Electronic supporting information

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Analysis of DNA-CalB

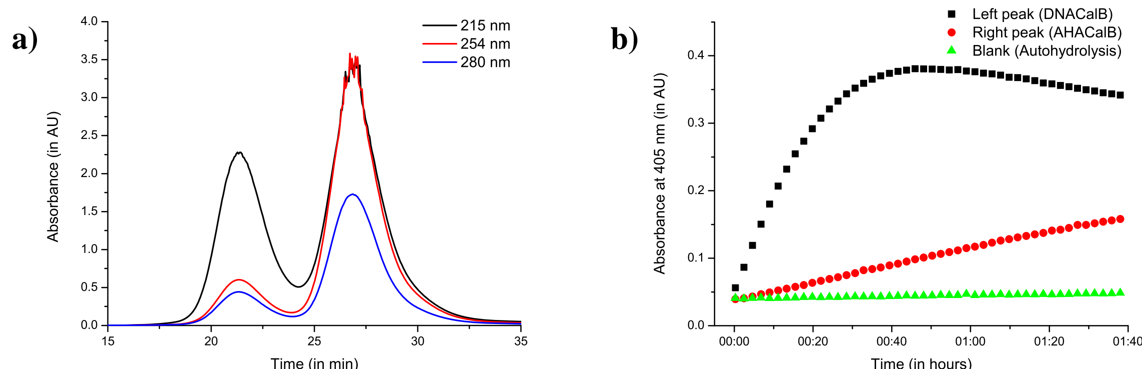

Figure S1. a) FPLC trace after purification of the copper catalysed reaction mixture of the conjugation of azido-functional CAlB to ss-DNA The left peak represents the DNA-CalB and the right peak the non-reacted AHA-CalB. **b)** The enzyme activity of each fraction after FPLC purification

As can be seen from the FPLC traces (Fig S1), the areas of the peaks do not correspond with the expected conversion of the click-reaction from the SDS-PAGE gels (Fig. 4a-c in article). For confirmation a sample was taken from each peak. The silver stained SDS-PAGE gel, Fig. 4c, shows that the left peak in Figure S1a is indeed the expected DNA-CalB and the right peak is confirmed to be the non-reacted AHA-CalB. Similar volumes at the maximum of each peak were taken and the enzyme activity was compared. As can be clearly seen in Figure S1b, the left peak contains more enzyme than the right peak, which is in accordance with the SDS-PAGE gel analysis. The high peak area of the right peak is therefore accredited to YodA (a stress protein of ~ 22 kDa, which cannot be separated from the AHA-CalB by FPLC), which is also present on the SDS-PAGE gel.

Calculation of the amount of immobilized DNA-CalB

The amount of immobilized DNA-CalB was back-calculated from the measured product conversion. By using the Lambert-Beer equation, where A = measured absorbance, ε = extinction coefficient, l = length of UV-cell, c = concentration of the solution,

$$A = \varepsilon l c$$

the concentration of the product pNP was determined. The extinction coefficient ε for pNP on the Knauer K2501 UV detector with a 45 nL flow cell and pathlength of 1 cm, was determined to be 2340 L.mol^{-1}.cm^{-1}. The maximum absorbance, when [S] = ∞, when the concentration of converted pNPB to pNP by CalB, was determined to be ~180 mAU. According to these numbers the concentration of converted pNP per min is

$$c = \frac{0.180 - 0.035}{2340 * 1} = 62\ \mu\text{mol/L (per min)}$$

Similar values were found in the Michaelis Menten curve and in the Eadie Hofstee plot (V_{max} = 61 ± 1 μM/min) (see Figure S2 and S3), which also indirectly implies that the immobilized enzymes are not limited by substrate diffusion and are seemingly behaving as in solution.[1]

As the flow in the capillary system was 0.5 μL/min, c has to be multiplied with the flow rate to obtain the amount of product formed per minute.

$$\frac{62\ \mu mol}{L} * \frac{0.5\ \mu L}{min} = 31 \times 10^{-6} \mu\text{mol product formed per min}$$

An important aspect is that it has to be assumed that the enzymes show the same activity in solution as when immobilized. The 6 cm patch of immobilized enzyme used in these experiments therefore contain:

$$\frac{31 \times 10^{-6}\ \frac{\mu\text{mol}}{\text{min}}\ \text{product}}{15.4\ \mu\frac{\text{mol}}{\text{min}}\ \text{product/mg enzyme}} = 2 \times 10^{-6}\ \text{mg enzyme}$$

A 6 cm patch of capillary, with r = 50 μm has a surface of

$$A = 2\pi r h = 0.188\ \text{cm}^2$$

To normalize the amount of enzymes to 1 cm^2 the numbers

have to be multiplied with

$$\frac{1\ \mathrm{cm}^2}{0.188\ \mathrm{cm}^2} = 5.3$$

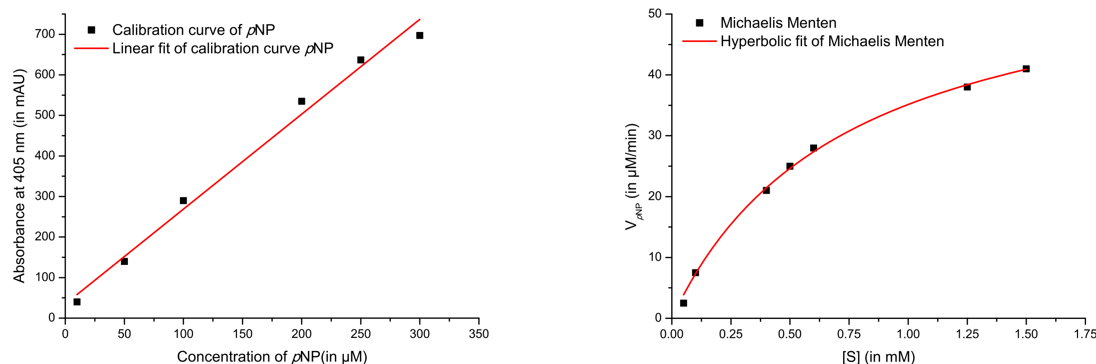

Figure S2. The calibration curve of *p*NP for the Knauer K2501 with a 45 nL flow cell, on the left, and the measured Michaelis Menten diagram on the right. Due to solubility issues of the substrate *p*NPB higher concentrations were not used.

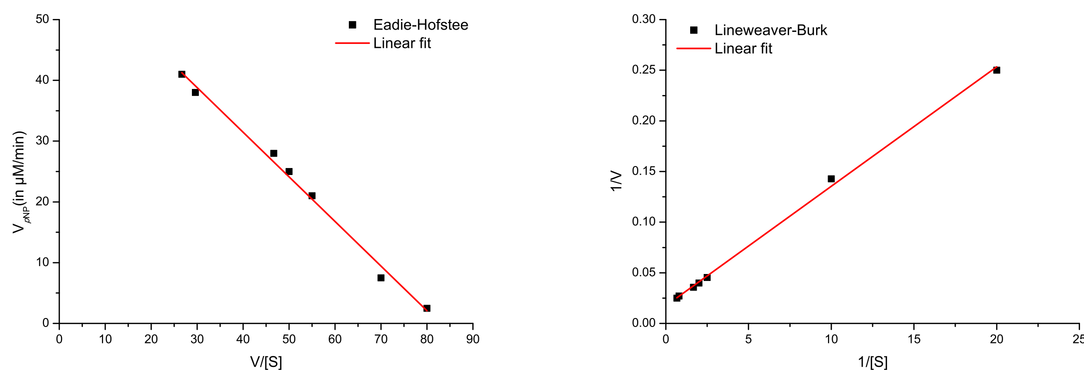

Figure S3. The Eadie Hofstee plot (left) and the Lineweaver-Burk plot (right) were derived from the Michaelis Menten diagram in Figure S2.

Which is equivalent to

$$\frac{2 \times 10^{-9}\mathrm{mg}}{34000\ \mathrm{Da}} * 6.02214\ \times\ 10^{23} * 5.3 = 2 \times 10^{11}\ \mathrm{enzymes/cm^2}$$

In our previous work, we have determined that on average 7×10^{11} DNA strands per square cm were available to bind the complementary strand.[2] This suggests that little less than 30 % of the available binding sites is occupied by DNA-CalB.

Calculation of the diffusion distance

The probability that any substrate molecule will move with a net diffusion distance $<x>$ within a given time t, in a solvent with concentration c and diffusion contant D can be calculated with[3]

$$< x > = 2\sqrt{\frac{Dt}{\pi}}$$

also known as the random-walk equation. The net diffusion distance or diffusion length $<x>$ will give insight whether the substrate is able to move from one side to the other side of the capillary to supply the immobilized enzymes of new substrate. However, in order to calculate $<x>$, D and t have to be determined. As for the diffusion constant, this can be derived from the Stokes-Einstein equation:

$$D = \frac{kT}{6\pi\eta\alpha}$$

where k stands for the Boltzmann constant and is = 1.38×10^{-23} kg.m^2.s^{-1}.K^{-1}, the temperature is T = 298 K, the viscosity of the eluent is $\eta_{water} = 0.891 \times 10^{-3}$ kg.m^{-1}.s^{-1} and the diameter α of the substrate, *p*NPB (*para*-nitrophenyl butyrate) is roughly 1 nm. Fillling in all the numbers will result in $D = 2.45 \times 10^{-10}$ m^2.s^{-1}

The residence time t is the time spent by the substrate floating along the 6 cm patch of immobilized enzyme. As the flow rate is in volume per min, it has to be determined what the total volume of a patch of 6 cm immobilized enzyme of a capillary

with an i.d. 100 μm (r = 50 μm) is:

$$V = \pi r^2 h = 471 \times 10^{-12}\text{m}^3 = 470\ \text{nL}$$

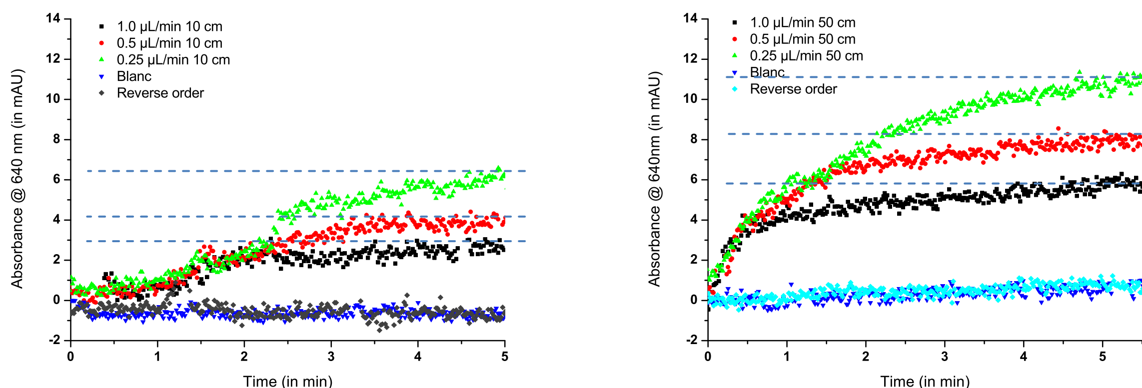

Figure S4 Absorbance increase during stabilization of the flow rate. The horizontal dotted lines indicate the maximum substrate conversion for each flow speed. a) The patches of CalB and HRP are 10 cm apart from each other. b)The distance between the patches of CalB and HRP is increased to 50 cm.

where V stands for volume in L, r is the radius and h is the length of the capillary in meters.

The residence time t, is then calculated by dividing this volume by the applied flow rate as calculated below for a flow rate of 0.25 μL/min:

$$t_{0.25\ \mu\text{L/min}} = \frac{0.470\ \mu\text{L}}{0.25\ \mu\text{L} * \text{min}^{-1}} = 1.88\ \text{min} = 113\ \text{s}$$

In the same way the following residence times are calculated for $t_{0.50\mu L/min}$ = 56.5 s and $t_{1.0\mu L/min}$ = 28 s.

Filling in the calculated diffusion rates and residence times for the designated flow rates in the random-walk equation, gives the respective diffusion distances of 188 μm, 133 μm and 94 μm. These distances are twice higher than the radius of the capillary and the diffusion of the substrate from the stock solution to the immobilized enyzme (CalB) is therefore not expected to be a limiting factor. N.B. This is most likely not applicable for the three-enzyme cascade case, as the diffusion of the substrates towards GOx (enzyme 2) and HRP (immobilized enzyme 3) is also dependent on the conversion of the previously involved enzyme.

Enzyme activity measurements in a microchannel

The activity of the enzymes immobilized inside a microchannel were followed on-line with a UV-VIS detector connected to the outlet of the modified capillary with zero dead-volume connectors. To obtain reproducible results, the initial flow was set to 10 μL/min and held at that speed for a minimum of five min until the flow had stabilized. After that, the flow was reduced to 0.25, 0.50 or 1.0 μL/min and the increase of absorbance by substrate conversion was observed, as the enzymes had more time to convert the substrate (see Figure S4). In this particular experiment, the substrate is not exhausted, but continuously supplied by pumping a constant concentration of substrate along the immobilized enzymes. This denotes that the increase in absorbance thus does not represent the conventional Michaelis-Menten kinetics (i.e. Figure S3), but the stabilization of the flow from a higher flow rate to the desired lower flow rate. The point where the absorbance does not further increase was taken as the maximum substrate conversion at that specific flow rate as shown in Fig. 8 in the main article.

References

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