Supporting Information

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The Archaeal Exosome: Identification and Quantification of Site-Specific Motions That Correlate with Cap and RNA Binding**

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Figure S1. Architecture of the exosome complex. Rrp42 is displayed as a cartoon (green), Rrp41 is shown in gray, Rrp4 in yellow and Csl4 in brown. The displayed structures are based on PDB-entries 2BR2 (exosome core)\(^1\), 2JEA (exosome Rrp4 complex)\(^2\) and 3M7N (exosome Csl4 complex, displayed structure is a homology model based on the archaeoglobus fulgidus structure of the complex)\(^3\).

The exosome core (Rrp41:Rrp42) can interact with substrate RNA and degrade this in a processive manner in the 3’ to 5’ direction. During this process, the exosome does not release the substrate. We thus expect that cap proteins will not be recruited to the processing exosome core: substrate RNA complex. It should be noted that the amounts of cap-free exosome (Rrp41:Rrp42) is expected to be very low in a cellular context as cap proteins, Rrp41 and Rrp42 are present in similar relative amounts\(^4\). The interaction between the free exosome and the cap proteins (Csl4 or Rrp4) is very tight. After formation of the exosome:cap complex RNA substrate can be recruited and degraded; the cap proteins will remain bound to the exosome core during this process.

To prepare the samples used in the current study, the *Sulfolobus solfataricus* Rrp41, Rrp42, Rrp4 and Csl4 DNA (a kind gift from E. Conti, MPI Munich) was cloned into pET vectors carrying a TEV cleavable N-terminal His6-tag. Point mutations were introduced using the Quikchange approach (Stratagene). \(\Delta^{2H,15N}\) Ile-\(\delta^{1}\), Leu-\(\delta^{1}\), Val-\(\gamma^{1}\) \(\Delta^{1H,13C}\) labeled Rrp42 proteins were obtained by overexpression of the corresponding gene in BL21(DE3) Codon Plus RIL (Stratagene) cells in 100 % D2O minimal medium, as previously described\(^5\). Purification of all constructs was achieved by using Ni affinity chromatography followed by cleavage of the histidine tag and size-exclusion chromatography. If required, amide protons of Rrp42 were back-exchanged by refolding the GuHCl denatured protein in H\(_2\)O based buffer. Exosome core complexes were reconstituted by combining separately purified components. Exosome-cap complexes were obtained by addition of purified Rrp4 or Csl4 to the exosome core complex. NMR samples contained between 0.05 and 1.5 mM protein (monomer concentration) in 30 mM KPO4 pH 6.8 (or 25 mM Hepes pH 7.5), 150 mM NaCl, 1 mM DTT in 100 % D2O or in 95:5 H2O:D2O. For the exosome RNA complex, excess of RNA was removed by size exclusion chromatography such that one RNA molecule was present per hexameric exosome core complex.

Substrate RNA (20 adenines linked to a hairpin structure) was produced by *in-vitro* transcription using a linearized pSP64 plasmid that contains the substrate RNA followed by a 3’ HDV ribozyme that auto-cleaves the RNA cotranscriptionally. The RNA was purified over a Dionex DNA Pac PA-100 column at 75 °C using a NaCl gradient in 5 M urea. Substrate RNA was not degraded during NMR experiments due to lack of phosphate in the buffer, the 2’,3’-cyclic phosphate at the 3’ end of the RNA and the hairpin structure at the 5’ end of the RNA.

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**Diagram:**

- **173 kDa exosome core**
- **257 kDa exosome:Rrp4 cap complex**
- **236 kDa exosome:Csl4 cap complex**

- **Rrp41:** Rrp42
- **Rrp41:** Rrp42
- **Rrp41:** Rrp42

**Figure S1.** Architecture of the exosome complex. Rrp42 is displayed as a cartoon (green), Rrp41 is shown in gray, Rrp4 in yellow and Csl4 in brown. The displayed structures are based on PDB-entries 2BR2 (exosome core)\(^1\), 2JEA (exosome Rrp4 complex)\(^2\) and 3M7N (exosome Csl4 complex, displayed structure is a homology model based on the archaeoglobus fulgidus structure of the complex)\(^3\).
**Figure S2.** Methyl group assignment strategy. (A) Building blocks used in the divide and conquer approach. First panel: $^1$H-$^{15}$N TROSY spectrum of $[^{1}H, ^{13}C, ^{15}N]$ labeled Rrp42 monomer. H-N groups are shown as green spheres. Second panel: $^1$H-$^{13}$C methyl TROSY spectrum of U-$[^{1}H, ^{15}N]$ Ile-δ, Leu-δ, Val-γ $[^{1}H, ^{13}C]$ Rrp42 as monomer. Third panel: Rrp42 within the exosome core complex. Last panel: Rrp42 within the exosome-Rrp4 complex. Labeled methyl groups are shown as red spheres. Exemplary assignments are indicated. Rrp42 backbone sequential assignments were completed using TROSY versions of HNCACB/HNCOCACB experiments. Methyl groups in the Rrp42 monomer were assigned using C(C)(CO)NH TOCSY, H-N and H-N-C NOESY spectra. C-C-H HMOC-NOE-HMQC and H-C-H NOE-HMQC spectra were used to assign methyl groups in the exosome-core and exosome-cap complexes. (B) Assignments by point mutations. Left: spectra of WT (black) and V197A (red) exosome complexes. The assignment for V197 is indicated in red. Residues that are in the vicinity of the mutation and that thus experience secondary chemical shift changes are labeled in blue. Right: Location of V197 and the residues that experience secondary chemical shifts on the crystal structure of the exosome core complex.
Figure S3. The cap proteins change the dynamics in the exosome core. MQ dispersion profiles observed for Ile 13, 19, 27 and 220. Note that the y-axis has the same range for all graphs for a specific residue to allow for direct comparison of the data. (A) Profiles in the exosome core (identical to Fig 2B in the main text). Blue and red correspond to state A and B respectively. See main text for details. The structure of the exosome core is indicated on the right. (B) Profiles in the exosome-Rrp4 complex. Note that only one state is present in the spectra. The structure of the exosome-Rrp4 complex is shown on the right. (C) Profiles in the exosome-Csl4 complex. Note that only one state is present in the spectra.
Figure S4. Predicted versus measured chemical shifts for all assigned isoleucine residues. The chemical shifts were predicted using shiftx\textsuperscript{2}[6] using the free exosome complex (2BR2) or the exosome-Rrp4 complex (2JE6) from which Rrp4 was removed as input. The methyl groups that show two conformations have been labeled; the Pearson R correlation coefficient is indicated. A red drawn line indicates the best fit between the predicted and measured shifts (y=x+A), where A corrects for an (potential) offset in chemical shift referencing. Note that none of the correlations is significant, most likely due to the large inaccuracies in the predicted values.
Figure S5. “State A mutant” (N9A) exosome complex. Location of N9 in the exosome complex. N9 is remote from the interaction with the cap structure. Mutations in this residue do thus not change the interaction between the exosome core and the cap directly, but rather indirectly through changes in exosome dynamics.

The identification of the N9A mutant was inspired by the spectra of the assignment mutants (Table S1), where we noticed that the relative intensities of the two sets of peaks varied. This indicated that the equilibrium between the two states could be modified. We then systematically mutated residues that were close, but not directly in the cap-interaction-helix and monitored the state A: state B peak ratio. In this process we identified that the N9A mutation yielded only a single set of resonances.
**Figure S6** Kinetic SPR analyses of cap protein Rrp4 with His-tagged wildtype (A) or “state A mutant” (B) exosome complex attached to a Ni NTA chip. The double-referenced sensorgrams (indicating that two controls experiment were performed: one without ligand and one without analyte) are overlaid with fits of a “1:1 binding with mass transfer” model.

|          | wildtype | SE * | state A mutant | SE * |
|----------|----------|------|----------------|------|
| $k_{on}$ (M$^{-1}$s$^{-1}$) | $1.7 \cdot 10^6$ | 2.1 $\cdot 10^4$ | $1.7 \cdot 10^6$ | 2.8 $\cdot 10^4$ |
| $k_{off}$ (s$^{-1}$) | $< 10 \cdot 10^{-7}$ | <10 $\cdot 10^{-7}$ | <10 $\cdot 10^{-7}$ | <10 $\cdot 10^{-7}$ |
| $K_D$ (M) | $< 10 \cdot 10^{-11}$ | <10 $\cdot 10^{-11}$ | <10 $\cdot 10^{-11}$ | <10 $\cdot 10^{-11}$ |
| $R_{max}$ (RU) | 51.2 | 0.01 | 57.7 | 0.01 |
| $\chi^2$ (RU$^2$) | 0.17 | 0.20 | 0.20 | 0.20 |

* Standard error (obtained from the Biaeval software kit)

* The off-rate is at the detection limit of the system

* Defined as $k_{off}/k_{on}$

* Theoretical maximum response that is reached when all ligand binding sites are occupied by the analyte; RU refers to response units.

The SPR analyses were performed on a Biacore 2000 system at 15 °C. Two consecutive flow cells (a measurement cell and a reference cell in which no ligand was immobilized) were used. In both the measurement and the reference cell an NTA chip (GE healthcare) was loaded with NiCl$_2$ following the manufacturer’s instructions. His-tagged wildtype or “state A mutant” exosome complex was diluted in running buffer (10 mM HEPES, pH 7.4, 150 mM NaCl, 5 % D20,
50 µM EDTA) and 100-110 RU of ligand were non-covalently bound to the experimental flow cell. Untagged cap protein Rrp4 was serially diluted in running buffer to concentrations ranging from 0.23 to 58 nM and injected for 200 s in both the experimental and the reference cell at a flow rate of 50 µl/min. The dissociation phase was followed for 1800 s. Additionally, we recorded a blank curve, where buffer (without analyte) was injected. Both the curve from the reference cell and the blank injection were subtracted from the SPR signal in the measurement cell. To regenerate the surface regeneration buffer (10 mM HEPES, 150 mM NaCl, 0.005% (v/v) P20, 0.35 M EDTA, pH 8.3) was injected for 3 minutes at a flow rate of 20 µl/min in both the reference and the measurement cell.

In addition to the interaction between the exosome complex and the Rrp4 cap we performed experiments to probe for the interaction between the exosome complex and the reduced Rrp4 cap (that lacks one of the domains; See Figure S7). Unfortunately, this protein interacted unspecifically with the sensorchip surface, which resulted in a strong signal from the reference cell. As a consequence, we were not able to extract any reliable interaction data for the exosome: reduced Rrp4 cap complex.
Figure S7 To move the Rrp4 binding affinity into a range where one can discriminate cap binding between WT and “state A mutant” exosome, we deleted one of the three domains from the cap structure (A). This reduced Rrp4 cap contains the domains that interacts with Rrp42 (the S1 and KH domains) but lacks the domain (the NTD; N-terminal domain) that interacts with Rrp41. We then used this reduced Rrp4 cap structure to probe for the interactions with the WT and “state A mutant” exosome. In NMR chemical shift titrations (B), where we added the unlabeled exosome to 15N-labeled reduced cap, we observed a faster decrease in resonance intensity upon addition of the “state A mutant” exosome than upon addition of the WT exosome (C). This implies that the “state A mutant” has a higher affinity for the cap than the WT exosome and establishes that state A plays an important role in the interaction with the Rrp4 cap structure.

(A) Left: Side view of the structure of the exosome-Rrp4 complex. The exosome core is drawn as a surface representation (Rrp41: gray; Rrp42: green), Rrp4 is shown as a ribbon. The three Rrp4 domains are colored separately; NTD (N-terminal domain) in yellow; S1 domain in orange; KH domain in red.
Middle: Top view of the complex, indicating that Rrp42 does not contact the Rrp4 NTD. The region that displays two conformations (blue) contacts both the S1 and KH domains. Selected Rrp4 residues that are in contact with Rrp42 are indicated (see below). Right: Cartoon representation of Rrp4. The S1-KH (reduced Rrp4 protein) region that is used to probe for binding with the exosome is circled.

(B) $^1$H$^1$N NMR spectra of the reduced Rrp4 protein in the absence (black) and presence of an equimolar amount of WT exosome (left, blue) or "state A mutant" exosome (red, right). Larger chemical shift perturbations are observed upon addition of the "state A mutant" complex, demonstrating a stronger interaction.

(C) Dependence of reduced Rrp4 peak intensities on the molar excess of exosome (blue-cyan scale) or "state A mutant" exosome (red-yellow scale). Four Rrp4 residues that contact Rrp42 are selected. The signals decrease more rapidly upon addition of the "state A mutant" due to the tighter interaction. Note that the decrease in peak intensity is largely due to fast relaxation in the high molecular weight complex that is formed, preventing accurate extraction of binding constants from the NMR data. After addition of a high excess of (WT or "state A") exosome the spectra of the reduced cap are no longer visible due to the formation of a large complex.
| Residue  | Rrp42 monomer | Rrp42 in exosome core | Point mutation |
|----------|---------------|-----------------------|----------------|
|          | $^{13}$C (p.p.m.) | $^1$H (p.p.m.) | $^{13}$C (p.p.m.) | $^1$H (p.p.m.) |                      |
| Ile 10 δ1 (A)² | 10.349 | 1.081 | 10.224 | 1.050 | yes |
| Ile 10 δ1 (B) | Not applicable³ | | 11.412 | 1.283 | yes |
| Ile 11 δ1 | 9.836 | 1.084 | 9.635 | 1.026 | yes |
| Ile 13 δ1 (A) | 10.394 | 1.127 | 10.402 | 1.153 | yes |
| Ile 13 δ1 (B) | Not applicable | | 10.004 | 1.117 | yes |
| Ile 14 δ1 | 9.972 | 1.062 | 10.576 | 1.079 | yes |
| Ile 19 δ1 (A) | 10.265 | 0.885 | 10.525 | 0.879 | |
| Ile 19 δ1 (B) | Not applicable | | 9.955 | 0.863 | |
| Val 20 γ1 | 19.456 | 1.325 | 19.86 | 1.219 | |
| Val 20 γ2 | 20.208 | 1.254 | 19.481 | 1.296 | |
| Leu 22 δ1 | 22.933 | 0.993 | 22.856 | 0.98 | |
| Leu 22 δ2 | 20.245 | 0.949 | 20.17 | 0.942 | |
| Ile 27 δ1 (A) | 11.088 | 1.076 | 11.066 | 1.062 | yes |
| Ile 27 δ1 (B) | Not applicable | | 11.337 | 1.049 | yes |
| Leu 34 δ1 | 22.451 | 1.14 | 22.385 | 1.118 | |
| Leu 34 δ2 | 22.933 | 1.045 | 22.356 | 1.007 | |
| Ile 42 δ1 | 10.674 | 0.786 | 10.659 | 0.756 | |
| Leu 44 δ1 | 23.109 | 1.305 | 22.995 | 1.274 | |
| Leu 44 δ2 | 21.238 | 1.087 | 21.066 | 1.043 | |
| Leu 55 δ1 | 20.712 | 0.761 | 20.731 | 0.681 | |
| Leu 55 δ2 | 22.422 | 0.664 | 22.084 | 0.473 | |
| Val 56 γ1 | 17.994 | 1.067 | 17.996 | 1.042 | |
| Val 56 γ2 | 19.587 | 1.04 | 19.567 | 1.011 | |
| Leu 58 δ1 | --- | --- | --- | --- | |
| Leu 58 δ2 | --- | --- | --- | --- | |
| Val 63 δ1 | --- | --- | --- | --- | yes |
| Val 63 δ2 | --- | --- | --- | --- | yes |
| Leu 64 δ1 | 21.136 | 1.165 | --- | --- | |
| Leu 64 δ2 | 23.605 | 1.11 | --- | --- | |
| Leu 69 δ1 | 22.232 | 0.615 | 22.176 | 0.603 | |
| Leu 69 δ2 | 23.035 | 0.8 | 23.129 | 0.772 | |
| Val 86 γ1 | 18.608 | 1.049 | --- | --- | yes |
| Val 86 γ2 | 18.914 | 1.096 | --- | --- | yes |
| Val 88 γ1 | 19.215 | 1.024 | --- | --- | |
| Val 88 γ2 | 18.068 | 0.996 | --- | --- | |
| Ile 85 δ1 | 10.304 | 0.23 | 10.159 | 0.087 | |
| Ile 85 δ2 | --- | --- | --- | --- | |
| Val 86 γ1 | 18.608 | 1.049 | --- | --- | yes |
| Val 86 γ2 | 18.914 | 1.096 | --- | --- | yes |
|     |      |      |      |      |
|-----|------|------|------|------|
| Leu 91 δ2 | --- | --- | --- | --- |
| Leu 93 δ1 | --- | --- | --- | --- |
| Leu 93 δ2 | --- | --- | --- | --- |
| Ile 108 δ1 | 9.878 | 1.153 | 1.123 | 10.615 | yes |
| Leu 110 δ1 | --- | --- | --- | --- | yes |
| Leu 110 δ2 | --- | --- | --- | --- | yes |
| Val 113 γ1 | 19.529 | 1.046 | --- | --- | yes |
| Val 113 γ2 | 20.172 | 1.215 | --- | --- | yes |
| Val 114 γ1 | 19.005 | 1.036 | --- | --- | yes |
| Val 114 γ2 | 19.938 | 1.023 | --- | --- | yes |
| Leu 118 δ1 | 23.912 | 0.664 | 24.015 | 0.856 | yes |
| Leu 118 δ2 | 19.573 | 0.81 | 19.543 | 0.786 | yes |
| Leu 124 δ1 | 21.034 | 0.841 | 20.822 | 0.809 |
| Leu 124 δ2 | --- | --- | --- | --- |
| Leu 126 δ1 | 23.001 | 1.205 | 22.981 | 1.197 | yes |
| Leu 126 δ2 | 20.61 | 0.857 | 20.554 | 0.85 | yes |
| Leu 129 δ1 | 24.014 | 1.07 | 23.981 | 1.045 |
| Leu 129 δ2 | 19.804 | 0.911 | 19.754 | 0.908 |
| Val 130 γ1 | 19.005 | 1.061 | 19.016 | 1.058 |
| Val 130 γ2 | 18.417 | 1.042 | 18.379 | 1.035 |
| Ile 131 δ1 | 11.027 | -0.082 | 11.018 | -0.067 |
| Val 137 γ1 | 19.689 | 1.018 | 19.702 | 1.005 |
| Val 137 γ2 | 14.955 | 0.71 | 14.945 | 0.707 |
| Val 140 γ1 | 18.184 | 0.883 | 18.129 | 0.871 | yes |
| Val 140 γ2 | --- | --- | --- | --- | yes |
| Leu 142 δ1 | 21.647 | 0.953 | 21.474 | 0.952 |
| Leu 142 δ2 | 23.328 | 0.978 | 23.246 | 1.006 |
| Val 144 γ1 | 18.637 | 0.966 | --- | --- | yes |
| Val 144 γ2 | 17.527 | 0.867 | --- | --- | yes |
| Val 146 γ1 | --- | --- | --- | --- | yes |
| Val 146 γ2 | --- | --- | --- | --- | yes |
| Leu 147 δ1 | 23.966 | 1.049 | --- | --- | yes |
| Leu 147 δ2 | --- | --- | --- | --- | yes |
| Val 153 γ1 | --- | --- | --- | --- | yes |
| Val 153 γ2 | --- | --- | --- | --- | yes |
| Leu 154 δ1 | 22.948 | 1.039 | --- | --- | yes |
| Leu 154 δ2 | --- | --- | --- | --- | yes |
| Leu 159 δ1 | 23.589 | 1.042 | 23.689 | 1.198 |
| Leu 159 δ2 | 21.574 | 1.133 | 21.449 | 1.137 |
| Val 162 γ1 | 19.733 | 1.378 | 19.821 | 1.345 |
| Val 162 γ2 | 21.222 | 1.517 | 21.265 | 1.502 |
| Leu 165 δ1 | 24.657 | 0.938 | 24.668 | 0.937 |
| Leu 165 δ2 | 21.267 | 0.876 | 21.441 | 0.86 |
| Val 170 γ1 | 18.41 | 0.657 | 18.356 | 0.642 |
| Val 170 γ2 | 18.184 | 0.386 | 18.138 | 0.365 |
| Val 173 γ1 | --- | --- | 19.702 | 1.018 |
| Residue | Y1 | Y2 | Y1 | Y2 |
|---------|----|----|----|----|
| Val 173 | 19.129 | 1.152 | 19.129 | 1.152 |
| Ile 180 | 10.997 | 0.906 | 11.008 | 0.899 |
| Val 182 | 19.653 | 1.274 | 19.856 | 1.317 |
| Val 182 | 19.14 | 1.158 | 19.106 | 1.148 |
| Val 187 | 19.683 | 1.254 | 19.106 | 1.158 |
| Val 187 | --- | --- | 19.585 | 1.148 |
| Val 188 | 18.785 | 1.166 | 18.824 | 1.162 |
| Val 188 | 16.446 | 0.945 | 16.356 | 0.941 |
| Leu 191 | 21.463 | 0.773 | 21.418 | 0.774 |
| Leu 191 | 24.283 | 1.01 | 24.356 | 1.018 |
| Leu 193 | 21.764 | 1.166 | 21.783 | 1.162 |
| Leu 193 | 16.446 | 0.945 | 16.356 | 0.941 |
| Val 197 | 17.322 | 1.02 | 17.904 | 0.954 |
| Val 197 | 18.288 | 1.055 | 18.389 | 1.02 |
| Val 198 | 19.719 | 1.008 | 19.816 | 0.97 |
| Val 198 | 16.285 | 1.082 | 15.932 | 1.071 |
| Ile 200 | 9.739 | 1.061 | 9.96 | 1.051 |
| Val 202 | 20.099 | 1.062 | 20.237 | 1.036 |
| Val 202 | 18.552 | 1.3 | 18.423 | 1.253 |
| Val 205 | 17.083 | 1.125 | 17.194 | 1.071 |
| Val 205 | 19.163 | 1.186 | 19.147 | 1.171 |
| Leu 209 | 23.591 | 1.057 | 23.697 | 1.032 |
| Leu 209 | 20.66 | 0.975 | 20.254 | 0.909 |
| Val 210 | 16.637 | 0.839 | 16.751 | 0.8 |
| Val 210 | 19.792 | 0.581 | 19.637 | 0.566 |
| Val 211 | --- | --- | 19.504 | 0.989 |
| Val 211 | --- | --- | 19.504 | 0.989 |
| Leu 215 | 22.561 | 1.1 | 22.122 | 1.143 |
| Leu 215 | --- | --- | 22.561 | 1.1 |
| Ile 220 | 13.178 | 1.06 | 13.031 | 1.01 |
| Ile 220 | Not applicable | 12.753 | 1.02 |
| Ile 225 | 13.093 | 0.945 | 12.911 | 0.796 |
| Leu 233 | 19.216 | 0.672 | 18.774 | 0.759 |
| Leu 233 | 22.564 | 0.893 | 22.668 | 0.927 |
| Ile 235 | 11.094 | 1.107 | 11.543 | 0.59 |
| Val 236 | 17.556 | 0.975 | 16.441 | 0.772 |
| Val 236 | 18.537 | 1.192 | 19.566 | 0.952 |
| Ile 238 | 11.284 | 1.115 | 10.106 | 0.486 |
| Ile 238 | 22.292 | 1.2 | 22.292 | 1.2 |
| Leu 248 | 20.941 | 1.14 | 20.941 | 1.14 |
| Ile 251 | 10.53 | 1.017 | 10.53 | 1.017 |
| Val 263 | 20.181 | 1.326 | 20.106 | 1.318 |
| Val 263 | 18.537 | 1.192 | 18.606 | 1.184 |
| Leu 265 | 20.508 | 0.669 | 19.918 | 0.625 |
| Leu 265 | 22.422 | 0.756 | 22.803 | 0.733 |
| Leu 266 | 22.82 | 0.919 | 22.619 | 0.881 |
|       | State A | State B | State A | State B |
|-------|---------|---------|---------|---------|
| Leu 266 δ2 | 21.659  | 0.935   | 22.043  | 0.93    |
| Leu 269 δ1 | 21.151  | 1.029   | 20.879  | 1.029   |
| Leu 269 δ2 | ---     | ---     | ---     | ---     |
| Leu 273 δ1 | 22.451  | 0.649   | 22.502  | 0.608   |
| Leu 273 δ2 | 20.04   | 0.982   | 19.957  | 0.94    |
| Ile 275 δ1 | 10.816  | 0.629   | 10.867  | 0.62    |

**Table S1:**
Assigned chemical shifts for Rrp42 as a monomer and in the exosome core.

1. Leu and Val methyl groups were not stereo-specifically assigned.
2. A and B refer to the states A and B in the exosome core.
3. The Rrp42 monomer only displays one state.
4. Indicated if a point mutation was made to assign (or check the assignment of) the residue.
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