Supplementary Information

Detection of C-reactive proteins using single cluster analysis of gold nanoparticle aggregates using dark-field microscope equipped with smartphone

Nanami Fukuzumi,[‡a] Takako Nakagawa,[‡b] Gen Hirao,[a] Atsushi Ogawa,[c] Mizuo Maeda,[d] Tsuyoshi Asahi [a] and Tamotsu Zako [*a,b]

[a]*Department of Chemistry, Graduate School of Science and Engineering, Ehime University, 2-5 Bunkyo, Matsuyama, Ehime 790-8577 Japan E-mail: zako.tamotsu.us@ehime-u.ac.jp*
[b]*Department of Chemistry, Faculty of Science, Ehime University, 2-5 Bunkyo, Matsuyama, Ehime 790-8577 Japan*
[c]*Proteo-Science Center, Ehime University, 2-5 Bunkyo, Matsuyama, Ehime 790-8577, Japan*
[d]*RIKEN Cluster for Pioneering Research, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan*
‡*Equal contribution.*

Table of Contents

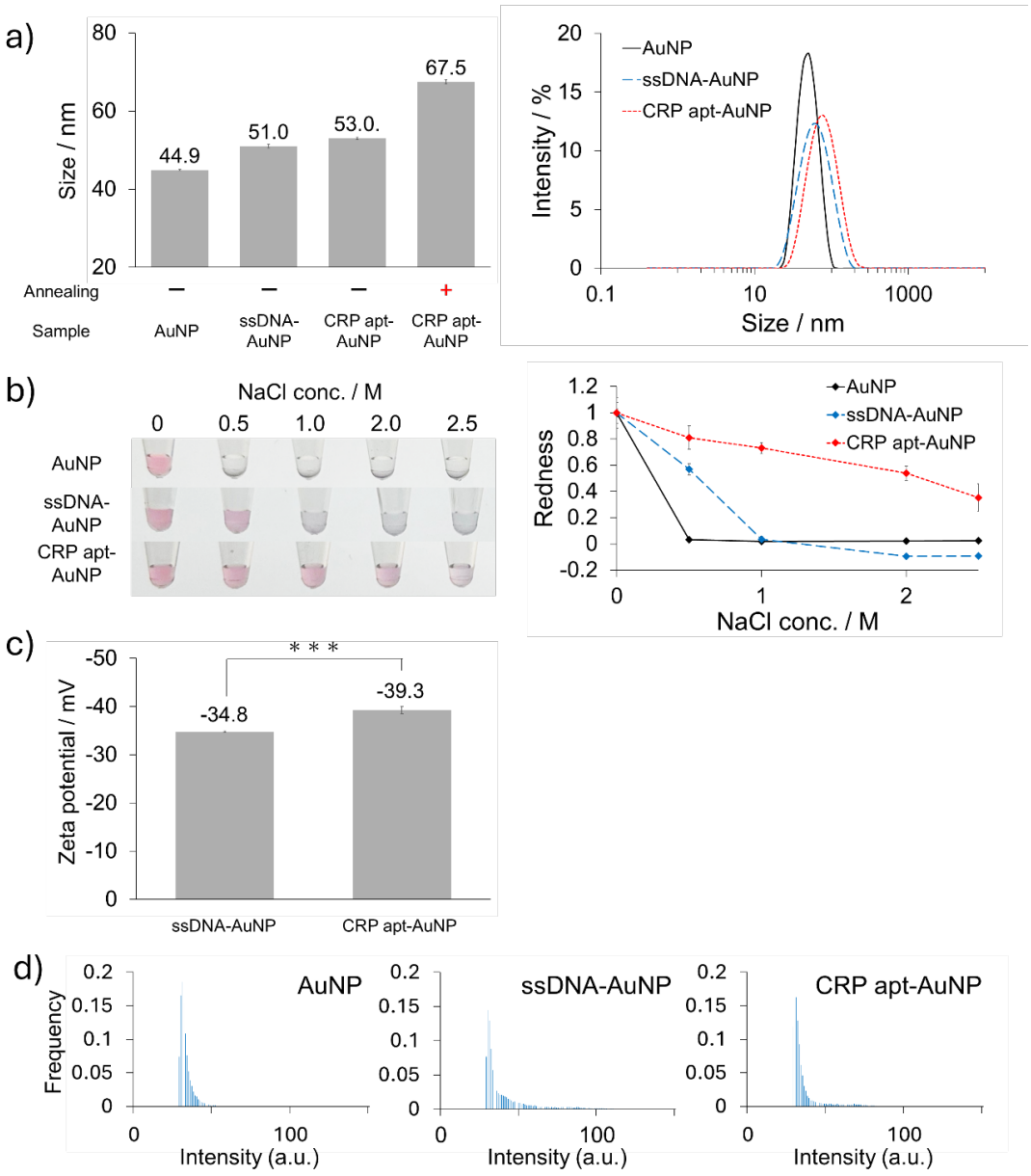

Figure S1 Characterization of ssDNA-AuNPs and CRP aptamer-AuNPs (CRP apt-AuNPs). a) Size of ssDNA-AuNPs and CRP apt-AuNPs. The size of 50 pM unmodified AuNPs, ssDNA-AuNPs, CRP-apt-AuNPs and annealed CRP apt-AuNPs diluted in MQ water was evaluated using a Zetasizer-Nano ZS (Malvern Worcestershire, UK). Size distribution graphs were shown right. b) Stability of ssDNA-AuNPs, CRP-apt-AuNPs against salt. 150 pM AuNP, ssDNA-AuNP and CRP-apt-AuNP were incubated with various concentrations (0–2.5 M) of NaCl for 60 min at room temperature. Redness values were shown right. Averaged values from three tubes were shown. c) Zeta potential of ssDNA-AuNPs and CRP apt-AuNPs. Zetapotential values of 75 pM ssDNA-AuNPs and CRP apt-AuNPs were evaluated using a Zetasizer-Nano ZS (Malvern Worcestershire, UK). The averaged values of the three measurements were shown. (***, $p < 0.005$). (d) DFM observation of unmodified AuNPs, ssDNA-AuNPs and CRP apt-AuNPs. Intensity histograms obtained from DFM images were shown.

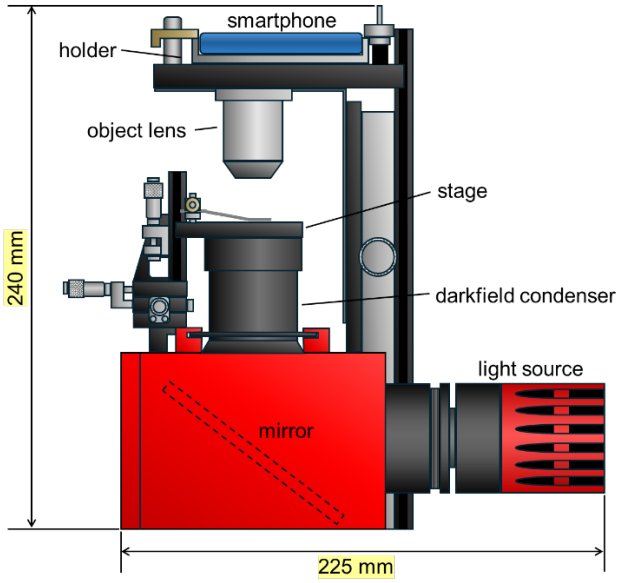

Figure S2 Set up of smartphone DFM. Smartphone DFM was equipped with a dark-field condenser (U-DCW, Olympus), a 50×objective lens (SLMPlan, Olympus), a LED light source (MCWHLP2, Thorlabs, Inc., NJ, USA) and a smartphone (HUAWEI P30, Huawei Device Co., Ltd, Shenzhen, China). The glass slides and smartphone were placed on commercial manual positioning devices and built with the optics into the main body fabricated by a 3D printer (Adventurer 4, FLASHFORGE). Dark-field images were taken using the smartphone.

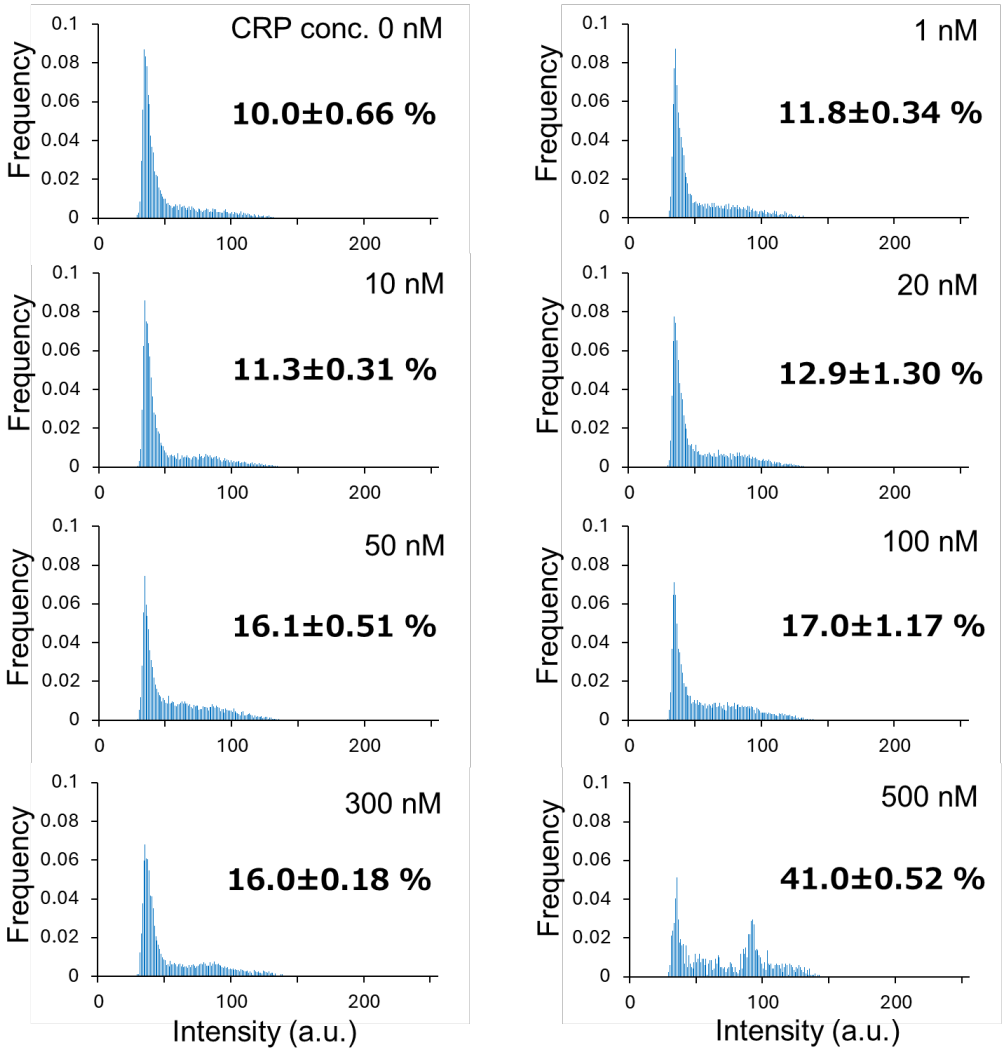

Figure S3 Histograms of the intensities of the spots in the DFM images of CRP aptamer-AuNPs incubated with CRP. CRP aptamer-AuNPs were incubated with various concentrations of CRP (0, 1, 10, 20, 50, 100, 300, 500 nM), and the intensities of the spots in the DFM images (Fig. 3a) were obtained to make histograms. Aggregation ratios obtained from three images were shown in each histogram.

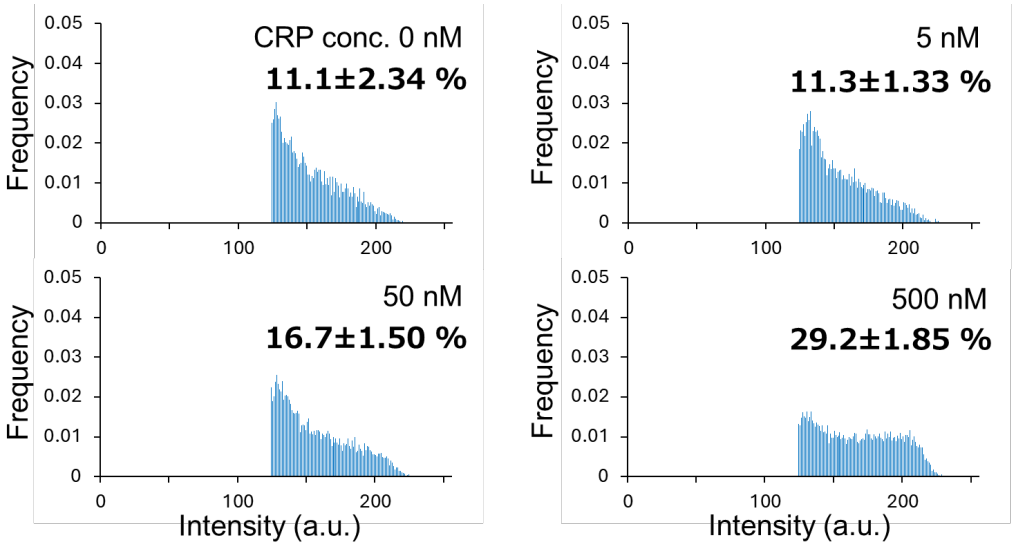

Figure S4 Histograms of the intensities of the spots in the smartphone DFM images of CRP aptamer-AuNPs incubated with CRP. CRP aptamer-AuNPs were incubated with various concentrations of CRP (0, 5, 50, 500 nM), and the intensities of the spots in the smartphone DFM images (Fig. 4b) were obtained to make histograms. Aggregation ratios obtained from three images were shown in each histogram. Threshold of this evaluation is 189 a.u.

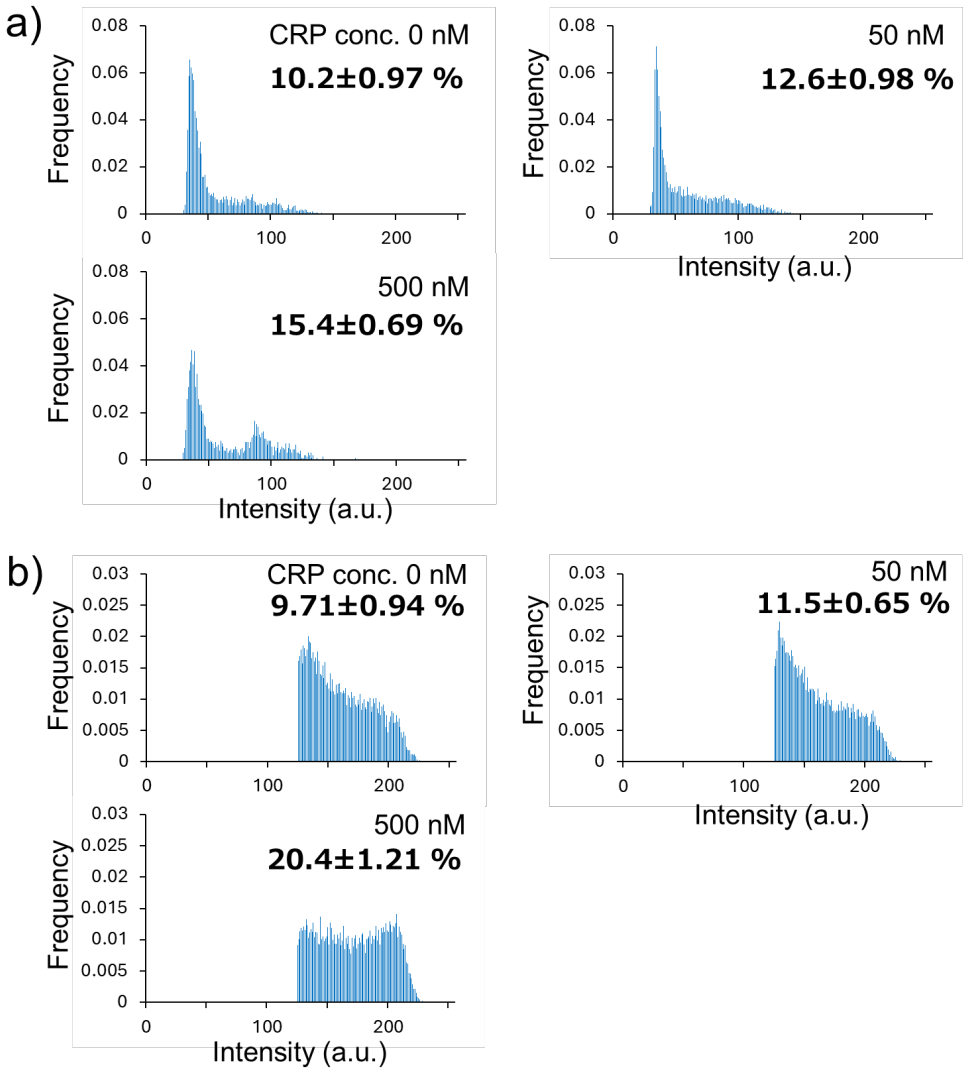

Figure S5 Histograms of the intensities of the spots in the DFM and smartphone DFM images of CRP aptamer-AuNPs incubated with CRP in the presence of FBS. CRP aptamer-AuNPs were incubated with various concentrations of CRP (0, 50, 500 nM) in the presence of FBS, and histograms of the intensities of the spots in the DFM images (a, Fig. 5a) and smartphone DFM images (b, Fig. 5c) were obtained. Aggregation ratios obtained from three images were shown in each histogram. Threshold of this evaluation is 98 a.u. (a) and 201 a.u. (b), respectively.

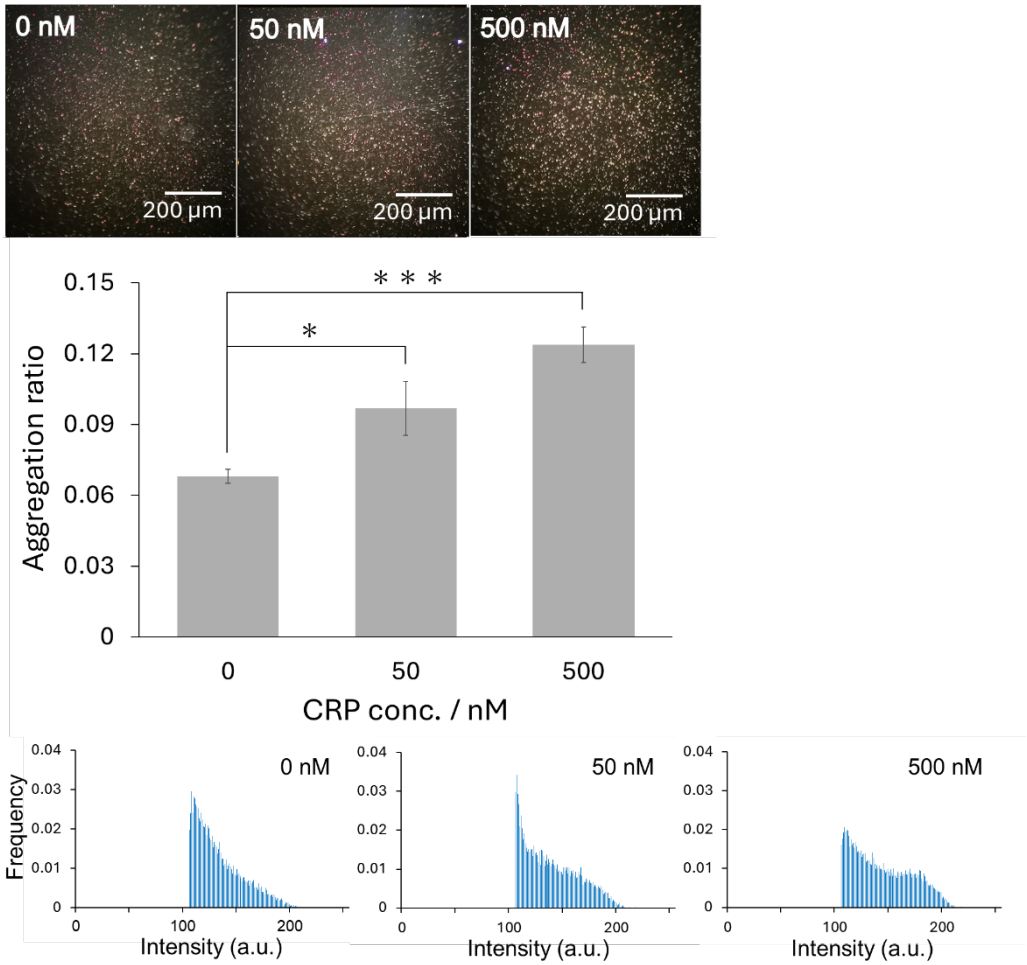

Figure S6 CRP detection in the presence of BSA using smartphone DFM. CRP aptamer-AuNPs were incubated with various concentrations of CRP (0, 50, 500 nM) in the presence of 1% BSA, and DFM images were obtained using smartphone DFM. DFM images (upper), intensity histograms (middle) and aggregation ratios (lower) were shown. Scale bars = 20 µm. The average values of three images are shown. (*, $p < 0.05$, ***, $p < 0.005$). Aggregation ratios obtained from three images were shown in each histogram. Threshold of the evaluation is 182 a.u..

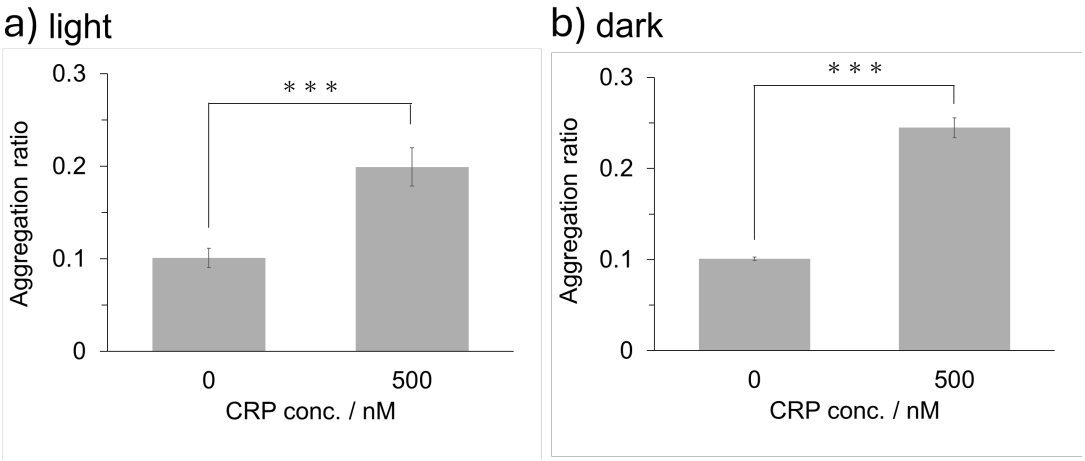

Figure S7 CRP detection under light-dark conditions using smartphone DFM. CRP aptamer-AuNPs were incubated with various concentrations of CRP (0, 500 nM) under light or dark conditions. The averaged aggregation ratios from three DFM images were shown (***, $p < 0.005$). Threshold of the evaluation is 199 a.u..