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Stimuli-responsive polymeric materials for separation of biomolecules

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Stimuli-responsive polymeric (SRP) materials undergo changes in their physical morphologies and chemical properties in response to small changes in their external environment, such as temperature, pH or light. When immobilised, SRP materials, fabricated in various formats and compositions, provide new opportunities for the separation of products generated by the biotechnology industry. This *Current Opinion* highlights the potential of these functional materials for the capture, purification and analysis of these products via batch capture methods, column chromatography or electrophoresis, drawing on break-through developments achieved particularly over the last five years.

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Introduction

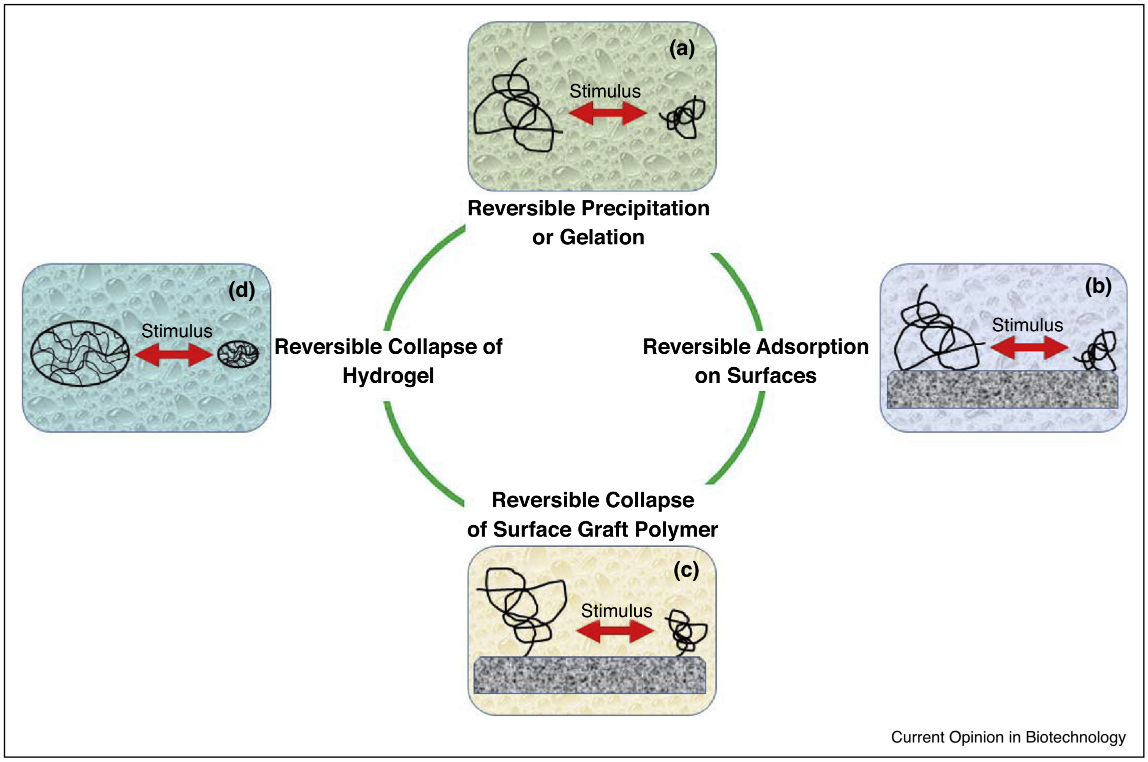
Separation technologies allow the resolution of complex mixtures of substances into two or more fractions having distinct composition, stereochemical structure or molecular mass and shape. Dating back to ancient times, people have used various methods to separate mixtures obtained from natural resources to improve the quality of their lives. Nowadays, separation techniques, based on affinity interactions, centrifugation, chromatography, crystallization, electrophoresis, extraction, floatation, precipitation, and many other methods, are widely employed across diverse fields of human endeavour with increasing emphasis on achieving molecular and economic sustainability within the life science and biotechnology industries [1••]. In this *Current Opinion*, the impact of different types of stimuli-responsive polymers (SRPs) and their fabrication as advanced separation materials is examined, drawing largely on developments that have been achieved during the past 5 years. Interested readers are referred to several informative reviews for prior developments of SRP technologies [2••,3••,4••,5••,6••,7••,8••], including those associated with the chemical, food, petrochemical, metallurgic, mining and environmental industries.

What are stimuli-responsive polymers?

Stimuli-responsive polymers (SRPs) — also known as 'smart', 'intelligent' or 'environmentally responsive' polymers — respond to changes in their external environment. The stimulus can be classified as due to physical (temperature, light, magnetic or electrical field, ultrasound or vibration), chemical (pH, ionic strength, solvent) or biological (associated with receptor signalling or enzymatic) effects. SRPs can be first, dissolved/suspended in aqueous solutions; second, chemi-adsorbed; third, covalently immobilized or grafted onto solid surfaces, or fourth, themselves be cross-linked, H-bonded and/or physically entangled in the form of hydrogels [9••]. As shown in Figure 1a, upon stimulation, the polymer chain of a SRP undergoes morphological changes from an extended state to a collapsed state. If the SRP is part of a block copolymer, then only the responsive portion undergoes an alteration in response to a stimulus. Application of stimuli to SRPs that have been chemi-adsorbed or covalently immobilised onto solid support materials enables the polymer chains of the SRP to collapse, expand or re-arrange (Figures 1b,c), resulting in significant changes in their adsorption properties, surface morphologies and stiffness. Furthermore, SRPs can be fabricated as hydrogels for use in a wide range of separation processes, including chromatography, with responsiveness to external stimuli as shown in Figure 1d. For all applications, the response of the SRP to the stimulus should be reversible, with the SRP able to re-equilibrate to its original state when the stimuli is removed.

Compared to conventional chromatographic materials, SRP materials have the advantage that as solid stationary phases their retention properties can be finely modulated with changes to their physical and/or chemical properties achieved without the need to change the composition of the mobile phase, which hitherto has often required the use of high salt concentrations or toxic and flammable organic solvents [1••,7••]. Separation processes with SRP materials thus tend to generate lower levels of waste, and operate under conditions that are compatible with the full preservation of structure and function of many biomolecules and biopharmaceuticals that otherwise could physically and/or chemically denature [2••,10••], and which

Figure 1



Schematic representation of the various types of induced transitions that stimuli-responsive polymers (SRPs) can undergo in response to an external stimuli. **(a)** Reversible precipitation or gelation; **(b)** reversible adsorption on a surface; **(c)** reversible collapse of SRP grafted onto a surface; **(d)** reversible collapse of a hydrogel.

permit the straightforward cleaning and recycling of the SRP. In addition, elaboration of SRP materials, as molecularly imprinted polymers (MIPs), expands the separation repertoire of SRP applications with enhanced opportunities to further fine-tune specific bind-and-release selectivities [6••,11••,12••,13••].

Types of stimuli-responsive polymers

Thermo-responsive polymeric materials

Thermo-responsive polymers are currently the dominant class of SRPs. Their wettability/solubility profiles change sharply, associated with prominent phase transitions particularly in aqueous environments, when the environment is heated or cooled to the lower critical solution temperature (LCST) [14]. Poly-(*N*-isopropylacrylamide) (PNIPAAm) is probably the most widely investigated thermo-responsive polymer (LCST of 32°C) with high sensitivity to small temperature changes. At the LCST, PNIPAAm polymers switch from a swollen (highly solvated, water-soluble) state to a shrunken or collapsed (less-solvated, water-excluded) state, that is, convert from a hydrophilic to a more hydrophobic state. The pendant amide groups of the PNIPAAm chains and water molecules form strong hydrogen bonds when the temperature is below the LCST, promoting solvation of PNIPAAm in water. When the temperature is increased above the LCST the hydrogen bonds, however, weaken and increased contributions from hydrophobic interactions lead to collapse of the chains to more compact structures [15••]. Some examples of common thermo-responsive polymers are summarized in Table 1.

Furthermore, LCST of thermo-responsive polymers can be manipulated by incorporating other hydrophilic or hydrophobic moieties into the SRP. For example, copolymerisation of *N*-isopropyl-acrylamide (NIPAAm) with hydrophobic monomers causes a decrease in the LCST. In contrast, the LCST increases when NIPAAm is copolymerized with hydrophilic monomers [16,17]. Discontinuous changes or even disappearance of the LCST can occur if ionisable or polar moieties, for example acrylic acid or *N*,*N*′-dimethylacrylamide, are grafted to PNIPAAm chains [18]. The LCST also depends on the mobile phase properties, such as ionic strength, pH or the type of ions present in the system [10••].

pH-responsive polymers

pH-Responsive SRPs contain weakly acidic or basic ionisable moieties (e.g. carboxylic acid or amino groups), which enable either protonation or deprotonation to occur