ENGINEERING THE INDIGOIDINE-SYNTHESISING ENZYME BPSA FOR DIVERSE APPLICATIONS IN BIOTECHNOLOGY

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Blue pigment synthase A (BpsA) is a single module non-ribosomal peptide synthetase (NRPS) originally isolated from the bacterium *Streptomyces lavendulae*. It synthesises an easily detectible blue pigment called indigoidine from two molecules of L-glutamine in an ATP powered reaction. BpsA is readily purified and amenable to *in vitro* assays that have a variety of useful applications. By spectrophotometrically quantifying indigoidine levels it is possible to accurately measure the amount of L-glutamine in complex biological fluids including urine, blood plasma and cell culture media. This method has several advantages over existing methods for glutamine measurement, including that it directly reports on glutamine levels. Existing commercially available enzymatic kits first convert glutamine into glutamate and then measure the level of glutamate, which requires additional sample processing and introduces complexity if glutamate may also be present in the target sample. Additionally, we have shown that BpsA can also be used to measure ATP concentrations in a similar manner. We have further developed a BpsA based assay to detect inhibitors of 4'-phosphopantetheinyl transferases (PPTases). PPTases are enzymes that attach a phosphopantetheine arm to fatty acid synthases, NRPSs and polyketide synthases, thereby switching them from an inactive *apo* form to an active *holo* form. PPTases have been validated as promising drug targets in several pathogenic bacteria including *P. aeruginosa* and *M. tuberculosis*. In order to detect PPTase inhibition, we have shown that BpsA can be purified in its inactive *apo* form and mixed with the target PPTase as well as a candidate inhibitor *in vitro*. The level of PPTase inhibition can then be calculated by measuring the rate of indigoidine production. The assay has been optimised for high throughput screening and used to identify several compounds from chemical libraries that inhibit essential PPTases of *P. aeruginosa* and *M. tuberculosis.*