Supplementary Materials: Overcoming Water Insolubility in Flow: Enantioselective Hydrolysis of Naproxen Ester

David Roura Padrosa, Valerio De Vitis, Martina Letizia Contente Francesco Molinari and Francesca Paradisi

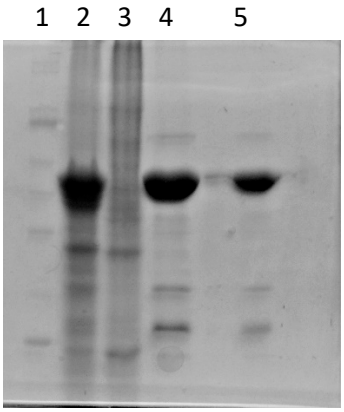

Figure S1. SDS-gel of the purification of HeEst. 1. Markers; 2. Whole cell after expression; 3. Insoluble fraction; 4. Soluble fraction; 5: Pure enzyme.

Table S1: kinetic study of HeE. Substrate concentrations ranging form 0.01 mM to 1 mM were used. The data was fitted to the Lineweaver-Burk plot to obtain K_M and K_{cat} values.

	HeE	
Substrate	pNPA	pNPB
***K*$_M$ (mM)**	2.213 ± 0.248	2.045 ± 0.472
K$_{cat}$ (s^{-1})	15.534 ± 0.888	29.526 ± 0.3.399

Table S2: physical parameters calculated for BCE, BS2m and HeE by Caver. Tunnel length describes the shortest distance between the solvent exposed surface and the catalytic serine oxygen group. Curvature is measured as the length divided by the distance, where length is the computed distance between the starting point and the ending point along the tunnel and distance the shortest possible distance between those two points. The bottleneck indicates the diameter of the narrowest part in the tunnel.

	Tunnel length (Å)	**Curvature**	**Tunnel bottleneck (Å)**
BCE	10.59	1.166	1.29
BS2m	1.98	1	2.56
HeE	7.15	1.37	1.53

Methods S1. NMR characterization of NSAIDs butyl esters:

The purity of butyl esters was assessed by HPLC and ^{1}H NMR. ^{1}H NMR spectra were recorded with a Varian Mercury (300 MHz) spectrometer. Chemical shifts (δ) are expressed in ppm.

1a. Naproxen butyl ester: white solid. 1H NMR (400 MHz, CDCl3) δ: 7.77– 7.12 (m, 6H), 4.09 (m, 2H), 3.93 (s, 3H), 3.86 (q, *J* = 7.1 Hz, 1H), 1.62 – 1.54 (m, 5H), 1.36 – 1.26 (m, 2H), 0.88 (t, *J* = 7.3 Hz, 3H) ppm.

1b. Ibuprofen butyl ester: clear oil. 1H NMR (400 MHz, CDCl3) δ: 7.0-7.3 (m, 4H), 4.1 (t, *J* = 6.6 Hz, 2H) 3.70 (q, *J* = 7.2 Hz, 1H), 2.46 (d, *J* = 7.2 Hz, 2H), 1.93 – 1.79 (m, 1H), 1.65 – 1.46 (m, 6H), 0.96 – 0.82 (m, 10H) ppm.

1c. Flurbiprofen butyl ester: clear oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.58 – 7.36 (m, 6H), 7.19 – 7.13 (m, 2H), 4.13 (t, *J* = 6.7 Hz, 2H), 3.77 (q, *J* = 7.2 Hz, 1H), 1.68 – 1.53 (m, 5H), 1.42 – 1.26 (m, 2H), 0.92 (t, *J* = 7.4 Hz, 3H) ppm.

1d. α-methyl benzeneacetic acid butyl ester: clear oil. ^{1}H NMR (400 MHz, CDCl3) δ: 7.42 – 7.16 (m, 5H), 4.1 (t, J = 6.6 Hz, 2H), 3.73 (q, J = 7.2 Hz, 1H), 1.79 – 1.56 (m, 5H), 1.53 – 1.31 (m, 2H), 0.9 (t, J = 7.3 Hz, 3H) ppm.