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Insights from the molecular docking aided interaction analysis of Hfq with small RNAs

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Abstract:

Hfq, RNA binding protein, is widely found in most of the prokaryotes. It plays a key role in gene regulation by binding with small RNA and facilitates mRNA pairing there by suppress or boost translation according to RNA structures. Interaction between sRNAs and Hfq in

Salmonella SL1344 were screened using Co-Immuno Precipitation (Hfq-CoIP) studies earlier. We have formulated an *In silico* approach, to model the 3D structures of 155 sRNA and studied their interactions with Hfq proteins. We have reported the key interacting PHE⁴², LEU⁷, VAL²⁷, PHE³⁹ and PRO²¹ residues of Hfq binds with many small RNAs. Further mutation of PHE⁴² in to ALA⁴² in Hfq leads to loss of sRNA binding efficiency. We have differentiated the interactions in to Hfq binding and non-binding sRNAs, based on Atomic Contact Energy and area. This methodology may be applied generically for functional grouping of small RNAs in any organism.

Key words: Hfq, small RNA, Co-IP, RNA-SEQ, reads

Background:

Hfq is a homo hexameric protein widely found in many Bacteria and Archaea [1]. Hfq was initially identified as host factor in replication process of Q β RNA bacteriophage [2]. Later, it was found to involve in the virulence of *Salmonella typhimurium* [3]. Hfq could be identified as post transcriptional regulator for small RNA-mRNA complexes in many Bacteria [4]. Hfq and Hfq dependent small RNAs and their network are major contributors of virulence and development in *Salmonella* [5]. Hfq suppress the translation by guarding sRNA so that it binds to 5' region of mRNA, thereby making 5' region inaccessible [4]. Hfq and RNaseE binds with similar region on RNA which facilitates coupled degradation of sRNA and its target mRNA in *Escherichia coli* [6]. Hfq promotes protein synthesis in similarly by directing sRNA to bind to 5' region of mRNA, in cases when 5' region of its mRNA forms a secondary structure which inhibit ribosome binding [7]. In such cases, secondary structure of mRNA is disrupted by the action of Hfq and sRNA. Hfq protects sRNA from ribonuclease cleavage while it can also promote mRNA cleavage [8]. Hfq's role seems to depend upon the structural information encoded in the RNA molecules which interacts with Hfq [4], [6]. Hfq (PDB ID: 2YLB) has ring like architecture and it has two faces for potential interaction with nucleic acids such as proximal face and distal face (Figure 1). The polymer chains of 2 YLB from N-terminus and C-terminal were marked from blue to red color (Figure 2). Face on which alpha helix exposed and near to centre is called proximal face and the one opposite to it is called distal face [9]. sRNAs interact with proximal and distal face differently. Hfq have highly conserved motif among 26 bacterial genomes. VYKHAIST are reported as the conserved motif in the Hfq in diverse genera [10]. Many Co-Immuno Precipitation studies show Hfq interacting with sRNAs. Therefore, it is of interest to document derived data from the molecular docking aided interaction of Hfq with small RNAs to gain functional insights.

Materials & Methods:

Already reported 155 sRNA sequences from SL1344 through Co-IP studies [3] were taken. Corresponding sequences were obtained and then 3D structures of sRNA were predicted using RNAComposer (<https://rnacomposer.cs.put.poznan.pl/>) [11]. With help of Literature survey, sRNAs which were supposed to bind with Hfq were identified and similarly non interacting sRNAs were also listed.

Protein-RNA docking:

PatchDock server was used to carry out the Protein-RNA docking. PatchDock returns Z score and Atomic contact Energy (ACE) for the complex [12], [13]. PatchDock server is based upon geometry-

based molecular docking algorithm [13]. PatchDock provides docking transformations that yield good molecular shape complementarities. Transformations had wide interface areas and small amounts of steric clashes. PatchDock algorithm divides the Connolly dot surface, a representation of surface where only atoms that are accessible to solvent [14] are shown, into concave, convex and flat patches.

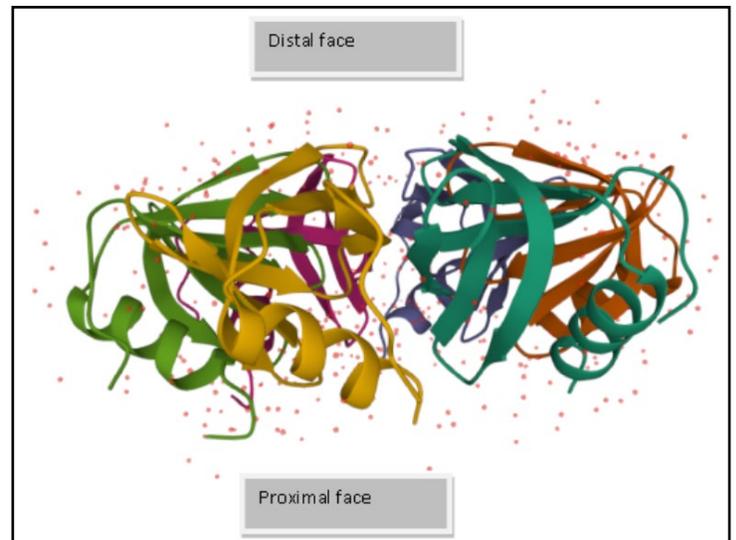


Figure 1: 3D structure of Hfq protein from *Salmonella typhimurium* PDB ID: 2YLB visualized using Mol (Javascript) available in pdb server.

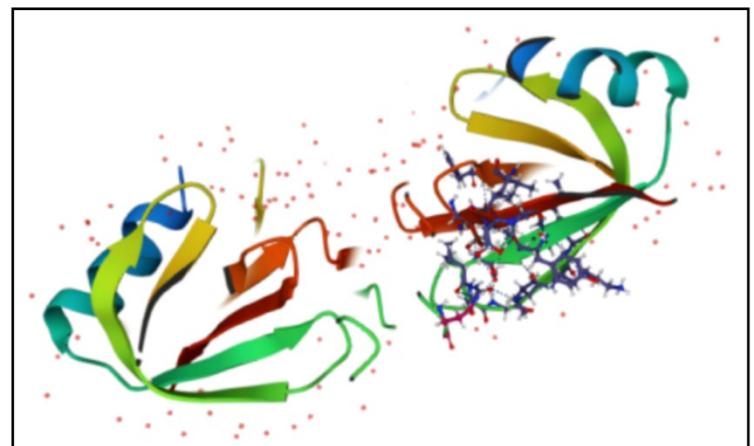


Figure 2: The polymer chains from N- to C-terminus were marked from blue to red color using residue property option and visualized using Mol (Javascript) available in pdb server.

Protein RNA interface of complex is identified using PRIDB of IOWA State University [15]. Conformations are generated by matching complementary patches. Scoring function evaluates each candidate transformation that considers both geometric fit and atomic desolvation. Based on geometric shape complementary score and conformations are ranked. Atomic contact energy is the

desolvation free energy required to transfer atom from water to a protein's interior. Approximate interface area of the Protein-RNA complex is returned as Area. sRNA passing through the pore of Hfq were examined using VMD [16] and the results were tabulated.

Table 1: List of interacting residues of sixteen sRNAs claim to bind with different chains of Hfq from this study.

Not Binding	chain A	chain B	chain C	chain D	chain E	chain F
IsrD	42 Phe	27 Val, 43 Val, 42 Phe	27 Val, 42 Phe		42 Phe	
ryhB-2				21 Pro, 67 Pro, 7 Leu	50 Val, 39 Phe	
IsrF			39 Phe	42 Phe	7 Leu, 4 Gly, 10 Pro, 39 Phe	42 Phe
IsrG	67 Pro, 10 Pro	67 Pro	50 Val		21 Pro, 50 Val	
IsrH-2	50 Val	68 Val, 20 Val			29 Gly, 30 Ile, 32 Leu	63 Val, 30 Ile
IsrH-1		12 Leu, 39 Phe, 10 Pro	4 Gly	67 Pro	50 Val	46 Leu, 34 Gly, 21 Pro
IsrJ		42 Phe, 10 Pro, 7 Leu	39 Phe			14 Ala
IsrK			67 Pro	30 Ile	29 Gly, 30 Ile	30 Ile, 32 Leu
IsrB-2	42 Phe, 7 Leu	10 Pro, 42 Phe, 45 Leu	42 Phe	42 Phe		
IsrL	7 Leu	42 Phe		42 Phe	42 Phe	
IsrM		7 Leu, 42 Phe, 10 Pro	42 Phe, 39 Phe	4 Gly, 42 Phe	42 Phe	
IsrN	53 Met, 42 Phe, 43 Val	39 Phe	42 Phe			42 Phe
IsrO	42 Phe	42 Phe		42 Phe	42 Phe	68 Val, 67 Pro, 7 Leu, 42 Phe, 14 Ala
IstR-1.2	67 Pro					32 Leu
SRaL	50 Val, 42 Phe			42 Phe	42 Phe	42 Phe, 67 Pro
IsrQ			4 Gly	68 Val, 67 Pro, 21 Pro, 34 Gly, 20 Val, 4 Gly	42 Phe, 4 Gly, 50 Val	42 Phe

Table 2: List of area and ACE (Atomic Contact energy) of sRNA which are proposed to bind with Hfq based on this study.

sRNA name	Score	Area	ACE	cDNA reads Control coIP	cDNA reads Hfq coIP	Pass around the pore
NC_016810_IsrG_2342448_2342729	14022	2737.5	-231.81	0	0	No
NC_016810_IsrJ_c2759646_2759717	14092	2208.4	-137.07	0	0	No
NC_016810_IsrH_1_c2392019_2392469	14670	4248.5	-319.72	0	0	No
NC_016810_IsrL_c2850244_2850588	15436	2583.6	234.31	0	0	No
NC_016810_IstR_1_2_c4019328_4019459	15628	2255.1	-48.3	0	0	No
NC_016810_IsrN_2929501_2929643	15814	3148.6	-177.71	0	0	No
NC_016810_SraL_c4526162_4526302	15934	3111.5	-350.23	0	0	No
NC_016810_IsrK_c2760480_2760556	16006	2175	-142.81	0	0	No
NC_016810_IsrM_2927616_2927944	16190	2580.2	-79.91	0	0	No
NC_016810_IsrH_2_c2392019_2392298	16204	2006.2	-315.62	0	0	No
NC_016810_IsrF_c1586724_1587013	16730	2969.8	-139.22	0	0	No
NC_016810_RyhB_2_c1309727_1309937	16990	2542.5	-91.77	0	0	No
NC_016810_IsrD_c1302591_1302641	17288	2460	-43.77	0	0	No
NC_016810_IsrQ_4783574_4783741	17328	2965.2	-694.78	0	0	No
NC_016810_IsrO_3219100_3219300	17470	2326	24.73	0	0	No
NC_016810_IsrB_2_c1060740_1060833	18316	2521.3	-79.03	0	0	No
NC_016810_IsrA_338816_339238	13594	2073	-17.17	0	0	No

Table 3: List of area and ACE (Atomic Contact energy) of sRNA reported to bind with Hfq through CoIP studies.

S. No	sRNA name	Score	Area	ACE	cDNA reads Control coIP	cDNA reads Hfq coIP	Passing through the pore of Hfq
1	NC_016810_IsrC_1285998_1286285	14660	2320.7	154.62	0	1	yes
2	NC_016810_SInc150_c1282521_1282677	15346	2373.3	-83.55	0	1	no
3	NC_016810_IsrI_c2759018_2759265	14596	3305.7	-288.38	0	2	no
4	NC_016810_IsrP_4328157_4328304	15744	2551.2	-124.67	0	2	no
6	NC_016810_RyhB_1_c3736722_3736817	16334	3154.1	-316.06	0	2	yes
7	NC_016810_RseX_2034743_2034837	15842	2624.7	-81.89	0	3	no
8	NC_016810_IsrB_1_1060741_1060835	16440	2638.5	-498.86	2	4	yes
9	NC_016810_RyfA_2672651_2672944	18042	3743.7	141.89	3	6	yes
10	NC_016810_OxyS_c4364305_4364423	17760	3203.2	-132.9	0	10	yes
11	NC_016810_RydB_1407278_1407371	12776	3372.4	-691.34	4	10	no

12	NC_016810_MicF_2364629_2364721	17378	3690.3	-	0	11	yes
13	NC_016810_MicC_c1702531_1702639	14972	2506.4	89.21	0	15	no
14	NC_016810_RygC_3243669_3243813	13288	1820.4	-107.92	14	17	no
15	NC_016810_STnc540_1376271_1376364	14910	2207.5	189.93	7	23	no
16	gi_378697983_stnc250_c2594737_2594511	17720	2756.6	374.3	6	24	no
17	NC_016810_SraF_3412774_3412961	15328	2207.7	-23.65	0	25	no
18	NC_016810_Spf_4230504_4230613	14002	2388	-255.68	2	33	no
19	NC_016810_STnc400_4072456_4072529	15640	2402.1	-182.94	112	42	no
20	NC_016810_OmrA_c3191584_3191670	16748	2035.7	-29.06	0	51	no
21	NC_016810_OmrB_c3191785_3191870	22398	3203.9	-395.73	1	52	no
22	NC_016810_SgrS_128577_1288084	13800	1878	392.54	3	61	no
23	NC_016810_CsrC_4231595_4231838	18286	2613.1	-230.81	63	64	no
24	NC_016810_CsrB_c3138160_3138522	17062	3233.2	-265.38	69	67	no
25	NC_016810_STnc290_c3215639_3215717	17394	2832.3	-31.61	2	72	yes
26	NC_016810_RyeC_2211623_2211760	16052	3548.3	-663.55	42	73	yes
27	NC_016810_STnc500_c1172561_1172641	14686	2506.7	23.21	7	84	no
28	NC_016810_GlmY_c2705381_2705564	12872	1879.4	186.77	20	92	no
29	NC_016810_STnc520_c1290590_1290674	14460	1919.3	3.36	12	100	no
30	NC_016810_RybB_c941815_941893	15470	2055.3	-192.69	3	103	no
31	NC_016810_RyxD_c3383000_3383144	13860	3014.3	-470.4	17	104	no
32	NC_016810_MicA_2989429_2989502	13630	2376.5	-132.39	1	128	no
33	NC_016810_DsrA_c2026216_2026302	15222	3687.6	-435.43	6	149	yes
34	NC_016810_GlmZ_4163192_4163399	15702	2829.3	-254.81	20	196	no
35	NC_016810_RyDC_1686526_1686591	15550	2421.8	-285.49	5	245	yes
36	NC_016810_RprA_c1401682_1401790	13460	2169	-251.49	37	286	yes
37	NC_016810_STnc560_MgrR_1550746_1550844	13878	1806.7	-32.2	10	290	no
38	NC_016810_STnc490_981622_981711	13902	2737.6	-348.07	75	385	yes
39	NC_016810_GcvB_3156779_3156979	18576	3324.8	-323.91	12	402	no
40	NC_016810_SsrS_3242594_3243000	14210	2818.7	299.81	836	451	yes
41	NC_016810_STnc440_4580485_4580565	16674	3071.1	-422.71	9	456	yes
42	NC_016810_RyeE_CyaR_2228847_2228932	15300	1804.4	101.3	31	659	no
43	NC_016810_SroC_c728172_728324	16846	2786.1	-343.4	26	898	no
44	NC_016810_SroB_ChiX_555450_555566	15892	2561.3	-251.22	27	1530	no
45	NC_016810_InvR_3067500_3067586	18948	3231.6	-818.65	113	3236	yes
46	NC_016810_FnrS_c1706784_1706905	17290	2126.4	-5.67	Hfq binding from Literature	Alexandra Sittka <i>et al.</i> 2008	yes
47	NC_016810_SdsR_c1925621_1925723	15832	2225.6	-127.08	Hfq binding from Literature	Sylvain Durand <i>et al.</i> 2010	no
48	NC_016810_SraC_1925543_1925853	17432	3585.5	-506.37	Hfq binding from Literature	Eric Massé <i>et al.</i> 2003	no
49	NC_016810_STnc710_3829661_3829727	14452	1930.1	-11.39	Hfq binding from Literature	Claudia Monteiro <i>et al.</i> 2012	no
50	NC_016810_STnc740_c2240283_2240366	14200	3116.9	-372.5	Hfq binding from Literature	Claudia Monteiro <i>et al.</i> 2012	no
51	STnc760_378697983_c3685117_3684975	14888	2121	-32.86	Hfq binding from Literature	Claudia Monteiro <i>et al.</i> 2012	no
52	NC_016810_ArcZ_3511800_3511917	14408	2496.7	-302.37	Hfq binding from Literature	Aixia Zhang <i>et al.</i> 2003	no

Table 4: List of area and ACE (Atomic Contact energy) of 87 sRNAs not given with any Hfq binding information. Highlighted sRNA is proposed to have interactions with Hfq from this study.

S No	sRNA NAME	Score	Area	ACE	Passing through pore
1	NC_016810_1_237091_237187	12820	1841.1	-219.2	No *
2	NC_016810_1_1931455_1931546	12928	1847.7	98.47	No
3	NC_016810_RyjB_c1662190_1662282	13100	2550.2	17.24	no
4	NC_016810_STnc1590_c3633247_3633424	13246	2174.8	27.37	no
5	NC_016810_STnc1390_2767704_2767767	13864	1863.8	-189.09	no
6	NC_016810_1_4467873_4467935	13976	2022.8	-125.43	No
7	NC_016810_STnc1160_c2760372_2760451	14096	2309.5	-10.41	no
8	NC_016810_1_2039984_2040056	14176	1977.2	52.77	No
9	NC_016810_RybA_c901301_901397	14192	1986.2	-262.63	no
10	NC_016810_STnc1400_c2770951_2771060	14228	2264.2	-239.56	no
11	NC_016810_STnc2160_c4683636_4683699	14294	2179.9	-420.86	No
12	NC_016810_RyBD_807345_807429	14322	2361.9	-158.31	no
13	NC_016810_STnc730_c1462356_1462445	14380	1853.2	-101.78	no
14	NC_016810_1_3633254_3633328	14472	2361	213.54	No
15	NC_016810_STnc1460_c4403810_4404049	14490	2317.7	79.04	No
16	gi_378697983_c924838_924769	14542	2779.6	-194.88	No

17	NC_016810_STnc840_1224244_1224316	14546	1755.5	67.47	no
18	NC_016810_STnc470_c94653_94741	14562	2682.4	-585.84	no
19	gi_378697983_c3489247_3489125	14570	2432.4	-21.23	No
20	NC_016810_STnc1300_2071271_2071409	14792	1903.1	180.56	no
21	NC_016810_STnc1060_c466718_466783	14812	2575.9	-117.99	no
22	NC_016810_STnc1290_c2070801_2070988	14842	3272.5	-198.28	no
23	NC_016810_STnc1340_c2319956_2320037	14846	1985.5	-119.47	yes
24	NC_016810_1_3048830_3048948	14900	2424.9	-28.05	No
25	gi_378697983_c3472312_3472142	14940	2054.2	-537.21	No
26	NC_016810_SroA_c126713_126802	14962	2931.1	-461.24	yes
27	NC_016810_STnc1150_2222979_2223134	15028	2479.8	250.7	no
28	gi_378697983_c3295994_3295818	15038	2085.5	251.8	No
29	NC_016810_STnc1640_1345883_1345970	15100	2733.9	-22.56	no
30	NC_016810_STnc750_c3261185_3261273	15170	2111.2	123.84	no
31	NC_016810_1_3382981_3383131	15216	2451	-153.72	No *
32	gi_378697983_c4237035_4236762	15226	3136	-115.59	No
33	NC_016810_RyeF_c1958513_1958819	15272	1982.1	64.24	no
34	gi_378697983_c3782856_3782695	15350	2190.8	-106.78	No *
35	NC_016810_STnc1220_c1448718_1448790	15506	2137.6	-119.19	no
36	NC_016810_1_254126_254264	15608	2881.5	-167.46	No
37	gi_378697983_c4559977_4559883	15610	2857.7	61.67	No *
38	NC_016810_STnc2130_4475777_4475867	15658	3094.4	-11.79	no
39	NC_016810_1_2449075_2449183	15718	2362.5	-76.02	No
40	NC_016810_STnc1650_2132959_2133102	15784	2450	-21.1	no
41	gi_378697983_c1937244_1937130	15846	2193.6	-188.65	No
42	NC_016810_STnc930_514484_514578	15878	2168.3	85.95	no
43	NC_016810_sRNA3_2808504_2808645	15940	2408.9	78.69	no
44	gi_378697983_c4209680_4209485	16090	3625.4	-828.54	yes
45	NC_016810_SraA_c504870_504925	16098	2783.6	-421.53	yes
46	gi_378697983_c2672567_2672469	16104	2588.5	-88.81	No
47	NC_016810_STnc1600_4356616_4356686	16138	2697.3	-243.87	yes
48	NC_016810_STnc2040_c1819352_1819414	16172	2636.3	-569.44	no
49	gi_378697983_c587414_587347	16176	2629.9	-413.5	No
50	NC_016810_STnc780_c3858665_3858992	16292	3136.6	-903.3	yes
51	NC_016810_STnc1110_1643465_1643658	16342	2726.6	-332.79	no
52	NC_016810_STnc850_1252598_1252673	16352	3334.5	-215.25	yes
53	NC_016810_STnc1080_c1062745_1062806	16376	2354.9	-217.96	no
54	NC_016810_1_14720_14783	16402	3220	-95.03	No *
55	NC_016810_1_523912_524027	16428	2765.3	-88.29	No
56	NC_016810_1_669642_669793	16472	2569.8	13.23	No
57	NC_016810_STnc970_4292540_4292597	16524	2375.1	-11.58	No
58	gi_378697983_c1553737_1553669	16560	2169.5	-490.22	No *
59	NC_016810_1_569023_569074	16568	2398.7	-157.08	No *
60	NC_016810_1_2268274_2268488	16602	2882.5	-856.04	yes
61	NC_016810_STnc700_2147102_2147280	16602	2444.7	-139.3	yes
62	NC_016810_STnc1380_c2729634_2729701	16604	2496.9	58.54	no
63	NC_016810_STnc770_c3778180_3778332	16778	2580.8	-761.1	yes
64	NC_016810_STnc1470_4797957_4798031	16808	2013.4	-322.01	yes
65	NC_016810_1_1316874_1317268	16820	2587.8	-175.25	No
66	NC_016810_STnc880_4486745_4486823	16820	2705.8	-230.37	no
67	NC_016810_STnc1010_41629_41708	16854	2492.9	-436.21	yes
68	NC_016810_1_2897019_2897102	16932	2715.9	-275.23	No *
69	NC_016810_1_259116_259164	16994	2612.7	-192.62	No
70	NC_016810_SraB_1231270_1231378	17026	2392.7	40.1	no
71	NC_016810_1_3593794_3593896	17114	2553.2	4.68	No
72	NC_016810_STnc1020_74847_74924	17198	2601.7	-61.57	no
73	NC_016810_sRNA10_c679315_679414	17214	2681.4	59.57	yes
74	NC_016810_sRNA1_257725_257790	17220	3844.9	-810.35	no
75	gi_378697983_c2321137_2321042	17230	2393.3	134.36	No
76	gi_378697983_c2759518_2759410	17232	2336.9	-334.37	No
77	NC_016810_STnc1210_1292956_1293014	17572	2699.3	98.6	no
78	NC_016810_1_2437665_2437806	18042	2676.7	-764.41	yes
79	NC_016810_1_1353832_1353959	18288	2679.5	172.96	No *
80	gi_378697983_c756436_756377	18346	2612.3	-234.44	Yes *
81	NC_016810_STnc2090_2808306_2808464	18394	3764.5	167.33	yes
82	gi_378697983_c1913755_1913615	18926	3354.3	-593.16	yes
83	NC_016810_STnc1270_c1789368_1789691	19134	2615.8	193.01	no
84	gi_378697983_c3312520_3312429	19486	2668.7	-475.42	No
85	NC_016810_1_1784673_1784725	19862	3195.9	-23.19	No *
86	NC_016810_1_1466054_1466240	20654	3380.7	-494.95	No *
87	NC_016810_1_4553813_4553972	20946	3406.2	-499.67	No

Results & Discussion:

Among the interacting residues, Phe⁴² of Hfq interacts well with sRNA. Residues interacting with sRNA are Phenyl alanine, Leucine, Glycine, Isoleucine, Valine, Proline, Alanine, Methionine which are hydrophobic and non-polar in nature. In most of the interactions, Phe⁴² is interacting sRNA. Phe⁴² forms a loop in Hfq protein. Hfq highly binds with AU rich regions of sRNA. Most of the interacting residues of sRNA of Hfq were PHE⁴², LEU⁷, VAL²⁷, PHE³⁹, and PRO²¹. This study has found Phe⁴² as a key residue for sRNA interactions. Alteration of Phe⁴² position with Ala⁴² has decreased the binding of Hfq with small RNAs and rpoS mRNA segment containing translation initiation region. There by marking the suppression of the phenotype [17]. On the other way, sRNA having cDNA reads Hfq coIP having lesser score. When the interacting residues were analyzed on hydrophobic interaction, Phenyl alanine takes place in most of the interaction with length less than 5 Å resolution. As suggested earlier Phe⁴² and Phe³⁹ were also important in virulence of *Salmonella*. Tyr⁴² plays a key role in RNA binding Hfq by stacking with the RNA bases in *Staphylococcus aureus* [17]. Entire *Salmonella*, genera has Phe at 42nd position. Both Tyr and Phe amino acids are Aromatic amino acids and similar in structure wise.

PatchDock Score and Deep sequencing reads were compared and sixteen sRNAs were proposed to interact with Hfq from this study (Table 1). The data are taken and analyzed with positive control shown with earlier Co-IP studies [18] (Table 2). IsrC sRNA exhibits less Hfq coIP reads but not having fewer score in PatchDock score (Table 3). But it is not the case with InvR, where it holds highest Patch Dock score among the Binding group sRNA and it also have high Hfq coIP read. Taking Patch dock score of InvR, sRNA of unknown group, with high scores which were highlighted in table are more likely to have interaction with Hfq. Those high scoring sRNA candidates are NC_016810_1_4553813_4553972, NC_016810_1_1466054_1466240, NC_016810_1_1784673_1784725, gi_378697983_c3312520_3312429, Stnc2090, Stnc1270, gi_378697983_c1913755_1913615, gi_378697983_c756436_756377 (Table 4). Other sRNA might have activity with Hfq, but sRNA not binding also has relatively good patchdock score comparing with other sRNA in binding group. But our interrogation studies has found too many false negatives among non-binding sRNA group. It is evident from certain sRNA has high PatchDock score also have high interface area of Protein-RNA complex. Hence only sRNA with very high PatchDock scores is proposed to have interaction with Hfq. The Hfq-RNA structure has binding mode of the RNA

within the central basic pore of Hfq. Binding of Hfq through central pore or along the central groove having electrostatic interaction, Hfq modulating RNA structure, thereby regulating translation, can be suggested [17]. This study has attempted to identify the key interacting residues of Hfq having binding with sRNAs. We have differentiated the Hfq binding and non-binding sRNAs based on their Atomic Contact Energy and Area value thresholds.

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