Protein glycosylation affects nano-bio interactions depending on particle size and surface functionalization

Rinea Barbir[a], Rafael Ramírez Jiménez[b], Rafael Martín-Rapún[b], Darija Domazet Jurašin[c], Jesus Martinez de la Fuente[b], Ivana Vinković Vrček[a]

[a] Institute for Medical Research and Occupational Health, Ksaverska cesta 2, 10 000 Zagreb, Croatia; *E-mail:rbarbir@imi.hr*
[b] Instituto de Nanociencia y Materiales de Aragón (INMA), Universidad de Zaragoza – CSIC and CIBER-BBN, Zaragoza, Spain
[c] Ruđer Bošković Institute, Bijenička cesta 54, 10 000 Zagreb, Croatia

INTRODUCTION

Understanding the interaction of nanomaterials (NMs) with biomolecules is of utmost importance for their succesful application in medicine. Despite numerous published data on nano-bio interactions, the role of protein glycosylation on the formation, characteristics, and fate of nano-bio complexes has been almost completely neglected, although most human serum proteins are glycosylated. Here, the binding affinity between different gold NMs to non-glycosylated protein (recombinant transferrin - non-glycoTRF) was compared with binding to glycosylated protein (human transferrin - glycoTRF).

MATERIALS AND METHODS

Study included citrate-coated AuNPs of three different sizes: small (sCITAuNPs), medium (mCITAuNPs) and large (lCITAuNPs). In addition, effect of particle shape was invastigated using polyethylene glycol (PEG)-coated AuNPs in spherical (PEGAuNPs), rod-like (PEGAuNRs) and prism-like (PEGAuNPrs) shapes. All NMs were characterized using dynamic light scattering (DLS), electrophoretic light scattering (ELS) and transmission electron microscopy (TEM). Fluorescent spectroscopy was employed to evaluate binding affinities and circular dichroism (CD) experiments to study protein conformational changes.

RESULTS

Table 1. Hydrodynamic diameter (d_H) obtained by DLS, primary diameter obtained from TEM images (d_{TEM}), and specific surface area (*SSA*, m^2g^{-1}) of different AuNMs. All experiments were done at 25°C and at NMs concentration of 10 mg of metal/L in ultrapure water (pH = 5.68).

AuNMs type	d_H/nm (% mean volume)	d_{TEM}/nm	*SSA*/m^2g^{-1}
sCITAuNPs	46.9 ± 1.7 (100)	13.1 ± 2.7	23.7
mCITtAuNPs	61.5 ± 0.6 (100)	36.0 ± 7.0	8.6
lCITAuNPs	82.9 ± 0.8 (100)	60.0 ± 7.4	5.2
PEGAuNPs	64.2 ± 0.8 (100)	35.7 ± 7.3	8.7
PEGAuNRs	154.9 ± 14.1 (100)	77.3 ± 17.7 13.52 ± 2.72	16.7
PEGAuNPrs	123.9 ± 1.4 (100)	125.0 ± 19.8	14.4

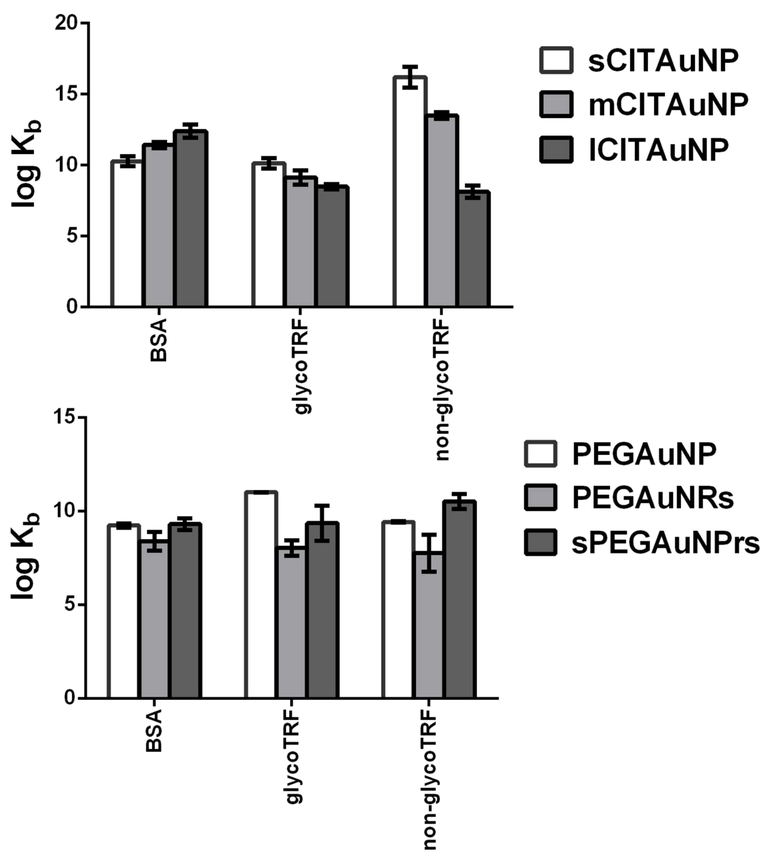

Figure 2. Binding affinities (logK_b) for interaction of glycoTRF, non-glycoTRF and bovine serum albumin (BSA) to different AuNMs.

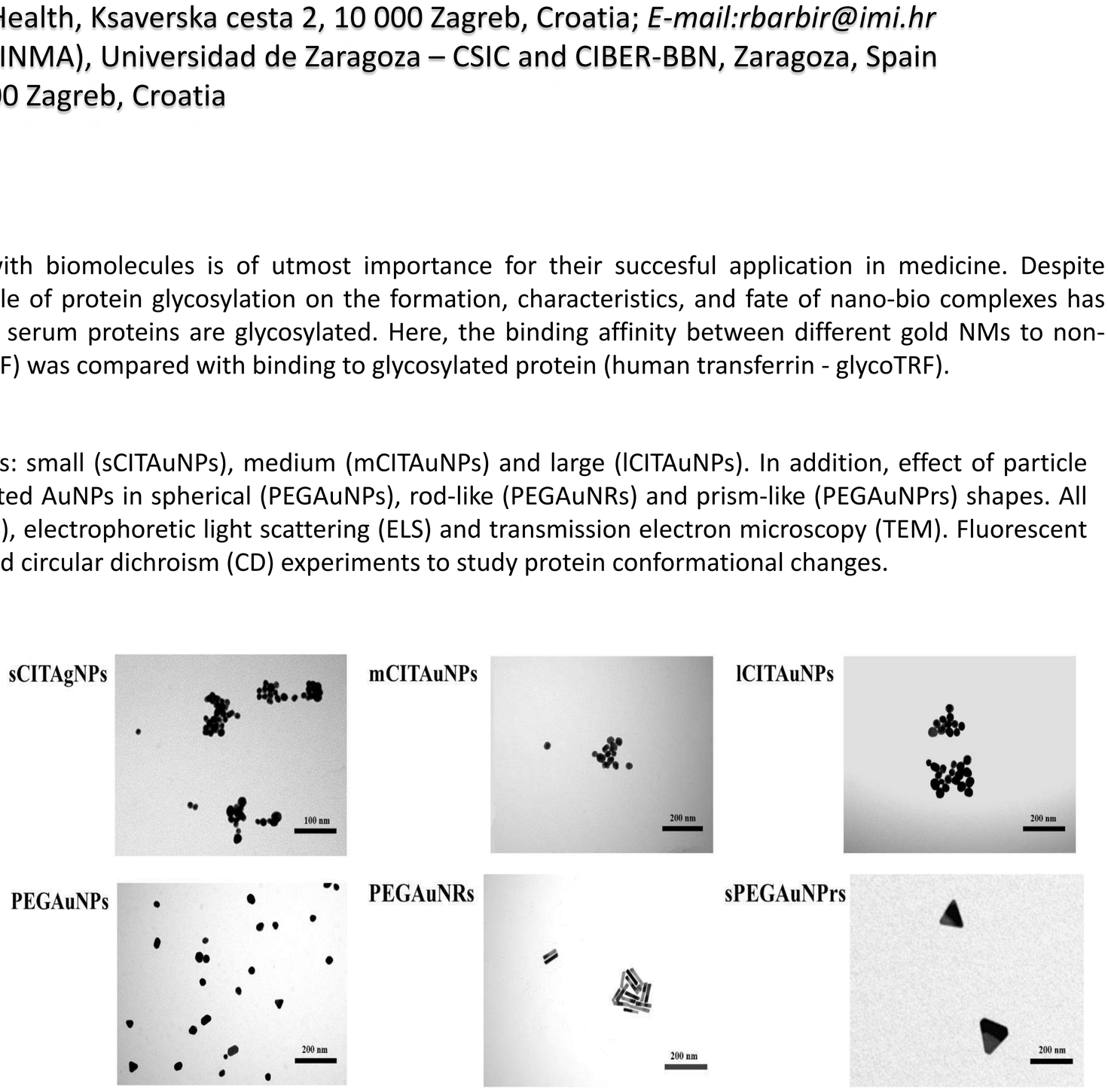

Figure 1. TEM micrographs of NPs.

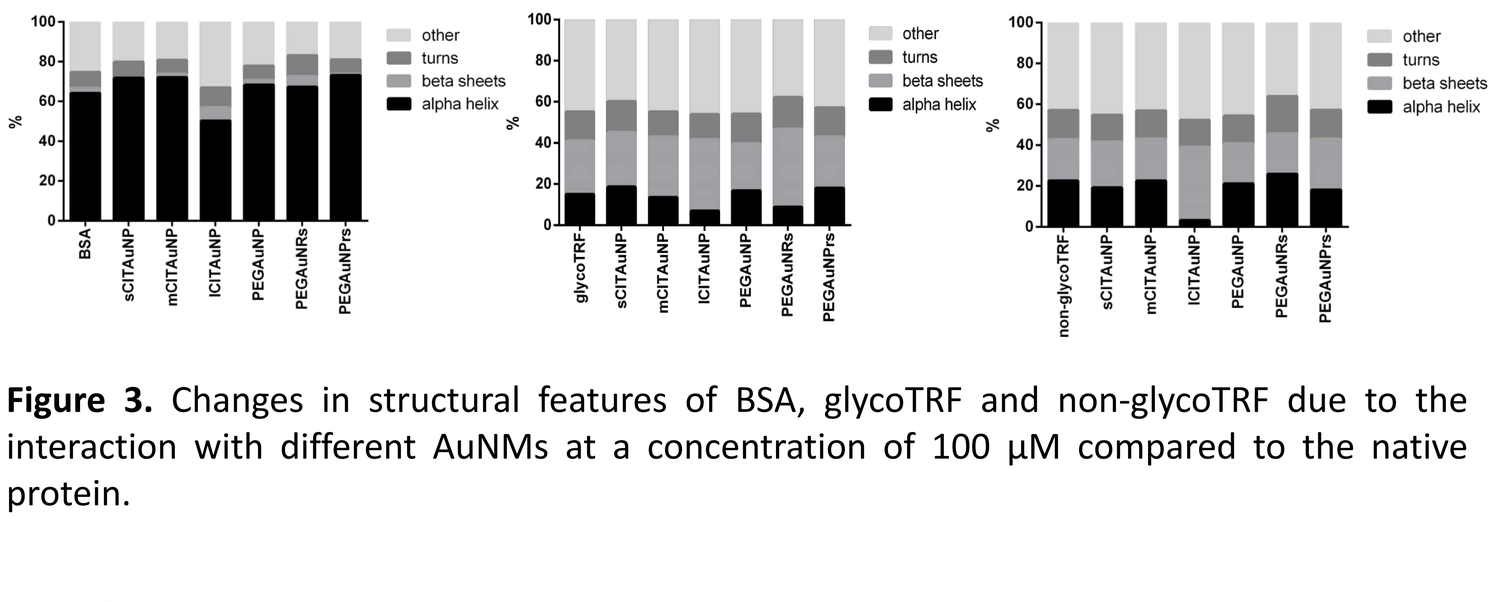

Figure 3. Changes in structural features of BSA, glycoTRF and non-glycoTRF due to the interaction with different AuNMs at a concentration of 100 µM compared to the native protein.

CONCLUSION

Binding of proteins to AuNPs and subsequent changes in the secondary protein structure depend not only on the physico-chemical properties of NMs but also on protein glycosylation. Glycosylation has significant impact on protein corona formation and thus on the fate of NMs in the body.

ACKNOWLEDGEMENT

This study was financially supported by the "Research Cooperability" Program of the Croatian Science Foundation funded by the European Union from the European Social Fund under the Operational Programme Efficient Human Resources 2014–2020 (grant HRZZ-PZS-2019-02-4323)