Supplementary Material (ESI) for Journal of Materials Chemistry


## Supplementary data

# Protein adsorbers from surface-grafted copolymers with selective binding sites

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### Syntheses of monomers

**Methacryloylamino-2-hydroxypropane (M1)**.[S1] A solution of methacryloyl chloride (2.72 g, 26.0 mmol) in dry dichloromethane (40 mL) was added dropwise to a mixture of 1-aminopropan-2-ol (4.21 g, 56.1 mmol) in dry dichloromethane (40 mL) at 8°C under an argon atmosphere. The precipitating solid was filtered off and the solvent was removed under reduced pressure. After purification by chromatography over silica gel, eluting with dichloromethane/methanol 14:1 v/v ($R_f$=0.32) a colorless solid was obtained. Yield: 1.80 g (12.6 mmol; 48%); 1H NMR (200 MHz, CDCl3): d=1.21 (d, 2J=6.4 Hz, 3 H), 1.98 (dd, 4J=1.5, 1.0 Hz, 3 H), 2.51 (br s, 1H), 3.18 (ddd, 2J=14.0, 3J=7.5, 5.3 Hz, 1 H), 3.51 (ddd, 2J=14.0, 3J= 6.5, 3.0 Hz, 1H), 3.96 (dqd, 3J=7.5, 6.4, 3.0 Hz, 1 H), 5.36 (qd, 4J=1.5, 2J=1.4 Hz, 1H), 5.74 (dq, 2J=1.4, 4J=1.0 Hz, 1H), 6.38 ppm (br s, 1H).

**5-Nitro-*m*-xylylene bisphosphonic acid tetramethylester.** [S2] 5-Nitro-*m*-xylene (9.52 g, 63.0 mmol, 1.0 eq) is dissolved in 165 mL of tetrachloromethane. *N*-Bromosuccinimide (24.83 g, 139.5 mmol, 2.2 eq) and a catalytic amount of α,α´-azobisisobutyronitrile are added and the mixture is refluxed for 13 hours. After filtering off the insoluble succinimide, the solvent is removed under reduced pressure. The remaining yellow oil is recrystallized from 7 mL of ethyl acetate and 15 mL of *n*-hexane. Subsequently the resulting yellowish solid (7.9 g crude product) is dissolved in an excess of trimethylphosphite (9.99 g, 80.5 mmol, approx. 3.1 eq) and the solution is refluxed for 5 hours. The volatile components are removed in vacuo and the product is purified by chromatography over silica gel eluting with dichloromethane/methanol (14:1 v/v, RF = 0.29). Yield: 4.10 g yellowish solid (11.2 mmol, 18 % over 2 steps). 1H-NMR (CDCl3; 200 MHz): δ(ppm) = 3.25 (d, 2JH,P = 22.0 Hz, 4H); 3.73 (d, 3JH,P = 11.0 Hz, 12H); 7.57-7.62 (m, 1H); 8.04-8.07 (m, 2H). 31P{1H}-NMR (CDCl3; 81 MHz): δ(ppm) = 27.3 (s).

**5-Amino-*m*-xylylene bisphosphonic acid tetramethylester.**[S2] Palladium on Carbon (0.93 g, 10% Pd = 93 mg, 0.9 mmol, 7.8 mol%) is added to a solution of 5-nitro-*m*-xylylene bisphosphonic acid tetramethylester (4.10 g, 11.2 mmol). The reaction mixture is stirred for 15 hours under a hydrogen atmosphere (atmospheric pressure). After filtering off the catalyst over celite, the solvent is removed under reduced pressure. Yield: 3.20 g slightly yellow solid (9.5 mmol, 85 %).1H-NMR (CDCl3; 200 MHz): δ(ppm) = 3.06 (d, 2JH,P= 21.8 Hz, 4H); 3.06 (sb, 2H); 3.68 (d, 3JH,P= 10.8 Hz, 12H); 6.53-6.58 (m, 2H); 6.56-6.61 (m, 1H).31P{1H}-NMR (CDCl3; 81 MHz): δ(ppm) = 29.5 (s).

**5-(Methacryloylamino)-*m*-xylylene bisphosphonic acid tetramethylester (M2).**[S2] 5-Amino-*m*-xylylene bisphosphonic acid tetramethylester (900 mg, 2.7 mmol, 1.0 eq), triethylamine (320 mg, 3.2 mmol, 1.18 eq) and catalytic amount of 4-(*N,N*-dimethylamino)-pyridine are dissolved in 30 mL of dichloromethane. A solution of methacryloyl chloride (0.42 g, 4.0 mmol; 1.51 eq) and 8 mL of dichloromethane is added dropwise at 0°C within 1

hour. Stirring is continued for 1 hour at room temperature. Subsequently the organic layer is washed with 30 mL 0.6 N NaOH and dried in vacuo. The received crude product is purified by chromatography over silica gel eluting with dichloromethane/methanol (19:1 v/v, $R_f$=0.09). Yield: 0.90 g colourless highly viscous oil (2.2 mmol; 83 %). 1H-NMR (CDCl3; 200 MHz): δ(ppm) = 2.06 (dd, 4JH,H= 1.5 Hz, 4JH,H= 1.0 Hz, 3H); 3.15 (d, 2JH,P= 22.0 Hz, 4H); 3.70 (d, 3JH,P= 10.8 Hz, 12H); 5.47 (qd, 1H); 5.79 (qd, 1H); 6.97-7.01 (m, 1H); 7.47-7.50 (m, 2H); 7.69 (sb, 1H). 31P{1H}-NMR (CDCl3; 81 MHz): δ(ppm) = 28.9 (s). MS (ESI pos., MeOH); m/z: 444 [M+K]+, 428 [M+Na]+, 406 [M+H]+. HRMS (ESI pos., MeOH) calcd. For C16H25NNaO7P2 [M+Na]+ m/z: 428.1004, found: m/z: 428.1011; elemental analysis: calcd: C 47.41%; H 6.22%; found: C 47.71%; H 6.41%.

**Test of reusability of protein adsorbers**

The reusability of neutral poly(M1-*co*-M2)-grafted membranes was tested in repeated bind-wash-elute-regenerate cycles (cf. Experimental Section); the results are summarized in Figure S1.

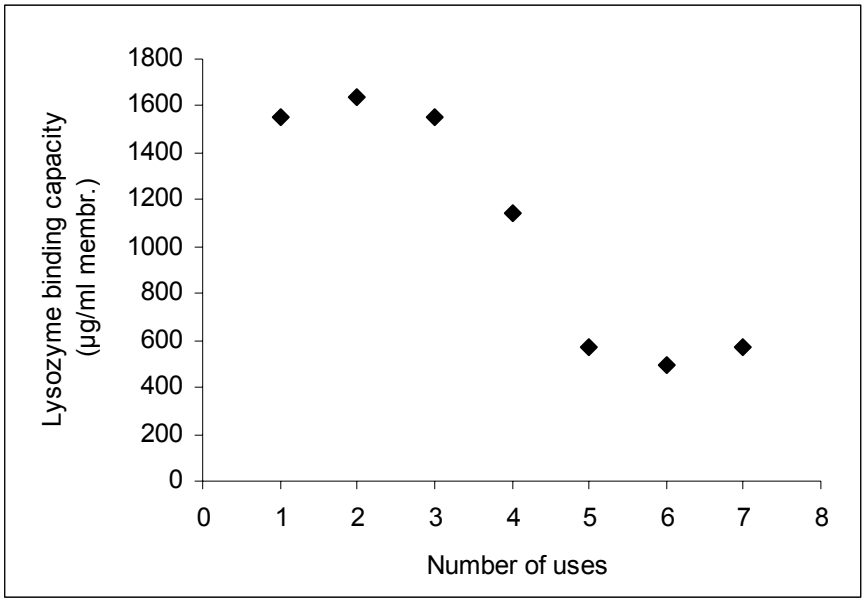


**Figure S1**. Lysozyme binding capacity in repeated bind-wash-elute-regenerate cycles (binding: from 1 mL protein solution with a concentration of 35 µg/mL in 25 mM HEPES buffer, pH 7.1; washing: 3 times with 2 mL buffer for 20 min; elution: with 1 mL 25 mM HEPES buffer containing 1 M NaCl overnight; regeneration: in excess of 25 mM HEPES buffer, pH 7.1 overnight).

**References**

S1 C. Renner, J. Piehler, T. Schrader, *J. Am. Chem. Soc.* **2006**, *128*, 620-628**.**

S2 W. Sun, H. Bandmann, T. Schrader, *Chem. Eur. J.* **2007**, 13, 7701-7707.