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A new HIV-1 Rev structure optimizes interaction with target RNA (RRE) for nuclear export

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Abstract

HIV-1 Rev mediates the nuclear export of unspliced and partially-spliced viral transcripts for the production of progeny genomes and structural proteins. In this process, four (or more) copies of Rev assemble onto a highly-structured 351-nt region in such viral transcripts, the Rev response element (RRE). How this occurs is not known. The Rev assembly domain has a helical-hairpin structure which associates through three (A-A, B-B and C-C) interfaces. The RRE has the topology of an upper-case letter A, with the two known Rev binding sites mapping onto the legs of the A. We have determined a crystal structure for the Rev assembly domain at 2.25 Å resolution, without resort to either mutations or chaperones. It shows that B-B dimers adopt an arrangement reversed relative to that previously reported, and join through a C-C interface to form tetramers. The new subunit arrangement shows how four Rev molecules can assemble on the two sites on the RRE to form the specificity checkpoint, and how further copies add through A-A interactions. Residues at the C-C interface, specifically the Pro31-Trp45 axis, are a potential target for intervention.

Keywords

HIV-1; nuclear export complex; Rev; Rev response element; RRE; specificity checkpoint

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Accession numbers

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Declaration of interests: none

1. Introduction

HIV/AIDS is projected by the World Health Organization to be among the leading contributors to global burden of disease by 2030 (Mathers and Loncar, 2006) (for 2015 updates see http://www.who.int/healthinfo/global_burden_disease/projections/en/). Despite the unquestionable success of combination antiretroviral therapies (cART) there remains a need for alternative means of intervention. In HIV-1, viral replication is regulated by two proteins; Tat (trans-activator protein) which stimulates transcription from the long terminal repeat, and Rev (regulator of expression of virion proteins) which controls the nuclear export of unspliced and partially-spliced transcripts required for the production of viral genomes and structural proteins. Rev binds to a 351-nt region within viral transcripts, known as the Rev response element (RRE), coupling them to Crm1, a nuclear export factor for certain host proteins and for RNAs (Karn and Stoltzfus, 2012). The importance of this interaction as a potential point of intervention has long been recognized (Baba, 2004; Giver et al., 1993; Xiao et al., 2001), but it has remained largely untargeted due in part to a lack of structural information about both Rev and the RRE.

Rev is a small (13 kDa) protein with two domains; an N-terminal domain (residues 1-65) with a simple anti-parallel helix-loop-helix hairpin-like structure, and a C-terminal domain (residues 66-116) that is generally thought to be unstructured (Daugherty et al., 2010a; DiMattia et al., 2010; Watts et al., 1998). The N-terminal domain engages in three types of homotypic interactions termed A-A, B-B and C-C. The first two involve the pairing of monomers through the A and B hydrophobic faces located on either side of the open ends of the hairpins (heterotypic A-B interactions have never been observed), whereas C-C interactions involve association between the closed (loop) ends of the hairpins. Observation of these three interfaces in numerous crystal structures (Daugherty et al., 2010a; DiMattia et al., 2010; Jayaraman et al., 2014), as well as in several cryo-EM helical reconstructions of tubular Rev polymers with different helical lattices (DiMattia et al., 2016), has revealed wide variation in the crossing-angles of the dimers. In the case of A-A dimers, the angle can vary, seemingly almost continuously, between 96° and 140° (DiMattia et al., 2016) whereas B-B dimers have been observed at angular settings of 50° and 120° (Daugherty et al., 2010a; Jayaraman et al., 2014). This variability is facilitated by the highly hydrophobic and rather featureless nature of these interfaces. Unlike the A-A and B-B interfaces, the C-C interface involves the proline-rich loop of one monomer hooking into a groove formed by the corresponding region of the opposing one (DiMattia et al., 2016). As pairing involves Pro-Trp stacking interactions and the formation of specific hydrogen bonds, the range of crossing angles at C-C interfaces is more restricted. A-A and B-B interactions are the dominant ways in which Rev monomers associate. By comparison, the C-C interface involves a smaller area (and a smaller calculated free energy of formation) and was initially considered a mere crystal contact. However, the repeated observation in several different crystal settings and helical lattices implied a functional role (DiMattia et al., 2016). It is these variable interactions of the N-terminal domain, and the apparently disordered nature of the C-terminal domain, combined with a strong tendency towards aggregation, that long hindered structure determination. Crystallization of full-length Rev was finally achieved by the use of Fab and scFv chaperones to block the B-faces, but the C-terminal domains

remained disordered in the crystal lattices (DiMattia et al., 2010; DiMattia et al., 2016). In helical reconstructions of full-length Rev, the C-terminal domains appeared only as poorly ordered bi-lobed densities (DiMattia et al., 2016). The N-terminal domain has also been crystallized, either alone or bound to a small RNA, but only when mutated at the A-face (Daugherty et al., 2010a; Jayaraman et al., 2014). In addition to the N- and C-terminal domains, Rev has several functional regions. Pertinent to this discussion is the Arg-rich motif (ARM) in helix 2 of the N-terminal domain and located near the closed end of the hairpin in the folded protein. It is the ARM by which Rev binds to the RRE (Battiste et al., 1996; Karn and Stoltzfus, 2012).

The RRE has been studied intently for 30 years, primarily by biochemical means, but it is still usually depicted either as a two-dimensionally folded RNA or simply as a cartoon. Nothing was known about the three-dimensional structure until recently when a SAXS-based model was advanced showing it as having a topology resembling an upper-case letter A (Fang et al., 2013). A single high-affinity binding site for Rev, located in Stem IIB of the RRE, was identified early on (Malim et al., 1990; Tiley et al., 1992), and this maps to one leg of the A-shaped RNA. The details of how Rev binds to the IIB site have previously been elucidated in a solution NMR study of an ARM peptide bound to a minimal IIB RNA (Battiste et al., 1996). More recently, a second site (IA) was identified in Stem I of the RRE, but this has been less well characterized and no structure has been reported (Daugherty et al., 2008; Jayaraman et al., 2014). This site maps onto the other leg of the A-shaped RNA. A third site has also been proposed at a distal location on Stem I on the basis of time-resolved SHAPE analysis (Bai et al., 2014).

Rev has been reported to bind to the RRE in *ca.* 4 – 13 copies (Rausch and Le Grice, 2015). However, how Rev assembles on the A-shaped RRE is not known. On the basis of the similar spacing between the two ARMs in A-A dimers (*ca.* 55 Å) and the distance between the IIB and IA sites, it was proposed that a single Rev dimer bridges the two sites and that subsequent dimers add in an analogous manner along the two legs of the A-shaped RRE (Fang et al., 2013). Following the discovery of the C-C interface, an updated model was proposed wherein one Rev dimer binds at each of the IIB and IA sites and these dimers are joined through a C-C interaction (DiMattia et al., 2016). Neither model showed how Rev engages the RRE, or how multiple copies of Rev might assemble given only two (or perhaps three) binding sites on the RRE. Attempts at three-dimensional reconstructions of the Rev-RRE-Crm1 export complex (Booth et al., 2014) have been hampered by the highly flexible nature of the assembly. In cases where traditional methods of structure determination experience difficulty, SAXS, though lower in spatial resolution, can still provide structurally useful information (Hura et al., 2009), with the HIV-1 RRE being a notable example.

Here we describe an X-ray crystal structure of the Rev assembly domain in an entirely new arrangement, obtained without either assembly-impeding mutations or chaperones. Docked with the SAXS-based model of the RRE, it shows how Rev can bind to and assemble on the RRE to form a stable four-Rev specificity checkpoint and also allow for the addition of further subunits through protein-protein interactions.

2. Materials and methods

2.1. Preparation of Rev¹⁻⁶⁹

Rev has two domains; an ordered N-terminal assembly domain (usually considered to be residues 1-65) and a disordered C-terminal effector domain (residues 66-116), however, in one instance (PDB: 3LPH) the N-terminal domain has been observed to be helically ordered up to Glu69 or possibly Pro70 (Daugherty et al., 2010a). To obtain crystals of the assembly domain we deleted the C-terminal domain. The resulting protein, Rev¹⁻⁶⁹, otherwise had the wild-type sequence and a molecular mass of 8.1 kD. We did not employ any mutations to impede oligomerization, nor did we add a surface entropy reducing mutation, as has sometimes been done (Jayaraman et al., 2014). Rev¹⁻⁶⁹ was expressed in *E. coli*. The cells were suspended in 50 mM Tris, pH 8.0, 0.1 M NaCl, 1 mM EDTA and 2 M urea supplemented with a protease inhibitor cocktail (Roche) then lysed by two passes through a French Press at 12,000 psi with one minute of sonication after each pass to reduce viscosity. The material was clarified by centrifugation in a JA-14 rotor at 13,000 rpm for two hours at 4 °C and then fractionated by DEAE anion exchange chromatography. Flow-through fractions were pooled, subjected to a 40% ammonium sulfate cut, and gel filtered on Superdex 75 (GE Healthcare) equilibrated with 4 M Guanidine HCl. The protein was refolded by sequential dialysis, first against 25 mM HEPES, pH 7.4, 0.15 M NaCl, 2 mM DTT and then 25 mM HEPES, pH 7.4, 0.15 M NaCl, 0.1 M Arginine, 10% glycerol and 2 mM DTT. The protein was concentrated using Millipore Ultra-15 centrifugal filters and any precipitate was removed by bench top centrifugation. The protein concentration was determined using UV spectroscopy: 1 mg/ml has an absorbance of 1.0.

2.2. Crystallization, Diffraction Data Collection and Structure Determination

Crystallization was performed by the hanging drop method. Protein, 1 µl (4-5 mg/ml), was mixed with 1 µl of 0.1 M sodium citrate (pH 5.6) containing 25% 2-methyl-2-propanol; 100 µl buffer was added to plate wells. Crystals were grown at 10 °C over ~ 2 weeks. Crystals were cryoprotected with 30% 2-methyl-2,4-pentanediol (MPD) and flash-frozen. Data were collected at the Advanced Photon Source (APS) beamline 22ID at 100 °K. Diffraction data were processed using XDS and scaled and merged using XSCALE (Kabsch, 2010). The HIV-1 Rev structure (PDB: 3LPH) (Daugherty et al., 2010a) was used for molecular replacement in Phenix (Adams et al., 2010). The crystal belonged to the P2₁2₁2₁ space group with a single copy of the Rev dimer per asymmetric unit. Iterative rounds of model building were done in Coot (Emsley and Cowtan, 2004) and refinement in Phenix. Interfaces were analyzed with PDBePISA (Krissinel and Henrick, 2007).

2.3 Model Building

The structure of the RRE, as determined by SAXS, has been described previously (Fang et al., 2013). All modeling of Rev on the RRE was performed with UCSF Chimera (Pettersen et al., 2004).

3. Results and discussion

3.1. Rev dimerizes in an arrangement opposite to that previously reported

We have determined a crystal structure for the Rev assembly domain (Rev¹⁻⁶⁹) at 2.25 Å resolution (Table 1), revealing a dimer (Fig. 1). Both monomers have a well folded core (residues 8-65) and are essentially superimposable. The two monomers associate *via* their B-faces in a V-shaped arrangement, where the two long helices are related by an outer angle of 299° (see Fig. S1 for an explanation of the Rev A and B interfaces). One monomer is, thus, rotated almost 180° relative to the corresponding one in PDB: 3LPH. Wide variation in the crossing angles of Rev dimers has been observed previously. A-A-dimers associate at angles ranging between 96° and 140° (DiMattia et al., 2016), and B-B-dimers at angular settings of 50° and 120° (Daugherty et al., 2010a; Jayaraman et al., 2014), but in all instances so far, the angle between the long helices of the monomers has been less than 180°. The interface in the current structure consists of the same set of hydrophobic residues that form the B-B interface (see below) but with one monomer in a “reversed” orientation.

The B-B interface in the current structure has an area and calculated free energy of formation (702 Å² and −11.4 kcal/mol, respectively) similar to that of the B-B interface in PDB: 3LPH (796 Å² and −11.1 kcal/mol), and larger than that of either the B-B interface in PDB: 4PMI (554 Å² and −9.1 kcal/mol) or the A-A interface in PDB: 2X7L (620 Å² and −11.4 kcal/mol). Like the PDB: 3LPH B-B interface, the current B-B interface involves six hydrophobic residues (Leu18, Phe21, Leu22, Ile52, Ile55, and Ile59), on each chain (Fig. 1 and Fig. S1). The current B-B interface has two hydrogen bonds, Asp11-Arg58 and Thr62-Arg58, versus three in PDB: 3LPH, and an Asp11-Arg58 salt bridge.

3.2. The Rev C-C interface is structurally important

Examination of the crystal lattice shows that two B-B dimers are joined at a third interface. This interaction involves residues Pro28, Pro29, Pro31, Trp45, and Arg46 where the proline-rich loop of one monomer hooks into a groove formed by the same region of the opposing subunit (Fig. 1). The interface, termed C-C, is stabilized by a Pro31-Trp45 stacking interaction and a Pro31-Arg46 hydrogen bond, and has been observed in several crystals and helical reconstructions of Rev (DiMattia et al., 2016). The crossing angle between monomers at the C-C interface is 61°, similar to 64° in PDB: 5DHV. We have previously proposed that this third interface, while not large in area (411 – 441 Å²), is required for higher order assembly of Rev on the RRE (DiMattia et al., 2016). Here, two B-B dimers, joined at a C-C interface, form a tetramer (Fig. 1). Wild-type full-length Rev forms tetramers *in vitro* (DiMattia et al., 2016; Zapp et al., 1991) and *in vivo* (Zapp et al., 1991).

3.3. Rev dimers bind to the IIB and IA sites on the RRE and interact through a C-C interface

As determined by SAXS, the RRE resembles an upper-case letter A (Fang et al., 2013), with one leg longer than the other (Fig. 2). Viewed from the side, the RRE is curved; in Fig. 2 the convex surface is oriented upward. The IIB high-affinity site is located on the inside of the short leg adjacent to the crossbar and the IA site is in a similar position on the long leg. Viewed down the long axes of their respective legs of the A, the IIB and IA sites are arranged almost at right angles with respect to one another. The IIB site corresponds to a

concave feature analogous to the major groove in A-form RNA. This groove is wider towards the convex surface of the RRE and access to the narrow end is obstructed by the IIB loop and stem loop IIC, which curls underneath the IIB region (Fig. 3), indicating that Rev would bind into the groove from the convex side. The IA site is located on the long leg at the point where the helix undergoes a bend and the major groove adopts a wide-open conformation, also facing the convex side (Fig. 3). Together, these observations indicate that Rev binds to the IIB and IA sites on the convex side of the RRE.

It has long been envisaged that Rev binds first at a high-affinity site and then assembles “along” the RNA, although no such contiguous binding sites have been demonstrated. More recently, it has been proposed that Rev dimers bridge between the IIB and IA sites. In one scenario (DiMattia et al., 2010; Fang et al., 2013), one dimer spans the two sites, with the arginine-rich motifs (ARM) of the monomers each making contact with one site. In the other scenario (DiMattia et al., 2016), two dimers bind, each to one site, and they are joined at a C-C interface. Observation of the above tetrameric complex in the Rev¹⁻⁶⁹ crystal lattice suggested to us an arrangement whereby the first two Rev dimers may assemble onto the RRE. Interactive docking of the complex onto the RRE revealed a good fit; the helices contacting the RNA have, to a close approximation, the correct center-to-center spacing, angles, and orientations for their ARMs to simultaneously contact the IIB and IA sites correctly.

3.4. Rev binding at the IIB site

The IIB site, though narrower than a typical major groove, is lined with most of the nucleotides previously determined to be involved in the binding of Rev (Battiste et al., 1996). As the RRE model is not an atomic structure, it is not possible to measure exact distances, therefore, we considered points apparently separated by only a few Angstroms as being “proximal”. As docked, the following residue-nucleotide pairs are proximal: Arg35-U66, Arg35-G67, Arg38-G67, Arg39-G70, Asn40-A73 and Asn40-G47 (Fig. 2). All of these are key interactions, according to NMR (Battiste et al., 1996). Arg44 has been shown by NMR to contact U45 in a minimal RNA (Battiste et al., 1996), but this is not possible in our model. It may be, for example, that the minimal 34-nt RNA used to represent the IIB stem loop (Battiste et al., 1996) does not have the same conformation as in the full-length RNA, or is more accessible (Fig. S2). The same arguments apply to the Junction site (Jayaraman et al., 2014), where a 40-nt RNA hairpin was taken to represent the junction of the IIA, IIB and IIC stem loops (Fig. S2).

3.5. Rev binding at the IA site

The IA site has been less extensively characterized than the IIB site and no structure has been reported. Rev residues Arg38, Arg41, Trp45 and Arg46 were proposed to be involved in binding, on the basis of mutational analysis, and in the case of Trp45, HSQC NMR data (Daugherty et al., 2008). In our model, Rev binds to the IA site similarly as to other RNA molecules, i.e. with the ARM of helix 2 facing into the major groove (Battiste et al., 1996). Also, the residue-nucleotide pairs Arg38-G199, Trp45-C200 and Arg46-G24 are proximal. Nucleotides G199 and C200 are adjacent to the internal loop constituting the IA site and G24 is within the loop. Arg46 projects deeply into the major groove directly towards G24,

and Trp45 is closely apposed to the side of the major groove (Fig. 2), potentially explaining the distinct chemical shift observed for this residue upon binding to the IA site (Daugherty et al., 2008). Asn40, which when mutated reduces binding to the IIB site but not the IA site (Daugherty et al., 2008) is here positioned directly away from the binding site. However, Arg41, which was reported to affect binding at this site (Daugherty et al., 2008), is not near the RNA. That Rev binds to the IA site with the side of helix 2 opposite to that with which it binds to the IIB site was proposed (Daugherty et al., 2008) prior to the determination of the Rev structure (Daugherty et al., 2010a; DiMattia et al., 2010). Given the now-known structure, it is unlikely that Rev binds to RNA with the opposite face of helix 2 as had been proposed.

3.6. Flexibility in Rev-RRE complex formation

Although strict rigid-body docking of the four-Rev¹⁻⁶⁹ crystal structure onto the RRE resulted in a suboptimal fit; that is, fully satisfying the requirements of either the IIB or IA sites but not quite both simultaneously, several points of flexibility in both Rev and the RRE would improve this substantially. The RRE itself is one great source of flexibility (Fang et al., 2013). In-depth SHAPE analyses have also shown that the initial binding of Rev induces tertiary structural changes in the RRE, enabling further Rev binding and additional long-range remodeling (Bai et al., 2014). In addition, it has been shown that the DEAD-Box helicase DDX1 functions as an RNA chaperone, inducing a persisting conformational change in the RRE in the region of the three-way junction (i.e. high-affinity site) that promotes the subsequent binding of the first Rev molecule (Hammond et al., 2017; Lamichhane et al., 2017). DDX21 appears to have similar RRE-chaperone activity (Hammond et al., 2018). The facility with which the Rev tetramer can be fitted to the RRE model does not mean that it does so either directly or in isolation. The arrangement proposed here would represent the state of the complex after these early events have already occurred.

Another source of flexibility lies within the Rev tetramer, particularly the angular flexibility at the two hydrophobic B-B interfaces, and to a lesser degree that at the C-C interface (Fig. 4). Additional sources of adaptive flexibility would include the two directions of lateral bending of helix 2 of the assembly domain, as observed in several crystal structures: PDB: 2X7L, 3LPH, 4PMI, 5DHV, 5DHX, 5DHY, 5DHz and 6BSY (reported here), as well as the coil-to-helix transition of the ARM, as observed by CD and NMR (Casu et al., 2013). Finally, merely selecting alternative side chain rotamers can substantially improve the fit of the four-Rev complex on the RRE, particularly at the IIB site, although we have not done so here.

3.7. Rev forms a four-molecule intermediate on the RRE and then assembles by protein-protein interactions

The number of Rev molecules binding to the RRE remains a matter of debate, ranging from 4 to 13 (Rausch and Le Grice, 2015), with 4 to 8 being common (Bai et al., 2014; Daugherty et al., 2010b; Pond et al., 2009; Zapp et al., 1991), and 4 was proposed as the specificity checkpoint (Bai et al., 2014). The internally stabilized arrangement shown in Fig. 2 may represent this checkpoint. The 3rd and 4th dimers could add directly to those already bound at the IIB and IA sites (Fig. S3). Notably, whereas no sites beyond IIB and IA have been

conclusively identified, indeed, as judged by quantitative functional reporter assays much of the RRE can be deleted as long as the two sites are properly opposed to one another (O'Carroll et al., 2017), it has repeatedly been found that Rev assembly on the RRE is dependent on protein-protein interactions (Daugherty et al., 2008; Pond et al., 2009). Our model indicates that: (a) Rev initially contacts the RRE at only two points, and then assembles through Rev-Rev interactions rather than “along” the RNA, (b) all the C-terminal domains are arrayed in a well-separated manner about the RRE to optimally engage Crm1 (Rausch and Le Grice, 2015), and (c) the ARMs of the two monomers involved in the C-C interaction are oriented outwards ready to engage the distal Region 3 of the RNA proposed to fold back at the checkpoint (Bai et al., 2014). Finally, we propose the C-C interface, and specifically the Pro31-Trp45 axis, as defined by our previous mutational analysis (DiMattia et al., 2016), as a target for intervention.

4. Conclusion

In this study, we have determined an X-ray crystal structure for the HIV-1 Rev assembly domain. This crystallization was achieved without employing either polymerization-impeding and energy-minimizing mutations, or crystallization chaperones. The structure revealed that the Rev dimer adopts a reflex crossing angle of 299° rather than one that is obtuse (120°) or even acute (50°), as previously reported. An important consequence of this new arrangement is that the arginine-rich RNA-binding regions on Rev are disposed on the convex rather than the concave surface of the dimer. Further, two such dimers join to form a tetrameric assembly resembling a lock-washer. Unlike previously reported structures for the Rev assembly domain, this tetrameric complex is readily docked with a SAXS-based model of the RRE in a manner consistent with numerous earlier observations. The new arrangement shows how multiple Rev molecules may assemble on the RRE and the point where this might best be disrupted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. Structure of the Rev assembly domain. (A) Domain arrangement of Rev. (B – E) Ribbon diagrams of Rev assembly domain dimers from PDB: 2X7L (B), 4PMI (C), 3LPH (D), and the current structure (E). The dimers associate via either an A-A (A) or B-B (C – E) hydrophobic interaction. Note that in (E) one monomer is rotated almost 180 degrees relative to (D). (F) Tetrameric arrangement of the current structure observed in the crystal lattice, showing two B-B dimers joined by a C-C interaction. Enlarged views into the B-B and C-C interfaces are indicated. Only residues on upper chains are labeled. Not shown, for clarity, are the: Fab chaperones in (B), RNA aptamer in (C), and A-A dimer in (D).

Fig. 2.
Model of the RRE-Rev₄ assembly complex. (A) Surface representation of the RRE (light blue, semitransparent) with the Rev assembly domain tetramer (red, green, yellow, blue) docked, shown in four orthogonal views. The IA and IIB Rev binding sites are indicated. (B) Enlarged view of Rev bound at the IA site. (C) Enlarged view of Rev bound at the IIB site.

Fig. 3.

The IA and IIB sites are both accessible from the convex (upper) side of the RRE. (A) Top view of RRE showing 1A and IIB sites, colored magenta and orange, respectively, as identified previously (Battiste et al., 1996; Daugherty et al., 2008). Stem loop IIC, yellow. Arrows show direction of view in the panels below. (B) The IA internal loop opens the major groove, which faces the convex side of the RRE. (C) The IIB site major groove is wider towards the convex side of the RRE, and the narrow end is partially obstructed by the IIB loop and the IIC stem loop.

Fig. 4.

Points of flexibility that may facilitate formation of RRE-Rev₄ assemblies. (A) Suboptimal fit between the monomers in the four-Rev¹⁻⁶⁹ crystal structure and the RRE at the IA and IIB sites. (B) Points of angular flexibility between monomers at B-B and C-C interfaces (solid arrows), and flexibility in helix 2 (open arrows). (C) Flexibility in helix 2 can occur in two directions; the chains were aligned on residues 46-62. (D) The IIB site (orange) with U66 (yellow) and G67 (cyan), which are contacted by Arg35 (Battiste et al., 1996), highlighted. The straight helix 2 would alleviate clash between Rev and RNA. The coil-to-helix transition of the ARM (Casu et al., 2013) is not illustrated. The conformational flexibility of the RRE has been demonstrated previously (see movie S5 in (Fang et al., 2013)).

Table 1

Diffraction data collection and refinement statistics

Data Collection	
Beamline	APS 22ID
Wavelength	1.00
Space group	P2 ₁ 2 ₁ 2 ₁
Cell dimensions	
a, b, c (Å)	43.80, 43.92, 70.80
α, β, γ (°)	90, 90, 90
Resolution (Å)	19.63-2.25 (2.31-2.25) *
Multiplicity	7.1 (7.25)
Completeness (%)	99.7 (100.0)
I/σ	13.73 (2.61)
R-meas	12.2 (100)
Refinement	
Resolution (Å)	19.63-2.25
No. reflections	12494
R _{work} /R _{free}	0.23/0.26
No. atoms	
Protein	980
Ion	5
Water	41
B-factors	
Protein	39.7
Ion	78.6
Water	44.04
RMS deviations	
Bond lengths (Å)	0.01
Bond angles (°)	1.36
Ramachandran (favored/allowed/outliers)	% 99.1/0.9/0.0

* Statistics for the highest-resolution shell are shown in parenthesis.