

## Supplementary Information

# Dynamic Contact Network between Ribosomal Subunits enables Rapid Large-Scale Rotation during Spontaneous Translocation

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## Supplementary Methods

### Choice of Occupancy Threshold for Clustering of Contacts

In the process of grouping contacts into intersubunit contact clusters (see Methods), in a first step, the contacts with an occupancy above a certain threshold were clustered (Supplementary Figure 1). This threshold was chosen on the basis of two criteria. First, the threshold should be in an interval where the number of clusters is independent of threshold variation. Second, the grouping of residues into clusters should depend as little as possible on the choice of the threshold.

To monitor the change of clustering of the residues, we applied the clustering protocol using thresholds ranging from 0.025 to 0.975. For each threshold, a  $N \times N$  matrix  $\mathbf{C}$  which describes the grouping of the  $N$  residues was constructed where  $C_{ij} = 1$  when residues  $i$  and  $j$  are in the same cluster and  $C_{ij} = 0$  otherwise. The difference of clustering  $d(\mathbf{C}^{\mathbf{A}}, \mathbf{C}^{\mathbf{B}})$  between two matrices  $\mathbf{C}^{\mathbf{A}}$  and  $\mathbf{C}^{\mathbf{B}}$  was defined as

$$d(\mathbf{C}^{\mathbf{A}}, \mathbf{C}^{\mathbf{B}}) = \frac{\sum_{i,j}^N |C_{ij}^{\mathbf{A}} - C_{ij}^{\mathbf{B}}|}{N^2 - N}.$$

The sum of differences is normalized by the largest possible difference  $N^2 - N$  between a matrix with all residues in one cluster and a matrix with each residue in a separate cluster. For each threshold, the difference between the matrix obtained using this threshold and the matrices using the two neighboring thresholds was calculated. The average of the two differences is shown in Supplementary Figure 2, along with the number of intersubunit contact clusters obtained for each threshold. Notably, for thresholds above 0.2 the number of clusters only fluctuates between 15 and 17 and also the difference of clustering is low. This result shows that the clustering protocol is robust with respect to the choice of the threshold. For further analysis, the clustering of contacts obtained with a threshold of 0.3 was chosen, because this threshold is in a region with constant numbers of clusters and with low difference of clustering to neighboring thresholds.

### Contact Pattern Comparison

Contacts between the two ribosomal subunits were extracted from MD simulation of the ribosome in pre- (pre1a–pre5b) and post-translocation (post1–post4)

states. To estimate how the trajectory length of 100 ns per state, influences the identification of intersubunit contacts, the sets of contacting residues extracted from different simulations was compared. To that aim, first, all the stable contacts with an occupancy larger than a certain threshold were recorded. The contact overlap between two simulation was defined as the ratio of the number of contacts found in both simulations by the number of contacts found in the first simulation. First, two sets of contacts obtained from two independent 100-ns simulations of the pre1a state were compared (Supplementary Figure 3, red line). Next, the contact overlaps between the first pre1a simulation and all other simulations (pre2–post4) was averaged (green line). The results suggests that the length of the simulations allows to capture differences in contact patterns between different states, despite the fact that the simulations are not fully converged.

All the contacts of 50S protein L5 with 30S proteins S13 and S19 (clusters 4 and 13, B1 bridges) extracted from our simulations were compared with contacts observed in X-ray structures of the ribosome in various states of rotation (1; 2; 3; 4). Two residues in the X-ray structures were considered to be in contact if the smallest distance between any of their atoms was below 5 Å. The contacts were compared with contacts from our simulations that have an occupancy above 50 %. The identified contacts common in X-ray structure and simulation are shown in Supplementary Table 26 for the state with the highest number of common contacts along with head and body rotation angles for the X-ray structure and for the simulation of the corresponding state.

### Interaction Enthalpy and Conformational Entropy

To check if the enthalpy between 30S and 50S residues of an intersubunit contact cluster is a reasonable measure for its relative contribution to 70S stability, we compared the changes of enthalpy and conformational entropy along the dominant mode of motion for each cluster. Since some of the clusters interact with other clusters (compare Figure 1d) for the following analysis the interacting clusters were merged (1+5, 4+13, 6+11, 7+10).

To obtain the dominant mode of motion, a principal component analysis (PCA) (5) was performed on the heavy atoms of the cluster residues involved

in contacts with an occupancy of at least 50 % in any translocation intermediate simulation (compare Table 1). To that aim, from each frame and each trajectory (pre1a–post4), the coordinates of these atoms were extracted, rigid-body fitted and concatenated. The first eigenvector resulting from a PCA of this concatenated trajectory describes the dominant mode of motion.

To describe changes along the dominant mode of motion, the frames were sorted based on their projection onto the first eigenvector into 50 equally sized bins.

To measure the change of enthalpy along this motion, first, for each frame the interaction enthalpy  $H_i$  between the 50S and 30S residues was calculated as described in the Methods section. Then, for each bin the mean and standard deviation of the interaction enthalpy of all the frames included in this bin was calculated.

To estimate change of the conformational entropy  $S_c$  along the motion, for all the bins, Schlitter’s formula (6) was applied to all frames included in this bin using the program `g_anaeig` from the GROMACS suite (7).

Finally, for each bin a free-energy estimate  $G_e$ , which does not contain solvent contributions, was calculated:

$$G_e = H_i - TS_c,$$

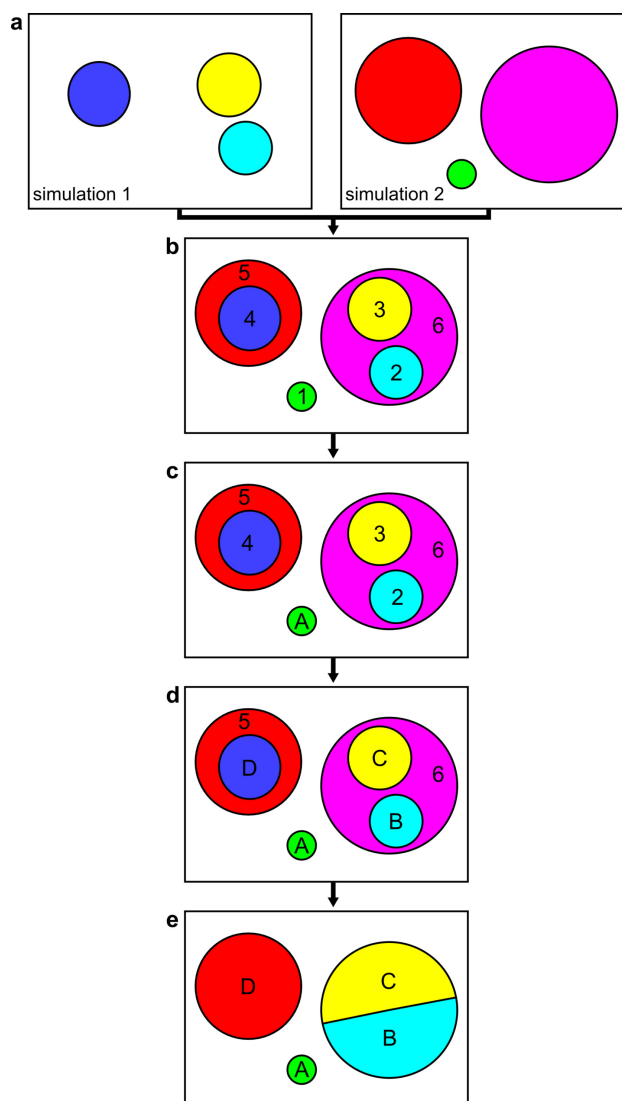
where  $T = 300$  K is the temperature used in the simulations. A correlation coefficient of 0.82 is obtained when comparing  $G_e$  and  $H_i$  values for all bins of all clusters. This high correlation suggests that the enthalpy follows the same trend as the free energy and therefore is a good measure of the relative contributions of the different intersubunit contact clusters in the different states.

## Supplementary Results

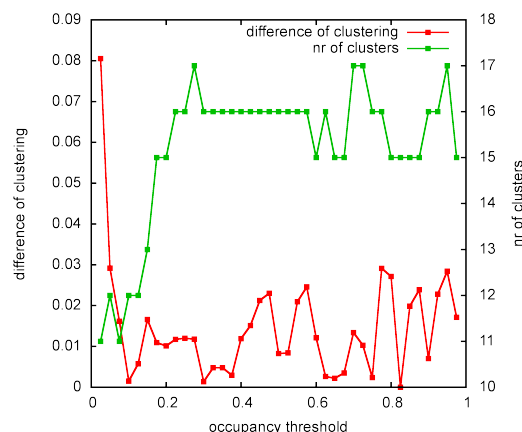
### Contact Pattern Comparison

For all intersubunit contact clusters, Supplementary Tables 1–15 list the frequencies of residue-residue contacts between the 30S and the 50S subunit for each intermediate state. Supplementary Tables 16–25 list residue-residue contact frequencies of contacts between residues from different contact clusters. The gray-scale level of the cells indicates the frequency of atom-atom contacts corresponding to the residue pairs, white

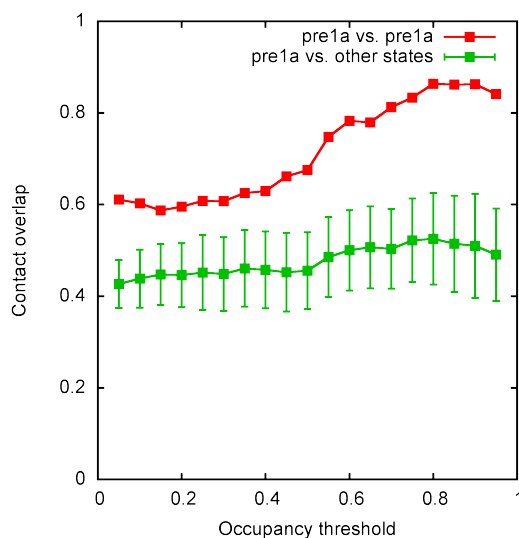
## Supplementary Figures



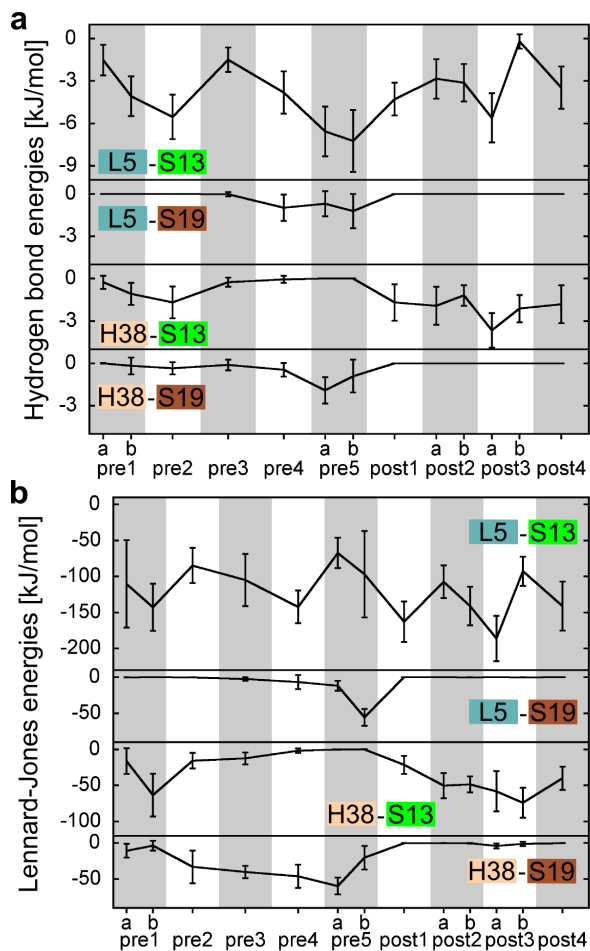
**Supplementary Figure 1:** Scheme of the clustering protocol applied to intersubunit contacts obtained from the simulations. (a) Clusters of contacts are represented by colored circles (from simulation 1: blue, orange, cyan; from simulation 2: red, magenta, green). (b) Clusters of different simulations were combined and sorted by size (small to large). (c) The smallest cluster was labeled new cluster A, and excluded from subsequent clustering. (d) With the remaining sorted clusters, this procedure was repeated until all clusters are assigned (clusters B, C, D). (e) Contacts from the remaining clusters were distributed to the closest new cluster, where distance was defined as the number of contacts which connect the residue to the cluster.



**Supplementary Figure 2:** Dependence of clustering of intersubunit contact on the threshold of contact occupancy. The difference of clustering is obtained by comparing the clustering at a specific threshold  $t$  with the clusterings at the two neighboring thresholds  $t - 0.025$  and  $t + 0.025$  (red line). For each threshold, the obtained number of contact clusters is shown (green line).



**Supplementary Figure 3:** Overlap of contacts contacts between two independent simulations of the pre1a state (red line) and between one pre1a simulation and the simulations of all other states (green, line, bars indicate standard deviation). The overlap was calculated for contacts with occupancies above different occupancy thresholds.



**Supplementary Figure 4:** Interactions of protein L5 and rRNA helix H38 with proteins S13 and S19 which contribute to intersubunit contact clusters 4 and 13 (B1 bridges). For each state and each pair of ribosomal parts the average hydrogen bond energies (a) and Lennard-Jones energies (b) are shown. Bars indicate standard deviation.

## Supplementary Tables

**Supplementary Table 1:** Contacting residues for cluster 1.

| 30S residue | 50S residue | 1a | 1b | 2 | pre |  | 3 | 4 | 5a | 5b | post |    |    |    |    |   |
|-------------|-------------|----|----|---|-----|--|---|---|----|----|------|----|----|----|----|---|
|             |             |    |    |   |     |  |   |   |    |    | 1    | 2a | 2b | 3a | 3b | 4 |
| h44 A1418   | H71 G1948   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 A1418   | H71 G1959   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 G1419   | H71 G1949   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 U1420   | H71 G1950   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 A1483   | H71 G1948   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 A1483   | H71 G1959   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 C1484   | H71 A1960   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 A1418   | H71 C1958   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 G1422   | L14 P48     |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 A1483   | H71 A1960   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h14 C339    | L14 R98     |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 G1421   | L14 K54     |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 A1483   | H71 C1947   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 U1472   | L14 R17     |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 U1485   | H71 C1961   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h14 C339    | L14 N13     |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 G1419   | H71 G1950   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 C1484   | H71 C1961   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 U1471   | L14 R17     |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 U1472   | L14 R18     |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h14 U340    | L14 R98     |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h14 U340    | L14 N13     |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h13 A338    | L14 R98     |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 U1420   | L14 K54     |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 A1418   | H71 G1949   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 U1420   | H71 G1949   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h14 C339    | L14 S14     |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 G1421   | L14 P48     |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 G1423   | L14 R49     |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 G1422   | L14 I47     |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h13 A338    | L14 N13     |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 G1419   | H64 C1768   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 G1422   | L14 R49     |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 G1423   | L14 P48     |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h14 U340    | L14 T97     |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 G1422   | L14 R17     |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h13 A338    | L14 F100    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h14 C339    | L14 T97     |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h13 A338    | L14 S14     |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 U1471   | L14 R49     |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h13 G337    | L14 R98     |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h14 C339    | L14 K51     |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h14 U340    | L14 K51     |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h14 C339    | L14 R49     |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h13 A338    | L14 R49     |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h13 A338    | L14 R17     |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 G1422   | L14 K54     |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h14 C339    | L14 R17     |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 C1484   | H71 C1947   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 U1485   | H71 A1960   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 C1484   | H71 G1959   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |

**Supplementary Table 2:** Contacting residues for cluster 2.

| 30S residue | 50S residue | 1a | 1b | 2 | pre |  | 3 | 4 | 5a | 5b | post |    |    |    |    |   |
|-------------|-------------|----|----|---|-----|--|---|---|----|----|------|----|----|----|----|---|
|             |             |    |    |   |     |  |   |   |    |    | 1    | 2a | 2b | 3a | 3b | 4 |
| h44 G1494   | H69 A1912   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 G1494   | H69 A1913   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 U1495   | H69 A1912   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 C1496   | H69 A1919   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 A1408   | H69 A1912   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 C1409   | H69 A1916   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h45 G1517   | H69 A1919   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 C1407   | H69 A1912   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 U1495   | H69 A1919   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 C1496   | H69 C1920   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 C1407   | H69 A1919   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 A1492   | H69 A1913   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 A1493   | H69 A1913   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 A1408   | H69 A1916   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 C1409   | H69 U1915   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 A1408   | H69 A1913   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 C1409   | H69 C1914   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 U1406   | H69 A1919   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h45 G1517   | H69 C1920   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 G1497   | H69 C1920   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h45 G1516   | H71 U1931   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 G1497   | H69 G1921   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 A1408   | H69 U1917   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 U1495   | H69 A1913   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 U1495   | H69 U1911   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 A1410   | H69 C1914   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 A1408   | H69 U1915   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 A1492   | H69 C1914   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 G1494   | H69 A1919   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 U1495   | H69 C1920   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h45 G1517   | H71 A1932   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 C1496   | H69 G1921   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 A1408   | H69 A1919   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h45 G1517   | H71 U1931   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h44 C1409   | H69 A1913   |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |

**Supplementary Table 3:** Contacting residues for cluster 3.

| 30S residue | 50S residue | 1a | 1b | 2 | pre |  | 3 | 4 | 5a | 5b | post |    |    |    |    |   |
|-------------|-------------|----|----|---|-----|--|---|---|----|----|------|----|----|----|----|---|
|             |             |    |    |   |     |  |   |   |    |    | 1    | 2a | 2b | 3a | 3b | 4 |
| S15 V59     | H34 A715    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| S15 L55     | H34 A715    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| S15 R62     | H34 A715    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| S15 Q39     | H34 A715    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| S15 I35     | H34 A715    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| S15 R88     | H34 A716    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| S15 L56     | H34 A715    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| S15 R52     | H34 A715    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| S15 R63     | H34 U714    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| S15 R87     | H34 U714    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| S15 R87     | H34 A716    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| S15 R63     | H34 A715    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| S15 R87     | H34 G713    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| S15 Q39     | H34 A716    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| S15 R88     | H34 A715    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| S15 R88     | H34 G712    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| S15 R88     | H34 G713    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| S15 R87     | H34 A715    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| S15 R88     | H34 U714    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h20 G763    | H34 A716    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h20 U762    | H34 A715    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| S15 R87     | H34 G712    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| h20 G763    | H34 A715    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| S15 R87     | H34 G711    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| S15 R52     | H34 A716    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| S15 F42     | H34 A715    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| S15 F42     | H34 A716    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| S15 R88     | H34 C717    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| S15 L55     | H34 A716    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| S15 R52     | H34 C717    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| S15 A43     | H34 A716    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| S15 K46     | H34 A716    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |
| S15 K46     | H34 C717    |    |    |   |     |  |   |   |    |    |      |    |    |    |    |   |

**Supplementary Table 4:** Contacting residues for cluster 4.

| 30S residue | 50S residue | 1a | 1b | 2 | 3 | 4 | 5a | 5b | 1 | 2a | 2b | 3a | 3b | 4 |
|-------------|-------------|----|----|---|---|---|----|----|---|----|----|----|----|---|
| S13 R70     | L5 D112     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 R69     | L5 R111     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 R70     | L5 F113     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 R70     | L5 R111     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 G66     | L5 D112     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 G66     | L5 R111     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 R70     | L5 R114     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 M74     | L5 R114     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 K77     | H38 C889    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 V59     | L5 V107     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 V59     | L5 I110     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 A60     | L5 I110     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 A60     | L5 F113     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 S73     | H38 C888    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 R70     | H38 C888    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 M74     | H38 C888    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 V59     | L5 F113     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 A60     | L5 R114     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 F62     | L5 R114     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 V63     | L5 R114     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 R69     | H38 C888    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 K77     | H38 C888    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 R92     | H38 C888    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 D67     | L5 R111     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 R92     | H38 C889    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S19 R80     | H38 U887    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S19 R80     | H38 C888    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 R69     | L5 E133     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 R91     | H38 C889    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 V64     | L5 R111     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 A60     | L5 R111     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S19 F60     | H38 U887    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 I76     | L5 R111     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 L79     | L5 R111     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 M80     | L5 D112     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 D81     | L5 R114     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 K77     | L5 R111     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 R78     | L5 R111     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 M80     | L5 R111     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S19 F60     | H38 C888    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 D81     | H38 C888    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 R78     | L5 F113     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 M80     | L5 F113     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 M80     | L5 R114     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S19 T62     | H38 C888    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 M80     | H38 C888    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 K77     | L5 E133     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 R70     | L5 R109     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 G66     | L5 I136     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 D67     | L5 I136     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 R70     | L5 I136     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S19 V57     | H38 C888    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S19 P75     | H38 C888    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S19 P58     | H38 C888    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 D67     | L5 R109     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 R69     | L5 R147     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S19 D63     | L5 R114     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 K77     | L5 R109     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 D81     | L5 R111     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 M74     | L5 R109     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S19 Q55     | H38 C888    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 R78     | L5 D112     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S19 V59     | H38 C888    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S19 T47     | H38 C888    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 R70     | L5 D146     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S19 K28     | H38 U887    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S19 E64     | L5 R114     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 S73     | L5 D146     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S19 M65     | L5 R111     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S19 E64     | L5 R111     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 K77     | L5 V148     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 E71     | L5 D146     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 K77     | L5 D146     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S19 H68     | L5 R111     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 R91     | H38 U887    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 Y22     | L5 R111     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 R69     | L5 R109     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 G66     | L5 P108     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 R91     | H38 C888    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 G66     | L5 I110     |    |    |   |   |   |    |    |   |    |    |    |    |   |

**Continued Supplementary Table 4:** Contacting residues for cluster 4.

| 30S residue | 50S residue | 1a | 1b | 2 | 3 | 4 | 5a | 5b | 1 | 2a | 2b | 3a | 3b | 4 |
|-------------|-------------|----|----|---|---|---|----|----|---|----|----|----|----|---|
| S13 S73     | L5 R114     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 S73     | L5 D112     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 D67     | L5 R114     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 R91     | H38 G883    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 R91     | H38 U884    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 M74     | H38 C889    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 Y22     | L5 E133     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 D57     | L5 R109     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 R70     | L5 L116     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 R78     | H38 U887    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| S13 A60     | L5 D112     |    |    |   |   |   |    |    |   |    |    |    |    |   |

**Supplementary Table 5:** Contacting residues for cluster 5.

| 30S residue | 50S residue | 1a | 1b | 2 | 3 | 4 | 5a | 5b | 1 | 2a | 2b | 3a | 3b | 4 |
|-------------|-------------|----|----|---|---|---|----|----|---|----|----|----|----|---|
| h14 G346    | L19 R38     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 G346    | L19 K36     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 C345    | L14 S117    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 C345    | L14 K114    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 C345    | L19 K36     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 C342    | L14 L118    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 C341    | L14 L118    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 C345    | L14 P120    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 G347    | L19 R38     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 C345    | L14 M113    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 U343    | L14 L118    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 G347    | L14 L118    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 G347    | L14 A119    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 G346    | L14 E121    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 G346    | L14 R108    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 G346    | L14 R105    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 G347    | L14 E121    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 G347    | L14 P120    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 C345    | L14 R108    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h8 G159     | L19 R38     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 C345    | L14 I116    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 C345    | L19 S35     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h8 G158     | L19 R38     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 U343    | L14 S117    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 G347    | L14 R105    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h8 G159     | L19 K36     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 C342    | L14 A119    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h8 A160     | L19 K36     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 G347    | L14 V122    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 C341    | L14 A119    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 C345    | L14 L118    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h8 G158     | L19 K36     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 G346    | L14 S117    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h8 G158     | L19 K37     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h8 G159     | L19 K37     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 A344    | L19 K36     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 U343    | L14 K114    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 C345    | L19 R38     |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 C345    | L14 R105    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 C345    | L14 V122    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 A344    | L14 S117    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 G346    | L14 L118    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 G347    | L14 X123    |    |    |   |   |   |    |    |   |    |    |    |    |   |
| h14 C345    | L14 E121    |    |    |   |   |   |    |    |   |    |    |    |    |   |

**Supplementary Table 6:** Contacting residues for cluster 6.

| 30S residue | 50S residue | pre |    |   |   |   | post |    |   |    |    |    |    |   |
|-------------|-------------|-----|----|---|---|---|------|----|---|----|----|----|----|---|
|             |             | 1a  | 1b | 2 | 3 | 4 | 5a   | 5b | 1 | 2a | 2b | 3a | 3b | 4 |
| S6 F80      | L2 P135     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S6 D82      | L2 R166     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S6 R24      | L2 D120     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S6 F80      | L2 A121     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S6 R79      | L2 P135     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S6 F80      | L2 L191     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S6 T76      | L2 I123     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S6 F80      | L2 I134     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S6 F80      | L2 L129     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S6 F80      | L2 N133     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S6 F80      | L2 S138     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S6 D82      | L2 N133     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S6 E73      | L2 I123     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S6 R24      | L2 V119     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S6 N81      | L2 N133     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S6 D82      | L2 R132     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h23 G713    | L2 R174     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S6 D82      | L2 P135     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h23 A712    | L2 V164     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S6 T77      | L2 I123     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h23 A712    | L2 A165     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S6 Q14      | L2 R132     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S6 F80      | L2 I123     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h23 G711    | L2 R268     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h23 A712    | L2 R174     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h23 A712    | L2 R268     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S6 N81      | L2 P135     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S6 D82      | L2 V136     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h23 G711    | L2 V136     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h23 G713    | L2 Q162     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h23 G711    | L2 V164     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h23 G711    | L2 A165     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h23 A712    | L2 V136     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S6 E73      | L2 K124     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h23 A712    | L2 Q162     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h23 G711    | L2 G137     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S6 F80      | L2 P125     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h23 G710    | L2 A165     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h23 G710    | L2 V136     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S6 D13      | L9 K89      | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S6 R24      | L9 D86      | █   |    |   |   |   |      |    | █ |    |    |    |    |   |

**Supplementary Table 7:** Contacting residues for cluster 7.

| 30S residue | 50S residue | pre |    |   |   |   | post |    |   |    |    |    |    |   |
|-------------|-------------|-----|----|---|---|---|------|----|---|----|----|----|----|---|
|             |             | 1a  | 1b | 2 | 3 | 4 | 5a   | 5b | 1 | 2a | 2b | 3a | 3b | 4 |
| h44 A1429   | H62 G1703   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 U1464   | L19 R108    | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 A1431   | L19 T103    | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 A1429   | H62 C1704   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 A1430   | L19 T103    | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 A1431   | L19 G104    | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 U1464   | L19 K105    | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 A1431   | L19 N65     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 A1433   | L19 N65     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 G1432   | L19 T103    | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 A1468   | L19 N65     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 A1465   | L19 K105    | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 G1432   | L19 N65     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 A1431   | L19 K105    | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 G1432   | L19 K105    | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 A1465   | L19 R108    | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 G1432   | L19 G104    | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 A1430   | H62 C1704   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 U1464   | H63 U1751   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |

**Supplementary Table 8:** Contacting residues for cluster 8.

| 30S residue | 50S residue | pre |    |   |   |   | post |    |   |    |    |    |    |   |
|-------------|-------------|-----|----|---|---|---|------|----|---|----|----|----|----|---|
|             |             | 1a  | 1b | 2 | 3 | 4 | 5a   | 5b | 1 | 2a | 2b | 3a | 3b | 4 |
| S11 R12     | H78 G2144   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S11 R12     | H78 C2143   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S11 K13     | H78 C2146   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S11 R12     | H78 C2145   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S11 R12     | H78 G2141   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S11 R12     | H78 A2142   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S11 K13     | H78 G2140   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S11 K74     | H78 G2141   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S7 R142     | H78 A2147   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S11 K74     | H78 G2140   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S11 E75     | H78 G2140   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S7 K130     | H76 G2116   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S7 R142     | H76 A2114   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S7 R110     | H78 U2167   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S7 K130     | H78 U2166   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S7 H141     | H76 G2115   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S7 R142     | H76 G2115   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S7 K148     | H78 A2147   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S11 Q37     | L9 T125     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S7 H141     | H76 G2116   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S7 K135     | H78 C2146   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S11 K74     | H78 C2146   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| S11 K13     | H78 G2144   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |

**Supplementary Table 9:** Contacting residues for cluster 9.

| 30S residue | 50S residue | pre |    |   |   |   | post |    |   |    |    |    |    |   |
|-------------|-------------|-----|----|---|---|---|------|----|---|----|----|----|----|---|
|             |             | 1a  | 1b | 2 | 3 | 4 | 5a   | 5b | 1 | 2a | 2b | 3a | 3b | 4 |
| h44 G1475   | H62 A1689   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 G1475   | H62 A1700   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 A1476   | H62 A1690   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 A1476   | H62 A1689   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 U1474   | H62 A1700   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 U1474   | H62 A1701   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 G1473   | H62 G1702   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 U1474   | H62 G1702   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 G1475   | H62 A1701   | █   |    |   |   |   |      |    | █ |    |    |    |    |   |

**Supplementary Table 10:** Contacting residues for cluster 10.

| 30S residue | 50S residue | pre |    |   |   |   | post |    |   |    |    |    |    |   |
|-------------|-------------|-----|----|---|---|---|------|----|---|----|----|----|----|---|
|             |             | 1a  | 1b | 2 | 3 | 4 | 5a   | 5b | 1 | 2a | 2b | 3a | 3b | 4 |
| h44 A1441   | L19 L113    | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 G1442   | L19 L113    | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 A1441   | L19 E111    | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 C1462   | L19 E111    | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 C1462   | L19 R87     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 G1461   | L19 R87     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 G1442   | L19 N114    | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 G1442   | L19 K86     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 G1442   | L19 R87     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 A1441   | L19 R112    | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 A1441   | L19 N114    | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 A1441   | L19 R87     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 A1441   | L19 K86     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 U1440   | L19 R87     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 U1440   | L19 L113    | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 C1443   | L19 K86     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 U1463   | L19 S64     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 C1462   | L19 S64     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 G1439   | L19 R87     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 G1461   | L19 L113    | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 C1443   | L19 N114    | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 G1442   | L19 R112    | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 A1441   | L19 K110    | █   |    |   |   |   |      |    | █ |    |    |    |    |   |
| h44 U1463   | L19 R87     | █   |    |   |   |   |      |    | █ |    |    |    |    |   |



**Supplementary Table 11:** Contacting residues for cluster 11.

| 30S residue | 50S residue | 1a | 1b | 2 | pre |   |    | post |   |    |    |    |    |   |
|-------------|-------------|----|----|---|-----|---|----|------|---|----|----|----|----|---|
|             |             |    |    |   | 3   | 4 | 5a | 5b   | 1 | 2a | 2b | 3a | 3b | 4 |
| h23 A702    | H68 G1846   | █  | █  | █ |     |   |    |      | █ | █  | █  | █  | █  | █ |
| h23 A702    | H68 C1895   | █  | █  | █ |     |   |    |      | █ | █  | █  | █  | █  | █ |
| h23 A702    | H68 A1848   | █  | █  | █ |     |   |    |      | █ | █  | █  | █  | █  | █ |
| h23 A702    | H68 A1847   | █  | █  | █ |     |   |    |      | █ | █  | █  | █  | █  | █ |
| h23 A681    | L2 V267     |    |    |   |     |   |    |      |   |    |    |    |    |   |
| h23 C680    | L2 V267     |    |    |   |     |   |    |      |   |    |    |    |    |   |
| h23 C680    | L2 M180     |    |    |   |     |   |    |      |   |    |    |    |    |   |
| h23 A702    | H76 U2099   |    |    |   |     |   |    |      |   |    |    |    |    |   |
| h23 A702    | H76 G2100   |    |    |   |     |   |    |      |   |    |    |    |    |   |
| h23 A681    | L2 T172     |    |    |   |     |   |    |      |   |    |    |    |    |   |
| h23 G682    | L2 K182     |    |    |   |     |   |    |      |   |    |    |    |    |   |
| h23 A681    | L2 K182     |    |    |   |     |   |    |      |   |    |    |    |    |   |
| h23 A681    | L2 M180     |    |    |   |     |   |    |      |   |    |    |    |    |   |
| h23 A702    | H76 U2192   |    |    |   |     |   |    |      |   |    |    |    |    |   |
| h23 A702    | H76 U2098   |    |    |   |     |   |    |      |   |    |    |    |    |   |
| h23 A702    | H76 A2191   |    |    |   |     |   |    |      |   |    |    |    |    |   |
| h23 G703    | H76 U2098   |    |    |   |     |   |    |      |   |    |    |    |    |   |
| h23 A702    | H76 G2193   |    |    |   |     |   |    |      |   |    |    |    |    |   |
| S6 K53      | L2 D167     |    |    |   |     |   |    |      |   |    |    |    |    |   |

**Supplementary Table 12:** Contacting residues for cluster 12.

| 30S residue | 50S residue | 1a | 1b | 2 | pre |   |    | post |   |    |    |    |    |   |
|-------------|-------------|----|----|---|-----|---|----|------|---|----|----|----|----|---|
|             |             |    |    |   | 3   | 4 | 5a | 5b   | 1 | 2a | 2b | 3a | 3b | 4 |
| h24 G773    | L2 M200     | █  | █  | █ |     |   |    |      | █ | █  | █  | █  | █  | █ |
| h24 U772    | L2 L201     | █  | █  | █ |     |   |    |      | █ | █  | █  | █  | █  | █ |
| h24 G774    | L2 R176     | █  | █  | █ |     |   |    |      | █ | █  | █  | █  | █  | █ |
| h24 U772    | L2 M200     | █  | █  | █ |     |   |    |      | █ | █  | █  | █  | █  | █ |
| h24 G774    | L2 Y160     | █  | █  | █ |     |   |    |      | █ | █  | █  | █  | █  | █ |
| h24 G775    | L2 R176     | █  | █  | █ |     |   |    |      | █ | █  | █  | █  | █  | █ |
| h24 G773    | L2 L201     | █  | █  | █ |     |   |    |      | █ | █  | █  | █  | █  | █ |
| h24 G775    | L2 Y160     | █  | █  | █ |     |   |    |      | █ | █  | █  | █  | █  | █ |
| h24 G774    | H66 U1820   |    |    |   |     |   |    |      |   |    |    |    |    |   |
| h24 G774    | L2 M200     |    |    |   |     |   |    |      |   |    |    |    |    |   |
| h24 U772    | L2 K4       |    |    |   |     |   |    |      |   |    |    |    |    |   |
| h24 U772    | L2 A1       |    |    |   |     |   |    |      |   |    |    |    |    |   |
| h24 G773    | L2 K4       |    |    |   |     |   |    |      |   |    |    |    |    |   |
| h24 C808    | L2 A1       |    |    |   |     |   |    |      |   |    |    |    |    |   |

**Supplementary Table 13:** Contacting residues for cluster 13.

| 30S residue | 50S residue | 1a | 1b | 2 | pre |   |    | post |   |    |    |    |    |   |
|-------------|-------------|----|----|---|-----|---|----|------|---|----|----|----|----|---|
|             |             |    |    |   | 3   | 4 | 5a | 5b   | 1 | 2a | 2b | 3a | 3b | 4 |
| S13 R2      | L5 D143     | █  | █  | █ |     |   |    |      | █ | █  | █  | █  | █  | █ |
| S13 I8      | L5 V145     | █  | █  | █ |     |   |    |      | █ | █  | █  | █  | █  | █ |
| S13 R2      | L5 Y142     | █  | █  | █ |     |   |    |      | █ | █  | █  | █  | █  | █ |
| S13 E65     | L5 I135     |    |    |   |     |   |    |      |   |    |    |    |    |   |
| S13 E65     | L5 Y142     |    |    |   |     |   |    |      |   |    |    |    |    |   |
| S13 R2      | L5 D141     |    |    |   |     |   |    |      |   |    |    |    |    |   |
| S13 I6      | L5 K144     |    |    |   |     |   |    |      |   |    |    |    |    |   |
| S13 I8      | L5 D143     |    |    |   |     |   |    |      |   |    |    |    |    |   |
| S13 I8      | L5 Y142     |    |    |   |     |   |    |      |   |    |    |    |    |   |
| S13 P9      | L5 D143     |    |    |   |     |   |    |      |   |    |    |    |    |   |
| S13 I8      | L5 P138     |    |    |   |     |   |    |      |   |    |    |    |    |   |
| S13 I8      | L5 K144     |    |    |   |     |   |    |      |   |    |    |    |    |   |
| S13 A1      | L5 Y142     |    |    |   |     |   |    |      |   |    |    |    |    |   |
| S13 I3      | L5 P138     |    |    |   |     |   |    |      |   |    |    |    |    |   |
| S13 E49     | L5 P138     |    |    |   |     |   |    |      |   |    |    |    |    |   |
| S13 R56     | L5 I135     |    |    |   |     |   |    |      |   |    |    |    |    |   |
| S13 P9      | L5 Y142     |    |    |   |     |   |    |      |   |    |    |    |    |   |
| S13 L47     | L5 Y142     |    |    |   |     |   |    |      |   |    |    |    |    |   |
| S13 L47     | L5 D141     |    |    |   |     |   |    |      |   |    |    |    |    |   |

**Supplementary Table 14:** Contacting residues for cluster 14.

| 30S residue | 50S residue | 1a | 1b | 2 | pre |   |    | post |   |    |    |    |    |   |
|-------------|-------------|----|----|---|-----|---|----|------|---|----|----|----|----|---|
|             |             |    |    |   | 3   | 4 | 5a | 5b   | 1 | 2a | 2b | 3a | 3b | 4 |
| h24 A784    | H68 C1837   | █  | █  | █ |     |   |    |      | █ | █  | █  | █  | █  | █ |
| h24 C783    | H68 C1836   | █  | █  | █ |     |   |    |      | █ | █  | █  | █  | █  | █ |
| h24 A784    | H68 C1836   | █  | █  | █ |     |   |    |      | █ | █  | █  | █  | █  | █ |
| h24 C783    | H68 G1835   | █  | █  | █ |     |   |    |      | █ | █  | █  | █  | █  | █ |

**Supplementary Table 15:** Contacting residues for cluster 15.

| 30S residue | 50S residue | 1a | 1b | 2 | pre |   |    | post |   |    |    |    |    |   |
|-------------|-------------|----|----|---|-----|---|----|------|---|----|----|----|----|---|
|             |             |    |    |   | 3   | 4 | 5a | 5b   | 1 | 2a | 2b | 3a | 3b | 4 |
| h27 C899    | H67 G1831   | █  | █  | █ |     |   |    |      | █ | █  | █  | █  | █  | █ |
| h27 A900    | H67 C1832   | █  | █  | █ |     |   |    |      | █ | █  | █  | █  | █  | █ |
| h27 A900    | H67 G1831   | █  | █  | █ |     |   |    |      | █ | █  | █  | █  | █  | █ |
| h27 C899    | H67 C1830   | █  | █  | █ |     |   |    |      | █ | █  | █  | █  | █  | █ |
| h27 C899    | H62 U1693   |    |    |   |     |   |    |      |   |    |    |    |    |   |

**Supplementary Table 16:** Contacting residues for cluster 4 (30S) and cluster 13 (50S).

| 30S residue | 50S residue | 1a | 1b | 2 | pre |   |    | post |   |    |    |    |    |   |
|-------------|-------------|----|----|---|-----|---|----|------|---|----|----|----|----|---|
|             |             |    |    |   | 3   | 4 | 5a | 5b   | 1 | 2a | 2b | 3a | 3b | 4 |
| S13 R69     | L5 P138     | █  | █  | █ |     |   |    |      | █ | █  | █  | █  | █  | █ |
| S13 G66     | L5 Y142     | █  | █  | █ |     |   |    |      | █ | █  | █  | █  | █  | █ |
| S13 D67     | L5 Y142     | █  | █  | █ |     |   |    |      | █ | █  | █  | █  | █  | █ |
| S13 R70     | L5 I135     |    |    |   |     |   |    |      |   |    |    |    |    |   |
| S13 R70     | L5 D143     |    |    |   |     |   |    |      |   |    |    |    |    |   |
| S13 R70     | L5 K144     |    |    |   |     |   |    |      |   |    |    |    |    |   |
| S13 S73     | L5 D143     |    |    |   |     |   |    |      |   |    |    |    |    |   |

**Supplementary Table 17:** Contacting residues for cluster 5 (30S) and cluster 1 (50S).

| 30S residue | 50S residue | 1a | 1b | 2 | pre |   |    | post |   |    |    |    |    |   |
|-------------|-------------|----|----|---|-----|---|----|------|---|----|----|----|----|---|
|             |             |    |    |   | 3   | 4 | 5a | 5b   | 1 | 2a | 2b | 3a | 3b | 4 |
| h14 C341    | L14 R98     | █  | █  | █ |     |   |    |      | █ | █  | █  | █  | █  | █ |
| h14 G347    | L14 R98     | █  | █  | █ |     |   |    |      | █ | █  | █  | █  | █  | █ |
| h14 G348    | L14 R98     | █  | █  | █ |     |   |    |      | █ | █  | █  | █  | █  | █ |
| h14 C342    | L14 R98     | █  | █  | █ |     |   |    |      | █ | █  | █  | █  | █  | █ |

**Supplementary Table 18:** Contacting residues for cluster 8 (30S) and cluster 6 (50S).

| 30S residue | 50S residue | 1a | 1b | 2 | pre |   |    | post |   |    |    |    |    |   |
|-------------|-------------|----|----|---|-----|---|----|------|---|----|----|----|----|---|
|             |             |    |    |   | 3   | 4 | 5a | 5b   | 1 | 2a | 2b | 3a | 3b | 4 |
| h23 G683    | L9 R123     | █  | █  | █ |     |   |    |      | █ | █  | █  | █  | █  | █ |

**Supplementary Table 19:** Contacting residues for cluster 9 (30S) and cluster 1 (50S).

| 30S residue | 50S residue | 1a | 1b | 2 | pre |   |    | post |   |    |    |    |    |   |
|-------------|-------------|----|----|---|-----|---|----|------|---|----|----|----|----|---|
|             |             |    |    |   | 3   | 4 | 5a | 5b   | 1 | 2a | 2b | 3a | 3b | 4 |
| h44 G1473   | L14 R18     | █  | █  | █ |     |   |    |      | █ | █  | █  | █  | █  | █ |

**Supplementary Table 20:** Contacting residues for cluster 9 (30S) and cluster 7 (50S).

| 30S residue | 50S residue | 1a | 1b | 2 | pre |  | 3 | 4 | 5a | 5b | 1 | 2a | post |  |  |  |  |  |  |  |
|-------------|-------------|----|----|---|-----|--|---|---|----|----|---|----|------|--|--|--|--|--|--|--|
| h44 G1473   | H62 G1703   |    |    |   |     |  |   |   |    |    |   |    |      |  |  |  |  |  |  |  |

**Supplementary Table 21:** Contacting residues for cluster 10 (30S) and cluster 7 (50S).

| 30S residue | 50S residue | 1a | 1b | 2 | pre |  | 3 | 4 | 5a | 5b | 1 | 2a | post |  |  |  |  |  |  |  |
|-------------|-------------|----|----|---|-----|--|---|---|----|----|---|----|------|--|--|--|--|--|--|--|
| h44 U1463   | L19 R108    |    |    |   |     |  |   |   |    |    |   |    |      |  |  |  |  |  |  |  |
| h44 U1463   | L19 K105    |    |    |   |     |  |   |   |    |    |   |    |      |  |  |  |  |  |  |  |

**Supplementary Table 25:** Contacting residues for cluster 13 (30S) and cluster 4 (50S).

**Supplementary Table 22:** Contacting residues for cluster 11 (30S) and cluster 6 (50S).

| 30S residue | 50S residue | 1a | 1b | 2 | pre |  | 3 | 4 | 5a | 5b | 1 | 2a | post |  |  |  |  |  |  |  |
|-------------|-------------|----|----|---|-----|--|---|---|----|----|---|----|------|--|--|--|--|--|--|--|
| h24 G776    | L2 R174     |    |    |   |     |  |   |   |    |    |   |    |      |  |  |  |  |  |  |  |
| h23 C680    | L2 R268     |    |    |   |     |  |   |   |    |    |   |    |      |  |  |  |  |  |  |  |
| h23 C679    | L2 R268     |    |    |   |     |  |   |   |    |    |   |    |      |  |  |  |  |  |  |  |
| h23 A681    | L2 A165     |    |    |   |     |  |   |   |    |    |   |    |      |  |  |  |  |  |  |  |
| h23 A681    | L2 R268     |    |    |   |     |  |   |   |    |    |   |    |      |  |  |  |  |  |  |  |
| h23 C680    | L2 V164     |    |    |   |     |  |   |   |    |    |   |    |      |  |  |  |  |  |  |  |
| h23 A681    | L2 V164     |    |    |   |     |  |   |   |    |    |   |    |      |  |  |  |  |  |  |  |

| 30S residue | 50S residue | 1a | 1b | 2 | pre |  | 3 | 4 | 5a | 5b | 1 | 2a | post |  |  |  |  |  |  |  |
|-------------|-------------|----|----|---|-----|--|---|---|----|----|---|----|------|--|--|--|--|--|--|--|
| S13 E65     | L5 R111     |    |    |   |     |  |   |   |    |    |   |    |      |  |  |  |  |  |  |  |
| S13 E65     | L5 R109     |    |    |   |     |  |   |   |    |    |   |    |      |  |  |  |  |  |  |  |
| S13 I6      | L5 R109     |    |    |   |     |  |   |   |    |    |   |    |      |  |  |  |  |  |  |  |
| S13 R2      | L5 P108     |    |    |   |     |  |   |   |    |    |   |    |      |  |  |  |  |  |  |  |
| S13 I3      | L5 V107     |    |    |   |     |  |   |   |    |    |   |    |      |  |  |  |  |  |  |  |
| S13 I3      | L5 P108     |    |    |   |     |  |   |   |    |    |   |    |      |  |  |  |  |  |  |  |
| S13 G5      | L5 R109     |    |    |   |     |  |   |   |    |    |   |    |      |  |  |  |  |  |  |  |
| S13 I8      | L5 I136     |    |    |   |     |  |   |   |    |    |   |    |      |  |  |  |  |  |  |  |
| S13 I6      | L5 R111     |    |    |   |     |  |   |   |    |    |   |    |      |  |  |  |  |  |  |  |
| S13 N7      | L5 I136     |    |    |   |     |  |   |   |    |    |   |    |      |  |  |  |  |  |  |  |
| S13 R56     | L5 P108     |    |    |   |     |  |   |   |    |    |   |    |      |  |  |  |  |  |  |  |
| S13 I6      | L5 I136     |    |    |   |     |  |   |   |    |    |   |    |      |  |  |  |  |  |  |  |
| S13 I8      | L5 R109     |    |    |   |     |  |   |   |    |    |   |    |      |  |  |  |  |  |  |  |
| S13 E65     | L5 P108     |    |    |   |     |  |   |   |    |    |   |    |      |  |  |  |  |  |  |  |
| S13 R2      | L5 I136     |    |    |   |     |  |   |   |    |    |   |    |      |  |  |  |  |  |  |  |
| S13 R56     | L5 R109     |    |    |   |     |  |   |   |    |    |   |    |      |  |  |  |  |  |  |  |
| S13 I3      | L5 I136     |    |    |   |     |  |   |   |    |    |   |    |      |  |  |  |  |  |  |  |
| S13 I6      | L5 P108     |    |    |   |     |  |   |   |    |    |   |    |      |  |  |  |  |  |  |  |
| S13 P9      | L5 I136     |    |    |   |     |  |   |   |    |    |   |    |      |  |  |  |  |  |  |  |
| S13 R2      | L5 D146     |    |    |   |     |  |   |   |    |    |   |    |      |  |  |  |  |  |  |  |

**Supplementary Table 23:** Contacting residues for cluster 11 (30S) and cluster 12 (50S).

| 30S residue | 50S residue | 1a | 1b | 2 | pre |  | 3 | 4 | 5a | 5b | 1 | 2a | post |  |  |  |  |  |  |  |
|-------------|-------------|----|----|---|-----|--|---|---|----|----|---|----|------|--|--|--|--|--|--|--|
| h24 G776    | L2 Y160     |    |    |   |     |  |   |   |    |    |   |    |      |  |  |  |  |  |  |  |

**Supplementary Table 24:** Contacting residues for cluster 12 (30S) and cluster 6 (50S).

| 30S residue | 50S residue | 1a | 1b | 2 | pre |  | 3 | 4 | 5a | 5b | 1 | 2a | post |  |  |  |  |  |  |  |
|-------------|-------------|----|----|---|-----|--|---|---|----|----|---|----|------|--|--|--|--|--|--|--|
| h24 G775    | L2 R174     |    |    |   |     |  |   |   |    |    |   |    |      |  |  |  |  |  |  |  |

**Supplementary Table 26:** Comparison of the contacts found in the trajectories for intersubunit contact clusters 4 and 13 (B1 bridges) with contacts obtained from X-ray structures.

| Reference            | body rotation <sup>a</sup> | head swiveling <sup>a</sup> | state <sup>b</sup> | body rotation <sup>c</sup> | head swiveling <sup>c</sup> | common contact(s)  |
|----------------------|----------------------------|-----------------------------|--------------------|----------------------------|-----------------------------|--|
| Dunkle et al. (2)    | 8.4°                       | 4.8°                        | pre4               | 8.2° to 11.2°              | -3.6° to 5.1°               | L5 109 - S13 70, L5 111 - S13 77, L5 111 - S13 78, L5 114 - S19 63, L5 133 - S13 77, L5 135 - S13 70, L5 136 - S13 70, L5 142 - S13 66 |
| Schuwirth et al. (1) | -2.3°                      | 16.4°                       | pre4               | 8.2° to 11.2°              | -3.6° to 5.1°               | L5 109 - S13 70, L5 111 - S13 78, L5 114 - S19 63, L5 135 - S13 70, L5 136 - S13 70, L5 142 - S13 66                                   |
| Zhou et al. (3)      | 16.3°                      | 6.8°                        | pre5a              | 11.0° to 14.3°             | 0.2° to 8.6°                | L5 144 - S13 70  |
| Brilot et al. (4)    | 9.7°                       | 4.8°                        | pre5a              | 11.0° to 14.3°             | 0.2° to 8.6°                | L5 111 - S13 81, L5 143 - S13 70<br>H38 888 - S19 47, H38 888 - S19 58,<br>H38 888 - S19 60  |

<sup>a</sup> angle taken from Mohan et al. (8)

<sup>b</sup> state for which the corresponding simulation showed the highest number of common contacts with the X-ray structure.

<sup>c</sup> range of angles observed in the simulation.

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