Supplementary Information

Mimicking the endothelial glycocalyx through supramolecular presentation of hyaluronan on patterned surfaces

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S1 Characterization of protected mercaptopropionic acid by NMR

^{1}H NMR spectra of starting materials (3-mercaptopropionic acid and 4-methoxytriphenylmethyl chloride) and the protected thiol product (3-(((4-methoxyphenyl)diphenylmethyl)thio)propanoic acid) were collected on a Bruker AV600 NEO spectrometer. $CDCl_3$ was used as solvent and its resonance signal in spectra was 7.26 ppm, which served as reference for the chemical shift. The loss of carboxylic acid in 3-mercaptopropionic acid was shown as the missing 10.52 ppm on the ^{1}H NMR of product. The shift in the two CH_2 groups in the backbone and the loss of thiol hydrogen were also observed.

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) of the protected thiol product = 7.40 (d, 4H, 8.4 Hz, *o*-CH), 7.30 (d, 2H, 8.9 Hz, *o*-CH), 7.27 (t, 4H, 7.4 Hz, *m*-CH), 7.20 (t, 2H, 7.3 Hz, *p*-CH), 6.80 (d, 2H, 8.9 Hz, *m*-CH), 3.78 (s, 3H, CH_3), 2.45 (t, 2H, 7.3 Hz, SCH_2), 2.25 (t, 2H,7.3 Hz, CH_2COOH).

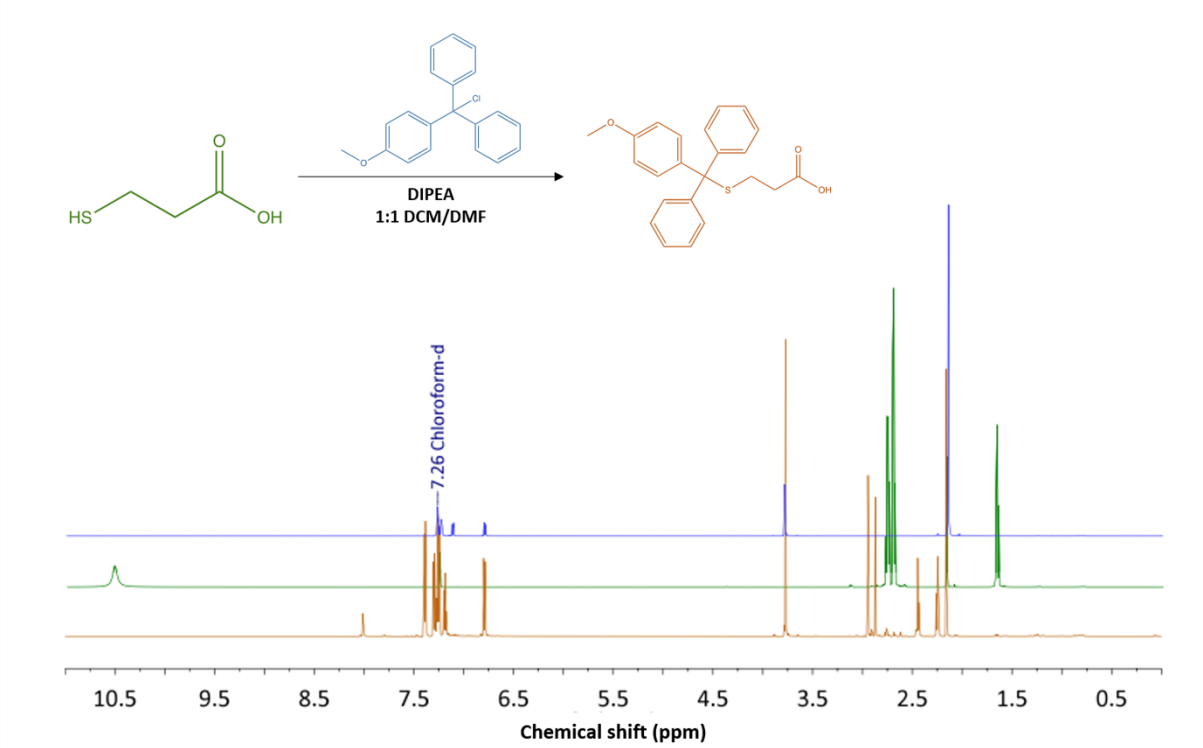

Fig. S1 ^{1}H NMR of 3-mercaptopropionic acid (green), 4-methoxytriphenylmethyl chloride (blue) and the protected thiol product (brown).

S2 Characterization of peptides by ESI-MS and RP-HPLC

All peptides were synthesized and purified successfully. ESI-MS was used to confirm the mass of pure peptides (Fig. S2-4, A). Analytical RP-HPLC was used to analyse the purity of peptides (Fig. S2-4, B-C).

S2.1 HS-Pep-1

The expected mass of HS-Pep-1 ($C_{67}H_{99}N_{21}O_{16}S$) is 1485.73, one main peak ($[M+2H]^{2+}$ m/z =744.9) was found in the ESI-MS (Fig. S2, A).

Table S1 RP-HPLC gradient for HS-Pep-1

Time (min)	Water (0.1% TFA) %	ACN (0.1% TFA) %
3	98	2
33	0	100
36	0	100
40	98	2
42	98	2

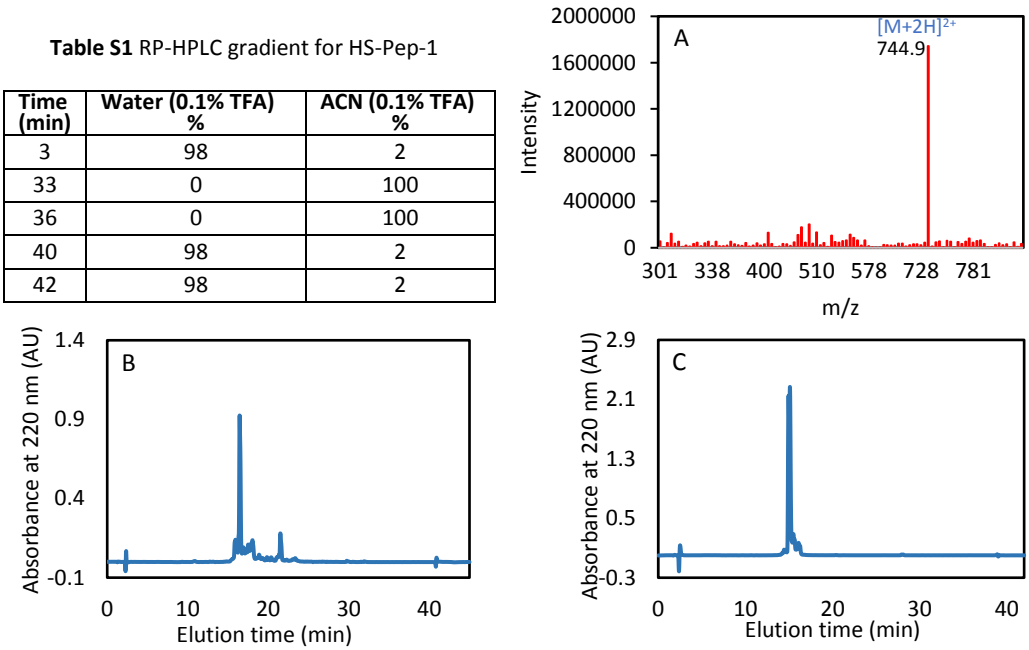

Fig. S2 Characterization of HS-Pep-1 in terms of mass and purity. (A) ESI-MS. Analytical RP-HPLC traces of crude HS-Pep-1 (B) and after purification (C).

S2.2 Ac-Pep-1

The expected mass of Ac-Pep-1 ($C_{66}H_{97}N_{21}O_{16}$) is 1439.74, one main peak ($[M+2H]^{2+}$ m/z =722.04) was found by ESI-MS (Fig. S3, A).

Table S2 RP-HPLC gradient for Ac-Pep-1

Time (min)	Water (0.1% TFA) %	ACN (0.1% TFA) %
5	98	2
35	0	100
40	0	100
43	98	2
45	98	2

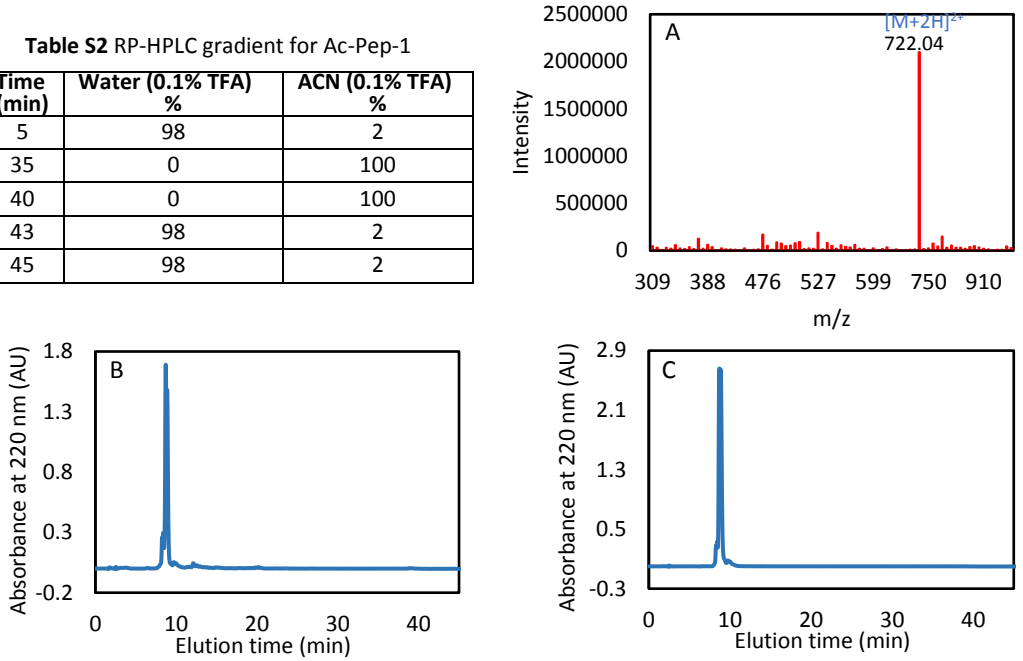

Fig. S3 Characterization of Ac-Pep-1 in terms of mass and purity. (A) ESI-MS. Analytical RP-HPLC traces of crude (B) and purified Ac-Pep-1 (C).

S2.3 HS-ScPep-1

The expected mass of HS-ScPep-1 ($C_{67}H_{99}N_{21}O_{16}S$) is 1485.73, two main peaks ($[M+2H]^{2+}$ m/z =744.68 and $[M+3H]^{3+}$ m/z =496.88) were found by ESI-MS (Fig. S4, A).

Table S3 RP-HPLC gradient for HS-ScPep-1

Time (min)	Water (0.1% TFA) %	ACN (0.1% TFA) %
3	98	2
33	0	100
36	0	100
40	98	2
42	98	2

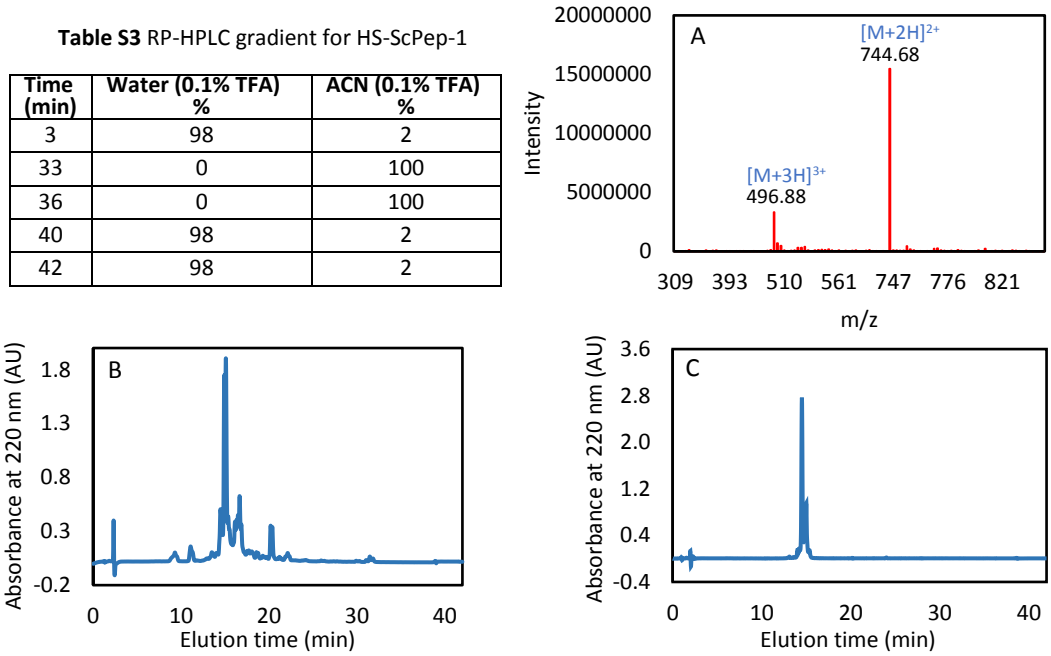

Fig. S4 Characterization of HS-ScPep-1 in terms of mass and purity. (A) ESI-MS. Analytical RP-HPLC trace of crude (B) and purified (C) HS-ScPep-1.

S3 µCP of HS-ScPep-1 SAM and incubation with fluorescently-labelled HA

HS-ScPep-1 was swabbed on PDMS moulds patterned with round holes and 200 µm diameter, then the loaded stamp was brought into contact with gold surface. The µContact printed HS-ScPep-1 substrate and bare Au were then immersed in 700 kDa fluorescein-HA aqueous solution (0.5 mg/mL) overnight, rinsed with ultrapure water and imaged under the Leica DMi8 Epifluorescence microscope.

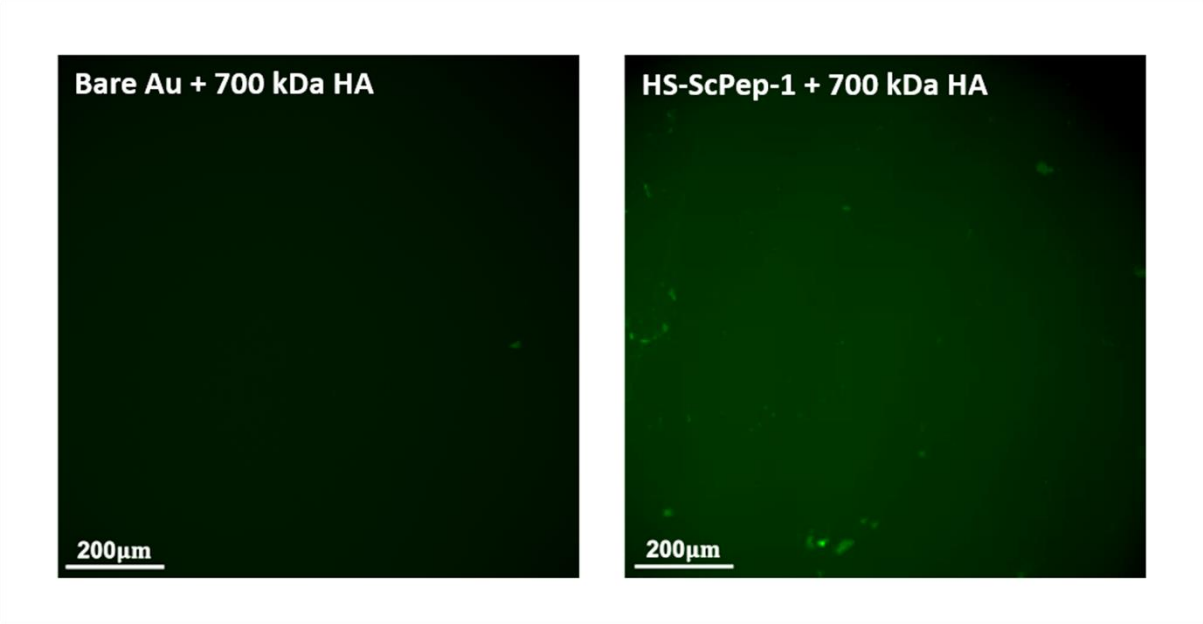

Fig. S5 Fluorescence image of bare Au (left) and µContact printed HS-ScPep-1 (right) after incubation with fluorescein-HA.

S4 QCM-D monitoring of peptide SAMs and HA deposition

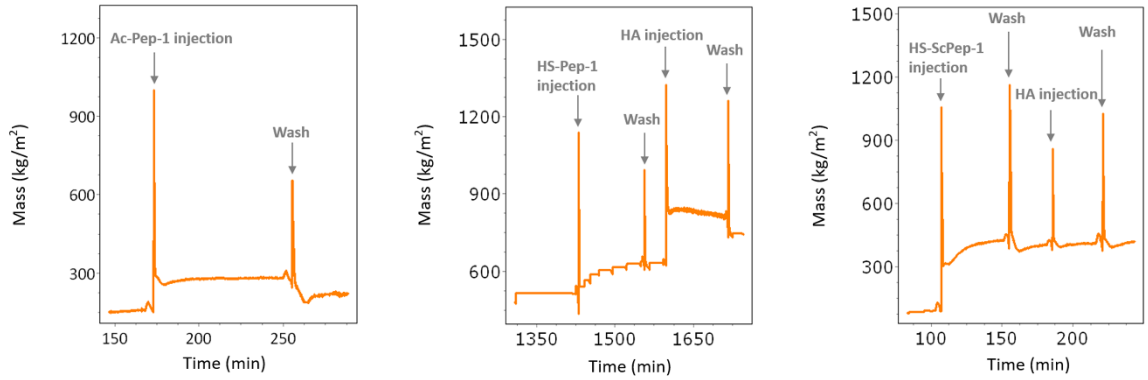

Fig. S6 Calculated mass change using Voigt model based on frequency and dissipation shift obtained by QCM-D monitoring.

S5 XPS survey of peptide SAMs

Table S4 Elemental composition of different concentration HS-Pep-1 modified gold surfaces

HS-Pep-1 concentration (mM)	Averaged elemental composition %				
	Au	**C**	**O**	**N**	**S**
0.01	36.63	42.15	9.10	12.12	0.00
0.05	36.63	41.37	9.53	11.21	1.26
0.1	34.95	41.71	9.54	10.91	2.89
0.5	22.51	49.73	11.36	14.32	2.08
1	32.09	43.18	9.97	11.96	2.80
1.5	29.95	44.59	10.41	12.19	2.86

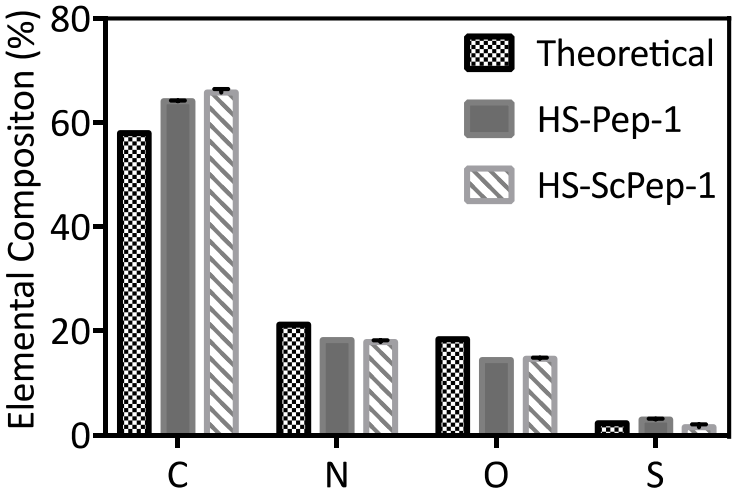

Fig. S7 Calculated theoretical elemental percent compositions for C, N, O and S for HS-Pep-1 and HS-ScPep-1 SAMs versus actual experimental percentages obtained from XPS.

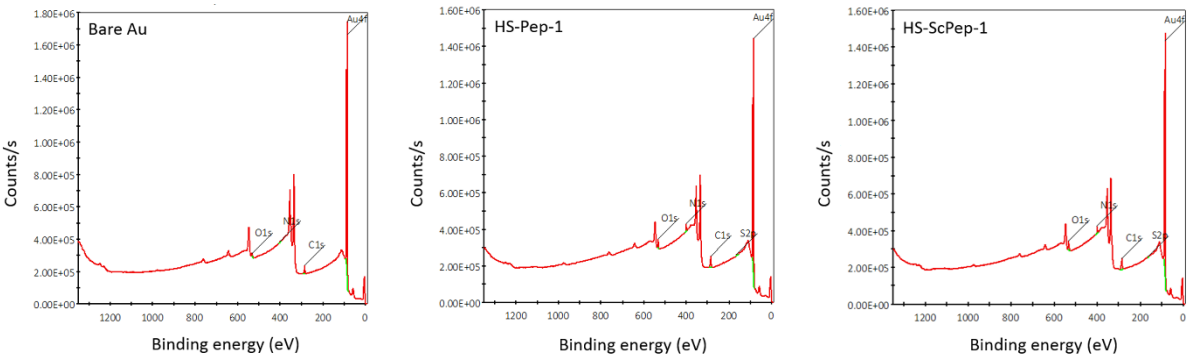

Fig. S8 XPS spectra of bare Au and coated surfaces.