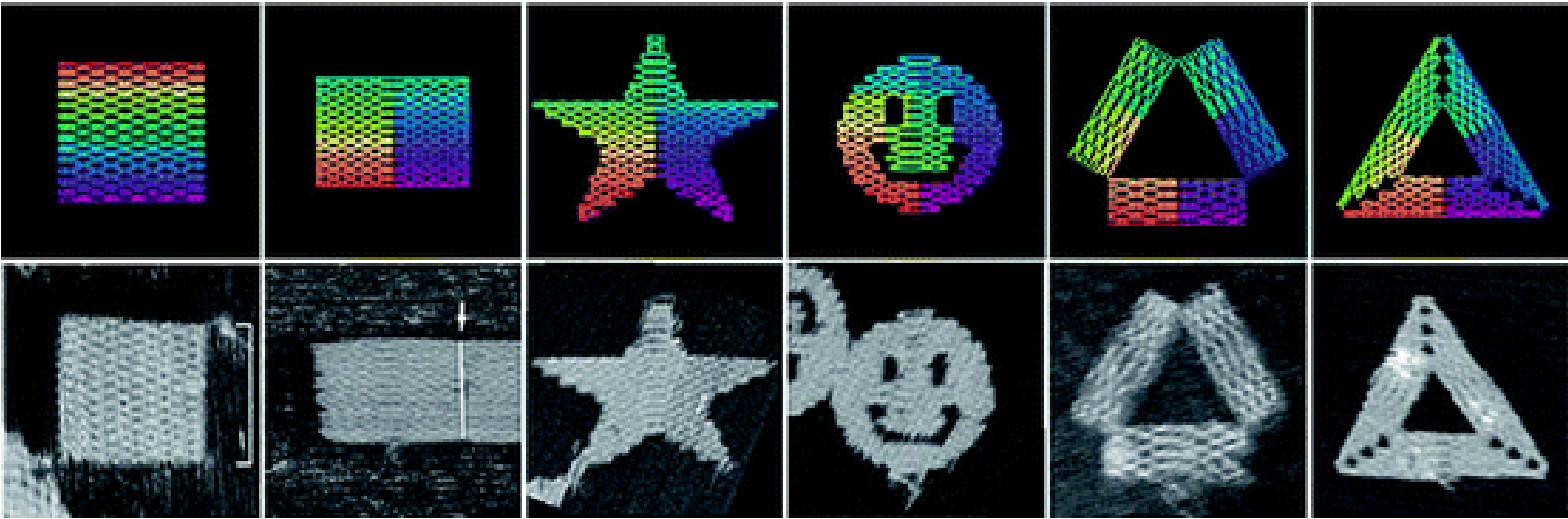
Elena De Cecco

01.12.2020

DNA nanodevices: applications and challenges

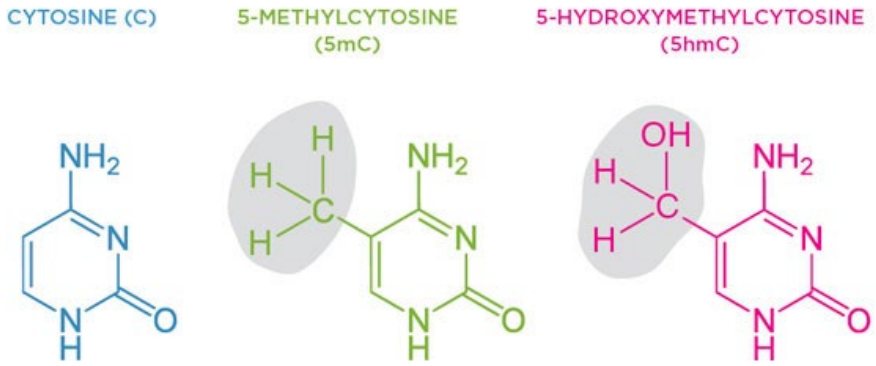


Why DNA?

Custom DNA sequences
available by chemical synthesis

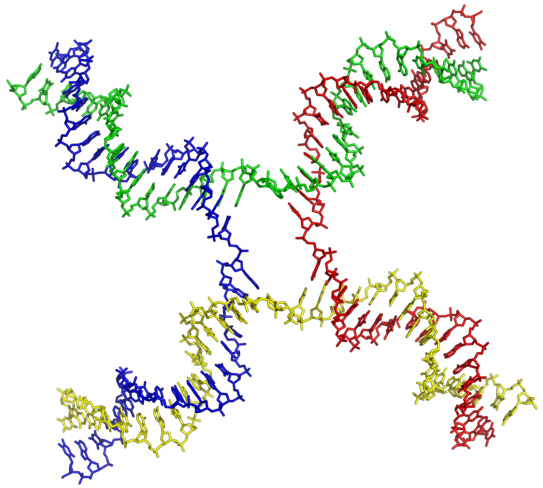
ssDNA is flexible, whereas
dsDNA are fairly rigid

Wide range of non-canonical,
modified nucleotides



Triplexes expand
chemical diversity

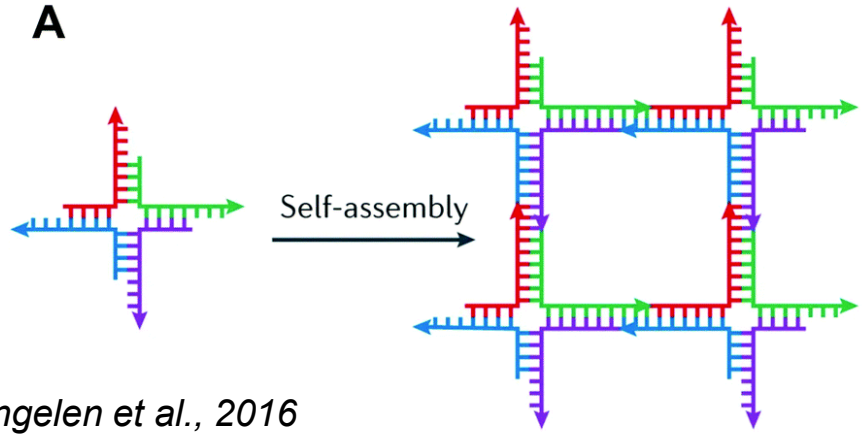
How do you fold DNA?



Holliday junctions are branched nuclei acid structures composed of four different ssDNA molecules

They play key roles in genetic recombination and double-strand break repair processes

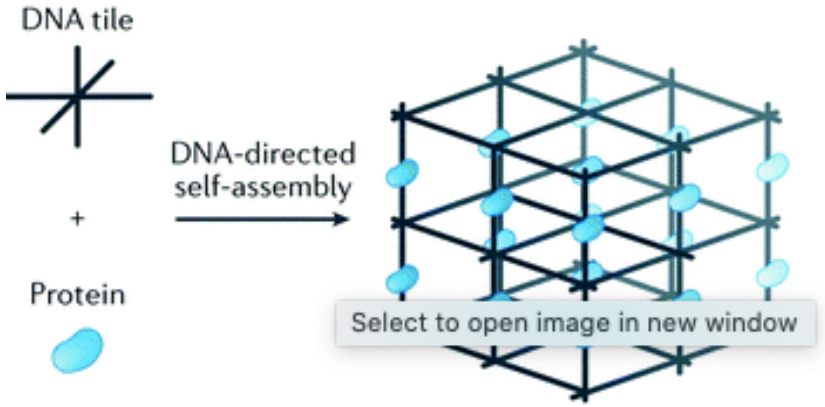
In natural Holliday junctions, DNA strands can slide through the junction. However, it is possible to use synthetic sequences with asymmetric base pairing that lock the strands in specific positions



Engelen et al., 2016

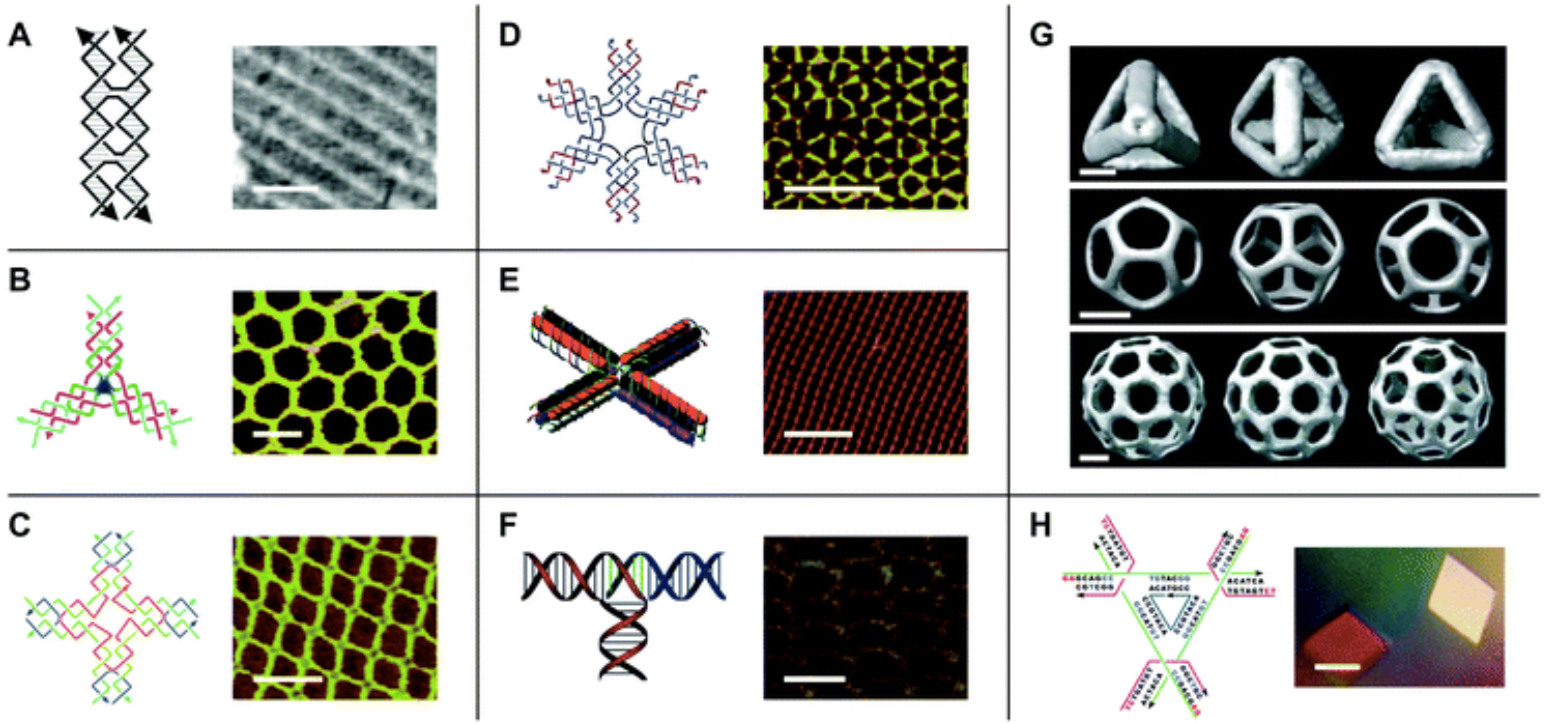
The immobile junctions are then joined through sticky ends

DNA tiles



When the structure involves at least two crossovers between the same strands, they are called tiles

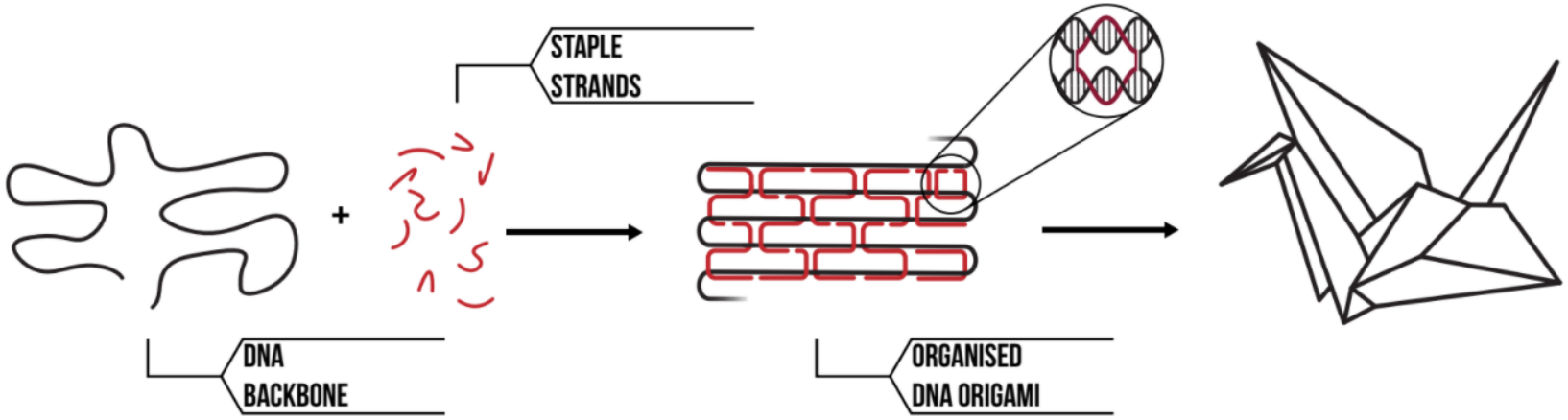
Tiles assembly allows the construction of 2D and 3D objects



Hill et al., 2020

DNA origami

Short oligonucleotides (**staples**) complementary to specific and non adjacent portions of the scaffold strand



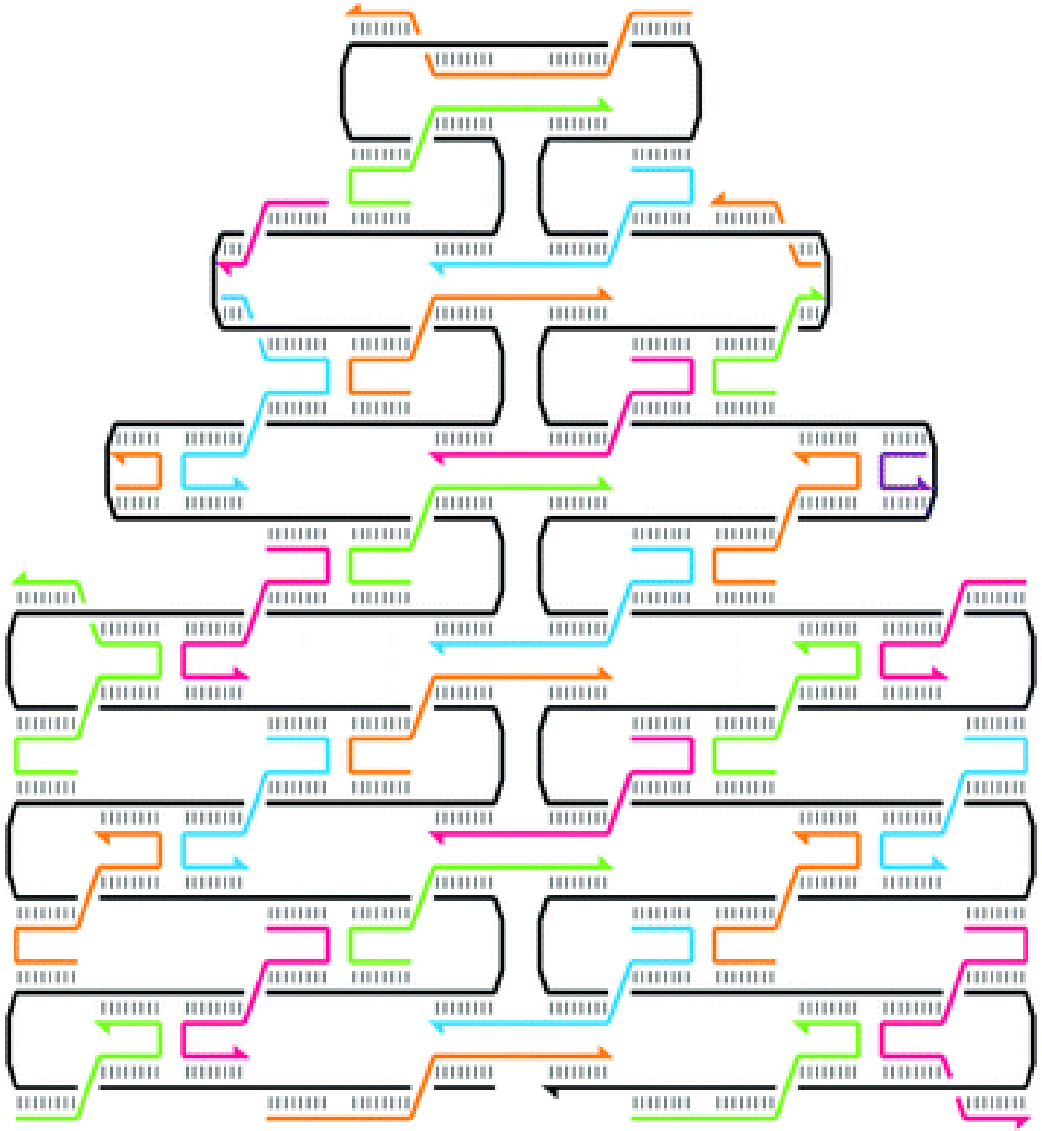
One ssDNA strand a few thousand bases long (**scaffold**)

The desired shape is achieved by virtually folding the dna scaffold and then using the staple strands to fix the strands in the desired conformations.
The scaffold is usually a phage genome, while the staples are synthesized chemically

The same approach can be applied to create more complex and three-dimensional structures

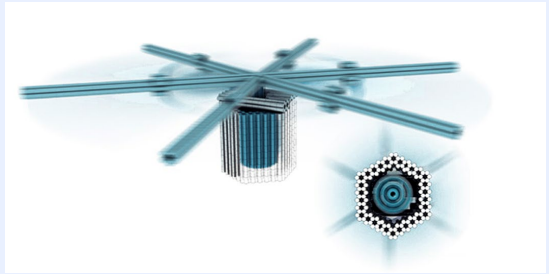
caDNAno and similar softwares are used to design the staple strands in the correct way

cadnano

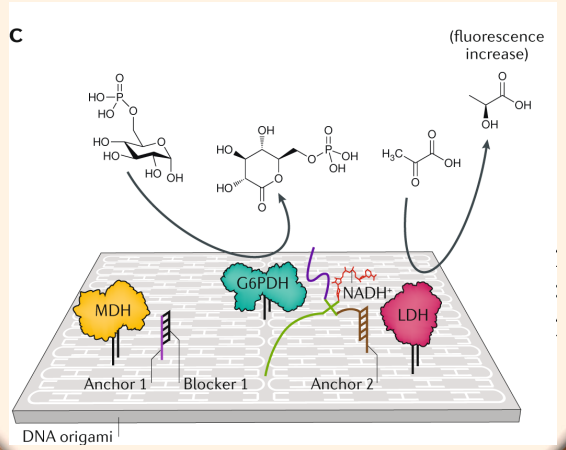


What can DNA origami do?

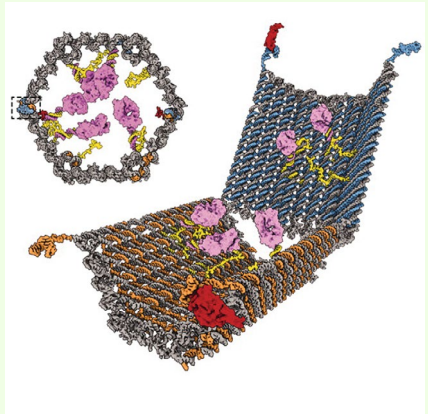
Robotics



Enzyme networks



Drug delivery



ACS NANO

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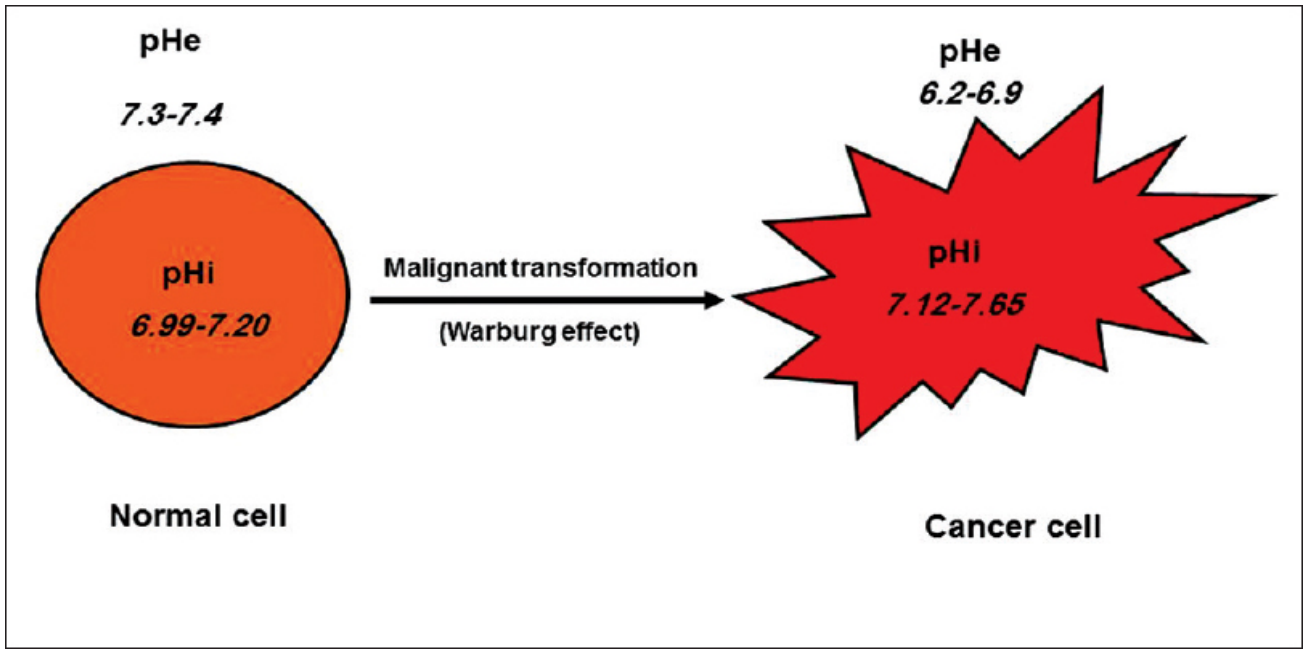
ARTICLE

Reconfigurable DNA Origami Nanocapsule for pH-Controlled Encapsulation and Display of Cargo

Heini Ijäs,[†,‡] Iiris Hakaste,[†] Boxuan Shen,[†] Mauri A. Kostiainen,[†,§] and Veikko Linko*[,†,§]

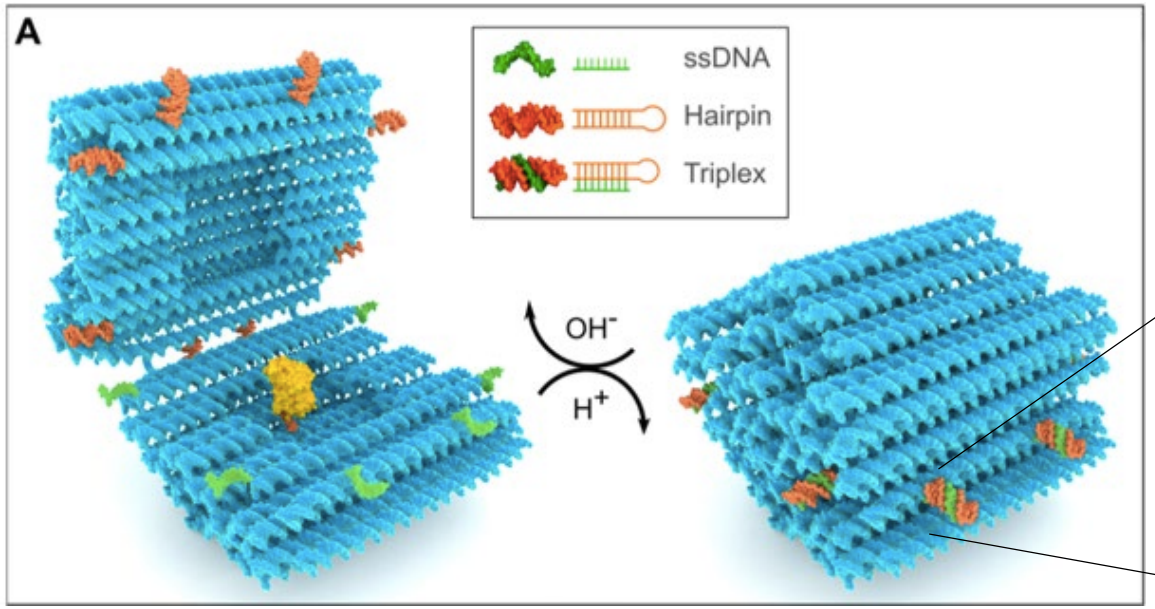
pH change of the environment is an intriguing trigger for the cargo release of drug-delivery systems



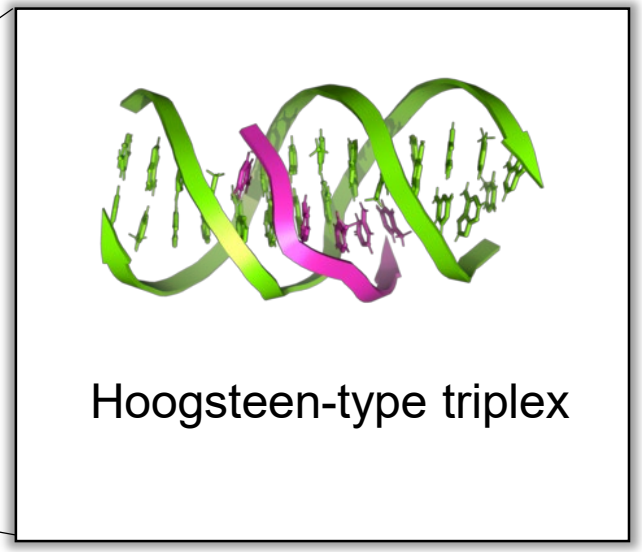
Normal cells have distinct pH values and any change might be linked to abnormal behaviour, i.e. presence of cancer cells

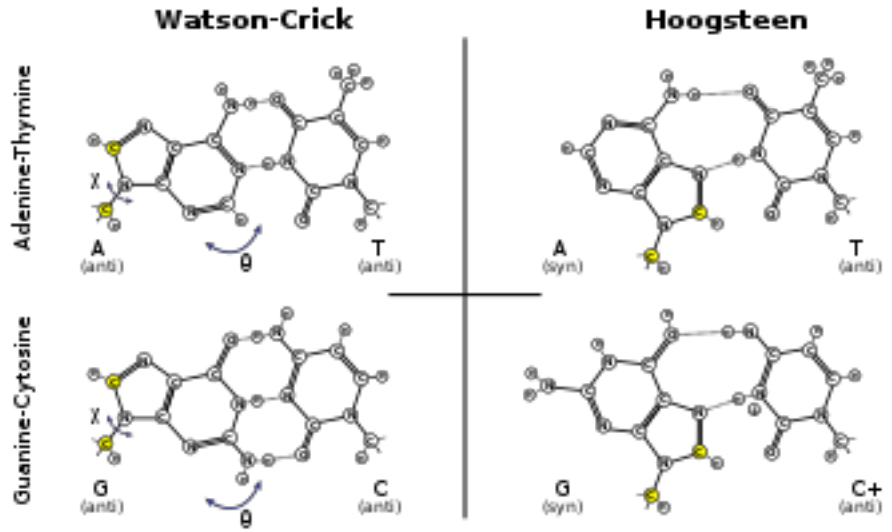
DNA origami nanocapsules that respond to sharp pH changes can be modified for targeted cargo delivery.

Moreover they do not rely on the addition of external elements i.e. oligonucleotides for strand displacement reactions



8 pH-sensitive latches



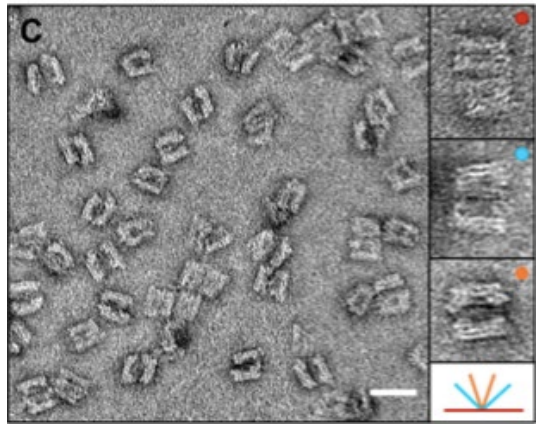


Hoogsteen bond is a variation of base-pairing where the hydrogen bond involves different groups of the nucleotides.

A third DNA strand can bind to a dsDNA via Hoogsteen bonds.

TEM analysis of the assembled structures

Open nanocapsules (pH 8)



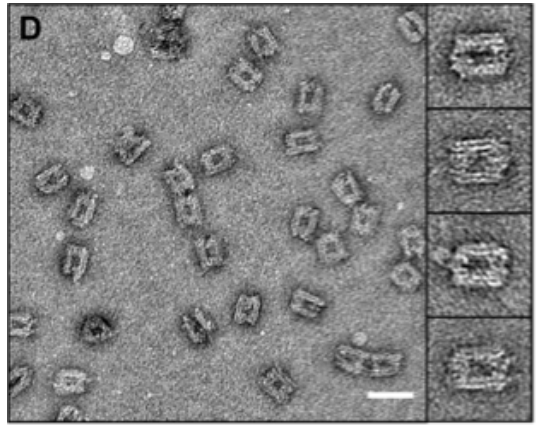
Almost no aggregation (~79% of monomers)

Clearly distinguishable open and closed conformations

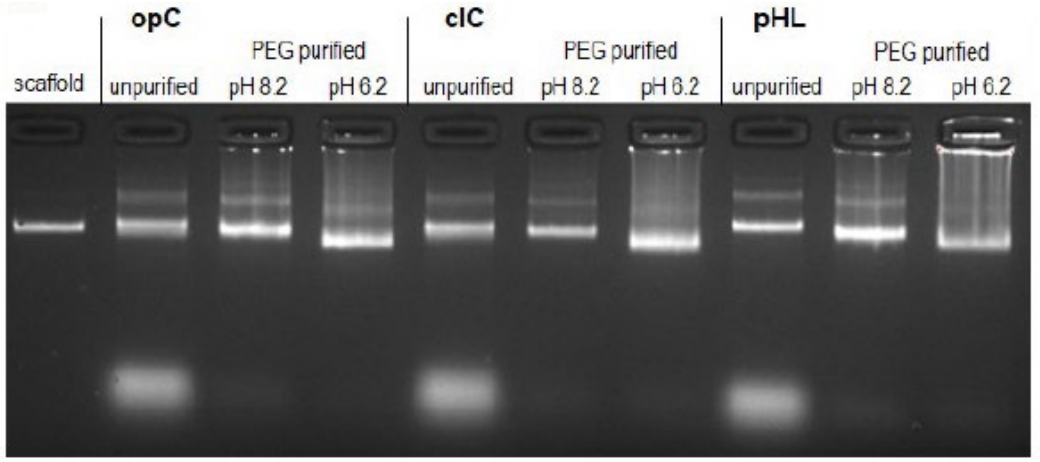
Closed nanocapsules are structurally homogeneous

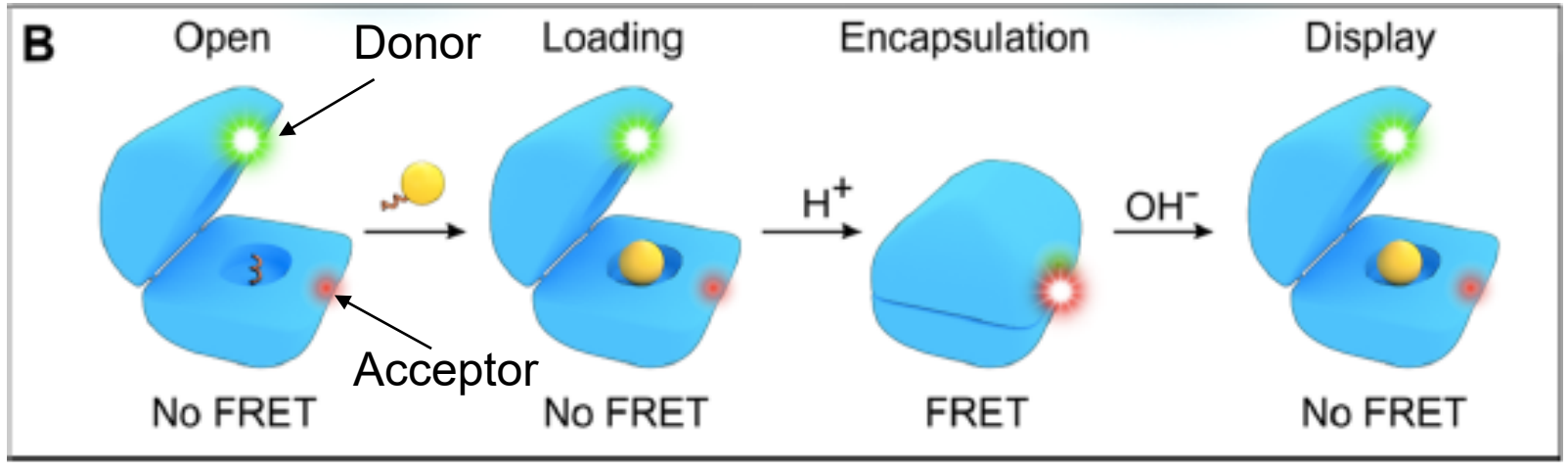
Open nanocapsules have a variety of opening angles

Closed nanocapsules (pH 6.2)



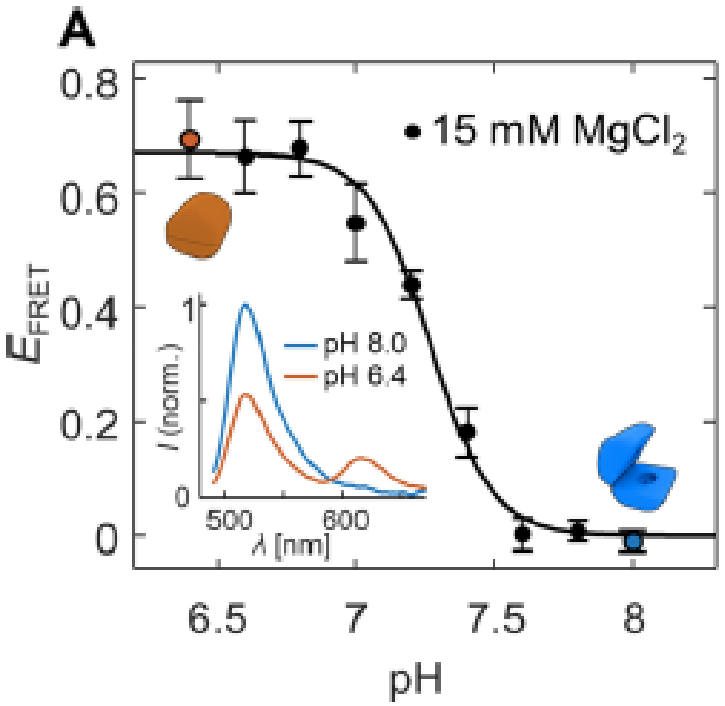
No different electrophoretic mobility





High FRET efficiency → donor and acceptor in close proximity → capsule closed

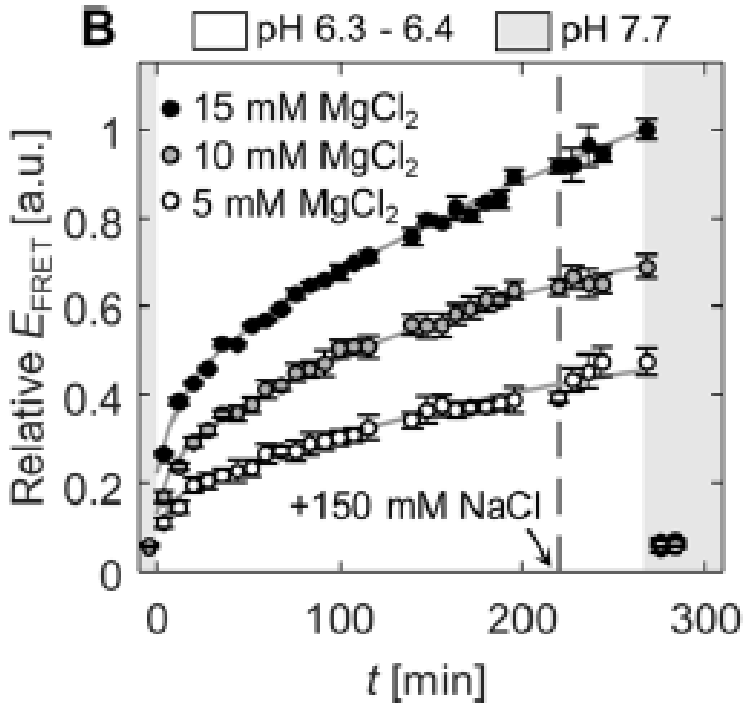
Low FRET efficiency → distance between donor and acceptor → capsule open



The FRET efficiency varies between pH 8, where the capsules are open, and pH 6.5, where they are almost all closed.

The pH-induced conformational change occurs very rapidly at pH around 7 – 7.2

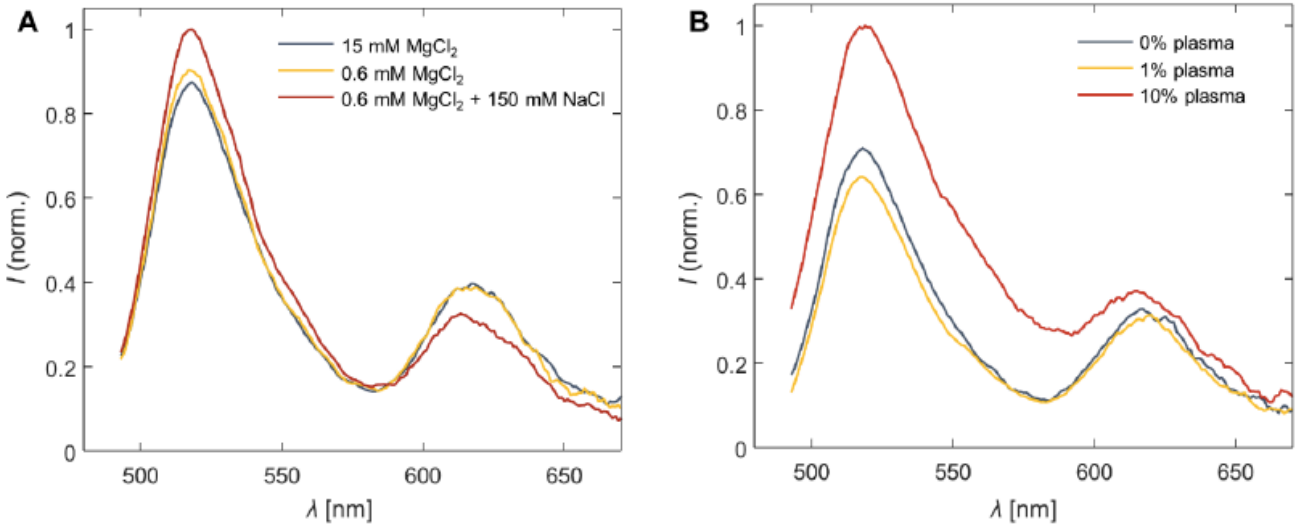
pKa determined by Hill equation (soid line) is around 7.27 and depends mainly on the T-A-T composition of the latches

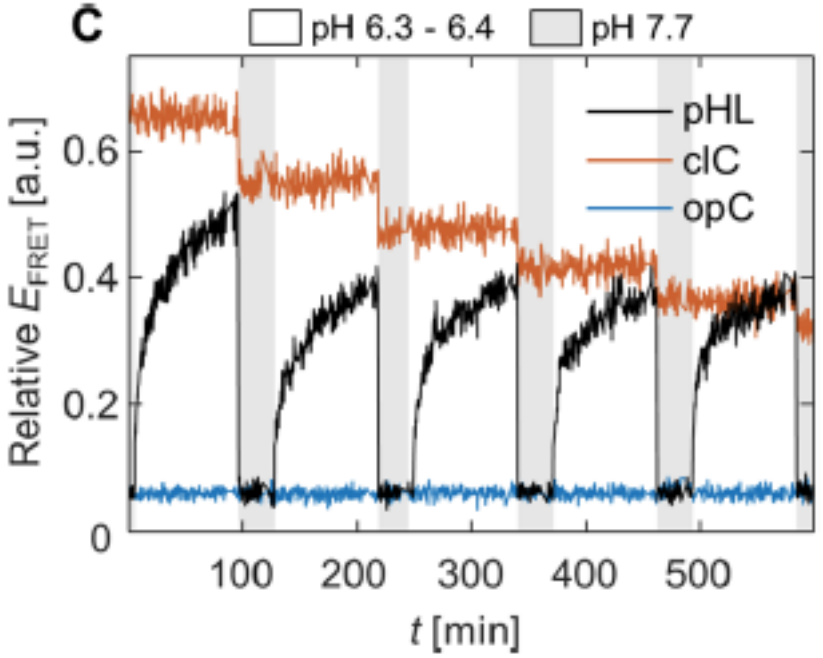


Divalent cation (Mg^{2+}) concentration affects the properties of the system by screening the repulsive interactions between the two nanocapsules halves

15 mM $MgCl_2$ is the optimal concentration for nanocapsules closing.

When the closed nanocapsules are put in a physyological environment (150 mM NaCl, 0.6 mM $MgCl_2$), there is only a small decrease in FRET efficiency



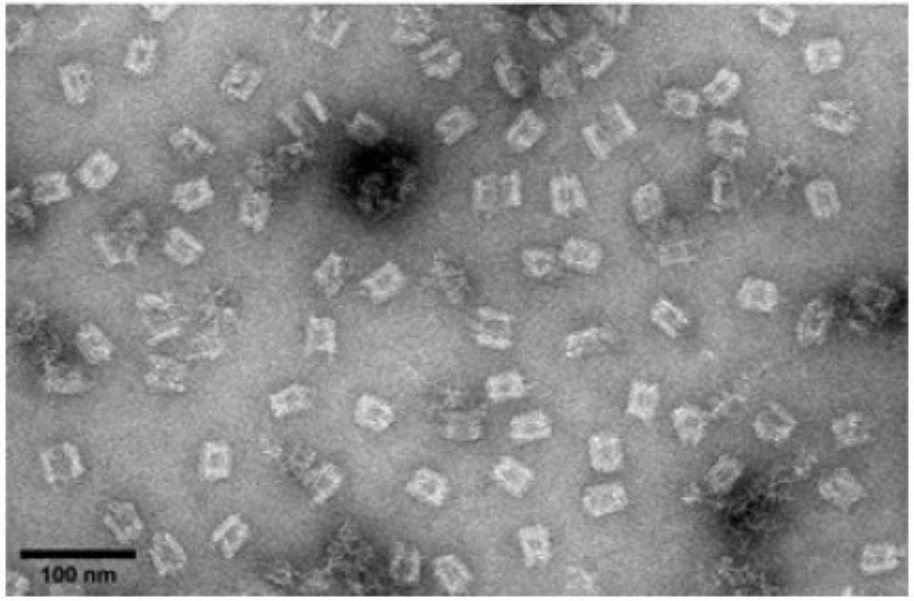


Nanocapsules can be opened and closed several times by changing the pH with acetic acid / sodium hydroxyde

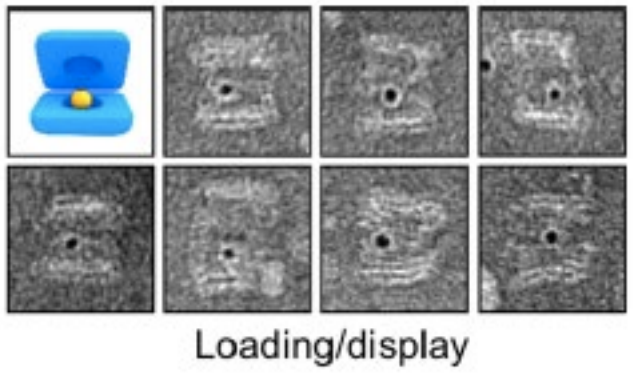
However, FRET efficiency decreases over time, indicating that only a portion of the capsules can close again

This effect is mainly visible in the clC control (always closed nanocapsules), but occurs also in the pH dependent structures (pHL).

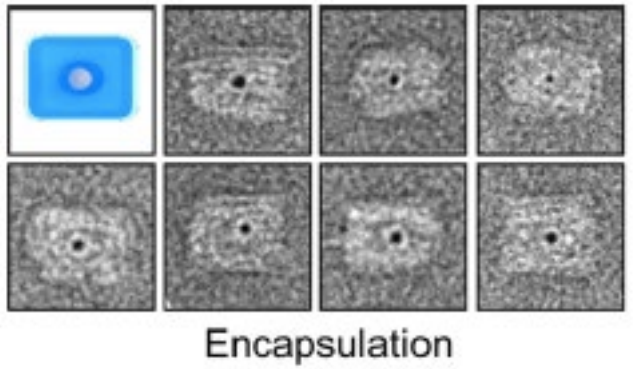
Indeed during pH changes there is accumulation of structural damage which disrupts the nanocapsules.



AuNPs and HRP were used as cargos to check the encapsulation efficiency and the cargo activity within the nanocapsules



pH 8 → open capsules
→ cargo loading



pH 6.4 → closed capsules
→ cargo encapsulation

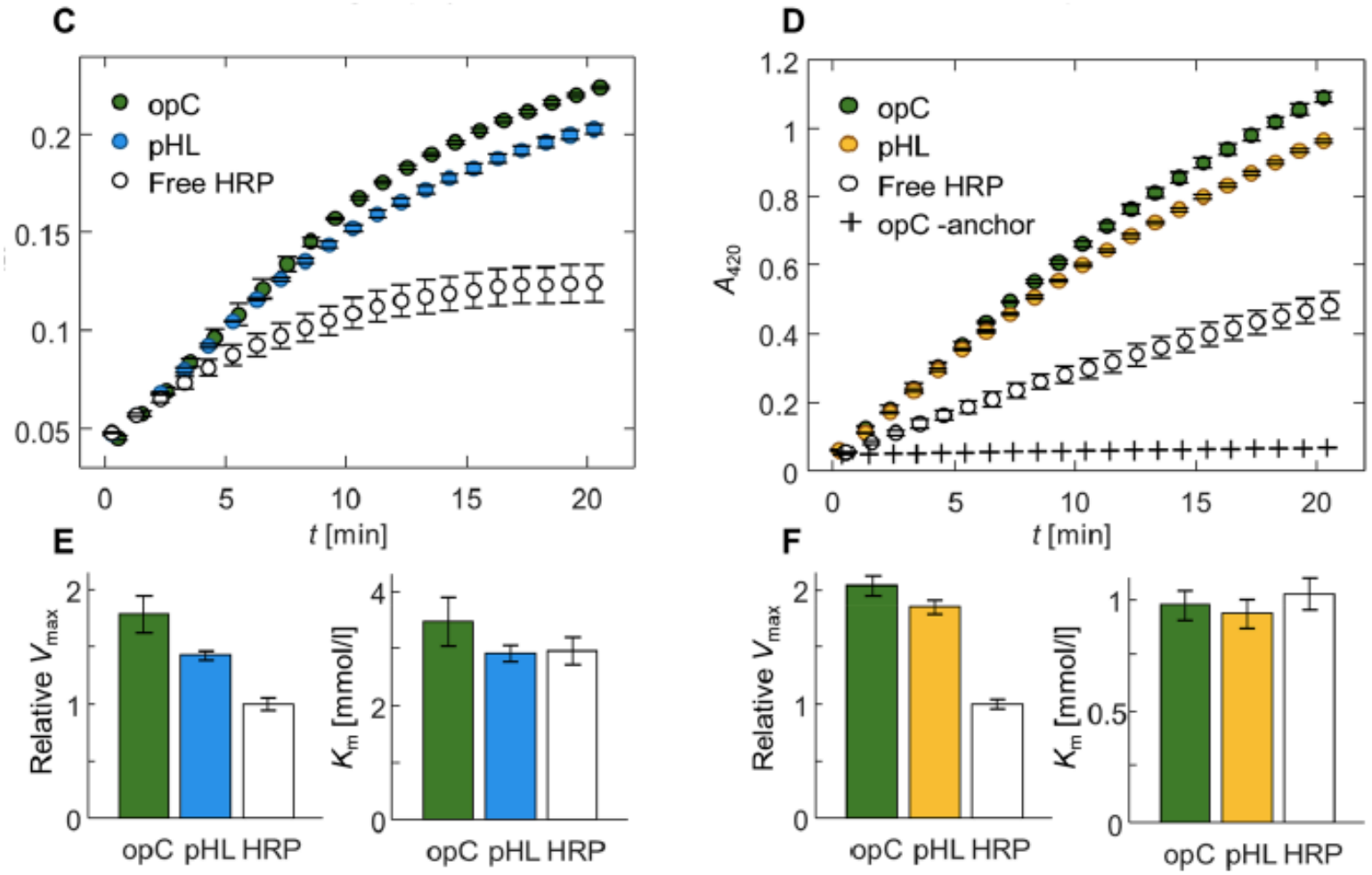
Encapsulation yield: 40-55%v

Cargos are linked to the nanocapsule bottom half by complementary ssDNA oligonucleotides

They are supposed to work from inside the capsule without being released

Catalytic activity of HRP-loaded pHL was compared with open nanocapsules and free HRP by measuring the absorbance of the reaction product.

The activity is higher at lower pH, as expected since HRP is known to be pH-dependent.



The increased catalytic activity of the encapsulated HRP can be due to the lower local pH near the origami surface.

Conclusions

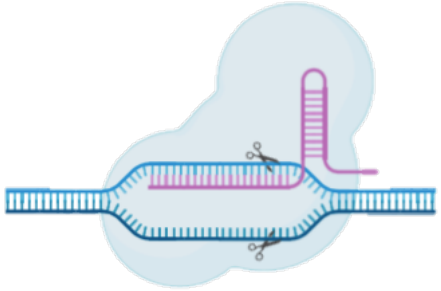
- DNA origami nanocapsules can undergo conformational changes upon pH shifts and no not need any external triggers.
- DNA latches form triplex structures at high pH. The pKa of the latches can be easily programmed by adjusting the T-A-T composition of the latches.
- The nanocapsules open and close very rapidly also in physyological environments (blood and plasma).
- The state of the system is highly dynamic and reversible.
- DNA nanocapsules can be functionalized to load and encapsulated cargos, which maintain their functionality also inside the cavity.
- This system can be further functionalized to increase the stability and to respond to other stimuli.

8870–8882 *Nucleic Acids Research, 2020, Vol. 48, No. 16* *Published online 18 August 2020*
doi: 10.1093/nar/gkaa683

Extracellular vesicles engineered with valency-controlled DNA nanostructures deliver CRISPR/Cas9 system for gene therapy

Jialang Zhuang[1,†], Jizhou Tan[2,†], Chenglin Wu[2,3,†], Jie Zhang[1], Ting Liu[2], Chunhai Fan[4], Jiaping Li[2,*] and Yuanqing Zhang[1,*]

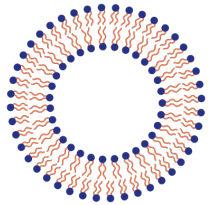
How to deliver CRISPR-Cas9 complexes?



CRISPR/Cas9 based genome editing

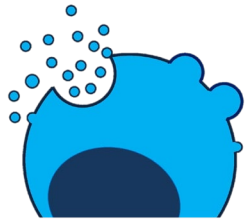
AAVs

- Very efficient delivery
- Immunogenicity issues



Liposomes

- High cell-type specificity
- Rapidly cleared



Extracellular vesicles (EVs)

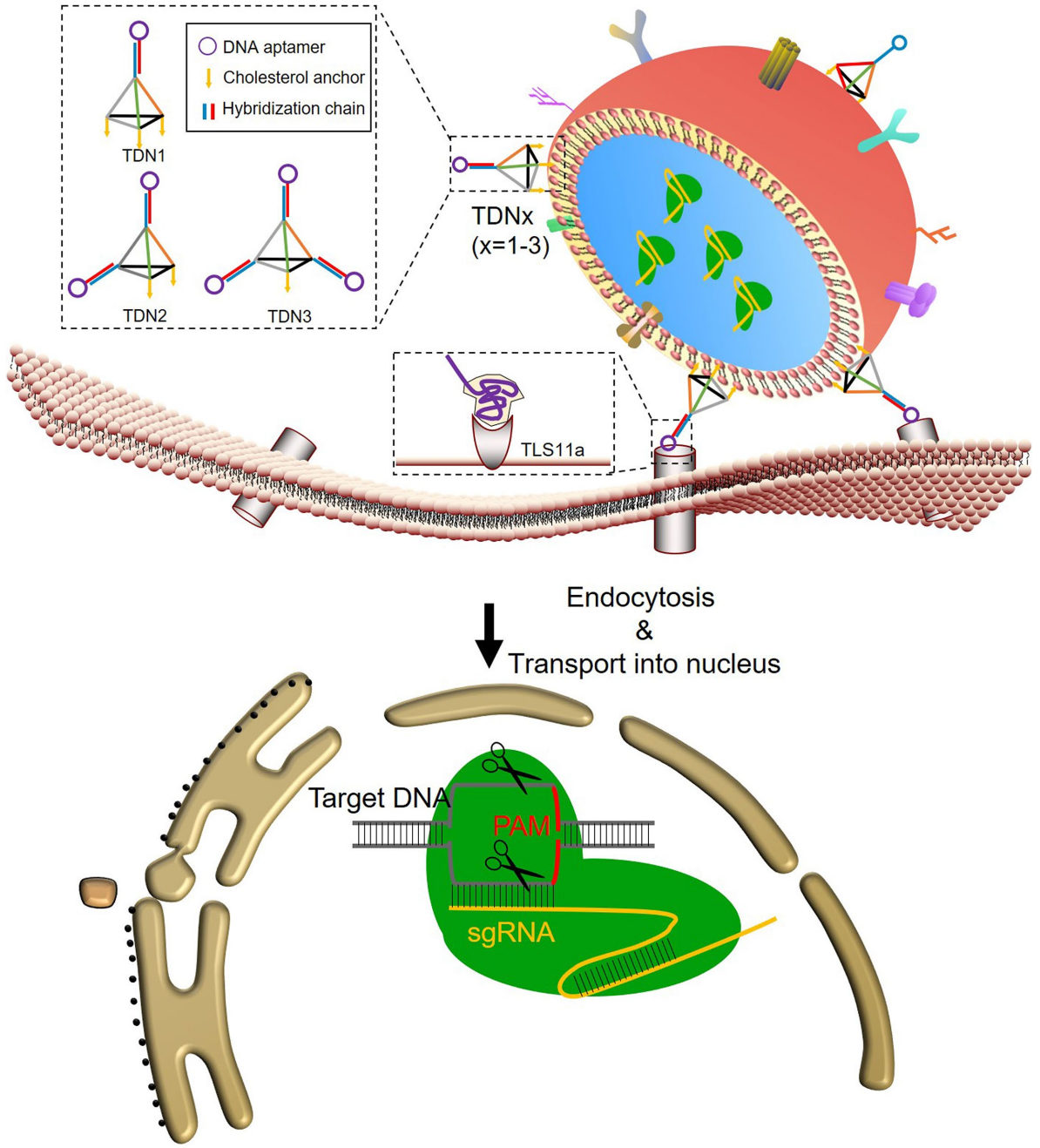
- Non immunogenic
- Efficient cellular interaction
- Poor cell specificity

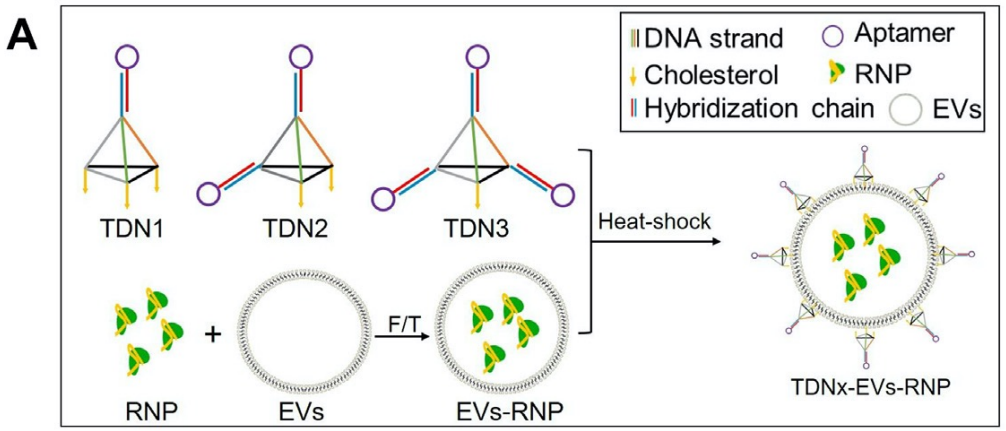
DNA aptamers are frequently used to direct EVs towards specific cell types.

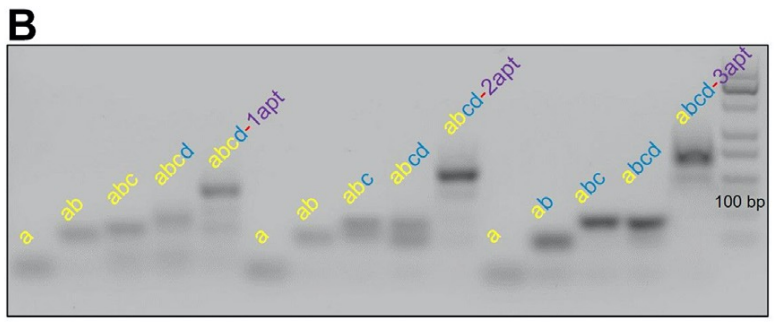
However, their efficacy relies on their specific orientation and may suffer from the interactions between adjacent ssDNA aptamers.

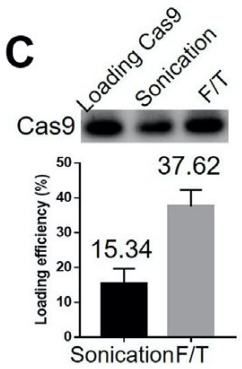
Aptamers were conjugated to tetrahedral DNA nanostructures (TDNs) together woth cholesterol to promote the display on the surface of EVs.

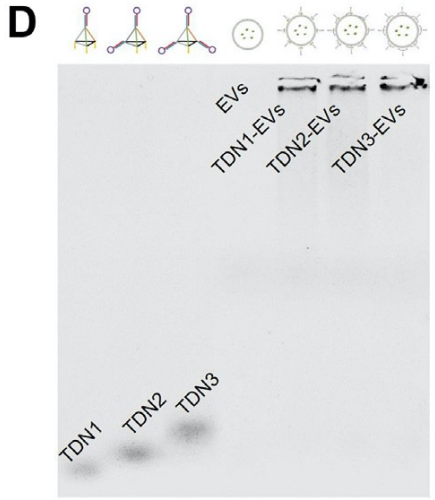
The use of TDNs gives the DNA aptamers a specific orientation.

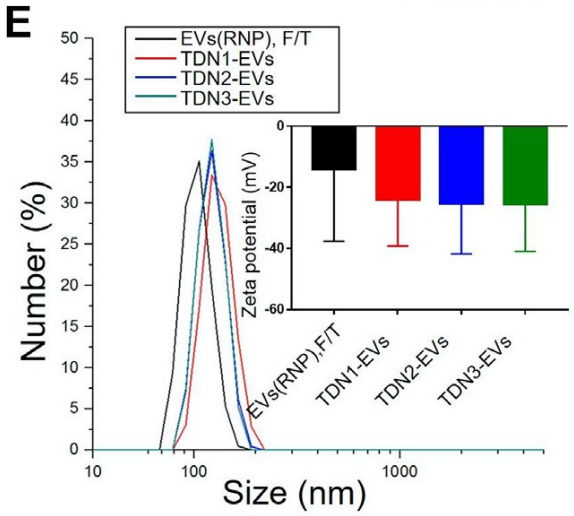










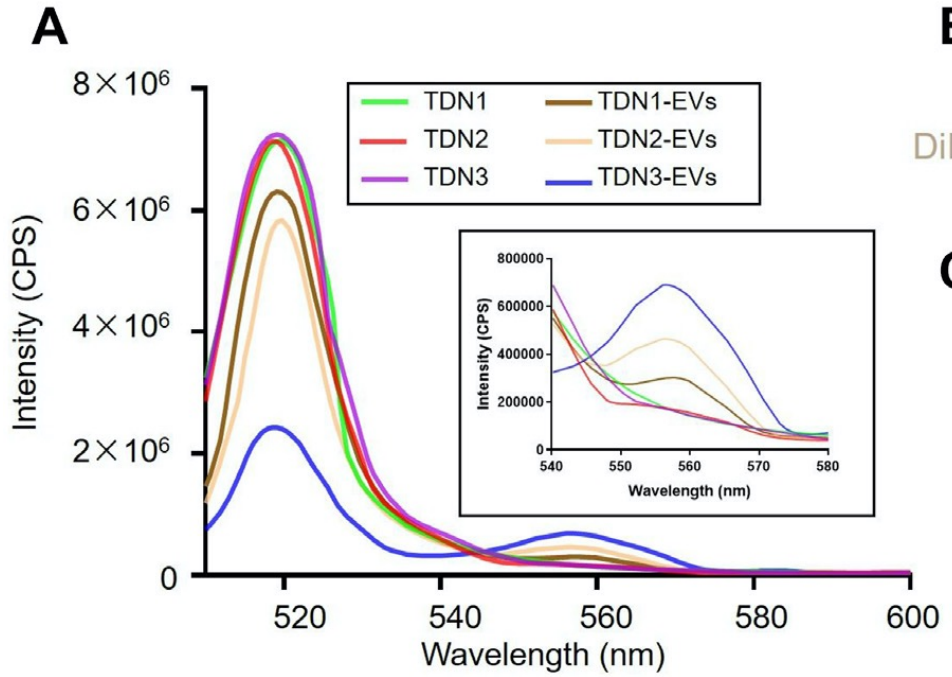


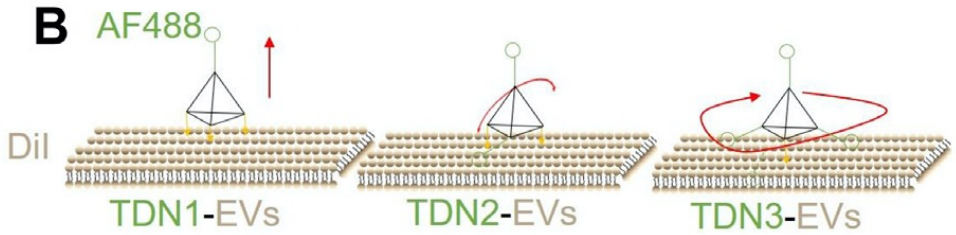
Three different TDNs were synthesized:

- TDN1: 1 aptamer and 3 cholesterol units;
- TDN2: 2 aptamers and 2 cholesterol units;
- TDN3: 3 aptamers and 1 cholesterol unit.

EVs were loaded with a RNP complex consisting of Cas9 and sgRNA against GFP/WNT10B (30% loading efficiency).

Assembled TDNs were attached to the surface of loaded EVs with a modified heath-shock protocol (50% of decorated EVs).
The addition of TDNs did not modify the shape of the EVs.





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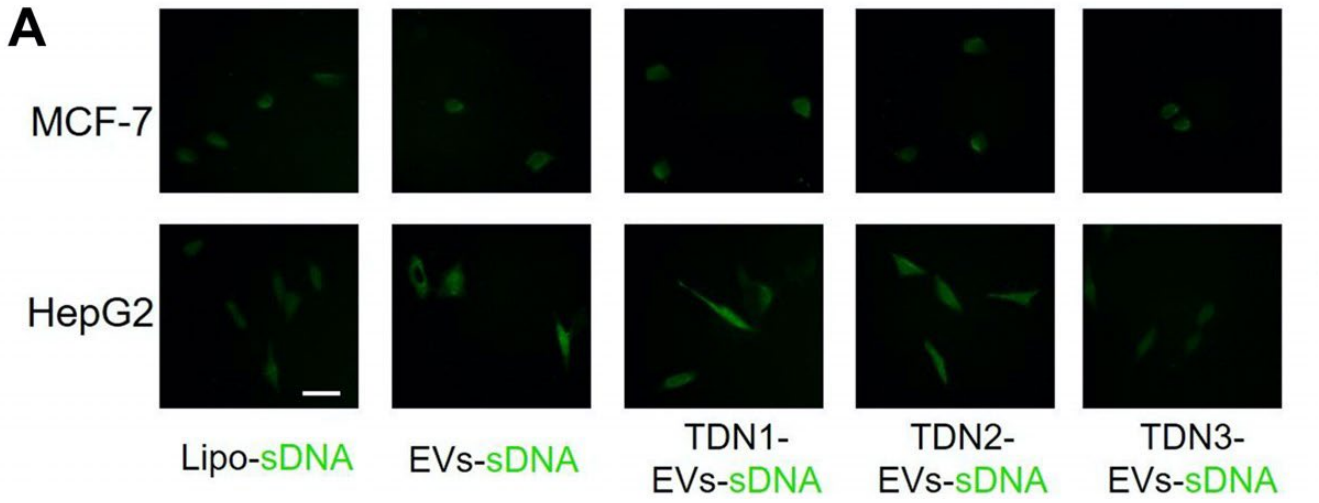
NPs	Ratio cholesterol /aptamer	FRET efficiency, %	TDNs loading efficiency, %	Interaction effect per TDNs
TDN1-EVs	3:1	9.09	77.43±12.95	0.12±0.02
TDN2-EVs	2:2	32.26	66.15±4.18	0.49±0.03
TDN3-EVs	1:3	66.44	49.83±6.71	1.35±0.19

To investigate the orientation of the aptamers, FRET efficiency between the donor-functionalized aptamers and acceptor-functionalized EV membrane was evaluated.

The highest the FRET efficiency, the stronger the interaction between the two partners.

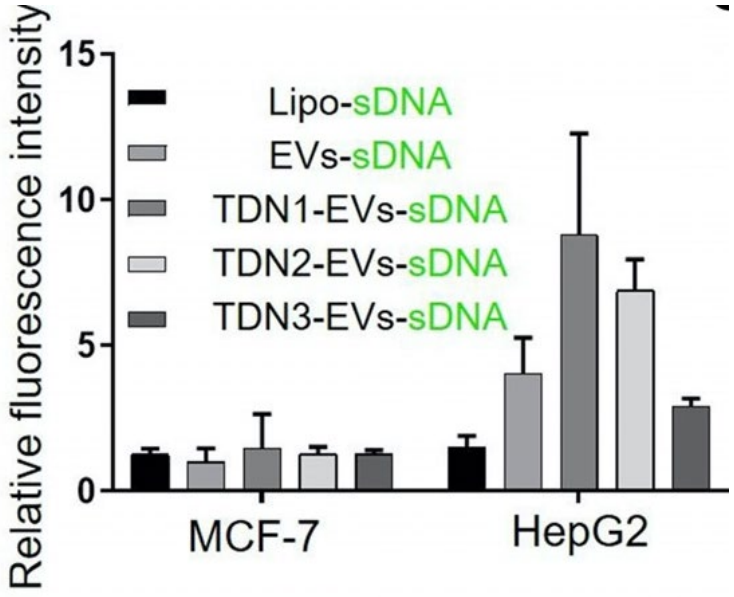
TDN1 showed the lowest FRET efficiency, indicating that the aptamer is not close to the EV membrane but is protruding from its surface.

The orientation of DNA aptamers can be controlled by changing the ratio aptamers:cholesterol.



TDNs with DNA aptamers that bind to HepG2 were administered to HepG2 positive cells and to MCF-7 positive cells (negative control).

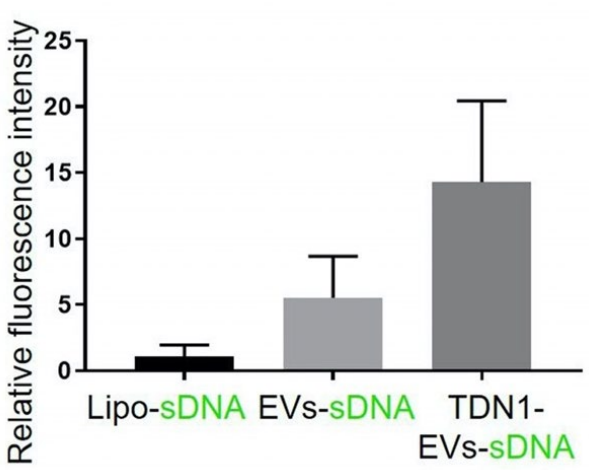
TDN1 displayed the highest cell-specific delivery.



The orthogonal orientation of the aptamer on TDN1 assures the highest interaction with the target cells.

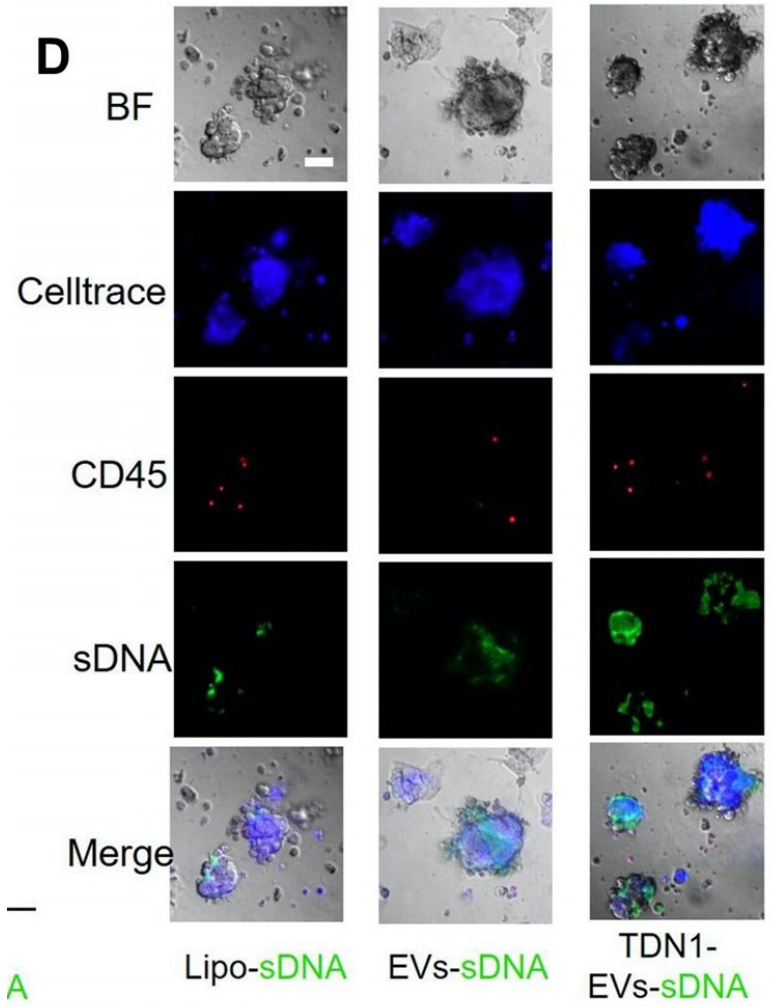
Therefore, TDN1 was selected as final delivery nanovehicle for further experiments

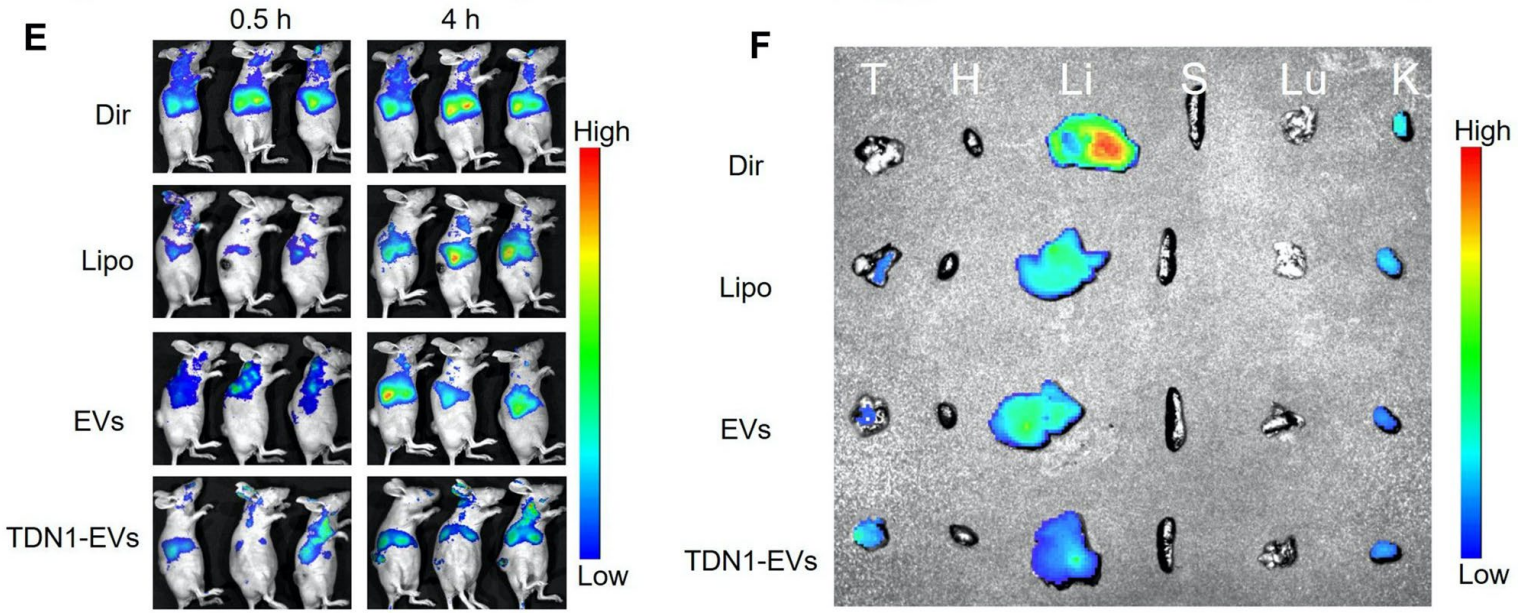
Cell targeting efficiency of TDN1-EVs was further evaluated in an organoid model of liver cancer.



TDN-decorated EVs exhibited a higher degree of accumulation in the organoids compared to bared liposomes and EVs.

CD45+ leukocytes present in the tumor organoids were used as controls. Less than 10% of the leukocytes were stained by TDN1-EVs, indicating a high cell-specificity.





HepG2 was injected subcutaneously into BALB/nude mice, and after 28 days fluorescently labelled liposomes, EVs or TDN1-EVs were administered.

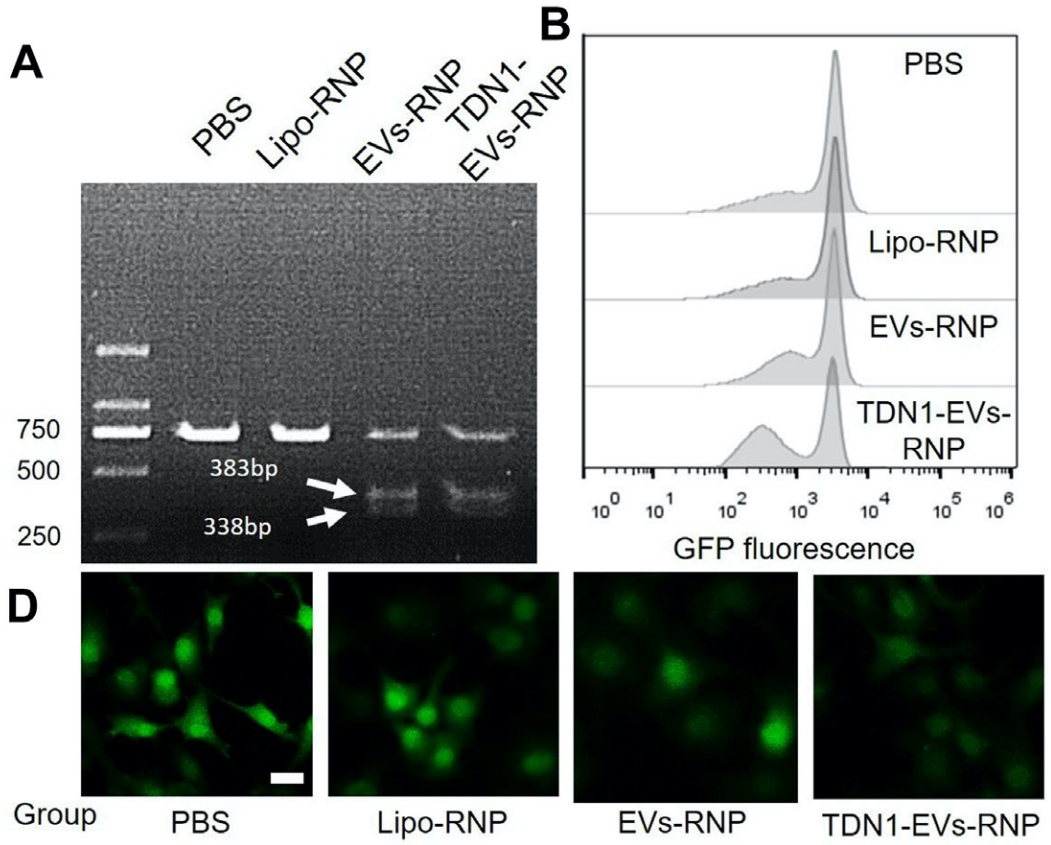
Alive tumor-bearing mice were subjected to fluorescence imaging.

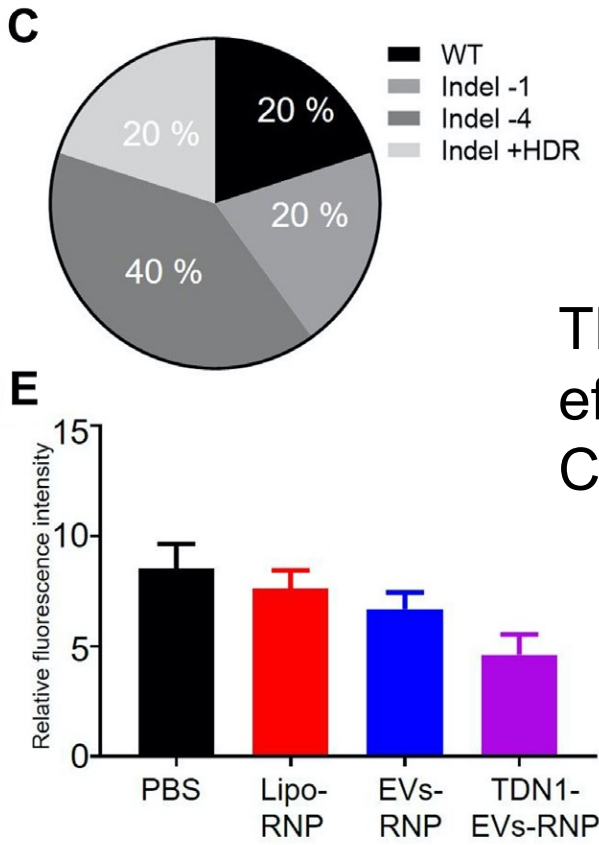
EVs formulations accumulated more than liposomes due to their longer half-life.

However, bare EVs failed to reach the tumor and accumulated mainly in the liver.

TDN1-EVs achieved successful delivery to the tumor site.

Liposomes, EVs and TDN1-EVs were loaded with RNP complexes targeting GFP, and were administered to HepG2 cells.
PCR-based sequencing and loss of fluorescence were used as readouts to quantify the efficiency of the CRISPR-mediated gene editing.





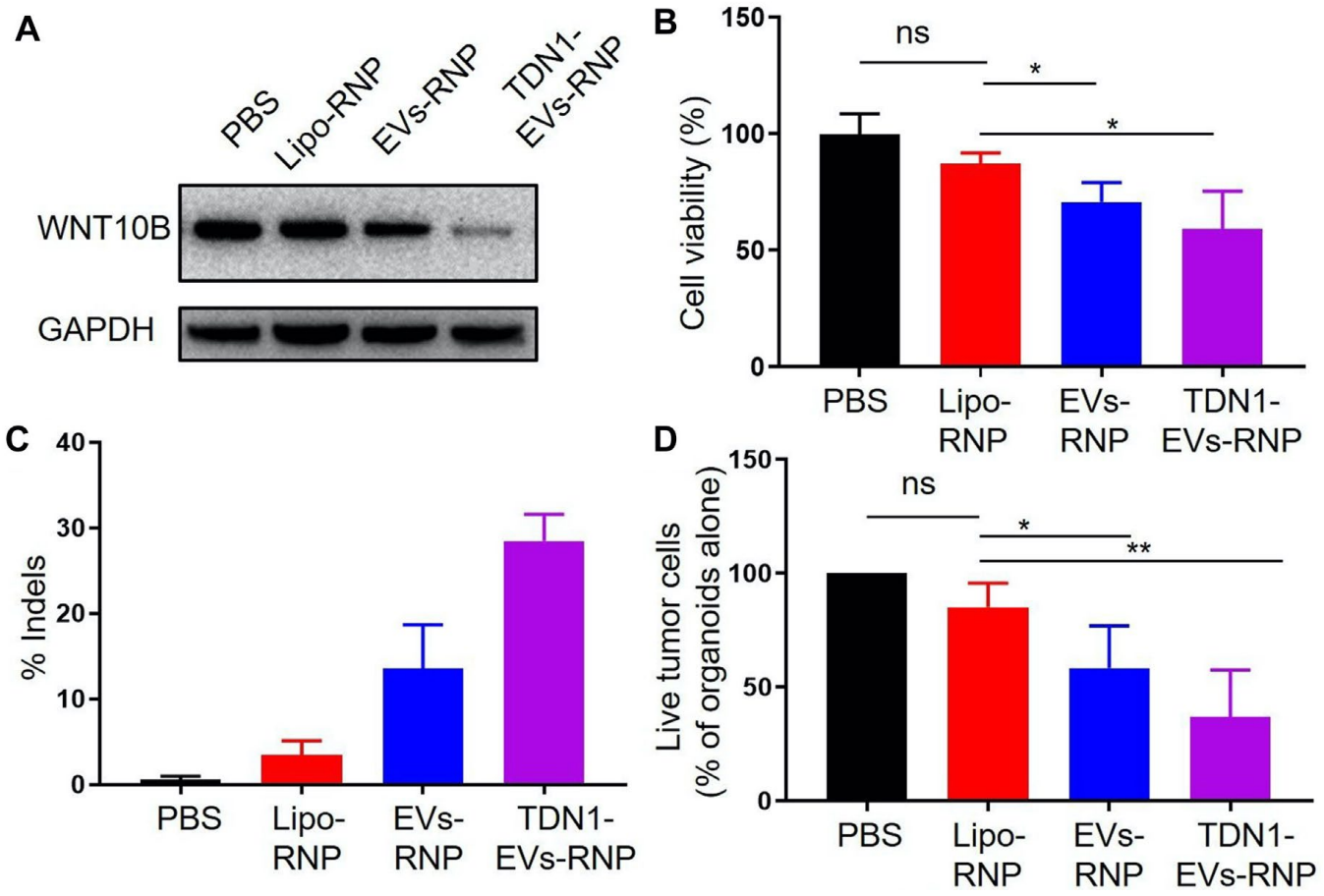
TDN1-EVs were the most efficient in delivering CRISPR/Cas9 complexes

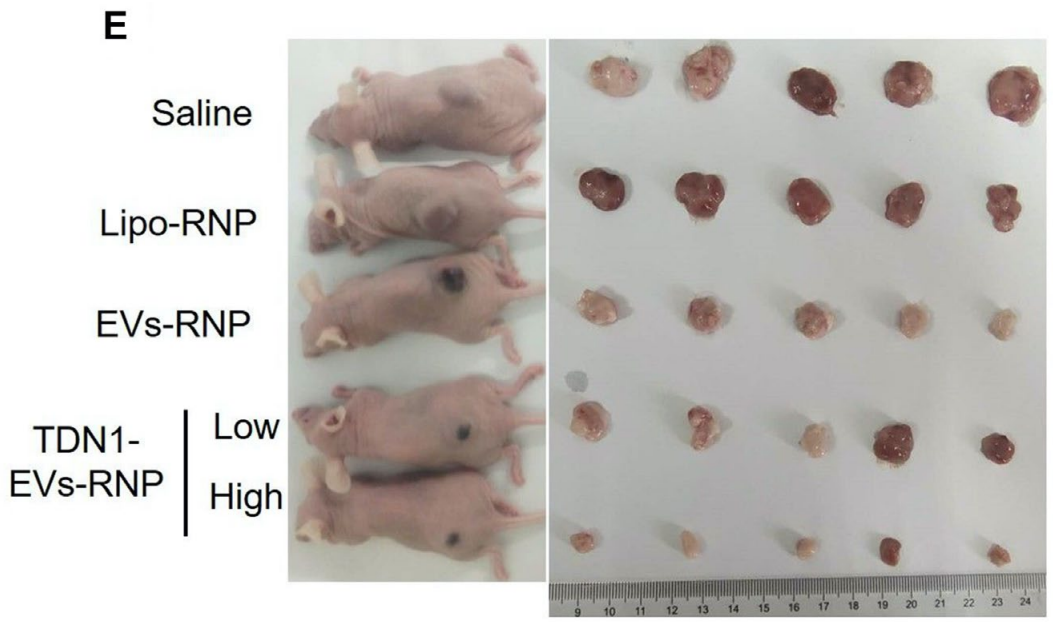
Hepatocellular cancer cells show increased expression of WNT10B, and its silencing reduces the migration of HepG2 cells.

Cas9/sgRNA targeting WNT10B was loaded as cargo on different nanodevices

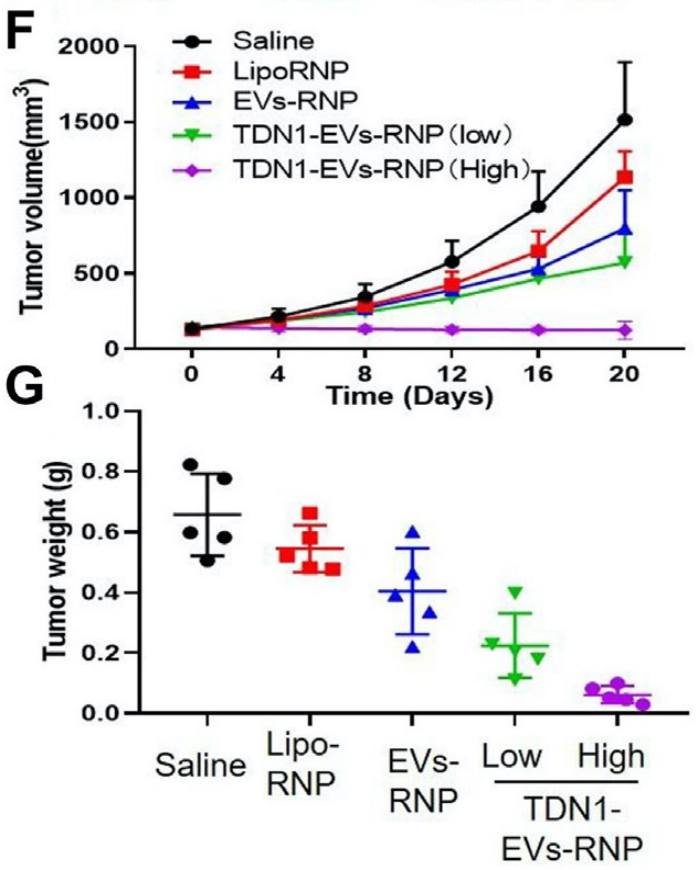
WNT10B expression decreased significantly only after treatment with TDN1-EVs.

The knock-out of WNT10B led to the growth inhibition of the tumor cells





BALB/nude mice bearing HepG2 xenografted tumors were injected with different Cas9/WNT10B nanocarriers



The growth of the tumor was monitored over time. Tumors treated with TDN1-EV nanocarrier remained small and confined.

No significant toxicity was detected in liver, kidneys, spleen, lung or heart.

Conclusions

Extracellular vesicles (EVs) hold great potential for gene therapy as they have evolved for transfer of molecules between cells.

However, they lack in cellular selectivity.

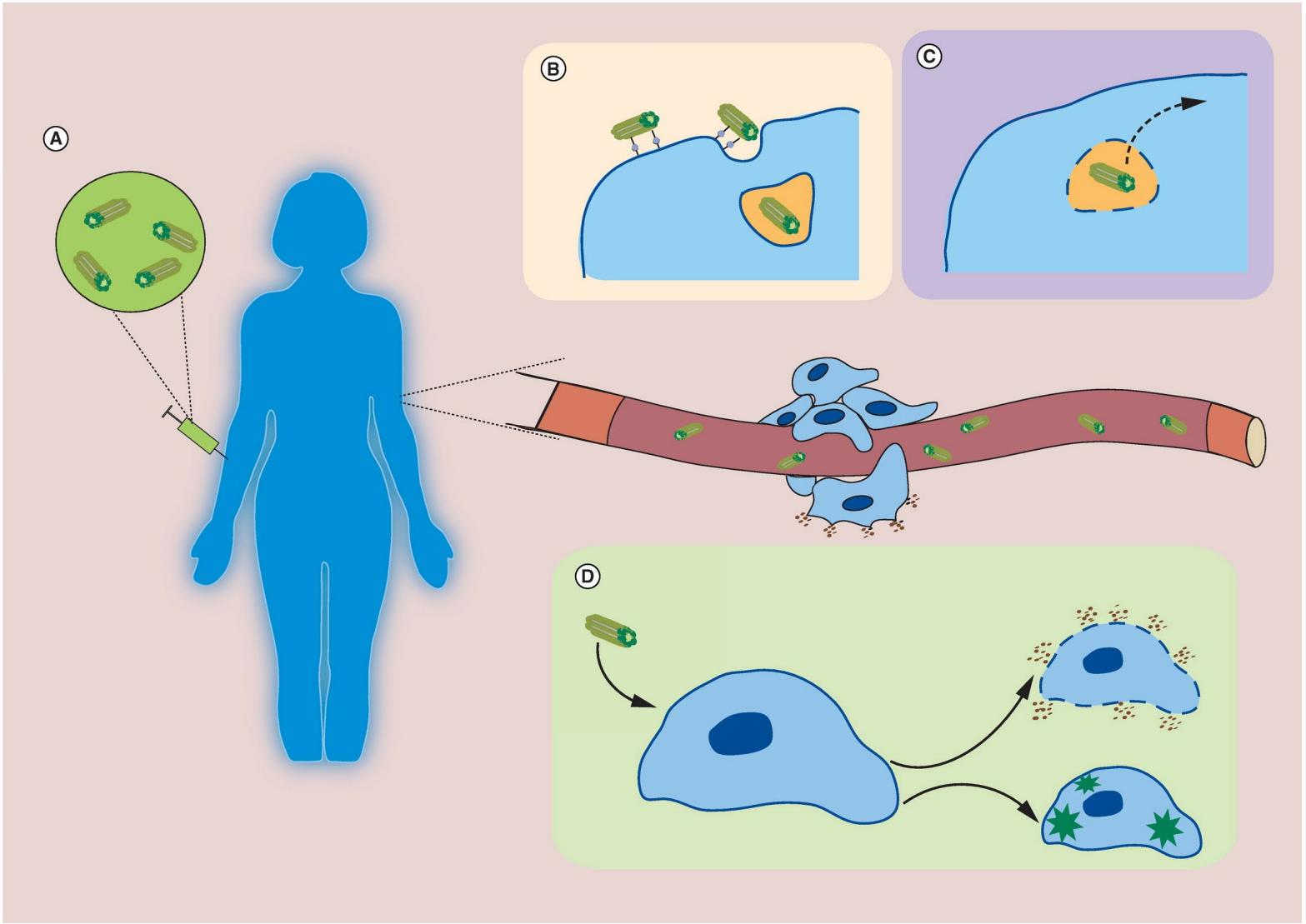
DNA aptamers can efficiently target EVs to desired cell types, but the orientation might affect their activity.

Tetrahedral DNA nanostructures (TDNs) can be attached to the surface of EVs and be used to correctly orient the DNA aptamers.

Functionalized TDNs-EVs can be used as nanocarriers for Cas9/sgRNAs against tumor-associated genes.

TDNs-EVs do not show significant toxicity when injected in vivo.

Delivering DNA nanostructures to the body



DNA nanostructures must:

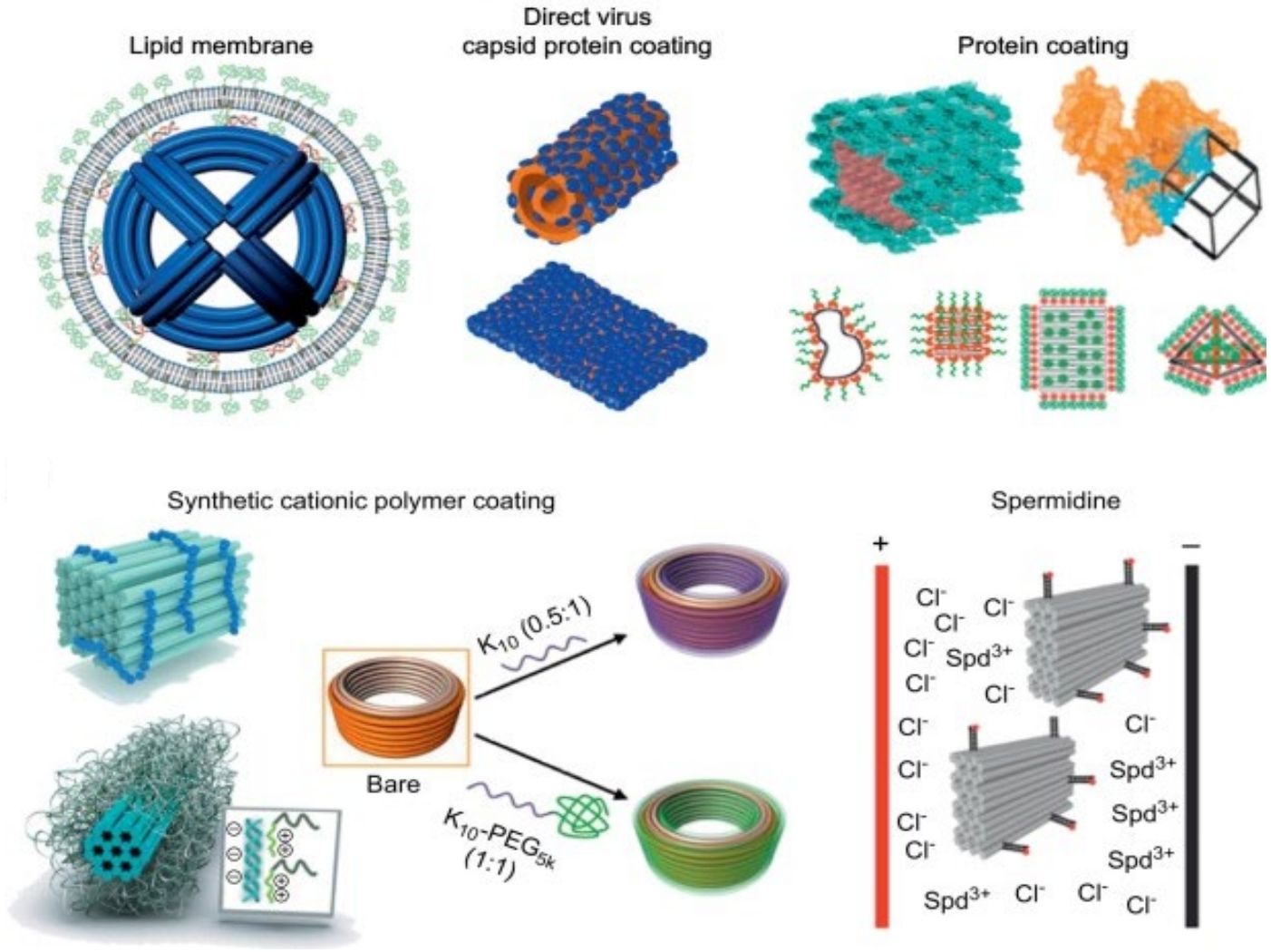
- Avoid clearance and degradation
- Be internalized in target cells
- Escape or release loaded cargo

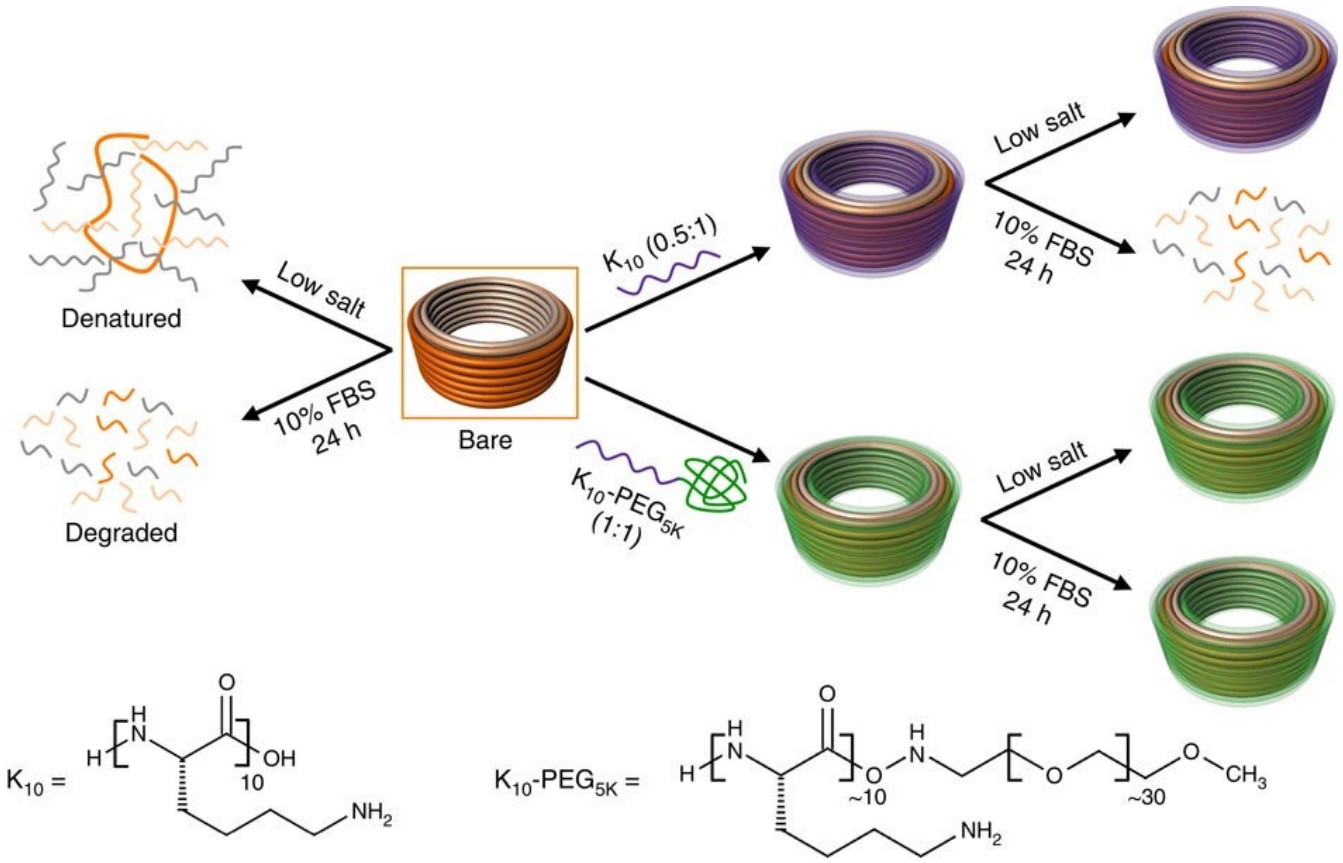
Balakrishnan et al., 2019

The application of DNA nanostructures in vivo is hampered by the susceptibility to nuclease-mediated degradation.

Some possible solutions:

- Lipid encapsulation
- Coating with proteins
- Coating with polymers
- UV photocrosslinking





Oligolysine polymers conjugated with PEG adhere to the negative DNA backbones and hold them together, replacing Mg^{2+} in low cation conditions

However, due to the weak nature of the electrostatic interactions, the binding is highly dynamic and reversible

Ponnuswamy et al., 2017

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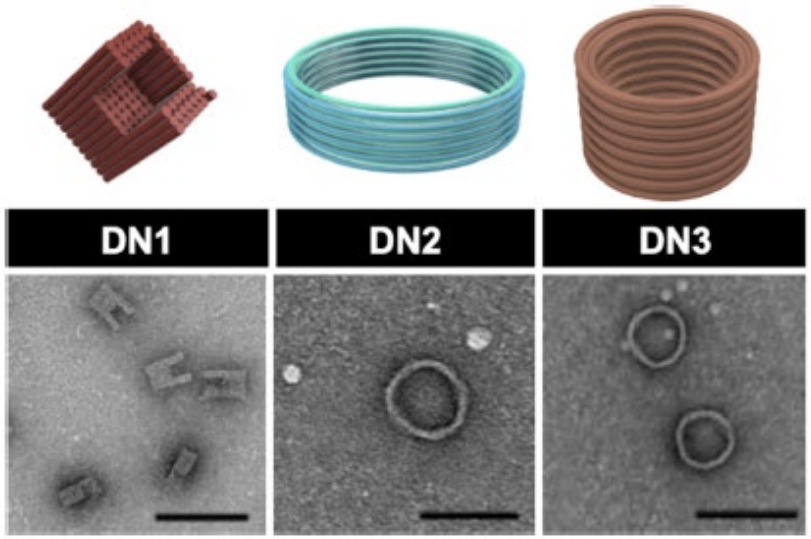
Communication

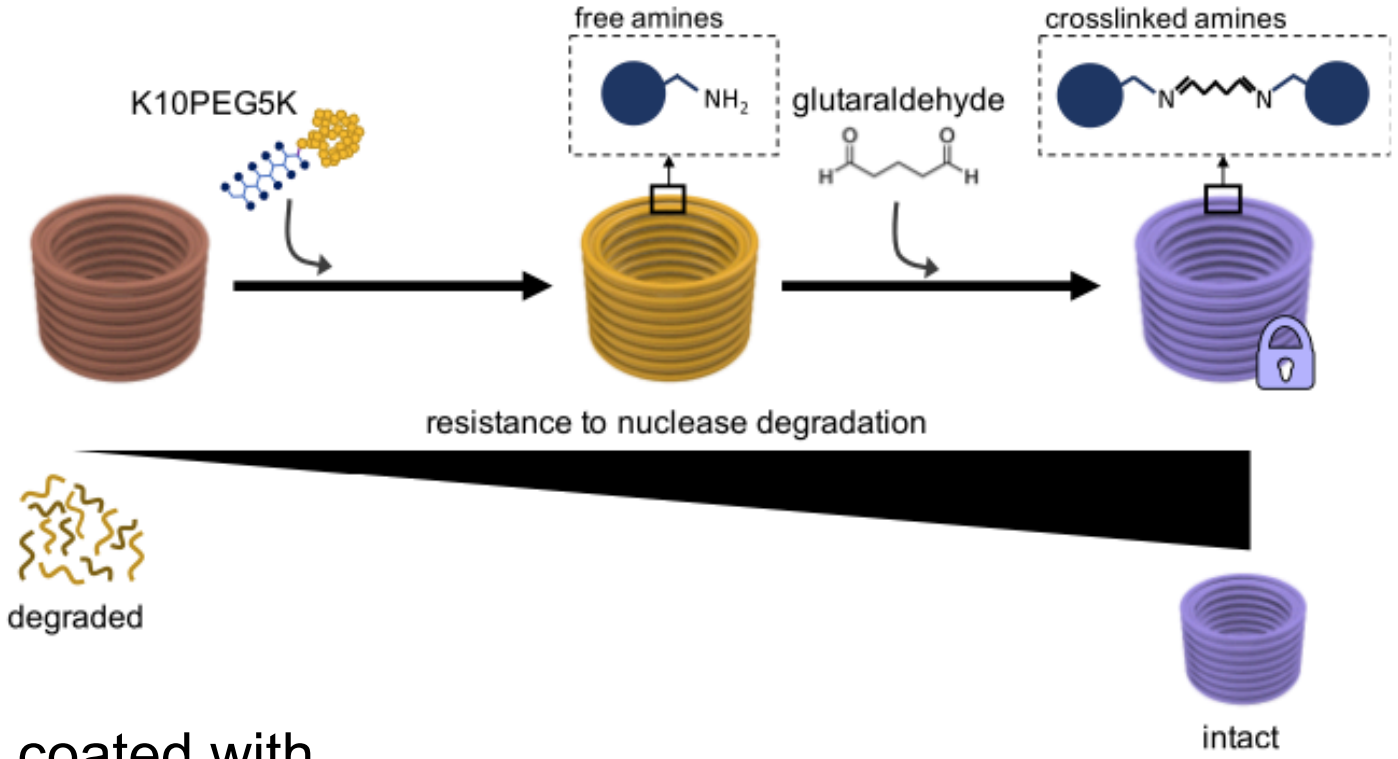
Glutaraldehyde Cross-Linking of Oligolysines Coating DNA Origami Greatly Reduces Susceptibility to Nuclease Degradation

Frances M. Anastassacos,[‖] Zhao Zhao,[‖] Yang Zeng, and William M. Shih*

Three DNA nanostructures (DNs) designed with caDNAno

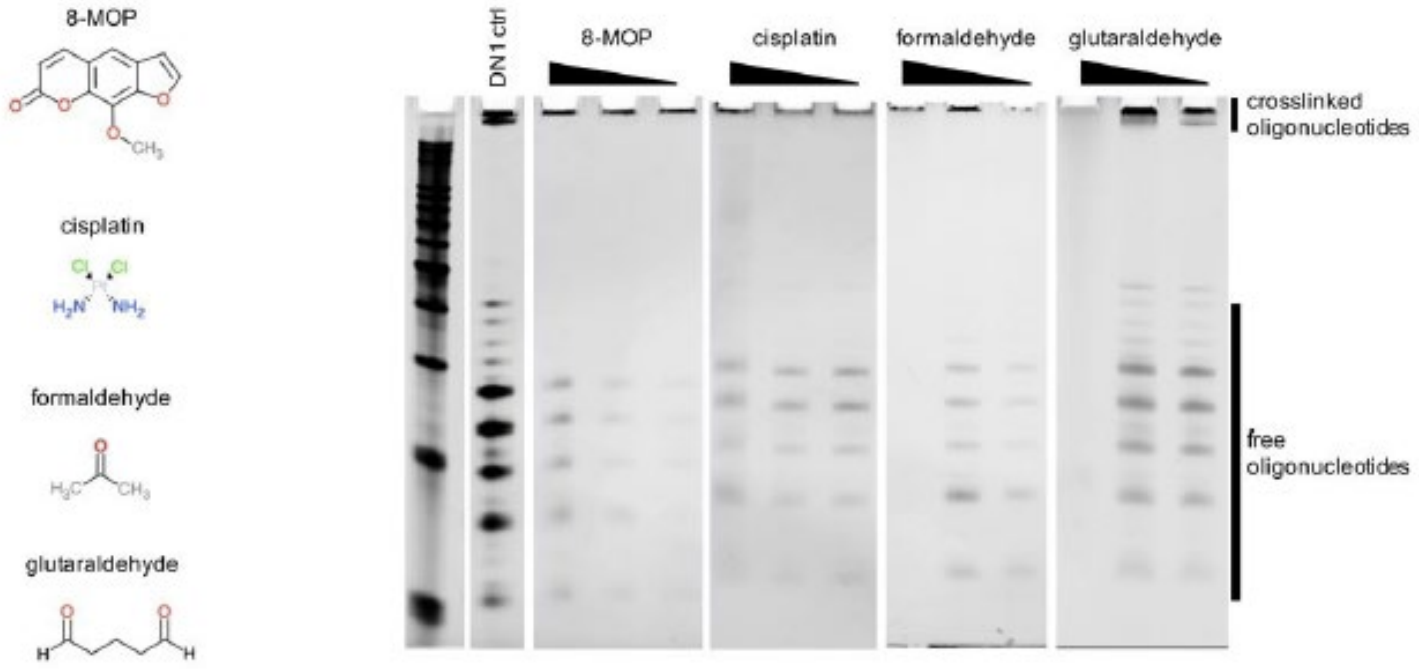


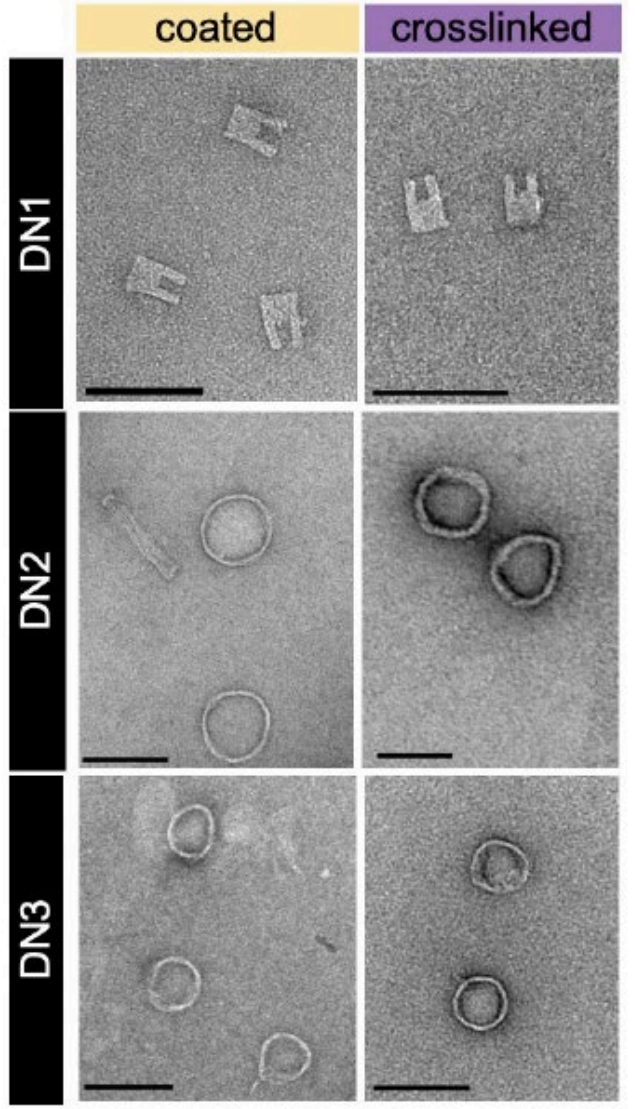
…and then crosslinked with glutaraldehyde which forms imine bonds with oligolysines



DNs are first coated with PEGylated oligolysines…

DNs display increased resistance to nuclease degradation

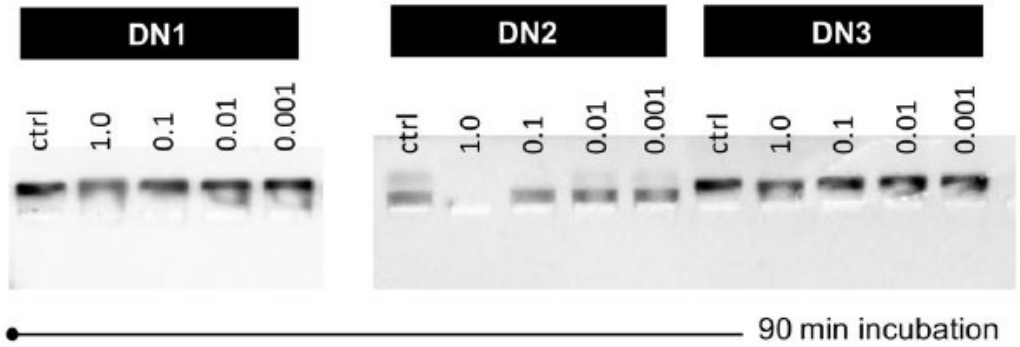




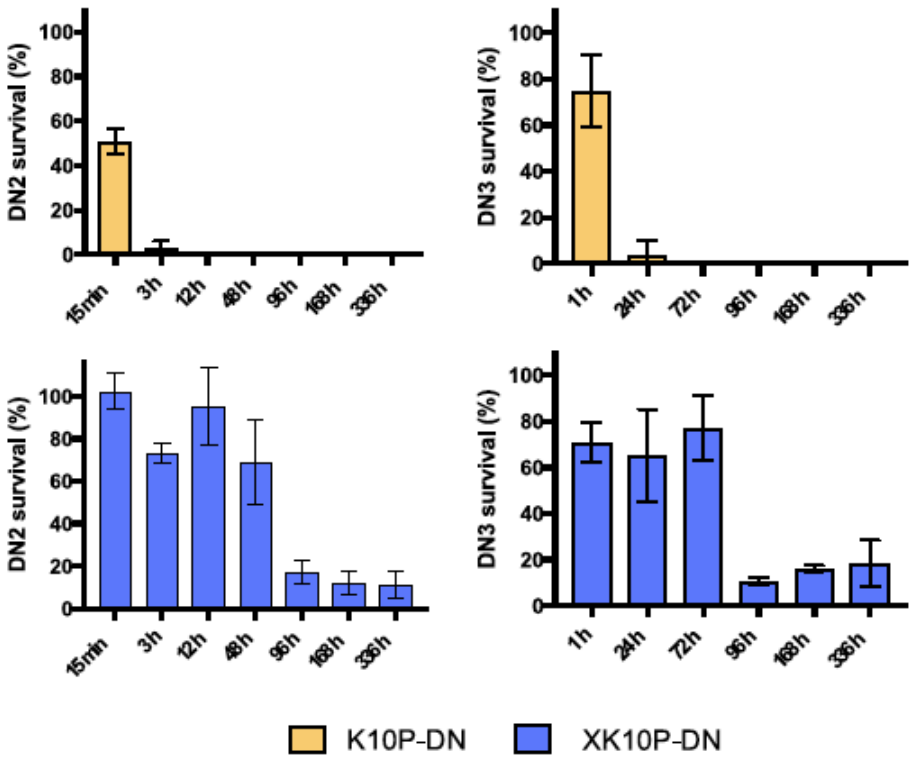
Of the cross-linkers tested, glutaraldehyde showed the greatest ability to prevent DNs denaturation and nuclease degradation.

No structural deformation of DNs occurs upon cross-linking.

Degradation rate of DNs by nucleases is conformation-dependent.



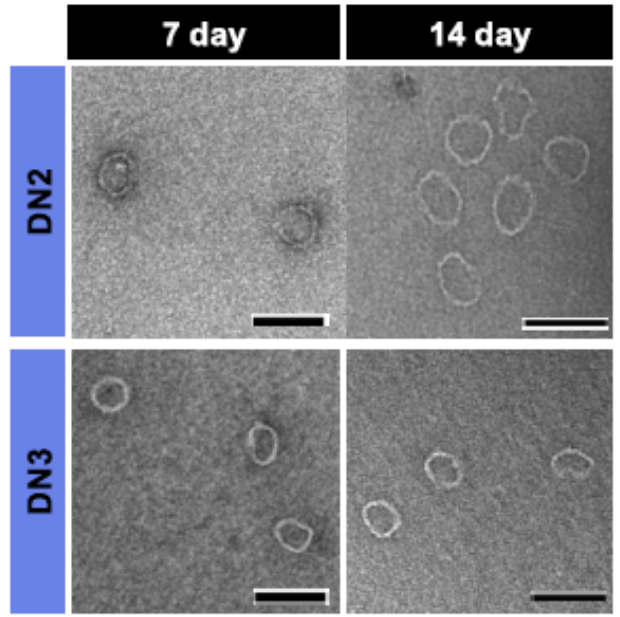
Cross-linked DNs were incubated with 1U/uL DNAse I → 2600-fold higher than the concentration found in blood



PEGylated DNs with no glutaraldehyde survived less than 1 h in strenuous conditions.

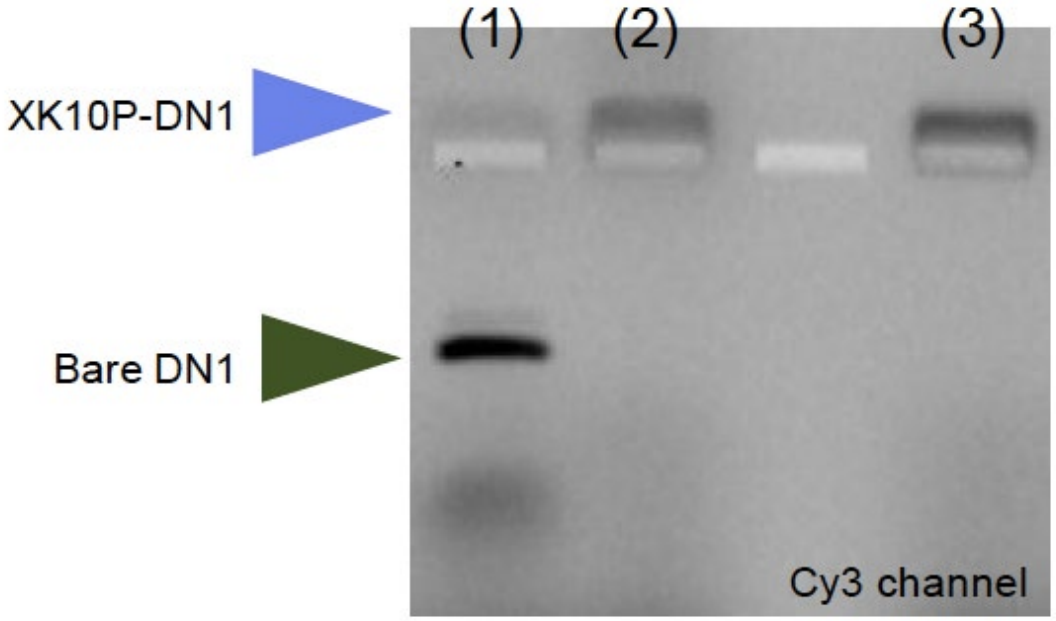
Cross-linked DNs could survive up to 14 days.

TEM images show intact DNs structures after the treatment

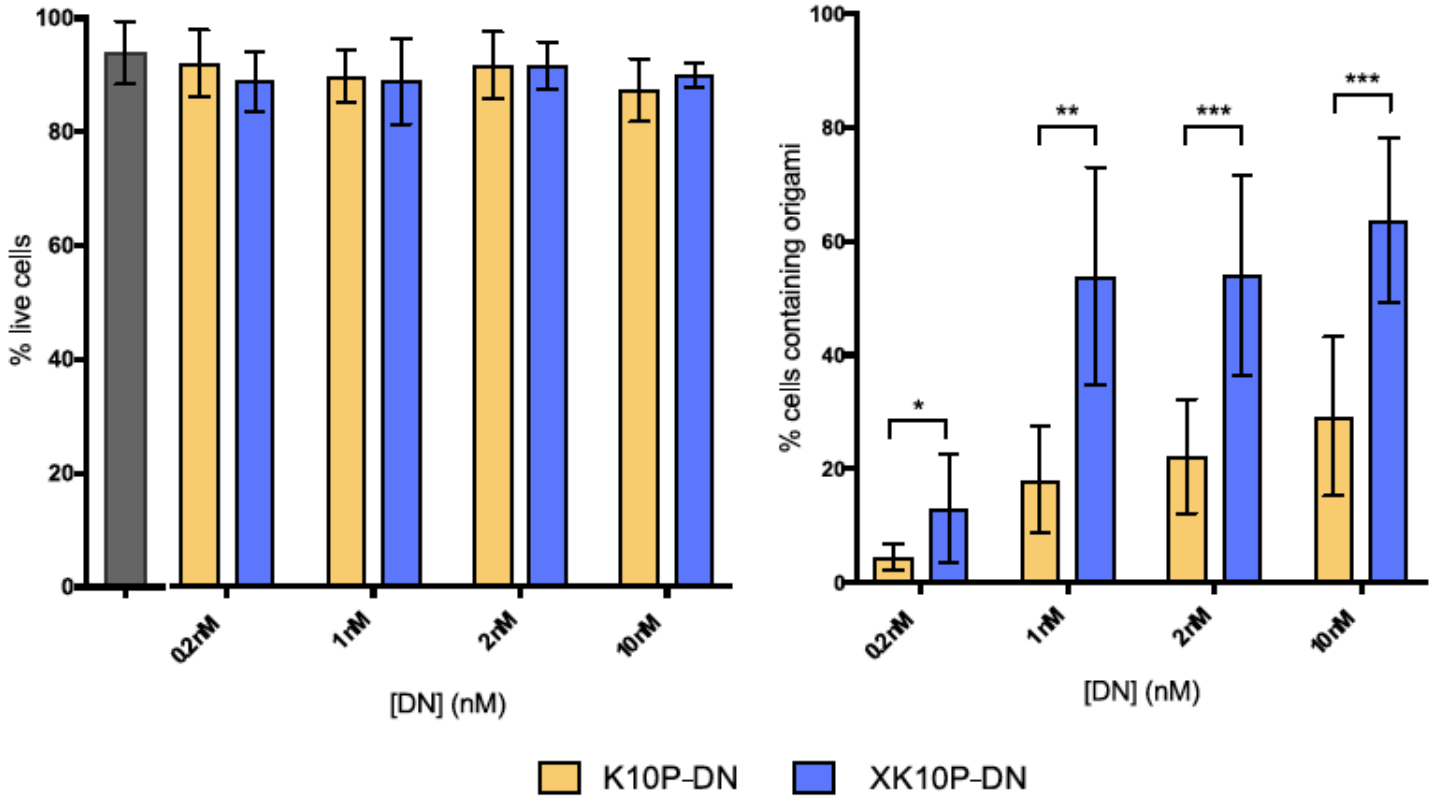


Glutaraldehyde cross-linking of PEGylated oligolysines-coated DNs increases their survival time of around 250 times.

DNs can be loaded with diverse cargos, and the loading occurs through Watson-Crick base pairing between a ssDNA of the DNs (handle) and a ssDNA conjugated to the cargo (antihandle).



Cross-linked DNs could be successfully loaded in a short incubation time, indicating that the handle is still accessible for binding with the antihandle.



Cross-linked DNs are taken up more easily when added to the cell culture medium

The increased uptake could be due to the rapid degradation of the PEGylated DNs

Cross-linked DNs did not show increased toxicity on HEK293T cells compared to PEGylated DNs

Conclusions

- Glutaraldehyde cross-linking is an inexpensive, scalable and generalizable method for protecting DNs in vivo.
- Cross-linking increases the resistance to nucleases up tp 250 fold compared to DNs coated with PEGylated oligolysines.
- Moreover, cross-linked DNs are taken up more easily by cultured cells.
- Cross-linking does not affect cargo loading on DNs.
- Unclear if the encapsulated cargo is still functional after cross-linking.


Thank you!