SUPPORTING INFORMATION

Reversible Social Self-Sorting of Colloidal Cell-Mimics with Blue Light Switchable Proteins

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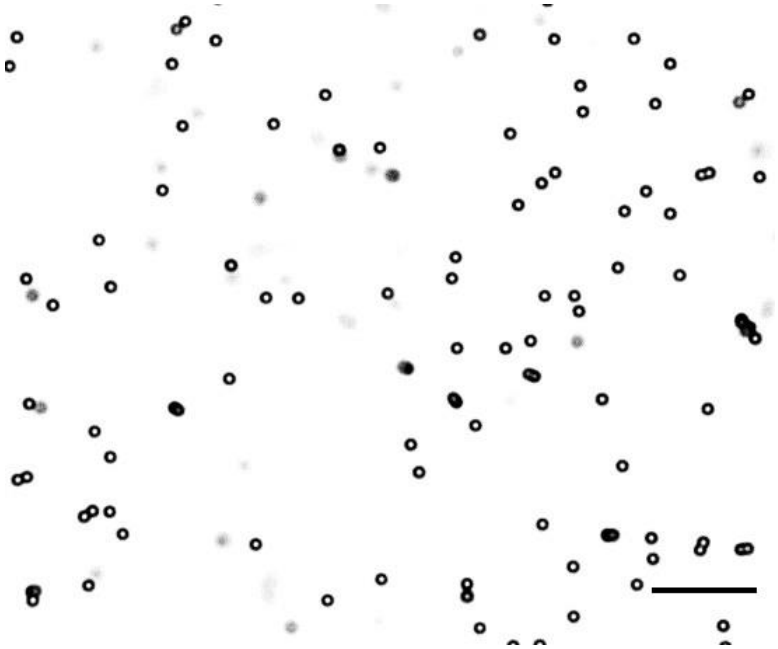
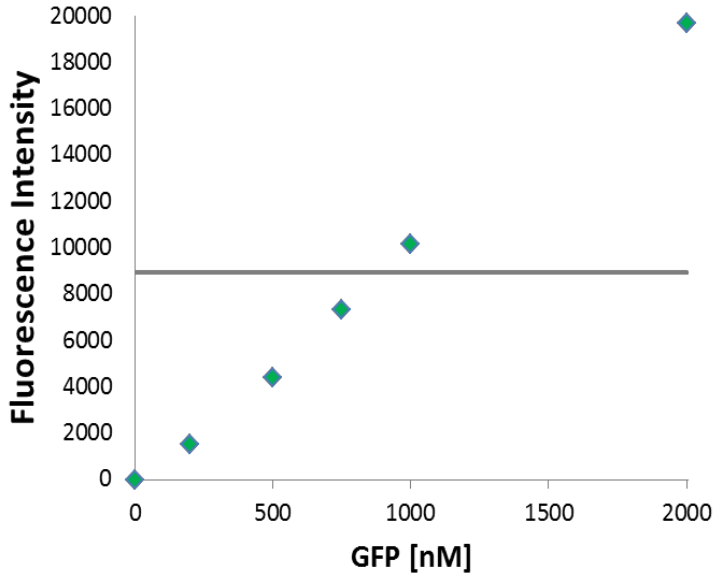


Figure S1. Exemplary bright field microscopy image of non-functionalized on 2 µm Ni^{2+}-NTA polystyrene beads. The scale bar is 15 µm.

a



	Protein in the supernatant [nM]	Binding [nM]	Number of proteins per bead
Beads	918	82	≈220

b

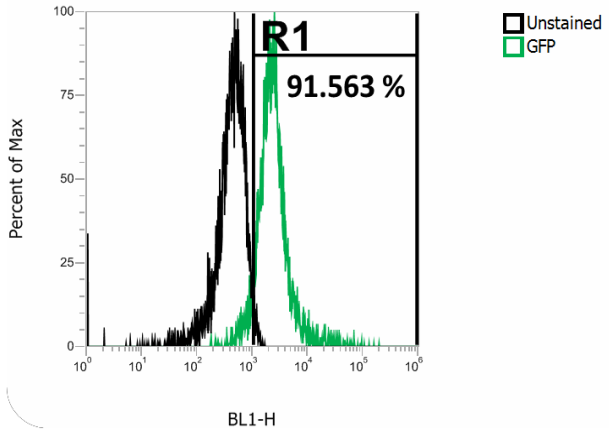


Figure S2. Bead functionalization with proteins. a) Estimation of the amount of GFP bound per bead. Calibration curve for GFP (green fluorescent protein). The fluorescence intensity of the protein that was not bound to the beads (50 mg/ml) and remained in the supernatant is depicted by the grey line. b) Analysis of functionalization efficiency of the Ni^{2+}-NTA polystyrene beads with His-GFP. A single population of GFP-functionalized beads (green) has higher fluorescence signal compared to unfunctionalized beads (black).

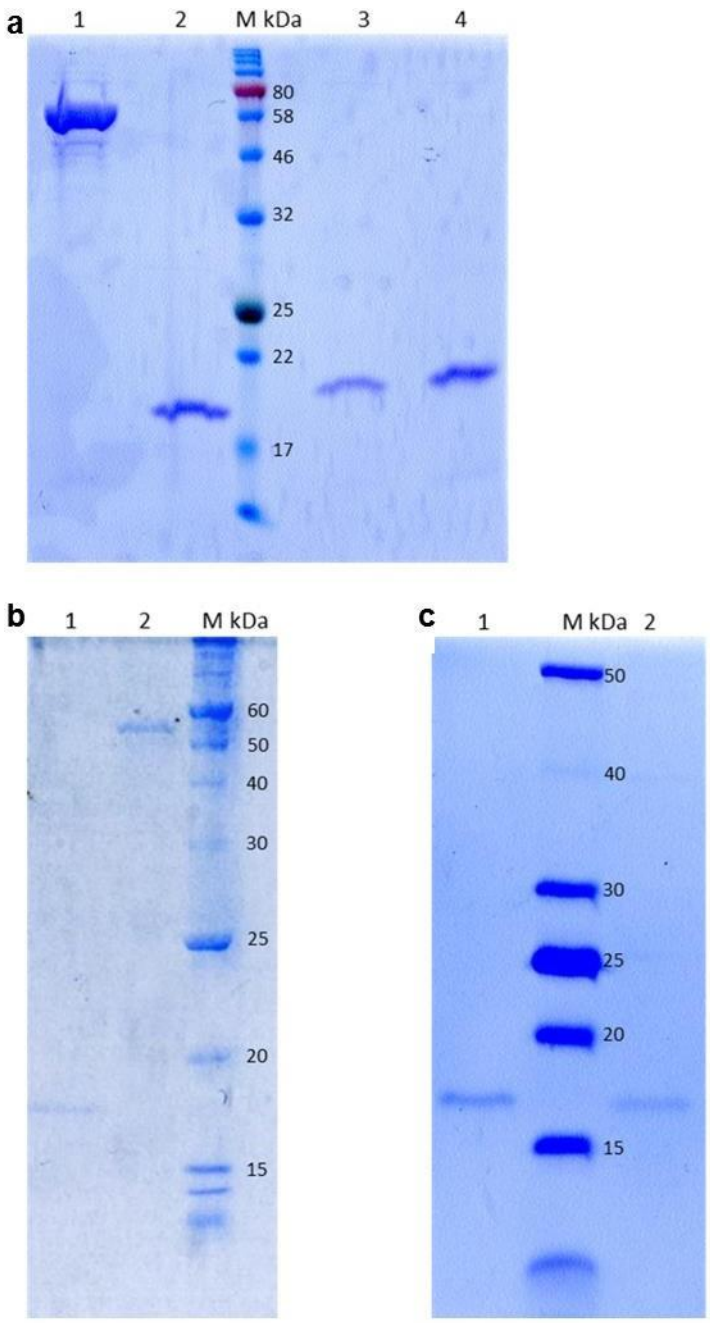


Figure S3. (a) SDS PAGE of the purified proteins: 1) Nano, 2) iLID, M) Marker, 3) nMagHigh, 4) pMagHigh; (b) iLID/Nano protein pair immobilized on the polystyrene beads: 1) iLID, 2) Nano, M) Marker; (c) nMagHigh/pMagHigh protein pair immobilized on the polystyrene beads: 1) nMagHigh, M) Marker, 2) pMagHigh.

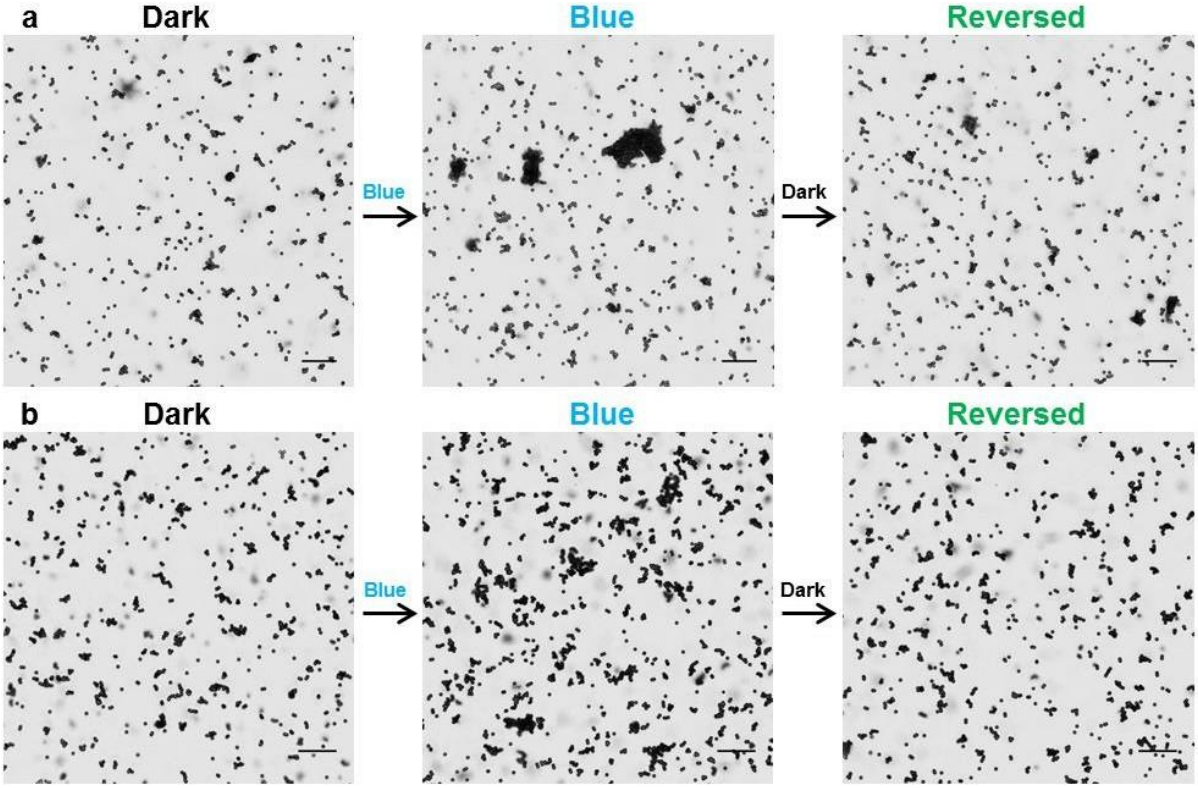


Figure S4. Exemplary bright field images of (a) iLID/Nano and (b) nMagHigh/pMagHigh functionalized beads in the dark for 2 hours, under blue light for 2 hours and reversed in the dark for 1 hour after 2 hour exposure to blue light. The scale bars are 25 µm.

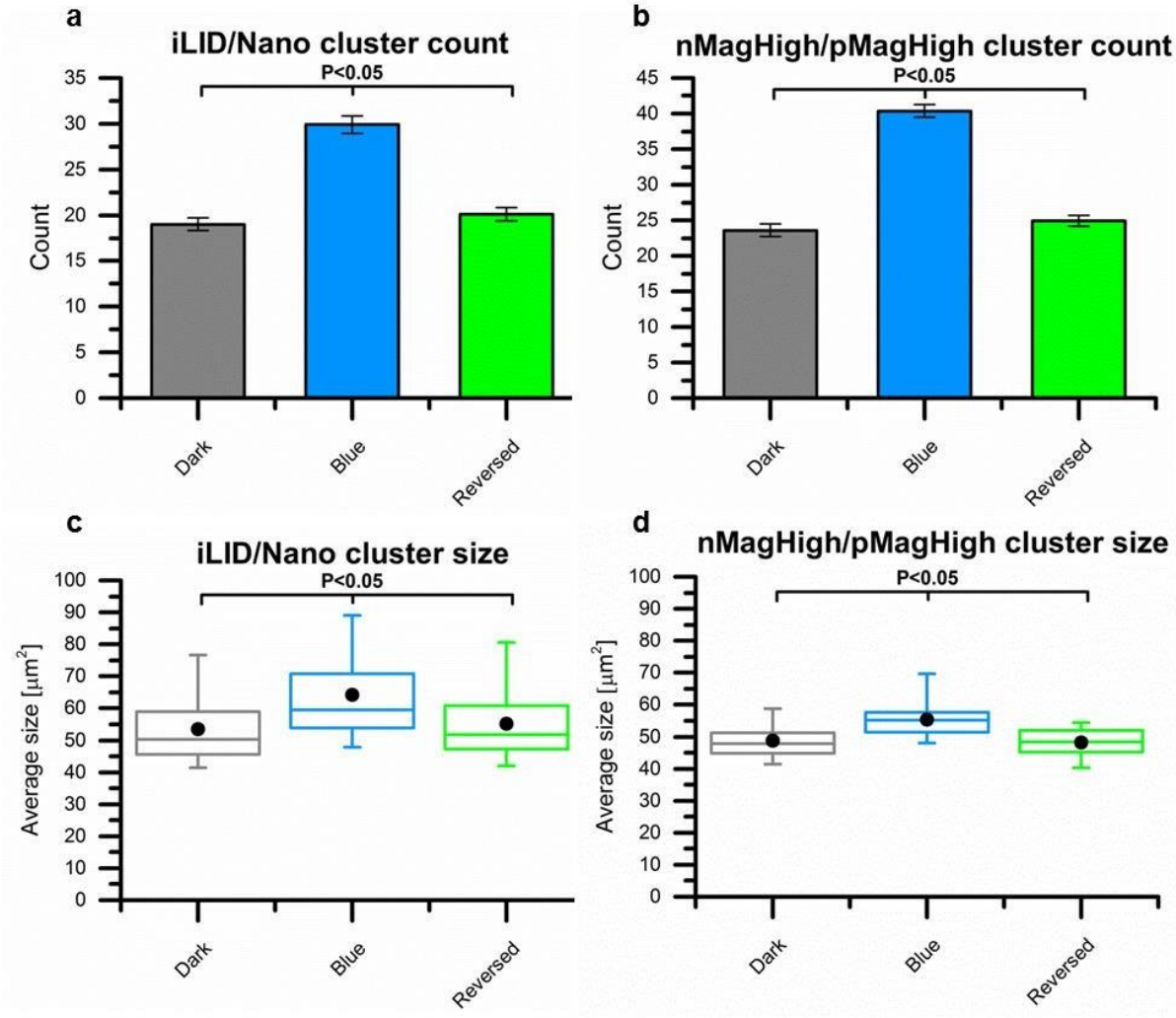


Figure S5. Light-induced reversible aggregation of the polystyrene beads. Cluster count for (a) iLID/Nano; (b) nMagHigh/pMagHigh. Cluster sizes for (c) iLID/Nano; (d) nMagHigh/pMagHigh. One-Way ANOVA test (significance level 0.05) was performed to analyze the statistical difference followed by Dunn-Sidak post hoc test (significance level 0.05). Error bars are the standard error from > 60 images.

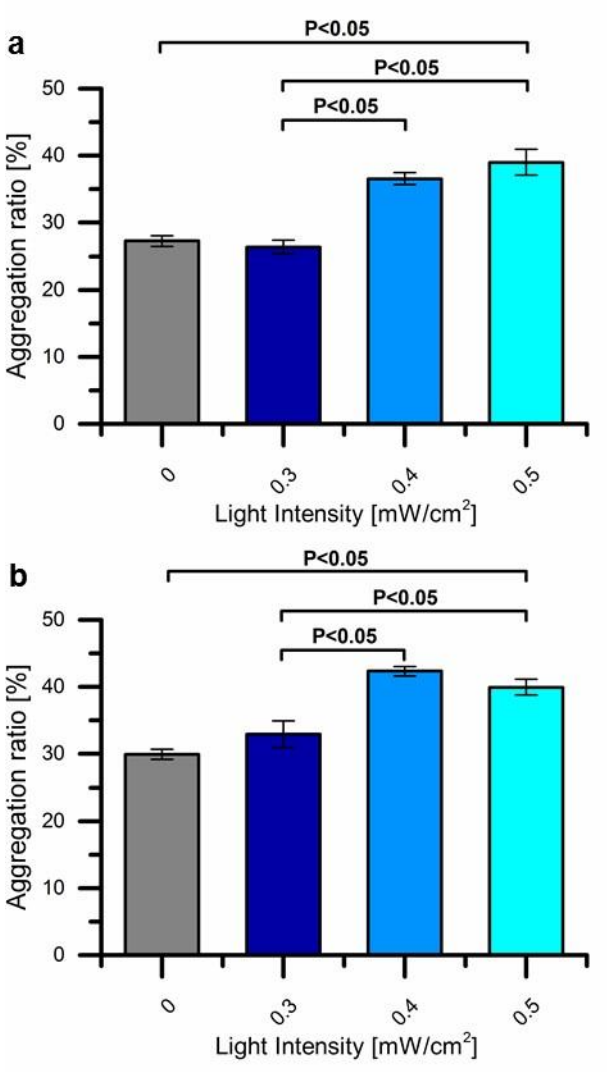


Figure S6. Dependence of bead aggregation on blue light intensity. The standard blue light intensity used in all other experiments is 0.4 mW/cm^2. One-Way ANOVA test (significance level 0.05) was performed to analyze the statistical difference followed by Dunn-Sidak post hoc test (significance level 0.05). Error bars are the standard error from > 15 images.

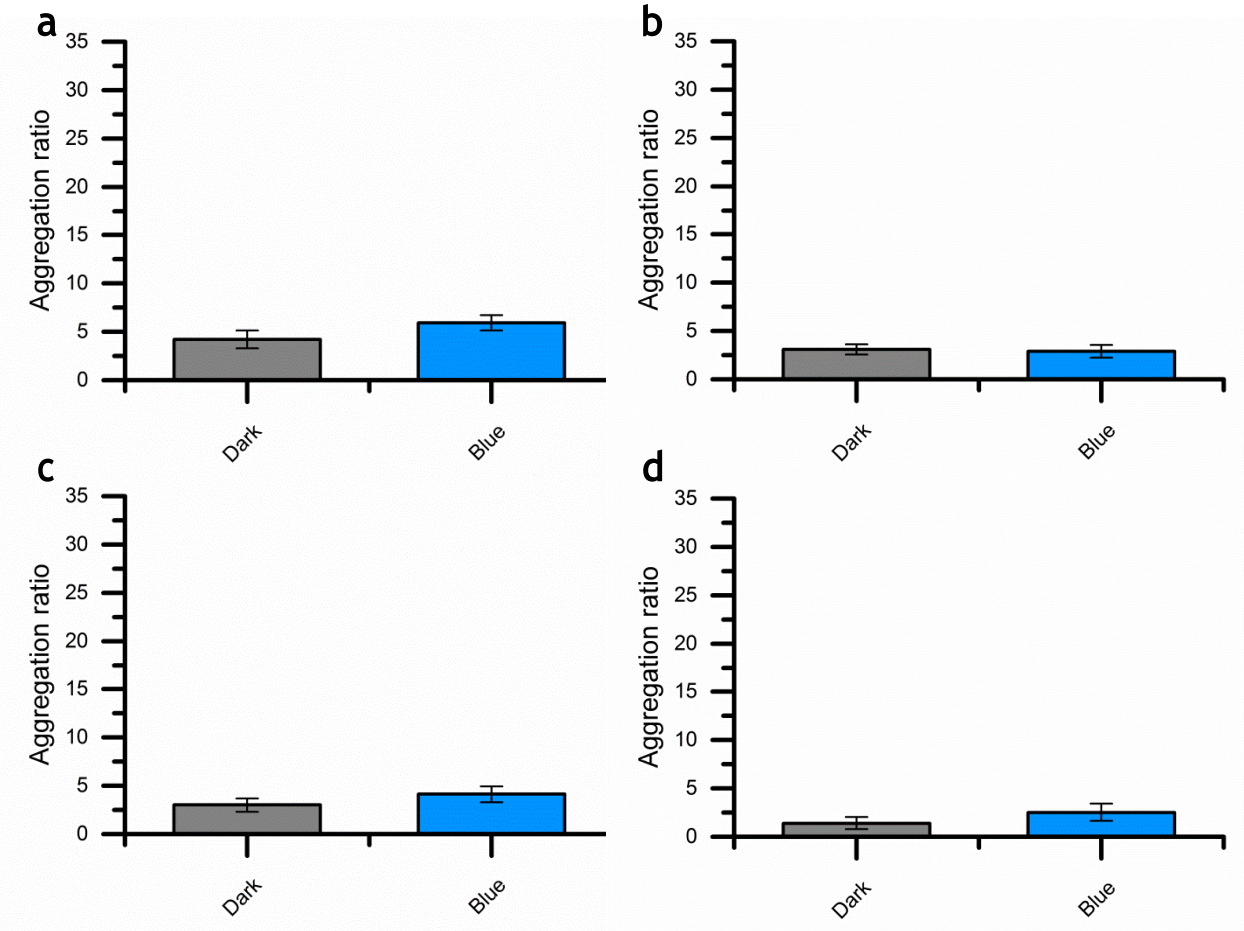


Figure S7. Light-dependent aggregation in presence of blocking agents: A mixture of a) iLID/Nano and b) nMagHigh/pMagHigh beads in the presence of 0.2 % (w/v) BSA; A mixture of c) iLID/Nano and d) nMagHigh/pMagHigh beads in the presence of 0.2 % (w/v) Pluronic® F-127. Mann-Whitney test (significance level 0.05) was performed to analyze the statistical difference, no significant difference was found between dark and blue light in any sample. Error bars are the standard error from 15 images.

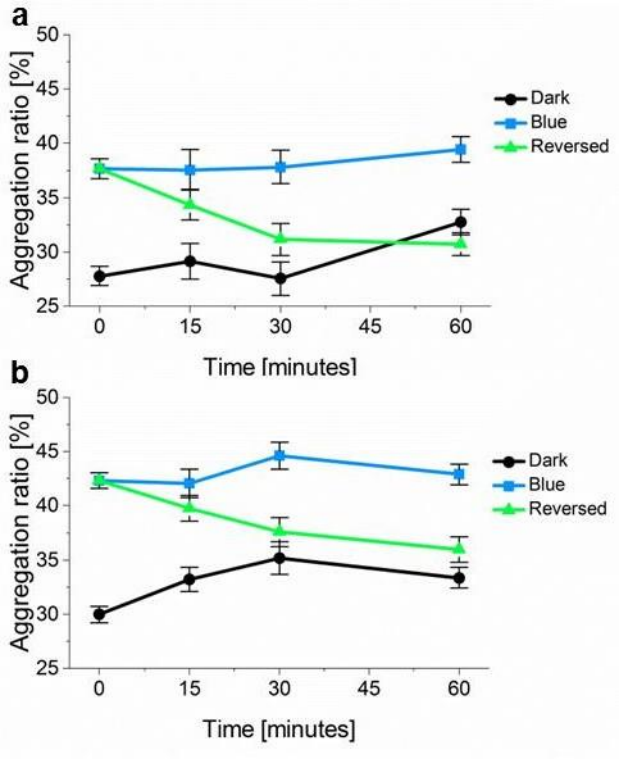


Figure S8. Reversion dynamics of blue light dependent bead aggregation in the dark for a) the iLID/Nano and b) the nMagHigh/pMagHigh protein pair. Reversed samples (green) were kept under blue light illumination for 2 hours before placing them into the dark. The dark (black) and blue (blue) samples were kept for 2 hours and during the data acquisition in the dark and under blue light, respectively. Error bars are the standard error from > 30 images.

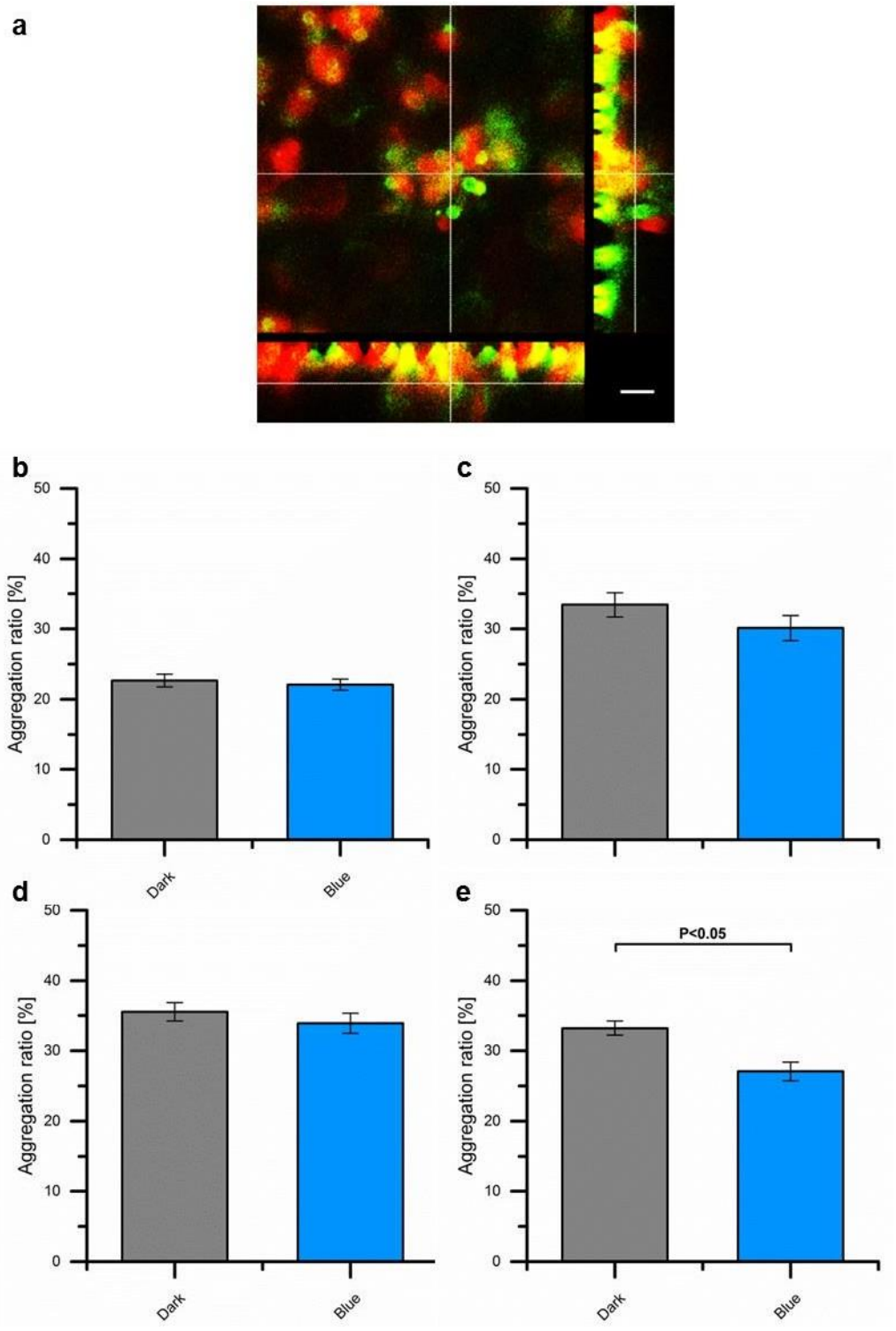


Figure S9. (a) Exemplary confocal fluorescence microscopy image of a 3D cluster formed under blue light with nMagHigh (red) and pMagHigh (green) functionalized beads. The scale bar is 5 µm. Homodimerization of (b) iLID, (c) Nano, (d) nMagHigh and (e) pMagHigh functionalized beads. Mann-Whitney test (significance level 0.05) was performed to analyze the statistical difference. Error bars are the standard error from > 40 images.

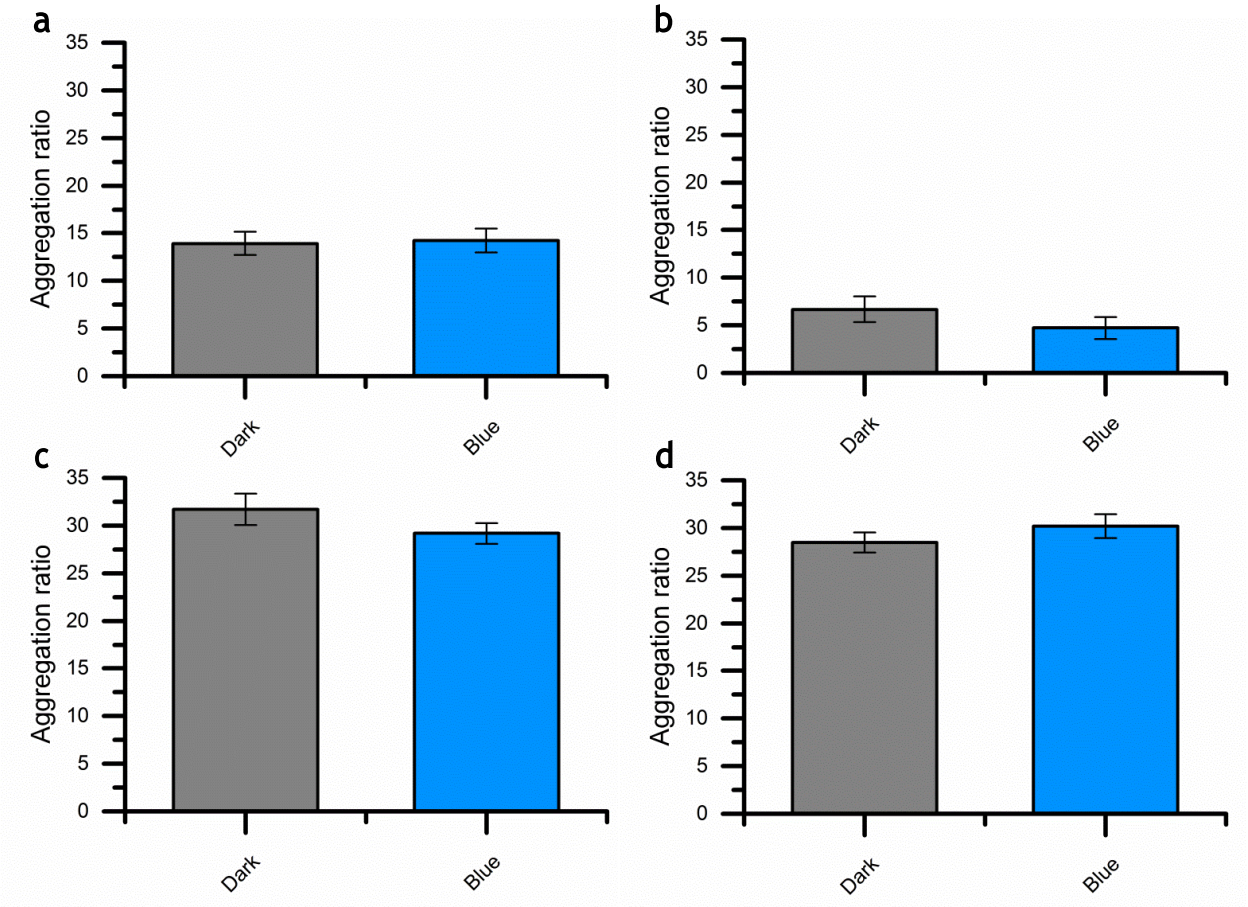


Figure S10. Light-dependent aggregation in presence of soluble protein binding partners: A mixture of iLID/Nano beads in the presence of a) 1μM iLID and b) 1μM Nano. A mixture ofnMagHigh/pMagHigh beads in the presence of c) 1μM nMagHigh and d) 1μM pMagHigh. Mann-Whitney test (significance level 0.05) was performed to analyze the statistical difference, no significant difference was found. Error bars are the standard error from 15 images.

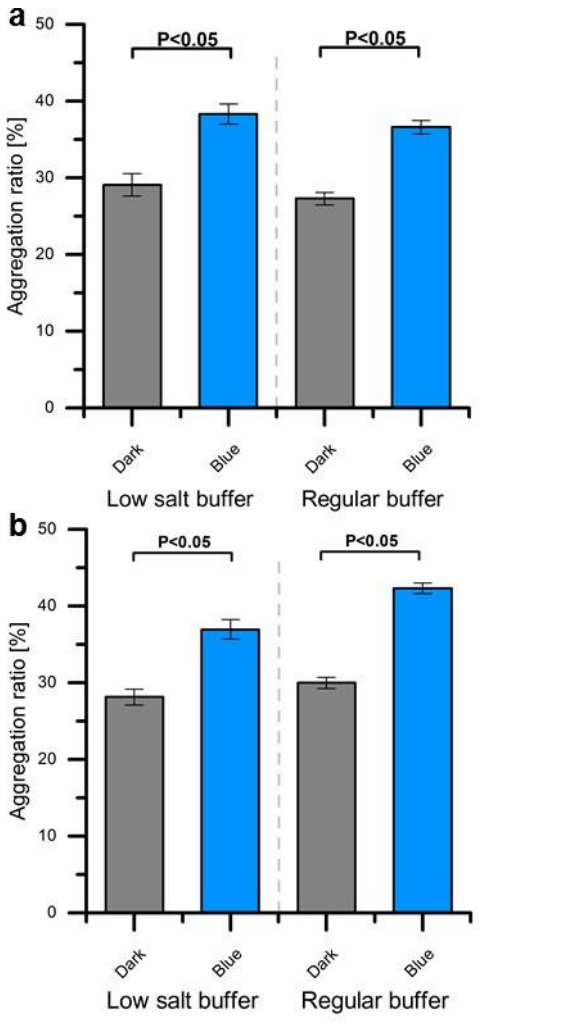


Figure S11. Light-dependent aggregation in buffers with different salt concentrations. Low salt buffer: 100 mM NaCl, 10 mM Tris, pH 7.4; regular buffer: 300 mM NaCl, 50 mM Tris, pH=7.4. Mann-Whitney test (significance level 0.05) was performed to analyze the statistical difference. Error bars are the standard error from 30 images.