Design, Optimisation and Characterisation of a *de Novo* gold hydroaminase

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The ability to design and produce enzymes from scratch would endow Chemists with a vastly superior biocatalytic toolbox than is currently accessible. Such an ability would allow for the creation of an ‘ideal' enzyme for a given transformation, which could be expressed in high yield and with high (thermal) stability. The field of enzyme design has, however, lagged behind that of protein design in recent years, owing principally to the more significant computational challenges involved, as well as the many unknowns we still face with respect to how an enzyme's structure relates to its function [1].

Combining designed protein scaffolds with transition metal catalysts to create *de novo* artificial metalloenzymes adds another rlayer of complexity but with it, a host of advantages. Herein, we showcase a thermostable *de novo* tandem repeat hydroaminase [2], designed to bind to an NHC-Au cofactor. We demonstrate strong binding affinity between the cofactor and the protein using crystallography and other biophysical methods. Furthermore, several rounds of directed evolution improved the enzyme's activity for the desired hydroamination reaction, resulting in significantly higher turnover numbers (TONs) and observed rates compared to the free cofactor.

[1] Lovelock, S. L.; Crawshaw, R.; Basler, S.; Levy, C.; Baker, D.; Hilvert, D.; Green, A. P., Nature 2022, 606, 49-58

[2] Doyle, L.; Hallinan, J.; Bolduc, J.; Parmeggiani, F.; Baker, D.; Stoddard, B. L.; Bradley., Nature 2015, 528, 585-588