FIGURE LEGENDS

Figure 1. (a) 2D diagram of a 4-way junction element composed of four helices labeled and color-coded by H_1 (red), H_2 (blue), H_3 (green) and H_4 (magenta), and the corresponding single stranded loop regions labeled J1/2 to J4/1 with nucleotides color-coded in yellow. Helices and loop regions are labeled in a unique way according to the 5′ to 3′ orientation of the entire RNA structure, by labeling H_1 as the first helix encountered, while entering the junction region, as one moves along the nucleotide chain in the 5′ to 3′ direction and so forth. Lines inside the helices represent the canonical WC basepairs G-C, A-U, and the wobble basepair G-U. (b) 3D diagrams containing two pairs of helices: H_1 with H_4, and H_2 with H_3, which are coaxial stacked. The 4-way junction illustrated corresponds to the 23S rRNA 1S72_2678 from Table 1.

Figure 2. Network interaction diagrams for the nine families of 4-way junctions. Family *H*, *cH* and *cL* contains two coaxial helices; family *cK* and π contains one coaxial stacking; while families *cW*, ψ, *X* and *cX* contains no coaxial stacking. The "*c*" before the capital letter in the family name denotes the crossing observed at the point of strand exchange. The network symbology follows the Leontis-Westhof notation[27] (see inset boxes).

Figure 3. A-minor interactions within junction domains. a) Secondary structure diagram for the most common interaction. b) Motif consensus for the most common interaction. c) Consensus motif for the less common interaction. *N* and *M* represent 0-3 and 2-3 nucleotides respectively. Often, a WC (GC) interaction appears next to the consecutive adenines at the n+2 position.

Figure 4. Interactions similar to sarcin/ricin motif (left box). The interactions are part of the junctions 23S rRNA 2AW4_267 and 23S rRNA 2J01_1832 of family ψ, and 16S rRNA 2AVY_942 of family *cX*. The interaction in 2AW4_267 was previously observed by Leontis *et al*[38]. RNA-Protein interactions (red font) are denoted by protein name followed by amino acid type and residue number.

Figure 5. Anatomy of a 4-way junction.

Table

Name	RNA Type	Coaxial stacks	Helical alignments	Family	Nomenclature	Domain	Helix Numbers
1U9S _78	Ribonuclease P_A	H1H4 , H2H3		H	2HS1HS2H		P7-8-9-10
2A2E _70	Ribonuclease P_A	H1H4 , H2H3		H	HS13HS1		P7-8-9-10
1NBS _89	Ribonuclease P_B	H1H4 , H2H3		H	2HS1HS2H		P7-8-9-10
2A64 _90	Ribonuclease P_B	H1H4 , H2H3		H	2HS1HS2H		P7-8-9-10
1M50 _13	Hairpin Ribozyme	H1H4 , H2H3		H	4H		A-B-C-D
1S72 _1827	23S rRNA	H1H4 , H2H3		H	HS3HS3HS3HS4	IV	H64-65-66-67
2AW4 _1771	23S rRNA	H1H4 , H2H3		H	HS3HS3HS2HS3	IV	H64-65-66-67
2J01 _1771	23S rRNA	H1H4 , H2H3		H	HS3HS3HS2HS3	IV	H64-65-66-67
1KH6 _4	HCV IRES	H1H4 , H2H3		cH	HS22HS1H		III-IIIa-IIIb-IIIc
2AVY _141	16S rRNA	H1H4 , H2H3		cH	HS3HS7HS4HS1	5'	H7-8-9-10
2J00 _141	16S rRNA	H1H4 , H2H3		cH	HS1HS4HS3HS1	5'	H7-8-9-10
1NKW _2621	23S rRNA	H1H4 , H2H3		cH	HS2HS1HS4HS2	VI	H94-95-96-97
1S72 _2678	23S rRNA	H1H4 , H2H3		cH	HS22HS2HS1	VI	H94-95-96-97
2AW4 _2642	23S rRNA	H1H4 , H2H3		cH	HS2HS1HS3HS1	VI	H94-95-96-97
2J01 _2642	23S rRNA	H1H4 , H2H3		cH	HS2HS1HS3HS1	VI	H94-95-96-97
3F2Q_7	Riboswitch (FMN)	H1H4 , H2H3		cH	HS6HS3HS1HS7		P1-P2-X-P6
3F2Q_31	Riboswitch (FMN)	H1H4 , H2H3		cH	2HS2HS3HS2		X-P3-P4-P5
1NKW _1457	23S rRNA	H1H2 , H3H4		cH	HS1HS3HS6HS4	III	H56-57-58-59
2AW4 _1443	23S rRNA	H1H2 , H3H4		cH	3HS3HS4	III	H56-57-58-59
2AVY _568	16S rRNA	H1H4 , H2H3		cL	HS7HS4HS10HS1	C	H19-20-24-25
2J00 _568	16S rRNA	H1H4 , H2H3		cL	HS7HS4HS10HS1	C	H19-20-24-25
1NKW _1282	23S rRNA	H1H4 , H2H3		cL	HS52HS2H	III	H47A-47-48-61
1S72 _1373	23S rRNA	H1H4 , H2H3		cL	HS72HS3H	III	H47A-47-48-61
2AW4 _1269	23S rRNA	H1H4 , H2H3		cL	HS52HS2H	III	H47A-47-48-61
2J01 _1269	23S rRNA	H1H4 , H2H3		cL	HS52HS2H	III	H49A-49-50-51
1EFW _6	transfer RNA	H1H4 , H2H3		cL	HS2HS1HS5H		H1-2-3-4
1EHZ _6	transfer RNA	H1H4 , H2H3		cL	HS2HS1HS5H		H1-2-3-4
1N78 _506	transfer RNA	H1H4 , H2H3		cL	HS2HS1HS4H		H1-2-3-4
1QRS _6	transfer RNA	H1H4 , H2H3		cL	HS2HS1HS5H		H1-2-3-4
1U08 _6	transfer RNA	H1H4 , H2H3		cL	HS2HS1HS4H		H1-2-3-4
2GIS _7	Riboswitch (SAM I)	H1H4 , H2H3		cL	HS6HS1HS8HS3		P1-2A-3-4
2AVY _114	16S rRNA	H1H4		cK	HS62HS2	5'	H7-11-12-13A
2J00 _114	16S rRNA	H1H4		cK	HS62HS2	5'	H7-11-12-13A

1NKW _2263	23S rRNA	H3H4		cK	$HS_4HS_4HS_1HS_1$	V	H82-83-86-87
1S72 _2318	23S rRNA	H3H4		cK	$HS_3HS_3HS_1HS_1$	V	H82-83-86-87
2AW4 _2284	23S rRNA	H3H4		cK	$HS_3HS_3HS_1HS_1$	V	H82-83-86-87
2J01 _2284	23S rRNA	H3H4		cK	$HS_3HS_3HS_1HS_1$	V	H83A-83-86-87
1NKW _1360	23S rRNA	H3H4		cK	$HS_1HS_3HS_4HS_2$	III	H51-52-53-54
1S72 _1452	23S rRNA	H3H4		cK	$HS_1HS_3HS_2HS_1$	III	H51-52-53-54
2AW4 _1346	23S rRNA	H3H4		cK	$HS_2HS_3HS_2HS_1$	III	H49A-49-50-51
2J01 _1347	23S rRNA	H3H4		cK	$HS_1HS_3HS_2H$	III	H51-52-53-54
2AVY _18	16S rRNA	H1H2		cK	$HS_7HS_{10}HS_1HS_3$	C	H2-3-19-27
2J00 _18	16S rRNA	H1H2		cK	$HS_7HS_{10}HS_1HS_3$	C	H2-3-19-28
1U9S _118	Ribonuclease P_A	H3H4	H1H2	π	$HS_{12}HS_7HS_1HS_4$		P11-12-13-14
2A2E _110	Ribonuclease P_A	H3H4	H1H2	π	$HS_{10}HS_7HS_2HS_4$		P11-12-13-14
1NKW _1682	23S rRNA		H1H4	cW	$HS_{15}2HS_{12}HS_5$	IV	H61-62-63-64
1S72 _1743	23S rRNA		H1H4	cW	$HS_{15}2HS_{12}HS_5$	IV	H61-62-63-64
2AW4 _1665	23S rRNA		H1H4	cW	$HS_{15}2HS_{12}HS_5$	IV	H61-62-63-64
2J01 _1665	23S rRNA		H1H4	cW	$HS_{15}2HS_{12}HS_5$	IV	H61-62-63-64
1S72 _42	23S rRNA		H2H4	ψ	$HS_6HS_4HS_5HS_1$	I	H4-5-8-10
1NKW _1824	23S rRNA		H2H4	ψ	$HS_1HS_2HS_{22}HS_{10}$	IV	H64-65-66-67
1S72 _1888	23S rRNA		H2H4	ψ	$HS_{12}HS_{20}HS_{10}$	IV	H67-68-69-71
2AW4 _1832	23S rRNA		H2H4	ψ	$HS_{12}HS_{20}HS_{10}$	IV	H64-65-66-67
2J01 _1832	23S rRNA		H2H4	ψ	$HS_{12}HS_{20}HS_{10}$	IV	H64-65-66-67
1NKW _244	23S rRNA		H2H4	ψ	$HS_5HS_6HS_6HS_2$	I	H14-16-21-22
2AW4 _267	23S rRNA		H2H4	ψ	$HS_2HS_8HS_6HS_2$	I	H14-16-21-22
1NKW_608	23S rRNA			X	$HS_{10}HS_9HS_3HS_{11}$	I	H27-28-29-31
2AW4 _600	23S rRNA			X	$HS_2HS_3HS_2HS_5$	I	H27-28-29-31
2J01 _600	23S rRNA			X	$HS_2HS_3HS_2HS_2$	I	H27-28-29-31
2IHX _166	Sarcoma Virus			cX	$HS_3HS_42HS_3$		A-B-C-O3
2AVY _942	16S rRNA			cX	$HS_2HS_5HS_{11}HS_{10}$	3'M	H29-30-41-42
2J00 _940	16S rRNA			cX	$HS_4HS_5HS_{11}HS_{12}$	3'M	H29-30-41-42

Table 1. List of RNA 3D structures containing 62 four-way junctions. The name describes the PDB code and the number of the first nucleotide of helix H_1 in the junction. The nomenclature is based on [10] and the helix number correspond to the labels given as published.

Size of $J_{i/(i+1)}$	0	1	2	3	4	5	6	7
Frequency	25	26	7	8	5	0	2	2

Table 2. Frequency of coaxial stacking between helices H_i and H_{i+1} joined by the size of the single stranded loop in between $J_{i/(i+1)}$ ranging from 0 to 7 as performed for the 75 coaxial stacking cases within our 62 4-way junction list (Table 1).

Figure1

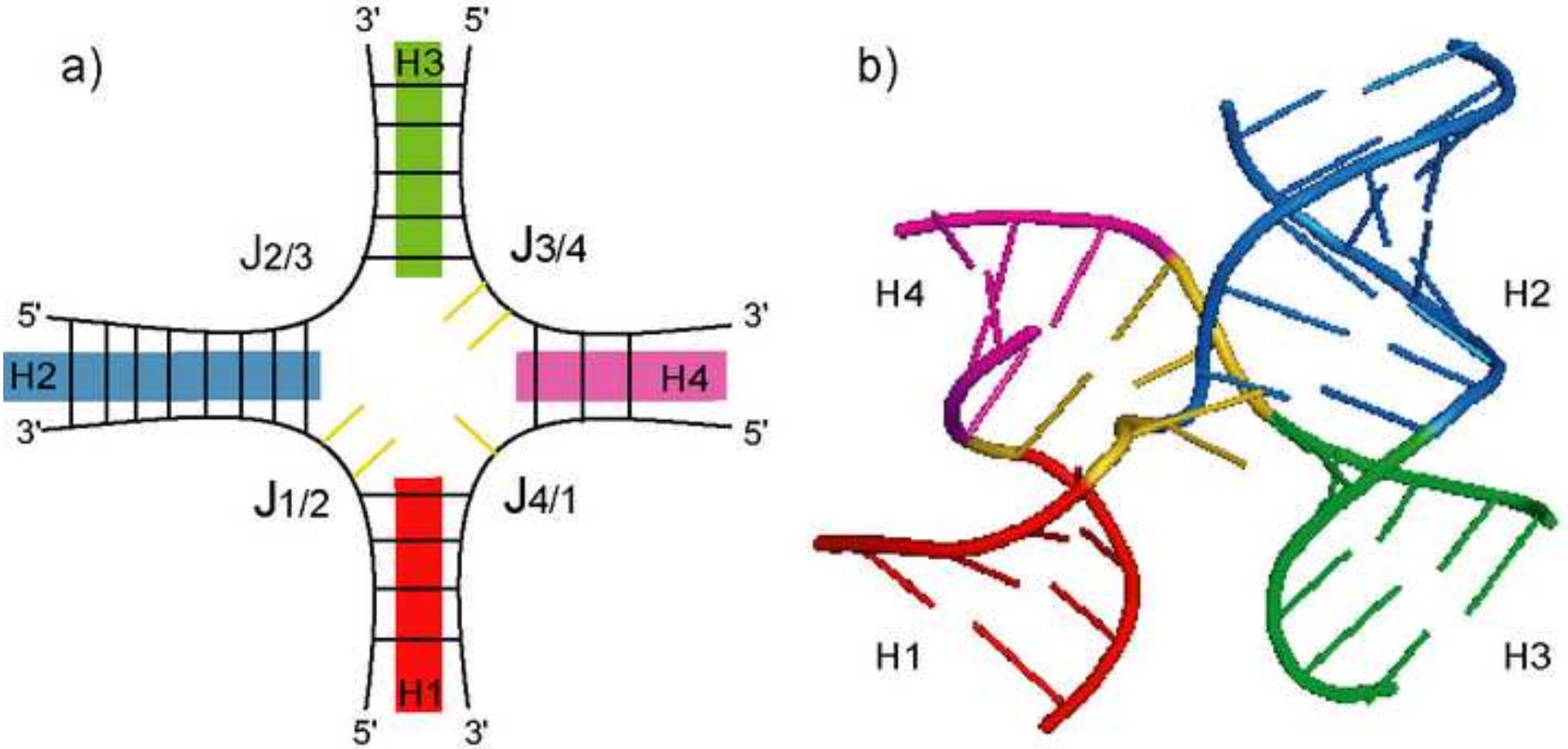


Figure2a-c

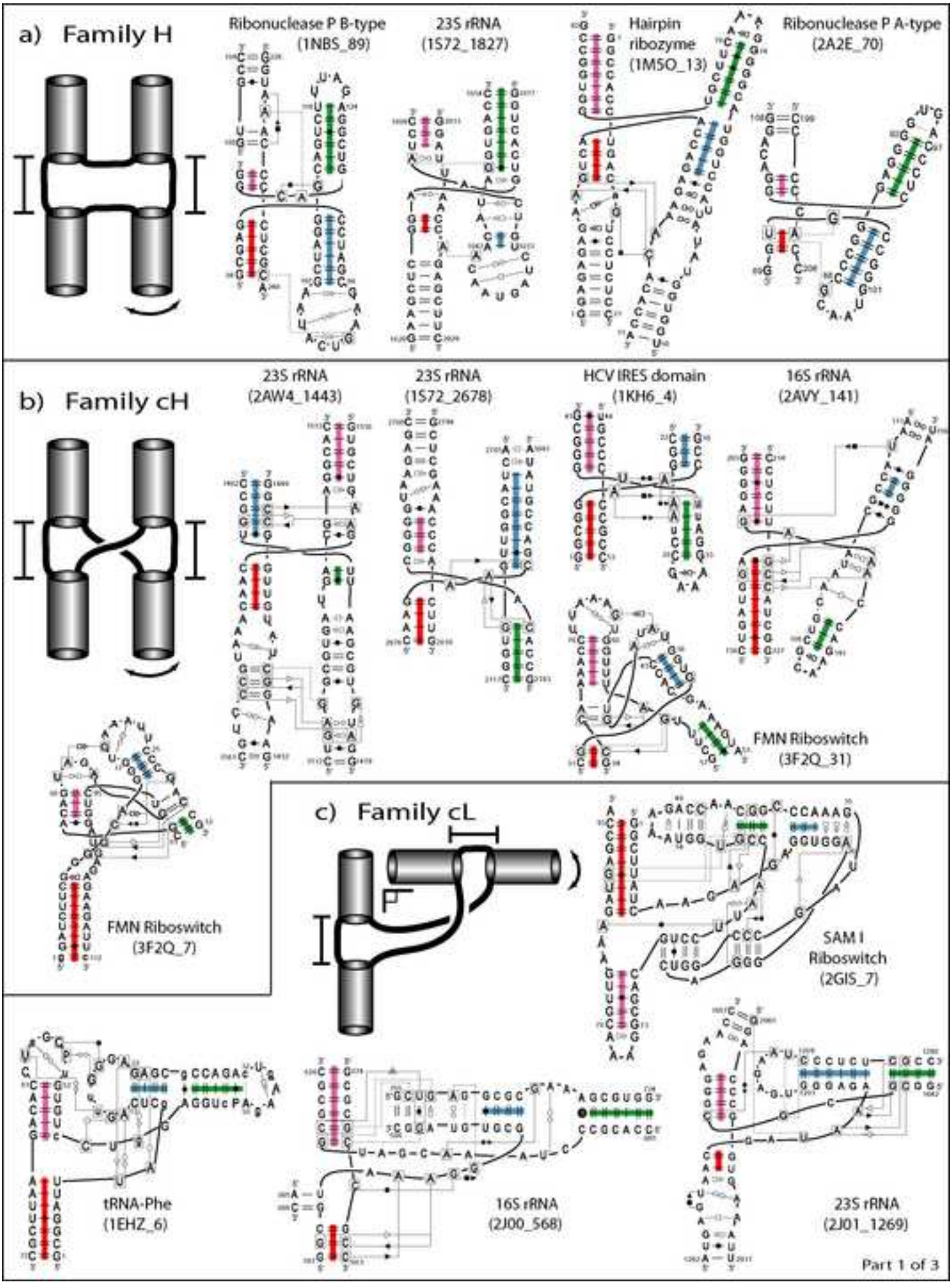

a) Family H
Ribonuclease P B-type (1NBS_89)
23S rRNA (1S72_1827)
Hairpin ribozyme (1M5O_13)
Ribonuclease P A-type (2A2E_70)
b) Family cH
23S rRNA (2AW4_1443)
23S rRNA (1S72_2678)
HCV IRES domain (1KH6_4)
16S rRNA (2AVY_141)
FMN Riboswitch (3F2Q_31)
FMN Riboswitch (3F2Q_7)
c) Family cL
SAM I Riboswitch (2GIS_7)
tRNA-Phe (1EHZ_6)
16S rRNA (2J00_568)
23S rRNA (2J01_1269)
Part 1 of 3

Figure2d-e

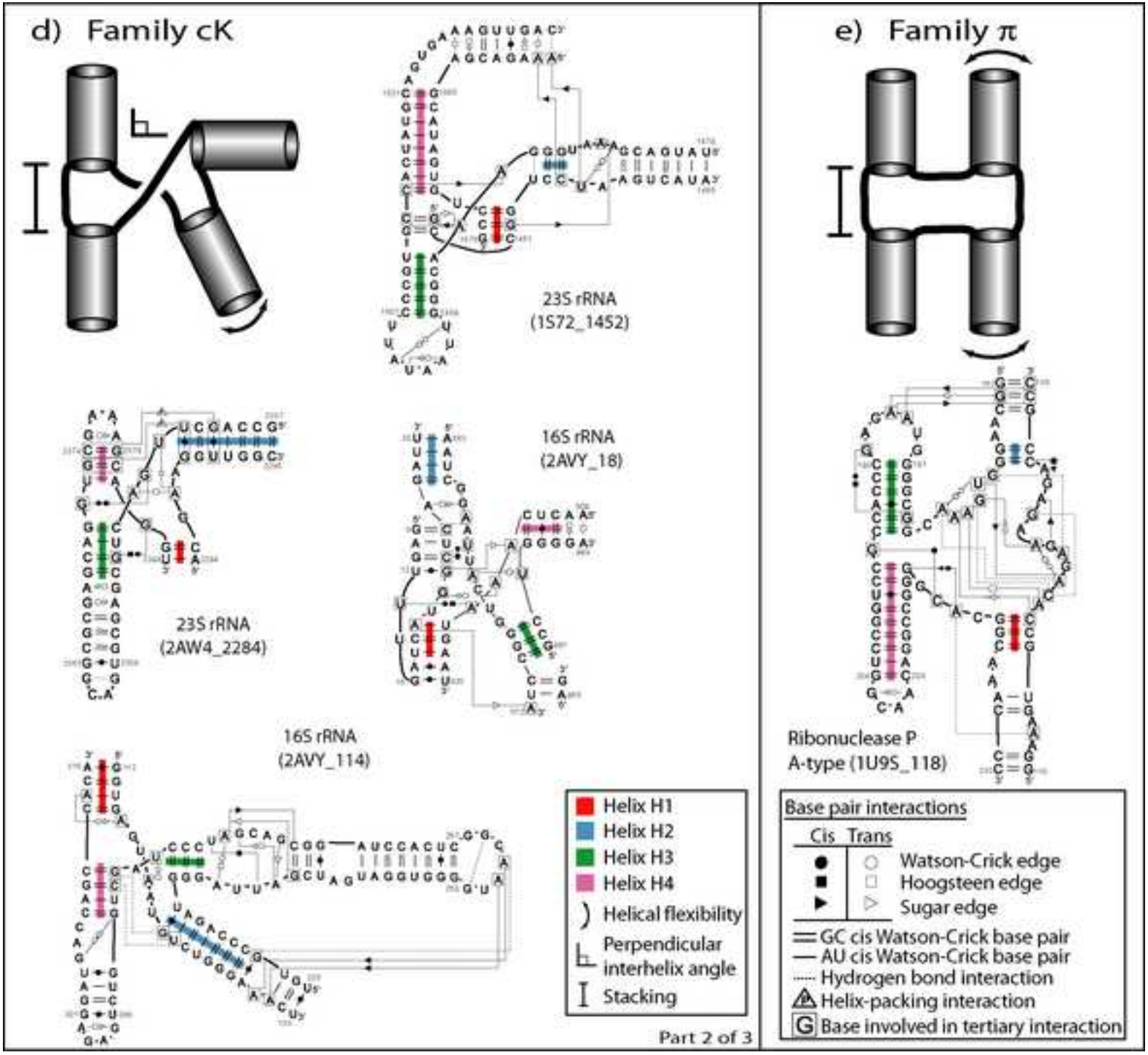

d) Family cK
23S rRNA
(1S72_1452)
23S rRNA
(2AW4_2284)
16S rRNA
(2AVY_18)
16S rRNA
(2AVY_114)
Helix H1
Helix H2
Helix H3
Helix H4
Helical flexibility
Perpendicular interhelix angle
Stacking
Part 2 of 3
e) Family π
Ribonuclease P
A-type (1U9S_118)
Base pair interactions
Cis Trans
Watson-Crick edge
Hoogsteen edge
Sugar edge
GC cis Watson-Crick base pair
AU cis Watson-Crick base pair
Hydrogen bond interaction
Helix-packing interaction
G Base involved in tertiary interaction

Figure2f-i

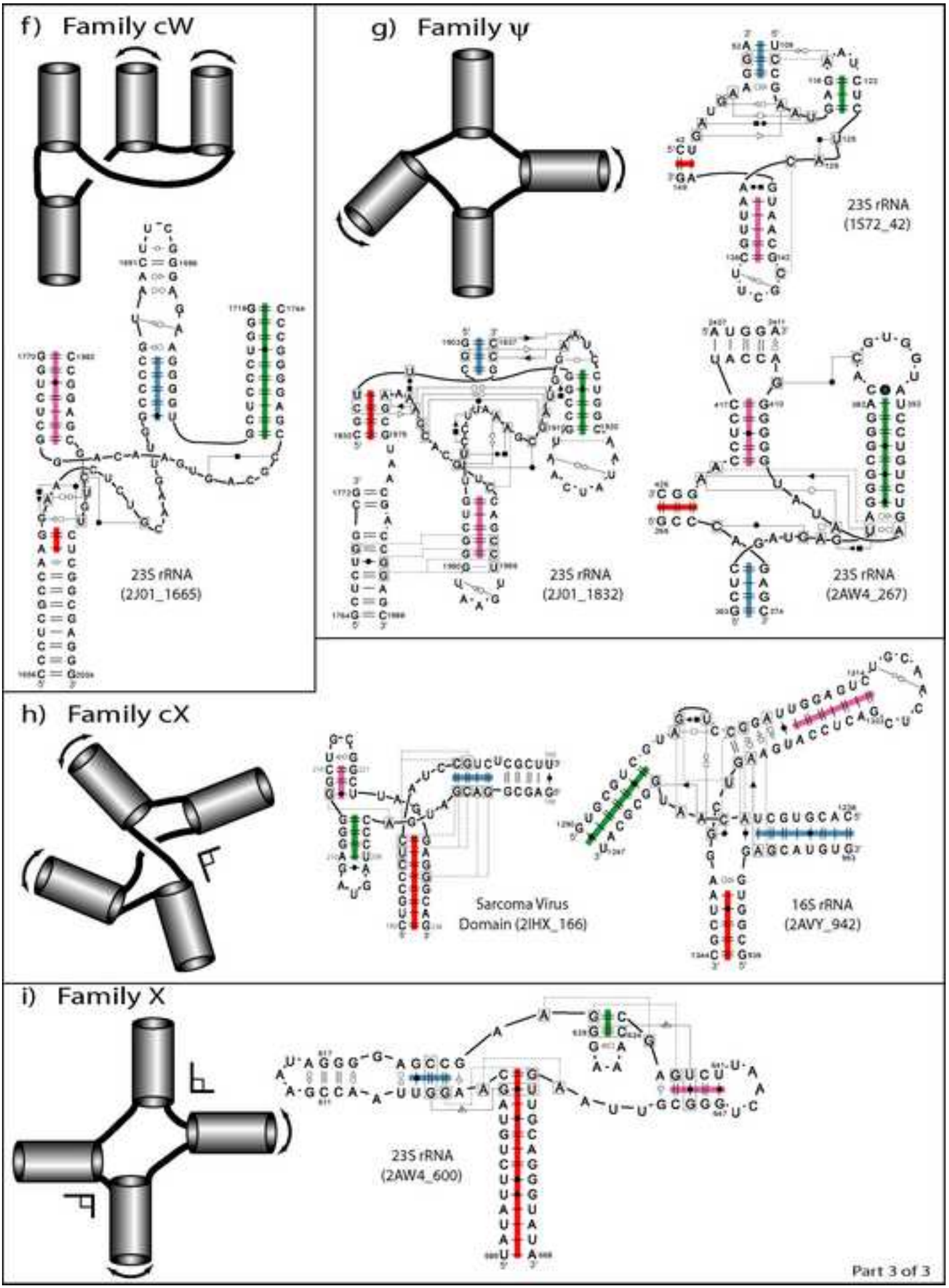

f) Family cW
23S rRNA
(2J01_1665)
g) Family ψ
23S rRNA
(1S72_42)
23S rRNA
(2J01_1832)
23S rRNA
(2AW4_267)
h) Family cX
Sarcoma Virus
Domain (2IHX_166)
16S rRNA
(2AVY_942)
i) Family X
23S rRNA
(2AW4_600)
Part 3 of 3

Figure3

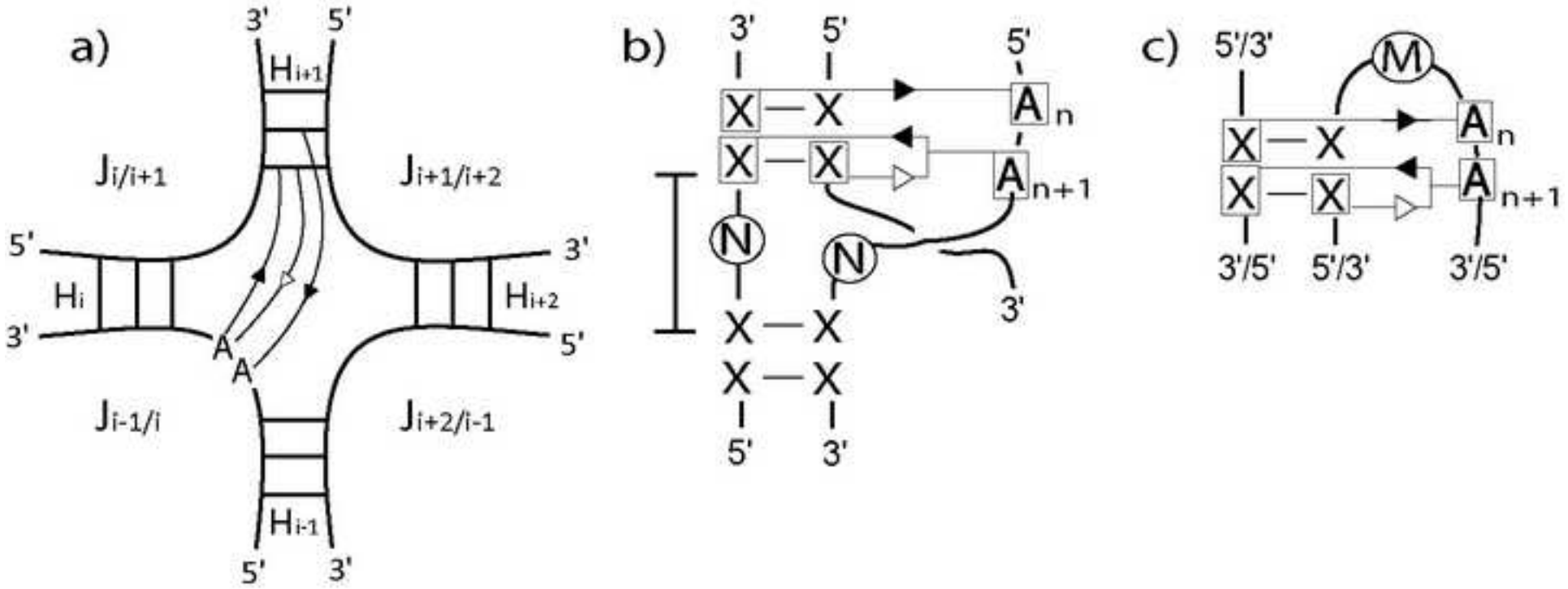

a)
3'
5'
Hi+1
Ji/i+1
Ji+1/i+2
5'
3'
Hi
Hi+2
3'
5'
A
A
Ji-1/i
Ji+2/i-1
Hi-1
5'
3'
b)
3'
5'
5'
X
X
An
X
X
An+1
N
N
3'
X
X
X
X
5'
3'
c)
5'/3'
M
X
X
An
X
X
An+1
3'/5'
5'/3'
3'/5'

Figure4

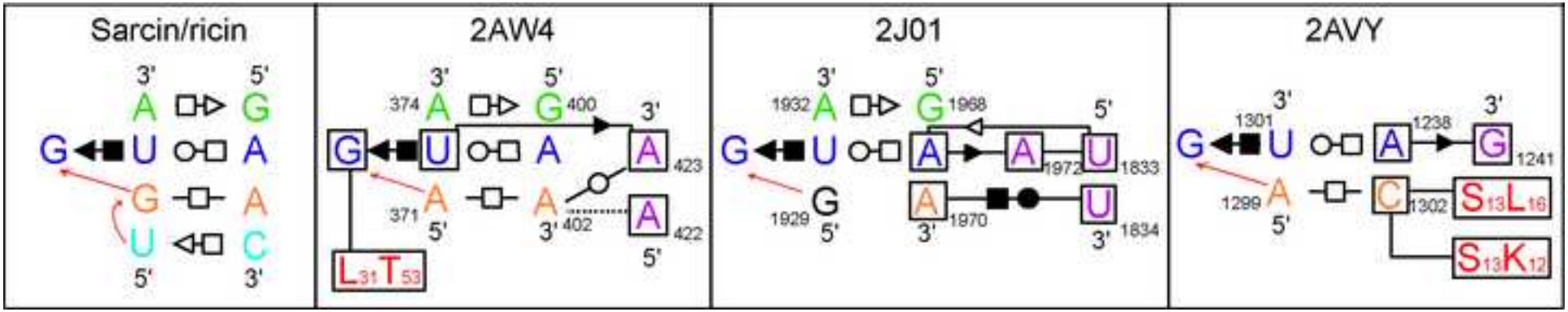
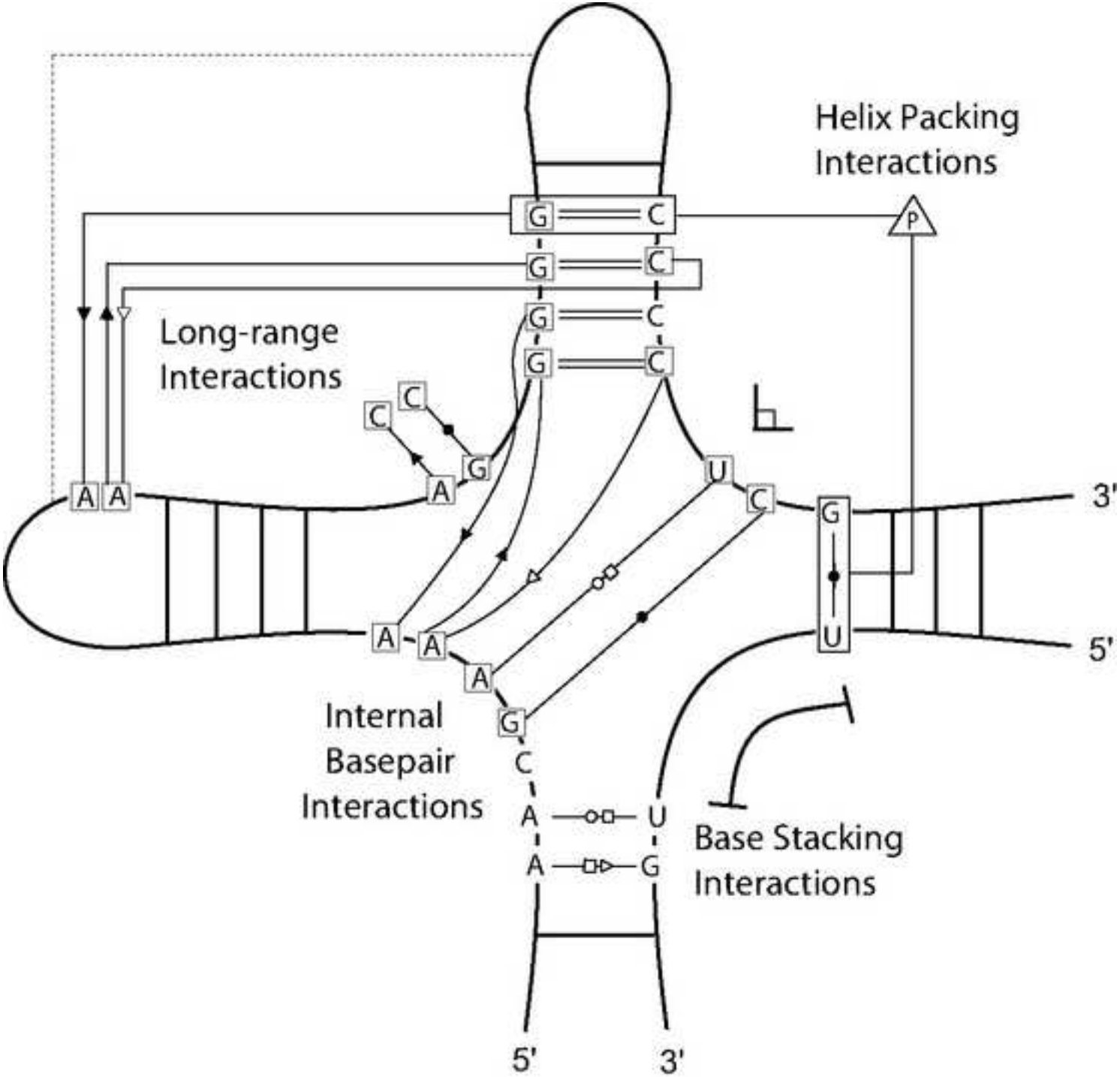

Sarcin/ricin
3'
5'
A
G
G
U
A
G
A
U
C
5'
3'
2AW4
3'
5'
374
A
G
400
3'
G
U
A
A
423
371
A
5'
A
3'
402
A
422
5'
L31 T53
2J01
3'
5'
1932
A
G
1968
5'
G
U
A
A
U
1833
1929
G
5'
A
1970
1972
3'
U
3'
1834
2AVY
3'
1301
3'
1238
G
U
A
G
1241
1299
A
5'
C
1302
S13 L16
S13 K12

Figure5


Helix Packing
Interactions
Long-range
Interactions
Internal
Basepair
Interactions
Base Stacking
Interactions
3'
5'
5'
3'