Supporting Information

Implementing Zn^{2+} ion and pH-value control into artificial mussel glue proteins by abstracting a His-rich domain from preCollagen.

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Contents

1. Materials

Calcium chloride dihydrate (≥99.5%), sodium citrate tribasic dihydrate (>98%), potassium chloride (≥99.5%), α-cyano-4-hydroxy-cinnamic acid (α-CHCA, 99%), formic acid (FA, ~98%), zinc chloride (≥98%), copper chloride (97%) and iron chloride (97%) were used as received from Sigma Aldrich (Seelze, Germany). Acetonitrile (HPLC-MS grade), ethanol absolute (>99.7%) and hydrochloric acid (37%) were obtained from VWR chemicals and have been used as received (Philadelphia, USA). Citric acid (≥99.5%), sodium sulfate (≥99%), sodium hydrogen carbonate (≥99.5%), sodium bromide (≥99%) and strontium chloride hexahydrate (≥99%) were used as received from Carl Roth GmbH (Karlsruhe, Germany). Boric acid (99%) and magnesium chloride hexahydrate (98%) were purchased from ABCR GmbH (Karlsruhe, Germany). Sodium chloride (≥99%) and potassium bromide (≥99%) were obtained from Acros Organics (Geel, Belgium) and have been used as received. Hellmanex III was acquired from Hellma GmbH (Müllheim, Germany).

All buffers and aqueous solutions were prepared with Milli-Q water. Ammonium acetate buffer was used for all experiments except for CD experiments, where water was used. Specified pH-values have been controlled by calibrated pH meters.

1.1 Peptide Synthesis

N-α-Fmoc protected amino acids: Fmoc-Cys(Trt)-OH, Fmoc-Gly-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(*t*Bu)-OH, Fmoc-His(Trt)-OH, Fmoc-Val-OH, Fmoc-Arg(Pbf)-OH, Fmoc-Ala-OH, Fmoc-Asn(Trt)-OH as well as coupling reagents 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronoium hexafluorophosphate (HBTU), benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate (PyBOP), and *N*-methyl-2-pyrrolidone (NMP, 99.9%, peptide synthesis grade) were used as received from IRIS Biotech GmbH (Marktredwitz, Germany). Fmoc-Rink Amide resin 100-200 mesh (loading: 0.74 mmol/g) was obtained from Rapp Polymere GmbH (Tübingen, Germany). *N, N*-diisopropyl ethylamine (DIPEA, peptide grade), piperidine (peptide grade) and 2,5-dihydroxybenzoic acid (99%) were purchased from Acros Organics (Geel, Belgium) and used without further purification. Triethylsilane (TES, 98+ %) was obtained from Alfa Aesar (Karlsruhe, Germany), trifluoroacetic acid (TFA, peptide grade) came from Acros Organics (Geel, Belgium). Dichloromethane (DCM, peptide grade) from IRIS Biotech GmbH (Marktredwitz, Germany) was distilled from CaH_2 prior to use.

Fmoc-Dopa(acetonide)-OH was synthesized using the procedure of Liu *et al.*[1]

1.2 SDS-PAGE

Dodecyl sulfate sodium salt (85%) was purchased from Acros Organics (Geel, Belgium). Glycine (≥99%) was obtained from Sigma Aldrich (Seelze, Germany). Tris(hydroxymethyl)aminomethane (Tris, ≥99.9%) was acquired from Carl Roth GmbH (Karlsruhe, Germany). The PageRuler Prestained Protein Ladder (10-180 kDa), the Pierce Lane Marker Non-reducing Sample Buffer and the Pierce Silver Stain Kit were purchased from Thermo Fisher Scientific (Waltham, MA, USA). The protein ladder and the lane marker were stored at -20 °C.

2. Instrumentation

Solid phase peptide synthesis (for HRD-based peptide) was carried out with a Liberty BlueTM Automated Microwave Peptide Synthesizer with a Liberty Blue HT12 resin loader from CEM (Kamp-Lintfort, Germany) and using Liberty standard Fmoc-procedure protocols (single coupling & capping) for synthesis in NMP at 75 °C. The first amino acid was double coupled and coupling time for the Dopa derivative was extended to 12 minutes.

MALDI-TOF. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry was performed on an autoflex III smartbeam system (Bruker, USA) with matrix assisted laser desorption/ionization and time of flight detector. On the sample plate, 2 µL of sample were mixed with 1 µL matrix solution, consisting either of 7 mg/mL α-cyano-4-hydroxy-cinnamic acid (CHCA) or 10 mg/mL 2,5-dihydroxybenzoic acid (DHB) in MQ-water-acetonitrile (1:1, v/v) with 0.1% TFA. Samples were air-dried at ambient temperature. Measurements were performed in linear positive mode. Gating and deflection modes were used for detection of the higher mass area (m/z > 10.000).

SDS-PAGE. Sodium dodecyl sulfate polyacrylamide gel electrophoresis measurements were performed in a Mini-PROTEAN tetra system cell (Bio-Rad, USA) with commercial 4-20% gel percentage precast polyacrylamide Mini-PROTEAN TGX gels (Bio-Rad, USA). As running buffer, a solution of 25 mM Tris, 192 mM glycine, 0.1% SDS in Milli-Q water was used. Staining was done according to standard protocol with a Pierce Silver Stain Kit (Thermo Fisher Scientific, USA).

Circular dichroism spectroscopy. CD measurements were done in a Jasco-720, using a Hellma™ quartz cuvette with 1.0 mm path length and 350 µL fill volume. The amounts of polymer used for CD measurements were 250 µM in phosphate buffer (10 mM) at different pH and T = 25°C.The results are shown in molar ellipticities Θ.

Fourier transformation infrared spectroscopy (FT-IR). FT-IR spectra were recorded on a Bruker Vertex 70v FT-IR spectrometer (Bruker Optics GmbH, Ettlingen, Germany) with an evacuable optics bench in a range from 4000-400 cm^{-1}. Samples were measured in solid form in ATR-IR modus under vacuum.

Gel permeation chromatography (GPC). Aqueous GPC was carried out on a NOVEMA Max analytical linear XL column (PSS, Germany) calibrated with pullulanes with an AS-100 autosampler, P-100 pump (TSP Thermo Separation Products, Germany) and Shodex RI-101 detector (VDS-optilab, Germany). As mobile phase acetate buffer (100 mM, pH 4.5):methanol, 4:1 (v/v) was used with a flow rate of 1.0 mL/min. Data were recorded and evaluated with the PSS-WinGPC UniChrom software package.

Quartz crystal microbalance (QCM). QCM measurements were conducted on a Q-sense Explorer E1 single-sensor QCM-D module with dissipation combined with a QE 401 Electronic Unit (Biolin Scientific, Sweden) and equipped with an IPC-N 4 multichannel pump (Ismatec, Germany). Piezoelectric sensor crystals coated with 100 nm aluminum oxide (QSX 309, Biolin Scientific, Sweden) were used for adsorption measurements.

Atomic force microscopy (AFM). For atomic force microscopy, the samples were spin-coated (3000 rpm) from solution (0.5-0.05 μM) on freshly cleaved Mica substrates. AFM imaging was done in tapping mode under a silicon nitride cantilever using Veeco Nanoscope VIII Multimode AFM. Commercial silicon tips (Type SCANASYST-AIR) were used with a tip radius 2-12 nm, employing a spring constant 0.4 Nm^{-1} at a resonance frequency of 50-90 kHz.

Depth sensing Nanoindentation. Mechanical studies were done using a TriboIndenter TI-950 (Hysitron-Bruker, MN, USA) equipped with a standard 2D transducer and a Berkovich tip. The tip was calibrated for the required contact depths using a standard Fused Quartz sample (E=69.6 GPa). A cyclic load function composed of 5 cycles, each cycle composed of a 5s-2s-5s loading-holding-unloading, and a max. load of 500 μN was used for measurements. The cyclic load function was used to make sure that the extracted indentation curves were not affected by the substrate or any inhomogeneity on the surface of the films. The Oliver-Pharr method was used for calculation of the elastic modulus and hardness of the samples. Three different locations per sample and 5-10 positions per location were used for measurements.

3. Methods

3.1 Peptide synthesis

Peptide synthesis was performed on Liberty BlueTM Automated Microwave Peptide Synthesizer with a Liberty Blue HT12 resin loader from CEM (Kamp-Lintfort, Germany). For synthesis standard protocol at 75 °C (coupling time: 4 min) was applied and for the first amino acid double coupling was done. Coupling time for Fmoc-Dopa(acetonide) was expanded to 12 min at 75 °C. For all amino acids, except of Fmoc-Dopa(acetonide), commercially available standard Fmoc-amino acid derivatives were used. Fmoc-Dopa(acetonide)-OH was synthesized using the procedure of Liu *et al.*[1]As solid support, Rink Amide resin (Sigma Aldrich, Seelze, Germany, loading 0.74 mmol/g, 0.1 mmol) was used. Each coupling step was carried out using DIC/oxyma as coupling and activation agent. Fmoc cleavage was done with 20 vol.% piperidine in NMP. After final Fmoc cleavage, the peptide carrying resin was transferred into a 15 mL syringe reactor and subsequently washed with NMP and DCM. Terminal amine functionality was capped with acetic anhydride (2x 5 min. Capping-solution: 9.5 mL acetic anhydride, 4.5 mL DIPEA and 0.4 g HOBt in 200 mL total solution volume, using NMP as solvent). After washing with NMP and finally with DCM, the peptides were cleaved from the solid support. Cleavage of the peptides was carried out with 95:2.5:2.5 vol.% TFA/H_2O/TES. Fully deprotected peptide was received after 3 h at room temperature. The resin was filtered and the supernatant was concentrated. The product was isolated by precipitation in cold diethyl ether followed by centrifugation and the peptides were obtained by lyophilization of the eluent.

3.2 SDS-PAGE

Samples for gel electrophoresis as well as the protein ladder were diluted with Milli-Q water to a volume of 20 µL and subsequently 5 µL of lane marker were added and mixed. The gel cassettes were clamped into the electrode assembly and the assembly and the tank was filled with approximately 800 mL of running buffer (25 mM Tris, 192 mM glycine, 0.1% SDS in Milli-Q water). Samples of 20 µL were loaded into the wells of the gel using a 10-100 µL pipette and runs were performed at 140 V until the lane marker reached the lower end of the gel. Staining was performed using silver stain (Thermo Fisher Scientific, USA) according to the manufacturer's standard protocol.

3.3 Quartz crystal microbalance

The piezoelectric sensor crystals coated with 100 nm aluminum oxide (QSX 309, Biolin Scientific, Sweden) were cleaned with 2% Hellmanex III solution (in Milli-Q water) for 15-30 min and ethanol (absolute, >99.7%) in an ultrasonic bath for 10 min prior to use. Subsequently, the sensors were thoroughly washed with Milli-Q water and dried under compressed air flow. Finally, the aluminum oxide coated crystals were cleaned by air plasma in a nanoETCH (Moorfield Nanotechnology) for 30 min 30W. The sensors were mounted into the QCM flow chamber and incubated with degassed sodium citrate buffer using a flow rate of 100 µL/min until the frequency signals were constant (1-3 h). The measurement was started and sample solutions were pumped into the flow chamber. Experiments were performed at 22°C in a stop-flow mode, and overtones 3, 5, 7, 9, 11 and 13 were recorded. The third overtones of all experiments were used for evaluation of the frequency shift.

4. Y*-HRD-C unimer characterization

The peptide unimer with the sequence **Y***-AVAHAHAHAHASAGANGRARAHARA-**GGC** (Y*-*HRD*-C, with Y* being the Dopa residue) was synthesized by fully automated solid-phase supported peptide synthesis (SPPS).

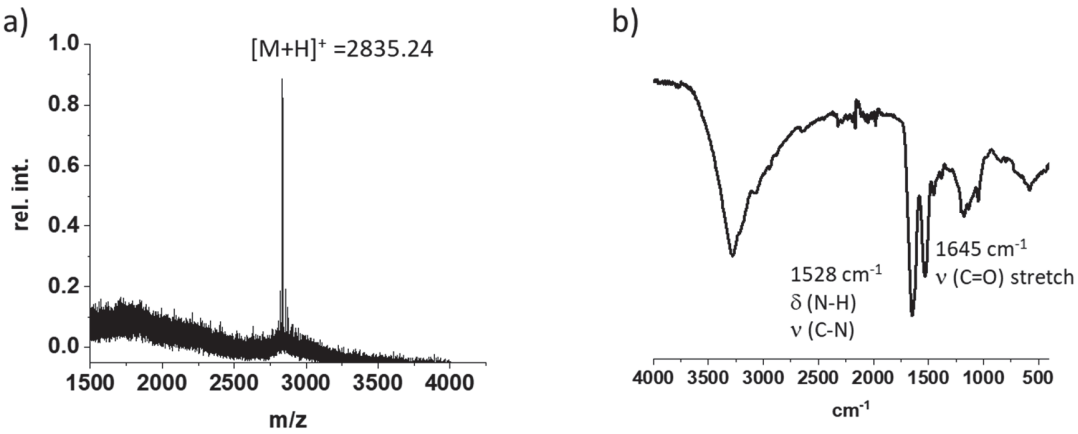


Figure S1. a) MALDI-TOF-MS analysis of Y*-*HRD*-C. Calculated: [M] = 2834.24 Da, found: $[M+H]^+$=2835.20. b) IR spectra of peptide (Y*-*HRD*-C).

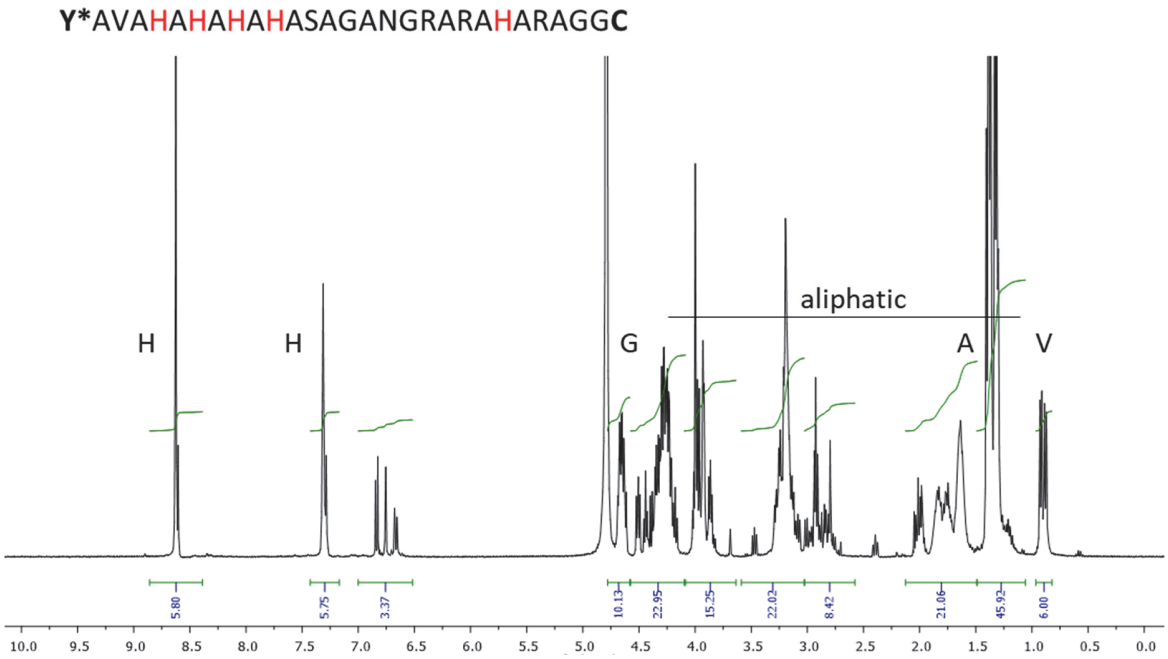


Figure S2. ^{1}H-NMR analysis of Y*-*HRD*-C (D_2O, 400 MHz).

5. Experiments

5.1 SDS-PAGE polymerization kinetics

For polymerization 50 µL of a peptide (Y*-*HRD*-C) solution (0.017µmol/mL in ammonium acetate buffer, 20 mM, pH 7) was mixed with 10 µL sodium periodate solution (2.53 mg/mL in Milli-Q water, 1.5 equiv.). Kinetic samples were set to finish at the same time and were directly analyzed using SDS-PAGE. Investigated polymerization times at room temperature were 1 h, 2 h, 4 h, 7 h and 24 h. Additionally one non-activated sample was analyzed as reference, where the molecular weight increase was not found (Figure S3a). All polymerization reactions were carried out in polypropylene reaction vessels. Moreover, the SDS-PAGE was additionally used to investigate the products obtained from polymerization reactions at different pH-values ranging from pH 3 to pH 8. Therefore, 50 µL of peptide (Y*-*HRD*-C) solution (0.017 µmol/mL in ammonium acetate buffer, 20 mM) at pH 3.0, 4.0, 5.0, 5.5, 6.0, 7.0 and 8.0 was treated with 1.5 equiv. of $NaIO_4$ (2.531 mg/mL in Milli-Q water). The comparison of the different samples after running SDS-PAGE shows that the average molecular weight reaches a maximum at pH 7.0.

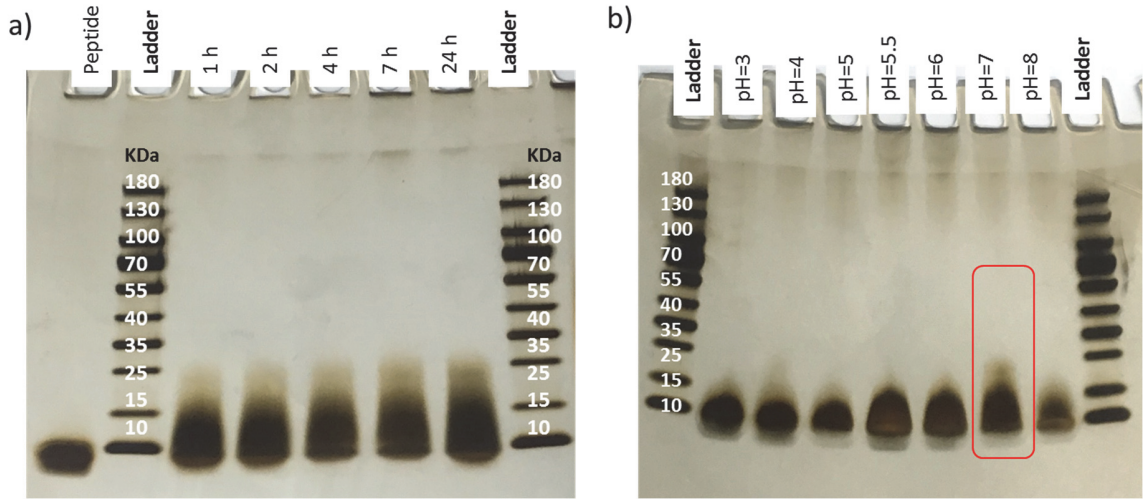


Figure S3. SDS-PAGE measurement of polymer reaction a) kinetics and b) at different pH conditions. (Conditions: 0.25 mM peptide(Y*-*HRD*-C) in ammonium acetate buffer 20mM at different pH range, 1.5 equiv. $NaIO_4$, in water).

5.2 Poly(Y*-*HRD*-C) characterization

a) GPC analysis of poly(Y*-*HRD*-C) obtained at pH 7

For GPC analysis larger reaction batches were synthesized. 1.5 mL of peptide(Y*-*HRD*-C) solution, 0.705 µmol/mL in ammonium acetate buffer (20 mM, pH 7) were activated using 300 µL sodium periodate solution (1.5 equiv., 1.06µmol/mL in Milli-Q water). The reaction was carried out for 4 h at room temperature and the mixture was directly analyzed with GPC.

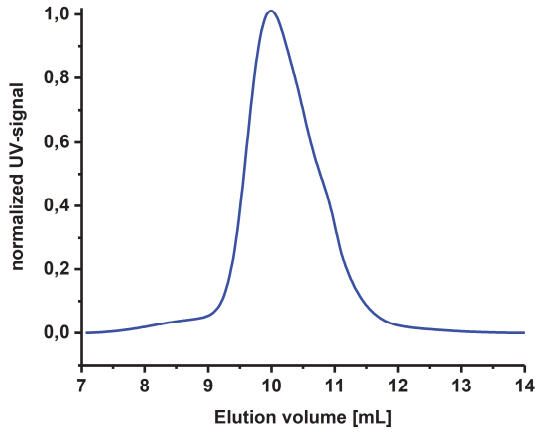


Figure S4. GPC trace of poly(Y*-*HRD*-C) obtained at pH 7: $M_{n,app}$ = 23700 g/mol, Đ = 1.4.

b) MALDI-TOF-MS

A 0.25 mM solution of peptide(Y*-*HRD*-C) in 50 mM sodium phosphate buffer at pH = 7 was treated with 1.5 equiv. of $NaIO_4$ for 15 min. Subsequently, 1.2 µL of the sample was mixed with 2 µL of matrix solution (7 mg/mL α-cyano-4-hydroxycinnamic acid (HCCA) in Milli-Q water-acetonitrile (1:1, v/v) + 0.1 % TFA) and spotted on a MALDI plate. The MALDI-TOF-MS measurement shows the formation of multimers up to DP = 4, that are linked via cysteine dopaquinone addition. The average difference of mas signals account for 2834.04Da, which corresponds to the repeat unit mass of peptide(Y*-*HRD*-C) (calc. 2834.24 Da).

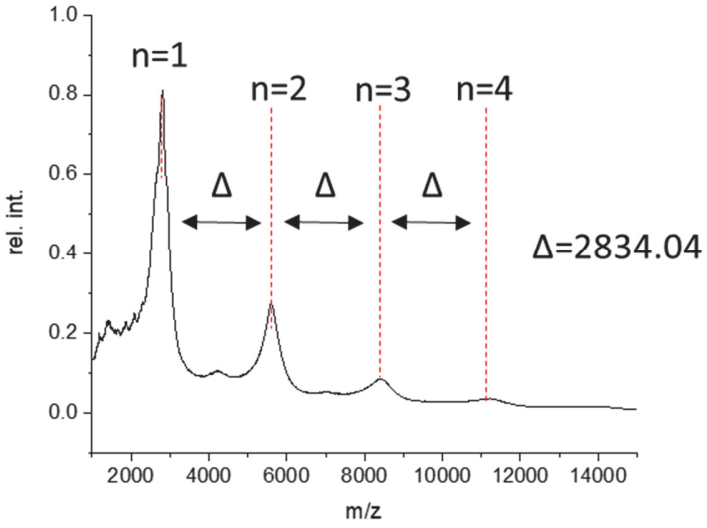


Figure S5. MALDI-TOF-MS spectrum of poly(Y*-*HRD*-C)

c) NMR analysis

For NMR analysis 2 mL of peptide(Y*-*HRD*-C) solution, 3.55 µmol/mL in ammonium acetate buffer, 20 mM, pH 7) were activated using 1 mL sodium periodate solution (1.5 equiv., 5,32 µmol/mL in Milli-Q water). The reaction was carried out for 4 h at room temperature and then was precipitated in acetone and dried in high vacuum.

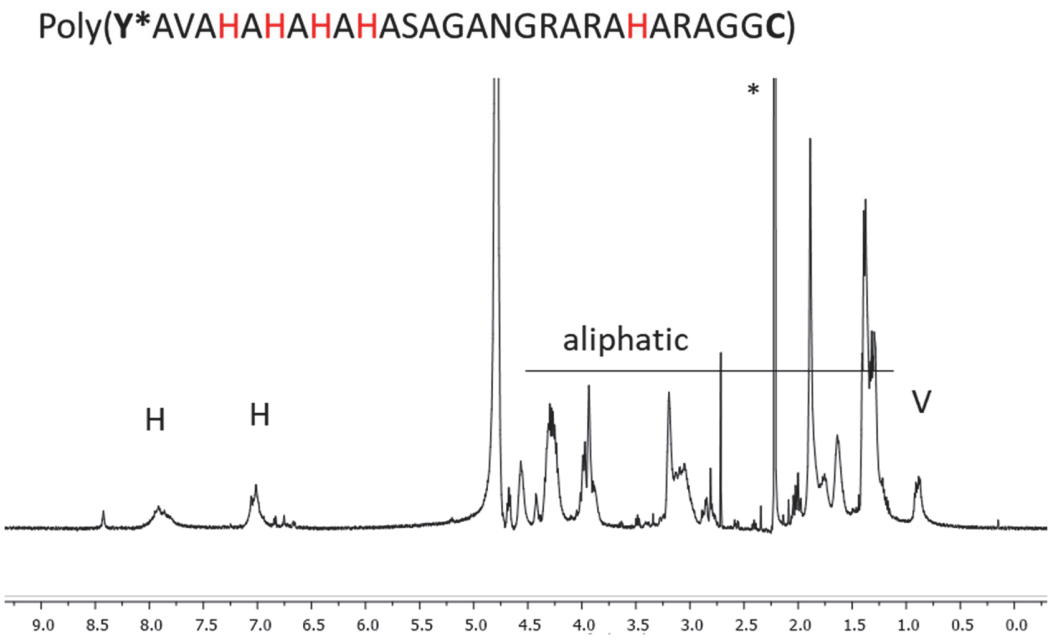


Figure S6. ^{1}H-NMR analysis of poly(Y*-*HRD*-C) in D_2O (400 MHz, *acetone).

5.3 Secondary interactions characterization

a) Monitored by IR

By FTIR analysis of the amide I band in the 1600−1700 cm^{-1} region (predominantly corresponding to the amide C=O stretch), it is possible to determine the secondary-structure composition of the sample and assign β-sheet structures or turns (Figure S7).[2, 3] The band shifts induced by hydrogen bonding indicates changes in the secondary structure.

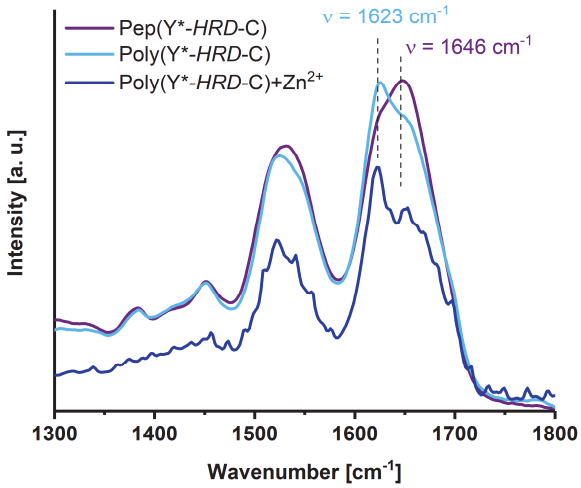


Figure S7. ATR-IR measurements.

b) Monitored by circular dichroism

- **HRD-based peptide (Y*-*HRD*-C)**

A 125 µM solution of peptide (Y*-*HRD*-C) in water at pH 4.0 and pH 7.0 was measured (Figure S8a). The measurements of the peptide in both acidic and basic conditions show the absence of β-sheet formation. This is due to the fact that the peptide at pH 7.0 is not able to form β-sheets with a signal intense enough to be seen in the CD spectrum (Figure S8a).

- **HRD-based poly(Y*-*HRD*-C)**

The polymerization was carried out using the protocol in section S5.1, using 0.705 µmol/mL peptide solution in ammonium acetate buffer (20 mM, pH 7), 300 µL sodium periodate solution (1.5 equiv., 1.06µmol/mL in Milli-Q water) and 4 h reaction time. The polymer was precipitated in acetone and dried in high vacuum. A solution of the polymer product with a concentration of 125 µM polymer in water at pH 4.0 or pH 7.0 was measured by CD spectroscopy. The CD spectra of the polymer at pH 4.0 show the absence of an organization in the secondary structure of the polymer (Figure S8b). However, after the adjustment of the pH to 7.0, a transition in secondary structure was evident as probably the histidine residues trigger self-assembly.[4] The presence of the typical Cotton effects for β-sheets at (-) 223 nm were evident in the CD spectra (Figure S8b). Besides these, random coil structures also remain visible in the CD spectra at (-) 203 nm even after 24 hours. This indicates the presence of intrinsically unstructured regions, probably as a result of the cysteinyldopa connectivities that act as structural defects (Figure S8b).

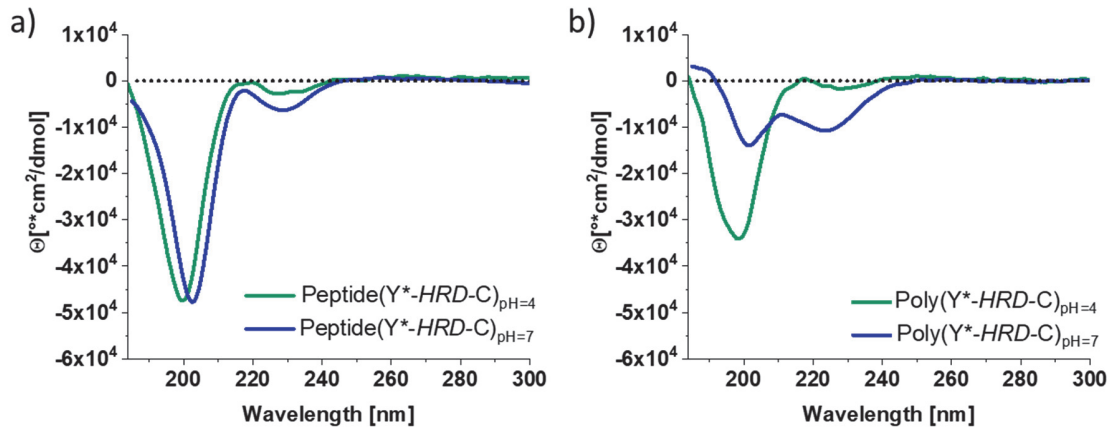
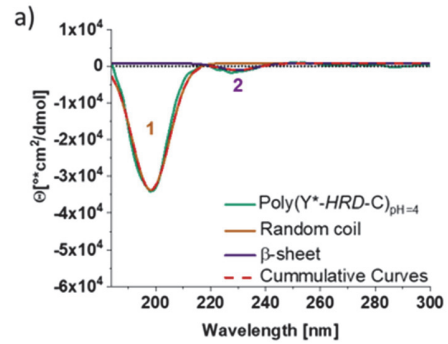
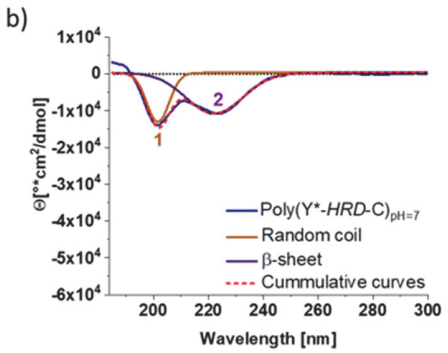


Figure S8. CD spectra of the (a) HRD-based peptide and (b) HRD-based polymer at different pH conditions.

Deconvolution of CD spectra of the polymer at different pH shows a decrease of 77% of the random coil structure from pH 4 to pH 7, while the presence of β-sheet increased at pH 7 with respect to the random coil fraction (Figure S9).



Model	Gaussian	
Equation	y = y0 + A/(w*sqrt(pi/(4*ln(2)))) * exp(-4*ln(2)*(x-xc)^2/w^2)	
Plot	**Random coil**	**β-sheet**
y0	741.78 ± 51.90	741.78 ± 51.90
xc	198.23 ± 0.03	229.86 ± 0.58
A	-581473.82 ± 2871.68	-30896.39 ± 2892.98
w	15.83 ± 0.08	15.83 ± 1.48
Reduced Chi-Sqr	1476767.35	
R-Square(COD)	0.98	
Adj. R-Square	0.98	



Model	Gaussian	
Equation	y = y0 + A/(w*sqrt(pi/(4*ln(2)))) * exp(-4*ln(2)*(x-xc)^2/w^2)	
Plot	**Random coil**	**β-sheet**
y0	308.91 ± 38.98	308.91 ± 38.98
xc	201.59 ± 0.06	222.15 ± 0.13
A	-137621.93 ± 2445.40	-288258.99 ± 3803.26
w	9.69 ± 0.14	24.43 ± 0.34
Reduced Chi-Sqr	431633.27	
R-Square(COD)	0.98	
Adj. R-Square	0.98	

Figure S9. Deconvolution curves and the correspond table of poly(Y*-*HRD*-C) at a) pH 4 and b) pH 7.

- **HRD-based poly(Y*-*HRD*-C) with metal ions**

Stock solutions of $ZnCl_2$, $CuCl_2$ and $FeCl_2$ were prepared with concentrations of 100 mM. After the polymerization of peptide (Y*-*HRD*-*C*), 125 µM of polymer solution in water at pH 4.0 with different metal ions was measured by CD spectroscopy (Figure S10). It was observed that the protonated histidine residues at pH 4 were able to coordinate to the metal ions and considering all presented results, the polymer containing Zn^{2+} shows the highest propensity to form β-sheets.

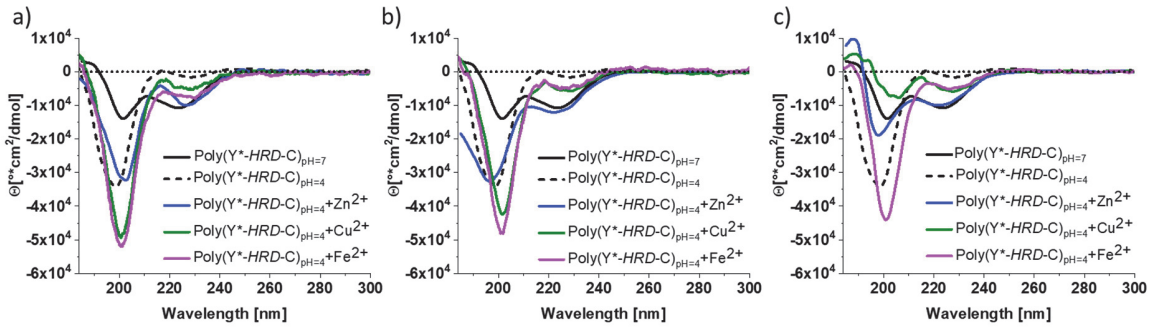


Figure S10. CD spectra of the polymer(Y*-*HRD*-*C*) at pH 4 with different metal ions. a) His/M^{2+} ~1:0.33, b) His/M^{2+} ~1:3.30 and c) His/M^{2+} ~1:33.

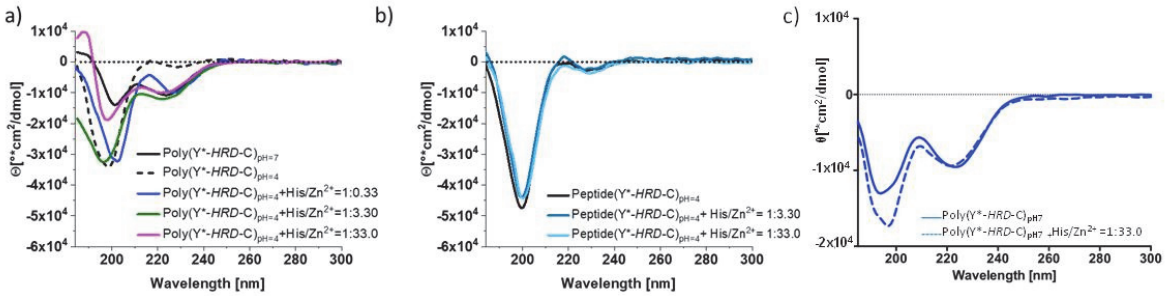
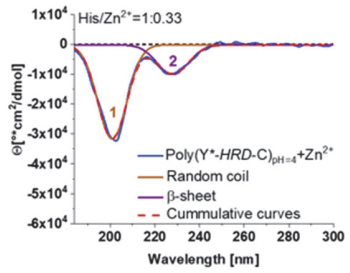


Figure S11. CD spectra of a) poly(Y*-*HRD*-C) at pH 4 with Zn^{2+}, b) non-polymerized peptide Y*-*HRD*-C at pH 4 with Zn^{2+} and c) poly(Y*-*HRD*-C) at pH 7 with and without Zn^{2+}.

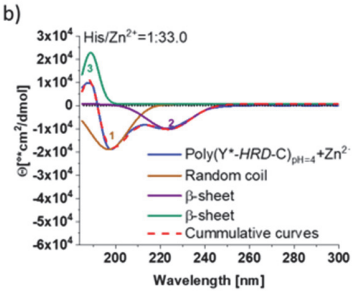
It is noteworthy that, for the peptide (Y*-*HRD*-C) at pH 4 with Zn^{2+} as a metal ion, the CD spectra of the peptide do not vary in the presence of Zn^{2+} (Figure S11b). However, when similar experiments were carried out with polymer (Y*-*HRD*-C) at pH 4 in presence of Zn^{2+} (His/Zn^{2+} ~1:0.33) the signal of the random coil structure decreases 12% respect to the polymer at pH 4 without metal ion and a β-sheet signal appears (Figure S11-S12a). Moreover, by increasing the amount of Zn^{2+} (His/Zn^{2+} ~1:33.0) the band of the random coil continually decreases and the β-sheet band at (-) 223 nm increases up to 34% compared with (His/Zn^{2+} ~1:0.33) and even the cotton effect at (+) 191 nm typical for β-sheets was not fully superimposed by the cotton effect of the random coil at (-) 190 nm (Figure S11a-S12b).

a)



Model	Gaussian	
Equation	y = y0 + A/(w*sqrt(pi/(4*ln(2)))) * exp(-4*ln(2)*(x-xc)^2/w^2)	
Plot	Random coil	β-sheet
y0	-189.79 ± 29.51	-189.79 ± 29.51
xc	200.90 ± 0.02	227.96 ± 0.07
A	-513866.66 ± 1661.83	-174126.83 ± 1756.27
w	15.35 ± 0.05	16.56 ± 0.17
Reduced Chi-Sqr	511201.98	
R-Square(COD)	0.99	
Adj. R-Square	0.99	

b)



Model	Gaussian		
Equation	y = y0 + A/(w*sqrt(pi/(4*ln(2)))) * exp(-4*ln(2)*(x-xc)^2/w^2)		
Plot	Random coil	β-sheet	β-sheet
y0	632.25 ± 90.25	632.25 ± 90.25	632.25 ± 90.25
xc	196.97 ± 1.62	223.85 ± 0.66	188.83 ± 0.15
A	-416378.52 ± 90571.40	-261093.19 ± 17170.63	202215.10 ± 62117.04
w	20.05 ± 2.98	22.87 ± 1.21	8.57 ± 0.85
Reduced Chi-Sqr	410817.22		
R-Square(COD)	0.99		
Adj. R-Square	0.99		

Figure S12. Deconvolution curves and the correspond table of poly(Y*-*HRD*-*C*) in presence of Zn^{2+} a) (His/Zn^{2+} ~1:0.3) and b) (His/Zn^{2+} ~1:33.3).

Table S1. Summary of the secondary structure ratios of poly(Y*-*HRD*-*C*) under different conditions as determined by CD spectra analysis.

Polymer	Random coil area (-190 nm)	%	β-sheet area (-223 nm)	%
poly(Y*-*HRD*-C)$_{pH\ 4}$	581474	95	30896	5
poly(Y*-*HRD*-C)$_{pH\ 7}$	137622	32	288259	68
poly(Y*-*HRD*-C)$_{pH\ 4}$ + Zn^{2+} (His/Zn^{2+} = 1:0.33)	513867	75	174127	25
poly(Y*-*HRD*-C)$_{pH\ 4}$ + Zn^{2+} (His/Zn^{2+} = 1:33.0)	214163	32	261093 202215	68

5.4 Microscopy studies

Microscopy analysis was performed in order to visualize nanostructures generated by the formation of β-sheet structures in the poly(Y*-*HRD*-C). The polymer was obtained according to the protocol in section 5.1, using a 0.705µmol/mL peptide solution (to avoid the formation of salts on the films), which were activated using 300 µL sodium periodate solution (1.5 equiv., 1.06µmol/mL in Milli-Q water). After 4 h the polymer was precipitated in acetone and frozen in liquid nitrogen and lyophilized. Subsequently, the samples were dissolved in a buffer solution at pH 4.0 and pH 7.0 with a concentration of 0.5 µM.

- **Atomic force microscopy (AFM)**

AFM samples were prepared by spin coating using 10 µL of a 0.5 µM solution of poly(Y*-*HRD*-C) at pH 7.0 and pH 4 onto freshly cleaved Mica substrates at 100 rpm followed by 2500 rpm to dry the substrate. A fibrillar aggregate at pH 7.0 confirms the formation of β-sheet while at pH 4.0 this aggregation structure is not overserved (Figure S13).

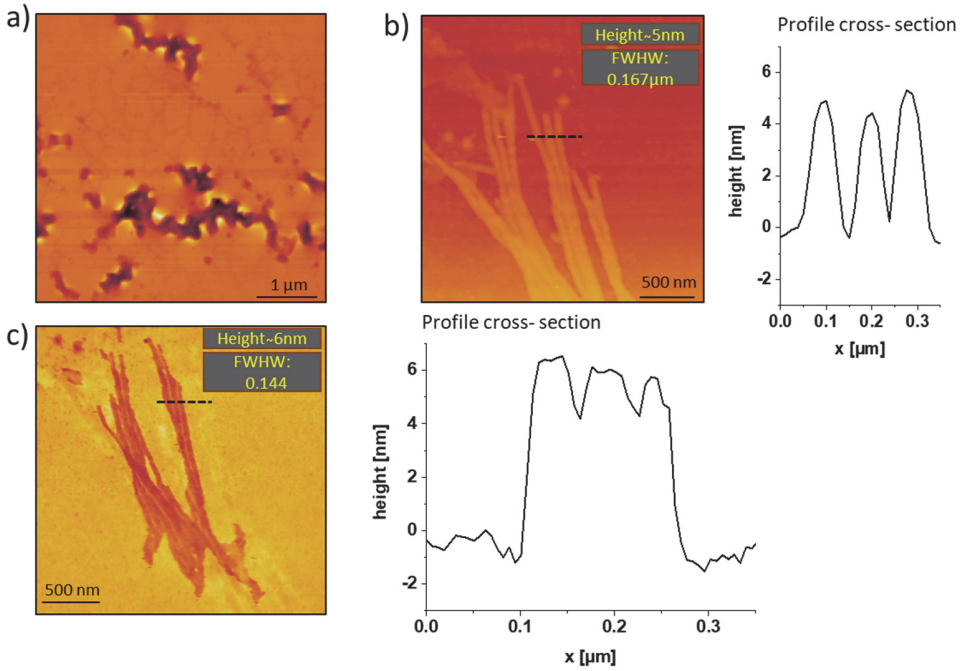


Figure S13. AFM images of poly(Y*-*HRD*-C) at (a) pH 4.0 and (b-c) pH 7.0 showing the absence (a) and presence (b-c) of fibrillar structures due to β-sheet formation (dotted line indicates section corresponding to the height profile cross-section).

5.5 QCM-D experiments on aluminum oxide surface

- ***HRD-based polymer at different pH***

For QCM measurements, the polymerization was carried out using the protocol in section 5.1, using 0.705µmol/mL peptide solution in ammonium acetate buffer (20 mM, pH= 7), 300µL sodium periodate solution (1.5 equiv., 1.06µmol/mL in Milli-Q water) and 4 h reaction time. The polymer was precipitated in acetone, dried and dissolved in 26 mL of a mixture of ammonium acetate buffer and degassed Milli-Q water (1:25, v/v) at pH 4.0 and pH 7.0, as appropriate (Figure S14a-b). All measurements were performed at 100 µL/min flow on an aluminum oxide-coated sensor (QSX 309, Biolin Scientific, Sweden). Changes in frequency (Δf) and energy dissipation (ΔD) were recorded for overtones n = 3, 5, 7, 9, 11 and 13. Calculations of adsorbed masses for the polymer coatings were performed with QSense Dfind software by approximation of the recorded data according to the Voight-based model.[5, 6]

For equilibration of the QCM-D system, 1 mL of the applied ammonium acetate buffer (20 mM, pH 7) was diluted in 25 mL Milli-Q water.

The poly(Y*-*HRD*-C) at pH 7.0 shows a remarkably rapid adsorption from aqueous solutions onto the alumina surface, resulting in a frequency shift of Δf = –30 Hz (Figure S14). The dissipation increases, indicating the presence of viscoelastic behavior of the coating. This requires the application of the Voight model to estimate mass deposition and film thickness. Taking the model into account, 5.31 g/m^2 of poly(Y*-*HRD*-C) at pH 7.0 was deposited during incubation, leading to a coating thickness of approximately 4.5 nm. Interestingly, the subsequent rinsing step with buffer resulted in further decrease of the frequency to reach –35 Hz after 3 h and a continuous decrease (Figure S14a). This effect was not expected, but is also not uncommonly observed for strong, high molecular weight binders, where coatings are formed by kinetically controlled deposition and film equilibration proceeds more slowly by taking up solvents/salts.

The poly(Y*-*HRD*-C) at pH 4.0 also shows a remarkably rapid adsorption from aqueous solution onto the alumina surface, resulting in a frequency shift of Δf = –6 Hz (Figure S14b). Due to the absence of the β-sheet formation under such acidic conditions, a coating with rather low mass deposition of 1.06 g/m² was estimated by applying the Voight model.[6] As in the previous case, the subsequent rinsing step with buffer resulted in further decrease of the frequency to reach –14 Hz. One detail appears to be notable in all cases, as the film deposition is highly rapid, the material coatings are seemingly not equilibrated with the aqueous surrounding (cf. reswelling while buffer rinsing). Potentially, this might indicate a "drying mechanism" where water is expelled from hydrated substrate surfaces, thereby reproducing one of the extraordinary effects found in the mussel adhesion process.[7]

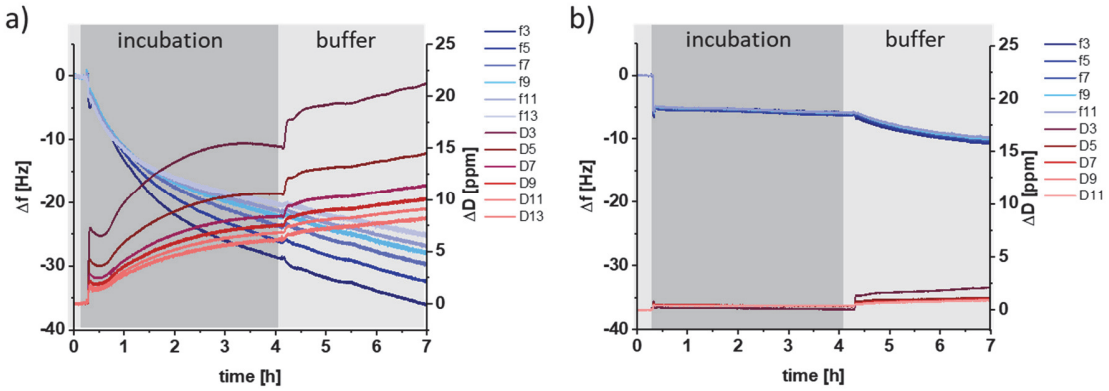


Figure S14. QCM-D experiments of poly(Y*-*HRD*-C) on alumina surfaces at pH 7.0 (a) and pH 4.0 (b).

- ***Rinsing of polymer coating***

After equilibration with buffer and incubation of the diluted polymer mixture, the stability of the coating was tested. For this, washing steps with 599 mM NaCl and 4.2 M hypersaline solution were carried out (Figure S15). The hypersaline solution used was modeled after salt concentrations of Dead Sea water[8] and contained $MgCl_2$•$6H_2O$ (368.0 g/L, 1.81 mol/L), NaCl (97.0 g/L, 1.66 mol/L), $CaCl_2$•$2H_2O$ (63.2 g/L, 0.43 mol/L), KCl (14.9 g/L, 0.20 mol/L), NaBr (6.82 g/L, 66.33 mmol/L), Na_2SO_4 (664.7 mg/L, 4.68 mmol/L) and $NaHCO_3$ (275.6 mg/L, 3.28 mmol/L). The calculated differences in adsorbed masses amounts to 5.1 g/m² (5.6%) for NaCl rinsing and 5.3 g/cm² (8.9%) for hypersaline rinsing, which demonstrates that the coating is highly stable against salinity (Figure S15 and Table S2).

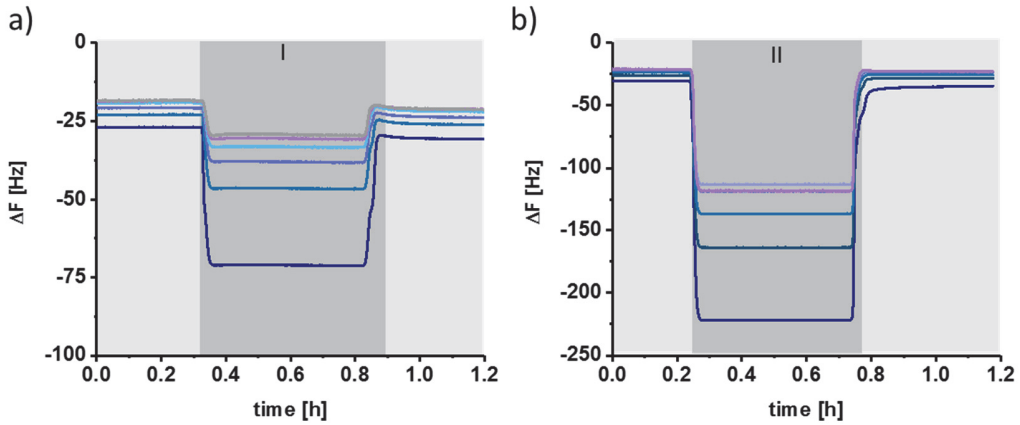


Figure S15. QCM-D adsorption and desorption kinetics of rinsing poly(Y*-*HRD*-C) modified Al_2O_3 surface at pH 7.0 with a) 599 mM NaCl solution (I) and b) 4.2 M hypersaline solution (II). Overtones 3, 5, 7, 9, 11 and 13 are shown.

- ***HRD-based polymer with ions***

The polymerization was carried out using 0.705 µmol/mL peptide solution, 300 µL sodium periodate solution (1.5 equiv., 1.05 µmol/mL in Milli-Q water) and 4 h reaction time. The polymer was precipitated in acetone, dried and after that, dissolved in 1.5 mL ammonium acetate buffer 20 mM at pH 4.0. His/Zn^{2+} ~1:3.3 equivalents of a $ZnCl_2$ (100 mM) and 26 mL degassed Milli-Q water (1:25, v/v) at pH 4.0 were added before starting the measurements (Figure 16a). The poly(Y*-*HRD*-C) at pH 4.0 with 20 equivalents of Zn^{2+} shows a remarkably rapid adsorption from aqueous solutions onto the alumina surface, resulting in a frequency shift of $\Delta f = -31$ Hz (overtone 3, Figure S16a). Taking the Voight model into account, 5.5 g/m^2 of poly(Y*-*HRD*-C)$_{pH=4}$ + Zn^{2+} (His/Zn ~3:1) was deposited during incubation, leading to a coating thickness of approximately 5.5 nm. The subsequent rinsing step with buffer resulted in frequency shift of $\Delta f = 26$ Hz, 4.6 g/m^2. After equilibration with buffer and incubation of the diluted polymer mixture, stability of the coating was tested by washing with 599 mM NaCl and 4.2 M hypersaline solution (Figure S14b-c). The calculated differences in adsorbed masses amounts to 3.9 g/cm² (9.6%) for NaCl rinsing and 3.7 g/m² (5.1%) for hypersaline rinsing, which demonstrates that the coating is highly stable against salinity (Figure S16b-c and Table S2).

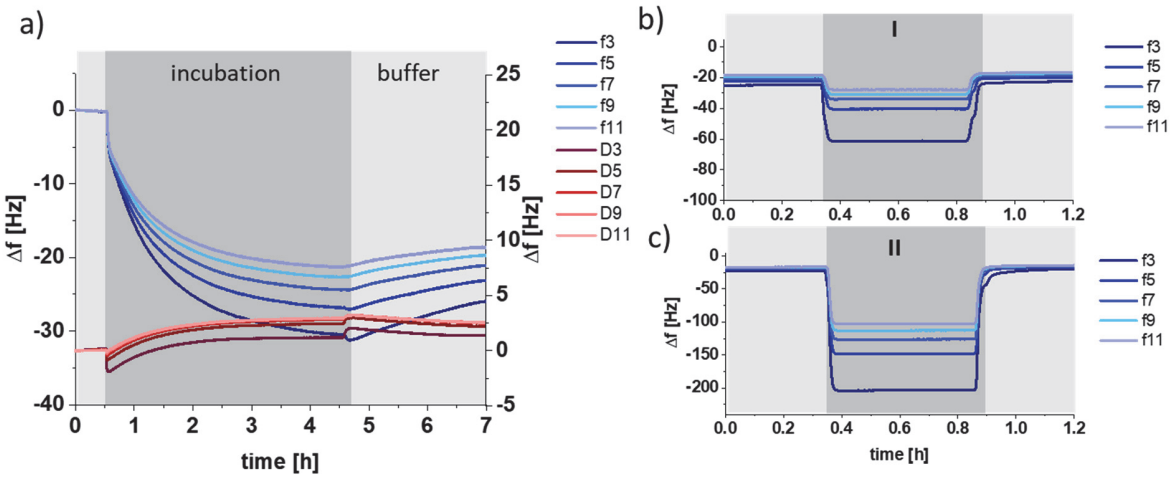


Figure S16. a) QCM-D adsorption and desorption kinetics of rinsing poly(Y*-*HRD*-C) modified Al_2O_3 surface at pH 4.0 in presence of Zn^{2+}(His/Zn ~3:1). b) 599 mM NaCl solution (I) and c) 4.2 M hypersaline solution (II). Overtones 3, 5, 7,9 and 11 are shown.

Table S2. Summary of mass deposition and washing stability of the poly(Y*-*HRD*-C) coatings under different conditions.

Polymer	Incubation	Buffer rinse	Saline solution	%	Hypersaline solution	%
poly(Y*-*HRD*-C)$_{pH\ 4}$	Δf = -6 Hz; 1.06 g/m²	-				
poly(Y*-*HRD*-C)$_{pH\ 7}$	Δf = -30 Hz; 5.31 g/m²	Δf = -27.5 Hz; 4.8 g/m²	Δf = -29 Hz; 5.1 g/m²	6	Δf = -32 Hz; 5.6 g/m²	9
poly(Y*-*HRD*-C)$_{pH\ 4}$ + Zn^{2+}	Δf = -31 Hz; 5.50 g/m²	Δf = -24.8 Hz; 4.4 g/m²	Δf = -22.4 Hz; 3.9 g/m²	10	Δf = -21 Hz; 3.7 g/m²	5

- ***HRD-based peptide control***

As a control experiment QCM-D measurement of a non-polymerized reference was carried out. 1.5 µL peptide Y*-*HRD*-C (0.705 µmol/mL in ammonium acetate buffer, 20 mM, pH 7) was mixed with 300 µL Milli-Q water. After 4 h at room temperature the reaction mixture was diluted with 25 mL degassed Milli-Q water and analyzed via QCM-D (Figure S17).

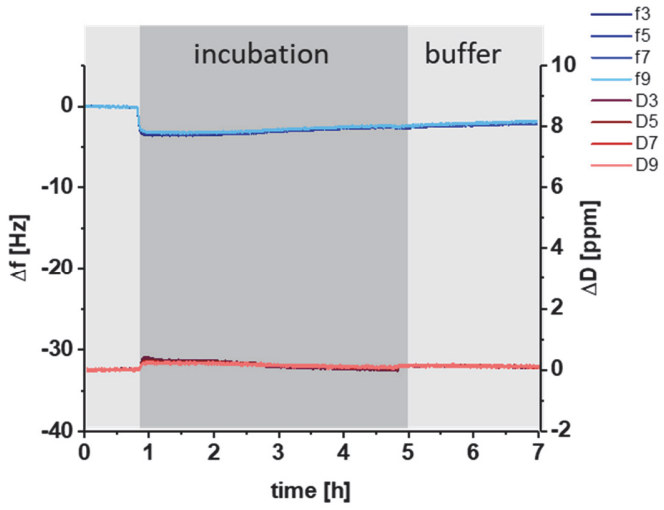


Figure S17. QCM-D analysis of non-polymerized peptide (Y*-*HRD*-C) as reference to the polymer system at pH 7.

5.6 Depth-sensing nanoindentation measurements

For nanoindentation analysis three different samples of 1.5 mL of peptide(Y*-*HRD*-C) solution, 0.705 μmol/mL in water at pH 7 (to avoid the formation of salts on the films), were activated using 300 μL sodium periodate solution (1.5 equiv., 1.06μmol/mL in Milli-Q water). After 4 h the three samples were purified by precipitation in cold acetone. Afterwards samples were frozen in liquid nitrogen and lyophilized.

- Film preparation for poly(Y*-*HRD*-C) at pH 7

One of the samples was dissolved in water at pH 7 (0.705 μmol/mL) and 10 μL of the solution were deposited onto the silicon wafer surface by drop casting.

- Film preparation for poly(Y*-*HRD*-C) at pH 4 with Zn^{2+}

The second sample was dissolved in water at pH 4 (0.705 μmol/mL). After 3 h, a solution of His/Zn^{2+} ~1:3.3 was added and after 20 min 10 μL were deposited onto the silicon wafer surface by the drop casting method.

- Film preparation for poly(Y*-*HRD*-C) at pH 4

The last sample was dissolved in water at pH 4 (0.705 μmol/mL). After 3 h, 10 μL of the solution were deposited onto the silicon wafer surface by the drop casting method.

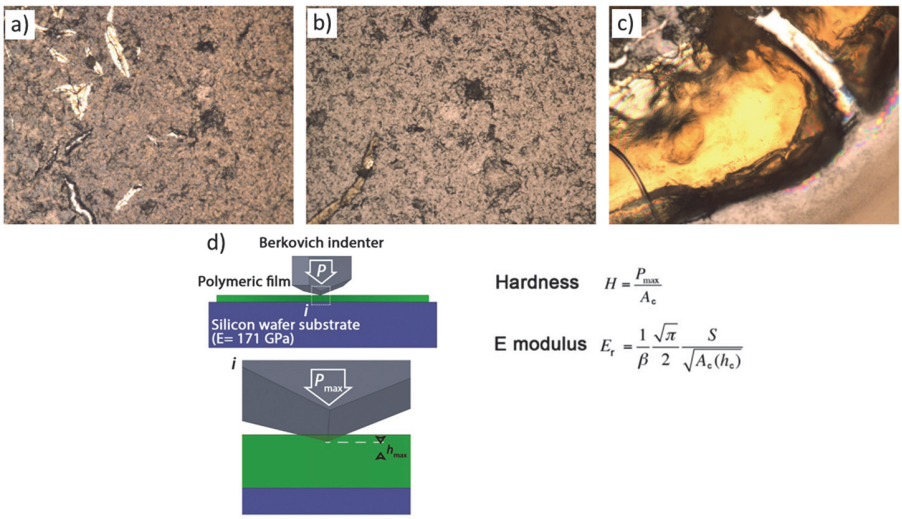


Figure S18. Illustration of the probed areas on films of (a) poly(Y*-*HRD*-C) at pH 7, (b) poly(Y*-*HRD*-C) at pH 4+ $Zn^{2+}$and (c) poly(Y*-*HRD*-C) at pH 4. d) Graphic concept of depth-sensing nanoindentation. The films formed on a silicon wafer substrate were probed using a Berkovich tip.

7 References

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