

Comparative evaluation of the asymmetric synthesis of (S)-norlaudanosoline in a two-step biocatalytic reaction with whole *Escherichia coli* cells in batch and continuous flow catalysis

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1. Expression of TAm and NCS in whole *Escherichia coli* cells

1.1 Expression of TAm

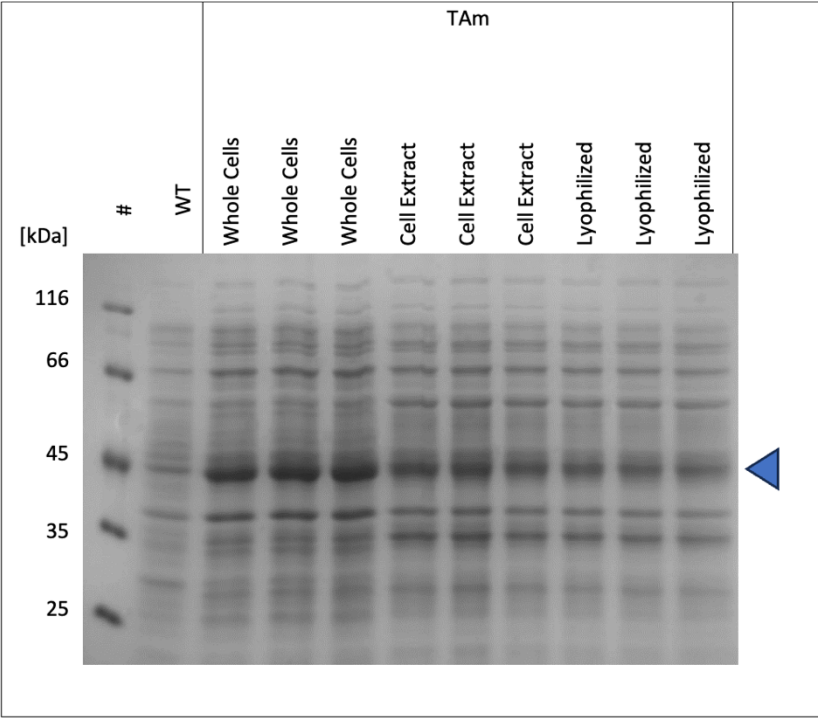


Figure S1: Analysis of the TAm expression in *E. coli* cells using SDS-PAGE (15 % gel). 1: left to right: pEqGold Protein Marker 1, wild type, 3x CV_TAm in whole cells, 3x CV_TAm in cell extract, 3x CV_TAm in lyophilized cells. The overexpressed TAm is marked by a blue arrow.

1.2 Expression of NCS

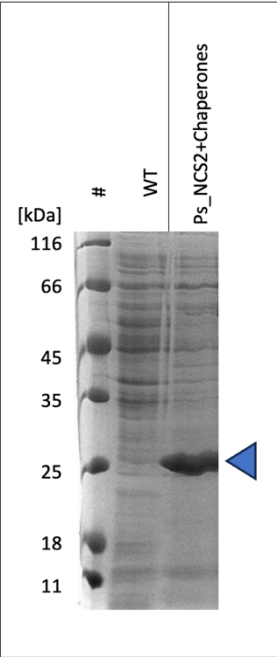


Figure S2: Analysis of the NCS expression in *E. coli* cells using SDS-PAGE (18 % gel). SDS-PAGE 2: left to right: pEqGold Protein Marker 1, wild type cells without plasmid, cells transformed with a plasmid coding for Ps_NCS2 and plasmid pG-Tf2 bearing the genes *groES-groEL-tig*. The overexpressed Ps_NCS2 is marked by a blue arrow. The chaperones GroEL (around 60 kDa), GroES (around 10 kDa) and trigger factor (around 56 kDa) are not visible.

2. Biocatalyst stability

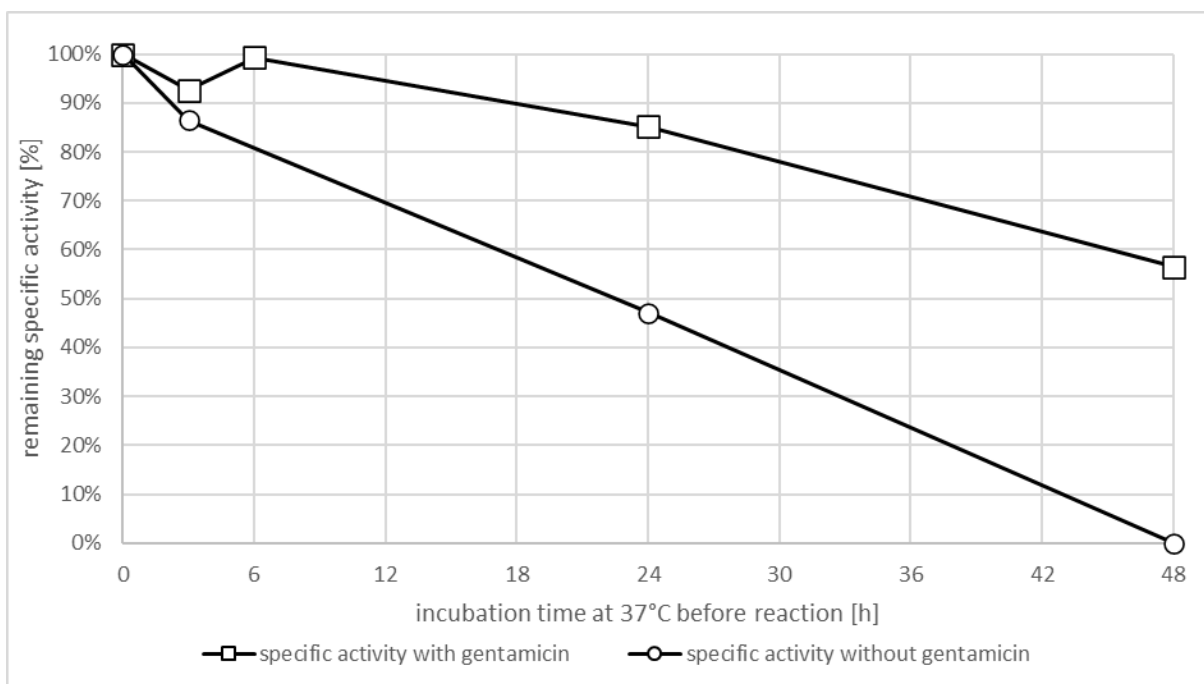


Figure S3: Time course of NCS activity in whole cells incubated at 37°C before substrate addition. Squares: Activity after incubation with 25 $\mu\text{g mL}^{-1}$ gentamicin during incubation and reaction. Circles: Activity without gentamicin during incubation and reaction.

Reaction conditions: 50 mM HEPES (pH 7.4), 50 mM dopamine, 50 mM pyruvate, 4 g L^{-1} ascorbate. 3 h; 37 °C. The activity determined by chiral HPLC.

3. Chiral HPLC analysis

3.1 (S)-NLS: chemical standard

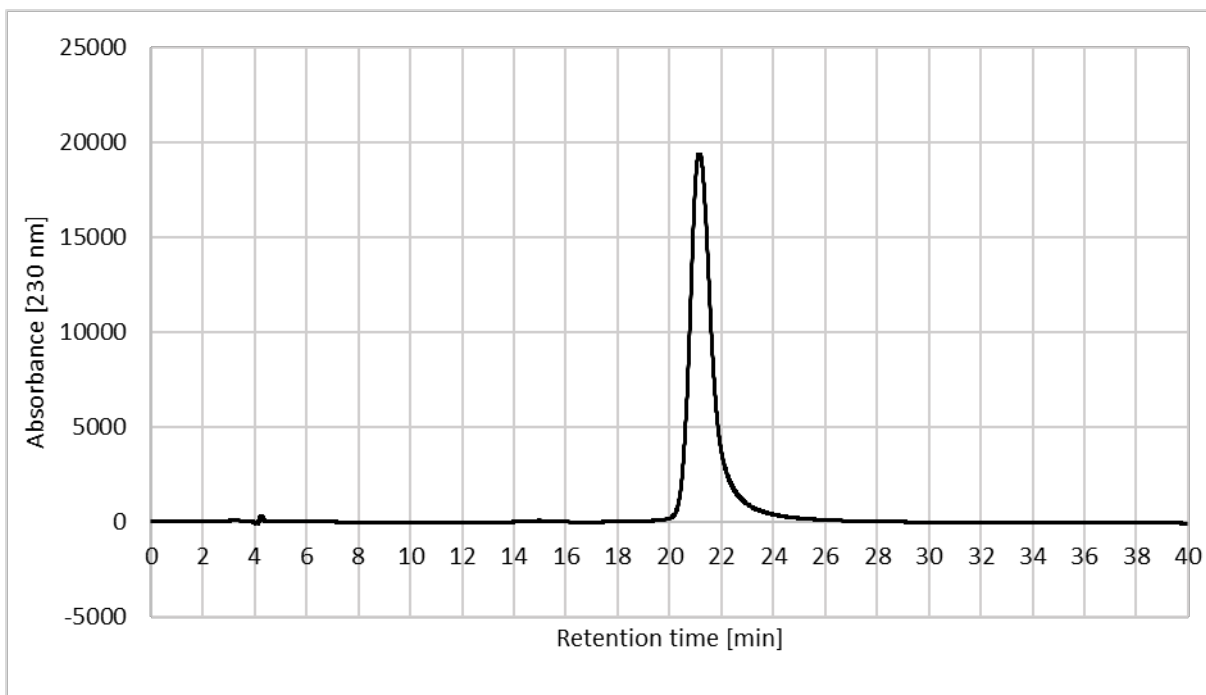


Figure S4: Chromatogram of (S)-norlaudanosoline analytics. An Astec Chirobiotic T column (25 cm) was used with 20:20:60 MeOH:ACN:25 mM phosphate buffer (pH 4.2) as mobile phase at a flow rate of 0.2 mL min⁻¹ at 20°C.

3.2 rac-NLS: chemical standard

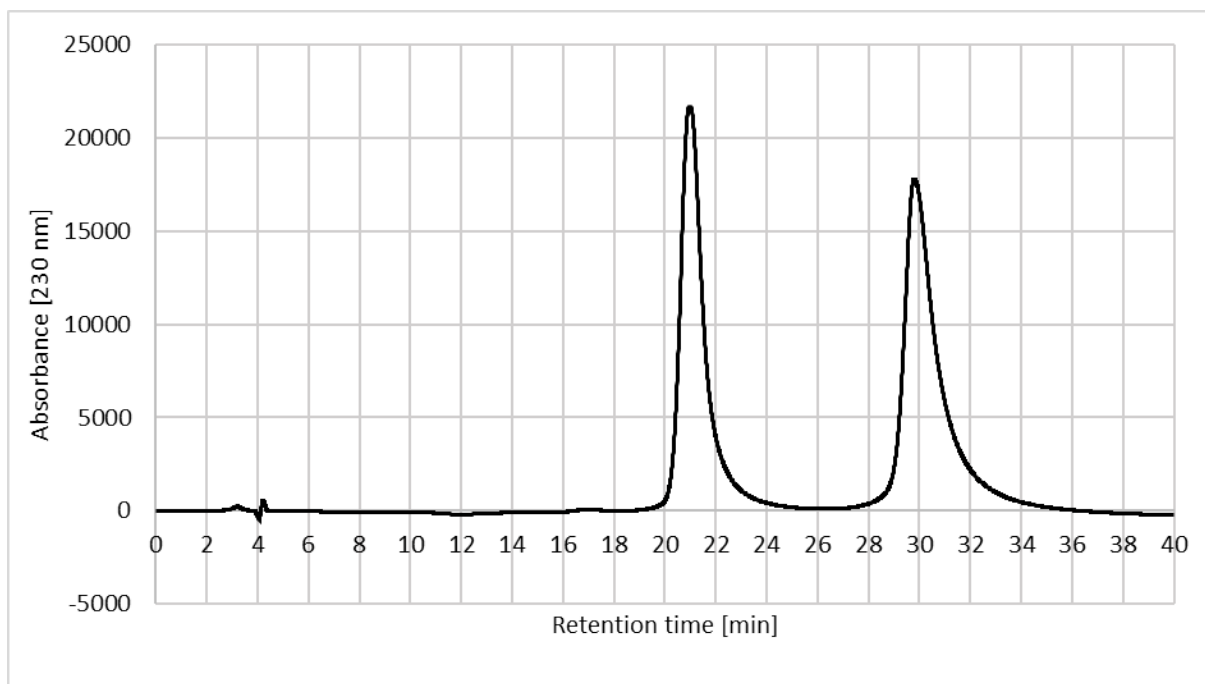


Figure S5: Chromatogram of rac-norlaudanosoline analytics. An Astec Chirobiotic T column (25 cm) was used with 20:20:60 MeOH:ACN:25 mM phosphate buffer (pH 4.2) as mobile phase at a flow rate of 0.2 mL min⁻¹ at 20°C.