Supplementary Information

Interaction of the Vascular Endothelial Growth Factor and Heparin Quantified by Single Molecule Force Spectroscopy

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■ Materials and reagents

$VEGF_{165}$ protein was supplied by Sino Biological Inc. (China). Heparin sodium with molecular weight of 6000 ~ 20000 was purchased from Sangon Biotech (Shanghai) Co., Ltd. (China). Silicon wafers were obtained from Shengxu Electronic Technology Co., Ltd. (China). NHS-PEG_{18}-acetal was supplied by Prof. Hermann J. Gruber (Johannes Kepler University, Austria). $NaCNBH_3$ was purchased from J&K Scientific Ltd. (China). 3-Aminopropyltriethoxysilane (APTES), trimethylamine (TEA) and ethanolamine were purchased from Sigma-Aldrich (USA). Anhydrous citric acid was obtained from Alfa Aesar (USA). N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDAC) and N-hydroxysuccinimide (NHS) were bought from Aladdin (China). Anhydrous ethanol and chloroform used in all experiments are guaranteed reagents. All other reagents were analytical-grade. Ultrapure water used for all solution was prepared by a Milli-Q water purification system. AFM cantilevers (MSCT, average spring constant is 0.045 ± 0.008 N m^{-1}, n = 11) were supplied by Bruker (USA), and spring constants of these cantilevers were calibrated by the thermal noise method.

■ Equations

To further ensure that the specific adhesion peaks observed correspond to the breaking of an interaction occurring between heparin and $VEGF_{165}$ protein attached at the free end of the PEG linker, the force-extension curves with clear unbinding events were fitted using worm-like chain (WLC) model [1] shown as the following Equation S1:

$$F(x) = \frac{k_B T}{l_P}\left[0.25\left(1 - \frac{x}{L}\right)^2 - \frac{x}{L} - 0.25\right] \quad \text{(S1)}$$

where k_B is the Boltzmann constant, T is the absolute temperature, l_p is the persistence length and L is the contour length.

The relationship between unbinding forces and loading rates can be described by the Bell-Evans model [2] as the following Equation S2:

$$F = \left(\frac{k_B T}{x_\beta}\right) ln\left(\frac{r x_\beta}{k_B T k_{off}}\right) \quad \text{(S2)}$$

where F is the most probable unbinding force, x_β is the distance of the transition state to the bound state, r is the loading rate derived from the product of retraction velocity and the effective spring constant, k_{off} is the kinetic off-rate constant.

The free energy barriers $\Delta G^{\ddagger}$ along the dissociation pathway can be quantified as following Equation S3 [3]:

$$\Delta G^{\ddagger} = - k_B T ln(\tau_D k_{off}) \quad \text{(S3)}$$

where R is the gas constant and τ_D is the diffusive relaxation time (10^{-9} s in our

calculation).

In MD simulation, the total binding free energy can be calculated by the Equation S4 [4]:

$$\Delta G_{bind} = \Delta E_{MM} + \Delta G_{sol} - T\Delta S$$

$$= \left(\Delta E_{vdW} + \Delta E_{ele} + \Delta E_{int}\right) + \left(\Delta G_{polar} + \Delta G_{nonpolar}\right) - T\Delta S$$

(S4)

Where ΔG_{bind} is the binding free energy the total of five energy items, ΔE_{MM} is the change of molecular mechanical energy, ΔG_{sol} is the solvation free energy and $T\Delta S$ considers the penalty of entropy. The ΔE_{MM} includes non-bonded interaction, van der Waals interaction ΔE_{vdW}, electrostatic interaction ΔE_{ele}, and local bonded interaction ΔE_{int}. The ΔG_{sol} includes polar solvation free energy ΔG_{polar} and nonpolar solvation energy $\Delta G_{nonpolar}$. Among them, ΔE_{vdW}, ΔE_{ele}, ΔG_{polar} and $\Delta G_{nonpolar}$ are calculated and decomposed using MM/PBSA method. The change of conformation entropy ($-T\Delta S$) is evaluated by normal mode analysis. ΔE_{int} can be counteracted in single trajectory approach, which contains contributions of bond, angle, and dihedral.

■ Figures

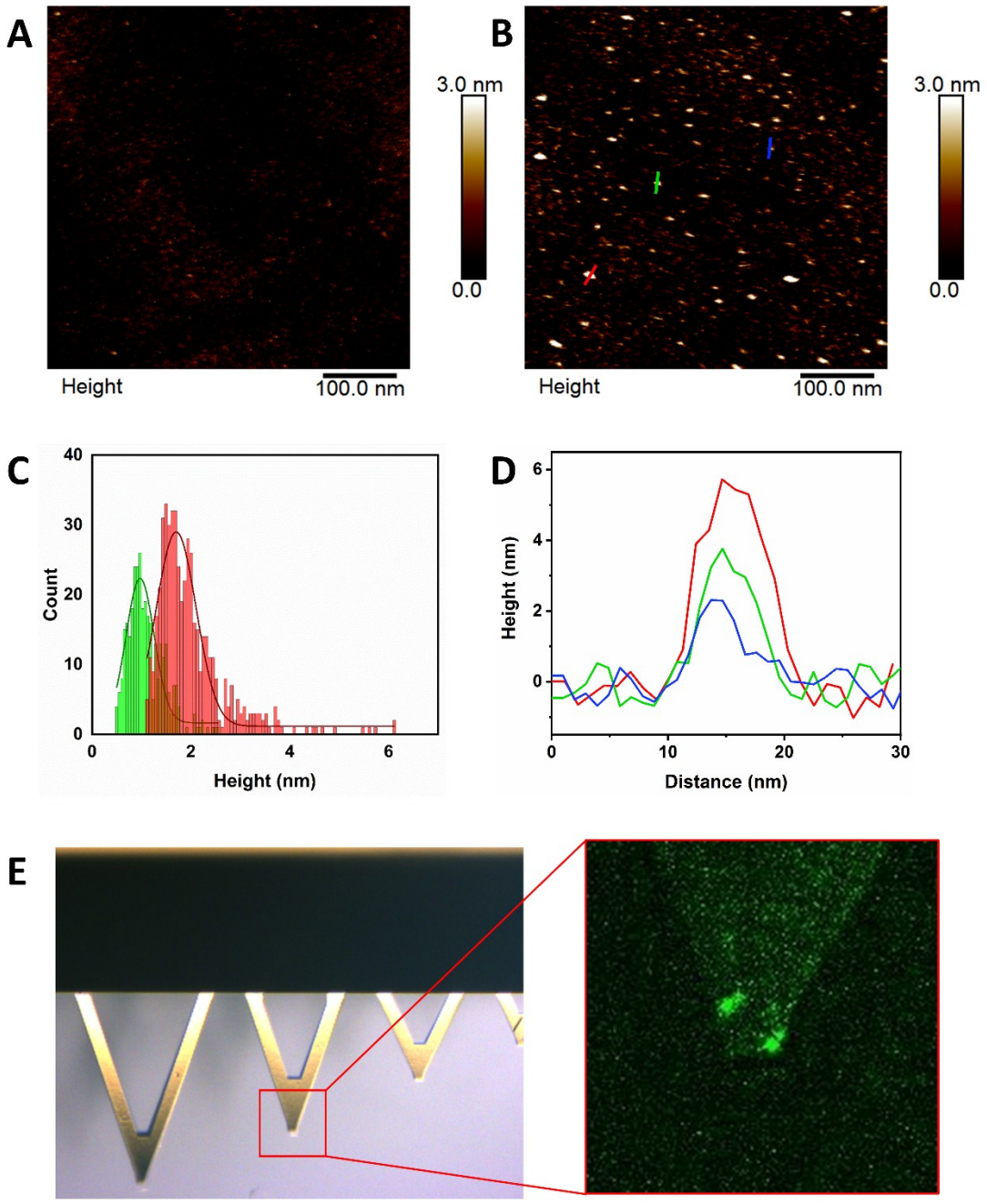


Figure S1 The characterization of functionalized silicon substrate and AFM probes. The AFM images of (A) aminated silicon substrate and (B) heparin-functionalized silicon substrate (Scan rate: 1 Hz; scan size: 1 μm; pixels: 512 × 512). (C) The histogram of height distribution on the aminated silicon substrate surface (green) and

the heparin-functionalized silicon substrate surface (red). (D) Three section lines shown the height of heparin molecules observed on the surface from (B). (E) The Optical (left) and fluorescence (right) images of the $VEGF_{165}$-functionalized AFM probe.

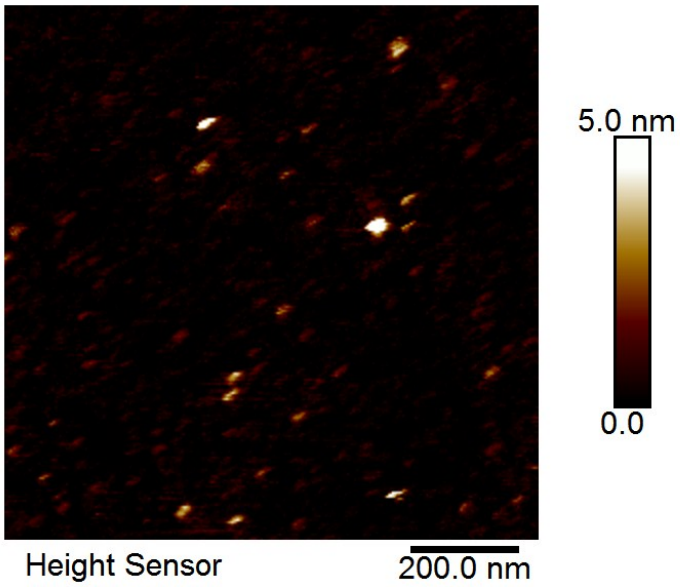
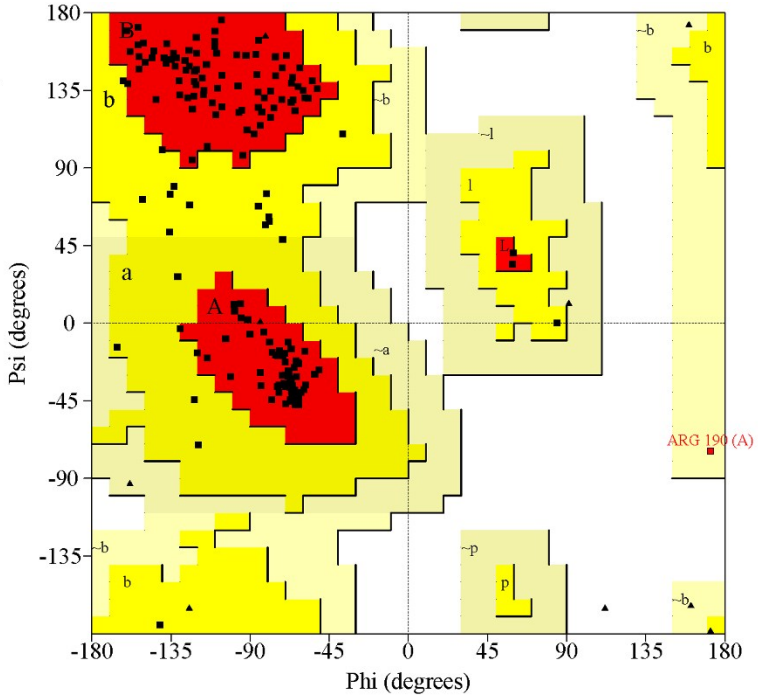


Figure S2 AFM image of $VEGF_{165}$ proteins on mica with a mean height of 1.67nm and mean diameter of 23.31nm. The protein can be approximated as a sphere with an effective diameter of 11 nm. Scan rate: 1 Hz; scan size: 1 μm; pixels: 512 × 512.

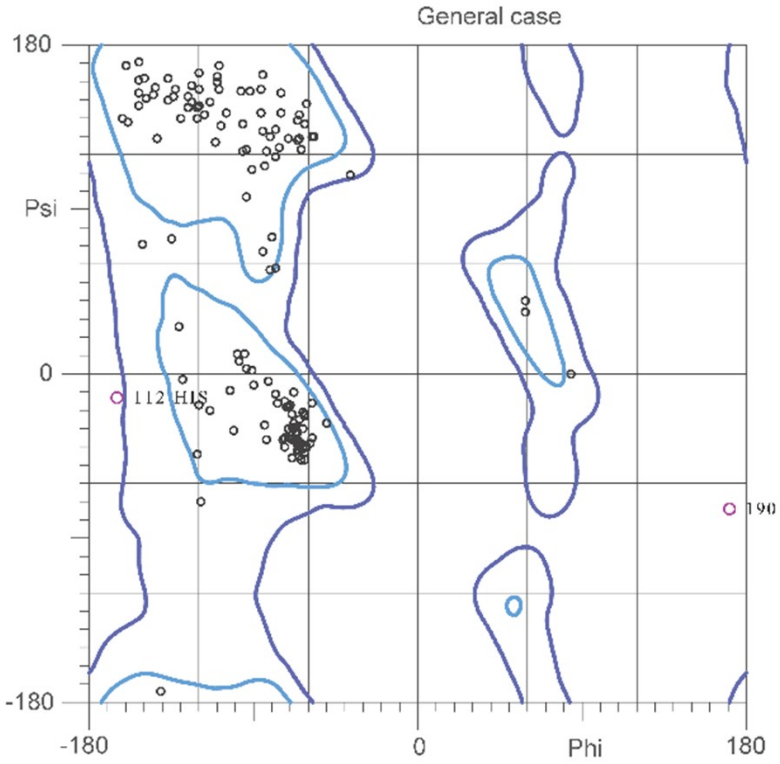
A



Plot statistics

Residues in most favoured regions [A,B,L]	148	88.1%
Residues in additional allowed regions [a,b,l,p]	19	11.3%
Residues in generously allowed regions [~a,~b,~l,~p]	1	0.6%
Residues in disallowed regions	0	0.0%
Number of non-glycine and non-proline residues	168	100.0%
Number of end-residues (excl. Gly and Pro)	2	
Number of glycine residues (shown as triangles)	9	
Number of proline residues	12	
Total number of residues	191	

B



94.2% (178/189) of all residues were in favored (98%) regions.
97.4% (184/189) of all residues were in allowed (>99.8%) regions.

Figure S3 PROCHECK and MolProbity servers both determined that most of the residues (>97%) were in allowed conformations as depicted in the Ramachandran plots shown above, indicating that the model built in the Robetta server is appropriate.

■ Table

Table S1 Unbinding forces at different loading rates

Loading rate (pN s^{-1})	350±28	660±62	800±118	840±42	850±48
Force (pN)	26.35±1.11	30.37±1.86	28.85±1.39	36.91±2.14	33.83±0.83
Loading rate (pN s^{-1})	850±83	940±74	980±60	1005±89	1100±77
Force (pN)	31.63±1.19	26.40±1.80	37.07±1.87	33.42±1.13	32.47±1.03
Loading rate (pN s^{-1})	1120±66	1140±97	1170±89	1240±83	1300±207
Force (pN)	28.05±3.07	32.43±1.71	31.77±1.38	34.31±1.41	33.84±1.90
Loading rate (pN s^{-1})	1440±134	1575±74	1760±99	1760±114	1830±102
Force (pN)	42.44±1.68	41.82±0.88	39.78±1.88	31.30±1.93	37.53±2.10
Loading rate (pN s^{-1})	1860±207	1940±95	1950±155	2000±195	2200±253
Force (pN)	28.53±1.55	43.61±1.10	31.41±1.55	40.93±2.20	34.50±1.64
Loading rate (pN s^{-1})	2670±100	3200±285	3300±563	7200±528	11400±1477
Force (pN)	36.65±3.00	43.09±2.62	45.49±2.04	50.84±3.09	57.76±4.19

Table S2 Binding free energy (kcal mol^{-1}) and the energy components for $VEGF_{165}$/heparin* complex

ΔG_{bind}	ΔE_{vdw}	ΔE_{ele}	ΔG_{polar}	$\Delta G_{nonpolar}$	$-T\Delta S$
-11.47	-57.25	-369.32	379.64	-6.81	42.27

■ References

1. Oesterhelt, F.; Oesterhelt, D.; Pfeiffer, M.; Engel, A.; Gaub, H.; Müller, D., *Science*, 2000**, 288**, 143-146.

2. Evans, E.; Ritchie, K., *Biophysical Journal*, 1999**, 76**, 2439-2447.

3. Li, W.; Kou, X.; Xu, J.; Zhou, W.; Zhao, R.; Zhang, Z.; Fang, X., *Analytical Chemistry*, 2018**, 90**, 4596-4602.

4. F. Cui, K. Yang and Y. Li, *PLoS One*, 2015, **10**.