Supporting Information to

Real Time Quantification of the Chemical Cross-Link Density of a

Hydrogel by in situ UV-vis Spectroscopy

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Table S1. Elementary reactions pertinent to the photoreaction of phenol groups and D.

Reaction	Note
(I) D + light \rightarrow D'	optical excitation of singlet dye
(II) $D' \rightarrow D^*$	conversion to triplet dye
(III) $D^* + D^* \rightarrow D + D$	nonradiative decay of triplet dye
(IV) D' + C ₆ H ₅ OH \rightarrow D + C ₆ H ₅ OH	phenol-quenching of excited singlet dye
(V) $D^* + C_6H_5OH \rightarrow DH^{\bullet} + C_6H_5O^{\bullet}$	reaction between phenol and triplet dye
(VI) $DH^{\bullet} + DH^{\bullet} \rightarrow DH_2 + D$	dismutation of reduce dye
$(VII) C_6H_5O^{\bullet} + C_6H_5O^{\bullet} \rightarrow C_6H_5O\text{-}OC_6H_5$	coupling reaction
$(VIII) D^* + O_2 \rightarrow D + O_2$	oxygen-quenching of triplet dye
(IX) $D^* + O_2 \rightarrow oxidized dye$	reaction between oxygen and triplet dye

This photoreaction is accomplished in a multiple-step process and the related reactions are listed in Table S1. With possible side reactions, the cross-linking reaction

may be not 100% in efficiency. The conversion of Eosin Y (D) to other two products (DH₂ and the oxidized dye) leads to the decrease of A₅₁₅. The reaction of $D \rightarrow DH_2$ is coupled with the cross-link reaction, whereas the reaction from D to oxidized dye is the side reaction. It is reported by Zwicker et al. that the k_{VIII} (1.6 x 10⁶ M⁻¹ sec.⁻¹) is much larger than k_{IX} (9.1 x 10³ M⁻¹ sec.⁻¹).^{1,2} Therefore, the decrease of A₅₁₅ is mainly contributed by the reaction of $D \rightarrow DH_2$. Thus, the efficiency of the cross-link reaction should be very high. Furthermore, the possible reaction between dissolved O₂ and D (Reaction IX in Table S1) and the possible reaction between SA and D are proved to be negligible, see main text and Fig. S2 for details. As a result, the effects of side reaction can be ignored. Thus, the photoreactions (reaction I-IX) can be summarily represented as Reaction 1 below:

$$D + 2 C_6 H_5 OH \rightarrow DH_2 + C_6 H_5 O - OC_6 H_5$$
(1)

Table S2. Rheological data (G') for three systems with 3w LED irradiation for about 30 min. The concentration of MSA was fixed at 0.5 wt.%, while concentrations of D were 0 μ M, 13.4 μ M and 24.4 μ M, respectively.

[D] (µM)	Average of G' (Pa)
0	1.3
3.4	51.2
24.4	97.9



Fig. S1. Possible pathways to consume D during the gelation process.



Fig. S2. The UV-vis spectra of D at 515nm in 0.1 M PBS buffer vs irradiation time. (a) D alone (D 13.4 μ M and 3w LED irradiation), (b) SA with D (SA 0.5 wt.%, D 13.4 μ M and 3w LED irradiation).



Fig. S3. The reaction kinetics of the reduction of D by phenol groups (Reaction 1) with light irradiation (n = 3) at different conditions. (a) [MSA] = 0.025 wt.% and LED power =3w. (b) [D] = 4.5μ M.

Fig. S3 demonstrates that Reaction 1 is a quasi first-order process. Upon visible light irradiation, the MSA hydrogels can be prepared. This result indicates that the coupling reaction of phenol groups (Reaction VII in Table S1) predominates over other possible reactions of phenol groups. The rate of Reaction 1 may be mainly determined by the oxidation process of photo excitation of singlet dye (Reaction I in Table S1). While the rate constant of Reaction 1 is associated with the specific condition, it is not in the scope of the present study and will not be discussed in details here.



Fig. S4. Standard curve of UV-vis absorbance of D (at 515 nm) in 0.1 M PBS buffers (pH = 7.0) (n=3).



Fig. S5. The cross-link density *vs* irradiation time in different conditions. Blue solid squares: D 24.4 μ M and 3w LED irradiation. Blue open squares: D 4.5 μ M and 3w LED irradiation. Red open circles: D 13.4 μ M and 3w LED irradiation. Red solid circles: D 13.4 μ M and 3w LED irradiation. Red solid circles: D 13.4 μ M and 3w×2 LED irradiation. The concentrations of MSA for all samples were 0.5 wt.%. The arrows point out the gelation time of the three profiles in different conditions (n=3). The gelation time is determined by test tube inverting method, see Fig. S6 b-d.



Fig. S6. (a) Storage modulus (G') of the sample under various LED irradiation time by rheological measurement. A remarkable change can be observed from 19 to 20 min. (b-d) Photos of the test tube inverting method under LED irradiation: (b) 0 min, (c) 19 min and (d) 21 min, respectively. For all experiments in this Figure, the concentrations of D was 24.4 μ M, and the concentration of MSA was fixed at 0.5 wt.%.

Fig. S6a shows the storage modulus (G') by rheological measurements of the samples with different time of LED irradiation. There is a jump of G' at \sim 0.21 Hz between 19 and 20 min, while G' shows no significant difference between 20 and 21 min. Thus, the gelation time is determined to be \sim 20 min. The gelation time

determined by test tube inverting method (~21 min) is similar to that by the rheological measurement, see Fig. S6 b-d.

References

- 1. L. I. Grossweiner and E. F. Zwicker, J. Chem. Phys., 1961, **34**, 1411-1417.
- 2. E. F. Zwicker and L. I. Grossweiner, J. Phys. Chem., 1963, 67, 549-555.