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Supporting Information

Facile Stereoselective Reduction of Prochiral Ketones by using an F_{420}-dependent Alcohol Dehydrogenase

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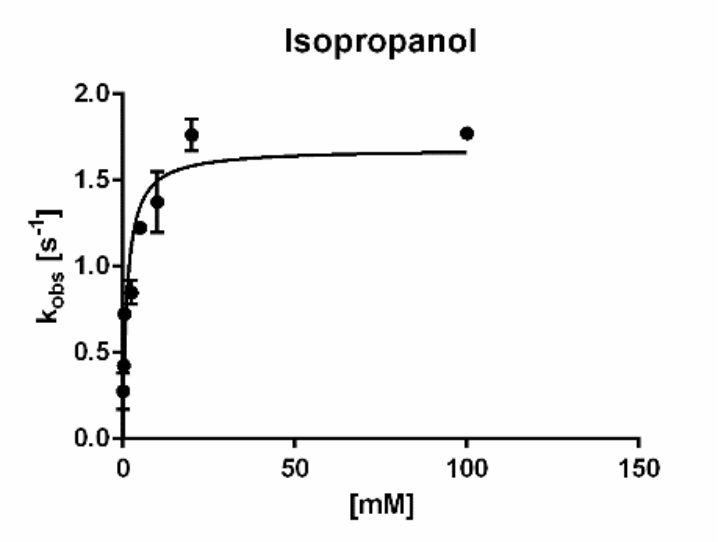
Expression and purification of F_{420}-dependent ADF and FGD

An Adf-encoding gene fragment was ordered codon optimized for *Escherichia coli* and cloned into a pBAD to have the construct expressed with a C-terminal His-tag. Expression was performed using *Escherichia coli* NEB 10β. Cells carrying the plasmid were grown in Terrific broth (TB) supplemented with 50 μg mL^{-1} ampicillin until OD_{600} was 0.8 and expression was induced with L-arabinose 0.02% at 24 °C overnight. Cells were harvested by centrifugation at 5000 × g for 20 min at 4 °C. Cell pellets were resuspended in 250 mM sodium phosphate buffer pH 7.0 containing 1 mM phenylmethylsulfonyl fluoride and 10% v/w glycerol. The cells were lysed by sonication, using a Sonics Vibra-Cell VCX 130 sonicator with a 3 mm stepped microtip (5s on, 5s off, 70 % amplitude, 7 min). Cell debris were pelleted by centrifugation at 12000 × g for 20 min at 4 °C. The supernatant was applied to Ni-Sepharose High Performance (GE Healthcare) pre-equilibrated with 250 mM sodium phosphate pH 7.0, 10% v/w glycerol. The washing buffer was 250 mM sodium phosphate pH 7.0, 10% v/w glycerol, 10 mM imidazole and the elution buffer was 250 mM sodium phosphate pH 7.0, 10% v/w glycerol, 500 mM imidazole. The eluted protein was desalted using a desalting column pre-equilibrated with 250 mM sodium phosphate pH 7.0, 10% v/w glycerol. FGD was purified as previously described. [1] Purity of ADF and FGD was assessed with SDS-PAGE analysis and protein concentrations were measured by using the Bradford assay.

Steady state kinetics analyses

The employed assay measures the rate at which F_{420} is reduced (ε_{400} =25.7 $mM^{-1}cm^{-1}$). The buffer used was 250 mM sodium phosphate (pH 7.0), 10% v/w glycerol. For obtaining K_M and k_{cat} values, the data were fit using a regular Michaelis-Menten equation: $k_{obs}=k_{cat}*[S]/K_M + [S]$.



K_M
1.3±0.5 mM
k_{cat}
1.7±0.1 s^{-1}
k_{cat}/K_M
1.3 $s^{-1}mM^{-1}$

Isopropanol tolerance

The tolerance of ADF towards isopropanol was probed by measuring its apparent melting temperature by ThermoFluor. [2] Using a real-time PCR the temperature at which ADF unfolds in the presence of different concentrations of isopropanol was measured. For the measurements, 250 mM sodium phosphate pH 7.0, 10% glycerol, 1x Sypro Orange, and 10 µM ADF was used.

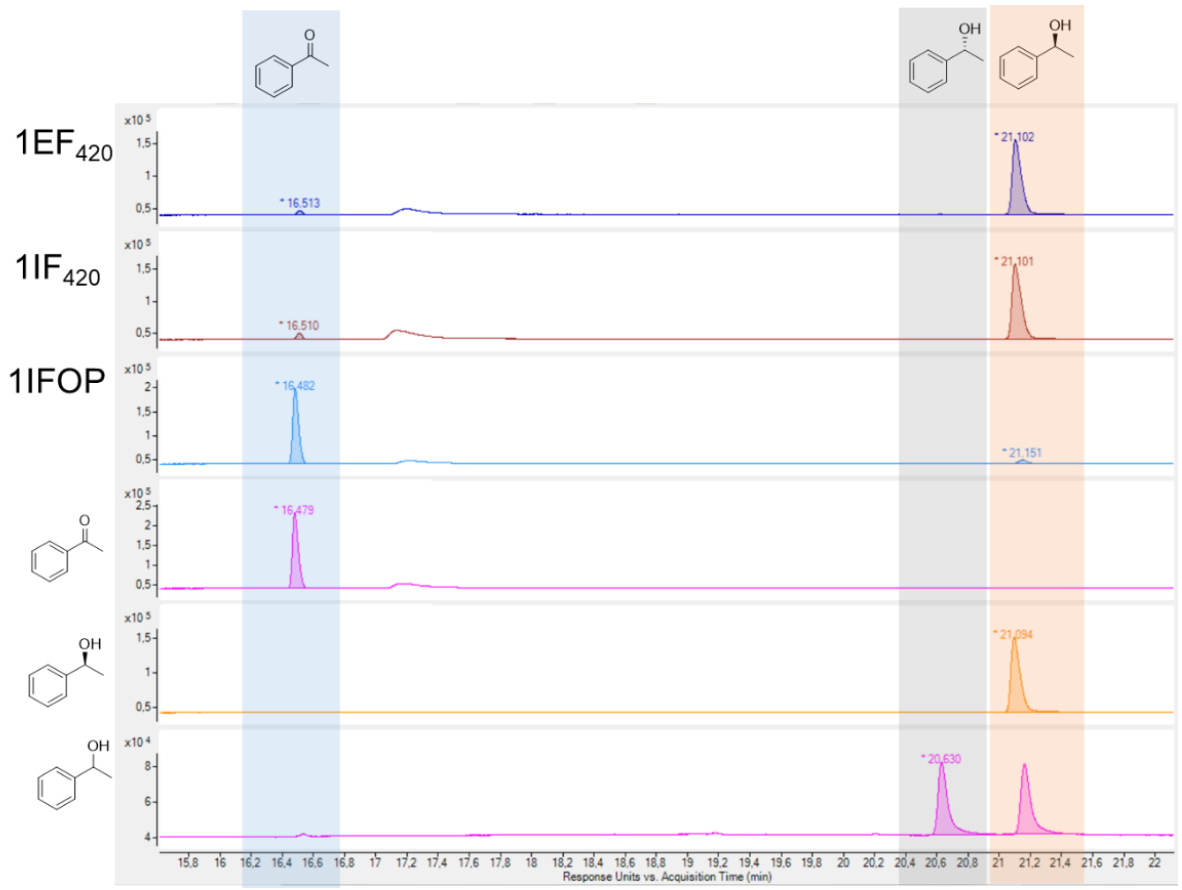
Table 1. Thermostability of ADF in the presence of isopropanol.

Isopropanol Concentration (mM)	T_m^{app} (°C)
50	57.5±0
100	57.5±0
200	56.0±0
500	53.5±0
1000	49.5±0

Conversions

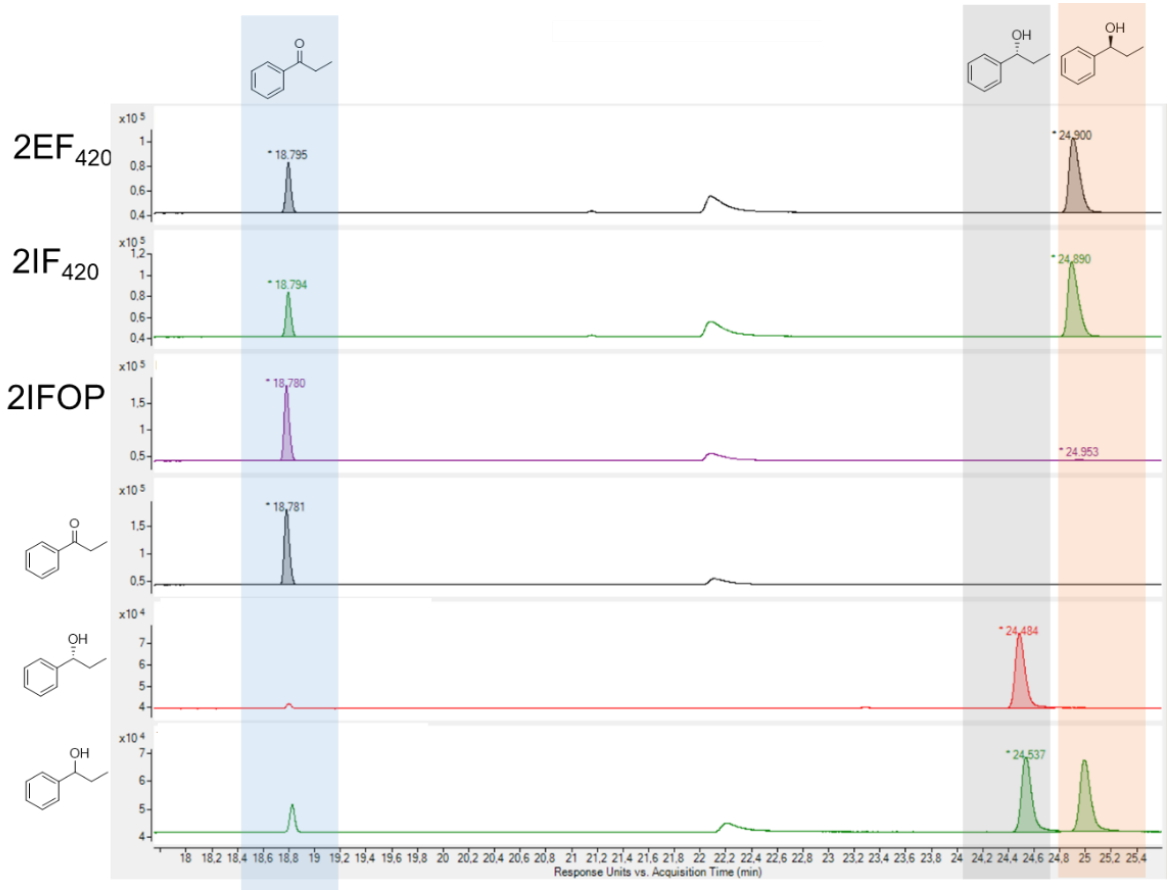
Substrate 1

Analyzed using: Agilent Technologies 7890A GC system with column CP Chiralsil Dex CB (Agilent). Program: 40 °C to 130 °C in 5 min, hold 130 °C 10 min, 130 °C to 180 °C in 10 min, hold 180 °C in 5 min.



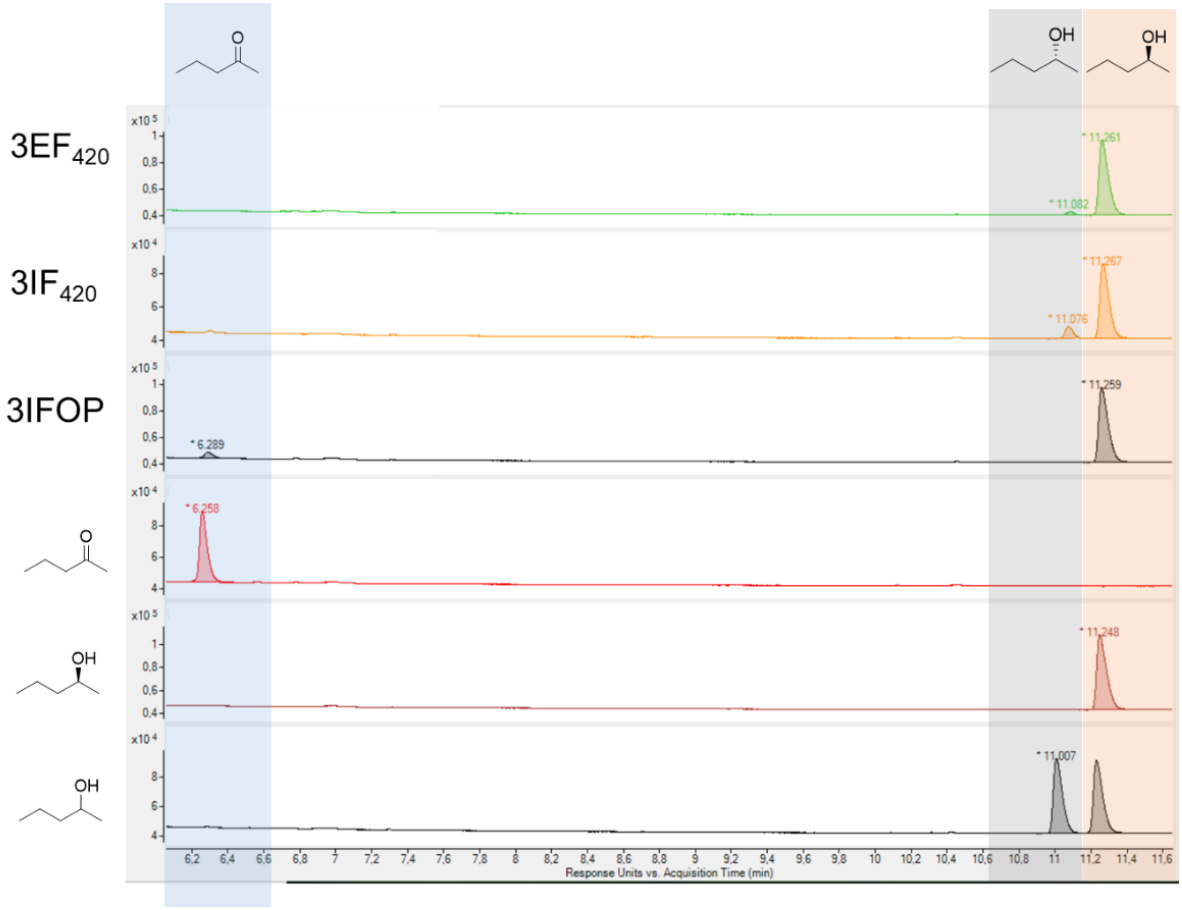
Substrate 2

Analyzed using: Agilent Technologies 7890A GC system with column CP Chiralsil Dex CB (Agilent). Program: 40 °C to 130 °C in 5 min, hold 130 °C 10 min, 130 °C to 180 °C in 10 min, hold 180 °C in 5 min.



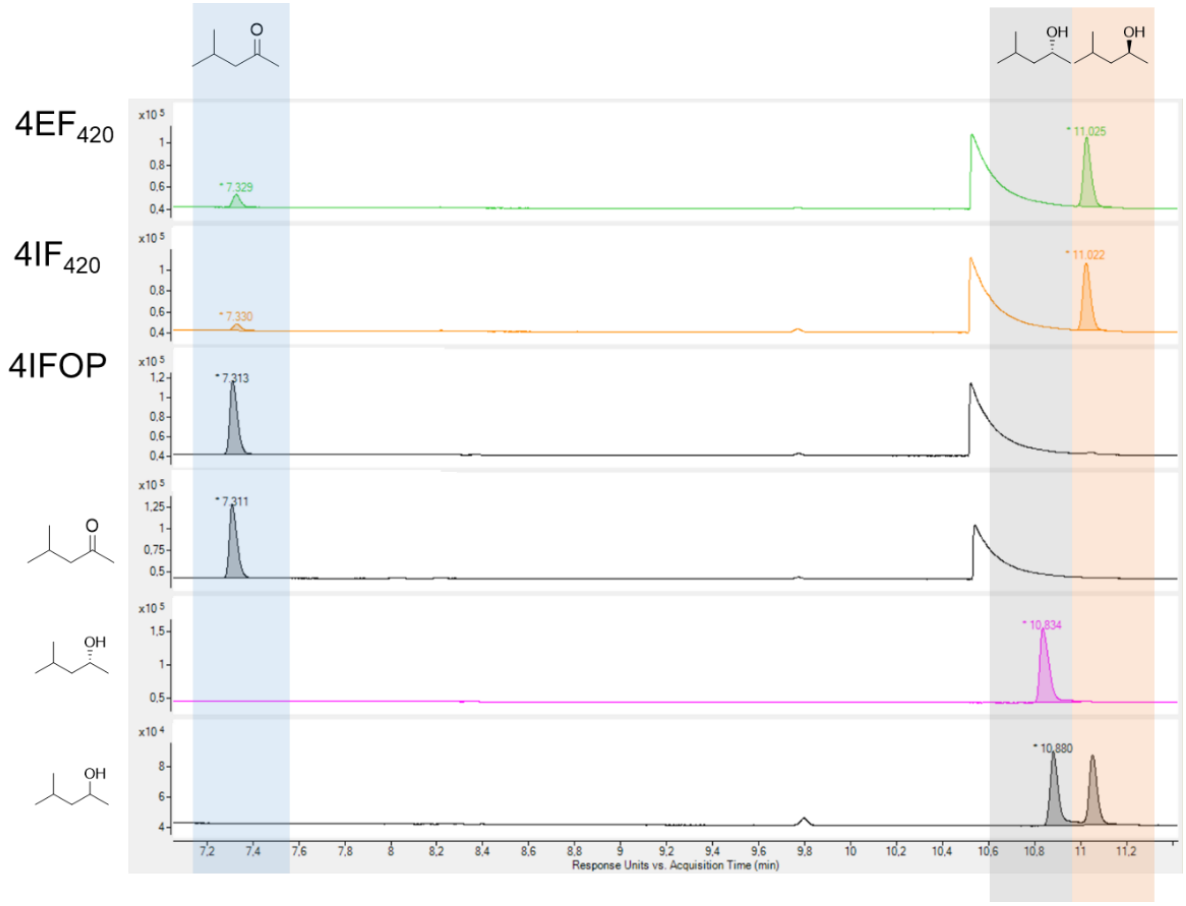
Substrate 3

Analyzed using: Agilent Technologies 7890A GC system with column CP Chiralsil Dex CB (Agilent).
Program: 40 °C to 120 °C in 3 min, 120 °C to 40 °C in 10 min.



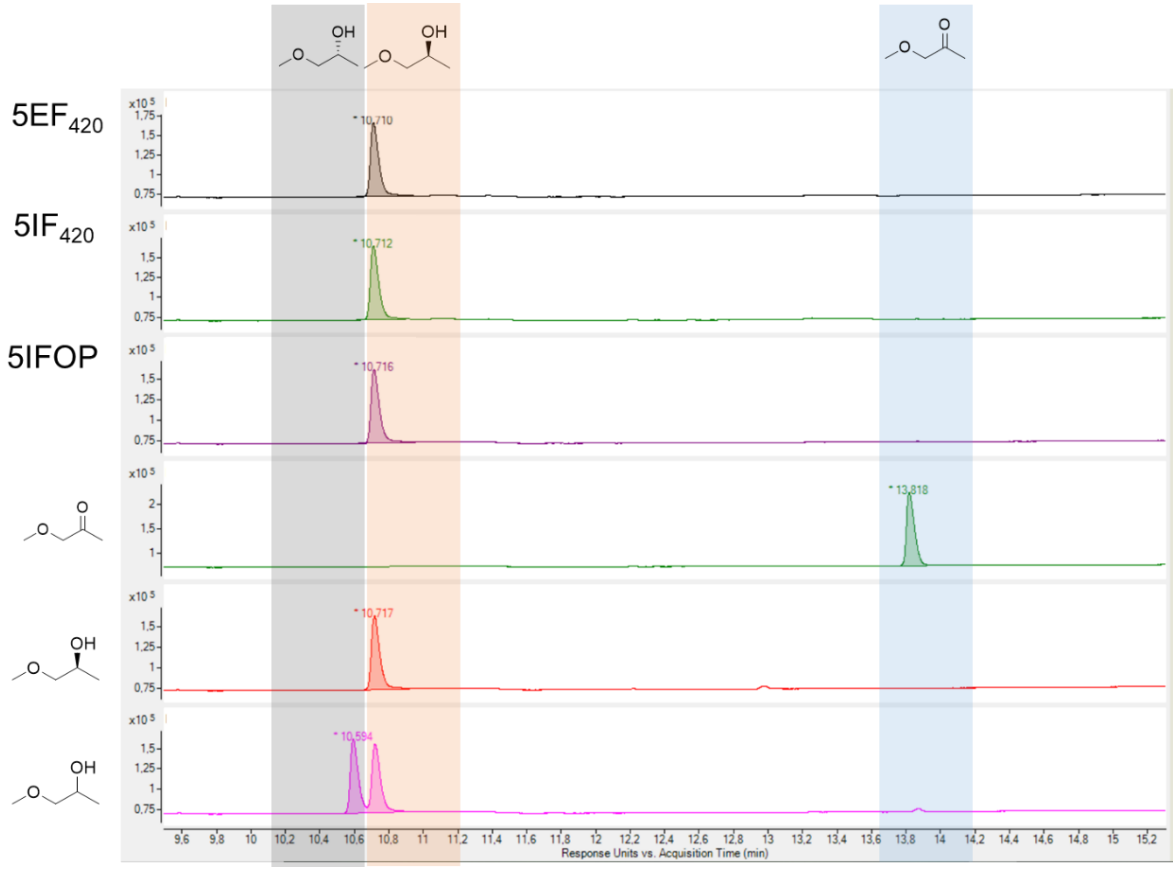
Substrate 4

Analyzed using: Agilent Technologies 7890A GC system with column CP Chiralsil Dex CB (Agilent).
Program: 40 °C to 140 °C in 5 min, 140 °C to 40 °C in 10 min.



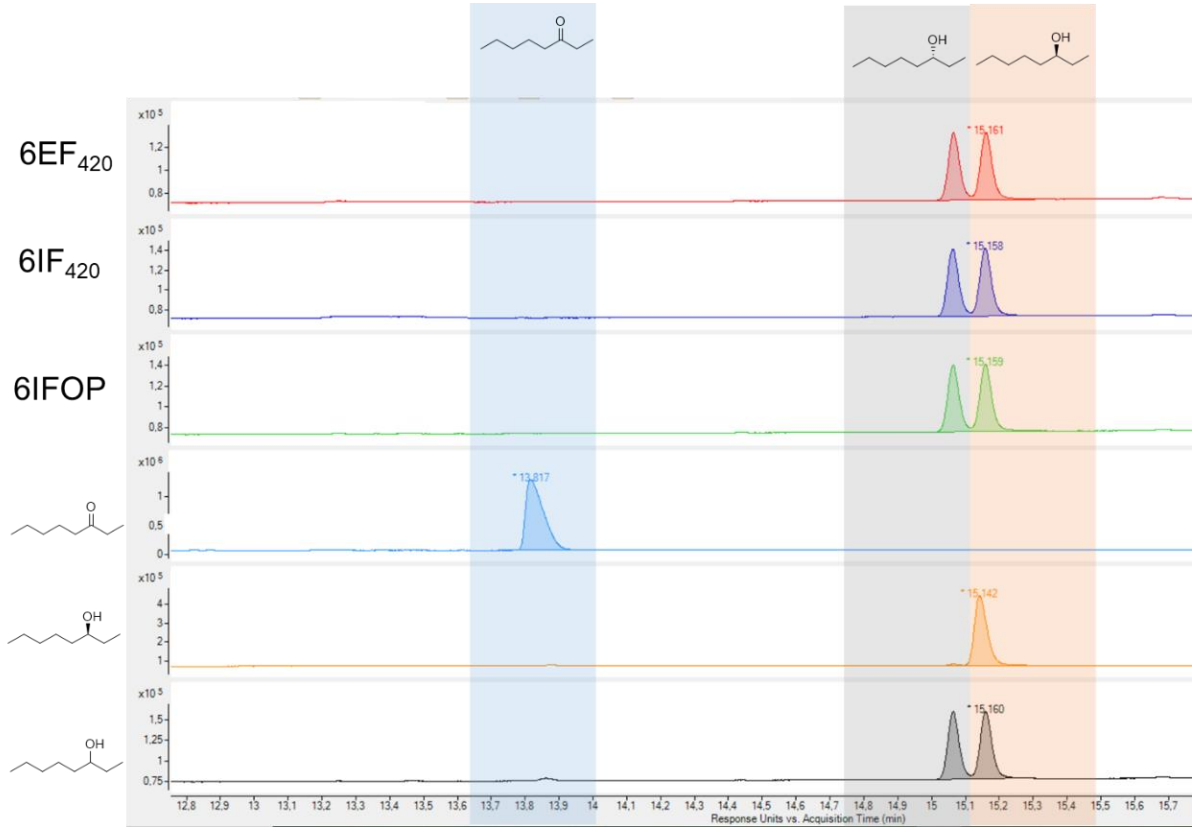
Substrate 5

Analyzed using: Agilent Technologies 7890A GC system with column FS-Hydrodex-B-TBDAc (Aurora Borealis) Program: 40 °C to 190 °C in 5 min, 190 °C to 40 °C in 10 min.



Substrate 6

Analyzed using: Agilent Technologies 7890A GC system with column FS-Hydrodex-B-TBDAc (Aurora Borealis) Program: 40 °C to 190 °C in 5 min, 190 °C to 40 °C in 10 min.



Substrate docking

Molecular docking was performed in YASARA Structure (version 19.12.14). [3] The crystal structure of ADF (1.8 Å resolution; PDB 1RHC [4]) was used. Substrates were built using YASARA, energy minimization was performed, and VINA was employed to perform the docking. [5] Docking was accomplished using the docking simulation macro 'dock_run.mrc' with a 5 Å cube cell size around the C5 atom of F_{420}, 100 runs, 2 Å cluster RMSD and using the YAMBER forcefield. [6] YASARA and UCSF Chimera were used to visualize the results. [7] Pymol was used for preparing the figures.

References

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