Preparation of Nanoparticle-Protein Conjugates

To produce NP-protein conjugates, NPs and protein in either 10% or 20% protein-NP ratio were coupled in 500 µL citrate buffer (10 mM, pH 4), sodium phosphate buffer (10 mM, pH 7.4), Tris buffer (10 mM, pH 9) or dH_2O for 16 hours on a rotational wheel at 4°C.

For couplings, the proteins Lysozyme (from hen egg white, lyophilized powder, Sigma-Aldrich), Ovalbumin (from hen egg white, lyophilized powder, Sigma-Aldrich), Beta-lactoglobulin a (from bovine milk, lyophilized powder, Sigma-Aldrich), Serotransferrin (apo-transferrin human powder, Sigma-Aldrich), birch pollen extract (Betula Pendula, ThermoFisher Scientific) and recombinant birch pollen allergen Bet v 1, were used.

For coupling of an artificial protein mix with pristine and APTES-coated SiO_2 NPs, the proteins Lysozyme, Ovalbumin, Beta-lactoglobulin a, and Serotransferrin were mixed. For coupling of birch pollen extract (Betula Pendula, ThermoFisher Scientific) with pristine and APTES-coated SiO_2 NPs, 100 mg of the birch pollen extract (BPE) were dissolved in 1 mL pure water for 1 hour on a sample shaker at 700 rpm. The BPE was then centrifuged for 10 min at 16 000 *g* and the supernatant was collected and used for the couplings in 1:2 dilution.

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 SDS-PAGE by comparing the samples quantitatively with control protein solutions without NPs, treated likewise the coupling samples.

SDS-PAGE (Sodium Dodecylsulfate-Polyacrylamide Gel Electrophoresis)

To determine the molecular weight of the applied proteins, the samples were compared to a molecular weight marker (Pierce™ Unstained Protein MW Marker, Thermo Scientific™) and to determine the amount of protein in the sample, control samples with known protein concentration were added to the gel for quantitative comparison.

To prepare four 15% polyacrylamide gels, 10 mL Acrylamide/Bis solution (Rotiphorese NF-Acrylamid/Bis-Solution 30% 29:1, Carl Roth) was added to 5 mL dH_2O and 5 mL lower buffer (1.5 M Tris-HCl, 0.4% SDS, pH 8.8), followed by 10 µL TEMED (Bio-Rad) and 60 µL 10% Ammonium persulfate (APS) and the pouring of the resulting solution in glass plates adjusted in the casting chambers. After addition of dH_2O to exclude oxygen, which disturbs the polymerization, the separating gel can stably polymerize. The stacking gel could then be prepared by adding 0.7 mL 30% Acrylamide/Bis solution to 2.6 mL dH_2O and 1.1 mL upper buffer (0.5 M Tris-HCl, 0.4% SDS, pH 6.8), followed by 7 µL TEMED and 50 µL APS. The

solution was poured on top of the separating gel and a 10-slot comb was added to exclude oxygen for polymerization and form the sample wells.
The investigated protein samples and control samples were mixed with 4x loading buffer (250 mM Tris, 8% SDS, 40% glycerol, 10% ß-mercaptoethanol, 0.04% Bromphenol blue) to dilute it to 1x and put in a reaction tube heating block at 95°C, 10 min for heat denaturation. 15 µL of the samples were loaded into the slots of the prepared gels, mounted in the electrophoresis chamber filled with 1x SDS running buffer (250 mM Tris, 1.92 M glycine, 1% SDS, pH 8.3) and constant current of 0.06 A per gel was applied for ca. 30 min until the samples were separated completely in the gel. The gels were then stained with Coomassie R250 Staining solution (0.1% Coomassie Brilliant blue R250, 50% methanol, 10% acetic acid) and imaged with the ChemiDoc Imaging system (ChemiDoc MP, Bio-Rad) and ImageLab™ (Version 6.0.1 build 34, Bio-Rad) was used for analysis to calculate the relative quantity of protein in the samples compared to the protein quantity in the control sample.

For analysis of the SDS-PAGE results of the NP-BPE conjugates, the five most prominent bands of the Gels were chosen for quantitative comparisons. They were assigned to the main birch pollen allergens according to their molecular weight: band 1 corresponds to Bet v 6 (MW 34.2 kDa), band 4 to Bet v 1 (MW 17.4 kDa) and band 5 to Bet v 2 (MW 14.1 kDa).