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Molecular imprinting using biopolymers as building Blocks: Sustainable and biocompatible metamaterials for smart recognition and selective biointerfaces

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ABSTRACT

The present review provides a comprehensive analysis of the development and application of molecularly imprinted polymers (MIPs) derived from natural biopolymers, covering literature from the early 1980s onward. The discussion is organized based on the chemical nature of the biopolymers utilized, covering glucans, chitosan, alginates, proteins, and nucleic acids. Each section offers a description of the respective biopolymer, reports synthetic approaches and applications, and attempt a critical evaluation of its effectiveness in molecular imprinting. The use of biopolymers in MIP technology is a promising approach for producing highly selective and sustainable recognition systems. The review underscores the potential of biopolymer-based MIPs in advancing molecular imprinting technology and their impactful contributions to future applications.

1. Introduction

Molecularly imprinted polymers (MIPs) are biomimetics with specific binding sites prepared by a template-assisted synthesis [1,2]. In MIPs, the specific molecular recognition for a defined molecular target is achieved through polymer synthesis in the presence of the molecular target, which acts as a template. As a result, binding cavities with stereochemical complementary to the template are formed in the material [1,3]. The majority of MIPs are composed of organic polymers [4], with silica-based MIPs remaining common though declining in popularity [5]. The general synthetic protocol is a radical polymerisation of acrylamides, acrylates, or methacrylate monomers, which has been followed with minor modifications for approximately 50 years [2]. While the dependence on the same, relatively small, selection of synthetic monomers has had the effect of consolidating the methodology, it partially subsided the drive towards innovative ventures, such as exploring diverse kinds of materials for the imprinting process. With the aim of highlighting research into MIPs made with non-conventional building blocks, the present review specifically focuses on the use of biopolymers for imprinting, while excluding biologically-sourced monomers.

Studying the kinds of biopolymeric materials which are most susceptible to imprinting, their physico-chemical properties, the degree of recognition that can be achieved, and the spectrum of molecular targets that can be imprinted in a particular class of material, is valuable in itself as a means of broadening the fundamental knowledge on molecular imprinting. However, there are two technological considerations which should direct the attention of the scientific community to imprinting with biopolymers: (i) the necessity of making MIPs that are biocompatible, which becomes essential for any intended clinical or biomedical application, and (ii) the necessity to move to more sustainable MIPs, in line with greener chemistry practices and environmentally-friendly materials [6].

For a better understanding of these affirmations, the general definitions of biocompatibility and sustainability are here briefly recalled. Biocompatibility is the ability of a medical device or material to perform with an appropriate host response in a specific application [7] More specifically for a material, this consists in interaction with biological systems without causing adverse effects, such as toxicity, immune rejection, or inflammation, or which degrades in a safe and functional manner, making it suitable for specific use in medical devices and implants, drug delivery systems, and tissue engineering applications. Referring to sustainability, a selected material should be produced, used, and disposed of in a way that minimizes environmental and human

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health impacts while ensuring long-term availability [7]. Concerning the chemical process to form sustainable materials, several factors are to be considered, such as whether they are derived from renewable or abundant resources, synthesized using energy-efficient, low-waste, and environmentally friendly processes; exhibit minimal toxicity and reduced exposure to hazardous substances; are biodegradable, recyclable, or easily repurposed at the end of its life cycle; can contributes to circular economy principles by reducing resource depletion and waste generation [8,9]. As biopolymers often exhibit biocompatibility and/or sustainability, such materials have the potential to redirect research trends in the MIP field. Table 1 provides a synoptical comparison between the characteristics of state-of-art monomers used in MIP synthesis *versus* natural macromonomers, taking into account the class of materials that can be chosen; the interactions between the monomers and the template; how this interaction and the formed MIP material is stabilized; how molecular recognition is achieved; whether the starting monomers or macromonomers are cheap or expensive; which issues are associated with the kind of starting material chosen; any additional or remarkable feature; how molecular recognition can be improved and/or modulated. Overall, Table 1 shows advantages and disadvantages of both approaches and can serve as a roadmap for a rational choice of the starting material for a MIP.

Prior to discussing in detail the imprinting with biopolymers, a consideration about the mechanistic of the imprinting process is required. Imprinting directly with polymers can be conceptualised as the creation of recognition sites in a material which previously displayed no selectivity for the imprinted target [3,10,11]. This is contrasted with 'traditional' imprinting, in which the recognition sites are formed along with the polymer during the synthesis from monomer reagents [3]. However, studies of MIP synthesis suggest that imprinting occurs through molecular interactions between the template and the nascent polymeric chains being formed in situ [12–14]. This would mean that traditional imprinting occurs by the same mechanism as that with biopolymers, but with the added limitations presented by the use of small monomer reagents, including monomer dimerization, variable solubility and differences in reactivity [15–17].

Table 1
General comparison between the characteristics of MIPs when synthesized starting from state-of-art small synthetic monomers (e.g. acrylates, methacrylates etc) or starting from natural macromonomers.

	Synthetic MIPs	Nature derived MIPs
Classes of materials	Polymethacrylates, Polyacrylamides, Conjugated polymers	Proteins, Nucleic acids, Polysaccharides, Polyphenols
Chemical interaction with template	Functional monomers	Intrinsic functionalities derived by constituting monomers (e.g. aminoacid lateral groups)
Stabilization of the interaction	Chemical crosslinking	Chemical and/or physical crosslinking
Costs	Generally cheap, Exceptions: when custom-synthesized monomers are requires; medical intended uses	Can be high depending on the recovery technology and on the final intended use (e.g. biomedical/drug delivery/in situ sensing requires high medical grade materials)
Source-dependent issues	Petrochemical derivation, environmental impact	Polymer fragmentation, Impurities and contaminations, Chemical sequence variability
Molecular recognition	Molecular imprinting	Molecular imprinting
Remarkable/ additional features	Functionalisation	Biocompatibility Biodegradability Functionalisation Low environmental impact
Imprinting optimization	Molecular modeling, chemometrics	Trials and errors

Finally, prior to discussing MIPs prepared starting from macromolecular building blocks, the concept of bioimprinting should be clarified. Bioimprinting, a term originating with Klaus Mosbach [18,19], can be defined as the method used to modify the recognition specificity of a macromolecule. It refers in particular to proteins and proceeds by (i) a partial unfolding, so as to make the 3D-structure of the protein more flexible, (ii) addition of the template followed by (iii) refolding into a tightly packed structure, and sometimes (iv) stabilization of such a non-natural fold by crosslinking. Bioimprinted macromolecules were observed to recognize and interact with their template, demonstrating that biopolymers could be tailored to selectively recognize and stabilize biological molecules, influencing both fundamental science and further practical biotechnological applications.

To conclude, an analytical consideration: the performance and the efficiencies of the various imprinted materials discussed herein were quantitatively compared using the imprinting factor (IF), which is defined as the quantity of analyte bound to a MIP relative to the quantity of the same analyte bound onto the respective non-imprinted, or control, polymer. The IF appears to be the most consistent and reliable qualification, despite some limitations. For the comparison across publications the IF was taken as the relative binding capacity (Q_{MIP}/Q_{NIP}) or as relative signal intensities (typically $\Delta I_{MIP}/\Delta I_{NIP}$).

2. Sugar-based materials

2.1. Glucans and cellulose

Glucans refer to polysaccharides composed of glucose monomers (Fig. 1), linked via glycosidic bonds, which play both structural and energy-storage functions. Among the energy-storage glucans, starch serves as the main energy-storage carbohydrate in plants and a major food source for humans. It is composed of amylose, which presents α-(1–4) glycosidic bonds, and of α-(1–6) branching networks, that is, amylopectin. Dextrins are low-molecular-weight carbohydrates obtained as the partial hydrolysis products of starch, containing both α-(1–4) and α-(1–6) linkages that serve as intermediates in digestion, and have industrial applications as food additives, adhesives, and in pharmaceutical formulations. Dextran meanwhile is a carbohydrate similar to dextrin with α-(1–6) glycosidic linkages, but with the addition of α-(1–3) branching and a broad mass range. Among the structural glucans, cellulose is a linear polysaccharide composed of β(1 → 4)-linked glucose units, forming the primary structural component of plant cell walls and a key material in paper and textiles.

Selected examples of glucan imprinting are reported in Table 2. In a landmark publication, Klibanov and Dabulis prepared a series of dextrans in the presence of malic acid, used as a template, by the lyophilisation of the polymer and template together and analysing the resulting imprinting in ethyl acetate [20]. Observing the mass of malic acid bound to the imprinted dextrans yielded high IF values (19 or more) suggesting a unique selectivity, especially for MIPs based on small dextrans (11 kDa). Later imprinting of dextrans has only been performed in blends with synthetic polymers or when modified with additional functionalisation [21–24].

Dabulis and Klibanov also imprinted dextrin, reporting zero binding to the control polymer [20], though this has also been generally neglected in more recent work. The exceptions however are cyclodextrins which have become common in MIP synthesis, particularly with protein analytes [25,26], though they function as monomers in these polymers [27].

Amylose imprinting was attempted by Kubik and Wulff by crosslinking in the presence of the template [28], while later amylose imprinting has been performed only after first modifying the biopolymer [29,30]. Imprinting of starch can be found early in a 1983 publication of Shinkai et al. in which the saccharide is crosslinked with cyanuric chloride in the presence of methylene blue as a template, resulting in

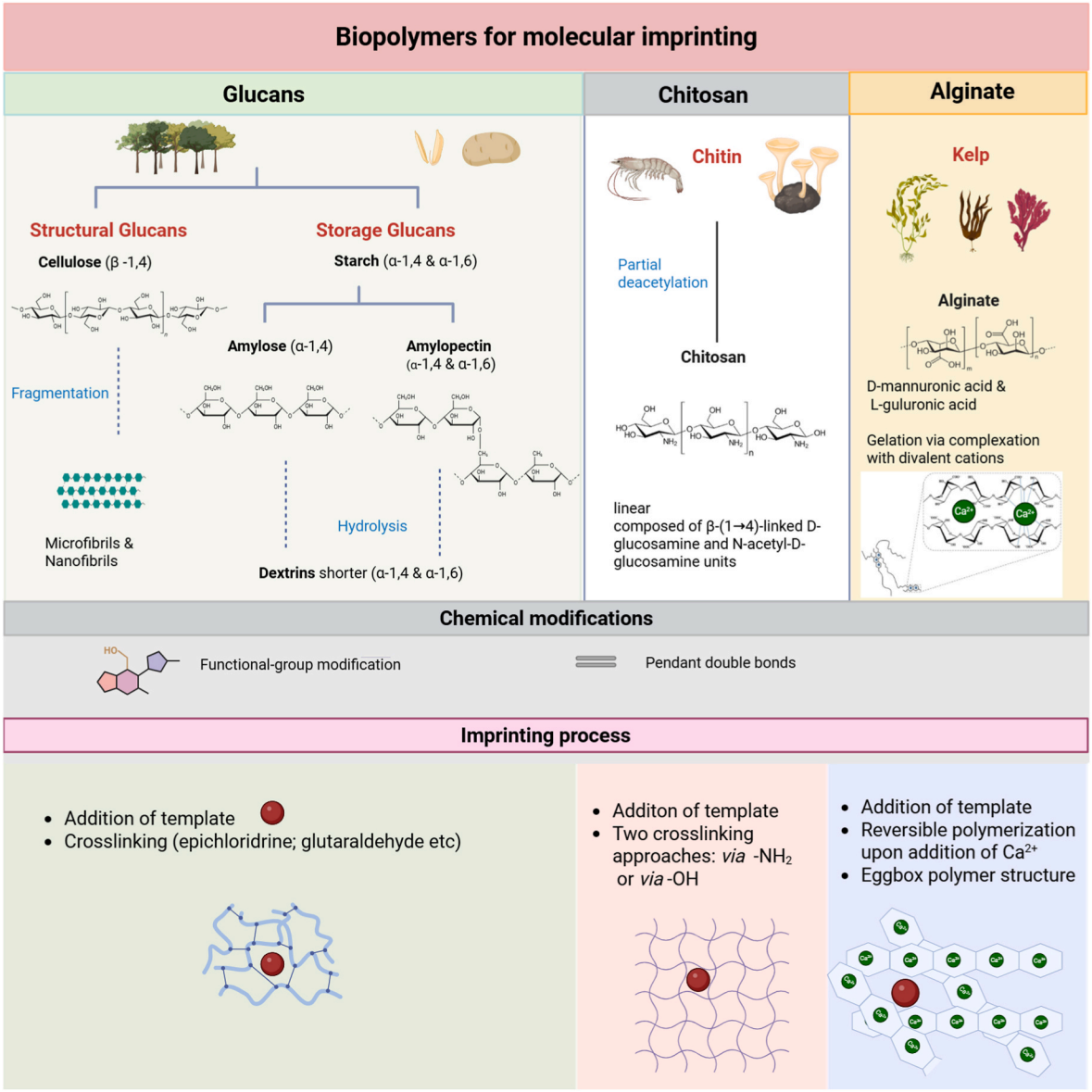

Fig. 1. Sugar-based biopolymers used for imprinting and their sources: glucans are reported with a map that highlighting the structural relationships, based on their glycosidic linkages and degree of polymerisation. The characteristics of chitosan and strategies to crosslink it are summarized. The general features of alginates and the strategy to form a polymeric hydrogel is reported.

high affinity binding sites [31]. More recently, Srivastava et al. described an electrochemical sensor for epinephrine prepared with glutaraldehyde crosslinked starch-graphene composite film [32]. The resulting sensor had a good IF of 3.9 and low response to interferents, with a limit of detection (LOD) of 40 ng/ml (218 nM). The same group later applied a modified procedure to imprint the glycoprotein transferrin [33], resulting in a sensor with a maximum response to interferents being less than 20 % that of the template, and a LOD of 20 ng/ml (i.e. ~250 pM). Mulyasuryani et al. produced a similar electrochemical sensor capable of simultaneously quantifying acetaminophen (paracetamol) and caffeine to millimolar LODs [34]. The sensor was prepared by imprinting the two templates in cassava starch and could be monitored due to the different oxidation potentials of the two targets. This illustrates the potential applications of starch electrochemical sensors, particularly in protein detection.

Cellulose, due to its abundance in nature and its widespread industrial use, is among the most referenced biopolymers in MIP literature. Bacterial cellulose is highly desirable due to its simple production process and mechanical properties similar to those of soft tissue [35]. In general, it has characteristics suitable for biomedical applications, such as wound dressing and artificial blood vessels and corneas, and several products are already commercially available [35,36].

Cellulose has been regularly used as a support material or carrier in the development of many applications of MIPs [44–46]. In contrast, direct cellulose imprinting is uncommon. Molecular imprinting was achieved by simple dissolution of bacterial cellulose with a template in buffer, as demonstrated by Trovatti et al. and Jantarat et al. in the proof of concept of a drug delivery system [37,38]. As this method often results in incomplete dissolution, improved solubilization was achieved with *N,N*-dimethylacetamide and lithium chloride solution [47]. Nakai and Yoshikawa dissolved cellulose derived from hardwood in *N,N*-dimethylacetamide, and succeeded in producing an enantioselective L-glutamic acid imprinted cellulose with IF of 4.9 and enantioselectivity of 2.8 without the necessity of a crosslinker [39]. The authors note that the total capacity of the imprinted cellulose is almost ten times higher when dissolved with additional LiCl, but the IF is lower (IF = 3.0). A similar protocol was later applied to produce a *permselective* membrane capable of separating *o*-xylene from *p*- and *m*-xylene structural isomers

Table 2
Selected imprinted glucans, including synthetic protocol and IF, where ∞ indicated zero observed binding to the NIP. Modified structures are given in italics.

Material	Shortened protocol	Template	IF	Ref.
Dextran 2000 kDa	Lyophilisation with template, disperse in organic solvent	Malic acid	19.3	[20]
Dextran 142 kDa			14.8	[20]
Dextran 11 kDa			∞[a]	[20]
Dextran sulfate 8 kDa			7.2	[20]
Dextrin			∞[b]	[20]
Starch	Starch-graphene composite crosslinked (glutaraldehyde), with template on electrode (aq)	Epinephrine	3.9	[32]
		Transferrin	4.2	[33]
Acryloylamylose	*Azo initiated radical polymerisation with methylenebisacrylamide*	*Bisphenol A*	*1.5*	[30]
Cellulose	Added dry to template solution (aq, pH 7.4, 30 °C) then desiccated	Lidocaine	1.4	[37]
	Template added to cellulose solution (7 % NaOH, 12 % urea, −20 °C); gelation in water	Quercetin	1.6	[38]
	Template added to cellulose solution (Dimethylacetamide, 100 °C), dried and washed with water	Glutamic acid	4.9	[39]
	Template with electrospun cellulose (aq, pH 6) and crosslinked with glutaraldehyde, washed with water	Methylene blue	1.4	[40]
Cellulose (furan functionalised)	*Dissolved with template in water-DMF (1:1 v/v,* pH *6) 3 h, filtered, washed with water-DMF*	*Indacrinone*	*2.5*	[41]
Cotton (amino functionalised)	*Crosslinked (glutaraldehyde) with template (aq,* pH *6.2, 25 °C)*	*Hemoglobin*	*7.6*	[42, 43]
	Crosslinked (glutaraldehyde) with template and Cu^{2+} (aq, pH *6.2, 25 °C)*		*7.9*	[42]

[a] Measured 20.3 mg/g malic acid bound to MIP, 0 mg/ml bound to NIP.
[b] Measured 32.7 mg/g malic acid bound to MIP, 0 mg/ml bound to NIP.

[10].

When glutaraldehyde is included as crosslinker, the imprinted cellulose can be electrospun into highly selective and extremely high capacity (>4000 mg/g) nanofibers and other precision structures, as in the work of Zhijiang et al. [40] More recent work reports the direct imprinting of amino-functionalised cotton with haemoglobin as a template and glutaraldehyde as a crosslinker, presenting an interesting potential for more raw reagents [43]. The imprinted cotton was reported to have a binding capacity for haemoglobin approximately 8 times that of the non-imprinted equivalent and good selectivity over other proteins [42,43]. Similar works report imprinting functionalised cotton with drugs and ions [48,49]. However, crosslinking with glutaraldehyde has some limitations, firstly in its reducing the green credentials of the synthesis and secondly in the potential for covalently coupling protein templates to the cotton.

As imprinting with unmodified cellulose can produce unsatisfactory results originating in its limited solubility, alternatives have been studied. Semi-synthetic cellulose acetate can be imprinted without additional reagents, due to its ability to self-entangle [50], and has yielded high-performance MIPs [51–53]. Similar results have been obtained with hydrazine and furan functionalised cellulose [41,54]. The biocompatibility and sustainability of these cellulose derivatives are, however, not easy to assess and context-specific [55–57].

Imprinting with cellulose and other glucans appears highly sustainable. However, the performance of these materials in selective binding is generally not encouraging. The main difficulty of using glucose-based polymers is their limited functionality, since template-saccharide interactions are largely dependent on bond formation through saccharide hydroxyl groups. Synthetic functionalisation is often therefore necessary to provide a more favourable electrostatic environment for specific target compounds.

2.2. Chitosan

Chitosan (Fig. 1) is a naturally occurring biopolymer, possessing hydroxyls and amino functionalities, that is produced by *N*-deacetylation of chitin, a common structural polysaccharide constituting arthropod exoskeletons and fungal cell walls [58]. The deacetylation degree defines the difference between chitin and chitosan, with materials with degree of deacetylation higher than 60 % being considered chitosan [58–60]. The production of chitosan can be sustainable on a large scale, either by cultivation or by recovery from industrial food waste processing [61,62]. Chitosan has been gaining attention as a future biomedical material due to its biocompatibility, non-allergenicity and biodegradability, combined with certain antibacterial and antifungal properties [58,59]. Formulated as a nanoparticle, chitosan is amongst the leading contenders for nanomedical drug delivery systems, with several empirical demonstrations of this capacity [63,64].

Chitosan is the most commonly applied biopolymer in MIP synthesis, in part due to its possession of greater functionality than many polysaccharides [15]. The presence of amino, ether, hydroxyl and hydrophobic regions, with variable additional acetyl functionality, provides environments electrostatically complementary to a broad range of analytes. The amino group also distinguishes chitosan from the most common polysaccharides, making chitosan highly basic and facilitating the formation of mesoscopic films and chelating structures [59].

Ohga et al. described an ion imprinting of chitosan in 1986, producing materials for the adsorption of Cu(II), Cd(II), Zn(II), Ni(II) and Fe(III) [65]. By dissolving chitosan and metal chlorides to produce a metal complex, followed by crosslinking with epichlorohydrin, the produced resin demonstrated enhanced adsorption for the specific metal ion over non-imprinted chitosan [65]. In addition to affinity, high selectivity for specific metal ions was demonstrated in crosslinked chitosan by this process [66]. This methodology provided the foundation for chitosan imprinting, though it was not explicitly referred to as such, until the turn of the millennium [67,68]. This was largely led by Tan's group publishing works describing the synthesis of crosslinked chitosan beads (Ø ~590 μm) and chitosan-coated particles imprinted for Ni(II) [67,68]. The material displayed a remarkable thermostability, over at least 120 °C, to pH extremes, and to repeated use, together with robustness at least comparable to that of many MIPs produced from synthetic reagents. Ion imprinting with chitosan has remained popular since these early publications and is highly represented in the literature, due to the relatively simple chitosan processing that results in materials which reversibly binding specific metal ions with very high capacity. In 2006, Jiang and colleagues reported the earliest example of molecularly imprinted chitosan materials, in the form of a film, for the enantiomeric separation of L-phenylalanine [69]. Binding analysis of the film revealed a 3.4-fold increase in L-phenylalanine retention compared with non-imprinted chitosan, and an affinity for its template approximately 7 times that for the template's enantiomer.

Crosslinking is likely a mandatory step to achieve the template-induced selectivity in chitosan [70,71], however the choice between crosslinking through hydroxyl groups (most commonly with epichlorohydrin) or amino groups (glutaraldehyde) remains an unresolved question. Chang et al. directly compared the effect of crosslinking with epichlorohydrin and glutaraldehyde in the synthesis of dibenzothiophene-imprinted chitosan [72]. It was observed that chitosan crosslinked through hydroxyl groups presented over double the adsorption capacity and distribution coefficient of the non-imprinted equivalent (IF 2.45), while chitosan crosslinked through amino groups

performed similarly to the non-imprinted material. This is consistent with the assertion that crosslinking should not involve the chitosan amino groups as they are often important in forming intermolecular template bonds [73–75]. Contrary to this are the publications describing the successful application of glutaraldehyde (GA) and research in which GA presents greater binding capacities than epichlorohydrin with little difference in IF [76]. Complicating this are reports that sulfuric acid, which crosslinks principally through amino groups, is superior due to its greater restriction of chitosan movement and reduced interaction with the template [77–80].

Table 3 evaluates the effect of crosslinker on imprinting chitosans [58–60,81,82]. The degree of deacetylation is also included in Table 3, where sufficient data was available, as it can greatly influence the properties of chitosan-based materials [81,83,84]. The effect of the degree of deacetylation on chitosan imprinting has apparently not been investigated, but some preliminary conclusions may also be reached from Table 3. An attempt was made to include every published example of molecular imprinting with chitosan in which no functionalisation of the chitosan is performed prior to imprinting. A selection of examples from ion imprinting are also included [92].

Comparing IFs, a strong difference cannot be observed between crosslinking through hydroxyl groups or amino groups, suggesting that the most suitable crosslinker is template-dependent. It also appears that generalised conclusions concerning the degree of deacetylation cannot be formulated, with the ideal degree of deacetylation also likely to be template- and application-specific.

In terms of sensor performance, the strong swelling of chitosan in response to template binding makes it an excellent basis for the development of stimuli-responsive sensors [152]. Several examples of imprinted chitosan electrochemical devices with sub-picomolar sensitivity can be noted, amongst which are the bisphenol A sensor of Chakroun Galai et al., [109] the aflatoxin B2 sensor of El Hassani [133], and glyphosate sensors of Zouaoui et al. [121–123].

Relative analyte specificity over interferents is difficult to evaluate when different templates/targets are being investigated, but enantiomeric selectivity is often useful as an indicator. Xiao et al., for example, prepared imprinted chitosan for the extraction of *S*-mandelic acid and found the material had an absorption capacity for its template 14.53 times that for *R*-mandelic acid, while this value for the non-imprinted equivalent was 1.01 [148]. Interestingly, however, it has been noted that, in certain cases, racemic imprinting can result in enantioselective materials [142]. With the appropriate target, therefore, it is possible that the inherent properties of chitosan contribute to the exceptionally low interference observed with many chitosan imprinted sensors, for example the dopamine sensors of Wang and Song [95,96], and the tryptophan sensor of Tian et al., [90] which required the interferent concentration to be greatly increased before the response could be observed and quantified.

From Tables 3 and it appears that most targets of chitosan-MIPs are relatively small molecules, while there are examples of chitosan imprinted with macromolecules and whole cells. Amongst these was the lysozyme imprinted sensor of Zouaoui et al., which showed an IF of 51.1 and a LOD of 5 pM [183]. The response to lysozyme was also an order of magnitude above the interferents. While the application of chitosan imprinting to macromolecular analytes is relatively rare, these first studies are rather promising.

2.3. Alginate

Alginate (Fig. 1) is commercially obtained from several species of kelp, extracted with a dilute alkaline solution, making it one of the most sustainably sourced biopolymers [198,199]. It is cultivated with minimal resources and shows rapid decomposition in soil [200]. It is typically prepared as sodium alginate, the salt with monovalent cations being highly water soluble [198,199]. The alginate structure is composed of D-mannuronic acid and L-guluronic acid, both of which

Table 3
Summary of chitosan's MIPs divided by application, crosslinker and degree of deacetylation (DD) where suitable. IF was taken as the relative binding capacity (Q_{MIP}/Q_{NIP}) or signal intensity (In most cases $\Delta I_{MIP}/\Delta I_{NIP}$). IF is presented here as a mean value with standard deviation, with exceptions isolated and cited in italics.

Application	Crosslinker	Template	IF
Sensors – medical	Glutaraldehyde	Rutin [85], albendazole [86], aspartame [87]	3.4 ± 0.4
	Sulfuric acid	Valsartan [88], losartan [88], tryptophan [89,90], baclofen [91], chlorogenic acid [92],	3.3 ± 0.5 *6.4* [89] *12* [90]
	None	*S*-Propranolol [93], dopamine [94–97], L-dopa [98], serotonin [99], urea [100,101], uric acid [102], glucose [103], L-cysteine [104], epinephrine [105], L-5-Hydroxytryptophan [106], glucose [107]	5.0 ± 2.5 *>10* [95,96]
	Other	Memantine [108]	6.2
Sensors – other	Glutaraldehyde	Bisphenol A [109], rutin [110], ketoprofen [111], nitrotriazolone [112], glutamate [113], lactic acid [114], imidacloprid [115], thiamethoxam [116], methyl paraben [117], trichlorphon [118], *p*-aminophenol [119]	2.2 ± 1.3 *16* [110]
	Sulfuric acid	2,4,6-Tribromophenol [120], glyphosate [121–124], bisphenol A [77,80,125], clenbuterol [126], melamine [127]	2.9 ± 0.8 *6.0* [77] *14.5* [123]
	None	2,4-Dichlorophenol [128], catechol [129,130], 4, 4′-methylene diphenyl diamine [131], parathion [132], aflatoxin B2 [133], butylated hydroxyanisole [134], 6-benzylaminopurine [135],	4.0 ± 2.1 *>10* [129, 133, 135]
	Other	Histamine [136], vanillin [137], tartrazine [137], perfluorooctane sulfonate [138],	2.5 ± 0.4 *4.5* [136]
Extraction and separation	Glutaraldehyde 75–90 % DD	Dibenzothiophene [139], Dibenzothiophene sulfoxide [140], benzothiophene sulfone [141], dibenzothiophene sulfone [141], 4,6-dimethyldibenzothiophene sulfone [141], ketorolac [142], 4-nitrophenol [143], l-glutamic acid [144], *p*-hydroxybenzoic acid [145]	1.8 ± 1.0 *6.0* [144] *9.4* [145]
	Glutaraldehyde >90 % DD	Citric acid–Cd complex [146], tartaric acid–Cd complex [147], *S*-mandelic acid [148], triclosan [149], epigallocatechin gallate [150], dibenzothiophene [151], dibenzothiophene sulfone [152]	*2.4* ± *1.1* *14.1* [148]
	Epichlorohydrin 75–85 % DD	4-Hydroxy benzoic acid [153], rose bengal [154], congo red [155], salicylic acid-Cd [156], bromate [157],	2.2 ± 0.1 *4.0* [156]
	Epichlorohydrin >85 % DD	Dibenzothiophene [72], urea [158], methandrostenolone [159], perfluorooctane sulfonate [160], α-lipoic acid [161],	2.3 ± 0.1 *12.5* [159]
	Sulfuric acid	Tryptophan [162], cyclic adenosine monophosphate [163], epigallocatechin gallate [164], chlorogenic acid [71], B vitamins [165], acrylamide [166], naringin [73]	2.3 ± 0.8 *3.5* [166] *14.3* [73]

(*continued on next page*)

Table 3 (*continued*)

Application	Crosslinker	Template	IF
	Other	L-Phenylalanine [69], *o*-xylene [167], l-aspartic acid [168], (−)-epigallocatechin gallate [169], S-mandelic acid [78], phenylalanine amide [170], dimethyl methylphosphonate [171]	2.2 ± 0.9 *5.3* [168] *28.4* [171]
	None	Cas9 epitopes [172], naringin [173]	2.1 ± 0.5
Drug delivery	Sulfuric acid	L-Tyrosine [79,174]	2.7, 7.5 [79]
	None	Cytosine [175], riboflavin [176]	1.5
Catalysts	Epichlorohydrin	Methyl orange [177] atrazine [178],	1.4
	Glutaraldehyde	Norfloxacin [179,180]	2, 3.5
Macromolecules	Glutaraldehyde	Ovalbumin [181], poly-γ-glutamic acid [182], lysozyme [183] sulfate reducing bacteria [184],	3.9 ± 0.9 *51.1* [183]
Ion Imprinting	Epichlorohydrin	Ni(II) [67,185–188], Ag(I) [189], Cu(II) [190], Pd(II) [191], Pb(II)	1.7 ± 1.0
	Glutaraldehyde	Pb(II) [192], As(V) [192,193], Cd (II) [193], U(VI) [194]	2.7 ± 1.0
	Other	Fe(III) [195], Hg(II) [196], Ce (III) [197]	1.7 ± 1.0

have a carboxylate functionality and hydroxyls. The peculiar stereochemistry of guluronate blocks in solution can form junctions with those of the adjacent polymer chains in the presence of divalent cations, which act as ionic attractors, determining the gelation of the solution. Among divalent ions, Ca^{2+} is usually chosen, since salts like calcium chloride ($CaCl_2$) and calcium lactate ($C_6H_{10}CaO_6$), can provide mild and fast gelation conditions, compared with those necessary to crosslink chitin and other materials [199,201,202]. Interest in alginate imprinting originates from the works of Zhang et al., who studied alginate-based porous hydrogels [201,203,204]. This, combined with earlier demonstrations of crosslinking in very mild conditions, attracted the attention of researchers interested particularly in imprinting protein templates [198,202]. Table 4 references the majority of indexed publications describing alginate imprinting. Bovine serum albumin (BSA) was often used as a template and rebinding target in initial studies [201,205,206]. Peppas and colleagues were early in demonstrating the possibility of BSA imprinted alginate MIPs by preparing materials with a binding capacity for the BSA target of 6.4 mg/g, contrasting strongly with the 0.1 mg/g obtained with the non-imprinted equivalent [202,207]. The alginate MIP was prepared by simply dissolving BSA and sodium alginate in mild acidic conditions and crosslinked with $CaCl_2$. The specificity of the BSA-imprinted alginate was very high, with the binding to interferent proteins (ovalbumin, haemoglobin and HSA) being similar to that observed with the non-imprinted [202].

Electrochemical sensors for protein detection and quantification have been prepared with alginate-based MIPs, but difficulties in maintaining structural stability have somewhat limited this research [208]. In fact, the crosslinking of the alginate material is easily reverted from the polymeric structure, simply by sequestering the divalent cations earlier used to trigger the polymeric formation. This makes alginate an interesting stimuli-responsive material but also limits its application due to low structural stability. Additionally, alginate lacks amino functionalities, meaning it lacks versatility in terms of chemical groups for pairing the template. These limitations resulted in modification of the alginate preparation protocol, typically by the introduction of additional structural components or crosslinkers [201,209,210]. Attempts have been made to improve the structural stability of alginate electrochemical devices by introducing additional structural components, with some success [211]. Particularly effective is the inclusion of carbon nanotubes for both structural support and conductivity to complement the alginate

Table 4
Selected examples of imprinted alginates divided by composition and application.

Material	Target category	Application	Template	Details
Alginate	Proteins	Drug delivery	Bovine serum albumin [207], insulin [215]	Ca^{2+} crosslinked microparticles
		Electrochemical sensors	Bovine serum albumin [208, 212]	IF: 3.5 ± 1.0 Ca^{2+} crosslinked films
		Optimization	Bovine serum albumin [201, 202,206]	IF: 2.2 [206], 64.0 [202] Ca^{2+} crosslinked materials
	Small molecules	Chiral resolution	Tryptophan [216,217], ketoprofen [218]	99 %ee [216, 217] Ca^{2+} crosslinked membranes
		Drug delivery	Tenoxicam [219]	Nanoparticles crosslinked with Ca^{2+}
Alginate + hydroxyethyl cellulose (~5 wt%)		Optimization	Bovine serum albumin [201, 220]	Highest IF (2.8) obtained with lowest HEC content [220]
Alginate + calcium phosphate (30 wt%)		Optimization	Bovine serum albumin [205, 221]	IF 3.7 [205] 4.9 [221]
Alginate + polyacrylamide		Heavy metal extraction	Pb(II) [210]	Powder with alginate functional monomer, abs. capacity 42 mg/g
Alginate + silica		enantiomeric separation	phenylalanine [209]	Membrane with alginate functional monomer, IF ~6

binding site, as in the work of Cheng et al. in their protein sensor [212]. Along a similar line, sensing of the glycoprotein biomarker CD44 was reported by Cui et al. [213], and an electrochemical device incorporating core-shell surface imprinted TiO_2 nanoparticles prepared by Cetinkaya et al. showed a 2.81 pM LOD for its target vancomycin [214].

The application of alginate to small molecules is relatively rarely reported, despite enantioselectivity being achieved in past studies [209, 216,217]. There is evidence that the cation crosslinked alginate is inherently enantioselective, and that, for a specific pair of enantiomers, the enantioselectivity can be changed by exchange of the crosslinking cation [218]. The majority of alginate imprinting (~65 % of indexed articles) concern protein templates, and this is the application in which the material is expected to have the greatest potential. Recent demonstrations of insulin drug delivery microparticles composed of alginate are promising [215], and build on established nanomedical technology [207,219].

Several studies with imprinted alginate include additional materials for stability, with hydroxyethyl cellulose and calcium phosphate being prominent biocompatible examples [201,205,221], but direct comparative analysis reveals an inverse relationship between stability and IF in these composites [205,220].

2.4. Other polysaccharides

There are several studies using other polysaccharides, though these are generally under investigated. Sulfated polysaccharides have been studied by Ferreira et al. who prepared chondroitin sulfate- and fucoidan-silicone composites imprinted with Pb(II) ions, finding a 5-fold increase in capacity by inclusion of the polysaccharides [222,223]. The IF with chondroitin sulfate was small however, and the authors note that attempts with other templates were less successful. Trials in imprinting

agarose with BSA were successful in terms of high IFs, but the strong binding to certain interferents inherent in agarose and the possible necessity of elevated temperatures during imprinting are not encouraging [224,225]. Direct imprinting of glucomannan using extracts from the konjac plant can be found in the work of Mi et al., who describe the preparation of papain and BSA imprinted membranes resulting in good specificity over several interferent proteins [226,227]. Later work by researchers at Hubei Minzu University demonstrates that glucomannan may also have useful properties as a support material [228–230].

Several naturally occurring polysaccharide blends, such as tragacanth, can be crosslinked with metal cations in the presence of drugs as templates to give pH responsive drug delivery systems [231]. There are a few reports of tragacanth being used as an effective imprinting material, though only following functionalisation or in combination with additional synthetic polymers [232–234]. Tara gum can also be imprinted and crosslinked with metal cations, and this method has been applied with modified tara gum to produce a tramadol drug delivery system with a release profile superior to a commercial equivalent [235].

Polysaccharides have great potential in MIP technological development and present great advantages over synthetic materials in sustainability. However, in general, these materials have inherently limited functionality, which is improved and expanded by functionalisation to offer a broader capability for complementary intermolecular interaction.

3. Protein building blocks

3.1. *Enzyme bioimprinting: a way to re-address substrate specificity*

Studies on enzyme catalysis in organic solvents, conducted earlier by Alexander Klibanov [236–238], proved that some organic solvents prevented the enzymes from unfolding, by reducing the overall flexibility of the protein and/or by restraining the flexibility of the catalytic site [237]. It was observed that when an enzyme was co-lyophilized in the presence of a non-natural substrate, its catalytic site could be 'tuned' to selectively bind the non-natural substrate and further catalyze its conversion into a product [237,239]. Soon after these reports, Klaus Mosbach began publishing similar descriptions of "bioimprinting" by placing enzymes in the presence of non-natural substrates, letting them interact and then precipitating, instead of lyophilising [240–242]. Indeed the process was extremely effective, resulting in a 65-fold increase in initial catalytic rate [243]. Mosbach's research originates the term "bioimprinting" to describe the process (Fig. 2) [18,19]. More important, however, was the discovery of techniques to disperse these materials in aqueous media without loss of the binding site [244]. This can be observed early in the work of Saraswathi and Keyes, who crosslinked a ribonuclease with glutaraldehyde in the presence of 3-indolepropionic acid in aqueous solution, resulting in a semi-synthetic acid-esterase [245–247]. Peiβker and Fischer then combined these

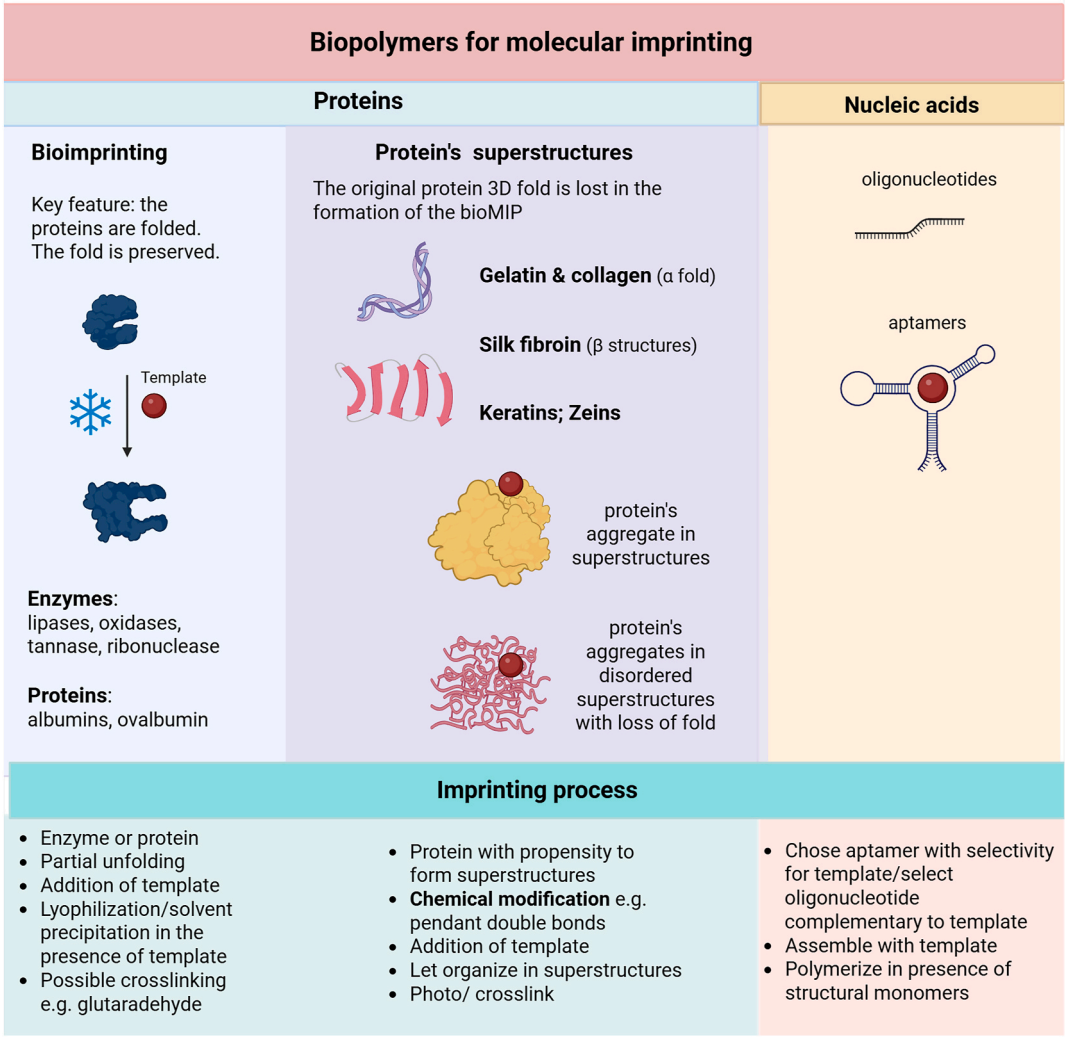

Fig. 2. The use of protein-based macromonomers can be both for the process of bioimprinting or for molecular imprinting. In the case of nucleic acid, both short oligonucleotides and aptamers can be employed.

processes and reproduced the effects observed in organic solvent by covalent cross-linking of the bio-imprinted enzyme [248]. The enzyme was first acylated with itaconic anhydride, which allowed cross-linking following precipitation with the selected substrate. The result was an aqueously dispersible enzyme, modified to react with an alternate compound, fixed in its organic-solvent dispersed conformation. Klibanov's group then demonstrated a bioimprinted myoglobin which catalysed the epoxidation of styrene, presenting the opportunity for the recycling of myoglobin from abattoir waste for the synthesis of industrially useful catalysts [249].

As enzymes provide superior selectivity over chemical catalysts [250], the bioimprinting concept to increase enzymatic activity was regarded as desirable. An example of this is found in the work of Aithal and Belur, in their study of propyl gallate synthesis [251]. Tannase catalyses the synthesis of propyl gallate from gallic acid and propanol, a highly economically valuable process. By imprinting with a small quantity of gallic acid in benzene, the researchers were able to increase the yield of propyl gallate by 2.65 times, an increase consistent with other tannase imprinting studies [251,252].

Kaulpiboon et al. induced preferential synthesis of larger cyclodextrins in cyclodextrin glycosyltransferases by imprinting with cyclomaltododecaose (CD_{12}) [253]. The cyclodextrin glycosyltransferases were acylated with itaconic anhydride and precipitated with CD_{12} before crosslinking with ethylene glycol dimethacrylate. The same authors demonstrated that the same selective synthesis could be induced for intermediate-sized γ-cyclodextrin (CD_8) by instead imprinting with this template [254]. These reagents were also used by Vaidya et al. to induce galactose oxidation in glucose oxidase [255].

Lipase bioimprinting has become relatively common due to the broad variety of industrial applications which would benefit from enhanced lipase catalytic activity [256–261], yielding to marked increase in the catalytic rates and in the total product yield. The origin of this increase was investigated by Liu et al. by circular dichroism, observing a higher proportion of β-sheet over α-helix upon bioimprinting, hence in a more open active site [262,263]. This change in the secondary structure appeared consistent with data from bioimprinting with other proteins [264].

Example applications of immobilised bioimprinted lipases include the synthesis of polyunsaturated fatty acids [265–272], oleate esters [273,274], sucrose-6-acetate [275], and biodiesel [276–279], the enantioselective acylation of secondary alcohols [280,281], and highly specific enantiomeric resolution [282,283]. The combination of imprinting and immobilisation is a highly effective strategy for increasing lipase activity, with the interfacial activation complementing the bioimprinting-induced site opening [263,284]. Much of this also draws influence from Braco, and Soler et al., who published work with immobilised bioimprinted lipase in the early development of the field, which proved to be significantly more stable than the dispersion [19, 238,250,265,277,285].

The bioimprinted materials also demonstrate potential in biosensors, in which they show improved stability over biologically occurring equivalents. [286–288]. In this regard, Willner et al. produced a photo-switchable bioimprinted chymotrypsin by the attachment of a reversibly photoisomerisable component which perturbs the protein backbone on isomerisation [289]. Enzymatic activity could, therefore, be controlled with irradiation of light of different wavelengths. The authors initially observed a small photo-switching effect with non-imprinted enzyme, but the effect was greatly augmented by bioimprinting and in non-aqueous solvent, or adopting an alternative photoisomerisable components, specifically nitrospiropyran [290].

Enzyme bioimprinting has however been primarily exploited to boost catalytic efficiency, and as such is increasingly obsolete as modification of the substrate specificity of an enzyme is now obtained via strategies of molecular design and recombinant approaches. The use of bioimprinted enzymes in alternative applications however resonates with circular economy approaches, due to the enzymes being byproducts of primary processes, such as agrifood sectors, and could therefore be valuable for future technologies.

3.2. Albumin imprinting: adding specific binding sites to a protein

Enzyme bioimprinting prompted investigations into whether selectivity could be induced into proteins without inherent binding sites. By simply lyophilising BSA with *p*-hydroxybenzoic acid, the resulting imprinted protein displayed a binding capacity approximately 10 times that observed for BSA lyophilized without the template [291]. Significant selectivity for *p*-hydroxybenzoic acid over *o*- and *m*-hydroxybenzoic acid was also demonstrated, in addition to a 20-fold binding relative to phenol and a 3-fold binding relative to benzoic acid. Repetition of the experiment with tartaric acid resulted in an IF of 30 relative to the non-imprinted protein, and similar results with malic acid (IF 12.0) [20]. However, these effects were only observable in organic solvents, with all evidence of imprinting being erased by redissolution in water [20], possibly due to steric hindrances that were tolerated by the protein when rigidized by the solvent, while reverted back to the most stable folded conformation when placed in the aqueous milieu. This principle has been applied in the development of catalytic 'enzymes' formed from non-catalytic proteins, as in the semi-synthetic glutathione peroxidase of Luo and colleagues prepared by the bioimprinting of albumins [264, 292–294].

The potentials of imprinted albumins in analytical technologies was explored by the research of Burmistrova et al. who produced an assay using bioimprinted ovalbumin immobilised on high surface area multichannel capillaries, resulting in a LOD for the mycotoxin cereal contaminant zearalenone of 0.12 ng/ml [295]. Beloglazova et al. bioimprinted bovine serum albumin with two mycotoxins simultaneously, with quantum dots as labelled [296]. The resulting assay was capable of simultaneous detection and quantification of the two toxins, with LODs of 5 ng/ml and 35 ng/ml, below regulatory requirements. The same research group has more recently published work describing sorbents for the same target by imprinting BSA and glucose oxidase [297–299], and imprinting BSA with 4-hydroxycoumarin, [300], demonstrating the versatility of the methodology.

Other contributions to analytical chemistry with this technique can be found in the work of Sakamoto et al., who produced an assay with kwakhurin-bioimprinted ovalbumin with a LOD of 2.5 μg/ml [301]. Interestingly, the authors also demonstrated bioimprinting of monoclonal antibodies with kwakhurin, in which the antibodies lost affinity for their original antigen in favour of the print molecule. Gutierrez et al. prepared an electrochemical aflatoxin B1 sensor by imprinting acid-denatured ovalbumin at elevated pH and crosslinking with glutaraldehyde [302]. The resulting device showed a picomolar LOD and high selectivity, with demonstrated application in real samples. While this represents a very promising direction for new biosensor development, the authors noted that attempts to prepare the sensor using bovine serum albumin were unsuccessful due to the higher hydrophobicity of certain BSA pockets [302].

Cao et al. presented a possible nanoparticle-based anti-cancer treatment using a bioimprinting strategy [303]. Glutathione imprinted human serum albumin was additionally functionalised and loaded with the anti-leukemic drug meclofenamic acid, resulting in a nanomedical drug delivery system for the treatment of acute myeloid leukemia. The *in vivo* studies on the distribution of the bioimprinted nanoclusters showed biodistribution in leukemic mice to be consistent with successful uptake by acute myeloid leukemic cells. A final interesting example can be found in Yoshikawa et al., who used bioimprinting to entail the desired specificity to membrane proteins isolated from thermophilic bacteria, permitting cultivation at high temperatures and thus reducing the risk of reagent contamination [304].

3.3. Imprinting emerging from proteins super-organizations

In biology the assembly of proteins into macro-structures yields specific and unprecedented functionalities, such as translation molecular machines etc. [305] Recently, specific molecular roles and properties were assigned directly to protein aggregates, hence independently from folding [306]. By extending this concept, new routes are open for imprinting, essentially based on assembling proteins into disordered super-structures for achieving recognition over a molecular template (Fig. 2) [307]. This approach forms fairly large superstructures, of a few tens of nanometers when prepared in the nanoparticle form, or macro-material with pores when prepares as bulk, that are able to recognize not only small molecules, but also entire proteins and macromolecules. The lack of necessity to maintain the original fold makes the process substantially easier and economically more convenient, but equally effective in the quality of imprinting. The macrostructures formed from aggregates of proteins (e.g., silk, gelatin, zeins) typically consist of entangled, hydrated networks with pore sizes that depend on the biopolymer's nature and crosslinking density; such materials can be used for effectively recognizing protein and large targets. The use of proteins to form nanometric molecular assemblies is a newly emerging field, but it inherently meets the criteria of sustainability and/or biocompatibility due to the selected protein sources.

3.3.1. Gelatin

Collagen is the most abundant structural protein in the extracellular matrix of animals, providing mechanical strength and support to tissues such as skin, tendons, and bones. It consists of a triple-helix structure composed of glycine, proline, and hydroxyproline-rich polypeptide chains, along with other amino acids. Gelatin is a denatured and fragmented form of collagen obtained through partial hydrolysis. Gelatin loses the organized triple-helix structure, resulting in a water-soluble polymer that forms a temperature-sensitive solution that can gellify below a critical temperature, varying from 30 to 40 °C to a few degrees depending on the animal source. Due to this property gelatin is widely used in food, but also has biomedical uses. Gelatin and collagen have attracted attention for their potential in drug delivery and other biomedical applications due to their high biocompatibility [308], with collagen being amongst the best materials for wound dressing [309–311].

Imprinting with gelatin as a base reagent is still relatively uncommon. Guan and colleagues prepared particles imprinted for TNT using layers of gelatin around a silica core, and crosslinking with glutaraldehyde [312]. The resulting particles showed an IF of 3.0 and good target selectivity, presenting a foundation for future gelatin-based analytical devices. Gelatin imprinting was also reported for 17β-estradiol in the form of nanoparticles for wastewater analysis by Hao et al. [313] The same author successfully prepared magnetic imprinted gelatin nanoparticles for the selective extraction of hemoglobin, a promising example of a protein-imprinted protein [314]. The nanoparticles were prepared by first covalently immobilising the hemoglobin template to the magnetic core, followed by the formation of a gelatin shell without the need of additional crosslinker. Elution of the protein gave selective and stable magnetic nanoparticles with a high binding capacity of 93.1 mg/g, 3.85 times that of the non-imprinted control nanoparticles [314].

Hao et al., in their description of extraction materials, discuss the benefits of gelatin's hydrophilicity and how this property makes it excellent as a drug delivery material [313,314]. Tang and colleagues prepared imprinted gelatin nanoparticles which could effectively enter cancer cells and selectively bind testosterone, presenting a plausible androgen deprivation therapy for prostate cancer patients [315]. While the binding capacity of the nanoparticles was not particularly high (<10 mg/g), *in vitro* studies demonstrated that they could freely enter cancer cells and sequester testosterone, blocking the testosterone androgen receptor pathway, and inhibiting prostate cancer cell proliferation [315].

Functionalised gelatin has also been applied in a similar manner in several successful demonstrations [316,317]. When gelatin is modified with methacryloyl groups (GelMA) it can be easily chemically or photo-polymerized to modify the rigidity of the final hydrogel. GelMA is a biomaterial regularly employed in cell scaffolding, drug delivery and tissue regeneration [318]. Furthermore, being a biomedically approved material, it can offer interesting advantages when prepared as a MIP. For example, Bossi and Maniglio prepared GelMA imprinted nanoparticles selective for the cytokine interleukin-6 (IL-6), with an IF ~8 and nanomolar affinity for IL-6 [307]. Additionally, these GelMA nanotraps were shown to sequester IL-6 directly in the cell culture medium, in a dose-response manner in an *in vitro* inflammatory model [307].

3.3.2. Silk

Silk is generally considered the most promising material for biomedical applications due to the high uniformity of its structure [319]. Silk nanoparticles are generally more stable than those produced from other biopolymers, while their regularity also makes them amongst the lowest risk materials discussed in terms of patient health [320]. Silk farming is also an ancient practice, where the source of the material is the silk worm. Silk is composed of intertwined proteins, fibroin and sericin, of which fibroin (SF) has been demonstrated to be non-immunogenic and highly biocompatible. At a structural level, SF exists in random coil and α-helix conformations in solution, with the capacity to self-assemble into β-sheets upon processing, which leads to mechanical property, stability, and controlled biodegradability [321]. SF facilitates cell attachment, growth, and maturation and is particularly useful for application in tissue engineering [322]. Its enzymatic degradation (i.e., by protease XIV, collagenase) is accomplished without the release of toxic byproducts, thus allowing controlled resorption in regenerative medicine [323]. Its reduced immunogenicity minimizes inflammatory responses relative to other natural polymers, thus allowing enhanced *in vivo* compatibility [324]. Due to its processability and versatility, SF can be processed into films, hydrogels, sponges, fibres, and nanoparticles, which allows its application in scaffolds, wound dressings, and drug carriers [325]. All these features place SF at the forefront as a biomaterial of choice in bioengineering and regenerative medicine.

The use of SF for imprinting was demonstrated by Bossi and Maniglio using the cross-linkable methacrylate silk fibroin SilMA [326,327], to imprint the protein human serum albumin [328]. The resulting imprinted SilMA nanoparticles displayed nanomolar affinity constant for their target protein, selectivity over other serum proteins and were then grafted onto silk nanofibers to produce functional nanofabrics that can find uses in sensing and in situ sensing [326].

3.3.3. Keratins

Keratin is a fibrous structural protein abundantly found in hair, wool, nails, and feathers, characterized by high cysteine content and strong disulfide bonding. Its intrinsic biocompatibility, biodegradability, and ability to self-assemble into nanostructures have made it an attractive biomaterial for tissue engineering and regenerative medicine, also considering its processability. For its hemostatic, antioxidant, and bioactive properties, keratin has been explored for wound healing, drug delivery, and scaffold fabrication. Its abundance from waste sources and tunable functional groups also supports sustainable and customizable biomedical applications [329]. Keratins have attracted some interest as a potential biomedical material [330], but demonstrations of imprinting with keratins are rare. Hassanzadeh and Ghaemy prepared magnetic MIP nanoparticles from keratin, extracted from chicken feathers, for the selective absorption of bisphenol-A from water sources [331]. The MIPs were formed with a combination of keratin and traditional synthetic monomers, but this work is likely the first successful use of such materials for imprinting. Further studies are needed to determine the future of keratin in molecular imprinting.

3.3.4. Zein

Zein is a maize storage protein, widely used as a biodegradable polymer, with proposed applications in drug delivery due to its relatively favourable intermolecular interactions relative to other biopolymers [332]. Zeins are insoluble in aqueous solution and bind strongly to small hydrophobic molecules, meaning that structural integrity can be obtained by changing the solvent from alcohol to water, with no requirement of a crosslinking agent [332,333]. Li et al. prepared imprinted zein magnetic nanoparticles for the extraction and analysis of curcumin [333]. The material, prepared from Fe_3O_4 nanoparticles, zein and curcumin, was cast onto a glassy carbon electrode, allowing electrochemical quantification of the target curcumin. The resulting device showed a 10 nM LOD and high IF, with the authors additionally demonstrating the accurate determination of curcumin content in real samples [333]. The same researchers have also experimented with including traditional synthetic monomers and deep eutectic solvents, including edible deep eutectic solvents, for additional functionality in aspartame, oxalic acid and tetracycline sensors [334–336]. Suriyanarayanan et al. prepared imprinted zein materials specific for biotin, using sacrificial nanoparticle beads and nanoporous alumina to form hyperporous nanostructures, nanowires and nanoparticles [337]. All devices showed a linear response to the target in the low millimolar range, with hyperporous films demonstrating stability when stored in aqueous medium for 6 months. The use of zein in molecular imprinting is relatively new, but this could be an important area in the future.

4. Nucleic acids

Nucleic acids used directly as imprinted recognition elements are relatively rare, while aptamers have attracted increasing interest due to their selectivity and the possibility of being custom-designed [338]. In aptamers however, the target-specificity is inherent in the specific nucleotide-sequence, due to its selection by a directed evolution approach, and are therefore produced without imprinting [338,339]. Nucleotide monomers have been investigated, as in the modified adenine used to produce a thymine sensor [339,340], though this is beyond the scope of this review. There are also examples of MIP-like devices, such as the highly selective G-quadruplex-based guanosine sensor devised by Li et al., which contain a binding site but are produced without an imprinting process [341].

There are however examples of oligonucleotide imprinting which involve trapping of the nucleotide-template complex in a synthetic polymer [342,343]. A relatively early example can be found in the work of Rezaei et al., who prepared an electrochemical sensor specific for dopamine by trapping a DNA-dopamine complex in an electropolymerized *o*-phenylenediamine film [344]. The device performed extremely well compared to the nonimprinted equivalent, with an IF of 6.6, and presented greater sensitivity and selectivity than an *o*-phenylenediamine film synthesized without DNA. Rezaei et al. further demonstrated the validity of this method by repeating the electropolymerisation of aminophenol with a Sudan II-oligonucleotide complex, resulting in excellent selectivity and sub-picomolar sensitivity [345]. Using a similar procedure, Zhang et al. prepared enantioselective electrochemical sensors for cimaterol and L-penicillamine, with the resulting MIP-sensors showing extremely high selectivity and sub-femtomolar sensitivity [346,347].

A variation of this can be found in the imprinted deoxyribonucleic acid aptamers of Zhang and Liu, 27 nucleotides in length, for adenosine and cytidine [348]. Fluorescently labelled and functionalised to give an acrydite group at the 5′ terminus, allowed integration of the sequence into an acrylamide crosslinking support matrix, with the resulting nanogels being found to illicit a stronger response than a free aptamer equivalent. Bai and Spivak similarly prepared a hydrogel for the measurement of whole viruses using polymerizable aptamers combined with conventional monomers [349]. The imprinted hydrogel was observed to shrink on target binding, which in combination with a simple laser-pointer based diffraction technique allowed the measurement of virus concentrations with LOD 10 ng/ml. The group used a similar protocol to produce protein sensors, suggesting this may be a versatile technique suitable for macromolecular detection [342].

The disadvantage of this method, from the perspective of this review is the need for a support structure. Relatively sustainable and biocompatible matrices are possible however, as demonstrated by Ghanbari and Roushani. The authors prepared an electrochemical sensor for a hepatitis C virus antigen using an imprinted 41 nucleotide aptamer immobilised on a carbon nanotube-chitosan composite with electropolymerized dopamine [350]. The sensor had a LOD of 1.67 fg/ml and demonstrated precise fg/ml quantification of the target antigen in real serum samples. Beiki et al. similarly prepared a lysozyme-specific sensor using imprinted aptamers and a carbon nanotube-chitosan composite but directly bound the aptamer to the chitosan covalently, though the structure was additionally secured by the electropolymerisation of methylene blue [351]. The device showed good selectivity for the target lysozyme, and a coherent response over a broad concentration range of 1 fM to 100 nM. Azadmehr and Zarei prepared a 2,4-dichlorophenoxyacetic acid sensor in a similar manner with chitosan and carbon nanotubes using phenylenediamine as an electopolymerisable monomer, resulting in a sensor with a 4 fM LOD [352].

Nucleic acids can therefore be employed in MIP sensor devices, with particular advantages in macromolecular sensing, however, a number of questions may be raised by this area of research which are not regularly addressed. Li et al. compared the effect of using an aptamer specifically optimized for the template vs a random DNA sequence in synthesis using aminophenol electropolymerized around a lincomycin-nucleic acid complex [353]. Unsurprisingly, the device formed using the specific aptamer showed a stronger response than that with the random sequence; however, the random sequence resulted in a similar response to the device prepared without any nucleic acid present, suggesting that the random DNA was not involved in the binding site formation. This raises questions regarding the ability of random sequences to form binding sites, particularly with smaller templates. Biopolymers are generally presumed to rearrange themselves into the optimal geometry for template binding, but nucleic acids may not have sufficient flexibility for this process [354]. In the absence of complementary geometry, a strong affinity would therefore depend on optimal electrostatic interactions, but in a nucleic acid, there are only 4 different nucleotide bases, and amongst these, there are several repetitions of the same chemical functionalities. Therefore, nucleic acid sequences that have not been previously optimized for a given template, i.e. those that would be obtained with minimal processing from a biological source, are unlikely to be broadly applicable in molecular imprinting, particularly for non-macromolecular templates.

5. Polyphenols and tannic acid

Polyphenols are a broad class of naturally occurring compounds characterized by multiple phenol groups. They include flavonoids, phenolic acids, lignans, and stilbenes, and are known for their antioxidant, antimicrobial, and anti-inflammatory properties. Among these, tannic acid is a specific type of hydrolyzable tannin, which is a subclass of polyphenols. It consists of multiple gallic acid units esterified to a glucose core. Tannic acid is commonly used for its astringency, metal-chelating ability, and antimicrobial properties, and it plays a role in leather tanning, medicine, and food preservation.

Asadi et al. prepared magnetic nanoparticles from crosslinked tannic acid as a possible cancer treatment by the controlled release of the 5-fluorouracil [355]. *In vivo* analysis demonstrated that the distribution of the nanoparticles could be controlled with an external magnetic field, with no effects of toxicity observed. Turan et al. prepared a biosensor for a prostate cancer biomarker from imprinted tannic acid crosslinked with diethylenetriamine [356]. The magnetic nanoparticles were applied in

combination with antibodies to form a plasmonic sandwich assay, resulting in a LOD of 0.9 pg/ml, high selectivity and demonstrated application in serum samples. Tannic acid has also been applied in the extraction of water pollutant following crosslinking with aminopropyltriethoxysilane [357] and successfully imprinted with several templates by copolymerisation with 1,6-diaminohexane [358]. In the copolymerisation with 1,6-diaminohexane it was noted that the IF was marginally lower with the tannic acid material than some other compositions in the study, but that it was much less expensive to produce than dopamine-based materials.

6. Vegetable oil-derived monomers for producing more sustainable, bio-sourced MIPs

Among the various non-conventional building blocks for the synthesis of bioMIPs, vegetable oils derived monomers are short-chained chemicals, thus cannot fit the definition of biopolymers, however this class of monomers is gaining increased attention largely due to the hydrophobicity, which is proposed to complement the hydrophilicity of most other monomers and of some biologically sourced polymers [359]. Lipid-based materials are also the most promising candidates for nanomedical therapy targeting the central nervous system [360]. An example in the field of imprinting can be found in the work of Le Goff et al., who prepared a biopesticide imprinted material from vegetable oil derivatives for sustainable crop protection [361]. Resveratrol is an effective pesticide obtained from several common berries, but is limited in application by its low aqueous solubility. Using epoxidized soybean oil acrylate, and functionalising with conventional synthetic monomers, the authors successfully prepared pesticide delivery microparticles composed of 80–90 % from plant derivatives. The lipid-based MIPs also showed an increase in resveratrol release in the presence of lipase, demonstrating their biodegradability.

Cao et al. produced magnetic nanoparticles coated with oleic acid and functionalised with methacrylic acid for the development of bisphenol A sensor [362]. The resulting oil-based imprinted magnetic fluid showed an IF of 5.12, and in combination with flow injection chemiluminescence gave a 3 nM LOD. Similar MIP particle systems stabilized with oleic acid have been prepared several times, with high-impact examples being those selective for cholesterol [363], metronidazole [364], thiabendazole and carbendazim [365]. Oleic acid has also been used directly as a functional monomer, as in 2,4-dichlorophenol imprinted microspheres of Li et al., which had a calculated high binding capacity of 183.8 mg/g and an IF of approximately 4.6 [366]. More recently, Katata et al. prepared an electrochemical sensor for methylene blue using a phospholipid fixed in an electropolymerized film [367]. The resulting device showed a nanomolar LOD and good selectivity, providing a foundation for further studies with such materials. Very recently, Cakir and colleagues prepared a sustainable monomer (acrylated methyl ricinoleate) from castor oil and used it for the synthesis of imprinted nanoparticles designed to recognize the cardiac failure marker cardiac troponin I [368]. Nanoparticles-based sensing, based on lifetime decay, permitted direct analysis of serum samples. These green-MIP-nanoparticles exhibited exceptional homogeneity in suspension (PDI = 0.064) and outstanding stability (1 year). Oil-based functional monomers are still rare, but their sustainability and ability to target hydrophobic environments means they are likely to become more prevalent in the future.

7. Discussion and conclusions

The advancement of molecular imprinting technology by utilizing biopolymers as starting materials is a promising avenue for developing highly selective and sustainable recognition systems. There are still several directions that the research should pursue to fully harness the potential of biopolymer-based MIPs. In particular, it would be essential to establish selection criteria for the biopolymers. To date, the use of a specific kind of biopolymer for defined analytical applications, as derived from a literature survey and as reported in Table 5, seems far from being optimized or motivated by rational choice. From the data available it is possible to make some preliminary observations of which materials appear most suited to different applications (Table 5). This is however based solely on the number and degree of successes reported with each specific biological material. In a few cases, such as in catalysis, it is clear that the emergence of a new catalytic specificity achieved by imprinting, started from an enzyme material. In the case of sensing, this can benefit from MIPs made starting from a variety of biopolymers, depending on the final intended use and its environment, despite the currents attempts having mainly involved chitosan-based MIPs.

The field of MIPs made starting from biopolymers should be optimized. This will be achieved by developing clear guidelines for choosing appropriate biopolymers. Factors such as molecular structure, functional group availability, and compatibility with imprinting processes should be considered to ensure optimal performance of the resulting MIPs. Additionally, expanding the range of biopolymers tested for the formation of MIPs represents another area of interest, and will enhance the versatility and applicability of sustainable “bio” MIPs. Systematic testing across various biopolymer classes can lead to the discovery of novel materials with superior imprinting capabilities. Ensuring the sustainability and the cost-effectiveness of these MIP materials is a key feature for their applicability in processes and analytical devices, therefore aligning the selection and utilization of biopolymers with sustainability and economic viability principles is crucial. Assessing the environmental impact and production costs in relation to the intended application niche will promote the development of eco-friendly and affordable MIPs, thus the use of web applications to define the degree of sustainability is a first key step in this process [6].

Obviously, achieving the required selectivity and affinity by these MIPs is of paramount importance for the MIP material. Attaining high selectivity and affinity in MIPs is key for their effectiveness.

Conventional molecular imprinting relies on numerous methods to select the most appropriate monomer combination. Often this can be sidestepped by referring to previously established protocols for the template or the broader molecular class to which the template belongs [369]. In some cases however it may be necessary to screen a large number of different functional monomers or specific compositions to achieve optimal molecular imprinting. While this process is most efficiently executed using computational methods, screening individual monomers has proven to be significantly less reliable than using polymer fragments or other polymer-based approximations [370,371]. Theoretically, the process of choosing the appropriate biopolymer for template imprinting is not substantially different from that of choosing a synthetic functional monomer. Computational screening of biopolymers for the design of molecularly imprinted polymers does not appear to have been performed thus far, given the complexity of such computational problems. Selecting the best biopolymers for synthesizing bioMIP necessitates a revision of the current rational design approaches, incorporating more specific computational tools and simulation techniques to predict and optimize the interactions between the template molecule and the

Table 5
Literature survey on the analytical applications so far described and the kind of biopolymer most commonly employed to prepare the bioMIP.

Analytical application so far described	Biomaterial selected for the bioMIP
Separation and extraction	Zein, dextrans, cellulose
Protein binding	Alginate, starch, silk fibroin, gelatin
Sensing	Chitosan, silk fibroin, lipid-based polymers
Catalysis	Imprinted enzymes
Assays and imaging	Imprinted albumin
Targeted drug delivery	Imprinted albumin, tannic acid, lipid-based polymers
Nanomedical sequestration	Silk fibroin, gelatin

specific biopolymer matrix. By addressing these areas, the field can progress towards creating biopolymer-based MIPs that are not only efficient and selective but also sustainable and cost-effective, thereby broadening their applicability across various sectors. An additional consideration relates to the measure for the efficiency of these bioMIPs. Throughout the present review the effectiveness of the imprinting process was estimated using the non-imprinted equivalent of the MIP as a control and reference for IF. This was solely for the reason that IF is available for most of the literature, however, it should be noted that in a discussion of biopolymers, it could be argued that the interaction between the analyte and the unmodified material should also be considered, as the material will have some inherent affinity for the material and crosslinking may simply reduce the available points of interaction. It is recommended therefore that, where possible, the effectiveness of the imprinting process, could be measured with more appropriate units. In the context of bioMIP optimization, it is proposed that blocking factors could be employed to enhance experimental reliability. A blocking factor is a variable that, while not of primary interest, is known to influence the outcome. In experimental design, such variables are used to form blocks—groups of similar experimental units—allowing researchers to control for extraneous sources of variability. This technique, originally developed to reduce experimental error and increase the precision of results by accounting for variability unrelated to the primary factors under investigation, may prove particularly valuable in the design of molecularly imprinted polymers (MIPs) derived from biopolymers. In the case, it is presumed that physical characteristics of the biopolymers, such as viscosity, entanglement, charges, might be considered for the blocks, so as to ultimately build a more suitable indicator to measure the relative imprinting effect with respect to the raw polymer and not just the NIP.

Concerning the key physical properties of the biopolymers so far used in molecular imprinting, these exhibit a degree of spatial deformability, allowing the contouring of target molecules during imprint formation. Cross-linking agents play a critical role by "locking in" these conformations, stabilizing the recognition cavities within the polymer network. This structural stabilization is conceptually similar to immobilised enzyme technology, where the functional conformation of an enzyme is preserved upon attachment to a support—thereby retaining bioactivity while improving operational stability. Indeed, both technologies share a common engineering principle: optimizing the balance between flexibility and structural integrity to maintain biological or recognition function under application-relevant conditions. Most of the bioMIPs mentioned form hydrogels, which are inherently soft and flexible, exhibiting mechanical properties that can be tuned for biointerface compatibility. For example, gelatin hydrogels have a Young's modulus ~10–50 kPa, hydrated silk fibroin up to ~1 MPa, chitosan ~0.1–2 MPa, alginate ~10–200 kPa, all depending on crosslinking density. These moduli fall within the range of many native biological tissues, supporting their application as biointerfaces, in biosensors, assays, or drug delivery. By blending different polymers (e.g., gelatin-chitosan or alginate-silk fibroin), one can further tailor mechanical stiffness, degradation rates, and biocompatibility, and at the same time fine tune the balance between conformational adaptability, for target recognition, and long-term structural stability, for application robustness. Finally, the advent of biorthogonal chemistry is expected to provide new platforms for crosslinking and for the functionalisation of biopolymers and bioMIPs. Reactions such as azide-alkyne cycloaddition or tetrazine-trans-cyclooctene ligation enable highly specific and efficient crosslinking or functional group incorporation under mild, physiologically compatible conditions, thereby enhancing the precision and tunability of the resulting material's structure and function, with advantages in the design of bioMIPs for tailored applications.

CRediT authorship contribution statement

Todd Cowen: Writing – review & editing, Writing – original draft, Data curation, Conceptualization. **Devid Maniglio:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Funding acquisition, Conceptualization. **Alessandra Maria Bossi:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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