Hydrodynamic study of gold nanoparticles carrying protein and PEG-like filler

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Introduction

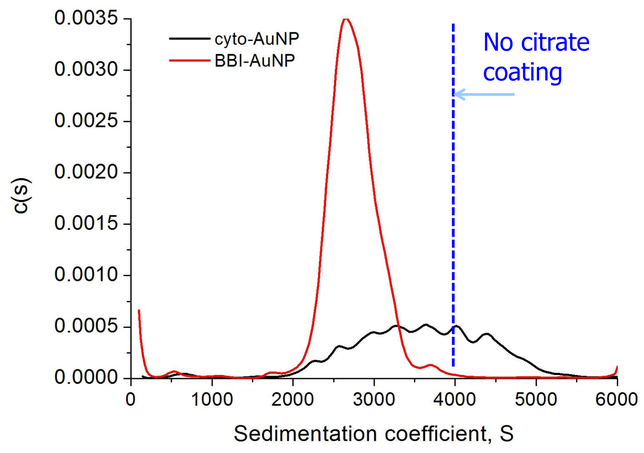
Gold nanoparticles (Au-NPs) offer great potential in variety of biomedical applications such as drug delivery, cancer treatment and many others (1). AuNPs represent an attractive carriers for functional biological matrix since they are biocompatible (2) and could be manufactured in well-defined sizes. Significant progress has been made in characterisation of ligand shell for coated nanoparticles (3), however, the characterisation of Au-NP interacting with complex biological moieties still remains a challenge. In particular, the correctly oriented biological coating is the matter of a great interest. It has been shown that outer membrane β-barrel proteins from *E.coli* can be engineered to form self-assembled monolayer on the gold planar surfaces (4,5). In this instance the direct coupling with gold is achieved by utilizing the gold-thiol chemistry in form of addition of a single cysteine residue into one of periplasmic loops of protein β-barrel along with thiolAlkylPEG molecules mimicking the membrane bilayer (6)

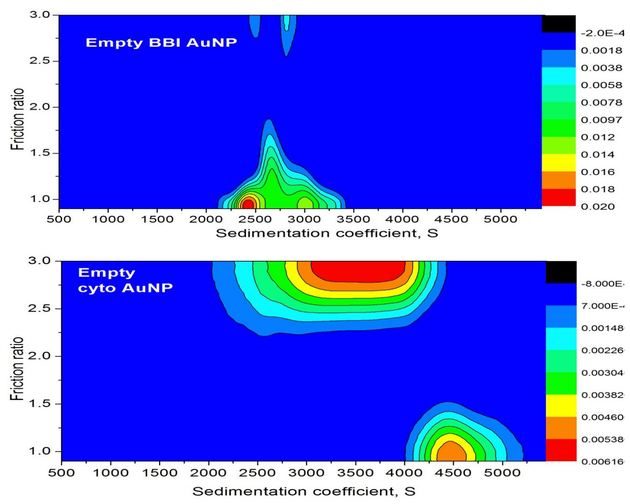
The aim of present study was to extend the previous work on protein self-assembling onto gold planar surfaces for the case of gold nanoparticles and to characterize outer membrane protein-AuNP conjugates using classical hydrodynamic methods.

Hydrodynamic characterization of commercial gold nanoparticles

Two types of commercial nanoparticles were characterised: cyto-AuNP (Cytodiagnostics, Canada) and BBI-AuNP (BBI Solutions, UK). Both samples demonstrate quite broad 1D distribution. Further analysis using 2D distribution shows that BBI are spherical and slightly variable only in terms of citration while cyto–AuNP had more complicated 2D distribution profiles.

All consecutive experiments were carried out using **BBI** AuNP





Gold nanoparticles and protein coating assembly

Citrate coating

Au

AuNP=Sphere with the diameter of 20 nm

Volume: $V = V_{Au} + V_{citrate\ shell} = \frac{4}{3}\frac{\pi N_A {R_h}^3}{\bar{v}_{particle}}$

Radius=Hydrodynamic Radius: $R_h = R_h^{Au} + R_h^{citrate\ shell}$

Mass: $M_{AuNP} = M_{AuNP} + M_{citrate\ shell} = \frac{sRT}{D(1-\bar{v}_{particle}\rho_{solvent})}$

Translation Diffusion Coefficient: $D = \frac{RT}{N_A f}$

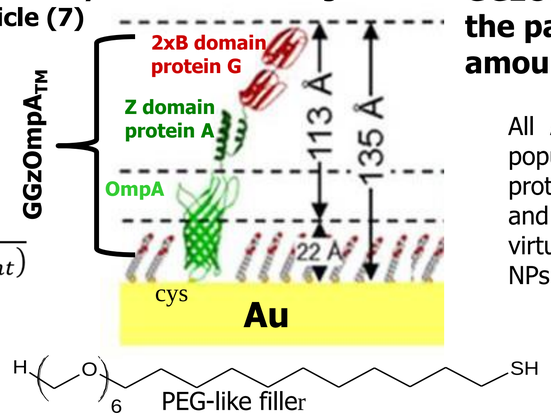
Friction coefficient: $f = 6\pi\eta R_h$

Friction ratio for a sphere f/f_0=1

Sedimentation coefficient: $s = \frac{M(1-\bar{v}_{particle}\rho_{solvent})}{N_A f} = \frac{M(1-\bar{v}_{particle}\rho_{solvent})}{6N_A\pi\eta R_h}$

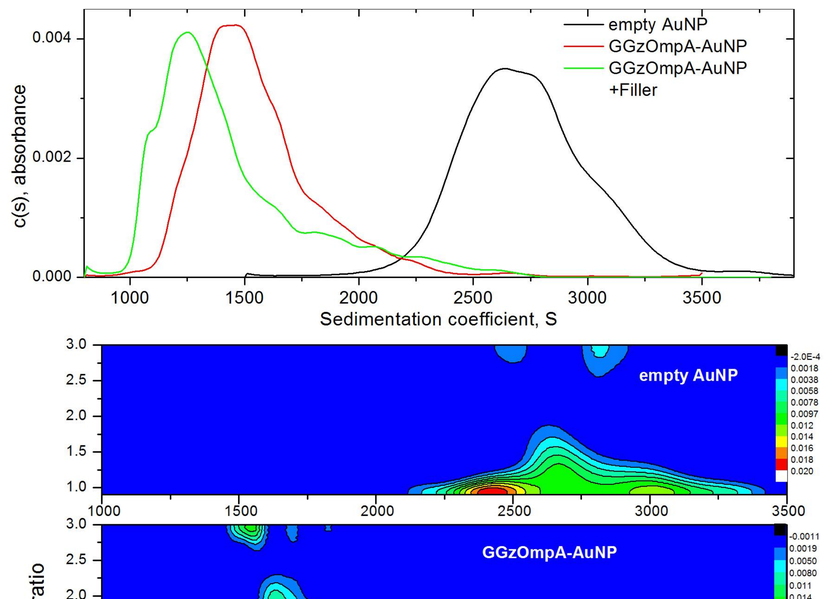
Partial Specific volume: $\bar{v}_{particle} = \frac{M_{Au}\bar{v}_{Au}+M_{citrate}\bar{v}_{citrate}}{M_{Au}+M_{citrate}} = \frac{(2R_h)^2}{(2R_h)^2\rho_{solvent} + 18\eta_{solvent}s}$ (8)

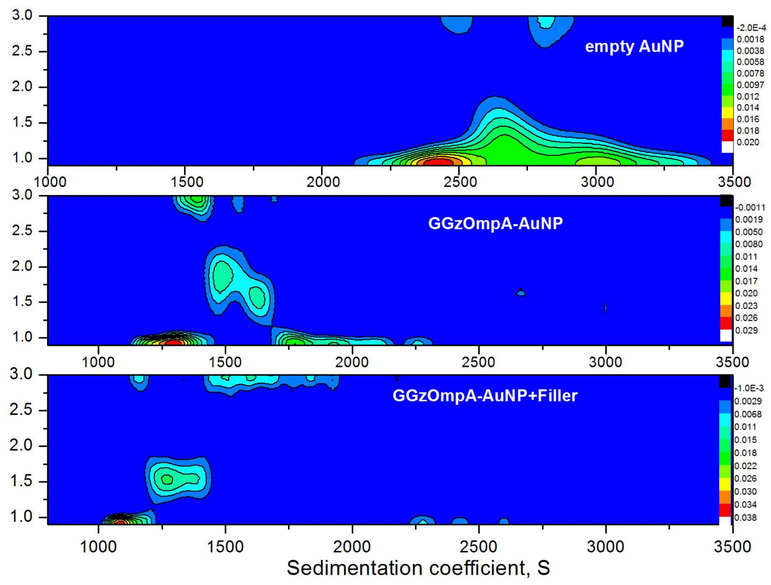
Protein assembly on the surface of gold nanoparticle (7)



Sedimentation velocity size-distribution of BBI Au-NP coated with GGzOmpA$_{TM}$+/-filler demonstrated changes in size and density of the particles. Filler presence resulted in some decrease in the amount of assymetrical/aggregated species

All Au-NP were populated with protein/protein and filler, virtually no free NPs were left



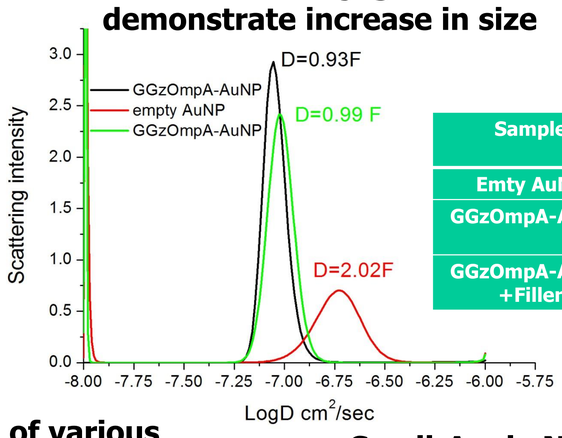


Dynamic Light Scattering

Analytical Ultracentrifugation

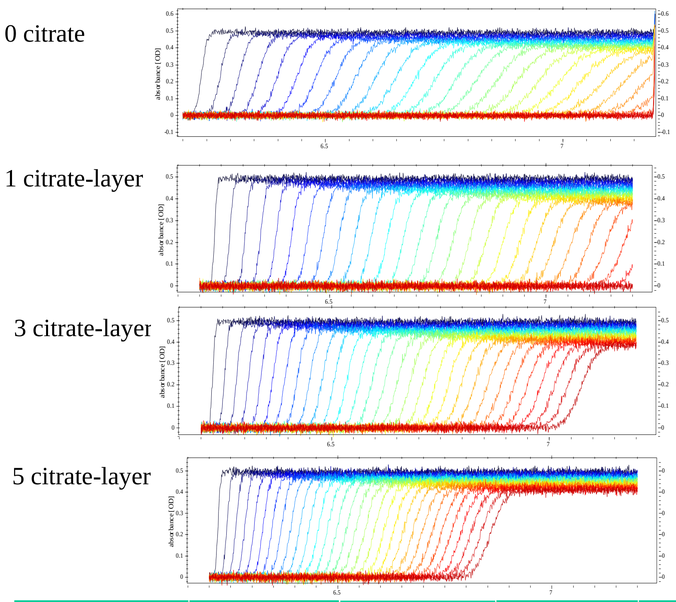
D_t, R_h → M, $\bar{v}_{particle}$ ← s, c(s), c(s, f/f_0)

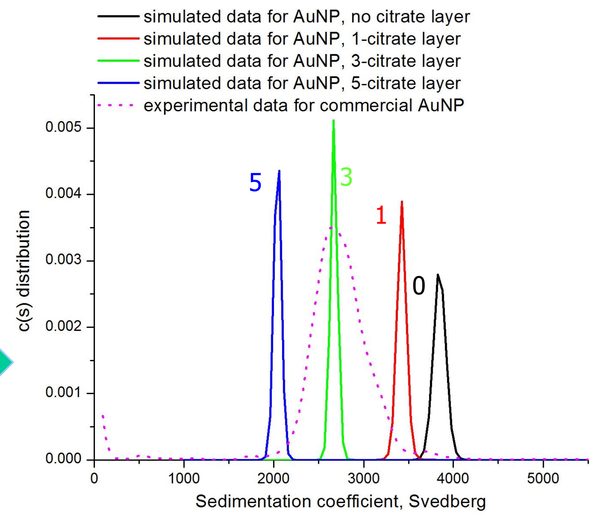
BBI Au-NPs conjugated with GGzOmpA$_{TM}$ demonstrate increase in size



Sample	Particle Diameter, nm
Emty AuNP	19.5
GGzOmpA-AuNP	43.7
GGzOmpA-AuNP +Filler	49.8

Simulated velocity boundaries clearly demonstrate effect of various thickness citrate coating to AuNP sedimentation behaviour



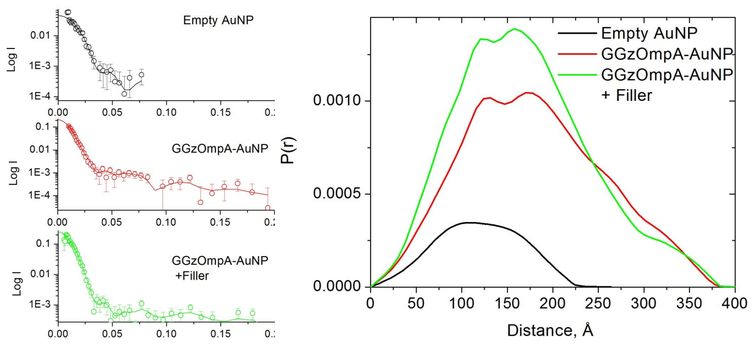


The combination of simulated data and experimental results suggests that it is in average three citrate molecules' coating thickness for BBI AuNP

Thickness of citrate coating, molecule	Citrate shell radius, nm	$\bar{v}_{citrate\ AuNP}$, ml/g	Mass of AuNP particle, kDa	Sedimentation coefficient of AuNP, S
0	0	0.050	48,692	4007
1	0.4	0.058	40,139	3424
2	0.8	0.065	35,790	3030
3	1.2	0.073	31,806	2669
4	1.6	0.083	28,168	2334
5	2.0	0.093	24,861	2941

Small-Angle Neutron Scattering data suggest the formation of less asymmetrical conjugate in presence of the filler

R_g, D_{max}

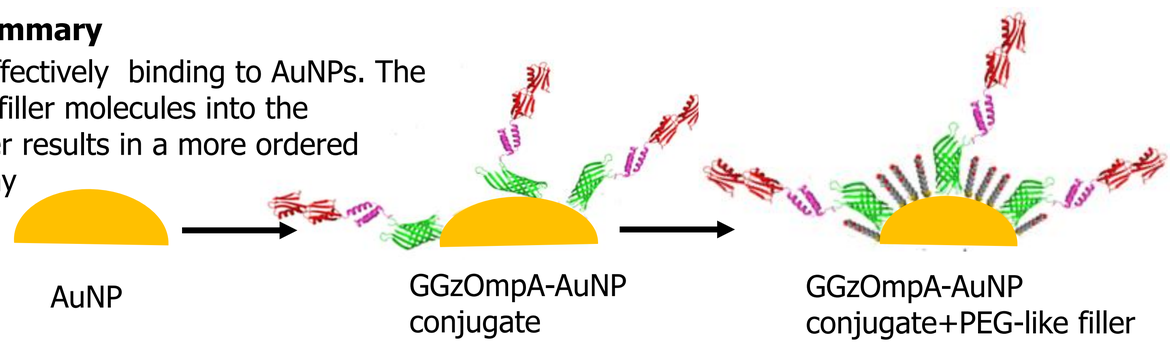


The mathpoint of gold is 73% D_2O.
100% D_2O contrast was chosen in present work as a sensible compromise to minimize signal from gold and maximize signal from both protein and filler

Sample	Radius of Gyration, (R_g) Å	Maximal Distance (D_{max}), Å
AuNP	90.62 ± 1.47	223 ± 13.0
GGzOmpA-AuNP	137.9 ± 1.64	370.2 ± 7.07
GGzOmpA-AuNP + Filler	129.8 ± 1.02	376.7 ± 5.23

Summary

GGzOmpA$_{TM}$ is effectively binding to AuNPs. The incorporation of filler molecules into the GGzOmpA$_{TM}$ layer results in a more ordered protein-filler array



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Acknowledgments
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