Supplementary data

What makes a lipase a valuable acyltransferase in water abundant medium?

M. Subileau *, A. H. Jan, J. Drone, C. Rutyna, V. Perrier E. Dubreucq

Equations in relation with scheme 2

The experiments conducted with various methanol concentration (R'OH in scheme 2) allowed the experimental determination of the initial transfer rate r_t. From the linear set of equations an expression for r_t as a function of the reactant concentrations ([R'OH], [EH], [AOR], [H_2O]) and the rate constants can be derived as described below.

First, according to scheme 2, the transfer rate is defined by:

$$r_t = k_t \,.\, [EA\cdots R'OH] \qquad \text{(S1)}$$

We assume that (i) substrate concentrations >> enzymes concentration, (ii) only the initial rates are measured, (iii) steady-state in the concentration of covalent and non-covalent intermediaries is obtained and (iv) reverse reactions from products can be neglected. This leads to the following equations:

$$\frac{d[EA\cdots R'OH]}{dt} = k_3 \,.\, [EA].\,[R'OH] - \left(k_{-3} + k_t + k_{h,R'OH} \,.\, [H_2O]\right).[EA\cdots R'OH] = 0$$

Thus,

$$[EA\cdots R'OH] = \frac{k_3 \,.\, [EA].\,[R'OH]}{k_{-3} + k_t + k_{h,R'OH} \,.\, [H_2O]} = K_{R'OH} \,.\, [EA].\,[R'OH] \qquad \text{(S2)}$$

With

$$K_{R'OH} = \frac{k_3}{k_{-3} + k_t + k_{h,R'OH} \,.\, [H_2O]}$$

At low [R'OH] and high [H_2O], [H_2O] can be considered constant and $K_{R'OH}$ is indeed a constant.

At high [R'OH] and low [H_2O], the reaction of hydrolysis from the intermediary EA…R'OH can be neglected, and $K_{R'OH}$ is constant and simplified as:

$$K_{R'OH} = \frac{k_3}{k_{-3} + k_t}$$

So equation (S1) becomes:

$$r_t = k_t \,.K_{R'OH} \,.\, [EA].\,[R'OH] \tag{S3}$$

Then,

$$\frac{d[EA]}{dt} = k_{-3} \,.\, [EA\cdots R'OH] + k_2 \,.\, [EH\cdots AOR] - (k_3 \,.\, [EA].[R'OH] + k_h \,.\, [EA].[H_2O]) = 0 \tag{S4}$$

and

$$\frac{d[EH\cdots AOR]}{dt} = k_1 \,.\, [EH] \,.\, [AOR] - (k_2 + k_{-1}) \,.\, [EH\cdots AOR] = 0 \tag{S5}$$

So,

$$[EH\cdots AOR] = \frac{k_1}{k_2 + k_{-1}} \,.\, [EH].[AOR] = K_{AOR} \,.\, [EH].[AOR] \tag{S6}$$

With

$$K_{AOR} = \frac{k_1}{k_2 + k_{-1}}$$

Using equations (S2), (S4) and (S6) we obtain:

$$[EA] = \frac{k_2 \,.[EH].[AOR].\, K_{AOR}}{[R'OH].\,(k_3 - k_{-3}.K_{R'OH}) + k_h \,.[H_2O]} \tag{S7}$$

Thus the transfer rate (S3) can be expressed as:

$$r_t = \frac{k_t \,.K_{R'OH} \,.\, k_2 \,.\, [EH].[AOR].\, K_{AOR}.\ [R'OH]}{[R'OH].\,(k_3 - k_{-3}.K_{R'OH}) + k_h \,.[H_2O]} \tag{S8}$$

For each experiment, in initial conditions, [R'OH], [AOR] and $[H_2O]$ can be considered constant, $[R'OH]=[R'OH]_0$, $[AOR]=[AOR]_0$ and $[H_2O]=[H_2O]_0$, subscript 0 for *t*=0) and only $[R'OH]_0$ and $[H_2O]_0$ vary between our experiments.

The free enzyme concentration [EH] cannot be considered constant because $[EH]_0 \ll [R'OH]_0$, $[AOR]_0$ and $[H_2O]_0$, and is defined by the equation (S9):

$$[EH] = [EH]_0 - [EH\cdots AOR] - [EA] - [EA\cdots R'OH] \tag{S9}$$

Using equations (S2), (S6) and (S7), equation (S9) can be modified as follows:

$$[EH] = [EH]_0 - K_{AOR} \cdot [EH] \cdot [AOR]_0 \left(\frac{[R'OH]_0 \cdot \left(k_3 + (k_2 - k_{-3}) \cdot K_{R'OH}\right) + k_h \cdot [H_2O]_0 + k_2}{[R'OH]_0 \cdot \left(k_3 - k_{-3} \cdot K_{R'OH}\right) + k_h \cdot [H_2O]_0} \right) \quad \text{(S10)}$$

So,

$$[EH] = \frac{[EH]_0 \cdot \left([R'OH]_0 \cdot \left(k_3 - k_{-3} \cdot K_{R'OH}\right) + k_h \cdot [H_2O]_0\right)}{[R'OH]_0 \cdot (k_3 - k_{-3} \cdot K_{R'OH} + K_{AOR} \cdot [AOR]_0 \cdot \left(k_3 + (k_2 - k_{-3}) \cdot K_{R'OH}\right)) + k_h \cdot [H_2O]_0 \cdot (1 + \quad \text{(S11)}$$

Then we replace [EH] in equation (S8) by its expression in equation (S11) :

$$r_t = \frac{k_t \cdot K_{R'OH} \cdot k_2 \cdot [EH]_0 \cdot [AOR]_0 \cdot K_{AOR} \cdot [R'OH]_0}{[R'OH]_0 \cdot (k_3 - k_{-3} \cdot K_{R'OH} + K_{AOR} \cdot [AOR]_0 \cdot \left(k_3 + (k_2 - k_{-3}) \cdot K_{R'OH}\right)) + k_h \cdot [H_2O]_0 \cdot (1 + \quad \text{(S12)}$$

After development and factorization, the following equation is obtained for the transfer rate:

$$r_t = \frac{k_{cat-t} \cdot [EH]_0 \cdot [R'OH]_0}{[R'OH]_0 + K_{M,\,R'OH}\left(1 + \frac{[H_2O]_0}{K_I}\right)} \quad \text{(S13)}$$

With the apparent kinetic constants:

$$k_{cat-t} = \frac{k_t \cdot K_{R'OH} \cdot k_2 \cdot [AOR]_0 \cdot K_{AOR}}{k_3 - k_{-3} \cdot K_{R'OH} + K_{AOR} \cdot [AOR]_0 \cdot \left(k_3 + (k_2 - k_{-3}) \cdot K_{R'OH}\right)} \quad \text{(S14)}$$

$$r_{t\,max}^{*} = (k_{cat-t})_{app} \cdot [EH]_0 \quad \text{(S15)}$$

$$K_{M,\,R'OH} = \frac{k_2 \cdot K_{AOR} \cdot [AOR]_0}{k_3 - k_{-3} \cdot K_{R'OH} + K_{AOR} \cdot [AOR]_0 \cdot \left(k_3 + (k_2 - k_{-3}) \cdot K_{R'OH}\right)} \quad \text{(S16)}$$

$$K_I = \frac{k_2 \cdot K_{AOR} \cdot [AOR]_0}{k_h \cdot (1 + K_{AOR} \cdot [AOR]_0)} \quad \text{(S17)}$$

and H_2O acting as a competitive inhibitor to the transfer reaction.

In our model reaction, $[H_2O]_0$ varies only in function of $[MeOH]_0$ (R'OH) and the increase of [MeOH] leads to a decrease of a_W (see equation (5)). The effect of methanol on enzyme activity and structure can thus be correlated to a_W. In the equation (13), a multiplication factor α, comprising water activity (a_W) in the medium and the minimal water activity ($a_{W\,min}$) from which the loss of enzyme activity is total (enzyme dependant), was applied to $r_t{}^*_{max}$:

$$r_t{}^*_{max} = r_{t\,max}.\alpha \qquad \text{with} \qquad \alpha = (a_W - a_{W\,min})/(1 - a_{W\,min}).$$

α varies between 0 (when $a_W=a_{W\,min}$) and 1 (when $a_W=1$ in the absence of MeOH), so that the activity is equal to 0 when $a_W=a_W$ min. Thus the equation we used to determine the kinetic parameters was the following (based on equation (13)):

$$r_t = \frac{r_{t\,max}.\alpha.[MeOH]}{[MeOH] + K_{M,MeOH}\left(1 + \frac{[H_2O]_0}{K_I}\right)} \qquad \text{(S18)}$$

In our conditions (methanol used as nucleophile and ethyl oleate as substrate), this model fitted most experimental data and determination of the kinetic constants $K_{M\,MeOH}$, K_I and $r_{t\,max}$ showed that K_I was sufficiently high to neglect the competitive inhibitory effect of water, so equation (18) could be simplified as follows:

$$r_t = \frac{r_{t\,max}.\alpha.[MeOH]}{[MeOH] + K_{M,MeOH}} \qquad \text{(S19)}$$

It can be noted that the effect of water activity decrease (correlated to methanol increase) encompassed in the α factor might be related to an apparent non-competitive inhibition.

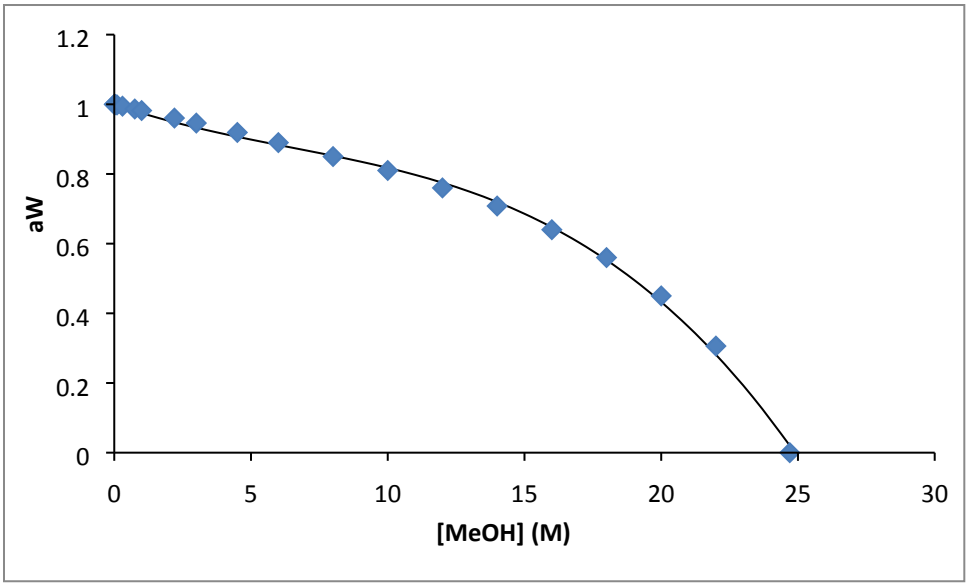
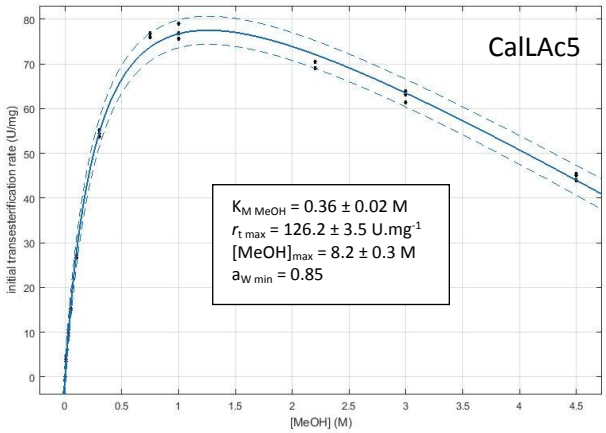
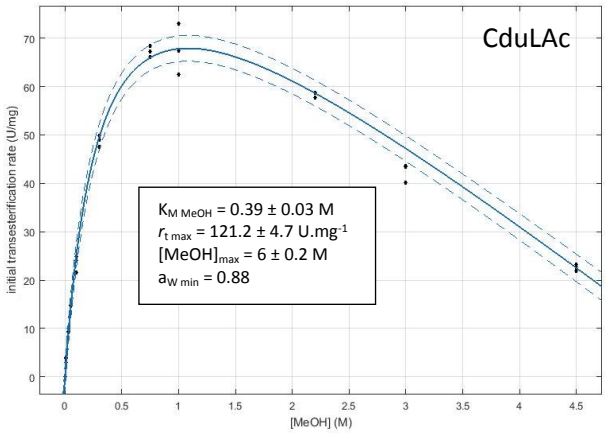
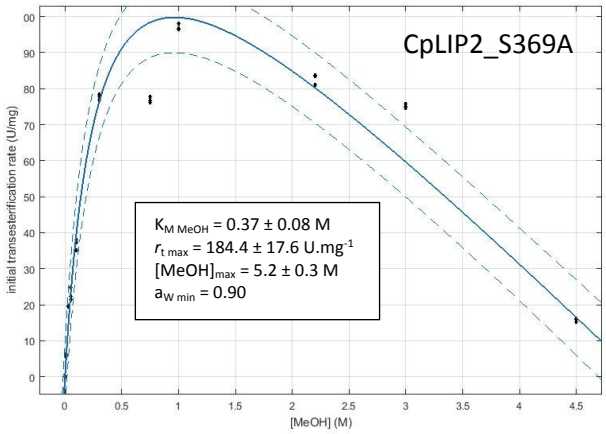
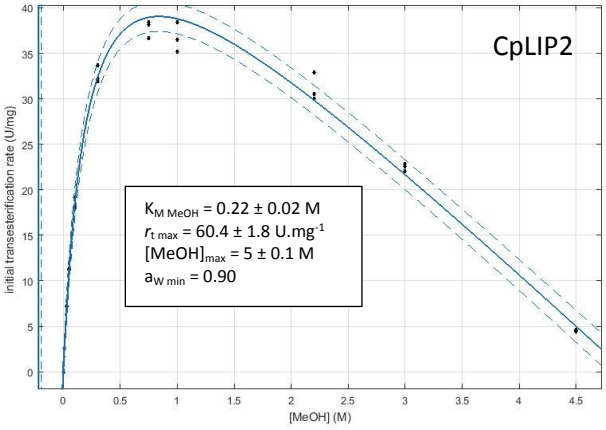


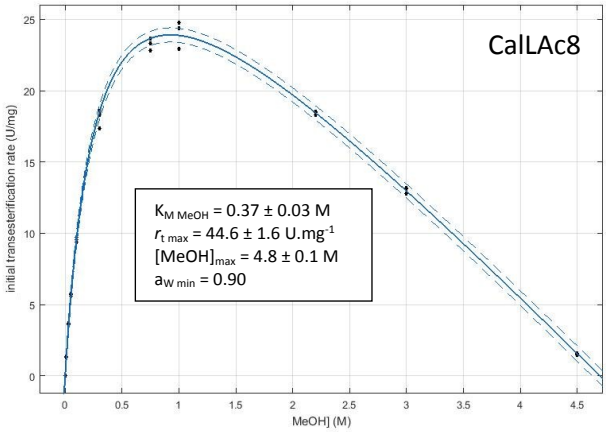
Figure S1 Determination of the correlation (in red) between the thermodynamic activity of water (y) and the methanol concentration (x) in the reaction medium consisting of 10 mM ethyl oleate as a substrate in PVA emulsion, and 900 µL of 50 mM sodium phosphate buffer pH 6.5 with various methanol concentrations at 30°C. Calculations of a_W values were made with the UNIFAC group contribution method (LLE parameters).

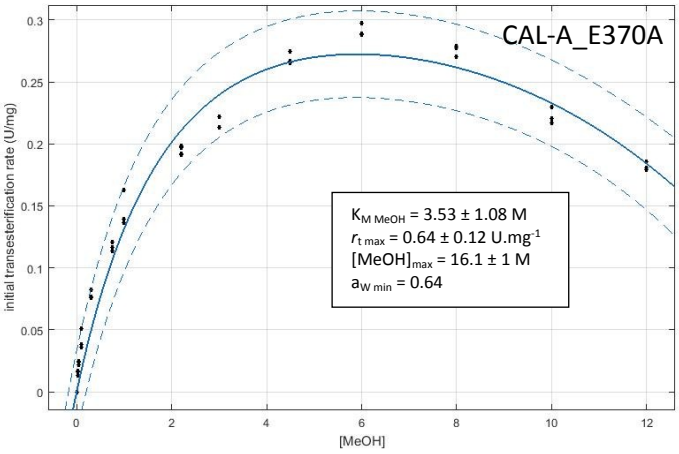

CalLAc5
$K_{M\ MeOH}$ = 0.36 ± 0.02 M
$r_{t\ max}$ = 126.2 ± 3.5 U.mg^{-1}
$[MeOH]_{max}$ = 8.2 ± 0.3 M
$a_{W\ min}$ = 0.85
initial transesterification rate (U/mg)
[MeOH] (M)

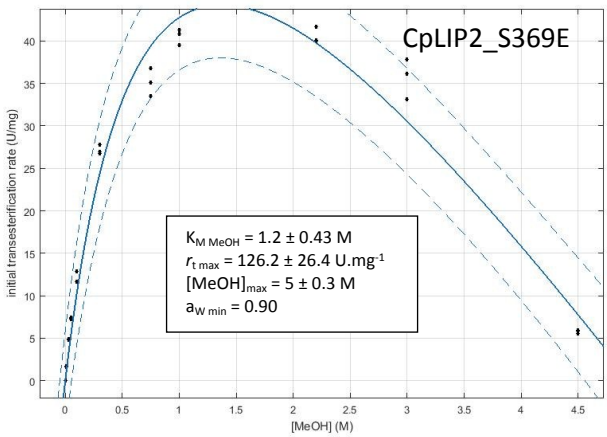

CduLAc
$K_{M\ MeOH}$ = 0.39 ± 0.03 M
$r_{t\ max}$ = 121.2 ± 4.7 U.mg^{-1}
$[MeOH]_{max}$ = 6 ± 0.2 M
$a_{W\ min}$ = 0.88
initial transesterification rate (U/mg)
[MeOH] (M)

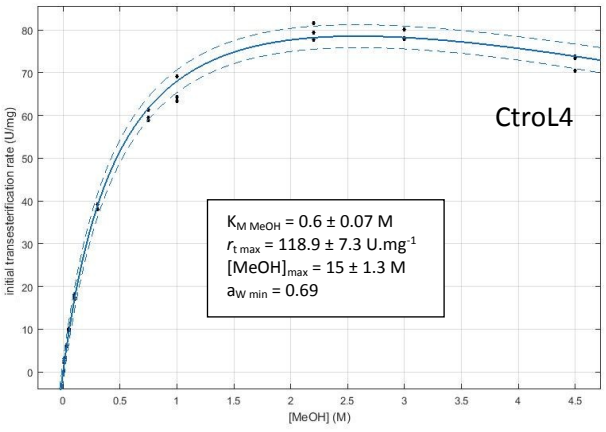

CpLIP2_S369A
$K_{M\ MeOH}$ = 0.37 ± 0.08 M
$r_{t\ max}$ = 184.4 ± 17.6 U.mg^{-1}
$[MeOH]_{max}$ = 5.2 ± 0.3 M
$a_{W\ min}$ = 0.90
initial transesterification rate (U/mg)
[MeOH] (M)

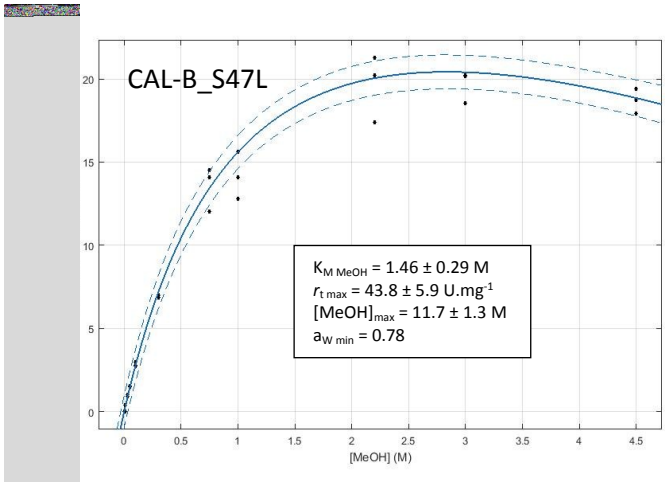

CpLIP2
$K_{M\ MeOH}$ = 0.22 ± 0.02 M
$r_{t\ max}$ = 60.4 ± 1.8 U.mg^{-1}
$[MeOH]_{max}$ = 5 ± 0.1 M
$a_{W\ min}$ = 0.90
initial transesterification rate (U/mg)
[MeOH] (M)

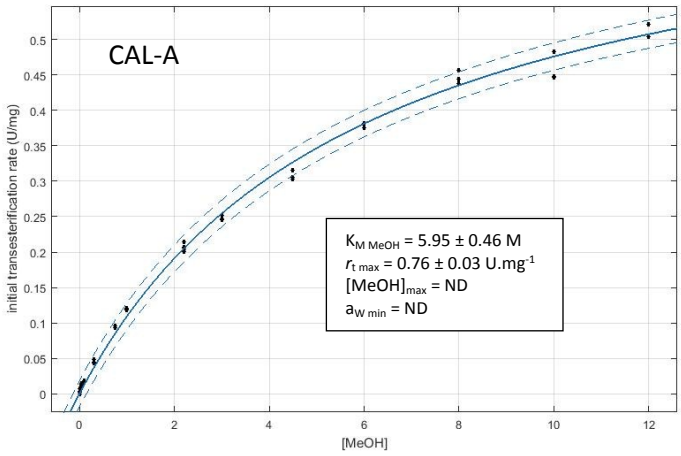

CalLAc8
$K_{M\ MeOH}$ = 0.37 ± 0.03 M
$r_{t\ max}$ = 44.6 ± 1.6 U.mg^{-1}
$[MeOH]_{max}$ = 4.8 ± 0.1 M
$a_{W\ min}$ = 0.90
initial transesterification rate (U/mg)
MeOH] (M)

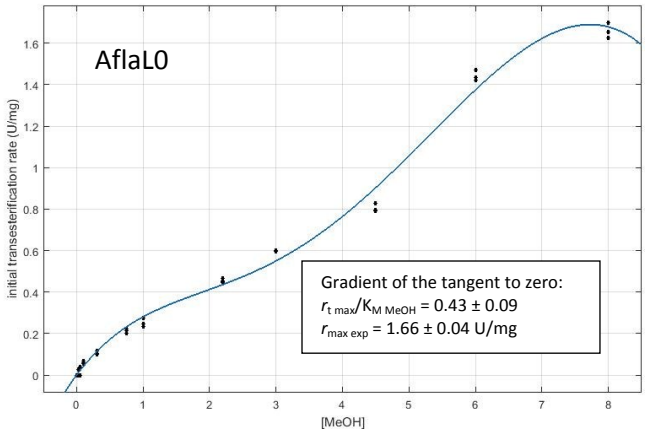

CAL-A_E370A
$K_{M\ MeOH}$ = 3.53 ± 1.08 M
$r_{t\ max}$ = 0.64 ± 0.12 U.mg^{-1}
$[MeOH]_{max}$ = 16.1 ± 1 M
$a_{W\ min}$ = 0.64
initial transesterification rate (U/mg)
[MeOH]

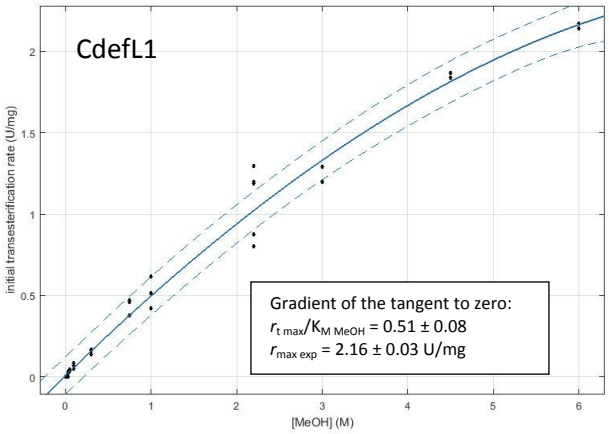

CpLIP2_S369E
$K_{M\ MeOH}$ = 1.2 ± 0.43 M
$r_{t\ max}$ = 126.2 ± 26.4 U.mg^{-1}
$[MeOH]_{max}$ = 5 ± 0.3 M
$a_{W\ min}$ = 0.90
initial transesterification rate (U/mg)
[MeOH] (M)


CtroL4
$K_{M\ MeOH}$ = 0.6 ± 0.07 M
$r_{t\ max}$ = 118.9 ± 7.3 U.mg^{-1}
$[MeOH]_{max}$ = 15 ± 1.3 M
$a_{W\ min}$ = 0.69
initial transesterification rate (U/mg)
[MeOH] (M)









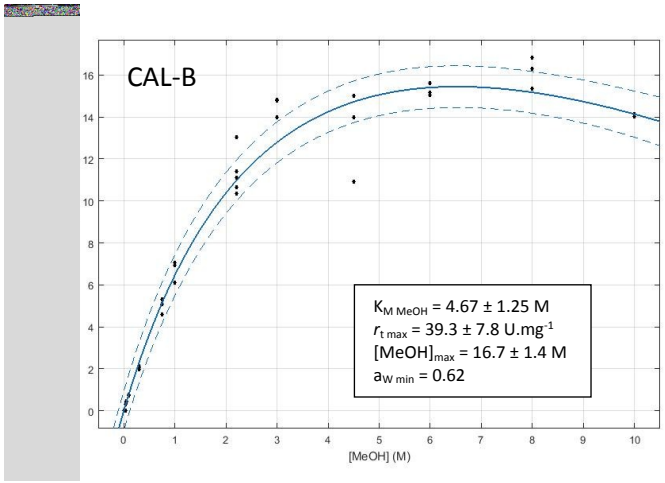


Figure S2 Graphical determination of the apparent kinetic constants $r_{t\ max}$ and $K_{M\ MeOH}$ and of the $a_{W\ min}$ using the kinetic model described by the following equation:

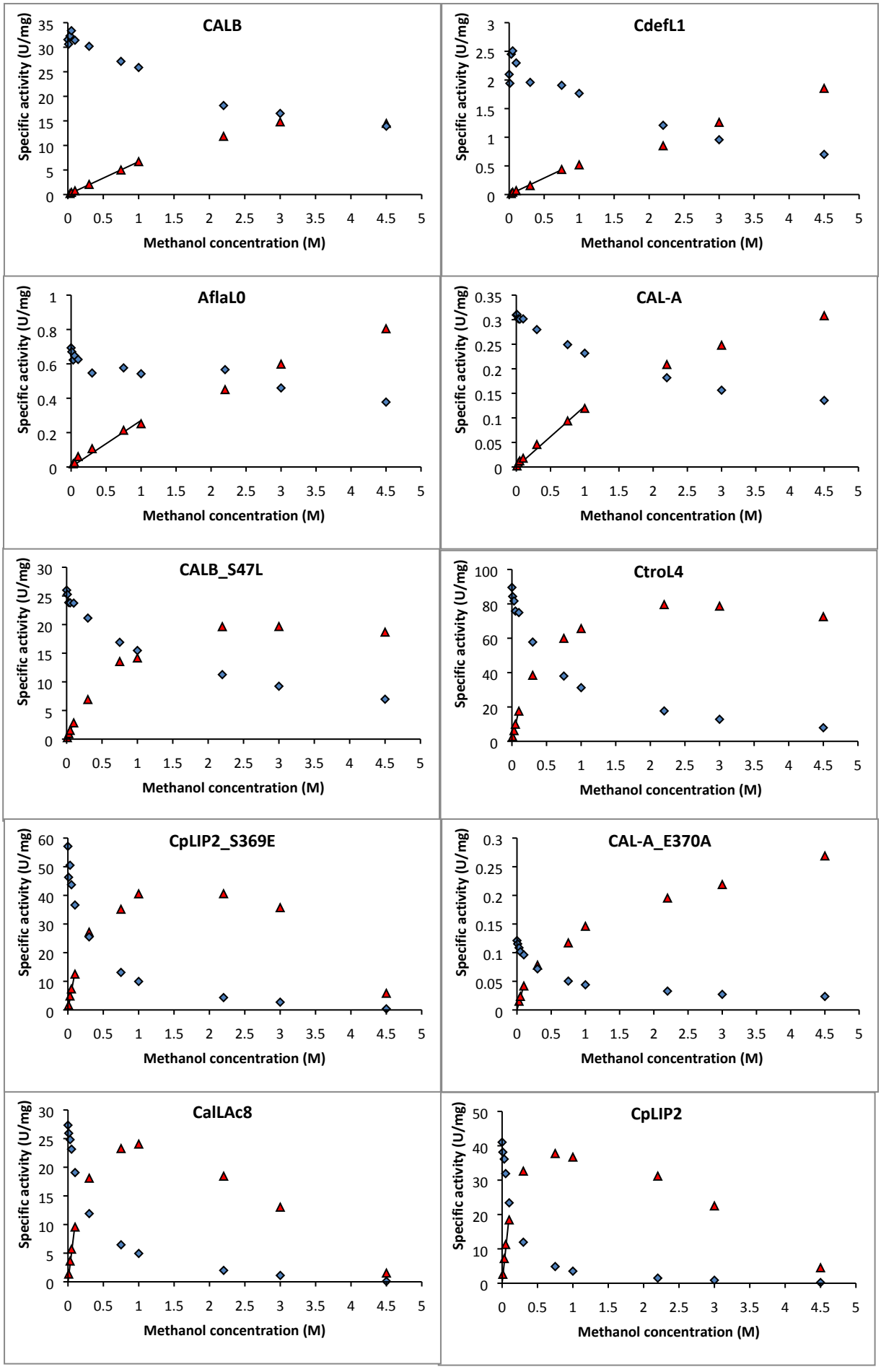
$$r_t = \frac{r_{t\,max} \cdot \frac{a_W - a_{W\,min}}{1 - a_{W\,min}} \cdot [MeOH]}{K_{M\,MeOH} + [MeOH]} \quad (6)$$

where a_w is defined by the equation (5)

$$a_W = 1 - 9.37.10^{-5}[MeOH]^3 + 1.79.10^{-3}[MeOH]^2 - 2.67.10^{-2} \quad (5)$$

For CdefL1 and AflaL0, we determined the catalytic efficiency $k_{cat\ t}/K_{M\ MeOH}$, by calculating the gradient of the tangent in 0 to the curve representing the initial transfer rate as a function of the methanol concentration. Then, we assumed that the $r_{t\ max}$ was at least the maximal experimental r_t. For these two enzymes we could only obtain the lower possible values of $r_{t\ max}$ and $K_{M\ MeOH}$.

The dotted lines represent the prediction bounds with 95% confidence.


CALB
Specific activity (U/mg)
Methanol concentration (M)
CdefL1
Specific activity (U/mg)
Methanol concentration (M)
AflaL0
Specific activity (U/mg)
Methanol concentration (M)
CAL-A
Specific activity (U/mg)
Methanol concentration (M)
CALB_S47L
Specific activity (U/mg)
Methanol concentration (M)
CtroL4
Specific activity (U/mg)
Methanol concentration (M)
CpLIP2_S369E
Specific activity (U/mg)
Methanol concentration (M)
CAL-A_E370A
Specific activity (U/mg)
Methanol concentration (M)
CalLAc8
Specific activity (U/mg)
Methanol concentration (M)
CpLIP2
Specific activity (U/mg)
Methanol concentration (M)

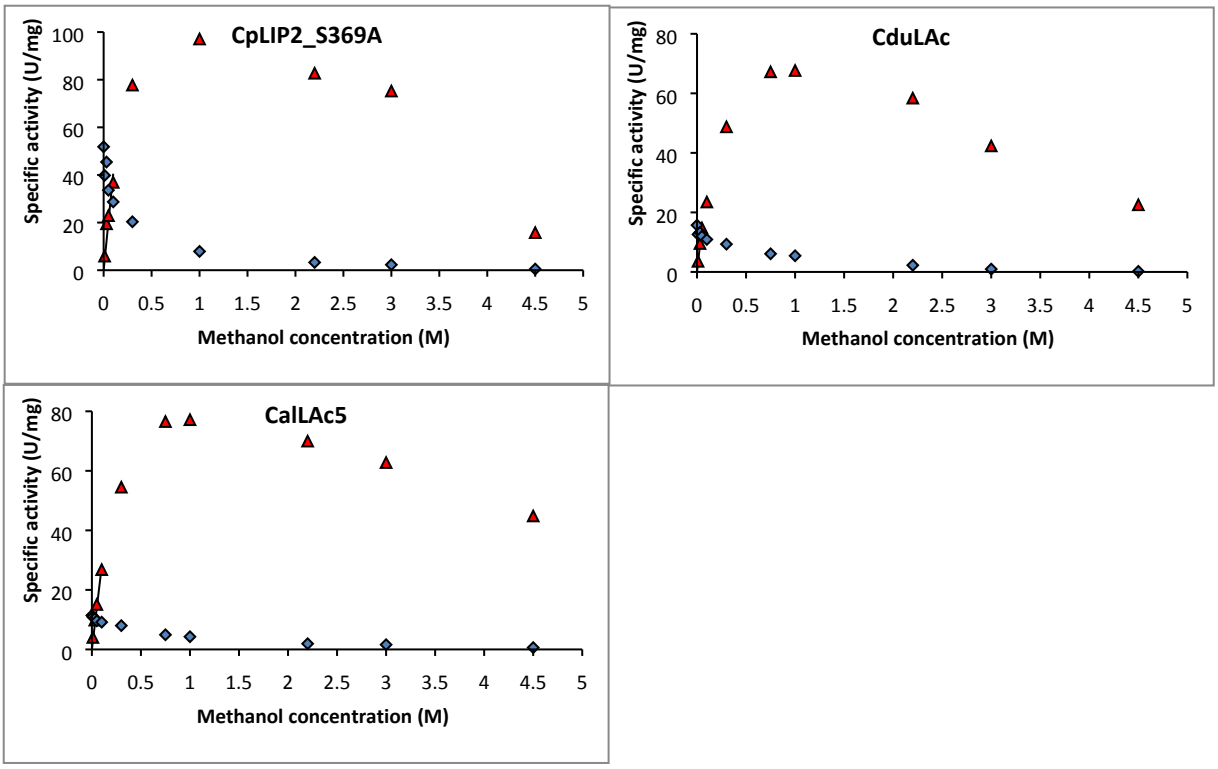
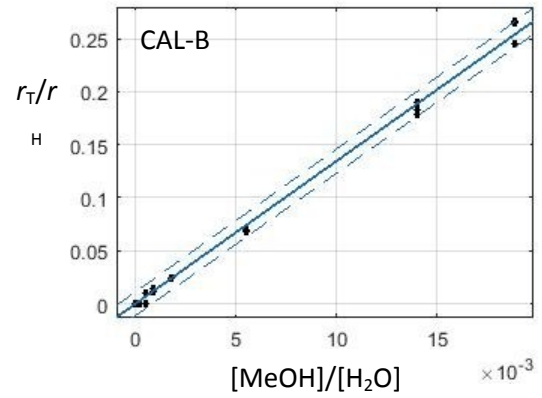
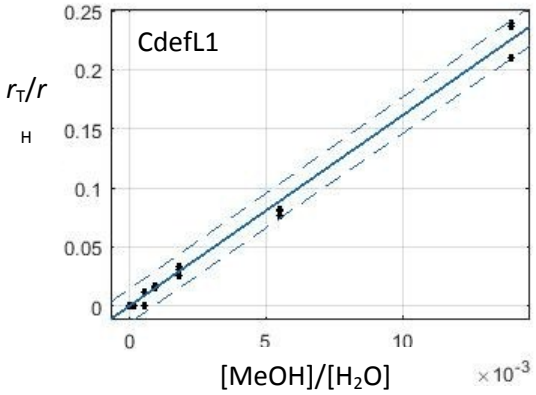
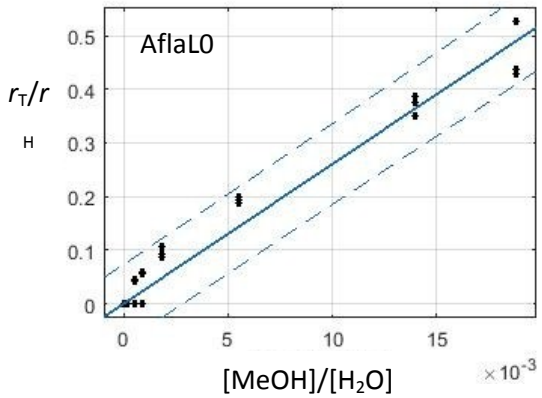
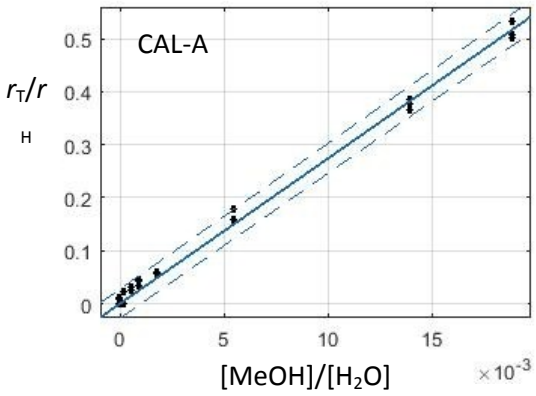


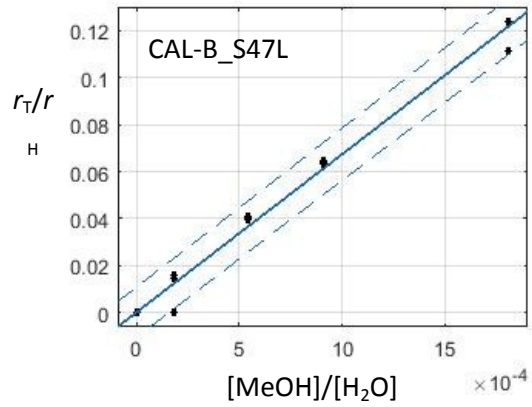
Figure S3 Experimental determination of the methanol concentration range where transfer rate is proportional to methanol concentration. Specific activities of hydrolysis (blue dots) and alcoholysis (red dots) were measured at various methanol concentrations. The reaction is performed at 30°C and pH 6.5, during 15 minutes, using 10 mM ethyl oleate in PVA emulsion as a substrate.

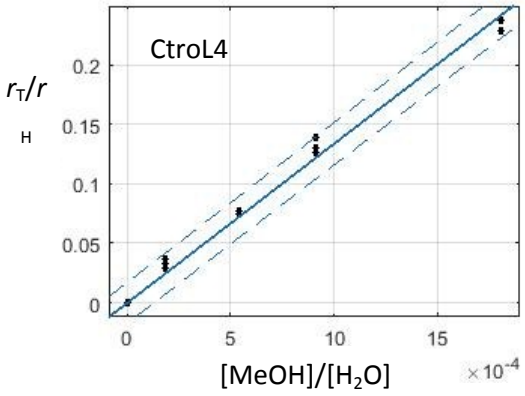

CAL-B
r_T/r_H
[MeOH]/[H2O]
×10-3

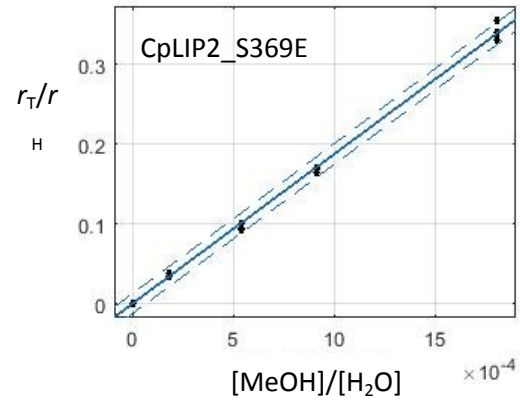

CdefL1
r_T/r_H
[MeOH]/[H2O]
×10-3

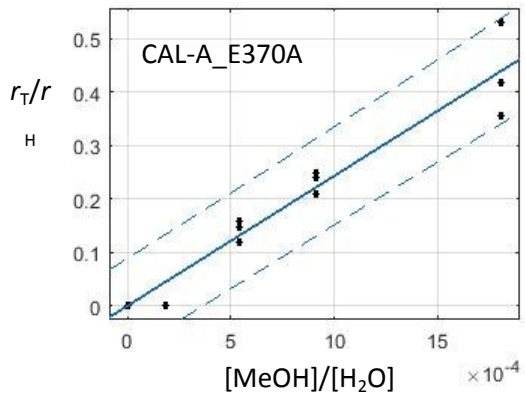

AflaL0
r_T/r_H
[MeOH]/[H2O]
×10-3


CAL-A
r_T/r_H
[MeOH]/[H2O]
×10-3


CAL-B_S47L
r_T/r_H
[MeOH]/[H2O]
×10-4


CtroL4
r_T/r_H
[MeOH]/[H2O]
×10-4


CpLIP2_S369E
r_T/r_H
[MeOH]/[H2O]
×10-4


CAL-A_E370A
r_T/r_H
[MeOH]/[H2O]
×10-4

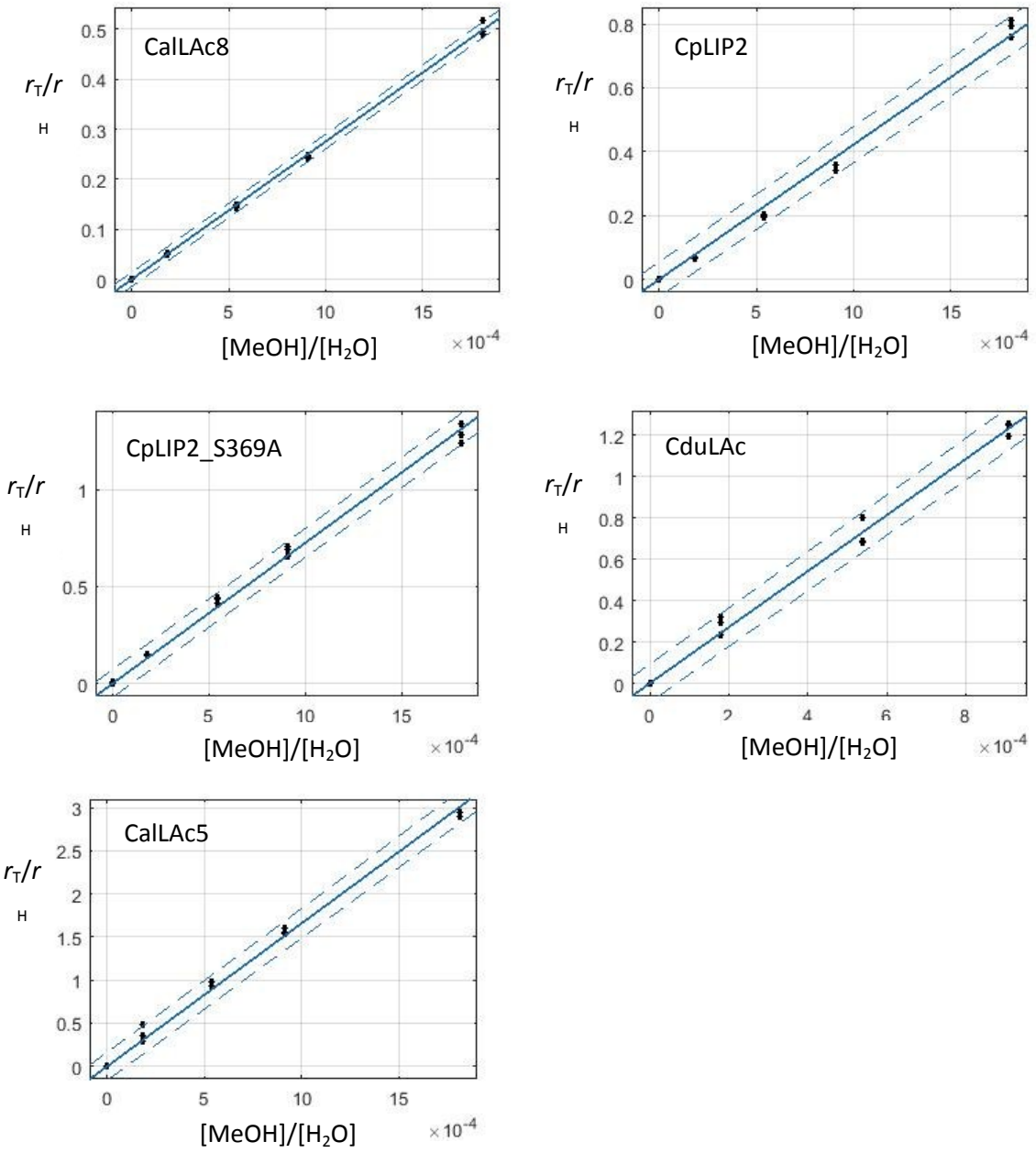


Figure S4 Graphical determination of the $(\frac{k_t}{k_h})_{app}$ as the slope of the initial rates ratio $\frac{r_t}{r_h}$ as a function of the ratio of methanol versus water concentrations $\frac{[MeOH]}{[H_2O]}$ using the methanol concentration range determined in figure 2. The dotted lines represent the prediction bounds with 95% confidence.