Supporting Information For:

Elucidating the Influence of Materials-Binding Peptide Sequence on Au Surface Interactions and Colloidal Stability of Au Nanoparticles

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Section S1: Additional Methodology

Simulation Details

System Setup:
Each system simulated consisted of a single peptide chain, a Au(111) slab, 6605 water molecules and a number of Cl^- counter-ions such as to ensure the overall charge neutrality of the system. The Au(111) slab was based on a *p*(20×24) supercell and was five atomic layers thick. The overall cell dimensions were 58.6×60.9×67.6 $Å^3$, resulting in an inter-slab distance of greater than 55 Å along the cell direction perpendicular to the slab surface. The density of water in the center of the system was equivalent to the density of liquid water at 300 K and 1 atm.

All peptide chains were modeled in the zwitterionic form, i.e. the N- and C- termini of the peptide were uncapped (i.e. modeled as NH_3^+ and COO^- respectively), consistent with the peptides used experimentally. At neutral pH the arginine residue will be protonated, however, the protonation state of the histidine residues is more complex, with both the protonated and non-protonated forms likely present in solution. Experimentally the pH of the solution during the QCM experiments was measured as pH 3.9-4.2. In addition, it is possible that the probability distribution of protonation states of the His residues in the sequence when the peptide is free in solution may differ from those when the peptide is in the surface-adsorbed state. To ensure the effect of protonation state was accounted for in the simulations, all possible protonation states of the histidine residues were considered, a total of seventeen systems. The naming convention of the different protonation states is as follows; PS0 – no His protonated; PS1 – one His protonated; PS2 – 2 His protonated. For those peptides containing two His residues (Pd4, Pd4-4 and Pd4-5) there are two PS1 states; PS1A – 1st His protonated, 2nd His unprotonated and PS1B – 1st His unprotonated, 2nd His protonated. In all simulations the side-chain of Arg was positively-charged.

REST Simulation Details:
All simulations were performed using Gromacs version 5.0.[1] The GolP-CHARMM FF[2,3] was used to model the interactions with the Au(111) surface, the peptide was modeled using the CHARMM22* FF[4,5] and the modified version of TIP3P water[6,7] (compatiable with the CHARMM FFs) was employed. All simulations were performed in the canonical (*NVT*) ensemble, at 300 K with temperature regulated by a Nose-Hoover[8,9] thermostat. Note that all replicas were run at the same thermal temperature of 300 K. The LJ non-bonded interactions were tapered to zero between 10 and 11 Å, while the electrostatic interactions were treated using a particle mesh Ewald (PME)[10] summation with a real-space cutoff of 11 Å. A timestep of 1 fs was used, each system simulated for 20×10^6 MD steps and exchanges between adjacent replicas attempted every 1000 steps (≡ 1ps). Coordinates were saved every 1000 steps.

The REST approach is a version of Hamiltonian replica exchange molecular dynamics (H_REMD) that provides advanced sampling of the conformational space of a system.[11-13] For fuller details of the technique, its implementation in GROMACS and investigation of the improvements sampling efficiency we refer readers to Terakawa *et al.*, Wright *et al.* and Tang *et al.*[11-13]

In the present study the 'effective temperature' window spanned 300-430 K across sixteen replicas. The λ (scaling) values used for the replicas were 0.000, 0.057, 0.114, 0.177, 0.240, 0.310, 0.382, 0.458, 0.528, 0.597, 0.692, 0.750, 0.803, 0.855, 0.930, and 1.000. The initial configuration of the peptide in each replica differed, covering a range of different secondary structure motifs, e.g. α–helix, β-turn, PPII helix and random coil.

Simulation Analysis:

The degree of residue-surface contact was determined by calculating the fraction of the total REST-MD reference trajectory (i.e. the trajectory that corresponds to the unscaled Hamiltonian, λ=0.000) that a reference site on a residue was less than or equal to a cutoff distance of the Au(111) surface. The reference sites and cutoff-distance are the same as used in previous work.[13] An anchor residue is defined as a residue that was in direct contact with the Au(111) surface for 60% or more of the trajectory.

To determine the Boltzmann-weighted ensemble of peptide conformations the Daura clustering algorithm,[14] with a 2 Å cutoff for the atoms in the peptide backbone was employed. The clustering analysis was performed over the full 20,000 frames of the reference trajectory. The percentage population of each cluster was determined from the fraction of the total 20,000 frames that were assigned to that cluster.

Table S1. Adsorption and desorption analysis for Au surface binding including k_a, k_d, K_{eq}, ΔG, and θ values for all peptides.

Peptide	Sequence	p*I*	k_a (M^{-1} s^{-1})	k_d (10^{-3} s^{-1})	K_{eq} (10^4 M^{-1})	ΔG (kJ mol^{-1})	θ (%)
Pd4	TSNAVHPTLRHL	9.47	3491 ± 22	9.86 ± 0.31	35.5 ± 1.2	-31.4 ± 0.1	79.8 ± 0.5
Pd4-1	TSNAVAPTLRHL	9.44	741 ± 36	11.80 ± 0.19	6.3 ± 0.4	-27.2 ± 0.2	41.2 ± 1.6
Pd4-2	TSNAVHPTLRAL	9.44	1609 ± 28	5.97 ± 0.18	27.0 ± 1.3	-30.7 ± 0.1	75.0 ± 0.9
Pd4-3	TSNAVAPTLRAL	9.41	4778 ± 554	10.89 ± 2.48	46.3 ± 14.8	-32.0 ± 0.8	82.8 ± 5.0
Pd4-4	SPHTTNHRALLV	9.51	4214 ± 56	5.93 ± 0.42	71.4 ± 5.5	-33.1 ± 0.2	88.8 ± 0.8
Pd4-5	HHTSNAVPTLRL	9.76	4204 ± 36	2.29 ± 0.19	184.4 ± 16.7	-35.5 ± 0.2	95.3 ± 0.4

Table S2. Residue-surface contact data (percentages) determined from the REST MD simulations. Data for all the different protonation states of the peptides are shown; PS0 (no His are protonated), PS1 (only one His is protonated; for those sequences with two histidines, in PS1A the first histidine in the sequence is protonated, in PS1B the second histidine in the sequence is protonated) and PS2 (both His are protonated). Data for protonated His residues are underlined. Anchor residues (defined as greater than 60% degree of contact) are highlighted in yellow.

Pd4	PS0	PS1A	PS1B	PS2	Pd4-1	PS0	PS1	Pd4-2	PS0	PS1	Pd4-3	
T	12	27	4	7	T	8	6	T	15	11	T	12
S	60	36	28	36	S	33	47	S	49	28	S	26
N	37	48	49	48	N	47	35	N	38	41	N	43
A	72	46	46	58	A	54	66	A	52	38	A	57
V	5	25	27	42	V	47	52	V	10	17	V	52
H	93	78	86	76	A	26	16	H	93	83	A	39
P	12	28	8	44	P	36	55	P	16	31	P	48
T	24	33	9	23	T	21	23	T	15	38	T	32
L	42	31	38	37	L	38	33	L	54	42	L	25
R	76	44	29	79	R	68	53	R	93	75	R	86
H	83	94	76	74	H	92	81	A	55	38	A	41
L	11	17	25	6	L	10	15	L	6	24	L	20

Pd4-4	PS0	PS1A	PS1B	PS2	Pd4-5	PS0	PS1A	PS1B	PS2
S	22	25	18	23	H	87	62	91	46
P	23	11	20	13	H	93	69	78	53
H	76	75	91	83	T	18	29	39	15
T	13	34	9	14	S	48	50	45	48
T	19	17	32	31	N	38	36	43	39
N	35	32	50	47	A	53	49	39	59
H	83	91	48	63	V	20	37	41	45
R	65	68	80	73	P	22	43	39	16
A	36	28	16	44	T	32	18	16	31
L	20	18	30	37	L	26	38	49	33
L	21	33	20	33	R	90	62	92	82
V	20	25	16	27	L	15	24	16	11

Table S3. Percentage population of the top ten most populated clusters of the Pd4 family of peptides adsorbed at the aqueous Au(111) interface. Data for all the different protonation states of the peptides are shown; PS0 (no His protonated), PS1 (one His protonated) and PS2 (two His protonated). In the case where there are two His present in the sequence, PS1A denotes that only the first His in the sequence is protonated, while PS1B denotes that only the second His in the sequence is protonated. The total number of clusters for each case is given in parentheses in the table header.

Cluster Rank	Pd4 PS0 (81)	PS1A (90)	PS1B (123)	PS2 (86)	Pd4-1 PS0 (135)	PS1 (129)	Pd4-2 PS0 (108)	PS1 (135)	PS4-3 (117)
1	18.7	19.9	12.4	25.9	12.1	15.2	20.2	12.4	19.7
2	14.2	10.8	12.2	8.0	8.5	9.2	15.4	10.2	12.9
3	6.8	8.0	8.9	7.5	8.3	9.2	10.2	8.6	8.9
4	6.0	7.9	6.8	6.2	6.7	8.6	9.0	5.5	6.4
5	4.9	6.8	6.1	5.3	5.5	6.3	4.7	5.3	6.0
6	4.5	5.1	5.5	5.1	5.4	4.4	3.5	4.3	4.6
7	4.2	4.5	4.0	4.4	5.2	4.4	3.0	3.4	4.2
8	4.0	4.2	3.2	3.7	5.6	2.9	2.9	3.1	3.2
9	3.7	3.2	3.2	3.0	3.3	2.9	2.8	3.0	2.5
10	3.6	2.8	2.7	2.5	2.5	2.9	2.4	2.7	2.5

Cluster Rank	Pd4-4 PS0 (151)	PS1A (133)	PS1B (133)	PS2 (130)	Pd4-5 PS0 (96)	PS1A (83)	PS1B (82)	PS2 (91)
1	12.2	18.9	11.1	19.5	13.0	18.2	17.3	22.4
2	9.5	12.4	9.0	9.4	12.0	14.3	16.5	13.8
3	7.4	10.0	7.0	8.6	11.1	10.5	10.4	7.4
4	7.3	6.5	5.3	4.8	7.1	7.2	5.6	7.2
5	6.9	6.2	5.0	4.4	6.9	6.4	4.9	5.6
6	5.6	5.6	5.0	3.9	4.8	5.8	4.8	5.2
7	3.9	2.9	4.7	3.8	4.4	4.3	3.4	4.3
8	3.4	2.4	4.1	3.1	4.0	3.3	3.3	3.7
9	2.9	2.3	3.4	2.8	3.6	2.9	3.3	3.5
10	2.3	2.3	3.4	2.5	3.4	2.7	3.0	3.7

Table S4. Comparison of cluster centroids of the top ten most populated clusters, comparing the Pd4 peptide backbone structure to each of the mutants. A matched peptide configuration has a RMSD less than 0.2 nm. Pairs where both structures come from one of the top five most populated clusters are highlighted in yellow.

Pd4	Pd4-1	RMSD / nm
1	1	0.176
1	8	0.162
2	3	0.199
2	10	0.171
6	8	0.159
8	2	0.199
9	1	0.193
10	6	0.195

Pd4	Pd4-2	RMSD / nm
1	5	0.160
8	1	0.166
2	10	0.192

Pd4	Pd4-3	RMSD / nm
1	1	0.150
1	7	0.182
2	2	0.188
2	9	0.193
6	1	0.140
8	7	0.157
9	4	0.152
10	10	0.078

Pd4	Pd4-4	RMSD / nm
2	2	0.178
8	1	0.191
10	10	0.171

Pd4	Pd4-5	RMSD / nm
-	-	-

Table S5. Conformational entropic contribution scores, S_{conf}, for the different peptides systems simulated.

Peptide	Protonation state	S_{conf}
Pd4	0	3.08
	1A	3.09
	1B	3.36
	2	3.01
Pd4-1	0	3.51
	1	3.38
Pd4-2	0	3.02
	1	3.57
Pd4-3		3.16
Pd4-4	0	3.57
	1A	3.27
	1B	3.64
	2	3.42
Pd4-5	0	3.15
	1A	2.99
	1B	3.03
	2	2.95

Table S6. Surface contact data (percentages) showing the breakdown of conditional probabilities of contact states for the two histidine residues in peptides Pd4, Pd4-4 and Pd4-5, determined from the REST MD simulations. Data for all the different protonation states of the peptides are shown; PS0 (no His protonated), PS1 (one His protonated) and PS2 (two His protonated). His A and His B are the first and second histidines as they appeared in the sequence, respectively.

Peptide	Protonation State	No His	His A only	His B only	Both His	Both His and Arg
Pd4	0	1	16	6	77	59
	1A	1	4	21	74	36
	1B	5	19	9	67	14
	2	7	19	19	55	44
Pd4-4	0	4	13	21	62	36
	1A	2	6	23	69	44
	1B	5	47	3	45	31
	2	3	34	14	49	30
Pd4-5	0	1	7	12	80	74
	1A	4	27	34	35	26
	1B	1	22	8	69	63
	2	8	40	46	6	5

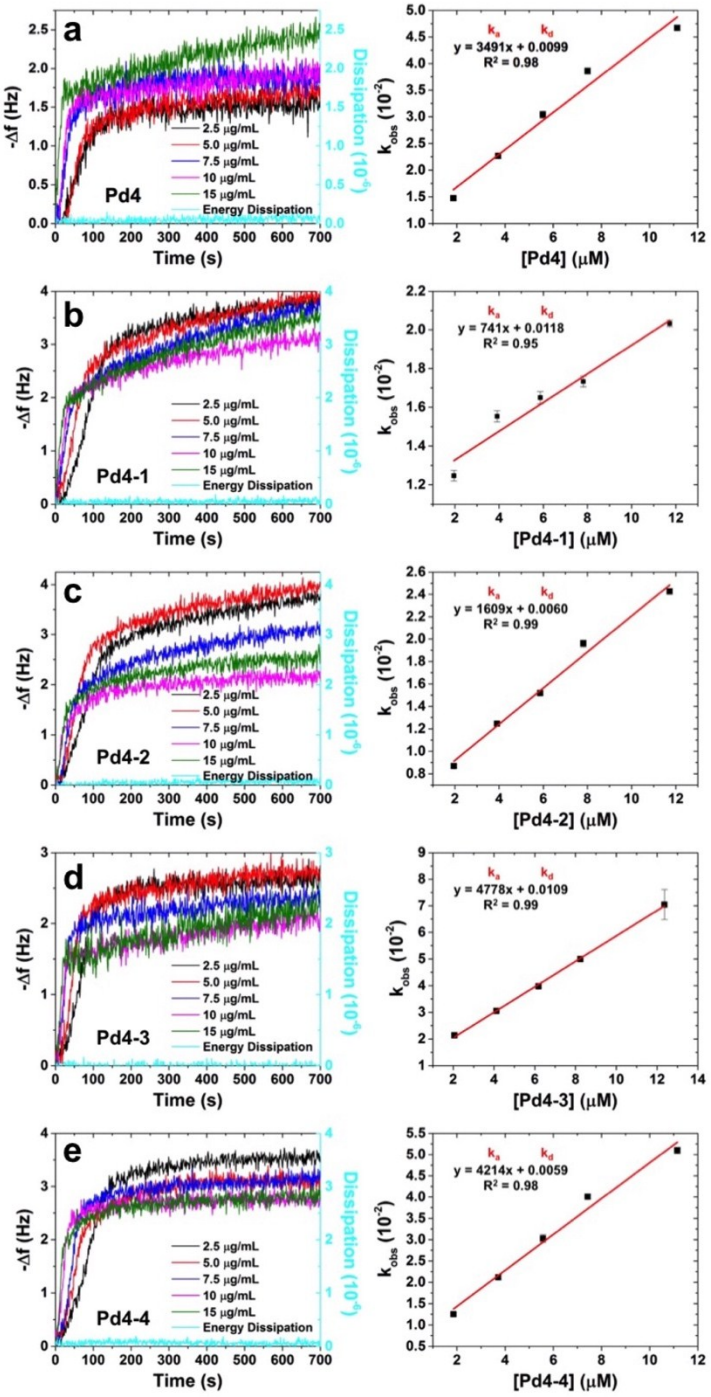

Figure S1. QCM analysis of the peptides to obtain k_a and k_d values: (a) Pd4, (b) Pd4-1, (c) Pd4-2, (d), Pd4-3, and (e) Pd4-4. The left panel shows the inverted frequency change vs. time, as well as the dissipation energy plot for the highest concentration studied. The right panel shows the plot of k_{obs} values vs. peptide concentration, obtained from the data in the left panel by fitting with Langmuir kinetics.

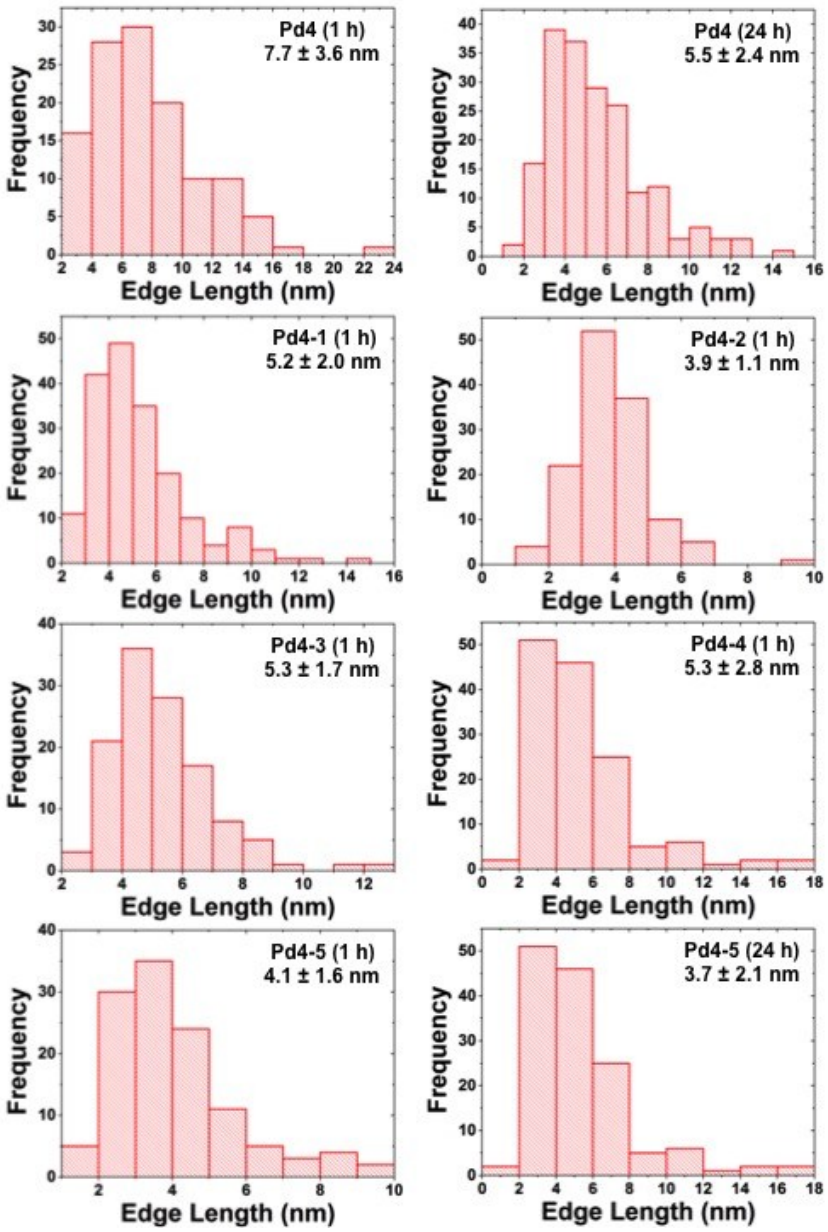

Figure S2. Size analysis of the Au nanoparticles prepared at a Au:peptide ratio of 2 capped with the indicated peptide.

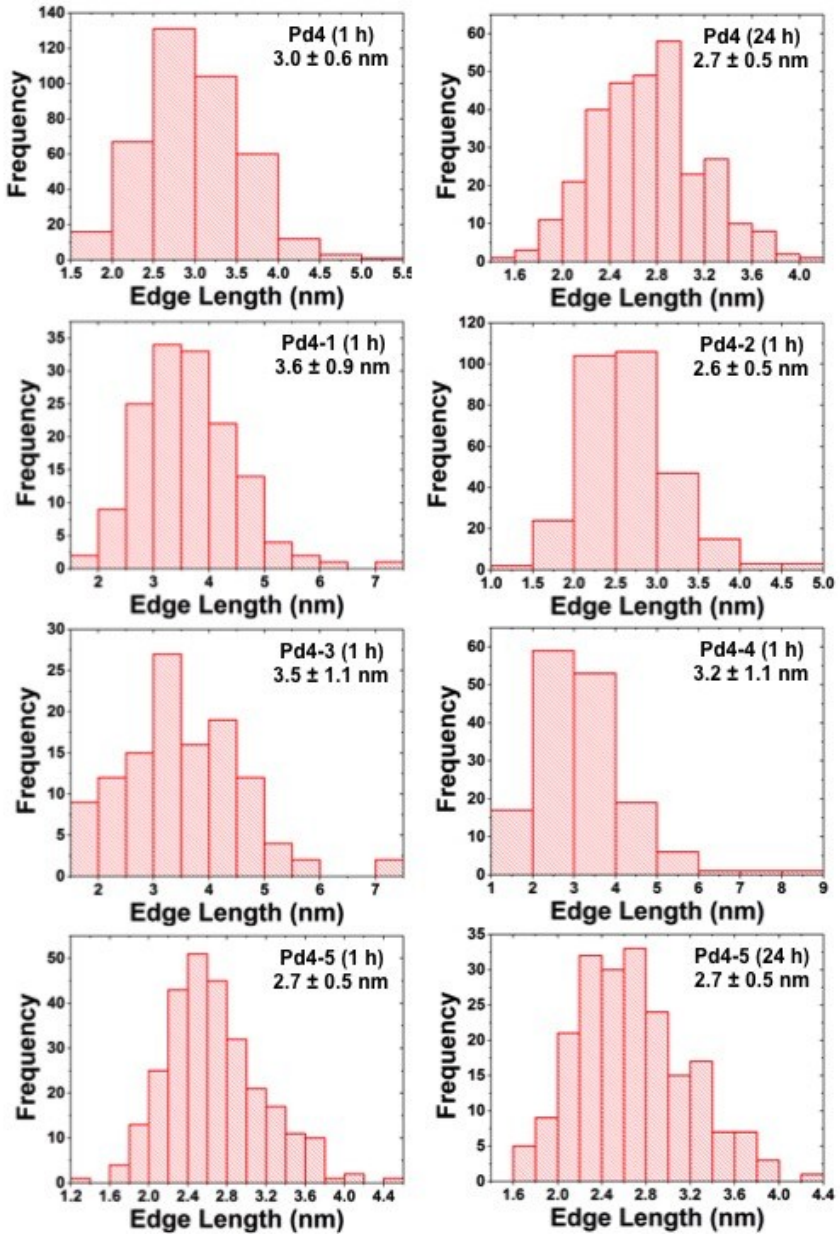

Figure S3. Size analysis of the Au nanoparticles prepared at a Au:peptide ratio of 1 capped with the indicated peptide.

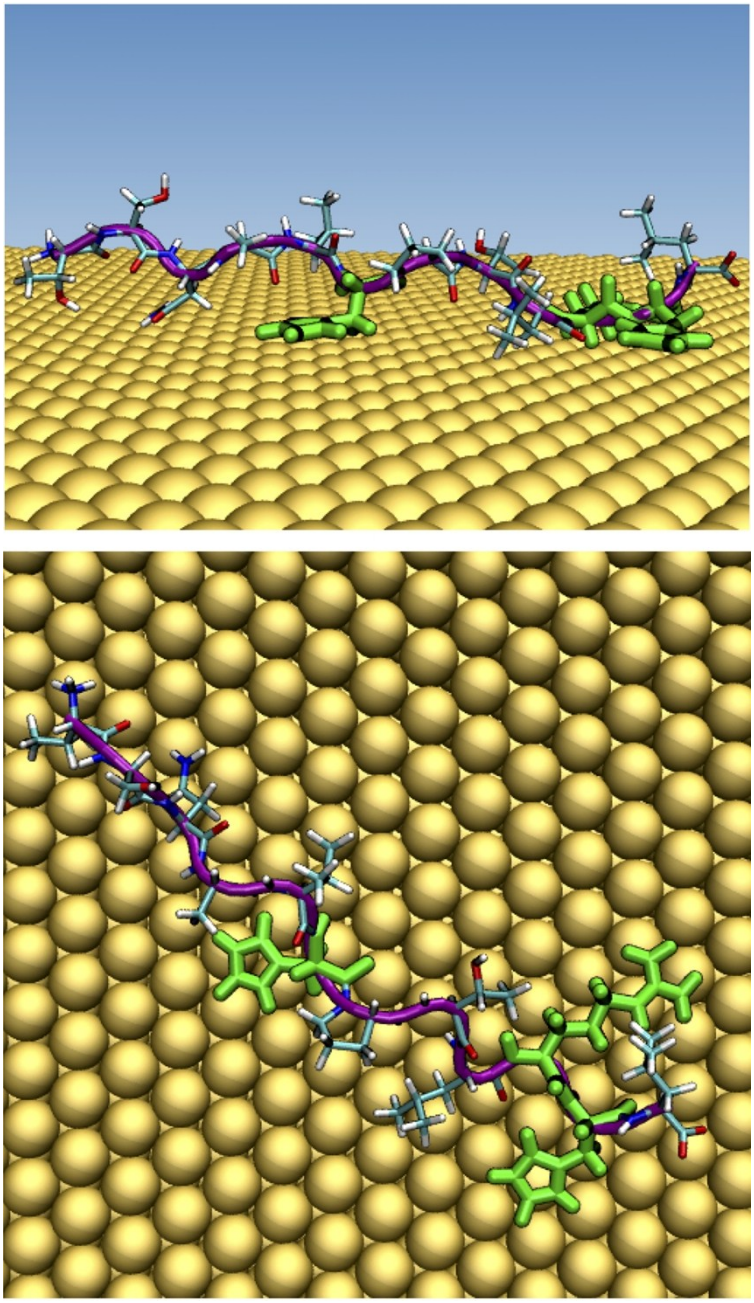

Figure S4. Representative snapshots of Pd4 adsorbed on the Au(111) interface. The anchor residues (H6, R10 and H11) are colored green and the peptide backbone is colored purple. Water molecules are not shown for clarity.

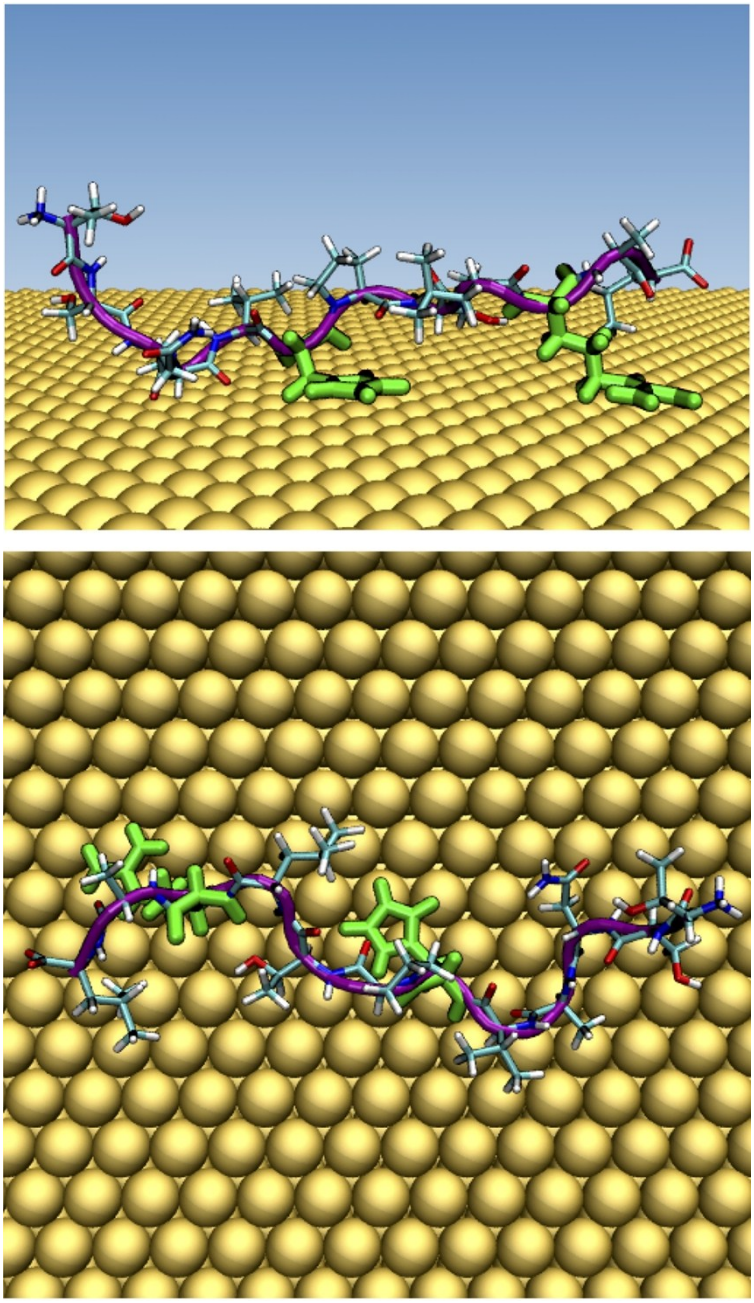

Figure S5. Representative snapshots of Pd4-2 adsorbed on the Au(111) interface. The anchor residues (H6 and R10) are colored green and the peptide backbone is colored purple. Water molecules are not shown for clarity.

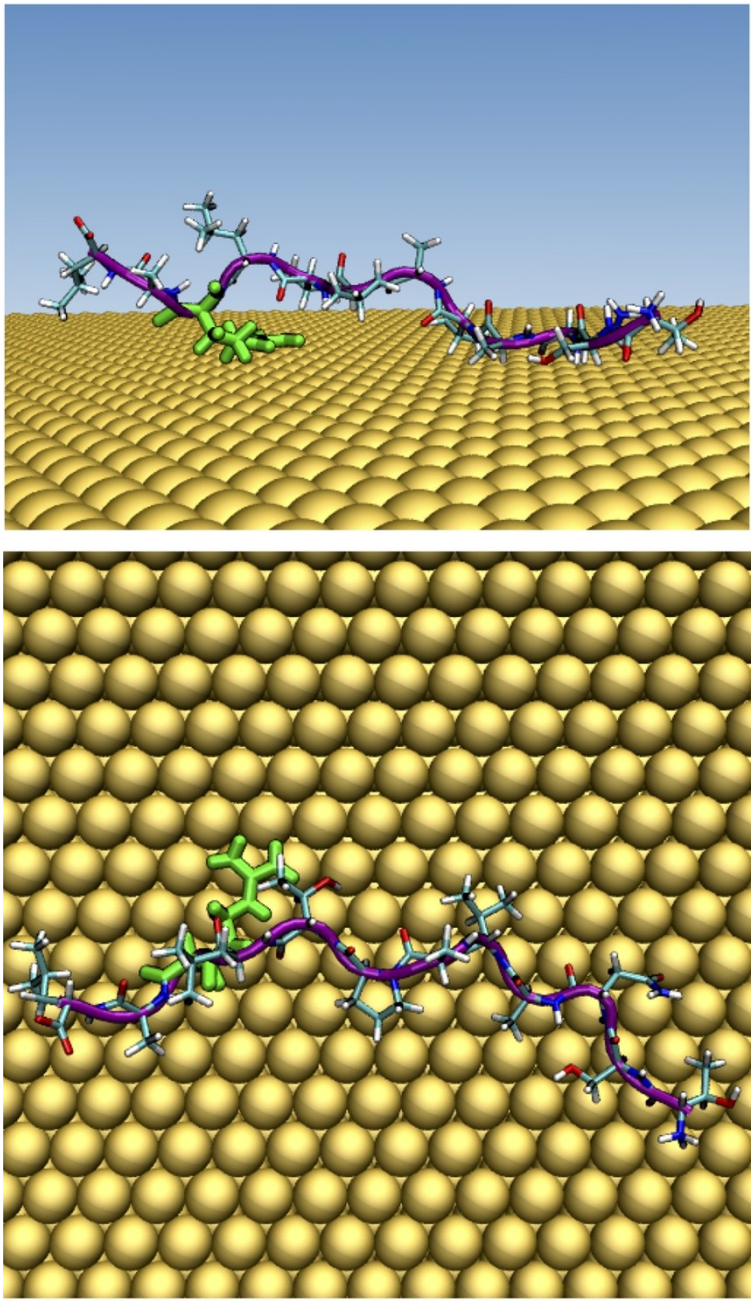

Figure S6. Representative snapshots of Pd4-3 adsorbed on the Au(111) interface. The anchor residue (R10) is colored green and the peptide backbone is colored purple. Water molecules are not shown for clarity.

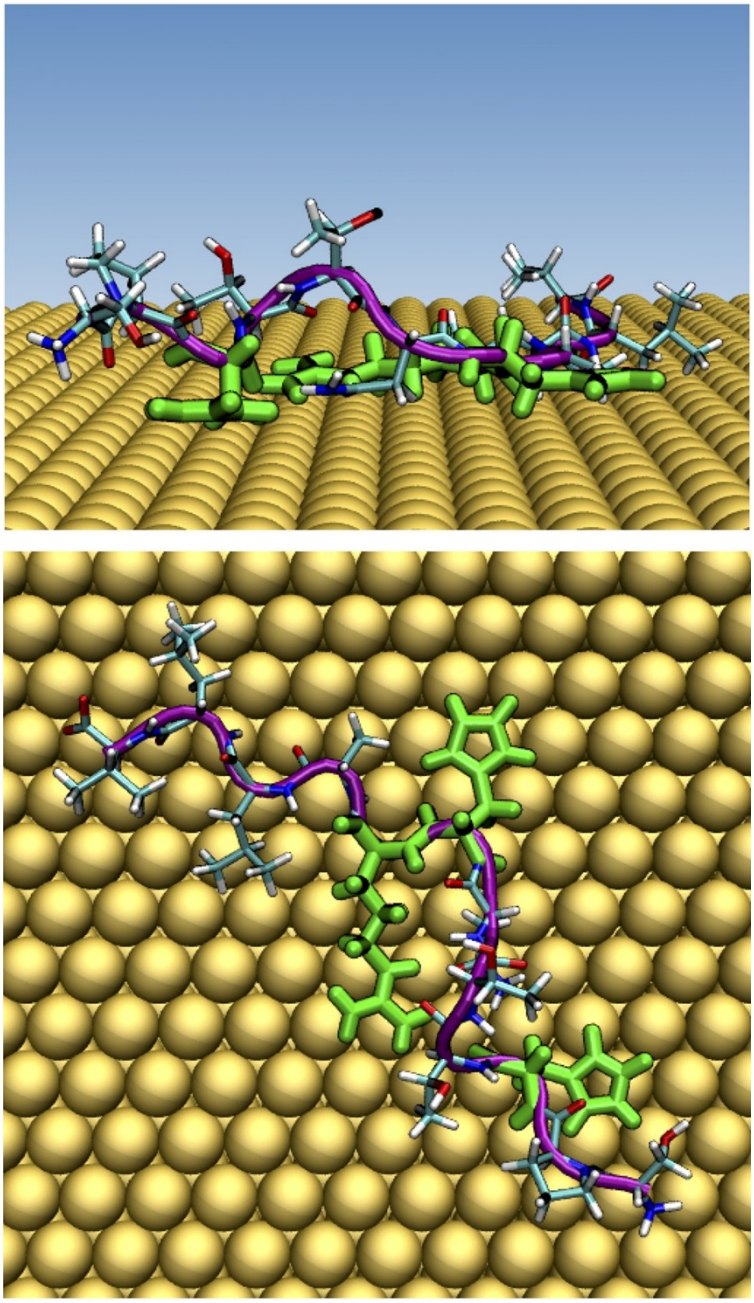

Figure S7. Representative snapshots of Pd4-4 adsorbed on the Au(111) interface. The anchor residues (H3, H7 and R8) are colored green and the peptide backbone is colored purple. Water molecules are not shown for clarity.

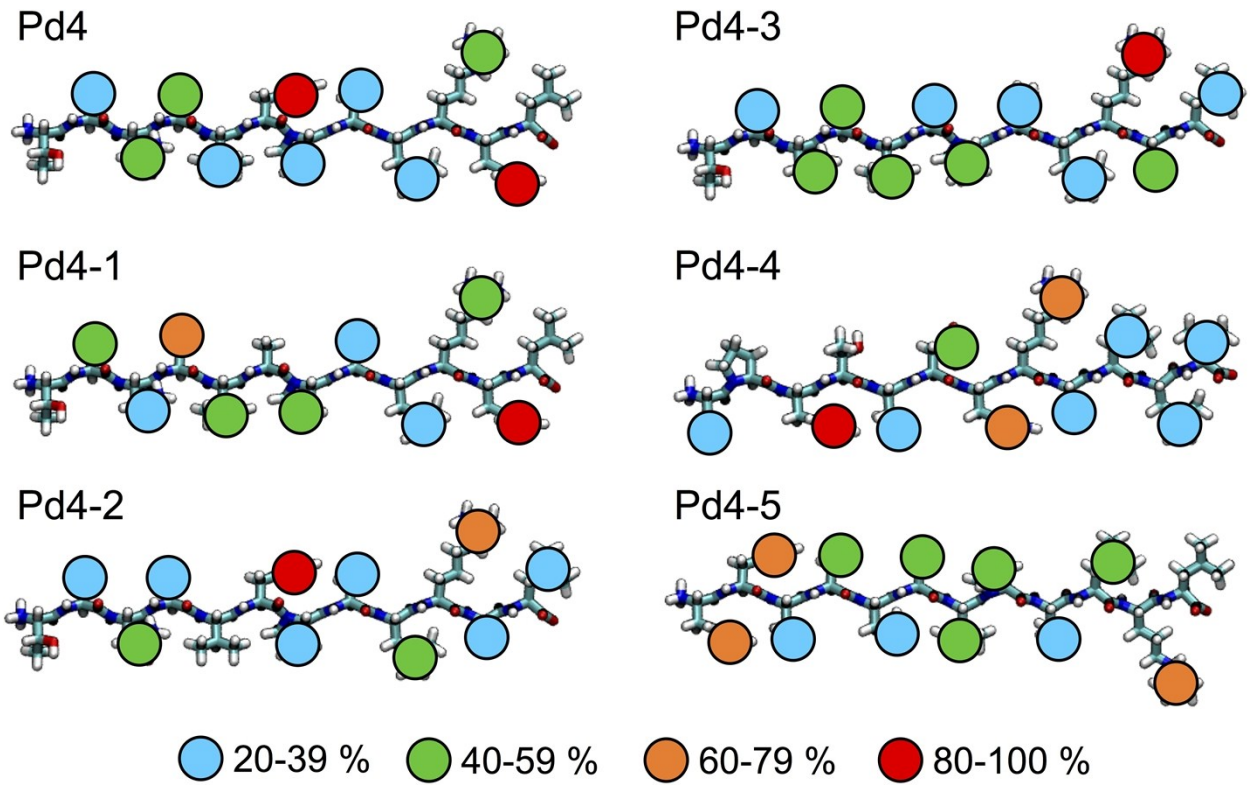

Figure S8. Degree of residue-surface contact for each of the 6 peptides, data is averaged over all three protonation states simulated for Pd4, Pd4-4 and Pd4-5.

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