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ARTICLE

Understanding the fate of DNA nanostructures inside the cell

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Structural DNA nanotechnology is poised to transform targeted therapeutic and theranostic delivery agents. Some of the most promising biomedical applications of DNA nanostructures include carriers for biosensing, imaging, and drug delivery. Additionally, the unique ability to precisely position inorganic and organic molecules on DNA-based substrates enables the spatially optimized high density interfacing of ligands with cell membrane receptors. To realize clinically viable biomedical products made from DNA nanostructures, it is necessary to fully understand the behavior of these systems inside and outside the cellular environment. To that end, cohesive and conclusive information on the physiological fate of DNA nanostructures at various time points - from the cell culture to the cell cytosol - is still lacking. In this highlight, we bring to attention efforts to understand DNA nanostructure behavior *in vitro* as well as some widespread disparities among studies on the subject. We also call for a discussion on the implementation of common standards and controls to address these disparities and consequently unify the scientific community's endeavours to build foundational knowledge on DNA nanostructure-cellular interaction.

Keywords: DNA, cells, hybridization, sensor, DNA origami, nanomedicine, drug delivery, endocytosis, drug delivery, FRET.

Introduction

Deoxyribonucleic acid (DNA) is generally appreciated as the molecule that encodes the genetic blueprint, however, the highly specific and non-covalent nature of DNA-DNA interactions through Watson-Crick base pairing, in combination with the predictable geometry of the DNA double helix, provides a system that is optimized for molecular self-assembly. Following the inception of structural DNA nanotechnology in the 1990's,[1-3] researchers have leveraged synthetic DNA to construct nanostructures with near limitless size and complexity through methods such as tiles[4-6] and origami.[7] Today, advancements in the field have revolutionized our ability to precisely engineer nanostructures from the bottom up with a vast library of chemical modifications and functionality.[8] This has generated significant interest within the biomedical community for use in applications such as targeted delivery of drugs, proteins, genes, nanoparticles, biological sensors and probes, as well as vaccine assembly and delivery, responsive diagnostic materials, and theranostics, particularly due to the ability to precisely position biomolecules and nanoparticles on the surface of DNA nanostructures.[9-13] In addition, the predictable structure and properties of DNA nanostructures provide a pathway to elucidate the complex nature of cellular uptake, the implications of which broadly span the biomedical community.

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To realize successful adoption of DNA nanostructures for the biomedical applications stated above, there are many factors that must be addressed. For example, we can list several factors which are suspected to play a role in the successful delivery and function of a payload in cells by DNA nanostructures:

- DNA nanostructure design (*size, shape, sequence, chemistry, position of payload, functional groups, dyes, etc.)*
- Linker/attachment chemistry for DNA-payload conjugation.
- Nanostructure stability in the cellular environment (*blood or serum, ionic conditions, presence of nucleases, etc.*)
- Desired uptake pathway and means of targeted uptake (*DNA nanostructure design, inclusion of receptor targeting species through designer receptor-ligand interactions, targeting aptamers, etc.*)
- Intracellular pathway, release of payload, and fate in the cell.
- Immunogenicity of components entering the cell.

Additionally, we must consider methods with which to observe/characterize nanostructures in the cellular environment and measure the outcome of cellular uptake. Living organisms and cells present challenging environments for detailed and systematic studies, but the precise nanomaterials synthesis enabled by DNA nanotechnology makes these challenges slightly more soluble.

Towards the goals outlined above, in the previous decade, the role of synthetic DNA nanostructures as potential vehicles for targeted diagnostic and drug delivery systems has been explored *in vitro* and *in vivo*.[14] Probing their stability in various biologically relevant environments, such as fetal bovine and human sera, blood, and cellular lysates, has shown that DNA nanostructures demonstrate a level of biocompatibility that

simple linear DNA strands do not possess, thereby suggesting that physical properties like size and shape of DNA nanostructure likely impart the biocompatibility in some manner.[15-20] Numerous studies have tested more than one kind of DNA nanostructure against different mammalian cell lines to understand their behavior along the putative endocytic trafficking pathway and have observed differing, and often even contradictory, results. Collectively, the field is still unable to offer fundamental knowledge about how to leverage or predictably control one of the most interesting properties of synthetic DNA nanostructures towards building therapeutic carriers – tunability. The versatility of DNA for constructing nanostructures with diverse shapes and sizes has resulted in a broad suite of potentially applicable biomedical tools, though such diversity has also made it challenging to compare the findings of individual investigations and deduce overarching rules that govern the interaction of DNA nanostructures with the cell. Moreover, the mammalian cell is inherently a "messy world" that has not been amenable to a deterministic and engineering ethos such as that found in the DNA nanotechnology community, and little attention has been given to consistency in the cellular environment between experiments which, among other factors, influences a cellular outcome. With the advent and application of DNA nanotechnology in biomedical applications, such a mindset might benefit the systematic studies needed to elucidate cellular uptake of DNA nanomaterials.

Notwithstanding the gaps in knowledge, we now know that DNA nanostructures have some interesting properties in cellular environments. A wide range of DNA nanostructures, varying in size, complexity, and surface functionalization, have been demonstrated to undergo receptor-mediated endocytosis;[14, 15, 21-23] **Figure 1** shows the various stages of cellular uptake of a DNA origami rod, decorated with gold

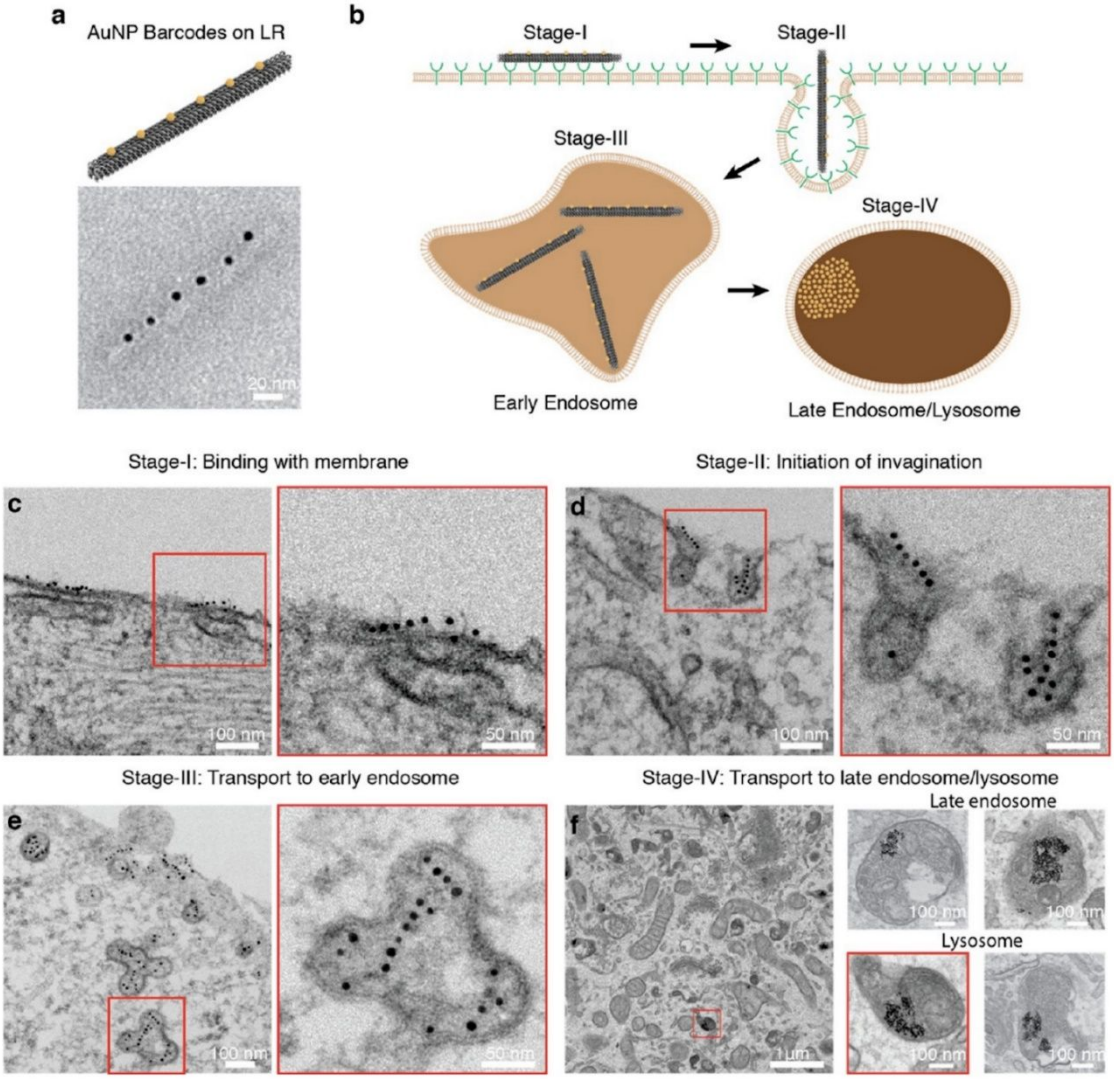

Figure 1 – Visualization of receptor-mediated DNA origami rod uptake by H1299 cells via transmission electron microscopy. (a) Schematic depiction and corresponding TEM image of a DNA origami rod discretely labelled with gold nanoparticles (AuNPs). (b) Depiction of four stages (Stages I, II, III, and IV) by which cellular uptake of the DNA origami nanorod was suspected to occur. (c-f) TEM visualization of DNA nanostructures undergoing Stages I through IV, respectively. Reproduced with permission from ref. [15]. Copyright 2018 American Chemical Society.

nanoparticles, into H1299 nonsmall lung cancer cells by scavenger receptor-mediated endocytosis.[15] Transmission electron microscopy shows the various stages of internalization of gold-labeled DNA rods, from binding to the cell membrane to the final lysosomal degradation. In particular, modifications with cell-specific ligands on the surface of DNA nanostructures enable recognition by cellular membrane receptors and induce nanostructure uptake.[24-34] The unique addressability of DNA nanostructures further enables high density and spatially precise immobilization of epitopes on their surface, consequently facilitating interaction with membrane receptors for enhanced targeting capabilities and vaccine development.[35-38] DNA nanostructures have also been shown to be preferentially internalized by tumors *in vivo* compared to other organs, thereby functioning as useful delivery agents.[32]

In this highlight we take a cautious but optimistic look at the recent progress in identifying the cellular fate of DNA nanostructures. We highlight exemplary results and challenges in a sequence illustrative of the putative voyage of a viable molecular carrier for *in vitro* and *in vivo* biomedical applications. Along this path, the DNA nanostructure-based carrier would be expected to demonstrate stability against serum, cell culture media, nucleases in and outside the cell, endosomal vesicles, lysosomes, and the cytosolic environment. For successful clinical products additional barriers could potentially pose a threat to DNA nanostructure stability, namely, the stomach, blood serum, multicellular tissues, and individual cells. We endeavor to highlight the key disparities and questions that require careful attention within the field in hopes of accelerating progress towards a cohesive and conclusive understanding of the interactions between DNA nanostructures, cells, and the cellular environment, as such understanding is foundational and needed before future clinical applications of DNA nanostructures are realized. Lastly, we suggest specific experimental standards and studies to help leverage the deterministic nature of DNA nanoengineering in the cellular environment, facilitating a systematic approach to research in this field as well as fruitful translation of information from DNA nanoengineering to cell biology.

Lessons learned and disparities in cellular behavior

Stability in serum. The long-term structural integrity of DNA nanostructures within the cellular environment is essential for structures to be able to reach their intended targets and function properly. Compact DNA nanostructures have been widely demonstrated to possess enhanced stability against degradation by nucleases in comparison to linear single stranded (ss) and double stranded (ds) DNA,[16] though large discrepancies have been reported for the stability of DNA nanostructures in 10% fetal bovine serum (FBS), a common ingredient of cell growth media and also a blood surrogate for *in vitro* studies of cells, that is often used to assess the resistance of nanostructures to nuclease degradation. In the literature, stability of DNA nanostructures in FBS can vary from 1 hour[22] to greater than 48 hours[23] depending on their physical properties and chemical modifications, though stability also varies significantly between studies for equivalent structures. For example, the stability of an unmodified DNA tetrahedron in serum is reported to be between 4 to 25 hours.[23, 39-42] Such variability might be attributed to the age/quality of FBS; the nuclease activity of the serum is strongly correlated with shelf life and is an often unaccounted factor in studies of DNA nanostructure uptake.[17]

While the serum stability of unmodified DNA nanostructures varies significantly between studies, chemical modifications, such as enzymatic ligation of nicks within DNA nanostructures and modified sugar backbones, have been consistently demonstrated to increase protection against nuclease degradation.[23, 43-45] Kim *et al.* evaluated a library of nucleic acid nanostructures and found that nanostructures with chemically-modified sugar backbones (enantiomeric L-DNA, 2'-fluoro-RNA, and 2'-O-methyl-RNA) had high resistance to nuclease degradation and significantly increased cellular uptake in HeLa cells relative to the equivalent DNA nanostructures, highlighted in **Figure 2**.[22] Stability in serum was observed to increase from 1 hour for unmodified DNA nanostructures to greater than 24 hours for structures with modified sugar backbones. Raniolo *et al.* compared the serum stability of unmodified and ligated forms of a DNA tetrahedron, octahedron, and chain-mail-like rod structure and observed that ligated DNA nanostructures maintained their structure in serum for nearly twice as long as unmodified structures.[23] The effects of size and shape on nanostructure stability in serum are less apparent due to variability between studies and the coarse-grained nature of stability experiments.

Uptake mechanism and pathway through the cell. Entry of naked ss and ds oligonucleotides into cells does not readily occur without transfection agents. Therefore, endocytosis of DNA nanostructures into cells has been considered a paradigm-shifting observation in advancing their potential applications in biomedicine.[46, 47] Recent studies, however, have produced contradictory results on uptake, endocytic pathways, and the intracellular fate of DNA nanostructures.[14, 21] It is still uncertain whether bare DNA nanostructures (lacking cell-specific ligands) can be efficiently internalized by any mechanism of endocytosis. DNA structures such as the tetrahedron, small wireframe cube and prism, larger DNA origami helix bundles and bricks have been reported to internalize *via* clathrin and/or caveolin dependent endocytosis.[15, 22-24, 40-42, 48, 49] In most cases the mechanism of tracking the movement of the DNA structures into and through the cell is suspected as a major source contributing to the uncertainty. Visualization of the uptake and progression of DNA structures across the cell membrane and into the cell is generally through a fluorescent dye attached to the structures, commonly cyanine 3 (Cy3) or cyanine 5 (Cy5), a method that is compatible with live cell imaging.[15, 22, 40-42, 49, 50] Another method employs biotin-streptavidin binding to label biotinylated nanostructures with fluorescently-tagged streptavidin after uptake experiments have been halted by cell fixation. Following incubation of biotinylated-DNA structures

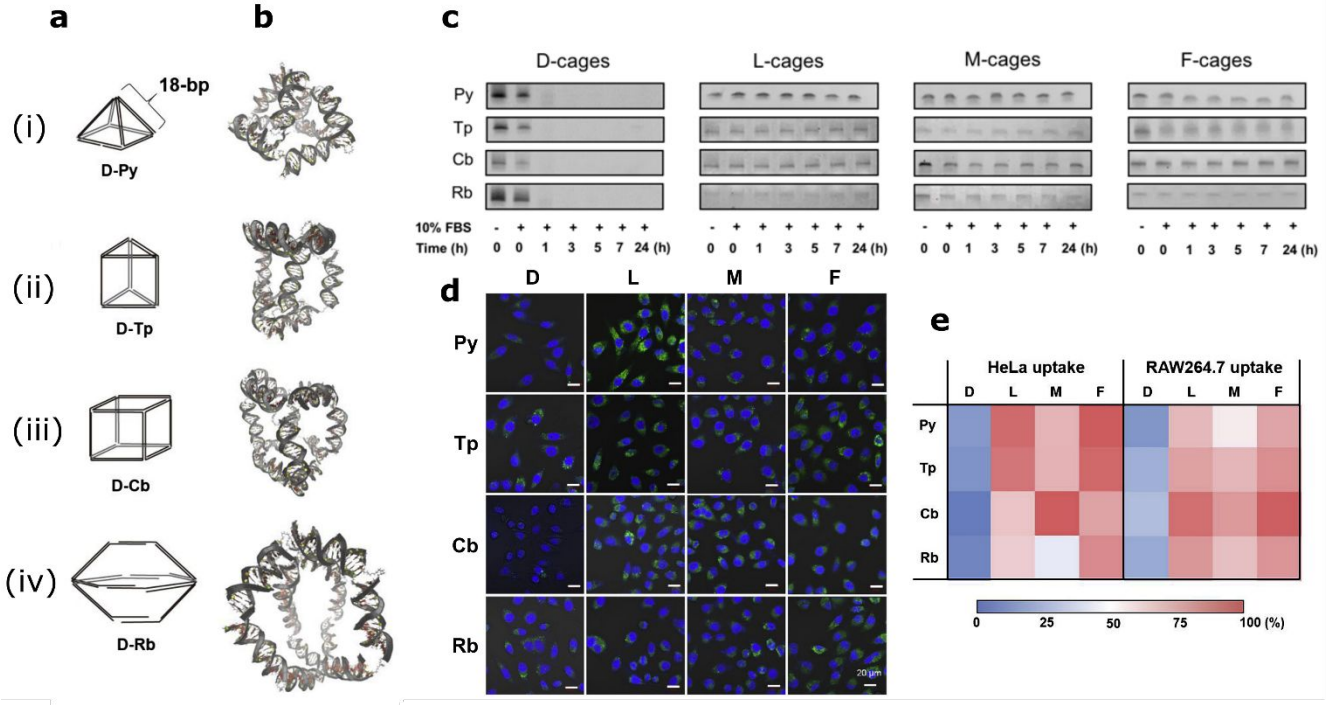

Figure 2 – Systematic studies of serum stability and cellular uptake of a library of nucleic acid nanostructures by HeLa cells. (a) Strand diagrams and (b) predicted helical structures of a DNA pyramid "Py" (i), triangular prism "Tp" (ii), cube "Cb"(iii), and rugby-ball shaped structure "Rb" (iv). (c) Nucleic acid nanostructure stability in 10% FBS, visualized by denaturing polyacrylamide gel electrophoresis (PAGE). (d) Confocal microscopy images of nucleic acid structures labelled with fluorescein. The images are arranged in a grid with the design shape (Py, Tp, Cb, and Rb) indicated on the left side, and the backbone type (DNA "D", enantiomeric L-DNA "L", 2'-O-methyl-RNA "M", and 2'-fluoro-RNA "F") indicated along the top. (e) Heat map of HeLa cell uptake (left) and RAW264.7 macrophage uptake (right) represented in grids spatially corresponding to the images in (d). Reprinted from ref. [22] with permission from Elsevier.

with cells, they are rinsed to remove unbound DNA structures and serum, fixed with paraformaldehyde, and permeabilized for internalization of streptavidin-labeled fluorescein-isothiocyanate (streptavidin-FITC) for fluorescent reporting of internalized DNA nanostructures. Both approaches to characterize DNA nanostructure uptake by cells, however, leave room for unaccounted cell behaviours to influence the outcome. Phosphate analogs of Cy3 and Cy5 have been shown to be directly endocytosed by HepG2 (human hepatocellular carcinoma) and HeLa (human cervical carcinoma) cells.[39] This is an interesting observation as it sheds a cautionary light on the use of cyanine dyes in general as a means to track DNA nanostructures inside cells; a byproduct of degradation of nanostructures labeled with these dyes would be their phosphate analogs which could be readily taken up by cells leading to a false positive signal of DNA nanostructure uptake. In the case of post-fixation labelling with streptavidin-FITC, both fixation and permeabilization can introduce spatial and compositional changes to cells and may affect the distribution of nanostructures within cells.[51, 52] Overall, detailed analytical techniques and parametric microscopic studies with adequate controls need to be adopted for a clearer understanding of the endocytic behavior of DNA nanostructures.

Cell specific activity of DNA nanostructures has also been shown with contradictory results with respect to endocytosis – with examples of no uptake as well as enhanced uptake in comparison to linear DNA oligonucleotides. For instance, in the case of small wireframe DNA nanostructures, HeLa cells in one report show little to no uptake when controlling for the uptake of free dyes,[39] whereas other groups have observed favorable internalization.[22, 40, 42, 49] In an effort to highlight these issues, a representative cross-section of several studies on DNA nanostructure uptake in COS cells, COS cells transfected with the oxidized low-density lipoprotein receptor LOX-1 (COS-LOX-1), and HeLa cells are summarized in **Table 1**. While reports of uptake are inconsistent, internalization by receptor-mediated endocytosis is commonly reported among studies which have observed favorable uptake.

Standardized experimental methods and controls

The complexity of the cellular environment, coupled with the vast design library of DNA nanostructures, introduces many uncertainties that can affect the outcome of otherwise well-designed experiments, producing potentially confusing results that hinder progress towards a unified understanding of the topic. We suggest the development of standardized controls and methods to ensure that the factors affecting uptake be isolated from experimental variations. The following sections briefly outline overarching ideas of unifying *in vitro* research undertaken on stability and uptake of DNA nanostructures.

Standardized DNA nanostructure for control studies. Novel DNA designs and structures are often employed in cellular uptake studies. Many unique nanostructures have been developed and

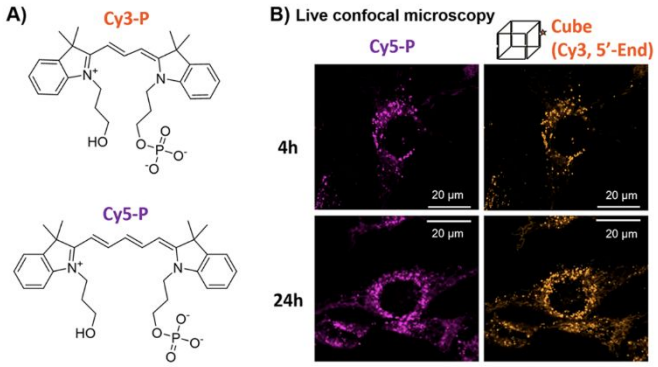

Figure 3 – Cellular uptake and colocalization of phosphorylated dyes with DNA nanostructures. (a) Structural formulas of phosphorylated cyanine 3 (Cy3-P, top) and cyanine 5 (Cy5-P, bottom), suspected free dye products of enzymatic degradation of dye-labelled DNA nanostructures. (b) Live, two-color confocal microscopy images of HeLa cells co-incubated with Cy5-P and a Cy3-labelled DNA cube structure. Signal from Cy5-P (left) and the DNA cube (right) strongly colocalize, suggesting similar or identical uptake mechanisms and efficiency. Reproduced with permission from ref. [39]. Copyright 2019 American Chemical Society.

tested *in vitro* and *in vivo*, though it is difficult to compare the results of individual studies due to variations in experimental conditions beyond nanostructure design alone. The DNA tetrahedron has been employed as a control structure in several studies, though often the design is intended to control only for shape and size, foregoing consistency in sequence, chemistry, or functionalization, factors which are suspected to affect stability and uptake.[15, 22, 23, 39, 53] To ensure that observed cellular uptake results from the properties of the nanostructure rather than variations in the experimental procedures, control experiments with a set of standardized DNA nanostructures would be facilitative in collectively reviewing different studies. This standard set of DNA nanostructure designs could account for consistent size, sequence, functionalization, and procedures for synthesis and handling and thus better serve to align the results of future experiments on DNA nanostructures within cellular environments. Taking into account the available suite of DNA nanostructures, the tetrahedron,[54] a two-dimensional DNA origami structure (triangle,[7] for instance), a three-dimensional DNA origami structure (DNA nanotube), and a wireframe DNA polyhedron (DNA icosahedron) provide a sufficient diversity in size and shape variability to approach this issue.

Reporter systems and uptake of free dyes. As reported recently,[39] nuclease digestion of dye-labeled DNA nanostructures may result in the release of free dyes into solution, which could potentially be taken up into cells and falsely signal nanostructure uptake. Lacroix *et al.* observed that certain fluorescent dye-phosphate analogs (not conjugated to DNA) displayed similar uptake efficacy by mammalian cells as dye-conjugated DNA nanostructures (**Figure 3**), suggesting that DNA nanostructures degrade due to extracellular nucleases and release dye-phosphate molecules which undergo endocytosis.[39] Stabilizing the dye-DNA nanostructure by increasing resistance to nuclease degradation with hexaethylene glycol protection groups on the 3' and 5' ends of oligomers further delayed the fluorescence signal inside the cell, suggesting that endocytosis of the bare DNA nanostructures (~10 nm in size) without any transfection agent does not readily occur. Moreover, the simultaneous introduction of Cy3-phosphate and Cy5-phosphate molecules to mammalian cells resulted in strong colocalization within the cells and displayed similar Förster resonance energy transfer (FRET) activity as when the dyes are conjugated in close proximity on the DNA nanostructures.[39] Several studies have employed FRET to differentiate free dyes from dyes immobilized on nanostructures, relying on the finely tuned spatial proximity of fluorophores on nanostructures to ensure their uptake and structural integrity within cells. While this was previously assumed to strongly reduce the possibility of signal falsely originating from free dyes, the results of Lacroix *et al.* demonstrate that free Cy3 and Cy5 dyes taken up into HeLa cells produced strong FRET signalling in live cell images despite a lack of fixed spatial proximity afforded by DNA nanostructures. It is worth noting that fixation was found to greatly reduce the concentration of intracellular free dyes relative to ssDNA-labeled dyes, potentially contributing to the discrepancies reported between studies.[39] Few studies have controlled for the uptake of free fluorophores into cells, and reports on the endocytosis of dye-conjugated linear oligos are also uncertain.[55, 56] Whether observed FRET is solely attributable to free dye uptake remains unclear. Until free dye uptake is better understood, we recommend the inclusion of experiments to control for this possibility, regardless of the reporter system employed. Additionally, dyes which have been demonstrated to readily undergo uptake, such as phosphorylated cyanine and rhodamine dyes, may be better substituted by other dye chemistries.[39, 57] This issue warrants focused studies in its own right to determine optimized dyes.

Activity of hydrolytic enzymes in solution. The stability of DNA nanostructures will inherently vary with the enzymatic activity of serum, and type of serum, used in stability experiments, but such variability exacerbates the challenge of differentiating the effects of size and design on resistance to nuclease degradation. Varying activities of nucleases in serum might also play a role in the discrepant accounts of nanostructure uptake in cells, which has been observed occurring at time scales closely correlated to nanostructure stability in serum.[15, 22-24, 40, 42, 48, 58] For example, uptake studies using identical design and preparation of a ligated DNA octahedron for uptake in COS and LOX-1-transfected COS cells reported serum stabilities of 5 and 27 hours with no uptake and slight uptake in non-transfected COS cells, respectively.[24, 48] As a point of reference between studies, stability reported as relative to a standardized DNA nanostructure in addition to total lifetimes would provide some indication of serum activity and whether it contributes to the varied behaviors reported in similar systems. In any case, the use of freshly prepared serum is a best practice that can help to minimize variations in the extracellular environments between and within studies.[17, 20]

Culturing, transfection, and permeabilization. Fibroblastic and epithelial-like cells, commonly used in uptake studies, are anchorage-dependent cells that require adhesion to a substrate for culturing. Trypsin, a proteolytic enzyme often used to release cells from cell culture plates, can potentially lyse cells (releasing nucleases) with excessive or prolonged exposure and has been shown to induce changes in the expression of proteins in cell membranes.[59] Such changes are expected to affect cellular uptake, though studies of nanostructure uptake rarely report the methods and conditions for plating and release. While many studies employ standardized methods for this process and chose instead to reference the work from which the methods were adopted, it is nonetheless important to report the conditions under which such plating and release are performed so that consistency can be assured externally.

Likewise, transfection and permeabilization are common techniques used in cell studies that are often underreported in nanostructure uptake studies. To observe the effects of a specific receptor on cellular uptake, cells are often transfected to express additional receptors on the cell membrane. For example, COS cell lines have been selectively transfected with LOX-1 for comparison to non-transfected COS cells in DNA nanostructure uptake.[23, 24, 48] While transfection is ubiquitous in cancer cell uptake studies as a means of expressing specific receptors that occur on cancer cell membranes, for DNA nanostructures, the process of transfecting cells may introduce uncertainties that offset the deterministic nature of DNA nanostructures in highly systematic studies of cellular uptake. In addition, studies of transfected cells often require repeated plating, permeabilization, and release prior to incubation with nanostructures, increasing the potential for variation in nanostructure uptake between identical cell lines. Nonetheless, the benefits of transfected cells in receptor-targeted studies outweigh the risks introduced by the process of transfection, and we only suggest that special care be taken in such studies to ensure that such benefits are retained through systematic reporting and use of common procedures.

Chemical fixation. Prior to characterization, chemical fixation is sometimes performed to halt cellular decay and preserve the structure of cells for imaging. Cross-linking fixatives, such as formaldehyde, and precipitating fixatives, such as methanol, are commonly employed under the assumption that fixation does not dramatically alter the distribution of intracellular fluorescent species despite much work suggesting the contrary.[39, 51, 52] Lacroix *et al.* compared confocal images of live cells to cells fixed with formaldehyde or methanol and found that the distribution of fluorescent species was altered by the process of fixation.[39] Fixation by formaldehyde was observed to remove free dyes from the mitochondria while retaining signal in endosomes/lysosomes; FRET signal from free dyes was also greatly reduced by fixation. In the case of methanol, fixation removed all fluorescent signal for free dyes but only resulted in precipitation of dye-conjugated ssDNA onto the cell membrane.[39] Beyond the results of Lacroix *et al.*, little work has been done to quantify the effects of fixation on intracellular fluorescent species, thus fixation stands as another potential contribution to discrepant results in the literature.

Conclusions

Synthetic DNA nanotechnology holds great promise in applications in biomedicine. The advancement from experiments in a test tube to clinical trials, however, requires a deeper knowledge about the fate of DNA nanostructures inside the cellular environment. To summarize, the behavior of DNA nanostructures during cell internalization and endocytosis remains elusive and the problem is compounded by equivocal reports on the stability of these structures in extracellular environments. It is difficult to galvanize the scientific community to unify around a common set of controls, cell types, and standards of biological samples in order to pursue the collective goal of answering these fundamental questions. The appropriate characterization tools to probe their behavior in a compelling manner are also lacking. A careful look at the state of metrology in the field is, therefore, also necessary. The most commonly used tools for characterizing and purifying DNA nanostructures still do not fully separate or confirm 100% well-formed structures from those that are partially formed.[60] This is a major issue since the U.S Food and Drug Administration (FDA) approval process typically requires completely defined active structures. There is a need for metrology that helps characterize DNA structures inside the cells, such as recovering cell lysates with intact DNA structures to determine their presence in different cellular fractions, electron tomography to track DNA structure intracellularly, and use of multi-step FRET to track DNA structure stability inside cells using dyes that have known uptake properties. Additionally, more studies are needed to provide a robust data set for analysis *via* machine learning.

Problems in DNA nanotech metrology and formulation are emblematic as other nanoparticle studies in healthcare are likely to struggle with the same issues in biomedical applications. The Nanotechnology Characterization Lab at the National Cancer Institute (NCL-NCI) is emerging as a valuable resource to leverage strong collaboration between various governmental branches such as the FDA and the NCI as well as academic laboratories for performing “preclinical efficacy and toxicity studies on nanoparticles.” The NCL-NCI focuses on assisting the transition of biomedical nanosystems from the lab to clinical phases by guiding in the development of strong and rigorous characterization tools, optimization of delivery formulas, and method development. DNA nanotechnology needs a vital resource such as the NCL-NCI to make bold advances in a unified approach to interfacing with the human body, towards which the NCL-NCI has already engaged efforts.[61]

Conflicts of interest

There are no conflicts to declare.

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Table 1 – Representative examples of reported DNA nanostructure uptake in COS, COS-LOX-1, and HeLa cells in the literature.

Cell line	DNA Structure	Size (kD)	(nm)	Modifications	Reporter (dye)	Serum Stability	Uptake	Mechanism	Destination	Ref.	Year	Notes
COS	Tetrahedron	82	7		Cy3	n/a	++	Ca	n/a	39	2014	
		98	8	Biotin-labeled	Streptavidin-FITC	25 hr	--	No uptake	n/a	23	2019	
				Biotin-labeled, ligated	Streptavidin-FITC	48 hr	--	No uptake	n/a	23	2019	Enzymatic ligation
	Chain-mail rod	180	18	Biotin-labeled	Streptavidin-FITC	17 hr	--	No uptake	n/a	23	2019	
				Biotin-labeled, ligated	Streptavidin-FITC	66 hr	--	No uptake	n/a	23	2019	Click-chemistry ligation
	Octahedron	180	18	Biotin & Folate labeled	Streptavidin-FITC	n/a	--	No uptake	n/a	24	2018	
				Biotin-labeled	Streptavidin-FITC	15 hr	--	No uptake	n/a	23	2019	
				Biotin-labeled, ligated	Streptavidin-FITC	5, 27 hr	- , --	n/a, No uptake	n/a	48,23	2016, 2019	Enzymatic ligation
	Rectangular origami	3300	80	Biotin-labeled	Streptavidin-FITC	24 hr	--	No uptake	n/a	23	2019	
COS-LOX-1	Tetrahedron	98	8	Biotin-labeled	Streptavidin-FITC	25 hr	++	SR	Lysosome	23	2019	
				Biotin-labeled, ligated	Streptavidin-FITC	48 hr	++	SR	Lysosome	23	2019	Enzymatic ligation
	Chain-mail rod	180	18	Biotin-labeled	Streptavidin-FITC	17 hr	++	SR	Lysosome	23	2019	
				Biotin-labeled, ligated	Streptavidin-FITC	66 hr	++	SR	Lysosome	23	2019	Click-chemistry ligation
	Octahedron	180	18	Biotin & Folate labeled	Streptavidin-FITC	n/a	++	SR	Cytoplasm	24	2018	
				Biotin-labeled	Streptavidin-FITC	15 hr	++	SR	Lysosome	23	2019	
				Biotin-labeled, ligated	Streptavidin-FITC	5, 27 hr	++	SR	Lysosome	48,23	2016, 2019	Enzymatic ligation
	Rectangular origami	3300	80	Biotin-labeled	Streptavidin-FITC	24 hr	++	SR	Lysosome	23	2019	
HeLa	Tetrahedron	82	7		Cy3, Cy5, FRET	8 hr	--	No uptake	n/a	39	2019	
					Cy3	n/a	++	Ca	Lysosome	40	2014	
					Cy3, Cy5, FRET	n/a	++	n/a	Cytoplasm	49	2012	1-arm single-stranded
					Cy5, TAMRA	4 hr	+	En	n/a	42	2014	
				AS1411 aptamers	Cy5, TAMRA	4 hr	++	R, En	n/a	42	2014	
				Non-targeting aptamers	Cy5, TAMRA	4 hr	+	En	n/a	42	2014	
				Nucleus-targeting peptides	Alexa488	n/a	++	n/a	Nucleus	40	2014	
		88	7	Lipofectamine-treated	Cy5	>6 hr	++	n/a	n/a	41	2011	20nt ssDNA Antisense
	Pyramid	94	10		Cy5.5, Fluoroscein	1 hr	-	Cl, Ca, MPn, SR	n/a	22	2019	HEG spacers
				2'-fluoro-RNA	Cy5.5, Fluoroscein	> 24 hr	+	Cl, Ca, MPn, SR	n/a	22	2019	HEG spacers
				2'-O-methyl-RNA	Cy5.5, Fluoroscein	> 24 hr	++	Cl, Ca, MPn, SR	n/a	22	2019	HEG spacers
				Enantiomeric L-DNA	Cy5.5, Fluoroscein	> 24 hr	++	Cl, Ca, MPn, SR	n/a	22	2019	HEG spacers
	Triangular prism	105	12		Cy5.5, Fluoroscein	1 hr	-	Cl, Ca, SR	n/a	22	2019	HEG spacers
				2'-fluoro-RNA	Cy5.5, Fluoroscein	> 24 hr	+	Ca, MPn, SR-iCl	n/a	22	2019	HEG spacers
				2'-O-methyl-RNA	Cy5.5, Fluoroscein	> 24 hr	++	Ca, SR-iCl	n/a	22	2019	HEG spacers
				Enantiomeric L-DNA	Cy5.5, Fluoroscein	> 24 hr	++	Ca, MPn, SR-iCl	n/a	22	2019	HEG spacers
	Cube	125	12		Cy3, Cy5, FRET	4 hr	--	No uptake	n/a	39	2019	8 of 12 sides ssDNA
		140	12		Cy5.5, Fluoroscein	1 hr	-	Ca, SR-iCl	n/a	22	2019	HEG spacers
				2'-fluoro-RNA	Cy5.5, Fluoroscein	> 24 hr	++	Ca, SR-iCl	n/a	22	2019	HEG spacers
				2'-O-methyl-RNA	Cy5.5, Fluoroscein	> 24 hr	++	Ca, SR-iCl	n/a	22	2019	HEG spacers
				Enantiomeric L-DNA	Cy5.5, Fluoroscein	> 24 hr	+	Ca, MPn, SR-iCl	n/a	22	2019	HEG spacers
	Rugby-ball	140	18		Cy5.5, Fluoroscein	1 hr	-	Cl, Ca, SR	n/a	22	2019	HEG spacers
				2'-fluoro-RNA	Cy5.5, Fluoroscein	> 24 hr	+	Ca, MPn, SR-iCl	n/a	22	2019	HEG spacers
				2'-O-methyl-RNA	Cy5.5, Fluoroscein	> 24 hr	++	Ca, SR-iCl	n/a	22	2019	HEG spacers
				Enantiomeric L-DNA	Cy5.5, Fluoroscein	> 24 hr	+	Ca, MPn, SR-iCl	n/a	22	2019	HEG spacers
	Octahedron	180	18	Biotin & Folate labeled	Streptavidin-FITC	n/a	+	R	Lysosome	24	2018	
	Small tetrahedron origami	250	11		Cy5	4-8 hr	+	Ca, SR	n/a	42	2018	
	Large rod origami	4500	127x8		Cy5	8-16 hr	+	Ca, SR	n/a	42	2018	

Cl--Clathrin-dependent endocytosis, Ca--Caveolin-dependent endocytosis, mPn--Micropinocytosis, MPn--Macropinocytosis, R--Receptor, SR--Scavenger receptor, SR-Cl--Clathrin-dependent Scavenger receptor, SR-iCl--Clathrin-independent Scavenger receptor, En--Endocytosis (nonspecific). Modifications to induce uptake. Modifications to increase stability.