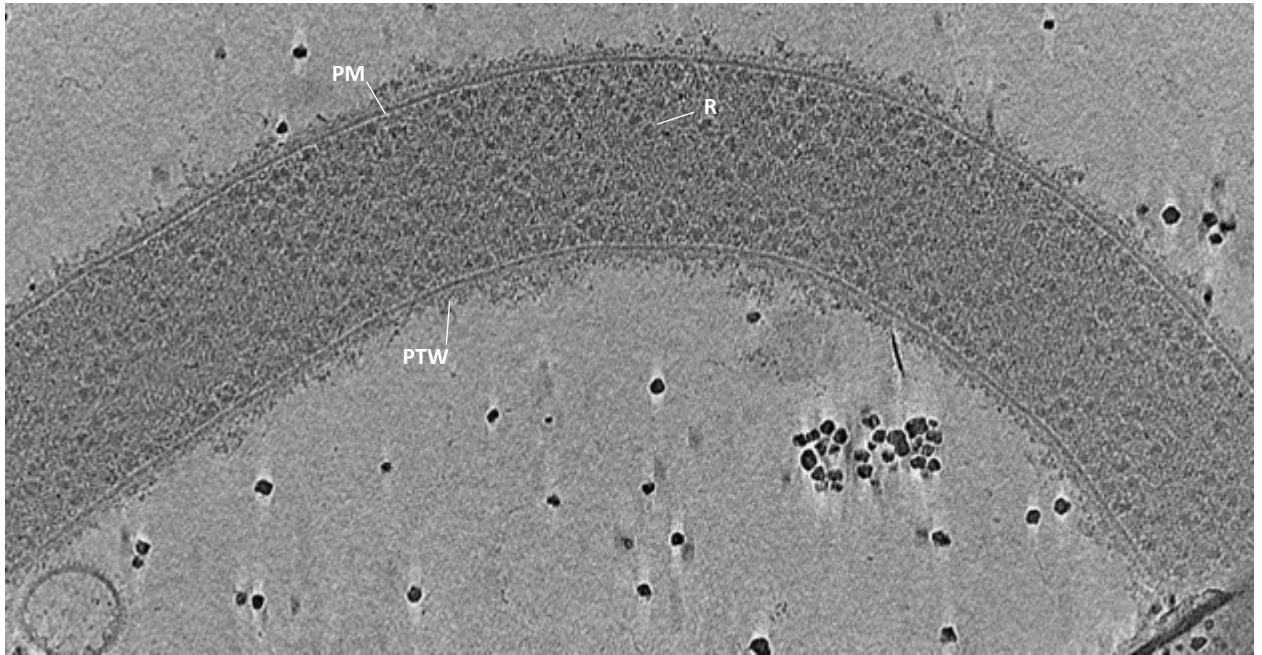




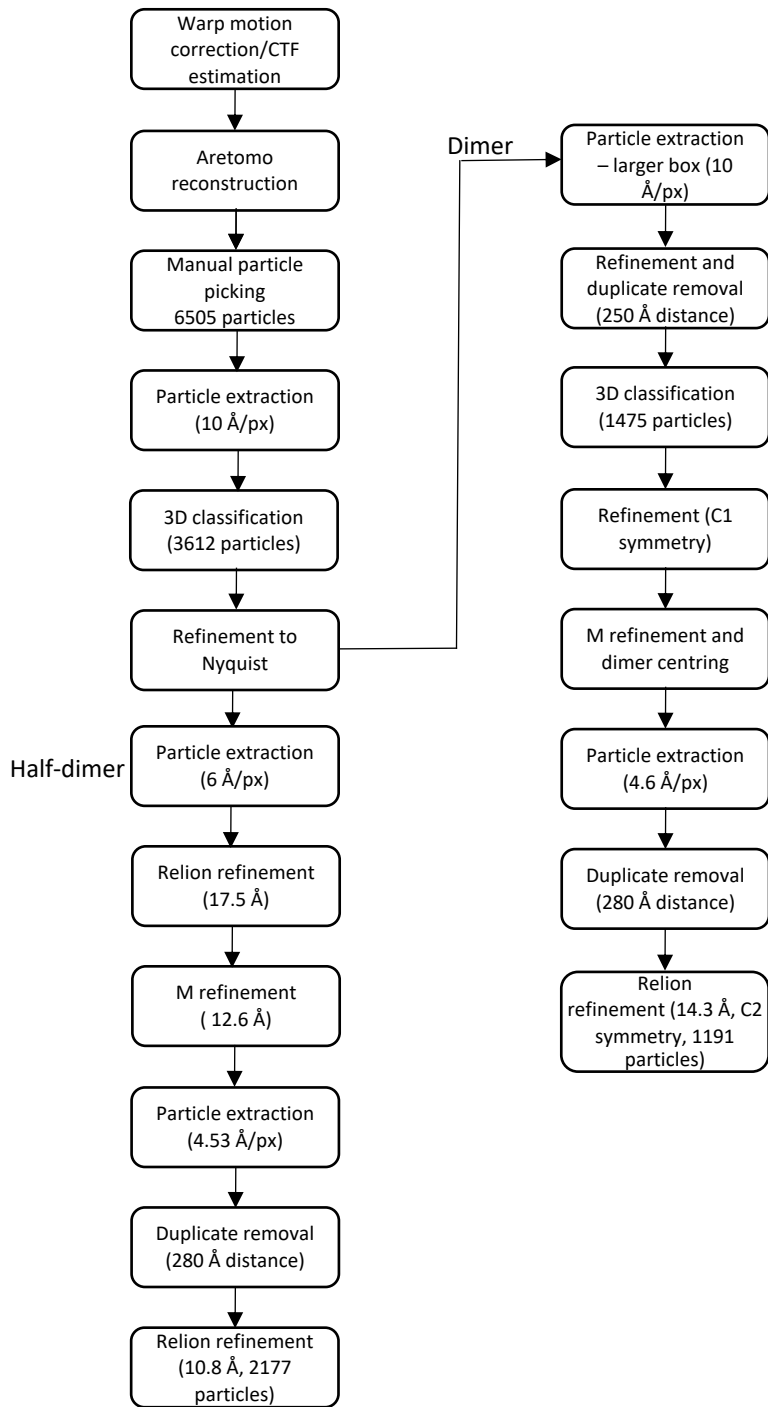
CryoEM reveals that ribosomes in microsporidian spores are locked in a dimeric hibernating state

In the format provided by the authors and unedited

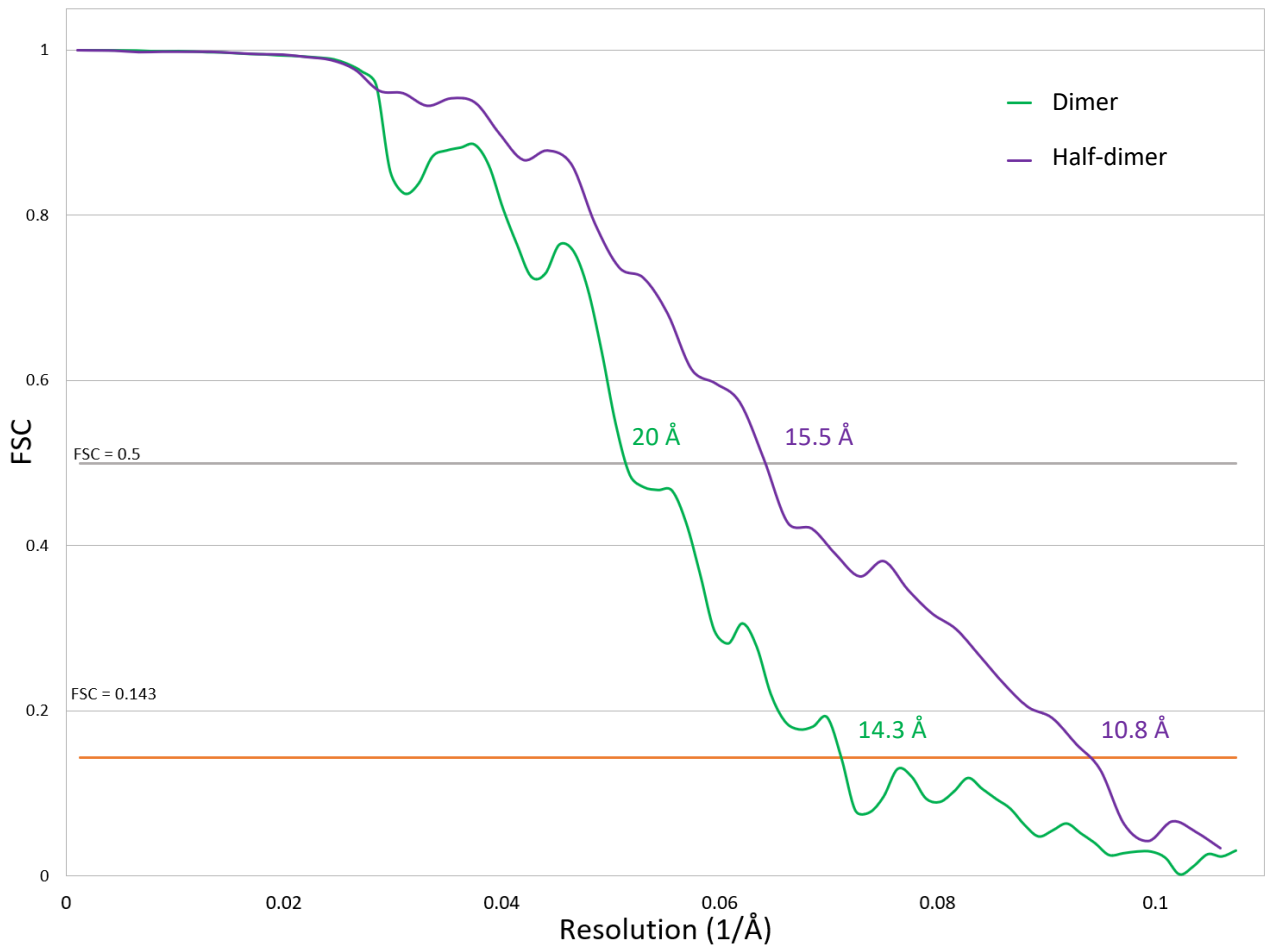


Supplementary Figure 1 – Slice through a tomogram of a polar tube of a germinated *S. lophii* spore.

PTW, polar tube wall, PM, plasma membrane, R, ribosome.



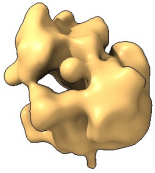
Supplementary Figure 2 - Flow chart of the subtomogram averaging pipeline.



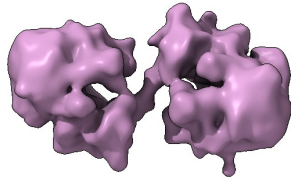
Supplementary Figure 3 - Resolution estimation of the *S. lophii* ribosome dimer and half-dimer maps from subtomogram averaging.

Gold Standard FSC of the dimer (green) and masked half-dimer within the average (purple).

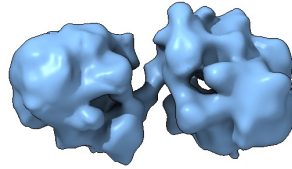
Class 1
(449 particles)



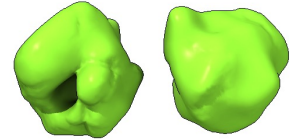
Class 2
(934 particles)



Class 3
(541 particles)

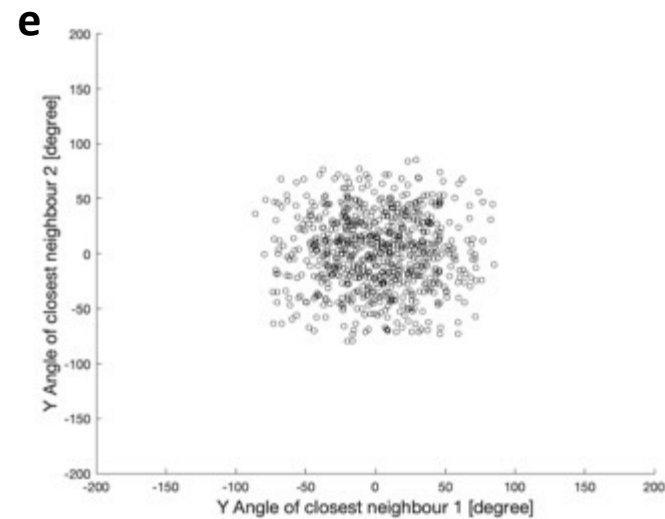
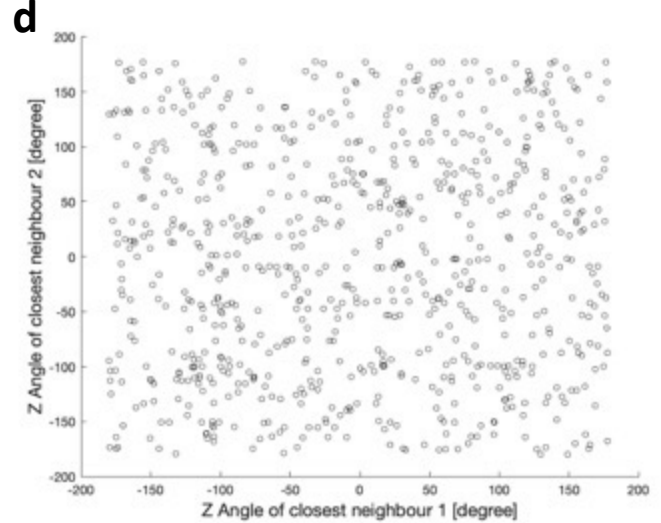
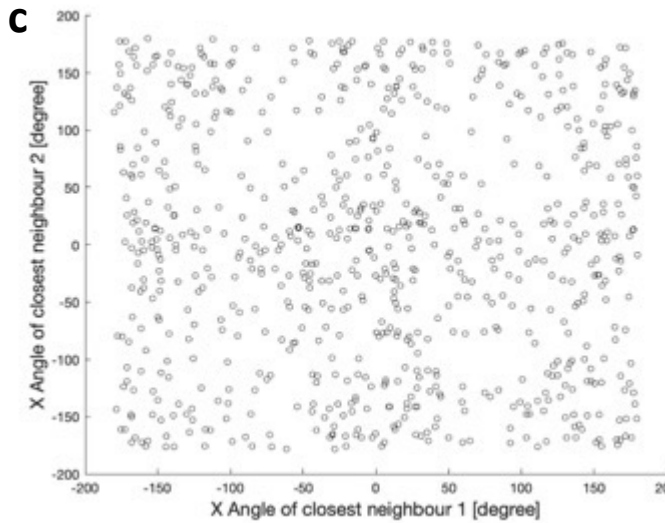
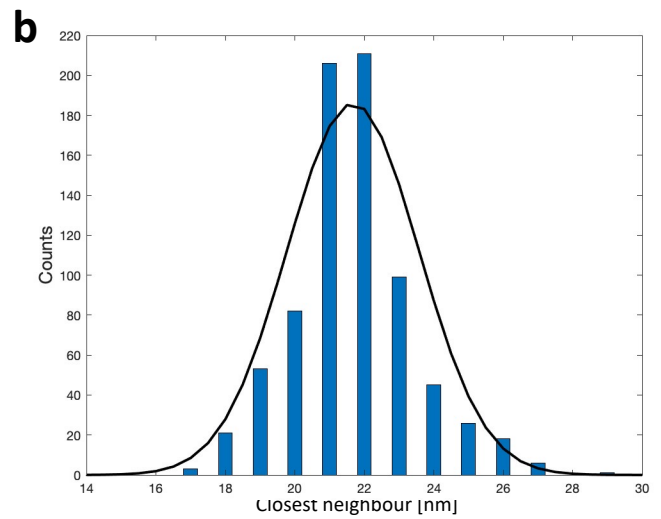
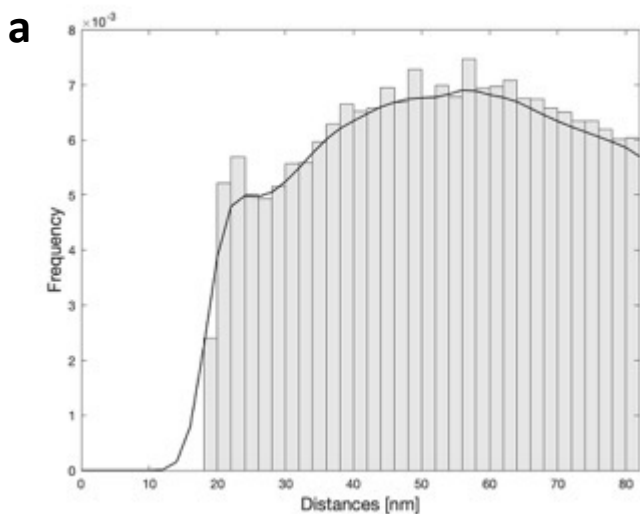


Class 4
(283 particles)



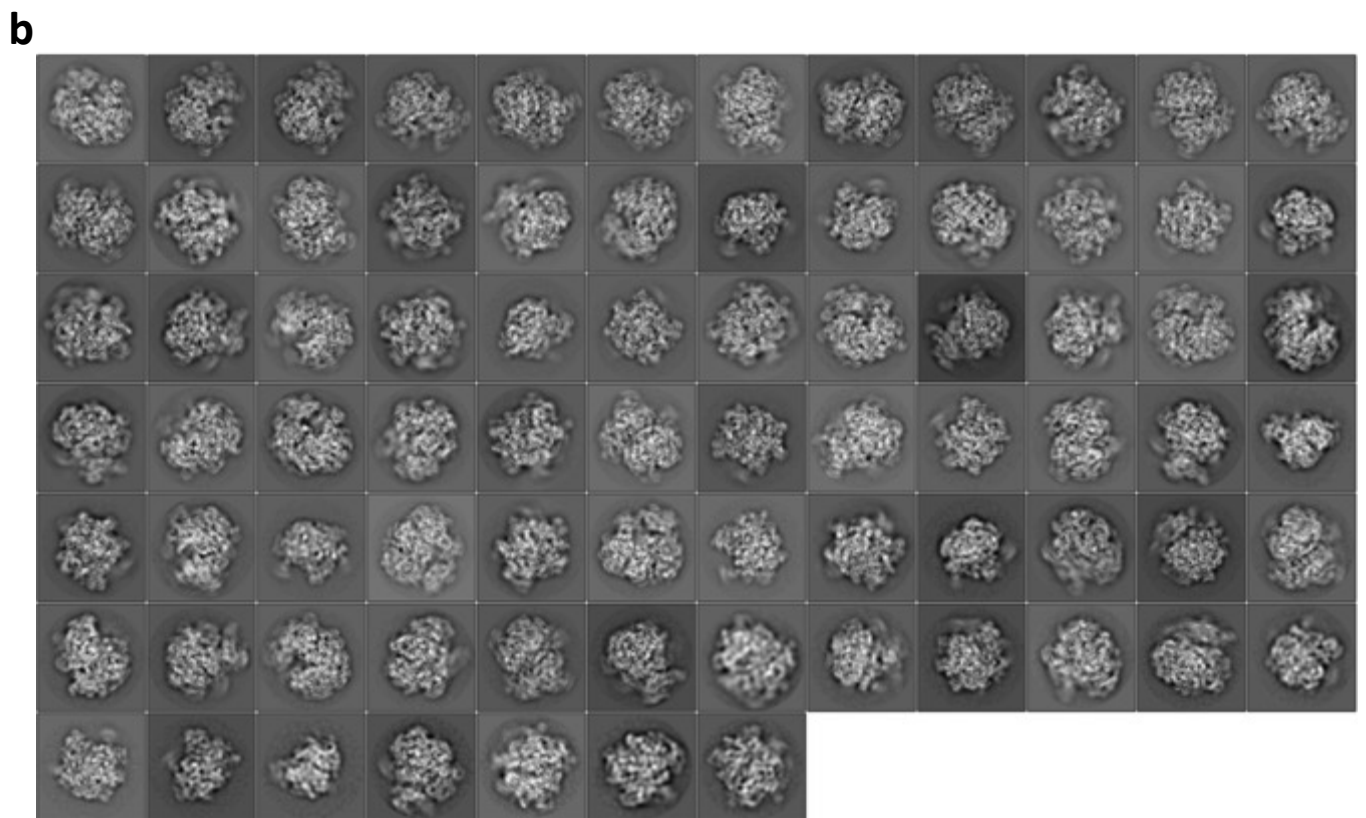
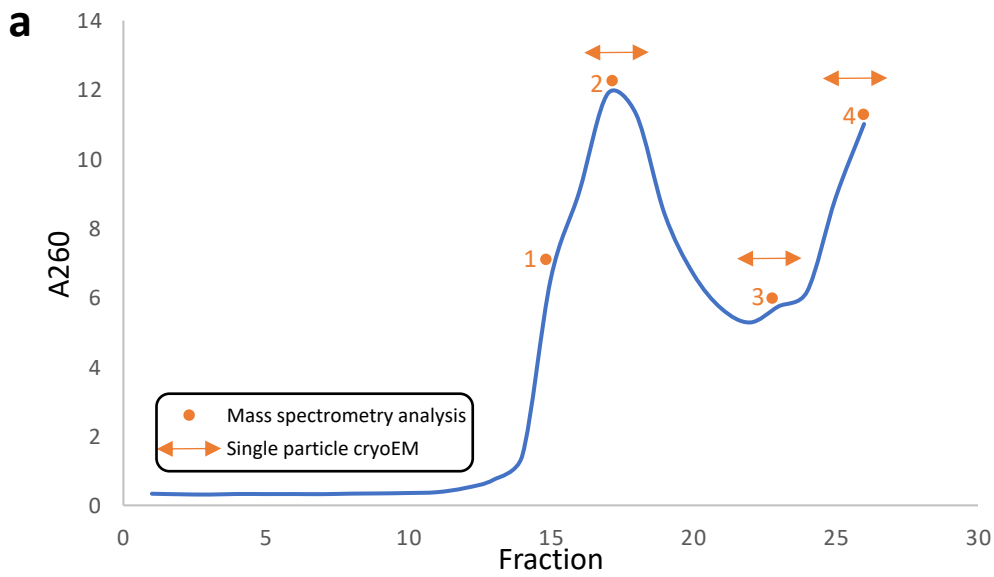
Supplementary Figure 4 – 3D classification of subtomogram average.

Out of a total of 2207 particles post duplicate removal, 449 particles contribute to a monomeric class (20%). In all 100 S dimers, the 70 S ribosomes adopt the same orientation with respect to each other. No obvious differences were observed between classes 2 and 3, and these were combined for further rounds of refinements.



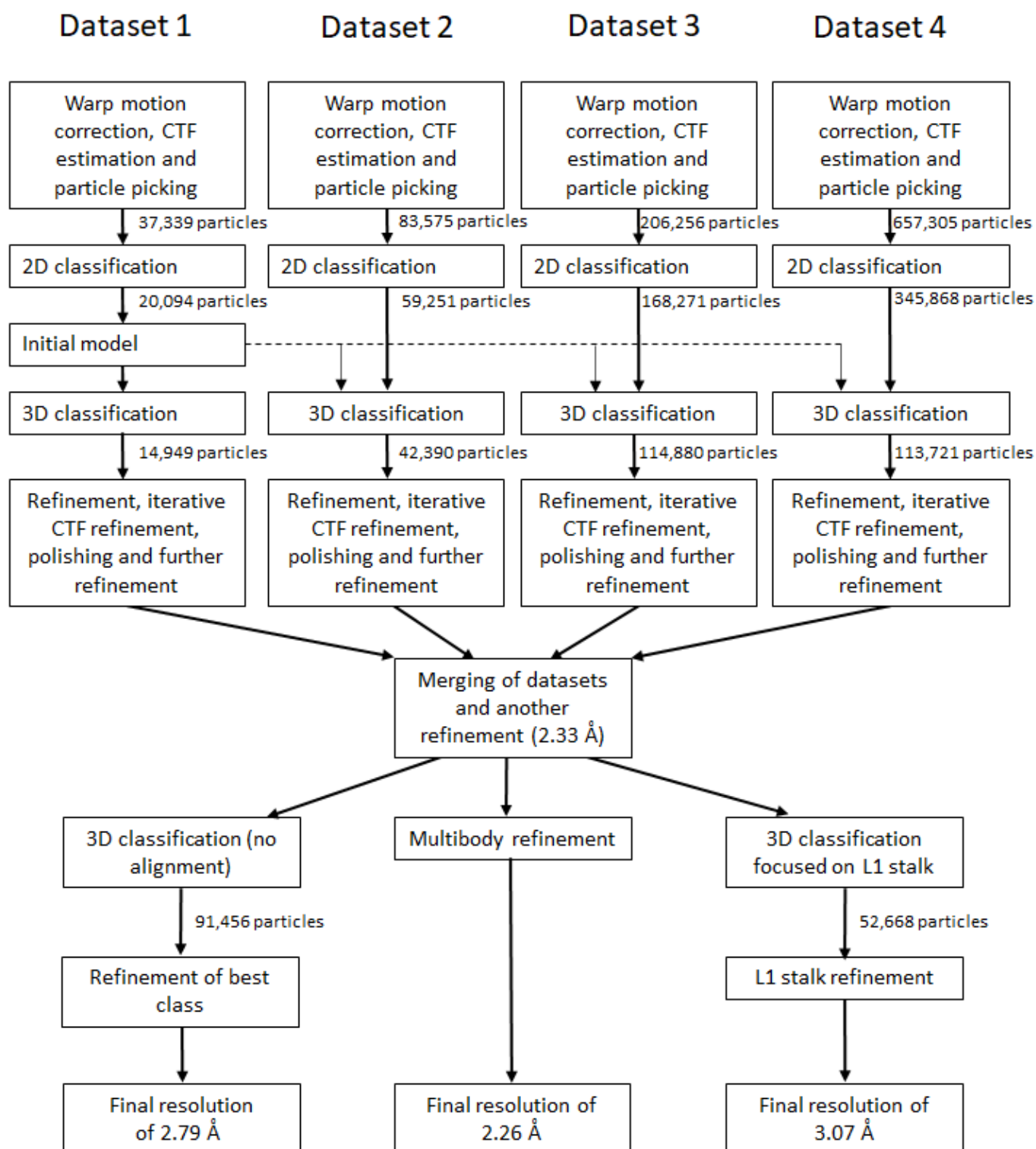
Supplementary Figure 5 – Statistical analysis of ribosome distribution within the polar tube.

a Histogram of distances between 70 S particles in a PT with a peak at around 21 nm, corresponding to the centre-to-centre distance between two partners in a dimer pair, or ribosomes in touching distance. No other peaks or multiples of the average distance between partners that could account for the presence of polysomes are evident. **b** Histogram showing only the distribution of distances between closest neighbours, with a peak at around 21 nm. **c-e** Angular relationships between closest 70 S neighbours of all particles. Scatter plots of the X and Z angles do not show any correlation and are randomly distributed between -180 and $+180^\circ$. The Y angles of closest neighbours (i.e. dimers) of all particles range only between -90 and $+90^\circ$, which is likely a manifestation of the relatively fixed angle between two 70 S particles within a dimer. The sample size was 771 ribosomes taken from one representative tomogram. Ribosomes were manually selected and subsequently aligned with an estimated accuracy of 10 \AA , corresponding to the resolution achieved by sub-tomogram averaging.

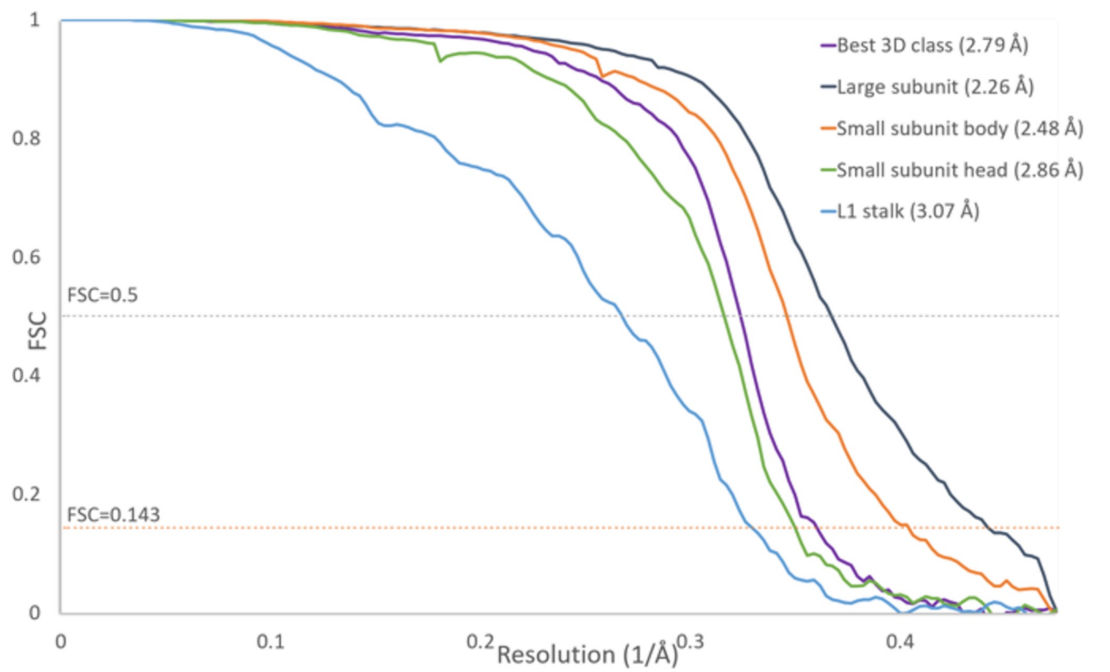


Supplementary Figure 6 – Purification profile of *S. lophii* ribosomes using a sucrose gradient.

a Purification profile. Samples taken for mass spectrometry analysis are marked with an orange dot, and the peaks investigated by cryoEM are shown with a double-headed arrow. **b** 2D classification of cleaned and polished single particle dataset.



Supplementary Figure 7 – Flow chart of the single particle processing in Relion .

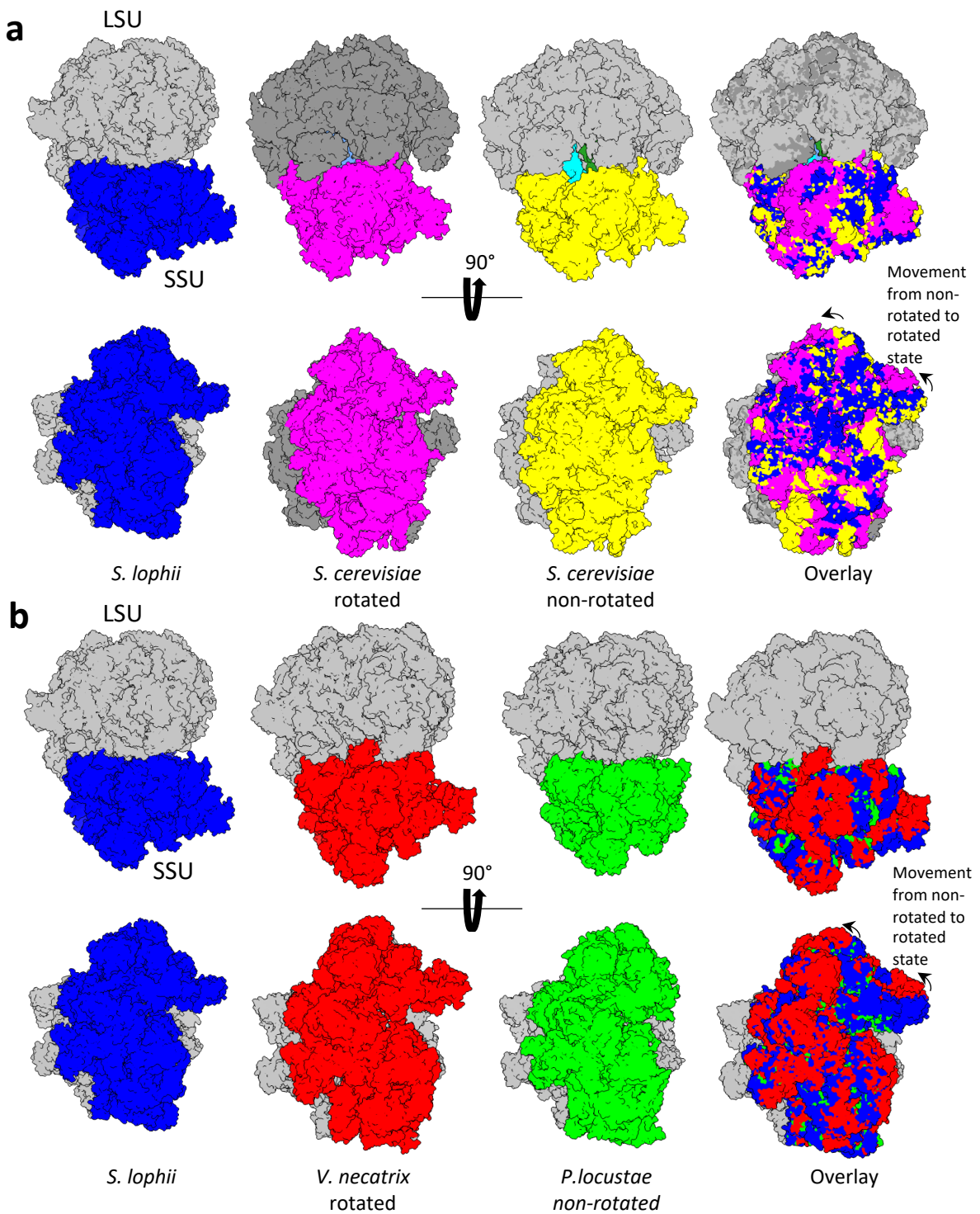


Supplementary Figure 8 – Gold standard Fourier Shell Correlation (FSC) of the single particle data. Purple, best 3D class; dark blue, large subunit; orange, SSU body; green, SSU head; blue, L1 stalk. Indicated resolution values correspond to the FSC 0.143 criterion.

Universal Chain nomenclature		Template	Sequence
rRNAs	L50	23S_rRNA	6RM3 AUACCACACACAAGGGAUCGUUUUGGCUCUUGGACGACGAGGAAGGGCGCAGCAGAGAGCGAUUAUGUGUGGGAGCAGC AGCAAUGACCACACGUCUCCCCGAAUACAGCAUAGUGCUGGAGUACUCCUUGGAAUUAAGCAUAUGAGUAAAAGGAAG GAAAAGAAACUAACGAGGAUCCCGUAGUAGCGGCGAGUGAACAGGGAAACAGUCCAACAGUGUAAUUCUCAAUUG AGAAGUUGUCAAAAAGGAAGAAGAUUGAUAUCAGAAAGAAAAGCUGAACGAUACAGGGUAGUAGUCCCGUAGUGU CUUCUUAUAUACGGUGAGUAGCUGUGCUCGGUAAUGCACAGUGAAGAGGUGGCAGUGUGCAUCUAAGACUAAAU ACAACAGGAGACCGAUAGCAAAGAAGUAGGUUGACCGAAAACAUUAAGUGAAAUUUGUGUGUGGACCCGAAUGGA UCGGGCCCGUCUUGAAACACGGACCAAGGAGUGCAUAACACUGCAAGUUGAGCAGAGGAAGUCUGUGAGGGCUA GUGAAGACGGAAAGCAGUGAGUGCGACCCGAUAGACUUGUGAACUAUACCUUGUUGCUGUGAAGGCUUGGCGA AAGCCAGCUGGAGGCGGCAAGCCGUUUUGAUCUGCAAUUAUGGCGUUAAGCAGGGUUAAGGGGCGAAAGACCAA UCGAACAGUCUAGUUGCUGGUUCCUCCGAAUUGUCUAUCAGGACAGCGCGCACGACAGAGAAGCAAGGUAGAG CAUGGGCCGGUACCUUAAGGAGACGGACGAACUUGCGAAUGUGCUUUUUCUUAUGCAAAGCGUAGUGGGCGAGU UCUGGUAAGCAGGACUGGCGAAGAGGAAUGAACCCUGGCACUGUGCUAAGGAACCCAAUGUCUGGACAGAGACCGA AAGAGGUAGGUAUAAGGACGGUAGGGCGGUAGCCAUGGAAGUCGGCAUCCGGUAAGAACCGUUAACAACGU ACCUACGGAAUUAUCUGCUUGAAGAAUUGAUGGCGUUAACAGACAUCCGAUUGCACAGUUAAGGAGGAGGG CGUGUGUCUACUGGCGAAGGUACCGAGUGAUCGGUGGGUAGAGUAGCAGAGCAGAUUCUGGUGGUAGUAG CGAUAAUUGUCUUCAGCGCAAAGACUGAGGAGGAGAAGGGUUUCUUCUAUAAAGAAGAGUGAGCCGGGUCUA AGCGAGUGUGUAGAAGCAUGAGCGAAGGAGAAACAGGUUGAUUAUCCUGUGCCUGUGCUGUCUACGGCAACGU GUUAUAGCUUGUCGACACGAACAUCUAGACAAAGCAGAAACACCUUUUCUGUUAAGGGGAAAAAGACCACUGAG ACGGAUCAUCUGGCGACGUGGUUUUAUCUCUGUAGCCAGGCCUGAGCCUGGUUUUGUUGAGGAGUGGUCUCCAU AAAAGACAACUAUUAUGGCAGCACAGUCCGUACCAACCGUAUCAGGACUCCAAGGUGAAGGCCUCUAGUGGAUGU UUUJACACAUUAAGGGAAUUCGGCAAAUUGGAUGCGUAACUUCGGGAGAAUUGGUUCAAUUGUAACUCAU UGUGACAAGGGAAUCUGACUGUUUAGUAAAAACAUAAGCUUCAUGAAGAGAGAUUAAGUUGAGUUCGCCGGUG CUCGGUCUGACACGGAGGAAUUGCCAGAAAGCAGGGUUAACGGCGGGAGUAAUCUAGACUCUUAAGGUAGC CAAAGCCUCUGUCAUUUAAUUGUGACGCGCAUUAUGGAAUGGAUAGCAUUCGCAUUCAGUUAAGACU GCGAACCACUUAUCCAGGGGAACGGGCCUGGACGGCCAGCGGGGAAAGAAGACCCUGUUGAGCUUAGACUCUAGUGU GGCCAAUGUUGAGCAUUGAGGCGAUUGAAGGAGGUGGAGAGCGCAAGCAGAAGUGAAAGACCACUGCGCCAU AUCUACACGACACACUUGUUUAAAGGACAAAGGCCAGAUUGGGGAGUUUGGCGUGGGCGGUACGACCACAGAAUCAG AACGUGGUCGACCGAAGGUACUCACAGCGAGACGAGAACACUCUGUAGAGCAUAAGGACUAAAGAGUGCCUGAG UGUGAGCACUACUGCUUGCAUAGAGGGGAAACCCGGGCCUJAGCGAUCCUACGCAUACGAGACACUUGUGGGCGUGGG UGUCAGAAAAGUUAACACAGGGAUAACUGGCUUGUGGCCCGCGAGCGUAGAAAGCGACGCGGCUUUUUGAUUCU UCGAUGUCGGCUCUUCUAGCAUUGGCCAUGCAGCCUGGCGAAGUGUUGGAUUUUUACCCACUUAACAGGGAAAC UGAGCUGGGUUUAGACCCUGUGAGACAGGUUAGUUUUUACUACUGUCCAACAGUUAAGGGGAGUGUGGAU UAGUACGAAAGGAAACCCACACGUGACCUCUGGUGCAGCGGGUGUGCGGAAGUGCAACUGCCAUGCUACGUCUCC UCAAGCAUAGCUGGAAAGCCUCUAAAGCUAGAAUUGAGUCCUGACUCUGGAAGACGGCCGGGAAGACGCCGUA UAACAGCGGUGUUGUACGAUAUGAGCUUUCUAUUUGUGUAGUAGCGAGUUAUUUGAGACACUGGUUUGUUAACAC CGUGUUUAU
	L70	5S_rRNA	6RM3 AGTTACGGCCATATCTACTGAAAAACACCGGATCCCGTCCGATCTCCGAAGTTAAGCCAATAAGAGCCATGCGAGTATTA AGGTGGGCGACTACTTGAGAAAGCGTGGTGTGTAGTTT
	S60	16S_rRNA	6RM3 ACCAGUUGAUUCUGCCUGACGUGGAGUAGUCUAGUUAAGCAUAGAUUUUAGCCAUUGAUUGUUAAGCGAAGCGAUUAGU GAAGCGGCGGAAGGCUAGUAACGGGCGAGUUAUUUAUCUCUAGAACCAGGACAACCUAGGAAACAGAGGGGAA ACGGAGUAAGAGCAUGUGUUUGUUAAGCAUUGAUGAGAAACAAGUAGCAGGAGAGUUAAGAAGCCAUCCAUAGU UAGUUAAGUAGGGUUAAGGGCCUUAUUUAGACGUAGACGGGUACGGGGGAAUUAAGGUUUGAUUCCGGAGAGGGAGC CUGAGAGACGGCUACCAGGUCCAAGGACAGCAGCAGGCGGAAAUAUACCGAAGCCUCCAAGAGGGCGGUAGUGA AGAGACGUGAAAAACAAGUUGUGUAAAAACCACUAGUAGUUAACUGGAGGUUAAGGCUUGUCCAGCUGUCCAGCAUCCGCGGUA AUACCAGCUCAGGAGUGUCUUAUGAUAUUGCUGCGAUUAAAAGGUCCGUAGUCGAGAAGCAGUACUUGUUUGU AAUGUGGCUUAUCGAGAGCCGAUAAGAACGAGAAGAGUUCAGAGUAAGGAGCGAAGGAGGGCUAGAUUAUUGAG CAGCGAGAGGUGAAAUUUGAUGACCUUCUUAAGGAGUAACAGAGGCGAAAGCGCUAGUCAAGAACGAAUCCGAUGA UCAAGGACGUAGGCUAGGAGUAGCAAGACGAUUAAGACCGUAGUAGUUAAGCAGUUAACGAGUCCUACGUCU UGUGUUUAUGACGACGGCAGAAAGAGAAUUCUAGUAGGGCUUUGGGGAGAGUACGCGCGCAAGCGAUAAAUUUA AGGAAUUUAGCGGAAGAACCACCAAGGAGUGGAGUGUGCGGCUUAUUUAGACUACACGCGGGACAGCUUACCAU ACCCGAUGGCCGUGUGAGCGUAGUACGCGAUAGGUACAGAGUGGUGCAUGGCCGUUAACGACGAGUGGGGUGA CCUUUGGGUUAAGUCCGGAAGUAGUGAGAUCCUAGGUAACGAAAGUUAUCGACAGGUGUUAAAAACACAGGA AGGAAGGGGCAAGAACAGGUCAGUAGUCCCUUAGAUUGGUUAUGGCUACGCGCACUACAGUGGUCACAGCAGA AACAAUAGAAGUAAAUGUGAUCGAGAGGGAAUAGCACUUAUUGUGCAAGGAAAGGAAUUGCUAGUAAU CGUUUAUCUAUUAAGAAGCGAUGAAUGUCCUUGUUCUUAUGUACACCGCCCGUCGUUAUCGAGAUGGAACC AGGUGCGAACAAGUGAAAGCGAGUGAGUGCAGGGUUCUAGAUUCGUAACAGUCGUAACAGGCAGCUGUAGGA GAACCUUGAUCUGGAUCAACUAGUUAUU

Large Subunit ribosomal proteins

LAO	uL2	6RM3	<u>MGKVIRTLRLQKEHVKRKHHSKSGIVGFPPTLKNCEATVEKIVHESGRPAPLAIINYKKEEKNTKGLLVAIEGIYEGQKIQIGENASLELGNVCPIKEPEGLVSMVEKPKDGGYKVSPPGCVATIVYHNKLTNQTTIKLPSGERVVRTGESRALLGIIAGGGKDDKPLLKAVNAYKYKTKGKPPWPRVGVAMNVPDHPHGGGNHQHVGHPTTVSRNAPVNGRVLVAARTRGRKKGSRKIVTVN</u>
LAA	uL15	6RM3	<u>MFNKKSKSRKLRGHVSHGYGRVGRKHKRHPAGRGKSGGLKHLRSLFORYPHDPHFRKLGIVMFHRNKNADFTRTVNSVNLWGLMKLEEQMKFKSSAEVPPVDCRNYGYLKVLLGGDLISVKKPIVVIARQFTKDAEEKINAVGGKCVVAA</u>
LBO	uL3	6RM3	<u>MSCRNYSAPRHGSLAYCPKRAKTKPAIKSFPKDDASKPVHLTGYLAKYVGMTHVVRLSTRRSKTERVLKETQKEVLDVAITILEVPAMRIFGMVGYKSTVNLGKAIKTVYAEHLDEQVRLARLKRNSRSSKLMFKLYPQKYENGDIKRDYELLKRESDVVRVLAHTQVSKIPSLNKKAHILEIQLNGGTIEEKIEWAIERLEKIEPVEVFSQQDLIDIVGVTKGGKGETTKRFGTITLQHKSRKGRKVCAGIWAHPANVRRTVARAGQLGFHRRTAQNKKIYMMNGKTFPSTEYDLTSTINPMGGFLRYGNVKSDFIMVKGGIQGSPKRVLALRKAFAFNSLGDNEEIVLKFVDASSKKGYGRFQTTEEKAYFGIKAEVVEQ</u>
LCO	uL4	6RM3	<u>MSRQVNIIEQDSSSRTTSVPAVFNLRVQDLISFTHKNVAMNSRQPYAVKPGAGMRHSASWGTGRAMARVPRVAGGTTRRAGQGANANFCRGGRMFAPTNVNRRVNRKTLISRRYASAMAVAASTIPAYVEGRGHRIEQIENIPLVLSNKIDTETKTEGKILYNLGLKEELDKEESKNIRAGKGWNRNRYVLKKGPLFVYTSSETSFTKAI RNIPGVDTERVE NLSILDAPGGQAGRLIWMEDAFEKLEIYEGEMNEESFKKGYTMGMDDVAENIDVENIFYSDEVEQFIDIPNFIKDKKMMVEKKNLAEIQQVVEIEERI</u>
LCC	eL30	6RM3	<u>MSRKRKREESFVDKLPALKTKGVNLGFKESLKSLEKTKLLILTSNFSPIKRKTLEYYAASNNTPIYYDGSNNDLSKNCCKGYFRIGVISIQDFGEADLMNAMAQA MVSNSINFQSDNKSYSYQRFQTKKRRRLRCKTNYRRRTKLKLDQKNVGEDKSRVVRITNSKVICQVVVSKLGGDMVVAQATS AELKEFGLKVGFTNYSAAYATG LLLCARKILMEKLDNIYKPKFCTD VDVVEDIEGKNA YRCYLDIGLSRNSKGA KFAAMK GASDGGIKIPYSPKIFPGYNKDEEFDQQLDRIFRIGKEVAN YMQLLKKEDETKFKKQFSEYIKQNIINAEDLEKMYENL FNSVITKEISKHEKKDYSAKKYKVMKLTKEERDARVOQKLVENN</u>
LDD	eL31	6RM3	<u>MAFKDDKAIELTIPMGKITIDVSKRWKCRIGIRRLKFKITFTFHDKAEAVQISPDNLKFLWGERMNRVPRKRVRVVNQEPYKPKDSKVVYKLSHVVVSTFKGLGTEAIAE MQSERQQRLNINIKNYTADDIPAEAKLQLHLKHPAPIELRTDVLVQGNVVVLEGEYASRVVYLSRTEDNKALCMGLPSINGIGLFEIDERFLRTSIVLDIRLDKKYRA KESKRSFKFNTKEKLVTEENDEI ELLLKEIENEKFMKYYFETPTYPEINNTVDVYIENH</u>
LEO	eL6	6RM3	<u>MDIVNPSLSKVIDIINKGSKKQKRFQSWRVKRVSESWRKRPRGIDNRMRKFGGAPDMPGIGYKKNPNAIRFLPNGRYKIMVACVKDEALISLNSFYCAEIRHAVGSKKRILEERA EELGILVINKEGLKVFEEEE</u>
LEE	eL32	6RM3	<u>MSNVDLIAKKEQYNLRMEELQKQEQEHLKRV EENKYYAEKRTVELLETYK KIAL EQQLSEERMQKENKIYAPKDPHFYAVVLIKSPLRASNKIKTLEFLRKHINNCV LVKNNESSRQMLQKIRNFVAYGELKLTRELIYKRGSC HIFSEKLNITNETLEDAFNKIRCEEFYELVFNDRFERKELNLFNLNPFKLT PPKGGYKGRKADIEGGAVGNH QDKIGELIKKMI</u>
LFO	uL30	6RM3	<u>MEVIKIKELKERVCAKGLTYSHKRSKNYPRYSLTIENVTREAA M KYVGN AVELFKPESTKEESHIGIIKAVHGRSGAVRAKFSRNI SPVEMGSKVYIKYKMNEDI MSDIQLTKQLLSSMNPRLTNKAMDMTRKEVIATRKEALVNAIKLPPALNQVTNYLSEEDLKEVSDLSKFRPETERKERQRLQNERKGPPIIVKQGIKHVTKLIEEKA KFYLIACDVPDIEVLYLPTLCVKGPIYALVTKEDLGKIIKKPTTCLCLCDVSSDIKSFENVI AKCNGQFKDNYESAMKVVWGSPEKVEKKE</u>
LFF	eL33	6RM3	<u>MVERQVVRGGSTFNTSRNKRKVRTPGNRLVFQDVKKKGGKPGCGQGIKLAGVKEARPAAFSRMRKSRKRVNRLIGVQCAKCVQEKVLDALFNAEEKRMELE V</u>
LGG	eL34	6RM3	<u>MVYVYVEEKVQIPEGCKVEVNVKVTVTGPKGTYSKDFSHIFVDIHEENVRCLWNCRKQERSKLITCSKLIKNMIDGSMGYFELKAAKYHPITFEIKENGLLIV KNFLGQKWAREFKMIGDTIIKQGVTKDHLILEGISKEDVSO TAGLIQNCLPKNLD RRRIFQDGVYIATKGLIEVK</u>
LHO	uL6	6RM3	<u>MKLLAKDLRNNISIEELEKNLVDAKSEMLQIRQKQSAILKPNELNRGNRRNVAVILTIIREKYYQLLEKYDADNIPKELKPRMNRKRQELTKKQKISKVGTKLYTKGNKLIYAF</u>
LHH	uL29	6RM3	<u>MGRRRPGRCYRYCKNPKYISRFRCRGVDPDKIRIHDLGNKNAPCVDLPLNVNLISYSKEQLSSESLESARIAV NKYMMVNAGKDNHYHRINVHPHYHLIRINKMLSCAGADRLOQTGMRLAYGKPYGAARVRFQRORISIRTKDSNLK MAMEALRRARYKFPKYEVQKSN EYGFSGIERSQFEELKNNKGLINMGDHWKIIKEKGTVERVYVLCENAIQ</u>
LII	eL36	6RM3	<u>MKINKLNNASKALSYPVKLAKTEKAPL TEKQQRSKELKFA MSIAREVGGLAPYELRATELLRRDEDKRCCKFMKRLGSLMAKKKIELLAEMRA MAEENPMNKIKIQKLCNICTGGGEILNASKVLEQLTGQKPRLSKSLRTIRSGIRREKIA CHVTVRGEKAMEILEKGIKEYELIDSNFSTQSGSFGIKEHIDMGIKY EPDVGIFGMNFYVLEKPGIRVSKRRKRVSRVGNKQRVTD EAKNVFVKYEGIVLKG</u>
LJO	uL5	6RM3	<u>MSKGTSPNGKKNRHHTCCRCGSVSKQKGCSSAYPEKMKMKMQRNKA MLKTTGTGKMRHMKKERISRRAGYPGNPILKEIRNKIKS MRGNQMLPNNHFNKSGMFKNWFNQPMRALRKRKEVREQSKICYPNPLKLRPVVRCSSMRYNKKVRLGRGFTKMEIEKAGMDIKYAVISGVIADLRRKNKNEE</u>
LJJ	eL37	6RM3	<u>GLEENVKRLMDYKNLKFETT VREARDNKIEYQKGEIMKPVNIIPEVKGIKVEEIIATYGN</u>
LLO	eL13	6RM3	<u>MGSIKTRRIKQVLSALKNRCIPQWKRACPGFKQYNYFTKRSWKRTLKAC</u>
LLI	eL39	6RM3	<u>KYFSYPLMYFIKPGCLIKKFSIYTSIVISVIDNNSVVIQSYDSDNGDVISIDREVENSVKIPIGNIDIKNKSKEIDGILVEENRNLKNKDDVLLMNDFERFEQLKKEVED</u>
LLL	eL14	6RM3	<u>MVIEEMA</u>
LLO	eL14	6RM3	<u>MQLFLKSPNGTSTIDLALENTLNVLTNEGTVSTENNRFIYNGKQMDTNRCLFSSYFGKLETIYTVTRIGCGKLTDDDKVLAITKFEKQVCRKCYARLPIRATHCRKRACG YSKLGRGKKRRDESK</u>
LMM	eL40	6RM3	<u>MGYQLYKIEIRRDQSSVMRYLLRVRVWEYRQNTAIHRASGPTYLDKARMLGYRNPICMFIFRARRVRRGARPKVKLGNTHGKPSKSGVYQRKPAKRLQFAEIRVGRKCSNMRVFNYSWVVGQDRVYKYFEVIMVDPYCDRIKNDKKNWLCPKVPMKHREARGLTNGTKTSRGLGKIRYNTQTKGGSKRAAWRNRRNTQSLRRYR</u>
LNO	eL15	6RM3	<u>MIGKRIVDAIGHVAGLAGHIAKELMCGIEVIVHAENVMFTGPIKRGVGFNSHLNKS CSYNPLRGPFFHLRSPSMHLMKVIKRLPHRKSQGIKALRLOVDFGCPTEYLSVEKMVCPDRDKLEYTTSPIRKS YLGDLMIKFGWKYESVVEKMNQNEEFQKIEETEKQVKVKEQKIEELKSNKFFQQRVEEIMVQMA</u>
LOO	uL13	6RM3	<u>MVNIPKTRRTYCIKAYHTEFKVGLLKKGKDSPRKQGARRYARKQRGVHGQTKPILKRAKATTKVKVILKIECTCKSSQLKVRGRAKHVEFGAEKVKGEALY MAHKYSCDISEATSVRSQYNNIKIFKNTREVCNIIKT MELNKA IAYLNDVLRFFKCIPIVFRFAKKGRTPAKAEFGTDKGGKWPVKSVA FVKMLLEDLLEAEKGLGT SELVIKHQIWNQAPKIYGRKHGAFGRIPYKNSPSHIEVI AVKREHNIKEGDVVEVKE</u>
LOO	eL42	6RM3/Alphafold2	<u>MSRKTKVGITGKYGSRYSGLKRRAMICMNAQSKRYCCSFCGKTTVKREVIGIWSRCFRGKGVSGGAYVITNQVEYKQVINRMKME</u>
LPO	uL22	6RM3	<u>MLKQKMFHEHYKKAIFDESRVPRTKNVFYQSLFSLYKVCENTENEIVHKITKRLTMSRTERHPKLSNIIIEQANGKEIVVFGVKVLDLDDKILEIPKITIVALKISQAKEL LTLNGGEFYLDLKFYSKLDKDALVKGDKNNRCKTRYFGASGDKSETYPRTRKGAARRGERRIKNKKK</u>
LPP	eL43	6RM3	<u>MKLPVVKLSADILKCGVRRYIYLDTFEPSKLSAATSRRDIRKLIKDGLIVRKAQKIHRSYRANKLMREKEKGRHS GPGKVGSKNARMPEKDKWIKRIRDLRNTLKLRLD</u>
LQO	eL18	6RM3	<u>KGEITKTEHKMYQKTKGNGYKNSAALMGAIKQKHDDERRMKEIEEQAKALKIAASEK</u>
LRO	eL19	6RM3	<u>MAINEYKVGIKIDDNGVGVDEVYAHIVFATNELLAKSFPNKILKQKIKPKNTKVIKVEIEKEDNKDLVKNYIDYIYKSKRCDHKYKFEFRGVS RCHAVDKLLCDIGGRHSVKNRILIFIMKVKELTVDEIKDKIKIFAMEDVEYPA YRNVLRNSKLFVSENEKLFN</u>
LSO	eL20	6RM3	<u>MVSSHGYYRRQTRNLFSQA FRKHGMPNTSYTLTVAYAGEYDIKVNPAIHGKMPHKHYHGRTGIIKEVNRGVSIVLFRNIRLANKTVERKVI GIEHIRKSKQDEYLRLEINKKIIIEAEKGEVPVLFKRQPKGPKPAVEMLMNIIELSYDKVESQ</u>
LTO	eL21	6RM3	<u>MSEAAVQDQSITYLTDCTKCTEQSLFSTDDLKEYLLGNIKYNGKKGQGLGKVLVLSNTGHAVDVFVFAKTSKRYLYKLIKFLRAKGLSDVWRPIAGDKQSYKFFSKQA GEE</u>
LUO	eL22	6RM3	<u>MPGKQKPLDAIKNRYGFTFRGIQVGTVLACADNSGAKMLKTIGVKVSGRLNRLPAAGPGDVIVASVVKGRSDMRKKIVLAVIIRKQKQKIRKDGVRLOFEESA AVI ISNKGDGLKGTQISGVLREVCDIWPKISSQAGSIY</u>
LVO	uL14	6RM3	<u>MFYEGTCVYSYVPRGKGTYSVFLDDKSSLFFSSKYKNSFDKIKAKRVKQTLMSKQFCGKVESVHTEKHKKIVVQKIVRGPALPDAVEKIREQNKDYKXKIVDEK VQHGGKVGKIEQKQMKQKQY</u>
LWO	eL24	6RM3	<u>MPKLNRRHKASRLDKKIKQKLIKPLGFSLLDIKYGIGHEGAALKIEEAGTLTFVVQDQADKINVKKAFEEIYGEKVKVNNINNTMGVKKAYIRLVELGNAAVVATKAG IL</u>
LXO	uL23	6RM3	<u>MKFNKNVTASRRNRKHAFTSIGLERTKLMNAPLSKDLKEEYGIKRIARVPGDMVVKVSGFEKKGEGVMVKNRHDKIEVEGFCVQKEDGKKINKGIHPSNLRILKLS MEKDRAEIIINKVEAMKSEVKA</u>
LYO	uL24	6RM3	<u>MKPTFIIRINLFFPKYFFLFIHFFLFFIFFCPMSETLFEKNMFLVLLNRRGFRAGNKGIIVDLENNRIIAGVSKIPKNNKRTIVFLKKNMNMHIMATTYKRDIGLSLDNLSIFEDKVKKAVALNRVREIFNKNKNNEGFAWLYKLEI</u>
LZO	eL27	6RM3	



Supplementary Figure 10 – The *S. lophii* monomeric ribosome is in a non-rotated state.

a Ribosome structures (left to right: *S. lophii* (7QCA), *S. cerevisiae* (3J77; rotated state), *S. cerevisiae* (3J78; non-rotated state) and all structures overlaid) shown in surface representation. Ribosomes are displayed in a side view in the upper panel, and then rotated by 90° to show a bottom view in the lower panel. Large subunits are coloured grey and small subunits blue (*S. lophii*), magenta (*S. cerevisiae*, rotated state) and yellow (*S. cerevisiae*, non-rotated state). tRNA molecules bound to the yeast non-rotated state are coloured in cyan and green. **b** Microsporidian ribosome structures (left to right: *S. lophii* (7QCA), *V. necatrix* (6RM3; rotated state), *P. locustae* (7ZU5; non-rotated state) and all structures overlaid) shown in surface representation. Ribosomes are displayed in a side view in the upper panel, and then rotated by 90° to show a bottom view in the lower panel. Large subunits are coloured grey and small subunits blue (*S. lophii*), red (*V. necatrix*, rotated state) and green (*P. locustae*, non-rotated state).

a

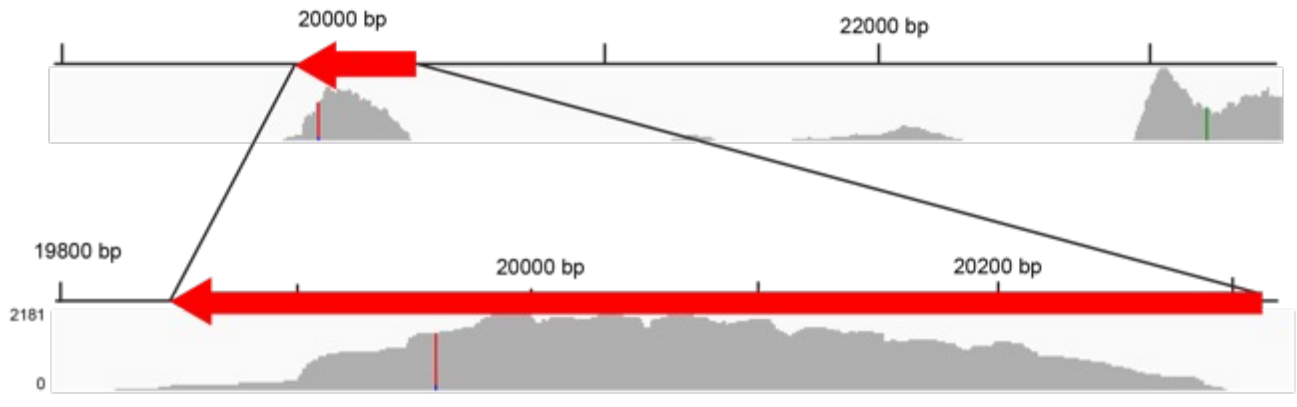
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Vn MDF1 1 MKYELIAKADFDNIKGLSCDQYPAILLSTACSFVHPKVVILTSESTIKEPNVYKCNWEM 60
Sl MDF1 1 MKYNVYIEGTFSGIQSIMVEQVYPITIKCSGCNLVHKKDVILSIDSINYTDKDEKVNLIK 60
      ***:: :. *.*::: :*.** :.:*.:** * ***: :* : * * ::

Vn MDF1 61 KCHSCKNDIKVSIYKSPSLSTVNIKDRYEDDLVLSYNPKNNECLLSILECSGGGEIREIK 120
Sl MDF1 61 KCASCTRQMYILI---KSMAT----ELYEEKFYCAKK--EDNRYLASQLQGRGCIYIEKIK 111
      ** *..::: : * *::: : *::.. : : :.:. * * *: * *.:**

Vn MDF1 121 NVPLNILTDDLRRLFNEKVVDEKRSLAEVYGNNKTYSIINYELEIRRVK 168
Sl MDF1 112 DANIHLVLESEKGRIPDIVFDEGYWREEDNMNN-TSSIEDFNIVLESVK 158
      :. :*:::: *.* * ** * ** :::: :. **

```

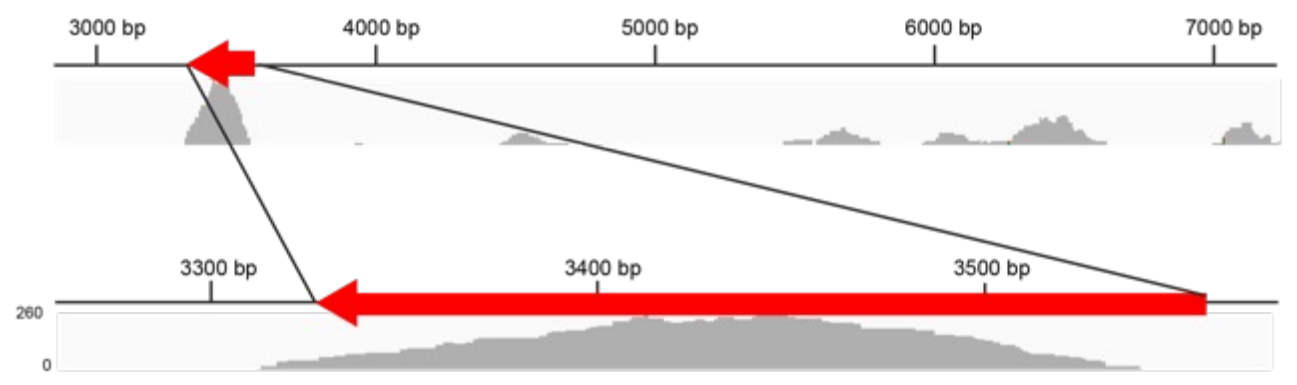
**b**

```

P1 Lso2 1 MSRTRNAQKKYEGMMRKLKAKEERMKEEELKEEEAKKFWISIGAKDESKKQALELKRQEK 60
Sl Lso2 1 MSRTRNYVKKKEGRRLRKLTKLEEAKRLEEEKKEEELKEYWSVGAKDYSEEMAKEEKRLAK 60
      ***** ** * :***. ** * *** ** ** *::*:*** *:: * * * *

P1 Lso2 61 IARRAMRKKIYEEELGL----- 77
Sl Lso2 61 IEARKQRMEE--QERMEMEEIEKMK 82
      * * * : :*.: :

```



Supplementary Figure 11 – MDF1 and Lso2 homologs are encoded in the genome of *S. lophii*.

Sequence alignments of ribosomal hibernation factors. **a** BLASTP search of the *S. lophii* protein database with the MDF1 protein sequence from *V. nectatrix* resulted in a hit for hypothetical protein SLOPH_1662 (EPR78075.1) with 29% sequence identity and 51% sequence similarity. **b** A putative Lso2 protein was identified in the *S. lophii* genome on contig ATCN01000495.1 using TBLASTN with the Lso2 from *P. locustae*. The sequences have 52% identity and 68% similarity. RNASeq reads from the *Spraguea lophii* transcriptome (SRX312311) were mapped onto the Celtic Deep *Spraguea lophii* assembly (GCA_001887945.1) using Bowtie2⁵¹ with default settings. Upper contigs show the context of the genes of interest and RNAseq coverage compared to immediately adjacent regions of the genome and lower contig regions focus on RNAseq coverage of the gene of interest.

Preliminary annotation	Protein name	Number of peptides	Peak area
60 S ribosomal protein L10	uL16	14	5.51E09
40 S ribosomal protein S4	eS4	29	4.90E09
60 S ribosomal protein L7	uL30	30	4.69E09
40 S ribosomal protein S0	uS2	24	4.38E09
40 S ribosomal protein S2	uS5	21	4.14E09
40 S ribosomal protein S3	uS3	21	4.08E09
Ribosomal protein L23	uL14	11	4.02E09
60 S ribosomal protein L4	uL4	30	3.85E09
60 S ribosomal protein L3	uL3	39	3.76E09
60 S ribosomal protein L12	uL11	8	3.71E09
60 S ribosomal protein L17	uL22	20	3.62E09
60 S ribosomal protein L10a	uL10	19	3.61E09
40 S ribosomal protein S14	uS11	11	3.52E09
60 S ribosomal protein L18	eL18	23	3.47E09
60 S ribosomal protein L5	uL18	26	3.29E09
40 S ribosomal protein S20	uS10	13	3.26E09
60 S ribosomal protein L8	eL8	14	3.05E09
Ribosomal protein L15	eL15	8	2.91E09
Ribosomal protein S19	eS19	17	2.90E09
60 S ribosomal protein L11	uL5	9	2.80E09
Ribosomal protein L29	uL29	8	2.73E09
40 S ribosomal protein S12	eS12	13	2.65E09
60 S ribosomal protein L6	eL6	7	2.62E09
60 S ribosomal protein L21	eL21	20	2.57E09
60 S ribosomal protein L20	eL20	18	2.55E09
S7XVN9 uncharacterised protein	-	10	2.50E09
60 S ribosomal protein L23a	uL14	10	2.45E09
40 S ribosomal protein S9	uS4	19	2.44E09
40 S ribosomal protein S7	eS7	12	2.43E09
40 S ribosomal protein S11	uS17	23	2.30E09
60 S ribosomal protein L26	uL24	14	2.30E09
60 S ribosomal protein L37a	eL43	6	2.30E09
60 S ribosomal protein L9	uL6	17	2.25E09
Guanine nucleotide binding protein β	RACK1	25	2.21E09
Ribosomal protein L34	eL34	6	2.17E09
40 S ribosomal protein S16	uS9	7	2.15E09
60 S ribosomal protein L8	eL8	19	2.11E09
40 S ribosomal protein S26	eS26	4	2.08E09

Preliminary annotation	Protein name	Number of peptides	Peak area
40 S ribosomal protein S5	uS7	17	2.06E09
40 S ribosomal protein S15A	uS19	13	2.03E09
60 S ribosomal protein L32	eL32	17	2.00E09
60 S ribosomal protein L27	eL27	11	1.94E09
40 S ribosomal protein S13	uS15	13	1.90E09
Ribosomal protein L13A	eL13	18	1.84E09
60 S ribosomal protein L35a	uL29	7	1.80E09
60 S ribosomal protein L31	eL31	11	1.76E09
60 S ribosomal protein L36	eL36	10	1.74E09
40 S ribosomal protein S25	eS25	8	1.70E09
60 S ribosomal protein L19	eL19	14	1.64E09
40 S ribosomal protein S24	eS24	16	1.62E09
Ubiquitin/40 S ribosomal prot S27a	eS31	9	1.61E09
60 S ribosomal protein L13	eL13	21	1.51E09
40 S ribosomal protein S8	eS8	11	1.51E09
40 S ribosomal protein S10	eS10	12	1.39E09
40 S ribosomal protein S6	eS6	13	1.33E09
60 S ribosomal protein L39	eL39	9	1.23E09
S7W9F9 uncharacterised protein	-	9	1.20E09
40 S ribosomal protein S29	uS14	8	1.10E09
60 S ribosomal protein L22	eL22	10	8.56E08
40 S ribosomal protein S18	uS13	8	8.50E08
60 S ribosomal protein L3	uL3	12	8.40E08
S7XRH7 uncharacterised protein	-	1	7.91E08
S7XL41 Uncharacterised protein	-	8	6.31E08
Ribosomal protein S21E	eS21	7	5.89E08
Ribosomal protein L24E	eL24	8	2.79E08
60 S ribosomal protein L44	eL42	6	2.52E08
S7W5K5 uncharacterised protein	MDF1	12	1.75E08
S7W996 uncharacterised protein	-	13	1.72E08
S7W9A1 uncharacterised protein	-	7	88280278
S7WEE5 uncharacterised protein	-	8	73470297
S7XUM1 uncharacterised protein	-	7	61605781
S7WA57 uncharacterised protein	-	2	51516554
60 S ribosomal protein P0	uL10	8	46350286
S7XJY0 uncharacterised protein	-	3	39028496
S7XJ25 uncharacterised protein	-	7	24881672

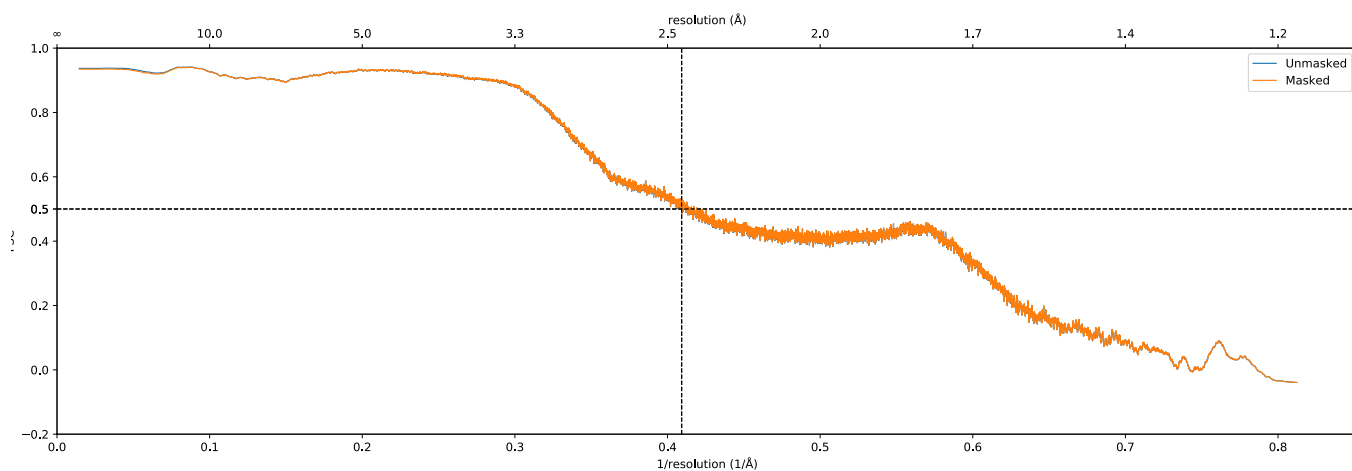
Preliminary annotation	Protein name	Number of peptides	Peak area
S7W9S4 uncharacterised protein	-	5	24124621
S7W6A0 uncharacterised protein	-	1	22330513
S7W535 uncharacterised protein	-	7	18090219
60 S ribosomal protein P2	P1/P2	3	16879465
Ubiquitin-40 S ribosomal prot S27a	eS31	4	16446609
S7W9Z4 uncharacterised protein	-	2	9578404
S7W8B4 uncharacterised protein	-	6	8240642
S7XIT1 uncharacterised protein	-	3	6803198
S7W633 uncharacterised protein	-	3	5718484
S7W7U9 uncharacterised protein	-	3	4781359
S7W9D1 uncharacterised protein	-	1	4668589
S7XF67 uncharacterised protein	-	1	3290196
60 S ribosomal protein	-	1	2760956
S7XUL4 uncharacterised protein	-	2	2582596
S7W4Q0 uncharacterised protein	-	2	2471210
S7XKV6 uncharacterised protein	-	1	1905871
S7W5D5 uncharacterised protein	-	1	1427322
Ubiquitin-60 S ribosomal prot L40	eL40	4	1401421
S7XLB7 uncharacterised protein	-	1	1030522
S7W9X9 uncharacterised protein	-	1	927439
S7XJ96 uncharacterised protein	-	1	-
S7XH02 uncharacterised protein	-	1	-

Supplementary Figure 12 – Mass spectrometry analysis of the purified *S. lophii* ribosome sample. List of ribosomal and uncharacterised proteins identified in the mass spectrometry analysis of main ribosomal peak (point 2 in Fig. S6a). The proteins are presented in peak height order.

a Single particle map and model (EMD-13892 / PDB-7QCA)

Masked: 2.4 Å

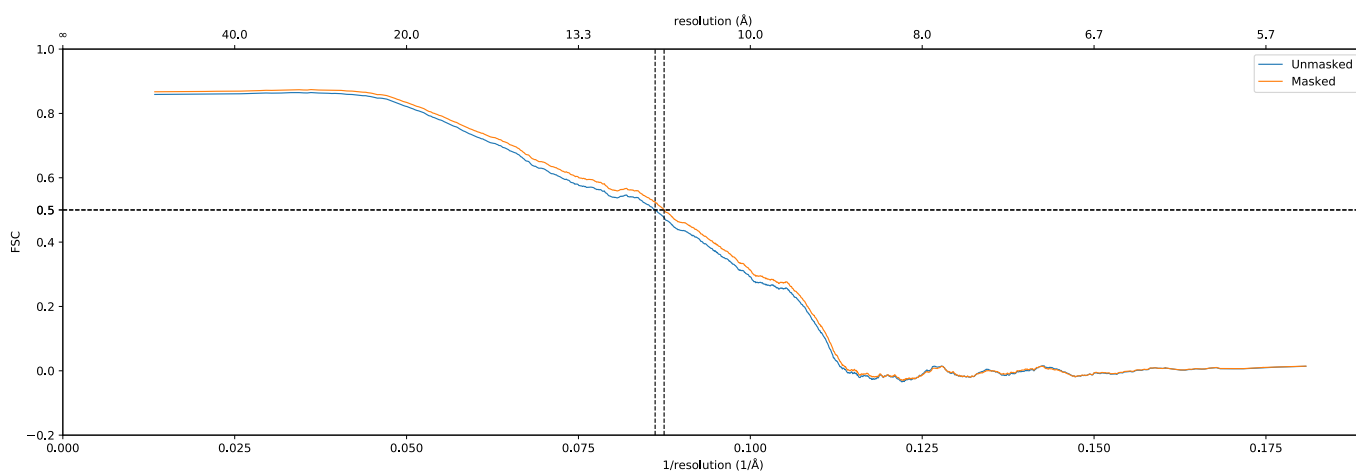
Unmasked: 2.4 Å



b Sub-tomogram average map of the half dimer and model (EMD-17448 / PDB-8P5D)

Masked: 11.4 Å

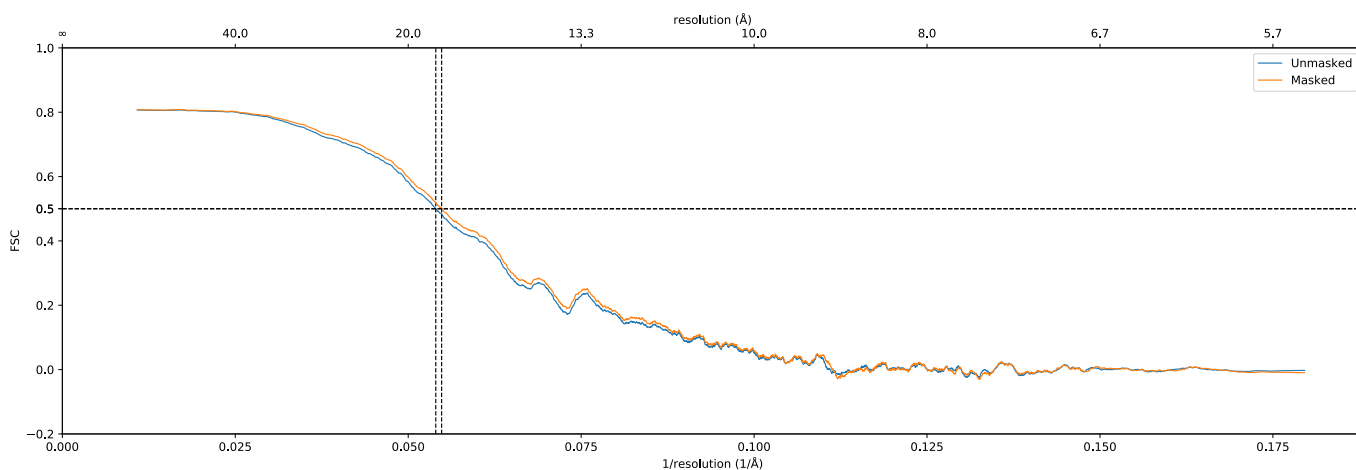
Unmasked: 11.6 Å



c Sub-tomogram average map of the full dimer and model (EMD-17457 / PDB-8P60)

Masked: 18.2 Å

Unmasked: 18.5 Å



Supplementary Figure 13 – Map/model FSCs.

a Map/model FSC for the single particle map. **b** Map/model FSC for the half-dimer subtomogram average. **c** Map/model FSC for the full dimer subtomogram average.

Data Collection	Dataset 1	Dataset 2
Acceleration voltage (kV)	300	300
Pixel size (Å)	4.377	4.53 (2.265 super res.)
Defocus range (µm)	-4 to -6 (0.5 step)	-2.5 to -4.5 (0.5 step)
Frames per tilt	10	26
Dose per tilt (e ⁻ /Å ²)	1.90	1.97
Tilt range (°)	-60 to +60	-60 to +60
Tilt step (°)	2	2
Total dose (e ⁻ /Å ²)	116	120

Depositions	
Dimer	
PDB ID	8P60
EMDB accession number	17457
Resolution (Å) (Masked FSC = 0.143 / 0.5)	14.3 / 20.0
Resolution (Å) (map/model; FSC = 0.5)	18.2
Half-dimer	
PDB ID	8P5D
EMDB accession number	17448
Resolution (Å) (Masked FSC = 0.143 / 0.5)	10.8 / 15.5
Resolution (Å) (map/model; FSC = 0.5)	11.4

Supplementary Table 1 – Electron cryo-tomography data collection and subtomogram averaging statistics

	Dataset 1	Dataset 2	Dataset 3	Dataset 4	
Acceleration voltage (kV)	200	200	200	300	
Pixel size (Å)	1.054	1.054 (0.525)	1.054 (0.525)	1.06 (0.53)	
Defocus range (µm)	-1.2 to -2.5	-1.2 to -2.5	-1.2 to -2.5	-1.2 to -2.5	
Frames per movie	40	40	40	40	
Total dose (e ⁻ /Å ²)	40	42	41.34	41.6	
Number of micrographs	513	1816	9192	15 989	
Number of particles	30 743	83 575	206 256	657 305	
Particles in final refinement	14 949	42 390	114 880	113 721	
4 datasets combined					
Maps	Overall	LSU focused	SSU head focused	SSU body focused	L1 stalk focused
Resolution (Å) (0.143 FSC / 0.5 FSC)	2.79 / 3.09	2.26 / 2.73	2.86 / 3.16	2.48 / 2.89	3.07 / 3.77
EMDB accession number	All submitted under EMDB-13892				
Model Refinement – Overall map					
PDB ID	7QCA				
EMDB accession number	13892				
Resolution (Masked FSC = 0.143) (Å)	2.79				
Resolution map / model (FSC=0.5)	2.4				
Model Composition					
Non-hydrogen atoms	170,634				
Protein residues	10,629				
RNA bases	3,972				
Ligands	368				
RMS Deviations					
Bond length (Å)	0.009				
Bond Angle (°)	1.609				
Validation					
Clash score	2.0				
Rotamer outliers (%)	1.1				
Ramachandran					
Favoured (%)	96.0				
Disallowed (%)	0.1				

Supplementary Table 2 – Single particle cryoEM statistics