Frustrations in Polymer Conformation in Gels and their Minimization through Molecular Imprinting

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We report an experimental realization of a gel system in which frustrations exist and can be minimized, thus meeting two crucial criteria predicted to enable memory of conformations in polymers. The gels consist of a thermosensitive major monomer component and two minor components. One minor component is positively charged and will form complexes around negatively charged target molecules placed in solution. The complexes can be imprinted into the gel by then cross-linking the second minor component, which will form cross-links additional to those in the major polymer matrix. The complexes are destroyed and reformed upon swelling and reshrinking of the gels, showing that memorization has been achieved.

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The recent development of the statistical mechanics of polymers has clarified the principles underlying the memory of conformation by macromolecules [1,2] (see recent review [3]). First, the polymer must be a heteropolymer, i.e., there should be more than one monomer species, so that some conformations are energetically more favorable than others. Second, the polymer must be condensed, in which case frustrations exist due to the interplay of chain connectivity and excluded volume [4]. Third, the sequence of monomers must be selected as to minimize these frustrations [1]. These three conditions allow the polymer to have its global energy minimum at one particular conformation, which thus can be said to be memorized.

We report an experimental implementation of the above principles, albeit at a primitive level, in the synthesis of a weakly cross-linked polymer gel. The gel can memorize a part of its molecular conformation—specifically, spatial assemblies of certain groups of its monomers.

Our gel consists of three types of monomers, one major and two minor. The major component is chosen such that taken alone it would form a weakly cross-linked network capable of undergoing a collapse transition. This component maintains our gel in the dense state. One of the minor components consists of monomers with thiol groups ($-$SH). After the gel has already been prepared, these monomers can be induced to form disulfide bonds ($-\mathrm{SH} + \mathrm{HS}- \rightarrow -\mathrm{S}-\mathrm{S}-$). As long as the gel is maintained under conditions in which this reaction is irreversible, the reaction should be viewed as effectively creating additional cross-links in the gel. We shall call this process *post-cross-linking* since it is done *after* polymerization and gelation of the major component. Monomers of the second minority component can also interact with each other, but in a very different fashion. Namely, we chose these monomers to have a positive charge, so that they can interact with special additional so-called *target* molecules, not connected to the gel network, but having several negative charges each. These positively charged minority monomers can form ionic complexes with the negatively charged target molecules. In other words, they assemble into groups in which their interaction is mediated by the target molecules.

Our major experimental strategy is to compare gels which are post-cross-linked in the presence or in the absence of target molecules. We call these gels imprinted and random, respectively. We argue that, by doing post-cross-linking in the presence of target molecules, we suppress frustrations and thus approach the minimal frustration situation. We view this approach as an implementation of the imprinting concept [5–9].

To test the effect of imprinting we swell both types of gels (removing target molecules from the imprinted gels) and then shrink them again. We then place target molecules in a solution with each type of gel and measure their absorption by the gels. For the randomly post-cross-linked gels, the random S–S bonds should partially inhibit the adsorbing monomers from forming complexes with the target molecules. The randomly post-cross-linked gels will therefore be frustrated. However, there should be less frustration in the imprinted gels. This is because the post-cross-linking for them has been done in a condition that favors the formation of the complexes. A difference in the absorption of target molecules by the two gels directly shows that the complexes were successfully memorized by the imprinted gels.

We now describe the actual experiments designed based on the above logic (Fig. 1). We chose as targets pyranine-3 with three negative charges (8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt) and pyranine-4 with four negative charges. The positively charged

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monomer, methacrylamido-propyl-trimethyl-ammonium-chloride (MAPTAC, 0 to 200 mM), was used as an adsorbing molecule. *N*-isopropylacrylamide (NIPA, 6 M) was chosen as a thermoresponsive majority monomer that allowed for the reversible swelling and shrinking of polymers [10–12]. We used N, N'-methylene-bis-acrylamide (BIS, 10 mM) as a cross-linker and *N*-(2-S-acetylthio)ethyl acrylamide (SAc, 0 to 256 mM) for the post-cross-linking into disulfide bonds, S–S. SAc was prepared from acryloyl chloride and 2-chloroethylamine monohydrochloride. The post-cross-linking monomers were capped with an acetyl group to avoid attack during the gelation process. These were all dissolved in methylsulfoxide and polymerized by free radical polymerization initiated by azobis-isobutylonitrile (10 mM) at 60 °C.

The overall monomer compositions were chosen so that the gels resumed the original polymer density at gelation, and so that the original state could be recovered in the equilibrium shrunken phase. Cylindrical gels of 300 μm diameter were washed with water, 100 mM HCl solution, 100 mM NaOH solution, and 100 mM NaCl solution. The SAc was decapped into SH groups by immersing the gels in 0.5 M NaOH solution. These were the common, original gels from which both random gels and imprinted gels were synthesized. Random gels were made by first letting them shrink at 60 °C and then placing them in 100 mM $NaBrO_3$ aqueous solution to oxidize the SH groups into disulfide bonds, S–S. The imprinted gels were made by first allowing them to adsorb the 70 μM pyranine-4 solution. It was confirmed that the gel was saturated with pyranine-4 in the molar ratio of [pyranine-4] : [MAPTAC] = 4 : 1 and that the total amount of adsorbed pyranine-4 was one-fourth that of MAPTAC, indicating that all of the MAPTAC monomers formed complexes with pyranine-4. Here and from now on [$**$] indicates the concentration of molecular species $**$. The gels were then post-cross-linked in the same way as the random gels.

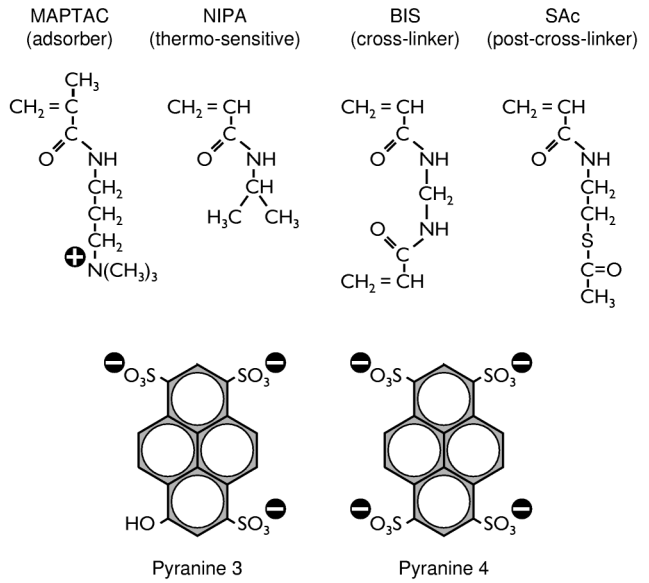

FIG. 1. The chemical structures of the major components, which are a thermoresponsive monomer (NIPA), adsorption sites with a positive charge (MAPTAC), cross-linker (BIS), and target molecules with three or four charges, pyranine-3 and pyranine-4, respectively. The recipe for synthesis is NIPA (6 M), MAPTAC (0 to 200 mM), and BIS (10 mM). SH groups (0 to 256 mM) are also incorporated into the gels to form S–S bonds after the gels are made in the presence and absence of pyranine. The monomers were dissolved in methylsulfoxide and polymerized by free radical polymerization initiated by azobis-isobutylonitrile (10 mM) at 60 °C.

The gels were placed in aqueous solutions of pyranine of concentrations ranging from 2.5 μM to 1 mM. In the swollen state, the MAPTAC monomers associate with more singly charged anions in solution, rather than form complexes to adsorb the pyranine molecules as they do in the shrunken state. All of the solutions had 100 mM NaCl, which was chosen so that the pyranine molecules and chloride ions could replace each other upon swelling and shrinking of the gel. The pyranine concentration adsorbed by the gel was determined by measuring the decrease of pyranine in the outer solution using fluorescence or UV spectroscopy.

Figure 2 shows the adsorption of pyranine per MAPTAC monomer as a function of pyranine concentration for imprinted and nonimprinted gels at 25 °C (swollen gel) and 60 °C (collapsed gel). The results and the interpretation of the experiments are summarized as follows.

(i) The pyranine adsorption per liter of gel, A, as a function of the equilibrium pyranine concentration in the external solution, P, fits well to the Langmuir isotherm: $A = SP/(P + 1/k)$ or $P/A = P/S + 1/Sk$, where S is the number of adsorption sites per unit volume of the gel, and k is the effective binding constant.

(ii) In the shrunken state, at high MAPTAC concentrations, the concentration of adsorption sites, S, was one-third of the MAPTAC concentration for pyranine-3 and one-fourth for pyranine-4: $S = [\text{MAPTAC}]/p$, where $p = 3$ for pyranine-3 and $p = 4$ for pyranine-4.

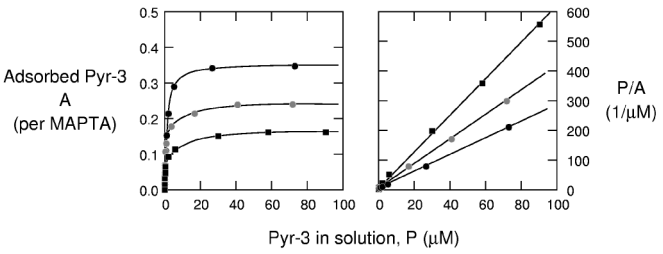

FIG. 2. The adsorption of pyranine-3 per MAPTAC monomer as a function of pyranine-3 concentration in the swollen state at 25 °C and in the shrunken state at 60 °C. The MAPTAC concentration was 30 mM. The plot of the reciprocal adsorption and the reciprocal pyranine concentration becomes linear, which indicates that the adsorption is well described by the Langmuir adsorption isotherm.

(iii) The affinity of the shrunken, nonimprinted gels has a power law relation with the MAPTAC concentration: $Sk \sim [\mathrm{MAPTAC}]^p$.

These relationships (Fig. 3) clearly showed that the adsorption sites were formed when three adsorbing molecules of MAPTAC gathered to capture one pyranine-3 having three charges, and four MAPTAC captured one pyranine-4 with four charges. This has been reported elsewhere [13].

(iv) At low concentrations of MAPTAC, the affinity Sk became very low and independent of MAPTAC, representing the adsorption by the polymer NIPA alone.

(v) When the gels were swollen, the affinity becomes proportional to MAPTAC for both pyranine-3 and -4: $Sk \sim [\mathrm{MAPTAC}]$.

This indicated that the MAPTAC monomers were separated and could not form adsorbing complexes. There was only a single-point adsorption of pyranine by MAPTAC with two or three counterions of Cl^- around them. The above results proved multiple point adsorption in the shrunken state and the reversible destruction of the adsorbing complexes upon gel swelling [13].

(vi) The affinity decreased exponentially as a function of S−S bond concentration: $A = A_0 \exp(-a \cdot [\mathrm{S{-}S}])$, where a and A_0 are constant (Fig. 4). This indicates the frustration.

This dependence can be derived by first noting that, below a certain length scale associated with the cross-link density, the gel behaved like a liquid, allowing the MAPTAC monomers to diffuse virtually freely [10,11]. Beyond that length scale, the gel behaves as an elastic solid body, and the MAPTAC monomers cannot diffuse over the distance. We may assume that each MAPTAC is at one end of a fictitious Gaussian chain with a length half the average polymer length between the nearest S−S cross-links, $l = nb = b([\mathrm{NIPA}]/[\mathrm{S{-}S}])/2$. Here n is the number of monomer segments of length b. There are $[\mathrm{MAPTAC}] \times N_A$ such polymers per volume whose other ends are fixed in space with an average distance, $R = 10\ \mathrm{cm}/([\mathrm{MAPTAC}] \times N_A)^{1/3}$, where N_A is the Avogadro number. This chain represents the restriction on MAPTAC to diffuse. We expect that the probability for three or four MAPTACs to meet should be proportional to the Boltzmann factor of the entropy loss associated with the formation of one assembly of MAPTACs.

$$P = P_0 \exp\left[-(p-1)\frac{R^2}{nb^2}\right] = P_0 \exp\left[-A\frac{[\mathrm{S{-}S}]}{[\mathrm{MAPTAC}]^{2/3}}\right], \quad (1)$$

where A is a constant.

Frustrations were thus created using post-cross-links that prevent MAPTAC from gathering and capturing pyranine to lower the energy.

(vii) The affinity of pyranine in the imprinted gel was 5 times larger than that of random gels at high S−S concentrations. This proved that some of the frustrations were indeed removed by imprinting. The fact that this high adsorption was recovered in the cycle of gel swelling and collapse shows that the MAPTAC complexes were destroyed and restored at the molecular level, involving the original sets of MAPTAC monomers. The imprinting provides much higher local MAPTAC concentrations and thus a lower decay of affinity with [S−S]. This post-cross-linking imprinting, however, was not able to make a

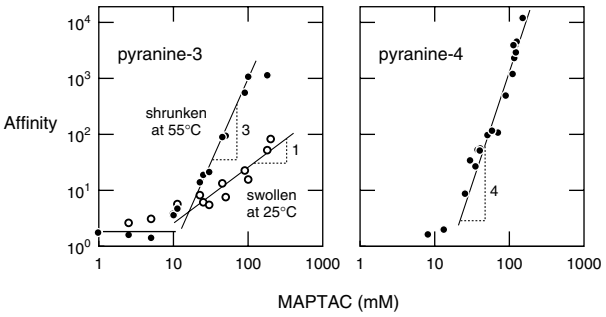

FIG. 3. The affinity of the random gels to pyranine-3 in the swollen state at 23 °C and in the shrunken state at 60 °C is plotted as a function of the MAPTAC concentration at the time of polymerization (in the left panel). They become linear in the log-log plot and roughly have a slope of 3 in the shrunken state and 1 for the swollen state. This indicates that the gels adsorb the targets at three contact points in the shrunken state, while single point adsorption occurs in the swollen state. The slope becomes 4 for pyranine-4, indicating that the MAPTAC captures pyranine-4 (right panel) in quartets. These prove the multiple point adsorption of pyranine and the formation of complexes. The results are taken from Ref. [13].

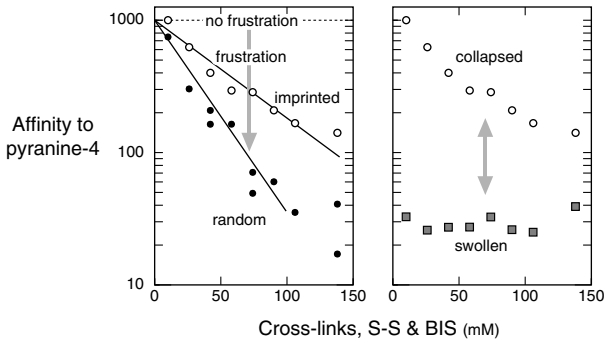

FIG. 4. The affinity of pyranine-4 is shown as a function of S−S bonds plus BIS (10 mM) concentration, within the gels in the collapsed state at 60 °C. For random gels the affinity decays exponentially with S−S concentration (the solid line is a guide to the eye). The loss of ability to form complexes indicates the frustration due to cross-links. For the imprinted gels the affinity increases 3 to 5 times at higher S−S concentrations. This indicates the partial resolution of frustration. When the gels are allowed to swell at 25 °C, the affinity becomes very low and independent of S−S concentration, indicating the destruction of the adsorption sites upon gel swelling (right panel). When the imprinted gels are allowed to collapse again, the pyranine adsorption resumes its original high values, indicating the restoration of the original complexes with the same MAPTAC monomers.

perfect imprinting, namely, the decay was not completely zero. The sequence of the majority and minority and SH groups had already been predetermined and randomly quenched. So the minimization of frustration was allowed only in the freedom of finding best partners among SH groups. Ideally the entire sequence of all of the monomers must be chosen so that the system is in the global energy minimum. Such efforts will be reported elsewhere.

It is important to confirm that the observed effect is not by trivial concentration fluctuations of cross-linking density induced by the presence of target molecules upon imprinting. The fact that the target molecules formed a complex with the adsorbing site in the stoichiometric ratio of 3 to 1 for pyranine-3 and 4 to 1 in the case of pyranine-4 means that all the target molecules are well stabilized and localized, and the complexes are homogeneously distributed within the gel at the molecular level. Furthermore, the target concentration was only 0.5 mol %, and it was well dissolved in the pregel solution. Thus the target molecules did not induce any density fluctuations in the gel upon polymerization. With these considerations, it may be reasonable to assume that the effect observed was indeed due to the molecular level imprinting.

In conclusion, polymer gels were synthesized that could memorize their original assembly of monomers [14,15]. In proving the memory of these assemblies, the concepts of attraction via target molecules and molecular "imprinting" played a crucial role. The frustration was introduced using covalent cross-links, and the memory of monomer complexes up to the cross-link density was achieved.

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