Electronic Supplementary Information

Defocused Dark-Field Orientation Imaging of Single Gold Microrods on Synthetic Membranes

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This document contains experimental methods and additional supplementary figures (Fig. S1 to S6).

Experimental Methods

1. **Sample Preparation and Characterization.** The AuNUs used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA) The AuNU colloid solution was first diluted with 18.2-MΩ pure water to a proper concentration. The diluted solution was then sonicated for 15 min at room temperature. Samples were prepared by spin casting the AuNU solution onto a pre-cleaned glass slide. Then, a 22 mm × 22 mm no. 1.5 coverslip (Corning, NY) was placed on the glass slide. In this study, the concentration of AuNUs on the glass surface was controlled to be ~1 μm^{-2} in order to facilitate single-particle characterization and to minimize inter-particle SPR coupling, which can result in a spectral shift. Structural characterization was carried out using a scanning electron microscope (SEM).

2. **Single-Particle Scattering Microscopy and Spectroscopy.** DF microscopy imaging was performed under a Nikon inverted microscope (ECLIPSE Ti-U). In DF mode, the microscope utilized a Nikon Plan Fluor 100× 0.5-1.3 oil iris objective and a Nikon DF condenser. An Andor iXon^{EM}+ CCD camera (iXon Ultra 897) was employed to record DF images of AuNUs. The collected images were analyzed with Image J. Furthermore, DF scattering spectra were acquired with an Andor spectrometer (SHAMROCK 303i, SR-303I-A) and an Andor CCD camera (Newton DU920P-OE). When obtaining a spectrum, the scanning stage moved the sample to the desired location so that only scattered light from the selected location was collected by the objective. The scattered light was directed to the entrance of the spectrometer, dispersed by a grating (300 l/mm,

center wavelength: 700 nm), and detected by the Newton CCD camera. The background was measured at a region without any particles. Data analysis was performed with specially designed Matlab programs.

3. Simulation of Scattering Image Patterns of AuMRs. We used the simulation program developed by Enderlein and Böhmer.[1] The program is designed to calculate the characteristic intensity distribution from an emitter with three perpendicular emission oscillations of different emission strength. It has been widely used to determine the spatial orientation of single dye molecules.[1, 2] The simulation program is a special Matlab based utility with a graphics user interface (GUI) for easy calculation. This program allows us to calculate exactly the defocused (or focused) images of single molecules. For using the GUI, one should download the files from the website (http://www.joerg-enderlein.de/imagingOfSingleMolecules.html).

The parameters that can be input are: the numerical aperture of the objective lens, magnification of imaging, extent of defocusing (or defocusing distance in micrometers), κ and R. For defining the emission strength ratios of the three independent oscillations (Fig. 1A), we input the parameter κ and R into the program. The ratio κ defines the ratio of the emission strength of the b- to the c-oscillation (transverse oscillations, Fig. 1A) as shown below.

$$I_b / I_c = (1-\kappa)/(1+\kappa)$$

In this study the emission strength of the b-oscillation is assumed to be same as that of the c-oscillation. In addition, the ratio R defines the emission strength of the a-oscillation

(or longitudinal oscillation) to the combined b and c oscillations (or transverse oscillations) as shown below.

$$R \times I_a + (1 - R) \times (I_b + I_c)$$

When *R* is 1, we only have the contribution from a-oscillation (longitudinal oscillation) to the image patterns. However, the other two transverse oscillations (b and c) start to contribute to the image patterns with decreasing the ratio *R*. That is, lower *R* values indicate more contributions from the two transverse oscillations. Therefore, we were able to calculate the scattering patterns of a AuNR by adjusting the important parameters.

4. **Preparation of Synthetic Lipid Bilayers on Glass Slides.** The phospholipid 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC, Avanti Polar Lipids) solution in chloroform was first dried by a nitrogen steam and followed by at least 3 hr drying under vacuum at room temperature to remove the residual chloroform. The dried lipids were stored in a -20°C freezer.

Phosphate buffered saline (1× PBS, pH 7.4) was used to bring the final concentration to 0.5 mg/mL. The cloudy solution containing multilamellar vesicles was obtained after swelling in the PBS buffer solution for min with several times vortexing. The suspension solution was then forcing through a 100 nm pore size polycarbonate membrane at least 21 times to prepare the solution with large unilamellar vesicles using a mini-extruder (Avanti Polar Lipids, Alabaster, AL). The resulted solution was kept in a 4°C fridge.

The planar bilayer was formed by incubating the large unilamellar vesicles solution on a freshly cleaned glass slide in a chamber created by two double-sided tapes and a clean coverslip for 10 min. After that, PBS was used to remove the excess lipids. AuMR solution was then introduced into the chamber with the membrane for optical imaging.

References

1. M. Böhmer and J. Enderlein, *J. Opt. Soc. Am. B*, 2003, **20**, 554-559.
2. M. A. Lieb, J. M. Zavislan and L. Novotny, *J. Opt. Soc. Am. B*, 2004, **21**, 1210-1215.

Supplementary Movies

Movie S1: This movie shows rotational motions of AuMR8 (in-focus) on synthetic membrane under focused DF microscopy. Temporal resolution is 100 ms.

Movie S2: This movie shows rotational motions of AuMR8 (out-of-focus) on synthetic membrane under defocused DF microscopy. A defocusing distance is 1 μm and temporal resolution is 100 ms.

Movie S3: This movie shows rotational motions and dispersion characteristics of AuMRs on synthetic membrane under focused DF microscopy. Temporal resolution is 100 ms.

Supplementary Figures

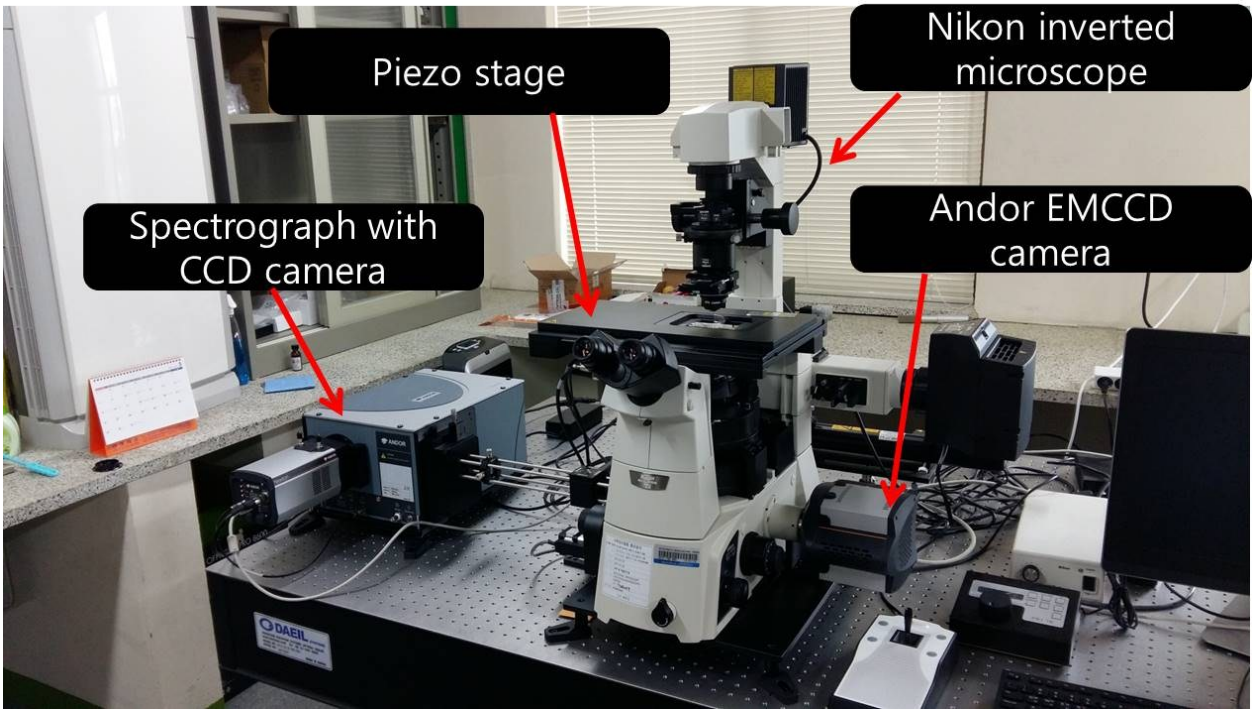

Fig. S1 A photograph of experimental setup for single particle scattering microscopy and spectroscopy.

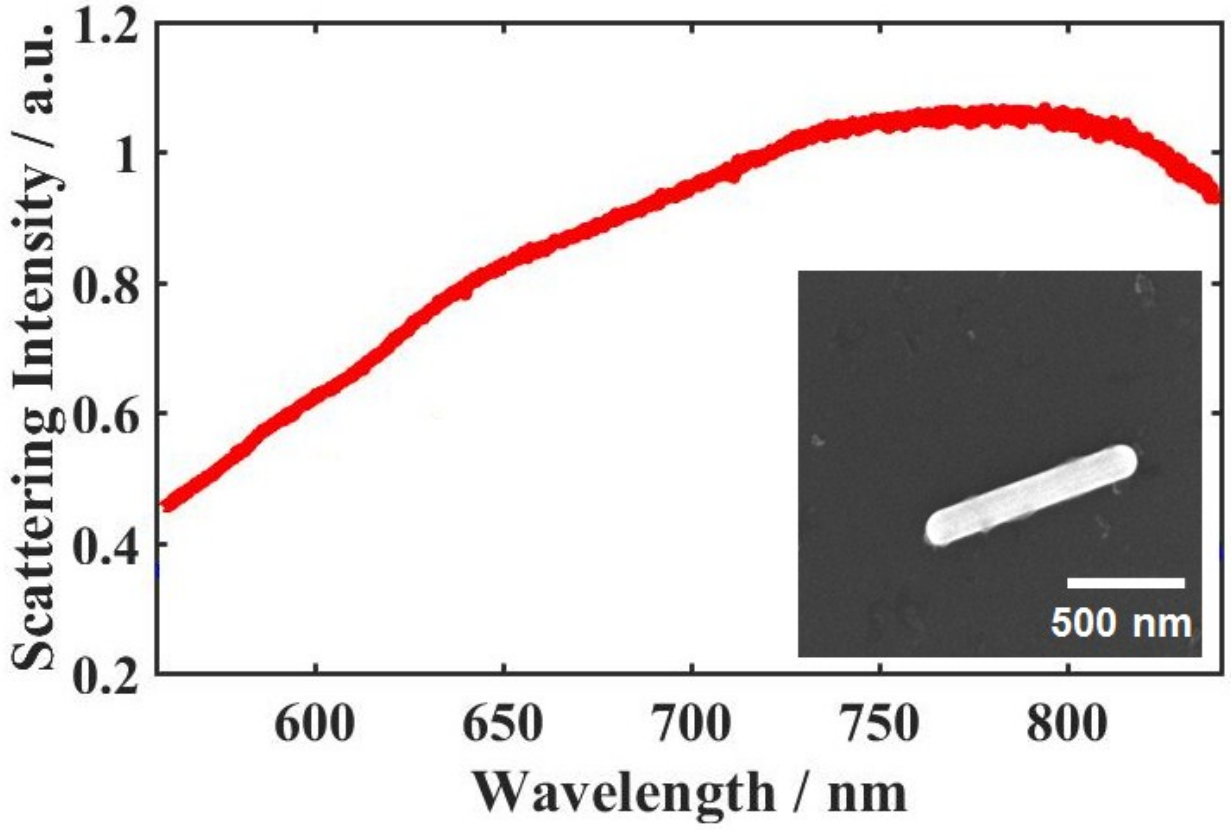

Fig. S2 Single particle scattering specrum of a AuMR. The inset shows a SEM image of single AuMR.

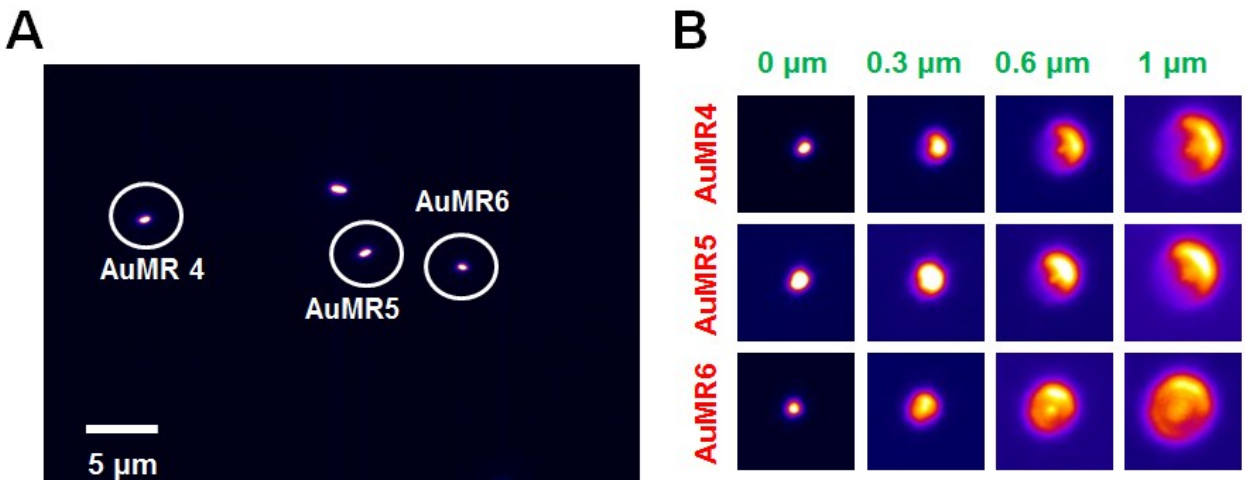

Fig. S3 (A) DF scattering image of single AuMRs. (B) Scattering images at four-different defocusing distances for the three AuMRs highlighted in (A).

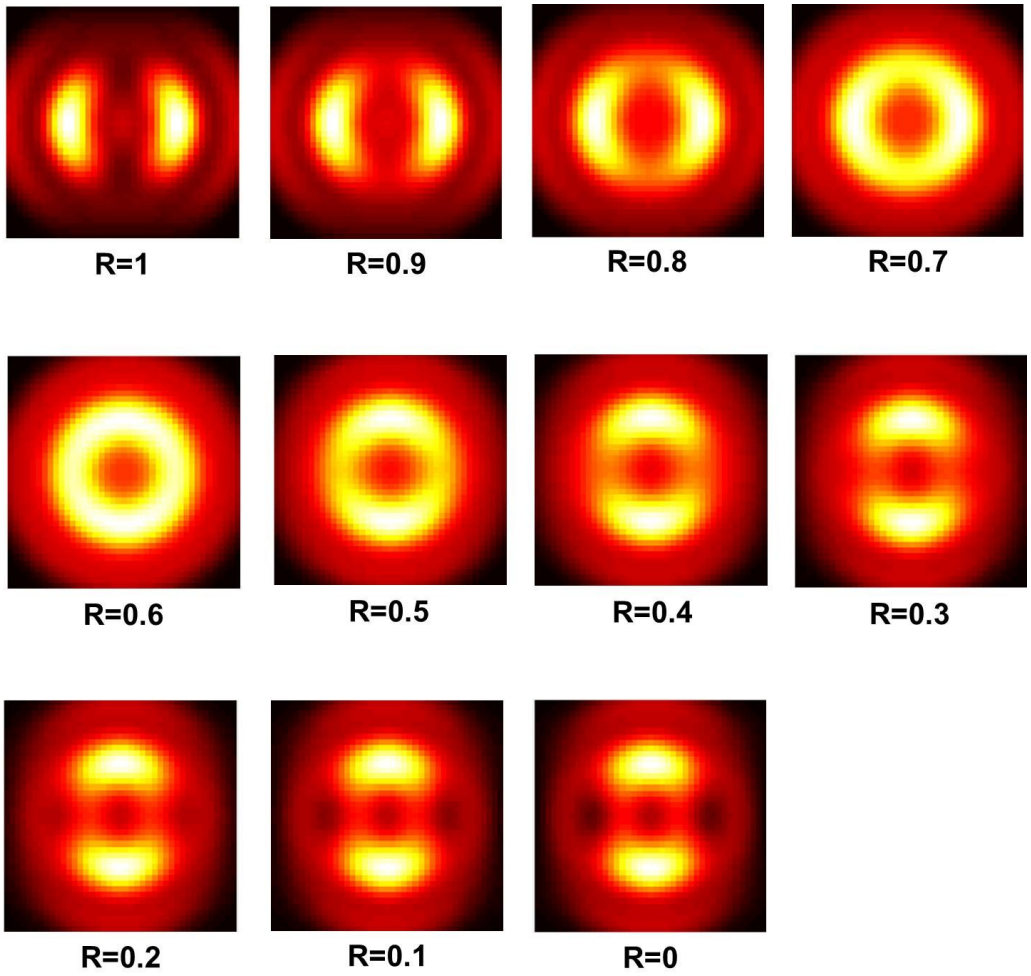

Fig. S4 The simulated scattering patterns of a AuMR by varying the parameter R from 1 to 0 in the focal plane. In this simulation, the polar angle θ of a AuMR was set to 90°.

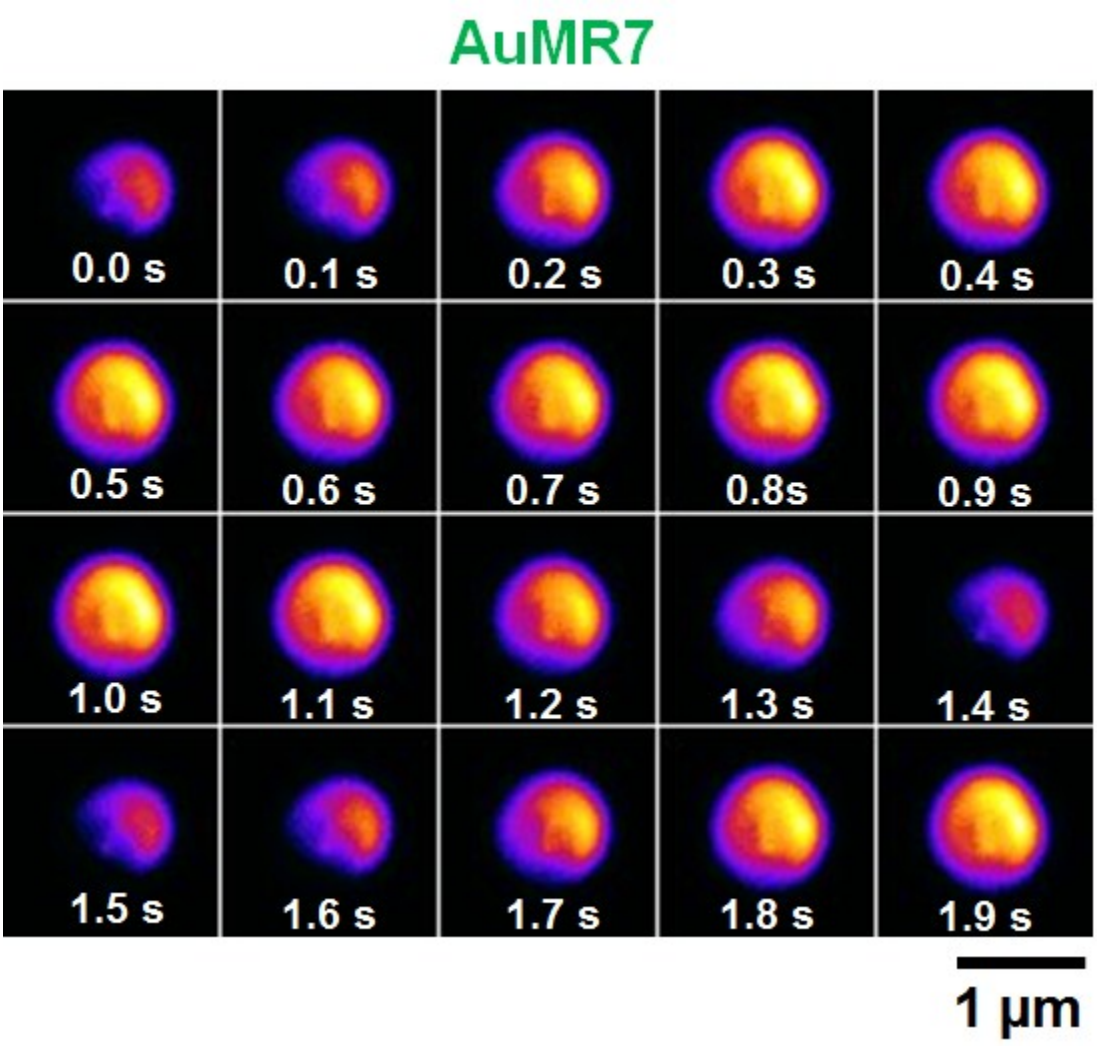

Fig. S5 20-consecutive defocused scattering images of single AuMR7 rotating on a synthetic membrane. The temporal resolution and a defocusing distance were 100 ms and 1 μm, respectively.

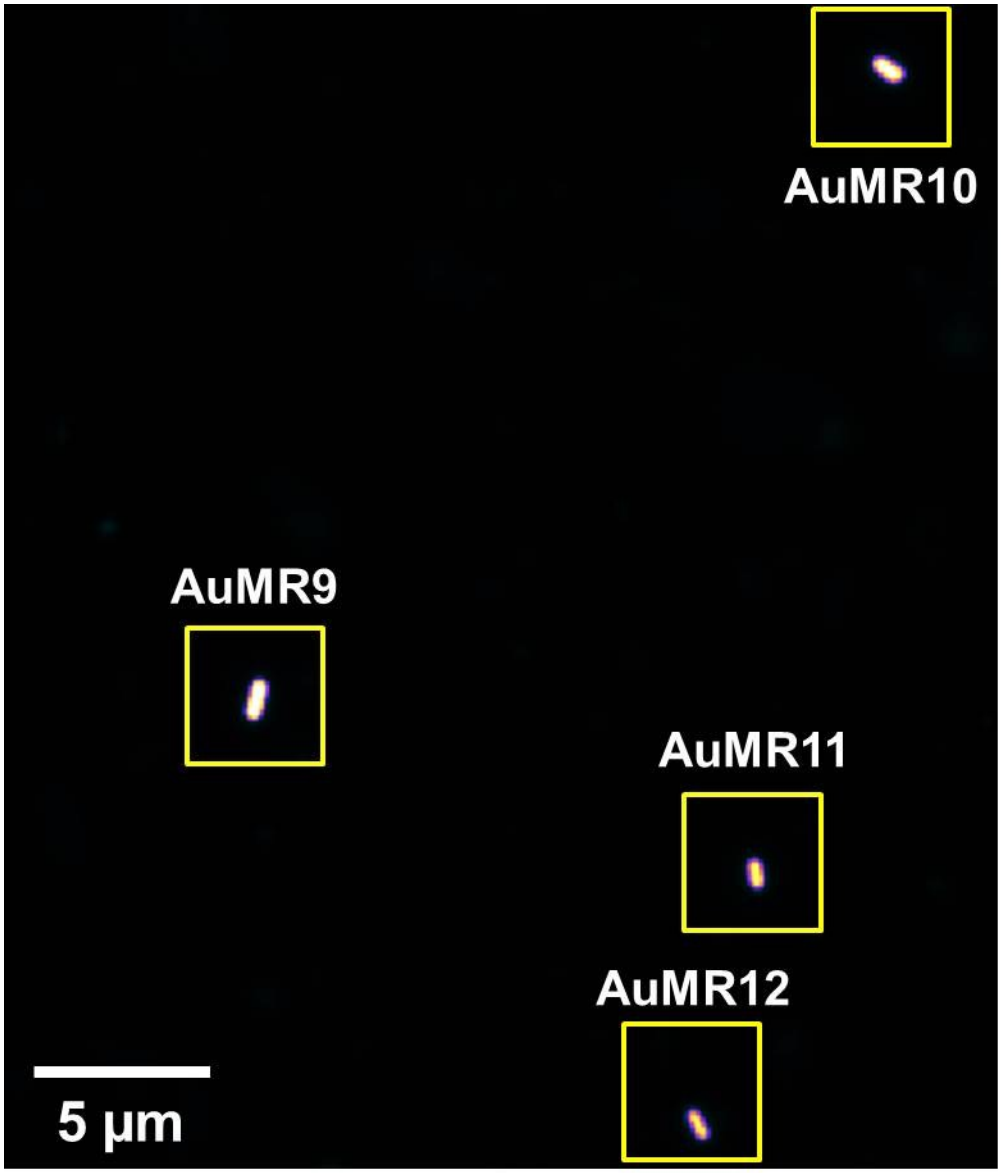

Fig. S6 DF image of single AuMRs bound onto the synthetic membrane. Single AuMRs are directly visualized with their size, shape and in-plane orientation.