

Supplemental Information

Structure, Dynamics, Evolution, and Function of a Major Scaffold Component in the Nuclear Pore Complex

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Supplementary Figure S1, Sampathkumar et al.,

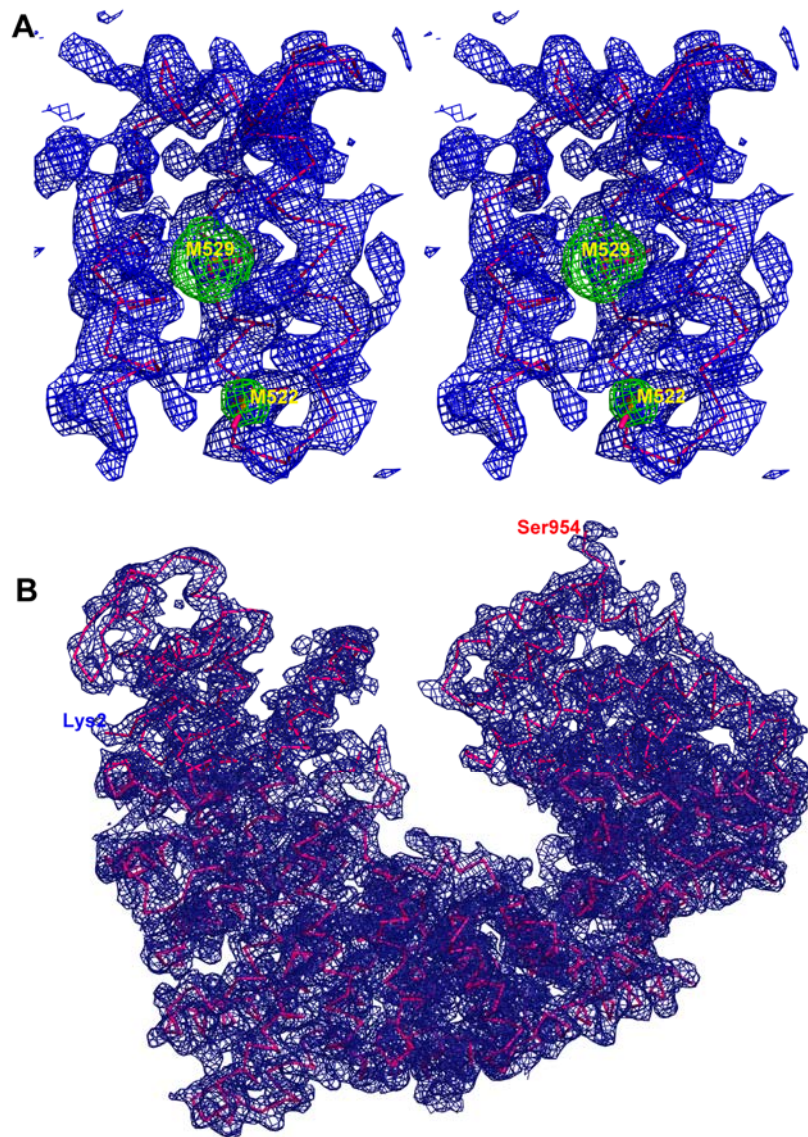
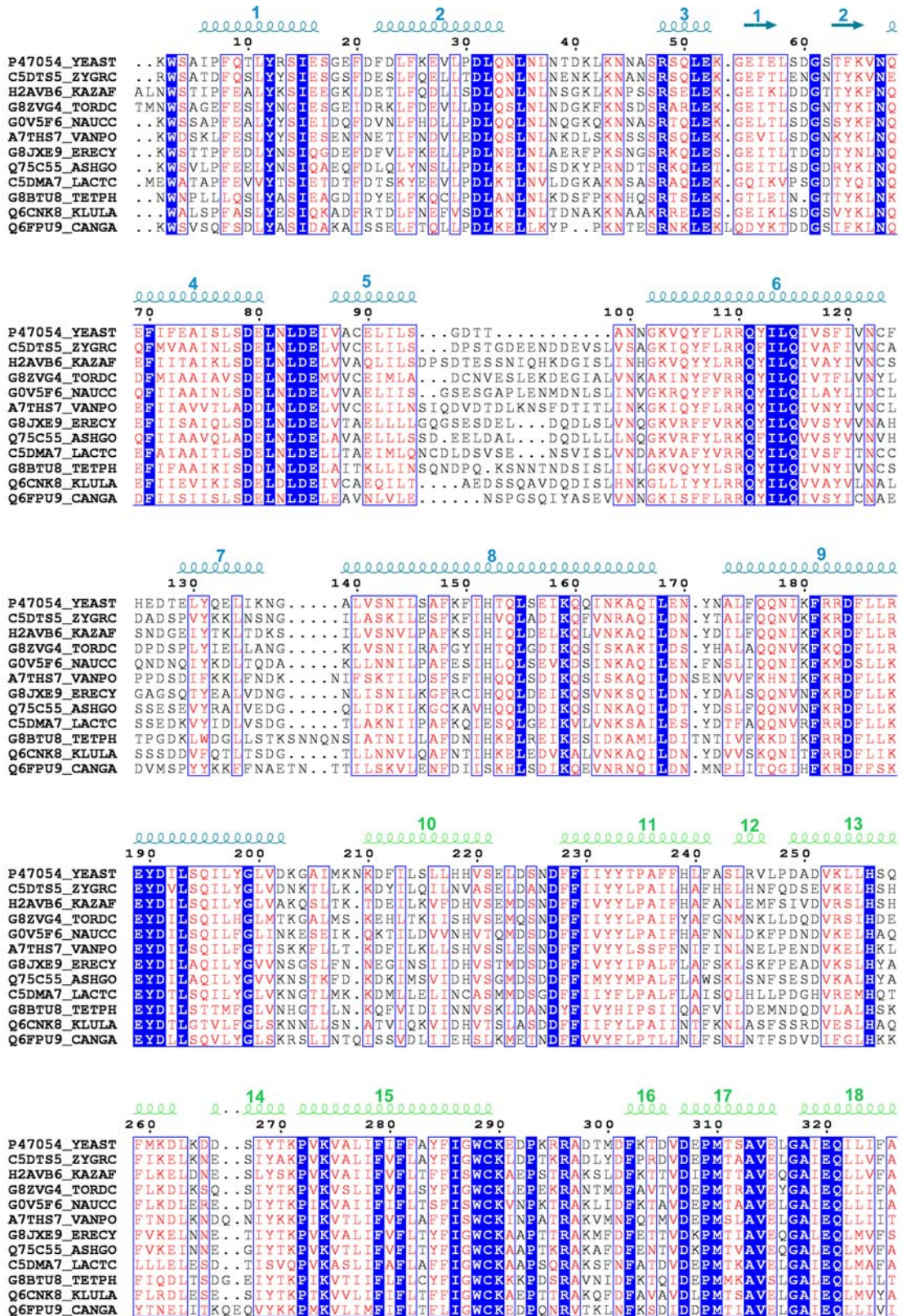


Figure S1, related to Figure 1. Electron density maps of ScNup192(2-960).

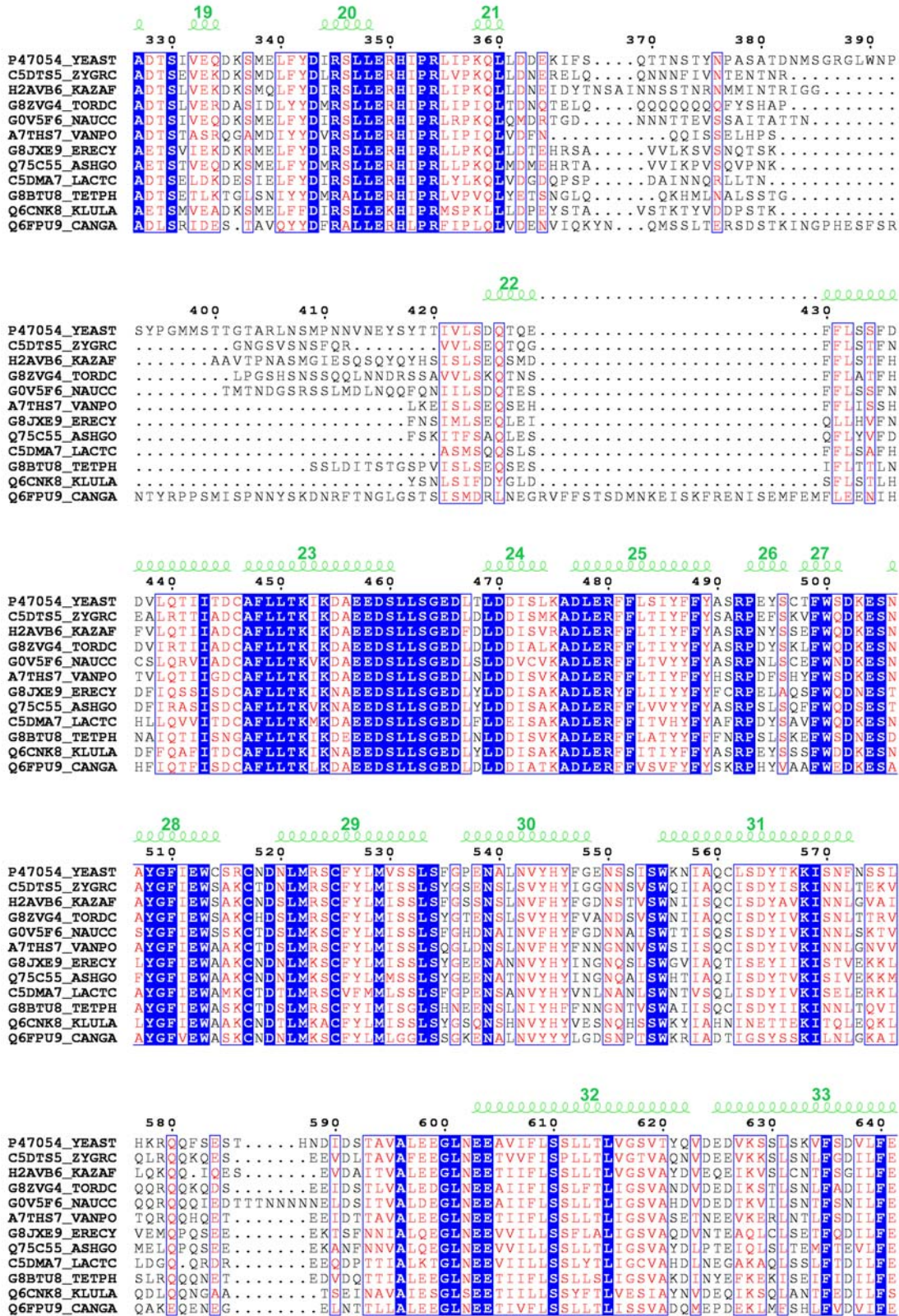
(A) Final mFo-DFc omit electron density map for a representative region of ScNup192(2-960) comprising residues Ala476 to Ala540 in blue displayed at 1.8 σ . Anomalous difference map obtained with final calculated phases shown in green for Se-Met522 (bottom) and Se-Met529 (middle) at 10.0 σ .

(B) The 2mFo-DFc electron density map for the entire length of ScNup192(2-960) at the end of the refinement displayed at 1.0 σ in blue. Molecular backbone is represented as ribbon with carbons in magenta.

Supplementary Figure S2, Sampathkumar et al.,



Supplementary Figure S2, Sampathkumar et al.,



Supplementary Figure S2, Sampathkumar et al.,

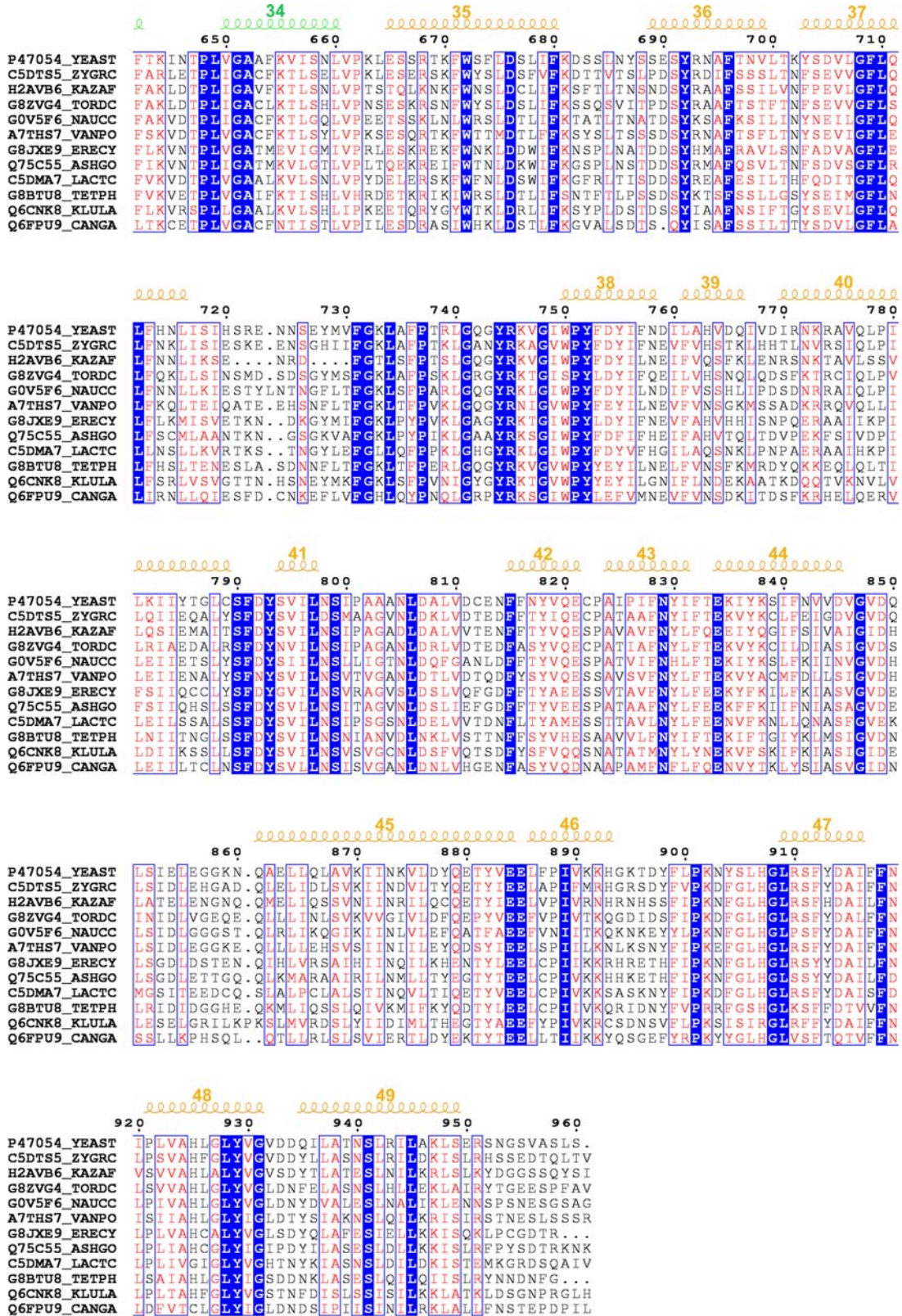


Figure S2, related to Figure 1. Conservation of Nup192(2-960) sequences.

Sequence alignment of ScNup192(2-960) with corresponding sequence-range from 11 other Nup192 of yeast / fungal origin labeled with respective uniprot entry names (www.uniprot.org). Conserved residues are shown in blue. Secondary structure elements are shown at the top with D1, D2, and D3 domain in cyan, green, and gold, respectively. Sequence alignment was performed using ClustalW (Larkin et al., 2007; <http://www.ebi.ac.uk/Tools/msa/clustalw2/>) and the illustration was prepared with ESPript (Gouet et al., 2003; <http://espript.ibcp.fr/ESPript/cgi-bin/ESPript.cgi>).

Supplementary Figure S3, Sampathkumar et al.,

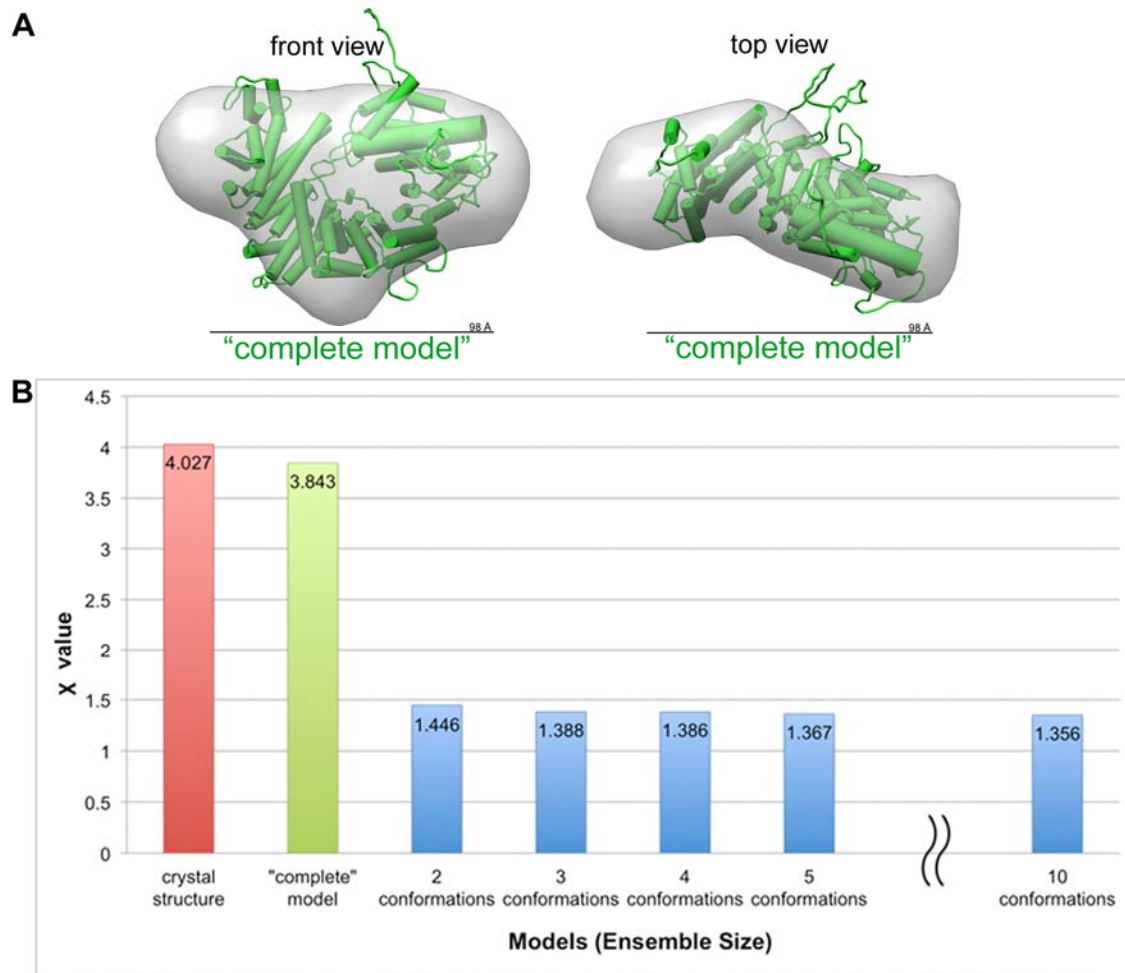
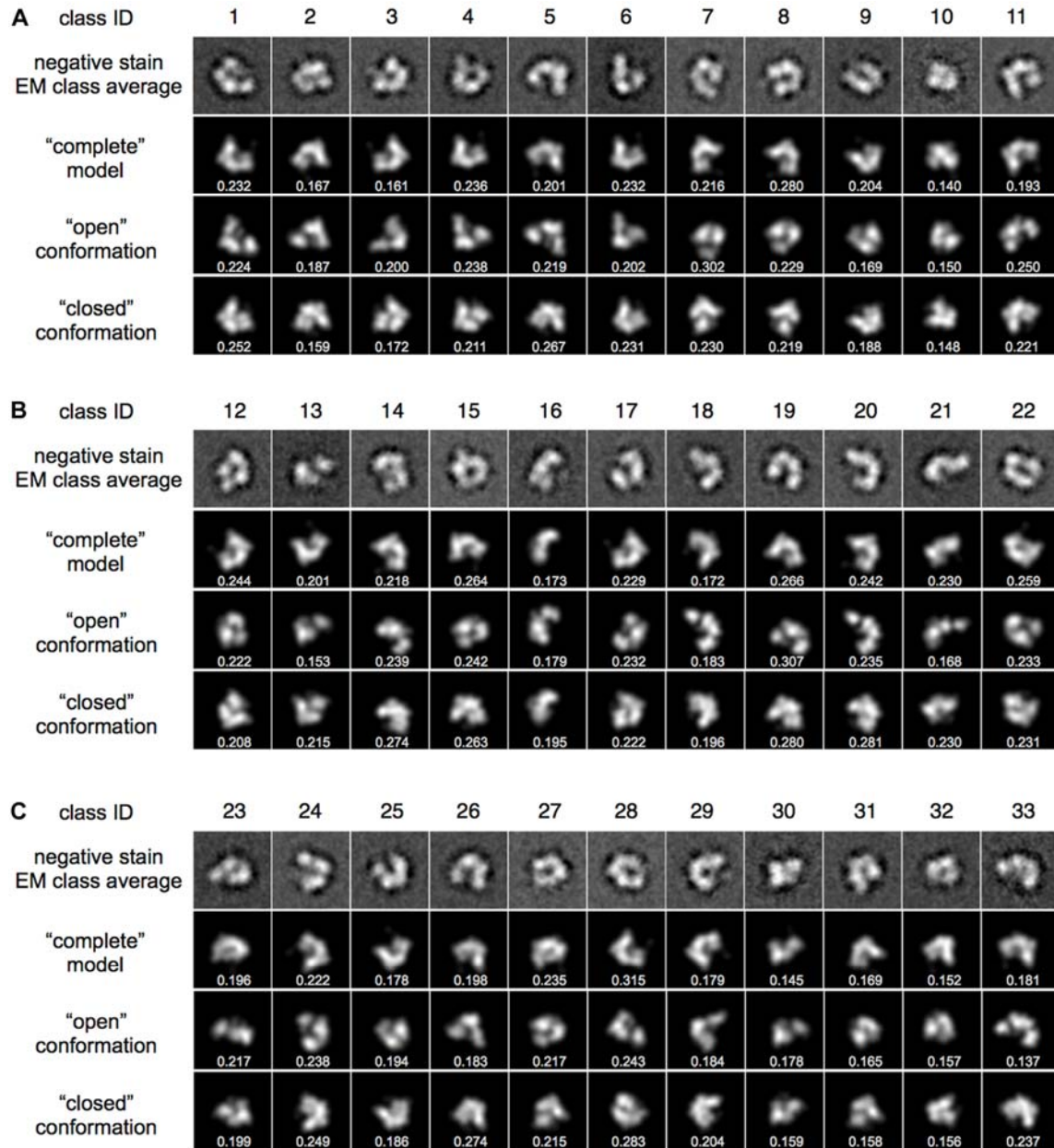


Figure S3, related to Figure 2. Conformational sampling analysis to reproduce the experimental SAXS profile

(A) Fit of the MODELLER derived “complete model” of ScNup192(2-960) to its *ab initio* SAXS shape in solution

(B) Comparison of the χ values (fit to the experimental SAXS profile) computed from the crystal structure, the “complete” model, and various sizes of ensemble of conformations (2 to 10 conformations). The χ values barely changes with larger sizes of ensemble. Thus, the ensemble of two conformations is sufficient to explain the experimental SAXS profile.

Supplementary Figure S4, Sampathkumar et al.,



Supplementary Figure S4, Sampathkumar et al.,

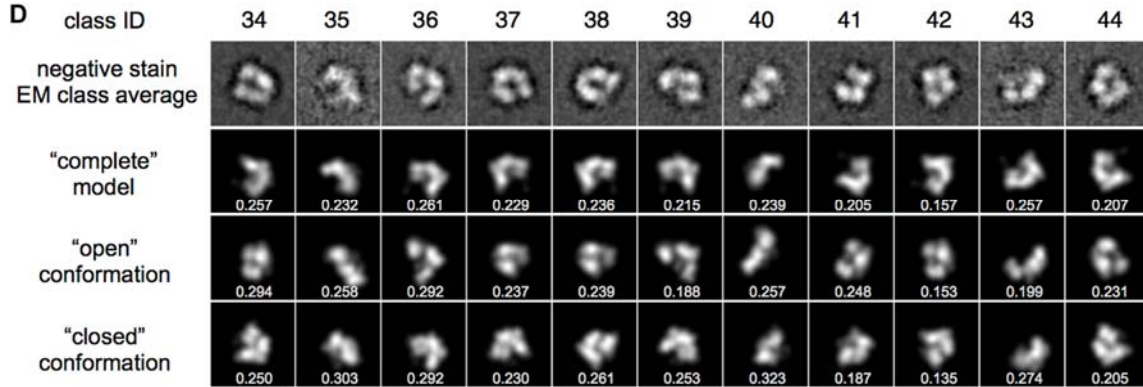
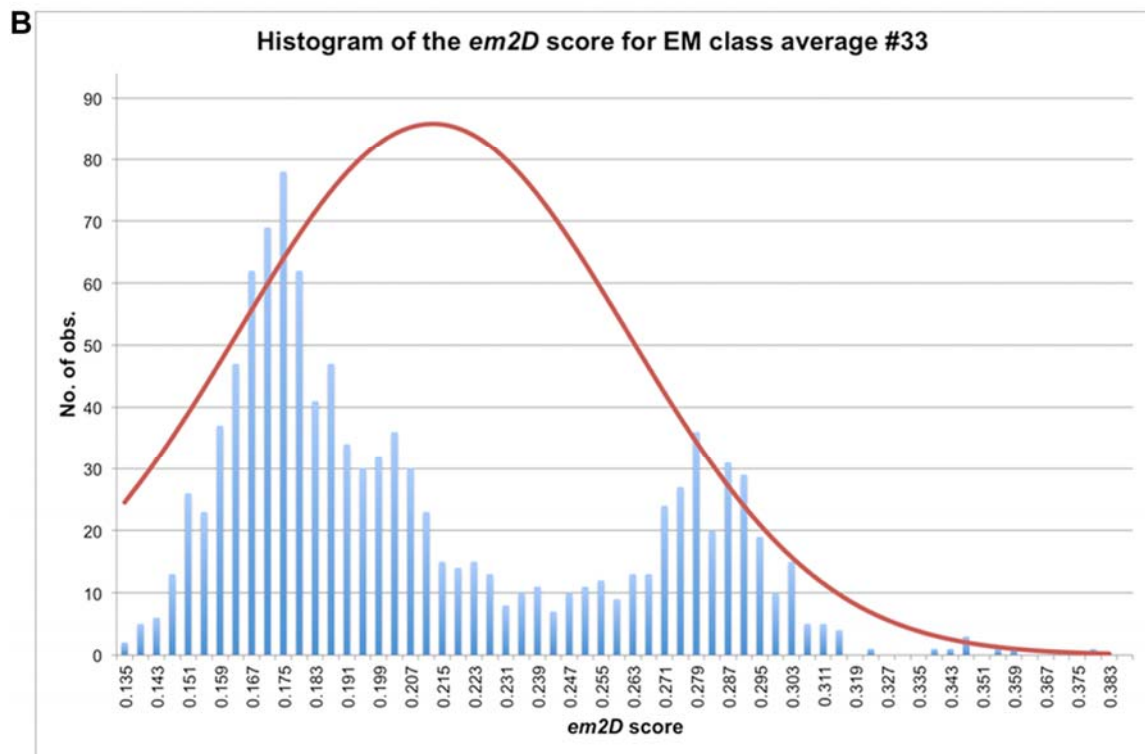
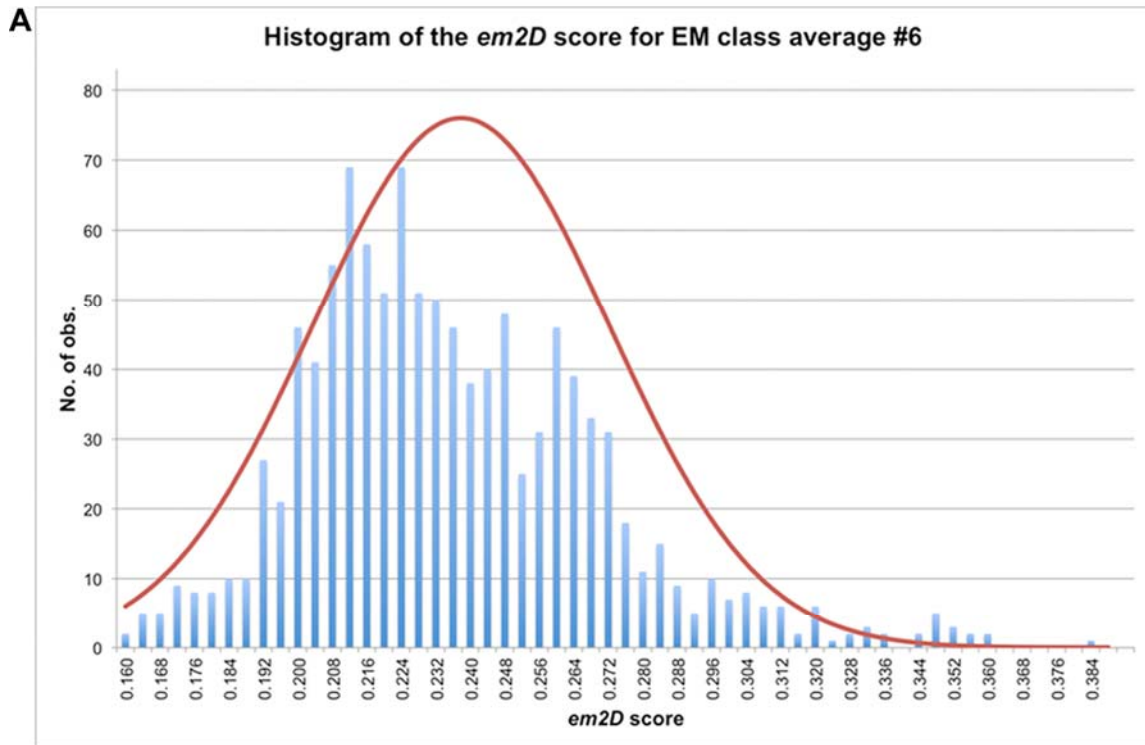


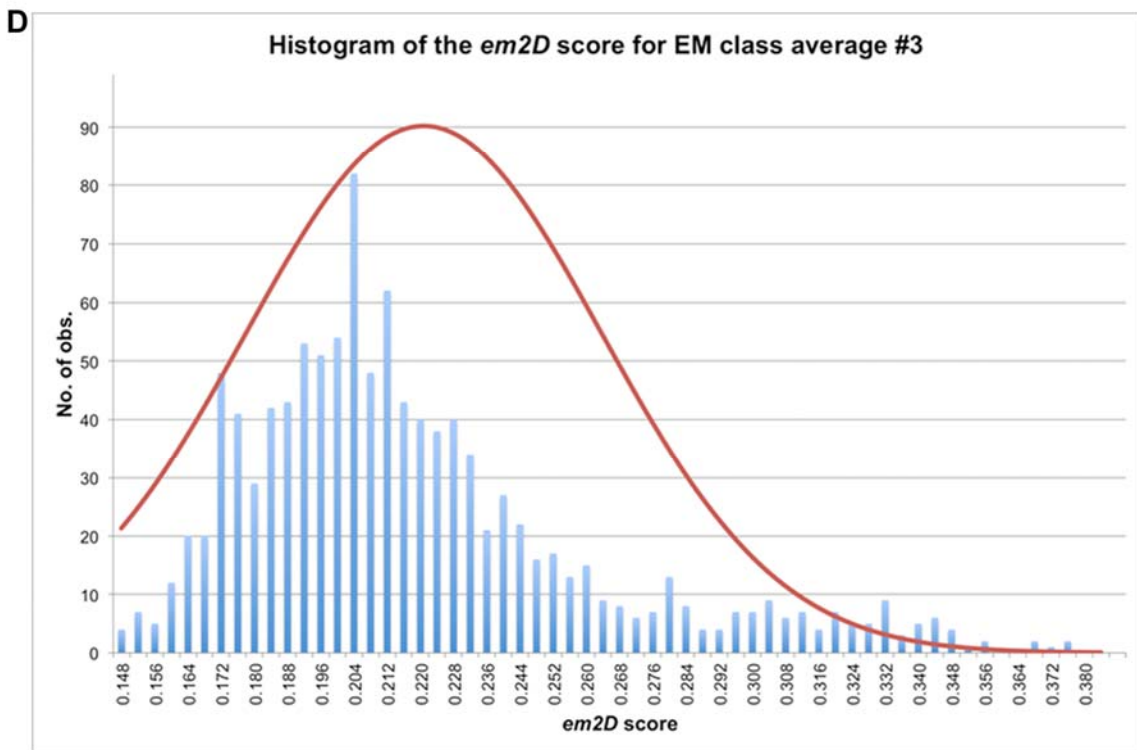
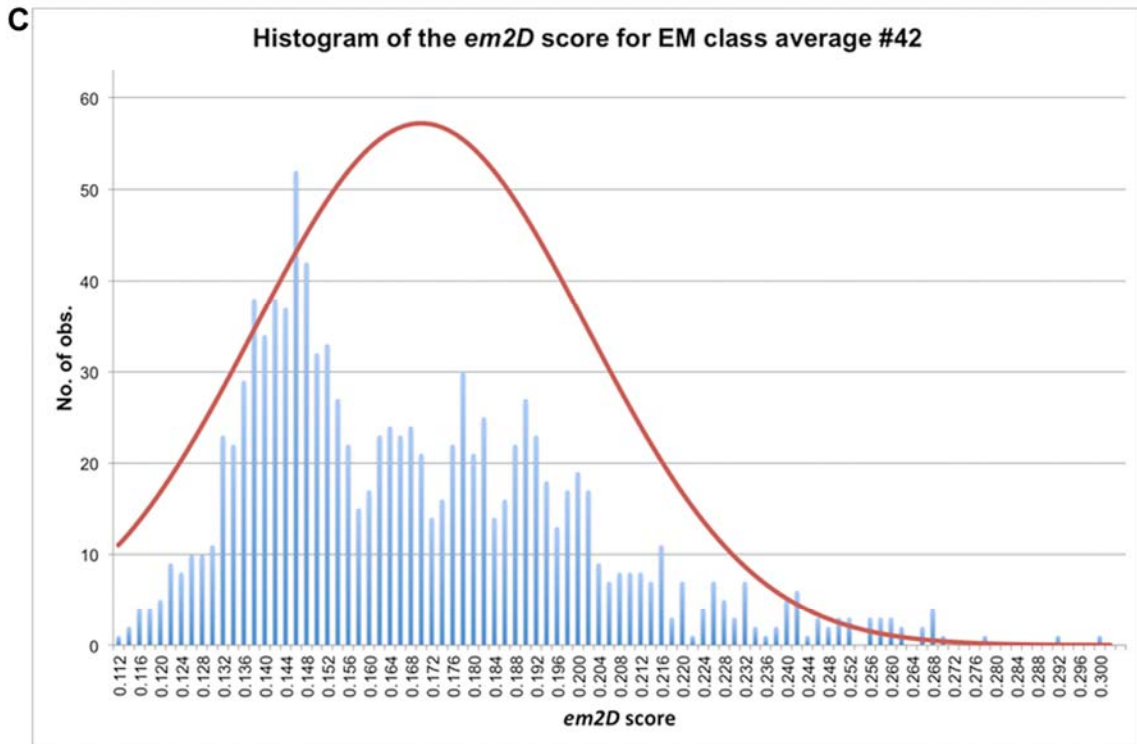
Figure S4 related to Figure 2. EM class averages and projections of ScNup192(2-960).

(A-D) The top row in each panels shows negative stain EM class averages from iterative stable alignment and clustering (ISAC) of 4,580 particles. After 7 generations, 3824 particles accounted for 44 classes. The number of particles comprising each class is indicated. Scale bar, 5 nm. Subsequent rows in each panel show projections of the "complete model", "open" conformation, and "closed" conformation of ScNup192(2-960). The *em2D* score (shown in the bottom of each projection) is defined as "one minus the cross-correlation coefficient between the image and the optimal model projection (1-ccc)", measuring the minimal difference between the image and a model projection. The projections and the *em2D* scores of the models are computed using *EMageFit* application of the Integrated Modeling Platform (IMP) package. In conclusion, the 44 class averages are not explained by any of a single-conformation alone, indicating heterogeneity of the ScNup192(2-960) on the EM grid.

Supplementary Figure S5, Sampathkumar et al.,



Supplementary Figure S5, Sampathkumar et al.,



Supplementary Figure S5, Sampathkumar et al.,

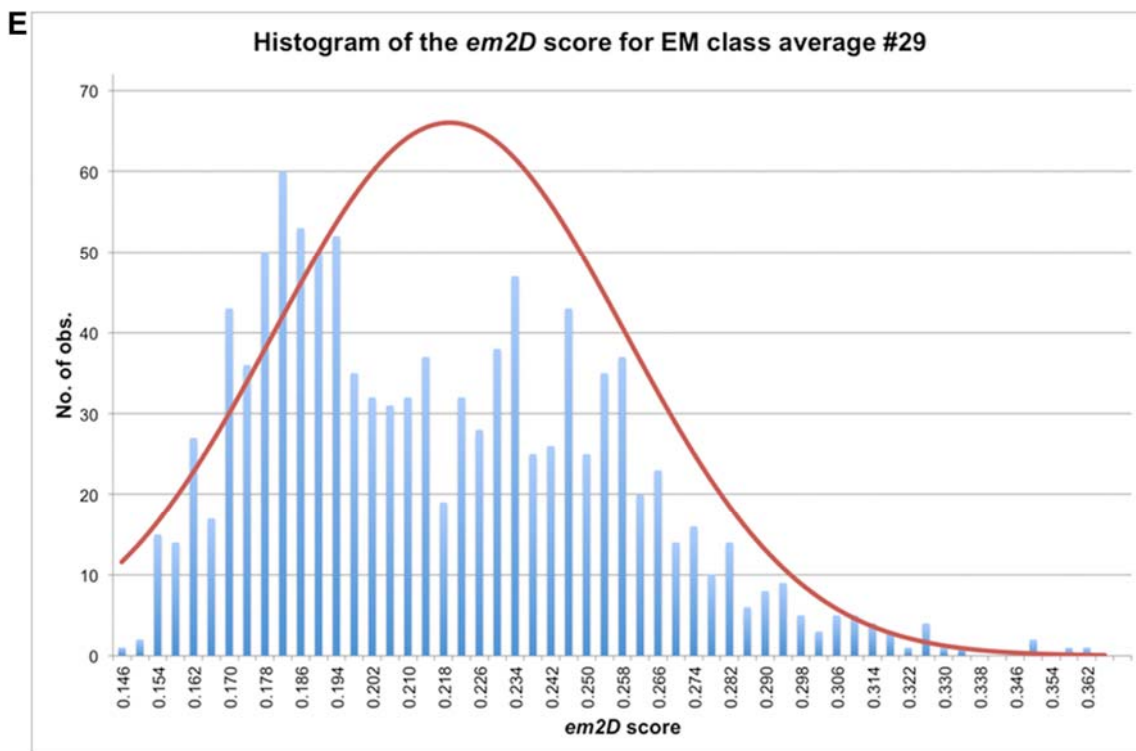


Figure S5, related to Figure 2. Histogram of *em2D* scores for selected class averages of ScNup192(2-960).

(A-E) The histograms (in blue bar) of the *em2D* scores (= 1 – the cross-correlation coefficient) for the EM class averages shown in Fig. 2b in the main text. The distributions of the *em2D* scores were determined from the pool of the crystal structure, the “complete model”, and the 110,000 conformations (generated by the conformational sampling analysis using Molecular Dynamics). The overlapped red curve represents the Gaussian distribution function, calculated from the statistics of the *em2D* scores for each EM class average. The EM class averages 6 and 33 correspond to the “open” conformation (*em2D* scores of 0.202 and 0.137, respectively); EM class average 42 corresponds to the “closed” conformation (*em2D* score of 0.135), and EM class averages 3 and 29 correspond to the “complete” model (*em2D* scores of 0.161 and 0.179, respectively).

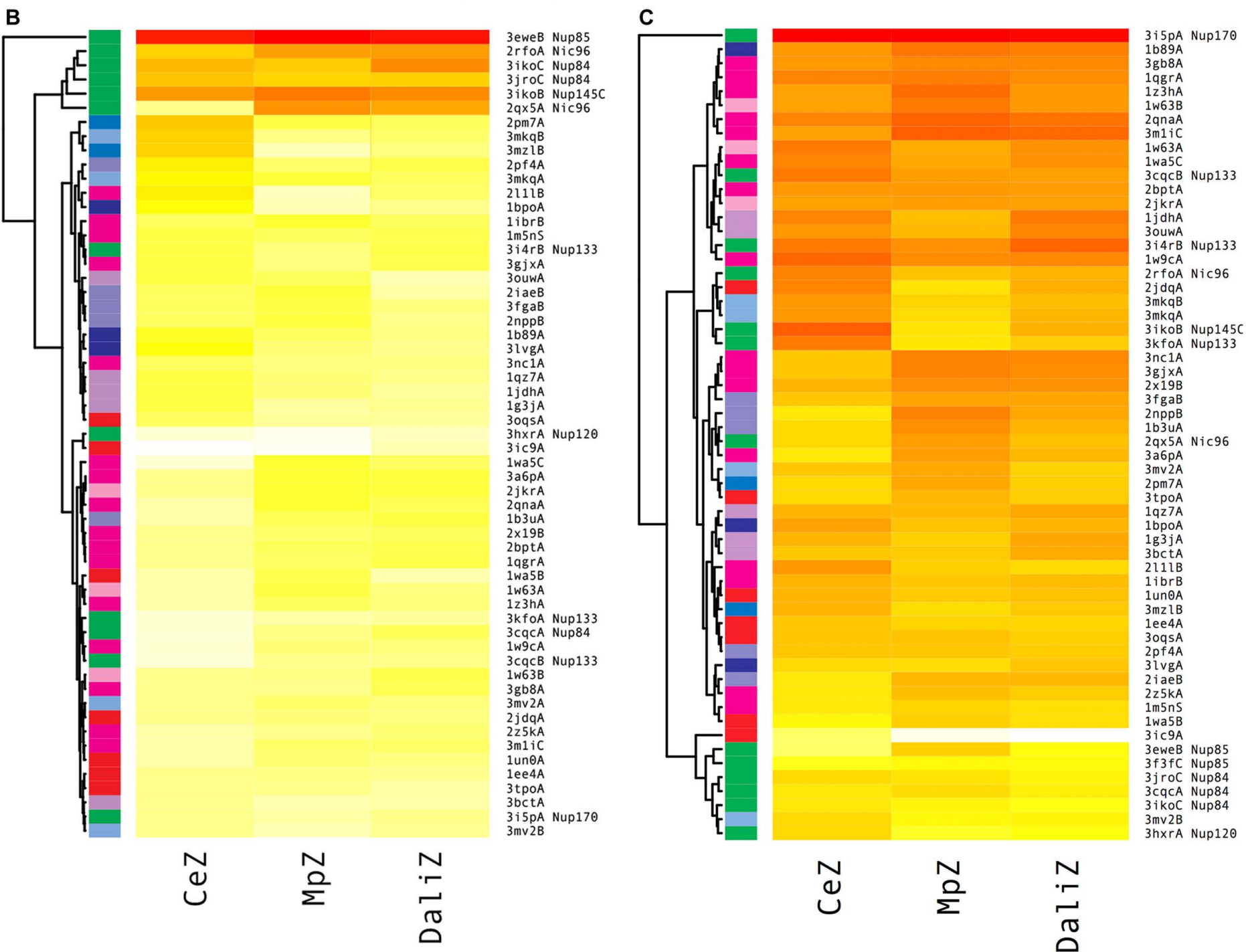
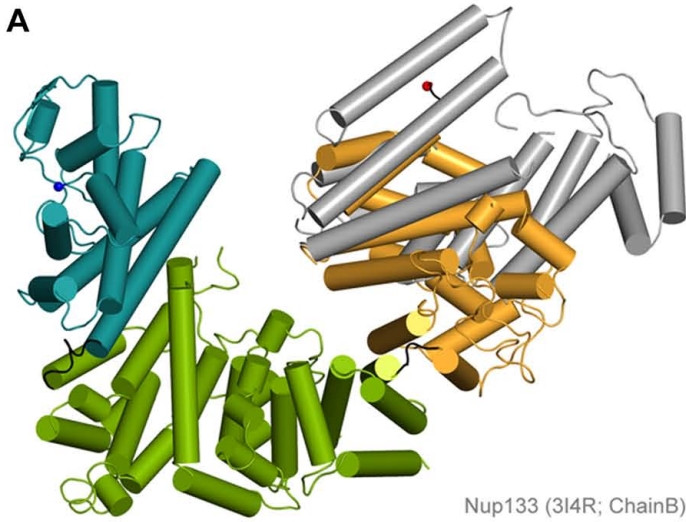


Figure S6 related to Figure 3. Structural alignment of ScNup192(2-960) with Nup133 and heatmaps of structural comparisons with Nup85 and Nup170.

(A) Structural superposition for DALI alignment of Nup192(2-960) with Nup133 (PDB: 3I4R, chain B). The D1, D2, and D3 domains of ScNup192(2-960) structure shown in cyan, green, and gold, respectively, and Nup133 in grey.

(B-C) Heat map for structural comparisons to Nup85 and Nup170, respectively. Standardized scores for Dali, CE, and Multiprot alignments, along with the composite Z-score are represented as a yellow to red gradient; red indicates stronger alignment scores (**Table S4 and S5** for Nup85 and Nup170, respectively). The structure dendrogram is computed by hierarchical clustering using pairwise distances between alignment Z-scores.

Supplementary Figure S7, Sampathkumar et al.,

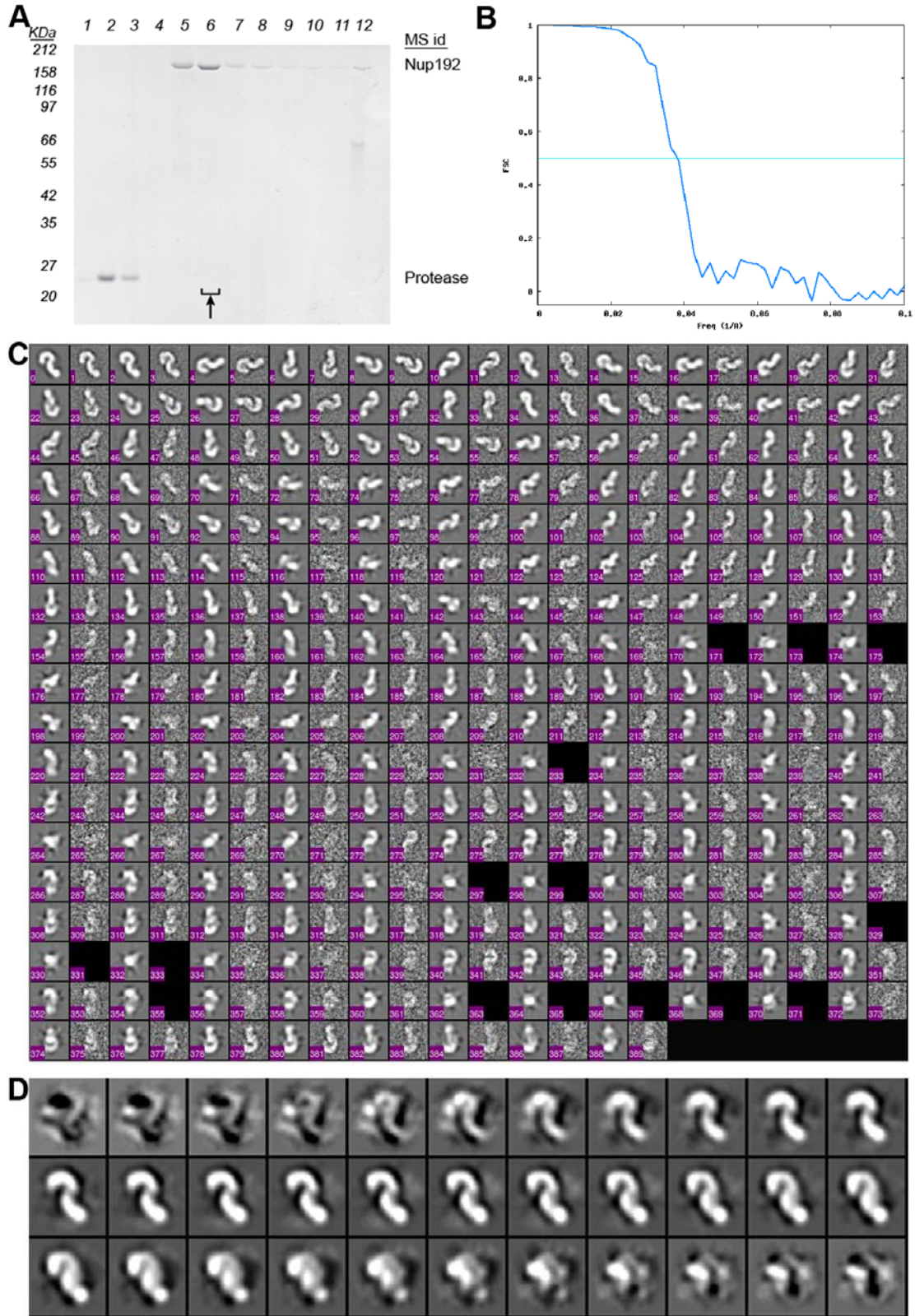


Figure S7 related to Figure 4. Structural model for the full length ScNup192 (ScNup192FL).

(A) SDS-PAGE image of ScNup192FL preparation stained with commassiae blue.

(B) Fourier shell correlation (FSC) plot for ScNup192FL. The FSC for the reconstruction was calculated by dividing the data set into equal halves and is plotted versus resolution ($1/\text{\AA}$) in blue. The resolution at which it falls below 0.5 (cyan line) is taken to be the resolution of the reconstruction, 26 \AA in this case.

(C) Image showing projections of the final 3D reconstruction versus class averages for each projection. The first image of each pair is the projection (even numbers) and the second is the associated class average (odd numbers). At 10 degree angular intervals there were 195 projections. Black images show where no data matched a projection. The angular coverage was nearly complete and the class averages matched the projections well.

(D) Slices of ScNup192FL made through its 3D volume in the Z-direction.

Supplementary Figure S8, Sampathkumar et al.,

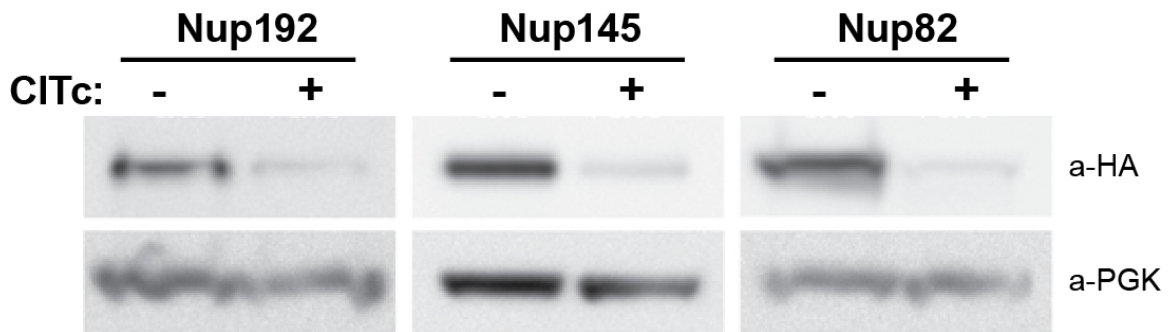


Figure S8 related to Figure 5. Analysis of the Nup protein levels after chlortetracycline treatment.

Western blot analysis showing the levels of HA-Nup192, HA-Nup145, and HA-Nup82 before (-) and after (+) treatment with chlortetracycline (CITc). The upper row shows the signal for anti-HA antibody and the lower row a loading control showing the signal from an anti-Pgk1 antibody.

Caption for Supplementary Movies:

Supplementary Movie S1, S2, S3, S4, related to Figure 2: The supplementary movies 1-4 illustrate how 110,000 conformations were generated by molecular dynamics simulation. All the movies start from an initial model (the “complete” model, generated by MODELLER), which was subjected to energy minimization and heated up to 1,500K in the CHARMM force field (version 33b). The D1 and D3 domains were allowed to move relative to the D2 domain about the inter-domain loop residues 204-211 and 663-670, subject to an R_g constraint of 30 Å to 50 Å. Disordered loops not present in the crystal structure were also allowed to move. In the movies, we enforced the R_g constraints of 32 Å (movie 1), 36 Å (movie 2), 40 Å (movie 3), and 50 Å (movie 4) to show the effect of the R_g constraints to the conformational sampling. All the movies were recorded by using VMD (Humphrey et al., 1996).

Supplementary Table S1: Data-collection and scattering-derived parameters for the SAXS experiment on ScNup192(2-960).

| | |
|---|----------------------------------|
| Data-collection parameters | |
| Instrument | SSRL BL4-2 |
| Defining slit size (mm) | 0.3(H) x 0.3(V) |
| Wavelength (Å) | 1.12709 |
| q range (Å ⁻¹) | 0.0133 – 0.3098 |
| Exposure time (sec) | 1 |
| Measurement repeats | 24 |
| Concentration range (mg ml ⁻¹) | 0.4 – 2.5 |
| Temperature (K) | 288 |
| Structural parameters † | |
| $I(0)$ (cm ⁻¹) [from $P(r)$] | 921.0 ± 2.872 |
| R_g (Å) [from $P(r)$] | 39.22 ± 0.090 |
| $I(0)$ (cm ⁻¹) (from Guinier) | 921.1 ± 6.850 |
| R_g (Å) (from Guinier) | 39.30 ± 0.450 |
| D_{\max} (Å) ‡ | 123.0 |
| Porod volume estimate (Å ³) | 198530 |
| Dry volume calculated from sequence (Å ³) | 134377 |
| Molecular-mass determination † | |
| Partial specific volume (cm ³ g ⁻¹) ¹ | 0.7586 |
| Contrast ($\Delta\rho \times 10^{10}$ cm ⁻²) | 2.67 |
| Molecular mass M_r [from $I(0)$] | 107.51 kDa |
| Calculated monomeric M_r from sequence | 111.06 kDa |
| Software employed | |
| Primary data reduction | <i>SASTOOL</i> |
| Data processing | <i>SASTOOL</i> and <i>PRIMUS</i> |
| <i>Ab initio</i> analysis (initial) | <i>DAMMIF</i> |
| <i>Ab initio</i> analysis (refinement) | <i>DAMMIN</i> |
| Validation and averaging | <i>DAMAVER</i> |
| Rigid-body modeling | N/A |
| Computation of model intensities | <i>FoXS</i> |
| 3D graphics representations | <i>UCSF Chimera</i> |

† Reported for the merged SAXS profile normalized at the concentration of 0.4 mg ml⁻¹.

‡ D_{\max} is a model parameter in the $P(r)$ calculation and not all programs calculate an uncertainty associated with D_{\max} . As such, it is reasonable to not cite an explicit error in D_{\max} , although it may be useful to provide some estimate based on the results of $P(r)$ calculations using a range of D_{\max} values.

Supplementary Table S2: The *em2D* scores (white background) with the rank percentage of a model (gray background) for each EM class average. The *em2D* score is defined as “one minus the cross-correlation coefficient between the image and the optimal model projection (1-ccc)”, measuring the minimal difference between the image and a model projection. Table S2 summarizes the *em2D* scores for the “complete model”, “open”, and “closed” conformations for each EM class average, along with the rank (in percentage) of a model that is its position in the sorted list values of the *em2D* score. A number of EM class averages (highlighted in bold, with red color) were assigned to either the “complete” model, “open”, or “closed” conformations. In general, a model of rank percentage of lower than ~20% can be presumably considered for the assignment of an EM class average.

| Class ID | "complete" model | | "open" conformation | | "closed" conformation | |
|----------|------------------|-------------|---------------------|--------------|-----------------------|-------|
| 1 | 0.232 | 31.9% | 0.224 | 21.8% | 0.252 | 52.8% |
| 2 | 0.167 | 4.0% | 0.187 | 26.3% | 0.159 | 0.8% |
| 3 | 0.161 | 1.4% | 0.200 | 34.0% | 0.172 | 6.1% |
| 4 | 0.236 | 33.3% | 0.238 | 35.7% | 0.211 | 12.9% |
| 5 | 0.201 | 6.8% | 0.219 | 18.9% | 0.267 | 57.6% |
| 6 | 0.232 | 48.8% | 0.202 | 12.3% | 0.231 | 47.8% |
| 7 | 0.216 | 9.9% | 0.302 | 92.1% | 0.230 | 22.1% |
| 8 | 0.280 | 91.7% | 0.229 | 54.7% | 0.219 | 43.6% |
| 9 | 0.204 | 33.7% | 0.169 | 0.6% | 0.188 | 10.4% |
| 10 | 0.140 | 15.4% | 0.150 | 31.4% | 0.148 | 26.0% |
| 11 | 0.193 | 3.4% | 0.250 | 40.7% | 0.221 | 19.7% |
| 12 | 0.244 | 50.5% | 0.222 | 23.6% | 0.208 | 12.0% |
| 13 | 0.201 | 64.6% | 0.153 | 11.5% | 0.215 | 75.1% |
| 14 | 0.218 | 19.1% | 0.239 | 49.4% | 0.274 | 86.8% |
| 15 | 0.264 | 53.4% | 0.242 | 24.9% | 0.263 | 52.2% |
| 16 | 0.173 | 16.6% | 0.179 | 26.3% | 0.195 | 54.8% |
| 17 | 0.229 | 39.6% | 0.232 | 42.9% | 0.222 | 33.1% |
| 18 | 0.172 | 5.5% | 0.183 | 10.5% | 0.196 | 18.4% |

| | | | | | | |
|---------|--------------|--------------|--------------|-------------|--------------|-------------|
| 19 | 0.266 | 35.1% | 0.307 | 65.1% | 0.280 | 43.3% |
| 20 | 0.242 | 41.2% | 0.235 | 34.7% | 0.281 | 65.3% |
| 21 | 0.230 | 50.2% | 0.168 | 2.2% | 0.230 | 50.1% |
| 22 | 0.259 | 44.2% | 0.233 | 22.8% | 0.231 | 21.7% |
| 23 | 0.196 | 21.2% | 0.217 | 64.1% | 0.199 | 24.0% |
| 24 | 0.222 | 31.4% | 0.238 | 49.4% | 0.249 | 61.3% |
| 25 | 0.178 | 18.7% | 0.194 | 50.1% | 0.186 | 34.4% |
| 26 | 0.198 | 18.3% | 0.183 | 8.5% | 0.274 | 89.5% |
| 27 | 0.235 | 32.2% | 0.217 | 18.2% | 0.215 | 15.3% |
| 28 | 0.315 | 84.5% | 0.243 | 6.0% | 0.283 | 49.7% |
| 29 | 0.179 | 14.5% | 0.184 | 20.9% | 0.204 | 42.8% |
| 30 | 0.145 | 8.1% | 0.178 | 72.4% | 0.159 | 29.8% |
| 31 | 0.169 | 40.9% | 0.165 | 37.4% | 0.158 | 30.6% |
| 32 | 0.152 | 28.4% | 0.157 | 44.3% | 0.156 | 40.8% |
| 33 | 0.181 | 36.1% | 0.137 | 0.0% | 0.237 | 70.2% |
| 34 | 0.257 | 47.1% | 0.294 | 83.4% | 0.250 | 37.4% |
| 35 | 0.232 | 8.2% | 0.258 | 36.2% | 0.303 | 76.6% |
| 36 | 0.261 | 25.0% | 0.292 | 55.6% | 0.292 | 55.5% |
| 37 | 0.229 | 33.3% | 0.237 | 41.6% | 0.230 | 34.7% |
| 38 | 0.236 | 24.7% | 0.239 | 28.2% | 0.261 | 50.2% |
| 39 | 0.215 | 31.3% | 0.188 | 6.1% | 0.253 | 68.3% |
| 40 | 0.239 | 44.8% | 0.257 | 61.0% | 0.323 | 82.6% |
| 41 | 0.205 | 51.7% | 0.248 | 88.6% | 0.187 | 24.1% |
| 42 | 0.157 | 43.8% | 0.153 | 39.4% | 0.135 | 8.5% |
| 43 | 0.257 | 47.4% | 0.199 | 3.8% | 0.274 | 54.6% |
| 44 | 0.207 | 11.8% | 0.231 | 28.4% | 0.205 | 9.7% |
| Average | 0.214 | 31.9% | 0.215 | 34.7% | 0.226 | 41.0% |

Supplementary Table S3. Structural similarity between ScNup192 and similar alpha-helical proteins.

| PDB | DALI score | CE score | MultiProt score | Composite Z-score | Name | Class |
|------------|-------------------|-----------------|------------------------|--------------------------|--------------|--------------|
| 1ee4A | 13.2 | 6 | 213 | 1.4 | SRP1_KAP60 | kap |
| 3oqsA | 11.8 | 6.1 | 206 | 1.3 | Impalpha2 | kap |
| 3tpoA | 11.9 | 6 | 206 | 1.3 | Impalpha2 | kap |
| 1un0A | 11.8 | 5.9 | 209 | 1.3 | SRP1_KAP60 | kap |
| 1wa5B | 13.2 | 5.7 | 201 | 1.3 | SRP1_KAP60 | kap |
| 1qz7A | 13.9 | 5.7 | 177 | 1.2 | Betacatenin | betacatenin |
| 2jdqA | 11.7 | 5.9 | 186 | 1.1 | Impalpha1 | kap |
| 1jdhA | 13.8 | 5.6 | 173 | 1.1 | Betacatenin | betacatenin |
| 3ouwA | 11.9 | 5.7 | 183 | 1.0 | Betacatenin | betacatenin |
| 3nc1A | 11.9 | 5.2 | 205 | 1.0 | CRM1_KAP124 | kap |
| 1g3jA | 13.8 | 5.3 | 172 | 1.0 | Betacatenin | betacatenin |
| 3bctA | 13.3 | 5.5 | 167 | 1.0 | Betacatenin | betacatenin |
| 2bptA | 11.8 | 4.9 | 217 | 1.0 | KAP95 | kap |
| 2qnaA | 13.3 | 5.2 | 173 | 0.9 | KAP95 | kap |
| 1w63A | 10 | 5.5 | 197 | 0.9 | AP1 | ap |
| 1z3hA | 10.5 | 5 | 211 | 0.9 | CSE1_KAP109 | kap |
| 1w9cA | 12.1 | 5.7 | 134 | 0.7 | CRM1_KAP124 | kap |
| 1wa5C | 13 | 3.9 | 218 | 0.7 | CSE1_KAP109 | kap |
| 3m1iC | 9.8 | 5 | 198 | 0.7 | CRM1_KAP124 | kap |
| 2z5kA | 12 | 4.4 | 199 | 0.7 | Transportin1 | kap |
| 1m5nS | 10.2 | 5.2 | 177 | 0.7 | KAP95 | kap |
| 1ibrB | 11.1 | 5.2 | 165 | 0.7 | KAP95 | kap |
| 1qgrA | 11.4 | 4.4 | 202 | 0.7 | KAP95 | kap |
| 3gjxA | 10 | 4.6 | 202 | 0.6 | CRM1_KAP124 | kap |
| 3gb8A | 11.3 | 3.9 | 198 | 0.4 | CRM1_KAP124 | kap |
| 2jkrA | 9.2 | 5.2 | 153 | 0.4 | AP2 | ap |
| 3a6pA | 8.8 | 5 | 158 | 0.4 | Expotin5 | kap |
| 2x19B | 9.6 | 4.7 | 160 | 0.3 | Imp13 | kap |
| 1b3uA | 7 | 5 | 151 | 0.2 | PP2A | pp2 |
| 2iaeB | 7.9 | 5 | 139 | 0.2 | PP2A | pp2 |
| 2pf4A | 6.3 | 4.7 | 171 | 0.1 | PP2A | pp2 |
| 1w63B | 8 | 4.6 | 152 | 0.1 | AP1 | ap |
| 3fgaB | 8 | 4.4 | 143 | 0.0 | PP2A | pp2 |
| 2nppB | 7.5 | 4.4 | 143 | -0.1 | PP2A | pp2 |
| 3cqcA | 3.2 | 5.2 | 73 | -0.6 | Nup84 | nup |
| 3mv2B | 3 | 4.6 | 94 | -0.7 | COP1 | copI |
| 3i4rB | 5.2 | 3.9 | 100 | -0.7 | Nup133 | nup |
| 1b89A | 3.5 | 3.9 | 87 | -0.9 | CHC1 | clathrin |
| 2rfoA | 4.9 | 3.3 | 95 | -1.0 | Nic96 | nup |
| 3hxrA | 2.4 | 4.1 | 84 | -1.0 | Nup120 | nup |

| | | | | | | |
|-------|-----|-----|----|------|---------|----------|
| 3i5pA | 3.7 | 3.7 | 87 | -1.0 | Nup170 | nup |
| 3lvga | 4.4 | 3.5 | 88 | -1.0 | CHC1 | clathrin |
| 3kfoA | 2.5 | 4.3 | 65 | -1.0 | Nup133 | nup |
| 1bpoA | 2.9 | 3.7 | 83 | -1.1 | CHC1 | clathrin |
| 3ikoB | 1.7 | 4.1 | 76 | -1.1 | Nup145C | nup |
| 3mzlB | 1.2 | 4.3 | 71 | -1.3 | SEC31 | copII |
| 2pm7A | 3.3 | 3.9 | 64 | -1.3 | SEC31 | copII |
| 3cqcB | 4 | 3.7 | 61 | -1.5 | Nup133 | nup |
| 3ikoC | 2.5 | 3.5 | 72 | -1.5 | Nup84 | nup |
| 2qx5A | 4.8 | 2.6 | 88 | -1.5 | Nic96 | nup |
| 3mv2A | 2.9 | 3.3 | 68 | -1.6 | COP1 | copI |
| 3mkqA | 2.2 | 2.8 | 82 | -1.8 | COP1 | copI |
| 3jroC | 4.2 | 2.6 | 66 | -1.3 | Nup84 | nup |
| 3mkqB | 1.9 | 3.1 | 64 | -1.3 | COP1 | copI |
| 3f3fC | 2.2 | 2.6 | 70 | -1.5 | Nup85 | nup |
| 3eweB | 3.2 | 2 | 69 | -1.5 | Nup85 | nup |

Supplementary Table S4. Structural similarity between ScNup85 and similar alpha-helical proteins.

| PDB | DALI Score | CE Score | MultiProt Score | Composite Z-score | Name | Class |
|------------|-------------------|-----------------|------------------------|--------------------------|-------------|--------------|
| 3ikoB | 14.3 | 5.9 | 141 | 1.44 | Nup145C | nup |
| 3ikoC | 14.2 | 5.2 | 105 | 1.02 | Nup84 | nup |
| 2rfoA | 13 | 4.7 | 124 | 0.93 | Nic96 | nup |
| 3jroC | 9.6 | 5 | 101 | 0.75 | Nup84 | nup |
| 2qx5A | 12.4 | 2.8 | 129 | 0.36 | Nic96 | nup |
| 2pm7A | 4.5 | 4.9 | 72 | 0.34 | SEC31 | copII |
| 3mkqB | 4.2 | 4.7 | 63 | 0.22 | COP1 | copI |
| 3mzlB | 3.7 | 4.7 | 56 | 0.16 | SEC31 | copII |
| 2pf4A | 4.9 | 4.1 | 67 | 0.09 | PP2A | pp2 |
| 3mkqA | 4.4 | 3.9 | 74 | 0.05 | COP1 | copI |
| 2l1lB | 4.3 | 4.1 | 55 | 0.00 | CRM1_KAP124 | kap |
| 3lvgA | 3.4 | 3.7 | 65 | -0.10 | CHC1 | clathrin |
| 1b89A | 3.6 | 3.5 | 68 | -0.13 | CHC1 | clathrin |
| 1m5nS | 4.8 | 3.3 | 69 | -0.14 | KAP95 | kap |
| 1bpoA | 3.4 | 3.7 | 56 | -0.15 | CHC1 | clathrin |
| 3i4rB | 4.9 | 3.3 | 64 | -0.16 | Nup133 | nup |
| 3gxA | 4.8 | 3.3 | 64 | -0.17 | CRM1_KAP124 | kap |
| 1ibrB | 4.5 | 3.1 | 75 | -0.18 | KAP95 | kap |
| 1qz7A | 3.5 | 3.3 | 65 | -0.21 | Betacatenin | betacatenin |
| 3fgaB | 3.8 | 3.1 | 72 | -0.22 | PP2A | pp2 |
| 3ouwA | 2.6 | 3.3 | 69 | -0.23 | Betacatenin | betacatenin |
| 2nppB | 3.5 | 3.1 | 73 | -0.23 | PP2A | pp2 |
| 1jdhA | 3.1 | 3.3 | 65 | -0.23 | Betacatenin | betacatenin |
| 2jkrA | 5.2 | 2.8 | 75 | -0.24 | AP2 | ap |
| 3a6pA | 5.2 | 2.8 | 75 | -0.24 | Expotin5 | kap |
| 2iaeB | 2.9 | 3.1 | 74 | -0.25 | PP2A | pp2 |
| 1g3jA | 3.3 | 3.3 | 59 | -0.26 | Betacatenin | betacatenin |
| 3nc1A | 3.7 | 3.1 | 64 | -0.27 | CRM1_KAP124 | kap |
| 2bptA | 4.8 | 2.8 | 69 | -0.29 | KAP95 | kap |
| 1qgrA | 4.9 | 2.8 | 68 | -0.29 | KAP95 | kap |
| 2x19B | 4.5 | 2.8 | 67 | -0.31 | Imp13 | kap |
| 2qnaA | 4.7 | 2.6 | 75 | -0.32 | KAP95 | kap |
| 1w63B | 4.9 | 2.8 | 63 | -0.32 | AP1 | ap |
| 3oqsA | 3.1 | 3.1 | 60 | -0.32 | Impalpha2 | kap |
| 1b3uA | 5.1 | 2.6 | 70 | -0.33 | PP2A | pp2 |
| 3gb8A | 4.7 | 2.8 | 62 | -0.33 | CRM1_KAP124 | kap |
| 3mv2A | 3.8 | 2.8 | 67 | -0.34 | COP1 | copI |
| 2jdqA | 3.7 | 2.8 | 65 | -0.36 | Impalpha1 | kap |
| 1w63A | 3.7 | 2.6 | 72 | -0.38 | AP1 | ap |
| 1ee4A | 3.4 | 2.8 | 63 | -0.38 | SRP1_KAP60 | kap |
| 3m1iC | 4.3 | 2.6 | 67 | -0.38 | CRM1_KAP124 | kap |

| | | | | | | |
|-------|-----|-----|----|-------|--------------|-------------|
| 1z3hA | 3.9 | 2.6 | 69 | -0.38 | CSE1_KAP109 | kap |
| 3i5pA | 3.5 | 2.8 | 58 | -0.40 | Nup170 | nup |
| 3tpoA | 2.9 | 2.8 | 62 | -0.41 | Impalpha2 | kap |
| 1un0A | 3.7 | 2.6 | 66 | -0.41 | SRP1_KAP60 | kap |
| 1wa5C | 4.4 | 2.3 | 75 | -0.42 | CSE1_KAP109 | kap |
| 2z5kA | 4 | 2.6 | 62 | -0.42 | Transportin1 | kap |
| 1wa5B | 2.7 | 2.6 | 71 | -0.42 | SRP1_KAP60 | kap |
| 3mv2B | 3.3 | 2.8 | 56 | -0.42 | COP1 | copI |
| 3bctA | 2.8 | 2.8 | 57 | -0.44 | Betacatenin | betacatenin |
| 3cqcA | 4.6 | 2.3 | 63 | -0.48 | Nup84 | nup |
| 1w9cA | 3.9 | 2.3 | 65 | -0.50 | CRM1_KAP124 | kap |
| 3cqcB | 3.5 | 2.3 | 62 | -0.53 | Nup133 | nup |
| 3kfoA | 3.1 | 2.3 | 58 | -0.57 | Nup133 | nup |
| 3hxrA | 2.4 | 2.3 | 48 | -0.66 | Nup120 | nup |
| 3ic9A | 2.6 | 2 | 47 | -0.74 | ExportinT | kap |

Supplementary Table S5. Structural similarity between ScNup170 and similar alpha-helical proteins.

| PDB | DALI Score | CE Score | MultiProt score | Composite Z-score | Name | Class |
|------------|-------------------|-----------------|------------------------|--------------------------|-------------|--------------|
| 3i4rB | 4.9 | 4.7 | 102 | 0.41 | Nup133 | nup |
| 1w9cA | 5 | 4.9 | 75 | 0.32 | CRM1_KAP124 | kap |
| 1jdhA | 3.9 | 4.6 | 102 | 0.30 | Betacatenin | betacatenin |
| 2qnaA | 5.9 | 4.6 | 83 | 0.28 | KAP95 | kap |
| 3m1iC | 6 | 4.3 | 96 | 0.24 | CRM1_KAP124 | kap |
| 1w63A | 4.3 | 4.7 | 82 | 0.23 | AP1 | ap |
| 3ikoB | 3 | 5 | 71 | 0.22 | Nup145C | nup |
| 3ouwA | 4 | 4.4 | 101 | 0.21 | Betacatenin | betacatenin |
| 1qgrA | 5.3 | 4.6 | 76 | 0.20 | KAP95 | kap |
| 1wa5C | 4.4 | 4.6 | 83 | 0.20 | CSE1_KAP109 | kap |
| 1b89A | 5.5 | 4.4 | 86 | 0.19 | CHC1 | clathrin |
| 3cqcB | 4.6 | 4.7 | 70 | 0.16 | Nup133 | nup |
| 3gb8A | 5.1 | 4.4 | 86 | 0.16 | CRM1_KAP124 | kap |
| 2jdqA | 3 | 4.6 | 82 | 0.11 | Impalpha1 | kap |
| 2bptA | 4.7 | 4.4 | 78 | 0.09 | KAP95 | kap |
| 2rfoA | 3.8 | 4.6 | 71 | 0.08 | Nic96 | nup |
| 1w63B | 5.4 | 4.3 | 75 | 0.06 | AP1 | ap |
| 1z3hA | 5.7 | 4.3 | 71 | 0.05 | CSE1_KAP109 | kap |
| 2jkrA | 4.6 | 4.3 | 79 | 0.04 | AP2 | ap |
| 2x19B | 4.9 | 4.1 | 89 | 0.03 | Imp13 | kap |
| 3mkqA | 3.2 | 4.4 | 80 | 0.01 | COP1 | copI |
| 3nc1A | 5.2 | 3.9 | 97 | 0.01 | CRM1_KAP124 | kap |
| 3kfoA | 2.9 | 4.7 | 62 | 0.01 | Nup133 | nup |
| 3gxA | 5.2 | 3.9 | 94 | -0.01 | CRM1_KAP124 | kap |
| 1g3jA | 3.5 | 4.1 | 94 | -0.01 | Betacatenin | betacatenin |
| 1bpoA | 3.8 | 4.3 | 77 | -0.02 | CHC1 | clathrin |
| 3mkqB | 3.4 | 4.4 | 73 | -0.02 | COP1 | copI |
| 1qz7A | 4 | 4.1 | 86 | -0.04 | Betacatenin | betacatenin |
| 3fgaB | 4.5 | 3.9 | 88 | -0.09 | PP2A | pp2 |
| 3bctA | 3.6 | 3.9 | 94 | -0.10 | Betacatenin | betacatenin |
| 1ibrB | 3.7 | 4.1 | 78 | -0.11 | KAP95 | kap |
| 1un0A | 3.6 | 4.1 | 75 | -0.14 | SRP1_KAP60 | kap |
| 2111B | 3.5 | 4.4 | 54 | -0.15 | CRM1_KAP124 | kap |
| 3mzlB | 3.2 | 4.1 | 76 | -0.16 | SEC31 | copII |
| 1b3uA | 4.9 | 3.7 | 80 | -0.22 | PP2A | pp2 |
| 2nppB | 5.2 | 3.5 | 88 | -0.24 | PP2A | pp2 |
| 2pf4A | 3.7 | 3.9 | 73 | -0.24 | PP2A | pp2 |
| 3oqsA | 3.8 | 3.9 | 70 | -0.26 | Impalpha2 | kap |
| 3lvga | 3.2 | 3.7 | 88 | -0.26 | CHC1 | clathrin |
| 2qx5A | 4.6 | 3.7 | 75 | -0.27 | Nic96 | nup |
| 1ee4A | 3.4 | 3.9 | 71 | -0.27 | SRP1_KAP60 | kap |

| | | | | | | |
|-------|-----|-----|----|-------|--------------|-------|
| 2iaeB | 4 | 3.5 | 92 | -0.28 | PP2A | pp2 |
| 3mv2A | 4.4 | 3.9 | 61 | -0.29 | COP1 | copI |
| 3a6pA | 4.6 | 3.5 | 83 | -0.31 | Expotin5 | kap |
| 3tpoA | 4.1 | 3.7 | 72 | -0.32 | Impalpha2 | kap |
| 2pm7A | 4.4 | 3.7 | 68 | -0.33 | SEC31 | copII |
| 2z5kA | 3.9 | 3.5 | 80 | -0.37 | Transportin1 | kap |
| 1m5nS | 3.4 | 3.5 | 75 | -0.43 | KAP95 | kap |
| 3mv2B | 2.4 | 3.7 | 63 | -0.48 | COP1 | copI |
| 3jroC | 3 | 3.7 | 58 | -0.48 | Nup84 | nup |
| 1wa5B | 3.5 | 3.3 | 78 | -0.50 | SRP1_KAP60 | kap |
| 3hxrA | 1.9 | 3.7 | 61 | -0.52 | Nup120 | nup |
| 3cqcA | 3.2 | 3.5 | 63 | -0.53 | Nup84 | nup |
| 3ikoC | 2.5 | 3.5 | 56 | -0.62 | Nup84 | nup |
| 3f3fC | 2.4 | 3.1 | 74 | -0.69 | Nup85 | nup |
| 3eweB | 3.5 | 2.8 | 67 | -0.81 | Nup85 | nup |
| 3ic9A | 0.4 | 2.8 | 45 | -1.15 | ExportinT | kap |

Table S6. Nup192 Segment Models

| Template PDB | Nup192 Segment | Alignment %id |
|-------------------------|---------------------------|----------------------|
| 1wa5C | 981-1070 | 11.7 |
| 1u6gC | 1075-1345 | 11.2 |
| 3grlA | 1371-1674 | 9.3 |

Supplementary Experimental Procedures

Cloning, Expression, Purification, and Crystallization of ScNup192(2-960)

The gene encoding Nup192 from *S. cerevisiae* was cloned from genomic DNA of strain 2601D-5 (ATCC, USA). The domain encoding residues 2-960 [ScNup192(2-960)] was PCR amplified using AAATGGTCTGCAATTCCTTTCC and CAGACAAAGACGCAACGGAGCCA as forward and reverse primers, respectively. The purified PCR product was TOPO[®] (Invitrogen, USA) cloned into pSGX3, a derivative of pET26b(+), yielding a protein with a non-cleavable C-terminal hexahistidine tag. The resulting plasmid was transformed into BL21(DE3)-Condon+RIL (Invitrogen, USA) cells for expression. Production of Se-Met protein (Van Duyne et al., 1993) was carried out in 1L of HY media at 22°C containing 50µg/mL of kanamycin and 35µg/mL of chloramphenicol. Protein expression was induced by addition of 0.4mM IPTG. Cells were harvested after 21 hours by centrifugation at 4°C.

For purification, 18g of *E. coli* cell pellet was resuspended in 200mL of cold buffer containing 20mM Tris HCl pH 8.0, 500mM NaCl, 25mM imidazole, 2mM MgCl₂, 0.5mM ATP, 0.5mM TCEP, and 0.1% (v/v) Tween20, and incubated with DNAaseI (Roche, USA) for 30 minutes in the presence of EDTA-free complete protease inhibitor cocktail tablets (Roche, USA) at 4°C. Cells were ruptured using an Avestin EmulsiFlex-C3 homogenizer and debris was removed by centrifugation at 4°C. The supernatant was batch bound to 7mL of Ni-NTA resin (Qiagen, USA) pre-equilibrated with 25mM imidazole in Buffer1 (20mM Tris HCl pH 8.0, 500mM NaCl, 10% (v/v) glycerol, 0.5mM TCEP). The sample was washed with 100 mL of 50mM imidazole in Buffer1, and subsequently eluted with 30 mL of 250mM imidazole in the Buffer1. Eluted protein was

further purified over a 120 mL Superdex 200 column equilibrated with 20 mM HEPES pH 8.0, 500 mM NaCl, 10% (v/v) glycerol, and 5mM DTT containing four complete protease inhibitor tablets in 400 mL (protein storage buffer). Elution fractions, analyzed by SDS-PAGE, were combined and concentrated and aliquots were frozen in liquid nitrogen for storage at -80°C.

Crystallization and Structure Determination of ScNup192(2-960)

Screening of ScNup192(2-960), (at 8.84 mg/mL), was carried out using MCSG1-4 crystallization formulations (Microlytic, USA), dispensed with a Phoenix Liquid Handling System (Art Robins, USA) *via* sitting drop vapor diffusion at 21°C (0.3 µL protein + 0.3 µL reservoir solution). Screen conditions which gave initial crystals were extensively optimized to improve diffraction. Diamond-like crystals were obtained from sitting drops with 10% to 20% PEG3350 in presence of variety of mono-valent ions (typically 1µL of reservoir solution combined with 1µL of protein). The sample (crystal number 1) used for Se-SAD structure solution appeared in the presence of 14 % PEG3350 and 200 mM potassium sulfate, and diffracted to 3.4 Å resolution in the presence of ~20 % (v/v) glycerol as cryoprotectant. The sample used for final refinement at 3.25 Å resolution (crystal number 2) grew in the presence of 10% PEG3350 and 100mM potassium iodide. This crystal was equilibrated against a solution of saturated ammonium sulfate for 2.5 hours, before being flash frozen in liquid nitrogen with 30% PEG400 and 25% of saturated ammonium sulfate as cryo-protectant.

Diffraction data sets were recorded at both the X29A (Brookhaven National Laboratory) and the LRL-CAT 31-ID (Advanced Photon Source) beamlines and processed with HKL3000 (Minor et al., 2006). Diamond-like crystals belong to the

$P4_32_12$ space group with one molecule of ScNup192(2-960) in the asymmetric unit. A selenium sub-structure solution was obtained using the AutoSol wizard (Terwilliger et al., 2009; Zwart et al., 2008) in Phenix (Adams et al., 2010) with resolution limit set to 3.7 Å for the heavy atom search. Subsequent density modification resulted in an electron density map with clearly defined secondary structural elements and discernable sidechain density features (**Figure S1**). Initial model building was carried out using both the AutoBuild wizard (Afonine et al., 2012; Terwilliger et al., 2008) in Phenix, and Buccaneer (Cowtan, 2008) as implemented in CCP4 (Potterton et al., 2003; Winn et al., 2011). The resulting models were manually edited and combined to assemble a model of ScNup192(2-960) which was complete except for N-terminal 145 residues and disordered loops. This model was refined against the dataset from crystal number 2. Subsequent model completion involved map improvement by atom update in ARP/wARP (Langer et al., 2008; Morris et al., 2003) and solvent flattening in Parrot (Zhang et al., 1997), and manual building in COOT (Emsley and Cowtan, 2004; Emsley et al., 2010) followed by refinement using Refmac5 (Murshudov et al., 1997). Illustrations were prepared using PyMol (DeLano, 2002) and Chimera (Pettersen et al., 2004).

Small Angle X-ray Scattering (SAXS)

SAXS measurements of ScNup192(2-960) were carried out at Beamline 4-2 of the Stanford Synchrotron Radiation Lightsource (SSRL), SLAC National Accelerator Laboratory. The beam energy and current were 11 keV and 300mA, respectively. A silver behenate sample was used to calibrate the q -range and detector distance. Data collection was controlled with Blu-Ice (McPhillips, et al., 2002). We used an automatic sample delivery system equipped with a 1.5 mm-diameter thin-wall quartz capillary within which

a sample aliquot was oscillated in the X-ray beam to minimize radiation damage. The sample was placed at 1.7 m from a Rayonix MX225-HE (MAR-USA, USA) CCD detector with a binned pixel size of 293 μm by 293 μm . For more details of other parameters, see Table S1.

All protein samples for the SAXS experiment were suspended in the protein storage buffer, composed of 20 mM HEPES at pH 8.0, 500 mM NaCl, 10 % (v/v) glycerol, and 5 mM DTT containing four complete protease inhibitor tablets in 400 mL. All the suspensions were filtered through 0.1 μm membranes (Millipore, Bedford, MA) and a total of 10% glycerol was added to reduce radiation damage (Kuwamoto et al., 2004). Each of the 24 scattering images was scaled by the transmitted beam intensity, using SASTool (<http://ssrl.slac.stanford.edu/~saxs/analysis/sastool.htm>, formerly MarParse), and averaged to obtain fully processed data in the form of intensity *versus* q [$q=4\pi\sin(\theta)/\lambda$, where θ is one-half of the scattering angle and λ is the X-ray wavelength]. The average of the lower scattering angle parts ($q < 0.15 \text{ \AA}^{-1}$) of the lower concentration profiles (0.4-1.0 mg/mL) and the average of the higher scattering angle parts ($q > 0.12 \text{ \AA}^{-1}$) of the higher concentration (1.5-2.5 mg/mL) profiles were merged to obtain the final experimental SAXS profile.

The merged experimental SAXS profile was compared with SAXS profiles calculated using the crystal structure of ScNup192(2-960) with FoXS program (<http://salilab.org/foxs>). In addition, 1,000 monomer models of ScNup192(2-960) which included a C-terminal hexa-histidine tag (Gly-His-His-His-His-His), 113 disordered residues (not modeled in the crystal structure), missing side-chains, and 8 Se-Met residues, were generated using the crystal structure with the automodel function of

MODELLER and customized scripts in IMP, and compared with the merged experimental SAXS profile. A “complete model” was chosen as having the smallest chi value ($\chi = 3.84$) among the 1,000 monomer models. The *ab initio* shape of ScNup192(2-960) was initially computed from the merged experimental SAXS profile by running DAMMIF (Franke, 2009) 20 times, and further refined with additional 50 DAMMIN (Svergun, 1999) runs, followed by superposition and averaging with DAMAVER (Volkov, 2003). Fittings of models into the *ab initio* shape were visualized by customized scripts in UCSF Chimera (Pettersen et al., 2004).

Negative Staining and EM analyses of ScNup192(2-960)

Purified recombinant ScNup192(2-960) was applied to glow-discharged carbon-coated copper grids. The grids were rinsed with four drops of 0.75-1% uranyl formate, then stained for a minute and air-dried. The images of ScNup192(2-960) were collected on a Tecnai F20 (FEI Inc., USA) transmission electron microscope operating at an accelerating voltage of 80 kV at 50,000x magnification and underfocus $\sim 1 \mu\text{m}$. Images were recorded on a Tietz F224 4096x4096 CCD camera (15 μm pixels) at 2X binning. The pixel size at the specimen level was 3.23 Å. Particles were selected using Boxer from EMAN (Ludtke et al., 1999). The contrast transfer function (CTF) of the images was determined using ctfit from EMAN and the phases flipped accordingly. The particles were normalized and were then subjected to the Iterative Stable Alignment and Clustering (ISAC; (Yang et al., 2012) technique to produce stable class averages. The program was run for 7 generations in total; after each generation stable particles were

removed from the stack and the program was re-run with unclassified particles until no new classes were found. A pixel error of $2\sqrt{3}$ was used for the stability threshold.

We quantified the overlap of projections of the “complete model” and the 110,000 MD generated conformations of ScNup192(2-960) with each of 44 EM class averages, using the *EMageFit* application (Velazquez-Muriel et al., 2012) of the Integrative Modeling Platform (IMP) software package (Russel et al., 2012) (**Figure S4; Table S2**). First, an atomic model was downsampled to a resolution of 15 Å and projected in all directions of the hemisphere. Second, a coarse registration between each projection and a class-average was performed. Finally, the best coarse match was refined by the Simplex algorithm to obtain the *em2D score*. The *em2D score* is defined as one minus the cross-correlation coefficient between the image and the best-matching projection. The histograms of the *em2D scores* for all class averages were generated to determine the best matching conformation (or subset conformations), while selected histograms are shown (**Figure S5**). In general, a model can be considered for assignment to a class average if its rank percentage is lower than ~20%.

Purification and 3D EM Construction of Native, full-length *S. cerevisiae* Nup192 (ScNup192FL).

To purify native Nup192, we constructed a strain in which the NUP encoding gene was genomically tagged with PrA preceded by the human rhinovirus 3C protease (ppx) target sequence (GLEVLFGGPS) as described previously (Fernandez-Martinez et al., 2012). The ScNup192FL was isolated by affinity purification using IP buffer (20mM Hepes, 300mM NaCl, 2mM MgCl₂, 0.5% Triton, 0.1% Tween 20, 1mM DTT) and released from the affinity matrix by protease digestion in digestion buffer (20mM Hepes,

300mM NaCl, 2mM MgCl₂, 0.01% Tween 20, 0.1mM DTT). The recovered sample was then centrifuged at 20,000 g for 10 min. The supernatant was loaded on top of a 5-20% sucrose gradient made with digestion buffer plus 1/1000 protease inhibitors. Gradients were ultracentrifuged on a SW55 Ti rotor (Beckman-Coulter) at 50,000 rpm and 5°C for 10 hours. Gradients were manually unloaded from the top into 12 fractions of 410 µL. Fractions were analyzed by SDS-PAGE, R250 Coomassie staining and mass spectrometry.

Purified, native ScNup192FL was applied to glow-discharged carbon-coated copper grids. The grids were rinsed with four drops of 0.75-1% uranyl formate, then stained for a minute and air-dried. The random conical tilt reconstruction method was used to create an initial model of ScNup192FL (Frank and Radermacher, 1992). A JEOL JEM-2100F transmission electron microscope (JEOL USA Inc., Peabody MA) operating at an accelerating voltage of 200 kV was used to image ScNup192FL. The image pairs were recorded at 50° and 0° under low-dose conditions and 50,000x magnification using underfocus values between 1 and 2 µm. Images were recorded on a Tietz F224HD 2048x2048 CCD camera with 24 µm pixels (Tietz Video and Image Processing Systems GmbH, Germany). The pixel size at the specimen level was 2.93 Å. Tilt pairs were selected using JWEB and windowed using SPIDER (Frank et al., 1996). The images were shrunk by a factor of 2 followed by one generation of classification of untilted images using the ISAC method. Three class averages comprising 120, 100 and 42 particles were chosen for 3D reconstruction using associated tilted images and calculated Euler angles. The three reconstructions were aligned using Chimera (Pettersen et al., 2004) and averaged, and this combined reconstruction was used as an initial model for reference

based alignment in SPIDER (Frank et al., 1996). A total of 3883 particles were used in the refinement: 676 tilted, 676 untilted, and an additional 2351 untilted particles picked with Boxer. Slices of ScNup192FL were made through its 3D volume in the Z direction using SPIDER (Frank et al., 1996).

Functional analysis of ScNup192

The Nab2NLS-mCherry-PrA reporter protein is a tandem fusion of the NLS of Nab2, mCherry and a single repeat motif of PrA, constructed from a Nab2NLS-GFP-PrA expression plasmid (pBT016; Timney, et al., 2006), and subcloned into a centromeric yeast plasmid for expression from a constitutive TEF1 promoter (pBT054). Conditional mutants of Nup192, Nup145 and Nup82 were engineered by inserting 3 tetracycline-binding aptamers (3tc-apt) and 3xHA tag upstream of the corresponding open reading frames with homologous recombination tagging in DF5 α strain. Recombination cassettes were PCR-amplified from pTDH3-tc3-3xHA (Euroscarf). Oligonucleotide sequences are available upon request. Tetracycline-repressible Nup192, Nup145 and Nup82 strains and WT cells, transformed with the NLS-mCherry reporter plasmid, were grown to log phase in synthetic complete media, diluted to 0.25×10^7 cells/mL, and treated with 0.2 mg/mL chlorotetracycline. Treated cultures were then incubated at 30°C for 24 hours. Samples of cultures for imaging were transferred to Concanavalin A coated glass-bottomed culture dishes for imaging (Zenklusen, et al., 2007). Images were collected of >100 cells after 0 hours and 24 hours of treatment. Z-stacks of cell-fields were collected on a Zeiss Axioplan 200 inverted microscope fitted with a Perkin-Elmer UltraView spinning disk confocal imaging head using a 100x 1.45NA objective collected with a Andor iXon

EMCCD camera. Image stacks were background subtracted, adjusted for flat field illumination and combined into single images using maximum-intensity projection. Nuclear and cytoplasmic regions from all cells in these images were segmented using purpose-built MatLab scripts, and the mean pixel intensities from the segmented images used to calculate the N/C reporter-protein ratio for each cell.

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