Supplementary Information

Reaction engineering of biocatalytic (*S*)-naproxen synthesis integrating in-line process monitoring by Raman spectroscopy

M. Aßmann[1], A. Stöbener[1], C. Mügge[2], S. K. Gaßmeyer[2], L. Hilterhaus[1], R. Kourist[2,3], A. Liese*[,1] and S. Kara*[,1]

[1]Institute of Technical Biocatalysis, Hamburg University of Technology, Denickestr. 15, 21073 Hamburg, Germany.

[2]Junior Research Group for Microbial Biotechnology, Ruhr-University Bochum, Universitätstr. 150, 44780 Bochum, Germany.

[3]Institute of Molecular Biotechnology, Graz University of Technology, Petersgasse 14, A8010 Graz, Austria

*** Correspondence:**
Dr. Selin Kara, Institute of Technical Biocatalysis, Hamburg University of Technology, Denickestr. 15, 21073 Hamburg, Germany, selin.kara@tuhh.de

Prof. Andreas Liese, Institute of Technical Biocatalysis, Hamburg University of Technology, Denickestrasse 15, 21073 Hamburg, Germany, liese@tuhh.de

^{1}H NMR analysis of naproxen synthesized by AMDase-CLGIPL

The analysis with ^{1}H NMR shows a high purity of the isolated naproxen product. Peaks:

1H NMR (400 MHz, MeOD) δ 7.76 – 7.65 (m, 3H), 7.40 (dd, J = 8.6, 1.8 Hz, 1H), 7.19 (d, J = 2.5 Hz, 1H), 7.10 (dd, J = 9.0, 2.5 Hz, 1H), 3.88 (s, 3H), 3.84 (q, J = 7.1 Hz, 1H), 1.52 (d, J = 7.2 Hz, 3H).

Contaminations:
[4.89 (s, H_2O), 3.35 (m, CH_3OH), 2.68 (s, 0.002 H) 1.99 (s, 0.09 H), 1.27 (s, 0.03 H, plasticizer)]

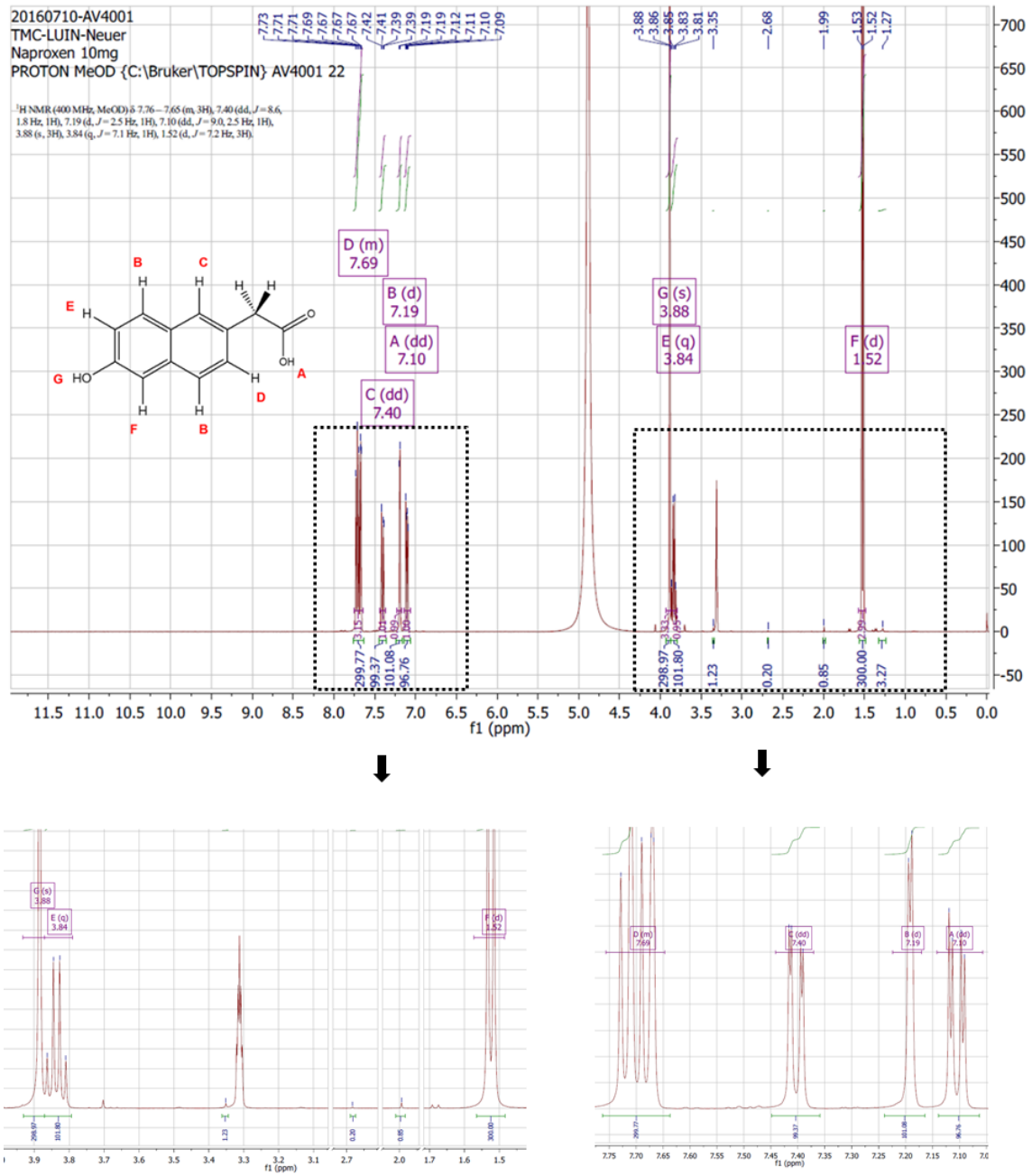

Figure S1: ^{1}H NMR spectra of isolated naproxen in MeOD.

Raman spectroscopy for in-line process monitoring with free and immobilized enzyme

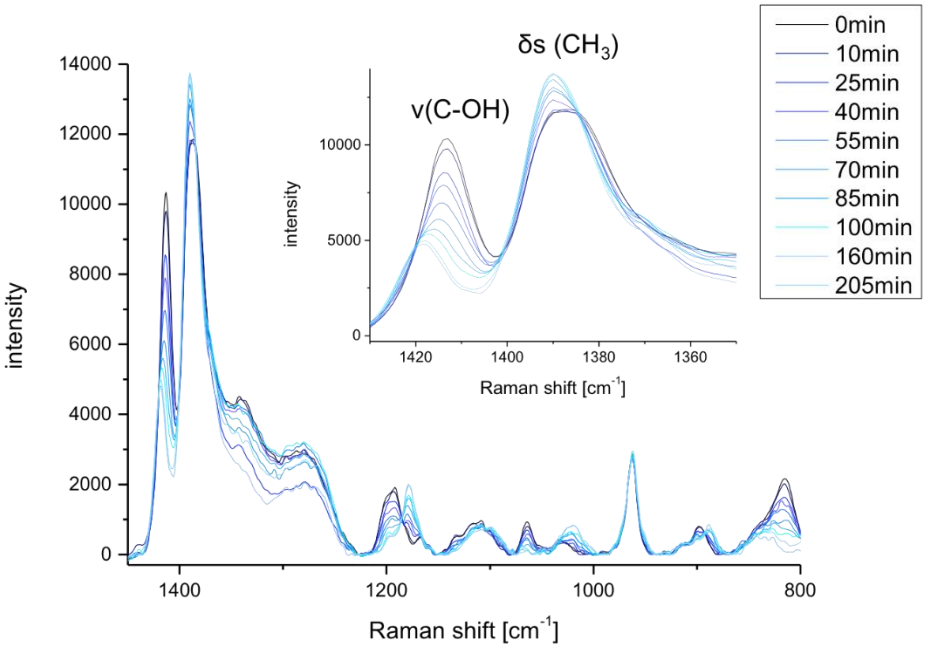

Figure S2: Raman spectra measured during naproxen synthesis, starting material naproxen malonate 25 mM in aqueous solution (water at pH 8.0, adjusted with 1 M NaOH), AMDase-CLGIPL free.

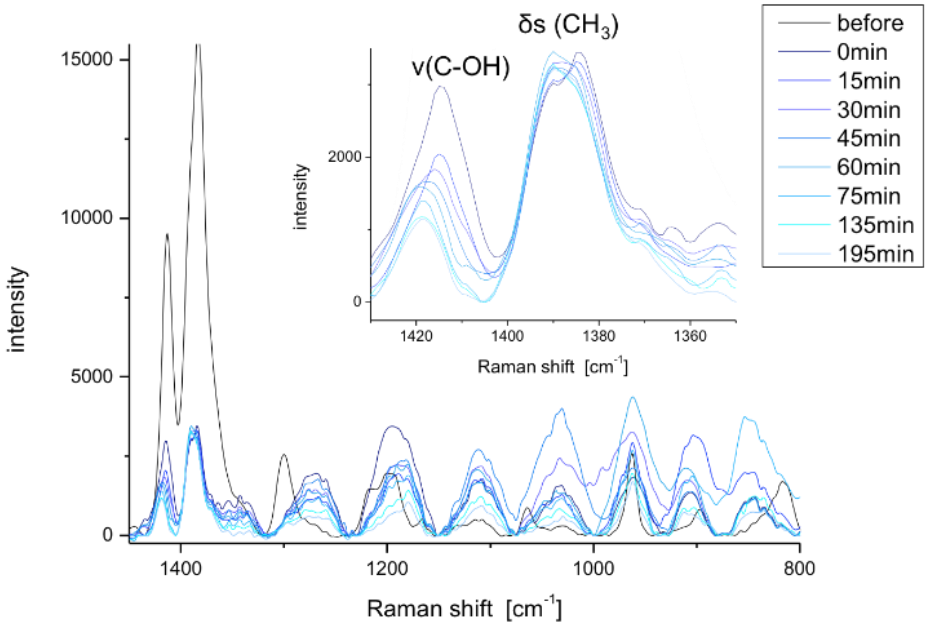

Figure S3: Raman spectra measured during naproxen synthesis, starting material naproxen malonate 25 mM in aqueous solution (water at pH 8.0, adjusted with 1 M NaOH), AMDase-CLGIPL immobilized.

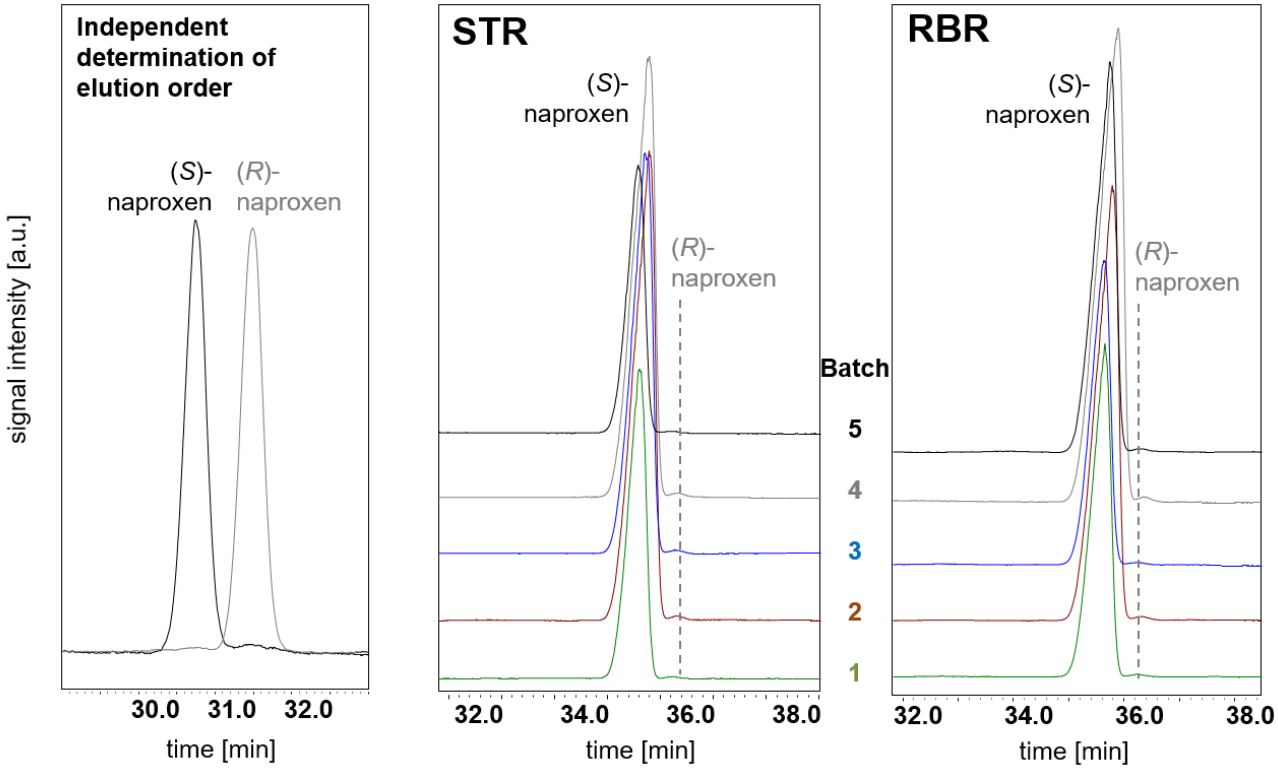

Figure S4: GC chromatograms of naproxen, measured with a chiral column (FS Hydrodex-β-6TBDM, Macherey-Nagel), and a method operating isothermal at 160°C with an injection split of 1/20. The elution order was (*S*)-naproxen (35.7 min) and (*R*)-naproxen (36.3 min) in the actual experiments. Left, previously determined retention order of (S)- and (R)-naproxen, confirmed with authentic standards and biocatalyses with (*R*)- and (*S*)-selective AMDase variants IPLL and GLG-IPL. Middle, 5 batch experiments with the STR; right, 5 batch experiments with the RBR system using (*S*)-selective AMDase CLG-IPL within this study.