Durable enzymes for a green economy

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→ **Reference**
M. Pelay-Gimeno, T. Bange, S Hennig and T.N. Grossmann, *Angew. Chem. Int. Ed. 57*, 11164–11170 (2018). doi: 10.1002/anie.201804506

→ It has become evident that we can no longer ignore the environmental changes mankind has been causing. We are facing pollution, climate change and reduced biodiversity, as well as resource shortages. The general awareness of the importance of moving our society towards an environment-friendly and sustainable lifestyle is increasing. The development of a 'green' economy requires a more efficient use of renewable raw materials along with the reduction and recycling of waste products, thereby leading to the transition from a linear to a circular economy.

The chemical and pharmaceutical industries are major players in our economies and are involved in the production of almost anything you can think of. Frequently, processes for the production of chemical intermediates and final products require harsh conditions and the use of hazardous reagents. Chemists are constantly improving yields, creating less hazardous alternative synthesis routes and developing more efficient catalytic systems. With recent advances in biotechnology, biocatalysts have become viable alternatives for classic chemical production. These enzymes are masters of chemical reactions: they are highly specific and efficient and often enable to shorten multi-step chemical synthesis routes. Evolved by nature, enzymes are renewable and non-hazardous, and already play an essential role in the food, textile and agriculture industries. Due to their specificity, enzymes are also applied in biomedical diagnostics.

The use of enzymes for the production of fine chemicals, materials and drugs is not fully harnessed due to their limited tolerance to non-physiological conditions such as elevated temperatures or unnatural microenvironments (e.g., presence of detergents, extreme pH). With the recent advances in protein engineering, highlighted by the 2018 Nobel prize in Chemistry for the 'Directed evolution of enzymes', enzymes can be evolved to be suitable for production purposes. Nevertheless, available stabilisation approaches often have limited effect and are difficult to implement (e.g., tedious interactive optimisation processes, incorporation of non-natural amino acids). In addition, current enzyme optimisation processes consider only a small subset of (bio)chemical structures for stabilisation which limits the stabilisation effect and results in small benefits. Overall, there is the need for straight-forward and highly efficient enzyme stabilisation technologies, which can make an impact in the field of green chemistry and contribute to drive the transition towards a circular economy.

We recently developed an entirely new stabilisation strategy for enzymes called INCYPRO (In situ Cyclisation of Proteins). INCYPRO enables a straight-forward, computationally guided and non-iterative design process that involves the introduction of three surface-exposed cysteines into the enzyme, which are subsequently reacted with a tris-electrophilic cross-linker. The resulting multi-cyclic protein exhibits a more robust core structure. Thus, we achieved a remarkable stabilisation effect towards elevated temperatures (e.g., increase in the protein melting temperature of 25 °C) through a single modification step instead of multiple optimisation cycles. Notably, INCYPRO can utilise enzymes entirely composed of natural amino acids that can be obtained rapidly, cost-efficiently and in large quantities. As an important feature, INCYPRO is orthogonal to existing stabilisation approaches and can therefore be applied to wild-type as well as pre-engineered and optimised enzymes. Given the straight-forward implementation and the durability of the resulting enzymes, INCYPRO supports current efforts towards green chemistry and has also the potential to enable completely novel biotechnological applications.

Currently, we are pursuing the implementation of INCYPRO for a variety of enzymes used in biotechnological applications. This will be a joint effort with the HIMS-Biocat group of Francesco Mutti at the University of Amsterdam. The valorisation of INCYPRO is supported by an ERC Proof-of-Concept grant and will be pursued by the Incircular Biotechnologies (www.incircular.com), a university spin-off. Ω

> **"Enzymes are masters of chemical reactions"**

↓ **Figure**
Conceptual scheme of increasing protein and enzyme stability using the INCYPRO technology.