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Review Article

Molecular Imprinting of Peptides and Proteins

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HIGHLIGHTS

- Fundamentals of peptides and proteins molecular imprinting.
- Essential elements and polymer formats of peptide/protein imprinted materials.
- Applications of peptide/protein imprinting.
- Challenges in peptide/protein imprinting.

ABSTRACT

Molecular imprinting described as a method utilized to create artificial receptors and antibodies by construction of selective recognition sites in a synthetic polymer can be a promising tool for generating peptide and protein artificial specific recognition sites. These materials, as potential antibody substitutes, have attracted great interest and attention in different fields such as peptide and protein purification and separation, chemical/electrochemical/optical sensors/biosensors, chromatographic stationary phases, and enzyme mimics. This review has focused on fundamentals of molecularly imprinted polymers in terms of selection of molecular template, functional monomer, cross linker, and polymerization format. Furthermore, several applications of peptide/protein-imprinted materials are highlighted and challenges regarding the intrinsic properties of peptide/ protein imprinting have been emphasized.

Keywords:
Artificial antibodies
Molecular imprinting
Peptides and proteins
Recognition

Introduction

Molecular imprinting can be described as a method utilized to create artificial receptors and antibodies by construction of selective recognition sites in a synthetic polymer where a template including atom, ion, molecule, macromolecule, and even a cell is introduced in order to facilitate interactions, such as hydrogen bonds, Van der Waals forces, hydrophobic, and electrostatic interactions as well as formation of recognition sites and spatial arrangements of monomers. The spatial arrangement is later fixed by a polymerization process of monomers and cross-linker. The subsequent removal of the template, completely or partly leaves chemical and steric spaces (imprint) vacated in the polymer network, with the ability of rebinding the template (Alexander et al., 2006; Whitcombe et al., 2014).

The pioneering study of Polyakov describing the formation of imprints using silica matrices was published in 1931 (Polyakov and Khim, 1931). However, The number of publications in design, construction, evaluation, and applications of molecularly imprinted polymers (MIPs) or plastic antibodies has been dramatically increased over recent years reflecting continuous development of trends and areas, broad interest of the scientific community, and gradual maturation of molecular imprinting technology (MIT) (Polyakov and Khim, 1931; Alexander et al., 2006; Castell et al., 2011; Chen et al., 2011; Hoshino and Shea, 2011; Nicholls et al., 2013; Whitcombe et al., 2014; Dai et al., 2015) . Therefore, it can be clearly concluded that molecular imprinting compared to the other recognition processes possesses unique features such as recognition

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specificity, structure predictability, straightforward preparation, high physical stability, remarkable robustness, low cost and application universality, which have attracted widespread attention in different fields, such as purification and separation, drug delivery, chemo/biosensing, artificial antibodies, and enzyme-like catalysis (Chen et al., 2016) .

MIPs have been particularly successful for low molecular weight template molecules with aqueous insolubility (Flavin and Resmini, 2009; Huang et al., 2009; Yang et al., 2012). Imprinting of peptides, proteins, DNA, viruses, cell receptors, and even whole cell has been less reported reflecting the truth of facing difficulties in large and sensitive biomolecules imprinting. However, recognition of biomolecules and cells has been recently considered as the next targets in molecular recognition (Turner et al., 2006; Takeuchi and Hishiya, 2008; Yang et al., 2012).

Highly cross-linked networks are used to ensure the imprint cavity of the low molecular weight templates. However, high cross-linked structure hinders mass transfer of the large templates which can lead to sluggish removal and rebinding kinetics of template and in the worst conditions, to permanent entrapment in the polymer network which can be due to physical immobilization. Furthermore, imprinting of biomacromolecules encounters difficulties due to the sensitive structure and the solubility properties. Hydrogen bonding in molecularly imprinted polymers strongly contributes to the affinity for low molecular weight compounds in organic and aprotic solvents; whereas is severely hampered in water and important biological molecules and macromolecules are mostly water soluble, and also natural recognition events including antigen-antibody interaction occur in an aqueous media. Moreover, peptides and proteins are vulnerable to harsh conditions such as high/low temperatures, pH, and high salt concentrations (Valdebenito et al., 2010). Therefore, there exists a strong need to create artificial receptors which are capable of recognizing peptides and proteins in aqueous media in order to create materials and devices capable of mimicking natural processes.

In this review, we focus on peptides and proteins molecular imprinting, emphasizing on literature studies published in recent years, including a brief overview of the fundamental aspects of molecular imprinting including templates, functional monomers, cross-linkers, polymer formats, and methods of preparation, and finally applications of peptide/protein MIPs.

Fundamentals

Molecular imprinting process involves the polymerization of a functional monomer and a cross-linker in presence of a template (Yan and Row, 2006). As it has been illustrated in Fig. 1 (Lofgreen and Ozin, 2014), after the template–monomer complex formation between a template and a complementary functional monomer, cross-linking polymerization is done around the complex. Later, by template extraction, the imprinted cavities remain containing a three-dimensional network including complementary pores to the functional groups of the templates. Considering two types of interactions between the template and the monomer which are mainly covalent and noncovalent interactions, methods of MIPs production can be classified into two main groups. Covalent imprinting is stoichiometric and monomer residues are only in the imprinted sites. This method utilizes reversible condensation reactions including boronate esters (Wulff et al., 1977), ketals/acetals (Shea and Sasaki, 1991), and Schiff's base (Takano et al., 2012). However, this method is less flexible due to the limitation of the reversible condensation reactions. Furthermore, it is really difficult to achieve thermodynamic equilibrium due to the strong covalent interactions and causing slow binding and later dissociation. Noncovalent imprinting proceeds by hydrogen bonding, ionic interactions, pi stacking, and van der Waals forces (Schirhagl, 2013). Noncovalent imprinting is more general synthesis method due to the simplicity of preparation and binding and removal rapidity. However, noncovalent imprinting method suffers from sensitivity to any slight disruption of complex formation such as water molecule. Semicovalent imprinting method has emerged to merge covalent imprinting durability and noncovalent imprinting rapidity. Therefore, in this new method, the template and the functional monomer are bound covalently, but template rebinds to MIP by noncovalent interactions (Lofgreen and Ozin, 2014).

There are three essential elements in MIP synthesis including template molecules, functional monomers, and cross-linkers. Moreover, polymerization initiator and a solvent (porogen), time and temperature control are also critical in MIP preparation. The most popular elements utilized in molecular imprinting, including templates (Hayden and Dickert, 2001; Cai et al., 2010; Zhang et al., 2011; Zhang et al., 2014; Fu et al., 2015; Zhang et al., 2015), functional monomers (Fasihi et al., 2011; Wu et al., 2011; Zhang et al., 2011; Banerjee and König, 2013; Kawamura et al., 2014), cross-linkers (Li et al., 2014; Li et al., 2014), solvents (Gladis and Rao, 2004; Saloni et al., 2013; Booker et al., 2014), and initiators (He et al., 2010; Walsh et al., 2011) have been summarized in Table1.

Peptides and proteins as templates

It is very challenging to consider peptides and proteins as targets for imprinted polymers, due to peptides and proteins purification, their physicochemical and conformational stability in polymerization reaction and organic solvents and their response in biological media. Molecular imprinting of peptides and proteins

Table 1. Typical examples of essential elemnets in molecular imprinting.

Elements	Examples			
Template	Ions: Pb(II); Sr(II); Hg(II); CH3Hg(I); Cd(II); Cu(II); Cr(III); Fe(III); Ni(II); UO_2^{2+}; Th(IV); Eu(III); As(III); PO_4^{3-}	Organic molecules: Pesticides, Endocrine disrupting chemical, Explosive: 2,4,6,-trinitrotoluene (TNT) Pharmaceuticals: tetracycline; quinolones; propranolol; digoxin; sulfonamides Amino acids and peptides: tyrosine; alanine; tripeptides; helical peptides; cinchona alkaloids; N-terminal histidine sequence of dipeptides Sugars: D-fructose; D-glucose; D-galactose	Biomacromolecules: Lysozyme; adenosine; 3,5-cyclic monophosphate (cAMP); bovine serum albumin(BSA)	Cells and viruses: Tobacco mosaic virus; bovine leukemia virus; dengue virus; gut-homing T
Functional monomer	Covalent: 4-Vinyl benzene boric acid 4-vinyl benzaldehyde 4-vinyl aniline tert-butyl p-vinylphenylcarbonate.	Non-covalent: acrylic acid (AA), methacrylic acid (MAA) trifluoromethyl acrylic acid (TFMAA) methyl methacrylate (MMA) p-vinylbenzoic acid itaconic acid 4-ethylstyrene 4-vinylpyridine (4-VP) 2-vinylpyridine (2-VP) 1-vinylimidazole acrylamide (AAm), methacrylamide 2-hydroxyethyl methacrylate (HEMA) 3-aminopropyltriethoxysilane (APTES) 3-methylacryloxyprolyl trimethoxysilane (3-MPTS) glycidoxypropyltrimethoxysilane (GPTMS)	Semi-covalent: 3-Isocyanatopropyltriethoxysilane (IPTS)	Ligand exchange: Cu(II)-iminodiacetate-derivatized vinyl monomer Fe^{2+}/MAA complex
Cross-linker	Covalent: triallyl isocyanurate (TAIC) bis-(1-(tert-butylperoxy)-1-methylethyl)-benzene(BIPB) dicumyl peroxide(DCP) trimethylpropane trimethacrylate (TRIM) pentaerythritol tetraacrylate	non-covalent: ethylene glycol dimethacrylate (EGDMA) N, N ‘-methylenediacrylamide (MBAA) divinylbenzene (DVB) 1,3-diisopropenyl benzene N, N ‘-1,4-phenylenediacrylamine 2,6-bisacryloylamidopyridine N, O-bisacryloyl-phenylalaninol; (3,5-bis(acryloylamido)benzoic acid 1,4-diacryloyl piperazine tetramethylene dimethacrylate N, O-bismethacryloyl ethanolamine (NOBE) glycidilmethacrylate (GMA)	cross-linkers in the sol–gel process: Tetramethoxysilan (TMOS) tetraethoxysilane (TEOS) phenyltriethoxy siliane (PTEOS) phenyltrimethoxy silane (PTMOS) (diphenyldiethoxysilane (DPDES)	new cross-linkers maleic rosin glycol acrylate (MRGA) ethylene glycol maleic rosinate acrylate (EGMRA)
Solvent	2-methoxyethanol, methanol, tetrahydrofuran (THF), acetonitrile, dichloroethane, chloroform, N,N-dimethylformamide (DMF), and toluene.			
Initiator	Azobisisobutyronitrile (AIBN); azobisdimethylvaleronitrile (ADVN); 4,4′-azo(4-cyanovaleric acid) (ACID); benzoylperoxide (BPO); dimethylacetal of benzyl (BDK); potassium persulfate (KPS).			

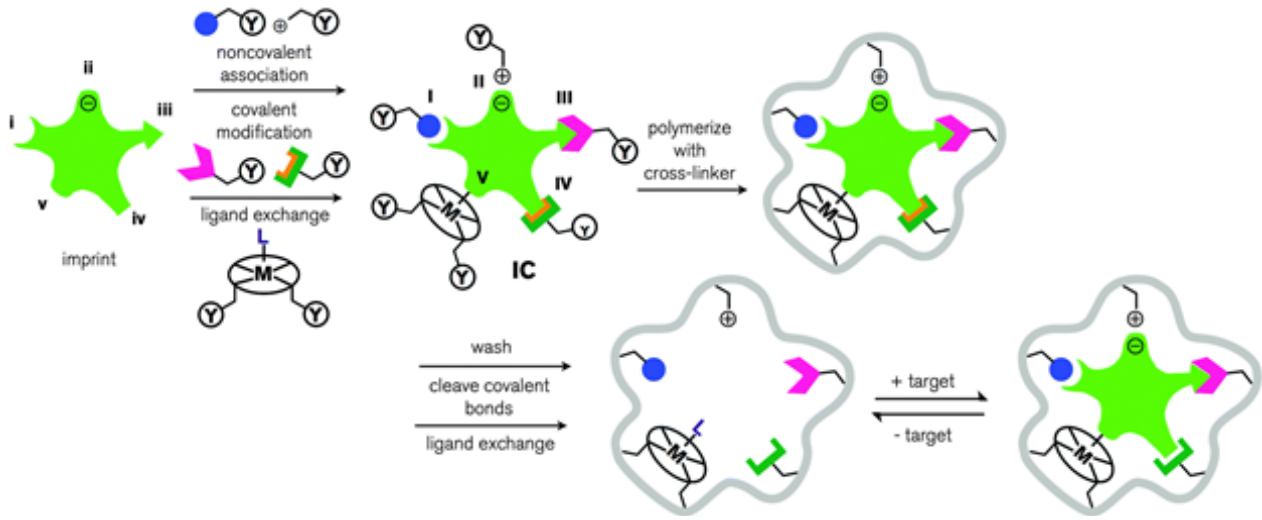

Figure 1. Five main types of molecular imprinting: (i) noncovalent, (ii) electrostatic/ionic, (iii) covalent, (iv) semicovalent, and (v) metal centre coordination. An imprint molecule is combined with an appropriately chosen functional monomer, through noncovalent, covalent, or ligand (L) to metal (M) interactions with complementary functional groups on the imprint. A complex of the imprint and functional monomer (IC) is formed, in which the functional monomer is bound to the imprint molecule (I) by hydrogen bonding or van der Waals interactions, (II) by electrostatic or ionic interactions (the charges on the imprint and functional monomer may be reversed), (III) through a covalent bond, (IV) through a covalent bond with a spacer (orange), or (V) by ligand–metal or metal–ligand coordination. The functional monomer contains a functional group, Y, which undergoes a cross-linking reaction with an appropriate cross-linker. After polymerization of the complex with a cross-linker to form the solid polymer matrix (grey), the imprint functional monomer interactions are intact. The imprint is removed through washing, cleavage of chemical bonds, or ligand exchange, and leaves behind an imprint cavity with functional groups on the walls. Subsequent uptake of a target molecule is achieved by noncovalent interactions (in types i, ii and iv), the formation of a covalent bond (in type iii), or by ligand exchange (in type v) with target molecules that fit into the cavity and possess the correct structure. The matrix may also participate in target recognition and binding through non-specific surface interactions that result from surface features created around the imprint molecule during cross-linking. Reproduced from Lofgreen and Ozin (2014) with permission of The Royal Society of Chemistry.

is really complicated by several parameters. Imprinting of small templates such as dipeptides, tripeptides can be straightforward. However, proteins are assumed to have a large number of conformations considering different parameters, which can include but not limited to pH, solution temperature, and ionic strength. Proteins have real difficulty to move freely in polymer networks with highly cross-linkage and the steric parameters make protein recognition difficult. In protein imprinting, the template can be chosen as whole proteins or epitopes. In order to reduce these complications with proteins and peptides imprinting "epitope approach" can be suggested (Rachkov and Minoura, 2000; Rachkov and Minoura, 2001; Nishino et al., 2006).

Epitopes can be defined as small active regions of a protein which can be recognized by natural receptors and can be selected as template instead of whole proteins. It is better to clarify that in biology, the epitope presence is essential in a conformation of antigen-binding regions. However, in artificial antibodies or imprinted polymers, an epitope is chosen based on the sequence, instead of the conformation to create the imprint which would be able to recognize the whole protein (Rachkov and Minoura, 2001; Nishino et al., 2006; Ye and Mosbach, 2008).

Polymerization formats and procedures

The polymerization procedure is really important to produce MIPs with desirable properties. The polymerization formats are dictated by the template presentation in peptide and protein imprinting. Peptide/Protein imprinting formats can be divided into 3D imprinting and 2D imprinting. In 3D imprinting, binding sites are formed throughout the bulk polymer while in 2D imprinting, binding sites are formed on the surface (Turner et al., 2006). 3D imprinting requires high porosity of polymer to transfer mass of the template, which results in less stability and rapid loss in imprinted property with environment changes. However, preparation of polymer with higher density, with the aim of increasing stability, results in template entrapment in polymer network and less capacity. 2D protein imprinting is classified into grafted and constrained imprinting according to the method of which the protein-recognition sites have been formed on the surface. In grafted protein-imprinting, target protein is immobilized on the film or particle

Table 2. Highlighted applications of peptide/protein imprinting.

Target/ template	Functional monomer	Application	Reference
RNase A	N-(4-vinyl) benzyliminodiacetic acid	HPLC	(Kempe et al., 1995)
Porcine serum albumin	Glycidoxypropyltrimethoxysilaneiminodiacetic acid (GLYMO-IDA)	SPE	(Liu et al., 2011)
Cytochrome c	Methacrylamide, methacrylic acid (MAA) / N,N′-bis-acryloylpiperazine (BAP)	HPLC	(Liu et al., 2010)
Urease and bovine serum albumin (BSA)	3-aminopropyltriethoxysilane, tetraethylorthosilicate	SPE	(Venton and Gudipati, 1995; Venton and Gudipati, 1995)
Hemoglobin, cytochrome c, and transferrin	Acrylamide / methylene-bis-acrylamide (MBA)	HPLC	(Hjerten et al., 1997; Tong et al., 2001)
Transferrin and hemoglobin	Acrylamide / MBA	Electrophoresis	(Takátsy et al., 2006; Takátsy et al., 2007)
YPLG, a tetrapeptide, similar to oxytocin at the C-terminal	MAA / ethylene glycol dimethacrylate (EGDMA)	SPE	(Rachkov and Minoura, 2000)
Epitopes of cytochrome c, alcohol dehydrogenase, and BSA	Acrylamide / ethylene-bis-acrylamide (EBA)	SPE	(Nishino et al., 2006)
Myoglobin	2-Acrylamido-2-methylpropanesulfonic acid, N-isopropylacrylamide/ EBA	SPE	(Turan et al., 2009)
Ovalbumin	MAA / poly (ethylene glycol) 400 dimethacrylate	SPE	(Su et al., 2009)
Creatine kinase	MAA / poly (ethylene glycol) 400 dimethacrylate	SPE	(Chen et al., 2009)
Epitopes of anthrax protective antigen PA(83), protein 1 of flavivirus and the dengue virus NS1 protein	Acrylic acid, acrylamide / EBA	Quartz crystal microbalance sensors	(Tai et al., 2010; Tai et al., 2012)
Human MIF, lysozyme, thaumatin, trypsin, HIV complex, hemoglobin, RECQ1, and catalase	MAA / MBA	Protein crystallization	(Saridakis et al., 2011)
Cytochrome c	Acrylamide / MBA, EBA, BAP	SPE	(El Kirat et al., 2009)
Myoglobin	Methyl methacrylate (MMA) / tetraethylene glycol dimethacrylate	SPE	(Lin et al., 2007)
Hemoglobin	Allyl bromide-modified glucosamine / poly(ethylene glycol) diacrylate	SPE	(Zhao et al., 2008)
RNase A	Methyl methacrylate / EGDMA	SPE	(Tan and Tong, 2007)
N-terminal protected neuropeptides	N,O-bis-methacryloyl ethanolamine	SPE	(Yoshimatsu et al., 2009)
tyrosine phosphopeptide	1-[3-(trimethoxysilyl) propyl] urea	fluorescent detection	(Li et al., 2015)
pro-gastrin releasing peptide (ProGRP) as a small cell lung cancer marker	N-(2-aminoethyl) methacrylamide hydrochloride (EAMA)	Extraction and detection	(Rossetti et al., 2014; Rossetti et al., 2016)
Soluble transferrin receptor (sTfR) in serum as a marker for breast cancer diagnosis	Methyl methacrylate (MAA), N-tert-butylacrylamide (TBAm)	Enrichment by MIPs and the quantification by targeted proteomics.	(Liu et al., 2015)
angiotensin I and II	methacrylic acid and N-t-butylacrylamide	magnetic separation	(Tan et al., 2015)
biotinylated structures	N, N'-methylenebisacrylamide and 2-acrylamido-2-methylpropanesulfonic acid	Quartz crystal microbalance (QCM) sensors	(Elmlund et al., 2014)

Table 2. (Continued).

Target/ template	Functional monomer	Application	Reference
trypsin	N-Isopropylacrylamide (NIPAM)/ N,N′-ethylenebisacrylamide (EbAm)	affinity purification column	(Ambrosini et al., 2013)
Cytochrome c (Cyt c, residues 96–104)	Scopoletin	Recognition film	(Dechtrirat et al., 2012)
bovine serum albumin	APTES	Bioseparation	(Yang et al., 2014)
cyclic citrullinated peptide antibody	acrylamide (AAm)	SPR sensor	(Matsunaga et al., 2007)

surface and is polymerized to form recognition sites on the surface (Matsunaga et al., 2007; Wang et al., 2008; Lu et al., 2009). However, the control of the thickness of the polymer layer is sophisticated and embedded template in the surface layer makes the template extraction difficult. The thickness of imprinted layers can be controlled by controlled polymerization methods; atom-transfer radical polymerization (ATRP) and reversible addition fragmentation chain transfer (RAFT) (Li et al., 2009; Pan et al., 2010). In constrained surface imprinting, the template is confined between polymer elements and a matrix. After polymerization, and removing the matrix support, a planar imprinted film is left. (Shi et al., 1999; Nematollahzadeh et al., 2011). 2D imprinting can be prepared as nanoparticles (on magnetic, silica nanoparticles), on carbon nanotubes, glass beads, nanowires, etc. A schematic representation of 2D (surface) and 3D (bulk) imprinting has been illustrated in Fig. 2.

Applications of peptide & protein-imprinted materials

MIPs have been prepared to separate and purify small molecules, pollutants and chiral compounds (Chen et al., 2016). MIPs have also been applied in solid-phase extraction (SPE) of pharmaceuticals and pesticides, drug delivery and library screening, chemical biosensors, and enzyme-like catalysis (Wulff, 2002; Alexander et al., 2006). Application of artificial bioreceptors or antibodies like peptide/protein/epitope-imprinted polymers has also been remarkably studied (Xu et al., 2010; Verheyen et al., 2011). MIPs have been applied in peptide/protein separation, SPE and HPLC of peptides and proteins (Guo et al., 2005; Lin et al., 2009; Qin et al., 2009; Xu et al., 2010). Lysozyme-imprinted polystyrene polymer beads, which were packed in an HPLC column to separate lysozyme from the competing proteins such as bovine hemoglobin, ovalbumin, and BSA, have been prepared and applied in chromatography (Qin et al., 2009). Moreover, the MIP monolithic column has been prepared and applied for lysozyme separation in real samples (Qin et al., 2011). Molecularly imprinted polymers have also been extensively applied as optical sensors (Valero-Navarro et al., 2009), QCM sensors (Reimhult et al., 2008) and electrical and electrochemical sensors (Fang et al., 2009; Liang et al., 2010; Scognamiglio et al., 2015). Another major application of peptide/ protein molecular imprinting can be enzyme mimicking (Sellergren, 2010). Haupt et al. prepared particles as enzyme inhibitors with enhanced selectivity and binding affinity (Cutivet et al., 2009). Furthermore, scientists claim that Peptide/protein imprinting can also be applied as an innovative technique to prepare bioactive scaffolds, and tissue regeneration due to the cell proliferation capability of peptide/protein MIPs (Yang et al., 2012). Some highlighted applications of peptide/protein imprinting have been summarized in Table 2.

Conclusion

This review presented the fundamental and design, highlighted applications, and also challenges of peptide/ protein-imprinted polymers as artificial receptors and antibodies. Rational selection of target template including epitope or whole protein approach, functional monomer, cross-linker and polymerization procedure and format should be carefully taken, to achieve highly selective peptide/protein recognition sites, and aimed application. Peptide/protein imprinting has been obtained by two approaches mentioned above in different format and types such as monoliths, hydrogels, films, nanoparticles, and nanofibers. In-depth promotion of peptide /protein imprinted polymer preparation has resulted in the vast applications of these artificial biomimetic receptors in versatile fields such as chromatographic media, sensors, artificial enzymes, protein crystallization, and tissue engineering. However, molecular imprinting of peptides and proteins still faces challenges considering the size, solubility, stability, biocompatibility, and aqueous media rebinding of peptide and proteins.

Competing Interests

The author declared that there are no competing interests.

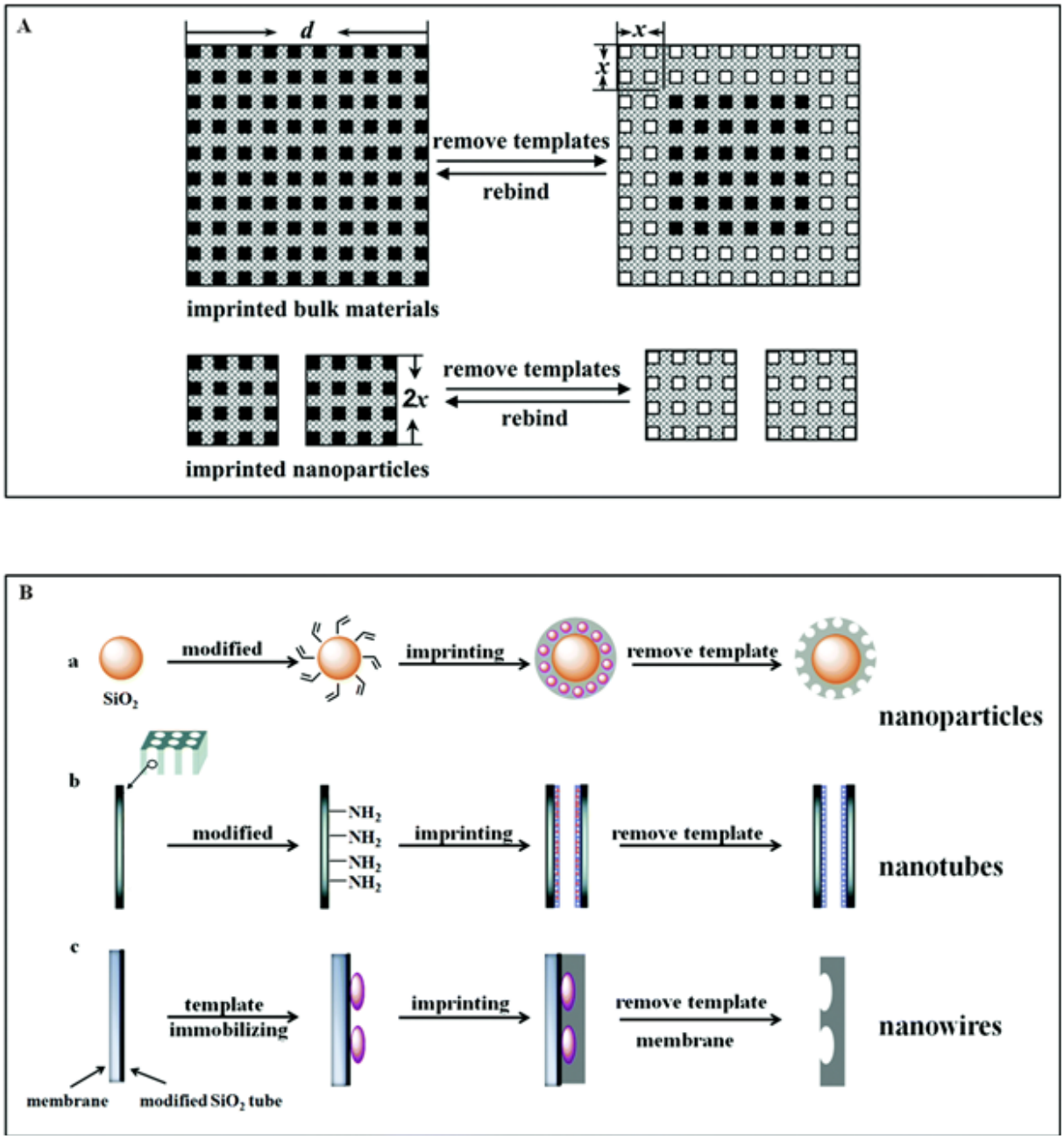

Figure 2. Schematic representation of nanoimprinting. (A) Schematic illustration of the distribution of effective binding sites in the imprinted bulk materials and the nanosized, imprinted particles after the removal of templates. Reprinted with permission from (Gao et al., 2007). Copyright (2007) by American Chemical Society. (B) Schematic diagrams of nanoimprinting process for different forms of N-MIPs. (a) Imprinting on the SiO2 support for the formation of core–shell imprinted nanoparticles. SiO2 core particles were first modified with the vinyl functional monomer, followed by initiating an imprinting polymerization reaction, leading to the formation of imprinted shells at the surface of silica particles. Reprinted with permission from (Gao et al., 2007) (b) Imprinting on silica nanotubes for the formation of imprinting nanotubes. SiO2 nanotubes were first modified with APTS, followed by the sol–gel process, leading to the formation of imprinted shells at the surface of SiO2 nanotubes. Adapted from (Xie et al., 2008). Copyright (2007) by American Chemical Society. (c) Imprinting on a sacrificial membrane support by employing an immobilized protein template approach for the formation of imprinted nanowires. The template molecule was firstly immobilized on the inner wall of a porous alumina membrane, followed by imprinting polymerization reaction, leading to the formation of nanowires by removing supporting alumina. Reprinted with permission from (Li et al., 2006). Copyright (2006) by American Chemical Society. The whole figure is reproduced from (Chen et al., 2016) with permission of The Royal Society of Chemistry.

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