# Supplementary Information - Docking of Ubiquitin to Gold Nanoparticles

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## Ubiquitin-AuNP Interaction Characterization

Gold nanoparticles (AuNP) were produced by $NaBH_4$ reduction of a starting solution of sodium citrate and $HAuCl_4$ in water following a standard protocol (see ref. 45) to provide AuNP in the 10-30 nm size range. The particle size distribution (PSD) of the resulting nanoparticles was measured by Dynamic Light Scattering (DLS). DLS measurements of free, freshly synthesized nanoparticles, showed that AuNP have near-monodispersed PSD with a mean hydrodynamic diameter of 12.0 nm and polydispersivity index of 0.1. Upon addition of human Ubiquitin the PSD of the AuNP-hUbq complex continued to show the presence of a near-monodispersed sample but with a mean hydrodynamic diameter of 17.1 nm and polydispersivity index of 0.21. This is an indication that AuNP were stable in the presence of Ubiquitin at pH 7.7 and the addition of protein did not cause the formation of nanoparticles aggregates. The increase in hydrodynamic diameter upon hUbq addition indicated that hUbq molecules interacted with the gold nanoparticles in solution. The direct interaction of hUbq with AuNP was confirmed by the shift in the plasmon resonance band of AuNP upon addition of hUbq. The UV-VIS spectrum of free AuNP showed a typical intense plasmon resonance band centered at 520 nm. After addition of human Ubiquitin this band shifted to 525 nm (as shown in Supplementary Information of ref. 45). This red shift of 5 nm in the surface plasmon resonance band was in agreement with results obtained on a similar system where azurin (a protein with size similar to Ubiquitin) interacted with gold nanoparticles of 20 nm.

## Analysis of the Presence of Amino Acid Residues with High Affinity for Gold in the Ubq Binding Patches

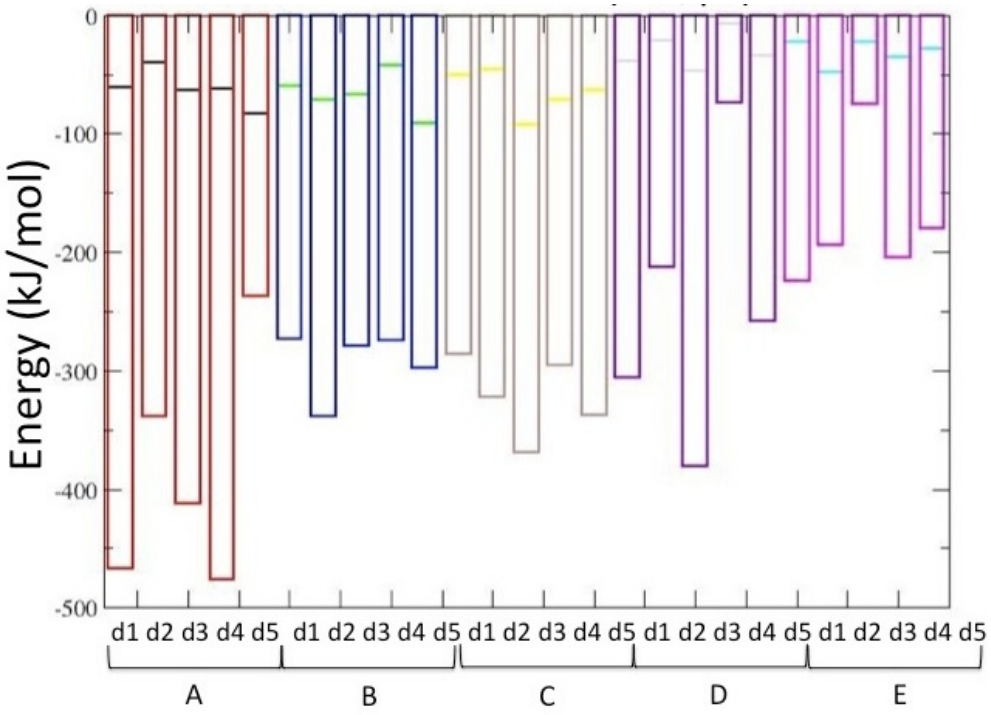
To quantify the presence of residues with high gold affinity (HIS, ARG, TRP, TYR, MET and PHE) in the proximity of the gold surface, we defined an orientation-dependent index, $I_{Aff}$. This index is a continuous, smooth function of the protein orientation and is large when many atoms from high

gold affinity residues are close to the Au surface. A definition that satisfies all the requirements is :

$$I_{Aff} = \sum_{i \in Protein, i \in HighAff.} \frac{1}{|z_i - z_{surf}|^3} \tag{1}$$

where $z_i$ is the $z$ coordinate of the protein atom $i$ belonging to one of the high gold affinity AAs ($z$ axis is perpendicular to the surface), $z_{surf}$ is the $z$-coordinate of the most external Au layer, and the third-power dependence is chosen to mimic dispersion interactions between an atom and the surface. The coordinates $z_i$ refer to the protein in contact with the surface (contact is here defined to take place when the protein atom nearest to the surface is 3 Å from it). The higher $I_{Aff}$ is, the higher is the protein-Au interaction mediated by high-affinity amino acid residues. The orientation(s) that maximize $I_{Aff}$ signals binding driven by high-affinity residues. To find such maxima, we scanned the orientation of the protein by varying the spherical coordinate angles $\theta$ and $\phi$ that define the protein orientation in the original 1UBQ PDB coordinate framework, with the origin at the geometric center of the protein. To perform this scan, we used a discrete grid having a spacing of $3^o$x$3^o$. We found that the global maximum of $I_{Aff}$ corresponds to an orientation in complex D. As discussed in the main text, Fig. 2, in complex D a single high gold affinity residue, ARG54, is able to approach the surface with the plane of its guanidinium group parallel to the Au surface and thereby to maximize its interaction with gold. Marginal stability of D is noted in the main text. Other local maxima of $I_{Aff}$ do not correspond to any of the orientations found by docking and MD in the main text.

Figure 1: Average Coulomb (Coul) and Lennard-Jones (LJ) components of the protein-Au(111) interaction, reported for all docking complexes from A to E. Energies are taken from the last 10 ns of the five independent MD runs for each complex. Complex A (Coul =black, LJ=red), Complex B (Coul=green, LJ=blue), Complex C (Coul= yellow, LJ=beige), Complex D (Coul=grey, LJ=violet) and Complex E (Coul=cyan, LJ=pourple). X-axis: reports the complex labels (e.g. starting from complex A: each histogram refers to the energies of complex A and velocities -d1, -d2, -d3, -d4, -d5 etc.)



**Standard MD Simulations**

For the standard MD simulations at T = 300 K, the root mean square deviation (RMSD) was computed at every 5 ps along the trajectory for all the Ubq atoms excluding the C-terminal tail (i.e. residues 1-70), relative to the respective equilibrated structure, (see Figure 2 (a)). The simulation protocol was as follows: (i) minimization of entire system including gold dipole charges for 2000 steps, (ii) 22 ns MD (T =300K) of the system (with frozen gold atoms and free dipoles) in the NVT ensemble. This analysis was aimed at investigating internal reorganization of the protein structure, i.e. surface-induced conformational changes and, therefore, the overall change of orientation of Ubq was not taken into account. The RMSD analysis reveals that Ubq has a rather stable conformation over the simulation time for all the simulated systems with an RMSD less than 3 Angstroms.

To compare possible induced orientations of the protein with respect to the surface, the initial conformations from rigid-body docking (Figure 2 (b)) were compared with the final orientations after the 20 ns MD simulations (Figure 2 (c)). Major changes in orientation were only seen for complexes D and E. The representative of complex D reoriented to an orientation corresponding to complex A, confirming that its stability, related to the favorable interaction of a single ARG residue with the surface, is only marginal. Complex E rotated to a new complex to allow the C-terminal tail to bind the gold (with residues 72-75); as a consequence, ARG42, LYS48, GLN49, ARG54 also contacted the surface.

Figure 2: Classical MD: (a) Root mean square deviation (RMSD) from the equilibrated structure for the selected complexes at each snapshot along the last 20 ns of MD trajectory, (b) initial protein-Au(111) surface complexes from rigid-body docking, (c) final orientations after the last 20 ns MD simulation (T = 300 K)

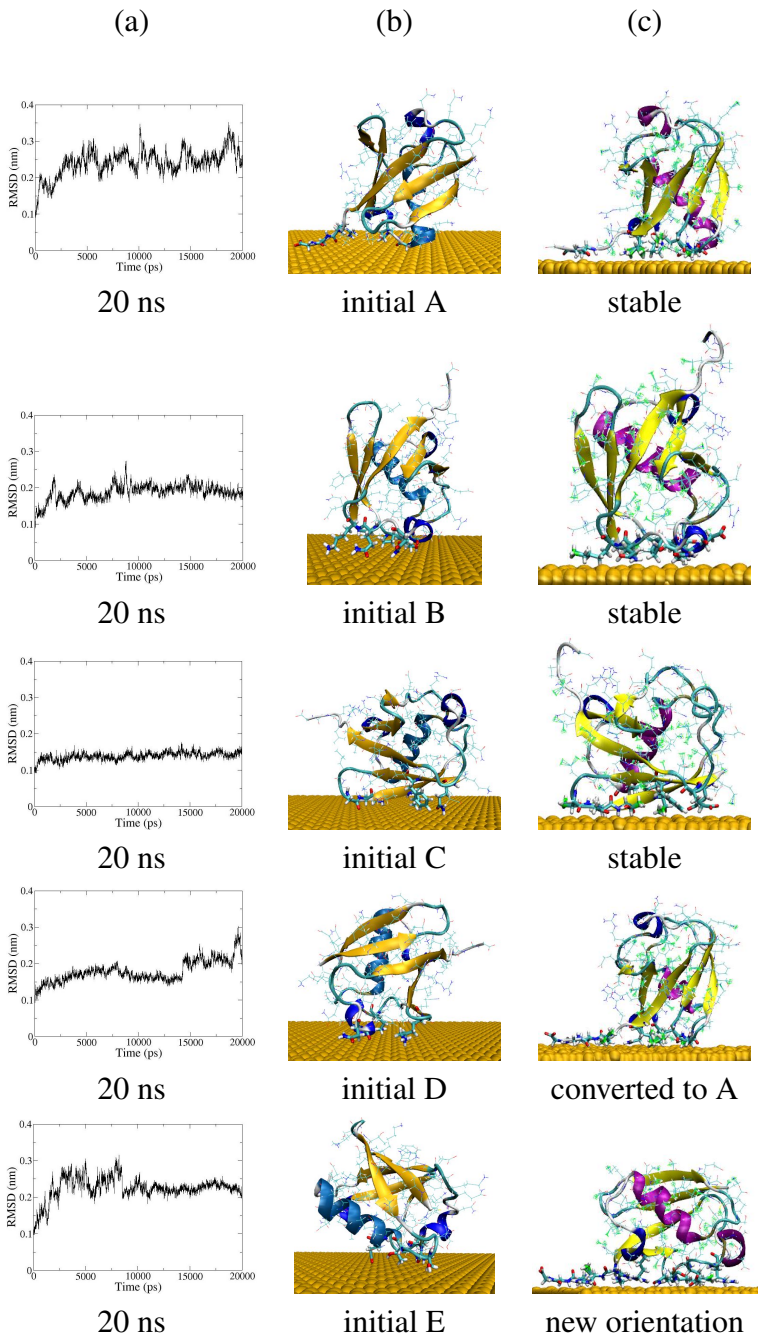


Figure 3: Test calculation: the stability of the free protein in solution (without the Au(111) surface) was examined by performing a 2 ns simulated annealing. Left Panel (top): Protein secondary structure representation of the Ubiquitin crystal structure. Right panel (top): Secondary structure evolution versus time of Ubiquitin in water during the 2 ns of simulated annealing. Left Panel (bottom): Superposition of the crystal structure and the final structure after 2 ns of simulated annealing (cyan). Right panel (bottom): RMSD at each snapshot along the first 2 ns of the simulated annealing trajectory. The stability of the structure is in agreement with experimental data (NMR and CD spectra) and with previous results reported in the literature.

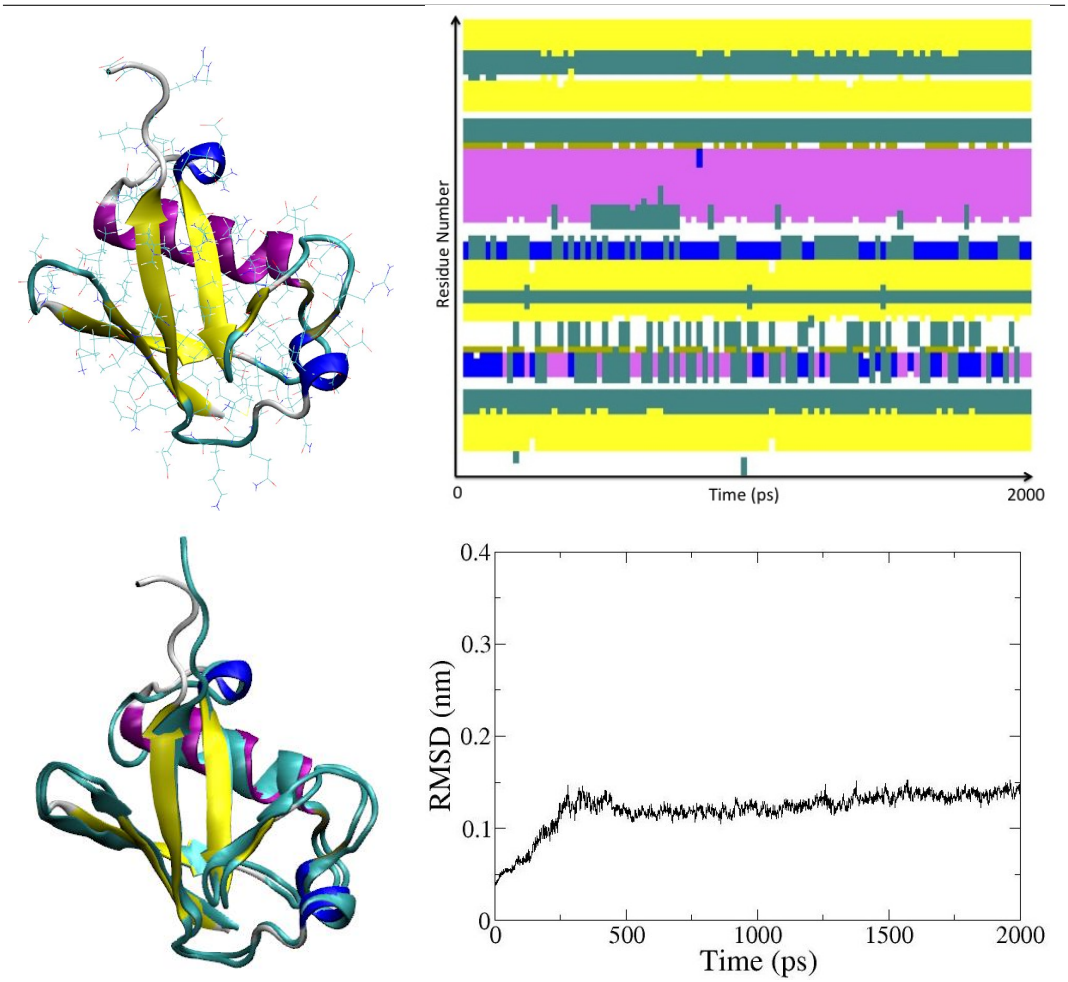


Figure 4: Clustering analysis of the selected complexes after 2 ns annealing and 20 ns MD at T =300 K. (a) Initial orientation from rigid-body docking, (b) Final orientation after annealing + MD (c) clustering analysis: the RMSD was computed only for the backbone atoms (excluding C-terminal tail) with respect to the representative structures of each cluster from annealing MD