Electronic Supporting Information

Fabrication of Rhythmic Assembly System based on Reversible Formation of Dynamic Covalent Bond in a Chemical Oscillator**

Enxiang Liang,^{ab} Hongwei Zhou,^{ab} Xiaobin Ding,^{*a} Zhaohui Zheng^{*a} and Yuxing Peng^a

^aChengdu Institute of Organic Chemistry, University of Chinese Academy of Sciences, Chengdu 610041, P. R. China.

E-mail: xbding@cioc.ac.cn; zhzheng@cioc.ac.cn.

^bGraduate University of the Chinese Academy of Sciences, Beijing 100049, P. R. China

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1. Materials

4-Carboxybenzeneboronic acid was purchased from Aladdin Co., Ltd (Shanghai, China). N-(2-Hydroxy-ethyl) acrylamide and methacryloyl chloride (both stabilised with 4-methoxyphenol) were obtained from TCL (Shanghai, China). 2-Amino-2-methyl-1, 3-propanediol (AMPO), 4-(dimethylamino)pyridine (DMAP) and N, N'-dicyclohexyl carbodiimide (DCC) were purchased from Adamas Reagent Co., Ltd (Shanghai, China). N, N'-methylenebis(acrylamide) (BIS) was obtained from Sigma-Aldrich (Shanghai, China). 2, 2'-Azobisisobutyronitrile (AIBN) (Sigma, 98%) was recrystallised from ethanol. Acetonitrile was distilled over P₂O₅ after refluxing until becoming colourless. Triethylamine (Et₃N) was distilled over calcium hydride after refluxing for 12 h. The water used in this study was distilled and further purified using a filtration system. All other reagents were commercial chemicals and they were used without further purification unless otherwise noted.

2. Characterisations

¹H and ¹³C NMR spectra were recorded on a 300 MHz Bruker Avance-300 NMR spectrometer. A Bruker AvanceII-600 spectrometer was used to collect solid ¹¹B NMR data using BF₃·OEt₂ as an external standard (0 ppm). Chemical shifts were reported in parts per million (ppm) with reference to the residual protons of the deuterated solvents. Infrared spectra were recorded on a Nicolet MX-1E FTIR spectrophotometer (USA) over the scanning range of 4000–400 cm⁻¹ using the KBr pellet method. The molecular weights of the copolymers were measured on a Waters 515 Pump equipped with a Waters 2410 differential refractive index detector. The columns (7.5×300 mm PLgel Mixed-C) were calibrated with PEG standards. Dimethyl formamide (DMF) was used as an eluent with a flow rate of 0.6 mL/min at 50 °C. A PHSJ-3F pH meter (Shanghai REX, China) was used to record the pH-time curve using the REX DC1.0 data collection software package (Shanghai REX, China). A ZDJ-4A automatic titrator (Shanghai REX, China) was used to determine the pKa value by potentiometric acid-base titration. The reactor for the pH oscillating system was fed by a Longer Dispensing Peristaltic Pump (BT100-1F, China). Stress-strain measurements for the assembled gel samples were analysed by a commercial test machine (Electronic Testing Machine, RGM-2001, China). Movies and pictures were obtained by a Canon IXUS 130 digital camera.

3. Synthesis of monomers and copolymers



Scheme S1 Synthesis of monomers and copolymers

3.1 Synthesis of (4-((2-acrylamidoethoxy)carbonyl)phenyl)boronic acid (M1)

N, N'-dicyclohexyl carbodiimide (DCC) (6.98 g, 33.5 mmol) in CH₂Cl₂ (50 mL) were added dropwise to a suspension of 4-carboxybenzeneboronic acid (5.01 g, 30.2 mmol), N-(2-hydroxyethyl)–acrylamide (4.18 g 36.3 mmol) and 4-(dimethylamino)pyridine (DMAP) (379 mg, 3.07 mmol) in CH₂Cl₂ (100 mL) over 3 h. After 24 h, the reaction solution was filtered and purified via flash chromatography (gradient 30:1 DCM:MeOH). A white solid was obtained (2.38 g; yield: 31.9 %). ¹H NMR (300 MHz, D₂O) δ 7.90 (d, *J* = 8.1 Hz, 2H), 7.82 (d, *J* = 8.2 Hz, 2H), 6.18 (dd, *J* = 17.0, 9.6 Hz, 1H), 6.08 (t, *J* = 8.5 Hz, 1H), 5.62 (dd, *J* = 9.7, 2.2 Hz, 1H), 4.28 (t, *J*=5.1 Hz, 2H), 3.51 (t, *J* = 5.0 Hz, 2H).¹³C NMR (75 MHz, DMSO-d₆) δ 165.90, 164.94, 134.17, 133.55, 131.57, 130.84, 128.33, 125.41, 63.49, 37.77.

3.2 2-Acryloylamino-2-methyl-propan-1, 3-diol (M2)

M2 was prepared according to a published method.¹ Methacryloyl chloride (2.4 ml, 20 mmol) was added in drops into a cold suspension of AMPO (2.10 g, 20 mmol) and triethylamine (8.4 mL, 60 mmol) in acetonitrile (200 mL) under nitrogen atmosphere. The reaction was allowed to proceed under stirring at 0 °C for 6 h. Subsequently, the suspension was heated to 60 °C and filtered while hot to remove the insoluble hydrochloride salt. The filtrate was concentrated to 20 mL, followed by being stored at room temperature (RT). Finally, the white crystal was formed and isolated by filtration (2.70 g; yield: 77.9%). ¹H NMR (300 MHz, DMSO-d₆) δ 6.82 (s, 1H), 5.58 (s, 1H), 5.30 (s, 1H), 4.81 (t, *J* = 5.9 Hz, 2H), 3.53 (dd, *J* = 10.8, 5.8 Hz, 2H), 3.43 (dd, *J* = 10.8, 6.0 Hz, 2H), 1.83 (s, 3H), 1.18 (s, 3H).¹³C NMR (75 MHz, DMSO-d₆) δ 168.02, 140.73, 118.81, 63.74, 58.34, 18.71, 18.32.

3.3 Water-soluble PBA-functionalised copolymer (P1)

The gelator (P1) bearing phenylboronic acid (PBA) moieties was prepared by the radical copolymerisation with acrylamide (AAm) and M1 in DMSO using AIBN as the initiator. The typical procedure can be described as follows: predetermined amounts of AAm (0.4 g, 5.63 mmol) and M1 (0.04 g, 0.152 mmol) were dissolved in DMSO. The solution was stirred for 30 min and degassed by bubbling nitrogen for 15 min. Then, 2 mg of AIBN was added immediately. The resulting solution was sealed in a cuvette with a magnetic stir bar and placed in a preheated oil bath (65 °C). The polymerisation continued for 24 h under vigorous stirring. The obtained viscous solution was exposed to air and cooled to RT. The reaction mixture was poured into excess cold acetone to yield a white precipitate, which was collected by filtration and washed with acetone, followed by drying under vacuum at RT for 24 h. Finally, P1 was obtained as a white solid. ¹H NMR (300 MHz, D₂O) δ 8.02 (s, 1H), 7.86 (s, 1H), 4.46 (s, 1H), 3.64 (s, 1H), 2.22 (s, 156H), 1.71 (d, 40H).

3.4 Water-soluble diol-functionalised copolymer (P2)

The gelator containing diol moiteies (P2) was prepared in the same manner as P1 in 3.3 except that M2 was used instead of M1. ¹H NMR (300 MHz, D₂O) δ 3.71 (t, 1H), 2.40–2.06 (m, 27H), 1.71 (d, 12H), 1.27 (d, J = 11.0 Hz, 1H), 1.16 (s, 1H). M_n = 3.77×10⁴, M_w = 2.69×10⁵.

3.5 General protection procedure for P1 (protected P1)

Prior to analysis by gel permeation chromatography (GPC), the PBA moieties of P1 were esterified with pinacol to prevent high adsorption of the P1 onto the GPC column materials. A typical

protection procedure was executed as follows: P1, pinacol and molecular sieves were placed in a Schlenk flask. Anhydrous DMF (10 mL) was added, and the mixture was stirred at 100 °C. After 16 h, the mixture was filtered, and the protected P1 was precipitated in cold acetone. Successful protection was confirmed via ¹H NMR spectroscopy due to the appearance of pinacol ester methyl protons of the protected P1 at δ = 1.23 ppm. ¹H NMR (300 MHz, D₂O) δ 7.96 (s, 1H), 7.84 – 7.66 (m, 1H), 4.50 – 4.35 (m, 2H), 3.64 (s, 2H), 2.24 (s, 147H), 1.67 (s, 32H), 1.23 (s, 12H). M_n=4.59×10⁴, M_w=2.78×10⁵.



Fig. S1 ¹H NMR spectrum of M1 in D_2O







Fig. S3 ¹³C NMR spectrum of M1 in DMSO











Fig. S8 GPC curves of the protected P1 and copolymer P2

4. Preparation of the gels

All of the gels were also prepared by radical polymerisation (Fig. 1a, Fig. S9). Predetermined amounts of AAm, BIS and M1 (or M2) were dissolved in DMSO (3 ml) (Table S1). The solution was purged with nitrogen for 15 min to remove oxygen, and then AIBN was added. The reaction mixture was transferred to a tetrafluoroethylene mould with a cavity (40 mm×6 mm×4 mm), and the mould was sealed immediately. Gelation was carried out in an oven at 70 °C for 24 h. The mould was then removed from the oven, and the bulk gel obtained from the mould was purified by washing with a large amount of DMSO to remove unreacted monomers and initiators. This was followed by soaking in water for hours, then repeatedly washing with water. Subsequently, the gels were cut into pieces measuring 3 or 4 mm in length with a knife. The gel pieces were then dyed with phenol red dye for visualisation. Above pH 8.2, phenol red turned a red color, but, it changed from red to light yellow below pH 6.8.



Fig. S9 Chemical structures of the (A) PBA(x)-co-diol(y)-gel (B) blank-gel prepared by radical polymerisation. The mol% of the BIS was set to 2.0 for all gels. The molar ratios of PBA moieties and diol moieties were denoted as *x* and *y*, respectively.

Table S1 Summary of the gel composition				
Sample	x (mol%)	y (mol%)		
Blank-gel	0	0		
PBA(2.5)-gel	2.5	0		
PBA(5)-gel	5	0		
Diol(2.5)-gel	0	2.5		
Diol(5)-gel	0	5		
PBA(2.5)-co-diol(2.5)-gel	2.5	2.5		

x, y indicate the molar percent of PBA and diol moieties, respectively

5. Determination of pKa value

The pH profiles of M1 and P1 were determined by potentiometric acid–base titration. In 150 mM of NaCl aqueous solution (10 ml) saturated with nitrogen gas, 0.25 mmol M1 and P1 were dissolved. The pH of all solutions were set to 2 with 1 M HCl prior to titration. Subsequently, potentiometric titration was carried out with 0.1 M NaOH using an automatic titrator. pKa values were obtained according to the plateau values of the titration curves (Fig. S10). The plateau values indicated that the pKa values of M1 and P1 were 7.8 and 8.2, respectively. The slightly larger pKa for P1 relative to that of M1 was likely due to the increased resistance of P1 to ionisation.²



Fig. S10 pH profiles of acid–base titration of M1 and P1 with 0.1 M NaOH.

6. Construction of an HPD pH oscillator

The pH-oscillating system used in our studies was based on the sulfur oscillatory mechanism developed by Ra'bai et al. under a continuously stirred tank reactor (CSTR).³ Sodium dithionite (7.5 mM) and hydrogen peroxide (23 mM) solutions were prepared separately using deionised water in 250 mL volumetric flasks. Throughout the experiments, the solution of sodium dithionite in its reservoir was bubbled' with nitrogen gas to prevent autooxidation. Therefore, the stock solution of sodium dithionite could be stabilised for at least 12 h. The experimental setup for the pH oscillator was shown in Scheme 1. The oscillating reactions were carried out at 22 °C in a cylindrical glass flow reactor (20.0 ml) equipped with one outlet pipe and two inlet pipes. The reactor was fed through two input pipes by a peristaltic pump at a rate of 3.4 mL/min. The contents of the reactor were mixed with a magnetic stirrer at a constant stirring rate. Excess solution was continuously overflowing from the reactor via an outlet pipe of a larger internal diameter. The chemical state of the pH oscillator was monitored by a pH meter connected to a computer. The change in the pH value was recorded on a computer using the REX DC1.0 data collection software package. The obtained pH-time curve was continuous, with a large amplitude of 6 pH units ranging from 3.45 to 9.52 and a period of 5 min (Fig. S11).



Fig. S11 pH-time curve of an HPD pH oscillator in a CSTR

7. Mechanism of RA system in response to pH value

To understand the mechanism of the assembly/disassembly behaviour in response to pH, the interaction between P1 and P2 was selected as a model instead of those between the PBA-gel and diol-gel for convenience. The system was investigated by means of the sol-gel transition. P1 and P2 dissolved in distilled water (20 mg/mL) were mixed, and the pH of the mixture was adjusted to 8.5. Gelation occurred within 10 s, and the obtained gel was denoted as $Gel_{(P1+P2)}$. Subsequently, acid solution (1 ml) was added, and the gel completely decomposed into a sol in 15 s with a final apparent pH 4 (Fig. S12, Movie S1). Meanwhile, the interaction between P1 and M2 was also investigated. Predetermined amounts of P1 and M2 were dissolved in DMSO, then, the pH of the solution was adjusted to 8.5 and stirred for 24 h. The reaction mixture was poured into excess cold acetone to yield a white precipitate, which was collected by filtration and washed with acetone, followed by drying under vacuum at RT for 24 h. Finally, a white solid obtained was denoted as Polymer_(P1+M2). FTIR analysis and solid-state ¹¹B NMR spectra provided evidences of the formation of PBEBs between PBA moieties and diol moieties (Fig. S13 and Fig. S14).



Fig. S12 Sol-gel transition between P1 and P2



Fig. S13 FTIR spectra of P1 and the $\operatorname{Gel}_{(P1+P2)}$ after drying



Fig. S14 Solid-state ¹¹B NMR spectra of P1, the Gel_(P1+P2) and Polymer_(P1+M2) after drying

8. Control experiments in buffer solution

To further understand the assembly mechanism, control experiments in buffer solution were carried out. When the PBA(2.5)-gel and the diol(5)-gel pieces were mixed in a solution of pH 8.5, followed by stirring at an appropriate speed, the PBA(2.5)-gel adhered to the diol(5)-gel to immediately form a combined gel, which could be picked up with tweezers (Movie S3). Then, the assembled gel was transferred to the other solution (pH 4), and disassembly took place. What was interesting was that the separated gels were able to reassemble upon reintroduction into the first solution (pH 8.5). However, the pairs PBA-gel/PBA-gel, diol-gel/diol-gel, blank-gel/blank-gel, PBA-gel/blank-gel, and diol-gel/blank-gel did not assemble together. The results are shown in Table S2. They indicate that the formation and breakage of the PBEBs are responsible for the assembly/disassembly behaviour in the RA system.

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	Blank gel	PBA(2.5)-gel	Diol(5.0)-gel	PBA(2.5)-co-diol(2.5)-gel
Blank gel	Ν	Ν	Ν	Ν
PBA(2.5)-gel	Ν	Ν	Y	Y
Diol(5.0)-gel	Ν	Y	Ν	Y
PBA(2.5)-co-diol(2.5)-gel	Ν	Y	Y	Y

Table S2 Control experiment in buffer solution

Y: assembly; N: can not assembly

9. Semi-quantitative estimation of the assembly dynamics

The tips of the PBA(x)-gel and diol(x)-gel samples ($35 \text{ mm} \times 6 \text{ mm} \times 4 \text{ mm}$) were in contact with each other at pH 8.5. After different adhesion times, stress-strain measurements were carried out using an electronic testing machine at the same strain rate (0.5 mm/s) at RT. Conversely, during the disassembly process, the assembled gels were immersed in buffer solution (pH=4). The stress-strain changes were measured over the same intervals.

10. References

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