Engineering a stable future for DNA-origami as biomaterial

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Engineering a stable future for DNA-origami as a biomaterial

Hale Bila,† Eva E. Kurisinkal† and Maartje M. C. Bastings*†

DNA as a biomaterial has evoked great interest as a potential platform for therapeutics and diagnostics and as hydrogel scaffolds due to the relative ease of programming its robust and uniform shape, site-specific functionality and controlled responsive behavior. However, for a stable self-assembled product, a relatively high cation concentration is required to prevent denaturation. Physiological and cell-culture conditions do not match these concentrations and present additional nucleases that cause a serious threat to the integrity of DNA-based materials. For the translation of this promising technology towards bioengineering challenges, stability needs to be guaranteed. Over the past years, various methods have been developed addressing the stability-related weaknesses of DNA-origami. This mini-review explains the common stability issues and compares the stabilization strategies recently developed. We present a detailed overview of each method in order to ease the selection process on which method to use for future users of DNA-origami as a biomaterial.

1. Introduction

Biomaterials, and in particular nanoparticles, can play a key role in providing controlled systems to stimulate cellular signalling and determine what molecular levers must be pulled, how hard, and when, in order to direct a desired state of action. Nanoparticles, because of their size similarity to cellular components, can enter living cells using cellular endocytosis mechanisms.

Synthetic materials have been used as immune adjuvants or delivery systems to amplify, regulate, or qualitatively alter cellular responses. Additionally, they provide tools for further understanding the basic mechanisms controlling cell signalling and can manipulate the bio-interface. Uniformity in particle size, shape and functionality is crucial to challenge cells with a well-defined input in order to measure a comprehensive output. Only when all material parameters are accurately controlled and systematically varied can changes in cellular response be linked to a unique material design.

Hale Bila is currently pursuing her PhD degree and working with DNA as a biomaterial to enhance the specificity of nanotherapeutics through programmable complex multivalency at École Polytechnique fédérale de Lausanne (EPFL). Upon graduation from the Middle East Technical University (METU), she completed her master’s in chemistry at Bilkent University, Turkey.

Eva Kurisinkal is a PhD student at the Programmable Biomaterials Laboratory at École Polytechnique fédérale de Lausanne (EPFL). She works with DNA as an ECM-mimicking programmable material to promote cell adhesion. Previously, Eva pursued a double international master’s degree in polymer science (IM-PolyS) at the Université de Strasbourg, France and Albert-Ludwigs-Universität Freiburg, Germany.
DNA nanotechnology offers the opportunity to design nanoparticles varying in composition, size, shape, and surface properties, with uniform control over all these parameters.\textsuperscript{13-16} The enormous variety in self-assembled nanoparticles based on DNA published in the past decade has demonstrated that this material can be programmed in virtually any desired shape.\textsuperscript{17} Producing synthetic DNA in large quantities required for materials and health applications is now possible thanks to scalable biotechnological approaches\textsuperscript{18} and thus we see the field quickly moving toward exploring many cellular and \textit{in vivo} targets. Recent reviews on the use of DNA nanocarriers for drug-delivery applications show the exciting potential of this powerful programmable biomaterial.\textsuperscript{19,20} Indeed, this unique platform has the potential to change our current bioengineering standards by the inherent uniformity that allows true quantification of complex biological questions. Yet, care should be taken in the design and assays using DNA-based materials in the biological context – the reason being the structural demands and limitations of DNA: (1) it is only stable below the melting temperature (typically around 50 °C, but is highly structure dependent); (2) it requires a significant ionic strength in the solvent (typically 5–20 mM MgCl\textsubscript{2}); and (3) it is sensitive to degradation by nucleases, present in the serum of cell culture media, and in physiological environments \textit{in vivo}.\textsuperscript{21,22}

Despite widespread literature studies describing the cellular effects caused by DNA-origami since its introduction in 2006\textsuperscript{23} (which we will not discuss in this work), over the past 5 years, the field has recognized and focused on careful quantification of the stability issues and limitations posed by using DNA as a building material. Currently, various methods for protection against temperature, non-optimal salt concentration and nucleases have been developed, all presenting a solution depending on the structure and intended application, as recently reviewed by Ramakrishnan \textit{et al.}\textsuperscript{24} In order to select the preferred method when targeting a cellular or \textit{in vivo} research question using DNA-based materials, we attempt to provide a comprehensive overview of the stability challenges and compare the performance of each method based on temperature, salt, and nuclease protection, as well as on generality and ease of use and translation.

2. Stability challenges

To validate the correct assembly of DNA-origami materials, the classic approach is to conduct gel electrophoresis. A resulting single tight band should correspond to the molecular weight of the DNA origami. This result is coupled with visual control via either atomic force microscopy (AFM) or transmission electron microscopy (TEM). This visual step is crucial in the determination of local structural errors, since these will generally not show up in the gel electrophoresis analysis.\textsuperscript{25,26} Additional techniques include dynamic light scattering (DLS),\textsuperscript{27} fluorescence assays,\textsuperscript{28} DNA-PAINT super-resolution imaging\textsuperscript{29} or fluorescence resonance energy transfer (FRET) assays.\textsuperscript{30} The latter set of methods is highly structure dependent and generally only used in the case of specific questions and interest. Regardless of the choice of initial characterization, the stability of the designed structure should be confirmed under conditions matching those of the experimental read-out. For instance, when a cellular effect is reported after 12 hours of incubation, the structural integrity should be confirmed in the medium and in cellular compartments for the same time period in order to draw conclusive results.

Divalent cation concentration and nuclease activity are the two main factors causing structural instability. Additionally, temperature, pH and adsorption of other medium components might influence the particle integrity. What complicates the matter is that the superstructure resulting from the DNA-origami design influences the level of susceptibility toward each parameter. The superstructure refers to the organization of DNA oligomers into the final structure. These can be designed as dense, lattice ordered origami structures with a high crossover density, elongated or wire-frame structures that present a low crossover density, and structures formed from just a few oligomers (polypods, tetrahedra or tile based materials). For the ease of comparison of the protection techniques in Table 1 in this review, we classify superstructures as 2D, 3D dense lattice, 3D sparse lattice, wireframe, and tile-based. The level of crossover density influences the amount of negative charges centred in a structure, and studies have demonstrated a direct correlation to stability. Hence, stability is highly structure specific and no general black-and-white answer can be given regarding the importance of each parameter. However, the literature has amassed an overall opinion on what is the general trend regarding cation concentration variations and nuclease resistance.

2.1 The effect of cation concentration

DNA-origami structures are traditionally assembled in TBE or TAE buffer, supplemented with 5–20 mM MgCl\textsubscript{2}. The Mg\textsuperscript{2+} has been heading the Programmable Biomaterials Laboratory at EPFL, Switzerland, developing precision materials as tools to quantify fundamental mechanisms in biology.

Maartje M. C. Bastings trained as a biomedical engineer at the Eindhoven University of Technology (TU/e) and obtained her PhD in 2012. She was honored with the University Academic Award. She moved to the Wyss Institute of Harvard University in Boston as a NWO Rubicon and Human Frontier Science Program postdoctoral fellow, where she focused on DNA-origami and their cellular interaction. Since 2017 she has been heading the Programmable Biomaterials Laboratory at EPFL, Switzerland, developing precision materials as tools to quantify fundamental mechanisms in biology.

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ions neutralize the negative charges of the phosphate backbone and those clustered at crossover sites. Structures with dense packing of helices or many staple crossovers generally require higher amounts of cations to be correctly folded whereas structures with a large interhelical spacing require significantly less salt. Physiological salt concentrations are however much lower than needed to ensure origami stability. The typical Mg2+ concentration in cell culture media is 0.04–0.8 mM and that of Na+ and K+ is ~150 mM and ~5.5 mM, respectively. In blood, these numbers are 0.6–1 mM, ~140 mM and ~4 mM, respectively, making physiological environments significantly destabilizing toward origami materials.22,33

In the Keller laboratory, a recent thorough study evaluates the stability of 3 different types of origami (a planar triangle, long “simple” tube with high interhelical spacing, and a densely packed shorter brick) in 8 different Mg2+ free buffers.21 Of importance for bioengineering targets are phosphate buffers, where the authors report that K2HPO4 is less damaging than Na2HPO4, yet both need to be supplemented with 100 mM KCl or 200 mM NaCl respectively to guarantee structural integrity for two months. Remarkably, the most complex and densely packed structure remained intact in Tris buffer, without an EDTA chelator. The authors hypothesize that EDTA chelates the remaining Mg2+ ions bound to the phosphate backbone and thus omitting this compound leads to a more stabilizing environment. What becomes clear from their many AFM images is that structures start to “open-up” or “breathe” more when situated in a low or zero cationic environment. Low cross-over structures inherently are more flexible and thus tolerant toward these conditions whereas lattice-based structures are much more rigid and quickly affected. However, the open structure does make the particles more sensitive toward nuclease degradation.21

### 2.2 The effect of nucleases

Despite abundant literature studies on DNA-origami use for cellular and in vivo targets, the low stability against nucleases and highly negative charge limit their circulation half time and efficient uptake into cells.22,33 Similar to the point made on salt concentration in section 2.1, the level of susceptibility to nuclease degradation is highly superstructure dependent. The stability of structures upon 12 h of incubation in cell lysate has been tested,26 however, the lysate is homogeneous and very different from the actual cellular environment. Under culture conditions as well as intracellularly, different endo- and exonucleases are present that act with varying destructive powers on DNA particles. In the Dietz lab, many variants were tested against a densely packed block-like structure.34

<table>
<thead>
<tr>
<th>Method</th>
<th>Salt stability</th>
<th>Nuclease stability</th>
<th>Origami structures</th>
<th>Availability</th>
<th>Non-toxic</th>
<th>Preserved functionality</th>
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<td>+</td>
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<td>5</td>
<td>In-house</td>
<td>N/A</td>
<td>Yes</td>
<td>65</td>
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</table>
that during the preincubation step, cationic species present in the medium have a chance to adhere electrostatically to the DNA origami, thereby generating a moderate protective coating that withstands nucleases up to a day. Although this effect has yet to be extensively studied, this theory could account for the variations in DNA origami stability and the results of cellular studies presented in early experiments within this context.

Importantly, nuclease activity is temperature dependent and all stability assays should therefore be performed at temperatures similar to culture conditions (e.g. 37 °C). We note that the FBS used in culture media is generally heat-treated at 56 °C by the vendor as a standard practice to inactivate immune factors, which obviously differs from in vivo conditions. Furthermore, frequent freeze–thaw cycles of the serum or prolonged storage when diluted in culture media at 4 °C rapidly decreases the activity of DNase I. Hence, as recommended by Hahn et al., when testing DNA stability, freshly prepared solutions should be used.

3. Strategies for controlled protection

Since the realisation of stability challenges of DNA-origami, the field has started exploring methods to prevent fatal destruction. The methods developed can be classified into 5 themes: (1) additives that electrostatically interact with the DNA backbone thereby forming a protective coating; (2) structural changes that covalently alter the interactions between individual DNA strands; (3) encapsulation of the structure in a protective shell; (4) modifications to the backbone and (5) changes to the environment rather than to the DNA structure itself (Fig. 1C). We will discuss the present examples per class in the following section and summarize in Table 1.

3.1 Enhanced stability using protective coatings

Adding a protective coating to DNA particles has become one of the main solutions to enhance origami stability. As always, there are many ways to Rome, yet all use the principle of a cationic molecule (dendrimer, polymer, sugar, protein) that electrostatically interacts with the negatively charged phosphate backbone of the DNA. From the solutions found, two classes can be observed: linear cationic (block co-) polymers and proteins with natural or synthetically attached cationic interfaces. In both classes, we find examples of commercially available solutions versus those needing in-house organic synthesis. It is important to note that, while earlier studies have published on the coating of DNA origami with cationic polymers, we only review examples where a demonstration of enhanced structural stability in biological media is presented.

Fig. 1 Examples of DNA superstructures, threats faced both in vitro and in vivo and protection strategies employed. (A) Cartoon representation of the different DNA superstructure formats used in this review. (B) The various threats DNA-origami particles can encounter in vitro and in vivo. (C) Summarized overview of the five categories of protection methods developed over recent years.
3.1.1 Protein coatings. Of the protein-based coating strategies we find two examples from the Kostai/kyn laboratory. The first method uses the cationic N terminus of the cowpea chlorotic mottle virus (CCMV) capsid protein to electrostatically bind to DNA origami structures.36 Interestingly, at a low concentration, their coated 2D rectangles are found to roll up like a cake roll. A 13-fold enhancement of cellular transfection was reported compared to unprotected structures. Although no specific nuclease stability data is presented, this change in cellular uptake is likely to result from the protective character of the protein coating on the origami.

The second method uses bovine serum albumin (BSA) with covalently attached first- or second-generation dendrons carrying 9 or 27 protonizable amines respectively.37 Only the second-generation dendron shows strong origami interaction. The choice of BSA leads to a perfect blending-in of the coated particles with the cell culture environment and indeed a significant reduction in immunogenic activity. The coated structures display full resistance toward DNase I compared to about 75% degradation for unprotected samples, yet only incubation for 1 hour at room temperature was tested.

Using the natural DNA-binding protein Sso7d of the hyperthermophilic archaeabacterium Sulfolobus solfataricus, the De Vries laboratory created a fusion protein by linking an octomer of the 99 amino acid hydrophilic collagen-inspired sequence C.38 Hereby, the final protein construct has a DNA binding function as well as a long shielding and solubilisation domain. Interaction with linear and coiled dsDNA and circular ssDNA was proven, as well as a moderate stabilization against DNase I when used as a coating for a 2D DNA rectangle.

3.1.2 Polymer coatings. Two studies report on the use of oligosilane-based polymer coatings as a protective layer against low-salt and high-nuclease conditions30,39 where the L-lysine block provides the positive charges to interact with the DNA backbone and the PEG block serves to shield the structure from nuclease attacks. The Schmidt lab reports a coating using in-house synthesized PEG12k–Lys1k that provided full protection during 16 hours of incubation in a cell medium and folding buffer supplemented with DNase I. To allow surface functionalization on the DNA origami structures to be accessible, a shorter coating similar to that reported by the Shih laboratory was used.30 Protection and stabilization using the commercially available ten-mer oligosilane K10 coupled to PEG3k was tested extensively by Ponnuswamy et al.30 Besides confirming a 1000×-improved stability under cell culture conditions, the integrity of the protected DNA origami structures was also confirmed after cellular uptake. The use of dendritic cells that are known for their efficient DNA degradation capacity provided a challenging test case. The coating method was shown to hold the structures intact after 12 hours of incubation, with over 50% still intact after 24 hours. Punctate fluorescence indicated the presence of the origami structures in the endo-lysosomal compartments. Additionally, a fivefold increase in the in vivo circulation halftime was observed upon intravenous injection in mice. In both studies, a variety of DNA origami designs were tested that covered all superstructure classes; hence, these coating methods provide a general solution. The K10PEG3k coating was used in a follow-up study by Bastings et al. to analyse the shape dependent cellular uptake efficiency of 11 different DNA origami structures in endothelial, epithelial and immune cells, confirming the stability and non-toxic character of this method.40

An alternative strategy has been recently reported by Ahmadi et al., who investigated linear polyethyleneimine (PEI) and the natural cationic polysaccharide chitosan as low-cost coating materials.28 Both polymers have been extensively used for in vivo gene delivery, with chitosan showing advantageous biocompatibility properties and PEI showing potential cytotoxic and haemolytic activity.41–43 Coated DNA origami showed largely enhanced stability under cell culture conditions for up to a week.

The Simmel lab used a spermidine coating to stabilize the origami structure for electrotansfection into Jurkat cells.44 A high concentration was required (700 μM) and a narrow function/aggregation window was found to be present. The Qian lab followed up the spermidine protection strategy and demonstrated the stability of DNA-origami for 12 hours in a cell culture medium and a doubling of the cellular uptake quantity.45 Spermidine was tested in the Shih laboratory and found to preserve the structural integrity only at very high N : P ratios.30 Samples did not withstand overnight dialysis in zero-Mg2+ buffer, presumably due to the dissociation of polyamines into the free solution.

3.2 Enhanced stability using chemical cross-linking

DNA-origami structures typically contain hundreds of small single strand “staple” oligos that interact with the long ssDNA “scaffold” strand. A rational solution toward increased stability therefore is the chemical cross-linking of the individual strands. Various methods to achieve covalent bonds between staple strands have been explored.

The first report dates back over 20 years and makes clever use of T4 DNA ligase, a natural enzyme that catalyses the covalent phosphodiester bond formation between the 5′ phosphate of one DNA strand and the 3′ hydroxyl of another.46 Since the 5′ phosphate is typically absent in synthetic DNA, the cross-linking can be controlled via chemical addition of this 5′ modification. Successful ligation was shown for a single-strand tile (SST) structure composed of 5 oligos that further assembles into long DNA nanotubes, upon incubation for at least one day at room temperature. The resulting products were shown to be stable at elevated temperatures (75 °C) and could be kept in pure water for over a month. No tests on nuclease stability were performed in this early work.

The first report on stabilization through chemical cross-linking using full DNA-origami structures was from the Sugiyama lab.37 Addition of the photo-crosslinker 8-methoxypsoralen (8-MOP, a natural product present in a variety of vegetables) allowed for cross-linking using 365 nm light. Irradiation for 1 hour using a 500 μM 8-MOP and 10 nM of origami placed on ice resulted in fully cross-linked structures that could withstand 1 hour of incubation at 90 °C.
Using again the SST method, a 24-oligonucleotide 6-helix tube structure was constructed by Cassinelli et al., where every oligo was functionalized with an alkyne on the 3’ end and an azide on the 5’ end. Using a mild click-reaction, the two ends were covalently interlocked into the so-called DNA catenanes. The structures displayed extreme stability in pure water and withstood heating to 95 °C. Additionally, incubation for 24 h at 37 °C in a DMEM cell medium (but without added FBS) did not affect the structural integrity. Incubation with exonuclease I caused some smearing in agarose gel analysis, yet TEM confirmed intact structures. Stability against DNase I was not tested. While extremely robust for tile-based DNA structures, translation toward origami type structures containing a long scaffold and many more oligonucleotides is difficult.

Gothelf and De Stefano reported the stabilization of DNA oligos and simple tiled structures using dynamic cross-linking of disulphide-terminated oligonucleotides. The cross-linked structures were proven to be stable under denaturing gel electrophoresis conditions and tested up to 60 °C. To cross-link the thiol-modified ends, a minimal incubation time of 12 h at room temperature was required and a yield of up to 80% was achieved. Although not tested, this method could have interesting advantages for future cellular delivery applications since the cytosolic environment is known to be strongly reducing. Therefore, structures stabilized by disulphide bridges would naturally fall apart or open up after internalization and deliver a programmed payload.

The latest and only method for full DNA-origami stabilization comes from the Dietz lab. It has long been known that ultraviolet (UV) irradiation causes DNA damage in the form of covalent bond formation (cyclobutane pyrimidine dimers, CPDs) in thymine (T) and cytosine (C) bases. In 1982 it was noted that CPDs could be formed between two terminal T residues; however, the realization to use them as an advantage to stabilize a structure was not made until this current year. By designing T residues at strategic places followed by irradiation with 310 nm UV light, the authors can link free strand termini, remove strand breaks and create additional interhelical connections that strengthen the structure through the extra T–T bridges at neighbouring locations. Once every single strand structure is cross-linked, an unmet level of stability is achieved. The structures are stable up to 90 °C which is far beyond the normal melting temperature, and withstand storage in double distilled water. The crosslinking time needs to be carefully optimized, since a too long crosslinking time would cause further unintended DNA damage, and a too short crosslinking time will not lead to complete cross-linking. The reported time for the dense block structure was 2 hours, but this highly depends on the light source and additionally should be verified for each structure.

3.3 Protection via structure encapsulation

Perrault and Shih developed a nature-inspired protection method based on virus lipid-membrane encapsulation. Although this method needs careful structural optimization and includes time-consuming preparation steps, the final result shows that 68% of the DNA-origami population is liposome-encapsulated at the end. This result was affected by the liposome formulation, where PEG-DOPE and cholesterol played key roles in successful and tight encapsulation. A significant protection against DNase I was obtained, measured at physiologically relevant activity levels and confirmed for 24 h at 37 °C. Additional benefits include the escape from immune surveillance as demonstrated by a largely reduced IL-6 and IL-12 expression from spleen cells and a largely extended in vivo circulation half-life of 6 hours compared to 20 minutes for non-protected structures. Although no specific study on salt stability was performed, the authors prove the presence of dsDNA after incubation in low-salt culture media.

3.4 Backbone modifications and non-natural bases

A different solution to obtain stability is found in chemical modifications of the phosphate backbone as well as the use of modified, non-canonical nucleobases. Backbone modifications are offered on synthetic oligos by vendors with various options. In the work of Fisher et al., it was shown that nucleolytic degradation of oligos can be prevented or limited using internucleotide linkage substitutions, such as phosphorothioate and phosphorimidate, or 2’-ribose substitutions, such as 2’-OMe.

Probably the best-known phosphate backbone modification to aid nuclease stability is the phosphorothioate (PS) bond that substitutes a sulphur atom for a non-bridging oxygen. Due to stereoisomers, 50% of the time this modification functions effectively; hence multiple modifications are recommended to play the statistics card. PS bonds throughout the entire oligonucleotide are known to help reduce attack by endonucleases as well. Although effective, these modifications are known to increase toxicity and should be evaluated in the resulting particle design. DNA oligonucleotides that include the 2’OMe modification are typically 5- to 10-fold less susceptible to DNases than unmodified DNA. The 2’OMe modification is commonly used in antisense oligonucleotides as a means to increase stability and binding affinity to target transcripts. Inverted dT can be incorporated at the 3’ end of an oligonucleotide, leading to a 3’–3’ linkage, and at the 5’ end, 2’,3′ dideoxy-dT base (5’ Inverted ddT) to inhibit degradation by exonucleases. Phosphorylation of the 3’ end of oligonucleotides, or a phosphoramidite C3 spacer can be incorporated to inhibit degradation by 3’ exonucleases. The latter modification can be made either internally, or at the ends of an oligo to introduce a long hydrophilic spacer arm that besides nuclease stability can be used as the attachment site of fluorophores or other functional groups. For all modifications, in order to avoid unanticipated side effects, including aggregation, toxicity, change in thermal stability due to disrupted Watson–Crick base pairing or unanticipated sequence-independent biological effects like triggering an innate immune response, structures including modifications should be carefully tested.

Unnatural nucleotides have been developed with favourable specificity and thermal stability properties. Base-pairing between 5-Me-isoC/isoG and A/2-thioT in a 6-arm junction...
DNA structure led to a doubling in time of the resistance against T7 exonuclease, up to 12 hours. Resistance toward other endo- and exonucleases was not reported.

Modifications can also be made at the strand terminus, as extensively studied in the Sleiman laboratory. A hexaethylene glycol (HEG) chain was added to both the 5′ and 3′ ends of oligos that assemble into tiled structures that displayed significantly enhanced serum stability. A half-life of 15 hours was measured, which proves a roughly 3-fold enhancement compared to similar tiled structures.⁶³ The modifications did not affect folding or cargo loading and release, as demonstrated by siRNA studies in follow-up work.⁶⁴ A variation of the end-modification strategy was reported by the same laboratory,⁶⁵ where the HEG was presented in a 4-arm dendritic confirmation. Cubes with this multivalent HEG modification showed a serum half-life of 8 hours. As a hydrophobic variant, a 4-arm dodecane DNA modification was introduced. The lipophilic resemblance of the C12 modifications led to high-affinity binding with human serum albumin (HSA) proteins. Coating with HSA resulted in an extensive increase in the half-life of up to 22 hours.⁶⁶

3.5 Enhanced stability via environmental changes

Besides modification of the origami structure, stability can also be achieved via environmental adaptations. As seen in the stability section, phosphate buffers can be supplemented with 100 mM K⁺ or 200 mM Na⁺ to counterbalance the absence of magnesium.⁶¹ Additionally, the same laboratory reported that supplementation with NaCl or MgCl₂ can help stabilize DNA origami in strongly denaturing buffers containing urea and guanidinium chloride (GdmCl).⁶⁷ Hereby, the advantages of the DNA origami platform toward single molecule protein folding studies could be anticipated.

To achieve a stable environment for cell culture, nucleases in cell media can be rendered inactive by extensive heat inactivation (5 min at 75 °C) or the addition of 200 nM monomeric actin.²² Obviously, these are only relevant for in vitro assays and all changes made could have unintended side-effects on viability, binding or target-processes since not all cell-types might accept the proposed alterations in their environment.

Recently, the Mao laboratory has reported the use of ethylene-nediamine (EN) as a reversibly chargeable molecule to substitute commonly used metal ions in DNA folding buffers.⁶⁸ EN can overcome the negative repulsions in DNA when charged, but thanks to its pH-responsiveness, creates a pH-triggered system of self-assembly. Since DNaseI uses metal cation cofactors for activity, the DNA tile tetrahedron structures folded under EN conditions (metal-ion free) showed improved stability against this nuclease. However, cations are present in sufficient amounts to re-activate nucleases in biological media to counteract this advantage.

4. Conclusion and recommendations

The DNA-origami technology has provided engineers with a toolbox of unique properties and advantages. Through the programmable control of self-assembly, control of spacing, and reproducibility, DNA-origami nanoparticles have the potential to outperform other nanoparticles when it comes to execution of specific tasks. When it comes to cellular and in vivo targets, this specificity is what could make a difference in the future steps of nano-therapeutics and diagnostics. Previous reports covering in vivo applications of DNA origami structures were often shown prior to testing without clear indication that the nanostructures were entering living cells and preserving their integrity. Realisation of the stability challenges of DNA in biological environments catalysed a design wave of protection strategies.

Many methods were shown to protect against salt instabilities and currently DNA origami can be stored for months in pure water. Nuclease and cellular stability, however, turned out to be a much more challenging parameter to master, and needs to be carefully addressed in a cell-type and in vivo experiment dependent matter. Of course, for a translation toward in vivo applications, the pharmacokinetic parameters need to be addressed. Two studies provide insights into circulation half times, ranging from about one hour for commercially available polymer coatings⁶⁰ to 6 hours for precision-engineered liposomal encapsulation of DNA structures.²⁷ DNA intercalators are mainly known from cancer therapy and are grouped as anthracyclines.⁶⁹ They intercalate with DNA via π–π stacking interactions and subsequently produce damaging reactive oxygen species. Doxorubicin is a member of this family and non-covalently binds to GC/CG base pairs.⁷⁰ We note that although a significant number of reports using intercalating drug molecules reported to stabilize and protect single stranded DNA exists,⁷¹ no experimental proof of enhanced stability of DNA-origami with intercalating compounds has been published. Future research toward this subject is needed and potentially a combination strategy with one of the following methods might turn out to be superior in in vivo therapeutics.

To select the optimal protection method for a cellular or in vivo application, Table 1 can be consulted as a starting point. The shelf life of synthetic polymers outperforms that of proteins and could play an important factor when deciding on a stability methodology. We re-emphasize that many protection strategies as well as stability sensitivity are structure dependent, and therefore a chosen method should always be verified. Additionally, some methods change the final dimensions and charge of the particle, which could impact cellular response. Lastly, for specific targeting or delivery, often functionalities will be added to the structures. It is important that the selected protection method maintains the accessibility of these engineered active sites. Besides the functionalization of the DNA-origami particle, some protection methods could be modified to display further functional groups in addition to its’ role as a protective shell for the DNA origami. Of course, this requires in-house modification of otherwise commercially available solutions.

Stability of DNA nanostructures both in the intracellular and extracellular environment is crucial for their biological
functions. Parameters affecting the integrity of the DNA-origami that are present in the intended biological application should always be analysed, with a stable structure as the control. With the options of protection now plentiful, we highly recommend all future reports on cellular and in vivo effects of DNA-origami to present adequate stability analysis data and to choose an appropriate protection method.

Conflicts of interest

MMCB holds a patent on the protection of DNA-origami structures using the K1-PEG5k method (Bastings, Ponnuswamy, Shih, Nucleic acid nanostructures for in vivo agent delivery. PCT/US2014/064659, 2015).

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Notes and references


