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Controlled assembly of covalent and supramolecular chemical modules: from engineering of complex structures to high-performance chromatography*

D. Alcor,[a] J.-F. Allemand,[a,b] I. Aujard,[a] J.-B. Baudin,[a] C. Benbrahim,[a] S. Charier,[a] E. Cogné-Laage,[a] V. Croquette,[b] A. Estevez Torres,[a] F. Ferrage,[a] L. Jullien,[a]★ A. Kononov,[a,c] A. Lemarchand,[d] H. Lemarchand,[d] and O. Ruel[a]

[a]Department of Chemistry, Paris Higher School**
Fax: +33 (0)1 44 32 33 25. E-mail: Ludovic.Jullien@ens.fr
[b]Laboratory of Statistical Physics, Paris Higher School**
[c]Chair of Molecular Optics and Biophysics,
Institute of Physics, St-Petersburg State University,
1 ul. Ul´yanovskaya, Peterhoff, 198516 St-Petersburg, Russian Federation
E-mail: Kononov.Alexei@pobox.spbu.ru
[d] Laboratory of the Theoretical Physics of Liquids,
Pierre and Marie Curie Paris University, Paris VI, CNRS UMR 7600,
4, Place Jussieu, 75252 Paris Cedex 05, France.

A biological approach to generating complex behaviors in chemistry is described. It is suggested theoretically that the assembly of "modules" composed of chemical reactions or molecular structures under appropriate external constraints can lead to features typical of the biological world like autocatalysis, kinetic proofreading, or oriented molecular motion. This approach may account for the primitive steps of molecular evolution and, in addition, it can find useful applications in chemistry such as high-performance chromatography.

Key words: complex structures, chromatography, supramolecular chemistry.

The search for structure—property relationships for a substance at the atomic scale is a major issue in chemistry. The abundance of experimental data and the existence of potent theoretical tools have enabled the syntheses of large molecules and supramolecular ensembles whose properties can reasonably be predicted. Thus, one can now synthesize "properties" instead of particular chemical species, *i.e.*, a behavior rather than a structure is the goal of the synthesis.

The living nature has been always a source of inspiration for chemists. Many aspects of the biological world are now understood. The main metabolic pathways in biology are known; they involve molecules whose structure and reactivity obey the canonical rules of chemistry.[1] The structures of numerous biological macromolecules and supramolecular ensembles (nucleic acids, proteins, lipid bilayers) have been elucidated and some of their functional properties such as catalysis and energy transfer during photosynthesis are understood, at least, qualitatively.[2] Many characteristics of these systems were reproduced by supramolecular chemists.[3] However, we still lack good chemical representations for several essential features of living organisms that are often regarded as "complex". The free energy transduction (transfer and conversion),[4] for instance, the conversion of electrical energy to chemical energy, light to chemical energy, and chemical energy to mechanical energy, remains obscure. Similarly, the origin of some other phenomena such as autocatalysis/cross-catalysis, self-reproduction, competition, and synergism, remains poorly understood. Chemists look for the tight connection between the structure, metabolism, and functioning of the components of living matter; in particular, they try to find out whether biological structures and the chemical reactions involving them can be regarded as the smallest chemical units able to fulfill the selection functions during molecular evolution.

In this study, we were interested to design, synthesize, and study simple artificial systems reproducing some of the poorly understood functions mentioned above. Macro-

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** Ecole Normale Supérieure, 24 rue Lhomond, F-75231 Paris, Cedex 05, France.

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scopic models exhibiting complex nonlinear behaviors have been already developed;[5,6] unfortunately, they do not provide useful tools for designing chemical structures at the microscopic level. To address this issue, we assume that the laws governing the molecular evolution before the appearance of living beings were basically identical to those involved in biological evolution; in particular, we consider that complex functions at the chemical level result from assembly (or symbioses) of elementary modules composed of molecular structures and/or chemical reactions. In our opinion, this novel "synthetic" frame could be of interest for elucidating structure—metabolism—function relationships in biology and could open new prospects in various fields of chemistry. The present paper gives a brief review of our recent theoretical advances in this field.

The processes occurring upon coupling of two chemical reactions through a molecule with two reaction sites

Auto- and cross-catalysis are among the most attractive types of complex behavior of chemical systems. Indeed, they are often claimed to have played a major role in the self-organization of living beings whose metabolism relies on organic reactions.[5—7] Meanwhile, simple autocatalytic reactions are hardly known in organic chemistry.[8] In fact, known autocatalytic behaviors involve either collective phenomena such as phase transitions,[9,10] rather sophisticated structures whose functioning resembles the self-replication of DNA,[11—19] the formation of homo-oligomeric chelates,[20,21] or feedback mechanisms related to physical properties.[22] As a possible alternative, we were interested in finding out whether autocatalysis could arise from coupling of two modules of classical reactions known from textbooks in organic chemistry.

To this end, we studied recently a combination of two chemical reactions with a C_{12} molecule involved in these two reactions and bearing two different reaction sites, **1** and **2**.[23] We assume that site **1** in C_{12} participates in the reaction of interest (1), either as a reactant or as a catalyst (Fig. 1). Under appropriate kinetic constraints, the state of site **2** is specified by an auxiliary reaction (2), due to exchange with a reservoir that maintains constant concentrations of some species. Under these conditions, the state of site **1** is determined by the mutual influence (coupling) between sites **1** and **2**. Variation of the state of site **1** induced by state **2** allows one to tune the rates of reaction (1) and, correspondingly, the dynamic properties and the steady state of the reactants involved in reaction (1). This approach has common features with the phenomenon of allostery, encountered in the living world[8] and reproduced in artificial systems.[24]

This simple two-site strategy provides a rich collection of systems that exhibit attractive dynamic and asymptotic properties. First, it can be used for local and continuous tuning of the product yields and the rate constants for a given reaction. This step is significant, as the most elaborated behaviors we report rely on appropriate scaling of the rate constants. In the case of two-site catalysis under conditions far from equilibrium, we showed that the reaction yield can be tuned as in the steady state, irrespective of the constraints imposed by the detailed balance at equilibrium.[25] We also proved that by using two-site catalysis, one can perform kinetic proofreading,[26] *i.e.*, the level of mismatch of the reactants becomes much smaller than that obtained near equilibrium. In addition, when considering two situations of autocatalysis, the simple two-site catalysis mechanism offers an alternative to the more intuitive cross-behavior between two-site reactants and products.

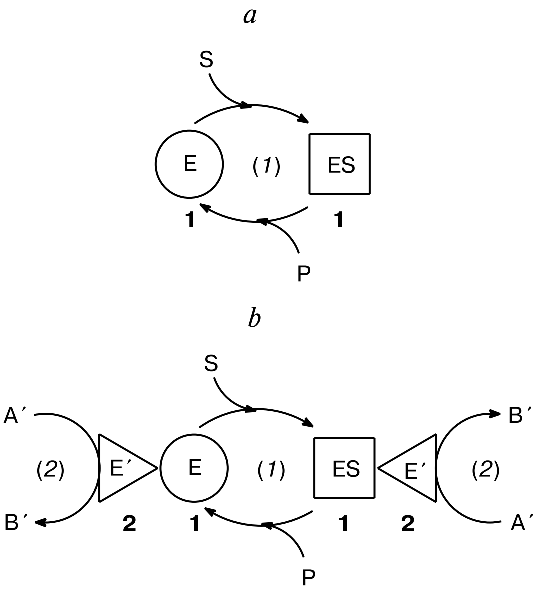

Fig. 1. Molecules with two reaction sites **1** and **2**, which are involved in coupling of two chemical reactions S $\rightleftharpoons$ P (1) and A´ $\rightleftharpoons$ B´ (2) in the absence of site **2** (*a*) and in the presence of site **2** coupled with site **1** (*b*).
Note. Sites **1** and **2** of molecule C_{12} function as catalysts for two reactions (*1*, the target reaction; *2*, activating reaction): (*a*) in the absence of site **2**, the concentrations in the states E and ES of catalytic site **1** are determined only by the reactant S and P concentrations; (*b*) in the presence of site **2** coupled to site **1** (E´), the concentrations in the states E and ES of the catalytic site **1** can depend on the activating reaction (*2*), which controls the state of site **2**; in this case, the concentrations of A´ and B´ influence the concentrations of S and P.

Oriented molecular motion induced by coupling of a chemical reaction with a molecular structure

The conversion of chemical energy into mechanical energy is one of the major goals of chemistry. The batteries and electric cells are familiar tools currently used for this purpose, although direct conversion of chemical energy into mechanical energy is more rare. In biological systems, this type of conversion is essential (processes involving mechanical coupling such as allostery or motion generation). Oriented motion is currently achieved by many living organisms but the underlying mechanisms at the molecular level still remain largely unknown. Several theoretical approaches accounting for conversion of chemical energy into mechanical energy have now been developed. One of the most satisfactory mechanisms is based on Feynman´s thermal ratchet.[27,28] In this model (Brownian molecular motor), oriented motion is due to a combination between the diffusion and a nonequilibrium chemical reaction in an appropriate potential.[29,30] More precisely, a molecular cargo would switch between two chemical states that interact with an external field in different ways (the rail). If the potential is spatially asymmetric and the characteristic times of the chemical reaction are commensurable with the times of diffusion/migration between two adjacent potential wells, anisotropic motion can be generated (Fig. 2). This principle probably governs the behavior of natural systems such as RNA polymerase/DNA.

Figure 2 shows how a nonequilibrium chemical reaction (for example, ATP → ADP + P_i in the case of the RNA polymerase/DNA system) controls the switch of a molecular cargo (in this case, RNA polymerase) that exhibits two distinct states interacting in different ways with a periodic and locally asymmetric potential, ψ, generated by the rail (DNA, in the system RNA polymerase/DNA, or an array of microelectrodes as envisaged in our laboratory). The molecular cargo existing initially in a bound state that "sees" the periodic potential (the black disk located at a given point; the probability of occurrence at this point is described by a Dirac function) switches to the free state that "sees" the flat potential (gray disk) due to a chemical reaction (*e.g.*, fixation of the phosphate residue on the cargo surface accompanied by ATP hydrolysis). When diffusion starts, the probability function becomes larger.

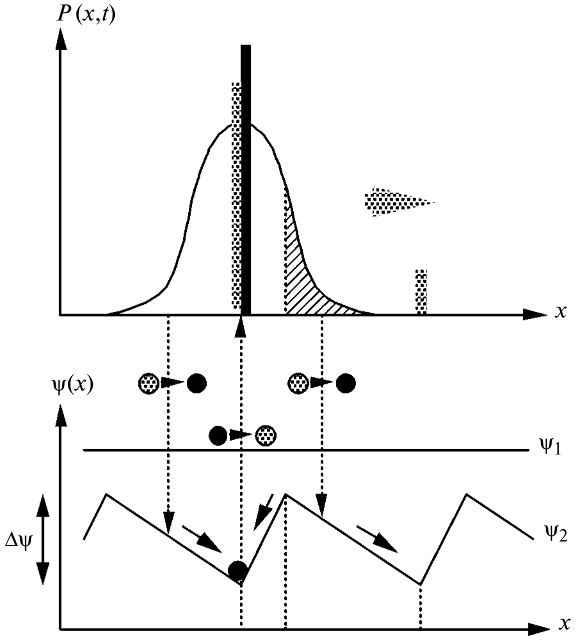

Fig. 2. Example of generating oriented motion by driving a system out of equilibrium under the action of a chemical reaction (ψ_1 — flat potential, ψ_2 — periodic and locally asymmetric potential).

If the lifetime of the free state is long enough, the molecular cargo can overcome the potential barrier. Having returned to the bound state (*e.g.*, by losing the phosphate residue upon hydrolysis), the molecular cargo experiences again the field action (one cycle results in a two-peak concentration profile). The asymmetry of the potential profile causes the oriented motion (in this case, to the right for a positively charged free state). The optimization of the average lifetime of the two states of the molecular cargo results in resonance relations that include chemical kinetics, diffusion, and field characteristics. Only those molecules that satisfy these resonance relations would move in a given direction; thus, motion generation is selective.

The model of molecular Brownian motor has already been addressed experimentally, in particular, the transition between the free and bound states was due to switching on and off of the external field rather than to a chemical reaction (the moving species was a nonreactive colloid).[31—35] Recently, we performed theoretical calculations in order to evaluate the model of the molecular Brownian motor in an experimental context that is closer to biological systems, for example, by relating the hydrolysis of ATP to motion. In this case, a nonequilibrium chemical reaction determines the switching between a free and a bound state. In our model, an asymmetric and stationary external field acts as the rail that restricts the cargo motion (actin filament, DNA, or microtubes in biological objects).[36] Our calculations provide relations to connect the parameters that are interpreted as stochastic resonances.[37—40] The speed of motion is optimized when the characteristic times associated with stochastic processes, such as reaction or diffusion, are close to the characteristic times imposed by the electric potential. These relations establish the orders of magnitude for numerous parameters involved in the model (diffusion coefficients, rate constants for chemical reactions, electric potential). We conclude that this strategy is easier to implement when the molecular cargo moves in a microscopic field (generated, for instance, by a set of microelectrodes) rather than in a nano-sized field generated by a molecular rail, as in natural systems. Then the range of spatial dimensions considered in the model is 0.1—10 μm rather than 1—10 nm, as is typical of DNA.

High performance chromatography using a chemical reaction in a variable periodic external field

The generation of molecular motion is closely related to chromatography, which is based on the selective motion of components. In fact, the first experiments related to the model of molecular Brownian motor dealt with separation of colloids on the basis of different diffusion coefficients.[31—35] This strategy is suitable for generating motion and organized structures but only within the scope of the difference between the diffusion coefficients of system components. In principle, our approach outlined in the preceding section may improve the separation process by adding reactivity as a further selectivity parameter.[36] In this case, even for molecules of similar size having similar diffusion coefficients, specific addressing that relies on fulfillment of the resonance conditions can generate motion and spatial organization. We were interested in applying the idea of resonance introduced in the previous section to explicitly address molecular separations under less constraining conditions than those associated with the Brownian molecular motors. In particular, we considered the question of whether it is possible to extract a molecule characterized by a particular rate constant for the reaction with a given target (*e.g.*, a drug for a receptor, or an oligonucleotide for a fragment of single-stranded DNA) from a mixture of molecules. The approach is based on using a uniform external field varying with time whose variation period is adjusted to the association kinetics of the molecule to be separated.

Diffusion controlled by conducting a chemical reaction in an external periodic field

Let us consider component **C** of a mixture, which reacts with target **P** to give complex **Q**

Scheme 1

$$\mathbf{P} + \mathbf{C} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathbf{Q},$$

k_1 is the rate constant for association and k_2 is the rate constant for dissociation.

The target **P** is assumed to be present in an excess and be uniformly distributed in the medium ($[\mathbf{P}]$ = const). The medium is exposed to a uniform electric field alternating according to a sinusoidal law (pulsation period ω and amplitude a) with a zero time-average value.

According to theoretical calculations,[40] after a short transient period, the average position of the {**C**, **Q**} species does not change, the motion of {**C**, **Q**} being diffusive. There exists a threshold of the amplitude a beyond which the apparent diffusion coefficient $D_{\{\mathbf{C},\mathbf{Q}\}}$ is described by the equation

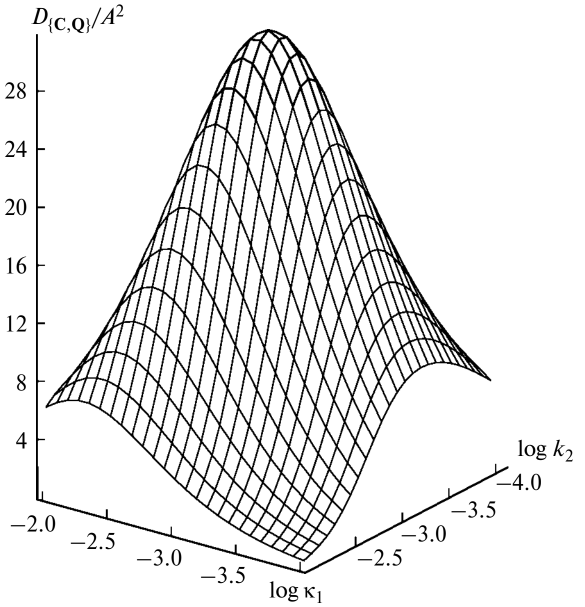

Fig. 3. Theoretical dependence of the $D_{\{\mathbf{C},\mathbf{Q}\}}/A^2$ value on $\kappa_1 = k_1[\mathbf{P}]$ and k_2 for $\omega = 0.002$ and $[\mathbf{P}] = 1$; $A = a(\mu_\mathbf{C} - \mu_\mathbf{Q})$.

$$D_{\{\mathbf{C},\mathbf{Q}\}} = [a(\mu_\mathbf{C} - \mu_\mathbf{Q})]^2 \cdot \frac{k_1[\mathbf{P}]k_2}{2(k_1[\mathbf{P}]+k_2)[(k_1[\mathbf{P}]+k_2)^2+\omega^2]}, \qquad (1)$$

which includes the rate constants k_1 and k_2, characteristics of the electric field, and the electrophoretic mobilities $\mu_\mathbf{C}$ and $\mu_\mathbf{Q}$. The dependence of the diffusion coefficient $D_{\{\mathbf{C},\mathbf{Q}\}}$ on k_1 and k_2 with constant ω and $[\mathbf{P}]$ is shown in Fig. 3.

The function $D_{\{\mathbf{C},\mathbf{Q}\}}$ is a symmetric function of $\kappa_1 = k_1[\mathbf{P}]$ and k_2. It exhibits a single maximum whose value can be changed by varying the amplitude a of the electric field. The position of this maximum is related to the resonance conditions: $k_1[\mathbf{P}] = k_2 = \omega/2$. A change in the rate constants k_1 and k_2 by an order of magnitude decreases appreciably the apparent diffusion coefficient. To evaluate these theoretical predictions, model systems involving charged azo dyes and α-cyclodextrin are currently under investigation.*

Controlled diffusion for the separation of molecules

In the above example, the apparent diffusion coefficient of a species can be selectively and considerably increased without any average displacement of its concen-

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* D. Alcor, J.-F. Allemand, E. Cogné-Laage, V. Croquette, F. Ferrage, L. Jullien, A. Kononov, and A. Lemarchand, unpublished results.

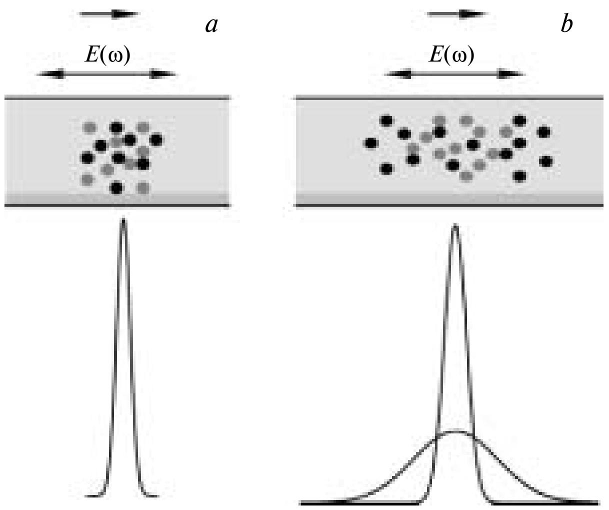

Fig. 4. Principles of separation of mixtures based on tunable diffusion: (*a*) in the chosen example, an equimolar mixture of gray and black particles is placed in a gel (initially, the concentration profiles of gray and black species coincide), (*b*) the application of a periodic electric field induces the diffusion of target particles (black, in this particular case); the diffusion profile is broader for black species than for gray ones.

tration profile. This feature can be used successfully for separation according to the following protocol: (*i*) spot a mixture in a medium with an appropriate receptor concentration $[\mathbf{P}] = 1/K = k_2/k_1$, (*ii*) apply a uniform periodic field with $\omega = 2k_2$ and with a relatively large amplitude a,[40] and (*iii*) collect the targeted species at the tails of the diffusion profile.

In affinity chromatography, the resolution of components eluted at different velocities is hampered by too pronounced diffusion. Here, in contrast, the enhancement of the effective diffusion in the absence of an average displacement of the mixture proves to be a key to the success (Fig. 4). In addition, this separation is very fast (shorter than a few oscillations of the periodic electric field, $\sim 10\pi/k_2$).

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This paper introduces a general theoretical frame for generation of several types of complex behavior observed in biology. The approach is based on assembling modules composed of chemical reactions and chemical structures under appropriate external constraints. The features like controllability of thermodynamic and kinetic properties, autocatalysis, kinetic proofreading, or molecular motion were predicted. In addition, this strategy stimulated the development of analogous approaches that rely on the exposure of a module of chemical reactions to an external field; a novel protocol for high performance chromatography was proposed. Experiments aimed at evaluating the scope of this approach are currently performed by our research group.

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