NMR STUDY OF NANO-BIO INTERFACE:

A CASE OF INTERACTION BETWEEN GOLD NANOPARTICLES AND BIOTHIOLS

Barbara Pem[a], Valerije Vrček[b], Marija Ćurlin[c], Darija Domazet Jurašin[d], Ivana Vinković Vrček[a,*]

[a] Institute for Medical Research and Occupational Health, Ksaverska cesta 2, 1000 Zagreb, Croatia; [b] Faculty of Pharmacy and Biochemistry, University of Zagreb, Ante Kovačića 1, 10000 Zagreb, Croatia; [c] School of Medicine , University of Zagreb, Šalata 3, 10000 Zagreb, Croatia; [d] Rudjer Boskovic Institute, Bijenička cesta 54, 10000 Zagreb, Croatia

INTRODUCTION

Gold nanoparticles (AuNPs) are being extensively researched for the development of new diagnostic and treatment methods for various diseases. [1] However, the exact nature of their interactions with biological systems has yet to be elucidated. Addition of NP suspensions to biological media results in the formation of a layer of adsorbed molecules on the surface of NPs. [2] One of the presumed binding mechanisms is through the thiol group present in many proteins. Small sulphur-containing molecules are utilized as models for probing the nano-bio interface. [3] This study examines the binding of endogenous biothiols cysteine and glutathione to the surface of spherical AuNPs.

SYNTHESIS

AuNPs were synthesised by reducing $HAuCl_4$ with $NaBH_4$ in the presence of cysteine (CYS) or glutathione (GSH) as stabilising agents. To the solutions of CYS or GSH, 100 mM $HAuCl_4$ was added and dissolved by constant stirring. To this solution, 300 mM $NaBH_4$ solution was added drop-wise (~1 drop/sec). The final concentrations of reagents were: 3 mM $HAuCl_4$, 3 mM coating and 30 mM $NaBH_4$ (1:1:10). The reaction mixture was mixed vigorously at room temperature for 45 min.

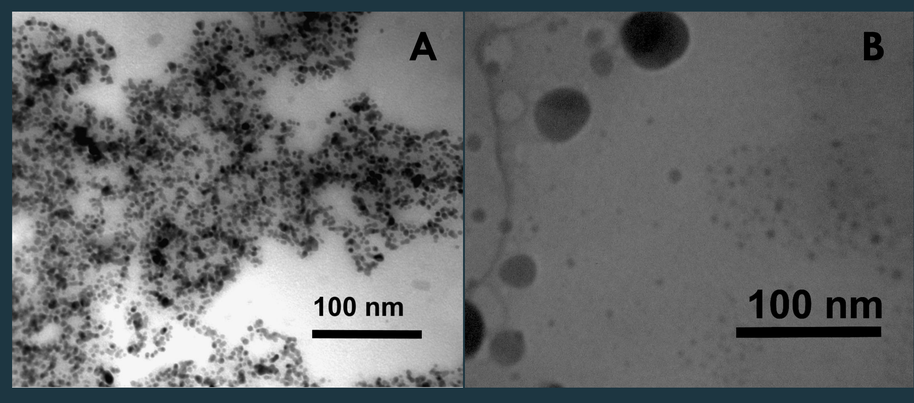

Figure 1. Transmission electrom micrographs of CYS-AuNP (A) and GSH-AuNP (B).

NP	d_H (nm)	% Volume	ζ (mV)
CYS-AuNP	24,2 ± 3,4	54,3	-46,8 ± 2,1
	80,0 ± 2,1	10,4	
	219,7 ± 48,9	35,3	
GSH-AuNP	6,4 ± 0,8	64,9	-58,0 ± 3,8
	65,1 ± 3,8	35,1	

Table 1. Hydrodynamic diameters (d_H) expressed as volume percentages of cysteine/ glutathione coated AuNPs, and their corresponding zeta potentials (ζ).

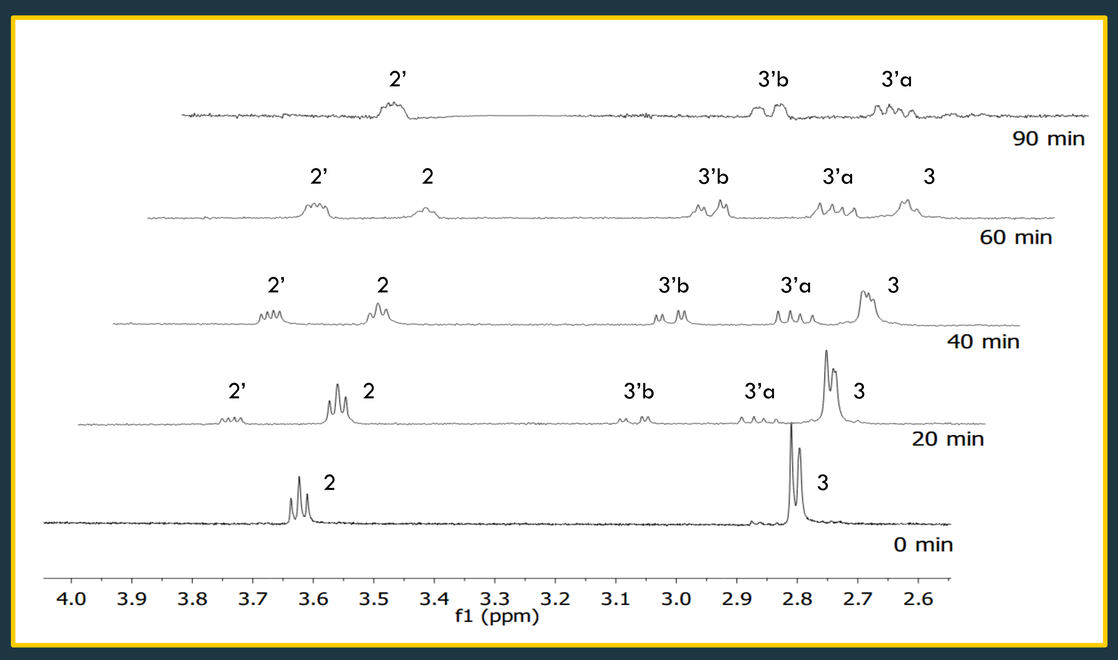

Figure 2. 1H NMR spectra of CYS in the reaction mixture with Au^{3+} and $NaBH_4$. The reaction was tracked in 20 min intervals.

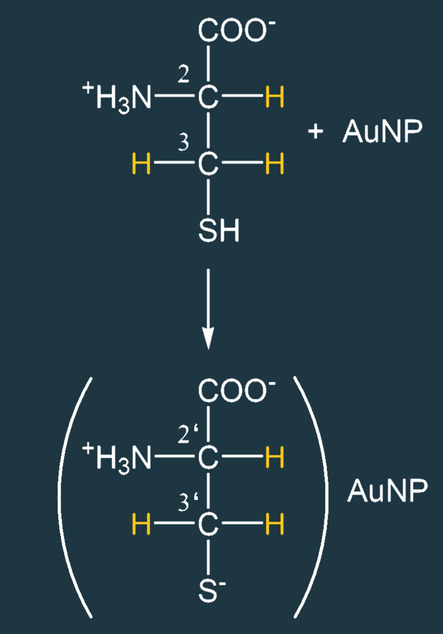

Figure 3. Proposed reaction of CYS binding to the surface of AgNPs.

METHODS

Size distribution and surface charge were obtained by dynamic and electrophoretic light scattering, respectively. AuNPs were visualized by transmission electron microscopy.

The 1H spectra in D_2O were recorded with a Varian INOVA 400 spectrometer, at 399.6 MHz. Chemical shifts in the 1H NMR spectra are expressed in parts per million (ppm) vs. TMS as the external standard. 1H NMR spectra were recorded immediately after mixing the reagents, and subsequently every 20 minutes until AuNPs formation finished.

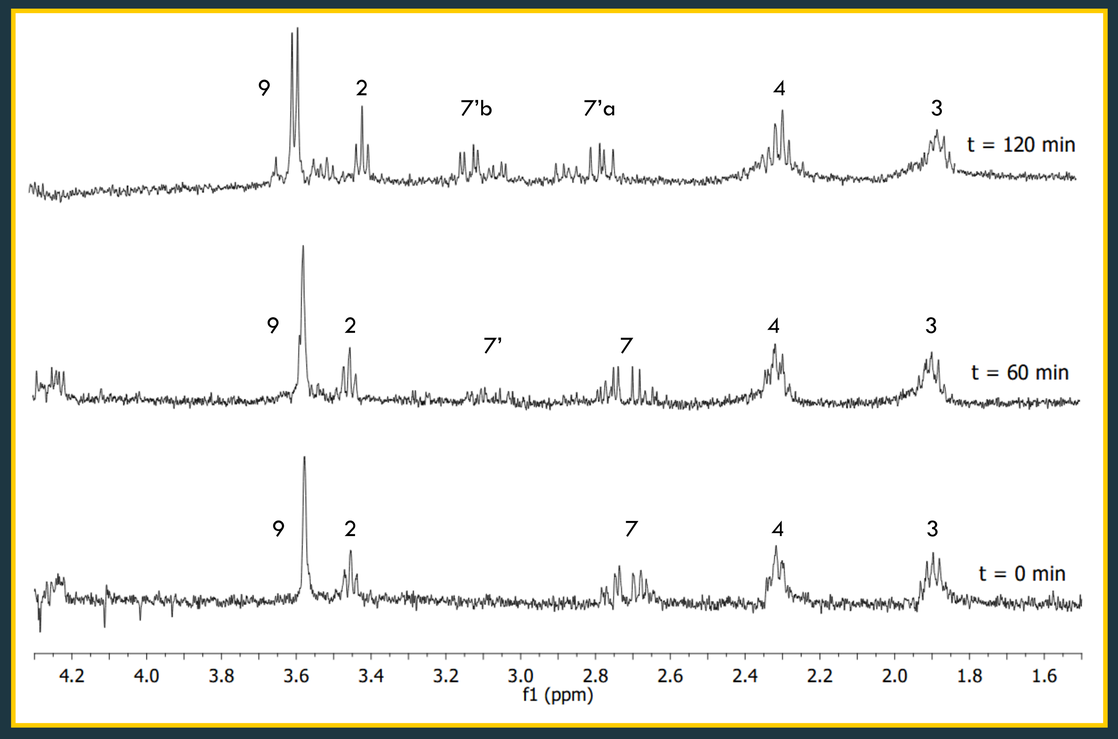

Figure 4. 1H NMR spectra of GSH in the reaction mixture with Au^{3+} and $NaBH_4$. The reaction was tracked in 60 min intervals.

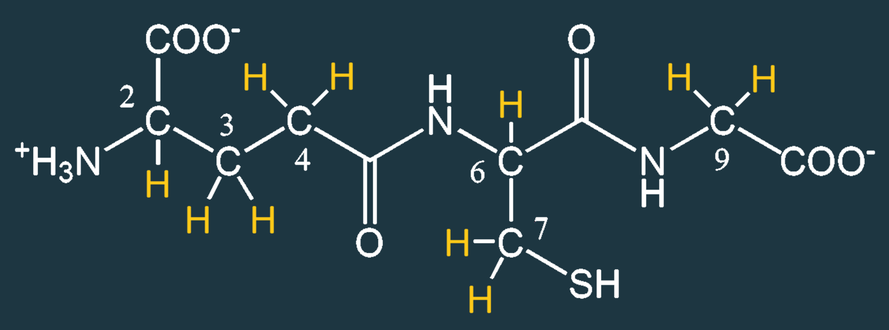

Figure 5. The structure of GSH.

RESULTS

Process of interaction between CYS/GSH and the AuNPs surface was observed by recording chemical shifts of signals, along with the loss of resolution and peak broadening. Furthermore, the intermediate spectra were captured, with distinct peaks corresponding to both bound and unbound biothiols.

CYS-AuNP spectra demonstrate a downfield shift of both peaks, as well as separation of the peak 3 into two distinct peaks. Spectra of GSH-AuNP show separation of peak 7. Both positions (3 in CYS and 7 in GSH) correspond to protons adjacent to the thiol group, which indicates that the thiol is responsible for binding to the NP surface. GSH-AuNP spectrum also shows splitting of the peak 9, which could indicate some interaction with the carboxyl group.

ACKNOWLEDGEMENT

This study was financially supported by the HRZZ-IP-2016_06_2436 grant.

Contact: bpem@imi.hr

REFERENCES

[1] J. A. Webb, R. Bardhan, *Nanoscale* **2014**, *6*, 2502.
[2] P. Del Pino, B. Pelaz, Z. Qian et al., *Mater Horizons* **2014**, *1*, 301.
[3] K. Vangala, F. Ameer, G. Salomon et al., *J Phys Chem C* **2012**, *116*, 3645.