

Electronic Supplementary Information

Application of an Enzymatic Cascade Reaction for the Synthesis of the Emeraldine Salt Form of Polyaniline

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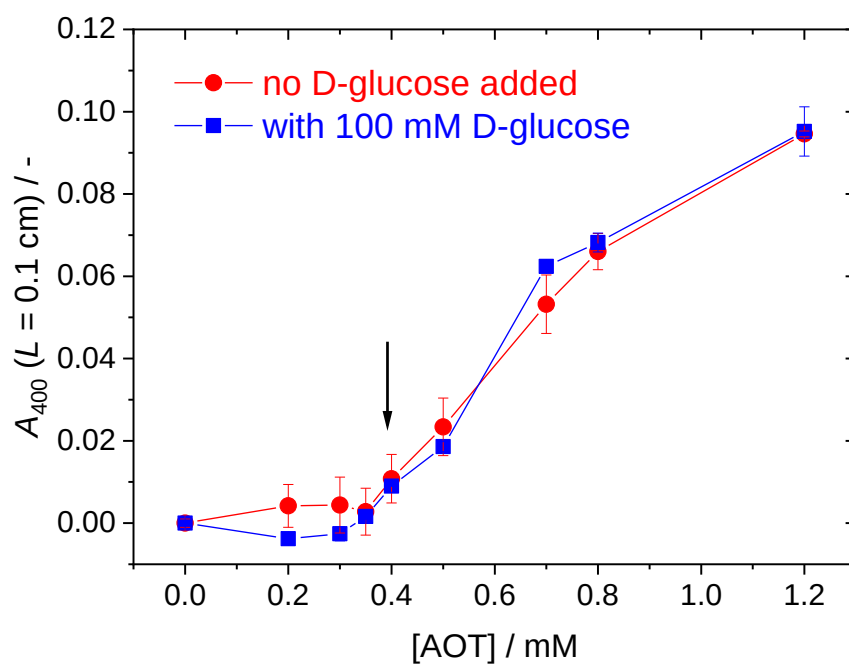


Fig. S-1.

Estimation of the critical concentration for AOT vesicle formation (CVC) in 100 mM NaH_2PO_4 solution ($\text{pH} = 4.3$) at $T \sim 25^\circ\text{C}$ by turbidity measurements in the absence of D-glucose (red filled circles) or in the presence of 100 mM D-glucose (blue filled squares). In both cases, CVC ~ 0.4 mM (arrow), see Experimental. The error bars represent standard deviations from three measurements. The lines between the data points are drawn for guiding the eyes.

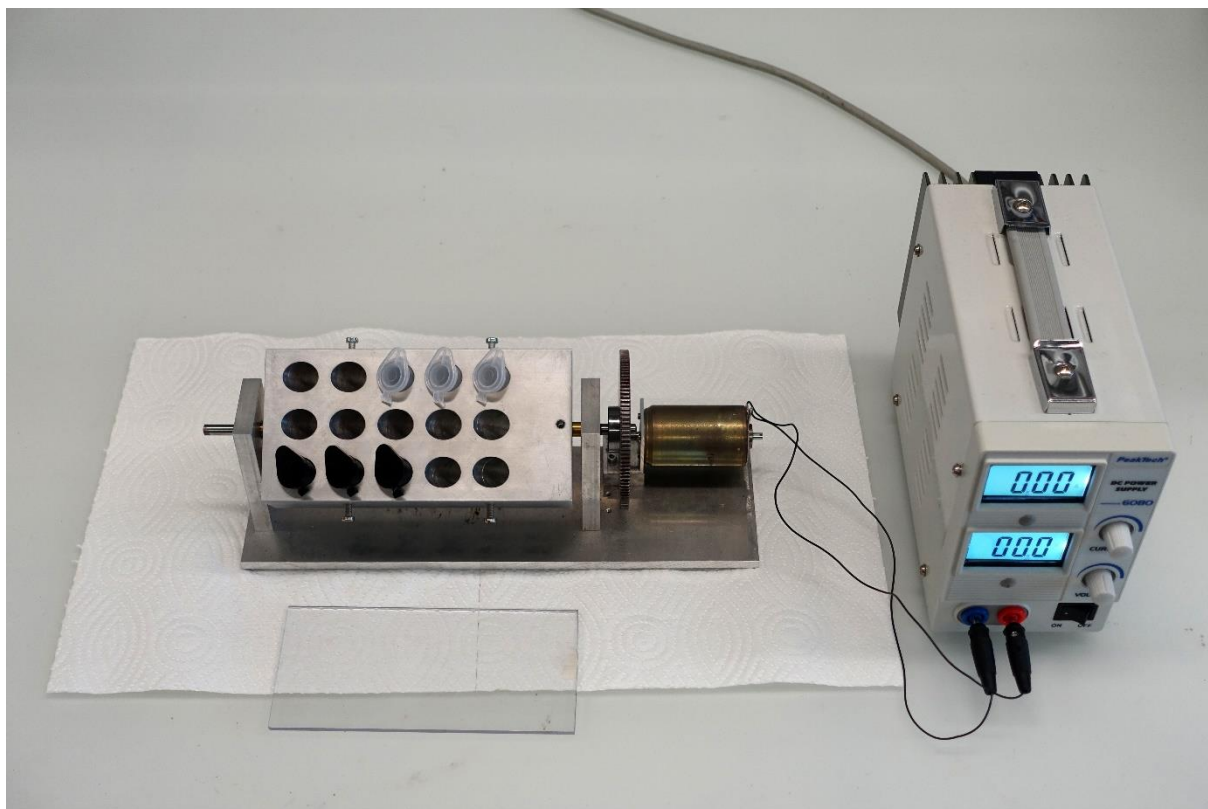


Fig. S-2.

Photograph of the home-made rotary device used for running cascade reactions. The cascade reaction mixtures were put inside 5 mL polypropylene Eppendorf tubes, which then were inserted into the holes drilled at a tilt angle of about 30° into a cuboid made of aluminium. The tubes were fixed by using a poly(methylmethacrylate) cover (in front) and rubber strings. Rotation was set to ~ 90 rpm by the controller (right hand side, DC power supply 6080 from PeakTech, Ahrensburg, Germany), and the device was kept at room temperature ($T \sim 25^\circ\text{C}$). The device was made by Dr. Thomas Schweizer, Department of Materials, ETH Zürich, Switzerland.

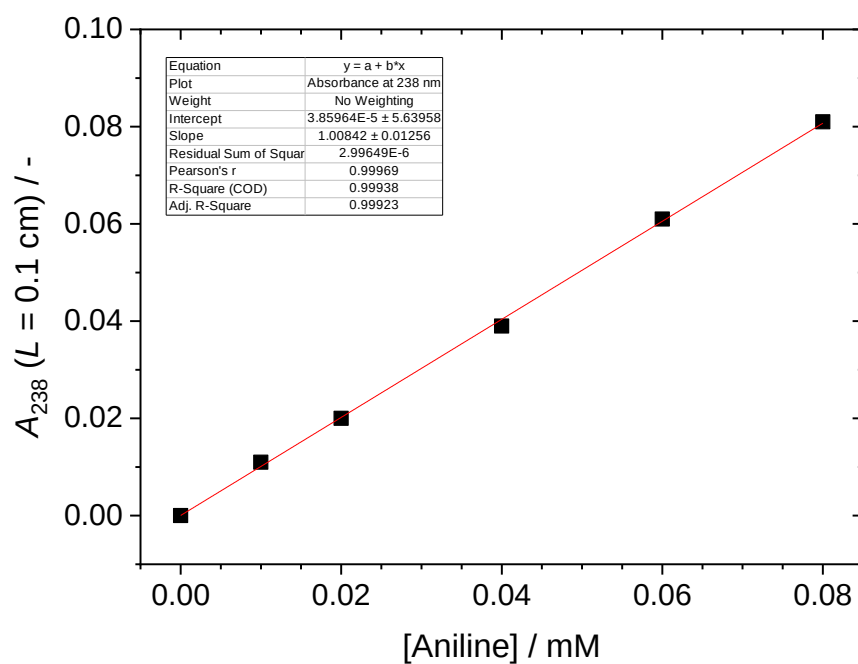


Fig. S-3.

Calibration curve for the quantification of remaining aniline, present in samples withdrawn from the cascade reaction mixtures and extracted into acetonitrile, determined from the linear fit of A_{238} vs. know [aniline], see Experimental. The concentrations given are the ones in the quartz cuvette used for the absorption measurements.

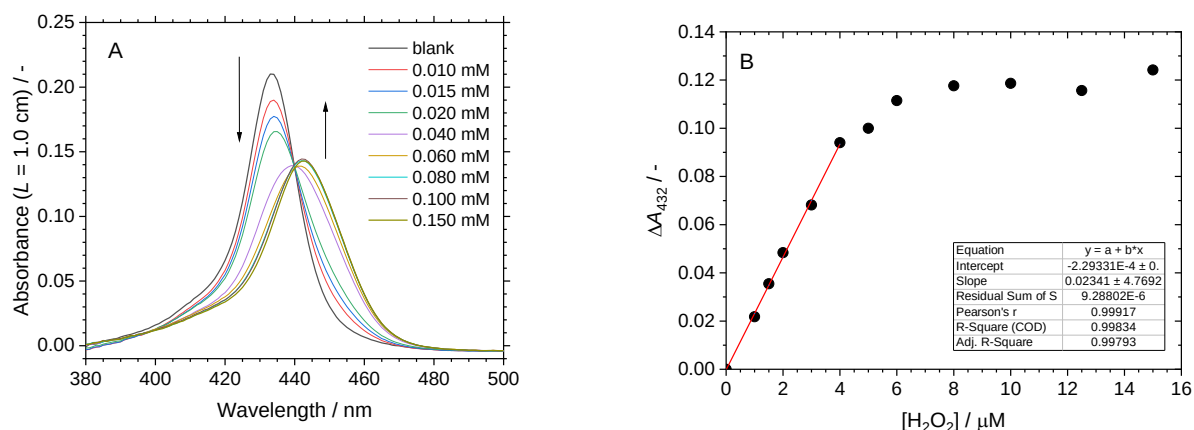


Fig. S-4.

Quantification of H_2O_2 with the Ti-TPyP assay using known amounts of H_2O_2 . Ti-TPyP stands for oxo[5,10,15,20-tetra(4-pyridyl)porphyrinato] titanium (IV), see Experimental.

(A) The absorption spectrum of the assay solution was measured as a function of H_2O_2 concentration in the sample solution ($0 \rightarrow 0.150 \text{ mM}$). The arrows indicate the changes in the spectrum with increase in H_2O_2 concentration.

(B) The measured differences in A_{432} (ΔA_{432}) between the absorption intensities at $\lambda = 432 \text{ nm}$ of the blank sample and the samples containing H_2O_2 are plotted as a function of H_2O_2 concentration in the cuvette. For the quantification of H_2O_2 , a linear fit was made between $[\text{H}_2\text{O}_2] = 0$ and $4.0 \mu\text{M}$.

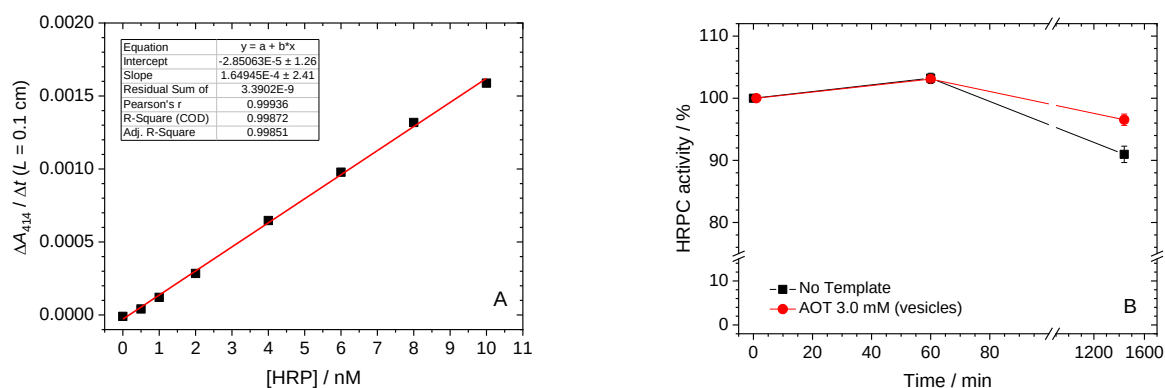


Fig. S-5.

(A) Calibration curve correlating the measured initial rate of ABTS^{2-} oxidation (monitored at $\lambda = 414$ nm) with known amounts of HRPc in the cuvette at fixed $[\text{ABTS}^{2-}]_0$ and $[\text{H}_2\text{O}_2]_0$. The activity measurements were performed in 10 mM MOPS buffer ($\text{pH} = 7.0$) at $T \sim 25$ °C. The HRPc activity is expressed as $\Delta A_{414} / \Delta t$ for the first 60 sec after starting the reaction by adding H_2O_2 . $[\text{ABTS}^{2-}]_0 = 1.0$ mM, $[\text{H}_2\text{O}_2]_0 = 0.2$ mM, see Experimental. The red line represents the linear fit.

(B) Stability of HRPc (0.92 μM) in 0.1 M NaH_2PO_4 solution ($\text{pH} = 4.3$) at $T \sim 25$ °C in the absence (black filled squares) and presence of AOT vesicles (red filled circles, $[\text{AOT}] = 3.0$ mM), as measured with $\text{ABTS}^{2-} / \text{H}_2\text{O}_2$ at $\text{pH} = 7.0$, $[\text{ABTS}^{2-}]_0 = 1.0$ mM, $[\text{H}_2\text{O}_2]_0 = 0.2$ mM, see Experimental. The error bars represent standard deviations from three measurements. The lines between the data points are drawn for guiding the eyes.

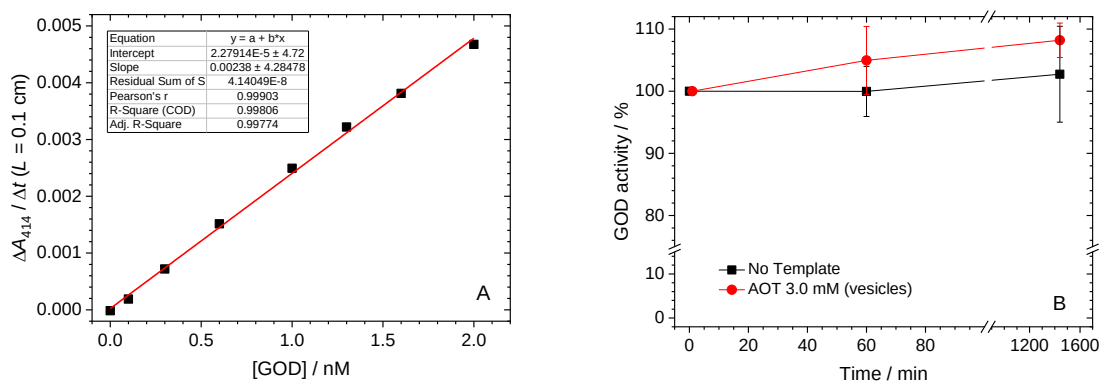


Fig. S-6.

(A) Calibration curve correlating the measured initial rate of ABTS²⁻ oxidation (monitored at $\lambda = 414$ nm) with known amounts of GOD in the cuvette at fixed [D-glucose]₀, [HRPC], and [ABTS²⁻]₀. The activity measurements were performed in 10 mM MOPS buffer ($pH = 7.0$) at $T \sim 25$ °C. The GOD activity is expressed as $\Delta A_{414} / \Delta t$ for the first 180 sec after starting the reaction by adding GOD. [D-glucose]₀ = 100 mM, [HRPC] = 0.1 μ M, [ABTS²⁻]₀ = 1.0 mM, in presence of dissolved O₂, see Experimental. The red line represents the linear fit.

(B) Stability of GOD (0.20 μ M) in 0.1 M NaH₂PO₄ solution ($pH = 4.3$) at $T \sim 25$ °C in the absence and presence of AOT vesicles ([AOT] = 3.0 mM), as measured with D-glucose / ABTS²⁻ / HRPC at $pH = 7.0$, [D-glucose]₀ = 100 mM, [HRPC] = 0.1 μ M, [ABTS²⁻]₀ = 1.0 mM, in presence of dissolved O₂, see Experimental. The error bars represent standard deviations from three measurements. The lines between the data points are drawn for guiding the eyes.

Whether GOD interacts with AOT vesicles is not clear. The reported isoelectric point of *Aspergillus niger* GOD is 4.2 (Pazur JH, Kleppe K (1964) The Oxidation of Glucose and Related Compounds by Glucose Oxidase from *Aspergillus niger*. Biochemistry 3: 578–583.) Therefore, at $pH = 4.3$, the enzyme has an overall negative charge, which prevents binding to the vesicles via electrostatic interactions.

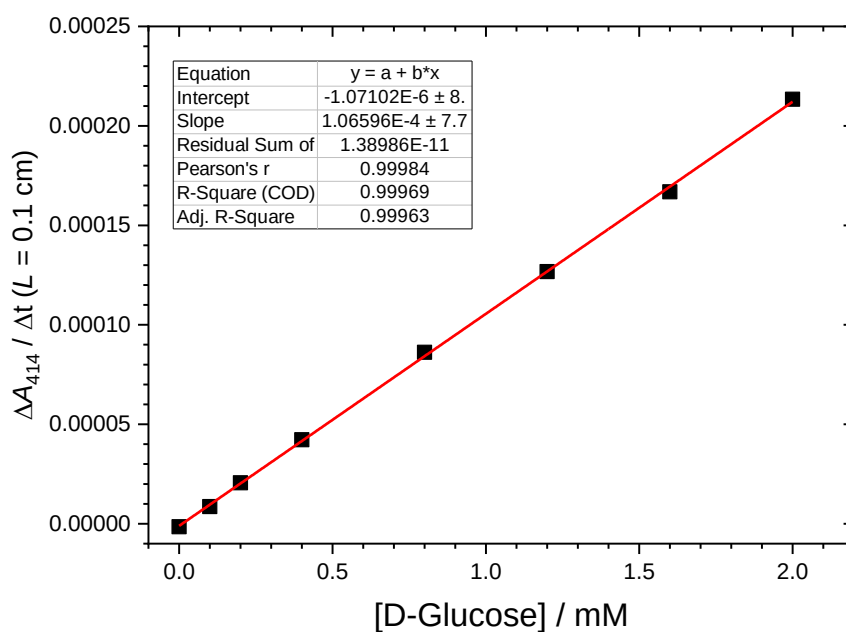


Fig. S-7.

Calibration curve correlating the measured initial rate of ABTS^{2-} oxidation (monitored at $\lambda = 414 \text{ nm}$) with known amounts of D-glucose in the cuvette at fixed $[\text{GOD}]$, $[\text{HRPC}]$, and $[\text{ABTS}^{2-}]_0$. $\Delta A_{414} / \Delta t$ was recorded for the first 180 sec after starting the reaction by adding D-glucose. $[\text{GOD}] = 1 \text{ nM}$, $[\text{HRPC}] = 0.1 \text{ }\mu\text{M}$, and $[\text{ABTS}^{2-}]_0 = 1.0 \text{ mM}$, in presence of dissolved O_2 , see Experimental. The red line represents the linear fit.

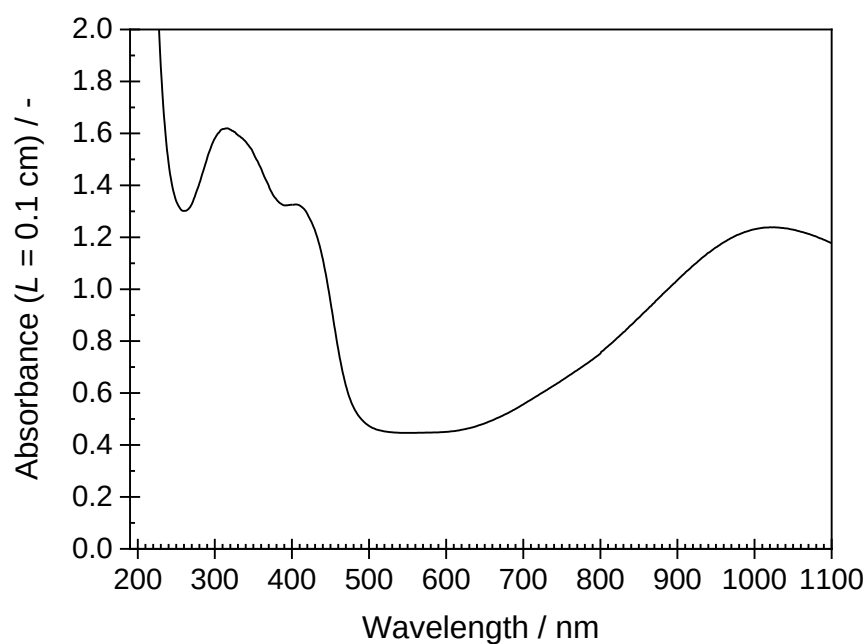


Fig. S-8.

UV/vis/NIR absorption spectrum of the “reference reaction” run at $T \sim 25\text{ }^{\circ}\text{C}$ with HRPC and initially added H_2O_2 , recorded after a reaction time $t = 24\text{ h}$. $[\text{AOT}] = 3.0\text{ mM}$; $[\text{aniline}]_0 = 4.0\text{ mM}$; $[\text{HRPC}] = 0.92\text{ }\mu\text{M}$; $[\text{H}_2\text{O}_2]_0 = 4.5\text{ mM}$; $pH = 4.3$ ($100\text{ mM NaH}_2\text{PO}_4$). See also (Junker et al. 2012; Pašti et al. 2017; Fujisaki et al. 2019).

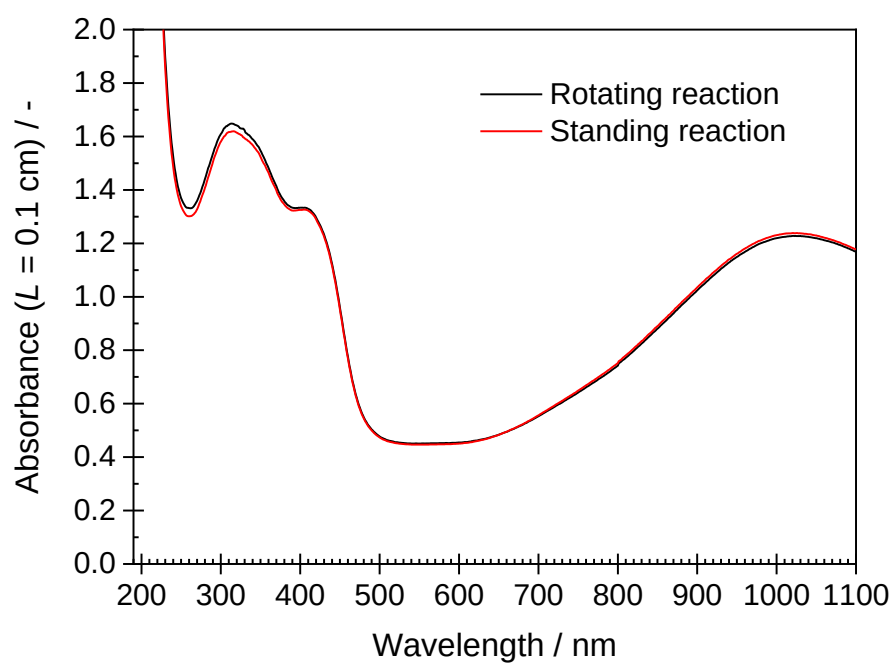


Fig. S-9.

Effect of reaction tube rotation on the UV/vis/NIR absorption spectrum of the “reference reaction”, recorded after $t = 24$ h at $T \sim 25$ °C. $[AOT] = 3.0$ mM; $[aniline]_0 = 4.0$ mM; $[HRPC] = 0.92$ μ M; $[H_2O_2]_0 = 4.5$ mM; $pH = 4.3$ (100 mM NaH_2PO_4). Red line: with reaction tube rotation. Blue line: without reaction tube rotation.

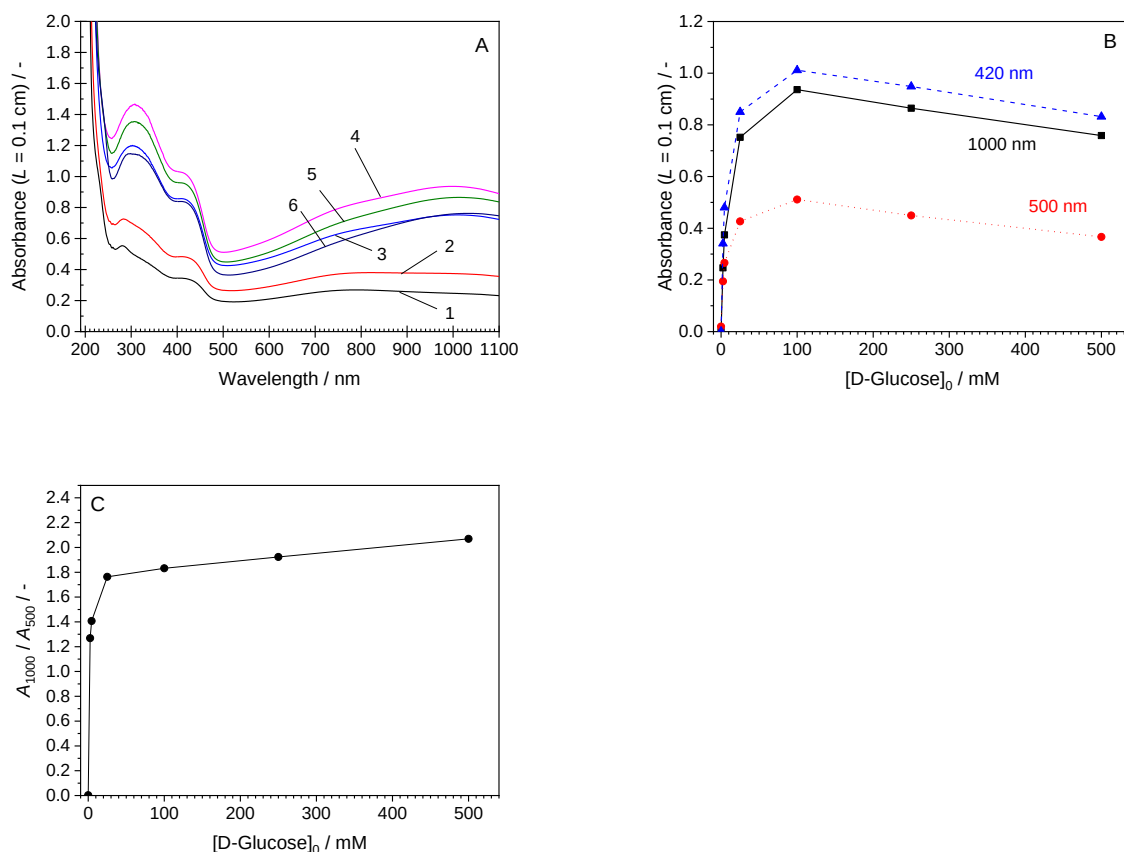


Fig. S-10.

UV/vis/NIR absorption spectra of different cascade reactions run at $T \sim 25 \text{ }^{\circ}\text{C}$ consisting of various amounts of initially added D-glucose and fixed amounts of GOD and HRPC, recorded after $t = 24 \text{ h}$. $[\text{AOT}] = 3.0 \text{ mM}$; $[\text{aniline}]_0 = 4.0 \text{ mM}$; $[\text{D-glucose}]_0 = 2.5$ (1), 4.5 (2), 25 (3), 100 (4), 250 (5), or 500 mM (6); $[\text{GOD}] = 0.15 \text{ } \mu\text{M}$; $[\text{HRPC}] = 0.92 \text{ } \mu\text{M}$; $pH = 4.3$ (100 mM NaH_2PO_4). **(A):** Measured spectra. **(B):** A_{1000} , A_{500} , and A_{420} vs. $[\text{D-glucose}]_0$. **(C):** A_{1000} / A_{500} vs. $[\text{D-glucose}]_0$. For **(B)** and **(C)**, the lines between the data points are drawn for guiding the eyes.

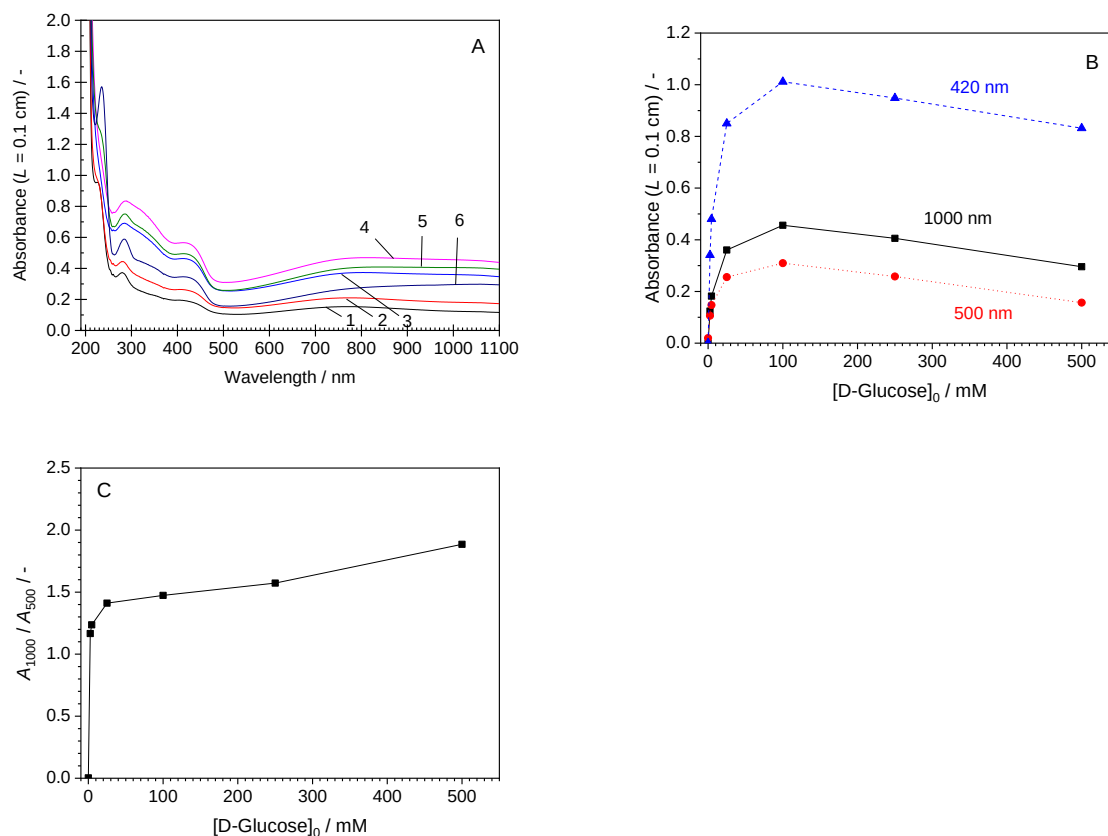


Fig. S-11.

UV/vis/NIR absorption spectra of different cascade reactions run at $T \sim 25 \text{ }^{\circ}\text{C}$ consisting of various amounts of initially added D-glucose and fixed amounts of GOD and HRP, recorded after $t = 24 \text{ h}$. $[\text{AOT}] = 3.0 \text{ mM}$; $[\text{aniline}]_0 = 4.0 \text{ mM}$; $[\text{D-glucose}]_0 = 2.5$ (1), 4.5 (2), 25 (3), 100 (4), 250 (5), or 500 mM (6); $[\text{GOD}] = 0.03 \text{ } \mu\text{M}$; $[\text{HRP}] = 0.92 \text{ } \mu\text{M}$; $\text{pH} = 4.3$ (100 mM NaH_2PO_4). **(A):** Measured spectra. **(B):** A_{1000} , A_{500} , and A_{420} vs. $[\text{D-glucose}]_0$. **(C):** A_{1000} / A_{500} vs. $[\text{D-glucose}]_0$. For **(B)** and **(C)**, the lines between the data points are drawn for guiding the eyes.

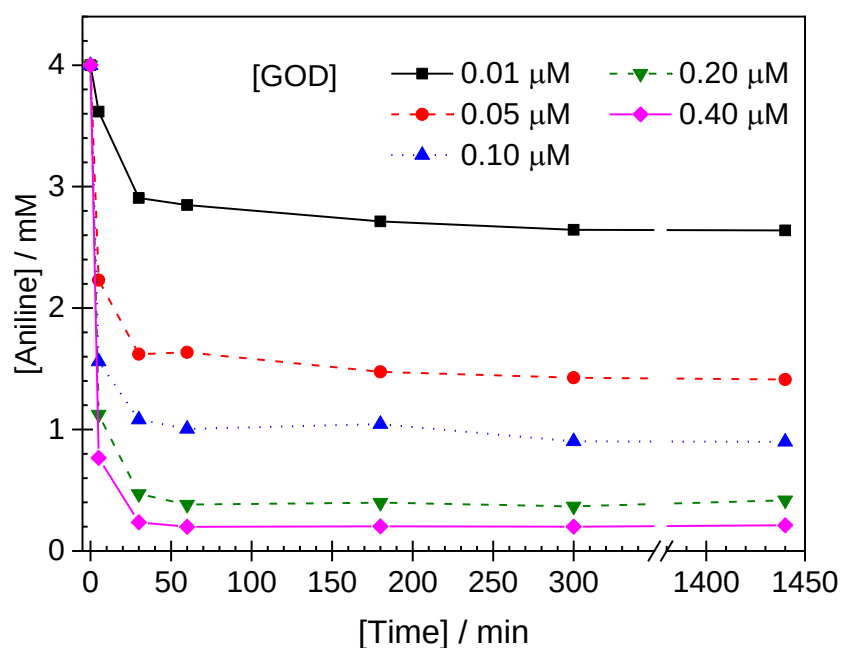


Fig. S-12.

Changes of the aniline concentration in different cascade reaction mixtures run at $T \sim 25\text{ }^{\circ}\text{C}$ with varying amounts of GOD and fixed amounts of initially added D-glucose and HRPC. $[\text{AOT}] = 3.0\text{ mM}$; $[\text{aniline}]_0 = 4.0\text{ mM}$; $[\text{D-glucose}]_0 = 100\text{ mM}$; $[\text{GOD}] = 0.01, 0.05, 0.10, 0.20, \text{ or } 0.40\text{ }\mu\text{M}$; $[\text{HRPC}] = 0.92\text{ }\mu\text{M}$; $pH = 4.3$ ($100\text{ mM NaH}_2\text{PO}_4$). The lines between the data points are drawn for guiding the eyes.

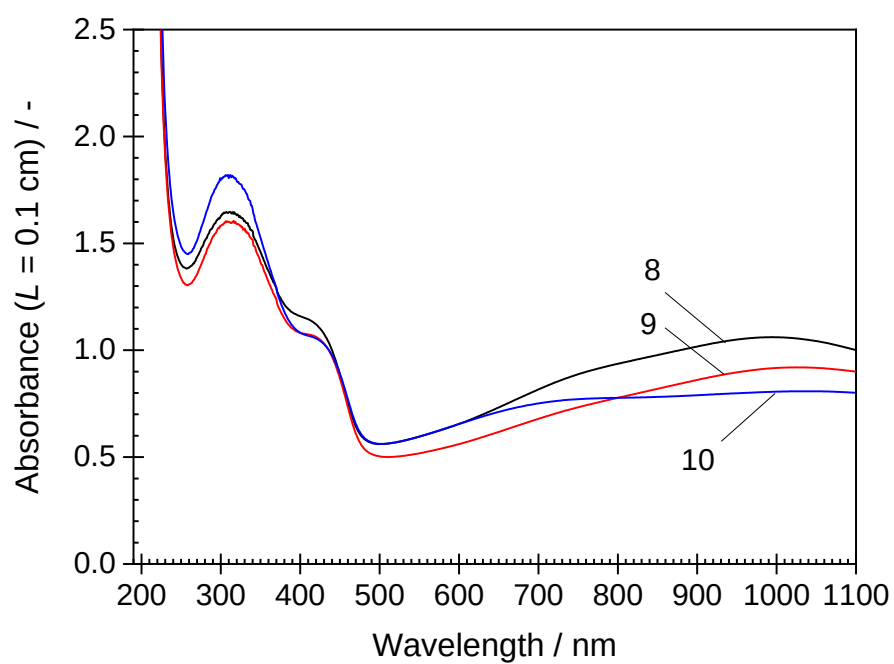


Fig. S-13.

UV/vis/NIR absorption spectra of the ideal cascade reaction mixture (see **Table 1**) for reaction times $t = 24$ h (8, data of **Fig. 4B**), $t = 72$ h (9), and $t = 2$ weeks (10). [AOT] = 3.0 mM; [aniline]₀ = 4.0 mM; [D-glucose]₀ = 100 mM; [GOD] = 0.20 μ M; [HRPC] = 0.92 μ M; $pH = 4.3$ (100 mM NaH₂PO₄).

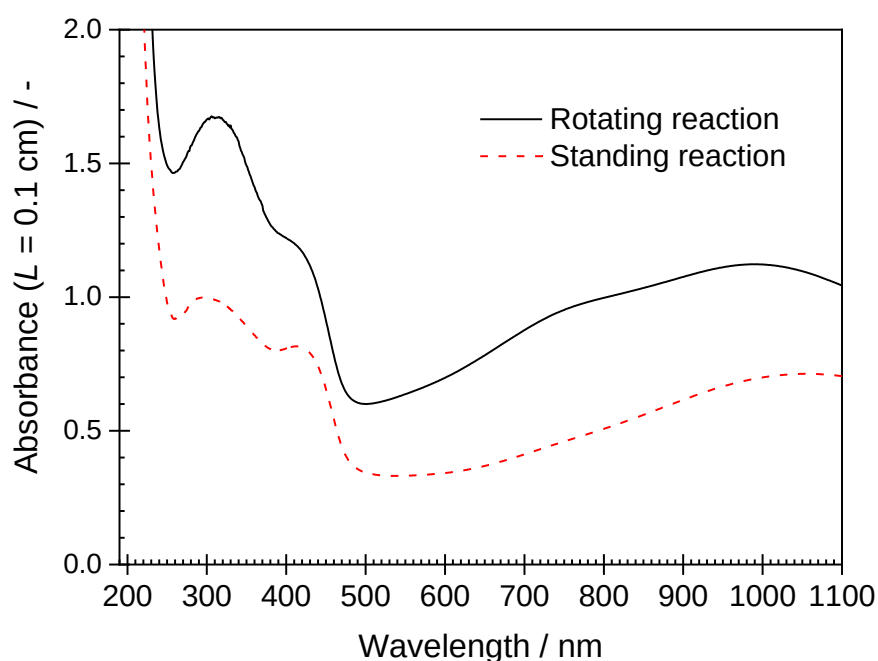


Fig. S-14.

Effect of reaction tube rotation on the UV/vis/NIR absorption spectrum of the ideal cascade reaction mixture (see **Table 1**), measured after a reaction time $t = 24$ h at $T \sim 25$ °C. $[AOT] = 3.0$ mM; $[aniline]_0 = 4.0$ mM; $[D\text{-}glucose]_0 = 100$ mM; $[GOD] = 0.20$ μ M; $[HRPC] = 0.92$ μ M; $pH = 4.3$ (100 mM NaH_2PO_4). The spectrum for the rotated reaction mixture (black solid line) is spectrum 6 of **Figure 2A**.

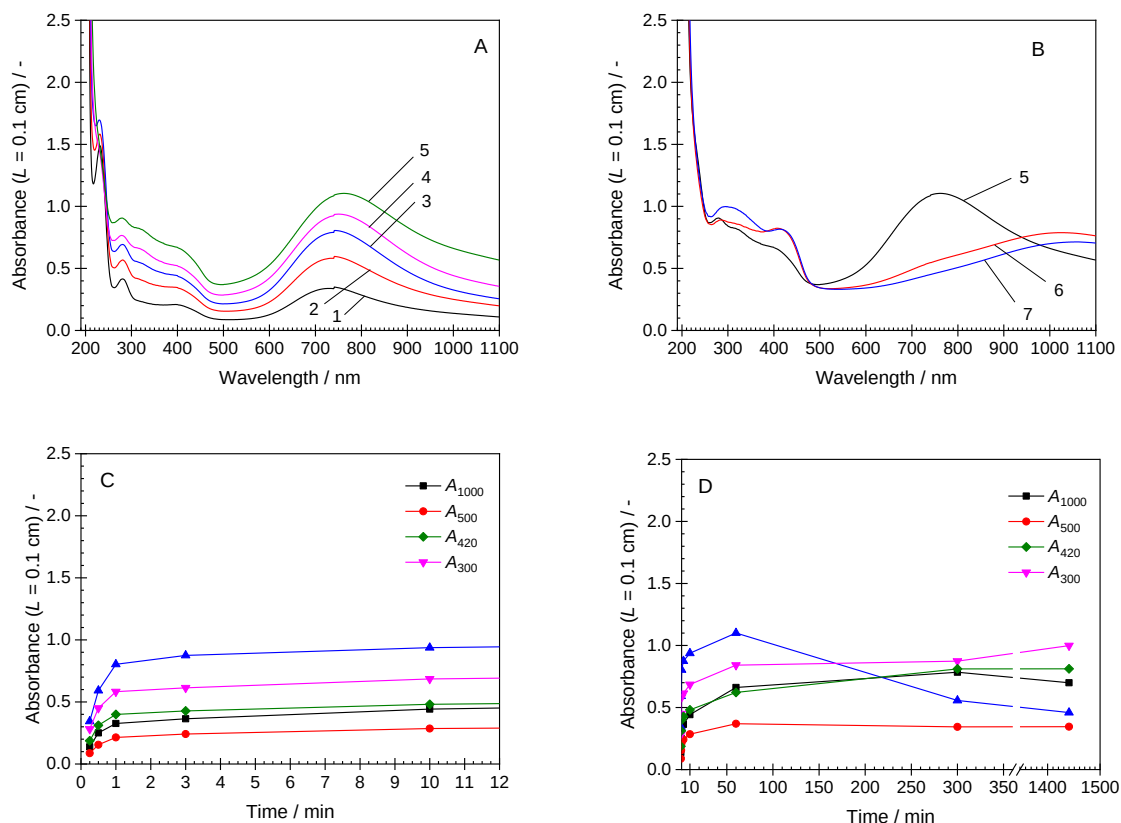


Fig. S-15.

UV/vis/NIR absorption spectra of samples which were withdrawn at predetermined times from the "ideal" cascade reaction mixture (see **Table 1**) kept in reaction tubes without tube rotation ("standing reactions"). [AOT] = 3.0 mM; [aniline]₀ = 4.0 mM; [D-glucose]₀ = 100 mM; [GOD] = 0.20 μM ; [HRPC] = 0.92 μM ; $pH = 4.3$ (100 mM NaH_2PO_4). The reaction times were $t = 15 \text{ s}$ (1), 30 s (2), 1 min (3), 10 min (4), 60 min (5), 5 h (6), and 24 h (7). **(A)**, **(B)**: Measured spectra for $t = 0 - 60 \text{ min}$ and 60 min – 24 h, respectively. **(C)**, **(D)**: Time dependent changes of A_{1000} , A_{750} , A_{500} , A_{420} , and A_{300} . For **(C)** and **(D)**, the lines between the data points are drawn for guiding the eyes.

Please note that the spectra measured for $t = 15 \text{ s}$ and $t = 30 \text{ s}$ are only approximate spectra due to the rapid changes during the first phase of the reaction, the reaction also taking place quite rapidly during the recording of the spectra.

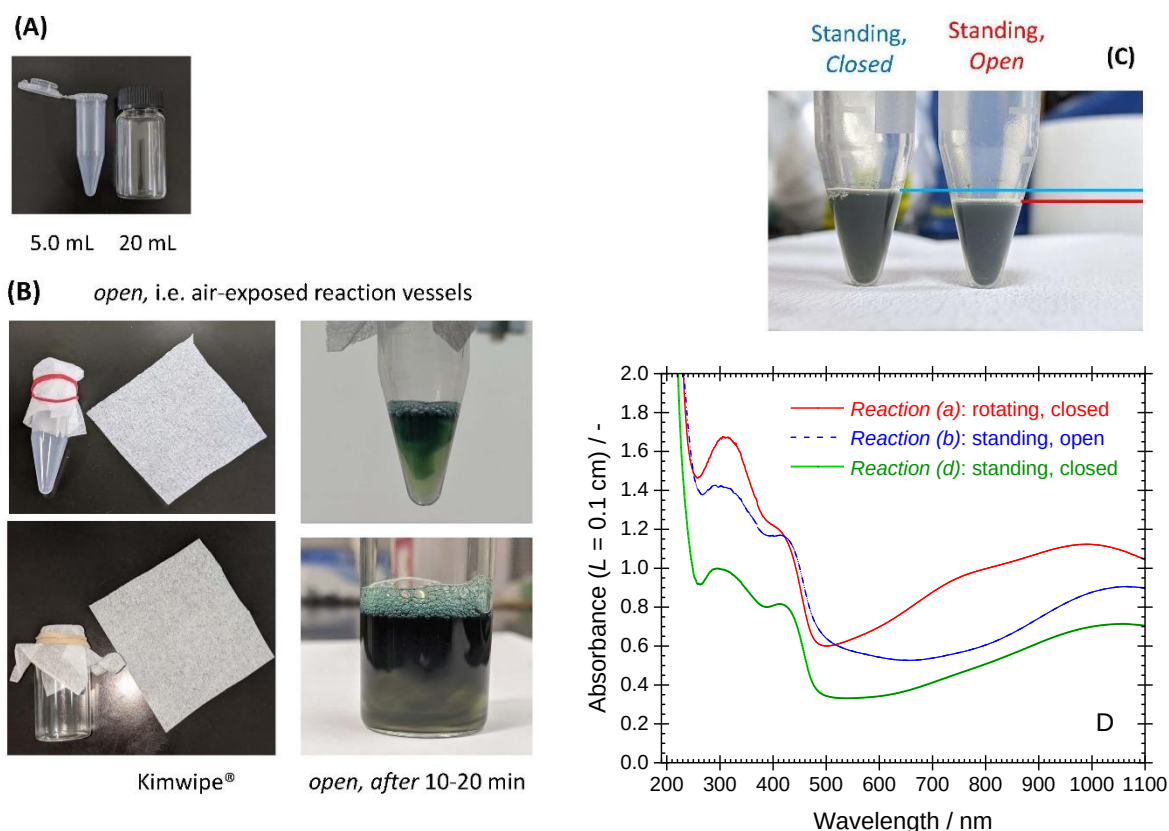


Fig. S-16.

Comparison of the cascade reaction run in **open** or **closed** reaction vessels. **(A)** The reactions were carried out with the initial composition considered to be ideal (**Table 1**) in either 5 mL polypropylene reaction tubes (with reaction volumes of 0.5 mL), or in 20 mL glass flasks (with reaction volumes of 10 mL). [AOT] = 3.0 mM; [aniline]₀ = 4.0 mM; [D-glucose]₀ = 100 mM; [GOD] = 0.20 μM; [HRPC] = 0.92 μM; pH = 4.3 (100 mM NaH₂PO₄); T ~ 25 °C t = 24 h. The reaction vessels were either **closed** (with a cap) for running the reaction, or they were **open**, air-exposed (covered with a Kimwipe®).

Reaction (a): 0.5 mL reaction mixture in 5.0 mL tube, **closed**, tube **rotating** (= "ideal")

Reaction (b): 10 mL reaction mixture in 20 mL flask, **open**, flask standing

Reaction (c): 0.5 mL reaction mixture in 5.0 mL tube, **open**, tube standing

Reaction (d): 0.5 mL reaction mixture in 5.0 mL tube, **closed**, tube standing

For *Reactions (b), (c), and (d)*, the reaction initially took place locally **(B)**, near the surface of the reaction mixtures. The reaction mixtures turned more and more green during the first 10-20 min after starting the reaction, then the whole reaction mixtures became homogeneously dark green without any precipitation. For *Reaction (c)*, significant water evaporation was evident after t = 24 h **(C)**. Therefore, this sample was excluded from further analysis.

The UV/vis/NIR absorption spectra of the mixtures of *Reactions (a), (b), and (d)* were recorded after t = 24 h **(D)**, and the pH was measured (**Table S-1**). The spectra for *Reactions (a) and (d)* correspond to the spectra shown in **Fig. S-14**. Highest intensities in the vis/NIR region of the spectrum were found for *Reaction (a)*, for the conditions we considered "ideal". *Reaction (b)* is between *Reaction (a)* and *Reaction (d)*.

Table S-1.

Measured pH-values of the reactions mixtures of *Reactions (a)*, *(b)*, and *(d)* after $t = 24$ h, see legend of **Fig. S-16**.

Reaction	Measured pH-value ^a
<i>Reaction (1)</i>	3.59±0.00
<i>Reaction (2)</i>	3.91
<i>Reaction (3)</i>	Not determined due to volume change
<i>Reaction (4)</i>	3.84±0.06
Reference reaction (with added H ₂ O ₂) ^b	4.02±0.02

^a: Values given with standard deviations ($n = 3$)

^b: Conditions as described by Junker K, Zandomenighi G, Guo Z, Kissner R, Ishikawa T, Kohlbrecher J, Walde P (2012) Mechanistic aspects of the horseradish peroxidase-catalysed polymerisation of aniline in the presence of AOT vesicles as templates. RSC Adv 2: 6478–6495.

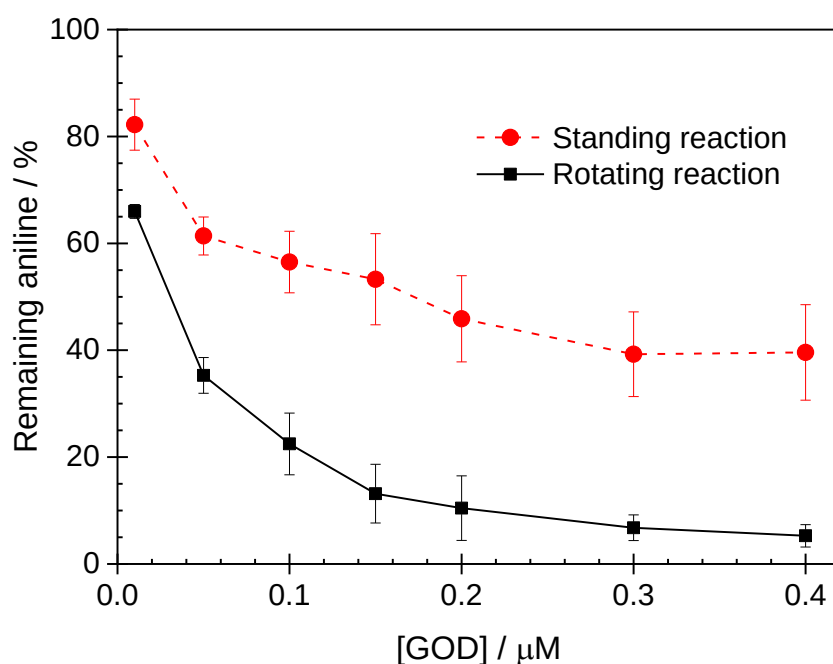


Fig. S-17.

Comparison of the relative amounts of remaining aniline after a reaction time of $t = 24$ h for reactions run at $T \sim 25$ °C kept in reaction tubes without tube rotation (red filled circles, "standing reactions") or with tube rotation (black filled squares, "rotating reaction"). The amount of GOD was varied at fixed amounts of initially added D-glucose and HRPC. [AOT] = 3.0 mM; [aniline]₀ = 4.0 mM; [D-glucose]₀ = 100 mM; [GOD] = 0, 0.01, 0.05, 0.10, 0.15, 0.20, 0.30, or 0.40 μM ; [HRPC] = 0.92 μM ; $pH = 4.3$ (100 mM NaH_2PO_4). The error bars represent standard deviations from two or three measurements. The lines between the data points are drawn for guiding the eyes.

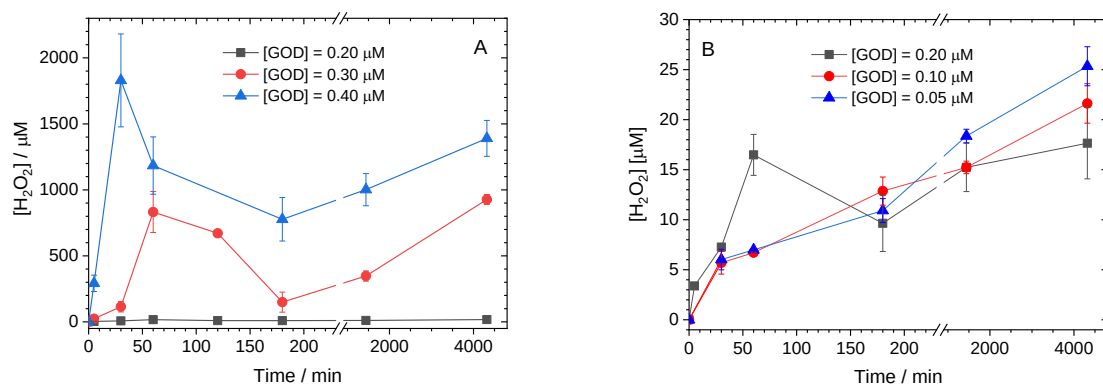


Fig. S-18.

Quantification of the *in situ* formed H_2O_2 for the "ideal cascade reaction mixture" ($[\text{GOD}] = 0.20 \mu\text{M}$, see **Table 1**) and for other concentrations of GOD during the course of the reaction, as determined by analyzing samples withdrawn from the reaction mixture, see Experimental. $[\text{AOT}] = 3.0 \text{ mM}$; $[\text{aniline}]_0 = 4.0 \text{ mM}$; $[\text{HRP}] = 0.92 \mu\text{M}$; $[\text{GOD}] = \text{variable}$; $[\text{D-glucose}]_0 = 100 \text{ mM}$; $\text{pH} = 4.3$ (100 mM NaH_2PO_4), $T \sim 25^\circ\text{C}$. The error bars represent standard deviations from three measurements. The lines between the data points are drawn for guiding the eyes.