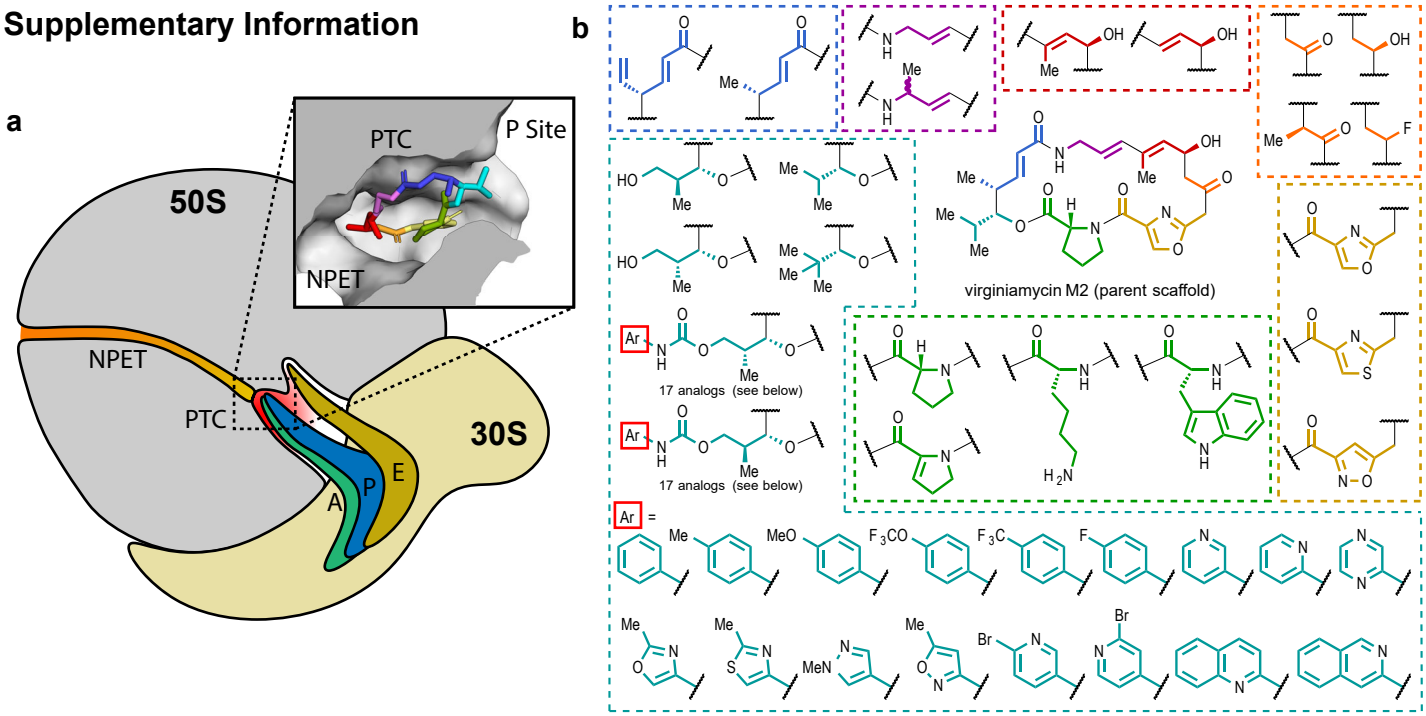
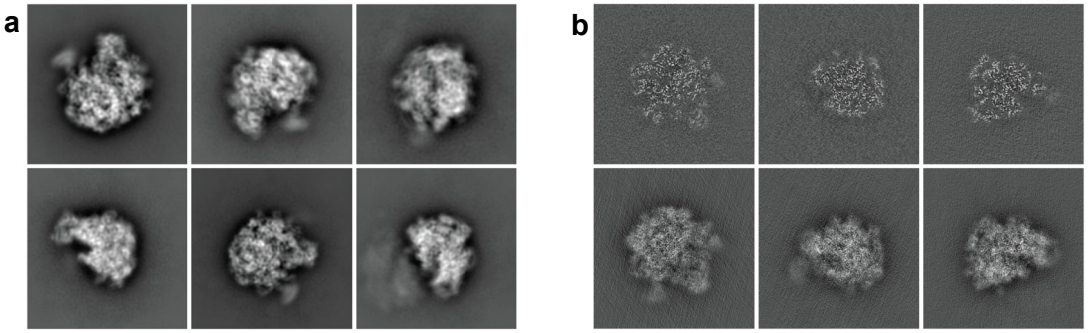
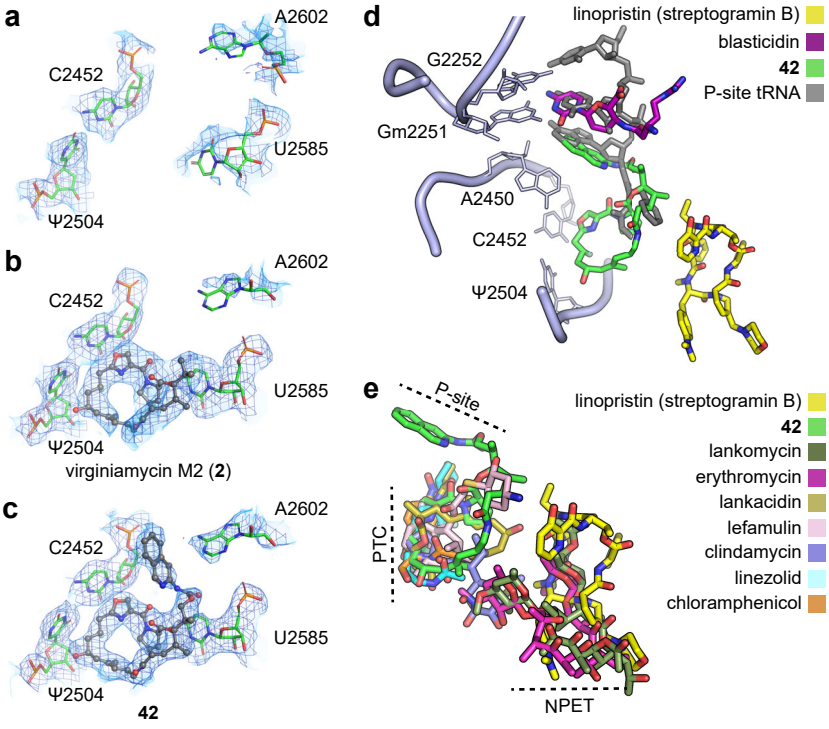
Supplementary Information



Supplementary Figure 1 | a. Cartoon of the ribosome, highlighting Streptogramin A binding in the peptidyl transferase center. **b.** Novel streptogramin analogs already produced by the Seiple lab's fully synthetic, modular route. Color coding identifies the structural diversity sampled at each position in the parent scaffold.



Supplementary Figure 2 | Preliminary CryoEM data. **a.** Representative 2D class averages. **b.** Three orthogonal slices (top) and three orthogonal projections (bottom) of the resultant 2.5 Å structure.



Supplementary Figure 3 | CryoEM characterization of virginiamycin M2 and analog **42** bound to the 50S subunit of the *E. coli* ribosome. **a.** Experimentally derived coulomb potential density map ofthe apo peptidyl transferase center (PTC) at 2.8-Å resolution. **b.** Density map of virginiamycin M2 bound to the PTC at 2.6-Å resolution, revealing stabilizing interactions with U2585. **c.** Density map of streptogramin analog **42** bound to the PTC at 2.4-Å resolution. Increased density of the mobile base A2602 indicates a stabilized conformation compared to streptogramin-bound and apo structures. **d.** Overlay of **42** (green), group B streptogramin linopristin (yellow, PDB 4U27), blasticidin (purple, PDB 1KC8), and P-site tRNA (gray, PDB 1VY5). The aryl carbamate sidechain from **42** partially overlaps with the terminal base in the P-site tRNA. **e.** Binding poses of 9 antibiotics that bind in the PTC and the nascent peptide exit tunnel (NPET), indicating the relative sidechain position.