Supplementary Tables

Table S1: Calibration of the small probe radius.

Index Small probe radius (Å)	2	3	4	11	12	13	23	26	32	35	36
1.50	**0.59**	**1.72**	**0.42**	**0.74**	**1.75**	**0.90**	**0.70**	**1.46**	**0.68**	**0.47**	**1.10**
2.50	5.74	1.72	1.14	0.74	2.23	1.14	0.72	1.46	0.39	0.47	1.10
3.50	7.05	2.40	2.39	6.74	3.41	1.47	0.72	4.81	0.39	0.47	0.62
4.50	7.05	2.61	2.39	8.80	3.77	7.67	0.72	4.98	1.41	0.47	5.01
5.50	8.46	2.61	2.39	20.04	4.49	9.18	2.11	8.13	2.53	10.50	11.49
6.50	12.42	13.83	7.51	20.08	22.45	15.78	3.94	9.54	5.77	10.54	12.57
7.50	14.22	15.05	48.12	20.08	32.30	21.46	47.02	45.25	46.52	47.36	45.05
8.50							66.58	62.85	61.70	48.11	52.80

Distances (Å) between known ABS to the closest computed binding pocket (background) as a function of the small and large probe radii (PDB IDs 1J5E and 1JJ2) are presented. Indices refer to the ribosome complexes listed in Table 1. The large probe radius was fixed to 9.5 Å. We consider a change in a distance equal or above 1 Å as significant. A small probe radius of 1.5 Å was chosen since a minimal distance between known ABSs and computed pockets was reached.

Table S2: Calibration of the large probe radius.

Index Large	2	3	4	11	12	13	23	26	32	35	36
9.50	0.59	1.72	0.42	0.74	1.75	0.90	0.70	1.46	0.68	0.47	1.10
8.50	0.59	1.72	0.42	0.74	1.75	0.90	0.70	1.46	0.68	0.47	1.10
7.50	0.59	1.72	0.42	0.74	1.75	0.90	0.70	1.46	0.68	0.47	1.10
6.50	0.59	1.72	0.42	0.74	1.75	0.90	0.70	1.46	0.68	0.47	1.10
5.50	**0.59**	**1.79**	**0.42**	**0.74**	**1.75**	**0.90**	**0.70**	**1.46**	**0.68**	**0.47**	**1.10**
4.50	0.59	1.79	0.42	0.74	1.75	0.90	1.24	1.46	0.68	1.43	1.10
3.50	0.59	2.03	0.42	0.74	1.75	0.90	1.90	1.46	1.14	2.53	2.56
2.50	0.59	2.55	0.42	5.83	3.59	8.21	3.09	3.08	2.79	5.47	3.23

Distances (Å) between know ABS to the closest computed binding pocket (background) as a function of the large probe radii (PDB IDs 1J5E and 1JJ2) are presented. Indices refer to the ribosome complexes listed in Table 1. The small probe radius was fixed to 1.5 Å according to Table S1. We consider a change in a distance equal or above 1 Å as significant. A large probe radius of 5.5 Å was chosen since a minimal distance between known ABSs and computed pockets was reached.

Table S3: Size of computed binding pocket as a function of the radius from a computed center of mass.

Index	Size of known binding site [number of nts]	Size of the closest computed pocket [number of nts]					
		R= 5 Å	R= 6 Å	R= 7 Å	R= 8 Å	R= 9 Å	R= 10 Å
2	9	3	5	7	10	14	14
3	11	1	7	9	10	11	14
4	14	5	7	10	12	14	18
11	11	8	8	12	14	17	19
12	16	3	3	4	9	13	18
13	16	6	7	9	12	12	16
23	9	3	5	6	9	11	13
26	11	3	3	3	4	7	11
32	14	2	3	5	8	12	15
35	10	3	4	4	7	9	11
36	15	3	6	10	11	14	17
Average	**12.36**	3.64	5.27	7.18	9.64	**12.18**	15.09

Size of a computed pocket, which is the closest to known ABS, was measured for different radius cutoffs. The complexes indices refer to Table 1. The size of ABS was measured as a number of rRNA nts it comprises. A radius of 9 Å was chosen due to the size similarity to known binding sites.

Table S4: Number of overlapping putative pockets in the small and the large ribosomal subunit

PDB code	Organism	No of pockets with a size of 12 nts
1J5E	*T. thermophilus*	5,724
IJJ2	*H. marismortui*	11,886
2AVY	*E. coli*	5,998
2AW4	*E. coli*	12,427
2ZJR	*D. radiodurans*	13,563

Number of overlapping putative pockets, in the small and the large ribosomal subunit. The pockets differ from each other in at least one nt. The comuted pockets were defined with a small probe radius of 1.5 Å (Table S1), large probe radius of 5.5 Å (Table S2) and a radius of 9 Å (Table S3) around the computed center of mass.

Table S5: Overlapping sensitivity and specificity between known ABSs and computed pockets.

Index	Size of known binding site [number of nts]	Size of putative pocket [number of nts]	Common [number of nts]	Percent specificity	Percent sensitivity
1	12	12	12	100.0	100.0
2	11	12	8	72.7	66.7
3	9	12	8	88.9	66.7
4	11	12	10	90.9	83.3
5	12	12	11	91.7	91.7
6	16	12	12	75.0	100.0
7	13	12	11	84.6	91.7
8	13	12	11	84.6	91.7
9	11	12	9	81.8	75.0
10	13	12	8	61.5	66.7
11	14	12	12	85.7	100.0
12	16	12	11	68.8	91.7
13	16	12	12	75.0	100.0
14	14	12	7	50.0	58.3
15	12	12	12	100.0	100.0
16	14	12	12	85.7	100.0
17	16	12	8	50.0	66.7
18	15	12	12	80.0	100.0
19	18	12	12	66.7	100.0
20	16	12	12	75.0	100.0
21	16	12	12	75.0	100.0
22	11	12	9	81.8	75.0
23	10	12	8	80.0	66.7
24	11	12	9	81.8	75.0
25	18	12	9	50.0	75.0
26	15	12	9	60.0	75.0
27	14	12	11	78.6	91.7
28	16	12	11	68.8	91.7
29	16	12	12	75.0	100.0
30	14	12	10	71.4	83.3
31	20	12	11	55.0	91.7
32	9	12	8	88.9	66.7
33	8	12	8	100.0	66.7
34	14	12	10	71.4	83.3
35	14	12	12	85.7	100.0
36	11	12	7	63.6	58.3

37	16	12	12	75.0	100.0
38	17	12	12	70.6	100.0
39	15	12	11	73.3	91.7
40	14	12	11	78.6	91.7
41	7	12	7	100.0	58.3
42	6	12	6	100.0	50.0
43	14	12	10	71.4	83.3
44	16	12	11	68.8	91.7
45	15	12	11	73.3	91.7
46	18	12	11	61.1	91.7
47	14	12	11	78.6	91.7
48	14	12	9	64.3	75.0
49	5	12	4	NA	NA
50	21	12	10	47.6	83.3
51	17	12	9	52.9	75.0
52	19	12	10	52.6	83.3
53	15	12	11	73.3	91.7
54	9	12	7	77.8	58.3
55	17	12	9	52.9	75.0
56	13	12	9	69.2	75.0
57	5	12	5	NA	NA
58	11	12	9	81.8	75.0
59	14	12	10	71.4	83.3
60	12	12	9	75.0	75.0
61	14	12	10	71.4	83.3
62	20	12	10	50.0	83.3
63	17	12	10	58.8	83.3
64	15	12	9	60.0	75.0
65	15	12	9	60.0	75.0

Sensitivity and specificity were calculated for antibiotics binding sites that consists more than 5 nucleotides. *Overlapping specificity* equals to the fraction of the common nts in the putative and the known pockets divided by the number of nts in the known ABS, and *overlapping sensitivity* equals to the fraction of common nts divided by the number of nts included in the putative pocket. Indices refer to the known ABSs as presented in Table 1.

Table S6: List of studied properties

Atomic level					
1.	P4HO	7.	N3H0	13.	C3H0
2.	O2H1	8.	N2H0	14.	Base donor
3.	O2H0	9.	C4H2	15.	Base acceptor
4.	O1H0	10.	C4H1	16.	Sugar oxygens
5.	N3H2	11.	C3H1	17.	Phosphate oxygens
6.	N3H1	12.	C3H1		

Nucleotide level			
1.	Base: A	16.	Base pair: WW trans
2.	Base: C	17.	Base pair: WH cis
3.	Base: G	18.	Base pair: WH trans
4.	Base: U	19.	Base pair: HH cis
5.	Sugar pucker: C1 exo	20.	Base pair: HH trans
6.	Sugar pucker: C1 endo	21.	Base pair: HS cis
7.	Sugar pucker: C2 exo	22.	Base pair: HS trans
8.	Sugar pucker: C2 endo	23.	Base pair: SS cis
9.	Sugar pucker: C3 exo	24.	Base pair: SS trans
10.	Sugar pucker: C3 endo	25.	Base Stack: adjacent
11.	Sugar pucker: C4 exo	26.	Base Stack: non- anjacent
12.	Sugar pucker: O4 endo	27	Centrality: Closeness
13.	Base conformation: syn	28.	Centrality: Betweenness
14.	Base conformation: anti	29.	Centrality: Degree
15.	Base pair: WW cis	30.	Evolutionary conservation

Abbreviations: W, Watson Crick; H, hoogsteen; S, sugar edge.

Table S7: Summary of properties that are over and under represented in *T. thermophilus* and *H. marismortui* ABSs

Over represented properties in binding site							Under represented properties						
	T. thermophilus			*H. marismortui*				*T. thermophilus*			*H. marismortui*		
Property	ABS	RP	Ligand Binding sites	ABS	RP	Ligand Binding sites	Property	ABS	RP	Ligand Binding sites	ABS	RP	Ligand Binding sites
Non-paired	NS	NS	1.3E-02	2.8E-05	NS	2.1E-03	WWcis	NS	NS	9.6E-03	2.0E-03	NS	2.1E-03
syn	NS	NS	3.2E-02	5.1E-02	NS	NS	adjacent	NS	NS	NS	1.6E-04	1.5E-03	3.2E-03
WHcis	1.0E-03	NS	NS	NS	NS	NS	GC	2.7E-02	NS	NS	5.2E-03	NS	1.7E-02
non-adjacent	NS	NS	NS	4.9E-02	1.4E-02	NS	UA	NS	NS	NS	2.1E-02	NS	8.1E-04
UC	NS	NS	NS	4.2E-02	NS	NS							

Abbreviations: RP, Ribosomal Proteins

Table S8: *p*-value table for structural properties that over and under represented in *T. thermophilus*, *H. marismortui* and *D. radiodurans* ABSs.

Overrepresented properties				Underrepresented properties			
	T. thermophilus	*H. marismortui*	*D. radiodurans*		*T. thermophilus*	*H. marismortui*	*D. radiodurans*
non-paired	NS	2.83E-05	1.33E-03	C3 endo	NS	NS	9.89E-03
syn	NS	5.06E-02	2.79E-02	WWcis	NS	2.02E-03	3.64E-06
WHcis	1.09E-03	NS	NS	adjacent	NS	1.60E-04	1.52E-09
non-adjacent	NS	4.93E-02	NS				

Non significant (NS) refer to P-value>0.05

Table S9: A-minor motif elements in ABSs

binding site index	Residue 1			Residue 2			Residue 3		
	base	number	chain	base	number	chain	base	number	chain
1	A	1492	A	A	35	Y	U	20	X
1,13	A	1493	A	A	36	Y	U	19	X
1,13	A	1519	A	G	1497	A	C	1404	A
2	A	696	A	C	796	A	G	786	A
6	A	1169	A	G	1088	A	C	1097	A
7	A	1375	A	C	1344	A	G	939	A
8	A	282	A	G	247	A	C	277	A
11	A	1191	A	G	1068	A	C	1107	A
12	A	915	A	C	912	A	G	885	A
24,25	A	767	0	G	2110	0	C	2477	0

Binding sites indices refer to Table 1.

Table S10: Known sites and their computed DI

Index	Antibiotics	Organism	PDB	Identifier	DI
1	Hygromycin B	T.t	1HNZ	HYG	0.29
2	Pactamycin	T.t	1HNX	PCY	0.46
3	Tetracycline	T.t	1HNW	TAC 1001	0.72
4	Tetracycline	T.t	1HNW	TAC 1002	0.59
5	Tetracycline	T.t	1I97	TAC 2001	0.65
6	Tetracycline	T.t	1I97	TAC 2003	0.34
7	Tetracycline	T.t	1I97	TAC 2004	0.25
8	Tetracycline	T.t	1I97	TAC 2005	0.40
9	Tetracycline	T.t	1I97	TAC 2006	0.55
10	Edeine	T.t	1I95	EDE	0.47
11	Spectinomycin	T.t	1FJG	SCM	0.34
12	Streptomycin	T.t	1FJG	SRY	0.39
13	Paromomycin	T.t	1FJG	PAR	0.29
14	Kasugamycin	T.t	2HHH	KSG	0.61
22	Erythromycin	H.m	1YI2	ERY	0.86
23	Azithromycin	H.m	1YHQ	ZIT	0.95
24	Telithromycin	H.m	1YIJ	TEL	0.86
25	Quinupristin	H.m	1YJW	SYB	0.95
26	Virginiamycin S	H.m	1YIT	VRS	0.95
27	Virginiamycin M	H.m	1YIT	VIR	0.95
28	Clindamycin	H.m	1YJN	CLY	0.86
29	Carbomycin	H.m	1K8A	CAI	0.91
30	Spiramycin	H.m	1KD1	SPR	0.91
31	Tylosin	H.m	1K9M	TYK	0.86
32	Sparsomycin	H.m	1M90	SPS	0.76
33	Chloramphenicol	H.m	1NJI	CLM	0.95
34	Anisomycin	H.m	1K73	ANM	0.98
35	Blasticidin S	H.m	1KC8	BLS 9001	0.59
36	Blasticidin S	H.m	1KC8	BLS 9002	0.44
37	Homoharringtonine	H.m	3G6E	HMT	0.91
38	Bruceantin	H.m	3G71	WIN	0.89
39	Tiamulin	H.m	3G4S	MUL	0.91
40	Oxazolidinone	H.m	3CXC	SLD	0.88
41	Girodazole	H.m	2OTL	GIR	0.59
42	13-deoxytedanolide	H.m	2OTJ	13T	0.61

The DI was calculated for the computed pocket with the highest degree of overlap.