DIRECTED EVOLUTION OF THE NON-RIBOSOMAL PEPTIDE SYNTHETASE BpsA TO ENABLE RECOGNITION BY THE HUMAN Sfp-LIKE PPTase

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Non-ribosomal peptide synthetases (NRPSs) are large, modular enzymes that have an assembly line architecture and synthesise a diverse range of compounds such as antibiotics, siderophores and immunosuppressants. Within the assembly line, the peptidyl carrier protein (PCP) domain has a crucial role in shuttling substrates between the different catalytic domains. The PCP domain is a small four-helix bundle that requires a phosphopantetheinyl moiety to be attached to a conserved serine on the second alpha helix for functionality. This post-translational modification is catalysed by a family of enzymes called the phosphopantetheinyl transferases (PPTases).

Due to their central role in activating enzymes involved in both primary (e.g., fatty acid synthetases) and secondary metabolism (e.g., NRPSs), PPTases have been identified as a promising antibiotic target in bacterial species such as *Mycobacterium tuberculosis*. We have previously developed a high-throughput enzymatic screen for PPTase inhibitors based on co-incubation of a target PPTase with a blue-pigment synthesising NRPS, BpsA. As part of the development of a complete screening platform, we also wanted to be able to rapidly counter-screen inhibitors for cross-inhibition of the endogenous human PPTase, as this is a potential source of toxicity. We found we were unable to use the native BpsA enzyme for this, as the human PPTase is incapable of recognising the PCP domain of BpsA.

To improve with the ability of BpsA to be activated by the human PPTase, a directed evolution campaign was undertaken. Firstly, error-prone PCR was used to introduce mutations into the PCP domain of BpsA. Approximately 200,000 variants were screened using a high-throughput plate-based assay. Forty ‘hits’ were then characterized in a semi-quantitative liquid assay. Based on the pattern of amino acid substitutions in the most active variants, specific combinations of substitutions were rationally introduced into BpsA. The top variant identified was now capable of being rapidly phosphopantetheinylated by the human PPTase and we have shown this can be used to quickly screen bacterial PPTase inhibitors for cross-reactivity with the human PPTase. This work illustrates the flexible nature of the PCP domain and provides further evidence that only a few point mutations may be sufficient to dramatically change the specificity of PCP domains for different PPTases.