, which provide only monodentate binding to the immobilized metal center of the D-phenylalanine imprinted matrix (Fig. 2). Although the D-**3** imprinted material was able to resolve the chiral amine **5** (Fig. 1c), the chiral carboxylic acid **6** still eluted early without being resolved. Further attempts to resolve the chiral carboxylic acid by reducing the pH were unsuccessful.

Furthermore, cross-selectivity for related amino acids depends on side chain size: materials templated with L- or D-phenylalanine exhibit good enantioselectivity when

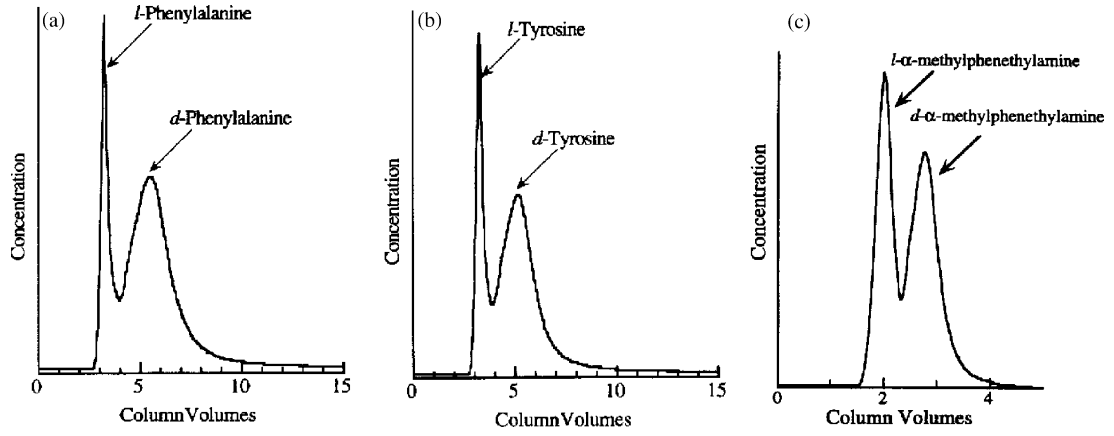
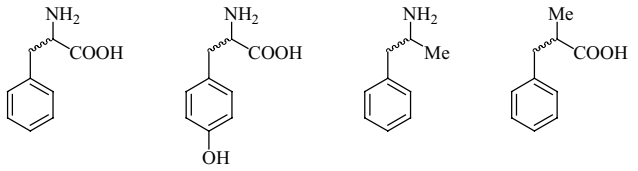


Fig. 1. Chromatographic resolution of (a) D,L-phenylalanine, (b) D,L-tyrosine and (c) D,L-α-methylphenethylamine on D-phenylalanine-imprinted polymer-coated silica (50 mm × 4.6 mm i.d. column). Peak identification was confirmed by comparison with the retention times of the pure enantiomers. Sample size: 100 μl of 1 mM solution. Running conditions: 1 ml min^{-1}, 50 °C, 1.5 mM glycine or 1.5 mM acetate, pH 8. Chromatographic separation factors are (a) 1.65 for D,L-phenylalanine, (b) 1.54 for D,L-tyrosine, and (c) 1.32 for α-methylphenethylamine. Reprinted with permission of Elsevier Science from Ref. [82].



3 4 5 6

Fig. 2. Phenylalanine (**3**), tyrosine (**4**) and monodentate phenylalanine analogs α-methylphenethylamine (**5**) and methylhydrocinnamic acid (**6**).

challenged with racemic tyrosine, but much reduced enantioselectivity towards D,L-tryptophan or aliphatic amino acids. To further improve the enantioselectivity of templated materials, Arnold suggests to choose other appropriate chiral functional monomers or, alternatively, to use co-monomers or cross-linkers that provide additional binding interactions with the side groups of the amino acid templates, provided the stabilization is preferential of the targeted enantiomers pairs [82].

Towards this end, Shea and Hart focused on the preparation of selective peptide receptors based on the coordination of histidine containing amino acids or oligopeptides and nickel(II)nitrilotriacetic acid **7** [84]. The strategy is taking advantage of the high affinity of N-terminal histidine and amino residues for Ni(II), which has been observed and used for the preparation of nonselective adsorbent for protein purification by Hochuli earlier [85–87]. The supporting ligand nitrilotriacetic acid (NTA) occupies four positions in the octahedral coordination sphere of the Ni(II) ions leaving the remaining two for selective interactions with a terminal amine of a pyridine nitrogen or a imidazole ring with high affinity [18]. Consequently, a His-Ala (HA) peptide was used for preparation of the templated receptors. Evidence for formation of a 1:1 **7**–HA complex was obtained by UV-Vis spectroscopy, negative ion electrospray mass spectrometry and calculations of the distribution of species based on potentiometric titrations prior to polymerization in solution [84] (Scheme 5).

Subsequently, the **7**–HA complex was immobilized from aqueous solution in poly(acrylamide) matrices with varying cross-link density, which were studied thereafter for peptide recognition. The investigation on the peptide rebinding capability reveals an interesting relationship between cross-link density and both selectivity and absolute capacity of the obtained polymers (Fig. 3).

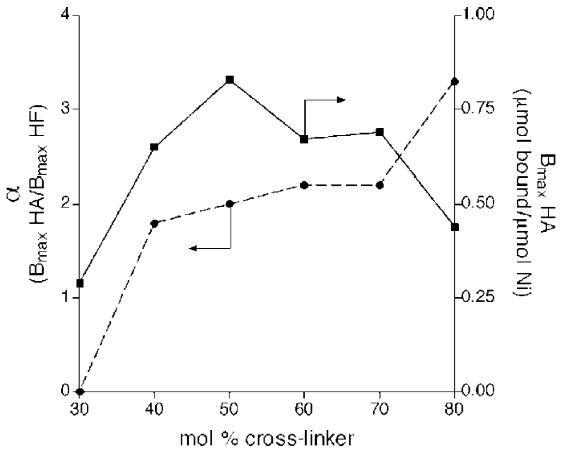
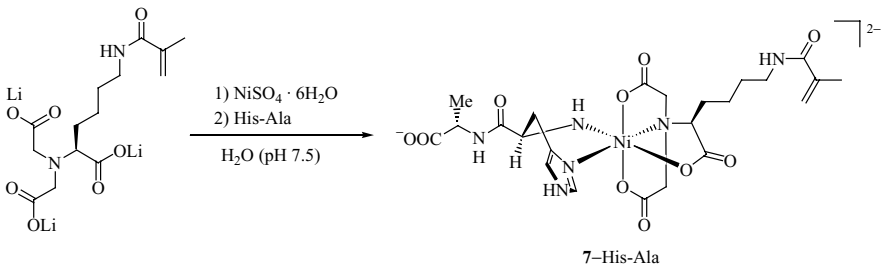


Fig. 3. Graph relating the change in selectivity α (left Y axis) and the maximal capacity B_{max} for the His-Ala (HA) peptide (right Y axis) with mol% cross-linker in the polymer. Reprinted with permission of the American Chemical Society from Ref. [84].

The capacity, as represented by B_{max} for His-Ala, is maximized at around 50 mol% cross-linker. The decrease in capacity at the higher cross-linking levels is ascribed to inaccessibility of some binding sites, while the decrease in uptake at lower levels of cross-linking may be due to hydrophobic collapse of the binding cavities. It seems likely that this phenomenon also results from some site inaccessibility within the polymer. However, Hart and Shea observed highest selectivity (α) for the largest cross-link content, which is in line with earlier investigations on the influence of the degree of cross-linking on the resulting material discrimination capability [88]. Additional batch rebinding analysis of the N-terminal histidine peptides His-Phe, His-Ala, and His-Ala-Phe on a His-Phe templated peptide showed no inherent difference in the affinity for the examined dipeptides. Extension of this methodology by using the pentapeptide His-$(Ala)_4$ showed a clear preference for the rebinding of small dipeptides substrates instead of the pentapeptide template. Furthermore, bulky residues, such as phenylalanine, do not cause a difference in uptake of peptides, even when they occupy the second position of the sequence. Thus, the results on oligopeptide recognition rather account for selective



Scheme 5. Synthesis of the **7**–HA complex [84].

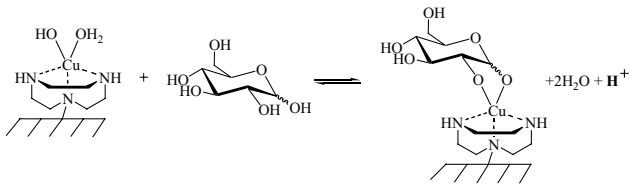
uptake in the polymer based on the overall size of the peptide applied only than for a template effect during material preparation. Nevertheless, the investigation revealed that the Ni(II)–NTA complex provides a strong histidine binding site that draws histidine dipeptides to the polymer surface [84].

3. Recognition sites for carbohydrates

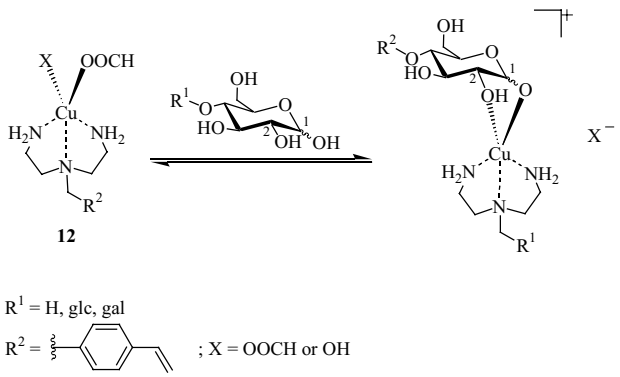
Metal coordinative bonds were also used to prepare a glucose sensing polymer by templating a poly(acrylamide) matrix with β-methyl-D-glucopyranoside [89]. At alkaline pH [90], the metal-complexing polymer binds glucose and instantly releases protons in proportion to the glucose concentration over the clinically relevant range (0–25 mM) (Scheme 6). The amount of liberated protons can be conveniently quantified by a pH electrode. The polymer's ability to function at nonphysiological pH, at which the buffer capacity of biological samples is small, has been also demonstrated for glucose determination in porcine plasma.

Discrimination of epimeric monosaccharides, such as D-glucose (**8**), D-mannose (**9**) and D-galactose (**10**), has been subsequently achieved by templating poly(acrylate) matrices with underivatized carbohydrates coordinated to the mononuclear copper(II) complex [(*N*-styryldiethylenetriamine)copper(II)] diformate (**11**). Using UV light for initiation of the radical formation, the preorganized carbohydrate–**11** complexes [91] were captured in the matrix at ambient temperature [92]. The selectivity factors *a* obtained for a D-glucose templated matrix in batch mode competition rebinding experiments with D-galactose and D-mannose were determined to be 8.3 and 9.0, respectively, at neutral pH. However, similarly prepared matrices failed to recognize disaccharides, such as D-lactose (**12**) or D-maltose (**13**) with sufficient capacity and selectivity [93]. Although the coordination mode of the carbohydrate–metal complexes remains 1:1 and the binding strength pK_{app} of the carbohydrates to the immobilized mononuclear copper(II) complex **11** remain comparable (p$K_{app} \approx 3.4$) for the investigated carbohydrates in aqueous alkaline solution (Scheme 7) [91], the rebinding of the monosaccharide D-glucose in a disaccharide templated material is always largely favored.

To overcome this shortcoming, oligosaccharide templated poly(acrylates) based on cross-linking monomers, which differ in the number of hydroxyl and acrylate groups (Fig. 4), are prepared to enable hydrogen bonding between the matrix



Scheme 6. D-Glucose (**8**) binding to an immobilized TACN–Cu^{2+} complex at alkaline pH results in the release of molar amounts of protons.



Scheme 7. Coordination of carbohydrates, such as D-glucose (**8**), D-maltose (**13**), D-lactose (**12**) or D-cellobiose (**14**) to mononuclear copper(II) complex (**11**).

and the carbohydrate substrate during rebinding [94]. While the metal coordination is strong, and therefore dominating, at alkaline conditions chosen for material preparation, chelation of the sugar with copper(II) complexes at neutral pH (as used for rebinding) is weak. Thus, hydrogen bonding in addition to coordinative bonds can noticeably contribute to the overall recognition capability during rebinding experiments.

While poly(acrylates) derived from pentaerythritol tetraacrylate (**15**) and pentaerythritol triacrylate (**16**) do not rebind considerable amounts of disaccharides or oligosaccharides [93], polymers derived from the more polar cross-linking monomer triglycerolate diacrylate (**17**) or templated matrices prepared from a combination of **17** and polymerizable monomer 2-hydroxyethyl acrylate (**18**) do. The capacity of disaccharide rebinding is increasing with enlarged polarity of the surrounding matrix. However, the selectivity of the templated material is still slightly in favor of the monosaccharide D-glucose, although a disaccharide template, such as D-lactose (**12**), is becoming increasingly competitive with enlarged polarity and flexibility of the binding site surrounding polymer backbone (Fig. 5).

Although the *highest capacity* for D-lactose rebinding was observed in templated polymers derived from a combination of cross-linking monomer **17** and **18**, the *highest selectivity* between structurally related disaccharides with identical binding site in the reducing sugar moiety, such as D-lactose

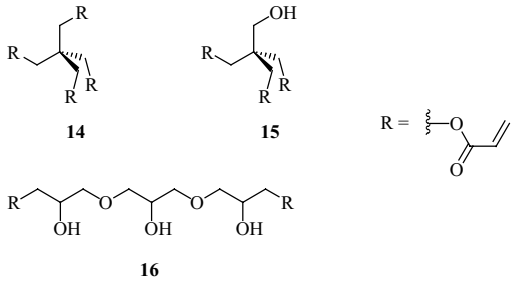


Fig. 4. Chemical structures of the cross-linking monomers pentaerythritol tetraacrylate (**15**), pentaerythritol triacrylate (**16**) and triglycerolate diacrylate (**17**).

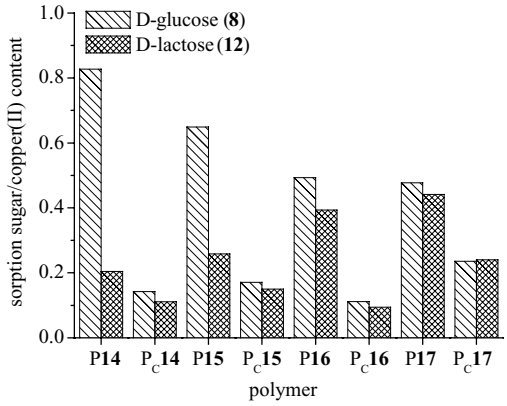


Fig. 5. Saturation rebinding experiments for the copper containing sugar binding sites under competition conditions using equimolar mixtures of D-glucose (**8**) and D-lactose (**12**) in D-lactose templated poly(acrylates) P**15**, P**16**, P**17**, P**18** with respect to the control polymers P_C**15**, P_C**16**, P_C**17**, P_C**18** templated with ethylene glycol.

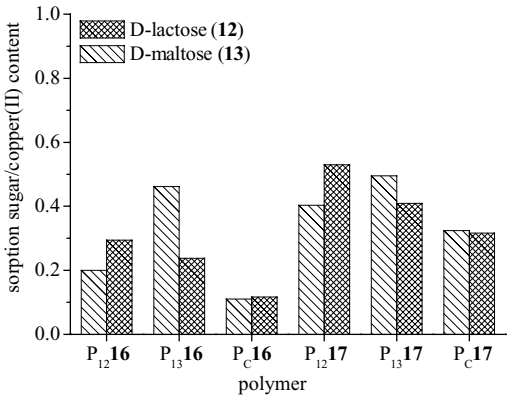


Fig. 6. Selectivity of D-lactose (**12**) and D-maltose (**13**) templated polymers P_{12}**17**, P_{13}**17**, P_{12}**18**, and P_{13}**18** for the saccharides **12** and **13** compared to control polymers P_C**17** and P_C**18** in competition, saturation rebinding experiments of equimolar mixtures of the disaccharides **12** and **13** at neutral pH.

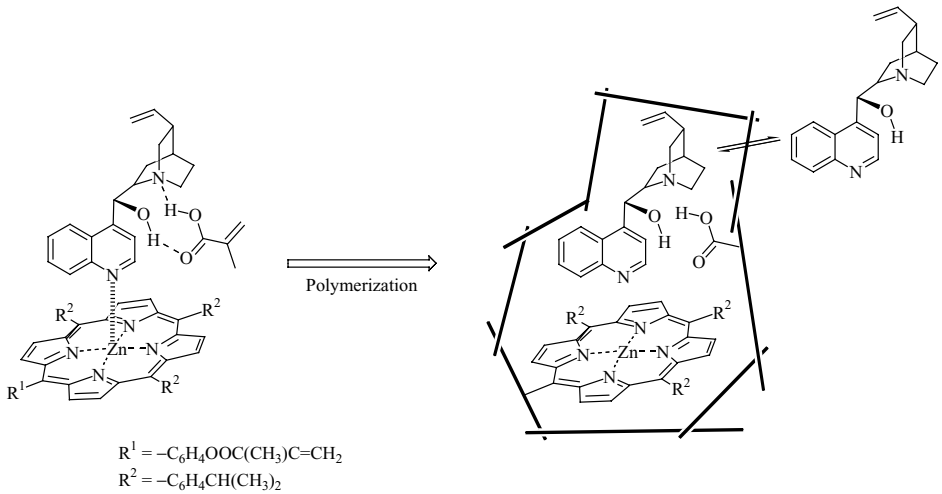
and D-maltose, is observed in the more rigid, but highly polar polymer derived from **17** only (Fig. 6).

The study therefore demonstrated a sufficient discrimination capability of polar polymers for two relevant and structurally closely related disaccharides, D-maltose, D-glucopyranosyl-(1 → 4α)-D-glucose, and D-lactose, D-galactopyranosyl-(1 → 4β)-D-glucose, in both selectivity and capacity towards the preparation of sugar-selective sites for polymeric mimics of glycoside transforming enzymes [91]. The application of aqueous environment for both the *polymerization and recognition experiments*, is similar to the approach of Shea, who reported this strategy to recognize oligopeptides first [18,84].

4. Recognition sites for other templates

In a similar context as discussed for the oligosaccharide recognition capability of templated matrices, Takeuchi and Matsui evaluated (–)cinchonidine templated poly (methacrylates). Combined interactions based on metal coordination and hydrogen bonding between template of matrix result in a material with superior rebinding capabilities as compared to control polymers based on either metal coordinative bonds or hydrogen bonds solely (Scheme 8) [95]. As only single point interaction of the template towards a Zn(II) porphyrins functional monomer is allowed by the design of the binding site, an even larger effect between the polymers incorporating different rebinding forces might be achieved, when template chelation to the metal site is enabled. Interestingly, the template retention properties of the cinchonidine templated sites are more than 20-times larger in polymers based on coordinative bonds than for that based on hydrogen bonds. Additionally, the polymer enabling hydrogen bonds between matrix and template shows only a slightly better performance on template recognition than a non-templated control polymer. In combination with other rebinding experiments, Takeuchi and Matsui conclude for both polymers based on metal coordination a similar number of high-affinity sites, while the overall number of both high- and low-affinity binding sites in the polymer based on hydrogen bonds solely appears significantly lower. Experiments in solution addressing the composition of the coordination compounds formed from methacrylic acid, Zn(II) porphyrins and the template in the pre-polymerization mixture are, however, not given [95].

Although the template removal from the templated sites is not generally problematic, when coordinative bonds are used for association, the distribution of binding sites resulting in heterogeneous matrices after polymerization certainly is. This problem tackles the concept of the molecular templating approach, as the chain growth during preparation of cross-linked material necessarily reaches the gel point after a certain time, at which the growing polymer chains starts to precipitate out of the polymerization mixture resulting in a heterogeneous system, even if the pre-polymerization mixture was homogenous at the polymerization start. Various attempts to characterize and overcome the often observed heterogeneity of sites have been recently described [96–99]. However, even more favorable than optimizing the problems associated with heterogeneity would be to circumvent the precipitation of the material out of the templating mixture and the responsible gel point of the system at all. A very elegant approach in this direction uses soluble dendritic hosts, which are also amenable to the incorporation of other functional groups [100]. Templating of a dendritic matrix with porphyrins produced macromolecules, from which the template is conveniently removed due to sufficient solubility of the dendrimer in common organic solvents. Moreover, separation of imperfectly assembled binding sites can be conveniently achieved by common chromatographic purification techniques resulting in a nearly homogenous distribution of templated sites. Current limitations of this approach include the need for multi-step synthesis of the material, which is in contrast to most convenient polymerization protocols of templated polymers. However, high efficiency with nearly



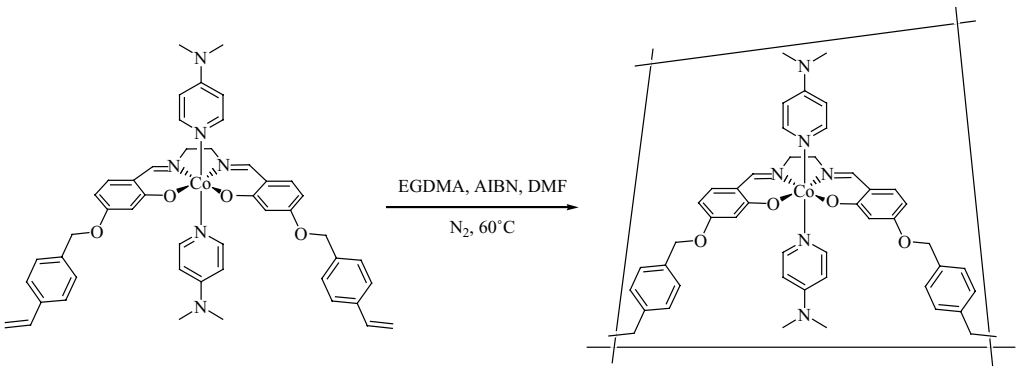
Scheme 8. Utilizing metal coordination and hydrogen bonding for preparation of (−)cinchonidine templated poly(methacrylates) [95].

all templates producing functional binding sites, and reasonable solubility of the templated material in common organic solvents as well as enabled separation of imperfectly assembled binding sites reimburse for the synthetic efforts making this approach very promising for future developments of templated polymers.

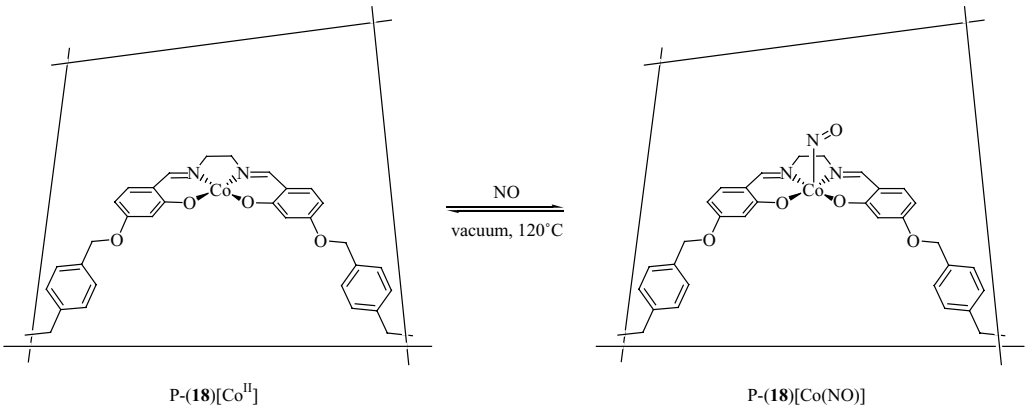
Taking advantage of the established rebinding properties achieved for carbon monoxide [19] and oxygen [20] due to site isolation of immobilized metal complexes in macroporous polymers [101], a polymer for *reversible* binding of the biologically important nitrogen oxide, NO, was prepared under similar conditions applying 4-dimethylaminopyridine, dmap, as template and a cobalt(III) complex of bis[2-hydroxy-4-(4-vinylbenzylmethoxy)benzaldehyde]ethylenediimine (**19**) as functional monomer (Scheme 9) [102].

The polymer has a significantly higher affinity for NO compared to oxygen, carbon dioxide and carbon monoxide. Spectroscopic measurements confirm that NO binding occurs at coordinatively unsaturated Co(II) sites, which are monodispersed within the porous host. Binding of NO causes an immediate, reversible color change of the material from orange to brown–green, indicating coordination of NO to the site-isolated Co(II) centers (Scheme 10).

It has been proposed that the formation of [Co(III)(salen)(NO_2)] proceeds in solution at room temperature through the readily formed dimeric intermediate [(salen)Co(III)(ON–O–O–NO)Co(III)(salen)] [102]. However, site isolation by the polymeric host, as demonstrated by Borovik et al., stabilizes the resulting immobilized [Co(II)(**19**)(NO)] complex and prevents the oxidation to an Co(III) nitrite complex, [Co(III)(**19**)(NO_2)]. The reversible coordination of NO has been confirmed by EPR, electronic absorbance, and X-ray absorption spectroscopies. At room temperature and atmospheric pressure, 40% conversion of the NO binding immobilized complex [Co(II)(**19**)(NO)] to [Co(II)(**19**)] is achieved in 14 days; under vacuum at 120 °C this conversion is complete in about 1 h. The binding of NO to immobilized [Co(II)(**19**)] is also observed when the polymer is suspended in liquids, includings water.



Scheme 9. Preparation of templated polymers derived from a cobalt(II) complex of the bis[2-hydroxy-4-(4-vinylbenzylmethoxy)benzaldehyde]ethylenediimine (**19**) ligand for reversible NO binding.



Scheme 10. Reversible binding of NO to immobilized cobalt sites [Co(II)(**19**)] with eye-visible color change of the templated material from orange to green–brown.

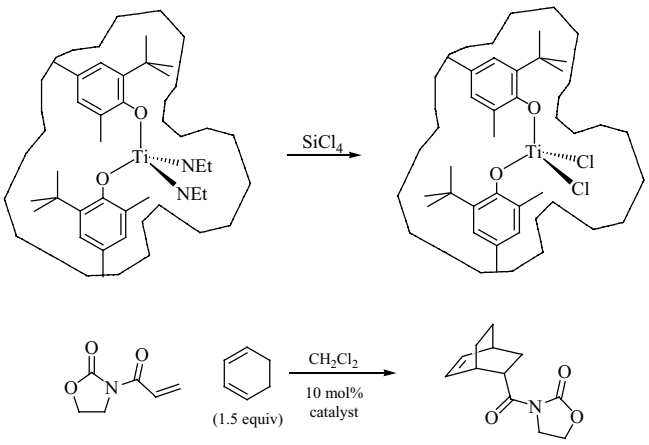
5. Metal ions in selective, catalytically active sites

The preparation of functional cavities with predefined shape and predetermined arrangement of functional groups is often based on the desire to perform stereoselective and regioselective reactions with the obtained material [103]. While the first coordination sphere of a transition metal ion is of high importance in homogeneous catalysis, while the design of the surrounding supporting ligands has been demonstrated to direct the stereo- and regioselectivity of the resulting complexes [104–106]. In heterogeneous catalysis, such as in imprinted polymers, the second coordination sphere might be utilized to impart additional points of interaction during subsequent recognition events, to facilitate selective substrate binding or transformation. Although these considerations are conceptually very attractive, only few molecularly imprinted catalysts, which aimed at installing active metal complexes into well-defined outersphere environments, are reported [3,13,14,107–109] and recently reviewed [13,110]. This is somewhat surprising, as the use of metal ion mediated recognition is certainly an area of great potential, special for selective transformations of substrates as required in asymmetric synthesis. The emphasis for preparation of catalytically active material, though, has been rather on creating templated polymers with active sites utilizing enzyme-like motifs to accelerate reactions [3,108,111,112] than on the incorporation of catalytic centers based on transition metals [110]. Initial model studies, such as the demonstration of carbon-carbon bond formation with correspondingly designed Co(II) sites, revealed that at least moderate reaction rates combined with sufficient selectivity can be achieved [1,113].

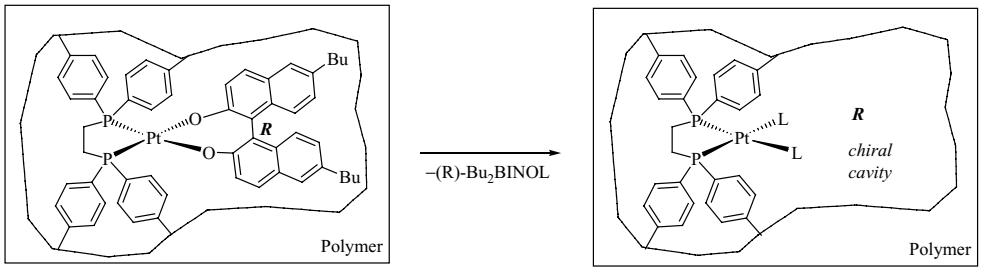
Gagne recently incorporated catalytical reactivity into polymers in form of a Lewis acid titanium complex, which has been combined with a templated environment to control the selectivity of the immobilized catalyst (Scheme 11) [108]. After activation of the precursor form of the dormant catalyst, the resulting polymer is only 3–5 times slower than a homogeneous catalyst in a Diels–Alder model reaction [108].

Encouraged by this result, the same group demonstrated with immobilized achiral platinum based complexes that the associated cavities can impart high selectivity to reactions on the metal center, when the surrounding matrix is templated with a bulky, axially chiral (*R*)-1,1′-bi-2-naphthol [(*R*)-BINOL] template (Scheme 12) [114]. In-depth investigation of the recognition capability of the prepared material revealed that the amount of templating ligand released by treatment of the templated matrix is inversely related to the steric bulk of that reagent, implying that a distribution of Pt sites with varying accessibilities exists within the polymer.

The template-free polymers contain chiral cavities, each associated with a reactive Pt center, which range from poor to high enantioselective recognition capability of imprinting ligand analogues during stoichiometric ligand-exchange reactions at the Pt centers [114]. The accessibility of a given Pt site depends on the level of outer-sphere definition of its associated chiral cavity. Platinum centers associated with loosely defined, open cavities are easily accessible to incom-



Scheme 11. Activation of the immobilized Lewis acid catalyst and employed model reaction.



Scheme 12. Preparation of chiral, (*R*)-Bu_2-BINOL templated cavities around catalytically active Pt centers in two different polymers, in which either L = Cl or OAr [114].

ing reagents, but poorly selective, whereas Pt centers with extremely well-defined cavities are so tightly bounded by the polymer as to be completely unreactive, although highly selective. Measurement of the kinetic selectivity of the *least reactive* Pt sites in the templated polymer reveals that selectivities up to 94% ee can be realized through predominantly *outer-sphere* effects. However, the aggregate selectivity of the templated polymer decreases considerably to 65% ee, when the effects of all sites, both reactive and unreactive, are taken into account. These observations imply dramatic consequences for utilizing metal complexes in templated material in asymmetric catalysis: the least selective sites will be the most reactive and will therefore dominate the templated catalyst's product output. To overcome this drawback, the most reactive sites have been selectively poisoned [115]. First experiments indicate that while the chiral cavity can differentiate the chiral poisons, it is the chiral diphosphine backbone ligand—and not a templating effect—which controls the enantioselectivity of the resulting ene product [115].

Applying the same principle of separating reactivity and selectivity around a catalytically active metal center, Polborn and Severin utilized polymers templated with organometallic transition state analoga of Rh and Ru based complexes to enable a selective hydrogen transfer for the reduction of aromatic ketones (Fig. 7) [13].

However, both similar reaction rates and selectivity for the enantioselective reduction of aromatic ketones can be obtained without the synthesis of Ru or Rh containing transition state analogues as demonstrated by Locatelli's group, who utilized a simple alcoholate template derived from (*R*)-1-phenylethanol earlier [116,117]. The best alcoholate templated catalyst (91% conversion after 9 days, 66% ee (*R*)) displays activity and selectivity, which can compete with the homogeneous catalyst (100% conversion after 7 days, 67% ee (*R*)). The catalyst performance even exceeds the homogeneous catalyst slightly, when embedded in carefully optimized and cross-linked matrix (70% ee (*R*)) and shows even better results in both reaction rates and enantioselectivity of the resulting alcohol produces, when compared to a related supported hydrogenation catalysts (96% conversion after 6 days, 47% ee (*R*) at 25 °C; 98% conversion after 1 day, 25% ee (*R*) at 60 °C) [104,110,118]. Other homogeneous catalysts, however, such as [Rh(norbornadien)Cl_2]/DIOP achieve 55–84% enantiomeric excess for the hydrogenation of acetophenon at 50 °C and 70 bar H_2 [119], while catalysts based on Ru and 2,2′-bis(dipheny1phosphino)-1,l′-dinaphthyl (BINAP) transform functionalized aromatic or aliphatic ketones with almost 100% ee [120]. The use of hydrogen gas, though, is elegantly avoided in the catalyst system used by Locatelli. Recently, templated palladium catalysts have been also applied for a Suzuki model reaction between *p*-bromoanisole and phenyl boronic acid (Scheme 13) [121].

The study demonstrated a comparable or superior performance of the templated catalysts in relation to homogeneous control experiments in respect to reaction rate acceleration, metal leakage off the catalyst and catalyst efficiency after continual use. This combination of properties in the templated polymers appears to be extremely attractive to industrial applications for a new generation of heterogeneous catalysts [121]. Further, in-depth discussions on catalytically active templated polymers [3,110], or templated silica [12,14,122–124], are out of the focus of this review and may be found elsewhere.

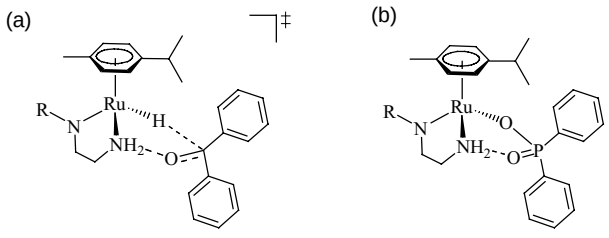
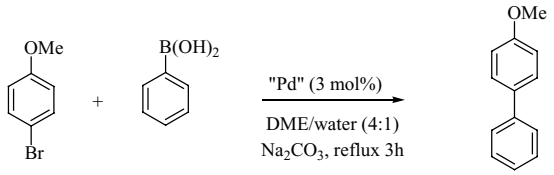


Fig. 7. (a) The transition state of the hydrogen transfer from the organometallic Ru(II) complex to benzophenone is mimicked (b) in a diphenylphosphinato templated polymer.



Scheme 13. Suzuki reaction between *p*-bromoanisole and phenyl boronic acid.

6. Concluding remarks

Although the preparation of templated polymers based upon metal coordinative bonds is conceptually very attractive for the design of selective recognition sites, the majority of investigations up to now rely on the application of hydrogen bondings as binding force between a substrate and the macromolecular surrounding. Nevertheless, the achievements summarized demonstrate that polymerization and recognition experiments performed in pure aqueous solution can be beneficially used for selective recognition of dipeptides or disaccharides. Further investigations demonstrate the advantageous combination of metal coordination and hydrogen bonds for selective substrate recognition. The current success in the selective recognition of larger molecules therefore promises fast achievements on biomolecule templated material utilizing metal coordinative bonds with exciting developments towards enzyme-like catalysis in the up-coming years.

Recent results on immobilized catalysts in molecularly templated polymers indicate high accessibility and reactivity of the incorporated active sites, which lead to chemoselectivities and enantioselectivities, and allow reaction modes that are inaccessible in solution. The combination of these properties, the efficiently incorporated metal ion in the material and the retained catalyst efficiency after continual use makes this new generation of heterogeneous templated catalysts extremely attractive for industrial applications.

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