Supplementary Information - Probing the Influence of Citrate-Capped Gold Nanoparticles on an Amyloidogenic Protein

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Validation of the Docking Model

To investigate the nature of the binding between β_2m and a citrate-coated gold surface, as well as the effect of a negative surface potential, we looked for the possible adsorption orientations of β_2m on citAu(111) (and the corresponding driving forces) by initially using an implicit electrostatic effects of negatively charged citrate molecules on the surface assigning a small negative charge density of (Au_{chg}^{net} = -0.01 e) per surface atom.

When this docking procedure was applied to the system, it yielded three different orientations accounting for more than 92 per cent of the total encounter complexes when the negative Au surface was considered (see Tab. 1). The representative structure of each computed complex is shown in Fig. 1. The protein residues contacting the surface differ in the various complexes, and are listed in Tab. 1.

In the case of the negatively charged state of the gold surface the preferred orientation are complex A which is still involving the residues at the N-terminal (ILE1, ARG3) tail and DE-loop (LYS58, ASP59 and TRP60) of the protein but complexes B (driven mostly by E_{LJ} interactions) and C (driven both by E_{LJ} and electrostatic terms) are also present. In both cases of a small negative charge density of (Au_{chg}^{net} = -0.01 e) per surface atom and a larger charge density of (Au_{chg}^{net} = -0.05 e) reported in the main text, pose A is present and it is the most populated, therefore suggesting a picture in which the layer of citrate covering the surface of the GNP does remain on the surface of the gold and interacts with the protein, as already predicted by the proposed atomistic model in Fig.1.
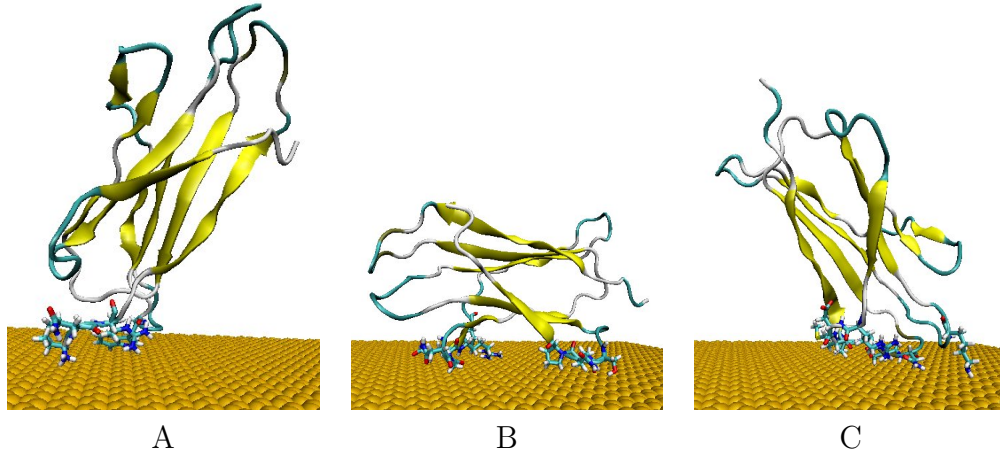
Au_{chg}^{neg} = -0.01 e

Figure 1: Most populated encounter complexes of β_2m on negatively charged gold nanocluster obtained by BD simulation. In the case of Au_{chg}^{neg} = -0.01 e, the structures of representative complexes for each of the three clusters are shown, ordered by decreasing cluster size. The reported complexes represent the 92 per cent obtained by BD simulation. The protein backbone is shown in cartoon representation. The residues contacting the gold surface are shown in stick representation.

Table 1: Resultant encounter complexes from rigid-body BD docking of β_2m (1JNJ) to an Au (111) surface. A hierarchical clustering algorithm (based on a minimum distance linkage function) was applied to the diffusional encounter complexes after docking to a bare negative gold (Au_{chg}^{neg} = -0.01 e) surface. The reported complexes represent 92 per cent obtained by BD simulation.

Label $Au_{chg}^{neg} = -0.01e$	RelPop % (a)	U_{Repr} (b)	$E_{LJ}+U_{ds}^p+U_{ds}^m$ (c)	U_{EP} (d)	spread (e)	Contact Residues (f)
A	45	-5.280	-0.053	-5.226	5.401	ILE1, ARG3, TRP60
B	28	-24.380	-30.71	6.294	0.128	ASN42, GLY43, GLU44, ARG45,
						GLU47, SER88, GLN89, PRO90
C	22	-30.260	-24.38	-5.879	5.545	PRO14, ALA15, GLU16, ASN17
						GLU74, LYS75, ARG97

Å [a] Relative population of this cluster

[b] U_{Repr}: total interaction energy of the representative of the given cluster in kT with T= 300 K

[c] E_{LJ}: Lennard-Jones energy term for the representative complex, U_{ds}^p: non-polar (hydrophobic) desolvation energy of the representative complex, U_{ds}^m: surface desolvation energy of the representative complex, in kT

[d] U_{EP}: total electrostatic energy of the representative complex, in kT

[e] RMSD of the structures within the cluster with respect to the representative complex

[f] Residues with atoms contacting gold at distances $\leq$ 3 Å

2D [^{1}H, ^{15}N] HSQC Experiments

2D [^{1}H, ^{15}N] HSQC NMR experiments have been used to characterize, at amino acid residue level, the interaction between β_2m and gold nanoparticles at various molar ratios. Different samples containing 130 nM of 5nm AuNP (Sigma-Aldrich) and variable β_2m concentrations ranging from 4 to 36 μM were analysed.

Figure 2: 2D [^{1}H, ^{15}N] HSQC: In blue and red are depicted the HN cross peaks of the protein 17μM in the absence and in the presence of 130 mM of gold nanoparticles, respectively. β_2m is dissolved in 25 mM phosphate buffer, pH 6.47 and at 298K.
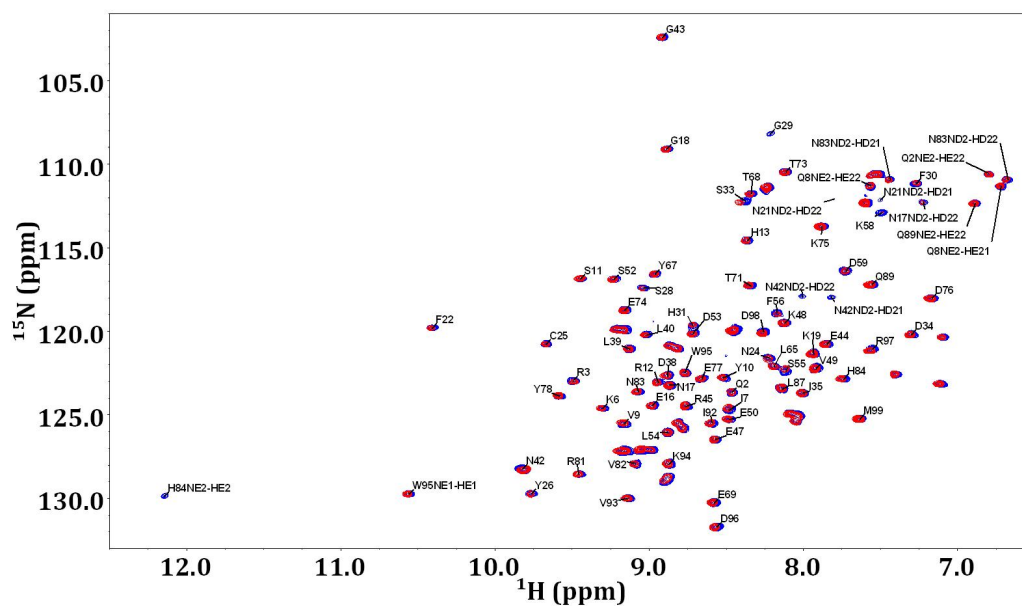
Validation of the Au surface with a positive charge density

As described in the main text, Section "Atomistic MD Simulations of β_2m on Citrate-Covered Au" we proposed an atomistic molecular mechanics surface model namely Cit-AuNPs, in which the fully deprotonated citrate anions $(C_3H_5O(COO)_3^{3-})$ are described as interacting adsorbed species on a positively charged AuNPs with few neutralizing counterions. To support the choice of a positively charged gold core of the AuNPs which was taken line with current experimental understanding and for the sake of completeness, we conducted a 10 ns MD simulation at 300 K of the protonated protein in the presence of a different surface model based on a neutral gold core with the counter ions included in aqueous solution over the citrate, namely Cit3Na-AuNPs (*i.e.*, three Na+ ions released from each sodium citrate when it is put in aqueous solution). Simulations results are summarized in Fig. 3 in which panel (a) is referring to protein on Cit-AuNPs and panel (b) is referring to protein on Cit3NA-AuNPs.

Top panel of Fig. 3(a) report the unique stable orientation for the protonated protein during the last 10 ns of 20 ns T-REMD and Fig. 3(b) report the final representative structures of the orientations found for the protonated protein during the 10 ns of standard MD preceded by 2 ns equilibration. Lowest panels of Fig. 3(a-b) report the time evolution of contacting residues for the same protonated protein with respect to the two model surfaces, (a) cit-AuNPs along 10 ns of T-REMD and (b) Cit3Na-AuNP along 10 ns of standard MD. Results are qualitatively the same showing that in both cases, there is an unique and well conserved binding patch lowest panels in Fig. 3(a-b), involving the same N-Terminal residues (ILE1, GLN2, ARG3) and DE-loop residues (LYS58, TRP60) proving that the charge of the gold core is not really crucial in determing the binding of the protein to the surface in the present case. In details, contacts in regions of residues 58-60 are lost with the ral gold core but they are clearly highlighted by NMR results shown here. Therefore, we believe that a positive

Figure 3: Panels (a) refers to the protein on Cit-AuNPs and panel (b) to the protein on Cit3NA-AuNPs. Top panels (a) report the orientation of the protein on Cit-AuNPs during T-REMD and top panel (b) the orientation for the protein on cit3Na-AuNPs during MD. Lowest panels (a-b) report the time evolution of contacting residues for the protein with respect to the surface of Cit-AuNPs and Cit3NA-AuNPs, extracted from the last 10 ns of T-REMD and MD, respectively.
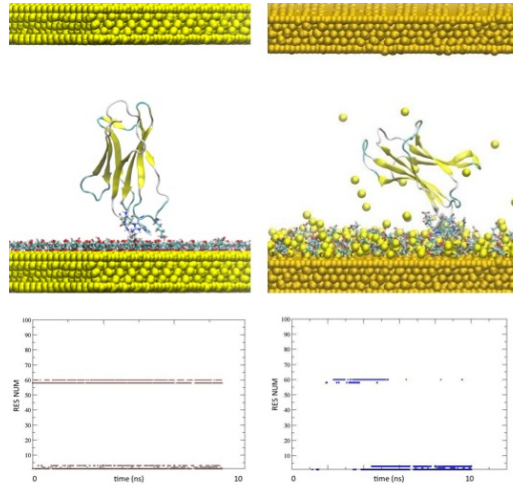
Positive Au vs Neutral Au

ILE1,GLN2, ARG3,HIS31, PRO32, LYS58,ASP59,TRP60

gold core is a more realistic model being in line with current understanding of citrate covered nanoparticles, as discussed in the main text and supported by references.