Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

How experimental details matter. The case of a laccase-catalysed oligomerisation reaction

Keita Kashima,[a,b] Tomoyuki Fujisaki,[a,b] Sandra Serrano-Luginbühl,[a] Abbos Khaydarov,[a] Reinhard Kissner,[c] Aleksandra Janošević Ležaić,[d] Danica Bajuk-Bogdanović,[e] Gordana Ćirić-Marjanović,[e] Lukas D. Schuler,[f] and Peter Walde *[a]

Affiliation:

[a] Department of Materials, ETH Zurich, Vladimir-Prelog-Weg 5, 8093 Zürich, Switzerland

[b] Department of Materials Chemistry and Bioengineering, National Institute of Technology, Oyama College, 771 Ohaza-Nakakuki, Oyama, Tochigi 323-0806, Japan

[c] Laboratory of Inorganic Chemistry, Department of Chemistry and Applied Biosciences, Vladimir-Prelog-Weg 2, 8093 Zürich, Switzerland

[d] University of Belgrade – Faculty of Pharmacy, Vojvode Stepe Street 450, 11221 Belgrade, Serbia

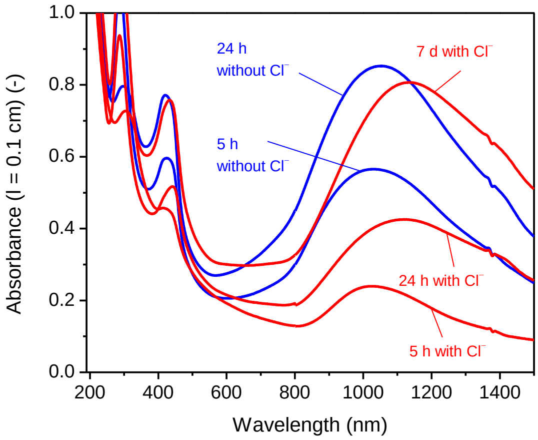
[e] University of Belgrade – Faculty for Physical Chemistry, Studentski trg 12-16, 11158 Belgrade, Serbia

[f] xirrus GmbH, Buchzelgstrasse 36, 8053 Zürich, Switzerland

Corresponding Author:

*E-mail: peter.walde@mat.ethz.ch

A)



B)

(B) Time course of A_{1000}

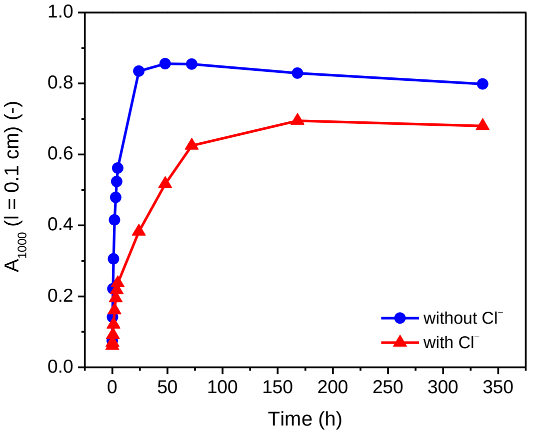
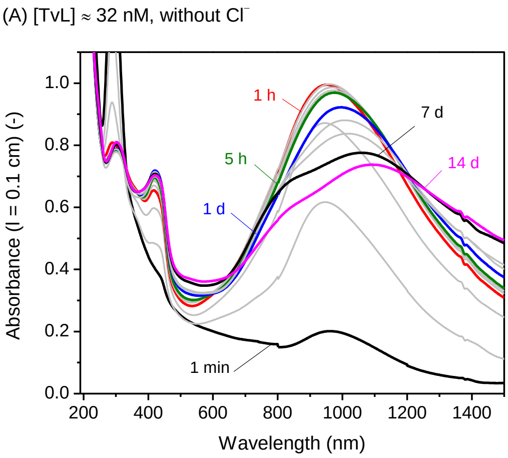


Fig. S-1. Direct comparison of the effect of chloride ions (≈2.4 mM) on the UV/vis/NIR absorption spectra of the reaction mixture recorded during the reaction with [TvL] ≈ 2.6 nM, $[PADPA]_0$ = 1.0 mM, [AOT] = 1.5 mM, $[H_2PO_4^-] + [H_3PO_4]$ = 0.1 M, pH = 3.5, and T = 25 °C. “Without Cl^-” means the use of the chloride-free “pH=3.5 solution” (*new optimal conditions*), “with Cl^-” means the use of the “pH=3.5 solution” containing chloride ions ($[Cl^-]$ ≈ 2.4 mM).

(A): Selected spectra taken from **Fig. 2** for t = 5 h, 24 h, and 7 d.

(B) Comparison of A_{1000} *vs.* t.

(A)



B)

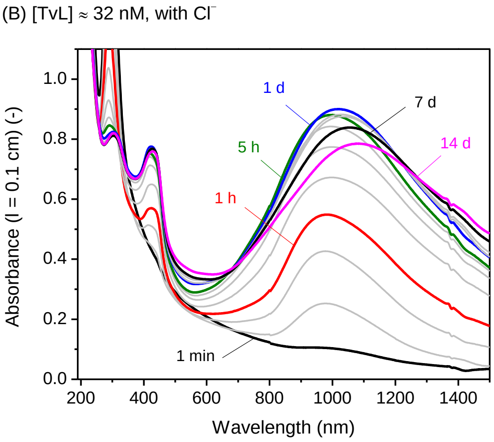
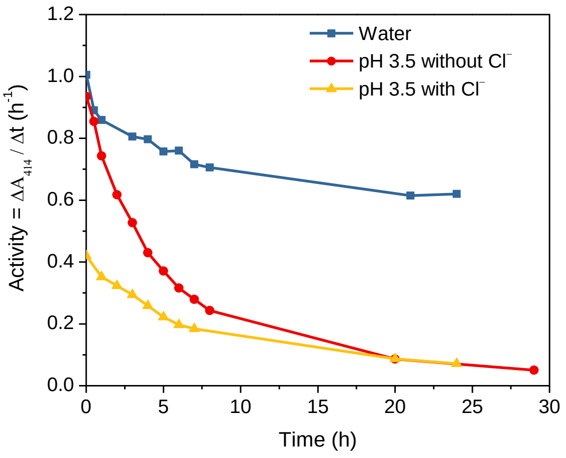


Fig. S-2. Comparison of the UV/vis/NIR absorption spectra of the two reaction mixtures recorded during the reaction with [TvL] ≈ 32 nM, $[PADPA]_0$ = 1.0 mM, [AOT] = 1.5 mM, $[H_2PO_4^-] + [H_3PO_4]$ = 0.1 M, pH = 3.5, and T ≈ 25 °C.

(A) Use of the chloride-free “pH=3.5 solution”. The grey lines are the spectra recorded after t = 10 min, 30 min, 2 h, 3 h, 4 h, 2 d, and 3d.

(B) Use of the “pH=3.5 solution” containing chloride ions (≈2.4 mM). These conditions were previously considered as optimal, see (Junker et al., 2014).[S1] The grey lines are the spectra recorded after t = 10 min, 30 min, 2 h, 3 h, 4 h, 2 d, and 3d.

(A) [TvL] ≈ 2.6 nM



(B) [TvL] ≈ 32 nM

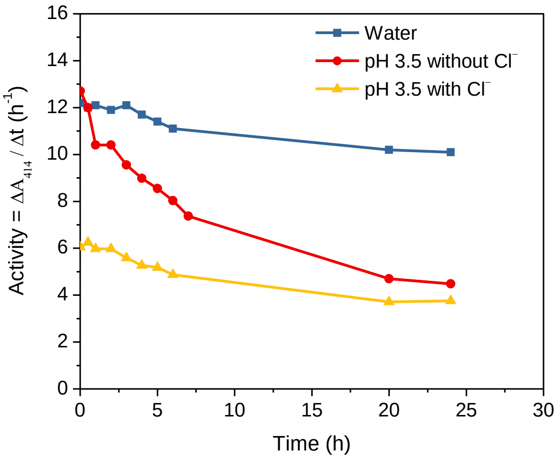


Fig. S-3. Stability of TvL dissolved either in deionized water (■), in the chloride-free “pH=3.5 solution” (●), or in the “pH=3.5 solution” containing chloride ions (≈2.4 mM) (▲), and stored at T ≈ 25 °C. For both “pH=3.5 solutions”, $[H_2PO_4^-] + [H_3PO_4] = 0.1$ M. The activity was measured with 0.25 mM $ABTS^{2-}$ at pH = 3.5 and T = 25 °C, as described before (Junker et al., 2014).[S1]

(A) [TvL] ≈ 2.6 nM.

(B) [TvL] ≈ 32 nM.

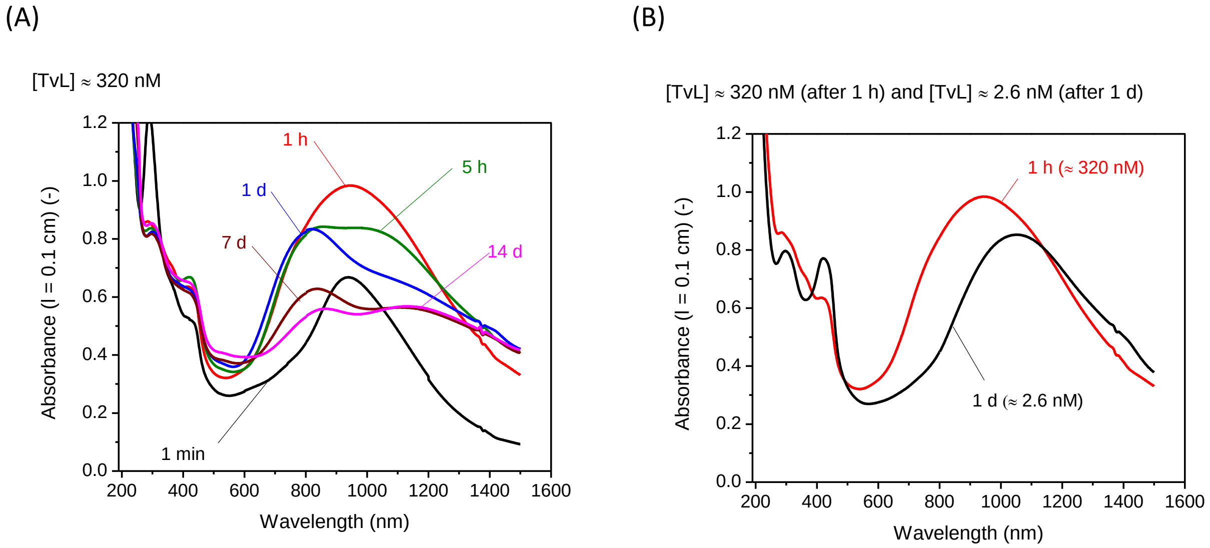


Fig. S-4. A: UV/vis/NIR absorption spectra of a reaction mixture recorded during the reaction with [TvL] ≈ 320 nM, $[PADPA]_0$ = 1.0 mM, [AOT] = 1.5 mM, $[H_2PO_4^-]$ + $[H_3PO_4]$ = 0.1 M, pH = 3.5, and T ≈ 25 °C. Use of chloride-free “pH=3.5 solution”. B: Comparison of the UV/vis/NIR spectra for [TvL] ≈ 320 nM (recorded after 1 h) – data taken from **Fig. S-4A** – and for [TvL] ≈ 2.6 nM (recorded after 1 d) – data taken from **Fig. 2A**.

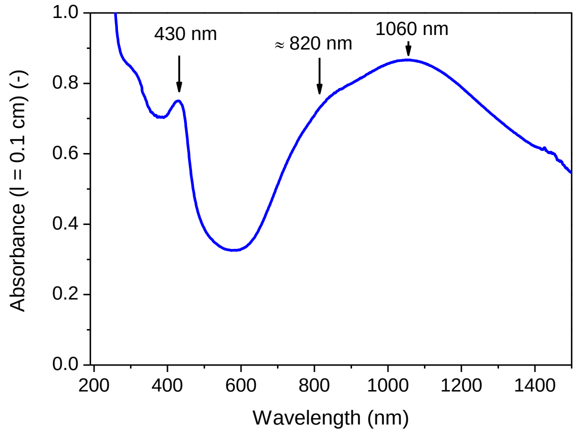
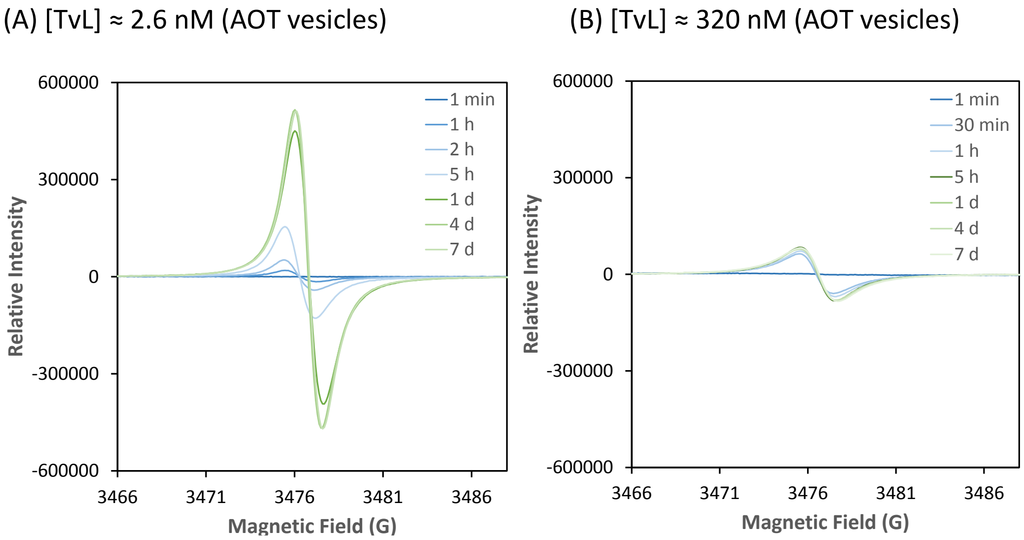


Fig. S-5. Example of the recorded UV/vis/NIR absorption spectrum of a reaction mixture after t = 24 h for the same conditions as described in the legend of **Fig. 3** for [TvL] ≈ 320 nM: $[PADPA]_0$ = 1.0 mM, [AOT] = 1.5 mM, $[H_2PO_4^-]$ + $[H_3PO_4]$ = 0.1 M, pH = 3.5, and T ≈ 25 °C; use of a chloride-free “pH=3.5 solution”.

In this spectrum, the highest absorption is at λ = 1060 nm and a shoulder is present at λ ≈ 820 nm. In the spectrum 2 of **Fig. 3**, the highest absorption is at λ = 817 nm and a shoulder is present at λ ≈ 1060 nm. Although in both cases the spectrum in this wavelength region is dominated by at least two absorption bands centered around λ ≈ 1060 nm and λ ≈ 820 nm, the ratio of the two bond intensities after t = 24 h shows great variations (please compare this spectrum with spectrum 2 of **Fig. 3**). The reason for such spectral differences is not clear.



(A) [TvL] ≈ 2.6 nM (AOT vesicles)

(B) [TvL] ≈ 320 nM (AOT vesicles)

Fig. S-6. EPR spectrum of two reaction mixtures recorded during the reaction with [TvL] ≈ 2.6 nM (A) or [TvL] ≈ 320 nM (B), $[PADPA]_0$ = 1.0 mM, [AOT] = 1.5 mM, $[H_2PO_4^-] + [H_3PO_4]$ = 0.1 M, pH = 3.5, and T ≈ 25 °C. Use of chloride-free “pH=3.5 solution”; ν = 9.766 GHz, $g_{final} = 2.0069 \pm 0.0002$ for both records; $g_{initial} = 2.0072 \pm 0.0002$ for [TvL] ≈ 2.6 nM (A).

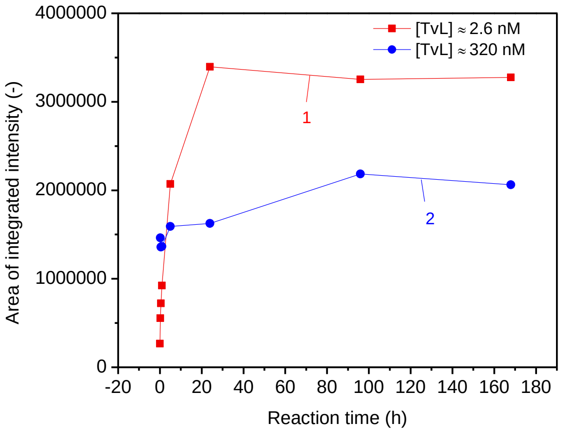


Fig. S-7. Comparison of the changes in the integrals of the EPR spectra of the reaction mixtures recorded during the reaction, as shown in **Fig. S-6**, for [TvL] ≈ 2.6 nM (**1**) or [TvL] ≈ 320 nM (**2**).

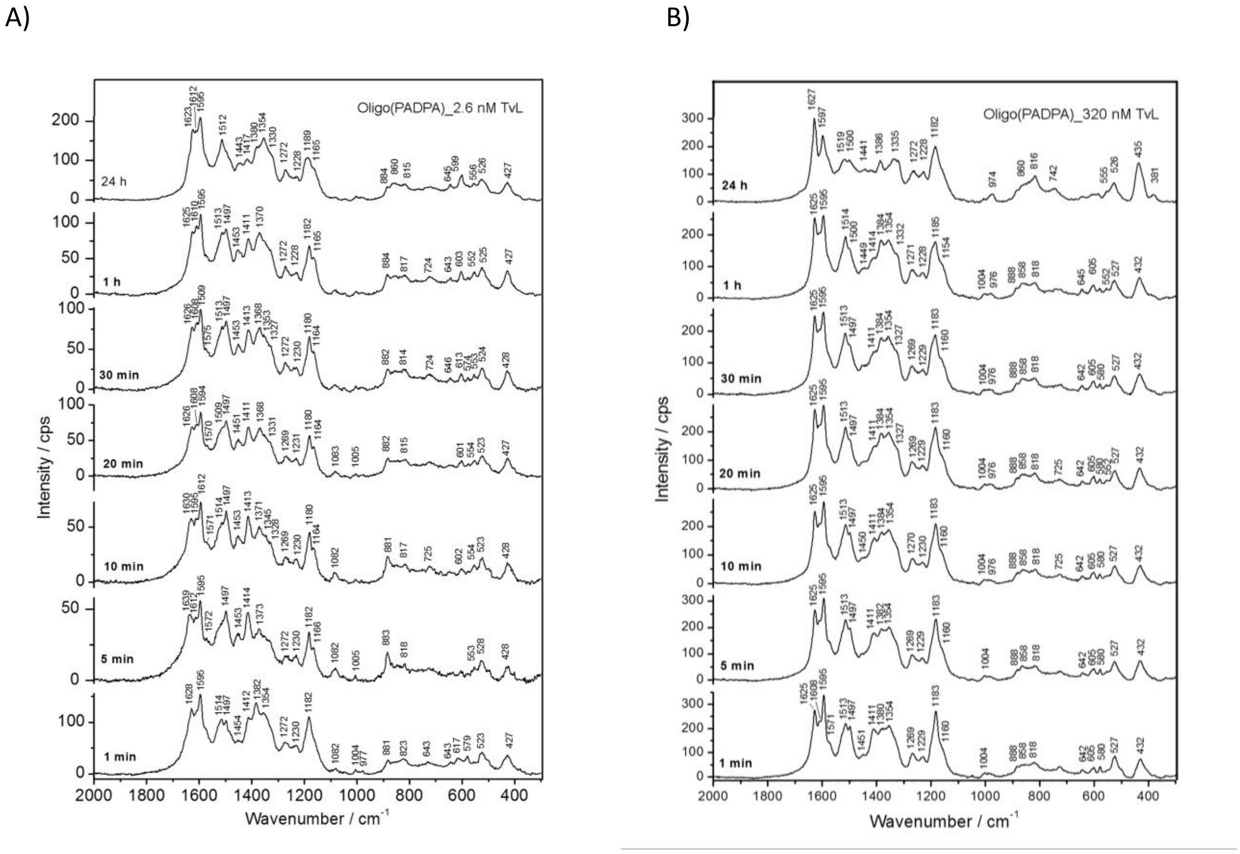


Fig. S-8. Raman spectra of the two reaction mixtures recorded during the reaction at [TvL] ≈ 2.6 nM (A) or [TvL] ≈ 320 nM (B). $[PADPA]_0$ = 1.0 mM, [AOT] = 1.5 mM, $[H_2PO_4^-] + [H_3PO_4]$ = 0.1 M (chloride ion-free), pH = 3.5, T ≈ 25 °C. Excitation wavelength: 633 nm.

The differences in the *in situ* Raman spectra related to the molecular structure of the products at various times during the reaction (from t = 1 min to t = 24 h) for the two reaction mixtures with TvL concentrations of ≈2.6 nM and ≈320 nM are obvious (**Fig. S-8**). Besides the main differences described in section 3.2.3, it is interesting to note some detailed differences during the early stages of the reaction. The earliest recorded spectra (at t = 1 min) are mutually similar, showing the same bands and indicating similarity in the structure of initial products for the two TvL concentrations. Virtually the only (but not insignificant) difference at t = 1 min is the relatively higher intensity of the $\nu(C{-}N^+)$/ $\nu(C{-}N^{\bullet+})$ band at about 1380 / 1350 cm^{-1} in the spectrum recorded for [TvL] ≈2.6 nM compared to the spectrum for [TvL] ≈320 nM, indicating *higher amounts of first-formed radical cations (polarons) in the case with low enzyme concentration*. In the following spectra (t=5 min, 10 min), the bands due to polarons, at about 1370, 1350 and 1330 cm^{-1}, are significantly weakened for [TvL] ≈2.6 nM, and later they again strengthen. Such intensity decrease is not observed for [TvL] ≈320 nM. For very long reaction times (t = 24 h) a significant increase in the relative intensity of the polaron band is observed for [TvL] ≈2.6 nM (the peak centred at 1350 cm^{-1} becomes one of the strongest in the spectrum), while the opposite trend is detected in the case of [TvL] ≈320 nM. Overall, these spectral differences indicate differences in the reaction course for the two conditions used.

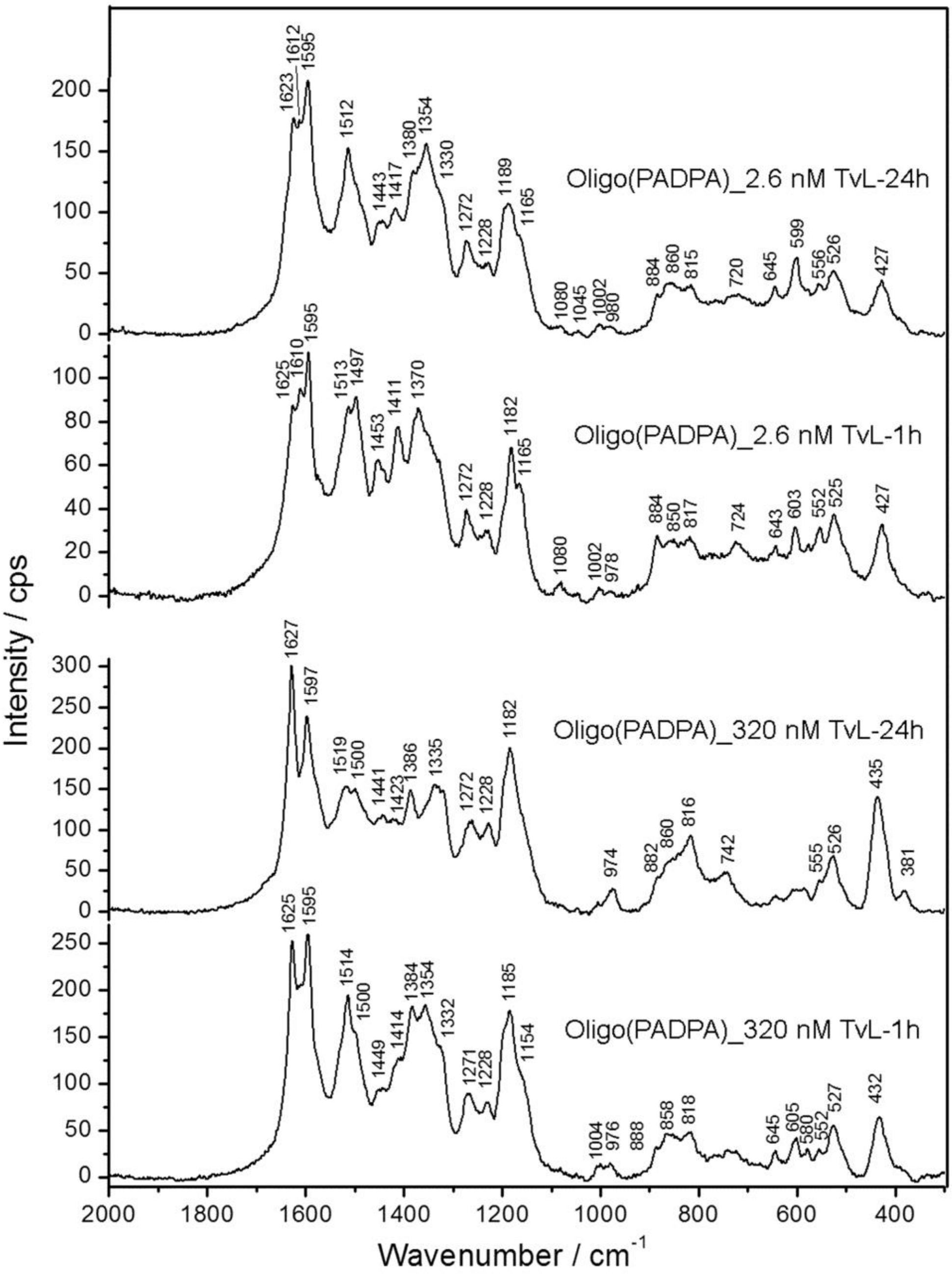
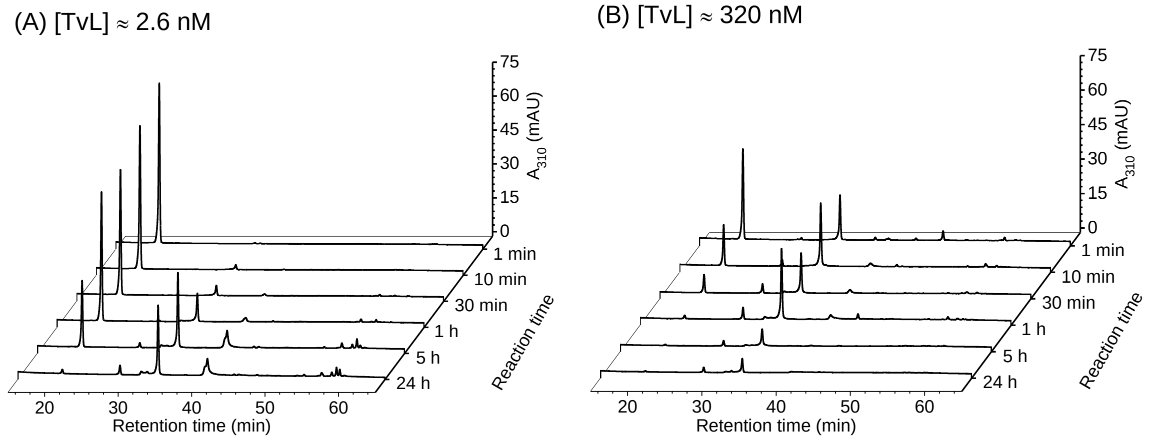


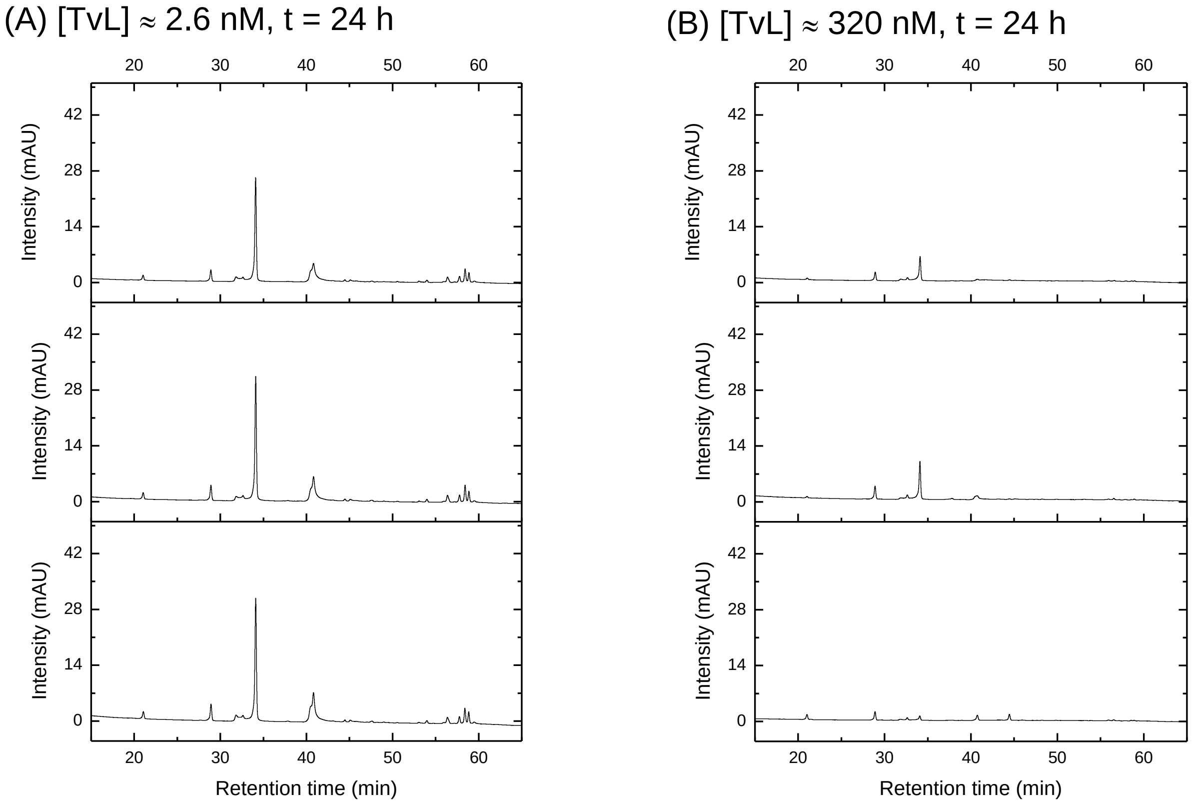
Fig. S-9. Comparison of the Raman spectra of the two reaction mixtures recorded after t = 1 h and t = 24 h for [TvL ≈ 2.6 nM (top) or [TvL] ≈ 320 nM (bottom). $[PADPA]_0 = 1.0$ mM, [AOT] = 1.5 mM, $[H_2PO_4^-] + [H_3PO_4] = 0.1$ M (chloride ion-free), pH = 3.5, T ≈ 25 °C. Excitation wavelength: 633 nm.


(A) [TvL] ≈ 2.6 nM

(B) [TvL] ≈ 320 nM

Fig. S-10. Comparison of the chromatograms for the HPLC-DAD analysis of the deprotonated and reduced reaction products which were extractable in MTBE (A) or $CHCl_3$ (B) at different reaction times for reactions run with [TvL] ≈ 2.6 nM (A) or [TvL] ≈ 320 nM (B). $[PADPA]_0 = 1.0$ mM, [AOT] = 1.5 mM, $[H_2PO_4^-] + [H_3PO_4] = 0.1$ M (chloride ion-free), pH = 3.5, T ≈ 25 °C. The relative absorbance at λ = 310 nm is plotted against the retention time.

Comments: For [TvL] = 320 nM and t = 24 h, the chromatogram consists of only a few peaks. This is due to incomplete extraction of the reaction products into the organic solvent ($CHCl_3$). For [TvL] ≈ 320 nM, the most intensive peak in the chromatogram for t = 1 h (rt ≈ 32 min) is very weak in the chromatogram for t = 24 h. This indicates that the product eluting at rt ≈ 32 min (linear tetraaniline) is no more present, or it could not be extracted due to strong interactions with other products present. In any case, the situation in the case of [TvL] ≈ 320 nM is very different from the reaction run with [TvL] ≈ 2.6 nM.


(A) [TvL] ≈ 2.6 nM, t = 24 h

(B) [TvL] ≈ 320 nM, t = 24 h

Fig. S-11. Comparison of the reproducibility of the chromatograms for the conditions described in the legend of **Fig. S-10** and a reaction time of t = 24 h. (A): [TvL] = 2.6 nM, extraction with MTBE; (B) [TvL] = 320 nM, extraction with $CHCl_3$. The relative absorbance at λ = 310 nm is plotted against the retention time.

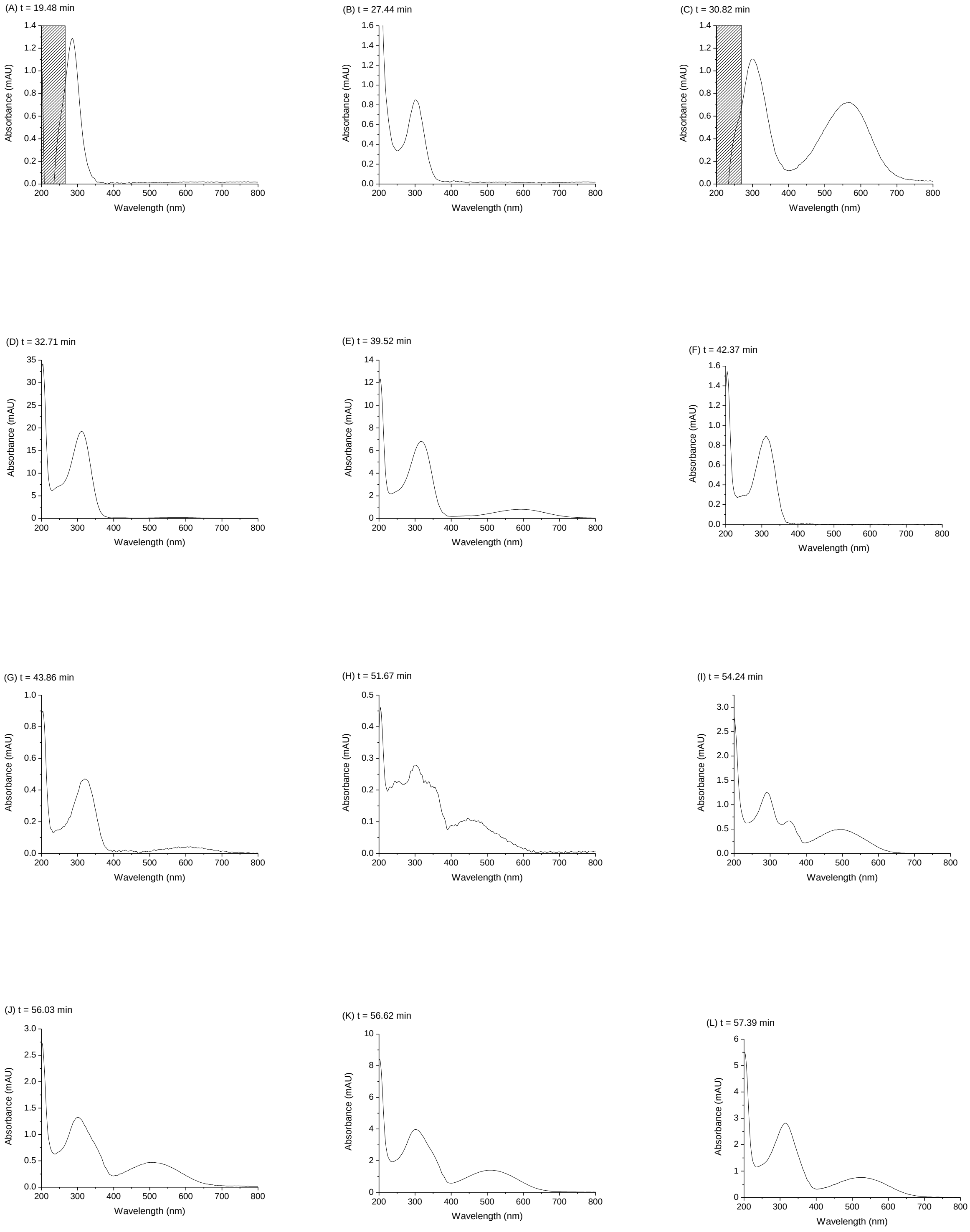


Fig. S-12. UV/vis absorption spectra of the molecules causing the different peaks in the chromatogram of **Fig. 6A**, as obtained from the HPLC-DAD analysis, for a reaction mixture with [TvL] = 2.6 nM, t = 24 h (*new optimal conditions*), see **Table 1**.

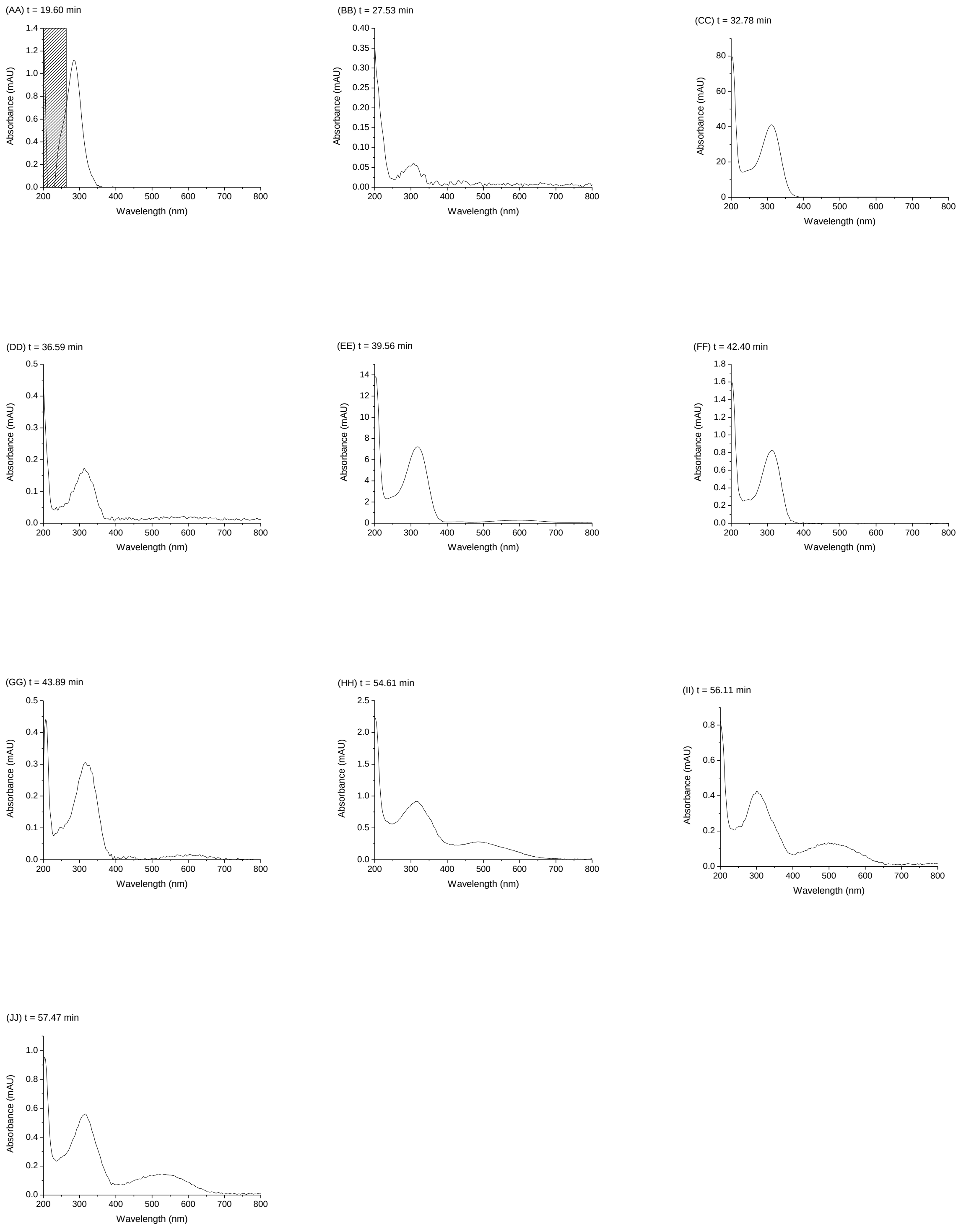
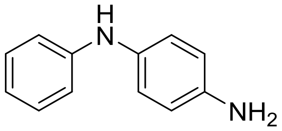


Fig. S-13. UV/vis absorption spectra of the molecules causing the different peaks in the chromatogram of **Fig. 6B**, as obtained from the HPLC-DAD analysis, for a reaction mixture with [TvL] = 320 nM, t = 1 h, see **Table 2**.

Chemical structures – or likely chemical structures - of the molecules responsible for the peaks in the chromatograms of Fig. 6

(1)

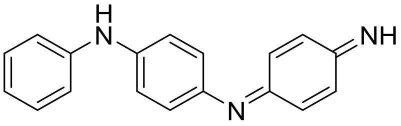


PADPA

PADPA·H^+

Chemical formula: $C_{12}H_{13}N_2^+$
Calculated molecular mass: 185.1073

(2)

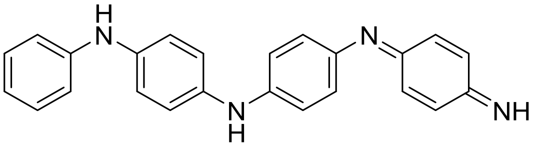


(PADPA)$_{1.5}$

(PADPA)$_{1.5}$·H^+

Chemical formula: $C_{18}H_{16}N_3^+$
Calculated molecular mass: 274.1339

(3)

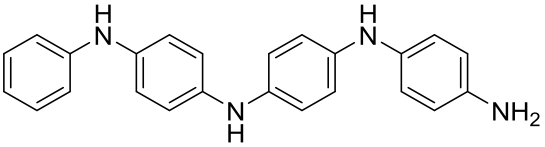


(PADPA)$_2^{\mathrm{I}}$ oxidized

(PADPA)$_2^{\mathrm{I}}$ oxidized·H^+

Chemical formula: $C_{24}H_{21}N_4^+$
Calculated molecular mass: 365.1761

(4)

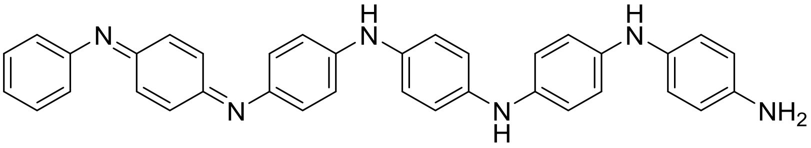


(PADPA)$_2^{\mathrm{I}}$

(PADPA)$_2^{\mathrm{I}}$·H$^+$

Chemical formula: $C_{24}H_{23}N_4^+$
Calculated molecular mass: 367.1917

(5)

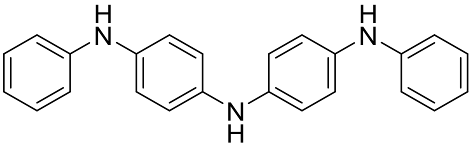


(PADPA)$_3^{\mathrm{I}}$ oxidized

(PADPA)$_3^{\mathrm{I}}$ oxidized·H$^+$

Chemical formula: $C_{36}H_{31}N_6^+$
Calculated molecular mass: 547.2605

(6)

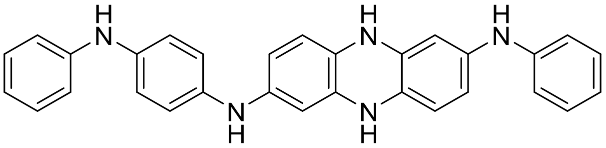


(PADPA)$_2^{\mathrm{II}}$–NH$_2$

(PADPA)$_2^{\mathrm{II}}$–NH$_2$·H$^+$

Chemical formula: $C_{24}H_{22}N_3^+$
Calculated molecular mass: 352.1808

(7)

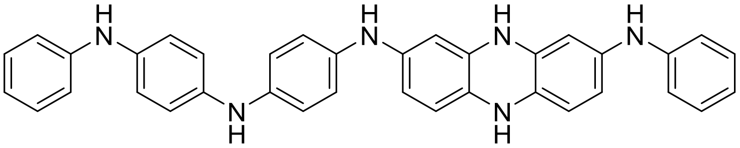


(PADPA)$_{2.5}$

(PADPA)$_{2.5}$·H^+

Chemical formula: $C_{30}H_{26}N_5^+$
Calculated molecular mass: 456.2183

(8)

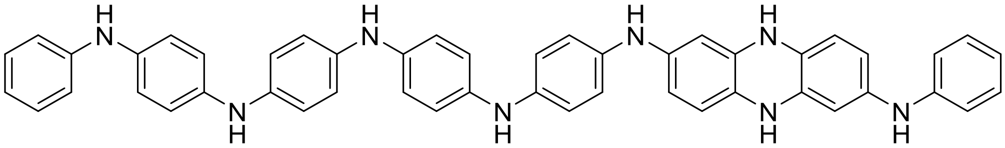


(PADPA)$_3^{II}$

(PADPA)$_3^{II}$·H^+

Chemical formula: $C_{36}H_{31}N_6^+$
Calculated molecular mass: 547.2605

(9)



(PADPA)$_4$

(PADPA)$_4$·H^+

Chemical formula: $C_{48}H_{41}N_8^+$
Calculated molecular mass: 729.3449

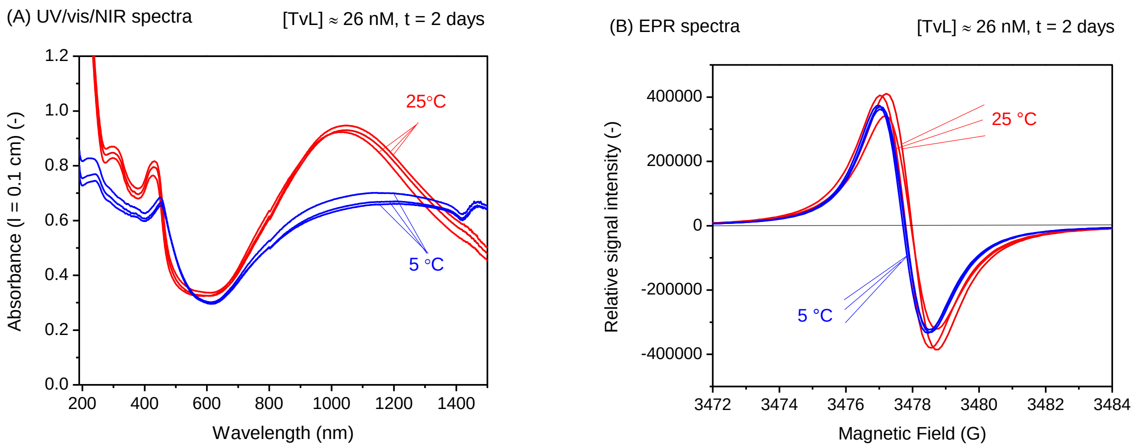


Fig. S-14. UV/vis/NIR (A) and EPR spectra (B) of reaction mixtures, as obtained for reactions carried out at either T ≈ 25 °C or T = 5 °C. $[PADPA]_0 = 1.0$ mM, [AOT] = 1.5 mM, $[H_2PO_4^-] + [H_3PO_4] = 0.1$ M (chloride ion-free), pH = 3.5, [TvL] ≈ 2.6 nM and t = 2 d. Red: T = 25 °C; blue: T = 5 °C; ν = 9.769 GHz, $g_5 = 2.0068 \pm 0.0002$, $g_{25} = 2.0069 \pm 0.0002$.

Remark: The lines of products obtained at T = 25 °C reaction temperature are slightly wider than those of products obtained at T = 5 °C. The reproducibility of the radical yield is also better at 5 °C.

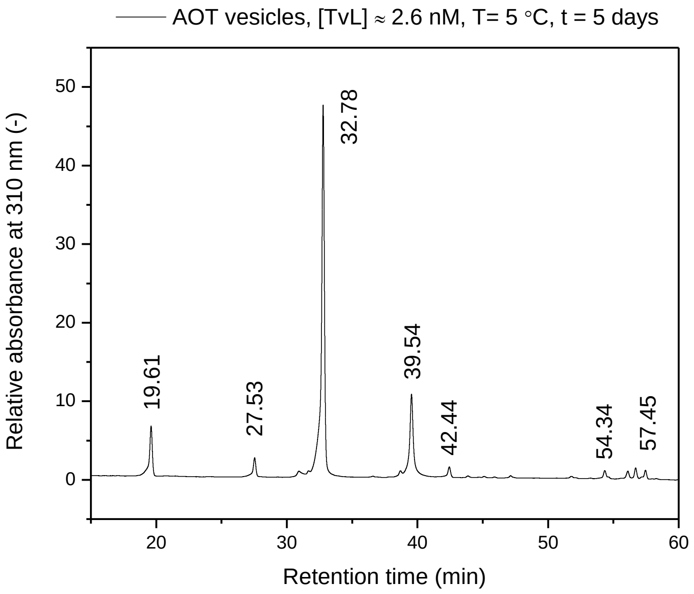


Fig. S-15. Chromatogram for the HPLC-DAD analysis of the deprotonated and reduced reaction products, as obtained from $[PADPA]_0 = 1.0$ mM, [AOT] = 1.5 mM, $[H_2PO_4^-] + [H_3PO_4] = 0.1$ M (chloride ion-free), pH = 3.5, T = 5 °C, and [TvL] ≈ 2.6 nM after t = 5 d. The relative absorbance at λ = 310 nm is plotted against the retention time. The peaks at t = 19.61, 27.53, 32.78, 39.54, and 42.44 min correspond to PADPA, $(PADPA)_{1.5}$, $(PADPA)_2^{I}$, $(PADPA)_3$, and $(PADPA)_2$-NH_2; the peaks between t ≈ 54 and 58 min correspond to products with phenazine units; see Luginbühl et al. (2016).[S2]

References

(S1) K. Junker, S. Luginbühl, M. Schüttel, L. Bertschi, R. Kissner, L. D. Schuler, B. Rakvin and P. Walde, *ACS Catal.*, 2014, **4**, 3421–3434.

(S2) S. Luginbühl, L. Bertschi, M. Willeke, L. D. Schuler and P. Walde, *Langmuir*, 2016, **32**, 9765–9779.