

# One Protein, Two Chromophores. Comparative Spectroscopic Characterization of 6,7-dimethyl-8-Ribityllumazine and Riboflavin bound to Lumazine Protein

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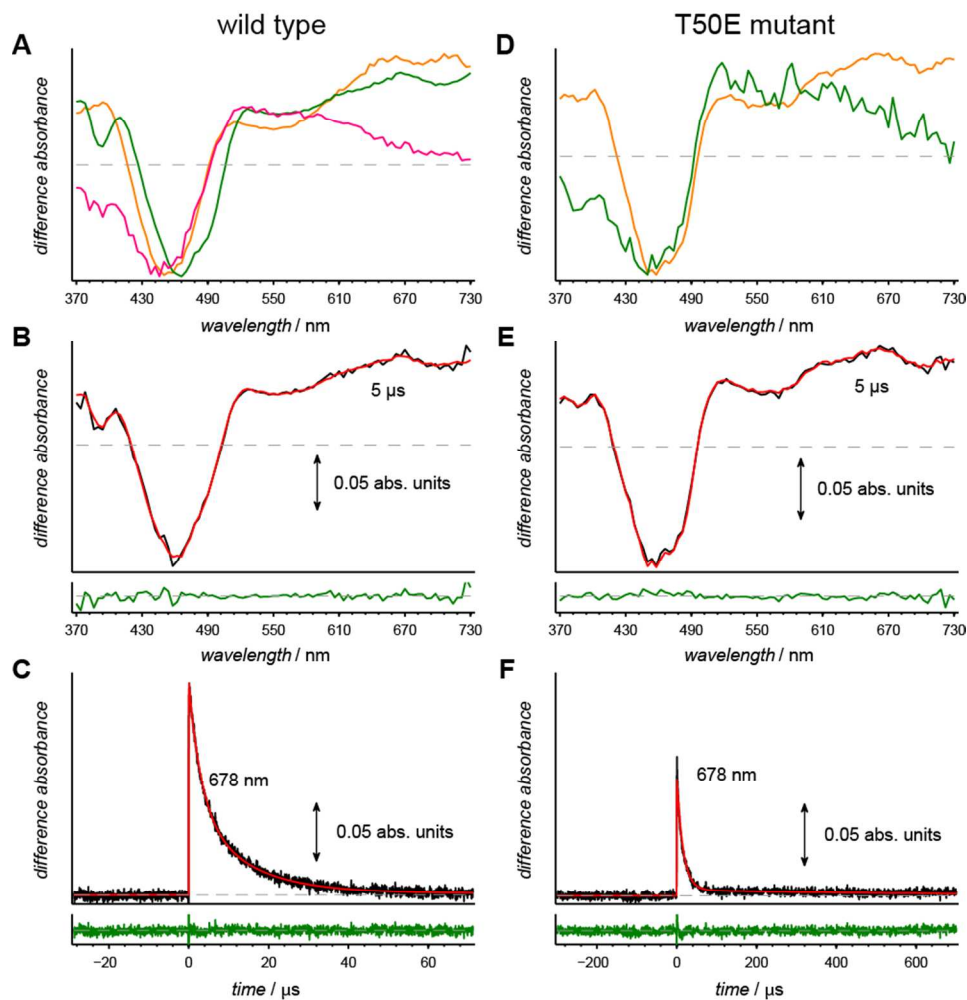
# 1. Transient Absorption Spectroscopy

**Supporting Table 1:** Rate constants  $k_i$  and lifetimes  $\tau_i$  from global analysis for samples with Rf cofactor.

Sample	Rate constants / $\mu\text{s}^{-1}$			Lifetimes / $\mu\text{s}$		
	$k_1$	$k_2$	$k_3$	$\tau_1$	$\tau_2$	$\tau_3$
Rf-LumP WT	$0.32 \pm 0.02$	$0.076 \pm 0.005$	$0.0028 \pm 0.0002$	$3.1 \pm 0.2$	$13.1 \pm 0.8$	$360 \pm 30$
Rf-LumP S48C	$0.146 \pm 0.009$	$0.00064 \pm 0.00004$	---	$6.9 \pm 0.5$	$1600 \pm 100$	---
Rf-LumP T50E	$0.063 \pm 0.004$	$0.00149 \pm 0.00009$	---	$16 \pm 1$	$670 \pm 40$	---
Rf-LumP T50W	---	---	---	---	---	---
Rf-LumP N101W	$0.10 \pm 0.02$	---	---	$10 \pm 2$	---	---

**Supporting Table 2:** Rate constants  $k_i$  and lifetimes  $\tau_i$  from global analysis for samples with ribolumazine cofactor. For all samples the kinetic scheme was a simple global analysis with a single decay.

Sample	Rate constants / $\text{ns}^{-1}$	Lifetimes / ns
	$k_1$	$\tau_1$
Lu-LumP WT	$0.028 \pm 0.003$	$36 \pm 3$
Lu-LumP S48C	$0.082 \pm 0.008$	$12 \pm 1$
Lu-LumP T50E	$0.089 \pm 0.008$	$11 \pm 1$
Lu-LumP T50W	$0.022 \pm 0.002$	$46 \pm 3$
Lu-LumP N101W	$0.023 \pm 0.002$	$45 \pm 3$



**Supporting Figure 1.** Exemplary analysis of the transient absorption spectra from Rf-LumP WT (left) and Rf-LumP T50E (right). The analysis of all other samples investigated in this contribution has been performed likewise. Experimental conditions: Temperature: 279 K, laser excitation: 460 nm. (A/D) Normalized SADS extracted from the global analysis of experimental Rf-Lump data. Either three (left) or two components are necessary to properly fit the data (see **Supporting Table 1** for details). (B/E) Difference between experimental spectra (black), recorded 5 μs after laser excitation, and the fit from global analysis (red) of Rf-Lump samples. (C/F) Comparison between experimental time signals (black) at indicated wavelengths and the global fit (red). All signals are true to scale.

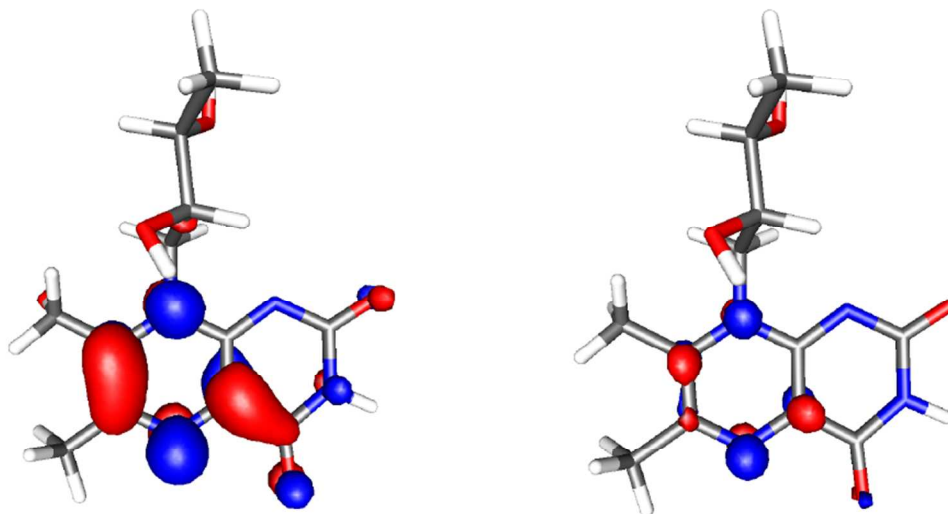
## 2. Transient EPR Spectroscopy

Transient EPR powder spectra have been analyzed using a program for simulation and fitting of spectra with isotropic  $g$ -factor and dipolar-coupling tensor  $\mathbf{D}$  as described previously<sup>1</sup>.

**Supporting Table 2:** The triplet parameters of Rf cofactor bound to various WT and mutant LumP samples ( $T = 80$  K) obtained by simulation of the time-resolved EPR spectra of **Figure 5**. The values of  $|D|$  and  $|E|$  are accurate to within  $\pm 6 \times 10^{-4} \text{ cm}^{-1}$ ; the zero-field populations  $p_i$  are accurate to within  $\pm 0.03$ .

	$ D  / (10^{-4} \text{ cm}^{-1})$	$ E  / (10^{-4} \text{ cm}^{-1})$	$p_X$	$p_Y$	$p_Z$
Rf-LumP WT	589	169	0.57	0.42	0
Rf-LumP S48C	595	195	0	0.60	0.40
Rf-LumP T50E	611	175	0.54	0.45	0
Rf-LumP T50W	---	---	---	---	---
Rf-LumP N101W	593	171	0.60	0.40	0

### 3. DFT Calculations



**Supporting Figure 2.** Singly occupied molecular orbitals (SOMOs) at  $0.05 \text{ e/au}^3$  (left) and  $0.1 \text{ e/au}^3$  (right) of the ribolumazine model. The red and blue areas denote regions of opposite sign of the wave function. The protein environment is not drawn, for clarity.

**Supporting Table 3:** Cartesian coordinates in Angstrom of the geometry-optimized supermolecular model of the neutral ribolumazine radical in the binding site of the lumazine protein.

number	atom	coordinate		
		x	y	z
1	N	16,37748	-4,83009	-19,53871
2	C	17,24545	-5,91782	-19,59775
3	C	18,38848	-5,83613	-20,3892
4	N	18,68852	-4,72177	-21,05851
5	C	17,84955	-3,62211	-20,96566
6	C	16,67678	-3,64331	-20,1982
7	N	15,83031	-2,63061	-20,09632
8	C	16,08285	-1,47365	-20,76369
9	N	17,25729	-1,4284	-21,53381
10	C	18,18831	-2,43111	-21,70335
11	O	15,334	-0,4715	-20,7205
12	O	19,20692	-2,28494	-22,43014
13	C	19,33461	-7,03608	-20,51531

14	C	16,88762	-7,17934	-18,88172
15	H	17,44359	-0,53415	-22,02317
16	H	20,1523	-6,75012	-21,18896
17	H	18,8386	-7,92164	-20,93669
18	H	19,77278	-7,33	-19,5549
19	H	16,42603	-6,99364	-17,90611
20	H	17,7734	-7,79587	-18,68939
21	H	16,1748	-7,77575	-19,47789
22	C	15,1191	-4,84143	-18,74438
23	C	15,24537	-4,11721	-17,37473
24	O	16,28185	-4,67428	-16,55137
25	C	13,921	-4,30687	-16,6122
26	O	12,85008	-4,00722	-17,52373
27	C	13,84824	-3,45986	-15,34688
28	H	17,09331	-4,12162	-16,66287
29	H	12,02419	-3,97934	-17,01035
30	H	14,36892	-4,3076	-19,33254
31	H	14,80657	-5,87989	-18,60778
32	H	15,4306	-3,0487	-17,55599
33	H	13,87443	-5,38126	-16,33669
34	H	13,86627	-2,38998	-15,59916
35	H	14,69541	-3,68222	-14,68369
36	H	12,92068	-3,66959	-14,7906
37	O	18,6267	-3,69798	-17,5993
38	C	19,79327	-3,86303	-17,19431
39	N	20,86615	-3,41152	-17,88391
40	C	20,78108	-2,62409	-19,11081
41	C	20,98373	-1,11273	-18,83176
42	C	19,97733	-0,43865	-17,86515
43	C	20,41937	1,01427	-17,63181
44	C	20,07738	-4,5926	-15,88662
45	C	19,60614	-6,0552	-15,98732
46	O	20,24023	-6,72918	-17,08102
47	C	21,87477	-3,17135	-20,07143
48	N	21,65773	-2,9532	-21,39834
49	C	22,60464	-3,37465	-22,44957
50	C	21,82045	-4,0475	-23,57975
51	O	21,00364	-5,07949	-22,99149
52	O	22,86497	-3,74907	-19,61013
53	C	18,52008	-0,50207	-18,36898
54	H	20,31976	-6,10778	-17,8252
55	H	21,14777	-4,55905	-15,63552
56	H	18,50977	-6,09259	-16,09624
57	H	19,88217	-6,60399	-15,07631
58	H	21,81192	-3,67738	-17,6075
59	H	19,77915	-2,78646	-19,53011
60	H	20,94588	-0,58644	-19,80066

61	H	22,00667	-0,97457	-18,4421
62	H	20,03075	-0,96444	-16,89619
63	H	18,12121	-1,51297	-18,22261
64	H	18,45814	-0,20687	-19,42578
65	H	17,89305	0,1896	-17,78878
66	H	19,76996	1,50704	-16,89385
67	H	20,36358	1,59628	-18,56524
68	H	21,45435	1,07208	-17,26239
69	H	22,50026	-4,53278	-24,29284
70	H	20,79449	-2,47018	-21,7072
71	H	21,17694	-3,32092	-24,09917
72	H	20,87181	-4,88384	-22,04595
73	H	23,15952	-2,50646	-22,83912
74	H	23,3196	-4,06819	-21,98652
75	H	19,52024	-4,07825	-15,08896
76	O	17,86001	1,03039	-22,68933
77	C	17,89859	2,23941	-22,93187
78	N	17,17338	3,19074	-22,31312
79	C	16,14889	2,87603	-21,32453
80	C	14,80203	2,88582	-22,04843
81	N	14,21327	1,67409	-22,17384
82	C	12,95663	1,47565	-22,88728
83	C	11,98518	0,52884	-22,19313
84	N	12,41507	-0,76711	-22,11227
85	C	11,68257	-1,75097	-21,32496
86	C	12,318	-3,11823	-21,52102
87	C	11,49233	-4,21606	-20,87056
88	C	12,25509	-5,52071	-20,87819
89	O	13,18266	-5,76195	-21,6526
90	O	14,37001	3,94143	-22,5202
91	O	10,89345	0,89524	-21,7705
92	C	11,59667	-1,33636	-19,84261
93	N	12,70316	-0,73144	-19,32076
94	C	12,77223	-0,34634	-17,90863
95	C	14,22529	-0,06587	-17,5265
96	O	10,58288	-1,5501	-19,16078
97	N	11,83839	-6,4309	-19,95804
98	C	11,87056	0,85962	-17,60636
99	H	11,89097	1,09092	-16,53015
100	H	10,83576	0,646	-17,89875
101	H	12,21446	1,74728	-18,15766
102	H	16,13857	3,65543	-20,55077
103	H	17,16135	4,12469	-22,71555
104	H	16,38949	1,90511	-20,87571
105	H	13,15781	1,10335	-23,9095
106	H	14,67827	0,87021	-21,73125
107	H	12,46941	2,45247	-22,9826

108	H	13,35995	-0,99955	-22,40663
109	H	10,63731	-1,75713	-21,67076
110	H	13,33445	-3,11533	-21,09683
111	H	12,42328	-3,32214	-22,59675
112	H	10,54582	-4,3639	-21,41818
113	H	11,21319	-3,93829	-19,84382
114	H	13,50626	-0,54309	-19,92615
115	H	12,39728	-1,20846	-17,33234
116	H	14,87523	-0,9212	-17,7551
117	H	14,29532	0,15891	-16,45255
118	H	14,61285	0,80468	-18,07712
119	H	12,26433	-7,35065	-19,93054
120	H	11,1086	-6,21364	-19,29064
121	H	18,56993	2,65131	-23,71611
122	O	20,43869	-0,47268	-23,92647
123	H	21,05367	0,21617	-24,22599
124	H	20,26668	-0,35285	-22,98005
125	O	15,69369	-7,3238	-15,66761
126	H	14,88216	-7,39118	-15,14252
127	H	15,90772	-6,37347	-15,8
128	O	20,15594	-7,11793	-24,03814
129	H	20,57383	-6,25115	-23,93298
130	H	20,0163	-7,4902	-23,15905
131	O	19,20356	-9,19408	-17,09644
132	H	20,11498	-8,86426	-17,11252
133	H	18,91234	-9,27641	-16,17484
134	O	18,44839	-10,67037	-19,08106
135	H	17,88213	-10,41771	-18,33885
136	H	18,57216	-9,90414	-19,65418
137	O	20,05539	-10,49795	-21,29371
138	H	19,88367	-9,68478	-21,79234
139	H	20,22745	-11,2171	-21,92049
140	C	22,48446	-7,64533	-18,84253
141	C	23,57613	-7,72942	-19,92974
142	C	23,01499	-7,77916	-21,33837
143	H	23,83202	-7,82852	-22,07153
144	H	22,38152	-6,9069	-21,5555
145	H	22,38226	-8,67123	-21,47665
146	H	21,89743	-6,72588	-18,9657
147	H	22,93564	-7,63562	-17,8416
148	H	21,79695	-8,50222	-18,91177
149	H	24,24934	-6,8631	-19,8238
150	H	24,1937	-8,62451	-19,74802
151	H	19,36192	-4,75263	-21,82398



## Literature

1. Kowalczyk, R. M.; Schleicher, E.; Bittl, R.; Weber, S., The photo-induced triplet of flavins and its protonation states. *J. Am. Chem. Soc.* **2004**, *126*, 11393-11399.