

Chris C. Broomell, Henrik Birkedal, Cristiano L. P. Oliveira, Jan Skov Pedersen,
Jan-André Gertenbach, Mark Young, and Trevor Douglas*

Protein Cage Nanoparticles as Secondary Building Units for the Synthesis of 3-Dimensional Coordination Polymers

Supporting Information

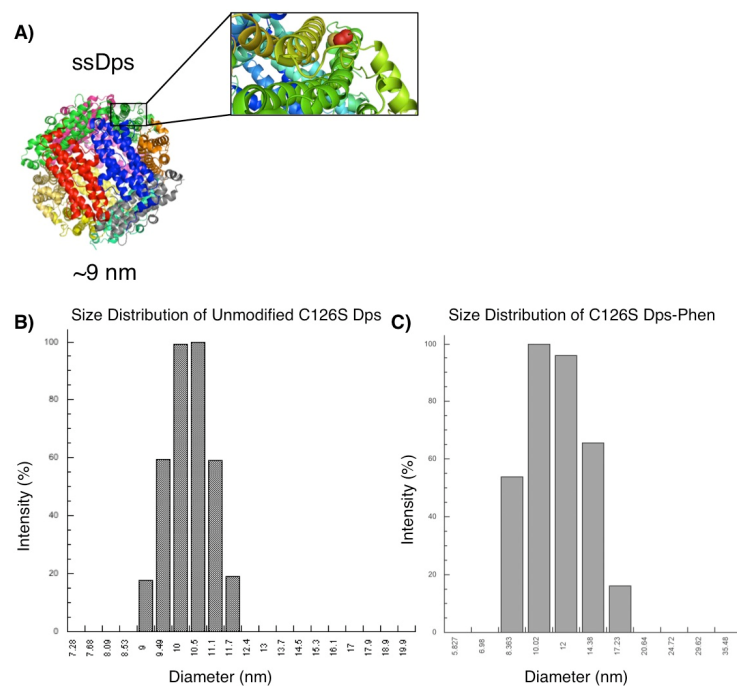


Figure S1. (A) Ribbon diagram representation of Dps. Inset indicates the position of Cys₁₀₁, which remains addressable by sulfhydryl-directed compounds in the C126S mutant. Dynamic light scattering profiles of (B) unmodified and (C) phenanthroline-labeled C126S indicate that diameters of both constructs are in relatively unchanged compared to native, unmodified cage.

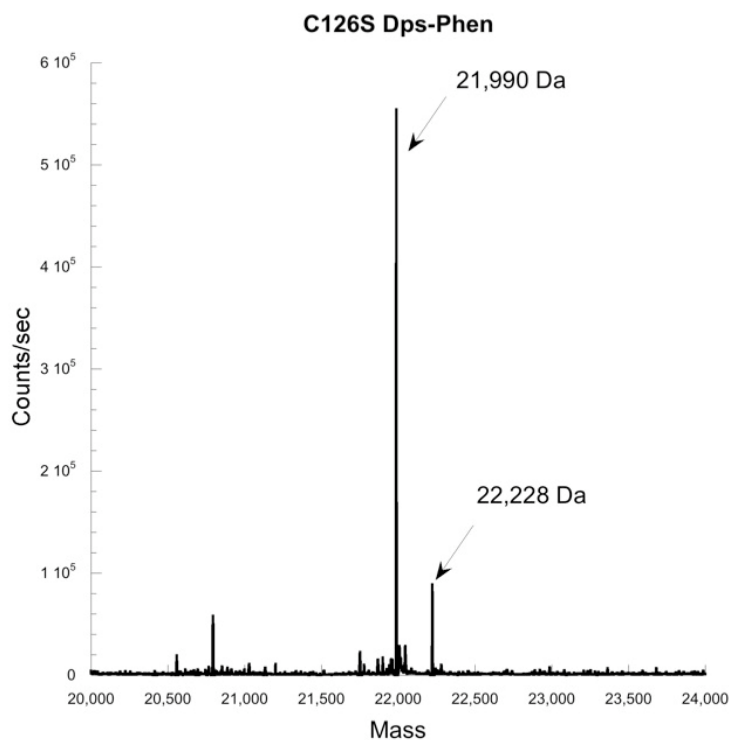


Figure S2. ESI-MS of IAP-treated C126S Dps. Peaks at 21,990 and 22,228 Da correspond to singly- and doubly-modified Dps subunits (respectively). Absence of native subunit (21,754 Da) indicates that all addressable cysteines were modified, yielding at least 12 phenanthroline pendant groups per dodecameric cage.

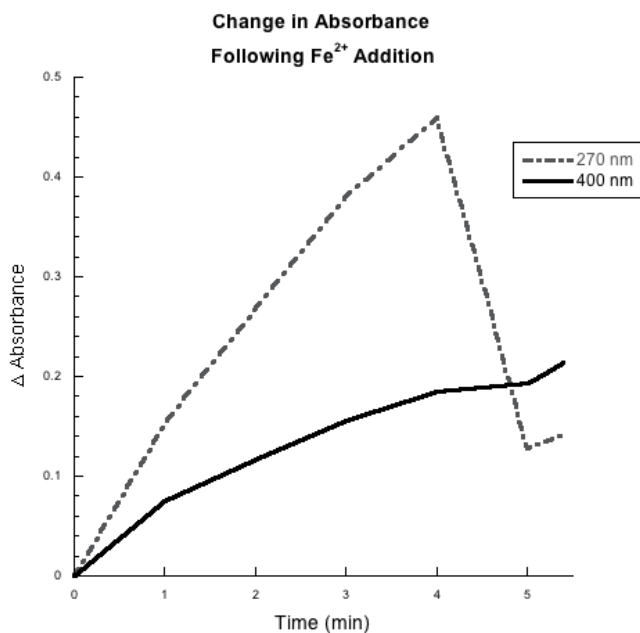


Figure S3. UV-vis spectroscopy of C126S Dps-phen after exposure to iron to follow absorbance and scattering. Addition of Fe²⁺ (as ferrous ammonium sulfate) at a final molar ratio of 2:1, subunit to iron) resulted in rapid aggregation as indicated by the increase in scattering at 400 nm and 270 nm. (Inset) (left) Addition of Fe²⁺ to free

Supplementary Material (ESI) for Soft Matter
This journal is (c) The Royal Society of Chemistry 2010

phenanthroline results in formation of a soluble pink complex. (Right) Addition of Fe^{2+} to C126S Dps-phen results in formation of a ruddy, flocculent aggregate (time = 5 minutes post metal addition).

SAXS analysis and modeling:

The characteristic real-space distance distribution functions $p(r)$ were determined from the scattering data using indirect Fourier transformation¹ using the implementations described elsewhere^{2,3}. This function corresponds to a histogram over all distances between pairs of points within the particle and it gives direct insight into the particle shape and size.

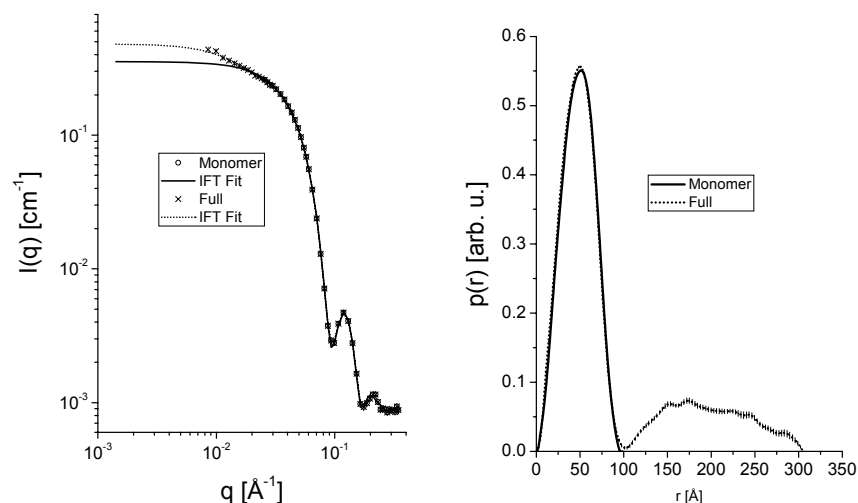


Figure S4. Left: Indirect Fourier Transform (IFT) fits to the data on C126S using a restricted (full line, cut a low q) or the full (dotted line) data range. Right: pair distribution functions calculated from the IFT fits for the restricted (full line) and full (dotted line) data range.

IFT fits to the C126S data are shown in Figure S4. At low q , the data show signs of aggregate formation and this was probed by making two IFT fits one to the full data range and one to a data set cut a low q . Indeed, the resulting pair distribution functions show a single clear peak at ~ 50 \AA radius, consistent with a spherical protein cage, for the restricted range and additionally peaks at larger radii for the full data range reflecting the presence of multimers.

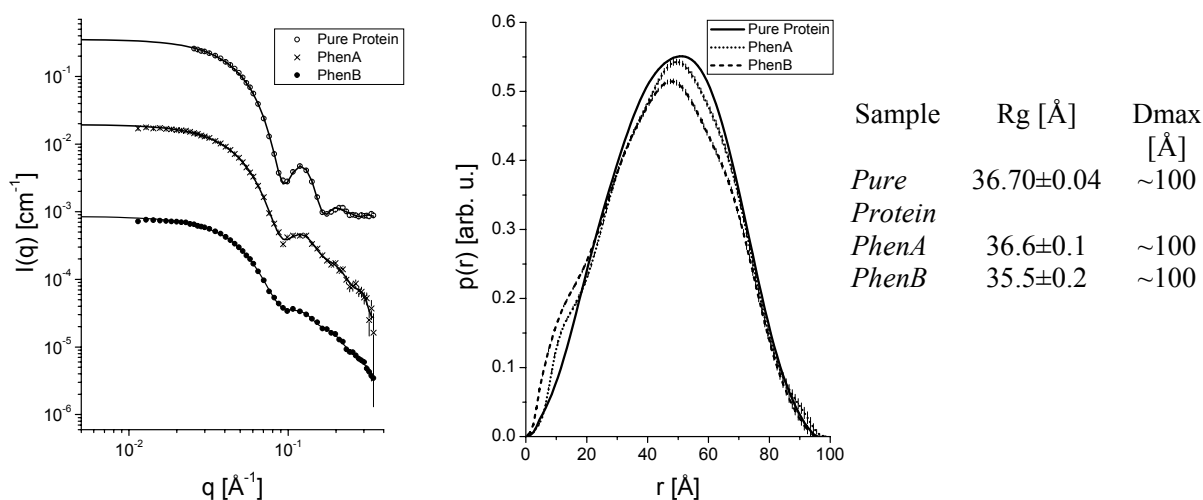


Figure S5. Comparison of IFT fits (left) and the resulting pair distribution function (center) of the pure C126S and two different formulations of C126S-phen. The right hand table shows the calculated radius of gyration and shows that the functionalized molecules are very close to the pure protein in size.

Figure S5 compares IFT fits data of C126S with two independent formulations of C126S-phen (PhenA and PhenB). While the SAXS data display some differences between the two C126S-phen data sets, the molecular size revealed by IFT is the same. This proves that the phen functionalized C126S molecules have the same size and shape as the unmodified protein.

The scattering intensity for the proteins was calculated using the atomic coordinates for the known crystallographic model structure (pdb entry *2CLB.pdb*⁴) in a dodecameric protein cage. Using the Debye formula⁵ it was possible to calculate the theoretical scattering intensity and compare with experimental data using a least squares procedure. Also, this approach enabled the addition of “impurities” as half-cages, free monomers, flexible parts and simple associations as dimers and trimers for the protein cages. A similar procedure was successfully applied on the study of glucagon fibrillation² and DNA self-assembly⁶.

For the study of the protein aggregation a new fractal structure factor model was developed⁶, which also takes into account the short-range correlation between the constituting subunits⁷. The new model was based on the analytical results for a freely rotating random walk chain with fixed step length⁸ and similar results for a linear rod-like arrangement of spheres. These two examples represent two- and one-dimensional objects, respectively. The new model combines, the generalized Debye-Bueche expression⁹ for describing the long-range correlations, with the hard-sphere structure factor¹⁰ for describing the short-range correlations. The model gives satisfactory agreement with the structure factor of the linear and random walks structures and provides a straightforward generalization to other values of the fractal dimension by varying the exponent in the generalized Debye-Bueche expression. For the final intensity calculation this structure factor was combined with the form factor calculated from the atomic coordinates for the protein dodecamer, as mention above.

- (1) Glatter, O. *J. Applied Crystallography* **1977**, *10*, 415-421.
- (2) Oliveira, C. L. P. B., M. A.; Pedersen, J. S.; Erlacher, K.; Otzen, D.; Pedersen, J. S. *A. J. Mol. Biol.* **2009**, *387*, 147-161.
- (3) Pedersen, J. S. H., S. and Bauer, R. *Eur Biophys J* **1994**, *22*, 379-389.
- (4) Gauss, G. H., Benas, P., Wiedenheft, B., Young, M., Douglas, T., Lawrence, C.M. *Biochemistry* **2006**, *45*, 10815-10827.
- (5) Debye, P. *Ann. Phys. Leipzig* **1915**, *46*, 809-823.
- (6) Oliveira, C. L. P., Juul, S., Jørgensen, H.L., Knudsen, B., Tordrup, D., Oteri, F., Falconi, M., Chillemi, G., Koch, J., Desideri, A., Pedersen, J.S., Andersen, F.F., Knudsen, B.R. **2009** (submitted).
- (7) Dimon, P., Sinha, S. K., Weitz, D.A., Safinya, C. R., Smith, G. S., Varady, W. A. and Lindsay, H. M. *Phys. Rev. Lett* **1986**, *57*, 595-598.
- (8) Buchard, W. a. K., *K. Proc. Roy. Soc. Lond.* **1970**, *185*, A316.

Supplementary Material (ESI) for Soft Matter
This journal is (c) The Royal Society of Chemistry 2010

- (9) Bungler, M. H., Foss, M., Erlacher, K., Hovgaard, M.B., Chevallier, J., Langdahl, B., Bungler, C., Birkedal, H., Besenbacher, F. and Pedersen, J.S. *Bone* **2006**, *39*, 531-541.
- (10) Kinning, D. J. a. T., E. L. *Macromolecules* **1984**, *17*, 1712-1718.