Supplementary Material (ESI) for Soft Matter

Supplementary Information of the Manuscript

Enzyme-Induced Graft Polymerization for Preparation of Hydrogels: Synergetic Effect of Laccase-Immobilized-Hydrogels for Pollutants Adsorption

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Table S1: Data resulting from fitting experimental adsorption isotherms obtained for different samples to Langmuir (grey boxes) and Freundlich (white boxes) equations.

Sample	b (L mg^{-1})	Q_{max} (mg g^{-1})	R^2	K_F (mg g^{-1})	n (g L^{-1})	R^2
PEG-g-F68(2:1-7)^{h}-lac1	0.008	40.6	0.985	1.51	2.14	0.903
PEG-g-F68(2:1-7)^{c}-lac1	0.014	74.5	0.997	7.27	2.80	0.941
PEG-g-F68(2:1-7)^{wc}-lac1	0.012	40.0	0.989	3.32	2.64	0.962
H_2O[PEG-g-F68(2:1-7)-lac1]	0.013	32.9	0.996	3.06	2.80	0.956
PEG-g-F68(2:1-7)^{h}-lac3	0.006	328.6	0.992	17.09	2.49	0.942
PEG-g-F68(2:1-7)^{c}-lac3	0.007	450.2	0.994	28.48	2.62	0.943
PEG-g-F68(2:1-7)^{wc}-lac3	0.006	235.6	0.996	11.15	2.40	0.965
PEG-g-F68(2:1-7)^{wc}-lac0	0.013	23.2	0.997	2.11	2.73	0.957
PEG-g-F68(2:1-7)^{wc}-lac2	0.009	126.3	0.998	10.31	2.54	0.951
PEG-g-F68(1:1-7)^{c}-lac1	0.006	55.8	0.997	3.10	2.46	0.950
PEG-g-F68(1:1-7)^{wc}-lac1	0.007	32.2	0.995	2.17	2.68	0.938
PEG-g-F68(3:1-7)^{c}-lac1	0.005	99.6	0.998	5.03	2.43	0.958
PEG-g-F68(3:1-7)^{wc}-lac1	0.020	51.8	0.990	9.38	4.01	0.935

Lac1 stands for 20 units, **lac2** stands for 80 units and **lac3** for 200 units. **Super index notation:** ^{h} stands for hydrogel, ^{c} stands for cryogels, ^{wc} stands for washed cryogel and ^{awc} stands for aged washed cryogel. [1] H_2O[PEG-g-F68] refers to water recovered after cryogel (eventually described into brackets) washing.

Figure S1: SEM micrographs of longitudinal sections of PEG-g-F68(2:1-7)^{c}-lac1 **(a)** and PEG-g-F68(2:1-7)^{c}-lac2 **(b)**. Bars are 20 μm in (a) and 50 μm in (b).

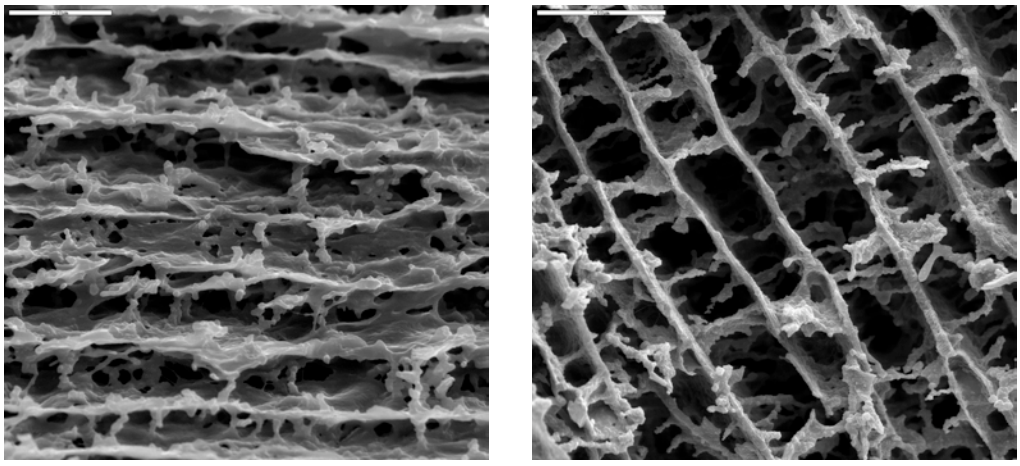


Figure S2: SEM micrographs of PEG(7)^{c}-lac1 (left column) and PEG(10)^{c}-lac1 (right column) cryogels at different magnifications. Bars are (from top to bottom) 200, 50 and 20 μm.

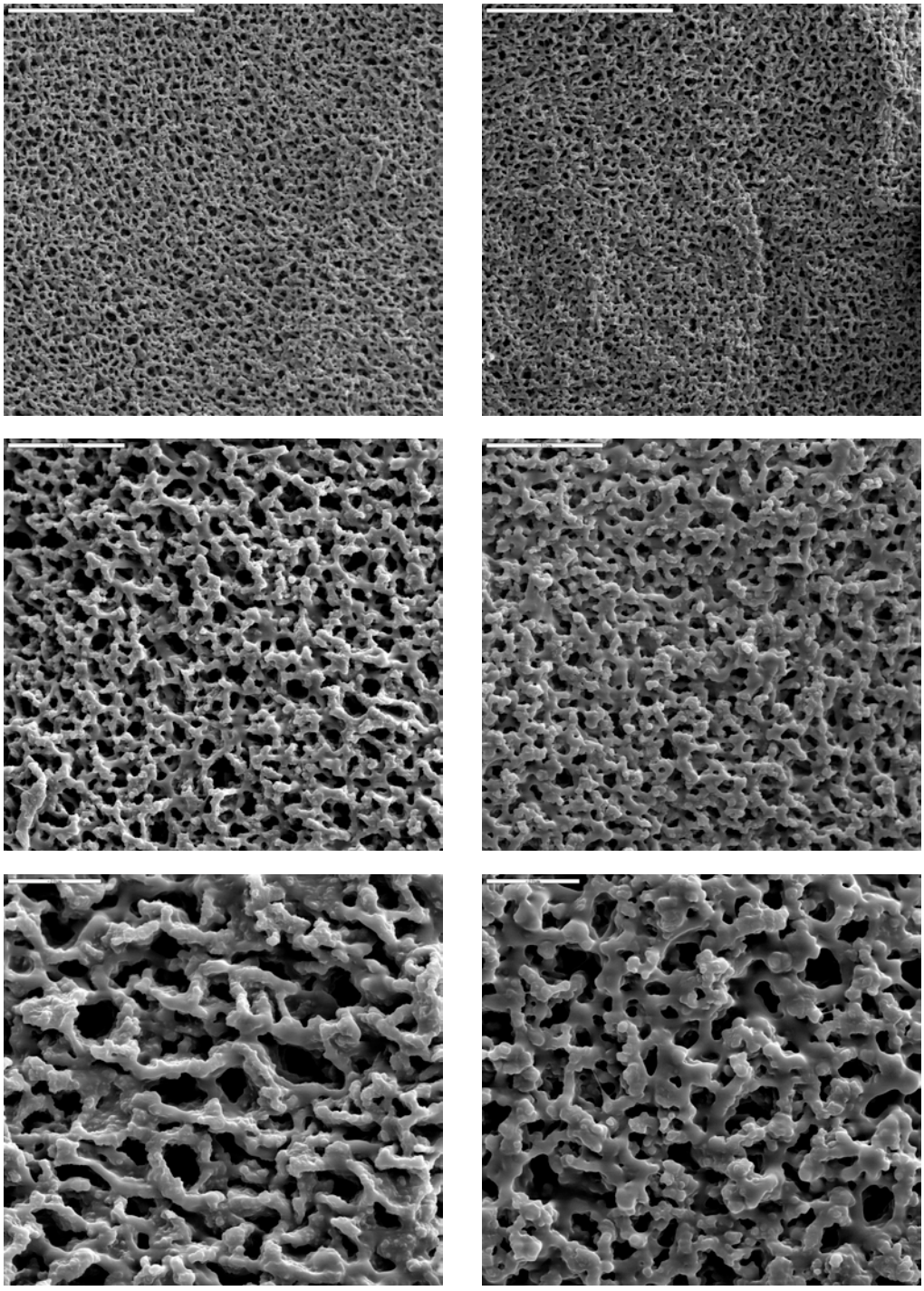
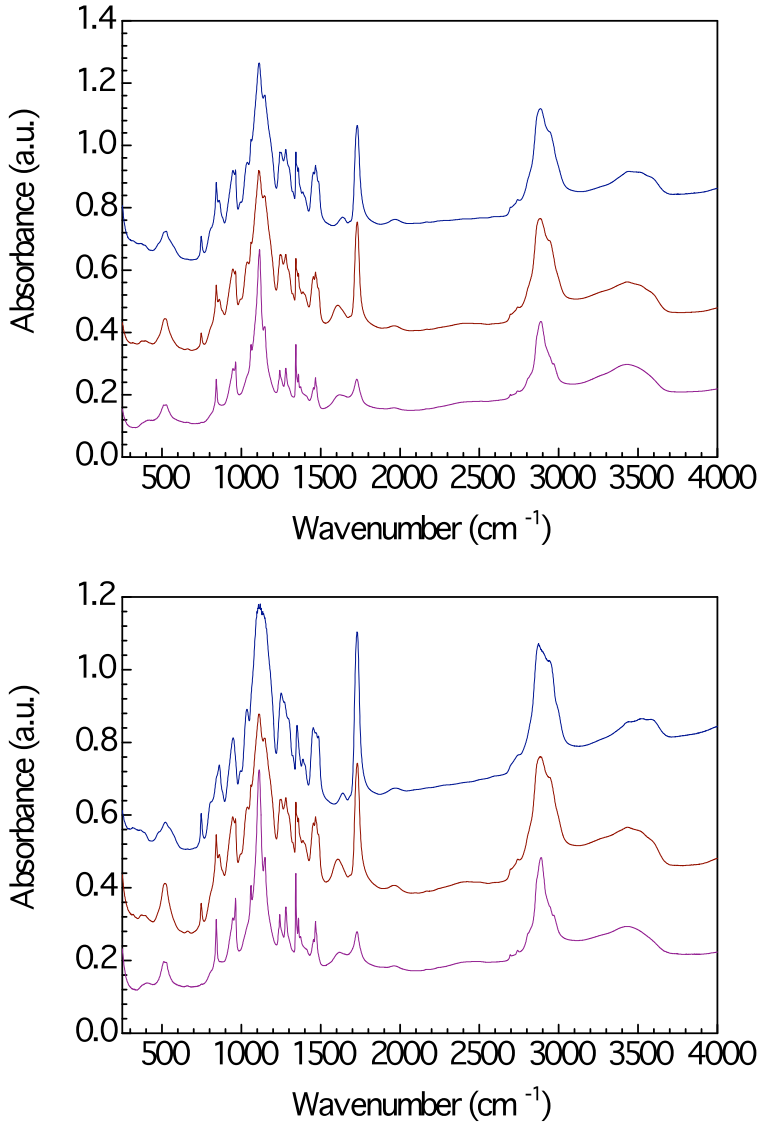


Figure S3: FTIR spectra of PEG-g-F68(1:1-7) (top) and PEG-g-F68(3:1-7) (bottom) cryogels before (red line) and after (blue line) extraction of un-reacted monomers (by keeping in distilled water at 25 °C for 72 hours). The FTIR spectrum of the extracted fraction is also represented (purple line).



Further discussion on FTIR spectra:

As mentioned in the principal text, the intensity increase of the bands at ca. 2950 cm^{-1} (ascribed to CH_3 symmetric and anti-symmetric stretching modes) as compared to those at ca. 2850 cm^{-1} (ascribed to CH_2 symmetric and asymmetric stretching modes) proofs the presence of PPO segments in PEG-g-F68 cryogels after extraction of non-reacted species.[1] The absorption band at ca. 3510 cm^{-1} is assigned to terminal hydroxyl groups in the copolymer and its was mostly observed in PEG-g-F68 cryogels after extraction of non-reacted species and confirming the presence of F68 in the resulting copolymer.[2a] FTIR spectrum of PEG-g-F68 cryogels after extraction of non-reacted species also confirmed the grafting of PEGDA onto F68 by the shift of the peak of carbonyl stretching resonance from 1724 (conjugated with acrylic groups) to 1734 cm^{-1} (non-conjugated) upon cross-liking.[1b] The decrease of the relative intensity of the peak assigned to C=C vibration (at 1636 cm^{-1}) versus that of the peak assigned to C=O stretching (within the 1724-1734 cm^{-1} range) in PEG-g-F68 cryogels after extraction of non-reacted species revealed efficient PEGDA polymerization for the formation of PEG-g-F68 cryogels.[2]

Moreover, PEO units (from both PEGDA and F68) exhibit strong bands at 844 and 950 cm^{-1}, and a shoulder at 962 cm^{-1} in the methylene rocking region. However, the band at 962 cm^{-1} is typically stronger than that at 950 cm^{-1} in polymers containing PPO units besides PEO units (e.g. F68). The intensities of the three bands decrease with increasing PPO/PEO ratios. Bands assigned to C–C and C–O stretching appear within the 1000–1200 cm^{-1} region. The intensity of the C–O stretching mode at 1115 cm^{-1} is very strong for PEO-PPO copolymers and PEO homopolymers. The 1060 cm^{-1} band is coupled C–O stretching and C–C stretching. The intensity of the band decreases in the FTIR spectrum of PEG-g-F68 cryogels after extraction of non-reacted species, which is ascribed to PPO/PEO ratio increase and revealed non-reacted species mostly correspond to PEG (only composed of PEO segments) rather than F68 (composed of both PEO and PPO segments).

[1] (a) C. Guo, H Liu, J Wang, and J Chen, *J. Coll. Interf. Sci.* 1999, **209,** 368-373, (b) C.-H. Choi, J.-H. Jung, T.-S. Hwang, and C.-S. Lee, *Macromol. Res.*, 2009, **17**, 163-167

[2] (a) Y. W., F.-B. Che, and J.-H. Chen, *J. Appl. Polym. Sci.* 2008, **110**, 1118-1128, (b) D. Biswal, and J. Z. Hilt, *Polymer* 2006, **47**, 7355-7360

Figure S4: Plot of absorbance decrease at 594 nm versus time as consequence of laccase induced degradation of Brilliant Blue dye Remazol. Experiments were carried out as follows: laccase immobilized PEG-g-F68 cryogels (35 mg of scaffold, 45 units of laccase) were soaked in a BBdB aqueous solution (3 mL, 0.08 mM) and the absorbance decrease at 594 nm was monitored versus time (solid line). For comparison, experiments were also carried in solution (dash line) by simple dissolution of laccase (45 units) in the BBdB aqueous solution (3 mL, 0.08 mM). The absorbance reached after 20 minutes in curve (b) was lower than in (a) due to the eventual adsorption of degradation by-products (also contributing to absorbance at 594 nm) on PEG-g-F68 hydrogels.