SUPPLEMENTARY METHODS

Analysis of gold cluster labeled FAS images. The first step in the analysis of the images was to align them to projections of a reference FAS structure (we assumed that labeling with the gold reagent would induce negligible structural changes). To keep it from interfering with the alignment procedure, the signal from the gold-particles had to be temporarily eliminated from the images. Because the Nanogold particles appeared as sharp, high-intensity spots, they could be identified as pixels above a certain threshold. Replacing information above 1.75 standard deviations above the average with random noise was sufficient to mask the gold particles without removing additional information. Thresholded images were aligned against 83 CTF-altered (at –1.8 μm, to match the average defocus of the images in the data set) projections of the unlabeled FAS structure. The resulting reference-based alignment parameters were validated by using a reference-free alignment algorithm to check the homogeneity and alignment of the particles assigned to each projection of the reference FAS structure. Finally, alignment parameters for each thresholded image (no gold signal) were applied to the corresponding original image, and 83 reference-based averages were obtained. Thresholded class averages were subtracted from the corresponding unthresholded averages and then compared to the reference structure projections. To better appreciate the results, a 3-D model of the gold-labeled FAS was reconstructed by back-projection from the unthresholded class averages. This model was Fourier normalized with respect to the CTF-altered reference and the reference was then subtracted from the gold-labeled model. This process revealed the location of the extra density of the gold clusters attached to the N-termini of the FAS molecule.

Refinement of the 3-D FAS reconstruction. The initial FAS reconstruction calculated from stained specimens was only used as an aid for classifying unstained particle images, and the alignment parameters based on the use of the reconstruction from stained specimens were discarded. In the next step of the analysis, the classification based on the stain reconstruction was exhaustively tested by subjecting the particles in each initial group to multiple (10) rounds of reference-free alignment. Groups that generated reference-free averages with consistent, well-defined projection maps were combined by back projection to obtain a new reference structure. A new round of classification based on this new structure was followed by calculation of new reference-free group averages. Finally, the relative orientation of these clean, reference-free averages was confirmed using a common-lines algorithm. This procedure has been described in detail previously[1]. A final volume was generated from the data set by refinement through iterative reference projection matching[2], and the resolution of the final reconstruction was estimated using the Fourier Shell Correlation method[3]. **Supplementary Figure 1** online shows that the projections used to calculate the FAS structure sample space evenly, and also show the Fourier Shell Correlation plot.

Calculation and refinement of DBP cross-linked FAS monomer structure. Reference-free alignment, multivariate statistical analysis, and hierarchical ascendant clustering revealed that zero-tilt images of the cross-linked monomers were divided into two major groups. A fraction of the images (519, or 32%) were too large to correspond to FAS monomers and reflected some contamination of the monomer preparation with cross-linked dimers revealed by SDS-PAGE analysis of the cross-linked monomer preparation. The remaining 1092 zero-tilt images were further classified, and the best-

aligned group, including a total of 459 particles, was used to calculate an initial reconstruction using the random conical tilt method. This initial reconstruction was subjected to seven cycles of refinement and, as before, reference-free alignment and back projection were used to confirm the validity of the refined random conical tilt reconstruction. The final random conical tilt reconstruction was then used as a reference for refinement by projection matching and back projection of the 1092 tilted images initially identified as corresponding to cross-linked FAS monomers. Reference-free alignment of the final group averages indicated that, at least to the limited resolution of the final DBP cross-linked FAS monomer structure (~30 Å), the monomer particle images represented different views of a single conformation.

References

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