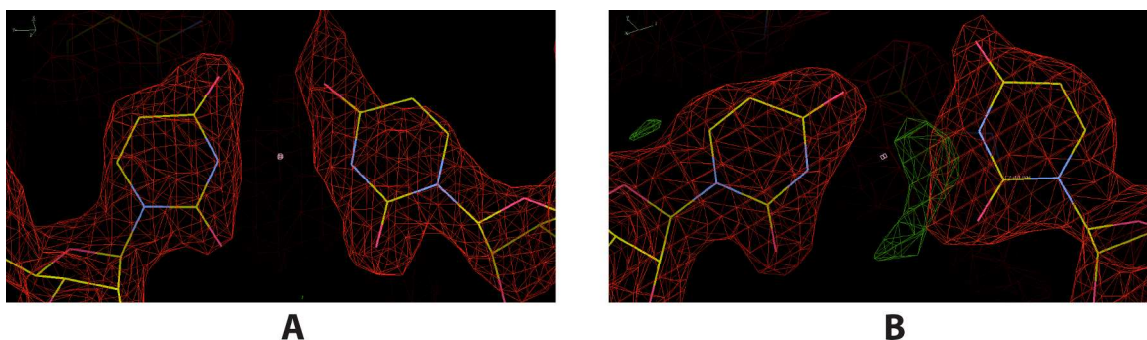


Supporting Information

Crystal Structure Studies of RNA Duplexes Containing  $s^2U:A$  and  
 $s^2U:U$  Base Pairs

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**Figure S1.** Experimental density map of (A) U5:U10 pair in chain EF (corresponding to Fig. 4D); and (B) U5:U10 pair in chain GH (corresponding to Fig. 4E). The red color represents the 2Fo-Fc map with  $\sigma$  level 1.3; the green color represents the Fo-Fc map with  $\sigma$  level 3.0.

**Table S1.** Local base-pair structural parameters in the  $s^2$ U-U 7mer ( $s^2$ UU): [5'-uagc( $s^2$ U)cc-3'/3'-aucgUgg-5'] and the native-UU 7mer: [5'-uagucc-3'/3'-aucgugg-5'], including all four duplexes in the asymmetric unit (N-UU-1, N-UU-2, N-UU-3 and N-UU-4).

		Local Base-Pairs							
		U-A	A-U	G-C	C-G	U-U	C-G	C-G	Ave.
Shear (Å)	N-UU-1	-0.34	0.16	-0.3	0.77	-2.45	0.33	0.33	-0.21
	N-UU-2	0.02	-0.02	-0.39	0.39	2.08	0.18	0.52	0.40
	N-UU-3	-0.3	-0.1	-0.25	0.34	0.68	0.12	0.17	0.09
	N-UU-4	-1.02	-0.03	-0.36	0.03	-1.87	0.06	0.66	-0.36
	$s^2$ UU	-0.33	0.06	-0.43	0.24	<b>2.26</b>	0.27	0.38	0.35
Stretch (Å)	N-UU-1	-0.07	-0.02	-0.17	-0.05	-1.66	-0.22	-0.19	-0.34
	N-UU-2	-0.22	-0.14	-0.13	-0.23	-1.8	-0.23	-0.2	-0.42
	N-UU-3	-0.24	-0.36	-0.31	-0.13	-1.36	-0.2	-0.18	-0.40
	N-UU-4	-0.14	-0.26	-0.19	-0.25	-0.74	-0.3	-0.36	-0.32
	$s^2$ UU	-0.05	-0.14	-0.23	-0.17	<b>-1.56</b>	-0.16	-0.17	-0.35
Stagger (Å)	N-UU-1	-0.33	-0.11	-0.13	-0.23	0.18	-0.46	-0.27	-0.19
	N-UU-2	-0.24	-0.18	0.08	-0.27	0.42	-0.11	0.11	-0.03
	N-UU-3	0.07	0	-0.17	-0.22	0.21	0.1	-0.31	-0.05
	N-UU-4	-0.29	0.04	-0.06	0.04	-0.39	-0.17	-0.1	-0.13
	$s^2$ UU	0.07	0.26	-0.02	-0.12	<b>0.12</b>	-0.12	-0.01	0.03
Buckle (°)	N-UU-1	6.68	0.92	-1	4.15	11.53	13.57	2.86	5.53
	N-UU-2	1.39	1.7	2.22	14.86	0.57	8.12	0.49	4.19
	N-UU-3	2.74	1.16	-0.6	3.32	1.81	2.81	8.14	2.77
	N-UU-4	5.57	-0.38	0.91	6.38	4.7	2.2	5.21	3.51
	$s^2$ UU	-3.60	6.66	4.95	6.43	<b>-6.89</b>	5.63	-0.17	1.86
Propeller (°)	N-UU-1	-15.02	-8.25	-12.97	-18.54	-9.36	-18.07	-1.28	-11.93
	N-UU-2	-10.01	-8.84	-11	-21.05	-20.79	-16.77	-6.4	-13.55
	N-UU-3	-6.81	-13.36	-11.66	-12.73	-13.28	-11.63	-5.75	-10.75
	N-UU-4	-1.99	-10.48	-14.96	-13.86	-16.02	-15.69	-3.66	-10.95
	$s^2$ UU	-2.51	-13.02	-11.90	-11.72	<b>-15.51</b>	-18.80	-1.41	-10.70
Opening (°)	N-UU-1	3.71	3.11	-1.54	-0.83	7.1	2.37	0.97	2.13
	N-UU-2	-0.43	-0.17	1.51	2.91	9.19	2.71	3.14	2.69
	N-UU-3	1.98	2.8	1.76	3.02	-4.62	3.7	-0.38	1.18
	N-UU-4	-4.66	-2.3	2.78	3.7	-11.61	2.22	2.64	-1.03
	$s^2$ UU	0.60	4.92	2.28	-2.44	<b>-4.86</b>	1.58	-1.30	0.11

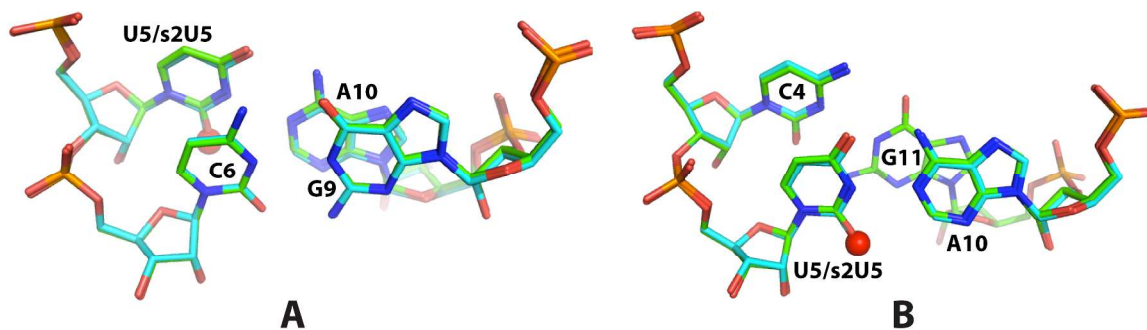
**Table S2.** Structural parameters of base pair steps in s<sup>2</sup>U-U 7mer (s<sup>2</sup>UU): [5'-uagc(s<sup>2</sup>U)cc-3'/3'-aucgUgg-5'] and Native-UU 7mer: [5'-uagucc-3'/3'-aucgugg-5'] including all the four duplexes in one asymmetric unit (N-UU-1, N-UU-2, N-UU-3 and N-UU-4).

		Base-Pair Steps						
		UA/UA	AG/CU	GC/GC	CU/UG	UC/GU	CC/GG	Ave.
X-disp (Å)	N-UU-1	-2.78	-4.3	-3.42	-6.86	-1.85	-4.44	-3.94
	N-UU-2	-3.67	-6.59	-3.35	-2.78	-4.4	-4	-4.13
	N-UU-3	-3.49	-3.36	-3.98	<b>-3.88</b>	<b>-4.23</b>	-4.17	-3.85
	N-UU-4	-3.74	-4.9	-3.56	<b>-7.24</b>	<b>-2.67</b>	-3.08	-4.20
	s <sup>2</sup> UU	-4.20	-4.45	-3.39	<b>-3.13</b>	<b>-4.24</b>	-4.38	-3.97
Inclination (°)	N-UU-1	13.16	11.47	8.35	47.25	9.49	16.55	17.71
	N-UU-2	18.49	12.23	7.65	16.74	14.44	9.5	13.18
	N-UU-3	13.59	13.76	10.3	20.78	9.74	8.71	12.81
	N-UU-4	12.18	13.6	15.11	31.96	7.11	10.68	15.11
	s <sup>2</sup> UU	19.27	17.69	12.79	<b>15.25</b>	<b>6.61</b>	14.24	14.31
Shift (Å)	N-UU-1	0.22	-0.55	-0.1	-0.36	-0.3	0.1	-0.17
	N-UU-2	0.1	0.42	-0.96	0.19	-0.17	0.18	-0.04
	N-UU-3	-0.01	-0.54	0.56	-0.38	0.27	0.39	0.05
	N-UU-4	0.35	-0.77	0.26	-0.55	0.47	0.18	-0.01
	s <sup>2</sup> UU	-0.01	-0.79	-0.37	<b>0.3</b>	<b>0.68</b>	-0.67	-0.14
Slide (Å)	N-UU-1	-0.99	-1.72	-1.55	-1.69	-0.94	-1.66	-1.43
	N-UU-2	-1.08	-2.42	-1.57	-1.1	-1.29	-1.85	-1.55
	N-UU-3	-1.33	-1.3	-1.6	-1.4	-1.71	-1.82	-1.53
	N-UU-4	-1.63	-1.86	-1.45	-1.93	-1.52	-1.36	-1.63
	s <sup>2</sup> UU	-1.43	-1.61	-1.35	<b>-1.53</b>	<b>-1.66</b>	-1.62	-1.53
Rise (Å)	N-UU-1	3.5	3.26	3.23	2.8	3.37	3.58	3.29
	N-UU-2	3.35	3.18	2.93	3.58	2.91	3.41	3.23
	N-UU-3	3.26	3.23	3.16	3.18	3.12	3.1	3.18
	N-UU-4	3.5	3.23	3.05	3.17	3.46	3.17	3.26
	s <sup>2</sup> UU	2.94	3.17	3.25	<b>3.67</b>	<b>2.71</b>	3.46	3.20
Tilt (°)	N-UU-1	-2.59	-0.57	0.65	-10.24	4.81	-1.43	-1.56
	N-UU-2	0.2	-3.21	-1.78	-2.64	9.32	-0.12	0.30
	N-UU-3	0.41	-0.33	1.67	-3.31	2.65	5.91	1.17
	N-UU-4	-2.97	-1.94	-0.26	0.03	-2.01	-0.46	-1.27
	s <sup>2</sup> UU	-1.89	-2.56	-0.55	<b>2.40</b>	<b>7.73</b>	-2.29	0.47
Roll (°)	N-UU-1	8.1	6.12	4.83	19.04	7.34	9.47	9.15
	N-UU-2	10.21	5.54	4.41	12.05	6.16	5.59	7.33
	N-UU-3	7.85	8.13	5.47	12.57	5.04	4.68	7.29
	N-UU-4	7.42	7.02	9.08	13.83	5.07	6.54	8.16
	s <sup>2</sup> UU	10.34	9.68	7.53	<b>11.50</b>	<b>3.00</b>	7.74	8.30
Twist (°)	N-UU-1	35.2	30.53	33.38	16.15	45.02	32.35	32.11
	N-UU-2	30.98	25.68	33.24	41.04	22.95	33.92	31.30
	N-UU-3	32.99	33.72	30.45	33.65	29.64	30.56	31.84
	N-UU-4	34.9	29.32	34.2	22.42	41.53	35.28	32.94
	s <sup>2</sup> UU	29.95	30.72	33.68	<b>43.28</b>	<b>25.42</b>	30.86	32.32

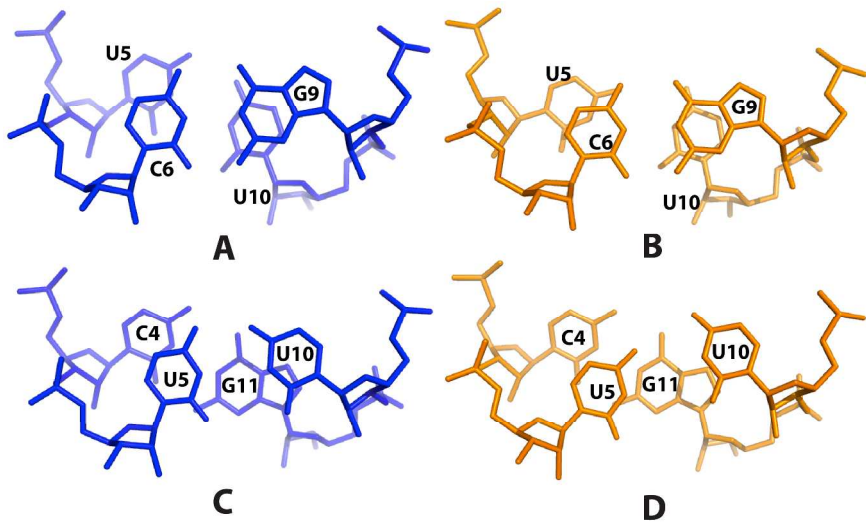
**Table S3:** Overlap area summary of base pair steps in s<sup>2</sup>U-U 7mer (s<sup>2</sup>UU): [5'-uagc(s<sup>2</sup>U)cc-3'/3'-aucgUgg-5'] and Native-UU 7mer: [5'-uagcucc-3'/3'-aucgugg-5'] including all the four duplexes in one asymmetric unit (N-UU-1, N-UU-2, N-UU-3 and N-UU-4).

	Steps	Total Overlap Area*				
		s <sup>2</sup> UU	N-UU-1	N-UU-2	N-UU-3	N-UU-4
1	UA/UA	2.19	1.85	2.27	2.41	1.98
2	AG/CU	1.55	1.50	3.10	2.48	1.26
3	GC/GC	13.04	13.74	11.57	12.71	12.05
4	CU/AG	2.04	2.26	3.12	1.88	0.73
5	UC/GU	4.85	10.53	6.32	5.55	7.69
6	CC/GG	4.62	3.89	3.86	3.40	5.01
	Overall	28.29	33.77	30.24	28.43	28.72

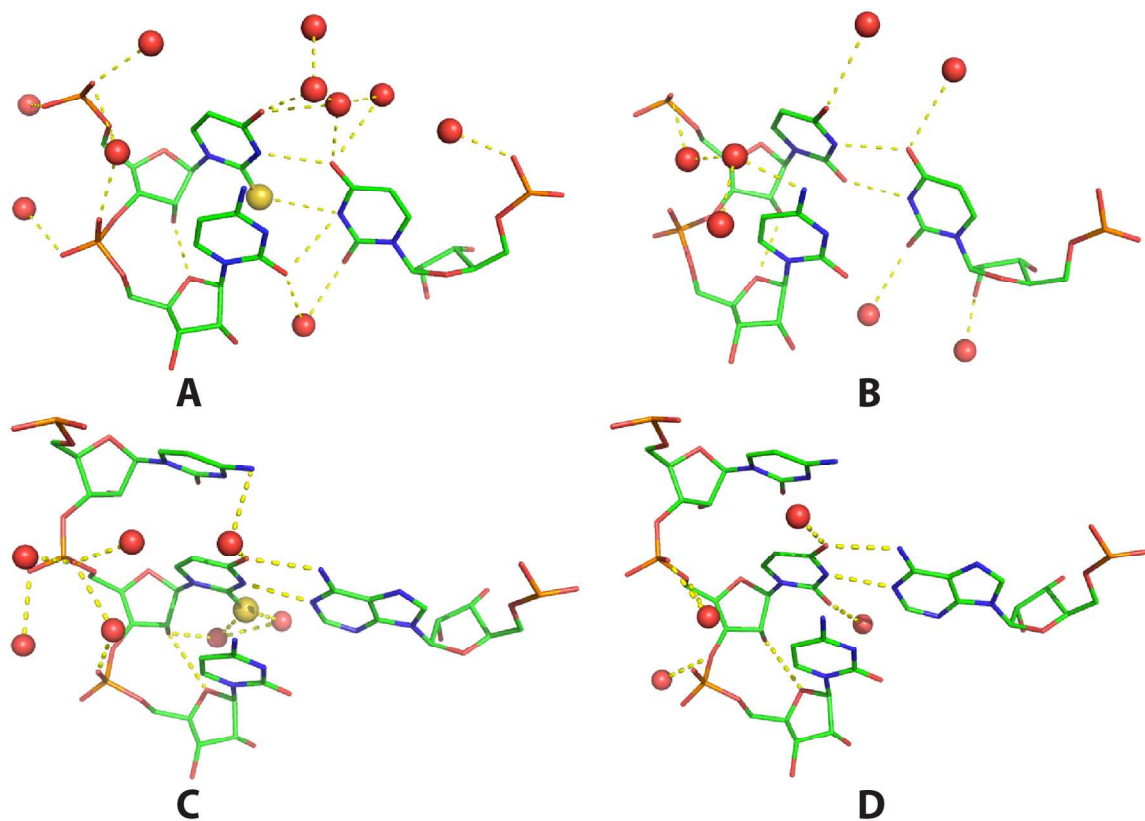
\*The total overlap area (in Å<sup>2</sup>) includes both intra-strand and inter-strand overlap within the four bases of each base pair step.



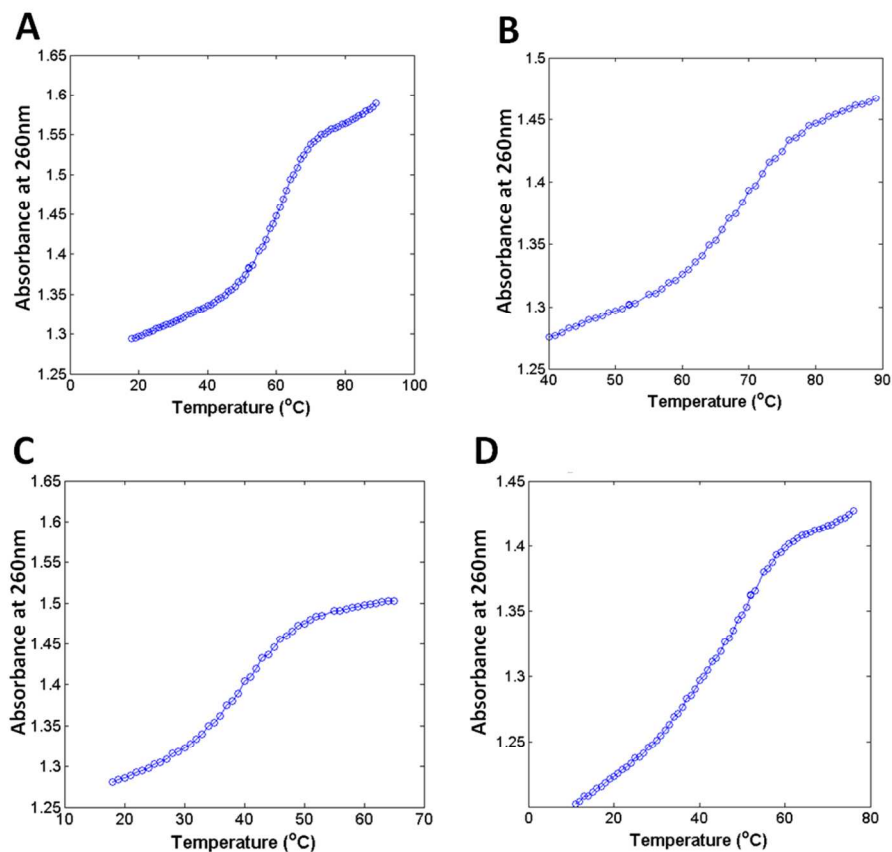
**Figure S2.** Comparison of base-pair steps in the native and s<sup>2</sup>U:A-containing duplex. (A) Superimposed views of the U5-C6/G9-A10 and s<sup>2</sup>U5-C6/G9-A10 steps; (B) Superimposed views of the C4-U5/A10-G11 and C4-s<sup>2</sup>U5/A10-G11 steps.



**Figure S3.** Stacking interactions of the two base-pair steps in the native UU-3 and UU-4 duplexes. (A) U5-C6/G9-A10 step in the native duplex UU-3. (B) U5-C6/G9-A10 step in the native duplex UU-4. (C) C4-U5/A10-G11 step in the native duplex UU-3. (D) C4-U5/A10-G11 step in the native duplex UU-4. The color code is same as in Fig. 3 and 4.



**Figure S4.** The hydration patterns discovered in (A)  $s^2$ U-U pair; (B) native U-U pair (in UU-1); (C)  $s^2$ U-A pair; (D) native U-A pair. (the yellow spheres represent the sulfur atoms)



**Figure S5.** Thermal denaturation curves of (A) U-A 7mer; (B)  $s^2$ U-A 7mer; (C) U-U 7mer; and (D)  $s^2$ U-U 7mer. Samples were prepared by dilution of stock solutions into buffers containing 100 mM  $MgCl_2$  and 200 mM HEPES, pH 7.5. Total strand concentration was 200  $\mu$ M (100  $\mu$ M duplex). Absorbance was monitored at 260 nm while the temperature was ramped at  $1^\circ C \text{ min}^{-1}$  between 4 and 89  $^\circ C$ . All melt data was collected in duplicate, from the first temperature ramp-up of independent samples.