SUPPORTING INFORMATION

A Biocatalytically Active Membrane Obtained from Immobilization of 2-Deoxy-d-ribose-5-phosphate Aldolase on a Porous Support

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RAFT copolymerization of NIPAm and DMMIBA with cysteine reactive chain transfer agent.

Scheme S-1 shows the reaction scheme of the RAFT copolymerization of NIPAm with DMMIBA using a pyridyl disulfide functionalized derivative of PABTC as chain transfer agent. The polymerization was carried out in dioxane at 90 °C using ABCVA as initiator. Comonomer ratios within the synthesized polymers as well as conversion of monomers were determined via $^1$H-NMR spectroscopy (Fig. S-1 and S-2). All spectra were recorded on a Bruker 500 MHz FT-NMR spectrometer using CDCl$_3$ as solvent.

Scheme S1. Synthesis of PNIPAm-DMMIBA via RAFT polymerization using a pyridyl disulfide-functionalized CTA.

Figure S1. Excerpts of $^1$H-NMR spectra taken from the synthesized p(NIPAm-co-DMMIBA) copolymers after purification. Spectra were recorded in CDCl$_3$. The sample codes refer to the different polymerization batches (see Table 1, main manuscript).
Figure S2. Excerpts of $^1$H-NMR spectra taken from the crude reaction mixtures of the RAFT copolymerization of NIPAm and DMMIBA after the reaction had been stopped. Spectra were recorded in CDCl$_3$. The sample codes refer to the different polymerization batches (see Table 1, main manuscript).

**Temperature dependent dynamic light scattering on aqueous solutions of the synthesized p(NIPAM-co-DMMIBA) polymers**

Figure S-3 shows the scattering intensity plotted as a function of temperature for the two types of polymers synthesized (see Table 1, main manuscript).

Figure S3. Derived count rate as a function of temperature of P-90 and P-350 (see table 1, main manuscript) dissolved in phosphate buffer (20 mM, pH = 7). The concentration of polymer was set to 0.05 mg/mL. The measurement specifications are given in the Exp. part of the main manuscript.
Details on the activity assay for DERAEC based on the conversion of the natural substrate

The reaction mechanism of the activity assay in use is depicted in Scheme S-2. First, 2-deoxy-D-ribose phosphate is converted to glyceraldehyde-3-phosphate by DERA followed by enzymatic reduction to glycerol-3-phosphate under consumption of NADH. The latter can be monitored by following the absorption at 340 nm.

Scheme S2. Schematic depiction of the reaction sequence that is utilized to assess the enzymatic activity of DERAEC and the respective conjugates.

Synthesis of 3,4-dimethyl maleic imidobutanoic acid (DMIA)

2 g 2,3-dimethylmaleic anhydride (15.9 mM) and 1.64 g 4-aminobutyric acid (15.9 mM) were transferred to a 25 mL round-bottomed flask equipped with a reflux condenser followed by heating to 170 °C for 6 h. The resulting liquid was cooled to room temperature and mixed with 20 mL dichloromethane followed by a washing step with HCl aq (0.1 M). The resulting product appeared as a viscous brown liquid. The $^1$H-NMR spectrum of DMIA is shown in Fig. S-4.

Figure S4. $^1$H-NMR spectrum (CDCl$_3$) of DMIA. The peak at ~ 7.2 ppm stems from the solvent.
Surface modification protocol for the polymeric supports COC and PAN/PEI

The surface modification for the COC supports was done in two steps (Scheme S-3). The first step included the coating with PEI. For that the film was plasma treated (O\textsubscript{2}) and immersed in an aqueous solution of branched polyethyleneimine (5 mg/mL). After 5 min, the COC films were transferred to an oven for drying at 80 °C for 1 h. Finally the film was washed with ethanol for 3 times. In the second step, which includes the introduction of UV-cross-linkable DMIA units, the modified COC films were immersed in 25 mL DMF solution containing 125 mg DMIA, 111 mg N,N'-dicyclohexylcarbodiimide (DCC) and 204 mg 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU). The films were kept in the solution overnight, and finally washed with ethanol for 3 times. The PAN/PEI membrane modification only required the second step, which was performed accordingly.

Wettability changes of the modified COC surfaces were assessed by contact angle measurements using a Dataphysics OCA 15 EC device equipped with a CCD video camera with a resolution of 752 × 582 pixels. The final contact angle data were calculated from an averaged of a series of 5 measurements each. Millipore water was used as testing liquid. The FT-IR spectra of the COC and PAN surfaces were recorded on a Thermo Nicolet spectrometer Nexus. The samples were measured with a resolution of 4 cm\textsuperscript{-1} and for 128 scans.

Upon the exposure of the PEI equipped materials to DMIA, the latter reacts with the abundant amino groups from the PEI via its carboxylic acid entity. For COC, it is also possible to attach DMIA without prior introduction of amino groups, as the O\textsubscript{2} plasma generates OH-groups that can be used for an esterification reaction. Yet, as the PAN/PEI blend membranes are already equipped with amino groups as received, it is more worthwhile to go the detour via a PEI layer for the COC supports in order to have an analogue modification protocol.

Performing contact angle measurements (Table S-1), the surface hydrophilicity change could be monitored upon each modification step. O\textsubscript{2} plasma activated the surface of COC and generated polar functional groups -C-O and -C=O\textsuperscript{1} which led to a contact angle drop from 69° to about 33°. The loading with PEI decreased the contact angle further while additionally leading to a respective pH dependence. FT-IR spectroscopy in attenuated total reflection (ATR) gave further insight (Figure S-5).\textsuperscript{2} The strong bands appearing after the first step of modification at 3200 to 3500 cm\textsuperscript{-1} and 1570 cm\textsuperscript{-1} correspond to the introduced amino groups. The introduction of the
rather hydrophobic DMIA comes along with a contact angle increase to 55 °. At the same time, FT-IR reveals a drop of the amino bands at 3200-3500 cm⁻¹ and 1570 cm⁻¹ to a very low level while a strong carbonyl group signal is found at 1670 cm⁻¹. Within the fingerprint region, a strong signal appears at 850 cm⁻¹, stemming from the substituted carbon-carbon double bonds.³

For the modification of the PAN/PEI blend membranes, an in-house made material with a pore size of 20 nm was applied in order to be able to prepare the active membrane with protein-polymer conjugates later on. As PAN can in principle dissolve in the modification medium DMF, the DMIA treated PAN was compared to unmodified PAN having been exposed to DMF for the same duration. FT-IR measurements of the modified material, in contrast to the unmodified counterpart, show a stronger signal at 1670 cm⁻¹ and a strong single peak at 850 cm⁻¹ which is same as the signal observed for the modified COC. These two changes indicated that the applied modification procedure also worked for PAN/PEI.

Scheme S3. Schematic depiction of the two-step, plasma backed surface modification of COC films for the introduction of UV-cross-linkable DMIA-units.
Figure S5. FT-IR spectra of A) COC films at different stages of functionalization (see scheme S-3); B) FT-IR spectrum of a PAN/PEI blend membrane equipped with DMIA (blue trace). The red trace corresponds to a membrane that has been exposed to the reaction medium DMF in the absence of DMIA for comparison.

Table S-1. Contact angles measured for the COC-substrates after each modification step (see Scheme S-3).

<table>
<thead>
<tr>
<th>Modifications</th>
<th>pH=7</th>
<th>pH=12</th>
</tr>
</thead>
<tbody>
<tr>
<td>untreated</td>
<td>69°</td>
<td>73°</td>
</tr>
<tr>
<td>O₂ plasma</td>
<td>33°</td>
<td>35°</td>
</tr>
<tr>
<td>Complete 1st step</td>
<td>20°</td>
<td>30°</td>
</tr>
<tr>
<td>2nd step</td>
<td>55°</td>
<td>58°</td>
</tr>
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Gas chromatography analysis of the permeate samples collected from the continuously operated membrane

**Figure S6.** Chromatograms obtained from the GC analysis of permeates collected during continuous operation of a PAN membrane equipped with crosslinked C-90-5. 5 fractions à 2 mL were collected over a course of 200 min. The lower row shows chromatograms of benchmark experiments: left: membrane bound conjugates in batch mode; right: non-immobilized conjugates in solution. The amount of enzyme in each experiment was the same (see Exp. part, Main manuscript).

**REFERENCES**

