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Blurring the Role of Oligonucleotides: Spherical Nucleic Acids as a Drug Delivery Vehicle

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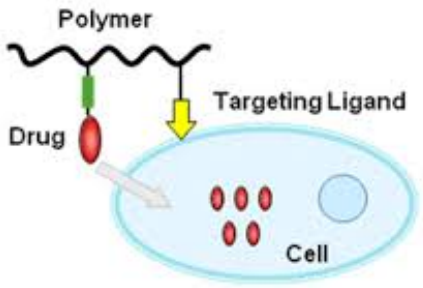
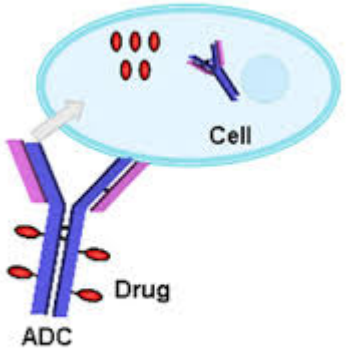
J. Am. Chem. Soc. 2016, 138, 10834–10837

Carrier-Drug Conjugation

- **Carrier-drug conjugation** is a method that brings unique benefits such as consistent drug formulation and increased stability through covalent binding of two components.
- Variety of drug conjugates have been reported, such as, **polymer-drug**, **peptide-drug**, **drug-drug** and **antibody-drug**.
- The conjugation showed **improved drug properties**, such as higher water solubility, longer blood circulation times, enhanced serum stability, cell uptake, and improved targeting.



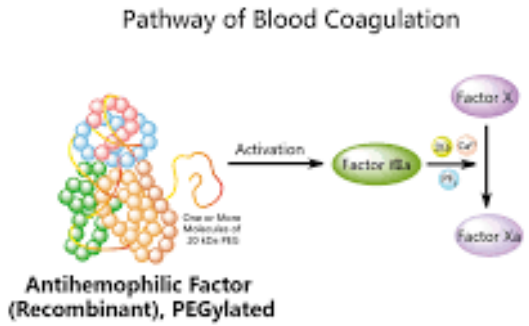
Polymer–Drug–Targeting Ligand Conjugation

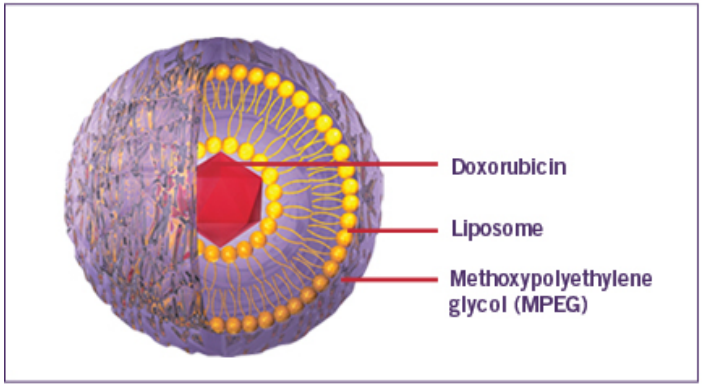


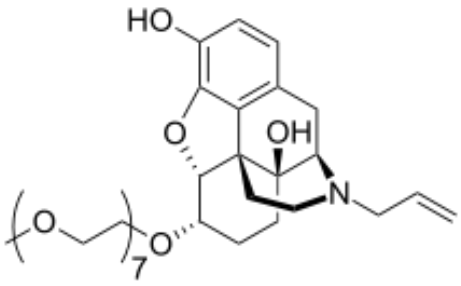
Antibody–Drug Conjugate (ADC)

Polymer Drug Conjugates

- The current polymer drug conjugates market has over 10 approved drug candidates for a wide range of molecular targets and disease areas.
- ➢ Adynovate by Baxalta,
- ➢ Doxil® by Pfizer/ Sun Pharmaceuticals
- ➢ Movantik™ by AstraZeneca
- ➢ Plegridy® by Biogen (pegylated form of **Interferon beta-1a**)







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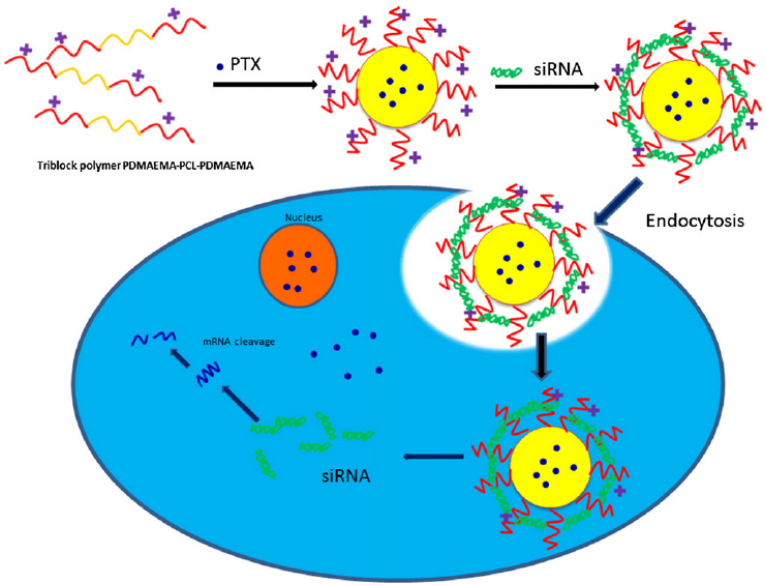
Nucleic Acid-Drug Conjugates (NADC)

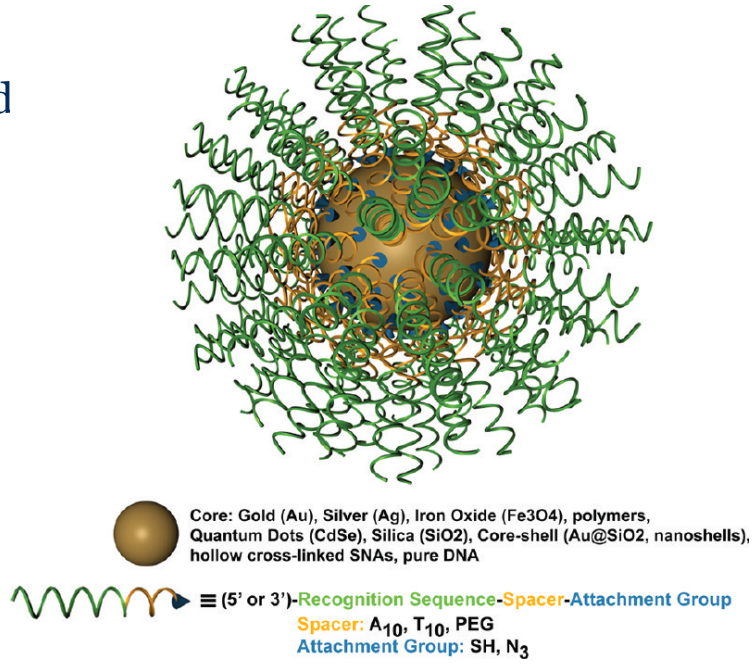
- **siRNA** and **antisence RNA**
- can work together with drugs to address difficult challenges such as **m**ulti**d**rug **r**esistance.
- In many cases, MDR is highly associated with P-glycoprotein (**Pgp**) or antiapoptotic B-cell lymphoma 2 (**Bcl-2**).
- Combination approaches with siRNA or antisence RNA dramatically increases the accumulation of chemotherapy drugs in tumors.



Li, J.; Wang, Y.; Zhu, Y.; Oupicky, D. J. Controlled Release 2013, 172, 589

Spherical Nucleic Acids (SNAs)

- By ordering oligonucleotides into a dense, spherical spatial arrangement (SNAs) nucleic acids can engage in scavenger receptor-mediated endocytosis and be rapidly taken up by essentially all cell types
- SNAs have improved nuclease stability and enhanced binding constant with a complementary sequence.
- These discoveries beg the reconsideration of nucleic acid's role from being a payload to being both a payload and a delivery vehicle.



Joshua I. Cutler, Evelyn Auyeung, and Chad A. Mirkin; J. Am. Chem.Soc., 2012, 134, 1376–1391

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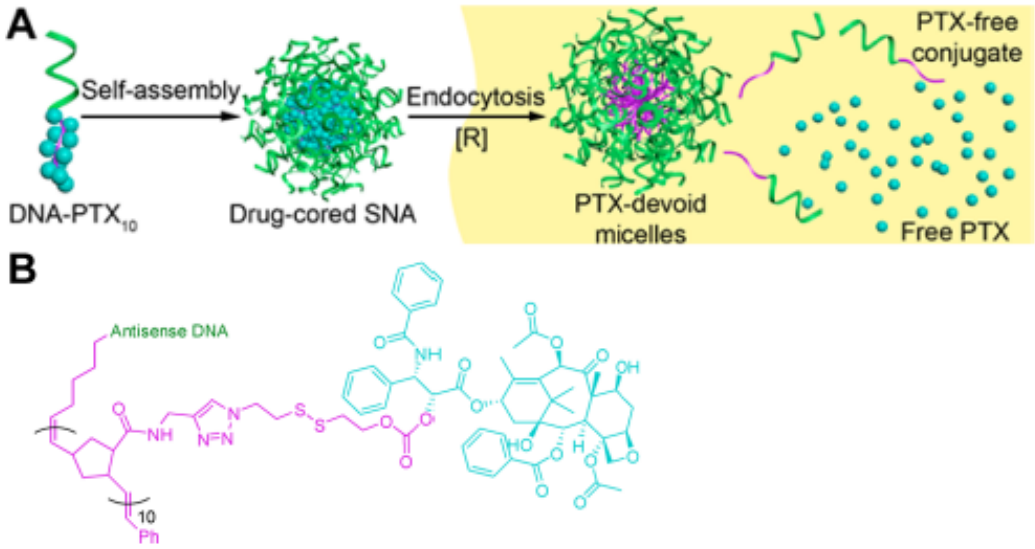
Design

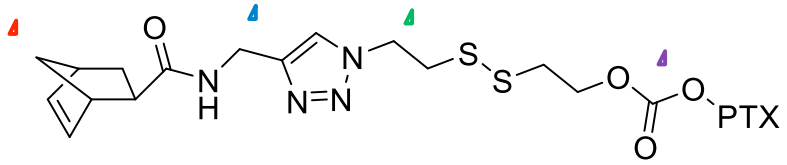
- **DNA-PTX10** - Drug-cored SNA, which exploits the opposing hydrophilicities of nucleic acids and the anticancer drug paclitaxel (PTX).
- By covalently joining the two payloads together, the amphiphilic **NADC** can self-assemble into micellar nanoparticles, which are structurally analogous to SNAs.



Monomer Design

Norbornenyl group for ring opening metathesis polymerization

Linker that contains a disulfide bond which can be cleaved under the reducing environment of the cell

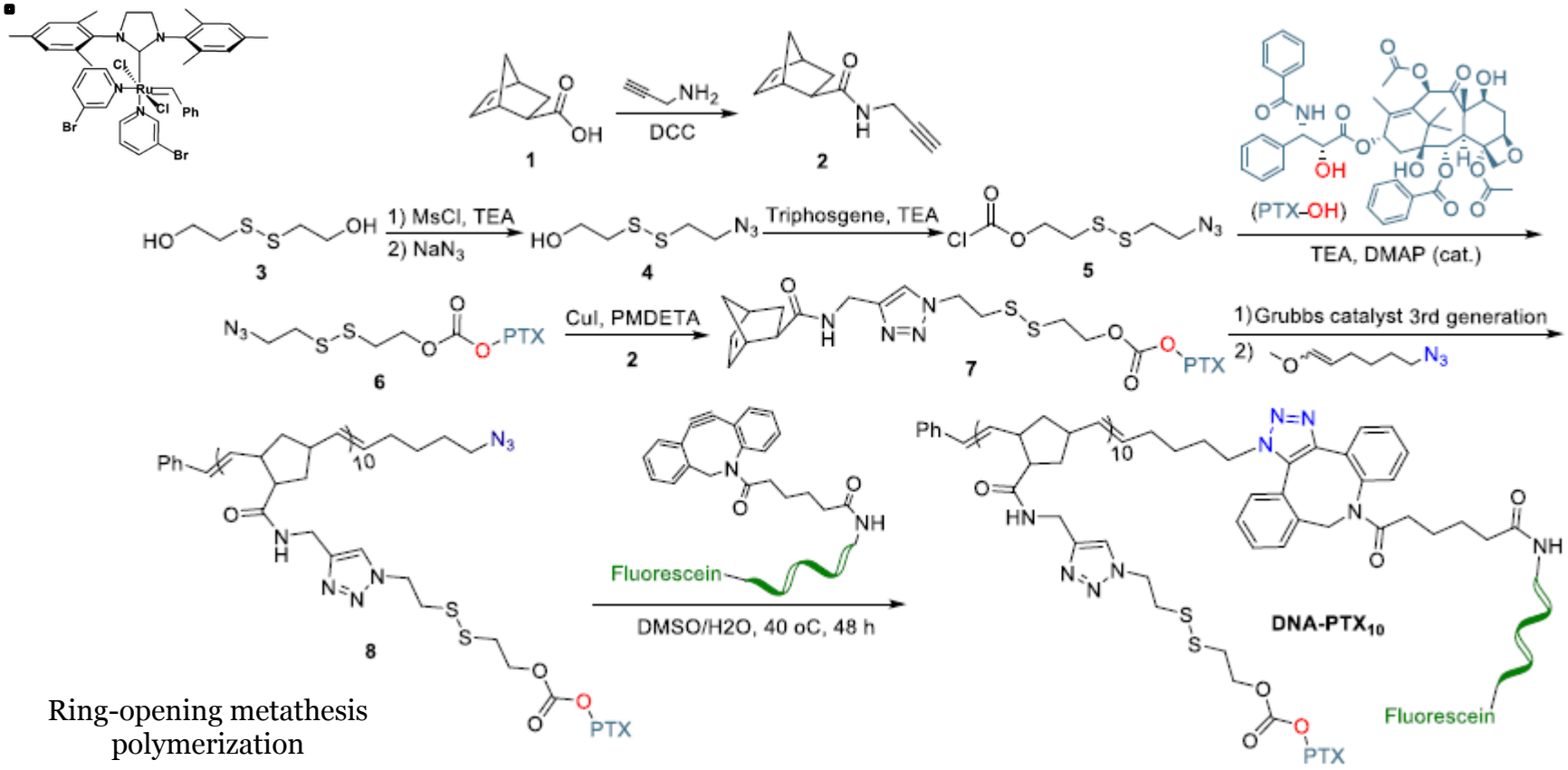


"click" product that connect between two parts of the monomer

Anticancer drug Paclitaxel

Monomer was synthesized in 5 steps with 20% overall yield

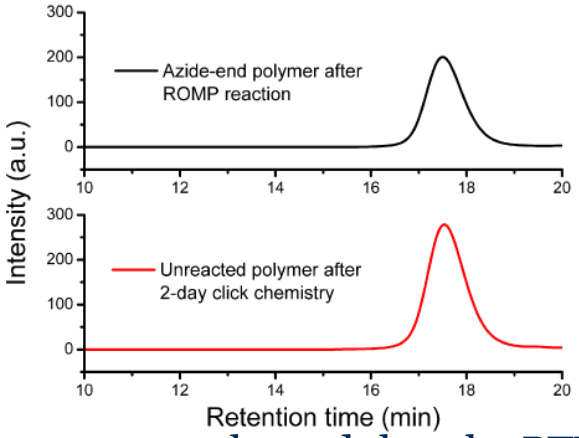
Synthesis



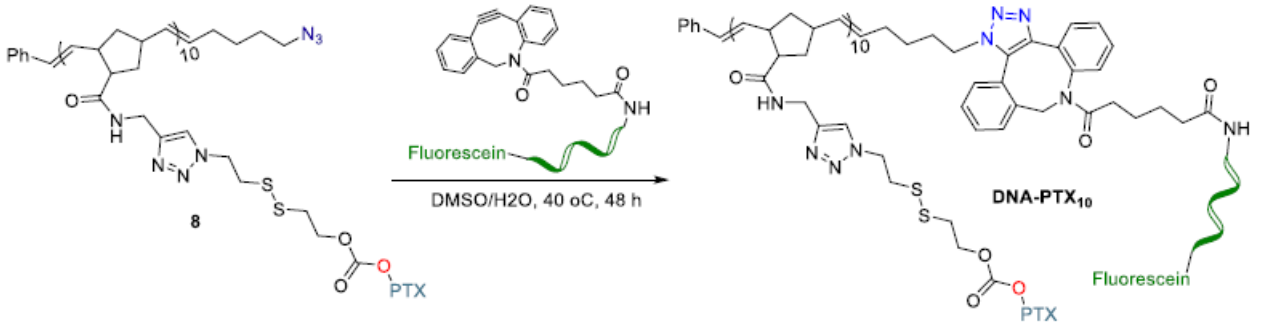
Ring-opening metathesis polymerization

DNA Segment

- For the DNA segment, antisense sequence that targets the antiapoptotic B-cell lymphoma 2 (**Bcl-2**) was used as a proof-of-concept.
- The choice of target stems from the observation that the Bcl-2 protein is often responsible for chemotherapeutic resistance



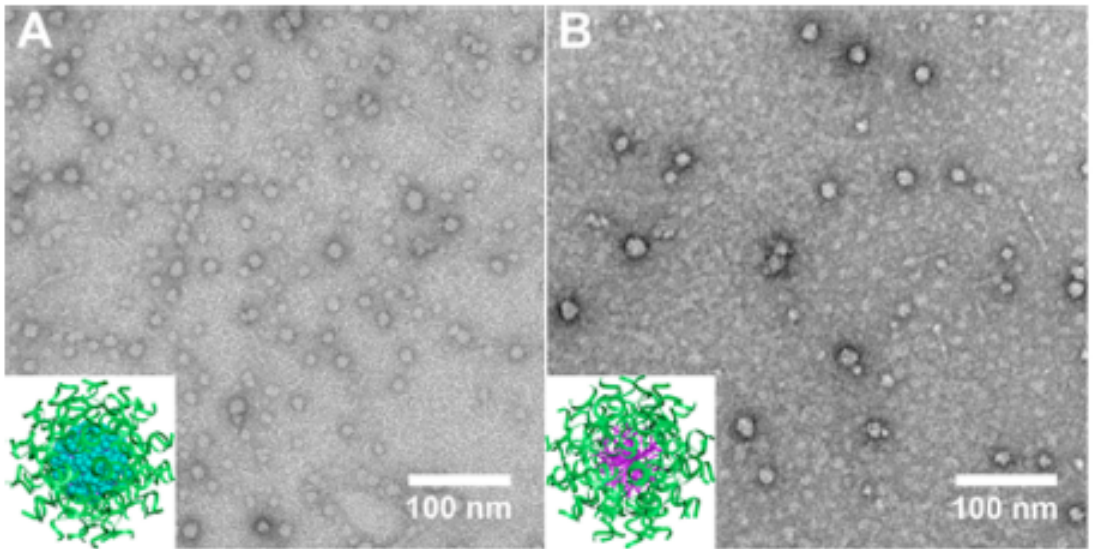
GPC chromatograms showed that the PTX10 polymer was narrowly dispersed (PDI = 1.2, Mw = 13 kDa



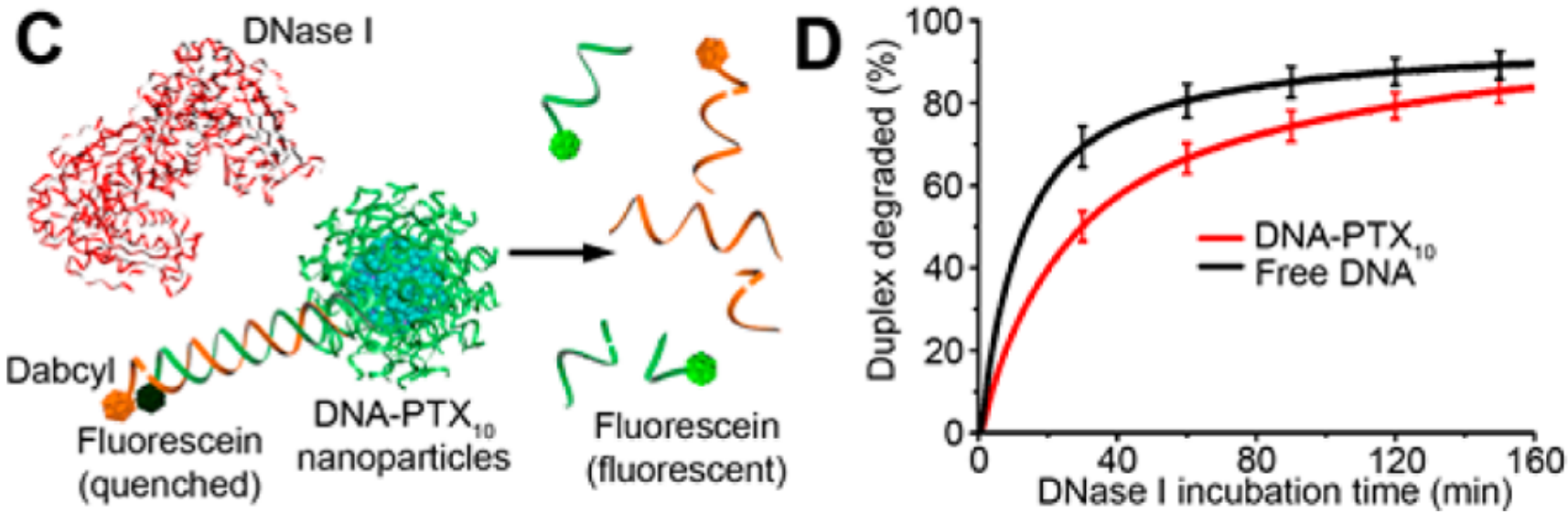
40%

TEM

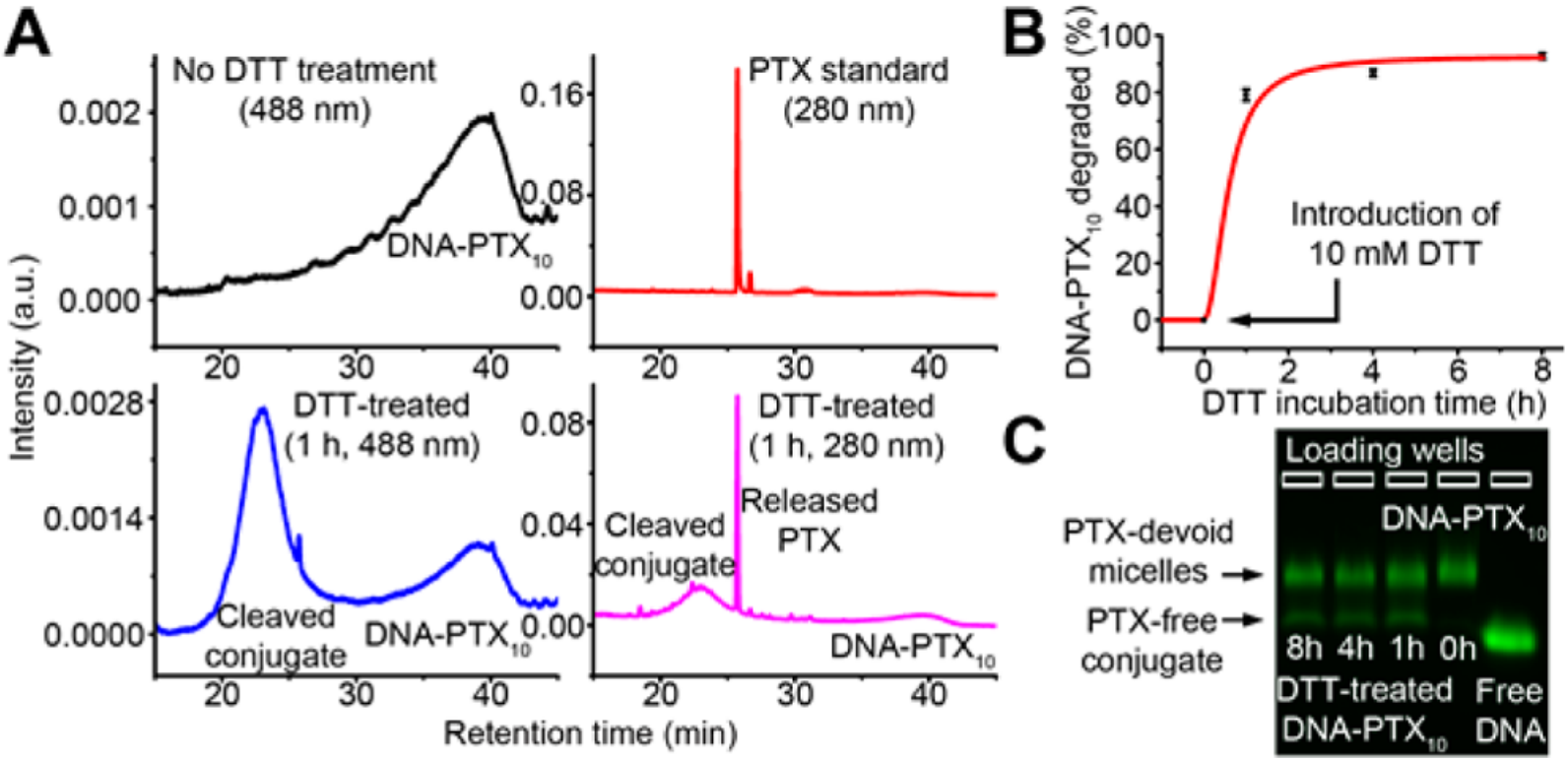
The size of the DNA-PTX10 micelles was characterized by TEM, that clearly shows the formation of uniform spherical nanoparticles with a number-average diameter of 14.2 ± 2.7 nm



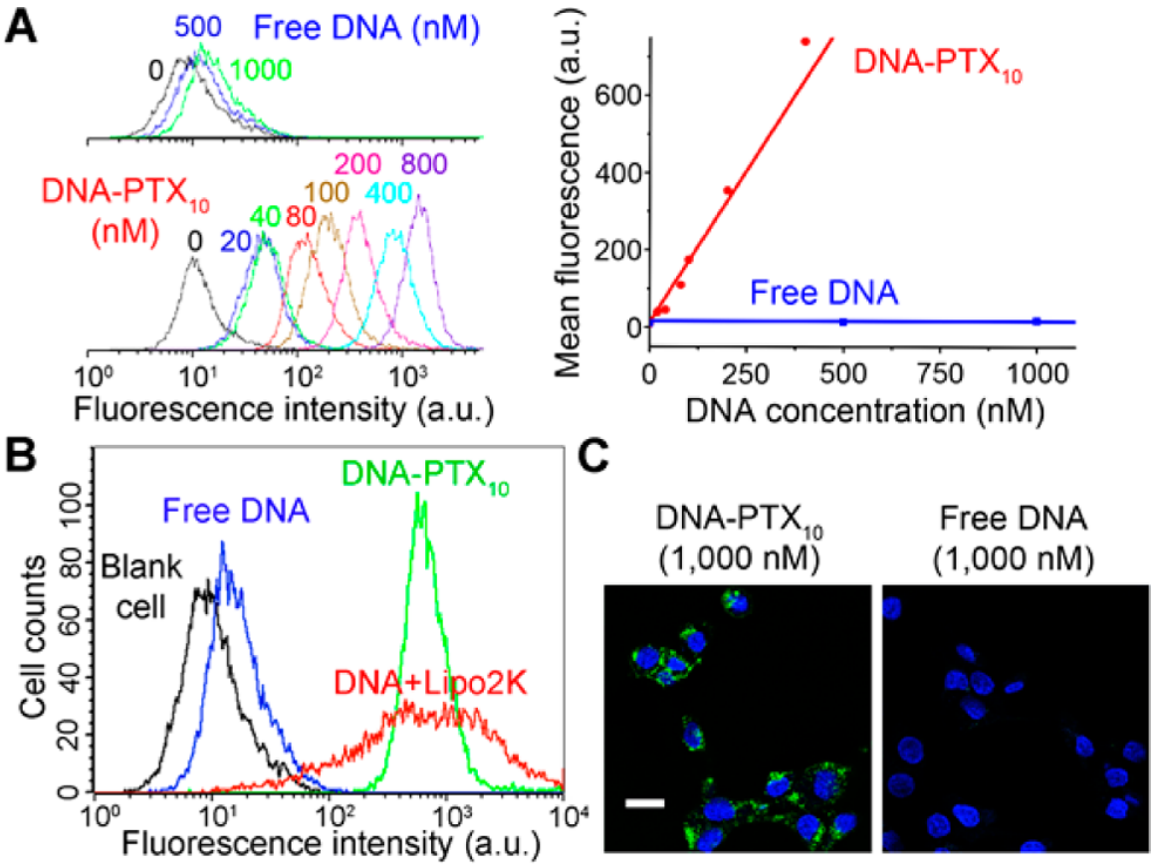
Increased nuclease stability



kinetics of payload release from DNA-PTX10 particles

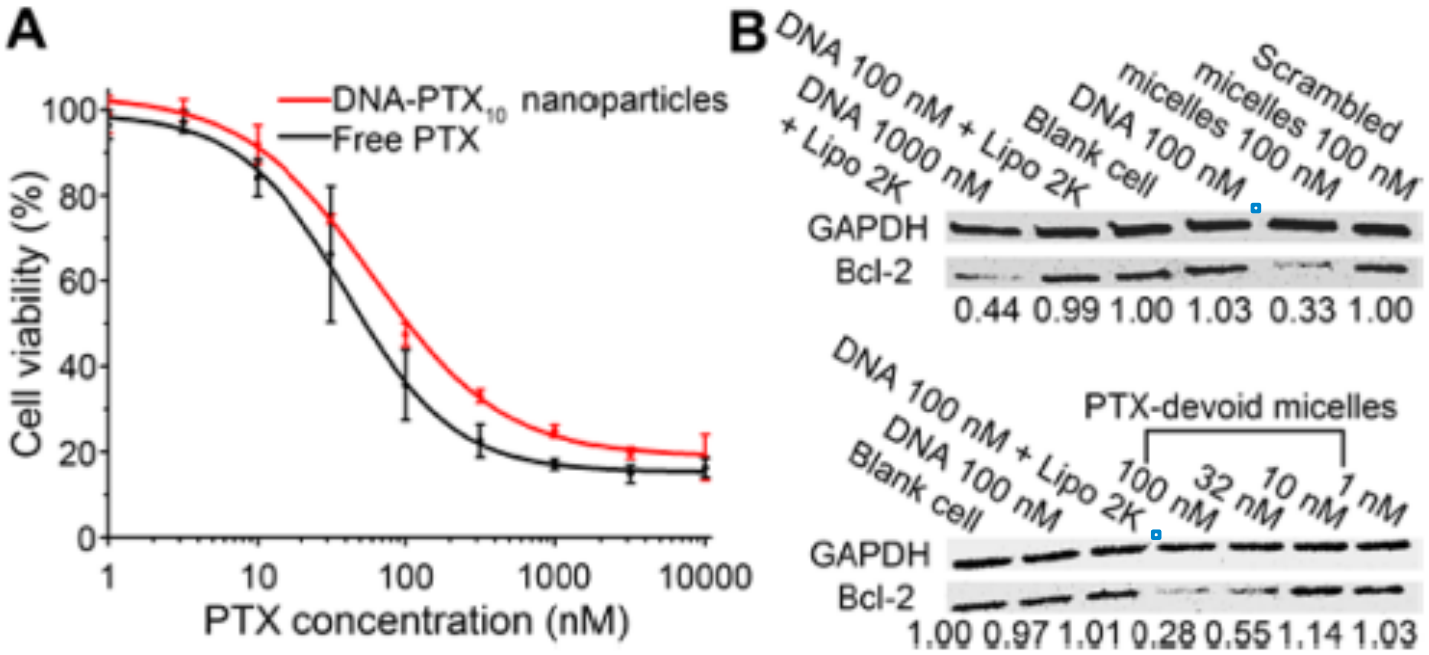


Cell uptake


A
500
Free DNA (nM)
0
1000
DNA-PTX10
(nM)
200
800
100
400
40
80
0
20
10⁰
10¹
10²
10³
Fluorescence intensity (a.u.)
Mean fluorescence (a.u.)
600
400
200
0
DNA-PTX10
Free DNA
0
250
500
750
1000
DNA concentration (nM)
B
DNA-PTX10
Free DNA
Blank
cell
DNA+Lipo2K
Cell counts
0
20
40
60
80
100
10⁰
10¹
10²
10³
10⁴
Fluorescence intensity (a.u.)
C
DNA-PTX10
(1,000 nM)
Free DNA
(1,000 nM)

In vitro efficacy

- IC50 values for free PTX and DNA-PTX10 are 41 nM and 59 nM
- DNA-PTX10 (100 nM) 70% reduction of Bcl-2 expression



Summary

- SNA-like DNA-drug nanostructure that bioreductively activated upon cell uptake was successfully synthesized.
- The drug component allows for the conjugate to self-assemble into a dense, spherical form, which enables otherwise noncellpenetrating nucleic acids to undergo rapid endocytosis.
- Covalently linking the two payloads together, and taking advantage of intracellular reducing environment and self-immolative chemistry, free drugs can be accumulatively released from inside the cell, resulting in excellent retention of the drug's cytotoxicity.
- With the recognition of the nucleic acid as both a vehicle and a payload, the authors anticipate that many more NADC structures will be developed to target a broad range of combination therapies.