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Electronic Supplementary Information

Generic High-Capacity Protein Capture and Release by pH Control

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Materials and Methods

Chemicals:

All chemicals and proteins used were purchased from Sigma-Aldrich unless stated otherwise. H_2O_2 (30%) and NH_4OH (28–30%) were from ACROS, while H_2SO_4 (98%) and ethanol (99.5%) were from SOLVECO. Water was ASTM research grade Type 1 ultrafiltered water (milli-Q-water). The chemicals employed in polymerization were *tert*-butyl acrylate, *tert*-butyl methacrylate, dimethylsulfoxide, dichloromethane, methane sulfonic acid, *N*,*N*,*N*′,*N*″-pentamethyldiethylenetriamine (PMDTA), $CuBr_2$ and L-ascorbic acid. Buffers used in this work were based on phosphate buffered saline (PBS) tablets (0.01 M phosphate, 0.13 M NaCl, pH 7.4) or disodium hydrogen phosphate and NaCl titrated to a specific pH with HCl (1 M aqueous solution) or NaOH (1 M aqueous solution). Polymers used as probes for brush height determination were PAA sodium salt (30,000 g/mol, 35 wt. % in H_2O), PAA (100,000 g/mol, 40 wt. % in H_2O) and PEG (35,000 g/mol). PEG-succinimidyl Valerate (PEG-SVA, 10,000 g/mol) was used for conjugation to BSA (Laysan Bio Inc.) DNA samples for testing binding (single-stranded and double-stranded) were available from collaborators in house. For enzyme activity assay 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and D-glucose were used. The proteins used in this study were avidin (AVI, ThermoFisher), bovine serum albumin (BSA), BSA-fluorescein isothiocyanate conjugate, fibrinogen (FIB) from bovine plasma, glucose oxidase (GOX) Type VII G2133 from *Aspergillus Niger*, horse radish peroxidase (HRP, ThermoFisher), insulin (INS), INS glargine, IgG antibodies from human serum, myoglobin (MYO) from horse skeletal muscle, lysozyme (LYS), NeutrAvidin (NAVI, Pierce), and ubiquitin (UBI) from bovine erythrocytes. Human serum (from human male AB plasma) was filtered through a 40 μm hydrophilic filter and diluted ten times in PBS prior to use. The carbohydrates used to study interactions with PMAA brushes were dextran (100,000 g/mol) from *Leuconostoc* spp., hyaluronic acid sodium salt from *Streptococcus equi* (15,000–30,000 g/mol). Oxytocin acetate salt hydrate was also tested.

Surface cleaning:

Prior to surface functionalization, QCM sensor crystals (standard Au, purchased from Biolin Scientific) and SPR sensor surfaces (standard Au, purchased from BioNavis) were cleaned with piranha wash (H_2SO_4:H_2O_2, 3:1 v/v) for 10 min followed by rinsing in milli-Q. Next, an RCA1 wash (H_2O:H_2O_2:NH_4OH 5:1:1 v/v at 75 °C) was performed for 20 min, followed by further rinsing in milli-Q, sonication in ethanol, and drying with N_2. For microelectrodes the piranha wash step was omitted to prevent destruction of the surface due to delamination of the gold film with nanoholes.

Surface activation:

Thiol-based initiators were either bis[2-(2bromoisobutyryloxy)undecyl]disulfide (DTBU) or HS-C_{11}-OC(O)-IzoButyrate-Br (Prochimia). The self-assembled monolayer was prepared as described previously.[1] Alternatively, an aryl diazonium salts was synthesized[2] and reduced in order to attached them to gold. To convert the diazonium monolayer into a polymerization initiator layer, the gold surfaces were exposed to α-bromoisobutyryl bromide and triethylamine in dichloromethane, after which surfaces were rinsed in ethanol and dried under N_2. Regardless of

the method, a thin (1-2 nm) and dense layer of tertiary Br groups is created (as verified by SPR) for ATRP initiation. No differences in results were observed with respect to initiator type.

Surface-initiated polymerization:

ATRP was used to prepare PMAA polymer brushes in a manner similar to published procedures.[1] Inhibitor was removed from the monomer *tert*-butyl methacrylate using an alumina column, after which it were stored at −20 °C, then warmed to room temperature immediately before use. Reactions were carried out using standard Schlenk line techniques under an inert atmosphere of N_2. $CuBr_2$ (0.006 g, 0.03 mmol), and PMDTA (0.052 mL, 0.246 mmol) were dissolved in dimethyl sulfoxide (20 mL) and, alongside a separate flask of *tert*-butyl methacrylate (20 mL, 0.1231 mol), was deoxygenated via vigorous bubbling of N_2 for 30 min. The reaction solution and monomer were then transferred via cannula into a screw-top jar (with rubber septa lid) containing initiator-prepared gold surfaces. The reaction was initiated by the addition of ascorbic acid (0.033 g, 0.185 mmol). The final concentrations of each component in the reaction medium were: [monomer] = 3.1 M, [$CuBr_2$] = 0.6 mM, [PMDTA] = 6.2 mM, and [ascorbic acid] = 4.6 mM. The reaction was placed under magnetic stirring. Reactions were quenched by immersing the samples in pure ethanol. Poly(*tert*-butyl methacrylate) (PTBMA) brushes were then converted to PMAA by exposure to 0.2 mM methane sulfonic acid in dichloromethane (10 mL) for 15 min, followed by rinsing in dichloromethane and ethanol. For PAA, *tert*-butyl acrylate was used as the starting monomer with an otherwise identical protocol.

Quartz crystal microbalance:

Sensor crystals coated with gold were used and measurements were performed using a Q-Sense E4 (Biolin Scientific). All data shown corresponds to the first or third overtone.

Surface plasmon resonance:

Measurements were performed on a SPR Navi 220A instrument (BioNavis), both in air and water. The total internal reflection (TIR) and SPR angle was recorded on three different laser wavelengths and in two different flow channels. The flow rate of buffer used was 20 µL/min. Fresnel modelling to quantify polymer and protein amounts was performed as described previously.[3] In brief, the refractive index of the dry polymer brushes was assumed to be equal for PAA and PMAA and set to 1.522. The refractive index of dry proteins bound in polyelectrolyte brushes was assumed to be equal to that of the polymer. To obtain surface coverage, the densities of the dry polymers and proteins were set to 1.22 g/cm^3 and 1.35 g/cm^3, respectively.

Plasmonic detection with nanohole arrays:

The nanohole arrays were prepared by colloidal lithography.[4] Extinction spectroscopy was performed on the microscale to detect the resonance shift of the peak.[5] In brief, the surface was imaged by a tungsten lamp in transmission mode and a fraction of the light was directed to the opening of an optical fiber in the focal plane. The detection spot (typically 50 µm) depends on the fiber diameter and the objective magnification. A fiber coupled photodiode array spectrometer (B&WTek) analyzed the spectrum. The final resolution is almost as high as in SPR (down to 0.1

ng/cm^2). Note that all data presented as resonance shifts in nm are from plasmonic nanohole arrays, while resonance shifts in degrees are from conventional angular SPR.

Binding and desorption of proteins:

For simplicity, in some experiments protein immobilization and release was not monitored in real-time. Instead, the surfaces were immersed in a solution of proteins for 30 min to ensure saturated binding, using the kinetics from SPR and QCM measurements as guideline. (PMMA was used at pH 5 unless otherwise stated.) The concentration was 0.3 g/L. Following protein loading the surface was rinsed in PBS pH 5.0 and then water and drying with N_2. Desorption was performed by immersing the samples in PBS with a pH high enough to fully desorb the protein in question (again based on SPR and QCM measurements). The ionic strength was always physiological except when rinsing with pure water. Only monovalent salts were used together with the buffering phosphate. The fluorescent proteins were immobilized in PMAA brushes in the same manner as the native proteins.

Fluorescence microscopy:

All fluorescence measurements were conducted using a Zeiss Axio Observer 7 inverted microscope equipped with a Axiocam506 camera. Surfaces were imaged using a 10× objective and with excitation/emission filters designed for the fluorophore.

Circular dichroism spectroscopy:

The polyelectrolyte brush surface was repeatedly loaded and unloaded with protein. The procedure was repeated until a sufficiently high concentration of protein was found in the collection solution. The quantification of desorbed protein was performed using a NanoDrop (ThermoFisher Scientific). When detectable quantities of protein were obtained, the sample was filtered through a 40 µm cellulose acetate filter and placed in a centrifuge column with a molecular size cut-off of 40 kDa (Sartorius). The sample was centrifuged for 10 min at 5000 rpm resulting in approximately 100–300 µL solution with a concentration around 0.3 g/L. CD was measured with a Chirascan spectrometer (Applied Photophysics). Each spectrum is an average of 10 scans. A quartz cuvette with path length of 0.05 cm was used. Spectra of desorbed proteins were compared to corresponding protein solutions with concentration of 0.3 g/L in PBS at pH 5.0 and 8.0.

Activity measurements:

The activity of GOX in bulk solution was measured using an ABTS and HRP assay. The reaction was initiated by the addition of GOX solution (diluted stock solution or desorbed from PMAA brush) to a mixture of ABTS, glucose and HRP in PBS pH 7.4. The final composition of the assay was 2 mM ABTS, 1 mM glucose, 20 nM HRP, and 2 nM GOX and the total volume for each measurement was 800 µL. To quantify the converted amount by GOX, a standard curve was produced where hydrogen peroxide was added instead of GOX (Fig. S12). Absorbance was recorded at 420 nm and the initial rate was determined by determining the slope of absorbance increase during the first 30 s of the reaction.

Protein conjugation:

PEG conjugation of BSA was performed by mixing PEG-SVA (100 mg) with BSA (5 mg) in PBS pH 8.0 for 16 h. Purification of PEG conjugated BSA from non-conjugated BSA and hydrolyzed PEG-SVA was performed by passing the reaction solution through a size exclusion column,16/600 Superdex 200 pg, connected to an ÄKTA Start protein purification system (GE Healthcare). Analysis of protein fractions by gel electrophoresis showed a molecular weight above 250,000 kg/mol (Fig. S8). Two different fluorophores were used in the cojugation of fluorescent dyes to the amines of BSA: Alexa Fluor 488 and 555 (with tetrafluorophenyl or *N*-hydroxysuccinimide ester groups). A BSA solution (100 μL, 10 mg/mL, pH 8.5) was mixed with Alexa Fluor dye (100 μg), and the resulting solution was inverted every 10 min for 1 h. The reaction was terminated by the addition of PBS pH 5.0, which reduced the pH to 5.0 and diluted the sample to a protein concentration of 0.2 mg/mL.

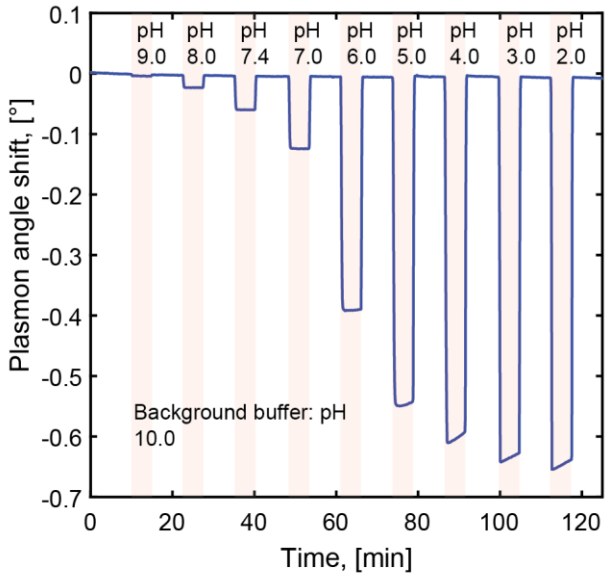

Fig. S1. SPR signals during titration for determination of polyelectrolyte brush pK_a (here PMAA). The resonance angle shift due to changes in pH is monitored. The signal originates mainly from protonation/deprotonation of the carboxylic acid groups[1] and can thus be used to determine pK_a.

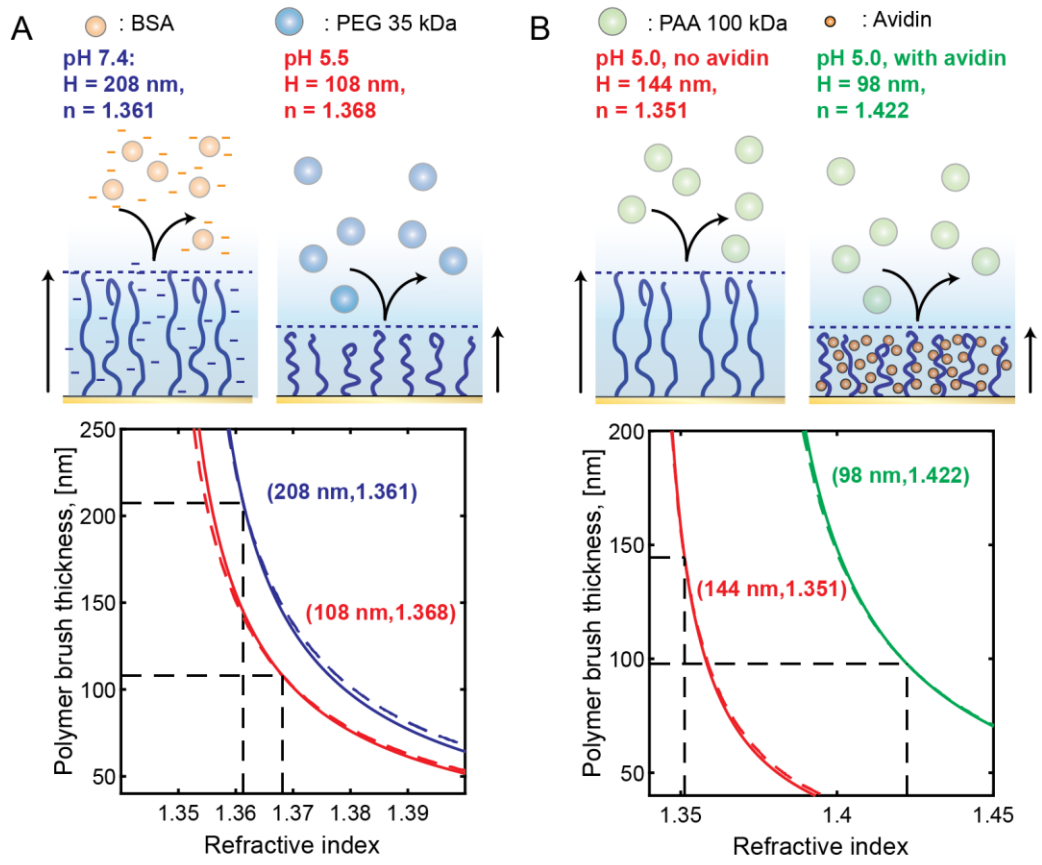

Fig. S2. Brush heights in liquid measured in SPR by injections of non-interacting probes. The TIR angle is used to calculate the bulk refractive index change and Fresnel models are used to find the thickness and refractive index which best describes the PMAA brush, at different pH and in the presence or absence of bound proteins. (A) Brush height at physiological pH (BSA as probe) and lower pH (PEG as probe). The dry height was 17 nm as determined by SPR scans in air.[1] (B) Brush height before and after avidin binding at pH 5 (PAA as probe). Further details on the methodology are given in previous work.[1] These results show qualitatively that the brush is compacted by protein binding (a more detailed quantitative analysis will require more measurements).

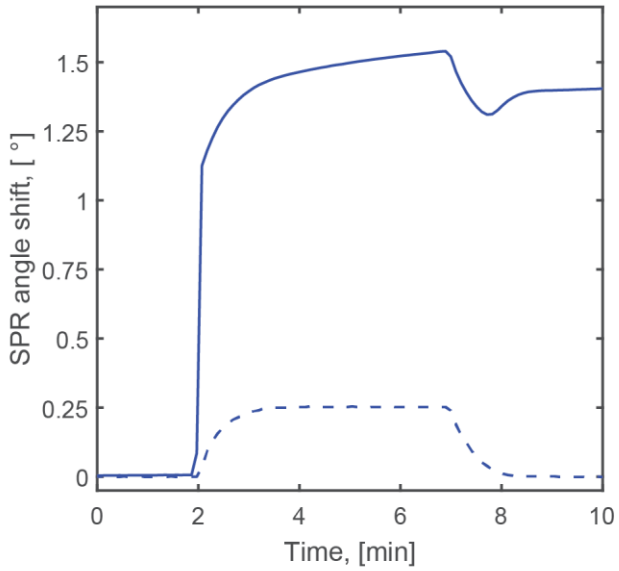

Fig. S3. Examples of protein binding to PAA. BSA introduced at pH 4 in SPR (dashed line is total internal reflection angle showing a bulk response).

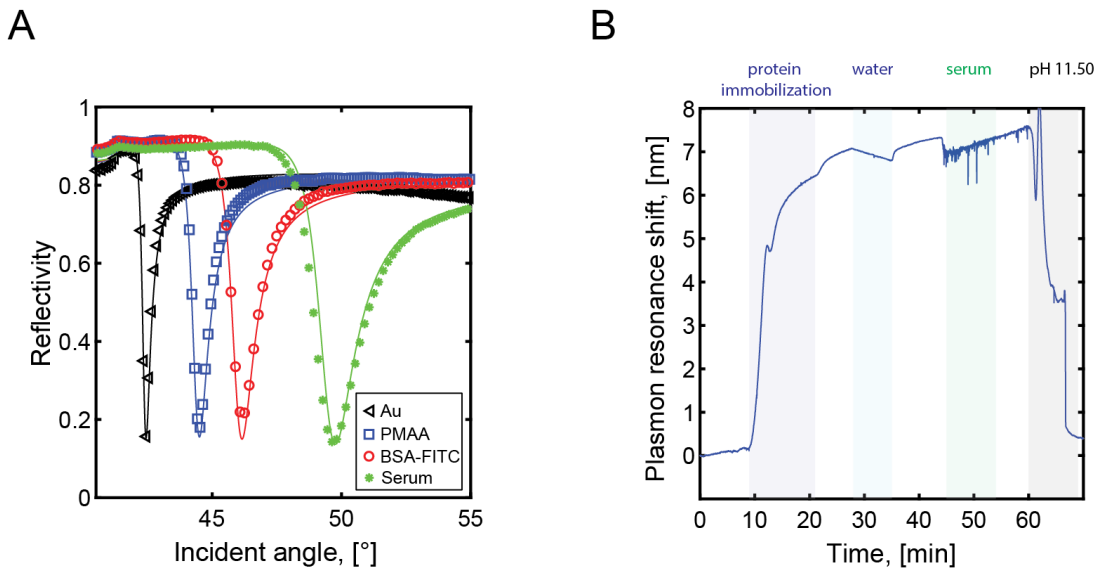

Fig. S4. Effects from exposure of immobilized proteins to serum. (A) Complementary data showing SPR scans in dry state after serum exposure. Even if the brush is first saturated with BSA, there is clearly an increase in total protein amount after exposure to serum. This is expected because the affinity of BSA is lower than for many of the other proteins in serum, especially larger ones. It should be kept in mind that the monomer density decreases with distance from the surface and thus it can be viewed as a heterogenous collection of "binding sockets". Some proteins will need to go deeper into the brush to bind, while others can bind also in the "upper" regions. However, it is still clear from the fluorescent signal (main text) that all BSA remains, i.e. there is no replacement. (B) Immobilization of avidin using PMAA on plasmonic nanohole arrays followed by rinsing with water and serum. No significant signal change occurs in serum. We attribute this to the fact that the loading capacity of serum is similar to that of avidin (main text). Avidin is eventually released in a buffer with very high pH (11.5).

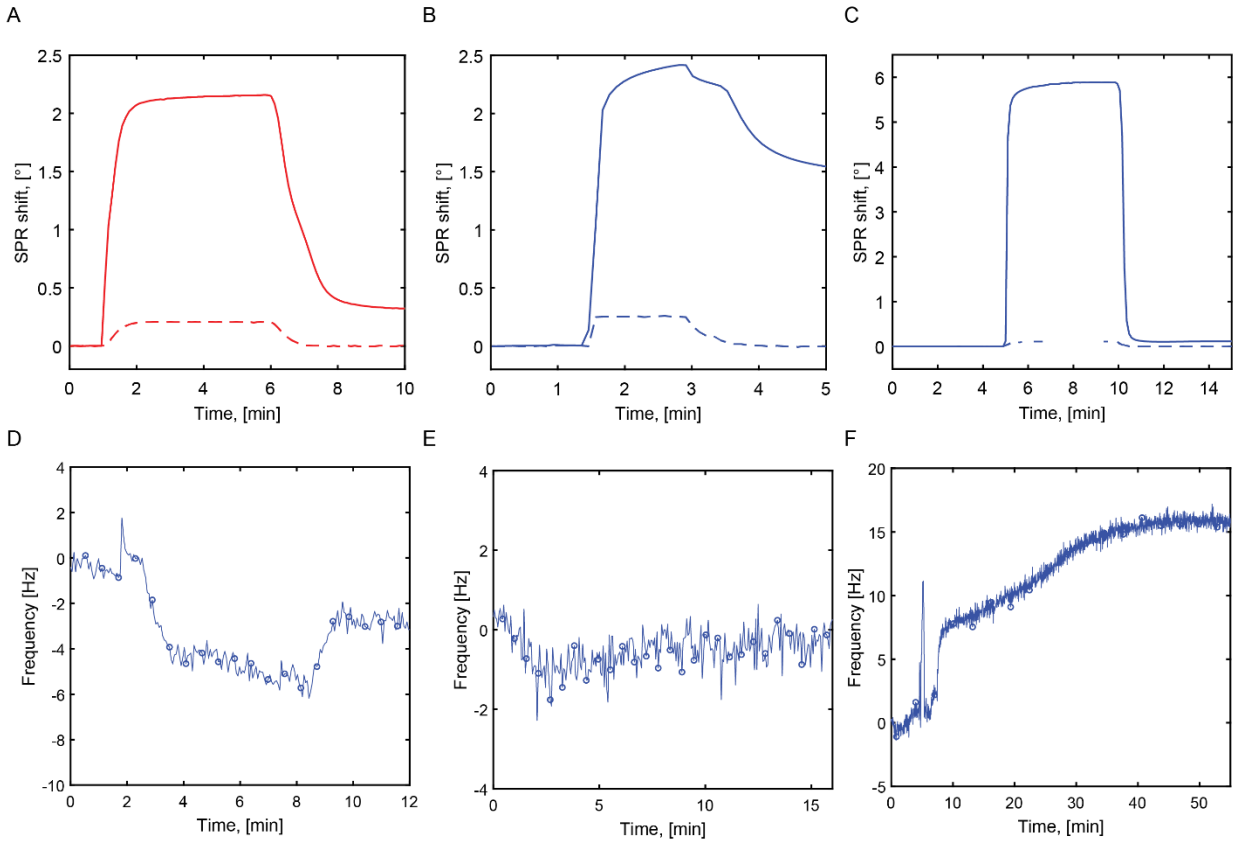

Fig. S5. Examples of interpolymer complexation by hydrogen bonds at pH 5. (A) PEG 35 kg/mol, (B) poly(*N*-isopropylacrylamide) ~20 kg/mol and (C) poly(hydroxy ethyl acylate) (unspecified molecular weight). In all cases extensive binding of polymers to the PMAA brush is confirmed since the SPR signal by far exceeds the total internal reflection angle responses (dashed lines). All these polymers are indeed supposed to interact with PMAA by hydrogen bonds according to literature.[6] In contrast, many other biopolymers did not bind: (D) Dextran (100 kg/mol), (E) plasmid DNA, and (F) single-stranded DNA-biotin, all measured in QCM, show insignificant changes in frequency compared to the proteins.

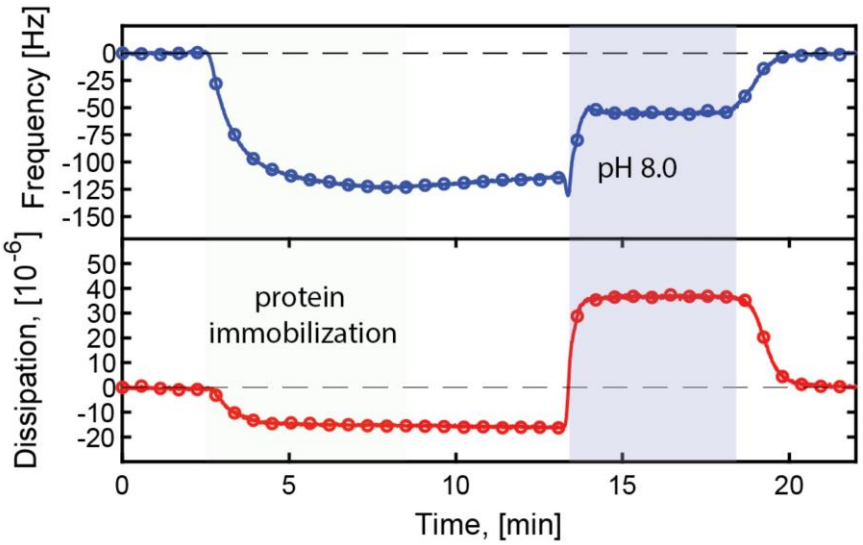

Fig. S6. Example of QCM data of protein capture and release with BSA binding to PMAA. The start and end points represent the same pH 5 buffer environment. Note that unlike for rigid systems, both frequency and dissipation decrease upon binding.

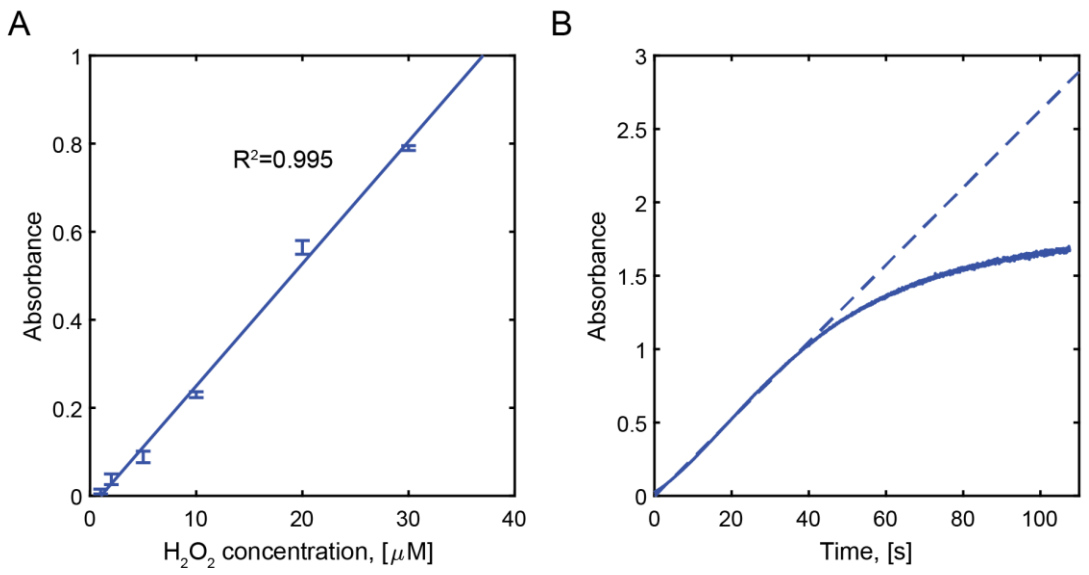

Fig. S7. GOX activity assay calibration. (A) Absorbance increase recorded at 420 nm as a function of hydrogen peroxide concentration. (B) Example initial rate determination (dashed line) for a glucose concentration of 50 mM.

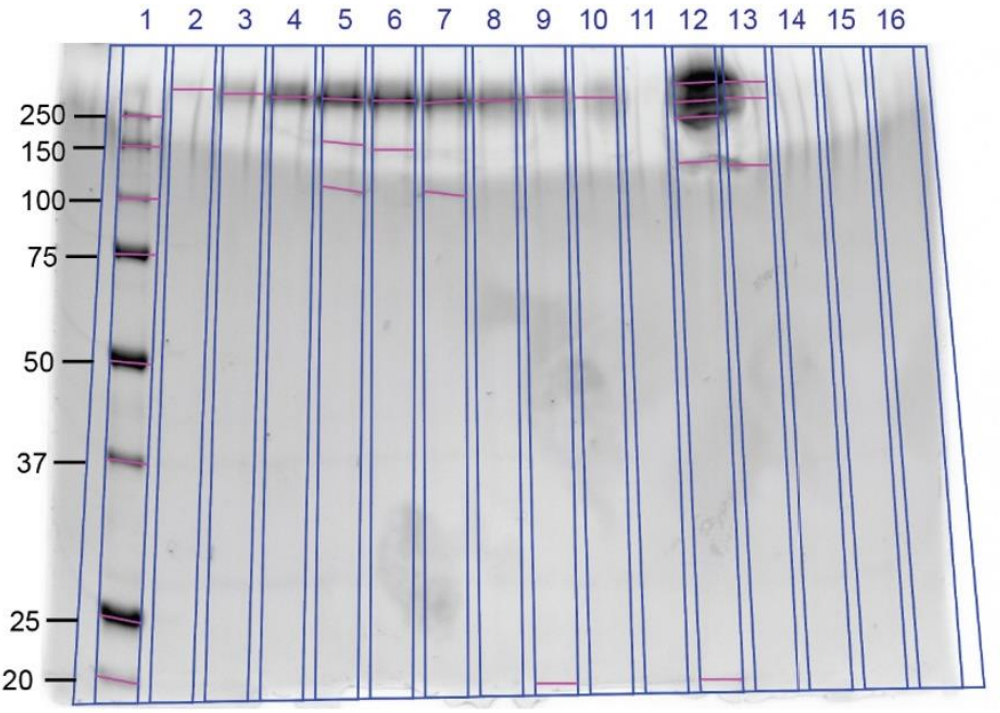

Fig. S8. Separation of PEG-conjugated BSA by size exclusion verified by SDS-PAGE. The fractions from under the eluted peak are lanes 2 to 11 and lane 12 is solution from the PEG conjugation reaction which was loaded on to the column. The molecular weight is beyond 250 kg/mol which corresponds to tens of PEG conjugated to each BSA.

Protein	PDB ID	MW (kDa)	Arg	Average	Asn	Average	Asp	Average	Cys	Average
HRP	1HCH	35	19	0,55	25	0,72	14	0,41	1	0,03
BSA	3V03	67	24	0,36	14	0,21	39	0,58	27	0,40
Lysozyme	1DPX	14	11	0,76	14	0,97	7	0,49	3	0,21
Ubiquitin	1D3Z	9	4	0,47	2	0,23	5	0,58	0	0,00
Insulin	1ZNI	6	1	0,17	3	0,52	0	0,00	6	1,04
IgG1 Mouse	1IGY	146	38	0,26	56	0,38	60	0,41	36	0,25
Protein	**Glu**	**Average**	**Gln**	**Average**	**His**	**Average**	**Lys**	**Average**	**Ser**	**Average**
HRP	6	0,17	12	0,35	2	0,06	6	0,17	21	0,61
BSA	56	0,84	18	0,27	16	0,24	59	0,88	23	0,34
Lysozyme	2	0,14	3	0,21	1	0,07	6	0,42	8	0,56
Ubiquitin	6	0,70	6	0,70	1	0,12	7	0,82	3	0,35
Insulin	4	0,69	3	0,52	2	0,35	1	0,17	3	0,52
IgG1 Mouse	64	0,44	52	0,36	30	0,20	86	0,59	150	1,02
Protein	**Thr**	**Average**	**Total**	**Average**						
HRP	23	0,67	129	3,74						
BSA	31	0,46	307	4,60						
Lysozyme	6	0,42	61	4,24						
Ubiquitin	7	0,82	41	4,78						
Insulin	2	0,35	25	4,34						
IgG1 Mouse	130	0,89	702	4,80						

Table S1. Analysis of the surface exposed amino acids with a polar or charged side chain residues capable of forming hydrogen bonds. The number of each amino acid is calculated together with the weight average with respect to the protein molecular weight. A relatively low presence (weight average <0.2) of a certain type of amino acid is marked in red. HRP contains the lowest relative number of surface exposed lysines. In addition HRP has the lowest overall weighted average number of amino acids with polar and charged side groups. It is important to note that this comparison can only provide an indication since protein structure may vary significantly depending on which species it was extracted from and post-translational modifications may alter the number of charged and polar functional groups present on the protein surface. The analysis was performed using Pymol and a script for calculating number of surface exposed residues:

findSurfaceResidues.pml, http://pymolwiki.org/index.php/FindSurfaceResidues

References (also cited in main text)

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