Electronic Supplementary Material (ESI) for Soft Matter.

Supporting Information for

Tracking Single-Particle Rotation during Macrophage Uptake

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Experimental Section

1. Fabrication of triblock patchy particles

Silica particles were etched with piranha solution (H_2SO_4: H_2O_2 = 3:1 v:v) for 15 minutes at 75 ˚C, and then rinsed a few times in Milli-Q water. Glass slides were pre-cleaned by sonicating in ethanol before being treated with piranha solution for 15 minutes. Etched glass slides were stored in milli-Q water prior to use. Monolayers of silica particles were made by using a solution evaporation method as previously described.[1] If the particle monolayer was not used immediately for micro-contact printing, it was etched again prior to use with 3:1 piranha solution for 15 minutes. Polydimethylsiloxane (PDMS) stamps were made by mixing Sylgard 184 base and curing agent at a 2:1 (w:w) ratio. The mixed base and curing agent were poured into a polystyrene petri dish and degassed in vacuum in order to remove air bubbles. PDMS stamps were cured for at least 19 hours in a vacuum oven at 70 ˚C. Prior to micro-contact printing, the PDMS stamps were cut into 1×1 cm^2 per piece and their stamping surfaces were treated with 1:1 (v:v) piranha solution for 3 minutes. A previous study has shown that this method is effective in removing the hydrophobic methyl groups from the PDMS surface.[2] The 1: 1 (v:v) piranha solution was used instead of the traditionally 3:1 (v:v) one to avoid the etch-induced cracking of the PDMS. The etched stamps were rinsed with milli-Q water multiple times before being incubated with fluorescently labeled proteins (20 μg/mL, a total of 200 μL) for 1 hour at room temperature. The first stamp was dried under a stream of filtered air and immediately placed against an etched particle monolayer with a pressure of $1.5x10^4$ Pa. The stamp was lifted off the silica monolayer 3 minutes later and placed onto a flat surface, with the particle-embedded side facing up. The printing step was repeated using a second stamp to generate the second protein patch on particles. Particles were sonicated off the stamps and collected in 1xPBS buffer containing 0.064 mg/mL BSA or BSA-biotin, for partially or uniformly coated particles, respectively.

To conjugate biotinylated IgG onto the particle surface, particles were incubated with 0.006 mg/mL of streptavidin and 0.25 mg/mL of BSA at room temperature for 1.5 hours, and subsequently with 0.0015 mg/mL of biotinylated-IgG and 0.1 mg/mL of BSA for another 1.5 hours. Functionalized particles were rinsed with 1xPBS before use. It is important that the particles are functionalized right before live cell

imaging, as the streptavidin-biotin conjugation dissociates gradually over time. The functionalized particles were stored in 1x imaging buffer.

2. Live Cell Imaging

RAW 264.7 macrophages were seeded at a density of 6.7 x 10^4 cells/mL in sterilized imaging chambers 24 hours before imaging. Seeded macrophages were serum starved for 3 hours, incubated with 0.002 mg/mL VivoTrack 680 in 1x imaging buffer for 30 minutes at room temperature, and gently rinsed with 1x imaging buffer before live cell imaging. All live cell imaging was done in 1x imaging buffer at 37 ˚C. A Nikon Eclipse-Ti epifluorescence microscope equipped with an Andor iXon3 EMCCD camera and a Nikon Plan Apo 100x/1.49 N.A TIRF objective was used for live cell imaging. Time-lapse multi-channel fluorescence images were acquired using Micro-Manager 1.4.15 software. Images were acquired with 2-second per frame interval time. Data acquisition started immediately after addition of triblock particles.

3. Single Particle Tracking

A single particle tracking algorithm was developed to identify and track the orientation and rotation of the Janus particles. Part of the algorithm that smoothes images and identifies particle positions was based on the PolyParticleTracker program by Rogers *et al*..[3] Because the particles displayed no obvious rotation within 6 consecutive images (including two channels), every 3 consecutive images of the same channel were averaged for further noise reduction. After image smoothing and noise reduction, the second step was to locate the center of each patch. The position of each patch was first estimated based on the local maximum of the fluorescence intensity. Pixels were discarded if the brightness is below the standard deviation of the pixel brightness of the entire image. The center position of each patch was refined by applying a two-dimensional polynomial fitting of the intensity around each intensity peak, weighted by a Gaussian function of the distance from the center. Algorithm for this step was also adapted from the PolyParticleTracker program by Rogers *et al*..[3] To remove random noise that may be falsely identified as an intensity peak, we applied a minimum brightness threshold, which was specified relative to the mean

and standard deviation of the pixel intensities of a given image. The threshold was set to be 1 and 0.25 standard deviation above the mean for tracking 3-μm and 1.6-μm particles, respectively. Intensity peaks below the brightness threshold were discarded.

The third step was to identify and link the same patch across different image frames, by applying two criteria based on fluorescence intensity and distance. Distance threshold of 50 pixels was used for tracking 3- and 1.6- μm particles, based on an estimation of the distance each particle moves from one frame to the next and the distance between two particles. Two patches in adjacent frames were identified as the same one if the proximity of their centers are within the set threshold. If multiple candidates were found, their peak intensities were compared to the one from previous frame and the closest match was identified as the same one. Others were discarded from the list of candidate peaks. Only patches that were identified in at least 5 consecutive frames were kept. The following step was to identify if a pair of red and green patches belong to a single particle by applying a proximity criterion. Only patches that are within the set proximity range will be considered a pair. We set 20 pixels and 10 pixels as the proximity range for analyzing 3- and 1.6-μm particles, respectively, based on the particle size appearing in the fluorescence images. Once patches are paired up, a final step was to draw a vector from the center of a green patch to the center of its paring red patch. The center of the vector was assigned as the center of the particle, assuming that the two patches are located exactly on opposite sides of a particle. The angle (θ) between the vector and the y-axis was calculated to quantify the orientation of each particle. Angular velocity at a given time t was an average velocity of the time internal $\Delta t = 20$ sec. Because particles did not rotate more than 360º in the entire process, their end-to-end rotational displacements of given time intervals were obtained for plotting the displacement probability distribution.

To estimate the tracking inaccuracy we imaged and analyzed triblock particles that were immobilized on glass surfaces. All immobilized particles were imaged using the same acquisition settings as for live cell imaging and four particles were analyzed using the single-particle rotational tracking algorithm. For each

particle, the standard deviation of all angles measured as a function of time was obtained. The maximum of all four standard deviations was 1 degree and used as the estimated tracking inaccuracy.

4. Simulation of rotation of Brownian particles

Simulations were performed in Matlab to verify that the significant deviation of the particle rotational displacements from a Gaussian distribution is not caused by the out-of-plane rotation. In the simulation, 10,000 particles were initialized with random orientations of the axis vector on a sphere. Each particle then underwent simulated Brownian rotation, with a diffusion constant of 0.75 deg^2/sec and simulated observations every 2 seconds for 400 frames. The observed in-plane angle displacement was then calculated for each 2 second step, resulting in nearly 4 million simulated in-plane displacements. Replicating the limits imposed on the out-of-plane angle from experiments, the displacements were recalculated after discarding any observations whose axis vector deviated from the image plane by more than 40 degrees. After this step, more than 2.5 million simulated in-plane displacements were left. As shown in **Figure S5 SI**, the displacement probability distribution for a particle undergoing simple Brownian rotation can be fitted with a single Gaussian even when the experimental observation limits are taken into account. The 40 degree angle employed in the simulation represents an upper-limit, and Gaussian fits were even better for simulations using smaller angular limits. The deviations from a single Gaussian fit observed in **Figures 3 and S7** cannot be explained by the observational technique, and must therefore represent non-Brownian rotational dynamics.

References

1. Q. Chen, E. Diesel, J. K. Whitmer, S. C. Bae, E. Luijten and S. Granick, *J. Am. Chem. Soc.*, 2011, **133**, 7725-7727.
2. D. Maji, S. K. Lahiri and S. Das, *Surf. Interface Anal.*, 2012, **44**, 62-69.
3. S. S. Rogers, T. A. Waigh, X. B. Zhao and J. R. Lu, *Phys. Biol.*, 2007, **4**, 220-227.

Supplementary Figures

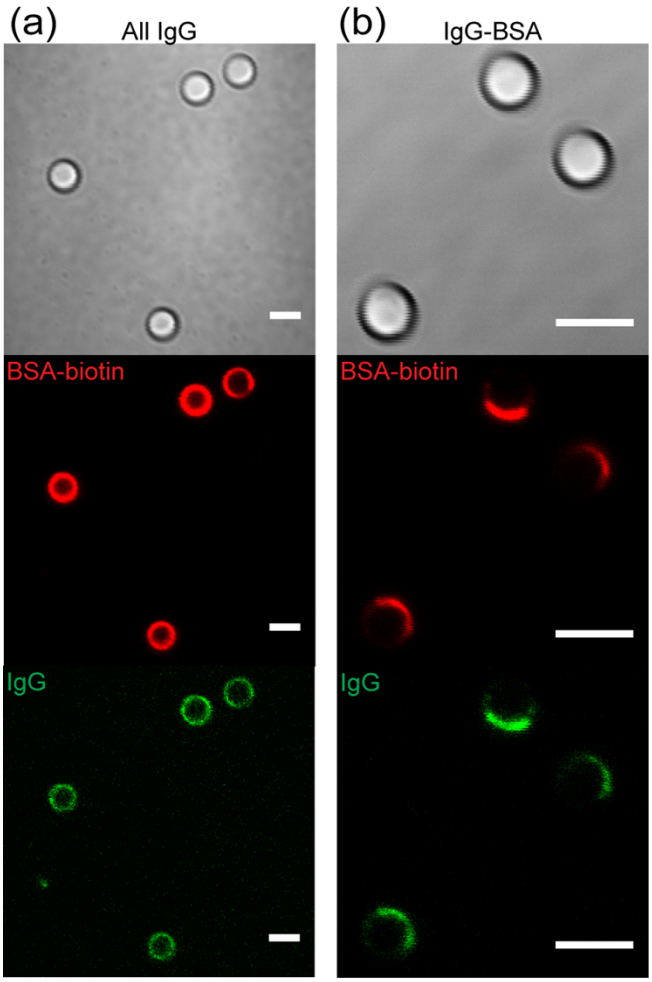

Figure S1. Characterization of the surface functionalization of the (a) all-IgG and (b) IgG-BSA particles. All particles shown are 3-μm in diameter. From top to bottom in both (a) and (b) are brightfield images, and fluorescence images of the Alexa568-BSA biotin and Alexa 488-IgG, respectively. Scale bars = 5 μm

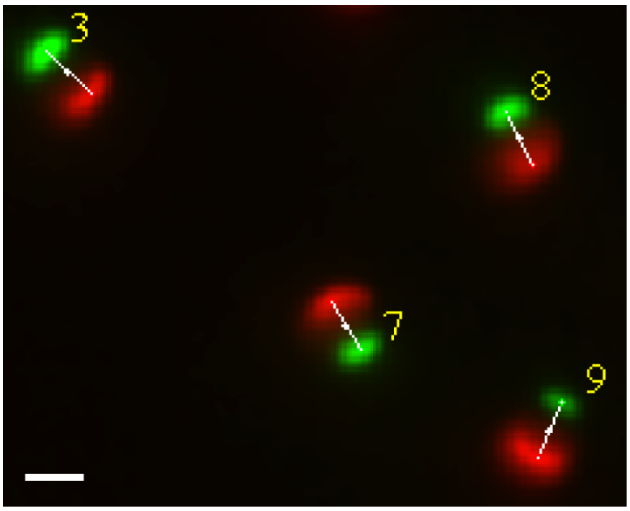

Figure S2. Single-particle tracking of multiple triblock Janus particles with a vector pointing from the green to the red patch. The numbers are arbitrarily assigned ID's for particles.

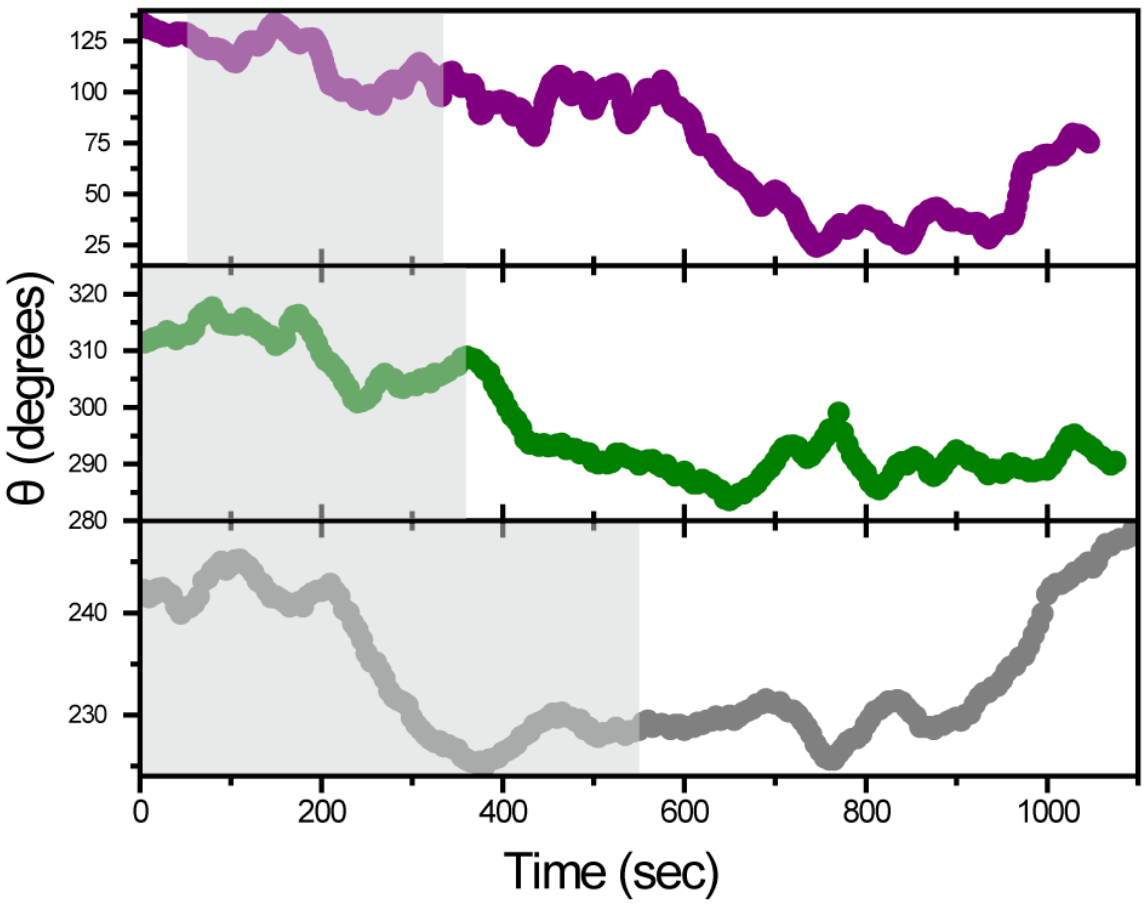

Figure S3. The particle orientation, defined by angle θ, is shown as a function of time for three representative 3-μm all-IgG triblock particles. Grey shades indicate the period from the initial cell-particle contact to the time when the particle is visibly engulfed by the cell membrane, which were estimated from fluorescence images. Plots are representative of N = 25 particles.

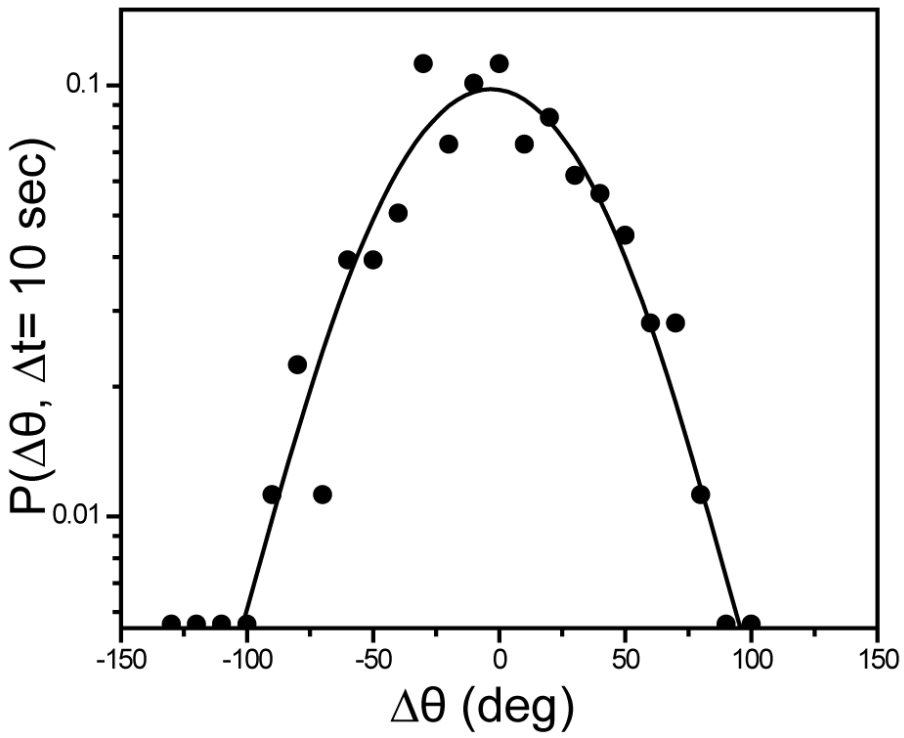

Figure S4. The probability distribution of particle rotational displacements, $P(\Delta\theta, \Delta t = 10\ sec)$, for 3-μm triblock particles dispersed in water containing 5% glycerol. The plot is an average of 612 steps from 2 particles. The solid line indicates a single Gaussian fit.

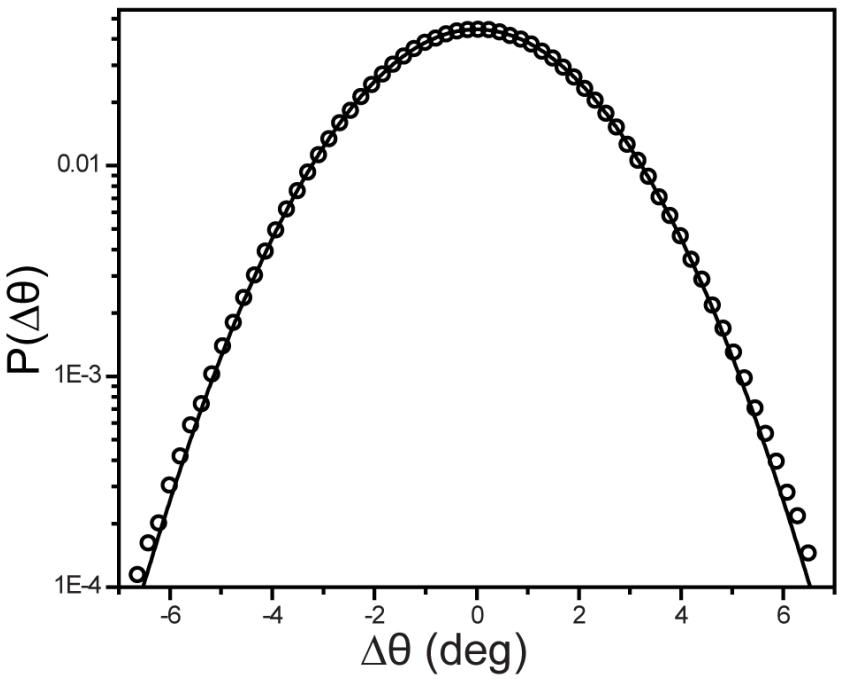

Figure S5. The probability distribution of particle rotational displacements, $P(\Delta\theta)$, from simulated Brownian particles. The plot is an average of 2,549,004 steps from 10000 particles. The solid line indicates a single Gaussian fit.

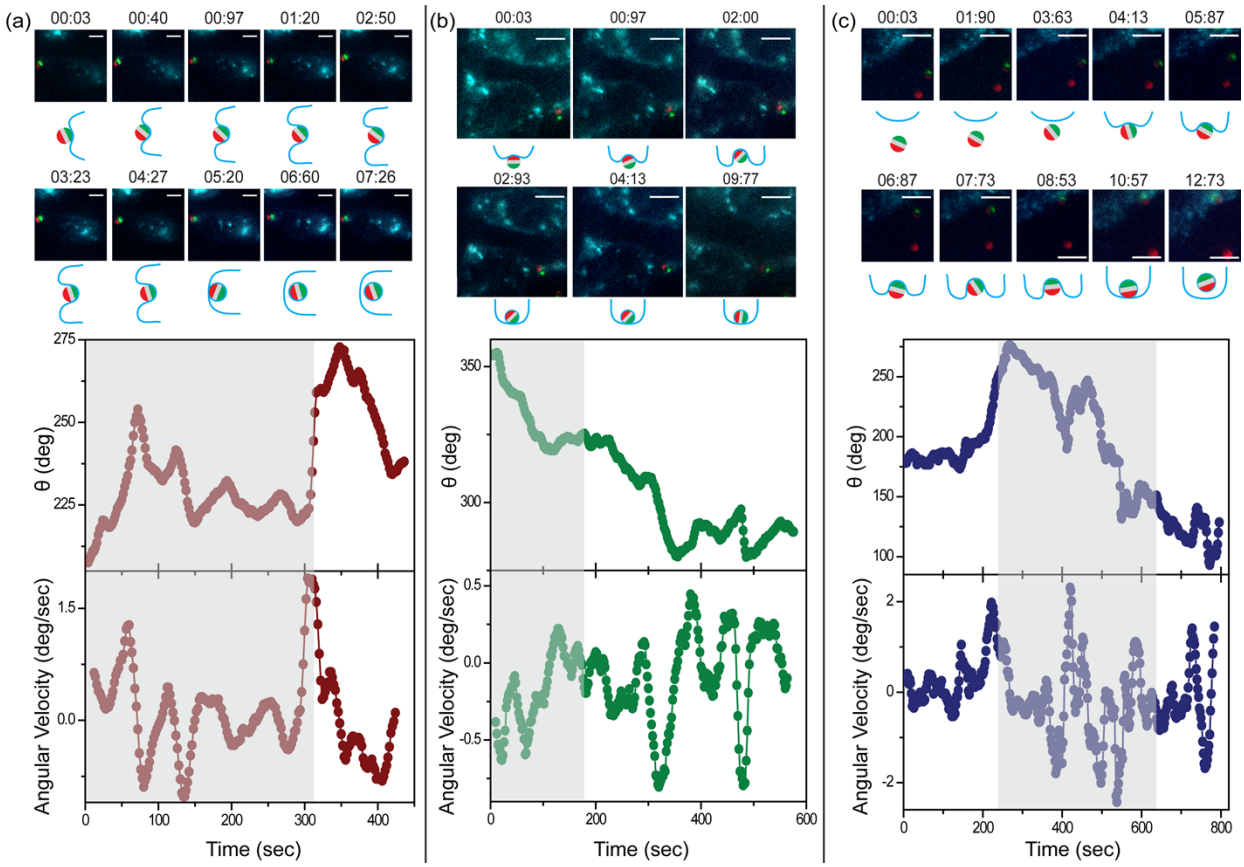

Figure S6. Fluorescence imaging and single-particle tracking of representative 1.6-μm (a) IgG-BSA, (b) all-IgG and (c) all-BSA particles during macrophage uptake. Overlaid epi-fluorescence images and schematic illustrations show particle uptake at various times as indicated. Scale bars: 5 μm. Angle θ and angular velocity of the triblock particles are plotted as a function of time. Grey shades indicate the period from the initial cell-particle contact to the time when the particle is visibly engulfed by the cell membrane. Both time points were estimated from the fluorescence images. Plots are representative of N=7 all-IgG, N = 12 IgG-BSA and N = 11 all-BSA particles, respectively.

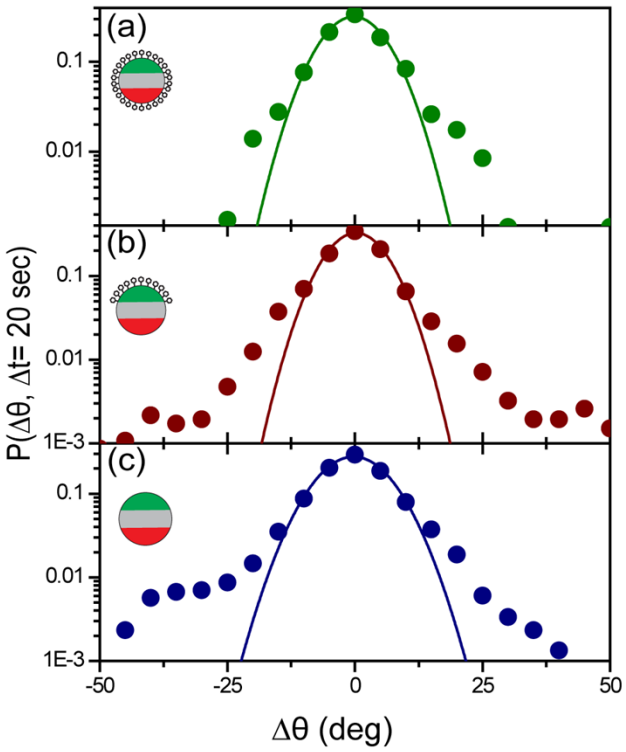

Figure S7. The probability distribution of particle rotational displacements, $P(\Delta\theta, \Delta t = 20\ sec)$, for 1.6-μm particles with different surface functionalities: (a) all-IgG (N =7), (b) IgG-BSA (N =12), and (c) all-BSA (N =11). Each plot is fitted with a single Gaussian function indicated by the solid lines. Approximately 3,000 steps were averaged for each $P(\Delta\theta, \Delta t = 20\ sec)$. Insets are schematic illustrations of the three types of particles.