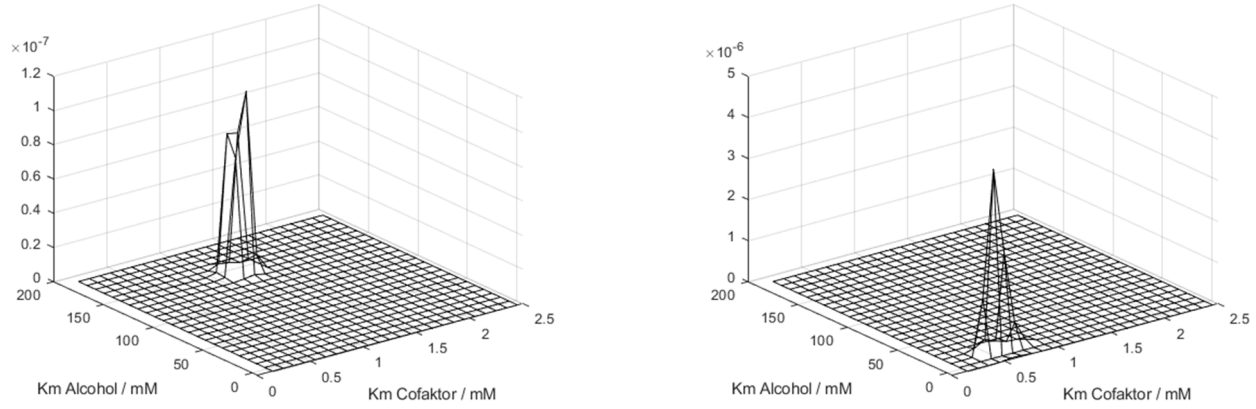
processes

**Supplementary Materials**

## Details to iteration

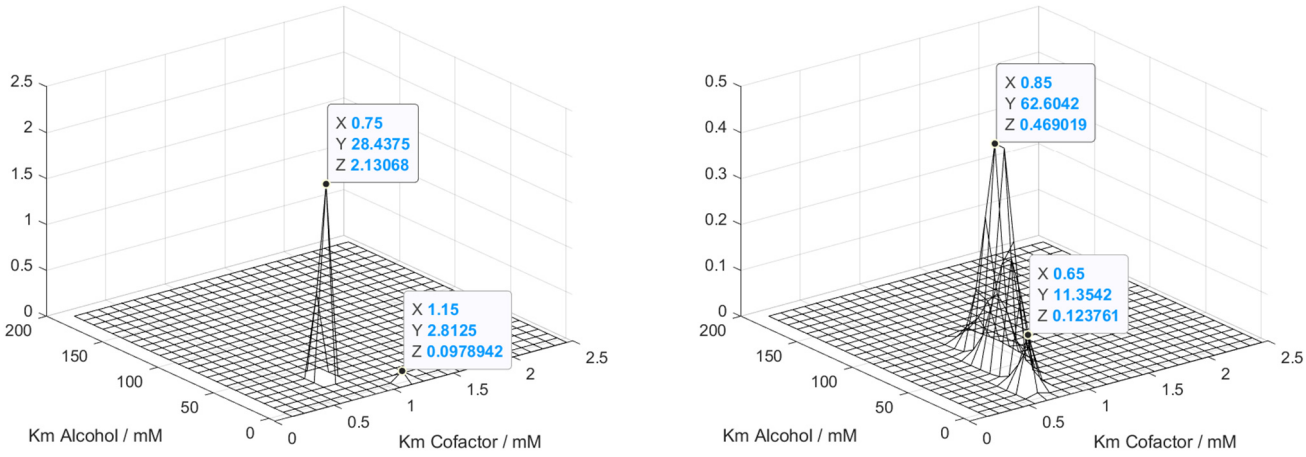
For application of the EM algorithm to calculate the AED the axes were set to 0.1 – 2.5 mM (25 grid points) for Km-cofactor and to -10 – 195 mM (25 grid points) for Km-alcohol, respectively, to cover the range of low Km values while allowing for the possibility of the peak going back to the base area and not cutting at 0. Otherwise, the volume would not be calculated correctly. However, the estimated parameters remain similar, if the axis is set to 0 – 195 mM (25 grid points). The volume of the peak was calculated by means of the trapz function. In all cases $5 \cdot 10^5$ iterations were done.

## Figures




(**a**) (**b**)

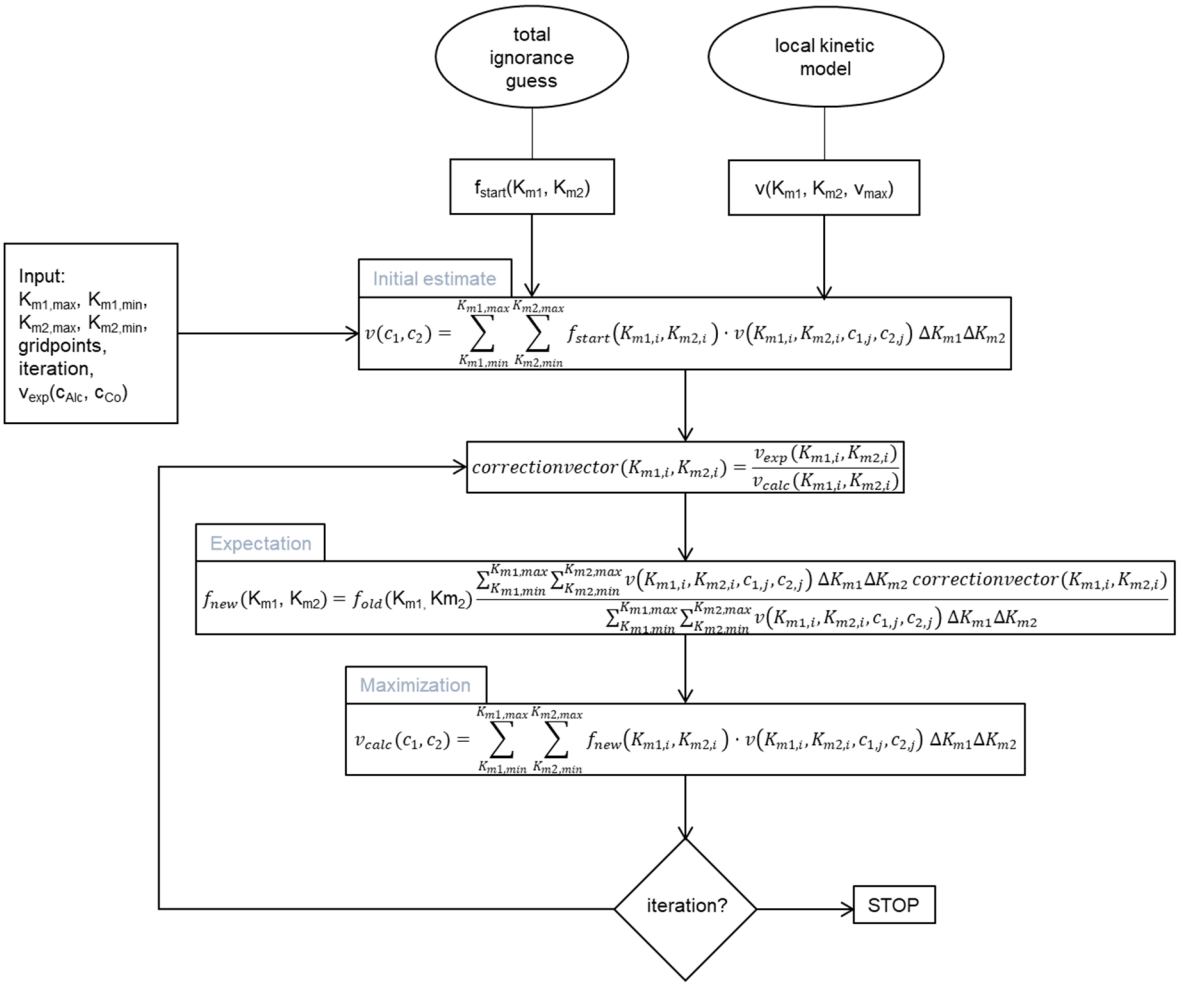
**Figure S1.** AED analysis of ADH with (**a**) substrate 2-butanol and cofactor NAD at ambient temperature in 100 mM phosphate buffer (pH 8.8) and (**b**) substrate propanol and cofactor NAD at ambient temperature in 100 mM phosphate buffer (pH 8.8).




(**a**) (**b**)

**Figure S2.** AED analysis of ADH with substrate propanol/2-butanol and cofactor NAD at ambient temperature in 100 mM phosphate buffer (pH 8.8) (**a**) 50/50 mol content (**b**) 25/75 mol content.

## Schemes




**Scheme S1:** Schematic representation of AED algorithm

## Tables

Table S1: Numerical data of initial slope experiments for ADH with 2-butanol or propanol as substrate at different substrate concentrations at ambient temperature in 100 mM phosphte buffer (pH 8.8)

| NAD [mM] | Alcohol [mM] | v with 2-butanol [mol $s^{-1}$ $g_{Enzym}^{-1}$] | S.D. [%] | v with propanol [mol $s^{-1}$ $g_{Enzym}^{-1}$] | S.D. [%] |
|---|---|---|---|---|---|
| 1.1 | 5 | 3.76E-09 | 15.42 | 1.70E-06 | 18.66 |
| 0.7 | 25 | 1.55E-08 | 44.32 | 3.08E-06 | 26.16 |
| 1.9 | 45 | 3.75E-08 | 22.70 | 4.90E-06 | 12.33 |
| 1.7 | 65 | 6.76E-08 | 10.10 | 5.26E-06 | 12.38 |
| 0.1 | 85 | 8.11E-09 | 13.58 | 1.06E-06 | 11.71 |
| 0.5 | 105 | 3.72E-08 | 11.58 | 3.51E-06 | 14.39 |
| 0.9 | 125 | 7.02E-08 | 6.55 | 4.40E-06 | 8.52 |
| 0.3 | 145 | 2.99E-08 | 3.14 | 2.54E-06 | 8.81 |
| 1.5 | 165 | 8.61E-08 | 16.73 | 5.34E-06 | 7.75 |
| 1.3 | 185 | 8.49E-08 | 10.12 | 5.34E-06 | 0.18 |
| 1.5 | 285 | 6.48E-08 | 11.11 | | |
| 1.5 | 225 | 6.11E-08 | 2.18 | | |
| 1.5 | 175 | 5.96E-08 | 11.78 | | |

| | | | |
|---|---|---|---|
| 1.5 | 100 | 4.98E-08 | 10.53 |
| 1.5 | 75 | 3.15E-08 | 21.48 |
| 1.5 | 40 | 2.58E-08 | 28.50 |
| 1.5 | 20 | 1.04E-08 | 48.72 |
| 1.5 | 10 | 6.80E-09 | 58.30 |
| 1.5 | 5 | 4.95E-09 | 17.03 |

Table S2: Numerical data of initial slop experiments for ADH with propanol/2-butanol mixture at different substrate concentrations at ambient temperature in 100 mM phosphate buffer (pH 8.8)

| **NAD [mM]** | **Sum Alcohol [mM]** | **v with mixture of propanol/2-butanol [mol $s^{-1}$ $g_{Enzym}^{-1}$]** | | | |
|---|---|---|---|---|---|
| | | **50/50 [mol content]** | **S.D. [%]** | **25/75 [mol content]** | **S.D. [%]** |
| 1.2 | 5 | 5.17E-07 | 35.04 | 2.40E-07 | 12.66 |
| 0.3 | 15 | 2.91E-07 | 55.34 | 2.56E-07 | 35.75 |
| 0.6 | 25 | 9.19E-07 | 4.26 | N.N. | N.N. |
| 1.0 | 35 | 1.69E-06 | 8.51 | 9.26E-07 | 18.03 |
| 1.8 | 45 | 2.14E-06 | 9.32 | 1.19E-06 | 6.38 |
| 0.8 | 55 | 1.65E-06 | 10.96 | 1.06E-06 | 2.94 |
| 0.5 | 65 | 1.41E-06 | 7.19 | 8.08E-07 | 17.18 |
| 2.0 | 75 | 2.66E-06 | 3.59 | 1.60E-06 | 2.98 |
| 0.1 | 85 | 3.40E-07 | 40.56 | N.N. | N.N. |
| 1.4 | 95 | 2.54E-06 | 1.05 | 1.55E-06 | 10.81 |
| 0.4 | 105 | 1.36E-06 | 10.12 | 7.91E-07 | 4.36 |
| 1.6 | 115 | 2.88E-06 | 3.48 | 1.79E-06 | 1.04 |
| 0.9 | 125 | 2.53E-06 | 10.69 | 1.53E-06 | 2.89 |
| 0.7 | 135 | 2.15E-06 | 0.86 | 1.30E-06 | 0.46 |
| 0.2 | 145 | 5.25E-07 | 45.10 | 5.48E-07 | 10.29 |
| 1.9 | 155 | 9.95E-07 | 7.20 | 2.04E-06 | 6.27 |
| 1.5 | 165 | 2.75E-06 | 8.69 | 1.86E-06 | 2.29 |
| 1.7 | 175 | 3.02E-06 | 7.13 | 1.97E-06 | 4.78 |
| 1.3 | 185 | 3.21E-06 | 7.33 | 1.82E-06 | 5.16 |
| 1.1 | 195 | 2.87E-06 | 10.41 | 1.70E-06 | 3.10 |

Table S3: Parameters obtained from NLR with different initial values for single substrate kinetics

| **Alcohol** | **Parameter** | **Initial value** | **NRL** | | |
|---|---|---|---|---|---|
| | | | **value** | **S.E. [%]** | **SSE** |
| 2-butanol | $v_{max}$ [mol $s^{-1}$ $g^{-1}$] | 8.61E-08* | 1.02E-07 | 76.50 | 2.5885 |
| | $K_{m,alc}$ [mM] | 1 | 1.02 | 431.72 | |
| | $K_{m,NAD}$ [mM] | 1 | 0.93 | 172.20 | |
| | $v_{max}$ [mol $s^{-1}$ $g^{-1}$] | 8.61E-08* | 2.08E-07 | 28.14 | 0.3060 |
| | $K_{m,alc}$ [mM] | 93** | 93 | 46.50 | |
| | $K_{m,NAD}$ [mM] | 0.81** | 0.65 | 48.17 | |
| | $v_{max}$ [mol $s^{-1}$ $g^{-1}$] | 3.06E-07*** | 3.07E-07 | 32.47 | 0.1652 |
| | $K_{m,alc}$ [mM] | 139.48*** | 139.48 | 39.67 | |
| | $K_{m,NAD}$ [mM] | 1.25*** | 1.23 | 45.32 | |
| propanol | $v_{max}$ [mol $s^{-1}$ $g^{-1}$] | 5.34E-06* | 8.37E-06 | 31.03 | 0.4421 |

| | | | | | |
|---|---|---|---|---|---|
| | $K_{m,alc}$ [mM] | 1 | 1 | 174.37 | |
| | $K_{m,NAD}$ [mM] | 1 | 0.89 | 71.28 | |
| | $v_{max}$ [mol $s^{-1}$ $g^{-1}$] | 5.34E-06* | 8.50E-06 | 12.50 | |
| | $K_{m,alc}$ [mM] | 5.7** | 5.7 | 38.32 | 0.1034 |
| | $K_{m,NAD}$ [mM] | 0.81** | 0.70 | 38.33 | |
| | $v_{max}$ [mol $s^{-1}$ $g^{-1}$] | 8.00E-06*** | 8.00E-06 | 2.90 | |
| | $K_{m,alc}$ [mM] | 11.35*** | 11.35 | 8.76 | 0.0056 |
| | $K_{m,NAD}$ [mM] | 0.55*** | 0.55 | 7.55 | |

* highest overserved experimental reaction rate

** literature values, see main text

*** AED values, see table 1

Table S4: Parameters for NLR estimation with different initial values for mixture substrate kinetics for a mixture of propanol/2-butanol

| Peak | Parameter | Initial value | 50/50 | | 25/75 | |
|---|---|---|---|---|---|---|
| | | | value | S.E. [%] | value | S.E. [%] |
| 1 | $v_{max}$ [mol $s^{-1}$ $g^{-1}$] | NLR values, see Table 1 | 2.51E-06 | 4E5 | 1.17E-5 | 1362 |
| | $K_{m,alc}$ [mM] | | 4.20 | 2E6 | 3.11 | 2796 |
| | $K_{m,NAD}$ [mM] | | 4.98 | 2E6 | 6.08 | 3007 |
| 2 | $v_{max}$ [mol $s^{-1}$ $g^{-1}$] | NLR values, see Table 1 | 5.60E-06 | 2E5 | 0 | ∞ |
| | $K_{m,alc}$ [mM] | | 1.92 | 8E5 | 1.32 | 3E25 |
| | $K_{m,NAD}$ [mM] | | 2.75 | 4E5 | 3.15 | 1E25 |
| | SSE | | 2.6146 | | 3.7487 | |
| 1 | $v_{max}$ [mol $s^{-1}$ $g^{-1}$] | AED values, see Table 2 | 3.35E-06 | 1290 | 3.32E-06 | 109 |
| | $K_{m,alc}$ [mM] | | 28.44 | 1288 | 62.60 | 144 |
| | $K_{m,NAD}$ [mM] | | 0.75 | 747 | 0.82 | 73 |
| 2 | $v_{max}$ [mol $s^{-1}$ $g^{-1}$] | AED values, see Table 2 | 1.354E-06 | 2992 | 5.35E-07 | 730 |
| | $K_{m,alc}$ [mM] | | 2.81 | 4633 | 11.35 | 538 |
| | $K_{m,NAD}$ [mM] | | 1.25 | 970 | 0.65 | 310 |
| | SSE | | 1.4954 | | 0.0235 | |