## Supporting Information for: A systematic comparison of different techniques to determine the zeta potential of silica nanoparticles in biological medium

Aneta Sikora,<sup>a</sup> Dorota Bartczak,<sup>b</sup> Daniel Geißler,<sup>c</sup> Vikram Kestens,<sup>d</sup> Gert Roebben, <sup>d</sup> Yannic Ramaye,<sup>d</sup> Zoltan Varga,<sup>e</sup> Marcell Palmai,<sup>e</sup> Alexander G. Shard,<sup>a</sup> Heidi Goenaga-Infante,<sup>b</sup> and Caterina Minelli <sup>a+</sup>



Figure S1. Particle size (DLS cumulants method) measurements of plain(A) and aminated (B) silica NPs at different pH.



**Figure S2**. Representative DLS normalised scattered light intensity-weighed particle size distributions obtained at 0 h (continuous line) and 24 h (dash line) after dispersing plain (A, C and E) and aminated (B, D and F) silica NPs in purified water (A and B), Tris-HCl (C and D) and 10 % FBS-EMEM (E and F) respectively.

 Table S1. Additional technical requirements for ζ–potential measurements.

| Properties                           | ELS  | TRPS  | zPTA   |
|--------------------------------------|--|---|--|
| NP size range                        | 3.8 nm to 100 μm   | 70 nm to 800 nm<br>(for larger particles the<br>recommendation is to<br>contact Izon)   | 10-20 nm to 1-2 μm<br>depending on particle<br>refractive index, medium,<br>sensitivity of the camera, and<br>wavelength and power of the<br>laser.  |
| Measurable<br>concentration<br>range | <10 nm: 0.5 g/L - limited by<br>sample material interaction,<br>e.g. aggregation.<br>10-100 nm: 0.1 mg/L to 5%<br>mass, assuming a density of<br>1 g/cm <sup>3</sup><br>100 nm-1 μm: 0.01 g/L -1%<br>mass assuming a density of<br>1g/cm <sup>3</sup><br>>1μm: 0.1g/L-1% mass) | 10 <sup>5</sup> -10 <sup>12</sup> NPs/mL  | 10 <sup>7</sup> -10 <sup>10</sup> NPs/mL   |
| Optimal<br>concentration             | Dependent on the scattering<br>properties of the measured<br>materials and the particle<br>size. Should be determined<br>experimentally  | 1 x 10 <sup>9</sup> NPs/mL to 5 x 10 <sup>10</sup><br>NPs/mL depending on size  | Dependent on the scattering<br>properties of the material.<br>The recommended number<br>of NPs in the field of view is<br>20-60. For silica NPs optimal<br>concentration was found<br>between 10 <sup>8</sup> to 10 <sup>9</sup> NPs/mL. |
| Type of<br>buffers                   | Aqueous/polar/non-polar.<br>Highly conductive samples<br>can lead to electrode<br>polarisation and<br>degradation.   | Variety of electrolytes with<br>molarity > 10 mM.<br>Applied voltage need to be<br>adjusted to have a current<br>>120 nA and a stable<br>baseline.<br>Membrane surface potential<br>need to be known or<br>estimated. | Variety of electrolytes inert<br>to glass, steel and rubber;<br>sample conductivities 0.005<br>mS/cm to 5 mS/cm.   |

**Table S2.** *ζ*-potential values of plain silica measured by z-PTA.

| Medium          | time | ζ-potential (mV) |
|-----------------|------|------------------|
|                 | 0 h  | -21 ± 1          |
| 150 mM Tris-HCl | 24 h | -21 ± 2          |