Electronic Supplementary Material (ESI) for Lab on a Chip.

Real-time observation of protein aggregates in pharmaceutical formulations using liquid cell electron microscopy†

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ELECTRONIC SUPPLEMENTARY INFORMATION†

FIGURES

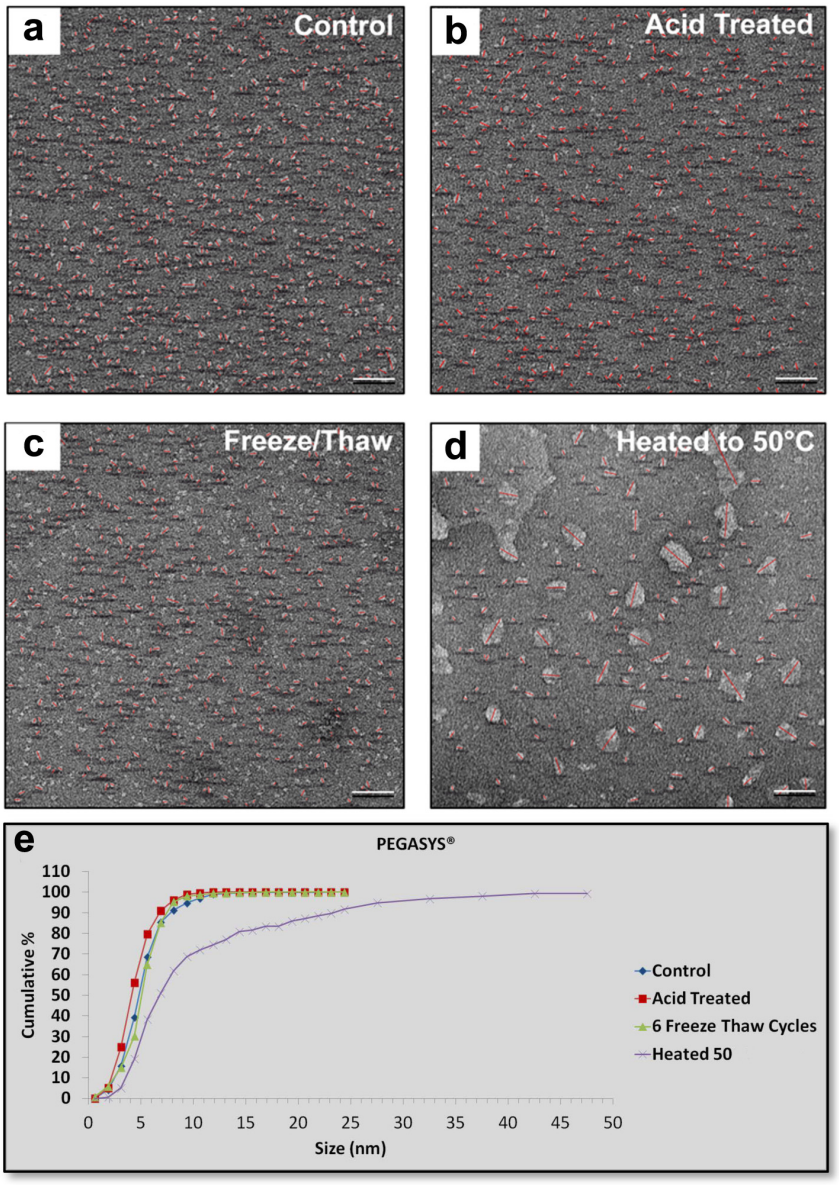

Fig. 1. Quantitative analysis of PEGylated interferon α_{2a} particles present in EM images. (a - d) For each stressed condition, we measured the length of each PEGylated interferon α_{2a} particle present in EM images according to previously defined procedures.[21] Dimensions across the long axis of each particle were measured using the Clemex image analysis system and validated using the SPIDER[22] and PARTICLE (http://www.image-analysis.net/EM/) software packages. Scale bar is 50 nm. Objects up to 50 nm were including in our statistical analysis. Items outside of this range (>10 times the size of the Pegasys® monomer) were excluded. **(e)** Plot to indicate the relationship between particle size and the percentage of particles present within each size range under stressed and control conditions.

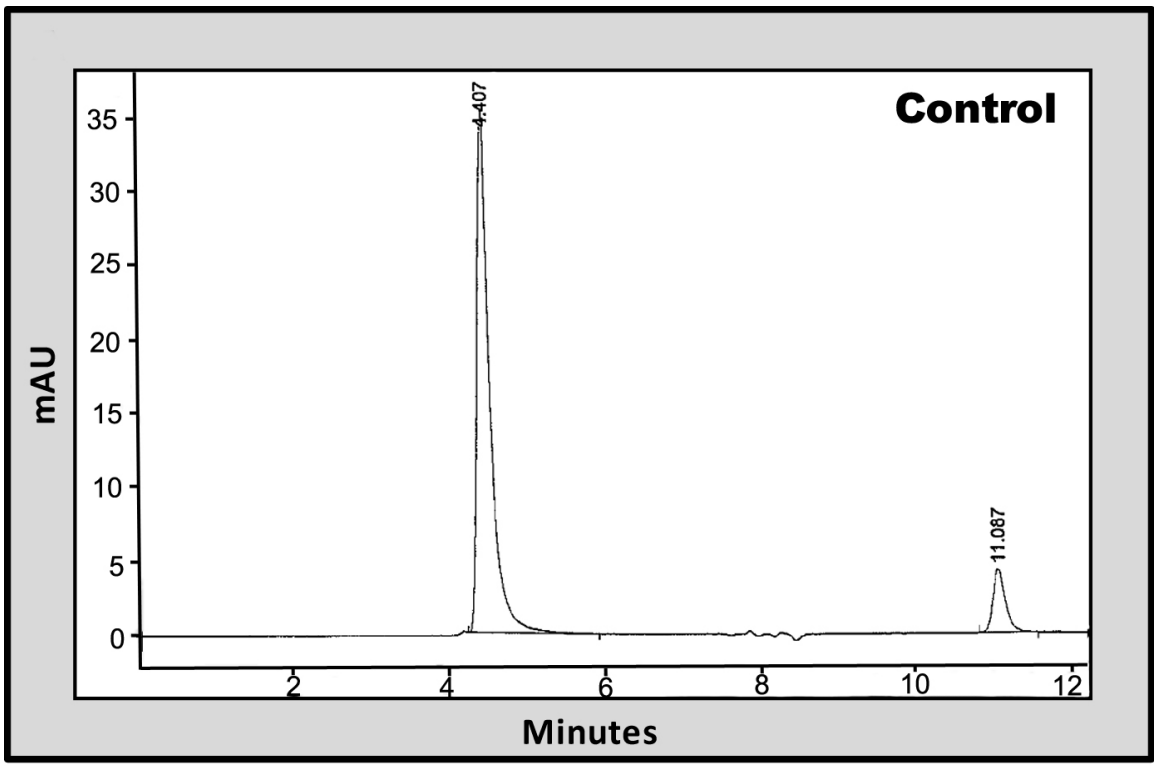

Fig. 2. Size exclusion chromatography (SEC) results of PEGylated interferon α_{2a} evaluated under control conditions. SEC chromatograph of untreated PEGylated interferon α_{2a} shows a major protein peak with a retention time of ~4.4 minutes.

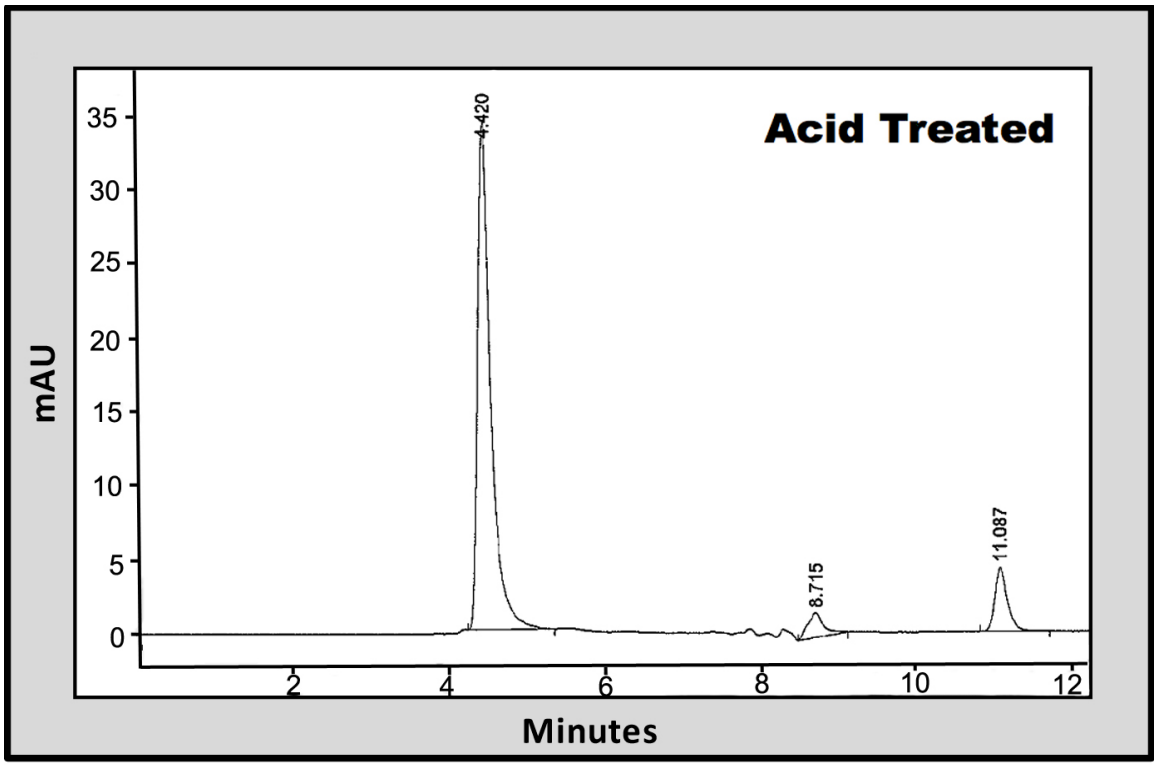

Fig. 3. SEC results of PEGylated interferon α_{2a} evaluated under acid treated conditions. SEC chromatograph of acid treated PEGylated interferon α_{2a} shows a major protein peak with a retention time of ~4.4 minutes.

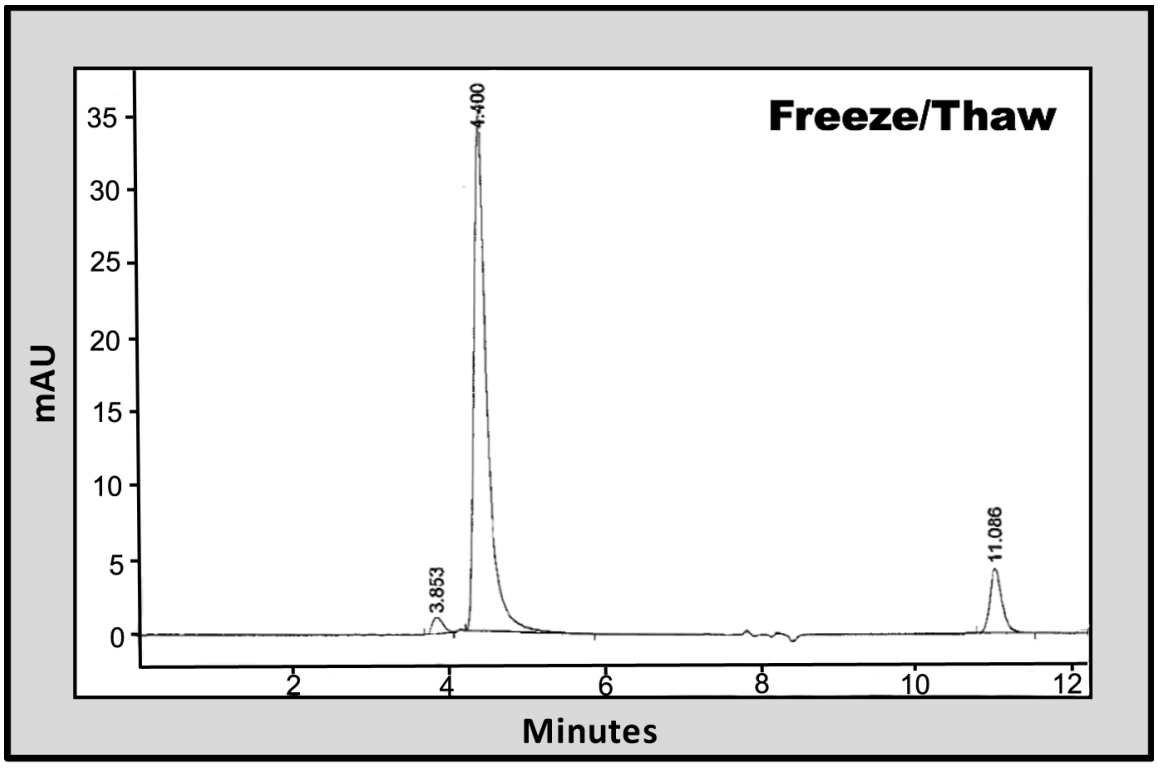

Fig. 4. SEC results of PEGylated interferon α_{2a} evaluated under freeze/thaw conditions. SEC chromatograph of PEGylated interferon α_{2a} following six freeze/thaw cycles shows a major protein peak with a retention time of ~4.4 minutes and a small quantity of higher molecular weight species at ~3.8 minutes.

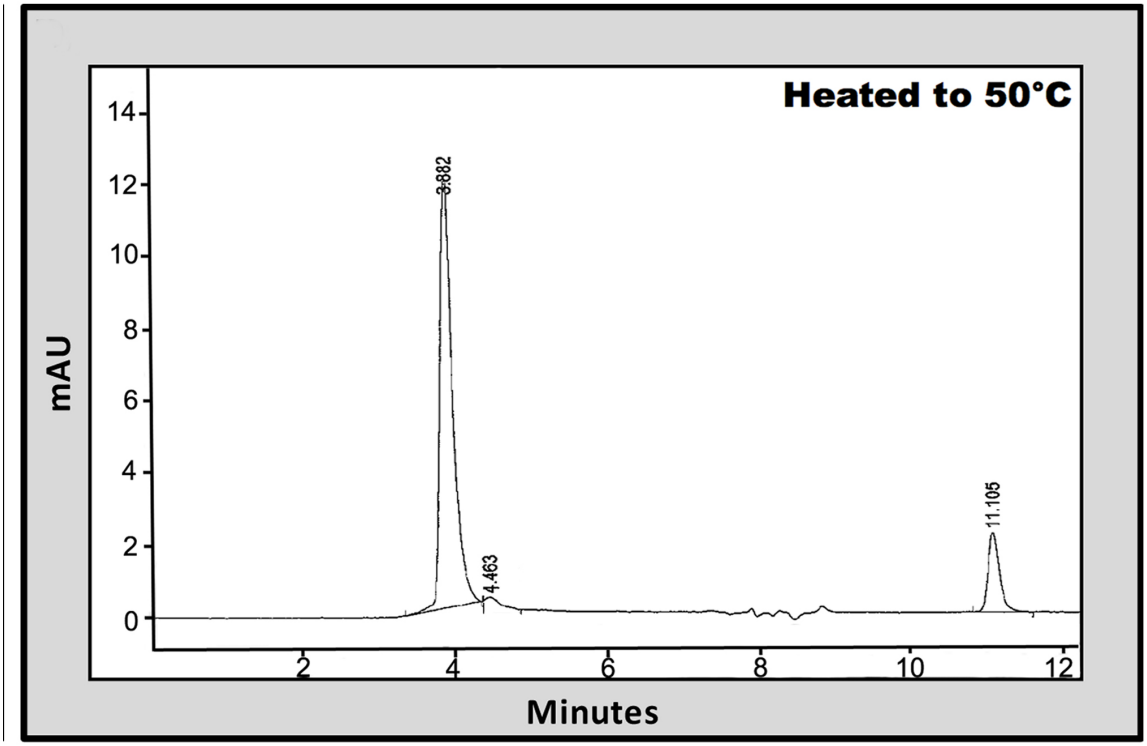

Fig. 5. SEC results of PEGylated interferon α_{2a} evaluated following heat stress. SEC chromatograph of PEGylated interferon α_{2a} after heating the agent at 50°C for 60 minutes shows a major peak of high molecular weight species at ~3.8 minutes retention time and a smaller peak at ~4.4 minutes.

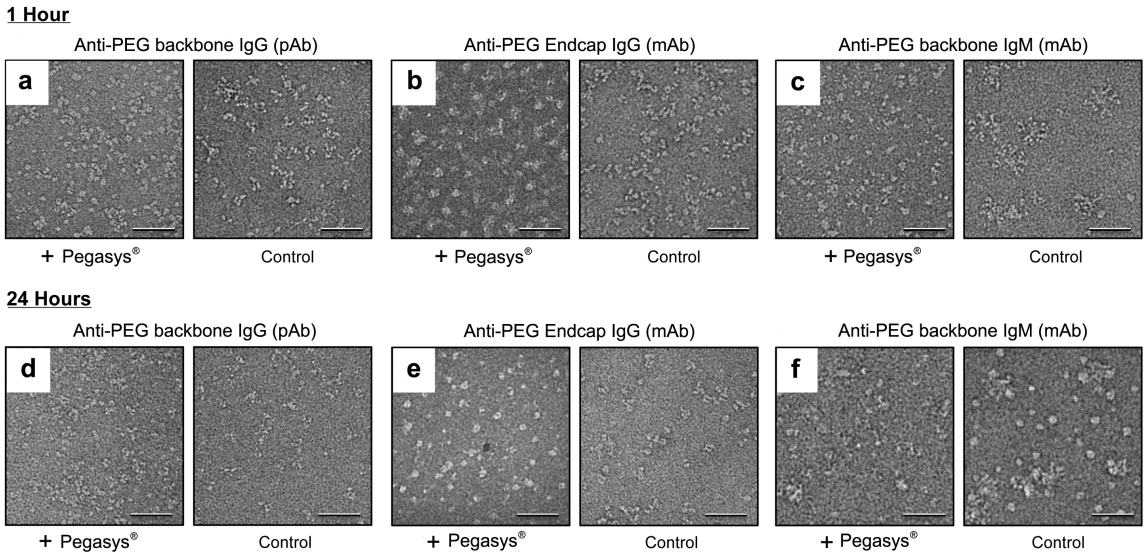

Fig. 6. EM analysis of antibody mixtures with the Pegasys® formulation. Antibodies against either the PEGylated backbone or endcap epitopes were mixed with the Pegasys® formulation and incubated over a 24-hour time course. The contrast in each image was enhanced by the use of uranyl formate on EM grids that were air-dried. Images revealed that particles in the unstressed antibody- Pegasys® mixtures were similar to control samples of antibodies alone (**a - f**). In general, no protein aggregates were formed within the time course tested for each unstressed antibody mixture. The branched PEGylated adduct arrangement engineered on the Interferon α_{2a} protein may help prevent aggregate formation. Scale bar is 100 nm. Polyclonal antibodies (pAb); monoclonal antibodies (mAb)

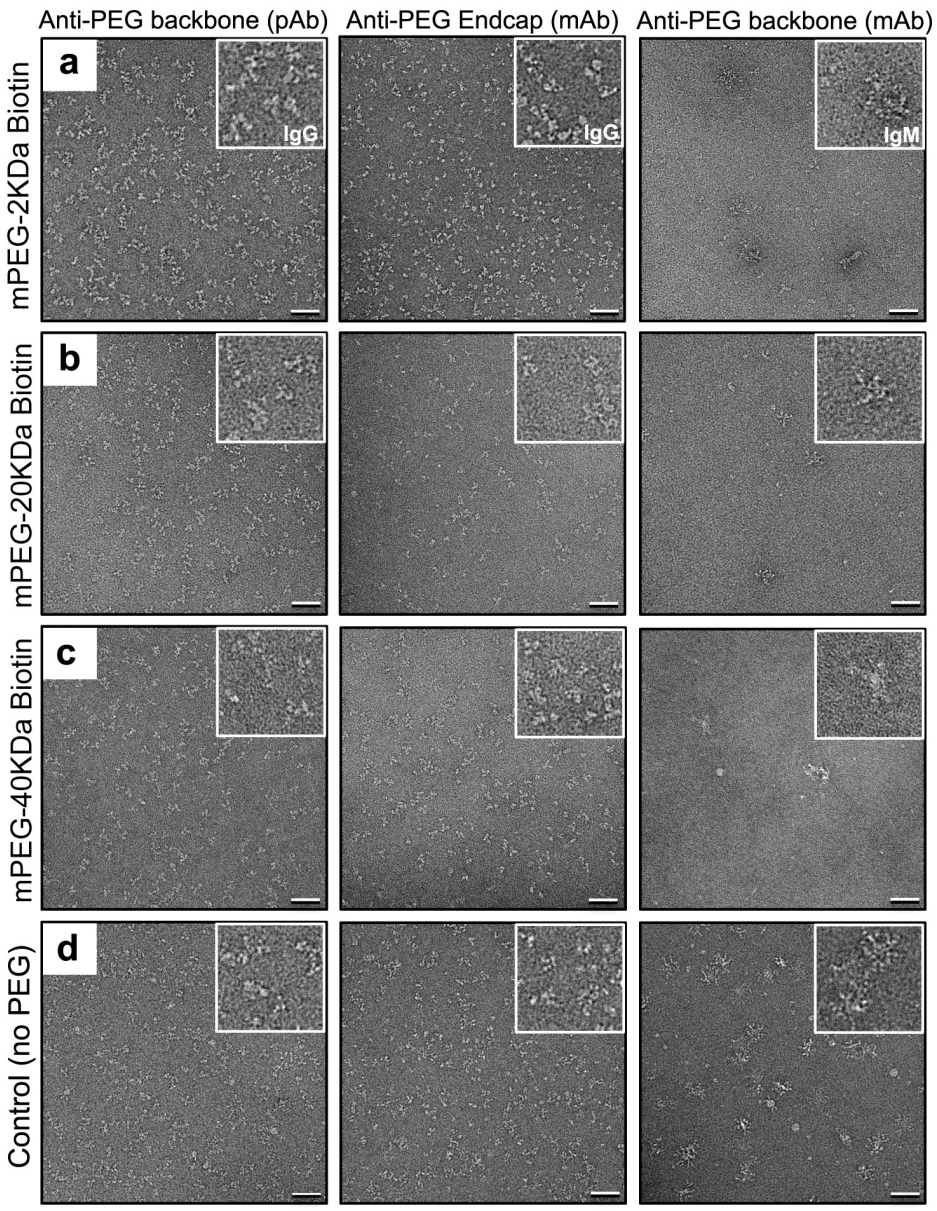

Fig. 7. EM analysis of antibodies interacting with different epitopes on PEGylated biotin substrates. TEM images revealed that particles of polyclonal IgG antibodies (pAb) against the different lengths of PEGylated adducts (**a-c, left panels**) are similar to particles in the control images of the antibodies lacking PEGylated substrates (**d, left panel**). The contrast in each image was enhanced by the use of uranyl formate on EM grids that were air-dried. Images of monoclonal IgG antibodies (mAb) against the PEG endcap (**a-c, middle panels**) have similar particles to control images lacking PEGylated substrates (**d, middle panel**). These images generally lacked protein aggregates. By contrast, IgM monoclonal antibodies against the PEG backbone tended to aggregate as the chain lengths of PEGylated adducts on biotin increased. Images (**a-c, right panel**) demonstrate this effect in comparison to images of control antibodies lacking PEGylated biotin (**d, right panel**). Scale bar is 100 nm in each panel.

MOVIES

Movie 1. Protein aggregates formed in the Pegasys® reagent show bulk migration properties in solution. Protein aggregates formed upon externally heating the Interferon α_{2a} drug in solution at 50°C reveal bulk migration properties in solution over a 40-second time course. Movie is accelerated by ~3×. For visualization purposes, the image contrast was inverted and the temperature range of each image was adjusted to the same value.

Movie 2. Migration properties of protein aggregates are displayed as contour maps. Contour maps generated from the frames included in Movie 1 simplify the visualization of aggregate movements. Movie is accelerated by ~3×. Darker colors represent a greater difference in electron density with respect to the image background. Lighter colors represent a lesser difference in electron density with respect to the image background.

Movie 3. Protein aggregates formed in the Pegasys® reagent show changes in association. Protein aggregates formed upon externally heating the Interferon α_{2a} drug in solution at 50°C show changes in bulk properties and associations within a 40-second time course. For visualization purposes, the movie is accelerated by ~3×. The image contrast was inverted and the temperature range of each image was adjusted to the same value.

Movie 4. Changes in association properties of protein aggregates are displayed as contour maps. Contour maps generated from the frames included in Movie 3 to simplify the visualization of aggregate associations. Video is accelerated by ~3×. Darker colors represent a greater difference in electron density with respect to the image background. Lighter colors represent a lesser difference in electron density with respect to the image background.

Movie 5. An additional selected region of protein aggregates formed in the Pegasys® reagent to demonstrate changes in bulk associations. Changes in the associations of the freely flowing aggregates are shown within a 40-second time course. For visualization purposes, the movie is accelerated by ~3×. The image contrast was inverted and the temperature range of each image was adjusted to the same value.