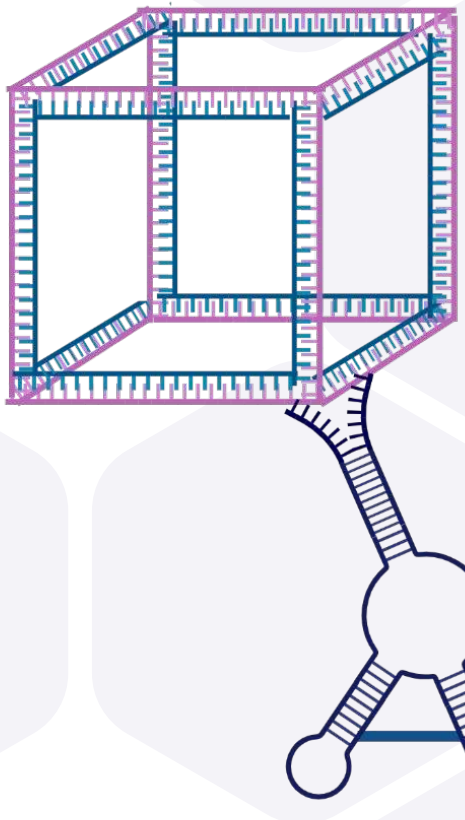


Aptagami

1 Cancer Background

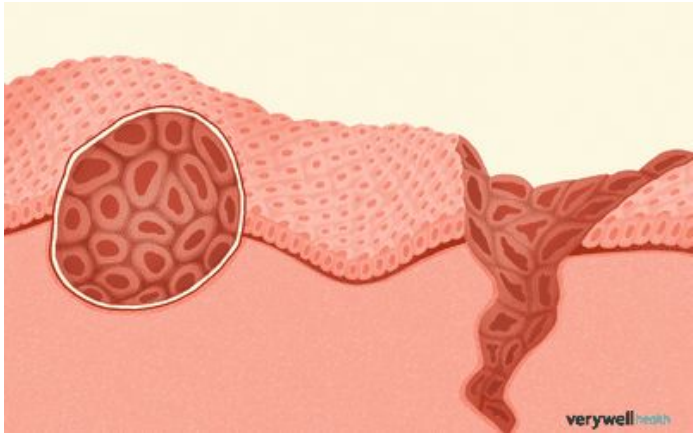
Cancer

Cancerous cells growing uncontrollably

Overexpression of proteins

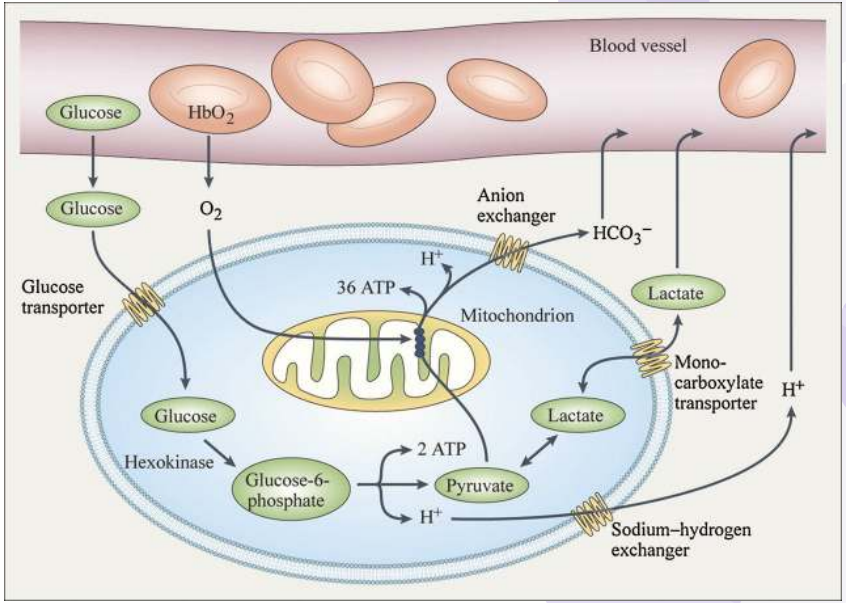
- HER2
- EGFR
- GRP78

pH of the tumours decrease



Cancer Tumour pH

- Production of Lactate & H+
- Increase uptake of glucose
- Warburg effect
- Hypoxia environmental
- Decrease of intracellular pH
- Lactate & H+ secreted
- Decrease of extracellular pH



	Cancer Tumour	Normal Tissue
pH intracellular	7.1-7.8	7.4
pH extracellular	6.4-7.1	7.4

2 Current Cancer Therapeutics

Chemotherapy

- Treat many types
- Fast growing cells
- Whole body

Advantages

- Kill cancer
- Reduce disease recurrence
- Slow progression

Disadvantage

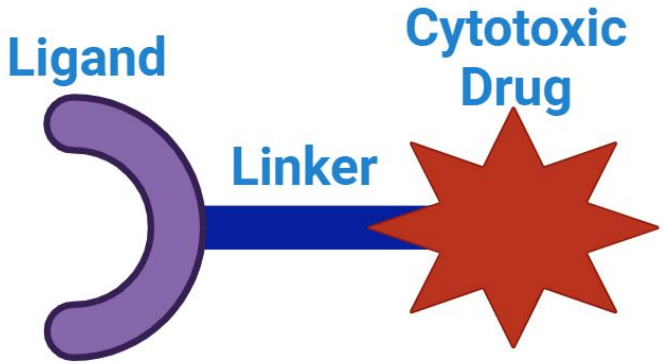
- Side effect
  - Nausea
  - Fatigue
  - Hair Loss
- Damage body

Drug Conjugate Therapy

Target cancer tumours

Comprised of three parts:

- Ligand
- Linker
- Cytotoxic



Antibody-Drug Conjugate

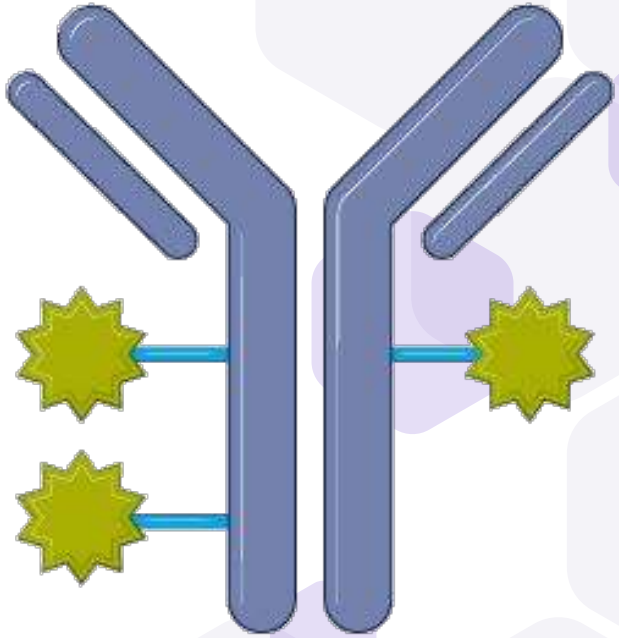
- Uses antibodies
- DAR 2-8
- First approved in 2000
- 13 approved

Advantages

- Target Delivery
- Reduce toxicity
- Enhanced efficiency

Disadvantage

- Off tumor toxicity
  - On target
  - Off target



Antibody- Drug Conjugate Drugs

Drug	Trade Name	Cytotoxin	Drug	Trade Name	Cytotoxin
Gemtuzumab ozogamicin	Mylotarg	Calicheamicin	Trastuzumab emtansine	Kadcyla	DM1
Inotuzumab ozogamicin	Besponsa	Calicheamicin	Mirvetuximab soravtansine	ELAHERE	DM4
Brentuximab vedotin	Adcetris	MMAE	Trastuzumab deruxtecan	Enhertu	Deruxtecan
Polatuzumab vedotin-piiq	Polivy	MMAE	Datopotamab Deruxtecan	Datroway	Deruxtecan
Enfortumab vedotin	Padacev	MMAE	Moxetumomab pasudotox	Lumoxiti	PE38
Tisotumab vedotin-tftv	Tivdak	MMAE	Loncastuximab tesirine-lpyl	Zynlonta	PBD
Sacituzumab govitecan	Trodelvy	Govitecan			

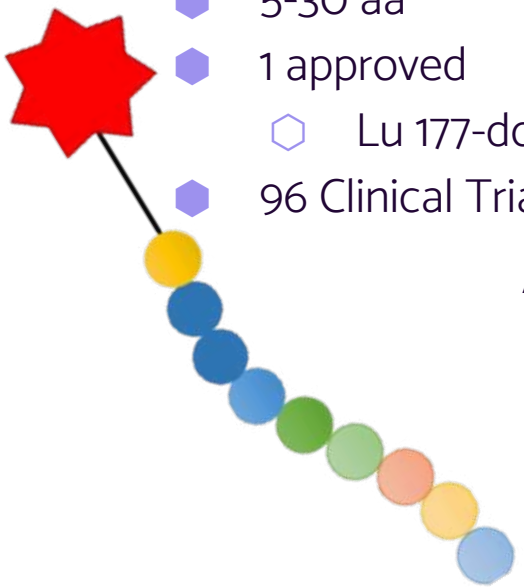
New Drug Conjugate Therapy

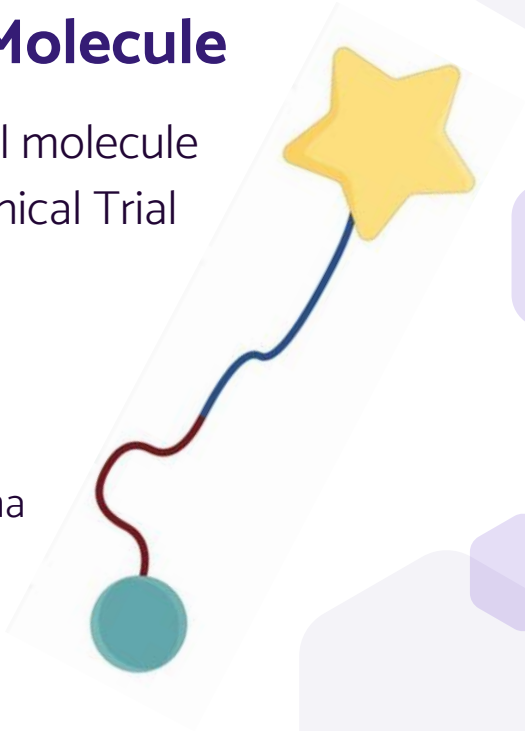
Peptide

- 5-30 aa
- 1 approved
  - Lu 177-dotatate (Lutathera)
- 96 Clinical Trial

Small Molecule

- Small molecule
- In clinical Trial





Advantage

- Penetrate tumour stroma

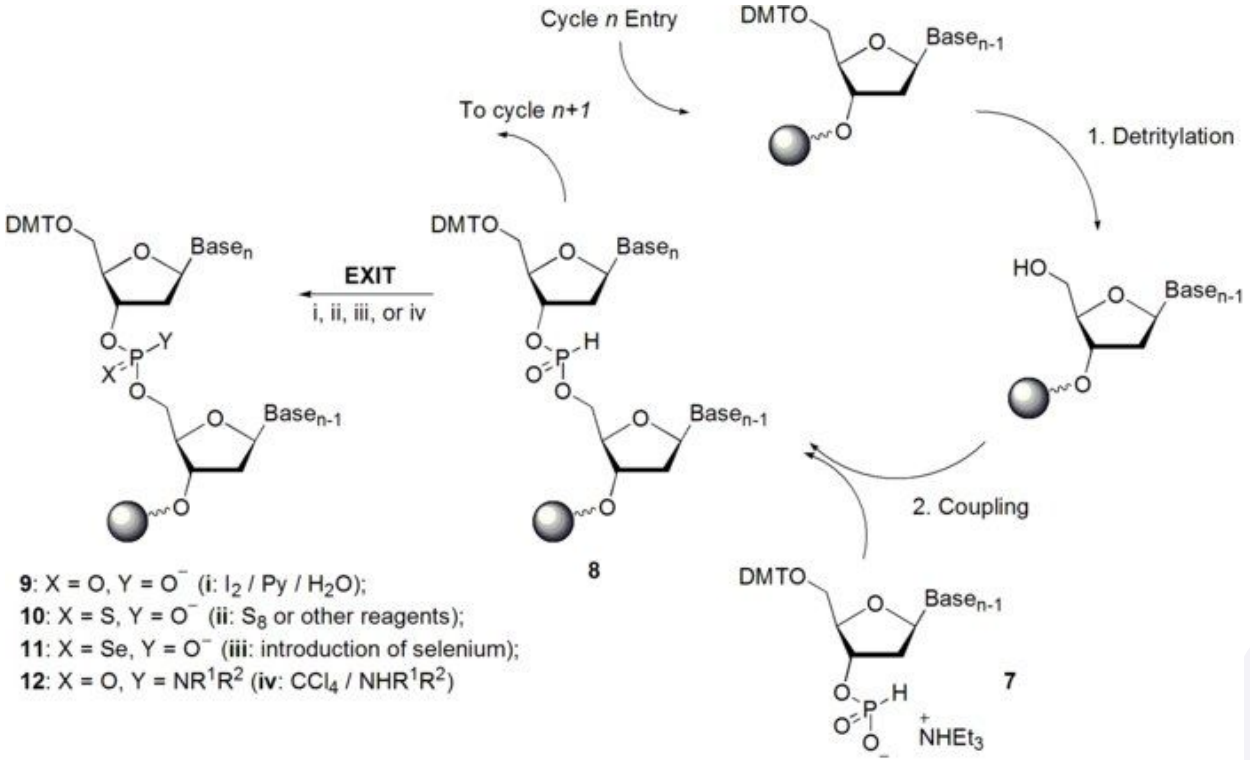
Disadvantage

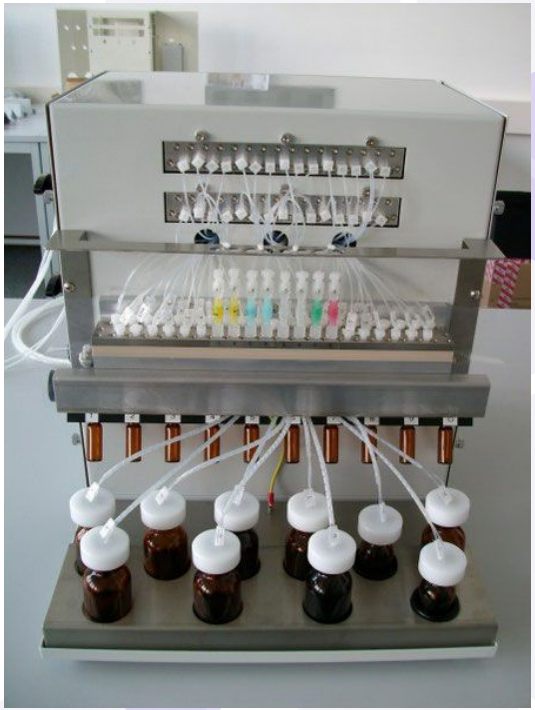
- Rapid clearance

3

Chemical Synthesis and Analysis of Oligos

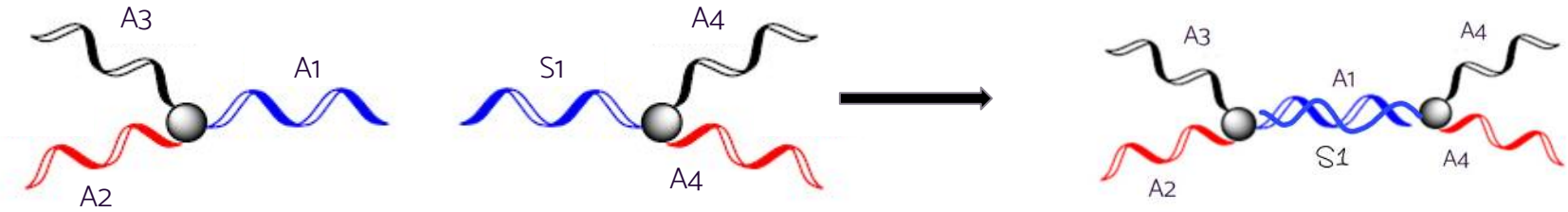
Chemical Synthesis Method



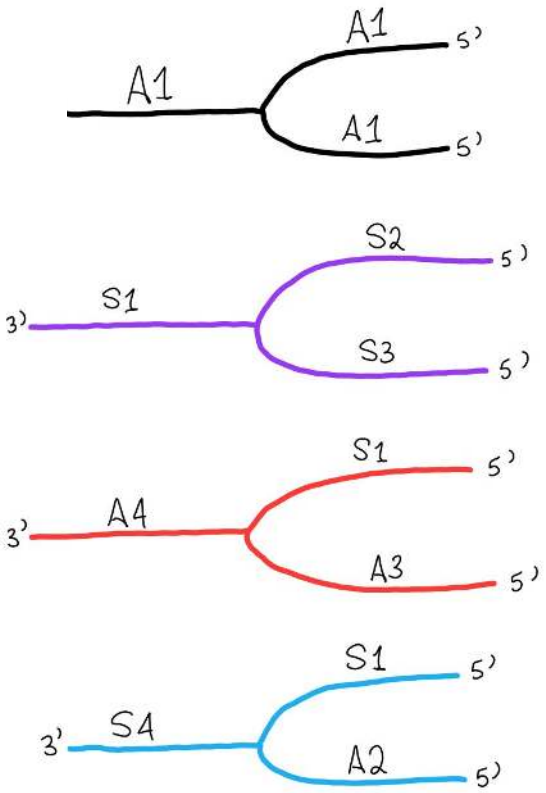


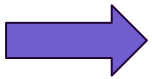
Modifications to Oligonucleotides

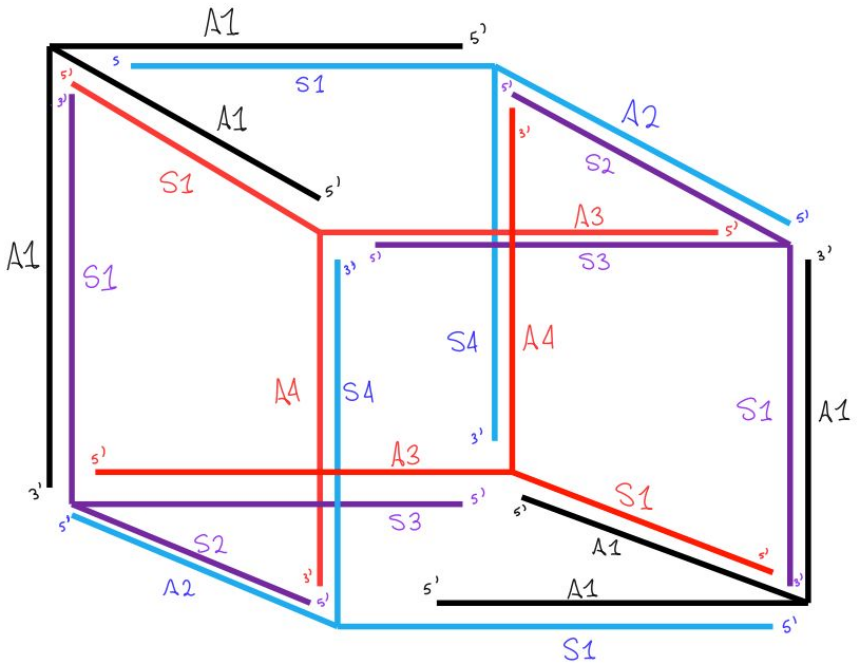
- **Fluorescent Labels:** Imaging and tracking
- **Linkers:** Chemical groups that enhance stability or controlled release
- **Branch points:** Multi-stranded oligonucleotides



Nanotube structure proposal



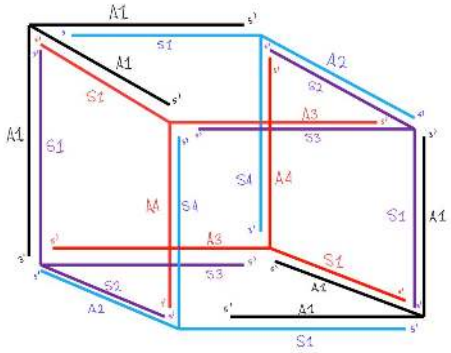


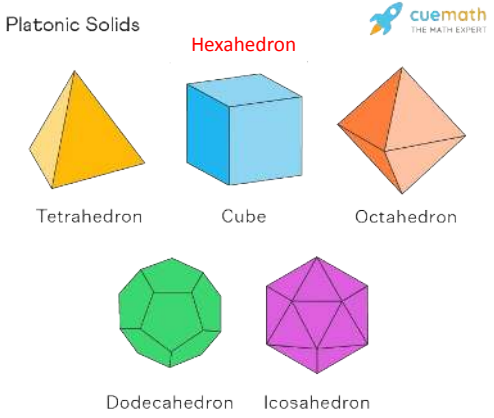


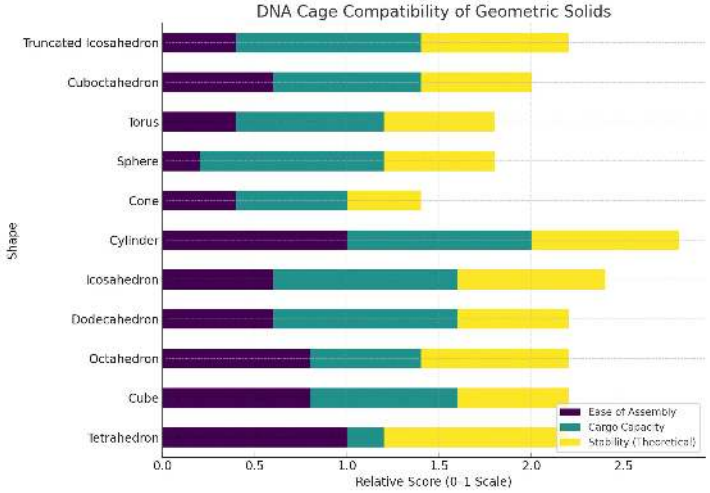
3.5 Energetics and Negotiations

A solid Justification

- Going for the Platonic Solids
- Regular polyhedral, same type of face [easier assembly]
- Archimedean solids have more than one type of polygon as face [Difficult for large scale]
- Prisms/Cylinders another good option, but has a surface [Expensive]
- We are going for an easy wireframe design







How many strands we need?

- Easy synthesis of Y-Shaped strands

Assumption

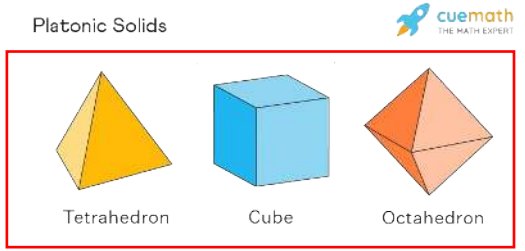
- 3 arm per Y strand
- No self binding; each arm connect to different arm

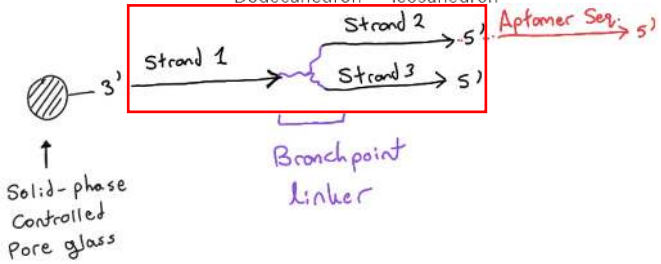
Proof

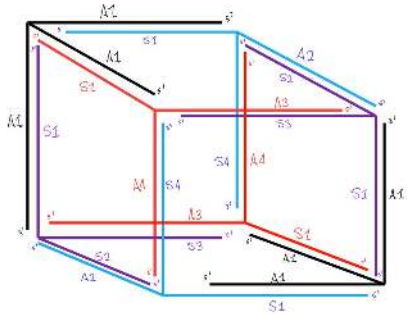
Each Y Strand contributes 3 arms
Each edge is made of 2 arms
Total Arms = 2 x Total number of edges (E)

Number of Y strands = 2E/3

- Tetrahedron needs 4, cube needs 8, octahedron needs 8, the remaining need 20
- By symmetry, we only need half the total number of strands as different strands.
- The first 3 solids are the best candidate for simplicity of folding and large-scale production

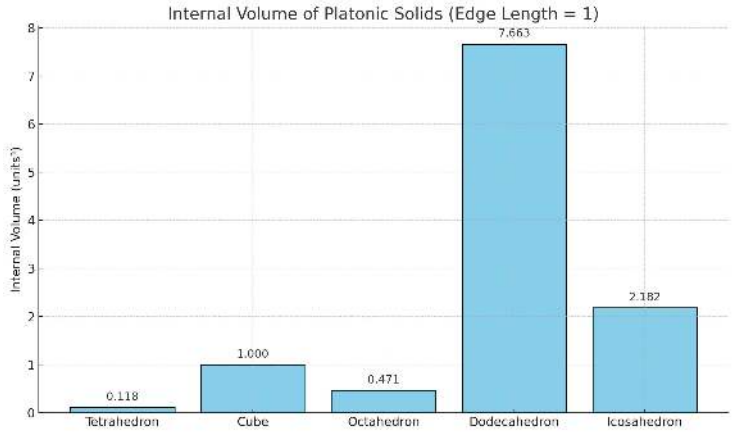






Internal Volume

- Tetrahedron has 12% volume of cube
- Octahedron requiring 8 strands too, has only 48% volume of cube
- Dodecahedron has highest volume
- But complex synthesis
- Non-specific binding becomes more important
- Cube is good enough



Solid	Volume
Tetrahedron	$\sqrt{2}\frac{a^3}{12}$
Hexahedron	a^3
Octahedron	$\sqrt{2}\frac{a^3}{3}$
Dodecahedron	$\frac{\left(15+7\sqrt{5}\right)a^3}{4}$
Icosahedron	$\frac{5a^3\left(3+\sqrt{5}\right)}{12}$

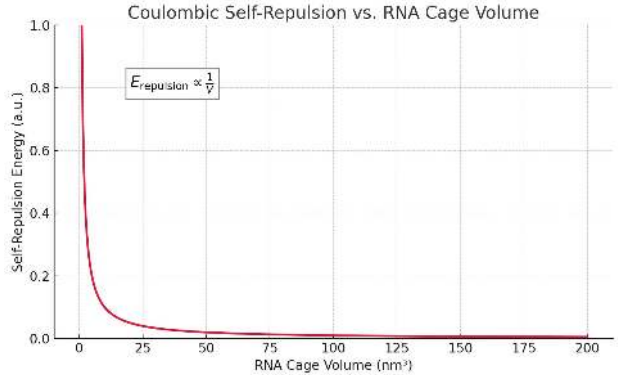
Energetics of the RNA Cage

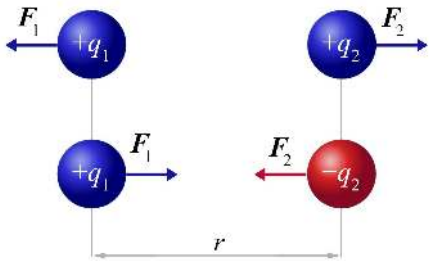
$$G = U - TS$$

- Free energy depends on 2 terms
- Enthalpy (H) (constant pv) [Simplifies to internal energy U]
- Entropy term (S)
- At minimum free energy, G = 0 [derivative wrt any desired parameter]
- U = TS
- Contributors of U are cosmos inducing phenomena
- Ensure cage will form around substrate and won't spontaneously unfold
- Contributors of S are chaos inducing phenomena
- Ensure cage has enough flexibility and won't brittle under noise

U-Coulombic considerations

- Did not find papers explicitly referring to charge considerations.
- Most made use of oxDNA simulation software to assess energetics
- Based on self-repulsion cause more instability to structure
- RNA backbone negatively charged
- Increasing volume decreases charge density, increasing stability
- Negative contributor to U
- Tetrahedron< Octahedron< Cube [For same edge length]
- Despite difference seeming small. At small scales it is important





$$|F_1| = |F_2| = k_c \frac{|q_1 \times q_2|}{r^2}$$

U-Mechanical Energies

- Bending, Stretching, Twisting
- Springy cage
- Watson-Crick Base pairing contributes to enthalpy by bond formation (exothermic)
- Increasing GC content increases rigidity of structure
- High rigidity given by shorter length

- Tightness/Strain given by high angle of polyhedron
- Strain from low vertex count
- Octahedron < Cube < Tetrahedron (Strain)

- These are positive contributors to U

Canham-Helfrich

$$U_b = \frac{1}{2}\kappa \int (\kappa_1 + \kappa_2 - 2\kappa_0)^2 dA$$

$$U_s = \frac{1}{2}k_s \sum_i (\ell_i - \ell_0)^2$$ Hooke

$$U_t = \frac{1}{2}k_t \sum_i (\theta_i - \theta_0)^2$$ Torsional Pendulum Potential

S- Length of Edges and Vertex Count

- What U loses, S gains
- Length increases number of microstates
- Cage retains identity in more ways
- Flexible and can bend without breaking apart (Adaptable structure)
- Entropy acts as a buffer to the cage
- Shock Absorption

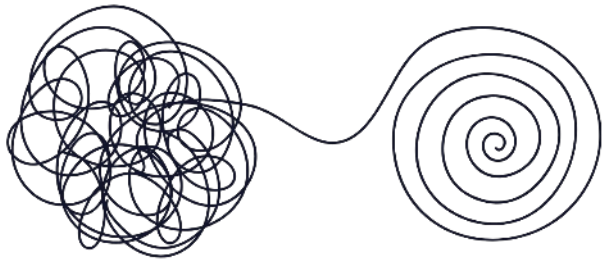
- Increase vertex count relaxes structure
- Octahedron > Cube > Tetrahedron (Relaxation)

$$S = k_B \ln \Omega$$

Balance

$$\frac{dG}{dV} = 0$$

- We desire rigidity for stability of structure but not too rigid so that it becomes fragile and brittle
- We need an optimum length
- We want high angles to reduce strain on molecule but not too high so that it becomes flimsy
- We need an optimum angle
- We also want a good amount of volume and simple synthesis



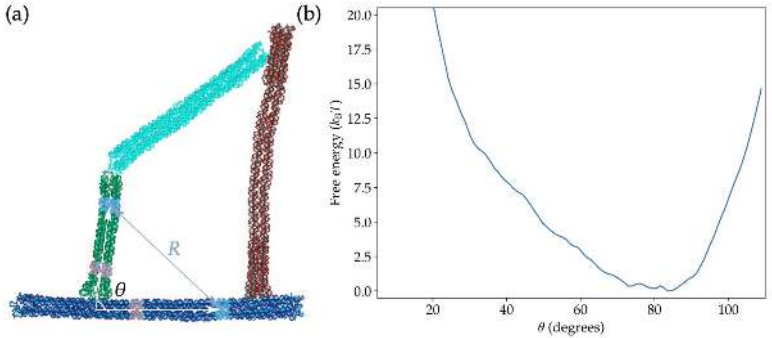
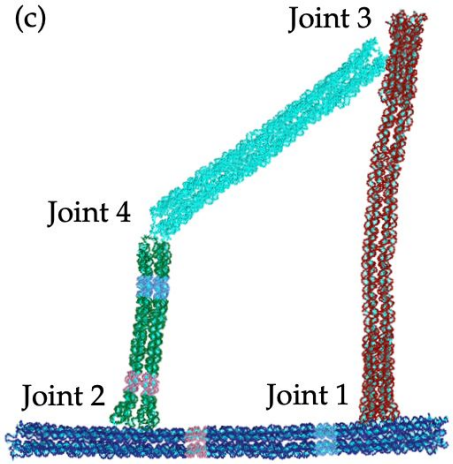
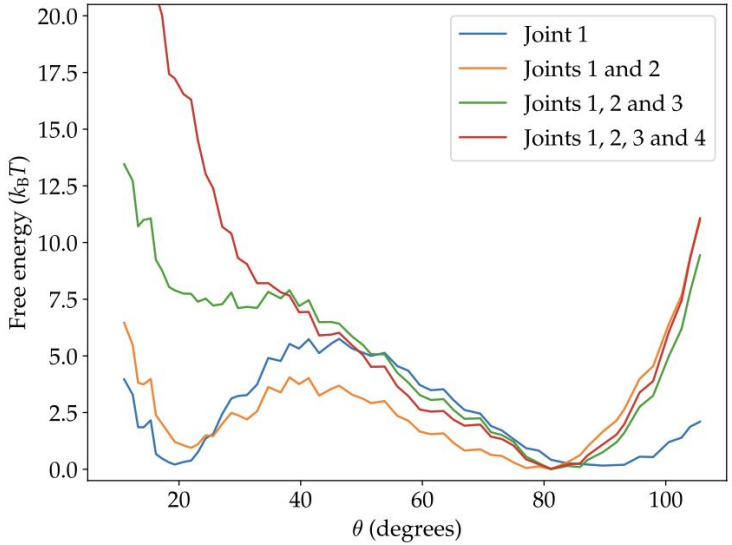


FIG. 2. **(a)** A configuration of the origami illustrating the definition of the order parameter R as the distance between the centres of mass of the two groups of nucleotides coloured in light blue. The value of θ was tracked in the simulations, and is defined as the angle between the two vectors coloured in white. The vectors pass through the centres of mass of the two groups of nucleotides coloured in light red and light blue in the crank block and the frame block, respectively. **(b)** The free-energy landscape of the origami as a function of θ.



- Paper designed an origami with different types of joints: rigid, hinges, crank, coupler
- Total free energy minima was still at 80°
- Despite RNA's relaxed structure closer to tetrahedron 109.47°
- Square at 90° is good enough
- Also, we get more volume



Wong, C. K., & Doye, J. P. K. (2022). The Free-Energy Landscape of a Mechanically Bistable DNA Origami. *Applied Sciences*, *12*(12), 5875. https://doi.org/10.3390/app12125875

Some minor problems and solutions

- Despite cube showing good angles
- It is prone to shearing and adopting a parallelogram form
- We increase rigidity by using short edge length of 20 bp
- We'll also experiment with linkers between arms to decrease strain and get closer to octahedron stability
- This with the stability at 80-90° will make structure stable during journey in plasma and only open at low pH by the cis-Acontyl linkage
- Loading cargo in the cube will also bulk it to prevent structural collapse
- Symmetric inclusion of aptamers will balance structure while providing guide to target

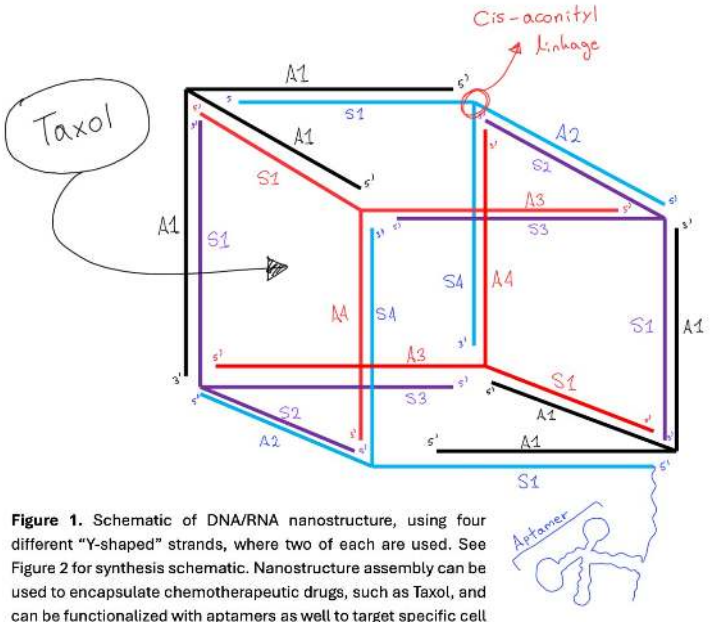
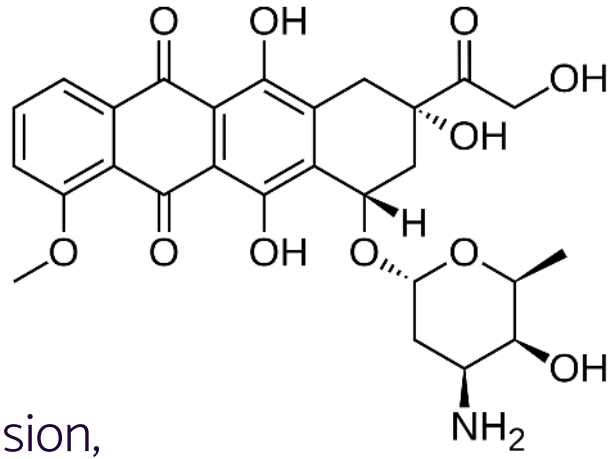


Figure 1. Schematic of DNA/RNA nanostructure, using four different "Y-shaped" strands, where two of each are used. See Figure 2 for synthesis schematic. Nanostructure assembly can be used to encapsulate chemotherapeutic drugs, such as Taxol, and can be functionalized with aptamers as well to target specific cell

Dimensions

- 20 bp x 20 bp x 20 bp
- 175.7 nm^3
- Vol.(Doxorubicin) = 1.3 nm^3
- Theoretical 135 molecules per cage
- Practically, 50 (Excluded volume, Steric Repulsion, Electrostatic Repulsion)
- Good Strand ΔG values
- Entropy driven folding will ensure enough drug is captured while maintaining stability



Drug Loading

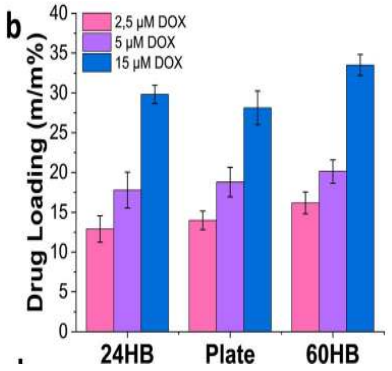
- Many previous studies mentioned intercalation loading
- Host Guest Chemistry
- Mixture containing Positively charged drug to remain in DNA cage
- Doxorubicin, Daunorubicin, ACT-D, Vincristine
- Mg^{++} to help Hybridization of arms, Tris-HCl, NaCl
- 80°C heat do disrupt secondary structures then cool down slowly to RT
- Good Strand ΔG values
- Entropy driven folding will ensure enough guest is captured while maintaining host structure stability

a

	24HB	Plate	60HB
Molecular Mass [MDa]	4.7	4.7	3.9
No. of base pairs	7560	7222	5460
CG-content [%]	42.5	42.2	41.4
Dimensions [nm]	12 x 115	40 x 55 x 6	20 x 20 x 31
Aspect ratio	9.6	9.2	1.6

24HB Plate 60HB

b



Paper showed loading was dependent on initial concentration of DOX and less on structure
Loading protocol like ours
34% mass/mass

Klose, A., Gounani, Z., Ijäs, H., Lajunen, T., Linko, V., & Laaksonen, T. (2024). Doxorubicin-loaded DNA origami nanostructures: stability in vitreous and their uptake and toxicity in ocular cells. *Nanoscale*, *16*(37), 17585–17598. https://doi.org/10.1039/d4nr01995d

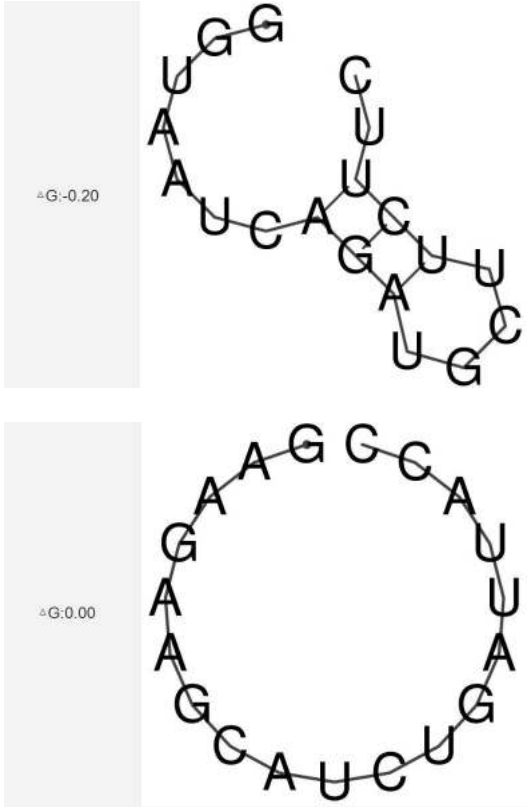
Potential Challenges with synthesis…

Secondary structures ⟶ hair pin loops!

- Avoid through minimizing mean free energy during sequence design

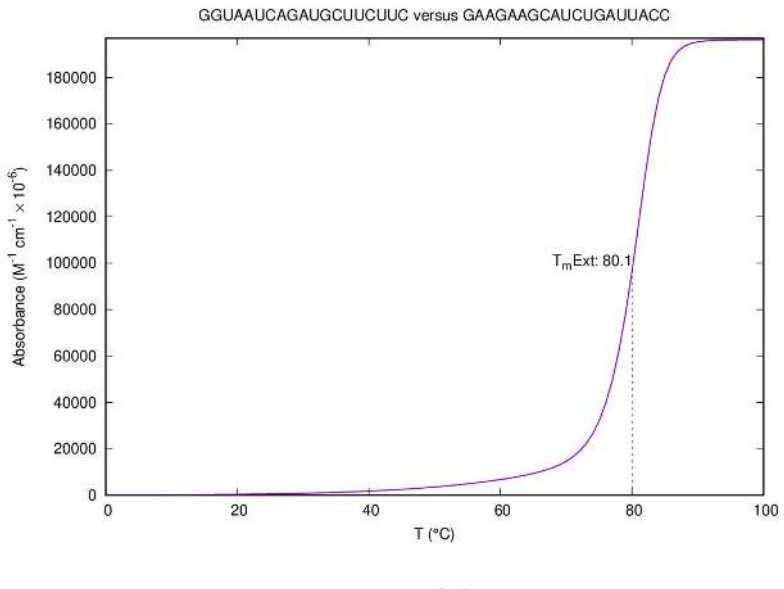
Ex. A1: 5′- GGU AAU CAG AUG CUU CUU C -3′

Ex. S1: 5′- GAA GAA GCA UCU GAU UAC C -3′



https://en.vectorbuilder.com/tool/dna-secondary-structure/fb45904b-a259-4927-8cde-26743ab21e3e.html

Determining Complementary Binding Kinetics



T_m(Conc): 80.8°C

ΔG: 45.9 kcal/mol ΔH: 201.2 kcal/mol ΔS: 570.8 cal/mol/K $T_m(C_p)$: 82.1°C

T_m(Ext1): 80.6°C T_m(Ext2): 80.1°C

$\Delta G_{Hybridization}$ = -192.05 kJ/mol

Very Stable!

Determining off-target hybridization

A1	5′- GGU AAU CAG AUG CUU CUU C -3′
S1	5′- GAA GAA GCA UCU GAU UAC C -3′
A2	5′- UUG UAU UCU CCG AGU CAG U -3′
S2	5′- ACU GAC UCG GAG AAU ACA A -3′
A3	5′- AUC AGA AUC UUC CAA CAC U -3′
S3	5′- AGU GUU GGA AGA UUC UGA U -3′
A4	5′- GCU CAA UAA GGC CAA GUU U -3′
S4	5′- AAA CUU GGC CUU AUU GAG C -3′

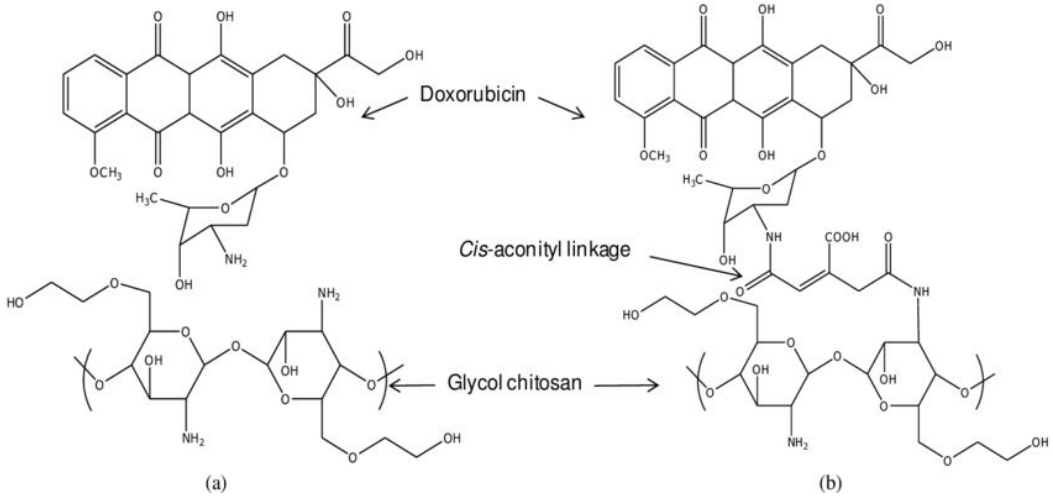
	A1	S1	A2	S2	A3	S3	A4	S4
A1	32.22	192.05	61.51	60.668	42.68	88.284	60.25	62.76
S1		24.27	53.97	53.13	72.38	69.88	52.3	54.81
A2			29.30	193.72	39.75	74.06	57.3	59.83
S2				28.87	39.33	73.22	56.48	59.41
A3					10.46	184.93	38.07	41.00
S3						44.77	72.38	75.31
A4								187.44
S4								

All values are in kJ/mol

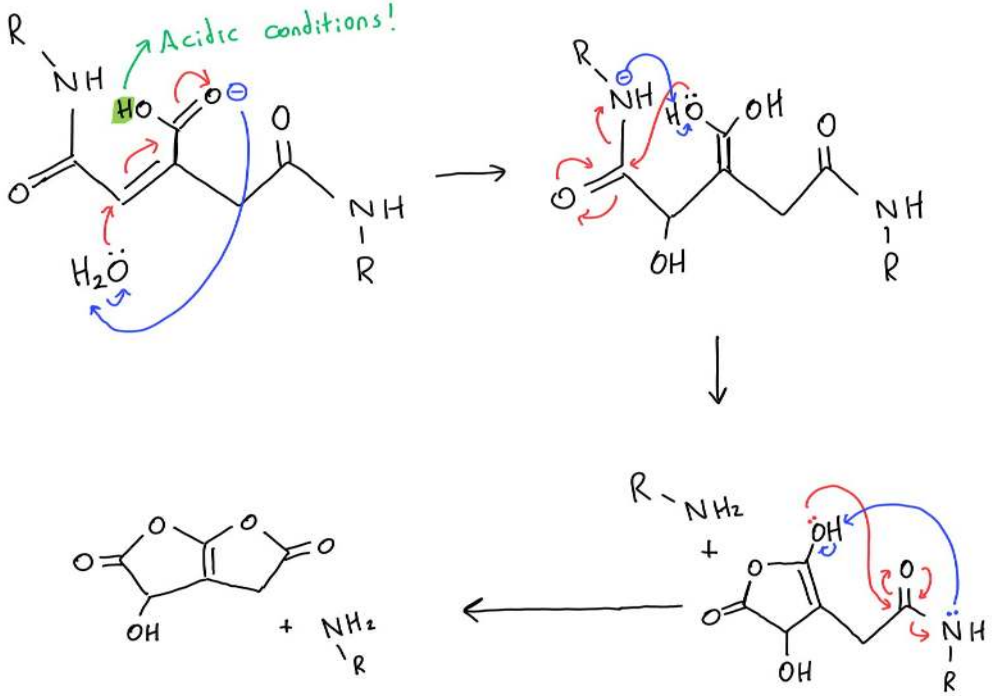
Off-target binding is not as favourable

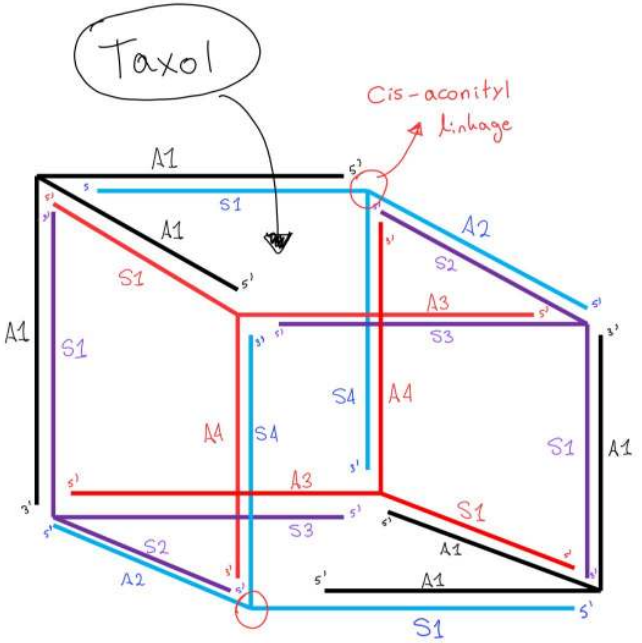
Controlled Release - Cis-aconityl Linkages

- Cis-aconityl linkages hydrolyze in slightly acidic conditions (pH 6.5)
  - Antibody-drug conjugates → Doxorubicin & Daunomycin
  - Protein modification of BSA conjugated to Doxorubicin
  - Doxorubicin conjugated glycol chitosan polymers

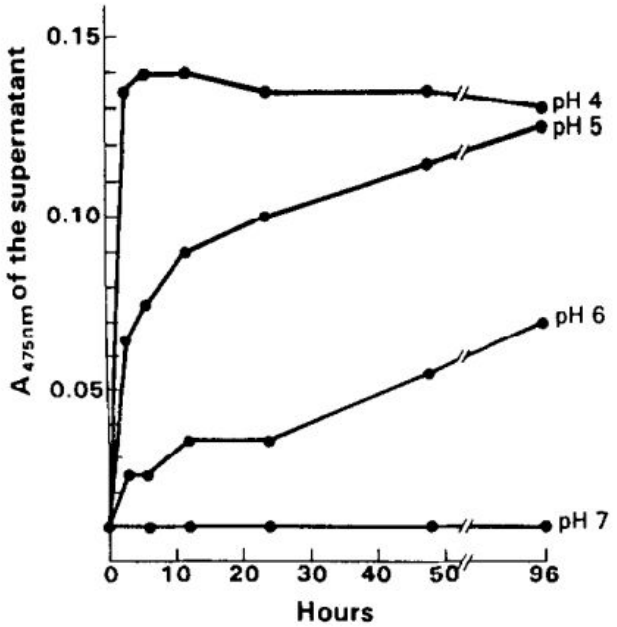


Cis-aconityl breakage mechanism





Linkage strength across a range of pH



Half-life of 96 hours at pH 6
Relatively stable at pH 7

Tumour microenvironment ~6.5
Normal tissue/blood pH = 7.35

Controlled, steady release of drugs

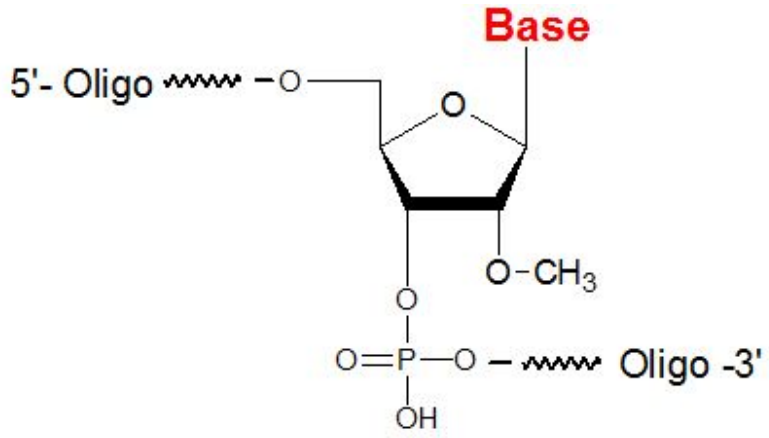
Reactions performed at 37°C in citrate-phosphate buffer conditions

Wei-Chiang Shen, Hugues J.-P. Ryser

Improving in-vivo stability of RNA Cube

Prevent premature degradation of RNA nanostructure by RNAses:

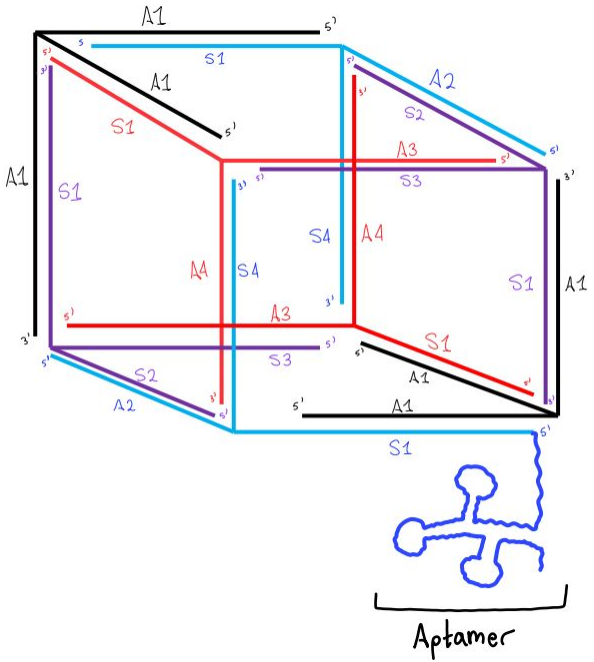
- 2’ O-methyl modifications



Shown to further increase RNA:RNA duplex stability

Connecting it all together…

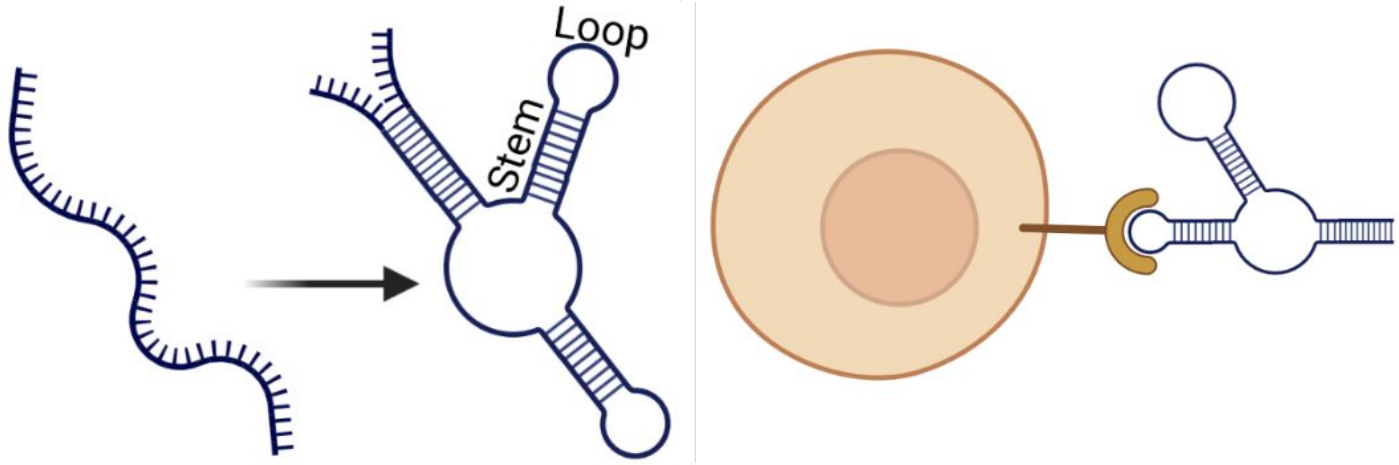
DNA Aptamer Modification to RNA nanostructure-drug payload



Ex. HeA2_3 DNA aptamer

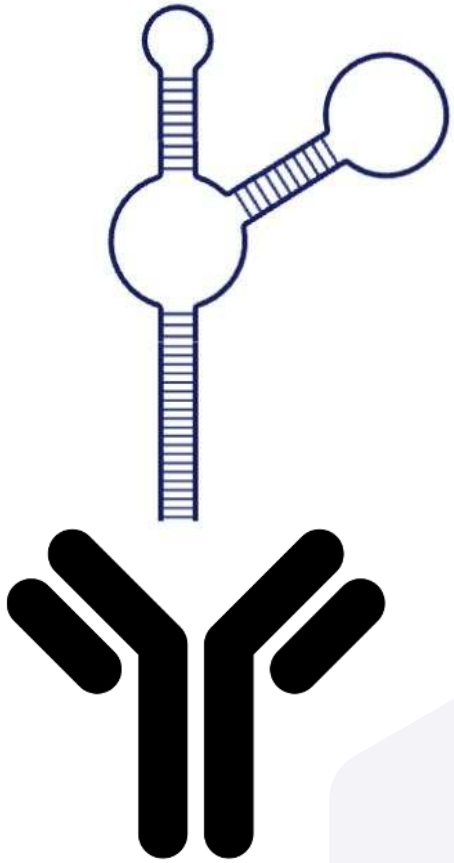
- Binds to HER2+ cancer cells
- Undergoes receptor-mediated endocytosis

Aptamers

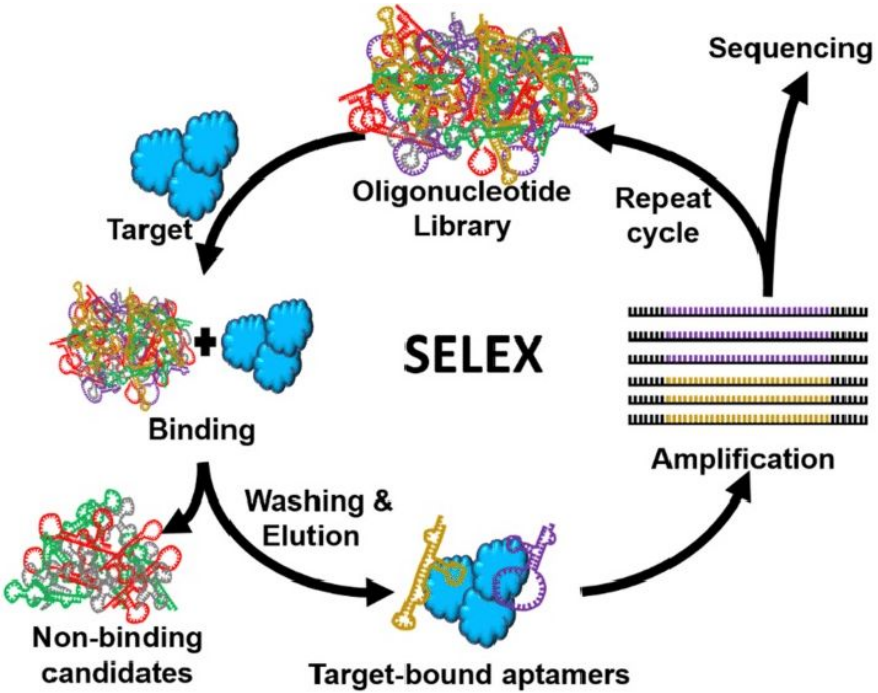


Why Aptamers?

- More stable
- Shorter time to generate
- Simple inexpensive process
- Smaller in size
- Introduce anywhere in nucleotide chain
- Optimize the recognition sequence



Systematic Evolution of Ligands by Exponential Enrichment (SELEX)

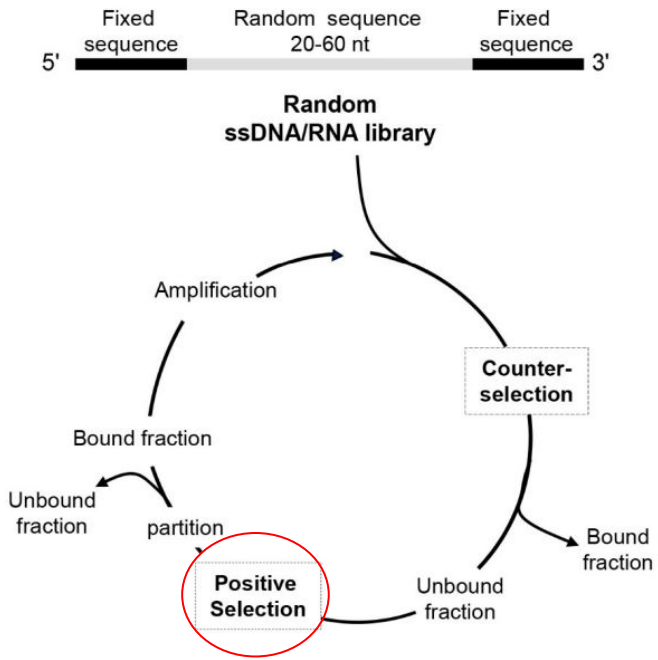


Library Preparation: known oligonucleotide primer sets

Binding: Protein (ie. VEGF) immobilized onto a surface

Selection: Sequences binding strongly to cancer proteins will stay attached, non-binders will wash away

Systematic Evolution of Ligands by Exponential Enrichment (SELEX)



Elution + Amplification: Using PCR/RT-PCR for the sequences with affinity for the target

Repetition: Repeat this cycle

Sequence: Next-Gen, or sanger, test for functionality, binding affinity

SELEX At Work: Altering Positive Selection

- Refining aptamer specificity to specific cancer cell proteins
- Discovering cancer biomarkers -> **PERSONALIZED MEDICINE**
- Applicable for stem cells, aggressive tumours, drug-resistant

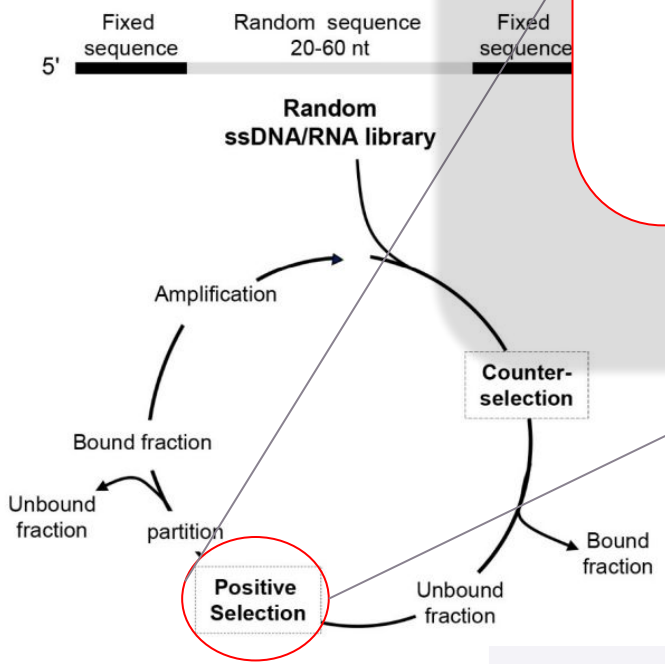


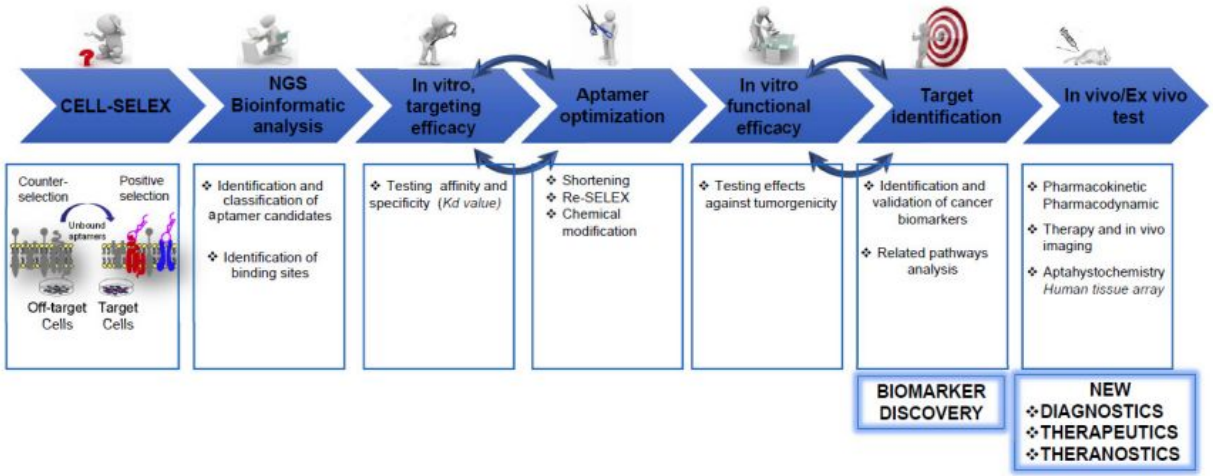
Table 1. Aptamer-based conjugates for targeted drug delivery in cancer.

Aptamer	Composition	Size (nt)	Cell Type for the Selection	Post-SELEX Identified Target	Kd (nM)	Aptamer-Based Targeted Conjugates for Therapeutic Applications
CL4	2′-F-Py-RNA	39	NSCLC	EGFR [75]	10	➢ 3WJ-CL4/anti-miRNA-21 [111] ➢ 3WJ-CL4/siRNA-XBP1 [112] ➢ 4WJ-CL4/paclitaxel [113] ➢ CL4-PNPs/cisplatin [114] ➢ CL4-antibody [41,42]
GL21.T	2′-F-Py-RNA	34	GBM	Axl [115]	13	➢ GL21.T-miRNAs [116]
Gint4.T	2′-F-Py-RNA	33	GBM	PDGFRβ [76]	9.6	➢ Gint4.T-PNPs/NVP-BEZ235 [117] ➢ Gint4.T-TDN/doxorubicin [118] ➢ Gint4.T-tFNA-GMT8/paclitaxel [107]
sgc8c	DNA	41	T-cell ALL	PTK7 [50]	0.78	➢ sgc8- SWNTs/daunorubicin [119] ➢ sgc8-AuNPs/daunorubicin [120] ➢ PA-AuNPs/daunorubicin [121] ➢ sgc8-hpDNA-AuNPs/doxorubicin [122] ➢ sgc8-NTRs/drug or AuNPs [123] ➢ sgc8-Ce6-AuNRs [124] ➢ sgc8-BP NS-PEG/doxorubicin [125] ➢ sgc8-MSN/doxorubicin [126] ➢ sgc8-BSA/PCL-ss-Ara prodrug [127]
GBI-10	DNA	69	GBM	Tenascin C [128]	150	➢ GBI-10-CPP/camptothecin prodrug [129] ➢ GBI-10-DGLs/zoledronic acid [130]
SQ-2	2′-F-Py-RNA	25	Pancreatic cancer	ALPPL-2 [15]	20	➢ SQ-2/5-fluoro-2′-deoxyuridine [131]

AuNPs: gold nanoparticles; AuNRs: gold nanorods; BP NS: black phosphorus nanosheets; BSA: bovine serum albumin; Ce6: chlorin e6; CPP: cell-penetrating peptide; DGLs: dendrigraft poly-*L*-lysines; hpDNA: hairpin DNA; MSN: mesoporous silica nanoparticles; miRNA: microRNA; NTRs: nanotrains; PA: polyvalent aptamers (sgc8 and anti-nucleolin AS1411 aptamers); PEG: polyethylene glycol; PNPs: polymeric NPs; siRNA: small interfering RNA; SWNTs: single-walled carbon nanotubes; TDN: tetrahedral DNA nanostructures; tFNA: tetrahedral framework nucleic acid; 3WJ: three-way junction.

Shigdar S, Agnello L, Fedele M, Camorani S, Cerchia L. 2021. Profiling Cancer Cells by Cell-SELEX: Use of Aptamers for Discovery of Actionable Biomarkers and Therapeutic Applications Thereof. Pharmaceutics 14(1):28.

Post Target Identification



Summary

- Expanding on Drug Conjugate Therapy
- RNA nanostructure to carry drug cargo
- pH-labile cis-aconityl linkage for controlled release cargo in acidic tumour microenvironments
- Aptamers used to target cell-surface proteins
- Allows for reduced toxicity to healthy tissue