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FEATURE ARTICLE

Supramolecular Functional Assemblies: Dynamic Membrane Transporters and Peptide Nanotubular Composites

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The fabrication of functional molecular devices constitutes one of the most important current challenges for chemical sciences. The complex processes accomplished by living systems continuously demand the assistance of non-covalent interactions between molecular building blocks. Additionally, these building blocks (proteins, membranes, nucleotides) are also constituted by self-assembled structures. Therefore, supramolecular chemistry is the discipline required to understand the properties of the minimal self-assembled building blocks of living systems and to develop new functional smart materials. In the first part of this feature article, we highlight selected examples of the preparation of supramolecular membrane transporters with special emphasis in the application of dynamic covalent bonds. In the second section of the paper we review recent breakthroughs in the preparation of peptide nanotubes hybrids with functional applications. The development of these devices constitutes an exciting process from where we can learn how to understand and manipulate supramolecular functional assemblies.

1. Introduction

A great number of different macromolecular architectures have been described since the emergence of supramolecular chemistry.[1-7] Chemists have realized about the potential application of some of these particular molecular constructions and they have designed and tested a variety of functional supramolecular systems.[4,5,8-10] In total synthesis, the requirements of a particular transformation trigger the development of new methodologies for new bond-forming reactions. Analogously, in supramolecular functional assemblies, the performance in a specific task is the driving force that dictates the design and methodological principles of the corresponding device. In other words, the function governs the supramolecular structure. Therefore, after the identification of a functional challenge, the supramolecular researcher envisions strategies to combine building blocks and weak interactions in order to modulate the shape and the properties of the final assembly. The functions that have been so far accomplished by supramolecular architectures are extensive and include applications in cellular delivery, molecular encapsulation and controlled release, energy conversion and storage, tissue regeneration, confined catalysis, self-healing materials and more.[4,5,10,11] The development of supramolecular and dynamic chemistry has triggered new opportunities for the preparation of functional devices.[9,12] The application of non-covalent interactions and dynamic bonds offers two critical advantages. On one hand it is the synthetic reward, as the target molecules are easily prepared by simple mixing appropriate counterparts such as hydrogen bond donors with acceptors or nucleophilic hydrazides with electrophilic aldehydes. On the other hand we have the reversibility of the ensembles, which represents an unmistakable advantage when preparing stimuli responsive functional materials. However, the comprehensive assessment of this myriad of functional macromolecular devices is far beyond the objective of this paper. Instead, in the first part of this feature article we will highlight a few selected examples of the design and synthesis of supramolecular membrane transporters with special attention in the implementation of dynamic covalent bonds. In the second section of the paper we will focus in the preparation of peptide nanotubular-shaped supramolecular polymers based on peptide hybrids, a simple tubular architecture with nanometric dimensions and a great potential for functional applications. Our group identifies these two important advantages (the synthetic and the

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responsiveness) as key features of working molecular devices. The implementation of dynamic bonds as part of functional supramolecular assemblies increases the molecular diversity and minimizes the synthetic efforts that are required to achieve the optimal functional architecture. Furthermore, the dynamic processes involved in membrane transport and delivery, make dynamic bonds particularly interesting for the design and preparation of novel materials for cellular internalization and drug delivery.

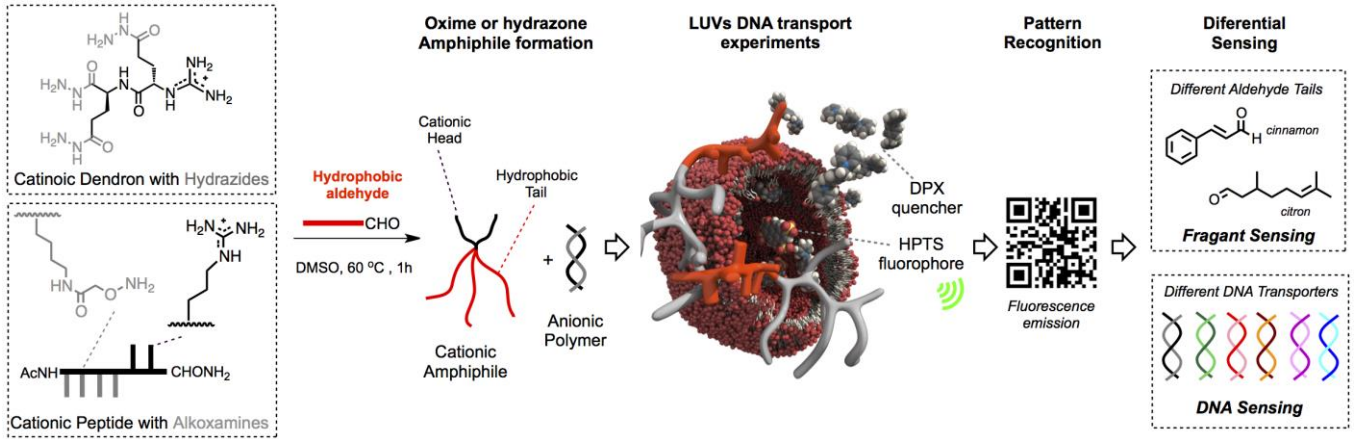

Fig. 1. Differential Sensing in Lipid Bilayer Membranes (from left to right): Reactive dendrons (or peptides) react with hydrophobic aldehydes to afford cationic amphiphiles. The amphiphilic dendron hydrazones (or peptide oximes) are transferred to transport experiments in fluorogenic vesicles.[19,20] The fluorescent transport data is tabulated and recognized as a unique fingerprint. In the case of hydrazone dendrons, the DNA is fixed (calf-thymus) and the aldehyde tails (odorants) are changed allowing the differentiation of odorants (fragrant sensing). In the case of the oxime peptides, the amphiphilic peptides are fixed and different nucleotides are employed in the vesicle transport experiments allowing nucleotide differentiation (DNA sensing).

2. Membrane Transporters

2.1. Dynamic covalent bonds for "*Differential Sensing*". The human olfactory reception operates through pattern recognition. Humans can detect thousands of different odours with only around three hundred olfactory receptors. This system is incompatible with a 1 to 1 recognition process. Therefore, a particular odorant substance activates a certain number of olfactory receptors with different intensity. This cross-responsive activation of olfactory receptors generates a pattern that is recognized as a unique fingerprint by the brain.[13] This fascinating natural mechanism has inspired chemists to design and develop different pattern recognition sensing systems.[14-18]

The amplification of the differences between similar molecules during membrane transport events has recently triggered new opportunities for differential sensing of small molecules[19] as well as high molecular weight biopolymers (Fig. 1).[20] This research program evolved from exciting results about conceptually new sensing assays based on fluorogenic vesicles developed in the group of Prof. Matile.[21-23] In lipid bilayer differential sensing, short hydrophobic volatile aldehydes (odorants) were covalently captured with a cationic reactive dendron head (Fig. 1). The resulting amphiphilic dendron activated the transport, across lipid membranes, of a anionic polymer (e.g. calf thymus DNA).[19] The fluorescence response obtained in the transport experiments, in large unilamellar vesicles (LUVs), was quantified and tabulated for each particular odorant in combination with several cationic heads (Fig. 1). This data was subjected to statistical analysis that confirmed the viability of the method to distinguish aldehydes with a single carbon atom difference, *cis/trans* isomers and even enantiomers.[19] The approach was tested for complex mixtures of odorants allowing the high-throughput differentiation of commercially available perfumes. This methodology was extended by charge inversion to create polyarginine artificial noses,[23] by the development of alkoxamine and disulfide reactive counterions[24-26] and by the implementation of polymersomes as more stable and robust devices.[27]

Following on this concept, we have recently adapted this technology to the challenging differentiation of polymers with biological relevance (Fig. 1).[20] In this case, we prepared small cationic peptides containing alkoxamine reactive residues. Conjugation with a variety of aldehydes provided cationic amphiphiles that performed as strong activators of the membrane translocation of different types of DNA molecules. Vesicle transport experiments of these cationic peptides with a collection of DNAs afforded the corresponding unique transport fingerprint for each of the DNA tested. Statistical analysis of this data allowed the DNA differentiation by pattern recognition with an impressive single base resolution (Fig. 1).[20]

2.2. Dynamic covalent amphiphiles for cellular internalization and delivery. The potential of dynamic covalent bonds for membrane transport and delivery purposes has triggered the attention of the chemistry community.[28] One of the first examples, by Jean-Marie Lehn, explored the hydrazone bond formation between a guanidinium (cationic hydrophilic) reactive hydrazyde and a cholestenone (hydrophobic) electrophile. The resulting amphiphile was employed as an efficient vehicle for the transfection of luciferase-expressing plasmid DNA (Fig. 2A).[28]

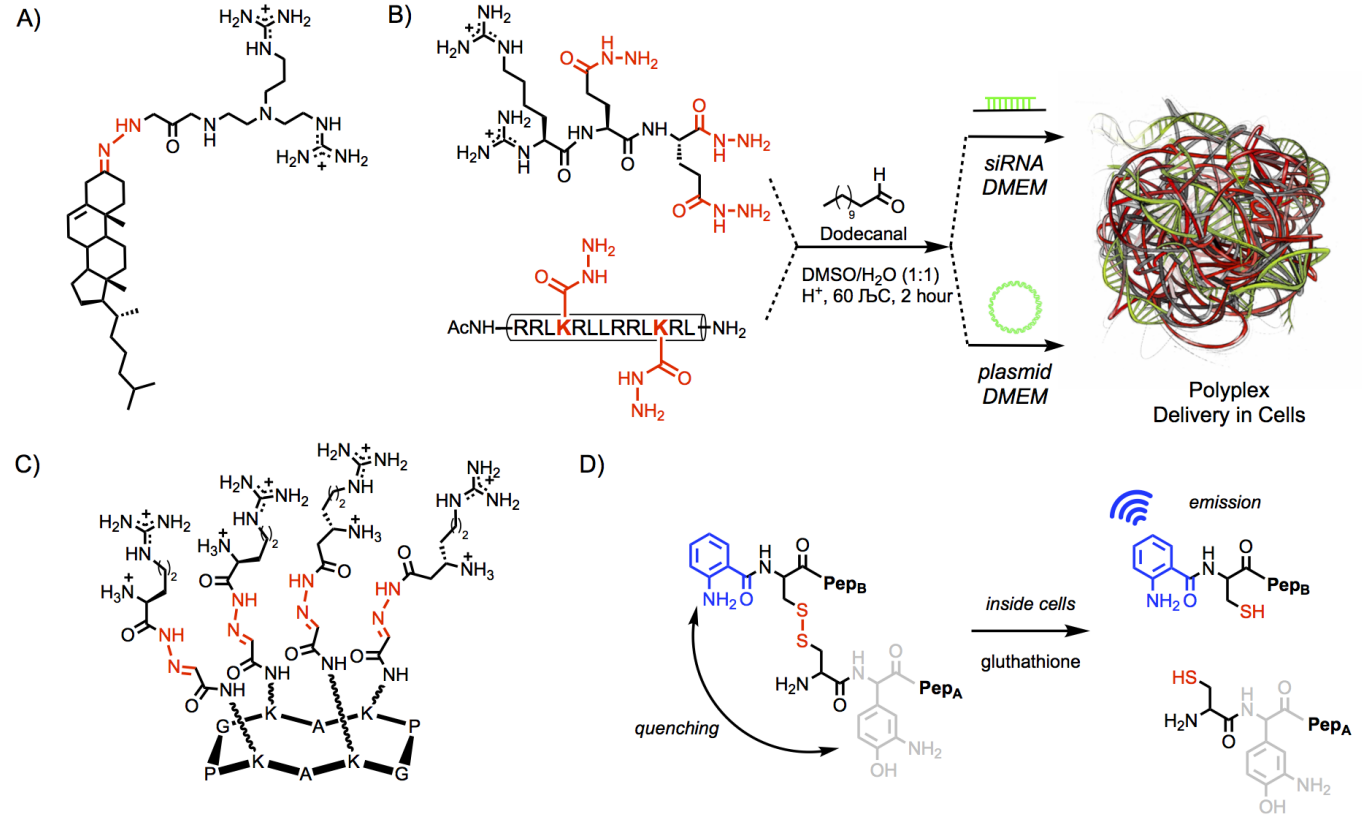

Fig. 2. Dynamic Bonds in Cell Delivery. A) Hydrazone linkage between a cationic guanidinium and cholestenone for DNA delivery. B) Differential sensing translated to delivery. Hydrazone Dendrons for the delivery of siRNA and oxime peptides for plasmid delivery. C) Cyclic peptide clusters for dynamic DNA complexation and release. D) Disulfide bond between a fluorophore and a quencher was applied to quantify gluthathione-mediated cytosol delivery kinetics.

The synthetic methodology developed for differential sensing in model membranes was also translated to the transport of functional DNA across the membrane of living cells (Fig. 2B).[29,30] The hydrazone connection between a collection of cationic hydrazides with different hydrophobic aldehyde tails allowed the preparation and screening of discrete libraries of dendronized amphiphiles for the transfection of siRNA into human cells.[29] The protocol allowed the identification of single-component formulations that performed as efficient vehicles for siRNA, in HeLa cells and human primary fibroblast, with better efficiency than the gold standard employed for siRNA routine transfection experiments (e.g. Lipofectamine RNAiMAX).[29]

We have recently implemented the similar hydrazone and oxyme bond formation strategy for the modulation of the hydrophobic properties of penetrating peptides.[30,31] The application of this approach to linear peptides allowed the straightforward modulation of the peptide hydrophobicity and, as a consequence, the peptide membrane translocation properties.[31] Structural studies showed that the introduction of hydrophobic tails into the backbone of pro-helical linear peptides increased both helicity and amphiphilicity. These amphiphilic peptides showed self-adaptive capabilities depending on the external conditions such as in the transition from water to the lipid bilayer. Three hydrazone-modulated peptides from a simple screening showed an excellent level of transfection of plasmid DNA in human HeLa cells.[30]

Cyclic peptides containing multiple aldehyde copies have been synthesized as scaffolds for the conjugation with hydrazides bearing guanidinium cations (Fig. 2C).[32,33] The resulting acyl-hydrazone cationic clusters were confirmed to dynamically complex and release DNA "*in vitro*" and to deliver functional nucleotides "*in cellulo*".[32,33]

Disulfide is another dynamic chemical bond that has been employed to study and improve cellular delivery (Fig. 2D).[26,34-40] In a very elegant approach, the group of Langel measured cytoplasm internalization kinetics by employing a disulfide linkage between a peptide cargo, labelled with a fluorophore, and a penetrating peptide carrier, bearing the corresponding fluorescent quencher (Fig. 2D).[35] In this smart molecular probe, fluorescence emission could only be detected after glutathione digestion at the cytoplasm. This assay allowed the simple quantification of the real cytosolic delivery kinetics of a selection of well-known sequences of penetrating peptides.

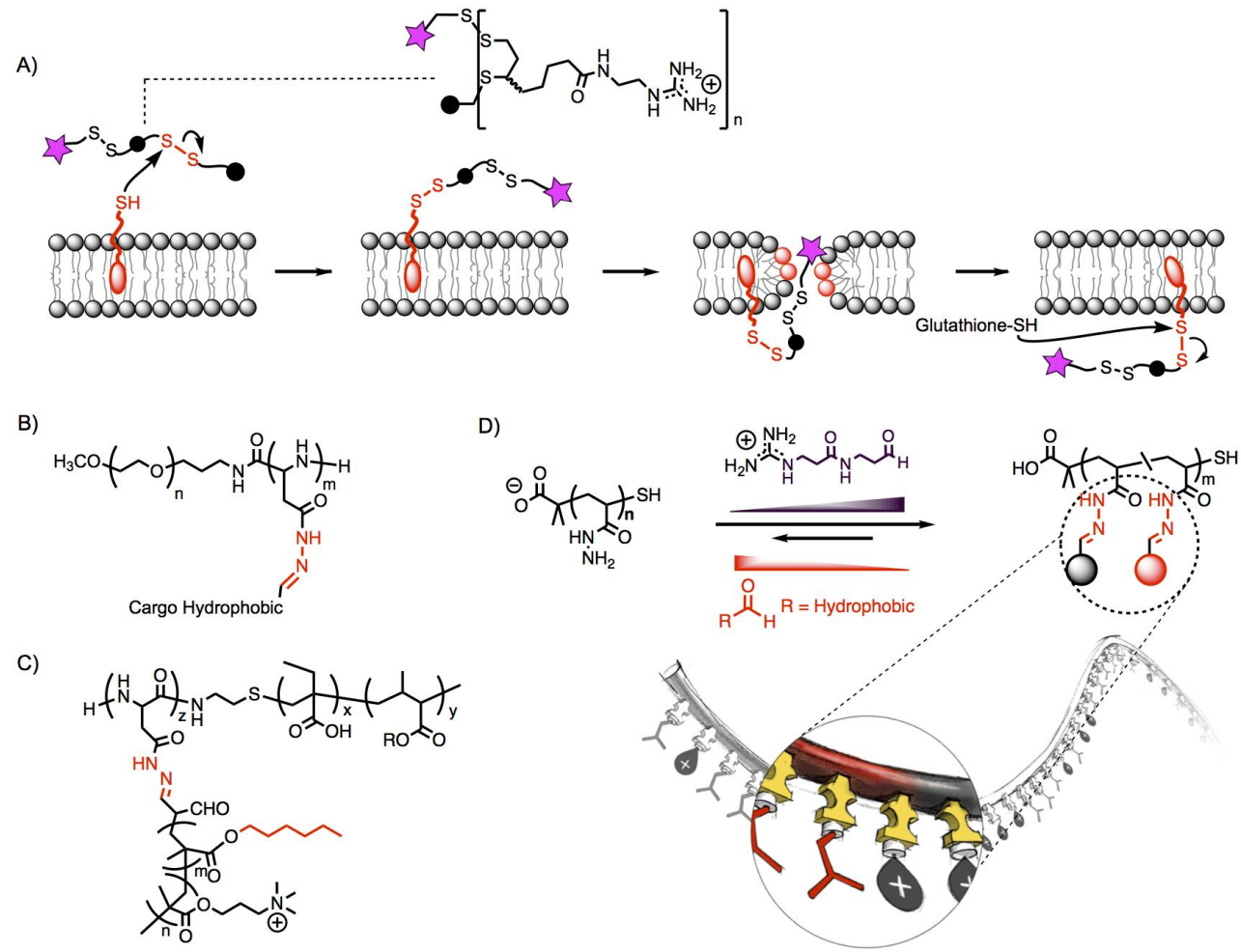

Fig. 3. Dynamic Bonds in Cell Delivery. A) Polydisulfides for dynamic covalent cell delivery. Exofacial cell membrane thiols trap the polydisulfide. The polymer is internalized by different mechanisms (i.e. micellar pores). The cytosolic glutathione releases the polymer in the cytosol. B) Polyethylene glycol hydrazone conjugates with hydrophobic cargo triggers micellar self assembly and enhanced delivery. C) Hydrazone grafted comb-like copolymers for gene delivery. D) Polyhydrazides can be *in situ* functionalized (i.e. in aqueous conditions and without further purification) with cationic and hydrophobic aldehydes. The resulting amphiphilic polymers allow high-throughput screening of polymers for siRNA delivery in human HeLa cells.

2.3. Dynamic polymers for delivery applications. As initially proposed by Gait[37] and confirmed by Matile,[38-41] the presence of exo-facial thiols, offers a great opportunity to exploit dynamic covalent chemistry, at the cell membrane, to enhance intracellular uptake (Fig. 3A). The presence of accessible cysteine residues in membrane proteins triggers thiol-disulfide exchange with disulfide containing molecules that approach the outer leaflet of the cell membrane (Fig. 3A). Following disulfide attachment, the exogenous molecule can be internalized by different mechanisms (i.e. direct translocation, micellar pores, receptor mediated endocytosis, etc.). After membrane translocation, the action of the glutathione releases the compound from the membrane leading to the cytosol (Fig. 3A).[37] After internalization, the reductive environment of the cytoplasm catalyses the digestion of the polydisulfide into the corresponding non-toxic monomers.[39]

Intriguingly, the depolymerisation kinetics affected the internalization mechanism. Lengthy polydisulfides, which spend longer times of disulfide dynamic exchange at the membrane level, undergo endocytic uptake. Shorter oligodisulfides are taken up faster and thus directly translocated.[39]

Hydrazone bond formation has also been explored for the controlled delivery of therapeutic molecules.[42] In a simple but very interesting design, Kataoka combined the polyethylene glycol (PEG) and the hydrazide functionalities in a block copolymer for intracellular delivery and controlled release (Fig. 3B). Hydrazone formation with a hydrophobic drug (e.g. doxorubicin) afforded the corresponding amphiphilic polymer. The amphiphilic properties of the resulting hydrazone hybrid triggered its self-assembly into polymeric micelles of nanometric dimensions, which were applied in the slow release of the drug in malignant cells.[42] Interesting grafted co-

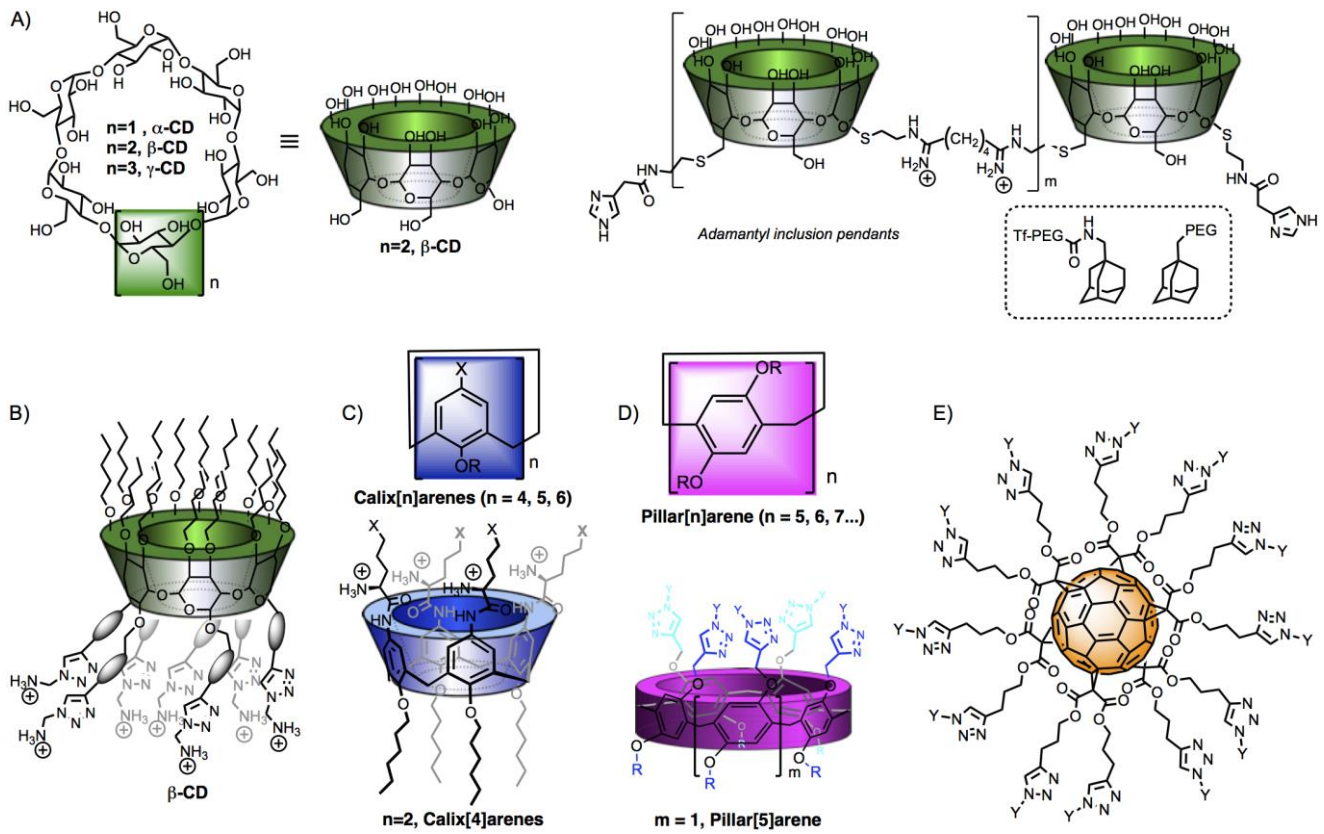

Fig. 4. Host/guest supramolecular complexes for delivery applications. A) In cationic cyclodextrin containing polymers, the adamantane guests tailor PEG (protection) and Dextran (targeting) functionalities for improved siRNA delivery. B) Cyclodextrins as templates for cationic and hydrophobic segregation for gene targeted delivery. C) Calixarenes templates for the circular clustering of cationic arginine and hydrophobic substituents. D) Pillararenes and Fullerene templates for the globular arrangement of multiple cations for DNA interaction and biological applications (In the scheme Y represents the cationic moiety).

polymers have also been synthesized in where the hydrazone bond was applied to graft, into the polymer backbone, the typical cationic and hydrophobic character from membrane penetrating molecules (Fig 3C).[43] These comb-like polymers showed excellent levels of cellular transfection of anti-GAPDH siRNA in the presence of serum.[43]

In collaboration with the group of Dr. Fernández-Trillo we have recently introduced the concept of "*in situ*" hydrazone functionalized polymers for intracellular delivery (Fig. 3D).[44] In this novel approach, poly-hydrazides are simultaneously functionalized with cationic and hydrophobic aldehydes under physiological compatible conditions (e.g. DMSO/H_2O) (Fig. 3D).[44] The resulting hydrazone-activated amphiphilic polymers were combined with the functional nucleotide cargo without any isolation or purification steps. These amphiphilic dynamic polymers turn out to be excellent transfection agents of siRNA in human HeLa cells. This modular methodology allows for the high-throughput screening of biologically relevant polymers over a consistent range of polymer length.[44] In the future, the variation of the structure and the molar ratio of the two (cationic and hydrophobic) aldehyde pendants, could be a potential excellent alternative for the straightforward preparation of customized polymeric transporters for different cargos (i.e. siRNA *versus* plasmid DNA).

2.4. Supramolecular host/guest delivery systems. Host/guest supramolecular entities have also inspired intriguing functional delivery systems. The cationic polymers containing cyclodextrin spacers, developed by Mark Davis, have shown great potential for siRNA therapeutic delivery (Fig. 4A). In these formulations, the cyclodextrin acts as biocompatible host for the inclusion of adamantane guests that are loaded together with stabilizers (e.g. PEG) and targeting motifs (e.g. transferrin) (Fig. 4A).[45-47] The resulting hybrid polymers condensate siRNA to form stable nanoparticles that exhibit great delivery efficiency and that have been successfully assayed in clinical trials.[47]

Cyclodextrins have also been employed as molecular templates for the controlled disposition of cationic charges and hydrophobic tails in a rigid molecular scaffold (Fig. 4B).[48,49] In this approach, an efficient acylation method was used to functionalize the secondary hydroxy rims of the cyclodextrins. After alkylation, it was possible to further modify the hydrophilic surface of the primary rim by "*click reactions*" to incorporate cationic moieties and condensate and deliver functional DNA (Fig. 4B).[48] This methodology was also used for macrophages targeting by decorating the cyclodextrins with mannosyl residues.[49] Calix[n]arenes are also widely studied supramolecules for the recognition of biological macromolecules.[50]

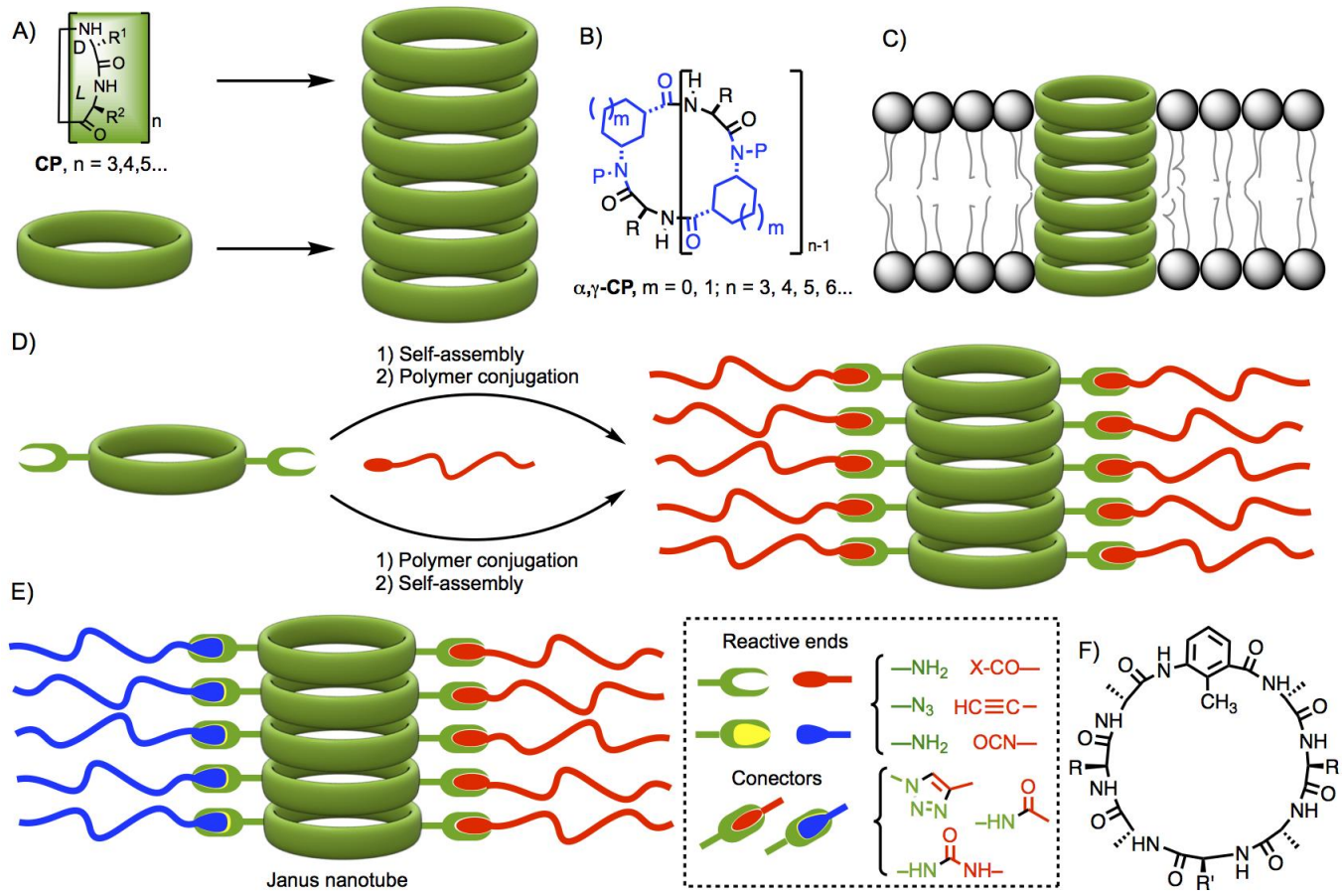

Fig. 5. Cyclic peptide nanotubes. A) Common cyclic peptide structure and nanotube formation through cyclic compound stacking. B) α,γ-cyclic peptide structures. C) Model of ion channel made by SCPN. D) Strategies for the formation of peptide/polymer nanotubular hybrids. E) Structure of Janus nanotubes made from a cyclic peptide bearing two orthogonal reactive ends and two polymers with different properties. F) Cyclic peptide containing a 3-amino-2-methylbenzoic acid that modifies the internal cavity of nanotubes used in gas transport films.

Calix[4]arenes templates have been recently employed for the circular clustering of four arginines and the condensation and delivery of DNA in different cell lines (Fig. 4C).[51] This study depicted, once again, that the topological disposition of the cationic arginine residues has important implications in the uptake and in the release of penetrating supramolecules, a situation also observed in recent studies with miniature proteins and penetrating peptides.[52-54] The Nierengarten group has applied the similar methodology of cationic clustering for biological applications with supramolecular templates such as pillar[5]arenes[55] and fullerenes (Fig. 4D,E).[56] The efficient "*click reactions*" employed for the preparation of fullerenes, allowed the incorporation of dendrons, also used with pillaarenes, with two, four and eight ammonium groups per clickable group. The 3D distribution of 96 amine residues for the third generation dendron over a globular template (Fig. 4E) provided a macromolecule that was efficiently employed for DNA complexation and delivery in cells.[56]

3. Peptide Nanotubes

3.1. Self-assembled peptide nanotubes (SCPNs), structural features. The particular architecture of cyclic peptide nanotubes allows the precise control over the nanotube diameter and the external properties by changing the number and the side chain of the amino acid monomers.[57,58] These hollow linear supramolecular polymers are formed by the stacking of cyclic peptide (CP) rings composed of an even number of amino acids of a specific chirality, mainly the alternation of residues with opposite chirality. These features allow the CP to adopt a well-defined planar conformation that orient the amide backbone groups in a perpendicular disposition to the plane of the macrocycle. Therefore, an extended β-sheet-like hydrogen-bond network between each pair of cyclic peptide subunits can be established, resulting in the successive stacking of cyclic peptides on top of each other. The number of amino acids that form the cycle (generally from 6 to 12) determine the diameter of the SCPN (Fig. 5A). Importantly, all the amino acid side chains are displayed radially from the nanotube longitudinal axis. This arrangement allows a precise

control over the external tubular decoration and potential reactive groups that would be displayed along the nanotube surface. Initial cyclic sequences of α-amino acids,[59] were followed by β,[60] δ[61] ε[62] or even the combination of two different types of amino acids (i.e. α, γ-CPs) (Fig. 5B).[63]

The self-assembling process of peptide nanotubes can be externally triggered by several approaches, generally by tuning the media conditions (i.e. pH modification).[59] For example, the octapeptide *c*-[(Gln-*D*-Xxx-)$_4$], in where Xxx can be Leu, Phe, Val or Ala, were soluble in acidic conditions and the assembly process was prompted by the slow increase of the pH.[64] Under acidic conditions (TFA), the amide groups (peptide backbone and Gln side chains) are protonated, which increases peptide solubility and reduces aggregation as a consequence of the repulsive interactions of the protonated rings. These particular features made peptide nanotubes optimal candidates for the preparation of membrane partitioned functional systems such as ion channels (Fig. 5C).[65] Our group has been continuously active in this field (*vide infra*).[65,66] Different recent experimental[67] and computational[68] contributions, from different research groups, keep on demonstrating the potential of the cyclic peptide ring for the preparation of ion channels. Of particular interest is the application of these peptide channels for the transmembrane transport of anticancer drugs.[67] Other helical self-assembled linear peptides have been also recently confirmed as functional membrane nanopores.[69]

Recent advances in electron microscopy techniques, combined with novel nanomechanical analysis allowed the insightful characterization of the mechanical properties and the aggregation propensity of the Leu substituted peptide.[70] This study revealed that the assembly of this CP, followed by a hierarchical bundling pattern, resulted in the formation of nanotube fibers. This bundling process gave rise to longer (several tens of μm) and thick (up to 1 μm) nanotubular bundles. The rigidity and the stretching resistance of these higher-order aggregates were determined to be among the best for protein nanofibers (i.e. collagen). Remarkably, these peptide bundles showed properties (i.e stiffness, rigidity) similar to the bone tissue with only a half of its density.

3.2. SCPNs/polymer functional hybrids. The modification of the properties of peptide nanotubes with different polymeric coatings has attracted a big deal of attention in the recent years (Fig. 5D). SCPNs are among the best platforms for the selective disposition of certain functional groups towards a particular aligned region of the space.[57] On the other hand, polymer science is experiencing a rapid growth in terms of new reactions, architectures and bio-conjugation.[4,71-73] Therefore, the self-assembly properties of cyclic peptides can be applied to control the spatial orientation of polymers, which can be covalently or non-covalently attached to the tubular ensemble. Along these lines, following the original work by M. Biesalski,[74] Perrier's group has developed several SCPN-polymer conjugates that allow the control of the nanotube formation under certain external conditions, such as pH[75] or temperature.[76] Different "*clickable*" polyacrylic acid derivatives or poly(2-ethyl-2-oxazoline)s (pEtOx) were attached as lateral chains of the peptide nanotube. The temperature sensitivity of similar poly(N-isopropylacrylamide)/SCPN hybrids has also been exploited to prepare ion channels whose trans-membrane transport activity can be externally controlled. The polymer/peptide hybrids, inactive at room temperature, can self-assemble and insert into lipid bilayers upon heating. These thermally induced channels showed proton transport ability in dye-entrapped liposomes (LUVs).[77] The radial disposition of two orthogonal reactive side chains in the cyclic peptides, allowed the preparation of Janus-like peptide/polymer nanotubes that could self assemble in the lipid bilayer forming membrane macro-pores (Fig. 5E).[78] At this respect a new clickable isocyanate/primary amine conjugation has been developed by Perrier group and used in the formation of new SCPN hybrids that provide a new tool for increasing the diversity and properties of SCPN (Fig. 5E).[79]

A SCPN/block copolymer hybrid has been developed, by Xu and co-workers, for the preparation of porous thin films of subnanometer dimensions.[80] The conjugated polymer guides the nanotube growth in a confined geometry, affording porous membranes that contained high-density arrays of channels. This film allowed the transport of small molecular gases (i.e. CO_2) or proton exchange through the interior of the nanotube pore.[80] This strategy is a proof of concept for the generation of porous thin films whose channels can be modulated by the dimensions and the properties of the cyclic peptide (CP) used in the process.

CPs attached to four norbornenes, through an ester linkage, allowed the preparation of robust polymers with lumens functionalized with carboxylate groups.[81] The self-assembling of the cyclic peptide was used to template the ring opening cross-metathesis polymerization process between norbonenes CPs and a diamine linker that contains two norbonene moieties. The hydrolysis of ester bond of the resulting polymer liberated the CP templates leaving the free carboxylic acid and provided the macropores (around 100 nm) with carboxylate lumens.

The use of SCPNs composites can also be used to reinforce the mechanical properties of polymeric systems (e.g. Lactic acid fibers).[82] The composite fibers that incorporate about 8% of bundles of SCPNs derived from *c*-[(Gln-D-Leu-)$_4$], turn out to be 5 times stiffer than the same polymer fibers by itself. This design allowed the control of the fiber dimensions depending on the processing conditions.[82]

3.3. Peptide nanotubes, internal cavity modulation. Several approaches using non-natural amino acids have been developed to modulate the properties of nanotube's internal pore. Initial examples consisted on engineering cyclic peptides that combine natural and non-natural amino acids such as cyclic γ[63] and ε[62] -amino acids. The ε-amino acid contained a triazol ring for cavity functionalization. Unfortunately, the hydrophobic CH of the triazol was not project towards the

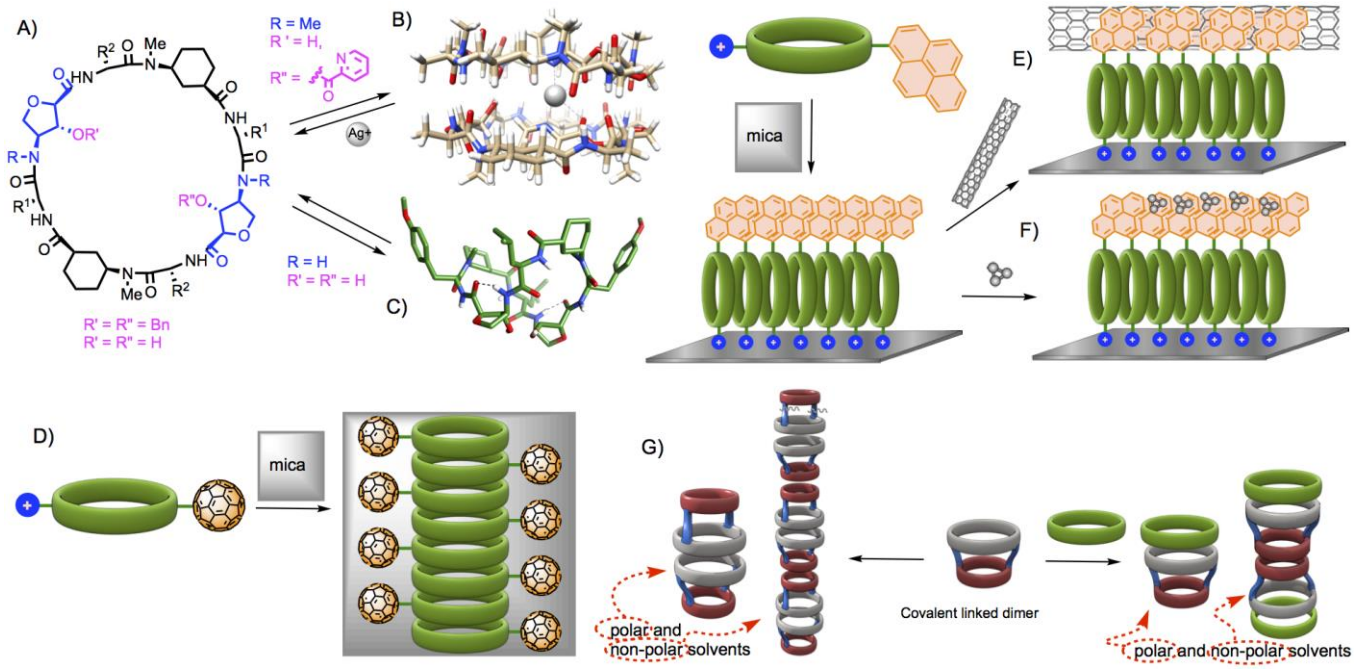

Figure 6. Peptide nanotubes internal functionalization and functional supramolecular hybrids. A) γ-amino acids with hydroxyls for the introduction of pyridyl for internal functionalization. B) The pyridine allows metal (silver) coordination. C) Without N-methylation the peptide folds in a v-shaped conformation that self-assembles into hexameric structures and coordinates and transport anion across the lipid bilayer. D) E) and F) Hierarchical surface deposition of peptide/fullerene (D), peptide/carbon nanotubes (E) and peptide/subnanometric metal clusters (F) hybrid nanotubes. G) Venturi-like peptide nanotubes.

inner cavity. Instead, the heterocycle remained parallel to nanotube longitudinal axis.[62] The approach, developed in our group, involving γ-amino acids employed 3-aminocyclohexanecarboxylic acids to ensure the flat conformation of the peptide ring and to project the β-carbon into the nanotube lumen.[62]

The incorporation of 3-aminobenzoic acid in D,L-α-CPs constitutes an alternative for nanotube lumen functionalization.[83] The presence of a methyl substituent in the aromatic ring (C2) gives a partial hydrophobic character to the nanotube interior without compromising the self-assembling properties (Fig. 5F). We have recently developed the first example in which a functional group was directed towards the ensemble cavity was based on non-aromatic γ-amino acids with a hydroxyl group in the β-carbon (Fig. 6A).[84] The octapeptide that contained two N-methylated γ-amino acids, requires the addition of a protic and polar solvent (e.g. MeOH, H_2O) to interact with the inner hydroxyl groups and properly assemble into the corresponding dimer.[66]

This hydroxy group can be chemoselectively modified to tune the cavity properties. Therefore, the incorporation of a pycolinic acid onto one of the hydroxyl groups transforms the properties of the resulting ensemble. The new peptide dimer generates a hollow structure that can entrap silver ions (Ag^+) or dicarboxilic acids (Fig. 6B).[66] Interestingly, a similar peptide, but without the N-methyl group in the hydroxylated γ-amino acid, folds in a "v-shaped" conformation. This folded peptide assembles in the presence of anions to form hexameric pseudo-spherical shaped structures (Fig. 6C).[85] This recognition of anions, which induced peptide assembly, was used to transport chloride in an antiporter fashion (exchange of chloride ion from the outside for nitrate) across a lipid bilayer. This was demonstrated by fluorescence experiments involving the quenching of an anion dependent fluorophore. This kind of supramolecular anion receptors could have important applications in organocatalysis, industrial anion separation, ion sensing or treatment of diseases, such as cystic fibrosis.[86,87]

The conjugation of SCPN with different nanostructures has been developed to expand the potential applications of the "naked" nanotubes. For example, the incorporation of electroactive moieties was studied to develop electronic nanocomponents.[88] The covalent incorporation of naphthalenetetracarboxylic diimide moieties into the cyclic peptide ring provided the first example of an electron activated peptide nanotube.[88] The resulting electronic delocalized nanotube was hundreds of nanometer in length. In an analogous approach, a fullerene moiety was also attached to a nanotube forming cyclic peptide (Fig. 6D).[89] In this case, perhaps as a consequence of the bulkiness of the C_{60} moiety, the deposition of the SCPN on surfaces was accomplished by alternating the fullerene moieties at both sides of a single nanotube. The parallel disposition of the two molecular wires obtained might found applications in electronic nanocircuits.

Small vertically oriented SCPNs were grown over gold by the layer-by-layer deposition method. This strategy was implemented to study the charge transfer processes along

nanotubular supramolecular motifs.[90] This special arrangement allowed the study of the dependence of the current as a function of nanotube length. The results showed that, in this architecture, the efficient charge transfer was achieved at the low voltage region. However, a transfer to field-assisted conductions was observed at voltages above 2.4 V.[90]

3.4. Peptide nanotubes functional supramolecular hybrids. The use of non-covalent interactions to prepare nanotubular functional supramolecular hybrids has been recently approached (Fig. 6E and 6F).[91,92] Our group has developed a cyclic peptide alternating α and γ aminoacids that was equipped with a pyrene on a lysine residue. This CP can self-assemble into nanotubes both in aqueous solution and over mica surface.[91] This pyrene "paddle" was used to interact and to assist in the aqueous dispersion of carbon nanotubes (CNTs).[91] The resulting peptide/carbon tubular twins combined the properties of both components, the biocompatibility and solubility of SCPNs and the strength and electronic properties of the carbon nanotube counterpart. Therefore, the aqueous dispersions of cyclic peptides and carbon nanotubes were used for the controlled deposition (for TEM and AFM analysis) of the corresponding hybrid tubes (Fig. 6E). The supramolecular nature of the approach maintained the properties of the carbon nanotubes unaltered and provided insights about new strategies for CNT manipulation and controlled deposition. Electron and AMF micrographs of the deposited nanotubes can be found in the corresponding article (and supporting information).[91]

Our group has recently implemented these peptide nanotubes decorated with pyrene paddles for the alignment of subnanometric silver-clusters (Ag_3) (Fig. 6F).[92] This pioneering work exhibited one of the first examples in which a long (several μm) 1D array of these subnanometric metal clusters were observed. This was achieved using previously synthesized atomic metal cluster lacking any ligand or stabilizer. These results depicted the importance that non-covalent interactions (i.e. van der Waals forces, π-stacking, hydrophobic effects, etc.) would have for the emerging field of subnanometric metal clusters.[92]

The potential to decorate peptide nanotubes with different components triggers intracellular delivery opportunities.[93-95] SCPNs bundles loaded with doxorubicin and further biocompatibilized by PEGylation have been tested for drug delivery.[94] The resulting complex showed a good level of cytotoxicity in human breast cancer cells. The authors proposed that this was probably due to an increased uptake and intracelular distribution of the doxorubicin payload.[94] Alternatively, a cationic (lysine) cyclic peptide, with a lysine residue modified with an artificial guanidiniocarbonyl-pyrrole moiety, allowed the formation of cationic nanofibers that complexed and delivered DNA.[95] A hydrophobic diketopiperazine made by D-Trp and Tyr was used in oral gene delivery.[93] The fibers derived from this CP were able to protect the DNA attached to the fibers surface from the DNase I and acidic digestion. The plasmid permeation took place through an energy-dependent process. The results indicated that these materials can act as nanovectors for the oral delivery to the duodenum, stomach liver and kidney.[93] Previously mentioned SCPN/polymer hybrids were also studied in drug conjugation.[96] In this study, the ruthenium-based anticancer drug (RAPTA) was conjugated to the copolymer that formed the nanotubular shell. The resulting structures exhibited a promising activity against *cis*platin-resistant ovarian cancer cell lines.

Nanotube-forming CPs have already found several applications as ion channels, antimicrobial or antiviral agents.[8,65] The similarity of the chemical and biochemical properties of amyloids with peptide nanotubes facilitate their cross-interaction. Recent studies have shown their ability to modulate the aggregation and toxicity of amyloid beta (Aβ).[97] Hexameric D,L-CPs interact with small oligomeric form of Aβ (1–3 mer), apparently through the stabilization of the parallel β-sheet conformation. As a result, the CP shifted the amyloid aggregation to yield less toxic structures that provide anti-amyloidogenic properties.[97] Recently, or group has achieved the click conjugation of two different dimer-forming cyclic peptides with different diameters (Fig. 6G).[98] To form the covalent heterodimer, one cyclic tetrapeptide was functionalized with a 3-propyl azide at the peptide skeleton while the cyclic octapeptide was modified with an propargyl moiety.[98] The click reaction afforded the final heterodimer that self-assembles, under controlled conditions, to yield different architectures (Fig. 6G). The difference between the association constant of two peptides, allowed the preparation of short nanotubes with precise length control (Fig. 6G). Changing parameters such as sample concentration or solvent properties modulated peptide length. In this way, nanotubes made of three, four or six ring stacks were precisely prepared.[98]

Conclusions

The mature field of supramolecular chemistry increases each day its potential applications in the development of functional devices. This is not surprising as functional properties emerge, in many occasions and especially in biology, from weak and non-covalent interactions between macromolecular counterparts. In this regard, it is important to remark that synthetic and organic chemistry will always be needed to create the new building blocks required for supramolecular functional devices. This feature article highlighted a few contributions from ours and other research groups to this emerging an exciting field. We have just focused in supramolecular delivery with special attention in dynamic bonded systems. In the first section of this paper we have commented examples related with differential sensing systems in lipid bilayers, dynamic bonds and cationic clustering for gene delivery and host/guest complexes with biological applications. In the second part of the article we reviewed different strategies for functional peptide nanotubes in the fabrication of porous films, molecular capsules, carbon

nanocomposites, ion channels and supramolecular templates among others. The insights extracted from this review, point out the strengths and improvements already accomplished as well as the key problems that still need to be overcome. As the synthetic methods improve and the field of supramolecular chemistry evolves, it seems essential, for self-assembled supramolecular architectures, to achieve the precise control over the spatial distribution and presentation of the functional molecular moieties. This paper subtly outlined some other future potential directions such as implementation of dynamic bonds in smart stimuli responsive delivery vehicles or the supramolecular recognition for protein delivery and cell targeting. There is still a lot to be done in this exciting field and we very much hope that these small lessons can help us to learn how the nano and the bio world behaves and how to control it for our own benefit.

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