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## A comparison of techniques for size measurement of nanoparticles in cell culture medium Electronic supplementary information

## I. COMPARISON OF DLS DATA FOR DIFFERENT WEIGHTINGS, LABORATORIES AND CONTAINER MATERIALS

Figure S1 displays the modal values extracted from the PSDs as measured by DLS under a variety of different conditions: two laboratories, three different media, two container materials, two points in time, two surface ligands, three different weightings. The results for particles in cell culture medium are entirely different from the results obtained in the simpler media. The data is non-reproducible and also not repeatable. Even though the same model of DLS device was used and all settings were equal, the data between the two laboratories do not match in the case of cell culture medium. This demonstrates that DLS data for particles in cell culture medium should be treated with care.

## II. 40 NM PARTICLE POPULATION AS MEASURED BY CLS

The fraction of smaller particles could be found by CLS in all measurements and is indicated by a minor peak at about 40 nm, which is best visible in the number-weighted PSD (Figure S2). For NP–NH<sub>2</sub>, the height of this peak seems to increase with agglomeration, but this is an artefact of the normalisation of the vertical axis of the PSD-graph. The PSDs are normalised to the highest peak in the histogram, which consists of the main fraction and the agglomerates. A broadening of this peak due to agglomeration leads to a different normalisation and consequently an apparent increase of the 40-nm peak.

## **III. UNCERTAINTY EVALUATION FOR CLS**

An alternative approach to the evaluation of the uncertainty of Stokes diameters measured with CLS measurements has been published before.[1] In Table I, the uncertainty contributions evaluated by a different laboratory with this approach are compared with the approach described in the main text, separating contributions related to trueness from contributions related to precision. The table illustrates the uncertainty budget for the case of the modal Stokes diameter values in the extinction intensity based PSD of NP-plain in water. The uncertainty budget for NP-NH<sub>2</sub>, as well as those for measurements of both materials in the Tris-HCl buffer and in the cell culture medium, result in similar values.

Both approaches agree within the combined expanded uncertainty of 5.3 nm (see figure S3). Because of the absence of significant bias between the labs, one can consider the values obtained at the two laboratories as independent estimates of the same value, and use the mean of their respective mean values as the best available estimate of the true Stokes diameters, with a combined uncertainty of 2 nm. However, when comparing the CLS results with results of other methods, this uncertainty value has to be combined with the trueness uncertainty contributions that both labs have in common, which varies between 2.7 nm and 1.3 nm. A general estimate of the combined standard uncertainty is then 4 nm.

[1] A. Braun, O. Couteau, K. Franks, V. Kestens, G. Roebben, A. Lamberty and T. P. J. Linsinger, *Adv. Powder Technol.*, 2011, **22**, 766–770.



FIG. S1. Modal diameters obtained from the scattered light intensity-weighted (a), volume-weighted (b) and number-weighted (c) PSD by DLS measurements in two laboratories. Round and squared symbols display the values obtained immediately after dispersion, and after 24 h, respectively. All individual values are plotted to illustrate the large range of the results from the same sample. Additional measurements were performed in Lab 2 using polycarbonate cells (PC) to exclude that the large variations observed in cell culture medium are due to the glass cells.





FIG. S2. The number-weighted PSD corresponding to for  $\mathrm{NP-NH}_2$  as measured by CLS.

|                                                            | to the measured Stokes diameter, |                     |
|------------------------------------------------------------|----------------------------------|---------------------|
| Sources of uncertainty                                     |                                  |                     |
|                                                            | $u_i(D)/\mathrm{nm}$             |                     |
|                                                            | Lab 1                            | Lab 2               |
| 1) Precision contributions                                 |                                  |                     |
| Repeatability                                              | 1.60                             | 0.20                |
| Intermediate precision                                     | (incl. in repeatability)         | 1.02                |
| Combined standard uncertainty from precision contributions | 1.6                              | 1.0                 |
| 2) Trueness contributions                                  |                                  |                     |
| Trueness (bias assessment against independent CRM)         | -                                | 1.71                |
| Diameter of PVC calibrant                                  | 0.92                             | 0.92                |
| Effective density of PVC calibrant                         | 1.21                             | (incl. in diameter) |
| Effective density of test particles                        | 2.24                             | 0.96                |
| Fluid gradient density                                     | 0.76                             | -                   |
| Sedimentation time of the calibrant                        | 0.01                             | -                   |
| Combined standard uncertainty from trueness contributions  | 2.8                              | 2.2                 |
| Combined standard uncertainty                              | 3.2                              | 2.4                 |

TABLE I. Uncertainty budget for the CLS method. For an intramethod comparison, the entries in italics are not considered.



FIG. S3. Modal diameters with the associated standard uncertainty for CLS obtained in Lab 1 (a) and Lab 2 (b). The error bars represent the standard uncertainty including the trueness contribution.