Metal-mediated molecular self-healing in histidine-rich mussel peptides

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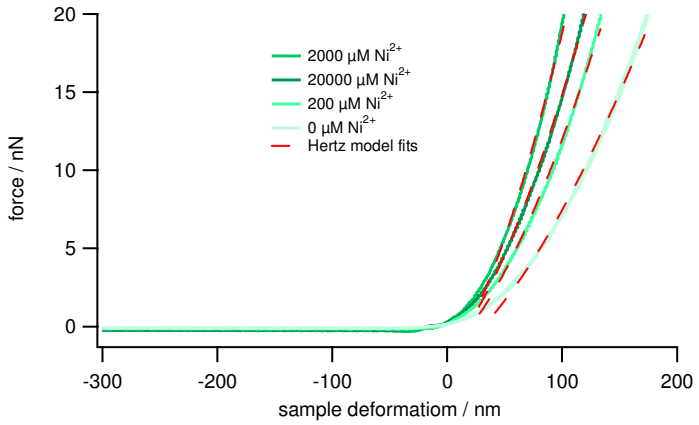
Supporting information

S1: SCP elastic moduli as a function of Ni^{2+} concentration. Typical force-deformation curves for His_5-bys functionalized SCPs as measured by colloidal probe AFM (5 µm probe diameter) at various concentrations of Ni^{2+}.



S2: Typical amino acid analysis data for His_5-bys functionalized SCPs

Amino Acid	Absolute Amount [nMol]	Rel. Perc. [Mol %]	Expected Rel. Perc. [Mol %]
Ser	0.8	6.4	7.7
Gly	5.6	45.0	46.2
Ala	3.0	23.6	26.9
His	3.1	25.0	19.2

The four amino acids comprising His_5-bys account for 98 mol% of the detectable amino acids.

Calculations of functionalization density of His_5-bys on SCPs:

Amount of His_5-bys = absolute amount His [nMol] / 5 His per peptide = **6.3×10^{-10} Mol His_5-bys**

Functionalization density in 1ml SCP solution with PEG concentration of 8.5 µg/ml (gravimetric) = **74 µmol/g His_5-bys in PEG SCPs**.

S3: Typical amino acid analysis data for His_5-bys functionalized glass surface

Amino Acid	Absolute Amount [nMol]	Rel. Perc. [Mol %]	Expected Rel. Perc. [Mol %]
Ser	0.4	14.4	7.7
Gly	0.9	34.8	46.2
Ala	0.6	23.3	26.9
His	0.7	27.5	19.2

The four amino acids comprising His_5-bys account for ~80 mol% of the detectable amino acids.

Calculation of functionalization density of His_5-bys on 25 mm diameter glass coverslips.
Amount of His_5-bys = absolute amount His [nMol] / 5 His per peptide **= $1.5x10^{-10}$ Mol His_5-bys**
Functionalization density on 25mm diameter coverslip = **$1.8x10^{17}$ His_5-bys molecules/m^2**

It was determined that the total amount of Ser, Gly, Ala and His detected (the amino acid components of His_5-bys) comprised ~80% of the total amino acid content, confirming the presence of our peptide on the surface at a relatively high purity. Deviation from expected values based on the peptide sequence may reflect that we are approaching the practical limit of quantification for the device.

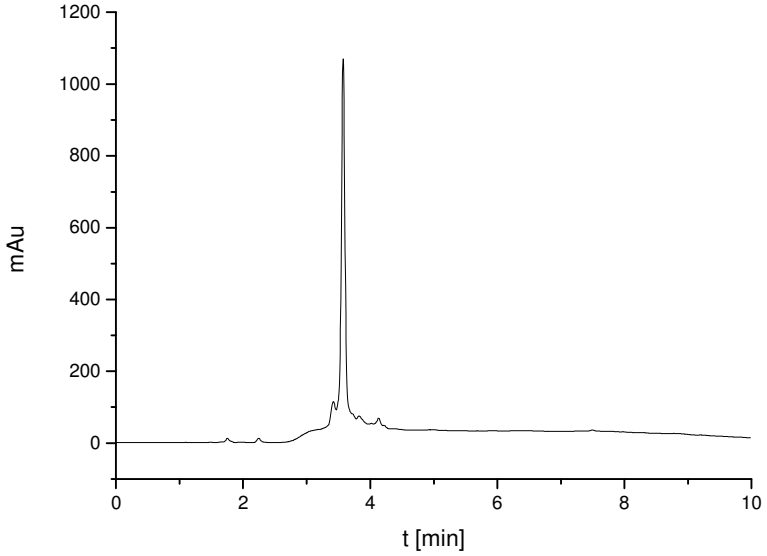
S4: Discussion of the peptide densities

The homogeneity and degree of SCP functionalization by His_5-bys was determined by TBO titration as well as amino acid analysis, providing an estimate for His_5-bys functionalization of 75±21 µmol/g of SCP. Considering the molecular weight of the PEG 8000, this translates into about one peptide for every 60-70 PEG repeat units or approximately one peptide per PEG chain. The density of the peptides in the SCPs can be estimated by assuming a PEG-chain hydrodynamic radius of 3.7 nm[1]. By taking into account that each PEG chain presents one peptide on average, the density amounts to one peptide per ~50 nm^2 of SCP surface (assuming close-packed stacking of the surface PEG-coils). The peptide functionalization density on the glass coverslip surface was estimated to be 1.8 $x10^{17}$±0.3 His_5-bys molecules per m^2 or one peptide per 5-6 nm^2 on the glass support. Thus, the peptide densities on glass are larger by one order of magnitude when compared to the densities on the soft colloidal probes. Considering an approximate radius of the His_5-bys of 0.9 nm, the theoretical maximum density could reach one peptide per 2.2 nm^2; roughly only 3-times larger than the measured density on the glass surface.

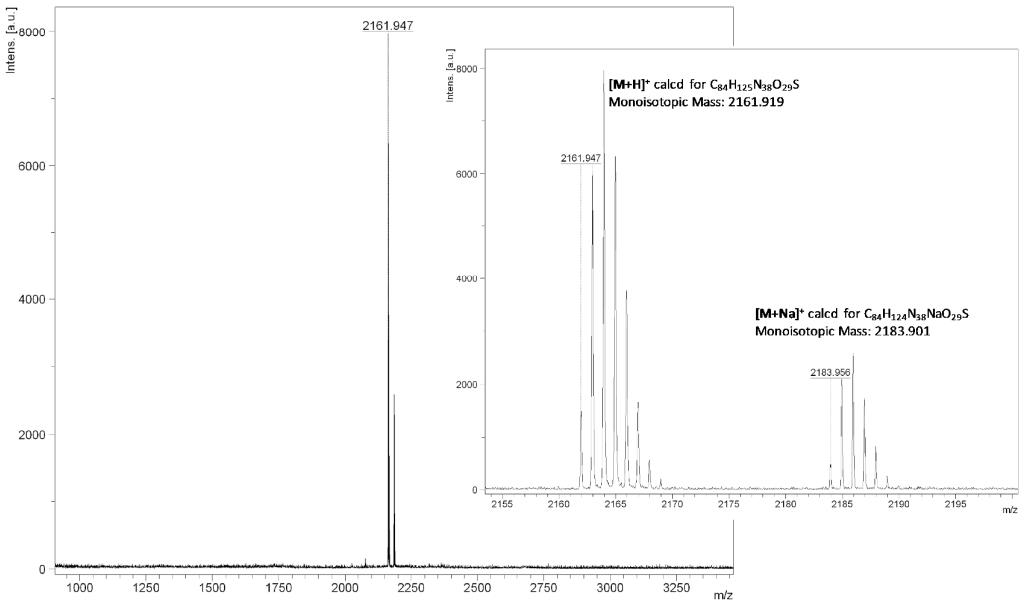
S5: HPLC and Maldi analysis of the His5-bys

Sequence: H_2N-GHGGGHGGGHGGGHGGSASAAAHAAAC-$CONH_2$

Eluent A: H_2O + 0.1% TFA; Eluent B: MeCN + 0.1% TFA
Gradient: Linear 5-95% B in 10 min

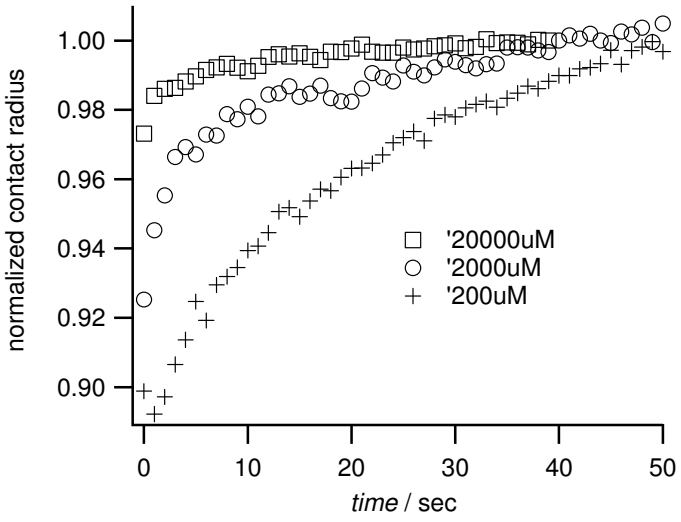


Sequence: H_2N-GHGGGHGGGHGGGHGGSASAAAHAAAC-$CONH_2$



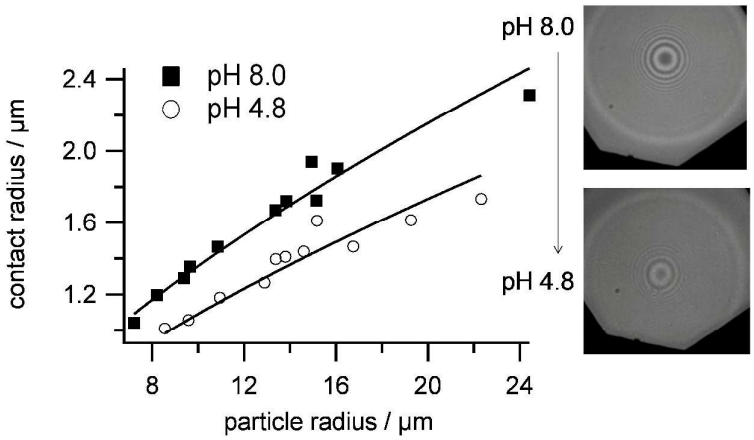
S6: Variation of the contact radius over time for different concentrations of Ni^{2+}. It should be noted that quantitative binding kinetics cannot be obtained from this data since the starting point of the

probe-surface contact was not well defined. Here, due to technical reasons only SCPs already in contact for more than 10 seconds could be imaged.



S7: pH dependence of functionalized SCP attachment

To test the effect of pH on the adhesion energy between His_5-bys functionalized colloidal probes we changed the pH from pH 8 (10 mM tris HCl) by exchanging the buffer in the measurement cell with 10 mM acetate buffer at pH 4.8, while maintaining the Ni^{2+} concentration at 1 mM. As a result the contact areas and adhesion energies decreased from 320 $\mu J/m^2$ to 50 $\mu J/m^2$. This is expected, as at low pH histidine binding to Ni^{2+} is drastically reduced due to protonation of the imidazole side chain.



S8: Determination of the hydrodynamic radius of a PEG chain in the SCPs

The calculation of the hydrodynamic radius of a PEG chain in the SCPs is based on the determination of the mass of dried PEG SCPs, which gives the total number of PEG8000 chains in the SCP. Then, under consideration of the total volume of the swollen SCP volume in buffer, the average volume per PEG chain in buffer can be calculated yielding the average hydrodynamic radius of PEG chains in the SCPs.

Example:

Measured volume of an SCP in liquid: 3.09×10^{-14} m^3 (via optical microscopy)

Measured volume of the SCP after drying on a coverslip: 1.25×10^{-15} m^3 (via AFM)

Mass of the dried SCP: 1.51×10^{-12} kg (considering a density of 1210 kg/m^3, MSDS Sigma)

Number of PEG chains (8000 g/mol) in the SCP: 1.89×10^{-13} mol or 1.14×10^{11} molecules

Volume per PEG chain: 2.71×10^{-25} m^3

Radius per PEG chain assuming a spherical coiled structure: 4 nm

The estimated hydrodynamic radius agrees well with literature values [1].

S9: Estimation of the surface binding energy per His_5-bys peptide

SCP surface energy as measured by SCP	0.00029 J/m^2
Densitiy of His_5-bys (estimated)	50 nm^2/peptide
Binding energy of His_5-bys self-interaction	**8.9 kJ/mol**

S10 The decrease of surface energy at elevated Ni^{2+} concentration

The decrease of surface energy between the His-rich surfaces at elevated Ni^{2+} concentration is not surprising – a similar effect was previously observed in metal binding DOPA-rich mussel proteins in the presence of excess Fe^{3+}.[2] However, it is surprising that saturation occurred at millimolar Ni^{2+} concentrations, in which the number of metal ions in the measurement cell far surpassed the number of histidines by many orders of magnitude. Maximum adhesion energies at these excess concentrations can be explained by the fact that the *local* histidine concentration in the contact area is rather large because histidine is present on the SCPs and on the glass surface, but not in the media, and therefore, oversaturation is reached only at excess of Ni^{2+} in the media. To roughly estimate the concentration of histidine in the PEG network, we consider that about one peptide is bound to a PEG chain, as

measured by amino acid analysis. Considering a hydrodynamic radius of the PEG8000 chain of 4 nm (S9) the peptide concentration would amount to ~8 mM within the SCPs. Therefore, locally stoichiometric conditions were achieved at about 1 mM concentration of Ni^{2+}, which is double the stoichiometric ratio for forming a single Ni^{2+}-$(His)^3$ complex[3].

S11 Calculation of dynamic adhesion energy from AFM-based force-distance curves

In principle, the critical pull-off forces *F* measured by force/distance data (Fig. 4) can be analyzed to yield the adhesive energy according to Chen et al. (1991)[4] with the equation: $W=F/(⅔\ \pi R)$, where R is the radius of the probe and W is the adhesive energy (Table S11). Unexpectedly, we found that the adhesion energies measured with RICM (Table 1) are larger by a factor of 2 to 5 at a given metal concentration compared to the pull-off adhesion energies calculated from the adhesion forces shown in the table below:

Table S11. Adhesion energy calculated from AFM pull-off forces

Ni^{2+} conc. [µM]	**force/distance (pull-off) adhesion energy [µJ/m2]**
50	23
250	59
2000	87
20000	74

(radius of AFM SCP probe R=9.9µm)

The reason for the reduced pull-off adhesion energies measured by AFM could be the reduced contact time of the SCP to the peptide surface. For the static RICM measurement the probes rested at least for at least 10 minutes on the peptide surface before taking images, whereas the contact time in AFM was only 20 sec. As indicated in S6, contact time has a pronounced effect on the interaction energies. We remain cautious in our interpretation of the significance of the dynamic pull-off values because not only contact time, but also load force and loading rate (see figure S11) of the highly deformable soft colloidal probe will undoubtedly contribute to the shape of the force deformation curve, and thus, may

affect the calculated values.[5] This is likely not seen for traditional setups like SFA or colloidal probe AFM that employ "hard" contacting surfaces like quartz/mica or glass spheres as evidenced by the very small deformation observed during retraction prior to the pull-off event (<10 nm)[6]. In contrast, the deformation occurring during retraction of the SCP prior to the pull-off event exceeds 100 nm in some cases (Fig. 4). As a result, the adhesive force was strongly dependent on the (un-)loading rate. When increasing the rate of retraction form 4nN/sec to 400nN/sec the critical pull-off forces increased by almost a factor of 3 (Fig. S11). This shows that the obtained adhesive forces are dependent on the dynamic deformation of the PEG-probe upon retraction and detailed analysis of adhesion energy from pull-off forces requires a more in-depth analysis.

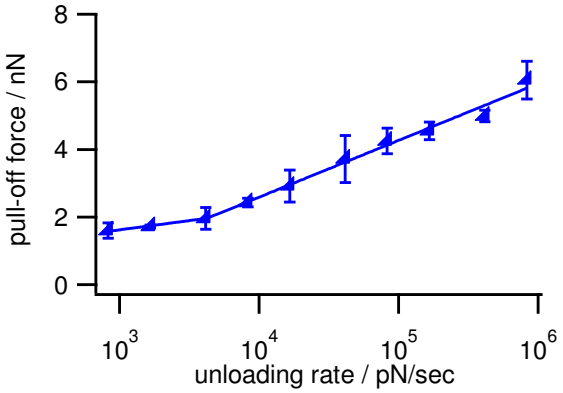


Figure S11. Pull of force vs. loading rate for His_5-bys SCP (R = 9.9 µm) on His_5-bys surface with 250 µM Ni^{2+} after 20 second contact time. The solid lines indicate the presence of a slow and fast loading rate regime. Analysis via Bell-Evans theory is not applicable in this case as pull-off occurred via rupture of multiple bonds.

Supporting References

1. Fagerlund, A.; Lindbäck, T.; Storset, A. K.; Granum, P. E.; Hardy, S. P., Bacillus cereus Nhe is a pore-forming toxin with structural and functional properties similar to the ClyA (HlyE, SheA) family of haemolysins, able to induce osmotic lysis in epithelia. *Microbiology* **2008,** 154, (3), 693-704.

2. Zeng, H.; Hwang, D. S.; Israelachvili, J. N.; Waite, J. H., Strong reversible Fe3+-mediated bridging between dopa-containing protein films in water. *Proc. Natl. Acad. Sci. U.S.A.* **2010,** 107, (29), 12850-12853.

3. Freeman, H. C., Crystal structures of metal-peptide complexes. *Adv. Prot. Chem.* **1967,** 22, 257-424.

4. Chen, Y. L.; Helm, C. A.; Israelachvili, J. N., Molecular mechanisms associated with the adhesion and contact angle hysteresis of monolayer surfaces. *J. Phys. Chem.* 1991, 95, (26), 10736-10747.

5. Buzio, R.; Bosca, A.; Krol, S.; Marchetto, D.; Valeri, S.; Valbusa, U., Deformation and Adhesion of Elastomer Poly(dimethylsiloxane) Colloidal AFM Probes. *Langmuir*, 2007, 23, (18), 9293-9302.

6. Zeng, H.; Hwang, D. S.; Israelachvili, J. N.; Waite, J. H., Strong reversible Fe3+-mediated bridging between dopa-containing protein films in water. *Proc. Natl. Acad. Sci. U.S.A.* 2010, 107, (29), 12850-12853.