

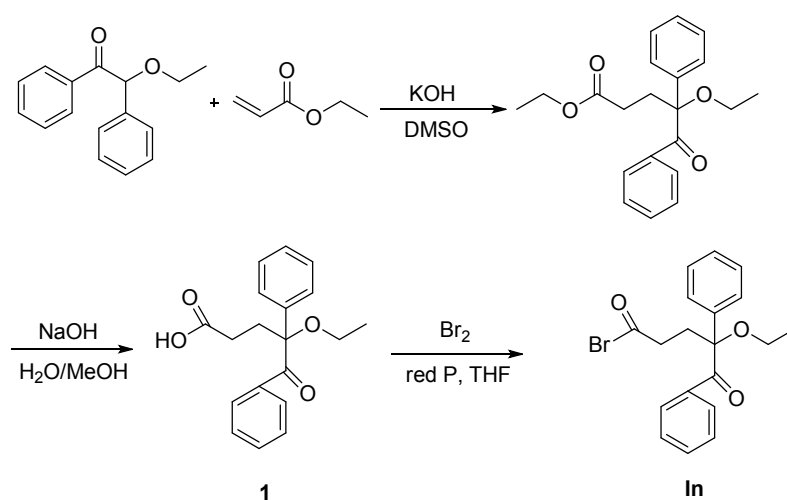
Supplementary data

Highly selective protein adsorber via a two-step surface-initiated molecular imprinting utilizing a multi-functional polymeric scaffold on macroporous cellulose membrane

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1.1. Photo-initiator synthesis

The synthesis of photo initiator (**In**) followed previous report¹ as shown below.



4-Ethoxy-5-oxo-4,5-diphenylpentanoic acid (**1**)

Benzoin ethyl ether (0.083 mol) was dissolved in 40 ml DMSO which contained 1.6 ml 4 M KOH solution and reacted for 4 h with ethyl acrylate (0.092 mol). Thereafter, the solvent was removed in vacuum and the remaining product was hydrolysed for 24 h at room temperature in 100 ml 1 M NaOH solution which contained 6% methanol. The solvent was removed again in vacuum and the remaining product was dissolved

in dichloromethane to remove the hydroxide. After removing dichloromethane in vacuum the product was obtained (yield 89.6%).

4-Ethoxy-5-oxo-4,5-diphenylpentanoyl bromide (**In**)

To obtain the bromide derivative of **1**, a Hell-Volhard-Zelinsky reaction was performed. In an argon atmosphere 6.4 mmol **1** was dissolved in 40 ml dry THF and ca. 4 mg of red phosphorous red were added. 6.4 mmol bromine were dissolved in 10 ml dry THF and added drop wise to the mixture and refluxed for 5 h. Thereafter, THF was removed in vacuum and the product (yield 95%) was analyzed with ^1H NMR (Figure S1). ^1H -NMR (D_2O ; 400 MHz): $\delta(\text{ppm}) = 1.06\text{-}1.10$ (t, $J = 8$ Hz, 3 H); 1.87-2.06 (m, 2 H); 2.50-2.62 (m, 2 H); 3.27-3.51 (m, 2 H); 7.36-7.55 (m, 9 H); 7.79-7.82 (m, 1 H).

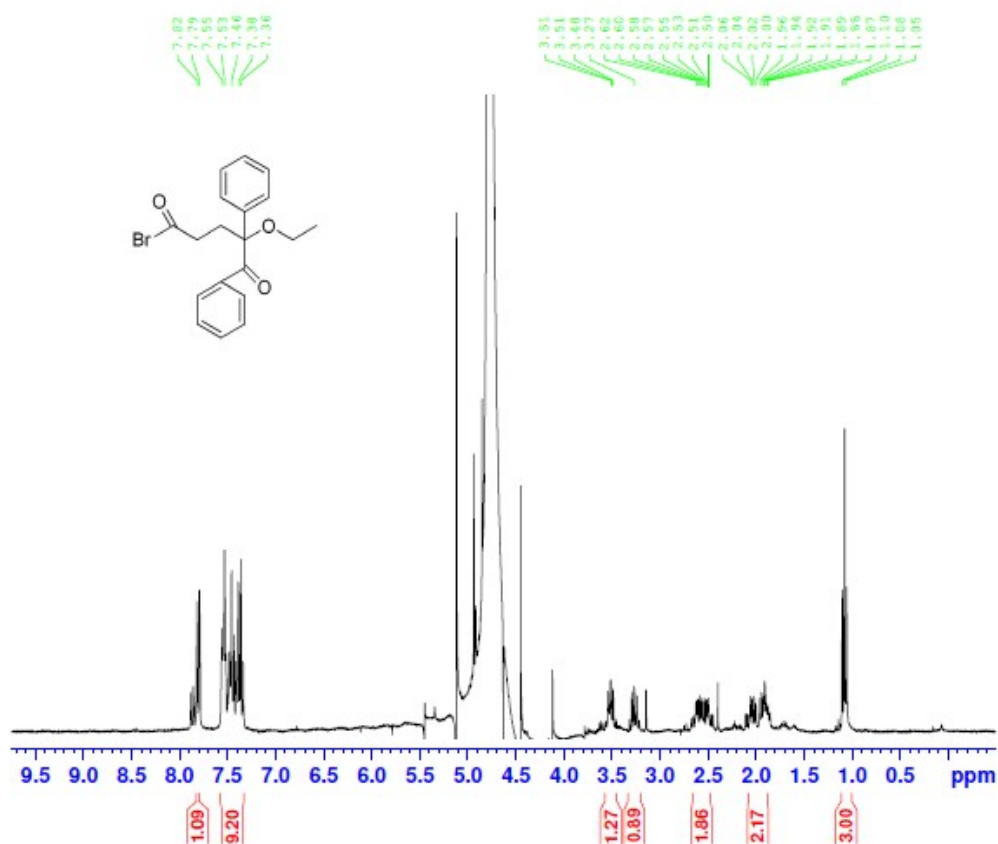
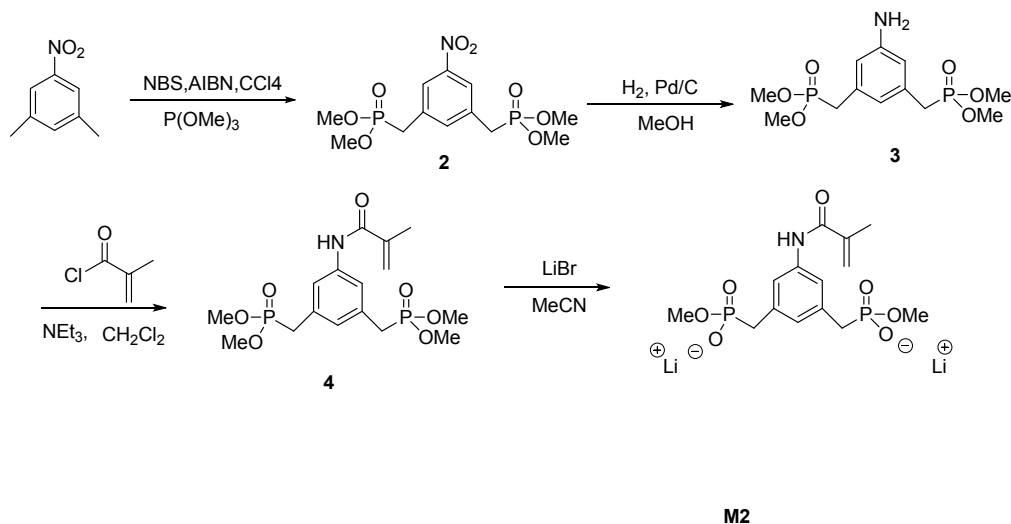


Figure S1. ^1H -NMR (400 MHz) of **In** in D_2O

1.2. M2 synthesis

The synthesis of M2 followed previous report² as shown below.



5-Nitro-m-xylene bisphosphonic acid tetramethylester (**2**)

5-Nitro-m-xylene (63.0 mmol) is dissolved in 165 mL of tetrachloromethane. N-Bromosuccinimide (139.5 mmol) and a catalytic amount of α,α' -azobisisobutyronitrile are added and the mixture is refluxed for overnight. After filtering off the insoluble succinimide, the solvent is removed under reduced pressure. The remaining yellow oil is recrystallized from ethyl acetate and n-hexane. Subsequently the resulting yellowish solid is dissolved in an excess of trimethylphosphite (80.5 mmol) 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. Yield: 4.70 g yellowish solid (19 %).

5-Amino-m-xylene bisphosphonic acid tetramethylester (**3**)

Palladium on Carbon (0.93 g, 10% Pd) is added to a solution of educt **2** (11.2 mmol). The reaction mixture is stirred overnight under a hydrogen atmosphere. After filtering

off the catalyst over celite, the solvent was removed under reduced pressure. Yield: 3.50 g yellow solid (85 %).

5-(Methacryloylamino)-m-xylene bisphosphonic acid tetramethylester (**4**)

Educt **3** (1.8 g), trimethylamine (640 mg) and catalytic amount of 4-(N,N-dimethylamino)-pyridine were dissolved in 50 mL of dichloromethane. A solution of methacryloyl chloride (0.9 g) and 10 mL of dichloromethane is added dropwise at 0°C within 1 hour. Stirring was continued for 1 hour at room temperature. Subsequently the organic layer was washed with 60 mL 0.6 N NaOH and dried in vacuo. The received crude product is purified by chromatography over silica gel. Yield: 1.70 g colorless oil (83 %).

5-(Methacryloylamino)-m-xylene bisphosphonic acid dimethylester dilithium salt (**M2**)

Educt **4** (1.8 g) was dissolved in 50 mL of absolute acetonitrile under argon. A solution of lithium bromide (283 mg) in 9 mL of acetonitrile is added and the reaction mixture is refluxed for 8 hours under argon. During this period the product precipitates from the reaction mixture. The solvent is decanted and the off-white solid was washed three times with acetonitrile. The pure compound is obtained after drying in vacuo and analyzed with ¹H NMR (Figure S2). Yield: 1.6 g colorless solid (90 %). ¹H-NMR (D₂O; 400 MHz): δ(ppm) = 1.89 (s, 3 H); 3.00-3.19 (m, 4 H); 3.53-3.57 (d, J= 4 Hz, 6 H); 5.53 (s, 1 H); 5.88 (s, 1 H); 7.53-7.57 (m, 1 H); 7.96 (m, 2 H).

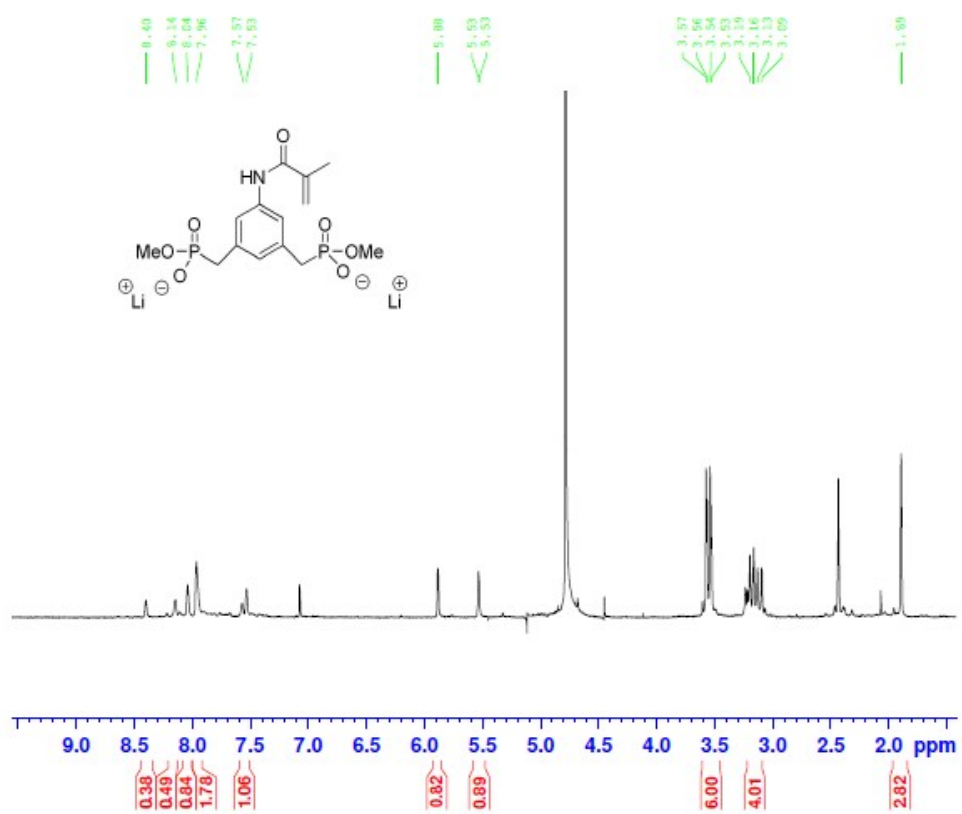


Figure S2. $^1\text{H-NMR}$ (400 MHz) of **M2** in D_2O

1.3. Additional data

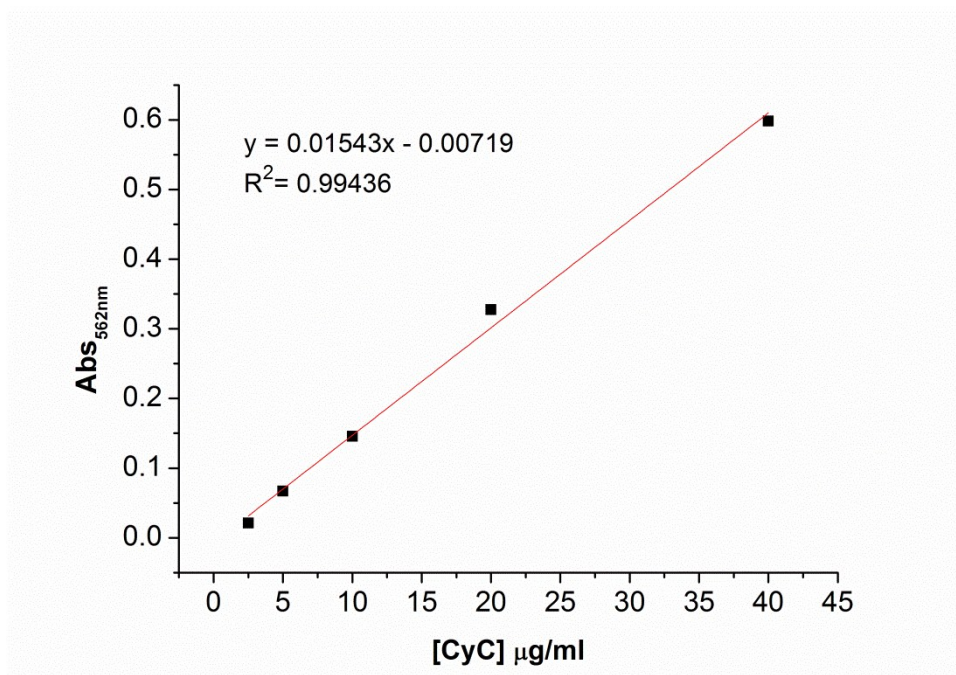


Figure S3. UV calibration curve (562 nm) for CyC concentration determination in static binding capacity assay using BCA method.

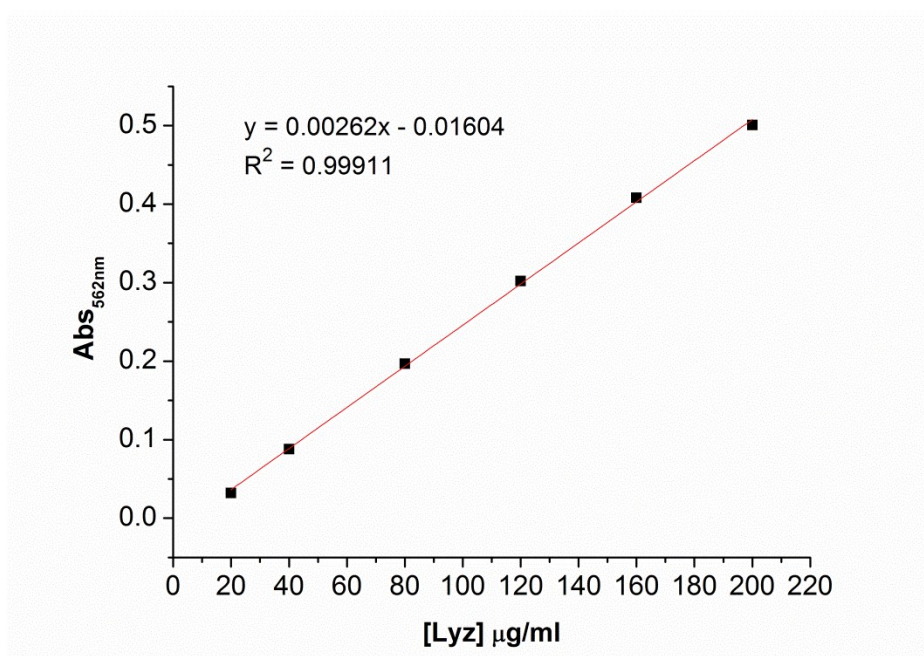


Figure S4. UV calibration curve (562 nm) for Lyz concentration determination in static binding capacity assay using BCA method.

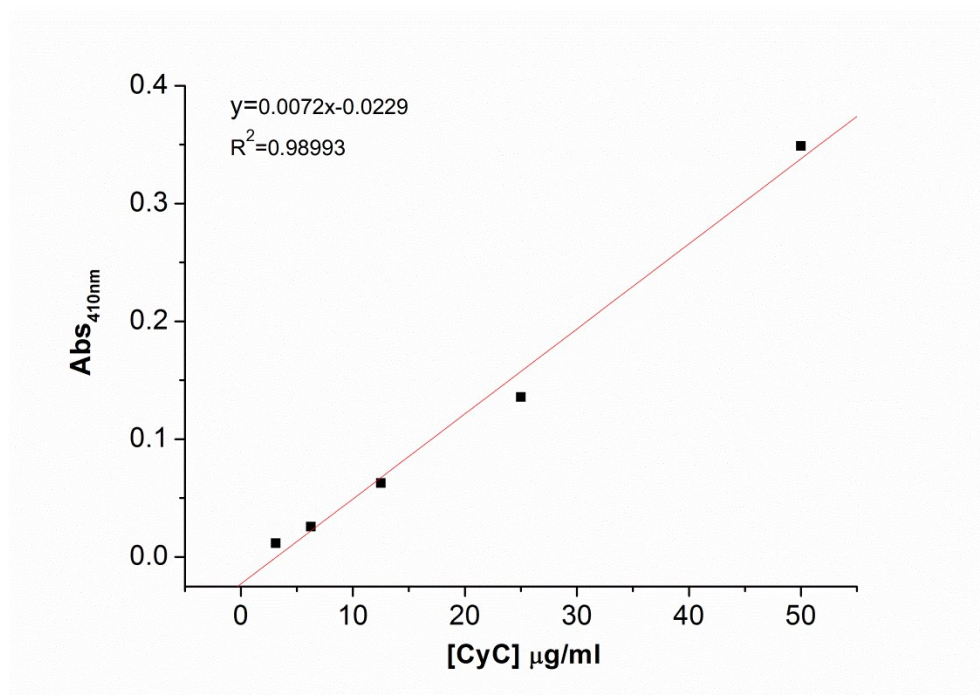


Figure S5. UV calibration curve (410 nm) for CyC concentration determination of *in situ* selective protein adsorption assay using direct UV-Vis absorbance measurement.

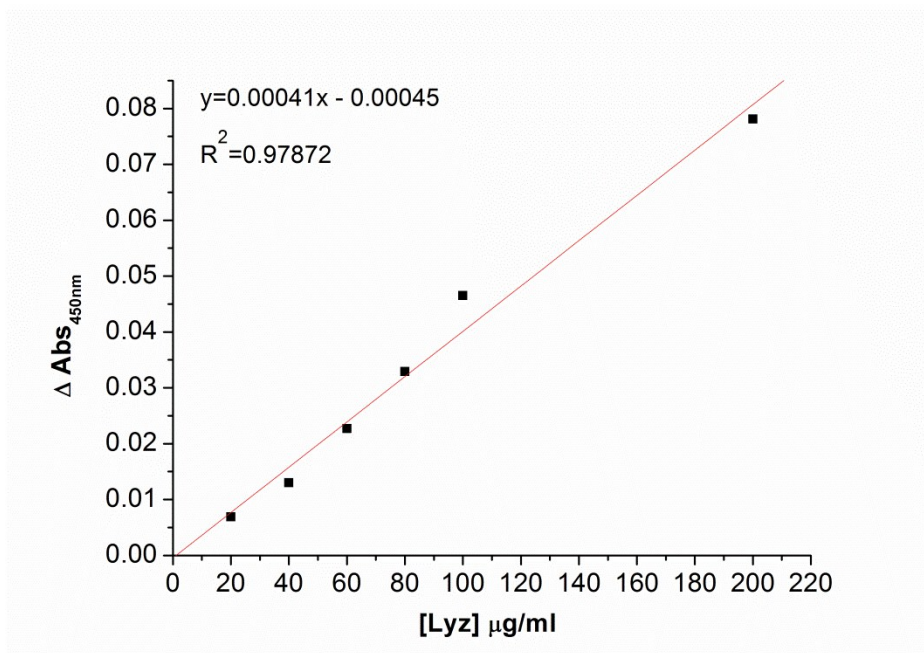


Figure S6. UV calibration curve (450 nm) for Lyz concentration determination of *in situ* selective protein adsorption assay using enzyme activity assay with *micrococcus lysodeikticus* as substrate (activity is related to decrease of turbidity of substrate dispersion).

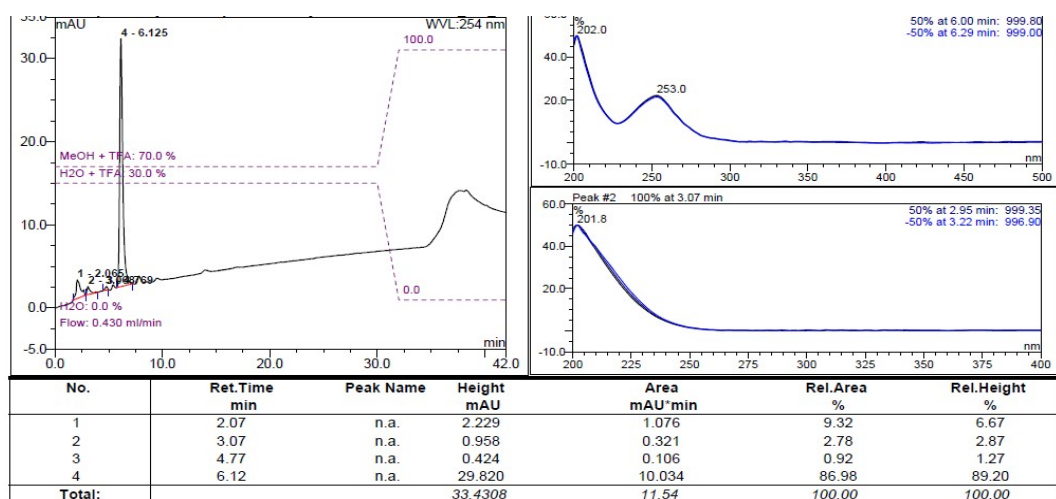


Figure S7. HPLC trace of photo-initiator derivative and benzoic acid cleaved from cellulose membrane

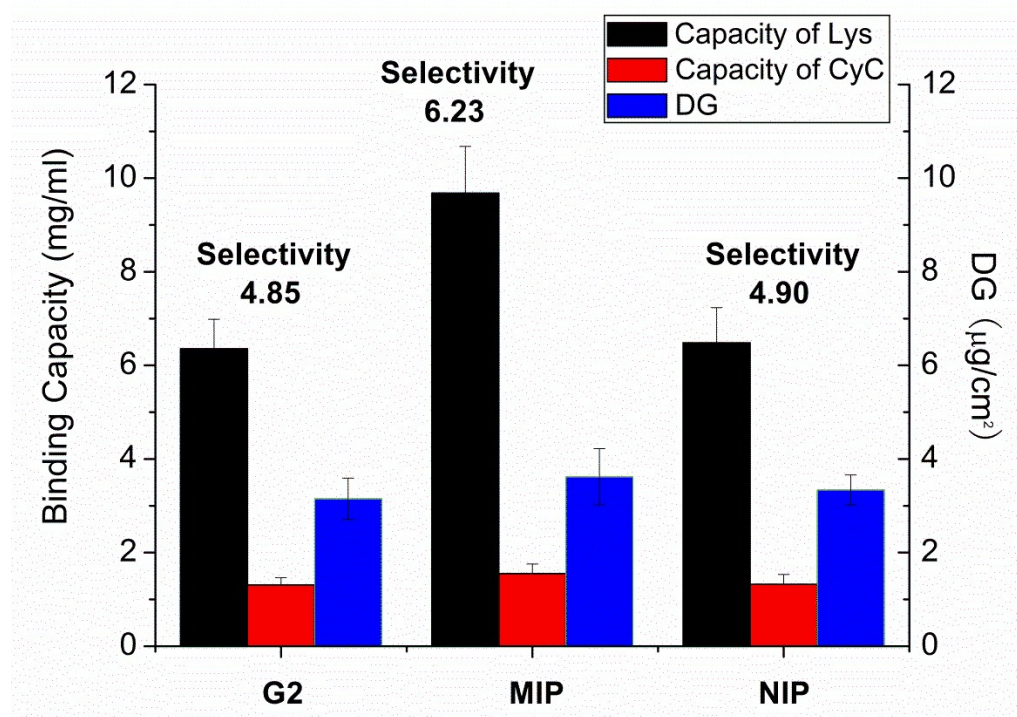


Figure S8. Degree of grafting (DG) as well as protein binding capacities and Lys vs. CyC selectivity of different grafted membranes in pH 7.4 PBS buffer solution (MIP and NIP were prepared using G2, i.e. scaffold-grafted membrane, as precursor; that membrane had been prepared at 0.56 M AM during optimization of monomer concentration in the imprinting step; cf. Figure 3).

Table S1. Weight changes of membranes after second PI immobilization and subsequent UV initiated grafting using M1 as monomer.

Membrane No.	Procedures				Δm in grafting-2
	PI – first modification	Grafting-1 condition	PI – second modification	Grafting-2 condition	
1.	PI 25 mg/mL	M1/M2 100 mg/mL	PI 25 mg/mL	M1 100 mg/mL	30 mg
2.	PI 25 mg/mL	M1/M2 50 mg/mL	PI 25 mg/mL	M1 100 mg/mL	12.3 mg
3.	PI 25 mg/mL	M1/M2 100 mg/mL	PI 12.5 mg/mL	M1 100 mg/mL	9.82 mg

1. S. Schwark and M. Ulbricht, *Eur. Polym. J.*, 2012, **48**, 1914-1922.
 2. C. Renner, J. Piehler and T. Schrader, *J. Am. Chem. Soc.*, 2006, **128**, 620-628.
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