# Hybrid $NH_2$-silica monolith as support for biocatalysis in flow: leveraging on a better control of the aminopropyl dispersion

Transaminases are enzymes that catalyze asymmetric transamination reactions. They are highly enantioselective and operate in mild conditions. They are potentially useful for preparing chiral amines, including industrially relevant drugs currently produced by organometallic complexes in relatively polluting batch processes [1]. The remaining challenge for a green and industrially practicable process is the design of solid transamination biocatalysts (Fig. A) that can be easily used in continuous flow mode to increase productivity and atom efficiency [2].

ATA-117 transaminase was immobilized by covalent grafting on highly porous silica monoliths, called Si(HIPE) (Fig. B, E and F) [3]. To that end, silica surface was functionalized with 3-aminopropyltriethoxysilane (APTES), and then pre-activated by glutaraldehyde. Functionalization was first performed by soaking the monoliths in a large excess of APTES solution for 24 h (as suggested by [3]).

Resulting biocatalysts were active (with high repeatability), recyclable, enantioselective, and showed no diffusional limitations.

However, a poor APTES dispersion in the monolith was macroscopically visible, and confirmed by thermogravimetry (TGA) and infrared (IR) analyses. This was assigned to the functionalization mode in which excess APTES in the solution accumulates at the outer regions of the monolith, causing an egg-shell dispersion (Fig. C).

Parameters known to affect the dispersion have been controlled in order to get better dispersion of amino groups and subsequently better catalytic properties: both wet and dry impregnation methods were tested for the functionalization step. As it is well known that both the availability of surface silanols and the presence of water in the medium greatly influence functionalization efficiency, preparation was conducted with a fine control on silanols (silica re-hydroxylation) and water availability (use of dried monolith and water saturated solvent). Materials used as supports for immobilized enzymes were characterized by IR and TGA.

In turned out that controlling the water content during the functionalization step allows obtaining a homogeneous APTES dispersion (Fig. D) and this was correlated with an increase in the biocatalytic activity of the monoliths.

In classical conditions, up to 40 % conversion was obtained, with a contact time (adjustable) of 10 min, with good repeatability. Immobilized enzyme residual activity reached 100 % (indicating that activity is totally retained when immobilized).

These results pave the way to the design of truly green flow mode biocatalytic synthesis processes.

**Keywords**

chiral amines, transaminases, silica monolith, functionalization, flow chemistry.

**References**

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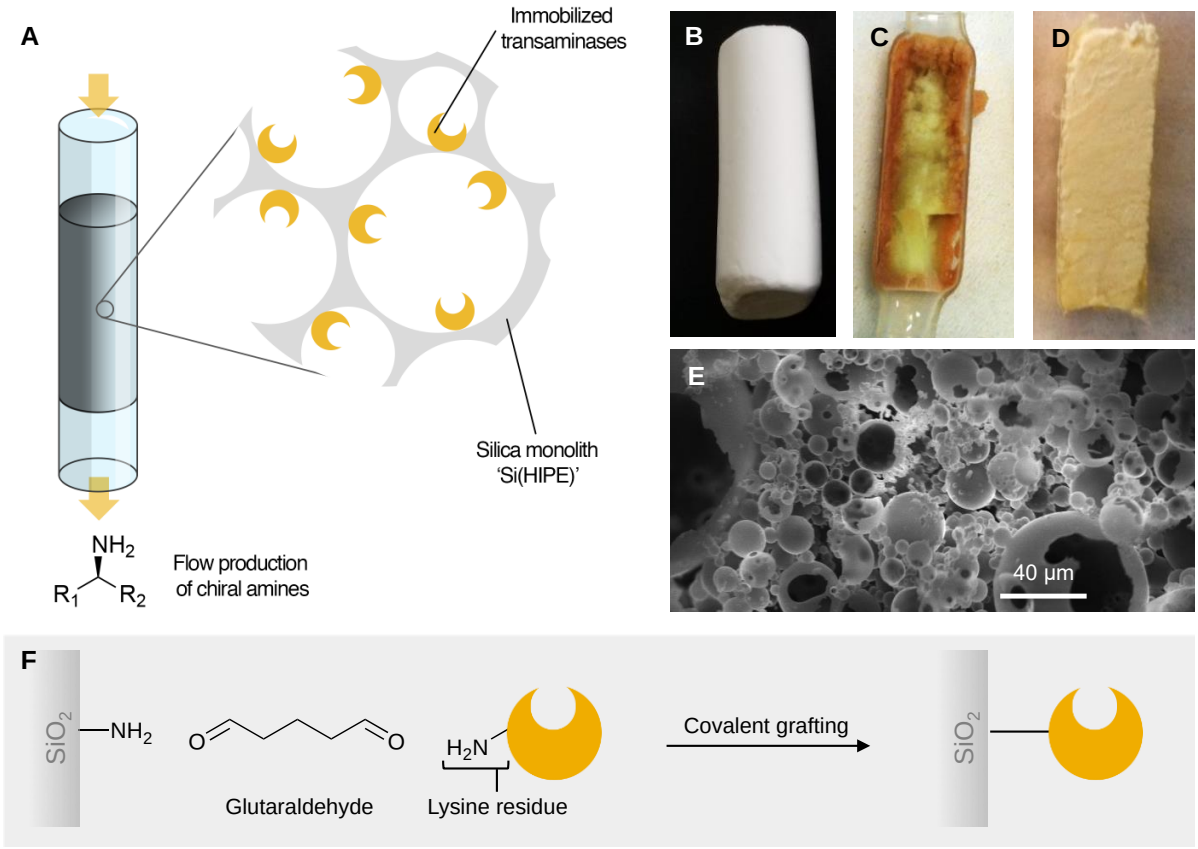
**Figure**




(A) Immobilized transaminases on Si(HIPE) monolith for flow chiral amine production. (B) Si(HIPE) monolith. (C) Poor and (D) good APTES dispersion (brown) in Si(HIPE). (E) SEM micrograph of Si(HIPE). (F) Covalent grafting of enzyme on amino moieties.