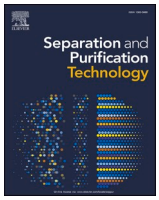
Separation and Purification Technology 337 (2024) 126288

Contents lists available at ScienceDirect

Separation and Purification Technology

journal homepage: www.elsevier.com/locate/seppur



Review

Polymer-based stimuli-responsive systems for protein capture: capacity, reversibility, and selectivity

Kieke de Boer *, Karin Schroën

Wageningen University, Laboratory of Food Process Engineering, Bornse Weilanden 9. 6708 WG Wageningen, the Netherlands

ARTICLE INFO

Keywords:
Stimuli-responsive materials
Protein capture
Interaction forces
Stimuli-responsive polymers
Switching behaviour

ABSTRACT

To develop effective (chromatography-like) separation systems, it's crucial to control protein interactions. Stimulus-responsive polymers, or "smart coatings," have emerged as a promising tool for achieving superior control by dynamically adjusting their physicochemical properties in response to stimuli like pH, temperature, salt, CO_2, or an electric field. This review provides an overview of protein capture systems that incorporate stimulus-responsive polymers and examines how conformational changes underlie the switch in protein-surface interactions. Specifically, we highlight the importance of a high adsorption capacity and selectivity for efficient capture, as well as reversibility for material reusability and sustainability. Our focus is on the key characteristics that determine the possibility of scaling up towards industrial separation systems with complex, diluted protein streams. Finally, we offer suggestions for further enhancement and identify critical investigation areas to drive the advancement of these innovative stimuli-driven separation techniques.

1. Introduction

Proteins are essential for human nutrition and play a pivotal role in medical and biotechnological applications. Separation and purification of proteins are important steps in scientific and industrial processes, requiring isolation up to varying extents. For example, separation strategies can be used to recover and recycle proteins from by-products or waste streams, thereby reducing industrial waste in the food industry and enhancing sustainable processing [1,2]. In general, the food industry employs a wide variety of separation techniques to extract and purify proteins. In the medical and biotechnical fields, purification is required for proteomics research, drug development, and the production of biopharmaceuticals [3–6]. Furthermore, environmental applications, particularly enzyme purification for effective waste management are worth mentioning [7].

To recover components from liquid streams, various methods can be applied depending on the required protein purity, including precipitation, centrifugation, membrane filtration, or chromatography [8–10]. Precipitation, for example by isoelectric point or organic solvent use, is economically attractive and convenient but may alter the structure and functionality of the proteins. Membrane filtration and centrifugation allow mild processing of the protein and do not interfere with its functionality. As these methods are largely dependent on the size of the protein, their ability to selectively separate a single protein from a mixture is low. Higher selectivity can be reached by chromatography, a high-precision separation technique that revolves around the interaction between the target molecule and a stationary phase (column) [8,11]. Separation of protein mixtures can be performed according to the retention time of the different proteins in the column. For some types of chromatography (e.g. affinity or ion-exchange chromatography), proteins have to be released from the column by changing the pH or ionic strength, which is often referred to as regeneration. The regeneration step allows the column to be used for a subsequent run. While a high protein purity can be reached, the costs of the column materials (resins) and regeneration chemicals are high, and often considerable secondary waste streams are produced [12].

A chromatography-like separation system with high selectivity and facilitated regeneration can be designed through the addition of smart responsive surfaces to the chromatographic materials [13,14]. In nature, many advanced responsive structures can be found (e.g., a Venus flytrap closing its leaf fast enough to catch insects; the motion of sunflowers towards sunlight; the swimming motion of jellyfish; the camouflage behaviour of chameleons in different environments) [15–18]. These naturally occurring structures form the inspiration for research into synthetic polymeric materials that mimic this responsive behaviour, based on internal stimuli, e.g., pH, temperature, ionic strength, and

* Corresponding author.
E-mail address: kieke.deboer@wur.nl (K. de Boer).

https://doi.org/10.1016/j.seppur.2024.126288
Received 25 September 2023; Received in revised form 28 December 2023; Accepted 2 January 2024
Available online 6 January 2024

external stimuli, e.g., light, electric field, or electromagnetic field. In this review, smart surfaces are described as materials that respond to an external or internal stimulus, allowing precise tuning of their chemical and physical properties, and through that their interactions with the target molecule(s) [18–20]. These materials are not only highly desirable for chromatographic-like separation applications but also more in general, as the molecular material properties can be controlled *in situ*, making them relevant for other processes as well.

These stimuli-responsive systems have been discussed in various reviews, which cover the notable publication increase over the last decade [15,16,21–28]. Their focus is often on the switching principle and the adsorption capacity, while reversibility over multiple cycles is hardly touched upon. The reversibility of adsorption is of utmost importance, as this allows product retrieval and material reusability [2,29]. We define reversible adsorption as the alternation between efficient capture and release mechanisms while preserving protein conformation and functionality. The collective process of capture and release over multiple cycles is referred to as separation, describing the complete approach to separate proteins from liquid streams. This review aims to demonstrate the possibilities of integrating stimuli-responsive systems into innovative protein separation processes. We first provide a general overview of stimuli-responsive systems and conditions relevant to protein adsorption and desorption. After this, we explore various types of polymers used in the development of stimuli-responsive separation systems for proteins and discuss their switching mechanisms and outcomes in terms of adsorption capacity, reversibility, and selectivity. We limit the review to systems that use stimuli-responsive polymers. The derived knowledge is then applied to suggest improvements and identify attention points for the development of novel responsive separation systems. Ultimately, this review aims to advance separation and purification strategies for proteins, with a particular focus on their removal from liquid streams.

1.1. Stimuli-responsive systems

Stimuli-responsive polymers are able to undergo a transition between two metastable energy minima in response to a stimulus [30]. A wide range of stimuli-responsive polymer systems has been researched, including polymer brushes, polyelectrolyte (multi)layers, self-assembled monolayers (SAMs), and conductive polymers (Table 1) [15,16,18,19,24,27,31]. Polymer brushes (Fig. 1A) are densely packed, surface-tethered polymer chains with controllable polymer density, composition, and morphology [18,19,32]. The polymer chains are anchored to the substrate by physisorption or covalent chemical attachment, of which the latter is preferred in terms of stability. Brushes are generally grown by either the grafting-to or grafting-from approach. In the grafting-to approach, pre-synthesized functional polymers are attached to the substrate. For the grafting-from method, polymer brushes are grown through surface-initiated polymerization. While grafted-to polymer brushes have a limited density due to steric hindrance between the chains, the grafted-from technique can be used to reach a higher grafting density. Between the fixed polymer chains, repulsive interactions occur, which can be relieved by the extension of the chains and swelling of the polymer brush [19]. When triggered by an external stimulus, the polymeric framework extends from or collapses onto the substrate surface [26].

A SAM is formed when molecules (in solution or vapor) adsorb and spontaneously self-organize in a well-defined thin monolayer on the surface [32,33]. While not limited to, most SAMs are made with long-chain hydrocarbons, of which alkanethiols on gold surfaces are most studied. The SAM structure is composed of an anchoring headgroup, backbone, and terminal group (Fig. 1 B). The anchoring group has a high affinity for the substrate and attaches strongly. Both the backbone and the terminal group can be modified with responsive moieties to gain unique responsive properties and interactions with the target molecule [31,34]. Additionally, low-density SAMs allow stimuli-responsive conformational transitions of the SAM chains, resulting in tuneable surface properties by a collapsing/extending motion [31,35]. The formation of SAMs is simple and well-defined functional layers can be created [32]. However, the layers are thin (one molecular layer) and have limited long-term stability. The formation and structure of polymer brushes are more complex, but better long-term stability is obtained. Additionally, polymer brushes can be tuned by the choice of starting monomers, and the method of preparation, which allows control over the brush length. For more information on the formation and structure of SAMs, we refer to Ulman [33].

Polyelectrolyte (multi) layers (Fig. 1 C) consist of one or more layers of polyions. In the case of a multilayer, layer-by-layer deposition of positively and negatively charged polyions is performed, allowing control over the layer thickness and the exposed surface charge [36,37]. Polyelectrolytes can be composed of negatively charged anionic monomers or positively charged cationic monomers, which can be sorted into two classes: strong and weak. Strong polyelectrolytes are permanently charged in aqueous solutions due to the presence of strong acid or base groups. Weak polyelectrolytes can reversibly protonate and deprotonate, allowing them to switch between charged and neutral states, based on the surrounding conditions. Stimuli-responsive polyelectrolyte multilayers are often associated with changes in the degree of swelling [37]. Besides polyelectrolyte multilayers, polyelectrolytes can be incorporated into polymer brushes as well [38–40].

For electrically responsive systems, conductive polymer layers (Fig. 1D) have been researched as well. These polymers are intrinsically conductive due to their conjugated backbone structure, consisting of a chain with localized carbon–carbon single bonds (σ bonds) and less localized carbon–carbon double bonds (π bonds) [18,41,42]. The p-orbitals of the π bonds overlap, allowing electrons to be delocalized more easily and move freely between the atoms. During synthesis, the polymer is created in its oxidized form, which requires the incorporation of dopants (mainly anions) to neutralize the charges and stabilize the polymer backbone. Besides, the dopant acts as a charge carrier that can remove or add electrons from or to the polymer chain, relocalizing them as polarons and bipolarons. When applying an electric field, the movement of the dopants disrupts the stable backbone which allows charge to pass along the polymer backbone. The most widely used intrinsically conductive polymers include poly[3,4-(ethylenedioxy)thiophene] (PEDOT) [43], polypyrrole (PPy) [44], and polyaniline (PANi) [45].

For all stimuli-responsive systems, the stimulus often results in one or more conformational changes in the polymer structure: swelling/collapsing, exposure of groups, (de)protonation, and internal restructuring. Consequently, these may induce a change in charge, wettability, exposed groups, and/or specific binding affinity, thereby affecting interaction with proteins in the solution.

Table 1
The main characteristic and potential switching behaviour of polymer brushes, SAMs, polyelectrolyte multilayers, and conductive polymers.

	Polymer brush	SAM	Polyelectrolyte multilayer	Conductive polymers
Main characteristic	Anchored polymer chains	Well-defined self-assembled monolayer	Alternating layers of polycations and polyanions	Intrinsically conductive polymer layer
Switching behaviour	• Chain conformation • (De)protonation • Hydration	• Chain conformation • (De)protonation	• (De)protonation • Swelling (hydration)	• Redox reactions • Internal restructuring

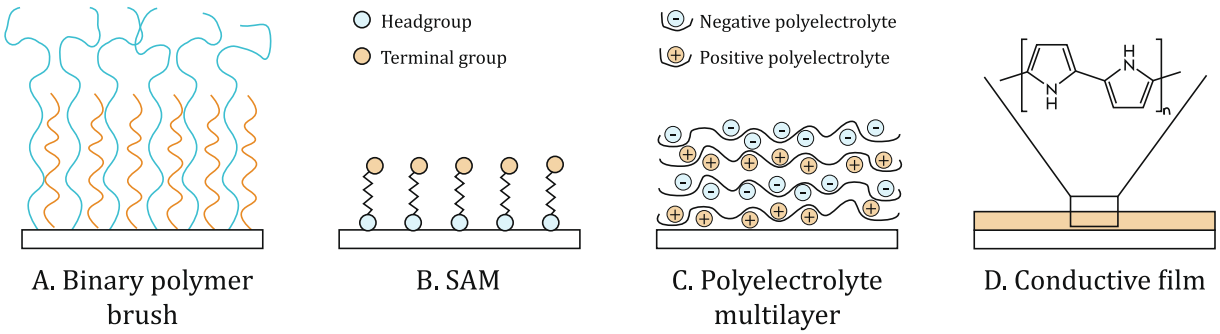


Fig. 1. Various polymer film types used in stimuli-responsive systems: A. binary polymer brush, B. SAM, C. polyelectrolyte multilayer (4 layers), D. conductive polymer film of polypyrrole.

1.2. Protein adsorption & desorption

The adsorption of proteins has been researched and reviewed extensively over the last decades but is still not completely understood [46–52]. The process is complex, as it is dynamic and controlled by the properties of the protein, surface, and environment in which it is present. The intricate composition and structure of proteins result in complex dynamic behaviour, like structural rearrangements or surface aggregation [46,52]. In general, the adsorption of proteins follows the overview given in Fig. 2. Firstly, the proteins will move towards the surface (1), either by diffusion or under an applied force. When in their vicinity, proteins can adsorb onto surfaces through various interaction forces (2). Depending on the strength of this initial adsorption step, several consecutive steps may occur. The protein can stay attached to the surface and undergo conformational rearrangements to increase the contact area further and minimize interaction energy (3a). On the other hand, desorption can occur due to repulsive forces (3b), competition with other adsorbing molecules (3c), or overcrowding, after which the protein leaves the surface. Desorption can be stimulated by displacement with another molecule which results in an overall lower interaction energy in the system [52,53]. The rate-determining steps are often: (i) transport towards the surface, due to diffusion limitations, or (ii) adsorption, which becomes dependent on the surface layer composition over time [53].

From a thermodynamic perspective, proteins will spontaneously adsorb to a surface if this results in a decrease of Gibbs free energy for the overall system [54]. It must be noted that the overall change in enthalpy and entropy during adsorption are not solely related to the interaction between proteins and the surfaces, but include those inside proteins (unfolding), between the proteins, and between the surrounding aqueous solution and the surface and proteins [20,49,55].

Time plays a crucial role in the capacity and reversibility of protein adsorption processes [56]. Increasing the protein-surface contact time has been shown to elevate adhesion forces and reduce reversibility, which suggests time-dependent physiochemical changes. This includes conformational changes to improve the interaction with the surface and lower the Gibbs free energy, or interactions with each other, thus forming films that are notoriously difficult to remove. Thermodynamically, most adsorption behaviour is considered to be reversible, however, this requires simultaneous multi-point detachment, which is unlikely to occur. Therefore, the chances for full desorption are approximately zero when sufficient contact time is allowed [53,57].

Besides time, the adsorption reversibility is determined by the type and strength of protein-surface interactions. Different interaction forces can play a role, including hydrophobic, electrostatic, van der Waals, and hydrogen interactions, dependent on the properties of the adsorbent material, protein, and solution [52,58]. Long-range forces between proteins and adsorbents are often well-described by DLVO theory, while non-classical DLVO contributions play a major role when proteins come close to the surface, determining the rate and strength of protein association [52,59–61]. For surfaces with stimuli-responsive polymers, the chosen stimuli will mainly influence the chemical and physical properties of the adsorbent, thereby influencing the interaction type and/or strength. Especially the charge and wettability of the adsorbent are known to be important parameters for the control over protein adsorption, which are discussed next.

1.2.1. Surface characteristics

Wettability. Protein adsorption can be regulated through the wettability of the substrate, which is often characterised by the contact angle that a liquid creates on the surface [62]. The wettability of a solid surface is dependent on the chemical composition and surface geometry and may be affected by a stimulus [63]. The wettability of a material determines the level of hydration in wetted systems. Here, the terms hydrophobic and hydrophilic are used to describe the surface properties, while

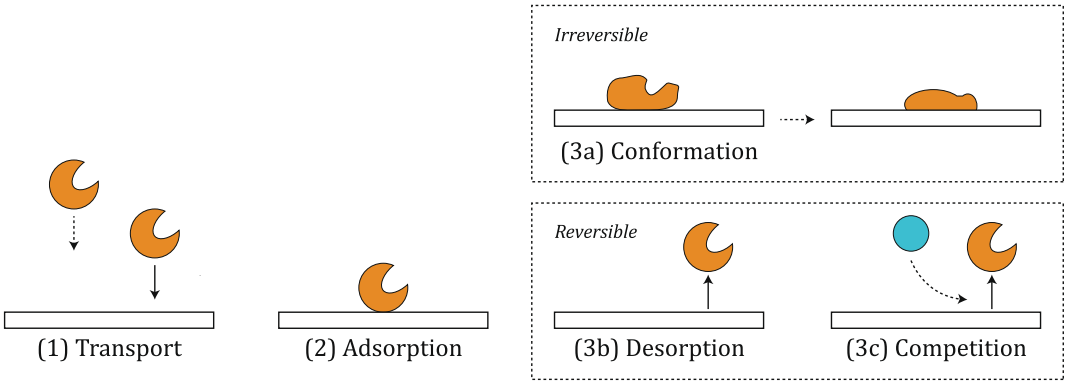


Fig. 2. Schematic overview of possible steps during protein adsorption.

hydration is seen as a consequence of this characteristic.

Protein adsorption can occur on hydrophobic and hydrophilic surfaces, but the interaction type and strength are dependent on the wettability, resulting in a different adsorption capacity and reversibility. On nonpolar surfaces, hydrophobic interactions are the driving force for protein adsorption. Upon protein adsorption, a reduction in Gibbs free energy is achieved by displacing the surface-bound water molecules, therewith decreasing the solvent-exposed area [47,49,52,64]. Additionally, moving water molecules surrounding the hydrophobic groups of the protein towards the bulk solution contributes to a decrease in free energy [47,49]. Contrarily to nonpolar surfaces, polar uncharged surfaces mediate protein interactions mainly through electrostatic, van der Waals interactions, and hydrogen bond formation [47,49,65]. Compared to their hydrophobic counterparts, these hydrophilic surfaces form strong hydrogen-bonded networks with water molecules [66,67]. The replacement of a hydrogen bond between the surface and a water molecule with a hydrogen bond between the surface and proteins correlates to a Gibbs free energy around zero and is therefore not thermodynamically favoured [49,52]. Still, in practice, adsorption of proteins does occur on polar surfaces, due to the numerous functional polar groups on the proteins [49]. Polar anti-fouling materials (e.g., polyethylene oxide (PEO)) are an exception, as they form a very strong water network which prevents replacement by proteins [49,68]. In general, higher levels of protein adsorption are seen on hydrophobic surfaces [56,64,69–71].

On both hydrophobic and hydrophilic surfaces, protein adsorption may result in conformational changes. For hydrophobic surfaces these are associated with competition over hydrophobic interactions which naturally stabilize the tertiary structure of proteins, resulting in unfolding [49,65]. On hydrophilic surfaces, conformational changes are related to the stabilizing hydrogen bonds in the protein structure [46,49]. Overall, conformational rearrangements occur more and faster on hydrophobic surfaces [64,72–74].

The wettability can be used as a tool to initiate protein desorption from the surface as well. Strong hydration repulsion forces, caused by the water network on polar surfaces, have been associated with driving protein desorption [52,75]. Due to the slower conformational changes and lower adhesive forces on hydrophilic surfaces, a higher desorption percentage could be reached by a rinsing step compared to hydrophobic surfaces [47,74].

Finally, the surface roughness influences surface wettability as well. In general, increasing the surface roughness will increase the surface hydrophobicity when the surface is intrinsically hydrophobic and reduces hydrophobicity when the surface is intrinsically hydrophilic [17,76]. For stimuli-responsive systems, the roughness often has a large impact on the final wettability of the surface [39]. For more information on the synergy between wettability and roughness, we refer you to the work of others [77].

Charge. For charged surfaces, it is generally assumed that electrostatic attraction and repulsion between the surface and proteins are the main interaction forces [49]. Electrostatic attraction is observed between surfaces and proteins of opposing charges and is strongly dependent on the pH and ionic strength [52,58,66,78]. When surface and protein have the same charge sign, electrostatic repulsive forces can create a strong electrostatic barrier to adsorption, overruling short-range attraction forces [58,64,74,79].

It is, however, important to consider the presence of charged salt ions in the solution. Their high diffusivity, compared to proteins, allows fast attraction to the surface, thus forming a so-called double layer [65]. This double layer consists of the Stern layer, a compact layer of ions close to the surface, and the Gouy-Chapman or diffuse layer, which extends into the solution and contains compensating ion charges. When proteins approach such a surface, the diffusive layers will overlap, resulting in the redistribution of ions [52,59,64]. In general, the situation is similar to protein adsorption on hydrophilic non-charged surfaces [49]. The replacement of electrostatic bonds between the surface and ions with electrostatic bonds between the surface and proteins is an ion-exchange process, driven by entropic effects. Spontaneous adsorption is further stimulated by the high charge density on protein surfaces, which can result in the simultaneous release of multiple ions. Additionally, distinct charge patches that may be used to control the orientation of proteins can play a role [80,81]. On charged surfaces, conformational rearrangements of the proteins occur due to competition for hydrogen-bonds and salt-bridges, which are both known for their stabilization role in protein structures [46].

Among charged surfaces, zwitterionic layers form an interesting exception to the described behaviour. Due to their closely spaced positive and negatively charged groups, they can bind water molecules and counterions strongly, thereby preventing displacement by proteins and exhibiting protein-repulsive behaviour (anti-fouling) [49,82].

1.2.2. Protein characteristics

Adsorption is known to be affected by characteristics of the proteins as well, including size, charge distribution, hydrophobicity, and structure stability [56,64,65,71,83]. Related to the latter aspect, proteins can be divided into ‘hard’ and ‘soft’ proteins, which show different affinities for hydrophobic and hydrophilic surfaces [64,71,84]. Adsorption of ‘hard’ proteins is dependent on the surface wettability, with preferred adsorption on hydrophobic surfaces, although they can adsorb on hydrophilic surfaces as well under electrostatic attraction. Their strong internal structure reduces the amount of conformational changes during adsorption. ‘Soft’ proteins have a lower structural stability and tend to have a higher driving force for adsorption due to structural conformations. These proteins show significant adsorption on both hydrophobic and hydrophilic surfaces. In general, the inherent stability of the protein structure influences the extent of conformational changes upon adsorption [85].

1.2.3. Stimuli-responsive interaction forces

For the design of stimuli-responsive systems that we consider in this review, adsorption of the target molecule needs to be as reversible as possible, as ideally, desorption is complete. Protein adsorption generally occurs through various non-covalent interactions, of which the strength, direction, or general presence should be altered by a switch in the adsorbent properties. In the case of polymer-based adsorbents, this may occur through reversible noncovalent and/or dynamic covalent interactions within or between polymer structures [86–88]. Ideally, the stimulus-initiated response of the polymer is rapid, specific, and reversible [18]. In this review, most systems will include polymers that change through noncovalent interactions including hydrogen bonding, hydrophobic interaction, and π-π stacking. In the discussed systems, the change in interaction forces is initiated by a stimulus that affects the material properties directly (e.g., electric field) or that affects the solution properties (e.g., pH). The latter generally influences both the characteristics of the adsorbent surface and the protein.

2. Protein capture with stimuli-responsive polymers

Polymers that can change their conformation in response to external stimuli can alternate between attractive and repulsive interaction with proteins in the solution. Multiple mechanisms might be in play during this ‘switch’, dependent on the type of polymer layer that is used. For this review, polymer conformations are divided into four groups: 1) swelling/collapsing; 2) exposure of groups; 3) (de)protonation; 4) internal restructuring. The properties of the polymer layer that change, have also been divided into four categories: a) charge; b) hydration; c) exposed groups; d) specific binding affinity.

2.1. Electrically-responsive polymers

In electrically-responsive systems, an electric field drives the adsorption and/or desorption of proteins towards or from the substrate surface. This approach requires materials to be electrically conductive (e.g., gold, Fig. 3A). Protein adsorption can be regulated on metal surfaces by an external electric field, with adsorption levels depending on the protein properties [89,90], surface properties [89,91], and electric potential [89,91,92]. Additionally, electrically induced protein desorption has been demonstrated on gold surfaces, for example for β-lactoglobulin [75] and fibrinogen [93,94]. In the case of β-lactoglobulin, partial release (up to 15 %) was reached by applying a negative potential, −0.4 V compared to open-circuit potential. This release was attributed to the increased double-layer potential and hydration repulsion [75]. However, it is important to note that the desorption percentage decreased over multiple cycles, suggesting that β-lactoglobulin predominantly binds irreversibly to the surface, leading to a reduction in separation efficiency. For fibrinogen, more efficient desorption was achieved (90–97.9 %), which was attained at higher electric voltages (−1.2 to −1.5 V vs Ag/AgCl) [93,94]. Here, the desorption mechanism was linked to hydrogen evolution reactions at the surface, which can negatively impact the structural integrity of the protein.

Improved adsorption capacity and reversibility, while maintaining protein functionality, can be realised by the addition of electrically-responsive polymers. Here, we evaluate non-conductive polymers and conductive polymers which show a response to an electric stimulus [41]. Electric conductive polymers are conductive due to the high electron mobility caused by constitutive bonds between atoms (intrinsic) or the presence of conductive particles (extrinsic). In Table 2, an overview of reversible electrically responsive protein separation systems is given.

2.1.1. Non-conductive polymers

For non-conductive polymers, the electric stimulus does not directly impact the inherent structure of the polymers. However, these polymers still show electrically-responsive behaviour due to attraction/repulsion between their charged moieties and the charged surface or due to pH-related (de)protonation and hydration. In the case of polyelectrolytes, the availability of charged groups allows adsorption of charged entities without the application of a potential difference between the electrodes [95]. This also applies to proteins, as shown in the work of Fritz et al. [96]. In that work, one of the electrodes was positively charged (coated with poly(diallyldimethylammonium chloride) (PDADMAC)), while the other was negatively charged (coated with poly(4-styrenesulfonate) (PSS)). Electrostatic interaction between negatively charged whey proteins and the PDADMAC electrode resulted in spontaneous adsorption (Fig. 3 B). Applying a negative potential to the PDADMAC electrode resulted in partial protein desorption (compared to the initial adsorbed amount) due to a change in interfacial surface charge of the carbon electrode, leading to repulsive electrostatic interactions. The importance of electrostatic interactions is highlighted in the work of Ladam et al. [97] as well, wherein human serum albumin is absorbed onto a polyelectrolyte multilayer. A benefit of polyelectrolyte layers is that polyelectrolyte-protein interaction is relatively weak, compared to e.g., carbon, leading to better reversibility of adsorption. In the work of Fritz et al. [96], the (partial) reversibility of protein adsorption is maintained when the adsorption time is increased. The duration of the adsorption and desorption steps solely determines the adsorption and desorption capacity.

In the work of Mu et al. [98], a mercaptohexadecanoic acid (MHA) low-density SAM was created, wherein the conformation of the MHA molecules could be altered by the application of an electric field (Fig. 3C). The surface could be covered with carboxylic acid or amino end groups to selectively adsorb avidin or streptavidin, respectively (Fig. 4A). Adsorption of avidin was achieved on a deprotonated COOH-terminated SAM, under a negative electric potential, driven by electrostatic attraction. Protein release was realised by collapsing the SAM under a positive electric field, by exposure of the hydrophobic uncharged backbone. For the NH_3-terminated SAM, the opposite behaviour was shown for streptavidin. The positively charged NH_3^+ groups were extended from the surface when a positive potential was applied, while the bent state was achieved by applying a negative potential. The conformational change of the SAM was said to be fully reversible, although the system does experience a decrease in adsorption efficiency over cycles. Finally, it is important to note that desorption of alkanethiols can occur at reductive potentials, which would be undesirable for a reversible protein separation process. The relation between reductive potential and desorption values depends on the surface material, the chain length, and the pH, among others [99–101].

In the work of Ferrand-Drake del Castillo et al. [102], protein capture and release were shown with poly(methacrylic acid) (PMAA) brushes in response to an electric stimulus. Here, the electrochemical switching was applied to change to local pH close to the surface, consequently affecting the charge and hydration level of the PMAA brush (Fig. 3D). Protein adsorption (1–4 μg/cm^2) could be realised at pH 7.4, driven by electrostatic interactions between deprotonated carboxylic groups and positively charged proteins. Reductive potentials resulted in protein desorption, with complete protein recovery at −0.75 V (vs Ag/AgCl). The strong protein repulsion is the result of the increased pH, which results in a combination of entropic effects (hydration and chain conformation) and electrostatic repulsion between the negatively charged PMAA brush and the now negatively charged proteins. The polyelectrolyte brush showed excellent switching reversibility (>100

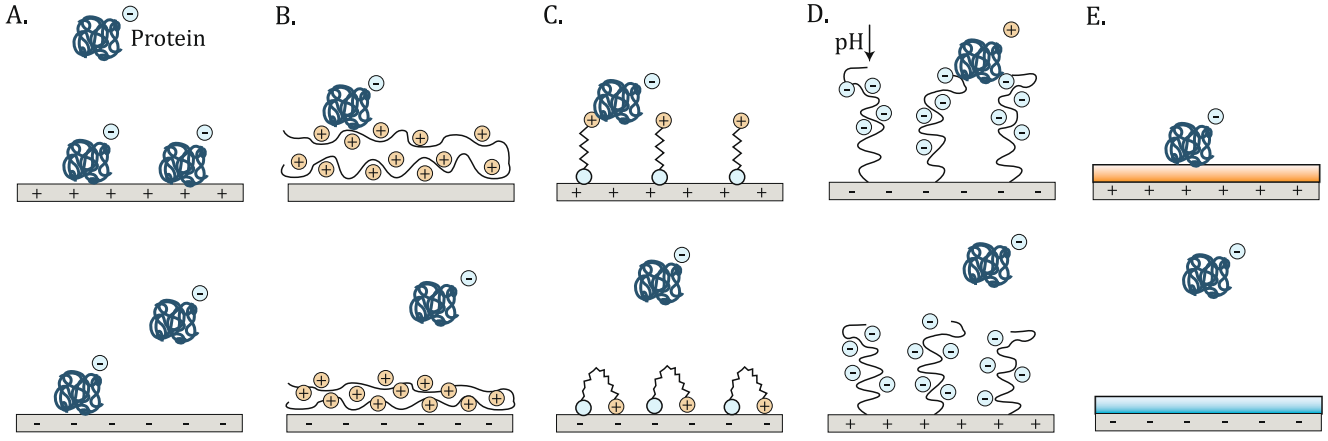


Fig. 3. Visualisation of protein adsorption and desorption in different electrically-responsive systems, which are all discussed in this review: A) conductive surface, no polymer layer, B) polyelectrolyte-covered, C) low-density SAM, D) polymer brush, E) internally conductive polymer film.

Table 2
Overview of electrically-responsive polymer systems for the separation of proteins. Conformational change: 1) swelling/collapsing; 2) exposure of groups; 3) (de) protonation; 4) internal restructuring; 5) no conformation. Responsive behaviour: a) charge; b) hydration; c) exposed groups; d) specific binding affinity. *Calculated from data/graph in article. [1] Reversibility shown with a change in current. N.D. = not determined.

Protein	Responsive polymer	Polymer type	Conformation	Response behaviour	Switch	Adsorption capacity	Desorption capacity	Reversibility percentage	Reference
Non-conductive polymers									
Avidin	SAM layer (–COOH end group)	SAM	1, 2	a, b, c	−0.3 V to +0.3 V	8 μg/cm^2	90.6 %	40 cycles, 30 % decrease adsorption	[98]
Streptavidin	SAM layer (–NH_2 end group)	SAM	1, 2	a, b, c	+0.3 V to −0.3 V	7 μg/cm^2	94.6 %	N.D.	[98]
Whey protein	PDADMAC	Polyelectrolytes	5	a	0 V to −1.2 V	10 mg/g	>100 % (compared to cyclic adsorption)	10 cycles, no loss	[96]
Serum proteins	PMAA	Polymer brush	3	a, b	0 V to −0.75 V	1–4 μg/cm^2	100 %	4 cycles, loss dependent on voltage	[102]
Conductive polymers									
Bovine serum albumin	Polypyrrole	Internally conductive polymer	2, 3, 4	a, b, c	+0.5 V to −0.8 V	0.27 μg/cm^2	63.0 %*	4 cycles, no loss	[104]
Protamine sulfate	Polypyrrole	Internally conductive polymer	2, 3, 4	b, c	−0.8 V to +0.5 V	1.1 μg/cm^2	81.8 %*	4 cycles, no loss	[104]
Fibronectin	Polypyrrole	Internally conductive polymer	2, 3, 4	a, b, c	+0.5 V to −0.8 V	0.16 μg/cm^2	62.5 %*	4 cycles, no loss	[104]
Fibrinogen	Polythiophene	Internally conductive polymer	1, 3, 4	a, b, d	0 V to +1.05 V	N.D.	N.D.	2 cycles, no change [1]	[103]

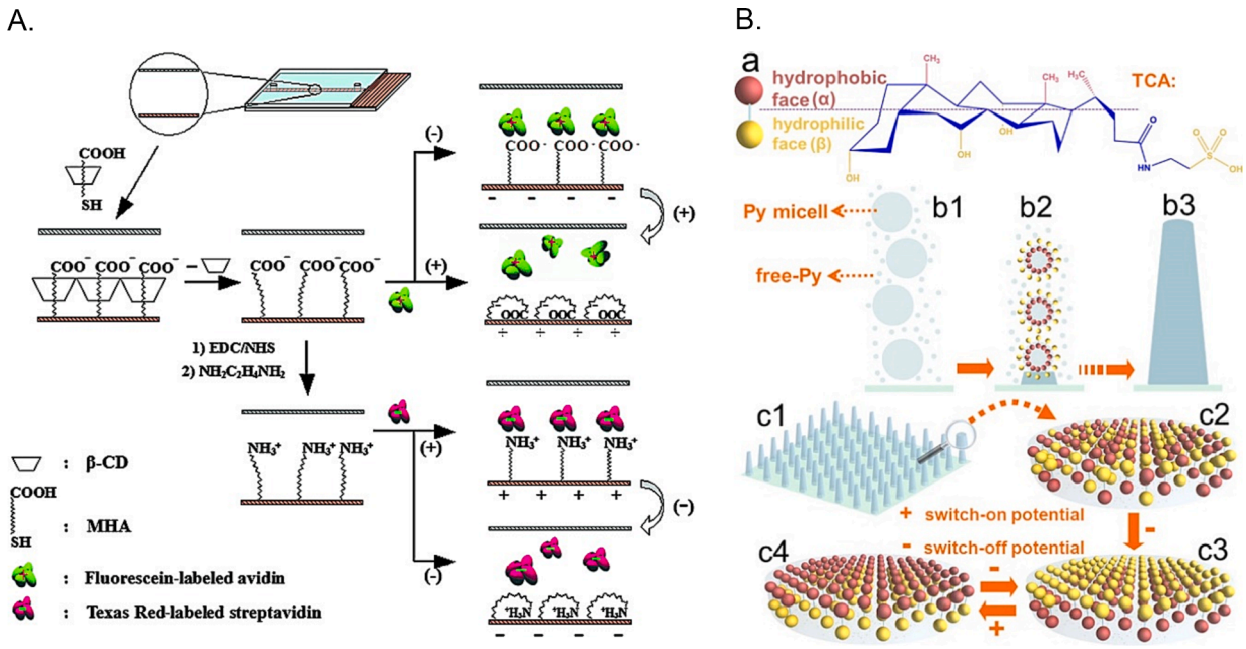


Fig. 4. Schematic illustration of two electrically-controlled polymer surfaces: SAMs of thiols with terminal carboxylic or amino groups (A), reproduced with permission from [98], and conductive polypyrrole films with taurocholic acid as dopant (B), reproduced with permission from [104].

cycles), for which they highlight the importance of grafting chemistry. Reversibility of protein adsorption (for a variety of proteins) was shown as well, which was voltage-dependent. This method shows promising results for future applications, which will be further discussed in chapter 3.3.

2.1.2. *Conductive polymers*

Intrinsically conductive polymers, such as polypyrrole, polyaniline, or polythiophene, have been used to functionalize electrically-driven separation systems as well (Fig. 3E) [103,104]. These polymers are conductive as a result of their conjugated backbone structure, which is stabilized by a dopant [105]. A switch between an oxidized state and a reduced state can be initiated by applying an oxidation or reduction

potential, respectively. Consequently, the chemical, physical, and electrical properties of the polymer film are impacted (e.g., conductivity, degree of swelling, and wettability), dependent on the type of conductive polymer that is used. Additionally, redox-active processes are thought to be an interesting route to achieve selectivity, as reduction or oxidation can alter the affinity of the active sites [106]. For more detailed information on conductive polymers and their properties, we refer to the work of others [76,105,107,108].

For electrically-responsive protein capture and release, polypyrrole [104] and polythiophene [103] films have been reported. For both polymers, proteins can reversibly adsorb on the conductive polymer film depending on the wettability and roughness of the substrate. The polythiophene film could be switched from rough, highly porous, and hydrophobic in the undoped state (0 V vs Ag/AgCl), to a doped state with reduced roughness, pore occupancy by counterions, and hydrophilic properties (1.05 V vs Ag/AgCl) [103]. The undoped surface was resistant to fibrinogen, which was linked to antifouling behaviour by the superhydrophobic surfaces when present in an aqueous solution. On the other hand, significant adsorption was observed in the doped state, which was thought to result from improved contact between the aqueous solution and the hydrophilic surface. Moreover, adsorption occurred due to electrostatic interaction between the positively charged conducting polymer in the hydrophilic state and the negatively charged protein [103]. For polypyrrole films, the properties showed to be tuneable when taurocholic acid (TCA) was used as a dopant (Fig. 4B) [104]. The surfactant-like properties of TCA allowed electrochemical switching between a hydrophilic and hydrophobic state by changing the orientation of the TCA molecule, caused by reduction and oxidation of the polypyrrole backbone. This switch was reported to initiate the adsorption and desorption of bovine serum albumin, protamine sulfate, and fibronectin. Adsorption of fibronectin and bovine serum albumin was stimulated on the hydrophobic substrate and prevented when hydrophilic, as opposed to the result found for the polythiophene film. This is likely the result of hydration repulsion, caused by intermolecular hydrogen bonds of the OH and SO_3H groups. Additionally, electrostatic repulsion is reported between the negatively charged protein and deprotonated OH groups at the hydrophilic side of the TCA molecule. In the hydrophobic state, these repulsion forces were diminished and adsorption occurred more easily. On the other hand, protamine sulfate was preferably adsorbed in the hydrophilic state due to attraction to –OH and –SO_3H groups on the surface of TCA. In the hydrophobic state, this attraction was greatly reduced and desorption was initiated. While protein adsorption seemed to be reversible over several cycles in both papers, complete protein recovery is not shown [103,104].

2.2. Temperature-responsive polymers

Temperature-responsive polymers (Table 3) rely on temperature-induced changes in the molecular conformation of the material [12,109–111]. While the solubility of some polymers increases with temperature due to enthalpic effects [110], other polymers show dehydration and eventually aggregation at higher temperatures [100]. At the so-called lower critical solution temperature (LCST), these polymers reversibly switch their conformation between collapsed/insoluble and swollen/soluble states. The most well-studied polymer exhibiting this behaviour is poly(N-isopropyacrylamide) (pNIPAM). This hydrophilic polymer forms intermolecular hydrogen bonds with water when the temperature is below the LCST (extended state), and intramolecular bonds between C═O and N-H groups of the polymer chain above the LCST (collapsed state) (Fig. 5) [18]. Not surprisingly, pNIPAM surfaces have been suggested for protein separation due to their switch between fouling (collapsed state) and anti-fouling (swollen state). While the extent of adsorption varies, it's generally accepted that proteins are adsorbed in greater quantities above the LCST [20]. Complete reversibility of adsorption has been observed for lysozyme in an adsorption column [112], while cytochrome *c* adsorption on a pNIPAM brush was only partly reversible (29.2 %) [113]. Full adsorption reversibility over multiple cycles is hardly achieved, which eventually will hamper continuous cycling as would be needed for large-scale processes.

As previously described by Cross et al. [20], the physical properties of pNIPAM films are expected to influence protein interactions, although consensus about the underlying mechanism has not been reached. Overall, it was suggested that wettability could not be used as a prediction for overall protein behaviour. This may indicate that local hydrophobicity changes affect protein interactions, or that other physical properties mediate adsorption behaviour [20]. Furthermore, the influence of the grafting density is under discussion: incomplete coverage will lead to direct binding to the substrate which will be irreversible [110,114,115]; when packed too densely, the decreased polymer mobility will prevent conformational changes and therefore the thermal switch [110]. Additionally, the exact thermal switch behaviour is affected by the molecular weight of the polymers and the addition of hydrophilic or hydrophobic moieties in the pNIPAM chain [27,110]. So far, the limited results prevent quantitative relationships from being established. A detailed overview of the effect of physical parameters can be found in Cross et al [20].

In the work of Balamurugan et al. [116], a SAM with oligo(ethylene glycol) (OEG) and methyl end chains was investigated as a temperature-responsive polymer film for reversible adsorption of lysozyme. Adsorption was seen above 32 °C while a lower temperature allowed protein removal. The switching behaviour is related to structural changes when going through a disordered-to-partially ordered state transition. This may lead to exposure of hydrophobic moieties and thus influence the hydration layer of the SAMs. Alternatively, it was suggested that the uptake of hydroxide ions from the solution could result in a negative surface charge [117], which would induce electrostatic repulsion towards the proteins. Additional work is required to deduce the precise impact of temperature on the structure of SAMs and the involved forces in protein repulsion. The reversibility between protein adsorption and release remains relatively stable over 4 cycles (±90 %), however, both adsorption and desorption capacity slightly decrease over multiple cycles, which may cause problems in a later stage [116].

The papers in Table 3 all report an LCST of 32 °C, and employ a temperature between 37 and 40 °C to initiate adsorption, which will not lead to serious denaturation of cytochrome *c* or lysozyme [118,119].

Table 3
Overview of temperature-responsive polymer systems for the separation of proteins. Conformational change: 1) swelling/collapsing; 2) exposure of groups; 3) (de) protonation; 4) internal restructuring; 5) no conformation. Responsive behaviour: a) charge; b) hydration; c) exposed groups; d) specific binding affinity. *Calculated from data/graph in article. N.D. = not determined.

Protein	Responsive polymer	Polymer type	Conformational change	Response behaviour	Switch	Adsorption capacity	Desorption percentage	Reversibility	Reference
Cytochrome *c*	pNIPAM	Polymer brush	1, 2	b, c	T > or < LCST (32° C)	0.65 mg/g	29.2 %	N.D.	[113]
Lysozyme	pNIPAM	Polymer brush	1, 2	b, c	T > or < LCST (32° C)	1.25 mg/column*	96.4 %	N.D.	[112]
Lysozyme	OEG	SAM	1, 2	a, b, c	T > or < LCST (32° C)	170 ng/cm^2	100 %	4 cycles, >90 % efficiency	[116]

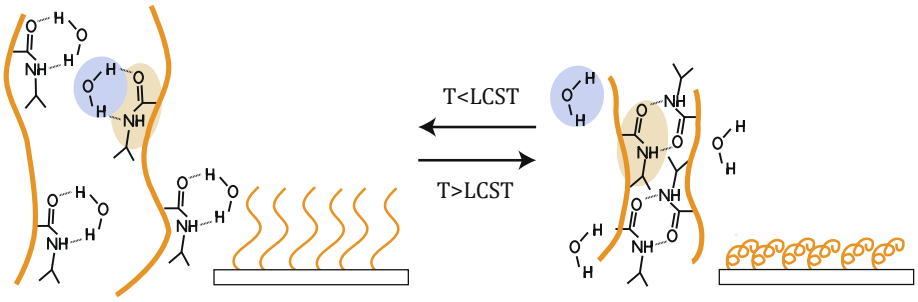


Fig. 5. Schematic illustration of thermal response of pNIPAM polymers upon heating and cooling above and below the LCST.

Other temperature-responsive polymers exhibit elevated LCST values and thus have a much bigger effect on protein denaturation [120–122]. Besides, the side groups and pH can affect the LCST [121,123], which makes us conclude that the selection of a polymer should be guided by its LCST under processing conditions and the temperature stability of the target protein.

2.3. pH-responsive polymers

The most direct way to create pH-responsive polymers is through incorporation of acidic and/or basic ionisable moieties (e.g., polyanions, polycations, and polyzwitterions) [16,27,124]. These ionizable groups can be (de)protonated in response to the pH, thereby influencing polymer conformation and solubilization, among others [16,27]. For polymers with polyanions or polycations, the charged state triggers electrostatic repulsion between the polymer chains, resulting in highly solvated and swollen brushes [39,40]. For neutral groups, the brush partially collapses due to a lack of electrostatic repulsion and increase in hydrophobic attraction. For zwitterionic polymers, which contain anionic and cationic moieties in close proximity, the pH determines whether the brush is negatively, positively, or neutrally charged, therewith influencing the final brush properties [49]. The pH has been used as a single external switch [39,40] (Table 4) and combined with other stimuli to tune protein adsorption and desorption [115,125–130], the latter is described in the section "Multi-stimulus responsive polymers".

In Yu et al. [39], reversible protein adsorption was achieved by protonation and deprotonation of carboxylic groups in a PMAA brush. At pH 4, the polymers partially collapsed, thus allowing hydrophobic and hydrogen bond formation between the polymer brush and proteins. At pH 9, repulsive interaction between the deprotonated carboxylic groups initiated swelling of the polymer brush, causing a high degree of hydration and consequently 90 % protein desorption, which seemed stable in a second cycle. Additionally, the use of 3D silicon nanowire arrays resulted in a higher adsorption capacity compared to a smooth silicon surface (220 compared to <4 μg/cm^2), which was attributed to the high specific surface area and enhanced local topographic interactions. The work of Ferrand-Drake del Castillo et al. [38] shows a similar strategy for protein adsorption and desorption on PMAA brushes. Here, protein capture is promoted in the neutral state of the polymer brush (pH < pK_a), which is attributed to multivalent hydrogen bond formation between the carboxylic acid groups on the polymer brush and various hydrogen acceptors on the protein surface. A high binding capacity could be reached for 13 types of proteins (±400–6000 ng/cm^2). Complete desorption of the proteins could be obtained by increasing the pH above the isoelectric point of the protein, as a result of electrostatic repulsion forces between deprotonated carboxylic groups and negatively charged proteins. A similar pH-regulated process was used to assist protein capture in a membrane system, in the work of Ye et al. [40], whereby tertiary amine groups of poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA)) were switched between protonated and deprotonated state. Protonation of the amine groups resulted in electrostatic attraction of the proteins, while deprotonation diminished electrostatic interactions, ultimately recovering 94 % of the bovine serum albumin. The recovery remained high during the first four sequential adsorption and desorption cycles, but decreased later [40], which could be due to salt accumulation in the polymer brushes, rendering them ineffective upon prolonged use [131].

As shown by Sundaram et al. [132], zwitterionic polymer layers can be switched from non-fouling behaviour at neutral pH, to adsorption behaviour at a pH below 4 or above 8, driven by (de)protonation (Fig. 6). The used carboxybetaine methacrylate (CBMA) monomer contains a carboxylic acid and amine group, which are both charged between pH 4 and 8, ensuring charge neutrality. At pH < 4, the carboxylic group protonates giving the zwitterionic polymer layer a positive charge, thus allowing negatively charged proteins (e.g., pepsin) to

Table 4
Overview of pH-responsive polymer systems for the separation of proteins. Conformational change: 1) swelling/collapsing; 2) exposure of groups; 3) (de)protonation; 4) internal restructuring; 5) no conformation. Responsive behaviour: a) charge; b) hydration; c) exposed groups; d) specific binding affinity. *Calculated from data/graph in article. N.D. = not determined.

Protein	Responsive polymer	Polymer type	Conformational change	Response behaviour	Switch	Adsorption capacity	Desorption capacity	Reversibility	Reference
Bovine serum albumin	PDMAEMA	Polymer brush	1, 3	a	pH 6.4 – pH 9.0	99 mg/g (at 50 % breakthrough)	94 %	6 cycles, 25 % loss in 5th and 6th cycle.	[40]
Lysozyme	PMAA	Polymer brush	1, 3	a, b	Low pH – high pH	220 μg/cm^2*	90 %	2 cycles, no loss	[39]
Fibrinogen	PMAA	Polymer brush	1, 3	a, b	Low pH – high pH	140 μg/cm^2*	N.D.	N.D.	[39]
Pepsin	CBMA	Polymer brush (zwitterionic)	3	a	Low pH – neutral pH	24 ng/cm^2	100 %	N.D.	[132]
Lysozyme	CBMA	Polymer brush (zwitterionic)	3	a	High pH – neutral pH	26 ng/cm^2	N.D.	N.D.	[132]
13 different proteins	PMAA	Polymer brush	1, 3	a, b	Neutral pH – pH > pI	400–6000 ng/cm^2	100 %	N.D.	[38]

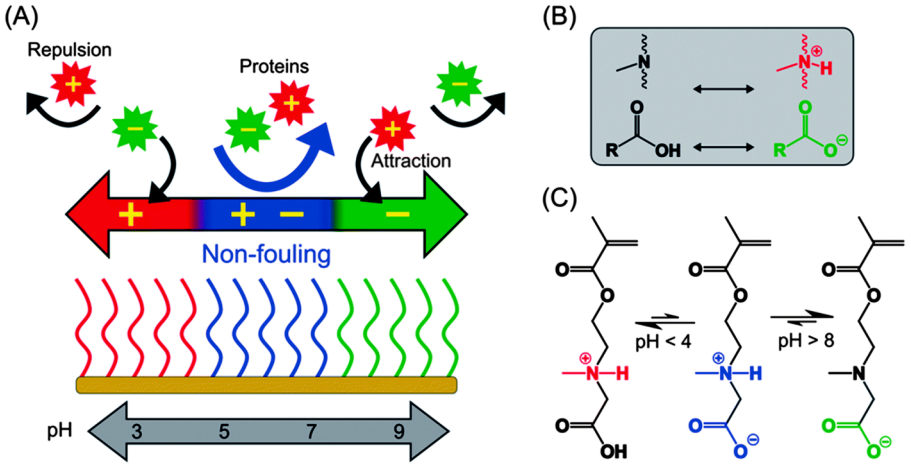


Fig. 6. Schematic representation of stimuli responsiveness of a zwitterionic polymer brush (A), and the chemistry behind this reversible switching behaviour (B and C). Reproduced with permission from [132].

adsorb. If the pH is increased above 8, the amino group deprotonates, leading to an overall negative charge that attracts positively charged proteins (e.g., lysozyme). Between pH 4 and 8, both proteins could be desorbed from the zwitterionic polymer brush, even up to 100 % for lysozyme. To allow this synergistic effect to happen, the number of carbon atoms (spacers) in between the amine and carboxylic groups should be small enough [132,133].

2.4. Gas-responsive systems

Reversible capture and release of proteins could be initiated by passing CO_2 and N_2 along CO_2-responsive polymer brushes (Table 5) [131,134]. Brushes made with poly(N,N-diethylaminoethyl methacrylate) (PDEAEMA) or PDMAEMA have been evaluated for this switch. These brushes are hydrophobic with a collapsed chain conformation but can be switched to a hydrated chain-extended state by bubbling CO_2. The dissolved CO_2 decreases the pH, causing tertiary amine groups on the polymer chain to react with the CO_2 and form charged ammonium bicarbonate groups [135–137]. The insoluble collapsed state can be retrieved by flushing with N_2 [131,134]. While this switch is driven by the exchange of gas inflow, the actual polymer conformation is the result of pH-responsive behaviour. In the work of Kumar et al. [131] and Liu et al. [134], the collapsed polymer brushes showed effective protein binding, while the swollen state initiated desorption. Adsorption was attributed to hydrophobic interaction, while desorption was initiated by an increase in hydration repulsion

Table 5
Overview of gas-responsive polymer systems for the separation of proteins. Conformational change: 1) swelling/collapsing; 2) exposure of groups; 3) (de)protonation; 4) internal restructuring; 5) no conformation. Responsive behaviour: a) charge; b) hydration; c) exposed groups; d) specific binding affinity.

Protein	Responsive polymer	Polymer type	Conformational change	Response behaviour	Switch	Adsorption capacity	Desorption capacity	Reversibility	Reference
Bovine serum albumin	PDEAEMA	Polymer brush	1, 3	a, b	N_2 – CO_2 bubbling	0.11 μg/cm^2	95 %	7 cycles	[131]
Bovine serum albumin	PDMAEMA	Polymer brush	1, 3	a, b	N_2 – CO_2 bubbling	30 mg/g	40 %*	5 cycles, no loss	[134]

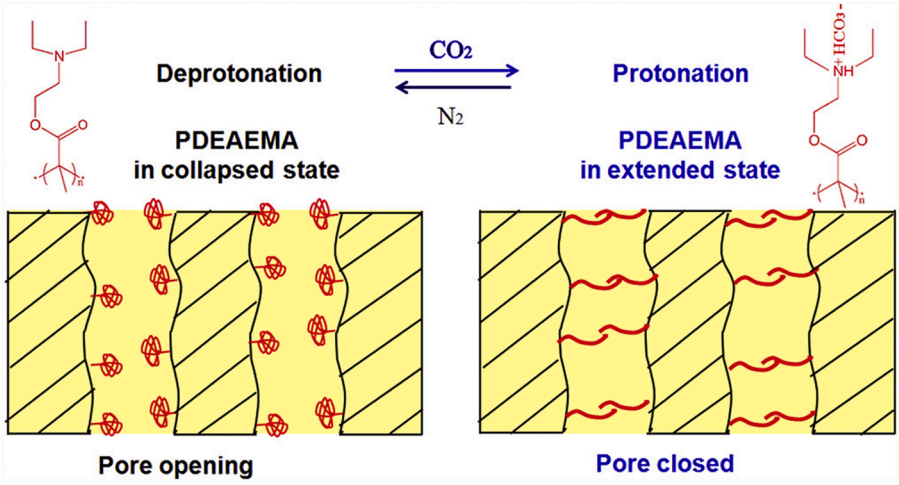


Fig. 7. Schematic representation of polymer conformations in a membrane covered with a PDEAEMA polymer brush upon CO_2/N_2 bubbling, reproduced with permission from [138].

[131,134]. In Kumar et al. [131], the desorption efficiency reached 95 %, while for Liu et al. [134], approximately 40 % of the initial protein concentration is retrieved. The desorption reversibility could be maintained over 7 [131] and 5 cycles [134]. The same CO_2-responsive system finds application in membranes as well. In the work of Zhang et al. [138], the switch from the collapsed to the extended state was used to remove attached proteins from the membrane surface. Additionally, it was observed that the change in polymer conformation affected the water flux of the membrane, with decreased values in the extended state due to pore closure (Fig. 7).

2.5. Salt-responsive polymers

For salt-responsive polymers, the ionic strength controls the adsorption behaviour, by tuning electrostatic interactions and steric effects [16]. In Table 6, mainly polyelectrolytes and zwitterionic polymer brushes are listed, which are pH-responsive systems as well. This dual response is further discussed in the section "Multi-stimulus responsive polymers". Polyelectrolyte brushes are known to fully extend in pure water while transforming to a collapsed state in a salt solution [139]. The same effect can be achieved by SAMs or polymer brushes made of polyelectrolytes [140]. When remaining in the osmotic regime, an increase in ionic strength enhances counterion condensation inside the brush without affecting the chain conformation. Upon increasing the ionic strength further, the Debye screening length is decreased until the salt regime is reached. Now, electrostatic repulsion between and within the polyelectrolyte chains is screened, causing them to partially collapse, and leading to the expulsion of water from the brush [139]. Zwitterionic brushes are known to show the complete opposite behaviour compared to polyelectrolyte brushes, which is known as the 'anti-polyelectrolyte effect'. In the salt regime, the attractive electrostatic interactions between zwitterionic groups are screened, which encourages polymer stretching. Besides the surface, the ionic strength influences the characteristics of the protein as well, for which we refer to the work of others [141–143]. For example, in the work of Skoda et al. [143], the salt concentration did not significantly influence the polymer film but did affect the protein properties, which consequently resulted in switchable adsorption.

In the work of Bratek-Skicki et al. [140], the polyelectrolyte polyacrylic acid (PAA) was mixed with PEO in a polymer brush used for adsorption of human serum albumin, lysozyme, and human fibrinogen. At a low salt concentration, PAA was extended in the solution and attracted the proteins. Changing to a high salt concentration caused the PAA chains to collapse and the PEO chains to invoke their antifouling behaviour. A mixed polymer brush like this is effective for switchable protein adsorption and is further discussed in the section "Multi-stimulus responsive polymers". In the work of Han et al. [144], salt-responsive cationic (poly([2-(methacryloyloxy) ethyl] trimethylammonium chloride (PMTAC)) and anionic (poly(3-sulfopropyl methacrylate potassium salt) (PSPMA)) polyelectrolyte brushes were prepared. Long-range attractive electrical double-layer forces were described to attract oppositely charged proteins, after which electrostatic interactions, van der Waals interactions, and hydrogen bonding enforced strong protein adhesion to the surface. At 1.0 M salt, not only electrostatic attraction was screened, but surface hydration and steric repulsion became so strong that they exceeded electrical double-layer attraction. This was sufficient to allow completely reversible BSA and lysozyme ad- and desorption over 10 cycles without noticeable losses.

In the work of Chen et al. [145], salt-responsive zwitterionic polymer brushes were made of poly(3-(1-(4-vinylbenzyl)-1H-imidazol-3-ium-3-yl) propane-1-sulfonate) (polyVBIPS) and assessed for switchable protein adsorption from human blood serum and human blood plasma. The zwitterionic polymer chains showed anti-polyelectrolyte behaviour, implying that the brush adopts a collapsed chain conformation at low ionic strength but extends in the solution at high ionic strength. Proteins adsorbed under low ionic strength conditions and were desorbed at high ionic strength, due to the enhanced hydration of the grafted chains. Although the results were interesting (around 99 % desorption and stable behaviour for 3 to 8 cycles), it is good to point out that the first rinsing step removed ~90 % of the proteins thus implying that the salt-induced conformational changes might not be the main desorption initiator. In the work of Wang et al. [146], adsorption and desorption of BSA and lysozyme were shown on a zwitterionic brush as well (poly (sulfobetaine methacrylate) (PSBMA)). They showed that protein adsorption is mainly driven by hydrophobic interactions and the extent of brush hydration, as both negatively and positively charged proteins were able to be adsorbed and desorbed from the same surface. Additionally, it was highlighted that the brush behaviour was anion-specific, with improved protein desorption for anions that could induce a weaker inter/intrachain association in the brush and a higher hydration level.

The work of Hyun et al. [147] explores the effect of salt on protein adsorption with temperature-responsive polymers (elastin-like

Table 6
Overview of salt-responsive polymer systems for the separation of proteins. Conformational change: 1) swelling/collapsing; 2) exposure of groups; 3) (de)protonation; 4) internal restructuring; 5) no conformation. Responsive behaviour: a) charge; b) hydration; c) exposed groups; d) specific binding affinity. *Calculated from data/graph in article. N.D. = not determined.

Protein	Responsive polymer	Polymer type	Conformational change	Response behaviour	Switch	Adsorption capacity	Desorption capacity	Reversibility	Reference
Bovine serum albumin	PMTAC	Polymer brush	1, 3	a, b	Low salt – high salt	2139.9 ng/cm^2	99.8 %*	10 cycles, no loss	[144]
Lysozyme	PSPMA	Polymer brush	1, 3	a, b	Low salt – high salt	3177.1 ng/cm^2	99.8 %*	10 cycles, no loss	[144]
Human blood plasma	Poly(VBIPS)	Polymer brush (zwitterionic)	1	b	Low salt – high salt	720 ng/cm^2 *	98 %*	3 cycles, slight decrease	[145]
Human blood serum	Poly(VBIPS)	Polymer brush (zwitterionic)	1	b	Low salt – high salt	900 ng/cm^2	99 %*	8 cycles, slight decrease	[145]
Lysozyme	PEO/PAA	Polymer brush	1, 2, 3	a, c	Low salt – high salt	937 ng/cm^2 (for PEO1/PAA 50/50)	100 %*	N.D.	[140]
Fibrinogen	PEO/PAA	Polymer brush	1, 2, 3	a, c	Low salt – high salt	530 ng/cm^2 (for PEO1/PAA 50/50)	100 %*	N.D.	[140]
Bovine serum albumin	PSBMA	Polymer brush (zwitterionic)	1	b	Low salt – high salt	N.D.	100 %	N.D.	[146]
Lysozyme	PSBMA	Polymer brush (zwitterionic)	1	b	Low salt – high salt	N.D.	100 %	2 cycles, no loss	[146]
Fusion protein	Elastin-like polypeptide	Polypeptide on SAM	1, 2	b	High salt – low salt	N.D.	N.D.	Loss over cycles	[147]

polypeptides). Salt lowered the critical temperature below ambient temperature, resulting in phase transitions from soluble to an aggregated and collapsed state, which allowed hydrophobic interactions with proteins. At low salt concentrations, the polymer layer became solvated and extended again, leading to desorption of proteins. Upon repeated adsorption and desorption, it was observed that some of the elastin-like polypeptides were lost or irreversibly collapsed.

2.6. Light-responsive polymers

This stimulus controls adsorption and desorption behaviour by irradiation with different light sources (Table 7) [28]. Photo-switched protein adsorption was achieved on a SAM of azobenzene by Zhang et al. [148]. A five-layered film with polyelectrolytes, (poly[1-[4-(3-carboxy-4-hydroxyphenylazo) benzenesul-fonamido]-1, 2-ethanediyl, sodium salt] (PAZO) and PDADMAC, was fabricated on a quartz slide, after which ten layers of PDACMAC and silica nanoparticles were added. Finally, the light-responsive layer, consisting of PAZO and PDADMAC, was created at the surface. Azobenzene can reversibly switch between two isomeric states, the meta-stable cis and stable trans form, upon irradiation with UV or visible light [18,149]. In the trans form, the surface is hydrophobic, which supports protein adsorption, while the hydrophilic cis form adsorbs only minor amounts of proteins. The incorporation of silica nanoparticles was especially important for this wettability and adsorption switch since the created surface roughness amplified the wettability [148]. Also, light-responsive supramolecular hydrogels have been used [149], which is out of the scope of this review.

2.7. Multi-stimulus responsive polymers

From the previous sections, it was already clear that multiple stimuli may be applied in one system. Synergistic effects can improve protein adsorption and desorption or even the selectivity of the system. An overview of multi-stimulus systems can be found in the work of Schattling, Jochum, and Theato [23]. Systems designed for protein separation are summarized in Table 8.

2.7.1. pH-salt responsiveness

The most popular multi-stimulus-responsive process is a combined pH- and salt-responsive system. These stimuli are relatively easy to combine as polyelectrolytes and zwitterionic polymers are both naturally pH- and salt-responsive [31]. Multiple articles have been published about the effect of pH and ionic strength on protein adsorption at polymer brushes containing weak polyelectrolytes such as poly (4-vinyl pyridine) (P4VP) [125], PDMAEMA [150], and PAA [126–130,140]. The configuration of the polyelectrolyte chains is controlled by the pH and ionic strength, through (de)protonation and the extent of screening. We must note that ionization and screening are strongly connected, for which we refer to a number of literature sources [151–156]. When polyelectrolytes are extended, adsorption is facilitated, while chain collapse initiates desorption due to a change in swelling, charge, and wettability of the polymer surface [128]. This is applied by Kusumo et al. [150] who showed reversible adsorption of bovine serum albumin on grafted PDMAEMA brushes. BSA adsorption was initiated in a 1 mM NaCl (pH 5.8) solution, by electrostatic attraction between negatively charged bovine serum albumin and the cationic amine groups of the extended brush. Up to 87 % desorption was reached upon rinsing with 1 M NaCl (pH 4), due to reduced electrostatic attraction, charge screening, and competitive Cl^- adsorption. It is good to mention that 83 % of adsorbed bovine serum albumin was already removed when rinsing with a 1 mM NaCl (pH 4) solution, indicating a larger influence of the degree of protonation.

To improve desorption efficiency, these polyelectrolyte chains can be incorporated in a mixed polymer brush with a second antifouling polymer. While the general mechanism is similar, specific switching conditions vary for every system in Table 8 and depend on the nature of the polymer surface and protein.

In Atif et al. [125], a combination of a weak polyelectrolyte P4VP and a non-fouling polymer (poly (2-methyl-2-oxazoline) (PMOXA)) is used for reversible adsorption of bovine serum albumin. Adsorption takes place at the extended P4VP chains at low pH and low ionic strength (pH = 3, I = 10^{-3} M), caused by electrostatic attraction between protonated pyridine and negatively charged BSA. To induce desorption, the pH is increased to 9 and the ionic strength to 10^{-1} M. The pH above pK_a induces deprotonation, while the increased ionic strength screens the electrostatic repulsion forces between the P4VP chains, resulting in chain collapse and reduced contact between P4VP and bovine serum albumin. Interestingly, the adsorption and desorption phase can be short, 2.5 and 2 min, respectively, while a similar adsorption and desorption capacity is achieved as for other comparable systems in Table 8, albeit that reversibility over multiple cycles was not shown.

Others used PAA as a weak polyelectrolyte, combined with either PMOXA or PEO as an antifouling chain [126–130,140]. At high pH and low ionic strength, PAA chains are extended in solution, while upon increasing the ionic strength, repulsive forces between the PAA chains are screened, causing the chains to collapse. The pH- and salt-dependent behaviour of PEO/PAA brushes is further shown in Fig. 8. In the work of Delcroix et al. [130], the adsorption and desorption capacity of pH- and salt-responsive mixed PEO/PAA brushes was determined for human serum albumin, lysozyme, and collagen. Partial reversibility of adsorption occurred for all proteins, but the required adsorption and desorption conditions varied among proteins. For human serum albumin and lysozyme, adsorption took place at very low ionic strength (10^{-5} M), under which the PAA brush is swollen. Desorption was initiated by increasing the ionic strength (10^{-1} M), by collapsing the PAA brushes and exposing the repelling PEO moieties. The pH is used to modulate the electrostatic interactions between proteins and polymers. Human serum albumin is desorbed at pH 9 when both PAA and human serum albumin are negatively charged and electrostatic repulsion is attained. Lysozyme desorption occurs at pH 3, in the absence of electrostatic interactions, as PAA is uncharged. The behaviour of collagen is more complex, since it aggregates under adsorption conditions leading to protein deposition, while under desorption conditions, these aggregates are solubilized and detach from the polymer brush. Collagen was unable to desorb from a pure PAA brush, which hints at synergic effects of the two polymers. Comparable adsorption and desorption behaviour, to human serum albumin and lysozyme [130], was found for bovine serum albumin [127,128] and lysozyme [126] when using a PMOXA/PAA mixed brush.

In most articles, the reversibility of these mixed polymer systems is shown for 2 to 5 cycles. Generally, an efficiency decline is shown over cycles indicating irreversible adsorption. However, improvement of the adsorption capacity is also reported to take place after the first cycle [127,130], which may be caused by rearrangements of the

Table 7
Overview of light-responsive polymer systems for the separation of proteins. Conformational change: 1) swelling/collapsing; 2) exposure of groups; 3) (de)protonation; 4) internal restructuring; 5) no conformation. Responsive behaviour: a) charge; b) hydration; c) exposed groups; d) specific binding affinity. N.D. = not determined.

Protein	Responsive polymer	Polymer type	Conformational change	Response behaviour	Switch	Adsorption capacity	Desorption capacity	Reversibility	Reference
Bovine serum albumin	Azobenzene and PDADMAC	SAM	4	b	UV irradiation	N.D.	N.D.	Yes, not quantified	[148]

Table 8
Overview of multi-stimulus-responsive polymer systems for the separation of proteins. Conformational change: 1) swelling/collapsing; 2) exposure of groups; 3) (de) protonation; 4) internal restructuring; 5) no conformation. Responsive behaviour: a) charge; b) hydration; c) exposed groups; d) specific binding affinity. N.D. = not determined.

Protein	Responsive polymer	Polymer type	Conformational change	Response behaviour	Switch	Adsorption capacity	Desorption capacity	Reversibility	Reference
Bovine serum albumin	PMOXA/ P4VP	Polymer brush	1, 2, 3	a, b, c	Low pH, low I – high pH, high I	1702 ng/cm^2 (for chain length 111)	94 %	5 cycles, no loss	[125]
Bovine serum albumin	PMOXA/ PAA	Polymer brush	1, 2, 3	a, b, c	Low pH, low I – high pH, high I	961 ng/cm^2 (for 90:10 PMG:PAG)	94 %	4 cycles, no loss	[127]
Lysozyme	PMOXA/ PAA	Polymer brush	1, 2, 3	a, b, c	Neutral pH, low I – Low pH, high I	1500 ng/cm^2 (for 90:10 PMG:PAG83)	91 %	3 cycles, desorption efficiency declines	[126]
Bovine serum albumin	PMOXA/ PAA	Polymer brush	1, 2, 3	a, b, c	Low pH, low I – high pH, high I	1175.6 ng/cm^2 (for Mw80)	87 %	N.D.	[128]
Human serum albumin	PEO/PAA	Polymer brush	1, 2, 3	a, c	Low pH, low I – high pH, high I	±1300 ng/cm^2 (For 63/37 PEO/PAA)	75 %	3 cycles, slight decrease	[130]
Lysozyme	PEO/PAA	Polymer brush	1, 2, 3	a, c	Neutral pH, low I – low pH, high I	±700 ng/cm^2 (For 63/37 PEO/PAA)	100 %	3 cycles, slight decrease	[130]
Collagen	PEO/PAA	Polymer brush	1, 2, 3	a	High pH, high I, low pH, low I	N.D.	85 %	2 cycles, slight decrease	[130]
Human serum albumin, lysozyme, and collagen	PEO/PAA	Polymer brush	1, 2, 3	a, c	High pH, low I – High pH, high I	1100 ng/cm^2 (for PEO1/PAA 50/50)	100 %	N.D.	[129]
Bovine serum albumin	PDMAEMA	Polymer brush	1, 3	a	Neutral pH, low I – Low pH, high I	2830 ng/cm^2	87 %	N.D.	[150]
Ovalbumin	pNIPAM copolymer with phenylboronic acid residues	Polymer brush	1, 2, 3	a, b, c, d	High T, medium pH, no sugar – low T, low pH, sugar	N.D.	46.8 %	N.D.	[115]

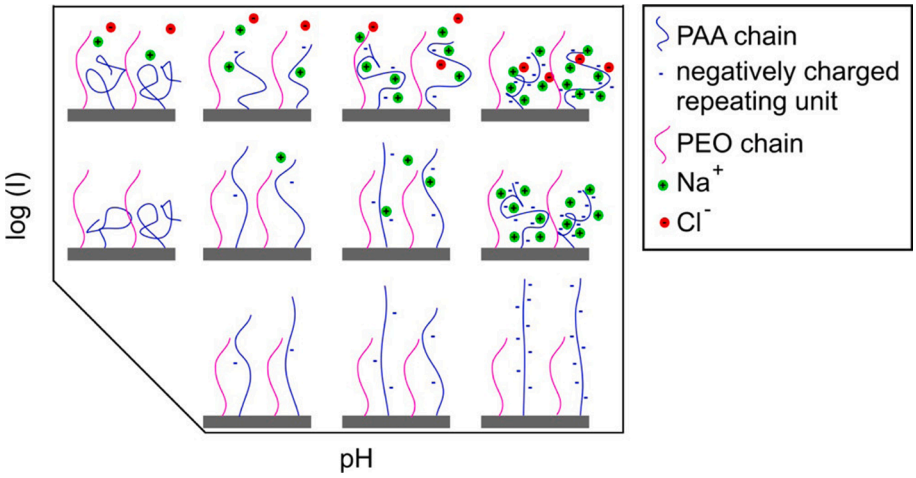


Fig. 8. Schematic illustration of a binary brush with PEO and PAA polymer chains as a function of pH and ionic strength. Reprinted with permission from Delcroix et al. [157]. Copyright (2014) American Chemical Society.

polyelectrolyte chains in the presence of salt. Several parameters are thought to impact the adsorption capacity and reversibility, including (i) the ratio between the two polymer brushes [126,127,129,130,140], (ii) the chain length and molar mass of the polyelectrolyte [125,126,128], and (ii) the chain length and molar mass of the antifouling polymer [140].

Ratio electrolyte:antifouling polymer. Increasing the density of the antifouling polymer enhances protein-repellence, and therefore leads to a lower adsorption capacity [126,127,129,140]. For fully reversible adsorption, a certain fraction of the polymer brush must be the antifouling unit. For a PEO/PAA brush, this was determined to be between 25 and 34 units/nm^2 for lysozyme and 25–27 units/nm^2 for fibrinogen [140]. On 50/50 PEO/PAA brushes, complete reversibility was achieved for both proteins [129,140]. For PMOXA/PAA mixed brushes, the highest reversibility was at 90/10 ratio, albeit that full reversibility was not reached [126,127].

Length of polymer chains. Adsorption capacity and reversibility could be improved by increasing the length of the polyelectrolyte chains [125–127]. In the work of Mumtaz et al. [126], the length of the PAA moiety varied between 6.8 and 21.1 nm, with longer PAA chains increasing the adsorption capacity. The work of Pan et al. [128] obtained similar results and suggested that the increased PAA chain length improved the reversibility as well. When the chain length of PAA was approximately 2.4 times that of PMOXA, higher adsorption values were found compared to the pure PAA brush, most probably because of better spacing, while maintaining a high desorption effficiency. Comparable results were found in Atif et al. [125]; enhanced adsorption and desorption were found when the P4VP chain was three times the length of the PMOXA chain. Furthermore, increasing the length of the anti-fouling moiety decreased adsorption capacity while it improved desorption efficiency [129,140]. The impact was dependent on the ratio between the fouling and antifouling moiety.

2.7.2. Thermal^{+}-responsiveness

In the work of Zhou et al. [115], a multi-stimulus responsive system was developed based on a temperature-responsive pNIPAM copolymer containing phenylboronic acid residues (PBA) (Fig. 9). The PBA groups were used for their ability to form complexes with diol-containing biomolecules through boronic ester bond formation. This complex is pH-dependent and can be affected by introducing competitive sugar molecules. It was observed that ovalbumin adsorbed on PBA groups could be partly (19.3 %) replaced when fructose molecules were introduced to the system. When the three stimuli, temperature, pH, and sugar addition, were switched simultaneously, a desorption capacity of 46.8 % was reached. When testing the same system for bacteria adhesion, is was found that fructose was able to release 75 % of the bacteria while the multi-stimuli system was able to release around 90 %, with reversibility over three cycles. The higher fraction of irreversible adsorption of proteins might have been a result of adsorption on the gold substrate in-between the polymer chains through hydrophobic interactions. This research clearly shows that the efficiency of the system is highly target-dependent, which should be taken as a starting point for the design of a larger-scale separation process.

3. Critical analysis of stimuli-responsive systems for protein capture and release

Stimuli-responsive systems are a great opportunity to capture proteins from liquid streams. Here, a wide variety of proteins with different sizes, charges, and sources has been described, showcasing the versatility of stimuli-responsive techniques. Given the wide variety of systems and their applications, it is logical that very diverse results have been published. Still, most papers described protein separation in terms of capacity, reversibility, and/or selectivity, which allowed comparison between systems.

For the design of the separation system of the future, it is important to look at various facets of the responsive systems. Here we look at the relevant interaction forces (3.1) of the stimuli-responsive separation systems, discuss characterization of the used responsive polymers (type of polymer, grafting density, layer thickness, etc.) (3.2), and explore novel multi-stimulus responsive systems (3.3) and surface structure designs (3.4). Although given less attention in literature, typical times for ad- and desorption, and system selectivity are very relevant when separating less defined feeds, as customary in bio-based and food applications. Their importance will be highlighted in sections 3.5 and 3.6. Finally, we showcase several chromatography applications using stimuli-responsive polymers and highlight other potential future applications (3.7).

3.1. Relevant interaction forces

The relevant interaction forces are determined by the type of polymer film, the type of stimuli, and the protein properties. For electrically-responsive polyelectrolyte (multi)layers and brushes, electrostatic attraction and repulsion are often mentioned as driving forces for adsorption and desorption [96,97,102]. To initiate adsorption, standard repelling forces caused by hydration and chain conformations have to be overcome [102]. For the electrically-driven SAM [98], adsorption was also attributed to electrostatic attraction, while desorption was the result of hydrophobic uncharged bent chains. For conductive polymers, adsorption was dependent on the wettability and charge of the surface, and the protein properties [103,104]. Fibronecton and BSA adsorption took place through hydrophobic interaction, while fibrinogen and protamine sulfate interacted with a hydrophilic surface through electrostatic interaction. Superhydrophobic surfaces showed efficient anti-fouling properties by strong dehydration of the surface [103]. From this follows that the actual adsorption mechanism is complex with multiple parameters playing a role (hydrophobic forces, electrostatic effects, the extent of hydration, and protein-specific properties). Temperature-responsive systems mainly relied on the switch between fouling (collapsed) and anti-fouling (swollen) state and were limited in terms of adsorption reversibility. Improved reversibility was achieved when the temperature affected multiple surface characteristics, including hydration, wettability, and conformation, which affected electrostatic interaction and hydration forces [116]. For pH- and/or salt-responsive systems, adsorption was attributed to electrostatic [40,132,144,150], hydrophobic interaction [39,146,147], or hydrogen bonds [38]. Desorption was predominantly caused by hydration of the polymer layer [39,144–147], by diminished electrostatic attraction [40,132], or by induced electrostatic repulsion [38]. For binary brushes, with

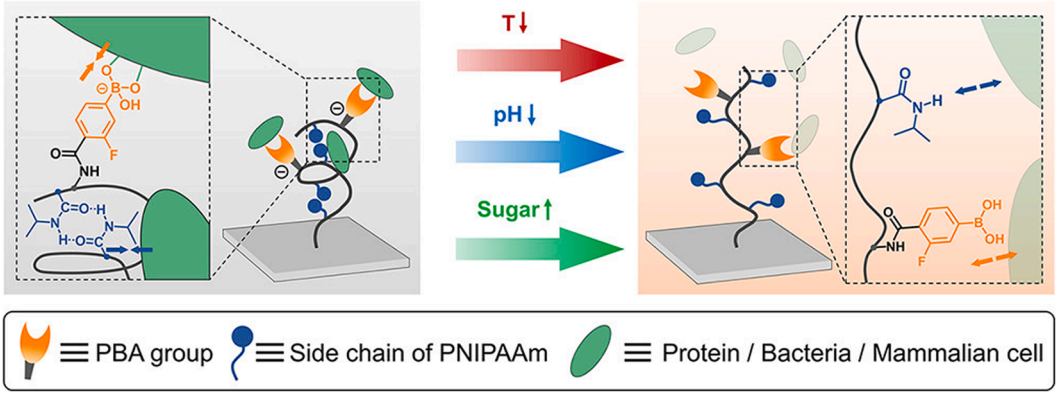


Fig. 9. Schematic illustration of a multi-stimulus responsive surface with switchable protein and cell adhesion. Reprinted with permission from Zhou et al. [115]. Copyright (2020) American Chemical Society.

polyelectrolyte and antifouling moieties, desorption is driven by the chain collapse of the polyelectrolyte and the exposure of the antifouling brush [125–130,140]. Overall, when the stimuli impacted multiple interaction forces, the reversibility of the system was improved.

We believe it is also important to point out that the target protein should determine the design of the polymer film. In the same system, proteins with varying properties exhibit completely different interaction behaviour [104,130]. Finally, in some instances, the mechanism behind adsorption of the same protein on the same polymer-coated surface was determined to be different [131,134].

3.2. Polymer layer design

The polymer type, functionality, and grafting density influence the final separation efficiency and should be chosen while keeping the target molecule in mind. The grafting density should be sufficient to prevent undesirable adsorption onto the bare substrate, as it may result in a low desorption efficiency [115] or a decrease in adsorption reversibility over cycles [98]. As shown in the section "Multi-stimulus responsive polymers", the combination of a responsive 'adsorption' polymer in conjunction with an anti-fouling polymer is an efficient way to achieve a high polymer density and assure effective desorption. Besides, the additional antifouling polymer can function as a spacer between the stimuli-responsive polymers, thereby reducing steric hindrance [34,128].

The adsorption and desorption efficiency for these binary polymer brushes is determined by the ratio between the two polymer brushes [126,127,129,130,140], the chain length and molar mass of the polyelectrolyte [125,126,128], and the chain length and molar mass of the antifouling polymer [129,140]. To illustrate this further, we have taken data from Bratek-Skicki et al. [129,140] and replotted them to highlight the effect of polymer-to-polymer ratio and length of the antifouling unit on the adsorption and desorption efficiency (Fig. 10). For both the 50/50 and 90/10 PAA/PEO brushes, the adsorption capacity decreased when the length of the antifouling unit was increased. The desorption efficiency was shown to rely on the length as well when the 90/10 ratio was used, with increased desorption efficiency for longer chains, while the 50/50 ratio resulted in complete desorption in all experiments. Additionally, increasing the length of the responsive polymer showed to improve the adsorption capacity, while maintaining efficient desorption efficiency due to strong stimuli responsiveness behaviour and the protein resistance of the antifouling polymer [125,126,128]. Lastly, Fig. 10 illustrates that differences in adsorption and desorption efficiency can arise from the type of protein (mixture).

3.3. Multi-stimulus

As discussed before, binary polymer layers, with an antifouling and stimuli-responsive moiety, showed a high desorption efficiency with reversibility over multiple cycles (for most cases). The improved efficiency of the dual stimuli points to a synergistic effect that may apply to other responsive systems as well.

A promising novel system consists of pH-dependent polymers in electrically-responsive systems, as described in the work of Ferrand-Drake del Castillo et al. [102]. Electrochemical switching at low voltages (-0.5 V to 1.0 V vs Ag/AgCl) was performed to tune the local pH at the electrode surface. Switching at reductive potentials was entirely reliant on the presence of ambient O_2, which resulted in a strong local pH increase (pH $\approx$ 12) causing changes in interaction forces between the polymer brush and proteins. To achieve reduced local pH values, a few mM of proton-producing redox-active species were added. Another option is to make use of water-splitting reactions. At the anode, water can be split into oxygen gas and protons, which lowers the surrounding pH. At the cathode, protons are consumed for the formation of hydrogen gas, and an increase in local pH is observed. This process occurs at higher voltages as shown by Fairclough [158], who observed a local pH increase at a cathodic current of 18 V, resulting in deprotonation of the tertiary amine groups and a collapse of the poly 2-diethylamino ethyl methacrylate (PDEAMA) brush. An anodic current decreased the local pH and led to protonation and brush extension. Another example is presented in the work of Tam et al. [159] wherein a P4VP-covered electrode was reversibly switched between an active and inactive state by electrochemical reduction and oxidation. In their case, the polymer brush was hydrophilic and swollen at pH $<$ 4.5, making the brush permeable for anionic redox species. When a negative potential was applied, the polymer brush switched to its hydrophobic shrunken state, forming a barrier for anionic redox species. The research above shows that a local pH-responsive switching system can be created without the need for large amounts of alkali or acid solutions.

Electrically-responsive conductive systems could also be combined with grafted polymer brushes [160]. The characteristics of these polymer brushes can be manipulated by an electric field or by another stimulus and might improve control over the surface properties.

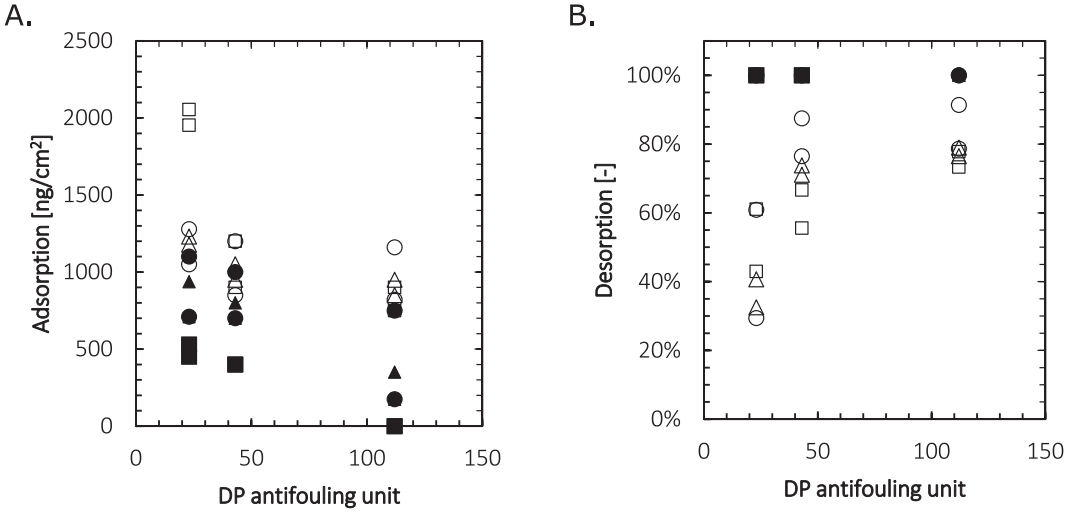


Fig. 10. The relation between the degree of polymerization (DP) of the antifouling unit and the adsorption capacity (A) and desorption efficiency (B) of fibrinogen (□), lysozyme (Δ), and a protein mixture (o), derived from the data of Bratek-Skicki et al. [129,140]. Adsorption unit PAA was mixed with antifouling unit PEO in a binary polymer brush in a ratio of 50/50 (filled symbols) and 90/10 (empty symbols).

3.4. Surface structure design

In addition to the inherent properties of polymer layers, structural features have been reported to be instrumental in improving stimulus-responsive separation systems [161]. Incorporation of nanoparticles has been shown to increase the roughness of the polymer film and therefore aid the wettability switch upon application of a stimulus [17,76,148]. In the work of Yu et al. [39], a polymer-modified nano-structured silicon nanowire array showed the highest adsorption capacity among all reported papers, 220 μg/cm^2, which indicates that nano-structure design is an interesting way to boost protein capture. Beyond surface structuring, the utilization of stimuli-responsive (nano-) particles holds considerable advantages, such as higher surface area, facilitated processing, and potential for delivery applications [162,163].

When transitioning towards industrial separation systems, it becomes a necessity to expand the surface area of polymers, which can be accomplished by coating porous materials [102]. This would require scalable polymer manufacturing techniques, as most techniques in this review are primarily tailored toward analytical separation [12,27]. This is one of the major challenges for bringing stimuli-responsive polymers towards industrial application, which must be tackled by interdisciplinary research in the chemical and material science field.

3.5. Selectivity design

In the systems described this far, selectivity was mostly not considered a priority, and most experiments were performed in pure protein solutions. However, we feel that this will become essential when using these systems for practical feed solutions. An example of selective capture can be found in the work of Fritz et al. [96], wherein an enrichment of β-lactoglobulin was reported when using whey protein isolate as feed in an electrically switched polymer-coated electrode system. In the work of Mu et al. [98], it was shown that tuning the SAM head group can induce selectivity for proteins. Much more advanced options to create selectivity are already available, such as incorporating immobilized ligands and molecular imprinting of polymer layers.

Affinity ligands allow molecular recognition through specific interactions between ligand and target protein while minimizing other non-specific interactions [164]. The options for ligands are versatile, ranging from biological (e.g. proteins, lectins, or peptides) to synthetic molecules, which have emerged as stable and cost-effective alternatives [165,166]. In affinity chromatography, traditionally used as final purification step, these ligands are bound to the column matrix [167–169]. The main challenges are to achieve high selectivity and efficient release, as well as maintaining selectivity in complex mixtures [6,165,169]. Advances include the conjugation of ligands with responsive polymers, offering dynamic control over ligand exposure and protein binding affinity. For example, stimuli-responsive ligand-polymer conjugates have been used for affinity precipitation, showing reversible switching of the complex between the soluble and insoluble state [6,170]. The work of Shastri et al. [171] presents a catch-and-release system with an aptamer-functionalized pH-responsive hydrogel for reversible capture of thrombin. About 95 % of the initial thrombin could be separated after 8 catch-and-release cycles, at high selectivity. Other examples include ligand-functionalized polymer brushes [172] and stimuli-responsive peptide ligands [165,173] that have significant potential for fine-tuning the selectivity of protein capture. An underexplored aspect of these systems is their capacity and reversibility over multiple cycles which is a necessity for large-scale separation applications [165]. Additionally, the process would need to be convenient in use, reproducible, reusable after regeneration, and cost-effective [6], which also requires more research.

Molecular imprinting of polymer layers has been known for decades but is used limitedly for protein separation. Template-assisted polymer growth with proteins incorporated into the polymer structure can be used to create selective cavities upon removal of the protein [174–178]. In the work of Pernites et al. [103], colloidal template-assisted electropolymerization of conductive polythiophene films was used to create cavities. Ideally, these cavities improve specific binding of the target protein and improve selectivity due to pre-organized functional groups [176].

In smart hydrogel systems, imprinting has been successfully implemented. These hydrogels are composed of stimuli-responsive polymers that alter their three-dimensional structure and functionality based on the chosen stimulus [179]. Protein separation has been reported by Wei et al. [180], in which a hydrogel with DMAEMA monomers was designed for reversible and selective adsorption of human serum albumin. The DMAEMA units are pH-responsive and react to local pH changes at the electrode surface caused by hydrogen formation. When a sufficiently large negative potential is applied, the increase in OH^- results in deprotonation of the tertiary amine groups of DMAEMA. In this uncharged state, hydrogen bonding and electrostatic interactions with the protein are suppressed. Additionally, imprinted nanocavities with specific recognition for the template protein were created in the hydrogel. Upon applying a negative potential, the recognition sites undergo a reversible structural transition from a less compact to a compact structure. The combined structural and chemical transitions result in the release of the template protein from the polymer structure, leading to very high recovery (92.4–101.2 %). Process reversibility over multiple cycles was unfortunately solely indicated by the obtained peak current. While molecular imprinting could offer the next step in terms of selectivity, it still suffers from drawbacks when working towards scale-up: limited availability of monomers and polymerization techniques, harsh conditions for template removal, limited loading capacity, and difficulties with macromolecule incorporation [181,182].

A major challenge is to create selective stimuli-responsive polymer systems while maintaining sufficient capacity and reversibility. This will, in the end, determine whether applications become economically feasible.

3.6. Time

Quite surprisingly, the factor time is hardly considered for any stimuli-responsive system in literature, while it is known to be very important for the reversibility of protein adsorption [56]. This is also a dilemma in the design of separation systems. On the one hand, one aspires to achieve a high protein loading of the system and use of the full capacity, while kinetic considerations may go against this because of the irreversibility of binding [183]. When looking at the percentage of irreversible adsorption as a function of time, it is observed that almost 80 % of protein adsorbs irreversibly on a standard silica surface within 20 min (Fig. 11A), while for most stimuli-responsive systems (collected in Fig. 11B), desorption is possible even if the adsorption time exceeds 20 min. This clearly illustrates the strength of stimuli-responsive systems, which can reverse protein-surface interactions and initiate desorption which would be impossible when using a non-responsive surface. Still, it is recommended to keep the adsorption time below 100 min to prevent slow processes (unfolding, aggregation) from taking place, therewith preventing irreversible binding and loss of capacity over multiple cycles. Much more in general, it would be highly advisable if adsorption times and desorption percentages were to be reported in future scientific work. This would help deconvolute the various effects that may play a role.

For upscaled processes, adsorption and desorption times need to be short and effective. The multi-stimulus-driven system discussed in Atif et al. [125] is the fastest technique discussed here, with an adsorption time of 2.5 min and a desorption time of 2 min. The adsorption and desorption efficiency are comparable to values reported by others for similar systems, which nicely illustrates that the processing time can be reduced when using efficient stimuli-driven polymer switches. In the work of Fritz et al. [96], the effect of adsorption and desorption time is evaluated. The stimuli accelerated adsorption and desorption, as the

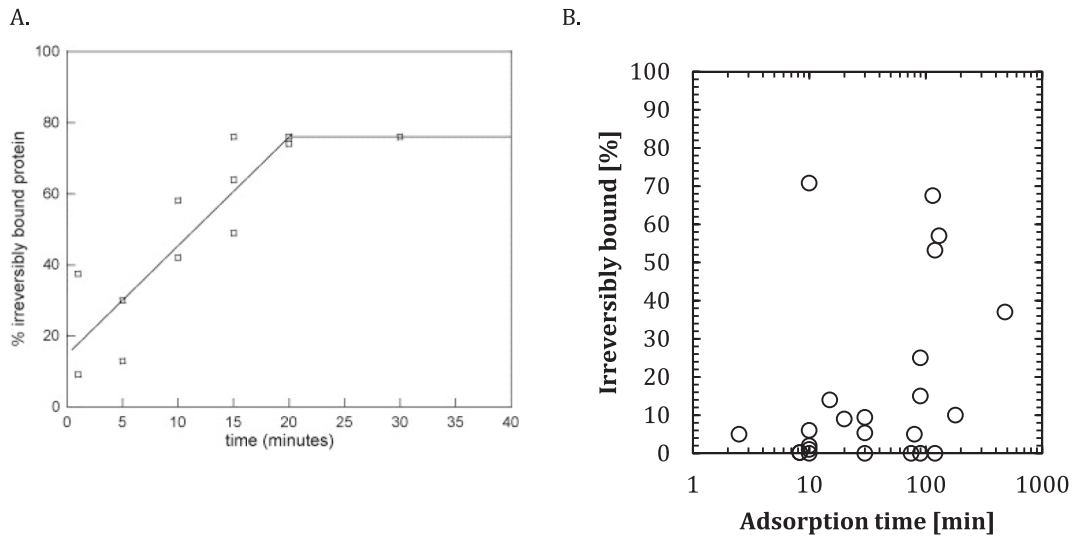


Fig. 11. The relation between adsorption time and the percentage of irreversibly bound proteins: on a silica surface (A), reproduced with permission from [183], and on stimuli-responsive polymer surfaces (B), data retrieved from [39,96,98,104,113,115,116,125–132,140,144,145].

driving force for mass transfer changed from diffusion to active transfer. However, the overall process was still relatively slow because of the previously mentioned desire to make use of the full capacity.

Besides adsorption and desorption times, the required time to initiate the switch in stimuli-responsive polymers should be considered. While hardly described, we can imagine that the speed of protein adsorption and desorption is determined by whether the polymer switch is direct or gradual. This is co-determined by the properties of the polymer structure [163,184–188], the type of stimuli [187] and the designed system. For pH- and salt-responsive systems, the liquid exchange efficiency of the used system becomes more relevant, as the internal solution has to be changed to initiate switching behaviour. For gas-responsive systems, the bubbling efficiency will determine the rapidness of environmental change inside the system. Temperature-responsive systems require heating and cooling, which generally cannot be applied instantaneously, causing a temperature gradient over time in the system. In these cases, the design of the system determines the speed at which the conditions change, giving an indication of the minimal time required to initiate (complete) polymer switching. An example of the effect of liquid exchange on the switching speed is shown in the work of Ferrand-Drake del Castillo et al. [102]. Here, the pH is controlled by either electrochemical switching or changing the bulk pH, of which the latter reaches the same response but slower.

Although challenging, we hope that the parameter time will be more extensively discussed in literature in the future, including the time required for switching, adsorption, and desorption. This information will aid the search for fast-responding stimuli-responsive systems.

3.7. Future applications

The systems discussed in this review are generally describing small, essentially flat systems. The incorporation of stimuli-responsive polymers for larger-scale protein separation systems brings along new challenges, including large-scale polymer synthesis. Still, there are some success stories to be found for responsive polymers used in chromatography columns for separation of proteins, which we describe next.

In the work of Sepehrifar et al. [26], PDMAEMA-b-PAA copolymers were grafted to modified silica beads, which showed temperature-responsive behaviour at selected pH conditions. The adsorption and elution behaviour could be modulated by pH and temperature, additionally allowing control over the separation selectivity.

The work of Qu et al. [189] showed temperature- and pH-responsive chromatographic separation of a protein mixture (trypsin, myoglobulin, bovine serum albumin), with poly(NIPAM-co-BMA-co-DMAPAAM) brushes (poly (N-isopropylacrylamide-co-butyl methacrylate-co-N,N-dimethylaminopropyl acrylamide)) on polystyrene microspheres. The three proteins could be separated by tuning the polymer charge and hydration. Besides, the proteins could be separated at a high flow velocity, showing promise for larger-scale chromatography.

Finally, in the work of Maharjan et al. [190], fractionation of whey proteins (α-lactalbumin, β-lactoglobulin, and lactoferrin) was performed with poly(N-isopropylacrylamide-co-acrylic acid-co-*tert*-butylacrylamide) (ItBA) on cross-linked agarose beads. At 20 °C the majority of proteins was directly eluted from the column, while at 50 °C, around 50 % of the lactoferrin was adsorbed while the other proteins were eluted. Lactoferrin could next be eluted (52 %) from the column by reducing the temperature to 20 °C.

It is good to keep in mind that the columns ranged from 50 to 150 mm in length, so are appreciable, but also far from large scale. Additionally, hardly any comparison was made to unmodified chromatography material. The current understanding of these systems does not yet allow comparison with benchmarking technologies, on process and economic performance, and environmental impact.

Although beyond the scope of the current review, we believe that stimuli-responsive technologies have the ability to find application in a variety of fields beyond protein separation. For example, controlled release [191], biosensing [192], tissue engineering [193], smart materials for architecture and civil engineering [194], water treatment [195], and soft robotics [196] have all been mentioned. For stimuli-responsive capture of proteins, we will especially benefit from the sensor and drug delivery field, where stimuli-responsive systems have been explored and tested over the last decades.

4. Conclusion and outlook

Stimuli-responsive polymers show significant potential for dynamically controlling interactions with proteins, offering an intriguing opportunity for protein separation through capture and release. This review presents a variety of polymer-based switchable systems, categorized by the (multi-)stimuli they respond to, leading to processes such as swelling/collapsing, exposure of groups, (de)protonation, or internal restructuring. Consequently, the properties of the polymer-coated surface can be dynamically altered, including the charge, wettability, and/or exposed groups at the surface. Additionally, the switch between protein adsorption and desorption can be the result of competition for binding spots or a change in specific binding affinity. The complexity of

protein adsorption makes it difficult to derive exact mechanisms and interaction forces. Overall, the electrostatic interaction, hydrophobic forces, and hydration forces seem to play a dominant role in the majority of stimuli-responsive systems.

So far, the primary focus of these stimuli-responsive systems has centred around reaching higher adsorption capacities. For further improvement of the adsorption capacity, structural adaptations of the nano topography have shown to be successful, and even multi-stimulus responsive systems (salt, pH, temperature) have been suggested.

To meet the demands of industrial processes, achieving full reversibility of adsorption is crucial. This reversibility can be tuned by changing the type of polymer and the grafting conditions, or by incorporation of antifouling moieties, which showed promising results in terms of desorption efficiency. Moreover, the reversibility of adsorption can be enhanced by the use of multi-stimulus responsive systems (especially pH-response behaviour generated through electrochemical reactions). Finally, for upscaled processes, adsorption and desorption times need to be short and effective, for which we require more information on the speed and directness of polymer switching for different stimuli-responsive systems.

Beyond achieving adequate adsorption capacity and reversibility, we expect that enhancing selectivity, potentially by immobilized ligands or molecular imprinting, will become more and more relevant, especially for less-defined feed solutions as would be used in bio-based and food applications.

CRediT authorship contribution statement

Kieke de Boer: Writing – original draft, Visualization, Investigation, Data curation, Conceptualization. **Karin Schroën:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Karin Schroen reports financial support was provided by Dutch Research Council.

Data availability

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

Acknowledgements

This publication is part of Targets 1 and 5 of the TTW Perspectief research programme ReCoVR: Recovery and Circularity of Valuable Resources which is (partly) financed by the Dutch Research Council (NWO).

References

[1] P. Alexander, C. Brown, A. Arneth, J. Finnigan, D. Moran, M.D.A. Rounsevell, Losses, inefficiencies and waste in the global food system, Agr. Syst. 153 (2017) 190–200, https://doi.org/10.1016/j.agsy.2017.01.014.
[2] C.M. Galanakis, Recovery of high components from food wastes: Conventional, emerging technologies and commercialized applications, Trends Food Sci. Technol. 26 (2012) 68–87, https://doi.org/10.1016/j.tifs.2012.03.003.
[3] B.K. Nfor, P.D.E.M. Verhaert, L.A.M. van der Wielen, J. Hubbuch, M. Ottens, Rational and systematic protein purification process development: the next generation, Trends Biotechnol. 27 (2009) 673–679, https://doi.org/10.1016/j.tibtech.2009.09.002.
[4] M. Du, Z. Hou, L. Liu, Y. Xuan, X. Chen, L. Fan, Z. Li, B. Xu, Progress, applications, challenges and prospects of protein purification technology, Front. Bioeng. Biotechnol. 10 (2022), https://doi.org/10.3389/fbioe.2022.1028691.
[5] I. Roy, K. Mondal, M.N. Gupta, Leveraging protein purification strategies in proteomics, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 849 (2007) 32–42, https://doi.org/10.1016/j.jchromb.2006.11.016.
[6] X. Fang, W.W. Zhang, Affinity separation and enrichment methods in proteomic analysis, J. Proteomics 71 (2008) 284–303, https://doi.org/10.1016/j.jprot.2008.06.011.
[7] S.K. Ahuja, G.M. Ferreira, A.R. Moreira, Utilization of enzymes for environmental applications, Crit. Rev. Biotechnol. 24 (2004) 125–154, https://doi.org/10.1080/07388550490493726.
[8] S. Liu, Z. Li, B. Yu, S. Wang, Y. Shen, H. Cong, Recent advances on protein separation and purification methods, Adv. Colloid Interface Sci. 284 (2020), https://doi.org/10.1016/j.cis.2020.102254.
[9] S. Mandal, B.D. Kulkarni, Separation strategies for processing of dilute liquid streams, Int. J. Chem. Eng. (2011) 1–19, https://doi.org/10.1155/2011/659012.
[10] A. Saxena, B.P. Tripathi, M. Kumar, V.K. Shahi, Membrane-based techniques for the separation and purification of proteins: An overview, Adv. Colloid Interface Sci. 145 (2009) 1–22, https://doi.org/10.1016/j.cis.2008.07.004.
[11] O. Coskun, Separation Tecniques: CHROMATOGRAPHY, North Clin Istanb. (2016). 10.14744/nci.2016.32757.
[12] N.S. Terefe, O. Glagovskaia, K. De Silva, R. Stockmann, Application of stimuli responsive polymers for sustainable ion exchange chromatography, Food Bioprod. Process. 92 (2014) 208–225, https://doi.org/10.1016/j.fbp.2014.02.003.
[13] P. Maharjan, B.W. Woonton, L.E. Bennett, G.W. Smithers, K. DeSilva, M.T. W. Hearn, Novel chromatographic separation - The potential of smart polymers, Innov. Food Sci. Emerg. Technol. 9 (2008) 232–242, https://doi.org/10.1016/j.ifset.2007.03.028.
[14] A. Kikuchi, T. Okano, Intelligent thermoresponsive polymeric stationary phases for aqueous chromatography of biological compounds, Prog. Polym. Sci. (Oxford) 27 (2002) 1165–1193. www.elsevier.com/locate/ppolysci.
[15] L. Hu, Y. Wan, Q. Zhang, M.J. Serpe, Harnessing the power of stimuli-responsive polymers for actuation, Adv. Funct. Mater. 30 (2020) 1903471, https://doi.org/10.1002/adfm.201903471.
[16] A. Bratek-Skicki, Towards a new class of stimuli-responsive polymer-based materials – Recent advances and challenges, Appl. Surf. Sci. Adv. 4 (2021) 100068, https://doi.org/10.1016/j.apsadv.2021.100068.
[17] F. Guo, Z. Guo, Inspired smart materials with external stimuli responsive wettability: A review, RSC Adv. 6 (2016) 36623–36641, https://doi.org/10.1039/c6ra04079a.
[18] L. Zhai, Stimuli-responsive polymer films, Chem. Soc. Rev. 42 (2013) 7148–7160, https://doi.org/10.1039/c3cs60023h.
[19] T. Chen, R. Ferris, J. Zhang, R. Ducker, S. Zauscher, Stimulus-responsive polymer brushes on surfaces: Transduction mechanisms and applications, Prog. Polym. Sci. 35 (2010) 94–112, https://doi.org/10.1016/j.progpolymsci.2009.11.004.
[20] M.C. Cross, R.G. Toomey, N.D. Gallant, Protein-surface interactions on stimuli-responsive polymeric biomaterials, Biomed. Mater. 11 (2016), https://doi.org/10.1088/1748-6041/11/2/022002.
[21] E.R. Gillies, Reflections on the evolution of smart polymers, Isr. J. Chem. 60 (2020) 75–85, https://doi.org/10.1002/ijch.201900075.
[22] M. Wei, Y. Gao, X. Li, M.J. Serpe, Stimuli-responsive polymers and their applications, Polym. Chem. 8 (2017) 127–143, https://doi.org/10.1039/c6py01585a.
[23] P. Schattling, F.D. Jochum, P. Theato, Multi-stimuli responsive polymers-the all-in-one talents, Polym. Chem. 5 (2014) 25–36, https://doi.org/10.1039/c3py00880k.
[24] J.K. Chen, C.J. Chang, Fabrications and applications of stimulus-responsive polymer films and patterns on surfaces: A review, Materials 7 (2014) 805–875, https://doi.org/10.3390/ma7020805.
[25] H. Musarurwa, N.T. Tavengwa, Thermo-responsive polymers and advances in their applications in separation science, Microchem. J. 179 (2022) 107554, https://doi.org/10.1016/j.microc.2022.107554.
[26] R. Sepehrifar, R.I. Boysen, B. Danylec, Y. Yang, K. Saito, M.T.W. Hearn, Design, synthesis and application of a new class of stimuli-responsive separation materials, Anal. Chim. Acta 963 (2017) 153–163, https://doi.org/10.1016/j.aca.2017.01.061.
[27] S. Tan, K. Saito, M.T. Hearn, Stimuli-responsive polymeric materials for separation of biomolecules, Curr. Opin. Biotechnol. 53 (2018) 209–223, https://doi.org/10.1016/j.copbio.2018.02.011.
[28] M. Bruneau, S. Bennici, J. Brendle, P. Dutournie, L. Limousy, S. Pluchon, Systems for stimuli-controlled release: materials and applications, J. Control. Release 294 (2019) 355–371, https://doi.org/10.1016/j.jconrel.2018.12.038.
[29] V. Orr, L. Zhong, M. Moo-Young, C.P. Chou, Recent advances in bioprocessing application of membrane chromatography, Biotechnol. Adv. 31 (2013) 450–465, https://doi.org/10.1016/j.biotechadv.2013.01.007.
[30] F. Liu, M.W. Urban, Recent advances and challenges in designing stimuli-responsive polymers, Prog. Polym. Sci. (Oxford) 35 (2010) 3–23, https://doi.org/10.1016/j.progpolymsci.2009.10.002.
[31] J. Zhang, Y. Han, Active and responsive polymer surfaces, Chem. Soc. Rev. 39 (2010) 676–693, https://doi.org/10.1039/b816231j.
[32] J.E. Raynor, J.R. Capadona, D.M. Collard, T.A. Petrie, A.J. García, Polymer brushes and self-assembled monolayers: versatile platforms to control cell adhesion to biomaterials (Review), Biointerphases 4 (2009) FA3–FA16, https://doi.org/10.1116/1.3089252.
[33] A. Ulman, Formation and structure of self-assembled monolayers, Chem. Rev. 96 (1996) 1533–1554. http://pubs3.acs.org/acs/journals/doilookup?in_doi=10.1021/cr9502357.
[34] E. Cantini, X. Wang, P. Koelsch, J.A. Preece, J. Ma, P.M. Mendes, Electrically responsive surfaces: experimental and theoretical investigations, Acc. Chem. Res. 49 (2016) 1223–1231, https://doi.org/10.1021/acs.accounts.6b00132.

[35] H. Nandivada, A.M. Ross, J. Lahann, Stimuli-responsive monolayers for biotechnology, Prog. Polym. Sci. 35 (2010) 141–154, https://doi.org/10.1016/j.progpolymsci.2009.11.001.
[36] E. Bittrich, A. Furchner, M. Koenig, D. Aulich, P. Uhlmann, K. Hinrichs, K. J. Eichhorn, Polymer brushes, hydrogels, polyelectrolyte multilayers: stimuli-responsivity and control of protein adsorption, in: K. Hinrichs, K. Eichhorn (Eds.), Ellipsometry of Functional Organic Surfaces and Films, Springer, cham, 2018, pp. 115–143, https://doi.org/10.1007/978-3-319-75895-4_13.
[37] K. Glinel, C. Déjugnat, M. Prevot, B. Schöler, M. Schönhoff, R.V. Klitzing, Responsive polyelectrolyte multilayers, Colloids Surf A Physicochem Eng Asp 303 (2007) 3–13, https://doi.org/10.1016/j.colsurfa.2007.02.052.
[38] G. Ferrand-Drake del Castillo, R.L.N. Hailes, Z. Adali-Kaya, T. Robson, A. Dahlin, Generic high-capacity protein capture and release by pH control, Chem. Commun. 56 (2020) 5889–5892, https://doi.org/10.1039/d0cc01250e.
[39] Q. Yu, H. Chen, Y. Zhang, L. Yuan, T. Zhao, X. Li, H. Wang, pH-reversible, high-capacity binding of proteins on a substrate with nanostructure, Langmuir 26 (2010) 17812–17815, https://doi.org/10.1021/la103647s.
[40] H. Ye, L. Huang, W. Li, Y. Zhang, L. Zhao, Q. Xin, S. Wang, L. Lin, X. Ding, Protein adsorption and desorption behavior of a pH-responsive membrane based on ethylene vinyl alcohol copolymer, RSC Adv. 7 (2017) 21398–21405, https://doi.org/10.1039/c7ra03206d.
[41] H. Palza, P.A. Zapata, C. Angulo-pineda, Electroactive smart polymers for biomedical applications, Materials 12 (2019) 277.
[42] G.G. Wallace, P.R. Teasdale, G.M. Spinks, L.A. Kane-Maguire, Conductive electroactive polymers: intelligent polymer systems, 3th ed., CRC Press, 2008.
[43] L. Xu, Q. Ye, X. Lu, Q. Lu, Electro-responsively reversible transition of polythiophene films from superhydrophobicity to superhydrophilicity, ACS Appl. Mater. Interfaces 6 (2014) 14736–14743, https://doi.org/10.1021/am5043627.
[44] Y. Ren, Z. Lin, X. Mao, W. Tian, T. Van Voorhis, T.A. Hatton, Superhydrophobic, surfactant-doped, conducting polymers for electrochemically reversible adsorption of organic contaminants, Adv. Funct. Mater. 28 (2018) 1–12, https://doi.org/10.1002/adfm.201801466.
[45] X. Zheng, Z. Guo, D. Tian, X. Zhang, L. Jiang, Electric field induced switchable wettability to water on the polyaniline membrane and oil/water separation, Adv. Mater. Interfaces 3 (2016) 1–6, https://doi.org/10.1002/admi.201600461.
[46] M. Rabe, D. Verdes, S. Seeger, Understanding protein adsorption phenomena at solid surfaces, Adv. Colloid Interface Sci. 162 (2011) 87–106, https://doi.org/10.1016/j.cis.2010.12.007.
[47] C.A. Haynes, W. Norde, Globular proteins at solid / liquid interfaces, Colloids Surf. B Biointerfaces 2 (1994) 517–566.
[48] E.A. Vogler, Protein adsorption in three dimensions, Biomaterials 33 (2012) 1201–1237, https://doi.org/10.1016/j.biomaterials.2011.10.059.
[49] R.A. Latour, Fundamental principles of the thermodynamics and kinetics of protein adsorption to material surfaces, Colloids Surf. B Biointerfaces 191 (2020) 110992, https://doi.org/10.1016/j.colsurfb.2020.110992.
[50] Z. Adamczyk, Protein adsorption: A quest for a universal mechanism, Curr. Opin. Colloid Interface Sci. 41 (2019) 50–65, https://doi.org/10.1016/j.cocis.2018.11.004.
[51] K. Nakanishi, T. Sakiyama, K. Imamura, On the adsorption of proteins on solid surfaces, a common but very complicated phenomenon, J. Biosci. Bioeng. 91 (2001) 233–244.
[52] T.S. Tsapikouni, Y.F. Missirlis, Protein – material interactions: From micro-to-nano scale, Mater. Sci. Eng. 152 (2008) 2–7, https://doi.org/10.1016/j.mseb.2008.06.007.
[53] M. Wahlgren, T. Arnebrant, Protein adsorption to solid surfaces, Trends Biotechnol. 9 (1991) 201–208.
[54] W. Norde, J. Lyklema, Thermodynamics of Protein Adsorption Theory with Special Reference to the Adsorption of Human Plasma Albumin and Bovine Pancreas Ribonuclease at Polystyrene Surfaces, 1979.
[55] A.N. Ebelegi, N. Ayawei, D. Wankasi, Interpretation of adsorption thermodynamics and kinetics,, Open J. Phys. Chem. 10 (2020) 166–182, https://doi.org/10.4236/ojpc.2020.103010.
[56] L.C. Xu, C.A. Siedlecki, Effects of surface wettability and contact time on protein adhesion to biomaterial surfaces, Biomaterials 28 (2007) 3273–3283, https://doi.org/10.1016/j.biomaterials.2007.03.032.
[57] G. Limousin, J.P. Gaudet, L. Charlet, S. Szenknect, V. Barthès, M. Krimissa, Sorption isotherms: A review on physical bases, modeling and measurement, Appl. Geochem. 22 (2007) 249–275, https://doi.org/10.1016/j.apgeochem.2006.09.010.
[58] S. Sakata, Y. Inoue, K. Ishihara, Molecular interaction forces generated during protein adsorption to well-defined polymer brush surfaces, Langmuir 31 (2015) 3108–3114, https://doi.org/10.1021/acs.langmuir.5b00351.
[59] P.C. Hiemenz, R. Rajagopalan, Principles of Colloid and Surface Chemistry, revised and expanded, 2016.
[60] C. Ruggiero, M. Mantelli, A. Curtis, P. Rolfe, Protein-surface interactions: An energy-based mathematical model, Cell Biochem. Biophys. 43 (2005) 407–417.
[61] D. Leckband, S. Sivasankar, Forces controlling protein interactions: theory and experiment, 1999. www.elsevier.nl/locate/colsurfb.
[62] T. Huhtamäki, X. Tian, J.T. Korhonen, R.H.A. Ras, Surface-wetting characterization using contact-angle measurements, Nat. Protoc. 13 (2018) 1521–1538, https://doi.org/10.1038/s41596-018-0003-z.
[63] Q. Zhang, F. Xia, T. Sun, W. Song, T. Zhao, M. Liu, L. Jiang, Wettability switching between high hydrophilicity at low pH and high hydrophobicity at high pH on surface based on pH-responsive polymer, Chem. Commun. (2008) 1199–1201, https://doi.org/10.1039/b716681h.
[64] W. Norde, Driving forces for protein adsorption at solid surfaces, Macromol. Symp. 103 (1996) 5–18.
[65] J. Barberi, S. Spriano, Titanium and protein adsorption: an overview of mechanisms and effects of surface features, Materials 14 (2021) 1590.
[66] D. Nagasawa, T. Azuma, H. Noguchi, K. Uosaki, M. Takai, Role of interfacial water in protein adsorption onto polymer brushes as studied by SFG Spectroscopy and QCM, J. Phys. Chem. C 119 (2015) 17193–17201, https://doi.org/10.1021/acs.jpcc.5b04186.
[67] L. Chen, X. He, H. Liu, L. Qian, S.H. Kim, Water adsorption on hydrophilic and hydrophobic surfaces of silicon, J. Phys. Chem. C 122 (2018) 11385–11391, https://doi.org/10.1021/acs.jpcc.8b01821.
[68] S.I. Jeon, J.H. Lee, J.D. Andrade, P.G. De Gennes, Protein-surface interactions in the presence of polyethylene oxide I, Simplified Theory (1991).
[69] S. Spriano, V. Sarath Chandra, A. Cochis, F. Uberti, L. Rimondini, E. Bertone, A. Vitale, C. Scolaro, M. Ferrari, F. Cirisano, G. Gautier di Confiengo, S. Ferraris, How do wettability, zeta potential and hydroxylation degree affect the biological response of biomaterials? Mater. Sci. Eng. C 74 (2017) 542–555, https://doi.org/10.1016/j.msec.2016.12.107.
[70] A. Sethuraman, M. Han, R.S. Kane, G. Belfort, Effect of surface wettability on the adhesion of proteins, Langmuir 20 (2004) 7779–7788.
[71] G.B. Sigal, M. Mrksich, G.M. Whitesides, Effect of surface wettability on the adsorption of proteins and detergents, J. Am. Chem. Soc. 120 (1998) 3464–3473.
[72] S. Guo, D. Pranantyo, E.T. Kang, X.J. Loh, X. Zhu, D. Jańczewski, K.G. Neoh, Dominant albumin-surface interactions under independent control of surface charge and wettability, Langmuir 34 (2018) 1953–1966, https://doi.org/10.1021/acs.langmuir.7b04117.
[73] J.M. Kleijn, D. Barten, M.A.C. Stuart, Adsorption of charged macromolecules at a gold electrode, Langmuir 20 (2004) 9703–9713, https://doi.org/10.1021/la049656m.
[74] R.J. Marsh, R.A.L. Jones, M. Sferrazza, Adsorption and displacement of a globular protein on hydrophilic and hydrophobic surfaces, 2002. www.elsevier.com/locate/colsurfb.
[75] P.A. Fritz, B. Bera, J. Van Den Berg, I. Visser, J.M. Kleijn, R.M. Boom, C.G.P. H. Schroën, Electrode surface potential-driven protein adsorption and desorption through modulation of electrostatic, van der Waals, and hydration interactions, Langmuir 37 (2021) 6549–6555, https://doi.org/10.1021/acs.langmuir.1c00828.
[76] T. Darmanin, F. Guittard, Wettability of conducting polymers: From superhydrophilicity to superoleophobicity, Prog. Polym. Sci. 39 (2014) 656–682, https://doi.org/10.1016/j.progpolymsci.2013.10.003.
[77] C.G. Jothi Prakash, R. Prasanth, Approaches to design a surface with tunable wettability: a review on surface properties, J. Mater. Sci. 56 (2021) 108–135, https://doi.org/10.1007/s10853-020-05116-1.
[78] K. Ishihara, K. Mitera, Y. Inoue, K. Fukazawa, Effects of molecular interactions at various polymer brush surfaces on fibronectin adsorption induced cell adhesion, Colloids Surf. B Biointerfaces 194 (2020), https://doi.org/10.1016/j.colsurfb.2020.111205.
[79] A.C. McUmber, T.W. Randolph, D.K. Schwartz, Electrostatic interactions influence protein adsorption (but not desorption) at the silica − aqueous interface, J. Phys. Chem. Lett. 6 (2015) 2583–2587, https://doi.org/10.1021/acs.jpclett.5b00933.
[80] Y.R. Dahal, M.O. de la Cruz, Controlling protein adsorption modes electrostatically, Soft Matter 16 (2020) 5224–5232, https://doi.org/10.1039/d0sm00632g.
[81] Y. Xie, Y. Pan, R. Zhang, Y. Liang, Z. Li, Modulating protein behaviors on responsive surface by external electric fields: A molecular dynamics study, Appl. Surf. Sci. 326 (2015) 55–65, https://doi.org/10.1016/j.apsusc.2014.11.078.
[82] A.T. Nguyen, J. Baggerman, J.M.J. Paulusse, C.J.M. Van Rijn, H. Zuilhof, Stable protein-repellent zwitterionic polymer brushes grafted from silicon nitride, Langmuir 27 (2011) 2587–2594, https://doi.org/10.1021/la104657c.
[83] M. Aramesh, O. Shimoni, K. Ostrikov, S. Prawer, J. Cervenka, Surface charge effects in protein adsorption on nanodiamonds, Nanoscale 7 (2015) 5726–5736, https://doi.org/10.1039/c5nr00250h.
[84] K. Wang, C. Zhou, Y. Hong, X. Zhang, A review of protein adsorption on bioceramics, Interface Focus 2 (2012) 259–277.
[85] C. Pinholt, R.A. Hartvig, N.J. Medlicott, L. Jorgensen, The importance of interfaces in protein drug delivery - Why is protein adsorption of interest in pharmaceutical formulations? Expert Opin. Drug Deliv. 8 (2011) 949–964, https://doi.org/10.1517/17425247.2011.577062.
[86] Z.M. Png, C.G. Wang, J.C.C. Yeo, J.J.C. Lee, N.E. Surat'man, Y.L. Tan, H. Liu, P. Wang, B.H. Tan, J.W. Xu, X.J. Loh, Q. Zhu, Stimuli-responsive structure-property switchable polymer materials, Mol. Syst. Des. Eng. 8 (2023) 1097–1129, https://doi.org/10.1039/d3me00002h.
[87] J. Hatai, C. Hirschhäuser, J. Niemeyer, C. Schmuck, Multi-stimuli-responsive supramolecular polymers based on noncovalent and dynamic covalent bonds, ACS Appl. Mater. Interfaces 12 (2020) 2107–2115, https://doi.org/10.1021/acsami.9b19279.
[88] X. Yan, F. Wang, B. Zheng, F. Huang, Stimuli-responsive supramolecular polymeric materials, Chem. Soc. Rev. 41 (2012) 6042–6065, https://doi.org/10.1039/c2cs35091b.
[89] E.E. Htwe, Y. Nakama, Y. Yamamoto, H. Tanaka, H. Imanaka, N. Ishida, K. Imamura, Adsorption characteristics of various proteins on a metal surface in the presence of an external electric potential, Colloids Surf. B Biointerfaces 166 (2018) 262–268, https://doi.org/10.1016/j.colsurfb.2018.03.035.

[90] T.E. Benavidez, D. Torrente, M. Marucho, C.D. Garcia, Adsorption of soft and hard proteins onto OTCEs under the influence of an external electric field, Langmuir 31 (2015) 2455–2462, https://doi.org/10.1021/la504890v.
[91] H. Ei Ei, Y. Nakama, H. Tanaka, H. Imanaka, N. Ishida, K. Imamura, Adsorption of lysozyme on base metal surfaces in the presence of an external electric potential, Colloids Surf. B Biointerfaces 147 (2016) 9–16, https://doi.org/10.1016/j.colsurfb.2016.07.042.
[92] B. Beykal, M. Herzberg, Y. Oren, M.S. Mauter, Influence of surface charge on the rate, extent, and structure of adsorbed Bovine Serum Albumin to gold electrodes, J. Colloid Interface Sci. 460 (2015) 321–328, https://doi.org/10.1016/j.jcis.2015.08.055.
[93] G. Mohanraj, C.M. Clark, B.S. Baboukani, P.C. Nalam, M.T. Ehrensberger, Electrochemical techniques to investigate adsorption and desorption behavior of fibrinogen on a gold surface, J. Appl. Electrochem. 52 (2022) 1525–1534, https://doi.org/10.1007/s10800-022-01720-y.
[94] C.T. Mallon, C. De Chaumont, N. Moran, T.E. Keyes, R.J. Forster, Electrochemical desorption of fibrinogen from gold, Langmuir 26 (2010) 293–298, https://doi.org/10.1021/la902115e.
[95] P.A. Fritz, R.M. Boom, K. Schroen, Polyelectrolyte-activated carbon composite electrodes for inverted membrane capacitive deionization (iMCDI), Sep. Purif. Technol. 220 (2019) 145–151, https://doi.org/10.1016/j.seppur.2019.03.053.
[96] P.A. Fritz, P. Zhang, T. Bruschinski, S. Sahin, L.C.P.M. de Smet, M.B. Chan-Park, R.M. Boom, C.G.P.H. Schroën, Steering protein and salt ad- and desorption by an electrical switch applied to polymer-coated electrodes, Sep. Purif. Technol. 250 (2020) 117195, https://doi.org/10.1016/j.seppur.2020.117195.
[97] G. Ladam, C. Gergely, B. Senger, G. Decher, J. Voegel, P. Schaaf, F.J.G. Cuisinier, Protein Interactions with polyelectrolyte multilayers:interactions between human serum albumin and polystyrene sulfonate / polyallylamine multilayers, Biomacromolecules 1 (2000) 674–687.
[98] L. Mu, Y. Liu, S. Cai, J. Kong, A smart surface in a microfluidic chip for controlled protein separation, Chemistry–A European Journal 13 (2007) 5113–5120, https://doi.org/10.1002/chem.200601624.
[99] S. Balasubramanian, A. Revzin, A. Simonian, Electrochemical desorption of proteins from gold electrode surface, Electroanalysis 18 (2006) 1885–1892, https://doi.org/10.1002/elan.200603627.
[100] J.A. Williams, C.B. Gorman, Alkanethiol reductive desorption from self-assembled monolayers on gold, platinum, and palladium substrates, J. Phys. Chem. C 111 (2007) 12804–12810, https://doi.org/10.1021/jp072869a.
[101] N.C. Ramos, J.W. Medlin, A. Holewinski, Electrochemical Stability of Thiolate Self-Assembled Monolayers on Au, Pt, and Cu, ACS Appl. Mater. Interfaces 15 (2023) 14470–14480.
[102] G. Ferrand-Drake del Castillo, M. Kyriakidou, Z. Adali, K. Xiong, R.L.N. Hailes, A. Dahlin, Electrically switchable polymer brushes for protein capture and release in biological environments, Angew. Chem. Int. Ed. (2022) 202115745, https://doi.org/10.1002/anie.202115745.
[103] R.B. Pernites, C.M. Santos, M. Maldonado, R.R. Ponnapati, D.F. Rodrigues, R. C. Advincula, Tunable protein and bacterial cell adsorption on colloidally templated superhydrophobic polythiophene films, Chem. Mater. 24 (2012) 870–880, https://doi.org/10.1021/cm2007044.
[104] J. Liao, Y. Zhu, Z. Zhou, J. Chen, G. Tan, C. Ning, C. Mao, Reversibly controlling preferential protein adsorption on bone implants by using an applied weak potential as a switch, Angew. Chem. 126 (2014) 13284–13288, https://doi.org/10.1002/ange.201406349.
[105] R. Balint, N.J. Cassidy, S.H. Cartmell, Conductive polymers: towards a smart biomaterial for tissue engineering, Acta Biomater. 10 (2014) 2341–2353, https://doi.org/10.1016/j.actbio.2014.02.015.
[106] X. Su, Electrochemical interfaces for chemical and biomolecular separations, Curr. Opin. Colloid Interface Sci. 46 (2020) 77–93, https://doi.org/10.1016/j.cocis.2020.04.005.
[107] Y. Li, Conducting Polymers, in: Y. Li (Ed.), Organic Optoelectronic Materials, Springer, Cham, 2015, pp. 23–50, http://www.springer.com/series/632.
[108] T.-H. Le, Y. Kim, H. Yoon, Electrical and electrochemical properties of conducting polymers, Polymers (Basel) 9 (2017) 150, https://doi.org/10.3390/polym9040150.
[109] M. Oak, R. Mandke, J. Singh, Smart polymers for peptide and protein parenteral sustained delivery, Drug Discov. Today Technol. 9 (2012) e131–e140, https://doi.org/10.1016/j.ddtec.2012.05.001.
[110] M.A. Cole, N.H. Voelcker, H. Thissen, H.J. Griesser, Stimuli-responsive interfaces and systems for the control of protein-surface and cell-surface interactions, Biomaterials 30 (2009) 1827–1850, https://doi.org/10.1016/j.biomaterials.2008.12.026.
[111] J. Kobayashi, M. Yamato, T. Okano, On-off affinity binding modulation on thermoresponsive polymer-grafted surfaces for capture and release of proteins and cells, J. Biomater. Sci. Polym. Ed. 28 (2017) 939–957, https://doi.org/10.1080/09205063.2017.1295508.
[112] K. Nagase, S.F. Yuk, J. Kobayashi, A. Kikuchi, Y. Akiyama, H. Kanazawa, T. Okano, Thermo-responsive protein adsorbing materials for purifying pharmaceutical protein on exposed charging surface, J. Mater. Chem. 21 (2011) 2590–2593, https://doi.org/10.1039/c0jm03453c.
[113] K. Kang, D. Lu, Z. Liu, Temperature-triggered protein adsorption and desorption on temperature-responsive PNIPAAm-grafted-silica: Molecular dynamics simulation and experimental validation, Chin. J. Chem. Eng. 20 (2012) 284–293, https://doi.org/10.1016/S1004-9541(12)60390-5.
[114] D.L. Huber, R.P. Manginell, M.A. Samara, B. Il Kim, B.C. Bunker, Programmed adsorption and release of proteins in a microfluidic device, Science 301 (2003) (1979) 352–354, https://doi.org/10.1126/science.1080759.
[115] Y. Zhou, Y. Zheng, T. Wei, Y. Qu, Y. Wang, W. Zhan, Y. Zhang, G. Pan, D. Li, Q. Yu, H. Chen, Multistimulus responsive biointerfaces with switchable bioadhesion and surface functions, ACS Appl. Mater. Interfaces 12 (2020) 5447–5455, https://doi.org/10.1021/acsami.9b18505.
[116] S. Balamurugan, L.K. Ista, J. Yan, G.P. López, J. Fick, M. Himmelhaus, M. Grunze, Reversible protein adsorption and bioadhesion on monolayers terminated with mixtures of oligo(ethylene glycol) and methyl groups, J. Am. Chem. Soc. 127 (2005) 14548–14549, https://doi.org/10.1021/ja054156g.
[117] S. Herrwerth, W. Eck, S. Reinhardt, M. Grunze, Factors that determine the protein resistance of oligoether self-assembled monolayers - Internal hydrophilicity, terminal hydrophilicity, and lateral packing density, J. Am. Chem. Soc. 125 (2003) 9359–9366, https://doi.org/10.1021/ja034820y.
[118] A. Blumlein, J.J. McManus, Reversible and non-reversible thermal denaturation of lysozyme with varying pH at low ionic strength, Biochim. Biophys. Acta Proteins Proteom 2013 (1834) 2064–2070, https://doi.org/10.1016/j.bbapap.2013.06.001.
[119] A. Naiyer, B. Khan, A. Hussain, A. Islam, M.F. Alajmi, M.I. Hassan, M. Sundd, F. Ahmad, Stability of uniformly labeled (13C and 15N) cytochrome c and its L94G mutant, Sci. Rep. 11 (2021), https://doi.org/10.1038/s41598-021-86332-w.
[120] D. Roy, W.L.A. Brooks, B.S. Sumerlin, New directions in thermoresponsive polymers, Chem. Soc. Rev. 42 (2013) 7214–7243, https://doi.org/10.1039/c3cs35499g.
[121] C. Weber, R. Hoogenboom, U.S. Schubert, Temperature responsive bio-compatible polymers based on poly(ethylene oxide) and poly(2-oxazoline)s, Prog. Polym. Sci. 37 (2012) 686–714, https://doi.org/10.1016/j.progpolymsci.2011.10.002.
[122] V.V. Khutoryanskiy, T.K. Georgiou, eds., Temperature-responsive Polymers, Wiley, 2018. 10.1002/9781119157830.ch5.
[123] X. Gao, Y. Cao, X. Song, Z. Zhang, C. Xiao, C. He, X. Chen, PH- and thermo-responsive poly(N-isopropylacrylamide-co-acrylic acid derivative) copolymers and hydrogels with LCST dependent on pH and alkyl side groups, J. Mater. Chem. B 1 (2013) 5578–5587, https://doi.org/10.1039/c3tb20901f.
[124] G. Kocak, C. Tuncer, V. Bütün, PH-Responsive polymers, Polym. Chem. 8 (2017) 144–176, https://doi.org/10.1039/c6py01872f.
[125] M. Atif, K. He, M. Irfan, Y. Wang, F. Hu, L. Chen, Y. Wang, pH responsive switchable protein adsorption properties of poly(2-methyl-2-oxazoline)/ poly(4-vinyl pyridine) based coatings: Influence of poly(4-vinyl pyridine) chain length and ionic strength, Prog. Org. Coat. 157 (2021) 106307, https://doi.org/10.1016/j.porgcoat.2021.106307.
[126] F. Mumtaz, C.S. Chen, H.K. Zhu, M. Atif, Y.M. Wang, Reversible Protein Adsorption on PMOXA/PAA Based Coatings: Role of PAA, Chinese Journal of Polymer Science (English Edition) 36 (2018) 1328–1341, https://doi.org/10.1007/s10118-018-2168-x.
[127] F. Mumtaz, C. Chen, H. Zhu, C. Pan, Y. Wang, Controlled protein adsorption on PMOXA/PAA based coatings by thermally induced immobilization, Appl. Surf. Sci. 439 (2018) 148–159, https://doi.org/10.1016/j.apsusc.2018.01.010.
[128] C. Pan, X. Liu, K. Gong, F. Mumtaz, Y. Wang, Dopamine assisted PMOXA/PAA brushes for their switchable protein adsorption/desorption, J. Mater. Chem. B 6 (2018) 556–567, https://doi.org/10.1039/c7tb02209c.
[129] A. Bratek-Skicki, V. Cristaudo, J. Savocco, S. Nootens, P. Morsomme, A. Delcorte, C. Dupont-gillain, Mixed polymer brushes for the selective capture and release of proteins, Biomacromolecules 20 (2019) 778–789, https://doi.org/10.1021/acs.biomac.8b01353.
[130] M.F. Delcroix, S. Laurent, G.L. Huet, C.C. Dupont-Gillain, Protein adsorption can be reversibly switched on and off on mixed PEO/PAA brushes, Acta Biomater. 11 (2015) 68–79, https://doi.org/10.1016/j.actbio.2014.09.010.
[131] S. Kumar, X. Tong, Y.L. Dory, M. Lepage, Y. Zhao, A CO2-switchable polymer brush for reversible capture and release of proteins, Chem. Commun. 49 (2013) 90–92, https://doi.org/10.1039/c2cc36284h.
[132] H.S. Sundaram, J.R. Ella-Menye, N.D. Brault, Q. Shao, S. Jiang, Reversibly switchable polymer with cationic/zwitterionic/anionic behavior through synergistic protonation and deprotonation, Chem. Sci. 5 (2014) 200–205, https://doi.org/10.1039/c3sc52233d.
[133] S. Fujii, M. Kido, M. Sato, Y. Higaki, T. Hirai, N. Ohta, K. Kojio, A. Takahara, pH-Responsive and selective protein adsorption on an amino acid-based zwitterionic polymer surface, Polym. Chem. 6 (2015) 7053–7059, https://doi.org/10.1039/c5py00783f.
[134] H. Liu, X. Yang, J. Wang, Q. Meng, L. Qian, H. Wu, C. Duan, Z. Li, H. Zhou, Gas responsive cellulose fibers for capturing and releasing of dyes and proteins from water by packing a smart separation column, Cellul. 27 (2020) 7127–7138, https://doi.org/10.1007/s10570-020-03277-5.
[135] B. Yan, D. Han, O. Boissière, P. Ayotte, Y. Zhao, Manipulation of block copolymer vesicles using CO2: Dissociation or “breathing”, Soft Matter 9 (2013) 2011–2016, https://doi.org/10.1039/c2sm27469h.
[136] J. Zhang, D. Han, H. Zhang, M. Chaker, Y. Zhao, D. Ma, In situ recyclable gold nanoparticles using CO2-switchable polymers for catalytic reduction of 4-nitrophenol, Chem. Commun. 48 (2012) 11510–11512, https://doi.org/10.1039/c2cc35784d.
[137] Z. Guo, Y. Feng, Y. Wang, J. Wang, Y. Wu, Y. Zhang, A novel smart polymer responsive to CO2, Chem. Commun. 47 (2011) 9348–9350, https://doi.org/10.1039/c1cc12388b.
[138] J. Zhang, Y. Liu, J. Guo, Y. Yu, Y. Li, X. Zhang, A CO2 -responsive PAN/PAN-co-PDEAEMA membrane capable of cleaning protein foulant without the aid of chemical agents, React. Funct. Polym. 149 (2020) 104503, https://doi.org/10.1016/j.reactfunctpolym.2020.104503.

[139] S. Xiao, B. Ren, L. Huang, M. Shen, Y. Zhang, M. Zhong, J. Yang, J. Zheng, Salt-responsive zwitterionic polymer brushes with anti-polyelectrolyte property, Curr. Opin. Chem. Eng. 19 (2018) 86–93, https://doi.org/10.1016/j.coche.2017.12.008.
[140] A. Bratek-Skicki, P. Eloy, M. Morga, C. Dupont-gillain, Reversible Protein Adsorption on Mixed PEO/PAA Polymer Brushes: Role of Ionic Strength and PEO Content, Langmuir 34 (2018) 3037–3048, https://doi.org/10.1021/acs.langmuir.7b04179.
[141] E.X.P. Almodovar, B. Glatz, G. Carta, Counterion effects on protein adsorption equilibrium and kinetics in polymer-grafted cation exchangers, J. Chromatogr. A 1253 (2012) 83–93, https://doi.org/10.1016/j.chroma.2012.06.100.
[142] S. Pasche, J. Vörös, H.J. Griesser, N.D. Spencer, M. Textor, Effects of ionic strength and surface charge on protein adsorption at PEGylated surfaces, J. Phys. Chem. B 109 (2005) 17545–17552, https://doi.org/10.1021/jp050431+.
[143] M.W.A. Skoda, N.F. Conzelmann, M.R. Fries, L.F. Reichart, R.M.J. Jacobs, F. Zhang, F. Schreiber, Switchable β-lactoglobulin (BLG) adsorption on protein resistant oligo (ethylene glycol) (OEG) self-assembled monolayers (SAMs), J. Colloid Interface Sci. 606 (2022) 1673–1683, https://doi.org/10.1016/j.jcis.2021.08.018.
[144] L. Han, B. Yan, L. Zhang, M. Wu, J. Wang, J. Huang, Y. Deng, H. Zeng, Tuning protein adsorption on charged polyelectrolyte brushes via salinity adjustment, Colloids Surf.A 539 (2018) 37–45, https://doi.org/10.1016/j.colsurfa.2017.12.004.
[145] H. Chen, J. Yang, S. Xiao, R. Hu, S.M. Bhaway, B.D. Vogt, M. Zhang, Q. Chen, J. Ma, Y. Chang, L. Li, J. Zheng, Salt-responsive polyzwitterionic materials for surface regeneration between switchable fouling and antifouling properties, Acta Biomater. 40 (2016) 62–69, https://doi.org/10.1016/j.actbio.2016.03.009.
[146] T. Wang, X. Wang, Y. Long, G. Liu, G. Zhang, Ion-specific conformational behavior of polyzwitterionic brushes: Exploiting it for protein adsorption/desorption control, Langmuir 29 (2013) 6588–6596, https://doi.org/10.1021/la401069y.
[147] J. Hyun, W.K. Lee, N. Nath, A. Chilkoti, S. Zauscher, Capture and release of proteins on the nanoscale by stimuli-responsive elastin-like polypeptide "switches", J. Am. Chem. Soc. 126 (2004) 7330–7335, https://doi.org/10.1021/ja049721e.
[148] J. Zhang, T. Hu, Y. Liu, Y. Ma, J. Dong, L. Xu, Y. Zheng, H. Yang, G. Wang, Photoswitched protein adsorption on electrostatically self-assembled azobenzene films, ChemPhysChem 13 (2012) 2671–2675, https://doi.org/10.1002/cphc.201200231.
[149] D. Wang, M. Wagner, H.-J. Butt, S. Wu, Supramolecular hydrogels constructed by red-light-responsive host–guest interactions for photo-controlled protein release in deep tissue, Soft Matter 11 (2015) 7656–7662, https://doi.org/10.1039/c5sm01888a.
[150] A. Kusumo, L. Bombalski, Q. Lin, K. Matyjaszewski, J.W. Schneider, R.D. Tilton, High capacity, charge-selective protein uptake by polyelectrolyte brushes, Langmuir 23 (2007) 4448–4454, https://doi.org/10.1021/la063660b.
[151] G. Ferrand-Drake Del Castillo, R.L.N. Hailes, A. Dahlin, Large changes in protonation of weak polyelectrolyte brushes with salt concentration-implications for protein immobilization, J. Phys. Chem. Lett. 11 (2020) 5212–5218, https://doi.org/10.1021/acs.jpclett.0c01289.
[152] M. Tagliazucchi, M.O. De La Cruz, I. Szleifer, Self-organization of grafted polyelectrolyte layers via the coupling of chemical equilibrium and physical interactions, PNAS 107 (2010) 5300–5305, https://doi.org/10.1073/pnas.0913340107.
[153] D. Beyer, P. Košovan, C. Holm, ExplaininG GIANT APPARENT pKa shifts in weak polyelectrolyte brushes, Phys. Rev. Lett. 131 (2023), https://doi.org/10.1103/PhysRevLett.131.168101.
[154] J.D. Willott, T.J. Murdoch, F.A.M. Leermakers, W.M. De Vos, Behavior of weak polyelectrolyte brushes in mixed salt solutions, Macromolecules 51 (2018) 1198–1206, https://doi.org/10.1021/acs.macromol.7b02244.
[155] J.D. Willott, B.A. Humphreys, G.B. Webber, E.J. Wanless, W.M. De Vos, Combined experimental and theoretical study of weak polyelectrolyte brushes in salt mixtures, Langmuir 35 (2019) 2709–2718, https://doi.org/10.1021/acs.langmuir.8b03838.
[156] O. Azzaroni, I. Szleifer, eds., Polymer and biopolymer brushes : for materials science and biotechnology, 1st ed., John Wiley & Sons, 2018.
[157] M.F. Delcroix, S. Demoustier-Champagne, C.C. Dupont-Gillain, Quartz crystal microbalance study of ionic strength and pH-dependent polymer conformation and protein adsorption/desorption on PAA, PEO, and mixed PEO/PAA brushes, Langmuir 30 (2014) 268–277, https://doi.org/10.1021/la403891k.
[158] G.J. Dunderdale, J.P.A. Fairclough, Coupling pH-responsive polymer brushes to electricity: Switching thickness and creating waves of swelling or collapse, Langmuir 29 (2013) 3628–3635, https://doi.org/10.1021/la3049949.
[159] T.K. Tam, M. Pita, O. Trotsenko, M. Motornov, I. Tokarev, J. Halámek, S. Minko, E. Katz, Reversible "closing" of an electrode interface functionalized with a polymer brush by an electrochemical signal, Langmuir 26 (2010) 4506–4513, https://doi.org/10.1021/la903527p.
[160] Y. Pei, J. Travas-sejdic, D.E. Williams, Reversible electrochemical switching of polymer brushes grafted onto conducting polymer films, Langmuir 28 (2012) 8072–8083.
[161] I. Firkowska-Boden, X. Zhang, K.D. Jandt, Controlling protein adsorption through nanostructured polymeric surfaces, Adv. Healthc. Mater. 7 (2018), https://doi.org/10.1002/adhm.201700995.
[162] K. Yoshimatsu, B.K. Lesel, Y. Yonamine, J.M. Beierle, Y. Hoshino, K.J. Shea, Temperature-responsive "catch and release" of proteins by using multifunctional polymer-based nanoparticles, Angewandte Chemie - International Edition 51 (2012) 2405–2408, https://doi.org/10.1002/anie.201107797.
[163] M.A.C. Stuart, W.T.S. Huck, J. Genzer, M. Müller, C. Ober, M. Stamm, G. B. Sukhorukov, I. Szleifer, V.V. Tsukruk, M. Urban, F. Winnik, S. Zauscher, I. Luzinov, S. Minko, Emerging applications of stimuli-responsive polymer materials, Nat. Mater. 9 (2010) 101–113, https://doi.org/10.1038/nmat2614.
[164] K. Mondal, M.N. Gupta, The affinity concept in bioseparation: Evolving paradigms and expanding range of applications, Biomol. Eng 23 (2006) 59–76, https://doi.org/10.1016/j.bioeng.2006.01.004.
[165] K. Bacon, A. Lavoie, B.M. Rao, M. Daniele, S. Menegatti, Past, present, and future of affinity-based cell separation technologies, Acta Biomater. 112 (2020) 29–51, https://doi.org/10.1016/j.actbio.2020.05.004.
[166] A.C.A. Roque, C.S.O. Silva, M.Â. Taipa, Affinity-based methodologies and ligands for antibody purification: Advances and perspectives, J. Chromatogr. A 1160 (2007) 44–55, https://doi.org/10.1016/j.chroma.2007.05.109.
[167] T. Shimanouchi, S. Morita, H. Umakoshi, R. Kuboi, Stimuli-responsive separation of proteins using immobilized liposome chromatography, 2000. www.elsevier.com/locate/chromb.
[168] S. Ashyüce, N. Idil, B. Mattiasson, Upgrading of bio-separation and bioanalysis using synthetic polymers: Molecularly imprinted polymers (MIPs), cryogels, stimuli-responsive polymers, Eng. Life Sci. 22 (2022) 204–216, https://doi.org/10.1002/elsc.202100106.
[169] K. Mondal, M.N. Gupta, I. Roy, Affinity-based strategies for protein purification, Anal. Chem. (2006) 3499–3504. https://pubs.acs.org/sharingguidelines.
[170] N. Malmstadt, D.E. Hyre, Z. Ding, A.S. Hoffman, P.S. Stayton, Affinity thermoprecipitation and recovery of biotinylated biomolecules via a mutant streptavidin-smart polymer conjugate, Bioconjug. Chem. 14 (2003) 575–580, https://doi.org/10.1021/bc020055l.
[171] A. Shastri, L.M. McGregor, Y. Liu, V. Harris, H. Nan, M. Mujica, Y. Vasquez, A. Bhattacharya, Y. Ma, M. Aizenberg, O. Kuksenok, A.C. Balazs, J. Aizenberg, X. He, An aptamer-functionalized chemomechanically modulated biomolecule catch-and-release system, Nat. Chem. 7 (2015) 447–454, https://doi.org/10.1038/nchem.2203.
[172] L. Jiang, L. Ye, Nanoparticle-supported temperature responsive polymer brushes for affinity separation of histidine-tagged recombinant proteins, Acta Biomater. 94 (2019) 447–458, https://doi.org/10.1016/j.actbio.2019.04.056.
[173] K. Day, J.D. Schneible, A.T. Young, V.A. Pozdin, L.A. Gaffney, R. Prodromou, D. O. Freytes, M. Daniele, S. Menegatti, Photoinduced reconfiguration to control the protein-binding affin- ity of azobenzene-cyclized peptides, J. Mater. Chem. B (2020), https://doi.org/10.1039/x0xx00000x.
[174] S. Xu, H. Lu, X. Zheng, L. Chen, Stimuli-responsive molecularly imprinted polymers: Versatile functional materials, J Mater Chem C Mater 1 (2013) 4406–4422, https://doi.org/10.1039/c3tc30496e.
[175] W. Chen, Y. Ma, J. Pan, Z. Meng, G. Pan, B. Sellergren, Molecularly imprinted polymers with stimuli-responsive affinity: Progress and perspectives, Polymers (Basel) 7 (2015) 1689–1715, https://doi.org/10.3390/polym7091478.
[176] Z. Chen, Z. Hua, L. Xu, Y. Huang, M. Zhao, Y. Li, Protein-responsive imprinted polymers with specific shrinking and rebinding, J. Mol. Recognit. 21 (2008) 71–77, https://doi.org/10.1002/jmr.870.
[177] S. Ramanavicius, A. Ramanavicius, Development of molecularly imprinted polymer based phase boundaries for sensors design (review), Adv. Colloid Interface Sci. (2021), https://doi.org/10.1016/j.cis.2022.102693.
[178] E. Mazzotta, T. Di Giulio, C. Malitesta, Electrochemical sensing of macromolecules based on molecularly imprinted polymers: challenges, successful strategies, and opportunities, Anal. Bioanal. Chem. 414 (2022) 5165–5200, https://doi.org/10.1007/s00216-022-03981-0.
[179] M.C. Koetting, J.T. Peters, S.D. Steichen, N.A. Peppas, Stimulus-responsive hydrogels: Theory, modern advances, and applications, Mater. Sci. Eng. R. Rep. 93 (2015) 1–49, https://doi.org/10.1016/j.mser.2015.04.001.
[180] Y. Wei, Q. Zeng, J. Huang, Q. Hu, X. Guo, L. Wang, An electro-responsive imprinted biosensor with switchable affinity toward proteins, Chem. Commun. 54 (2018) 9163–9166, https://doi.org/10.1039/c8cc05482g.
[181] L. Zhang, H. Yu, H. Chen, Y. Huang, I. Bakunina, D.P. de Sousa, M. Sun, J. Zhang, Application of molecular imprinting polymers in separation of active compounds from plants, Fitoterapia 164 (2023), https://doi.org/10.1016/j.fitote.2022.105383.
[182] L. Chen, X. Wang, W. Lu, X. Wu, J. Li, Molecular imprinting: Perspectives and applications, Chem. Soc. Rev. 45 (2016) 2137–2211, https://doi.org/10.1039/c6cs00061d.
[183] M. van der Veen, M.C. Stuart, W. Norde, Spreading of proteins and its effect on adsorption and desorption kinetics, Colloids Surf. B Biointerfaces 54 (2007) 136–142, https://doi.org/10.1016/j.colsurfb.2006.08.017.
[184] S. Qi, L.I. Klushin, A.M. Skvortsov, M. Liu, J. Zhou, F. Schmid, Tuning transition properties of stimuli-responsive brushes by polydispersity, Adv. Funct. Mater. 28 (2018), https://doi.org/10.1002/adfm.201800745.
[185] R. Ravichandran, S. Sundarrajan, J.R. Venugopal, S. Mukherjee, S. Ramakrishna, Advances in polymeric systems for tissue engineering and biomedical applications, Macromol. Biosci. 12 (2012) 286–311, https://doi.org/10.1002/mabi.201100325.
[186] S. Dai, P. Ravi, K.C. Tam, Thermo- and photo-responsive polymeric systems, Soft Matter 5 (2009) 2513–2533, https://doi.org/10.1039/b820044k.
[187] M.R. Kim, I.W. Cheong, Stimuli-triggered Formation of Polymersomes from W/O/W Multiple Double Emulsion Droplets Containing Poly(styrene)-block-poly(N-isopropylacrylamide-co-spironaphthoxazine methacryloyl), Langmuir 32 (2016) 9223–9228, https://doi.org/10.1021/acs.langmuir.6b02178.

[188] C.S. Linsley, B.M. Wu, Recent advances in light-responsive on-demand drug-delivery systems, Ther. Deliv. 8 (2017) 89–107, https://doi.org/10.4155/tde-2016-0060.
[189] J.B. Qu, Y.L. Xu, J.Y. Liu, J. Bin Zeng, Y.L. Chen, W.Q. Zhou, J.G. Liu, Thermo- and pH-responsive polymer brushes-grafted gigaporous polystyrene microspheres as a high-speed protein chromatography matrix, J. Chromatogr.A 1441 (2016) 60–67, https://doi.org/10.1016/j.chroma.2016.02.072.
[190] P. Maharjan, M.T.W. Hearn, W.R. Jackson, K. De Silva, B.W. Woonton, Development of a temperature-responsive agarose-based ion-exchange chromatographic resin, J. Chromatogr. A 1216 (2009) 8722–8729, https://doi.org/10.1016/j.chroma.2009.04.037.
[191] A. Aghabegi Moghanjoughi, D. Khoshnevis, A. Zarrabi, A concise review on smart polymers for controlled drug release, Drug Deliv.Transl. Res. 6 (2016) 333–340, https://doi.org/10.1007/s13346-015-0274-7.
[192] J. Song, Q. Cheng, S. Zhu, R.C. Stevens, "Smart" Materials for Biosensing Devices: Cell-Mimicking Supramolecular Assemblies and Colorimetric Detection of Pathogenic Agents, 2002.
[193] K. Zhang, S. Wang, C. Zhou, L. Cheng, X. Gao, X. Xie, J. Sun, H. Wang, M.D. Weir, M.A. Reynolds, N. Zhang, Y. Bai, H.H.K. Xu, Advanced smart biomaterials and constructs for hard tissue engineering and regeneration, Bone Res. 6 (2018), https://doi.org/10.1038/s41413-018-0032-9.
[194] M. Sobczyk, S. Wiesenhütter, J.R. Noennig, T. Wallmersperger, Smart materials in architecture for actuator and sensor applications: A review, J. Intell. Mater. Syst. Struct. 33 (2022) 379–399, https://doi.org/10.1177/1045389X211027954.
[195] K. Dutta, S. De, Smart responsive materials for water purification: An overview, J Mater Chem A Mater 5 (2017) 22095–22112, https://doi.org/10.1039/c7ta07054c.
[196] Y.L. Yap, S.L. Sing, W.Y. Yeong, A review of 3D printing processes and materials for soft robotics, Rapid Prototyp. J. 26 (2020) 1345–1361, https://doi.org/10.1108/RPJ-11-2019-0302.