Color-tunable and high quantum-yield luminescence from a biomolecule-inspired single species emitter of white light

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Single-species light emitters with high photoluminescence quantum yields (PLQYs) and broad-spectrum color tunability are sought-after for applications ranging from bio-imaging to artificial lighting. We explore a new strategy to design such emitters, inspired by bioluminescent fireflies and click-beetles. These organisms use a single molecular substrate, D-Luciferin (LH₂), to emit light ranging in color from green to red. By combining LH₂ with metals, we synthesize new bio-analogous, color-tunable, luminescent metal complexes. The copper complex forms an organic molecule of intrinsic microporosity (OMIM), which crystallizes into a stable structure with intermolecular voids. By changing the composition of guest molecules in the voids, we can tune the emitted color. The optimum composition gives nearly perfect white light, with the highest PLQY reported for a single-species white-light emitter. Similarities between our OMIM and the luciferase active site provide a new approach to investigating the heavily-debated mechanisms underlying *in-vivo* bioluminescence color variations. Moreover, as a proof of principle, we show that these materials can be used in a new type of light-emitting device (LED). The current generation of LEDs requires at least two active layers to achieve color tunability. The tunability is intrinsic in our materials, and therefore may lead to simpler device fabrication.

Introduction

The reason why we can see fireflies and click beetles at night is that these organisms are bioluminescent. Both use the same light-emitting substrate, D-Luciferin (LH_2), which is oxidized in the active site of largely homologous luciferase proteins during the bioluminescence reaction. And yet, fireflies emit green, and click beetles, red light.^[1, 2] Several theories have been proposed to elucidate these color variations.^[3–7] Studies show that the polarity, hydrophobicity, and conformation of the luciferase protein surrounding LH_2 play key roles, as they influence the emitter's chemical form,^[4, 6] and the species proximate to its terminal groups.^[8–10] Most such studies rely on investigating the photochemical properties of the isolated emitter, or the emission from bioluminescent reactions in proteins engineered with point mutations. However, conclusive explanations have eluded the community, due to the multitude of contributing factors, and the dynamic nature of the bioluminescence reaction. As a result, no generally-accepted explanation for this exists thus far.^[11, 12]

What is known, however, is that LH_2 , its analogues, and derivative molecules, possess fascinating photochemical properties. Here, we take inspiration from LH_2 -based bioluminescent systems to develop a new class of synthetic emitters for a range of potential applications, including artificial lighting. These emitters exploit the photochemical properties of LH_2 to harness both structural and microenvironment-triggered emission color tuneability. To achieve this, we use copper and zirconium ions to fuse LH_2 molecules into stable complexes. The reaction of zirconium with LH_2 produces a Zr-based complex. Based on the analysis of mass spectra and DFT calculations (see Supplementary Information for structural details), this complex is an OH-bridged dinuclear complex, with the formula $\text{Zr}_2(\text{LH}_2)_2(\mu\text{-OH})_2\text{Cl}_4$, which we named ZrLH_2 (see Supplementary [Figure S2](#)). While amorphous in the solid phase, ZrLH_2 is freely soluble in methanol, which is particularly advantageous for the formation of thin films from solution for the fabrication of light-emitting devices.

1. Cu-LH₂ metal complex

The copper reaction on the other hand, results in a mixed-valence, porous, crystalline copper complex, which we named Cu₂L₃. A detailed characterization of the crystal structure of Cu₂L₃ revealed the presence of periodic intermolecular voids, characteristic of an emerging class of materials known as Organic Molecules of Intrinsic Microporosity (OMIMs).^[13, 14] OMIMs are extrinsically porous crystalline materials, formed from molecules with bent, angular, or otherwise ‘awkward’ shapes, that pack together leaving intermolecular void spaces. Cu₂L₃ forms as the result of a redox reaction between D-LH₂ and Cu(NO₃)₂·2.5H₂O. When combined in an appropriate ratio at 373 K in a H₂O/DMF/EtOH solution (See Supplementary methods for full synthesis details), Cu^{II} is partially reduced to Cu^I, while LH₂ is simultaneously oxidized to dehydroluciferin (L) (**Figure 1a**). The relatively insoluble complex consists of three L ligands linked together via two Cu ions; Cu^I and Cu^{II}, in the Cu^I and Cu^{II} oxidation states respectively (**Figure 1b**). The 6'-OH hydroxyl terminals of the ligands, with their ground-state pK_a of ~8,^[15] remain protonated during synthesis and do not take part in coordination to the Cu ions.

Figure 1c shows the inefficient but regular packing of Cu₂L₃, characteristic of porous, crystalline OMIMs. The void spaces of Cu₂L₃, account for 13.1% of the unit-cell volume, and manifest as 1D channels that extend along the *c* axis (**Figure 1c, inset**). X-ray photo-electron spectroscopy (XPS, **Figure 1d** and **Figure S5**) analysis confirms the presence of both Cu^{II} and Cu^I in the complex, in a 1:1 ratio.

Figure 1: The structure of Cu_2L_3 . **a** Chemical transformation of luciferin (LH_2 , top) into dehydroluciferin (L , bottom) by means of an oxidizing agent, Cu^{II} . **b** Asymmetric unit of Cu_2L_3 consisting of three L units bridged by two Cu ions. **c** Spacefill model of Cu_2L_3 shows 1D channels in the c -axis direction; inset: Views of internal surfaces of the channels in the crystal highlight the accessibility of ligand terminal groups. **d** Cu 2p XPS spectrum of as-synthesized Cu_2L_3 . Atom color scheme: C = grey, H = white, O = red, N = blue, S = yellow, Cu = orange.

2. Biomimetic optical properties of LH_2 -based metal complexes

The optical properties of Cu_2L_3 are influenced by its structural aspects. Calculated and experimentally measured absorption and emission spectra of Cu_2L_3 are shown in **Figure 2a and b** respectively. The UV-visible absorption spectrum of Cu_2L_3 follows a similar profile to that of the free ligand L , with a narrow absorption band at 340 nm corresponding to the sum of local π - π^* transitions in each of the three ligands, L_1 , L_2 , and L_3 (Labels indicated in **Figure S4**). Both computed and measured spectra show that the absorption peak of Cu_2L_3 is red-shifted

with respect to that of the isolated ligand. When excited at 350 nm, as-synthesized crystals of Cu_2L_3 show a broad PL spectrum consisting of two distinct contributions; a blue peak centered at 430 nm, and a green-yellow 550 nm peak. Since the UV-visible absorption of Cu_2L_3 is ligand-centered, these two emission peaks are attributed to the neutral and anionic forms of the complexed L ligands, respectively. The ligand precursor, D-LH₂ (and many of its derivative molecules) is a strong photoacid.^[16] Photoexcitation of the molecule therefore lowers the pK_a of its 6'-OH terminal group from ~ 8 to ~ -0.5 ,^[15] allowing proximate water molecules deprotonate them via fast excited-state intermolecular proton transfer (ESPT).

Figure 2: Optical properties of Cu_2L_3 . Calculated, **a**, and experimentally measured, **b**, UV-vis absorption (via diffuse reflectance) and photoluminescence (PL) emission spectra of Cu_2L_3 . Solid red lines: absorption of Cu_2L_3 ; dashed red lines: absorption of free ligand L; Solid blue lines: PL emission of neutral Cu_2L_3 ; dashed blue lines: PL emission of fully deprotonated ($6-\text{O}^-$) Cu_2L_3 . **c** Dependence of PL spectrum (normalized to the 550 nm peak) on the concentration of H_2O in the pores of Cu_2L_3 , and **d** Corresponding emission colors. Black and blue circles/dashed lines correspond to signature 6-OH and 6-O⁻ spectra seen in pure ethanol and pure water, respectively. CIE coordinates of Cu_2L_3 synthesized in 25% H_2O (red circle) are (0.29, 0.33). **e** Photograph of Cu_2L_3 crystals suspended in solvent mixtures with varying amounts of water, from the left, 0, 5, 15, 25, 50, 75, and 100%. **f** stability of Cu_2L_3 in air and in aqueous solutions is demonstrated via PXRD patterns of the material as-synthesized, after three months in open air, and after soaking overnight in an aqueous ethanol solution.

Typically, neutral LH₂-like molecules emit in the blue region, and deprotonation of the 6'-OH group, which results in the anionic phenolate form of the molecule, lowers the emission energy, resulting in a characteristic 100 nm redshift.^[8] Since the Cu₂L₃ complex is charge-neutral, the 550 nm 6'-O⁻ peak in the experimentally measured emission spectrum of as-synthesized crystals is attributed to the void-facing position of its 6'-OH terminals, which are easily deprotonated by water. While Cu₂L₃ crystals suspended in pure water exhibit only the deprotonated 6'-O⁻ peak at 550 nm (Figure 2b, dashed blue line), crystals suspended in non-aqueous solvents exhibit only the 430 nm 6'-OH peak characteristic of the neutral ligand (Figure 2b, solid blue line). This assignment of the optical absorption and emission peaks in Cu₂L₃ was confirmed by density functional theory (DFT) calculations of the molecular orbitals associated with photoexcited transitions (Figure S9 and Figure S10).

3. Color tunability of LH₂-based metal complexes

Since changes in the protonation state are influenced by the concentration of water molecules in the voids, we expect to use this mechanism to fine-tune the emission color of Cu₂L₃, for example by changing the ethanol-water composition. Indeed, Figure 2c shows how the relative intensity of the two characteristic peaks in the emission spectrum can be tuned by changing the composition of the solvent. We observe that as the ratio of water is increased, the emission coordinates of the crystals follow the white region of the *International Commission on Illumination* (CIE) map from cool to warm (Figure 2d and e), narrowly crossing the coordinates for ideal white light. The change in color emitted from Cu₂L₃ as water is added to a suspension of the complex in ethanol can be seen in Supplementary Movie S1. The emission of Cu₂L₃ in 25% water is significantly broad (FWHM = 182.1 nm), and consists of the 450 and 550 nm peaks in a 0.75:1.00 intensity ratio (Figure 2c, red line). This corresponds to nearly pure-white CIE coordinates of (0.29, 0.33) (Figure 2d, red circle). The PLQY is 57%; amongst the highest reported for a single-species white light emitter, and to the best of our knowledge, the highest

quantum yield of an emitter closest to the equal energy point of the CIE map.^[17] We attribute this to rigidity of the complex, due to the ligand C2–C2', OH, and COOH bonds being locked in position by the Cu centers.^[18] Often the performance of an emitter is seen as a compromise between tunability and quantum yield. In our biologically-inspired complex, we have found a way to have both. Stability of the complex in air and water is demonstrated via PXRD measurements of the complex after three months in air, and after soaking in a H₂O:EtOH (0.25:0.75) mixture overnight (Figure 2f). Using DFT calculations, we also identified how LH₂-based modified ligands^[19, 20] could form OMIM-like, complexes, similar to Cu₂L₃, with even more extensive spectral tunability (Figure S11).

To gain insights into the interplay between water and ethanol molecules in the voids of Cu₂L₃, we carried out Grand Canonical Monte Carlo (GCMC) and NTV Monte Carlo simulations for different solvent concentrations. As seen in Figure 3a-f, one of the preferential adsorption sites of water (site 1) lies next to the 6'–OH groups. Ethanol molecules, however, show a stronger affinity to this particular site (Figure S12 and Figure S13), and displace the water molecules, making it increasingly difficult for the 6'–OH to deprotonate when ethanol is present.

Figure 3: Bioanalogous solvent behaviour in Cu₂L₃ pores. a–f Solvent adsorption probability density plots viewed from x-direction computed in the 3x3x2 supercell of DFT optimized Cu₂L₃ at 298.15 K and 0.02 bar. Blue and pink clouds represent the free energy grid corresponding to O atoms in H₂O and in EtOH, with the H₂O compositions of a, d 100% b, e 50% and c, f 25%. Black and green dots correspond to minimum energy basins for H₂O adsorption site 1 and 2, respectively. Black arrows in a indicate the O atoms associated with those –OH terminals that are subject to deprotonation. g, h, Water adsorption surfaces of Cu₂L₃ and the ATP-bound molecule in the *L. cruciata* protein pocket. For visual clarity, we omit the amino acids that form the protein but they are considered for the calculation of the adsorption surface.

Interestingly, the packing of the ligand units in Cu₂L₃ bears a remarkable resemblance to that of LH₂ in the luciferase active site of the *L. cruciata* firefly^[6, 8] (Figure 3g and h). In particular, analysis of the water adsorption surface within Cu₂L₃ voids show that in both structures, the 6'–OH can, from all hydrogen bonding active functional groups, most strongly interact with water (full details in Supplementary Information). Based on similarity analyses between the structure of our compound and the Oxy-LH₂ molecule embedded in the luciferase protein scaffold (PDB 2D1T), the maximum distance of the oxygen of the water molecule to oxygen the phenolic OH group is 3.3 Å in our simulations, which is close to the distances of 2.73 Å and 3.3 Å that are found in the protein. The challenge in studying the color-tuning mechanism observed in nature has been to disentangle the different factors contributing to it. In our material, we locked the

solvent-emitter interactions into a stable synthetic binding pocket of Cu_2L_3 , and were thus able to confirm the drastic effect of changes in solvent composition on the emission color, while all other variables are fixed. By introducing systematic modifications to the ligand, we can mimic some of the point mutations introduced in luciferase proteins and systematically study their behavior.

4. Bioluminescence-inspired proof-of-concept thin-film LEDs

While the molecular mechanism that determines the color of light emitted by bioluminescent organisms remains under debate, the bioluminescence reaction process is well understood. In this reaction, the enzyme, luciferase, converts the molecular substrate, LH_2 , into an adenosine monophosphate (AMP)-bound intermediate.^[21–23] Following oxidation by molecular oxygen, a basic residue in the luciferase pocket then promotes the abstraction of a proton from the C-4 carbon of the intermediate. Subsequent loss of CO_2 results in electronically excited OxyLH_2 that decays to produce light. In this way, nature succeeds in perfectly balancing the bipolar electron and hole injection into a luminescent molecular substrate to generate light, almost like a biological LED. Our detailed characterization of the structure of Cu_2L_3 facilitated the use of DFT to calculate the energetic positions of its Highest Occupied and Lowest Unoccupied Molecular Orbitals (HOMO and LUMO, respectively), which are crucial characteristics in designing the architecture of an LED. Our calculations, described in detail in the Supplementary Information, suggest that upon injecting charge carriers, the first reduction and oxidation of Cu_2L_3 takes place in the Cu_2 and Cu_1 copper centers. This is unsurprising given the low redox potential of Cu ions,^[24, 25] however it means that careful design of device architecture is required to balance the electron- and hole-injection rates and obtain electroluminescence from Cu_2L_3 .

Figure 4: Simple tunable device from ZrLH₂ complex. **a** Photograph of ZrLH₂ dissolved in methanol-water mixtures with varying amounts of water, from the left, 0, 25, and 100% by volume. **b** ZrLH₂ @PVP layers without (left) and with (right) the ionic liquid EAN showing colour tunability in dried substrates. **c**, **d** photographs of a working device made with ZrLH₂ when switched off (**c**) and on (**d**). **e** schematic of the simple working device made with ZrLH₂. ITO = indium-tin oxide, Active layer = ZrLH₂@PVP, EGaIn = gallium-indium eutectic.

Since Zr is known to have a higher redox potential,^[26, 27] the Zr analogue of Cu₂L₃ was used to demonstrate the applicability of our strategy towards tunable emitters for LEDs. Also, relying on water to deprotonate the 6'-OH groups may be complicated from a device point of view, as it requires careful sealing to avoid evaporation. Instead, low-vapor pressure ionic liquids (ILs), which are typically used as electrolytes in batteries or fuel cells, can substitute water. Since ILs can be relatively bulky, it is preferable to have a freely soluble complex rather than a porous crystal in the active layer of a device. The IL ethylammonium nitrate (EAN) was selected based on its chemical similarities to water, and is capable of deprotonating the 6'-OH groups of the ligand. This indicates that the OH groups are accessible to the IL, which strongly suggests a similar color tuning mechanism.

Indeed, **Figure 4b** shows that adding EAN to a dry film of ZrLH₂ gives rise to a color tunability similar to that in Cu₂L₃. Another advantage of having a freely soluble is much easier processability. By dissolving ZrLH₂ into a polyvinylpyrrolidone (PVP) host, we were able to make a simple single-layer LED entirely from solution. To do this, the ZrLH₂@PVP mixture was drop-cast onto indium-tin-oxide (ITO)-coated glass substrates and left to dry in open air before depositing a gallium-indium cathode. Similar to its copper-based counterpart, ZrLH₂ exhibits ligand-centered optical properties, including emission color tunability that can be exploited by changing the water content surrounding its 6'-OH terminals (**Figure 4a**). Dropcast layers of ZrLH₂ in PVA on ITO form a simple working LED, processed entirely from solution, that emits a cold white light at an applied voltage of 5V (**Figure 4c-e**).

Remarkably, this rudimentary working device was fabricated and operated in open air, without the need for any charge-injection or -blocking layers. The simple, low-cost synthesis, single-species tunability, high quantum yields, and potentially straightforward implementation into working LEDs, all demonstrate the promise for a new family of LH₂-based light emitters reported in this manuscript.

5. Conclusion

We report a novel strategy for designing high-stability, high quantum-yield, color-tunable light emitters. Taking lessons from nature, we mimic the bioluminescence of fireflies and click beetles in a series of metal complexes based on the bioluminescent molecule, luciferin (LH₂). Using a low-cost, low-temperature, water-based synthetic protocol, we obtain highly stable electroluminescent materials, whose emission colors can be tuned by varying the concentration of small-molecule ionic liquids in their immediate environment.

Our copper complex in particular, crystallizes to form a porous structure with regular voids that mimic the binding pocket surrounding naturally-occurring LH₂ in bioluminescent organisms.

These striking similarities provide a unique molecular model, through which we can gain insights into the elusive bioluminescence color-determining mechanisms that remain under debate.

Using our materials, we prepare a rudimentary proof-of-principle LED, which was fabricated in open air at room temperature, consists of only a single active layer, and exhibits a stable emission for over ten hours under a continuous applied voltage of 6 V.

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References

1. H. Fraga, Firefly luminescence: A historical perspective and recent developments. *Photochem. Photobiol. Sci.* **7**, 146–158 (2008).
2. D. T. Amaral, G. Oliveira, J. R. Silva, V. R. Viviani, A new orange emitting luciferase from the Southern-Amazon *Pyrophorus angustus* (Coleoptera: Elateridae) click-beetle: structure and bioluminescence color relationship, evolutionary and ecological considerations. *Photochem. Photobiol. Sci.* **15**, 1148–1154 (2016).
3. E. H. White, E. Rapaport, T. A. Hopkins, H. H. Seliger, Chemi- and bioluminescence of firefly luciferin. *J. Am. Chem. Soc.* **91**, 2178–2180 (1969).
4. B. R. Branchini, T. L. Southworth, M. H. Murtiashaw, R. A. Magyar, S. A. Gonzalez, M. C. Ruggiero, J. G. Stroh, An Alternative Mechanism of Bioluminescence Color Determination in Firefly Luciferase. *Biochemistry*. **43**, 7255–7262 (2004).
5. N. N. Ugarova, L. G. Maloshenok, I. V. Uporov, M. I. Koksharov, Bioluminescence Spectra of Native and Mutant Firefly Luciferases as a Function of pH. *Biochemistry (Moscow)*. **70**, 1262–1267 (2005).
6. T. Nakatsu, S. Ichiyama, J. Hiratake, A. Saldanha, N. Kobashi, K. Sakata, H. Kato, Structural basis for the spectral difference in luciferase bioluminescence. *Nature*. **440**, 372–376 (2006).

7. Y. Ando, K. Niwa, N. Yamada, T. Enomoto, T. Irie, H. Kubota, Y. Ohmiya, H. Akiyama, Firefly bioluminescence quantum yield and colour change by pH-sensitive green emission. *Nature Photonics*. **2**, 44–47 (2008).
8. P. Naumov, Y. Ozawa, K. Ohkubo, S. Fukuzumi, Structure and Spectroscopy of Oxyluciferin, the Light Emitter of the Firefly Bioluminescence. *J. Am. Chem. Soc.* **131**, 11590–11605 (2009).
9. V. R. Viviani, G. V. M. Gabriel, V. R. Bevilaqua, A. F. Simões, T. Hirano, P. S. Lopes-de-Oliveira, The proton and metal binding sites responsible for the pH-dependent green-red bioluminescence color tuning in firefly luciferases. *Scientific Reports*. **8**, 17594 (2018).
10. M. Kheirabadi, Z. Sharafian, H. Naderi-Manesh, U. Heineman, U. Gohlke, S. Hosseinkhani, Crystal structure of native and a mutant of *Lampyrus turkestanicus* luciferase implicate in bioluminescence color shift. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*. **1834**, 2729–2735 (2013).
11. Z. M. Kaskova, A. S. Tsarkova, I. V. Yampolsky, 1001 lights: luciferins, luciferases, their mechanisms of action and applications in chemical analysis, biology and medicine. *Chemical Society Reviews*. **45**, 6048–6077 (2016).
12. C. Carrasco-López, N. M. Lui, S. Schramm, P. Naumov, The elusive relationship between structure and colour emission in beetle luciferases. *Nature Reviews Chemistry*. **5**, 4–20 (2021).
13. J. R. Holst, A. Trewin, A. I. Cooper, Porous organic molecules. *Nature Chemistry*. **2**, 915–920 (2010).
14. Porous Molecular Solids and Liquids | ACS Central Science, (available at <https://pubs.acs.org/doi/10.1021/acscentsci.7b00146>).
15. Y. Erez, D. Huppert, Excited-State Intermolecular Proton Transfer of the Firefly's Chromophore d-Luciferin. *J. Phys. Chem. A*. **114**, 8075–8082 (2010).
16. L. P. da Silva, R. Simkovitch, D. Huppert, J. C. G. E. da Silva, Oxyluciferin Photoacidity: The Missing Element for Solving the Keto–Enol Mystery? *ChemPhysChem*. **14**, 3441–3446 (2013).
17. Z. Chen, C.-L. Ho, L. Wang, W.-Y. Wong, Single-Molecular White-Light Emitters and Their Potential WOLED Applications. *Advanced Materials*. **32**, 1903269 (2020).
18. L. L. Bras, K. Chaitou, S. Aloïse, C. Adamo, A. Perrier, Aggregation-caused quenching versus crystallization induced emission in thiazolo[5,4-b]thieno[3,2-e]pyridine (TTP) derivatives: theoretical insights. *Phys. Chem. Chem. Phys.* **21**, 46–56 (2018).
19. D. C. McCutcheon, M. A. Paley, R. C. Steinhardt, J. A. Prescher, Expedient Synthesis of Electronically Modified Luciferins for Bioluminescence Imaging. *J. Am. Chem. Soc.* **134**, 7604–7607 (2012).
20. N. R. Conley, A. Dragulescu-Andrasi, J. Rao, W. E. Moerner, A Selenium Analogue of Firefly D-Luciferin with Red-Shifted Bioluminescence Emission. *Angewandte Chemie International Edition*. **51**, 3350–3353 (2012).

21. M. DeLuca, in *Advances in Enzymology and Related Areas of Molecular Biology* (John Wiley & Sons, Ltd, 1976; <https://onlinelibrary.wiley.com/doi/abs/10.1002/9780470122891.ch2>), pp. 37–68.
22. M. DeLuca, W. D. McElroy, Kinetics of the firefly luciferase catalyzed reactions. *Biochemistry*. **13**, 921–925 (1974).
23. N. Wada, M. Honda, H. Suzuki, Theory of D-(-)-Luciferin and Dehydroluciferin in Acidic or Basic Solution. *J. Phys. Soc. Jpn.* **54**, 4851–4860 (1985).
24. J. D. Cope, H. U. Valle, R. S. Hall, K. M. Riley, E. Goel, S. Biswas, M. P. Hendrich, D. O. Wipf, S. L. Stokes, J. P. Emerson, Tuning the Copper(II)/Copper(I) Redox Potential for More Robust Copper-Catalyzed C–N Bond Forming Reactions. *European Journal of Inorganic Chemistry*. **2020**, 1278–1285 (2020).
25. J. Qiu, K. Matyjaszewski, L. Thouin, C. Amatore, Cyclic voltammetric studies of copper complexes catalyzing atom transfer radical polymerization. *Macromolecular Chemistry and Physics*. **201**, 1625–1631 (2000).
26. S. Imdadul Hossain, M. Abdul Aziz, D. Han, P. Selvam, S. Shanmugam, Fabrication of SPAEK–cerium zirconium oxide nanotube composite membrane with outstanding performance and durability for vanadium redox flow batteries. *Journal of Materials Chemistry A*. **6**, 20205–20213 (2018).
27. J. Su, S. Yuan, T. Wang, C. Tori Lollar, J.-L. Zuo, J. Zhang, H.-C. Zhou, Zirconium metal–organic frameworks incorporating tetrathiafulvalene linkers: robust and redox-active matrices for in situ confinement of metal nanoparticles. *Chemical Science*. **11**, 1918–1925 (2020).
28. D. C. McCutcheon, M. A. Paley, R. C. Steinhardt, J. A. Prescher, Expedient Synthesis of Electronically Modified Luciferins for Bioluminescence Imaging. *J. Am. Chem. Soc.* **134**, 7604–7607 (2012).
29. E. H. White, F. McCapra, G. F. Field, W. D. McElroy, THE STRUCTURE AND SYNTHESIS OF FIREFLY LUCIFERIN. *J. Am. Chem. Soc.* **83**, 2402–2403 (1961).
30. P. Ciuffreda, S. Casati, G. Meroni, E. Santaniello, A new synthesis of dehydroluciferin [2-(6'-hydroxy-2'-benzothiazolyl)-thiazole-4-carboxylic acid] from 1,4-benzoquinone. *Tetrahedron*. **69**, 5893–5897 (2013).
31. Global Spectral Deconvolution Based on Non-Negative Matrix Factorization in GC × GC–HRTOFMS | Analytical Chemistry, (available at <https://pubs.acs.org/doi/full/10.1021/ac5038544>).
32. L. Patiny, M. Zasso, D. Kostro, A. Bernal, A. M. Castillo, A. Bolaños, M. A. Asencio, N. Pellet, M. Todd, N. Schloerer, S. Kuhn, E. Holmes, S. Javor, J. Wist, The C6H6 NMR repository: An integral solution to control the flow of your data from the magnet to the public. *Magnetic Resonance in Chemistry*. **56**, 520–528 (2018).
33. F. Lu, R. A. Zarkesh, A. F. Heyduk, A Redox-Active Ligand as a Reservoir for Protons and Electrons: O₂ Reduction at Zirconium(IV). *European Journal of Inorganic Chemistry*. **2012**, 467–470 (2012).

34. Summary: B3LYP proper reference, (available at <http://www.ccl.net/chemistry/resources/messages/2002/05/22.008-dir/>).
35. Basis Sets | Gaussian.com, (available at <https://gaussian.com/basissets/>).
36. Citation | Gaussian.com, (available at <https://gaussian.com/citation/>).
37. G. M. Sheldrick, SHELXT – Integrated space-group and crystal-structure determination. *Acta Cryst A*. **71**, 3–8 (2015).
38. G. M. Sheldrick, Crystal structure refinement with SHELXL. *Acta Cryst C*. **71**, 3–8 (2015).
39. O. V. Dolomanov, L. J. Bourhis, R. J. Gildea, J. a. K. Howard, H. Puschmann, OLEX2: a complete structure solution, refinement and analysis program. *J Appl Cryst*. **42**, 339–341 (2009).
40. P. van der Sluis, A. L. Spek, BYPASS: an effective method for the refinement of crystal structures containing disordered solvent regions. *Acta Cryst A*. **46**, 194–201 (1990).
41. (IUCr) PLATON SQUEEZE: a tool for the calculation of the disordered solvent contribution to the calculated structure factors, (available at <https://onlinelibrary.wiley.com/iucr/doi/10.1107/S2053229614024929>).
42. C. F. Macrae, I. J. Bruno, J. A. Chisholm, P. R. Edgington, P. McCabe, E. Pidcock, L. Rodriguez-Monge, R. Taylor, J. van de Streek, P. A. Wood, Mercury CSD 2.0 – new features for the visualization and investigation of crystal structures. *J Appl Cryst*. **41**, 466–470 (2008).
43. Conquering Aggregation-Induced Solid-State Luminescence Quenching of Carbon Dots through a Carbon Dots-Triggered Silica Gelation Process | Chemistry of Materials, (available at <https://pubs.acs.org/doi/10.1021/acs.chemmater.6b05375>).
44. D. G. Congrave, B. H. Drummond, V. Gray, A. D. Bond, A. Rao, R. H. Friend, H. Bronstein, Suppressing aggregation induced quenching in anthracene based conjugated polymers. *Polym. Chem.* (2021), doi:10.1039/D1PY00118C.
45. W. Kohn, L. J. Sham, Self-Consistent Equations Including Exchange and Correlation Effects. *Phys. Rev.* **140**, A1133–A1138 (1965).
46. C. Adamo, D. Jacquemin, The calculations of excited-state properties with Time-Dependent Density Functional Theory. *Chem. Soc. Rev.* **42**, 845–856 (2013).
47. C. Adamo, G. E. Scuseria, V. Barone, Accurate excitation energies from time-dependent density functional theory: Assessing the PBE0 model. *J. Chem. Phys.* **111**, 2889–2899 (1999).
48. K. Raghavachari, G. W. Trucks, Highly correlated systems. Excitation energies of first row transition metals Sc–Cu. *The Journal of Chemical Physics*. **91**, 1062 (1998).
49. A. D. McLean, G. S. Chandler, Contracted Gaussian basis sets for molecular calculations. I. Second row atoms, Z=11–18. *J. Chem. Phys.* **72**, 5639–5648 (1980).

50. V. Barone, M. Cossi, Quantum Calculation of Molecular Energies and Energy Gradients in Solution by a Conductor Solvent Model. *J. Phys. Chem. A.* **102**, 1995–2001 (1998).
51. M. Cossi, N. Rega, G. Scalmani, V. Barone, Energies, structures, and electronic properties of molecules in solution with the C-PCM solvation model. *Journal of Computational Chemistry.* **24**, 669–681 (2003).
52. Gaussian 09 Citation | Gaussian.com, (available at <https://gaussian.com/g09citation/>).
53. UFF, a full periodic table force field for molecular mechanics and molecular dynamics simulations | Journal of the American Chemical Society, (available at <https://pubs.acs.org/doi/abs/10.1021/ja00051a040>).
54. Applicability of Tail Corrections in the Molecular Simulations of Porous Materials | Journal of Chemical Theory and Computation, (available at <https://pubs.acs.org/doi/full/10.1021/acs.jctc.9b00586>).
55. J. L. F. Abascal, C. Vega, A general purpose model for the condensed phases of water: TIP4P/2005. *J. Chem. Phys.* **123**, 234505 (2005).
56. Monte Carlo Calculations for Alcohols and Their Mixtures with Alkanes. Transferable Potentials for Phase Equilibria. 5. United-Atom Description of Primary, Secondary, and Tertiary Alcohols | The Journal of Physical Chemistry B, (available at <https://pubs.acs.org/doi/abs/10.1021/jp003882x>).
57. D. Dubbeldam, S. Calero, T. J. H. Vlugt, iRASP: GPU-accelerated visualization software for materials scientists. *Molecular Simulation.* **44**, 653–676 (2018).
58. S. Iwano, R. Obata, C. Miura, M. Kiyama, K. Hama, M. Nakamura, Y. Amano, S. Kojima, T. Hirano, S. Maki, H. Niwa, Development of simple firefly luciferin analogs emitting blue, green, red, and near-infrared biological window light. *Tetrahedron.* **69**, 3847–3856 (2013).

Supporting Information

Color-tunable and high quantum-yield luminescence from a biomolecule-inspired single species emitter of white light

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Materials and Methods

Materials

All of the starting chemicals are commercially available and were used without any further purification. D-Cysteine was purchased from Fluorochem. 2-Cyano-6-hydroxybenzothiazole and potassium carbonate were purchased from Acros. $\text{Cu}(\text{NO}_3)_2 \cdot 2.5\text{H}_2\text{O}$ (98%) was purchased from Alfa Aesar.

Synthesis of D-Luciferin (LH₂)

D-Cysteine (1.444 g, 11.92 mmol) and 2-cyano-6-hydroxybenzothiazole (2.000 g, 11.35 mmol) were suspended in MeOH-H₂O 2:1 mixture (80 mL) in a 100 mL Schlenk flask. After addition of potassium carbonate (1.6448 g, 11.92 mmol) to the mixture the color changed to bright yellow-green and the resulting solution was stirred under N₂ overnight. Methanol was removed *in vacuo* and the remaining aqueous solution was acidified with 1M HCl solution to maximize the amount of precipitate, which was extracted with EtOAc (3×50 mL). The combined organic phase was dried over Na₂SO₄ and evaporated to afford D-luciferin (LH₂) as a pale yellow solid (2.630 g, 96%). ¹H NMR (400 MHz, Methanol-*d*₄) δ 7.89 (d, *J* = 8.9 Hz, 1H), 7.33 (d, *J* = 2.4 Hz, 1H), 7.06 (dd, *J* = 8.9, 2.4 Hz, 1H), 5.38 (t, *J* = 9.1 Hz, 1H), 3.75 (m, 2H). (1) ¹³C NMR (101 MHz, MeOD) δ 173.34, 167.64, 159.00, 158.50, 148.14, 139.11, 125.91, 118.18, 107.32, 79.47, 35.84. (28) ¹H (400.13 MHz) and ¹³C (100.62 MHz) NMR spectra were recorded on a Bruker Avance II 400 spectrometer at 298 K.

Synthesis of dehydroluciferin(29, 30)

D-luciferin (115 mg, 0.41 mmol) was dissolved in 250 mL of aqueous NaOH (1M) and the resulting solution was heated to 100°C. Air was bubbled through the solution for 6 h with vigorous stirring, then the reaction mixture was cooled, acidified with 1M HCl and extracted with EtOAc (3×25 mL). The product was subjected to column chromatography (DCM-MeOH, 4:1), resulting in dark violet solid (47 mg, 41%). ¹H NMR (400 MHz, DMSO-*d*₆) 10.17 (s, 1H), 8.67 (s, 1H), 7.91 (d, *J* = 8.8 Hz, 1H), 7.46 (d, *J* = 2.4 Hz, 1H), 7.04 (dd, *J* = 8.8, 2.4 Hz, 1H). ¹³C NMR (DMSO-*d*₆): δ = 162.1, 161.8, 157.5, 157.0, 148.8, 146.8, 137.2, 131.6, 124.7, 117.6, 107.5. ESI MS *m/z* 276.9768 (calcd 276.9742 [M-H]⁻).

Synthesis of the Cu_2L_3 complex

$\text{Cu}(\text{NO}_3)_2 \cdot 2.5\text{H}_2\text{O}$ (20 mg, 0.068 mmol) and 20 mg (0.071 mmol) of LH_2 were introduced into borosilicate glass scintillation vials, dissolved in a DMF- H_2O -EtOH 3:1:1 mixture (6 mL), and then sealed and heated to 100 °C for 16 hours. Reddish-brown crystals of Cu_2L_3 were isolated by centrifugation with the yield of ~70% (15 mg). The purity of the product was confirmed by elemental analysis and PXRD of the solid crystals.

Synthesis of ZrLH_2 complex

ZrCl_4 and D- LH_2 were combined in a 2:1 ratio and refluxed in methanol for 3 hours. The solvent was left to evaporate resulting in a low yield of the yellow solid product.

DFT optimization of ZrLH_2 structure

Based on these findings, we manually enumerated some dinuclear zirconium complexes, also inspired by the literature (using a search in the CSD with dinuclear Zr bridged by OH/Cl as pattern)(33), and optimized their structure using DFT (B3LYP)(34) hybrid functional with LANL2DZ basis set(35), and ultrafine integral settings in Gaussian 16(36). To estimate the stability we calculated the formation energies. We find that both a OH and Cl bridged dinuclear complex are stable, with the OH bridge one being slightly more stable. Based on these DFT calculations and our analysis of the mass spectra, the OH bridge dinuclear complex best represents the structure of ZrLH_2 (see [fig. S2](#))

Single-crystal X-ray diffraction

Diffraction data were collected on a BRUKER D8 VENTURE four-circle diffractometer equipped with a MoK α microfocus sealed X-ray tube and a Photon 100 2D CMOS detector. The crystal structure was solved with SHELXT(37) and refined with SHELXL programs integrated within the Olex2 program suite(38, 39). All non-H atoms were found upon solution and refined anisotropically. Aromatic H atoms were introduced based on the molecular geometry with the AFIX 43 command, while those belonging to the hydroxy groups were found from the Fourier–difference maps and refined either independently (H3) or using AFIX 83 or AFIX 147 commands (H6 and H9). No additional H atoms in proximity to N or S atoms were found from the Fourier–difference maps. Several restraints were imposed on the atomic displacement parameters of the non-H atoms. The contribution of the disordered solvent molecules found in the structural voids to the measured structure factors was quantified using the solvent masking procedure implemented in Olex2 equivalent to PLATON SQUEEZE(40–42). Structure-derived pore volume was calculated from the hypothetical solvent-free structure with the program Mercury(43) using the probe radius of 1.2 Å and approx. grid spacing of 0.7 Å.

Material characterization

Powder X-ray diffraction (PXRD) data on all samples were collected on a Bruker D8 Advance diffractometer at ambient temperature using monochromated Cu K α radiation ($\lambda = 1.5418$ Å), with a 2θ step of 0.02° and a 2θ range of ~ 2 to 70° . Simulated PXRD patterns were generated from the corresponding crystal structures using Mercury 3.0. X-ray photoelectron spectroscopy (XPS) was performed using a PHI 5000 Versaprobe-II instrument from Physical Electronics. Samples were deposited on an insulating double sided, vacuum compatible, taped, and charge neutralization was applied during the XPS measurements. The binding energy scale was then corrected with the C-C bound of the C1s photoelectron peak located at 284.6 eV. Peak fitting of the Cu 2p $_{3/2}$ photoelectron peak, along with the kinetic energy of the Cu L $_3$ M $_{4,5}$ M $_{4,5}$ peak, were used to evaluate the chemical state of copper.

Optical characterization

Optical characterization was carried out on crystals in the solid state, and in dilute suspensions. For measurements in suspension, the crystals were crushed and suspended in ethanol/water solvent mixtures with varying ratios of water. For solid measurements, crystals were grown on glass pieces placed inside the reaction vials, and the optical properties of the films measured. PXRD patterns were measured after all measurements to ensure stability of the material. Similarity between the emission spectra of **Cu₂L₃** films grown on glass substrate and **Cu₂L₃** crystals dispersed in dilute suspensions can be seen in [fig. S1](#) below. Stability of **Cu₂L₃** crystals after suspension in aqueous solution is demonstrated in [Fig. 2f](#) in the main manuscript.

Fig. S1.

a Photoluminescence emission spectra and **b** corresponding CIE coordinates of as-synthesized **Cu₂L₃** crystals, formed on a glass substrate (in red), and suspended in a 25% H₂O solution (in black). It should be noted that the emission intensity of **Cu₂L₃** in the solid film form is lower due to aggregation quenching(16, 17). Emission intensity of the film is normalized to that of the suspension for a comparison of the peak positions.

UV-visible absorption and diffuse reflectance spectra were recorded on a PerkinElmer LAMBDA 1050 spectrometer equipped with a praying mantis. Steady state photoluminescence spectra and absolute quantum yields were measured using a Horiba Fluorolog-3 equipped with an integrating sphere. Reported quantum yield value is an average of five independent measurements.

Device Fabrication

5 mg of ZrLH_2 was dissolved in 1 mL of methanol, and then mixed in a 1:5 volume ratio with a 30 mg/mL methanol solution of polyvinylpyrrolidone. The solution was drop-cast onto cleaned indium-tin-oxide-(ITO)-coated glass substrates and left to dry in open air to form the active layer. A gallium-indium (GaIn) eutectic paste was applied to the dried active layer through a mask to form the cathode. Copper wire was contacted to the GaIn eutectic and sealed with epoxy or Kapton tape. An applied voltage of 5 V across the simple device gives rise to cold white light emission shown in Fig. 4d of the main manuscript.

Density Functional Theory calculations

Absorption spectra calculations were done with Kohn-Sham DFT(45) and linear response Time-Dependent DFT (TDDFT)(46) using the PBE0(20) functional and 6-311g(d) basis set(48, 49) including implicit solvent (ethanol) by means of the CPCM(50, 51) method as implemented in Gaussian 09 code(52), for the isolated ligand and for the Cu_2L_3 complex being all 6'-OH groups protonated.

Molecular simulations

For all our simulations we used the DFT-optimized unit cell. Van der Waals interactions between the framework atoms were modelled using Universal Force Field(53) with the tail corrections(54). Charges on the framework atoms were calculated with DDEC method using the electronic density calculated from geometrically optimized structure. Lennard-Jones parameters for water and ethanol molecules were taken from TIP4P2005(55) and TraPPE(56), respectively. Pairwise interactions were calculated using Lorentz-Barthelot mixing rules. The interactions between an atom and the atoms beyond the cut-off 12.5 Å were computed using a tail correction.

The Grand Canonical Monte Carlo (GCMC) simulations were carried out at 298.15 K and 0.02 bar. We utilized 500,000 equilibration cycles and at least 140,000 production cycles with combination of insertion/deletion, reinsertion, rotation, and translation moves. To increase the efficiency of the simulations, identity change moves were also used. One cycle consists of max (20, N) steps where N is the number of adsorbate molecule in the system. In addition to performing a single component simulation for water adsorption, we also considered binary mixture simulations due to the competitive adsorption effect. Binary mixture simulations were carried out for the feed mixture with the compositions of 50:50 and 25:75 (water: ethanol).

NVT Monte Carlo simulations were performed by starting from the last snapshot of the equilibrated GCMC simulation corresponding to the composition of interest. For the bulk composition of 25:75, this corresponds to 20 ethanol and 39 water molecules in the pores of the $3 \times 3 \times 2$ supercell, and for the 50:50 bulk composition, 14 ethanol and 67 water molecules. For the single adsorption of water, 122 molecules were loaded into the supercell. All of these loadings were achieved by inserting the molecules into the positions we took from the last snapshot of the GCMC simulations. Since we started from already equilibrated system, we utilized 300,000 production cycles with combination of reinsertion, rotation, and translation moves. One cycle consists of max (20, N) steps where N is the number of adsorbate molecule in the system. All Monte Carlo simulations were performed on RASPA software package(57).

Density plots and radial distribution functions

Cartesian coordinates of the oxygen atoms in the adsorbate molecules (water and ethanol) were taken from the snapshots that were stored during the simulations. The positions of the atoms were binned to a 3-D grid by counting the number of times that a grid point has been visited during the simulation. This grid data was used to calculate free energies from the Boltzmann distribution. These data were used to create the free energy grids shown in [Fig. 3a-f](#), where the blue and pink areas are the iso-surfaces corresponding to an 80% probability to find the oxygen of water and ethanol inside this surface. To provide a more detailed picture of the water adsorption, we defined two principal adsorption sites and emphasized the corresponding minimum energy basins with different colors (black: site 1; green: site 2). Then, we calculated the probability of having a water molecule around the two adsorption sites for each composition, see [fig S12](#).

To further quantify the effect of ethanol on the probability to find a water molecule next to the 6' oxygen of the luciferin that is in a protonated (6'-OH) or non-protonated (6'-O-), see, we computed the radial distribution function of 6'-O with the oxygen of the water ([fig. S13](#)).

Molecular model for luciferin@luciferase

To quantify the similarity between our compound and the luciferin molecule in the protein, we performed similarity analyses between the structure of our compound and the OxyLH₂ molecule embedded in the protein scaffold (PDB 2D1T).

To visualize the interface of **Cu₂L₃** with the environment shown in [Fig. 3g-h](#), we created energy surfaces using the iRASP program⁽⁵⁷⁾ (using the Ow parameters of the TIP5P-Ew model as a probe molecule for water at 298 K and the default force field of iRASP, for the calculation of the adsorption surface (blue) of the molecule in the protein pocket we used the same parameters).

Supplementary Discussion

ZrLH₂ structural details

Fig. S2.

Structure of Zr₂LH₂ metal complex. OH-bridged dinuclear Zr complex with uncoordinated color-tuning 6'-OH groups. Atom color scheme: C = grey, H = white, O = red, N = blue, S = yellow, Cl = green, Zr = cyan

To solve the structure of ZrLH₂, we used a non-negative matrix factorization technique, related to the one described in(31) and implemented in [cheminfo ELN](<http://cheminfo.github.io>)(32), to deconvolve the mass spectrum. For this, we perform a peak picking (centroid) of the mass spectrum, generate all the theoretical isotopic distribution of all entities to look for, align the theoretical and experimental peaks and calculate a non-negative matrix factorization to obtain the weights for the different entities. We enumerated theoretical isotope distributions for:

- deprotonated firefly luciferin ligand (in the range 0 to 3)
- chloride (in the range 0 to 4)
- Zr(IV) (in the range 0 to 3)
- hydroxy (in the range 0 to 2)
- methyl ester of the luciferin ligand (in the range 0 to 1)

Fig. S3.

Mass spectrum of Zr-LH₂ reaction product

We considered the following ionizations: H⁺, Na⁺, NH₄⁺. The mass spectrum is dominated by the ammonium adduct of the ligand at m/z=298 and we also observe peaks of the methyl ester, such as the [M+H]⁺ at m/z=295. Moreover, many of the most relevant fragments from the reconstructed spectrum lead us to the working hypothesis of a dinuclear Zr complex:

- [Zr₂L(OH)₂Cl₃²⁺ + NH₄⁺] at m/z=204.9
- [Zr₂L(OH)₂Cl₂⁴⁺ + NH₄⁺] at m/z=112.5527
- [Zr₂L₂Cl₃³⁺ + Na⁺] at m/z=215.9169
- [Zr₂L₂(OH)₂Cl³⁺ + Na⁺] at m/z = 206.9338
- [Zr₂(LH)₂(OH)Cl₃⁴⁺ + H⁺] at m/z = 172.1407
- [Zr₂Cl₂(OH)L(LH)⁴⁺] at m/z = 205.9299
- [Zr₂(LH)LCl₄(OH)₂²⁺ + NH₄⁺] at m/z = 216.9043
- [Zr₂(LH₂)Cl₃(OH)⁴⁺ + NH₄⁺] at m/z = 214.9240
- [ZrL(OH)₂Cl + NH₄⁺] at m/z = 454.8948
- [ZrLCl(OH)⁺ + NH₄⁺] at m/z = 218.9458
- [ZrCl₂(OH)₂(LH)⁺ + NH₄⁺] at m/z = 236.9341

Cu₂L₃ crystal structure description

During the synthesis, the LH₂ precursor partially reduces some of the Cu^{II} to Cu^I, while simultaneously being oxidized to its planar aromatic 2-(6'-hydroxy-2'-benzothiazolyl)-thiazole-4-carboxylic acid (dehydroluciferin, L) form (Fig. 1a). The oxidation of LH₂ to form L removes the chirality of the ligand precursor. Cu₂L₃ thus crystallizes in the triclinic centrosymmetric space group $P\bar{1}$ with one metal complex present in the asymmetric unit. Each metal complex of Cu₂L₃ is composed of three L molecules acting as ligands linked by two Cu ions (fig. S4). Cu1 links L1 to L2 via coordination bonds to the L1 3N, L1 4-COO, L2 3S, and L2 3'N. Bridging of L2 3S, and L2 3'N by Cu2 fixes the C2-C2' bond, presumably contributing to the enhanced photoluminescence quantum yield of the Cu₂L₃ complex with respect to the free ligand. Cu2 links L2 to L3 via the 3N and 4-COO groups of both ligands. Both Cu1 and Cu2 display distorted tetrahedral coordination geometries, with Cu1 in the 1+ oxidation state and Cu2 in the 2+ oxidation state. Both Cu ions form coordinate bonds with the N atoms of all three thiazole rings, as well as with the O atoms of all the ligand terminal carboxylate groups. Cu1 additionally bridges the thiazole and benzothiazole fragments of the central ligand via the S and N atoms.

Fig. S4.

Asymmetric unit of the Cu₂L₃ metal complex. Metal coordination bond lengths are indicated. The structure consists of two metal centers, Cu1 and Cu2, linking three ligands, L1, L2, and L3. Cu1 is in the 1+ oxidation state, and Cu2, coordinated to the carboxylate O of L2 and L3, is in the 2+ oxidation state. Atom color scheme: C = grey, H = white, O = red, N = blue, S = yellow, Cu = orange

Despite the ligands of Cu_2L_3 being planar, the coordination geometries of Cu^{I} and Cu^{II} impose a zigzag shape on the complex, with outer ligands rotated 90 degrees with respect to the central ligand. Ligands L1 and L3 , positioned at the extremities of the metal complex (Fig. S4), bind to the Cu centers through thiazolate N (N1-Cu1 , N5-Cu2) and carboxylate O (O1-Cu1 , O7-Cu2) atoms, whereas the L2 molecule positioned in the center of the metal complex, apart from the thiazolate N (N4-Cu2) and the carboxylate O (O4-Cu2), also uses the thiazolate S (S3-Cu1) and the benzothiazolate N (N3-Cu1) atoms to coordinate to the Cu centers. L2 is furthermore partially disordered as in the crystal structure a part of it assumes two discrete positions of unequal occupancy. The occupancies of the 2 components of the disorder were refined to approximately 1/3 and 2/3. Hydrogen bonds $\text{O9-H9}\cdots\text{O8}$ (2.577(3) Å) and $\text{O3-H3}\cdots\text{O2}$ (2.608(3) Å) join neighboring metal complexes of Cu_2L_3 into chains extending along the b axis, while a hydrogen bond $\text{O6-H6}\cdots\text{O7}$ (2.864(5) Å) and a short contact $\text{S3}\cdots\text{N6}$ (3.1188(1) Å) cross-link these chains, thus forming a 3D supramolecular aggregate. Strong cohesion of this aggregate presumably contributes to its low solubility in water and common organic solvents.

To confirm the assignment of Cu^{I} and Cu^{II} oxidation states, the presence of Cu^{I} and Cu^{II} in the crystal structure was confirmed by X-ray photoelectron spectroscopy (XPS). XPS measurements confirmed the presence of Cu^{I} and Cu^{II} in a 1:1 ratio. Fitting of the $\text{Cu } 2\text{p}_{3/2}$ line (fig. S5 a) shows two components with similar integrals, at binding energies of 932.5 eV and 934.4 eV, which are assigned to Cu^{I} and Cu^{II} respectively. Since a binding energy of ~933 eV may typically correspond to either Cu^{I} or Cu^0 , assignment of the 932.5 eV peak to Cu^{I} rather than Cu^0 was further verified by Auger transitions (Fig. S5 b). Cu LMM transitions are similar (~916 eV) in Cu^{II} and Cu^{I} , but higher in energy (~918 eV) in Cu^0 . The presence of a single peak at 916 eV in the Cu LMM line of Cu_2L_3 confirms the absence of Cu^0 in the complex. XPS data thus confirm that Cu_2L_3 contains about 50% Cu^{II} and 50% Cu^{I} .

Fig. S5.

XPS and kinetic energy spectra **a** $\text{Cu } 2\text{p}$ XPS spectrum of as-synthesized Cu_2L_3 **b** The presence of a single peak at 916 eV in the kinetic energy spectrum of Cu LMM transition in Cu_2L_3 confirms the absence of Cu^0 in the material.

Fig. S6.
a SEM image, and **b** corresponding Cu EDX of as-synthesized Cu_2L_3 . Scale bar corresponds to 10 μm

Scanning electron microscopy (SEM) and Energy Dispersive X-ray (EDX) images ([Fig. S6a, b](#)) of Cu_2L_3 show the flat, rectangular shape of the copper-rich crystals.

Table S1.Crystal data and structure refinement for Cu₂L₃.

Identification code	Cu2L3
Empirical formula	C ₃₃ H ₁₆ Cu ₂ N ₆ O ₉ S ₆
Formula weight	959.96
Temperature/K	127.93
Crystal system	triclinic
Space group	$P\bar{1}$
$a/\text{\AA}$	10.6952(5)
$b/\text{\AA}$	13.6637(7)
$c/\text{\AA}$	15.4144(8)
$\alpha/^\circ$	114.8850(10)
$\beta/^\circ$	98.494(2)
$\gamma/^\circ$	100.936(2)
Volume/ $\text{\AA}^3$	1939.77(17)
Z	2
$\rho_{\text{calc}}/\text{g/cm}^3$	1.644
μ/mm^{-1}	1.480
$F(000)$	964.0
Crystal size/ mm^3	$0.225 \times 0.05 \times 0.04$
Radiation	MoK α ($\lambda = 0.71073$)
2θ range for data collection/ $^\circ$	4.358 to 52.898
Index ranges	$-13 \leq h \leq 13, -17 \leq k \leq 17, -19 \leq l \leq 19$
Reflections collected	57298
Independent reflections	7962 [$R_{\text{int}} = 0.0460, R_{\text{sigma}} = 0.0331$]
Data/restraints/parameters	7962/331/543
Goodness-of-fit on F^2	1.069
Final R indexes [$I \geq 2\sigma(I)$]	$R_1 = 0.0431, wR_2 = 0.0907$
Final R indexes [all data]	$R_1 = 0.0567, wR_2 = 0.0949$
Largest diff. peak/hole / $\text{e \AA}^{-3}$	0.76/−0.74

Optical properties of Cu_2L_3

Cu_2L_3 crystals are emissive, porous molecular crystals, which mimic bioluminescence color-tuning in response to changing chemical environment within the pores. Cu_2L_3 crystals exhibit stable, tunable, and broad-spectrum emission. The optical properties of Cu_2L_3 are ligand-based and therefore similar to those of the LH_2 precursor. The neutral complex emits at 430 nm, and deprotonation of the photoexcited 6'-OH groups by water molecules present in the pores of Cu_2L_3 crystals results in a redshifted emission peak centered at 550 nm (Fig. 2c).

Dispersions of Cu_2L_3 in a range of non-aqueous solvents, including ethanol, dimethylformamide (DMF), N-methyl-2-pyrrolidone (NMP), and benzene, exhibit only the 430 nm phenol signature peak in their emission spectra, (Fig. S7), indicating full protonation of the benzothiazole terminals. In pure water and aqueous sodium hydroxide (NaOH) and buffers of pH 9 and 4, only the 550 nm phenolate emission is present, indicating complete deprotonation of the benzothiazole terminals (Fig. S7). Notably, the effect of pH on the protonation state of the phenol terminals is negligible compared to that of H_2O , due to the low pK_a of these terminals in the excited state, as confirmed by dominance of the 6'-O⁻ characteristic peak at pH 4.

Fig. S7.

Photoluminescence emission spectra of Cu_2L_3 crystals dispersed in a range of non-aqueous (purple lines) and aqueous (blue lines) solvents

Stability of Cu_2L_3

PXRD spectra of Cu_2L_3 crystals after being soaked in aqueous solution overnight, then centrifuged and dried (Fig. 2f) confirm that the insoluble complex is stable and retains its crystallinity after being dispersed in water-rich solvent mixtures.

Fourier Transform Infra-red (FTIR) spectra before and after soaked (fig. S8) further confirm the stability of the complex. The more pronounced phenol–OH band around 3200 cm^{-1} in the FTIR of the soaked material suggests that water molecules in the solution approach the –OH groups of the complex. The fingerprint region of the spectra is the same before and after soaking, which confirms that the material remains stable.

Fig. S8.

FTIR spectra of Cu_2L_3 crystals before (blue) and after (yellow) being dispersed in aqueous solution and soaked overnight.

Density Functional Theory calculations

Absorption spectra calculations were done with Kohn-Sham DFT(18) and linear response Time-Dependent DFT (TDDFT)(46) using the PBE0(47) functional and 6-311g(d) basis set(35, 45) including implicit solvent (ethanol) by means of the CPCM method as implemented in Gaussian 09 code(52), for the isolated ligand and for the **Cu₂L₃** complex being all 6'-OH groups protonated. Analysis of the active excited states allowed determining that S₁ is the low-lying absorbing state of the ligand corresponding to the HOMO-LUMO excitation. The computed peak of **Cu₂L₃** complex is slightly red-shifted with respect to the isolated ligand in agreement with the experiments (fig. S9) and corresponds to the sum of the local excitations of the three L ligands (fig. S10). Low-lying charge transfer states involving transitions from the d-orbitals of Cu to the ligands are found with weak intensities at lower energies.

Fig. S9.

a Computed absorption (thin lines) and emission (dashed lines) spectra of L ligand (orange) and **Cu₂L₃** complex (blue). Protonated (grey) and deprotonated (green) emission states (O⁻) of **Cu₂L₃** have been considered. A width of 0.3 eV was used in the spectra, **b** Diffuse reflectance spectra of L and **Cu₂L₃** show a comparable redshift in the peak absorption of the ligand L in the free and complexed form.

Fig. S10.

Molecular orbitals associated with the electronic transition corresponding to the absorption peak of **L** computed at 340 nm, and with the three main excitations contributing to the absorption band of **Cu₂L₃** complex computed at 390, 380 and 370 nm.

Optimization of S₁ excited state of **L** ligand (in its deprotonated form) was done to obtain the simulated emission spectrum. Evaluation of the emission spectrum of **Cu₂L₃** complex was performed by means of substituting the three **L** ligands in the complex with its S₁ optimized geometry. Both the protonated and deprotonated state of the OH groups of **L** were considered.

The same methodology was applied to evaluate the emission properties of the Se-substituted Cu_2L_3 complex, and with an extended ligand that substitute the benzene ring by naphthalene (fig. S11). Comparison of the computed and experimental emission spectra of Cu_2L_3 complex indicates that the emission energies are overestimated by 0.31 eV in the calculations. Therefore, a shift of 0.31 eV is applied to the computed emission spectra in order to predict the emission properties of the modified compounds in terms of CIE coordinates.

Fig. S11.

Structure and emission of predicted Cu_2L_3 -like complexes. Computed geometry and emission spectra (blue lines) of Cu_2L_3 complex with Se-substituted L ligands (left) and naphthalene L (right). The computed emission spectra of Cu_2L_3 is also given for comparison (grey dotted lines). Both protonated and deprotonated forms of 6'-OH are considered. A width of 0.3 eV was used in the spectra representation.

Adsorption behavior of water in Cu₂L₃ synthetic binding pockets

To obtain a qualitative molecular understanding of the behavior of water and ethanol inside the pores, we carried out molecular simulations. To determine the selective adsorption, we carried out grand-canonical Monte Carlo simulations and NVT Monte Carlo simulations.

Simulation results: Fig. 3 a-f and fig. S12 show that ethanol has a pronounced effect on the water molecules next to the 6'-O. If we increase the ethanol concentration, the probability of a water molecule next to the 6'-O site decreases. Fig. S13 shows that while the probability of having a water molecule next to adsorption site 1 decreases with the increasing ethanol composition in the mixture, exact opposite happens for the adsorption site 2, which clearly supports the hypothesis that water molecules are pushed away from the adsorption site 1 in the presence of ethanol.

Fig. S12

Probability distribution of the oxygen atoms of water and ethanol molecules over the enumerated adsorption sites highlighted in fig. S13. Percentages in the legend represent water compositions. Blue and pink colors represent water and ethanol, respectively.

Fig. S13

Radial distribution plot of oxygen atoms in the water molecules from the oxygen positions of -OH bonds that are expected to be deprotonated by water.

Molecular model for luciferin@luciferase complexes

Luciferase/luciferin pairs are extensively investigated for use in bioimaging and analysis applications(58). Near- and far infra-red emitting systems in particular are of interest for *in vivo* bioimaging of hemoglobin-rich or high-density tissue such as bones. These applications drive the search for an understanding of bioluminescence color tuning mechanisms. Such an understanding may help guide the design of luciferase/luciferin variants that support high quantum yield bioluminescence well into the red.

Investigations of luciferase variants have unveiled a range of factors that may contribute to determining the bioluminescence emission color. The most commonly proposed factors include tautomerization of keto (red-emitting) and enol (green-emitting) forms of the thiazole fragment in luciferin(3), non-specific solvent interaction effects(9), interactions between residues of the luciferase enzyme, and the confirmation of the active site(8). While the community has yet to converge on a hypothesis that explains precisely how the emitted color is determined, recent studies have provided substantial mechanistic clues. For example, it was found that both green and red bioluminescence can be observed when the keto-like 5,5'-dimethyl-oxyluciferin (dimethoxyluciferin), which cannot undergo tautomerism, is used as a substrate. Subsequent experimental and theoretical studies have served to dispute the impact of tautomerization within the luciferase binding site³⁶. Residue interactions, conformation of the active site, and solvent interactions with the 6'-OH group can then be considered to play significant roles in determining whether the emission is green or red.

In-depth studies into the effects of site-directed mutations on a range of luciferase variants have shed light on the complex interplay between different factors present in bioluminescent systems. For example, even substitutions of randomly-distributed residues, distant from the luciferase binding site, have resulted in changes of the bioluminescence emission color, possibly due to effect of inter-residue interactions on the conformation and/or chemical environment of the binding site. Studies have shown that changes in the binding site conformation, whereby it takes on a more 'open' configuration, result in redshifting of the luminescence. This redshift has been attributed to the open-form microenvironment allowing excited oxyluciferin to rotate about its C2-C2' bond, thus lowering the energy of its radiative deexcitation. Several examples reported in the literature demonstrate that enlarging of the binding site or its surrounding cavity also result in redshifted emissions. Besides geometric relaxation, an alternative hypothesis to explain this tendency points to the effect of solvent interactions. Recent comprehensive structural studies of pH-sensitive and pH-insensitive luciferases suggest that increased accessibility of solvent molecules to the luciferase binding site result in red emissions. Interestingly, these studies also suggest that the 6'-OH group of oxyluciferin lies close to the luciferase binding site region that is responsible for color determination. Within this region of the binding site, potential non-specific solvent effects that can result in redshifted emissions include changes in pH, the concentration of metal ions, or the concentration water molecules.

Fig. S14.
Crystal structure of the crystalized oxyluciferin illustrates the comparable intraligand interactions of the external ligands in Cu_2L_3

As seen in Fig. S14, the geometry-optimized structure of Cu₂L₃ (red) agrees almost perfectly (RMS < 0.067 Å) with the crystal structure of Oxy-LH₂ reported by Naumov et al.³² (blue). In the protein scaffold, the thiazole half of the benzothiazole ring can π - π interact with the phenyl ring from Phe249, which is at a distance of 4.16 Å. In our compound, we find a stacking distance of approximately 3.7 Å, and similar to Naumov et al. we observe that the dimers are offset to each other. We also find a similar packing fraction of 0.12 as in the crystal of enol-Oxy-L₂(0.11).

Therefore, by proposing Cu₂L₃ and similar porous crystals as molecular models for bioluminescence color tuning effects, we are able to disentangle and separately investigate the different contributing factors that are currently under debate, and thus potentially contribute to the design of application-specific luciferases. In Cu₂L₃, the thiazole rings are fixed in a planar aromatic (“enol-like”) structure, and the COOH terminals, which take part in coordination, are unavailable to interact with the pore environment. Rigidity of the packed structure prevents rotation about the C2–C2' bond, eliminating any effects of geometry relaxation on the radiative deexcitation energy. Access of the 6'-OH groups of all three ligands in the asymmetric unit of Cu₂L₃ allows us to monitor the impact of the pore environment on the emission wavelength, in the absence of any other structural or chemical modifications.

Key structural similarities between the ligands in Cu₂L₃, crystallized oxyluciferin, and AMP-complexed oxyluciferin in the *Luciola cruciata* binding site affirm the use of Cu₂L₃ as a molecular model. Binding of the thiazole N3 to Cu in all three ligands of Cu₂L₃ is comparable to N3–O binding to a 6'-OH in crystallized oxyluciferin, and to a proximate water molecule in AMP-complexed luciferin of the *L. cruciata* species. N3–Cu bond lengths in Cu₂L₃ range from 1.927 Å to 1.983 Å, while in both, crystallized oxyluciferin and LH₂@*L. cruciata* the N–O bonds range from 2.782 Å to 2.878 Å. To quantify the similarity between our compound and the luciferin molecule in the protein, we performed similarity analyses between the structure of our compound and the OxyLH₂ molecule embedded in the protein scaffold (PDB 2D1T). The geometry of our PBE optimized structure of Cu₂L₃ (red) agrees perfectly (RMS < 0.067 Å) with the crystal structure of OxyLH₂ reported by Naumov *et al* (blue). In the protein scaffold, the thiazole half of the benzothiazole ring can π - π interact with the phenyl ring from Phe249, which is at a distance of 4.16 Å. In our compound, we find a stacking distance of approximately 3.7 Å, and similar to Naumov et al. we observe that the dimers are offset to each other. We also find a similar packing fraction of 0.12 as in the crystal of enol-Oxy-OH₂ (0.11).

To visualize the interface of Cu₂L₃ with the environment, we created energy surfaces using the iRASPA program (using the Ow parameters of the TIP5P-Ew model as a probe molecule for water at 298 K and the default force field of iRASPA, for the calculation of the adsorption surface (blue) of the molecule in the protein pocket we used the same parameters). It is clearly observable that, similar to the structure embedded in the protein, the phenolic OH can from all hydrogen bonding active functional groups most strongly interact with water.

We also find similar water coordination environments in our GCMC simulations. The maximum of the distribution of distances of the oxygen of the water molecule to oxygen the phenolic OH group is 3.3 Å in our simulations, which is close to the distances of 2.73 Å and 3.3 Å that are found in the protein.

Movie 1.

Real-time change of luminescence color from Cu₂L₃ crystals suspended in ethanol, upon addition of water.