

Table S1. Representation of the Nup82 holocomplex components. Related to Figure 1.

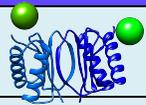
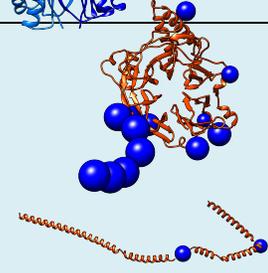
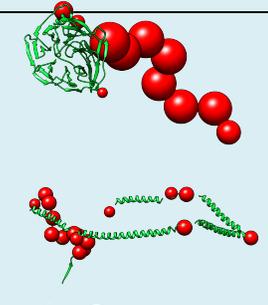
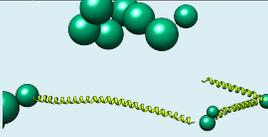
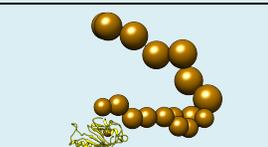
| Components | Rigid domains | Segments | | | | | |
|---|---|--|---|---|---------|----------------|---|
| | | structured segments with templates | | disordered and/or unknown segments without a template | | | |
| | | begins at | ends at | begins at | ends at | flexible beads | |
| Dyn2 |  | Dyn2 dimer (7-92) 7-92: X-ray 4DS1_A / C | 7 | 92 | 1 | 6 | 1 |
| | Nup82 |  | NTD (7-452), beta-propeller 7-452: X-ray 3PBP_G (100% seq id) | 7 | 16 | 1 | 6 |
| Flexible linker region | | | 23 | 120 | 17 | 22 | 1 |
| CTD (522-713), 3 coiled-coils 522-713: Model 5CWS_E (15% seq id, HHpred) Model 5C3L_A (14% seq id, HHpred) | | | 123 | 452 | 121 | 122 | 1 |
| | | | | | 453 | 521 | 7 |
| Nup159 |  | NTD (1-381), beta-propeller 2-381: X-ray 1XIP_A (100% seq id) | 2 | 347 | 1 | 1 | 1 |
| | | Disordered FG region | 362 | 381 | 348 | 361 | 2 |
| | | CTD (1117-1460), 3 coiled-coils (1211-1412) 1117-1126: X-ray 4DS1_B / D (bound to Dyn2) 1211-1412: Model 5CWS_D (12% seq id, HHpred) Model 5C3L_B (10% seq id, MUSCLE) 1429-1456: X-ray 3PBP_I (bound to Nup82 NTD) | 1117 | 1126 | 1127 | 1210 | 9 |
| | | | 1211 | 1239 | 1240 | 1265 | 3 |
| | | | 1266 | 1321 | 1322 | 1331 | 1 |
| | | | 1332 | 1372 | 1373 | 1381 | 1 |
| Nsp1 |  | Disordered FG region | | | 1 | 636 | 7 |
| | | CTD (637-823), 3 coiled-coils 637-823: Model 5CWS_C (32% seq id, HHpred) Model 5C3L_C (26% seq id, HHpred) | 637 | 727 | 728 | 741 | 2 |
| | | | 742 | 778 | 779 | 787 | 1 |
| | | | 788 | 823 | | | |
| Nup116 |  | Disordered FG region | | | 1 | 750 | 8 |
| | | Flexible linker region | | | 751 | 965 | 9 |
| | | CTD (966-1113) 966-1111: X-ray 3PBP_H (bound to Nup82 NTD) X-ray 3NF5 (59% seq id) | 966 | 1111 | 1112 | 1113 | 1 |

Table S3. Unique inter-subunit DSS cross-links identified connecting components of the cytoplasmic mRNA export platform. Related to Figures 3 and 6.

| Protein1 (residue1) | Protein2 (residue2) | Cross-linked peptides (residue) | Modification | P-value | mass | ppm |
|---------------------|---------------------|--|--------------|---------|----------|------|
| Nup159(1384) | Seh1(198) | RLDDNPLVAKLAK(10)-GKDGK(2) | | 1.E-03 | 2093.184 | 0.5 |
| Nup159(1367) | Seh1(244) | INHTEELLNILKLFVTK(12)-IFKITEK(3) | | 5.E-06 | 3039.762 | 2.4 |
| Nup159(1387) | Seh1(198) | LAKESLAR(3)-GKDGK(2) | | 4.E-02 | 1527.862 | 1.5 |
| Seh1(244) | Nup159(1372) | IFKITEK(3)-LFTVKNK(5) | | 6.E-04 | 1864.107 | -1.3 |
| Nup82(649) | Nup85(2) | NQILQFNSFVHSQKSLQQDLSYLK(14)-TIDDSNR(1) | | 7.E-07 | 3821.922 | 2.6 |
| Nup82(659) | Nup85(2) | SLQQDLSYLKSELTR(10)-TIDDSNR(1) | | 2.E-09 | 2737.377 | 5.3 |
| Nup85(231) | Nup82(622) | SDGEPDEEYIEQVFSVKDSTAGK(17)-KISHGEMK(1) | | 2.E-04 | 3595.687 | 0.7 |
| Nup82(671) | Nup145(773) | IEAETIKVDKK(7)-DIVGKSGVSIK(5) | | 4.E-03 | 2512.436 | 1.7 |
| Nup145(696) | Nup159(1397) | LDVSKDWVEQLILAGSSLR(5)-DGLLKEIK(5) | | 4.9E-02 | 3192.839 | 2.5 |
| Nup82(251) | Gle1(48) | NVIKQLQFVSK(4)-LPSFTKVGTK(6) | | 2.E-05 | 2517.457 | 3.8 |
| Nup116(1070)/(1067) | Gle1(48) | KPIKDPNHQLVK(4)-LPSFTKVGTK(6) | | 4.E-06 | 2630.516 | -2.7 |
| Gle1(53) | Nup82(251) | KLVNESVILDPALLENALR(1)-NVIKQLQFVSK(4) | | 3.E-02 | 3533.023 | 1.9 |
| Gle1(115) | Nsp1(613) | ASHSSLLDNAKNSNATAPLLEAIEESFQR(11)-STADVKSDDLK(6) | | 9.E-07 | 4487.230 | -3.7 |
| Nup159(1198) | Gle1(134) | EYYTSAKVSNIQFVSNSTLR(7)-KMQLVLANQK(1) | | 8.E-17 | 3826.993 | 0.9 |
| Gle1(144) | Nsp1(613) | MQNLVLANQKEIQSIR(10)-STADVKSDDLK(6) | | 5.E-07 | 3258.713 | 0.9 |
| Gle1(144) | Nsp1(607) | KMQNLVLANQKEIQSIR(11)-TEESSTGKSTADV(8) | | 1.E-09 | 3588.867 | -1.2 |
| Gle1(144) | Nsp1(599) | KMQNLVLANQKEIQSIR(11)-KTEESSTGK(1) | | 2.E-08 | 3115.655 | 1.8 |
| Gle1(144) | Nsp1(574) | MQNLVLANQKEIQSIR(10)-AAISFGAKPEEQK(8) | | 7.E-02 | 3396.807 | 4.1 |
| Gle1(144) | Nsp1(585) | MQNLVLANQKEIQSIR(10)-SSDTSKPAFTFGAQK(6) | | 1.E-06 | 3592.856 | 0.6 |
| Nup42(407) | Gle1(231) | FVQGLSSEKDGILELADLAEETLKIFR(24)-EHEAKLLQQK(5) | | 4.E-04 | 4381.342 | 2.3 |
| Nup42(392) | Gle1(259) | FVQGLSSEKDGILELADLAEETLK(9)-DKIAQIK(2) | | 5.E-06 | 3556.913 | 0.5 |
| Nup116(952) | Nsp1(370)/(465) | QEYSEDDKAVFADVAEK(9)-KDGDAKPAFSGAK(1) | | 4.E-04 | 3733.800 | -5.3 |
| Nup116(1067) | Nup159(1460) | KPIKDPNHQLVK(1)-NLNMAK(6) | Oxi | 1.E-05 | 2259.241 | 3.7 |
| Nup116(935) | Nup82(587) | HHSRNMDEENKENVADLQK(11)-KWDAQLSR(1) | | 2.E-02 | 3433.643 | 9.6 |

Table S4. Summary of SAXS experiments. Related to Figure 2.

Data collection

| | |
|---------------------------------------|---|
| Beamline | SSRL Beam Line BL4-2, SLAC National Accelerator Laboratory |
| Defining Slits size (mm) | 0.3 (H) × 0.3 (V) |
| Beam energy (keV) | 11 |
| Sample-Detector Distance (m) | 1.7 |
| Detector | Rayonix MX225-HE |
| Pixel binning | 8 × 8 |
| Pixel size (μm) | 292 |
| Exposure time (sec) | 1 |
| Images | Images taken for duration of run = ~20 images per concentration |
| Type of sample Cell | Quartz capillary (Diameter = 1.5mm) |
| Temperature (K) | 288 |
| Calibrant | AgBe |
| Final q range (\AA^{-1}) | 0.01 up to 0.45 |

Data analysis

| | |
|-------------------------------|--|
| Programs | SASTOOL (SasTool, 2013), PRIMUS (Konarev et al., 2003) |
| Buffer | 10mM HEPES, 150mM NaCl, 10% (v/v) glycerol, 5mM DTT, pH 7.5 |
| Concentration range (mg/mL) | 0.5 - 5.0 (SAXS profiles at 1 mg/mL were shown in Figure S4) |
| Guiner R_g (\AA) | 23.0 (Nup82 ⁴⁻²²⁰), 26.6 (Nup82 ⁴⁻⁴⁵²), and 38.7 (Nup82 ⁵⁷²⁻⁶⁹⁰) |
| Guiner qR_g limits | 1.236, 1.032, and 1.293 (in the same order as above) |
| D_{max} (\AA) | 72.2, 89.7, and 110.3 (in the same order as above) |

Modeling

| | |
|-------------------------------------|--|
| Programs | FoXS, DAMMIF, DAMMIN, DAMAVER |
| NSD* of the <i>ab initio</i> shapes | 0.540 (\pm 0.012), 0.597 (\pm 0.027), and 0.853 (\pm 0.027) |
| Data plotting | GNUPLOT, OPTIMUS, SAXS MOW, UCSF Chimera 1.10 |

*Normalized spatial discrepancy