Supplementary Information:

Non-Linear and Linear Enhancement of Enzymatic Reaction Kinetics using a Biomolecule Concentrator

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Fig. S1 Numerical simulation results for a concentration-enhanced enzyme-substrate reaction in the trapped plug with non-linear enhancement as modelled in the system of equations (1)-(5) with an accumulation rate, $\alpha = 1$, with increasing starting substrate concentrations. The quadratic enzyme-limited and subsequent linear substrate-limited phases of the product curve can be clearly seen.
Fig. S2 Variation of the mean product fluorescence of the plug in the non-linear enhancement mode with time at different initial substrate concentrations. Within a certain range of initial substrate concentrations, the initial quadratic and subsequent linear phases are observed. The initial phase is fitted to a quadratic polynomial in time while the later phase is fitted to a linear polynomial in time.
**Fig. S3** Fluorescence to product concentration calibration curve for the reaction of the substrate fluorescein di-β-galactopyranoside (FDG) with the enzyme β-Galactosidase. This curve was obtained by reacting various initial concentrations of substrate with a relatively high concentration of enzyme \(1 \mu\text{g/ml}\) for a long time (3hrs) at room temperature. From the slope of this curve the fluorescence to concentration calibration factor of \(~4.6 \text{ AU/µM}\) is obtained which is then used to scale the product-time curves for obtaining reaction parameters.
Fig. S4 Variation of product formation rate in the linear enhancement mode as measured by the rate of variation of mean fluorescence in the closed chamber and a fitting of the expected Michaelis-Menten form to the experimental data in order to obtain reaction parameters. The rate of product formation \( \frac{d[P]}{dt} \) on the y-axis here was obtained by differentiating the product fluorescence data — after scaling it to product concentration using the calibration factor and filtering it to remove high frequency noise to enable numerical differentiation. This is plotted against the product concentration \([P]\) itself on the x-axis. This curve is then fitted to the Michaelis-Menten expression shown above which assumes that only a small fraction of substrate is in the intermediate at any time. The value of product concentration at long times during the experiment is used as \([P_{\infty}]\) and the two fitted parameters \(V_{\text{MAX}}\) and \(K_M\) can be extracted from this fit.