First results of 20 kV EFTEM of core-shell QDS with an albumin-derived polypeptide surface coating on graphene

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Electrons with an energy of 20keV show significant different scattering behaviour compared to commonly used faster electrons with an energy between 80keV and 300keV as: (1) the inelastic (as well as the elastic) scattering cross section increases, and therefore an significant increase in image contrast of EFTEM (as well as TEM) images can be expected; (2) the elastic (electron-nucleus) damage mechanisms decrease significantly and the inelastic (electron-electron) damage mechanisms increase, therefore specimens and specimen conditions need to be selected carefully; (4) the penedration depth of slower electrons decreases significantly and therefore ultra-thin substrates and/or specimens have to be used.

Functionalized Qds are of emerging interest nowadays in the broad field of ultrastable contrast agents for bioimaging applications [1] . Herein, CdSe/CdZnS QDs were coated with a albumin-derived polypeptide coating that interact via multipe interaction sites with the QD core surface. Such coated CdSe/CdZnS core-shell nanoparticles reveal high emission intensities, excellent stabilities in aqueous solution as well as efficient cell uptake enabling their use as sensors inside cells. Here we report the 20kV EFTEM investigation of core shell CdSe/CdZnS QDs with an albumin polymere surface coating [2] on single-layer graphene, the thinnest substrate possible. The free supporting graphene flakes were prepared by micro-mechanical cleavage and transferred onto a quantifoil grid [3]. The investigations were performed with the monochromized and imaging aberration-corrected SALVE1 microscope [4] operating at 20 kV equipped with a 4kx4k CMOS Tietz camera (F416) with an energy slit width of 1.1 eV, an energy resolution of 0.18 eV (FWHM of ZLP). A series of energy filtered images was taken up to an energy loss of 40 eV with an energy delta of 0.5 eV between each image and an illumination time of 5s per image. This datacube was used to obtain EEL spectra by selecting specific sample positions and integrating over 20x20 pixel. Fig. 1a) show a cutout @ 22,5 eV energy loss where the EELS positions are marked. The extacted spectra reveal significant changes in the peak shape and position for the different sample positions. The peak positions of single layer graphene (see Figure 1b) spectrum 1 around 5 eV and 15 eV) agee very well with the expected peak positions of 4,7 eV and 14,8 eV for the π– and π–σ plasmon modes respectively in free standing single sheets of graphene [3]. Also the expected difference of the EELS of single layer and double layer graphene is visible, where the triangle shaped plasmon loss around 15 eV changes to a more plateau shaped peak figure 1 b) spectra 1 and 2. The following spectra 3 up to 7 display peaks correlated to the contamination of graphene, the protein as well as the QDs and their surface plasmons, respectively. In figure 2 a) the starting zero loss filtered image is shown, where single and agglomerated QDs as well as contamination and protein accumulations are separately visible. Figure 2 (b-e) show background corrected distribution images (b) of the plasmon loss of graphene at approximately 5 eV (π– mode) (c) the surface plasmon loss of the QDs at approximately 6 eV (d) broad plasmon losses of contamination and protein at 22 eV and (e) ELNES correlated to pure protein at 22 eV.

We discus the feasibility of 20kV EFTEM studies of functionalized Qds on graphene showing high contrast of the different species at their defined energy losses. Our ambitious goal for the future are higher resolution EFTEM studies to further understand the protein structure as well as the binding between protein and QDs.This may open new avenues for the analysis for low contrast objects also in biological science at voltages as low as 20kV, which conventionally needed to be imaged stained at much higher voltage.

References

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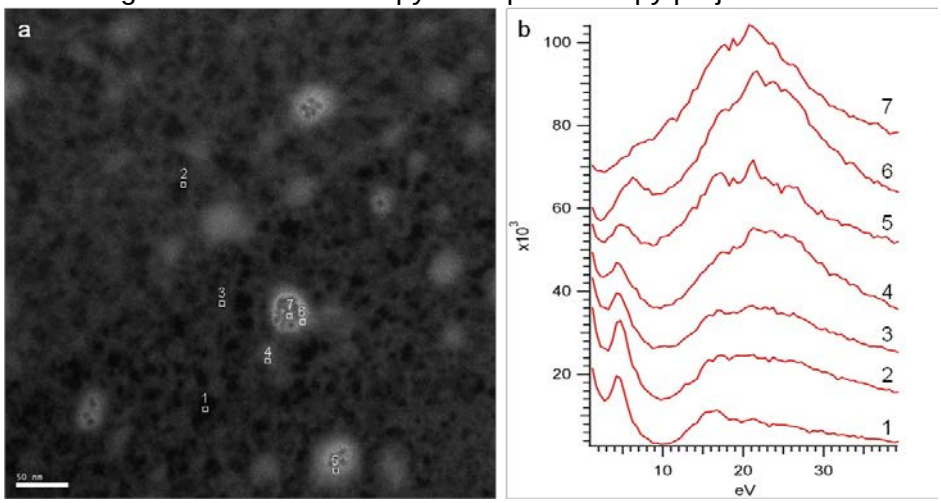

Figure 1: *Datacube of CdSe core shell particles funcionalized with an albumin-polymer coating on single and double layer graphene. (a) cutout @ 22,5 eV energy loss (b) extracted EELS, where position 1 is single graphene 2 double layer graphene, 3 graphene dirt, 4 graphene dirt and pure protein, 5 QD , 6 shell of the QD and 7 in between the QDs.*

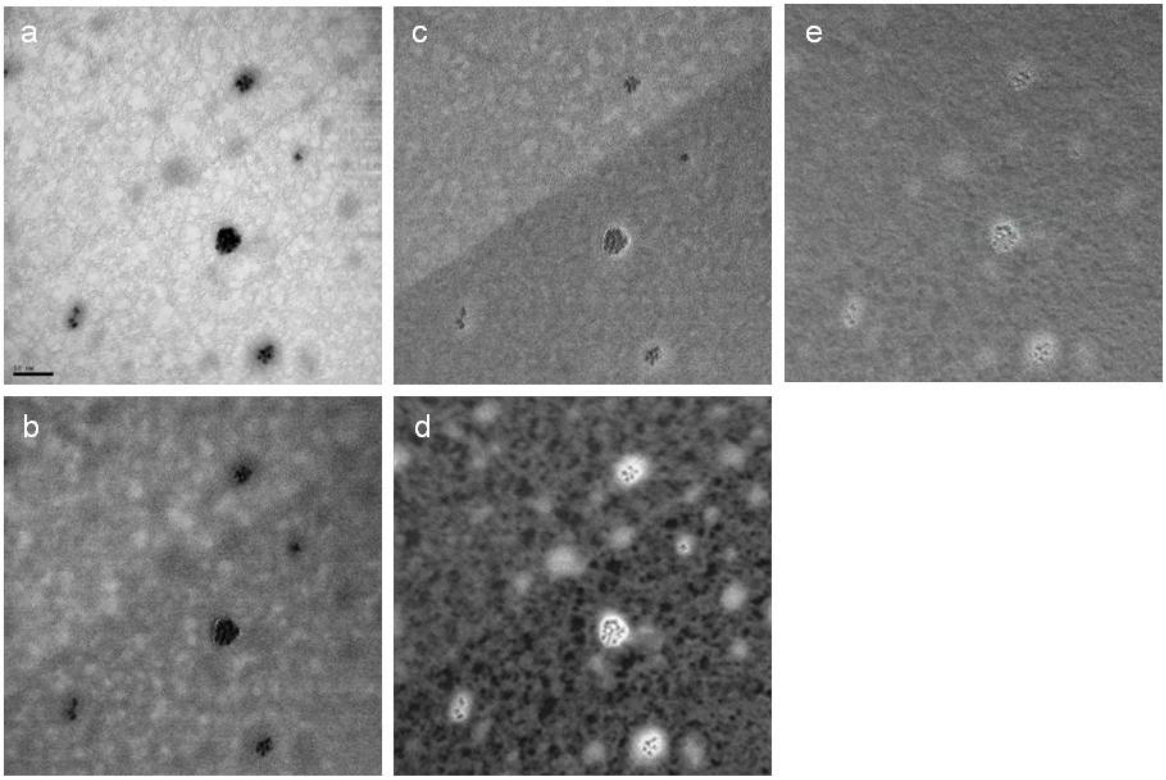

Figure 2*: CdSe core shell particles funcionalized with an albumin-polymer coating on single and double layer graphene. (a) zero loss filtered image (b-e) background corrected distribution images (b) plasmon loss of graphene 5 eV (π– mode) (c) surface plasmon loss of the QDs 6 eV (d) broad plasmon losses dirt and protein 22 eV (e) ELNES correlated to pure protein at 22 eV.*