ChemistryOPEN

# Supporting Information



# Bio-inspired Dynamic Gradients Regulated by Supramolecular Bindings in Receptor-Embedded Hydrogel Matrices

Xinglong Luan,[a] Yihe Zhang,*[a] Jing Wu,[b] Pascal Jonkheijm,[c] Guangtao Li,[d] Lei Jiang,[e] Jurriaan Huskens,*[c] and Qi An*[a]

open_201600030_sm_miscellaneous_information.pdf

Supporting Information

## 1. Experimental

### 1.1 Materials

Poly(acrylic acid) (PAA, Mw≈240 000, 25wt% water solution) was purchased from Alfa Aesar, 4-aminoazobenzene, 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC) and N-hydroxysuccinimide (NHS) was obtained from J&K. 6-mono-amino-6-mono-deoxy-β-cyclodextrin was obtained from Shandong BinzhouZhiyuanBio-technology Co., Ltd, China. Dialytic bag (Mw≈8,000~14,000) was purchased from Thermo Fisher. PAA was lyophilized before use. PDMS was purchased from Dow Corning, Sylgard 184, Midland, MI, USA. Other reagents were obtained fromSinopharm Chemical Reagent Beijing Co., Ltd. (Beijing, China).

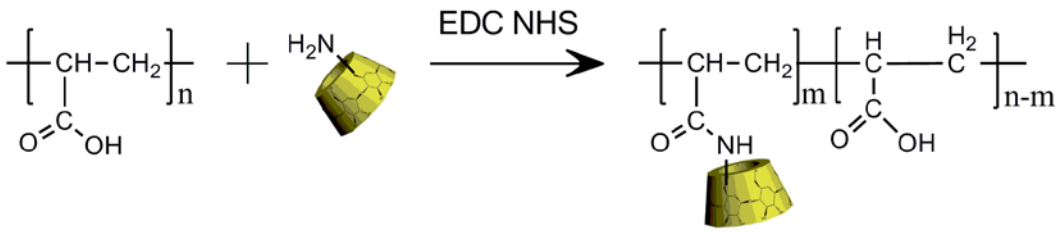
### 1.2 Characterizations

Normal $^{1}$H-NMR was measured by BRUKER AV600 and variable-temperature $^{1}$H-NMR was carried out using BRUKER DMX 300. Rheological measurements were performed by Anton Paar MCR 301 using the CP50-0.3 rotor. XRD data were obtained using Rigaku D/max-B withascanning rate of 8°/min. UV-vis spectra were obtained by UV-6100 double beam spectrophotometer (MAPADA, China). Lyophilization was realized using FD-1A-50 Lyophilizer (Bo d Kang, Beijing, China).UV irradiation was provided by a 365 nm lamp of 20 W from a distance of 5 cm. The diffusion phenomena were observed and recorded with GAOSUO digital microscope and Webex. The snapshots were analyzed using Image-J, and the detection limits using the pixel analysis were determined to be around 0.15 mM.

### 1.3 Preparation of microfluidic channels

The fluidic chip with T-shaped fluidic channels with across-sectionwidth of 2 mm and a depth of 500 μmwas fabricated using standard soft lithography and replica molding techniques. Briefly, negative photoresist SU-8 1075 (Microchem, Newton, MA) was spin-coated onto the silicon wafer which was cleaned by piranha solution beforehand. The spinning speed was set as 1000 rpm for 60 s. After baking to dry, the silicon master mold was fabricated under UV irradiation through a mask. According to the manufacturer instructions, a premixed 10:1 (by mass) poly-dimethylsiloxane (PDMS) prepolymer and curing agent were prepared and poured onto the mold. It had been cured in an oven for 2 h at 75 °C after degassed under vacuum. The PDMS was peeled off carefully and cut into the designed shape with a surgical scalpel. The inlet and outlet of channels were punched with a flat-tip syringe needle and then the PDMS replica was sealed with a glass slide via oxygen plasma (PDC-32g, Harrick Plasma, Ithaca, NY, USA) treatment for 90 s.

## 2. Synthesis scheme of PAA-CD




Scheme S1. Reaction scheme of poly(acrylic acid) and 6-mono-amino-6-mono-deoxy-β-cyclodextrin using EDC and NHS as coupling reagents.

**3. Digital image and structural schematic illustration of PAA-CD hydrogel.**

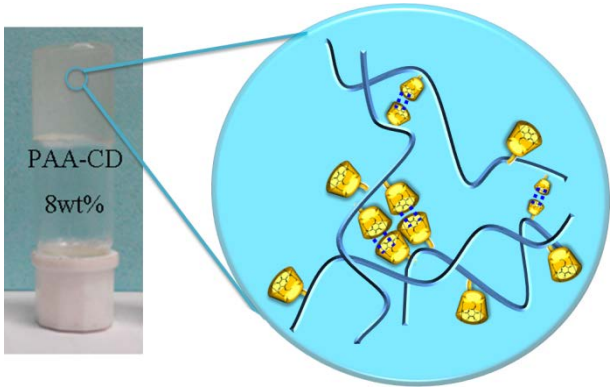



Figure S1.Digital image of PAA-CD hydrogel (grafting ratio 4.3%, concentration 8wt%) sticking to the upper part of a vial and its structural schematic illustration.

**4. Theological characterization of PAA-CD hydrogel.**

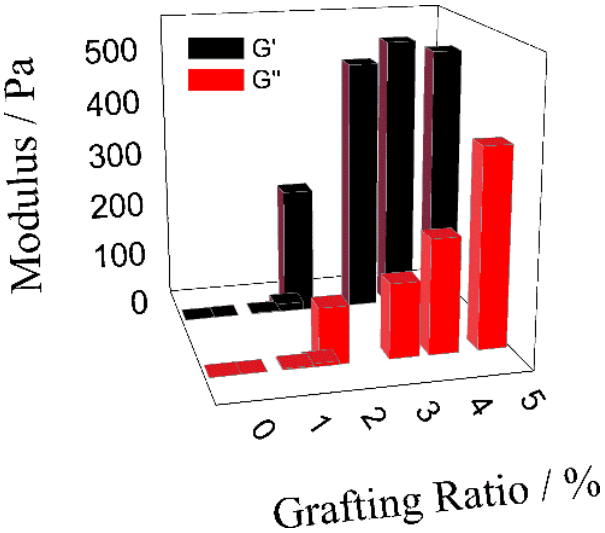



Figure S2. Histogram of the moduli of PAA-CD at different grafting ratios at concentration of 8wt%, with black block for storage modulus (G') and red for loss modulus (G") at oscillation frequency 1.13 Hz.

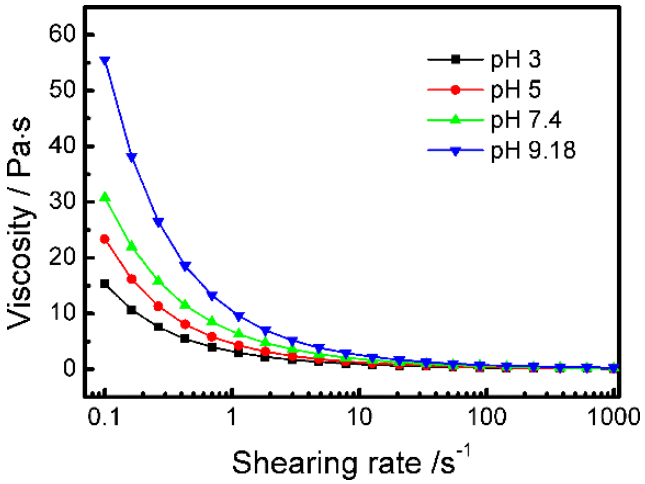



Figure S3. Viscosities of PAA-CD hydrogel (grafting ratio 2.9, concentration 8 wt%) at varying pH values and different shear rate.

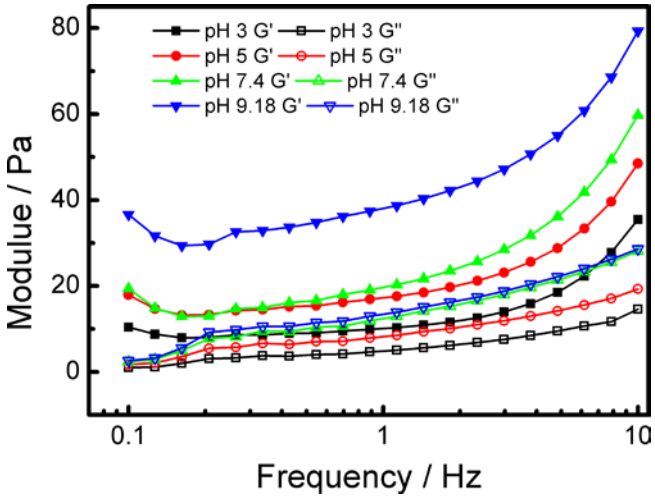



Figure S4. Storage and loss moduli of PAA-CD hydrogel (grafting ratio 2.9, concentration 8 wt%) at varying pH values and different shear rate.

## 5. Variable-temperature $^{1}$H-NMR spectra of PAA-CD

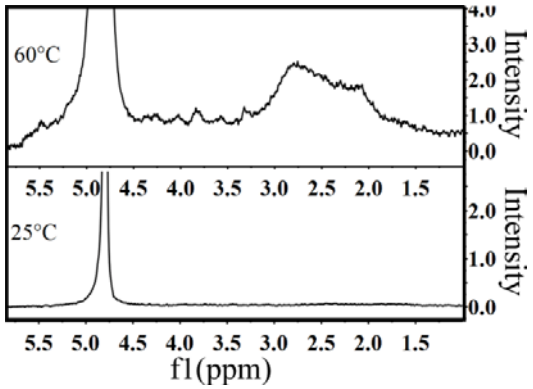



Figure S5. Variable-temperature $^{1}$H-NMR spectra of PAA-CD with a grafting ratio of 2.1%. The hydrogel displayed no signals at 25° C but presented characteristic PAA-CD signals at 60° C, because at 60° C hydrogen bonds were broken and PAA-CD polymers were freed for rotation to give an NMR signal.

## 6. Moduli of PAA-CD hydrogel with different amount of aminoazobenzene.

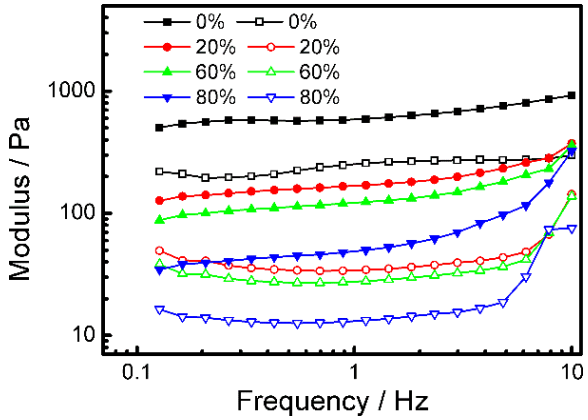



Figure S6. Storage modulus (solid ) and loss modulus (hollow) of PAA-CD hydrogel (concentration 8wt%, grafting ratio 5.3%) with different amount of aminoazobenzene loading.

## 7. Calculation of the binding constant between CDs

The concentration of unoccupied CDs in PAA-CD was derived according to the multivalence model proposed by Huskens and coworkers.[S1] The critical gelling point corresponded to the critical additive molarity of CD in multivalence. Since CD dimers were the basic building blocks of the physical cross-linkages in PAA-CD hydrogel. According to the equilibrium

$$CD+CD \rightleftharpoons CD_2 \quad (S1)$$

At the critical additive concentration, $K_a \cdot [CD]_c = 1$ ($K_a$, the binding constant between CDs; $[CD]_c$, the critical gelling concentration of CD). Thus $K_a$ equals $1/[CD]_c$

From gelling phase diagram in Figure 2a, the average of three CD concentration were determined as the critical gelling concentration: 1) grafting ratio 2.1%, concentration 7wt%, 2) grafting ratio 1.9%,concentration7wt% and 3) grafting ratio 1.9%, concentration 8wt%.

Table S1. Molecular weight employed in the calculation

| molecule | molecule weights(Da) |
|---|---|
| CD | 1135 |
| Acrylic acid(AA) | 72 |
| CD-AA | 1189 |

Table S2. "Effective molecular weight" per CD (the molecular weight of PAA was averaged into each CD moiety) in PAA-CD at grafting ratios 1.9% and 2.1%.

| grafting ratio | molecular weight per CD |
|---|---|
| 1.9% | 4906 |
| 2.1% | 4546 |

Table S3. Calculated $K_a$.

| Grafting ratio (%) | Concentration of PAA-CD (wt%) | concentration of CD (mol/L) | $K_a$ |
|---|---|---|---|
| 1.9 | 7 | 1.43E-02 | 69.9 |
| | 8 | 1.63E-02 | 61.3 |
| 2.1 | 7 | 1.54E-02 | 64.9 |
| **Average** | | **1.53E-02** | **65.4** |

## 8. Calculation of the equilibrium concentration of unoccupied CD moiety in PAA-CD matrices (polymer concentration 8 wt%, grafting ratio 3.5%).

I. When the concentration of 4-aminoazobenzene is zero.

According to equation S1:

$$Ka \cdot [CD]^2 = [CD_2] \quad (S2);$$

The total amount of CD moiety in PAA-CD hydrogel:

$$[CD]+2[CD_2] = 2.52\times10^{-2}\,M \quad (S3)$$

Combining (2) and (3):

$$[CD]=1.06\times10^{-2}\,M$$

II. When the concentration of trans-aminoazobenzene is $1.7\times10^{-3}$M (correspond to total

aminoazobenzene concentration $2.07\times10^{-3}$M, as in the diffusion experiment in ambient light).

$$CD + Azo \rightleftharpoons CD\cdot Azo \quad (S4)$$

Thus

$$K_1\cdot[CD][Azo] = [CD\cdot Azo] \quad (S5)$$

where $K_1$ is the binding constant between trans-aminoazobenzene and CD moiety in PAA-CD.

$$[CD]+2[CD_2]+[CD\cdot Azo]=2.52\times10^{-2}\ M \quad (S6)$$

According to S1, S5 and S6,

$[CD]=1.02\times10^{-2}$ M;

$[CD\cdot Azo]=1.5\times10^{-3}$ M;

$[Azo]= 1.5\times10^{-4}$ M.

From the above calculations, it's clear that during diffusion experiments, the concentrations of free CD moiety in the matrices were almost constant. Even at the hydrogel channel entrance, where [Azo] was the highest throughout the diffusion matrices, the concentration of free CD moiety was only slightly changed from $1.06\times10^{-2}$ M (when [Azo]=0) to $1.02\times10^{-2}$M (when $[Azo]_{total}$ $=2.07\times10^{-3}$M, [Trans-Azo]= $1.7\times10^{-3}$M). Thus [CD] as $1.06\times10^{-2}$ M was viewed as constant in diffusion experiments.

## 9. Mass transport consideration

As previously discussed, the bindings between 4-aminoazobenzene and CD are fast reversible and as a result, the coupled binding-diffusion is diffusion-limited. So, before a dissociated 4-aminoazobenzene could diffuse away from a CD cavity, re-association, if favored by probability, took place first. In other words, only already dissociated 4-aminoazobenzene was able to diffuse away from the CD cavity to which it was previously bound. Thus, the amount of 4-aminoazobenzene residing inside CD cavities was in equilibrium with the "free" molecules in diffusion matrices but remained outside the CD cavities. This phenomenon, in turn, indicated that thermodynamic equilibrium equations could be used to predict diffusion behaviors in this combined diffusion-association case.[S2]

## 10. The relationship between apparent diffusion coefficient and equilibrium concentration of unoccupied CD

Statistically, the fraction free time to bond time of a 4-aminoazobenzene molecule in the hydrogel matrices is expressed by the following equation: $(K_a\cdot[CD])^{-1}$ where $K_a$ is the association constant and [CD] is the equilibrium concentration of unoccupied cyclodextrin inthehydrogel. And a diffusive 4-aminoazobenzene can only diffuse in its free fraction ofthetime. Thus, the apparent diffusion coefficient of 4-aminoazobenzene in the matrices is $D_a = (D_o\cdot\text{free time fraction}) = D_o\cdot(K_a\cdot[CD]+1)^{-1}$

## 11. Numerical simulation:

### I. Worksheet simulation of the diffusion data

The diffusion under ambient light and 365 nm light throughout 0-40 min were simulated using Excel and compared with experimental data.

Table S4: Parameters employed for worksheet simulation

| Concentrations | | |
|---|---|---|
| $C_{CD}$ | concentration of free CD moiety in diffusion matrices [a] | $1.06\times10^{-2}$ M |
| $C_{AZO}$ | normalized concentration of AZO | 1 |

| | | |
|---|---|---|
| $C_{1\text{-trans-AZO}}$ (Ambient light) | normalizedconcentration of *trans*-AZO[b] | 0.8 |
| $C_{1\text{-cis-AZO}}$ (Ambient light) | normalizedconcentration of *cis*-AZO | 0.2 |
| $C_{2\text{-trans-AZO}}$ (365 nm light) | normalized concentration of *trans*-AZO [b] | 0.2 |
| $C_{2\text{-cis-AZO}}$ (365 nm light) | normalizedconcentration of *cis*-AZO | 0.8 |
| Binding constants | | |
| $K_1$ | Binding constant between trans-aminoazobenzene and CD [c] | 1000 $M^{-1}$ |
| $K_2$ | Binding constant between cis-aminoazobenzene and CD [c] | 200 $M^{-1}$ |
| Diffusion coefficient | | |
| $D_0$ | Regular diffusion coefficient [d] | $1.0\times10^{-10}$ $m^2/s$ |
| $D_1$ | Apparent diffusion constant of trans-aminoazobenzene [e] | $1.0\times10^{-11}$ $m^2/s$ |
| $D_2$ | Apparent diffusion constant of cis-aminoazobenzene [f] | $5.0\times10^{-11}$ $m^2/s$ |

[a] Calculated in section 7 in SI; [b] chosen according to previous report[S3,S4]; [c] determined using calorimetry and considerations in previous studies[S5,S6]; [d] the same order as most small molecules in hydrogel matrices[S7] and verified using fluorescence recovery after photobleaching employing FITC as the probe (data not shown); [e,f] calculated according to section 9 in SI.

In Excel, the built-in function (S7) was used in simulation as the expression of Fick's diffusion law. The total normalized intensity at a specific location and time was calculated as the sum of intensities contributed by both trans-aminoazobenzene and cis-aminoazobenzene.

$$n(x,t) = \mathrm{erfc}\left(\frac{x}{2\sqrt{Dt}}\right) \quad \text{(S7)}$$

**II. Finite element simulation.**

The diffusions for longer time span up to 90 h for a single type of diffusive molecule were simulated using finite element simulation.

Finite element simulation of the binding-diffusion process was simulated as slowed diffusions, using Comsol Multiphysics 5.2 with *Transport of Diluted Species* in Chemical *Species Transport* Module. The geometry of diffusion matrix was a rectangular block with length, width and height of 5 mm, 2 mm and 0.5 mm, respectively. The mesh was built in a sweeping manner with size "extremely fine". Transport properties in the rectangular block are isotropic and the diffusion coefficients $D_1$ and $D_2$ were used as listed in the parameter Table S4. One of the facets composed with cross-section dimensions 2 × 0.5 mm was set as the boundary with a constant concentration normalized to 1. All the other surfaces were set as *Flux* before the study carried out. The preset equation for diffusion was used as shown below:

$$\frac{\partial c_i}{\partial t} + \nabla \cdot (-D_i \nabla c_i) = 0 \quad \text{(S7)}$$

The time-dependent study was conducted from 0 to 90 h with an interval of 30s and concentration profiles were obtained from the calculated results.

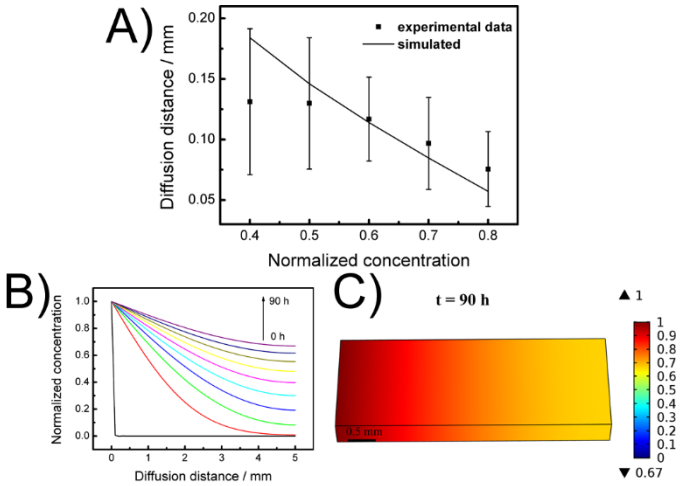



Figure S7. Simulated concentration distributions of aminoazobenzeneunder 365 nm light in PAA-CD hydrogel. (a) Experimental (solid square) and simulated dislocations during diffusions for each particular normalized concentration from 0.4 to 0.8. The disparities at normalized concentration 0.4 and 0.5 might result from discoloration caused by extended UV irradiation.Simulatedspatial-temporal concentration profiles (b) and concentration distributions at t=90 h (c).

References:


S1 Huskens, J. et al. A model for describing the thermodynamics of multivalent host-guest interactions at interfaces. *J. Am. Chem. Soc.***126,** 6784-6797 (2004).

S2 Perl, A. *et al.* Gradient-driven motion of multivalent ligand molecules along a surface functionalized with multiple receptors. *Nature Chem.* **3**, 317-322 (2011).

S3 Beharry, A. A. & Woolley, G. A. Azobenzene photoswitches for biomolecules. *Chem. Soc. Rev.* **40**, 4422-4437 (2011).

S4 Sierocki, P. *et al.* Photoisomerization of azobenzene derivatives in nanostructured silica. *J. Phys. Chem. B***110**, 24390-24398 (2006).

S5 Liu, Y., Zhao, Y. L., Chen, Y. & Guo, D. S. Assembly behavior of inclusion complexes of beta-cyclodextrin with 4-hydroxyazobenzene and 4-aminoazobenzene. *Org. Biomol. Chem.***3**, 584-591 (2005).

S6. Yamaguchi, H. *et al.* Photoswitchable gel assembly based on molecular recognition. *Nature Comm*. **3**, 603 (2012).

S7. Loren. N. *et al.* Fluorescence recovery after photobleaching in material and life sciences: putting theory into practice. *Q. Rev. Biophys.***48**, 323-387 (2015).