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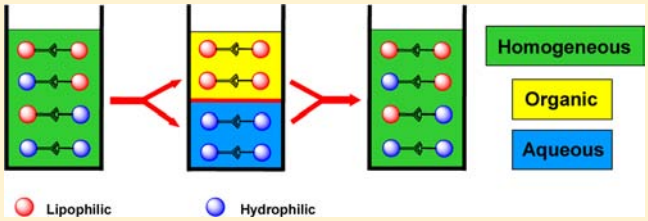
Adaptation of Dynamic Covalent Systems of Imine Constituents to Medium Change by Component Redistribution under Reversible Phase Separation

Nema Hafezi and Jean-Marie Lehn*

Institut de Science et d'Ingénierie Supramoléculaires, ISIS Université de Strasbourg, 8 allée Gaspard Monge, Strasbourg 67000, France

Supporting Information

ABSTRACT: A dynamic covalent library of interconverting imine constituents, dissolved in an acetonitrile/water mixture, undergoes constitutional reorganization upon phase separation induced by a physical stimulus (heat) or a chemical effector (inorganic salt, carbohydrate, organic solvent). The process has been made reversible, regenerating the initial library upon phase reunification. It represents the behavior of a dynamic covalent library upon reversible phase separation and its adaptation to a phase change, with up-regulation in each phase of the fittest constituents by component selection. Finally, the system exemplifies the splitting of a 2D (square) constitutional dynamic network into a 3D (cube) one.



INTRODUCTION

Constitutional dynamic chemistry (CDC)[1] encompasses chemical systems based on noncovalent interactions as well as on reversible covalent reactions, the latter defining dynamic covalent chemistry (DCC).[2] Such systems may respond to the application of physical stimuli or chemical effectors by undergoing adaptation of their constituents through constitutional variation via component exchange and selection. Physical stimuli that have been applied to dynamic covalent systems include temperature,[3] crystallization,[4] mechanical stress,[5] light,[6] and an electric field,[7] while chemical effectors comprise protons,[3] metal cations,[8] and medium/solvent[9] as well as molecular recognition interactions,[10] such as hydrogen bonding.[10b] These effectors induce the expression of the 'fittest' entity from a distribution of all possible constituents. DCC is also making a profound impact on the development of novel, dynamic entities, such as dynamic polymers (dynamers)[11] as well as self-sensing devices[7] and self-healing materials,[12] which can adapt in response to outside stimuli. Recent work from our laboratory has in particular examined the effects of gel formation, resulting from the self-assembly of guanine quartets, on a dynamic library of acylhydrazones, which demonstrated three constitutional dynamic features: supramolecular recognition interactions, dynamic covalent bond connection, and gel/sol distribution.[13] The latter represents a response of the system to the formation of an organized assembly, the gel. In general terms, it points to the behavior of a dynamic system in response to a phase change, a feature that deserves closer exploration, as it represents a physicochemical transition of the system, an attractive step toward the design of systems out of equilibrium. It relates to component selection and constituent expression and adaptation[1,3] under the pressure of self-organization on one hand[1b,13] as well as to phase transfer and transport processes involving adaptation of a dynamic set of constituents to a change in medium.[14] Phase changes may act on a constitutional dynamic system undergoing gas/liquid, liquid/liquid, or liquid/solid transfer. Thus, the latter occurs on crystallization of a specific entity from an interconverting set.[4] Also along these lines, we have shown earlier that a change from organo-aqueous to aqueous medium was associated with a strong, hydrophobicity-driven monomer selection in the generation of a dynamic polymer.[15]

Herein, we report on adaptation of a dynamic covalent system to phase change, via component selection within sets of interconverting imine constituents, caused by the separation of a homogeneous liquid phase into two nonmiscible phases through the action of a physical stimulus or the addition of a chemical effector (Figure 1). It results in the reorganization of a chemical effector (Figure 1). It results in the reorganization of a dynamic library based on the medium preference of the library members. Moreover, we demonstrate reversibility in these phase separations by switching between the biphasic state and the homogeneous solution. Finally, we note that such processes may provide access to dynamic nonequilibrium systems.

RESULTS AND DISCUSSION

The separation of an organo-aqueous mixture into two separate phases leads to the formation of two distinct solvent environments from a single one. These two media differ considerably from each other and from the homogeneous phase in their physicochemical properties, such as polarity and viscosity. As a consequence, they may be expected to affect the distribution of the reversibly interconverting constituents of a dynamic covalent system and drive the amplification/up-

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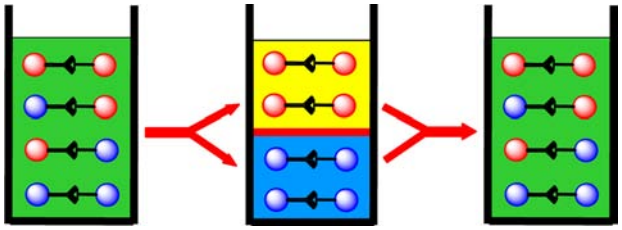


Figure 1. Schematic representation of the component redistribution occurring in a dynamic covalent library on phase separation and recombination of a binary organo-aqueous solvent mixture. (Left) Library constituents in the single homogeneous phase (green). (Center) Distribution after phase separation and equilibration, (yellow) organic phase, and (blue) aqueous phase. (Right) regeneration of the initial library after phase reunification. Red and blue spheres: lipophilic and hydrophilic components. In the present case, a 3/2 (v/v) CH_3CN/H_2O mixture was used. Phase separation was induced by treating the mixture with either NaCl, sucrose, diethyl ether, KF, or by cooling, and phase recombination was achieved by heating, fractional evaporation, $LiClO_4$, or warming to room temperature, respectively.

regulation of the fittest compounds in their respective environments. We herein describe such effects induced by phase separation of acetonitrile/water (AN/W) mixtures on two different dynamic covalent libraries of imines.

Medium, Phase Separation Procedures, and Components of the Dynamic Covalent Library. We selected AN/W mixtures (3/2, v/v) as a medium, in view of the miscibility of the two solvents, their physicochemical properties, and their extensive use.[16−24] Phase separation was accomplished by either a physical stimulus (temperature) or the addition of a chemical effector of one of three different types: an inorganic salt, such as sodium chloride or potassium fluoride, a hydrophilic organic molecule, such as sucrose, or a water immiscible organic liquid, such as diethyl ether.[25] The AN/W composition of the separated phases, which is expected to affect phase distribution, varied depending on the procedure used for causing separation. The strongly hydrophilic inorganic salts caused higher differentiation in composition of the aqueous and organic phases than agents which present less pronounced solubility preferences between AN and W (see Supporting Information (SI) for exact compositions, pages S27−S28). The sets of imines investigated here were generated from the aldehyde and amine components **1**−**4** and **1**, **9**−**11** giving imines **5**−**8** and **12**−**15**, respectively.

Dynamic libraries of imines were selected because of their facile formation and dissociation in neutral aqueous media.[2] They consisted each of four components: a hydrophilic aldehyde and amine and a hydrophobic aldehyde and amine. Salicylaldehyde **1** was chosen in view of the high rate of formation and exchange of its imines and its appreciable hydrophobicity.[26] In addition, for the first library (Scheme 1), a charged ammonium analogue of salicylaldehyde **2** was selected as the hydrophilic counterpart, while heptylamine **3** and the sodium salt of taurine **4** were chosen as the organo-soluble and hydrophilic amine components, respectively.

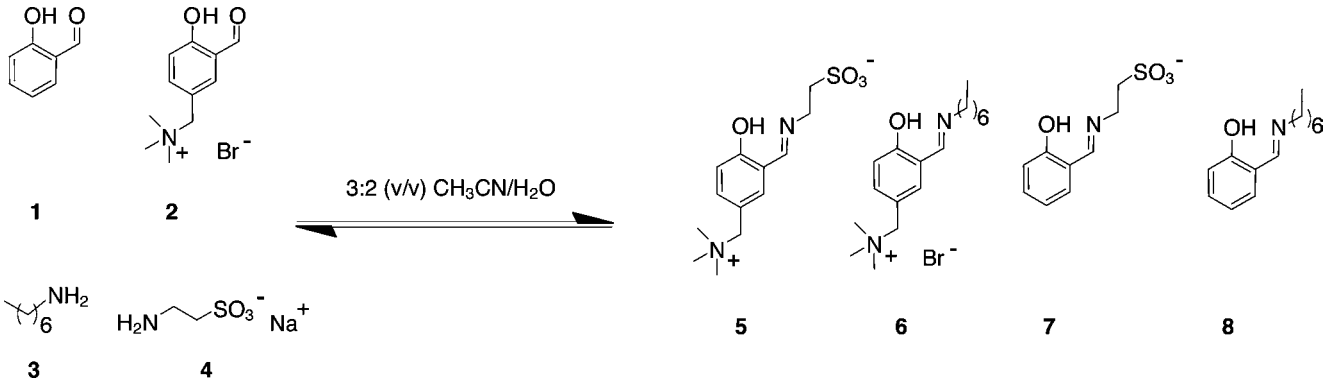
Effects of Reversible Phase Separation on the Dynamic Covalent Library 5−8. Dissolution of compounds **1**−**4** (20 mM each) in 3:2 (v/v) AN/W generated a single phase solution containing a given distribution of imines **5**−**8**, as observed by ^{1}H NMR and quantified by integrating the distinct CH═N protons against an external standard (Figure 2). The four imines, possessing either hydrophilic−hydrophilic (**5**), hydrophobic−hydrophobic (**8**), or amphiphilic characteristics (**6** and **7**), were present in almost statistical distribution (but see also below, the case of **12**−**15**).

Phase separation was induced upon treatment with sodium chloride, potassium fluoride, sucrose, or diethyl ether[27] as well as by cooling to 0 °C, leading to a marked reorganization of the dynamic library, whereby the hydrophilic **5** was strongly distributed into the aqueous phase, whereas the agonistic (see Figure 6) hydrophobic constituent **8** was dominant in the organic phase. As expected, this reorganization was more pronounced when the separated phases had a more pronounced difference in AN/W composition (see SI page S27−S28).

Two parameters may be used to characterize the changes introduced by the phase separation: the distribution of the different constituents between the two phases and the amplification (or conversely the reduction) of specific constituents (summed over the two phases), with respect to their fraction in the homogeneous phase. One notes that the amplification cannot exceed 50% for any constituent; the other 50% being its agonist. Furthermore, one must take into account the fact that some hydrolysis is present and may slightly change between homogeneous and separated states, thus reducing the total amount of library imine members.

Sodium chloride was found to induce the most bias in the product distribution, whereas separations by temperature change were the least biased. The distributions were similar when other salts were used, such as $CaCl_2$, KCl, and KF.

Scheme 1. Dynamic Covalent Library of Imines 5−8, Generated by Reaction of Salicylaldehydes 1 and 2 with Amines 3 and 4



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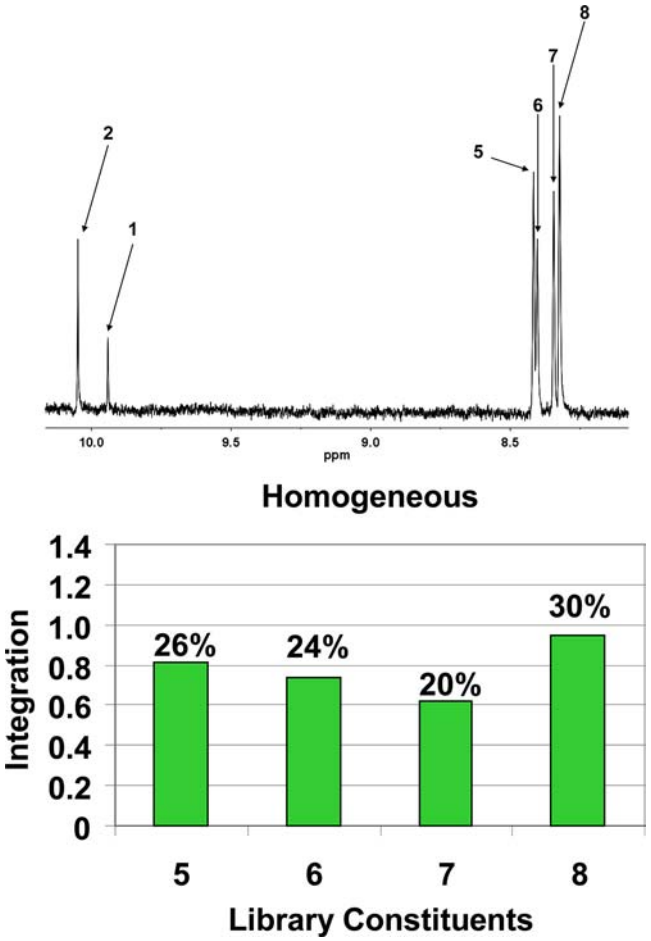

Figure 2. Distribution of a dynamic covalent library of imines **5**–**8** (Scheme 1) in a single phase of AN/W 3/2 (v/v). (Top) 400 MHz ^{1}H NMR spectrum of the mixture: −CH═N− proton signals of imines **5**–**8** in the 8.3–8.5 ppm region; and −CHO signals of **1** and **2** around 10 ppm . (Bottom) Amounts and fractions of the four different imine constituents **5**–**8**, determined by integration of the corresponding −CH═N− proton signals against an external standard (see Experimental Section).

Thus, when phase separation was performed with NaCl, the compositions were 93% W for the aqueous phase and 85% AN for the organic phase (see SI page S27–S28). The corresponding distributions were 77% for **5** and 72% for **8** in the aqueous and organic phases, respectively (Figure 3, bottom left). The total fractions (summed over both phases) amounted to 35% for **5** and 43% for **8** (Figure 3, bottom right), as compared to relative values of 26% for **5** and 30% for **8** in the homogeneous phase (Figure 2), indicating amplification of these two constituents on phase separation.

The organic as well as the aqueous phases strongly disfavor the formation of both amphiphilic **6** and **7**, which are antagonistically related to **5** and **8**, and thus present in only small amounts.

When one of the charged species was replaced with a water-soluble neutral component, such as 2-pyridine carboxaldehyde or ethanolamine, the selection driven by phase separation was far less pronounced. Furthermore, when the homogeneous mixture was treated with the chaotropic tetrabutylammonium bromide, no phase separation occurred, and no perturbation of the equilibrium distribution was observed.

Phase reunification was achieved by different procedures depending on the phase separation agents used. In the case of phase separations induced by NaCl or sucrose, the biphase was reunited by warming the solution to 70 °C, leading to the reformation of imines **6** and **7**. Although the imines were found to give a statistical distribution, there was a slightly greater degree of overall hydrolysis at the higher temperature. Comparison of the NMR spectra of the homogeneous phase before and after phase separation indicated that the systems were reversible, with the initial and final phases having similar compositions (see SI page S24–S25).

Whereas NaCl-induced phase-separated mixtures were re-united using temperature, the recombination of a KF-induced biphase was achieved by a chemical approach. Treatment of the latter mixture with solid $LiClO_4$ resulted in rapid precipitation of both LiF (solubility product in water, $Ksp_{[water]} = 1.84 \times 10^{-3}$) and $KClO_4$ ($Ksp_{[water]} = 1.02 \times 10^{-2}$), which were subsequently removed by filtration, to yield a single phase solution. Remarkably, in this case the effector, the salt KF, used to cause phase separation is eliminated by double precipitation, so that the initial single phase is restored, therefore regenerating also the initial imine distribution (within experimental accuracy). This process represents repeatable switching between single phase and phase-separated states.

For separations using diethyl ether, fractional distillation caused reunification of both phases. The trace amount of ether remaining in the newly formed homogeneous phase (less than 3%) had no observable effect on the product distribution of the dynamic library.

Modulation of phase separation by cooling–heating cycles represents a fully reversible procedure without use of any chemical additive. Thus, cooling a solution of compounds **5**–**8** to 0 °C led to phase separation and to the distribution of imines shown in Figure 6 of SI. Upon warming to 21 °C, the phases reunited, and the initial distribution of imine constituents was restored (Figure 2 of SI).

Effects of Reversible Phase Separation on the Dynamic Covalent Library 12–15. We extended our dynamic libraries to include biogenic amines and aldehydes (Scheme 2). The neurotransmitter tryptamine **10**, an important precursor to a myriad of biogenic alkaloids, was selected as the organic amine component. While α-amino acids are an ideal choice for an aqueous amine component, they form only low amounts of imines. However, the sodium salt of the parasympathetic neurotransmitter γ-aminobutyric acid (**11**) gave satisfactory amounts of imines. Salicylaldehyde was again selected as the organic aldehyde component.[26] Whereas water-soluble aldehydes have a propensity to form hydrates and acetals, which interfere with imine formation,[30] one notable exception is pyridoxal-5-phosphate (**9**), which does not give a hydrate detectable by ^{1}H NMR and yields imines at a high conversion and with fast rate.[26]

Thus, a solution of **1** and **9**–**11** (20 mM each) was prepared in 3:2 (v/v) AN/W to give a distribution of the imines **12**–**15**, as shown in Figure 4. It should be noted that, in this case, formation the AN/W medium influences the distribution of the library even in a single phase. In line with this inference, increasing the water content was found to decrease the imbalance and approach to a statistical distribution of the four imines (Figure 28, SI). Another factor could be the micro-heterogeneity of AN/W mixtures,[16] an aspect that is beyond the present study but which, if confirmed, could be of much

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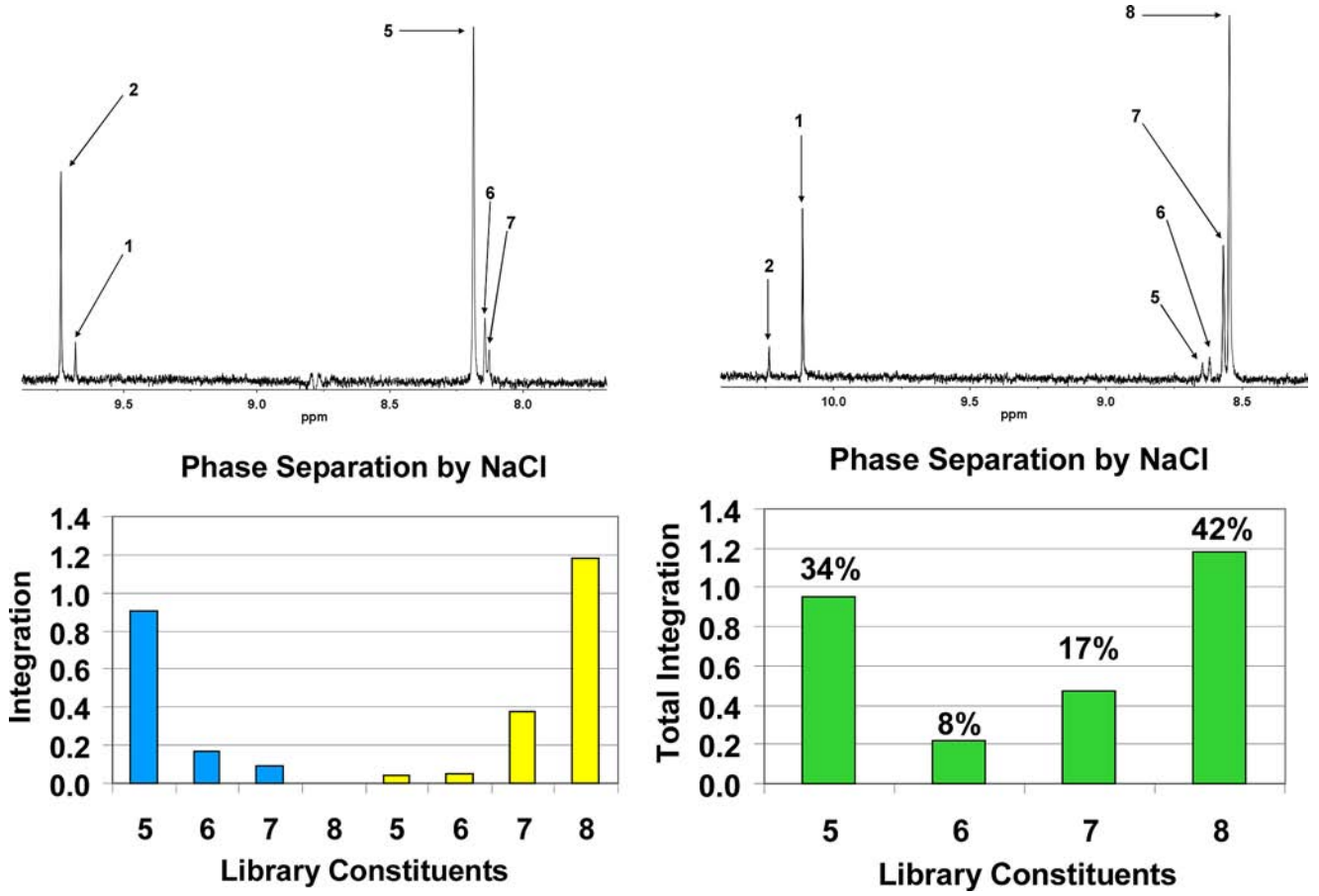
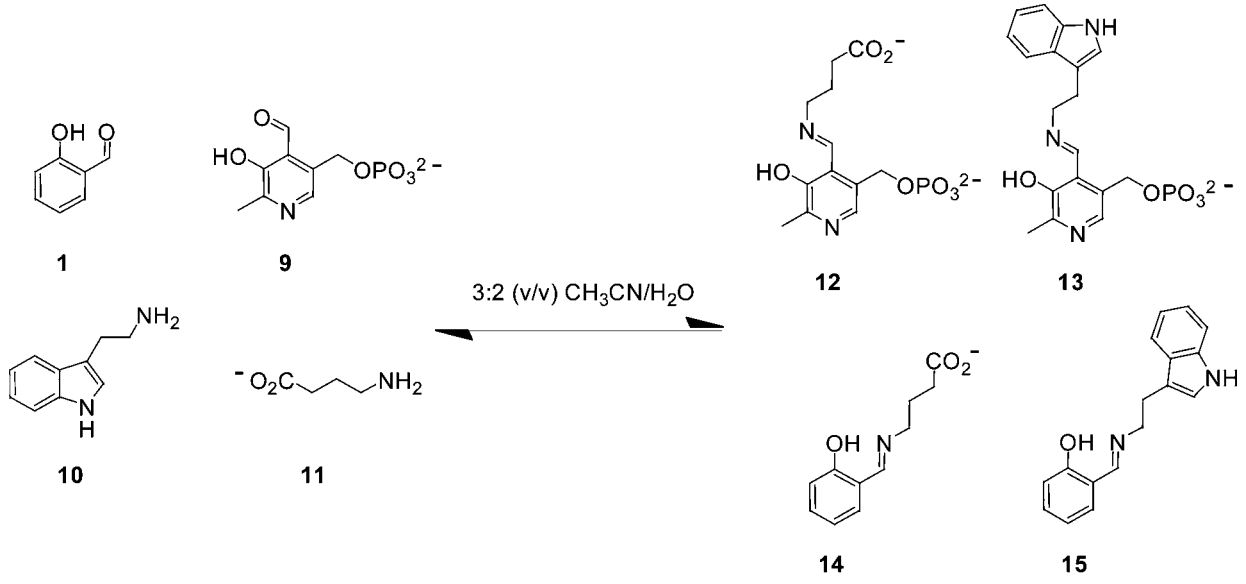


Figure 3. (Top) 400 MHz ^{1}H NMR spectrum of the aqueous (left) and organic (right) phases generated by NaCl-induced phase separation of an AN/W 3/2 (v/v) mixture containing the imines **5**–**8** into two separate phases; −CH═N− and −CHO proton signals in the 8.1–8.7 and 9.6–10.3 ppm regions, respectively. (Bottom left) Distributions of the four different imine constituents in the respective phases, aqueous (left) and organic (right), determined by integration of the corresponding −CH═N− proton signals against an external standard. (Bottom right) Integrated amounts and fractions of the constituents **5**–**8** summed over both phases (see Experimental Section).

Scheme 2. Dynamic Covalent Library of Imines 12–15 Generated by Reaction of the Biogenic Amines 10 and 11 with the Aldehydes 1 and 9



interest with respect to the general question of the physicochemical structure of solutions.[31]

Upon phase separation with NaCl, sucrose, or diethyl ether, a similar, even more pronounced recombination trend was

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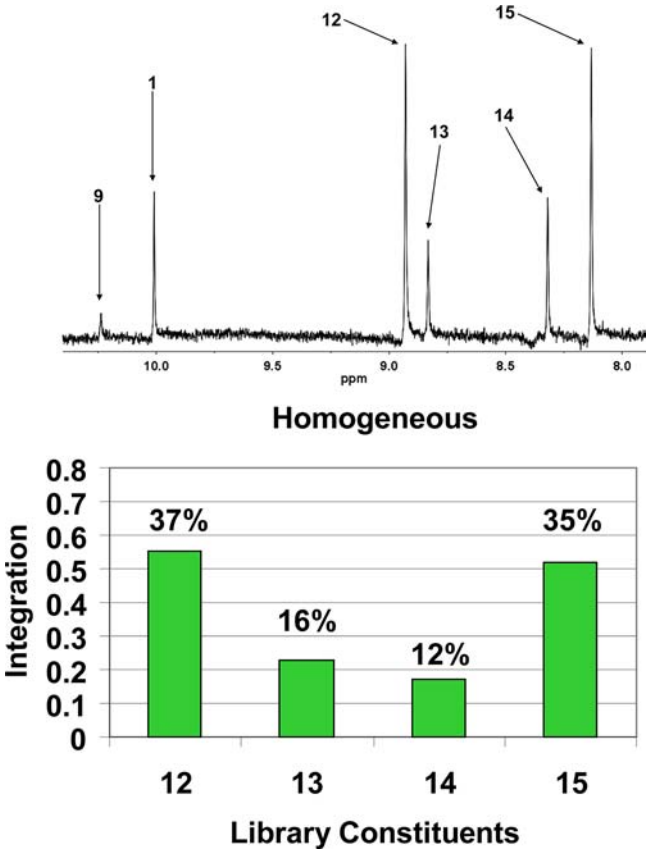

Figure 4. Distribution of a dynamic covalent library of imines **12**–**15** (Scheme 2) in a single phase of AN/W 3/2 (v/v). (Top) 400 MHz ^{1}H NMR spectrum of the mixture: −CH═N− proton signals of imines **12**–**15** in the 8.0–9.0 ppm region; and −CHO signals of **1** and **9** between 10.0 and 10.3 ppm. (Bottom) Amounts and fractions of the four different imine constituents **12**–**15**, determined by integration of the corresponding −CH═N− proton signals against an external standard (see Experimental Section).

observed as found in the aforementioned system, giving the hydrophilic–hydrophilic imine **12** as the largely dominant species in the aqueous phase and the hydrophobic–hydrophobic imine **15** almost exclusively in the organic phase (Figure 5). Again, sodium chloride was found to be superior to sucrose and diethyl ether in promoting the most bias in the product distribution.

Indeed, phase separation using NaCl gave compositions of 93% W for the aqueous phase and 85% AN for the organic phase (see SI page S27–S28). The distributions were respectively 84% for **12** and 99% for **15** in the aqueous and organic phases (Figure 5, bottom left). The total fractions (summed over both phases) amounted to 40% for **12** and about 50% for **15** (Figure 5, bottom right) as compared to relative values of 37% for **12** and 35% for **15** in the homogeneous phase (Figure 4), indicating here also amplification of these two constituents on phase separation. The organic as well as the aqueous phases strongly disfavor the formation of both amphiphilic **13** and **14**, which are antagonistically related to **12** and **15**, and thus present in only small amounts.

Attempts to reunite the phases separated with either NaCl or sucrose with heat were unsuccessful, as it was found that the charged components raised the phase integration temperature to near the boiling point of acetonitrile, rendering it difficult to analyze by variable-temperature NMR. However, fractional distillation of diethyl ether from the phase-separated mixture successfully reunited the phases and resulted in the restoration of the original product distribution. In this case again, phase separation by KF was reversed with removal of all added salts by double decomposition on treatment with $LiClO_4$. The changes in environment described above bear relation to processes that may occur in a membrane system, with dynamic redistribution taking place at the interface between the aqueous medium and a lipid membrane.[28] The analogy is even closer when liquid membranes are considered, as represented here by the organic (acetonitrile-rich) phase. In this respect, the present set up may be considered as a switchable liquid membrane system, that may be implemented for introducing reversible modulation of membrane processes, such as (selective) substrate pumping by coupling to a chemical effector (e.g., proton or redox gradient) or physical stimulus[29] (e.g., light)[29b] as well as establishing switched chemical potentials with generation of out-of-equilibrium conditions. Thus, one may imagine to drive transport systems[29] by creating nonequilibrium conditions via phase separation. Such work is being pursued in this laboratory.

From 2D to 3D Constitutional Dynamic Networks. In the general context of complex networks,[32] the data reported here can be represented within the framework of constitutional dynamic networks.[1b,3] The four constituents in the single, homogeneous phase define a square, that splits, for the separated two phase system, into two squares, whose corners are connected through the interface, thus defining a cube (Figure 6). The system exemplifies the splitting of a 2D (square) into a 3D (cube) constitutional dynamic network. The trans-phase edges of the cube connect identical constituents, distributed in the two phases and made antagonistic by the phase separation, an increase in one phase causing a depletion in the other phase. The trans-phase diagonals connect the agonists between the two phases, in particular those two agonists that are amplified on phase separation and represent the fittest constituent in each phase. Thus, the generation of the fittest in one phase is linked to that of the fittest in the other phase, each of them occupying their environmental "ecological" niche.

■ CONCLUSIONS

The results described herein lead to the following conclusions: (1) We have demonstrated that a dynamic library of reversibly interconverting entities undergoes constitutional reorganization when subjected to liquid–liquid phase separation of a binary solvent system. In the present case, a set of imines in AN/W solution displays component exchange upon phase separation induced by temperature change as well as by the addition of a salt, a hydrophilic agent (a carbohydrate), or a hydrophobic solvent. (2) The phase separation leads to the up-regulation/amplification of the best suited/fittest constituents for each phase with down-regulation of the amphiphilic ones through medium-induced component selection. (3) The process is reversible as the biphasic mixture can be reunited, e.g., by gentle heating, ion exchange, precipitation, or fractional distillation, causing the reformation of the original single-phase library, in particular of the amphiphilic components that were repressed in the phase-separated conditions. It may also be considered as self-sorting[33] modulated by reversible phase change. (4) The processes described couple the behavior of a constitutional dynamic library to a phase change, a step in the induction of

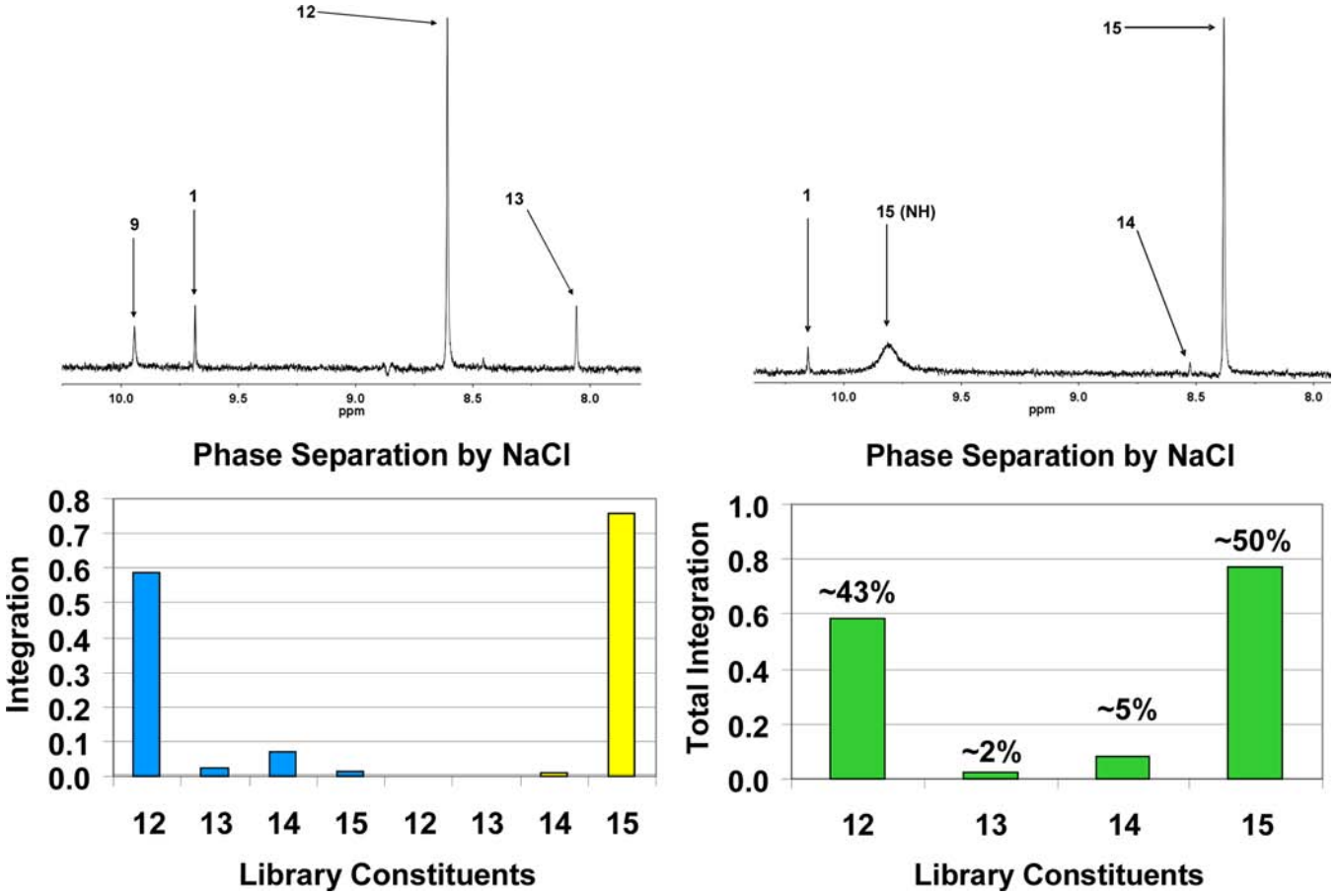

Figure 5. (Top) 400 MHz ^{1}H NMR spectrum of the aqueous (left) and organic (right) phases generated by NaCl-induced phase separation of an AN/W 3/2 (v/v) mixture containing the imines **12**−**15** into two separate phases; −CH═N− and −CHO proton signals in the 8.0−8.7 and 9.6−10.3 ppm regions, respectively. (Bottom left) Distributions of the four different imine constituents in the respective phases, aqueous (left) and organic (right), determined by integration of the corresponding −CH═N− proton signals against an external standard. (Bottom right) Integrated amounts and fractions of the constituents **12**−**15** summed over both phases (see Experimental Section).

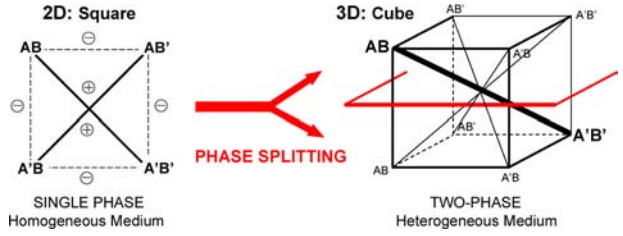

Figure 6. Splitting of a 2D (square) into a 3D (cube) constitutional dynamic network by liquid/liquid phase separation. Phase transfer of the interconverting constituents AB, A′B′, A′B, and AB′ across the interface (horizontal plane) occurs with adaptation to each phase through component exchange. The diagonals and the edges of the square link constituents presenting respectively agonistic (+) and antagonistic (−) relationships. The diagonals of the cube link agonistic constituents, and the vertical edges link antagonistic constituents across the interface. The larger size and bold letters of AB and A′B′ as well as the thicker diagonal line linking them indicate simultaneous up-regulation of these two agonistic constituents across the interface.

constitutional change by a phase transition. They allow in principle to subject such libraries to nonequilibrium conditions. They also touch upon the interesting question of the behavior of a dynamic covalent library at an interface.[14] Such behavior represents an adaptation of the dynamic system to the medium by constitutional variation, with simultaneous up-regulation of the fittest agonist constituents in the respective phase through component selection under the pressure of the environment. In terms of constitutional dynamic networks,[1b,3] the system described exemplifies the splitting of a 2D (square) network into a 3D (cube) one. (7) Finally, phase change-induced modulation of chemical constitution may also bear relation to the selective evolution of biologically significant molecules, whereby they adapt their constitution to specific interconnected environmental niches in prebiotic conditions.

■ EXPERIMENTAL SECTION

General. All reagents were purchased from chemical suppliers (Sigma-Aldrich, Merck, Fluka) and used without further purification unless mentioned otherwise. Acetonitrile (CHROMOSOLV for HPLC, gradient grade) was purchased from Sigma-Aldrich and used as received. Water was purified using a Millipore Elix 10 filtration system. Salicylaldehyde was freshly distilled prior to use. ^{1}H NMR spectra were recorded on a Bruker Avance 400 MHz NMR spectrometer. These samples were equilibrated at 298 K and consisted of nondeuterated solvents equipped with a sealed capillary charged with DMSO-d_6 and a small amount of DMSO-h_6 as an external standard. The preparation of **2** is described in the SI. (Caution: Perchlorate salts are known to detonate on exposure to organic matter and heat, thus great care must be taken in their handling.)

Phase Separation Experiments. An NMR tube containing a capillary, as mentioned above, was charged with 250 μL of 80 mM **1**−**4** in 3:2 (v/v) AN/W, resulting in a 1 mL solution of 20 mM of each

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component. The mixture was gently agitated for 5 min. Phase separations were accomplished by treating the above solution with either 25 mg NaCl, 25 mg KF, 100 mg of sucrose, or 250 μL of diethyl ether. The biphase was gently agitated for 10 min, and the tube was allowed to stand until clean phase separation was observed. The aqueous phase could be measured by ^{1}H NMR without any observable interference from the top layer and gave the same results when the phases were analyzed independently. The organic layer was carefully separated using a syringe and transferred to a new NMR tube containing the same external standard used previously.

For phase separation experiments involving imines **12**−**15**, a modified approach was used. An intimate stock solution of 80 mM **9** and **10** was prepared, as it was found that **9** alone was insoluble in 3:2 (v/v) AN/W. Thus, pyridoxal-5-phosphate (**9**) (79 mg, 0.320 mmol) and tryptamine (**10**) hydrochloride (63 mg, 0.320 mmol) were suspended in 2.4 mL of acetonitrile, diluted with 640 μL of water, and treated with 960 μL of a 1.0 M NaOH volumetric standard. This solution was stored at −20 °C to prevent phosphate hydrolysis.

An NMR tube containing an external standard was charged with 250 μL of 80 mM **1**, 250 μL of 80 mM of **9** and **10**, 250 μL of 80 mM **11**, and 250 μL of 3:2 (v/v) AN/W, resulting in a 1 mL solution of 20 mM of each component. The mixture was gently agitated for 5 min. Phase separation experiments were carried out in the same manner as described for the library consisting of **5**−**8**.

Phase Recombination Experiments. To demonstrate the reversibility of these phase separations, an NMR tube containing a phase-separated library (induced by addition of 25 mg NaCl or 100 mg sucrose) consisting of imines **5**−**8** and an external standard was placed inside an NMR probe equilibrated at 70 °C. The sample was allowed to stand for 10 min. Spinning of the sample was found to be insufficient for mixing the organic and aqueous layers. Thus, the sample was quickly removed from the probe and carefully agitated. Once homogenization was achieved, the sample was quickly placed into the probe and analyzed.

For phase separations induced by KF, a 1 mL solution containing imines **5**−**8**, phase separated by addition of 25 mg KF, was treated with 46 mg of $LiClO_4$ (1 equiv with respect to KF). This resulted in precipitation of both LiF and $KClO_4$, which were filtered off, leaving a single phase filtrate.

For phase separations using diethyl ether, the reaction described above was scaled up 4-fold and treated with 1 mL of diethyl ether, upon which a phase separation occurred. The biphasic mixture was then placed on a rotary evaporator, and the ether evaporated at 50 °C at 1 atm until the phases had reunited.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Preparation of **2**, tables describing product distributions, and mole fractions of AN/W. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

lehn@unistra.fr

Notes

The authors declare no competing financial interests.

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at the molecular scale and consist of water- and acetonitrile-rich clusters. Treatment of these binary mixtures with ionic kosmotropes, such as sodium chloride,[23] or nonionic kosmotropes, like carbohydrates,[24] can force water molecules to coalesce and thus induce a phase separation. Conversely, a phase separation may be induced by the addition of a water-immiscible organic solvent, which consolidates acetonitrile clusters and expels water.

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