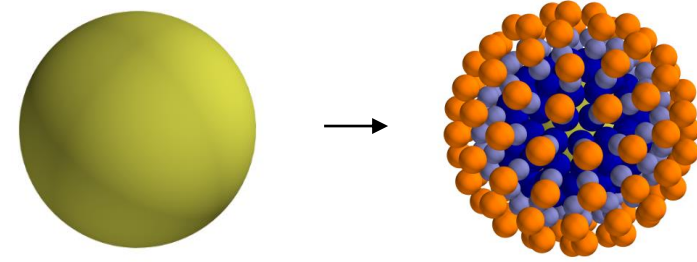


Design of nanomedicines by smart decoration of gold nanoparticles

Enrico Ferrari

School of Life Sciences, University of Lincoln

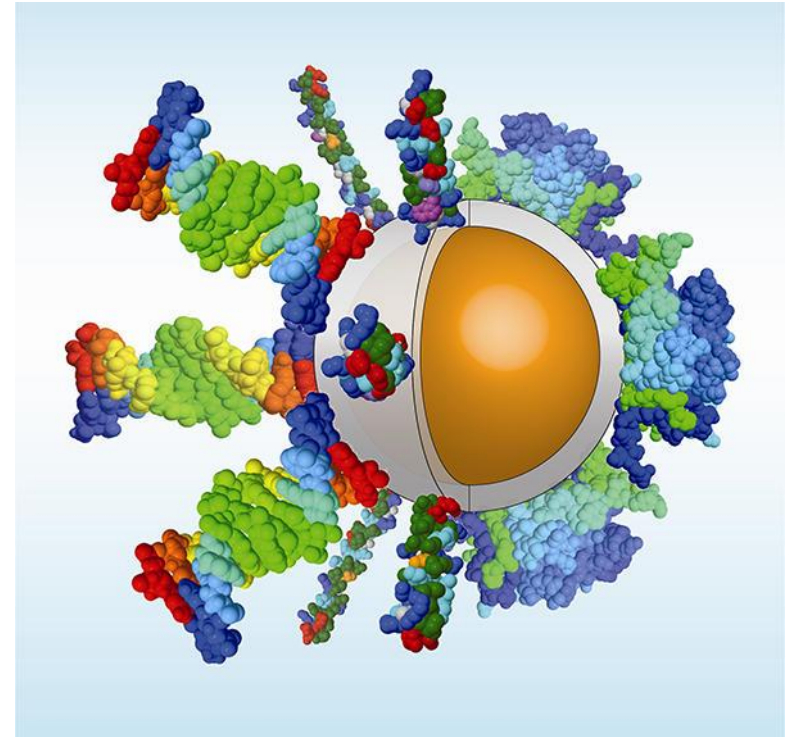


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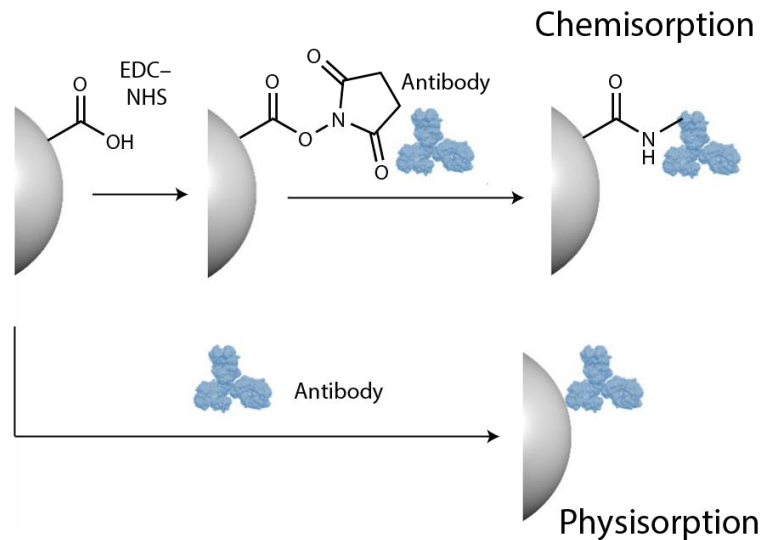
Decoration of nanoparticles with proteins

- Proteins can have various potential roles in nanomedicine:
 - targeting and crossing biological barriers (transferrin, antibodies),
 - bio-therapeutics (antibodies, blood factors, growth factors),
 - nanoparticle-based diagnostic kits (antibodies, enzymes)
- Proteins are very diverse
- They interact differently with different materials
- Protein-nanoparticle conjugates potentially present very high regulatory complexity



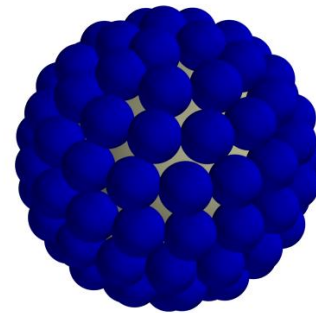
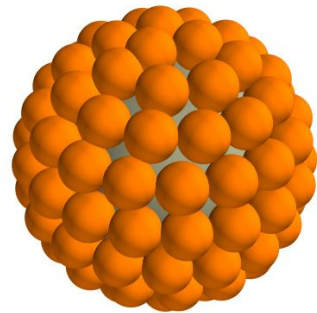
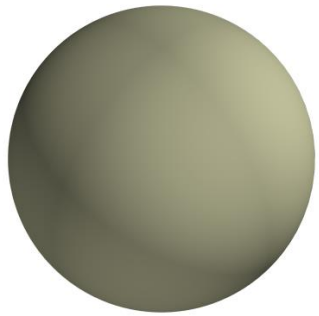
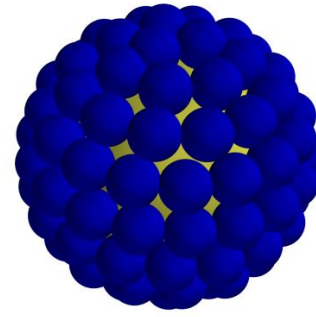
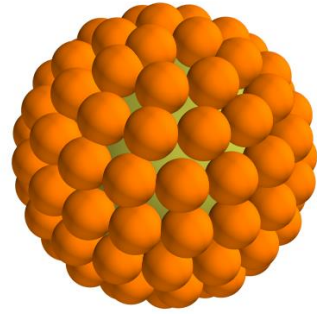
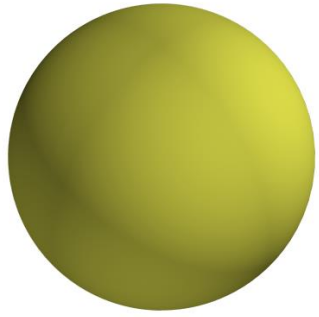
K. E. Sapsford *et al.*, “Functionalizing Nanoparticles with Biological Molecules: Developing Chemistries that Facilitate Nanotechnology” *Chem. Rev.*, vol. 113, no. 3, pp. 1904–2074, 2013.

Decoration of nanoparticles with proteins



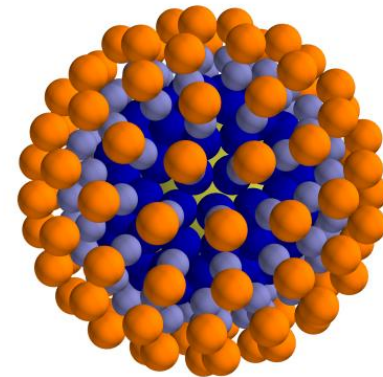
Chemisorption	Physisorption
Near-permanent	Leaky
Harsh on the protein	More gentle

M. Tonigold et al. “Pre-Adsorption of Antibodies Enables Targeting of Nanocarriers despite a Biomolecular Corona” *Nat. Nanotechnol.* **2018**, 13 (9), 862–869.



Modular approach to nanoparticle decoration with proteins

- Material-specific protein adaptor
- Also presents universal binding site
- Bonds are all covalent
- Reactions are efficient and happen in mild conditions
- Preserves the function of the protein

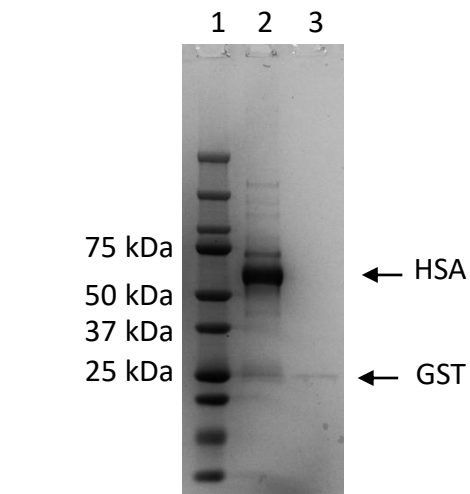
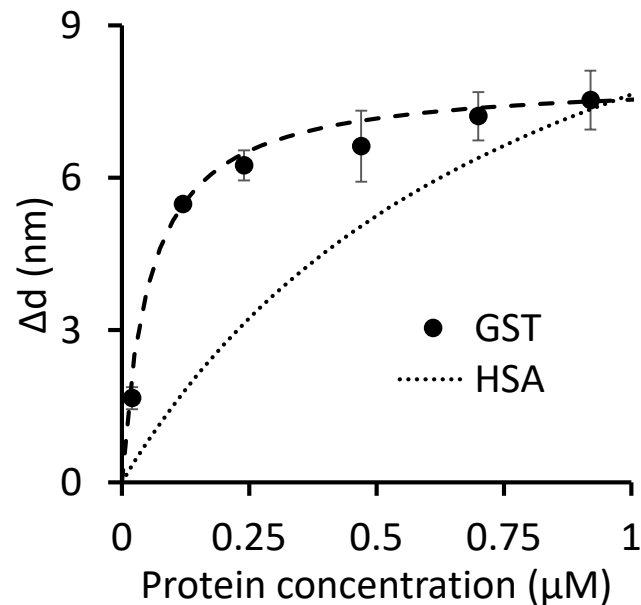


W. Ma, A. Saccardo, D. Roccatano, D. Aboagye-Mensah, M. Alkaseem, M. Jewkes, F. Di Nezza, M. Baron, M. Soloviev, E. Ferrari, "Modular assembly of proteins on nanoparticles," *Nature Communications*, vol. 9, p. 1489, 2018.

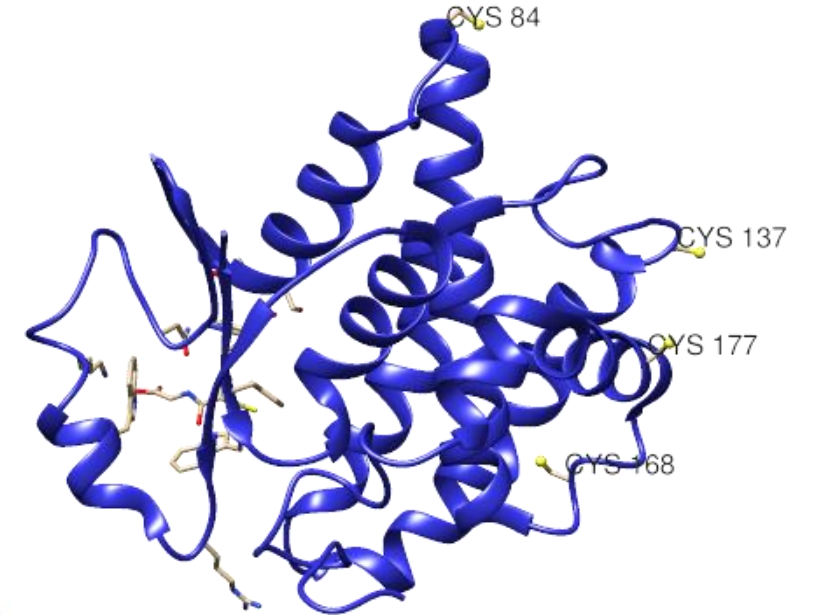
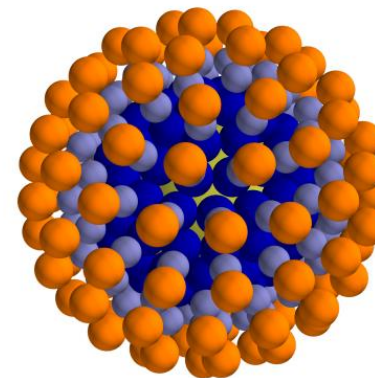
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Citrate-capped gold nanoparticles (GNPs) binding protein

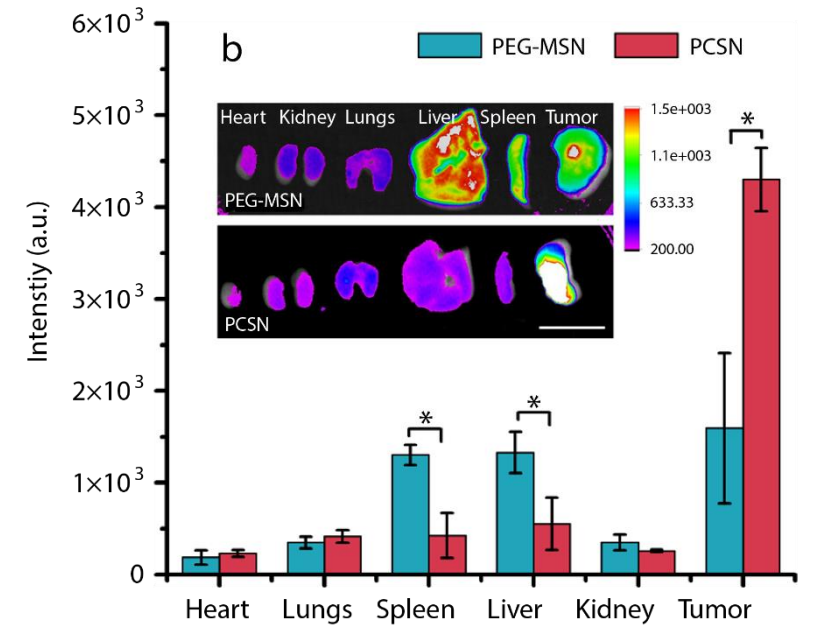
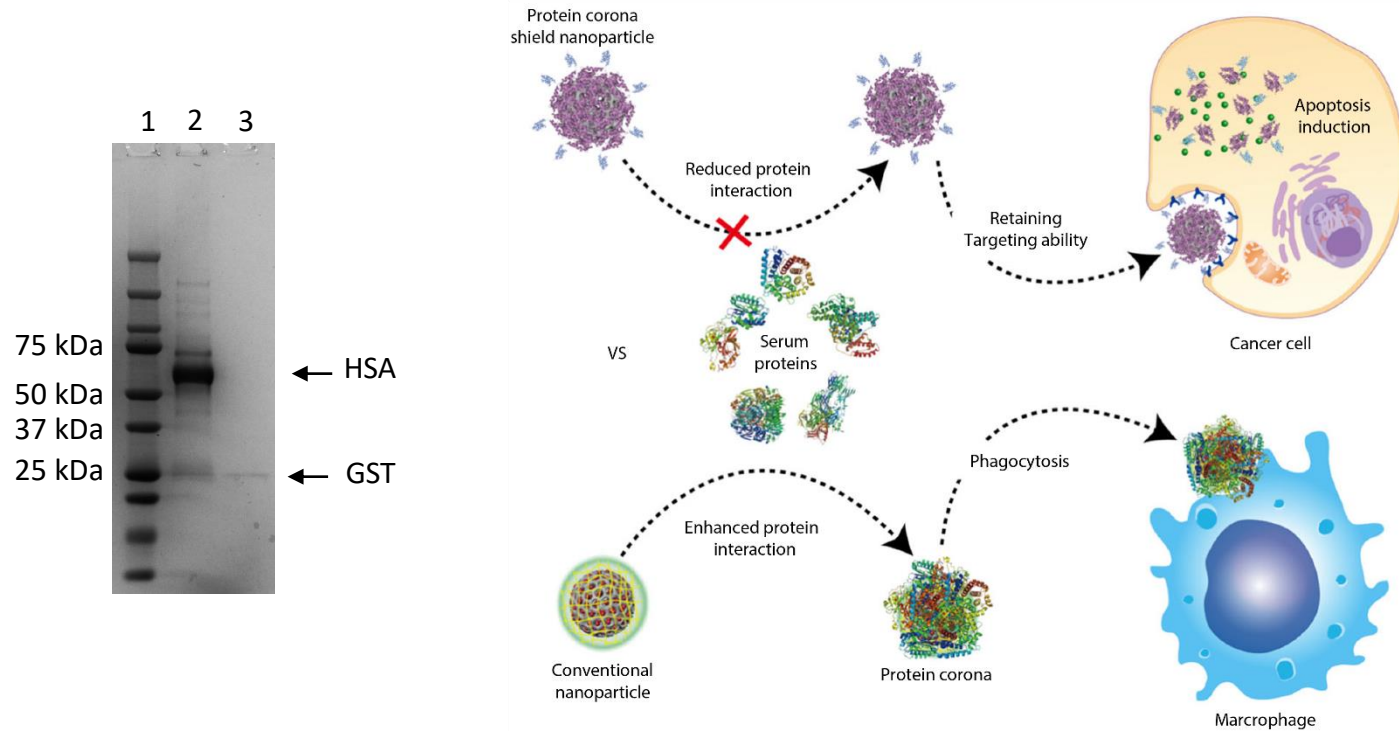
- Glutathione S-Transferase (GST)
- Ubiquitous, we use the one from *Schistosoma japonicum*



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Protein corona



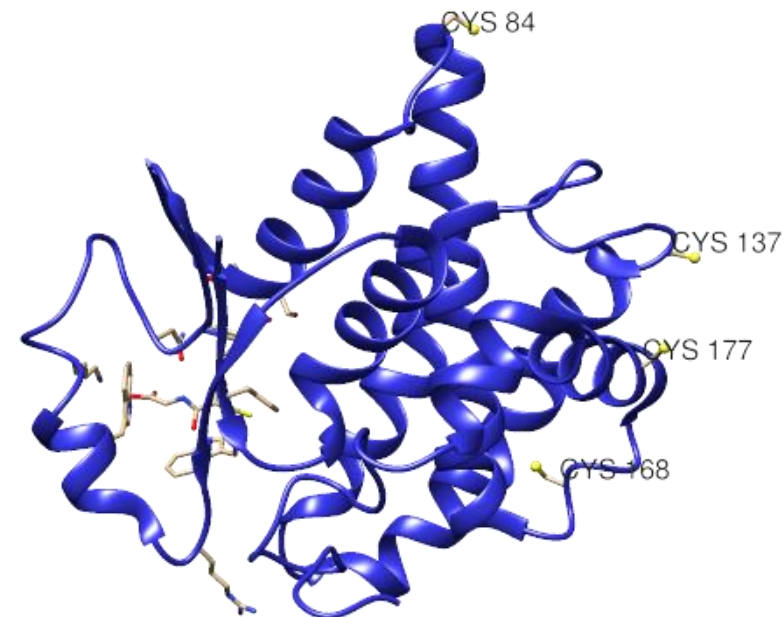
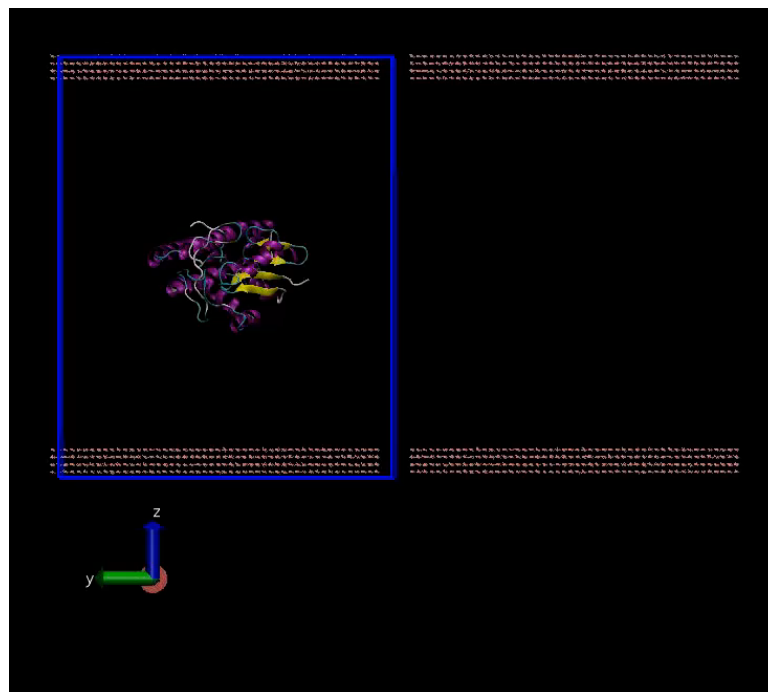
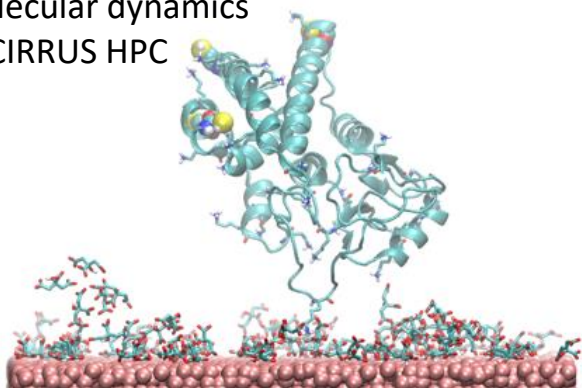
*Ulsan National Institute of Science and Technology (UNIST),
Republic of Korea*

Oh J. et al. "Cloaking Nanoparticles with Protein Corona Shield for Targeted Drug Delivery" *Nat. Commun.* **2018**, 9 (1), 4548.

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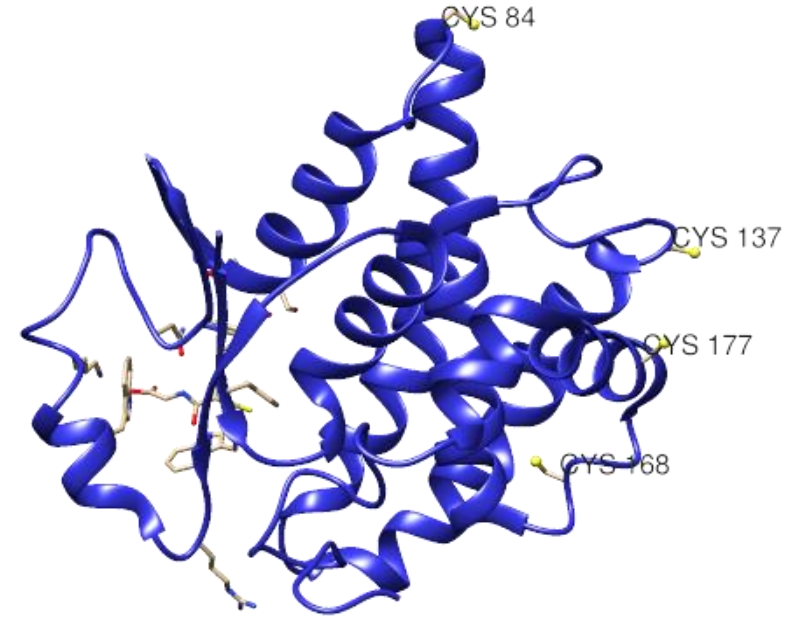
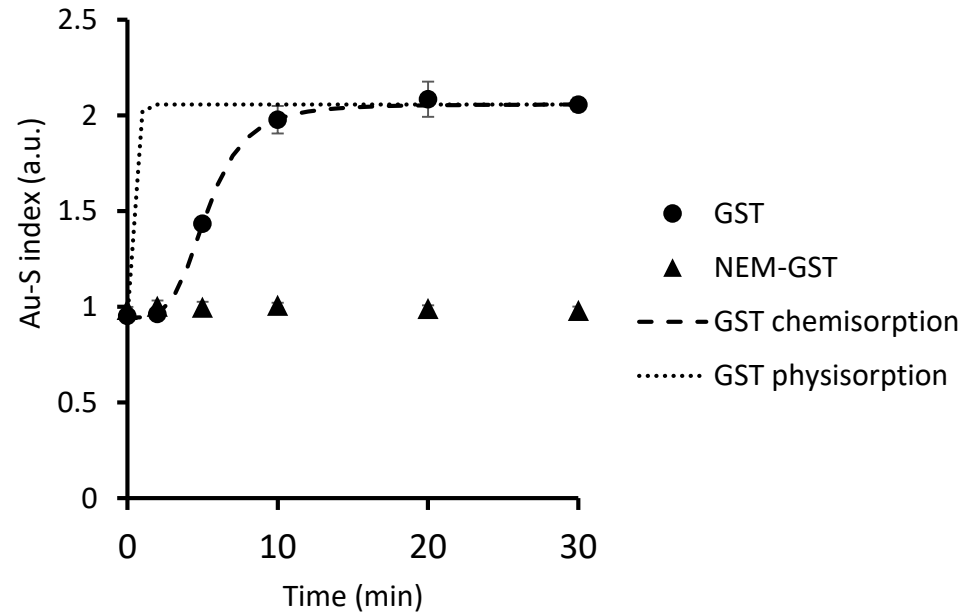
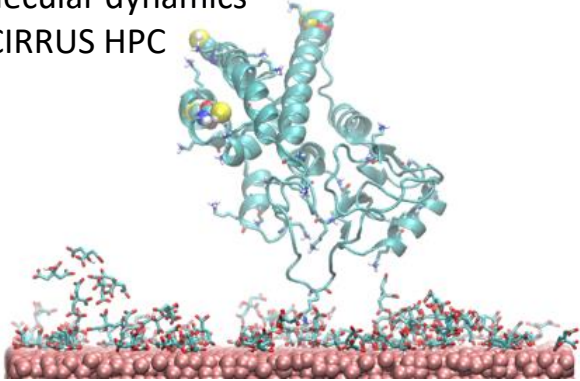
Mechanism of binding of GST to GNPs

Molecular dynamics
@ CIRRUS HPC

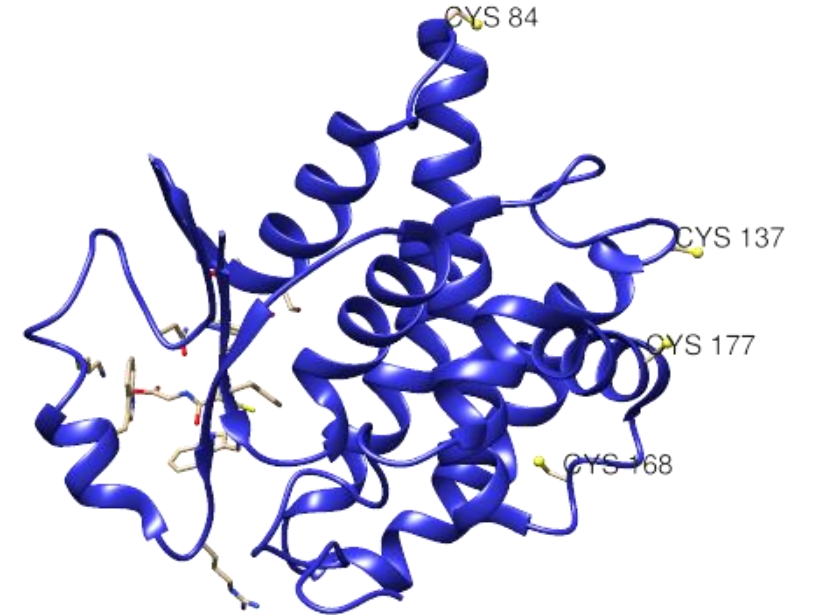
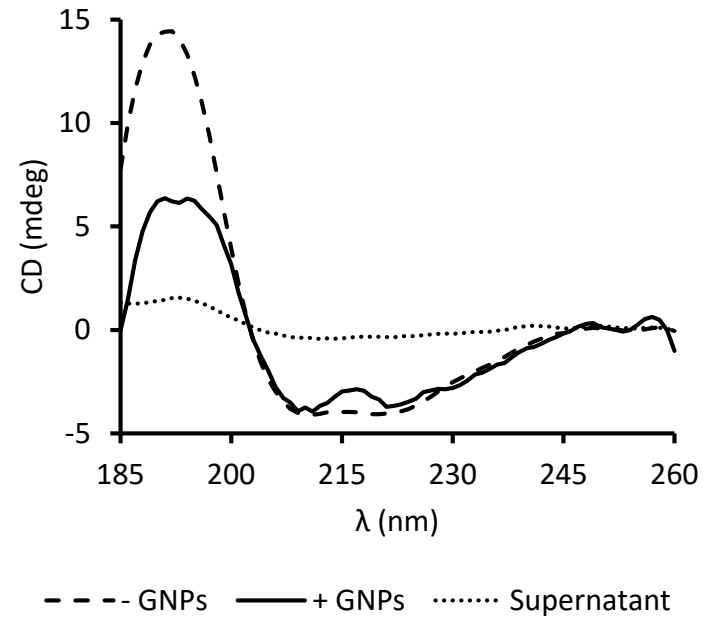


Mechanism of binding of GST to GNPs

Molecular dynamics
@ CIRRUS HPC



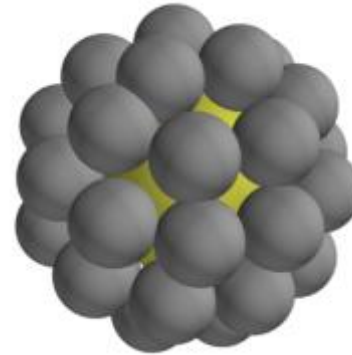
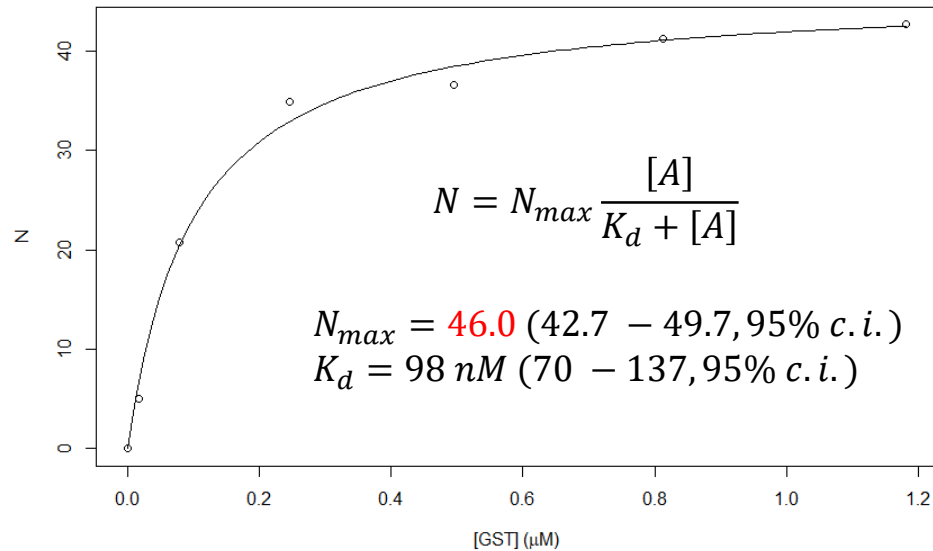
Mechanism of binding of GST to GNPs



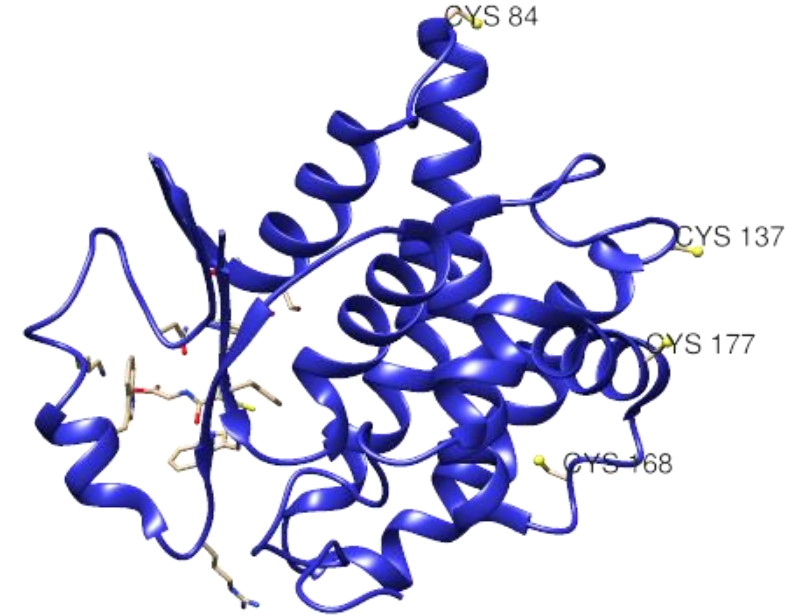
Synchrotron Radiation Circular Dichroism
B23 beamline of Diamond Light Source

Mechanism of binding of GST to GNPs

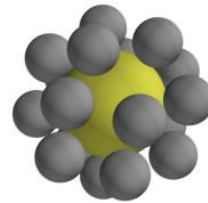
GST + 10 nm AuNP



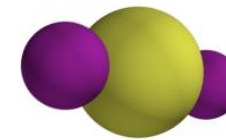
$N = 48$



Retention after extensive washing:

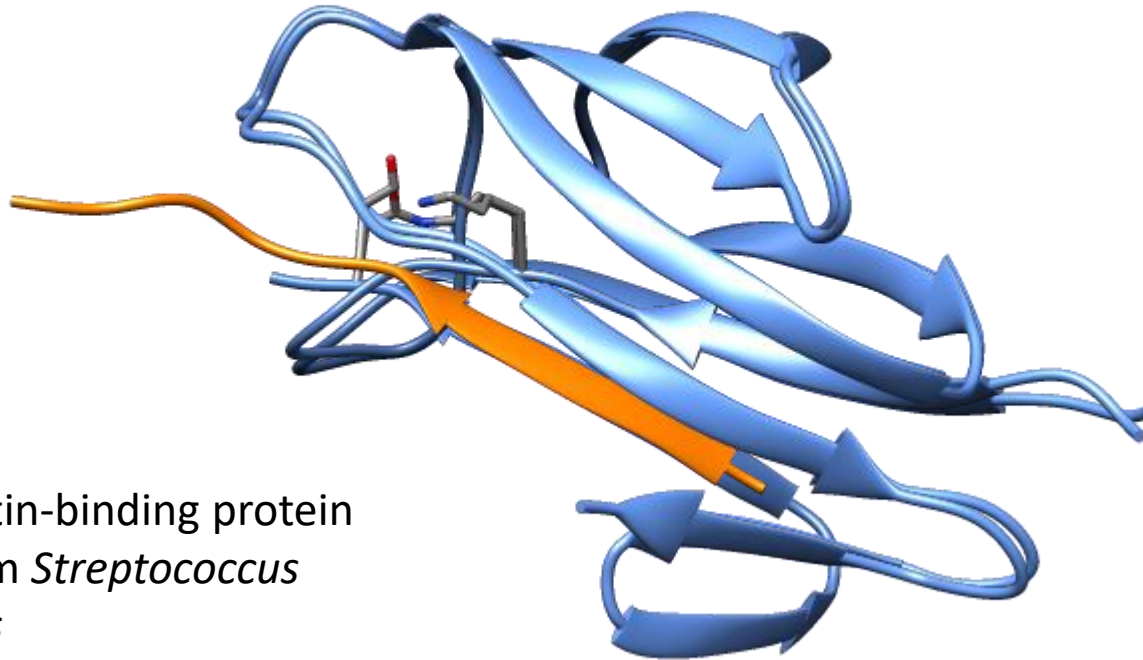


GST ~50%



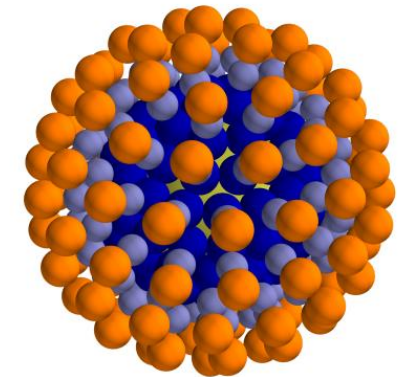
BSA ~10%

Protein adaptor: SpyCatcher/SpyTag



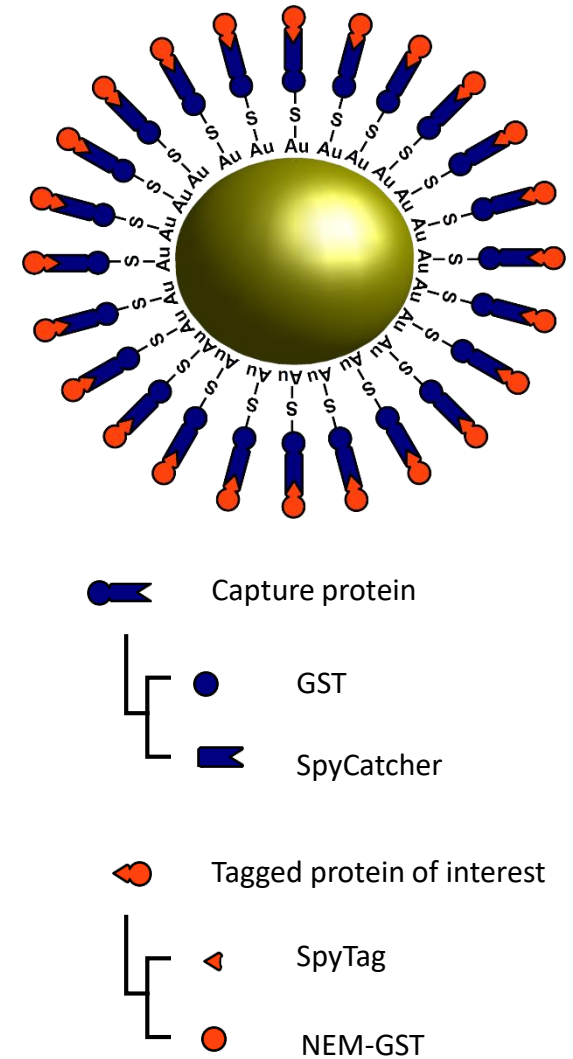
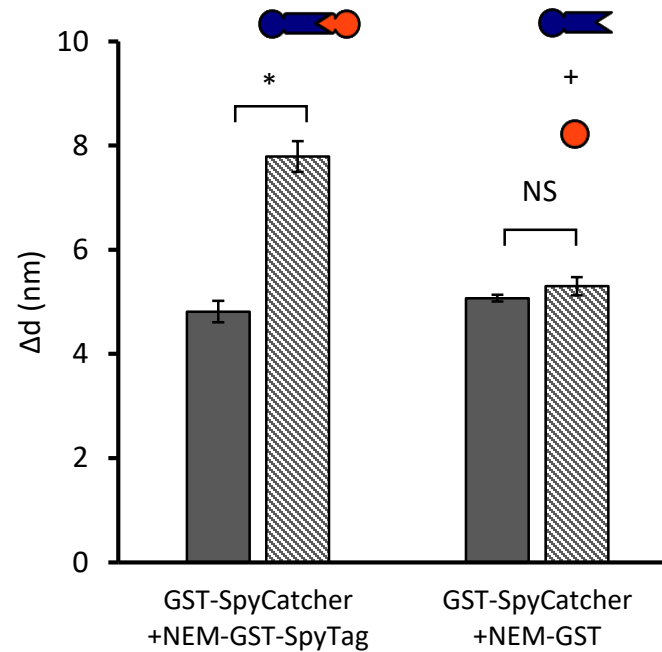
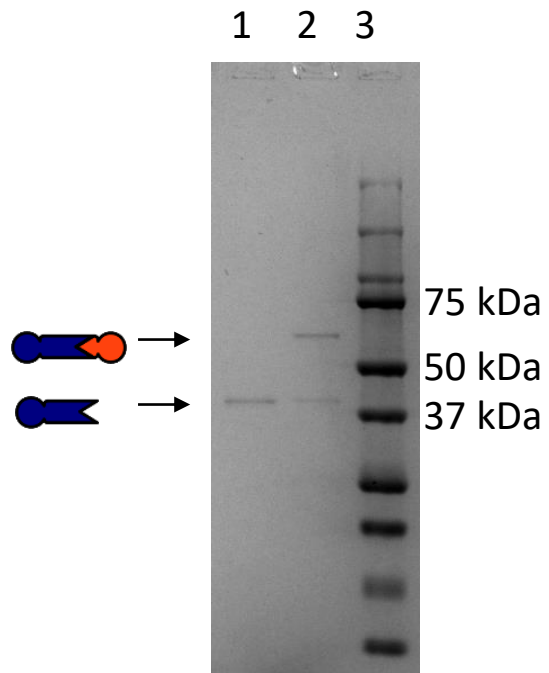
Fibronectin-binding protein
FbaB from *Streptococcus
pyogenes*

NH₂-AHIVMVDAYKPTK-COOH



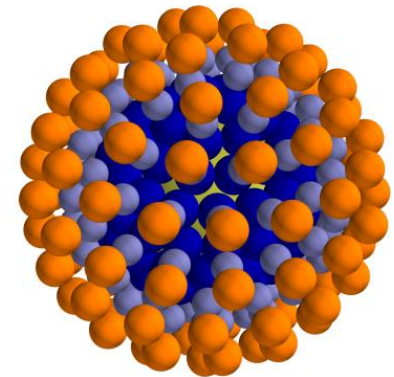
B. Zakeri, J.O. Fierer, E. Celik, E.C. Chittock, U. Schwarz-Linek, V.T. Moy, M. Howarth, "Peptide tag forming a rapid covalent bond to a protein, through engineering a bacterial adhesin.," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 109, no. 12, pp. E690–E697, 2012.

Protein adaptor: SpyCatcher/SpyTag



Summary

- GST identified as an excellent gold-binding protein
- SpyCatcher/SpyTag provides a convenient adaptor
- The protein layers assemble spontaneously by simple mixing in mild conditions
- All covalent bonds (Au-S and iso-peptidic)
- The “USB” plug is the 13 aa peptide SpyTag: it is possible to plug in many different “devices”
- The approach conforms to the “**plug-n-play**” paradigm of synthetic biology
- The ability to engineer the particle at the interface with biological fluids might be critical to determine the **fate of nanoparticles** or overcome **biological barriers**



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- Lee-Anne McCarthy

Undergrad students

- Matthew Jewkes

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- Beatrice Morandotti

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 - Mark Baron (University of Lincoln)
 - Mohammad Alkaseem (University of Lincoln)
 - Mikhail Soloviev (Royal Holloway University of London)
- Enrico Ferrari



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W. Ma, A. Saccardo, D. Roccatano, D. Aboagye-Mensah, M. Alkaseem, M. Jewkes, F. Di Nezza, M. Baron, M. Soloviev, E. Ferrari, "Modular assembly of proteins on nanoparticles," *Nature Communications*, vol. 9, p. 1489, 2018.