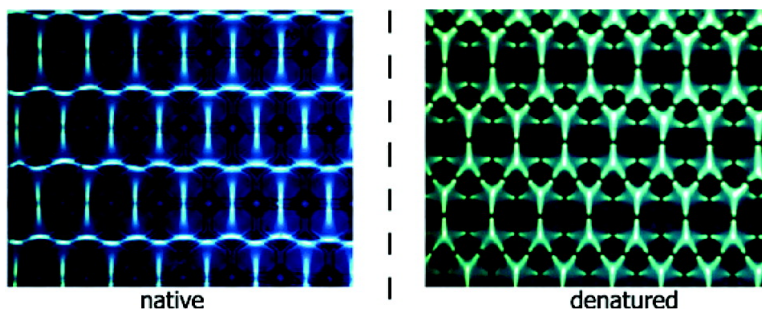## Communication

Amplification of Changes of a Thin Film's Macromolecular Structure into Macroscopic Reaction−Diffusion Patterns

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Amplification of Changes of a Thin Film's Macromolecular Structure into Macroscopic Reaction−Diffusion Patterns

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To study phase transitions occurring at molecular scales, it is first necessary to translate the changes that accompany them into a form perceptible to our senses. While some transitions (e.g., those involving separation of phases,[1] changes in order parameter,[2] or in a system's symmetry[3]) manifest themselves by pronounced visual changes detectable even by a naked eye, others (e.g., conformational transitions in macromolecules[4]) alter only the physical/material properties of a substance without visual effects. This work provides an example of a system, in which relatively small changes in the material properties (here, water absorptivity) of a thin gelatin film undergoing a phase transition are amplified and translated into color patterns by reaction−diffusion (RD). The RD process is initiated from a network of connected microfeatures placed onto the film. When a helix-to-coil phase transition in the gel occurs, its absorptivity changes and the micronetwork reconfigures itself internally, and as a result, the color patterns that emerge by RD switch from symmetry-broken to symmetric ones. Pattern switching is a consequence of an interplay between the RD processes and the system's geometry.

A series of ionically doped gelatin solutions were prepared by first dissolving fixed amounts (3 g) of gelatin (Gelatin B, 225 bloom, Sigma-Aldrich) in 27 mL of deionized water, heating for 30 min at temperatures (T) ranging from 30 to 65 °C, adding 0.3 g of potassium hexacyanoferrate, $K_4[Fe(CN)_6]$, to each solution, and heating for another 10 min. Equal aliquots (3 mL) of each solution were cast against flat-bottom Petri dishes, allowed to gelate, and left to dry for 4 days under ambient conditions to give 30 μm thick films. Next, agarose stamps (8% w/w OmniPur Hi-Strength Agarose, Darmstadt, Germany) micropatterned in bas relief[7,8] with a regular network of connected, pentagonal features and uniformly soaked for 25 min in a 10% w/w solution of iron(III) chloride were placed onto each of the films (Figure 1A).

As the iron chloride "ink" penetrated into the dry films, it reacted with the $K_4[Fe(CN)_6]$ "indicator" contained therein to give a deeply colored Prussian blue precipitate and to develop into regular spatial patterns.[5,8] Importantly, for gels prepared by dissolving gelatin at $T < 39 \pm 1$ °C, patterns originated "asymmetrically" only from the higher-degree ("four") nodes of the pentagonal tiling, while for solutions prepared at $T > 39 \pm 1$ °C, they propagated "symmetrically" from all nodes of the stamped network (Figure 1B). In an independent microcalorimetric study (Figure 1D), we verified that at a $T_C = 38 \pm 1$ °C, the solutions of doped gelatin underwent a phase transition from partly folded triple helices (below T_C) to random coils (above T_C).[6] These observations suggest that the crossover between the two types of RD patterns reflects the changes in the molecular structure of the gel.

To show that, it is first necessary to understand the nature of RD processes occurring in the system and their relationship to the geometry of the imposed network. When the tops of the stamped
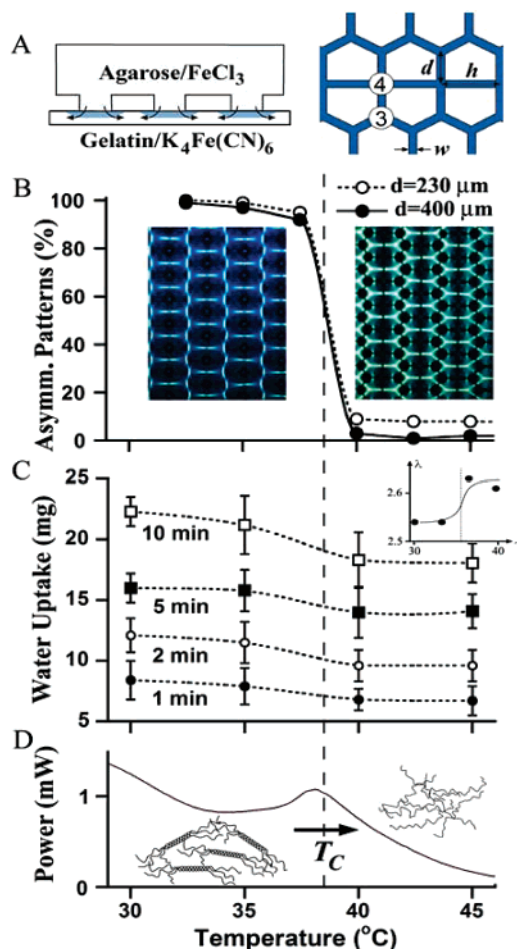

Figure 1. (A) Schematic of the experimental setup ($w = 50$ μm, $h = 300$ μm, $d = 230$−400 μm). (B) Percent of asymmetric solutions as a function of T. (C) Water uptake of gels prepared at different temperatures. For each temperature, the amounts of absorbed water fit to an exponential dependence, $w(\tau) = w_0[1 - \exp(-\tau/\alpha)]$. The insert has the plot of water uptake rates, α, versus the temperature. (D) DSC scan of a gelatin/hexacyanoferrate solution showing helix-to-coil transition at temperature T_C equal to that of the crossover between asymmetric and symmetric RD patterns on dry gel films.

features come into conformal contact with gelatin, water rapidly wets its dry surface by capillarity. This outflow decreases the initially uniform water content, ρ_{init}, in the network's features and establishes its spatial gradients along which the ions contained in the stamp migrate prior to entering the gel. We have recently shown[8] that in networks having one type of nodes, these nodes lose less water than the edges, and that for appropriate dimensions of the network and the rate of water outflow, gradients of ρ can be strong
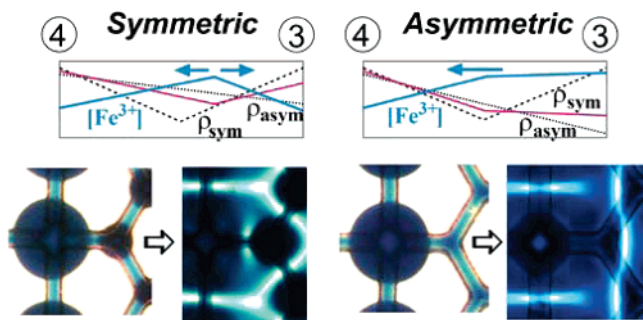
Figure 2. The schemes illustrate instantaneous concentrations of water (red lines) and iron cations (blue lines) along a *3−4* edge, leading to symmetric (left) and asymmetric (right) RD solutions. Pictures in the bottom row are optical micrographs showing how both types of patterns develop.

enough to cause all Fe^{3+} cations to diffuse toward the nodes and enter the substrate only from these locations (for more details, see Supporting Information).

In networks having nodes of different types, the amount of water flowing out of a node per unit time decreases with the node's degree.[9] In the pentagonal network used in this work, the nodes are of two types and the 4-fold nodes (*4*) lose less water than the 3-fold (*3*) ones. Consequently, the concentration gradients that the Fe^{3+} cations experience are asymmetric, and depending on their relative steepness, cations can migrate either to both types of nodes or to only the 4-fold ones (Figure 2). Mathematically, the two scenarios can be explained in terms of the gradients of water content created in the edges connecting the *3* and *4* nodes. The water content profile, $\rho(x, \tau)$, along a *3−4* edge at time τ can be approximated as a sum of two contributions (i) asymmetric, $\rho_{asym}(x, \tau)$, due to the difference in the water contents between the nodes, $\rho_{asym}(x, \tau) \approx \rho_4(\tau) - [\rho_4(\tau) - \rho_3(\tau)]x/d$, and (ii) symmetric, triangle-like correction, ρ_{sym}, accounting for the fact that nodes lose less water than the edges,[7] $\rho_{sym}(x, \tau) \approx -f(\tau)x/d$ for $x < d/2$ and $f(\tau)(x - d)/d$ for $x \geq d/2$, with $f(\tau)$ being a monotonically increasing function of time. Furthermore, the water content at the nodes decreases approximately exponentially with τ (cf. Figure 1c, insert), $\rho_{3,4}(\tau) \approx \rho_{init} \exp(-\sigma_{3,4}\alpha\tau)$, where $\sigma_{3,4}$ are some positive constants ($\sigma_3 > \sigma_4$), and α is the rate of water absorption by the gelatin film. The type of pattern that emerges is determined by the rate of water outflow (α), for low values of α, the overall water profile (red line in Figure 2) exhibits a minimum between the nodes, and the Fe^{3+} cations diffuse toward both types of nodes; when α is large, the steep asymmetric term dominates the overall water profile, and the cations migrate only toward the *4* nodes. For a fixed geometry of the network, the crossover between the two types of patterns occurs at some critical value, α^*, corresponding to the gradient of water content, $d\rho(x)/dx$, at the *3* node being equal to zero. This condition leads[10] to the following approximate expression for α^*: $(\sigma_3 - \sigma_4) \alpha^* \approx \gamma$, in which γ is a positive constant (the asymmetric solution corresponds to $\alpha > \alpha^*$). In other words, substrates absorbing water rapidly give rise to asymmetric RD patterns, while those absorbing water slowly give rise to symmetric ones.

This condition for pattern switching can be related to the macromolecular structure of the gel by noting that dry gelatin containing partly folded collagen-like helices absorbs water more readily than denatured one;[11] this property reflects the fact that collagen-like helices coordinate hydration waters more efficiently than corresponding (denatured) random coils. In our experiments, we have verified that the rate of water uptake by ionically doped dry gels prepared at different temperatures decreases near T_C (Figures 1C). From this observation, we infer that gel films prepared at $T < T_C$ are composed of nondenatured gelatin, while those made at $T > T_C$ are composed of denatured one. At around T_C, the value of α drops below α^*, causing a change in the sign of the water content gradient near the 3-fold nodes of the stamped network. This gradient reversal, in turn, manifests itself by the visual change in the RD patterns.

We briefly note that although water outflow is essential for establishing concentration gradients in the network, neither its absolute value, w, nor its rate, α, are by themselves accurate "predictors" of the film's macromolecular structure. While the RD patterns switch completely upon phase transition, w changes by ~15% and α by only ~2.5% (Figure 1C, insert). In other words, w and/or α can be viewed as control parameters whose small changes are amplified by the reaction−diffusion readout.

In summary, we have described a model chemical system, which by virtue of the imposed microgeometry, autonomously detects a macromolecular phase transition occurring in a thin film and reports it in a visual form of color patterns. We suggest that micronetwork RD systems similar to the one described here might be useful in rapid assessment of absorptivities of other materials, likely using different indicator/ink pairs and possibly with different solvents.[12] Finally, we believe that this work will stimulate new theoretical research on RD processes coupled by mass transfer and occurring in complex microgeometries.

Supporting Information Available: Comparison with networks having one type of nodes, and Network design rules. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) This follows from geometrical arguments: The direction of the flux, **q**, of water flowing out of the stamp and wetting the surface of the gel is perpendicular to the borderline of a feature. The amount of water transferred from a given element of the feature is thus proportional to the density of the flux lines. Because this density decreases with the degree of a node, the *4* nodes lose less water than the *3* ones. For details, see ref 8.
(10) The type of the solution is determined at the early stages of the dehydration process (see ref 7) when both $\rho_{3,4}(\tau)$ and $f(\tau)$ change approximately linearly with time, $\rho_{3,4}(\tau) \approx \rho_{init}(1 - \sigma_{3,4}\alpha\tau)$ and $f(\tau) \approx \gamma\tau$.
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