Supplementary Material

The manuscript " **Elastic properties of ribosomal RNA building blocks: Molecular dynamics of the GTPase associated center rRNA** " is accompanied by supplementary information:

Supplementary text: Restrained dynamics

Structure and dynamics of *Hinge2*

Conservation and isostericity of *Hinge2*

Structure and dynamics of base pairing in the rGAC area

Results of the 3D motif search for *H.m.* triad U1164-A1192/C1182

Supplementary Figures and Tables: Figure S1-S13, Table S1

Supplementary Animations (animated .gif files): Kt-42-rGAC-NMA1x.gif

Kt-42-rGAC-NMA1y.gif

Kt-42-rGAC-NMA1z.gif

Kt-42-rGAC-NMA2x.gif

Kt-42-rGAC-NMA2y.gif

Kt-42-rGAC-NMA2z.gif

Kt-42-rGAC-NMA3x.gif

Kt-42-rGAC-NMA3y.gif

Kt-42-rGAC-NMA3z.gif

Kt-42-rGAC-NMA4x.gif

Kt-42-rGAC-NMA4y.gif

Kt-42-rGAC-NMA4z.gif

Supplementary PDB: Kt-42+rGAC initial structure of *H.m.* (Kt-42+rGACinitial-Hm1JJ2.pdb)

Kt-42+rGAC initial structure of *E.coli* (Kt-42+rGACinitial-Ecoli2AW4.pdb)

Kt-42+rGAC initial structure of *E.coli* (Kt-42+rGACinitial-Ecoli2AWB.pdb)

Restrained dynamics.

We performed 11 ns of control restrained simulation where base pairs in the *Hinge2* region (*H.m.*) were restrained according to the initial structure. The distance restraints were applied to following residues of *Hinge2* region (G1158=C1209/A1188, G1159=C1208/A1189 and A1207/G1160…G1190/C1186 (Figure 1)):

A1188(O2')-C1209(O3') = 3.93 Å
A1188(O2')-C1209(O2') = 3.60 Å
A1188(N3)-C1209(O2') = 2.64 Å
A1188(C2)-C1209(O2) = 3.48 Å
A1189(O2')-C1208(O2') = 2.95 Å
A1189(O3')-C1208(O2') = 3.09 Å
A1189(O2')-C1208(O2) = 3.09 Å
G1159(O2')-A1189(N6) = 2.95 Å
G1159(O2')-A1189(N7) = 3.21 Å
A1207(O2P)-A1207(C8) = 3.36 Å
A1207(N7)-G1160(N2) = 3.68 Å
G1190(C4')-A1207(N1) = 3.57 Å
A1207(N6)-G1190(O2') = 2.98 Å
A1207(N6)-G1160(O2') = 2.93 Å
G1160(O2')-G1190(C8) = 3.07 Å
G1190(N2)-C1186(N3) = 2.83 Å
G1190(N1)-C1186(O2) = 3.41 Å

A force constant 50 kcal/mol/Å^2 was used to clamp the particular distance within the range ± 0.5 Å. Additionally, a force constant 100 kcal/mol/Å^2 was applied when particular distance changes more than ± 0.5 Å.

Structure and dynamics of *Hinge 2*.

The junction between Helix 42 and Helix 43/44 comprises the bases localized at the border between the extended Kt-42 *NC-stem* and the rGAC, namely base triads G1158=C1209/A1188 and G1159=C1208/A1189 and tetrad A1207/G1160…G1190/C1186 (Figure 1). The first triad forms type II A-minor interaction with *cis* SE/SE base pair between C and A (S1,S2). In the second triad the A1189 is flipped out (*syn* orientation) from the A-minor like orientation and forms *trans* H/SE interaction with the G1159 in all available *H.m.* structures (Figure S11). This interaction is, however, classic type I A-minor motif in the available x-ray structures of large subunits of *E.coli* and *Deinococcus radiodurans* (*D.r.*). The base tetrad A1207/G1160...G1190/C1186 (G1190 in *syn* orientation) comprises *trans* H/SE A1207/G1160 base pair, *trans* WC/WC G1190/C1186 base pair and bifurcated H-bonds between O2' of G1160 and O2' and C8 of G1190 (Figure S7). The adenosines A1188 and A1189 protrude to the bulk solvent without any visible role in rigidifying the RNA geometry. Their role is probably to provide molecular contacts with surrounding L10/L7/L12·L11 protein complex, as revealed by chemical probing (S3).

This flexible region *Hinge2* represents the pivoting point of the initial displacement (Figure 2A) of rGAC. Then it becomes the center of the reversible directional bending (Figure 2D) between the upper part of Helix 42 and rGAC. The intrinsic *Hinge2* bendability is, however, not localized as in case of Kt-42, where the motion is pivoting around a single H-bond (S4). In *Hinge2*, the structural dynamics of the A-minor interaction, the second base triad, and the base tetrad (see below) are directly coupled with neither the initial displacement of rGAC nor with motion of rGAC revealed by EDA mode 3.

Detailed analysis of the *Hinge2* region and the adjacent base steps reveals that the movement of rGAC (the initial displacement and the EDA mode 3) correlates only with change of major groove width of the RNA duplex formed by the upper part of Helix 42 and bottom of rGAC. Spontaneous initial straightening of this long rRNA duplex from its bent form (x-ray) to straight canonical-like form is indicated by increase of the following inter-phosphate distances: G1210(P)-A1152(P) from 9.5 Å (x-ray) to 16.0 Å (relaxed MD), C1209(P)-C1153(P) from 9.2 Å to 19.1 Å, C1208(P)-A1154(P) from 12.4 Å to 22.9 Å and A1207(P)-G1155(P) from 11.3 Å to 23.0 Å (Figure S4). We tentatively suggest that the adjacent L10/L7/L12·L11 protein complex

(disordered in the x-ray structure) may deform the conformation of this RNA duplex, which seems to be easily deformable especially within the *Hinge2* region.

We performed 11 ns of control restrained MD simulation (see above) where base pairs in the *Hinge2* region were restrained according to the initial structure. The results indicate that the motion around *Hinge2* is fully independent of the dynamics of the triads in this region. The overall dynamics is very similar to the free MD simulation and the initial displacement of rGAC can be best described as a spontaneous return of an initially bent duplex to a straight, relaxed conformation. Additionally, two free control MD simulations of corresponding rRNA segment in *E.coli* structures (2 x 10 ns, see Material and methods), reveal similar initial dynamical behavior of *Head,* qualitatively in line with motions occurring during standard MD and control restrained MD of Kt-42+rGAC rRNA of *H.m.*

Conservation and isostericity of *Hinge2*.

We compared the sequence, base pairing and 3D architecture of the *Hinge2* region in crystal structures of large ribosomal subunits of *H.m*., *D.r*., and *E.coli* (codes 1JJ2, 1NKW, 2AW4 and 2AWB). We also examined sequence alignments obtained from the latest release of the European Ribosomal Database (S5). Repeats of identical organisms were removed in order to obtain divergent and evolutionarily unbiased alignments. The final "unique" alignment included 24 archaeal, 184 bacterial, and 137 eukaryal sequences. The isosteric sequence analysis was carried out by Ribostral (S6).

The A-minor type II adenine of the first triad takes the same shape in all three structures, forming G1158=C1209/A1188 triple with *cis* SE/SE base pair between C/A in *H.m.,* A1065-U1116/A1095 triple with *cis* SE/SE U/A base pair in *D.r.* and identical A1054-U1105/A1084 triple in *E.coli*. Almost all sequences (>96%) have isosteric substitutions to the regular *cis* WC subfamily, and also have A as the third base in this triad. In 4% of archaeal sequences the nearly isosteric G/U replaces the *cis* WC base pair. Since all base pairs forming *cis* SE/SE interactions belong to the same isosteric subgroup, any combination of nucleotides for this base pair is sterically acceptable (S1).

The second triad is G1159=C1208/A1189 in *H.m.,* G1066=C1115/A1096 in *D.r.* and G1055=C1104/A1085 in *E.coli*. Thus the sequence is identical in all three structures. However, this triad forms type I A-minor motif in *D.r.* and *E.coli* structures, but not in *H.m.* due to the above-noted *syn* flip of A leading to interaction similar to *trans* H/SE with the G (see Figure 1A, S9). More than 95% of all sequences in all domains have G=C/A at this position and the observed covariations isosterically agree with both types of interactions seen in the x-ray structures. Since the overall compactness of the triad is similar in both cases perhaps the formation of the triad as a whole outweighs the role of local base pairs giving rise to it, as long as the formed structure does not impede the movement at this hinge. However, some refinement problems cannot be ruled out.

The base tetrad A1207/G1160…G1190/C1186 according to *H.m.* numbering represents two non-WC base pairs connected by bifurcated H-bonds between the two G's (Figure 1A, S7). The *trans* H/SE A1207/G1160 base pair is identical in all three structures (*trans* H/SE A1114/G1067 and A1103/G1056 in *D.r*. and *E.coli,* respectively), but covaries from A/G to A/A in sequences, being A/G in 74% of archaea and 93% of bacteria and >92% A/A in eukarya. Since A/G and A/A are isosteric in the *trans* H/SE family, the interaction is structurally conserved. The

trans WC/WC interaction between G1190/C1186 in *H.m.* is *trans* WC/WC A1097/U1093 in *D.r.* and *tran*s WC/WC A1086/U1082 in *E.coli*. In this position 99% of bacteria have A/U, 96% of eukrarya have G/C and archaea have 58% of G/C and 42% of A/U. The G/C and A/U *trans* WC/WC base pairs are nearly isosteric and their covariation is not surprising. The tertiary contact G1160…G1190 is made by bifurcated H-bonds between O2' of G1160 and O2' and C8 of G1190. This is replaced by identical G…A contacts in both bacterial organisms. Sequences belonging to the three domains are GA, AG, GG, and some AA with no clear preference. Isosteric sequence analysis of this position is not straightforward since it does not form any of the characterized types of base pairs (S1) but the requirement for purine bases is clearly seen. In conclusion, covariations of all the elements forming *Hinge2* core region conform to the isostericity rules, suggesting that the 3D structure of this region is universally conserved in all organisms even if individual nucleotides are not the same. This further indicates the vital importance of this area of the ribosome.

Structure and dynamics of base pairing in the rGAC area.

To investigate the substantial internal breathing of rGAC (Helix 43/44, EDA mode 2) we carried out detailed analysis of the dynamics of all base pairs. While all canonical base pairs were entirely stable the non-canonical interactions reveal considerable structural dynamics and primarily contribute to the internal breathing of the rGAC.

The stable base pairs are *cis* WC/WC A1161-U1185, G1162=C1184, G1163=C1183, A1166-U1180, G1167=C1179, C1168=G1178 and U1169-A1177 localized in Helix 43 and *cis* WC/WC U1206-A1191, *trans* H/SE A1202/G1197 and *trans* H/SE C1201/U1198 localized in Helix 44 (Figure 1A).

The most dynamical are base triads and tetrads compacting the Helix 43/44 structure. The first two base triads G1158=C1209/A1188 (Figure S12A) and G1159=C1208/A1189 (Figure S12B) form A-minor type II and unusual A-minor I like (with A1189 *syn*) interactions, respectively. Both triads reveal stable G=C interactions and dynamic C/A interaction. The first triad forms an A-minor type II which reversibly oscillates (*anti-syn* transition) between optimally paired geometry (0-2 ns; 3.5-15.5 ns) and geometry when A1188 (interacting only with C1209) is orthogonal to the plane of G1158=C1209 (2-3.5 ns and 15.5-31 ns intervals). The key interaction between C1209/A1188 sugars remains unchanged (Figure S12A). Note that the C1186 – G1190 sequence forms structural element that is known as IB type lonepair triloop which stacks with Helix 43 and structurally supports the „neck“ region between the *NC-stem* of Helix 42 and the compact *Head* of the rGAC (S7). The G1159=C1208/A1189 interaction reveals water mediated dynamics of the C1208/A1189 base contacts while the A/G contacts remain stable. The C1208(O2')-A1189(O2') distance oscillates between *open* geometry when water molecule mediates the C/A sugar - sugar interaction and direct H-bonding. The water-inserted substate is more populated during MD (with maximal binding time of individual water molecules up to 1.9 ns) than the *closed* arrangement with direct H-bond between C1208(O2')-A1189(O2'). It resembles A-minor type I dynamics seen in Kt-38 or Kt-42 simulations (Figures S12B and S11) (S4).

The *trans* H/SE A1207/G1160 … *trans* WC/WC G1190/C1186 base tetrad (Figure S12C) mediates another contact between Helices 43 and 44. It is stabilized by bifurcated H-bond between O2' of G1160 and O2' and C8 of G1190. The G1160…G1190 interaction is stable (Figure S12C). The *trans* H/SE A1207/G1160 base pair shows a series of substates when the

A1207(O2P)-G1160(N2) distance gradually and reversibly fluctuates between 2.9 Å, 4.7 Å, 5.3 Å and 7.3 Å, with lifetimes of the individual substates up to 2 ns (Figure S12D). The 4.7 Å distance is the most populated one and is accompanied with presence of A1207(O2P)-G1160(N1,N2) long-residency water bridges formed by two water molecules with maximal individual binding time up to 1.3 ns. Further increase of the distance is coupled with formation of quite common hydration site with water exchange times 100-300 ps (Figure S12D). The A1207(N7)-G1160(N2) and A1207(N6)-G1160(N3) H-bonds are stable. The *trans* WC/WC G1190/C1186 base pair of the base tetrad samples an alternative geometry when the contact between G1190(N2)-C1186(N3) is slightly shifted, and G1190(N1)-C1186(O2) and G1190(N1)-C1186(N3) distances increase due to insertion of water molecule or counter ion. The presence of individual Na^+ ions (binding time up to 1.5 ns, inner shell occupancy 44%) or water molecules (binding time up to 0.7 ns) between G1190(O6)-C1186(O2) stabilizes the *open* geometry of this base pair which alternates with *closed* (life time up to 2 ns) conformations when bifurcated G1190(N1)-C1186(O2) and G1190(N1)-C1186(N3) H-bonds occur. The *opening* and *closing* is pivoting at G1190(N1)-C1186(N3) H-bond (Figure S12E). We conclude that the interaction pattern seen in the triad/tetrad area of the simulated molecule is amazingly dynamical, being intimately associated with highly specific structural interactions with the solvent and ions.

Helix 43 then continues with three stable *cis* WC base pairs (1161-1163 and 1183-1185) followed by base triad U1164-A1192/C1182 (Figure S12F). The triad comprises entirely stable *cis* WC/WC U1164-A1192 and dynamical A1192…C1182 interaction (see below).

The x-ray arrangement of A1192...C1182 interaction (i.e. coplanar) is very similar to the G1160…G1190 interaction seen in the tetrad of *Hinge2* (Figure S7). We thus suggest that this interaction may represent a new type of base pairing which includes the bifurcated H-bond between O2' atom of one base and O2' and C8 atoms of second base.

MD simulation revealed, in contrast to x-ray, that the dynamical A…C interaction, which involves base-sugar A1192(C8)-C1182(O2') H-bond, A1192(C2')-C1182(O2) and A1192(C3')-C1182(O2) contacts, and some sugar-sugar contacts, is not coplanar but perpendicular (comparison between x-ray arrangement and MD geometry is shown in Figure S8). This uncommon perpendicular geometrical arrangement (lifetime up to 4 ns) additionally alternates with geometry (lifetime up to 1.2 ns) when the nucleotide C1182 shifts downwards with C1182 base closer to the A1192 sugar (i.e., the A/C base-sugar interaction is converted to A/C sugar-

base interaction). This shift disrupts all original base-sugar contacts and new A1192(O2')-C1182(N4) H-bond with some additional base-base and sugar-base contacts are formed (Figure S12F) without breaking the perpendicularity.

Moreover, the relative abundance of the two structural states [x-ray (coplanar) vs. MD (perpendicular), Figure S8] is further supported by crystal structure data. Using the motif search program FR3D (S8), we exhaustively searched the best ribosomal structures for interactions that fall within a small discrepancy of U1164-A1192/C1182 seen in *H.m.*. Thus, general search of similarly shaped contacts was preferred. Obtained results included both types of conformations discussed above (i.e. coplanar and perpendicular), confirming that these structural alternatives are both viable and structurally sound. In addition, the perpendicular form was found more than twice as often as the coplanar form, supporting the stable arrangement observed in the simulation. It suggests that the perpendicular form may indeed be stable enough to be considered as a new class of base pairs (see 3D motif search below, Table S1).

Helix 43 ends with UAGA tetraloop 1170-1173 stacked on *cis* WC/WC U1169-A1177 base pair. All hairpin bases are unpaired and accessible to mediate intermolecular contacts with other components of ribosome. Bases U1170 and A1173 are positioned to form *trans* SE/H interaction, but they are significantly twisted and out of plane and thus cannot form the hydrogen bonds required for base pairing. The unpaired bases A1171 and G1172 are stacked. This stacking is reversibly disrupted when unpaired base A1171 flips out (*anti-syn* transition), becoming more exposed to the solvent (lifetime up to 2.5 ns). Such flipping of A1171 (A1067 in *E.coli* and *T.maritima*) may play important role in the dynamic process of decoding and tRNA accommodation (S9).

Helix 44 continues after the A1207/G1160…G1190/C1186 tetrad (Figure S12C) with stable *cis* WC/WC U1206-A1191 base pair and three base triads U1205-A1194/A1193, C1204=G1195/G1175) and G1203=C1196/C1176 (Figure S13). (Note that the G1175 and C1176 belong to the Helix 43, to its sequence following the 3' end of the apical tetraloop). The first triple comprises the *cis* WC/WC U1205-A1194 and *cis* H/SE A1194/A1193 base pairs. The third triple comprises *cis* WC/WC G1203=C1196 and *cis* H/WC C1196/C1176 base pairs. All these interactions are stable during the simulation. In contrast, the second base triple C1204=G1195/G1175 (G1175 in *syn* orientation) reveals dynamical behavior of *cis* H/H G1195/G1175 base pair while its *cis* WC pair C1204=G1195 remains stable. The *cis* H/H

G1195/G1175 base pair slightly differs from its ideal description (S1) due to insertion of multiple exchanging monovalent Na^+ ions into the base pair. The ions occupy (85% of trajectory) the G1195(N7)-G1195(O6) binding pocket [50% of trajectory G1195(N7, O6)-G1175(N7)] with individual binding times up to 1.8 ns. The ions alternate with long-residency water molecules with maximal binding times up to 1.3 ns. The base pair is further stabilized by G1195(O6)-G1175(O6) water bridge mediated by long-residency water molecules with individual binding times up to 4 ns. This G/G geometry alternates with another arrangement when the positions of bases remain unchanged while the distance between them increases [G1195(O6)-G1175(N7) distance from 4.3 Å to 6.3 Å] what is necessary for accommodation or releasing of water molecule. This G/G base pair is ca. 50% of trajectory in *open* geometry when the water molecule is inside. Then Na^+ binding site alternates between G1195(N7, O6) (ca. 70% of *open* geometry) and G1175(N7, O6) (ca. 30% of *open* geometry). The dynamical behavior of this G/G pair can be thus considered as *open/closed* dynamics pivoting around the G1195(O6)-G1175(O6) link, coupled with dynamical insertion of solvent molecules (Figure S13B).

Helix 44 ends with UCAA tetraloop 1198-1201 where A1199 and A1200 are unpaired and stacked, accessible for interactions with adjacent ribosomal elements. The domain around A1199 (A1095 in *E.coli*) forms part of the binding domain for EF-G (S10).

Both terminal tetraloops of Helices 43 and 44 are additionally stabilized by inter-strand contacts G1172(O2')-A1200(C4'), G1172(C1')-A1200(O4') and A1173(C5')-A1200(O2'). As a result, the backbone dynamics is quite restricted, contrasting the unpaired bases A1171, G1172 and A1199 stochastically sampling different positions (e.g. bulged out and mutually stacked conformations).

rGAC contains also two mutually stacked unpaired bulged out bases G1165 and A1174 protruding to the solvent. Their positions are, however, stabilized by A1173(O1P)-G1165(N1), A1173(O1P)-G1165(N2) and A1174(N7)-A1200(O2') H-bonds which remain stable during the whole simulation. The highly conserved domain around A1174 (A1070 in *E.coli*) is well known as the site where rGAC interacts with elongation factor EF-G and L10/L7/L12·L11 protein complex (S3,S11,S12).

Results of the 3D motif search for *H.m.* triad U1164-A1192/C1182.

We used the motif search program FR3D (S8) to look for motifs similar to the triad U1164-A1192/C1182 observed in *H.m.* (Figure S8A). The search was carried out on six different ribosomal structures representing *H.m.*, *E.coli (E.c. in table)*, and *T.th. (T.t.* in the Table*)*. Thirty one candidates were found, but upon closer visual analysis, only fifteen of those hits contained an undistorted *cis* WC/WC interaction equivalent to the U-A in the query. These were further subdivided into two groups based on how the third nucleotide of the triad positioned itself against the *cis* WC/WC base pair: In the first group (11 hits), the third nucleotide was not coplanar with the *cis* WC/WC base pair but still formed H-bonds with it. In the second group (4 hits), the third nucleotide was coplanar with the *cis* WC/WC base pair. This group included two *cis* SE/SE and two *trans* SE/SE interactions. The Table S1 below summarizes these results.

Note that the search was carried out assuming the whole U1164-A1192/C1182 triad. Thus the occurrence of novel base pairs like G1160/G1190 or A1192/C1182 is likely much more frequent in the ribosome (the G1160/G1190 base pair was actually not detected by the search!). When attempting to directly search for the new base pair type (which does not correspond to any established base pair) the FR3D program was not able to discriminate a meaningful number of base pairs, there were thousands of hits.

PDB ID	Molecule	Resolution (A)	Reference	NT1	NT2	NT3	Result
2AW7	16S E.c.	3.50	(S13)	U 12	G 22	G 885	NT3 not coplanar with the *cis* WC/WC basepair formed by NT1 and NT2
1s72	23S H.m.	2.40	(S14)	C1104	G1239	A1078	
2J00	16S T.t.	2.80	(S15)	U 12	G 22	G 885	
2J01	23S T.t.	2.80	(S15)	C1007	G1136	A 980	
2AWB	23S E.c.	3.50	(S13)	C1007	G1136	A 980	
1J5E	16S T.t.	3.05	(S16)	U 12	G 22	G 885	
2J01	23S T.t.	2.80	(S15)	G 855	G 921	A2269	
2AWB	23S E.c.	3.50	(S13)	U2404	G2413	G 389	
1s72	23S H.m.	2.40	(S14)	C2556	G2579	A2680	
1s72	23S H.m.	2.40	(S14)	U 210	G 229	C 197	
2AWB	23S E.c.	3.50	(S13)	G1601	C1345	U1396	
1s72	23S H.m.	2.40	(S14)	U1164	A1192	C1182	NT3 forms *trans* SE/SE with NT2
2AWB	23S E.c.	3.50	(S13)	G 969	A 947	U 569	
2AWB	23S E.c.	3.50	(S13)	G2674	C2646	G2544	NT3 forms *cis* SE/SE with NT2
1s72	23S H.m.	2.40	(S14)	U1338	G1319	U 27	

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