SUPPORTING INFORMATION

Smart Hydrogels Containing Adenylate Kinase: Translating Substrate Recognition into Macroscopic Motion

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Materials

All reagents were purchased from Sigma-Aldrich unless otherwise specified.

Mutagenesis, Expression and Purification of Triple-Mutant Adenylate Kinase (AKtm)

Starting from the (C77S, A55C)-AKe plasmid (a kind gift of Dr. E. Haas), the (C77S, V169C, A55C)-AKe (AKtm) triple mutant was prepared using the Quick Change® II site-directed mutagenesis kit (Strategene). AKtm construct was then transformed to HB101 competent cells (Zymo Research) and grown in 420 mL LB medium containing100 mg/L ampicillin. The bacteria were harvested after 16 h shaking at 37 ^{o}C and centrifuged at 6000 rpm for 10 min. The bacteria pellet was suspended in 10 mL of 50 mM Tris-HCl, pH 7.5, 0.1 M NaCl buffer and disrupted by sonication followed by centrifugation at 15000 rpm for 60 min. The supernatant was applied on a Hitrap™ Blue HP affinity column (GE Healthcare). The column was washed by equilibrium buffer (50 mM Tris-HCl, 1 mM *tris*(2-carboxyethyl)phosphine (TCEP), 0.2% NaN_3, pH 7.5) and proteins were eluted with a linear gradient of KCl (0-1 M) in 20 mL of the buffer. Fractions containing AKtm (verified by SDS-PAGE for absence of contamination with other proteins) were concentrated by membrane centrifugation cell (Amicon) equipped with YM-3 ultrafiltration membrane (Millipore, Amicon) and further purified by size-exclusion chromatography on a HiLoad™ 16/60 Superdex™ 75 Prep grade column (Pharmacia). The fractions eluted by 50 mM Tris-HCl, 1 mM TCEP, 0.2% NaN_3, pH 7.5 were collected, concentrated, desalted (PD-10 column, GE Healthcare), and lyophilized. The identity of AKtm was ascertained by electro-spray ionization (ESI) mass spectrometry on Quattro II Triple Quadrupole Mass Spectrometer (Micromass) (Figure S1). The yield of enzyme was about 10-15 mg/L of LB medium.

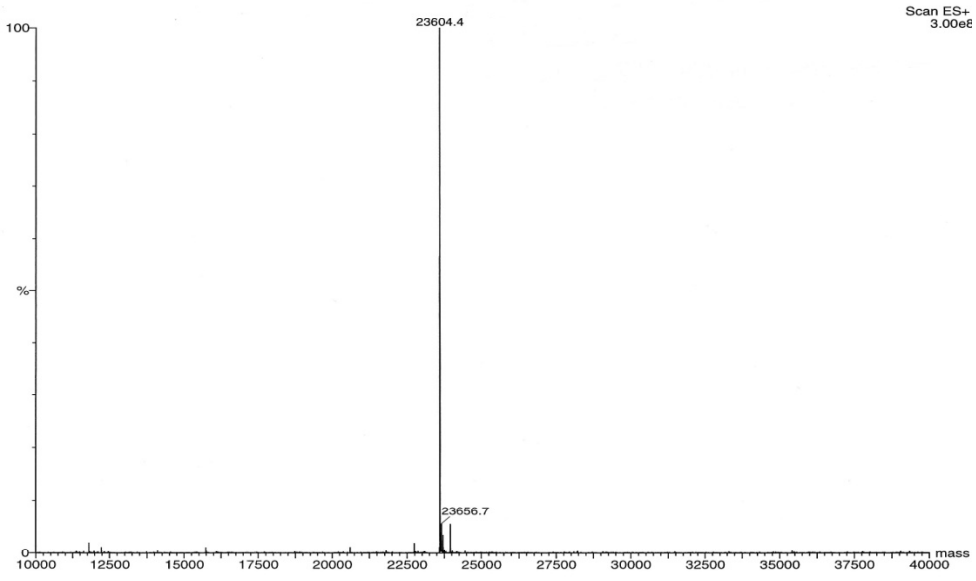


Figure S1. ESI mass spectrum of the triple mutant adenylate kinase (AKtm) purified by FPLC. The mass of AKtm was calculated to be 23606 Da and the experimental peak was observed at 23604 Da.

Labeling of AKtm by Alexa Fluor® 488 TFP Ester

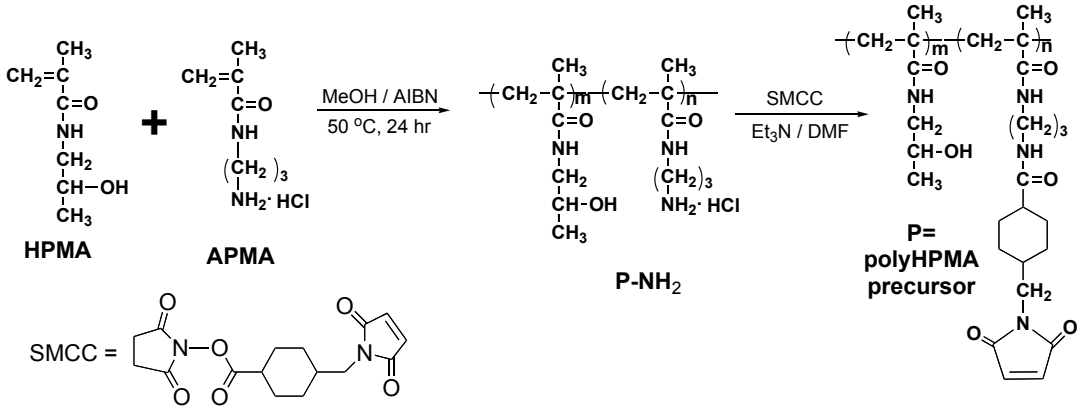
To a degassed solution of AKtm in MES buffer (0.5 mL, 2.36 mg/mL), 50 μL of 1 M sodium bicarbonate was added to adjust the pH of the reaction mixture. The solution was then transferred to a vial which contained 0.33 mg Alexa Fluor® 488 TFP ester (Invitrogen). The reaction mixture was stirred at room temperature for 1 h and unreacted Alexa Fluor® 488 TFP ester was removed on a PD-10 column with deionized water as the elutant. The macromolecular fraction was collected and freeze-dried.

Enzymatic Activity of Adenylate Kinase

The enzymatic activities of AKe and AKtm were measured at 37°C using a spectrophotometric assay [1] which couples the production of ATP from ADP to the production of NADPH, by first phosphorylating glucose using hexokinase, and then oxidizing glucose-6-phosphate using glucose-6-phosphate dehydrogenase and $NADP^+$. The final concentrations of reactants were: 2 mM ADP, 58 mM glycylglycine, 2.3 mM β-$NADP^+$, 10 mM $MgCl_2$, 10 mM glucose, 23 nM hexokinase, and 21 nM of glucose-6-phosphate dehydrogenase. The reaction was triggered by the addition of wild-type or mutated adenylate kinase (final concentration 44 nM). The formation of β-NADPH was recorded by continuous increase of absorption at 340 nm using a Cary 400 Bio UV-Visible Spectrophotometer (Varian). The enzymatic activity of AKtm was 92.6±1.9% of wild-type adenylate kinase (Sigma) as determined by comparison of initial slopes of β-NADPH production.

Synthesis of *N*-(2-Hydroxypropyl)methacrylamide (HPMA) Copolymer with Pendant Maleimide Groups (P)

Scheme S1



Radical copolymerization of HPMA with *N*-(3-aminopropyl)methacrylamide hydrochloride (APMA) was performed in methanol using 2,2'-azobisisobutyronitrile as the initiator (Scheme S1) [2]. The linear HPMA copolymer with amine side groups (P-NH_2) was reacted with succinimidyl *trans*-4-(maleimidylmethyl) cyclohexane-1-carboxylate (SMCC) resulting in HPMA copolymer with side chains terminated in maleimido groups (**P**). Two copolymers with different molecular weight and side chain content were used. A typical procedure is briefly described. In a dry and clean vial, 200 mg P-NH_2 (534 nmol NH_2/mg polymer determined by ninhydrin assay) was dissolved in

1.5 mL dimethylformamide (DMF). SMCC solution (0.5 mL) in DMF (54 mg, 0.162 mmol) and 44 μL triethylamine (0.321 mmol) were added sequentially into the vial. The reaction mixture was stirred at room temperature. Reversed-phase HPLC (Agilent Technologies, 1100 series) with Zorbax C8 column 4.6×150 mm and gradient elution (2 to 60% acetonitrile containing 0.1% TFA in 30 min) was used to monitor the reaction. The peak of P-NH_2 (elution time 12.53 min; Figure S2) disappeared after 3 h and a new peak emerged (24.35 min). The product was precipitated into a large excess of acetone/ether (3:2), filtered, re-dissolved in methanol, and precipitated into acetone again. Excess SMCC was washed out during this process and its absence was confirmed (no peak at 21.79 min). The product (**P2**) was filtered and dried under vacuum. The maleimide content was measured using modified Ellman's assay (Table S1; **P2**).

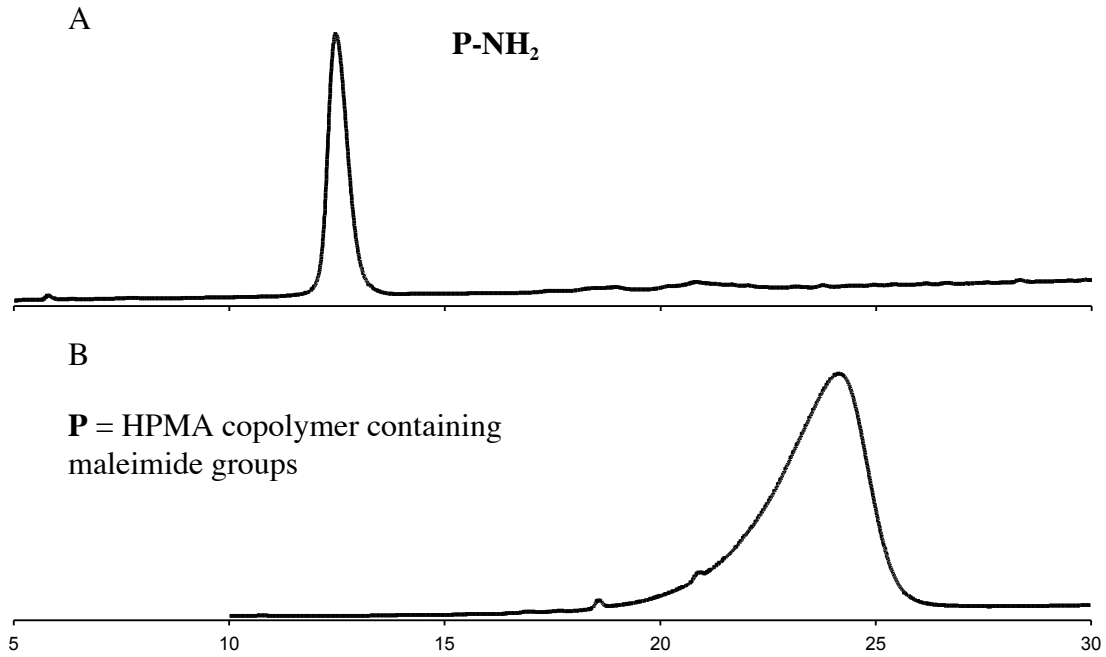
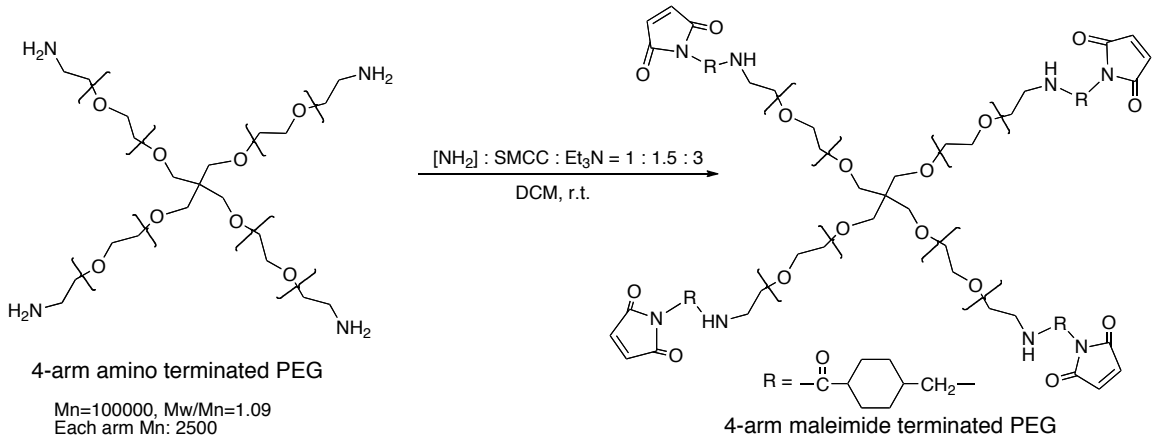


Figure S2. RP-HPLC chromatograms of HPMA copolymers containing amine side groups (A) and maleimide groups (B).

Synthesis of 4-Arm PEG Terminated in Maleimide Groups (PEG-Mal)

A similar procedure (as with HPMA copolymers) was followed to prepare maleimide terminated 4-arm PEG (Scheme S2). Briefly, 100 mg PEG-4NH_2 (Polymer Source, Canada) was dissolved in 1 mL dichloromethane (DCM). SMCC solution (0.5 mL) in DCM (21 mg, 0.06 mmol) and 20 μL triethylamine (0.12 mmol) were added sequentially into the vial. The reaction mixture was stirred at room temperature. Reversed-phase HPLC (Agilent Technology, 1100) was used to monitor the occurrence of the product, whereas Kaiser test was used to test the existence of NH_2 groups. When Kaiser test gave a negative result, the product was precipitated into a large excess of acetone/ether (3:2), filtered and dried under vacuum. The maleimide content was measured using modified Ellman's assay (Table S1).

Scheme S2



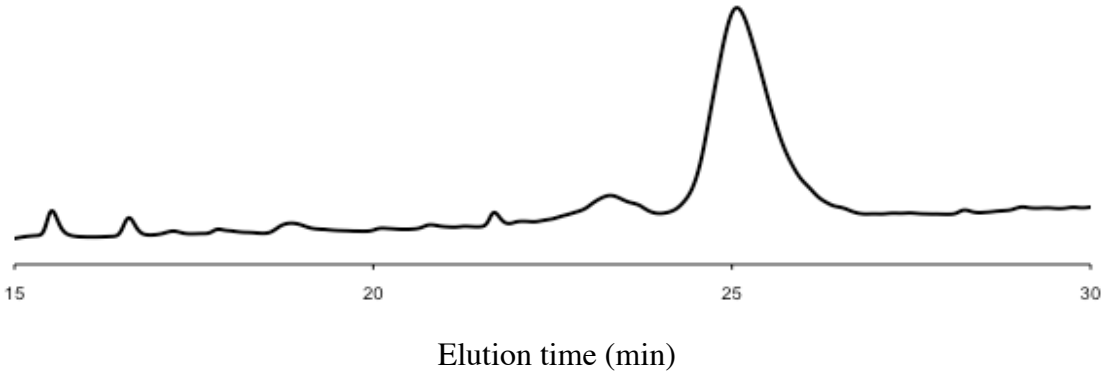


Figure S3. RP-HPLC chromatograms of 4-arm PEG terminated in maleimide groups

Preparation of Hybrid Hydrogels and Control Gels (Table S1)

Hybrid hydrogels were made in a mold by crosslinking of HPMA copolymer **P** or 4-arm PEG-Mal with AKtm or with AKtm and additional crosslinking agent - dithiothreitol (DTT). Control gels were prepared by crosslinking with DTT only. The synthesis of Gel **5** is described as an example: AKtm (2.9 mg, 124 nmol) was dissolved in 8 µL of N_2 purged deionized H_2O, then transferred to a mold with a diameter of 4.0±0.1 mm. A solution of 2 mg **P** in 8 µL H_2O (822 nmol maleimide) was added to the protein solution (molar ratio of maleimide to thiol groups = 3.3:1) and the mixture homogenized by pippeting up and down. Gelation occurred in minutes. The hydrogels were retrieved after 8 h reaction (Figure S4). The composition of hydrogels used in this study is listed in Table S1.

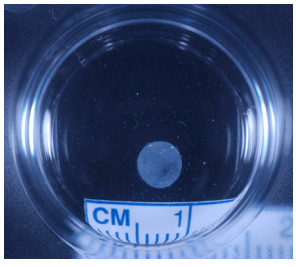


Figure S4. HPMA-based hybrid hydrogel **5** containing 100% AKtm incubated in ATP-free buffer.

Table S1. Composition of Hybrid Hydrogels

Sample	Polymer backbone				Crosslinker	AKtm %
	Polymer	Mn	PDI	[Mal] % (mol)		
1	**P 1**	27 kD	1.33	3.0	AKtm	100
2	**P 1**	27 kD	1.33	3.0	AKtm+DTT	50
3	**P 1**	27 kD	1.33	3.0	AKtm+DTT	25
4	**P 1**	27 kD	1.33	3.0	DTT	0
5	**P 2**	43 kD	1.92	7.9	AKtm	100
6	**P 2**	43 kD	1.92	7.9	AKtm*	100
7	**PEG**	10 kD	1.09	3.0	AKtm+DTT	50
8	**PEG**	10 kD	1.09	3.0	DTT	0

*AKtm labeled with Alexa Fluor® 488 was used

Measurements of Equilibrium Swelling Ratios

The hydrogels were equilibrated in deionized H_2O at room temperature for at least 24 h. Then the hydrogels were removed from the water and blotted with filter paper to remove surface water of the hydrogels. The masses of the swollen hydrogels were measured gravimetrically. The hydrogels were dried under the vacuum. The masses of dried hydrogels were measured at different time points until the last three measurements had no significant difference. The equilibrium swelling ratios were calculated according to the equation:

$$\text{Equilibrium Swelling Ratio} = \frac{m_s - m_d}{m_d}$$

where m_s and m_d are the average of three measurements of masses of the swollen and dried hydrogels, respectively. The equilibrium swelling ratios of the hydrogels are listed in Table S2.

Table S2. Equilibrium Swelling Ratios of Hydrogels*

AKtm%	HPMA copolymer-based				PEG-based	
	0**	25***	50	100	0	50
Equilibrium Swelling Ratio	26.9	24.5	16.7	12.7	22.9	18.7

* The error of the determination was ± 10%.

** 0% AKtm +100% DTT as crosslinkers

*** 25% AKtm + 75% DTT as crosslinkers

Hydrogel Response in the Presence of Substrate

Hydrogels were placed in a 12-well polystyrene tissue culture plate and equilibrated in 1 mL of ATP-free buffer (50 mM Tris-HCl, 50 mM $MgCl_2$, pH 7.5) for at least 1 h. Gels were examined under an optical microscope (Nikon Eclipse E800) before and after addition of ATP (substrate) containing buffer. Images of gels were captured by a CCD camera (MTI) at different time points. Assuming isotropic swelling, the V/V_0 *(%)* was calculated as $(L/L_0)^3$, where L is the one-dimensional size of the hydrogel in buffer containing substrate and L_0 is the size of the original hydrogel equilibrated in ATP-free buffer. L and L_0 were measured using the Image-Pro® Plus software (Media Cybernetics).

Re-swelling of Hydrogels during the Washing Step

The rate of re-swelling of hydrogels when the ATP-containing buffer was replaced with ATP-free buffer was evaluated. As an example, the repeated re-swelling of Gel **5** is shown in Figure S5.

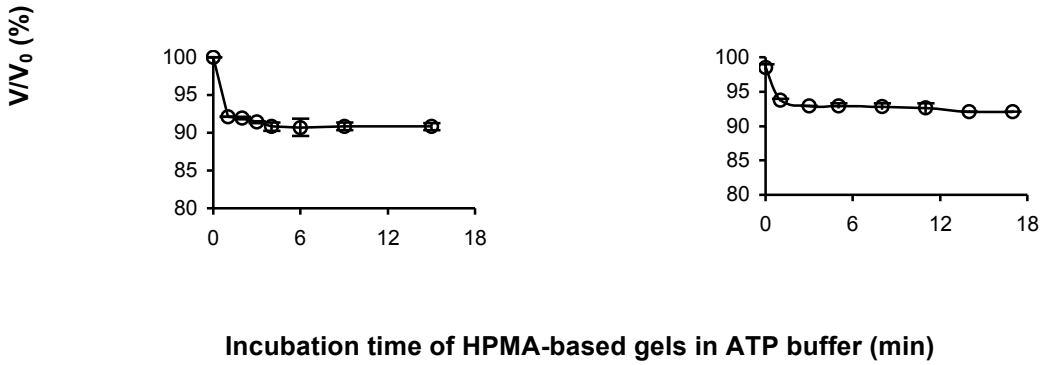


Figure S5. Repeated exposure of Gel **5** to ATP-containing buffer and ATP-free buffer.

References

[1] Bergmeyer, H. U. *Methods in Enzymatic Analysis*, 2nd edition; Academic Press: New York, 1974; Vol. 2, p. 486.

[2] Yang, J.; Xu, C.; Kopečková, P.; Kopeček, J. *Macromol. Biosci.* **2006**, 6, 201.