

Real-time investigation of dynamic protein crystallization in living cells

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SUPPLEMENTAL MATERIAL

FIGURE S1

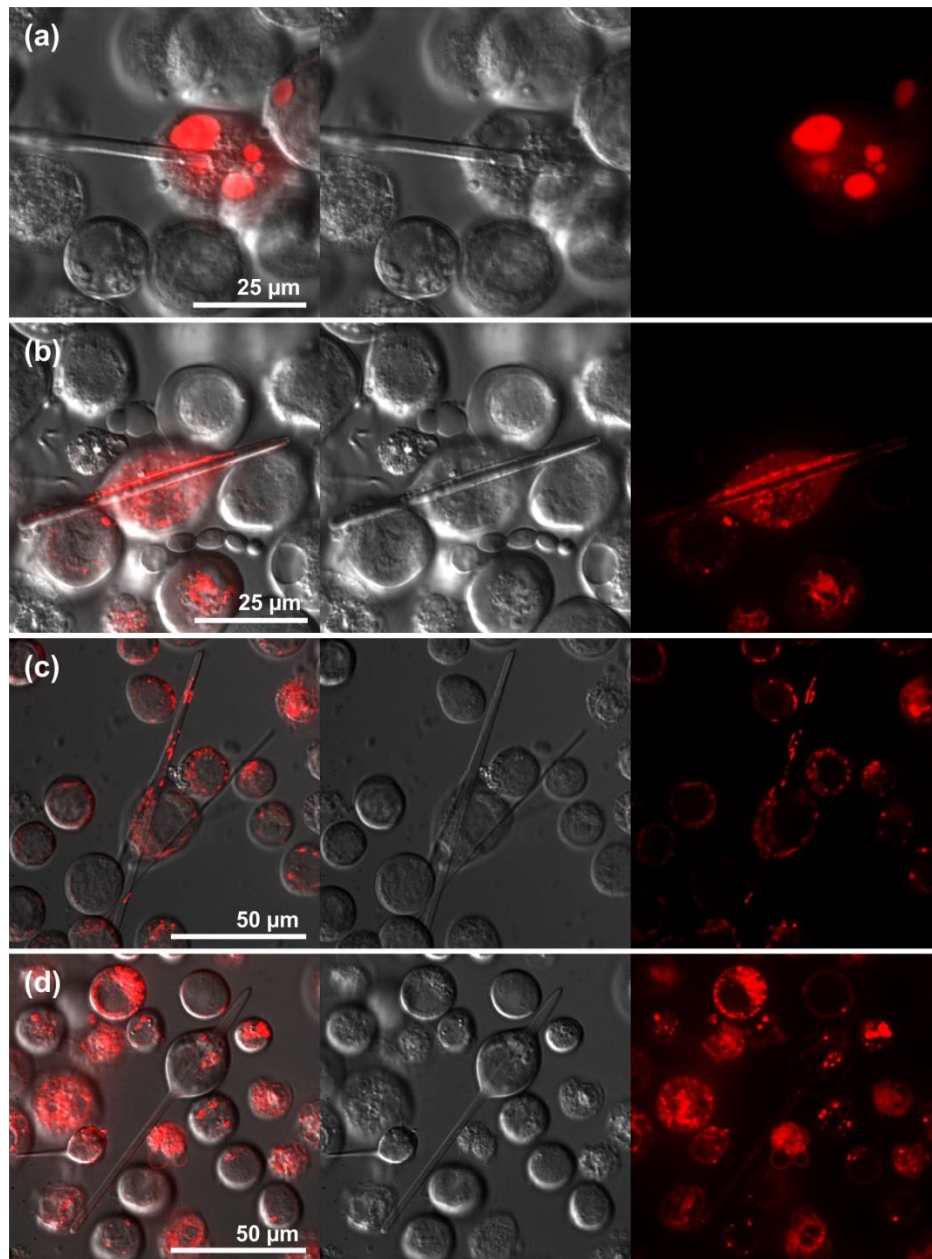


FIG. S1. Localization of *in vivo* firefly luciferase crystals. (a) Cells were co-infected with recombinant luciferase and mCherry-KDEL baculovirus. Confocal images with DIC optics and the mCherry fluorescence channel were taken 3 days p.i. Crystals are not surrounded by mCherry fluorescence, ruling out the endoplasmic reticulum as the compartment of crystallization for luciferase. (b) Cells were co-infected with recombinant firefly luciferase and Pex3 baculovirus. Pex3 was fused to mCherry protein to act as a peroxisomal membrane marker. Confocal images with DIC optics and the mCherry fluorescence channel were taken 4 days p.i. The internal membrane surrounding the crystal is clearly labeled with the peroxisomal marker protein. (c) Mitochondria were stained with MitoTracker, as expected. Note that MitoTracker does not stain the membrane surrounding the crystal. Numerous mitochondria are visible alongside the crystal, even near the far tip of the crystal that is spiking out of the cell body. (d) Lysosomal structures were stained with LysoTracker. In virus infected cells, lysosomes are visible together with a weak plasma membrane staining. Note that no labeling of the membrane surrounding the crystal was observed. Small lysosomes are visible along the full length of the crystal.

FIGURE S2

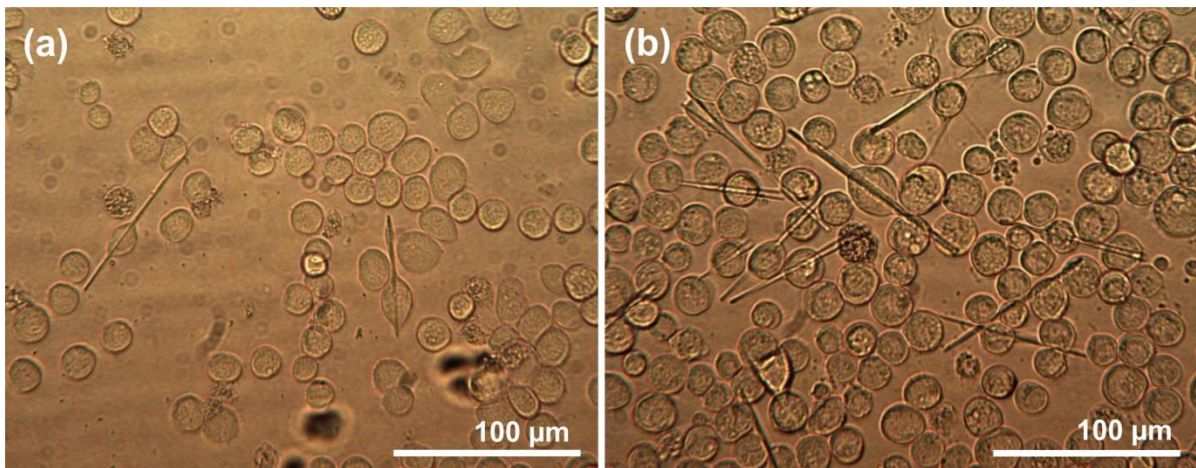


FIG. S2. Virus-free vs. baculovirus induced *in vivo* crystallization of luciferase. (a) A pIEX4 vector containing the gene for the firefly luciferase was transfected into 0.5×10^6 Sf9 insect cells by lipofection with ESCORT reagent and grown in serum-free medium. After 24 hours, the first needle like crystals were observed. Images were taken three days after transfection with phase contrast optics on a Brunel SP95I microscope. (b) 1×10^6 Sf9 insect cells were infected with recombinant baculovirus expressing luciferase and incubated for 3 days in parallel to the virus-free transfection experiment.

FIGURE S3

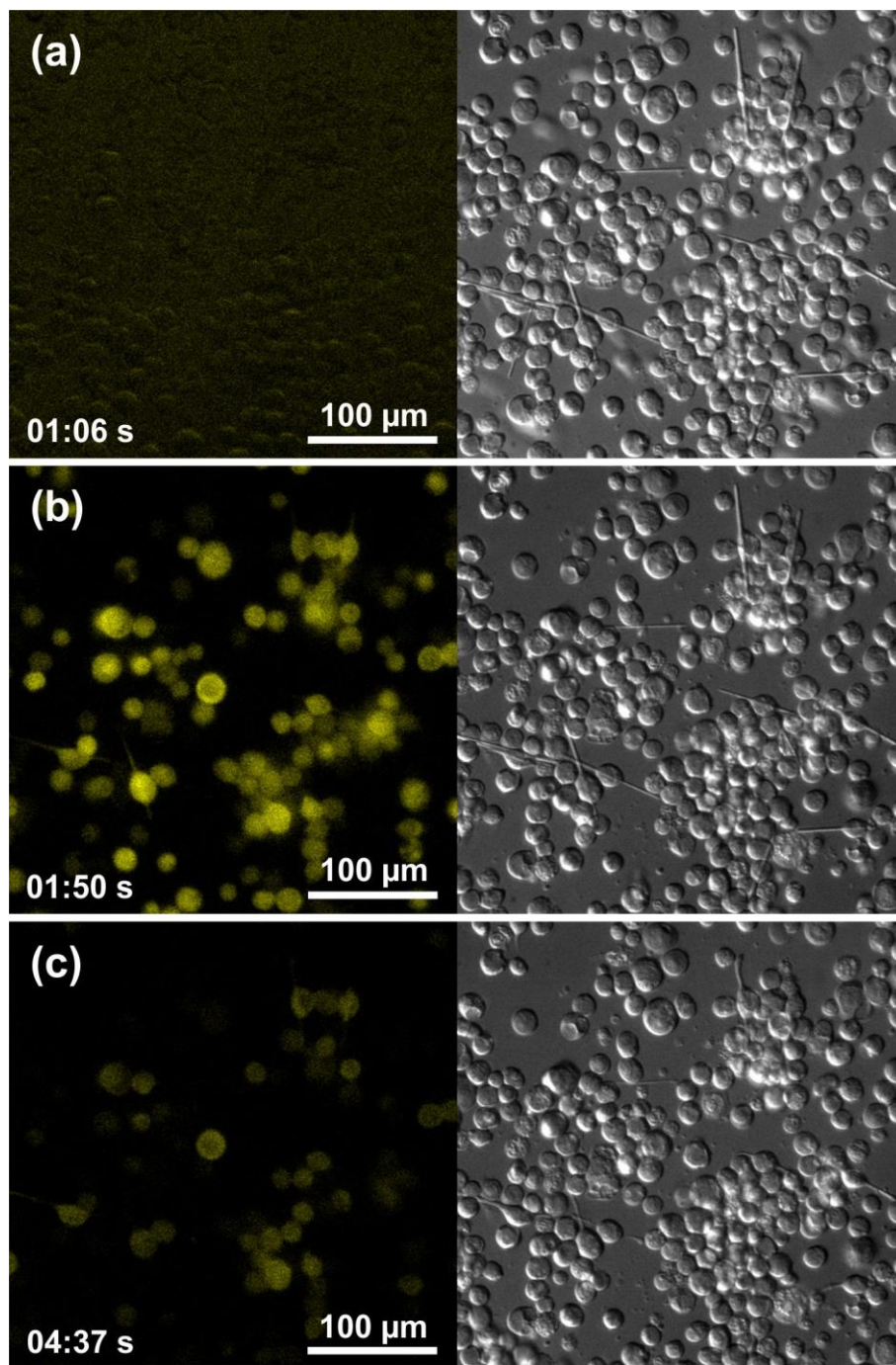


FIG. S3. Soluble recombinant firefly luciferase is still enzymatically active within the cells but no longer when it enters into the crystalline state. At 4 days p.i. with Bac-luc, luciferase activity was tested *in vivo* with a membrane permeable luciferin ester. During image acquisition 100 μ M luciferin ester was added to the cells. (a-c) On the left side of the panels luciferin bioluminescence is shown. A significant peak in bioluminescence is observable. As is clearly visible from the DIC images on the right side of the panels, the luciferase crystals in the intact cells dissolve very quickly upon addition of D-Luciferin ethyl ester. Note that only a fraction of the luciferase expressing cells show crystals in the beginning. See the corresponding movie in the supplemental material (Fig. S3 movie).

FIGURE S4

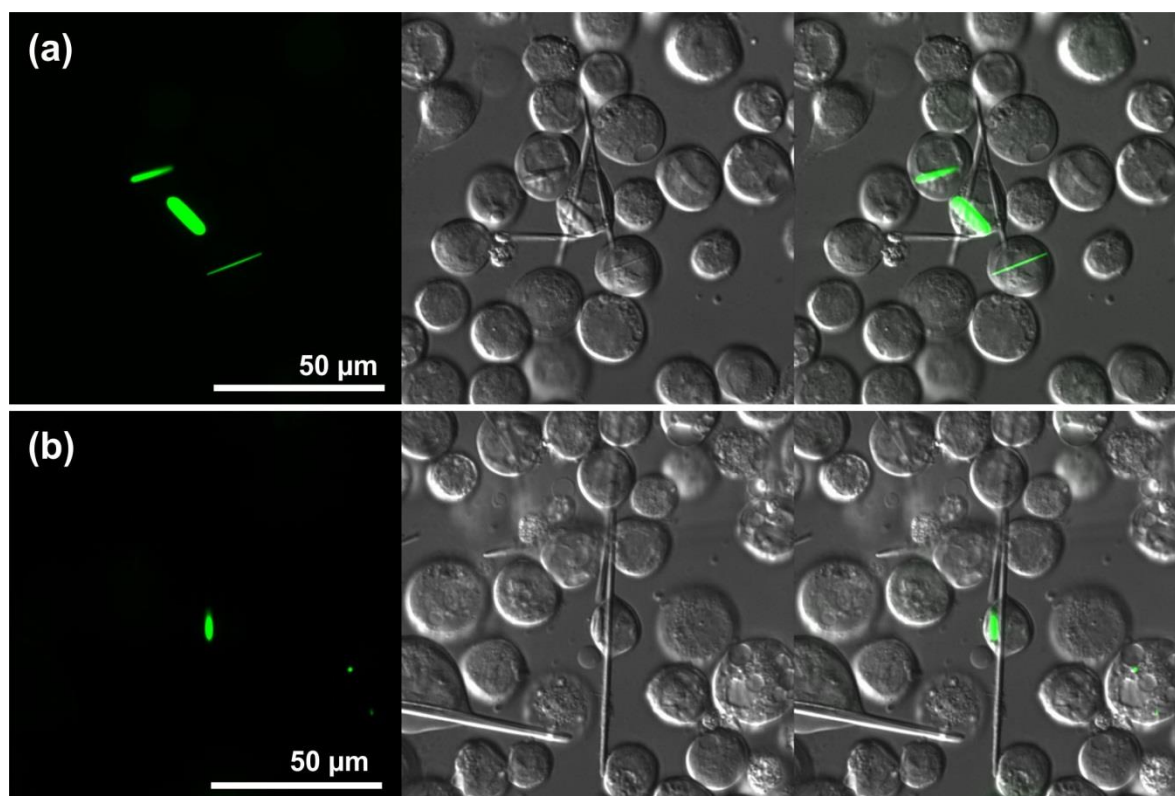


FIG. S4. GFP- μ NS crystallizes together with firefly luciferase in the same cell. (a, b) Cells were co-infected with recombinant luciferase and GFP- μ NS baculovirus. Confocal images with DIC optics and the GFP fluorescence channel were taken at 4 days p.i. Crystals of both proteins can develop in the same cell without obvious changes to the crystal morphology of either of the proteins.

FIGURE S5

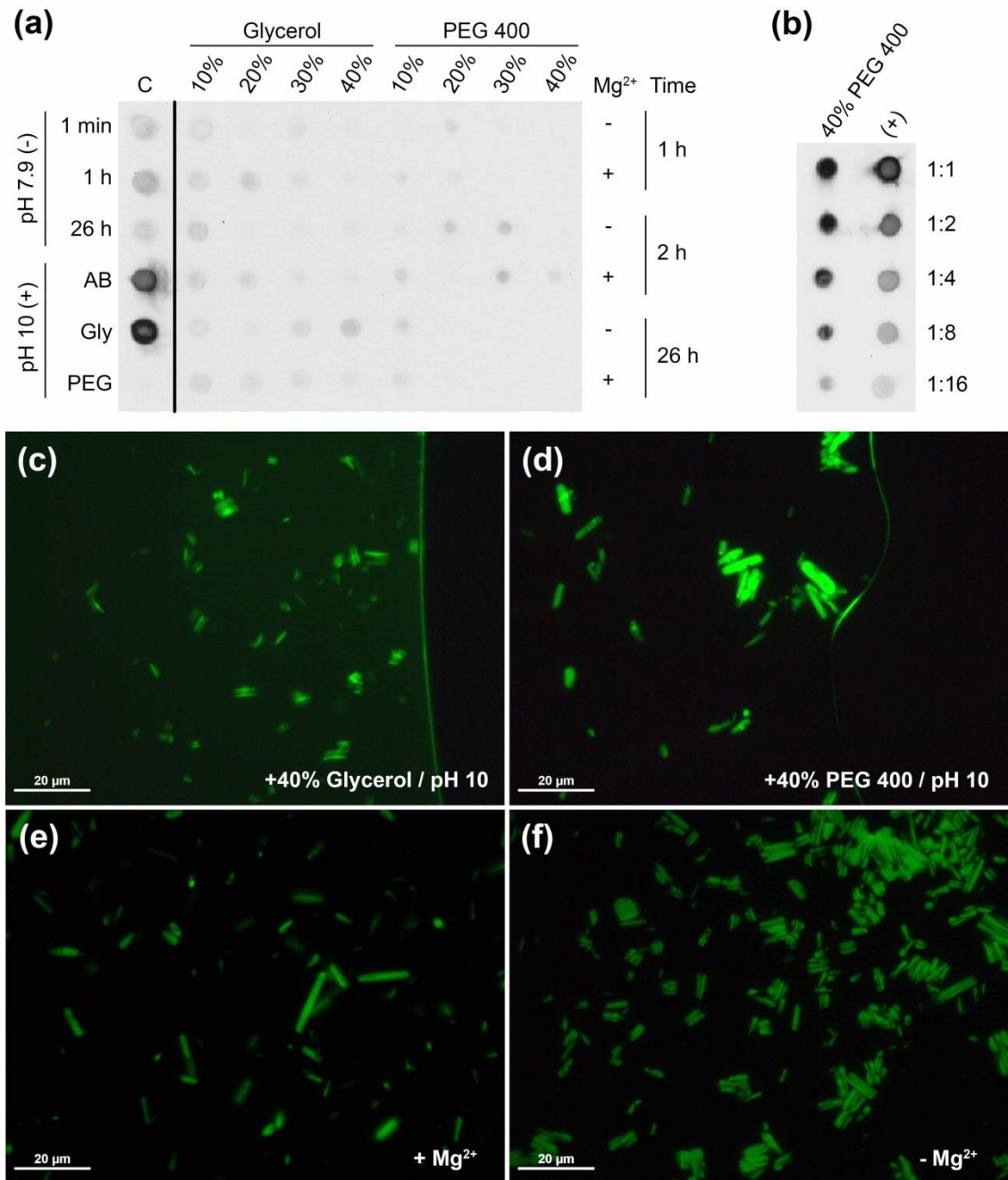


FIG. S5. Stability analysis of isolated GFP-μNS crystals preliminary to x-ray diffraction experiments. (a) Dot Blot analysis of to test the impact of the cryo-protectants glycerol and PEG 400 on GFP-μNS crystal stability. Crystals were incubated in storage buffer (10 mM HEPES pH 7.9, 10 mM KCl, 5 mM MgCl₂) containing different amounts of glycerol or PEG 400 (10-40%), respectively. After 1, 2, and 26 hours the crystal suspensions were centrifuged and samples were taken from supernatant and spotted on a nitrocellulose membrane. Mouse-α-GFP and HRP-conjugated α-mouse antibodies were used for detection of solubilized GFP-μNS by ECL. Dot Blot analysis shows no significant impact of glycerol and PEG 400 concentrations of up to 40% on crystal stability at pH 7.9. Controls (C): Negative controls (pH 7.9 (-)): Crystals incubated in storage buffer for 1 min, 1 h and 26 h. Positive controls (pH 10 (+)): Crystals incubated for 1 h in alkaline buffer (50 mM CAPS pH 10, 10 mM KCl, 5 mM MgCl₂) without cryo-protectant (AB), with 40% glycerol (Gly), or with

40% PEG 400 (PEG). Crystals dissolved in alkaline buffer with and without glycerol, as high signal intensity shows. When 40% PEG 400 was present at pH 10 no signal was detected, hence the crystals remained stable. (b) Dot Blot analysis of GFP- μ NS crystals under crystal dissolving conditions. Crystals were first dissolved in alkaline buffer (50 mM CAPS pH 10, 10 mM KCl, 5 mM MgCl_2) without cryo-protectant (right, (+)) before PEG 400 was added up to a final concentration of 40% (left). Sample preparation and detection was performed as described in (a). Samples with and without PEG 400 show comparable intensities, hence membrane- and antibody binding were not affected by 40% PEG 400. (c – f) Fluorescence microscopic images (GFP channel) of GFP- μ NS crystals under different buffer conditions reveal higher crystal stability in presence of PEG 400 and Mg^{2+} . At pH 10, GFP- μ NS crystals instantly started to dissolve if 40% glycerol was used as cryo-protectant (c) but remained stable in the presence of 40% PEG 400 (d). Crystals were stable under storage buffer conditions (10 mM HEPES pH 7.9, 10 mM KCl) in the presence of 5 mM Mg^{2+} (e), but fanned out in the absence of Mg^{2+} (f).