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Softmatter interactions at the molecular scale: Interaction forces and energies between single hydrophobic model peptides

Philipp Stock[1,2*], Thomas Utzig[1] and Markus Valtiner[1,2*]

[1] Max-Planck-Institut für Eisenforschung GmbH, Department for Interface Chemistry and Surface Engineering, D-40237 Düsseldorf, Germany

[2] Technische Universität Bergakademie Freiberg, Physical Chemistry II, Freiberg, D-09599, Germany

*Corresponding authors email: stock@mpie.de and Markus.Valtiner@chemie.tu-freiberg.de

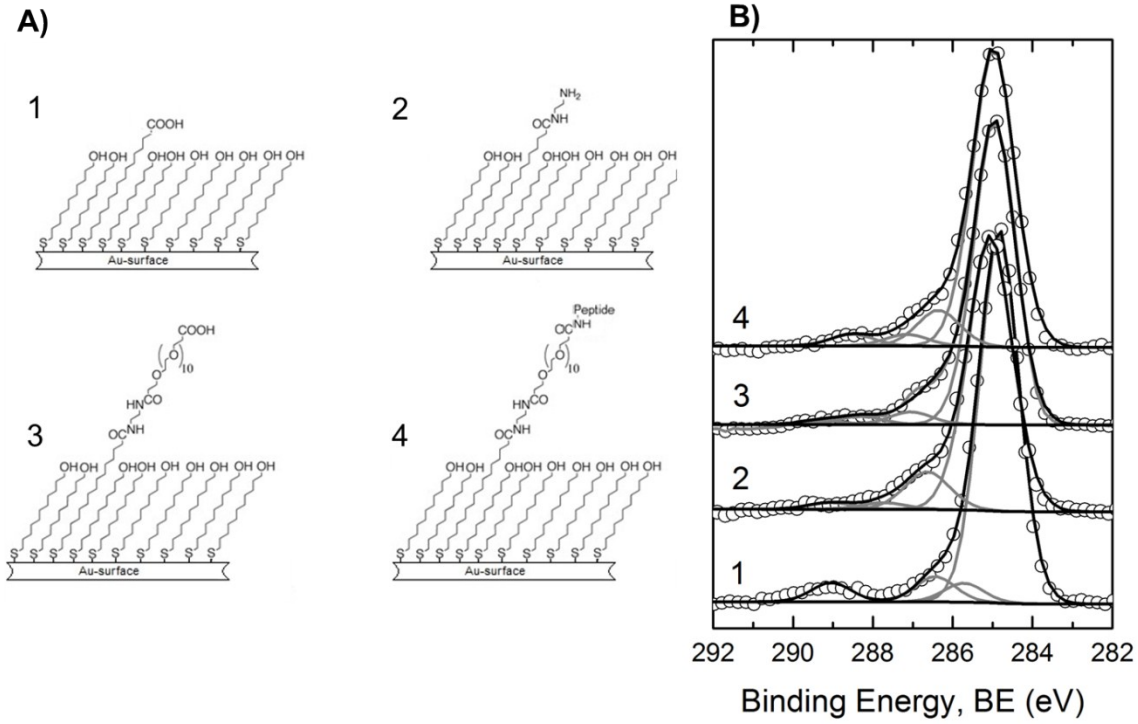

Figure S1 A) reaction steps on surface, 1 preparation of mixed SAM (for XPS COOH/OH ratio 1:1); 2 reaction mixed SAM (COOH/OH, 1:1) functionalized with ethylenediamine; 3 mixed SAM (COOH/OH, 1:1) functionalized with ethylenediamine and dicarboxylic acid; 4 mixed SAM (COOH/OH, 1:500) functionalized with ethylenediamine, dicarboxylic acid and –N–GSGSGSGSGS. B) The corresponding C 1s spectra.

Table S1) Assignment of features in the C 1s and N 1s XPS spectra on the surface. . Atomic ratios are given relative nitrogen.

Reaction Step	Core level	Binding energy [eV]	classification	Theoretical atomic ratio	Experimental atomic ratio

1	C 1s	284.85	$\underline{C}H_2$	-	-
		285.83	$\underline{C}H_2COOH$	1	1
		286.62	$\underline{C}H_2OH$	1	1.3
		289.26	$\underline{C}OOH$	1	1
2	C 1s	285.25	$\underline{C}H_2$	-	-
		286.64	$\underline{C}H_2OH$	-	-
		287.73	$\underline{C}H_2CO$	1	1.5
		289.07	$\underline{C}ONH$	1	1.5
	N 1s	400	$–\underline{N}H_2$	1	1
		401.2	$CO–\underline{N}HR$	1	1
3	C 1s	284.96	$\underline{C}H_2$	-	-
		286.25	$PEG + \underline{C}H_2OH$	-	-
		287.05	$\underline{C}H_2CO$	3	4.2
		288.31	$\underline{C}O$	2	2.8
		289.31	$\underline{C}OOH$	1	1.4
	N 1s	400.89	$–\underline{N}H–CO$	2	1
4	C 1s	284.97	$\underline{C}H_2$	-	-
		286.36	$PEG + \underline{C}H_2OH$	-	-
		287.15	$\underline{C}H_2CO$	1	1.22
		288.52	$\underline{C}O$	1	1.22
	N 1s	400.70	$–\underline{N}H–CO$	1	1

Fitting parameters for all approach curves

For fitting the approach profiles of the force profiles an extended DLVO equation, which describes interactions as a linear superposition of van der Waals interactions, electric double layer forces and hydration forces was used[1–3]:

$$\frac{F}{2\pi R} = \frac{-A_H}{6(D-D0)2} + \frac{\lambda}{\varepsilon\varepsilon 0}\left[2\sigma A\sigma B e^{-\frac{D-D0}{\lambda_D}} - (\sigma A2 + \sigma B2)e^{-\frac{2(D-D0)}{\lambda_D}}\right] - 2\gamma Hy e^{-\frac{D}{\lambda H}}$$

A_H = Hamaker constant ($A_H = 4.5 \cdot 10^{-20}$ J), R = radius of the AFM tip, D_0 = parameter that quantifies any shift of the hard wall by e.g. compression of the SAMs or shift of the effective plane of origin of the VDW interaction. ε is the relative permittivity, ε_0 the vacuum permittivity, σ_A and σ_B the surface potential of the AFM tip and functionalized gold surface with the solutions Debye-length λ_D. The last part of the formula describes the hydrophobic

interaction. Here, γ is the interfacial tension of the interface, Hydra, *Hy* is a parameter that describes the effective hydrophobic interaction with a decay length λ_H.
In all cases hydration forces and electric double layer interactions are effectively 0. Both surfaces are mainly hydrophilic, this results in *Hy* ~ 0, also all surfaces are intrinsically uncharged. This leaves the radius, R and the shift of the plane of origin of the VDW forces as only two fitting variables, if the Hamaker constant is fixed to a known value. Fitted values are tabulated in **Table S1**.

Table S2: Parameters for fitting of AFM-force vs. distance profiles.

Mutation sequence	Figure	R (nm)	D_0 (Å)
L_0/L_0	S1	10	0
L_4/L_4	2	16	0
L_4/L_2	S3	11	0
L_2/L_2	S4	16.3	0
backbone/backbone	S2	10.4	0

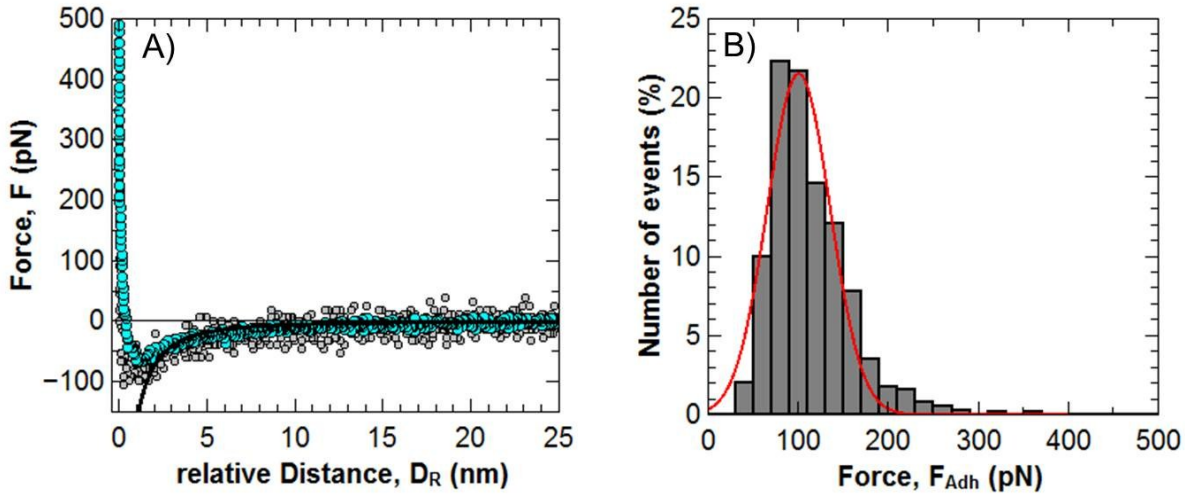

Figure S2 A) Typical force vs distance profile (approach only, single profile in grey) and mean curve of about 15 approach profiles (blue) measured for the L_0/L_0 setup. B) Adhesion measured for the same setup.

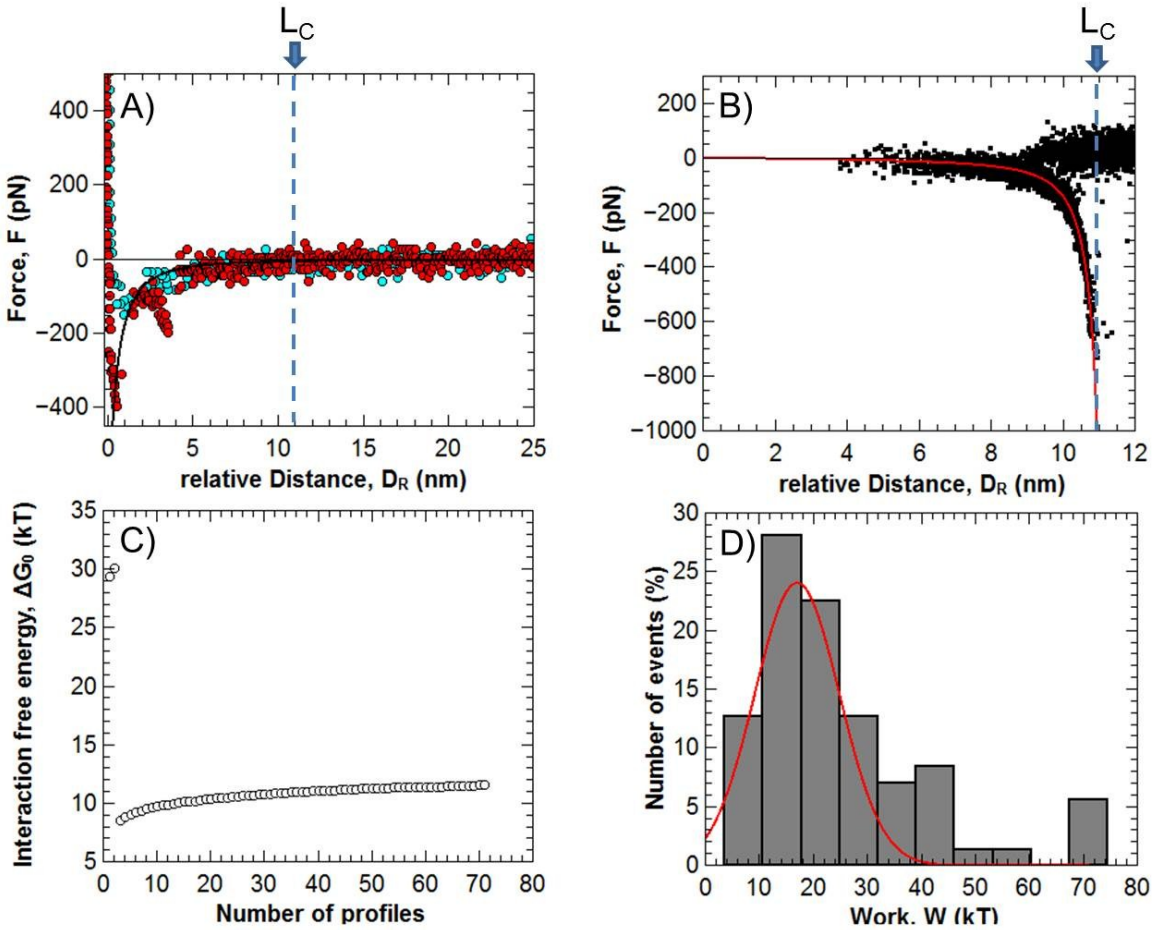

Figure S3 A) A typical individual SM-AFM force distance profile with both a primary adhesive minimum and a single molecular rupture signature at 44% full extension of the linker. The fit in the inset shows a fit by an extended DLVO theory (for fit details see **table S1**). B) Typical master curve with about 70 individual rupture events measured between two NHS activated backbones, aligned by the best worm like-chain fit with a contour length of ~11 nm and a persistence length of 0.37 nm. C) A plot of Jarzynski's free energy ΔG_0 as a function of the number of force trajectories. D) Normalized histogram of the measured work distribution.

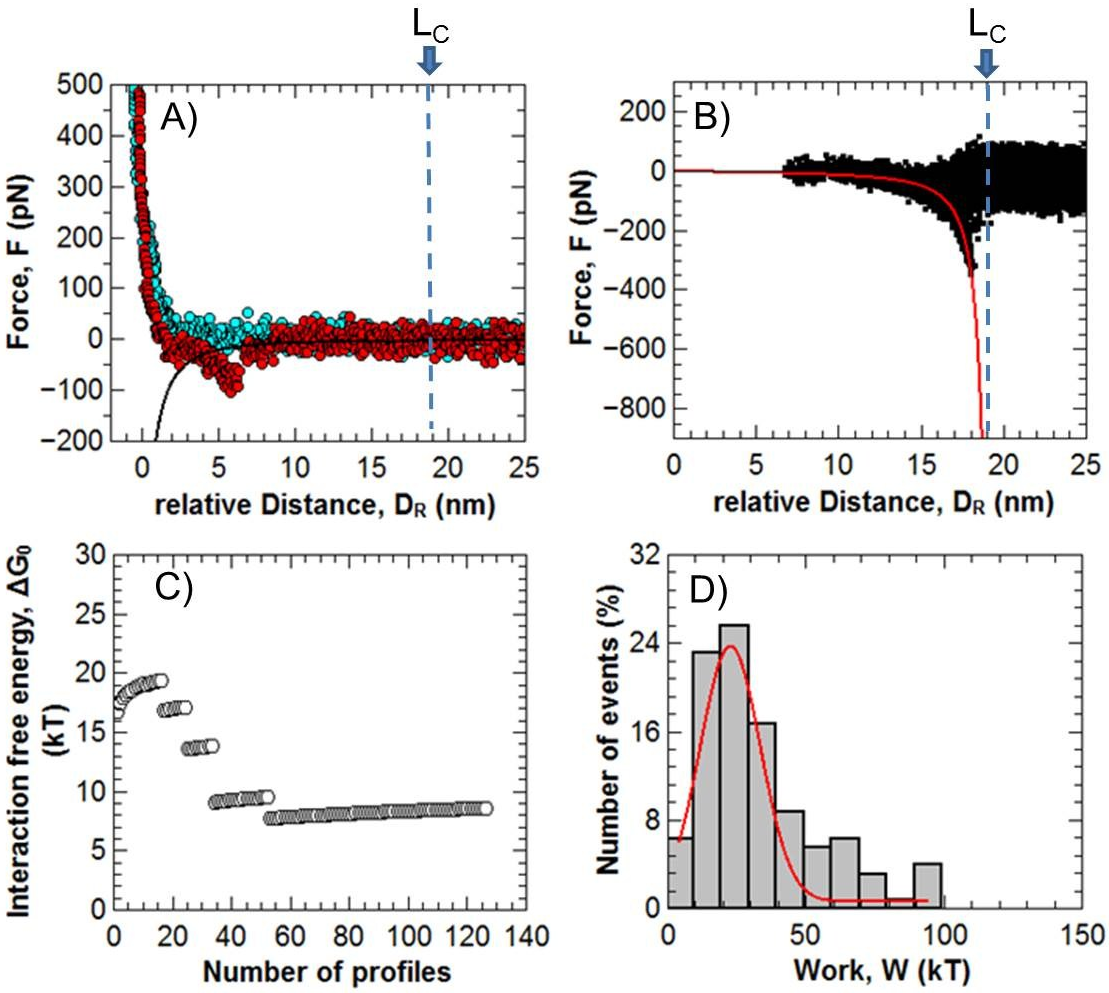

Figure S4 A) A typical individual SM-AFM force distance profile with both a primary adhesive minimum and a single molecular rupture signature at 32% full extension of the linker for L_4/L_2 setup. The fit in the inset shows a fit by an extended DLVO theory (for fit details see **table S1**). B) Typical master curve with about 125 individual rupture events measured between L_4 and L_2, aligned by the best worm like-chain fit with a contour length of ~19 nm and a persistence length of 0.37 nm. C) A plot of Jarzynski's free energy ΔG_0 as a function of the number of force trajectories. D) Normalized histogram of the measured work distribution.

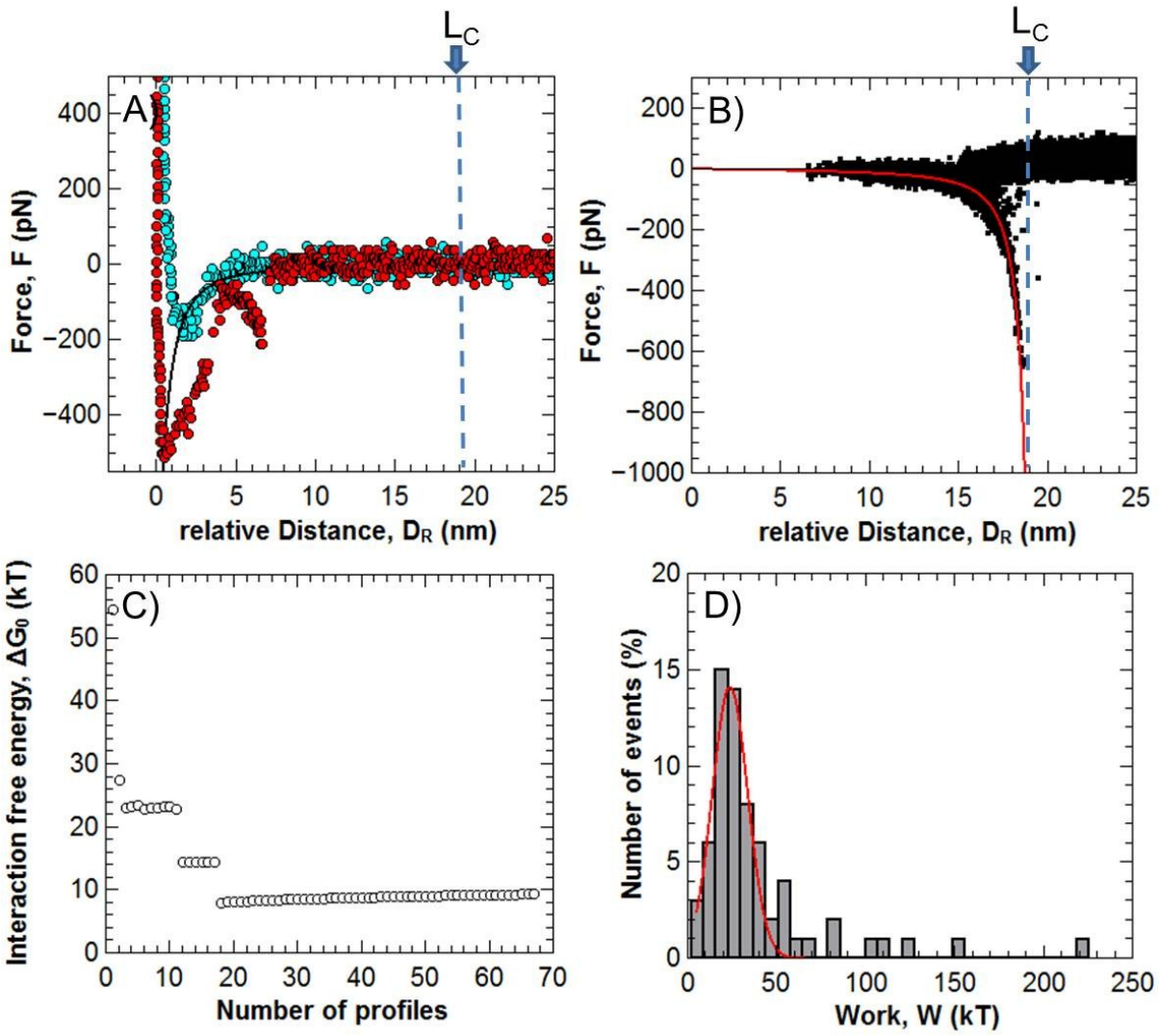

Figure S5 A) A typical individual SM-AFM force distance profile with both a primary adhesive minimum and a single molecular rupture signature at 37 % full extension of the linker for L_2/L_2 setup. The fit in the inset shows a fit by an extended DLVO theory (for fit details see **table S1**). B) Typical master curve with about 70 individual rupture events measured between two L_2 peptides, aligned by the best worm like-chain fit with a contour length of ~19 nm and a persistence length of 0.37 nm. C) A plot of Jarzynski's free energy ΔG_0 as a function of the number of force trajectories. D) Normalized histogram of the measured work distribution.

Table S3 Calculated free energy using Jarzynski's equality as well as the lowest measured work value in SM-AFM as a function of the peptide sequence, indicating a sufficient sampling and no excessive bias by the lowest measured work values.

Mutation sequences:	**lowest measured work value (k_BT)**	**JE interaction free energy (k_BT)**
L_4/L_4	13.2	16.3 ± 2
L_2/L_2	5.2	9.4 ± 2
L_4/L_2	4.1	8.6 ± 1