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Electronic Supplementary Information

Tailoring uptake and release of ATP by dendritic glycopolymer/PNIPAAm hydrogel hybrids: First approaches towards multicompartment release systems

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Content of Electronic Supplementary Information

Materials, synthesis and methods

Used buffer solutions

10 mM phosphate buffer pH 2.0 + 154 mM NaCl (Figures 5, 7, 9, ESI-6)

0.245 g of H_3PO_4 were mixed with 0.288 g of NaH_2PO_4 and diluted up to 500 ml with MQ water. If it was required, 3.728 g of NaCl were added to the solution.

100 mM stock acetate buffer pH 5.4

0.44 ml of acetic acid were mixed with 3.460 g of sodium acetate and diluted up to 500 ml with MQ water.

10 mM acetate buffer pH 5.4 + 154 mM NaCl (Figures 5, 7, 9, ESI-6)

50 ml of 100 mM stock acetate buffer were dissolved with 450 ml of MQ water, and 3.728 g of NaCl were added to the solution.

10 mM PIPES buffer pH 7.4 + 154 mM NaCl (Figures 5, 7, 9, ESI-6)

1.512 g of PIPES free acid in approx. 450 ml of pure water were titrated with 0.1 M NaOH to pH 7.4. Volume was made up to 500 ml with MQ water, and 3.728 g of NaCl were added to the solution.

10 mM phosphate buffer pH 7.4 + 154 mM NaCl (Figure 1, 3)

0.2208 g of NaH_2PO_4 were mixed with 0.4473 g of Na_2HPO_4 and diluted up to 500 ml of MQ water. If it was required, 3.728 g of NaCl were added to the solution.

Synthesis of acrylamidophenylboronic acid (AAPBA)

AAPBA was synthesized according to the preparation previously described in literature.[13] In brief, 3-aminophenylboronic acid (1.3 g, 8.4 mmol) was dissolved in NaOH (16.9 ml, 2 M) and cooled in an ice bath. Acryloyl chloride (1.4 ml, 17.2 mmol) was added dropwise to the solution with intensive stirring for 15 min. Hydrochloric acid (1 M) was added dropwise to adjust the pH to ca. 1. The pH value was controlled with a pH meter. The white-yellow precipitate of the product was filtrated on a glass filter (Schott, Duran, No. 4) and then washed on the filter with cold water. The resulting brown to light brown precipitate was recrystallized by dissolving the crude product in ca. 35 ml water on heating to 90°C. The product was obtained as white-beige needles by crystallization of the solution overnight in the fridge. The crystals were filtrated (Schott, Duran, No. 4), washed with cold water and then dried under vacuum for 2 days. The yield of AAPBA was 0.7 g (35.8%).

Synthesis of fluorescein-modified PEI-Mal-B25 (fl-PEI-Mal-B25)

PEI-Mal-B25 (5 g; $1.4*10^{-4}$ mol) was dissolved in 500 ml of MQ water. A solution of fluorescein 5(6)-isothiocyanate (53.8 mg; $1.4*10^{-4}$ mol) in anhydrous dimethylsulfoxid (27 ml) was added to the PEI-Mal solution and the corresponding reaction solution was stirred overnight at room temperature under light protection. Product fl-PEI-Mal-B25 was separated by dialysis using 1k regenerated cellulose membrane against MQ water for three days under light protection followed up by a freeze drying step (ready for uptaking and releasing experiments).

Synthesis of DABITC-modified PEI-Mal-B25 (d-PEI-Mal-B25)

PEI-Mal-B25 (0.1 g; $2.8*10^{-6}$ mol) was dissolved in 100 ml of MQ water. A solution of 4-(4-isothiocyanatophenylazo)-N,N-dimethylaniline (282.36 g/mol; 1.6 mg; $5.6*10^{-6}$ mol) in

anhydrous dimethylsulfoxid (6 ml) was added to the PEI-Mal solution and the corresponding reaction solution was stirred overnight at room temperature under light protection. Product d-PEI-Mal-B25 was separated by dialysis using 5k regenerated cellulose membrane against MQ water for three days under light protection followed up by a freeze drying step ready for uptaking and releasing experiments.

Differential scanning thermal calorimetry analysis (DSC) for studying the LCST behaviour of hydrogels

Differential scanning calorimetry was utilised in order to detect the LCST of different hydrogels and PEI-Mal-B25@Hydrogel complexes. The measurements were carried out with a Setaram micro-DSC III heat conduction scanning microcalorimeter (Setaram, France).

The pure swollen hydrogels **µA**, **µAB3** and **µAB5** were investigated in order to check the impact of AAPBA content on the transition temperature. To study the influence of the hb polymer carrier materials (PEI-Mal-B25) on the LCST of **µA** or **µAB3**, respectively, the PEI-Mal-B25@hydrogel complexes were prepared in the same way as the samples for uptake measurements.

The water saturated samples were transferred to a 1 mL measurement batch vessel (Hastelloy C). Absorbed or released heat was recorded relative to the 1 mL reference vessel filled with pure water as blank sample. The vessels were allowed to stabilize at 20 °C for 60 min prior to the initiation of the scanning experiment over the temperature range of 20 °C to 80 °C. The heating rate was 0.5 °C min^{-1} for all experiments. Cooling of the vessel to the initial conditions and rescanning of the sample were done in order to check whether the polymer transitions were reversible. We focus the attention principally on the evaluation of the transition temperature, which is an intensive parameter, i.e. it does not depend on the actual content of hydrogel in the sample considered. All measurements were repeated at least once.

Scanning electron microscopy (SEM) of swollen hydrogel samples A and AB3

Swollen hydrogel samples were lyophilized and broken into pieces. The SEM images were taken on a Gemini microscope (Zeiss, Germany) at an accelerating voltage of 4kV. In order to increase the contrast and quality of images the samples were coated with a thin Au layer before the measurements were carried out.

Cryo scanning electron microscopy (cryo SEM) of swollen hydrogel samples μA

Suspension of mechanically crushed hydrogel **μA** was frozen by plunging it into liquid nitrogen and subsequently grounded with mortal and pestle. Using sputter-coater (Leica EM SCD500, Leica Microsystems GmbH, Germany) particles were covered with Pt layer (5 min, 20 mA). Leica EM VCT100 (Leica Microsystems GmbH, Germany) attached to a scanning electron microscope was used. Samples were imaged in a Neon 40EsB (Carl Zeiss NTS GmbH, Germany).

Determination of size and electrokinetic properties

Zetasizer Nano (Malvern Instruments/UK) was used for the determination of the hydrodynamic diameter of ATP@PEI-Mal complexes. The zetasizer was equipped with a 633 nm He/Ne laser and a non-invasive back scatter (NIBS®) technology. The zetasizer was also used for electrophoretic experiments to determine the electrophoretic velocity of the ATP@PEI-Mal complexes. The complex velocity in an electric field, using 120 V/cm, was measured by laser Doppler anemometry employing the He/Ne laser. The apparent electrokinetic potential (zeta-potential, ζ) values were calculated from the complex velocity according to the Smoluchowski equation.[14]

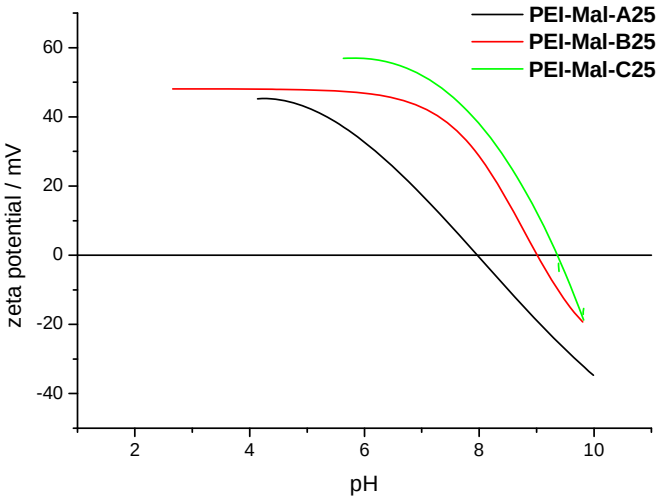

Figure S1. pH-dependency of zeta potential for PEI-Mal-A25, PEI-Mal-B25 and PEI-Mal-C25 (concentration of polymers 0.5 mg/ml).

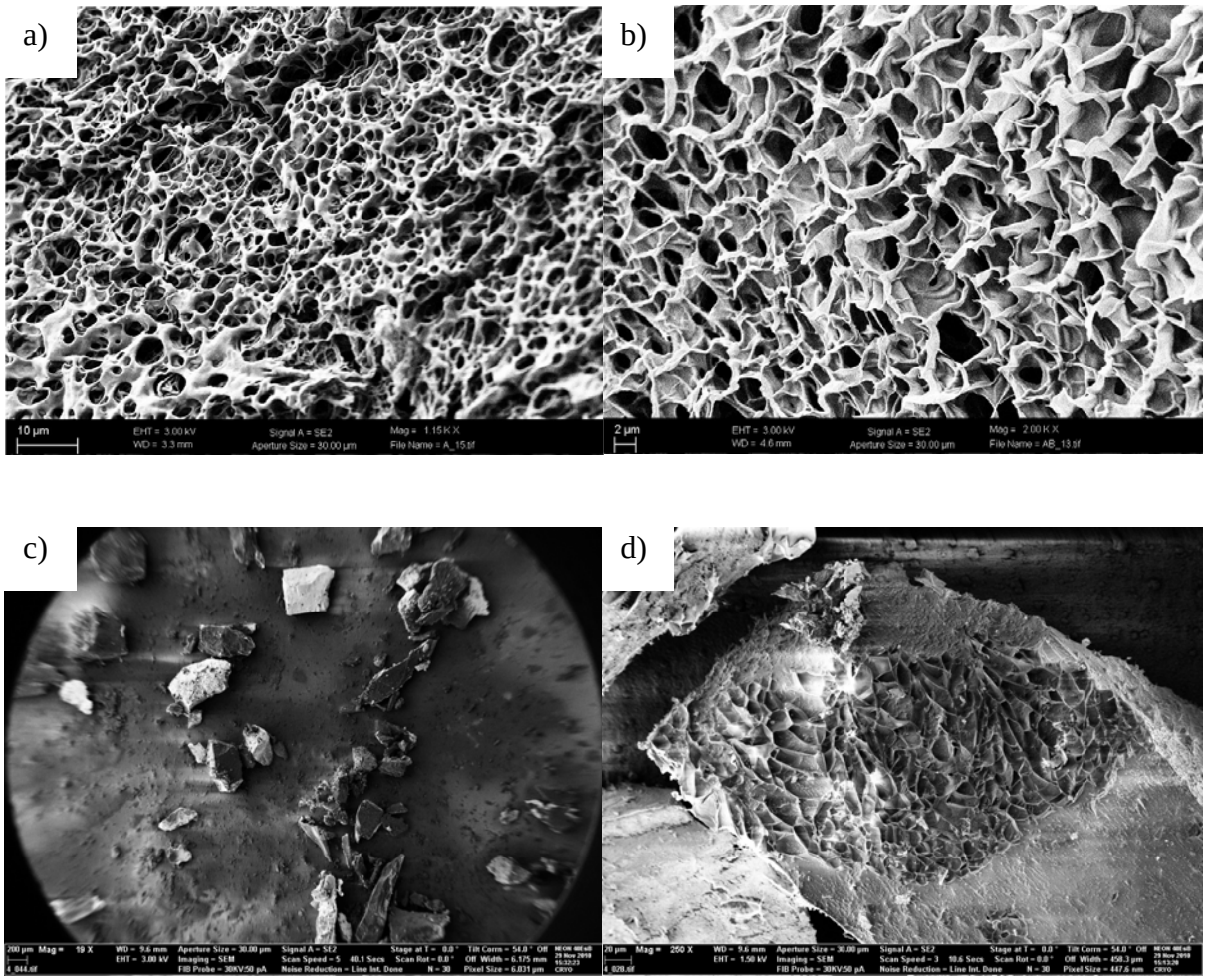

Figure S2. SEM micrographs of hydrogels **A** (a) and **AB3** (b) and cryo-SEM micrographs of mechanically crushed hydrogel **A** mentioned as **µA** in the paper (c, d).

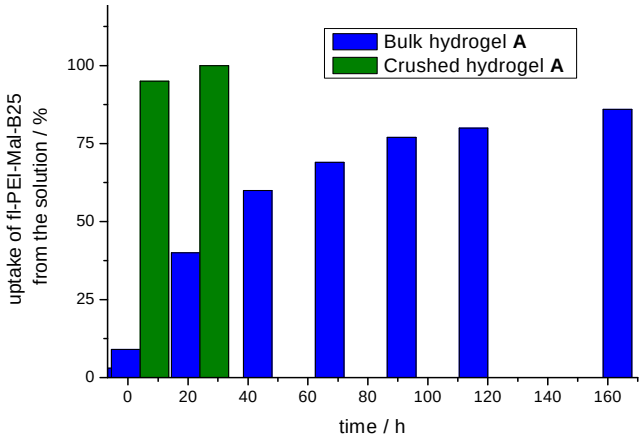

Figure S3. Comparison of uptake efficiency of fl-PEI-Mal-B25 by bulk hydrogel **A** and mechanically crushed hydrogel **A** as **μA**.

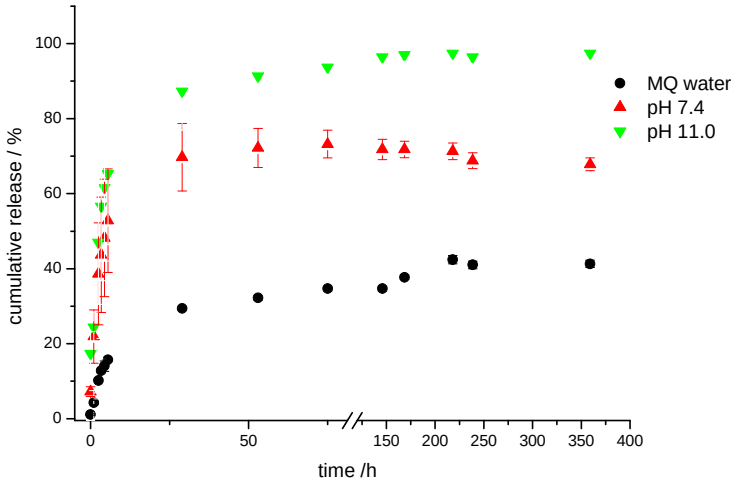

Figure S4. Release of PEI-Mal-B25 by hydrogel **A** in different media.

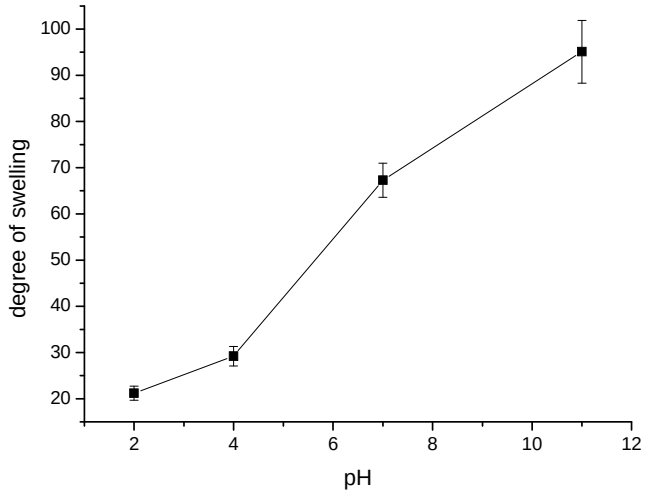

Figure S5. pH-dependent degree of swelling (DS) of hydrogel **A**.

With increasing pH hydrogel **A** shows an increase in the DS caused by the increasing negative charge of acid groups in the hydrogel **A** under basic conditions. In opposite to that, once mixing aqueous solution with defined amount NaCl or $CaCl_2$, then a dramatic reduction of DS for hydrogel **A** (**Table S4**) can be observed.

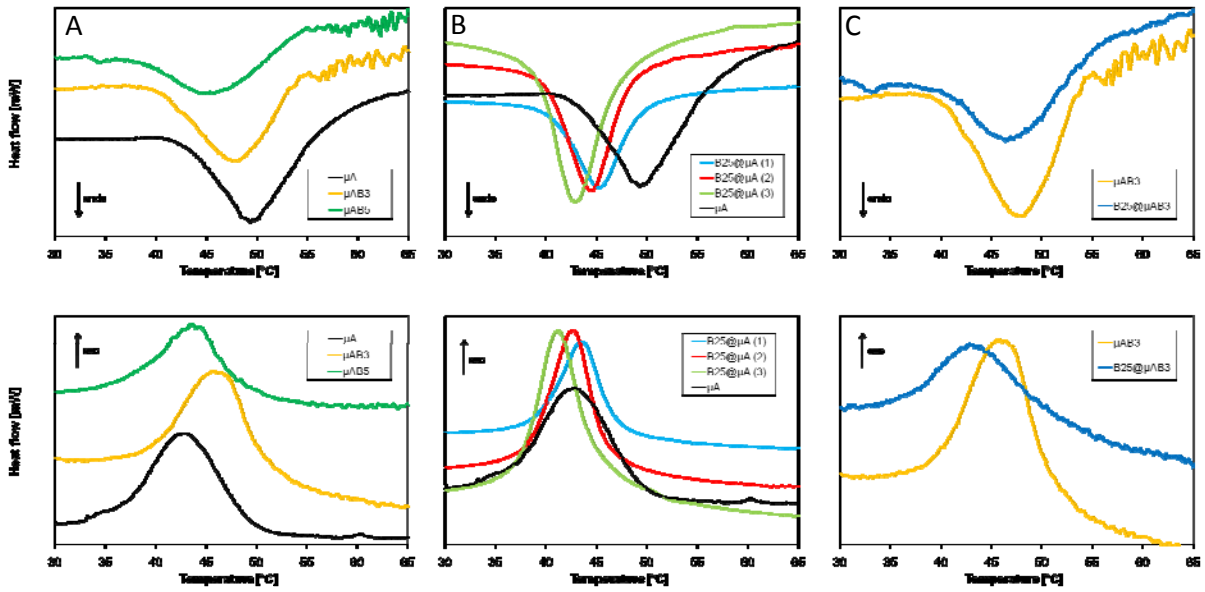

Figure S6. DSC heating (top) and cooling (bottom) curves for the swollen hydrogels **μA**, **μAB3** and **μAB5** (A), for PEI-Mal-B25@**μA** complexes of various composition (PEI-Mal-B25 concentration (1): 7.5, (2): 13.8, (3): 24.2 wt%, g/g shrunken hydrogel) in comparison to pure **μA** hydrogel (B) and for a PEI-Mal-B25@**μAB3** complex (PEI-Mal-B25 concentration: 42 wt%, g/g shrunken hydrogel) in comparison to pure **μAB3** hydrogel (C).

Generally, repeated heating (top) and cooling (bottom) cycles yielded reproducible peak shapes and temperatures. The LCST shown in Table 3 equals the transition midpoint temperature, $T_{max,\ heat}$, considered as the temperature at which the local extremum occurs in the excess heat capacity during heating. Additionally the temperature at the respective extremum from the DSC cooling curves was evaluated ($T_{max,\ cool}$). We observed the typical hysteresis between the calorimetric curves obtained during heating and during cooling (Wang et al. *Macromolecules*, 1998, **31**, 2972; Shpigelman et al. *Journal of Polymer Science: Part B: Polymer Physics*, 2008, **46**, 2307). It was less pronounced for hydrogels **μAB3** and **μAB5** than for **μA**. Incorporation of PEI-Mal-B25 also reduced the hysteresis for **μA**, but increased it for **μAB3** in comparison to the pure hydrogels.

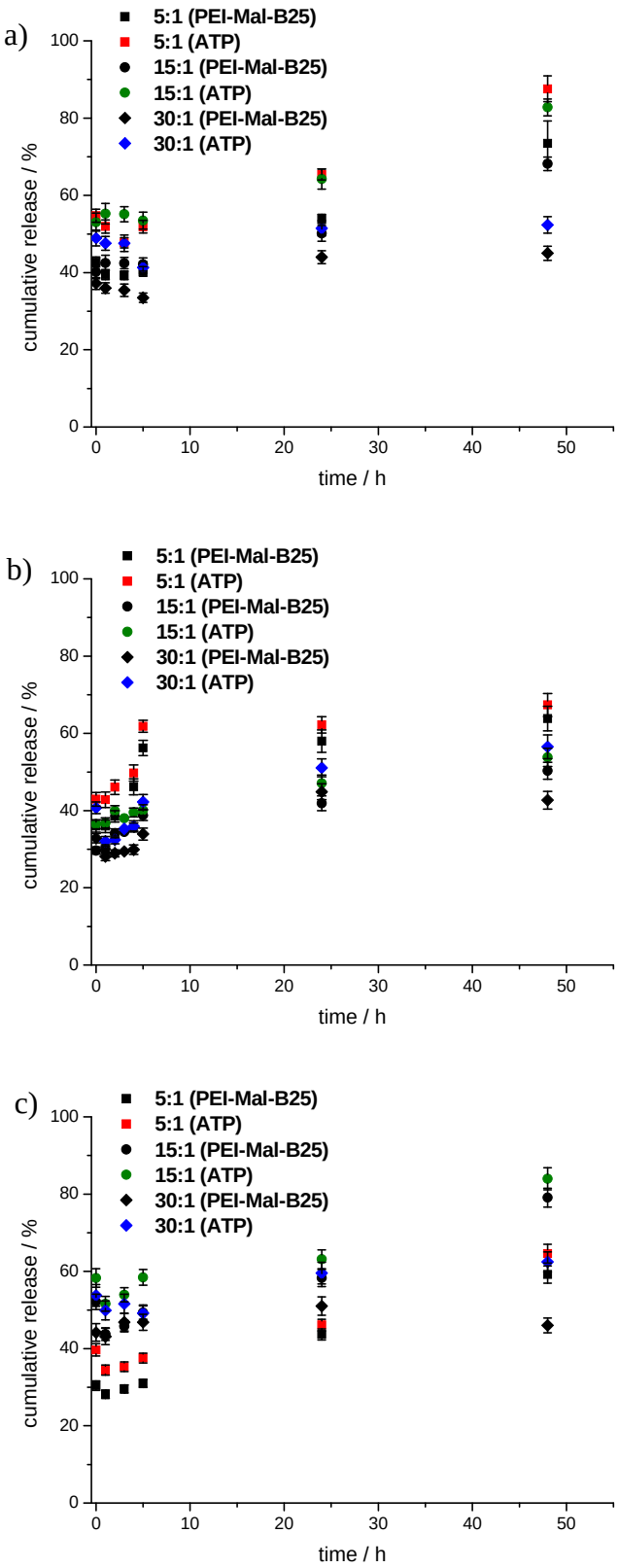

Figure S7. Release of ATP from ATP@PEI-Mal-B25@**μA** hydrogel multi-release system with different ratios between excess ATP and PEI-Mal-B25 (5:1, 15:1 and 30:1) at pH 2.0 (a), 5.4 (b) and 7.4 (c).

Table S1. Masses of ATP and PEI-Mal structures in various encapsulation experiments. For example, results for encapsulated ATP by PEI-Mal are presented in **Figure 3**.

Ratio ATP: PEI-Mal	ATP, ***mg***	PEI-Mal-A5**,** ***mg***	PEI-Mal-B5, ***mg***	PEI-Mal-A25, ***mg***	PEI-Mal-B25, ***mg***
1:1	4.6	-	-	-	300.0
7:1	88.0	-	300.0	-	-
8:1	35.7	-	-	-	300.0
18:1	69.5	-	-	-	250.0
21:1	45.0	-	-	295.5	-
30:1	305.4	-	250.0	-	-
32:1	135.0	-	-	-	271.8
32:1	187.5	300.0	-	-	-
36:1	75.0	-	-	282.0	-
64:1	198.4	-	-	-	200.0
121:1	244.0	-	50.0	-	-
128:1	625.0	250.0	-	-	-
128:1	148.8	-	-	-	75.0
146:1	319.3	-		300.0	-

Table S2. Masses of ATP@PEI-Mal-B25 complexes in defined volume (V) in various release experiments.

Ratio ATP: PEI-Mal-B25	ATP@PEI-Mal-B25 ***mg***	V solution ***ml***
15:1	57.1	250
45:1	46.5	500

Table S3. Compositions of the mixtures for the preparation of ATP@PEI-Mal-B25@Hydrogel complexes.

Ratio ATP: PEI-Mal-B25	0.5 mg/ml ATP water solution ***ml***	PEI-Mal-B25@Hydrogel complex ***mg***
5:1	5.0	100.0
15:1	8.0	69.6
30:1	23.25	56.8

Table S4. Degree of swelling of hydrogel **A** as a function of pH and salt concentration.

Solution	Degree of swelling
MQ water	67.3
MQ water adjusted with HCl to pH 2	21.2
MQ water adjusted with HCl to pH 4	29.2
MQ water adjusted with NaOH to pH 11	95.1
MQ water + 0.5 M $CaCl_2$	7.8
MQ water + 0.5 M NaCl	14.2

Table S5. Masses of adsorbed PEI-Mal-B25 into 1 g of shrunken hydrogel **A** after 120 hours and zeta potential values of PEI-Mal-B25 at the same pH.

	Mass of absorbed PEI-Mal-B25	Zeta potential of PEI-Mal-B25
	mg	***mV***
pH 5	364.7 ±45.8	47.78
pH 7	915.0 ±47.1	42.83
pH 9	1466.7 ±12.8	0.12
pH 11	6.1 ±5.6	Less then -19.3

Table S6. Masses of adsorbed PEI-Mal-B25 into 1 g of shrunken hydrogel **µA** and **µAB3** after 24 hours.

	PEI-Mal-B25	PEI-Mal-B25
	mg	***%***
µA	725 ±3.3	99.9 ±0.5
µAB3	724 ±4.1	99.8 ±0.6