

Electronic Supplementary Materials to:

## Measuring rotational diffusion of colloidal spheres with confocal microscopy

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### 1) Experimental details

Monodisperse fluorescent SiO<sub>2</sub> colloids were synthesized according to the reported procedure<sup>[27]</sup>. Briefly, 12 mg of rhodamine B isothiocyanate (RITC) and 40 µl of 3-aminopropyl triethoxysilane (APS) were dissolved into 3.2 g of absolute ethanol and were allowed to react for around 4 hours in the dark before use. 7.6 g of ammonia aqueous solution (NH<sub>3</sub>·H<sub>2</sub>O) was mixed with 63 g of absolute ethanol in a round flask, and 3.2 g of tetraethyl orthosilicate (TEOS) was added under a gentle magnetic stirring. And then the RITC solution was added. The mixture was stirred overnight. Afterwards, another 0.2 g of TEOS was added into the flask for depositing a thin layer of silica. The synthesized SiO<sub>2</sub> colloids were washed with ethanol by repeated centrifugation.

To introduce functional double bonds onto SiO<sub>2</sub> surface, 0.1 g of as-prepared SiO<sub>2</sub> colloids were dispersed in 20 ml of ethanol containing 0.5 ml of H<sub>2</sub>O and 0.5 ml of ammonia, and 0.6 ml of 3-methacryloxypropyl trimethoxysilane (MPTS) was added. The coating reaction was performed for 12 h. The coated SiO<sub>2</sub> particles were washed with ethanol by repeated centrifugation.

To coat a thin layer of polystyrene (PS) onto SiO<sub>2</sub> surface, 0.1 g of MPTS-coated SiO<sub>2</sub> was dispersed in 30 ml of 1.0 - wt% of (sodium dodecyl sulphate) SDS aqueous solution, and 0.8 ml of styrene (St) and 0.04 ml of divinyl benzene (DVB) was added. After the temperature reached

to 75°C, 0.5 ml of 0.02 g/ml of potassium persulfate solution was injected into the flask. The polymerization was performed for around 12 hours. The coated SiO<sub>2</sub> particles were washed with water and ethanol by repeated centrifugation.

The eccentric SiO<sub>2</sub>/Poly(MPTS) core-shell particles was prepared according to the reported procedure<sup>[28]</sup>. Briefly, 5 ml of MPTS was hydrolysed in 50 ml of deionized water by stirring for 5 h before use. PS-coated SiO<sub>2</sub> colloids were dispersed into 3 ml of deionized water to prepare ca. 8.0-10.0 w/v % suspension, and 10 µl of ammonia solution was added. Then 3ml of hydrolyzed MPTS aqueous solution was added. After 1 hour, 1 ml of hydrolyzed MPTS was added into this solution every 1 hour till the expected colloidal size was obtained. The particle was polymerized by adding 2 mg of azobisisobutyronitrile (AIBN) and then stirring for 3 hours at 75°C. The SiO<sub>2</sub>/Poly(MPTS) core-shell particles was purified by washing with water and repeated centrifugation.

To mark the shell with a fluorescent dye, 1.2 mg of coumarin 153 was dissolved in 10 ml of toluene. Then an emulsion toluene in water was prepared by ultrasonication with SDS as surfactant. 1 ml of SiO<sub>2</sub>/Poly(MPTS) dispersion was mixed with 1 ml of toluene emulsion, and then the suspension was stirred for 5 ~ 6 hours. Afterwards, the flask was left open to evaporate toluene for two days. The particles were washed with water for five times to remove the surfactant. After a solvent replacement for 2~3 time, the model particles was re-dispersed into the two solvents we used for confocal imaging.

## 2) Supplementary figures

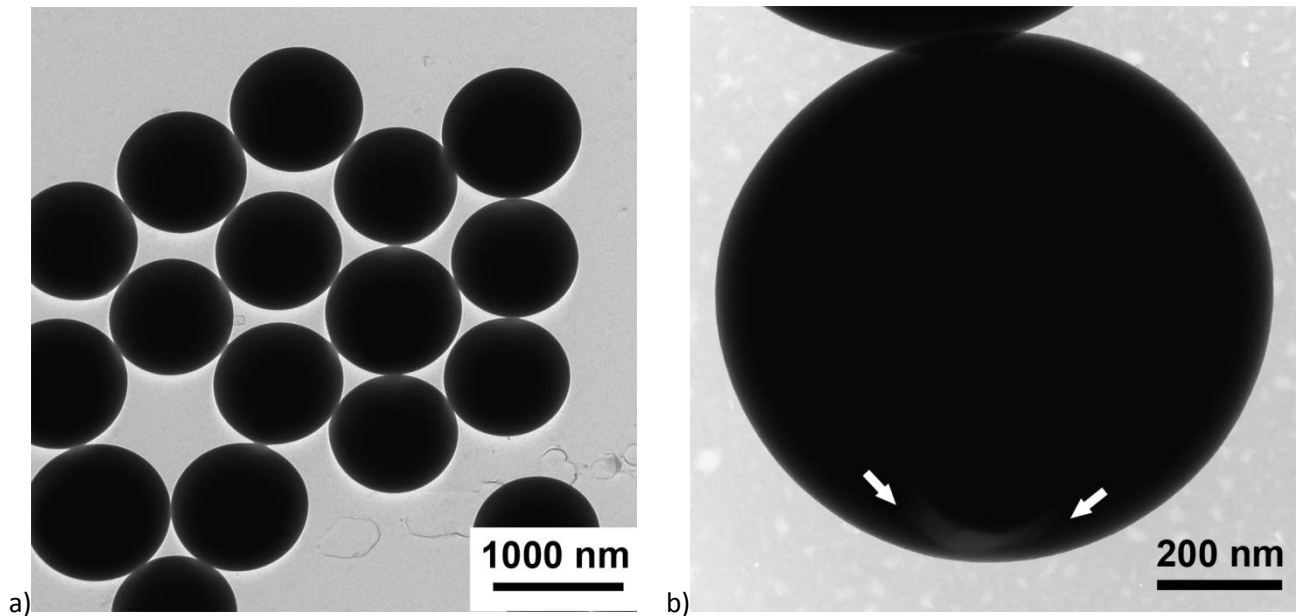


Figure S1. a)  $\text{SiO}_2/\text{Poly}(\text{MPTS})$  core-shell particles and b) the particle shown in a) in high magnification. The difference in contrast is indicated by two white arrows, which indicates the position of the core.

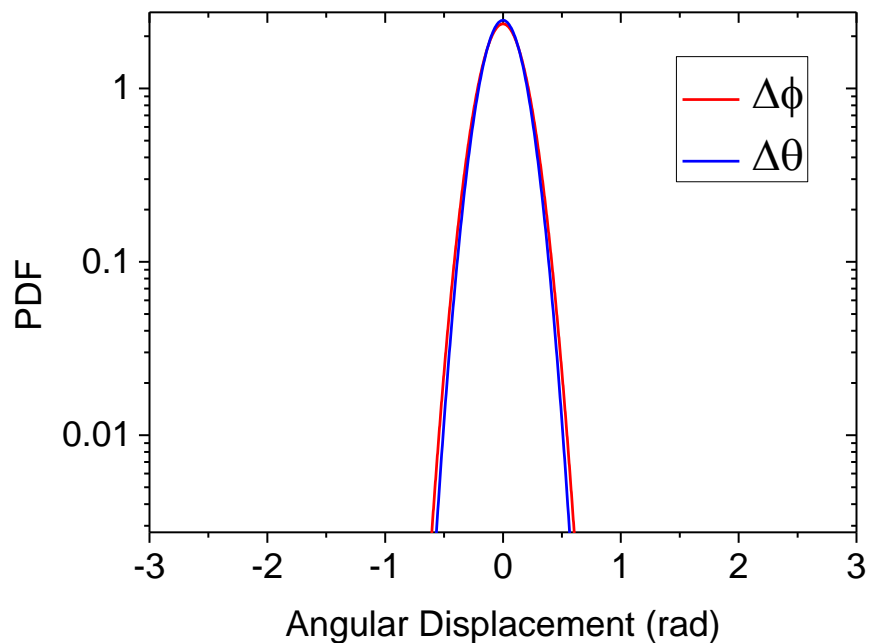


Figure S2. A comparison of the fitted Gaussian curves between two rotational displacements, corresponding Figure 3e-f in the main text. It shows that the two rotational displacements have almost identical probability distribution.