Electronic Supporting Information for

Mechanistic aspects of the horseradish peroxidase-catalysed polymerisation of aniline in the presence of AOT vesicles as templates

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1. NMR and MS analysis of 4-deuteroaniline and 2,6-dideuteroaniline

4-deuteroaniline

\[
\begin{array}{c}
\text{NH}_2 \\
\text{H} \\
\text{H} \\
\text{D} \\
\text{H} \\
\text{H}
\end{array}
\]

4-deuteroaniline

(a)

\(^1\)H NMR (CDCl\(_3\)) \(\delta/\text{ppm}: 7.20 (\text{d, } J = 7.8 \text{ Hz} , 2\text{H, meta}); 6.73 (\text{d, } J = 8.4 \text{ Hz} ,2\text{H, ortho}); 3.63 (\text{br. s, } 2\text{H, amine}).\)
13C NMR (CDCl₃) δ/ppm: 146.61; 129.50; 118.66 (t, J = 24.9 Hz); 115.45.

HRMS (Magnet EI⁺) calculated for C₆H₆DN[M]^+: 94.0641, found 94.0633.

Fig. S1:
1H NMR spectrum (a), 13C NMR spectrum (b) and MS analysis (c) of 4-deuteroaniline.
Solvent for the NMR spectra: CDCl₃.
2,6-dideuteroaniline

\[
\begin{align*}
\text{H NMR (CDCl}_3\text{) } & \delta/\text{ppm: } 7.20 \ (d, \ J = 7.5 \ \text{Hz}, \ 2\text{H, meta}); \\
& 6.80 \ (t, \ J = 7.4 \ \text{Hz}, \ 1\text{H, para}), \\
& 3.62 \ (\text{br. s, } 2\text{H, amine}).
\end{align*}
\]
$^{13}\text{C NMR (CDCl}_3\text{)}\ \delta/\text{ppm}: \ 146.53; \ 129.50; \ 118.89; \ 115.17 \ (t, \ J = 23.9 \text{ Hz}).$

HRMS (Magnet EI$^+$) calculated for C$_6$H$_5$D$_2$N [M$^+$]: 95.0704, found 95.0701.

**Fig. S2:**

$^1\text{H NMR spectrum (a), }^{13}\text{C NMR spectrum (b) and MS analysis (c) of 2,6-dideuteroaniline.}$

Solvent for the NMR spectra: CDCl$_3$. 

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2. Calibration curve for the determination of unreacted aniline

Defined amounts of aniline were added to the reaction system, followed by addition of the reaction system (30 μL) to acetonitrile (1470 μL). After centrifugation, the UV/VIS spectrum of the supernatant was measured and $A_{238}$ was plotted against aniline concentration, assuming complete extraction of aniline into acetonitrile.

**Figure S3:**

Calibration curve for the quantitative determination of aniline, extracted from the reaction system into acetonitrile.
3. Calibration curve for the determination of H$_2$O$_2$ with the Ti-TPyp assay

Figure S4:
Quantification of H$_2$O$_2$ with the Ti-TPyp assay.\textsuperscript{7} Ti-TPyP stands for oxo[5,10,15,20-tetra(4-pyridyl)porphyrinato]titanium(IV).

a): The absorption spectrum of the assay solution was measured as a function of H$_2$O$_2$ concentration, [Ti]$_{\text{total}}$ = 6.25 μM. The arrows indicate the changes in the spectrum with increase in H$_2$O$_2$ concentration.

b): ΔA$_{432}$ = A$_{432}$ (blank) – A$_{432}$ (sample, i.e. in presence of H$_2$O$_2$) is plotted as a function of [H$_2$O$_2$] in the final assay solution. A linear regression was made for [H$_2$O$_2$] = 0.1 – 4.0 μM.
4. Pinacyanol chloride test for the detection of surfactant aggregate formation in aqueous solution

![Graph showing absorbance at 606 nm against sulfosuccinate concentration for Sodium di-n-hexylsulfosuccinate and Sodium di-n-butylsulfosuccinate.]

**Figure S5:**
Pinacyanol chloride test for the detection of aggregate formation in aqueous solution, pH = 4.3, 0.1 M NaH₂PO₄. [Pinacyanol chloride] = 2.9 μM.

▲: Sodium di-n-butylsulfosuccinate
■: Sodium di-n-hexylsulfosuccinate
5. Stoichiometric equations for the chemical polymerisation of aniline into the emeraldine salt form of PANI with \((\text{NH}_4)_2\text{S}_2\text{O}_8\) as oxidant

**Scheme S1:**
Stoichiometric equation for the chemical polymerisation of aniline into the emeraldine salt form of PANI with the peroxydisulfate anion as oxidant; the bipolaron state of the emeraldine salt form of PANI is shown (see Scheme 2).

a) Reaction formulated with the neutral form of aniline (Ar-NH$_2$).

![Diagram of the reaction](image)

b) Reaction formulated with the anilinium cation (Ar-NH$_3^+$).

![Diagram of the reaction](image)

c) The chemical polymerisation of aniline often is carried out with \((\text{NH}_4)_2\text{S}_2\text{O}_8\) as oxidant in strongly acidic solution (1 M HCl)\(^1\) with Ar-NH$_3^+$Cl$^-$, therefore – in absence of any template/dopant - the counter ions of the emeraldine salt mainly are Cl$^-$.
6. Reaction kinetics in the presence of AOT vesicles

**Figure S6:**
Overall time dependent changes of the UV/VIS/NIR absorption spectrum during the HRP-catalysed polymerisation of aniline in the presence of AOT vesicles carried out at $T = 25 \, ^\circ\text{C}$; $[\text{AOT}] = 3.0 \, \text{mM}$; $[\text{aniline}] = 4.0 \, \text{mM}$; $[\text{HRP}] = 0.92 \, \mu\text{M}$; $[\text{H}_2\text{O}_2] = 4.5 \, \text{mM}$; pH = 4.3 (0.1 M H$_2$PO$_4^-$), path length: 0.1 cm. Absorption spectrum of the reaction system as a function of reaction time; the first spectrum shown was recorded 5 min after start of the reaction (after addition of H$_2$O$_2$); the following spectra were recorded in intervals of 5 min; the arrows indicate the direction of the changes of the intensities with time; the last spectrum was recorded after 24 hours. The discontinuity at 840 nm is due to an artefact of the instrument.
Figure S7:

Initial phase of the time dependent changes of the UV/VIS/NIR absorption spectrum during the HRP-catalysed polymerisation of aniline in the presence of AOT vesicles carried out at T = 25 °C; [AOT] = 3.0 mM; [aniline] = 4.0 mM; [HRP] = 0.92 μM; [H₂O₂] = 4.5 mM; pH = 4.3 (0.1 M H₂PO₄⁻), path length: 0.1 cm. Absorption spectrum of the reaction system as a function of reaction time; the first spectrum shown was recorded 26 s after start of the reaction (after addition of H₂O₂); the following spectra were recorded in intervals of 1.5 s; the arrow indicates the direction of the change of the intensity with time; the upper most spectrum was recorded after 45.5 s. Instrument used: diode array spectrophotometer (Specord S 600 from Analytik Jena AG).
7. Effect of the AOT concentration of the absorption spectrum of the reaction system after reaching reaction equilibrium (t = 24 h)

Figure S8:
HRP/H$_2$O$_2$-catalysed polymerisation of aniline at pH = 4.3, 0.1 M NaH$_2$PO$_4$, room temperature. Influence of the AOT concentration on the UV/VIS/NIR absorption spectrum of the reaction system at reaction equilibrium (t = 24 h). [HRP] = 0.92 μM, [aniline] = 4.0 mM, [H$_2$O$_2$] = 4.5 mM. The determined reaction yields were 90.4 % (for 2 mM AOT), 90.7 % (for 3 mM), 94.0 % (for 4 mM), and 92.6 % (for 5 mM).
8. Stability of HRP in absence and presence of H$_2$O$_2$ as a function of time

Figure S9:
Changes of the HRP activity during storage at T = 25 °C in the presence of 4.5 mM H$_2$O$_2$ or in the absence of H$_2$O$_2$. [HRP] = 0.92 μM, pH = 4.3 (0.1 M NaH$_2$PO$_4$), T = 25 °C. The activity of HRP was measured with ABTS$^2$ as substrate, [ABTS$^2$]$_0$ = 0.25 mM, [H$_2$O$_2$]$_0$ = 0.05 mM, pH = 6.0, T = 25 °C, see Materials and methods.

■: 0.92 μM HRP
▲: 0.92 μM HRP, 3 mM AOT
◆: 0.92 μM HRP, 3 mM AOT, 4 mM aniline, 4.5 mM H$_2$O$_2$
▲: 0.92 μM HRP, 3 mM AOT, 4.5 mM H$_2$O$_2$
★: 0.092 μM HRP, 3 mM AOT, 4 mM aniline, 4.5 mM H$_2$O$_2$
●: 0.225 μM HRP, 3 mM AOT, 4 mM aniline, 4.5 mM H$_2$O$_2$
●: 1.8 μM HRP, 3 mM AOT, 4 mM aniline, 4.5 mM H$_2$O$_2$
9. Stability of HRP which was added to the reaction system after reaching reaction equilibrium (t = 18 h) as a function of time

Aniline first was polymerised by HRP/H$_2$O$_2$ in the presence of AOT vesicles under the optimal reaction conditions until the green emeraldine salt form of polyaniline formed (reaction time t = 18 h). Afterwards, a small portion of the reaction system was removed and tested for remaining HRP activity with ABTS and H$_2$O$_2$ as substrates. In agreement with data presented in Fig. S6, no active HRP was present anymore. To this reaction system, a new HRP solution was added and the activity was again measured as a function of time. The results are plotted in Fig. S10, indicating that the added HRP was rapidly inactivated.

**Reaction mixture**

*Before* new HRP addition (see 2.4. for details):
714.4 μL sodium dihydrogen phosphate solution (0.1 M, pH = 4.3)
150 μL AOT vesicle suspension (20 mM)
99.8 μL aniline solution (40 mM)
13.6 μL HRP solution (67.45 μM, spectrophotometrically determined)
22.5 μL H$_2$O$_2$ solution (200 mM)
Total reaction volume: 1 mL
3 mM AOT, 4 mM aniline, 0.92 μM HRP, 4.5 mM H$_2$O$_2$, pH = 4.3 (0.1 M NaH$_2$PO$_4$)

*After* t = 18 h at T = 25 °C:
addition of 13.6 μL HRP solution (67.45 μM)

**Activity measurements** (see 2.5. for details):
2692 μL 0.1 M sodium phosphate solution (pH = 6)
150 μL ABTS solution (5 mM)
8 μL reaction solution
150 μL H$_2$O$_2$ solution (1 mM)
Total assay volume: 3 mL
250 μM ABTS, 50 μM H$_2$O$_2$, 2.45 nM HRP (or 4.9 nM HRP)
T = 25 °C
Figure S10:
Time dependent changes of the relative activity of HRP which was *newly added* to a reaction mixture which reached reaction equilibrium. The activity measurements were made with ABTS$^2-$ (0.250 mM) and H$_2$O$_2$ (0.050 mM) as substrates at pH = 6.0. See text for details.
10. FTIR spectrum of the reaction products and of commercial polyaniline

The reaction products obtained were isolated by acetone precipitation and washing with water and then 1 M HCl, as described in Materials and methods. The commercial polyaniline samples were analysed as received. For all FTIR spectra, KBr pills containing the products were pressed and analysed.

(a)

(b)

Figure S11:

a) FTIR spectra (1800 – 400 cm$^{-1}$) of the reaction products isolated after a reaction time of 48 hours at the optimal reaction conditions at $T = 25$ °C in the presence of AOT vesicles ([AOT] = 3.0 mM) or in the absence of AOT; [aniline] = 4.0 mM; [HRP] = 0.92 μM; [H$_2$O$_2$] = 4.5 mM; pH = 4.3 (0.1 M H$_2$PO$_4$).
b) FTIR spectra (1800 – 400 cm\(^{-1}\)) of commercial polyaniline samples, emeraldine base (EB) with \(M_w = 10,000\) g/mol and \(M_w = 65,000\) g/mol and emeraldine salt (ES) with \(M_w > 15,000\) g/mol, all from Sigma-Aldrich.

For the tentative assignment of the peaks, see Dmitrieva and Dunsch (2011)\(^{10}\) and references cited therein. Some of the main peaks present in the product isolated from the reaction mixtures containing AOT vesicles (a) are assigned as follows:

\[
\approx 1580\ \text{cm}^{-1}: \nu (\text{C}=\text{C}) \text{ in } N=Q=N \\
\approx 1500\ \text{cm}^{-1}: \nu (\text{C}=\text{C}) \text{ in } N–B–N \\
\approx 1296\ \text{cm}^{-1}: \nu (\text{C}–\text{N}) \text{ in secondary aromatic amines or N-H bending} \\
\approx 1236\ \text{cm}^{-1}: \nu (\text{C}–\text{N}^{+}) \text{ in polarons} \\
\approx 1129\ \text{cm}^{-1}: \delta (\text{C}–\text{H}) \text{ of } Q=N^+\text{H–B or B–NH–B} \\
\approx 800\ \text{cm}^{-1}: \gamma (\text{C}–\text{H}) \text{ in 1,4-disubstituted ring or NH}_2^+ \text{ rocking} \\
\approx 502\ \text{cm}^{-1}: \text{out-of-plane ring deformation vibrations in 1,4-di- or monosubstituted ring}
\]

The bands present at \(\approx 1718\ \text{cm}^{-1}\) and \(\approx 1033\ \text{cm}^{-1}\) in the product isolated from the reaction mixture containing AOT are probably due to \(\nu (\text{C}=\text{O})\) and \(\nu (\text{S}=\text{O})\) of AOT.

Comment: Two intensive bands located at \(1072\ \text{cm}^{-1}\) and \(933\ \text{cm}^{-1}\) were previously found to be present in the FTIR spectrum of a product isolated from a reaction mixture containing AOT vesicles;\(^6\) these peaks were not present anymore in the samples prepared in this work for the reaction carried out under optimal conditions in presence or absence of AOT.

According to Dmitrieva and Dunsch (2011),\(^{10}\) IR bands at \(\approx 1470\ \text{cm}^{-1}, \approx 1414\ \text{cm}^{-1},\)
\(\approx 950\ \text{cm}^{-1}, \approx 830\ \text{cm}^{-1}, 750\ \text{cm}^{-1},\) and \(\approx 600\ \text{cm}^{-1}\) are attributed to phenazine rings present in the polyaniline chain. Bands with high intensity in these regions of the FTIR spectrum could not be detected, indicating that the amount of phenazine rings must be low, although their presence can not be excluded completely.

Abbreviations:
\(\nu:\) stretching; \(\delta:\) in-plane bending; \(\gamma:\) out-of-plane bending; \(B:\) benzene ring; \(Q:\) quinoid ring
11. Control measurements: no AOT

Figure S12:
(a) Time dependent changes of the UV/VIS/NIR absorption spectrum during the HRP-catalysed polymerisation of aniline in the absence of AOT vesicles carried out at $T = 25^\circ C$; [aniline] = 4.0 mM; [HRP] = 0.92 μM; [H$_2$O$_2$] = 4.5 mM; pH = 4.3 (0.1 M H$_2$PO$_4$), path length: 0.1 cm. Absorption spectrum of the reaction system as a function of reaction time; the first spectrum was recorded immediately after starting the reaction (after addition of H$_2$O$_2$); the following spectra were recorded in intervals of 3 min (until 147 min); the arrows indicate the direction of the changes of the intensities with time. Product precipitation occurred during the reaction, increasing the turbidity of the reaction system.

(b) UV/VIS/NIR absorption spectrum of the supernatant, obtained after removal of the precipitate by centrifugation. Path length: 0.1 cm
Figure S13:

*Initial* phase of the time dependent changes of the UV/VIS/NIR absorption spectrum during the HRP-catalysed polymerisation of aniline in the *absence of AOT vesicles* carried out at \( T = 25 \, ^\circ\text{C} \); [aniline] = 4.0 mM; [HRP] = 0.92 μM; [H\(_2\)O\(_2\)] = 4.5 mM; pH = 4.3 (0.1 M H\(_2\)PO\(_4\)), path length: 0.1 cm. Absorption spectrum of the reaction system as a function of reaction time; the first spectrum shown was recorded 26 s after start of the reaction (after addition of H\(_2\)O\(_2\)); the following spectra were recorded in intervals of 1.5 s; the arrow indicates the direction of the change of the intensity with time; the upper most spectrum was recorded after 45.5 s. Instrument used: diode array spectrophotometer (Specord S 600 from Analytik Jena AG).
12. Changes of the amount of remaining aniline during the polymerisation reaction

**Figure S14:**
Changes of the relative amount of remaining aniline during the HRP/H$_2$O$_2$-catalysed polymerisation of aniline under different experimental conditions at pH = 4.3 (0.1 M NaH$_2$PO$_4$), room temperature.

- ▲: 3 mM AOT (vesicles), 4.5 mM H$_2$O$_2$, 0.92 µM HRP
- ●: 3 mM AOT (vesicles), 4.5 mM H$_2$O$_2$, 0.092 µM HRP
- ▼: no AOT, 4.5 mM H$_2$O$_2$, 0.92 µM HRP
- ■: 3 mM di-$n$-butylsulfosuccinate (no vesicles), 4.5 mM H$_2$O$_2$, 0.92 µM HRP
13. Control measurements: no H$_2$O$_2$

Figure S15:
UV/VIS/NIR absorption spectrum of a reaction mixture that did not contain H$_2$O$_2$, T = 25 °C; [AOT] = 3.0 mM; [aniline] = 4.0 mM; [HRP] = 0.92 μM; pH = 4.3 (0.1 M H$_2$PO$_4^-$), path length: 0.1 cm. The spectrum was recorded after preparation of the reaction mixture (1) and after 24 h (2).
14. Control measurements: no HRP

**Figure S16:**
UV/VIS/NIR absorption spectrum of a reaction mixture that *did not contain HRP*, T = 25 °C; [aniline] = 4.0 mM; [H₂O₂] = 4.5 mM; pH = 4.3 (0.1 M H₂PO₄), path length: 0.1 cm. The spectrum was recorded after preparation of the reaction mixture (t = 0), after 24 h or after 48 h, either in the absence of AOT or in the presence of 3 mM AOT vesicles. Differences in the two set of absorption spectra are due to the turbidity caused by the vesicles.
15. Reaction with non-aggregated sodium di-\(n\)-butylsulfosuccinate instead of AOT vesicles

**Figure S17:**

Time dependent changes of the UV/VIS/NIR absorption spectrum during the HRP-catalysed polymerisation of aniline in the presence of di-\(n\)-butylsulfosuccinate carried out at \(T = 25\, ^{\circ}\)C; [di-\(n\)-butylsulfosuccinate] = 3.0 mM; [aniline] = 4.0 mM; [HRP] = 0.92 \(\mu\)M; \([\text{H}_2\text{O}_2]\) = 4.5 mM; pH = 4.3 (0.1 M H\(_2\)PO\(_4\)), path length: 0.1 cm. Absorption spectrum of the reaction system as a function of reaction time; the first spectrum was recorded 3 min after starting the reaction (after addition of \(\text{H}_2\text{O}_2\)); the following spectra were recorded in intervals of 3 min (until 15 min); the arrows indicate the directions of the change of the intensity with time. *Product precipitation* occurred during the reaction.
Figure S18:

*Initial* phase of the time dependent changes of the UV/VIS/NIR absorption spectrum during the HRP-catalysed polymerisation of aniline in the presence of di-n-butylsulfosuccinate carried out at $T = 25 \, ^\circ\text{C}; [\text{di-n-butylsulfosuccinate}] = 3.0 \, \text{mM}; [\text{aniline}] = 4.0 \, \text{mM}; [\text{HRP}] = 0.92 \, \mu\text{M}; [\text{H}_2\text{O}_2] = 4.5 \, \text{mM}; \text{pH} = 4.3 \,(0.1 \, \text{M H}_2\text{PO}_4^-)$, path length: 0.1 cm.

Absorption spectrum of the reaction system as a function of reaction time; the first spectrum shown was recorded 28 s after start of the reaction (after addition of H$_2$O$_2$); the following spectra were recorded in intervals of 1.5 s; the arrow indicates the direction of the changes of the intensities with time; the uppermost spectrum was recorded after 46 s. Instrument used: diode array spectrophotometer (Specord S 600 from Analytik Jena AG).
16. The “Nonclassical or reactivation chain polymerisation mechanism”

HRP-catalysed oxidation of aniline:

\[
2 \text{NH}_2 - \text{H}_2\text{O}_2 \rightarrow 2 \text{NH}_3 + 2\text{H}_2\text{O}
\]

Protonation of aniline radical:

\[
2 \text{NH}^+ + 2\text{H}^+ \rightarrow 2 \text{NH}_2^+
\]

PADPA formation:

\[
2 \text{NH}_2 \rightarrow \text{PADPA}
\]

PSQ formation:

\[
\text{PADPA} + \text{H}_2\text{O}_2 + 2\text{H}^+ \rightarrow \text{PSQ} + 2\text{H}_2\text{O}
\]

One aniline addition to PSQ:

\[
\text{PSQ} + \text{NH}_2 \rightarrow \text{reduced form of trimer}
\]

Partial oxidation of reduced form of trimer:

\[
\text{reduced form of trimer} + \text{H}_2\text{O}_2 + 2\text{H}^+ \rightarrow \text{partially oxidised form of trimer}
\]

One aniline addition to partially oxidised form of trimer:

\[
\text{partially oxidised form of trimer} + \text{NH}_2 \rightarrow \text{reduced form of tetramer}
\]

Partial oxidation of reduced form of tetramer:

\[
\text{reduced form of tetramer} + \text{H}_2\text{O}_2 + 2\text{H}^+ \rightarrow \text{half-oxidised form of tetramer}
\]

**Overall reaction for the formation of a half-oxidised tetramer:**

\[
4 \text{NH}_2 + 4\text{H}_2\text{O}_2 + 2\text{H}^+ \rightarrow \text{half-oxidised form of tetramer} + 8\text{H}_2\text{O}
\]

**Overall reaction for the formation of half-oxidized oligomers:**

\[
4n \text{NH}_2 + (3n+1)\text{H}_2\text{O}_2 + 2n\text{H}^+ \rightarrow \text{half-oxidised form of oligomer} + (10n+2)\text{H}_2\text{O}
\]
Scheme S2:
Possible individual steps of the HRP/H₂O₂-catalysed polymerisation of aniline via polymer chain elongation if the reaction would follow the “nonclassical or reactivation chain polymerisation mechanism” proposed by Wei et al.² for the chemical polymerisation of aniline. The key step in this mechanism is the addition of a neutral aniline monomer to the oxidised (activated) end of the chain (iminium or nitrenium ions).

In the reaction steps listed below, it is assumed that HRP is only involved at the beginning of the reaction through the formation of two anilino radicals to trigger the growth of the polymer chain. Chain growth occurs through the neutral form of aniline (not oxidised). The overall stoichiometry of the reaction to obtain one repeating unit of the emeraldine salt form of PANI is given at the end (half-oxidised form of tetramer). The stochiometric equation for obtaining the half-oxidised form of oligomers is also given (see Scheme 4).

PADPA: p-aminodiphenylamine = (N-phenyl-1,4-phenylenediamine)
PBQ: N-phenyl-1,4-benzoquinonediimine

Comment:
There is no support for this mechanism to take place in the HRP/H₂O₂ – AOT system investigated.
17. Reaction mixtures containing PADPA, aniline and H₂O₂ in presence of AOT vesicles

Figure S19:
To mimic a possible intermediate reaction situation in the HRP-catalysed polymerisation of aniline and to test whether oligomer and polymer chain growth is possible without HRP, the aniline dimer PADPA was incubated with aniline in the presence of H₂O₂ and AOT vesicles without HRP at pH = 4.3 (0.1 M H₃PO₄) and T = 25 °C. UV/VIS/NIR absorption spectra before H₂O₂ addition (t = 0) and 48 h after H₂O₂ addition were recorded for the following initial conditions.

(a) [PADPA] = 0.08 mM; [aniline] = 3.84 mM; [H₂O₂] = 4.42 mM; [AOT] = 3.0 mM. Spectrum 1 was recorded after t = 48 h.
(b) [PADPA] = 0.2 mM; [aniline] = 3.60 mM; [H₂O₂] = 4.30 mM; [AOT] = 3.0 mM. Spectrum 2 was recorded after t = 48 h.

The reported pKₐ values for the protonated forms of aniline⁸ and PADPA⁹ are 4.6 and 4.7 (in CH₃CN:H₂O, 1:1 v/v).
18. The “Radical cation mechanism”

HRP-catalysed oxidation of aniline:

\[
\text{4 } \text{NH}_2 + 2 \text{H}_2\text{O}_2 \rightarrow 4 \text{NH} + 4 \text{H}_2\text{O}
\]

Protonation of aniline radical:

\[
\text{4 } \text{NH} + 4 \text{H}^+ \rightarrow 4 \text{NH}_2^+
\]

PADPA formation:

\[
\text{2 } \text{PADPA} \rightarrow \text{PADPA} + \text{H}_2\text{O}
\]

PBQ formation:

\[
\text{PADPA} + \text{H}_2\text{O}_2 + 2 \text{H}^+ \rightarrow \text{PADPA} + \text{PBQ} + \text{2H}_2\text{O}
\]

One anilinium radical cation addition to PBQ:

\[
\text{PBQ} + \text{NH}_3^+ \rightarrow \text{radical cation of trimer}
\]

Partial oxidation of radical cation of trimer:

\[
\text{radical cation of trimer} + \text{H}_2\text{O}_2 + 2 \text{H}^+ \rightarrow \text{partially oxidized radical cation of trimer} + \text{2H}_2\text{O}
\]

One anilinium radical cation addition to partially oxidized radical cation of trimer:

\[
\text{partially oxidized radical cation of trimer} + \text{NH}_3^+ \rightarrow \text{half-oxidized form of tetramer}
\]

OVERALL REACTION FOR THE FORMATION OF A HALF-OXIDIZED TETRAMER:

\[
\text{4 } \text{NH}_2 + 4 \text{H}_2\text{O}_2 + 2 \text{H}^+ \rightarrow \text{half-oxidized form of tetramer} + \text{8H}_2\text{O}
\]

OVERALL REACTION FOR THE FORMATION OF HALF-OXIDIZED OLIGOMERS:

\[
\text{4n } \text{NH}_2 + (5n+1) \text{H}_2\text{O}_2 + 2n \text{H}^+ \rightarrow \text{half-oxidized form of oligomer} + (10n+2) \text{H}_2\text{O}
\]
Scheme S3:
Possible individual steps of the HRP/H₂O₂-catalysed polymerisation of aniline *via* polymer chain elongation according to the “*radical cation mechanisms*”, originally proposed by Genies and Tsintavis³ and Ding et al.⁴ for the electrochemical polymerisation of aniline. The key step in this mechanism is the *addition of an anilinium radical cation* to the oxidised radical cation of the growing chain.

In the reaction steps listed below, it is assumed that HRP is involved in the reaction by providing anilinium radical cations for the initial formation of PADPA as well as for the growth of the chain. Chain growth occurs through the oxidised form of aniline, i.e. the anilinium radical cation. The overall stoichiometry of the reaction to obtain one repeating unit of the emeraldine salt form of PANI is given at the end (half-oxidised form of tetramer). The stochiometric equation for obtaining the half-oxidised form of oligomers is also given (see Scheme 4).

PADPA: *p*-aminodiphenylamine = \((N\text{-phenyl}-1,4\text{-phenylenediamine})\)

PBQ: *N*-phenyl-1,4-benzequinonediimine

Comment:
There is experimental evidence supporting this mechanism to take place in the HRP/H₂O₂ – AOT system investigated.
19. Reactions with (NH₄)₂S₂O₈ as oxidant instead of HRP/H₂O₂

**Figure S20:**
UV/VIS/NIR absorption spectrum of the supernatant solution obtained upon reacting aniline with (NH₄)₂S₂O₈, either in the presence of AOT vesicles (spectrum 1, 3.0 mM AOT) or in the absence of AOT (spectrum 2). The reaction was carried out at T = 25 °C by using the following initial conditions: [aniline] = 4.0 mM; [(NH₄)₂S₂O₈] = 4.5 mM; pH = 4.3 (0.1 M H₂PO₄⁻). The reaction time was 48 hours. The reaction turned to yellow-brown and some of the products obtained precipitated. These precipitates were removed by centrifugation and the spectrum of the supernatant solution was recorded by using a cell with a path length of 0.1 cm.
20. Calculation of the expected pH change during polymerisation

For the optimal conditions, the expected change in pH during polymerisation was estimated by using the following data and assumptions.

**Optimal conditions** at the beginning of the reaction:

\[
\text{[aniline]}_{\text{total}} = [\text{Ar-NH}_2] + [\text{Ar-NH}_3^+] = 4.0 \text{ mM}
\]

\[
\text{pK}_a (\text{Ar-NH}_3^+) = 4.6
\]

\[
\text{pH} = 4.3 = \text{pH}_{\text{start}}
\]

\[
[H_2PO_4^-] + [H_3PO_4] = 0.1 \text{ M}
\]

\[
\text{pK}_{a1} (H_3PO_4) = 2.15
\]

**Room temperature**

Assumption: all activity coefficients \( \gamma_i = 1 \).

At \( \text{pH}_{\text{start}} = 4.30 \): \([H_2PO_4^-] \gg [H_3PO_4] \)

\[
\text{pH}_{\text{start}} = \text{pK}_{a1} (H_3PO_4) + \log ([H_2PO_4^-] / [H_3PO_4]) = 2.15 + \log ([H_2PO_4^-] / [H_3PO_4]) = 4.30
\]

\[
\rightarrow \frac{[H_2PO_4^-]}{[H_3PO_4]} = 1.4125 \cdot 10^2
\]

\[
\rightarrow [H_2PO_4^-] = 9.9297 \cdot 10^{-2} \text{ M and } [H_3PO_4] = 7.0299 \cdot 10^{-4} \text{ M}
\]

For a net release of 0.68 mM \( H^+ \) during the reaction (100 % reaction yield), \( \approx 0.68 \text{ mM} \) \( H_2PO_4^- \) are getting protonated.

\[
\rightarrow [H_3PO_4] = 7.0299 \cdot 10^{-4} \text{ M} + 6.8 \cdot 10^{-4} \text{ M} = 1.3830 \cdot 10^{-3} \text{ M}
\]

\[
\rightarrow [H_2PO_4^-] = 9.9297 \cdot 10^{-2} \text{ M} - 6.8 \cdot 10^{-4} \text{ M} = 9.9249 \cdot 10^{-2} \text{ M}
\]

\[
\rightarrow \text{pH}_{\text{end}} = 2.15 + \log (9.9249 \cdot 10^{-2} / 1.3830 \cdot 10^{-3}) = 2.15 + 1.86 = 4.01
\]

For 100 % reaction yield, the expected drop in pH is 0.29, from \( \text{pH}_{\text{start}} = 4.30 \) to \( \text{pH}_{\text{end}} = 4.01 \)

Accordingly, for 90 % reaction yield: \( \text{pH}_{\text{end}} = 4.03 \)
21. Geometric considerations of the AOT vesicles

The average number of AOT molecules in the outer and inner monolayers of a unilamellar vesicle with a diameter of 80 nm was calculated based on the assumptions listed.

AOT average head group area: \(567 \text{ Å}^2 = 0.67 \text{ nm}^2\)

Bilayer thickness: \(19.5 \text{ Å}^2 \approx 20 \text{ Å} = 2 \text{ nm}\)

Outer vesicle radius: \(r_o = 40 \text{ nm}\); outer vesicle surface, \(4\pi r_o^2 = 2.0106 \times 10^4 \text{ nm}^2\)

Inner vesicle radius: \(r_o - 2 \text{ nm} = 38 \text{ nm}\); inner vesicle surface, \(4\pi r_i^2 = 1.8146 \times 10^4 \text{ nm}^2\)

Calculated number of AOT molecules in the outer monolayer, \(N_{AOT,o} = 3.0009 \times 10^4 (53\%)\)
Calculated number of AOT molecules in the outer monolayer, \(N_{AOT,i} = 2.7083 \times 10^4 (47\%)\)
Total number of AOT molecules per vesicle, \(N_{AOT,\text{total}} = N_{AOT,o} + N_{AOT,i} = 5.7092 \times 10^4 (100\%)\)

22. Calculated AOT-vesicle concentration for the optimal conditions

\([\text{AOT}]_{\text{total}} = 3.0 \text{ mM}\)

Concentration of non-associated AOT molecules at pH 4.3 (0.1 M H\(_2\)PO\(_4\)):\(^6\)

\([\text{AOT}]_{\text{in solution}} \approx 0.4 \text{ mM}, (= \text{critical concentration for vesicle formation})\)

\([\text{AOT}]_{\text{as vesicle membrane}} = 3.0 \text{ mM} - 0.4 \text{ mM} = 2.6 \text{ mM}\)

\([\text{vesicle}] = (2.6 \times 10^{-3} \text{ mol·L}^{-1}) / (5.7092 \times 10^4) = 4.5541 \times 10^{-8} \text{ M} = 46 \text{ nM}\)

23. Calculated number of HRP molecules per vesicle for the optimal conditions

\([\text{HRP}] = 0.92 \mu\text{M}\)

\([\text{HRP}] / [\text{vesicle}] = 920 \text{ nM} / 46 \text{ nM} = 20\)

If all added HRP molecules would bind to the vesicles, every vesicle would have 20 bound HRP molecules on the outer vesicle surface, i.e. one HRP molecule per 1500 AOT molecules (= \(N_{AOT,o} / 20 = 3.0009 \times 10^4 / 20\)).
24. HRP activity measurements with ABTS\textsuperscript{2-} as substrate

For a direct comparison with other commercial peroxidase samples, the activity of the used horseradish peroxidase isoenzyme C (HRPC Grade I, 280 purpurogallin U/mg, RZ ≥ 3, Lot. number 0240160000 from Toyobo Enzymes) was determined spectrophotometrically with ABTS\textsuperscript{2-} as substrate. The details of the assay and the definition of one \textit{ABTS unit} are as follows.

Stock solutions
- Sodium phosphate solution (0.1 M, pH = 6.0)
- ABTS\textsuperscript{2-} stock solution: 5 mM, prepared by dissolving ABTS\textsuperscript{2-} (NH\textsubscript{4})\textsubscript{2} in sodium phosphate solution (0.1 M, pH = 6.0)
- HRP stock solution: 67.45 nM, prepared in sodium phosphate solution (0.1 M, pH = 4.3), taking into account $\varepsilon_{403} = 1.02 \cdot 10^5$ M\textsuperscript{-1} cm\textsuperscript{-1}\textsuperscript{[11]}
- H\textsubscript{2}O\textsubscript{2} stock solution: 1 mM, prepared in water from 30% H\textsubscript{2}O\textsubscript{2} (= 9.79 M)

Assay (total volume = 1.0 mL)
The following solutions were directly mixed in the sequence given in a quartz cuvette with a path length of 1 cm.
- 850 μl sodium phosphate solution (0.1 M, pH = 6.0)
- 50 μl ABTS\textsuperscript{2-} stock solution (final concentration: 250 μM)
- 50 μl HRP stock solution (final concentration: 3.37 nM)
- 50 μl H\textsubscript{2}O\textsubscript{2} stock solution (final concentration: 50 μM)

Directly after H\textsubscript{2}O\textsubscript{2} addition, the rate of ABTS\textsuperscript{+} formation was determined by measuring the increase in absorbance at $\lambda = 414$ nm every second during a period of $t = 1$ min at $T = 25$ °C. $\Delta A_{414}/\Delta t$ was taken as a measure for the activity of HRP ($\varepsilon_{414}(\text{ABTS}^+) = 3.6 \cdot 10^4$ M\textsuperscript{-1} cm\textsuperscript{-1}),\textsuperscript{[12]} Each measurement was carried out in triplicates.

\textbf{ABTS unit (ABTS U)}
We define one \textit{ABTS unit} as the amount of enzyme that oxidises 1 μmol ABTS\textsuperscript{2-} per minute under the conditions given above.

As a result, the HRPC used throughout the work (from Toyobo, Lot. number 0240160000) had an activity of 84.4±5.8 ABTS U/mg. This means that 3.89±0.27 ABTS U/ml were used in the reaction mixture for the polymerisation of aniline under the optimal conditions (0.92 μM).
25. References


