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Engineering metal-dependent biotin synthase for the biotechnological production of biotin

"This project laid the foundation for an efficient high-throughput workflow towards understanding and engineering one of nature's most challenging enzymes biotin synthase. A better and faster approach with much broader scope than ever before will allow us improving the synthesis of the important vitamin B7 more efficiently." Christof Jäger, University of Nottingham

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The robotics suite at the University of Nottingham

In order to set the stage for an effective high-throughput enzyme screening approach for the enzyme biotin synthase we have developed and tested two biotin assays. One was based on a previously described *Corynebacterium glutamicum* indicator strain and the second made use of a biotin analogue bound to avidin (HABA/avidin). Both assays have the potential to be adapted for automated screening of large libraries in multi-well plate format. We showed that the *C. glutamicum*-based bioassay measured cell growth using biotin prepared from stock or produced in *E. coli*. Assays in which multi-well plates were manually loaded or prepared using the liquid handling unit of the robotic suite both showed that the biotin concentration was directly proportional to the ability to support *C. glutamicum* growth. In the second affinity-based assay, the amount of free HABA reflects the amount of biotin that is present once it is displaced from avidin. The assay was optimised for use in96-well plate format. The computational approaches focused on targeting potential mutation sites of biotin synthase that influence the redox reactivity of one or both iron–sulfur clusters and thus potentially influence sulfur insertion and cluster repair kinetics. For that reason we developed scripts to analyse the electrostatic effect of the protein environment (due to directional polarity) on the cluster. This type of analysis makes it possible to spot individual amino acids that influence the reactivity significantly, without being directly in contact with the active site. These amino acids will be taken forward to mutation studies.

INITIAL AIMS: Biotin (vitamin B7) is used primarily for the enhancement of animal feed and also in vitamin food supplements. Its production is challenging and hence the product is expensive (around $1600/kg). This project aims to demonstrate a potential way towards a sustainable and cost-effective production of biotin. The enzyme biotin synthase, which contains a delicate iron-sulfur cluster, is a key bottleneck in the biosynthetic pathway of biotin production. We will investigate the first steps into the rational computational design of this enzyme, together with the development of automated high-throughput, multidimensional *in vivo* assays. Our approach will act as starting point for not only for rational informed directed evolution strategies, but also will integrate regulatory elements for the repair mechanisms of the host cells.

- Project continued during doctoral training programme rotations
- BBSRC responsive mode grant application in preparation