Electronic Supplementary Information for "Probing the influence of crosslinkers on the properties, response, and degradation of enzymatic hydrogels for electrochemical glucose biosensing through fluorescence analysis"

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1. *Hydrogel profilometries*

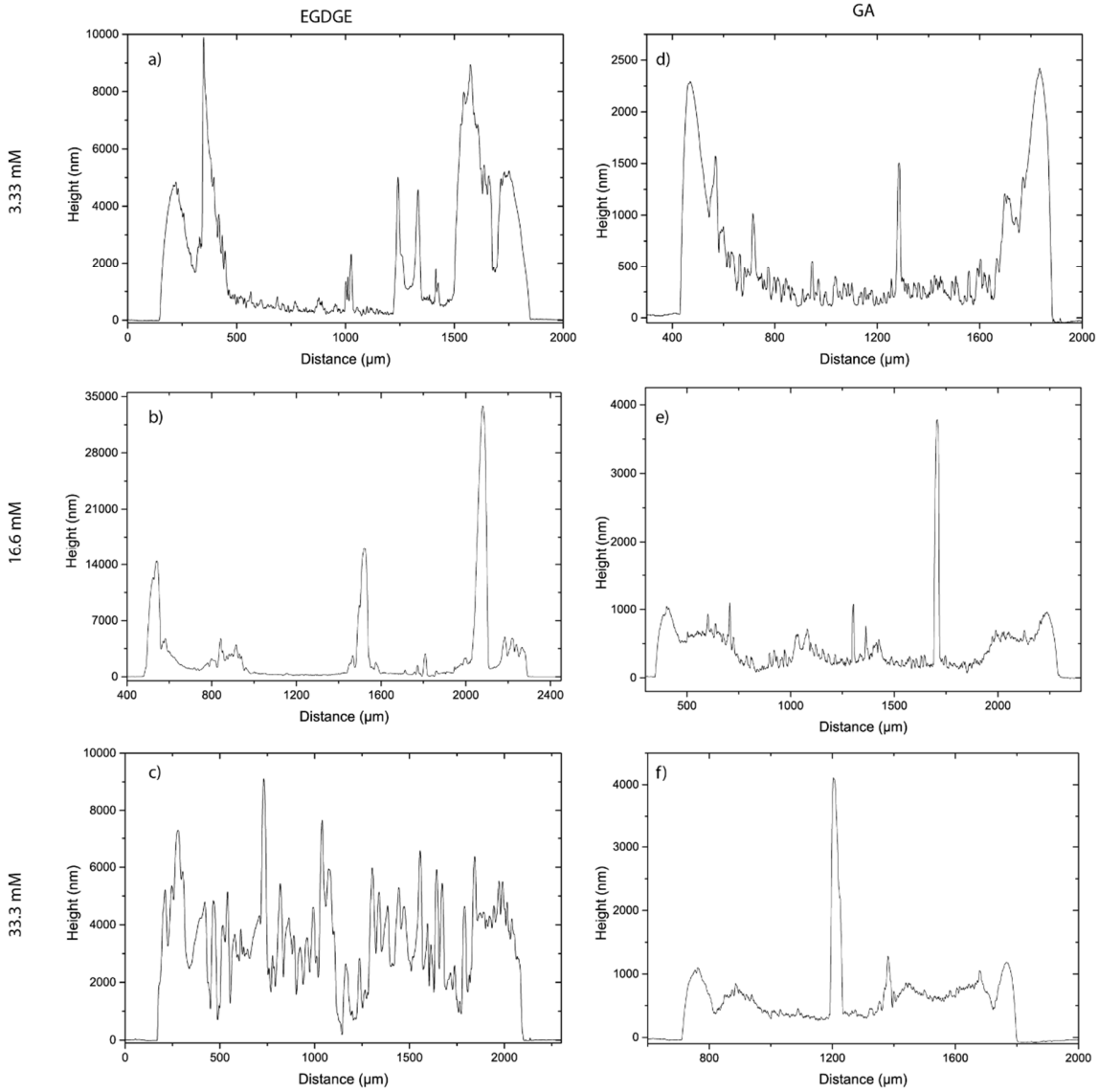

Figure S1. Profilometries of (a-c) GA-based and (d-f) EGDGE-based GOx/BPEI hydrogels prepared using cross-linkers at (a,d) 3.33 mM, (b,e) 16.6 mM and (c,f) 33.3 mM deposited on glass surfaces.

2. *Hydration of the hydrogel*

The video file titled "Video_S1" presents the sequence of fluorescence images of a hydrogel deposited on glass prepared with 33.3 mM glutaraldehyde (GA) upon the addition of a drop of phosphate buffer (PB) 0.1 M pH = 7.4. The video is displayed at half of the actual speed. The appearance of the folds and change in the deposit footprint can be clearly observed.

3. *Stability of the deposited hydrogels*

Table S1. Stability evaluation (adhesion failure) for 30 days in 0.1 M PB pH 7.4 on glass and gold surfaces of crosslinked hydrogels with different concentrations of GA and EGDGE.

	Adherence failure at 15 days		**Adherence failure at 30 days**	
Gel	**Gold**	**Glass**	**Gold**	**Glass**
BPEI/GOx/GA 3.33 mM	0/5	3/5	0/5	3/5
BPEI/GOx/GA 16.65 mM	0/5	1/5	0/5	1/5
BPEI/GOx/GA 33.3 mM	0/5	0/5	0/5	0/5
BPEI/GOx/EGDGE 3.33 mM	1/5	3/5	1/5	3/5
BPEI/GOx/EGDGE 16.65 mM	1/5	4/5	1/5	4/5
BPEI/GOx/EGDGE 33.3 mM	2/5	4/5	2/5	4/5

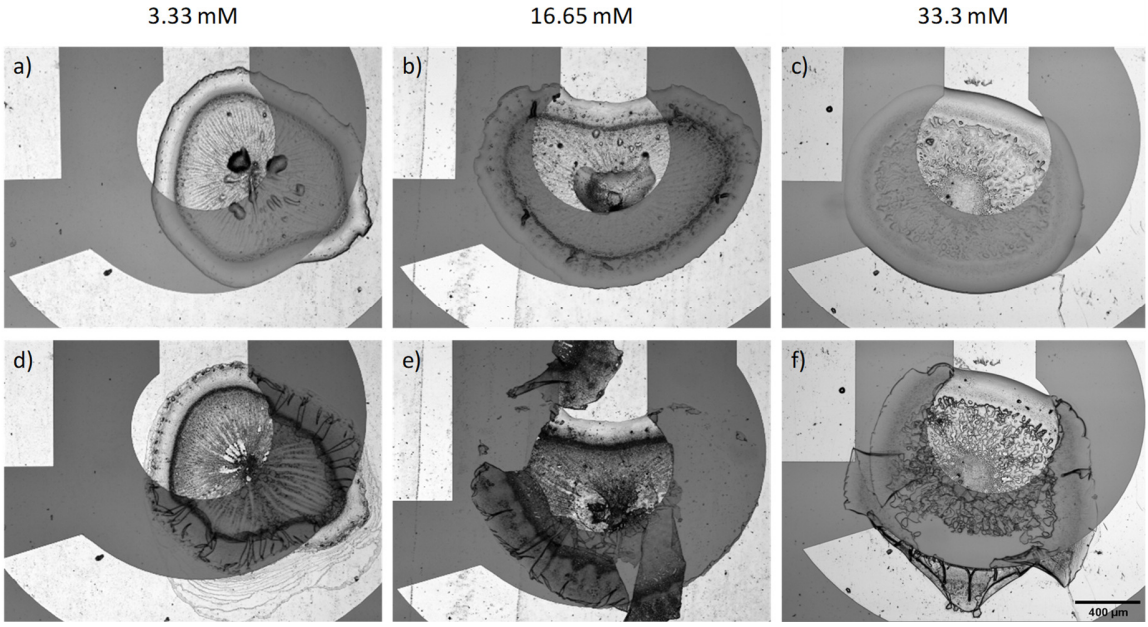

Figure S2. Structural damage after the swelling process due to the swelling degree/water absorption related to the EGDGE concentration. Dehydrated (a-c) and hydrated (d-f) state of the gels.

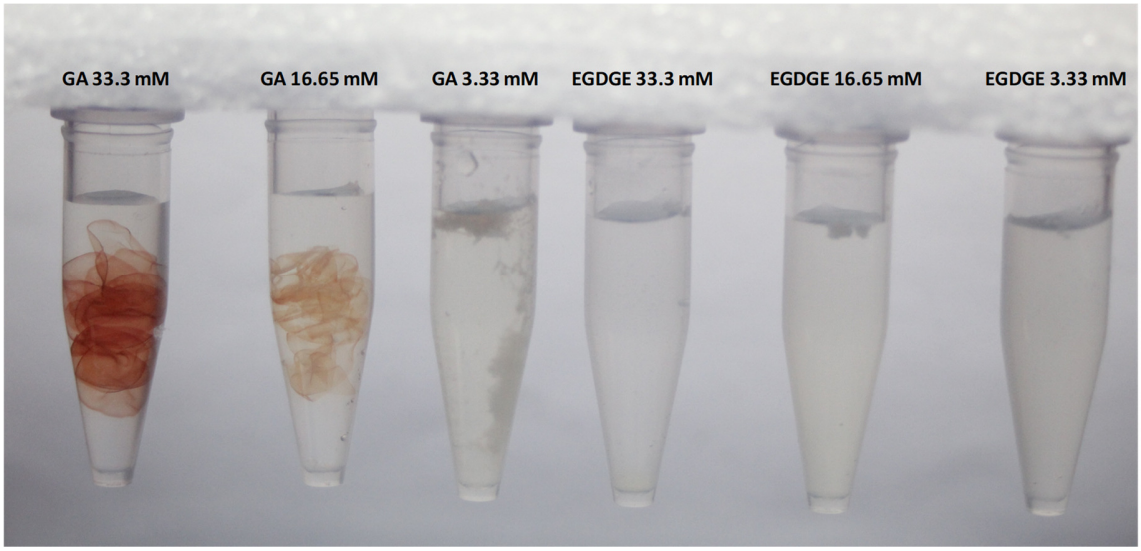

Figure S3. Physical appearance of unsupported EGDGE and GA crosslinked gels immersed in 0.1 M PB pH 7.4 for 1 h.

4. Enzyme kinetic parameters

Table S2. Maximum current densities (J_{max}) and apparent Michaelis-Menten constants (K_m^{app}) determined for crosslinked gels with different concentrations of EGDGE and GA.

Gel	J_{max} (μA cm²)	K_m^{app} (mM)
BPEI/GOx/GA 3.33 mM	56.07±2.45	1.62±0.22
BPEI/GOx/GA 16.65 mM	77.18±2.11	1.52±0.13
BPEI/GOx/GA 33.3 mM	66.39±4.25	1.95±0.36
BPEI/GOx/EGDGE 3.33 mM	27.06±4.62	13.43±3.51
BPEI/GOx/EGDGE 16.65 mM	26.12±2.64	4.43±0.99
BPEI/GOx/EGDGE 33.3 mM	43.70±1.75	1.61±0.20

5. Spectroscopic studies

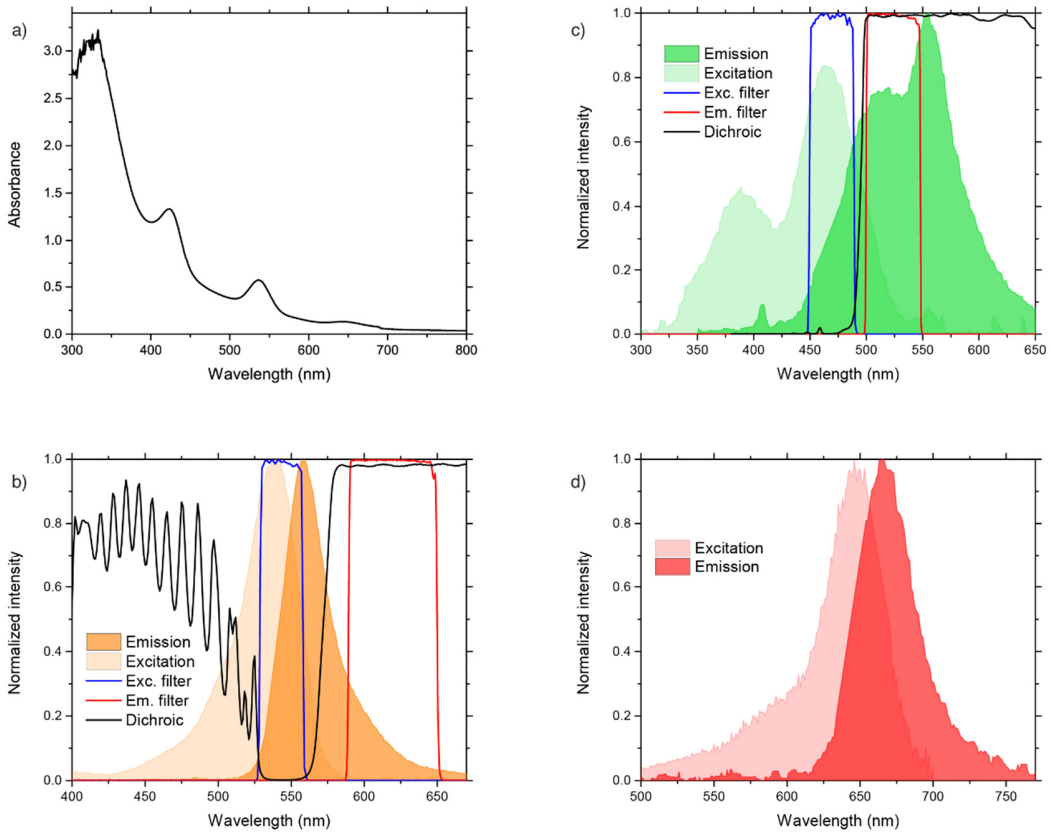

Figure S4. Spectroscopic characterization of GA-based hydrogels. a) UV-vis absorption spectrum. b-d) Excitation and emission spectra in three different wavelength regions. In (b) and (c), the normalized transmission spectra of the blue and green fluorescence filter sets are included.