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at 590 nm (orange emission) with a fluorescent quantum yield of 0.15 and a lifetime of 2.47 ns.

The sensorial ability of **1** was evaluated in aqueous solution towards Na^+, Ag^+, Ca^{2+}, Zn^{2+}, Cd^{2+}, Cu^{2+}, Pb^{2+}, Co^{2+}, Ni^{2+}, Fe^{2+}, Hg^{2+}, Al^{3+}, Cr^{3+}, Ir^{3+}, Tl^{3+} metal ions. Among all metal ions studied were only observed spectral changes with Hg^{2+} and Ir^{3+} metal ions.

The addition of Hg^{2+} induces to spectral changes only in the excited state, with quenching in the emission intensity of ca. 40% at 590 nm. However, the most significant alterations were observed with Ir^{3+} (see figure 1).
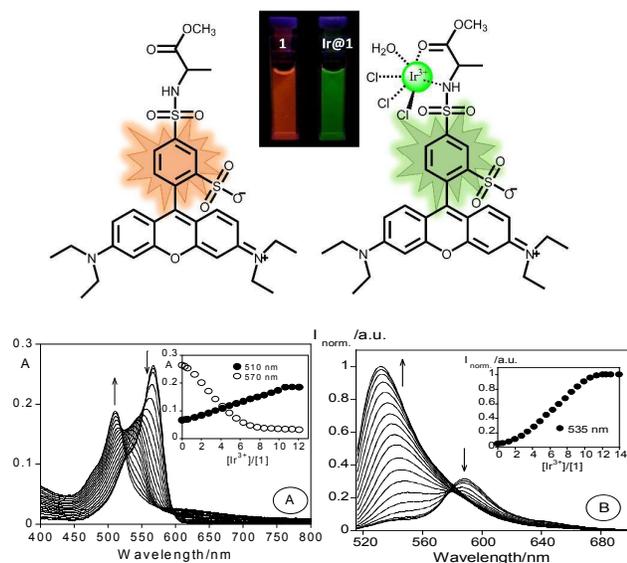

Figure 1. (Above) Schematic representations of **1** and **1**@Ir^{3+} and their colour observed under UV lamp. (Bellow) Spectrophotometric (A) and spectrofluorimetric (B) titrations of compound **1** with Ir^{3+} metal ions in aqueous solution. ([**1**] = 1.50×10^{-5} M, $\lambda_{exc.}$ = 510 nm, T = 298 K).

In the ground state a blue shift from 570 nm to 510 nm was detected, accompanied by a decrease in the absorbance at 570 nm and an increase at 510 nm. Consequently, a change of colour from *pink* to *pale green* was naked eye visualized. Concerning the excited state, a blue shift from 590 nm to 535 nm was observed, as well as, enhancement in the emission intensity at 535 nm. At the same time, under a UV lamp, a change of colour from *orange* to *green* was visualized. Therefore, from the results obtained, a new water-soluble iridium@**1** complex was achieved, with an absorption band at 510 nm and an emission band at 535 nm, with a highly fluorescent quantum yield of 0.47. The association constant was calculated using HypSpec program (17), and a value of $logK_{ass.}$ = 3.30 ±0.01 was determined with a stoichiometry of one ligand per metal ion.

In order to shed light on our knowledge about the system formed the same molar ratio Ir^{3+}/**1** was added to the parent compound **A** (Ir^{3+}/**A**) (lissamine rhodamine B sulfonyl chloride). Compound **A** present a pink and orange colours to the naked eye and under UV lamp, respectively. As can be seen in figure 2, in A@Ir^{3+} complex no colour change was observed with the naked eye with respect to compound **A**. However, in the emission, a change of

colour from *orange* to *yellow* was visualized. From these results, we might conclude that the Ir^{3+} coordination takes place at the amino acid alanine, involving probably the carbonyl and the amide groups (fig. 1).
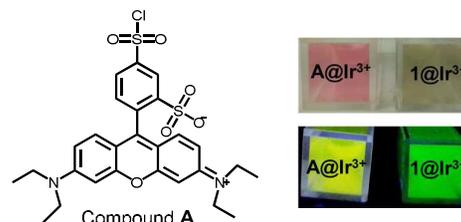

Figure 2. Right: Image of colour at the naked eye (at the top) and under UV lamp, $\lambda_{exc.}$ = 365 nm, (at the bottom) of **A** + Ir^{3+} and of **1** + Ir^{3+} in aqueous solution.

The ^1H NMR titration of compound **1** with one equivalent of Ir^{3+} in $CDCl_3$, a displacement of the NH signal (alanine group) from 5.4 to 6.0 was observed (see S3). This result confirms that the amine group was not deprotonated, and direct interaction with the metal ion takes place. Thus, the modifications observed are attributed to metal ion coordination and not to deprotonation of the amine group of the alanine. Modification of the signals assigned to the protons located in the proximity of the NH group, such as OCH_3 (3.8 to 3.4 ppm), methyl group (1.6 to 1.9 ppm) and αH (4.2 to 3.9 ppm) of the alanine amino acid unit, supports the coordination mode proposed.

Compound **1** shows to be pH independent, whereas no changes in the absorption and the emission were observed in the pH range 2 to 11 (see S8).

The interaction of Ir@**1** complex was also carried out towards Na^+, Ag^+, Ca^{2+}, Zn^{2+}, Cd^{2+}, Cu^{2+}, Pb^{2+}, Co^{2+}, Ni^{2+}, Fe^{2+}, Hg^{2+}, Al^{3+}, Cr^{3+}, Ir^{3+}, Tl^{3+} metal ions, demonstrating to be highly selective for Hg^{2+} metal ion. Figure 3 shows the spectrofluorometric titration of Ir@**1** complex with Hg^{2+} (fig.3A) and IrHg@**1** with cysteine (fig.3B).

In the ground state no significant alterations were noticed, on the other hand, in the excited state a total quenching in the emission intensity at 535 nm was verified. This behavior is not unexpected due to Hg^{2+} "heavy" metal ion nature, which increases the probability to occur non-radiative deactivation processes, such as intersystem crossing, responsible for chelation enhancement of quenching (CHEQ) in the emission intensity (18).

Consequently, the addition of cysteine leads to a recovery of the emission intensity. Nevertheless, this effect was due to the higher affinity of Hg^{2+} metal ion to the sulphur atom present in the cysteine, thereby removing the Hg^{2+} metal ion from the Ir@**1** complex.
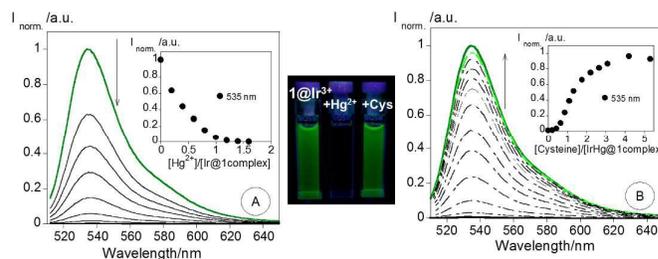
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Figure 3. Spectrofluorimetric titrations of compound Ir@**1** complex with Hg^{2+} and cysteine in aqueous solution, and final image under UV lamp, $\lambda_{exc.}$ = 365 nm ([**1**] = 1.50×10^{-5} M, $\lambda_{exc.}$ = 510 nm, T = 298 K).

Atomistic insights into the coordination geometry of Ir^{3+} to the lissamine derivative **1** were obtained from density functional theory calculations with the B3LYP functional.

The lowest energy complex conformation, depicted in Figure 4, involves coordination of the Ir^{3+} cation to the amine nitrogen ($d_{N\cdots Ir}$ = 2.102 Å) and carbonyl oxygen ($d_{O\cdots Ir}$ = 2.109 Å) from the apical amino acid fragment. The octahedral coordination sphere of the metal is completed upon coordination to three Cl^{-} anions (at $d_{Cl\cdots Ir}$ = 2.377, 2.409 and 2.442 Å) and one water molecule ($d_{H2O\cdots Ir}$ = 2.128 Å). This coordination mode is *ca.* 120 kJ/mol more stable than the coordination involving the sulfonic oxygens, indicating a lack of prearrangement of the sulphonic oxygens to accommodate an octahedral coordination of the cation. Simultaneously, the availability of the nitrogen electron lone pair from the amine and the flexibility of the entire apical amino acid provides the necessary prearrangement for the efficient coordination of the metal to the receptor through the carbonyl and the amine. This result supports the experimentally inferred coordination mode of the metal to the receptor (vide supra). Additional evidence were obtained from the calculated complexation induced ^{1}H NMR chemical shifts of the amino acid NH, OCH$_3$, βCH$_3$ and αH and comparison with the experimental ones (see Table S1). Both the sign and magnitude of the change in the ^{1}H chemical shifts closely follows the experimental ones, apart from the NH resonance for which the magnitude is considerably larger than the measured one, probably due to the absence of H-bonding stabilisation of that proton.

Calculation of vertical excitation energies by TDDFT also supports the suggested coordination mode of Ir^{3+} to the receptor. The lowest energy singlet excitation shifts from 636 nm (1.95 eV) to 593 nm (2.09 eV) upon complexation (see Table S2), in line with the data from Figure 1. In both cases, the excitation in mainly described by a transition from HOMO to the LUMO (see supporting information); the HOMO is mainly located on the sulphonic group and the Ir^{3+} metal (in Ir@**1**), whereas the LUMO is mainly located on the rhodamine aromatic fragment in both cases (see Figure S9).
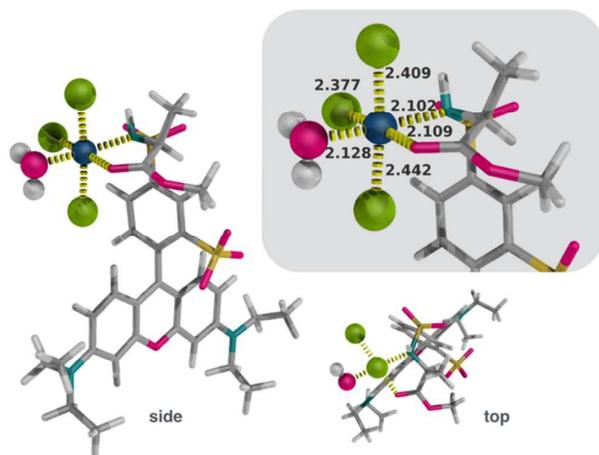
Figure 4. Side and top view of the lowest energy coordination mode of Ir^{3+} to the lissamine receptor, with the inset highlighting the interaction sites and coordination distances (color code: Cl - green; S - yellow; O - pink; C - gray; Ir - petroleum; H - white).

Further, from the molecular logic gate viewpoint, a logic operation G1 was created, considering the three inputs defined as chemical species (input 1: Ir^{3+}, input 2: Hg^{2+}, input 3: cysteine) and the output 1 and 2 were defined as fluorescence emission at 535 nm (O1) and 590 nm (O2). All possible combinations of input data and the corresponding output sequences are gathered in figure 5. The combinational logic circuit (figure 5) was designed through the truth table presented in figure 5A, in a Logisim 2.7.1 tool for designing and simulating logic circuits. The molecular switches transduce the inputs into outputs through AND, OR and NOT operations.

Concerning the emission technique, compound **1** produce the output O1, and the O2 by the minimal amount of 0.16 ± 0.03 nM of the input 1; the Ir@**1** complex produce the output O1 by the minimal amount of 13.63 ± 0.11 μM of input 2, followed by 6.56 ± 0.10 μM of input 3 in water.

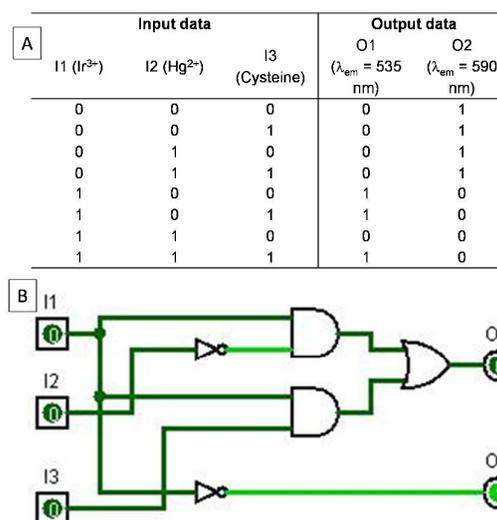|	Input data			Output data	
A			I3 (Cysteine)	O1 (λ_{em} = 535 nm)	O2 (λ_{em} = 590 nm)
	I1 (Ir^{3+})	I2 (Hg^{2+})			
	0	0	0	0	1
	0	0	1	0	1
	0	1	0	0	1
	0	1	1	0	1
	1	0	0	1	0
	1	0	1	1	0
	1	1	0	0	0
	1	1	1	1	0

Figure 5. (A) Truth table for the logic gate G1. (B) The logic circuit equivalent to the molecular switches transduces the inputs into outputs through AND, OR and NOT operations.

Conclusions

In summary, a new lissamine rhodamine-alanine derivative, **1**, was successfully designed and obtained. After that, unusually a new green luminescent iridium@**1** complex was achieved in aqueous solution at room temperature, with a fluorescence quantum yield of 0.47. The complex obtained can detect the minimal amount of 13.63 ± 0.11 μM of the toxic metal ion mercury (II) and 6.56 ± 0.10 μM of cysteine. Based on that, a combinational logic circuit was created, and the molecular switches transduce the inputs into outputs through AND, OR and NOT operations.

Methods

Physical measurements. Elemental analyses were carried out on a Thermo Finnigan-CE Instruments, Flash EA 1112 model, CHNS series, and the ^{1}H-NMR, ^{13}C-NMR, HSQC, COSY in a Bruker Avance III 400 at an operating frequency of 400 MHz at REQUIMTE facility, Chemistry Department from Faculty of Sciences and Technology of University NOVA of Lisbon.

The MALDI-MS analyses have been performed in a MALDI-TOF-TOF-MS model Ultraflex II Bruker, Germany, equipped with nitrogen, from BIOSCOPE group, PROTEOMASS Scientific Society, Chemistry Department FCT-UNL (Portugal).

The MALDI mass spectra of the soluble samples (1 or 2 mg/mL), were recorded using the conventional sample preparation method for MALDI-MS. 1μL of the compound was put on the sample holder. In all spots in the holder, a sinapic acid matrix was added. Afterthought the sample holder was inserted into the ion source.

All calculations were performed with Gaussian09 (19) using Density Functional Theory with the B3LYP functional and the 6-31G** (C, H, N, O, Cl, S) and LanL2DZ (Ir) basis set, as implemented in Gaussian09. NMR parameters were calculated using implicit acetonitrile with the PCM model. Default parameters were used throughout the calculations.

Chemicals and starting materials. Na(BF$_4$).xH$_2$O, NaCl, Ag(NO$_3$).xH$_2$O, Ca(CF$_3$SO$_3$)$_2$, Zn(BF$_4$)$_2$.xH$_2$O, Cd(CF$_3$SO$_3$)$_2$, Cu(BF$_4$)$_2$.6H$_2$O, Pb(CF$_3$SO$_3$)$_2$, Co(CF$_3$SO$_3$)$_2$, Ni(BF$_4$)$_2$.6H$_2$O, Fe(NO$_3$)$_3$.H$_2$O, Hg(NO$_3$)$_2$.xH$_2$O, Al(NO$_3$)$_3$.9H$_2$O, Cr(NO$_3$)$_3$.H$_2$O, IrCl$_3$.xH$_2$O, Tl(NO$_3$)$_3$ salts, L-Alanine, L-cysteine, Cesium carbonate, triethylamine, were purchased from Stream Chemicals, Sigma-Aldrich or Solchemar and used without further purifications. Lissamine Rhodamine B sulfonyl chloride was from Exciton company were used without previous purification. Water was always used purified and deionized (Milli-Q grade, Millipore).

Spectrophotometric and spectrofluorometric measurements. Absorption spectra were recorded on a JASCO V-650 spectrophotometer and a fluorescence emission by an HORIBA Scientific FLUOROMAX-4 spectrofluorimeter The spectrophotometric characterizations and titrations were performed as follows: the stock solutions of the compounds (*ca.* 10^{-3} M) were prepared by dissolving an appropriated amount of the compounds in a 10 ml volumetric flask and diluting them to the mark with miliQ water. The solutions were prepared by appropriate dilution of the stock solutions still 10^{-5} – 10^{-6} M. Titrations of the ligand **1** was carried out by the addition of microliter amounts of standard solutions of the ions in water. All the measurements were performed at 298 K.

Luminescence quantum yields were measured using a solution of rhodamine B in water as standard [ϕ_F = 0.31] (20) for compound **1**; a solution of fluorescein in ethanol [ϕ_F = 0.79] (21) for complex Ir@**1**. Lifetime measurements were recorded in TEMPRO Fluorescence Lifetime System, from HORIBA JOBIN YVON.

All solvents used were of the highest purity from Merck.

The Synthesis and physical characterization of compound **1** are in supporting information.

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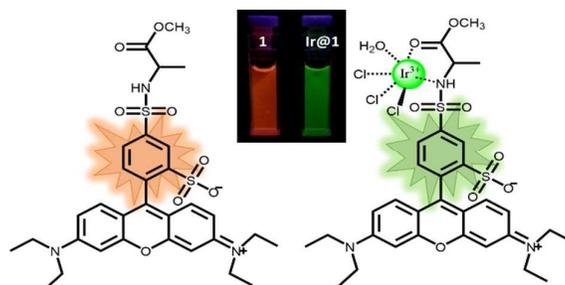
Green highly fluorescence iridium lissamine- alanine-based complex (**1**) to selectively sense Hg^{2+} and cysteine in aqueous solution.