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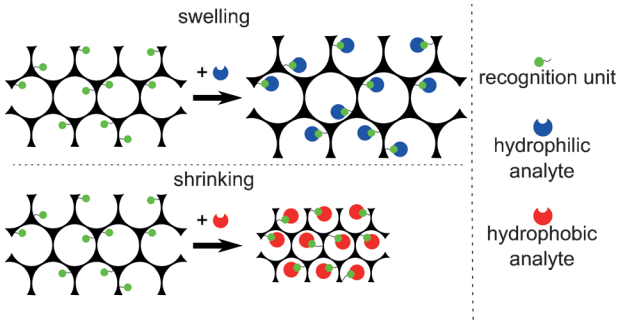
Responsive Inverse Opal Hydrogels for the Sensing of Macromolecules**

Jean-Philippe Couturier, Martin Sütterlin, André Laschewsky, Cornelia Hettrich, and Erik Wischerhoff**

Abstract: *Dual responsive inverse opal hydrogels were designed as autonomous sensor systems for (bio)macromolecules, exploiting the analyte-induced modulation of the opal's structural color. The systems that are based on oligo(ethylene glycol) macromonomers additionally incorporate comonomers with various recognition units. They combine a coil-to-globule collapse transition of the LCST type with sensitivity of the transition temperature toward molecular recognition processes. This enables the specific detection of macromolecular analytes, such as glycopolymers and proteins, by simple optical methods. While the inverse opal structure assists the effective diffusion even of large analytes into the photonic crystal, the stimulus responsiveness gives rise to strong shifts of the optical Bragg peak of more than 100 nm upon analyte binding at a given temperature. The systems' design provides a versatile platform for the development of easy-to-use, fast, and low-cost sensors for pathogens.*

Three-dimensional photonic crystals, namely synthetic opals and inverse opals, are attractive platforms for autonomous chemical sensing and colorimetric bioassays. Characteristically, their structural color is modified by the binding of an analyte to appropriate functional groups incorporated into the photonic crystal.[1] This changes the conditions for the constructive interference of reflected light. While the color changes may be induced either by modulating the refractive index or the spacing of the grating, the latter is more effective.[2] In the case of bioassays, the colloidal crystal is favorably made of a hydrogel, which swells or shrinks in response to changes of the overall hydrophilicity of the system because of the binding of the analyte (Scheme 1). Such sensor devices are of striking simplicity, enabling a priori even visual detection without the need for complicated equipment or especially trained operators. Accordingly, hydrogel-based photonic crystal sensors have been explored successfully for several low-molar-mass analytes.[1b–3]



Scheme 1. Analyte binding induced volume changes of three-dimensional photonic crystal sensors, exemplified by inverse opal hydrogels (IOHs).

However, systems for the sensing of large analytes, such as macromolecules, viruses, or even bacteria, are virtually missing.[1b,d,e] In the few reported cases, the maximal shifts of the interference maximum were small, hardly exceeding 20 nm at the outmost.[4]

This is presumably due to a limited change of the hydrophilicity of the IOHs upon the binding of the analyte, as well as to the problem of fast and effective diffusion of macromolecules into the photonic crystal, as needed to induce a sufficiently big change of the grating, and thus of the optical signal. To overcome these problems, we speculated on the use of dual responsive inverse opal hydrogels (IOH), which can undergo a volume phase transition. While the IOH structure will facilitate the infiltration of the sensor hydrogel by the analyte through large channels, the stimulated phase transition will induce a strong signal.

Accordingly, we explored new IOH systems based on copolymers of oligo(ethylene glycol) monomers **1** and **2** (Figure 1) for sensing macromolecules. Such copolymers are known to be biocompatible and to show low-fouling behavior,[5] a prerequisite for every bioassay. Importantly, they exhibit also a lower critical solution temperature (LCST) type phase transition in aqueous media, which can be tuned by the copolymer composition.[5b,c,6] Moreover, they can easily incorporate additional functional comonomers, which bear recognition units for binding specific analytes. If the analyte

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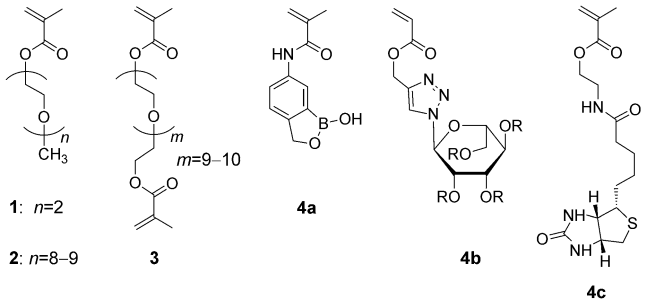


Figure 1. Monomers used for producing the hydrogel matrix (**1** and **2**), its cross-linking (**3**), and functionalization (**4a–c**). The mannose moiety of **4b** was protected (R = acetyl) during polymerization, and deprotected (R = H) in the final copolymer and IOH systems.

Table 1: Composition of selected responsive copolymers and IOHs based on **1** and **2** (fixed molar ratio 85/15).

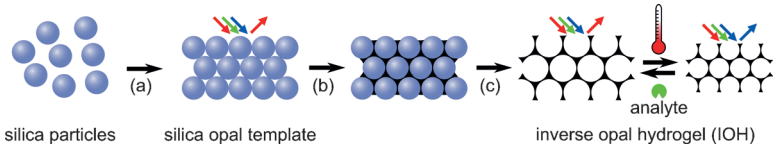
Sample	functional comonomer	comonomer content [mol %]	cross-linker content [mol %]	cloud point [°C][a]
5a	**4a**	15	0	27.0
IOH-a	**4a**	7	19	
5b*	**4b**	10	0	39.2[b]
5b	**4b**	10	0	42.4[c]
IOH-b	**4b**	14	19	
5c	**4c**	10	0	34.5
IOH-c	**4c**	13	19	
5d	none	–	0	30.9
IOH-d	none	–	19	

[a] Cloud points measured by turbidimetry in 0.3 wt% PBS solution (pH 7.4). [b] Before deprotection. [c] After deprotection.

binding modifies the overall hydrophilicity, the LCST of such systems is shifted.[7]

This dual responsiveness allows an induced phase transition under isothermal conditions, if the binding of the analyte shifts the phase transition temperature from below to above the sensing temperature, or vice versa.[7b] Within an appropriately chosen temperature window, this should produce much larger volume changes than simple swelling/deswelling effects because of the primary modulation of the hydrophilicity of the system by the binding of an analyte.

For a proof of concept, we focused on established model recognition systems, such as the binding of vicinal diols as in certain glycopolymers by benzoboroxol,[8] of lectins by specific sugars,[9] and of avidin by biotin.[7b,10] Hence, we synthesized the recognition group bearing comonomers **4a–c**, functionalized by a boronic acid half-ester,[8a] an azidomannose derivative, and a biotin moiety,[11] respectively (Figure 1). The functional IOHs were produced in three steps. First, monodisperse silica nanoparticles were prepared through the Stöber process[12] and assembled into a colloidal crystal. Then, the voids were infiltrated by appropriate monomer mixtures and polymerized. Finally, the template nanoparticles were removed (Scheme 2).



Scheme 2. Synthesis of inverse opal hydrogels (IOHs): a) Monodisperse silica particles (diameter 420 nm) are assembled into a synthetic opal of about 5 μm thickness. b) The template opal is infiltrated by the precursor monomer mixture, which is subsequently photopolymerized to give a filled hydrogel. c) Removal of the template particles by dilute HF produces the dual responsive IOH.

The nature and content of the cross-linker in the sensor IOH must be carefully adapted, to allow effective contact/penetration of the macromolecular analytes onto/into the hydrogel walls, while ensuring a still sufficient refractive index difference between the hydrogel matrix and the water-filled voids. Good results were obtained with the oligomeric cross-linker **3** in quantities of 19 mol % of the total comonomer content (Table 1). Moreover, in order to obtain IOH films of sufficient mechanical stability for handling, the thin monomer-filled opal template films were covered by a comparatively thick comonomer film of about 100 μm thickness, resulting in about 5 μm thin IOH layer supported by the corresponding bulk hydrogel film after curing and template removal.

In addition to the functional inverse opal series IOH-a to IOH-c, we prepared analogously IOHs devoid of recognition units as references (series IOH-d, Table 1). Also for comparative model studies in aqueous solution, we synthesized the analogous soluble copolymers without cross-linker (samples **5a–d**, Table 1).

Cloud points of the soluble copolymer analogues of the IOHs in aqueous buffer solution (phosphate buffered saline, PBS) are listed in Table 1. They indicate an LCST-type phase transition of the basic comonomer composition at 30.9 °C. Functionalization by monomers **4a–c** inevitably modulated the overall hydrophilicity of the copolymers and thus changed the cloud points to a small extent. Importantly, the addition of the corresponding ligands (see Figure 2 for a selection) induced a notable shift of the cloud points. For instance, the addition of fructose or of the xylose-derived copolymer **PXM** (see below) increased the cloud point of 0.3 wt % solutions in PBS of copolymer **5a** by 8.4 °C or by 5.9 °C, respectively, while the weakly or non-binding related molecules ribose, maltopentaose (**PMA**), or poly(vinylalcohol) (**PVA**) produced only marginal shifts (< 0.5 °C). Similarly, the addition of the protein avidin increased the cloud points of **5c** by 6.7 °C, while the addition of other, non-binding proteins, such as bovine serum albumin (BSA), does not affect the cloud point. These results demonstrate the basic double responsiveness of the functionalized copolymers toward the temperature as well as molecular recognition processes.

When appropriately cross-linked, the thermoresponsiveness of these copolymer systems is preserved in inverse opal hydrogels. This results in their pronounced shrinking upon heating, with the concomitant marked shift of the Bragg peak position and of the corresponding color. This is exemplified for IOH-a in Figure 3. Characteristic for copolymers of **1** and

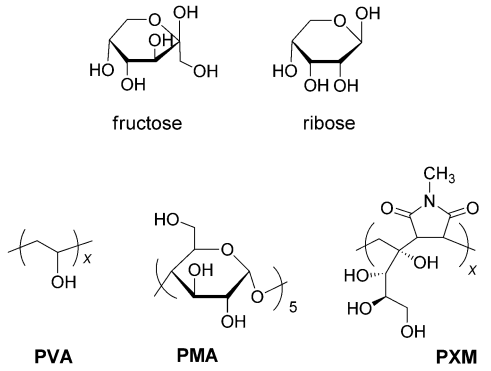


Figure 2. Selection of potential low-molar-mass and polymeric ligands tested for binding to boroxol-containing copolymer **5a** and IOH-a (sugars shown in their preferential β-pyranose form).

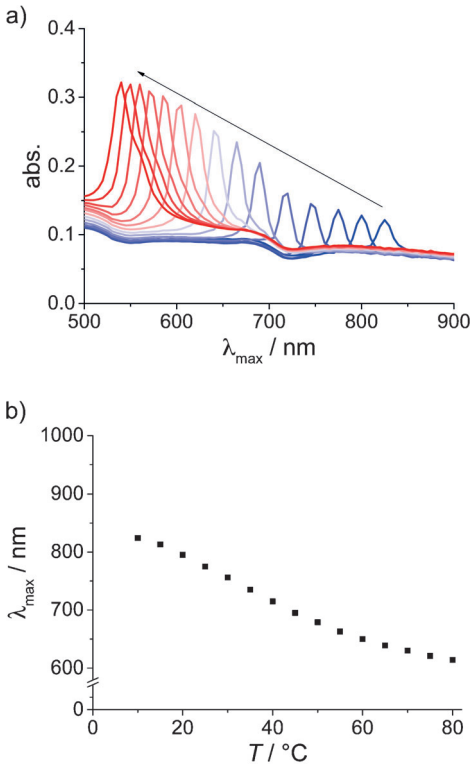


Figure 3. Temperature (*T*)-dependent optical properties of IOH-a swollen in PBS buffer: a) absorbance spectra, b) wavelength of the position of the Bragg peak maximum (λ_{max}).

2, the deswelling transition of the hydrogels stretches over a rather broad temperature range,[13] in contrast to the sharp cloud points that indicate the onset of the phase transition of analogous soluble copolymers.

When adding specific polymeric ligands to the functional IOHs, the temperature-induced shift of the Bragg peaks is strongly modulated (Figure 4,5). This behavior is remarkably selective. For instance, the strongly binding polymeric ligand **PXM** induces a strong and sensitive shift of the deswelling transition of IOH-a, which bears a boroxol moiety as recognition group,[14] while the effects of the non-binding or

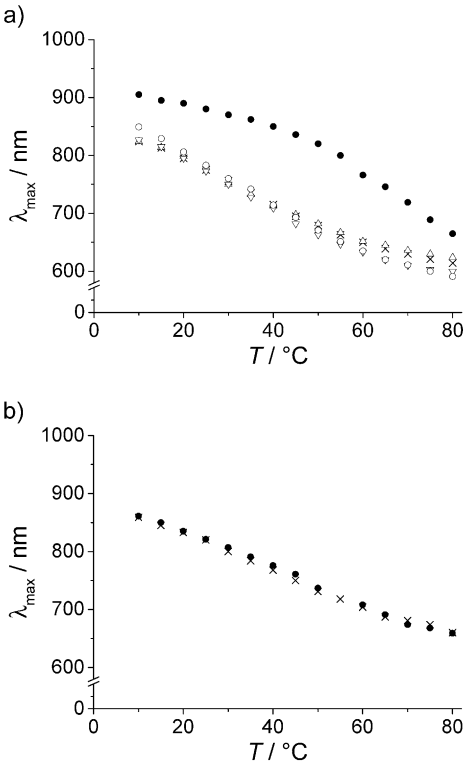


Figure 4. Temperature (*T*)-dependent swelling of functionalized IOHs, in the presence of potential ligands, followed by the shift of the Bragg peak (wavelength of maximum absorbance λ_{max}). a) IOH-a: × = in PBS buffer, ○ = in the presence of fructose, △ = in the presence of **PVA**, ▽ = in the presence of **PMA**, ● = in the presence of **PXM** (IOH-specific analyte). b) Reference IOH-d (no recognition units): × = in PBS buffer, ● = in the presence of **PXM** (all analyte concentrations 50 mM repeat unit).

weakly binding polymers **PVA** and **PMA** on the deswelling transition are negligible (Figure 4a). Remarkably, even fructose hardly affects the transition of IOH-a, although this moderately binding low-molar-mass ligand induces a notable shift of the cloud point for the soluble copolymer analogues (see above). In contrast, even the addition of the strongly binding polymer **PXM** does not modulate the reference inverse opal hydrogel IOH-d, which lacks recognition units (Figure 4b). All these results indicate the successful and specific sensing of macromolecules by the IOHs.

If the IOHs are kept at a constant temperature within the transition window, the phase-transition-enhanced sensing can be conducted under isothermal conditions. Importantly, biologically relevant water-soluble polymers tend to add to the hydrophilicity of the overall system, thus increasing its phase-transition temperature. The resulting swelling transition of the hydrogels is much faster and easier to detect than an induced collapse transition. For instance, the binding of the specific ligand **PXM** to IOH-a at 37 °C induces a shift of the Bragg peak by 130 nm. In fact, in the optimal temperature range of 50–55 °C for the particular IOH, the shift amounts to up to 200 nm. Such strong shifts are easy to detect by a simple photometer, or even by the naked eye.

The IOH system based on comonomers **1**–**3** seems to be a rather general platform for the sensing of macromolecules.

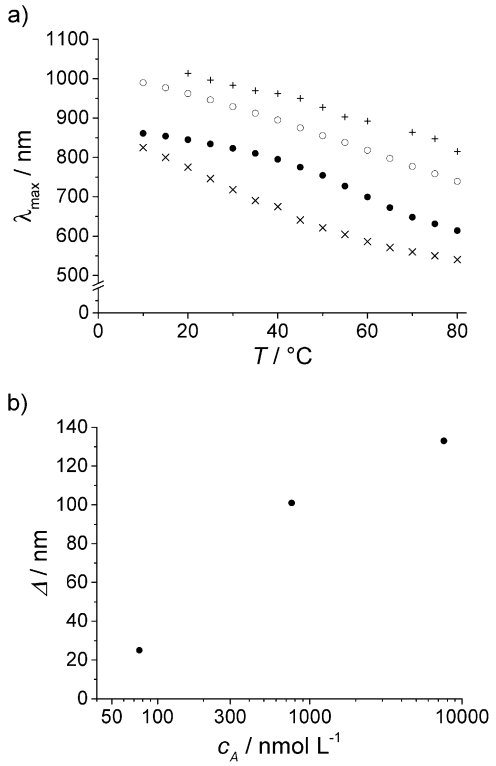


Figure 5. a) Temperature (*T*)-dependent swelling of functionalized IOHs, in the presence of potential ligands, followed by the shifting of the Bragg peak maximum (λ_{max}): + = IOH-b in PBS buffer, ○ = IOH-b in the presence of ConA, × = IOH-c in PBS buffer, ● = IOH-c in the presence of avidin (analyte concentration 0.5 g L^{-1}). b) Shift Δ of the optical Bragg peak maximum of biotin-functionalized IOH-c with increasing concentration of avidin C_A at 35 °C.

If comonomer **4a** with the boroxol recognition unit is replaced by other functional comonomers, for example, by mannose-functionalized **4b** or biotin-bearing **4c**, proteins such as the lectin concanavalin A (ConA) or avidin, respectively, can be specifically detected as well (Figure 5a). Again, their specific binding induces strong shifts of the temperature window for the deswelling process, and thus of the Bragg peak maximum at a given temperature, here of inverse opals IOH-b and IOH-c. Even at concentrations in the nanomolar region, the spectral shift is still larger than 20 nm (Figure 5b).

In summary, tailored dual responsive inverse opal hydrogels, which combine thermosensitivity with sensitivity toward molecular recognition processes, enable the versatile sensing of macromolecular analytes by simple optical means. Appropriately designed systems afford effective diffusion even of large analytes into the photonic crystal, and undergo a big change of the grating upon analyte recognition to produce a strong optical signal, which is easy to detect. Such systems represent a promising and versatile platform for the development of simple and low-cost sensors for pathogens.

Keywords: hydrogels · photonic crystals · polymers · responsive materials · sensors

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Communications

Responsive Materials

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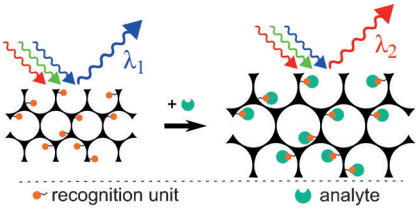
Responsive Inverse Opal Hydrogels for the Sensing of Macromolecules



A successful marriage: The combination of smart hydrogels and inverse opal structures unites simplicity with efficacy for sensing macromolecules. While the inverse opal structure provides structural color and a large accessible interface for binding, the induced phase transition of the analyte-responsive hydrogel produces strong optical effects. The resulting spectral shifts can surpass 100 nm and are easily detected.