Final project report
(KH 129578)

The aim of the project was to investigate how a single layer of polyethylene glycol (PEG) performs in a colloidal system as steric repulsion layer. This steric repulsion originating from the limited compressibility of polymer chains located at the surface of nanoscale objects is of crucial importance in various fields (biomedical application, food industry, etc.) as it can prevent clogging or aggregation of nanoscale object and macromolecules in a complex environment. In this regard PEG is of central importance as it is one of the most used material due to its biocompatibility and can provide nanoscale objects a "bio-stealth" capability.

The main concept of the proposed research was to investigate the heteroaggregation of two different nanoparticle types. One of them has charged PEG grafted on its surface, while the other displays only permanent charges on the surface. By combining these two types of particles, the equilibrium distance between them is determined by colloidal interactions. The unique aspect of the project was that in contrast to the usually applied ensemble approach, we targeted to read out this distance in-situ upon dimer formation at the individual dimer level relying on the plasmon ruler effect. As the particles are made out of gold, the interaction between their localised plasmon resonance modes will modify their optical scattering spectrum and this change is very sensitive to the separation distance. By the controlled preparation of the dimers and the in-situ investigation at the single dimer level we hoped to gain a deeper insight into the colloid chemical process could be obtained.

As some of the important results of the work could not been published in peer-reviewed journals yet, the section below also shown some measurement data; we restricted ourself, however, to show only essential data.

In agreement with the project workplan, we have successfully prepared the prototypical gold nanoparticle types by seed-mediated wet-chemical synthesis. We used several different nanoparticle batches that had a diameter between 20 and 80 nm, in each case the DLS derived polydispersity index was around 0.1. The nanoparticles' surface modified by various molecules to arrive at the model systems: for the permanent positive surface charged particles MTAB ((11-Mercaptoundecyl)-*N*,*N*,*N*-trimethylammonium bromide) was used, while for the other type pH responsive carboxylated PEG (COOH–PEG–HS) was applied. The electrophoretic mobility measurements confirmed the successful surface modification: for MTAB covered particles a pH independent, large positive zeta-potential was measured (+40 mV), for the PEGylated particles the zeta-potential varied between -36 mV@pH=6 and 0 mV@pH=3. To verify the particles' affinity for heteroaggregation, successful self-assembly has been carried out in the bulk, and the process followed by ensemble optical spectroscopy. As can be seen in Figure 1, at pH values where the PEGylated particles are negatively charged they aggregate with the MTAB coated particles, indicated by the redshift of the plasmon resonance wavelength. By taking samples from these bulk experiments for electron microscopy, the formation of aggregates has been confirmed independently as well.

It has to be mentioned, that as written in the periodic reports, we also experimented with other surface ligands for the negatively charged particles (11-mercaptoundecanoicacid (MUA), mercaptohexanoicacid (MHA), mercaptopropionicacid (MPA), 2-Mercaptoethanesulfonic acid monosodium salt (MESNa), thiolated DNA), but these were not as successful as expected. Nevertheless we obtained nice results on the differences in the ligand exchange procedure using the different thiol molecules [1,2].

As written in the Workplan, we also prepared mixed ligand layers to play around with the coverage of the particles. Using this approach we could successfully prepare heterodiers of

nanorods and spheres. We explored this direction as it allowed us to study the orientational (re)arrangement of the heterodimers due to the complex scattering spectrum of the dimers based on their in-situ measured, polarization resolved scattering spectra. [3] These spectra were, however so complex, that these type of heterodimers were not suitable for the unambiguous determination of the interparticle distances.

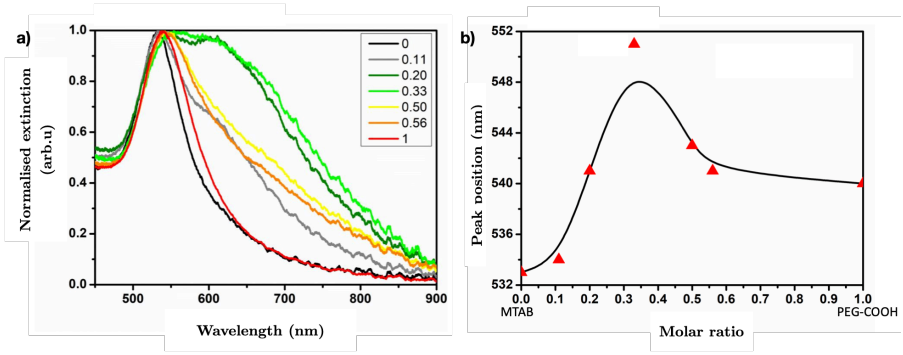

Figure 1: Change of the main resonance peak position during the bulk-assembly by using MTAB and PEG-COOH covered AuNPs at different molar rations (a) and the extracted main peak position (b).

We performed the optical simulations written in the Workplan, by customizing a freely available Toolbox for Matlab (MNPBEM Toolbox). The results of these simulations are optical scattering spectra of the substrate supported nanoparticle dimers. From the simulated spectra the plasmon coupling related scattering peak-position shifts can be extracted and plotted against the interparticle separation (plasmon ruler). This gives the possibility to conclude on the particle separation in a real experiment. The simulations were performed to account for our specific measurement setup (geometry of the illumination and detection cones). As shown below, our simulation results differ significantly from the classical plasmon ruler equation developed in 2007 by Jain et al. (10.1021/nl071008a). In our simulation we can take retardation effects also into account, which explains that even fitting of the ruler equation will fail for large particle sizes. We found that an empirical exponential in the form of $\lambda = a\left(1 - e^{-bx^c}\right)$ can provide a reasonably good fit to enable extraction of separation distance from the optical spectra.

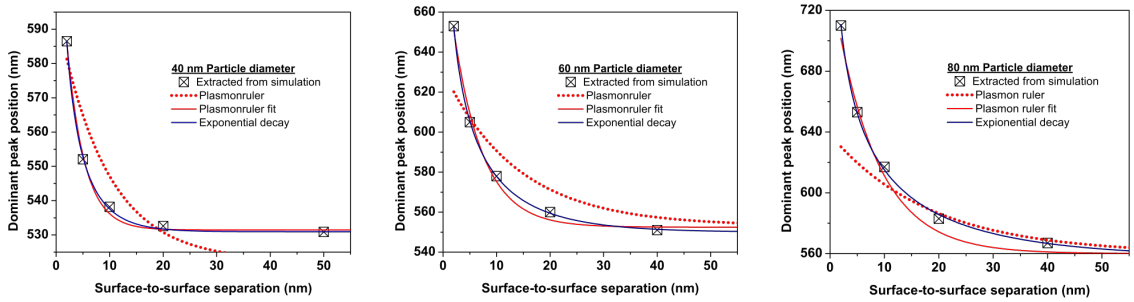

Figure 2: Comparison of the simulation results with the classical plasmon ruler equation from the literature, and empirical fitting of the results.

As the next step we performed the flow cell-based experiment. The aim here was to use the particle types developed earlier (and for which the necessary affinity to each other was already confirmed during the bulk experiments), detect their assembly in the cell at the level of individual heterodimers, and extract the interparticle separation by comparing the experimental and simulated optical spectra. We introduced a commercially available, closed flow cell, that allowed the use of a peristaltic pump for the liquid handling. In this way the experiment could be carried out more conveniently and in a "cleaner" way. The MTAB covered particles have been deposited on the substrate at sufficient low coverage, so that the separation distance between them was bove 10 microns. This facilitated the measurement of individual scatterers. The second, PEGylated

nanoparticle was introduced into the cell and allowed to equilibrate for half an hour to form dimers. We developed a simulation code in Matlab, so that we could check the potential energy landscape in terms of self-assembly driving force and colloidal interactions. This takes not only particle-particle, but also substrate-particle interactions into account. We incorporated the dispersion, electric double layer and the PEG associated steric interactions into the code. An example of the simulation results is given in Figure 3. It shows the total interaction energy in *kT* units for an PEGylated particle approaching from the solution an MTAB covered particle (blue circle) that is located at the liquid/solid interface. For the simulations, actual measurement data was used as an input. This simulation approach was very useful in selecting those systems, where self-assembly in the flow-cell could be expected at all. A big lesson learned was that in a flow cell experiment, the chance for assembly in a given time frame – even if all necessary requirements are met – is much lower in contrast to the bulk. The effective volume, where the attractive interaction dominates and hence provides a chance to "capture" a particles, is rather small (blue half-circle above the particle), and the particles are not as freely exploring the volume as in the bulk.

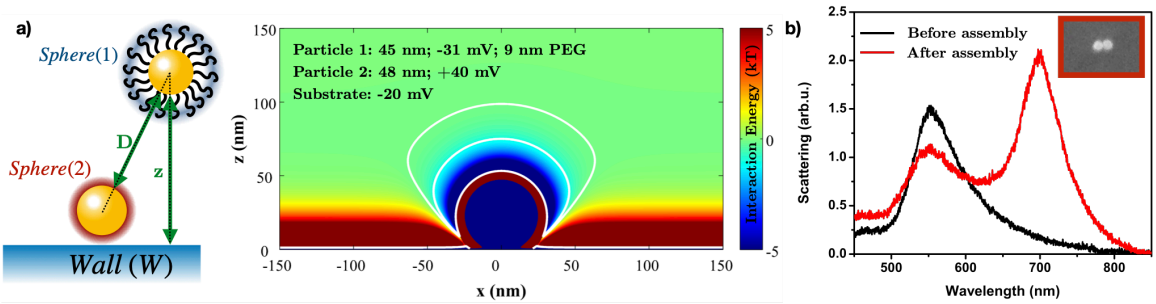

Figure 3: Colloid interaction potential map for a carboxyPEGylated gold particle approaching an MTAB covered nanosphere from the bulk. The blue half-circle above the solid supported particle represents the region where particle trapping can occur (negative interaction potential), while the dark-red aura stems from the steric repulsion of PEG (a). In-situ individual particle spectra of a dimer formation event and the corresponding ex-situ SEM after the assembly (b).

By using MTAB coated and carboxyPEGylated particles, it was possible though to capture the heterodimer formation in situ in the flow cell (Figure 3b). The spectrum of a single nanoparticle is substantially changed by the arrival of a second, PEGylated particle. By comparing the measurement with the on the optical simulation, an equilibrium particle distance of 3.4 nm could be extracted. Later, ex situ electronmicroscopy investigation confirmed the formation of a particle dimers. Other dimer-formation events have been detected successfully as well, but to arrive at a reliable statistics, additional measurements would be needed. With French colleagues (Doru Constantin, CNRS, Orsay) we also undertook combined ensemble SAXS and spectroscopy measurements for the quantification of the interparticle distance of the PEGylated particles. We successfully determined the equilibrium distance between 750 Da PEG covered particles, the measurements for the larger PEGs that are more relevant for the dimer formation are still under way. We have an accepted abstract for the 2021 EMRS Spring Meeting.

Also related to the proposal is a side-project, that evolved naturally over time, but was not explicitly included in the original Workplan. Here we used the gold nanoparticles and PEGylated nanospheres as a support layer for graphene. One paper has been already published [4], while another has just been submitted beginning of April 2021 [5].

The project results were disseminated in peer-reviewed journal and at conferences, seminars. University students were also involved in the work, Rita Némedi from Budapest University of Technology and Economics and Dávid Kovács from ELTE contributed. They wrote BSc theses and work now on their MSc. TDK works also have been prepared. Independently from the fact that the project has to be closed now, we will continue to work on this topic, as we have still very

promising aspects. We learned especially a lot by implementing the solid supported particle colloidal interactions and the bulk assembly experiments.

Papers:

(1) Szekrényes, D. P.; Kovács, D.; Zolnai, Z.; Deák, A. Chemical Interface Damping as an Indicator for Hexadecyltrimethylammonium Bromide Replacement by Short-Chain Thiols on Gold Nanorods. *J. Phys. Chem. C* **2020**, *124* (36), 19736–19742. https://doi.org/10.1021/acs.jpcc.0c04629.
(2) Zámbó, D.; Szekrényes, D. P.; Pothorszky, S.; Nagy, N.; Deák, A. SERS Activity of Reporter-Particle-Loaded Single Plasmonic Nanovoids. *The Journal of Physical Chemistry C* **2018**, *122* (41), 23683–23690. https://doi.org/10.1021/acs.jpcc.8b06716.
(3) Szekrényes, D. P.; Pothorszky, S.; Zámbó, D.; Deák, A. Detecting Spatial Rearrangement of Individual Gold Nanoparticle Heterodimers. *Phys. Chem. Chem. Phys.* **2019**, *21* (19), 10146–10151. https://doi.org/10.1039/C9CP01541H.
(4) Piszter, G.; Kertész, K.; Molnár, G.; Pálinkás, A.; Deák, A.; Osváth, Z. Vapour Sensing Properties of Graphene-Covered Gold Nanoparticles. *Nanoscale Adv.* **2019**, *1* (6), 2408–2415. https://doi.org/10.1039/C9NA00110G.
(5) Zoltán Osváth, Dániel Zámbó, Attila Sulyok, András Pálinkás, and András Deák: Tuning the nanoscale rippling of graphene with PEGylated gold nanoparticles and ion irradiation, submitted to *Carbon*, 2021.

Conferences:

1. Dániel P. Szekrényes, Cyrille Hamon, András Deák, Doru Constantin: Temperature and ionic strength triggered aggregation of PEGylated gold nanospheres, Submitted to symposium K : Advanced small-scale mechanical characterization: strength, plasticity, fracture and fatigue of the E-MRS 2021 Spring Meeting, which will be held at in a virtual format from May 31 to June 4, 2021.
2. András Deák: Felületmódosított arany nanorészecskék önszerveződése és optikai tulajdonságai, EK-MFA Seminar/MTA Colloid Chemistry Workgroup Meeting, 2021.03.10 (on-line meeting, in Hungarian).
3. Dániel, P. Szekrényes, Zámbó Dániel, Zolnai Zsolt, Nagy Norbert, and Deák András. "Detecting Short-Chain Thiol Binding on CTAB-Stabilised Gold Nanorods at Single Particle Level." In *Proceedings of Anyagtudományi Szimpózium*, 34–36.
4. András Deák: Self-assembly and microspectroscopic characterization of nanoparticles (in Hungarian) Invited Seminar - ELTE Department of Materials Physics 10.03.2020
5. Rita Némedi: Self-assembly of electric double-layer stabilized nanospheres (in Hungarian), BME VBK Scientific Students' Associations Conference, 2019.
6. Szekrényes Dániel P., Pothorszky Szilárd, Zámbó Dániel, Hajnla Zoltán, Osváth Zoltán, Deák András, Surface chemical patch formation and self-assembly studied at the single-nanoparticle level, 25th International Conference on Chemistry (2019).
7. D.P. Szekrényes, S. Pothorszky, D. Zámbó, Z. Osváth, Z. Zolnai and A. Deák, Nanoscale inhomogeneities characterized by the optical scattering spectra of individual gold nanoparticles, 33rd Conference of the European Colloid and Interface Society, KU Leuven, 8-13.09.2019.