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Single-molecule study of the synergistic effects of positive charges and Dopa for wet adhesion

Yiran Li, Tiankuo Wang, Lei Xia, Lei Wang, Meng Qin, Ying Li*, Wei Wang* and Yi Cao*

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Experimental Section

AFM Cantilever Modification.

The Cantilever modification procedure was similar as our previous study[1]. Briefly, the MLCT cantilever (from Bruker company) was treated with chromic acid to remove the impurities and generate hydroxyl groups for 20 minutes. After extensive resining with pure water, the cantilever was transferred into 0.5% (vol/vol) (3-mercaptopropyl)trimethoxysilane (MPTMS) in toluene solution for 2 h, resulting in an thiol group functionalized cantilever. The unreacted MPTMS was removed by resining with large amount of toluene solution. Then the cantilever was stored in an oven at 100 °C to immobilize silane conjugation. Next the cantilever was immersed in a dimethyl sulfoxide (DMSO) solution containing 1mg ml^{-1} of maleimide-polyethylene glycol-N-hydroxysuccinimide (MAL-PEG-NHS) (purchased from Nanocs, MW: 5000 Da) for 3 h to functionalize with the NHS group. Finally, after rinsing with pure water, the cantilever was immersed into 10 μM lysine-Dopa PBS solution for 1h.

It is technically difficult to measure the density of the dipeptide on the cantilever tip. Instead, we controlled the density of the conjugated dipeptides in a trial-and-error fashion, based on the pickup rate of the single molecule AFM measurements. We gradually lower the concentration of the peptide till the pickup rate is ~2%, which means most events either show no interactions or only nonspecific interactions which can be easily excluded based on the polymer elasticity of the PEG linker. Moreover, Keeping the pickup rate of ~2% or even lower is a common practice in single molecule AFM community[2, 3], which is essential to ensure that most of the events we measured are single bonds. Assuming the adhesion bond formation obeyed Poisson statistics, an adhesion frequency of ~2% in the force measurements implies that among the observed force events, the probabilities of forming a single, double, and triple adhesion bonds between the dipeptide and the surfaces were 99%, 0.99%, and 0.01%, respectively[4]. Therefore, our experimental condition ensured there was a > 99% probability that the binding event was mediated by a single bond[5]. Moreover, multiple-bonding interactions can be excluded in the force traces by using Worm-like chain fitting to the elastic properties of the PEG linker. When multiple bonds are formed, the persistence length measured will be significantly shorter as force is distributed to all PEG chains. On the contrary, higher pickup rates indicates that there are more dipeptides labeled on the cantilever tip and the chance to pick up multiple bonds also increases.

Surface Preparation.

Titania (TiO_2) and mica surfaces were glued on a glass slide for single-molecule force spectroscopy experiments. The substrates were first immersed into methanol and cleaned with ultrasonic cleaners for 10 min to remove impurities. Next, the substrates were treated with chromic acid for 2h. After thoroughly rinsing with pure water, the substrates were dried with argon.

Single-Molecule Force Spectroscopy Experiments and Data Analysis.

All data was collected on a commercial AFM (ForceRobot 300 from JPK). The spring constants of individual cantilever (MLCT, D type, from Bruker), calibrated by thermo fluctuation method, were in the range of 0.03-0.05 N m^{-1}. All AFM experiments were conducted in Millipore water or PBS buffer (containing 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4 and 2 mM KH_2PO_4 and 0.1 mM ascorbic acid, pH 7.4) at room temperature. The force-extension curves were recorded by a commercial software from JPK (JPK data processing) and were further analyzed by a home-written procedure based on Igor pro 6.12 (Wavemetric, Inc.).

Statistical Analysis.

All data are expressed as means ± standard error of the mean (S.E.M). Significant differences between groups were indicated by *: not significantly different; **:$P<0.05$; ****: $P<0.0001$. respectively

Statistical analysis of rupture force distribution

We used probability density distribution of rupture force to depict the dipeptides binding strength in Figure 2, Figure 3 and Figure S3b. The probability density distribution is the normalized results of the occurrences (rupture force events). As depicted by equation (1), the probability of rupture force at a certain range can be given by the interval. The probability density distribution of each group is calculated by Igor pro 6.12 (WaveMetrics Inc.).

$$Pr(f) = \int_a^b p(f)df \tag{1}$$

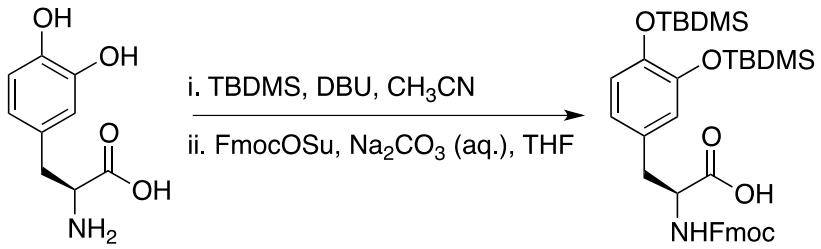
Where Pr(f) is the probability of rupture force at range [a,b], p(f) is the probability density distribution and f is rupture force.

Cleavage of dde protecting groups.

Dde protecting groups on lysine residue were cleaved by treating the tip in 2% hydrazine hydrate (vol/vol in dimethyl formamide) for 30 min. Then the AFM cantilever was rinsed with pure water thoroughly.

Dipeptides synthesis

Fmoc-Dopa(TBDMS)$_2$-OH



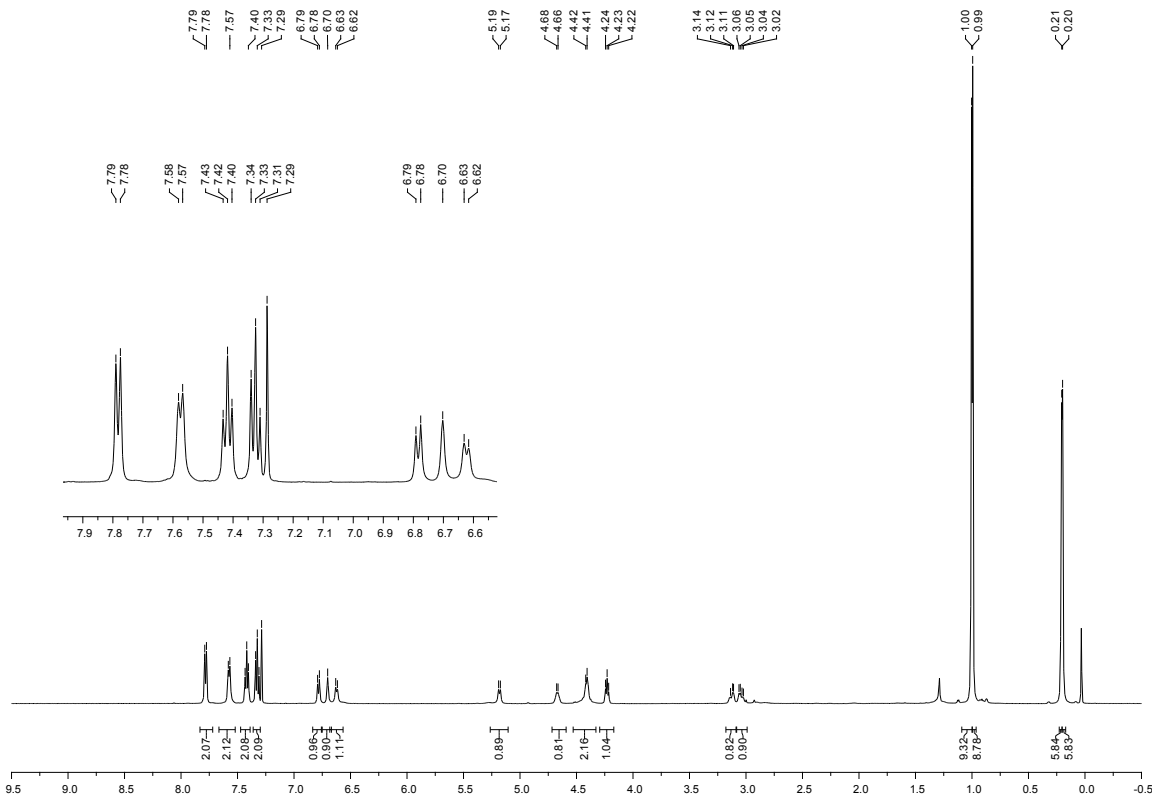
***i.Dopa(TBDMS)$_2$-OH*:**

The compound was prepared according to the literature[6, 7] with some modifications. TBDMS-Cl (4.6g,30.5mmol) in anhydrous CH_3CN (23.0mL) was added with dihydroxylphenylalanine (Dopa) (2.0 g, 10.1 mmol). The mixture was cooled on an ice-water bath for 10 min. Then DBU (4.6 mL, 30.2 mmol) was dropwise added to the mixture over 10 min. The reaction was stirred on ice bath for 4 h and after that at room temperature for an additional 20 h. After the reaction was complete, the solvent was removed *in vacuo* and the residue was loaded onto a silica gel column(2.5 cm×30 cm) and eluted with MeOH:CH_2Cl_2 (1:9 to 3:7) to give pale white solid as the desired product.

ii:Fmoc-Dopa(TBDMS)$_2$-OH:

Dopa(TBDMS)$_2$-OH(8.62g,31.3mmol) was added in 113 mL THF and 40 mL 10% Na_2CO_3 solution was combined.This procedure was taken on an ice bath. The mixture was stirred 10 min and then FmocOSu (6.39 g, 19.0mmol) was added. The reaction was stirred on ice bath for 4 h and after that at room temperature for an additional 18 h. The volatile was removed *in vacuo* and the residue was added with 50 mL H_2O and acidified with 1 N HCl to pH ~1. Then the aqueous slurry was extracted with CH_2Cl_2 (3 × 50 mL). The CH_2Cl_2 layers were combined, washed with H_2O (3 × 50 mL) and brine (50 mL), and dried over anhydrous Na_2SO_4. Finally the solution was filtered and concentrated. The residue was subject to flash chromatograph with 0.5 % MeOH in CH_2Cl_2 to get white powder as the desired product (2.8 g, 4.4 mmol).
^{1}H NMR (500 MHz, $CDCl_3$, r.t.): *δ (ppm)* 0.20 (s, 6 H), 0.21 (s, 6 H), 0.99 (s, 9 H), 1.00 (s, 9 H), 3.04 (m, 1 H), 3.12 (m, 1 H), 4.23 (t, *J*= 6.93 Hz, 1 H), 4.42 (m, 2 H), 4.67 (d, *J*= 6.15 Hz, 1 H), 5.18 (d, *J*= 7.79 Hz, 1 H), 6.62 (d, *J*= 7.54 Hz, 1 H), 6.70 (s, 1 H), 6.78 (d, *J*= 7.95 Hz, 1 H), 7.33 (t, *J*= 7.44 Hz, 2 H), 7.42 (t, *J*= 7.36 Hz, 2 H), 7.57 (d, *J*= 6.84 Hz, 2 H), 7.78 (d, *J*= 7.51 Hz, 2 H).

ESI-MS: [$C_{36}H_{50}NO_6Si_2^+$] calcd. 648.95, found: 648.41.



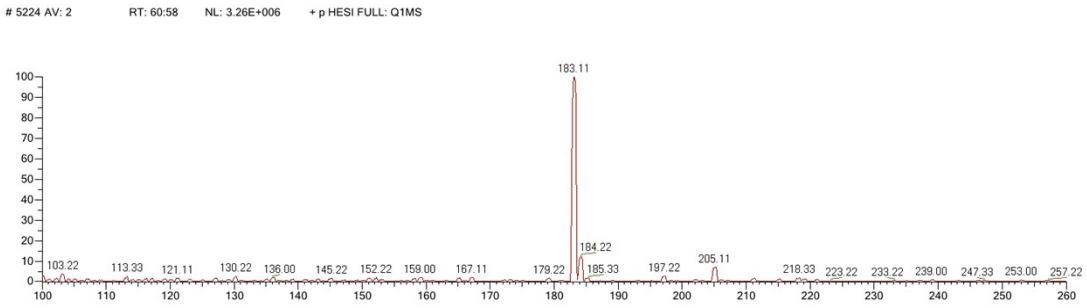
Fmoc-Lys(Dde)-OH

i:Dde-OH

HOAc (1.13 mL, 19.7 mmol) was slowly added to 38 mL DMF solution of DMAP (2.40 g, 19.6 mmol), EDC-HCl (3.75 g, 19.6 mmol), and 5,5-dimethyl-1,3-cyclo hexanedione (2.50 g, 17.8 mmol). The resulting mixture was stirred at room temperature overnight and then concentrated under reduced pressure. The residue was dissolved in EtOAc (100 mL), washed with 1 N HCl (2 × 50 mL), H_2O (2 × 50 mL) and brine, and dried with anhydrous Na_2SO_4. The solution was filtered and concentrated in vacuo. The desired product was purified via flash chromatography (EtOAc:PE 1:19 to 1:9) (R_f=0.68 in 1:3 EtOAc:PE) which solidified upon cooling. Yield: 2.48 g, 76%.

ESI-MS: $C_{10}H_{14}O_3^+$ calcd. 182.09, found. 182.11.

H^1 NMR (500 MHz, d_1-$CDCl_3$): *δ(ppm)* 1.09 (s, 6 H), 2.37 (s, 2 H), 2.54 (s, 2 H), 2.62 (s, 3 H).

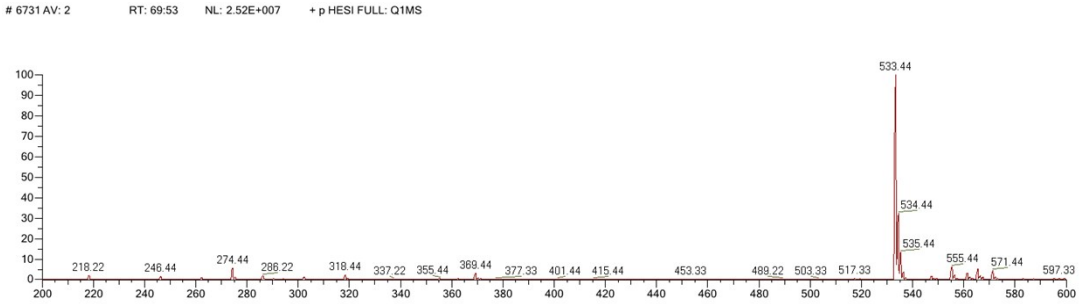


ii:Fmoc-Lys(Dde)-OH

Fmoc-Lys(Boc)-OH (2.00 g, 4.27 mmol) in 50% TFA in CH_2Cl_2 (60 ml) was stirred at rt overnight and the solvent was removed under reduced pressure. The residue was subject to flash chromatography (MeOH:CH_2Cl_2 1:99 to 1:19) to give pale yellow oil as the desired product, which soon solidified upon standing (R_f= 0.06 in 10% MeOH/CH_2Cl_2). Yield: 1.92 g 91%. ESI-MS: calcd for $C_{21}H_{25}N_2O_4^+$: 368.43, found: 369.00. Then, Fmoc-Lys-OH (1.92 g, 5.22 mmol) and Dde-OH (2.0g 10.98 mmol) in EtOH (50 mL) was heated to reflux for 48 hr and concentrated under reduced pressure. The residue was subject to flash chromatography (1:99 to 3:97) to provide a white solid as the desired product was isolated via flash chromatography (MeOH:CH_2Cl_2) (R_f=0.50 in 10％ MeOH/CH_2Cl_2).Yield: 1.16 g, 60%.

ESI-MS: $C_{31}H_{36}N_2O_6^+$, calcd. 532.26, found 532.44.

H[1] NMR (500 MHz, d_1-$CDCl_3$): *δ(ppm)* 1.03 (s, 6 H), 1.55 (m, 2 H), 1.74 (m, 3 H), 1.98 (m, 1 H), 2.37 (s, 4 H), 2.56 (s, 3 H), 3.43 (m, 2 H), 4.44 (t, *J*= 7.04 Hz, 1 H), 4.40 (dd, J_1= 7.04 Hz, J_2= 2.05 Hz, 2 H), 4.48 (dd, J_1= 12.87 Hz, J_2= 7.08 Hz, 1 H), 5.70 (d, *J*= 7.97 Hz, 1 H), 7.30 (m, 2 H), 7.40 (t, *J*= 7.44 Hz, 2 H), 7.76 (d, *J*= 7.57 Hz, 2 H), 13.33 (s, br, 1 H).



Dopa-Lys(Dde)-$CONH_2$

Fmoc-DOPA(TBDMS)$_2$-OH (1 eq.) and DIPEA (4 eq.) was dissolved in CH_2Cl_2 (20 mL g^{-1} resin). Followed by the addition of Wang resin (1.3 mmol/g, 1 eq.). Then the mixture was stirred at room temperature for 1 h. After that the solvent was filtered off and the unreacted resin was capped by using 17:2:1 CH_2Cl_2 : MeOH : DIPEA (v:v:v, 3 × 20 mL/g resin). Then the resin were thoroughly washed with CH_2Cl_2, DMF, and CH_2Cl_2 and dried over vacuum. The bead loading was measured by using 2%

DBU/DMF method to be ~ 0.5 mmol g^{-1} . Then the resin was swollen in DMF at room temperature for about 0.5 h. After that the solvent was removed via filtration. 20 % Piperidine/DMF (3 × 5 mL) was added to remove the Fmoc protecting group and the mixture was stirred for 5 min, followed by using CH_2Cl_2 and DMF wash 3 times respectively. Fmoc-Lys(Dde)-OH(4 eq.), HBTU (4 eq.), and DIPEA (8 eq.) in DMF (5 mL) was added to the de-Fmoc resin and the mixture was shaken at room temperature for 2 h. Then the solvent was filtered and the resin was washed 3 times with DMF, CH_2Cl_2, and DMF respectively.After that the resin was incubated in 15 mL of 0.13 M TBAF in THF to remove the TBDMS protecting group at room temperature for 45 min. Finally, 30% TFA in CH_2Cl_2 (20 mL g^{-1} resin)was incubated with the resin for about 3 hr at room temperature to cleave the peptide.The solvent was concentrated *in vacuo* and cold Et_2O was added to precipitate the peptide. The product was collected by filtration, washed with Et_2O, and dried over high vacuum.

Lys(Dde)-Dopa-CONH$_2$

Fmoc-Lys(Dde)-OH (1 eq.) and DIPEA (4 eq.) was dissolved in CH_2Cl_2 (20 mL g^{-1} resin). Followed by the addition of Wang resin (1.3 mmol/g, 1 eq.). Then the mixture was stirred at room temperature for 1 h. After that the solvent was filtered off and the unreacted resin was capped by using 17:2:1 CH_2Cl_2 : MeOH : DIPEA (v:v:v, 3 × 20 mL/g resin). Then the resin were thoroughly washed with CH_2Cl_2, DMF, and CH_2Cl_2 and dried over vacuum. The bead loading was measured by using 2% DBU/DMF method to be ~ 0.5 mmol g^{-1} . Then the resin was swollen in DMF at room temperature for about 0.5 h. After that the solvent was removed via filtration. 20 % Piperidine/DMF (3 × 5 mL) was added to remove the Fmoc protecting group and the mixture was stirred for 5 min, followed by using CH_2Cl_2 and DMF wash 3 times respectively. Fmoc-Dopa(TBDMS)$_2$-OH(4 eq.), HBTU (4 eq.), and DIPEA (8 eq.) in DMF (5 mL) was added to the de-Fmoc resin and the mixture was shaken at room temperature for 2 h. Then the solvent was filtered and the resin was washed 3 times with DMF, CH_2Cl_2, and DMF respectively.After that the resin was incubated in 15 mL of 0.13 M TBAF in THF to remove the TBDMS protecting group at room temperature for 45 min. Finally, 30% TFA in CH_2Cl_2 (20 mL g^{-1} resin)was incubated with the resin for about 3 hr at room temperature to cleave the peptide.The solvent was concentrated *in vacuo* and cold Et_2O was added to precipitate the peptide. The product was collected by filtration, washed with Et_2O, and dried over high vacuum.

Dopa-Gly-CONH$_2$

Fmoc-DOPA(TBDMS)$_2$-OH (1 eq.) and DIPEA (4 eq.) was dissolved in CH_2Cl_2 (20 mL g^{-1} resin). Followed by the addition of Wang resin (1.3 mmol/g, 1 eq.). Then the mixture was stirred at room temperature for 1 h. After that the solvent was filtered off and the unreacted resin was capped by using 17:2:1 CH_2Cl_2 : MeOH : DIPEA (v:v:v, 3 × 20 mL/g resin). Then the resin were thoroughly washed with CH_2Cl_2, DMF, and CH_2Cl_2 and dried over vacuum. The bead loading was measured by using 2% DBU/DMF method to be ~ 0.5 mmol g^{-1} . Then the resin was swollen in DMF at room temperature for about 0.5 h. After that the solvent was removed via filtration. 20 %

Piperidine/DMF (3 × 5 mL) was added to remove the Fmoc protecting group and the mixture was stirred for 5 min, followed by using CH_2Cl_2 and DMF wash 3 times respectively. Fmoc-Gly-OH(4 eq.), HBTU (4 eq.), and DIPEA (8 eq.) in DMF (5 mL) was added to the de-Fmoc resin and the mixture was shaken at room temperature for 2 h. Then the solvent was filtered and the resin was washed 3 times with DMF, CH_2Cl_2, and DMF respectively.After that the resin was incubated in 15 mL of 0.13 M TBAF in THF to remove the TBDMS protecting group at room temperature for 45 min. Finally, 30% TFA in CH_2Cl_2 (20 mL g^{-1} resin)was incubated with the resin for about 3 hr at room temperature to cleave the peptide.The solvent was concentrated *in vacuo* and cold Et_2O was added to precipitate the peptide. The product was collected by filtration, washed with Et_2O, and dried over high vacuum.

Supporting Figures

1. Representative force-extension curves for other dipeptides

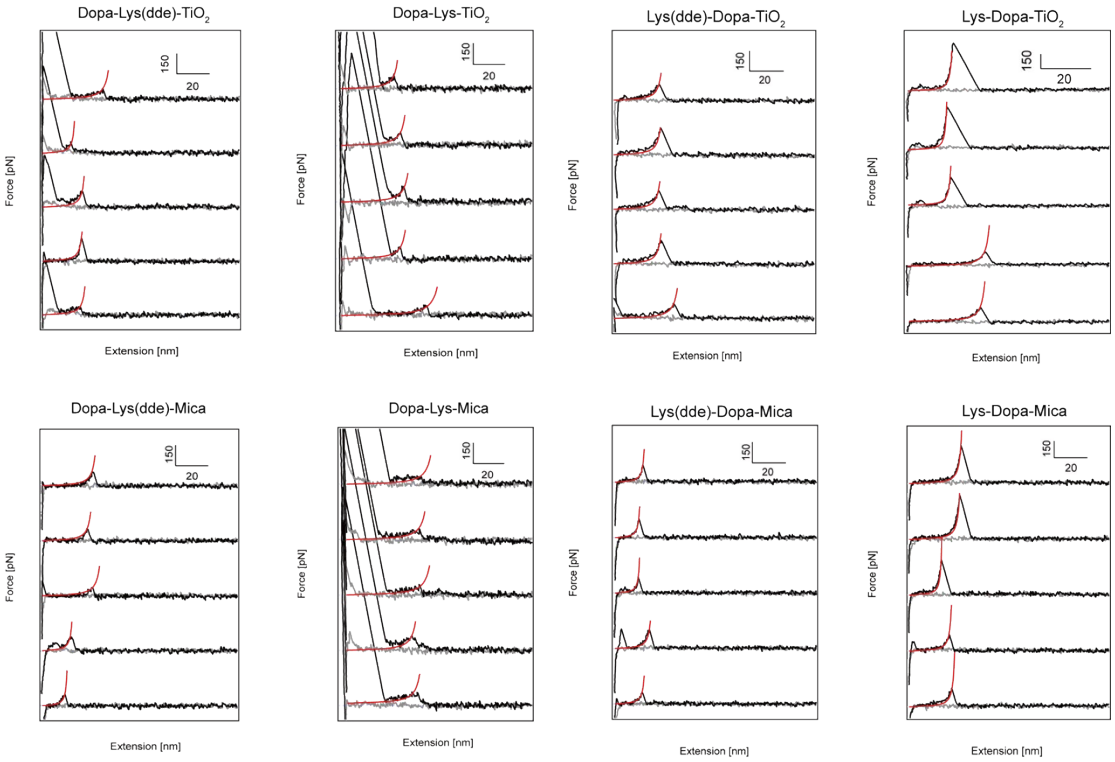


Figure S1. Representative force-extension curves for other dipeptides and surfaces. The number of events refers to the number of unbinding events. In the force curves only the last detatching force peak that can be fitted with Worm-like chain model was counted as the unbinding events. Therefore, only one unbinding event per force curve.

2. Pulling speed dependent experiments

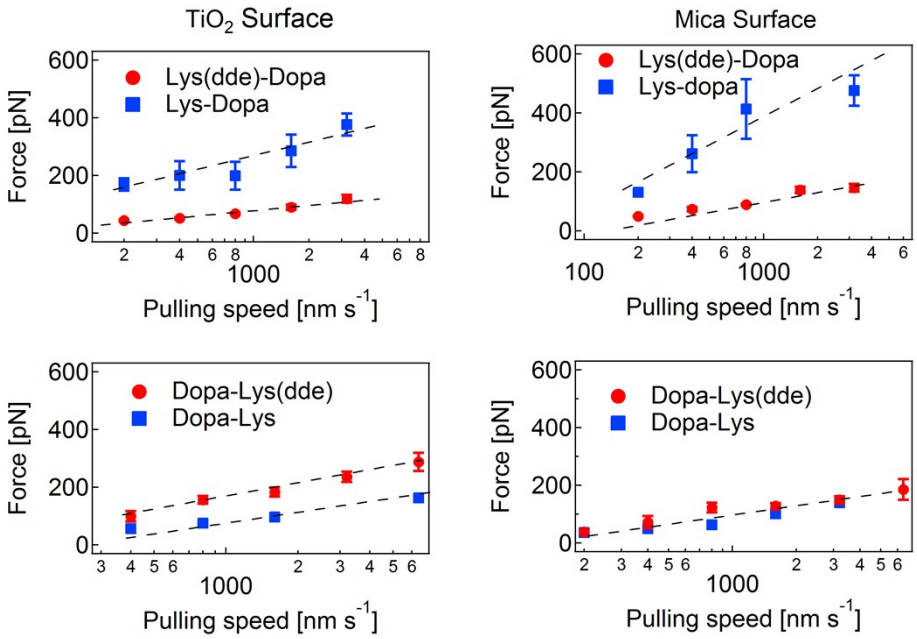


Figure S2. Pulling speed dependent experiments. The rupture forces for all dipeptides are pulling speed dependent, the larger the pulling speed is, the higher the rupture forces are. The red circles represent Lys(dde)-Dopa dipeptides and the blue squares correspond to Lys-Dopa dipeptides. The dashed lines are used to guide the eyes for data trends.

3. Control experiments of Gly-Dopa and TiO_2 or mica surfaces

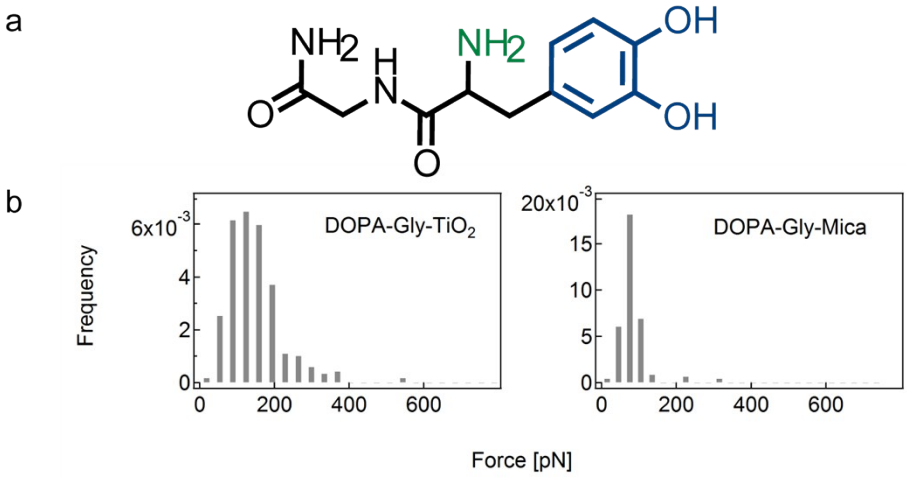


Figure S3. Detaching force distribution of Dopa-Gly on TiO_2 and Mica surfaces. (a) Molecular structure of Dopa-Gly. The amino group used to conjugate to AFM tip and Dopa residues are labeled in green and blue, respectively. (b) The average detaching forces are 106 pN (for TiO_2 surface) and 76 pN (for mica surface), respectively.

4. Statistic comparison of the force distributions

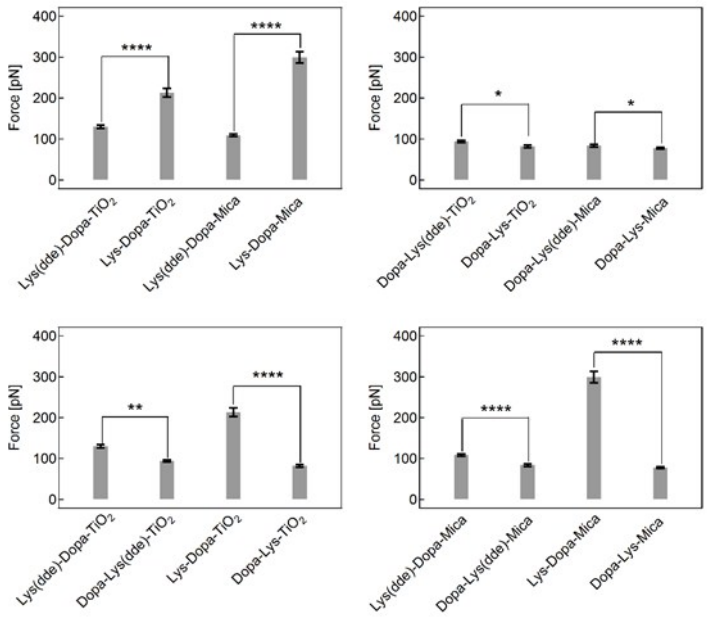
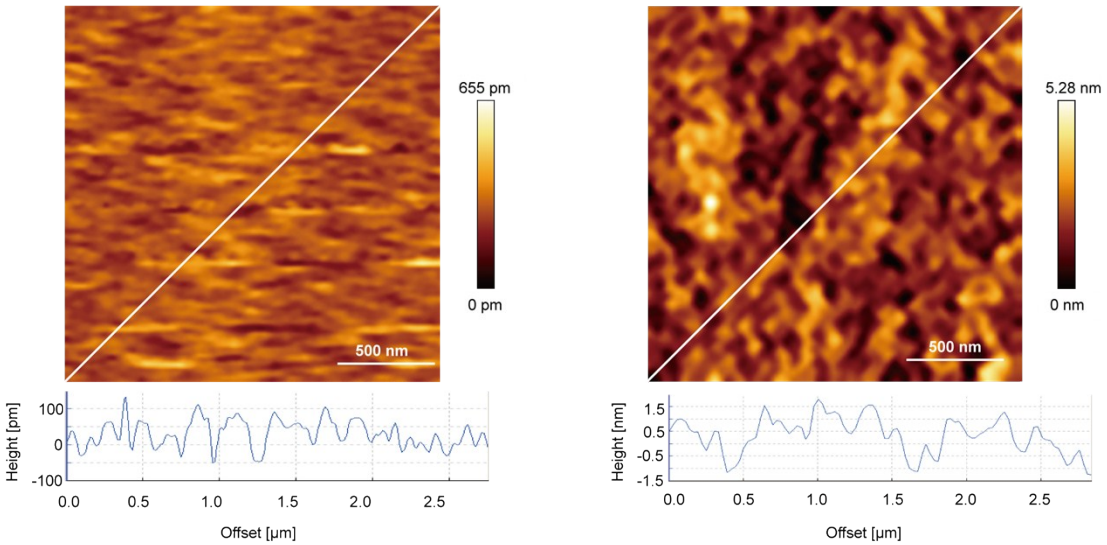


Figure S4 Student's T test on the force distributions of different peptide-surface binding. *: not significantly different; **:P<0.05; ****: P<0.0001.

5. Influence of surface roughness on synergistical binding

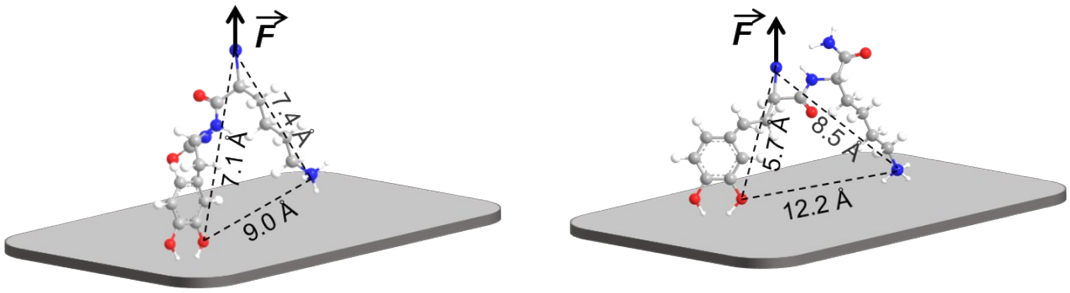


	Mica	TiO_2
Average roughness [Å]	0.3	5.8

Figure S5. Surface roughness of Mica (left) and TiO_2 (right) evaluated by AFM imaging. The images were obtained at room temperature by a NanoWizard II AFM (JPK, Germany) operating in intermittent contact mode. Surface roughness of the two substrates were automatically evaluated by a commercial

software (JPK data processing). The height profile of the white line is shown below each image.

6. 3D drawings for the stereoscopic structure of Lys-Dopa and Dopa-Lys dipeptides.



Lys-Dopa Dopa-Lys

Figure S6. 3D drawings for the stereoscopic structure of lys-Dopa and Dopa-lys. 3D structures of the two dipeptides were generated and optimized in ChemBio 3D Ultra. The distances between the catechol of Dopa, the amine of lysine and the force applied atom are also measured in ChemBio 3D ultra.

Supporting Table

Table S1. Statistic data for Figure 2.

	Average detaching force [pN]	Standard error of the mean [pN]	Number of events*
Lys(dde)-Dopa-TiO_2	130	4	158
Lys-Dopa-TiO_2	213	10	239
Lys(dde)-Dopa-Mica	109	3	445
Lys-Dopa-Mica	299	14	127
Dopa-Lys(dde)-TiO_2	94	3	135
Dopa-Lys-TiO_2	82	3	179
Dopa-Lys(dde)-Mica	84	3	168
Dopa-Lys-Mica	78	2	243
Dopa-Gly-TiO_2	106	4	276
Dopa-Gly-Mica	76	2	161

* The number of events refers to the number of unbinding events. In the force curves only the last detatching force peak that can be fitted with Worm-like chain model was counted as the unbinding events. Therefore, only one unbinding event per force curve.

Reference:

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