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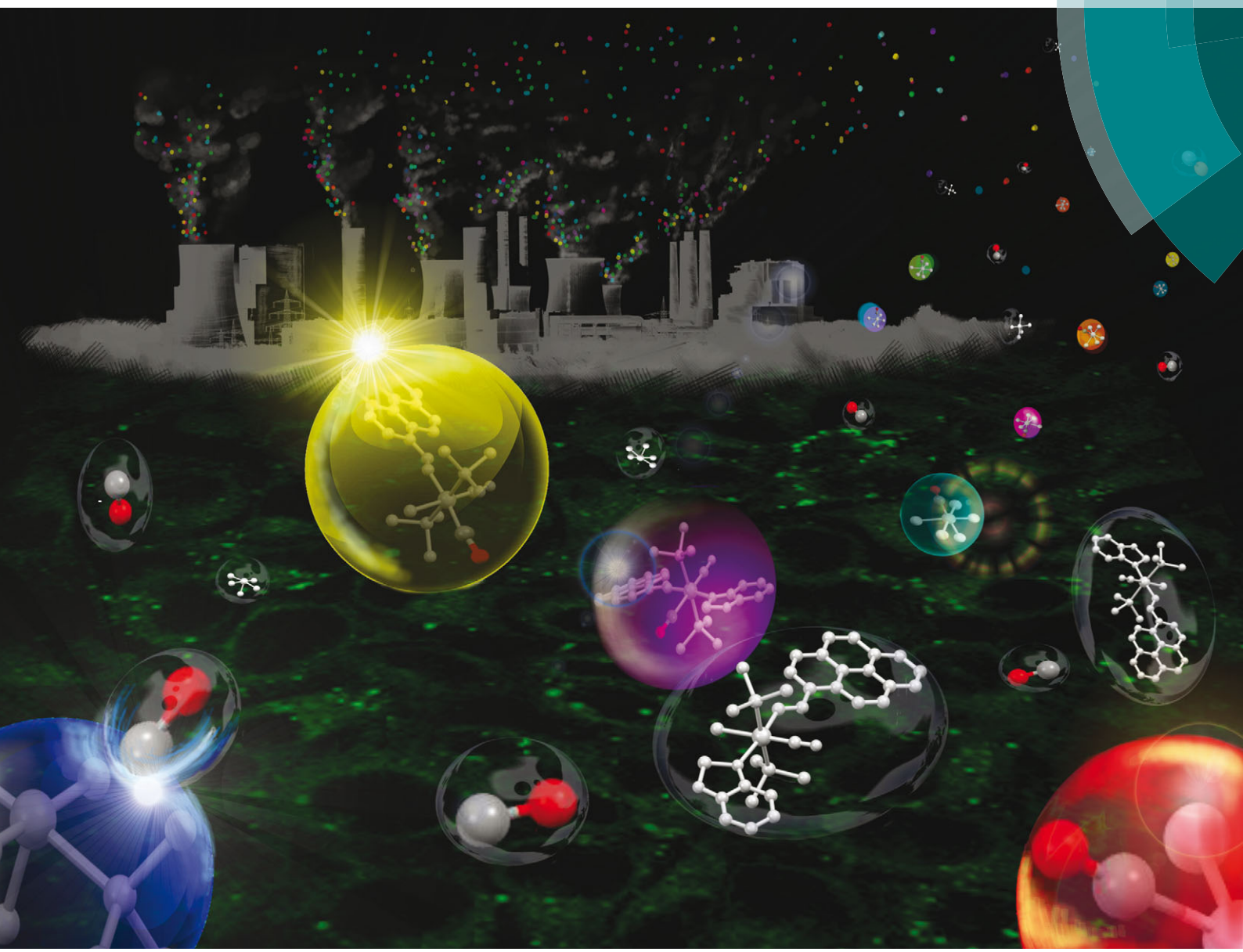

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James D. E. T. Wilton-Ely, Ramón Martínez-Máñez *et al.*

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Chromo-fluorogenic probes for carbon monoxide detection

Cristina Marín-Hernández,†[abc] Anita Toscani,†[d] Félix Sancenón,[abc] James D. E. T. Wilton-Ely*[d] and Ramón Martínez-Máñez*[abc]

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The sensing of carbon monoxide (CO) using electrochemical cells or semiconducting metal oxides has led to inexpensive alarms for the home and workplace. It is now recognised that chronic exposure to low levels of CO also poses a significant health risk. It is perhaps surprising therefore that the CO is used in cell-signalling pathways and plays a growing role in therapy. However, the selective monitoring of low levels of CO remains challenging, and it is this area that has benefited from the development of probes which give a colour or fluorescence response. This feature article covers the design of chromo-fluorogenic probes and their application to CO sensing in air, solution and in cells.

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Introduction

Over the past two decades, interest in carbon monoxide (CO) has intensified in part due to the discovery of the therapeutic and cell-signalling role that this small molecule plays in biological systems.[1,2] Previous to these findings, CO was most widely known as a silent, invisible and odourless threat that could lead to fatal consequences on inhalation.[3]

The presence of CO in domestic and work environments is frequently due to the incomplete combustion of gas, oil or solid fuels by improperly maintained appliances in poorly ventilated spaces. While death can occur after a few minutes at extreme exposure to the gas (2000 ppm), confusion and tiredness as well as migraine-like symptoms and dizziness can arise from long-term exposures even at low levels of CO (30–50 ppm). It is a concern that these symptoms are often misdiagnosed as those of common viral infections. It has also been reported that

[a] *Instituto Interuniversitario de Investigación de Reconocimiento Molecular y Desarrollo Tecnológico (IDM), Unidad Mixta Universidad Politécnica de Valencia-Universidad de Valencia, Spain*
[b] *Departamento de Química, Universidad Politécnica de Valencia, Camino de Vera s/n, E-46022 Valencia, Spain. E-mail: rmaez@qim.upv.es; Fax: +34 96 387 9349*
[c] *CIBER de Bioingeniería Biomateriales y Nanomedicina (CIBER-BBN)*
[d] *Department of Chemistry, Imperial College London, SW7 2AZ, London, UK. E-mail: j.wilton-ely@imperial.ac.uk*
† Both authors contributed equally to this work.

Cristina Marín-Hernández

Cristina Marín-Hernández graduated in Chemistry at the University of Valencia, Spain, in 2011 and received her Master's Degree at the same institution in 2012. She is a PhD Student at the Polytechnic University of Valencia in the group of Prof. Ramoń Martínez-Máñez and her main area of interest is the development of chemosensors for cationic, anionic and neutral molecules. She has also carried out research with the Wilton-Ely group at Imperial College London.

Anita Toscani

Anita Toscani obtained her MSci degree in Chemistry (2011) at the University of Turin, Italy, where she studied cobalt carbonyl complexes as potential carbon monoxide releasing molecules (CO-RMs) under the supervision of Prof. Roberto Gobetto. She then remained at the University of Turin for a further year in the group of Prof. Claudia Barolo, where she worked on the development of long-lived luminescent iridium complexes for water-splitting applications. In 2012, she moved to Imperial College London, where she is currently completing her PhD focused on multimetallic and nanoparticle assemblies for sensing applications under the supervision of Dr James Wilton-Ely.

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chronic exposure to carbon monoxide may often have a significant impact on the central nervous system, leading to depression and memory loss.[4] The interest in detecting this gas at subtoxic concentrations (lower than 50 ppm) is therefore becoming important to avoid harm from its presence as a chronic poison. The majority of sensors used for the detection of CO in air are based on electrochemical cells and on semiconducting metal oxides (typically tin dioxide).[5] However, significant progress has been made recently towards the development of new, inexpensive CO sensors using different technologies. In this context, colorimetric and fluorimetric probes for carbon monoxide, that exhibit striking optical changes visible to the 'naked eye' at low concentrations of the analyte, are seen as a powerful alternatives to the established systems.

Since the discovery of the benign role of carbon monoxide, a whole new research field, focused on the real-time detection of CO in living cells and tissues, has emerged. This coincides with interest in the therapeutic potential of CO-releasing molecules (CORMs and PhotoCORMs)[1] that are able to release CO in a controlled manner *via* hydrolysis or photoactivation. The growth of these fields has led to attempts to develop luminescent chemosensors capable of quantifying the production of endogenous CO or the generation of CO by CORMs in cells.[2] However, despite this attention, few systems capable of the fluorogenic detection of CO in cells and tissues have been reported.

Most of the probes developed for the optical sensing of CO, both for detection in air or in cells, are based on metal complexes, as they possess many promising attributes suited to the design of chromo- or fluorogenic probes.[6] The possibility of tuning the steric and/or electronic properties of metal complexes enables a subtle control to be exerted over the selectivity and reactivity of the metal towards CO.[7] In terms of CO-detection, the sensing mechanism is intrinsically related to the reactivity of the metal centre towards carbon monoxide and in most cases the detection is often unsurprisingly related to the direct coordination of CO to the metal. The binding of CO to the metal centre can occur at a vacant coordination site or through the displacement of a labile ligand (*vide infra*).

When using metal complexes the optical response can be triggered in two different ways. It can arise from (a) the OFF–ON switching of the optical response from the metal complex, which acts as both receptor and signalling unit, or (b) *via* the revival of the optical signal of a displaced ligand, which was previously quenched by its close proximity to the metal centre.[7] Alternatively, transition-metal catalysed carbonylation, hydrocarboxylation and azidocarbonylation reactions in the presence of CO have also proved to be useful in generating highly fluorescent derivatives from poorly emissive organic precursors. Ideally, such reactions should be immediate and need to take place under mild temperatures and at atmospheric pressure.

This review covers the key developments to date in the design of molecular chromogenic probes for the detection of

Félix Sancenón

Félix Sancenón was born in Manises, Valencia, Spain, and graduated in Chemistry in 1991. He received his PhD degree in 2003. Afterward, he worked with Professor Luigi Fabbrizzi at the University of Pavia, Italy. He then joined the Department of Chemistry at the Polytechnic University of Valencia with a Ramón y Cajal contract. He became a lecturer in 2006. He is the co-author of more than 185 research publications. His current research interests comprise the synthesis of molecular chemosensors and the use of hybrid materials for the development of new sensing protocols.

James D. E. T. Wilton-Ely

James Wilton-Ely received his BSc and PhD degrees from Imperial College London, UK, before conducting postdoctoral research at TU Munich and the University of St. Andrews, UK. He held independent research fellowships at University College London (Ramsay Fellow) and Oxford University (Fitzjames Fellow, Merton College) before returning to the Department of Chemistry at Imperial College in 2009. He is the co-author of more than 90 research publications based on transition metal coordination chemistry and functionalized nanostructures. His current research interests are focused on sensing, medical imaging and the catalytic transformation of biomass into platform chemicals.

Ramón Martínez-Máñez

Ramoń Martínez-Máñez was born in Valencia, Spain. He received his PhD from the University of Valencia and went on to postdoctoral work at Cambridge University, UK. He is a full professor in the Department of Chemistry at the Polytechnic University of Valencia where he directs the IDM Research Institute. He is also Scientific Director of the Biomedical Research Networking Centre in Bioengineering, Biomaterials and Nanomedicine (CIBER-BBN). He is the co-author of more than 330 research publications and ten patents. His current research interests involve the preparation of gated hybrid materials for on-command delivery applications and the design of chemical sensors.

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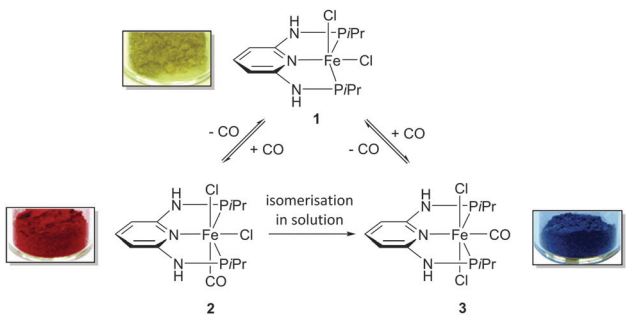
CO including our own contributions in the area. Some of these species have also been incorporated into solid supports, ultimately showing their versatility in detecting CO in air and in solution. The review is divided into two main sections according to the sensing response (*i.e.* chromogenic or fluorogenic). Within each section, structural features, the response observed and details of the sensing mechanism used by the probe are highlighted.

Chromogenic CO sensors

All examples in this section are based on the use of transition metal complexes that show a clear colour change in the presence of CO in air, or when air containing CO is bubbled through solutions of the complexes. For all complexes detailed below, the coordination of CO to the electron-rich metal centre induces a variation of the electron density and/or change of the spin-state of the metal, thus modifying the coordination environment, which ultimately results in a clear colour change. At this point it is worth noting that colour modulation due to the reaction of CO with metal complexes has often been described with little import being attached to this observation. Only recently has awareness grown of the potential application of this behaviour (colour modulation) to the design of probes for CO sensing. However, it is also noteworthy that few systems which exhibit such colour changes can be applied to the more challenging task of sensing CO in air on a solid support.

An early example from 2008 is based on the use of the coordinatively-unsaturated iron pincer complex **1** [$FeCl_2$(PNP-iPr)], (PNP-iPr = *N*,*N*′-bis(diisopropylphosphino)-2,6-diaminopyridine), which binds CO in a stereospecific and reversible manner.[8] Complex **1** was found to react selectively with CO in both solution and the solid state, affording the *cis* or *trans* isomers **2** and **3**. In the presence of CO, the light yellow complex **1** converts to the deep red complex **2**, while retaining its crystallinity. Moreover, in the absence of CO, the heating of complex **2** under vacuum results in the complete regeneration of **1** and the recovery of the initial yellow colour. This remarkable solid-state reaction is fully reversible with no decomposition reported. Alternatively, the deep blue *trans* complex **3** can be obtained by bubbling CO through an acetone solution of **1** for 2 minutes or by a *cis*–*trans* isomerisation reaction of complex **2** in DMSO (Scheme 1).

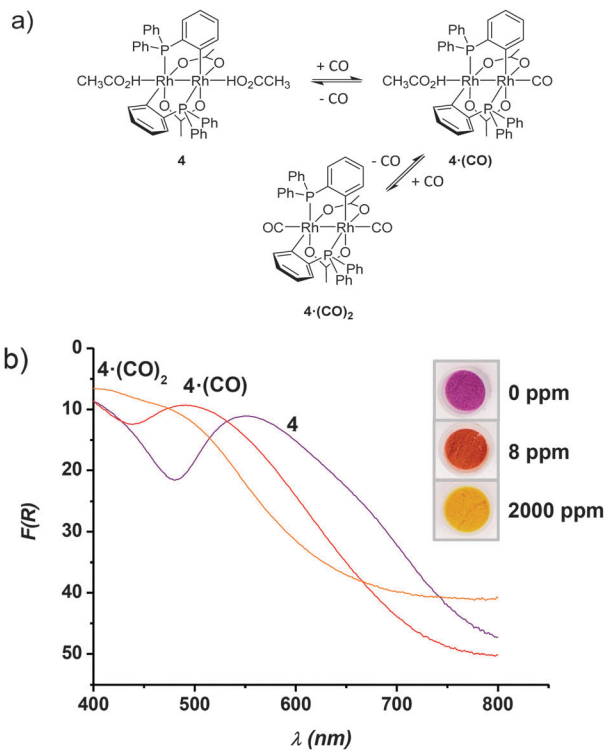


Scheme 1 Reaction of the iron complex **1** with CO, showing the associated colour changes. Adapted and reprinted with permission from ref. 8. Copyright 2008 Wiley-VCH.

Although these complexes show outstanding optical properties in the presence of CO, they appear to require a high concentration of CO to function effectively.

For obvious practical reasons, ideal chromogenic sensors should not only display both selectivity and sensitivity towards the desired analyte, but also be air- and photo-stable. In this context, our 2010 report of an efficient chromogenic system based on a binuclear rhodium complex was notable for the unambiguous colour change it showed in the presence of increasing concentrations of CO.[9]

Complex **4** enables the detection of CO as its colour varies from violet to orange-yellow when air samples containing CO are bubbled through chloroform solutions of the complex. Colour modulations are due the displacement of the carboxylic acid ligands in the axial positions and the consequent coordination of up to two molecules of CO (compounds **4·CO** and **4·(CO)$_2$** in Scheme 2). The system is reversible and, when carbon monoxide is removed from the atmosphere, the initial violet colour is recovered without significant degradation of the compound. The operational advantage of this system rests on the intrinsic reactivity of this probe in the solid state. Once probe **4** is immobilised on silica gel, the reaction with CO in air takes place in just a few minutes, resulting in a change in colour visible to the naked eye at subtoxic concentrations of CO (50 ppm). Moreover, using diffuse reflectance methods, the limit of



Scheme 2 (a) Binuclear rhodium complex **4** and the formation of the corresponding complexes upon coordination of one (**4·CO**) or two (**4·(CO)$_2$**) molecules of CO in axial positions; (b) diffuse reflectance UV-Vis spectra for the complexes and the colour changes in the presence of various concentrations of CO. Adapted and reprinted with permission from ref. 10. Copyright 2011 American Chemical Society.

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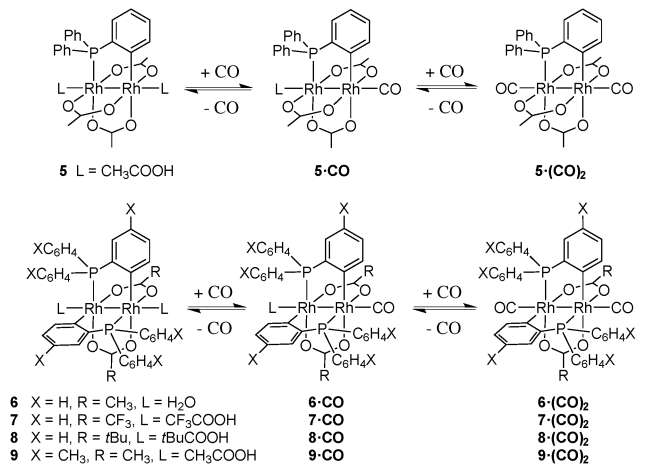
detection is calculated to be as low as 0.5 ppm. The probe shows high selectivity towards carbon monoxide over other common gases (CO_2, N_2, O_2, Ar, SO_2, NO, NO_2 and water vapour) and volatile organic compounds (acetone, chloroform, ethanol, formaldehyde, hexane, toluene and xylene). This aspect allows molecular sensors to avoid the drawbacks of many commercial devices based on semiconducting metal oxides, which have to be sited carefully to avoid exposure to high levels of steam or particulates. False alarms can also be caused by solvents from cleaning or cosmetic products (*e.g.*, hairspray), so screening for potential interferents is particularly important if the probe is to be used in domestic or workplace settings for CO detection in air.

Following these promising results, a family of binuclear rhodium complexes of general formula $[Rh_2\{(XC_6H_3)P(C_6H_4X)_2\}_n(O_2CR)_{4-n}]\cdot L$, bearing one ($n$ = 1) (**5**) or two (n = 2) (**6** to **9**) differently *meta*-substituted (X = H, CH_3) cyclometallated phosphines was then reported in 2011. Further tuning proved possible using various different equatorial ligands (R = CH_3, CF_3, or *t*Bu) and axial groups (L = CH_3CO_2H, CF_3CO_2H, *t*$BuCO_2H$, or H_2O).[10] In a similar manner to complex **4**, this family of dirhodium complexes shows remarkable selectivity and sensitivity towards CO in air, with 'naked eye' detection limits as low as 0.2 ppm when immobilised on solid supports (silica gel and cellulose strips). As in the case of **4**, colour changes were observed due to the coordination of one (**5·CO** to **9·CO**) or two (**5·(CO)$_2$** to **9·(CO)$_2$**) carbonyl ligands (depending on the structure of the complex) at the axial sites (Scheme 3). Our studies based on these rhodium complexes clearly show that, by combining the electron-withdrawing/donating properties of the equatorial and axial ligands, it is possible to tune the level of electron density on the rhodium centre, and thus modulate the d–π* back-donation. It was then possible to demonstrate that these properties are connected to the kinetic rate of CO release of **5·(CO)$_2$** to **9·(CO)$_2$**, as stronger back-donation corresponds to a weaker ability to release the two carbonyl groups and to recover the initial colour.



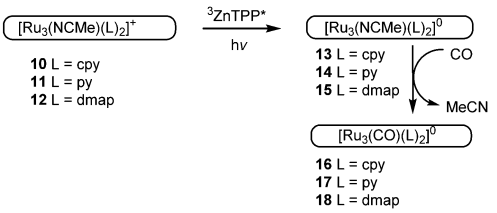
Scheme 3 Binuclear rhodium complexes **5–9** and the corresponding products obtained upon coordination of one and two molecules of CO in the axial positions.

For a probe to become a CO detector comparable to those available commercially, it is necessary to convert the colour change into a numerical readout and audible alarm. We were able to show that **7** could be incorporated into a bespoke optoelectronic device, which can serve as a portable detector of carbon monoxide in air.[11] The device consists of a small chamber that accommodates the probe adsorbed on a cellulose strip, which is illuminated by a tricolour 624/525/470 nm LED. The variations in colour of the cellulose strip in the presence of CO are detected by a photodiode, followed by conversion into RGB values, and then transformed into numerical readings. With this apparatus, it is possible to determine in approximately 7 minutes the ambient concentration of CO in the range 0 to 7000 ppm and with a limit of detection of 11 ± 4 ppm.

In related work, we were able to demonstrate that the binuclear rhodium complexes **6·(CO)$_2$** and **9·(CO)$_2$** displayed a low rate of CO-release and were tested as CO-Releasing Molecules (CO-RMs).[12] The inhibition of nitrite levels in iNOS-expressed RAW 264.7 macrophages was proven to be effective even after 30 days from initial incubation of the macrophages with those complexes.

Another example of chromogenic CO detection involves the use of the oxo-acetato-bridged triruthenium complex $[Ru_3(NCMe)(cpy)_2]^+$ (cpy = 4-cyanopyridine) (**10**). This complex exhibits a cyan colour in acetonitrile solutions due to the presence of the main broad absorption band centred at 705 nm.[13] The presence of zinc tetraphenylporphyrin (ZnTPP) under irradiation induces the formation of $[Ru_3(NCMe)(cpy)_2]^0$ (**13**) (Scheme 4). This new compound shows an orange colour with bands at 480 and 943 nm. When orange acetonitrile solutions of complex **13** are saturated with CO, a colour change to blue is observed due to the formation of $[Ru_3(CO)(cpy)_2]^0$ (**16**). The same results are obtained for complexes $[Ru_3(NCMe)(py)_2]^+$ (**11**) and $[Ru_3(NCMe)(dmap)_2]^+$ (**12**) (py = pyridine; dmap = 4-dimethylaminopyridine) which were transformed into **14** and **15** in the presence of ZnTPP under irradiation and gave the corresponding complexes **17** and **18** in the presence of CO. Despite the remarkable colour changes observed upon CO addition, the authors did not report any data on detection limits or the response towards CO in the presence of other possible interfering species.

Some of the binuclear rhodium probes described above show a remarkable colour change with CO in air when adsorbed on silica. This reflects the increasing use of inorganic supports for molecular probes, following the widespread use of 'smart materials' with organised active surfaces in a number of research fields.[14]



Scheme 4 The reaction of trinuclear ruthenium complexes with CO.

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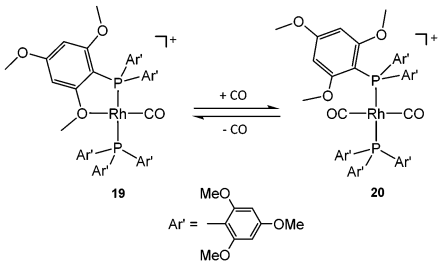
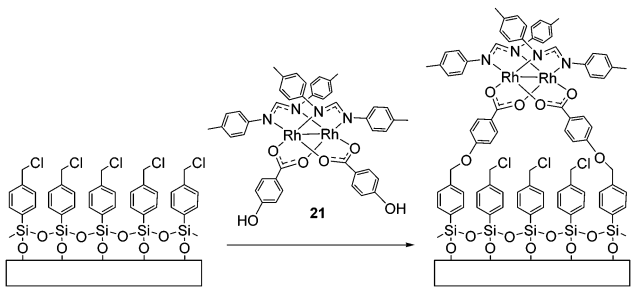


Scheme 5 Reversible reaction of the rhodium complex **19** with CO to form **20**.

From a sensing perspective, the organisation, fine control and distribution of the probes on a host support/matrix can maximise the detection response and enable the incorporation of sensing molecules into optoelectronic devices. Moreover, the attachment of a molecular probe to a physical support can improve the stability of the chemosensor itself and facilitate handling without losing the specificity of the probe. Dunbar *et al.*[15,16] adopted this approach to investigate the synthesis and CO binding properties of zirconia and titania glasses impregnated with the rhodium(I) complex $[Rh(CO)(TMPP)_2][BF_4]$ (**19**, TMPP = tris(2,4,6-trimethoxyphenyl)phosphine). This unusually air-stable rhodium(I) complex undergoes a reversible reaction with carbon monoxide to form the dicarbonyl species **20** (Scheme 5). The incorporation of **19** into porous sol–gel derived titania and zirconia composite films enables the use of these sensing molecules for CO detection in the solid state using both spectroscopic and electrochemical techniques. The appearance of spectral bands at 2013 cm^{-1} in the infrared spectrum and at 435 nm in the UV-Vis spectrum are consistent with the conversion of **19** into **20** after 15 min of exposure to the gas. Mixtures of CO and argon containing less than 2% of CO are detectable with the quantitative interconversion between the two species. However, this conversion appears to be incomplete at lower concentrations. The material is not only suitable for the immobilisation of the probe *via* facile impregnation with a dichloromethane solution but, most importantly, it facilitates the diffusion of CO and its reversible binding to the probe.

Another relevant example of a chromogenic probe for CO is the bimetallic rhodium complex (**21**) attached to a siloxane-based monolayer *via* reaction with a *p*-chlorobenzyl moiety (Scheme 6).[17] The surface formed can be used for selective and sensitive CO monitoring by UV-visible measurements. Glass monolayers of complex **21** (recorded in the transmission mode) show a broad absorption band (ranging from 280 to 750 nm) with a maximum centred at *ca.* 290 nm. Exposure of the monolayers to air containing 5 ppm of CO induces an increase in the absorption intensity of the entire spectrum. This behaviour is ascribed to an enhancement of the ligand-to-metal charge transfer (LMCT) promoted by CO coordination to the metal centres. Moreover, a low-intensity absorption at 560 nm also appears, which is indicative of the formation of Rh–CO bonds. The response of the monolayer is relatively selective because only CO, among all the other gases tested (Ar, N_2, N_2O, O_2, NO_x, H_2, CO_2, CH_4, ethylene and propylene), is able to induce the appearance of the 560 nm band. Importantly, air and water-saturated air fail to induce changes within the UV-visible region. The authors did not report a limit of detection but indicate that monolayers are able to respond to CO concentrations of 0.3 ppm in air. The regeneration of the sensor can be achieved by heating or by purging the monolayers with air, argon or nitrogen.

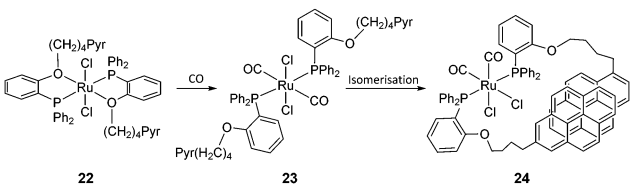


Scheme 6 Schematic representation of the bimetallic rhodium complex **21** and its attachment to a siloxane-based monolayer.

Fluorogenic CO sensors

To the best of our knowledge, the first fluorogenic CO probe was reported in 2002 by Wolf and co-workers.[18] The authors reported that the ruthenium(II) complex **22**, bearing a hemilable phosphine pyrene ether, reacts rapidly at room temperature with carbon monoxide to produce a remarkable monomer-to-excimer emission. In this reaction, a *trans*-dicarbonyl complex **23** is formed initially, but then undergoes isomerization to form the *cis*-dicarbonyl complex **24**. The change from blue (pyrene) to blue-green (excimer) emission is due to the formation of the more thermodynamically stable isomer **24** *via* the partial dissociation of the hemilable ligand (Scheme 7). The strong excimer emission is observed in both concentrated (10^{-2} M) and dilute (10^{-6} M) solutions and is due to the favoured inter- and intramolecular π-stacking of the pyrene units. Air samples containing 0.05% (*ca.* 850 ppm) of CO can be analysed by measuring the emission variations in a 10^{-6} M dichloromethane solution of **22**. Despite the remarkable emission response, and the colour change from red to greenish yellow occurring within seconds after exposure to CO, selectivity issues are likely in the presence of other Lewis-basic small molecules (*e.g.*, SO_2).[19]

Chung and co-workers have also exploited the reactivity of transition metals (rhodium and iridium) towards CO by developing two families of bimetallic complexes, all bearing a bifunctional 1,10-phenanthroline N-heterocyclic carbene



Scheme 7 Reactivity of the ruthenium complex **22** with CO.

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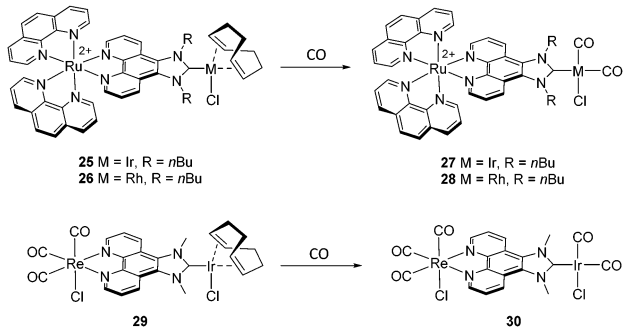


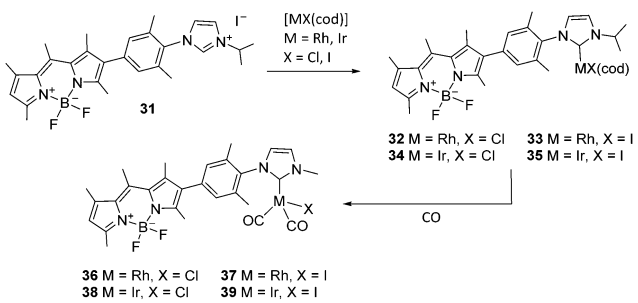
Scheme 8 Reactivity of the bimetallic complexes **25**, **26** and **29** with CO.

ligand (compounds **25**, **26** and **29**).[20,21] These metal complexes exhibit high molar extinction coefficients, moderately long-lived lifetimes and strong metal-to-ligand charge transfer (MLCT) emission. The photophysical properties are dependent on the different metals. Solutions of the bimetallic complexes **25**, **26** and **29** in solvents such as acetonitrile, DMF and DMSO are quantitatively converted into the respective dicarbonyl complexes **27**, **28** and **30** when carbon monoxide is present (Scheme 8). Generally, the resulting dicarbonyl complexes show higher emission intensities and quantum yields than the metal-cod precursors (cod = 1,5-cyclooctadiene). In the case of **25**, the conversion rate to **27** was found to be quite slow and proportional to the concentration of CO added. In the same manner, complex **29** reacts only slowly with high concentrations (1000 ppm) of carbon monoxide to afford the final product **30**. In all cases, the reaction with CO is reported to be completely irreversible.

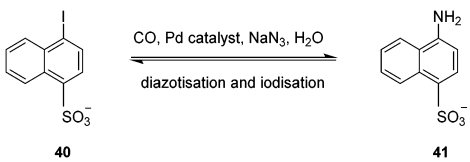
More recently, Kos and Plenio described the luminescence behaviour upon reaction with carbon monoxide of a series of metal complexes (from groups 8–11) bearing an N-heterocyclic carbene coupled to a boron dipyrromethene (BODIPY) fluorophore (**31**) in ethyl acetate solutions (complexes **32** to **35**).[22] The absorption and emission spectra of the complexes display the typical electronic transitions of the BODIPY unit, which correspond to the $S_0 \rightarrow S_1$ low-energy transition at 510 nm. However, the quantum yields of complexes **32** to **35** were found to be very small and this was attributed to fluorescence quenching caused by a photoinduced electron transfer (PET) mechanism. Compounds **32** to **35** were found to react with carbon monoxide *via* a ligand-exchange reaction whereby the 1,5-cyclooctadiene (cod) ligand is quantitatively substituted by CO (Scheme 9) to give the dicarbonyl complexes **36–39**. The exchange of the electron-rich diene ligand with the stronger π-acceptor CO leads to the formation of dicarbonyl species with less electron density on the metal and the revival of a strong emission (Φ = 0.58–0.65). The relationship between the difference in electron densities at the metal centre and the quantum yields obtained was confirmed by cyclic voltammetry measurements. These findings also provide support for the existence of quenching by PET in **32–35**, though internal charge transfer (ICT) processes are also known to operate in many BODIPY systems.[23] The complexes in this study were found to show a dose-related fluorescence response within seconds upon exposure to carbon monoxide and a detection limit of 1 nanomole of CO. No details were provided regarding competition from potential interferents in this particular study.

In contrast to the approaches described so far based on transition metal probes, a recently reported fluorescent chemosensor developed by Liu and co-workers (Scheme 10)[24] exploits the reactivity of CO with an organic substrate (albeit in the presence of a metal catalyst). In this system, the detection of CO in aqueous solution is achieved *via* a Grushin azidocarbonylation reaction[25] with sodium azide and a palladium catalyst. Starting from a weakly emissive naphthalene iodide precursor (**40**), the resulting strongly fluorescent naphthalene amine product **41** can be readily obtained at room temperature in the presence of moderate to high levels of CO. The sensing mechanism is based on the palladium-catalysed formation of the intermediate azidocarbonylated moiety which undergoes a Curtius rearrangement and subsequent hydrolysis to form the final product. In addition to the concentration of CO, the conversion is also dependent on the type of catalyst, the temperature and the pH of solution. By exploiting the 100-fold enhancement in the fluorescence quantum yield, CO can be detected with the naked eye in the range 50–1000 ppm when the compound is either impregnated onto a paper strip or simply in solution. The bright blue fluorescence emission is clearly visible to the naked eye upon irradiation with a UV lamp and the modulations in intensity can also be distinguished in the presence of different concentrations of CO. Moreover, no significant fluorescence enhancement was observed in the presence of other gases (CO_2, SO_2, NO and NO_2) or volatile organic compounds.

Very recently, work in our own laboratories has produced a ruthenium(II) pyrenylvinyl complex **42**, which shows an exceptional chromo-fluorogenic response towards CO in both solution and in solid state. Using **42**, CO can be detected at extremely low levels (5 ppb) thanks to the striking colour change from orange to bright yellow in the presence of carbon monoxide.[26] An even lower detection limit (1 ppb) is achieved *via* the fluorescence



Scheme 9 Synthesis of the bimetallic complexes **32–35** and their reaction with CO.



Scheme 10 Structure of the weakly fluorescent probe **40** and its reaction with CO.

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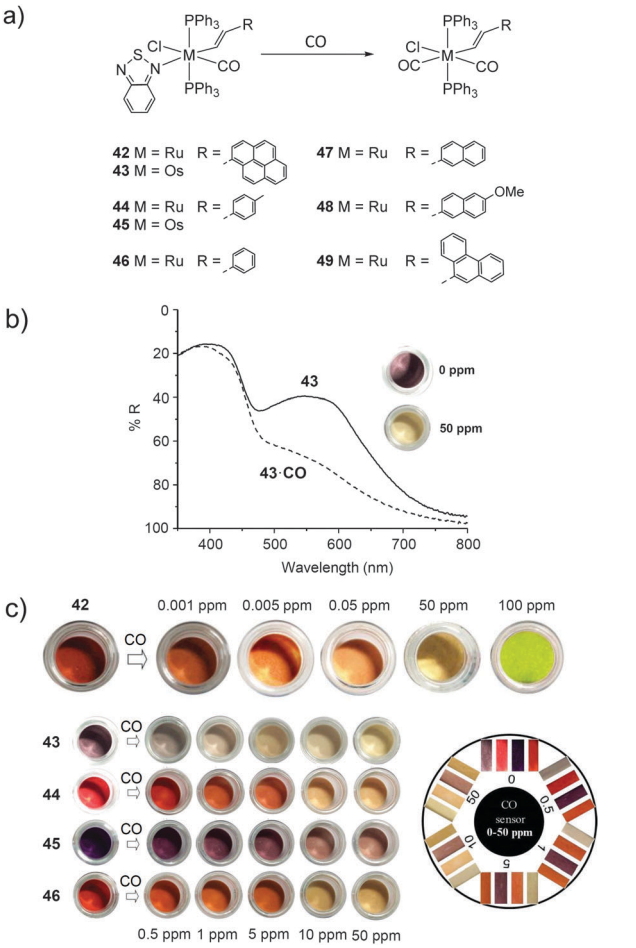
response generated by the system. At the centre of the sensing mechanism are two mutually *trans* ligands attached to the metal centre. One is the pyrenylvinyl group, responsible for the fluorescence response, and the other is the labile N-donor ligand 2,1,3-benzothiadiazole (BTD). In the presence of CO, the initial complex is converted to the corresponding pyrenylvinyl dicarbonyl compound *via* displacement of the BTD ligand. The change in the coordination environment produces not only the chromogenic effect (even at subtoxic concentrations) but also the concomitant revival of the emission of the pyrenyl group, which was previously quenched by the interaction with the BTD ligand. This represents a subtly different mechanism to the heavy atom effect usually used to suppress the fluorescence in the 'off' complex, as the metal-fluorophore connectivity remains unchanged during sensing. In terms of selectivity, no response is observed with other common gases, steam or volatile organic compounds. The immobilisation of the chemosensor on a cellulose strip also enables the use of this inexpensive probe in the portable device described above.[11]

In view of these results, a family of ruthenium(II) and osmium(II) vinyl analogues (**42–49**), was prepared and the sensing ability of the red (Ru) or purple (Os) complexes tested (Scheme 11).[27] Chloroform solutions of these complexes undergo a rapid colour change (Ru, red to yellow; Os, purple to yellow) in the presence of CO. Analogous colour modulations are observed when the compounds are adsorbed on silica supports. These colour variations allow the immediate naked-eye detection of CO in air at very low concentrations (ranging from 0.005 to 5 ppm). In a similar manner to compound **42**, the reaction of carbon monoxide (in both solution and air) with **48** and **49** results in the revival of a strong fluorescence emission in the 450 to 540 nm range. These systems are characterised by very low detection limits of 1.1 (**48**) and 10 (**49**) ppb of CO in air. Importantly, the chromo-fluorogenic response of this family of chemosensors is combined with a remarkable selectivity towards carbon monoxide over other gases and solvent vapours (including steam). Some change in colour is observed with extremely high concentrations of acetonitrile and NO_x (well above levels considered acutely toxic). However, neither acetonitrile nor NO_x can be considered plausible airborne interferents because of their low content in the atmosphere. The coordination of CO to these chemosensors appears to be essentially irreversible for the majority of the compounds. However, the related phosphavinyl compound $[Ru(P{=}CHBu^t)Cl(CO)(PPh_3)_2]$[28] demonstrates that the coordination of CO can be achieved in a reversible manner but, disappointingly, this behaviour was only observed in solution over a useful timescale.

Recent studies indicate that CO is a very important biological signalling molecule (gasotransmitter), which is endogenously produced during the haem catabolism and is implicated in vasodilatation, neurotransmission, anti-inflammatory and anti-apoptotic processes.[1,29] In order to unravel the biochemistry of CO within biological systems, researchers have been investigating fluorescence imaging and sensing as potential techniques to detect and monitor the presence of carbon monoxide in living cells. Despite the fact that the beneficial intracellular properties of carbon monoxide have been known for over a decade, it is only in the last few years that the detection of CO in living systems has been addressed.



Scheme 11 (a) Reaction of the ruthenium complexes **42–49** with CO; (b) diffuse reflectance UV-vis spectra for **43** and **43·CO**; (c) colour changes with CO on silica. Adapted and reprinted with permission from ref. 27. Copyright 2015 Wiley-VCH.

He and co-workers[30] were the first to describe a genetically encoded fluorescent protein (**50**) capable of the selective imaging of CO in living cells. Despite the complexity of the probe, the sensing mechanism is relatively simple and is based on the affinity of CO towards the iron(II) centre of the haem cofactor situated in the binding domain (Scheme 12). While the quaternary structure of this type of protein normally consists of only two major domains (the haem effector-binding domain and a DNA-binding domain), in this genetically encoded system a new domain (yellow fluorescent protein) is introduced in the sequence to induce the fluorogenic response in the presence of carbon monoxide. Upon reaction of the haem–iron(II) unit with CO to form **51**, the encoded protein undergoes a conformational change that exposes the yellow fluorescent protein, resulting in a 2-fold fluorescence enhancement at 528 nm (Fig. 1). The probe response is selective over other known gasotransmitters such as NO and H_2S.

Shortly after this contribution, Chang and co-workers[31] reported a palladium-BODIPY complex (**52**) with a CO-sensing

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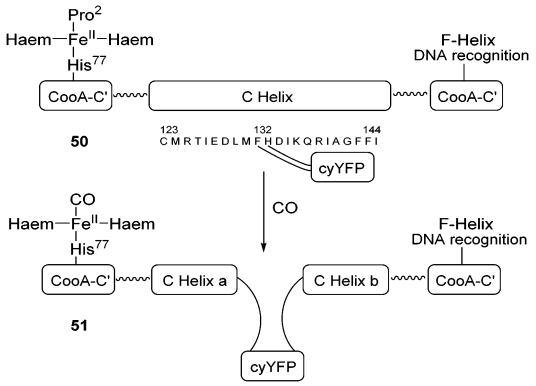


Scheme 12 Changes in the long C helix of CooA (a dimeric CO-sensing haem protein from *Rhodospirillum rubrum*) after CO binding (probe **50**).

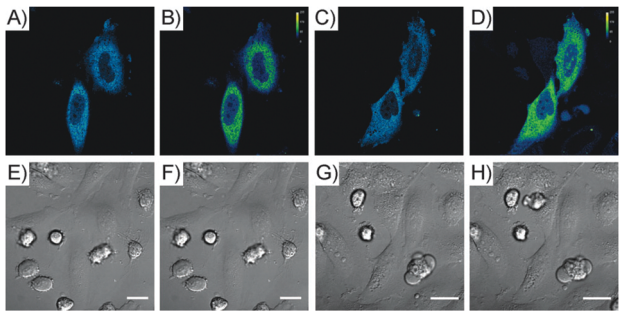
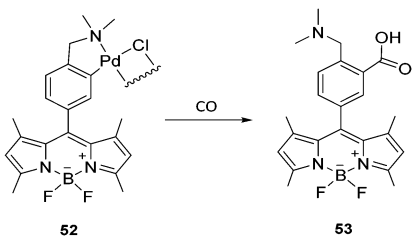


Fig. 1 Response of **50** to different concentrations of CO in living HeLa cells. (A–D) HeLa cells expressing **50** without added CO (A and C; control experiments), and with added CO (B: 5 μM; and D: 10 μM). (E–H) differential interference contrast images of live HeLa cells before and after CO. Scale bars: 20 μm. Reprinted with permission from ref. 30. Copyright 2012 Wiley-VCH.

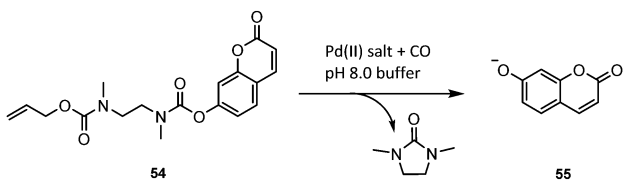
mechanism based on a selective, palladium-mediated carbonylation conducted under mild conditions to release the modified BODIPY ligand **53** (Scheme 13). The removal of the partial quenching provided by the palladium centre in **52** *via* the heavy-atom effect leads to the revival of the fluorescence of the BODIPY unit. The fluorescence properties and reactivity towards CO of **52** were monitored in aqueous solution buffered to physiological pH. The addition of 50 μM of [RuCl(glycinate)$(CO)_2$] (an easily handled CO source, CORM-3) resulted in a 10-fold increase in fluorescence within 60 min of reaction. The experiment, run in a Dulbecco's phosphate-buffered saline (DPBS) showed a detection limit as low as 1 μM (≈28 ppb CO). Overall, the probe also exhibits a good selectivity for CO over other small molecules such as H_2S, glutathione, NO, O_2, CN^-, imidazole and other species. When HEK293T cells were incubated with CORM-3 and treated with **52**, a significant, dose-dependent increase in the intracellular fluorescence was observed. In addition, the palladium-based probe **52** was found to be non-toxic to the cells tested.

As has been recently highlighted,[32] although these two fluorescent probes (**50** and **52**) display highly promising properties for the imaging of CO in living systems, improvements in terms of response time, signal-to-noise ratio and reversibility need to be improved. Based on these premises, Dhara *et al.* investigated the selective detection of CO in aqueous media using the coumarin-based probe **54**, which undergoes intramolecular cyclisation and elimination reactions in the presence of palladium(0) species (Scheme 14).[33] The luminescence of the organic derivative **54** is initially quenched due to the presence of the carbamate bond. However, when probe **54** is incubated with zero- or divalent palladium species (*e.g.*, $Pd(PPh_3)_4$, $PdCl_2$, $Pd(OAc)_2$, Na_2PdCl_4) and CO as a reducing agent, the highly fluorescent 7-hydroxycoumarin (**55**) is generated, resulting in a strong emission at 460 nm. In their experiments the authors used CORM-3 as source of CO. The highest fluorescence response was found at pH 8 when the 7-hydroxycoumarin is deprotonated. To simulate the cell environment, the probe was dissolved in a 10 mM HEPES solution (0.4% DMSO) and its fluorescence was measured at 37 °C. Under these conditions, the CO-induced turn-on fluorescence was found to be dependent on the dose of CORM-3 down to 1 μM (28 ppb). Furthermore, the response was found to be highly selective towards CO over various biologically relevant species such as NO, H_2S, NaOCl, H_2O_2, O_2^- and other small molecules containing sulfur and nitrogen. The probe was tested in human lung carcinoma cells, where a clear fluorescence response was observed on treatment with different concentrations of CORM-3.

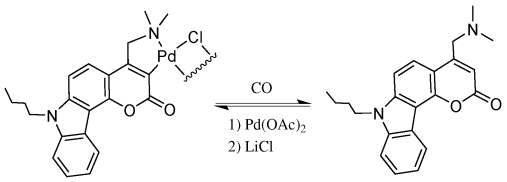
Inspired by the pioneering work of Chang, a cyclometallated palladium complex bearing a carbazole–coumarin-fused unit (**56**) has recently been reported for imaging carbon monoxide in living tissues (Scheme 15).[34] The metal-free carbazole–coumarin derivative (**57**) shows two-photon fluorescence due to the presence of a carbazole electron-donor group as well as a carbonyl electron-acceptor group, which together create a highly conjugated push–pull structure. The functionalisation of the coumarin derivative at the 4′-position allows the facile (re)generation of the cyclometallated palladium probe **56** from **57**. Due to the heavy atom effect of the palladium, a solution of **56** in PBS buffer and DMSO (9 : 1 v/v) at pH 7.4 and 37 °C



Scheme 13 Reactivity of the palladium complex **52** with CO.



Scheme 14 Reactivity of **54** with CO to give fluorescent **55**.



56 57

Scheme 15 Synthesis of the palladium complex **57** and its reaction with CO.

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displays a very weak one-photon fluorescence (Φ = 0.07) and a negligible two-photon emission. However, after the addition of $[Ru_2Cl_4(CO)_6]$ (CORM-2), a remarkable emission due to **57** is observed. The probe shows high sensitivity towards CO with a detection limit of 0.653 μM. Unlike the Pd-BODIPY probe (**52**) reported by Chang, **56** undergoes a protonolysis reaction in the presence of CO leading to the formation of **57**. This is strongly fluorescent, affording a one-photon fluorescence spectrum at 477 nm (Φ = 0.51) and a high two-photon cross section (σ = 50.1 GM) at 740 nm. Further studies at different pH values indicated that the probe is sufficiently stable with a maximum fluorescence response in the pH range of 7.0–9.0. The authors demonstrated that other biologically relevant analytes such as anions, reactive oxygen species (ROS), reducing agents, NO and H_2S induced no emission changes indicating that the probe is highly selective towards CO. Moreover, the incubation of the probe in the presence of CORM-2 with HeLa, MCF-7 and MKN-28 cells was carried out and resulted in a strong one-photon fluorescence response. Additionally, probe **56** was also found to be capable of detecting CO in living tissue slices of liver incubated with CORM-2 when subjected to two-photon fluorescence microscopic analysis.

Conclusions and outlook

The systems described above provide examples of the key design features currently employed in chromogenic and chromofluorogenic probes for CO detection in solution and air. Most of the reported probes are based on metal complexes that act as suitable centres for CO coordination. However, metal complexes can act as both the coordinating site and the luminophore upon CO binding. Alternatively, the sensing response can arise from the displacement by CO of a fluorophore, which was previously coordinated to, and quenched by, the metal centre. A few examples have also been shown in which the optical changes are induced by transition-metal catalysed reactions with CO. Despite these advances, the probes reported to date still display certain drawbacks such as poorly resolved changes in colour or emission, modest selectivity or long response times.

The design of a reversible probe which remains selective for CO over the other species present remains a challenge. The selectivity towards CO is often driven by the strong metal–carbon bond formed, but it is the same aspect which inhibits reversibility. Depending on the sphere of application and the interferents present, systems could be tuned electronically to bind CO less strongly, though some form of purging might still be necessary.

The immobilisation of molecular probes on supports (*e.g.*, silica, titania, zirconia, cellulose strips) shows great promise and appears the simplest way to provide an interface between these systems (especially chromogenic ones) and devices that could be used as alternatives to those available commercially. However, it is important not to overlook the benefits that a simple, low-cost, system (*e.g.*, cellulose strips) with a clear, visible colour change could deliver in certain settings.

The biological detection of carbon monoxide is still in its infancy and urgently needs probes with an improved fluorescence response over a shorter timeframe in order to monitor the role of endogenous CO in cells and tissues. However, the sensing systems reported so far show substantial promise, suggesting that an understanding of this fascinating role of carbon monoxide will soon be aided by further improvements in probe design.

Acknowledgements

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References

1 (*a*) R. Motterlini and L. E. Otterbein, *Nat. Rev.*, 2010, **9**, 728–743; (*b*) C. Szabo, *Nat. Rev. Drug Discovery*, 2016, **15**, 185–203.
2 S. H. Heinemann, T. Hoshi, M. Westerhausen and A. Schiller, *Chem. Commun.*, 2014, **50**, 3644–3660.
3 (*a*) J. J. McGrath, *Carbon Monoxide*, CRC, Boca Raton, 2nd edn, 2006, p. 695; (*b*) L. J. Wilkinson, in *Molecules of Death (Carbon Monoxide—the Silent Killer)*, ed. R. H. Waring, G. B. Steventon and S. C. Mitchell, Imperial College Press, London, 2007, pp. 37–46.
4 I. Blumenthal, *J. R. Soc. Med.*, 2001, **94**, 270–272.
5 (*a*) B. Wang, Y. Zhao, D. Yu, L. M. Hu, J. S. Cao, F. L. Gao, Y. Liu and L. J. Wang, *Chin. Sci. Bull.*, 2010, **55**, 228–232; (*b*) H. Ge and J. Liu, *Sens. Actuators, B*, 2006, **117**, 408–414.
6 K. K.-W. Lo and S. P.-Y. Li, *RSC Adv.*, 2014, **4**, 10560–10585.
7 C. W. Rogers and M. O. Wolf, *Coord. Chem. Rev.*, 2002, **233–234**, 341–350.
8 D. Benito-Garagorri, M. Puchberger, K. Mereiter and K. Kirchner, *Angew. Chem., Int. Ed.*, 2008, **47**, 9142–9145.
9 J. Esteban, J. V. Ros-Lis, R. Martínez-Máñez, M. D. Marcos, M. E. Moragues, J. Soto and F. Sancenón, *Angew. Chem., Int. Ed.*, 2010, **49**, 4934–4937.
10 M. E. Moragues, J. Esteban, J. V. Ros-Lis, R. Martínez-Máñez, M. D. Marcos, M. Martínez, J. Soto and F. Sancenón, *J. Am. Chem. Soc.*, 2011, **133**, 15762–15772.
11 M. E. Moragues, R. Montes-Robles, J. V. Ros-Lis, M. Alcañiz, J. Ibáñez, T. Pardo and R. Martínez-Máñez, *Sens. Actuators, B*, 2014, **191**, 257–263.
12 M. E. Moragues, R. Brines, M. C. Terencio, F. Sancenón, R. Martínez-Máñez and M. J. Alcaraz, *Inorg. Chem.*, 2013, **52**, 13806–13808.
13 M. Itou, Y. Araki, O. Ito and H. Kido, *Inorg. Chem.*, 2006, **45**, 6114–6116.

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14 (*a*) L. Basabe-Desmonts, D. N. Reinhoudt and M. Crego-Calama, *Chem. Soc. Rev.*, 2007, **36**, 993–1017; (*b*) A. B. Descalzo, R. Martínez-Máñez, F. Sancenón, K. Hoffmann and K. Rurack, *Angew. Chem., Int. Ed.*, 2006, **45**, 5924–5948.
15 J. I. Dulebohn, S. C. Haefner, K. A. Berglund and K. R. Dunbar, *Chem. Mater.*, 1992, **4**, 506–508.
16 S. C. Haefner, K. R. Dunbar and C. Bender, *J. Am. Chem. Soc.*, 1991, **113**, 9540–9553.
17 A. Gulino, T. Gupta, M. Altman, S. Lo Schiavo, P. G. Mineo, I. L. Fragalà, G. Evmenenko, P. Dutta and M. E. van der Boom, *Chem. Commun.*, 2008, 2900–2902.
18 (*a*) C. W. Rogers and M. O. Wolf, *Angew. Chem., Int. Ed.*, 2002, **41**, 1898–1900; (*b*) K. M. Matkovich, L. M. Thorne, M. O. Wolf, T. C. S. Pace, C. Bohne and B. O. Patrick, *Inorg. Chem.*, 2006, **45**, 4610–4618.
19 E. Lindner, M. Geprägs, K. Gierling, R. Fawzi and M. Steimann, *Inorg. Chem.*, 1995, **34**, 6106–6117.
20 H.-J. Park and Y. K. Chung, *Inorg. Chim. Acta*, 2012, **391**, 105–113.
21 H.-J. Park, K. Kim and Y. K. Chung, *Inorg. Chim. Acta*, 2014, **410**, 214–220.
22 P. Kos and H. Plenio, *Chem. – Eur. J.*, 2015, **21**, 1088–1095.
23 (*a*) L. Gai, J. Mack, H. Lu, H. Yamada, D. Kuzuhara, G. Lai, Z. Li and Z. Shen, *Chem. – Eur. J.*, 2014, **20**, 1091–1102; (*b*) L. Gai, J. Mack, H. Liu, Z. Xu, H. Lu and Z. Li, *Sens. Actuators, B*, 2013, **182**, 1–6; (*c*) H. Lu, J. Mack, Y. Yang and Z. Shen, *Chem. Soc. Rev.*, 2014, **43**, 4778–4823.
24 T. Yan, J. Chen, S. Wu, Z. Mao and Z. Liu, *Org. Lett.*, 2014, **16**, 3296–3299.
25 F. M. Miloserdov and V. V. Grushin, *Angew. Chem., Int. Ed.*, 2012, **51**, 3668–3672.
26 M. E. Moragues, A. Toscani, F. Sancenón, R. Martínez-Máñez, A. J. P. White and J. D. E. T. Wilton-Ely, *J. Am. Chem. Soc.*, 2014, **136**, 11930–11933.
27 A. Toscani, C. Marín-Hernández, M. E. Moragues, F. Sancenón, P. Dingwall, N. J. Brown, R. Martínez-Máñez, A. J. P. White and J. D. E. T. Wilton-Ely, *Chem. – Eur. J.*, 2015, **21**, 14529–14538.
28 R. B. Bedford, A. F. Hill, C. Jones, A. J. P. White, D. J. Williams and J. D. E. T. Wilton-Ely, *Organometallics*, 1998, **17**, 4744–4753.
29 P. Fagone, K. Mangano, M. Coco, V. Perciavalle, G. Garotta, C. C. Romao and F. Nicoletti, *Clin. Exp. Immunol.*, 2012, **167**, 179–187.
30 J. Wang, J. Karpus, B. S. Zhao, Z. Luo, P. R. Chen and C. He, *Angew. Chem., Int. Ed.*, 2012, **51**, 9652–9656.
31 B. W. Michel, A. R. Lippert and C. J. Chang, *J. Am. Chem. Soc.*, 2012, **134**, 15668–15671.
32 L. Yuan, W. Lin, L. Tan, K. Zheng and W. Huang, *Angew. Chem., Int. Ed.*, 2013, **52**, 1628–1630.
33 S. Pal, M. Mukherjee, B. Sen, S. K. Mandal, S. Lohar, P. Chattopadhyay and K. Dhara, *Chem. Commun.*, 2015, **51**, 4410–4413.
34 K. Zheng, W. Lin, L. Tan, H. Chen and H. Cui, *Chem. Sci.*, 2014, **5**, 3439–3448.