Molecular Devices

A Sleeping Host Awoken by Its Guest: Recognition and Sensing of Imidazole-Containing Molecules Based on Double Cu^{2+} Translocation inside a Polyaza Macrocycle**

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Recently, a lot of attention has been focused on artificial molecular machines,[1] in part for their potential to help understand and mimic natural biological systems but mainly from the perspective of developing molecular devices capable of information processing and data storage.[2] However, a different use of this kind of molecule can be envisaged, which relies on one of their typical properties: when a molecular machine moves as a consequence of an external stimulus, an overall rearrangement of the molecular system takes place and its shape changes dramatically. According to the well-established lock-and-key concept, the shape of a molecule, and in particular the shape of a cavity inside a large molecule, is connected to its ability to act as a selective receptor, that is, its ability to recognize a complementary substrate. Provided that only one of the possible shapes assumed by a given molecular machine presents a cavity in which a chosen substrate can selectively fit, molecular machines could advantageously enter the field of molecular recognition by acting as receptors with a useful implemented function: to recognize and bind a given substrate only when the proper external stimulus is applied. Few examples have been published that consist of relatively simple photoisomerizable molecules, in which the stimulus required to make the system change towards the correct shape and recognize a substrate is provided by light.[3]

Herein we report the first example of a new kind of molecular machine capable of molecular recognition. The stimulus required by the system for changing towards the correct shape for selective binding can be provided by the substrate itself. Accordingly, the molecular machine described in this paper behaves as a receptor, which merges the advantages of the lock-and-key principle (selectivity) with those of its adaptive behavior (activation in the presence of substrates). Moreover, the drastic color change associated with movement and recognition events turns this system in a very efficient colorimetric sensor.

Macrocycle LH_4[4] contains two couples of polydentate compartments capable of binding Cu^{2+} ions, and comprises two diamide–diamine (DADA) tetradentate and two pyridine–diamine (PDA) tridentate binding sets, which share the four secondary amino groups.

Potentiometric titrations carried out with standard base, first on a solution containing LH_4 and excess acid, then on a solution containing LH_4, two equivalnents of Cu^{2+} ions, and excess acid[5] followed by nonlinear least-squares treatment of the obtained electrode potential versus added base data,[6] allowed us to calculate the protonation constants of LH_4 and the formation constants of the copper-containing species present in solution in the pH range of 2–12 (see caption of Figure 1).[7] From these data, a distribution diagram for the $LH_4/2\,Cu^{2+}$ system was drawn (Figure 1a). Subsequently, coupled pH-metric and spectrophotometric titrations were carried out. Two distinct colors and types of band are observed as a function of pH. A band centered at around 660 nm, responsible for the blue color of the solutions, starts to form at pH 3 and predominates without dramatic changes up to pH 9.5. At this pH, the intensity of the band at 660 nm decreases relative to the band centered at 515 nm then disappears leaving a purple–pink solution. The interpretation is straightforward: if the molar absorbance at 660 and 515 nm versus pH profiles are superimposed onto the distribution diagram (Figure 1a, filled and empty triangles, respectively): the relatively short wavelength band at 515 nm correlates with the $[Cu_2(L)]$ neutral complex (form **a** in Scheme 1)[8] and

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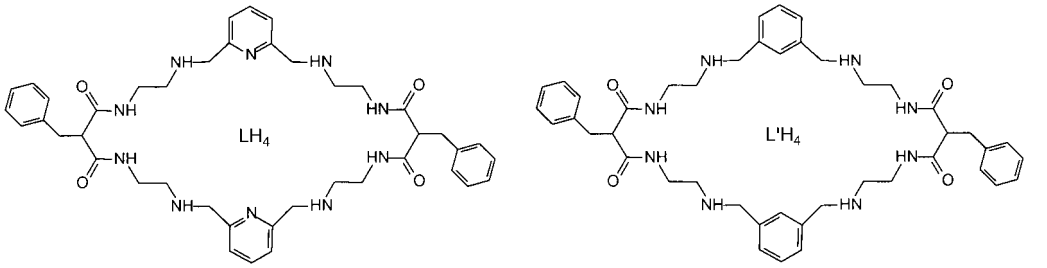


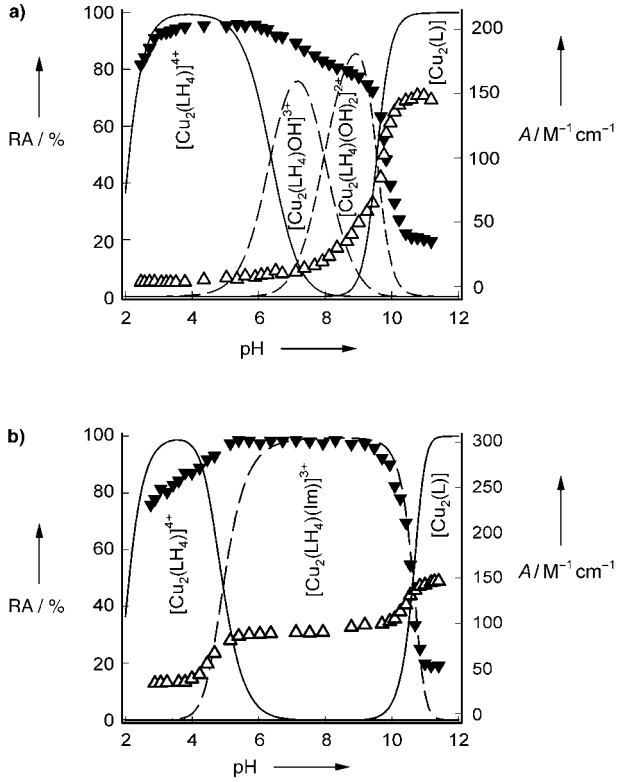
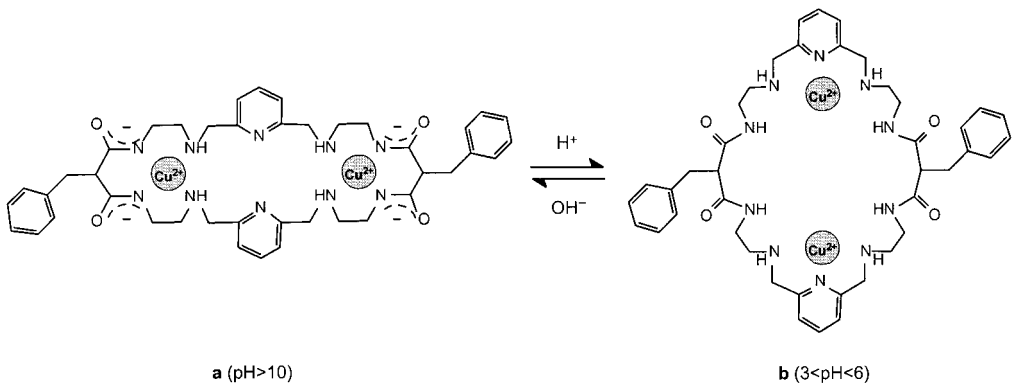


Figure 1. a) Distribution diagram (relative abundance % (RA), of species vs pH) for a system that contains $Cu(CF_3SO_3)_2$ at 10^{-3} M and LH_4 at 5×10^{-4} M. The calculated formation constants (expressed as logβ) for the species indicated (with uncertainties in parenthesis) are: $LH_5^{+}=7.66(0.01)$, $LH_6^{2+}=14.80(0.01)$, $LH_7^{3+}=21.55(0.01)$, $LH_8^{4+}=27.73(0.01)$, $[Cu_2(LH_4)]^{4+}=25.31(0.02)$, $[Cu_2(LH_4)(OH)]^{3+}=18.95(0.02)$, $[Cu_2(LH_4)(OH)_2]^{2+}=10.97(0.02)$, $[Cu_2(L)]=-8.17(0.02)$. The full and empty triangles report the molar absorptivity of the complex at 660 and 515 nm vs pH, respectively, relative to $[Cu_2(LH_4)]^{4+}$ and to $[Cu_2(L)]$; b) Distribution diagram (RA of species vs pH) for a system that contains $Cu(CF_3SO_3)_2$ at 10^{-3} M, and LH_4 and IMH at 5×10^{-4} M. The full and empty triangles report the molar absorptivity of the complex at 650 and 515 vs pH profiles, respectively, relative to $[Cu_2(LH_4)(Im)]^{3+}$ and to $[Cu_2(L)]$. The calculated formation constants are the same as in (a), to which these equilibria and constants have been added: $LH_4 + 2\,Cu^{2+} + ImH = [Cu_2(LH_4)(Im)]^{3+} + H^+$, $\log K = 38.05(0.02)$; $Im^- + H^+ = ImH$, $\log K = 10.82(0.02)$; $Im^- + 2\,H^+ = ImH_2^+$, $\log K = 19.2(0.02)$.

arises from the typical d–d transition for a tetra coordinated Cu^{2+} ion with square-planar geometry inside a bis-deprotonated DADA donor set.[9]

On changing pH to lower values (3–6), the deprotonated amide groups are reprotonated, thus becoming noncoordinating, and the two Cu^{2+} ions move to the two available separated tridentate PDA units. This form is the complex $[Cu_2(LH_4)]^{4+}$, form **b** in Scheme 1, in which each Cu^{2+} is tricoordinated by the ligand ($\lambda_{max}=660$, deep blue), with the other coordination positions of the Cu^{2+} ion occupied by water molecules.[10] On raising the pH (6–9.5), two water molecules are deprotonated to give the blue species $[Cu_2(LH_4)(OH)]^{3+}$ and $[Cu_2(LH_4)(OH)_2]^{2+}$. According to these data, when a 1:2 molar ratio of LH_4/Cu^{2+} is chosen, this system is capable of a pH-driven double cation translocation; in a solution with $4<pH<5$ the two Cu^{2+} ions are coordinated by the two PDA moieties to give $[Cu_2(LH_4)]^{4+}$ (form **b** in Scheme 1, 95% of the relative abundance, RA, in the distribution diagram), but if the pH is raised above 10.5 the four amide protons are released and each Cu^{2+} moves inside one of the two deprotonated DADA moieties to give the neutral complex $[Cu_2(L)]$ (form **a** in Scheme 1, 99% in the distribution diagram). The movement is reversible and several translocation cycles have been carried out in various experiments, without significant variations (except those due to dilution) in the spectra of the **a** and **b** forms. To check the correctness of the interpretation of spectral and potentiometric data, we carried out the same measurement set on the reference compound $L'H_4$, which lacks the pyridine nitrogen atoms and should not give rise to the same behavior. Infact, no blue was seen in the whole pH range, and only a very weak shoulder in the spectra between 600–700 nm was observed at low pH, which completely disappeared before pH 7. On the other hand, pink, pertinent to the band centered at 510 nm, started to appear at a pH of around 5 and the intensity of this band reached a plateaux at pH 7 (see the Supporting Information), which did not change with an increase in pH, thus showing that: 1) no blue dicopper complex is formed; 2) a pink complex $[Cu_2(L')]$ is completely formed (>95%) at pH 7, which is more than two pH units below the pH at which the analogous complex with LH_4 exists. These results clearly show that the nitrogen atom of the pyridine moiety is essential to the formation of the blue complex, giving rise to two binding cavities in which the two copper ions can be positioned in a wide pH range. These cavities have such a good affinity for Cu^{2+} ions that they prevent them from entering the DADA ligand sets before pH 9.

$[Cu_2(L)]$ is a neutral, quite-rigid molecule in which each Cu^{2+} centre is four-coordinated and square-planar. Moreover, it is well-established that inside a similar donor set the Cu^{2+} ion is coordinatively saturated, that is, it does not interact with any ligand added to the solution:[11] $[Cu_2(L)]$ can be considered the "closed" form of the system. On the other hand, in the $[Cu_2(LH_4)]^{4+}$ form, each Cu^{2+} is only three-coordinated by the ligand and the water molecules that complete the coordination sphere can be easily substituted by stronger ligands. Thus, the system is in its "open" form and resembles a whole category of dicopper complexes inside ditopic macro-



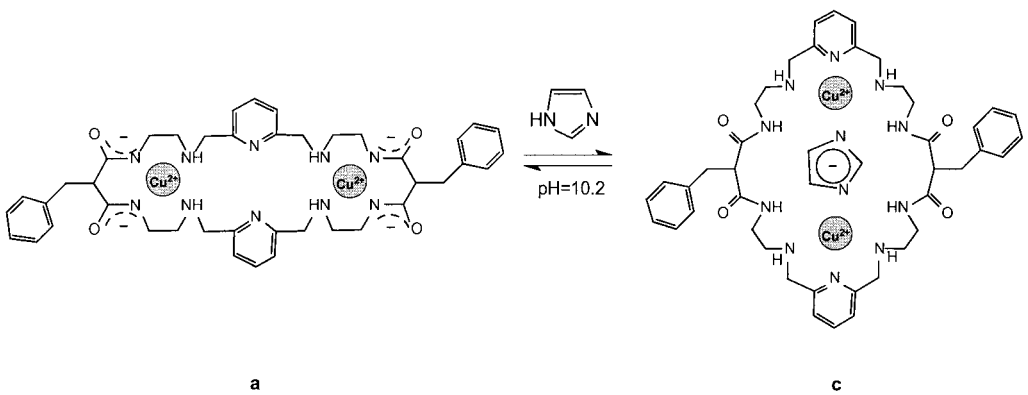
Scheme 1. pH dependent intramolecular dislocation of the Cu^{2+} ions.

cyclic ligands,[12] which are able to bind in cascade several bidentate ligands in a bridging fashion. Consequently, we investigated the behavior of the system in the presence of typical copper-coordinating bridging substrates.

A solution of the complex, buffered at pH 7 with HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (0.05 M) was titrated with several anions such as dicarboxylates (oxalate and malonate), phosphates (phosphate and pyrophosphate), azide and imidazolate. At the chosen pH the $[Cu_2(LH_4)(OH)]^{3+}$ species is predominant (>70%). In all cases, (except azide) the formation of 1:1 adduct was observed. Following our interests on imidazole (ImH) and histidine detection,[13] we decided to further investigate the behavior of the complex in the presence of imidazole.

Potentiometric titrations were carried out with standard base on solutions containing LH_4, Cu^{2+}, and ImH in a 1:2:1 molar ratio with excess acid. The formation constants of the species in solution for this system have been calculated and the relative distribution diagram drawn (Figure 1b, see caption for additional equilibrium and the relative constants).[14] In the range of $3 < pH < 4.5$ $[Cu_2(LH_4)]^{4+}$ is predominant, as it is in the absence of ImH, whereas an increase in pH above 4.5 result in the gradual formation of $[Cu_2(LH_4)(Im)]^{3+}$ (form **c** in Scheme 2), which predominates in the pH range of 6.0 to 10.0. This latter species contains a bridging imidazolate anion (Im^-), which forms thanks to the particularly stable[15,13c] $\{Cu^{2+}\text{-}(Im^-)\text{-}Cu^{2+}\}$ disposition. Coupled pH–spectrophotometric titrations evidence the formation of the blue complex $[Cu_2(LH_4)]^{4+}$ ($\lambda_{max} = 660$ nm, $A = 204\,M^{-1}cm^{-1}$) in the expected range of $3 < pH < 4.5$, while a rise in the pH up to 6 induces the shift of the maximum to 646 nm and an increase in the intensity of this band ($A = 302\,M^{-1}cm^{-1}$), which displays λ and A values consistent with a $\{Cu^{2+}\text{-}(Im^-)\text{-}Cu^{2+}\}$ moiety.[16] This band persists in the pH range in which $[Cu_2(LH_4)(Im)]^{3+}$ is prevalent (Figure 1b, full triangles). On further increasing the pH over 10.0, the blue disappears, leaving the pink of the $[Cu_2(L)]$ species, which displays a band with the same λ and ε values found when no ImH is present. It has to be stressed that in the presence of imidazole no OH-containing species are found in the 2–12 pH range and that $[Cu_2(LH_4)(Im)]^{3+}$ is so stable that it exists in a very large pH range (<90% in the 5.8–10.2 pH range), thus pushing the formation of $[Cu_2(L)]$ to a pH value one unit higher than in the absence of imidazole. Double cationic translocation also occurs in the presence of imidazole, as the correct and reversible spectral changes are observed on changing pH from, for example, 11.0 to 7.0 and back again. Moreover, it has to be pointed out that at pH 11.0 ImH is free in solution and does not interact with $[Cu_2(L)]$ (form **a**, closed), while at pH 7.0 the two Cu^{2+} ions translocate, the system is in it open form and it is able to bind bridging anions such as Im^-, thus representing an example of controllable binding action obtained inside a molecular machine after a chemical (ΔpH) stimulus.

A comparison of the distribution diagrams in the absence and presence of imidazole (Figure 1a and b) shows that in a sharp pH range ($10.0 < pH < 10.4$) in the absence of imidazole the $[Cu_2(L)]$ species is at 95%, whereas in the presence of imidazole, it is at 10% and the $[Cu_2(LH_4)(Im)]^{3+}$ species is around 90%. Thus, we can expect that when a pink solution buffered at pH 10.2 is treated with one equivalent of imidazole, translocation takes place with imidazolate binding, according to the equilibrium described in Scheme 2. Although it is somewhat slow, this process is indeed observed, as can be seen by the series of spectra obtained from a solution of $[Cu_2(L)]$ buffered at pH 10.2 after addition of 1 equivalent of ImH.



Scheme 2. The imidazole-induced translocation equilibrium at pH 10.2.

Figure 2 shows the formation of the band typical of $[Cu_2(LH_4)(Im)]^{3+}$ with time. The absorbance versus time profile corresponds to a first-order kinetic, with $k = 0.00037\ s^{-1}$ ($\tau = 45$ min). According to this result, in this system it is the substrate itself that makes the cations translocate and causes the system to open, thus allowing binding to take place. A high degree of selectivity is found: when pH is fixed at 10.2 the system is in its closed form (form **a**), and the addition of potentially bridging anions (N_3^-, PO_4^{3-}, $P_2O_7^{4-}$, $C_2O_4^{2-}$) give no spectral change even after the addition of up to fivefold excess of anions. The same behavior is observed when several equivalents of representative aminoacids and other biologically relevant substrates are added to the solution: no color change is observed upon addition of several equivalents of glycine, arginine, proline, glutamate, ADP, ATP. On the other hand, as expected, the addition of histidine or histamine gives the same effect already observed with imidazole. The same response (switch from pink to blue) is observed when titrations with imidazole, histidine, and histamine are repeated in the presence of a fivefold excess of the potential interferents. Only histidine and histamine are recognized and sensed colorimetrically. The receptor is a sleeping host that is closed to all guests we have tried so far, except imidazole-containing molecules, which have the key (the imidazole fragment itself) to switch the host cavity to the open, binding form. This recognition is associated to a neat color change, thus providing a signal of the selective inclusion. The peculiar, extreme case of selectivity relies on the unique energy gain obtained from the particularly stable $\{Cu^{2+}\text{-}(Im^-)\text{-}Cu^{2+}\}$ moiety, which more than compensates the energy barrier necessary for the movement of the molecular machine, that is, double Cu^{2+} translocation. The design and synthesis of devices based on this approach are being performed in our laboratory. This peculiar kind of selectivity can be exploited by simply changing the groups appended between the two amide functions for a broader range of applications such as fluorimetric sensing (appending fluorogenic fragments), transport across apolar membranes (by using lipophilic tails), histamine removal from biological fluids or its controllable release from nanodevices.

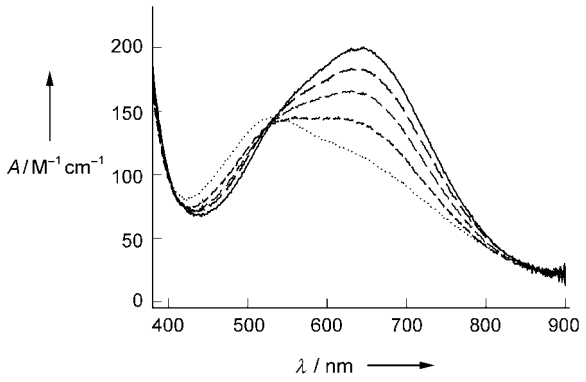


Figure 2. The effects on absorption spectra of addition of one equivalent of imidazole to a solution (7.5×10^{-4} M) of the complex buffered at pH 10.2 with CAPS (3-cyclohexylamino-1-propanesulfonic acid) (0.05 M). Sample spectra showed are taken at $t = 0$ (dotted line), 20, 50, 90, 150 min (solid line).

The Supporting Information for this article (distribution diagram for ligand LH_4 and pH–spectrophotometric titration of the reference compound $L'H_4$ in presence of two equivalents of Cu^{II} ions) is available on the WWW under http://www.angewandte.org or from the author.

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[4] Macrocyclic ligand LH_4 and $L'H_4$ were synthesized through a 2+2 condensation via Schiff base formation of 6-benzyl −1,4,8,11-tetraazaundecane-5,7-dione and, respectively, 2,6-pyridine dicarboxyaldehyde (for LH_4) and isophtaladehyde (for $L'H_4$). A solution of 6-benzyl-1,4,8,11-tetraazaundecane-5,7-dione (0.8 mmol) in acetonitrile (30 mL) was added dropwise to a solution of the corrseponding dialdheyde (0.8 mmol) in the same solvent (60 mL) at room temperature under a nitrogen atmosphere over a period of 30 min. The reaction mixture was stirred overnight, then was reduced in situ with an excess of $NaBH_4$ (0.6 g). The solvent was removed from the reaction mixture on a rotary evaporator and the solid residue was treated with water (50 mL). Extraction with CH_2Cl_2, drying with $MgSO_4$, and removal of the solvent under vacuum gave the desired product as a solid in 72% yield (LH_4) and 73% ($L'H_4$). Elemental analysis calcd (%) for LH_4 $C_{42}H_{54}N_{10}O_4$: C 66.12, H 7.13, N 18.36; found C 66.48, H 7.06, N 18.15; for $L'H_4$ $C_{44}H_{56}N_8O_4$: C 69.45, H 7.42, N 14.72; found C 69.32, H 7.16, N 14.11. MS(ESI): m/z: 764 [LH_4+H^+], 762 [$L'H_4$+H^+].

[5] Measurements were carried out in a solution of $NaClO_4$ (0.05 M) in 1:4 v/v water/ethanol. The water/ethanol mixture was chosen instead of pure water for the low water solubility of the less protonated or neutral forms of the ligand. All the presented experiments and data were obtained in the aforementioned solvent mixture. However, it has to be stressed that both the $[Cu_2(L)]$ and the $[Cu_2(LH_4)]^{4+}$ complexes are fairly soluble also in pure water (up to 5×10^{-3} M).

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[7] The distribution diagram for protonated species (i.e., in the absence of Cu^{2+}) is available in the Supporting Information.

[8] The two metal/one ligand nature of the complexes responsible of the 515 and 660 nm bands, that is, $[Cu_2(L)]$ and $[Cu_2(LH_4)]^{4+}$, has been checked also by means of spectrophotometric titrations: A solution of KOH (0.01 M; i.e., at pH ≈ 12) in water/ethanol (1:4 v/v) that contained 5×10^{-4} M LH_4 were titrated with standard aqueous $Cu(CF_3SO_3)_2$; a linear increase of the 515 nm band and a clear end point at 1:2 ligand/metal molar ratio was observed; on the other hand, solutions buffered at pH 4.5 (acetic/acetate buffer) that contained 5×10^{-4} M LH_4 were titrated with standard aqueous $Cu(CF_3SO_3)_2$; a linear increase of the 660 nm band with a sharp end point at 1:2 ligand/metal molar ratio was observed. Solid samples of $[Cu_2(L)]$ and $[Cu_2(LH_4)(OH)](CF_3SO_3)_3 \cdot 5H_2O$, which gave satisfactory CHN analysis, were obtained by slow

evaporation of solutions at pH 11.5 and 7.0, respectively. Mass spectra (ESI) carried out for $[Cu_2(L)]$ gave the expected m/z peak m/z: 887 $[[Cu_2(L)] + H^+]$.

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