



Figure S4, related to Figure 4. Binding of PACT to the 70S ribosome. (A) Chemical structure of pactamycin. (B, C) Unbiassed ($F_{\text{obs}} - F_{\text{calc}}$) difference Fourier maps of PACT in complex with the *T. thermophilus* 70S ribosome contoured at 2.2σ . PACT is colored in light blue for the soaking experiment and in dark blue for the co-crystallization experiment. Nitrogens are colored blue, oxygens are colored red. (D) Comparison of the superimposed structures of PACT. The previously published structure of PACT in complex with only the 30S ribosomal subunit is shown in green (PDB entry 1HNX (Brodersen et al., 2000)). The structure of PACT from the current work in complex with the 70S ribosome from the soaking experiment is in light blue and from the co-crystallization experiment is in dark-blue. All structures were aligned based on h24 of the 16S rRNA (nucleotides 769-810). (E, F) Close-up views of the PACT binding site on the *T. thermophilus* 70S ribosome. The coloring of PACT is the same as in (D). The 16S rRNA is shown in light yellow, mRNA is in magenta, and the anticodon stem loop (ASL) of the E-site tRNA is in orange. The typical location of the mRNA in the absence of cognate tRNA in the E site is shown in pink as reference. The black contour of the E-site tRNA in (F) indicates that it is absent from the structure. Steric clashes between the antibiotic and parts of the ribosome are indicated by red arrows. Note, that binding of PACT to the ribosome causes displacement of the mRNA from its normal

location (dashed arrows). Also note, that co-crystallization and soaking experiments yielded non-identical orientations of PCT in its binding site on the small ribosomal subunit. See also Movie S2.