Al Consiglio Nazionale delle Ricerche
Direzione Generale, Ufficio Paesi Industrializzati e Organismi Internazionali
Piazzale Aldo Moro 7, 00185 Roma

CNR SHORT-TERM MOBILITY 2010

Application Period: 26/04/2010 – 18/05/2010
Applicant: Dr. Giorgia Brancolini

Oggetto: relazione scientifica finale per la liquidazione di missione short-term mobility, missione CNR-S3 **MO090/10**

Proposed Activities.

1. Benchmark calculations of the Ubiquitine (protein) – Gold (surface) interactions by using a docking protocol based on a suite of codes recently developed in the group of R. Wade at HITS, in Heidelberg (http://projects.villa-bosch.de/mcmsoft/sda/6.00/)
2. Prediction of the most likely orientations of the Ubiquitine on the Gold surface by performing Brownian Dynamics simulations including a continuum water solvent, followed by clustering of the resulting structures.
3. Analysis of results: what features of the surface and of the protein (electronic, structural, morphological) determine the protein ability to bind to the surface and how? How relevant is the environment? Comparison with experimental NMR and Dynamics Light Scattering measurements on Ubiquitine-Gold Nanoparticles complexes, obtained by F. Rossi at EU Joint Research Center in Ispra (IT).
4. Improving of the computational test-set according to the experiments. Since Au(111) is covered with a layer of negatively charged Citrate anions the strategy was to implement in the SDA6.00 code the possibility to investigate the docking of Ubiquitine with charged Au atoms of the Au(111) surfaces.
5. Start writing of a joint publication with staff members at the host institution at HITS and identify future prospective collaborations.

WORK CARRIED OUT DURING THE VISIT

The work was very successful and went even farther the intended plan.

- The files for two systems illustrated in Fig.1 were prepared: UBQ with its complete sequence (76 residue, HUMAN UBIQUITINE, X-RAY structure) and UBQ without the flexible tail (70 residue, without 71-76 residues). In our opinion the presence of charged termini at both NTRM and CTRM of UBQ, and in particular at the end of the flexible tail of the CTRM, can affect the final binding site, by performing rigid docking between the protein and the surface. The influence of the flexible tail at the CTRM of UBQ has been extensively discussed together with the Host. The final decision was to perform docking on

both systems with and without tail (Fig. 1). Additionally, for UBQ1-70, both charged CTRM (COO-) and neutral CTRO (COOH) termini were considered. Whenever obtaining the same orientations by docking in the presence and in the absence of the flexible tail, we could be more confident in discussing and understanding the binding mechanism.

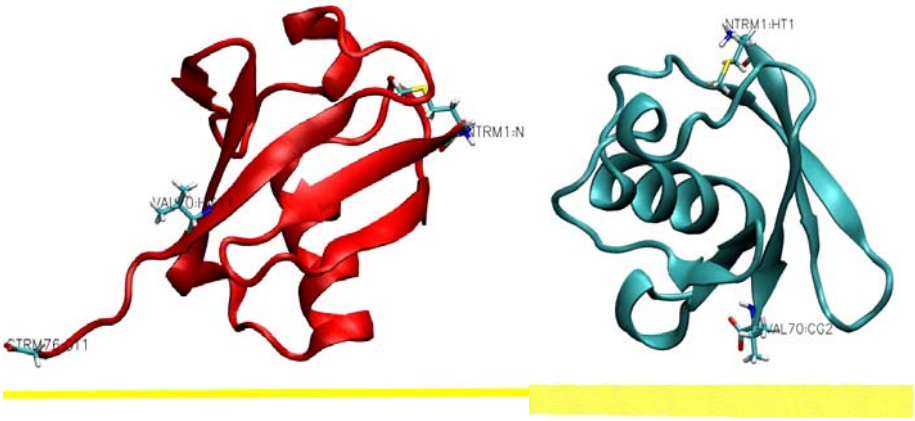
- Preparing and analysing all of the necessary electrostatics grids (Fig.2) for the Docking of UBQ and Au(111) with the newly released tools of SDA6.00 published on the website of Prosurf. Comparison of behaviors of different software and different procedures to get the correct grids in order to define an automated procedure.

- Testing the value for the hydrophobic desolvation factor parameter (hdfct=-0.019) recently re-defined by comparing with new experimental data available for another system.

- Preparing files of both positively and negatively Charged Surfaces for the Docking with UBQ within SDA6.00. Defining a criteria to assign an average atomic charge to each Au atom by taking into account that in the experiments the Au(111) surface is covered with a layer of Citrate anions at pH=7.5 (Fig. 3c). As a starting point, a total net charge for each Au atom was taken of ≈ - 0.1 and +0.1, respectively. The idea is to model two different scenario: the negative charges of citrate anions covering the surface before the binding and the positive polarization of the gold surface below the layer of anions during the binding.

- Extensive test and production runs followed by clustering, post-processing and analysis of the results and comparing with NMR experimental data.

- A joint publication with staff members at the host institution at HITS on this topic is currently being finalized.



UBQ1-76-Au(111) **UBQ1-70**-Au(111)

Fig. 1 UBQ with its complete sequence (76 residue, HUMAN UBIQUITINE, X-RAY structure) and for the UBQ without the flexible tail (70 residue, without 71-76 residues).

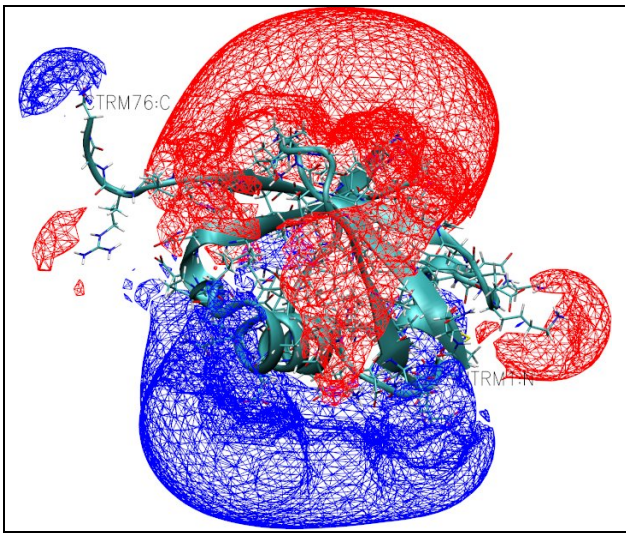


Fig. 2 Electrostatic Potential Grid computed for **UBQ1-76** (**Blu** is negatively and **Red** is positively charged)

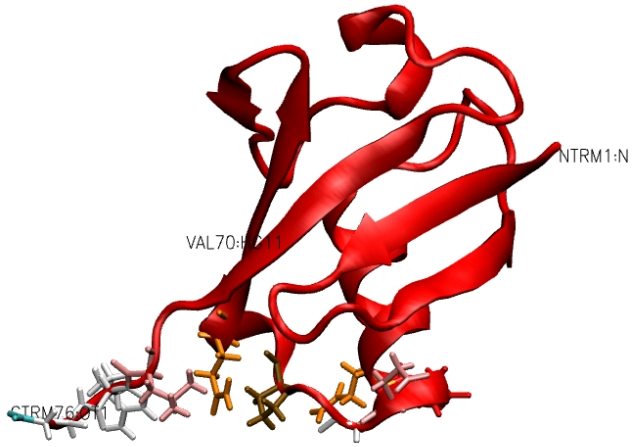
MAIN RESULTS

1. The energetically most favourable cluster we get for the binding of the UBQ to the neutral Au(111) surface is reported in Fig. 3a and it has the residues of the NTRM facing the surface. The reported name of the residues, refers to residues lying at a distance smaller than 4 Å from the Gold surface (**Blu name** refers to negatively charged residues and in **Red name** to positively charged residues). It is moreover evident from Fig. 3d that by changing the total net charge of the Au surface atoms i.e. from neutral to negative, the binding of the UBQ is affected by the change of the charges on the surface and new residues are approaching the Au(111) at the small distances. In particular, a binding site of the protein quite rich in negatively charged residues (Fig. 3a) at neutral Au(111) is then substituted by a binding fragment more rich in positively charged residues (Fig. 3d) at negatively charged Au(111).
2. By comparing those two clusters (Fig. 3a, 3d) with the NMR experimental data (Fig. 4), we interestingly found that both docked complexes are involving residues at the NTRM of UBQ, in the binding. Those residues are belonging to the region (coloured in YELLOW in Fig.4) responsible for most of the strongest NMR shifts. By including negative charges to the Au(111) atoms we are able to find a better agreement with the experimental NMR chemical shift mapping. On the contrary, much less good agreement is found when including positively charged Au(111) atoms (Fig. 3e, 3f). For this reasoning we are now aiming at introducing an interplay between the present docking runs (treating solvent and citrate implicitly) with MD runs (including explicit solvent and citrate anions) in order to more deeply understand the effects of the environment on the binding.
3. From Tab. 1 we can observe which are the driving forces involved in the UBQ-Au(111) binding for the complexes we obtained from the Docking. In particular, different contributions to the total binding energy are reported separately, and we could conclude that Lennard-Jones (LJ) term is the driving force if residues are in optimal binding position and Electrostatics term is the driving force if charged residues contact directly with the metal surface.

3a. Neutral Au (111) Complex1

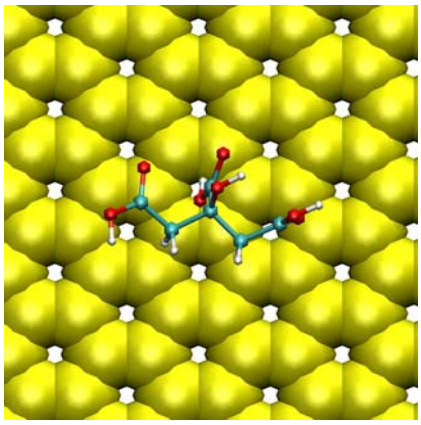
ASP-32, ALA-28, **LYS**-29, ASN-25, **GLU**-24, THR-22, **ASP**-21, SER-20, **GLU**-18 (< 4 Å)

3b. Neutral Au(111) Complex2



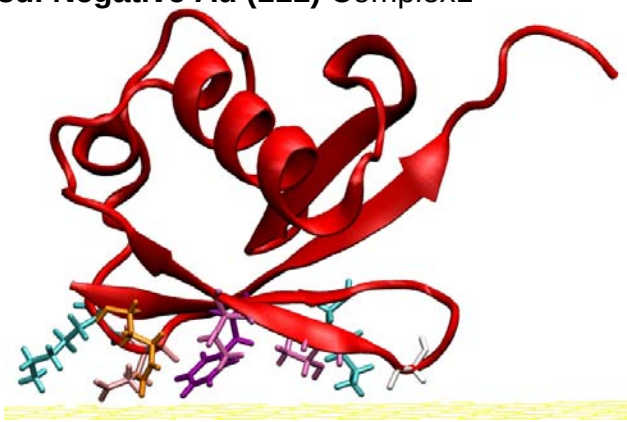
ARG-74, GLY-75, GLY-76, LEU-73, GLN-40, PRO-37, GLN-31, **ASP**-32, **GLU**-34, GLY-35 (< 4 Å)

3c. Citrate anion on Au (111)



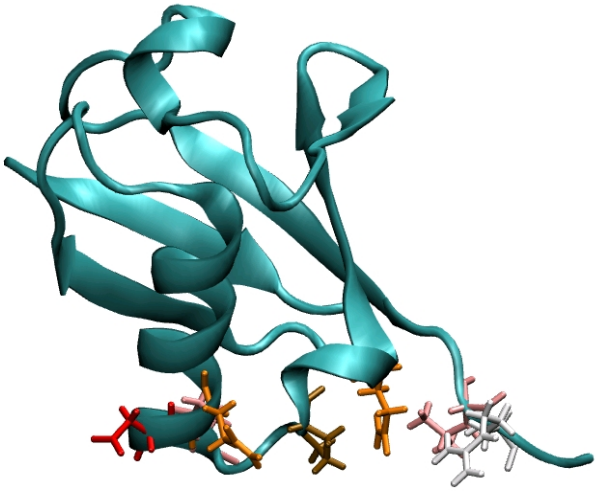
J. Kunze et al. *Journal of Electroanalytical Chemistry* 599 (**2007**) 147-159

3d. Negative Au (111) Complex1



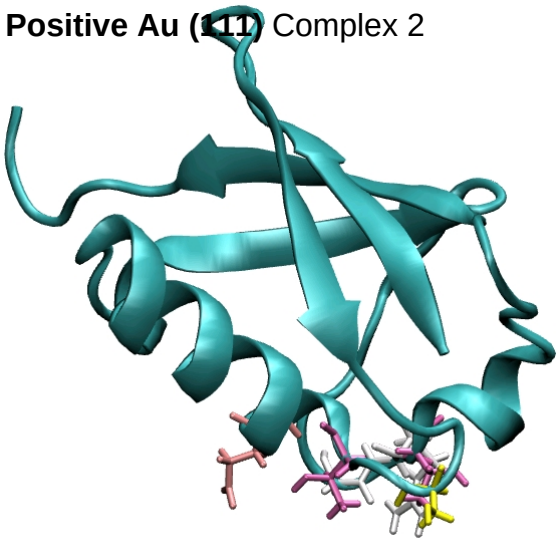
GLN-2, PHE-4, **LYS**-6, GLY-10, THR-12, THR-14 , **GLU**-64, **LYS**-63 (< 4 Å)

3e. Positive Au (111) Complex1



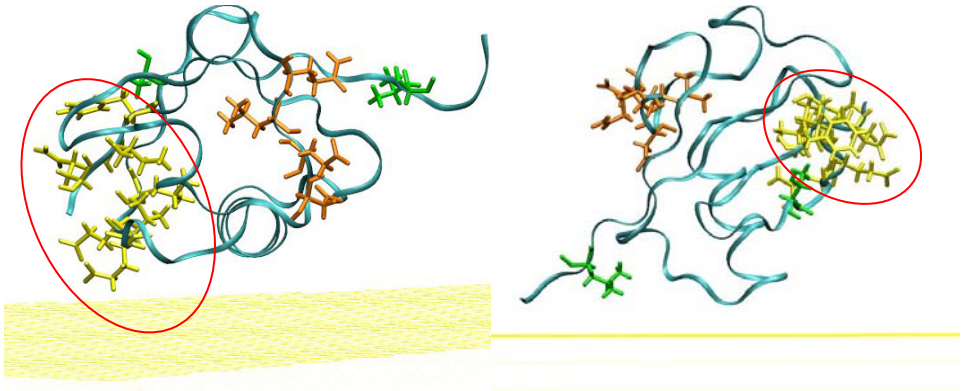
GLN-31, **ASP**-32, **GLU**-34, GLN-40, PRO-37, **ARG**-74, LEU-73 (< 4 Å)

3f. Positive Au (111) Complex 2



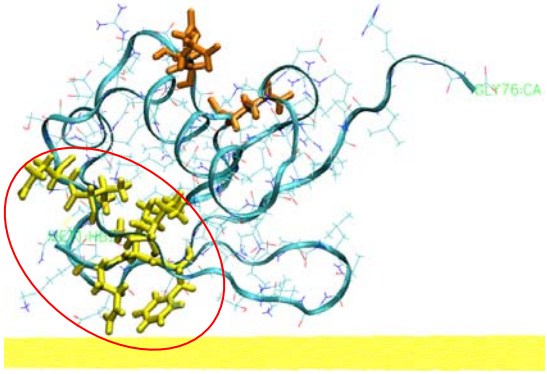
SER-20, THR-22, **GLU**-24, GLY-53, **ARG**-54, THR-55 (< 4 Å)

Fig. 3 Docked structures with neutral and charge Au(111) surfaces.



UBQ1-76-neutral Au(111)
Docked Complex1

UBQ1-76- neutral Au(111)
Docked Complex 2



UBQ1-76- negative Au(111)
Docked Complex1

Fig. 4 NMR-chemical shifts mapping. NMR Strongest Shifts: **RES 2,3,4 (YELLOW)** and **RES 15,17,18 (YELLOW).** NMR Minor Shifts: **RES 49,51,52 (ORANGE)**

CHARGE STATE OF GOLD	NEUTRAL Au(111)	NEUTRAL Au(111)	NEGATIVE Au(111)	POSITIVE Au(111)	POSITIVE Au(111)
BINDING ENERGY	-83.84	-58.60	-69.96	-63.85	-63.38
LJ ENERGY	-84.78	-100.5	-76.42	-97.96	-56.46
ELECTROSTATIC ENERGY	-64.16	-17.02	-46.69	-24.98	-49.27

Tab.1 Driving forces of the UBIQUITINE-GOLD Binding: energy contributions to binding energy are reported separately. Comparison between complexes binding to neutral, negatively and positively charged Au(111) surface.

FUTURE COLLABORATION WITH THE HOST INSTITUTION

♦ As a result of the present SHORT-TERM MOBILITY, the applicant (Giorgia Brancolini) with Stefano Corni and the HITS staff members (Rebecca Wade and Daria Kokh) are now in the process to further investigate the influence of the Protein-Protein interactions to the binding of the Protein to the Gold. From experiments, the region responsible for most of the UBQ protein-protein interactions seems to refer to the fragment 50-59 [1]. The idea is to use the SDA6.00 code to perform Docking of [UBQ] with $[UBQ\text{-}Au(111)]^{docked}$ or of $[UBQ]_2^{docked}$ –[Au(111)] systems.
This is supported by the experimental observation, trough Dynamics Light Scattering measurements, that around 120 UBQ systems can be surrounding each gold nano-particle in solution. Based on this observation, the interaction of UBQ with the nearby proteins could most probably, not be neglected in the overall interaction with the metal nano-particles.

[1] P. Pasikowski, M. Cydzic, A. Kluczyk, P. Stefanowicz, Z. Szewczuk, BioMol Concepts, Vol.1 (2010), pp. 67-83

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..... Modena, 8 Giugno 2010