Table S1. Components and modeling of yeast 90S structure

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Components | Alias | Functional group | Residue number | Modeling remark | Key crosslinks for AFs or MS signals of r-proteins |
| 5' ETS |  |  | 700 | 462 nt modeled |  |
| 18S |  |  | 1800 | 1103 nt modeled |  |
| ITS1 |  |  | 361 | 12 nt modeled |  |
| U3 RNA |  |  | 333 | 157 nt modeled |  |
| Utp15 |  | 5' ETS factor, UTPA | 513 | WD domain (about 1-376): poly-A model (2YMU, residues 5-13 and 302-577)  CTD (about 377-513): de novo poly-A model | EMG1\_2-UTP15\_244  EMG1\_2-UTP15\_243  UTP15\_341-UTP30\_170 |
| Utp5 |  | 5' ETS factor, UTPA | 643 | WD domain (about 1-365): poly-A model (2YMU, residues 5-13 and 302-577)  CTD (about 366-553): de novo poly-A model | NOP1\_168-UTP5\_419 |
| Utp9 |  | 5' ETS factor, UTPA | 575 | CTD (about 381-518): de novo poly-A model |  |
| Utp8 |  | 5' ETS factor, UTPA | 713 | WD domain (about 1-402): poly-A model (2YMU, residues 5-13 and 302-577)  CTD (about 403-688): de novo poly-A model | UTP4\_371-UTP8\_700  UTP4\_773-UTP8\_700 |
| Utp4 |  | 5' ETS factor, UTPA | 776 | WD domain (about 1-776): poly-A model (2YMU, residues 9-577) | UTP18\_418-UTP4\_316  UTP10\_644-UTP4\_561  UTP4\_371-UTP8\_700  UTP4\_773-UTP8\_700  NOP58\_93-UTP4\_217 NOP1\_229-UTP4\_217 |
| Utp17 | Nan1 | 5' ETS factor, UTPA | 896 | WD domain (about 1-786): poly-A model (2YMU, 8-577)  Residues 807-848: poly-A model of ctUtp10-ctUtp17 complex (5WYL, Chen R. and Ye K. unpublished data) | NAN1\_163-UTP10\_644 |
| Utp10 |  | 5' ETS factor, UTPA | 1769 | N domain (residues 3-421): homology model of ctUtp10-ctUtp17 complex (5WYL, Chen R. and Ye K. unpublished data)  M domain (residues 463-808): homology model of ctUtp10 M domain (5WY3, Chen R. and Ye K. unpublished data)  C domain (about 809-1769): poly-A model based on two copies of importin Kap60 (2C1T) | UTP10\_249-UTP18\_219  UTP10\_271-UTP21\_245  UTP10\_644-UTP4\_561  NAN1\_163-UTP10\_644  UTP10\_1754-UTP20\_27  UTP10\_1754-UTP20\_36 |
| Utp1 | Pwp2 | 5' ETS factor, UTPB | 923 | WD domain (about 1-706): homology model (2YMU)  Residues 707-852: homology model of the ctUtp1 CTD (5ICA). | PWP2\_27-UTP21\_730  PWP2\_27-UTP21\_766  PWP2\_85-UTP21\_661  PWP2\_85-UTP21\_9  PWP2\_123-UTP21\_9  PWP2\_129-UTP21\_661  PWP2\_129-UTP21\_9 |
| Utp21 |  | 5' ETS factor, UTPB | 939 | Residues 20-671: Utp21 WD domain (4NSX).  Residues 793-939: homology model of ctUtp21 CTD (5ICA). | PWP2\_27-UTP21\_730  PWP2\_27-UTP21\_766  PWP2\_85-UTP21\_661  PWP2\_85-UTP21\_9  PWP2\_123-UTP21\_9  PWP2\_129-UTP21\_661  PWP2\_129-UTP21\_9  UTP10\_271-UTP21\_245  UTP18\_219-UTP21\_288  RPS16A\_107-UTP21\_533 |
| Utp12 | Dip2 | 5' ETS factor, UTPB | 943 | WD domain (about 1-685): homology model (2YMU)  Residues 747-913: homology model of the ctUtp1 CTD (5ICA). | DIP2\_740-EMG1\_213 |
| Utp13 |  | 5' ETS factor, UTPB | 817 | WD domain (about 13-644): homology model (2YMU)  Residues 660-810: homology model of the ctUtp1 CTD (5ICA) | RPS14A\_2-UTP13\_181  RPS14A\_49-UTP13\_555 |
| Utp18 |  | 5' ETS factor, UTPB | 594 | WD domain (235-591): homology model of the ctUtp18 WD domain (5IC7) | UTP18\_219-UTP21\_288  UTP10\_249-UTP18\_219  UTP18\_418-UTP4\_316 |
| Utp6 |  | 5' ETS factor, UTPB | 440 | Unassigned |  |
| Nop56 |  | 5' ETS factor, U3 snoRNP | 504 | Residues 8-417: homology model of Nop5 (3PLA) | NOP1\_212-NOP56\_164  NOP1\_299-NOP56\_32 |
| Nop58 |  | 5' ETS factor, U3 snoRNP | 511 | Residues 3-403: homology model of Nop5 (3PLA) | NOP58\_93-UTP4\_217 |
| Snu13 |  | 5' ETS factor, U3 snoRNP | 126 | Residues 5-126: yeast Snu13 (2ALE) |  |
| Nop1 |  | 5' ETS factor, U3 snoRNP | 327 | Residues 84-322: homology model of Fibrillarin (3PLA) | NOP1\_212-NOP56\_164  NOP1\_299-NOP56\_32  FCF1\_35-NOP1\_168  FCF1\_35-NOP1\_299  FCF1\_133-NOP1\_299  NOP1\_229-UTP4\_217 |
| Rrp9 |  | 5' ETS factor, U3 snoRNP | 573 | Residues 130-570: yeast Rrp9 WD domain (4J0X) | RPS4A\_53-RRP9\_114  RPS24A\_11-RRP9\_114  RPS24A\_21-RRP9\_122  RPS24A\_49-RRP9\_122  RPS4A\_245-RRP9\_350  RPS24A\_11-RRP9\_350  RPS9A\_90-RRP9\_430 |
| Bud21 |  | 5' ETS factor | 214 | Unassigned |  |
| Utp7 |  | 5' ETS factor | 554 | WD domain (about 1-333): poly-A model (2YMU, residues 5-13 and 302-577) | UTP22\_745-UTP7\_518 SOF1\_449-UTP7\_478  SOF1\_449-UTP7\_476 |
| Utp11 |  | 5' ETS factor | 250 | N-terminal α-helix (about 2-74): de novo poly-A model | BMS1\_1075-UTP11\_75  BMS1\_1075-UTP11\_71  RPS23A\_70-UTP11\_2  RPS23A\_70-UTP11\_3 |
| Mpp10 |  | 5' ETS factor | 593 | Residues 45-64: poly-A model of the Rpf1-Rrs1 complex (5WXL, Zheng S. and Ye K. unpublished data)  Residues 429-454: structure of Imp3-Mpp10 complex (5WXM, Zheng S. and Ye K. unpublished data) | IMP3\_73-MPP10\_392  IMP3\_73-MPP10\_459 |
| Imp3 |  | 5' ETS factor | 183 | Residues 29-161: structure of Imp3-Mpp10 complex (5WXM, Zheng S. and Ye K. unpublished data) | IMP3\_73-RPS16A\_140  IMP3\_73-IMP4\_63  IMP3\_73-MPP10\_459  IMP3\_73-MPP10\_392 |
| Imp4 |  | 5' ETS factor | 290 | Residues 84-267: homology model of Rpf1-Rrs1 complex (5WXL, Zheng S. and Ye K. unpublished data) | IMP3\_73-IMP4\_63 |
| Sas10 |  | 5' ETS factor | 610 | Unassigned |  |
| Sof1 |  | 5' ETS factor | 489 | WD domain (about 1-376): poly-A model (2YMU, residues 5-13 and 302-577) | RPS7A\_148-SOF1\_143  SOF1\_449-UTP7\_478  SOF1\_449-UTP7\_476 |
| Fcf2 |  | 5' ETS factor | 217 | Unassigned |  |
| Fcf1 | Utp24 | 5' ETS factor | 189 | Residues 57-182: homology model of Utp23 (4MJ7) | FCF1\_35-NOP1\_168  FCF1\_35-NOP1\_299  FCF1\_93-RPS9A\_92  FCF1\_112-RPS22A\_71 FCF1\_133-NOP1\_299 |
| Enp2 |  | 5' domain factor | 707 | WD domain (about 1-311): poly-A model (2YMU, residues 5-13 and 302-577) |  |
| Bfr2 |  | 5' domain factor | 534 | Unassigned |  |
| Lcp5 |  | 5' domain factor | 357 | Unassigned |  |
| Efg1 |  | 5' domain factor | 233 | Unassigned |  |
| Krr1 |  | Central domain factor | 316 | Residues 38-212: structure of the Krr1-Faf1 complex (4QMF) | KRR1\_87-RPS14A\_2  KRR1\_102-RPS14A\_92  KRR1\_127-RPS14A\_92  KRR1\_245-RPS13\_107  KRR1\_245-RPS13\_107 |
| Rrp5 |  | Central domain factor | 1729 | Residues 1408-1721: structure of the Rrp5 TPR domain (5WWM, Chen X. and Ye K. unpublished data). |  |
| Utp22 |  | Central domain factor | 1237 | Residues 81-1237: structure of the Utp22-Rrp7 complex (4M5D) | RPS1A\_33-UTP22\_732  RPS1A\_219-UTP22\_1007  UTP22\_745-UTP7\_518  RRP7\_2-UTP22\_1205  RRP7\_292-UTP22\_1018 |
| Rrp7 |  | Central domain factor | 297 | Residues 3-189: structure of the Utp22-Rrp7 complex (4M5D)  Residues 190-216: de novo poly-A model | RPS13\_100-RRP7\_255  RPS13\_43-RRP7\_255  RPS13\_43-RRP7\_244  RRP7\_2-UTP22\_1205  RRP7\_292-UTP22\_1018 |
| Rok1 |  | Central domain factor | 564 | Unassigned |  |
| Emg1 |  | 3' major domain factor | 252 | Residues 28-252: structure of yeast Emg1 dimer bound to substrate RNA (3OIN) | EMG1\_177-RPS5\_213  DIP2\_740-EMG1\_213  EMG1\_2-UTP15\_244  EMG1\_2-UTP15\_243 |
| Utp30 |  | Late factor | 274 | Residues 8-255: homology model of r-protein L1 (1MZP) | UTP15\_341-UTP30\_170 |
| Bms1 |  | Late factor | 1183 | Residues 33-284 and 787-1016: homology model of Tsr1 (5IW7, Wang B. and Ye K. unpublished data)  Residues 547-636: structure of the Rcl1-Bms1 complex (4CLQ) | BMS1\_602-RCL1\_46  BMS1\_610-RCL1\_46  BMS1\_646-RCL1\_213 |
| Rcl1 |  | Late factor | 367 | Residues 7-361: structure of the Rcl1-Bms1 complex (4CLQ) | BMS1\_602-RCL1\_46  BMS1\_610-RCL1\_46  BMS1\_646-RCL1\_213 |
| Kre33 |  | Late factor | 1056 | Unassigned |  |
| Nop14 |  | Late factor | 810 | Unassigned |  |
| Noc4 |  | Late factor | 552 | Unassigned |  |
| Utp20 |  | Late factor | 2493 | de novo poly-A model | UTP10\_1754-UTP20\_27  UTP10\_1754-UTP20\_36  RPS4A\_198-UTP20\_1596  RPS6A\_131-UTP20\_643  RPS8A\_142-UTP20\_1245  RPS11A\_102-UTP20\_1646  RPS24A\_83-UTP20\_27 |
| Utp14 |  | Late factor | 899 | Unassigned |  |
| Enp1 |  | Late factor | 483 | Residues 205-465: structure of Enp1 (5WWO, Zheng S. and Ye K. unpublished data) |  |
| Pno1 |  | Late factor | 274 | Residues 94-266: homology model of aDim2 (3AEV) |  |
| Rrp12 |  | Late factor | 1228 | Unassigned |  |
| Rrt12 |  | Late factor | 206 | Unassigned |  |
| Faf1 |  | Late factor | 346 | Unassigned |  |
| S0 | uS2 | 3' major domain | 252 | Unassigned | Weak MS signal. |
| S1 | eS1 | Central domain | 255 | Residues 20-233: yeast ribosome structure (4V88) | Strong MS signal. |
| S2 | uS5 | head-body junction | 254 | Unassigned | No MS signal. |
| S3 | uS3 | 3' major domain | 240 | Unassigned | Weak MS signal. |
| S4 | eS4 | 5' domain | 261 | Residues 9-245: yeast ribosome structure (4V88) | Strong MS signal. |
| S5 | uS7 | 3' major domain | 225 | Residues 20-225: yeast ribosome structure (4V88) | Strong MS signal. |
| S6 | eS6 | 5' domain | 236 | Residues 1-226: yeast ribosome structure (4V88) | Strong MS signal. |
| S7 | eS7 | Central domain | 190 | Residues 4-187: yeast ribosome structure (4V88) | Strong MS signal. |
| S8 | eS8 | 5' domain | 200 | Residues 2-200: yeast ribosome structure (4V88) | Strong MS signal. |
| S9 | uS4 | 5' domain | 197 | Residues 12-186: yeast ribosome structure (4V88) | Strong MS signal. |
| S10 | eS10 | 3' major domain | 105 | Unassigned | No MS signal. |
| S11 | uS17 | 5' domain | 156 | Residues 2-142: yeast ribosome structure (4V88) | Strong MS signal. |
| S12 | eS12 | 3' major domain | 143 | Residues 20-143: yeast ribosome structure (4V88) | Strong MS signal. |
| S13 | uS15 | Central domain | 151 | Residues 9-142: yeast ribosome structure (4V88) | Strong MS signal. |
| S14 | uS11 | Central domain | 137 | Residues 11-119: yeast ribosome structure (4V88) | Strong MS signal. |
| S15 | uS19 | 3' major domain | 142 | Unassigned | Weak MS signal. |
| S16 | uS9 | 3' major domain | 143 | Residues 3-127: yeast ribosome structure (4V88) | Strong MS signal. |
| S17 | eS17 | 3' major domain | 136 | Unassigned | Strong MS signal. |
| S18 | uS13 | 3' major domain | 146 | Unassigned | Strong MS signal. |
| S19 | eS19 | 3' major domain | 144 | Unassigned | Strong MS signal. |
| S20 | uS10 | 3' major domain | 121 | Unassigned | No MS signal. |
| S21 | eS21 | head-body junction | 87 | Unassigned | No MS signal. |
| S22 | uS8 | Central domain | 130 | Residues 2-130: yeast ribosome structure (4V88) | Strong MS signal. |
| S23 | uS12 | 5' domain | 145 | Residues 43-145: yeast ribosome structure (4V88) | Weak MS signal. |
| S24 | eS24 | 5' domain | 135 | Residues 2-102: yeast ribosome structure (4V88) | Strong MS signal. |
| S25 | eS25 | 3' major domain | 108 | Unassigned | Weak MS signal. |
| S26 | eS26 | Central domain | 119 | Unassigned | Weak MS signal. |
| S27 | eS27 | Central domain | 82 | Residues 4-82: yeast ribosome structure (4V88) | Weak MS signal. |
| S28 | eS28 | 3' major domain | 67 | Residues 5-67: yeast ribosome structure (4V88) | Strong MS signal. |
| S29 | uS14 | 3' major domain | 56 | Unassigned | No MS signal. |
| S30 | eS30 | 5' domain | 63 | Residues 23-52: yeast ribosome structure (4V88) | No MS signal. |
| S31 | eS31 | 3' major domain | 152 | Residues 102-152: yeast ribosome structure (4V88) | No MS signal. |
| Asc1 | RACK1 | 3' major domain | 319 | Unassigned | No MS signal. |

The table includes 51 AFs that are present in the mature 90S ([Zhang et al. 2016b](#_ENREF_74)) and all 33 r-proteins in 40S. The mass spectrometry (MS) signals for r-proteins in a reference state of 90S (ITS1-239/Noc4-TAP particle) are included. The PDB codes of the fitted structures and the template structures for poly-A or homology models are indicated.

Table S2. Statistics of data collection, structural refinement and model validation

|  |  |  |  |
| --- | --- | --- | --- |
|  | Noc4-TAP | ΔMtr4/Enp1-TAP | ΔDhr1/Enp1-TAP |
| **Data collection** |  |  |  |
| EM equipment | FEI Titan Krios | FEI Titan Krios | FEI Titan Krios |
| Voltage (kV) | 300 | 300 | 300 |
| Detector | Falcon II | Falcon III | Falcon III |
| Grid | Quantifoil 2/2 | Quantifoil 1.2/1.3 | Quantifoil 1.2/1.3 |
| Micrographs | 1769 | 1102 | 2055 |
| Particles for 3D classification | 127,198 | 195,317 | 420,755 |
| Pixel size (Å) | 1.42 | 1.76 | 1.76 |
| Defocus range (μm) | 1.5-5 | 1.5-3.5 | 2.0-4.0 |
| Electron dose (e-/Å2) | 40 | 40 | 50 |
| **Map refinement** |  |  |  |
| Particles for refinement | 12,643 | 73,543 | 30,995 (state 1) |
| Overall resolution of map (Å) | 8.7 | 4.5 | 8.7 |
| Map sharpening B-factor (Å2) |  | -135 | -629 |
| **Model composition** |  |  |  |
| Protein chains |  | 55 | 61 |
| Protein residues |  | 16547 | 19955 |
| RNA chains |  | 3 | 3 |
| RNA bases |  | 1619 | 1734 |
| **Structural refinement** |  |  |  |
| Map CC (whole unit cell) |  | 0.399 | 0.513 |
| Map CC (around atoms) |  | 0.491 | 0.682 |
| **Rms deviations** |  |  |  |
| Bonds (Å) |  | 0.0057 | 0.0052 |
| Angles (°) |  | 1.16 | 1.02 |
| **Validation (protein)** |  |  |  |
| Molprobity score |  | 1.96 (78th percentile) | 1.94 (79th percentile) |
| Clashscore |  | 8.23 (81th percentile) | 8.61 (79th percentile) |
| Good rotamers (%) |  | 95.99 | 96.07 |
| Ramachandran plot favored (%) |  | 91.16 | 92.23 |
| Ramachandran plot Outliers (%) |  | 0.27 | 0.25 |
| **Validation (RNA)** |  |  |  |
| Correct sugar puckers (%) |  | 98.52 | 98.44 |
| Good backbone conformations (%) |  | 74.61 | 77.87 |