Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information

Three steps to gold:

Mechanism of protein adsorption revealed by Brownian and molecular dynamics simulations

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METHODS

S1. Clustering methods

During the Brownian dynamics (BD) simulations, the coordinates of the protein at each timestep at which the protein had at least two non-hydrogen atoms within 6 Å distance of the surface were recorded. If the coordinates at a given timestep had an RMSD value less than 2 Å to a complex that had already been recorded, they were considered to belong to the same docked complex and only the coordinates with the most favorable interaction energy were retained and the number of occurrences of this complex was incremented by 1. The 2000 distinct docked complexes with the most favorable interaction energies obtained in each BD trajectory were recorded. The docked complexes were then subjected to two different hierarchical procedures to cluster them: a single-linkage method and an average-linkage clustering method. An inter-configuration distance matrix was created using the continuous backbone and continuous C_α RMSD values between the docked complexes for the average-linkage and the single-linkage methods, respectively. In the single-linkage clustering, for the RMSD values of the C_α atoms, a 3Å RMSD cutoff was chosen to assign an orientation to a particular cluster. The clusters obtained were ranked by the interaction energy values of their representatives. The representative of a cluster was defined as the configuration with the smallest RMSD to every other member of the cluster. In the average-linkage method described by Motiejunas et al.[1], encounter complexes were clustered with a predefined number of clustering cycles and the clusters obtained were afterwards ranked according to size, i.e. the number of encounter complexes in each cluster. The number of clusters was chosen such that the RMSD between any two members of a cluster (the range) did not exceed 5 Å and the standard deviation of RMSD values for the cluster in any of the clusters did not exceed 1.0 Å Euclidean distance metrics were used to determine cluster assignment.

RMSD is a commonly used measure of the three-dimensional structural similarity between the molecular structures. For a protein structure, a C_α RMSD value of 2 Å usually corresponds to high structural or orientational proximity. An RMSD value of about 5 Å, points at a similarity between the structures or orientations. Applying a cutoff in the range of 3-5 Å in the clustering was found to give reasonably robust results.

To assess the clustering methods, we compared the results with another method: the k-means algorithm. We used the same 2000 docked complexes that we obtained from BD simulations for the k-means clustering. An inter-configuration distance matrix was created using the continuous backbone RMSD values between the docked complexes. The Forgy method was used for initialization, in which n center locations were chosen randomly. Euclidean distance metrics were used to determine cluster assignment.

The trajectories obtained from 25 ns MD simulations were clustered using the single-linkage method implemented in the GROMACS software package. Each structure was added to the corresponding cluster when its distance was less than 1 Å from any of the structures in that cluster. Euclidean distance metrics were again used to determine cluster assignment. The same input feature vector was used for clustering of both BD and MD trajectories.

S2. Discussion of average-linkage clustering.

For a second evaluation of the probabilities of the encounter complex orientations of 3H-BLIP-Au(111), we applied an average-linkage clustering method as explained in the previous section. In Table S3, the 3 largest clusters obtained using this method are listed. The energetically most favorable binding orientation of 3H-BLIP obtained by this method (cluster 2) corresponds to the first cluster obtained by the single-linkage method (the representatives of the two clusters have a root mean squared deviation (RMSD) of 0.1 Å). Although the representative of the cluster has a stronger interaction energy than the representatives of the other clusters, the percentage of similar binding orientations in this cluster is less than 4% of the total number of encounter complexes observed. On the other hand, the first and the third clusters obtained with the average-linkage clustering method correspond to the second and the third from the single-linkage method, with RMSDs between representatives (see Fig.1) of 0.66 Å and 0.67 Å respectively. Finally, clustering of the wtBLIP-Au(111) docked structures by the average linkage clustering method also resulted in the same three major binding orientations revealed by the single-linkage clustering.

S3. Discussion of k-means clustering.

The results of k-means clustering showed that most of the clusters obtained by the other two linkage methods were also obtained by the k-means method (see Table S4). Since the k-means method depends

strongly on the initialization method, the results with different center locations led to obtaining distinct cluster sets. Moreover, since the k-means method tends to produce equi-sized clusters, the clusters obtained had larger RMSD standard deviations, and did not reveal distinct clusters with smaller sizes. In many of the trials, for instance, we were unable to reproduce orientation *e* of wtBLIP, which is geometrically very distant from the other clusters (data not shown). Also, for 3H-BLIP, even choosing as many as 10 different initial clustering centers was not sufficient to reduce the large RMSD range of the 8th cluster (See Table S5), thus leaving many similar sized clusters with small intra-cluster deviations and geometrically close cluster centers.

Overall, the results did not produce any significantly different clusters owing to the small RMSD threshold used in all three clustering methods employed in this study. However, due to the reasons mentioned above, the linkage methods seem to be much more robust than the k-means method. Finally, we conclude that, due to the small RMSD deviations and ranges (See Tables S1, S3 and S5) produce valid results.

S4. Potential of mean force (PMF) calculations.

The PMF calculations were done using the method described in the paper by Kokh et al.[2] and implemented in SDA 6 software. The PMF was computed by placing the center of geometry of the protein 10 Å from the surface and increasing its separation in 0.1 Å increments up to 30 Å in the z direction. The atomic structure of the surface may have an effect on the overall adsorption energy. To take this effect into account, at each separation in the z direction, the center of the protein was placed at different x and y coordinates within a region of 6 Å x 6 Å in increments of 1 Å. The interaction energies were then computed at these (x, y) locations for all orientations of the protein sampled in rotational increments of $d\Omega_1=d\Omega_2=1.5^o$ and $d\Omega_3=3^o$ and averaged for every separation in the z direction.

S5. Computation of the relative permittivity of the solvent.

The relative permittivity of the water in the binding region was computed as follows. First, a region centered on the middle on the surface was defined with a box of size 20 Å x 20 Å x 10 Å in the x, y and z directions, respectively. At each time step, the water molecules present in the box were identified and used to compute the total dipole moment. The relative permittivity was then computed from the mean

value at each time step using a frame of 100 ps around each of the steps. The following formula was used to compute the relative permittivity, ε, in each Cartesian direction:

$$\frac{(\varepsilon - 1)(2\varepsilon + 1)}{3\varepsilon} = \frac{4\pi}{VkT}\left(\left\langle (M.e)^2 \right\rangle_{Ed} - \langle M.e \rangle_{Ed}^2\right)$$

Here, ε is the relative permittivity; V (m^3), k (J/K) and T (K) are the volume of the sample, the Boltzmann constant and the temperature, respectively; and **M** (Debye), **e** and $\mathbf{E_d}$ are the total dipole moment of the sample, a unit vector and the directing field, respectively. For details of the method, see Ref.[3].

References

(1) Motiejunas, D.; Gabdoulline, R.; Wang, T.; Feldman-Salit, A.; Johann, T.; Winn, P. J.; Wade, R. C. Protein-Protein Docking by Simulating the Process of Association Subject to Biochemical Constraints. *Proteins* **2008**, *71*, 1955–1969.

(2) Kokh, D. B.; Corni, S.; Winn, P. J.; Hoefling, M.; Gottschalk, K. E.; Wade, R. C. ProMetCS: An Atomistic Force Field for Modeling Protein−Metal Surface Interactions in a Continuum Aqueous Solvent. *J. Chem. Theory Comput.* **2010**, *6*, 1753–1768.

(3) Ahmad, M.; Gu, W.; Geyer, T.; Helms, V. Adhesive Water Networks Facilitate Binding of Protein Interfaces. *Nat. Commun.* **2011**, *2*, 261.

FIGURES

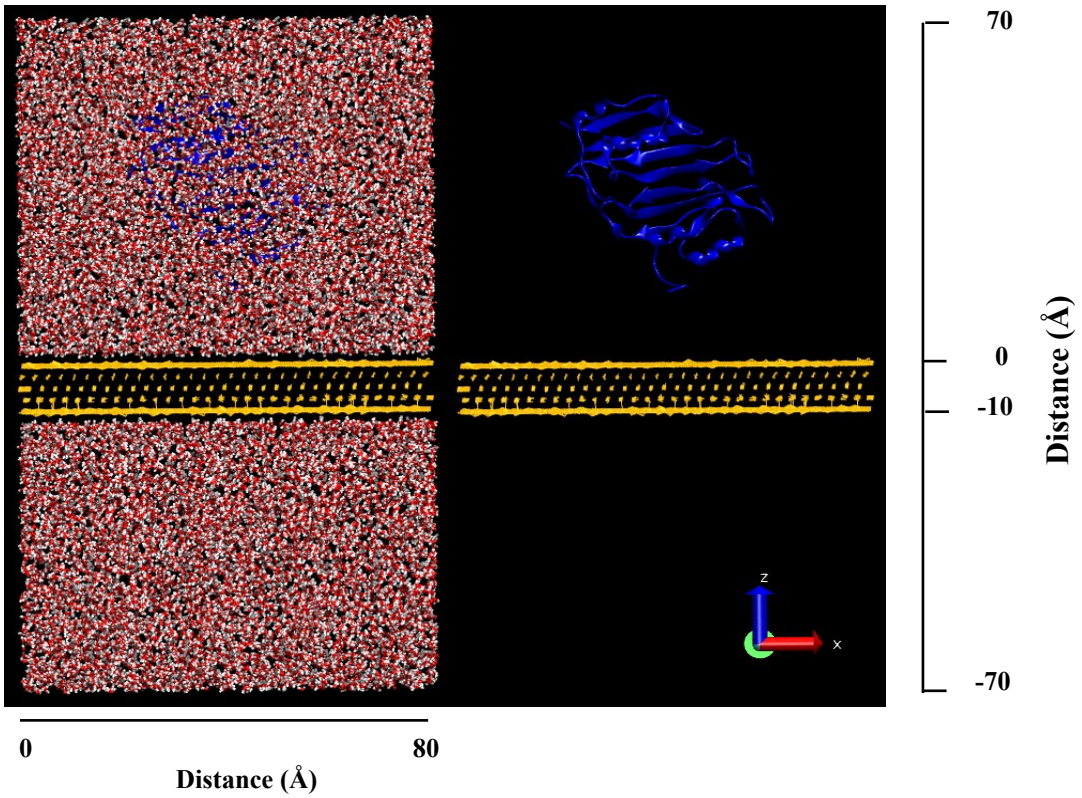


Fig. S1 The simulation box created for the MD simulation of 3H-BLIP-Au (111) system started with a representative orientation obtained from the BD simulations. The solvated (left) and the unsolvated (right) boxes are shown with their size.

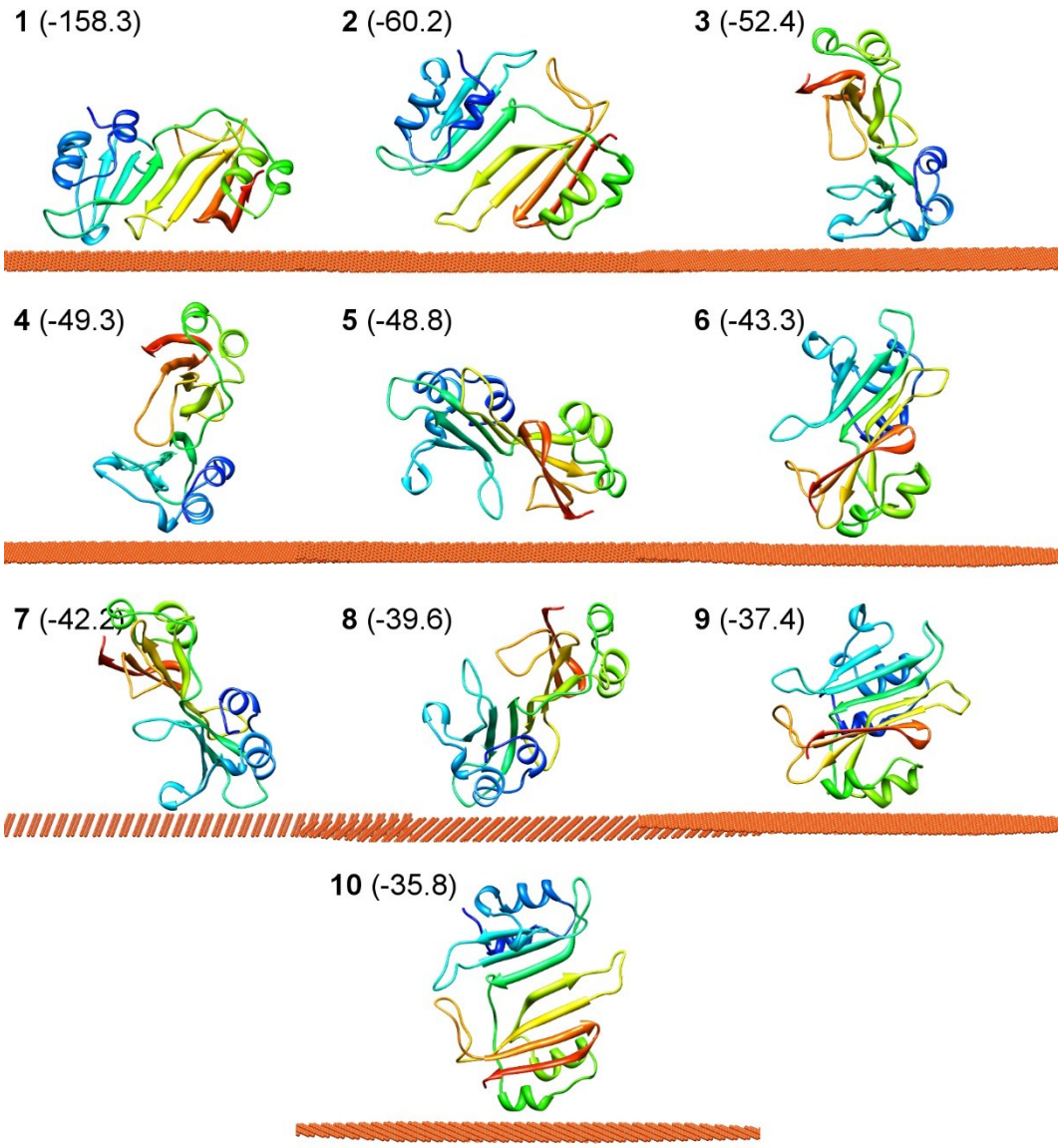
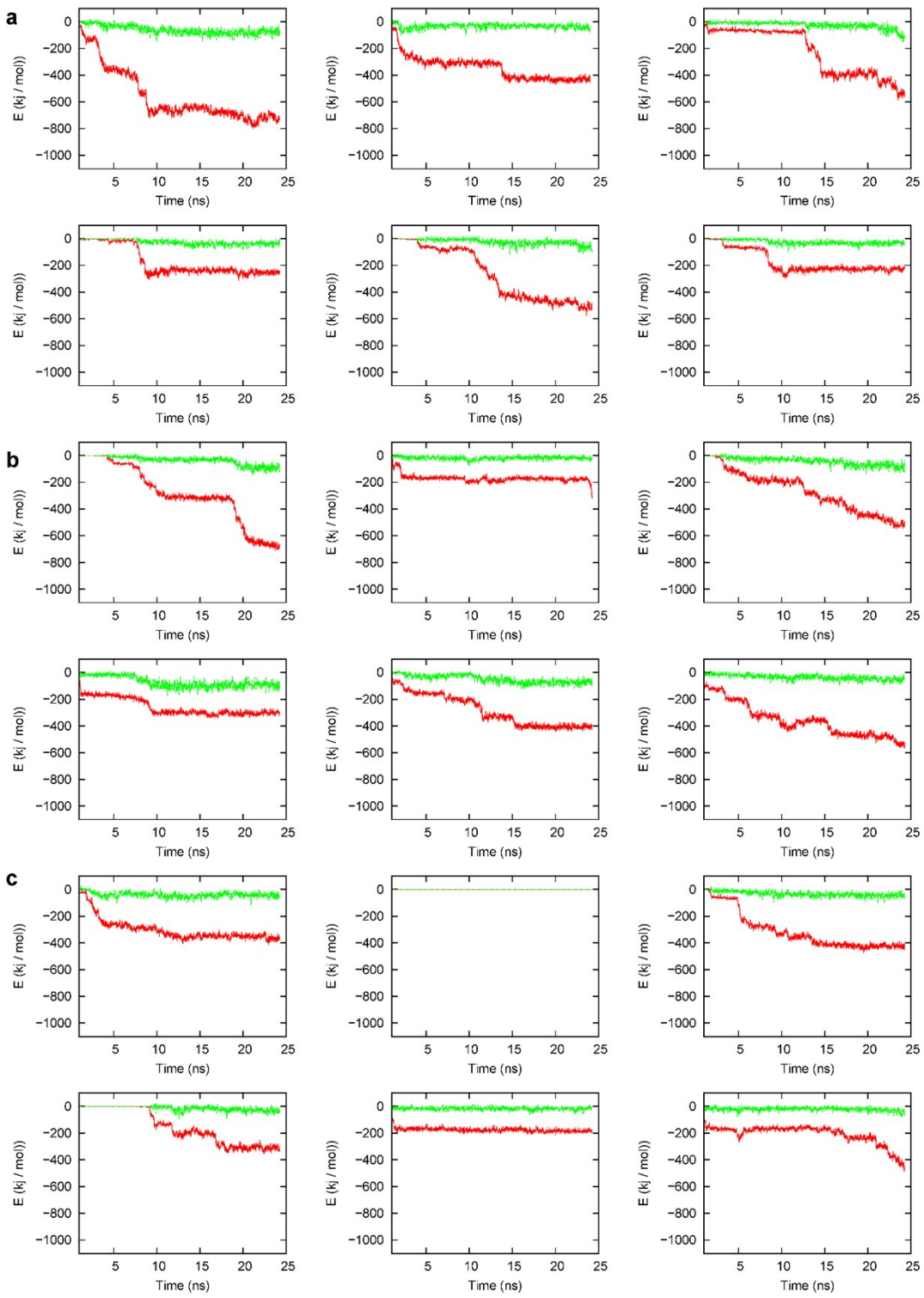


Fig. S2 The binding orientations of wtBLIP to the Au (111) surface. The 10 representatives were obtained by clustering of the BD trajectories by the single linkage clustering method and ranking the clusters by protein-surface interaction energy (in kJ/mol units) shown in parentheses.


a
b
c
E (kj / mol))
Time (ns)
0
−200
−400
−600
−800
−1000
5
10
15
20
25

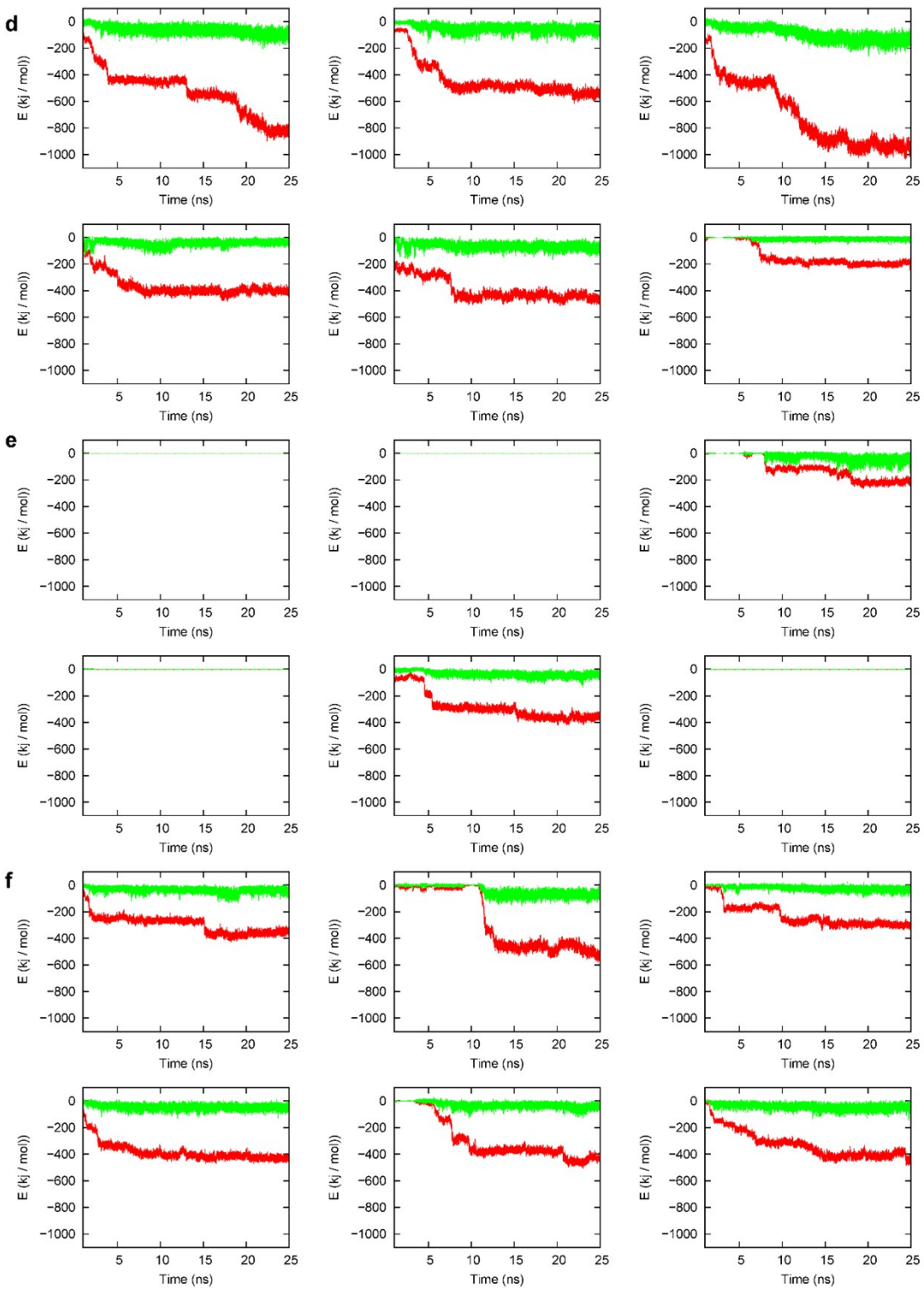


Fig. S3 Time evolution of the LJ (red) and the electrostatic (green) components of the interaction energies during 25 ns-long MD simulations of 3H-BLIP/wtBLIP and the Au (111) surface. Figures a, b and c correspond to the 3H-BLIP simulations started with orientations *a*, *b* and *c,* respectively and d, e and f correspond to the wtBLIP simulations started with orientations *d*, *e*

and *f,* respectively. For each of the starting orientations, the proteins were initially translated by 3 Å (first row) or 5 Å (second row) away from the surface.

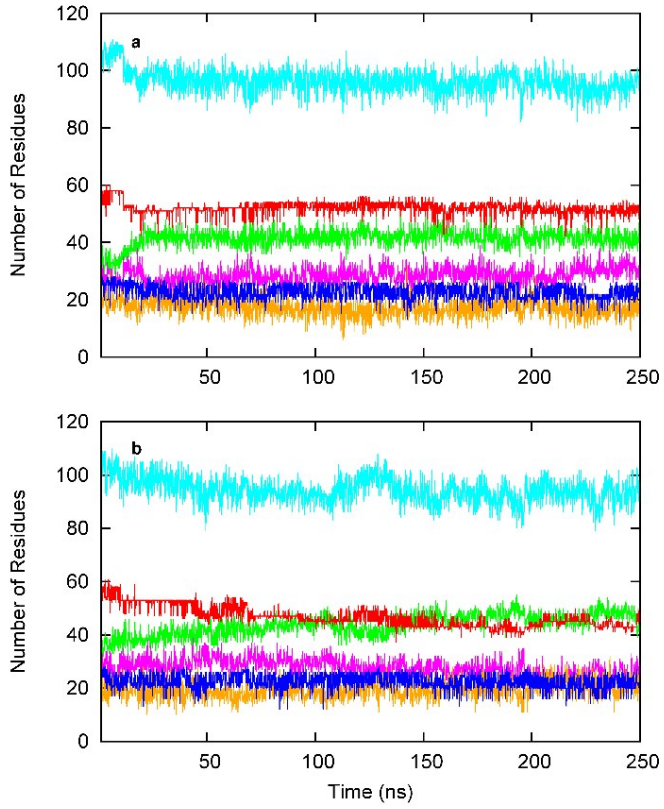


Fig. S4 Time evolution of the number of residues in different secondary structure elements during the MD simulations of 3H-BLIP binding the Au (111) surface started with orientation *a* (a) and *b* (b). The number of residues involved in a beta-sheet (red), in an alpha-helix (blue), in a coil (green), in a bend (magenta) and in a turn (orange) are shown. Note that in (b) the total number of residues involved in a beta-sheet or in a turn (cyan) decreases gradually with time.

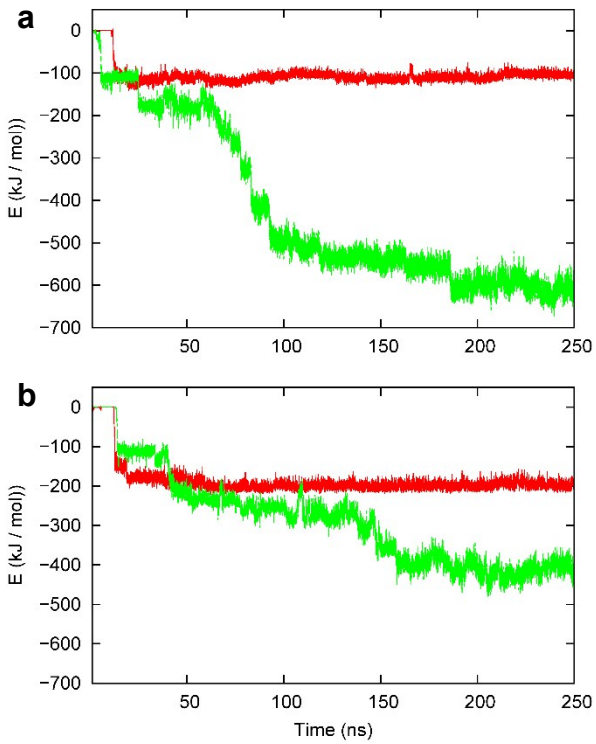


Fig. S5 LJ interaction terms of the aromatic residues (green) and of the 3H residues (red) with the surface for the 250 ns simulations of 3H-BLIP started with orientations *a* (a) and *b* (b) separated by 12 Å from the Au (111) surface.

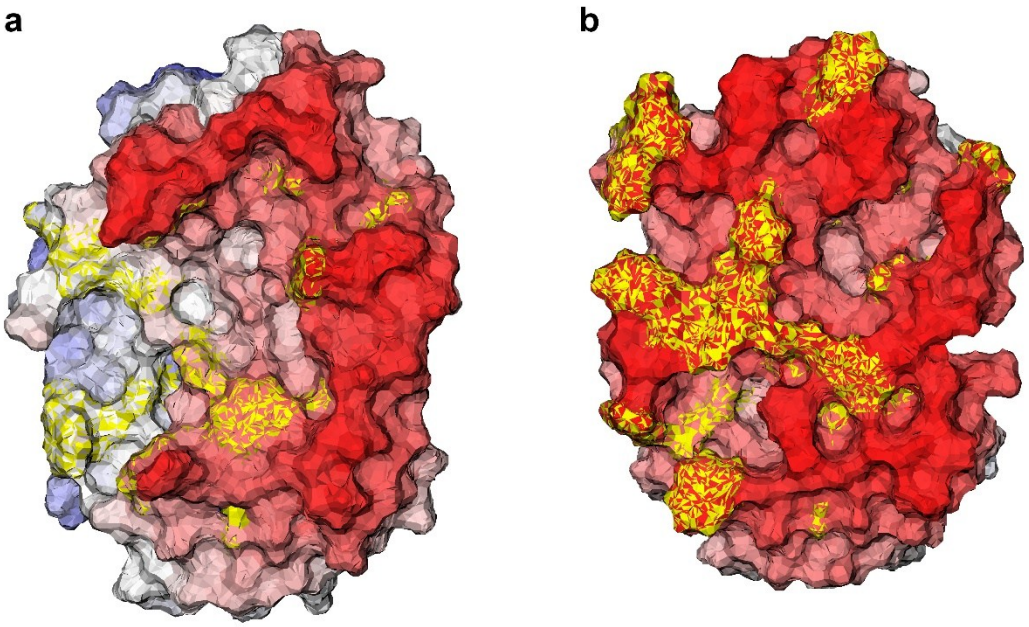


Fig. S6 The surface area exposed by the aromatic residues in the crystal structure of BLIP (a) and from the MD simulation started from orientation *a*, viewed from the Au (111) surface (b). The surface of the aromatic residues is shown in yellow, and the distance of the surface points from the Au (111) surface in the z direction increases from red to blue (from 2 Å to 30 Å, see Figure S1).

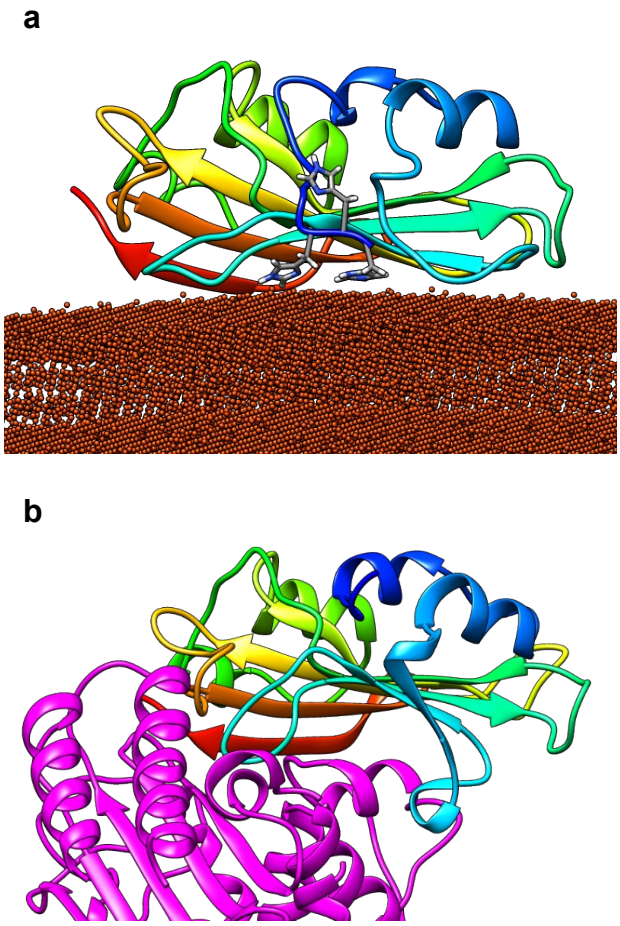


Fig. S7 Binding orientation of 3H-BLIP to the Au (111) surface at the end of the 250 ns simulation starting with orientation *a* (a) and to TEM1 in the crystal structure of the complex (PDB ID: 2B5R) (b).

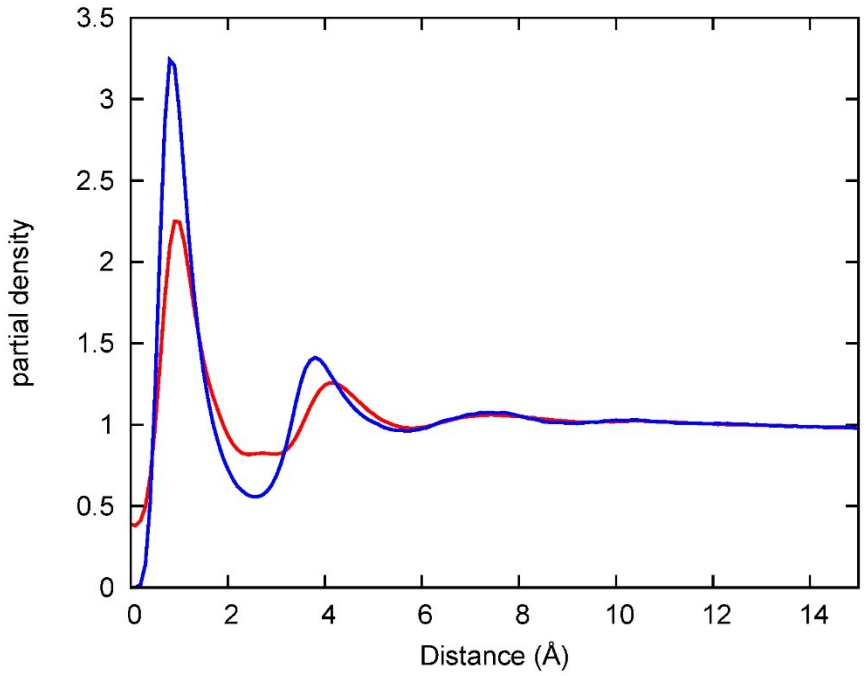


Fig. S8 Ordering of three layers of water next to the Au (111) surface with the water dipoles pointing towards the metal surface. Partial density of the oxygen atoms (blue) and of the hydrogen atoms (red) of the water on the metal surface along the *z* axis of the simulation box computed from MD simulations. The densities are normalized to the values of the bulk water.

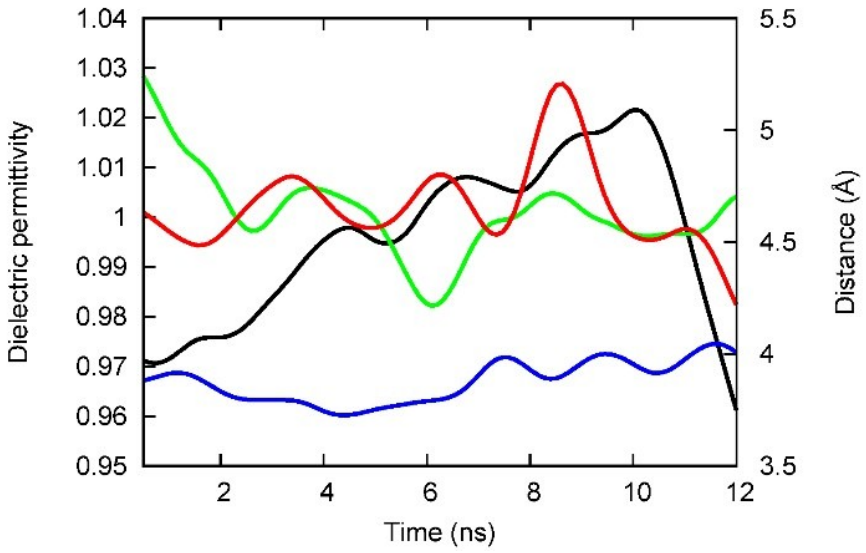


Fig. S9 Anisotropy of the dielectric constant of the interfacial water during the adsorption of 3H-BLIP to the Au(111) surface in the first 12 ns of the 250 ns-long MD simulation starting from orientation *b*. Computed directional relative dielectric constants of the interfacial water along the *x* (green), *y* (red) and *z* (blue) axes (with the plane of the gold surface at *z*=0 Å as defined in Figure S1 and the distance in the *z*-direction between the 3H-BLIP 3H tag and the Au(111) surface (black) are shown. The values of the relative dielectric constants are normalized relative to the values for bulk water. The relative dielectric constants in the *x* and *y* directions (parallel to the surface) in the interfacial gap region fluctuate around the value of the bulk water. On the other hand, the relative dielectric constant in the *z* direction (perpendicular to the surface) is lower than that of the bulk water and of the dielectric constants in the *x* and *y* directions.

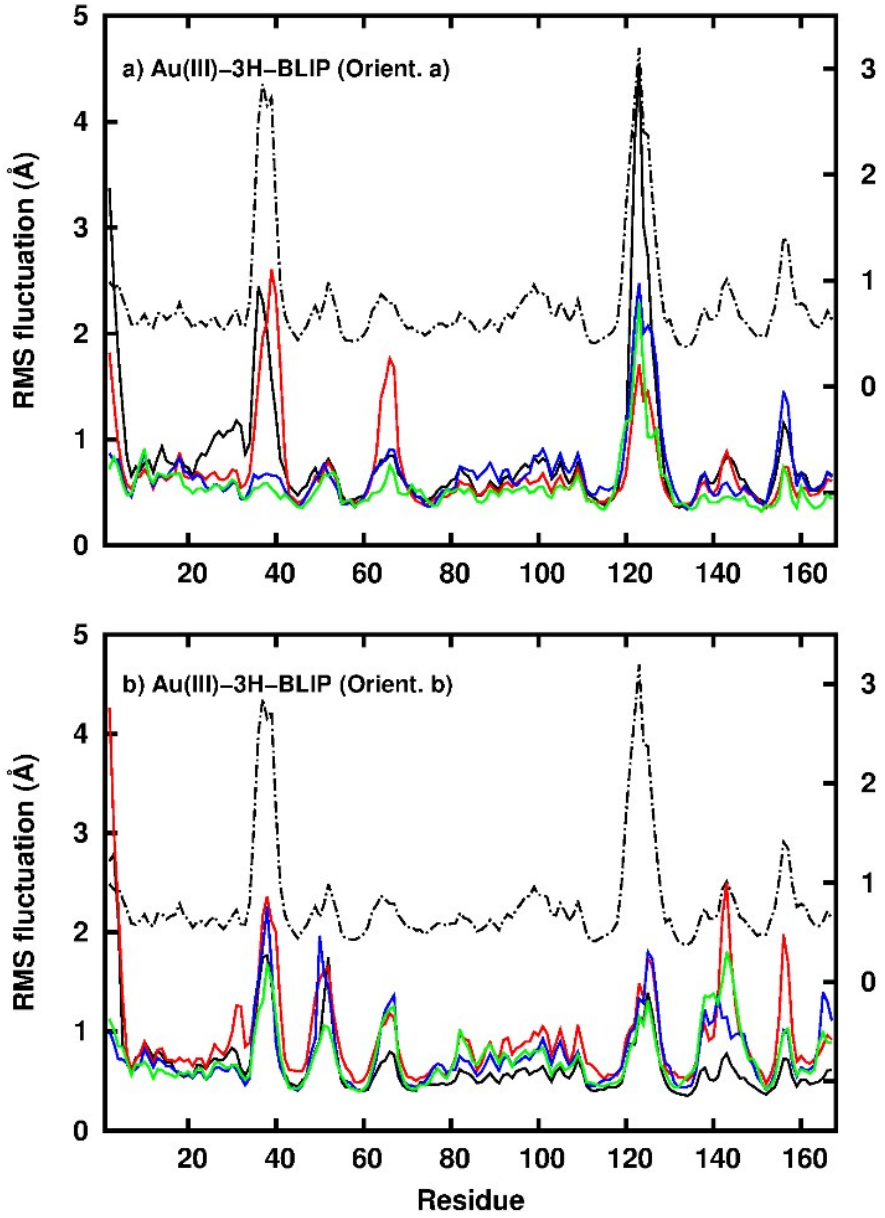


Fig. S10 RMS fluctuations of the backbone atoms of 3H-BLIP in MD simulations for the protein in the presence of an Au(111) surface with starting orientations *a* (a) and *b* (b) compared to the free protein in solution shown by dashed lines (right-hand *y*-axis). The fluctuations are shown for the periods from 10 to 25 ns (black) of the three simulations, and from 25 to 55 ns (red), from 85 to 115 ns (blue) and from 145 to 175 ns (green) for the two 250 ns-long simulations with the surface). Comparisons of the fluctuations with those of 3H-BLIP simulated in the presence of the surface (orientations *a* and *b*) show two different behaviors. The RMS fluctuations in the period from 10 to 25 ns for the simulation that started with orientation *a* are rather similar to those of

free 3H-BLIP whereas for the simulation started with orientation *b*, the RMS fluctuations are smaller. The RMS fluctuations tend to decrease for the simulation started with orientation *a* and to increase for the simulation started with orientation *b* after adsorption takes place. Increasing RMS fluctuations upon adsorption in the simulations that started with orientation *b* suggest an energy barrier that traps the protein. This difference may be due to the different initial binding interactions formed in the two simulations.

TABLES

Table S1 Computed properties of the BD-docked clusters of the 3H-BLIP and wt-BLIP proteins on the gold surface

Protein	Cluster No.[a]	Total Interaction Energy	Electrostatic Interaction Energy[b]	Non Polar Interaction Energy[b]	Cluster Size[c]	Mean Interaction Energy [d]	Mean RMSD	Orientation
3H-BLIP	1	-146.2	-20.1	-126.3	0.8%	-122.0 (min: -146.3 max: -113.5 std: 8.2)	0.2 (range: 0.3 std: 0.1)	*a*
	2	-124.2	-47.7	-76.6	99.0%	-106.6 (min: -124.2 max: -82.6 std: 9.4)	0.9 (range: 4.9 std: 0.6)	*b*
	3	-96.3	-63.9	-32.4	0.2%	-91.5 (min: -96.2 max: -86.0 std:4.0)	0.4 (range: 0.5 std: 0.2)	*c*
wtBLIP	1	-158.3	-30.5	-127.7	6.9%	-136.9 (min: -158.2 max: -114.3 std: 9.9)	0.1 (range: 0.4 std: 0.1)	*d*
	2	-60.2	-2.0	-58.2	50.4%	-49.6 (min: -60.2 max: -35.0 std: 5.2)	2.9 (range: 4.4 std: 1.1)	*e*
	3	-52.4	11.0	-63.3	3.6%	-42.3 (min: -52.3 max: -35.0 std: 5.7)	0.2 (range: 0.7 std: 0.2)	*f*

[a]Cluster number represents the rank of the corresponding cluster. The ranking is based on the interaction energy of the cluster representatives. [b]The electrostatic interaction energy is the sum of the electrostatic interaction and desolvation terms, whereas the nonpolar interaction energy is the sum of the LJ and the non-polar desolvation terms. [c]Cluster size is given as the fraction of docked structures recorded. [d]Mean of the total interaction energies of the docked structures within the corresponding cluster, with minimum (min), maximum (max) and standard deviation (std) values in parentheses. All energy values are given in kJ/mol and root mean square deviation (RMSD) values, indicating the variation in orientation within each cluster, are given in Å.

Table S2 Interaction energy components at selected time points in the beginning of the 250 ns-long MD simulations of 3H-BLIP at Au (111) started with orientations *a* and *b*. The interactions of 3H-BLIP with the gold surface are divided into those of the 3H tag and of BLIP itself

Time (ns)	3H				BLIP			
	Orientation *a*		Orientation *b*		Orientation *a*		Orientation *b*	
	ELE	LJ	ELE	LJ	ELE	LJ	ELE	LJ
10.0	0.5	0.0	0.8	0.0	-55.6	-222.3	-0.2	0.0
10.5	-1.3	-0.7	1.4	0.0	-59.5	-242.1	-0.5	0.0
11.0	-9.9	-5.9	3.7	0.0	-55.9	-228.0	-1.7	0.0
11.5	-7.7	-16.0	3.2	0.0	-44.7	-214.7	-2.1	0.0
12.0	-62.2	-78.5	1.1	-11.6	-47.9	-221.8	-4.1	-0.1
12.5	-54.8	-105.2	-78.6	-133.7	-51.6	-245.4	0.3	-1.8
13.0	-54.3	-98.1	-40.4	-145.4	-58.8	-237.2	-20.0	-11.9
13.5	-60.1	-103.3	-35.6	-145.9	-54.9	-250.5	-41.2	-114.5

All energy values are given in kJ/mol and RMSD values are given in Å.

Table S3 Computed properties of the BD-docked clusters of the 3H-BLIP and wt-BLIP proteins on the gold surface using the average-linkage clustering method.

Protein	Cluster No.[a]	Total Interaction Energy	Electrostatic Interaction Energy[b]	Non Polar Interaction Energy[b]	Cluster Size[c]	Mean Interaction Energy [d]	Mean RMSD	Orientation[e]
3H-BLIP	1	-107.7	-39.4	-68.3	95.0%	-108.8 (min: -124.2 max: -82.6 std: 9.4)	0.6 (range: 2.0 std: 0.4)	***b***
	2	-132.8	-24.2	-108.5	3.7%	-125.1 (min: -146.3 max: -113.5 std: 8.2)	0.1 (range: 0.3 std: 0.1)	***a***
	3	-98.3	-60.5	-37.8	0.6%	-95.3 (min: -101.9 max: -86.0 std: 4.5)	0.6 (range: 0.9 std: 0.3)	***c***
wtBLIP	1	-49.1	-2.9	-46.2	29.0%	-51.6 (min: -60.2 max: -35.0 std: 5.2)	1.2 (range: 4.5 std: 1.0)	***e***
	2	-138.0	-28.5	-109.3	24.3%	-145.9 (min: -158.2 max: -114.3 std: 9.9)	0.1 (range: 0.4 std: 0.1)	***d***
	4	-41.4	10.9	-52.2	9.8%	-42.8 (min: -52.3 max: -35.0 std: 5.7)	0.3 (range: 0.6 std: 0.1)	***f***

[a]Cluster number represents the rank of the corresponding cluster. The ranking is based on the size of the clusters. [b]The electrostatic interaction energy is the sum of the electrostatic interaction and desolvation terms, whereas the nonpolar interaction energy is the sum of the LJ and the non-polar desolvation terms. [c]Cluster size is given as the fraction of docked structures recorded. [d]Mean of the total interaction energies of the docked structures within the corresponding cluster with minimum, maximum and standard deviation in parentheses. [e]The clusters obtained by the average-linkage method that have similar orientations to those obtained by the single-linkage method are shown by the same letter.

All energy values are given in kJ/mol and RMSD values, showing variation within each cluster, are given in Å.

Table S4 RMS deviations in Å of the cluster representatives, obtained by the k-means method, from those obtained by single and average linkage methods.

		Single-linkage cluster number[a,b]					
		Average-linkage cluster number[a,b]					
Protein	**k-means cluster number[b]**	**1**	**2**	**3**	**4**	**5**	**6**
3H-BLIP	**1**		0.6 (*b*)				
		0.1 (*b*)					
	2		0.1 (*b*)				
		0.6 (*b*)					
	3						
		0.8 (*b*)					
	4						0.9
		0.5 (*b*)					
	5		0.3 (*b*)				
		0.5 (*b*)					
	6		0.9 (*b*)				
		0.3 (*b*)					
	7		0.5 (*b*)				
		0.2 (*b*)					
	8		0.7 (*b*)	4.5 (*c*)	4.0	2.5	
				1.3 (c)			
	9		1.0 (*b*)				
		0.5 (*b*)					
	10	0.1 (*a*)					
			0.1 (*a*)				
wtBLIP	**1**						
		0.8 (*e*)					
	2						
		0.3 (*e*)					
	3				0.3		
	4						
					0.0 (*f*)		
	5		0.3 (*e*)				
	6	0.0 (*d*)					
						0.0	
	7						
		0.6 (*e*)					
	8						
			0.1 (*d*)				
	9						
			0.1 (*d*)				
	10					0.1	
			0.3 (*d*)				

[a]RMSD values of the cluster representatives from the representatives obtained by the single- and average-linkage methods are shown with light gray and dark gray backgrounds respectively. [b]Cluster number represents the rank of the corresponding cluster and the ranking is based on the interaction energy of the cluster representatives (for the average-linkage methods) or the size of the clusters (for the single-linkage and k-means methods). The corresponding binding orientations are shown in brackets.

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Table S5 Computed properties of the BD-docked clusters of the 3H-BLIP and wt-BLIP proteins on the gold surface obtained by the k-means algorithm.

Protein	Cluster No.[a]	Total Interaction Energy	Electrostatic Interaction Energy[b]	Non Polar Interaction Energy[b]	Cluster Size[c]	Mean Interaction Energy [d]	Mean RMSD
3H-BLIP	1	-115.3	-40.0	-75.3	15.5%	-108.2 (max: -90.7 min: -121.0 std: 6.1)	0.3 (range: 0.8 std: 0.1)
	2	-122.0	-47.0	-75.0	13.3%	-114.3 (max: -100.6 min: -124.2 std: 5.2)	0.3 (range: 0.9 std: 0.1)
	3	-98.9	-30.6	-68.3	13.2%	-94.2 (max: -114.8 min: -98.9 std: 7.7)	0.4 (range: 1.0 std: 0.2)
	4	-102.4	-37.4	-64.9	12.7%	-103.6 (max: -117.7 min: -102.4 std: 7.3)	0.4 (range: 1.8 std: 0.3)
	5	-114.0	-41.8	-72.2	11.1%	-113.2 (max: -124.0 min: -114.0 std: 5.6)	0.3 (range: 0.9 std: 0.1)
	6	-103.3	-33.7	-69.6	10.3%	-102.3 (max: -118.7 min: -103.3 std: 7.3)	0.2 (range: 0.6 std: 0.1)
	7	-117.7	-40.4	-77.3	10%	-109.3 (max: -121.5 min: -117.7 std: 6.5)	0.2 (range: 0.4 std: 0.1)
	8	-123.4	-50.9	-72.5	6.9%	-114.4 (max: -124.1 min: -123.4 std: 6.1)	0.8 (range: 4.6 std: 1.0)
	9	-99.2	-31.5	-67.7	6.0%	-97.6 (max: -116.5 min: -99.2 std: 7.4)	0.4 (range: 1.2 std: 0.2)
	10	-116.8	-15.5	-101.3	0.8%	-121.9 (max: -146.2 min: -116.8 std: 8.3)	0.2 (range: 0.6 std: 0.2)
wtBLIP	1	-58.0	-2.9	-55.1	22.9%	-49.5 (max: -35.8 min: -60.0 std: 5.2)	0.5 (range: 1.8 std: 0.3)
	2	-52.2	-2.6	-49.6	18.7%	-51.3 (max: -36.8 min: -58.9 std: 4.1)	0.4 (range: 1.0 std: 0.2)
	3	-42.1	-14.8	-27.3	11.8%	-39.2 (max: 34.9 min: -49.3 std: 3.3)	0.4 (range: 1.0 std: 0.2)
	4	-36.1	-17.4	-18.8	10.7%	-36.4 (max: -34.8 min: -39.6 std: 1.0)	0.2 (range: 0.6 std: 0.1)
	5	-52.3	-1.6	-50.8	7.7%	-47.7 (max: -34.9 min: -60.2 std: 6.0)	1.0 (range: 2.3 std: 0.6)
	6	-138.0	-28.5	-109.3	7.6%	-136.9 (max: -114.4 min: -158.3 std: 9.8)	0.2 (range: 0.7 std: 0.1)
	7	-43.1	-2.6	-40.5	6.8%	-47.5 (max: -36.5 min: -58.0 std: 5.1)	0.7 (range: 2.1 std: 0.5)
	8	-43.3	-47.6	4.3	5.1%	-40.2 (max: -34.9 min: -47.5 std: 2.9)	0.2 (range: 0.4 std: 0.1)
	9	-39.4	-46.6	7.2	4.3%	-39.9 (max: -34.8 min: -48.8 std: 3.0)	0.2 (range: 0.5 std: 0.1)
	10	-36.3	-44.2	7.9	4.1%	-38.5 (max: -35.1 min: -48.8 std: 2.5)	0.2 (range: 0.6 std: 0.1)

[a]Cluster number represents the rank of the corresponding cluster. The ranking is based on the size of the clusters. [b]The electrostatic interaction energy is the sum of the electrostatic interaction and desolvation terms, whereas the nonpolar interaction energy is the sum of the LJ and the non-polar desolvation terms. [c]Cluster size is given as the fraction of docked structures recorded. [d]Mean of the total interaction energies of the docked structures within the corresponding cluster, with minimum, maximum and standard deviation in parentheses.

All energy values are given in kJ/mol and RMSD values, showing the variation within each cluster, are given in Å.