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# Nucleic-Acid-Templated Synthesis of Smart Polymer Vectors for Gene Delivery

José García Coll<sup>[a]</sup> and Sébastien Ulrich<sup>\*[a]</sup>

Nucleic acids are information-rich and readily available biomolecules, which can be used to template the polymerization of synthetic macromolecules. Here, we highlight the control over the size, composition, and sequence one can nowadays obtain by using this methodology. We also highlight how

templated processes exploiting dynamic covalent polymerization can, in return, result in therapeutic nucleic acids fabricating their own dynamic delivery vector – a biomimicking concept that can provide original solutions for gene therapies.

## 1. Introduction

The printing industry relies on templates that are used multiple times to copy text and images. Johannes Gutenberg revolutionized the European printing press by introducing movable types in the XV<sup>th</sup> century, and the technique rapidly spread around the world due to its simplicity, efficacy and high-quality that outperformed all previous technologies. Similarly, Nature makes exquisite use of templated processes to copy and translate the molecular information stored in biopolymers: DNA is copied, maintained and repaired in our cells through replication processes where a single strand of DNA templates the formation of its complementary strand; RNA is enzymatically transcribed from DNA templates; and proteins are synthesized by the ribosomes starting from mRNA templates through a process called translation. These examples continue to inspire chemists to build biomimetic compounds and machines.<sup>[1]</sup> Self-replication of synthetic polymers was originally observed about 70 years ago in a process coined “replica” polymerization,<sup>[2]</sup> and the enzyme-free replication of natural biopolymers, such as nucleic acids<sup>[3]</sup> and peptides,<sup>[4]</sup> is considered to be a key step in the origin of life.

Although IUPAC has precisely defined “template” as “The nucleic acid single strand that is copied during replication or transcription”, the term has vastly propagated across chemical sciences to evoke a more general process where molecular information is copied or translated in new chemical entities. For instance, dense and small cations,<sup>[5]</sup> followed later by larger inorganic anions<sup>[6]</sup> or rigid organic compounds,<sup>[7]</sup> have been

used to template the formation of macrocycles and interlocked species (Scheme 1, top). In a bioinspired approach, translating the information encoded in nucleic acids – which can be rapidly engineered at will and now at low cost using solid-phase oligonucleotide synthesis – represent a fascinating way to produce controlled and functional polymers (Scheme 1, bottom). Yet, it requires (bio)chemists to apprehend the structural and interactional complexity of biomolecular templates that greatly exceed that of small and rigid templates. Herein, we highlight the recent progresses made in using nucleic acids as templates to produce bioactive polymers, emphasizing the potential of such an approach for making polymer vectors for gene delivery applications.

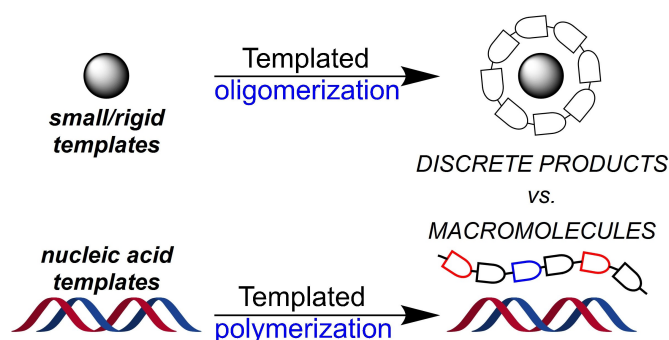
## 2. Principles of Nucleic-Acid Templating

We build here on previous accounts that discussed the molecular and supramolecular basis of DNA-templated self-assembly,<sup>[8]</sup> and focus on the use of nucleobase and phosphodiester recognition events to trigger a polymerization process.

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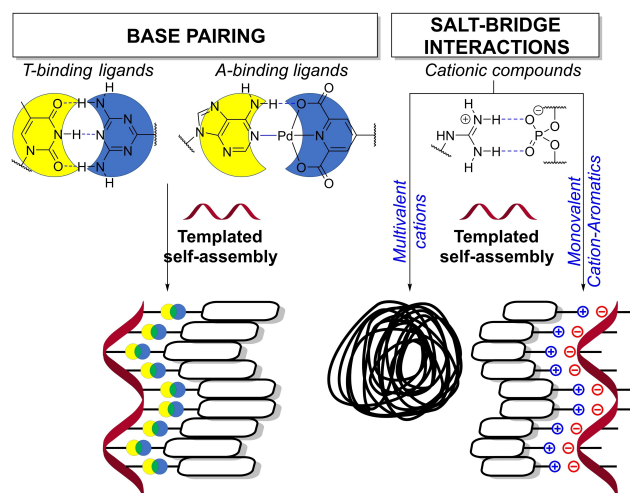
**Scheme 1.** General representation of templated processes yielding discrete assemblies with small/rigid templates (top) and macromolecules through nucleic-acid-templated polymerization (bottom).

## 2.1. Self-assembly through base pairing

The formation of base pairs through complementary hydrogen bond patterns is at the basis of the well-known DNA double helix, notwithstanding the less-popular but important contributions arising from  $\pi$ - $\pi$  interactions and steric complementarity.<sup>[9]</sup> In addition to natural nucleobases, synthetic compounds can also selectively bind to nucleobases. In pioneering works, Schenning, Meijer *et al.* identified naphthalene-bearing diamino-triazines able to bind to oligo-thymine single-stranded DNA (ssDNA) through a combination of hydrogen-bond and  $\pi$ - $\pi$  stacking,<sup>[10]</sup> leading to the helical self-organization of multiple small molecules around the templating ssDNA (Figure 1).<sup>[11]</sup> The supramolecular polymerization process can also be converted into a templated dynamic covalent polymerization.<sup>[12]</sup> More recently, Galindo *et al.* have extended this approach by reporting a palladium chelidamic acid complex that bind to oligo-adenine ssDNA through nucleobase recognition, leading to helical aggregates (Figure 1).<sup>[13]</sup>

## 2.2. Self-assembly through salt-bridge interactions

DNA is a worm-like random coil which can locally be seen as a rod-shape double-helix having a diameter  $\sim 2.4$  nm and a persistence length  $\sim$  of 50 nm (ca.  $\sim 150$  base pairs).<sup>[14]</sup> The local rigidity stems from electrostatic repulsion between neighboring anionic phosphodiester groups that are separated by 0.34 nm. However, even at pH above pKa ( $\sim 2.5$ ), not all the phosphodiester groups are deprotonated. Binding to monovalent cations provides charge compensation and the Manning-Oosawa condensation theory provides a framework explaining that, in the end, a linear density of one free negative charge per 0.7 nm is reached.<sup>[15]</sup> At this distance, called the Bjerrum length, electrostatic repulsion equals thermal energy  $k_B T$ . Therefore, around 75% of phosphodiester groups are bound to monovalent cations in order to decrease electrostatic repulsion. Multivalent cations enhance this number, reaching for instance 94% with tetra-cationic ligands.<sup>[16]</sup> Above 90%, the charge compensation leads to DNA condensing into toroid-shape nanoparticles that aggregate several molecules of DNA and condensing agents (Figure 1).<sup>[17]</sup> The valency of the cationic



**Figure 1.** Examples of nucleic-acid-templated self-assembly processes using nucleobase pairing (left) or salt-bridge interactions (right), leading to either organized helical arrays of DNA-supported small molecules, or to condensed assemblies.

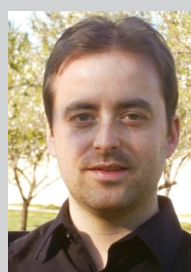
ligands is therefore a key parameter, and DNA condensation can be observed above a threshold valency of 3–4 for oligonucleotides longer than  $\sim 400$  base pairs.<sup>[16,18]</sup> Another strategy is to combine salt-bridge interactions (most often enforced using guanidiniums<sup>[19]</sup>) with additional secondary forces (Figure 1). Following this approach, we<sup>[20]</sup> and others<sup>[21]</sup> have shown that  $\pi$ - $\pi$  stacking interactions can further promote the organization of monovalent organic cations onto ssDNA through the recognition of the phosphodiester backbone.

## 2.3. Mechanism of templated polymerization

Through the study of the templated self-assembly of diamino-triazines onto oligo-thymine ssDNA, the group of Schenning developed a model which explains the distribution of bound molecules on the nucleic acid template as a function of i) the free energy of guest binding onto a site of the template ( $g$ ), ii) the free energy of guest-guest interactions bound to the template ( $\epsilon$ ), iii) the temperature, and iv) the length of the



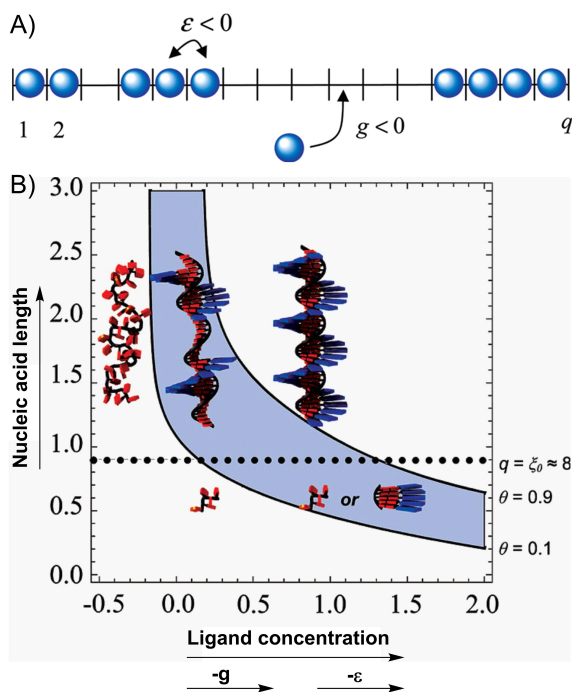
José García Coll started his chemistry studies at the Universitat de València (Spain), then he moved to the Ecole Européenne d'ingénieurs de chimie, polymers et matériaux (ECPM) at the Université de Strasbourg (France) to obtain a double international master's degree in molecular chemistry and supramolecular chemistry in 2021. He is currently a PhD student at the Institut des Biomolécules Max Mousseron (IBMM) in Montpellier under the supervision of Dr. Sébastien Ulrich and Dr. Nadir Bettache. His research project is focused on the design and synthesis of smart delivery vectors for gene therapeutics.



Sébastien Ulrich carried out his PhD with Prof. Jean-Marie Lehn (Université de Strasbourg, France), and post-docs with Prof. Harry L. Anderson (Oxford University, UK) and Prof. Eric T. Kool (Stanford University, CA, USA). In 2011, he was awarded an ANR starting grant and joined the group of Prof. Pascal Dumy in Grenoble, before moving in 2012 to Montpellier, France where he was recruited by the CNRS to develop his research interests in the field of supramolecular bioorganic chemistry. Since then, he has contributed to developing dynamic self-assemblies for nucleic acid recognition and delivery applications. In 2017, he was awarded the CNRS Bronze Medal.

nucleic acid template ( $q$ ).<sup>[10a,11d]</sup> A negative  $g$  means favorable binding to the nucleic acid. A cooperative templated self-assembly occurs when  $g < \epsilon < 0$ , while a non-templated supramolecular polymerization occurs first prior to nucleic acid binding when  $\epsilon < g < 0$  (Figure 2). The case of  $g < 0 < \epsilon$  correspond to a situation where ligands will occupy distant sites on the nucleic acid because of repulsive inter-ligand forces.

The difficulty in mastering templated processes when the templates are biomolecules, as opposed to rigid/small molecules, lies in the different conformations they can adopt depending on the environmental conditions. And this is clearly the case for nucleic acids which can be seen as floppy (ssDNA), rigid rods (double-stranded DNA, dsDNA), or folded 3D structures (RNA, condensed DNA). The nature of the interaction between monomers and the nucleic acid template may also have a role as it can affect its conformation. In general, compounds that interact through base pairing reinforce the helical structure and rigidity of nucleic acids by enhancing  $\pi$ - $\pi$  interactions. On the other hand, templated polymerization with monomers that bind nucleic acids through salt-bridge interactions is a more complex case to apprehend. Indeed, when multivalent cations bind and neutralize the negative charges of nucleic acids, DNA condensation may occur.<sup>[16]</sup> Applied to the case of a templated polymerization, it implies that DNA condensation would be elicited as the templated polymerization proceeds, meaning that the templating effect may operate differently in the initial and final phases of the process.



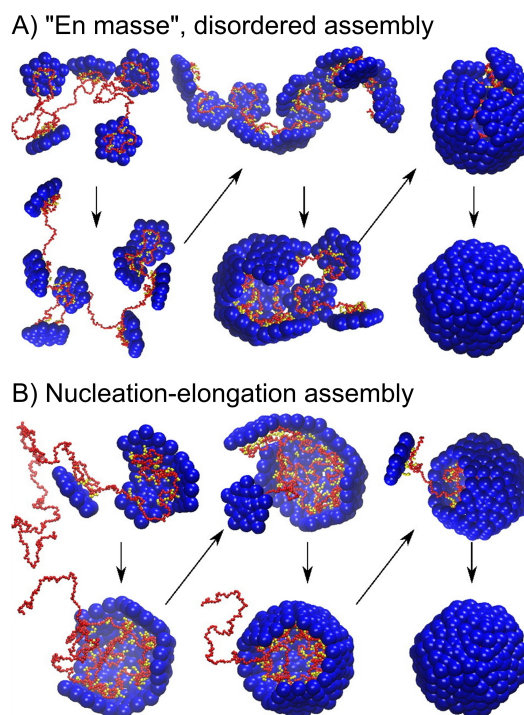
**Figure 2.** Model for binding multiple ligands onto nucleic acids. A) schematic representation of free energy of guest binding onto a site of the template ( $g$ ) and free energy of guest-guest interactions bound to the template ( $\epsilon$ ); B) distribution of species as a function of nucleic acid length ( $q$ ), ligand concentration,  $g$ , and  $\epsilon$ . Adapted with permission from reference [10a]. Copyright 2009, American Chemical Society.

This situation happens similarly in viruses during capsid formation.

#### 2.4. Nucleic-acid templating in natural systems

DNA condensation by multivalent basic proteins takes place hierarchically in the nucleosome/chromatin/chromosome formation,<sup>[22]</sup> and is also orchestrated by viruses packing their viral genomes within capsids.<sup>[23]</sup> Understanding the latter may provide useful approaches for building a bioinspired gene delivery technology.

Viruses are diverse in function (different cargoes can be transported, short to long nucleic acids, single- or double-stranded), come in very different shapes (filamentous or spherical), and display different organ/organelle tropisms.<sup>[23]</sup> Interestingly, the mechanisms of assembly of viral capsids are also diverse and involve, for the tobacco mosaic virus, influenza virus, SV40 and HIV, a co-assembly process where the viral nucleic acid templates the formation of its own capsid through either "nucleation-and-growth" or "en masse" mechanisms (Figure 3). A common feature is that assembly is directed by electrostatic and hydrophobic interactions, with capsids typically growing until reaching charge ratios (ratio of total negative charge on nucleic acid cargo and total positive charge of capsid proteins) between 1 (neutral capsid) and 2 (negatively-charged capsid).<sup>[24]</sup> The size of the nucleic acid encapsulated within the capsid has also been shown to depend upon the net charge of the polypeptide arm of capsid proteins.<sup>[24b]</sup> The tobacco mosaic virus is a well-studied representative



**Figure 3.** Viral capsid assembly through either A) "en masse", or B) "nucleation-and-growth" mechanisms. Adapted from reference [26]. Copyright 2014, with permission from Elsevier.

example of a filamentous capsid composed of 2130 protein repeats which self-assemble by a nucleation-growth mechanism. It results from the templating effect of the ~6390 bases viral ssRNA and operates by electrostatic interactions with protruding basic arginine-rich loops in the protein subunits and "Caspar" carboxylate pairs between adjacent protein subunits that contribute to neighboring contacts while also making the disassembly pH-sensitive.<sup>[25]</sup> Regarding their disassembly which can occur stepwise or in a concerted manner, viruses use multiple cellular cues, depending on the final destination of the nucleic acid cargo (cytosol vs. nucleus), such as receptor binding, acidic pH of endosomes, cytosolic protein contacts, and intracellular reductive environment.<sup>[23]</sup>

### 3. Precision Polymers

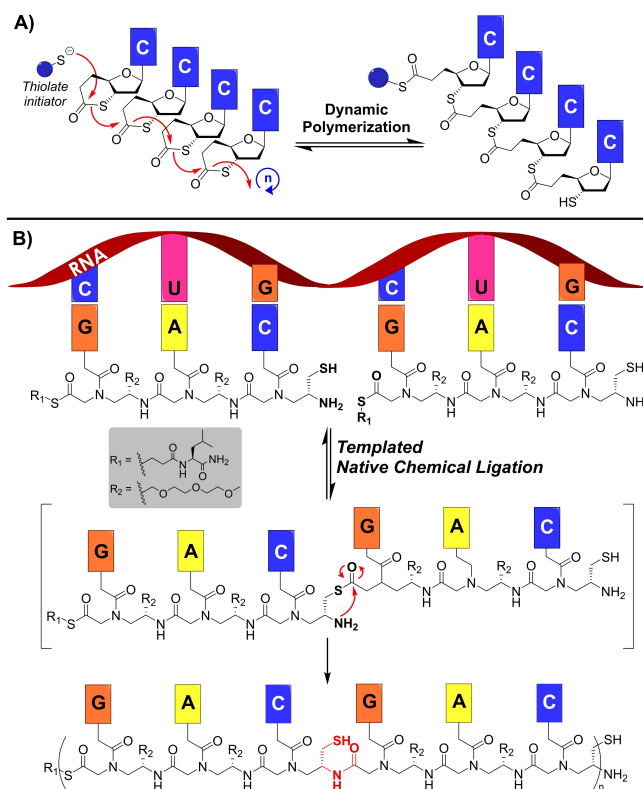
Nature uses complex molecular machinery to convert the molecular information carried by nucleic acids into sequence-defined biopolymers, and giant leaps have been made in designing analogous artificial processes.<sup>[27]</sup>

The Lynn group reported an inspiring case of DNA-templated polymerization of modified nucleoside through reduction amination.<sup>[28]</sup> The Liu group quickly extended the concept for making sequence-selective peptide nucleic acids through the same reduction amination methodology.<sup>[29]</sup> New polymerization chemistries applicable to nucleic acid templating have recently been reported. For instance, the Sleiman group applied ring-opening metathesis polymerization (ROMP) to translate a poly-adenine template into a poly-thymine polymer.<sup>[30]</sup> Interestingly, they showed that the daughter polymer has a chain length that match the parent polymer ( $M_w = 18700$  vs.  $17700 \text{ g mol}^{-1}$ , respectively) as well as a similar narrow molecular weight dispersity ( $\mathcal{D} = 1.20$  vs.  $1.07$ , respectively). As comparison, the non-templated or mismatched-templated polymerization yielded shorter and polydisperse polymers ( $M_w = 13300 \text{ g mol}^{-1}$ ,  $\mathcal{D} = 2.77$  and  $M_w = 7200 \text{ g mol}^{-1}$ ,  $\mathcal{D} = 2.25$ , respectively). More recently, the Feng group described the templated polymerization of non-natural nucleobase-containing polymers into complementary polymers via a reversible addition-fragmentation chain transfer (RAFT) polymerization.<sup>[31]</sup> The templated polymerization was well-controlled, with parent polymer templates ( $M_n = 6400 \text{ g mol}^{-1}$ ,  $\mathcal{D} = 1.09$ ) yielding precisely defined daughter polymers ( $M_n = 10900 \text{ g mol}^{-1}$ ,  $\mathcal{D} = 1.21$ ). Again, using a non-complementary template led to smaller and polydisperse polymers ( $M_n = 7000 \text{ g mol}^{-1}$ ,  $\mathcal{D} = 2.30$ ). In a seminal contribution, the O'Reilly group showed the importance of segregating processes in favoring such templated polymerization process, producing high molecular weight polymers ( $M_w$  up to  $400,000 \text{ g mol}^{-1}$ ) with very low polydispersity ( $\mathcal{D} \leq 1.08$ ).<sup>[32]</sup>

Besides the control over the length and dispersity of templated homopolymers, nucleic acids can also impose preferences over the sequence of the templated polymers. For instance, Ghadiri and co-workers showed that the composition and sequence of peptide nucleic acids, where the nucleobase is appended through a reversible covalent reaction (thioester),

adapt to the presence of a templating nucleic acid (DNA, RNA) through a base-filling mechanism.<sup>[33]</sup> More recently, Bowman and co-workers used this thioester chemistry as a polymerization technique and demonstrated the homo-polymerization of cytosine and thymine based monomers (Figure 4A).<sup>[34]</sup> The process follows a thioester ring-opening polymerization which is initiated by thiolates. Interestingly, since the polymers are made of reversible thioester linkages, adaptation by rearrangement (e.g. monomer exchange, polymer growth and shrinkage) was shown by the authors to occur through thiol-thioester exchange reactions. Despite no templated polymerization has been reported yet, this proof of concept may lead to the synthesis of sequence-selective DNA-like polymers by templated polymerization. Ly and co-workers have reported a different design with monomers made of tri-nucleotide (three codon recognition system) and a native chemical ligation based polymerization process (Figure 4B).<sup>[35]</sup> The system is capable of undergoing RNA-templated polymerization and spontaneous depolymerization when the template is removed. Although the aforementioned works all use similar base-pairing interactions, they also show that the nucleic-acid-templated polymerization can operate with multiple and diverse chemical reactions.

To turn such templated approach into a useful technique for producing synthetic polymers, one must address the turnover challenge. Indeed, in general the binding affinity for the growing polymer toward its template increases during the



**Figure 4.** Dynamic polymers amenable to sequence-selective nucleic acid-templated polymerization: A) homo-polymerization of cytosine-based nucleoside derivatives through thiol-initiated ring-opening polymerization; B) sequence-controlled RNA-templated polymerization through native chemical ligation polymerization.

process, meaning that in the end the template has to be used in stoichiometric amount. Chemical post-modifications or thermal cycling can promote dissociation of the templated polymer in order to release the native template that can then re-enter a new cycle.<sup>[12,36]</sup> The Matile group recently described a peptide-templated disulfide-based ring-opening polymerization where the helical peptide template and the growing oligomer are linked through reversible covalent reactions (semicarbazone).<sup>[37]</sup> Dynamic covalent exchange of semicarbazones with oxyamines enables to detach the templated oligomer and release the template which can then enter a new cycle. Previously, the Liu group reported a more complex system made of an adapter linking a DNA template to the growing sequence-controlled polymers which can be covalently detached through disulfide cleavage.<sup>[38]</sup>

#### 4. Smart Polymers for Gene Delivery

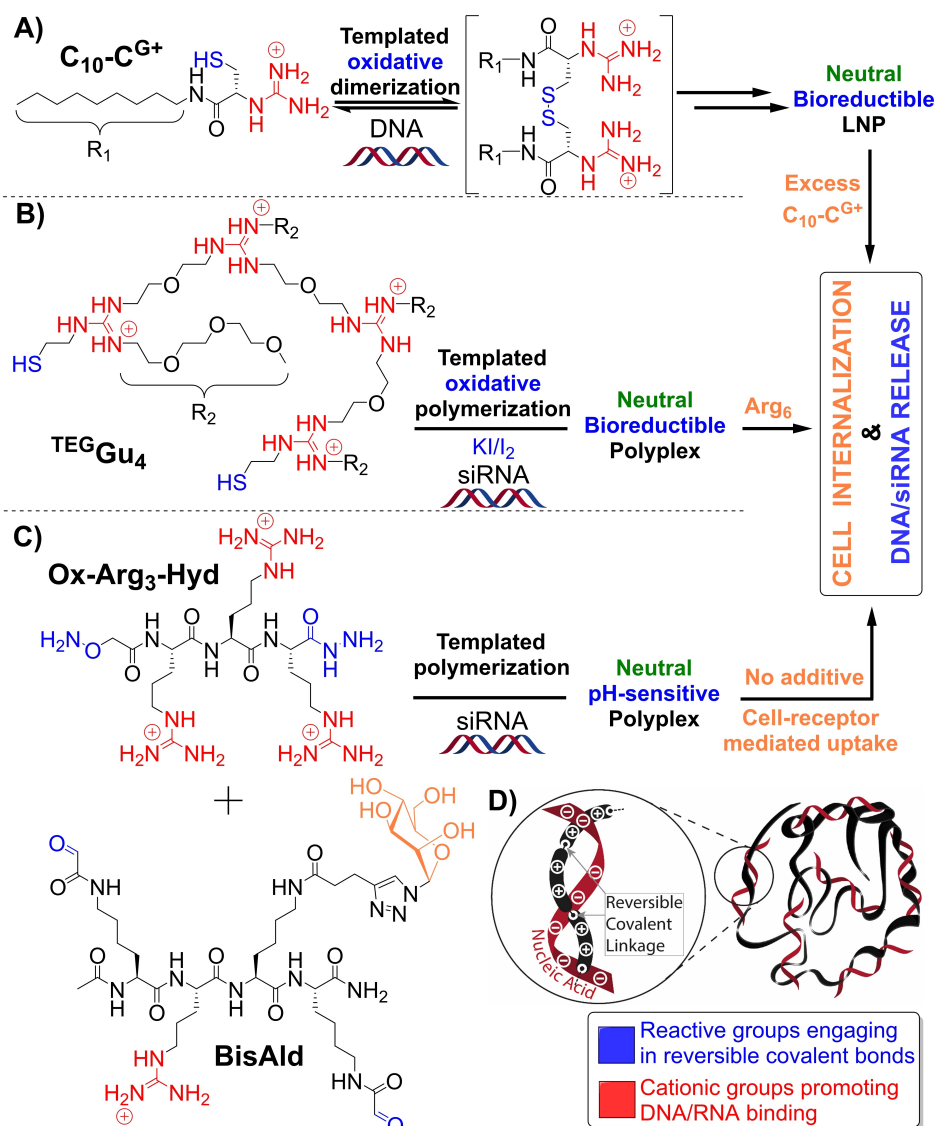
There is one application where turnover is not necessary: gene delivery. In this case, the templated polymer could serve as a delivery vector and the nucleic acid would thus fabricate its own delivery vehicle. This approach represents a new paradigm complementary to the combinatorial screening of large libraries of polymer vectors.<sup>[39]</sup> Since nucleic acids are becoming important active ingredients of drugs (e.g. antisense DNA,<sup>[40]</sup> siRNA,<sup>[41]</sup> mRNA,<sup>[42]</sup> CRISPR-Cas9<sup>[43]</sup>), it is essential to develop specific vectors and a templated approach can indeed be best for producing adaptive vectors that are self-fitted to one type of nucleic acid. However, a central challenge is to perform a polymerization at low concentration which, without the assistance of a template, is very unlikely to occur.

Since the overall delivery process requires at the end the active release of the therapeutic nucleic acid, the exploration of responsive systems has become an important endeavor.<sup>[44]</sup> In this line, while there are recent reports of beautiful virus-inspired capsid-mimicking self-assemblies<sup>[45]</sup> and nucleic-acid-templated supramolecular vectors,<sup>[46]</sup> we believe there is a strong interest in developing dynamic vectors assembled through reversible covalent reactions because they will offer a better chemical control over their assembly and disassembly processes.

An early example of templated polymerization onto plasmid DNA (pDNA) was reported in 1998.<sup>[47]</sup> Although the process involved an irreversible polymerization of cationic acrylate monomers, the authors demonstrated that the template DNA remained biologically active in cells. The same year, the Behr group showed the DNA-templated covalent dimerization of amphiphilic compounds through an aerobic oxidative disulfide formation ( $C_{10}-C^{Cu+}$ , Figure 5A).<sup>[48]</sup> The resulting stable spherical DNA condensate nanoparticles of 20–30 nm in size being devoid of excess positive surface charges ( $\zeta$  potential ca. –40 mV), the authors rightfully noticed that they were unable to penetrate cells, and therefore resorted to deliberately using an excess of cationic monomers in order to elicit a moderate effect of pDNA delivery. Similarly, in 2000, the Rice group reported short cationic cross-linking peptides inserting multiple

cysteines which can undergo a DNA-templated polymerization, triggering DNA condensation (nanoparticles < 100 nm, with positive  $\zeta$  potential 18–37 mV) and pDNA delivery in cells, with biological results indicating that intracellular release was responsible for the observed 5–60-fold enhanced efficacy.<sup>[49]</sup> In 2015, the group of Aida reported a system made of abiotic monomers which undergo siRNA-templated polymerization through oxidative disulfide formation mediated by KI/I<sub>2</sub>.<sup>[50]</sup> They used triethylglycol-bearing oligo-guanidinium monomers ( $^{TEG}Gu_4$ , Figure 5B) where the cationic groups are regularly spaced by a 7.4-Å long ether spacer, matching the 6–7 Å distance between phosphate groups in RNA. The templated assembly leads to the formation of polyplex “nanocaplets”, measuring around 7 nm in size. Analysis of the templated polymers after dialysis and siRNA hydrolysis confirmed that polymerization is driven by electrostatics (1:1 guanidinium: phosphodiester stoichiometry) and reached low degree of polymerization (~6) necessary for the charge compensation of the 21-base pair siRNA ( $\zeta$  potential 1.7 mV). In contrast, only dimers formed when the polymerization was carried out without the siRNA. Similarly to Behr *et al.*, the authors noticed a 5-fold-lower cell uptake using these neutral nanoparticles, and subsequently resorted to appending a short arginine-rich peptide ( $Arg_6$ ) to endow an excess of cationic surface charges that was found effective to promote cell internalization (*vide infra*). Once inside the cytosol, the “nanocaplets” are cleaved through the action of glutathione, releasing siRNA through the rapid reductive depolymerization of the vector. The interest in poly-disulfide vectors is that they undergo intracellular depolymerization within minutes,<sup>[51]</sup> and can also promote cell-internalization through thiol-mediated uptake.<sup>[52]</sup> This approach was later extended by Li, Yang and co-workers who designed cationic cyclic disulfide monomers, and showed that they undergo a nucleic-acid-templated ring-opening polymerization, removing the need of oxidative polymerization.<sup>[53]</sup>

We have been exploring a pH-sensitive polymerization involving acylhydrazone formation. While disulfide-based redox-sensitive delivery systems are interesting for their degradability once in the cytosol, pH-sensitivity is interesting to harness the acidification of endosomes during endocytosis in order to trigger cell internalization and cargo release.<sup>[54]</sup> We started off our journey by first reporting in 2014 the spontaneous formation of dynamic covalent polymers through a polycondensation assembly, showing that they are able to complex DNA through multivalent interactions while undergoing a pH-controlled hydrolytic depolymerization ( $t_{1/2}$  < 20 hours at pH 4 vs.  $\gg$  200 hours at pH 7).<sup>[55]</sup> After a proof of concept showing effective delivery of siRNA in live cells with cationic dynamic covalent polymers pre-formed in the absence of nucleic acids,<sup>[56]</sup> we set out to address the targeted delivery challenge with *in-situ* templated vectors. Using complementary modified cationic peptides flanked with a hydrazide C-terminus, oxyamine N-terminus, and complementary bisaldehyde peptides ( $Ox-Arg_3-Hyd$  and  $BisAld$ , Figure 5C), we showed that a siRNA-templated polymerization takes place through the *in situ* formation of acylhydrazone and oxime bonds, leading, over the course of a 16 hours incubation, to neutral polyplex nano-



**Figure 5.** Dynamic covalent polymer vectors which formation is templated by the DNA/RNA cargoes: A) amphiphilic dimers leading to neutral bioreducible lipid nanoparticles (LNP); B) redox-sensitive guanidinium-rich dynamic covalent polymers; C) pH-sensitive peptide-based dynamic covalent polymers; D) Schematic representation of polyplexes generated using dynamic covalent polymers. Note that neutral polyplexes are formed and cell internalization require either using cationic helpers (A, B) or a ligand promoting cell-receptor-mediated uptake (C).

particles ( $\zeta$  potential 1.3 mV).<sup>[57]</sup> Increasing the number of cationic groups in monomers was found to accelerate the process which is complete within 30 minutes incubation with trisarginine building blocks.<sup>[58]</sup> This phenomenon most likely originates from an increased residence time of the cationic monomers onto nucleic acid when their positive charges are increased.

Beyond the nucleic-acid-templated formation of dynamic covalent polymers that result in neutral polyplexes, a central challenge is then to promote cell internalization, ideally with tissue/cell selectivity. Indeed, neutral polyplexes usually display a poor intrinsic ability to penetrate cell membranes. One common strategy mentioned above is to use an excess of positive charges to promote cell uptake through electrostatic interaction with the anionic phospholipids of the cell membrane.<sup>[59]</sup> The Behr group used this approach to transfect

the plasmid DNA pCMV-Luc into BNL CL.2 epithelial cancer cells, detecting the expression of the Luciferase protein after 24 hours of incubation.<sup>[48]</sup> Taking advantage of the “magic” guanidinium recognition of anions,<sup>[52b]</sup> the Aida group improved the internalization capability of their siRNA-templated nanocaplets by appending a short peptide containing 6 arginine ( $\text{Arg}_6$ ), showing an improved cell internalization (one-order of magnitude higher in the presence of  $\text{Arg}_6$ ) into human hepatocellular carcinoma Hep3B-Luc cells after 72 hours incubation, and a silencing activity revealed by the knockdown of the luciferase gene down to ca. 10% achieved at a 200 nM dose (N/P = 30) of siRNA vs. 29% without  $\text{Arg}_6$  and 27% using the commercial transfection reagent DharmaFECT.<sup>[50]</sup> However, relying solely on electrostatics for promoting cell uptake can be risky when it also mediates nucleic acid recognition. Electrostatic binding to the cell membrane can then lead to premature release of the

nucleic acid prior to cell penetration. Therefore, a second approach is to tether ligands that recognize cell membrane receptors. In a subsequent work, the Aida group covalently photo-immobilized transferrin onto their siRNA-templated nanocaplets.<sup>[60]</sup> Interestingly, they found that binding to the transferrin receptor not only promote cell uptake but also transcytosis which is the ability to escape and re-enter surrounding cells. The biological experiments carried out on a 3D Hep3B cancer spheroid model revealed siRNA delivery up to a depth of 70  $\mu\text{m}$ . Our own recent contribution has been to harness multivalent carbohydrate recognition to promote cell entry. Using a clickable peptide building block, we were able to append a *D*-Mannose as targeting group. Studying the delivery of a siRNA against luciferase onto HCT-116-Luc colorectal cancer cells successfully revealed the receptor-dependent intracellular delivery of siRNA, leading to a knockdown luciferase activity of 47% at a 200 nM dose (N/P=20) of siRNA, with no apparent cytotoxicity.<sup>[57]</sup> This activity was found to be specific to these colorectal cancer cells – compared to MCF-7 human breast adenocarcinoma cells – because they were known to over-express the membrane-bound mannose receptors. Key to success was the ability to self-assemble dynamic covalent copolymers, simply by mixing different monomers, in order vary the density of targeting ligands which has to match the multivalent presentation of the corresponding cell membrane receptors.

## 5. Summary and Perspectives

Understanding the templating effect of nucleic acids has a basic importance linked to the RNA world hypothesis of the origin of life.<sup>[61]</sup> As highlighted in this article, it also bears a strong technological potential, either to manufacture polymers by copying/translating the information encoded in nucleic acid templates, or to use the templated polymers as vectors for the delivery of therapeutic nucleic acids. The latter approach, where the cargo fabricates its own vector, mimics the templated self-assembly of viral capsids. When looking at the different examples highlighted here, it turns out that different molecular designs were successful in eliciting a nucleic acid-templated polymerization, leaving much room for further works. The future challenges revolve around achieving a better control of the dynamics of polymerization and depolymerization of the templated vectors in complex biological media, as well as increasing their complexity going toward multi-component vectors. Last but not least, modern characterization techniques will be needed to decipher the fine structure of the selected vectors which in many cases remain elusive.

Finally, the knowledge gained through advances in this field is more general and will definitely expand beyond nucleic acids. For instance, electrostatic binding to proteins has been used to template self-immolative macromolecules that assist the intracellular delivery before undergoing a depolymerization inside cells.<sup>[62]</sup> Even bacteria were shown to template polymer formation, leading to the discovery of strain-selective antimicrobials.<sup>[63]</sup> The manipulation of the dynamic assembly of

polymers that form through an adaptive templated polymerization and a responsive depolymerization shall therefore find wide therapeutic applications (e.g. protein/enzyme/antibody/nucleic acids delivery).<sup>[64]</sup>

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## Conflict of Interests

The authors declare no conflict of interest.

**Keywords:** Template · Polymer · Nucleic acid · Dynamic covalent chemistry · Gene Delivery

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