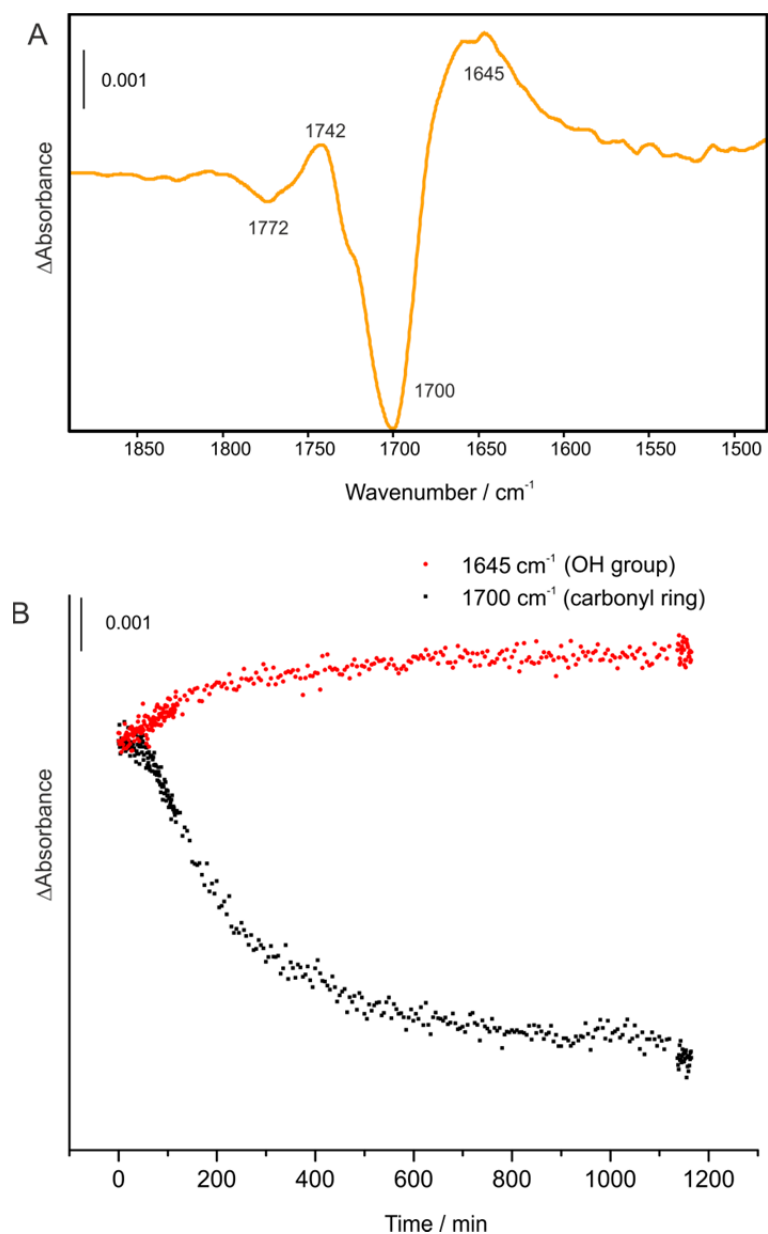


## Highly stable Protein Immobilization via Maleimido-Thiol Chemistry to Monitor Enzymatic Activity

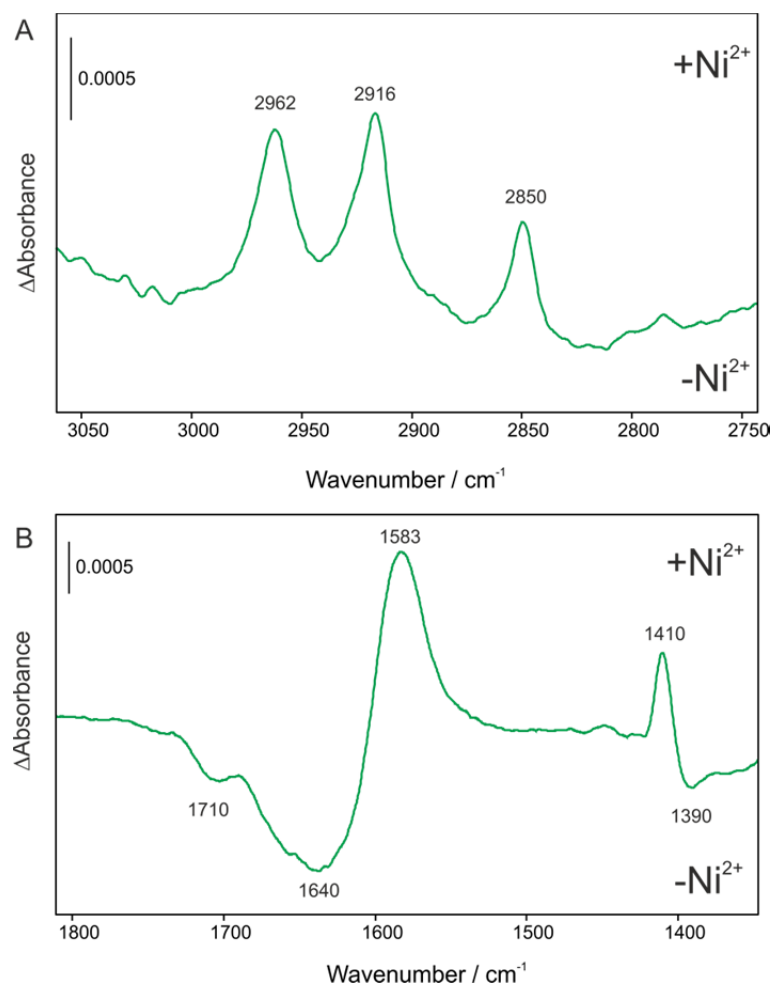
Jonas Schartner,<sup>†</sup> Jörn Güldenhaupt,<sup>†</sup> Sarah Gaßmeyer,<sup>‡</sup> Katharina Rosga,<sup>†</sup> Robert Kourist,<sup>‡</sup> Klaus Gerwert,<sup>†</sup> and Carsten Kötting<sup>\*,†</sup>

<sup>†</sup>Department of Biophysics, Ruhr-Universität Bochum, Universitätsstrasse 150, 44801 Bochum, Germany

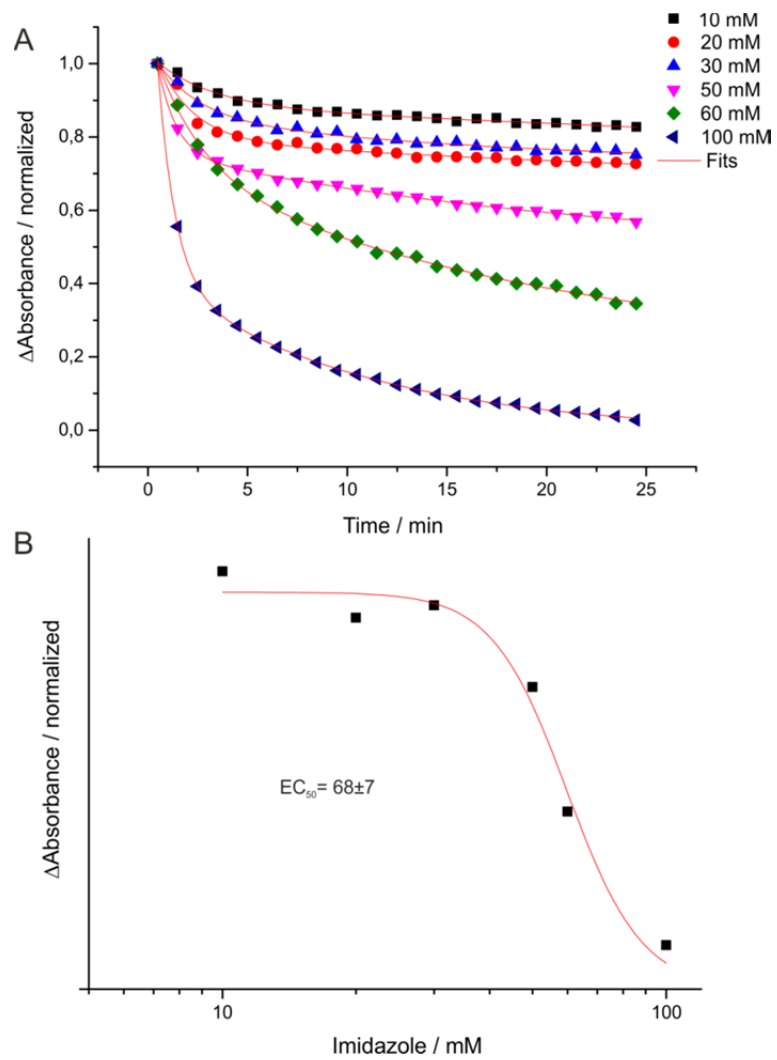
<sup>‡</sup>Junior Research Group for Microbial Biotechnology, Ruhr-Universität Bochum, Universitätsstrasse 150, 44801 Bochum, Germany



**Figure S1. (A) Reaction of a maleimido-terminated germanium surface with 3-mercaptopropanol and (B) the corresponding kinetics of the major bands. The obtained spectrum and the kinetics show changes in the carbonyl region of the maleimido group due to the successful addition of the 3-mercaptopropanol.**



**Figure S2. Difference spectra of the nickel complexation by the maleimido-NTA surface. (A)** Three signals in the CH-region at 2962, 2916 and 2650  $\text{cm}^{-1}$  were detected, probably indicating a structural change in the NTA-molecule. **(B)** Three negative bands at 1710  $\text{cm}^{-1}$ , 1640  $\text{cm}^{-1}$ , and 1390  $\text{cm}^{-1}$  present the previous state of carboxylic acids. Positive bands at 1583 and 1410  $\text{cm}^{-1}$  can be assigned to the asymmetric and symmetric stretching vibration of the carboxylic acids.



**Figure S3. Determination of the EC<sub>50</sub>-value. (A) Different imidazole concentration were tested and fitted ranging from 10 till 100 mM. (B) The data was plotted and the EC<sub>50</sub>-value with determined.**

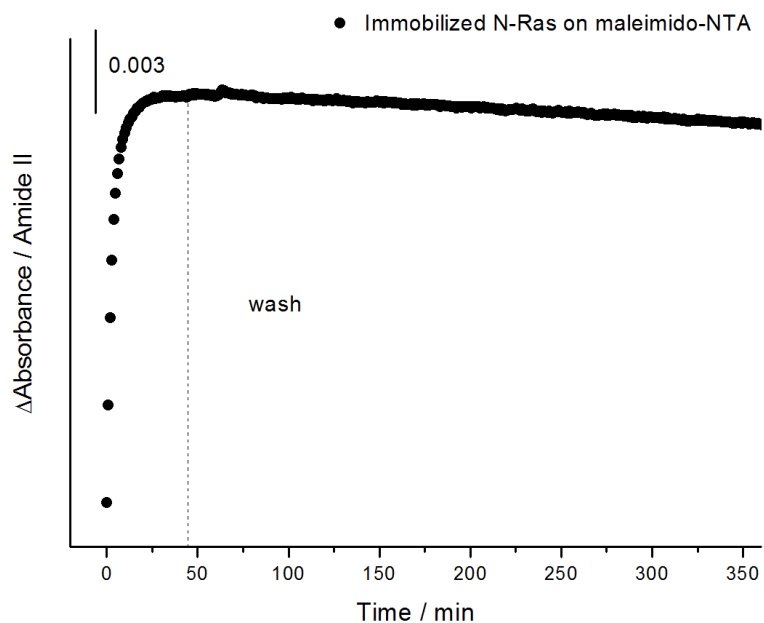


Figure S4. Long term analysis of the immobilized Ras protein by washing the surface for six hours with buffer.

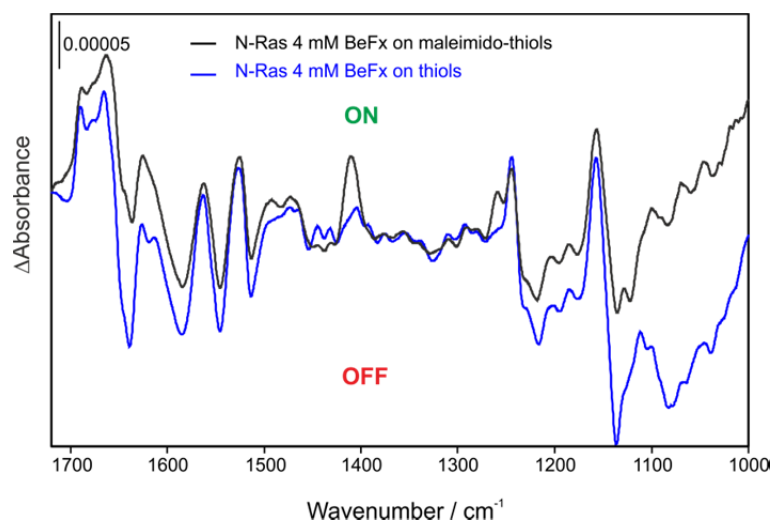


Figure S5. ATR-FTIR Difference spectra of immobilized Ras with beryllium fluoride mimicking the switching of the small GTPase from the **OFF** to the **ON** state.<sup>1</sup>

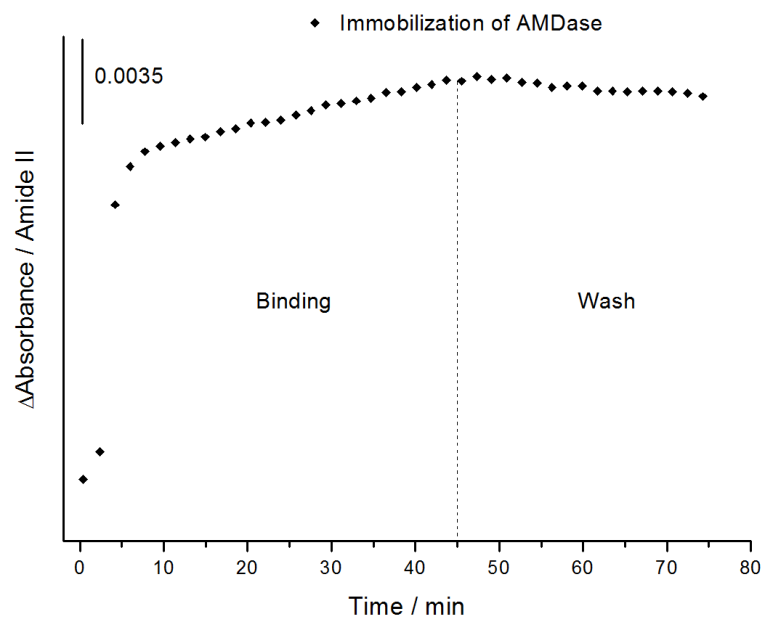


Figure S6. Immobilization kinetics of the AMDase on the maleimido-Ni-NTA surface.

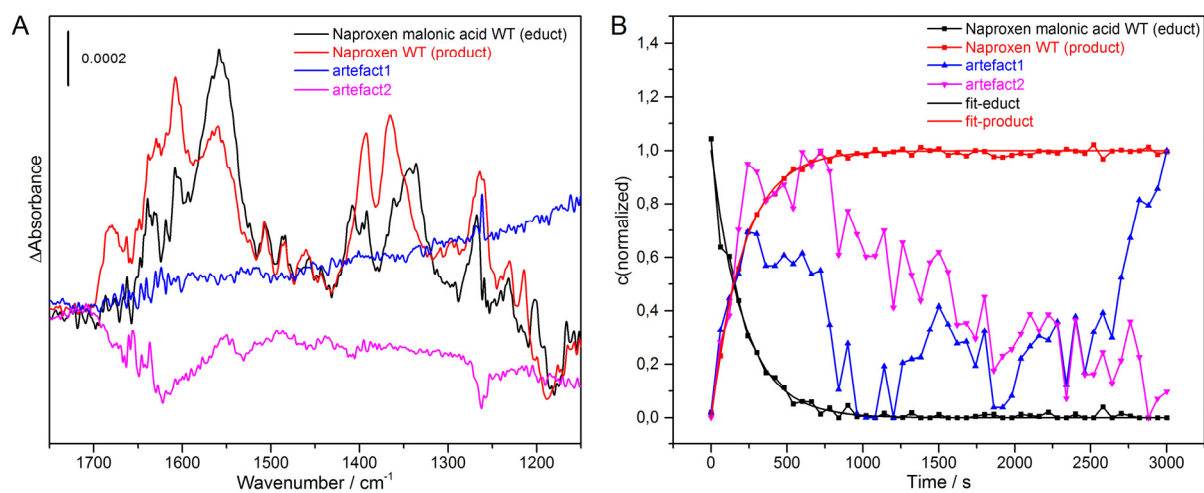


Figure S7. MCR-ALS (HS-MCR) decomposition of the IR spectra time series of the AMDase WT catalyzed decarboxylation reaction of naproxen malonic acid. This MCR result consists of educt, product and two artefact spectra (A) and the corresponding concentration profiles (B).

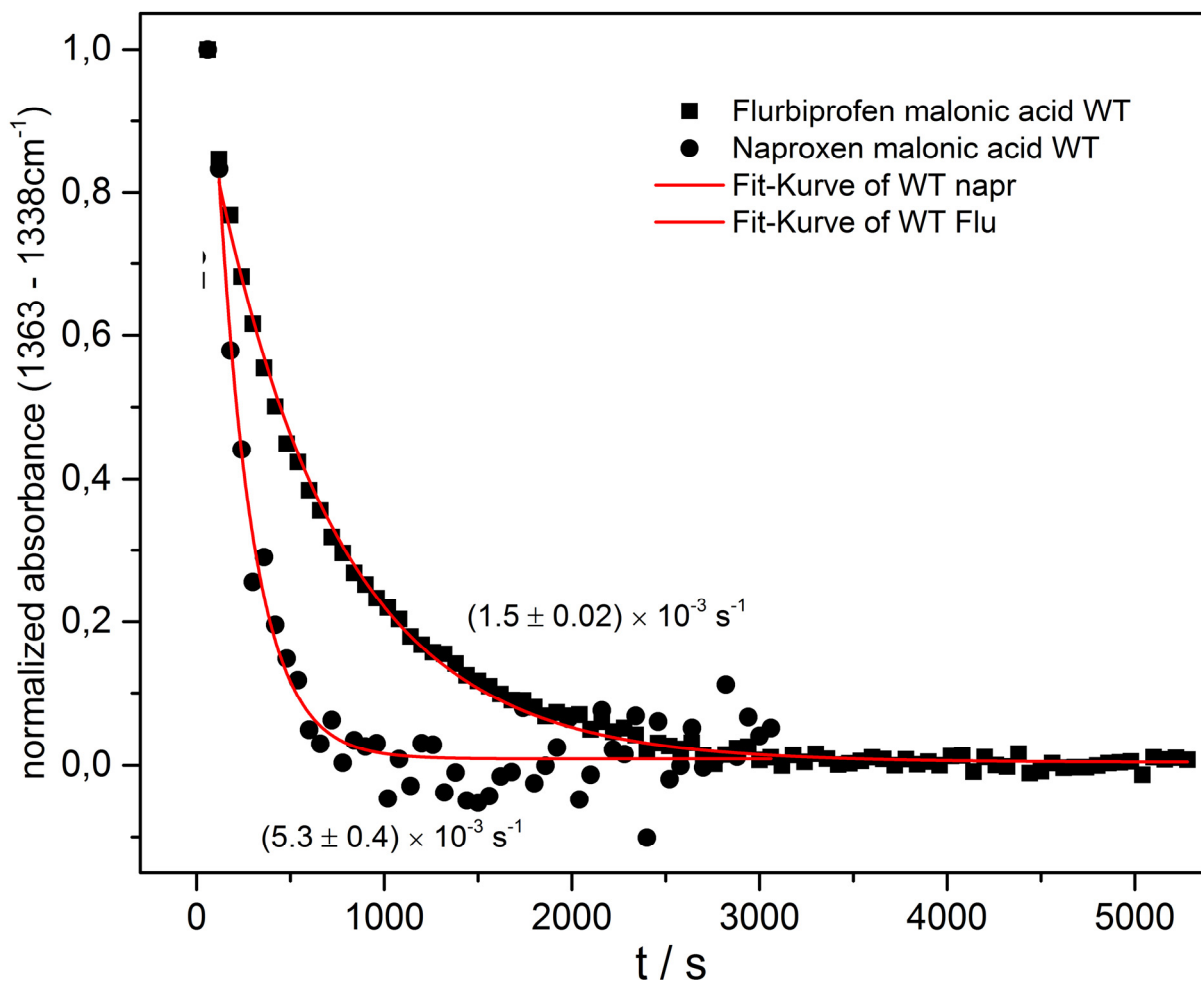


Figure S8. Comparison of the kinetics of AMDase WT based on absorption differences without application of HS-MCR.

Table S1: Decarboxylation kinetics of immobilized AMDase with different substrates. Obtained by global fit analysis.

k [s <sup>-1</sup> ]	Difference kinetics	HS-MCR
WT naproxen malonic acid	$(5.3 \pm 0.4) \times 10^{-3}$	$4.73 \times 10^{-3}$
WT flurbiprofen malonic acid	$(1.5 \pm 0.02) \times 10^{-3}$	$1.7 \times 10^{-3}$