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pH-Controlled Aggregation of Au-NPs and Impact of Aggregation Size on Cell Uptake and Toxicity

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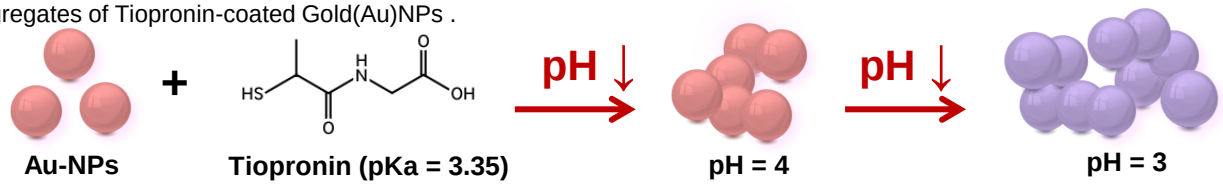
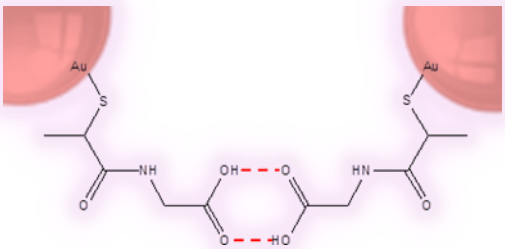
BACKGROUND & MOTIVATION

It is known that nanoparticle(NP)-cell interaction strongly depends on the physicochemical properties of the investigated NPs. In addition, medium density and viscosity influence the colloidal behavior of NPs and aggregation appears to be a common phenomenon, when suspended in biological fluids.
Here we present a systematic study to evaluate the impact of aggregation on cell uptake and cytotoxicity by controlling the size of aggregates of Tiopronin-coated Gold(Au)NPs .



SYNTHESIS & AGGREGATION OF Au-NPs

AuNPs were prepared by boiling aqueous $HAuCl_4$ (0.5 mM) in the presence of sodium citrate, followed by surface modification with Tiopronin (0.5 mM).
Aggregation was induced by adding drops of a HCl solution, recorded by UV-Vis spectroscopy and ended by adding a polymer coating (polyvinyl alcohol, PVA).
Finally the suspensions were transferred to PBS (pH 7.4) and up-concentrated via centrifugation.



Scheme 1. Illustration of pH induced aggregation via hydrogen bonding of Tiopronin-coated AuNPs.

PHYSICO-CHEMICAL CHARACTERISATION

Au-NPs and aggregates of Au-NPs were characterized in terms of **Size** (Dynamic Light Scattering, DLS), **Surface Charge** (Zeta-Potential) and **Morphology** (Transmission Electron Microscopy, TEM).

Table 1. Size and Zeta-Potential (in PBS)

Aggregates	Size [nm]*	Polydispersity [%]	Zeta [mV]
AuNPs	13.0	9.9	-21.2 +/-3.7
AuTio @pH4	115.9	66.3	
AuTio @pH3	262.2	44.4	

*Schultz-Zimm mean, measured at 90°

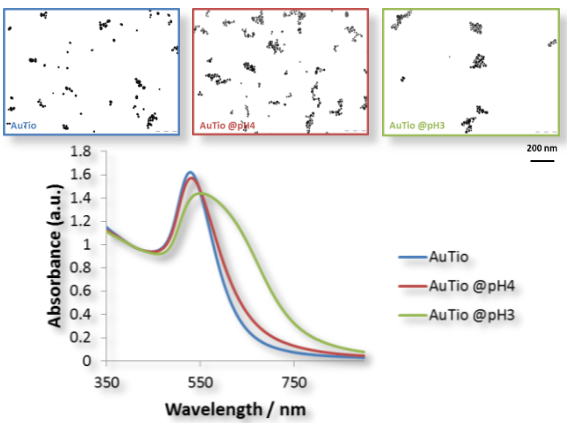


Fig.1. UV-Vis spectra and **TEM images** after transfer to PBS

CELL UPTAKE & CITOTOXICITY

Cell uptake of single particles (AuTio) and aggregates of Tiopronin-coated AuNPs (at pH 4 and pH 3) were measured by inductively coupled plasma optical emission spectroscopy (ICP-OES) after exposure to HeLa cells at 100 µg Au/mL.
Cytotoxicity was assessed by quantifying the activity of Lactate Dehydrogenase in the supernatant of HeLa cells (Detection Kit (Roche, Germany). Cell culture media only was used as the negative control. The detergent Triton X-100 acted as the positive control (0.2% in PBS).

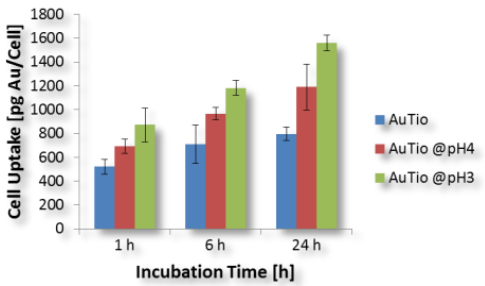


Fig. 2. Cell Uptake (1 h, 6 h and 24 h)

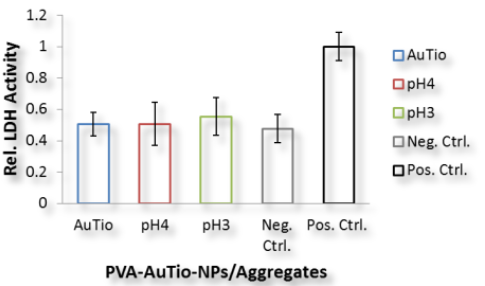


Fig. 3: Cytotoxicity (Rel. LDH Activity after 24 h)

CONCLUSION

- ✓ We showed pH-controlled aggregation of Tiopronin-coated Au-NPs
- ✓ Aggregates of different sizes were successfully transferred to PBS (pH 7.4)
- ✓ We showed a direct correlation between size of aggregates and cell uptake
- ✓ Increased cell uptake by increased aggregation is hereby independent of sedimentation rates (data not shown)
- ✓ Neither single NPs nor aggregates showed any cytotoxicity, independent of aggregation size or time of exposure

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