Supporting Information

Effect of template type on the preparation of the emeraldine salt form of polyaniline (PANI-ES) with horseradish peroxidase isoenzyme C (HRPC) and hydrogen peroxide

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Content

A. Experiments with vesicles, micelles and dissolved polyelectrolyte as soft templates

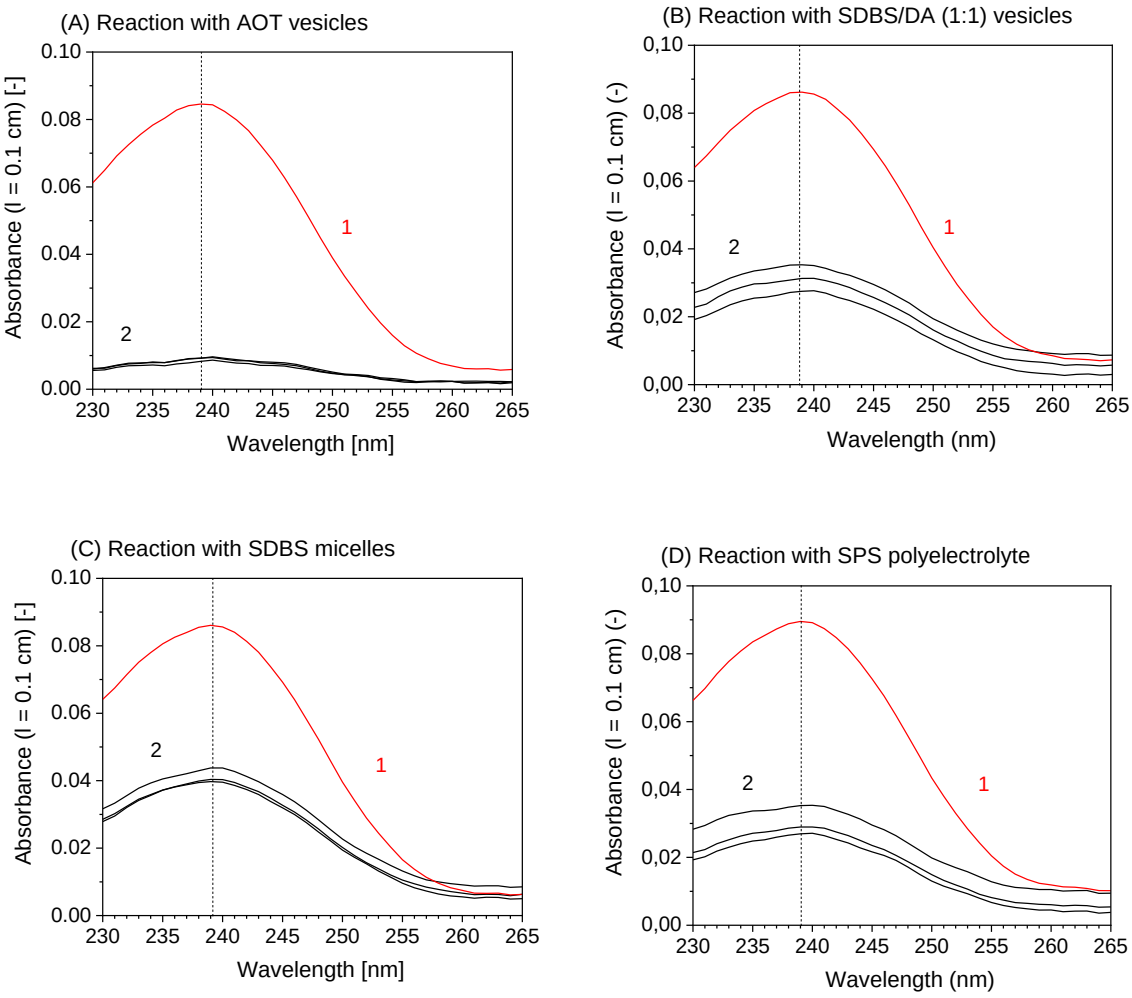


Fig. S-1. Quantification of unreacted aniline in the reaction mixtures (for the optimal reaction conditions, see **Table 1**): 0.03 mL reaction mixture, 1.47 mL acetonitrile; centrifugation; spectrum of the supernatant. See Materials and Methods (section 2. 5.) for details.

For each reaction mixture, the spectrum of unreacted aniline is shown before starting the reaction (**1, red**) and after a reaction time of *t* = 24 h (**2, black**, three independent reactions and determinations). Each spectrum shown represents a difference spectrum between the spectrum which was measured for the actual reaction mixture (**Fig. S-2**) minus the reference spectrum measured for the reaction mixture without any aniline (**Fig. S-3**, contribution of template molecules).

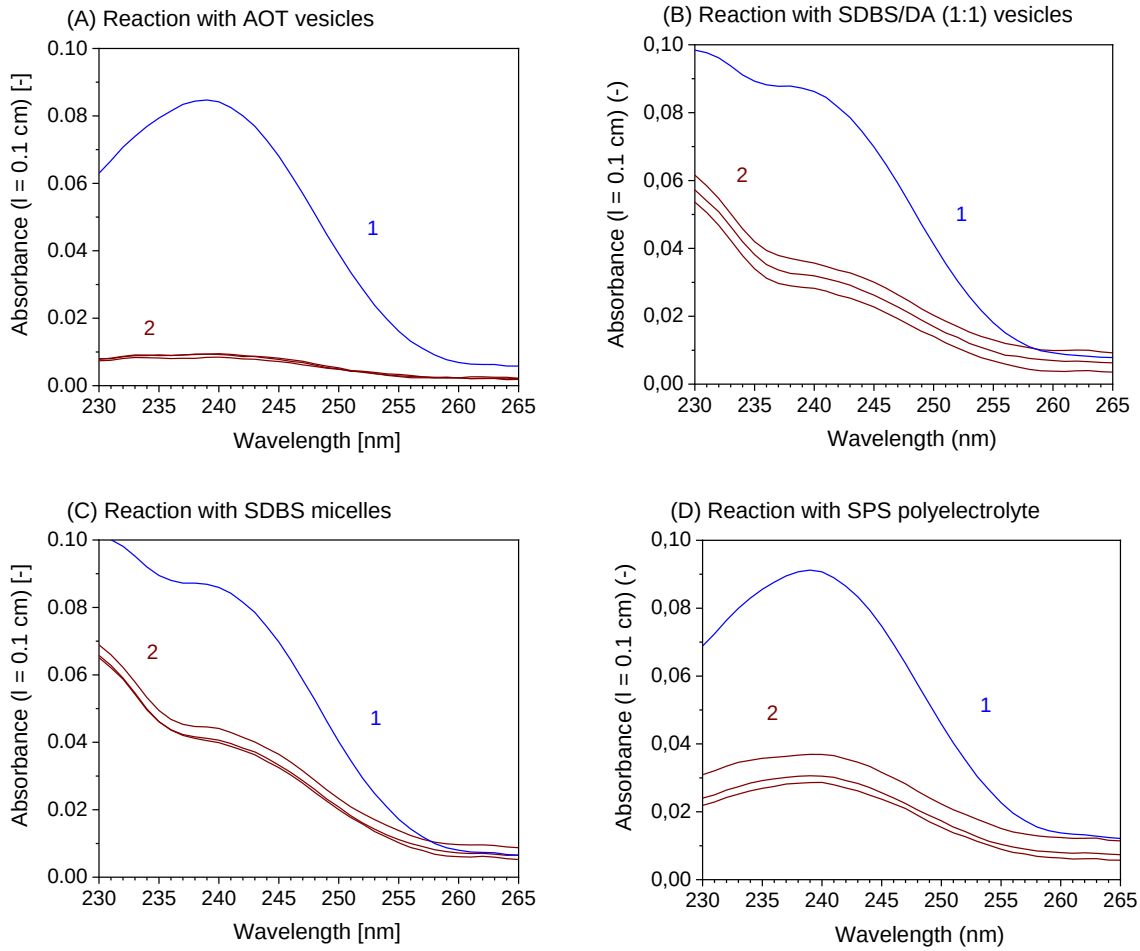


Fig. S-2. Quantification of unreacted aniline in the reaction mixtures (for the optimal reaction conditions, see **Table 1**): 0.03 mL reaction mixture, 1.47 mL acetonitrile; centrifugation; spectrum of the supernatant. See Materials and Methods (section 2. 4. 3.) for details.

Measured spectra before starting the reactions (**1, blue**) and after *t* = 24 h (**2, brown**, three independent reactions and determinations), without considering the contribution from the template molecules.

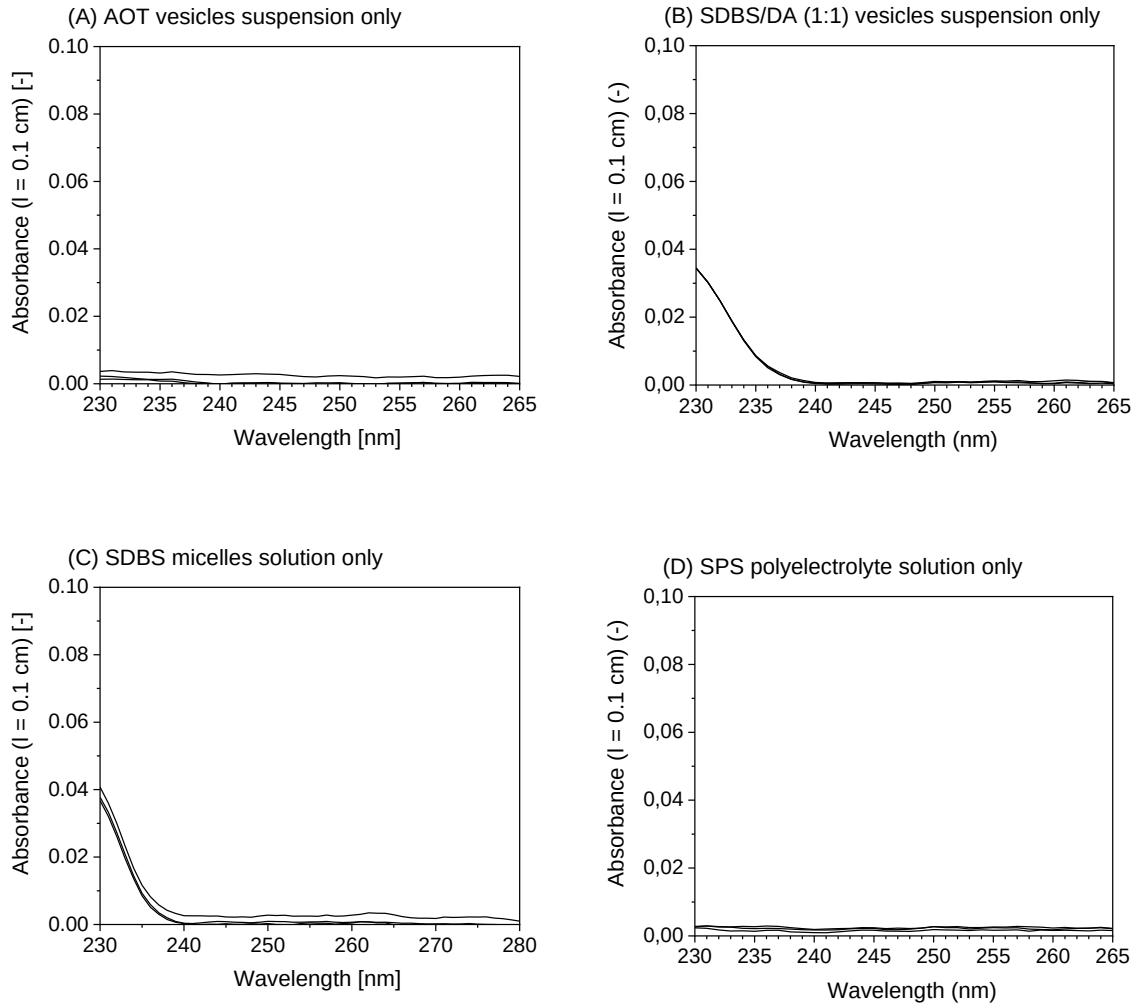


Fig. S-3. Quantification of unreacted aniline in the reaction mixtures (for the optimal reaction conditions, see **Table 1**): 0.03 mL reaction mixture, 1.47 mL acetonitrile; centrifugation; spectrum of the supernatant. See Materials and Methods (section 2. 4. 3.) for details.

Reference spectra for mixtures which did not contain aniline (contribution from the template molecules).

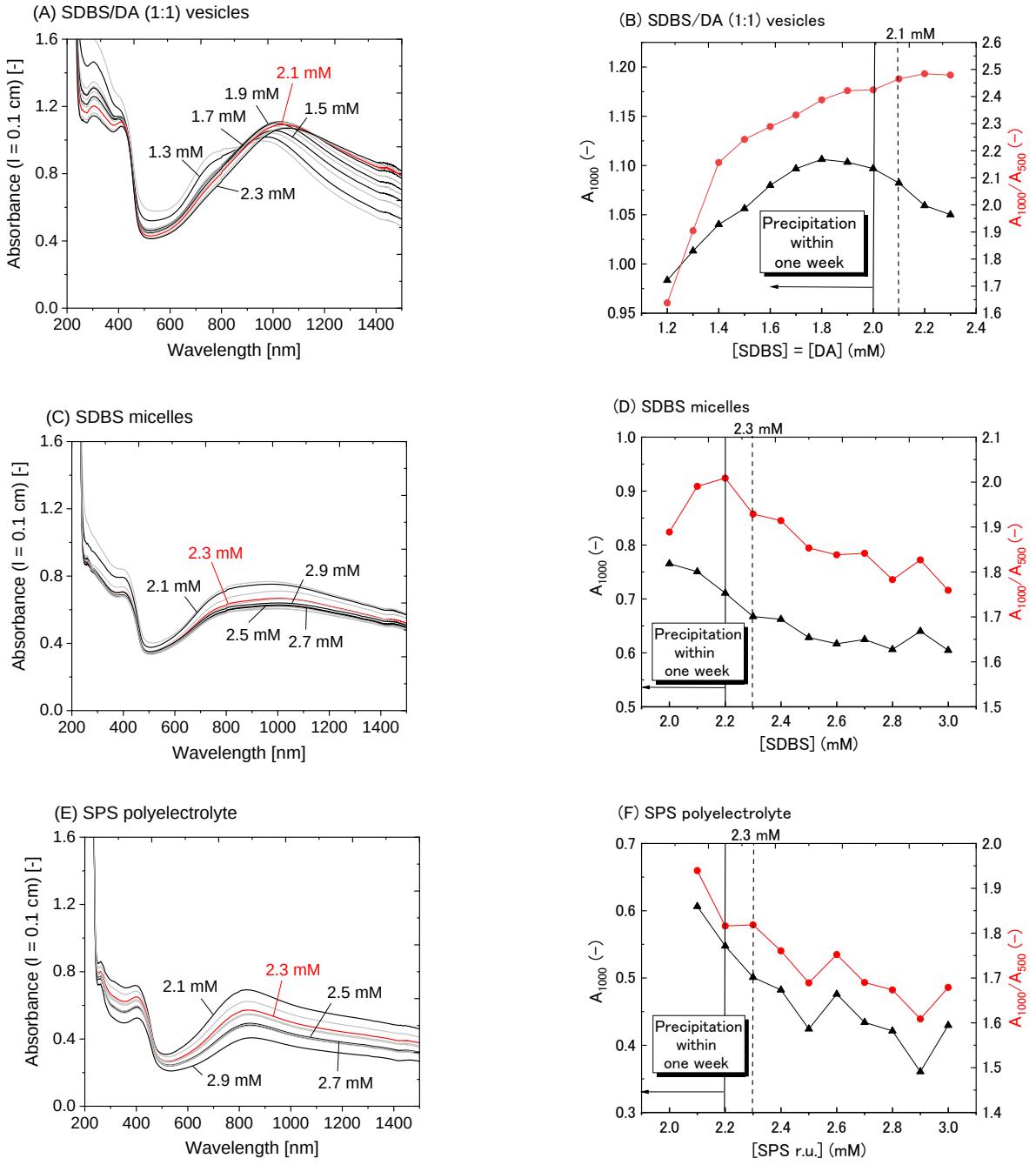


Fig. S-4. Determination of the optimal reaction conditions for reaction mixtures containing one of the three templates (A, B) SDBS/DA (1:1) vesicles, (C, D) SDBS micelles, and (E, F) SPS polyelectrolyte. All reactions were carried under the following conditions: [aniline] = 4.0 mM, [HRP] = 0.92 µM, and [H_2O_2] = 4.5 mM, see Materials and Methods (section 2.3.) and **Table 1** for details. The following template molecule concentrations were used.

SDBS/DA (1:1) vesicles: [SDBS] = [DA] = 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, **2.1**, 2.2, and 2.3 mM.
SDBS micelles: [SDBS] = 2.0, 2.1, 2.2, **2.3**, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9 and 3.0 mM.
SPS polyelectrolyte: [SPS r.u.] = 2.1, 2.2, **2.3**, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9 and 3.0 mM.

In (A), (C), and (E), only some of the measured spectra are shown. The ones referring to template molecule concentrations we considered optimal are drawn **in red**, see section 3.2.1.

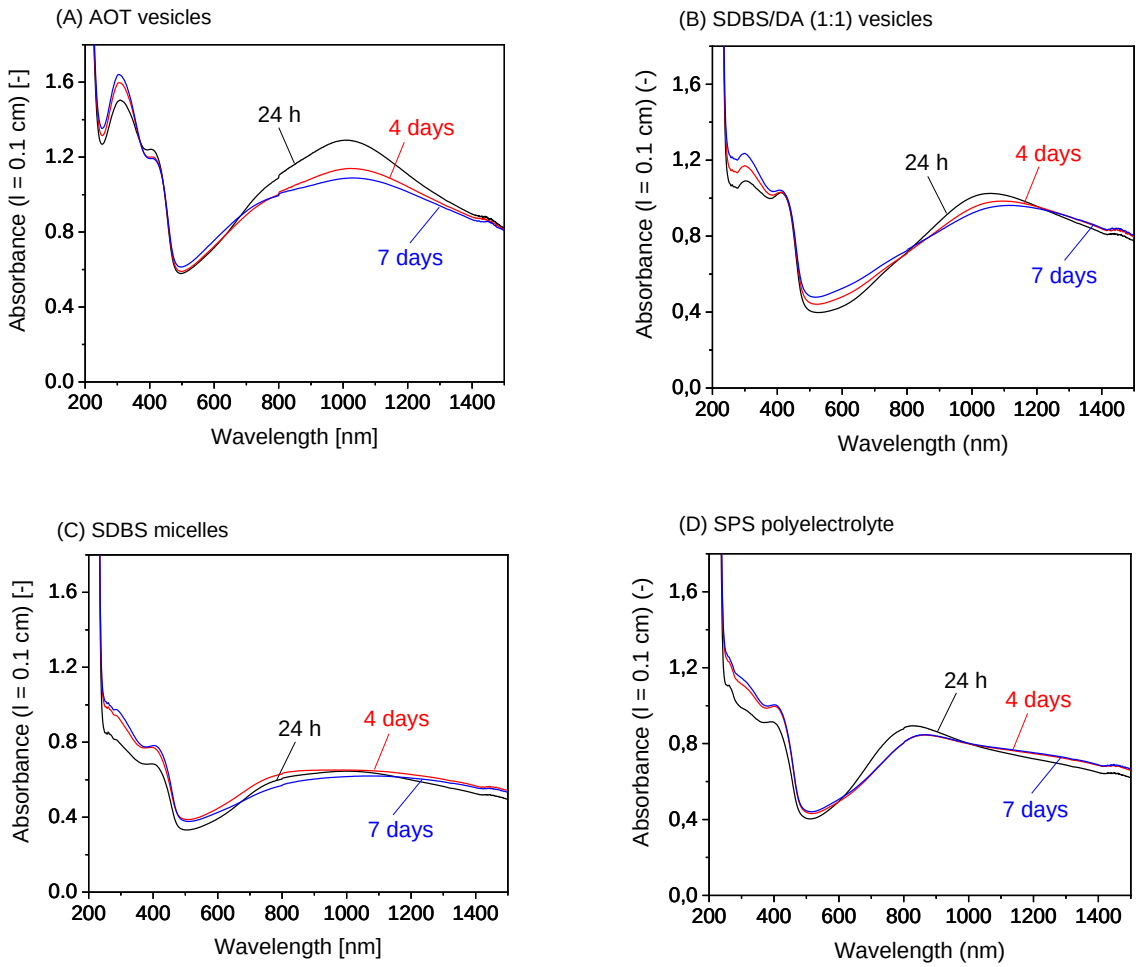


Fig. S-5. Changes in the UV/vis/NIR spectra of the reaction mixtures, recorded after long term storage at RT for reactions run under optimal conditions.

A. In the presence of AOT vesicles as templates. [AOT] = 3.0 mM, [aniline] = 4.0 mM, [HRPC] = 0.92 µM, [H_2O_2] = 4.5 mM.

B. In the presence of SDBS/DA (1:1) vesicles. [SDBS] = [DA] = 2.1 mM, [aniline] = 4.0 mM, [HRPC] = 0.92 µM, [H_2O_2] = 4.5 mM.

C. In the presence of SDBS micelles. [SDBS] = 2.3 mM, [aniline] = 4.0 mM, [HRPC] = 0.92 µM, [H_2O_2] = 4.5 mM.

D. In the presence of SPS polyelectrolyte. [SPS r.u.] = 2.3 mM

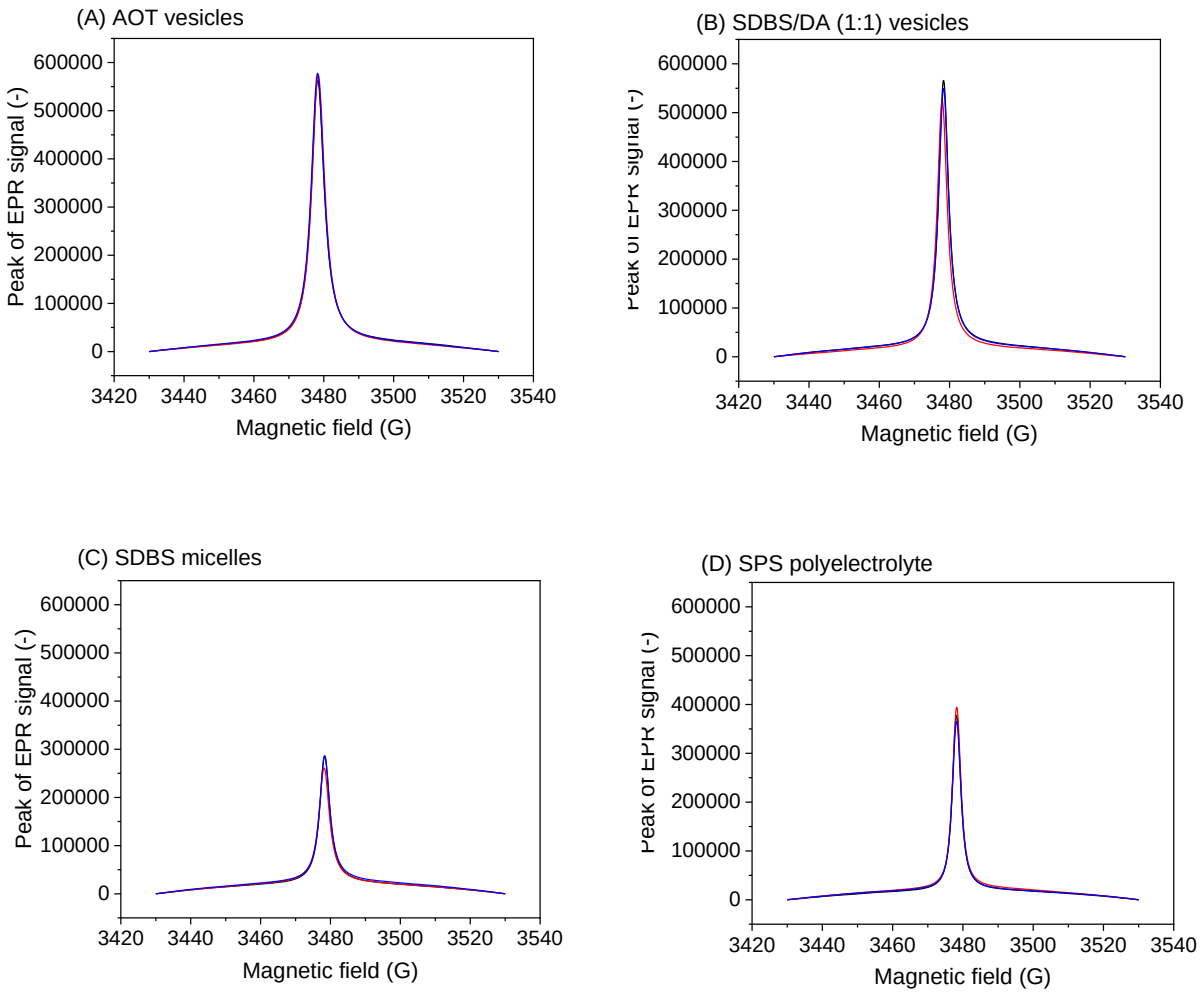


Fig. S-6. Integrals of the EPR spectra shown in **Fig. 1B** (AOT vesicles, A), **Fig. 2B** (SDBS/DA (1:1) vesicles, B), **Fig. 3B** (SDBS micelles, C), and **Fig. 4B** (SPS polyelectrolyte, D). For a summary of the experimental details, see **Table 1**. Three integrals are shown for each system, as obtained from three separate reactions to illustrate the reproducibility.

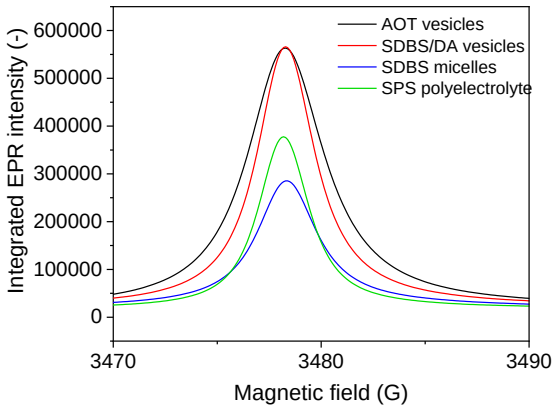
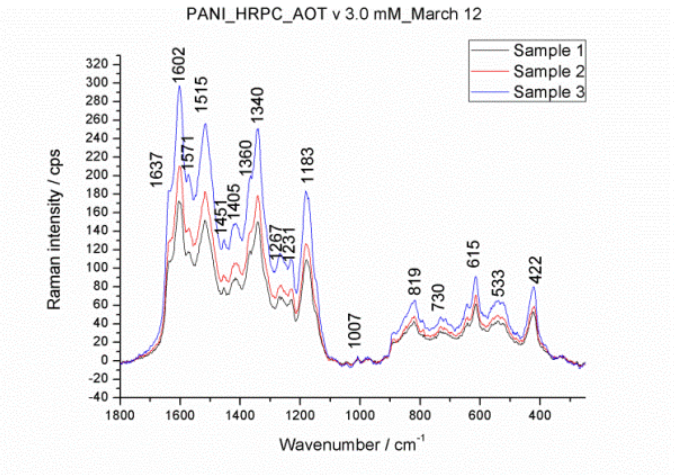
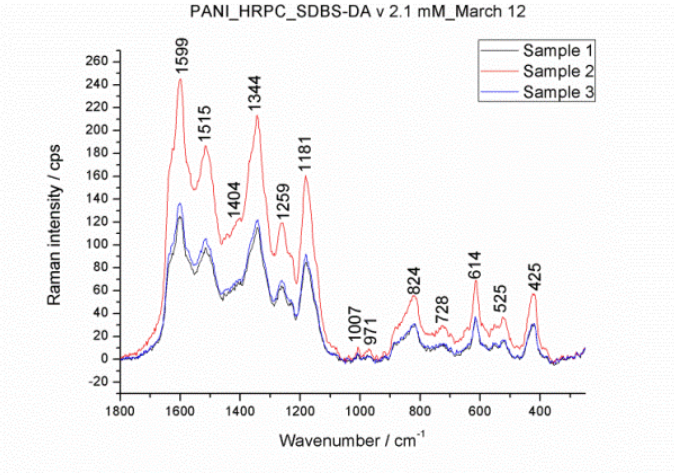


Fig. S-7. Direct comparison of the integrals of the EPR spectra of **Fig. S-6**.

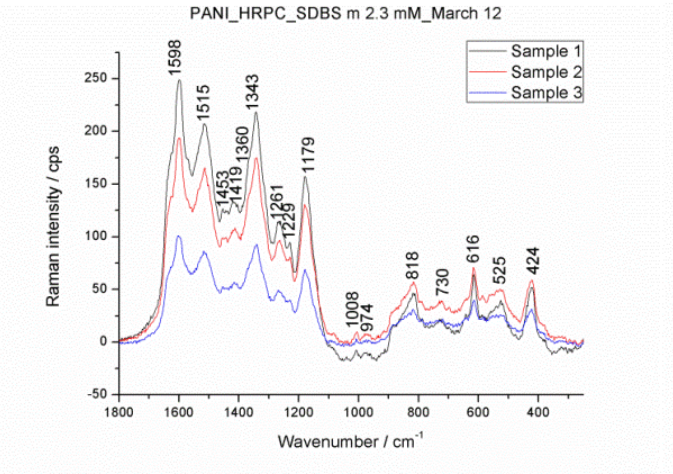
(A)



(B)



(C)



(D)

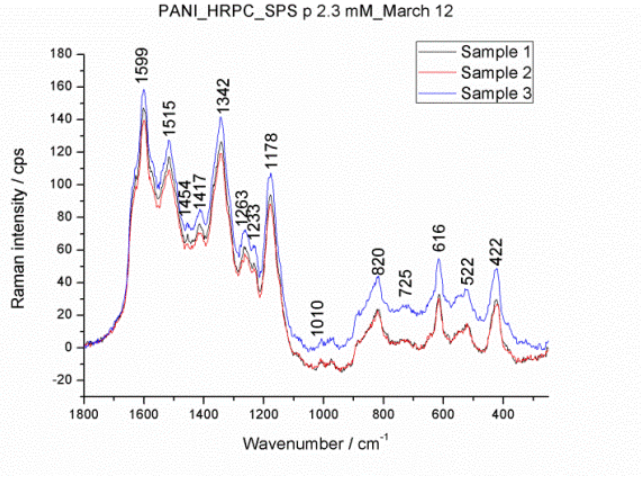


Fig. S-8. Raman spectra of the different reaction mixtures run for *t* = 15 d at T ≈ 25 °C, either in the presence of AOT vesicles (A), SDBS/DA (1:1) vesicles (B), SDBS micelles (C), or SPS polyelectrolyte (D) and aniline, HRPC and H_2O_2 at pH = 4.3. For a summary of the experimental details, see **Table 1**. Three spectra are shown as obtained from three separate reactions to illustrate the reproducibility.

B. Experiments with CNCs as rigid templates

(A) (B)

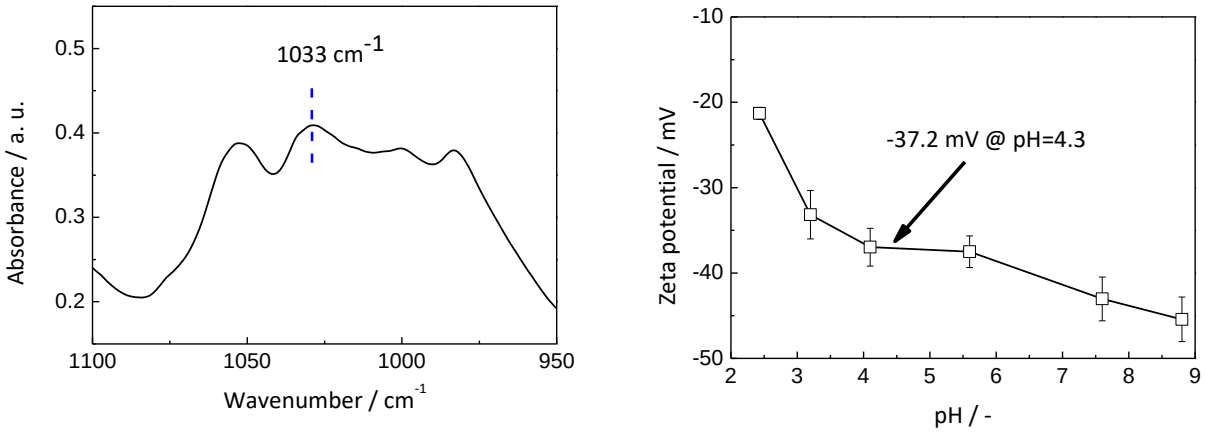


Fig. S-9. Characterisation of the synthesised CNCs. (A) Region of the ATR-FTIR spectrum indicating the presence of sulfate groups (at about 1033 cm^{-1}); (B) Zeta potential of CNCs dispersed in water at different pH values, recorded at a concentration of 0.1 $mg \cdot mL^{-1}$.

Table S-1. Preparation and composition of samples reaction mixtures 1-13 (rxn # 1-13), 1'-4' (rxn # 1'-4') and 9'-13' (rxn # 9'-13') of **Fig. 9**. The concentrations given in parenthesis are the initial concentrations in the reaction mixtures at the start of the reactions. Rxn # **3** is the reaction mixture which was considered optimal; rxn # **3'** is the same reaction mixture but without CNC, see **Table 2**.

Total reaction volume for all reaction mixtures: 400 µL
Diluted pH = 4.3 solution used: 0.05 M
CNC stock suspension: 2 wt% (0.01 M phosphate (450 µL 2.23 wt% CNC + 50 µL 0.1 M phosphate)
Aniline stock solution: 40 mM (182.42 µL in 50 mL), pH adjusted to 4.3
HRPC stock solution: 6.67 µM (0.34 mg·mL^{-1})
H_2O_2 stock solution: 20 mM
For cationic CNC analogue: Amount added as calculated to yield the same final amount as for CNC

Rxn # (total volume: 400 µL)	Diluted pH = 4.3 solution (µL)	CNC stock suspension (µL)	Aniline stock solution (µL)	HRPC stock solution (µL)	H_2O_2 stock solution (µL)
1	272.9		20.0 (→ 2.0 mM)	5.3 (→ 0.09 µM)	9.0 (→ 0.45 mM)
2	238.6		40.0 (→ 4.0 mM)	10.6 (→ 0.18 µM)	18.0 (→ 0.9 mM)
3	**169.9**	**92.8 (→ 0.46 wt%)**	**80.0 (→ 8.0 mM)**	**21.3 (→ 0.36 µM)**	**36.0 (→ 1.8 mM)**
4	32.6		160.0 (→ 16.0 mM)	42.6 (→ 0.71 µM)	72.0 (→ 3.6 mM)
5	308.3	23.1 (→ 0.12 wt%)	40 (→ 4.0 mM)	10.6 (→ 0.18 µM)	18 (→ 0.9 mM)
6	285	46.4 (→ 0.23 wt%)			
7	238.6	92.8 (→ 0.46 wt%)			
8	145.8	185.6 (→ 0.93 wt%)			
9	260.0	92.8 (→ 0.46 wt%)	40 (→ 4.0 mM)	2.65 (→ 0.04 µM)	4.5 (→ 0.23 mM)
10	252.9			5.3 (→ 0.09 µM)	9.0 (→ 0.45 mM)
11	238.6			10.6 (→ 0.18 µM)	18 (→ 0.9 mM)
12	209.9			21.3 (→ 0.36 µM)	36.0 (→ 1.8 mM)
13	152.6			42.6 (→ 0.71 µM)	72.0 (→ 3.6 mM)
1'	365.7	-	20.0 (→ 2.0 mM)	5.3 (→ 0.09 µM)	9.0 (→ 0.45 mM)
2'	331.4	-	40.0 (→ 4.0 mM)	10.6 (→ 0.18 µM)	18.0 (→ 0.9 mM)
3'	262.7	-	**80.0 (→ 8.0 mM)**	**21.3 (→ 0.36 µM)**	**36.0 (→ 1.8 mM)**
4'	125.4	-	160.0 (→ 16.0 mM)	42.6 (→ 0.71 µM)	72.0 (→ 3.6 mM)

Rxn # (total volume: 400 µL)	Diluted pH = 4.3 solution (µL)	CNC stock suspension (µL)	Aniline stock solution (µL)	HRPC stock solution (µL)	H_2O_2 stock solution (µL)
9'	352.9	-	40 (→ 4.0 mM)	2.65 (→ 0.04 µM)	4.5 (→ 0.23 mM)
10'	345.7	-		5.3 (→ 0.09 µM)	9.0 (→ 0.45 mM)
11'	331.4	-		10.6 (→ 0.18 µM)	18 (→ 0.9 mM)
12'	302.7	-		21.3 (→ 0.36 µM)	36.0 (→ 1.8 mM)
13'	245.4	-		42.6 (→ 0.71 µM)	72.0 (→ 3.6 mM)
Control 1: As **rxn # 3**, but without aniline	249.9	92.8 (→ 0.46 wt%)	-	21.3 (→ 0.36 µM)	36.0 (→ 1.8 mM)
Control 2: As **rxn # 3**, but with µ-cellulose	169.9		80.0 (→ 8.0 mM)		
Control 3: As **rxn # 3**, but with cationic CNC analogue	93.7	169.9			

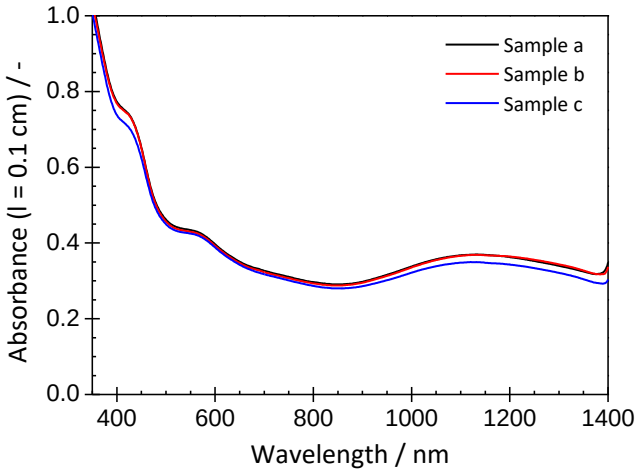


Fig. S-10. Test of the reproducibility of the formation of PANI-ES products from aniline with HRPC/H_2O_2 in the presence of CNC. Three samples were prepared (sample a, b, and c), all consisting of the same initial composition (rxn # 3 of **Fig. 9**). The UV/vis/NIR spectrum was measured after a reaction time of *t* = 24 h.

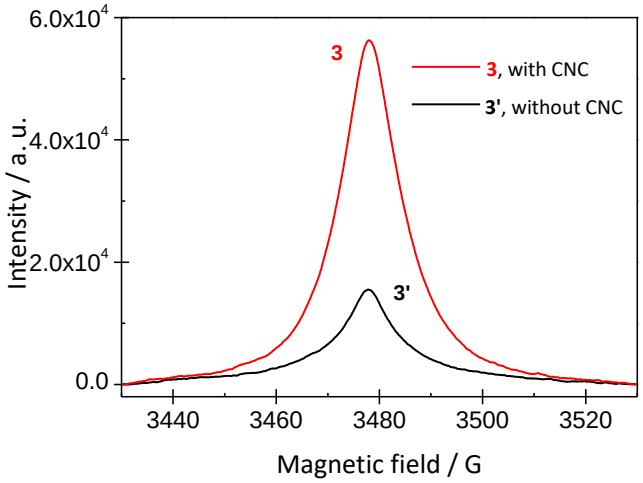


Fig. S-11. Integrals of the EPR spectra shown in **Fig. 12.** Reaction mixture 3 ("optimal" conditions for CNCs as templates) and reaction mixture 3' (identical initial composition as 3, but without CNC). The reaction time was *t* = 24 h at T ≈ 25 °C.

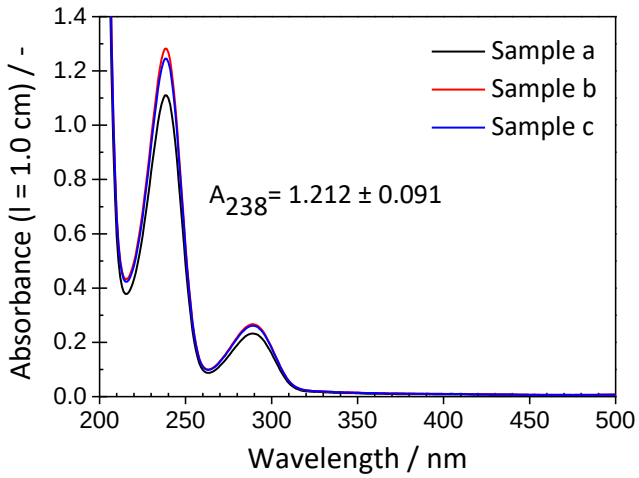


Fig. S-12. Quantification of the amount of unreacted aniline after a reaction time *t* = 24 h. Three samples were prepared (sample a, b, and c), all consisting of the same initial composition (reaction mixture 3 of **Fig. 9**). A volume of 30 µL of the reaction mixture was added to 1470 µL acetonitrile. After centrifugation, the UV/vis spectrum of the supernatant was measured and quantified, as described in Materials and Methods (section 2. 4. 3.).

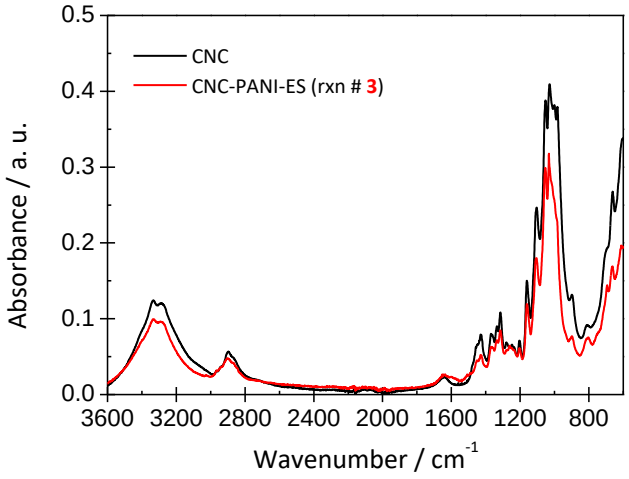


Fig. S-13. Comparison of the ATR-FTIR spectrum of the prepared CNCs (black, see **Fig. 8B**) with the CNCs containing PANI-ES (red), as obtained from the reaction mixture 3 (see **Fig. 9** and **Fig. 10**).

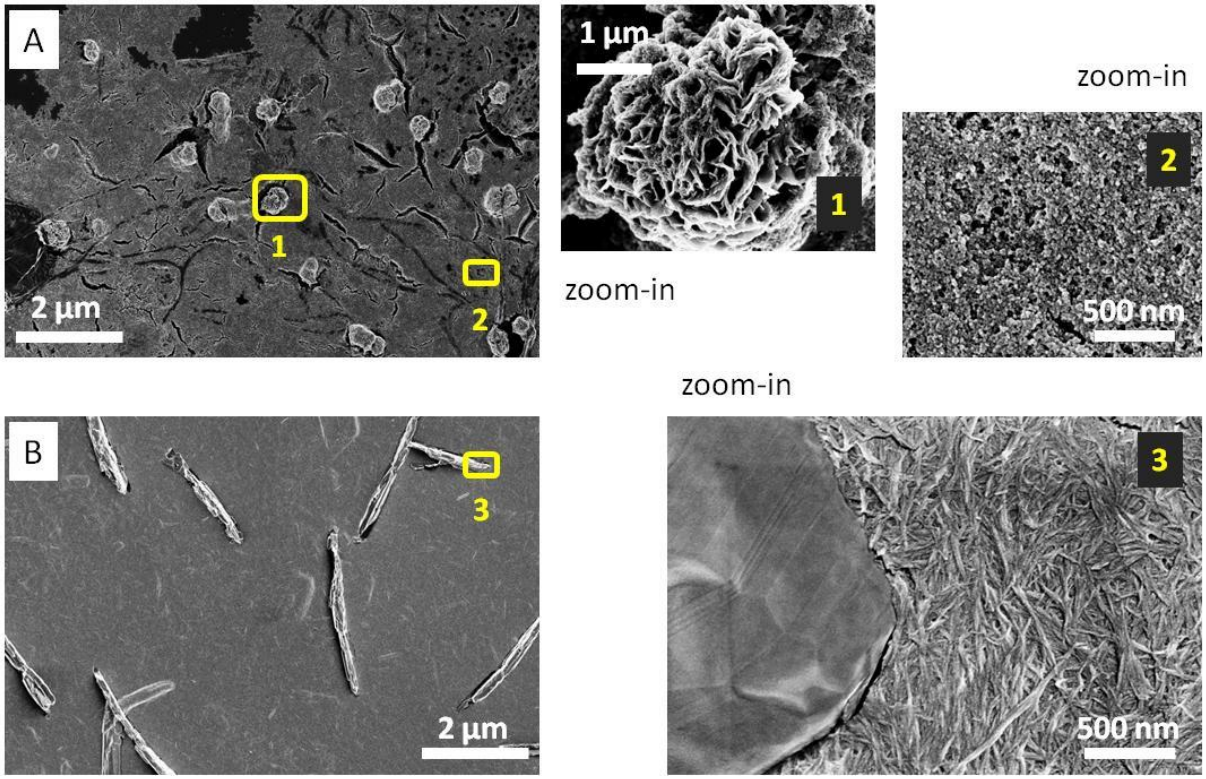


Fig. S-14. Representative SEM micrographs of PANI obtained from aniline with HRP and H_2O_2 without any template (rxn # **3'**, see **Table 2** and **Fig. 9**) (A) and of a mixture of CNC and aniline before enzymatic polymerization (B).

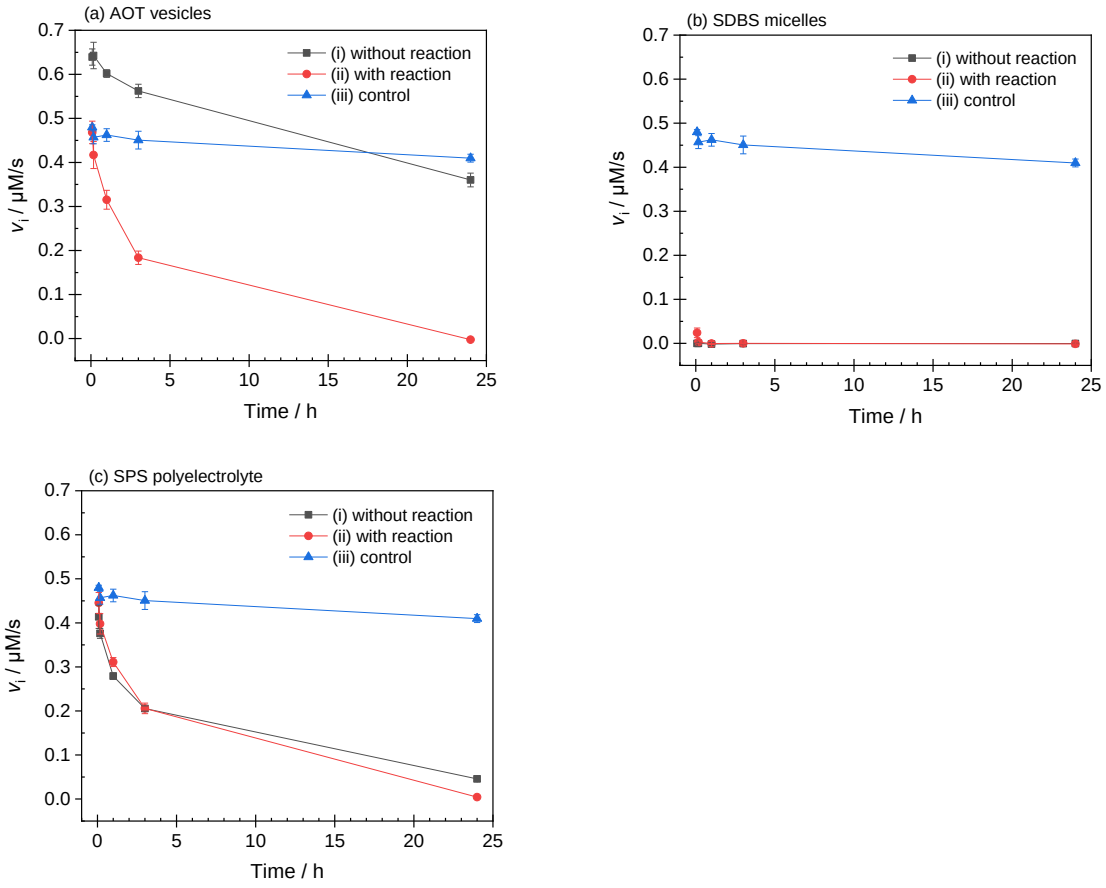


Fig. S-15. Stability of HRPC at *pH* = 4.3 (0.1 M NaH_2PO_4) and T ≈ 25 °C in reaction mixtures (ii) containing as templates either AOT vesicles (a), SDBS micelles (b), or SPS polyelectrolyte (c). For comparison, the stability of HRPC was also measured in the presence of the templates without reaction (i) and in the pH = 4.3 solution only (iii). Data obtained from three samples are shown as averages with standard deviations.

The HRPC activity was measured at pH = 7.0 and room temperature with $ABTS^{2-}$ as substrate (see below); v_i, initial rate of $ABTS^{2-}$ oxidation.

(i) Without reaction: Samples of 0.5 mL were prepared, containing either 3.0 mM AOT (a), 2.3 mM SDBS (b), or 2.3 mM SPS r.u.; *pH* = 4.3 (0.1 M NaH_2PO_4).

(ii) With reaction: Samples of 0.5 mL were prepared, containing 0.92 µM HRPC, 4.0 mM aniline, 4.5 mM H_2O_2 and either 3.0 mM AOT (a), 2.3 mM SDBS (b), or 2.3 mM SPS r.u.; *pH* = 4.3 (0.1 M NaH_2PO_4).

(iii) Control: HRPC was stored at 0.92 µM in the pH = 4.3 solution, stored at room temperature. In (a), (b), and (c) the same data are shown.

As already known from previous studies with SPS polyelectrolyte[S-1] or AOT vesicles,[S-2] HRPC is inactivated during the template-assisted oxidation of aniline to PANI-ES at *pH* = 4.3. The direct comparison shows that the enzyme is rapidly inactivated *during the reaction* in the presence of SDBS micelles (b). *Without reaction* but in the presence of template, HRPC is very stable in the case of AOT vesicles (a), unstable in the case of SPS polyelectrolyte (c) and very unstable in the case of SDBS micelles (b).

HRPC activity measurements

HRPC activity measurements were carried out with the substrate $ABTS^{2-}$ (using $ABTS^{2-}(NH_4^+)_2$, the diammonium salt of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) from Sigma).[S-3]

Aliquots of 10 µL volume were withdrawn from each mixture or from the control solution at t = 5 min, 10 min, 1 h, 3 h, 24 h and added to the assay solution prepared from 960 µL of a 0.1 M sodium phosphate buffer solution (pH = 7.0), 20 µL of $ABTS^{2-}$ stock solution (50 mM in the pH = 7.0 buffer solution), and 10 µL of H_2O_2 solution (20 mM in deionised water) in a quartz cuvette with a path length of 0.1 cm. $[ABTS^{2-}]_0$ = 1.0 mM, $[H_2O_2]_0$ = 0.2 mM, [HRPC] = 9.2 nM, room temperature.

After gentle mixing, the increase of A_{414} corresponding to the formation of $ABTS^{\bullet-}$ was measured for 60 seconds using a spectrophotometer (UVmini-1240 from SHIMADZU). The values of A_{414} were converted to the concentration of the formed $ABTS^{\bullet-}$ with $\varepsilon_{414}(ABTS^{\bullet-}) = 3.6 \times 10^4\ M^{-1}\ cm^{-1}$).[S-4] Initial reaction rate v_i [µM s^{-1}] were calculated from the slope of the concentration of formed $ABTS^{\bullet-}$ *vs.* time.

References

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