IntaRNA 2.0 - enhanced and customizable prediction of RNA-RNA interactions - Supplementary material -

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1 Energy computation details

An RNA molecule consisting of n nucleotides is described by its sequence of bases encoded by $\mathcal{S} \in \{\mathtt{A},\mathtt{C},\mathtt{G},\mathtt{U}\}^n$ indexed from 5' to 3' end.

For a given pair of two RNAs $\mathcal{S}^1, \mathcal{S}^2$, we will denote with $E^{\text{hybrid}}\left[\begin{smallmatrix} i,k \\ j,l \end{smallmatrix}\right]$ the minimal energy of any interaction of the subsequences $\mathcal{S}^1_i..\mathcal{S}^1_k$ and $\mathcal{S}^2_l..\mathcal{S}^2_j$ under the additional condition that the subsequence ends form each a base pair, i.e. $(\mathcal{S}^1_i, \mathcal{S}^2_j)$ and $(\mathcal{S}^1_k, \mathcal{S}^2_l)$ are Watson-Crick or $\mathtt{G}$-$\mathtt{U}$ base pairs. This energy term also includes the RNA-RNA interaction initiation energy penalty as well as closing base pair penalties (if the final base pairs are not $\mathtt{G}$-$\mathtt{C}$).

The energy penalty $ED^1[i,k]$ to make the subsequence $\mathcal{S}^1_i..\mathcal{S}^1_k$ accessible is given by

$$ED^1[i,k] = -RT \cdot \log(Pr^u[i..k]), \tag{1}$$

where $Pr^u[i..k]$ denotes the unpaired probability for subsequence $\mathcal{S}^1_i..\mathcal{S}^1_k$ (e.g. computed via McCaskill's algorithm [1]), R the gas constant and T the temperature of the system. ED^2 is defined analogously.

For an interaction with left/right-most base pairs $(\mathcal{S}^1_i, \mathcal{S}^2_j)/(\mathcal{S}^1_k, \mathcal{S}^2_l)$, resp., and an hybridization energy given by E^{hybrid}, the overall interaction energy in IntaRNAv1 is defined by

$$\begin{aligned} E\left[\begin{smallmatrix} i,k \\ j,l \end{smallmatrix}\right]^{v1} &= E^{\text{hybrid}}\left[\begin{smallmatrix} i,k \\ j,l \end{smallmatrix}\right] \\ &+ \min \begin{cases} ED^1[i-1,k+1] + E^{\text{dangle}}\left[\mathcal{S}^1_{i-1}\right] + E^{\text{dangle}}\left[\mathcal{S}^1_{k+1}\right] & \text{both ends free} \\ ED^1[i-1,k] + E^{\text{dangle}}\left[\mathcal{S}^1_{i-1}\right] & \text{5'-end free} \\ ED^1[i,k+1] + E^{\text{dangle}}\left[\mathcal{S}^1_{k+1}\right] & \text{3'-end free} \\ ED^1[i,k] & \text{no end free} \end{cases} \\ &+ \min\left\{\text{dangling end cases for } \mathcal{S}^2 \text{ and interval } j..l\right\}. \end{aligned} \tag{2}$$

Here, all possibilities of free ends and according dangling end contributions $E^{\text{dangle}}[..]$ are considered while the ED penalty is extended to cover the free end positions as well.

The new IntaRNAv2 dangling end treatment always takes free dangling end contributions into account by weighting them with according conditional probabilities Pr^u that the position is unpaired given that the interaction site is accessible, i.e. not involved in intramolecular base pairs. For instance, the 5'-dangling-end probability for the first sequence (position $\mathcal{S}^1_{i-1}$), is given by

$$\begin{aligned} Pr^u[i-1 \mid i..k] &= Pr^u[(i-1)..k] \;/\; Pr^u[i..k] \\ &= \exp(-ED^1[i-1,k]/RT) \;/\; \exp(-ED^1[i,k]/RT) \\ &= \exp((ED^1[i,k] - ED^1[i-1,k])/RT) \end{aligned} \tag{3}$$

using the ED^1 values for $\mathcal{S}^1$ given Eq. 1.

Given these probabilities, IntaRNAv2 computes the overall interaction energy using

$$\begin{aligned} E\left[\begin{smallmatrix} i,k \\ j,l \end{smallmatrix}\right]^{v2} &= E^{\text{hybrid}}\left[\begin{smallmatrix} i,k \\ j,l \end{smallmatrix}\right] \\ &+ ED^1[i,k] \\ &+ Pr^u[i-1 \mid i..k] \cdot E^{\text{dangle}}\left[\mathcal{S}^1_{i-1}\right] + Pr^u[k+1 \mid i..k] \cdot E^{\text{dangle}}\left[\mathcal{S}^1_{k+1}\right] \\ &+ ED^2[j,l] \\ &+ Pr^u[j-1 \mid j..l] \cdot E^{\text{dangle}}\left[\mathcal{S}^2_{j-1}\right] + Pr^u[l+1 \mid j..l] \cdot E^{\text{dangle}}\left[\mathcal{S}^2_{l+1}\right]. \end{aligned} \tag{4}$$

2 Minimal energy profiles of Spot42-*sthA* interactions

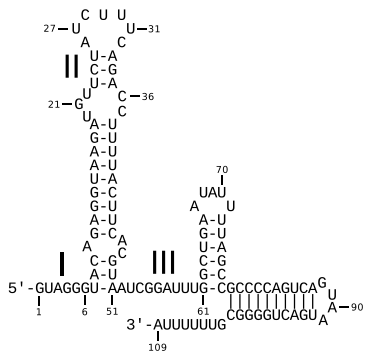

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Figure 1: (Left) Secondary structure of Spot42 [2] with its three accessible regions I, II and III that are known to interact with target mRNAs [3]. Figure adapted from [4]. (Right) Spot42 primary sequence and mutated versions used by [3]. Mutated regions are highlighted (capital and red).

Spot42 is known to interact with its targets via three conserved accessible regions I (positions 1-10), II (20-37), and III (47-60) [2, 3] that are depicted in Figure 1. It has been shown that mainly sites I and III are important for the interaction with the target mRNA encoded by the *sthA* gene. The mutated sequences in Figure 1 (mutation in capital red letters) were employed in the original study [3].

Mutating sites I and III has been reported to show the highest effect while the mutation of region II exhibited only minor effects. Figure 2 shows the according minimal energy profiles for the Spot42 wildtype and all three mutants. The individual mutations on their own do not completely break the regulation of *sthA* by Spot42 [3], which suggests additive effects of regions I and III.

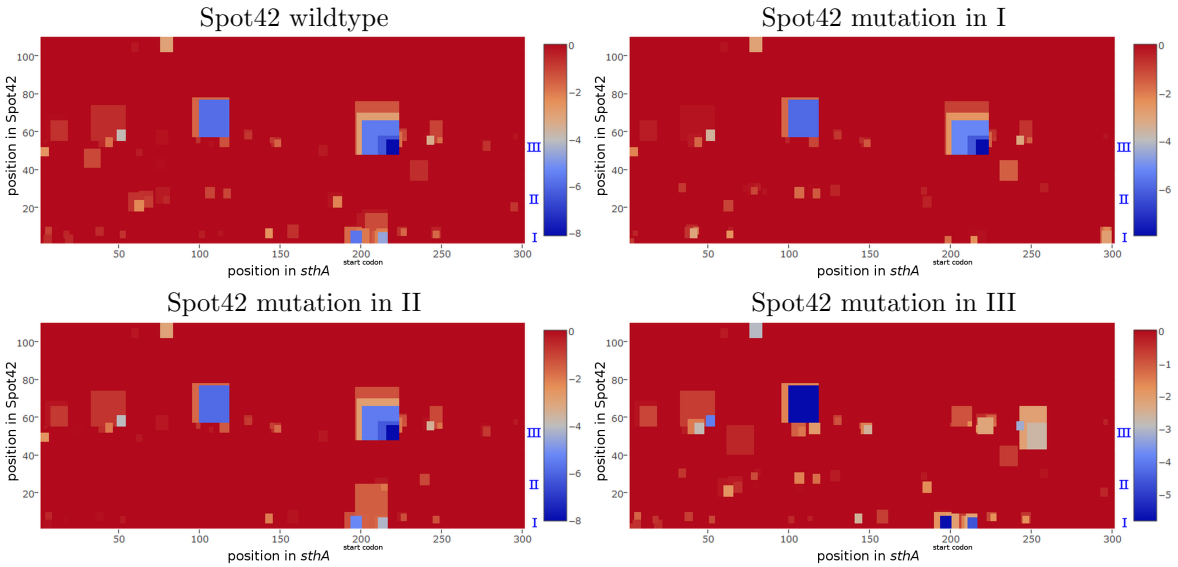

Figure 2: Minimal energy profile for all intermolecular index pairs covered by any predicted interaction of Spot42 and the *sthA* mRNA (with E<0) for different sequence variants of Spot42. Conserved accessible regions I, II and III of Spot42 known to interact with target RNAs are tagged on the right.

While the mutation of region II shows (as experimentally shown) no significant effect (only a minor minimal energy increase for sites I and III of 0.5 kcal/mol), mutating region I or III breaks the respective predicted interaction site completely. On the other hand the non-mutated site stays intact and may be responsible for the remaining partial regulation of *sthA* by the mutant [3].

3 Supplementary Table 1

The following table contains the results for the benchmark. The first, second and third columns specify the sRNA name, locus tag and name of the target, respectively. The fourth column indicates the rank produced by an IntaRNAv1 whole genome target prediction (arguments `-p 7 -w 150 -L 100`). The fifth column shows the rank produced by an IntaRNAv2 whole genome target prediction with standard parameters (arguments `--seedBP 7 --tAccW 150 --tAccL 100 --qAccW 0 --qAccL 0`) and the sixth column shows the rank for IntaRNAv2 whole genome target predictions which enforce a seed energy $\leq$ -4.8 kcal/mol (additional argument `--seedMaxE=-4.8`). Rank '-' indicates that no prediction could be made for a specific sRNA-target pair. The last column contains the references to the articles reporting the RNA pairs.

sRNA	tar locus tag	tar name	v1	v2	v2 seed≤-4.8	reference
ArcZ	STM1682	tpx	1678	1995	1191	[5]
ArcZ	STM2970	sdaC	4433	4420	-	[5]
ArcZ	STM3216	-	3616	3662	-	[5]
ArcZ	b1892	flhD	2322	2525	1726	[6]
ArcZ	b2741	rpoS	192	423	256	[7]
ArcZ	b3546	eptB	2665	4276	-	[8]
ChiX	STM0687	ybfM/chiP	8	8	6	[9]
ChiX	STM1313	celB	2	4	3	[9]
ChiX	b0619	dpiB/citA	6	4	4	[10]
ChiX	b0681	chiP	3	3	3	[11]
ChiX	b1737	chbC	2	2	2	[12]
CyaR	STM0833	ompX	129	83	59	[13]
CyaR	b0723	sdhA	243	376	260	[14]
CyaR	b0814	ompX	118	70	58	[15]
CyaR	b1740	nadE	2332	1813	-	[15]
CyaR	b1824	yobF	60	54	46	[14]
CyaR	b2416	ptsI	77	53	45	[14]
CyaR	b2666	yqaE	405	661	448	[15]
CyaR	b2687	luxS	690	556	379	[15]
DsrA	b1237	hns	14	8	2	[16]
DsrA	b2741	rpoS	1	1	-	[16]
DsrA	b3251	mreB	1786	1683	-	[17]
FnrS	b0723	sdhA	31	30	543	[14]
FnrS	b0755	gpmA	1873	1852	1059	[18]
FnrS	b0887	cydD	418	315	174	[19]
FnrS	b1107	nagZ	427	395	-	[14]
FnrS	b1479	maeA	330	329	2341	[18]
FnrS	b1531	marA	54	96	-	[14]
FnrS	b1656	sodB	667	118	64	[19]
FnrS	b1841	yobA	5	5	5	[19]
FnrS	b2153	folE	512	539	284	[18]
FnrS	b2303	folX	807	515	271	[18]
FnrS	b2531	iscR	1	1	1	[14]
FnrS	b3829	metE	211	61	37	[19]
FnrS	b3908	sodA	770	1095	591	[19]
GcvB	STM0002	thrA	168	83	47	[20]
GcvB	STM0245	metQ	620	367	562	[20]
GcvB	STM0399	brnQ	77	55	171	[20]
GcvB	STM0602	ybdH	573	227	125	[20]
GcvB	STM0665	gltI	109	131	100	[21]
GcvB	STM0959	lrp	165	368	194	[20]
GcvB	STM1299	gdhA	116	110	1824	[20]
GcvB	STM1452	tppB	819	924	457	[20]
GcvB	STM1746.S	oppA	147	130	1187	[21]
GcvB	STM2355	argT	35	18	330	[21]
GcvB	STM2526	ndk	55	78	2060	[20]
GcvB	STM3062	serA	119	177	131	[20]
GcvB	STM3064	iciA	209	61	35	[20]
GcvB	STM3225	ygjU/sstT	459	447	232	[20]
GcvB	STM3564	livK	47	133	80	[21]

GcvB	STM3567	livJ	75	99	60	[21]
GcvB	STM3630	dppA	16	20	13	[21]
GcvB	STM3903	ilvE	1519	2122	957	[20]
GcvB	STM3909	ilvC	6	4	3	[20]
GcvB	STM3930	yifK	299	233	129	[22]
GcvB	STM4351	-	978	1598	736	[21]
GcvB	STM4398	cycA	20	9	6	[20]
GcvB	b1040	csgD	240	520	326	[23]
GcvB	b1130	phoP	851	713	417	[24]
GcvB	b3089	sstT	287	126	374	[25]
GcvB	b4208	cycA	44	36	30	[26]
GlmZ	b3729	glmS	392	202	77	[27]
MicA	STM4231	lamB	89	161	-	[28]
MicA	b0411	tsx	661	419	275	[29]
MicA	b0814	ompX	398	186	131	[29]
MicA	b0957	ompA	57	54	-	[30]
MicA	b1130	phoP	50	61	46	[31]
MicC	STM1572	ompD	137	92	58	[32]
MicC	b2215	ompC	2	1	1	[33]
MicF	STM0366	yahO	174	126	65	[34]
MicF	STM0959	lrp	2	3	2	[34]
MicF	STM1328	lpxR	388	306	158	[34]
MicF	b0241	phoE	V167	189	97	[35]
MicF	b0889	lrp	1	2	1	[35]
MicF	b0929	ompF	10	8	5	[36]
MicF	b3912	cpxR	401	287	371	[35]
OmrA	b0565	ompT	38	52	42	[37]
OmrA	b1040	csgD	52	85	68	[38]
OmrA	b1892	flhD	2590	2144	1457	[6]
OmrA	b2155	cirA	2779	2735	1860	[37]
OmrA	b3405	ompR	69	110	84	[37]
OmrB	b0565	ompT	145	206	71	[37]
OmrB	b1040	csgD	27	23	11	[38]
OmrB	b1892	flhD	3192	2793	-	[6]
OmrB	b2155	cirA	1492	3458	-	[37]
OmrB	b3405	ompR	268	330	118	[37]
OxyS	b1892	flhD	488	385	254	[6]
OxyS	b2731	fhlA	2444	2923	-	[39]
RprA	b1040	csgD	9	10	9	[40]
RprA	b1341	ydaM	300	436	260	[40]
RprA	b2741	rpoS	29	35	-	[41]
RybB	STM0413	tsx	313	51	46	[42]
RybB	STM0687	ybfM/chiP	981	660	484	[43]
RybB	STM0999	ompF	613	647	475	[42]
RybB	STM1070	ompA	825	1048	732	[42]
RybB	STM1473	ompN	79	58	51	[44]
RybB	STM1530	-	71	60	53	[45]
RybB	STM1572	ompD	986	1196	813	[42]
RybB	STM1732	ompW	2150	2498	-	[42]
RybB	STM1995	ompS	525	791	1640	[42]
RybB	STM2267	ompC	139	83	71	[42]
RybB	STM2391	fadL	548	902	641	[42]
RybB	b0081	mraZ	38	50	39	[14]
RybB	b0721	sdhC	2113	1569	1036	[46]
RybB	b0805	fiu	410	193	146	[29]
RybB	b1256	ompW	643	292	219	[47]
RybB	b2215	ompC	199	133	108	[47]
RybB	b2594	rluD	840	418	344	[29]
RyhB	b0118	acnB	237	620	319	[48]
RyhB	b0156	erpA	157	153	228	[14]
RyhB	b0288	ykgJ	552	623	323	[49]
RyhB	b0592	fepB	216	197	-	[49]
RyhB	b0683	fur	3825	3923	-	[50]
RyhB	b0721	sdhC	177	157	92	[46]
RyhB	b0723	sdhA	225	1329	1813	[14]

RyhB	b0894	dmsA	14	14	7	[49]
RyhB	b1107	nagZ	18	17	9	[14]
RyhB	b1200	dhaK	786	905	-	[49]
RyhB	b1452	yncE	2950	3495	-	[49]
RyhB	b1531	marA	1662	1221	-	[14]
RyhB	b1588	ynfF	316	182	101	[49]
RyhB	b1612	fumA	446	447	230	[51]
RyhB	b1656	sodB	780	842	439	[52]
RyhB	b1778	msrB	358	298	152	[53]
RyhB	b1981	shiA	570	515	-	[54]
RyhB	b2069	yegD	3166	2944	-	[49]
RyhB	b2155	cirA	153	149	87	[49]
RyhB	b2206	napA	324	270	108	[49]
RyhB	b2530	iscS	357	250	130	[55]
RyhB	b3365	nirB	46	63	40	[14]
RyhB	b3607	cysE	83	74	45	[56]
RyhB	b3942	katG	882	1083	-	[49]
RyhB	b4070	nrfA	214	694	360	[49]
RyhB	b4122	fumB	241	249	129	[49]
SgrS	STM2945	sopD	1287	747	450	[57]
SgrS	STM3962	yigL	95	60	36	[58]
SgrS	b1101	ptsG	8	7	6	[59]
SgrS	b1817	manX	1531	1696	1004	[60]
SgrS	b2416	ptsI	73	15	12	[14]
Spot42	STM2190	mglB	97	94	50	[61]
Spot42	b0039	caiA	1765	1043	-	[62]
Spot42	b0720	gltA	179	326	163	[3]
Spot42	b0721	sdhC	44	42	28	[46]
Spot42	b0728	sucC	203	188	85	[14]
Spot42	b0757	galK	1	7	9	[2]
Spot42	b1136	icd	50	82	41	[14]
Spot42	b1302	puuE	3	1	1	[62]
Spot42	b1398	paaK	10	14	8	[62]
Spot42	b1761	gdhA	49	49	31	[14]
Spot42	b1901	araF	134	152	67	[63]
Spot42	b2221	atoD	166	137	61	[62]
Spot42	b2702	srlA	456	758	-	[3]
Spot42	b2715	ascF	135	216	102	[62]
Spot42	b2801	fucP	29	31	22	[62]
Spot42	b2802	fucI	1634	2016	1041	[3]
Spot42	b3224	nanT	6	5	3	[62]
Spot42	b3566	xylF	108	181	417	[3]
Spot42	b3927	glpF	67	74	39	[62]
Spot42	b3962	sthA	454	542	280	[3]
Spot42	b4311	nanC	51	35	24	[3]

4 Supplementary FASTA

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gaccgauugaagccaauugcagacaucaugugugacugaguauuugguguaggcgauagccuaaaaucac
ccgccagcagauaauaucugcuggcuuuuuuu
>Spot42_stm
guaggguacagagguaagauguucuaucuuucagaccuuuuacuucacguaaucggauuuggcugaaua
uuuuagccgccccagucaguuuaugacuggggcguuuuuua
```

5 Target sequence generation

Input: The target sequences that were used for the benchmark can be generated by performing a whole genome target prediction using the IntaRNA webserver interface. For this, "Get target RNA sequences from NCBI Genome" should be selected on the input page. Then, any FASTA file can be pasted into the query area. Next, either NC_000913 or NC_003197 need to be specified in the "Target NCBI RefSeq ID" field. NC_000913 retrieves the sequences for *Escherichia coli* and NC_003197 does the same for *Salmonella*. In the last input step, "nt up" needs to be set to 200, "nt down" needs to be set to 100 and the job can be started.

Output: On the result page "Show Input Parameters" can be selected. Here, "Target RNA (long) in FASTA" shows a link to a FASTA file with the target sequences of interest.

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