SUPPORTING INFORMATION for
“Promoting RNA helical stacking via A-minor junctions”
by Cody Geary, Arkadiusz Chworos and Luc Jaeger

A. Supplemental Tables

Table S1 A: List of A-minor junction motifs identified in known X-ray atomic structures.

The location of the A-minor junction is specified by the nt positions of it (type I) A-minor bp interaction. The A-minor junction motif has been identified at **16** independent locations in **7** different families of RNA molecules (16S and 23S rRNA, RNase P RNA, group IA3 and group II introns and TPP and M-box riboswitches). Ec: *Escherichia coli*; Tt: *Thermus thermophilus*; Hm: *Haloarcula marismortui*. Bs: *Bacillus subtilis*; Bst: *Bacillus stearothermophilus*; Oi: *Oceanobacillus iheyensis;* 2x_bulge; double bulge motif; 3WJ and 4WJ: three-way and four way junctions, respectively; a-d: at the level of their 2D structures, these junctions are part of more extended junctions (a, H30-31-32 is part of a 4WJ; b, H36-37-38-39 is part of a 6WJ; c, H67-68-69-70-71 is part of a 5WJ; d, these junctions are part of pseudoknots).

RNA molecule	**PDB ID**	**Location A-minor A(n1):g(n2):c(n3)**	**Loop/receptor A-minor junction (A-min.J) motif (type of A-minor)**	**Junction type**	**Figure**
16S_rRNA					
Ec_16S_rRNA	2AVY	A196:G142:C221	GA/A-min.J (type I/II)	4WJ_ABCD	*S3*
Tt_16S_rRNA	1J5E	A196:G142:C221	GA/A-min.J (type I/II)	4WJ_ABCD	*S3*
Ec_16S_rRNA	2AVY	A959:G1221:C984	U(UAA)U triloop/helix A-min.J (type I/II)	3WJ_ACD[a]	*4*
Tt_16S_rRNA	1J5E	A959:G1221:C984	U(UAA)U triloop/helix A-min.J (type I/II)	3WJ_ACD[a]	*4*
23S_rRNA					
Ec_23S_rRNA	2AW4	A819:G942:C837	Sarcin loop/helix A-min.J (type I)	4WJ_ABCD[b]	*S3*
Tt_23S_rRNA	2J01	A819:G942:C837	Sarcin loop/helix A-min.J (type I)	4WJ_ABCD[b]	*S3*
Hm_23S_rRNA	1JJ2	A912:U1041:C930	Sarcin loop/helix A-min.J (type I)	4WJ_ABCD[b]	*S3*
Ec_23S_rRNA	2AW4	A1085:G1055:C1104	U(UAA)A triloop/helix A-min.J (type I/II)	4WJ_ABCD	*4*
Tt_23S_rRNA	2J01	A1085:G1055:C1104	U(UAA)A triloop/helix A-min.J (type I/II)	4WJ_ABCD	*4*
Hm_23S_rRNA	1JJ2	A1189:G1159:C1208	C(UAA)G triloop/helix A-min.J (type I/II)	4WJ_ABCD	*4*,S8C
Ec_23S_rRNA	2AW4	A1545:G1465:C1446	GA:NAA/helix A-min.J (type I/II)	4WJ_ABCD	*4*, 5B, 7C, *S7A*, S8D
Tt_23S_rRNA	2J01	A1545:G1465:C1446	GA:NAA/helix A-min.J (type I/II)	4WJ_ABCD	*4*
Hm_23S_rRNA	1JJ2	A1632:G1568:C1553	GNRA/helix A-min.J (type I/II)	4WJ_ABCD	3A, *4*, 7A, S6B, S8B
Ec_23S_rRNA	2AW4	A1928:G1904:C1836	C(UAA)G triloop/2x_bulge A-min.J (type I/II)	4WJ_ABCD[c]	*4*, 6, S8H
Tt_23S_rRNA	2J01	A1928:G1904:C1836	C(UAA)G triloop/2x_bulge A-min.J (type I/II)	4WJ_ABCD[c]	*4*, 6
Hm_23S_rRNA	1JJ2	A1969:G1945:C1892	U(UAA)G triloop/2x_bulge A-min.J (type I/II)	4WJ_ABCD[c]	*4*, 6, 7B
Ec_23S_rRNA	2AW4	A1969:C1833:G1972	Bulged-G/helix A-min.J (type I/Ib)	3WJ_ACD[c]	*S10*
Tt_23S_rRNA	2J01	A1969:U1833:A1972	Bulged-G/helix A-min.J (type I/Ib)	3WJ_ACD[c]	*S10*
Hm_23S_rRNA	1JJ2	A2010:C1889:G2013	Bulged-G/helix A-min.J (type I/Ib)	3WJ_ACD[c]	*S10*
Ec_23S_rRNA	2AW4	A2388:G2280:C2260	A:A internal loop/helix A-min.J (type I/I)	3WJ_ACD[d]	*S10*
Tt_23S_rRNA	2J01	A2388:G2280:C2260	A:A internal loop/helix A-min.J (type I/I)	3WJ_ACD[d]	*S10*
Hm_23S_rRNA	1JJ2	A2425:G2314:C2294	A:A internal loop/helix A-min.J (type I/I)	3WJ_ACD[d]	*S10*
Hm_23S_rRNA	1JJ2	A243:G274:C376	Internal loop/helix A-min.J (type I/II)	3WJ_ACD	*S10*, 7G
Ec_23S_rRNA	2AW4	A2565:G2545:C2520	U(UAA)A T-loop/helix A-min.J (type I/II)	4WJ_ABCD	*4*
Tt_23S_rRNA	2J01	A2565:G2545:C2520	U(UAA)A T-loop/helix A-min.J (type I/II)	4WJ_ABCD	*4*
Hm_23S_rRNA	1JJ2	A2600:G2580:C2555	U(UAA)A T-loop/helix A-min.J (type I/II)	4WJ_ABCD	*4*, 5B
RNase P type B					
Bs	1NBS	A178:G132:C234	U(GAA)A triloop/helix A-min.J (type I/II)	4WJ_ABCD	*4*
Bst	2A64	A188:G139:C244	U(GAA)A triloop/helix A-min.J (type I/II)	4WJ_ABCD	*4*
TPP riboswitch					
	2CKY	A72:C38:G8	GA:AA@helix-end/helix A-min.J (type I/I) (Expanded kink turn)	3WJ_ABC	*S10*, 7D
	2GDI	A84:C50:G16	GA_shared/helix A-min.J (type I/I) (Expanded kink turn)	3WJ_ABC	*S10*
	2CKY	G30:G11:G34	T-loop/helix A-min.J (type I/II)	3WJ_ACD	*S3*
	2GDI	G42:G19:A47	T-loop/helix A-min.J (type I/II)	3WJ_ACD	*S3*
M-Box riboswitch					
	2QBZ	A117:G84:C57	UA_h/ helix A-min.J (type I/Ib)	3WJ_ABC	*S10*
Group I intron					
Twort intron	1Y0Q	A157:U125:A189	Bulged-G/helix A-min.J (type I/Ib)	3WJ_ACD[d]	*S10*
Group II intron					
Oi intron	3BWP	A49:C197:A189	Bulged-G/helix A-min.J (type I)	3WJ_ACD[d]	*S10*, 7F

Table S1 B: List of 2bp_double_bulge motifs (2bp_2x_bulge) identified in known X-ray atomic structures. The location of the 2x_bulge is specified by the two middle WC bps. The S8-like and P12-like 2bp_2x_bulge motifs have been identified at **2** and **4** independent locations, respectively. Tma: *Thermatoga maritima*.

RNA molecule	PDB ID	RNA motif (location with 2D structure)	WC bps location Xa(n1)Ya:Yb(n2)Xb	Modularity	Figure
		S8-like 2bp_2x_bulge			
16S_rRNA					
Ec_16S_rRNA	2AVY	H21	A596G:C643U	S8 binding site	*S7A*
Tt_16S_rRNA	1J5E	H21	C596G:C643G	S8 binding site	*S7A*
23S_rRNA					
Ec_23S_rRNA	2AW4	H89	A2459U:U2492U	GNRA-like/receptor interaction	*S7A*
Tt_23S_rRNA	2J01	H89	A2459U:U2492U	GNRA-like/receptor interaction	*S7A*
Hm_23S_rRNA	1JJ2	H89	G2494U:U2527U	GNRA-like/receptor interaction	*S7A* ,S7B, S8I
		P12-like 2bp_2x_bulge			
23S_rRNA					
Ec_23S_rRNA	2AW4	H68	G1840U:A1901C	triloop/2x_bulge A-minor junction	6, S8H
Tt_23S_rRNA	2J01	H68	G1840U:A1901C	triloop/2x_bulge A-minor junction	6
Hm_23S_rRNA	1JJ2	H68	G1896U:A1942C	triloop/2x_bulge A-minor junction	6, 7B
5S_rRNA					
Ec_5S_rRNA	2AW4	H2	G0016C:G0067C	Loop A 3WJ-BCD	
Tt_5S_rRNA	2J01	H2	G0016C:G0067C	Loop A 3WJ-BCD	
Hm_5S_rRNA	1JJ2	H2	G0014C:G0066C	Loop A 3WJ-BCD	
RNase P RNA					
Bs Type B	1NBS	P10/P11	G0180U:A0321C	triloop/helix A-minor junction	*4*
Bst Type B	2A64	P10/P11	G0190U:A0241C	triloop/helix A-minor junction	*4*
Tma Type A	2A2E	P10/P11	G0107G:C0199C		
Tt Type A	1U9S	P10/P11	G0115U:A0227C		
Tt Type A	1U9S	P12/P13	C0139G:C0160G	triloop/2bp_2x_bulge interaction	5B, S8G

Table S2: List of the sequences of all the tectoRNA molecules used in this study

Name	**Sequence**
GAAA/CA:UG_A (wt)	GGAUGGGAAACGUGGUCCGAUCUGAAGGAGGCACGGAUUGGACUACGCUAAGUCGAUGAAGUGGACACGUCGAUUUAGUCAUUCUU
GAAA/CA:UG_B (wt)	GGAUGGGAAACGUGGUCCGAUCUGAAGUCCACACGGAUUGGACUACGCUAAGUCGAUGAAGCCUCCACGUCGAUUUAGUCAUUCUU
GUGA/CA:UG_A	GGAUGGGUGACGUGGUCCGAUCUGAAGGAGGCACGGAUUGGACUACGCUAAGUCGAUGAAGUGGACACGUCGAUUUAGUCAUUCUU
GUGA/CA:UG_B	GGAUGGGUGACGUGGUCCGAUCUGAAGUCCACACGGAUUGGACUACGCUAAGUCGAUGAAGCCUCCACGUCGAUUUAGUCAUUCUU
UUCG/CA:UG_A	GGAUGGUUCGCGUGGUCCGAUCUGAAGGAGGCACGGAUUGGACUACGCUAAGUCGAUGAAGUGGACACGUCGAUUUAGUCAUUCUU
UUCG/CA:UG_B	GGAUGGUUCGCGUGGUCCGAUCUGAAGUCCACACGGAUUGGACUACGCUAAGUCGAUGAAGCCUCCACGUCGAUUUAGUCAUUCUU
GAAA/GG:CC_A	GGAUGGGAAACGUGGUCCGAUCUGAAGGAGGCACGGAUUGGACUACGCCAAGUCGAUGAAGUGGACACGUCGAUUUGGUCAUUCUU
GAAA/GG:CC_B	GGAUGGGAAACGUGGUCCGAUCUGAAGUCCACACGGAUUGGACUACGCCAAGUCGAUGAAGCCUCCACGUCGAUUUGGUCAUUCUU
GUAA/GG:CC_A	GGAUGGGUAACGUGGUCCGAUCUGAAGGAGGCACGGAUUGGACUACGCCAAGUCGAUGAAGUGGACACGUCGAUUUGGUCAUUCUU
GUAA/GG:CC_B	GGAUGGGUAACGUGGUCCGAUCUGAAGUCCACACGGAUUGGACUACGCCAAGUCGAUGAAGCCUCCACGUCGAUUUGGUCAUUCUU
UUAAA/GG:CC_A	GGAUGUUAAAGUGGUCCGAUCUGAAGGAGGCACGGAUUGGACUACGCCAAGUCGAUGAAGUGGACACGUCGAUUUGGUCAUUCUU
UUAAA/GG:CC_B	GGAUGUUAAAGUGGUCCGAUCUGAAGUCCACACGGAUUGGACUACGCCAAGUCGAUGAAGCCUCCACGUCGAUUUGGUCAUUCUU
CUAAG/GG:CC_A	GGAUGCUAAGGUGGUCCGAUCUGAAGGAGGCACGGAUUGGACUACGCCAAGUCGAUGAAGUGGACACGUCGAUUUGGUCAUUCUU
CUAAG/GG:CC_B	GGAUGCUAAGGUGGUCCGAUCUGAAGUCCACACGGAUUGGACUACGCCAAGUCGAUGAAGCCUCCACGUCGAUUUGGUCAUUCUU
UUAAG/GG:CC_A	GGAUGUUAAGGUGGUCCGAUCUGAAGGAGGCACGGAUUGGACUACGCCAAGUCGAUGAAGUGGACACGUCGAUUUGGUCAUUCUU
UUAAG/GG:CC_B	GGAUGUUAAGGUGGUCCGAUCUGAAGUCCACACGGAUUGGACUACGCCAAGUCGAUGAAGCCUCCACGUCGAUUUGGUCAUUCUU
GAAA/11nt motif_A	GGAUGGGAAACGUGGUCCGAUCUGAAGGAGGCACGGAUUGGACUACGCUAAGUCGAUGAAGUGGACACGUCGAUAUGUCAUUCUU
GAAA/11nt motif_B	GGAUGGGAAACGUGGUCCGAUCUGAAGUCCACACGGAUUGGACUACGCUAAGUCGAUGAAGCCUCCACGUCGAUAUGUCAUUCUU
GGAA/11nt motif_A	GGAUGGGGAACGUGGUCCGAUCUGAAGGAGGCACGGAUUGGACUACGCUAAGUCGAUGAAGUGGACACGUCGAUAUGUCAUUCUU
GGAA/11nt motif_B	GGAUGGGGAACGUGGUCCGAUCUGAAGUCCACACGGAUUGGACUACGCUAAGUCGAUGAAGCCUCCACGUCGAUAUGUCAUUCUU
GGAA/R1_A	GGAUGGGGAACGUGGUCCGAUCUGAAGGAGGCACGGAUUGGACUACGCUGUGUCGAUGAAGUGGACACGUCGAAUCUGUCAUUCUU
GGAA/R1_B	GGAUGGGGAACGUGGUCCGAUCUGAAGUCCACACGGAUUGGACUACGCUGUGUCGAUGAAGCCUCCACGUCGAAUCUGUCAUUCUU
GAAA/R1_A	GGAUGGGAAACGUGGUCCGAUCUGAAGGAGGCACGGAUUGGACUACGCUGUGUCGAUGAAGUGGACACGUCGAAUCUGUCAUUCUU
GAAA/R1_B	GGAUGGGAAACGUGGUCCGAUCUGAAGUCCACACGGAUUGGACUACGCUGUGUCGAUGAAGCCUCCACGUCGAAUCUGUCAUUCUU
UUAAG_H68_Hm_A (wt)	GGAUGUUAAGGUGGUCCGAUCUGAAGGAGGCACGGAUUGGACUACGCCCAGUGCGAUGAAGUGGACACGUCGUCAACGGUCAUUCUU
UUAAG/H68_Hm_B (wt)	GGAUGUUAAGGUGGUCCGAUCUGAAGUCCACACGGAUUGGACUACGCCCAGUGCGAUGAAGCCUCCACGUCGUCAACGGUCAUUCUU
CUAAG/H68_Hm_A	GGAUGCUAAGGUGGUCCGAUCUGAAGGAGGCACGGAUUGGACUACGCCCAGUGCGAUGAAGUGGACACGUCGUCAACGGUCAUUCUU
CUAAG/H68_Hm_B	GGAUGCUAAGGUGGUCCGAUCUGAAGUCCACACGGAUUGGACUACGCCCAGUGCGAUGAAGCCUCCACGUCGUCAACGGUCAUUCUU
UUAUG/H68_Hm_A	GGAUGUUAUGGUGGUCCGAUCUGAAGGAGGCACGGAUUGGACUACGCCCAGUGCGAUGAAGUGGACACGUCGUCAACGGUCAUUCUU
UUAUG/H68_Hm_B	GGAUGUUAUGGUGGUCCGAUCUGAAGUCCACACGGAUUGGACUACGCCCAGUGCGAUGAAGCCUCCACGUCGUCAACGGUCAUUCUU
CUAAG/H68_Ec_A (wt)	GGAUGCUAAGGUGGUCCGAUCUGAAGGAGGCACGGAUUGGACUACGCCCGGUGCGAUGAAGUGGACACGUCGUAAACGGUCAUUCUU

CUAAG/H68_Ec_B (wt)	GGAUGCUAAGGUGGUCCGAUCUGAAGUCCACACGGAUUGGACUACGCCCGGUGCGAUGAAGCCUCCACGUCGUAAACGGUCAUUCUU
CUAAG/H68_Tt_A (wt)	GGAUGCUAAGGUGGUCCGAUCUGAAGGAGGCACGGAUUGGACUACGCCCGGUGCGAUGAAGUGGACACGUCGUGAACGGUCAUUCUU
CUAAG/H68_Tt_B (wt)	GGAUGCUAAGGUGGUCCGAUCUGAAGUCCACACGGAUUGGACUACGCCCGGUGCGAUGAAGCCUCCACGUCGUGAACGGUCAUUCUU
UUAAG/H68_Sc_A (wt)	GGAUGUUAAGGUGGUCCGAUCUGAAGGAGGCACGGAUUGGACUACGCCCAGUGCGAUGAAGUGGACACGUCGUAAACGGUCAUUCUU
UUAAG/H68_Sc_B (wt)	GGAUGUUAAGGUGGUCCGAUCUGAAGUCCACACGGAUUGGACUACGCCCAGUGCGAUGAAGCCUCCACGUCGUAAACGGUCAUUCUU
CUAAG/P12_(#A)_A	GGAUGCUAAGGUGGUCCGAUCUGAAGGAGGCACGGAUUGGACUACGCCAGCGGCGAUGAAGUGGACACGUCGUGCGGGUCAUUCUU
CUAAG/P12_(#A)_B	GGAUGCUAAGGUGGUCCGAUCUGAAGUCCACACGGAUUGGACUACGCCAGCGGCGAUGAAGCCUCCACGUCGUGCGGGUCAUUCUU
CUAAG/P12_neg(#A)_A	GGAUGCUAAGGUGGUCCGAUCUGAAGGAGGCACGGAUUGGACUACGCCACGGCGAUGAAGUGGACACGUCGUGCGGGUCAUUCUU
CUAAG/P12_neg(#A)_B	GGAUGCUAAGGUGGUCCGAUCUGAAGUCCACACGGAUUGGACUACGCCACGGCGAUGAAGCCUCCACGUCGUGCGGGUCAUUCUU
CUAUG/P12_(#A)_A	GGAUGCUAUGGUGGUCCGAUCUGAAGGAGGCACGGAUUGGACUACGCCAGCGGCGAUGAAGUGGACACGUCGUGCGGGUCAUUCUU
CUAUG/P12_(#A)_B	GGAUGCUAUGGUGGUCCGAUCUGAAGUCCACACGGAUUGGACUACGCCAGCGGCGAUGAAGCCUCCACGUCGUGCGGGUCAUUCUU
CUAAG/P12_(#B)_A	GGAUGCUAAGGUGGUCCGAUCUGAAGGAGGCACGGAUUGGACUACGCCGAUGGCGAUGAAGUGGACACGUCGAUCAGGUCAUUCUU
CUAAG/P12_(#B)_B	GGAUGCUAAGGUGGUCCGAUCUGAAGUCCACACGGAUUGGACUACGCCGAUGGCGAUGAAGCCUCCACGUCGAUCAGGUCAUUCUU
CUAAG/P12_(#C)_A	GGAUGCUAAGGUGGUCCGAUCUGAAGGAGGCACGGAUUGGACUACGCCGAUGGCGAUGAAGUGGACACGUCGCACAGGUCAUUCUU
CUAAG/P12_(#C)_B	GGAUGCUAAGGUGGUCCGAUCUGAAGUCCACACGGAUUGGACUACGCCGAUGGCGAUGAAGCCUCCACGUCGCACAGGUCAUUCUU
Internal_GNRA/H57_Ec_A	GGAUGCGAGCUUCGGUGAAGGUGGUCCGAUCUGAAGGAGGCACGGAUUGGACUACGCCAAGUCGAUGAAGUGGACACGUCGAUUUGGUCAUUCUU
Internal_GNRA/H57_Ec_B	GGAUGCGAGCUUCGGUGAAGGUGGUCCGAUCUGAAGUCCACACGGAUUGGACUACGCCAAGUCGAUGAAGCCUCCACGUCGAUUUGGUCAUUCUU
Internal_GNRA/H57_Ec_neg_A	GGAUGCGAGCUUCGGUGACGGUGGUCCGAUCUGAAGGAGGCACGGAUUGGACUACGCCAAGUCGAUGAAGUGGACACGUCGAUUUGGUCAUUCUU
Internal_GNRA/H57_Ec_neg_B	GGAUGCGAGCUUCGGUGACGGUGGUCCGAUCUGAAGUCCACACGGAUUGGACUACGCCAAGUCGAUGAAGCCUCCACGUCGAUUUGGUCAUUCUU
UA_h_GNRA/U999_A	GGAUGCGAAGCUUCGGCUAAGGUGGUCCGAUCUGAAGGAGGCACGGAUUGGACUACGCCAAGUCGAUGAAGUGGACACGUCGAUUUGGUCAUUCUU
UA_h_GNRA/U999_B	GGAUGCGAAGCUUCGGCUAAGGUGGUCCGAUCUGAAGUCCACACGGAUUGGACUACGCCAAGUCGAUGAAGCCUCCACGUCGAUUUGGUCAUUCUU
UA_h_GNRA/U999_neg_A	GGAUGCGAAGCUUCGGCUACGGUGGUCCGAUCUGAAGGAGGCACGGAUUGGACUACGCCAAGUCGAUGAAGUGGACACGUCGAUUUGGUCAUUCUU
UA_h_GNRA/U999_neg_B	GGAUGCGAAGCUUCGGCUACGGUGGUCCGAUCUGAAGUCCACACGGAUUGGACUACGCCAAGUCGAUGAAGCCUCCACGUCGAUUUGGUCAUUCUU
Hairpin Junction (fiber)_A	GGGAACCUGAGGUUCGCUUCAACCGUCCGAUCUGAAGGAGGCACGGAUUGGACGGUACGAAGUCGAUGAAGUGGACACGUCGAUUUCGUGGUUCCUU
Hairpin Junction (fiber)_B	GGGAACCUGAGGUUCGCUUCAACCGUCCGAUCUGAAGUCCACACGGAUUGGACGGUACGAAGUCGAUGAAGCCUCCACGUCGAUUUCGUGGUUCCUU
Hairpin Junction (dimer)_A	GGGACGUGGUGGUUCGCUACCUGAGUCCGAUCUGAAGGAGGCACGGAUUGGACUCAACCAAGUCGAUGAAGUGGACACGUCGAUUUGGUACGUCCUU
Hairpin Junction (dimer)_B	GGGACGUGGUGGUUCGCUACCUGAGUCCGAUCUGAAGUCCACACGGAUUGGACUCAACCAAGUCGAUGAAGCCUCCACGUCGAUUUGGUACGUCCUU

B. Supplementary References

1. Leontis, N.B. and Westhof, E. (2001) Geometric nomenclature and classification of RNA base pairs. *RNA*, **7,** 499-512.
2. DeLano, W.L. (2002) The PyMOL Molecular Graphics System.

C. Supplementary Information Figure Captions

Figure S1: Three-dimensional models of RNA four-way junction conformers. The conformers I, II, III, IV and O predicted for RNA four-way junctions presented in Figure 1 were modeled in Swiss PDB viewer and rendered as three-dimensional stereo-view images.

Figure S2: Study of hairpin ribozyme junction. (A) The four-way junction of the hairpin ribozyme within its natural context, including the adjacent tertiary interaction. **(B)** H40-H41-H45 consensus from the 23S rRNAs from Archaea and Bacteria has similarities to the hairpin ribozyme junction, including a neighboring tertiary interaction at a similar distance from the junction. **(C)** Self-assembly native PAGE experiments with 4WJ-tectoRNAs taking advantage of hairpin ribozyme 4WJ junction in two different orientations. The gels were measured in presence of 1 mM $Mg(OAc)_2$ and TB 1X (see Materials and Methods). Lane m corresponds to the monomer A alone.

Figure S3: Extended A-minor junction network. Extended version of the network described in Figure 4, containing all identified A-minor junctions. Submotifs combine in multiple ways to generate a variety of functionally equivalent A-minor junction motifs. The lines on the network indicate which motifs can be combined. Interactions are indicated according to the Leontis-Westhof nomenclature (1) (see in box Legend).

Figure S4: Kissing loops used for 4WJ-tectoRNAs self-assembly. (A) Three-dimensional view of the DIS HIV kissing-loop (KL) interaction (PDB_ID: 1JJM) and motif diagram. **(B)** Schematic of the two different KL interactions L1-L1' and L2-L2' used in this study.

Figure S5: AFM analysis. (A) Representative AFM images of GUGA/CU:AG 4WJ-tectoRNA nano-rings. **(B)** Histogram of the size distribution of nano-rings observed in the images from A. **(C)** The periodicity observed of bumpy features along one-dimensional tectoRNA fibers by AFM can vary related to the bending of the fibers. In the model of the conformer **I** fibers the 4WJ motif creates a bulky density with a periodic spacing between units of approximately ~9 nm. By AFM analysis, this periodic spacing is closer to ~10 nm, likely due to bending. To describe the shape expected for the rings composed of end-to-end assembled units, products of the intermediate-length assembly were expected to have a circumference of length approximately 2N*(periodicity), where N is an integer greater than 1. A value of ~11 nm was measured for the periodicity in ring-circumferences. Experiments were performed as described in Materials and Methods.

Figure S6: Additional PAGE gels. (A) Self-assembly PAGE assay for composite GGAA/R(1)-receptor 4WJ tectoRNAs. **(B)** PAGE gels demonstrating the effect of combining different A+B units in the same assembly. Experiments were performed as described in Materials and Methods. (left to right) GAAA/CU:AG (A+B), GAAA/CU:AG (A) + GUAA/GG:CC (B), GUAA/GG:CC (A) + GAAA/CU:AG (B), GUAA/GG:CC (A+B). Lane m corresponds to the monomer A alone.

Figure S7: Study of GNRA-like/receptor interactions. (A) GNRA-like/receptor motif network showing the modularity of GA:NAA/helix, UAA:GAN/helix and L39-H89 motifs. Interactions are indicated according to the Leontis Westhof nomenclature (1) (see in box Legend in Figure S3). **(B)** Corresponding gel experiments with 4WJ-tectoRNAs taking advantage of GNRA-like motifs.

Figure S8: Three-dimensional representations of various A-minor interactions. On the left are shown a series of loop/helix A-minor interactions and junctions, on the right are loop/receptor A-minor interactions and junctions. In the stereo images, the interacting adenine loop is colored green. The Type I

A-minor binding site is colored blue while the Type II A-minor binding site is orange. All the A-minor loop/receptor interactions depicted include nt platforms shown in yellow with surface. Note that the color code on 2D diagrams does not correspond to the one on the 3D stereo images. **(A)** GyRA/helix A-minor interaction (Type I/IIP) (PDB_ID: 1JJ2); **(B)** GNRA/helix A-minor junction (Type I/IIP) (PDB_ID: 1JJ2); **(C)** triloop/helix A-minor junction (Type I/IIP) (PDB_ID: 1JJ2); **(D)** GA:NAA/helix A-minor junction (Type I/IIP) (PDB_ID: 2AW4); **(E)** UAA:GAN/helix A-minor interaction (Type I/IIT) (PDB_ID: 2AW4); **(F)** GAAA/11nt-motif A-minor interaction (Type I/IIT) (PDB_ID: 1U6B); **(G)** L13-loop/P12-receptor A-minor interaction (Type I/IIT) (PDB_ID: 1U9S); **(H)** L70-triloop/H68-receptor A-minor junction (Type I/IIT) (PDB_ID: 2AW4); **(I)** GNRA-like/S8-like receptor A-minor interaction (Type I/IIT) (L39-H89) (PDB_ID: 1JJ2). Images were rendered in Pymol (2).

Figure S9: Non-stoichiometric assemblies. Native PAGE assembly of non-stoichiometric mixes of tectoRNA fiber units A+B. By increasing the ratio of molecule B to molecule A, the self-assembly equilibrium is shifted towards the formation of B-A-B trimers. This was used to differentiate between the opened and cyclic dimers, and linear trimer. The experiment highlights differences between two functional classes of 4WJ motifs: more-flexible and more-rigid.

Figure S10: Three-way junction A-minor network. Extended A-minor motif network describing all the various A-minor junctions (3WJ) and their relationship with the Kink turn motif. Interactions are indicated according to the Leontis-Westhof nomenclature (1) (see in box Legend).

D. Supplementary Figures

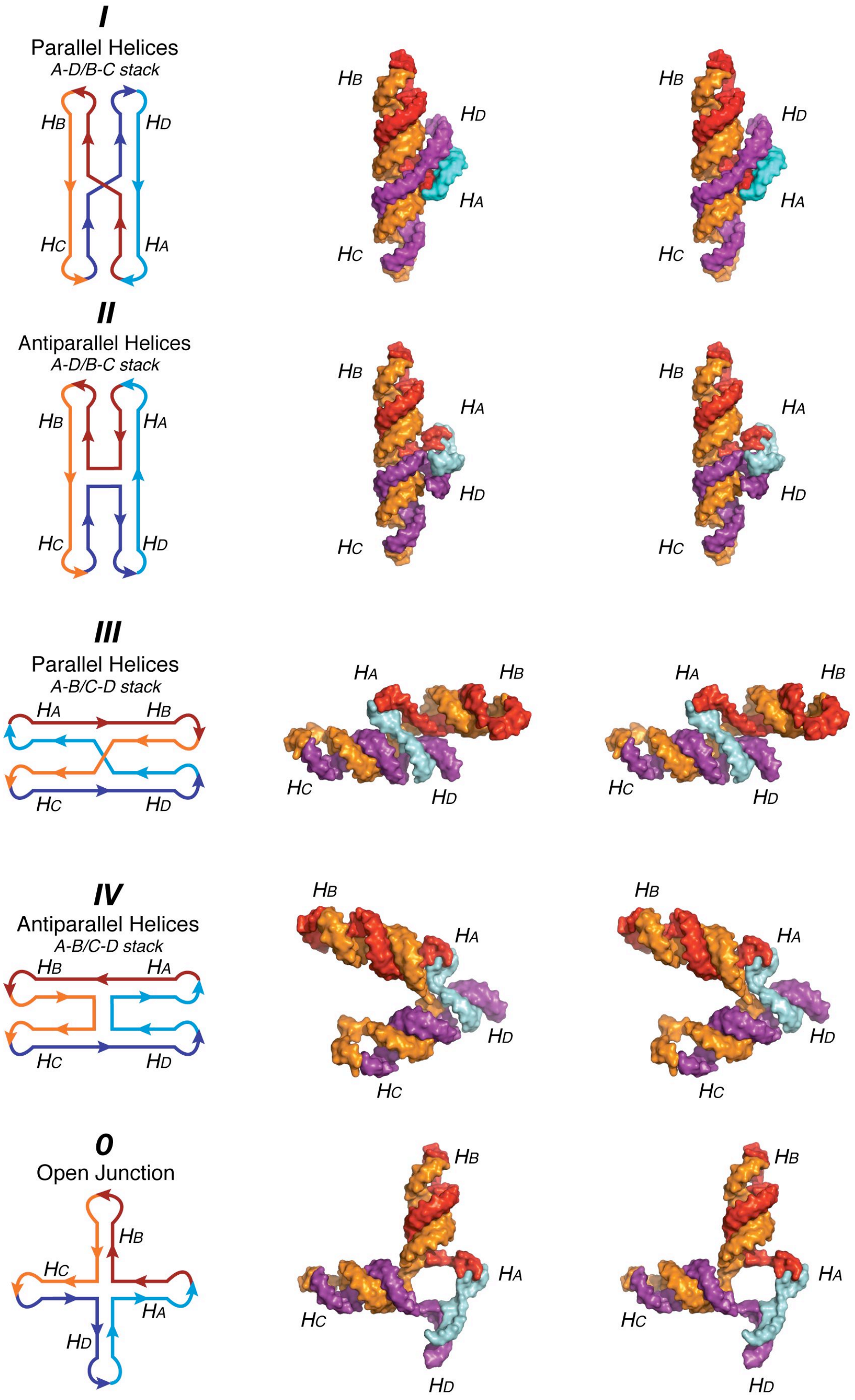
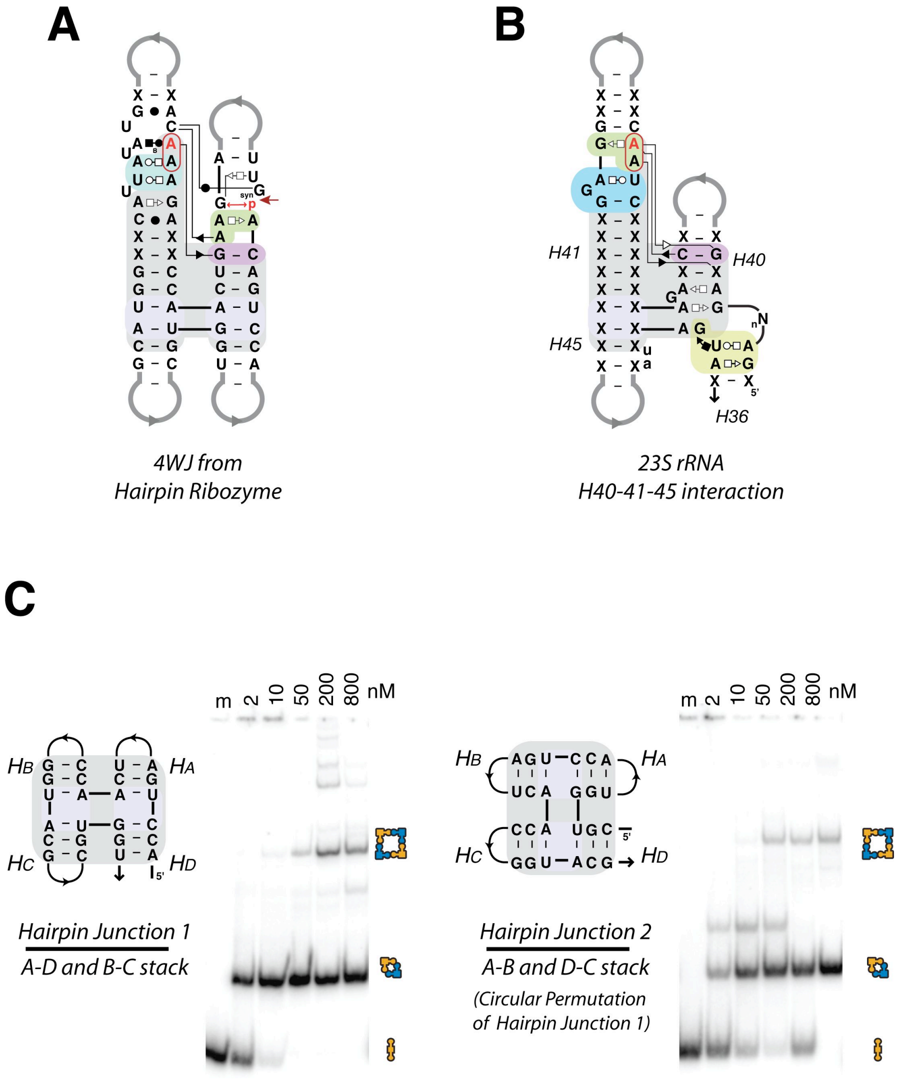


Figure S1



4WJ from
Hairpin Ribozyme

23S rRNA
H40-41-45 interaction

Hairpin Junction 1
A-D and B-C stack

Hairpin Junction 2
A-B and D-C stack
(Circular Permutation
of Hairpin Junction 1)

Figure S2

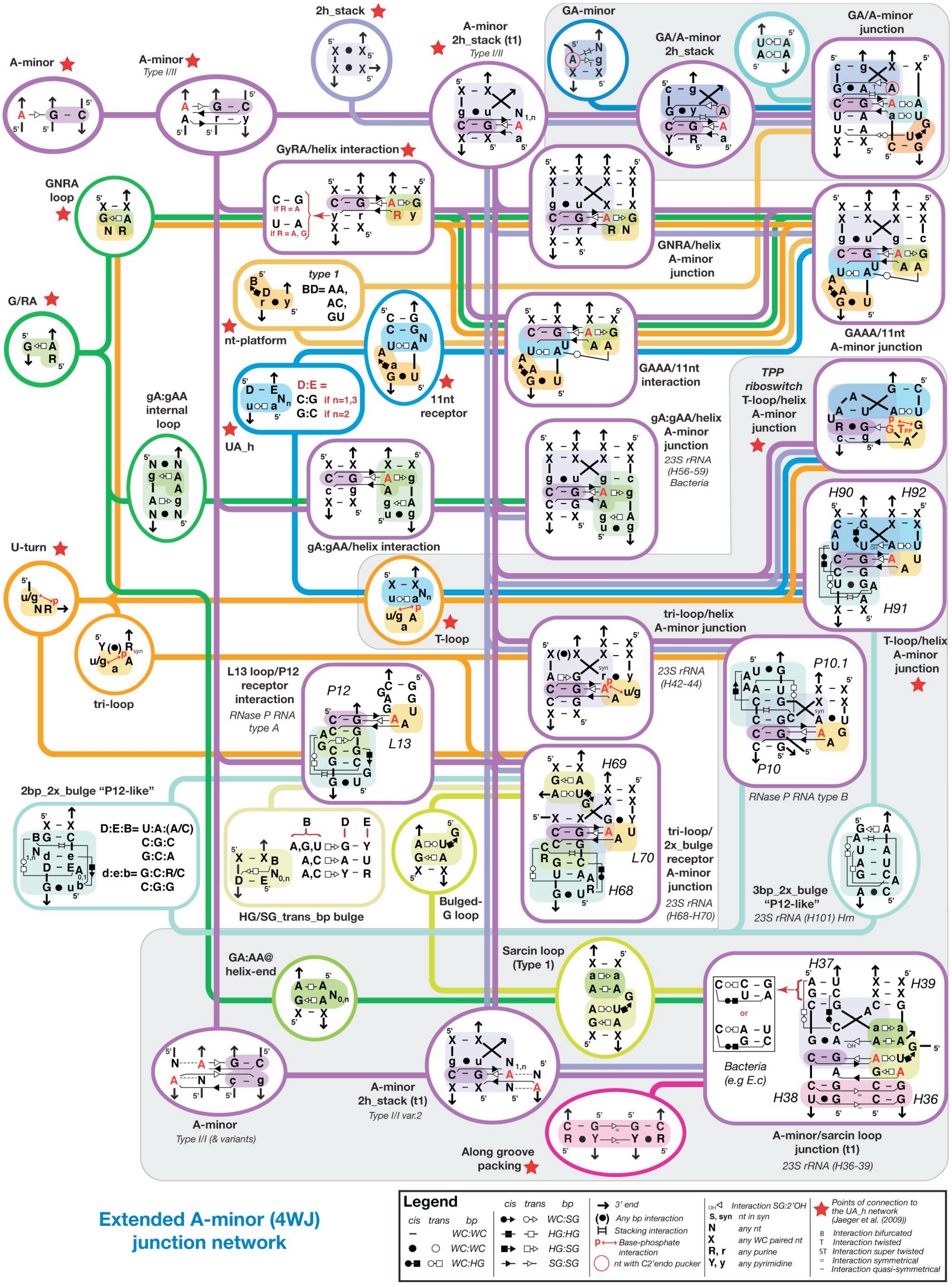

2h_stack
A-minor
A-minor
Type I/II
A-minor
2h_stack (t1)
Type I/II
GA-minor
GA/A-minor
2h_stack
GA/A-minor
junction
GyRA/helix interaction
if R = A
if R = A, G
GNRA
loop
GNRA/helix
A-minor
junction
G/RA
type 1
BD= AA,
AC,
GU
nt-platform
11nt
receptor
GAAA/11nt
interaction
GAAA/11nt
A-minor junction
D:E =
C:G if n=1,3
G:C if n=2
UA_h
gA:gAA
internal
loop
gA:gAA/helix interaction
gA:gAA/helix
A-minor
junction
23S rRNA
(H56-59)
Bacteria
TPP
riboswitch
T-loop/helix
A-minor
junction
H90
H92
H91
U-turn
T-loop
tri-loop
tri-loop/helix
A-minor junction
23S rRNA
(H42-44)
T-loop/helix
A-minor
junction
L13 loop/P12
receptor
interaction
RNase P RNA
type A
P12
L13
P10.1
P10
RNase P RNA type B
2bp_2x_bulge "P12-like"
D:E:B= U:A:(A/C)
C:G:C
G:C:A
d:e:b= G:C:R/C
C:G:G
HG/SG_trans_bp bulge
Bulged-
G loop
H69
L70
H68
tri-loop/
2x_bulge
receptor
A-minor
junction
23S rRNA
(H68-H70)
3bp_2x_bulge
"P12-like"
23S rRNA (H101) Hm
GA:AA@
helix-end
Sarcin loop
(Type 1)
A-minor
Type I/I (& variants)
A-minor
2h_stack (t1)
Type I/I var.2
Along groove
packing
H37
H39
H38
H36
Bacteria
(e.g E.c)
A-minor/sarcin loop
junction (t1)
23S rRNA (H36-39)
Extended A-minor (4WJ)
junction network
Legend
cis trans bp
WC:WC
WC:WC
WC:HG
WC:SG
HG:HG
HG:SG
SG:SG
3' end
Any bp interaction
Stacking interaction
Base-phosphate interaction
nt with C2'endo pucker
Interaction SG:2'OH
S, syn nt in syn
N any nt
X any WC paired nt
R, r any purine
Y, y any pyrimidine
Points of connection to the UA_h network (Jaeger et al. (2009))
B Interaction bifurcated
T Interaction twisted
ST Interaction super twisted
= Interaction symmetrical
~ Interaction quasi-symmetrical

Figure S3

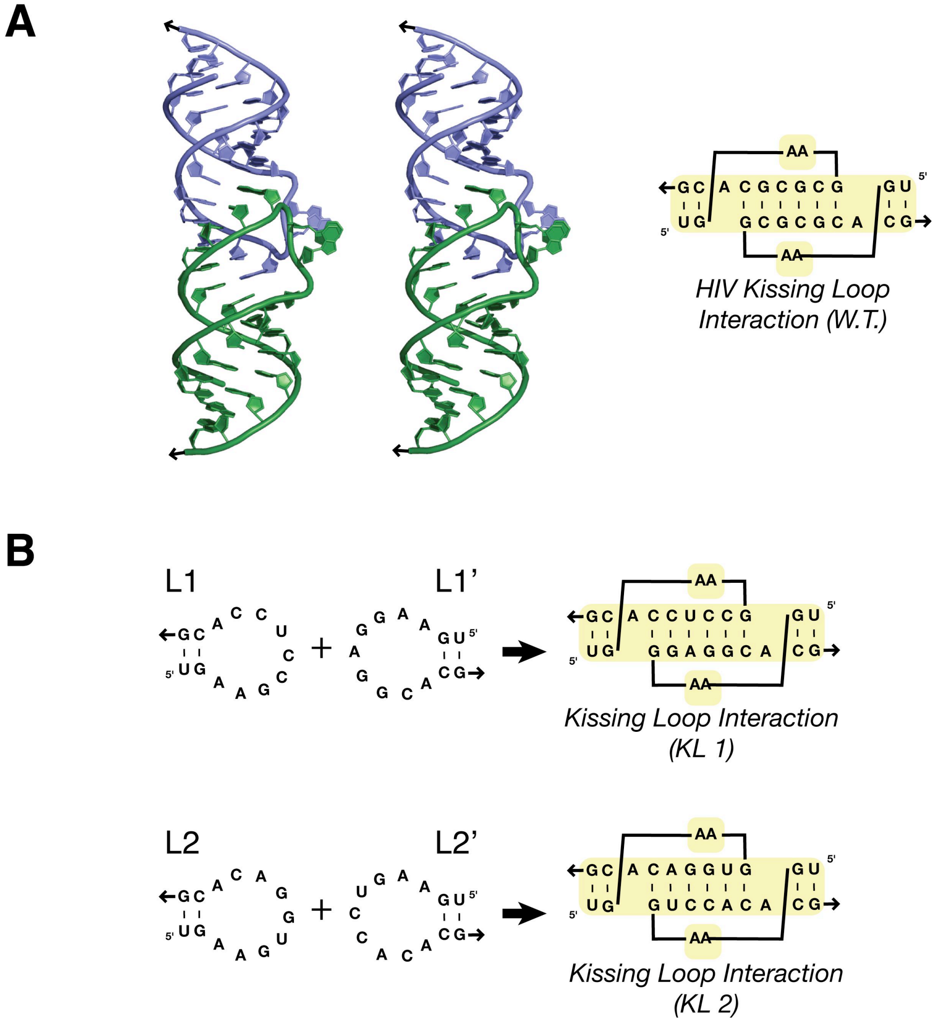

A
AA
←GC A C G C G C G GU 5'
5' UG G C G C G C A CG→
AA
HIV Kissing Loop Interaction (W.T.)
B
L1
L1'
←GC A C C U C A G A A G 5' UG
G G A A GU 5' A G G C A CG→
AA
←GC A C C U C C G GU 5'
5' UG G G A G G C A CG→
AA
Kissing Loop Interaction (KL 1)
L2
L2'
←GC A C A G G U G A A 5' UG
U G A A GU 5' C C C A C A CG→
AA
←GC A C A G G U G GU 5'
5' UG G U C C A C A CG→
AA
Kissing Loop Interaction (KL 2)

Figure S4

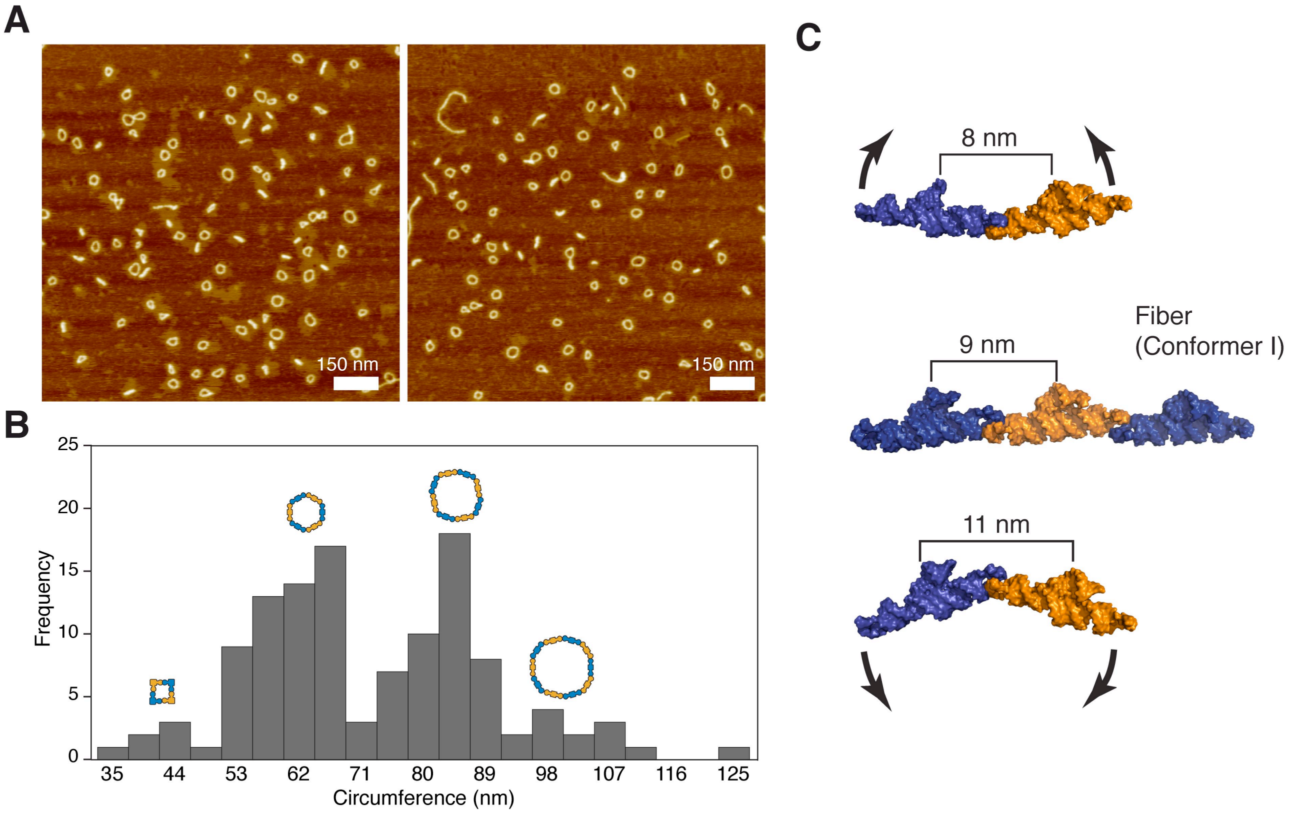

A
150 nm
150 nm
B
25
20
15
10
5
0
Frequency
35
44
53
62
71
80
89
98
107
116
125
Circumference (nm)
C
8 nm
9 nm
Fiber
(Conformer I)
11 nm

Figure S5

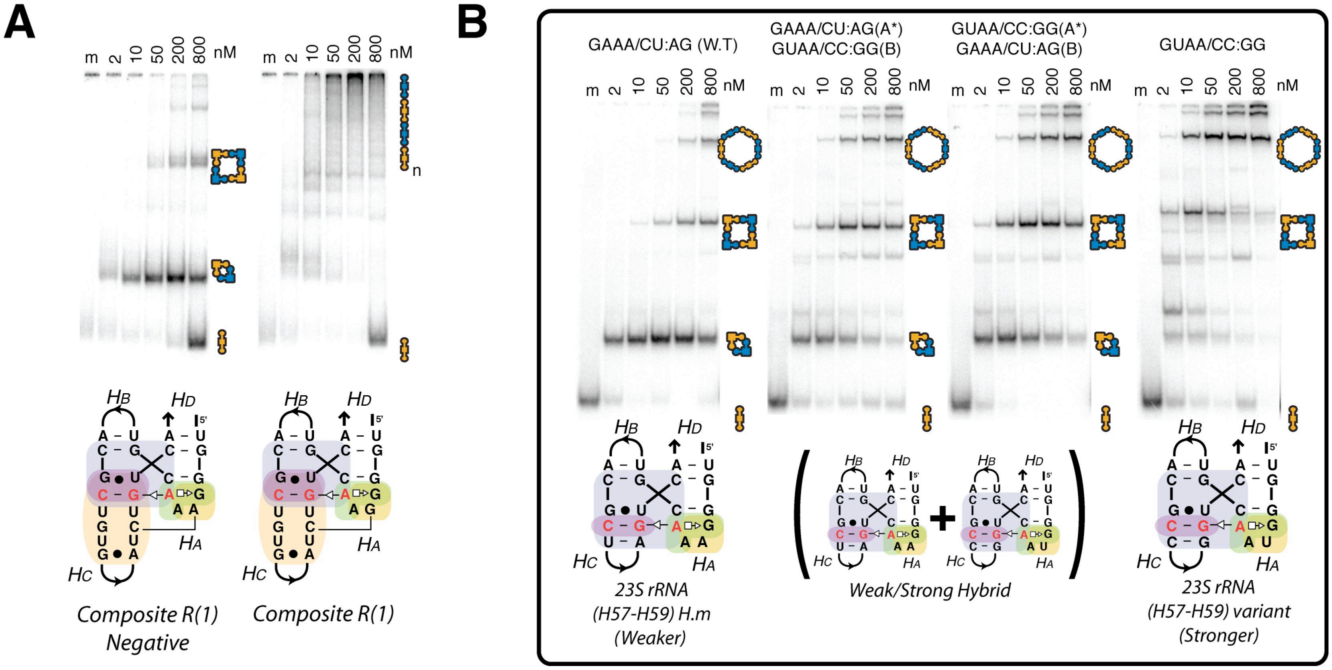

A
m
2
10
50
200
800
nM
n
HB
HD
HC
HA
5'
Composite R(1) Negative
Composite R(1)
B
GAAA/CU:AG (W.T)
GAAA/CU:AG(A*)
GUAA/CC:GG(B)
GUAA/CC:GG(A*)
GAAA/CU:AG(B)
GUAA/CC:GG
23S rRNA (H57-H59) H.m (Weaker)
Weak/Strong Hybrid
23S rRNA (H57-H59) variant (Stronger)

Figure S6

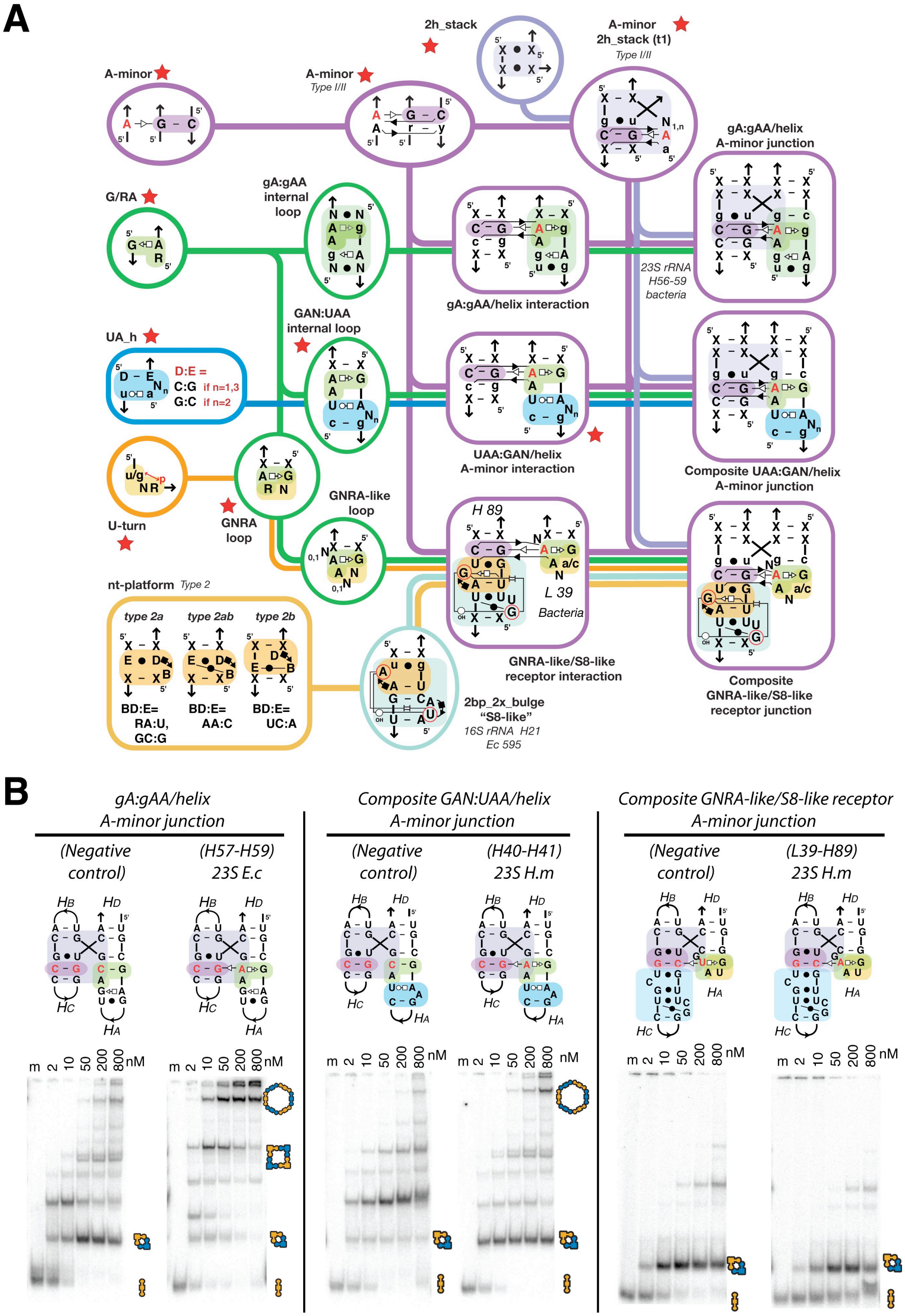

A
A-minor
A-minor
Type I/II
2h_stack
A-minor
2h_stack (t1)
Type I/II
G/RA
gA:gAA
internal
loop
gA:gAA/helix
A-minor junction
gA:gAA/helix interaction
23S rRNA
H56-59
bacteria
UA_h
D:E =
C:G if n=1,3
G:C if n=2
GAN:UAA
internal loop
UAA:GAN/helix
A-minor interaction
Composite UAA:GAN/helix
A-minor junction
U-turn
GNRA
loop
GNRA-like
loop
H 89
L 39
Bacteria
GNRA-like/S8-like
receptor interaction
Composite
GNRA-like/S8-like
receptor junction
nt-platform Type 2
type 2a
type 2ab
type 2b
BD:E=
RA:U,
GC:G
BD:E=
AA:C
BD:E=
UC:A
2bp_2x_bulge
"S8-like"
16S rRNA H21
Ec 595
B
gA:gAA/helix
A-minor junction
(Negative
control)
(H57-H59)
23S E.c
Composite GAN:UAA/helix
A-minor junction
(Negative
control)
(H40-H41)
23S H.m
Composite GNRA-like/S8-like receptor
A-minor junction
(Negative
control)
(L39-H89)
23S H.m
HB
HD
HC
HA
m
2
10
50
200
800
nM

Figure S7

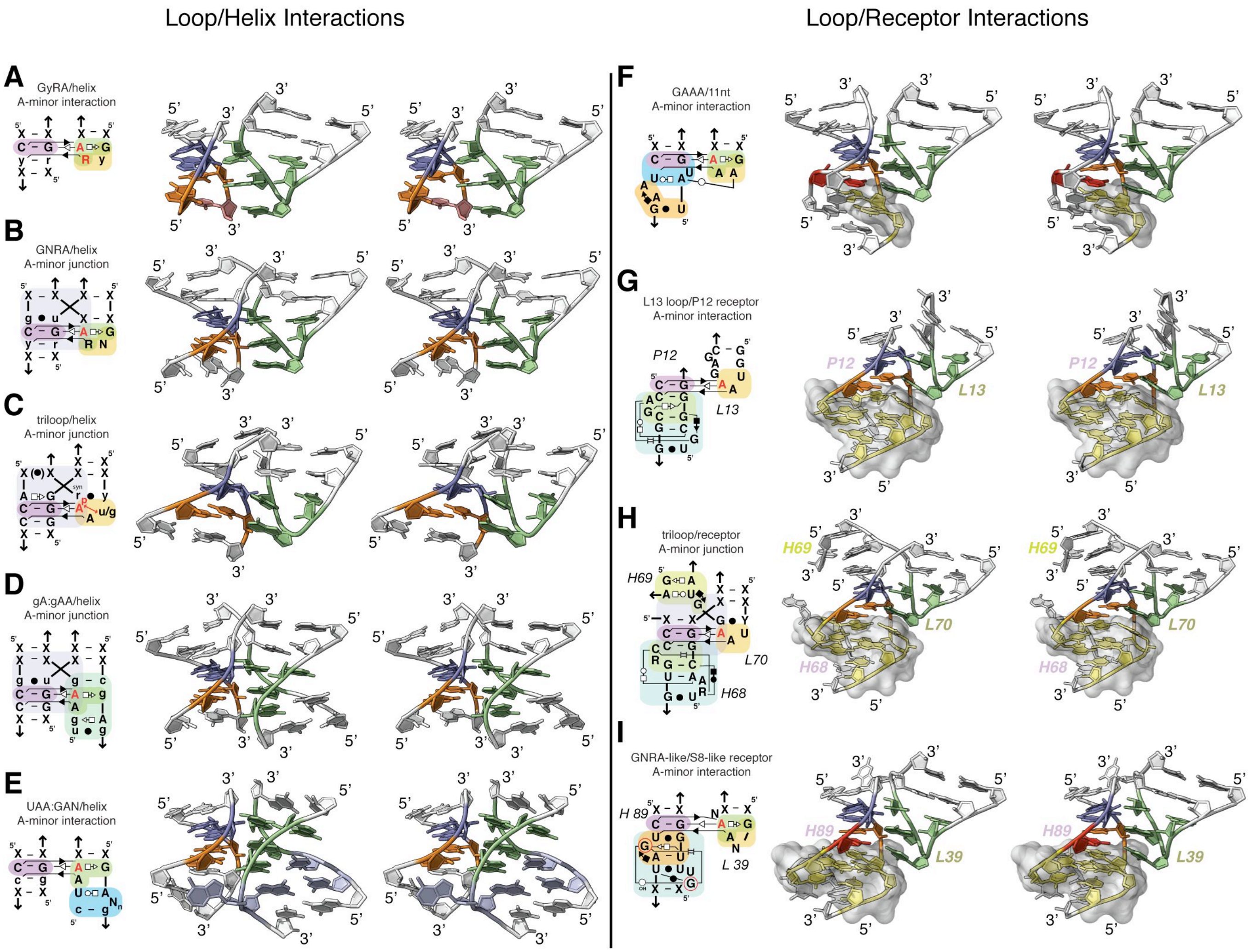

Loop/Helix Interactions
Loop/Receptor Interactions
A
GyRA/helix
A-minor interaction
B
GNRA/helix
A-minor junction
C
triloop/helix
A-minor junction
D
gA:gAA/helix
A-minor junction
E
UAA:GAN/helix
A-minor interaction
F
GAAA/11nt
A-minor interaction
G
L13 loop/P12 receptor
A-minor interaction
P12
L13
H
triloop/receptor
A-minor junction
H69
L70
H68
I
GNRA-like/S8-like receptor
A-minor interaction
H 89
L 39
5'
3'

Figure S8

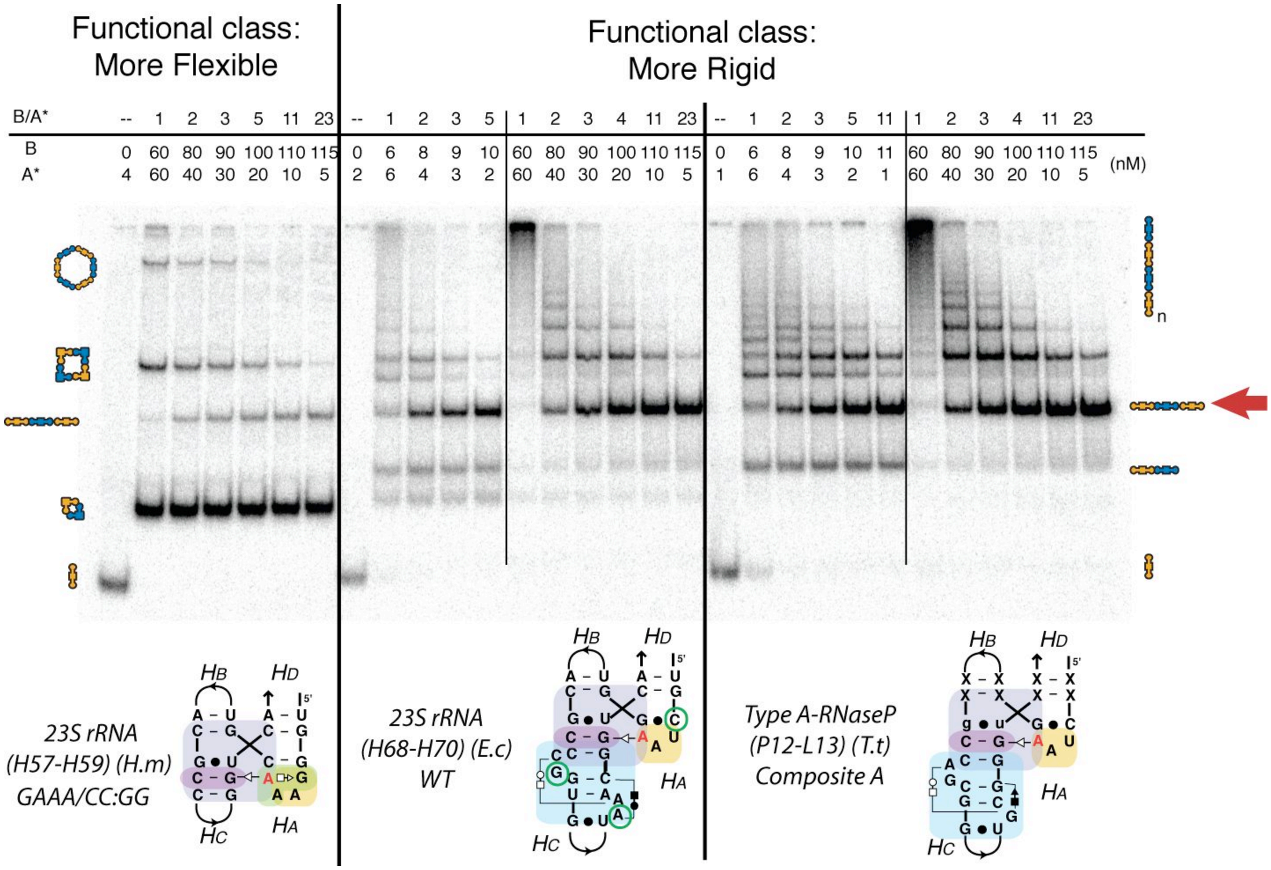

Functional class: More Flexible
Functional class: More Rigid
B/A* -- 1 2 3 5 11 23 -- 1 2 3 5 1 2 3 4 11 23 -- 1 2 3 5 11 1 2 3 4 11 23
B 0 60 80 90 100 110 115 0 6 8 9 10 60 80 90 100 110 115 0 6 8 9 10 11 60 80 90 100 110 115
A* 4 60 40 30 20 10 5 2 6 4 3 2 60 40 30 20 10 5 1 6 4 3 2 1 60 40 30 20 10 5
(nM)
n
23S rRNA (H57-H59) (H.m) GAAA/CC:GG
23S rRNA (H68-H70) (E.c) WT
Type A-RNaseP (P12-L13) (T.t) Composite A
HB
HD
HC
HA
5'

Figure S9

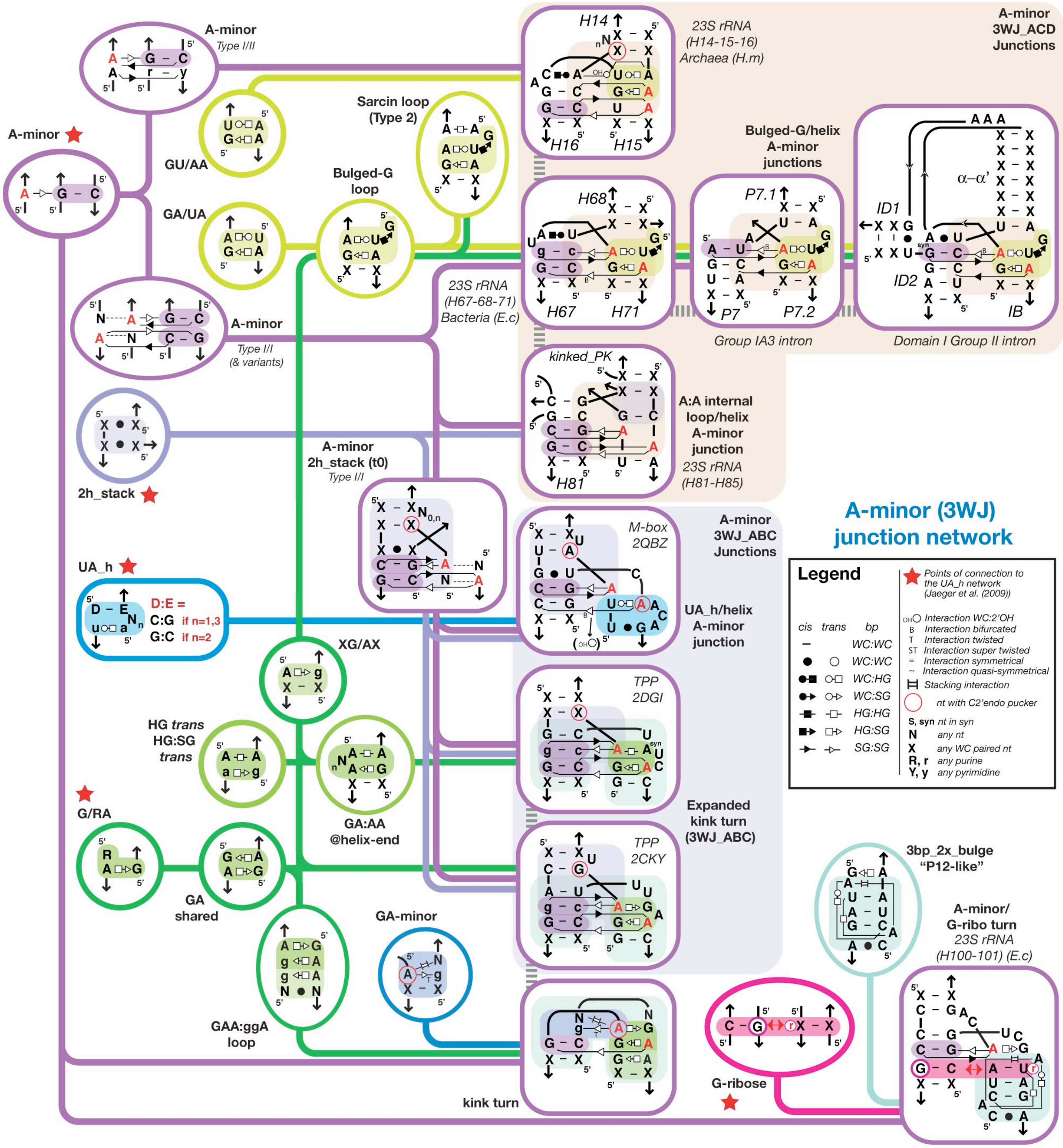

A-minor
Type I/II
A-minor
GU/AA
GA/UA
Sarcin loop
(Type 2)
Bulged-G
loop
A-minor
Type I/I
(& variants)
23S rRNA
(H67-68-71)
Bacteria (E.c)
23S rRNA
(H14-15-16)
Archaea (H.m)
H14
H15
H16
A-minor
3WJ_ACD
Junctions
Bulged-G/helix
A-minor
junctions
H68
H67
H71
P7.1
P7
P7.2
Group IA3 intron
ID1
ID2
IB
α–α'
Domain I Group II intron
kinked_PK
H81
A:A internal
loop/helix
A-minor
junction
23S rRNA
(H81-H85)
2h_stack
A-minor
2h_stack (t0)
Type I/I
M-box
2QBZ
A-minor
3WJ_ABC
Junctions
UA_h/helix
A-minor
junction
A-minor (3WJ)
junction network
UA_h
D:E =
C:G if n=1,3
G:C if n=2
XG/AX
HG trans
HG:SG
trans
GA:AA
@helix-end
TPP
2DGI
Expanded
kink turn
(3WJ_ABC)
TPP
2CKY
G/RA
GA
shared
GA-minor
GAA:ggA
loop
kink turn
G-ribose
3bp_2x_bulge
"P12-like"
A-minor/
G-ribo turn
23S rRNA
(H100-101) (E.c)
Legend
Points of connection to the UA_h network (Jaeger et al. (2009))
cis trans bp
WC:WC
WC:WC
WC:HG
WC:SG
HG:HG
HG:SG
SG:SG
Interaction WC:2'OH
Interaction bifurcated
Interaction twisted
Interaction super twisted
Interaction symmetrical
Interaction quasi-symmetrical
Stacking interaction
nt with C2'endo pucker
S, syn nt in syn
N any nt
X any WC paired nt
R, r any purine
Y, y any pyrimidine

Figure S10