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# **Supplementary Information**

## **Biocatalytic PEI-PSS membranes through aqueous phase separation:**

## influence of casting solution pH and operational temperature

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**Fig. S1** Photographs of the prepared membranes without lysozyme (a) M-pH<sub>11.4</sub>, (b) M-pH<sub>10.9</sub>, (c) M-pH<sub>10.5</sub>, and with lysozyme (d) M-L-pH<sub>11.4</sub>, (e) M-L-pH<sub>10.9</sub>, (f) M-L-pH<sub>10.5</sub>.



**Fig. S2** Cross-section SEM images of the PEI-PSS membranes without lysozyme (a) M-pH<sub>11.4</sub>, (b) M-pH<sub>10.9</sub>, (c) M-pH<sub>10.5</sub>, and with lysozyme (d) M-L-pH<sub>11.4</sub>, (e) M-L-pH<sub>10.9</sub>, (f) M-L-pH<sub>10.5</sub>.



**Fig. S3** The full cross-section SEM images of the PEI-PSS membranes without lysozyme (a) M-pH<sub>11.4</sub>, (b) M-pH<sub>10.9</sub>, (c) M-pH<sub>10.5</sub>, and with lysozyme (d) M-L-pH<sub>11.4</sub>, (e) M-L-pH<sub>10.9</sub>, (f) M-L-pH<sub>10.5</sub>.

### **Porosity calculation**

The porosity ( $\epsilon$ ) of the membrane is calculated using the Equation S1:

$$\varepsilon(\%) = \frac{v_{pore}}{v_{total}} * 100 = \left(1 - \frac{v_{pure}}{v_{total}}\right) * 100 = \left(1 - \frac{m_{pure}/\rho_{pure}}{s*h}\right) * 100$$

Where  $m_{pure}$  and  $\rho_{pure}$  is the dry weight and density (1.1 g·cm<sup>-3</sup>) of the pure dry membrane without pores. Here we assume that the density of the pure membrane is similar with the polyelectrolyte complexes.<sup>1</sup> s and h are the surface area and thickness (measured from SEM images in Fig.S3) of the dry membrane.

Membranes	M-pH <sub>11.4</sub>	M-pH <sub>10.9</sub>	M-pH <sub>10.5</sub>	M-L-pH <sub>11.4</sub>	M-L-pH <sub>10.9</sub>	M-L-pH <sub>10.5</sub>
Porosity (%)	81.4	78.7	67.1	81.8	77.1	66.0

**Table S1.** Porosity of the membranes prepared with different pH casting solution.



**Fig. S4** Absorbance at 450 nm of 2.5 mL substrate suspension with 100  $\mu$ L free lysozyme solution (5 mg·L<sup>-1</sup>) and 100  $\mu$ L PBS buffer (blank sample) measured at different temperatures.



**Fig. S5** Absorbance at 450 nm of the substrate suspension after the biocatalytic membranes (a)  $M-L-pH_{11.4}$ , (b)  $M-L-pH_{10.9}$ , and (c)  $M-L-pH_{10.5}$  were treated in the suspension for 1 hour at different temperatures. All values are shown as averages of three samples, and the error bar represents the standard deviation.



**Fig. S6** Enzymatic activity of the biocatalytic membranes (a)  $M-L-pH_{11.4}$ , (b)  $M-L-pH_{10.9}$ , and (c)  $M-L-pH_{10.5}$  after being treated in substrate for 1 hour at different temperatures, and then put in new 0.15 mg·mL<sup>-1</sup> substrate suspension.



**Fig. S7** Absorbance at 281.5 nm of the supernatant water of the PEI-PSS membranes without and with lysozyme stored in water for 7 days.

Membranes	PAH-PSS	M-L-pH <sub>11.4</sub>	M-L-pH <sub>10.9</sub>	M-L-pH <sub>10.5</sub>
Casting solution pH	pH~13	pH~11.4	pH~10.9	pH~10.5
Coagulation bath pH	pH~1	pH~4	pH~4	pH~4
Water permeability (L·m <sup>-2</sup> ·h <sup>-1</sup> ·bar <sup>-1</sup> )	12±2	3.6±0.2	50±16	452±83
Lysozyme loading (µg∙cm <sup>-2</sup> )	4.49±0.41	~7.5	~7.5	~7.5
Highest activity (U·cm <sup>-2</sup> )	2.47±0.49	4.29±0.15	3.80±0.17	2.35±0.30
7 days activity (U·cm <sup>-2</sup> )	1.23±0.47	3.25±0.12	4.31±0.03	2.64±0.09
7 days stability	50.2%	75.8%	100%	100%
60 days activity (U·cm <sup>-2</sup> )	١	1.85±0.07	2.67±0.07	1.57±0.04
60 days stability	١	43%	62%	59%

**Table S2**. Overview comparation between the biocatalytic PEI-PSS and PAH-PSSmembranes.

### Reference

1. R. Köhler, I. Dönch, P. Ott, A. Laschewsky, A. Fery and R. Krastev, *Langmuir*, 2009, **25**, 11576-11585.