

Size of the Lactase from *Kluyveromyces lactis*: 124 kDa

Calculation example for activity:

Slope from the machine:

(imaginary value, careful with the unit – the machine gives it in **AU/min** not AU/s)

$$\frac{dAbs}{dt} = 0.123 \frac{AU}{min}$$

Concentration (Lamber-Beer-Equation)

$$\frac{dC}{dt} = \frac{dAbs}{\epsilon * l} = \frac{0.123 \text{ min}^{-1}}{3296 \text{ M}^{-1} \text{ cm}^{-1} * 1 \text{ cm}} = 3.73 * 10^{-5} \frac{\text{mol}}{\text{l} * \text{min}}$$

Initial velocity

$$\begin{aligned} v_0 &= \frac{dC}{dt} * \text{volume} = 3.73 * 10^{-5} \frac{\text{mol}}{\text{l} * \text{min}} * 0.001 \text{ l} = 3.73 * 10^{-8} \frac{\text{mol}}{\text{min}} \\ &= 37.3 \frac{\text{nmol}}{\text{min}} \text{ bzw } 0.621 \frac{\text{nmol}}{\text{s}} \end{aligned}$$

(Without the dilution factor)

Calculation of kcat

$$k_{cat} = \frac{V_{max}}{[E_0]}$$

Let's assume for example

$$V_{max} = 1.23 \frac{dC}{dt} = 1.23 \frac{\text{mol}}{\text{l} * \text{min}}$$

And

$$[E_0] = 10 \frac{\text{mg}}{\text{ml}}$$

Then we first have to calculate E_0 in mol/l

$$MW(\text{lactase}) = 124\,000 \text{ Da} = 124\,000 \frac{\text{g}}{\text{mol}}$$

$$[E_0] = \frac{10 \frac{mg}{ml}}{124\,000 \frac{g}{mol}} = 8.065 * 10^{-5} \frac{mol}{l} = 80.65 \mu M$$

Let's come back to k_{cat}

$$k_{cat} = \frac{V_{max}}{[E_0]} = \frac{1.23 \frac{mol}{l * min}}{80.65 * 10^{-6} \frac{mol}{l}} = 15252 \text{ min}^{-1} = 254 \text{ s}^{-1}$$