SUPPORTING INFORMATION

Bioinspired Molecular Lantern: Tuning the Firefly Oxyluciferin Emission with Host-Guest Chemistry

Na'il Saleh,[†]* Abdul Rahman Ba Suwaid,[†,#] Ahmad Alhalabi,[†,#] Ahmed Z. A. Abuibaid,[†,#] Oleg V. Maltsev,[‡] Lukas Hintermann,[‡] Panče Naumov[§]*

[†]*Department of Chemistry, College of Science, United Arab Emirates University, P.O. Box 15551, Al Ain, United Arab Emirates*
[‡]*Department Chemie, Technische Universität München, Lichtenbergstrasse 4, 85748 Garching bei München, Germany*
[§]*New York University Abu Dhabi, P.O. Box 129188, Abu Dhabi, United Arab Emirates*

Supporting methods section

Determination of the binding constants

The binding constants were extracted from the changes of UV-Visible absorption or fluorescence of oxyluciferin with the addition of different amounts of CB7 at a given pH. The measurements were carried out in a rectangular cuvette (1 cm optical path length) before and after adding small amounts of CB7 stock solution to a known concentration of the guest using an automatic pipette until a plateau was reached. While total concentrations of the guest were kept constant, the concentrations of CB7 were gradually increased. The analysis of the resulted spectra involves the assumption of 1:1 equilibrium between the firefly compounds and the host molecule, as follows:

$$\mathbf{OxyLH_2} + \text{CB7} \rightleftharpoons \mathbf{OxyLH_2}/\text{CB7} \quad (1)$$

where K is the association equilibrium constant:

$$\boldsymbol{K} = \frac{[\boldsymbol{OxyLH_2/CB7}]}{[\boldsymbol{OxyLH_2}][\boldsymbol{CB7}]}$$

Using the laws of mass balance:

$$C_{\mathbf{OxyLH2}} = [\mathbf{OxyLH_2}] + [\mathbf{OxyLH_2}/\text{CB7}] \quad (2)$$

$$C_{\text{CB7}} = [\text{CB7}] + [\mathbf{OxyLH_2}/\text{CB7}] \quad (3)$$

where $C_{\mathbf{OxyLH_z}}$ and C_{CB7} are the total concentrations of **OxyLH$_2$** and CB7, respectively.

If we denote the optical density (OD) from the UV-Visible absorption spectral measurements or the fluorescence intensity (Int.) from the fluorescence measurements with *Y*, then

$$Y^{\lambda} = \Delta\varepsilon_{\mathbf{OxyLH2}}\,[\mathbf{OxyLH_2}] + \Delta\varepsilon_{\mathbf{OxyLH2}/\text{CB7}}\,[\mathbf{OxyLH_2}/\text{CB7}] \quad (4)$$

where $\Delta\varepsilon_{\mathbf{OxyLH2}}$ and $\Delta\varepsilon_{\mathbf{OxyLH2}/\text{CB7}}$ are the molar extinction coefficients ($M^{-1}\,cm^{-1}$) for the free and complexed chemical form, respectively. A combination of Eqs.1–4 gives

$$\Delta Y^{\lambda} = \frac{\frac{\Delta(\Delta\varepsilon)C_{CB7}}{2}}{\frac{KC_{\mathbf{OxyLH2}}-1-KC_{CB7}+\sqrt{(1-KC_{\mathbf{OxyLH2}}+KC_{CB7})^2+4KC_{\mathbf{OxyLH2}}}}{2}+1} \quad (5)$$

where ΔY^{λ} is the spectral changes at a given λ as a function of CB7 concentration, $\Delta(\Delta\varepsilon$ is the difference between the molar extinction coefficients of uncomplexed and complexed **OxyLH$_2$**

and *K* is the binding constant. The binding constants (*K*) in Table 1 were then obtained by using the nonlinear formula of Eq. (5), utilizing Levenberg-Marquardt algorithm which is available in SigmaPlot software (version 6.1; SPCC, Inc., Chicago, Illinois, USA). The molar extinction coefficient of the CB7-complexed **OxyLH$_2$** was left as a floating parameter in the analysis.

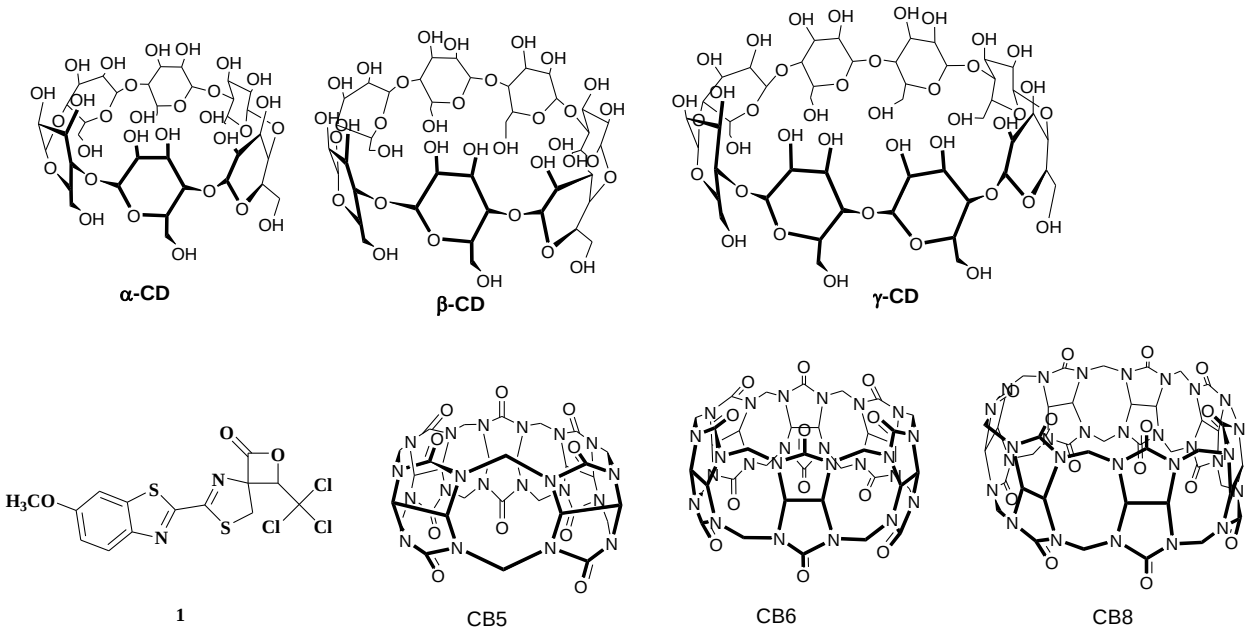

Chart S1. The structure of the model compound (**1**) used to assign the dissociation constant of the carboxyl group of **LH$_2$** and the structures of the cyclodextrins and cucurbiturils macrocycles (except CB7) that were tested as hosts in this study.

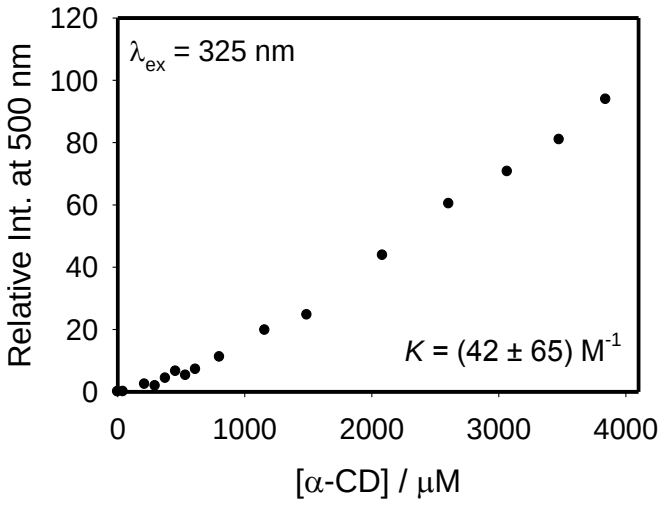

Figure S1. Titration curve that confirms the formation of a weakly bound complex between firefly oxyluciferin (**OxyLH$_2$**) and α-cyclodextrin (α-CD).

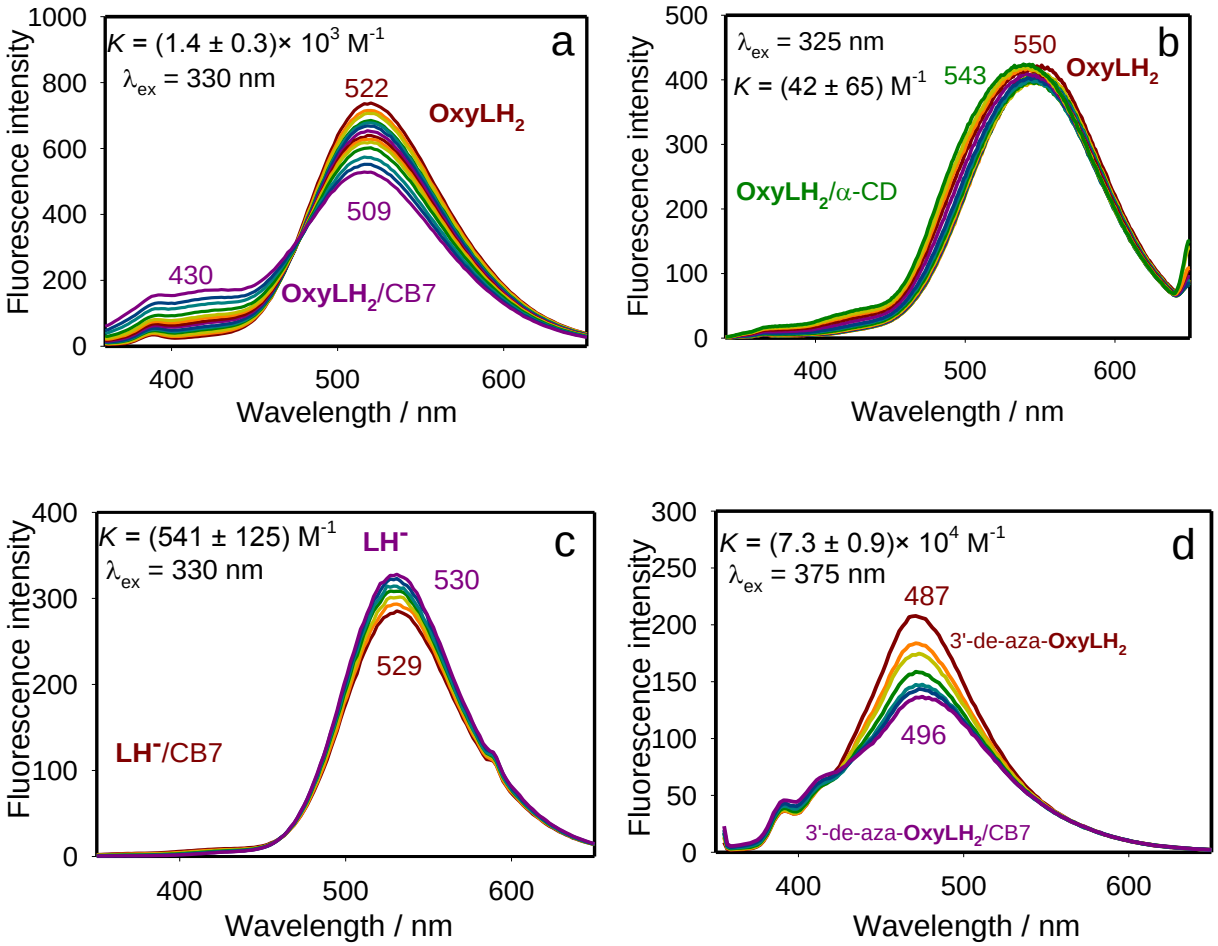

Figure S2. Dependence of the fluorescence spectra of **OxyLH$_2$** (a, b), **LH$^-$** (c), and 3'-de-aza-**OxyLH$_2$** (d) on the concentration of CB7 (pH 5.5; a, c, d) and α-CD (pH 3; b). For clarity, the initial and final spectra are shown in matching colors with the dominant chemical species. The numbers are the corresponding maxima (in nanometers).

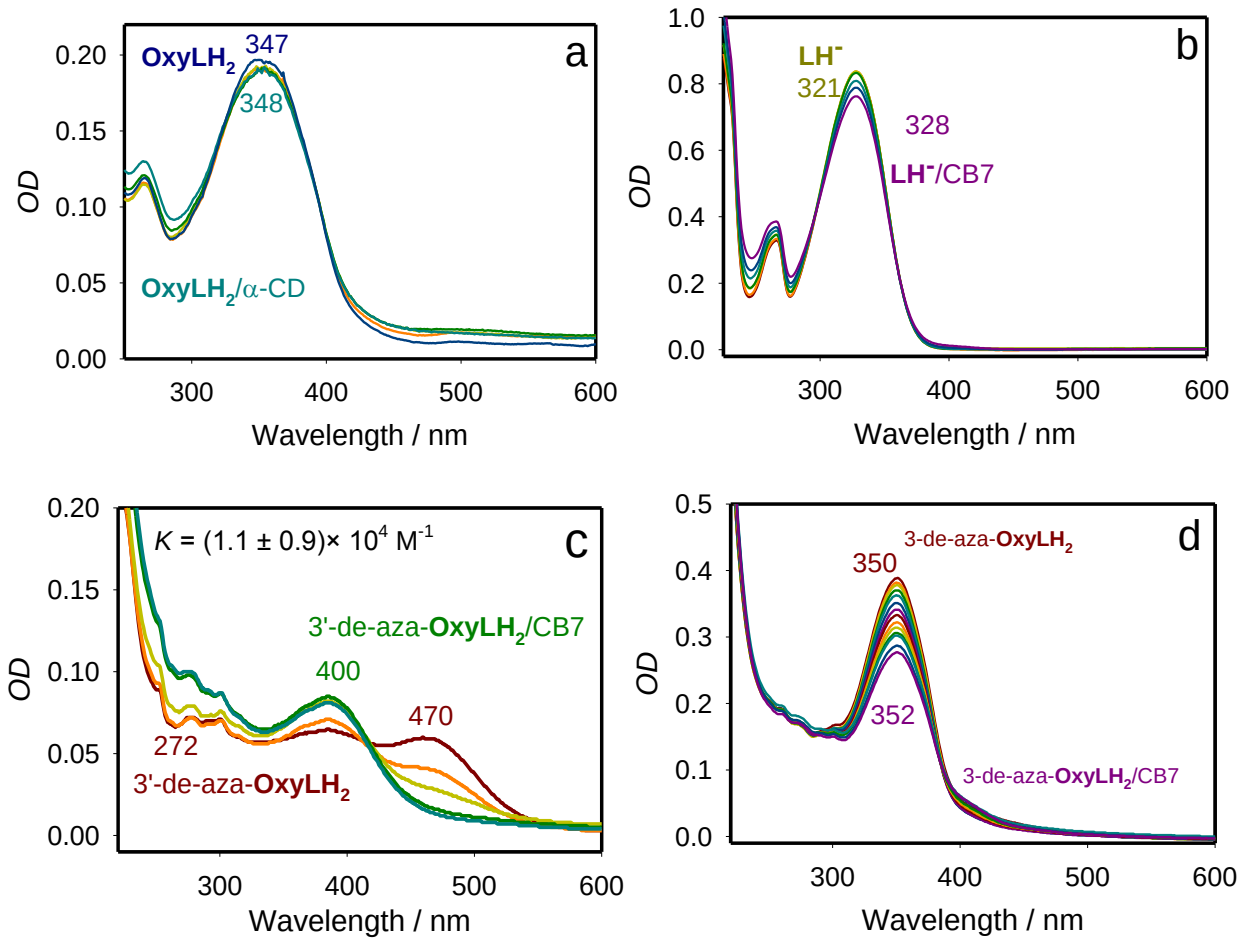

Figure S3. Dependence of the UV-Visible absorption spectra of selected chemical forms of firefly oxyluciferin on the concentration of CB7 (pH 5.5) and α-CD (pH 3). Except in the case of 3'-de-aza-**OxyLH2**, no binding constants could be extracted due to small changes in intensities upon complex formation. The numbers are the corresponding maxima (in nanometers).

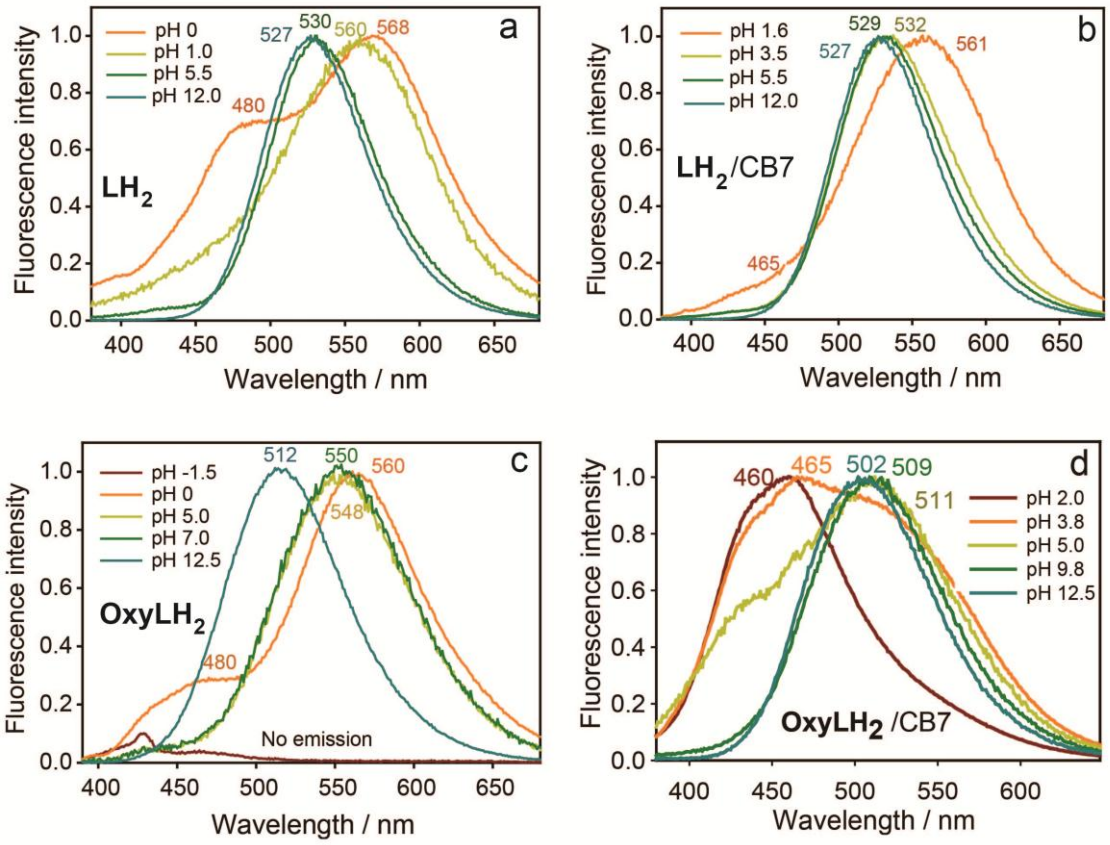

Figure S4. The emission spectra of firefly luciferin (**LH2**) and oxyluciferin (**OxyLH2**) at different pH values recorded with and without CB7. The species were excited at 350 nm (a,b) for **LH2**; 375 nm (c) and 330 nm (d) for **OxyLH2**. All emission spectra were normalized, except for the spectrum of **OxyLH2** at pH –1.5 in panel c. The numbers are the corresponding maxima (in nanometers).

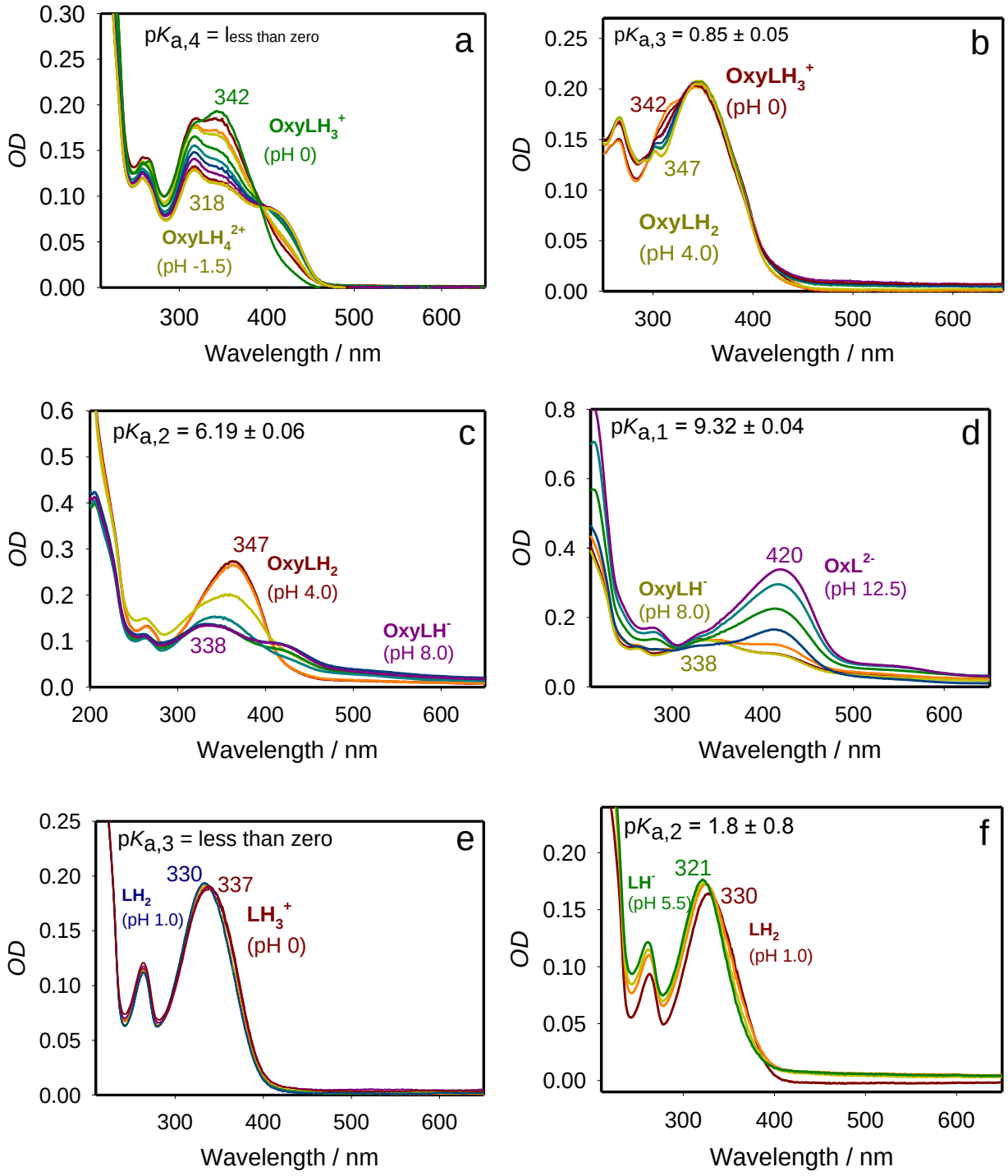

Figure S5. Dependence of the UV-Visible absorption spectra of the chemical forms of firefly luciferin (**LH2**) and oxyluciferin (**OxyLH2**) on the pH. For clarity, the initial and final spectra are shown in matching colors with the dominant chemical species. The numbers are the corresponding maxima (in nanometers).

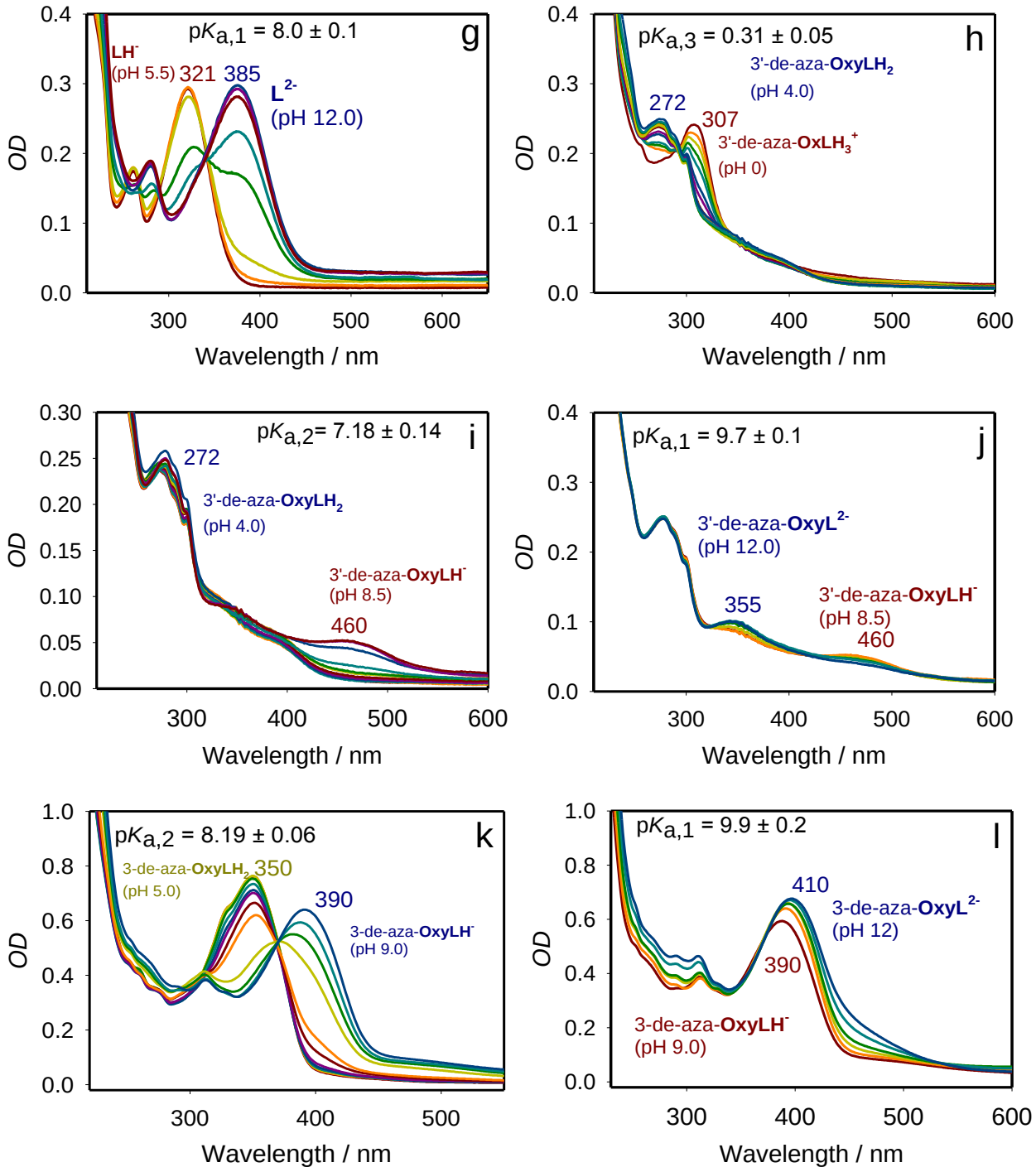

Figure S5 (continued)

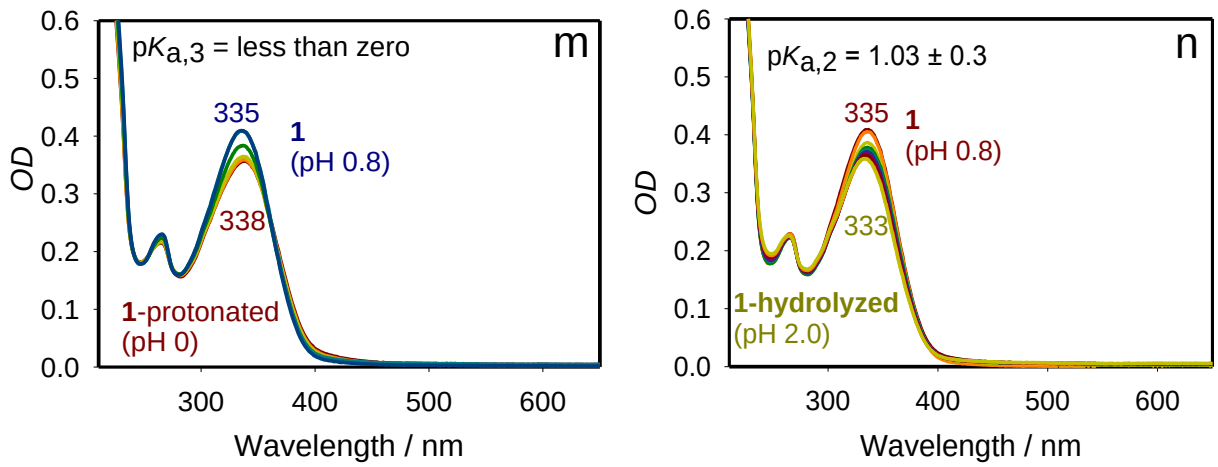

Figure S5 (continued)

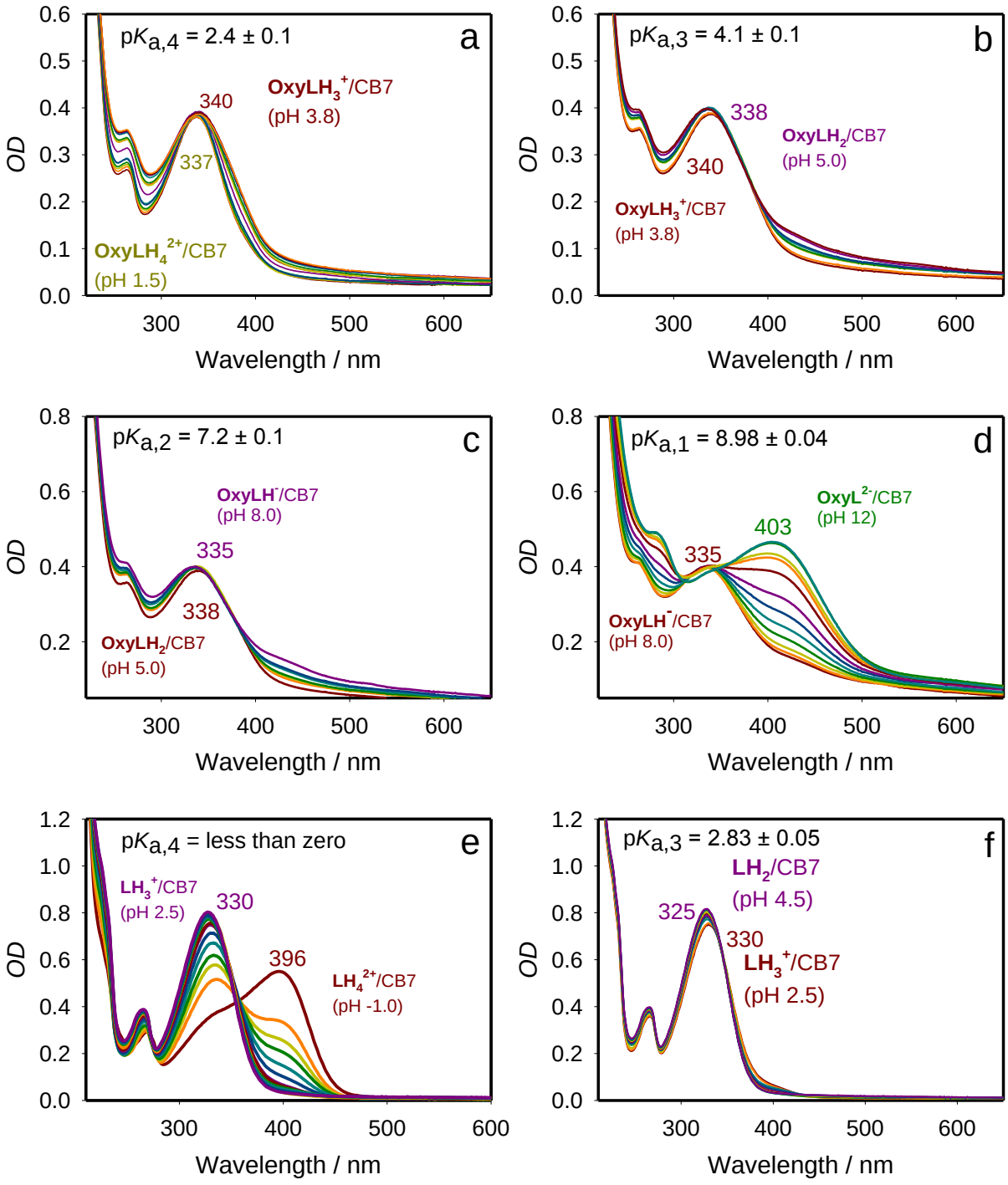

Figure S6. Dependence of the UV-Visible absorption spectra of the chemical forms of firefly luciferin (**LH_2**) and oxyluciferin (**$OxyLH_2$**) complexed with CB7 on the pH. For clarity, the initial and final spectra are shown in matching colors with the dominant chemical species. The numbers are the corresponding maxima (in nanometers).

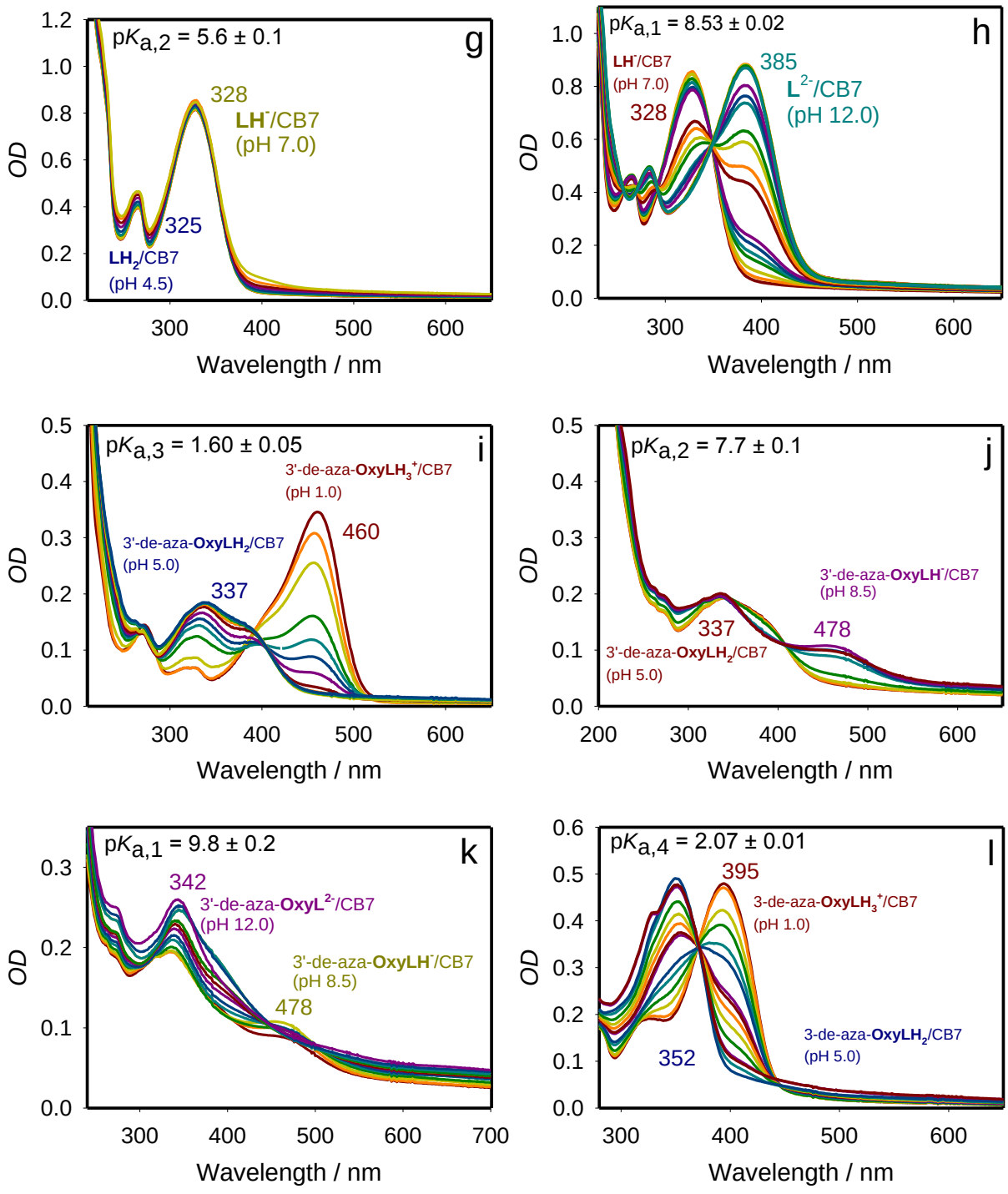

Figure S6 (continued)

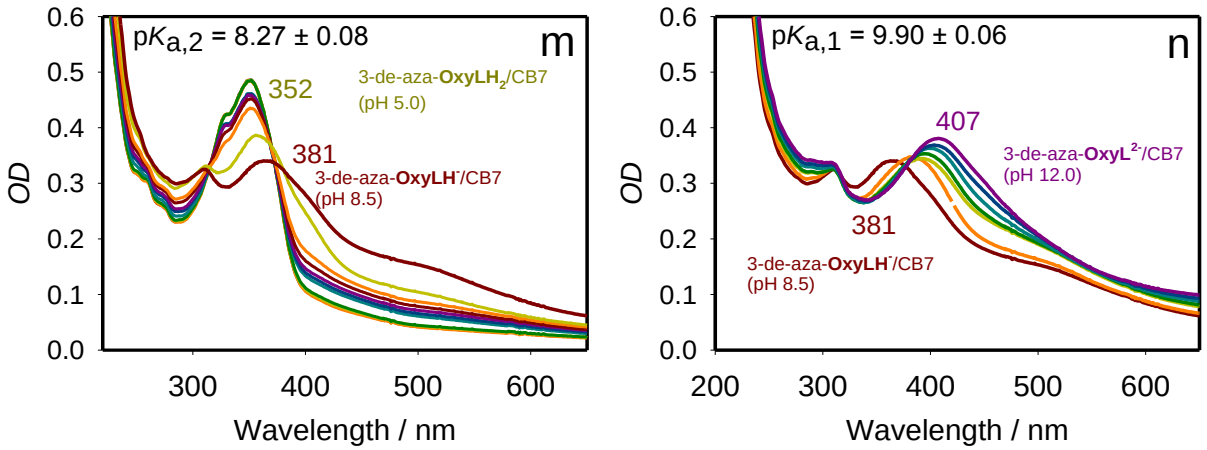

Figure S6 (continued)

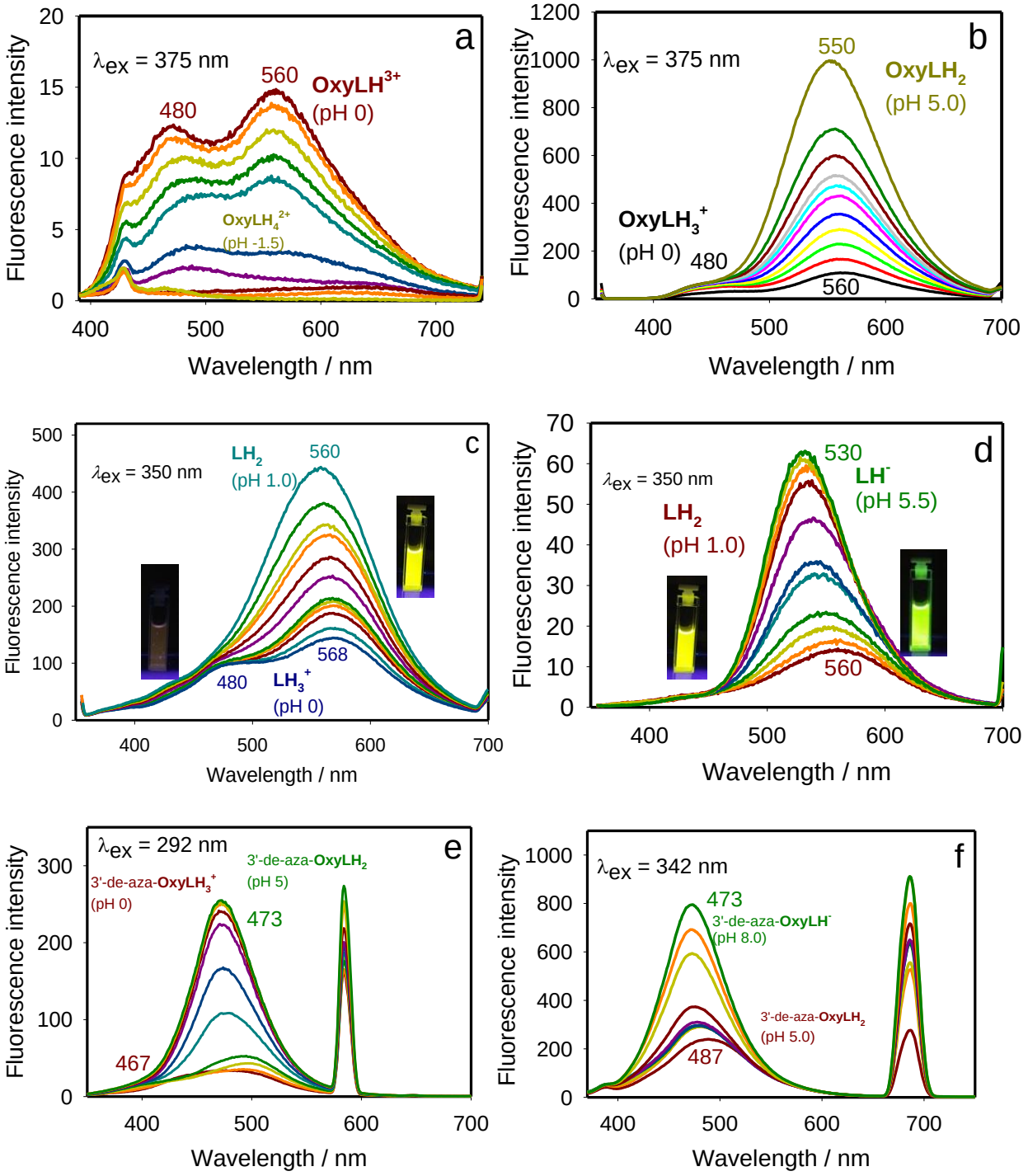

Figure S7. Dependence of the emission spectra of the chemical forms of firefly luciferin (**LH$_2$**) and oxyluciferin (**OxyLH$_2$**) on the pH. For clarity, the initial and final spectra are shown in matching colors with the dominant chemical species. The numbers are the corresponding maxima (in nanometers). Insets show images of emission from the excited samples.

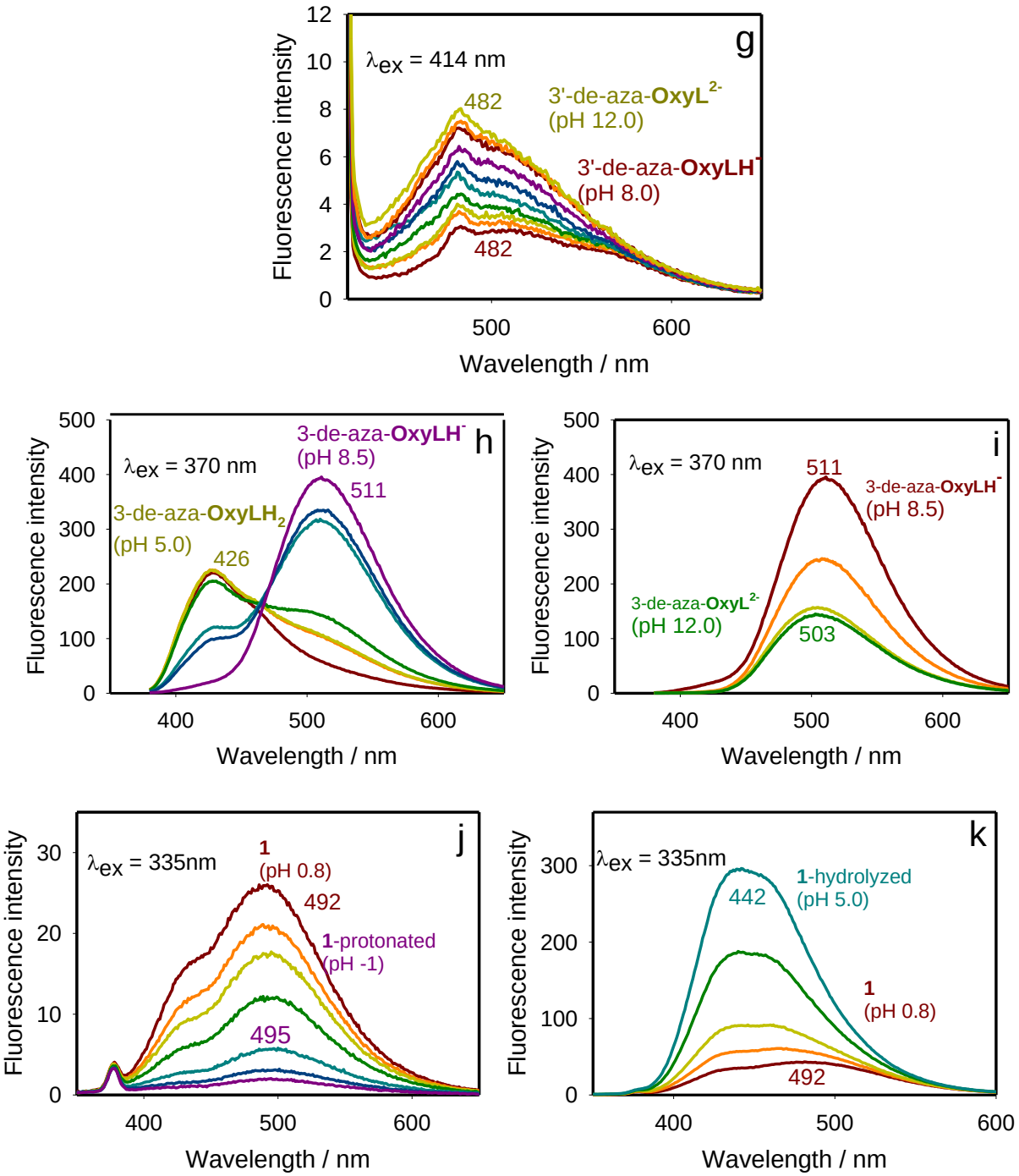

Figure S7 (continued)

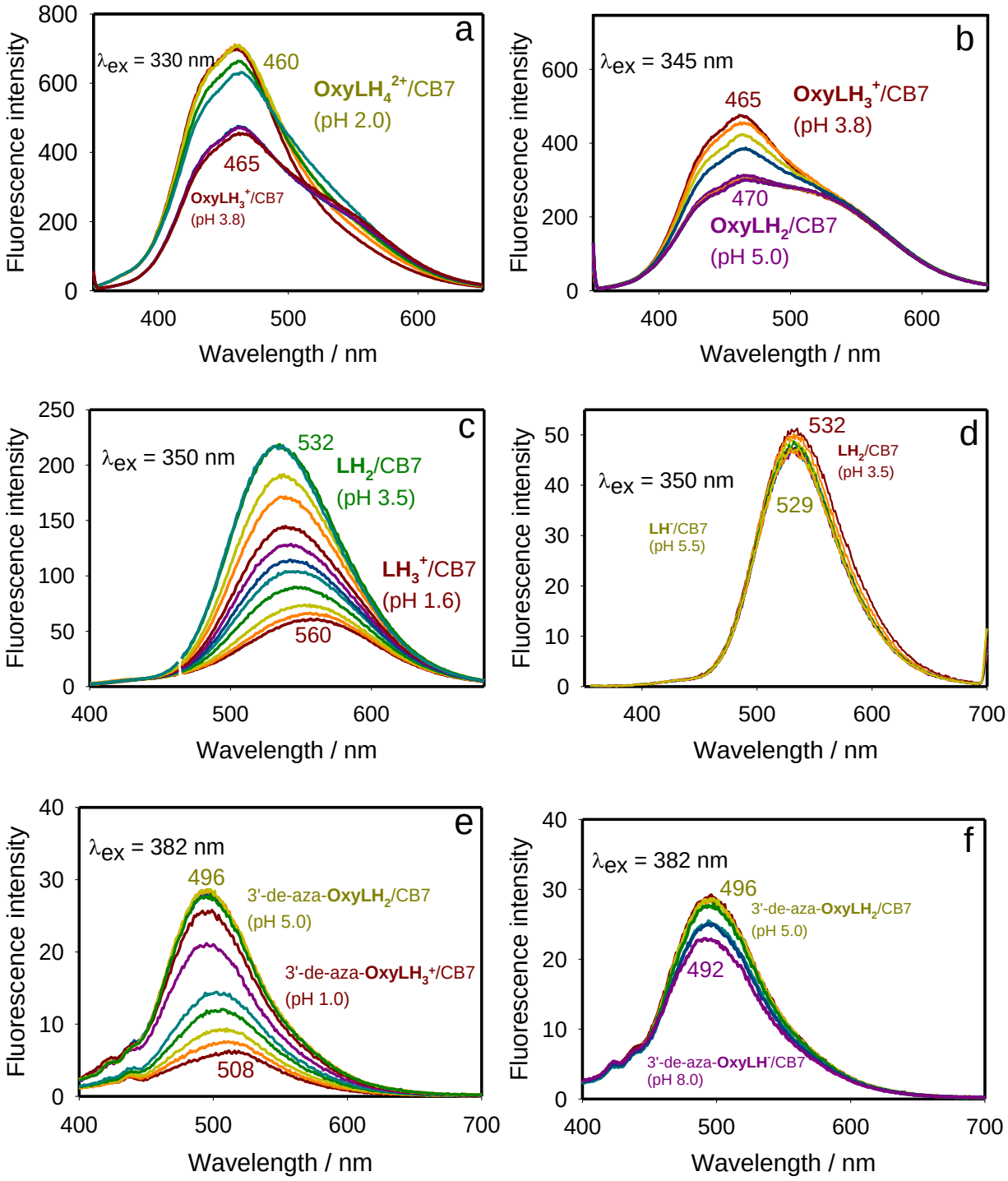

Figure S8. Dependence of the emission spectra of the chemical forms of firefly luciferin (**LH_2**) and oxyluciferin (**$OxyLH_2$**) complexed with CB7 on the pH. For clarity, the initial and final spectra are shown in matching colors with the dominant chemical species. The numbers are the corresponding maxima (in nanometers).

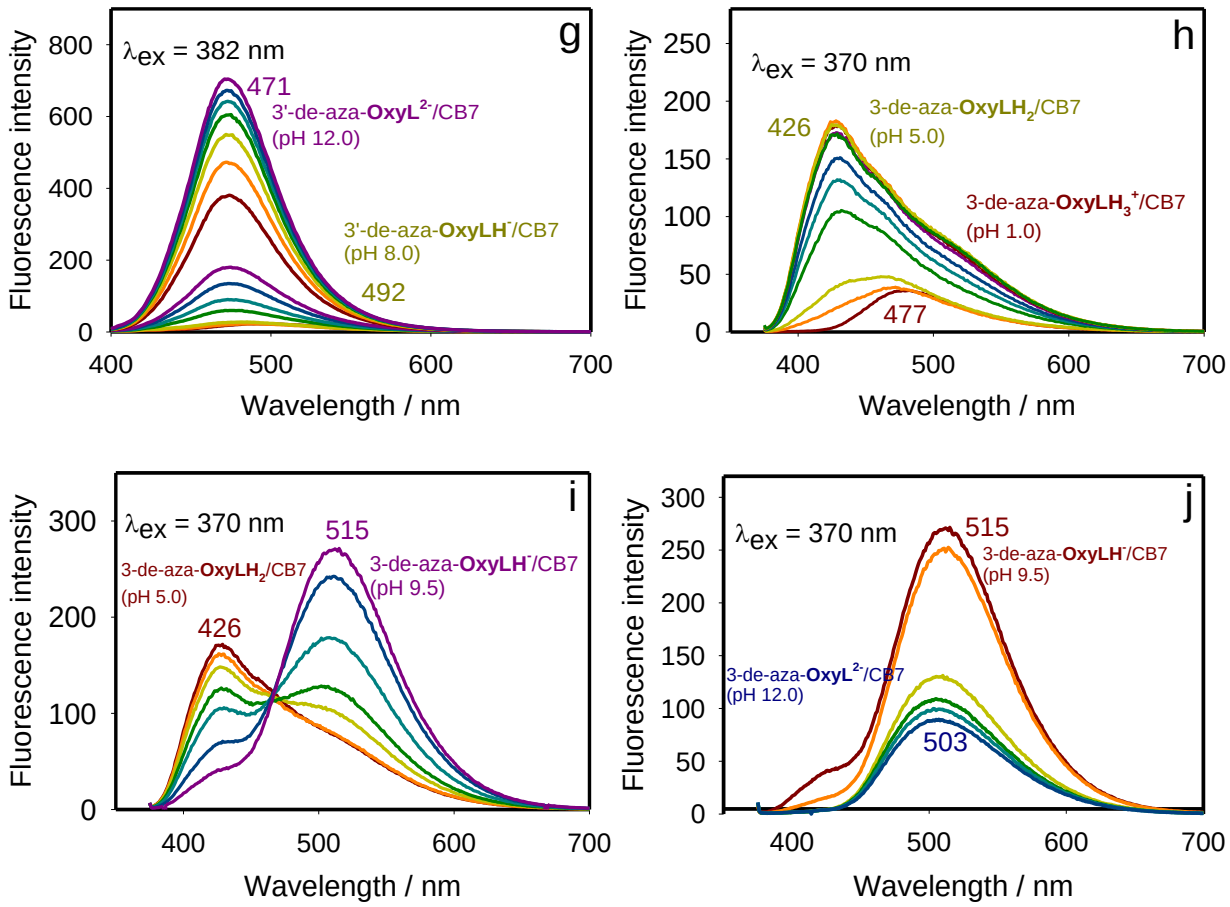

Figure S8 (continued)

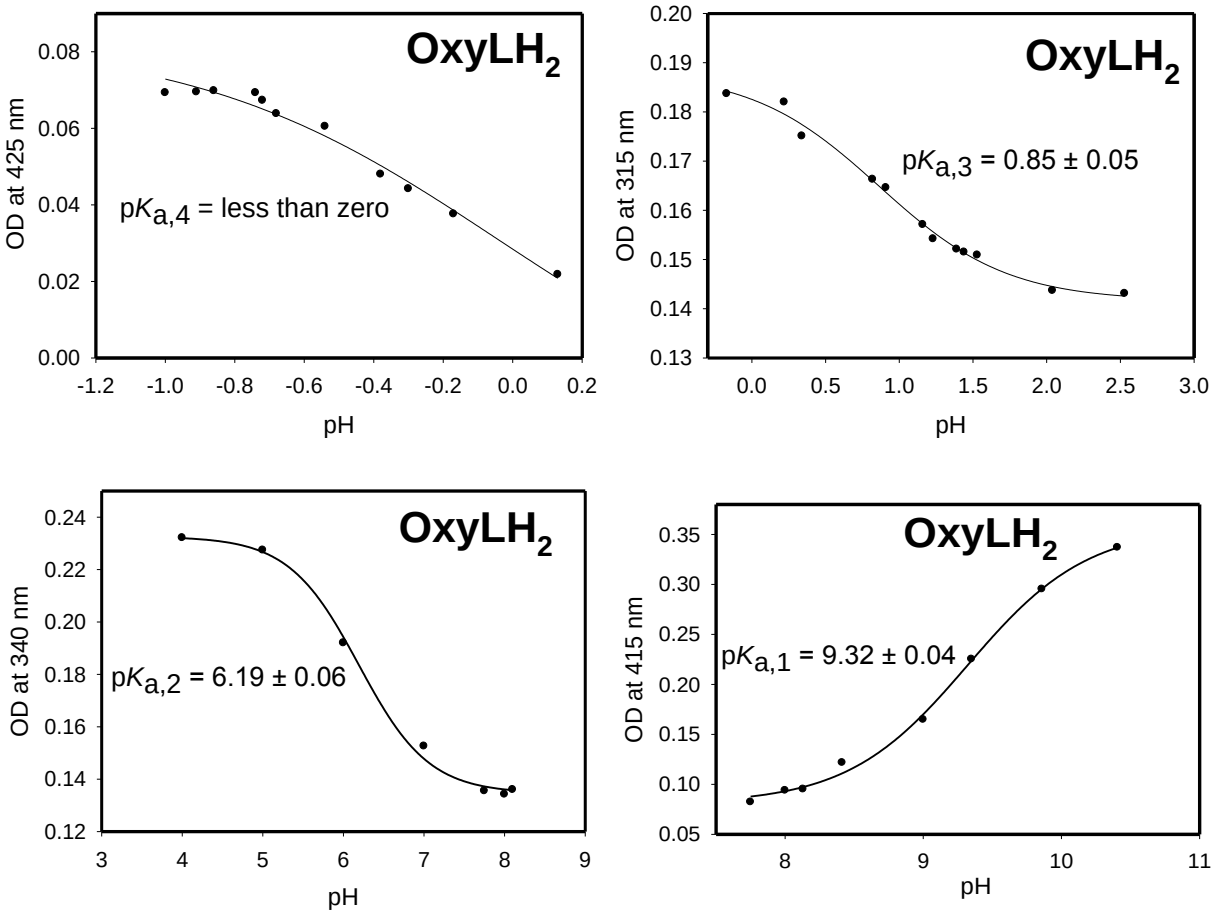

Figure S9. pH-concentration profile of the optical density extracted from the UV-Visible absorption spectra and the respective fits obtained by using the Henderson-Hasselbalch equation for free and CB7–bound firefly luciferin, oxyluciferin and model compounds. The experimental values of the constants are given on the plots.

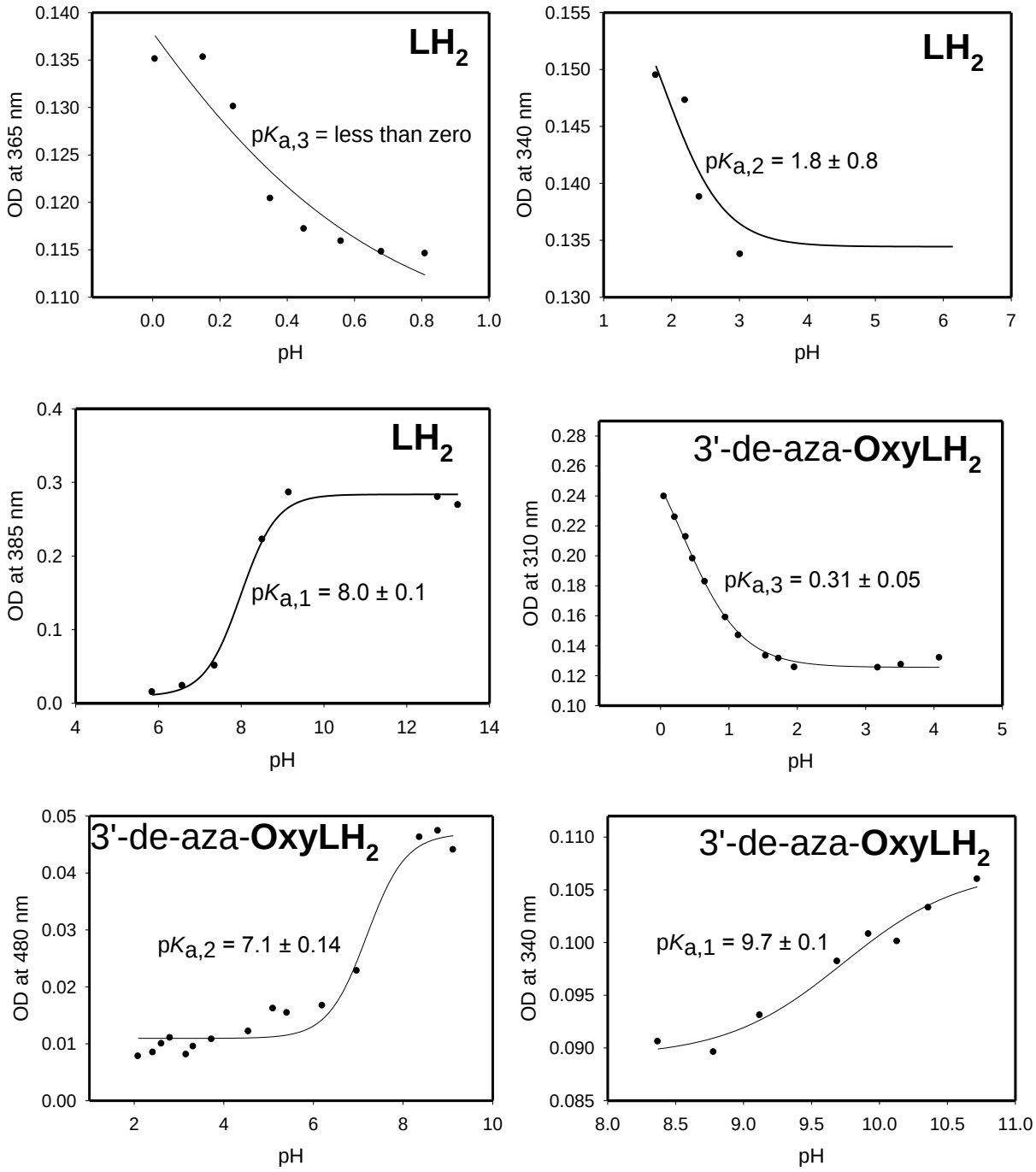

Figure S9 (continued)

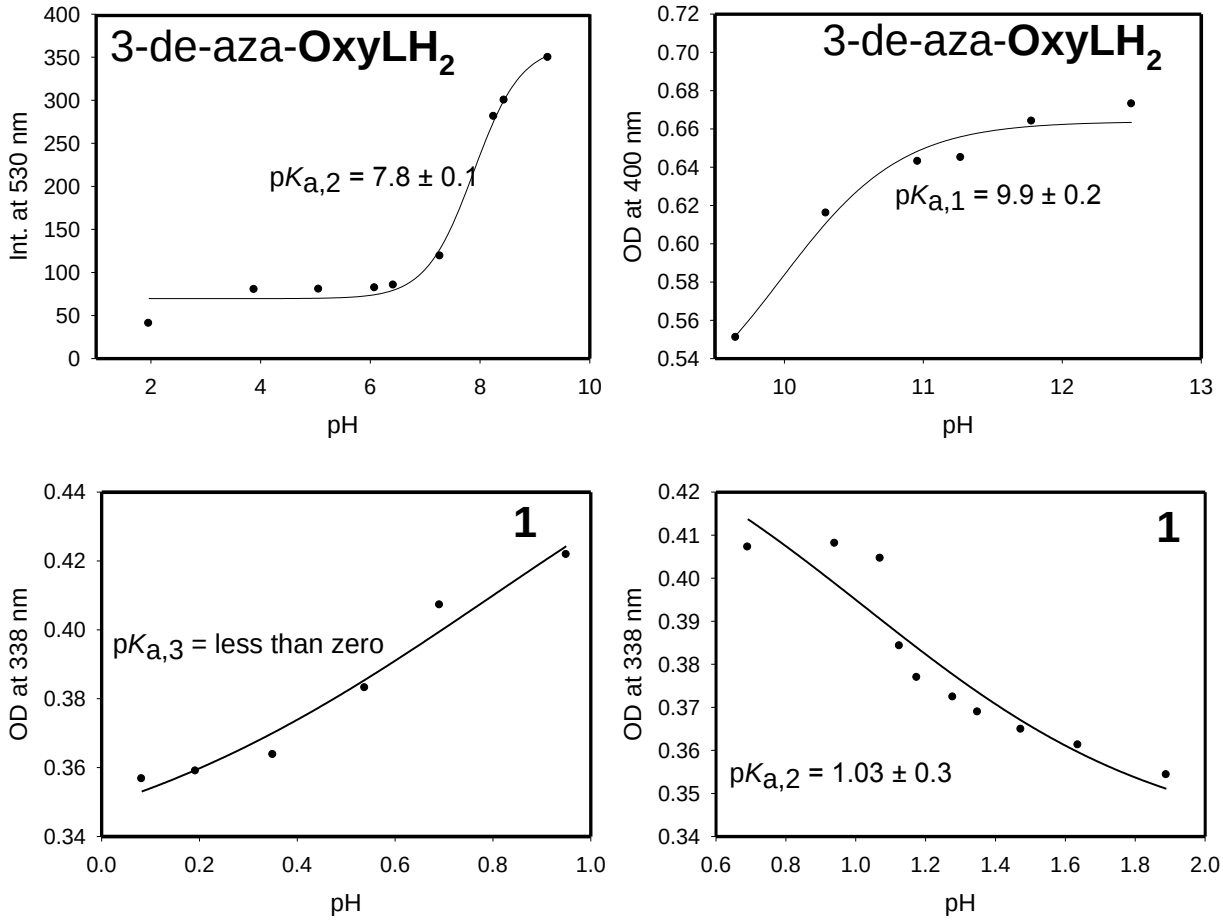

Figure S9 (continued)

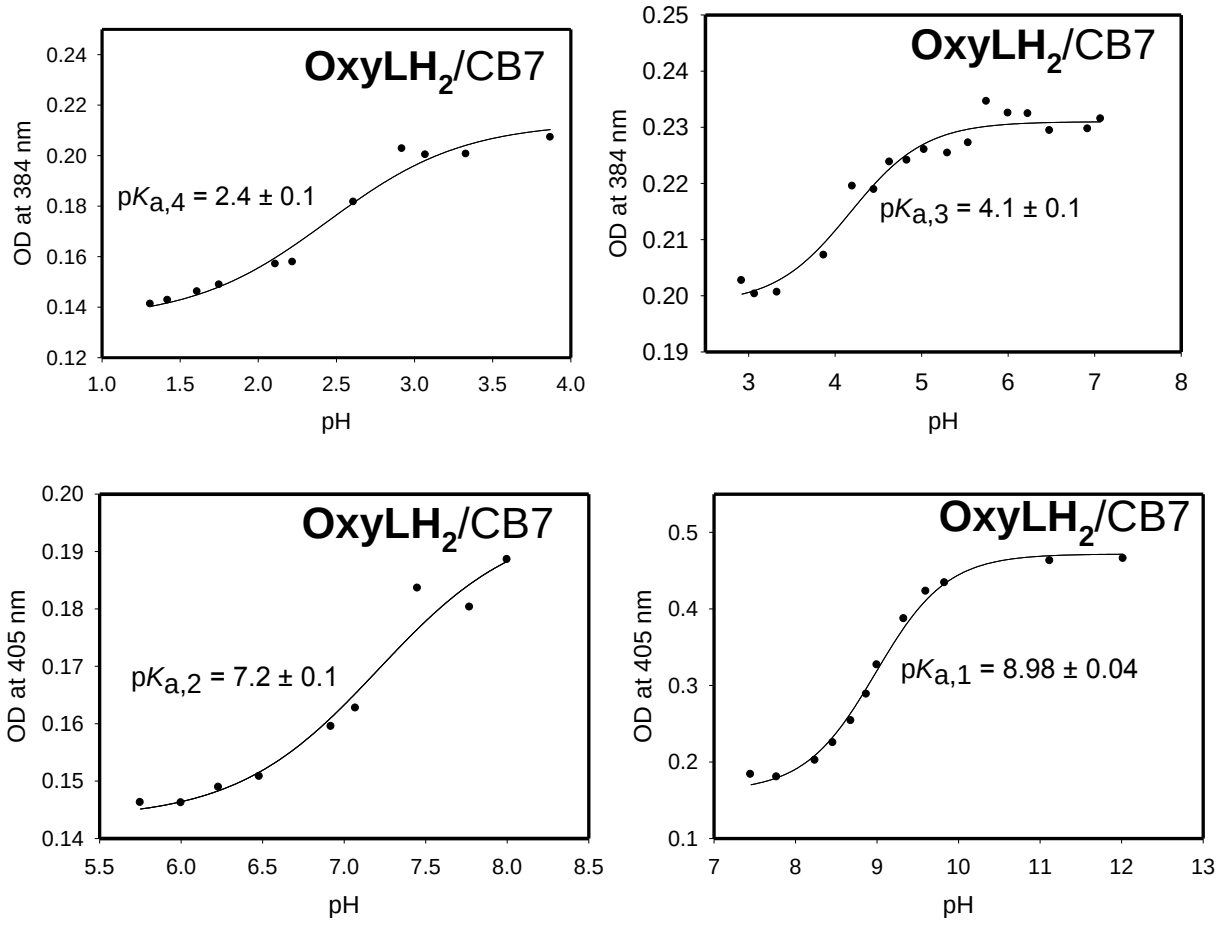

Figure S9 (continued)

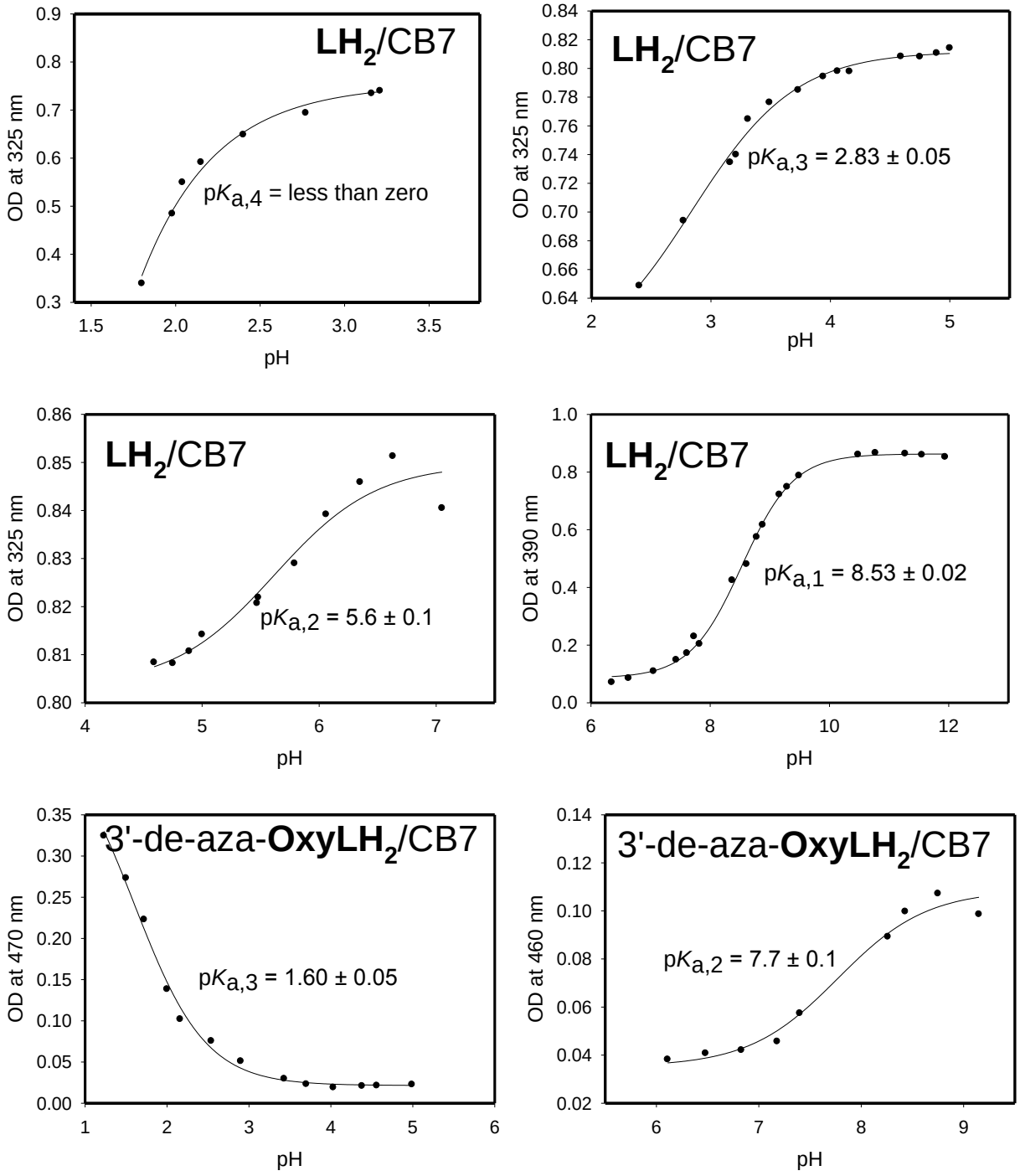

Figure S9 (continued)

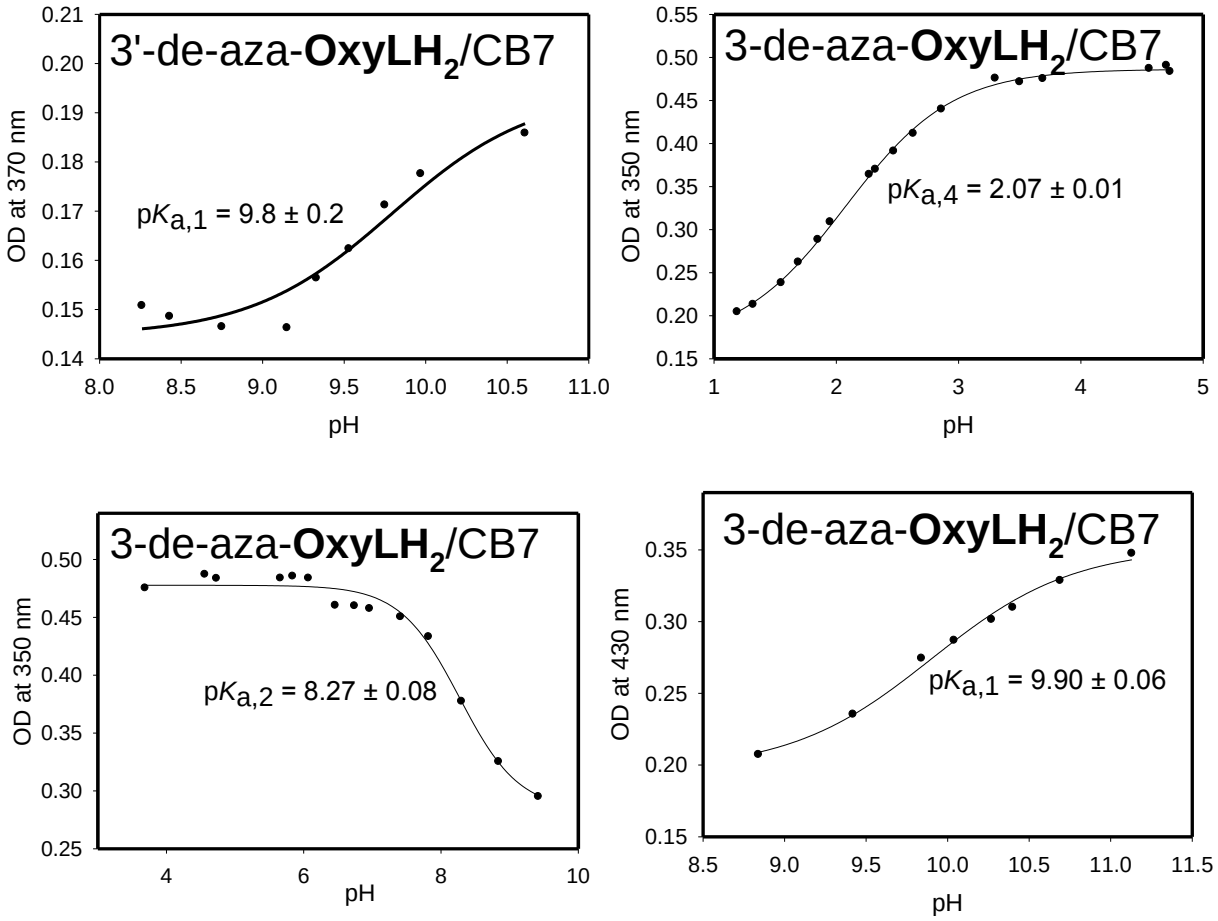

Figure S9 (continued)

Table S1. Time-resolved fluorescence data for **OxyLH$_2$** recorded upon addition of 0 to 100 equiv. of CB7 in aqueous solution at pH 5.5. The excitation was at 375 nm, and the time resolution was 30 ps

# Equivalents of CB7	$\lambda_{abs/}$ nm	$\lambda_{em/}$ nm	Emitted color	$\lambda_{obs/}$ nm	τ_i (ns)	%	Chi-Square	$\bar{\tau}$ (ns)
0	347	550	Yellow green	550	1.34 5.29	12 88	1.190	5.15
2				550	1.42 3.78 7.84	28 42 30	1.199	5.77
4				550	0.77 2.77 7.24	11 43 46	1.127	5.97
16				550	0.68 2.61 7.25	9 48 43	1.167	5.85
25				550	0.97 2.87 7.41	13 42 44	1.230	6.04
100	338	-	-	540	0.25 3.41 7.93	3 15 82	1.248	7.59

Table S2. Time-resolved fluorescence data for 3'-de-aza-**OxyLH$_2$** recorded upon the addition of 0 to 25 equiv. of CB7 in aqueous solution at pH 5.5. The excitation was at 375 nm, and the time resolution was 30 ps

# Equivalents of CB7	$\lambda_{abs/}$ nm	$\lambda_{em/}$ nm	Emitted color	$\lambda_{obs/}$ nm	τ_i (ns)	%	Chi-Square	$\bar{\tau}$ (ns)
0	272	-	-	480	0.09 3.83	30 70	1.317	3.79
2				480	0.06 1.84 4.10	31 13 55	1.073	3.85
4				480	0.08 2.07 4.39	37 24 38	1.201	3.81
16				480	0.11 1.77 4.30	34 30 36	1.298	3.59
25	337	496	Cyan	480	0.17 1.98 4.93	44 37 19	1.377	3.49

Table S3. Time-resolved fluorescence data for the chemical forms of **$OxyLH_2$** in aqueous solution at different pH values. The excitation was at 375 nm, and the time resolution was 30 ps. Selected images of samples are shown.

pH	$\lambda_{abs/}$ nm	$\lambda_{em/}$ nm	Emitted Color	$\lambda_{obs/}$ nm	τ_i (ns)	%	Chi-Square	$\bar{\tau}$ (ns)
0.97 **$OxyLH_3^+$**	342	560, 480	Yellow green 	450	0.05 0.63	85 15	1.940	0.45
0.97				570	0.29 0.69	30 70	0.799	0.63
2.00 **$OxyLH_2$**	347	550	Yellow green 	450	0.07 2.93	88 12	1.251	2.51
2.00				570	0.25 1.62 3.74	6 38 56	1.084	3.26
2.85				570	0.41 2.23 6.50	6 32 62	1.212	5.70
3.60				570	0.32 2.13 7.33	5 31 64	1.100	6.67
5.11				570	1.75 7.51	32 68	1.281	6.94x
6.31				570	0.47 2.21 7.52	6 40 54	1.087	6.54
7.28 **$OxyLH^-$**	338	548	Green 	570	0.84 2.88 7.44	11 36 53	1.247	6.39
8.80				570	0.48 2.44 5.95	6 20 74	1.138	5.57
10.51 **$OxyL^{2-}$**	420	512	Pale green 	570	0.81 5.46	7 93	1.261	5.41
11.62				570	0.56 5.42	3 97	1.106	5.41
12.97				570	5.49	100	1.117	5.49

Table S4. Time-resolved fluorescence data for **OxyLH$_2$**/CB7 in aqueous solution at different pH values. The excitation was at 375 nm and the time resolution was 30 ps

pH	$\lambda_{abs/}$ nm	$\lambda_{em/}$ nm	Emitted color	$\lambda_{obs/}$ nm	τ_i (ns)	%	Chi-Square	$\bar{\tau}$ (ns)
1.61				430	0.24 0.90 4.69	66 30 4	0.979	1.88
2.40 **OxyLH$_3^+$** /CB7	340	-	-	430	0.23 0.91 4.90	66 30 4	1.197	2.00
2.40				560	0.19 1.06 3.10	11 78 11	1.310	1.63
3.31				430	0.29 1.54 7.64	47 48 5	0.955	3.26
4.90 **OxyLH$_2$** /CB7	338	-	-	430	0.24 1.49 6.95	49 44 7	1.256	3.48
4.90				560	1.76 3.56 7.75	21 24 55	1.212	6.69
5.80				560	0.32 2.53 7.50	4 36 60	1.137	6.65
8.98 **OxyLH$^-$** /CB7	335	-	-	430	0.32 1.55 5.91	26 34 40	1.052	4.98
8.98	335			560	0.32 2.63 5.81	3 28 69	1.139	5.31
10.20				560	2.03 5.51	17 83	1.246	5.27
12.27 **OxyL^{2-}** /CB7	403	-	-	430	5.81	100	1.323	5.81
12.27				560	5.51	100	1.187	5.51

Table S5. Time-resolved fluorescence data for the chemical forms of **LH_2** in aqueous solution at different pH values. The excitation was at 375 nm, and the time resolution was 30 ps. Selected images of samples are shown.

pH	$\lambda_{abs/}$ nm	$\lambda_{em/}$ nm	Emitted Color	$\lambda_{obs/}$ nm	τ_i (ns)	%	Chi-Square	$\bar{\tau}$ (ns)
1.08 **LH_2**	330	560	Yellow Green 	510	1.86 0.76 3.44	46 50 4	1.116	1.71
1.08				580	0.27 0.78	21 79	0.822	0.74
1.82 **LH^-**	321	530	Green 	580	0.28 1.50	8 92	1.001	1.48
2.03				510	0.29 1.99	13 87	1.473	1.95
2.03				580	0.33 1.71	8 92	1.374	1.69
5.85				510	0.33 4.71	5 95	1.128	4.69
5.85				580	0.39 4.71	7 93	1.118	4.68
6.81				580	0.39 4.97	3 97	1.393	4.96
8.52 **L^{2-}**	385	527	Green 	580	4.96	100	1.256	4.96
11.10				510	4.93	100	1.451	4.93
11.10				580	4.84	100	1.062	4.84

Table S6. Time-resolved fluorescence data for **LH_2**/CB7 in aqueous solution at different pH values. The excitation was at 375 nm, and the time resolution was 30 ps

pH	$\lambda_{abs/}$ nm	$\lambda_{em/}$ nm	Emitted color	$\lambda_{obs/}$ nm	τ_i (ns)	%	Chi-Square	$\bar{\tau}$ (ns)
1.20 **LH_3^+** /CB7	330	560	Yellow green	570	0.36 0.90 3.77	47 44 9	1.118	1.88
1.20				430	0.10 0.65 3.78	44 45 11	1.251	2.35
1.44				570	0.17 0.80 2.60	15 78 7	1.242	1.17
1.64				570	0.35 1.07 3.33	17 81 2	0.960	1.18
1.81				570	0.31 1.19 3.03	12 85 3	0.999	1.31
2.08				570	0.34 1.52 3.64	8 90 2	1.012	1.60
3.40 **LH_2** /CB7	325	532	Green	570	1.53 4.21	38 62	1.414	3.72
4.57				570	1.37 4.80	20 80	1.482	4.57
5.09 **LH^-**/CB7	328	529	Green	570	1.02 4.99	5 95	1.153	4.95
5.62				570	0.49 4.95	5 95	1.254	4.93
8.84 **L^{2-}** /CB7	385	527	Green	570	5.00	100	1.436	5.00
11.54				570	5.03	100	1.363	5.03

Table S7. Time-resolved fluorescence data for the chemical forms of 3'-de-aza-**OxyLH$_2$** in aqueous solution at different pH values. The excitation was at 375 nm, and the time resolution was 30 ps

pH	$\lambda_{abs/}$ nm	$\lambda_{em/}$ nm	Emitted color	$\lambda_{obs/}$ nm	τ_i (ns)	%	Chi-Square	$\bar{\tau}$ (ns)
0.09 3'-de-aza-**OxyLH$_3^+$**	307	-	-	470	0.28 1.39 6.04	44 41 15	1.112	3.94
0.53				470	0.24 1.37 6.90	45 40 15	1.195	4.68
0.53				530	0.16 1.17 5.45	38 43 19	1.271	3.90
1.01				530	0.04 2.34	88 12	1.290	2.08
2.11 3'-de-aza-**OxyLH$_2$**	272	-	-	530	0.04 2.79	77 23	1.424	2.66
5.84				480	0.09 3.83	30 70	1.317	3.79
5.84				530	0.04 3.36	81 19	1.251	3.20
8.36 3'-de-aza-**OxyLH$^-$**	460	-	-	470	0.47 3.91	4 96	1.371	3.89
8.36				530	0.24 3.90	16 84	1.391	3.86
8.83				530	0.22 3.88	12 88	1.541	3.85
11.10 3'-de-aza-**OxyL^{2-}**	355	-	-	530	0.44 3.90	4 96	1.452	3.88
11.85				430	0.64 3.89	3 97	1.230	3.87
11.85				530	0.44 3.90	6 94	1.416	3.87

Table S8. Time-resolved fluorescence data for the chemical forms of 3'-de-aza-**OxyLH$_2$**/CB7 in aqueous solution and at different pH values. The excitation was at 375 nm, and the time resolution was 30 ps

pH	λ_{abs}/ nm	λ_{em}/ nm	Emitted color	λ_{obs}/ nm	τ_i (ns)	%	Chi-Square	$\bar{\tau}$ (ns)
1.23 3'-de-aza-**OxyLH$_3^+$** /CB7	460	508	Pale green	430	0.12 1.85 6.73	54 23 22	0.860	5.46
1.23				570	0.03 1.55 4.89	68 12 19	1.168	4.26
1.91				430	0.12 1.31 5.33	62 17 21	0.999	4.42
3.04 3'-de-aza-**Oxy LH$_2$** /CB7	337	496	Cyan	430	0.13 1.50 5.27	64 21 15	0.953	3.91
4.09				430	0.12 1.67 6.43	63 22 15	0.984	4.85
6.47				430	0.15 1.78 6.77	57 24 19	1.033	5.27
6.47				570	0.07 1.00 3.34	71 13 16	1.001	2.69
7.08				430	0.12 1.87 6.84	53 27 20	1.069	5.32
8.20 3'-de-aza-**OxyLH$^-$** /CB7	478	492	Cyan	430	0.12 2.51 6.08	37 36 27	0.930	4.73
8.20				570	0.07 1.25 4.62	40 31 29	1.080	3.80
8.89				430	0.11 3.38 6.65	22 63 15	1.025	4.39
10.30				430	0.28 3.88	5 95	1.209	3.87
13.23 3'-de-aza-**OxyL^{2-}** /CB7	342	471	Blue	430	0.64 3.89	3 97	1.230	3.87
13.23				570	0.34 4.01	6 94	1.380	3.99

Table S9. Time-resolved fluorescence data for the chemical forms of 3-de-aza-**OxyLH$_2$** in aqueous solution and at different pH values. The excitation was at 375 nm, and the time resolution was 30 ps

pH	$\lambda_{abs/}$ nm	$\lambda_{em/}$ nm	Emitted color	$\lambda_{obs/}$ nm	τ_i (ns)	%	Chi-Sqaure	$\bar{\tau}$ (ns)
1.20 3-de-aza-**OxyLH$_2$**	350	426	Pale blue	430	0.13 0.38	43 57	1.571	0.33
1.99				430	0.26 0.64	70 30	1.520	0.46
3.88				430	0.34 0.76	89 11	0.924	0.43
5.06				560	0.62 5.21	34 66	1.356	4.94
7.27				560	0.62 5.21	34 66	1.356	4.94
8.25 3-de-aza-**OxyLH$^-$**	390	511	Pale green	560	0.52 5.20	8 92	1.131	5.16
9.24				560	0.89 5.17	18 82	1.215	5.01
10.05				560	1.07 5.01	51 49	1.264	4.29
11.38				560	0.99 2.41	74 25	1.431	1.63

Table S10. Time-resolved fluorescence data for the chemical forms of 3-de-aza-**OxyLH$_2$**/CB7 in aqueous solution at different pH values. The excitation was at 375 nm and the time resolution was 30 ps

pH	$\lambda_{abs/}$ nm	$\lambda_{em/}$ nm	Emitted color	$\lambda_{obs/}$ nm	τ_i (ns)	%	Chi-Sqaure	$\bar{\tau}$ (ns)
1.19 3-de-aza-**OxyLH$_3^+$** /CB7	395	477	Blue	430	0.30 1.09	87 12	1.721	0.56
1.19				560	0.27 1.39	81 19	1.785	0.88
1.61				430	0.30 1.09	87 12	1.721	0.56
3.85 3-de-aza-**OxyLH$_2$** /CB7	352	426	Pale blue	430	0.39 2.08	96 4	1.254	0.70
3.85				560	0.66 4.98	37 63	1.081	4.67
6.77				430	0.38 2.23	95 5	1.091	0.82
7.77				430	0.38 2.05	93 7	1.690	0.86
7.77				560	0.68 5.17	21 79	1.120	5.04
8.10 3-de-aza-**OxyLH$^-$** /CB7	381	515	Pale green	560	0.65 5.19	12 88	1.285	5.11
9.10				560	0.70 5.07	12 88	1.565	4.99
11.58				560	1.27 3.71	75 25	0.210	2.47