

Use of a new family of biomarker : fluorescent noble metal (Au, Ag) nanoclusters for protein-labelling

Xavier Le Guével^a, Benjamin Hötzer^b, Gregor Jung^b, and Marc Schneider^a

^aPharmaceutical Nanotechnology, Saarland University, Saarbrücken, Germany-
x.leguevel@mx.uni-saarland.de

^bDepartment of Biophysical Chemistry, Saarland University, Saarbrücken, Germany

Fluorescent labelling techniques have been used extensively in both biological research (in vivo imaging & targeting) and clinical diagnosis because of its high sensitivity, simplicity, and diversity. Biological studies are often limited by existing fluorophores which suffer from inherent deficiencies. For example, organic fluorophores, which are most commonly used in fluorescence spectroscopy, are easily photobleached. Large fluorescent tags can also perturb labelled biomolecules, causing artificial movement within cells and altered protein-protein interactions. Another fluorophore, quantum dots, shows great promise in biolabelling due to their unique optical properties which cause them to emit light of different colours depending on their size. Unfortunately, quantum dots are commonly synthesized using harsh conditions and toxic precursors, are difficult to surface passivate, have large physical size comparable to proteins after biocompatible surface modification, and tend to photoblink.

Noble metal nanoclusters (NCs) have gained a tremendous interest in the last five years due to their photophysical properties and their applications in nanomedicine. Several groups have studied the origin of the fluorescence of NCs and their structure–property inside different cavities such as polymers^[1], dendrimers^[2] and proteins^[3, 4]. Few-atom nanoclusters differ from gold or silver nanoparticles in that they can be highly fluorescent, do not support a surface plasmon, and do not have the metallic and bulk-like properties of nanoparticles/nanocomposites. This fluorescence is likely due to the transition of molecule-like electronic levels when subnanometre sizes are smaller than the Fermi wavelength (i.e. < 1 nm) and the Jellium $E_{\text{fermi}}/N_{1/3}$ energy scaling law was used as a model to describe the size dependent electronic structure and relative electronic transitions of the small clusters^[5, 6].

Gold and silver clusters were labelled to three common proteins : bovine serum albumin (BSA) and human transferrin (Tf) by wet chemistry, and glutathione by the etching process. Structural investigations indicate a covalent binding between the metal cluster to the proteins via the sulfur groups. Photophysical studies suggest a relative high quantum yield with a fluorescence emission tuneable in the visible range (Figures 1&2). Cytotoxicity and cell studies performed on gold labelled BSA and Tf exhibit biocompatibility and the cell uptake in lung tumor cell lines, A549 (Figure 3). This survey highlights the potential of a new type of non-toxic fluorescent proteins for imaging and targeting.

References

- [1] H. W. Duan, S. M. Nie, *Journal of the American Chemical Society* **2007**, 129, 2412.
- [2] J. Zheng, J. T. Petty, R. M. Dickson, *Journal of the American Chemical Society* **2003**, 125, 7780.
- [3] J. P. Xie, Y. G. Zheng, J. Y. Ying, *Journal of the American Chemical Society* **2009**, 131, 888.
- [4] X. Le Guevel, B. Hötzer, G. Jung, M. Schneider, *Journal of Materials Chemistry* **2011**, 10.1039/c0jm02660c.
- [5] Z. K. Wu, R. C. Jin, *Nano Letters* **2010**, 10, 2568.
- [6] J. Zheng, P. R. Nicovich, R. M. Dickson, *Annual Review of Physical Chemistry* **2007**, 58, 409.

Aknowledgements

Xavier Le Guevel would like to thank the Andalusian Regional Ministry of Health the “Andalusian Initiative for Advanced Therapies” – Fundacion Progreso y Salud for supporting the research activity. Thanks to Dr. Klaus Hollemeyer for performing MALDI-TOF MS experiments, Vanessa Trouillet for the XPS measurement. Petra König is thanked for help with the cytotoxicity evaluation and Leon Muijs for the CLSM imaging.

Figures

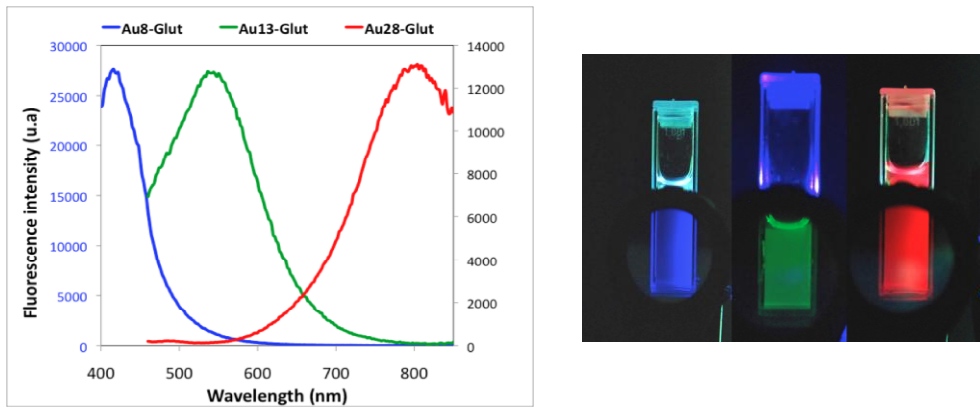


Figure 1. Different size gold nanoclusters (Au NCs) labelled to Glutathione prepared by the etching process.

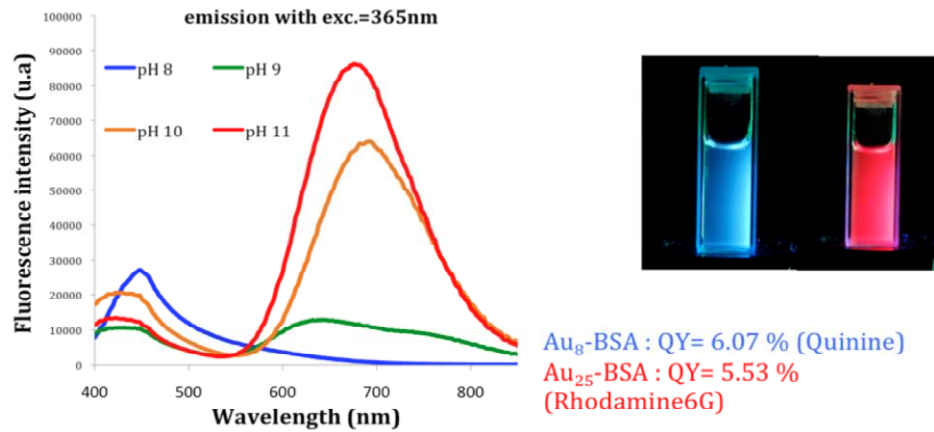


Figure 2. pH dependent synthesis of blue and red fluorescent gold nanocluster labelled BSA.

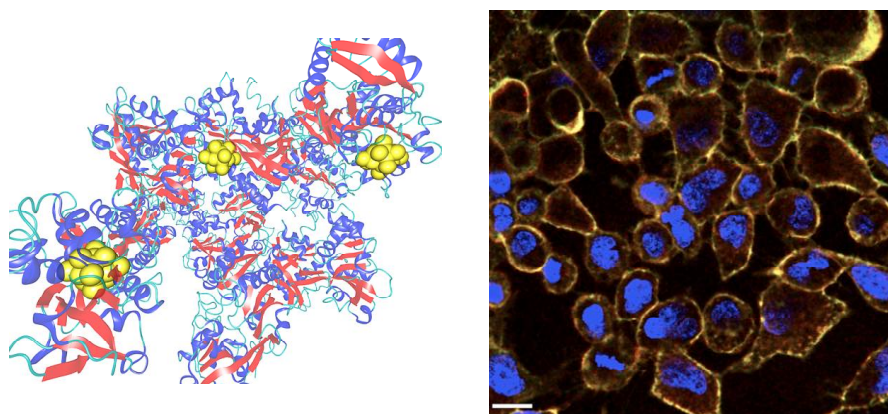


Figure 3. Uptake of gold labelled iron loaded human transferrin (with a red fluorescence emission) in lung tumor cells line, A549. Bar 21 μ m.