Biocatalytic chiral amine synthesis coupled with in-situ chemo-catalytic substrate production

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One alluring approach to improve the sustainability of drugs production is the synthesis of chiral amines using enzymes [1]. Particularly, the use of enantioselective transaminases (ATAs) has the potential to significantly reduce the environmental burden of synthesis and purification, compared to traditional chemical routes [2]. A frequently utilized ATA is the R-selective transaminase commercialized by Codexis know as ATA-117. However, a significant issue with this transaminase is substrate inhibition at low concentrations, which limits its use large scale [3]. One strategy to overcome this inhibition is the in-situ synthesis of the ketone substrate. This approach also allows the use of a cheaper substrate (e.g. an alcohol).

Here, we present a hybrid cascade in which the substrate of the enzyme (acetophenone) is synthesized by the oxidation of 1-phenylethanol using a heterogeneous catalyst made of gold nanoparticle dispersed onto a porous silica support (Fig. 1). This catalyst is synthesized through a straightforward one-pot aerosol route, resulting in mesoporous Au/SiO_2 catalysts with small gold nanoparticles (3 nm) [4].

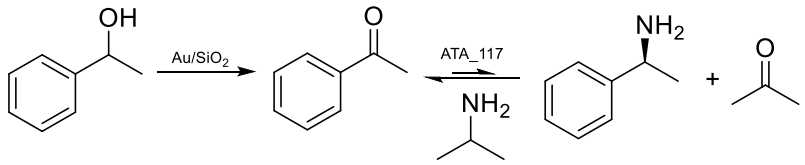

Figure 1: Cascade reaction of oxidation of 1-phenylethanol by Au/SiO_2 follow by transamination acetophenone by ATA_117

Regarding the first (chemo-catalytic) step of the reaction (Fig. 2A), the acetophenone yield after 24 hours is 80%. However, the gold catalyst is strongly deactivated by the enzyme in its free form, which leads to an acetophenone yield as low as 6%. Thus, we immobilized the enzyme onto Sepabeads EC-HFA resins (ATA_117-HFA), achieving a high immobilization efficiency (>90%). Enzyme immobilization minimizes the deactivation of the Au/SiO_2 catalyst achieving to 50% acetophenone yield. Concerning the second (biocatalytic) step of the cascade (Fig. 2B), the immobilization of the enzyme enhances the 1-phenylethylamine yield from 12% to 16% by enzyme stabilization. Furthermore, enzyme immobilization enables operation to 1M of isopropylamine (IPA), a more elevated temperatures (50°C), and in the presence of 10% methanol (Fig. 2C).

In the complete cascade reaction, the large IPA concentration and the external addition of cofactor (PLP) also resulted in a dramatic deactivation of the gold catalyst. Therefore, we back tracked to a “one-pot, two-step” hybrid catalysis system in which Au/SiO_2 first selectively oxidizes 1-phenylethanol during 24h (80% acetophenone yield), followed by addition of ATA, PLP and IPA to start the transamination. This strategy led to a final 15% yield for the chiral amine. Increasing the loading of immobilized enzyme on the resin from 1 mg/mL to 5 mg/mL led us to achieve a 1-phenylethylamine yield of 56%.

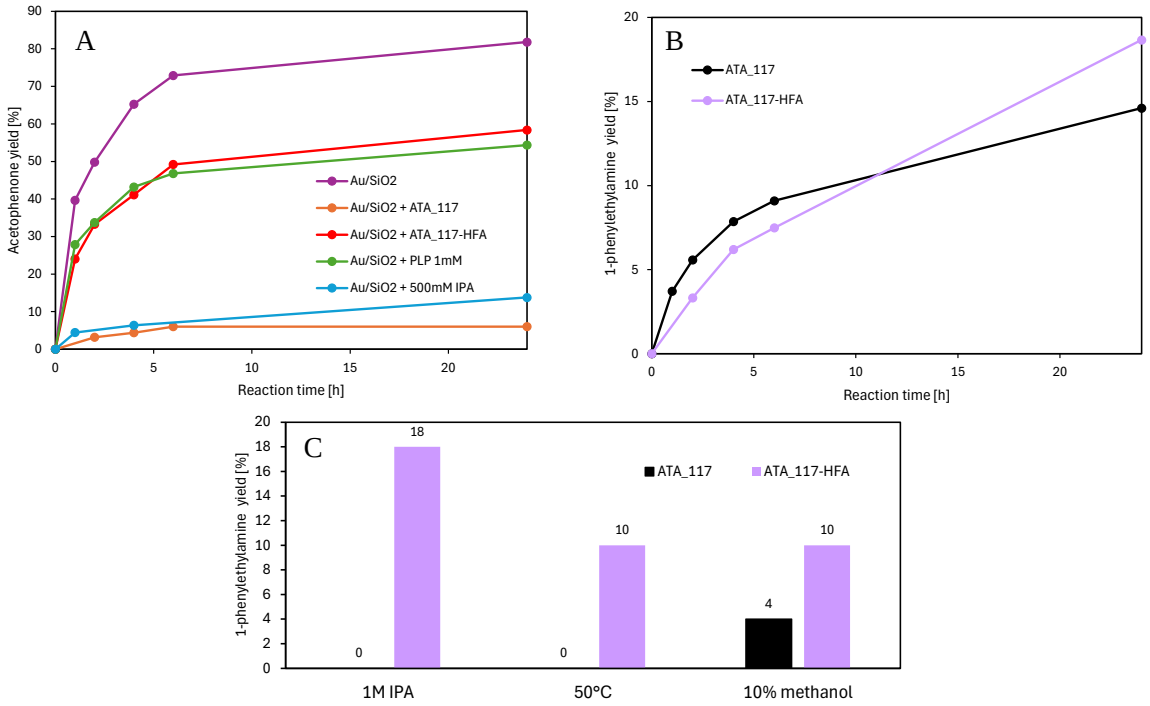

*Figure 2 : **A)** Acetophenone yield of from oxidation of 1-phenylethanol by Au/SiO_2 , Au/SiO_2 with free ATA_117, Au/SiO_2 with ATA_117-HFA, Au/SiO_2 with 1 mM of PLP and Au/SiO_2 with 500 mM of IPA (20 mM 1-phenylethanol, 100 mM Kpi, 20 mg/ml AU/SiO_2, 35°C at atmospheric pressure) **B)** 1-phenylethylamine yield from the transamination of acetophenone by ATA_117 and ATA_117-HFA (20 mM acetophenone, 100 mM Kpi, 500mM isopropylamine, 1 mM of PLP, 35°C, 1 mg/mL enzyme concentration). **C)** Yield of 1-phenylethylamine from the transamination of acetophenone by ATA_11 and ATA_117-HFA with 1M of isopropylamine or at 50°C or with 10% of methanol (standard condition: 20 mM acetophenone, 100 mM Kpi, 500mM isopropylamine, 1 mM of PLP, 35°C, 1mg/mL enzyme concentration).*

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