



Nanoparticle-based drug delivery vehicles

The Molecular
Foundry
A Nanostructures User
Facility

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The Molecular Foundry

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Berkeley, CA*





In-vitro research reagent business

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Company	Market Cap
Amersham	division of GE Healthcare
Bio-Rad	\$2.4 B
Invitrogen	\$4.1 B
Promega	private
Qiagen	\$3.9 B
Sigma-Aldrich	\$6.4 B
Thermo Fisher	\$26.1 B



Transfection reagents

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- Huge need for the intracellular delivery of:
 - plasmid DNA
 - antisense oligonucleotides
 - siRNA
- Short-term application areas: Diagnostics & Engineering
 - Gene silencing in cells/animals
 - Target identification for biopharmaceuticals
 - Genetic manipulation of organisms
 - Introduction of foreign DNA into cells
- Long-term application area: Therapeutics
 - Non-viral gene therapy
 - Oral protein delivery



Toxicity is a major problem

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- Current reagents are based on mixtures of cationic lipids/polymers
- Most suffer from deleterious interactions with the cell membrane
- Limits utility *in vitro*, prevents use *in vivo*



Bio-inspired polymers for drug delivery

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- Develop a new class of polymers that can be:
 - efficiently synthesized
 - precisely engineered
 - bio-friendly
 - monodisperse
 - chemically diverse alphabet
 - highly purified
 - low-cost
- Peptoid polymers have unique properties:
 - Precise sequence control
 - Properties in between polymers and proteins
 - Combinatorial discovery of new materials

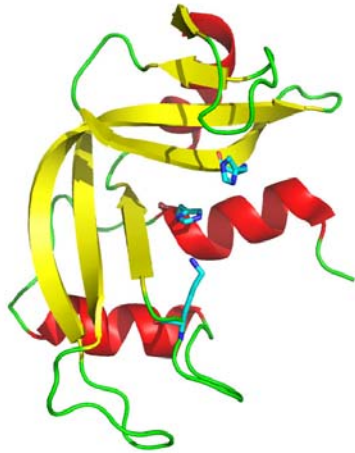
New smart biomaterials

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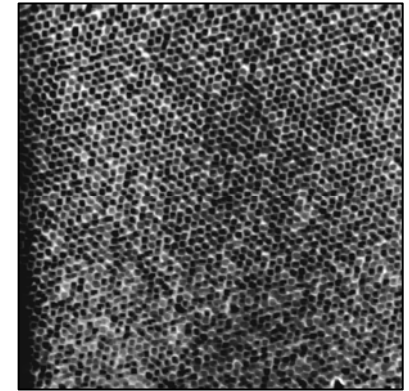


Linear polymer chain

self-assembly



Precisely folded tertiary structure
(proteins, nucleic acids)



Nanoscale patterning
(diblock copolymers)

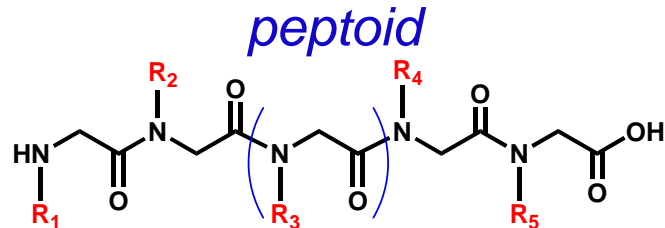
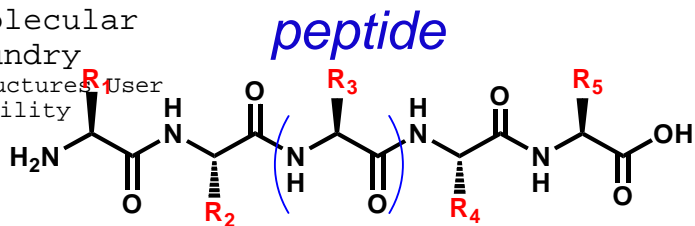


- Sequence complexity
- Side-chain diversity

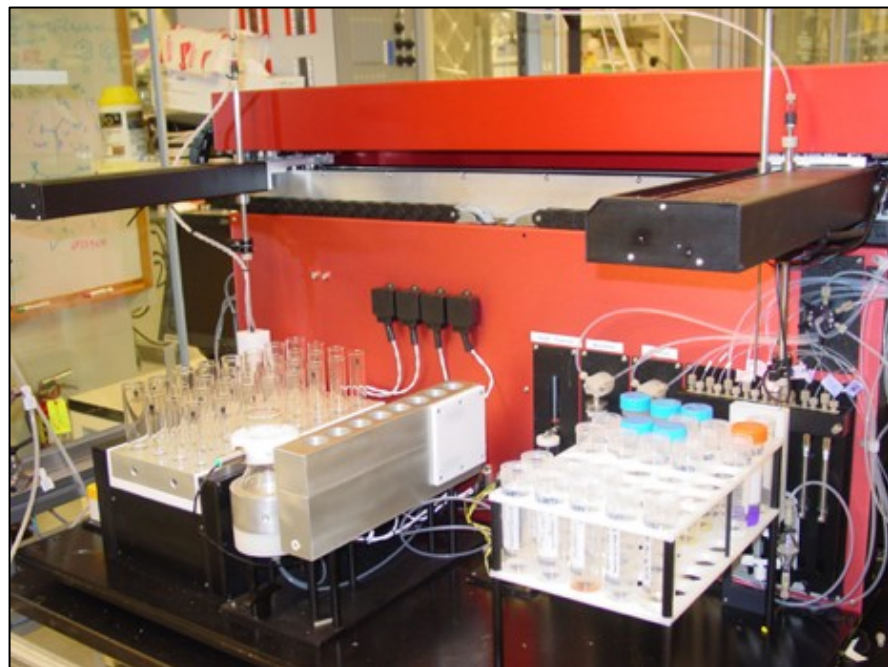
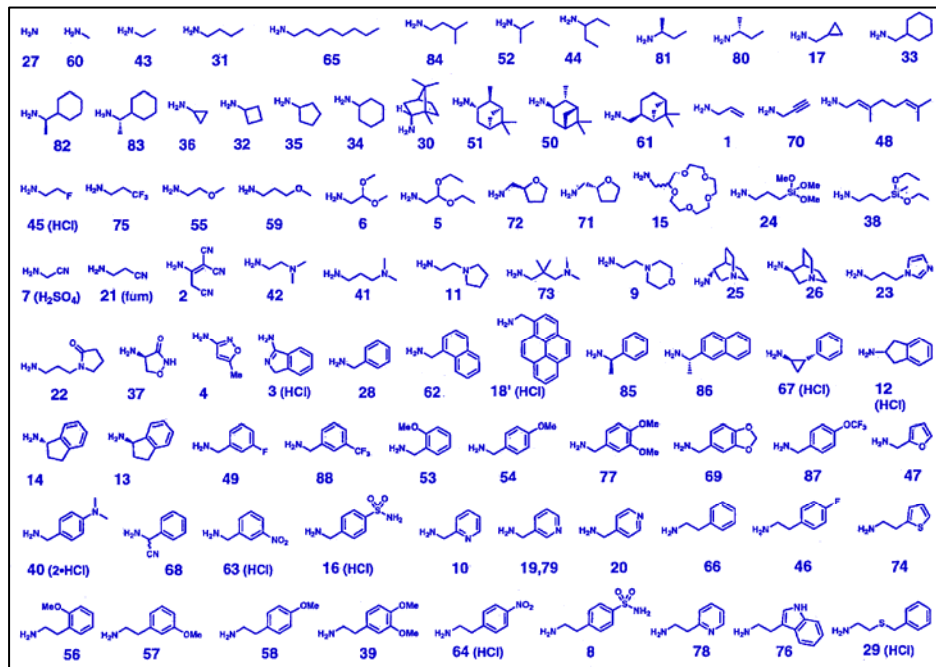
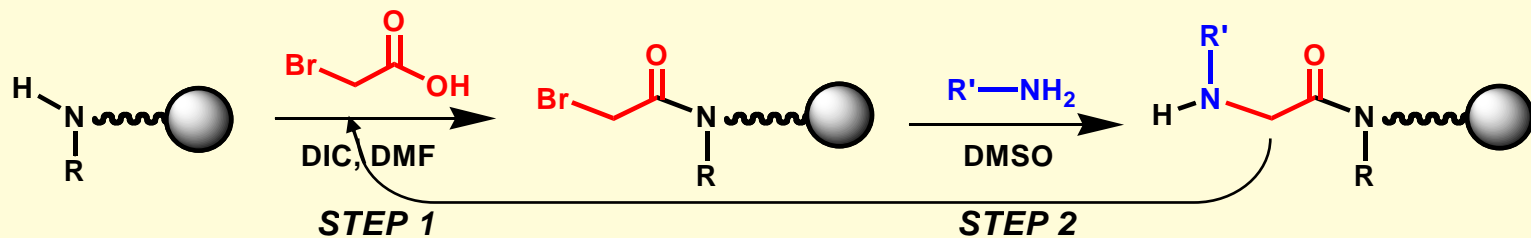


Bio-inspired polymers: peptoid synthesis

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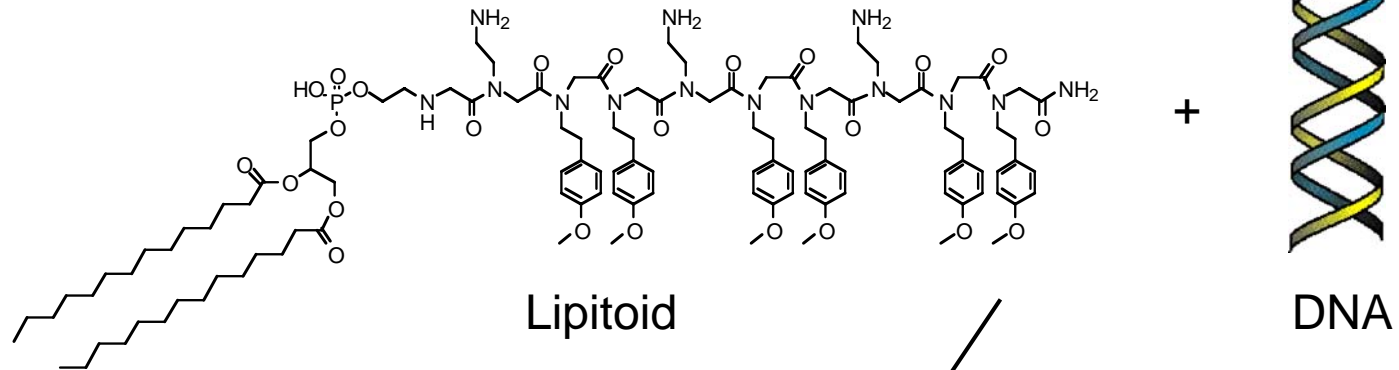


Solid-phase submonomer synthesis

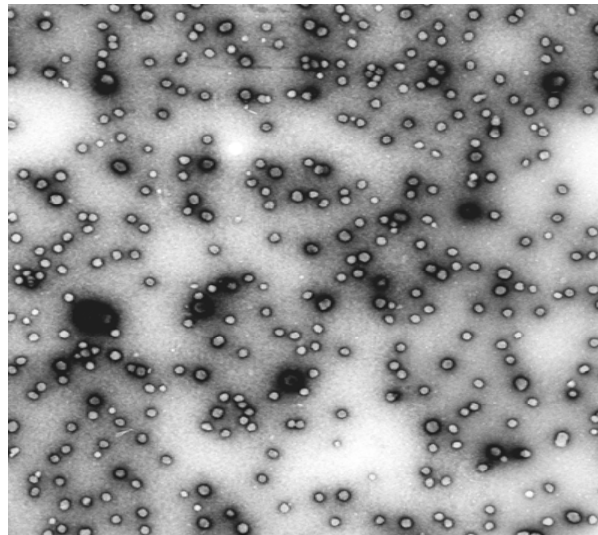


Nanoparticle-based transfection reagents

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Complex formation

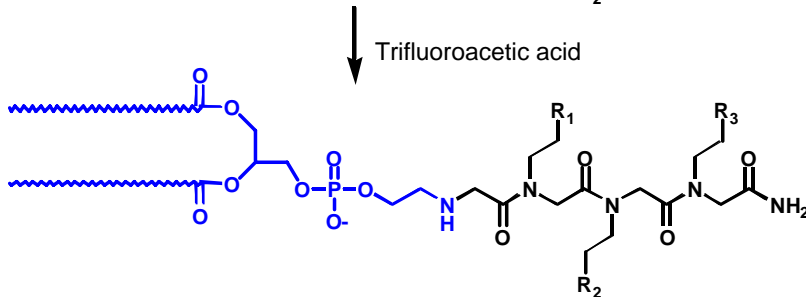
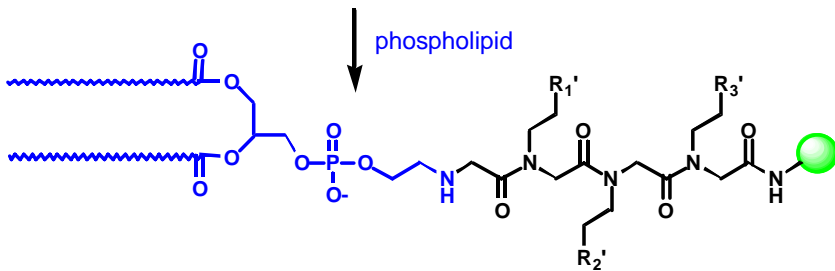
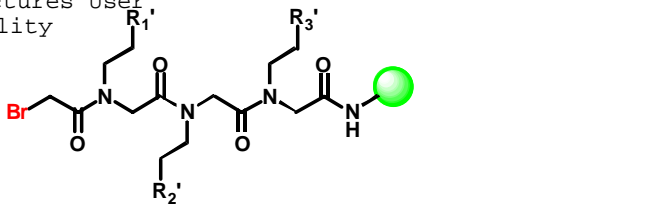


Electron Micrograph of
peptoid/DNA complex

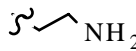
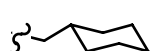
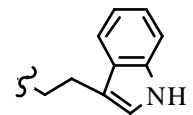
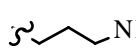
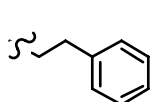
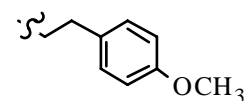
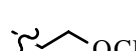
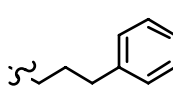
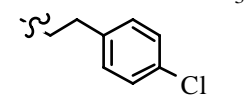
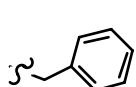
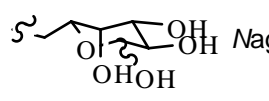
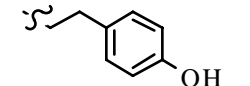
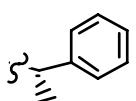
PNAS, **95**, 1517 (1998)
Chem. Biol., **5**, 345 (1998)
Mol. Biosys., **2**, 312 (2006)

Combinatorial synthesis of cationic lipids

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Sidechain^a/Designator

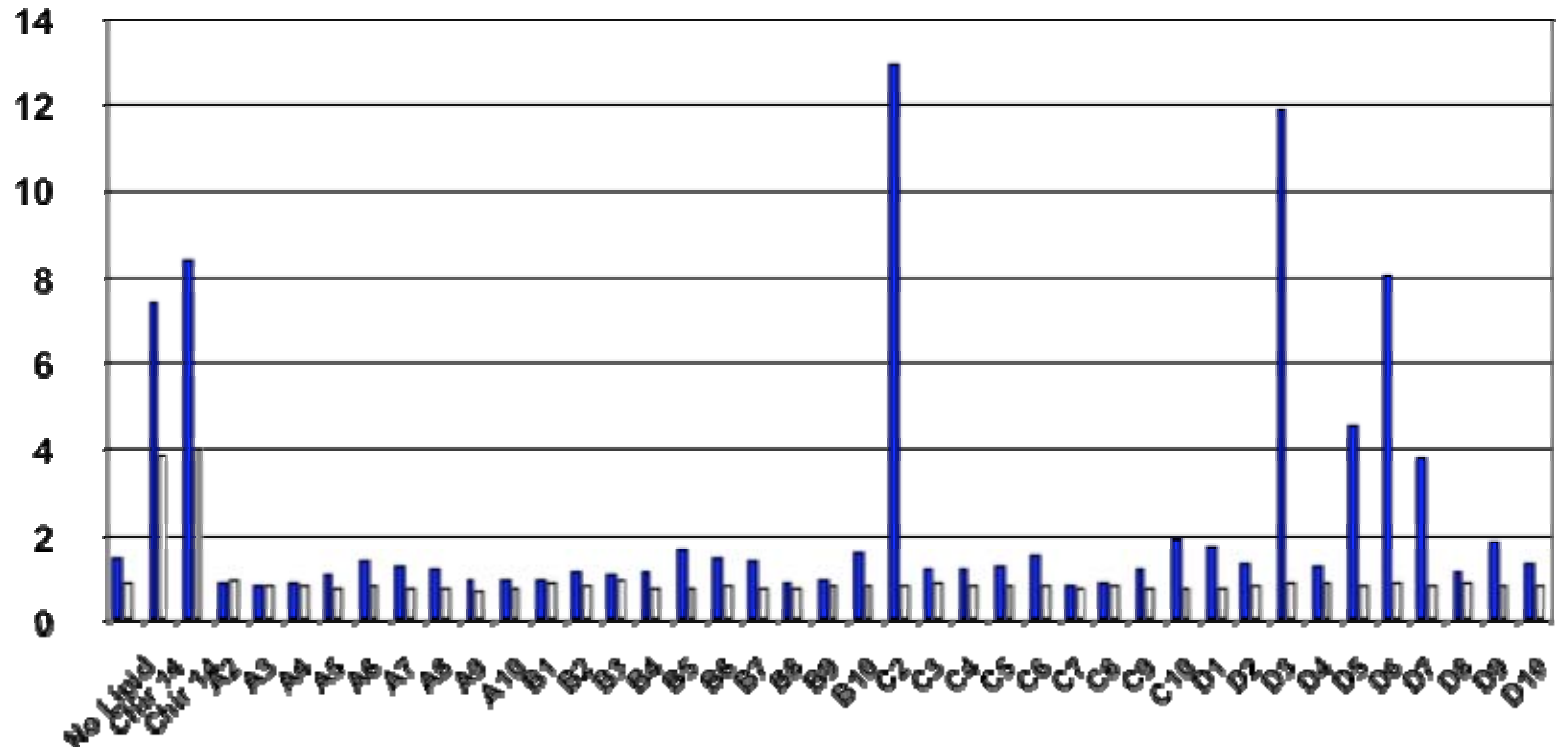
 NH ₂	Nae		Nchm		Me
 NH ₂	Napr		Npe		Nmpe
 OCH ₃	Nme		Npp		Ncpe
	Nbn		Nag		Mhpe
	Nspe				

^aThe indicated bond is the site of attachment to the mainchain glycine nitrogen.



Antisense delivery screening

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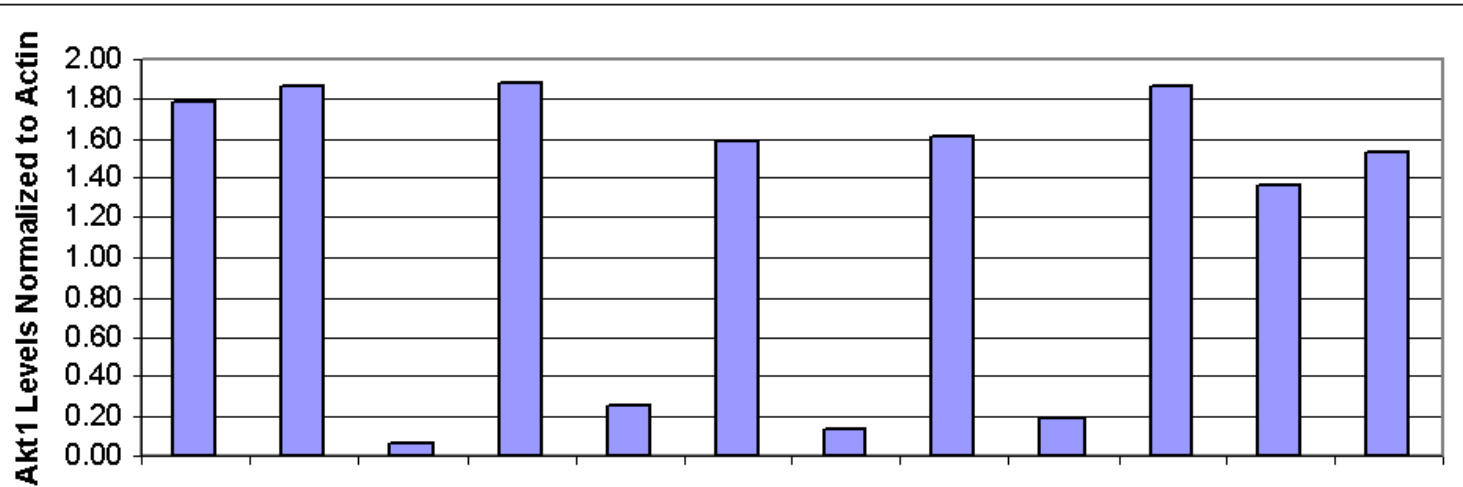
 Activity Ratio: Reverse Control/ Anti-Sense
 Toxicity Ratio: No Lipid/ Reverse Control

Comparison with commercially available reagents

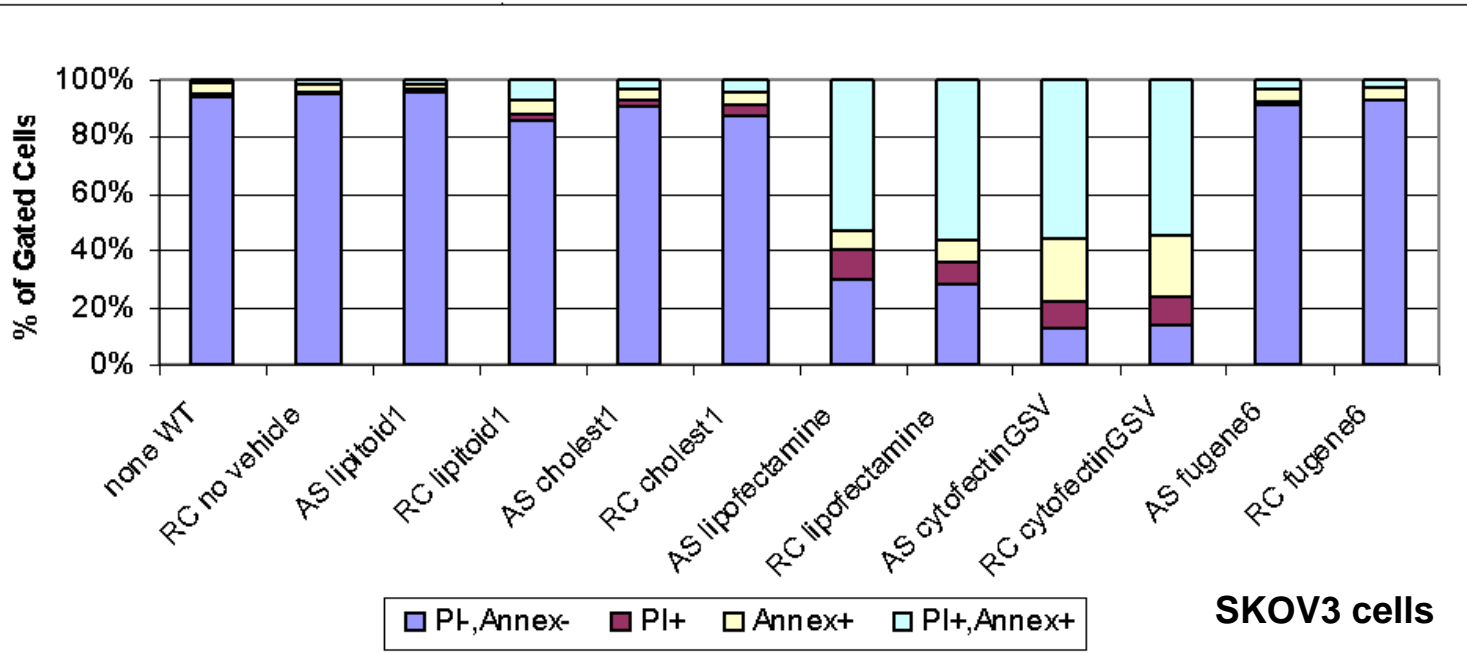
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A Nano

A



B

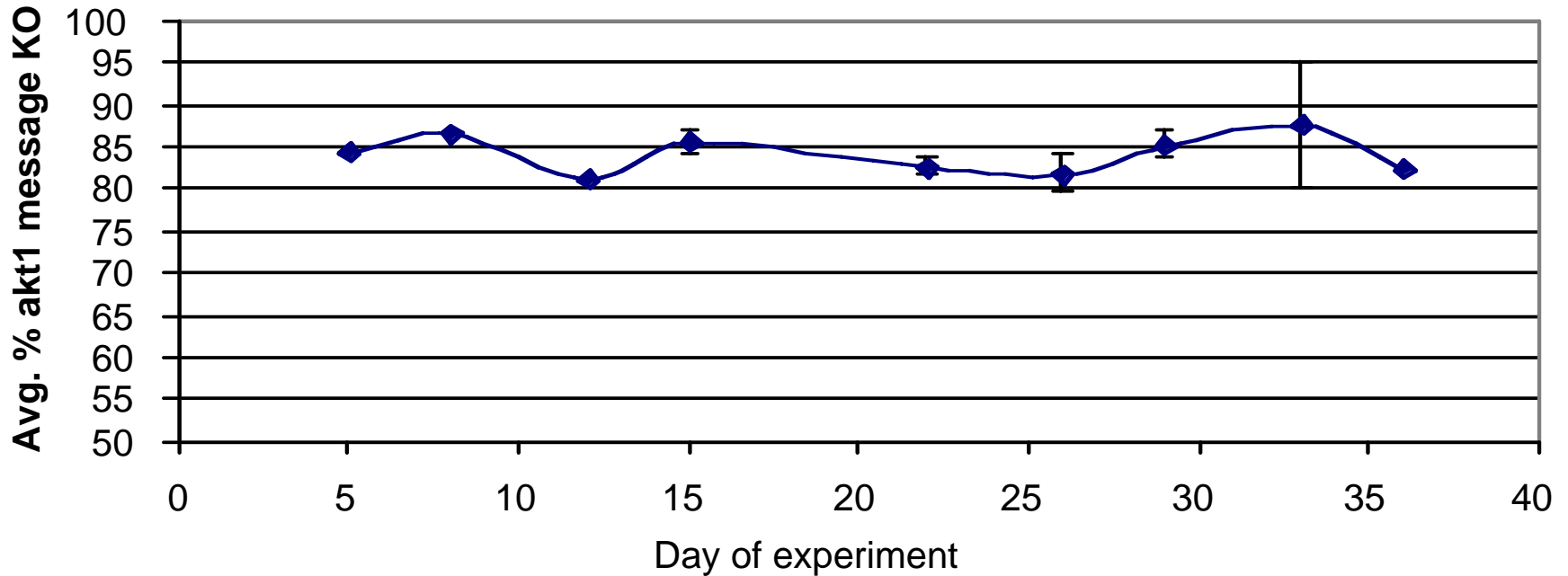


SKOV3 cells



Long-term Chir 169/akt1 message knockout

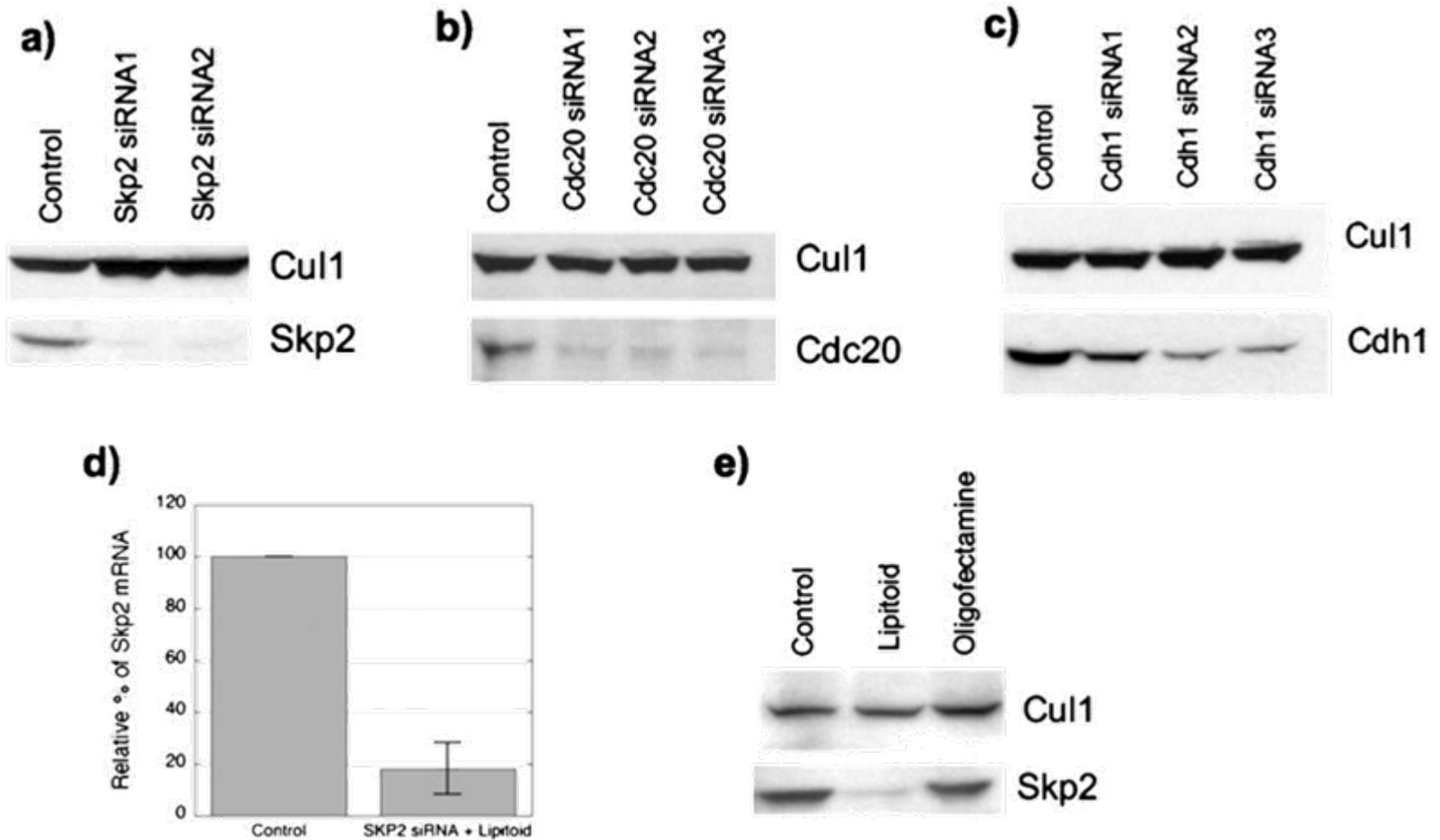
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Sustained mRNA knockout can be achieved without cell growth inhibition

Efficient siRNA delivery to primary cell lines

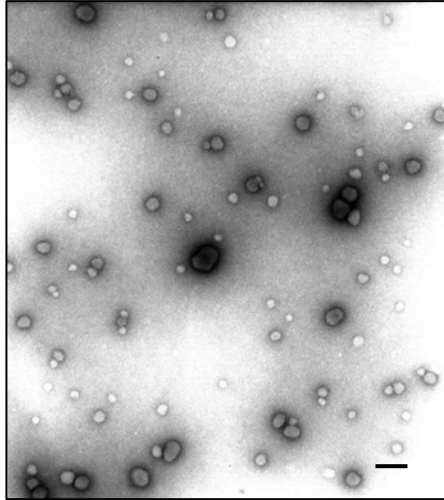
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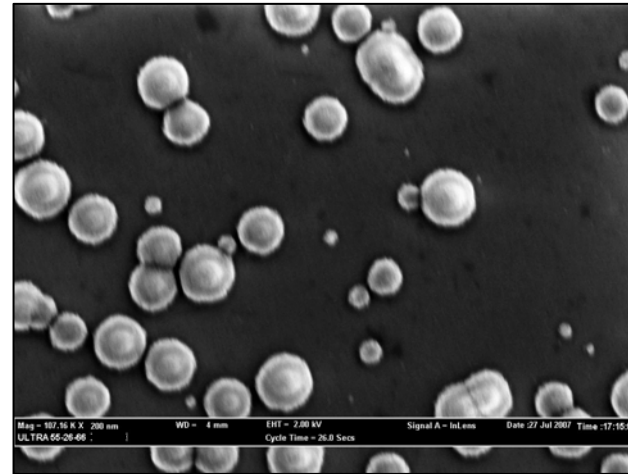
T98G cells (human brainglioblastoma cells)

Lipitoid complexes with pDNA and siRNA

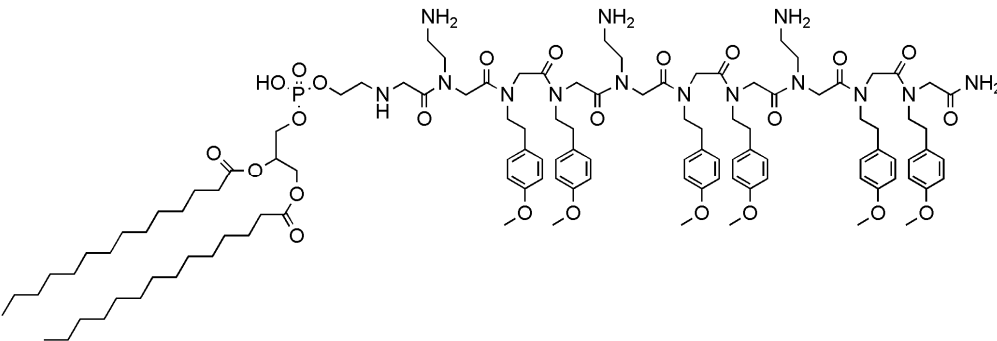
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pCMV (5kb)



siRNA (25bp)



- Structure activity relationship
- Formulation stability
- Intracellular delivery mechanism

Future work: Artificial proteins

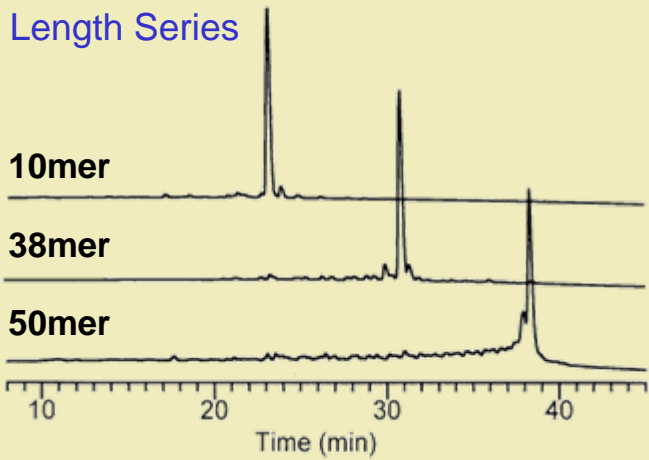
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Length Series

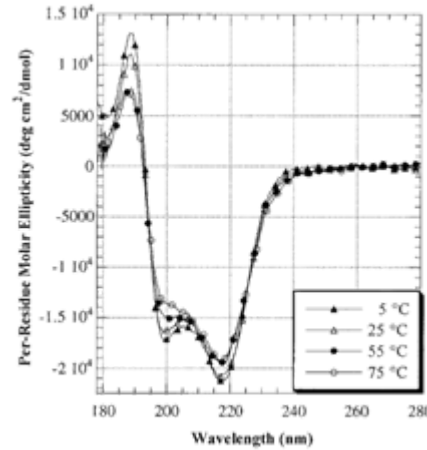
10mer

38mer

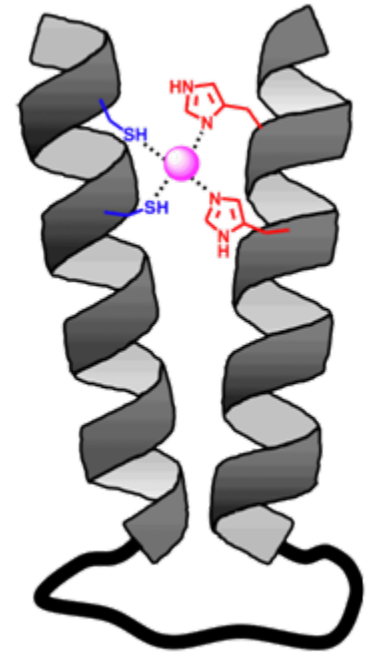
50mer



Helix stability:

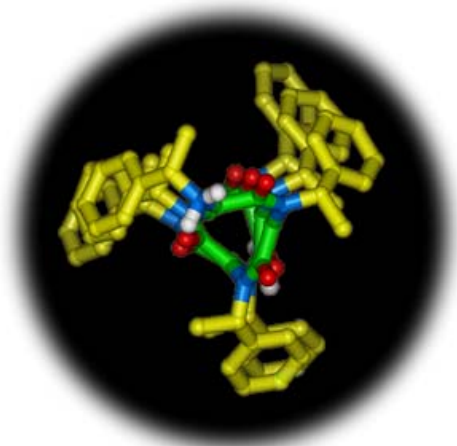


Folded nanostructures:

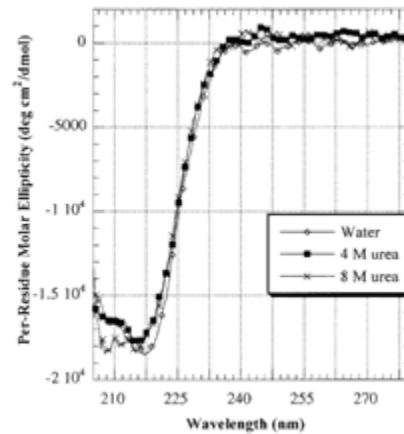
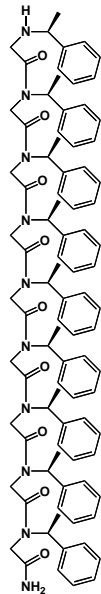


Nat. Chem. Biol. In review

Peptoid helix:



Folding & Design, **2**, 369 (1997)
JACS, **125**, 13525 (2003)



PNAS, **95**, 4303 (1998)
Biopolymers **63**, 12 (2002)