SUPPLEMENTARY MATERIAL

Characterization of a Highly Flexible Self-Assembling Protein System Designed to Form Nano Cages

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Figure S1

SDS PAGE of samples of unmodified KDPG aldolase (A-WT), A-(+), A-(-); and the complexes formed by a 1:1 mixture of A-(+) and A-(-). Addition of the highly charged coiled coil linker domains to A-WT introduces some heterogeneity into their electrophoretic properties.



Figure S2

Representative sedimentation velocity traces and van Holde-Weischet analyses for: A: unmodified KDPG aldolase (A-WT); B: A-(+); C: A-(-); D: complexes formed by a 1:1 mixture of A-(+) and A-(-)



Figure S2 A. Sedimentation velocity analytical ultracentrifugation data and van Holde-Weischet anlaysis for KDPG aldolase (A-WT). *Top* Sedimentation velocity data scans for KDPG aldolase. The red line indicates where back diffusion occurs and data after that line is omitted from van Holde-Weischet analysis. *Bottom* van Holde-Weischet extrapolation plot for scan data in A. Intersection by all lines to one point indicates that the sample contains a single species with s_{20w} of ~4.8 S.



Figure S2 B. Sedimentation velocity analytical ultracentrifugation data and van Holde-Weischet anlaysis for A(+). *Top* Sedimentation velocity scans for A(+). The red line indicates where back diffusion occurs and data after that line is omitted from van Holde-Weischet analysis. *Bottom* van Holde-Weischet extrapolation plot for scan data in A. Extrapolations intersect at varying points showing that more than one species was present in the sample



Figure S2 C. Sedimentation velocity analytical ultracentrifugation data and van Holde-Weischet anlaysis for A(-). *Top* Sedimentation velocity scans for A(-). The red line indicates where back diffusion occurs and data after that line is omitted from van Holde-Weischet analysis. *Bottom* van Holde-Weischet extrapolation plot for scan data in A. Extrapolations intersect at varying points showing that more than one species was present in the sample.

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Figure S2 D. Sedimentation velocity analytical ultracentrifugation data and van Holde-Weischet analysis for 1:1 A(-):A(+) mixure. *Top* Sedimentation velocity scans for 1:1 A(-):A(+) mixture. The red line indicates where back diffusion occurs and data after that line is omitted from van Holde-Weischet analysis. *Bottom* van Holde-Weischet extrapolation plot for scan data in A. Extrapolations intersect at multiples points with sedimentation coefficients much larger than seen for A(-) and A(+). These results indicate that upon mixing of A(-) and A(+) a heterogeneous mixture of aggregates much larger than the individual A(-) and A(+).

Figure S3. Raw negative stain EM micrograph showing of A-(+):A-(-) protein complexes collapsed into patches.



Figure S4.

Three hundred seventy six protein cage particle projections were selected and subjected to reference-free classification and averaging into 50 classes. Many of the averages present surface features compatible with the formation of cages.



