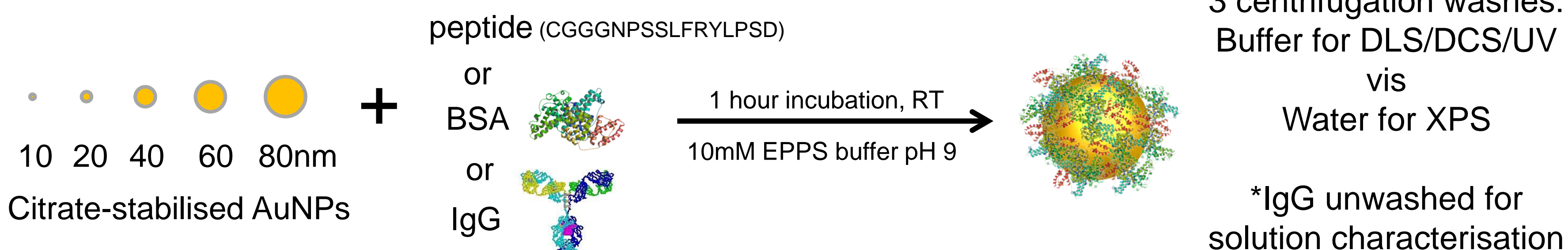


# Shell thickness determination of core-shell nanoparticles

## Introduction

The attachment of proteins to nanoparticles is of increasing interest in medicine for applications such as drug delivery and diagnostics, and for understanding how performance and potential toxicity are affected by the unintentional acquisition of a protein corona from biological mediums. All of the above require a thorough characterisation of protein layers on nanoparticles with both accuracy and precision for efficacy and safety. Our efforts are focussed upon developing measurement techniques to enable useful characterisation using model systems.

## Preparation of model NP systems

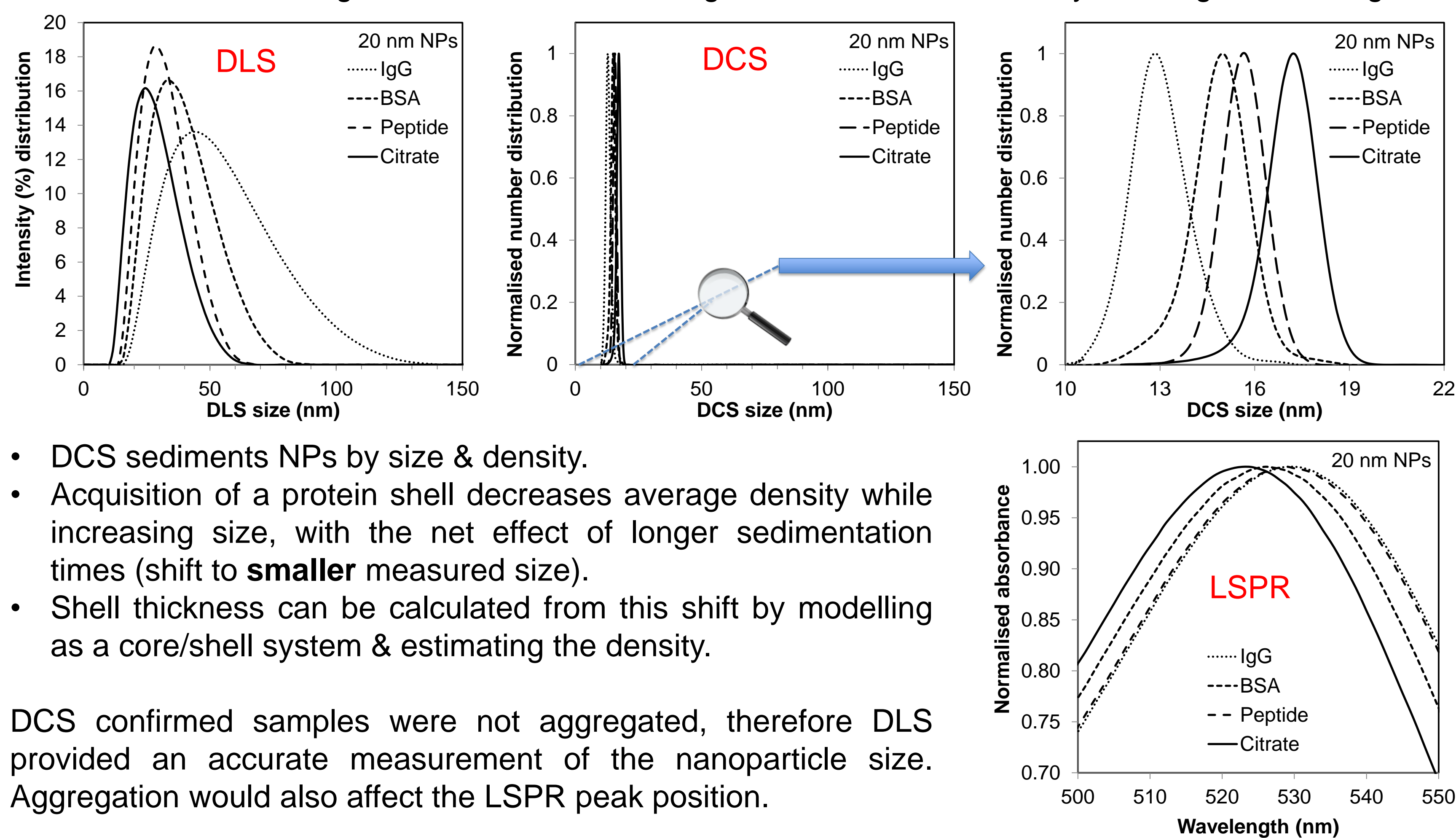


## Why?

- Different techniques provide complementary information.
- Not all NPs are suitable for characterisation by all techniques.
- Vacuum techniques can be expensive; there is a need for validation of reliable, cost effective and high throughput techniques.

## Solution-based techniques: DLS, DCS & UV vis (LSPR)

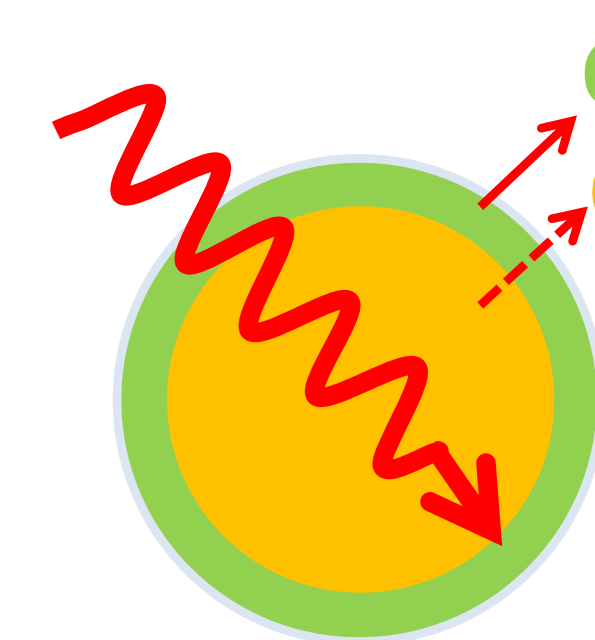
Differential centrifugal sedimentation offers higher size resolution than dynamic light scattering:



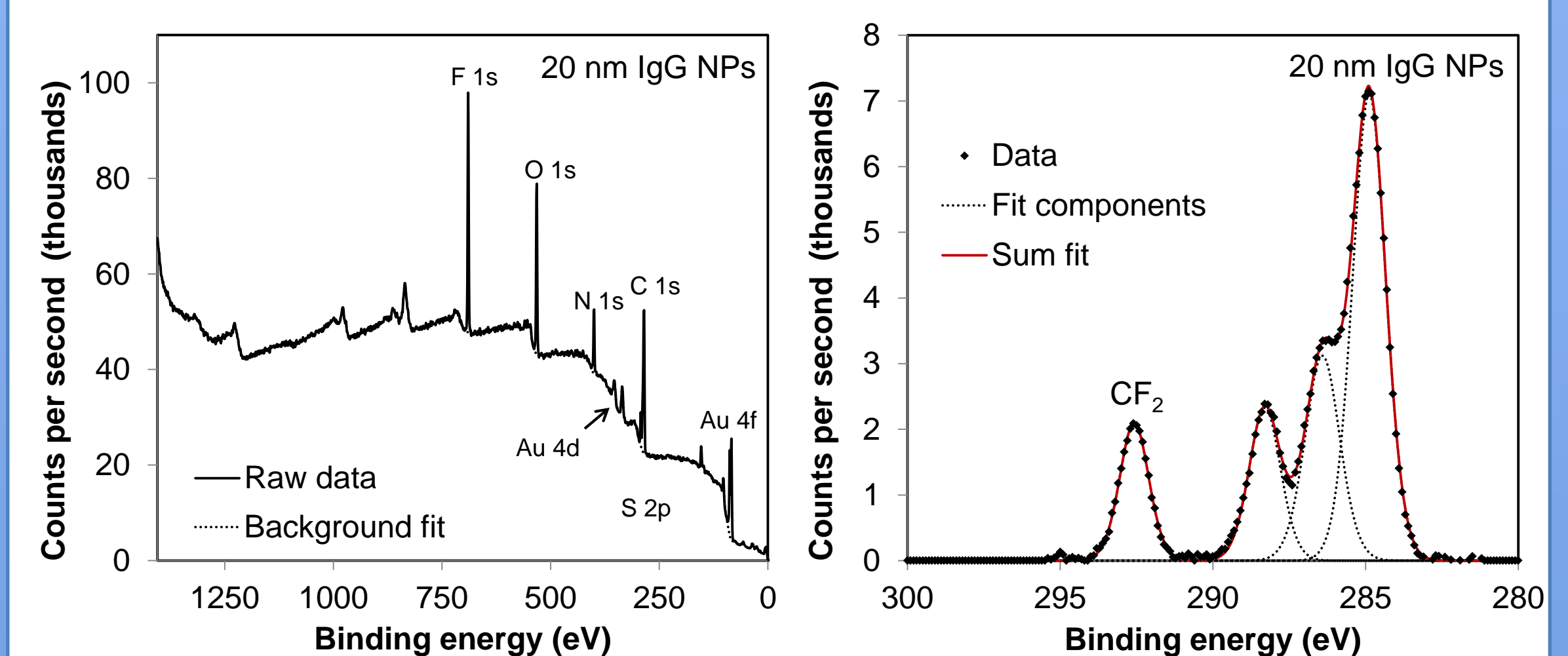
- DCS sediments NPs by size & density.
- Acquisition of a protein shell decreases average density while increasing size, with the net effect of longer sedimentation times (shift to **smaller** measured size).
- Shell thickness can be calculated from this shift by modelling as a core/shell system & estimating the density.

DCS confirmed samples were not aggregated, therefore DLS provided an accurate measurement of the nanoparticle size. Aggregation would also affect the LSPR peak position.

## Determination of shell thickness by XPS



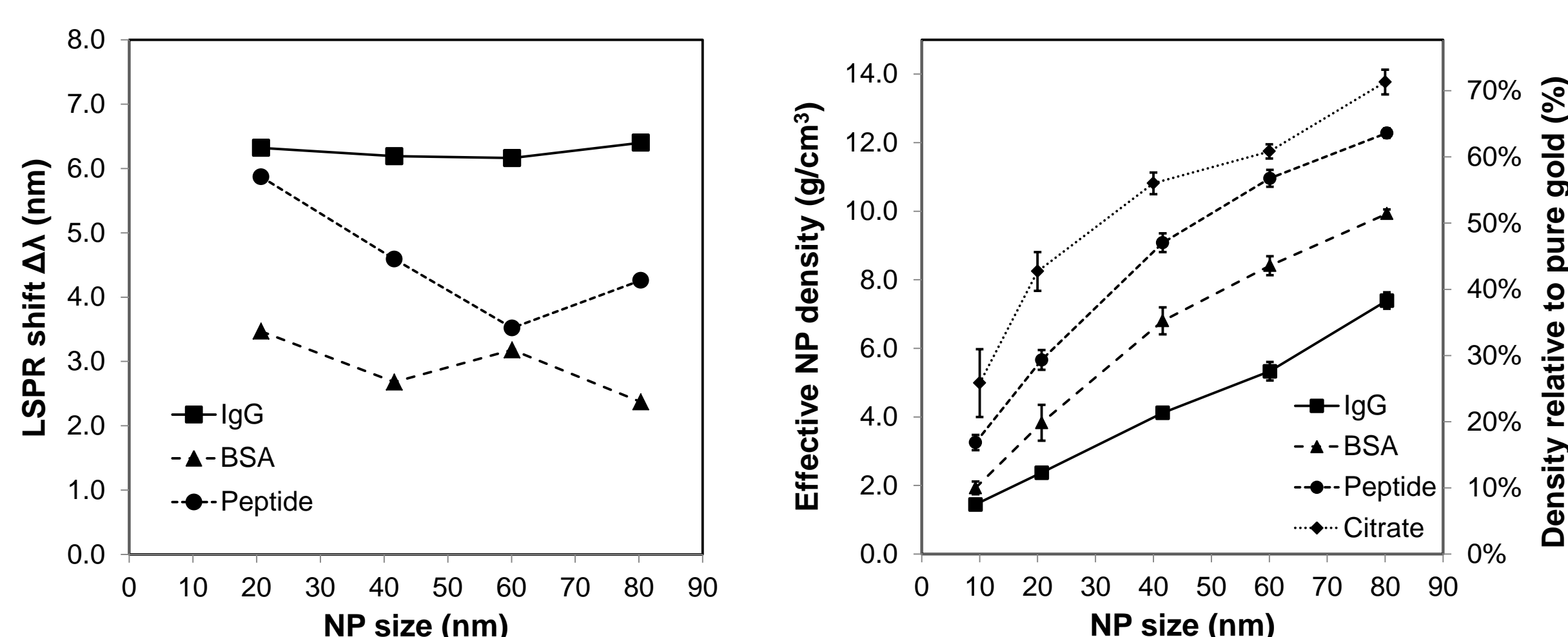
- Samples on PTFE-wrapped Si-wafer.
- Shell thickness calculated based on the attenuation of Au electrons with increasing protein thickness and on geometry of the sample.<sup>2</sup>
- CF<sub>2</sub> signal from the PTFE substrate easily separated due to large energy shift.



XPS is performed in ultra-high vacuum, therefore reports on the dehydrated shell thickness.

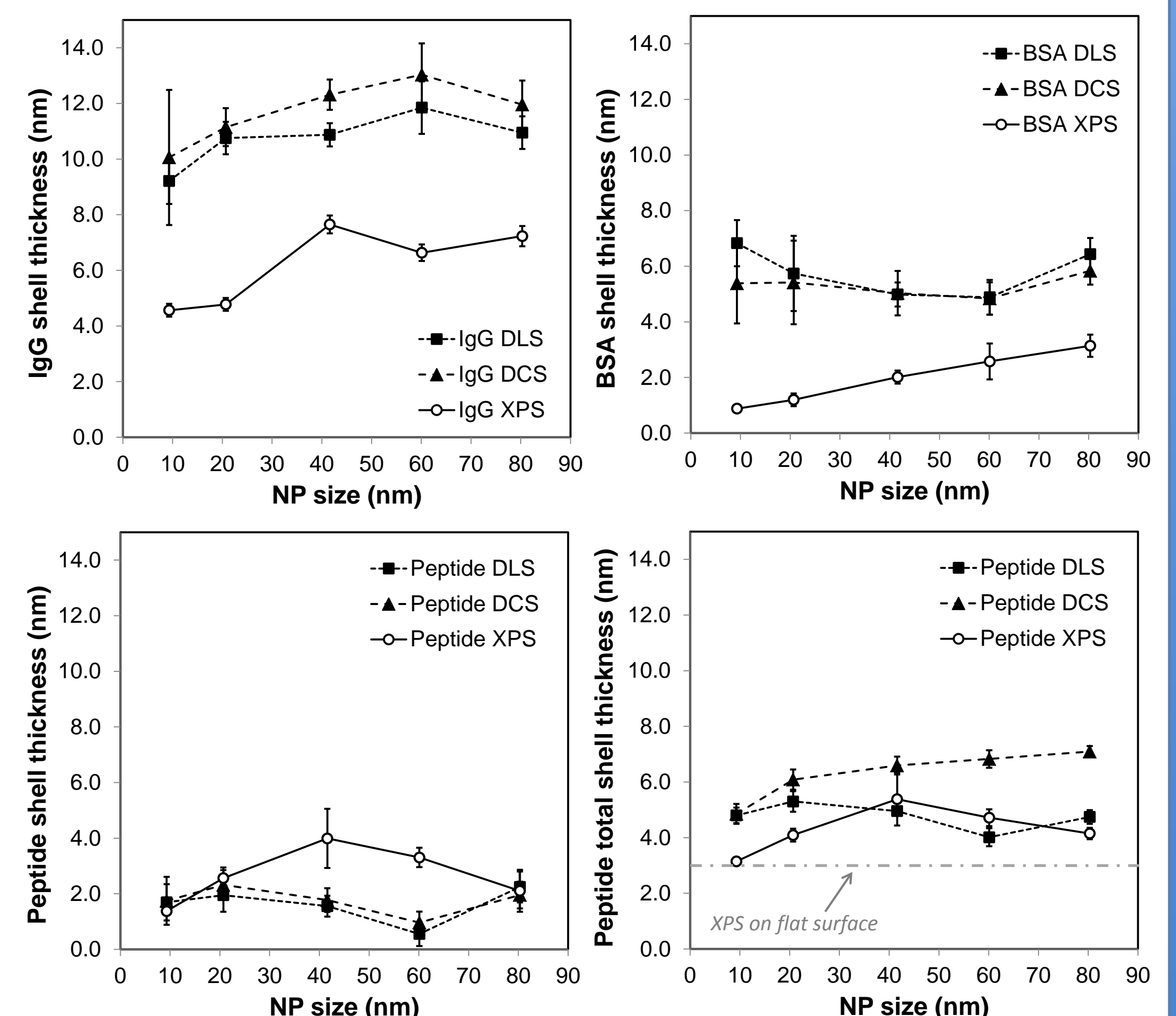
Protein	Molecular mass	Average thickness (nm)*	Average n <sub>s</sub>	Average water % (v/v) (UV-Vis & DLS)	Average water % (v/v) (XPS & DLS)
IgG	160k	11.1 ± 1.0 (14.3 ± 1.1)	1.45	51%	50%
BSA	66.5k	5.5 ± 0.9 (8.7 ± 0.7)	1.42	63%	64%
peptide	1.7k	1.6 ± 0.6 (4.8 ± 0.5)	1.48	38%	30%

\* Values in brackets:  $T = \frac{1}{2}(D_{DLS} - D_{TEM})$



## Analysis

- Lower thickness calculated from XPS data reflects the loss of water under vacuum.
- Thicknesses are in-line with a monolayer formation.
- BSA and IgG most likely adsorb on top of the citrate shell. The peptide binds via a terminal cysteine, so would more likely displace the citrate.
- XPS data (right) show thicknesses less the measured thickness of the citrate shell; except the bottom right, following the assumption that the peptide displaces the citrate.



## Conclusions

- XPS is an excellent technique to measure not only elemental composition, but also shell thickness.
- XPS measurements were in good agreement with solution based-measurements.
- Complementary techniques enabled cross-validation & information 'jigsaw'.
- DLS accurately measures hydrodynamic radius but lacks resolution to detect small aggregates.
- DCS is a very valuable tool to verify monodispersity; even doublets are clearly identifiable.

## References

1. Bell, N.C; Minelli, C; Shard, A.G. Analytical Methods, 2013, 5, 4591.
2. Shard, A.G. Journal of Physical Chemistry C, 2012, 116, 16806.
3. Minelli, C; Garcia-Diez, R; Sikora, A.E; Gollwitzer, C; Krumrey, M; Shard, A.G. Surface and Interface Analysis, 2014.
4. Bell, N.C; Minelli, C; Tompkins, J; Stevens, M.M; Shard, A.G. Langmuir, 2012, 28, 10860.

## Acknowledgement: