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Controlling allostery using redox chemistry†

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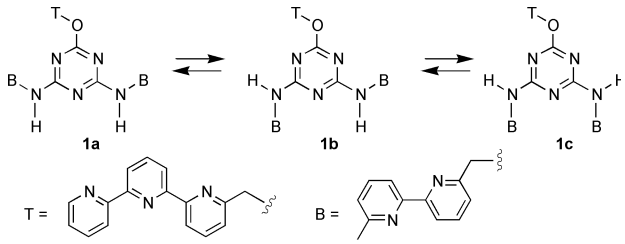
The binding of a hydrogen-bonding receptor to its substrate is reversibly regulated by varying the oxidation state of a copper allosteric cofactor.

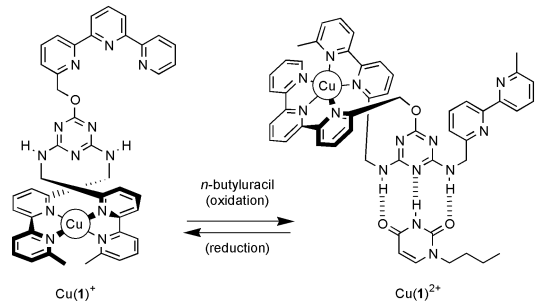
Negative allostery is the outcome when a cofactor binds to a receptor at a location removed from the active site to induce a conformational change in the receptor that reduces its ability to complement its substrate.[1,2] We have recently reported how the Cu^{I} ion acts as a negative allosteric cofactor and significantly diminishes the binding of uracil derivatives by a receptor based on the triaminotriazine scaffold.[2] This is feasible because the coordination of the metal ion by two bipyridine ligands is accompanied by the distortion of the receptor's hydrogen-bonding surface. Here we report the first example of a receptor that exhibits controllable allostery due to the delicate balance of the receptor's electronic complementarity to the oxidation state of copper.

Diaminotriazine receptor **1** provides the donor–acceptor–donor hydrogen bond site needed to target imide guests. The receptor also contains one tridentate terpyridine and two bidentate bipyridine ligands. In the absence of metal ions, three conformations of **1** coexist as a result of the limited rotation of the triazine's exocylic C–N bonds (**1a**, **1b** and **1c**).[3] Only **1a**



possesses a hydrogen bond site where both N–H donors are projecting in a fashion suitable to bind imide guests. Rotation around one or both C–N bonds generates the non-binding rotomers **1b** and **1c**.[3] The complexation of the bipyridine ligands to a Cu^{I} ion produces a 4-coordinate complex which locks the system into a single non-effective conformation ($Cu(\mathbf{1})^{+}$ in Scheme 1). The Cu^{II} ion, on the other hand, prefers to reside in a 5- or 6-coordinate fashion, the former of which is provided by the terpyridine ligand and one of the bipyridine ligands. In order to achieve this mode of coordination, one of the C–N bonds is locked into a single orientation while the other remains free to rotate. The result is the regeneration of the productive hydrogen-bonding surface ($Cu(\mathbf{1})^{2+}$ in Scheme 1).

Receptor **1** was prepared in two steps (43% overall yield) from cyanuric chloride, 6-aminomethyl-6′-methyl-2,2′-bipyridine[4] and 2-hydroxymethyl-2,2′-6′;2″-terpyridine.[5]† Copper complexes were prepared by treating solutions (CH_3CN/$CHCl_3$) of **1** with $Cu(CH_3CN)_4PF_6$ for $Cu(\mathbf{1})^{+}$ and $Cu(OTf)_2$ for



Scheme 1

$Cu(\mathbf{1})^{2+}$ followed by the addition of ether to precipitate the coordination compounds in greater than 90% yield.

The structural heterogeneity of the two copper complexes was immediately apparent when their ^1H NMR spectra in CD_3CN were compared. The signals corresponding to the protons on the 2,2′-bipyridine arms and the N–H protons appear as a single set of peaks confirming the *C*2 symmetry of $Cu(\mathbf{1})^{+}$. The symmetrical nature of the Cu^{I} complex and the tetrahedral geometry around the metal are supported by the X-ray analysis of single crystals of $Cu(\mathbf{1})^{+}$ (Fig. 1).§ This structure highlights that both exocyclic C–N bonds have rotated almost 180° from their original positions in order to form the coordination compound. The structural changes that accompany the coordination event perturb the hydrogen bond sites and only two low-affinity donor–acceptor recognition sites[2,3] remain in $Cu(\mathbf{1})^{+}$.

In the case of $Cu(\mathbf{1})^{2+}$, the signals corresponding to the N–H protons and those of the methyl groups on the bipyridines appear as two independent sets of peaks in the ^1H NMR spectrum.¶ This doubling of signals is certainly an outcome of the dissymmetric nature of the Cu^{II} complex, where the two bipyridine ligands cannot simultaneously coordinate to the metal cation. Instead, only one bipyridine ligand partners with the terpyridine ligand to provide the 5-coordinate binding site better suited to the oxidized metal.

The binding abilities of receptor **1** and its corresponding metal complexes for imide substrates were studied using ^1H NMR spectroscopy. The downfield shifting of the signals corresponding to the N-H protons in **1** and $Cu(\mathbf{1})^{2+}$ when $CDCl_3/CD_3CN$ solutions (1 : 1) of the receptors were titrated with *n*-butyluracil indicate effective hydrogen bonding. These

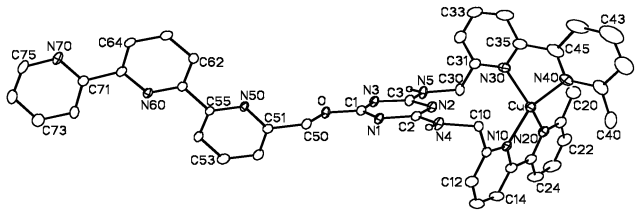


Fig. 1 Molecular structure of $Cu(\mathbf{1})^{+}$ in the crystal. The hydrogen atoms attached to N4 and N5 are shown with arbitrarily small thermal parameters; all other hydrogen atoms and the PF_6^- counterion have been omitted. The thermal ellipsoids are drawn at the 20% probability level.

† Electronic supplementary information (ESI) available: Experimental and X-ray structure determination and crystal data for $[Cu(\mathbf{1})][PF_6]$. See http://www.rsc.org/suppdata/cc/b1/b109051h/

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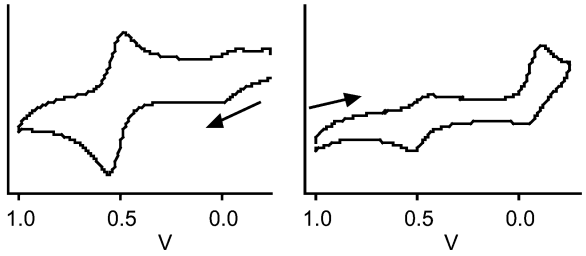

Fig. 2 Cyclic voltammograms of CH_3CN solutions of Cu(**1**)$^+$ (left) and Cu(**1**)$^{2+}$ (right).

shifts were monitored and, in both cases, the data correlate well with calculated curves using 1:1 binding models‖ and give association constant (K_a) values of 65 ± 3 M^{-1} for **1** and 64 ± 5 M^{-1} for Cu(**1**)$^{2+}$. When the titration experiments were performed using Cu(**1**)$^+$, there were only minor observable changes in the ^{1}H NMR spectra, none of which fit to a binding model implying that the association was too weak to be characterized. These results clearly support the claim that the metal ion acts as a negative allosteric cofactor only in the Cu^I oxidation state.

The changes in coordination as the metal ion was varied between its two oxidation states were monitored using UV–VIS absorption spectroscopy. When a CH_3CN solution of Cu(**1**)$^+$ was treated with bromine (4 equivalents), the solution immediately changed from the red–brown color (λ_{max} = 450 nm, ε = 5700) characteristic of the 4-coordinated complex to yellow–green (λ_{max} = 690 nm, ε = 102) indicating that chemical oxidation of the metal ($Cu^I \rightarrow Cu^{II}$) was accompanied by transformation to the 5-coordinate complex Cu(**1**)$^{2+}$.[6] The reverse reaction ($Cu^{II} \rightarrow Cu^I$) was also tracked by the color change (yellow–green to red–brown) as hydrazine (4 equivalents) was added to a solution of Cu(**1**)$^{2+}$.

The cyclic voltammogram†† of Cu(**1**)$^+$ exhibits the characteristic redox couple for a 4-coordinate complex ($Cu^{II/I}N_4$) at +0.52 V (Fig. 2).‡‡ The subsequent reduction wave has a slight decrease in intensity with the concomitant appearance of an additional peak at −0.08 V corresponding to the reduction of 5-coordinate $Cu^{II}N_5$ to Cu^IN_5. This indicates that, within this time-scale, a small amount of the 4-coordinate Cu^{II} complex ($Cu^{II}N_4$) rearranges to the 5-coordinate complex ($Cu^{II}N_5$) after the initial oxidation of Cu(**1**)$^+$. On the other hand, the voltammogram of Cu(**1**)$^{2+}$ displays a significant decrease in the oxidation peak of Cu^IN_5 with the appearance of the oxidation peak of Cu^IN_4. This can be explained by the rapid and almost quantitative rearrangement of Cu^IN_5 to Cu^IN_4 immediately after it is generated.[6]

The reversible interconversion between Cu(**1**)$^+$ and Cu(**1**)$^{2+}$ is also feasible using electrochemical means. Oxidation of 10^{-4} M solutions of Cu(**1**)$^+$ at a constant potential of +800 mV led to a change in the color of the solutions from red–brown to yellow–green as a result of the reduction in intensity of the absorption at 450 nm and the appearance of a new absorption at 690 nm. After 9 min of oxidation, the UV–VIS absorption spectrum was identical to that of preformed Cu(**1**)$^{2+}$. The original spectrum and the color of Cu(**1**)$^+$ were restored after the solutions were reduced for 24 min at a potential of −200 mV. Several cycles of the redox processes (at +800 mV and −200 mV) starting with either Cu(**1**)$^+$ or Cu(**1**)$^{2+}$ can be achieved with quantitative conversion (Fig. 3).

^{1}H NMR spectroscopy was used to evaluate how effectively the allosteric process can be regulated. The addition of **1** (2 equivalents) to a solution of *n*-butyluracil (2.0 mM in 1:1 $CDCl_3/CD_3CN$) resulted in the expected downfield shift of the signal corresponding to uracil's N–H protons. When 1 equivalent of $Cu(CH_3CN)_4PF_6$ was added directly to the NMR tube, the peaks corresponding to receptor **1** were replaced by those corresponding to Cu(**1**)$^+$ and the signal for the N–H proton of *n*-butyluracil returned to its original position. A similar spectrum was obtained when preformed Cu(**1**)$^+$ was used. When

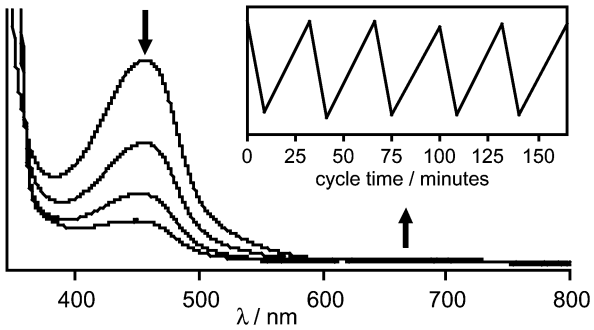

Fig. 3 Changes in the UV–VIS absorption spectra of a CH_3CN solution of Cu(**1**)$^+$ (1×10^{-4} M) upon electrochemical oxidation at +800 mV. Electrolysis periods are 0, 3, 6, and 9 min. The inset shows the modulated absorption spectra (λ = 450 nm) during alternating electrolysis at +800 mV for 9 min periods and −200 mV for 24 min periods.

$Cu(CH_3CN)_4PF_6$ was replaced with $Cu(OTf)_2$ in the above experiments, the signal corresponding to the N–H protons of *n*-butyluracil once again shifted downfield. A similar spectrum was obtained when preformed Cu(**1**)$^{2+}$ was added to a solution of the substrate.

When bromine (4 equivalents) was added to the above solutions of Cu(**1**)$^+$ and *n*-butyluracil, the signals for the Cu^I complex were replaced by those for Cu(**1**)$^{2+}$. The peak corresponding to the N–H proton of *n*-butyluracil also shifted downfield in a similar manner as was observed for the previously formed Cu(**1**)$^{2+}$·U complex. Alternatively, the hydrazine-induced (4 equivalents) reduction of Cu(**1**)$^{2+}$ to Cu(**1**)$^+$ shifted the signal of uracil's N–H proton upfield to the position already recorded for Cu(**1**)$^+$·U. Thus, the allostery can be switched off and on using bromine ($Cu^I \rightarrow Cu^{II}$) and hydrazine ($Cu^{II} \rightarrow Cu^I$).

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Notes and references

§ *Crystal data*: $C_{60}H_{44}CuF_6N_{12}OP$, M = 1037.48, triclinic, space group $P\bar{1}$, a = 9.9312(12), b = 15.733(2), c = 17.743(2) Å, α = 72.366(2), β = 80.042(3), γ = 88.735°, U = 2600.8 Å^3, T = 193 K, Z = 2, D_c = 1.325 g cm^{-3}, μ(Mo-Kα) = 0.521 mm^{-1}, 13 106 (10 515 independent) reflections, R = 0.0979 and Rw = 0.2428 for 4973 reflections with $I > 2\sigma(I)$. CCDC reference number 174651. See http://www.rsc.org/suppdata/cc/b1/b109051h/ for crysallographic data in CIF or other electronic format.

¶ Despite the fact that the signals in the ^{1}H NMR spectrum of Cu(**1**)$^{2+}$ are uniformly broad due to the paramagnetic effect of the Cu^{II} ion, structural information was readily obtained.

‖ The ^{1}H NMR titration data were analyzed using Christopher A. Hunter's 1:1 complexation program (Department of Chemistry, University of Sheffield, UK). All titrations were performed in triplicate.

†† Cyclic voltametry was performed on CH_3CN solutions using platinum working and counter electrodes, a Ag/AgCl reference electrode and Bu_4NPF_6 as the electrolyte. Electrolysis was performed by replacing the platinum electrode with platinum coil.

‡‡ The 4-coordinate complexes are labeled with the notation 'N_4', while the 5-coordinate complexes are labeled with the notation 'N_5'.

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