Protein Nanocapsules for Therapeutic Applications

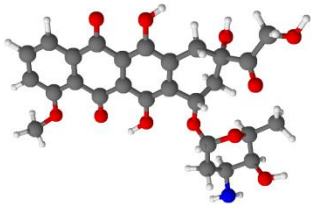
Yi Tang

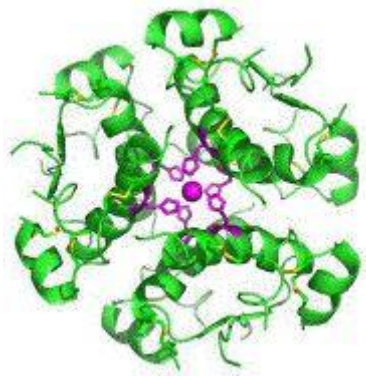
Department of Chemical and Biomolecular Engineering
Department of Chemistry and Biochemistry
University of California, Los Angeles

UCLA

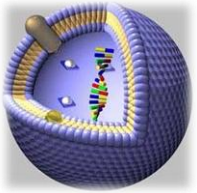
Delivery of Therapeutic Molecules to Biological Systems

Therapeutics





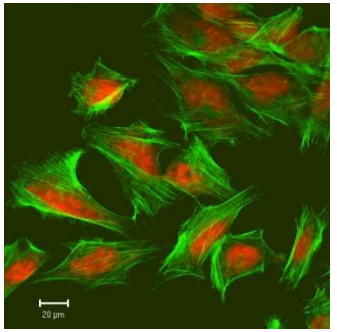
Carriers/Vehicles



Requirements

Effective
Efficient
Safe
Controlled Release

Targets



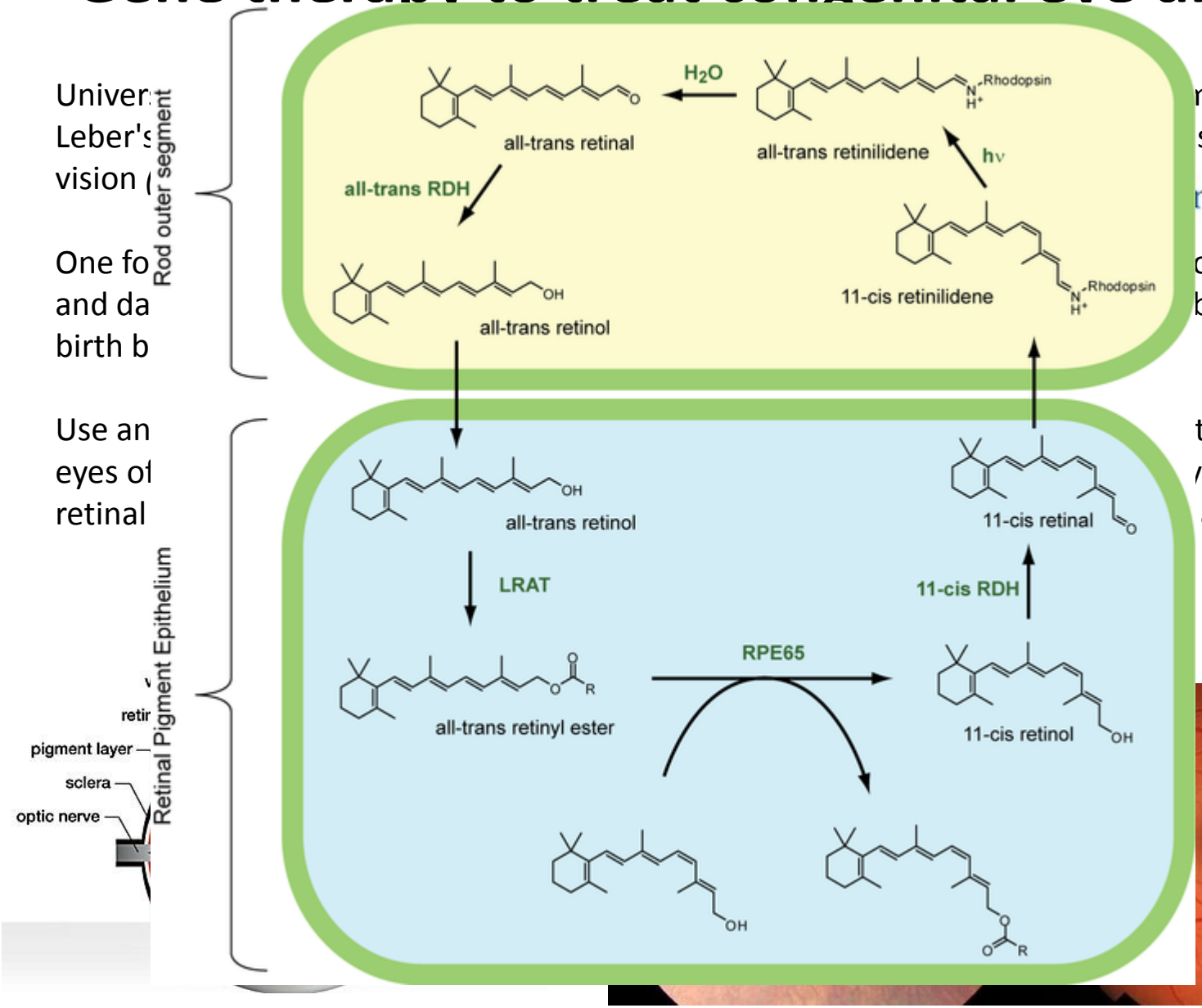
Gene therapy to treat congenital eye diseases

Univers… methods to treat
Leber's… sion loss or no
vision (…

n Medicine

One fo… oss of 11-cis retinal
and da… ble. Poor vision at
birth b…

Use an… the RPE65 into the
eyes of… produced 11-cis
retinal… as legally blind.



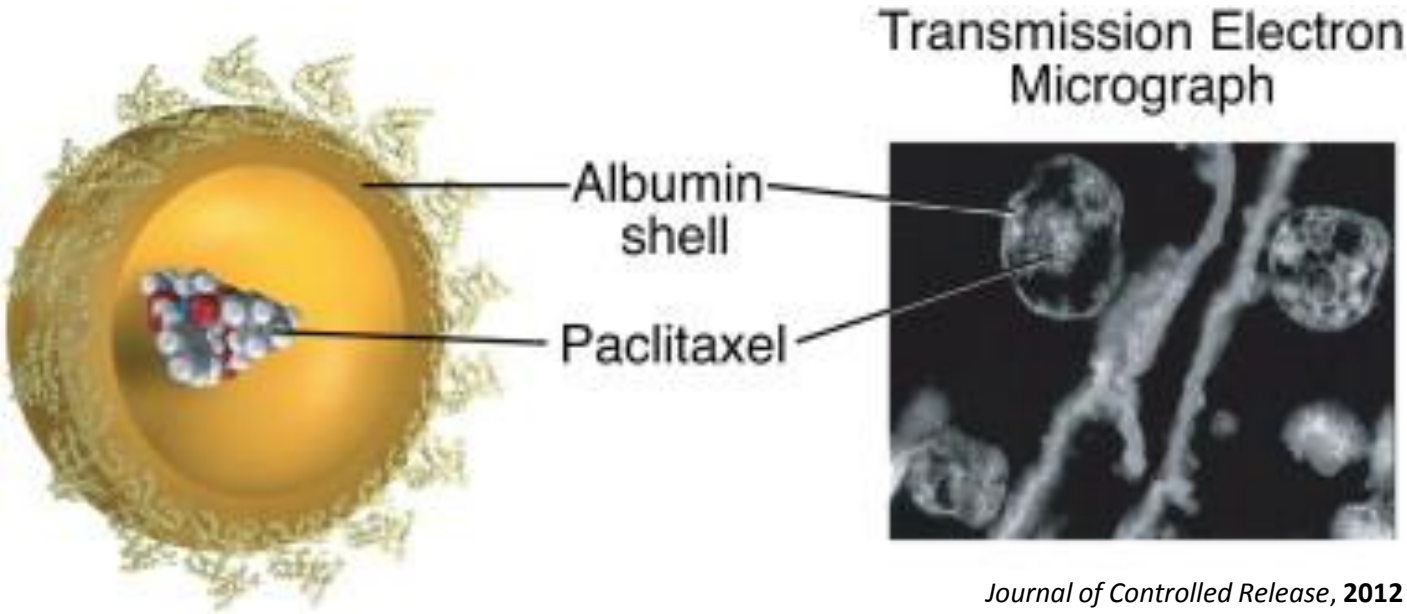
After

Credit: Moorfields Eye Hospital & University College London

NEJM, **2008**, p2240

Drug delivery using Albumin Nanoparticles

Paclitaxel is a small molecule mitotic inhibitor that used for treating different cancers. Its poor water solubility and toxicity to normal tissues however, had resulted in poor bioavailability and major side effects



Journal of Controlled Release, **2012**

By conjugating paclitaxel containing nanoparticles to human serum albumin. It is more water soluble and more efficiently transported to tumor cells through albumin-mediated transcytosis. The resulting formulation (Abraxane®) is significantly more effective compared to paclitaxel itself.

Protein-based Therapeutics

- **Advantages**
  - **Specificity**
  - **Biocompatibility**
  - **High potency**
  - **Unique in form and function**

- **Approved Protein Therapeutics**
  - **Diabetes** (Insulin: Humulin, Novolin, Symlin…)
  - **Cancer** (Herceptin, ELSPAR, Avastin, Vectibix…)
  - **Cardiovascular** (Natrecor, Angiomax, Retavase…)
  - **Immunoregulation** (Adagen, Infergen, Intron A…)
  - **Growth regulation** (Sandostatin, Kepivance…)

  …~$77 billion in 2010

Nearly all current protein therapeutics act on extracellular targets

Intracellular Protein Delivery Opportunities

- **Potential Applications**
  - **Catalyze intracellular reactions**
  - **Restore loss-of-function genetic conditions**
  - **Maintain normal cellular life cycles**
  - **Artificial control of gene expression levels**
  - **Imaging**
  - **Vaccination**

Protein delivery adds functions to cells without modifications to the host genome

B. Leader, Q. J. Baca, D. E. Golan, ***Nat Rev Drug Discov***, **7**, 21, 2008.

Intracellular Protein Delivery

Challenges

Extracellular Challenges

Intrinsically unstable
(aggregation/denaturation)
Prone to proteolysis
Rapid clearance of small proteins
(< 30kDa)
Elimination by immune system
Targeting specific cells

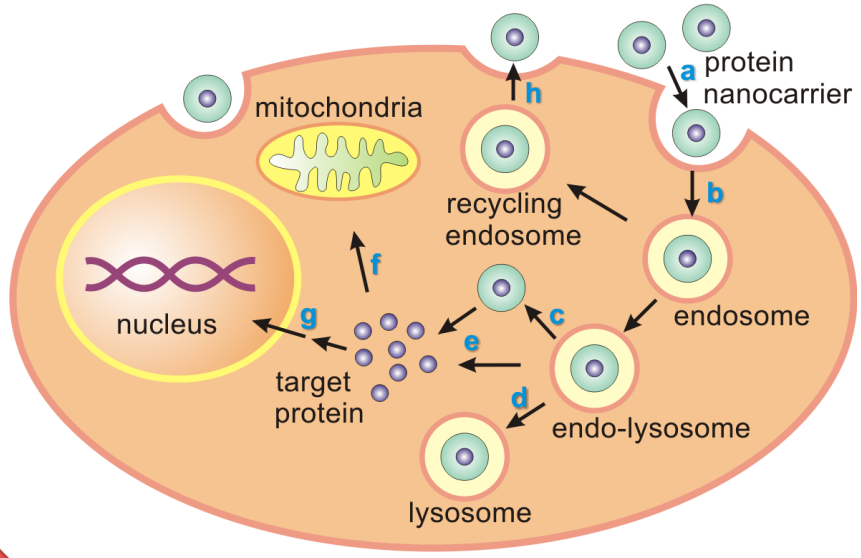
Entry Challenges

Surface charge (negative)

Intracellular Challenges

Escape from delivery vehicles
Release into cytosol
Maintain structure and function

Nanocarriers



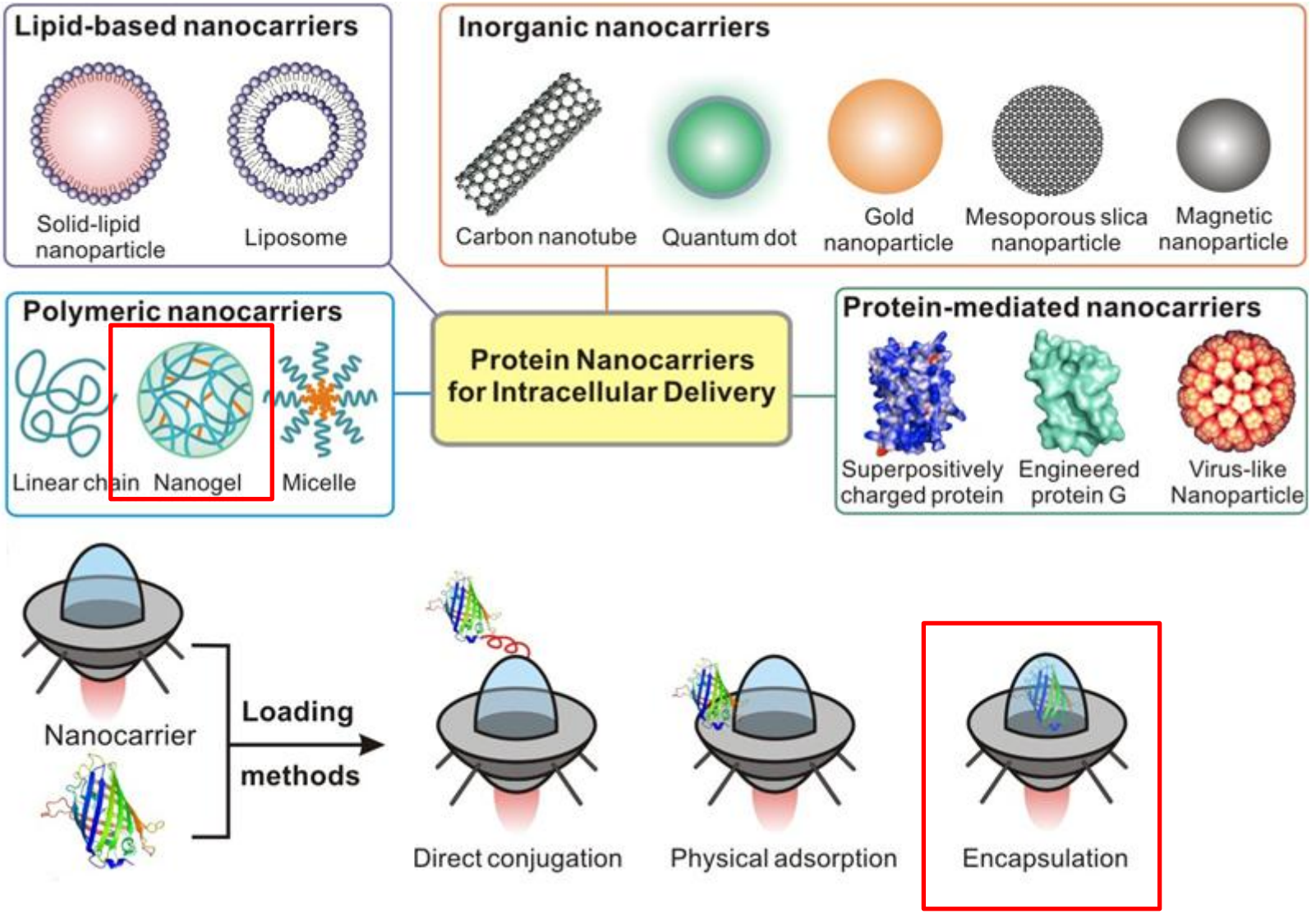
Advantages

Increase protein stability
Shield protein from protease
Increase circulation time
Concealing immunogenic epitopes
Allow surface modification
Tailor carrier surface charge

Many of the delivery challenges can be addressed using nanocarriers

Choice and design of vehicle is crucial

Nanocarriers for Intracellular Delivery



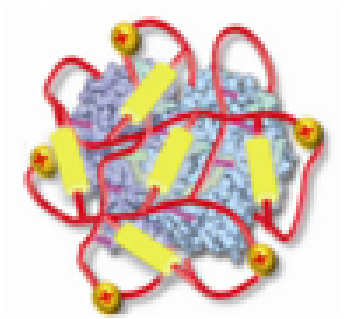
D. Peer, J. M. Karp, S. Hong, O. C. Farokhzad, R. Margalit, R. Langer, *Nat. Nanotechnol.*, *2*, 751, **2007**.

Z. Gu, A. Biswas, M. Zhao, Y. Tang, *Chem. Soc. Rev.*., **2011**.

Engineering Protein Nanocapsule

Outline of the Different Generations of Nanocapsules Designed in Our Lab

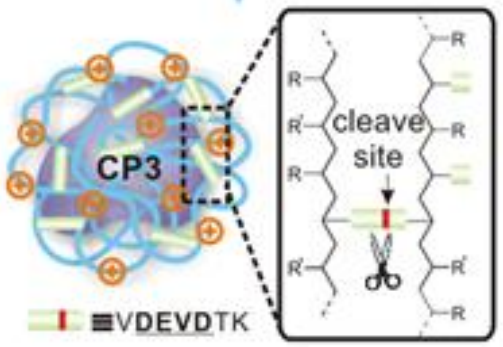
Surface modified NCs



covalent modification

Degradable

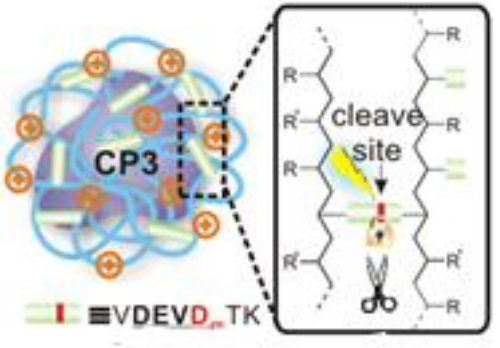
Self-degradable NCs



non-covalent modification

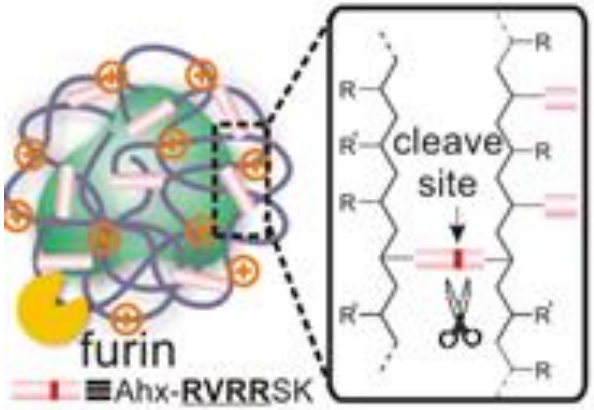
Controllable

Photo-caged self-degrading NCs

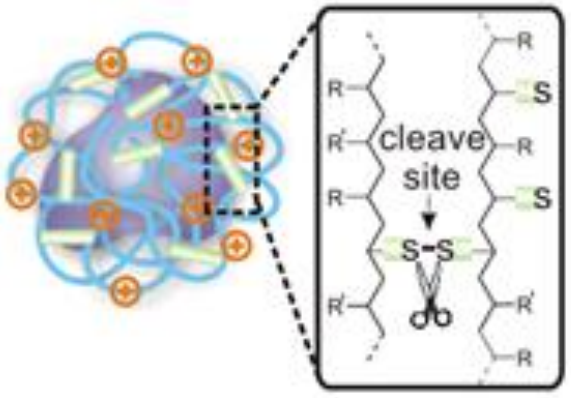


Generalizable

Endoprotease-degradable NCs

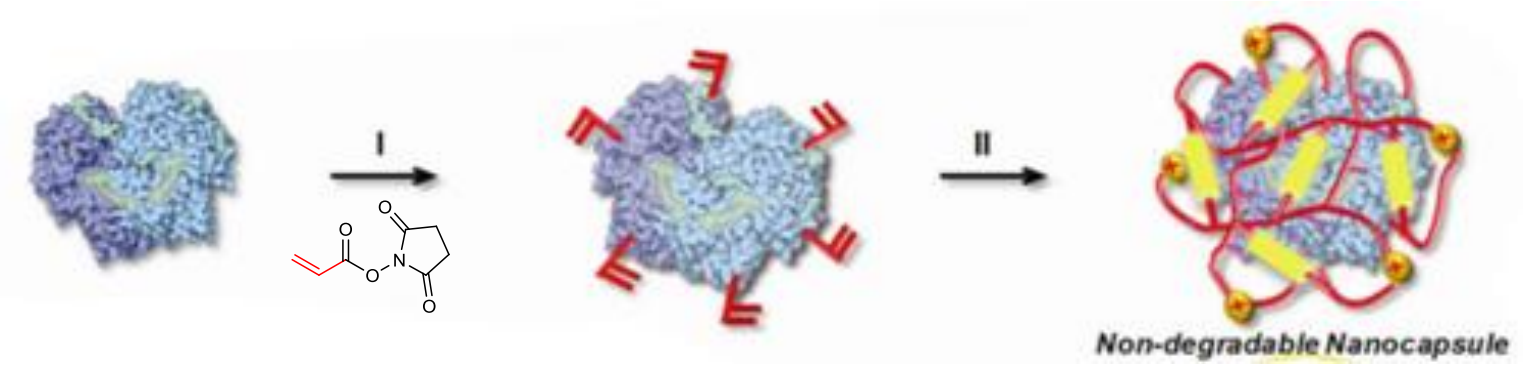


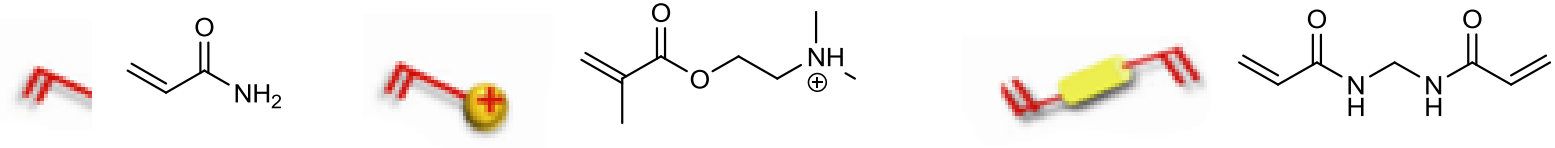
Redox-responsive NCs



Single-Protein Based Nanocapsules

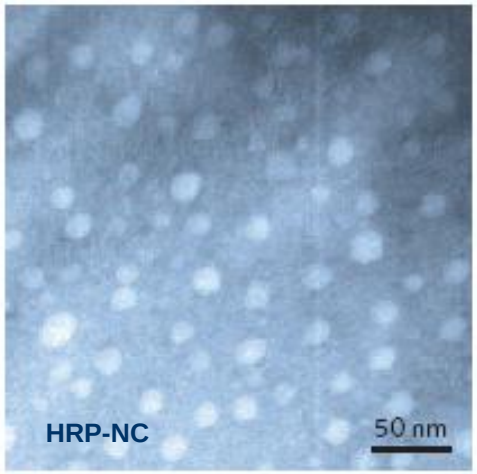
M. Yan, et al *Nat. Nanotechnol.*, 5 **2010.**



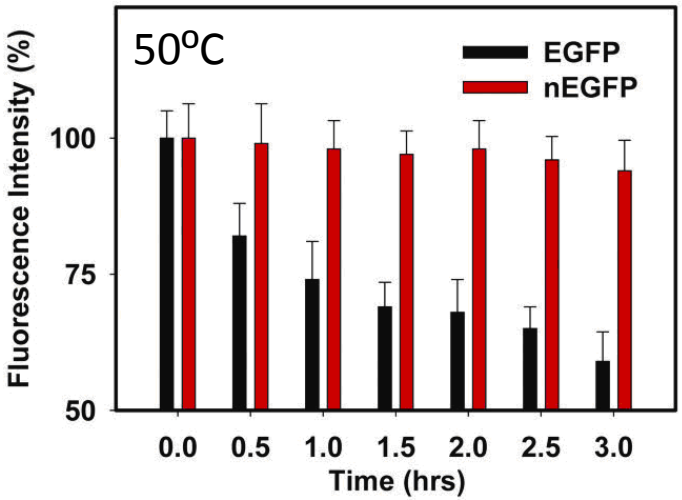


monomer **positive monomer** **crosslinker**

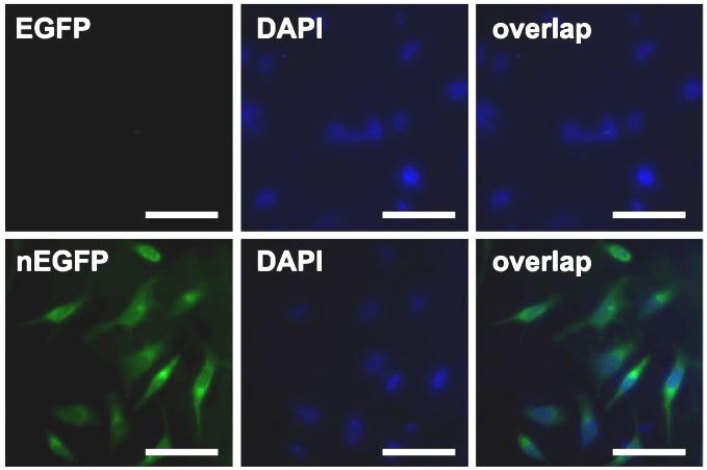
Uniform size



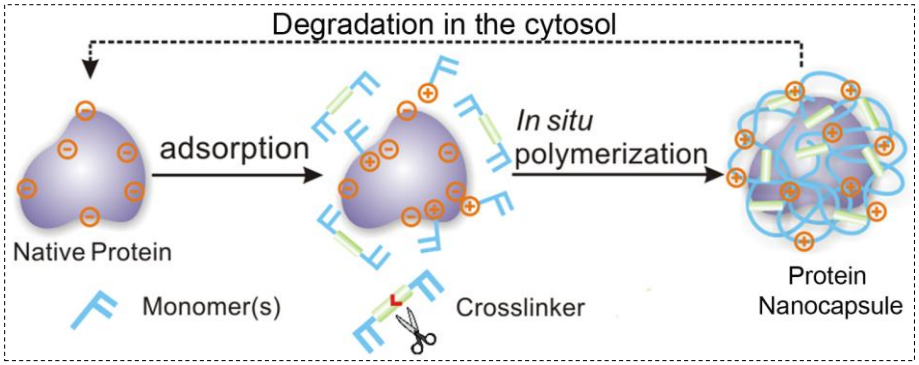
Increased stability

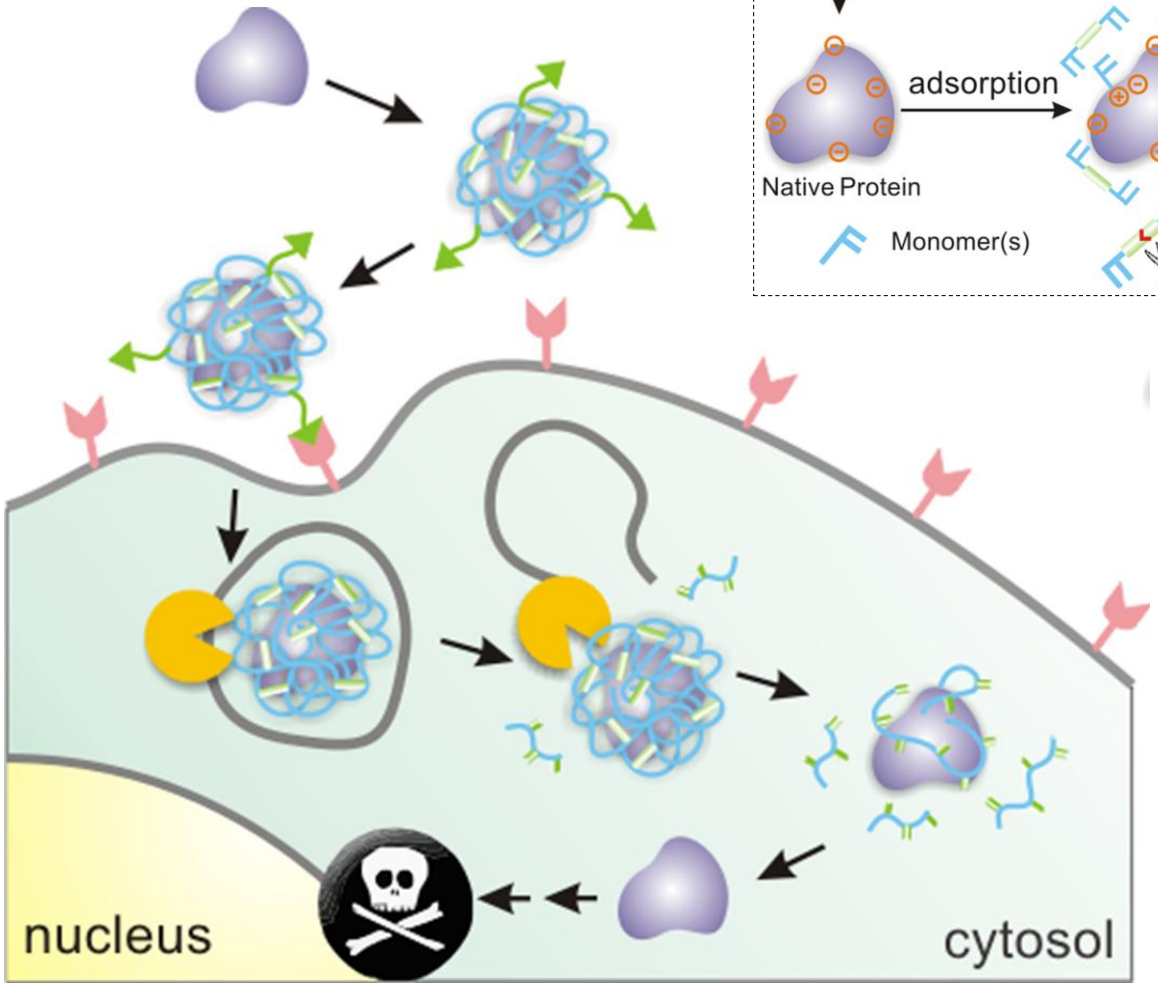


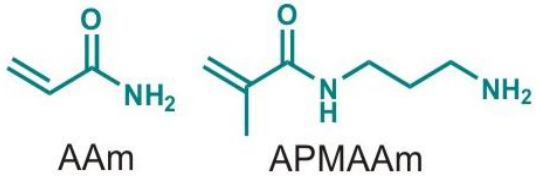
Efficient cell entry



Degradable Nanocapsules







Shown here is the delivery of a protein that can cause apoptosis in cells.

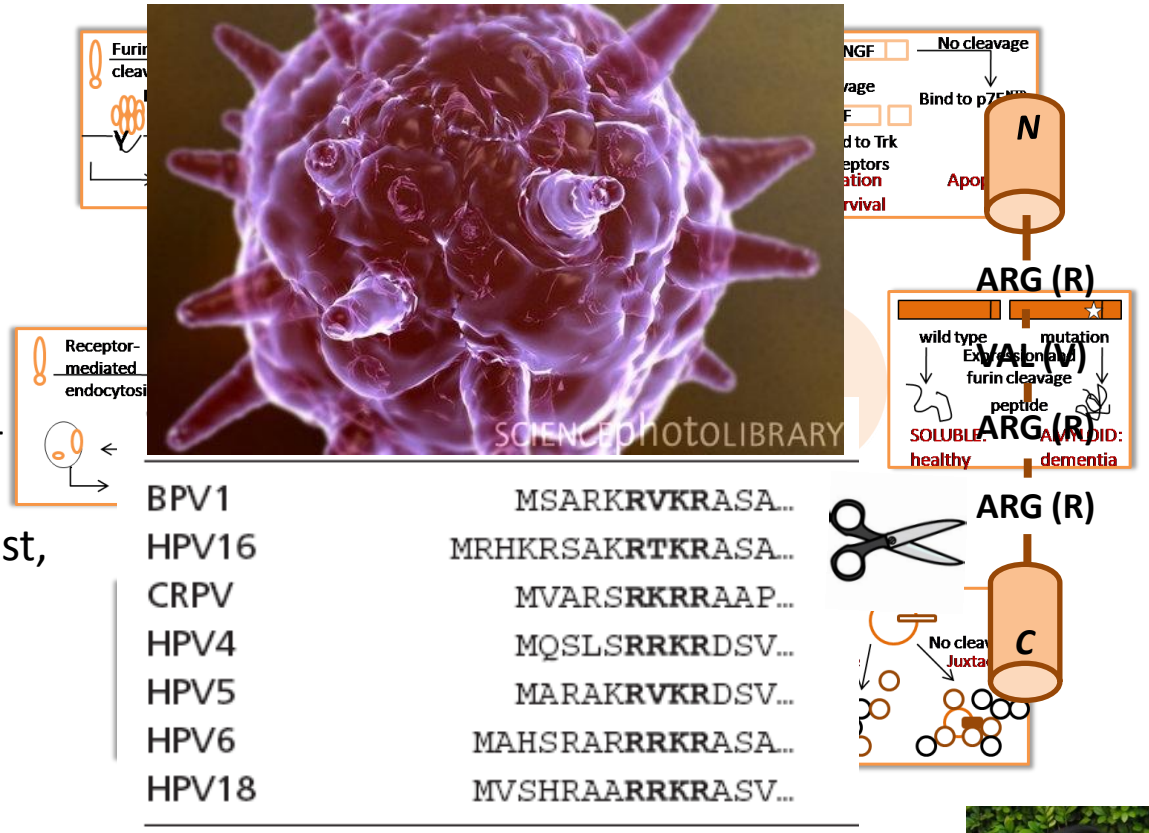
Endoprotease-degradable Nanocapsules

For cytosolic delivery, the actions of ***intracellular*** proteases can be explored

Furin –Proprotein convertase

Richards *et al*, *PNAS.* **2002**

- Required for the maturation of various cellular proteins.
- Found in various intracellular locations, including cell membrane, endosomal compartments, and trans-Golgi network.
- Increased furin expression in breast, ovarian, brain, lung and neck cancers.



Furin-mediated cleavage of papillomaviruses is necessary for dissociation of capsid, release of viral DNA and infection.

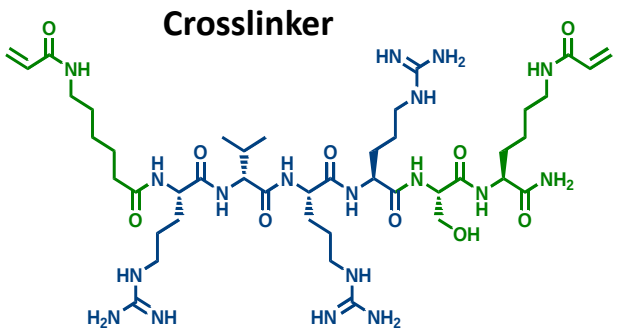
Can we mimic some of these natural infection processes in our carrier design?

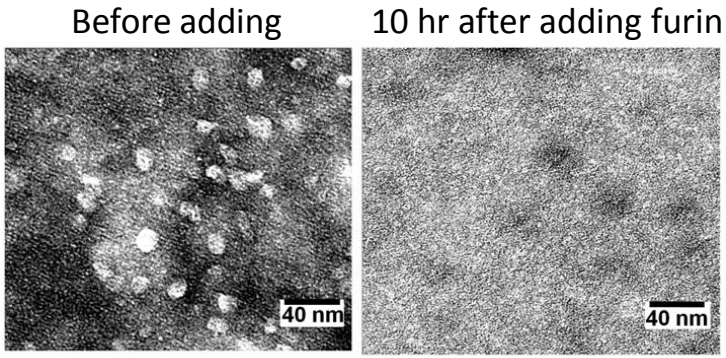
Natural role of furin facilitated release of foreign cargo.

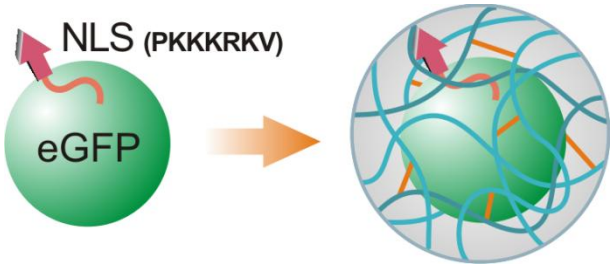
Biswas *et al*, *ACS Nano.* **2011**

Anuradha Biswas

Furin-Mediated Release of Protein Cargo

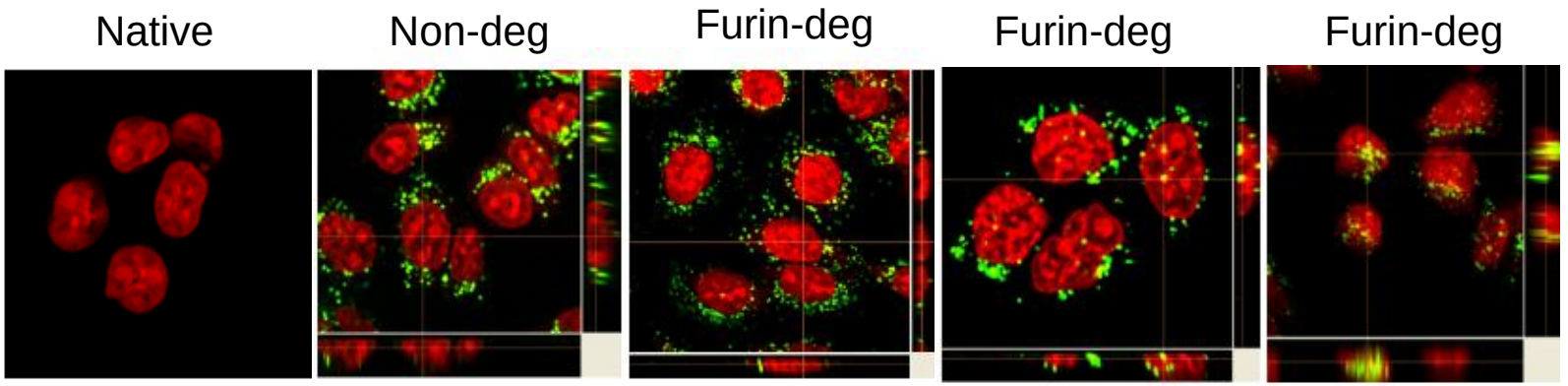






Degradation → **NUCLEAR LOCALIZATION**

No degradation → **RETENTION IN CYTOSOL**

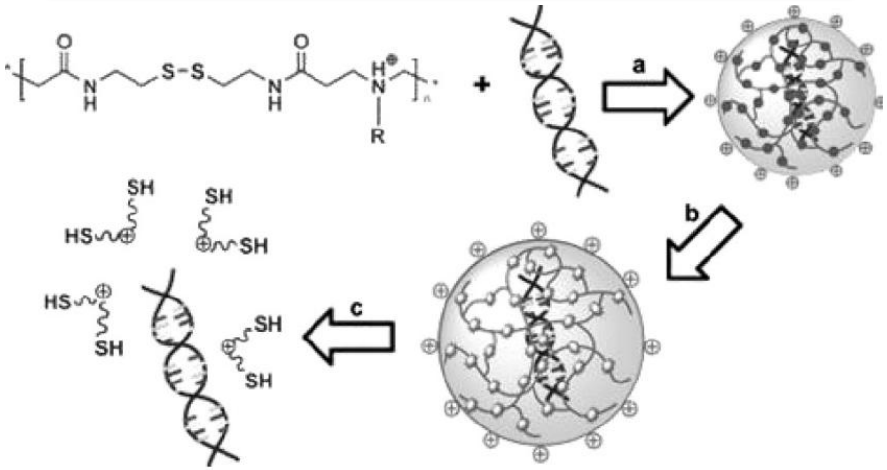


Biswas, A. et al, *ACS Nano,* **2011**.

Strategy for Chemo-Degradation

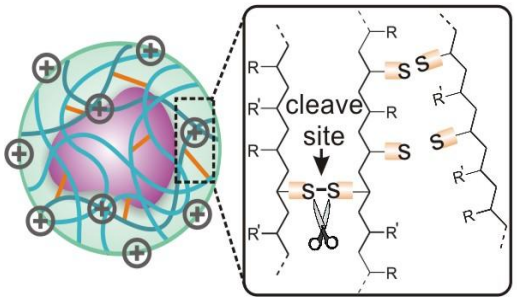
- The difference in the redox environment between extracellular (oxidizing) and intracellular (reducing) can also be explored for degradation of the polymeric shell.
- The high intracellular concentrations of glutathione (GSH) can rapidly trigger reduction of disulfide (S-S) crosslinked matrices.

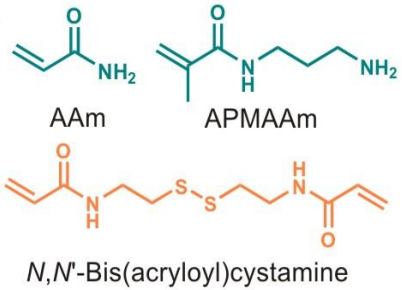
Gene delivery from redox degradable polyplexes



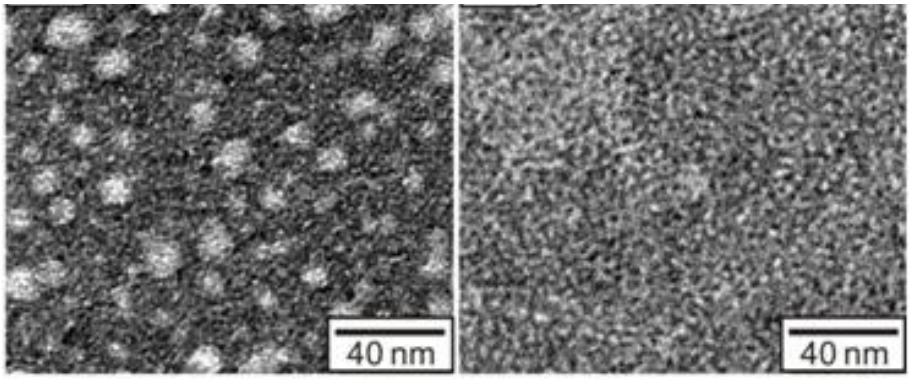
Engbersen and coworkers, *Bioconjug. Chem.* **2007**

Redox-Responsive Nanocapsules





Before GSH Treatment After GSH Treatment



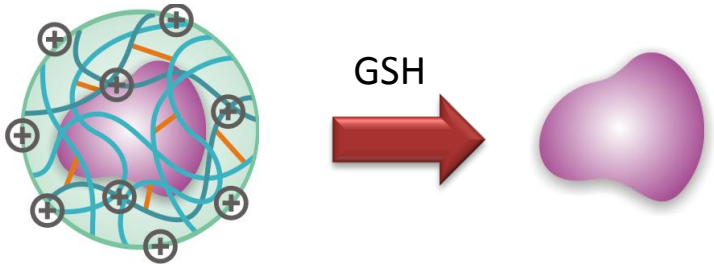
ζ-potential ~ 4 mV

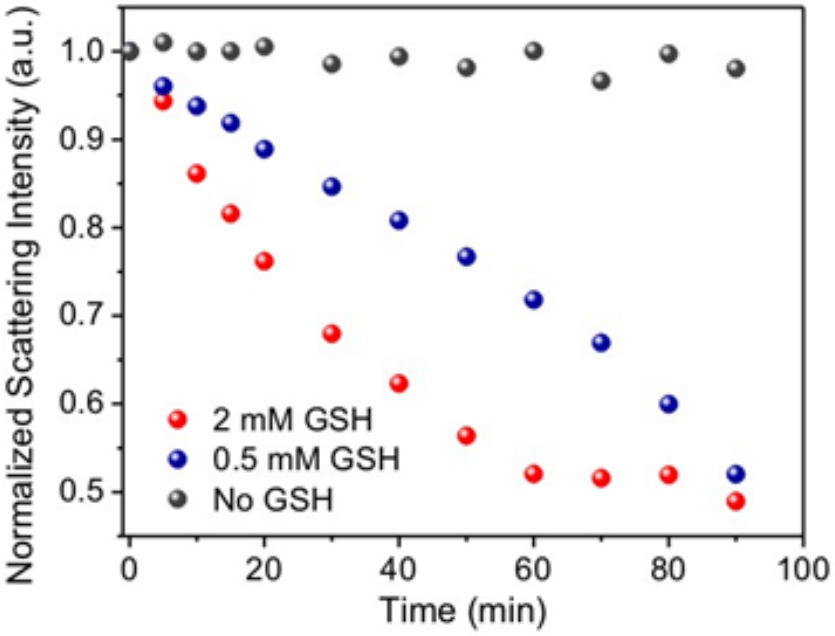
2 mM GSH, 2 hr, 37°C

Zhao, M. *et al*, *Biomaterials,* **2011**

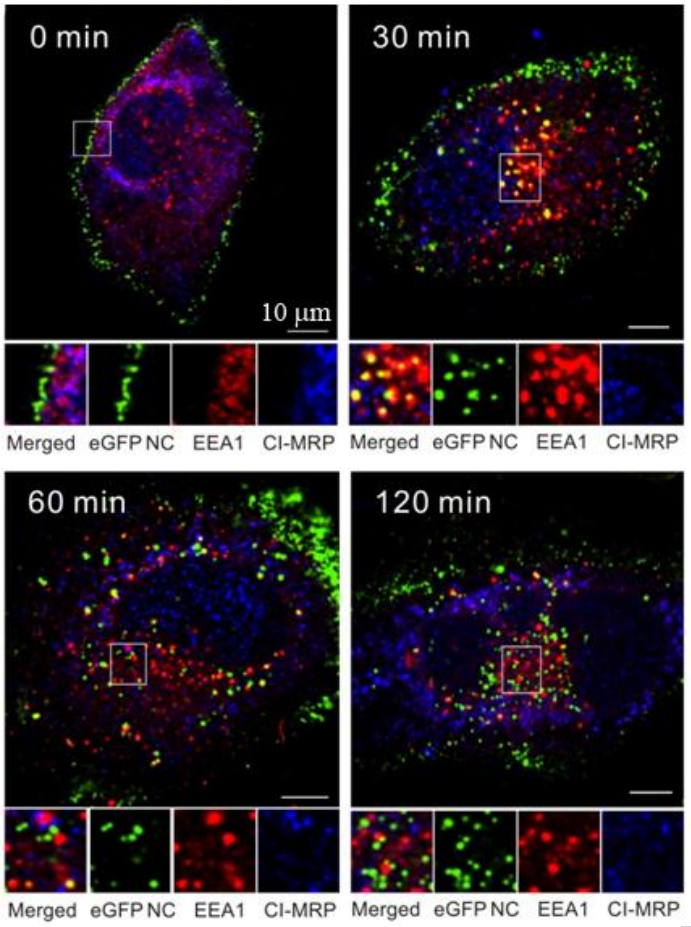
Redox-Responsive Nanocapsules

Time course of GSH-mediated degradation





S-S eGFP NC Trafficking in HeLa

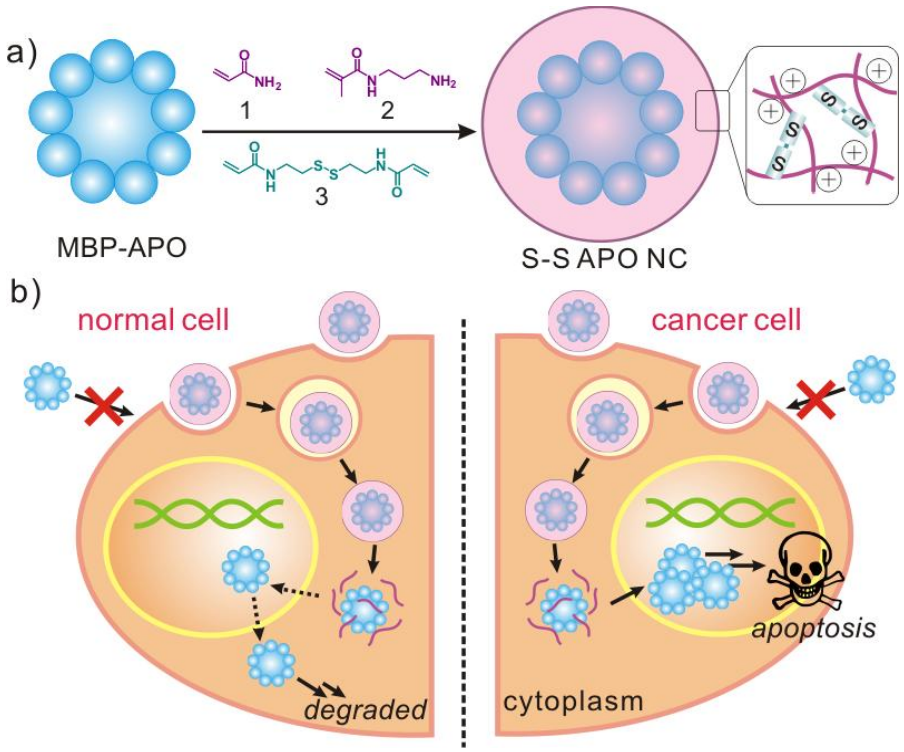


Muxun Zhao

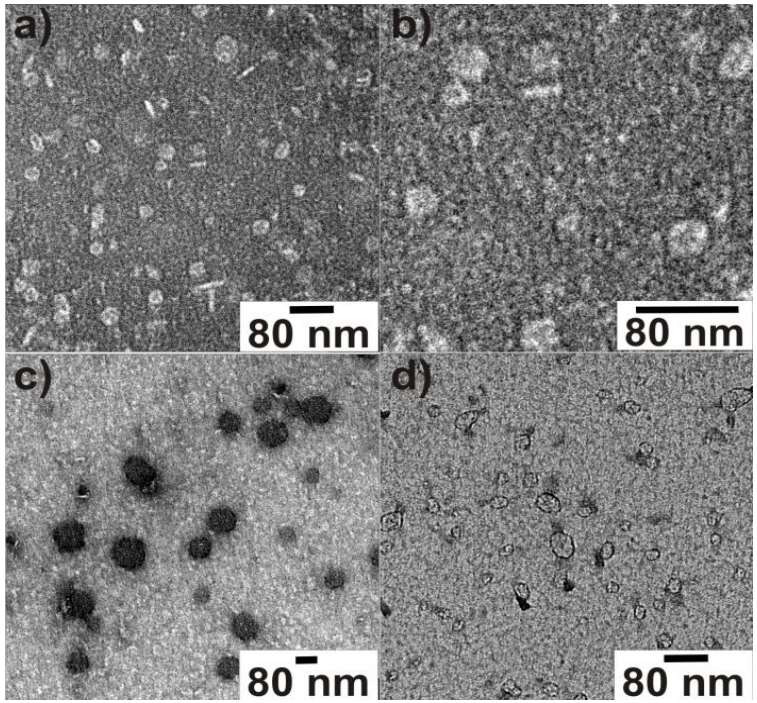
Zhao, M. *et al*, *Biomaterials,* **2011**

Application: Apoptin

Apoptin induces apoptosis selectively in tumor cells, but not in normal cells



Native MBP-APO



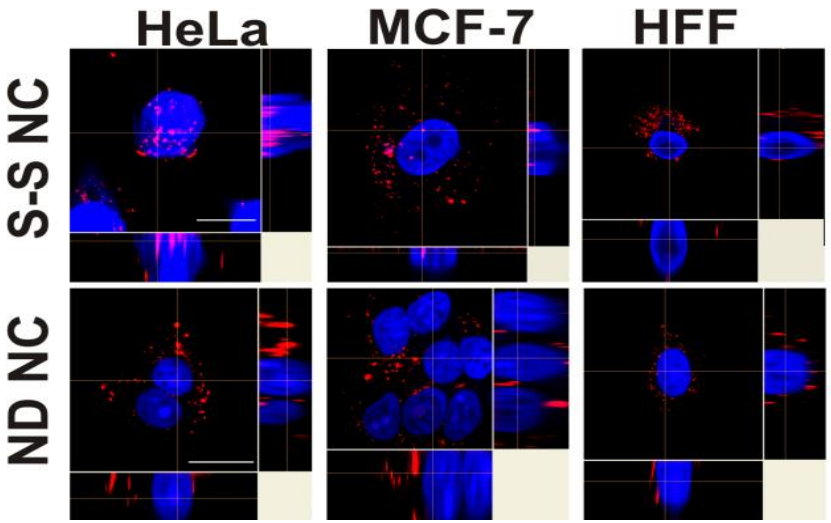
After encapsulation After degradation

Potential antitumor therapeutic if the correct delivery vehicle can be designed.

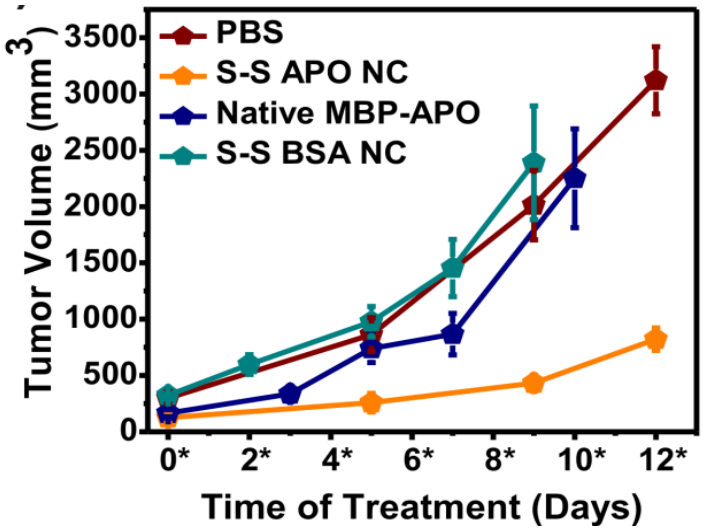
Zhao, M. et al, *submitted*, **2012**

Delivery of Apoptin to Selectively Kill Tumor Cells

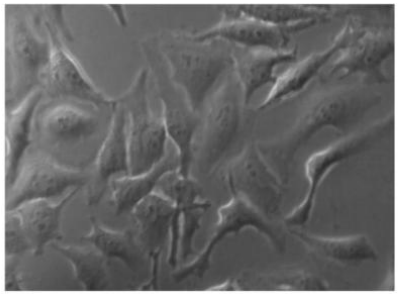
Nanocapsule delivered-apoptin localization



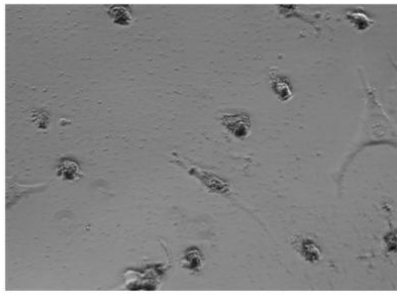
Nanocapsule can slow down tumor growth in mouse MCF-7 breast cancer xenografts.

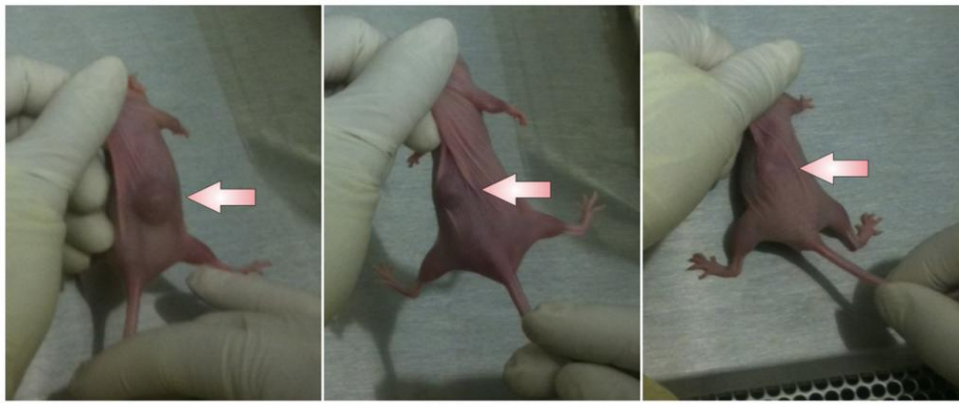


Normal Cell (HFF)



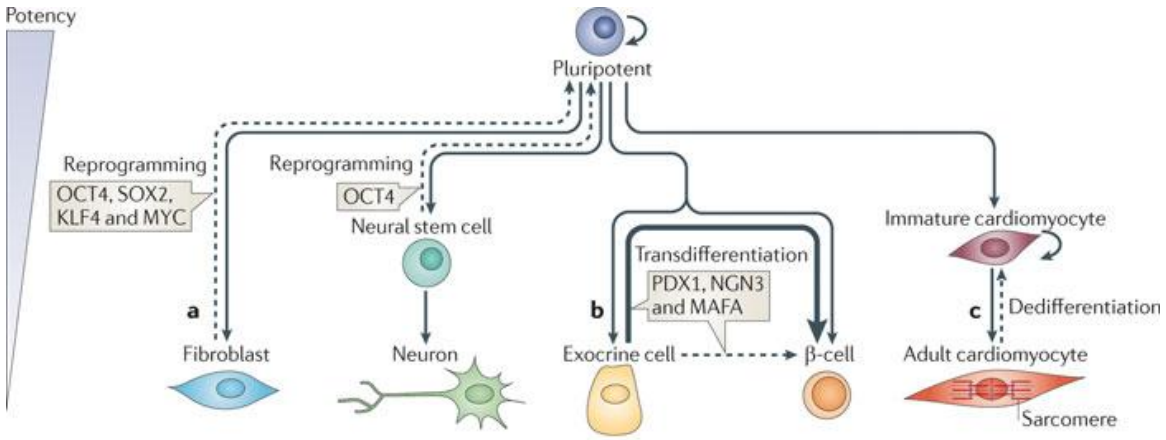
Cancer Cell (MCF-7)



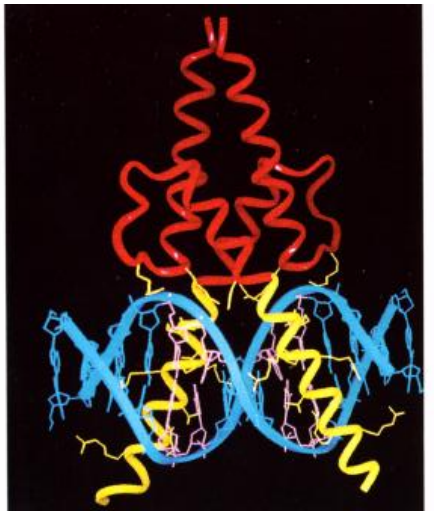


No Treatment | IV PBS | IT S-S APO NC

Application: Transcription Factors

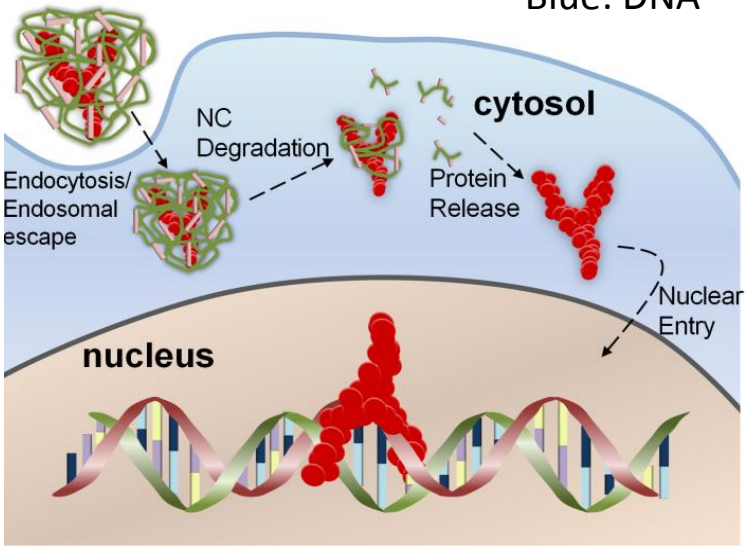


Jopling et al. *Nature Reviews Molecular Cell Biology*, **2011**



Red/yellow: TF
Blue: DNA

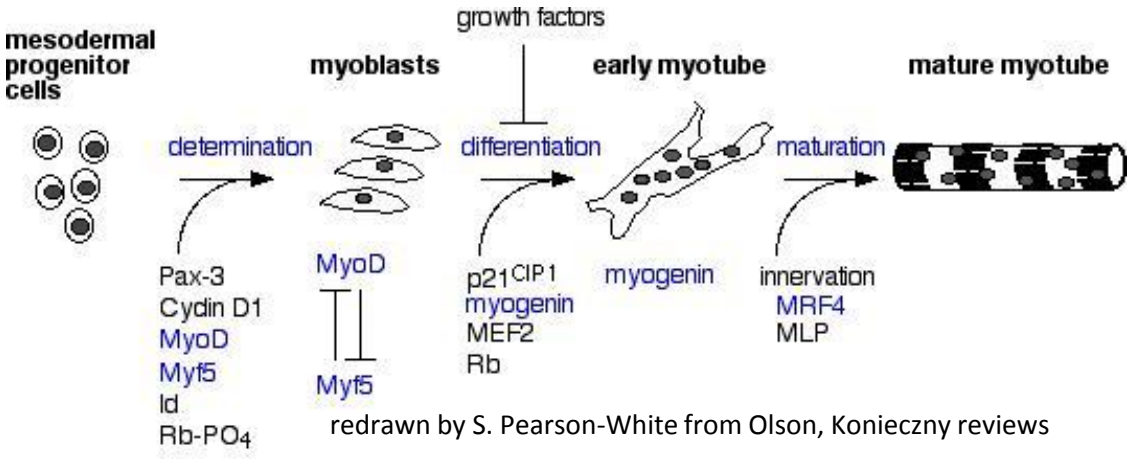
- Regenerative medicine aims to help repair diseased or damaged tissues by replacing affected cells with healthy functional cells
- Transcription factors are driving forces for directing cellular fate
- Effective TF delivery requires efficient nuclear delivery of proteins in active form



Delivery of MyoD

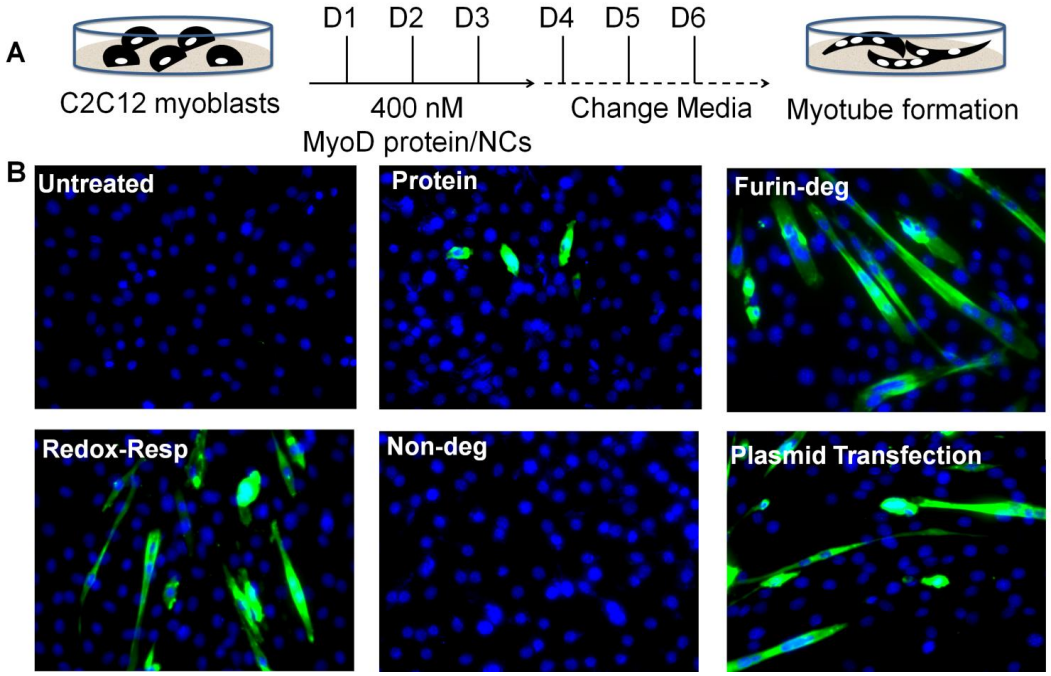
MyoD is one of myogenic regulatory factors which act sequentially in myogenic differentiation

Key role of MyoD is to commit mesoderm cells to a skeletal lineage, and then to regulate that process



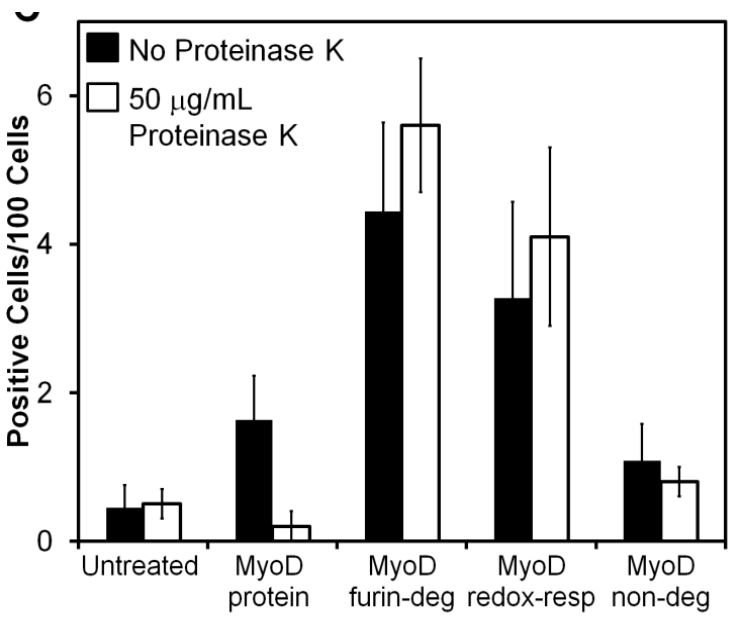
redrawn by S. Pearson-White from Olson, Konieczny reviews

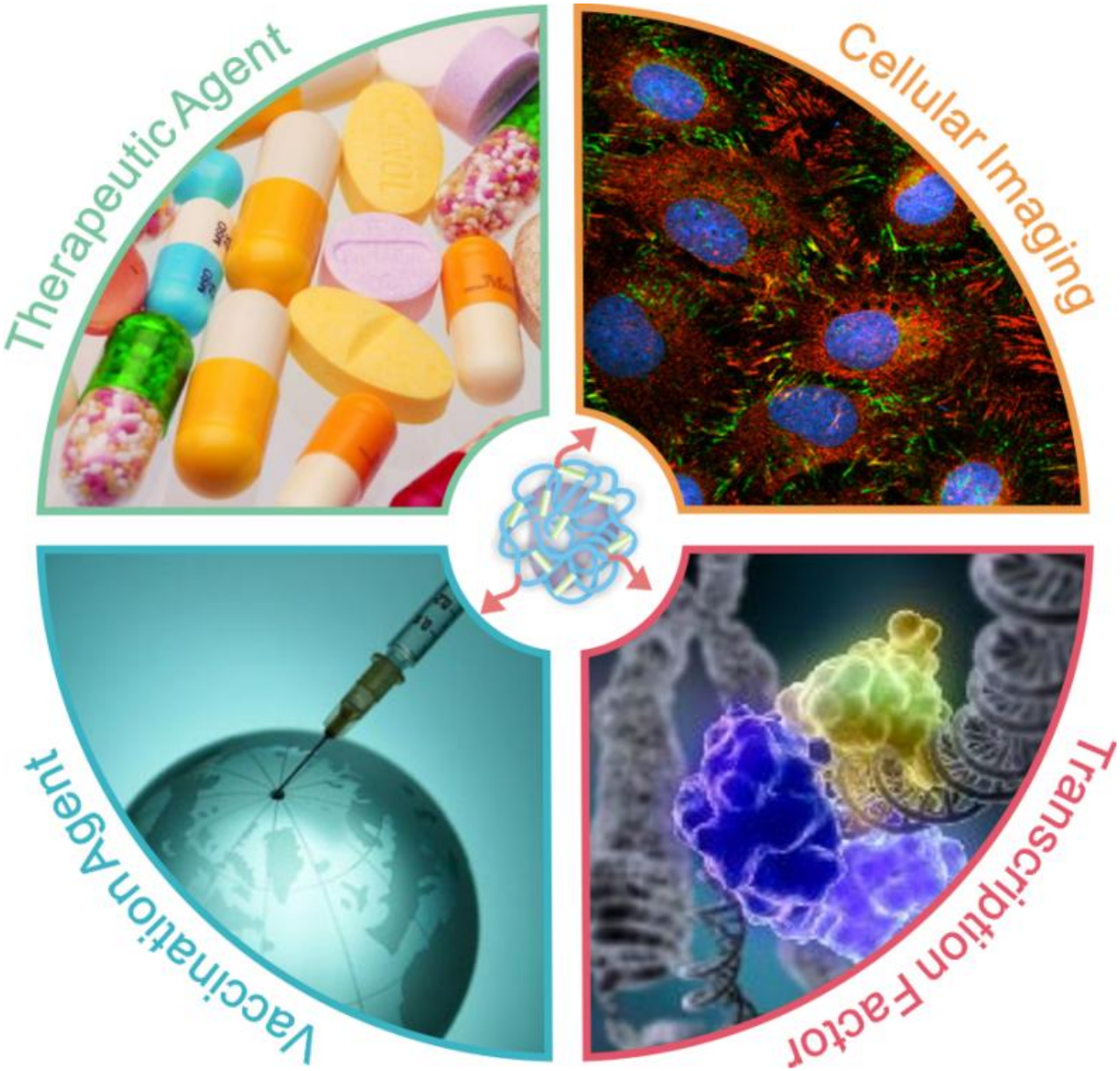
MyoD nanocapsule drives myotube formation



Green: MyHC Ab; Blue: DAPI-stained nuclei

MyoD nanocapsule is resistant to protease




Therapeutic Agent
Cellular Imaging
Vaccination Agent
Transcription Factor

Acknowledgement

Current Members

- Anuradha Biswas
- Muxun Zhao

Former Members

- Dr. Zhen Gu

Collaborators

- Prof. Guoping Fan (UCLA)
- Prof. Pin Wang (USC)

Funding Sources

Defense Threat Reduction Agency (DTRA)

Department of Defense BCRP

UCLA Broad Stem Cell Research Center

Carved by Caspase 3
Φ = 600 μm