Preparation of Nanoparticle-Protein Conjugates – Coupling

To produce NP-protein conjugates with a protein of interest, here Bet v 1 (major birch pollen allergen, recombinant), NPs and protein in either 10% or 20% protein-NP ratio were coupled in 500 µL of either 10 mM citrate buffer pH 4, sodium phosphate buffer pH 7.4 or Tris buffer pH 9 for 16 hours on a rotational wheel at 4°C.

Protein Quantification – Determining the Binding Capacity of SiO_2 NPs

To determine the amount of protein bound to the NPs after the coupling reaction, the samples were centrifuged twice for 1 hour, 16 000 *g* at 4°C. This two-step collection of the supernatant diminishes contamination risk of the supernatant with parts of the pellet. The resulting pellet was dissolved in dH_2O and the protein content of the pellet (bound protein) and supernatants (unbound protein) was quantified by using the BCA (bicinchoninic acid) protein assay. The binding capacity in weight percent was calculated using following equation:

$$\frac{\text{Concentration of Bound Protein x 100}}{\text{Applied NP Concentration}} = \text{Binding Capacity in wt\%}$$

BCA (Bicinchoninic Acid) Protein Assay

This protein assay is based on the biuret reaction, which is the reduction of cupric ions (Cu^{2+}) to cuprous ions (Cu^{1+}) in the presence of peptide bonds, followed by the chelation of one cuprous ion with two BCA molecules. This results in an intense purple complex with its peak absorbance at 562 nm [1].

The assay was performed according to the microplate procedure protocol provided by the manufacturer (Pierce™ BCA Protein Assay Kit) [2]. 40 µL sample were pipetted into a microplate well followed by addition of 200 µL BCA working reagent (50:1 BCA Reagent A and Reagent B). A serial dilution of known protein concentrations of the protein of interest was added to the plate in duplicates as standard curve, and treated likewise the samples. The 96-well plate was then sealed and put on a shaker for 30 sec. to ensure sufficient mixing of the samples and the working reagent, followed by a 30 min incubation at 60°C, according to the enhanced protocol for samples in the expected low working range of 5-250 µg/mL protein. After the plate was cooled down to RT, the absorbance was measured at 562 nm with a microplate reader (Tecan M200pro).

References:

1. Smith, P.e., et al., *Measurement of protein using bicinchoninic acid.* Analytical biochemistry, 1985. **150**(1): p. 76-85.
2. Walker, J.M., *The bicinchoninic acid (BCA) assay for protein quantitation*, in *The protein protocols handbook*. 2009, Springer. p. 11-15.