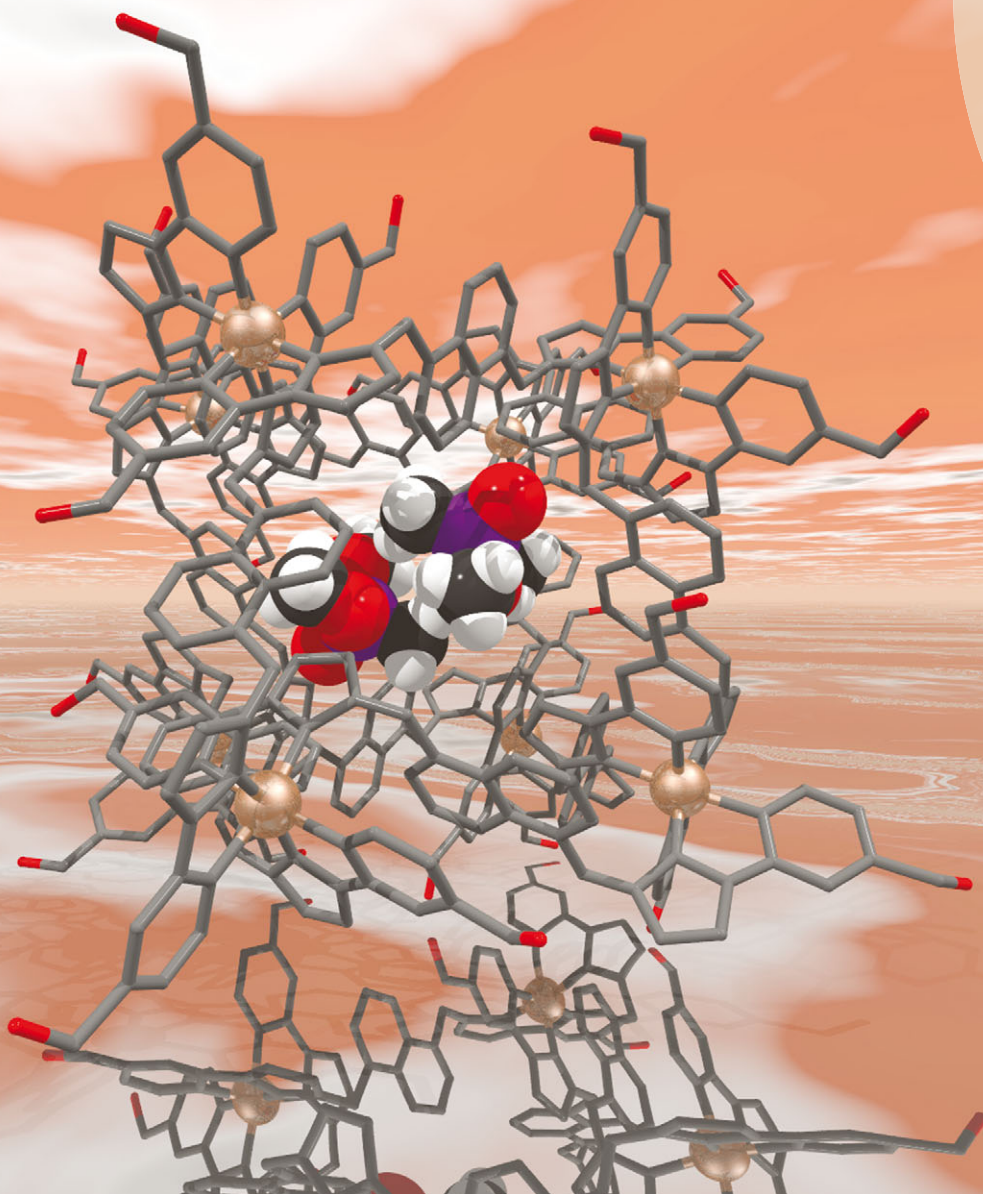


ChemComm

Chemical Communications

www.rsc.org/chemcomm



ISSN 1359-7345



COMMUNICATION

Michael D. Ward *et al.*

Binding of chemical warfare agent simulants as guests in a coordination cage: contributions to binding and a fluorescence-based response

175 YEARS



Cite this: *Chem. Commun.*, 2016, 52, 6225

Received 7th March 2016,
Accepted 22nd March 2016

DOI: 10.1039/c6cc02021f

www.rsc.org/chemcomm

Binding of chemical warfare agent simulants as guests in a coordination cage: contributions to binding and a fluorescence-based response†

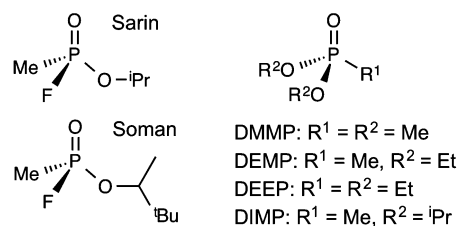
Christopher G. P. Taylor, Jerico R. Piper and Michael D. Ward*

Cubic coordination cages act as competent hosts for several alkyl phosphonates used as chemical warfare agent simulants; a range of cage/guest structures have been determined, contributions to guest binding analysed, and a fluorescent response to guest binding demonstrated.

Organophosphorus chemical warfare agents (CWAs; see Scheme 1 for examples) were developed during and immediately after the second world war. Their mode of action is well understood: they are derived from organophosphonates but substituted with excellent leaving groups which make them highly reactive phosphorylating agents, and in humans and animals they act as potent acetylcholinesterase inhibitors.¹ The relatively recent use of this type of CWA in a terrorist attack in Japan in 1994,² and their very recent use in Syria,³ graphically illustrates that they still constitute a significant threat. As such, strategies for detection, analysis and destruction of CWAs remain of high importance. As the chemistry of these relatively simple and reactive molecules is well understood there exist very many methods for their deactivation and destruction which have been comprehensively reviewed.⁴

Recently interest has turned to the supramolecular interactions of CWA molecules which have been, in contrast, relatively little explored.⁵ Molecular recognition of a CWA by a suitable host bearing a reporter group may be the basis of optical sensing.⁶ In addition the recently-developed ability of self-assembled hollow capsules and cages to effect catalytic transformations of bound guests offers interesting possibilities for selective recognition of a target molecule, binding in a cavity, and subsequent catalytic destruction.⁷ Whilst chemical destruction of CWAs by chemical methods such as treatment with powerful oxidants is undoubtedly effective,⁴ the selectivity and mild conditions that supramolecular catalysis can provide has obvious advantages.⁵

This, in turn, requires greater understanding of the supramolecular behaviour of CWA molecules. As CWAs themselves cannot



Scheme 1 Examples of organophosphorus CWAs and simulants.

be used outside of specialist facilities most work is done on 'simulants', which are generally alkyl phosphonates of a similar size/shape to CWAs but lacking the highly reactive leaving group (Scheme 1). Examples of the measurement and exploitation of supramolecular complexes with CWAs or simulants are relatively rare. Gale and co-workers have examined how phosphonate-based CWA simulants interact with hydrogen-bonded gels, which provides possibilities for both optical sensing and remediation.⁸ The same group has also shown how the hydrogen-bonding based recognition of some CWA simulants by 1,3-diindolylurea receptors leads to increased rates of hydrolytic destruction of the substrates.⁹ The catalytic destruction of CWA simulants in the cavities of metal-organic frameworks which contain strongly Lewis-acidic metal sites has been reported by Farha, Hupp and co-workers.¹⁰ Other examples of supramolecular complexes in which CWA simulants act as guests have been reported based on the use of cyclodextrin or cavitand-based hosts.⁵

Here we report the use of coordination cages as hosts for binding of a range of alkyl-phosphonate CWA simulants as guests and providing a luminescent response for detection purposes. The well-developed host-guest chemistry associated with the relatively rigid, hydrophobic cavities in such pseudo-spherical metal/ligand assemblies¹¹ means that such cages are particularly appealing targets as hosts for CWAs and their simulants, offering size/shape selective guest uptake and the possibility of enhanced reactivity of the guest in the unusual environment. To date however there is a just single example, from Nitschke's group, of a chlorophosphate insecticide acting as a guest in the cavity of a coordination cage and undergoing accelerated hydrolysis as a result.¹²

Department of Chemistry, University of Sheffield, Sheffield, S3 7HF, UK.

E-mail: m.d.ward@sheffield.ac.uk

† Electronic supplementary information (ESI) available: Experimental and crystallographic data. CCDC 1458105–1458107. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c6cc02021f



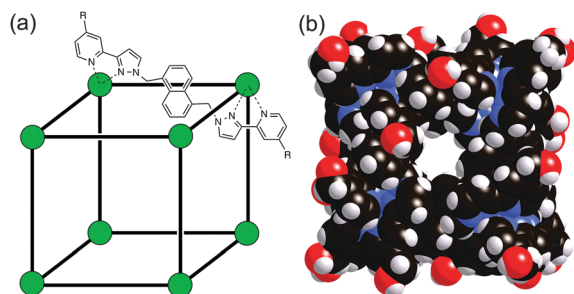


Fig. 1 (a) Sketch of the cubic host cages showing the arrangement of ligands along the edges (for Co-H^0 , $R = \text{H}$; for Co-H^W and Cd-H^W , $R = \text{CH}_2\text{OH}$); (b) a space-filling view of the Co-H^W cation, showing the O atoms of the hydroxyl groups in red (reproduced from ref. 14a).

The host cages that we describe here are the two M_8L_{12} cubic cages, with an $\text{M}(\text{II})$ ion at each vertex ($\text{M} = \text{Co}, \text{Cd}$) and a bis(pyrazolyl-pyridine) ligand along each edge, shown in Fig. 1: the guest binding properties have been studied by us in some detail in previous work.^{13,14} The central cavity, with a volume of *ca.* 400 \AA^3 , can accommodate a wide range of small molecule guests which have access through the pores in the centre of each face. In the unsubstituted cage Co-H^0 which is soluble in polar organic solvents such as MeCN, the dominant contribution to guest binding arises from hydrogen-bonding between an electron-rich site on the guest and a hydrogen-bond donor site on the interior surface of the cage.¹³ This affords binding constants in the range 10^3 – 10^4 M^{-1} in the best cases. In water, in contrast – using the derivatised cage Co-H^W (with 24 externally-directed HO groups to make it water-soluble, but otherwise isostructural to Co-H^0) the hydrophobic effect dominates guest binding, with binding constants of up to 10^8 M^{-1} being observed.¹⁴

The phosphonate-based guests that we used (Scheme 1) vary only in the size of the alkyl substituents. Their molecular volumes (calculated using Spartan-06) are: DMMP, 119 \AA^3 ; DEMP, 157 \AA^3 ; DEEP, 175 \AA^3 ; and DIMP, 193 \AA^3 . Based on the Rebek 55% rule¹⁵ we expect the optimal size for guest binding to be around 220 \AA^3 so there should be no significant steric problem associated with binding any of these. Binding constants were measured by conventional ^1H NMR titrations in both MeCN and water using the parent or functionalised cages respectively. As we have shown in other papers,^{13,14} the paramagnetism of the $\text{Co}(\text{II})$ ions disperses the ^1H NMR signals over a range of around 200 ppm, making it very easy to see changes in individual signals associated with guest binding. The smallest guest DMMP was in fast exchange between free/bound states in both MeCN and water, showing a steady shift in the signals for the cage as guest binding reached saturation, giving a binding curve which could be fit to a 1:1 or 2:1 host:guest isotherm. The larger two guests DEEP and DIMP were in slow exchange between free/bound states, giving separate signals for empty and bound cage which could be integrated to determine the K values. DEMP showed fast exchange in MeCN but slow exchange in water. Binding constants are in Table 1: each is the average of three independent measurements with quoted errors being two standard deviations.

In MeCN the binding constants are all quite small ($< 15 \text{ M}^{-1}$), with differences between them being marginal. In water a more obvious progression occurs with K increasing from $7(2) \text{ M}^{-1}$ for

Table 1 Binding constants (M^{-1}) at 298 K for the cage/guest complexes

	DMMP	DEMP	DEEP	DIMP
$\text{Co-H}^0/\text{CD}_3\text{CN}^a$	4(1)	14(3)	14(3)	9(1)
$\text{Co-H}^W/\text{D}_2\text{O}^a$	7(2) ^c	26(23)	160(30)	390(80)
$\text{Cd-H}^W/\text{H}_2\text{O}^b$	7(1) ^c	20(9)	31(9)	46(17)

^a Measured by NMR spectroscopic titrations (see ESI). ^b Measured by luminescence titrations (see ESI). ^c There are two DMMP guests and this is the K value for each – see ESI.

DMMP to $390(80) \text{ M}^{-1}$ for DIMP, corresponding to an increase in the magnitude of ΔG from $-4.8(6)$ to $-15.0(5) \text{ kJ mol}^{-1}$ per guest. Starting from DMMP, in order of increasing size the guests contain two, then three, and then four additional methylene groups which should contribute in an approximately stepwise manner to the strength of hydrophobic binding. We demonstrated recently with a series of aliphatic cyclic ketones of increasing size from cyclopentanone to cyclotridecanone that each additional CH_2 group added 4.7 kJ mol^{-1} to guest binding in water arising from the hydrophobic effect, until the point at which the guests became too large.^{14b} With these new examples we see similar behaviour but with the average increase in ΔG per CH_2 group being *ca.* 2.5 kJ mol^{-1} . This smaller hydrophobic contribution to binding of the alkyl phosphonates compared to the cyclic ketones could occur due to the greater flexibility of the alkyl chains in the former case compared to the latter, resulting in a greater entropic penalty for binding of the alkyl phosphonates due to less preorganisation compared to the more rigid cyclic ketones. It would also occur if the alkyl phosphonates are not fully desolvated on binding; there is structural evidence for this below. We note that for the $\text{Co-H}^W/\text{DMMP}$ system only, the NMR titration curve fitted best to a 2:1 guest: host stoichiometry with the two guests binding with equal affinity ($K_1 = K_2 = 7 \text{ M}^{-1}$, giving a global binding constant $K_1 \cdot K_2$ of *ca.* 50 M^{-2} for the pair of guests). The volume of two DMMP guests (238 \AA^3) is close to the optimal volume based on the Rebek 55% rule.¹⁵

To understand more about the specific interactions responsible for guest binding we determined the crystal structures of a series of the cage/guest complexes. These could be prepared either by growing crystals using conventional solvent-diffusion methods in a mixture containing both cage and guest; or by pre-growing crystals of the cage and then treating them with a concentrated solution of the guest, which resulted in guest molecules being taken up into the cage cavities without loss of crystallinity. The structure of $\text{Co-H}^W \cdot 2\text{DMMP}$ is in Fig. 2. In Fig. 2(a) is shown a view of the complete cage in which only the guests are shown in space-filling mode. The cage itself has the usual structure^{13,14} which requires no further comment. In nice agreement with the solution binding data, we see two molecules of DMMP in the cavity. The whole assembly is centrosymmetric with the two guest molecules (and two halves of the host cage) equivalent. Each DMMP guest is oriented such that the O atom from the $\text{P}=\text{O}$ bond is directed into one of the two pockets in opposite corners of the cage, at either end of a long diagonal, where there is a hydrogen-bond donor site arising from a convergent set of C–H protons associated with a *fac* tris-chelate $\text{Co}(\text{II})$ centre.^{13a,14b,c,16} As we showed recently these two pockets are the regions of the highest positive electrostatic potential on the cage



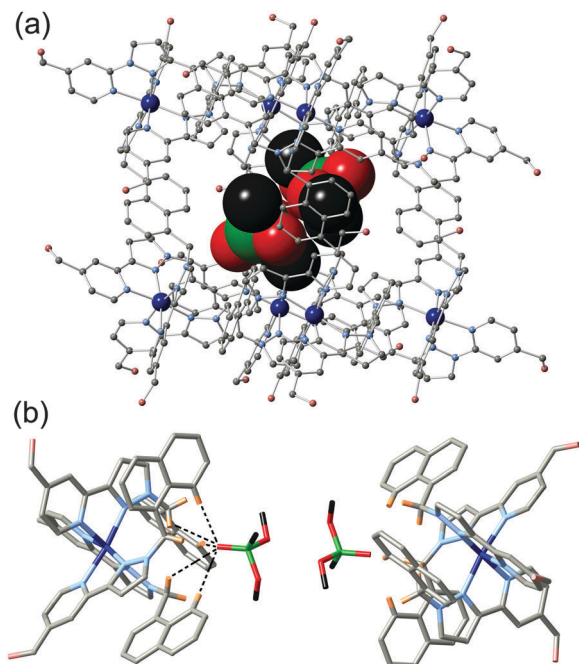


Fig. 2 Two views of the structure of **Co-H^w-2DMMP**: (a) the cubic cage showing both guests (shown space-filling); (b) the two guests showing how the P=O groups interact with the H-bond donor pockets at the *fac* tris-chelate vertices (P, green; O, red; N, blue; C, grey).

interior surface^{13a} and are invariably where the electron-rich atoms of guests lie as they can get close to the positively-charged metal vertex^{14b,c,16} [the P=O oxygen atom of the guest is just 5.40 Å from the *fac* tris-chelate Co(II) ion]. Fig. 2(b) is a view showing only these two vertices of the cage where the H-bonding pockets lie: both halves are equivalent, but on the left are shown with dotted lines those O...H interactions that are less than 3 Å, with the two shortest interactions both being 2.58 Å (the associated C...O distances in these CH...O hydrogen bonds are *ca.* 3.5 Å). The DMMP guests are rotationally disordered about the P=O bond such that the methyl and two methoxy groups are mutually disordered, but the P=O group is ordered and clearly defined. The two DMMP guests are mutually staggered about their P=O bonds to minimise steric problems.

The structures of **Co-H^p-DEEP-0.5H₂O** and **Co-H^p-DIMP** are in Fig. 3 and 4. In **Co-H^p-DEEP-0.5H₂O** the larger volume of the guest molecule compared to DMMP means that only one lies in the cavity. It is oriented in the same way as in the previous structure with DMMP, with the P=O group directed towards one of the *fac* tris-chelate vertices such that it participates in multiple CH...O hydrogen-bonds with the convergent array of CH protons at this site (O...H distances of under 3 Å are shown with dotted lines in Fig. 3(b)). The second binding pocket is occupied by a water molecule (50% occupancy), whose O atom occupies a similar position to the P=O oxygen atom in the other pocket: the O...Co separations are 5.55 Å (to water) and 5.45 Å (to the DEEP guest). The two guests are mutually disordered over both binding sites such that the asymmetric unit contains one DEEP and one water molecule. The ethyl groups of the DEEP guest also show disorder, only one component of which is shown in Fig. 3, but again the P=O group is

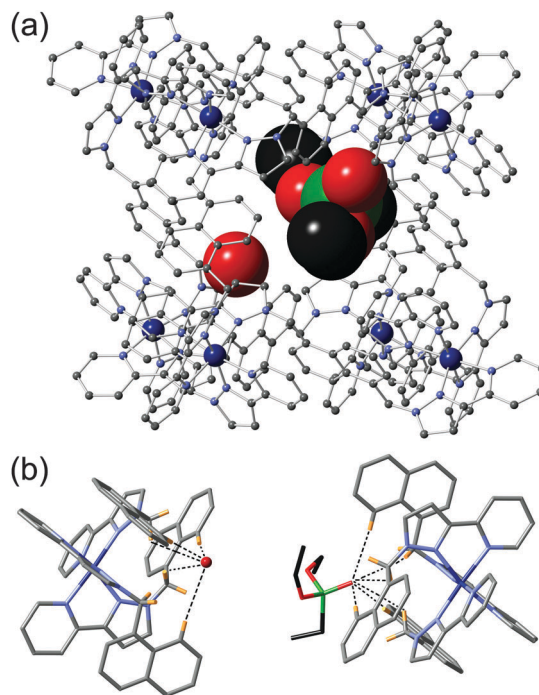


Fig. 3 Two views of the structure of **Co-H^p-DEEP-0.5H₂O**: (a) the host cage showing both guests (space-filling); (b) the two guests showing how the P=O group and the H₂O molecule interact with the H-bond donor pockets at the *fac* tris-chelate vertices (P, green; O, red; N, blue; C, grey).

ordered and well behaved. In **Co-H^p-DIMP** there is only one DIMP guest in the cavity, again oriented in the same way with the P=O group interacting with the hydrogen-bond donor pocket and an O...Co separation of 5.48 Å, and again disordered over the two possible binding pockets at diagonally opposite corners.

From these structures, and the binding constant data, we have a good picture of how these phosphonate guests bind. The hydrogen-bonding interaction between the P=O oxygen atom and the polar pocket on the cage interior surface provides orientational control and also, in MeCN, contributes to the driving force for guest inclusion.^{13a} In water the solvent will provide a better medium for hydrogen-bonding than the cage interior surface,^{14a,b} so the binding is predominantly driven by the hydrophobic effect as it scales with the number of methylene groups in the guest.^{14b} but once the desolvated guest is bound, the H-bonding to the cage surface provides the orientational control that we see in these and other^{14b,c,f} crystal structures of cage/guest complexes. We do not reach the point with these relatively small phosphonates where the guests become too large to bind.

The presence of naphthyl fluorophores in the cage¹⁷ provides a possible mechanism for an easily-visible optical response to guest binding. As water provides stronger binding than MeCN, and is of course a far more appropriate solvent for any potential real-world application involving sensing of CWA binding, we were interested to see if the binding that we have seen translates into luminescence-based sensing in water. For this application the cage **Co-H^w** is not ideal as the luminescence for the naphthyl groups is substantially quenched by the Co(II) ions. However, we can replace Co(II) by Cd(II) which is non-quenching due to its d¹⁰ configuration, to make an



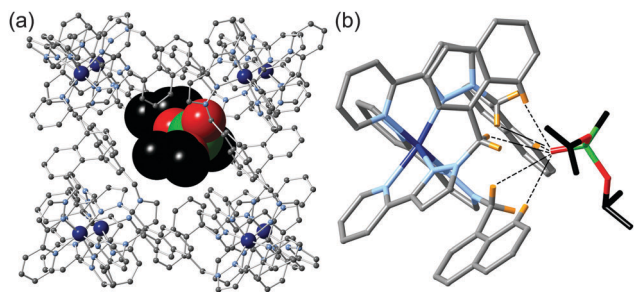


Fig. 4 Two views of the structure of Co-H^{w} -DIMP: (a) the host cage showing the guest (space-filling); (b) the guest showing how the $\text{P}=\text{O}$ group interacts with the H-bond donor pockets at the *fac* tris-chelate vertices (P, green; O, red; N, blue; C, grey).

isostructural water-soluble cage which will bind the CWA simulants with the possibility of a luminescent response.

The Cd(II) -based cage $[\text{Cd}_8(\text{L}^{\text{w}})_{12}](\text{BF}_4)_{16}$ was prepared from the ligand and $\text{Cd}(\text{BF}_4)_2$ in precisely the same way as the Co(II) analogue (see ESI[†]); to improve its water solubility further the anion was exchanged to nitrate to give $[\text{Cd}_8(\text{L}^{\text{w}})_{12}](\text{NO}_3)_{16}$ (Cd-H^{w}). The luminescence spectrum in water shows a broad band at 400 nm, substantially red-shifted from the usual naphthalene luminescence profile due to the participation of the naphthyl groups in extended π -stacked arrays around the cage periphery, as we have described before.¹⁷ Titration with the phosphonate guest series was accompanied by a steady reduction in luminescence as the phosphonate was incorporated into the cage cavity. The quenching of cage luminescence on titration with DIMP is shown in Fig. 5; the intensity variation here (and with DEEP and DEMP) fitted well to a 1:1 binding model with the values given in Table 1. With DMMP/ Cd-H^{w} a 2:1 guest:cage model fit the data, as we saw earlier for Co-H^{w} , and Table 1 includes the individual K value per guest. These K values are somewhat different from the values observed for binding in Co-H^{w} by ^1H NMR spectroscopy, though the general trend is the same. Given the different ionic radius of Cd(II) compared to Co(II) , and the presence of a different anion, some variation in the binding constants between Cd-H^{w} and Co-H^{w} is not surprising.

In conclusion we have shown that the small alkyl phosphonates commonly used as CWA simulants can bind in the cavity of

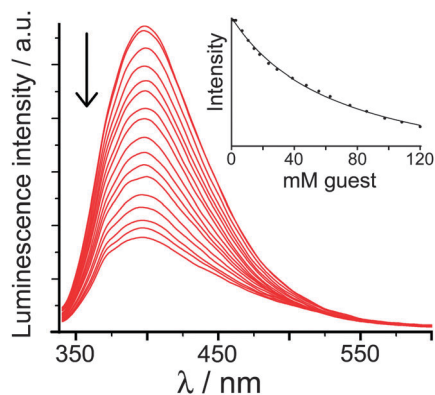


Fig. 5 Decrease in luminescence intensity of Cd-H^{w} (10 μM in water) as DIMP is added. Inset: Change in luminescence intensity during the titrations fitted to a 1:1 binding isotherm [$K = 46(17) \text{ M}^{-1}$, see Table 1].

the cubic coordination cages Co-H^{w} , Co-H^{w} and Cd-H^{w} and we have identified both polar (hydrogen-bonding to the interior surface of the cage) and hydrophobic contributions to binding. This binding results in partial quenching of the fluorescence of Cd-H^{w} , providing a possible basis for luminescence sensing of CWAs using supramolecular methods.

We thank the EPSRC for financial support (grant EP/J00124/1), Dr Will Cullen for assistance with ligand syntheses, and Dr Sandra van Meurs for the NMR data (see ESI[†]).

Notes and references

- 1 S. M. Somani, *Chemical Warfare Agents*, Academic Press, San Diego, 1992.
- 2 (a) N. Masuda, M. Takatsu, H. Morinari, T. Ozawa, H. Nozaki and N. Aikawa, *Lancet*, 1995, **345**, 1446; (b) T. Suzuki, H. Morita, K. Ono, K. Maekawa, R. Nagai, Y. Yazaki, H. Nozaki, N. Aikawa, Y. Shinozawa, S. Hori, S. Fujishima, K. Takuma and M. Sagoh, *Lancet*, 1995, **345**, 980.
- 3 (a) E. Dolgin, *Nat. Med.*, 2013, **19**, 1194; (b) *Science*, 2013, **341**, 1324 (editorial material).
- 4 K. Kim, O. G. Tsay, D. A. Atwood and D. G. Churchill, *Chem. Rev.*, 2011, **111**, 5345.
- 5 M. R. Sambrook and S. Notman, *Chem. Soc. Rev.*, 2013, **42**, 9251.
- 6 Z. Lei and Y. Yang, *J. Am. Chem. Soc.*, 2014, **136**, 6594.
- 7 C. J. Brown, F. D. Toste, R. G. Bergman and K. N. Raymond, *Chem. Rev.*, 2015, **115**, 3012.
- 8 (a) J. R. Hiscock, F. Piana, M. R. Sambrook, N. J. Wells, A. J. Clark, J. C. Vincent, N. Busschaert, R. C. D. Brown and P. A. Gale, *Chem. Commun.*, 2013, **49**, 9119; (b) J. R. Hiscock, I. L. Kirby, J. Herniman, G. J. Langley, A. J. Clark and P. A. Gale, *RSC Adv.*, 2014, **4**, 45517; (c) F. Piana, M. Facciotti, G. Pileio, J. R. Hiscock, W. V. Rossom, R. C. D. Brown and P. A. Gale, *RSC Adv.*, 2015, **5**, 12287.
- 9 (a) M. R. Sambrook, J. R. Hiscock, A. Cook, A. C. Green, I. Holden, J. C. Vincent and P. A. Gale, *Chem. Commun.*, 2012, **48**, 5605; (b) A. Barba-Bon, A. M. Costero, M. Parra, S. Gil, R. Martínez-Mañez, F. Sancenón, P. A. Gale and J. R. Hiscock, *Chem. – Eur. J.*, 2013, **19**, 1586.
- 10 (a) S.-Y. Moon, Y. Liu, J. T. Hupp and O. K. Farha, *Angew. Chem., Int. Ed. Engl.*, 2015, **54**, 6795; (b) J. E. Mondloch, M. J. Katz, W. C. Isley III, P. Ghosh, P. Liao, W. Bury, G. W. Wagner, M. G. Hall, J. B. DeCoste, G. W. Peterson, R. Q. Snurr, C. J. Cramer, J. T. Hupp and O. K. Farha, *Nat. Mater.*, 2015, **14**, 512.
- 11 (a) M. Yoshizawa, J. K. Klosterman and M. Fujita, *Angew. Chem., Int. Ed.*, 2009, **48**, 3418; (b) T. R. Cook, Y.-R. Zheng and P. J. Stang, *Chem. Rev.*, 2013, **113**, 734; (c) M. D. Pluth, R. G. Bergman and K. N. Raymond, *Acc. Chem. Res.*, 2009, **42**, 1650; (d) M. M. J. Smulders, I. A. Riddell, C. Browne and J. R. Nitschke, *Chem. Soc. Rev.*, 2013, **42**, 1728; (e) S. Zarra, D. M. Wood, D. A. Roberts and J. R. Nitschke, *Chem. Soc. Rev.*, 2015, **44**, 419; (f) M. D. Ward and P. R. Raithby, *Chem. Soc. Rev.*, 2013, **42**, 1619; (g) C. J. Brown, F. D. Toste, R. G. Bergman and K. N. Raymond, *Chem. Rev.*, 2015, **115**, 3012.
- 12 J. L. Bolliger, A. M. Berlunguer and J. R. Nitschke, *Angew. Chem., Int. Ed.*, 2013, **52**, 7958.
- 13 (a) S. Turega, M. Whitehead, B. R. Hall, A. J. H. M. Meijer, C. A. Hunter and M. D. Ward, *Inorg. Chem.*, 2013, **52**, 1122; (b) S. Turega, M. Whitehead, B. R. Hall, M. F. Haddow, C. A. Hunter and M. D. Ward, *Chem. Commun.*, 2012, **48**, 2752.
- 14 (a) M. Whitehead, S. Turega, A. Stephenson, C. A. Hunter and M. D. Ward, *Chem. Sci.*, 2013, **4**, 2744; (b) S. Turega, W. Cullen, M. Whitehead, C. A. Hunter and M. D. Ward, *J. Am. Chem. Soc.*, 2014, **136**, 8475; (c) W. Cullen, S. Turega, C. A. Hunter and M. D. Ward, *Chem. Sci.*, 2015, **6**, 625; (d) W. Cullen, S. Turega, C. A. Hunter and M. D. Ward, *Chem. Sci.*, 2015, **6**, 2790; (e) W. Cullen, K. A. Thomas, C. A. Hunter and M. D. Ward, *Chem. Sci.*, 2015, **6**, 4025; (f) W. Cullen, M. C. Misuraca, C. A. Hunter, N. H. Williams and M. D. Ward, *Nat. Chem.*, 2016, **8**, 231.
- 15 (a) M. R. Ams, D. Ajami, S. L. Craig, J. S. Yang and J. Rebek, *J. Am. Chem. Soc.*, 2009, **131**, 13190; (b) S. Mecozzi and J. Rebek, *Chem. – Eur. J.*, 1998, **4**, 1016; (c) J. Rebek, *Acc. Chem. Res.*, 2009, **42**, 1660.
- 16 A. J. Metherell, W. Cullen, A. Stephenson, C. A. Hunter and M. D. Ward, *Dalton Trans.*, 2014, **43**, 71.
- 17 (a) N. K. Al-Rasbi, C. Sabatini, F. Barigelli and M. D. Ward, *Dalton Trans.*, 2006, 4769; (b) I. S. Tidmarsh, T. B. Faust, H. Adams, L. P. Harding, L. Russo, W. Clegg and M. D. Ward, *J. Am. Chem. Soc.*, 2008, **130**, 15167.

