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IN SITU ANALYSIS OF THE PROTEIN CORONA BY CRYO-TRANSMISSION ELECTRON MICROSCOPY

Jéril DEGROUARD[1,*], Laurent MARICHAL[1], Stéphanie DEVINEAU[2], Gaël GIRAUDON-COLAS[4], Yves BOULARD[3], Jean-Philippe RENAULT[4], Serge PIN[4]

[1] Université Paris-Saclay, CNRS, UMR 8502, Laboratoire de Physique des Solides, 91405 Orsay Cedex, France

[2] Université de Paris, BFA, UMR 8251, CNRS, F-75013 Paris, France

[3] Université Paris-Saclay, CEA, CNRS, I2BC, B3S, Gif-sur-Yvette 91190, France

[4] Université Paris-Saclay, CEA, CNRS, NIMBE, Gif-sur-Yvette 91190, France

In a biological environment, the adsorption of proteins on nanoparticles (NPs) is an early process that leads to major changes of the physicochemical properties and the biological effects of NPs. The formation of the so-called protein corona drives NP-cell interaction, NP biodistribution, and NP toxicity *in vivo*. However, few experimental techniques allow for the analysis of the protein corona *in situ*. As a result, little is known on its structure.

Using silica NPs and a library of purified hemoproteins with sizes ranging from 2.8 to 22 nm in diameter (17 to 3,600 kDa in MW), we investigated the effect of protein size on the protein corona assembly by cryo-TEM. Our observations revealed that larger proteins, composed of more than a hundred subunits, form open networks with NPs rather than a homogeneous monolayer of proteins decorating the surface as observed for smaller ones (Figure 1) [1]. The ability of each protein to bind to several NPs drives the formation of novel supramolecular assemblies, while preserving protein structure and function, here in terms of oxygenation properties. By combining cryo-TEM and synchrotron radiation circular dichroism, we further showed that NPs can stabilize partially unfolded protein conformations, thereby driving the formation of a 'soft protein corona' consisting of small weakly bound proteins directly adsorbed to the surface, a dynamic process associated with the partial loss of protein structure and stability (Figure 2) [2]. These results highlight the need for *in situ* analytical approaches to investigate the structuring and driving forces of protein adsorption on nanomaterials to better our understanding of their effects in biologically relevant conditions.

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References :

[1] From Protein Corona to Colloidal Self-Assembly: The Importance of Protein Size in Protein−Nanoparticle Interactions. Marichal L. *et al*. Langmuir (2020) 36, 8218-8230.
[2] *In situ* analysis of weakly bound proteins reveals molecular basis of soft corona formation. Sanchez-Guzman D. *et al*. ACS Nano (2020) 14, 9073-9088.

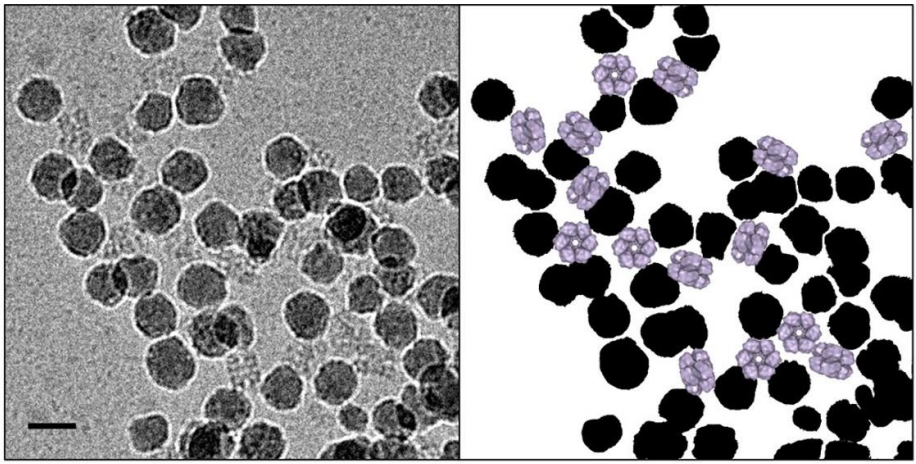

Figure 1: Cryo-TEM image of Arenicola marina hemoglobin (22 nm in diameter) interacting with silica nanoparticles in phosphate buffer (100 mM, pH 6). The corresponding schematic highlights the orientation of the proteins (in purple) relative to the NPs (in black). Scale bar 30 nm. From [1]

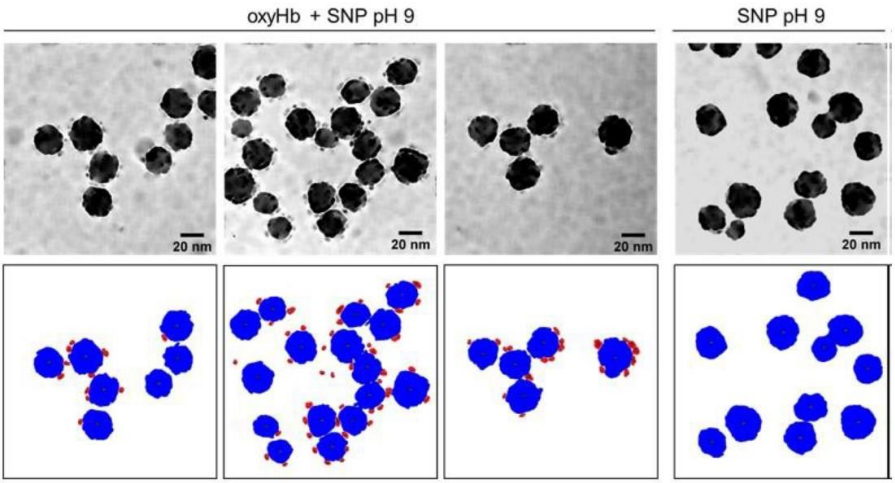

Figure 2: Analysis of the soft protein corona formed by human hemoglobin (oxyHb) on silica nanoparticles (SNPs) by cryoTEM. Automated image analysis was performed to identify proteins adsorbed on silica nanoparticles (in red and in blue respectively). Scale bar is 20 nm. From [2]

* jeril.degrouard@universite-paris-saclay.fr