Studies at the Nanoparticle-Biomolecular Interface and Beyond

Jie An Yang Final Seminar October 29, 2013

The ease of surface functionalization and the excellent optoelectronic properties of gold nanoparticles (Au NPs) have attracted much interest in biological applications.[1] However, while Au NPs are positioned to revolutionize nanotechnology based biomedical applications, our fundamental understanding at the nanoparticle-biomolecular interface and its resultant impact on cells is still limited. In particular, analysis of the spatial arrangement of nanoparticle's surface ligands using scanning tunneling microscopy (STM) is a highly controversial topic.[2,3] Addition of nanoparticles to cell culture media was shown to result in a hard and soft protein corona formation,[4,5] which acted to mitigate/reduce the proposed chemical capabilities of nanoparticles.[6] More critically but even less well understood is the precise orientation of proteins upon adsorption as well as the possible change in protein's conformation, which can alter the protein's intrinsic function. This seminar will thus focus on developing our understanding at this interface, by probing the chemistries at this nanoparticle-biomolecular interface and subsequently, how the Au NPs influence cellular responses.

The question of spatial location of different ligands on nanoparticle surfaces with diameters less than 100 nm is an important one that is difficult to quantitatively address. To investigate the spatial arrangement of biomolecules on Au NPs, the surface of 20, 50, and 90 nm Au NPs were functionalized with two different lipids, both single and mixed, using two different surface chemical procedures utilizing electrostatic or hydrophobic interactions.[7] Mass spectrometry supported the presence of both lipids in the mixed-lipid systems on nanoparticles, and it was observed that the surface chemistry of Au NPs influenced the relative ratios of mixed lipids incorporated. Electron microscopy evidence showed domain sizes for one lipid apparently a quarter to a half the projected diameter for 50 and 90 nm particles; but for 20 nm particles, there is no evidence for the existence of patches of the two lipids.

To study the potential use of Au NPs to limit α-synuclein (α-syn) misfolding, the binding and orientation of α-syn on anionic and cationic Au NPs were investigated.[8] On anionic Au NPs, α-syn was determined to interact with 20 and 90 nm Au NPs via multilayered adsorption, consisting of a strong electrostatic interaction between α-syn and Au NPs in the hard corona and a weaker noncovalent protein−protein interaction in the soft corona. On cationic PAH Au NPs, titration of α-syn into cationic Au NP at >2000 α-syn/cationic Au NP caused the flocculation and sedimentation of α-syn coated PAH Au NPs. The orientation of α-syn onto Au NPs was studied using protease digestion method, revealing that α-syn absorbs onto anionic Au NPs via its N-terminus while on cationic Au NPs, a random orientation of α-syn was adopted (Figure 1). Comparison of the digestion pattern of α-syn on both Au NP with respect to free α-syn reveal an increase in the release of peptides from the N-terminus (amino acid 1–23, lysine position 10) and a decreased number of peptides in the non-amyloid component region (amino acid 59–97, lysine position 80) when adsorbed onto Au NPs, suggesting that the adsorption and binding orientation of α-syn depends on the surface charge of Au NPs.

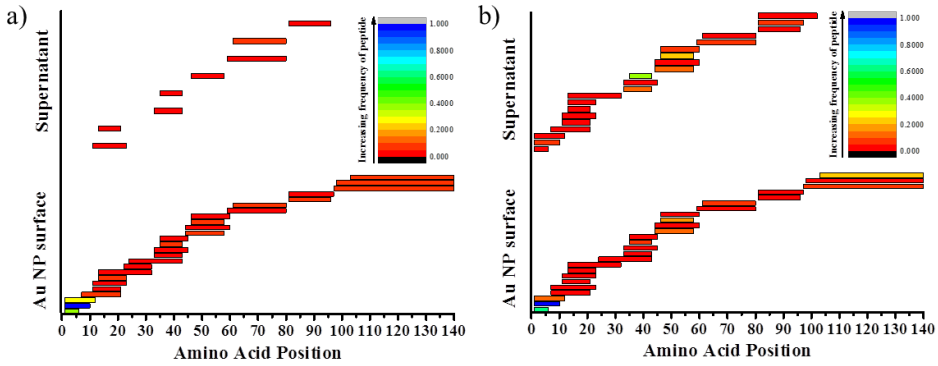

Figure 1. Digestion pattern of α-syn using trypsin when absorbed onto a) anionic Au NPs and b) cationic Au NPs. The peptide fragments (horizontal bars) in the supernatant are separated from those on the Au NP surface and are shown separately. Color scale (normalized) represents the number of each peptides detected.

Differently functionalized Au NPs were found to induce different cellular responses when incubated with cells in vitro. Darkfield microscopy demonstrates that both prostate cancer cells (PC3) and human dermal fibroblast (HDF) cells can "vacuum" Au NPs from the surface.[9] Mean cumulative square distance of cells shows that PC3 migration decreases in the presence of Au NPs while for HDF, migration is dependent on the surface charge and shape of Au NPs (Figure 2). Preliminary investigations on the global impact of Au NPs with cells, based on gene expression analysis, demonstrated that genes related to cell proliferation were up-regulated while genes related to metabolism were down-regulated in HDF. In contrast, gene expression changes in PC3 were observed to strongly depend on surface functionalization of Au NPs, suggesting that Au NPs impact PC3 on at more fundamental molecular level.

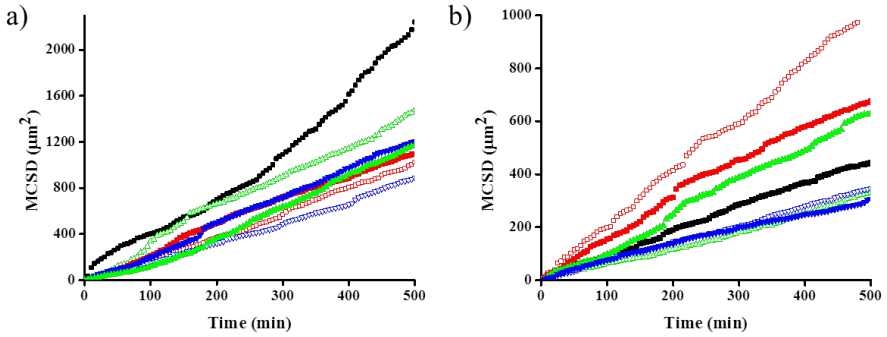

Figure 2. Influence of Au NPs on the migration of PC3 and HDF cells. Plot of the mean cumulative sqaure displacement (MCSD) of a) PC3 and b) HDF cells with and without Au NPs with respect to time. Legends are as follows: black solid square, PC3 or HDF alone; red hollow square, PAH NPs; green hollow triangle, PAA NPs; blue hollow triangle, mPEG NPs; red solid square, PDADMAC Au NRs; green solid triangle, PSS Au NRs; blue solid triangle, mPEG Au NRs.

References

(1) Dreaden, E. C.; Alkilany, A. M.; Huang, X.; Murphy, C. J.; El-Sayed, M. A. The Golden Age: Gold Nanoparticles for Biomedicine. *Chem. Soc. Rev.* **2012**, *41*, 2740–2779.

(2) Jackson, A. M.; Myerson, J. W.; Stellacci, F. Spontaneous Assembly of Subnanometre-Ordered Domains in the Ligand Shell of Monolayer-Protected Nanoparticles. *Nat. Mater.* **2004**, *3*, 330–336.

(3) Cesbron, Y.; Shaw, C. P.; Birchall, J. P.; Free, P.; Lévy, R. Stripy Nanoparticles Revisited. *Small* **2012**, *8*, 3714–3719.

(4) Cedervall, T.; Lynch, I.; Lindman, S.; Berggard, T.; Thulin, E.; Nilsson, H.; Dawson, K. A.; Linse, S. Understanding the Nanoparticle-Protein Corona Using Methods to Quantify Exchange Rates and Affinities of Proteins for Nanoparticles. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 2050–2055.

(5) Lundqvist, M.; Stigler, J.; Elia, G.; Lynch, I.; Cedervall, T.; Dawson, K. A. Nanoparticle Size and Surface Properties Determine the Protein Corona with Possible Implications for Biological Impacts. *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 14265–14270.

(6) Salvati, A.; Pitek, A. S.; Monopoli, M. P.; Prapainop, K.; Bombelli, F. B.; Hristov, D. R.; Kelly, P. M.; Åberg, C.; Mahon, E.; Dawson, K. A. Transferrin-Functionalized Nanoparticles Lose their Targeting Capabilities when a Biomolecule Corona Adsorbs on the Surface. *Nat. Nanotechnol.* **2013**, *8*, 137–143.

(7) Yang, J. A.; Murphy, C. J. Evidence for Patchy Lipid Layers on Gold Nanoparticle Surfaces. *Langmuir* **2012**, *28*, 5404–5416.

(8) Yang, J. A.; Johnson, B. J.; Wu, S.; Woods, W. S.; George, J. M.; Murphy, C. J. Study of Wild-Type α-Synuclein Binding and Orientation on Gold Nanoparticles. *Langmuir* **2013**, *29*, 4603–4615.

(9) Yang, J. A.; Phan, H. T.; Vaidya, S.; Murphy, C. J. Nanovacuums: Nanoparticle Uptake and Differential Cellular Migration on a Carpet of Nanoparticles. *Nano Lett.* **2013**, *13*, 2295–2302.