Electronic Supplementary Information (ESI)

pH-Stable Hyperbranched Poly(ethyleneimine)-Maltose Films for the Interaction with Phosphate Containing Drugs

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Methods

Preparation of the pre-coating. Si wafers (with thermal oxide film of 30 nm thickness) were freshly cleaned in a mixture of aqueous solutions of ammonia (Acros Organics) and hydrogen peroxide (Merck, Darmstadt Germany). Thereafter the Si wafers, prior dried in N_2 stream, were surface-modified with 3-aminopropyl-dimethylethoxysilane (ABCR, Karlsruhe, Germany). Then, poly(ethene-*alt*-maleic anhydride) (PEMA) with M_w = 125 000 g/mol (Aldrich, Munich, Germany) was used to complete the formation of the pre-coating. For thin film preparation 0.15 wt% PEMA was dissolved in an acetone/ tetrahydrofuran (THF) mixture with ratio of 1:2. After spin coating process covalently attached PEMA on aminosilane film was achieved by annealing at 120 °C for 2 h in the drying oven. Final step to realize desired pre-coating with acid groups was to hydrolyze covalently attached PEMA on aminosilane film in MilliQ water overnight. Besides the conversion of the anhydride groups in PEMA into acid groups, traces of non-covalently attached PEMA was removed from the surface of the pre-coating. Thus, pre-coating, dried in N_2 stream, was directly used for preparing PEI-Mal films.

Stability experiments for PEI-Mal films. All PEI-Mal films, attached on Si wafers, were rinsed in MilliQ water for 2 h to remove non-attached PEI-Mal from the surface. After that the thin films were dried under N_2 stream prior to use them directly or to use them later after storage in closed atmosphere. The film thickness (***d***) and refractive index (***n***) of each dry film were calculated from ellipsometric data. Then, they were placed in buffer solution at different pH values (4, 6.5, 7.4 and 10) for 48 h and 96 h. After each deposition time the films were dried as described above and ***d*** and ***n*** were determined again. The results are shown in Figure 1, Figure S2 and Table S2.

Interaction study with phosphate containing drugs by spectroscopic ellipsometry. For interaction experiments with swollen PEI-Mal films the same sample preparation was used as mentioned in swelling experiment in different solutions. After deposition and fixation of PEI-Mal film substrate in the quartz cell, first films were allowed to swell in MilliQ water. After reaching an equilibrium the aqueous drug solution (ATP, AMP, CMP, CTP, c= 10 mg/ml) was injected into the MilliQ water to reach an end concentration of 0.5 mg/ml (ATP, CTP, AMP and CMP) or 5 mg/ml (AMP and CMP) within the measurement cell (final pH values around 6.5). After that the dynamic scan of ellipsometric measurements was immediately started. After reaching the equilibrium of Δ and Ψ, the drug solution in the measurement cell was exchanged two times by MilliQ water to remove non interacting drugs

with swollen PEI-Mal films (This process is described as rinsing process in the paper and in the Figure 2 in the paper).

PEI-Mal films characterized by AFM. AFM measurement was carried out in a Dimension^{TM} 3100 Nanoscope IV (Digital Instruments Inc, Santa Barbara, CA). For the image editing and roughness determination (R_{ms}) of the polymer films, the WSXM software (software packages for scanning probe microscopy and a tool for nanotechnology: *Rev. Sci. Instrum.* 2007, **78**, 013705; doi:10.1063/1.2432410, published 31 January 2007) was used. The swelling behaviour of the films in water and other solutions was measured in "contact mode" after the establishment of the equilibrium of swelling.

Determination of ATP in PEI-Mal film by XPS. XPS analysis was performed with a AXIS Ultra spectrometer (Kratos Analytical, Manchester, United Kingdom) equipped with a monochromatic Al Kα X-ray source of 300W at 20 mA. The maximum information depth of the XPS method is not more than 8 nm. Further details of method and analysis of the data are described in Synytska et al. (*Macromolecules*, 2007, **40**, 297).

Charging of the PEI-Mal films. In order to study the charging of the PEI-Mal films in aqueous environment, the streaming current vs. pressure gradient was determined by applying the in-house developed Microslit Electrokinetic Setup (Zimmermann et al. *Microfluid Nanofluid*, 2006, **2**, 367). The streaming current was measured across a rectangular streaming channel formed by two parallel sample surfaces (length 20 mm, width 10 mm, separation distance 30 µm). The measurements were started at an alkaline pH-value. After each titration step the system was equilibrated for about 45 minutes. As electrolyte solution potassium chloride with a concentration of 1 mM was used.

In order to make the results of the electrokinetic measurements comparable with those obtained for other surfaces, apparent zeta potentials were calculated applying the Smoluchowski equation (Lyklema, J., *Fundamentals of Interface and Colloid Science*. Academic Press: London, 1995.). For electrokinetically ideal surfaces, i.e. surfaces without roughness, porosity, hairy layers or a patch-like distribution of chemical properties at the surface, the *zeta potential* is defined as the potential of an imaginary hydrodynamic shear plane separating an inner region where no fluid motion occurs and an outer region where fluid velocity takes non-zero values. However, in the case of soft surfaces, as for the PEI-Mal films, such hydrodynamic shear plane does not exist. Therefore, the quantity calculated by the Smoluchowski equation is called apparent zeta potential.

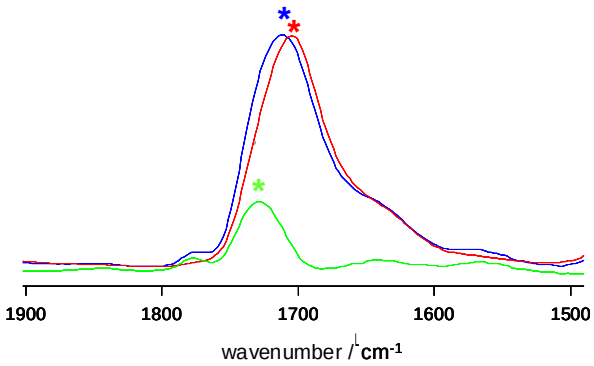


Figure S1. Model reaction between maltose and PEMA in thicker films to exhibit ester bond formation. ATR-IR spectra of mixture between maltose and PEMA as film on Si wafer. Molar ratio between maltose and PEMA is 2. Red IR spectrum belongs to mixture of maltose and PEMA which was dried at room temperature. Blue IR spectrum belongs to mixture of maltose and PEMA which was annealed at 120°C for 2h. Green IR spectrum belongs to the subtraction of red spectrum from blue spectrum.

Both spectra (blue and red) provides IR bands at about 1712 (blue) and 1704 (red) cm^{-1} assignable for acid groups in PEMA. Generally, IR band for acid group in blue spectrum is slightly shifted to higher wavenumbers after thermal treatment. Thus, no identical IR bands for acid groups can be stated implying that there is hidden one additional functional group. Subtraction of red spectra from blue spectrum reveal the appearance of a new IR band in the green IR spectrum. The IR band at 1729 cm^{-1} in the green IR spectrum can be assigned to the ester group which is formed during the thermal treatment.

One additional observation was obtained that the storage of the film without thermal treatment outlined no stability in water after 16h (red IR spectrum). In contrast to this, storage of thermally treated film (blue IR spectrum) in water revealed stable film after 16h. Thus one can conclude that chemical cross-linking took place and the formation of ester bonds is preferentially responsible for the stability of maltose-PEMA film in aqueous phase.

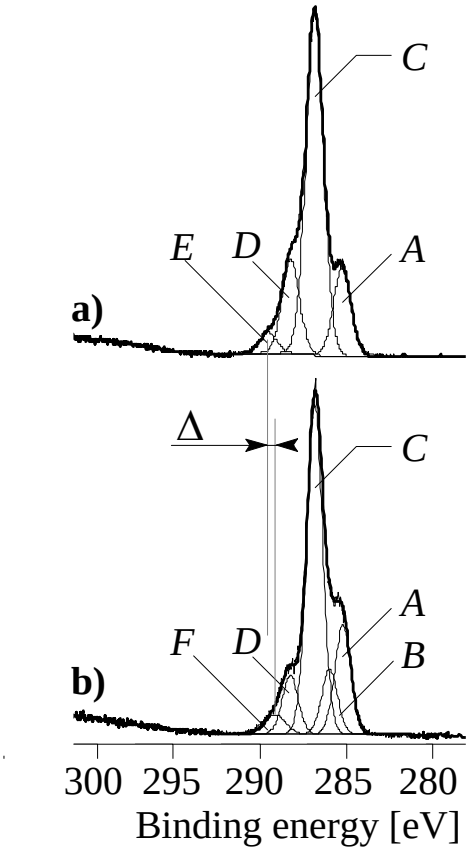


Figure S2. High-resolution C 1s X-ray photoelectron spectra of a maltose reference sample (a) and maltose reacted with PEI and subsequently cross-linked with hydrolyzed poly(ethylene-*alt*-maleic anhydride) (b). The difference between component peak *F* and component peak *E* (Δ) illustrates the presence of carbonic ester groups.

The shape of the two C 1s spectra is very typical for saccharide molecules (Figure S2a: maltose). The spectrum of the reference sample was deconvoluted into four component peaks (A, C, D and E). Beside the saccharide maltose, sample **B** also contains poly(ethylene imine), PEI (Figure S2b). Hence it was necessary to introduce an additional component peak *B* showing the C–N bond amino groups (Figure S2b). Component peak *A* shows saturated hydrocarbons resulting from surface contaminations typically observed in surface analysis (Figure S2: for both samples). Component peak *C* shows the C–OH groups of the maltose molecules and the C–O–C bridge in the cyclic form of the maltose. The corresponding cyclic hemiacetal group O–C–O is represented by component peak *D*. Aldehyde groups, which are present in the oxo-form of maltose, also contribute to component peak *D*. According to the stoichiometric ratio the relative intensity $[D]:[C]|_{stoich}$ should equal $D]:[C]|_{stöch}$ = 1:5. The reference sample showed a slight excess of the component peak *D* ($[D]:[C]|_{maltose}$ = 0.275)

(Figure S2a). Obviously, oxidized species are present on the maltose powder surface. Oxidation also produced carbonic acid groups on the maltose powder surface. They were analyzed as component peak *E* (Figure S2).

The shape C 1s spectrum of sample **B** (Figure S2: PEI-Mal cross-linked with PEMA) is similar to the C 1s spectrum recorded from unmodified maltose powder (Figure S2a). The origin of the additional component peak *B* was discussed above. Its intensity is approximately the double of the [N]:[C] ratio, which was determined from the wide-scan spectrum. Hence, it can be concluded that preferably secondary and tertiary amines contribute to component peak *B* (Figure S2b).

The intensity ratio [*D*]:[*C*] of the PEI/maltose/PEMA film was significantly decreased ($[D]:[C]|_{\text{PEI-maltose}} = 0.177$). Aldehyde groups of the oxo-form were reacted with the amino groups of PEI forming in the first step azomethine groups. The decrease of the relative intensity ratio [*D*]:[*C*] can be considered as proof of conversion between PEI and maltose.

Esterification reactions between the OH groups of the maltose molecules and hydrolyzed poly(ethylene-*alt*-maleic anhydride), PEMA, should form carbonic ester groups. In the C 1s spectrum carbonic ester groups were expected at binding energies of BE < 289 eV, while carbonic acids usually found at BE > 289 eV. In the C 1s spectrum of the PEI/maltose/PEMA film a component peak *F* arises at BE = 288.88 eV (Figure S2b). The full width at half maximum of this peak (FWHM = 1.334 eV) is significantly wider as the values observed for the other component peaks (FWHM = 1.051-1.067 eV). From the wideness of component peak *F* can be concluded that the component peak represents two kinds of functional groups, namely carbonic acid groups and ester groups.

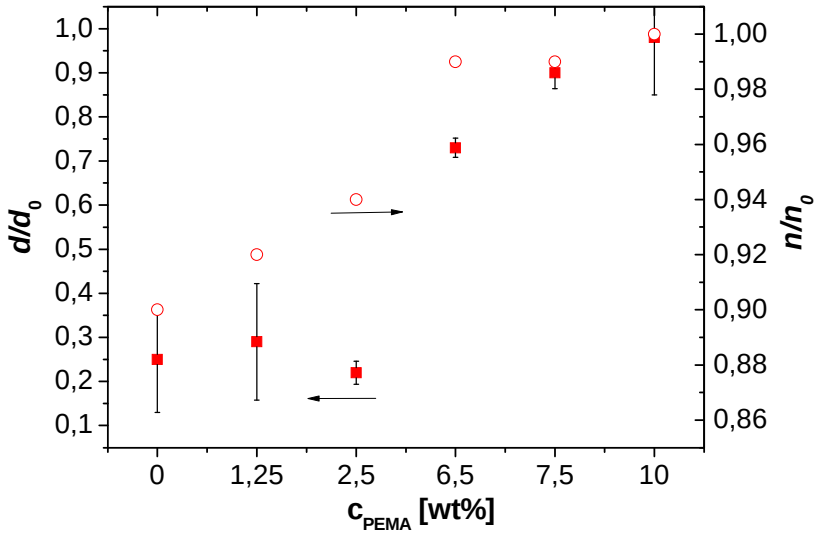


Figure S3. Stability of dry PEI-Mal films in dependence of the cross-linker concentration for poly(ethene-*alt*-maleic anhydride) (PEMA) hydrolyzed.

Comparison of the film thickness ratio d/d_0 with d (after step ii in Scheme 1 meaning film thickness d after annealing, washing and drying steps) and d_0 (after step ii in Scheme 1 meaning film thickness d_0 after annealing step) and refractive index ratio n/n_0 with n (after step ii in Scheme 1 meaning refractive index n after annealing, washing and drying steps) and n_0 (after step ii in Scheme 1 meaning refractive index n_0 after annealing step) for dry PEI-Mal films. **Full symbols** belong to film thickness ratio d/d_0, and **open symbols** belong to refractive index ratio n/n_0.

For PEMA dependence one can conclude that the most stable films can be realized at 7.5 wt% for PEMA. Here, no decrease of the film thickness occurs using concentration of 7.5 wt% related to the total amount of PEI-Mal in aqueous solution. Further concentration of 10 wt% for PEMA could not be used from the practical point view due to the preferred gelation process between the negatively charged PEMA and cationic PEI-Mal.

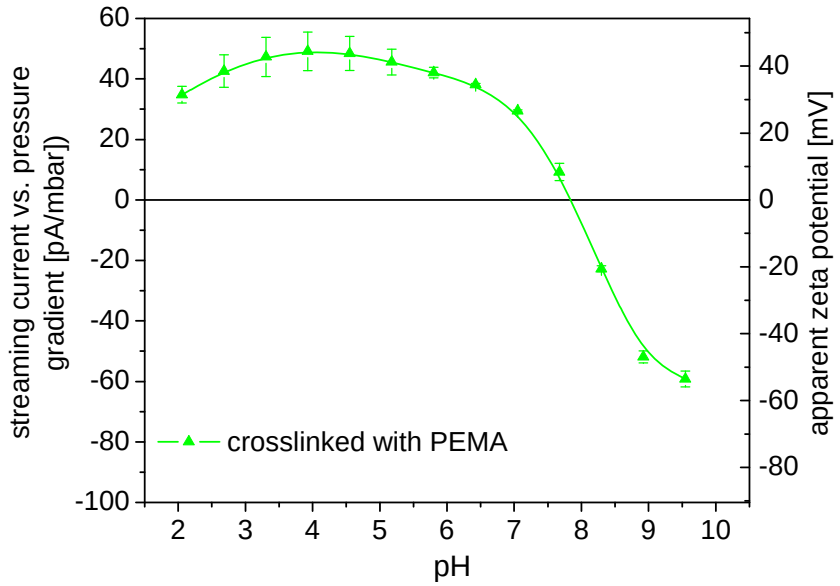


Figure S4. pH dependence of the streaming current versus pressure gradient and apparent zeta potential for PEI-Mal film with cross-linker poly(ethene-*alt*-maleic anhydride) (PEMA) (film thickness 110 nm) at 1 mM KCl solution. The measurements were performed at a channel height of 30 µm. Explanation for apparent zeta potential is presented in the method section in ESI.

The pH-dependence of streaming current vs. pressure gradient shown in Figure 4-ESI is typical for amphoteric surfaces. The isoelectric point was found at about pH 7.8 suggesting that protonated amino groups (only secondary amino groups) of the PEI determine the charging of the interface at lower pH values. As the interaction studies were performed at pH 6.4, electrostatic attraction between PEI-Mal films having positive charge and phosphate containing drugs possessing negative charge under these conditions can be considered to be the driving force. The sign reversal of the streaming current to negative values above pH 7.8 can be attributed to the increasing ionisation of remaining carboxyl groups of the PEMA (cross-linker) in the PEI-Mal films. In order to further unravel the contribution of the different groups in the layer to the interfacial charge formation we performed a XPS study. However there was no chance to separate between ester and acid groups (Figure S2).

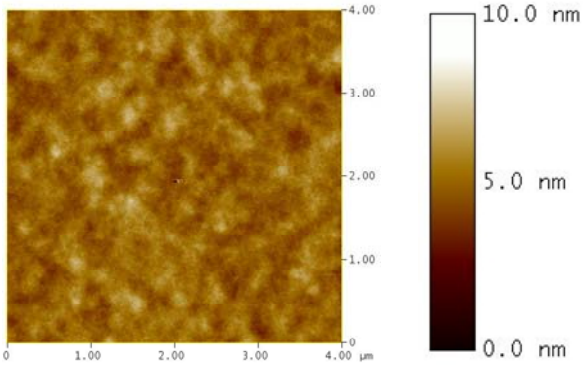
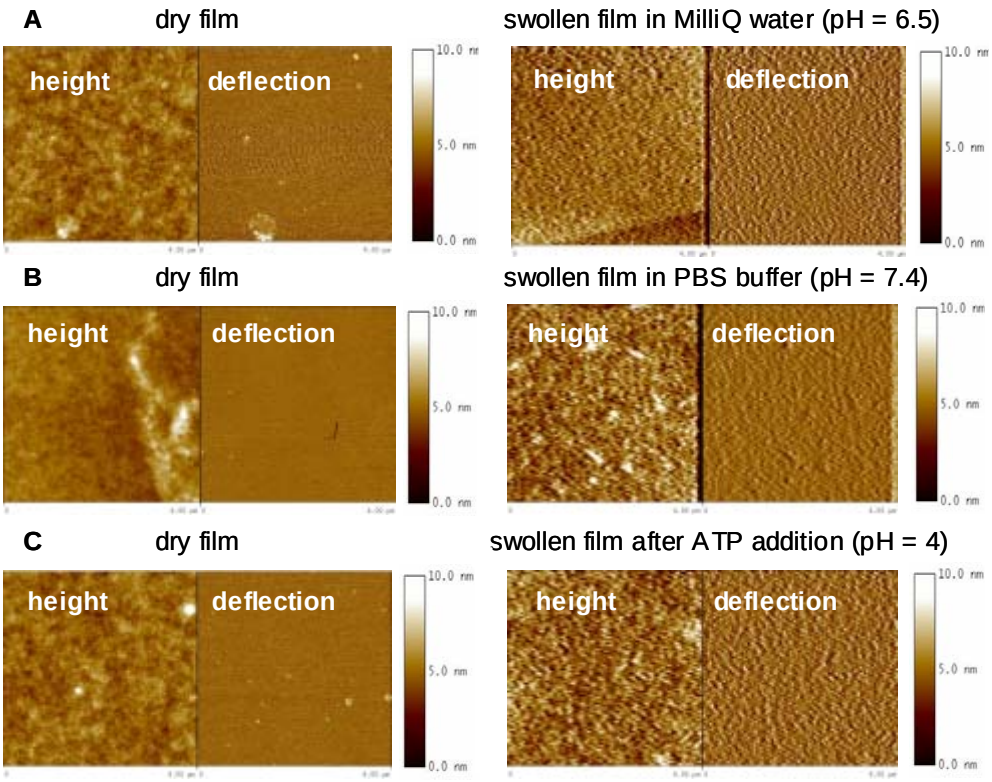


Figure S5. AFM height images of dry PEI-Mal film (***d*** = 100 nm) after annealing and washing/drying step (after step (ii) in Scheme 1); PEI-Mal film cross-linked with PEMA hydrolyzed.

Generally, one can conclude that closed and homogenous films are available in dry state investigated various films.



Comparison	Mal-PEI film	d / nm	Rms / nm	SD
A	dry	62.5	0.35	
	MilliQ water (pH 6.5)	77.6	2.53	0.25
B	dry	56.6	0.69	
	PBS (pH 7.4)	73.2	1.35	0.30
C	dry	65.4	0.38	
	ATP (pH 4)	72.1	1.32	0.10

Figure S6. AFM study of the surface morphology of PEI-Mal films cross-linked with PEMA possessing very thin film thickness of about 60 nm in dry state; (A) PEI-Mal film in dry state and swollen in MilliQ water, (B) PEI-Mal film in dry state and swollen in PBS buffer; (C) PEI-Mal film in dry state and swollen in ATP solution (0.5 mg/ml). AFM images show the height and deflection images of each investigation step.

In each comparison for A – C at first PEI-Mal film, prepared as thin films, was investigated as dry film and then used directly for swelling experiment.

Here, we have compared the surface morphology of films under different media to determine, may be, their different responses. Generally, it gives no significant differences regarding to their surface morphology. Additionally, one can recognize, that the swelling behaviour after

ATP addition is lower in comparison to the cases A and B. Nevertheless, the thinner PEI-Mal films behave similarly as thicker PEI-Mal films after the addition of AMP in this paper.

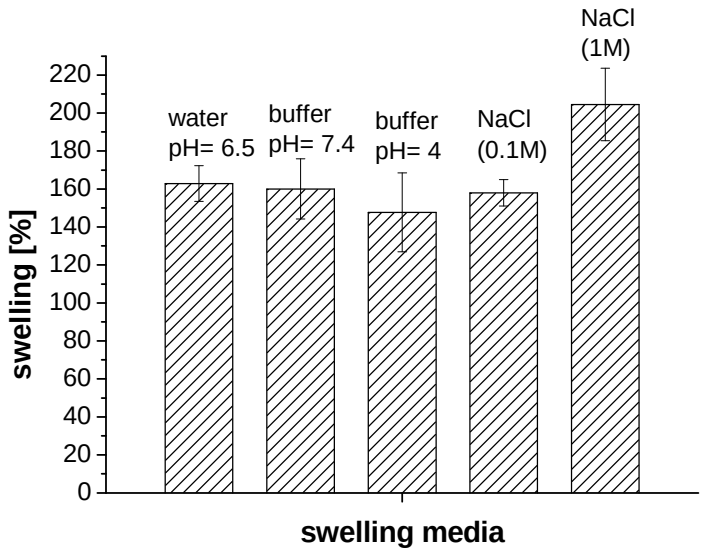


Figure S7. Swelling behavior of thin PEI-Mal film series cross-linked with PEMA deposited in different media.

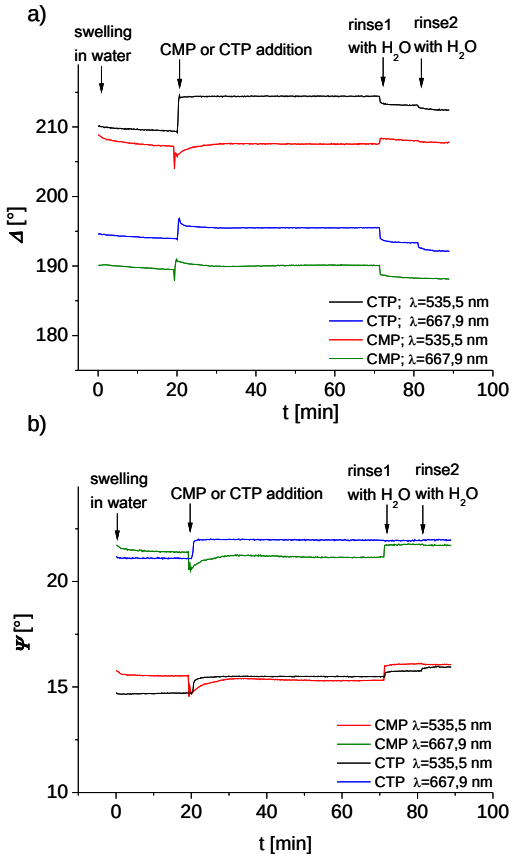


Figure S8. Dynamic scan of the experimental ellipsometric scan of the parameters Δ (a) and Ψ (b) (e.g. for λ = 535.5 and 667.9 nm) during the swelling in water, addition of CTP and CMP (both: c = 0.5 mg/ml) and rinsing process with water of PEI-Mal films cross-linked with PEMA.

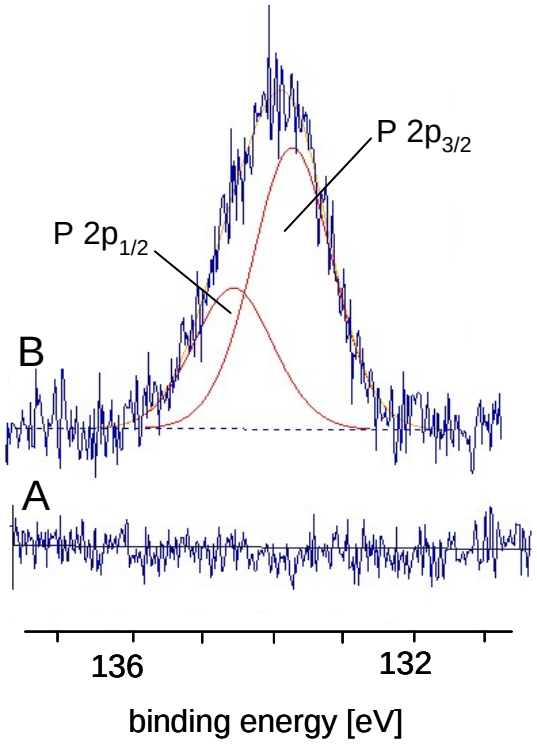


Figure S9. High-resolution P 2p X-ray photoelectron spectrum of PEI-Mal film (cross-linked with PEMA) with ***d*** = 100 nm showing the phosphorus atoms on the topmost surface of dried PEI-Mal film (A) before ATP adsorption and (B) after the ATP adsorption followed by two rinsing steps with water.

Table S1. PEI-Maltose films without cross-linker (NON) and with cross-linker hydrolyzed poly(ethene-*alt*-maleic anhydride) (PEMA) - Comparison of film thickness (*d*) and refractive index (*n*) at λ = 667.9 nm between dry state and swollen state in MilliQ water and swelling degree (SD) of different PEI-Mal films.

	cross linker	d_{dry} [a] [nm]	d_{water} [b] [nm]	n_{dry} [a]	n_{water} [b]	SD [c]
1	NON [d]	16	18	1.511	1.460	0.2
2	NON [d]	21	29	1.529	1.476	0.4
3	PEMA [e]	154	371	1.530	1.371	1.4
4	PEMA [e]	158	395	1.529	1.407	1.5
5	PEMA [e]	142	362	1.500	1.402	1.5

[a] In air. [b] In MilliQ water. [c] Swelling degree was calculated from the formula: $(d_{dry} - d_{water})/d_{water}$. [d] $c_{PEI\text{-}Mal}$ = 1,5 wt% in MilliQ water. [e] $c_{PEI\text{-}Mal}$ = 8 wt% in MilliQ water; c_{PEMA} = 7.5 wt% related to the total amount of PEI-Mal in solution.

Table S2. Stability of film thickness (***d***) for PEI-Mal films with cross-linker poly(ethene-*alt*-maleic anhydride) (PEMA) at different pH deposited for 0, 48 and 96 hours (h) and their film thickness ratio $\boldsymbol{d/d_0}$ after 0, 48 and 96 hours.

Cross linker	pH	***d*** / nm				film thickness ratio $\boldsymbol{d/d_0}$		
		before washing/ drying step	0 h	48 h	96 h	0 h	48 h	96 h
PEMA	4	115	106	95	97	1	0.89	0.91
	6.5	132	132	131	134	1	0.99	1
	7.4	106	100	100	95	1	0.99	0.95
	10	124	117	0	0	1	0	0

Table S3. Interaction/encapsulation of phosphate containing drugs (PCD) (CTP = cytidine 5´-triphosphate disodium salt; CMP = cytidine 5´-monophosphate disodium salt; AMP = adenosine 5´-monophosphate disodium salt) with swollen PEI-Mal films cross-linked with PEMA – Comparison of film thickness and refractive index at λ = 667.9 nm for each step in the interaction process (dry film, swelling in water, addition of PCD to swollen film, and two rinsing steps with water) at different concentration for PCD.

	c mg/ml	PCD	film thickness / nm[a]					refractive index[a]				
			d_{dry}	d_{H2O}	D_{PCD}	d_{rinse1}	d_{rinse2}	n_{dry}	n_{H2O}	D_{PCD}	n_{rinse1}	n_{rinse2}
1	0,5	CTP	99	267	259	258	257	1.540	1.386	1.389	1.391	1.393
2	0,5	CTP	104	284	276	275	274	1.538	1.431	1.428	1.428	1.428
3	0,5	CMP	101	280	281	276	276	1.539	1.375	1.376	1.378	1.378
4	0,5	CMP	61	119	123	120	119	1.530	1.434	1.431	1.433	1.433
5	5	CMP	101	285	328	280	285	1.539	1.373	1.359	1.376	1.372
6	0,5	AMP	99	271	268	268	268	1.544	1.379	1.381	1.381	1.381
7	0,5	AMP	58	123	127	124	123	1.429	1.426	1.429	1.430	1.430
8	5	AMP	96	273	296	277	271	1.543	1.376	1.361	1.376	1.376
9	5	AMP	101	290	330	303	283	1.538	1.372	1.358	1.366	1.368

[a] d_{dry} = film is dried, d_{H2O} = swollen film in water, d_{PCD} = addition of phosphate containing drug, d_{rinse1} = first rinsing with water to extract non-complexed phosphate containing drug from swollen, film, d_{rinse2} = second rinsing with water to extract non-complexed phosphate containing drug from swollen film, n_{dry} = film is dried, n_{H2O} = swollen film in water, n_{PCD} = addition of phosphate containing drug, n_{rinse1} = first rinsing with water to extract non-complexed phosphate containing drug from swollen film, n_{rinse2} = second rinsing with water to extract non-complexed phosphate containing drug from swollen film.

Release properties of CTP. Release properties of CTP is partly different against ATP. Here, there is no release of CTP under the rinsing process. Maybe, the cytidine unit exhibits additionally H-bonds in the PEI-Mal films which is more usable in biological experiments.