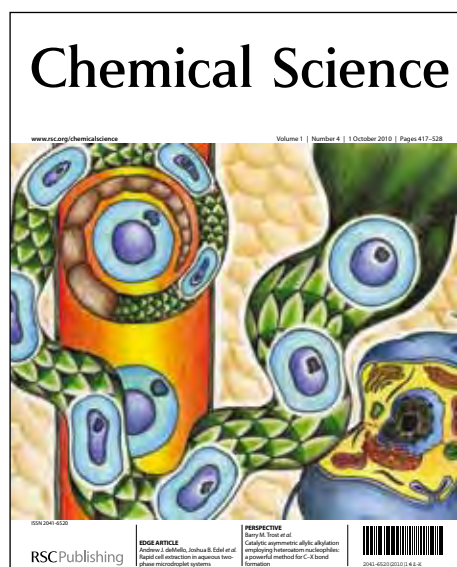


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## ARTICLE

# A Light-induced Reversible Phase Separation and its Coupling to a Dynamic Library of Imines

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Ghislaine Vantomme, Nema Hafezi and Jean-Marie Lehn\*

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Irradiation of a 3:2 acetonitrile/water solution of the bis-pyridyl hydrazone **1** and calcium chloride causes a photo-induced phase separation, due to an increase in free calcium cations as a consequence of the photo-conversion of **1** from its E form to the Z configuration. Complexation studies confirmed that the former binds calcium more strongly than the latter. Acid-catalyzed back-conversion from **1-Z** to **1-E**, followed by basification, leads to the regeneration of the initial single phase. The process thus involves a photo-thermal cycle with reversible interconversion between monophasic and biphasic states. In presence of a dynamic library of imines generated from hydrophilic and hydrophobic aldehyde and amine components, a coupling to the phase interconversion processes is achieved, leading to a dynamic redistribution of the imine constituents, whereby amplification of the most lipophilic and the most hydrophilic imine constituent, respectively in the organic and in the aqueous phase, is achieved by component selection. The system thus undergoes an adaptation by up-regulation of the fittest constituent for its specific phase. The process is reversible, regenerating the initial distribution of constituents upon phase reunification. It also represents the coupling of a dynamic covalent library to out-of-equilibrium conditions resulting from the photo-generation of a kinetically trapped entity.

## Introduction

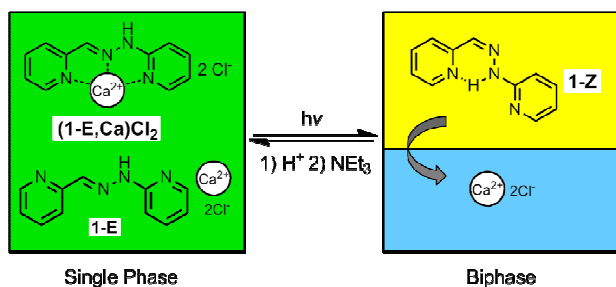
Binary liquid mixtures which can separate into two distinct phases by action of a physical stimulus or a chemical effector have been extensively used over the years in the purification of components of mixtures,<sup>[1]</sup> the development of bulk membranes,<sup>[2]</sup> the extraction of proteins,<sup>[3]</sup> etc. They may also be implemented for the modulation of the composition of dynamic libraries, generated by dynamic covalent processes,<sup>[4]</sup> subjected to biphasic as well as to three-phase transport conditions.<sup>[5]</sup> We have recently described the coupling of such dynamic covalent systems to reversible phase separation and shown that they respond by adaptation of their constituents to phase distribution through component selection.<sup>[6]</sup>

On the other hand, the induction of a phase separation by light may attract particular interest due to its potential applications in light controlled actuators,<sup>[7]</sup> photo-driven drug delivery,<sup>[8]</sup> and transport across bulk liquid membranes,<sup>[9]</sup> involving for instance photo-induced electron transport.<sup>[9d]</sup> Unrelated cases of photo-induced phase separation have been reported.<sup>[10]</sup> Furthermore, we have shown recently that photoisomerization of a ligand in a supramolecular solid-state assembly leads to the release of the metal cations,<sup>[11]</sup> a process that may allow for phase separation of a binary liquid mixture.<sup>[6]</sup>

In our earlier exploration of the response of dynamic covalent systems of imine constituents to phase separation,<sup>[6]</sup> the phase

separations were produced by addition of a chemical effector (such as an inorganic salt or a carbohydrate) or by a physical stimulus (e.g. temperature) to partition a mixture of acetonitrile (AN) and water (W). On the other hand, prior work from our laboratory demonstrated the photorelease of potassium ions from photoswitchable acyl hydrazones.<sup>[11]</sup> We now realized the merging of these two processes by coupling phase separation through photo-induced cation release with a dynamic covalent system undergoing constituent redistribution by component exchange.

More specifically, we demonstrate herein, i) the use of light as a non-invasive technique towards inducing a phase separation of a binary mixture of two *bulk solvents*, AN and W, by the light-induced release of a divalent cation (calcium) from its complex (**1-E**,Ca)Cl<sub>2</sub> with the photo-responsive ligand **1**, that undergoes photo-isomerization from its E form, **1-E**, to the weaker binding Z state, **1-Z**,<sup>[12,13]</sup> ii) the reversibility of this system by acid catalysed thermal back-isomerization followed by basification leading to reunification of the binary mixture into a single phase (Fig. 1), and iii) the successful coupling of the photo-thermal reversible process to a dynamic covalent library of imines that responds to medium change by adaptation of its composition.



**Fig. 1** Photoisomerization of the ligand **1-E** forming the  $(\mathbf{1-E}, \text{Ca})\text{Cl}_2$  complex, to the **1-Z** form, inducing an increase of free  $\text{Ca}^{2+}$  cations in solution, thus causing phase separation. Phase reunification by back conversion of **1-Z** to **1-E** via sequential protonation and restoration of initial pH with triethylamine (see text). For clarity, only the **1-Z** form and the generation of free calcium ions are represented on the right hand side. The single phase used had the composition AN/W 3:2 v/v, i.e. 34% AN and 66% W mole percent in all experiments.

## Results

### Calcium binding and photo-induced reversible phase separation by the hydrazone **1**.

**Rationale.** The photo-induced phase separation produced in the present work utilizes the ability of a pyridyl hydrazone ligand (**1**) to form complexes with calcium ions (see below). This hydrazone presents orthogonally responsive features,<sup>[12]</sup> comprising 1) constitutional dynamics as well as double-configurational dynamic properties, 2) complexation to a cation with its NNN tridentate binding site,<sup>[14]</sup> and 3) E to Z photochemical isomerization about the CH=N bond, stabilized by an internal hydrogen bond to the pyridine moiety.<sup>[12,15]</sup> The latter two are exploited here to produce a reversible phase separation induced by light. Upon irradiation of a 1:1 solution of **1** and calcium chloride in a 3:2 (v/v) AN/W mixture, photoisomerization of **1-E** to **1-Z** decreases the amount of  $(\mathbf{1-E}, \text{Ca})\text{Cl}_2$  complex. As a consequence, calcium ions are expected to be released, since they bind more weakly to the photogenerated form **1-Z** (see below), thus leading to an increase in the concentration of free cations (Fig. 1). Reaching the ionic threshold is expressed visibly at the macroscopic scale by the phase separation of the AN/W mixture into a biphasic system. In all experiments and results reported below the composition of the homogenous medium was 3:2 AN/W v/v, i.e. 34% AN and 66% W mole percent. In order to substantiate these expectations, the calcium complexation ability of both forms **1-E** and **1-Z** were first investigated.

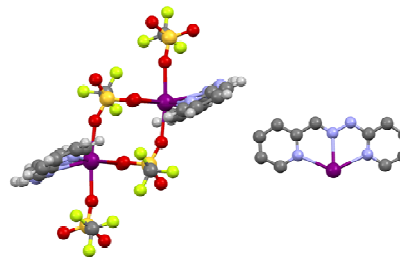
#### Calcium binding by hydrazone **1**.

**Isolation and structures of complexes of 1-E with calcium cations.** Pyridyl-hydrazone **1-E** had been examined over forty years ago as a multidentate ligand for transition metals.<sup>[14]</sup> It was noted in passing that no observable strong complex was formed between **1-E** and magnesium or calcium ions in dilute aqueous solutions.<sup>[14e]</sup>

For the present purpose, as well as in view of the broader significance of the complexation of the biologically important calcium cation, a closer examination was warranted. Indeed, crystals of three different complexes of **1-E** with calcium cations could be obtained, depending on the counterion, by vapor diffusion of diisopropylether into a solution of **1-E** and a calcium salt in AN or methanol (see captions of Fig. 2, 3 and

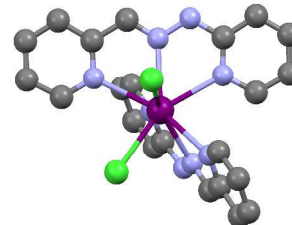
4). The X-ray crystal structures of all three were determined, unequivocally demonstrating the ability of **1-E** to complex calcium cations.

One of the complexes was found to be a dimeric anion bridged complex of 1:1 stoichiometry (Fig. 2).



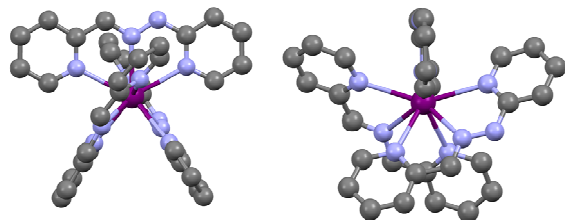
**Fig. 2** (Left) Solid state molecular structure of the dimeric complex formed from two  $(\mathbf{1-E}, \text{Ca})\text{OTf}_2$  1:1 complexes by bridging through two triflate counterions. (Right) View of an isolated 1:1  $(\mathbf{1-E}, \text{Ca}^{2+})$  complex. Protons and triflate counterions are omitted for clarity. Crystals were obtained by vapor diffusion of diisopropylether into a solution of **1-E** and  $\text{Ca}(\text{OTf})_2$  in AN.† (gray = carbon; red = oxygen; blue = nitrogen, purple = calcium, yellow = fluoride, orange = sulfur)

The second complex has a 2:1 stoichiometry  $[(\mathbf{1-E})_2, \text{Ca}]\text{Cl}_2$ , with each  $\text{Ca}^{2+}$  ion bound in an octa-coordinated fashion involving the six nitrogen sites of two ligands **1-E** and two chloride anions (Fig. 3). In addition, each chloride anion is hydrogen bonded to one of the N-H sites of the pyridyl-hydrazone of another complex (see Fig. 1, SI).



**Fig. 3** Solid state molecular structure of the  $[(\mathbf{1-E})_2, \text{Ca}]\text{Cl}_2$  complex. Protons are omitted for clarity. Crystals were obtained by vapor diffusion of diisopropylether into a solution of **1-E** and  $\text{CaCl}_2$  in methanol.† (gray = carbon; green = chloride; blue = nitrogen, purple = calcium)

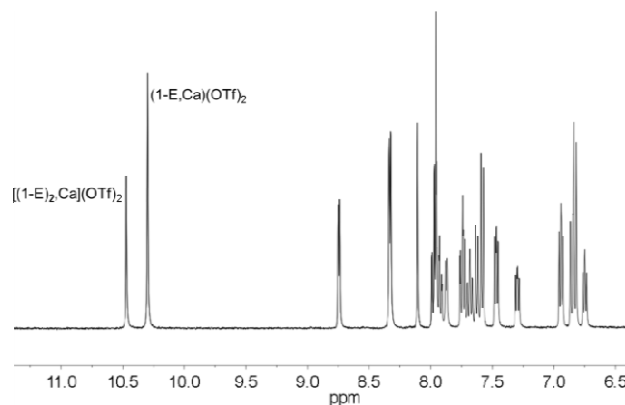
Finally, other crystals were obtained which yielded a 3:1 complex (Fig. 4), in which the calcium ion interacts with the nine nitrogen sites of the three **1-E** ligands. Complexes of all three stoichiometries have been observed by <sup>1</sup>H NMR (see Fig. 5 below and Fig. 5 in SI).



**Fig. 4** Two views of the solid state molecular structure of the 3:1 [(1-E)<sub>3</sub>,Ca](ClO<sub>4</sub>)<sub>2</sub> complex. (*Left*) One ligand **1-E** lies in plane and the two others are in perpendicular planes. (*Right*) View perpendicular to that on the left. Perchlorate anions and protons are omitted for clarity. The C17 and N6 atoms are disordered over two identical positions. Crystals were obtained by vapor diffusion of diisopropylether into a solution of **1-E** and Ca(ClO<sub>4</sub>)<sub>2</sub> in methanol.<sup>†</sup> (gray = carbon; blue = nitrogen, purple = calcium)

*Binding constants of 1-E and 1-Z with calcium cations.* When a suspension of **1-E** (355 mM) in 3:2 AN/W was treated with CaCl<sub>2</sub> (305 mM), instead of the CaCl<sub>2</sub>-induced phase separation expected on the basis of our previous work,<sup>[6]</sup> a rapid uptake of **1-E** into solution was observed, giving rise to a single-phase homogeneous solution at 25°C. The reverse experiment was also performed, wherein a solution of 3:2 AN/W was phase separated with CaCl<sub>2</sub> and subsequently treated with 1.2 eq. of **1-E** with respect to CaCl<sub>2</sub>. Again, a rapid uptake of **1-E** was observed, accompanied now by phase reunification of the AN/W biphasic. Thus, it became clear that the observed behaviour was due to the complexation of the calcium ions by **1-E**, in homogeneous AN/W solution. The stability constant of the complex (1-E,Ca)Cl<sub>2</sub> in 3:2 AN/W was found to be 11.0 ± 1.4 M<sup>-1</sup> at 25°C, as determined by analysis of the UV-vis spectroscopy and confirmed by <sup>1</sup>H NMR titration data obtained on progressive addition of CaCl<sub>2</sub> to **1-E** (see Fig. 2-3, SI). The 1:1 stoichiometry in AN/W agreed, within experimental accuracy, with a study by Job's method using <sup>1</sup>H NMR data at 25°C (see Fig. 4, SI). It is also in line with NMR data in pure AN (see below). While the crystal structures confirm the formation of complexes of Ca<sup>2+</sup> with **1-E**, the coordination of the cation with two or three ligands is not apparent in solution, due presumably to the weakness of the binding of a second and third ligand in the AN/W medium and to the low sensitivity of the detection methods.

In order to obtain more information about the relative calcium binding abilities of the **1-E** and **1-Z** forms, the complexation was also studied in pure AN, instead of the binary 3:2 AN/W medium, where the binding by **1-Z** is too weak for reliable determination in the experimental conditions used. <sup>1</sup>H NMR titration measurements on progressive addition of Ca(OTf)<sub>2</sub> to **1-E** in AN at -40°C showed the presence of three complexes of 1:1, 2:1 and 3:1 ligand:cation stoichiometry and of the free ligand in slow exchange (Fig. 5 and in SI Fig. 5). Simple integration of the N-H proton signals provided the corresponding binding constants of **1-E** with Ca<sup>2+</sup>: log β([(1-E)<sub>n</sub>,Ca](OTf)<sub>2</sub>) = 3.5 (n=1), 6.3 (n=2), 7.9 (n=3) ± 0.3. The Job plot from the <sup>1</sup>H NMR titration data showed two complexes of 3:1 and 1:1 stoichiometry for increasing amounts of Ca(OTf)<sub>2</sub> in AN at 25°C (see Fig. 7, SI).



**Fig. 5** A portion of the 400 MHz <sup>1</sup>H NMR spectrum of a solution of **1-E** and Ca(OTf)<sub>2</sub> (10 mM each) in AN-*d*<sub>3</sub> at -40°C showing the presence of the two complexes (1-E,Ca)(OTf)<sub>2</sub> and [(1-E)<sub>2</sub>,Ca](OTf)<sub>2</sub>. The 3:1 complex and the free ligand are observable at different stoichiometries of **1-E** and Ca(OTf)<sub>2</sub> (see Fig. 5, SI).

The binding constants of **1-Z** with Ca<sup>2+</sup> was found to be log β((1-Z,Ca)(OTf)<sub>2</sub>) = 2.6 ± 0.3 at 25°C in AN as determined by <sup>1</sup>H NMR titration on progressive addition of Ca(OTf)<sub>2</sub> to **1-Z** (see Fig. 8, SI). In this case, slow exchange was not observed at -40°C, so that the binding constants could not be directly evaluated as in the case of the **1-E** form. The 1:1 stoichiometry of (1-Z,Ca)(OTf)<sub>2</sub> in AN agreed with a Job's plot of the <sup>1</sup>H NMR data at 25°C (see Fig. 9, SI). To confirm the stronger binding of Ca<sup>2+</sup> to **1-E** compared to **1-Z** in the same conditions, a <sup>1</sup>H NMR competition experiment has been performed at -40°C on an AN solution of Ca(OTf)<sub>2</sub>, **1-E** and **1-Z** (10 mM each). The resulting spectrum was compared to that obtained with **1-E** alone in presence of Ca(OTf)<sub>2</sub> (10 mM each in AN; see also Fig. 10, SI). The results showed a change in the relative integration values of the <sup>1</sup>H NMR signals corresponding to the loss of 0.19 eq. Ca<sup>2+</sup> from the (1-E,Ca)(OTf)<sub>2</sub> and [(1-E)<sub>2</sub>,Ca](OTf)<sub>2</sub> complexes, due to binding by **1-Z**, in agreement with the binding constants calculated above. The binding of Ca<sup>2+</sup> by **1-Z** may be expected to occur in a bidentate fashion, in line with the observations made for the acylhydrazone analogue **3** of the hydrazone **1** (see below).

#### Photo-induced reversible phase separation with the hydrazone **1**.

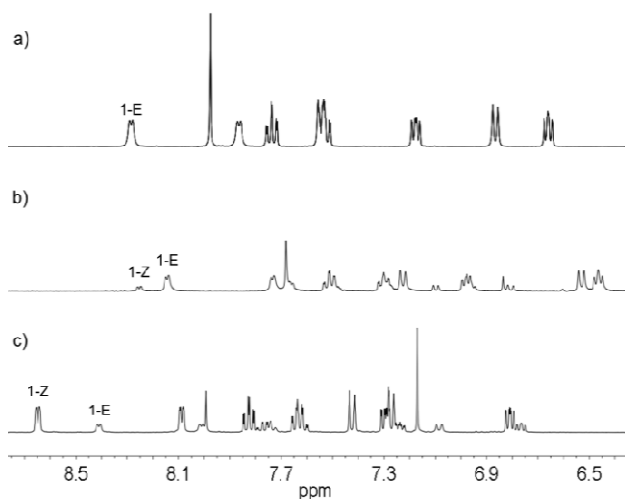
It was mentioned previously, that **1-E** undergoes photo-isomerization to the metastable **1-Z** configuration, stabilized by an intramolecular hydrogen bond.<sup>[12]</sup> As shown above, calcium binding is weaker for **1-Z** than for **1-E**, so that on irradiation the amount of (1-E,Ca)Cl<sub>2</sub> complex decreases and calcium cations are released, thus restoring their kosmotropic properties and inducing a phase separation (Fig. 1). After four hours of light exposure (see Experimental, SI) of a 3:2 AN/W solution of 355 mM **1** and 305 mM CaCl<sub>2</sub> in a thermostated bath at 25°C, 68% of **1-E** was isomerized into **1-Z** and the AN/W mixture had separated into two phases due to the release of 115 mM of free calcium ions into solution, as calculated from the amounts of **1-E** and **1-Z** present in solution (obtained by integration of the corresponding <sup>1</sup>H NMR signals), using the calcium binding constant of **1-E** obtained above in AN/W and assuming that binding of Ca<sup>2+</sup> by **1-Z** is negligible in these conditions. The contents of each phase in **1**, (1-E,Ca)Cl<sub>2</sub> complex and calcium cations before and after light irradiation are given in Table 1. The isomerization of **1-E** was followed by

$^1\text{H}$  NMR (Fig. 6). The compositions of the separated phases were 84% W (in mole percent) (both v/v and mole % are used) for the bottom aqueous phase and 57% AN for the top organic phase. The phase separation was visible after one hour of irradiation but the difference in AN/W composition of the two phases increased further on longer irradiation.

**Table 1** Concentrations of **1**,  $(\mathbf{1-E,Ca})\text{Cl}_2$  complex and calcium cations before and after light irradiation<sup>a</sup>

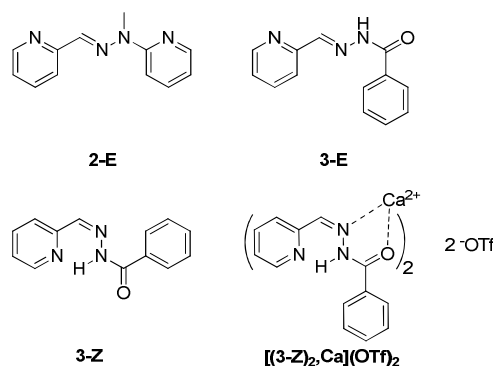
	Before irradiation	After irradiation
[ <b>1</b> ] total (mM)	355	355
[Ca <sup>2+</sup> ] total (mM)	305	305
[ <b>1-E</b> ] (mM)	355	(45 OPh, 540 APh)
[ <b>1-Z</b> ] (mM)	0	115
[ <b>1-E</b> ] free (mM)	160	(160 OPh, 60 APh)
[ $(\mathbf{1-E,Ca})\text{Cl}_2$ ] (mM)	195	240
[Ca <sup>2+</sup> ] free (mM)	110	(440 OPh, 30 APh)
[Ca <sup>2+</sup> ]-released (mM)		35
		80
		225
		115

<sup>a</sup> Irradiation for 4 h for an initial single phase solution in 3:2 AN/W. Concentrations calculated using the stability constant determined (see text). After photo-induced phase separation, the two phases had the composition: 57% AN in the top organic phase and 84% W in the aqueous bottom phase (in mole percent). The total fractions summed over the two phases were 68% of **1-Z** and 32% of **1-E** in part free and in part complexed by  $\text{CaCl}_2$ . The concentrations in each phase (organic, OPh, and aqueous, APh) are also indicated. The amount of calcium in each phase was determined by atomic absorption (values in parentheses, second line,  $\pm 2\%$ ).



**Fig. 6** Aromatic proton portion of the 400 MHz  $^1\text{H}$  NMR spectra of a solution of 355 mM **1-E** and 305 mM of  $\text{CaCl}_2$  in 3:2 AN/W before light irradiation (a) and after light irradiation in the aqueous (b) and organic (c) phases.

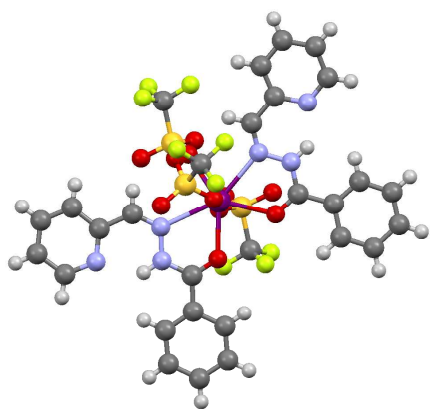
*Phase reunification* is expected to be obtained by back conversion of **1** from *Z* to *E* configuration. However, such reunification of the biphasic solution did not take place in the course of one month at 25°C, indicating high kinetic stability of **1-Z**. Nevertheless, on acidification by addition of hydrochloric acid (>37% solution, about 2.5 eq. with respect to **1-E** to reach pH about 1.0), the back conversion from **1-Z** to **1-E** occurred in less than 5 minutes, as observed by  $^1\text{H}$  NMR at 25°C. The two phases were then still separated due to the protonation of **1-E**, which is incapable of binding  $\text{Ca}^{2+}$  ions. Subsequent basification of the solution by addition of triethylamine (1.1 eq. with respect to hydrochloric acid, to reach the initial pH of 9.5), allowed for the regeneration of the complex  $(\mathbf{1-E,Ca})\text{Cl}_2$  in solution and hence, produced a single AN/W phase. The newly reunified solution can be irradiated again to obtain a phase separation, thus confirming the reversibility of the process. The *N*-methylated hydrazone **2-E** did not cause the reunification of the AN/W biphasic induced by  $\text{CaCl}_2$ , underscoring the importance of the acidic, ionisable N-H bond,<sup>[17]</sup> which reinforces the electronic density at the NH nitrogen site in **1-E**, whereas the presence of the methyl group in **2-E** leads to weaker complexation of calcium cations (see SI, Fig. 11-12 for more details).<sup>[18]</sup>



### Calcium binding and photo-induced reversible phase separation by the acyl-hydrazone **3**.

In view of the constitutional analogies of acyl-hydrazones with pyridyl-hydrazones and their wide range of possible structural variations as well as for comparison purposes, some studies were also conducted with the acyl-hydrazone **3**.

*Isolation and structures of complexes of 3-E and 3-Z with calcium cations.* Solid state molecular structures were determined by X-ray crystallography for two complexes of the acyl-hydrazone **3**. One of them is a dimeric association of two neutral 2:1 **3-E**: $\text{Ca}^{2+}$  complexes with the ligands in their ionised form at the N-H site (see more details and Fig. 14 in SI). The other one is a 2:1 **3-Z**: $\text{Ca}^{2+}$  complex,  $[(\mathbf{3-Z})_2,\text{Ca}](\text{OTf})_2$ , that confirms in this case the binding of  $\text{Ca}^{2+}$  by the *Z* form of the ligand. It shows the formation of a dimer between two ligands **3-Z** and two triflate anions with the  $\text{Ca}^{2+}$  cation bound to each ligand in a bidentate N,O fashion (Fig. 7).



**Fig. 7** Solid state molecular structure of the  $[(3-Z)_2Ca](OTf)_2$  complex. Crystals obtained by slow vapor diffusion of diisopropylether into a solution of **3-Z** and  $Ca(OTf)_2$  in AN.† (gray = carbon; white = hydrogen; red = oxygen; blue = nitrogen, purple = calcium, yellow = fluoride, orange = sulfur)

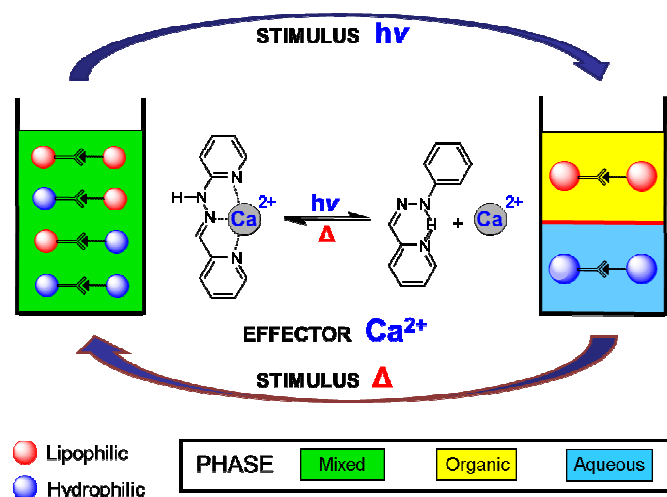
**Binding constants of 3-E and 3-Z with calcium cations.** In order to obtain information about the binding of calcium cations by **3-E** and **3-Z**, spectroscopic experiments were performed. The stability constant of the complex  $(3-E,Ca)Cl_2$  in 3:2 AN/W was found to be  $13.2 \pm 1.34 M^{-1}$  at 25°C, from the analysis of the UV-vis spectroscopy titration data obtained by progressive addition of  $CaCl_2$  to **3-E** (see Fig. 15, SI). The 1:1 stoichiometry in AN/W was confirmed by Job's method using  $^1H$  NMR data at 25°C (see Fig. 16, SI). No evidence of formation of a complex between **3-Z** and  $CaCl_2$  was obtained in AN/W mixture by analysis of the UV-vis spectroscopy titration data obtained by progressive addition of  $CaCl_2$  to **3-Z**. Therefore, as for **1-E** and **1-Z** above, the binding constants and stoichiometries for the calcium complexes of **3-E** and **3-Z** were also studied in pure AN. Binding constants of **3-E** and **3-Z** with  $Ca^{2+}$  were found to be respectively  $\log \beta[(3-E)_nCa](OTf)_2 = 3.1$  ( $n=1$ ),  $6.4$  ( $n=2$ )  $\pm 0.3$  and  $\log \beta[(3-Z)_nCa](OTf)_2 = 2.6$  ( $n=1$ ),  $5.0$  ( $n=2$ )  $\pm 0.3$  as determined by  $^1H$  NMR titration on progressive addition of  $Ca(OTf)_2$  to **3** at 25°C in AN (see Fig. 17-18, SI). In this case, exchange was fast on the NMR time scale, so that the evaluation of the stability constants was based on the shifts of specific signals on calcium addition as well as on a competition experiment between the two forms (see Fig. 17-19, SI). The 2:1 stoichiometry of  $[(3-E)_2Ca](OTf)_2$  and  $[(3-Z)_2Ca](OTf)_2$  in AN was confirmed by Job's method using  $^1H$  NMR data at 25°C (see Fig. 20-21, SI).

**Photo-induced reversible phase separation with the acylhydrazone 3.** Photo-induced phase separation was also obtained with the pyridyl-acylhydrazone **3-E**, as with **1-E** (see above), by conversion to the corresponding **3-Z** form. However, a larger amount of **3-E** (400 mM, 1.3 eq. with respect to  $CaCl_2$ ) was needed to reunify the phase separated by 305 mM of  $CaCl_2$ . After 4 hours of light irradiation, 71% of **3-E** were isomerized into **3-Z**. The compositions of the two phases were 85% W for the aqueous phase and 44% AN for the organic phase, showing a less efficient phase separation than with **1-E** confirmed by the quantity of  $CaCl_2$  found in each phase by atomic absorption (100 mM OPh, 455 mM APH, compared with data for **1-E** in Table 1). This behaviour may be attributed to the occurrence of binding of  $Ca^{2+}$  to **3-Z**, as indeed seen in the solid state (see above) and to the interaction between **3-Z** and water. In contrast with the case of **1-Z**, phase reunification was

achieved after 48 hours at 40°C without addition of acid, by purely thermal **3-Z** to **3-E** back-conversion. Thermal Z to E conversion is thus easier for the acylhydrazone than for its pyridyl analog, a property that may be of broader interest for establishing photo-thermal E-Z interconversion cycles. The temperature of 40°C was low enough so as not to produce phase reunification by itself.

### Coupling of photo-induced phase separation to component selection and constitutional adaptation in a dynamic library of imines.

The phase separation of an organo-aqueous AN/W mixture leads to the formation of two distinct solvent environments from a single one. These two media differ considerably from the homogenous phase in their physico-chemical properties, and promote in a constitutional dynamic system, the formation of the fittest compounds in their respective environments.<sup>[6]</sup> Thus, we extended the previous study to the coupling of the present photo-induced phase separation process to a dynamic covalent library responding to these different solvent environments, along the overall process represented in Fig. 8.

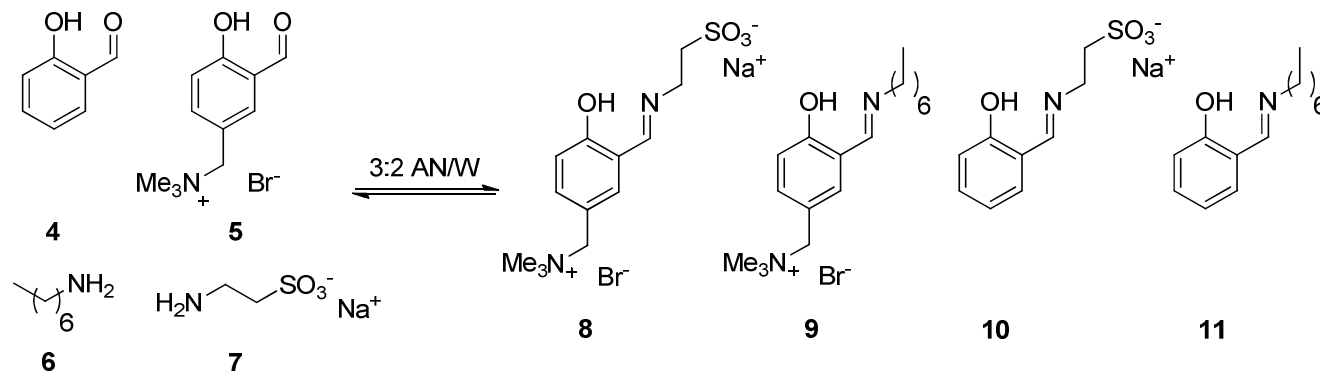


**Fig. 8** Representation of the coupling of a dynamic library of imines, derived from lipophilic and hydrophilic amines and aldehydes, to the photo-thermal reversible phase separation cycle comprising: (left to right) the photo-isomerization (stimulus  $h\nu$ ) of ligand **1-E** to its **1-Z** form leads to a decrease in the amount of  $(1-E,Ca)Cl_2$  complex, inducing the release of free  $Ca^{2+}$  cations (effectors) into solution and the simultaneous phase separation, and (right to left) the thermal (stimulus  $\Delta$ ) back-conversion of **1-Z** to **1-E**, causing phase reunification. The dynamic library responds by reversible component selection and adaptation to the single/double phase interconversion. For clarity, the small amounts of mixed (amphiphilic) constituents distributed in both phases are not represented in the phase separated state at the right. Similarly, the free **1-E** and calcium ions are not shown (middle).

A dynamic covalent library of imines was generated from hydrophilic/hydrophobic pairs of aldehydes and amines. Thus, the aldehydes **4** and **5** were reacted with the amines **6** and **7** to give the imines **8-11** (Scheme 1).<sup>[6]</sup> A 40 mM solution of equimolar amounts of compounds **4-7** in 3:2 AN/W, together with 305 mM  $CaCl_2$ , produced a given distribution of imines, as observed by  $^1H$  NMR and quantified by integrating the  $CH=N$

proton signals against an external standard. The four imines possess either hydrophilic-hydrophilic (**8**), hydrophobic-hydrophobic (**11**), or amphiphilic (**9-10**) properties. The choice

of aldehydes and amines was determined by the high formation propensity of salicylaldehydes<sup>[19]</sup> and their stability towards light irradiation.



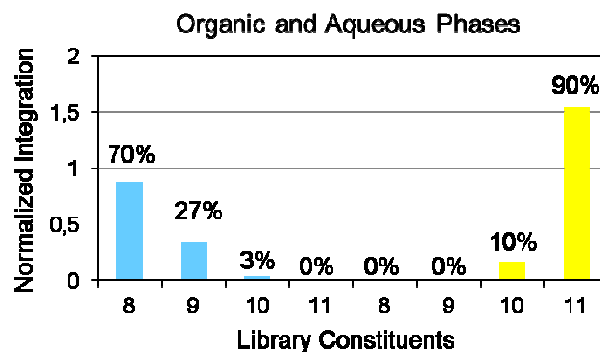
**Scheme 1** Dynamic covalent library of the imine constituents **8** (hydrophilic-hydrophilic), **11** (hydrophobic-hydrophobic) and **9-10** (amphiphilic), generated by reaction of the hydrophobic/hydrophilic pairs of salicylaldehyde **4** and **5** and amine **6** and **7** components.

The derivative of **1** deuterated in positions  $\alpha$  and 6 (see SI) was prepared to facilitate the integration of the CH=N proton NMR signals which would otherwise overlap with proton signals of **1-E**. In 3:2 AN/W in presence of ( $\alpha$ -*d*,6-*d*)**1-E** (8.8 eq. with respect to the library components, 355 mM) and CaCl<sub>2</sub> (7.7 eq. with respect to the library components, 305 mM), 72% of imine constituents **8-11** were formed and 28% of aldehydes and amines **4-7** were left due to hydrolysis. The library presented a 20/18/26/36 % distribution for the imine constituents **8/9/10/11** respectively (see Fig. 22 in SI).

*Phase separation* was performed by irradiating a solution of 355 mM ( $\alpha$ -*d*,6-*d*)**1-E** and 305 mM CaCl<sub>2</sub> in presence of 40 mM of each of the library components **4-7** for 9h at 25°C, which gave a 49% conversion of **1-E** into **1-Z**, causing the release of Ca<sup>2+</sup> cations into the solution, as shown above. The composition of the separated phases obtained had then the same AN and W contents as in the absence of the library after 4h irradiation. Thus, a longer irradiation time was required in presence of the library to achieve a similar composition of the two separated phases.

The dynamic library underwent reorganization on phase separation to promote the bis-hydrophilic **8** in the aqueous phase and the bis-hydrophobic **11** in the organic phase. The distribution obtained was 0/0/10/90 % in the organic phase and 70/27/3/0 % in the aqueous phase for the imine constituents **8/9/10/11** respectively (Fig. 9). The total fraction (summed over both phases) amounted to 30% **8** and 52% **11**, as compared to 20% of **8** and 37% of **11** in the starting homogeneous phase (see Fig. 23 in SI). These percentages indicate an overall amplification/up-regulation of **8** and **11** on photo-induced phase separation and represent an adaptation of the system to the creation of two separate media. One may note that there was somewhat more hydrolysis after phase separation, which reduced the total amount of library imine constituents (The hydrolysis was of 28% before phase separation and of 37% after phase separation). To minimize the hydrolysis, the experiments described here were performed at the highest concentration

of the library components that could be used (40 mM). Higher concentration of the library caused a phase separation due to the charged components. The distribution of the library in the two phases was depended on the contents in AN and W in each phase, and was modified by a change in their AN and W contents.



**Fig. 9** Distribution of the dynamic covalent library of the four different imine constituents **8-11** (40 mM each), in the presence of 305 mM CaCl<sub>2</sub>, upon phase separation of a 3:2 AN/W solution into a biphasic system by light irradiation for 9h. The relative amounts in the respective phases, aqueous (blue, left) and organic (yellow, right), were determined by integration of the corresponding -CH=N proton signals against an external standard.

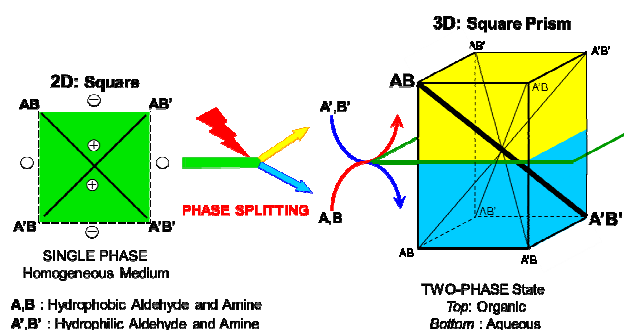
*Phase reunification* was thereafter obtained by back conversion from **1-Z** to **1-E**. First, on addition of hydrochloric acid (>37% solution, about 2.5 eq. with respect to **1-E** to reach a pH of about 1.0) into the biphasic AN/W at 25°C **1-Z** was converted to **1-E** in its protonated form. In this acidic medium, the library of imines was totally hydrolysed. Thereafter, basification of the biphasic with triethylamine (1.1 eq. with respect to hydrochloric acid, to reach the initial pH) led to the restoration of the single phase with again, as initially, a distribution of 24/19/27/29 % of

8/9/10/11 respectively. The quantity of charged species in solution was increased by addition of acid and base, leading to a slight change in the distribution of imines. The entire process was repeated in the same set up, underlining its reversibility. On multiple cycling, the salt content changes, thus limiting the number of cycles.

## Conclusions

The results reported above provide a system performing a reversible modulation of a liquid mixture between single and double phase states and demonstrate its coupling to a constitutional dynamic library by a combination of photochemical and thermal processes (Fig. 8). They lead to several conclusions.

- 1) Light-irradiation of a single phase solution of the photoswitchable hydrazone **1-E** and calcium chloride in AN/W results in the isomerization of **1-E** into its isomer **1-Z** and causes a photo-induced phase separation, resulting from an increase in free  $\text{Ca}^{2+}$  cations in the solution, due to a weaker binding of these cations to **1-Z**. The ionic threshold of the cation concentration is visible at the macroscopic scale by the separation of the single AN/W phase.
- 2) The phase separation is reversible and may be repeated. The light-triggered biphasic AN/W solution was reunified by back-conversion from **1-Z** to **1-E**, in presence of acid. Subsequent basification restores the initial single phase solution.
- 3) Acyl-hydrazones such as **3** undergo similar interconversions and give in principle access to a wide range of potential modulating ligands.
- 4) The overall process consists in a *photo-thermal cycle*, whereby a photo-induced out-of-equilibrium state, at both the molecular (metastable **Z** form) and macroscopic levels, is restored to thermodynamic equilibrium by a thermal process (it is strictly so for the acyl-hydrazones **3-E/3-Z** and aided by acid catalysis for the pyridyl analogues **1-E/1-Z**).
- 5) The reversible phase separation cycle may be coupled to a dynamic covalent library of imines allowing for the photomodulation of the expression and distribution of the different library constituents, with amplification of the fittest constituents with respect to a specific phase and restoration of the initial library upon phase reunification. The system thus performs an adaptation by up-regulation of the fittest constituents through component selection.
- 6) The behaviour of the coupled system described here may be represented within the framework of constitutional dynamic networks<sup>[4],6,20]</sup> as a photo-induced switching between a 2D (square) and a 3D (square prism) network state (Fig. 10).



**Fig. 10** Splitting of a 2D (square) into a 3D (square prism) constitutional dynamic network by photo-induced liquid/liquid phase separation and phase transfer of the interconverting constituents **AB**, **A'B'**, **A'B** and **AB'** across the interface (green plane) with adaptation to each phase through component exchange. The diagonals of the square prism link agonistic (+) constituents, the vertical edges link antagonistic (-) constituents across the interface. 2D relationships are conserved in each phase.

7) The light-induced changes in environment described above may be considered as a photoswitchable liquid membrane system where the organic phase represents the membrane. Exchanges take place at the interface leading to a redistribution of the dynamic library of imines. This process may in principle be implemented in a three phase active transport system, whereby the phase separation establishes a photo-induced non-equilibrium state to drive a substrate through the membrane, eventually by a carrier mechanism.<sup>[21]</sup> Such a transport system would present the attractive feature of creating a potential by a clean stimulus, light, acting via a macroscopic phase separation.

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## Notes and references

<sup>a</sup> Laboratoire de Chimie Supramoléculaire, Institut de Science et d'Ingénierie Supramoléculaires (ISIS), Université de Strasbourg, 8 allée Gaspard Monge, 67000 Strasbourg, France

† CCDC 971152-971155 contain the supplementary crystallographic data for this paper.

Electronic Supplementary Information (ESI) available: [Synthesis of ( $\alpha$ -*d*,6-*d*)**1-E**, UV spectra, <sup>1</sup>H NMR spectra, Job plots, fitting for determination of binding constants, X-ray structures]. See DOI: 10.1039/b000000x/



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addition of **1-E** when phase separation had been caused by means of these salts. On addition of 1 eq. of LiCl, MgCl<sub>2</sub> or BaCl<sub>2</sub>, no <sup>1</sup>H NMR shifts were observed. The stability constant of the complex (**1-E,Sr**)Cl<sub>2</sub> was found to be about 4.3 ± 0.7 M<sup>-1</sup> at 25°C in 3:2 AN/W (v/v) as determined by analysis of the UV-vis spectroscopy titration data obtained on progressive addition of SrCl<sub>2</sub> to **1-E** (see Fig. 13, SI). The binding of **1-E** with transition metal cations is much stronger<sup>[14]</sup>, but such salts (for instance ZnCl<sub>2</sub>, PbCl<sub>2</sub> ...) were not able to cause a phase separation, as they are soluble in AN.

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