Mechanistic insights on riboflavin synthase inspired by selective binding of the 6,7-dimethyl8-8ribityllumazine exomethylene anion

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SUPPORTING INFORMATION

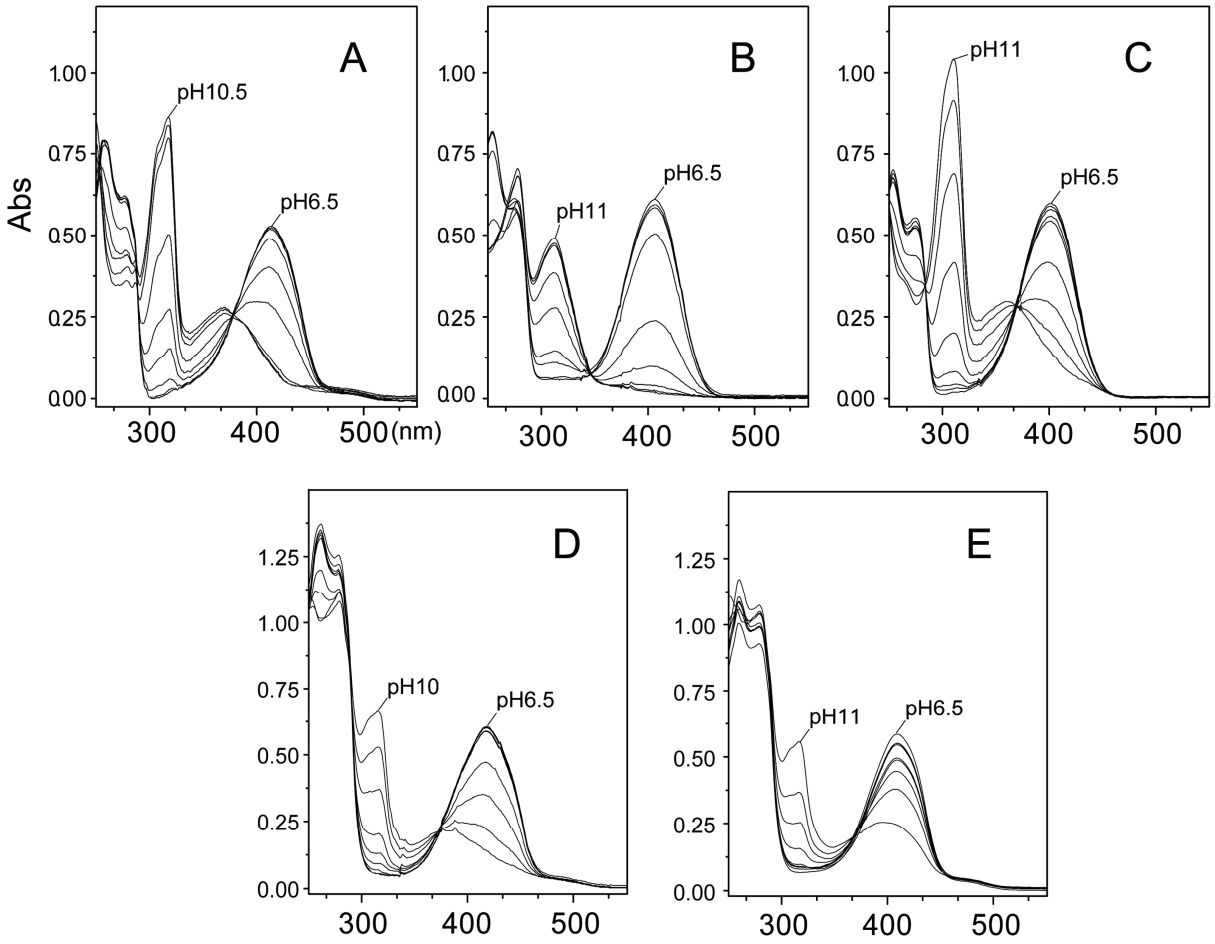


Fig. S1. Photometric titration of lumazine derivatives. pH of the samples was varied from neutral to alkaline values. A, **1** in complex with the N-terminal domain of riboflavin synthase (NRS); B, **1** in aqueous solution; C, **6** in aqueous solution; D, **1** in complex with the T67A mutant of NRS; E, **1** in complex with the T50A mutant of NRS.

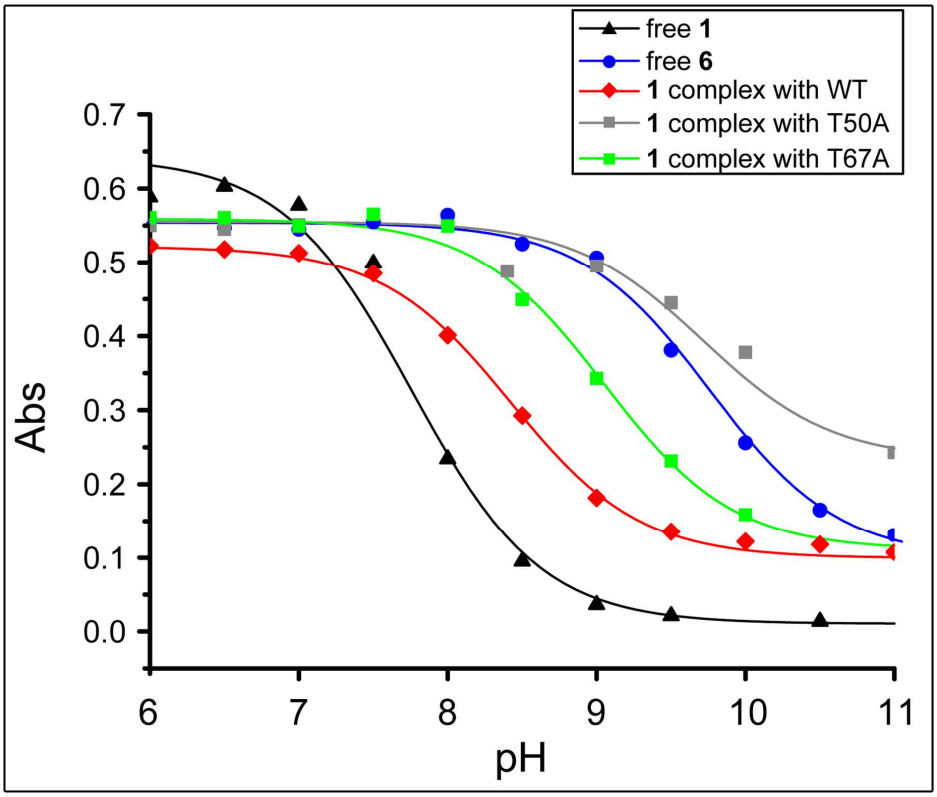


Fig. S2. Photometric titration curves of lumazine derivatives at 410 nm. NRS, wild type *E. coli* riboflavin synthase, N-terminal domain; T67A and T50A are respective mutants of NRS.

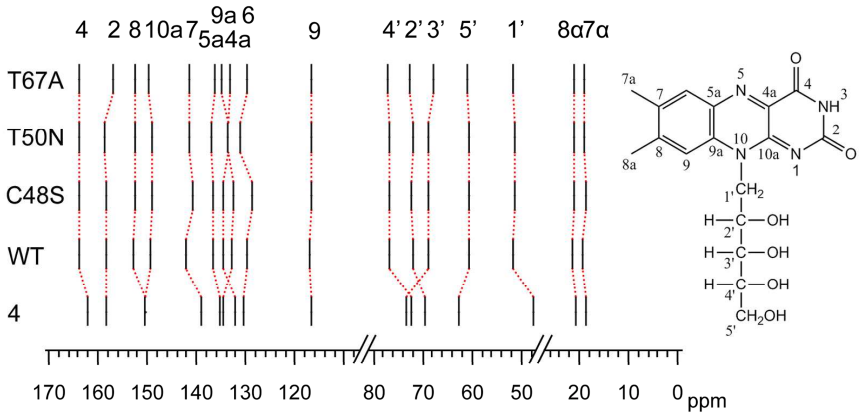
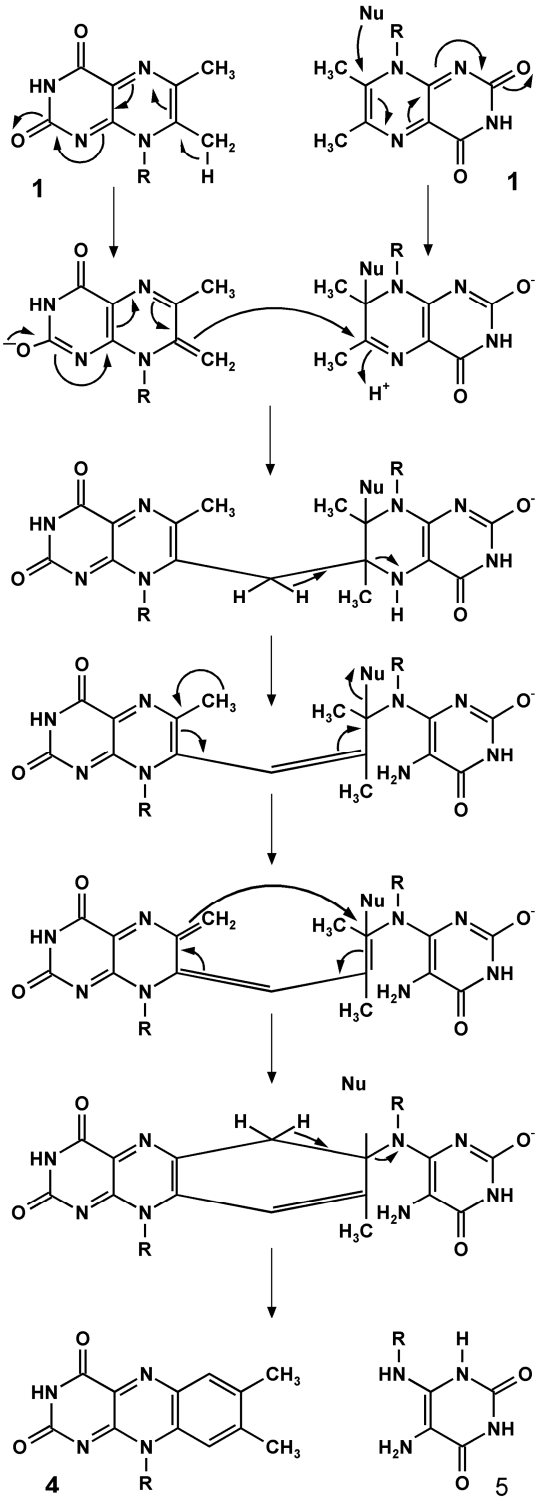


Fig. S3. ^{13}C chemical shifts of riboflavin in aqueous solution (**4**) and in complex with the N-terminal domain of riboflavin synthase [wild type (WT) and mutants T67A, T50N, C48S] at pH 6.9.



Scheme S1. Hypothetical reaction mechanism proposed in the 1960s by Plaut, Wood and their coworkers.[10,13] Nu, unknown nucleophile.

Table S1. NMR data of [U-$^{13}C_{13}$]-**1-**, [5-$^{15}N_1$]-**1-,** [8-$^{15}N_1$]-**1-,** and [U-$^{15}N_4$]-**1**-complex with the N-terminal domain of riboflavin synthase wild type (WT) or mutants at pH 6.9 or 11.0. **A,** ^{13}C-NMR chemical shifts (δ^{13}C, ppm) **B,** ^{15}N-NMR chemical shifts (δ^{15}N, ppm).

A.

Position	WT		C48A		C48S		T50A		T67A	
	pH 6.9	pH 11	pH 6.9	pH 11	pH 6.9	pH 11	pH 6.9	pH 11	pH 6.9	pH 11
4	165.9	164.0	165.9	164.1	166.0	164.0	165.7	163.8	165.9	164.0
2	159.4	160.2	159.4	160.2	159.4	160.2	159.7	160.1	158.2	159.3
7	157.1	143.2	157.5	142.3	158.3	142.9	155.7	143.1	156.6	143.0
8a	151.5	155.2	151.5	155.2	151.4	155.2	151.3	154.6	151.9	155.4
6	148.6	152.0	148.2	152.2	149.5	153.3	148.6	150.6	147.5	151.5
4a	130.8	106.0	130.6	105.6	130.2	105.7	131.4	106.0	130.8	105.9
4'	78.2	78.3	78.1	78.1	78.1	78.1	78.3	77.9	78.7	78.7
2'	73.9	75.3	74.1	75.5	74.1	75.6	73.9	75.1	74.4	75.8
3'	70.2	70.0	70.4	70.1	70.4	70.0	70.2	69.8	69.2	69.3
5'	62.6	62.5	62.3	62.5	62.2	62.5	62.4	62.2	62.6	62.5
1'	57.1	50.6	56.6	49.4	56.7	49.4	56.9	50.5	57.0	50.8
6α	23.3	22.8	22.5	22.4	22.3	22.0	24.1	23.7	23.2	22.7
7α	19.9	88.9	20.4	89.2	20.1	89.4	19.7	87.1	19.8	88.0

B.

Position	[5-$^{15}N_1$]**1** with WT		[8-$^{15}N_1$]**1** with WT		[U-$^{15}N_4$]**1** with WT		[U-$^{15}N_4$]**1** with T50A		[U-$^{15}N_4$]**1** with T67A	
	pH 6.9	pH 11	pH 6.9	pH 11	pH 6.9	pH 11	pH 6.9	pH 11	pH 6.9	pH 11
1					183.9	183.2	183.0	181.5	185.8	185.5
3					162.4	150.3	162.1	150.6	162.7	149.9
5	327.6	274.0			327.5	273.8	340.8	284.0	326.5	274.7
8			197.9	118.5	197.7	118.5	196.5	118.3	196.9	118.3

Table S2. NMR data of [U-$^{13}C_{17}$]-**4** in complex with the N-terminal domain of riboflavin synthase [wild type (WT) and mutants T67A, T50N, C48S].

Position	Chemical shifts, $\delta^{13}C$ (ppm)			
	WT	C48S	T50N	T67A
4	165.5	165.5	165.3	165.5
2	159.9	159.8	160.1	158.7
8	154.4	154.1	153.9	154.2
10a	150.9	150.8	150.6	151.3
7	143.7	142.5	142.9	143.2
5a	138.3	138.5	138.7	137.9
9a	136.3	136.3	135.3	136.4
4a	134.4	134.2	135.2	134.7
6	131.4	130.5	132.9	131.4
9	118.6	118.4	118.3	118.4
4'	78.5	78.3	78.4	78.7
2'	73.6	73.8	73.6	74.2
3'	70.3	70.3	70.4	69.4
5'	62.1	62.2	62.2	62.6
1'	53.1	52.9	52.9	53.3
8α	22.8	22.5	22.6	22.7
7α	20.8	20.3	20.7	20.8

Table S3. Plasmids and bacterial strains used in this study.

Strains/Plasmids	Relevant characteristics	Source
Strains		
E.coli XL-1 Blue	Cloning strain	Stratagene
E.coli M15[pREp4]	Expression strain	Qiagen
Plasmids		
pQE30	Expression vector	
pERN	pNCO113 containing the gene for the wild type N-terminal domain of *E.coli* riboflavin synthase	Eberhardt et al.,2001[19]
pERN-C48S	pNCO113 containing the gene for the C48S mutant N-terminal domain of *E.coli* riboflavin synthase	Eberhardt et al.,2001[19]
NRS-WT	pQE30 containing the gene for the wild type N-terminal domain of *E.coli* riboflavin synthase	This study
NRS-mutant variants	pQE30 containing the gene for the mutant variants N-terminal domain of *E.coli* riboflavin synthase	This study

Table S4. Oligonucleotides used in this study.

Primer	Amino acid replacement	Novel restriction site	Nucleotide sequence (5'→3') [a]
Forward	None	*Bam*H I	GAGGAGAAAGGATCCATGTTTACGG
Reverse	None	*Pst* I	GTCCTGCAGTTAGTGTCCGCC
1	C48→A	*Hph* I	CCGTCACGGTGAG**GGC**GCAACCGTTATG
2	T50→A	*Hinf* I	GACATGATTCCCGTTAATTTCCGTCAC**GGC**CAGGCA
3	T67→A	*Sna*BI	CGCCAAGATTGGTAATACGTAA**CGC**TTCTTTCAT

[a] Codons specifying modified amino acid residues are shown in bold type. Restriction sites are underlined.