Electronic Supplementary Material (ESI) for RSC Advances.

Biocatalytic transamination in a monolithic flow reactor: improving enzyme grafting for enhanced performance

Electronic Supplementary Information

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Figure S1 – (top) Picture depicting the casting of the monolith into the heat-shrinkable Teflon tube and connexion wit the PTFE tubing. (bottom) Picture of the experimental set-up for flow reaction. Monolithic reactors are connected to syringe-pumps and outflow collectors via PTFE tubes. Monoliths are and immerged in a temperature-controlled water bath.

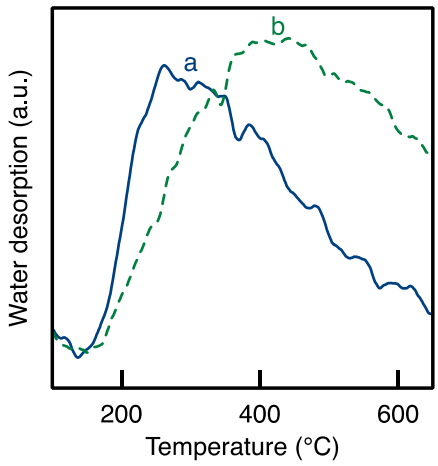

Figure S2 – Thermo programmed water desorption of (a) Si(HIPE) and (b) rehydroxylated Si(HIPE).

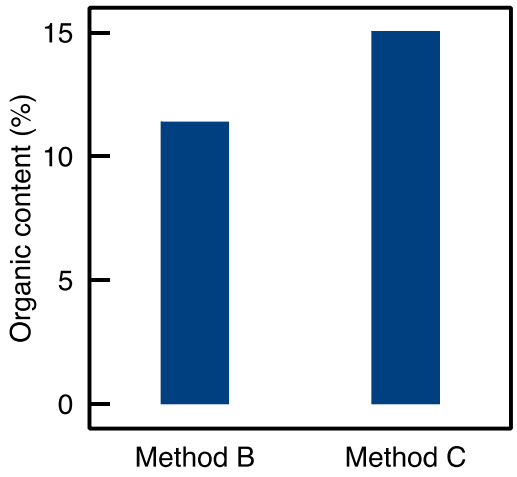

Figure S3 – TGA analyses of $A500_B$ and $A500_C$ samples. More organic functions were detected on the rehydroxylated samples. This suggested that the rehydroxylation process allowed featuring more silanols at the surface.

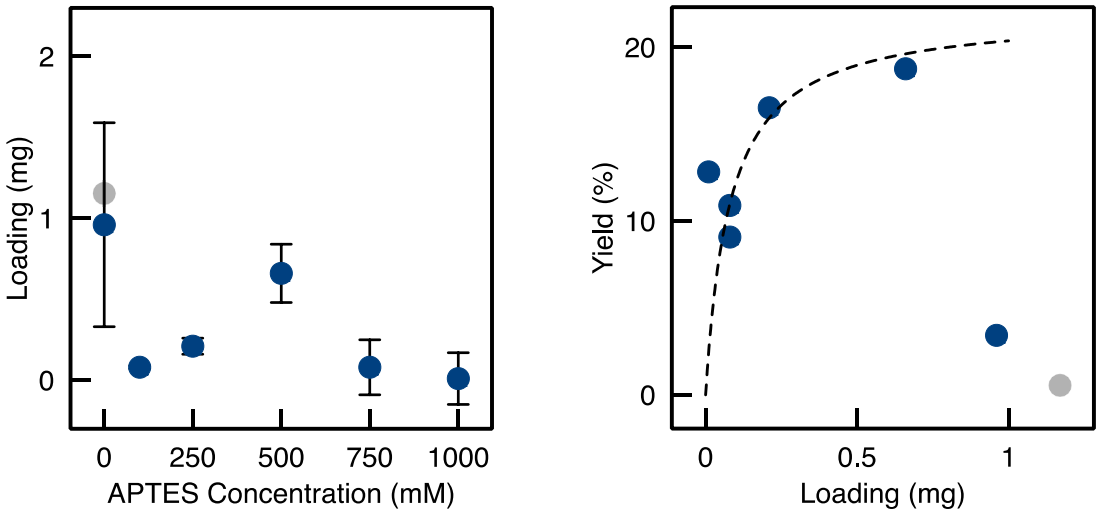

Figure S4 – (Left) Enzyme loading as a function of the APTES concentration used for functionalization in Method D. (Right) BAP yield as a function of the enzyme loading for the samples prepared by Method D. Dotted curve is only a guide to the eye. Shaded dots (●) represents the yield and loading for TA-Si(HIPE) sample

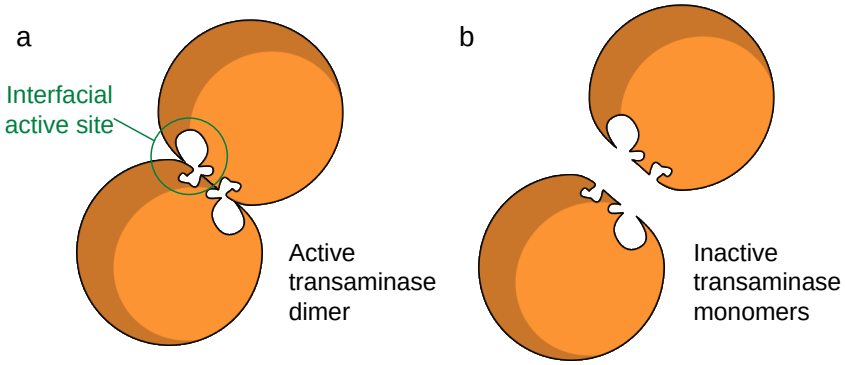

Figure S5 – Transaminases are dimeric enzymes. (a) Active sites are located on the interfacial area. (b) When the environment gets colder, monomers are suspected to undergo a reversible dissociation, leading to a "cold dissociation".[1]

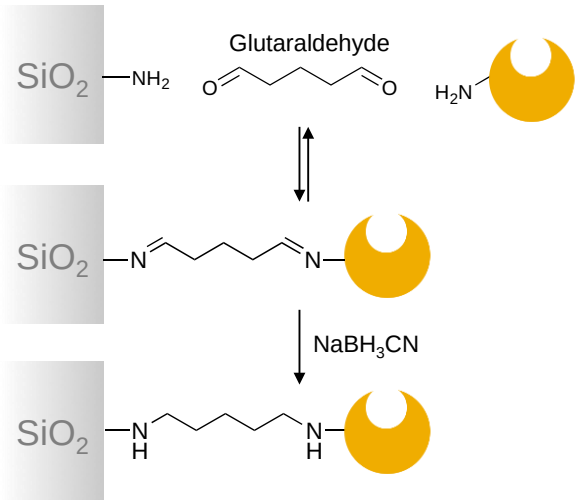

Figure S6 – Transaminases are immobilized through a reversible covalent bound (amines activated with glutaraldehyde, forming imines). Reducing imines into secondary amines lead to irreversible bonds.

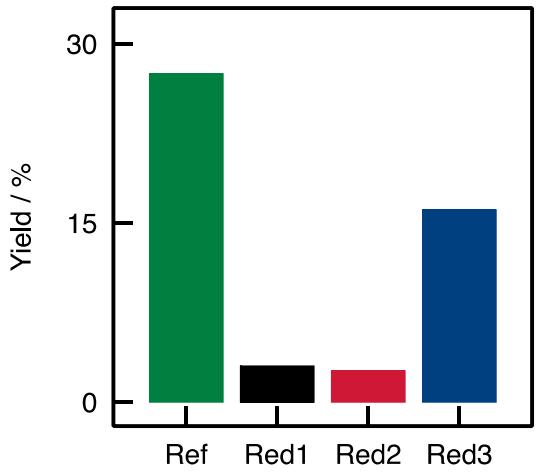

Figure S7 – Effect of imines reduction on A250D-30d samples yields. "Ref" stands for reference sample (no reduction).

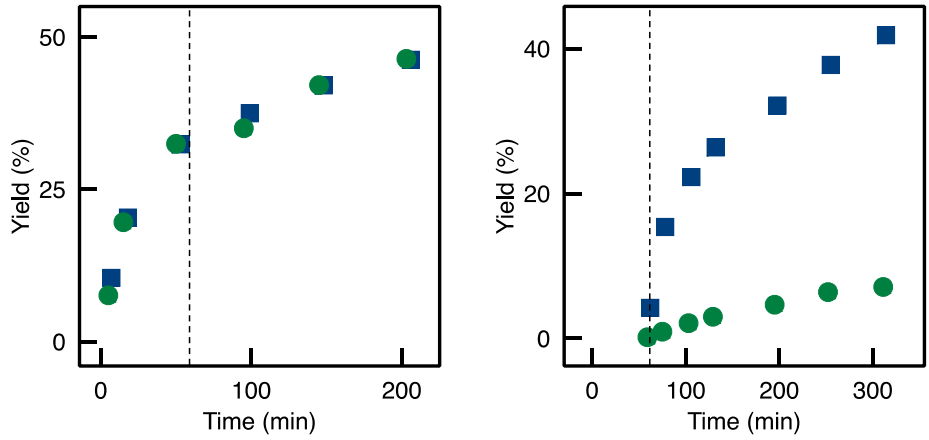

Figure S8 – (Left) Effect of the addition of $NaBH_3CN$ during the transamination in batch reactors (1h after starting the reaction, dotted line): (■) blank (no addition of $NaBH_3CN$); (●) addition of $NaBH_3CN$. Reactions conditions: 10 mM rac-BMBA, 10 mM pyruvate, 0.5 g/L PLP, 4.4 µM ATA-117, 5 % DMSO, $NaBH_3CN$ 50 mM, 30 °C. No deactivation occurred in batch reactors when reducing agent was added after 1 hour reaction. (Right) Effect of pre-incubation with 50 mM $NaBH_3CN$ on transamination reactions in batch reactors (1 hour pre-incubation, dotted line): (■) blank (no pre-incubation with $NaBH_3CN$); (●) one hour pre-incubation with $NaBH_3CN$. Reactions conditions: 10 mM rac-BMBA, 10 mM pyruvate, 0.5 g/L PLP, 4.4 µM ATA-117, 5 % DMSO, 30 °C. When the reducing agent was added to transaminases solutions prior to the batch reaction (one hour pre-incubation before the transamination reaction), a strong deactivation was observed.

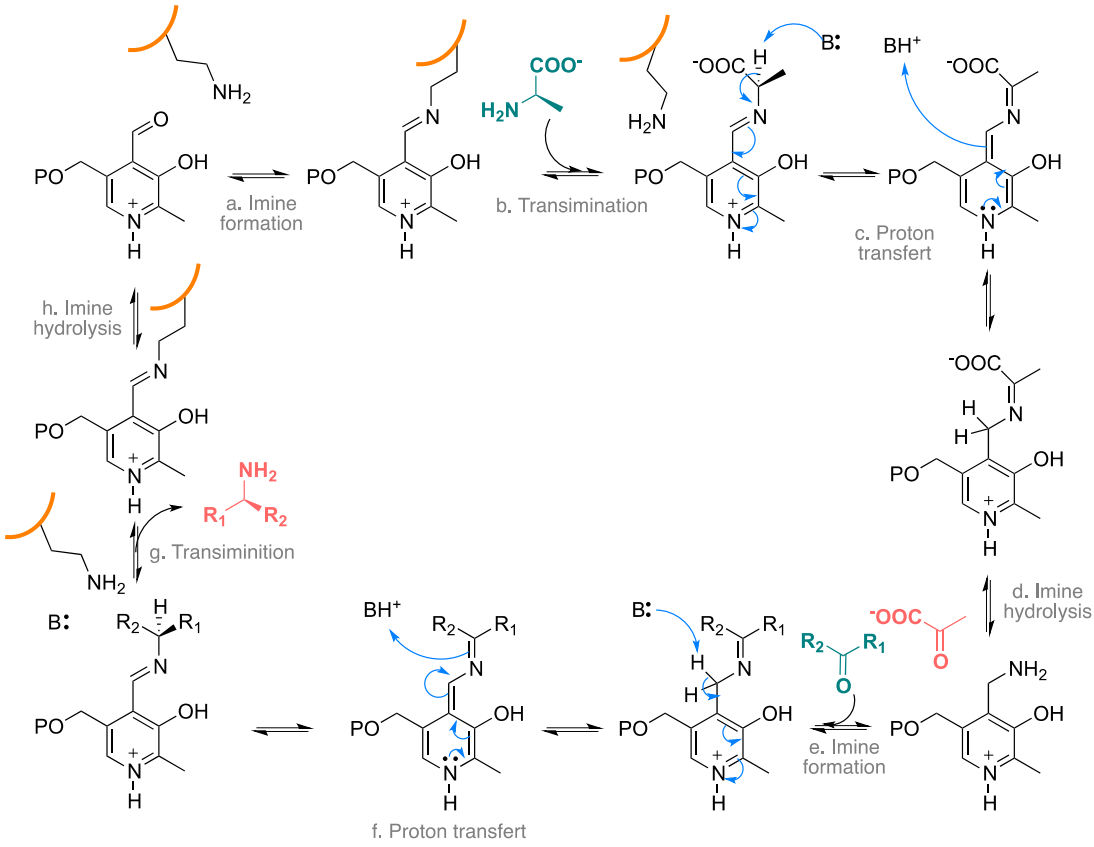

Figure S9 – Transamination mechanism. Two half-reactions execute the ping-pong bi bi mechanism: from (a) to (d), the amine reacts with the PLP cofactor to form the ketone by-product and a PLP amine-derivative (PMP, Pyridoxamine phosphate); from (e) to (h), the ketone reacts with the PMP to form the amine and release the PLP. PO stands for phosphate, and B for basic molecule or residue. Adapted from refererences[2-4].

References

1. P. L. Privalov, *Crit. Rev. Biochem. Mol. Biol.*, 1990, **25**, 281-306.
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