HaloTag® immobilization of novel **α-ketoacid-dependent dioxygenases** increases **initial rate activity and process stability**

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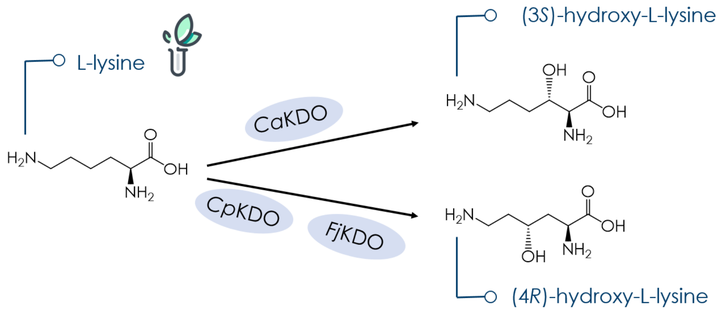
The **reaction**

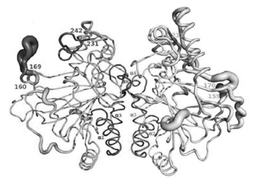


*Cp*KDO: α-ketoacid dependent dioxygenase from *Chitinophaga pinensis*
*Fj*KDO: α-ketoacid dependent dioxygenase from *Flavobacterium johnsoniae*
*Ca*KDO: α-ketoacid dependent dioxygenase from *Catenulispora acidiphila*

- insertion of an oxygen atom in non-activated C-H bond in the absence of harmful oxidizing agents is chemically challenging
- novel α-ketoacid dependent dioxygenases (KDOs) catalyze the stereoselective hydroxylation of amino acids[1–3]
- L-lysine derivatives hydroxylated in 3- and 4-position find versatile application as chiral building block for active pharmaceutical ingredients

The **catalyst**

α-ketoacid-dependent dioxygenases



cofactor	Fe^{2+}
reducing agent	L-ascorbic acid
cosubstrate	α-ketoacid
side reaction	α-ketoacid to succinate
reaction	hydroxylation
mechanism	dioxygen activation (O_2 dependent)

The **challenge**

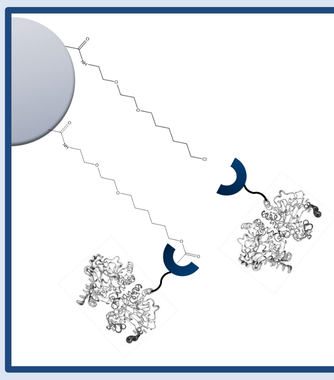
precipitated and inactive *Cp*KDO after metal affinity chromatography in TRIS buffer

- general instability
- loss of activity
- loss of iron in active site
- precipitation during purification

→ purification with constant supply of iron (II), reducing agent and cosubstrate increases stability

→ laborious and expensive

The **solution:** HaloTag® immobilization

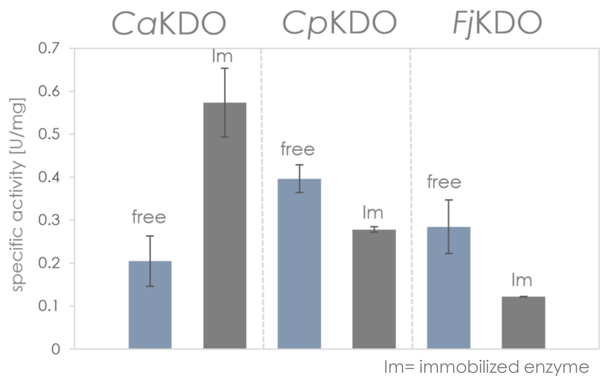


- mutated dehalogenase fused to target enzyme via linker [4,5]
- commercially available carriers: sepharose & magnetic beads

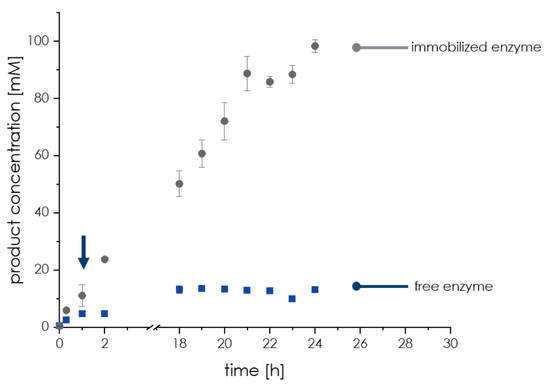
Site-specific covalent immobilization
no enzyme leaking
immobilization from cell-free extract
high residual activity

The **results**

residual activity



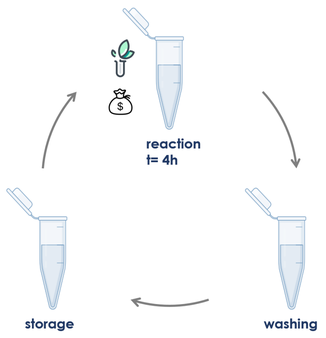
*Ca*KDO - process stability



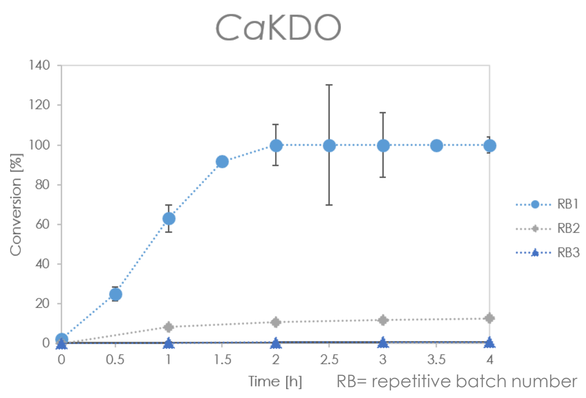
- ***Ca*KDO:** immobilization increases specific activity and process stability
- ***Cp*KDO:** 70 % residual activity, good process stability with free and immobilized enzyme
- ***Fj*KDO:** 32 % residual activity, good process stability with free and immobilized enzyme

100 mM L-lysine, 750 rpm, 0.5 mg/ml – 1 mg/ml catalyst 200 mM HEPES, 1 ml reaction volume, measured by HPLC analytics, data from two independent reactions

recyclability- repetitive batch studies

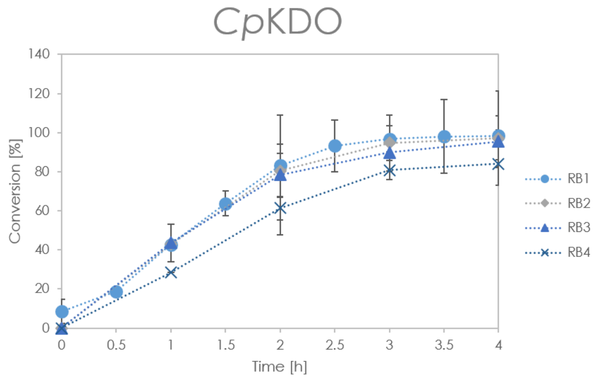


→ decrease of overall process costs
→ more sustainable

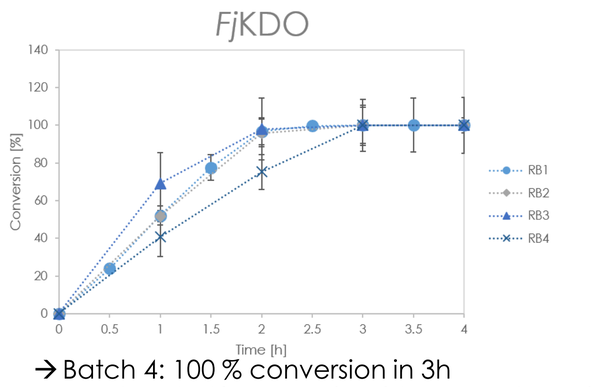


→ Not recyclable

RB= repetitive batch number



→ Batch 4: 84 % conversion



→ Batch 4: 100 % conversion in 3h
→ Batch 7: 27 % conversion (data not shown)

100 mM L-lysine, overhead shaker, 5 mg/ml immobilized KDO, 200 mM HEPES, 1 ml reaction volume, measured by HPLC analytics, data from two independent reactions. Immobilized catalyst was washed and stores at 4°C in-between batches.

preparative lab scale - *Ca*KDO

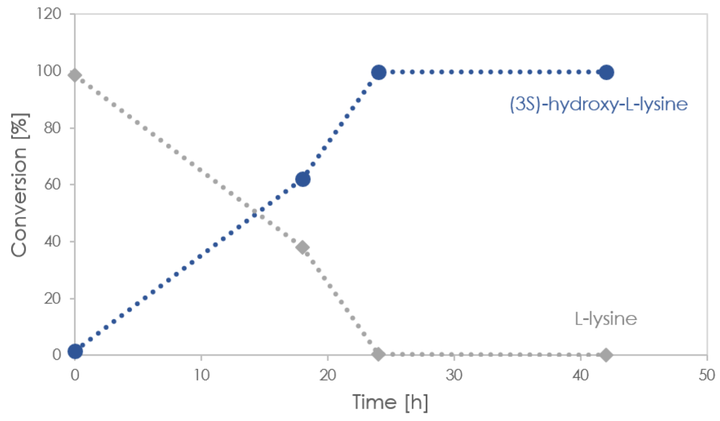
1 ml reaction volume

100 mM substrate
1 mg/ml *Ca*KDO

reaction optimization

10 ml reaction volume

200 mM substrate
1.94 mg/ml *Ca*KDO



200 mM L-lysine, 200 mM HEPES, 150 rpm, 1.94 mg/ml *Ca*KDO, 10 ml total volume, measured by HPLC analytics, single measurement

*Ca*KDO

→ Upon immobilization and reaction optimization **200 mM** conversion in **42 h** possible with moderate enzyme concentration

references

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