

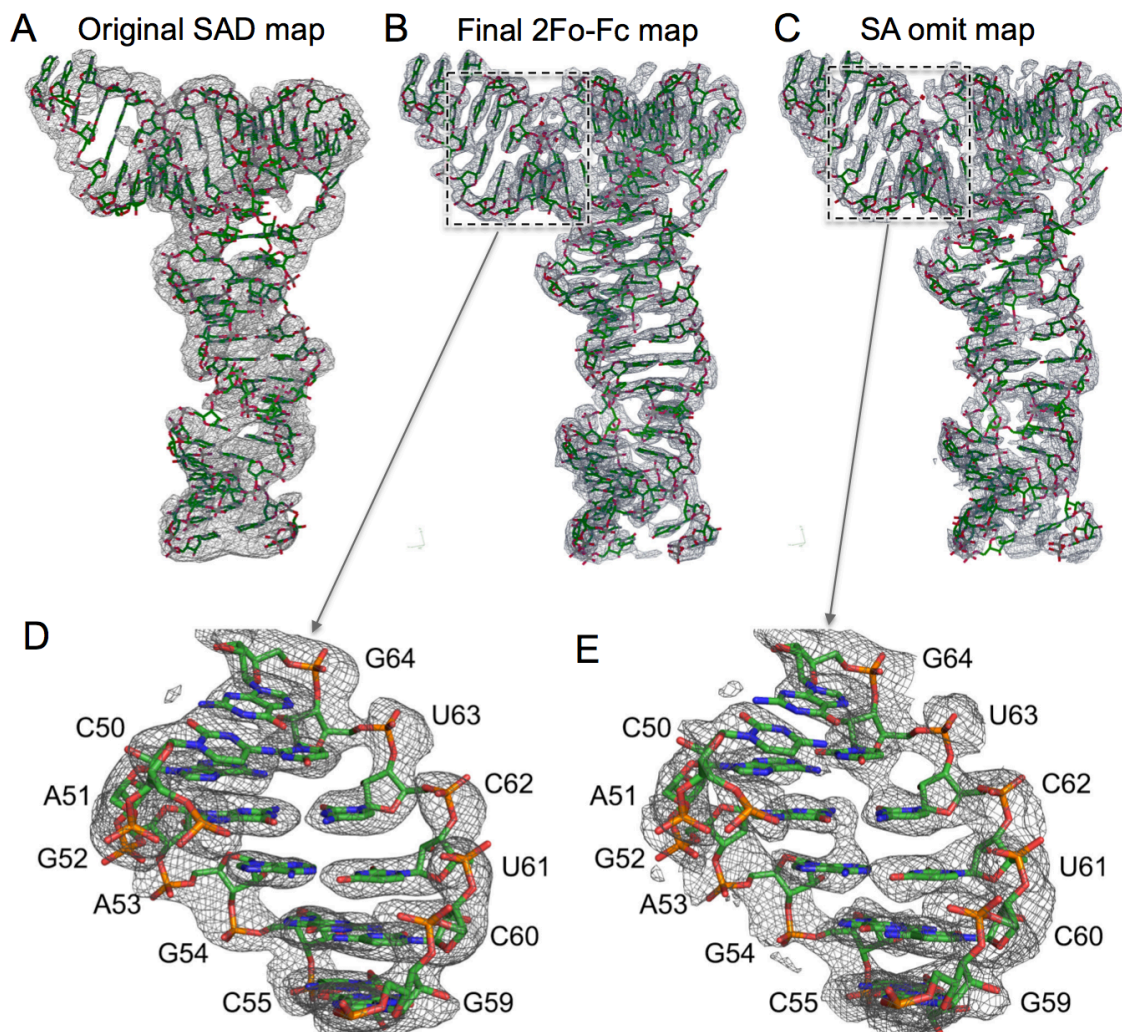
- 1 Table S1. The best crystallization conditions for YrlA constructs. Related to STAR
- 2 Method and Table 1.

	YrlA WT	YrlA Tetraloop	YrlA Tetraloop 3C
Space group	I4	C222 ₁	P6 ₃
Diffraction limit	4.9 Å	3.0 Å	5.0 Å
Crystallization conditions	0.05M Sodium Cacodylate pH6.0 10mM MgSO ₄ 1.8M Li ₂ SO ₄	0.05M Sodium Succinate pH5.5 20mM MgCl ₂ 3.0M (NH ₄) ₂ SO ₄	0.05M Sodium Cacodylate pH6.5 0.2M KCl 0.1M Mg(OAc) ₂ 10% PEG8000

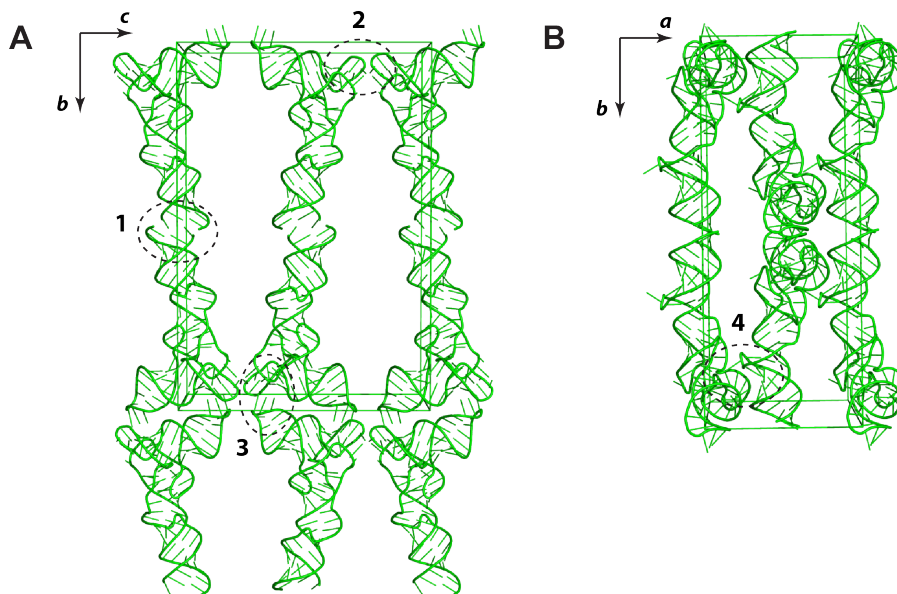
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5 Figure S1. Quality of electron density maps. Related to Table 1 and Figure 1. (A)
 6 Experimental electron density map from SAD phasing and solvent flattening (grey, 1
 7 σ level) with the final model overlaid (sticks). (B) and (D) 2Fo-Fc electron density
 8 map (grey, 1 σ level, sharpened with B-factor of -70\AA^2) with the final model overlaid
 9 (sticks). The boxed region is amplified in (D). (C) and (E) Simulated annealing (SA)
 10 omit map (grey, 1 σ level, sharpened with B-factor of -70\AA^2) with the final model
 11 overlaid (sticks). The map is generated with the standard composite omit map
 12 procedure implemented in Phenix (torsion angle simulated annealing with 5% of
 13 model omitted at a time). The boxed region is amplified in (E).



15 Figure S2. Crystal contacts of *S. Typhimurium* YrlA Tetraloop molecules. Related to
16 Table 1 and Figure 1. (A) Packing of YrlA molecules perpendicular to the *a* axis. For
17 clarity, only one layer of molecules in the unit cell is shown. The interactions include
18 1) stacking interaction between symmetry-related AC/Connector stem termini of
19 two neighboring molecules; 2) stacking interaction between symmetry-related base
20 pair C40-G75 in the TSL-DSL regions of two neighboring molecules 3) hydrogen
21 bonds between one ⁵⁶GAAA^{58a} tetraloop and the TSL-DSL region of a symmetry
22 related YrlA. (B) Crystal packing of YrlA molecules perpendicular to the *c* axis. Only
23 one layer of molecules in the unit cell is shown. Interaction 4) consists of the minor
24 groove surface of one YrlA T-stem packing against the Connector duplex of a
25 symmetry-related YrlA molecule.



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