Figure 1

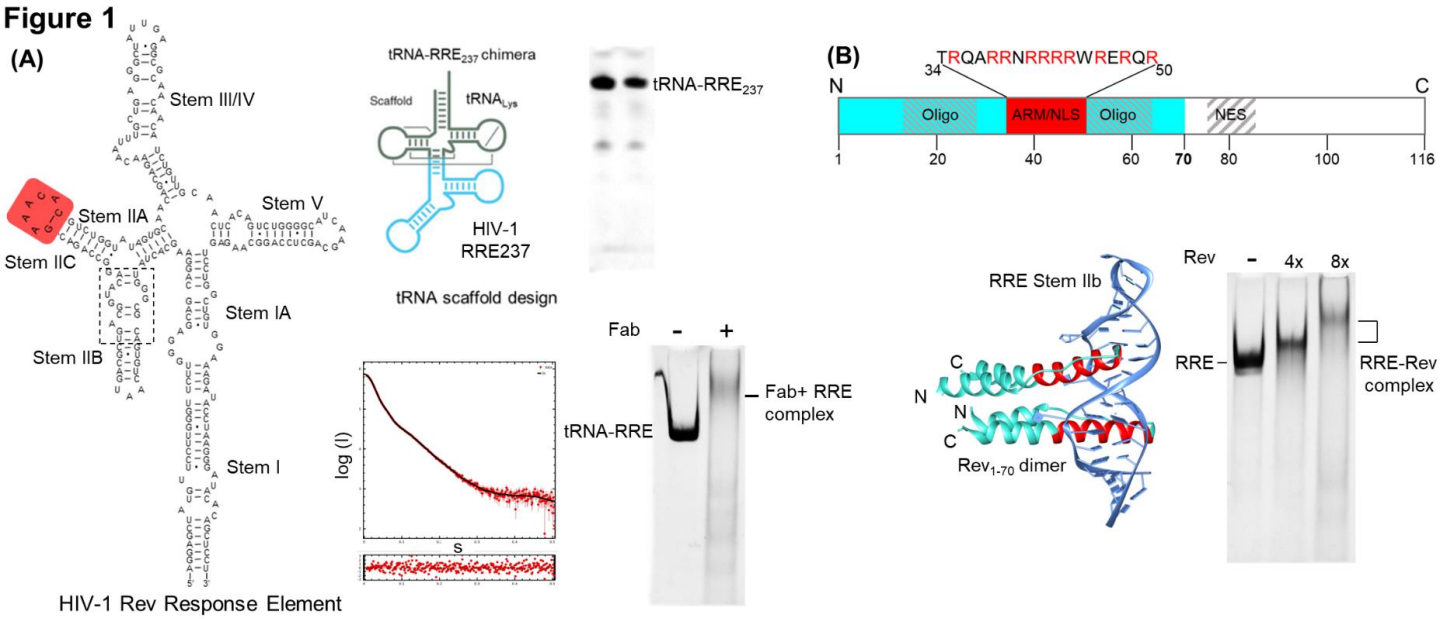

Figure. 1 HIV-1 Rev Response Element. (A) Secondary structure of HIV-1 RRE (**left**). The five stems are labeled. The high-affinity Rev binding site on Stem IIb is enclosed in dotted lines. Fab BL3-6 binding site mutation in Stem IIC is shown in red The construct used for cryo-EM contains 237 nt of the 350 nt sequence of RRE inserted into the anticodon loop of $tRNA_{Lys}$ to generate $tRNA\text{-}RRE_{237}$ that is purified recombinantly (**top right**). SAXS analysis (**bottom middle**) showed a monodisperse scattering profile and a good Guinier fit. EMSA of tRNA-RRE and Fab-BL3,6 shows discrete complex formation between tRNA-RRE and Fab fragment (**right**). **(B)** Domain organization of full-length HIV-1 Rev protein (1-116). Functional domains of Rev viz., oligomerization domains (Oligo), Arginine-rich motif (ARM), nuclear localization signal (NLS), nuclear export signal (NES) are labeled with amino acid sequence of ARM shown above. **(C)** Crystal structure of Rev1-70-RNA complex (PDB: 4PIM). N-terminal deletion mutant Rev1-70 (cyan) dimer bound to stem IIb of RRE (blue in structure, dotted inset in A). ARM is shown in red. EMSA of tRNA-RRE and Rev70 shows binding of Rev to RRE to form discrete complexes in solution.

Figure 2

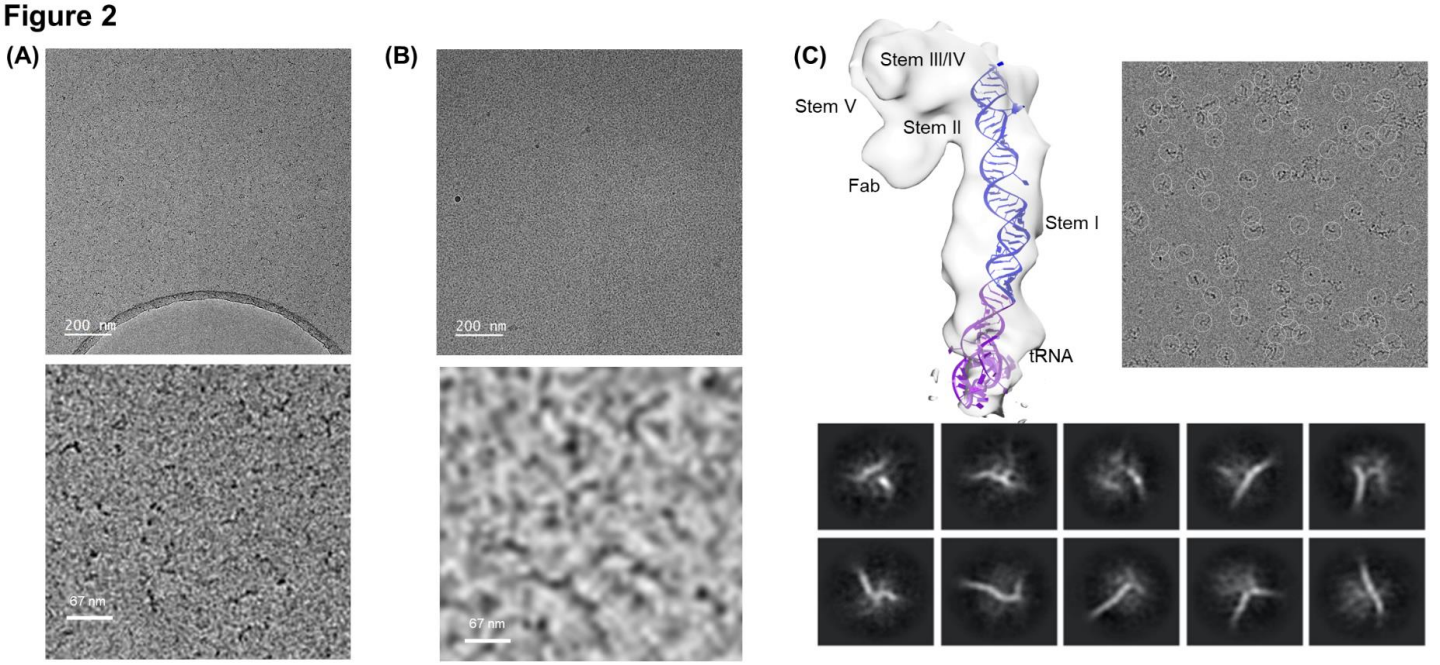

Figure. 2 Cryo-EM of tRNA-RRE. (A) Micrograph of tRNA-RRE alone. RNA was prepared in 50mM Hepes pH 7.4, 150 mM Sodium Acetate and 10 mM $MgCl_2$. Specimen was vitrified using Vitrobot on R2/1 holey-carbon grids, at 22 °C and 100% humidity. Prepared grids were screened on JEM 2100. RNA is localized to the carbon support film on the grid. Zoomed in view of the carbon with RNA sample is shown below. **(B)** Micrograph of tRNA-RRE Fab complex. Specimen was prepared by coating the grid with Fab BL3-6 followed by addition of Fab binding mutant of tRNA-RRE. Sample buffer conditions and vitrification conditions are the same as in A. Zoomed in view of the RNA sample in ice (dark lines) is shown below. **(C)** Preliminary cryo-EM reconstruction of tRNA-RRE Fab complex. Cryo-EM data was collected on Titan-Krios™ at UTMB. 136651 particles were obtained from 8000 images. Preliminary assignment of the RRE stem-loops is indicated on the reconstruction. Representative image with particles circled (right) and representative 2D-classes (below) are shown