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# SUPPORTING INFORMATION

# pH-dependent Fluorescence from Firefly Oxyluciferin in Agarose Thin Films

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## Contents

## 1. Experimental details

### 1.1. UV-Vis absorbance spectroscopy of OxyLH$_2$ thin films

**OxyLH$_2$**-doped thin films were fabricated from oxyluciferin samples provided and synthesized by O.V. Maltsev and L. Hintermann.[S1] The spectra were recorded as described in the experimental section of the main text. As seen in Figure S1a/b, films containing 0.2% and 0.4% **OxyLH$_2$** did not produce a detectable signal. At slightly higher concentrations (0.6%, Figure S1c) there are two distinct absorbance bands: one at 378 nm that shifts to 434 nm in alkaline conditions, representing the phenol-enolate and the phenolate-enolate forms, respectively. The isosbestic point gives p$K_a$ = 9.91 ± 0.91, in agreement with the value produced by the 1.0% thin film described in the main text. No clear isosbestic point could be identified in the 0.8% thin film (Figure S1d).

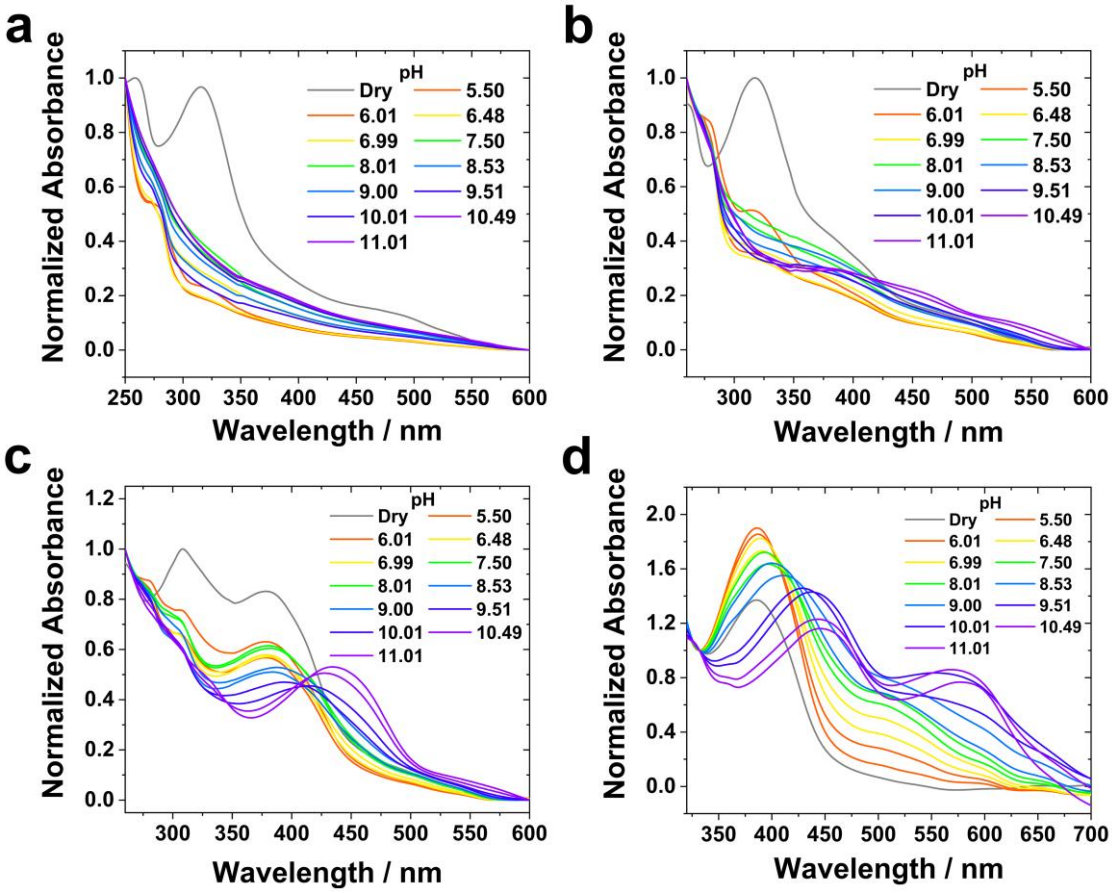


**Figure S1.** Absorbance spectra of a) 0.2, b) 0.4, c) 0.6, and d) 0.8% **OxyLH$_2$**-agarose thin film at several pH values.

## 1.2. Fluorescence spectroscopy of OxyLH$_2$ thin films

The spectra were recorded as explained in the experimental section of the main text. Despite the poor absorbance signals fluorescence profiles were able to be recorded for low weight films (0.2 – 0.4%, Figure S2a/b). All thin films showed a characteristic dual-banded fluorescence profile when they were dry (Figure S2, Table S1) as well as a maximum at approx. 522 nm (wet, Table S1) that was quenched under alkaline conditions (Figure S2). The 0.8% thin film (Figure S2d) displayed the strongest signal.

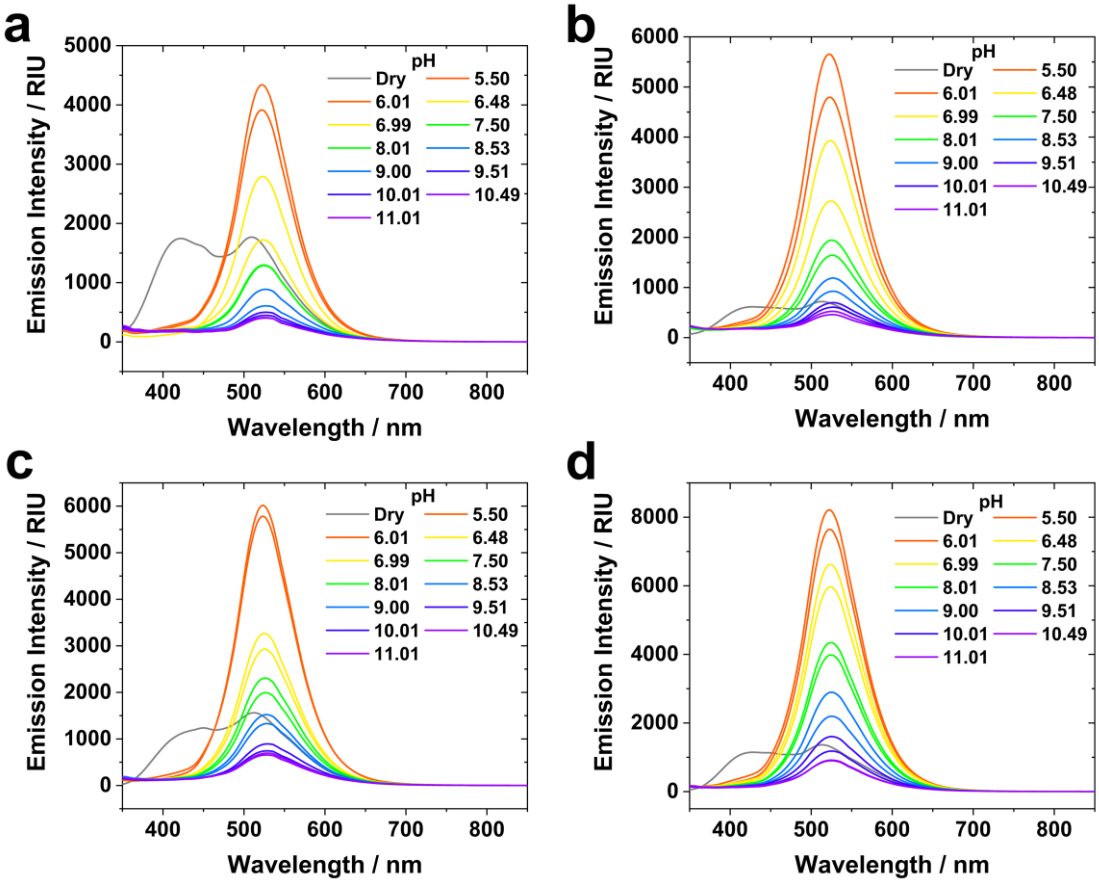


**Figure S2.** Fluorescence spectra of a) 0.2, b) 0.4, c) 0.6, and d) 0.8 *wt* % oxyluciferin-agarose thin film at several pH values. $\lambda_{ex}$ = 326 nm.

**Table S1.** Emission maxima of **OxyLH$_2$** thin films.

| wt % OxyLH$_2$ | $\lambda_{em}$ (Dry) / nm | | $\lambda_{em}$ (pH 5.50) / nm | $\lambda_{ex}$ / nm |
|---|---|---|---|---|
| 0.2 | 422 | 510 | 522 | 326 |
| 0.4 | 428 | 510 | 522 | 326 |
| 0.6 | 448 | 512 | 524 | 326 |
| 0.8 | 428 | 514 | 522 | 326 |
| 1.0 | 448 | 514 | 524 | 326 |

### 1.3. UV-Vis absorbance spectroscopy of Me-OxyLH thin films

The UV-Vis absorption spectra of the **Me-OxyLH** thin films were carried out as described in section 1.1, with films cast at 0.8%. The absorbance spectra of the methylated **OxyLH$_2$** are generally insensitive to changes in pH (Figure S3). This is expected as the p$K_a$ of the first hydroxyl group (p$K_a$ ≈ 7.1) is much lower than that of the phenol to phenolate deprotonation identified in the main text.[S2] Thus, no isosbestic point exists, rendering the change undetectable.

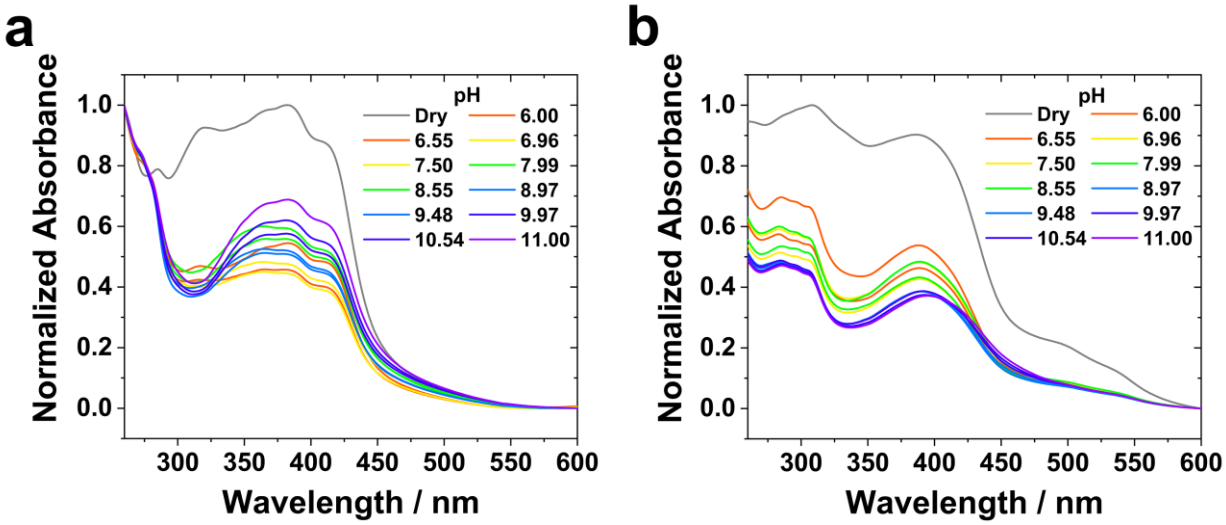


**Figure S3.** Absorbance spectra of a 0.8 *wt* % a) **4-MeOxyLH**-agarose b) **6'-MeOxyLH**-agarose thin film at several pH.

### 1.4. Humidity-responsive spectroscopy of OxyLH$_2$ thin films

In addition to the described pH response, the films displayed a change in fluorescence profile once submerged into the buffers. Upon the introduction of moisture, the minor signal in the blue region of the emission spectra disappeared completely, suggesting that the water selectively induces change in the composition of the thin films, shifting from a mix of the possible ground state structures in the dry film to a single form of the emitter in the wet film. To explore this process, films were exposed to dry (20% relative humidity @ 19 °C, RH), moderate (59% RH), and wet (99% RH) environments for several days.

To measure the thin films' response to humidity, they were placed in a vacuum desiccator with a hygrometer (±0.1%) over anhydrous calcium chloride (Sigma) and evacuated using a Schlenk line. The films were left for two days and removed for analysis. The lowest achievable relative humidity using this drying method was 20%. A moderate humidity level was achieved in a similar manner by replacing the calcium chloride with a saturated solution of ammonium chloride (Sigma) achieving a stable RH of 59%.[S3] Finally, a fully saturated environment (99%) was formed by placing pure water in the lower compartment of the desiccator. Upon removal from the desiccator the films were sealed twice with parafilm (Bemis Co., Neenah, WI, USA) and analyzed immediately. Absorbance and fluorescence spectra were collected after separate

rounds of incubation. All spectroscopic measurements were carried out at room temperature in the manner described in the experimental section of the main text.

The absorbance spectra of the thin films are invariant to changes in relative humidity (Figure S4a/b/c) suggesting that the wetting of the films has little effect on the chemical form of oxyluciferin and thus the acidochromism described in the main text. Furthermore, the fluorescence spectra (Figure S4d/e/f) show a clear equilibrium between two emitting species that is strongly affected by the humidity.

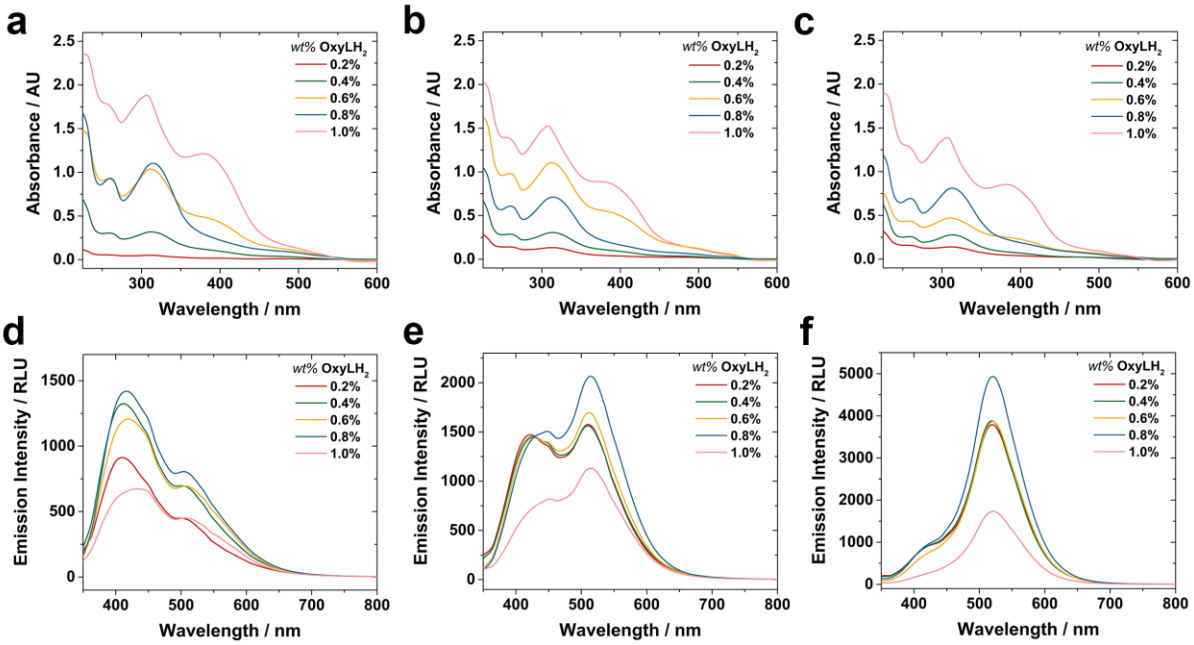


**Figure S4.** Top: Absorbance spectra of **OxyLH$_2$** thin films at a) 20%, b) 59%, and c) 99% relative humidity. Bottom: Fluorescence spectra of **OxyLH$_2$** thin films at d) 20%, e) 59%, and f) 99% relative humidity. $\lambda_{ex}$ = 326 nm.

### 1.5. Full optical images of OxyLH$_2$ thin films

A small section of the 1.0% **OxyLH$_2$** thin film was cut from a larger piece and affixed using an alligator clamp. The film was photographed under standard indoor fluorescent lighting (Fig. S5a) and illuminated using a handheld ultraviolet LED torch (Fig. S5b). Fig. 1 was created from these two images.

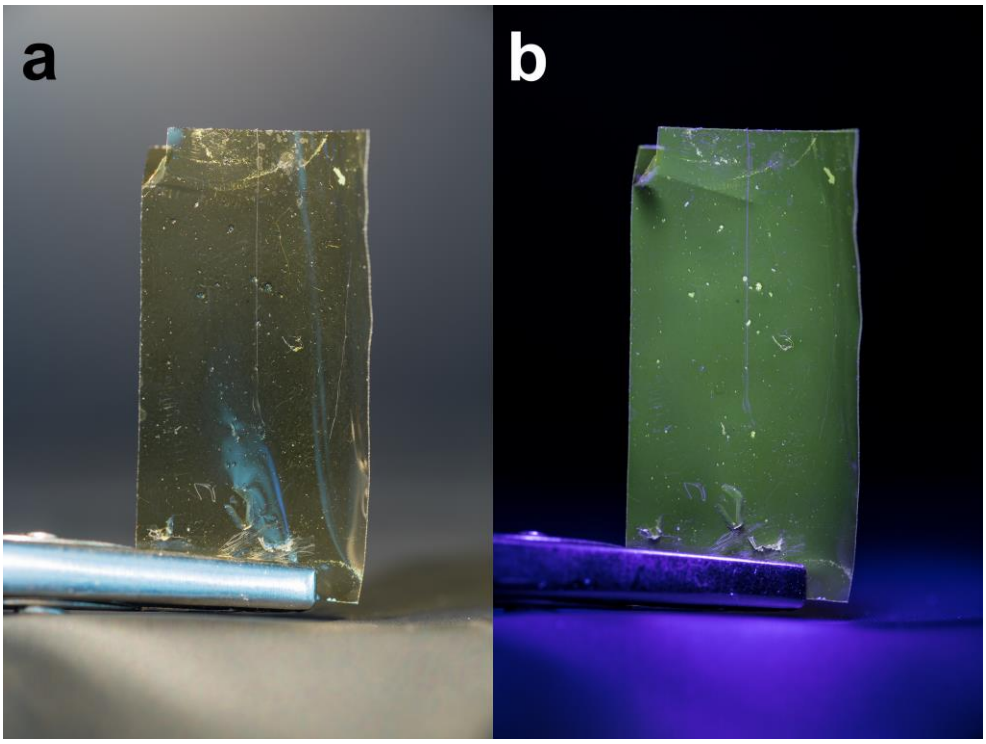


**Figure S5**. Full photograph of a dry 1.0 wt% oxyluciferin-agarose thin film under a) white light and b) UV illumination. The film was cut from a larger sheet and is approximately 10 mm x 22 mm.

## 2. Supporting references

S1. O. V. Maltsev, N. K. Nath, P. Naumov and L. Hintermann, *Angew. Chem. Int. Ed.*, 2014, **53**, 847.
S2. A. Ghose, O. V. Maltsev, N. Humbert, L. Hintermann, Y. Arntz, P. Naumov, Y. Mély and P. Didier, *J. Phys. Chem. B*, 2017, **121**, 1566.
S3. Greenspan, L., *J. Res. Natl. Bur. Stand., Sect. A,* 1976, ***81***, 89.