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 $C$ 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 $d(C^A, C^B)$ between two matrices $C^A$ and $C^B$ was defined as

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

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:

$$G_e = H_i - T S_c,$$

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 grayscale level of the cells indicates the frequency of atom-atom contacts corresponding to the residue pairs, white (0–12.5 %), light gray (12.5–25 %), dark gray (25–50 %), and black (50–100 %).
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.

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

**Supplementary Table 3:** Contacting residues for cluster 3.
Supplementary Table 11: Contacting residues for cluster 11.

| 30S Residue | 50S Residue | pre 1a 1b 2 3 4 5a 5b | post 2a 2b 3a 3b 4 1 |
|-------------|-------------|-------------------------|-----------------------|
| h23 A702    | H68 G1846   | 1a 1b 2 3 4 5a 5b       | 2a 2b 3a 3b 4 1       |
| h23 A702    | H68 C1845   |                         |                       |
| h23 A702    | H68 A1848   |                         |                       |
| h23 A702    | H68 A1847   |                         |                       |
| h23 A681    | L2 V267     |                         |                       |
| h23 C680    | L2 V267     |                         |                       |
| h23 G682    | L2 K182     |                         |                       |
| h23 A681    | L2 K182     |                         |                       |
| h23 A681    | 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 G2193   |                         |                       |
| 36 K53      | L2 D167     |                         |                       |

Supplementary Table 12: Contacting residues for cluster 12.

| 30S Residue | 50S Residue | pre 1a 1b 2 3 4 5a 5b | post 2a 2b 3a 3b 4 1 |
|-------------|-------------|-------------------------|-----------------------|
| h24 G773    | L2 M200     | 1a 1b 2 3 4 5a 5b       | 2a 2b 3a 3b 4 1       |
| h24 U772    | L2 M201     |                         |                       |
| h24 G774    | L2 R176     |                         |                       |
| h24 U772    | L2 M200     |                         |                       |
| h24 G774    | L2 Y160     |                         |                       |
| h24 G775    | L2 R176     |                         |                       |
| h24 C773    | L2 Y160     |                         |                       |
| h24 G774    | H68 U1820   |                         |                       |
| h24 G774    | L2 M200     |                         |                       |
| h24 U772    | L2 K4       |                         |                       |
| h24 U772    | L2 A1       |                         |                       |
| h24 C808    | L2 A1       |                         |                       |

Supplementary Table 13: Contacting residues for cluster 13.

| 30S Residue | 50S Residue | pre 1a 1b 2 3 4 5a 5b | post 2a 2b 3a 3b 4 1 |
|-------------|-------------|-------------------------|-----------------------|
| S13 R69     | L5 P138     | 1a 1b 2 3 4 5a 5b       | 2a 2b 3a 3b 4 1       |
| S13 G66     | L5 Y142     |                         |                       |
| S13 D67     | L5 Y142     |                         |                       |
| S13 H70     | L5 H135     |                         |                       |
| S13 H70     | L5 D143     |                         |                       |
| S13 S73     | L5 D143     |                         |                       |

Supplementary Table 14: Contacting residues for cluster 14.

| 30S Residue | 50S Residue | pre 1a 1b 2 3 4 5a 5b | post 2a 2b 3a 3b 4 1 |
|-------------|-------------|-------------------------|-----------------------|
| h24 A784    | H68 G1846   | 1a 1b 2 3 4 5a 5b       | 2a 2b 3a 3b 4 1       |
| h24 A784    | H68 C1836   |                         |                       |
| h24 A784    | H68 C1836   |                         |                       |
| h24 A784    | H68 G1835   |                         |                       |

Supplementary Table 15: Contacting residues for cluster 15.

| 30S Residue | 50S Residue | pre 1a 1b 2 3 4 5a 5b | post 2a 2b 3a 3b 4 1 |
|-------------|-------------|-------------------------|-----------------------|
| h27 A899    | H67 G1831   | 1a 1b 2 3 4 5a 5b       | 2a 2b 3a 3b 4 1       |
| 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 | pre 1a 1b 2 3 4 5a 5b | post 2a 2b 3a 3b 4 1 |
|-------------|-------------|-------------------------|-----------------------|
| S13 R69     | L5 P138     | 1a 1b 2 3 4 5a 5b       | 2a 2b 3a 3b 4 1       |
| S13 G66     | L5 Y142     |                         |                       |
| S13 D67     | L5 Y142     |                         |                       |
| S13 H70     | L5 H135     |                         |                       |
| S13 H70     | L5 D143     |                         |                       |
| S13 S73     | L5 D143     |                         |                       |

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

| 30S Residue | 50S Residue | pre 1a 1b 2 3 4 5a 5b | post 2a 2b 3a 3b 4 1 |
|-------------|-------------|-------------------------|-----------------------|
| h14 C341    | L14 R98     | 1a 1b 2 3 4 5a 5b       | 2a 2b 3a 3b 4 1       |
| h14 C341    | L14 R98     |                         |                       |
| h14 C341    | L14 R98     |                         |                       |
| h14 C341    | L14 R98     |                         |                       |

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

| 30S Residue | 50S Residue | pre 1a 1b 2 3 4 5a 5b | post 2a 2b 3a 3b 4 1 |
|-------------|-------------|-------------------------|-----------------------|
| S13 R69     | L5 P138     | 1a 1b 2 3 4 5a 5b       | 2a 2b 3a 3b 4 1       |
| S13 G66     | L5 Y142     |                         |                       |
| S13 D67     | L5 Y142     |                         |                       |
| S13 H70     | L5 H135     |                         |                       |
| S13 H70     | L5 D143     |                         |                       |
| S13 S73     | L5 D143     |                         |                       |

Supplementary Table 19: Contacting residues for cluster 9 (30S) and cluster 1 (50S).
**Supplementary Table 20:** Contacting residues for cluster 9 (30S) and cluster 7 (50S).

| 30S residue | 50S residue | pre | post |
|-------------|-------------|-----|------|
| h44 G1473   | L19 R105    | 1a  | 1b  |
| h44 U1463   | L19 R105    | 2   | 3    |
| h44         | h44         | 4   | 5a   |
|             |             | 5b  | 1    |
|             |             | 2a  | 2b   |
|             |             | 3a  | 3b   |
|             |             | 4   | 5    |

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

| 30S residue | 50S residue | pre | post |
|-------------|-------------|-----|------|
| h44 U1463   | L19 R105    | 1a  | 1b  |
| h44 U1463   | L19 R105    | 2   | 3    |
| h44         | h44         | 4   | 5a   |
|             |             | 5b  | 1    |
|             |             | 2a  | 2b   |
|             |             | 3a  | 3b   |
|             |             | 4   | 5    |

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

| 30S residue | 50S residue | pre | post |
|-------------|-------------|-----|------|
| h23 G775    | L2 R174     | 1a  | 1b  |
| h23 C680    | L2 P268     | 2   | 3    |
| h23 C679    | L2 R268     | 4   | 5    |
| h23 A681    | L2 A165     | 5a  | 5b   |
| h23 A681    | L2 R268     | 1   | 2    |
| h23 C680    | L2 V164     | 2a  | 2b   |
| h23 A681    | L2 V164     | 3a  | 3b   |
|             |             | 4   | 5    |

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

| 30S residue | 50S residue | pre | post |
|-------------|-------------|-----|------|
| h23 G775    | L2 V164     | 1a  | 1b  |
| h23 C680    | L2 P268     | 2   | 3    |
| h23 C679    | L2 R268     | 4   | 5    |
| h23 A681    | L2 A165     | 5a  | 5b   |
| h23 A681    | L2 R268     | 1   | 2    |
| h23 C680    | L2 V164     | 2a  | 2b   |
| h23 A681    | L2 V164     | 3a  | 3b   |
|             |             | 4   | 5    |

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

| 30S residue | 50S residue | pre | post |
|-------------|-------------|-----|------|
| h23 G775    | L2 R174     | 1a  | 1b  |
| h23 C680    | L2 P268     | 2   | 3    |
| h23 C679    | L2 R268     | 4   | 5    |
| h23 A681    | L2 A165     | 5a  | 5b   |
| h23 A681    | L2 R268     | 1   | 2    |
| h23 C680    | L2 V164     | 2a  | 2b   |
| h23 A681    | L2 V164     | 3a  | 3b   |
|             |             | 4   | 5    |

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

| 30S residue | 50S residue | pre | post |
|-------------|-------------|-----|------|
| s13 E65     | L5 R111     | 1a  | 1b  |
| s13 E65     | L5 R109     | 2   | 3    |
| s13 G4      | L5 R109     | 4   | 5    |
| s13 G4      | L5 R109     | 5a  | 5b   |
| s13 I3      | L5 P108     | 1   | 2    |
| s13 I3      | L5 P108     | 2a  | 2b   |
| s13 I3      | L5 P108     | 3a  | 3b   |
| s13 I3      | L5 P108     | 4   | 5    |

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**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\(^a\) | head swiveling\(^b\) | state\(^b\) | body rotation\(^c\) | head swiveling\(^c\) | 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, H38 888 - S19 47, H38 888 - S19 58, H38 888 - S19 60 |

\(^a\) angle taken from Mohan et al. (8)  
\(^b\) state for which the corresponding simulation showed the highest number of common contacts with the X-ray structure.  
\(^c\) range of angles observed in the simulation.
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