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pharmaceutics



Development of anti-TNFR antibody-conjugated nanoparticles

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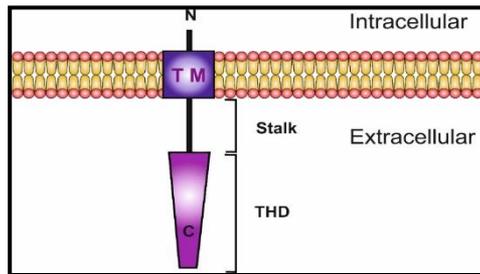
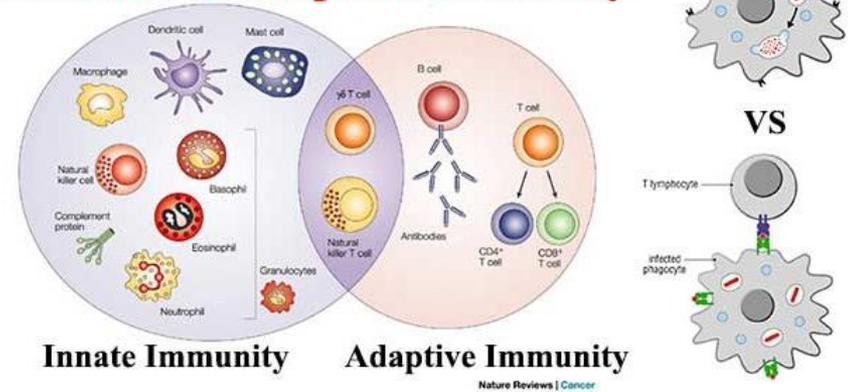
Abstract: Immunotherapy is considered as a new pillar of cancer treatment. However, the application of some promising immunotherapeutic antibodies, such as antibodies against certain immune-stimulatory receptors of the TNF receptor superfamily (TNFRs) including CD40, 41BB, CD27 and anti-fibroblast growth factor-inducible 14 (anti-Fn14) are limited due to their low bioactivity. It has been previously shown that the bioactivity of such anti-TNFR antibodies could be improved by crosslinking or attachment to the plasma membrane by interaction with Fc γ receptors (Fc γ R). Both result in proximity of multiple antibody-bound TNFR molecules what allows activation of proinflammatory signaling pathways. In this work, we have grafted antibodies on gold nanoparticles to simulate the “activating” effect of Fc γ R-bound and thus plasma membrane-presented anti-TNFR antibodies. We have developed and optimized the method for the preparation of gold nanoparticles, their functionalization with poly-ethylene glycol (PEG) linkers, and grafting of antibodies on the surface. We showed here that antibodies, including the anti-Fn14 antibody PDL192, can be successfully attached to nanoparticles without affecting antigen binding. We hypothesize that conjugation of monoclonal anti-TNFR antibodies to the inorganic nanoparticles is a promising technique to boost the efficacy of these immunotherapeutic antibodies.

Keywords: Nanoparticles; Surface modification; Drug-delivery, agonistic anti TNFRSF receptor (TNFR) antibody

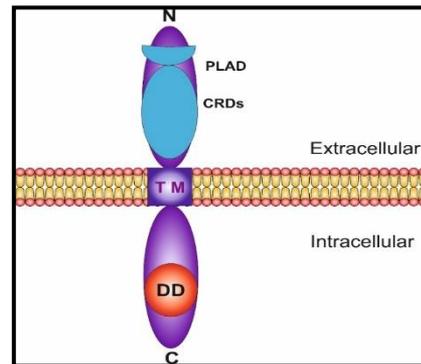
Introduction and keywords

1. Addaptive and Innative Immunoresponse
2. TNFSF ligand and TNFRSF receptors
3. Single chaine variable domaine scFv as an anchoring domain

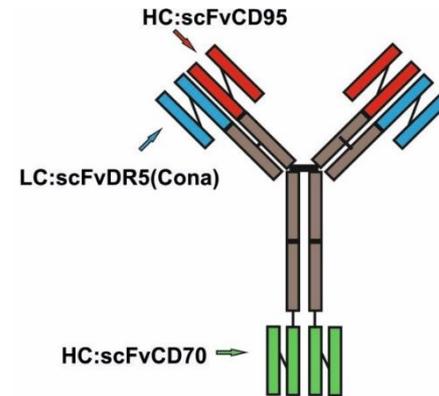
Difference between Innate and Adaptive Immunity



Domain architecture of the TNF ligand



Domain architecture of a TNFRSF death receptor



HC:scFvCD95-LC:scFvDR5-IgG1-HC:scFvCD70

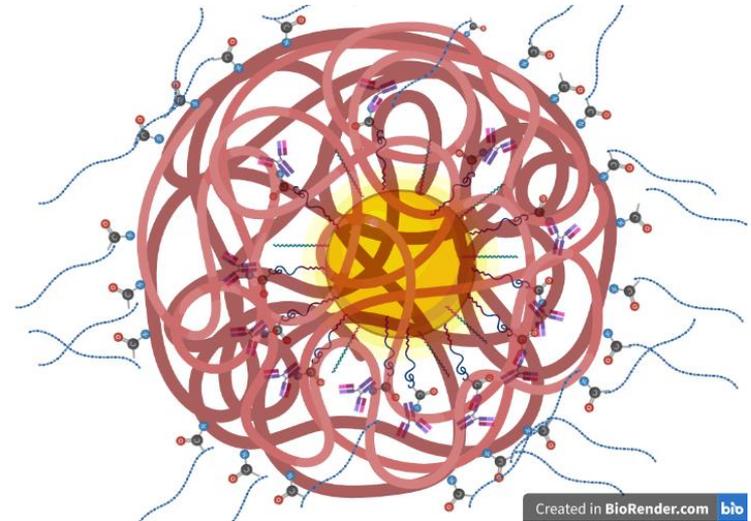
Aim of the work

1. Immobilizing the bio molecules.

- To enhance the activity of the antibody

2. Encapsulation of the bio molecules:

- To enable release-on-demand (cleavable peptides, etc.)
- To eliminate systemic side effect



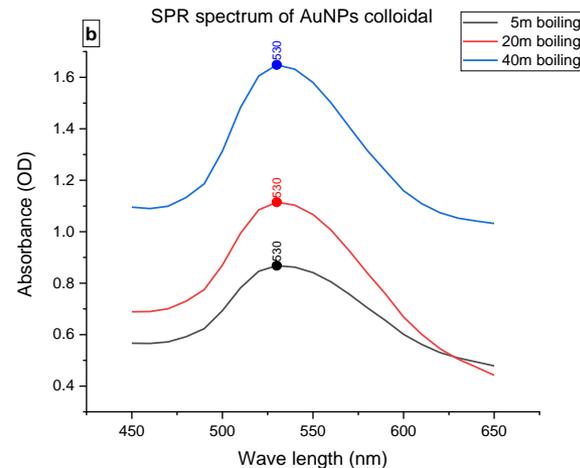
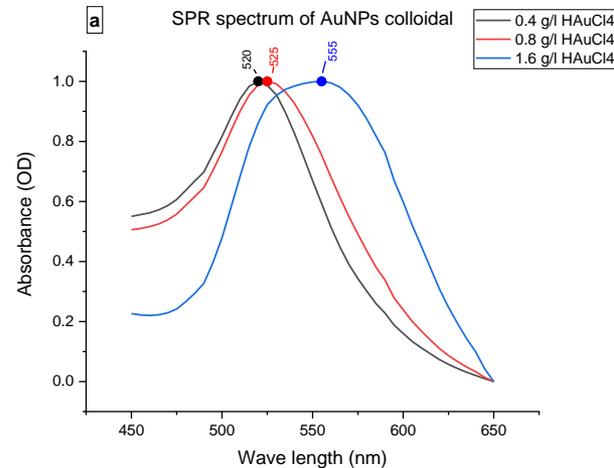
Results and Discussion

➤ Optimized protocol of synthesis and C-AuNPs ca. 86 nm .

- Reduction of Au^+ (HAuCl_4) with Trisodium citrate.

1. The size of the particles can be controlled by the concentration of Auric salt or Trisodium citrate.

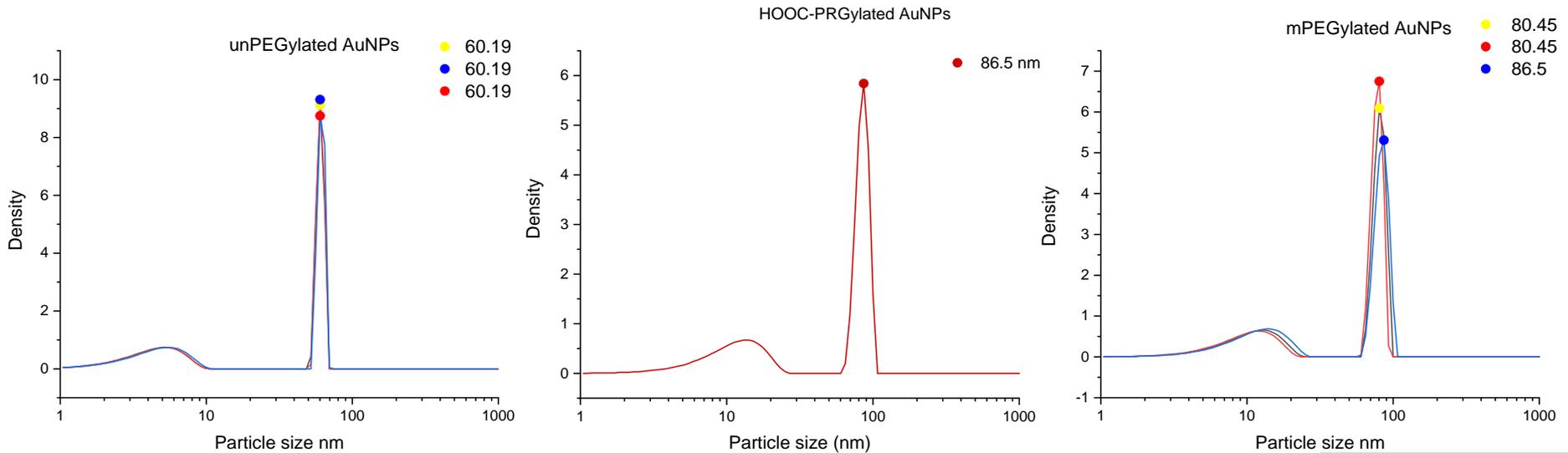
2. Longer boiling duration increases the concentration with the same size.



Results and Discussion

➤ AuNPs Colloid's stability can preserved by functionalization with carboxyl-PEG.

- The size of AuNPs increases after the PEGylation.



Results and Discussion

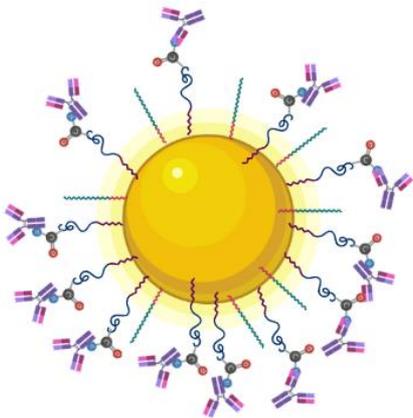
- AuNPs Colloid's stability can preserved by functionalization with carboxyl-PEG.
 - The negative charge on the surface of the particles increases after the PEGylation.

Sample structure	Particles Size	ζ potential
Trisodium citrate - AuNPs	60.19 nm	-14 mv
mPEG-AuNPs	80.45 nm	-7 mv
HOOC-PEG-AuNPs	86.5 nm	-20 mv

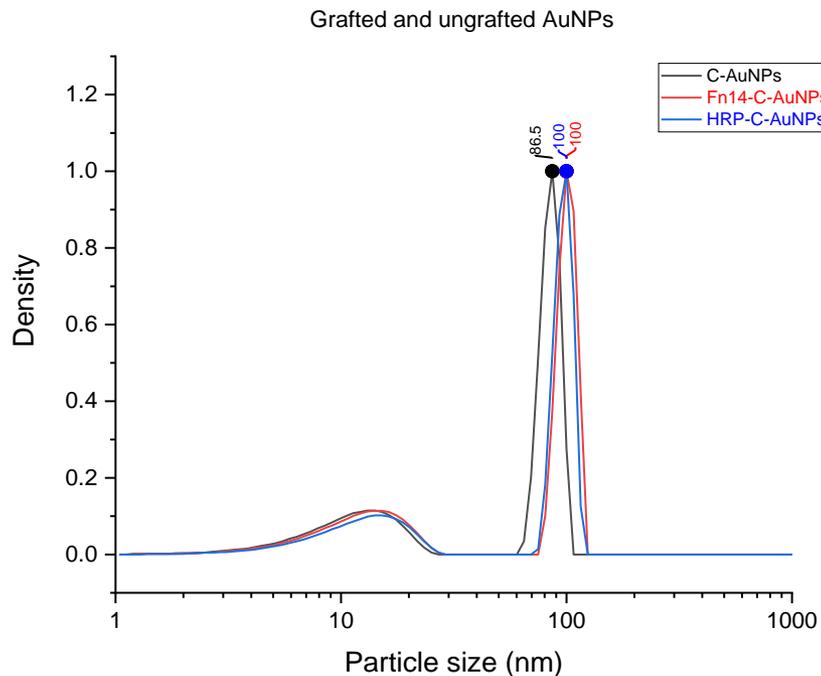
Results and Discussion

➤ Grafting the carboxyl-modified AuNPs with the protein of interest

- Using EDC/NHS Covalent Coupling Procedure
- The size of the AuNPs has been slightly increases after the grafting

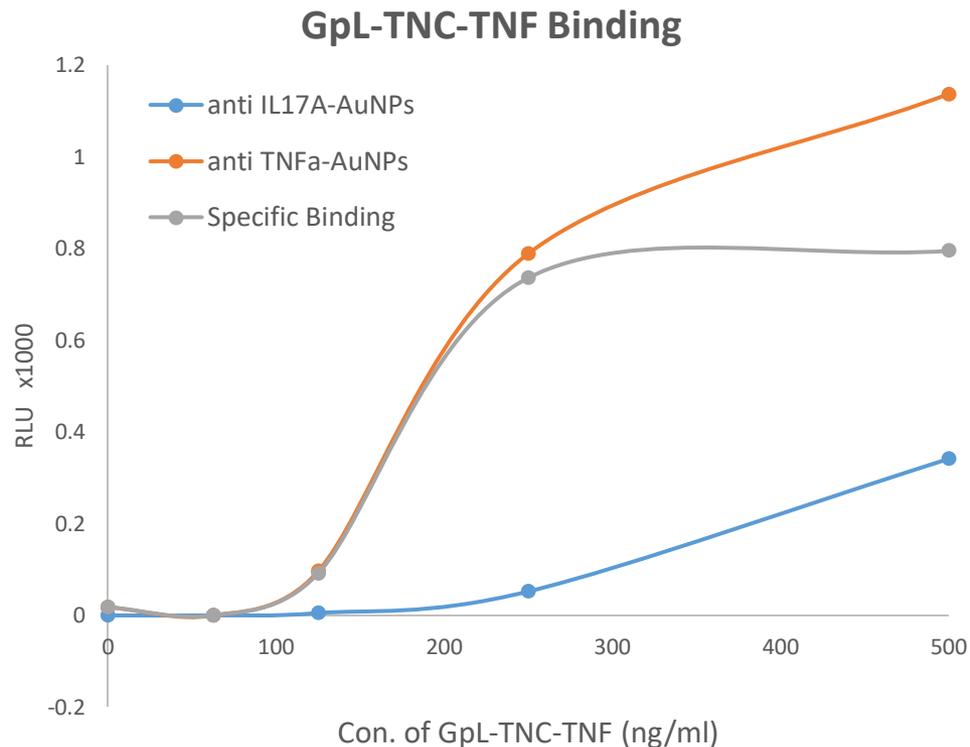


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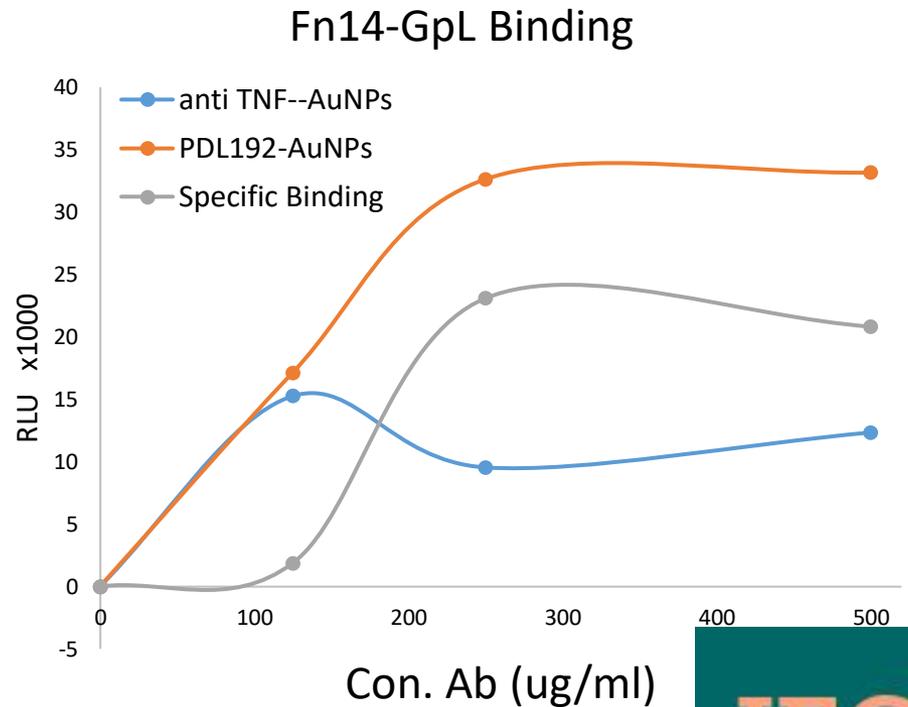
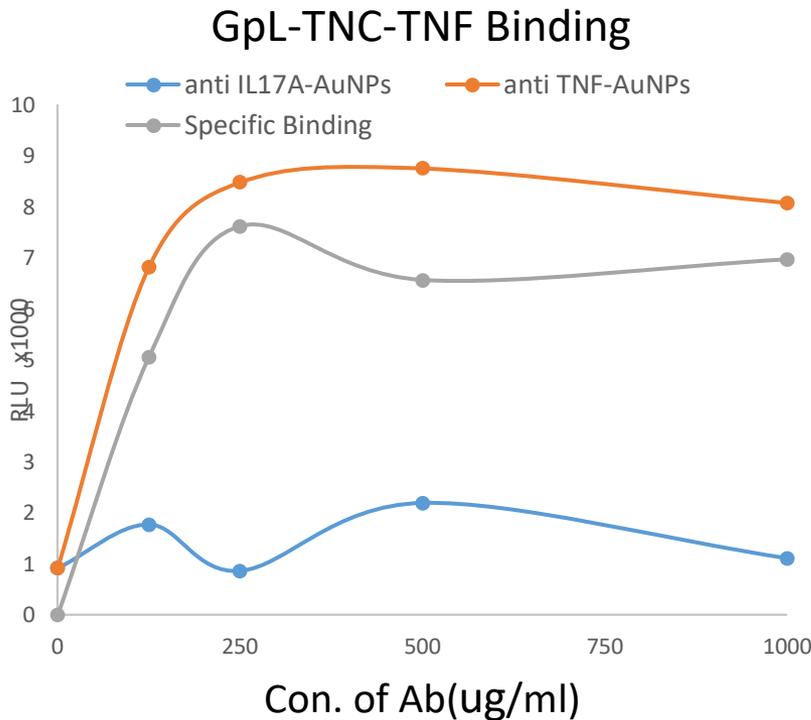
Results and Discussion

- C-AuNPs can be coupled with different types of therapeutic antibodies without affecting their activity.
 - Using EDC/NHS Covalent Coupling Procedure



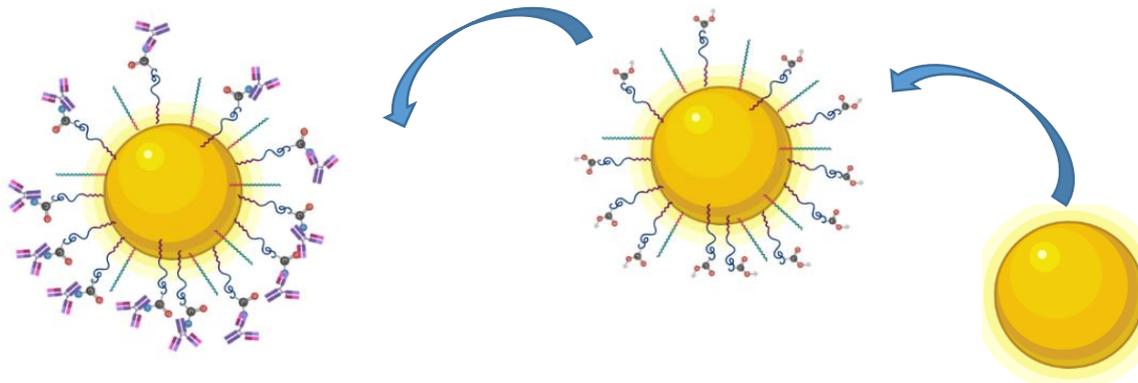
Results and Discussion

- The max grafting capacity on AuNPs (25mg/ml-60nm) is around 250 (µg/ml)



Conclusions

- ✓ Gold nanoparticles of diameter ca. 60 nm have been synthesized by sodium citrate reduction of gold chloride.
- ✓ Functionalization with COOH-PEG-SH stabilize the colloidal solution of the gold nanoparticles and help to cross link them with antibodies.
- ✓ The carboxyl-modified gold nanoparticles can be coupled with the antibodies of interest using the EDC/NHS coupling procedure without affecting their activity. Our future work will focus on the in vitro assays to compare the activity of the conjugated antibodies and their soluble variants.



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