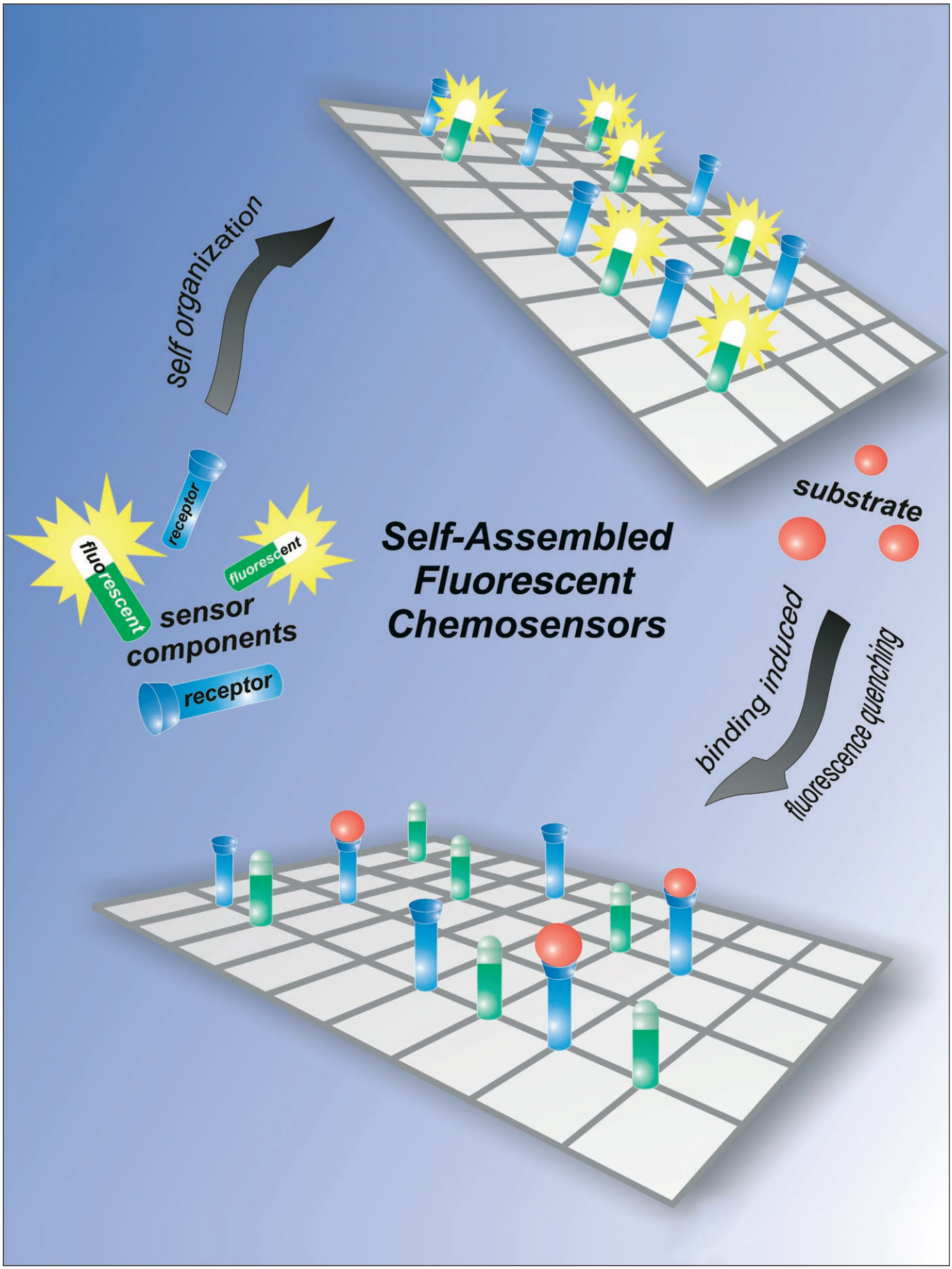

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Self-Assembled Fluorescent Chemosensors

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Abstract: Self-assembling and self-organizing methodologies are powerful tools for the "bottom-up" approach for the realization of complex structure with functional properties. Recently, this concept has been extended to the design of fluorescent chemosensors providing new exciting potentialities for the development of innovative sensing systems. This Concept Article deals mainly with this new approach and discusses its evolution, applications, and limitations.

Keywords: chemosensors · fluorescence · nanostructured materials · self-assembly · supramolecular chemistry

Introduction

The design of chemosensors, molecules that can selectively recognize and signal the presence of a specific analyte, is one of the main achievements of supramolecular chemistry[1] and quite a number of reports indicate the great attention devoted to fluorescent chemosensors.[2] In fact, among the different possible signaling methods, fluorescence offers several advantages, such as high sensitivity and low-cost instrumentation. In addition, the molecular dimensions of chemosensors, combined with the availability of techniques such as confocal microscopy, allow high spatial resolution in the detection of the analyte, which makes intracellular monitoring of selected species for medical and biochemical studies feasible.

The design of fluorescent chemosensors has continuously evolved. In the first approach explored, the sensors feature the donor atoms for substrate complexation as part of the fluorophore π system.[2,3] In these systems, which have been named *intrinsic chemosensors*,[1a] the interaction between the bound substrate and the fluorophore leads directly to the modification of its emission properties (Figure 1). These chemosensors are relatively easy to design, but they are intrinsically rigid, as they have to be designed around the substrate; any modification of the binding site, in order to modulate its selectivity or affinity for the substrate, may result in a change in the emission properties of the dye. For this reason, the evolution and the optimization of this type of sensor may not be easy from the synthetic point of view. A second strategy, which has been widely pursued owing to the new perspectives brought forward by supramolecular chemistry, is based on the construction of a sensor in which the ligand is electronically insulated from the π system of the fluorophore, although the two subunits are kept in close

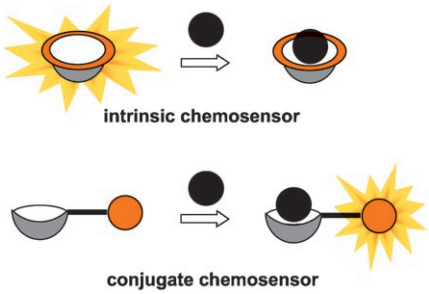

Figure 1. The cartoon represents the design of intrinsic and conjugate chemosensors. In the *intrinsic chemosensors*, the binding unit (gray cup) is fluorescent and the complexation of the substrate (black balloon) modifies directly the emission intensity (switching it off in the cartoon). In *conjugate chemosensors*, the binding subunit (empty cup) is electronically insulated from the fluorescent subunit (orange balloon) by an inert spacer (black line). Binding of the substrate activates a transduction mechanism that modifies the emission of the dye (switching it on in the cartoon).

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proximity by means of covalent links (Figure 1).[1a,2] In these kinds of sensors, referred to as *conjugate chemosensors*, the two subunits can be designed, separately optimized, and then eventually connected. Such a modular approach clearly allows more flexibility than in the previous one, but still the fluorophore and the ligand must be covalently linked, which often results in a considerable synthetic effort and in a structural rigidity. Moreover, the overall design of the system must integrate a transduction mechanism, needed to allow the proper communication between the two subunits in order to convert the recognition of the analyte into the generation of the signal.

Self-assembling and self-organizing methodologies have attracted increasing attention during the last years in the chemistry of complex systems with functional properties.[4] Indeed, they are at the basis of the so called "bottom-up" approach, as the building of complex structures, following this strategy, simply requires the design and synthesis of a limited number of relatively simple building blocks that are then allowed to self-organize. As a result of the molecular organization into a supramolecular assembly, novel properties and functions may result and lead to possible important applications.

On these bases, self-organization of receptors and fluorescent dyes to form organized assemblies can, at least partially, overcome the synthetic problems connected to the classical covalent systems, and provide an efficient strategy for the easy realization and optimization of fluorescent chemosensors. In this case, there is no need for covalent links between the essential subunits; they only have to be designed in such a way as to favor their assembly in solution. Among the different strategies[5] that can be followed to exploit such principles at the present, two approaches appear to be the most promising and are well established. The first one, which has been initially proposed by Anslyn and his coworkers and then developed and applied to the detection of a large number of substrates by the same author and by other groups, is based on a competitive assay in which the fluorophore and the substrate compete for the receptor.[6] The displacement of the dye from the complex results in a change in the local environment experienced by the fluorophore, which influences its emission properties. These self-assembled chemosensors are made by assembling a dye, a substrate, and a receptor that is able to interact with both chemical species, but with different binding strengths and, following this design, they have been named *chemosensing ensembles*.

The second approach to the realization of self-assembled chemosensors, proposed by us[7] in 1999, is based on the self-organization of the essential sensor subunits on a proper template. In this case, the dye and the receptor do not interact directly, and the communication between the bound substrate and the dye is ensured only by the spatial proximity of the two subunits on the template, provided that a transduction mechanism is present. The template employed in the original work was a surfactant aggregate, but this approach was further developed by us and other groups and extended to the use of more suitable templates, such as glass surfaces, monolayers, and nanoparticles. For their mode of construction these systems can be named *template-assisted self-organized chemosensors*.

This Concept Article will briefly describe the principles behind chemosensing ensembles, and will then focus mainly on template-assisted chemosensors, discussing their evolution, applications, and limitations.

The Chemosensing Ensemble Paradigm

The chemosensing ensemble strategy is based on a competition assay and works in a manner similar to that of many antibody-based biosensors in competitive immunoassays:[8] a solution containing the unlabeled antigen is added to the antibody receptor that is associated with a tagged antigen. Upon displacement of the tagged antigen, a signal modulation is observed. Likewise, the supramolecular version of this assay uses a recognition unit, designed for selective interaction with a desired analyte, along with an external indicator (a UV-visible chromophore or a fluorescent dye) that associates with the recognition unit in the absence of the analyte. When the analyte is added, the indicator is displaced from the cavity, thus leading to a measurable change in its optical properties (Figure 2). This methodology has several

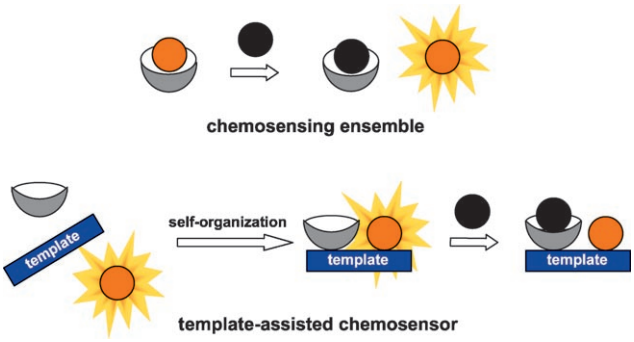

Figure 2. The cartoon represents the design of chemosensing ensemble and template-assisted chemosensor. In the first class the substrate (black balloon) and the dye (orange balloon) are in competition for the receptor (gray cup). The displacement of the dye from the complex results in a change of its fluorescence emission (switching on in the cartoon). In the template-assisted chemosensor the dye and the receptor self-organize on a template surface. Upon binding to the receptor the substrate is hold in close proximity to the dye and may influence its fluorescence emission (switching off in the cartoon).

useful features, related mainly to the self-assembling nature of the ensemble. It can be applied to a variety of receptors without need for covalent attachment of the indicator, which, in turn, can be selected in a large pool of commercially available fluorescent or UV-visible chromophores. The indicators may be chosen on the basis of their optical properties or on their association ability compared with that of the analyte, and the indicator–receptor ratio can be varied, according to specific needs allowing a fine tuning of the

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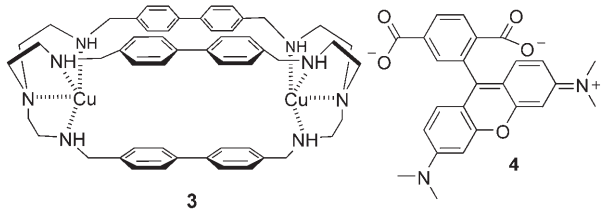
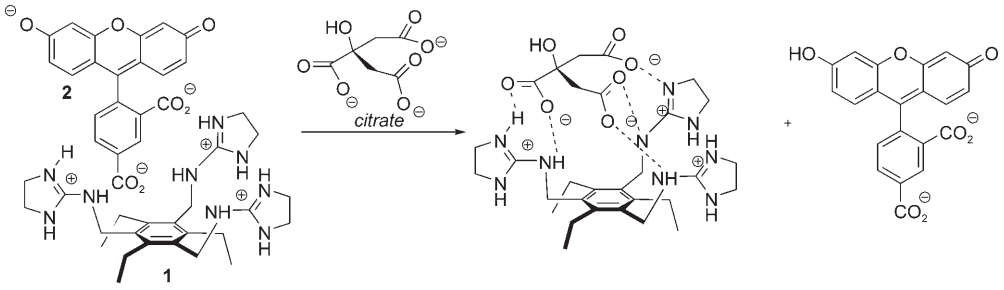
system.[9] Moreover, in this set-up, the indicator does not interact directly with the analyte, but only with the receptor through non-covalent interactions. Upon release from the complex, the indicator experiences a change of interactions, from those within the complex to those with solvent molecules, and such a change of the indicator environment originates in the modulation of the signal. Therefore, the analyte is not involved in the transduction mechanism; this feature is particularly important, because it allows the detection of substrates that are not active directly in perturbing the optical properties of the indicator.

Although the use of chemosensing ensembles is easy and convenient, it has been developed only in the last ten years; after two earlier examples reported by Inouye[10] and Shinkai[11] and their co-workers, it has been pioneered by Anslyn and co-workers, who reported a chemosensing system for the detection of citrate in aqueous media.[12] Receptor **1** (depicted in Scheme 1) was found to be selective for citrate over dicarboxylates, phosphates, sugars, and simple salts in water. Due to the preorganization of the three guanidinium moieties on the same face of the receptor and on the ability to form multiple hydrogen-bonding and charge-pairing interactions, it binds citrate better than simple dicarboxylic and monocarboxylic acid by factors of around 35 and 700, respectively. The anionic fluorescent dye 5-carboxyfluorescein (**2**) was used as indicator in a methanol/water solution buffered at pH 7.4. Binding between **1** and **2** ($K_a = 4.7 \times 10^3\,\text{M}^{-1}$) lowers the p$K_a$ of the phenol moiety of **2**, due to the positively charged microenvironment presented by the receptor, causing its deprotonation. Upon addition of citrate to the ensemble ($K_a = 2.9 \times 10^5\,\text{M}^{-1}$), the carboxyfluorescein is released as a phenol-protonated species, and, as a result, a decrease of the indicator's luminescence is observed that allows the quantitative detection of citrate. The ensemble was used to determine citrate concentration in commercial beverages that contain high concentrations of potentially competitive anions, including malate, ascorbate, lactate, benzoate, and phosphates.

Following this approach, Anslyn and other research groups have reported the realization of chemosensing ensembles for the detection of several organic or inorganic substrates, such as tartrate,[13] gallic acid,[14] heparin,[15] phosphates,[16] carbonate,[17] amino acids,[18] and short peptides.[19] To mediate the interaction between the indicator and the binding site of the receptor, electrostatic interactions, hydrogen bonding, formation of boronic esters, and/or metal–ligand interactions have been employed. The last mode of interaction has the advantage to provide sufficiently high association constants, even in polar solvents such as water, a solvent that is strongly desired for several applications, but is also highly demanding. One example of such a class of chemosensing ensembles can be taken from the work of Fabbrizzi and his co-workers, who pioneered the application of metal–ligand interaction in the field of chemosensors.[20] The dicopper(II) cryptate complex **3** forms a long ellipsoidal cavity in which the two copper ions are rigidly held at a distance of 11.3 Å.[21] Therefore, **3** is particularly well suited for



the inclusion of ambidentate anions, such as dicarboxylates, the donor groups of which are well separated and able to interact with both metal ions. Carboxyrhodamine (**4**), which contains two carboxylate groups and emits at 571 nm (orange fluorescence), was chosen as indicator. In water at pH 7, it binds to the receptor ($\log K_a = 7.0$) and, in the resulting (receptor/copper/indicator) complex, its fluorescence emission is completely quenched, probably through a photoinduced electron transfer or an electronic energy transfer from the metal center to the excited fluorophore. Addition of dicarboxylate derivatives, such as phthalate isomers and aliphatic α,ω-dicarboxylate, results in the displacement of the rhodamine dye from the complex and in the recovery of its fluorescence emission. Due to the geometric constraints imposed by the rigid structure of the receptor, the system is able to discriminate between the different substrates on the basis of the distance between the two carboxylate functions. Thus, among the phthalate isomers only the 1,4-derivative, which has the correct distance between the two carboxylates to interact with both the metal ions without inducing any endoergonic rearrangement of the cage, binds to the receptor more strongly ($\log K_a \approx 8$) than the dye, and can efficiently displace it from the complex. The other derivatives



Scheme 1. The chemosensing ensemble for the detection of citrate reported by Anslyn and co-workers.

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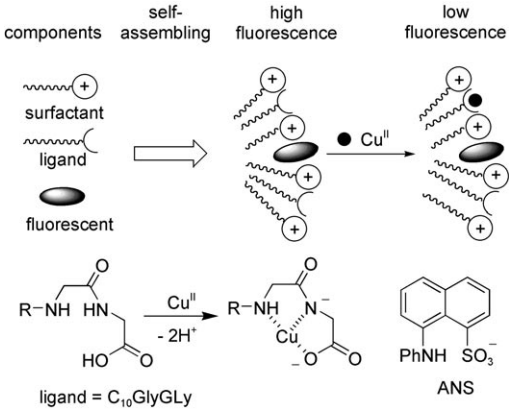
bind to the receptor more weakly ($\log K_a < 4.5$) and, as a consequence, the 1,4-derivative can be detected selectively. A similar behavior is observed for aliphatic α,ω-dicarboxylic acids with the derivatives that contain five or six carbon atoms detected selectively over the shorter or longer analogues. The strong metal–ligand interactions established between the receptor and the anion easily compensate for the unfavorable dehydration effects and allows recognition in pure water at neutral pH. Again, the key feature of the chemosensing ensemble assay is the displacement of the dye from the receptor due to competition with the substrate; the fluorescence modulation is the result of the sole receptor–dye interaction, thus allowing the detection of substrates that are unable to perturb the electronic properties of the indicator.



Scheme 2. Self-assembled fluorescent chemosensors based on surfactant aggregate.

The Template-Assisted Self-Organized Chemosensing Strategy

This approach is based on the self-assembling or self-organization of the fluorescent dye and the receptor on a proper template forming an organized assembly. In the assembly, the two subunits do not interact directly and the communication between the bound substrate and the dye is ensured only by their spatial closeness ensured by the template. The method is simple and the main advantages are related to the choice of the template and the availability of easy methods for its functionalization. Indeed, depending on the template, little or no synthetic modifications of the ligand and the dye are needed, and this allows the easy formation of the sensor and the rapid screening of a large number of receptors and dyes in order to optimize its properties for a given application. Moreover, due to the spatial proximity of a large number of subunits in the assembly, new collective effects and properties may arise and contribute to the improvement of the sensors' performances. In the last few years, different types of template have been used to guide the self-organization of the chemosensor spanning from micellar aggregates to monolayers, to glass surfaces, and, more recently, to nanoparticles. Each of these templates has its own peculiar properties that are reflected in a characteristic performance of the resulting sensor.

Self-assembling in surfactant aggregates and monolayers: A few years ago, we reported a novel methodology to self-assemble a fluorescent chemosensor for Cu^{II} ions that exploits the self-aggregation of sensor components within surfactant aggregates.[7] Following this approach, lipophilic ligands and fluorophore molecules dispersed in an aqueous solution containing micelles move into the surfactant aggregates to generate a co-micellar assembly. The concentration of the species due to the partition within the submicroscopic micellar pseudophase ensures proximity between the ligand and the dye, so that the complexation of copper(II) ions by the ligands leads to the quenching of the dye fluorescence emission, as indicated in Scheme 2. By employing the lipophilic ligand *N*-decylglycylglycine, which binds Cu^{II} ions strongly and selectively, due to the deprotonation of the amide nitrogen,[22] the fluorophore 8-anilinonaphtalensulfonic acid (ANS), and the inert surfactant CTABr (CTABr = cetyltrimethylammonium bromide), a self-assembled chemosensor was obtained that can detect metal-ion concentrations down to the micromolar range. The sensitivity of the system can be improved by increasing the ligand/total surfactant (ligand + CTABr) molar ratio, reaching the best performances at a limiting value of 1:2, and by decreasing the surfactant concentration down to values approaching the critical micellar concentration (cmc) value of the resulting co-micelles. The main advantages of such a system are:

1) Selectivity, mainly due to the ligand choice.
2) Simplicity: the sole mixing of the components (two of them, CTABr and ANS are commercially available) in water is required to prepare the sensor.
3) The possibility to tune the detection range just by the modification of the components ratio.
4) Modularity, which allows the modification or the optimization of the system by simply substituting one of the components.

The last point was demonstrated by setting up combinatorial experiments, in which, keeping the ligand constant, sixteen combinations of surfactant and dye were tested by employing four different surfactants and four different dyes, all commercially available. Recently, another example of the application of such an approach to the detection of Cu^{II} that employs a different ligand and dye has also been reported.[23]

One of the limits of the previous system was the use of the inert surfactant to form the micellar aggregates: on one hand, it is needed in its micellar form to take up the neutral and poorly soluble $C_{10}GlyGly{\cdot}Cu^{II}$ complex, but on the other hand, it implies dilution of the ligand in the aggregate and hence a decrease in the sensitivity. To improve the system we designed a family of ligands that are anphiphilic both in the free and in the complexed form.[24] These ligands produce stable homoaggregates, also in the presence of the

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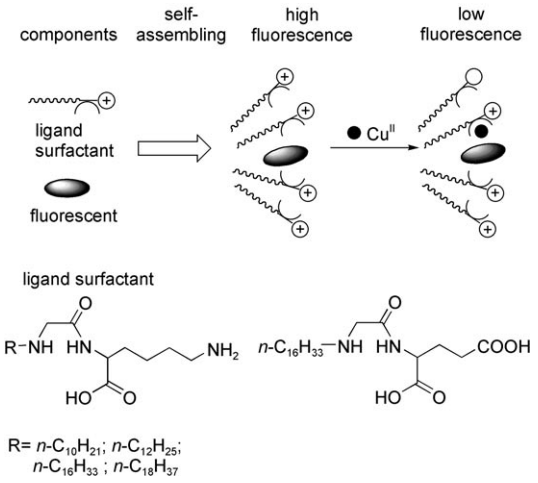
metal ion, thus avoiding the use of any added surfactant and allowing to reduce the system from three to two essential components, as indicated in Scheme 3. The ligands were prepared by substituting one of the two Gly residues with



Scheme 3. Second generation of self-assembled fluorescent chemosensors based on surfactant aggregate. In this case the ligand is anphiphilic and forms homoaggregates, making superfluous the use of the inert surfactant.

lysine (Lys) and glutamic acid (Glu), bearing an ionizable group in the side-chain, on the assumption that this could ensure amphiphilicity to the ligands and hence the capability to form homoaggregates also when complexed to the copper(II) ion. Lipophilic fluorophores, like ANS or Rodamine 6G, are effectively bound into the aggregate pseudophase, and the binding of Cu^{II} ions to the dipeptide units causes a strong fluorescence quenching. The sensor system is very sensitive to Cu^{II} (concentrations in the submicromolar range are detected), is promptly reversible and no interference is observed due to the presence of many metal ions. The sensitivity of the system improves by decreasing the ligand concentration and (up to a point) the ligand's cmc by changing the size of the lipophilic alkyl chain.

A similar concept was exploited by Leblanc and his coworkers for the development of self-assembled Cu^{II} chemosensors onto Langmuir and Langmuir–Blodgett (LB) films.[25] The system is based on the use of lipophilic peptides as selective receptors for the metal ion and two different approaches have been explored. In the first one, a covalent approach, both the ionophore and the fluorophore are linked together in the same molecule (lipid A of Figure 3a) so that an intramolecular interaction is responsible for the fluorescence quenching of monolayers of lipid. In the second one, a self-assembled approach (Figure 3b), the ionophore (lipid B) and the dye (lipid C) are located on different molecules that self-assemble in the film. In this case, fluorescence quenching is due to a through-space interaction mechanism favored by the close proximity of the sensor components on the layer. The sensor can be assembled on Langmuir films and transferred to monolayer LB films, maintaining its Cu^{II}-sensing properties. Moreover, on LB films, the system is endowed with high sensitivity (10^{-5}–10^{-6} M detection limit), good selectivity in comparison with several other transition-metal ions, and it is fully reversible as the quenched fluorescence is restored by simply washing the film with HCl.

The driving force for the self-assembling of these chemosensors based on surfactant aggregates is the hydrophobic interaction of the lipophilic building blocks; this results in an usually simple chemical synthesis of the sensor components and in the easy preparation of the sensor.

However, the actual applicability of such systems is limited by several factors. In particular, surfactant aggregates are delicate objects owing to their dynamic nature. They form

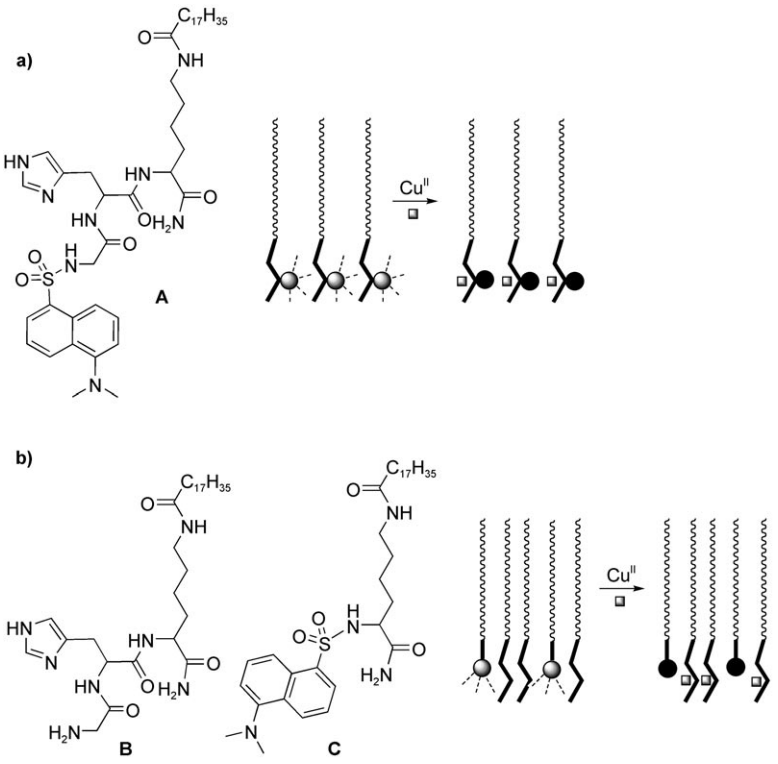


Figure 3. Structure of the lipids and proposed mechanism of the fluorescence quenching on Langmuir monolayers caused by Cu^{II} ions: a) intramolecular quenching for monolayers of lipid A; b) intermolecular quenching for monolayers of lipids B/C.

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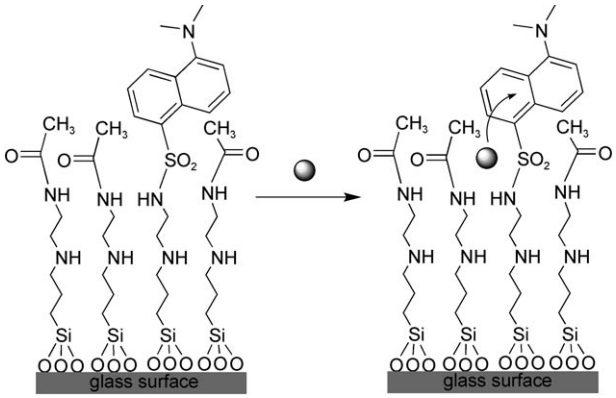
only above the cmc, the fraction of non-micellized components may be significant, and they are very sensitive to environmental conditions, such as temperature and ionic strength, which can affect the reproducibility of the sensor response and, in the case of the system based on LB-films, also its duration. To address these limitations, the use of other templates has been explored.

Self-organization on glass surfaces: As in the previously discussed example, the concept of proximal but spatially separated receptor–fluorophore communication can be transferred from dispersible aggregates in solution to suitable surfaces, which might prove more practical in terms of actual device implementation. Self-assembled monolayers (SAMs) provide a more convenient way to produce surfaces with specific chemical functionalities that allow a precise tuning of surface properties.[26] SAMs have been successfully used to demonstrate that a molecular recognition process is feasible at the monolayer–solution interface;[27] however, the realization of such fluorescent a chemosensor has been limited, mainly because most of the systems so far reported were laid on surfaces of gold, which causes fluorescence quenching.[28] As a step forward toward fluorescent chemical sensing, attention is being addressed to glass (SiO_2) as the supporting surface, since it is transparent to light and does not alter the fluorescence emission. Crego-Calama and Reinhoudt have introduced a new methodology that involves the sequential chemical modification of amino-terminated SAMs on glass with a fluorescent probe and a specific amino-capping functionality.[29] Such a chemically modified surface can be used as a simple recognition material for metal ions, while the fluorophore acts as a reporter. The initial monolayer is formed by reaction of the glass surface with amino-functionalized trialkoxysilane and, subsequently, the free amino groups are treated with the proper derivative to form amide, urea derivative, and sulfonamide moieties. The resulting SAMs are not strictly "self-assembled",[30] because the coupling to the surface is covalent and not reversible. However, the new properties of the material derive from a spontaneous assembly of the subunits on the surface and, therefore, these systems might properly be referred to as "self-organized".[31] By following this method (Scheme 4), a chemosensor able to detect Pb^{II} has been prepared. However, the lack of strong metal-ion binding sites and the relatively low intensity of fluorescence emission due to the small surface area resulted in low selectivity and sensitivity.

The intrinsic low selectivity of these SAMs turned out to be advantageous in the realization of microsensor arrays. Following a combinatorial approach, Reinhoudt and his coworkers have prepared a library of SAMs functionalized with different ligands and different fluorophores.[32] The ligands are individually poorly selective, but the response of the whole library to different metal ions and organic anions is characteristic for each substrate, allowing their detection. This methodology is transferable from the macro to the microscale through microcontact printing, in which the fluorophore is printed onto a glass surface, and through direct attachment of the fluorophore to microchannel walls. The detection limit is still relatively high (10^{-4}–10^{-5} M), but the system is fully reversible and the response is fast. The ease of miniaturization using this technology is really promising and may allow the realization of a wide variety of simple and yet efficient microarrays.



Scheme 4. Schematic representation of a fluorescent self-organized monolayer. The metal ion (black dot) interacts with the layer and switches off the fluorescence of the dye.

Self-organization on nanoparticles: The grafting of the active components of the sensor on a glass surface allows one to overcome the limitations deriving from the dynamic nature of the surfactant-based sensors and to open the way to a variety of optodes or sensor arrays. However, for several biological applications, nanometric-sized particles are strongly preferred. In medical and biochemical research, as the size of the sample is reduced to micrometer dimensions compatible with those of living cells or their sub-compartments, the real-time measurements of chemical and physical parameters with high spatial resolution and negligible perturbation of the sample becomes extremely important and challenging. Kopelman, Rosenzweig, and their co-workers[33] have recently proposed the use of polymer nanoparticles as chemically inert matrices to entrap fluorescent chemosensors for intracellular noninvasive real-time analysis. These water-soluble nanoparticles, dubbed PEBBLEs (probes encapsulated by biologically located embedding), are based on matrices of cross-linked polymers (e.g., polyacrylamide, polydecylmethacrylate, sol–gel silica) with a fluorescent chemosensor embedded and not linked to the polymer. These matrices have been used to make sensors for pH, metal ions, and for some nonionic species. The small size of the PEBBLE sensors (from 20 to 600 nm) enables their noninvasive insertion into a living cell. The semipermeable and transparent nature of the matrix allows the analyte to interact with the indicator dye that reports the interaction through a change in the emitted fluorescence. Moreover, when compared to the "naked" chemosensor, the nanoparticle can shelter the indicator from interferences, such as protein or membrane binding, thus minimizing its toxicity. Strictly speaking, PEBBLEs are not self-assembled sensors, but complex sensing systems can be created by simply com-

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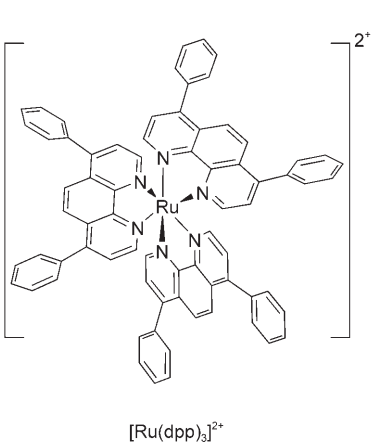
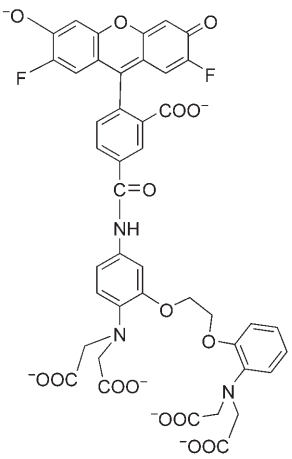
bining multiple dyes and ionophores within the polymeric matrix. These sensing schemes can include reference dyes that allow ratiometric sensing, or ionophore/chromo-ionophore combinations that allow the use of highly selective, nonfluorescent ionophores. For example, Kopelman and his co-workers[34] have reported the realization of a ratiometric sensor for intracellular oxygen obtained by including in silica nanoparticles, with diameters ranging from 100 to 400 nm, a ruthenium complex $[Ru(dpp)3]^{2+}$ (dpp = 4,7-diphenyl-1,10-phenanthroline) and the dye Oregon Green



$[Ru(dpp)_3]^{2+}$



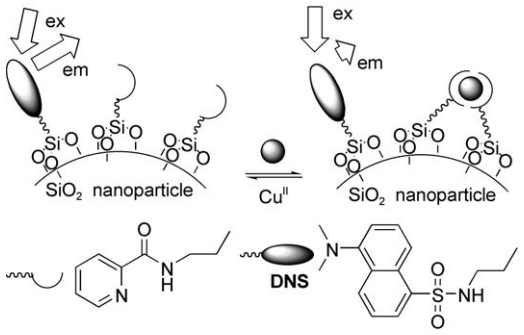
Oregon Green 488

488. In the presence of oxygen, the fluorescence emission of $[Ru(dpp)3]^{2+}$ is strongly quenched, while the emission of Oregon Green is not affected thus allowing ratiometric quantification of the level of oxygen. These PEBBLES have been inserted in living cells by using gene gun delivery techniques and have been used for the monitoring of variations of the oxygen level in the cytosol.

A different approach, introduced by Rosenzweig and his co-workers,[35] utilizes CdS quantum dots (QDs), fluorescent semiconductor nanocrystals with size-tunable fluorescence emission,[36] capped on the surface with weak ligands, such as polyphosphate, L-cysteine, and thioglycerol, as water-soluble metal-ion sensors. The ligands have an important effect on the luminescence response of CdS QDs to physiologically relevant metal cations, showing also some degree of selectivity depending on the capping used. Polyphosphate-capped CdS QDs were sensitive to nearly all mono- and divalent cations, but without selectivity. In contrast, thioglycerol-capped CdS QDs are selective toward copper and iron, while L-cysteine-capped CdS QDs respond selectivity to zinc ions without interferences by other biologically relevant metal ions and with detection limits below 1 μM. Later Gattàs-Asfura and Leblanc[37] reported a similar system in which CdS QDs (2.4 nm diameter) were capped with a pentapeptide (Gly-His-Leu-Leu-Cys), designed specifically for the binding of copper and silver ions. The resulting sensor responds selectively to these two metal ions, allowing their detection at concentrations down to 0.5 μM. These systems are peculiar because the nanoparticle is at the same time the fluorophore and the template for the construction of the sensor. As a consequence, a simplification is introduced that reduces the essential components of the self-organized sensor from three (receptor, fluorescent dye, and template) to two (receptor and fluorescent template). The simplicity is, however, counterbalanced by a decrease of flexibility, since the fluorophore cannot be easily changed, even though the fluorescence emission of the quantum dots may be tuned by changing their chemical nature and their dimensions.

The onset of cooperative or collective processes is one of the most intriguing and peculiar aspects related to nanoparticles as template for the creation of self-assembled chemosensors. These features arise from the relatively high degree of organization of the sensor components in an extended and not dynamic network and, indeed, cooperation has been described in dendrimers,[38] in nanoparticles,[39,40] and in self-assembled monolayers on gold;[41] however, it has not been observed in surfactant aggregates,[42] probably due to the dynamic and poorly organized nature of these systems.

Recently, Montalti and co-workers have studied fluorophore-functionalized silica nanoparticles and reported evidence that collective processes are at play.[43] Silica nanoparticles are well suited for the realization of fluorescent chemosensors: they are transparent to light, photophysically inert, and their surface can be easily modified by reaction with alkoxysilane derivatives. Following the studies of Montalti and co-workers,[43] we have recently described the realization of self-organized fluorescent chemosensors for Cu^{II} ions obtained by surface modification of silica nanoparticles.[44] Commercially available particles (20 nm diameter) were functionalized with the triethoxysilane derivatives of the ligand picolinamide, selective for Cu^{II}, and of the fluorophore dansylamide (Scheme 5). The grafting of the sensor components to the particle surface ensures the spatial proximity required to signal Cu^{II} by quenching of the fluorescence emission. In a 9:1 DMSO/water mixture, the coated silica nanoparticles (CSNs) selectively detect copper ions down to micromolar concentrations, and the operative range of the sensor can be tuned by the simple modification of the



Scheme 5. Self-organized fluorescent chemosensors on silica nanoparticles.

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components' ratio. Moreover, clear evidence of cooperation of the ligand subunits bound to the particles' surfaces to form binding sites with an increased affinity for the substrate (Scheme 5) was obtained.

Preparing a small library of CSNs coated with the same ligand and different fluorophores proved the versatility of this approach.[39] The emission spectra of these CSNs span over a large wavelength range from 300 to 600 nm (correspondingly, excitation wavelengths are in the range 285–466 nm), allowing the choice of the more suitable sensor for the desired application. Moreover, using a stronger ligand for the Cu^{II} ion, we were able to exploit collective processes in which one single metal ion is capable of quenching the emission of about ten surrounding dyes, thus lowering the detection limit of the sensor to the nanomolar range.[39]

Following a similar approach, a three-component, nanometer-sized, self-assembled chemosensor has been reported by Larpent and her co-workers.[40] The authors used polymeric nanoparticles of vinylbenzene (15–20 nm diameter prepared by polymerization in microemulsion) as a template, decorated with cyclam as metal-ion ligand, and the fluorescent reporter BODIPY (Figure 4). The hydrophobic

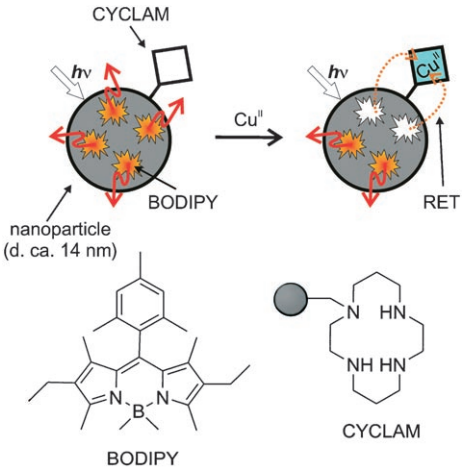

Figure 4. Schematic representation of the self-organized Cu^{II} sensor in polymeric nanoparticles. The picture shows the cooperative RET quenching process in which one single metal ion quenches the emission of several fluorescent dyes.

dye was entrapped within the polymeric matrix by impregnation and the ligand was covalently attached to the polymer backbone. Binding of the Cu^{II} ions to the cyclam ligand results in a strong quenching of the BODIPY fluorescence, probably by means of resonance energy-transfer (RET) process from the dye to the metal-ion complex. Although the ligand cyclam readily binds Zn^{II}, Ni^{II}, and other transition-metal ions, the sensor responds selectively to Cu^{II}, since the absorption band of the complexes formed with the other metal ions and the emission of the dye do not overlap, thus allowing the RET process with the Cu^{II} ion alone to dominate. Interestingly, the organization of several fluorescence units in the nanoparticle allows cooperative quenching of the fluorescence with one single metal ion able to quench the emission of up to 44 dye molecules. This cooperative behavior results in a very high sensitivity, the detection limit being in the nanomolar range.

Conclusions and Outlook

Since their appearance in the early 1980s, with the series of intracellular calcium probes proposed by Tsien and his coworkers,[3] the interest in fluorescent chemosensors boosted. They are not only the subject matter of an increasing number of scientific publications every year, but also successful commercial tools for biomedical applications. Still, most fluorescent chemosensors are complex molecules that require wearisome synthetic processes, and the modification and optimization of which, to match the requirements for a specific application, are often difficult and laborious.

Self-assembled and self-organized systems could open new perspectives to a wider application of such systems. Libraries of receptors and fluorescent dyes could be made available and then easily combined to produce the most suitable system for the desired use. Of course, the great potentialities of such a strategy must face some problems that have to be positively solved before obtaining really useful systems.

In self-organized systems, receptor and fluorophore units are not chemically connected, and this makes the implementation of a transduction mechanism more difficult. Such a problem has been brilliantly solved in the case of the *chemosensing ensemble* approach. In this case, the factor that triggers the emission of the signal is simply the location of the fluorophore, as its properties are different when it is bound to the receptor or free in solution. As said before, such a mechanism is particularly attractive, as it is completely independent of the photophysical properties of the analyte. As a consequence, at least in principle, it allows the realization of a fluorescent chemosensor for any desired target, provided that a receptor capable to recognize that substrate is available, without any further synthetic work. Moreover, grafting of the receptor units on resin beans leads to the realization of sensing devices that can be recovered and recycled after dye reloading.[45] Unfortunately, chemosensing ensembles do not appear to be suitable for several potential applications. For example, their use in flow analysis, even employing supported systems, is hampered by the fact that, once the recognition event has occurred, the reported dye is brought away by the sample flow. Intracellular applications have not yet been tested, but they appear troublesome too, if it is taken into account that cell permeabililty and reversibility are key requisites of the systems used in this field.

Template-assisted systems, with the exception of those based on surfactant aggregates, may cover a much wider application field. Nanoparticles or functionalized optical fibres tips could be used for intracellular studies, while SAMs or thin-films based systems could be the basis for the development of devices for flow monitoring. Also the realization of

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the analyte recognition site can be made much easier, as demonstrated by the elegant work of Crego-Calama and Reinhoudt,[32] in which the simple assembling of many functional groups on the template leads to the formation of binding sites for the substrate. Of course, such randomly formed recognition sites cannot be really selective, but such problems can be overcome by the realization of a sensor array in which fingerprint responses can take the place of selectivity. Moreover, the several functional subunits, kept close by the template, can operate collectively and give new properties to the material. As we found in the case of coated silica nanoparticles systems, the ligand units can cooperate to form binding sites with an improved affinity for the substrate, and many fluorescent units can respond simultaneously to the analyte recognition, thus producing an amplified signal. Other collective phenomena between the photoactive units, such as energy transfer or antenna effects, could be useful to improve the sensor features. Unfortunately, all these potentials are counterbalanced by the intrinsic difficulty in the individuation of effective transduction mechanisms. As there is no direct interaction between the recognition and the signaling units, the modification of the fluorophore state as consequence of analyte binding must occur by long-range interactions. Copper(II) and other transition-metal ions can quench fluorescence emission either by electron or energy transfer, and the latter mechanism can operate even at relatively large distance. For this reason, almost all the template-based systems so far reported are based on Cu^{II}-induced fluorescence quenching. Other transduction mechanisms used in conjugate sensors, such as photoinduced electron transfer (PET) fluorescence quenching, are much more complex to be implemented in such systems, as electron transfer requires direct contact or chemical linkage to take place. However, they would open the way to the sensing of many other substrates rather than solely the Cu^{II} ion.

In conclusion, self-assembled and self-organized fluorescent chemosensors appear to be a promising answer to the need of systems with larger applications in sensing and detection problems. Two main strategies have been investigated to date. On one hand, chemosensing ensembles are well studied and mature for practical applications; on the other hand, template-assisted self-organized systems are probably better suited for a more general use, but they are still making the first steps and more research is needed. Of course, these are not the only possible approaches to the realization of self-assembled systems: other strategies will be explored in the future and will give new insights to this research field.

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