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Combinatorial Chemistry

Dynamic Combinatorial Evolution within Self-Replicating Supramolecular Assemblies[**]

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Dynamic combinatorial chemistry (DCC) rests on the design and the study of libraries of species connected by reversible (supra)molecular bonds.[1,2] It represents a very attractive domain of modern chemistry because it associates combinatorial features together with the spontaneous self-organization of molecules.[3–5] Dynamic combinatorial libraries (DCLs) are governed by thermodynamics and are consequently subjected to the influence of internal or external parameters that can reversibly modify the expression of their constituents through selection/adaptation. In bioinspired chemistry, other efforts to understand molecular evolution are focused on minimal autocatalytic and self-replicating systems that are governed by kinetics.[6,7] Herein we show that by coupling DCC with the autocatalytic formation of specifically designed supramolecular assemblies, a self-replicating selection can occur at two length scales with a sigmoid concentration–time profile. Indeed, we have found that by using a new kind of molecular objects, namely dynamic amphiphilic block copolymers (dynablocks), in which a hydrophobic block is reversibly linked to a hydrophilic one, the formation of micelles can have autopoietic[8,9] growth in water. Moreover, when different hydrophilic blocks compete for the same hydrophobic block in coupled equilibria, the differential thermodynamic stabilities and autocatalytic efficiencies of the resulting mesoscopic structures lead to the selection of the most efficient self-replicator and to the depletion of its competitors.

We have recently shown that DCC could be associated with self-replicating systems to increase the concentration of a single product, by duplication from a pool of reshuffling constituents competing in a series of coupled thermodynamic equilibria.[10] Although this reported DCL has both kinetic and thermodynamic biases that amplify the best duplicator and decrease its competitors, it does not present a strong autocatalytic behavior. Herein we describe another DCL which avoids the drawback of product inhibition by taking advantage of the growth/division cycles of micellar self-assemblies, and which displays a particular case of autocatalysis, namely autopoiesis. This concept appeared in the mid-1970s, when Maturana, Varela, and Uribe proposed that living systems are essentially characterized by their aptitude to continuously organize the generation of their own components, thus maintaining the very network process that produces them.[11] The minimal criteria defining autopoiesis should verify whether 1) the system has a semipermeable boundary that is 2) produced within the system, and 3) that encompasses reactions which regenerate the components of the system.[8] The seminal work of Luisi et al. brought to light the first examples of minimal chemical autopoietic systems that produce surfactants inside micelles or vesicles built by these very constituents.[12,13] Whereas the definition of life is controversial, and is more popularly defined by self-replication according to the prebiotic RNA world view,[14] autopoiesis remains at least a complementary approach[9,15] and defines a very interesting conceptual framework that encompasses collective properties, such as self-assembly, self-organization, and emergence. Thus, the possible association of autopoiesis with selection processes—for instance, those occurring through the network of coupled equilibria in a DCL—constitutes a very attractive pathway in the field of molecular evolution.

To set up our study, we first designed a new type of amphiphilic molecular objects that, because of their reversible connections and through molecular recombination, allow the production of various types (in size and shape) of micellar self-assemblies in water. These objects were constructed by using the reversible connection of a single imine bond between hydrophilic and hydrophobic blocks, thus leading to dynamic amphiphilic blocks (dynablocks).[16,17] The individual condensations of aliphatic, benzylic, aromatic, and hydroxy amines **1**–**8** (having PEO units of different lengths) with the *p*-substituted benzaldehyde **A** having a hydrophobic tail of 8 carbons lead to the formation of dynablocks **1A**–**8A**. These compounds have different Hydrophilic/hydrophobic ratios ($r_{H/h}$) that are related to surfactant shape parameters[18] (Figure 1 and Supporting Information, Table S1 and Figure S1). The equilibrium constants were determined by ^{1}H NMR spectroscopy in deuterated acetonitrile at 298 K ($[\text{aldehyde}]_{\text{init}} = [\text{amine}]_{\text{init}} = 50$ mM). These experiments show that, as expected, the condensation of imines depends on the

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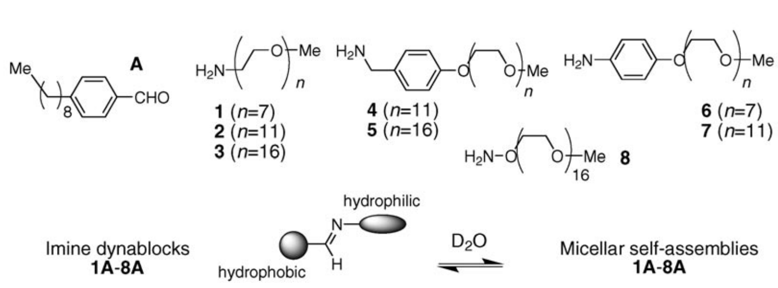

Figure 1. Structures of dynablocks. The individual reactions of hydrophilic amines **1**–**8** with hydrophobic aldehyde **A** lead to imines **1A**–**8A**. In D_2O, these dynablocks self-assemble into supramolecular micellar structures.

nucleophilicity of the amine reacting groups (hydroxy amine $\gg$ aliphatic $\geq$ benzylic $\gg$ aromatic; Supporting Information, Table S1). The dynablocks were then diluted in deuterated water and the acetonitrile was evaporated, keeping a final concentration of 50 mM (the residual quantity of acetonitrile was not detectable by ^{1}H NMR spectroscopy).[19] The clear solutions (except for **2A** and **3A**) were studied by ^{1}H NMR and DOSY NMR spectroscopy and by light and neutron scattering. In most cases, ^{1}H NMR spectroscopy indicates that the signals for the imine groups are present in water ($\delta = 7.5$–8.5 ppm) together with the free aldehyde ($\delta = 9.8$ ppm). The equilibrium constants were measured, and although the presence of water as a solvent should favor the hydrolysis of the imine bonds, a high degree of condensation was observed for compounds **4A**–**8A** (Supporting Information, Table S1). This increase of the equilibrium constant can be attributed to the self-assembly of the dynablocks in highly stable supramolecular structures, except for **2A** and **3A**, which quickly hydrolyze and release the insoluble free aldehyde. Only product **1A** has an equilibrium constant that is lower in water than in acetonitrile, which reveals a relatively weak thermodynamic stability of its amphiphilic self-assembled superstructure, probably because of the partial lack of π–π stacking interactions. The micellar structures were confirmed for **1A**, **4A**, **5A**, **7A**, and **8A** by using DOSY NMR spectroscopy[20] with which the diffusion of the imine self-assemblies in water can be correlated to their hydrodynamic radii (Supporting Information, Table S1). The results indicate the formation of micellar self-assemblies having hydrodynamic radii comprised between 5 and 7.1 nm, and which vary inversely with $r_{H/h}$. For instance, dynablock **4A** ($r_{H/h}$=2.1) has a hydrodynamic radius of 6.9 nm, whereas **5A** ($r_{H/h}$=2.9) shows a smaller hydrodynamic radius (6.5 nm). The evolution from cylindrical to spherical micelles depending on $r_{H/h}$,[18] was shown by light- and neutron-scattering experiments for compounds **6A** and **8A** (Supporting Information, Figure S2 a–b). For compound **6A** ($r_{H/h} = 1.3$), the size of the structure is too large to be observed by DOSY experiments, but neutron scattering reveals the presence of cylindrical micelles having a mass of 507000 g mol^{-1}, a number of aggregation of 783, and a size of 6 nm diameter and 34 nm in length. For compound **8A** ($r_{H/h} = 4$), neutron scattering data support the presence of spherical micelles. In this study, another asset of the DOSY NMR spectroscopy technique is to discriminate, within mixtures, the components with different diffusion coefficients.[21] For example, when studying dynablock **7A** in water (Supporting Information, Figure 3), the correlation between the signals in the first dimension with the diffusion values in the second dimension lead to the following conclusions. The imine dynablock diffuses with a rate of 28 $\mu m^2 s^{-1}$, corresponding to a micellar object of 14.2 nm in diameter that contains in its core all the remaining free aldehyde whereas the free amine stays outside of the micellar system with a diffusion of 250 $\mu m^2 s^{-1}$. This location of the free aldehyde within the boundary of the structure is the first requirement to give rise to autopoietic behavior.[8]

We then turned to the kinetic and thermodynamic studies of the formation of compound **7A** by mixing **7** and **A** directly in deuterated water (equimolar ratio of 50 mM each). The condensation of the product, measured by ^{1}H NMR spectroscopy, reveals a sigmoid concentration–time profile, which is characteristic of an autocatalytic system (Figure 2 a). To determine the origin of the autocatalytic process, we set up

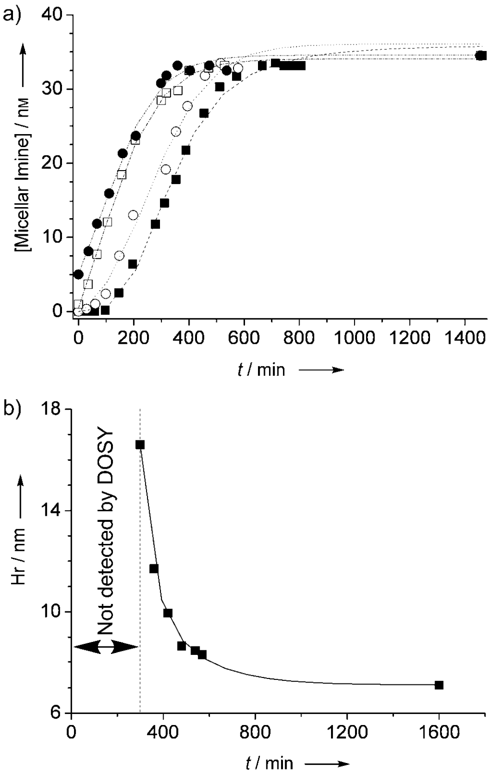

Figure 2. Autopoietic behavior of dynablock **7A**. a) Concentration of micellar imine **7A** versus time starting from **7** and **A** in D_2O (50 mM each), and as a function of the quantity of initially added micellar imine **7A**: ■ 0, ○ 5.1×10^{-4}, □ 51×10^{-4}, ● 102×10^{-4} mmol. The micelle concentrations were determined by the integration of the micellar imine ^{1}H NMR spectroscopy signal (8.12 ppm), which differs from the free imine signal (8.23 ppm). Maximum standard deviation of the data presented 5%, determined by setting up the reaction three times under the same conditions. Dotted lines connecting the experimental results drawn to guide the eye. b) Hydrodynamic radii Hr of the micellar structure formed from **7A** as a function of the course of the condensation reaction from **7** and **A**.

the same experiment but in the presence of increasing initial amounts of preformed micelles of **7A**. The progressive loss of the sigmoid shape clearly indicates that the micelle catalyses its own formation through the condensation of **7** and **A** with a $v_{\max}$ of 72 10^{-1} mmol h^{-1} (autocatalytic efficiency $\varepsilon \approx 80$; Supporting Information, Table S2). This maximum rate is reached for a concentration of micellar imine of 5 mM, and this saturation effect suggests the assistance of the micelle to solubilize the hydrophobic aldehyde until attaining the maximum rate of the templated imine condensation itself (Supporting Information, Figure S4a). For low quantities of catalyst (5.1×10^{-4} mmol $< x <$ 51×10^{-4} mmol), the plot of $\log(V_0)$ against the logarithm of the initial micellar concentration shows a linear dependence of rate on catalyst concentration, demonstrating that the rate of the uncatalyzed reaction is comparatively negligible (Supporting Information, Figure S4b). We also determined the size of the micellar structures as a function of the advancement of the condensation reaction between **7** and **A**. After nucleation, we expected to observe a constant average size of the micellar self-assembly as the structure grows and divides constantly because of the sheer forces leading to a thermodynamic instability above a critical size.[22] However, DOSY NMR studies show a more complex behavior, with the decrease of the average hydrodynamic radii of the micelles from 16 nm with a condensation of 36% to 7 nm with a condensation of 70% (Figure 2b). The reason is that the size and shape of the micelles are determined by both $r_{H/h}$ and the intramicellar free aldehyde/imine ratio (ia/i). At the beginning of the reaction the ia/i ratio is high because the micelles solubilize a high quantity of aldehyde, thus producing larger objects with a (probably) cylindrical structure.[18] This effect on the micellar dimensions was confirmed by the addition of free aldehyde **A** (up to 300%) to a preformed solution of micellar **7A** (Supporting Information, Figure S5a). However, as the critical size of the spherical structure is reached, an expected growth/division cycle with a constant average size of the population takes place, as was shown by increasing the concentration of both **7** and **A** from 2 mM to 75.5 mM (Supporting Information, Figure S5b). Finally, the importance of the micellar structure to the thermodynamic stability of dynablock **7A** was demonstrated by mixing acetonitrile and water. For the lower molar fractions in water, the dynablocks lose their supramolecular stabilization and start hydrolyzing (Supporting Information, Figure S6a–b). The combined results of the kinetic and thermodynamic studies clearly demonstrate that the self-replication process of the dynablocks described herein amounts to a minimal autopoietic system without any other reagent than the two building blocks and water.

Finally, we set up two competition experiments between **1A** and **7A** by mixing **1**, **7**, and **A** (50 mM each), and we determined the corresponding concentration–time profiles by ^{1}H NMR spectroscopy (Figure 3). In the first experiment, the beginning of the competition was performed in deuterated acetonitrile, showing a clear domination of **1A** ($V_0 =$ 15 mM h^{-1}, and $c = 31$ mM at equilibrium) over **7A** ($V_0 =$ 2.1 mM h^{-1}, and $c = 16$ mM). The acetonitrile was then exchanged for D_2O, keeping the concentration at a constant 50 mM. The concentration–time profile shows a dramatic evolution of the selectivity in favor of **7A**, owing to the formation of the most stable micellar self-assembly.[23] The formation of **7A** ($V_0 = 15$ mM h^{-1}, and $c_{eq} = 32$ mM) is achieved by the destruction of its competitor **1A** ($V_0 =$ -15 mM h^{-1}, and $c_{eq} = 4.5$ mM). The second competition was performed directly in deuterated water from **1**, **7**, and **A** (50 mM each). The sigmoid concentration–time profile indicates a highly selective self-replicating process in favor of **7A**, with **1A** being formed in quantities that are always less than 5 mM and reaching a concentration at the equilibrium similar to that shown in Figure 3a. Moreover, the half-time reaction for this experiment (720 min, conversion of 16 mM) is twice the half-time of the formation of neat **7A** (340 min, conversion of 17.5 mM), illustrating the competition between the coupled equilibria. The final sizes, measured by DOSY NMR spectroscopy, of the micellar self-assemblies produced from these two competitions are similar to one another (7.2 nm) but also near equal to the structure of neat **7A** (7.1 nm; Supporting Information, Table S1).

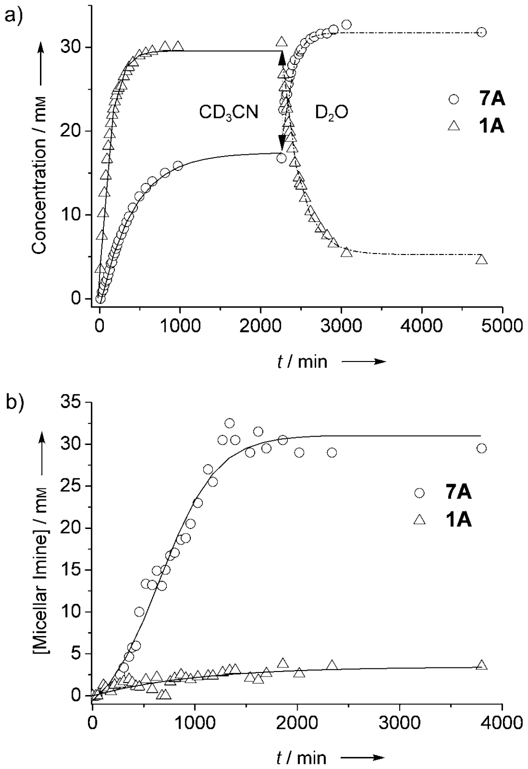

Figure 3. Molecular selection in coupled equilibria through the self-replication of a specific mesostructure. a) Concentration of imines **1 A** and **7 A** versus time starting from an equimolar mixture of **1**, **7**, and **A** ($c = 50$ mM each) in CD_3CN and, after reaching the thermodynamic equilibrium, by changing the solvent to pure D_2O. b) Concentration of imines **1 A** and **7 A** versus time starting from an equimolar mixture of **1**, **7**, and **A** in D_2O ($c = 50$ mM each).

This work describes a general concept for the synergistic relationships that exist at two length scales within a self-replicating DCL (Figure 4). The molecular constituents compete at the subnanometer scale for the reversible production of dynablocks having different $r_{H/h}$. This ratio, together with

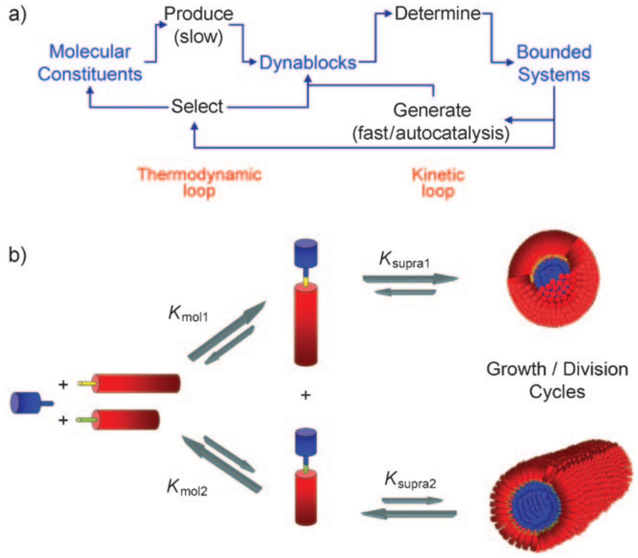

Figure 4. a) Synergistic constitutional relationships observed at two length scales within b) a model minimal self-replicating DCL. For clarity, the growth/division cycles of micellar structures are not represented.

the stacking effect, mainly determines the formation and the thermodynamic stability of the bounded structures at the tens of nanometer scale. Then, in a first autocatalytic loop with a sigmoid concentration–time profile, these self-assemblies are able to generate their own formation by increasing the rate of the dynablock condensation and they entirely fulfill the required characteristics of a minimal autopoietic process. Moreover, in a second thermodynamic loop, the self-assemblies discriminate between the incorporated dynablocks and thus favor the preferential synthesis of their own blocks. Such a system, combining cooperative processes at different length scales in networks of equilibria and displaying autocatalysis within DCLs, is of interest for the understanding of the emergence of self-organizing collective properties but also for the design of responsive systems.[24–26] We are currently working on the development of more complex dynamic combinatorial networks of competing self-replicating assemblies.

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