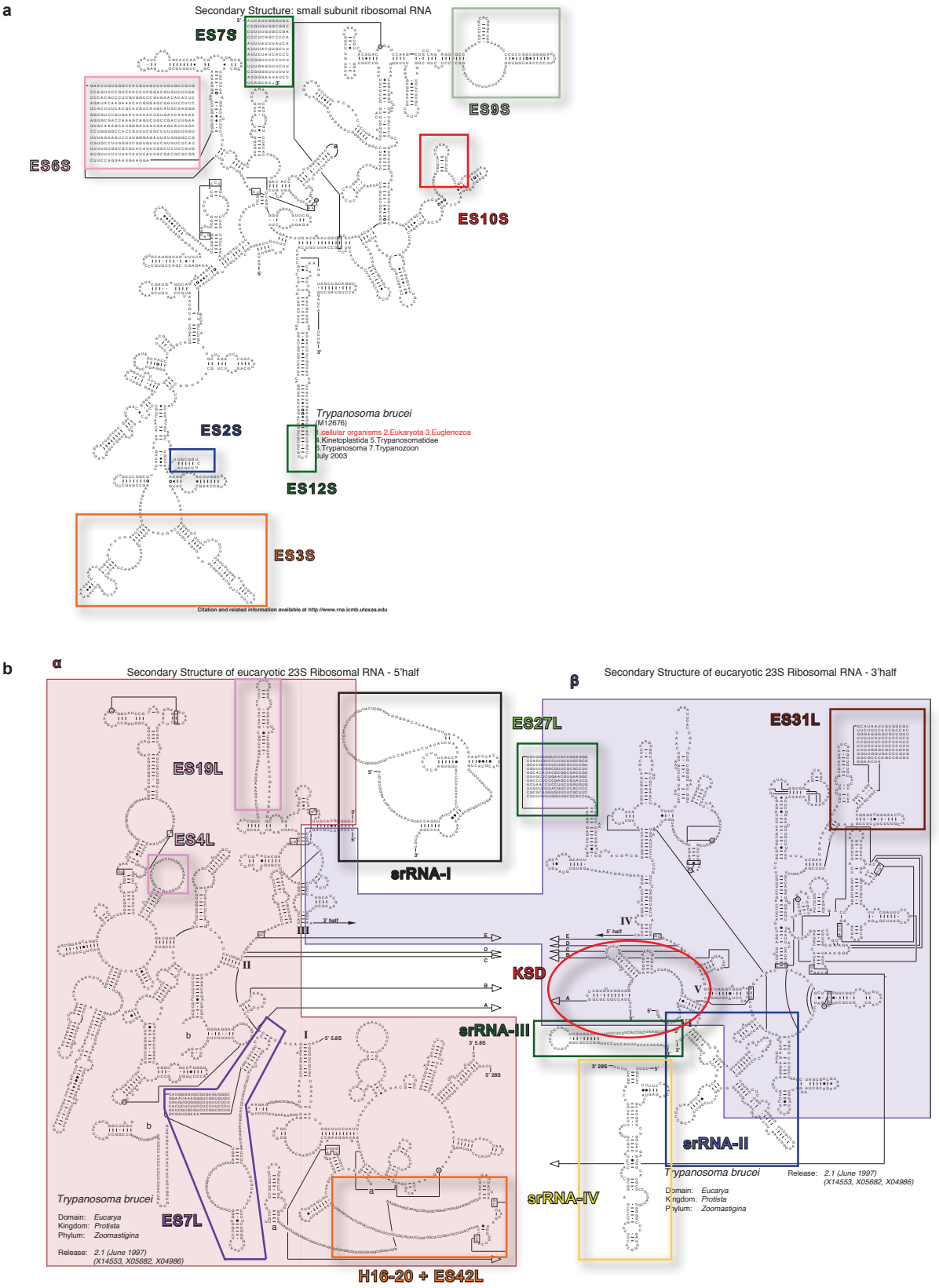
Supplementary Figures



Supplementary Figure 1. Secondary structures (2D) of both subunits rRNAs of the T. brucei ribosome. The secondary structures are fetched from the CRW site[26]. (**a**) Secondary structure of the SSU rRNA chain (18S), highlighted in colored frames are the largest expansion segments. (**b**) Secondary structure of the LSU rRNA chains, a (orange transparent area), b (purple transparent area), srRNA-I (black frame), srRNA-II (blue frame), srRNA-III (forest frame) and srRNA-IV (yellow frame). Largest expansion segments are highlighted in colored frames and the Kinetoplastid-Specific Domain (KSD) is encircled in red.

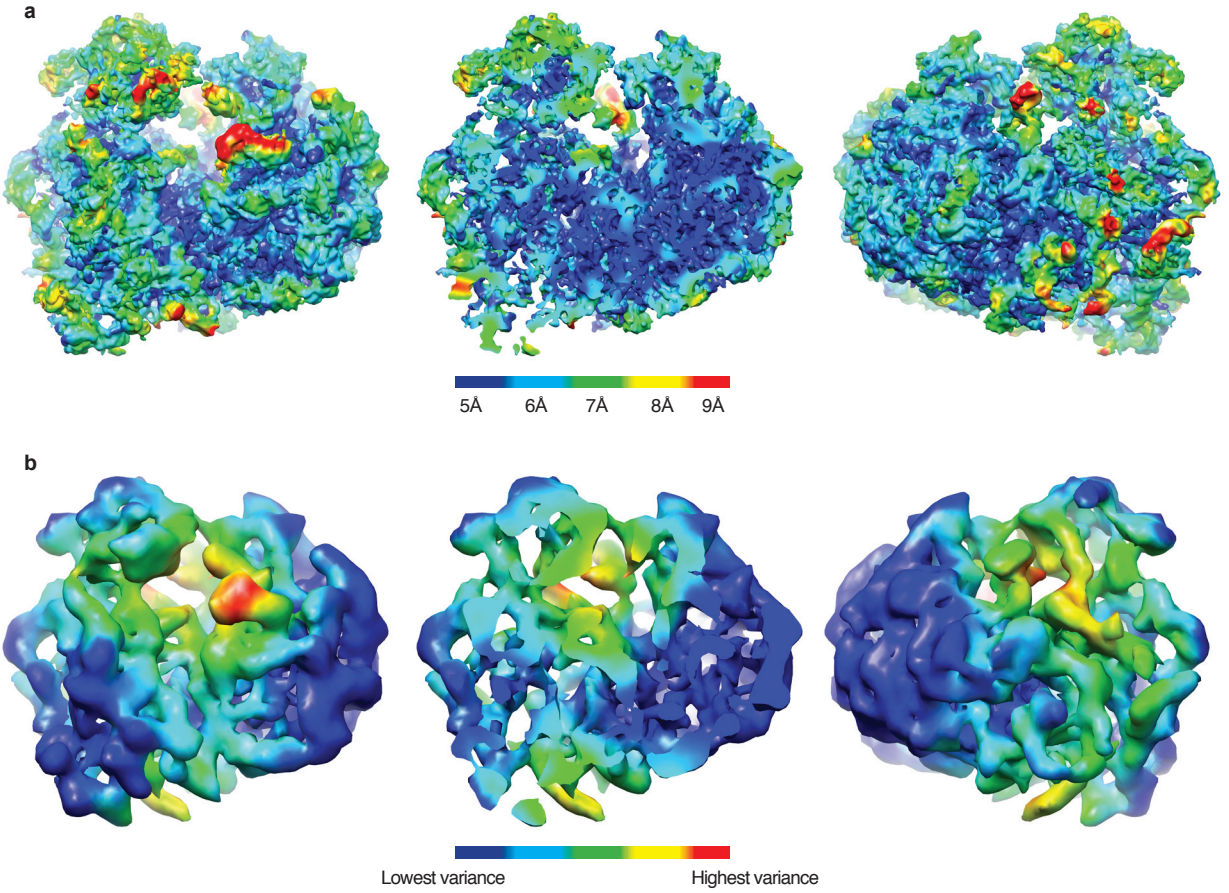
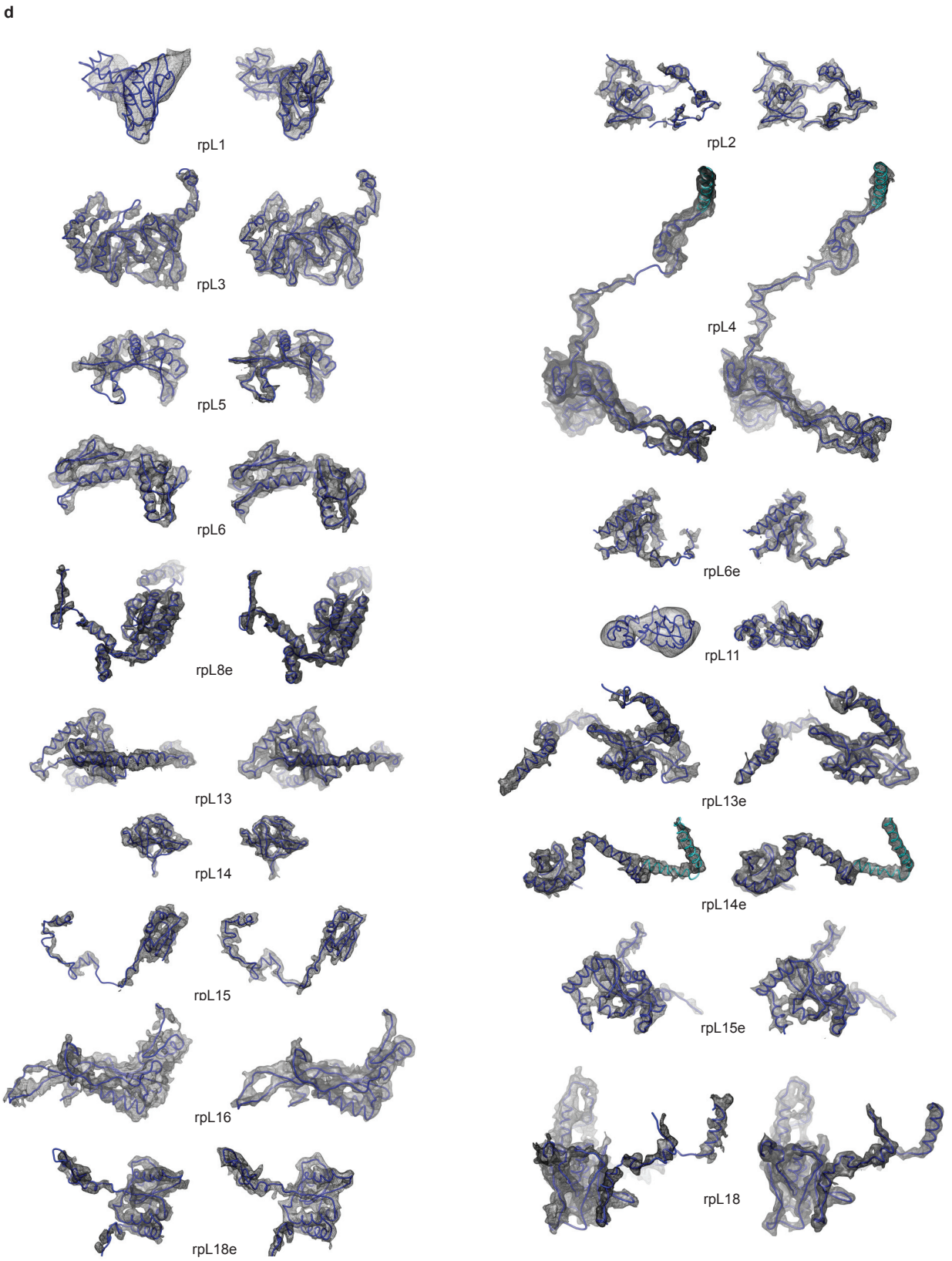


Fig. S2 continued on next 4 pages...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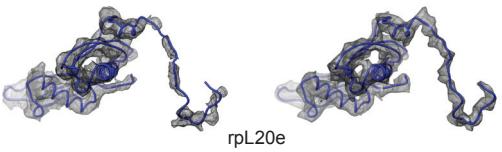
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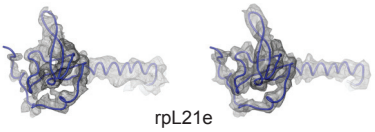
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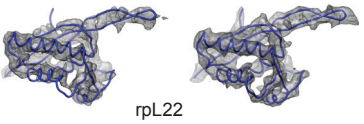
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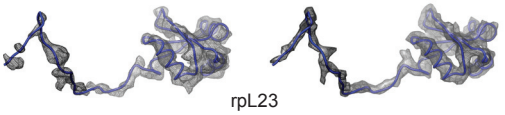
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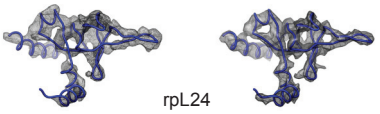
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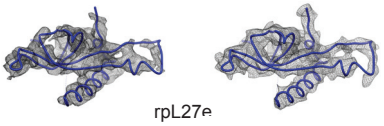
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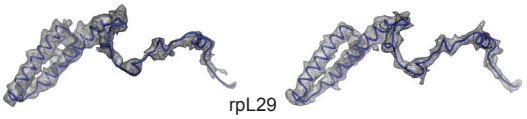
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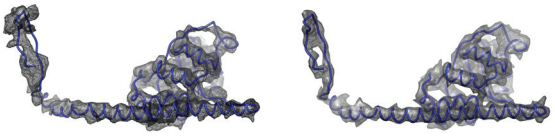
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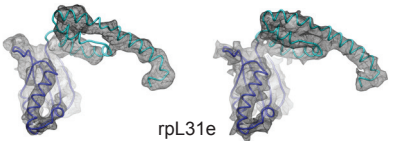
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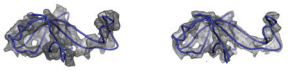
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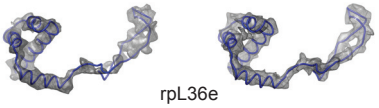
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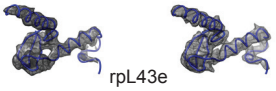
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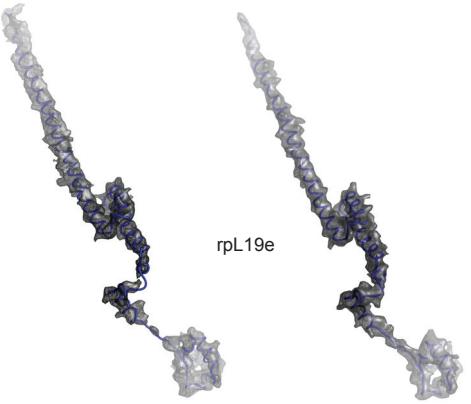
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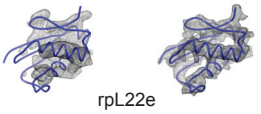
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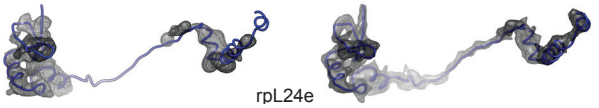
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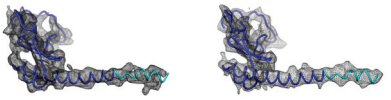
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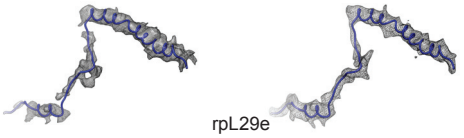
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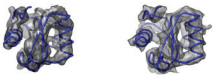
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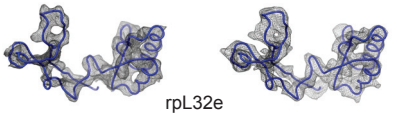
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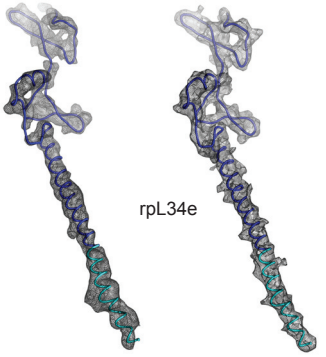
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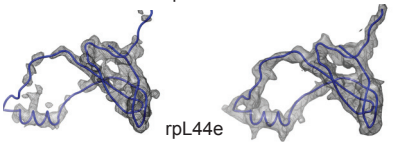
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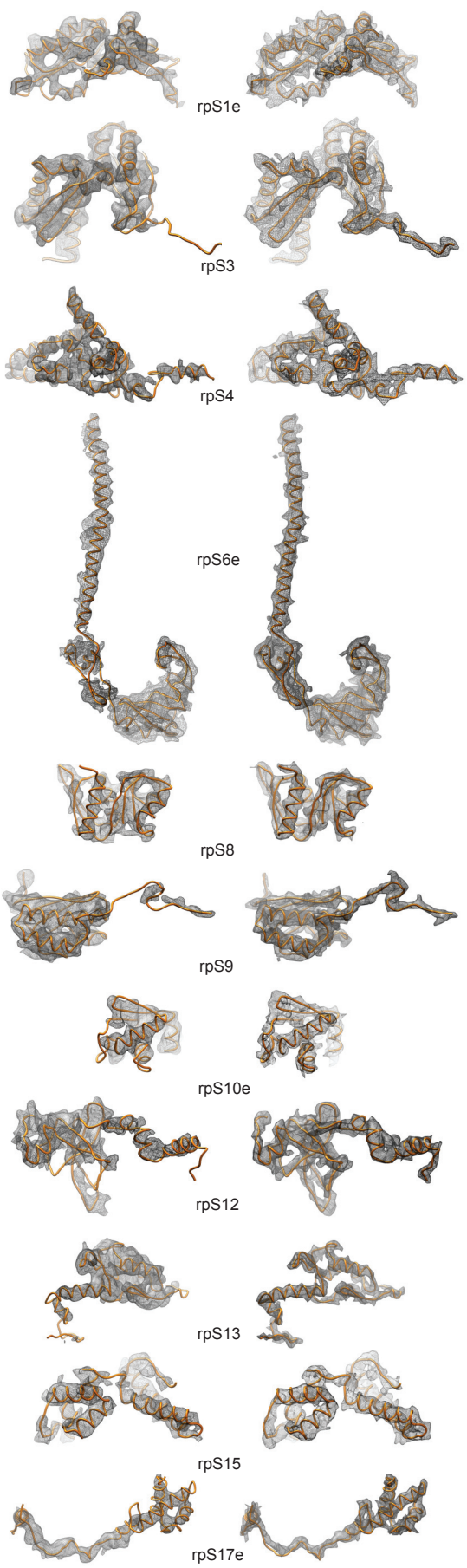
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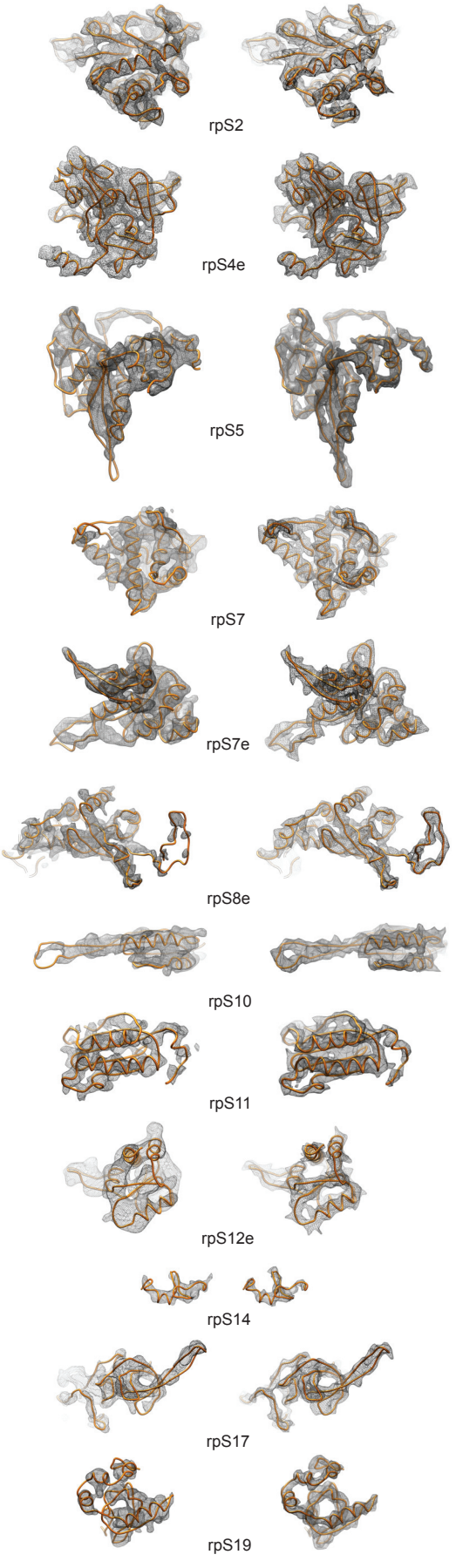
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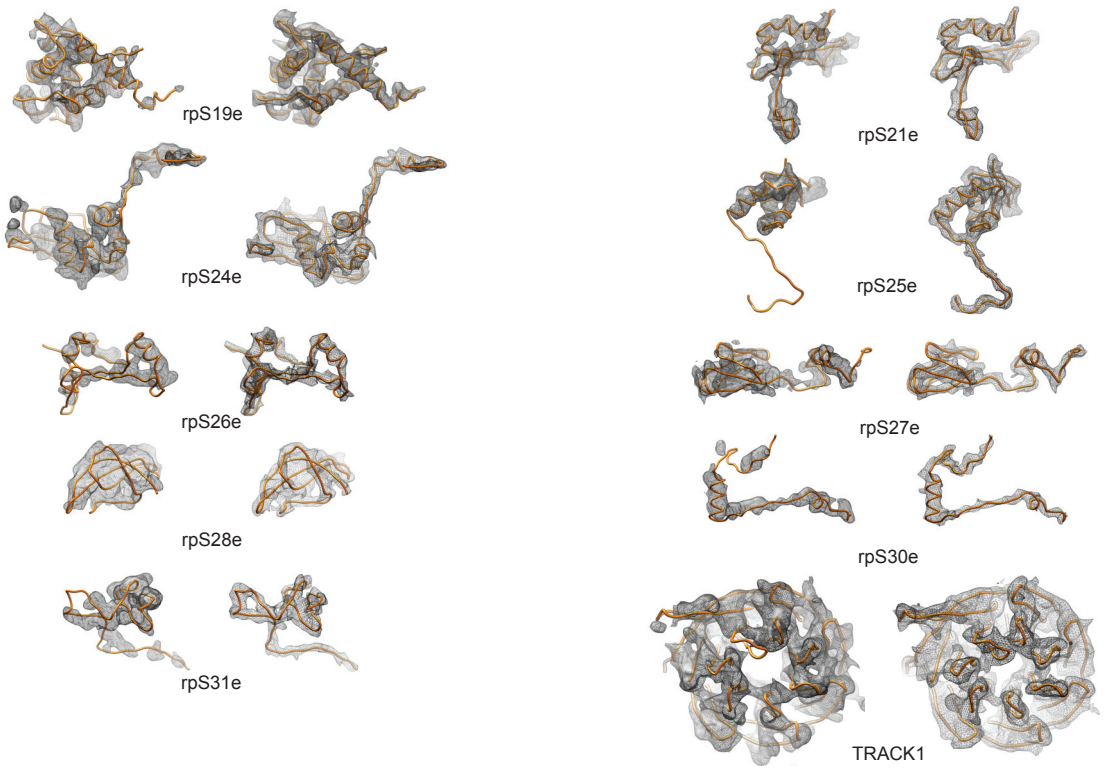
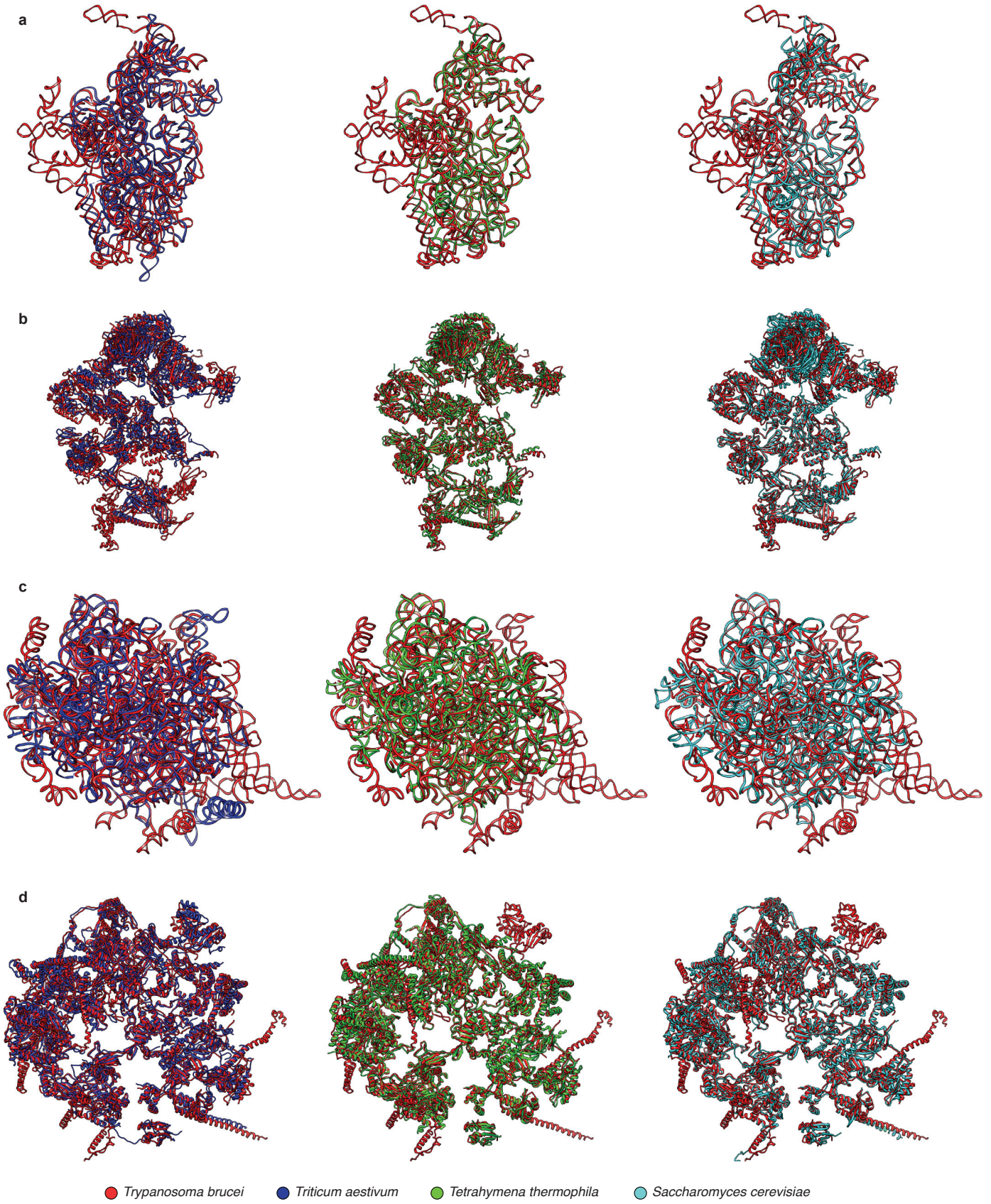
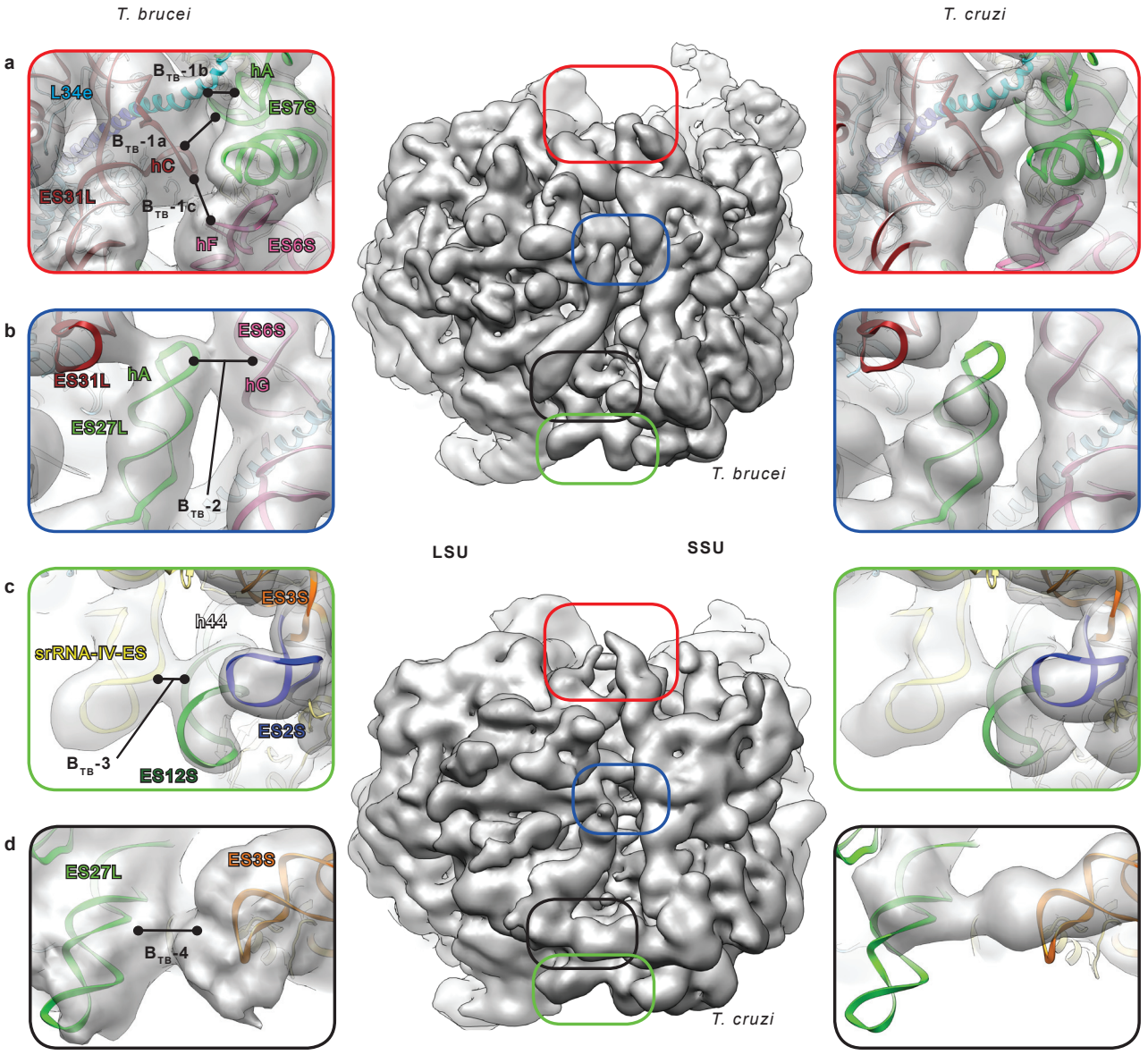


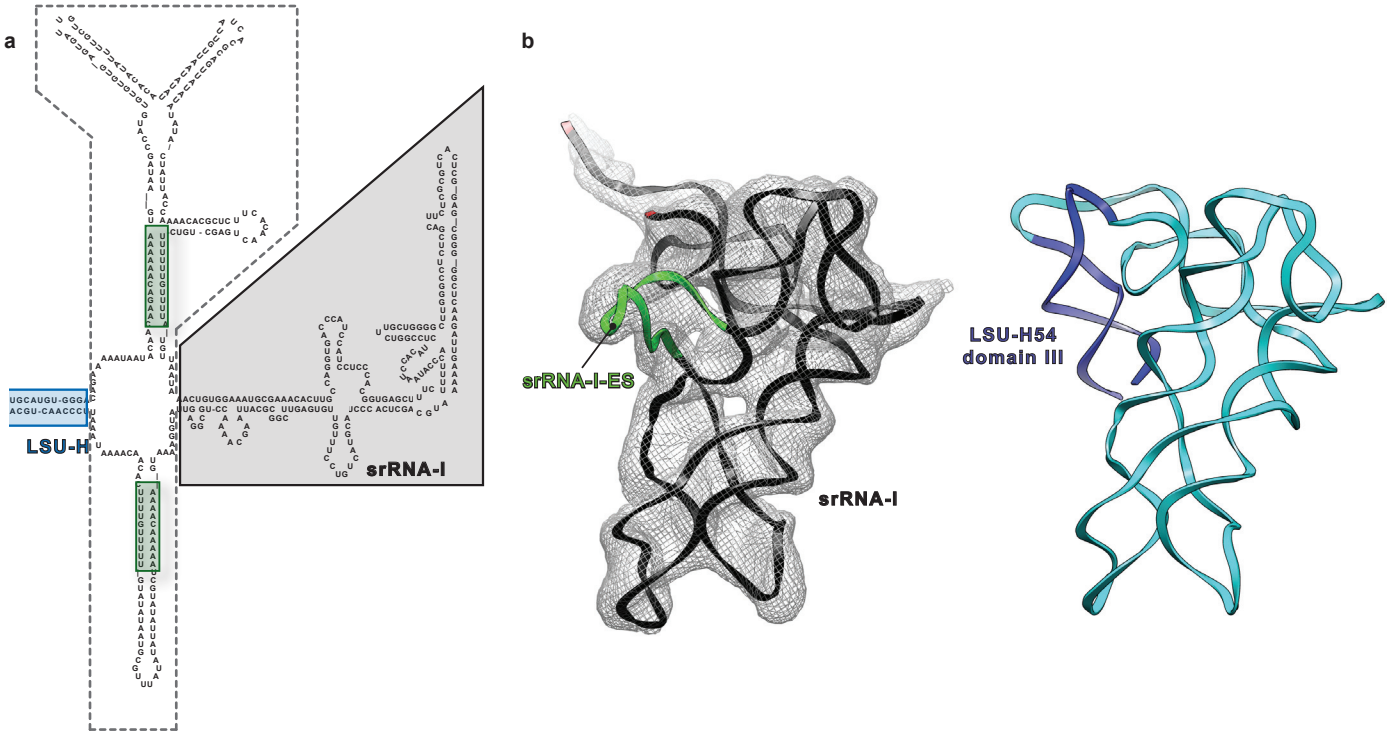
Supplementary Figure 2. Resolution of the T. brucei cryo-EM reconstruction. (**a**) *T. brucei* ribosome Cryo-EM map filtered and colored according to the measured local resolution (see Methods). The map displays a variable resolution from 5Å (dark blue) to 9Å (red), see color key. Left, front view (P-stalk side). Middle, a section of the map viewed from the front. Right, back view (L1-stalk side). (**b**) *T. brucei* ribosome Cryo-EM map colored according to the computed 3D variance (see Methods), from dark blue for the lowest variance to red for the highest variance. The map is filtered to the resolution at which the 3D variance was estimated (~ 20 Å). Left, front view (P-stalk side). Middle, a section of the map viewed from the front. Right, back view (L1-stalk side). (**c**) Up-left: Fourier Shell Correlation (FSC) curve displaying the average nominal resolution of the entire 3D reconstruction using FSC=0.5. x-axis = spatial frequency, y-axis = FSC. Pixel size is 1.09 Å. Up-right: an image of the *T. brucei* cryo-EM map, at the KSD region, showing the rRNA phosphate density bumps and indications of the proteins side chains, typical for the measured resolution (~5.0 Å). Bottom: selected images demonstrating the quality of the atomic model fitting in the map. (**d**) Segments of all the ribosomal proteins of the *T. brucei* cryo-EM map, with their atomic models fitted in (see Methods). In each column, the cryo-EM map segments (left) are compared to simulated cryo-EM maps, generated from their atomic models (using Chimera[12]), at a resolution of 5.0 Å (right) with the atomic models fitted in. This comparison validates the measurements of the local resolution but also shows discrepancies in the resolution of various ribosomal proteins as some segmented proteins display less molecular details than their simulated density map. Proteins of the LSU are colored in blue, the SSU proteins are in orange.



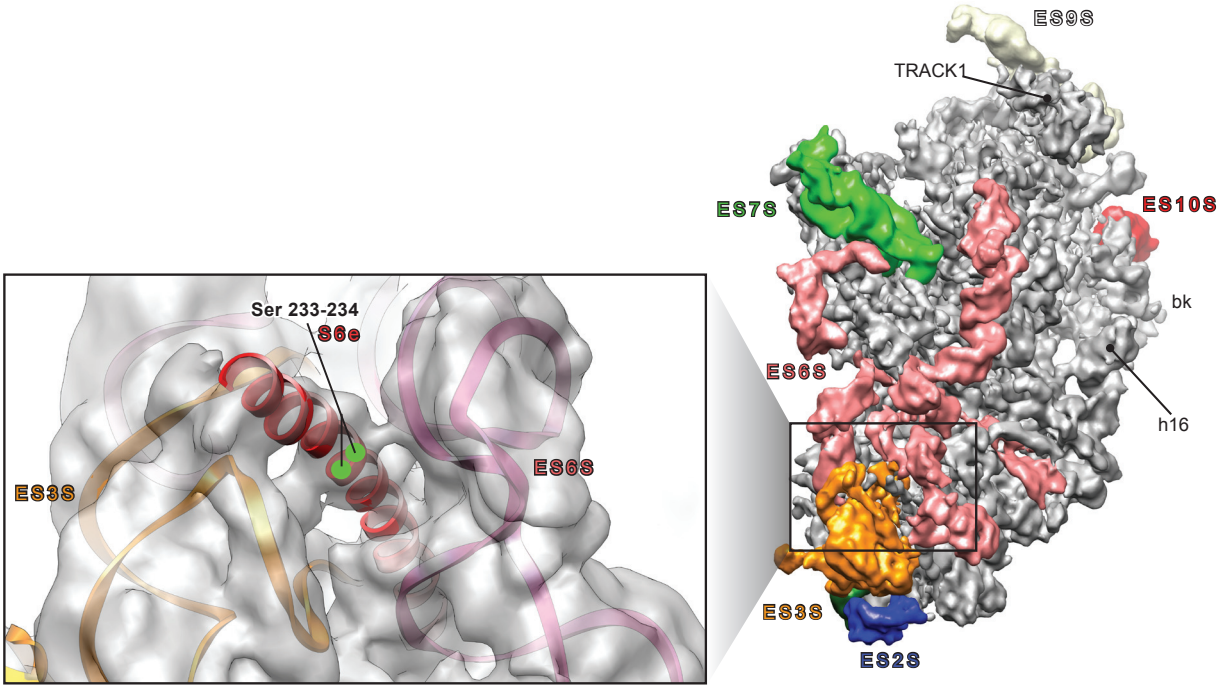
Supplementary Figure 3. Comparison between the T. brucei ribosome (this study) with other available eukaryotic ribosomal structures. Superimposition of the *T. aestivum* (left), *T. thermophila* (middle), *S. cerevisiae* (right) and the *T. brucei* ribosomal RNA and protein components: (**a**) SSU rRNA. (**b**) SSU ribosomal proteins. (**c**) LSU rRNA. (**c**) LSU ribosomal proteins.



Supplementary Figure 4. Comparison between T. brucei and T. cruzi additional ribosomal intersubunit bridges. Cryo-EM maps of ribosomes from *T. brucei* (middle top) and *T. cruzi* [14] (middle bottom), displayed at the resolution of 12Å. The *T. brucei* 80S atomic model was rigid-body fitted into the previously published *T. cruzi* map. The trypanosome-specific bridges are highlighted on each map by colored frames for both, *T. brucei* (left) and their equivalents in *T. cruzi* (right). (**a**) B_{TB}-1, formed by three contacts, a, b and c, (**b**) B_{TB}-2, (**c**) B_{TB}-3 and (**d**) B_{TB}-4. Despite the similarity of the ribosomes from the two species, the *T. brucei* 80S model fitted into the *T. cruzi* cryo-EM map indicates differences in the conformation of some trypanosome-specific expansion segments.



Supplementary Figure 5. Short rRNA-I secondary and tertiary structures. (**a**) Predicted secondary structure of the immature rRNA containing srRNA-I (according to ref. 12) (transparent gray area), adjacent to LSU helix 54 (transparent blue area), including srRNA-I flanking cleaved sequences (delimited by dashed lines). Although the predicted secondary structure of srRNA-I was revealed to be inaccurate by this study, it allows locating a conserved base-paired region (green transparent rectangles) symmetrically arranged with respect to the srRNA-I cleavage sites that might be part of the cleavage signal. (**b**) Comparison between *T. brucei* srRNA-I and its homologous rRNA region, adjacent to LSU helix 54 in domain III, in yeast.



Supplementary Figure 6. Interaction of S6e C-terminal tail with T. bruci-specific segments of SSU ESs 3S and 6S. Close-up on a region in the SSU from the *T. brucei* cryo-EM map displaying the interaction of S6e C-terminal tail with SSU ESs 3S and 6S (left). The atomic model shows that segments of SSU ESs 3S and 6S form a tight rRNA tunnel surrounding serine residues 233 and 234 (their positions are highlighted by green spheres), part of the S6e C-terminal tail phosphorylation site. This tight rRNA tunnel could render the phosphorylation site inaccessible to the S6e kinases (RSK and S6K). The blown-up region in the *T. brucei* SSU is indicated by a black frame on the segmented map (right).

eIF3 subunit 2 Tb11.01.1370

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            ++ + L GH +P+T IK+NREGDLLF+ AK+P V+VWY+  GERLGTY GH+    C D
Sbjct  1    MKPILLQGHERPITQIKYNREGDLLFTVAKDPIVNVWYSVNGERLGTYMGHTGAVWCVDA  60

Query  62   NNYSTLLVTGGMDFKAKLWCVETGEELANIMLRTPARAVGFSHDDNLLMVSTSRKMGQKS  121
            +  +  ++TG  D   +LW  ETG++LA +   +  R  GF    N +M ST ++MG +
Sbjct  61   DWDTKHVLTGSADNSCRLWDCETGKQLALLKTNSAVRTCGFDFGGNTIMFSTDKQMGYQC  120

Query  122  AVQLYNLPFLPPKDGYSIHPVNTVFNPCTEFVSENDDVTFAIWGPTNDTIYYSTSDGSVA  181
             V  ++L    P    +  P   +  PC +       +T A+WGP  + I      G +
Sbjct  121  FVSFFDL--RDPSQIDNNEPYMKI--PCND-----SKITSAVWGPLGECIIAGHESGELN  171

Query  182  ILDVETMSTVCTHKPHEETINRISFDSNYYTLITASKDKTARLLDSRDLSVVQTYTSDVP  241
                ++   +   K H   IN I    +    +TASKD TA+L DS  L   +T+ ++ P
Sbjct  172  QYSAKSGEVLVNVKEHSRQINDIQLSRDMTMFVTASKDNTAKLFDSTTLEHQKTFRTERP  231

Query  242  VNDASISPRGDHVIIGGGMDAQDVTTQGGQT-TFEVKFYHKVHEKQLGQVRCHFGTINSV  300
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Sbjct  232  VNSAALSPNYDHVVLGGGQEAMDVTTTSTRIGKFEARFFHLAFEEEFGRVKGHFGPINSV  291

Query  301  CFFPDGRGFASGAFDGLVKLHRFDDKY  327
             F PDG+ ++SG  DG V++H FD +Y
Sbjct  292  AFHPDGKSYSSGGEDGYVRIHYFDPQY  318
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eIF3 subunit 4 Tb427.04.1930

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Sbjct  57   KEVINGNIKTVTEYKIDEDGKKFKIV-RTFRIETRKASKAVARRKNWKKFGNSEFDPPGP  115

Query  89   ADQKDLVSRDPPIVLELG--------EVDPFERMAREEVMRL-MNEVERYTVEV--KDVH  137
                 VS D  +             E DP  ++  ++++   + + + +T     KD
Sbjct  116  NVATTTVSDDVSMTFITSKEDLNCQEEEDPMNKLKGQKIVSCRICKGDHWTTRCPYKDTL  175

Query  138  LARYAKVKE-------EQERAAKE----AAAPDDQGKE-----RTWAAARGDKTSVQHKE  181
                 ++ E       E+E+   E     A  +  GK       R  A+ RG+      +
Sbjct  176  GPMQKELAEQLGLSTGEKEKLPGELEPVQATQNKTGKYVPPSLRDGASRRGESMQPNRRA  235

Query  182  DTDRRLRITNISDDISREELYNIFNTDEYRIDKLFLPTDGKTSNYRGFAFITFETPEQAE  241
            D +  +R+TN+S+D    +L  +F      I +++L  D  T   +GFAFI+F   E A
Sbjct  236  DDNATIRVTNLSEDTRETDLQELFRP-FGSISRIYLAKDKTTGQSKGFAFISFHRREDAA  294

Query  242  RCLSRTKGVARFKNTVMHIVRALP  265
            R ++   G   + + ++++  A P
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eIF3 subunit 5 Tb10.61.2180

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            G  +P   P G  V +HP++L S+VD Y R N   +   RV G LLG    H      ++
Sbjct  79   GPALPGPFPGGRVVRLHPVILASIVDSYERRNEGAA---RVIGTLLGTVDKHS-----VE  130

Query  97   INNSFAVPFDEDPQNPDVWFLDTNYAEEMFLMFRRVHPKVRIVGWYSAGPSCAVQPNDML  156
            + N F+VP +E   + D   +D  +A+ M+ + ++V P   I+GWY+ G    +  + +L
Sbjct  131  VTNCFSVPHNE---SEDEVAVDMEFAKNMYELHKKVSPNELILGWYATGHD--ITEHSVL  185

Query  157  LHLLIADRFSLNPVYCIVNTDPNNKGVPVLAY-TTVHGRDG-ARSLEFRNIPTHLGAEEA  214
            +H   + R + NP++  V+T   N  + + AY +T+ G  G    + F  +        +
Sbjct  186  IHEYYS-REAPNPIHLTVDTSLQNGRMSIKAYVSTLMGVPGRTMGVMFTPLTVKYAYYDT  244

Query  215  EEIGIEHLLRD-LTDSTITTLSAQVQERELSLDHLGRVLQSIEEYLNDVAEGTMPISEDV  273
            E IG++ +++   + + +  LS+ +Q+   +   +   L ++ +Y  DV  G +     V
Sbjct  245  ERIGVDLIMKTCFSPNRVIGLSSDLQQVGGASARIQDALSTVLQYAEDVLSGKVSADNTV  304

Query  274  LSVLQEVISLQPHIYHLKTSTDMVRHANDQAIAMFIAAIGRCVCTLYDVIAN  325
               L  +++  P I      T +  + ND  +  ++A + +    L + + N
Sbjct  305  GRFLMSLVNQVPKIVPDDFETMLNSNINDLLMVTYLANLTQSQIALNEKLVN  356
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eIF3 subunit 6 Tb11.01.3420

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Query  107  EKEGITPKVMTAVMDLAFLYYDAARYGDASELLSLLQC-VTGYELGESKLLWGRLVCDTC  165
            +K G   + +  +   A   Y+   Y  A+E L   +  V  +      LWG+L  +
Sbjct  119  DKHGFRQEYLDTLYRYAKFQYECGNYSGAAEYLYFFRVLVPATDRNALSSLWGKLASEIL  178

Query  166  SCRWPSAIAAAEKLWKQQGADGSENKSGKTTLRGDNGTSVTERVWLLHWALFPFF---KG  222
              W +A+   +L      +  +N S  + L+      S+ +R WL+HW+LF FF    KG
Sbjct  179  MQNWDAAMEDLTRL-----KETIDNNSVSSPLQ-----SLQQRTWLIHWSLFVFFNHPKG  228

Query  223  GNQYSTHLLNIVFDNKTDSIYQCVVETVCPHYLRYICAAAILNT---HRRSALRRAAEMV  279
             +       NI+        Y   ++T+CPH LRY+  A I N     RR  L+   +++
Sbjct  229  RD-------NIIDLFLYQPQYLNAIQTMCPHILRYLTTAVITNKDVRKRRQVLKDLVKVI  281

Query  280  GR-IYEYSDPLTQLVREITNYRSFEDTLELLPKVSELAQGDYFLNLHADNLVENAKRLIF  338
             +  Y Y DP+T+ V  +     F+   + L +   +   D+FL    ++ +ENA+  IF
Sbjct  282  QQESYTYKDPITEFVECLYVNFDFDGAQKKLRECESVLVNDFFLVACLEDFIENARLFIF  341

Query  339  TQYVVTHSVVSIPYMAERLEMSAAGAEVWLADLISETKQRAKIDAVTGQMFVGSQVRSVH  398
             +   H  +SI  +A++L M+   AE W+ +LI   +  AKID+  G + +G+   S +
Sbjct  342  ETFCRIHQCISINMLADKLNMTPEEAERWIVNLIRNARLDAKIDSKLGHVVMGNNAVSPY  401

Query  399  QTVLDR  404
            Q V+++
Sbjct  402  QQVIEK  407
```

eIF3 subunit 7 Tb427.06.4370

```
Score =  129 bits (325),  Expect = 2e-29, Method: Compositional matrix adjust.
 Identities = 119/403 (30%), Positives = 187/403 (46%), Gaps = 55/403 (14%)

Query  103  KSTRVKERRQVPDWSAPRRHQNSRRNFQNKPKRNITLPPDT-VKVPSDAVILEQFRQAEL  161
            KS + KER ++      ++     R+ +  K ++    P D+ V+V SD  + E+     +L
Sbjct  124  KSAKQKERERIRLQKKFQKQFGVRQKWDQKSQK----PRDSSVEVRSDWEVKEEMDFPQL  179

Query  162  AKMPNLTSLPTVSDISQHNRPPVYKNEMDKASCKAPIPLNEKETKVDFTRSDSFTDNVLR  221
             KM  L  +    DI        Y    D+ + ++  PL  +  K  F    +  D V+R
Sbjct  180  MKMRYL-EVSEPQDIECCGALEYYDKAFDRITTRSEKPL--RSIKRIFHTVTTTDDPVIR  236

Query  222  GILKSEPPGTYPIVVATDEVLALLMTCSRSVYSWHLHFYRVGRFYFISKVDGCNVEKQWV  281
             + K++       V ATD +LA LM+C+RSVYSW +   RVG   F  K D  + +   V
Sbjct  237  KLAKTQGN-----VFATDAILATLMSCTRSVYSWDIVVQRVGSKLFFDKRDNSDFDLLTV  291

Query  282  DETADVSRVPSETEVVETDRTSSLEAESSKVNNFFVAQSCTAARYQMDCEKSPFPGKHPR  341
             ETA+    P + E    +   +L  E++ +N+ F +Q C     +M  E+  FP  +P
Sbjct  292  SETAN---EPPQDEGNSFNSPRNLAMEATYINHNF-SQQCL----RMGKERYNFPNPNPF  343

Query  342  L-------------YRYRRFVMHADTKDRYDLIVRCEVDAMQGDKH-----IRLFGLLE-  382
            +             YRYRR+ +  D     DLIVRCE D +     +     I +  L E
Sbjct  344  VEDDMDKNEIASVAYRYRRWKLGDDI----DLIVRCEHDGVMTGANGEVSFINIKTLNEW  399

Query  383  ---HCIKGEENDWRKMLASQTATCISEEYRRNAQKMARWIALCHLSGA-HMKIGFISRCR  438
               HC      DWR+ L SQ    I+ E + N+ K+ARW     L+G+ ++K+G++SR
Sbjct  400  DSRHC---NGVDWRQKLDSQRGAVIATELKNNSYKLARWTCCALLAGSEYLKLGYVSRYH  456

Query  439  KGAGVFDPLRHEVLATFTNDPSPLAAQLGIKVANMWTVADTII  481
                V D  RH +L T    P+  A+Q+ + V N W +   +I
Sbjct  457  ----VKDSSRHVILGTQQFKPNEFASQINLSVENAWGILRCVI  495
```

eIF3 subunit 8 Tb427.10.8290 or Tb427.10.8270

```
Score =  142 bits (357),  Expect = 3e-32, Method: Compositional matrix adjust.
 Identities = 145/591 (25%), Positives = 262/591 (44%), Gaps = 63/591 (11%)

Query  172  ELTEDDIVKQLRESVTCTGKKASK----------YRKLANECKRKGYKALQITTCGIVAD  221
            E+T   ++K+L E +   GKK +            ++A E    G   +      I+A
Sbjct  323  EITHAVVIKKLNEILQARGKKGTDRAAQIELLQLLVQIAAE-NNLGEGVIVKIKFNIIAS  381

Query  222  ALLEEDNREVYVSTKTWAKSCDTLEECFGLIVENPGIRLSDKF---SDKLNKRDA--FIK  276
                 N   Y+  + W K  D + E    ++  NP I + +     S+ L+  D    ++
Sbjct  382  LYDYNPNLATYMKPEMWGKCLDCINELMDILFANPNIFVGENILEESENLHNADQPLRVR  441

Query  277  GGLHALLQSLSKHLRRITQFKDGIPSDYIEIVHLENRLVAIADALFGYYRDNSRGRAVCC  336
            G +  L++ + +  +I Q  D    +Y+E +  E ++ AI + +  Y  +      VC
Sbjct  442  GCILTLVERMDEEFTKIMQNTDPHSQEYVEHLKDEAQVCAIIERVQRYLEEKGTTEEVCR  501

Query  337  QILVDILGSRRQ---EAHEILFGKMPPGQREAVSDSVI---ETVRSLYEQL---LLIGD-  386
              L+ IL +  +   +AH+      P G  ++  D      E    L E+L  +   D
Sbjct  502  IYLLRILHTYYKFDYKAHQRQLTP-PEGSSKSEQDQAENEGEDSAVLMERLCKYIYAKDR  560

Query  387  -DESKSLALLHLVYQMGLEGKYREGRDLIRRSGGAEKLCNSNHN-SVLYNRAVAQLGLAS  444
             D  ++ A+L  +Y   L  ++ + RDL+  S   + + +++     +LYNR + QLG+ +
Sbjct  561  TDRIRTCAILCHIYHHALHSRWYQARDLMLMSHLQDNIQHADPPVQILYNRTMVQLGICA  620

Query  445  FIMGDIMQAYELLSPLWNSWEGPEVL-IGQKLPNLKDEKGDEELRYRDLLLLPPHAHIPYS  503
            F G    A+  L  + +S    E+L  G  L +L++    ++E   R   +P H HI
Sbjct  621  FRQGLTKDAHNALLDIQSSGRAKELLGQGLLLRSLQERNQEQEKVERRRQVPFHLHINLE  680

Query  504  QLELATMLSTLVVGTVDEAKXPYEVTHHHRYFYRVINQMQF---------QPLLGEPIEF  554
             LE   ++S +++       + PY   H       R+I++ QF          QPLLG P
Sbjct  681  LLECVYLVSAMLL------EIPYMAAHESDARRRMISK-QFHHQLRVGERQPLLGPPESM  733

Query  555  REQLTAAYTALKLGDYARSSEVI----KNMKVWDNMPRGTEARDTFLRRLKEAALQIFCY  610
            RE + AA  A+K+GD+      I     N KVWD  P   + R    +R+++E +L+ + +
Sbjct  734  REHVVAASKAMKMGDWKTCHSFIINEKMNGKVWDLFPEADKVRTMLVRKIQEESLRTYLF  793

Query  611  NSRRSFATISVEIMAKKFDITESTVKHVINGIISENNTPLIAVWDRDDQYLHVDRSNISR  670
                 + +IS+E ++  F++    TV  +I+ +I   N  L+A  D+  Q + + R+  +
Sbjct  794  TYSSVYDSISMETLSDMFELDLPTVHSIISKMII--NEELMASLDQPTQTVVMHRTEPTA  851

Query  671  LQYLVEATARSVENIAHYCEK---------GGHGNDFRGG--RGQGYMRGG  710
             Q L     A  + ++     E+          GG+  D + G  + +GYMR G
Sbjct  852  QQNLALQLAEKLGSLVENNERVFDHKQGTYGGYFRDQKDGYRKNEGYMRRG  902
```

Fig. S7 continued on next page...

eIF3 subunit 9 Tb427.05.2570

```
Score =  144 bits (362),  Expect = 5e-33, Method: Compositional matrix adjust.
 Identities = 152/657 (23%), Positives = 262/657 (40%), Gaps = 88/657 (13%)

Query  2    EDDGLMSNIIIVNGLPARVTPEKRAMFLRHMTKKVSDVLGHDKFTIHPVLDEETEHVAGA  61
            E DG+ S +I+V+ +P +V P+ R   L+++  K+    G      +P  D +T+
Sbjct  179  EADGIDS-VIVVDNVP-QVGPD-RLEKLKNVIHKIFSKFGKITNDFYPEEDGKTKGYI--  233

Query  62   FLTFATVNSAEDALARLNRFPFTKTDILSTYRWCALKAASEPPEEYKPPEMEQDTDADFA  121
            FL +A+   A DA+   + +   K        +          +E+  PE +   D
Sbjct  234  FLEYASPAHAVDAVKNADGYKLDKQHTFRVNLFTDFDKYMTISDEWDIPEKQPFKDLGNL  293

Query  122  HTMAEDSMARPQFFIKQGESFDVEWYWFNYTTLKAELYRKPRPLKTDSVGQWTEMDRRQK  181
                E++  R Q+ +          +W +           P+  +   +WTE
Sbjct  294  RYWLEEAECRDQYSVIFESGDRTSIFWNDVKD----------PVSIEERARWTE------  337

Query  182  RLDPGLVYGALTSVRPMPAWSTFGRIMVSQHMGGLKLWGGRKMHMLFEVTELDIKAFYIS  241
                        T VR    WS  G  + + H  G+ LWGG K   +   +   ++    S
Sbjct  338  -----------TYVR----WSPKGTYLATFHQRGIALWGGEKFKQIQRFSHQGVQLIDFS  382

Query  242  PQEKYLVVKSP--------KEVSVWNIRLSKKIRVLGGLDLADSDKWPIARYNAEDELVA  293
             P E+YLV  SP        + + +W+I    K R   G     S  WPI +++ + +  A
Sbjct  383  PCERYLVTFSPLMDTQDDPQAIIIWDILTGHKKR---GFHCESSAHWPIFKWSHDGKFFA  439

Query  294  ISHACLEPMGQGKLFLYRAETMRALQVESNSETPVHSLVIPGLKVAEWNPAVGNQMAILV  353
                    M    L +Y   +M  L  +S        L I G+K   W+P  GN +A  V
Sbjct  440  R-------MTLDTLSIYETPSMGLLDKKS--------LKISGIKDFSWSPG-GNIIAFWV  483

Query  354  QGGSSEGWKIIIQNLVVKDDVVRAEVIEQRNFLQAQRLDLLWHPQGTHLVVKVTKTNS--  411
                   ++ +  L  + +      I  RN        L W   G +L VKV +T
Sbjct  484  PEDKDIPARVTLMQLPTRQE------IRVRNLFNVVDCKLHWQKNGDYLCVKVDRTPKGT  537

Query  412  ----TEYSIFSVGVKSAAAYQLKVENGL---TPGRFAWKPSGPHFAVICEDRARTGKLGD  464
                T + IF +  K      ++++  L   T   FAW+P+G  FAV+  +  R
Sbjct  538  QGVVTNFEIFRMREKQVPVDVVEMKGKLYFKTIIAFAWEPNGSKFAVLHGEAPRIS----  593

Query  465  TSEIRIYCIKK--QLKLIGHYP-TSATHLFWAPRGSRLVATNYDKSTLHFYGINDSGACV  521
               +  Y +K   +++LI  +    A  +FW+P+G  +V             ++ S   V
Sbjct  594  ---VSFYHVKNNGKIELIKMFDKQQANTIFWSPQGQFVVLAGLRSMNGALAFVDTSDCTV  650

Query  522  QLERVTSPVTDTXWDPTGRFYAAWVSALRNSGDNQFRIFDLNGRELMQKSVRQLSHFAWR  581
                    +D  WDPTGR+    VS   +  DN + ++   GR L + +  +     WR
Sbjct  651  MNIAEHYMASDVEWDPTGRYVVTSVSWWSHKVDNAYWLWTFQGRLLQKNNKDRFCQLLWR  710

Query  582  PLAPPVLTAAEIKHIQDNLREYSQRYQNEVKEQKEREEAELQSKEREKEEQYKKRMK  638
             P  P +L+  +IK I+ +L++YS+ ++ + +  + +   EL  + R   E ++K  K
Sbjct  711  PRPPTLLSQEQIKQIKKDLKKYSKIFEQKDRLSQSKASKELVERRRTMMEDFRKYRK  767
```

eIF3 subunit 10 Tb927.7.6090

```
Score = 70.9 bits (172),  Expect = 7e-10, Method: Compositional matrix adjust.
 Identities = 108/498 (22%), Positives = 216/498 (43%), Gaps = 60/498 (12%)

Query  127  LILSSLSGISIDQRVKDLYLVPAEKIVGEVAREMLTFNVIGHSGKLLPIYLETTEKLIEL  186
             ++LS++SG     R   L L P  K + E  R+ L  +++ ++ ++  +Y +  ++  +
Sbjct  127  VLLSAVSGEDTQDRTDRLLLTPWVKFLWESYRQCL--DLLRNNSRVERLYHDIAQQAFKF  184

Query  187  CRTYKFRAAIGHVADSFVRFFLRFLLYPIRPKTNKAYSTRAADALKIDRESFHRDVTAAQ  246
             C  Y  +A    + D+     LR  L  I+   N++ +    +    + +S H +    Q
Sbjct  185  CLQYTRKAEFRKLCDN-----LRMHLSQIQRHHNQSTAINLNNP---ESQSMHLETRLVQ  236

Query  247  KTVSVFCQLLEALIAVSNWQGAWRTLECFTKVLAKTKQHEDFRKSQSDAYLVMATLFWEC  306
                      L++ I++  WQ A++ +E    + + +K+     +  ++ Y  ++T+FW+
Sbjct  237  ---------LDSAISMELWQEAFKAVEDIHGLFSLSKKPPK-PQLMANYYNKVSTVFWKS  286

Query  307  SCYSFHAHCLLSAAFLADDERK-------ESLLSRAVLAALCVPNIKGRESFARGSDS--  357
                FHA  L     L+ + RK       + + +R +LA L +P    R   AR  D
Sbjct  287  GNALFHASTLHRLYHLSREMRKNLTQDEMQRMSTRVLLATLSIPITPERTDIARLLDMDG  346

Query  358  -FFQKNEQIAKLLDLKEAPSRNFLVQRMQQMQLLQ-AAP--KGVVAVVELLRNEVFDGEA  413
               +K  ++A LL L+  P+R  L+  M +  +LQ   P  K +   +E+  N +   E
Sbjct  347  IIVEKQRRLATLLGLQAPPTRIGLINDMVRFNVLQYVVPEVKDLYNWLEVEFNPLKLCER  406

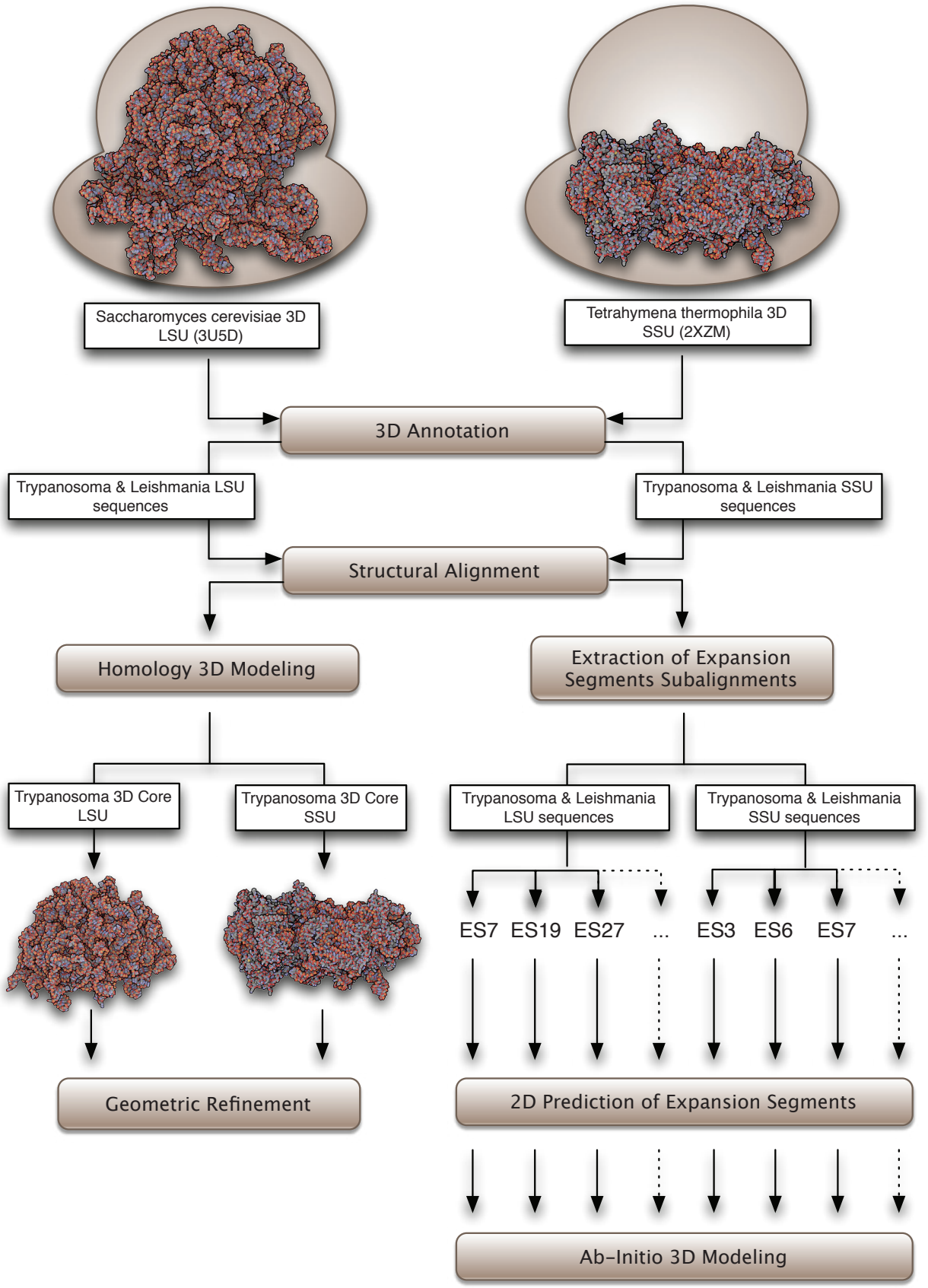
Query  414  SSRAIAQVSQVVQKDQSLEKYQQPLRKVVMKRFLEYMATKVTRVEASSLR----IWESEQ  469
              ++ +  V +  +K+  L++Y   L+   + R L+ ++     +E S L       ++ Q
Sbjct  407  VTKVLNWVREQPEKEPELQQYVPQLQNNTILRLLQQVSQIYQSIEFSRLTSLVPFVDAFQ  466

Query  470  SEGAYVNEIEPYILHESGITVEIDHKTNSITFSNATKIKVLEAFDTLAQHVQLQPAAS-R  528
              E A V+           + V IDH + +++F +       E    +  H+Q  P+   R
Sbjct  467  LERAIVDAA-----RHCDLQVRIDHTSRTLSFGSDLNYATRED-APIGPHLQSMPSEQIR  520

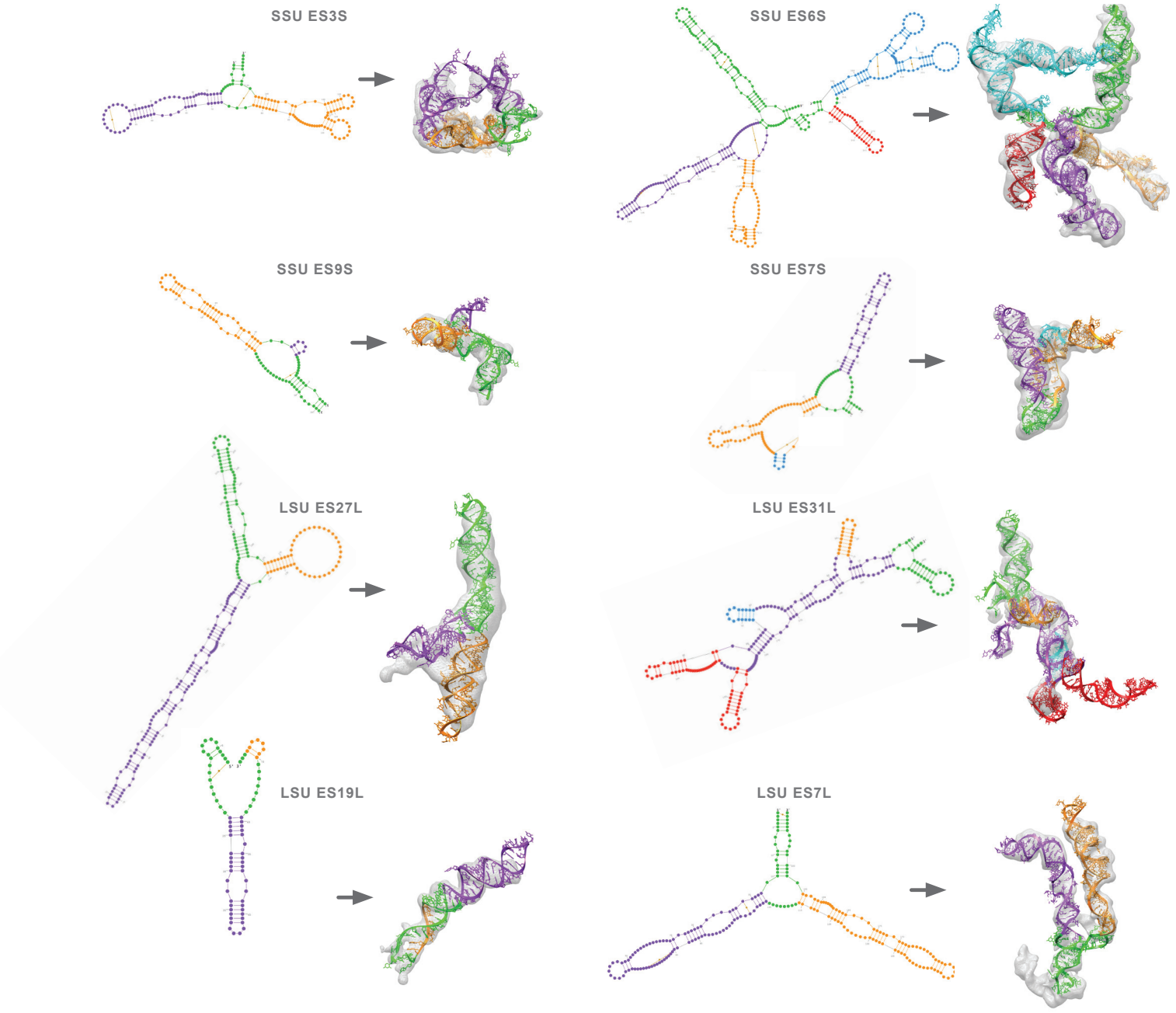
Query  529  RKLD------------IKPDHLRLVHERTRNLYNQQQSCEEAAEQRRKDAKLREREKRSK  576
              +L             IKP H+  + E+     +Q          R++  ++  R +  +
Sbjct  521  NQLTAMSSVLAKALEVIKPAHI--LQEKEEQ--HQLAVTAYLKNSRKEHQRILARRQTIE  576

Query  577  ERAERIENEKKKKEAADL  594
             ER ER+E+   ++E  +L
Sbjct  577  ERKERLESLNIQREKEEL  594
```

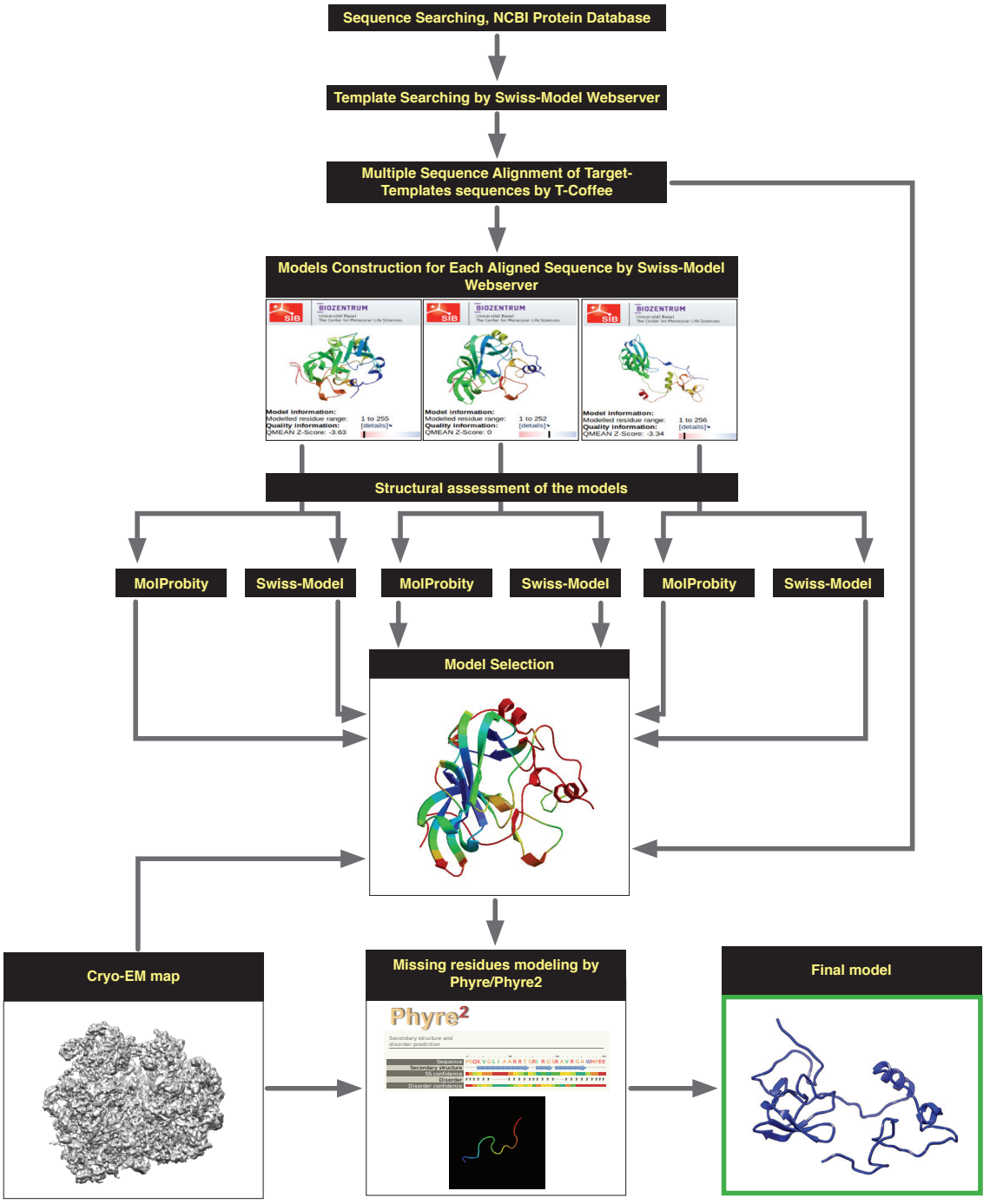
Supplementary Figure 7. Alignment of conserved eIF3 subunits with their human homologs. 8 subunits in total were found after running a blast against human eIF3 subunits. The *T. brucei* eIF3 subunits annotations are consistent with the blast results except for eIF3 subunit 5 that was found annotated as subunit 8 of the *T. brucei* proteasome, and without further analysis it is unclear to which complex it really belongs. Subunits names are in blue, in red the gene names in *T. brucei* and highlighted in green the identity and homology cores. Query = *T. brucei*, sbjct = *Homo sapiens.*



Supplementary Figure 8. rRNA modeling workflow. The *T. brucei* LSU conserved RNA core was modeled by homology to the X-ray structure of the yeast ribosome and the SSU RNA conserved core was modeled by homology to the *T. thermophila* 40S in complex with eIF1. The *T. brucei* ESs were modeled *ab initio* based on covariation analysis from orthologous rRNA sequences found in other kinetoplastids. Only few ESs from each subunit are mentioned as an example.



Supplementary Figure 9. T. brucei rRNA ESs modeling. For all displayed *T. brucei* ESs: (Left column) secondary structure models calculated based on the alignment and the covariation analysis. (Right column) Based on these secondary structure models, 3D models are then constructed interactively directly into the ES density maps (Not yet fitted by MDFF). Almost in all cases, some parts of the 2D models had to be modified in order to better fit the topology displayed by the cryo-EM map. The list of ESs displayed is not exhaustive, only largest ESs on each subunit are displayed. For every displayed ES, each secondary structure element conserves the same color in both columns, the secondary and 3D structures. Some parts of the displayed segmented ESs density maps can cover fully the fitted atomic model only when displayed at a lower density threshold due to the dynamic variability of the concerned part of the ES.



Supplementary Figure 10. Ribosomal proteins modeling workflow. The *T. brucei* proteins were modeled by homology to several X-ray structures. The ribosomal protein L2 is shown as an example, the modeling of all the ribosomal proteins follows the same workflow.

Supplementary Tables

Ribosomal proteins models

Yeast name	T. thermophila name	NCBI GI#	size, aa	Template's PDB ID	Range modeled, aa	Percentage modeled
rpL1	rpL1	70833218	214	1DWU_D	1-214	100%
rpL2	rpL2	74026238	260	3U5E_A	2-251	96%
rpL3	rpL3	72387810	480	3U5E_B	53-480	89%
rpL4	rpL4	72387341	374	3U5E_C	3-370	98%
rpL5	rpL11	71744962	194	3U5E_J	13-180	86%
rpL6	rpL9	71746626	189	3U5E_H	1-189	100%
rpL6e	rpL6	71748584	192	3U5I_E	2-105, 142-192	80%
rpL8e	rpL8 (7a)	72392339	276	3U5E_G	37-276	87%
rpL11	rpL12	72393265	164	3O5H_L	7-144	84%
rpL13	rpL13a	72389080	222	4A17_I	22-222	90%
rpL13e	rpL13	72387001	218	4A18_U	1-214	98%
rpL14	rpL23	71745556	139	3U5E_V	2-139	100%
rpL14e	rpL14	2500363	186	4A18_F	2-184	98%
rpL15	rpL27a	71745558	145	4A17_K	2-145	100%
rpL15e	rpL15	71746212	221	3U5E_N	19-221	91%
rpL16	rpL10	71745090	213	3U5I_I	2-213	100%
rpL18	rpL5	71746208	308	3U5E_D	2-308	100%
rpL18e	rpL18	71755183	193	3U5E_Q	2-193	100%
rpL19e	rpL19	72391662	260	3U5E_R	1-200	77%
rpL20e	rpL18a	71747154	179	3U5E_S	1-179	100%
rpL21e	rpL21e	71754491	159	3U5E_T	2-159	100%
rpL22	rpL17	71755285	166	4A17_Q	2-155	93%
rpL22e	rpL22	74025028	130	4A18_M	21-123	83%
rpL23	rpL23a	62359061	164	3U5E_X	44-164	73%
rpL24	rpL26	71746108	143	4A17_S	2-125	87%
rpL24e	rpL24	71747474	125	3U5I_W	1-125	100%
rpL27e	rpL27	74026162	133	3U5E_Z	2-133	100%
rpL28e	rpL28	71755559	146	4A18_O	5-144	95%
rpL29	rpL35	71744004	127	3U5E_h	2-127	100%
rpL29e	rpL29	74025002	71	3U5E_b	2-71	100%
rpL30	rpL7	62176425	257	3U5I_F	1-257	100%
rpL30e	rpL30	71747210	114	4A18_G	19-114	83%
rpL31e	rpL31	71745684	188	3U5E_d	1-186	100%
rpL32e	rpL32	71746464	132	3U5E_e	2-129	97%
rpL33e	rpL35a	72387888	149	3U5E_f	34-149	78%
rpL34e	rpL34	74024942	170	3U5E_g	2-152	89%
rpL36e	rpL36	71746214	109	4A18_Q	2-107	97%
rpL37e	rpL37	71754935	84	3U5E_j	2-84	100%
rpL38e	rpL38	71744028	82	3U5E_k	2-82	100%
rpL39e	rpL39	72393111	51	3U5E_l	2-51	100%
rpL40e	rpL40	302393731	52	3U5E_m	1-52	100%
rpL43e	rpL37a	71746454	93	3U5E_p	2-92	98%
rpL44e	rpL44	62359897	106	3U5E_o	2-106	100%

Supplementary Table 1. LSU ribosomal proteins models. Exhaustive list of the modeled *T. brucei* LSU proteins. Yeast name = names of the *T. brucei* LSU proteins according to the new nomenclature used in yeast X-ray structure[13]. *T. thermophila* name = names of the LSU proteins according to the nomenclature used in *T. thermophila* ribosomal LSU structures in complex with eIF6[15]. NCBI GI# = the numbers of the *T. brucei* ribosomal protein sequences in the NCBI database. Size is in amino acids (aa). Template PDB ID = the ID of the template structures in the PDB used for the modeling of the homologous *T. brucei* proteins. Range modeled = the modeled part(s) of the *T. brucei* LSU proteins, in amino acids (aa).

Ribosomal proteins models

Yeast name	T. thermophila name	NCBI GI#	size, aa	Template's PDB ID	Range modeled, aa	Percentage modeled
rpS1e	rpS1	71747174	256	3U5G_B	20-237	85%
rpS2	rpS0	74025178	277	3U5C_A	41-243	73%
rpS3	rpS3	71748612	214	3U5C_D	3-214	99%
rpS4	rpS9	72392295	190	3U5C_J	2-187	98%
rpS4e	rpS4	71755053	272	3U5C_E	2-255	90%
rpS5	rpS2	71749200	266	2XZM_E	40-262	84%
rpS6e	rpS6	71746448	250	3U5C_G	2-250	100%
rpS7	rpS5	71755579	190	2XZM_G	4-190	98%
rpS7e	rpS7	71744362	202	3U5G_H	2-192	94%
rpS8	rpS15a	72387207	130	3U5C_W	2-130	100%
rpS8e	rpS8	72393291	220	3U5C_I	2-219	99%
rpS9	rpS16	72390870	149	3U5G_Q	2-149	100%
rpS10	rpS20	71402828*	117	2XZM_J	12-115	89%
rpS10e	rpS10	71747454	172	3U5C_K	1-101	59%
rpS11	rpS14	62359856	144	3U5G_O	7-144	96%
rpS12	rpS23	71746622	143	3U5C_X	2-143	100%
rpS12e	rpS12	71755181	144	2XZM_U	24-144	83%
rpS13	rpS18	71747448	153	3U5C_S	1-146	95%
rpS14	rpS29e	71402016*	57	3U5C_d	16-57	74%
rpS15	rpS13	84043888	151	3U5C_N	2-151	100%
rpS17	rpS11	71746518	174	3U5C_L	5-174	98%
rpS17e	rpS17	74025356	142	3U5C_R	2-127	89%
rpS19	rpS15	72391126	152	3U5G_P	22-148	84%
rpS19e	rpS19	72387824	167	2XZM_T	19-167	89%
rpS21e	rpS21	71755621	194	3U5C_V	6-85	40%
rpS24e	rpS24e	71747828	137	3U5C_Y	2-137	100%
rpS25e	rpS25	62175407	113	3U5C_Z	27-111	75%
rpS26e	rpS26	71755959	111	2XZM_5	2-101	90%
rpS27e	rpS27	74024940	86	3U5C_b	2-83	95%
rpS30e	rpS30	72390075	66	3U5G_e	2-66	100%
rpS31e	rpS31e (27a)	72391394	155	3U5C_f	86-152	43%
rpS28e	rpS33	72390708	103	3U5C_c	36-103	66%
RACK1	RACK1	74025270	318	3DM0_A	1-314	99%

Supplementary Table 2. SSU ribosomal proteins models. Exhaustive list of the modeled *T. brucei* SSU proteins. Yeast name = names of the *T. brucei* SSU proteins according to the new nomenclature used in yeast X-ray structure[13]. *T. thermophila* name = names of the SSU proteins according to the nomenclature used in *T. thermophila* ribosomal SSU structure in complex with eIF1[14]. NCBI GI# = the numbers of the *T. brucei* ribosomal protein sequences in the NCBI database. * = the sequences of these proteins couldn't be found for *T. brucei* and were taken from *T. cruzi* instead due to the evolutionary proximity of these two organisms, and the homology to *T. brucei* is further confirmed by the fit in the density map (see figure S2D). Size is in amino acids (aa). Template PDB ID = the ID of the template structures in the PDB used for the modeling of the homologous *T. brucei* proteins. Range modeled = the modeled part(s) of the *T. brucei* SSU proteins, in amino acids (aa).