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Achieving Biopolymer Synergy in Systems Chemistry

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Complete List of Authors:	Bai, Yushi; Emory University, Chemistry Chotera, Agata; Ben Gurion University of Negev, Department of Chemistry Taran, Olga; Emory University, Chemistry Liang, Chen; Emory University, Chemistry Ashkenasy, Gonen; Ben Gurion University of the Negev, Department of Chemistry Lynn, David; Emory University,

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ARTICLE

Achieving Biopolymer Synergy in Synthetic Systems

Yushi Bai,[a] Agata Chotera,[b] Olga Taran,[a] Chen Liang, [a]Gonen Ashkenasy*[b] and David G. Lynn*[a]

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Synthetic and Materials Chemistry initiatives have enabled the translation of the macromolecular functions of biology into synthetic frameworks. These explorations into alternative chemistries of life attempt to capture the versatile functionality and adaptability of biopolymers in new orthogonal scaffolds. Information storage and transfer however, so beautifully represented in the Central Dogma of biology,require multiple components functioning synergistically. Over a single decade, the emerging field of Systems Chemistry is beginning to catalyze the construction of mutualistic biopolymer networks, and this review begins with the foundational small-molecule based dynamic chemical networks and peptide amyloid based dynamic physical networks on which this effort builds. The approach both contextualizes the versatile approaches that have been developed to enrich chemical information in synthetic networks and highlights the properties of amyloids as potential alternative genetic elements. The successful integration of both chemical and physical networks through β-sheet assisted replication processes further informs the synergistic potential of these networks. Inspired by the cooperative synergies of nucleic acids and proteins in biology, synthetic nucleic-acid-peptide chimeras are now being explored to extend their informational content. With our growing range of synthetic capabilities, structural analyses, and simulation technologies, this foundation is radically extending the structural space that might cross the Darwinian threshold for the origins of life as well as create an array of alternative systems capable of achieving the progressive growth of novel informational materials.

1. Introduction – Systems Chemistry

Systems Chemistry[1-5]has emerged over the last decade as an experimental approach to capture the complex, dynamic, and emergent properties of biological systems using synthetic networks. Such networks exhibit system-level properties that cannot be found simply in the linear sum of the individual constituents. To date, three thermodynamic models have been established[4, 6] for characterizing complex chemical systems: (i) systems under thermodynamic control, where a minimum energy state is reached, (ii) systems under kinetic control that can be trapped in local kinetic minima or driven by irreversible processes, and (iii) systems sustained far-from-equilibrium propelled by continuous energy input. Each of these models can generate unique molecular networks, and have proven useful in the construction of receptors, sensors, and catalysts[7]. Reaction networks involving kinetic feedback loops can show adaptive behaviours[8], and out-of-equilibrium networks can exhibit unique functions such as selective information storage and propagation, molecular oscillation, and fueled unidirectional macromolecular motions[4].

A living cell is a remarkably complex network that integrates the metabolic potential for producing energy and constructing materials within the informational context necessary for replicative potential. The cell houses these chemical networks within a complex web of physical networks that range from internal and external membranes, scaffolding such as actin and tubulin, multi-biopolymer complexes, and various organelles. For example, the tricarboxylic acid cycle[9] is a coordinated chemical network physically confined to the mitochondrial matrix. On the other hand, the ribosome is a protein/RNA co-assembly[10] that functionally translates RNA sequence information into protein scaffolds. This convolution of the chemical network within their physical confines must be considered in our attempts to build alternative synthetic systems.

The conceptual framework for this review then focuses on constructing the mutualistic synergy of biology in synthetic systems. Our discussion is developed thematically, initially highlighting the thermodynamic and kinetic properties of synthetic dynamic combinatorial libraries and replication networks. As such, we endeavor to present the versatile approaches that have been developed to enrich the chemical information in artificial networks. Unlike natural systems, most small-molecule based dynamic networks do not broadly capture the formation of large-scale physical phase space that

[a] Emory University, 1521 Dickey Drive, Atlanta, Georgia 30322, United States. Email: dlynn2@emory.edu
[b] Department of Chemistry, Ben-Gurion University of the Negev, Beer Sheva 84105, Israel. Email: gonenash@bgu.ac.il
† Footnotes relating to the title and/or authors should appear here.
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is so prevalent in biology. This section is consequently followed by the structure-dynamics of amyloid assembly as a platform to understand higher ordered functional assemblies in the regulation of dynamic physical networks. In the fourth section, the chemical and physical networks are integrated, building in the mutualistic behaviors of polymers that appear to be critical in achieving progressive autocatalytic and self-organizing behaviors. Finally, we provide perspective on how the information of the networks can be expanded by utilizing simple co-assemblies of nucleic acids and peptides. We conclude that such efforts will extend the designs for new dynamic information-rich materials and inform the potential for emergent scaffolds in early chemical evolution under different environmental conditions.

2. Dynamic chemical networks

Inspired by natural systems, chemical networks have been constructed with reversible covalent bonds including imines, disulfides, thioesters, etc. and noncovalent interactions such as metal coordination and hydrogen bonding[7]. The resulting dynamic chemical networks (DCN) adapt to changes in the free-energy landscape. Early studies achieved the translation of information from DNA templates into amine nucleoside polymers via templated polymerization[11-14]. Unique structures[15-17], difficult to prepare using conventional synthesis, were achieved by reversible ligation of monomers along an informational template. Extensions of these approaches have led to the development of new sensors and receptors[2, 18], some of industrial and bio-medicinal value, and to the development of transition state binding catalysts[19].

The DCN formed under initial reversible ligation conditions or the irreversible chemical ligations first shown to make phospodiesters for self-replicating DNA,[20] set the stage for extending these strategies to a wide variety of chemical scaffolds (Fig. 1)[21-23]. In each cycle of the autocatalytic process, the replicating molecule (i.e., the template-T) binds two shorter molecules, A and B, consisting of its own fragments, to form a ternary complex (A·B·T), thus positioning A and B reactive ends in close proximity to facilitate covalent bond formation. The coupling of the fragments results in the formation of another copy of the template, and the template-product complex (T·T) then dissociates to provide two free copies of T, which can ideally re-enter the next cycle of replication. Several parameters have been identified as crucial to prepare systems that exhibit efficient autocatalytic behaviour[22]. First, the templates (T) should bind tightly and selectively to the substrates A and B to produce a significant population of the A-B-T intermediate. Second, the ligation step that transforms the A-B-T complex to T-T complex should be enhanced by the template, relative to the template free reaction. And third, release of the newly formed template occurs via some energy input to provide an accessible template for the next cycle.

Larger synthetic networks, containing several replicating molecules, require the molecular components to mutually interact

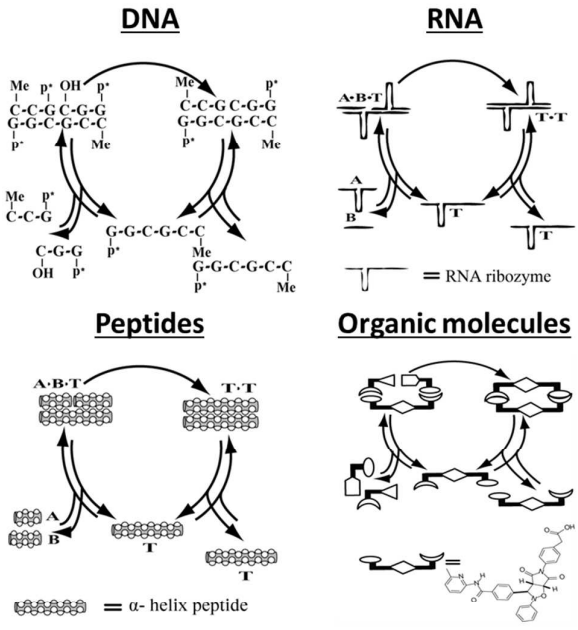

Figure 1. Schematic representation of non-enzymatic self-replication cycles of hexanucleotide DNA, ribozyme RNA, helical peptides and synthetic abiotic organic molecules. In each case, T represents template and A, B represent substrates.

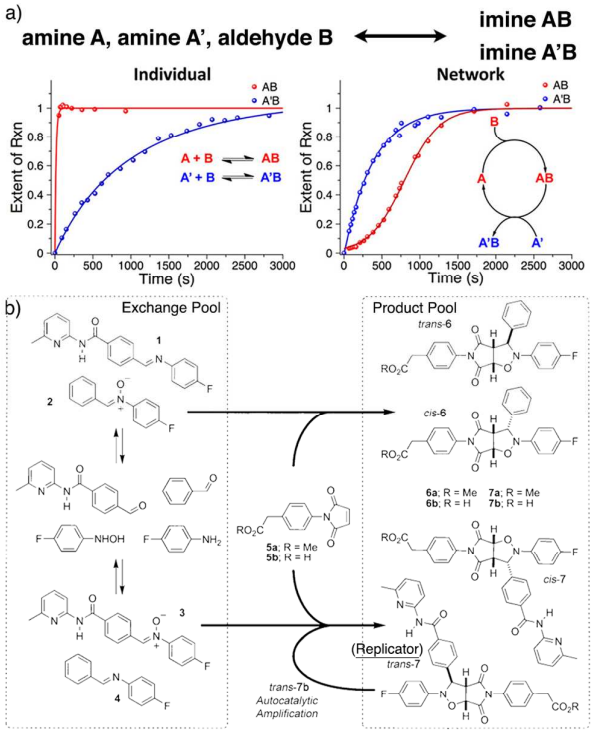

Figure 2. (a) Reversible bond formation within a dynamic covalent library. Individual formation rates for AB and A'B (*left*) were found significantly different from their formation rates in the network (sigmoidal rate profile – *right*) under the same conditions. Adapted with permission from Ref. 33. Copyright 2016 American Chemical Society. (b) Synthetic replicator amplifies itself from a dynamic combinatorial library. A pool of compounds containing imines 1 and 3 and nitrones 2 and 4 can exchange freely, while material is transferred irreversibly products, present in the same solution. When a specific maleimide (5b) is used, a specific replicator, trans-7b, is formed in the product pool, and this species amplified as a catalyst for its own formation. Adapted with permission from ref. 29. Copyright 2008 John

either physically (i.e., via covalent or non-covalent bonding) or functionally (e.g., contributing to certain catalytic processes). Various simple networks have been constructed by simultaneous implementation of autocatalysis and crosscatalysis,[24-27]and such rationally designed self-organized synthetic networks provide useful models for understanding of and exploiting complex systems behaviors, as well as predicting connectivity and global topology. Moreover, the synthetic networks can also be manipulated in various ways to show that just like cellular networks, their rewiring following changes in the environmental conditions is substantial, and that they carry out chemical transformations via multiple programmed pathways.

Due probably to practical rather than conceptual limitations, irreversible ligation reactions have been used to drive replication network evolution under kinetic control. However, it has been postulated recently that in order to better mimic natural selectivity, one should utilize molecules that replicate efficiently and reversibly. In that case, the less complementary molecule(s) within the mixture decompose back to feed the precursors pool, or are simply degraded as waste. Eschenmoser has defined such a process as 'combinatorial synthesis proceeding under (partial) thermodynamic control'[28]. Several groups have since then studied novel experimental systems evolving by partial thermodynamic control, practiced by incorporating catalysis and molecular replication into DCLs[29-32] New dynamic phenomena have been demonstrated using these systems, including for example, non-linear kinetic behaviour on the pathway to equilibrium (Figure 2a) [33], strong replication-dependent selections (Figure 2b)[29, 34], and bi-stable product formation far-from-equilibrium environments[35].

3. Dynamic physical networks

Biological systems beautifully couple dynamic metabolic and biomolecular networks with supramolecular interactions and ordered physical phases to achieve diverse cellular functions. Subcellular organelles and local asymmetries in the distribution of macromolecular condensates may be governed by complex phase transitions. Indeed, all biopolymer assemblies may go through liquid-liquid phase transition in aqueous environments during assembly[36]. Here we focus on amyloid peptides as a model because of the extensive work on assembly relative to protein misfolding diseases.

Peptides and proteins alone assemble into many large-scale ordered phases with amyloid assemblies being capable of conformational evolution in health and disease[37]. Their extended arrays of β-sheets stabilized by hydrophobic interactions of the sheet interfaces give a characteristic "cross-β" diffraction signature[38] and have proven to present a robust scaffold for materials engineering. Extensive work with this architecture now reveals peptide folding energetics that control both amyloid assembly and conformational mutagenesis[39]. These assemblies have recently been effectively used to investigate the coupling of chemical networks with physical phase networks for protein folding and self-assembly[40].

Even the simplest peptides undergo an initial liquid-liquid transition into a symmetrical phase that provides a concentrated and dehydrated environment favorable for the nucleation of various cross-β paracrystalline structures[41]. The nucleating core of the Aβ peptide of Alzheimer's disease, Ac-KLVFFAE-NH_2, or Aβ(16−22), follows this pathway and shows a particle size dependence for the transition from molten particle to para-crystalline phases.[42] Once a nucleus forms, it templates the propagation of supramolecular assemblies and shear forces or other surfaces in solution can result in the formation of additional nuclei, catalyzing propagation rates.[41, 43]

Subtle differences in peptide sequence dictate assembly dynamics, and understanding these association energies underlie a molecular code for assembly. For example, assembly of the extensively studied Aβ(16−22) is sensitive to single amino acid mutations,[44-47] and forces such as electrochemical interactions, steric packing, and hydrogen bonding all contribute significantly to the final assembled structure. Metha et.al revealed the competing effect between electrostatic interactions and steric effects in controlling the conformation of Ac-KLVFFAE-NH_2,[48] and introduced the term facial complementarity as a critical determinant of cross-β folding dynamics. This peptide forms anti-parallel in-register strands

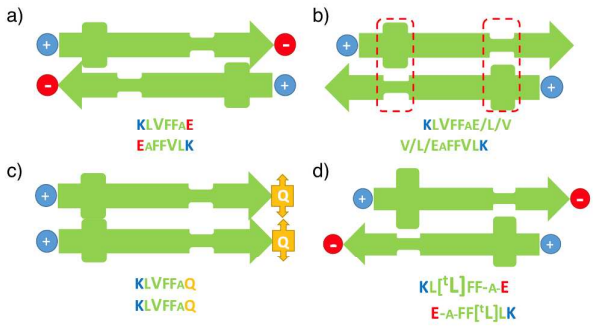

Figure 3. Schematic representation of the alignment of the Ac-KLVFFAE-NH_2 nucleating core and its variants. a) Ac-KLVFFAE-NH_2 molecules assemble in an anti-parallel in-register fashion under neutral pH dictated by the electrostatic inter-strand K-E pairing. b) Ac-KLVFFAE-NH_2 under acidc pH, and Ac-KLVFFAL-NH_2/ or Ac-KLVFFAV-NH_2 variants, under both pH solutions assemble in an anti-parallel out-of-register fashion, where the inter-strand V-A becomes the dominant factor. c) Ac-KLVFFAQ-NH_2 variant assemble in a parallel in-register alignment due to strong inter-strand hydrogen bonds. d) KL[^{t}L]VFFAE variant assemble in anti-parallel out-of-register fashion under neutral pH due to significant steric effect.

(Figure 3a) under neutral pH where the inter-strand K/E salt-bridges dictate assembly, or out-of-register arrangements determined by V-A cross-strand paring under acidic pHs (Figure 3b). When the glutamic acid is substituted with valine or leucine, the attractive electrostatic interactions are eliminated and the peptides forms anti-parallel out-of-register tubes independent of pH (Figure 3b)[44, 49], reflecting the tension between environment and polymer sequence during assembly. When the terminal glutamic acid (E) is replaced with glutamine (Q), parallel in-register fibers are the final

assembly because of strong inter-strand hydrogen bonds Q-tracks along the sheet (Figure 3c)[45]. When the internal valine is replaced with the bulky tert-leucine (^{t}L), the larger side chain forces out-of-register tubes to form even in neutral pH conditions (Figure 3d)[46]. To the extent that such interactions dominate assembly, they can be used as key molecular determinants, or codes, of the assembly for the rational design and construction of these dynamic nanomaterials[50].

This coding 'alphabet' is further expanding through the introduction of non-natural amino acids and chimeric motifs into the peptide backbone, thus greatly expanding the structural and informational content of the peptide assembly networks. For example, the amyloid/lipid chimeras[51] provide self-assembly morphologies ranging from fibers and ribbons to hollow tubes controlled by the length of the introduced acyl chain. The C12 (N-lauroyl) (Figure 4a) chimera assembles as homogeneous tubes (Figure 4b, 4c), similar to the well-characterized Aβ(16−22) tubes, but with a distinct β-sheet packing distance as a result of the need to accommodate the lauroyl alkyl chain within the lamination

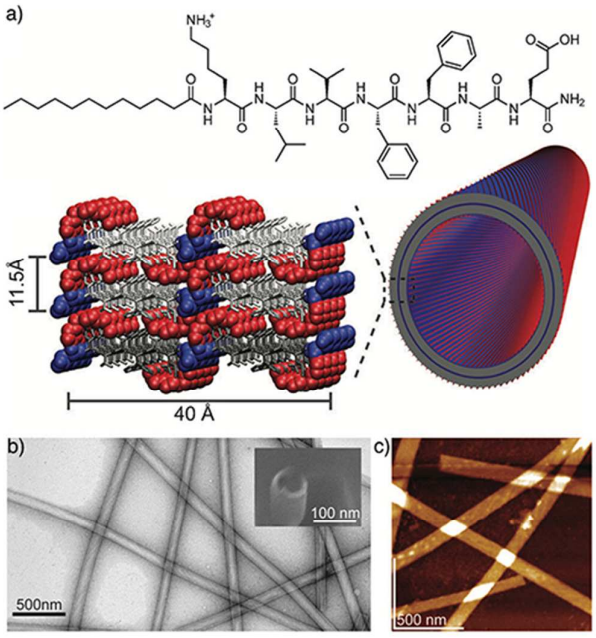

Figure 4. a) Structure of N-lauroyl-Aβ (16-22) and the packing of N-lauroyl-KLVFFAE peptides within tube.; b) TEM, cryo-SEM (inset), and c) AFM micrographs of the N-lauroyl-Ab(16-22) assembly nanotubes. Adapted with permission from ref. 51. Copyright 2012 John Wiley and

interface (Figure 4a).

While amyloid nucleation and propagation templates function with high fidelity, conformational changes are also possible and highly susceptible to environmental conditions.[45, 52, 53] During the two-step nucleation process, once the propagating amyloid template is exposed to solvent, the aqueous environment changes the energetic contributors for assembly. Liang et. al[45] provided the first detailed quantitative analysis of the striking conformational changes with KLVFFAQ during assembly, and established that within the oligomer phase the assembly is dominated by facial complementarity to form anti-parallel out-of-register ribbons, but later, the assembly switches during propagation into parallel structures because of the greater thermodynamic stability of side chain H-bonding in Q-tracks (Figure 5). Ac-KLVFFAE-NH_2 also assembles under neutral pH conditions as anti-parallel out-of-register ribbons, dictated by facial complementarity nucleation in the particle phase, followed by the transition into in-register fibers during propagation[52]. Such conformational changes also occur in co-assembly processes [44]. Other than pH and local solution conditions, metal coordination also serves as an environmental input to expand the amyloid self-assembly landscape. With peptide Aβ(13-21) HHQKLVFFA[54] containing both the nucleation core and a metal bind dyad (HH), metal ions control the rate of assembly as well as amyloid morphologies. In summary, the dynamic nature of the structural features underlying peptide backbone energetics in an ever-changing environment creates a versatile platform for

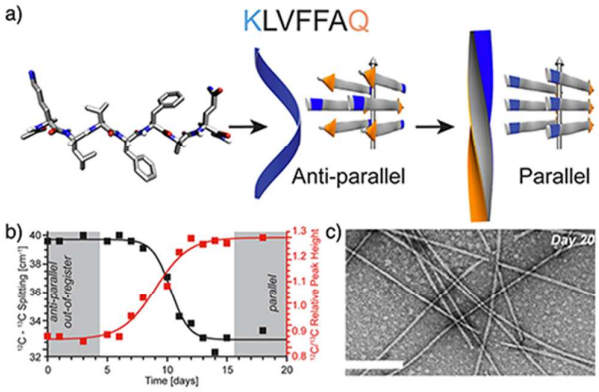

Figure 5. a) Model for the progressive transitions observed for KLVFFAQ over time. b) Changes in the frequency and amplitude of the 12C/13C amide-I bands of the IR spectra indicating the conformational changes overtime. c) TEM image of the mature fibers at 20 days, scale bar=200 nm. Adapted with permission from Ref. 45. Copyright 2014 American Chemical Society.

understanding dynamic physical phase transitions and provides a powerful scaffold for the development of functional supramolecular materials.

4. Integrating chemical and physical networks

While biology beautifully integrates dynamic chemical networks with dynamic phase networks, only recently have systems integrating both been successfully constructed. Otto's group[55, 56] has exploited aromatic thiols containing peptide chains to construct replicating chemical networks made of a range of cyclic oligomer sizes (Figure 6a). As the network matures, the product distribution is significantly affected by β-sheet assisted replication processes, and mechanic forces control the selective formation of specific products (typically hexamers or heptamers). As with amyloids, physical agitation of this system generates new fiber ends, promoting autocatalytic self-replication (Figure 6b). Structure replication within these networks can also be controlled by mutations to the peptides, where the more-hydrophobic building block gives rise to smaller replicators, because fewer peptides are required for sheet stabilization. When network complexity is increased with two different building blocks,[57] two distinct sets of assemblies compete for resources.

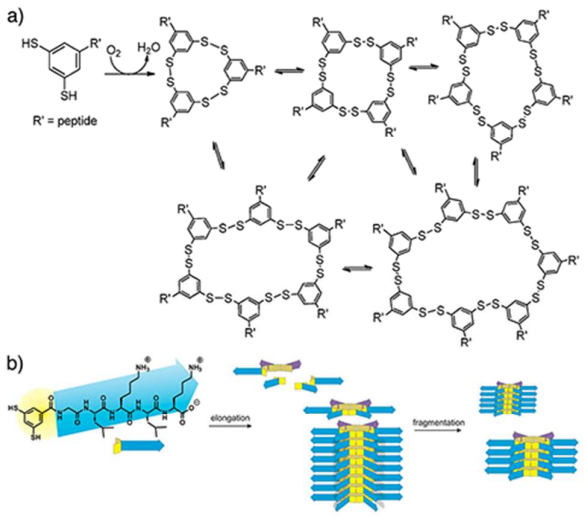

Figure 6. a) Dynamic Combinatorial Library of different macrocyclic disulfides, and b) illustration of β-Sheet replication-elongation from the end, as well as fragmentation enhanced under mechanic agitation. Adapted with permission from Ref. 56. Copyright 2013 American Chemical Society.

Rubinov et al. have recently investigated peptide replication and amyloid fibril reproduction with short amphiphilic peptides (Figure. 7a).[58, 59] Various soluble β-sheet assemblies, from β-plates, to fibrils and hollow nanotubes, contribute to a physical network that templates the ligation of simpler building blocks to form new assemblies. A detailed kinetic analysis of both the self-assembly and self-replication processes shows that only specific transient structures, primarily the fibrils that are stable for less than a few hours, can serve as active catalysts. Here again, similar to prion proteins, propagation is exploited for the enrichment of replication products. Recently, Nanda et al.[60] analyzed a related system, driven by a complex web of ligation processes that forms a native peptide and three undesired isomeric side products. Nevertheless, it was found that the formation of several products in such a mixture is not always harmful for production of the native template/product since efficient and selective template-assisted reactions serve as a backbone correction mechanism, keeping the concentration of the desired peptide equal to, or even higher than, the concentrations of the other products (Figure 7b).

In a final example[40] developed by Chen et al., the C-terminus of a small peptide was reduced from carboxylic acid to aldehyde, thus attenuating the energetic barrier for ligation to that of an imine. This strategy allows for direct rapid exchange between the peptide building blocks and the progressive growth of chain length as well as the selective choice of the N-terminal asparagine to participate in this acid catalyzed N,N-acetal cyclic ligation to trap the intermediate. The peptide building blocks create a dynamic chemical network consisting of diverse oligomeric species (Figure 8a) that exploit the peptide two-step nucleation phase transition (Figure 8b, 8c) ensuring the self-generation of templates and the subsequent template-directed growth into homogeneous fibers.

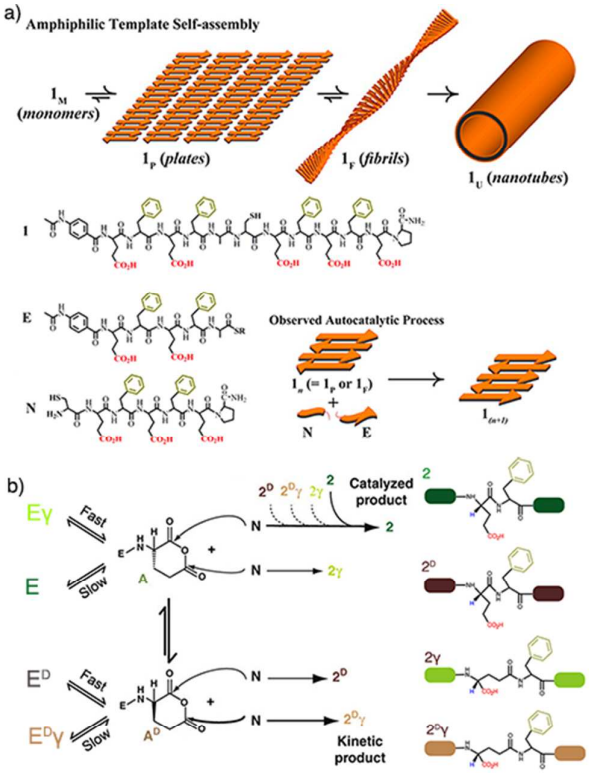

Figure 7. a) Presentation of the two processes that take place in parallel during the peptide replication: *top* – dynamic self-assembly of monomers to sheets, fibers, and nanotubes; *bottom* – autocatalytic reaction in which the β-sheet structure serves as template for in-register association of E and N, which then react to form a new copy of peptide 1 and the larger aggregate, available to serve again as a template. Adapted with permission from Ref. 56. Copyright 2012 American Chemical Society. (b) General mechanism of a network reaction initiated by a nucleophilic peptide N and an electrophile isomer Ex, leading to spontaneous production of four isomeric products. The scheme shows the kinetically favored pathways leading to an undesired product $2^{D}\gamma$ and the catalyzed pathways leading to product 2 containing the native backbone. Adapted from Ref. 60.

Taken together, these systems underpin a new strategy for exploiting the tension created by combining chemical and physical dynamic networks and using the complex nature of the system to achieve amplification of specific chain length, backbone

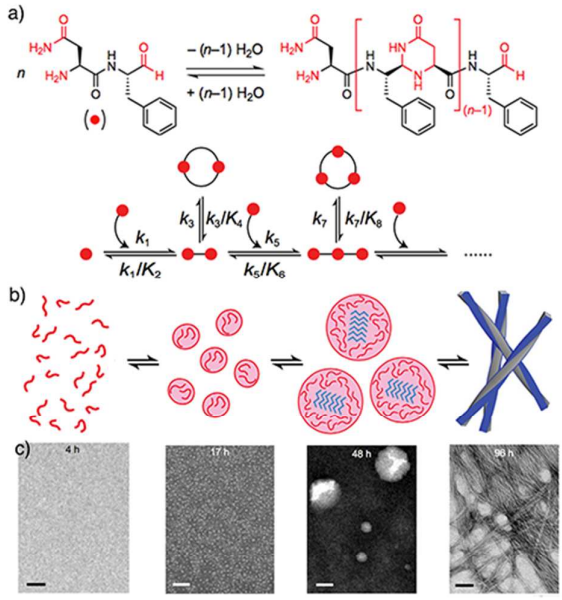

Figure 8. a) Condensation scheme of H-Asn-Phe-CHO (NF-CHO) peptides to give oligomers with N,N-acetal (4-pyrimidinone) linkages and kinetic modes of equilibrium between different oligomers. b) schematic representation of the multiple physical phase transitions of the dynamic chemical network. c) corresponding TEM images at different time points. Scale bar=100 nm. Adapted with permission from ref. 38. Copyright 2017 Nature Publishing Group.

configurations, and ring sizes of templates for molecular selection. Notable in all cases is the cooperation between chemical and physical networks, setting in motion the autocatalytic processes essential for molecular replication even in an hypothetical pre-RNA world[61].

5. Building towards function

An important implication underlying the construction of alternative molecular networks would be extending the physical and chemical limits on these adaptable and functionally versatile scaffolds. Even though the field of integrated molecular networks remains young and advanced functionalization is just beginning, the design of amphiphilic peptide networks that enable self-guided assembly into well-defined architectures is yielding new functional peptide scaffolds. During the past decade, peptide fibril models have been used for scaffolding cell tissues[62, 63], drug delivery[64], catalysis[65], self-replication[55, 59], and bio-electronics[66]. While peptide-based functionalization efforts provide a great starting point, they appear to be merely the very tip of a vast repertoire of future more complex integrated networks that are either compatible with or orthogonal to biology. Some critical investigations have now begun to address the three dimensions required for the design of such dynamic scaffolds: *covalent macromolecular interactions* → *supramolecular non-covalent assembly* → *emergent functionality.* In addition, adaptability to a changing environment can actively influence all three dimensions, providing a reasonable approximation to biological systems.

The remarkable catalytic activity of enzymes has often been associated with an ability to achieve a stable, folded conformation. With the aim of developing enzyme-like catalysts, short amyloid-forming peptides have been explored for various different bond breaking and bond making reactions. Hydrolysis of (reactive) ester substrates has been demonstrated using various self-assembling peptide models containing catalytically active His side chains[67, 68]. An esterase was developed from a seven-residue Zn^{2+}-binding peptide where Zn^{2+} stabilized the fibril and also served as a cofactor for acyl ester hydrolysis [69](Fig. 9a). Fibril assemblies made of peptides that contained Pro residues at their n-termini have been investigated by Ashkenasy, Escuder, and coworkers and found to catalyze C-C bond forming reactions via direct aldol coupling[70]. In all cases, the peptides that form fibrils are more active than the non-assembling analogues containing the respective catalytically active group(s). This observation recently guided fabrication of an artificial hydrolase with pH-switchable activity, achieved by introducing a catalytic histidine residue at the terminus of a pH-responsive peptide[71]. The peptide exhibits a conformational transition from random coil to β-sheet by changing the pH from acidic to alkaline. The β-sheet self-assembles to form long fibrils with the hydrophobic edge and histidine residues extending in an ordered array to create a catalytic microenvironment with significant esterase activity. Catalytic activity is reversibly accessed by pH-induced assembly/disassembly of the fibrils into random coils (Fig. 9b).

In a recent study, Omosun et al. extensively characterized the 3D structure of peptide-based nanotube structures (Fig. 10a) and used that structural insight to develop a catalyst for various

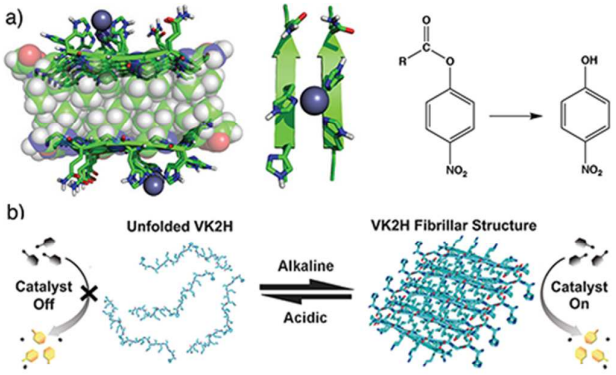

Figure 9 (a) Computationally derived model of fibrils formed by a designed Zn^{+2} binding peptide (Ac-IHIHIQI-$CONH_2$) and the studied para-nitrophenylacetate (p-NPA) hydrolysis reaction. Adapted with permission from ref. 69. Copyright 2017 Nature Publishing Group. (b) Schematic representation of the pH-switched artificial hydrolase based on conformation change of an amphiphilic β-sheet forming. Adapted with permission from ref. 71. Copyright 2012 John Wiley and Sons.

reactions including imine polymerization (Figure 10b) and retro-aldol C-C bond cleavage (Figure 10c).[49] The cross-β laminate grooves running along the KLVFFAL nanotube surface serve as extended arrays of binding sites containing the N-terminal K residue amine as a base catalyst. This extended co-linear array of many binding/active sites over the internal and external surfaces of hollow nanotubes exhibits distinct catalytic activity. Maybe most importantly, the catalytic activity is readily modulated by subtle changes of the peptide sequence and supramolecular architecture, highlighting the robust catalytic potential of these dynamic and environmentally responsive supramolecular assemblies.

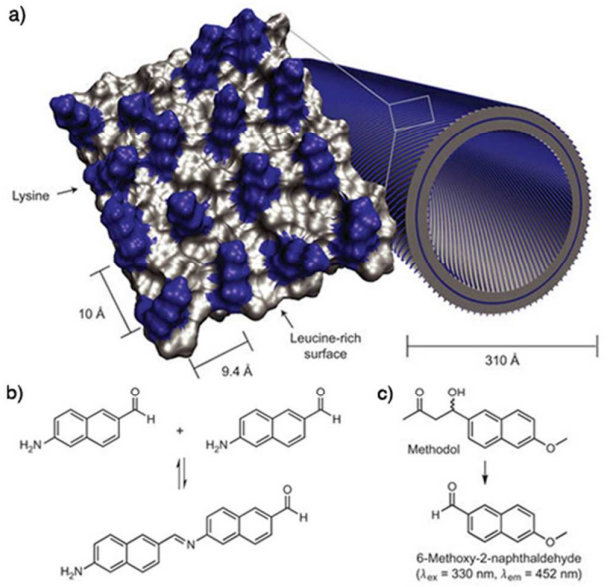

Figure 10. (a) Structural model for the KLVFFAL nanotube with a van der Waals surface expansion displaying the solvent exposed lysine (blue) and leucine (grey) residues. The nanotube surface can be utilized as catalyst to b) convert 6-amino-2-naphthaldehyde into condensed dimer and for c) retro-aldol reaction. Adapted with permission from ref. 49. Copyright 2017 Nature Publishing Group.

Further efforts to exploit the supramolecular function of these assemblies has resulted in the fabrication of conductor and semi-conductor devices for long range energy[47, 72, 73] and electron transfer (ET)[66, 74]. For example, replacing the lysine residue of the Aβ(16–22) peptide with Rhodamine 110 produced the Rh17-22 peptide, which provided valuable information about peptide self-assembly as well as the first demonstration of resonance energy transfer along a peptide nanotube.[75] The ability to sustain intermolecular ET has been exploited in solution, as well as utilizing various device architectures, including planar devices in which specifically designed peptides self-assemble into a monolayer between two remotely located electrodes (Figure 11a)[76]. The conductivity of fibers of de-novo designed phenylalanine containing peptides was found to be much higher than that of a film of non-assembled peptides, attesting to the importance of self-assembly and the resulted π-stacking in promoting conductivity. The ability to influence the conductivity by the nature of peptide side chains suggested that it can be further tuned by the incorporation of non-natural aromatic groups. Replacement of the phenylalanine side chain with other aromatic groups, such as naphthalene diimide (NDI), thienyl and furyl, was shown to significantly promote ET in peptide fibrils[76-78]. One of the first demonstrations of promoting electron delocalization by stacking large aromatic groups was that of cyclic peptide nanotubes to which NDI groups were incorporated by conjugation to Lys side chains[79]. ET promoting aromatic groups can also be conjugated to the peptide termini, or appended to the backbone in a bola-amphiphilic design[66]. The Ashkenasy group has recently designed an amphiphilic peptide (Figure 11b. 11c) that enabled ET that depended on different fibril polymorphs accessed under varying environmental conditions (water and co-solvent mixtures).[76] This peptide was equipped with a single aromatic NDI moiety and its fibril showed a significant increase (≥2 orders of magnitude) in conductivity relative to an analog lacking the NDI (KFE-8; Figure 11b). Molecular dynamics (MD) simulations, spectroscopy, and microscopy studies have shown that the intimate interactions between adjacent NDI moieties differ significantly in assemblies formed under different conditions, affecting both the degree of π-stacking and their chiral arrangement. The different interaction patterns, in turn, affected fibril morphology, leading to substantial differences in fibril width, as well as twist and directionality. Finally, the polymorphic self-assemblies exhibited distinctly different conductivities, and those containing protonating/deprotonating side chains can be useful for charge transport via different mechanisms, i.e., proton transport along the formed fibrils[80]. While several charge transport peptide-based nanostructures were reported recently, some challenges remain in order to make those

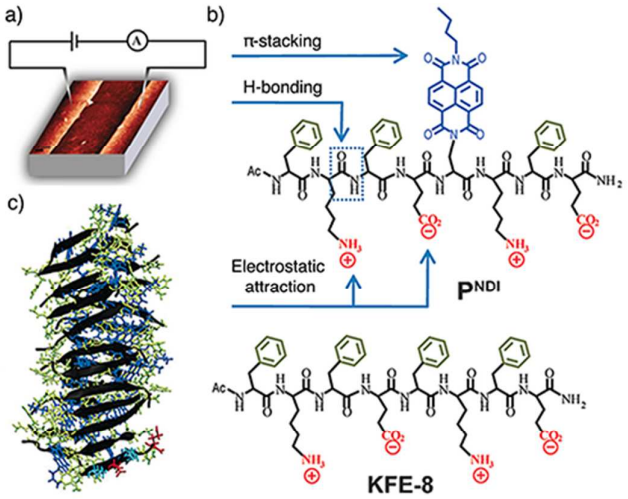

Figure 11. a) AFM image of a peptide fibril network covering chip electrodes and the (5 μm) gap between them. The measurement setup is also represented schematically. b) Molecular structures of the PNDI and KFE-8 peptides. c) side view of the most stable fibrillar structure of self-assembled PNDI (black arrows=fibril β-strands; green=Phe residues, blue=NDI moieties; most other residues omitted for clarity. Adapted with permission from ref. 76. Copyright 2016 John Wiley and Sons.

relevant for real-life applications, primarily the need for designing aromatic peptide conjugates presenting higher conductivity and the ability to construct and measure single-fibril architectures as the conducting element.

6. Expanding information content through cooperative co-assembly

Hybrid materials based on synthetic peptides and nucleic acids (NA) can potentially combine the advantages of both building blocks and lead to new functional assemblies. While the synergistic activities of proteins and nucleic acid observed in all extant living systems,[61] strategies for building truly mutualistic functions into synthetic assemblies are just beginning to emerge. New synthetic architectures have however emerged from synthesized NA-pep chimeras and the co-assembly of NA and pep segments containing groups that exploit complementary interactions. Regarding chimeras, the covalent conjugation of peptides/proteins to nucleic acids has been exploited for drug delivery, antisense technology and intracellular fluorescence imaging.[81-85]. Only recently has the self-assembly of such chimeras into vesicle or fibril architectures been described as new bio-materials. Remarkably, some of the recently developed synthetic system discussed below clearly show synergistic behavior, where assembly and stability depends on the interaction between NA and pep segments. One of the simplest and more interesting examples comes from the conjugation of the widely studied diphenylalanine peptide motif with a hexanucleotide amino modified sequence. While the FF dipeptide forms fibril structures in aqueous solution, conjugation of a nucleotide sequence induced a morphological transition from fibrillar to vesicular structures (Figure 12).[86] Similar studies performed with ditryptophan-DNA hybrids take advantage of greater aromatic π-stacking and superior photo-physical properties.[87] These conjugates form spheres in dilute aqueous solutions and fibers at higher concentrations, and tryptophan intrinsic fluorescence provides evidence that the hybrid organization is similar to that of the nucleation polymerization in the corresponding peptide fibrils. The controlled aggregation of a series of short-peptide–DNA hybrids composed of different short peptide fragments [88] in aprotic solvents revealed formation of spherical structures at low concentrations and ambient temperature, which under increased temperature, concentration, and incubation time, assemble into long β-sheet fibers. However, in pure water, only tryptophan containing chimeras assemble into fibers due presumably to stronger π–π stacking and intermolecular hydrogen bonding.

NA-pep co-assembly has been investigated by several groups in order to develop new virus-inspired mimics and novel hybrid materials for nanotechnology and medicine. A synthetic mimic of viral structure was developed by the synergistic co-assembly of a short peptide and plasmid DNA (Figure 13a)[89]. The peptide had

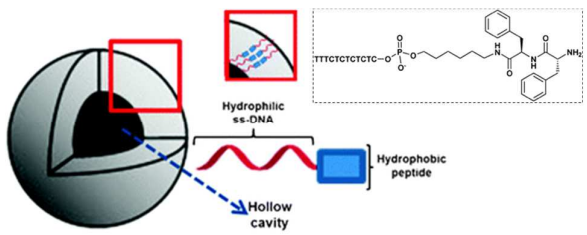

Figure 12. Proposed structure of a vesicle self-assembled by DNA-FF short peptide hybrids. Adapted from ref. 86 with permission from The Royal Society of Chemistry.

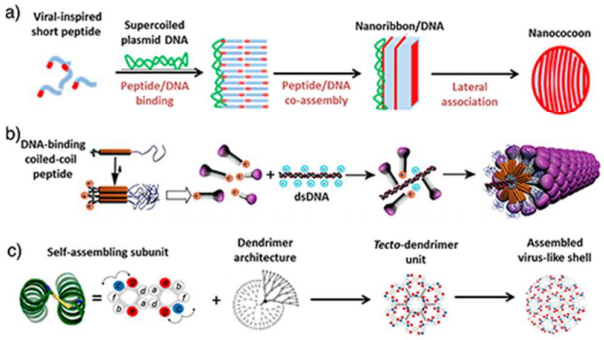

Figure 13. Various structural models of viral-inspired peptide/NA co-assembly nanostructures. Adapted with permission from Ref. 89., Ref. 91. and Ref. 92. Copyright 2014, 2013, 2016 American Chemical Society.

been designed to mimic capsid protein motifs by: (1) a positively charged region responsible for protein-DNA interaction, (2) a hydrophobic region stabilizing assembly through secondary structure and aromatic interactions, and (3) a hydrophilic region helping to solubilize the capsid in aqueous solution. Formation of nanoparticles of virus-like dimensions has been observed, and the particles were called 'nanococoon' due to the display of repeating stripes. In the co-assembly process, plasmid DNA interacted electrostatically with positively charged peptide segments, which helped to organize peptide strands, while the bilayer β-sheets stabilized the entire capsid mimic and induced the DNA organization. Moreover, the authors have shown that their design results in a stable structure, protecting the nucleic acid from enzymatic degradation as seen in viral structures. The same group demonstrated the ability to regulate the morphology of the co-assembled "capsids" by tuning the inter-nanofibril interactions. Strengthening of these interactions has been achieved by extending peptide side chain complementarity and/or elongating the peptide chain. Additionally, structural adjustments gave more stable nanococoons for higher cellular uptake, but they did not have a negative impact on gene transfection efficiency. [90] Virus-like particles have also been developed by the encapsulation of DNA templates with positively charged nanostructures, built by self-assembly of coiled-coil peptides conjugated to cationic spermine units and poly(ethylene glycol) (PEG) segments (Figure 13b).[91] This approach provided precise control over shape and dimensions of the particles, thus offering a strategy to create one-dimensional structures of defined length, which was achieved by the synergy of a peptide forming heptameric aggregates, entropy-limited aggregation mediated by the long PEG chains, and the electrostatic repulsion among spermine segments. This precise templating indeed offers possibilities for future applications in therapies and material sciences.

Another example of *de novo* virus-like topology comes from the design of a trifaceted coiled-coil peptide helix, which self-assembles into small anionic shells that encapsulated and transfer nucleic

acids (Figure 13c).[92] Relatively short peptide sequences were used and assembled on a dendrimer template that supported symmetry, mono-dispersity, and hierarchical assembly of the structures. The obtained shells promoted gene transfer into live cells without cytotoxicity effects common in commercial non-viral systems, and thus offered a promising solution for engineering artificial viruses with desired functions.

DNA origami, the most exploited approach for bottom-up DNA self-assembly, has also been used for co-assembly with collagen-mimetic peptides.[93] These peptides are designed to carry a central block with two positively charged domains at both termini. Co-assembly of peptides and DNA origami led to the formation of nanowires with DNA nanosheets stacking face-to-face and peptide motifs aligned perpendicularly to the sheet surfaces, creating new 1D hybrid material (Figure 14a). These new biomaterials may have future applications in the creation of novel nanodevices that synergistically combine properties of both peptides and nucleic acids. In another advance, DNA origami nanotubes were utilized as a platform for nucleation and organization of amyloid fibrils (Fig. 14b).[94] In this more complex system, covalently attached DNA staple strands and synthetic amyloid peptide sequences served as starting components in very specific self-assembly of peptide fibrils on a DNA surface. The DNA part of the conjugate created staples for origami nanotube construct and sticks out of the inside of the tube. After mixing with the peptide solution, amyloid fibers form and the protruding staples are hybridized with DNA segments at specific sites on the DNA platform, thus enabling control over orientation and location of the nanotubes. This work was the first example showing how amyloid fibrils can be nucleated and arranged by using cylindrical DNA motifs.

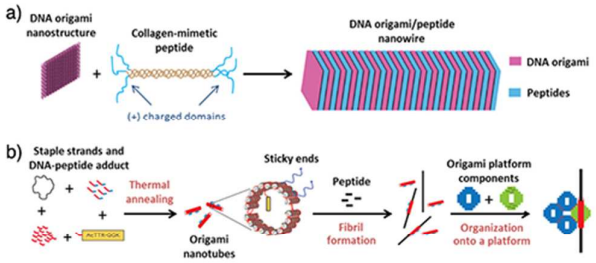

Figure 14. DNA-origami-peptide nanostructures. Adapted with permission from Ref. 93. and Ref. 94. Copyright 2017 American Chemical Society& Copyright 2014 Nature Publishing Group.

Self-assembly of NA-pep covalent conjugates has been demonstrated using β-suRGD peptide and two complementary DNA strands.[95] These dual-mode conjugates self-assembly into a toroidal global minimum energy state (supramolecular deoxyribonucleoprotein; suDNP) (Figure 15), and two scenarios were hypothesized for the formation of the thermodynamically stable supramolecular complex. First, a condition that enables the formation of a peptide β-sheet followed by DNA hybridization was investigated (pathway 1). For that, equimolar amounts of both conjugates were dissolved in a nonpolar environment (HFIP) that has a strong propensity to induce the formation of peptide α-helices in any peptide, destabilizing the β-sheet self-assembly mode of the β-suRGD segment. After prolonged incubation, the HFIP was evaporated and the dried mixture was re-dissolved in TE buffer. The aqueous mixture was vigorously sonicated, incubated at a temperature above the melting temperature (Tm) of the duplex, and subsequently cooled slowly to anneal the duplex. Second, the duplex was hybridized first, and then β-sheet formation was induced (pathway 2). An equimolar mixture was dissolved in aqueous solution at a concentration below the critical aggregation concentration of the peptide β-sheets. After annealing the duplex at this low concentration, the solution was slowly concentrated using a centrifugal filtration device to induce assembly of the peptide. Investigation of the assembly status using various techniques revealed that the two pathways resulted in the formation of identical nanostructures with both pathways leding to the formation of thermodynamically stable β-suRGD-AS/β-suRGD-S complexes. By comparison, simple dissolution of the aqueous mixture resulted in the formation of a heterogeneous population of nanostructures, suggesting the coexistence of kinetically trapped states and a thermodynamically stable state. This study provided an example for how the covalent linkage between the two

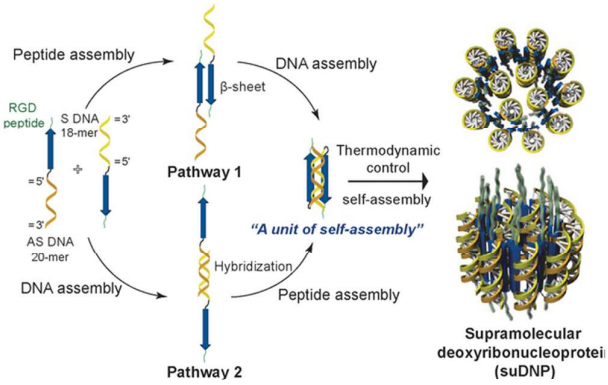

Figure 15. Pathways for the dual-mode self-assembly of complementary peptide–DNA conjugates into a Programmable DNP. Adapted with permission from ref. 95. Copyright 2016 John Wiley and Sons.

macromolecular segments constrains structures formed by reducing the number of degrees of freedom, thus decreasing nonspecific aggregation. Additionally, it opens the possibility of thermodynamically programing self-assembling structures for optimizing gene regulation.

7. *Future outlook*

Using extant biology as a guide, the strategies for combining dynamic chemical and physical networks of mutualistic biopolymers

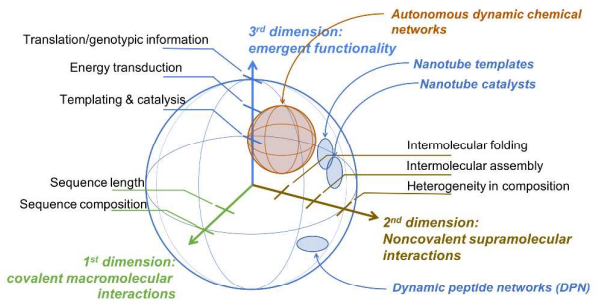

Figure 16. The three dimensions proposed for exploring the structural space of potential autonomous chemical networks. Adapted from ref. 96.

has been realized. While dynamic peptide networks (DPN) and DNA origami have set the standard for these studies, they may well represent only a small portion of the available structural space that can be synthetically accessed for the autonomous growth of new function (Figure 16). Despite hypotheses for an RNA (only) world transition to living systems, biological systems are marvelously mutualistic networks of biopolymer co-assemblies and life's origins must reflect these synergies[96].

NA-pep conjugates[97-99] and peptide nucleic acid (PNA)[100] oligomers can radically increase the complexity of the dynamic networks and have been utilized in the medical sciences for drug delivery or gene silencing. Fewer studies have focused so far on analyzing the mutualistic functions associated with NA-pep conjugate assemblies and NA-pep co-assemblies. We propose that each of the new amyloid-like, spherical or globular NA-pep architectures can now be mapped into Figure 16. For example, stiff linear fibrils have been recently formed by mixing small concentrations of NA-pep conjugates with amphiphilic peptides, and these assemblies might be useful for long range electron transfer in molecular electronics or living cells.[101] It has also been demonstrated recently for spherical architectures that NA-pep co-assembly, or the assembly of conjugates following DNA hybridization, confers additional duplex stability. The dsDNA motifs within these assemblies are less susceptible than their soluble counterparts to changes in temperature or pH, as well as more stable against enzymatic degradation. The NA-pep spherical co-assemblies can also serve as efficient hosts for small molecules, binding to either the DNA or peptide motifs, and thus highly protected from the aqueous environment. Upon changes in conditions, *in-vitro* or within cells, these small molecules can be released and serve as diagnostics, as drugs, or maybe most importantly, for new cellular elements extending biology into new area of functional space. As a future target, not accomplished so far, we suggest that the NA-pep elongated fibril architectures, or the spherical co-assemblies, might serve as templates and catalysts for various reactions including the replication of their own DNA and peptide segments for the progressive evolution of new function.

While both peptide and nucleic acid precursors can form in prebiotic environments from simple building blocks, it is the synergistic assembly and mutualistic functions that must emerge to achieve the Central Dogma that provided the Darwinian threshold of cellular life. Finding those spaces in Figure 16 poses a significant challenge, but several recent studies are now revealing unexpected aspects of synergism in NA–pep systems.[102-108] For example, simple peptide catalysts, containing Pro residues, have been used to form enantiopure RNA precursors[109], and His containing peptides catalyzed RNA phosphodiester bond formation and oligomerization[110]. Synergy between simple prebiotically relevant molecules from other biopolymer families have also been reported. Among other examples, interactions between NA and lipids resulted in the formation of multi-lamellar architectures,[111] fatty acid vesicles enhanced and controlled the stereochemistry of peptide oligomerization,[112-114]and chiral pentose sugars were found useful in mediating enantioselective synthesis of amino acid precursors[115]. Taken together, harnessing the increased molecular information observed in NA-pep networks, as well as in other Pep-x networks relevant for the origin of life, may pave the way to the future grand challenges of systems chemistry, extending synthetic systems to support compartmentalization, replication and metabolism maintained far-from equilibrium on different scaffolds to achieve autonomous dynamic chemical networks capable of emerging functional order for molecular evolution.

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