Table S1. DNA Primers

Primer Name	Sequence (5' to 3')	Description
Y25Df	gtc gtg aac gta tcc ctg ttt cta tcg acc ttg tga acg gca tta aac tgc aag g	Mutagenic primers to construct distal face RNA-binding mutant of *V. cholerae* Hfq (Y25D)
Y25Dr	cct tgc agt tta atg ccg ttc aca agg tcg ata gaa aca ggg ata cgt tca cga c	
K56Af	gct gaa gaa cac agt aaa cca aat ggt tta cgc gca tgc gat ttc tac tgt ggt tcc tg	Mutagenic primers to construct proximal face RNA-binding mutant of *V. cholerae* Hfq (K56A)
K56Ar	cag gaa cca cag tag aaa tcg cat gcg cgt aaa cca ttt ggt tta ctg tgt tct tca gc	
Qrr1S1	gtg acc cgc aag ggt cac cta gcc aac tga cgt tgt tag tga ata atc	Gene synthesis primers to construct Qrr1 template for *in vitro* transcription
Qrr1AS1	gaa tga gtc tat tgg ctg tta ttt gtg aac att gat tat tca cta aca acg tca gtt ggc	
Qrr1S2	taa tac gac tca cta tag ggt gac ccg caa ggg tcac	
Qrr1AS2	aaa aaa ata gcc aat aga atg agt cta ttg gct gtt att tgt gaa c	
Qrr2S1	ggt gac cct tgt taa gcc gag ggt cac cta gcc aac tga cgt tgt tag tg	Gene synthesis primers to construct Qrr2 template for *in vitro* transcription
Qrr2AS1	cgc gat tgg ctt ata tat gat gtg aac aat act att cac taa caa cgt cag ttg gct agg	
Qrr2S2	taa tac gac tca cta tag ggt gac cct tgt taa gcc gag	
Qrr2AS2	aaa aaa ata gcc aat cgc aag aac cgc gat tgg ctt ata tat gat gtg aac	
Qrr3S1	ggg tga ccc tta att aag ccg agg gtc acc tag cca act gac gtt gtt ag	Gene synthesis primers to construct Qrr3 template for *in vitro* transcription
Qrr3AS1	gat tgg ctg ata aaa caa atg tga aca att tca ttc act aac aac gtc agt tgg cta ggt g	
Qrr3S2	taa tac gac tca cta tag ggt gac cct taa tta agc cga g	
Qrr3AS2	aaa aaa gcc aat cac aaa agg gtg att ggc tga taa aac aaa tgt gaa caa ttt cat tc	
Qrr4S1	gtg acc ctt cta agc cga ggg tca cct agc caa ctg acg ttg tta gtg aac acc	Gene synthesis primers to construct Qrr4 template for *in vitro* transcription
Qrr4AS1	gtg tga ttg gcc gtc tat aag tgt gaa caa tgg tgt tca cta aca acg tca gtt gg	
Qrr4S2	taa tac gac tca cta tag ggt gac cct tct aag ccg agg	
Qrr4AS2	aaa aaa aag gcc aac cac aag aag tgt gat tgg ccg tct ata agtgtg	

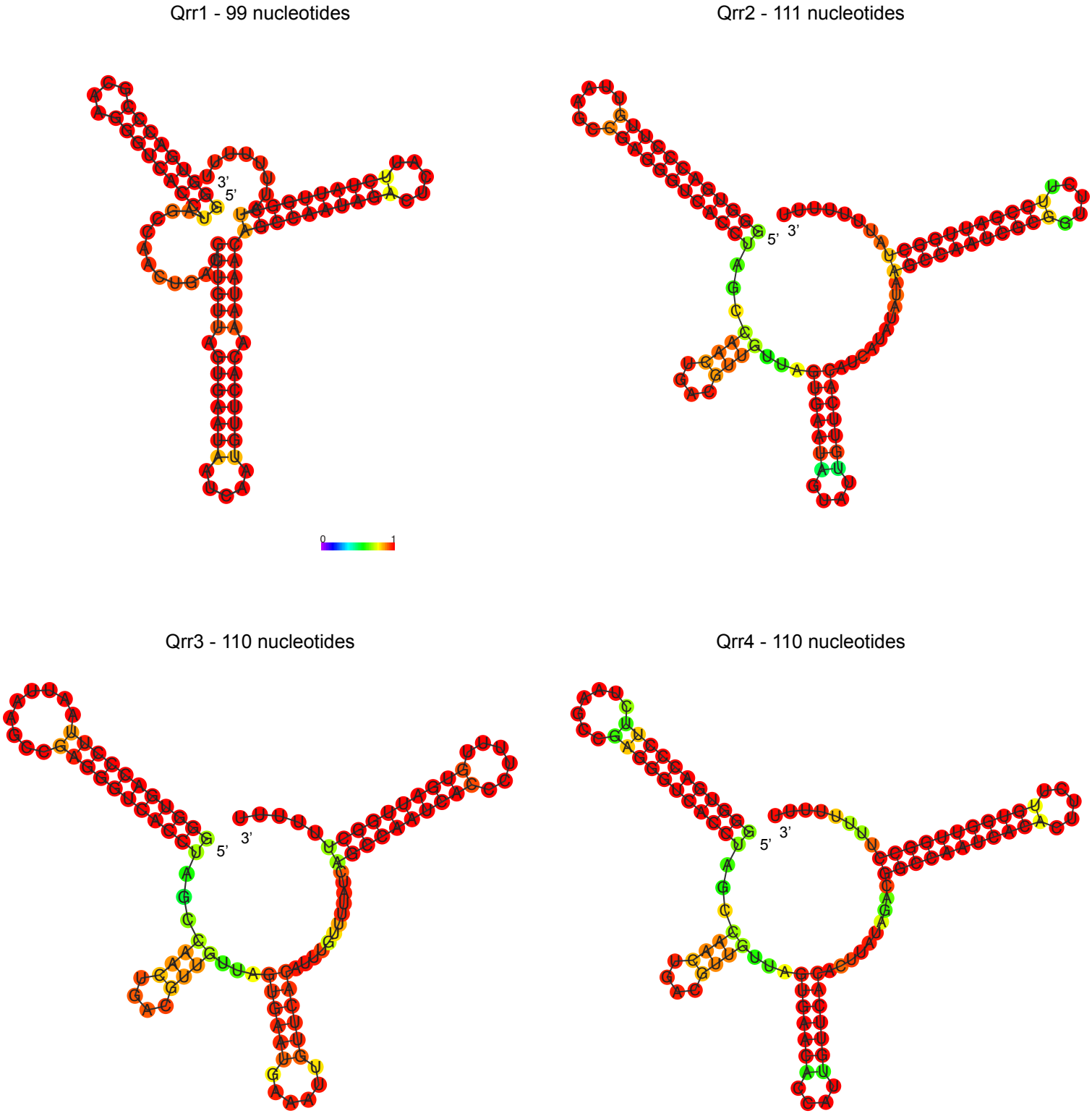

Figure S1. Secondary structure of Qrrs1-4

Secondary structures were predicted using the Vienna RNAfold server (RNA stuff @ tbi.univie.ac.at). Nucleotides are coloured according to the probability of being present in the shown secondary structure with blue corresponding to a probablility of zero an red to a probability of one. The RNA sequences shown include the three additional guanosines that were added to the 5' end of the RNAs in this work in order to ensure efficient transcription by T7 RNA polymerase. The structures are similar to those reported by Lenz *et al.* (6) suggesting that the addition of these nucleotides does not significantly alter the secondary structures.

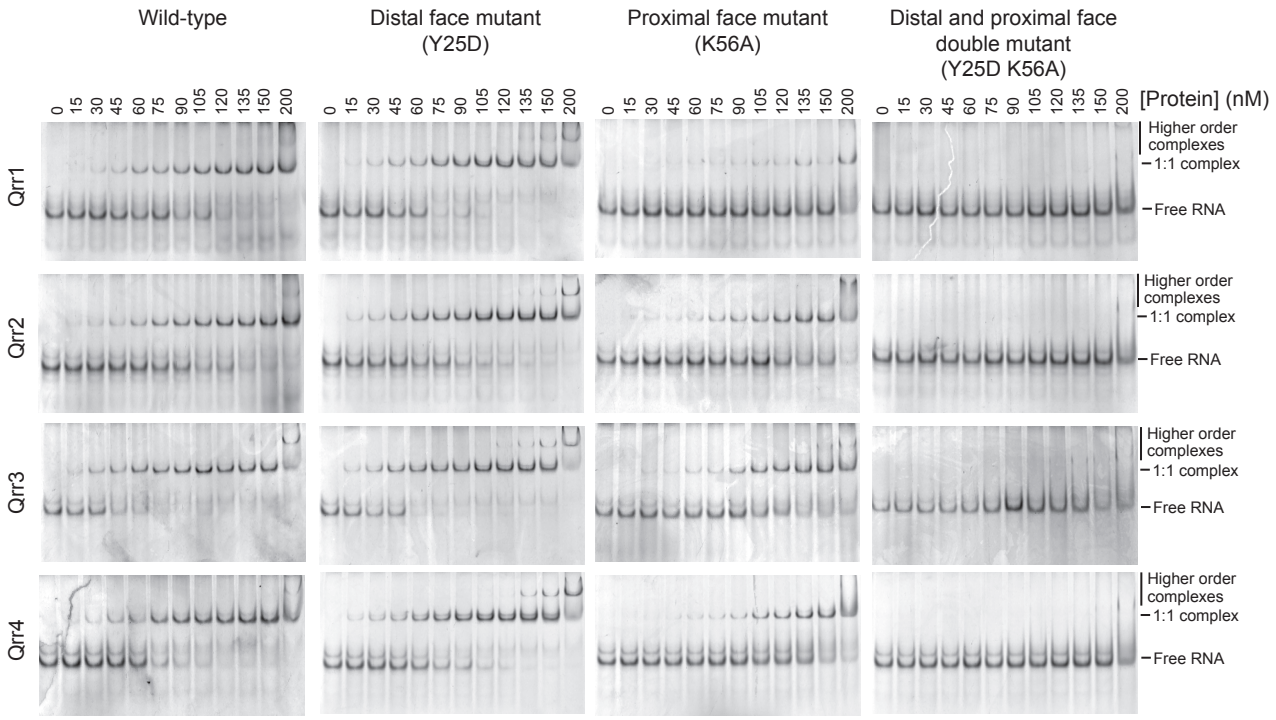

Figure S2. VcHfq binding to Qrrs 1-4

Representative EMSAs for wild-type VcHfq, a distal site mutant (Y25D), a proximal site mutant (K56A) and a distal and proximal site double mutant (Y25D K56A) binding to Qrr1, Qrr2, Qrr3 and Qrr4. RNAs were present at 30 nM in each case and the concentration of VcHfq present is indicated above the lane. The mobilities of free Qrr RNA, the 1:1 VcHfq:Qrr complex and higher order complexes are indicated on the right of the gels.

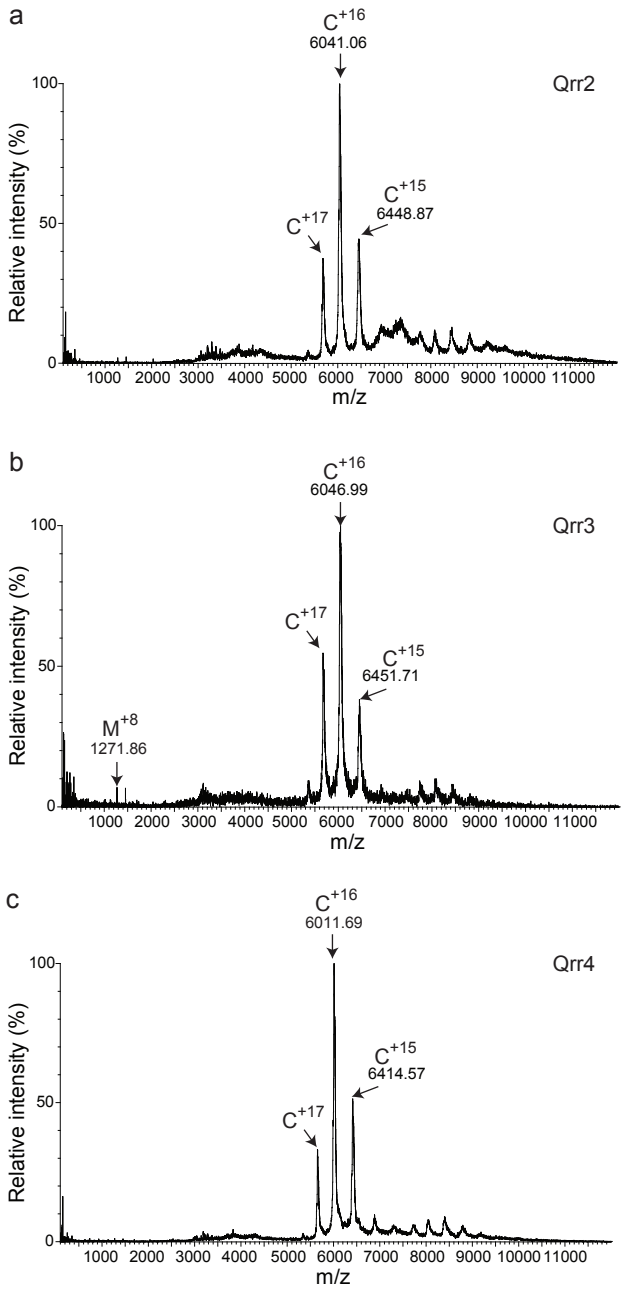

Figure S3. Stoichiometry of VcHfq-Qrr complexes

Non-denaturing MS spectra of VcHfq with Qrr2 (a), Qrr3 (b) and Qrr4 (c). VcHfq and the respective Qrr were mixed a equimolar concentrations. Peak series corresponding to the 15+ to 17+ charge states of the 1:1 VcHfq (hexamer):Qrr complexes are labelled with C in each spectrum. (a) VcHfq:Qrr2 1:1 complex (theoretical mass: 96,714.8 Da; experimental mass: 96,615 Da). (b) VcHfq:Qrr3 1:1 complex (theoretical mass: 96,291.6 Da; experimental mass: 96,750 Da). A smaller peak series, centred on the 8+ charge state, corresponds to a small amount of monomeric VcHfq and is labelled with M (theoretical mass 10,163.6 Da; experimental mass: 10,164.9 Da). (c) VcHfq:Qrr4 1:1 complex (theoretical mass: 96,278.6 Da; experimental mass: 96,185 Da).

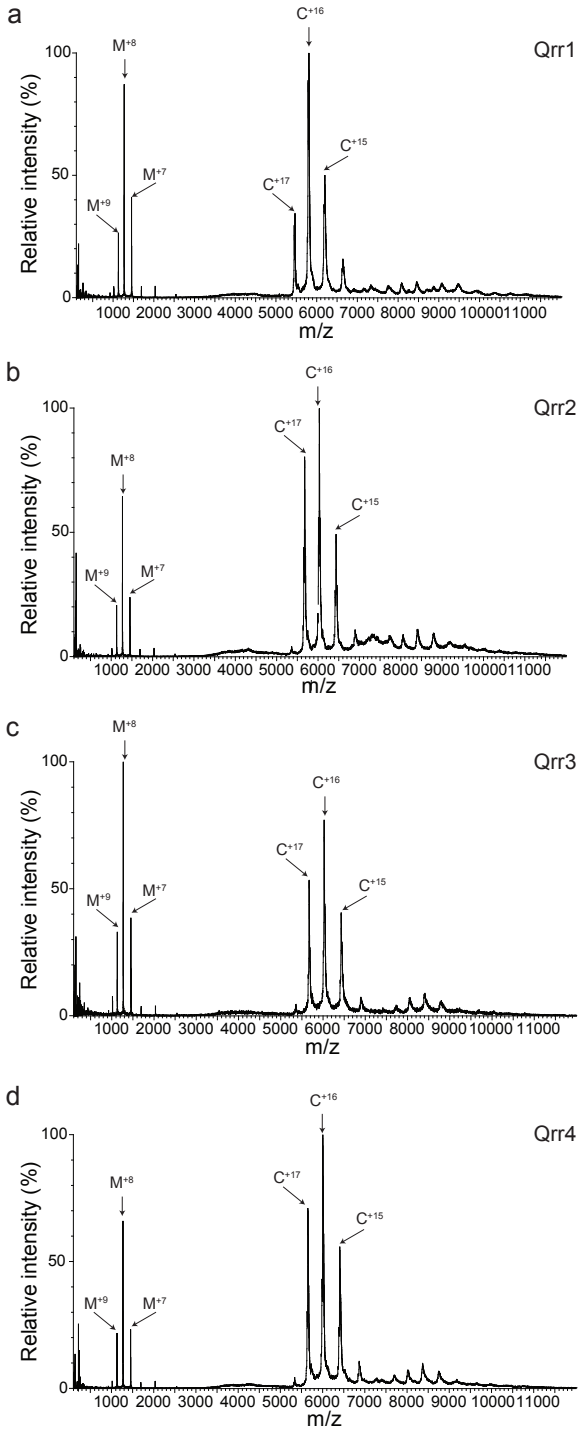

Figure S4. Collision induced dissociation of VcHfq-Qrr complexes

CID MS spectra of (a) VcHfq-Qrr1, (b) VcHfq-Qrr2, (c) VcHfq-Qrr3 and (d) VcHfq-Qrr4 complexes. The peak series corresponding to the VcHfq:Qrr 1:1 complex is labelled with C and the peak series corresponding to free VcHfq (monomer) is labelled with M in each spectrum.

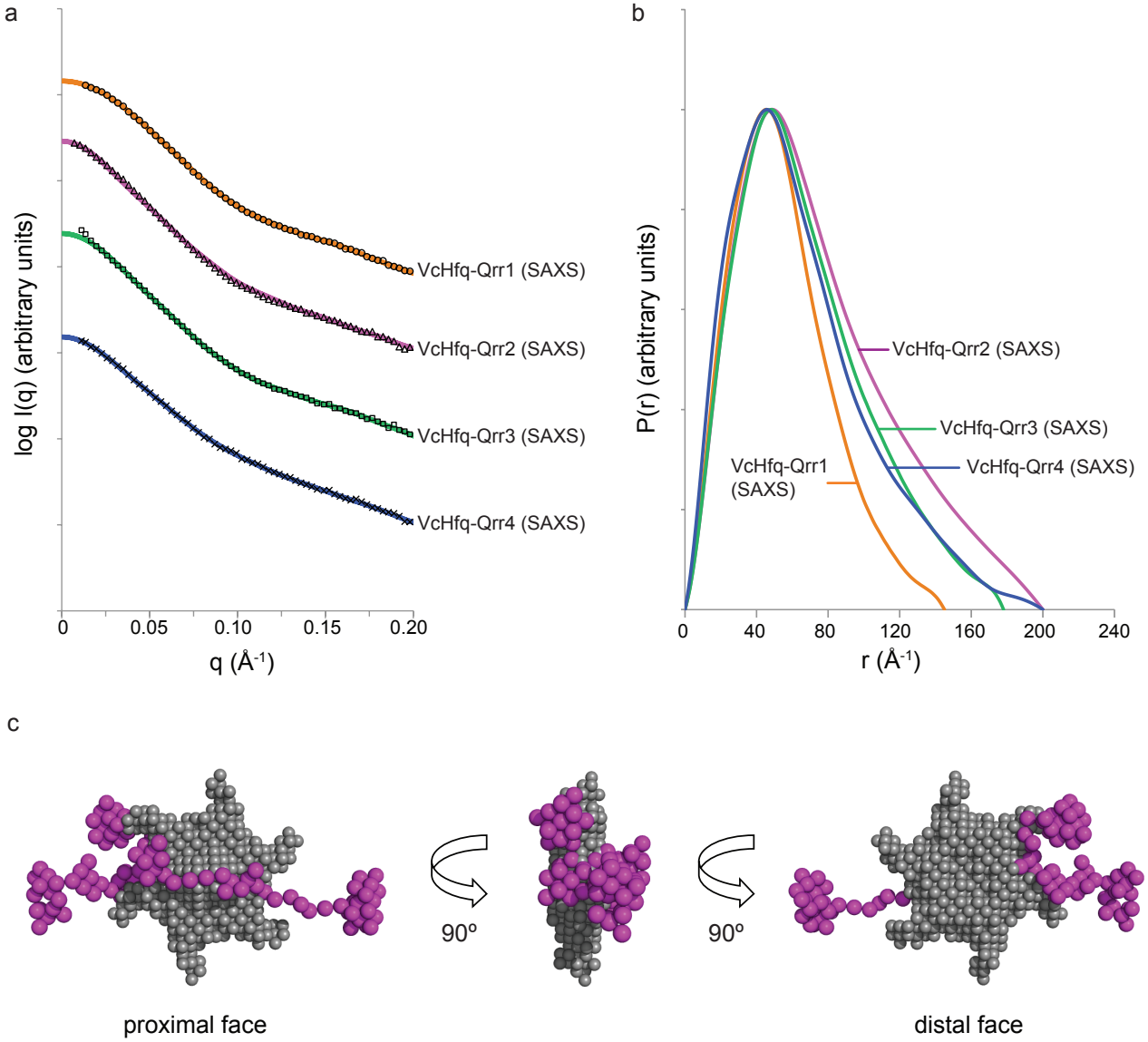

Figure S5. Comparison of VcHfq-Qrr complexes

(a) SAXS scattering curves for VcHfq-Qrr1 (circles), VcHfq-Qrr2 (triangles), VcHfq-Qrr3 (squares) and VcHfq-Qrr4 (crosses). The VcHfq-Qrr1 scattering curve is the same as in Figure 5a but is shown again here for comparison. Back-transformed distance distribution functions, P(r), are shown for VcHfq-Qrr1 (orange), VcHfq-Qrr2 (magenta), VcHfq-Qrr3 (green) and VcHfq-Qrr4 (blue).

(b) P(r) functions for the SAXS data of VcHfq-Qrr1 (orange), VcHfq-Qrr2 (magenta), VcHfq-Qrr3 (green) and VcHfq-Qrr4 (blue). The VcHfq-Qrr1 P(r) function is the same as in Figure 5b but is shown again here for comparison. Since some evidence of aggregation was evident for the VcHfq-Qrr3 complex, a q_{min} of 0.019 $Å^{-1}$ was chosen to obtain a P(r).

(c) *Ab initio* model of the VcHfq-Qrr2 complex generated in MONSA (29,30) and visualized in PyMOL. VcHfq is shown as grey spheres and the Qrr2 sRNA as magenta spheres. The proximal and distal faces labels are derived from the data for the RNA-binding mutants presented in Figure S2.

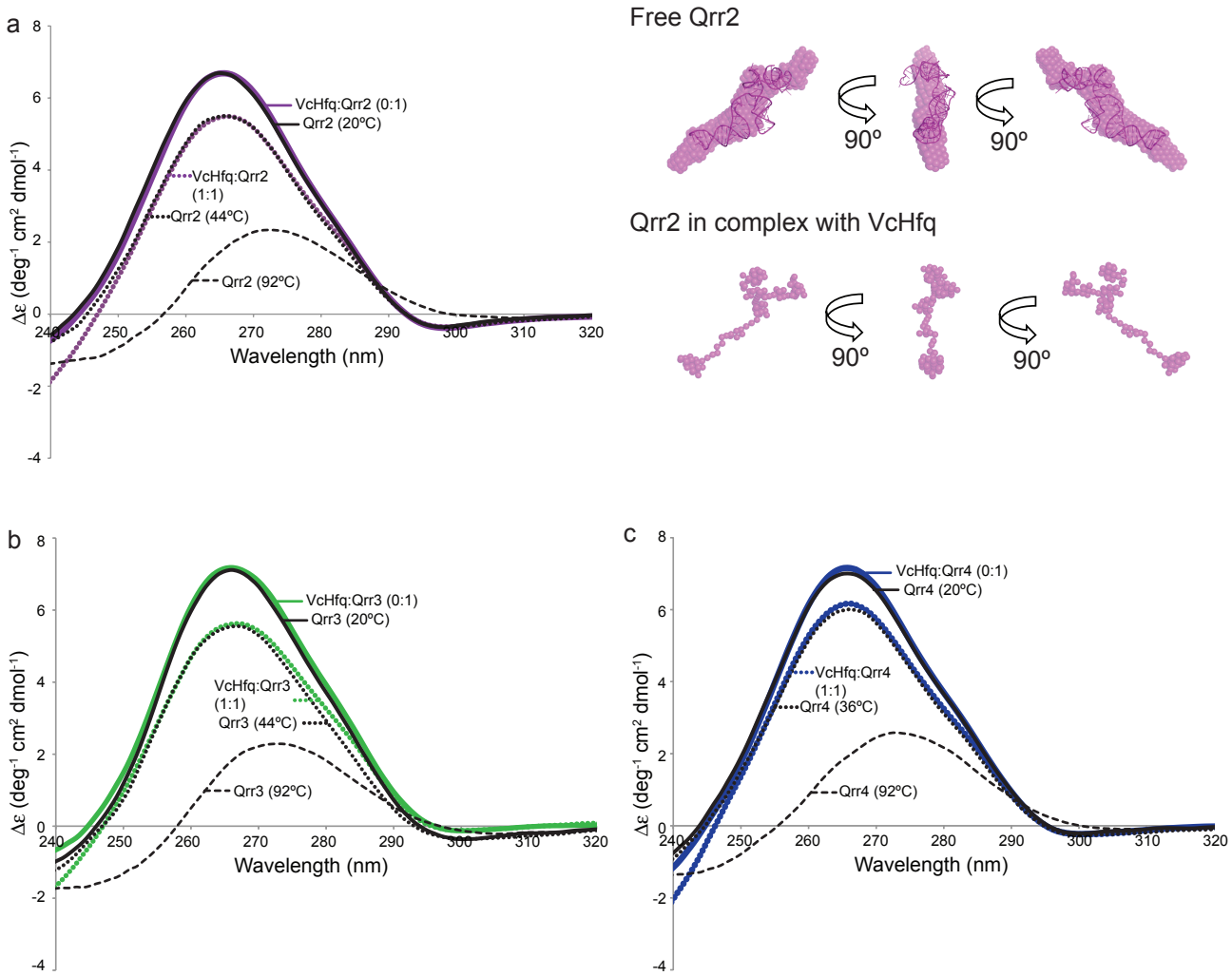

Figure S6. The conformation of the RNA changes upon complex formation

(a) On the left are the CD spectra for VcHfq-Qrr2 complexes (magenta lines) at 20ºC at VcHfq:Qrr2 ratios of 0:1 (solid magenta line) and 1:1 (dotted magenta line) and for free Qrr2 sRNA (black lines) at 20ºC (solid black line), 44ºC (dotted black line) and 92ºC (dashed black line). Qrr2 was present at 1.4 µM in each case. On the right, the upper panels show a ribbon representation of Qrr2, predicted by iFoldRNA (26,27), superimposed with SUPCOMB (28) on the *ab initio* model of the sRNA (spheres) generated from the SAXS data of free Qrr2 with DAMMIF (24) and averaged with DAMAVER and DAMFILT (25). The lower panels show the *ab initio* model of the sRNA, as it appears in complex with VcHfq, generated from the SAXS data of the complex with MONSA (29,30). All models were visualized with PyMOL.

(b) CD spectra for VcHfq-Qrr3 complexes (green lines) at 20ºC at VcHfq:Qrr3 ratios of 0:1 (solid green line) and 1:1 (dotted green line) and for free Qrr3 sRNA (black lines) at 20ºC (solid black line), 44ºC (dotted black line) and 92ºC (dashed black line). Qrr3 was present at 1.8 µM in each case.

(c) CD spectra for VcHfq-Qrr4 complexes (blue lines) at 20ºC at VcHfq:Qrr4 ratios of 0:1 (solid blue line) and 1:1 (dotted blue line) and for free Qrr4 sRNA (black lines) at 20ºC (solid black line), 36ºC (dotted black line) and 92ºC (dashed black line). Qrr3 was present at 1.6 µM in each case.