Biodegradable Hollow Nanospheres

For

Drug Delivery

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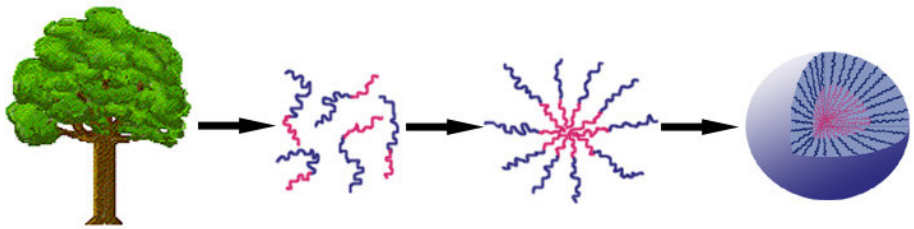
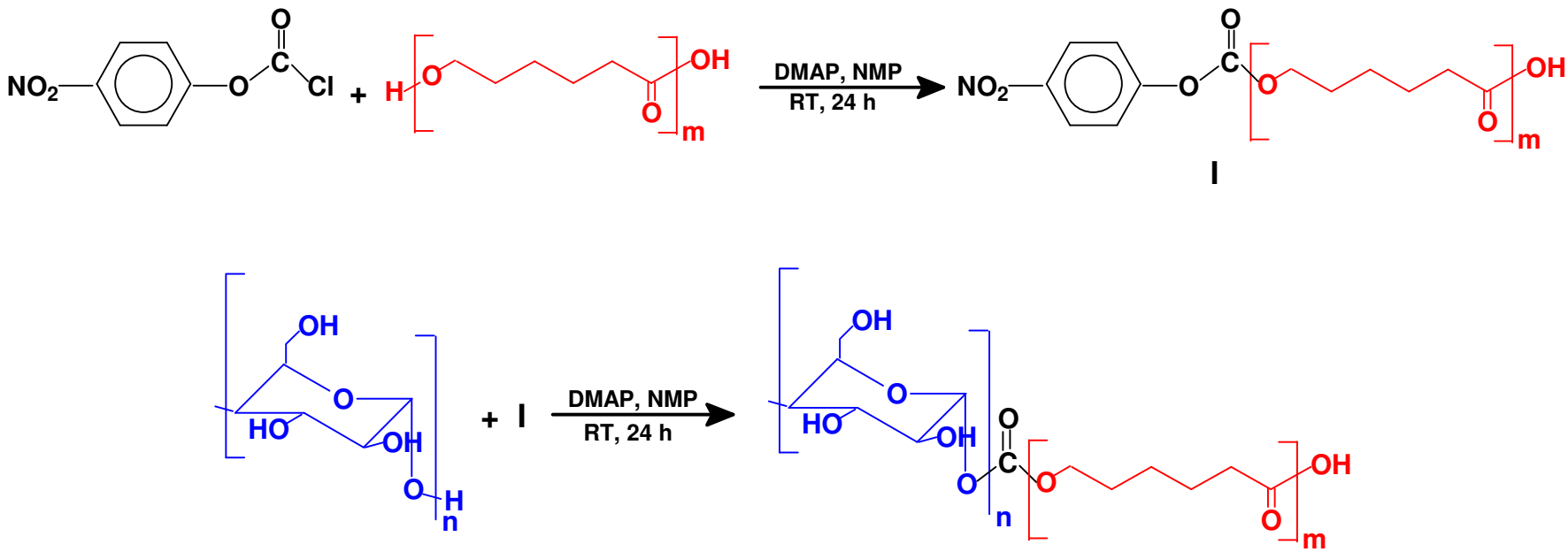


Characteristics of Polysaccharide-based Nanoparticles

- *Nontoxic*
- *Available from renewable resources*
- *Biodegradable*
- *"Green" synthesis*
- *Small size/large specific surface area*
- *Crosslinkable shell*
- *Core can be removed by selective hydrolysis*
- *Core and shell can be functionalized independently*

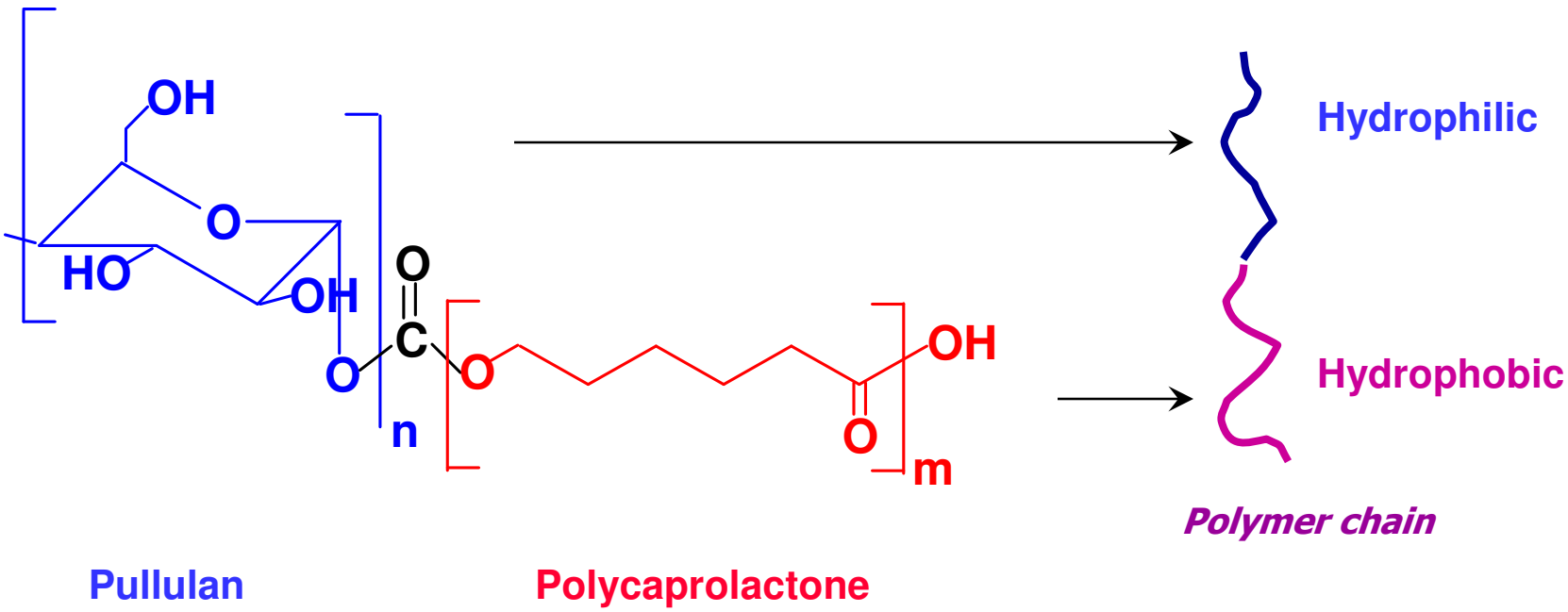
Synthesis



Novel "green" synthesis of linear block copolymers of pullulan and polycaprolactone with > 90 % yield

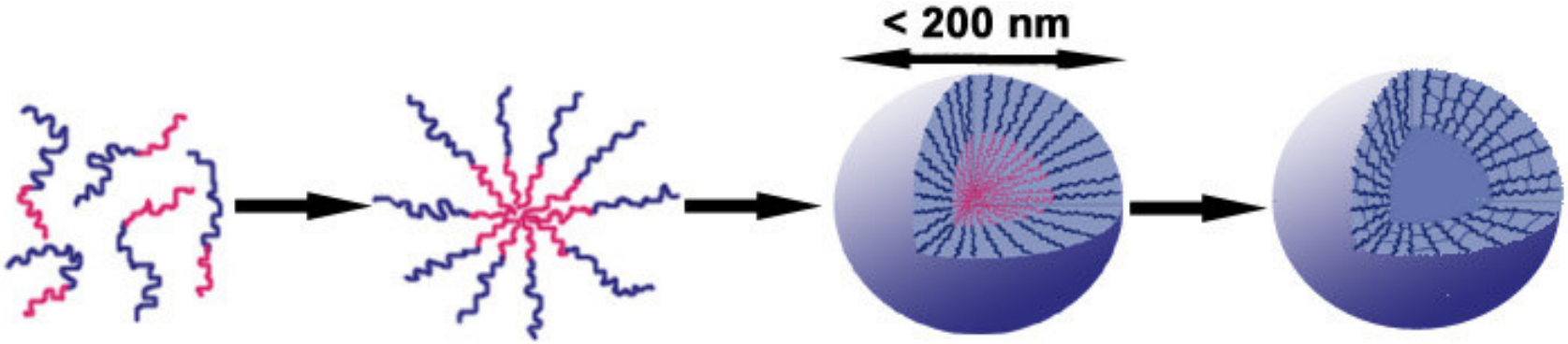
General method for polysaccharides and polyesters

Linear Amphiphilic Block Copolymers of Pullulan and Polycaprolactone as Building Block

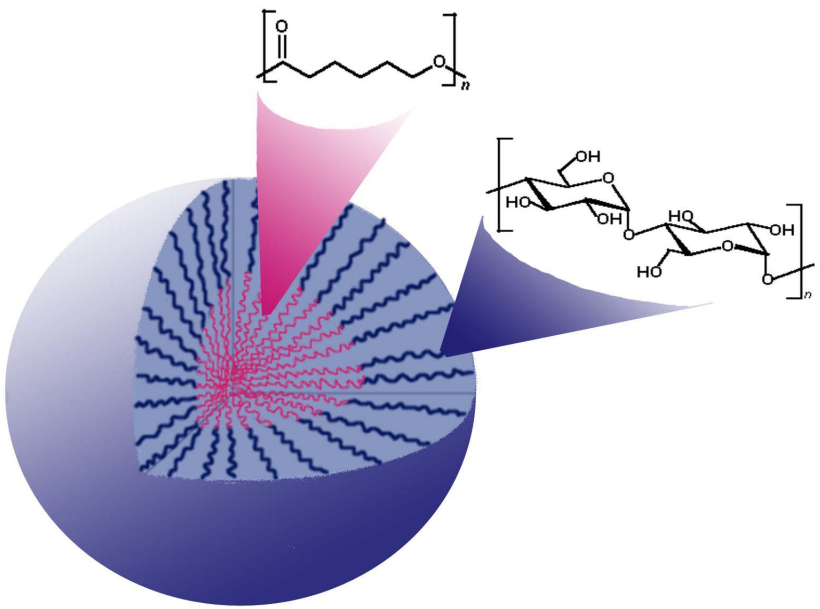


Amphiphilic Block Copolymers

Self-assembly of Polymer Micelles



Self-assembly in water ***Optional removal of core***



- ***Core-shell structure***
- ***Shell can be crosslinked***
- ***Core can be selectively removed***
- ***Shell and/or core can be functionalized selectively***

Nanoparticles

Proof

Fluorescence study shows critical aggregation concentration

Atomic Force Microscopy detected deposited spheres on surface

Transmission Electron Microscopy detected nanoparticles

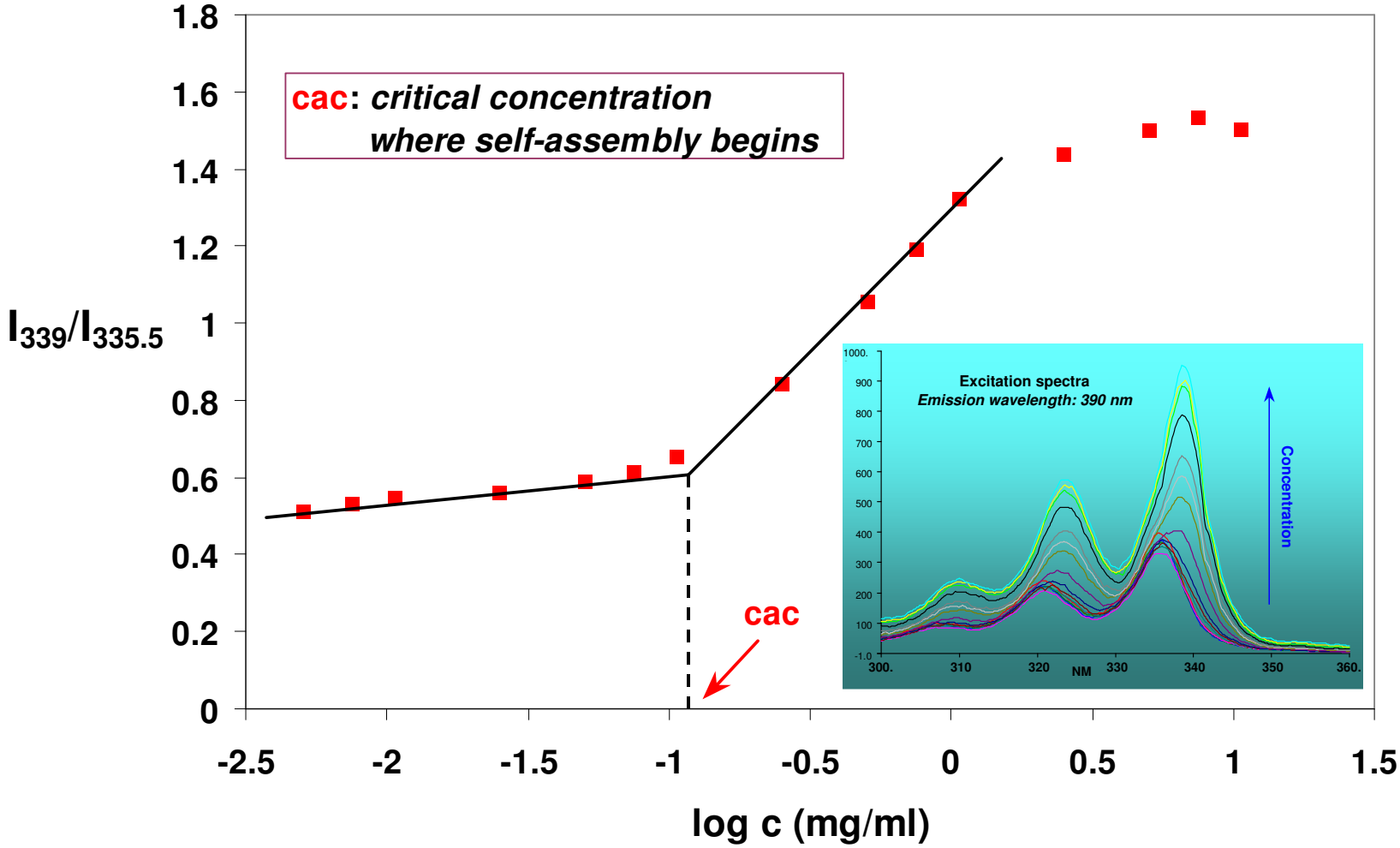
Spheres

Rods

Size

~ 60 nm diameter

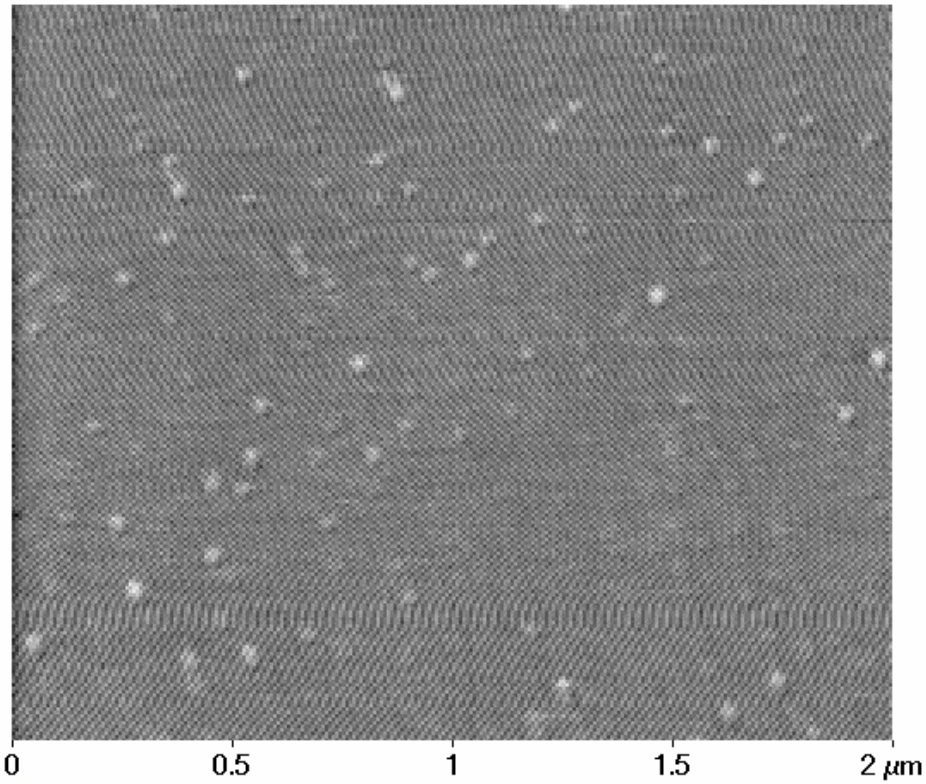
Fluorescence Study of Self-Assembly by Pyrene Partitioning



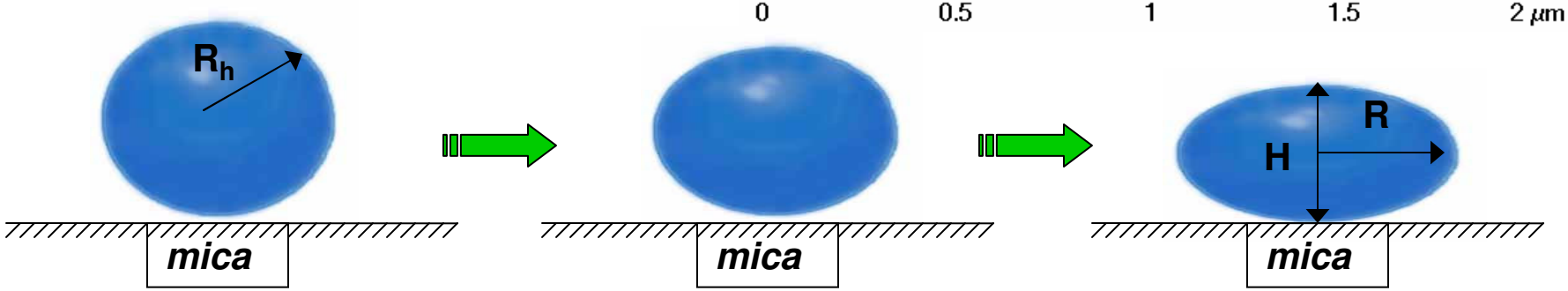
Typical curve with expected value of cac

Atomic Force Microscopy Analysis of Nanoparticles

Well-defined spherical nanoparticles observed



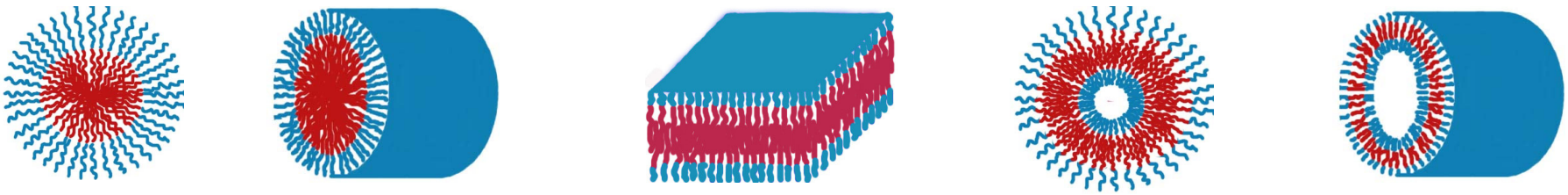
Soft nanoparticles flatten on surfaces upon drying



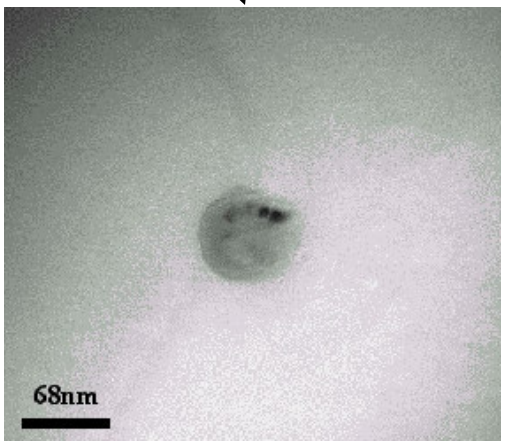
Morphology of Nanoparticles

Morphology = F(relative length and hydrophilicity/hydrophobicity of blocks)

Hydrophobic block length

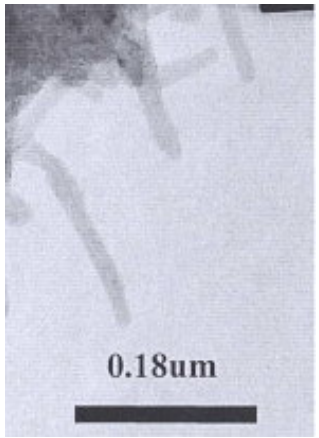


Sphere **Rod** **Lamella** **Vesicle** **Hollow rod**

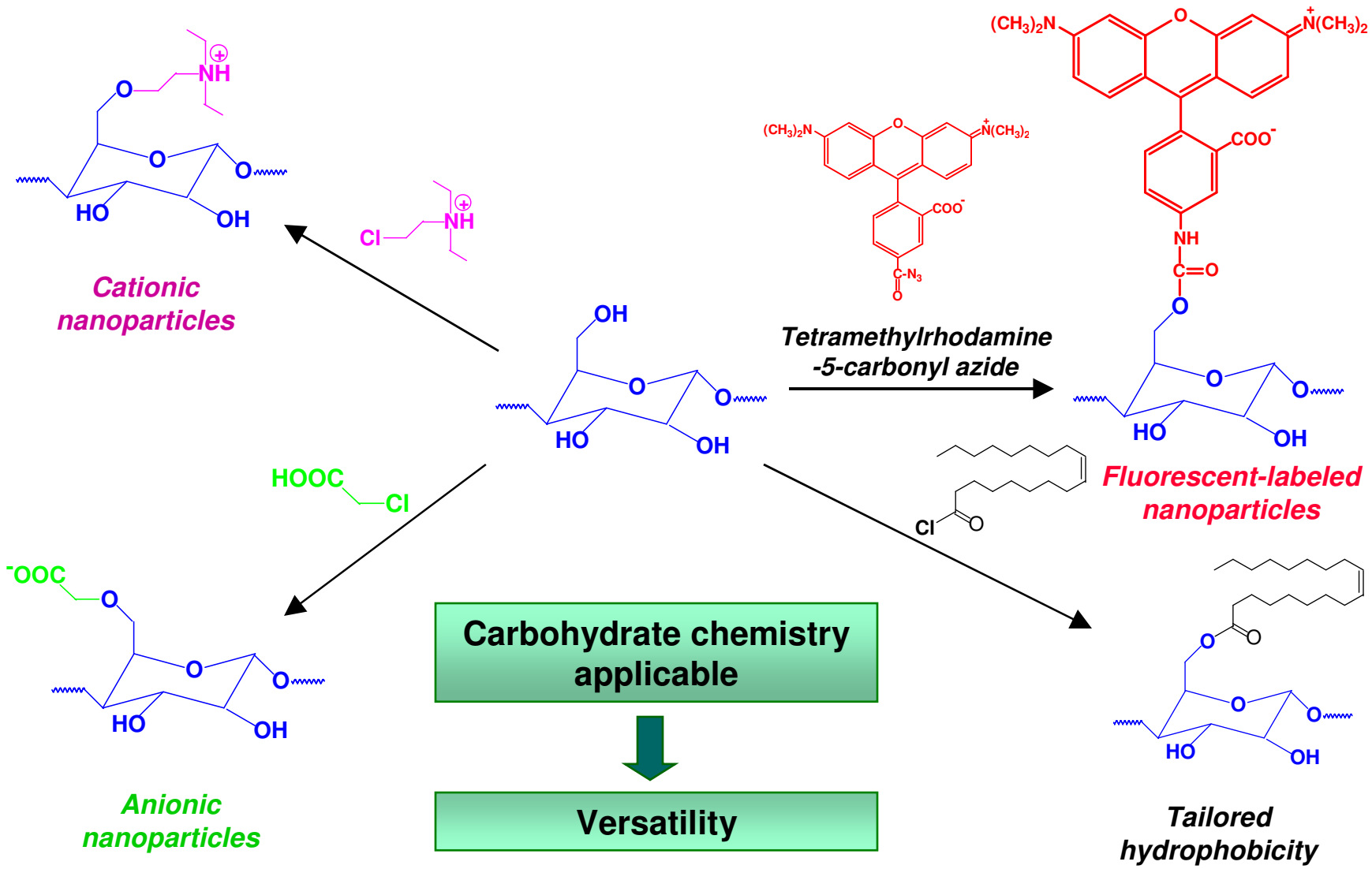


Pullulan-*block*-polycaprolactone

MW		
	~ 2,000	~ 10,000
	~16,000	~ 2,000



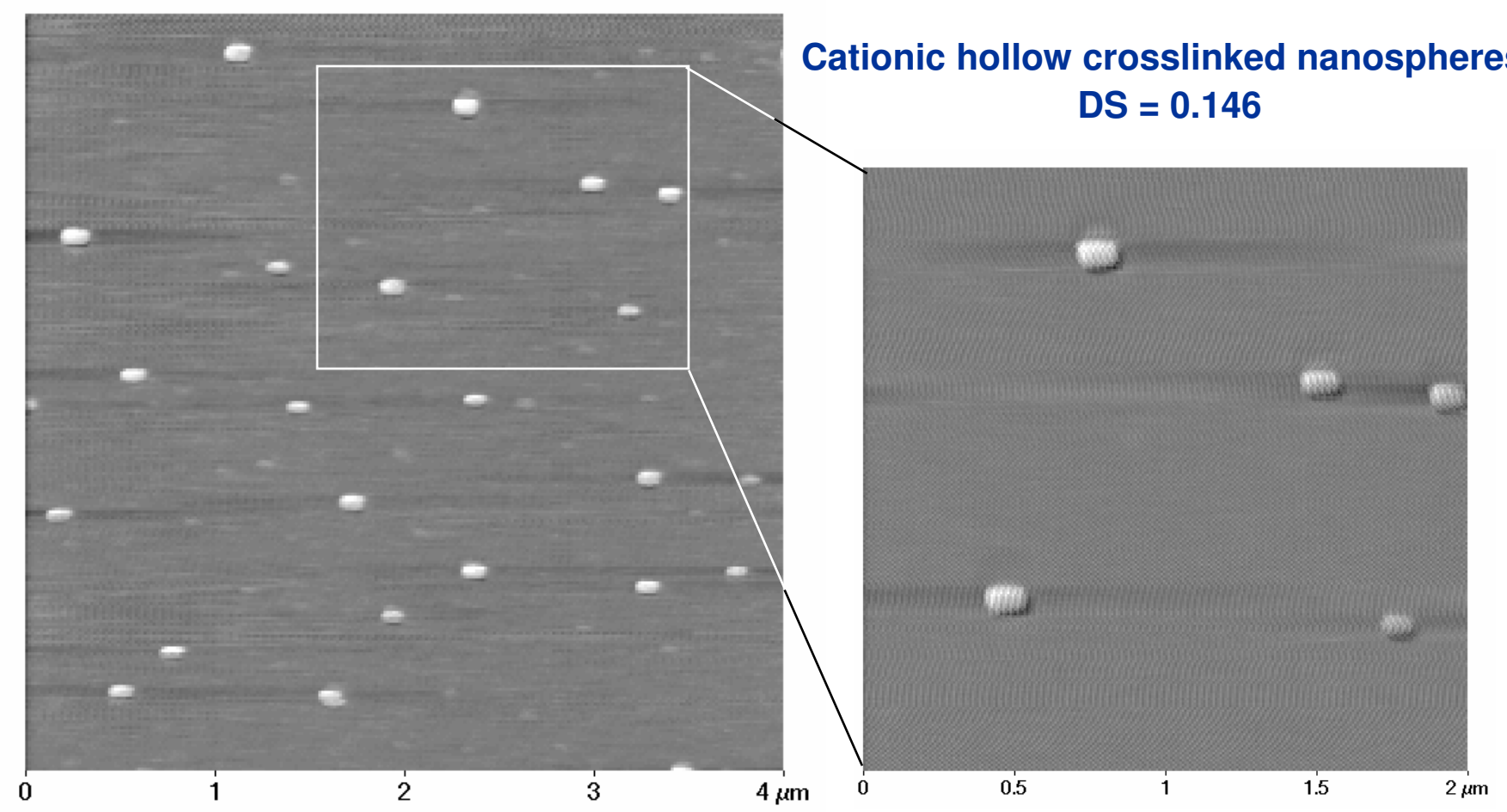
Functionalization of Nanoparticles



Carbohydrate chemistry applicable

Versatility

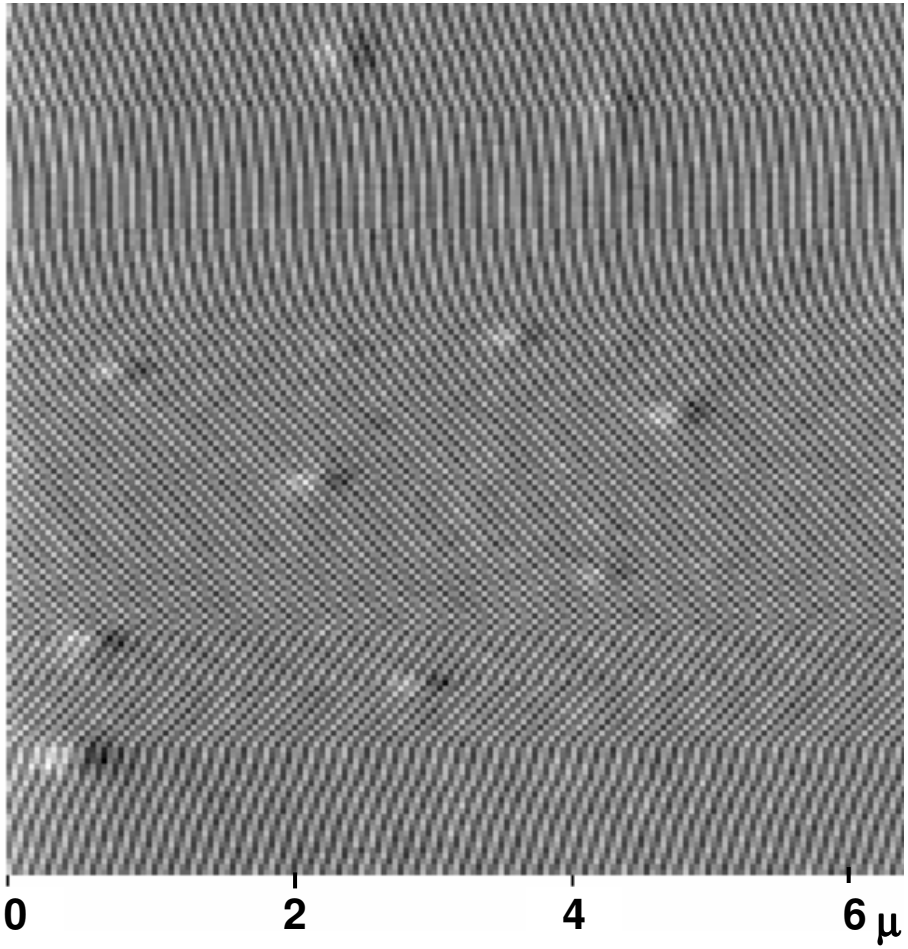
Atomic Force Microscopy Analysis of Cationic Nanospheres



Cationic hollow crosslinked nanospheres
DS = 0.146

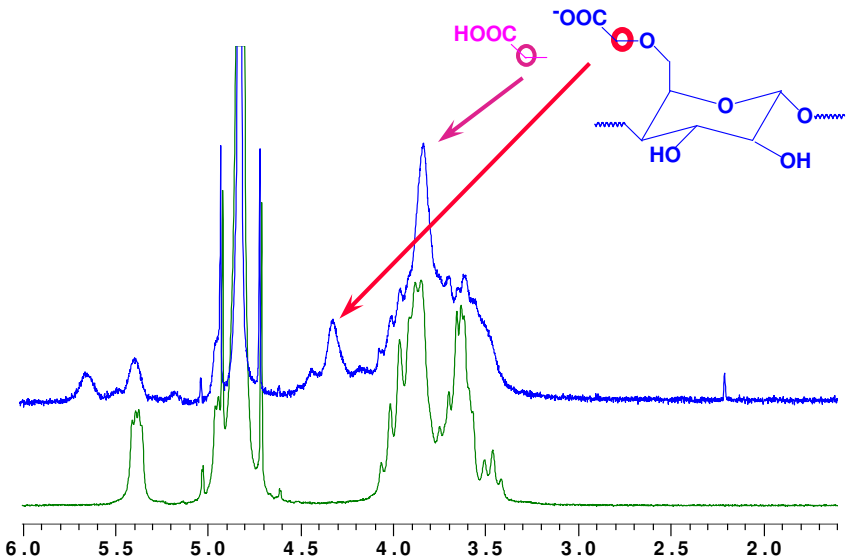
Well-defined spherical particles (horizontal distortion due to drag by probe)

Atomic Force Microscopy Analysis of Anionic Nanospheres



Anionic hollow crosslinked nanospheres

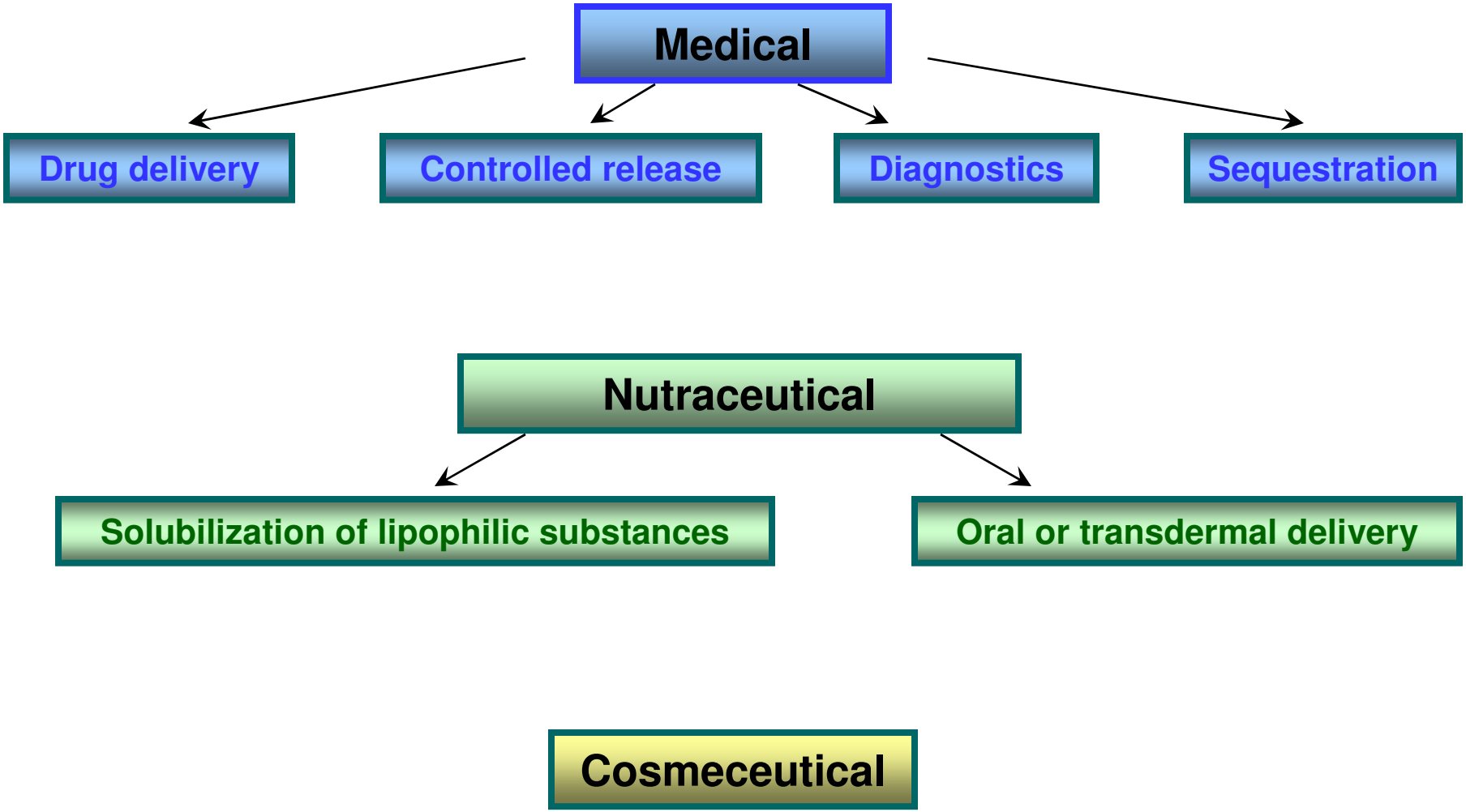
DS = 0.611



^{1}H NMR spectrum

Applications

General Overview

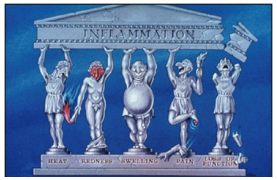


Highlights of Results

Oral and transdermal delivery of insulin

Encapsulation of hydrophobic and hydrophilic substances

Transdermal delivery of antiinflammatory agents



Encapsulation of nutrients, such as Vitamin E

Antibacterial properties of cationic nanospheres

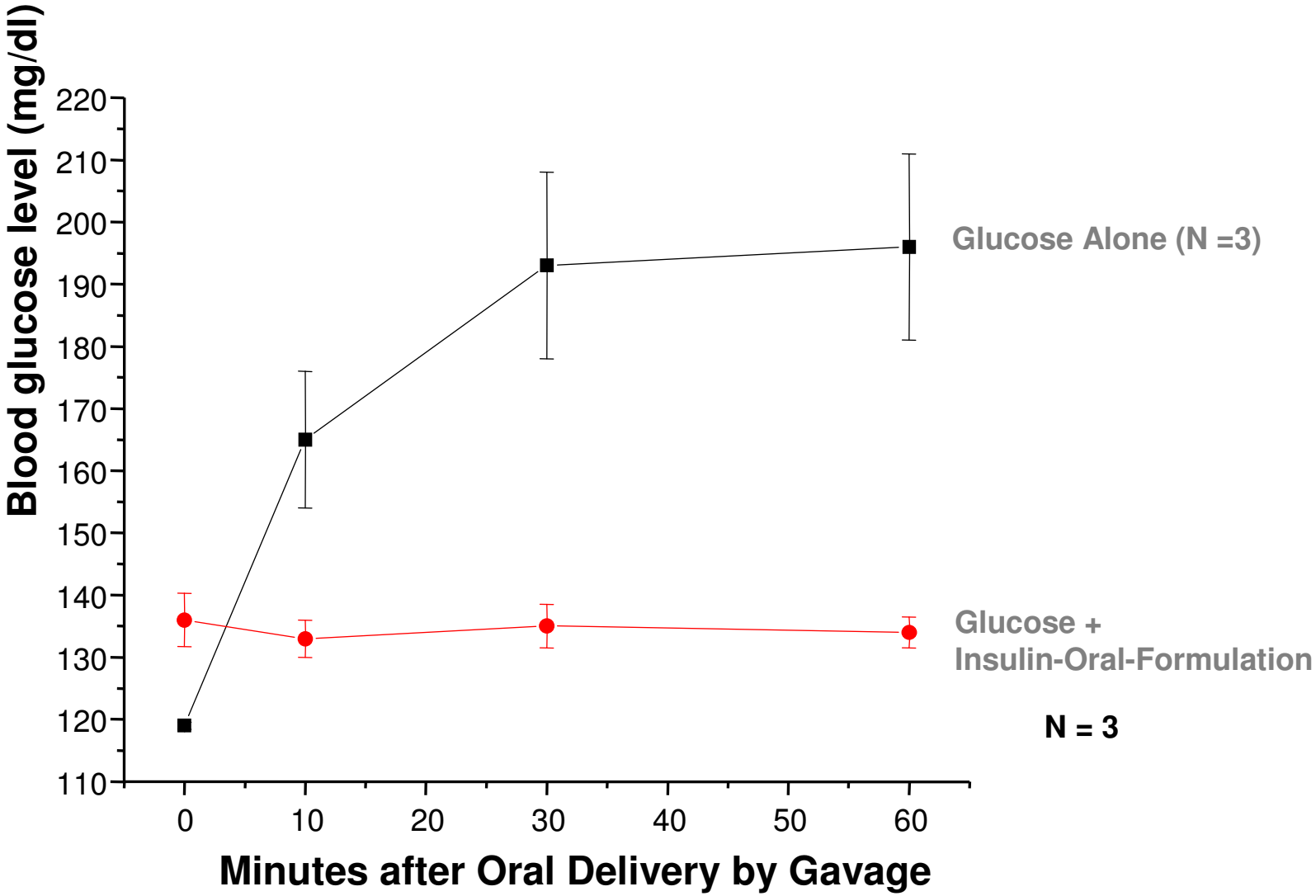
- **Challenge: More convenient non-injection dosage form for insulin delivery.**
  - **Avoid side-effects of inhalants**
  - **Avoid complexities of current attempted transdermal approaches (micro-puncture, iontophoresis)**
  - **Improve bioavailability**

Experimental data based on Charles River rats

- Used bovine insulin
- Experimental design:
  - Day #1:
    - Animals anesthetized by 50:50 O_2/CO_2
    - Gavaged with .4 grams of glucose in water
    - Made serial measurements of blood glucose with Freestyle glucometer
  - Day #3:
    - Same animals anesthetized by 50:50 O_2/CO_2
    - Gavaged with .4 grams of glucose plus Insulin-Oral-Formulation
    - Made serial measurements of blood glucose with Freestyle glucometer

- Insulin concentration (17 ug/ml) in Insulin-Oral-Formulation measured by radioimmunoassay.

Effect of Oral Formulation of Insulin on Rat Blood Glucose



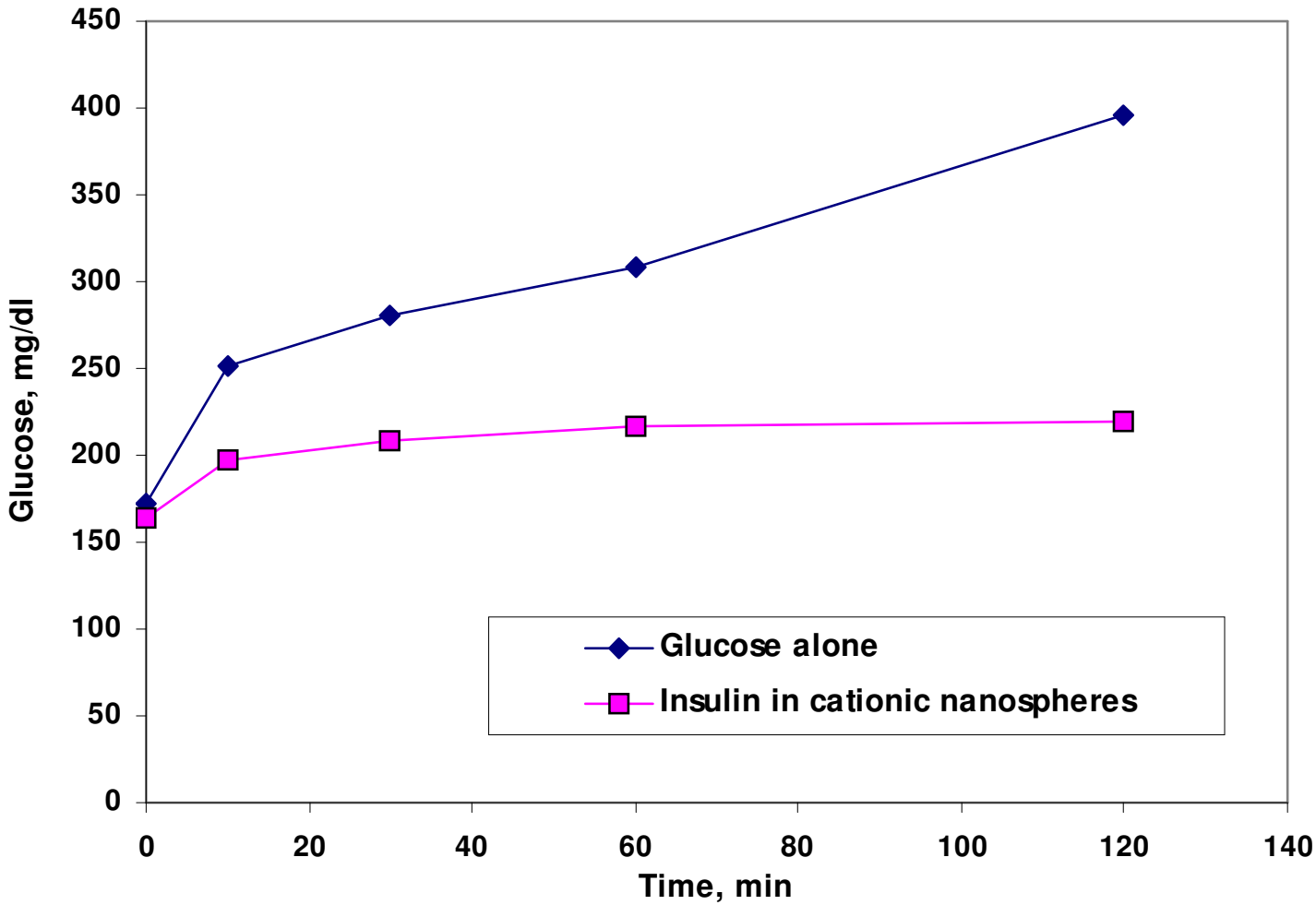
Transdermal Formulation of Insulin – Comparison to Empty Nano-Particle/Saline Controls

Experimental data based on Charles River rats

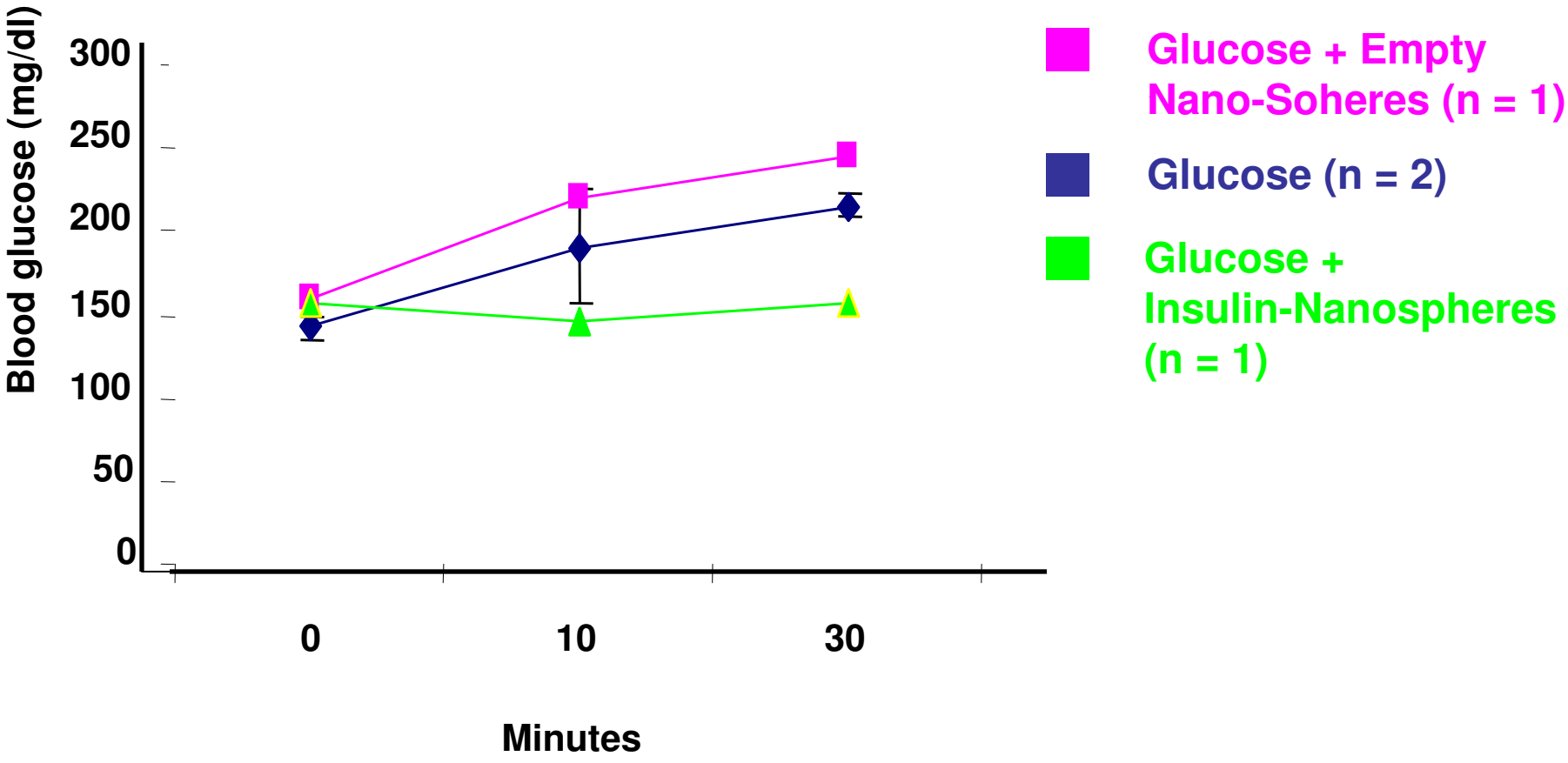
- Used bovine insulin
- Experimental design:
  - Day #1:
    - Animals anesthetized by 50:50 O_2/CO_2
    - Gavaged with 0.4 grams of glucose in water
    - Made serial measurements of blood glucose with Freestyle glucometer
  - Day #3:
    - Animals anesthetized by 50:50 O2/CO2
    - Gavaged with .4 grams of glucose
    - Simultaneously applied Insulin-Nano-Formulation formulation applied to 1.25 sq. inches of skin
    - Made serial measurements of blood glucose with Freestyle glucometer
- Insulin measured by radioimmunoassay.
- Insulin concentration (9 ug/ml) in Insulin-Nano-Formulatio

Transdermal Delivery of Insulin with Cationic Shell-xlinked Hollow Nanospheres in Hyperglycemic Rats

Glucose tolerance test in normal rats



Transdermal Proprietary Formulation of Insulin – Comparison to Empty Nano-Particle/Saline Controls

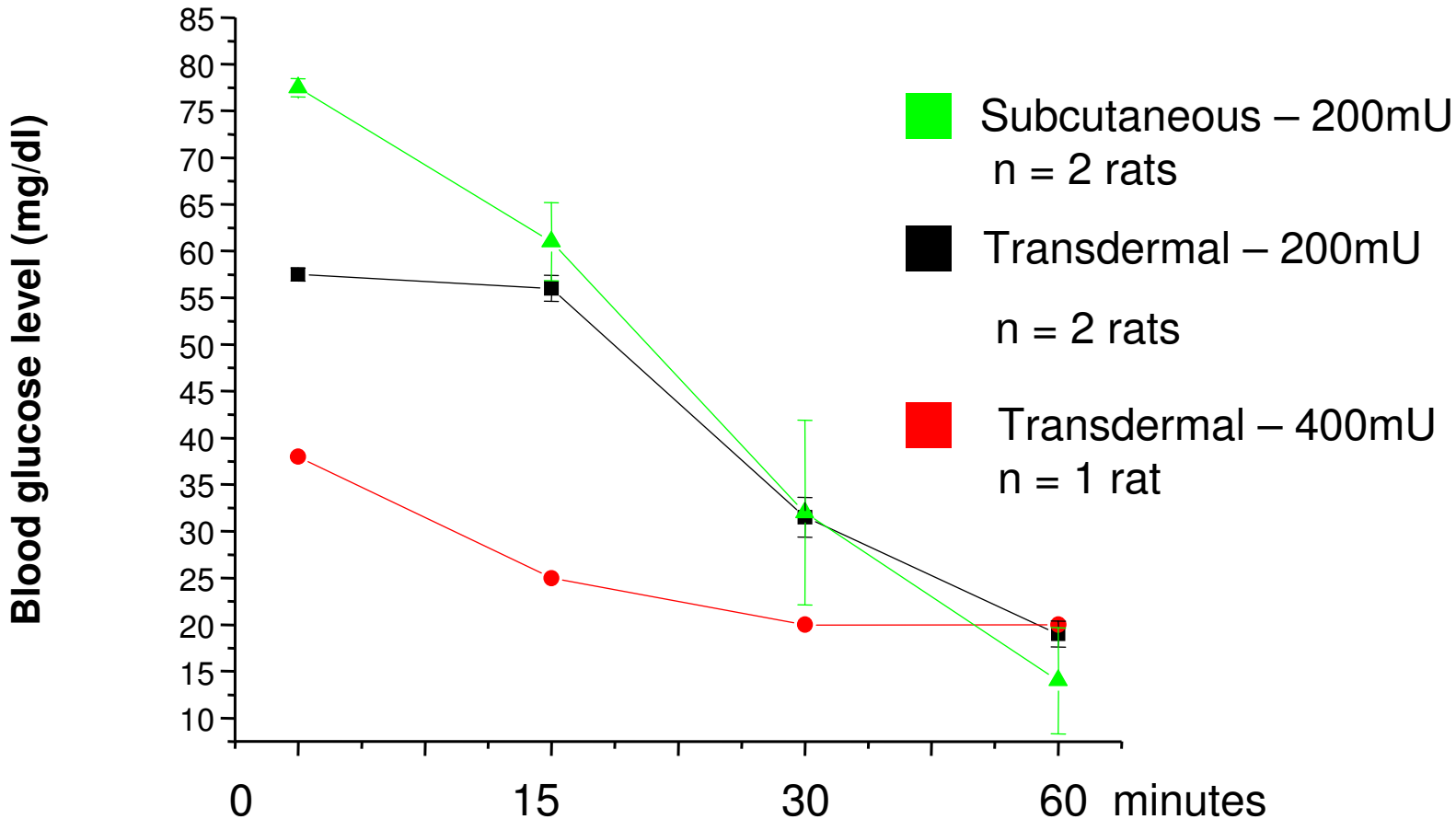


Transdermal Formulation of Insulin – Comparison to Subcutaneous Insulin

- Experimental data based on WAG nude rats
- Used bovine insulin
- Experimental design:
  - Subcutaneous:
    - Animals fasted for 18 hrs then anesthetized by 50:50 O_2/CO_2
    - Injected with SC insulin (200mU)
    - Made serial measurements of blood glucose with Freestyle glucometer
  - Transdermal
    - Animals fasted for 18 hrs then anesthetized by 50:50 O_2/CO_2
    - Applied Transdermal-Insulin formulation (200mU or 400mU) in drop-wise fashion on approximately 1.25 sq. inches of skin
    - Made serial measurements of blood glucose with Freestyle glucometer

- Insulin concentration (200 mU or 400 mU) in Transdermal-Insulin Formulation measured by radioimmunoassay.

Transdermal Proprietary Formulation of Insulin – Comparison to Subcutaneous Insulin



Insulin Delivery

Summary of Results

Transdermal

- Insulin encapsulated during self-assembly was delivered transdermally
- Nanosphere without insulin did not decrease blood glucose.
- Encapsulated insulin decreased blood glucose in hypoglycemic rats, as well.
- Experiments carried out on nude rats showed that the nanospheres can pass through undamaged skin.
- Nanosphere preparations can be dried and redissolved without affecting activity

Oral

- Neutral noncrosslinked nanospheres delivered insulin orally and significantly decrease blood glucose.

Additional Drug Delivery Applications

Anti-Cancer

- **Challenge: Higher efficacy (with less off-target side-effects)**
- **ASF (neuroblastoma xenograft)**
- **Dacarbazine (skin cancer xenograft)**
- **[Tamoxifen (breast cancer cell line)]**

- **Anti-Inflammatory**
  - **Challenge: Local delivery (to reduce systemic side-effects)**
  - **Celebrex**

Materials & Methods

- **Nude Mice**
  - since they lack a fully-functional immune system
- **Injection of neuroblastoma**
  - by subcutaneous injection of mouse neuroblastoma cells (300,000 cells /mL)
- **Injection of testing solution**
  - once tumors were detected
- **Measure the tumor diameter**
  - quantified daily in accordance with the longest diameter

Fig 1. Treatment Effects of Tumor Growth

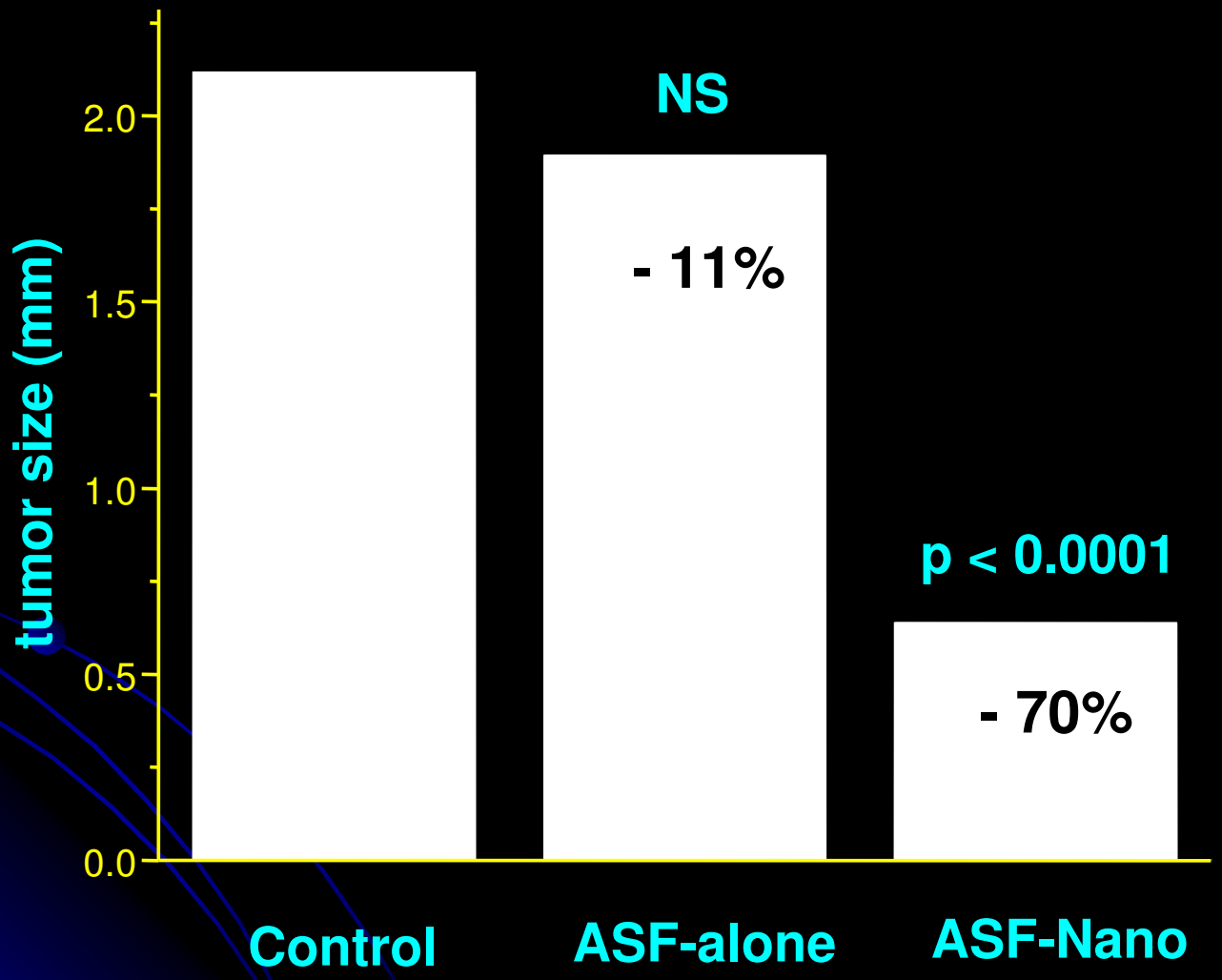
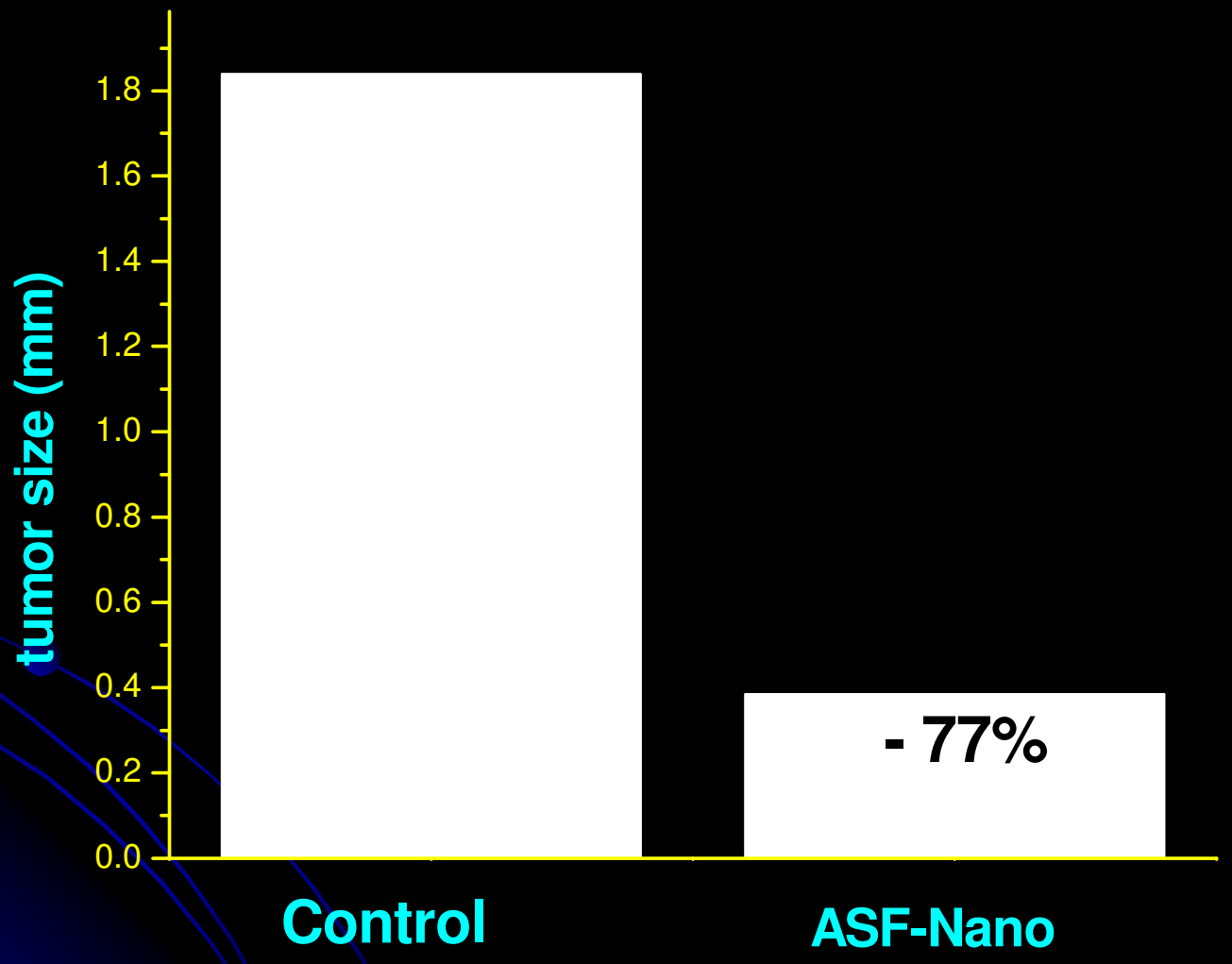
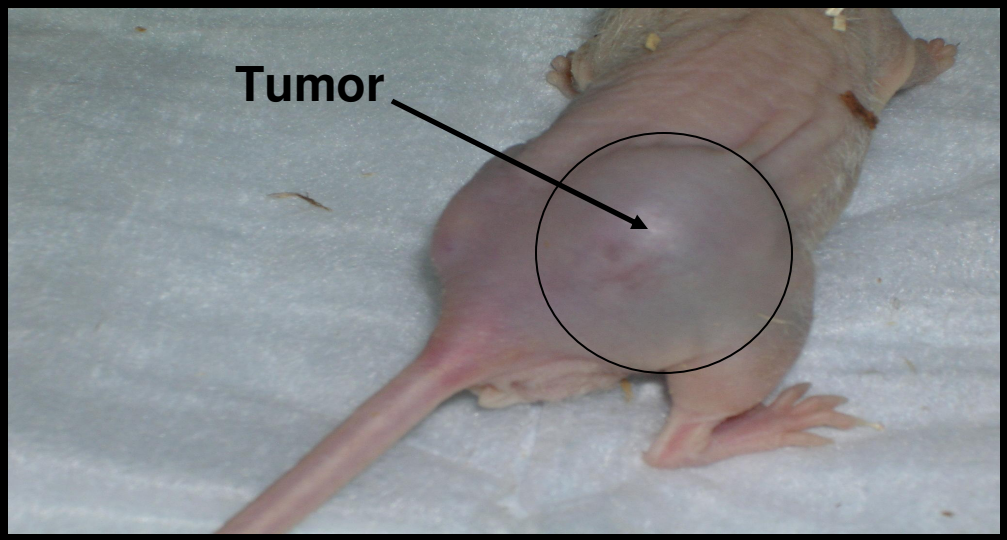


Figure 2 - Prevention Effects on Tumor Growth

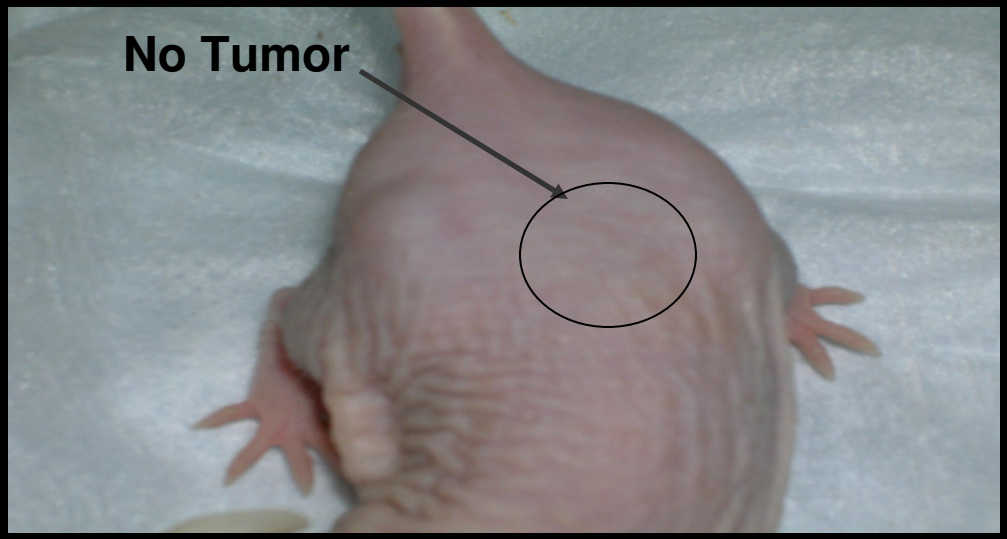


The neuroblastoma in the nude mice

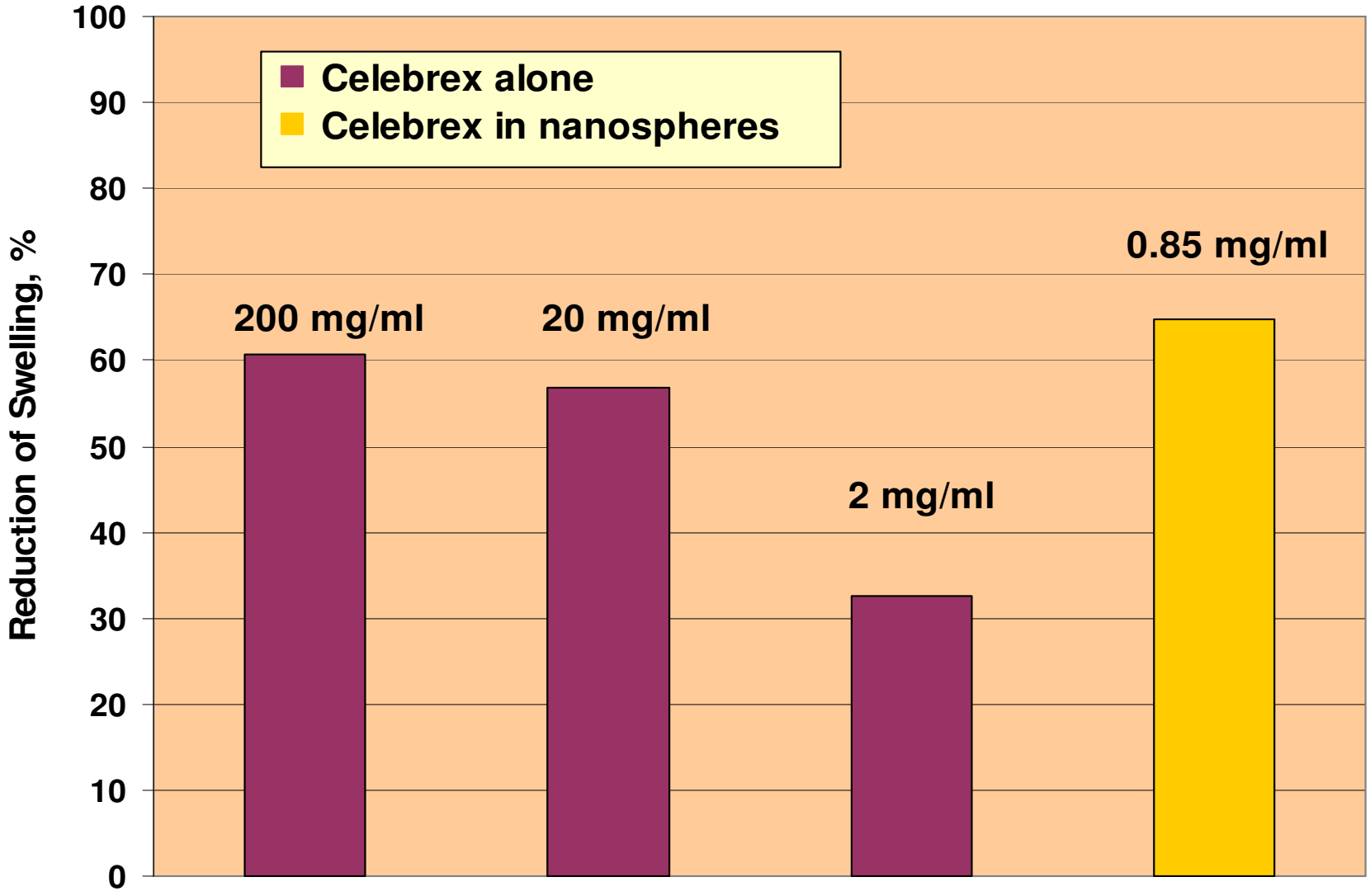
Control
(no treatment)



ASF
Nano-emulsion



Dose Response Study of Celebrex in CD-1 Mice (n = 5) Applied Topically in Nanospheres



Conclusion

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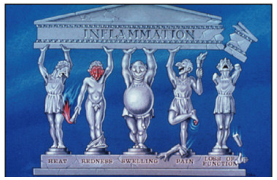
Encapsulation of hydrophobic and hydrophilic substances

Transdermal delivery of antiinflammatory agents



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