Supporting Information

Directed evolution of the nonribosomal peptide synthetase BpsA to enable recognition by the human phosphopantetheinyl transferase for counter-screening antibiotic candidates

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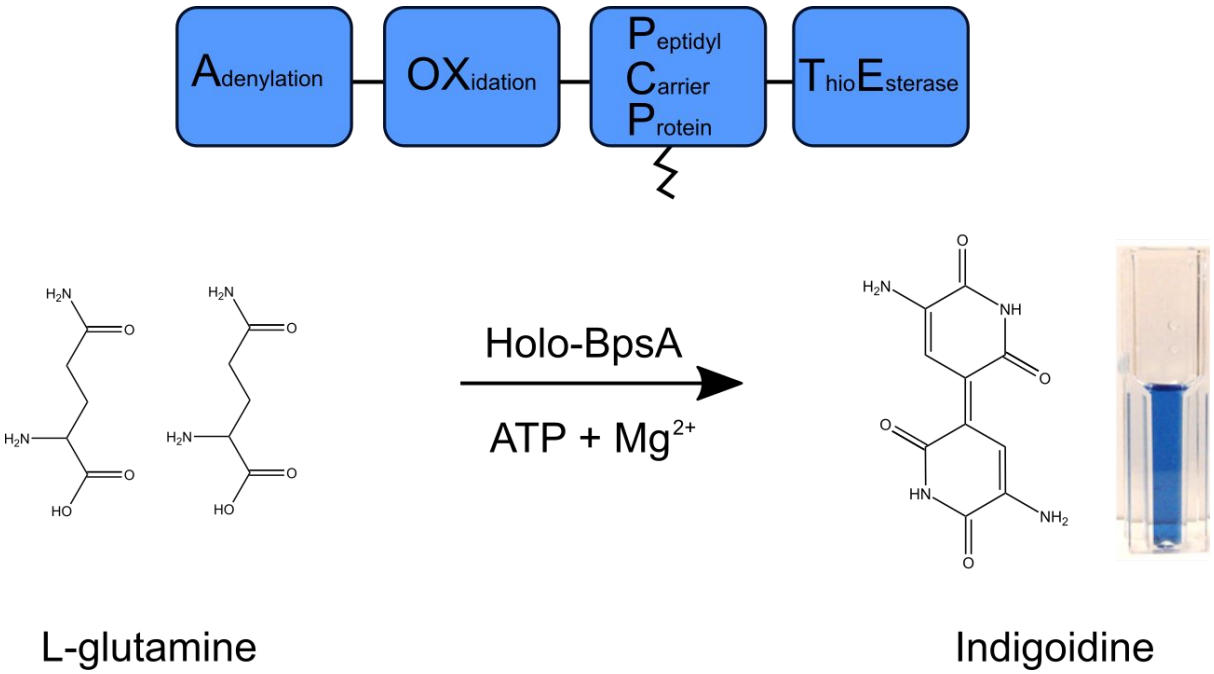


Figure S1: BpsA is a single module NRPS (domain structure above) that cyclizes a single molecule of L-glutamine in an ATP powered reaction. Two cyclized glutamines then dimerize to form the vibrant blue pigment indigoidine (fully solubilised in DMSO in the image).

A) B)

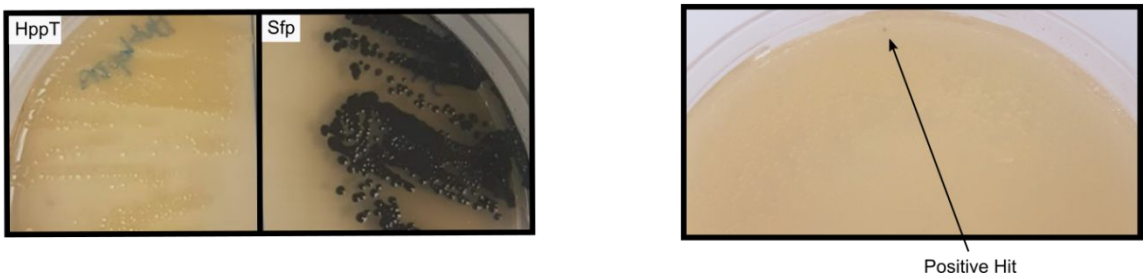


Figure S2: A) Indigoidine production by *E. coli Δ entD* BL21 co-expressing *bpsA* and either hPPTase ("HppT") or *sfp* ("Sfp"), plated on pigment production agar. The photo was taken 24 hours post induction with IPTG. B) A representative positive 'hit' from a screening plate approximately eight hours post-induction.

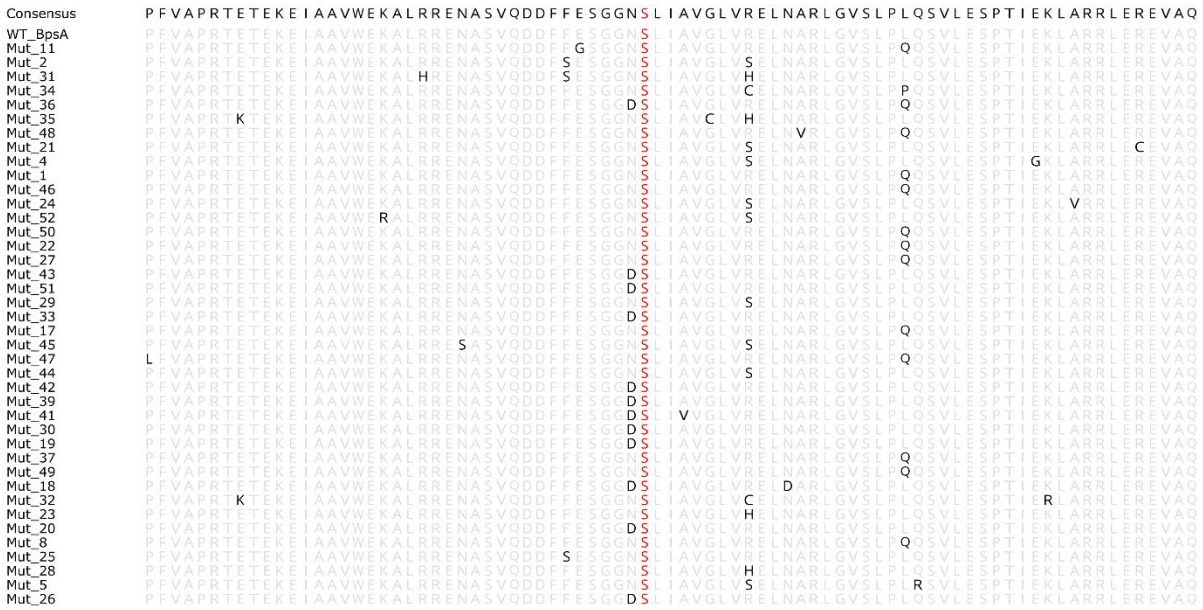
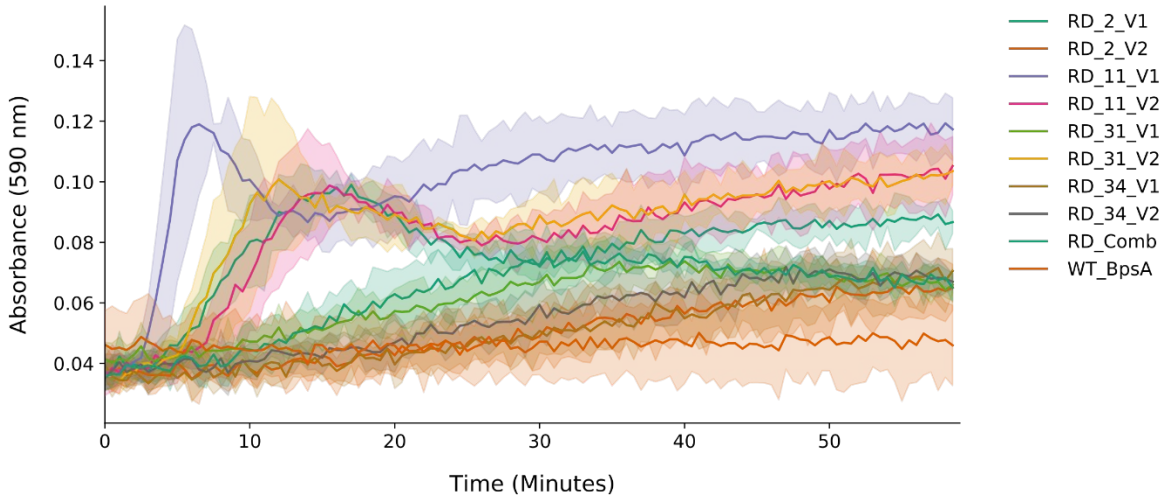


Figure S3: The amino acid sequences of the top 30 PCP domain variants that were assayed for activity in the liquid screen. The variants are ranked by activity in the assay, with the conserved serine residue marked in red, and changes from the wild-type sequence highlighted in black.

A)



B)

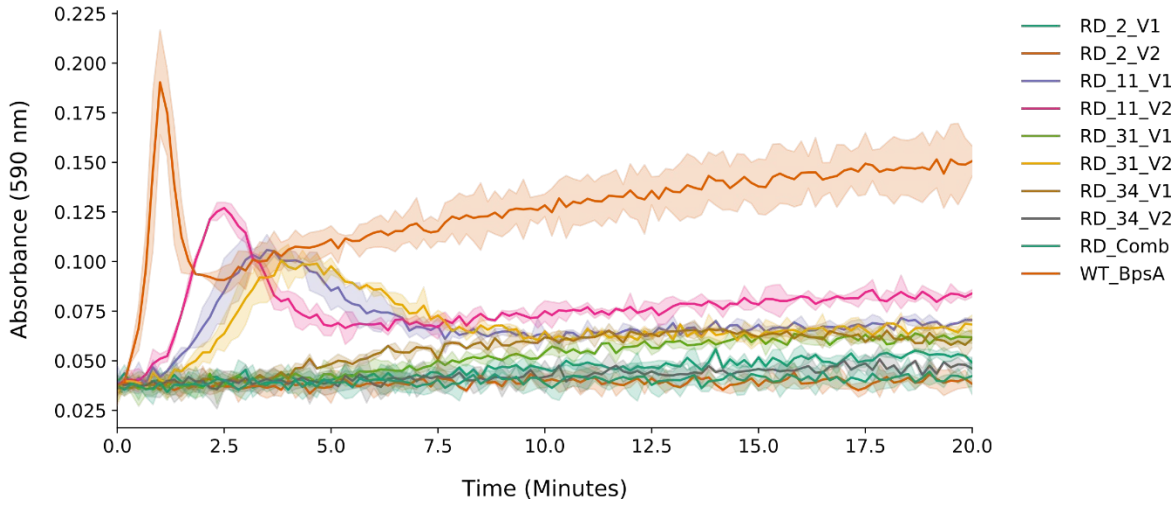


Figure S4: A) Raw A_{590} values, monitoring indigoidine synthesis by the nine second generation variants and wild type BpsA (WT_BpsA) during real-time activation by hPPTase. Data is the average of three replicates and the lighter shaded boundary represents one standard deviation. B) Raw A_{590} values for the nine second generation variants and WT_BpsA following their pre-activation by Sfp. Data is the average of three replicates and the lighter shaded boundary represents one standard deviation.

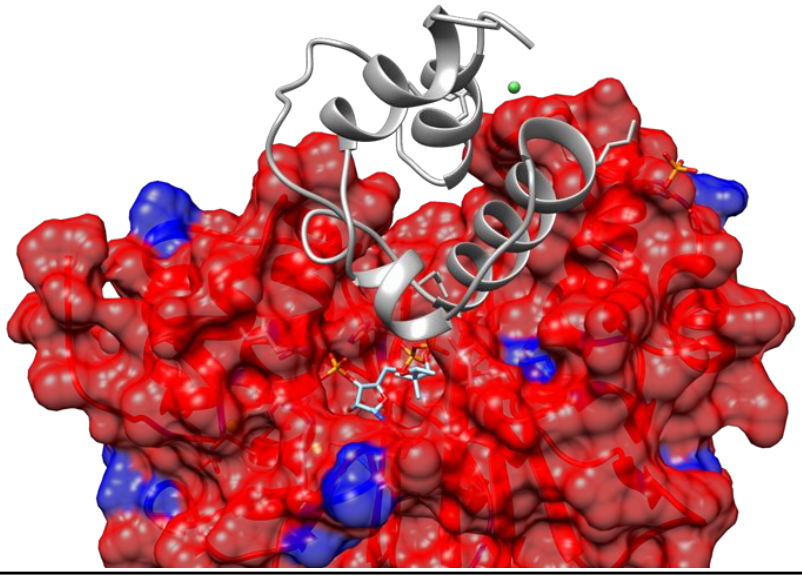
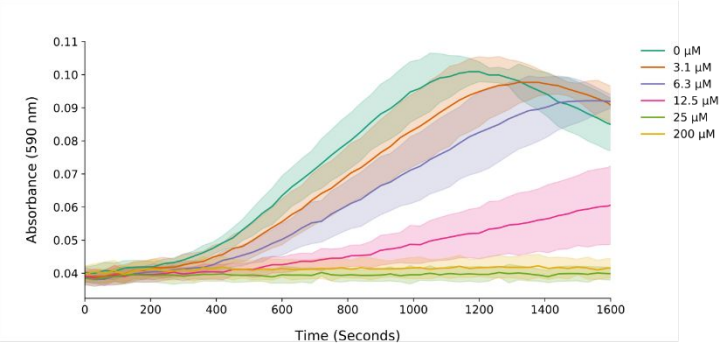


Figure S5: A model of rPPTase was built using the SWISS-MODEL homology server with the hPPTase (PDB:1CG5) as a template. The rPPTase model and 1CG5 were aligned in UCSF Chimera using the matchmaker function and differences were coloured in blue. The ACP domain is displayed as a ribbon in grey.

A)



B)

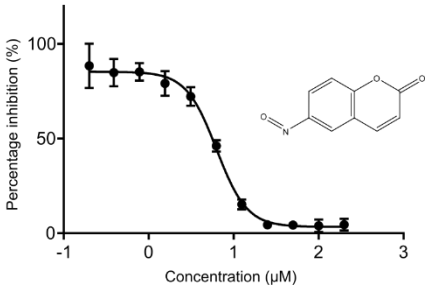


Figure S6: A) The rate of activation of RD_11_V1 by rPPTase is diminished in the presence of increasing concentrations of 6-NOBP, a broad spectrum PPTase inhibitor. Data was recorded every 30 s and is the average of three replicates. The lighter shaded boundaries represent one standard deviation. For clarity, not all concentrations of 6-NOBP that were used to derive the hPPTase EC_{50} are represented here**. B)** Graph used to derive EC_{50} values for rPPTase inhibiton by 6-NOBP. Data is the average of three replicates and the error bars represent one standard deviation. The structure of 6-NOBP is inset in the graph

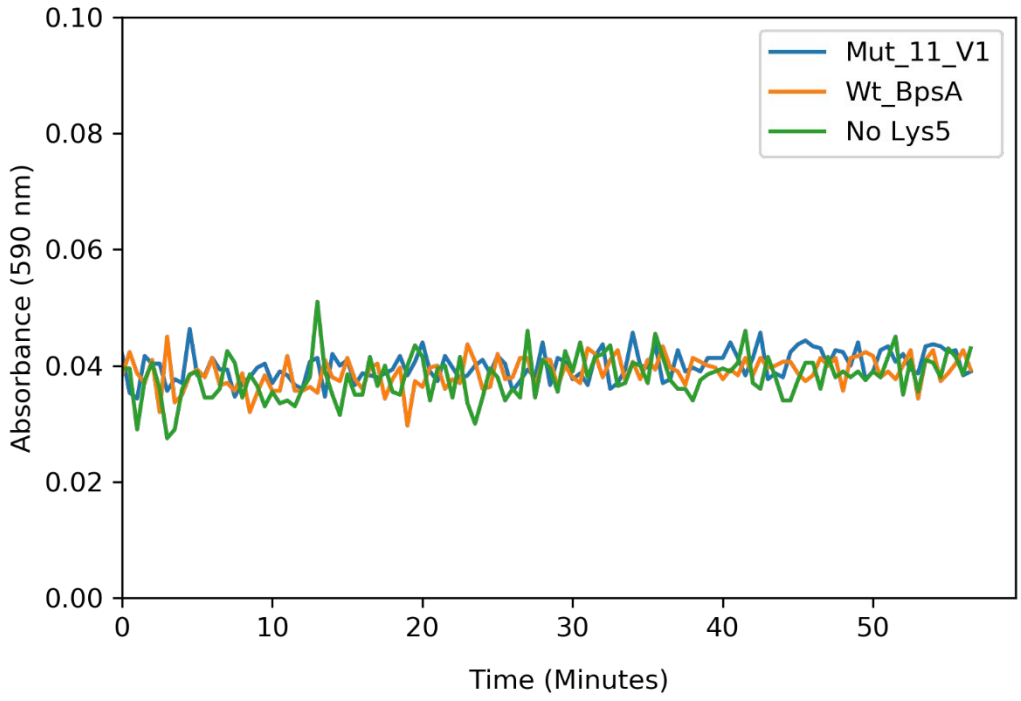


Figure S7: Raw A_{590} values for *apo*-RD_11_V1 and *apo*-Wt_BpsA incubated with Lys_5 and everything required for the synthesis of indigoidine. No_Lys5 is a negative control, identical to the other reactions but lacking Lys_5. Data are the averages of three technical replicates.