



A. GliP Domain Architecture and Reaction The NRPS GliP (7 domain protein) catalyzes the reaction between activated aminoacids phenylalanine and serine to form a diketopiperazine scaffold for subsequent production of Gliotoxin

B. Purification of GliP Final stock of GliP (236kDa) on 10% SDS-PAGE, **(right)** Homology Model for GliP overlayed with envelope from SAXS. Red represents region of domain flexibility for execution of catalysis. The envelope fits extremely well to the model, which also corroborates with MD simulation results.

C. SEC: 16/60 superdex-200 chromatography profile. **(right)** – Comparison of coenzyme-A, aminoacyl-coenzyme-A analog, and PPT-arm conjugated to a generic T-domain

D. 2D Classification with cryoSPARC EM imaging with 120keV Glacios cryo-TEM at Columbia University (CEC).