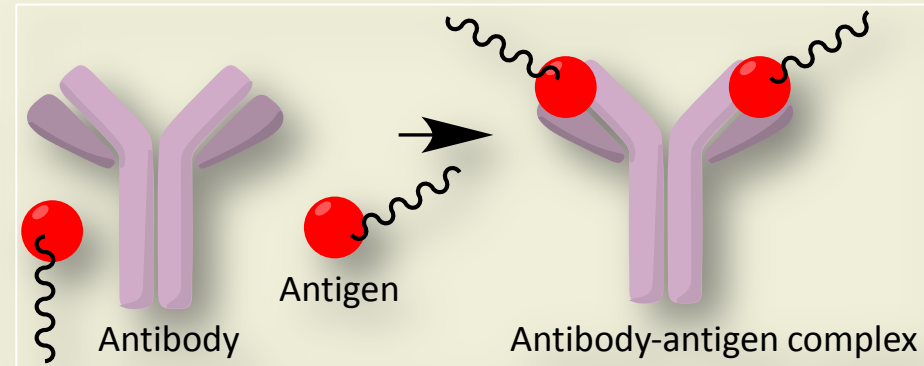
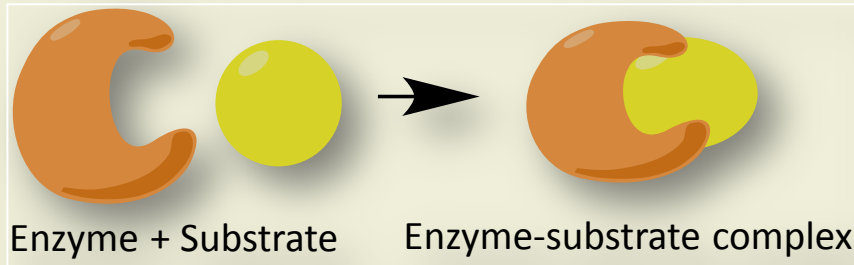


Protein-functionalised Gold Nanoparticles

Shin Yiing Lim & Matthew Gibson

Introduction

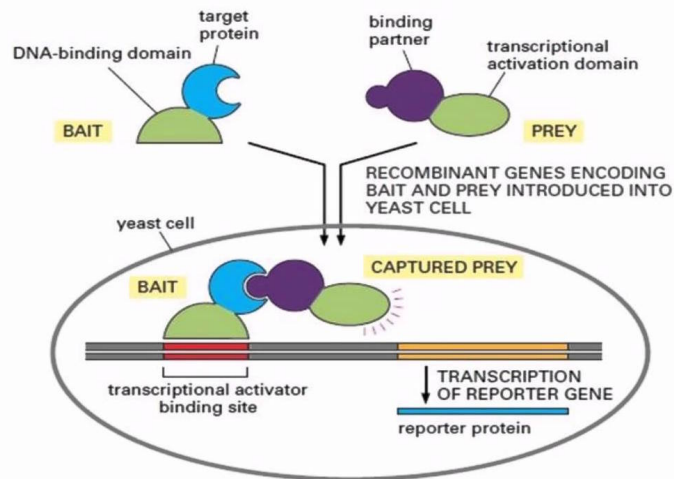
- Protein-mediated interactions are one of the main interaction in biological systems.



- Examples: enzyme-substrate relationship, antibody-antigen relationship

Monitoring Protein-mediated Interactions

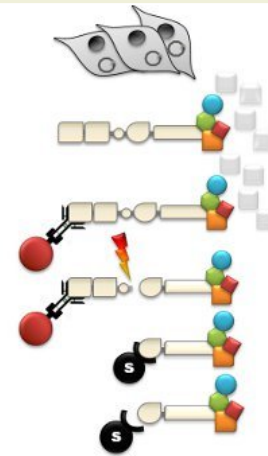
- Protein-protein interactions as therapeutic targets
- Traditional methods in monitoring protein-protein interactions



Yeast Two Hybrid System

www.technologysciences.com

Yeast two hybrid



HEK293 pMEP4 TAP/bait fusion TEV protease

Rabbit IgG agarose Interaction partners Streptavidin beads

Tandem affinity purification

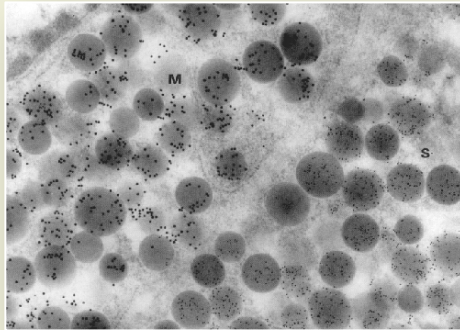
Nanotechnology in Monitoring Molecular Interactions

Bioimaging
and
visualisation

Diagnostics

Targeted
therapy

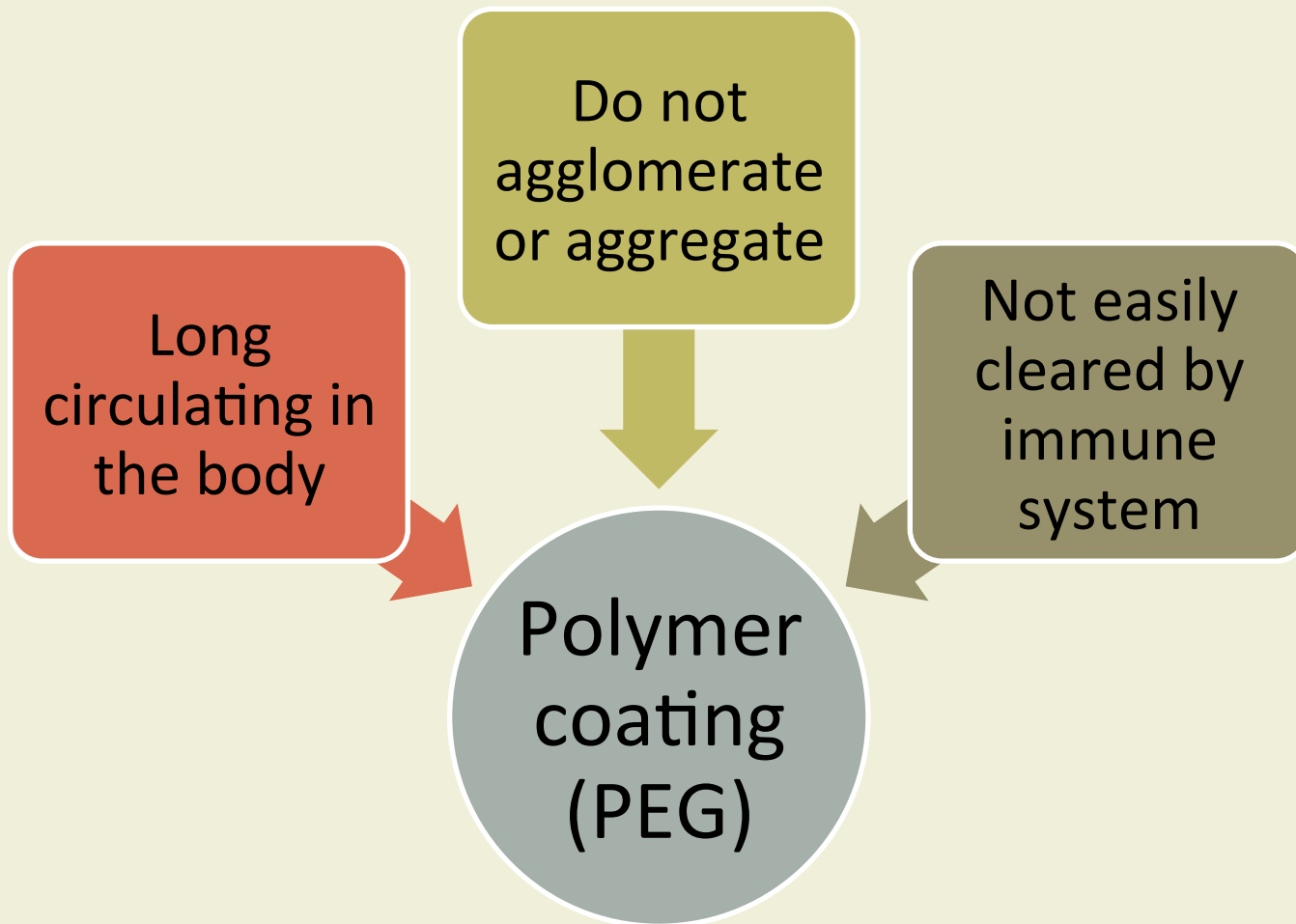
Therapeutic
agent/Drug
delivery



<https://www.emsdiasum.com/microscopy/products/immunogold/micrograph.aspx>



Challenge for Nanotechnology

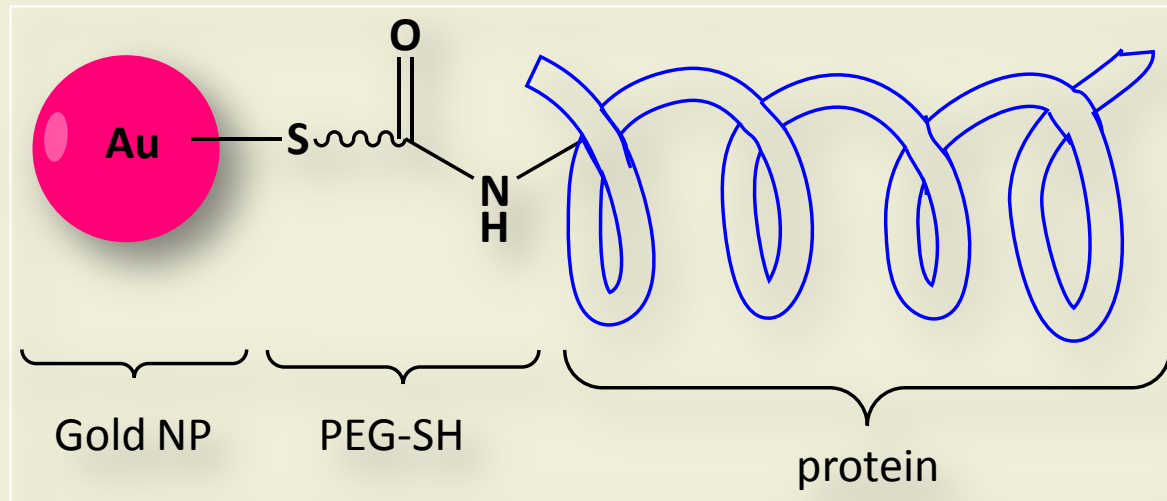


Poly (ethylene glycol) gives particles a
“stealth” character!¹

¹D. E. Owens III and N. A. Peppas, *Int. J. Pharm.*, 2006, **307**, 93 – 102.

Aim

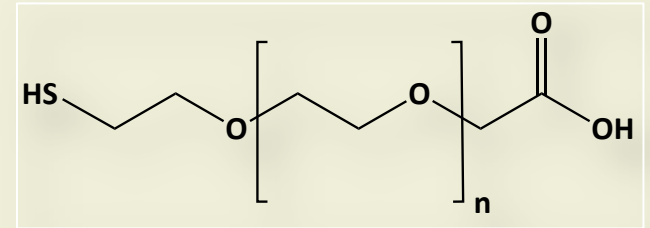
- To develop a strategy to attach proteins onto polymer coated gold NP



- To use them to monitor biological interactions

Experimental Method

- Use of 40nm gold colloid
- Attach bifunctional PEG with a thiol and carboxylic acid group
- Test for saline stability
- Activation using EDC/NHS
- Attachment with proteins
- Treatment with glycosylated surfaces



PEG-2-mercaptoethyl ether acetic acid

Results

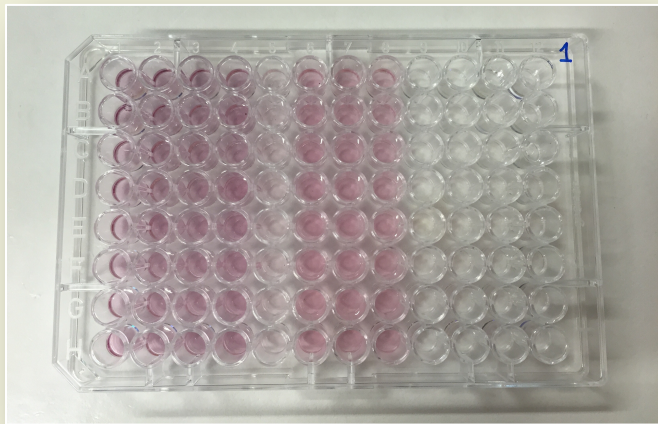
PEGylation of 40nm gold NP

DLS measurement

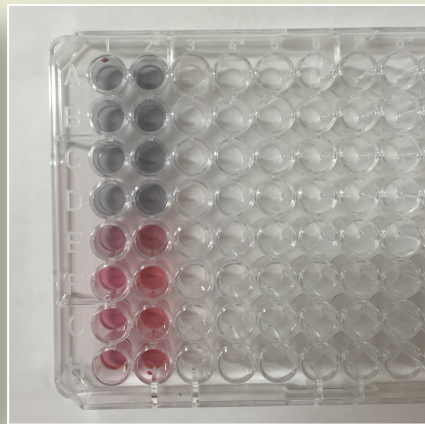
Particle	Hydrodynamic diameter (nm)
Gold	44.8 ± 0.4
PEGylated gold	55.4 ± 0.1

Table 1 Hydrodynamic diameter measurements of particles from DLS

Saline stability test



(a)



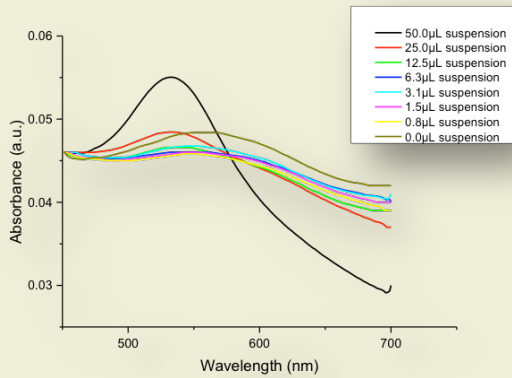
(b)

Figure 1 Saline stability test with (a) PEGylated gold and (b) normal gold colloid in decreasing saline concentration

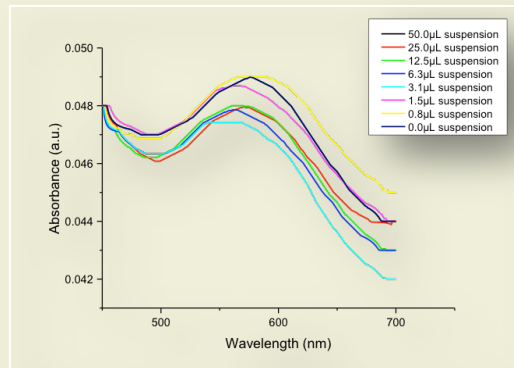
Results

Attachment of proteins onto PEGylated gold NP

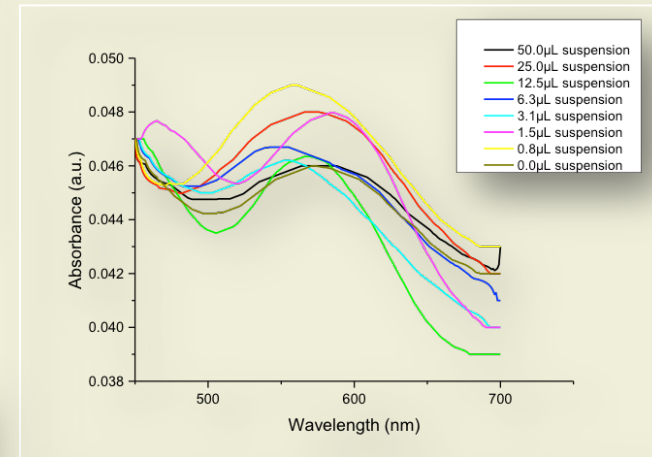
Galactose Surface



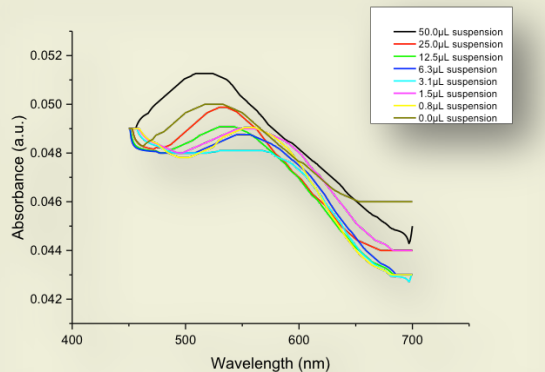
Graph 1.1 PNA-particle conjugate



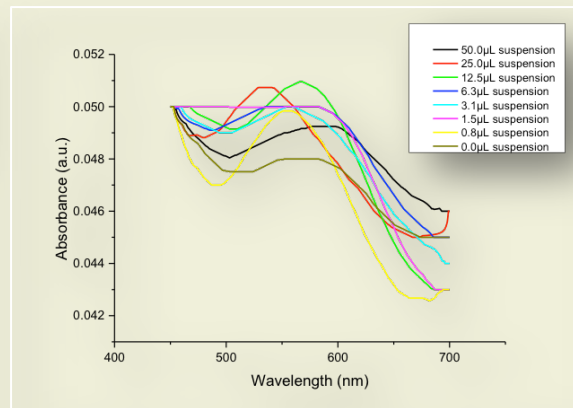
Graph 1.2 BSA-particle conjugate



Graph 1.5 PEGylated gold particle



Graph 1.3 DBA-particle conjugate



Graph 1.4 Con A-particle conjugate

Results

Attachment of proteins onto PEGylated gold NP

Galactose Surface

Surface	Percentage coverage of droplet (%)
Unfunctionalised surface	30.5 ± 2.4
Galactose-functionalised	40.1 ± 1.6
PNA-gold	55.5 ± 6.6
BSA-gold	52.7 ± 1.2
DBA-gold	55.2 ± 6.4
Con A-gold	45.0 ± 4.9
PEGylated gold	39.8 ± 1.5

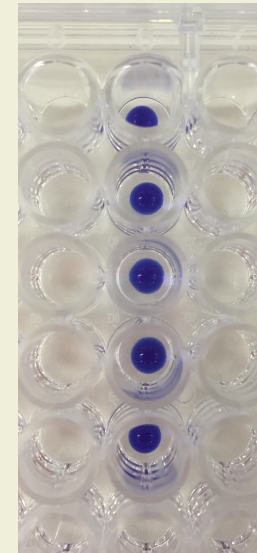


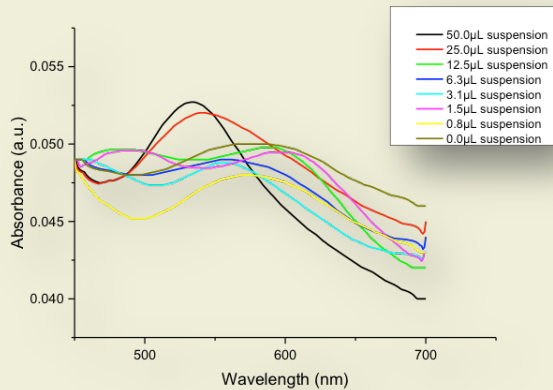
Figure 2 Modified drop shape analysis

Table 2 Modified drop shape analysis results on galactose surface

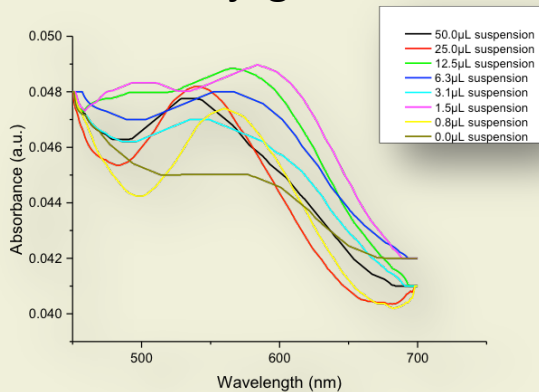
Results

Attachment of proteins onto PEGylated gold NP

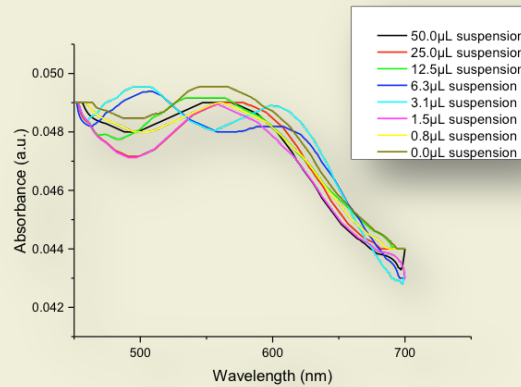
Mannose Surface



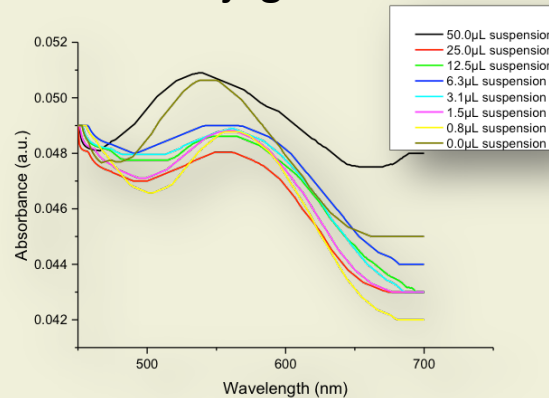
Graph 2.1 PNA-particle conjugate



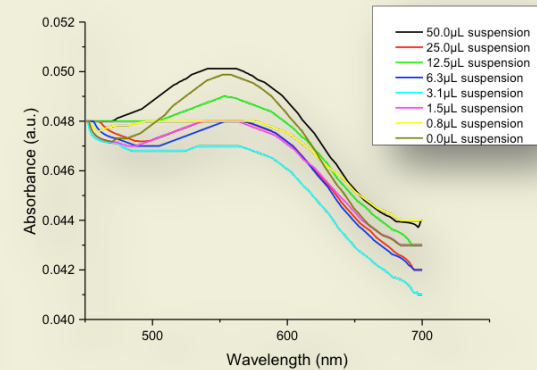
Graph 2.3 DBA-particle conjugate



Graph 2.2 BSA-particle conjugate



Graph 2.4 Con A-particle conjugate



Graph 2.5 PEGylated gold particle

Results

Attachment of proteins onto PEGylated gold NP

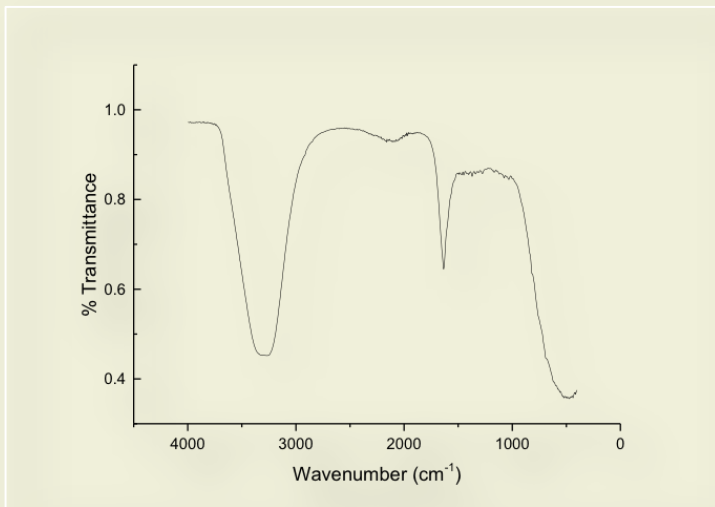
Mannose Surface

Surface	Percentage coverage of droplet (%)
Unfunctionalised surface	30.5 ± 2.4
Mannose-functionalised	40.7 ± 2.1
PNA-gold	43.3 ± 2.5
BSA-gold	51.8 ± 2.9
DBA-gold	54.3 ± 7.5
Con A-gold	49.3 ± 3.8
PEGylated gold	39.1 ± 1.7

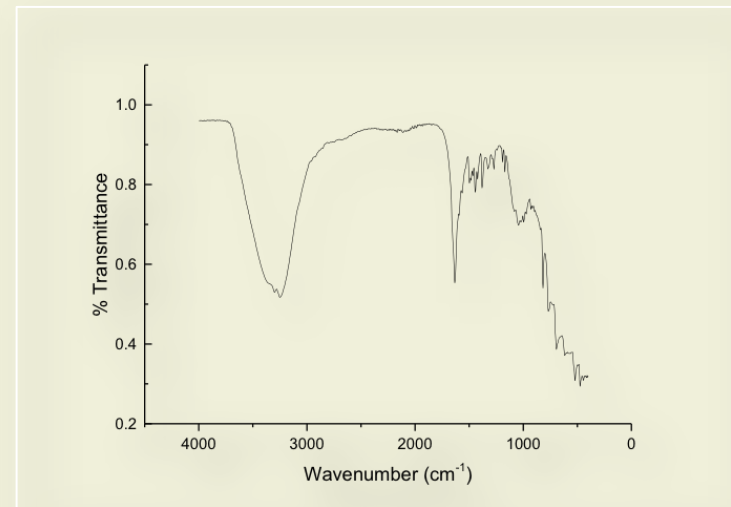
Table 3 Modified drop shape analysis results on mannose surface

Challenges

- How to confirm the conjugation of protein onto gold particle?



IR spectra of PNA-gold conjugate



IR spectra of DBA-gold conjugate

- Uncontrolled loading of protein onto gold particle

Next Steps

- DLS on conjugated samples
- XPS analysis