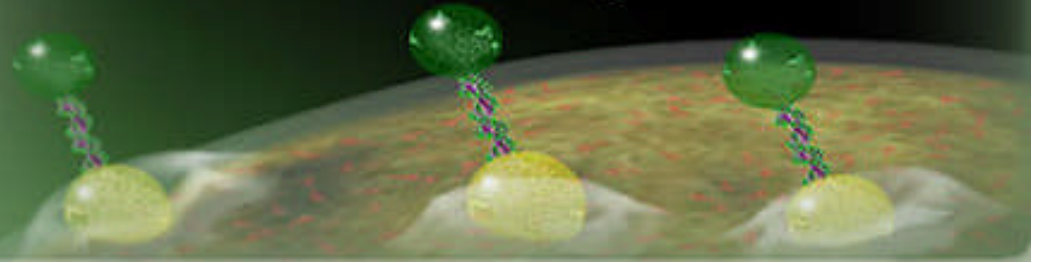


Michigan Nanotechnology Institute for Medicine and Biological Sciences



# Encapsulation of Submicrometer-sized 2-Methoxyestradiol crystals into Polymer Multilayer Capsules for Biological Applications

Suhe Wang, Xiangyang Shi, James Baker Jr

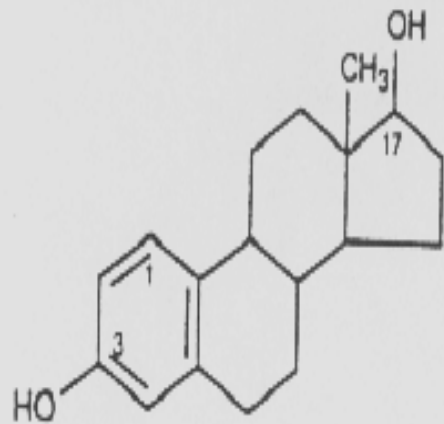
Michigan Nanotechnology Institute of Medicine and Biological Sciences  
University of Michigan, Ann Arbor, MI 48109

Presented at the International Congress of Nanotechnology, October 31-November 3, 2005- San Francisco

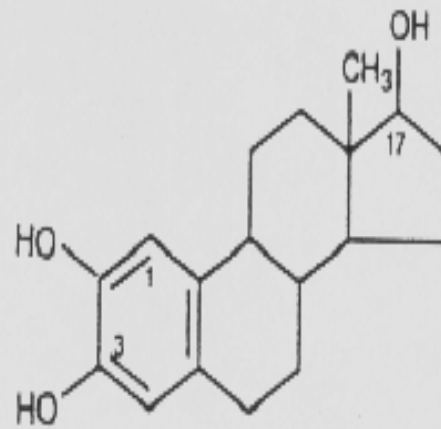
## What is 2-methoxyestradiol (2-ME)?

- metabolite of estrogen
- elevated level as a result of estrogen increase
- low affinity for estrogen receptor

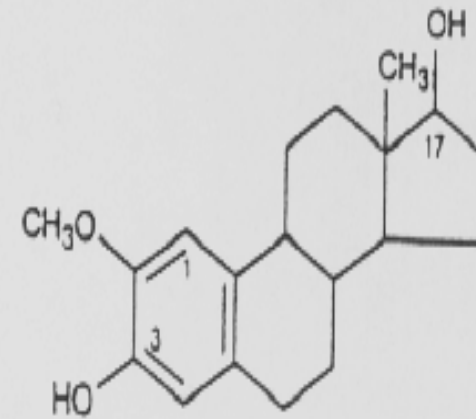
# Chemical Structure of Estradiol and its Metabolites



Estradiol-17 $\beta$



2-hydroxy-17 $\beta$ -estradiol



2-methoxyestradiol (2-ME)

# Characterization of 2-ME

- a low-toxic, anticancer agent
- insoluble in water
- soluble in ethanolic solutions
- requires sustained release for effect

# **Mechanism of 2-ME as an anti-tumor agent**

## **Microtubule activity**

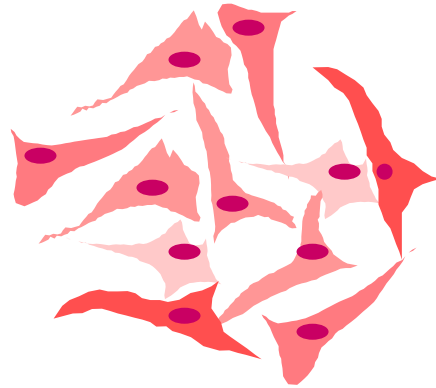
2-ME caused selective disruption of microtubules, tubulin.

## **Apoptosis**

Death receptor pathway: DR5, TRAIL, FLIP.

Mitochondrial apoptotic pathway: cytochrome c, Cdc6.

# Outline of experiment



**Primary human  
thyrocytes and  
FRTL-5 cells**



**treatment either with  
estradiol or 2-methoxyestradiol**



**morphology**



**MTT  
assay**

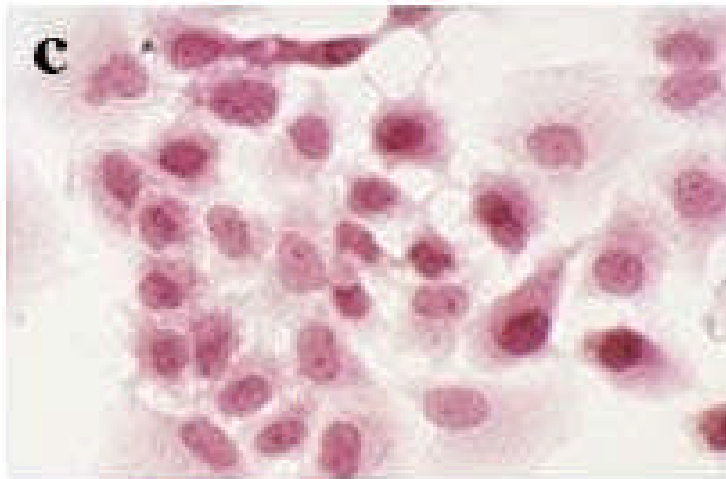
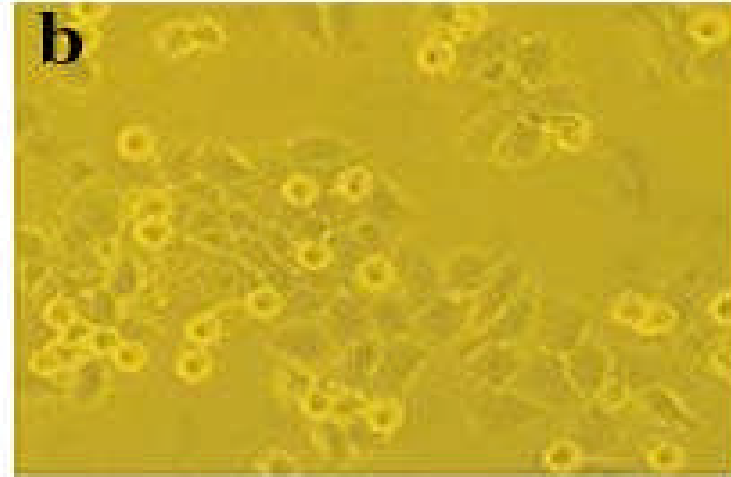
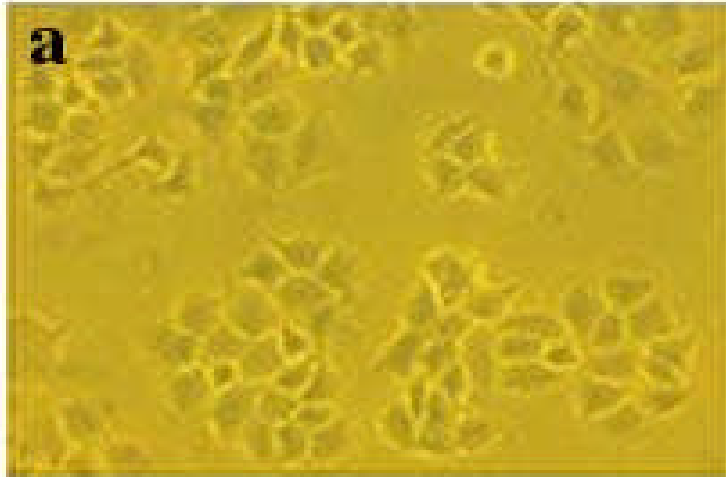


**cell cycle  
analysis**

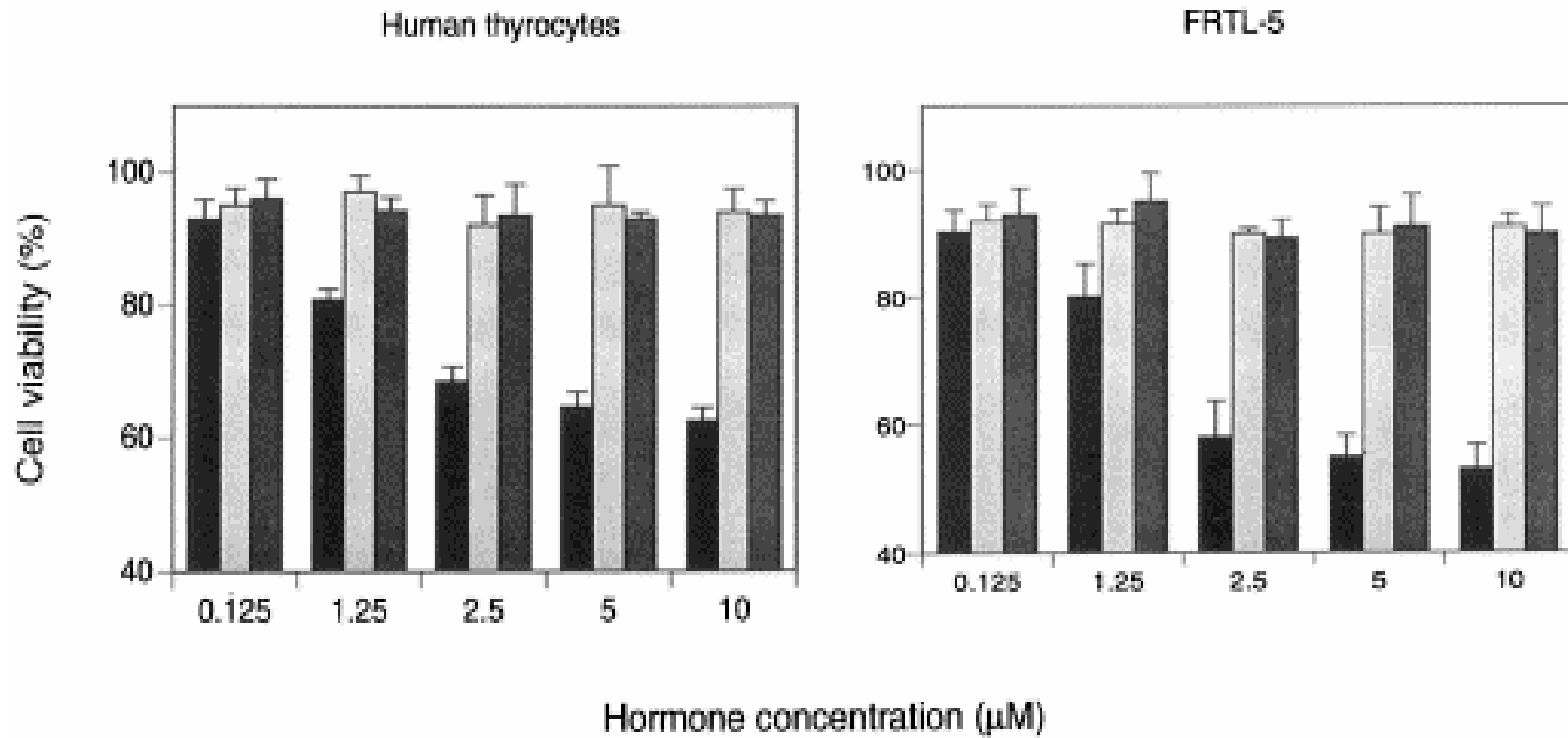


**DNA  
fragmentation**

## Effect of 2-ME on FRTL-5 Cells

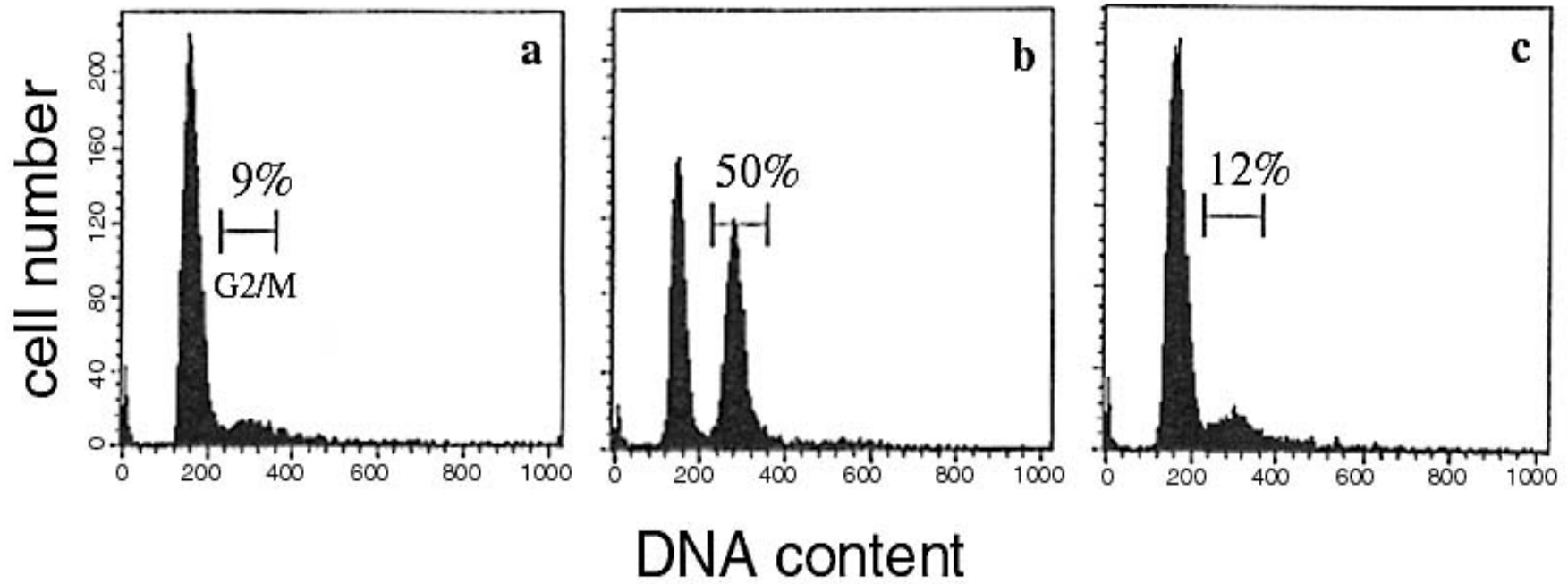


# Cell viability assayed by MTT

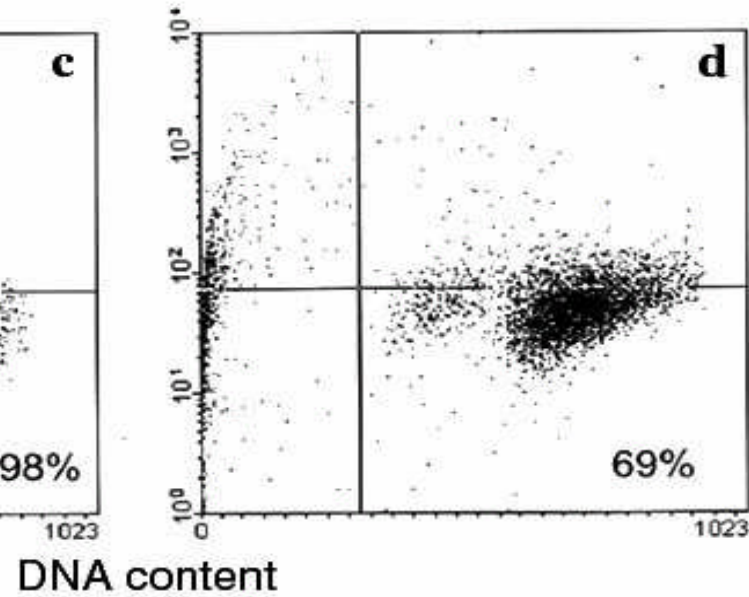
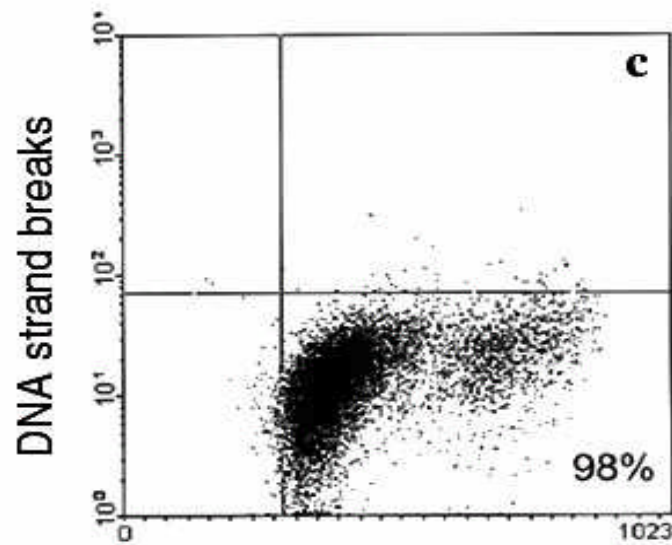
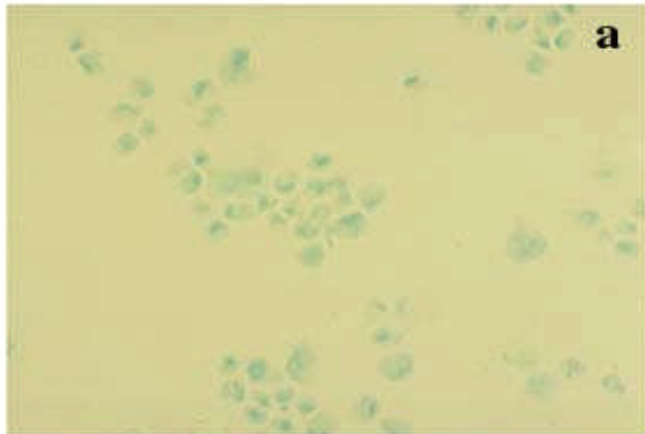




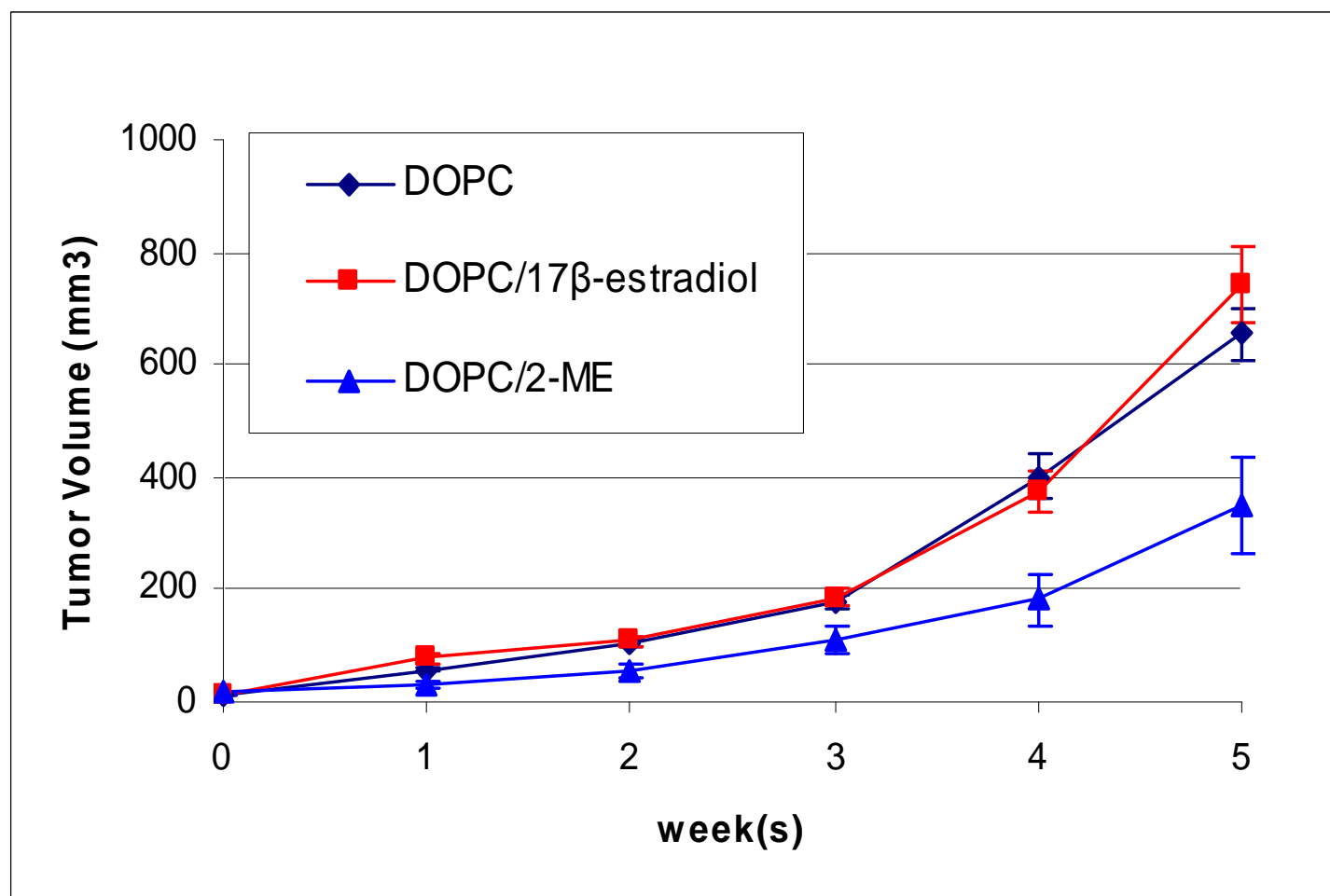
# Cell cycle analysis



# Apoptosis induced by 2-ME



# Inhibition of tumor growth



# **Reasons to have a new formulation of 2-ME - Encapsulation**

- Low bioavailability: 2-ME without further modification can quickly be cleared.
- High doses of 2-ME or frequent administration may cause hot flashes, fatigue, diarrhea, and reversible liver enzyme elevations.

# **Reasons to have a new formulation of 2-ME - Encapsulation**

Avoid toxicity

Deliver a prolonged therapeutic level of the drug

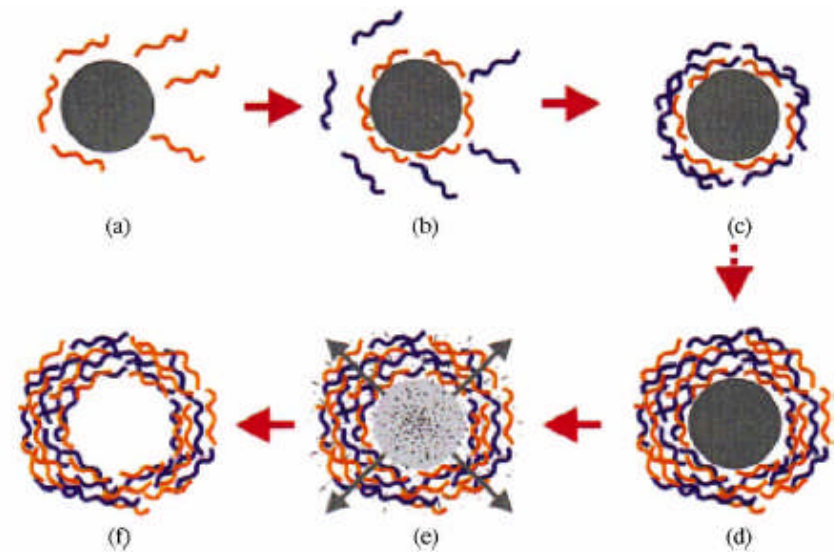
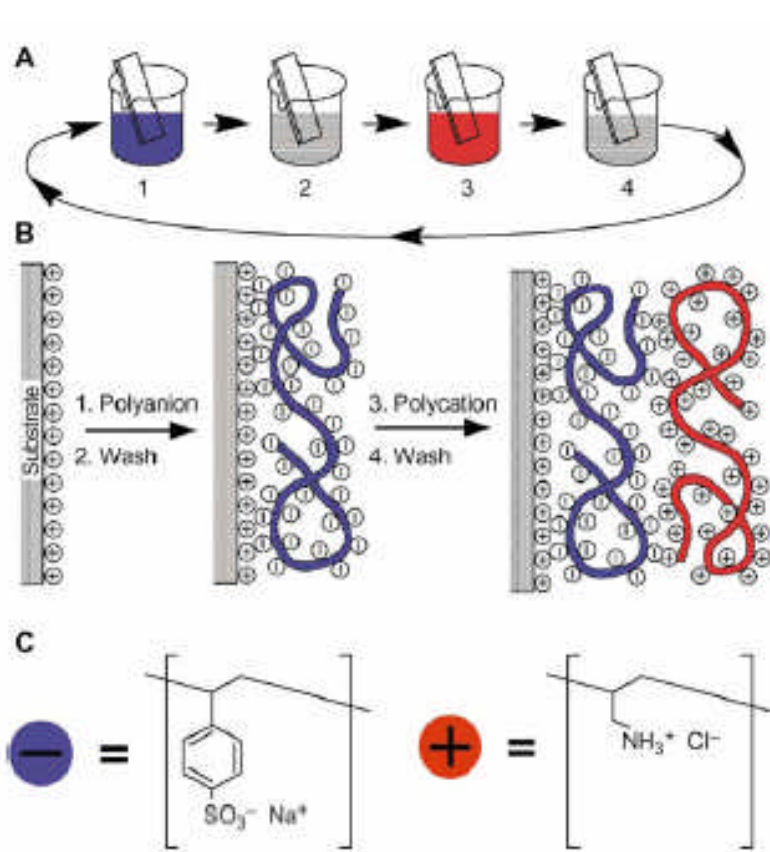
Increase therapeutic efficiency

## Formulations of 2-ME

- Concentrated ethanol solution
- Liposome-encapsulated 2-ME
- **Additional formulations?**

# Self-Assembled Polymeric Capsules

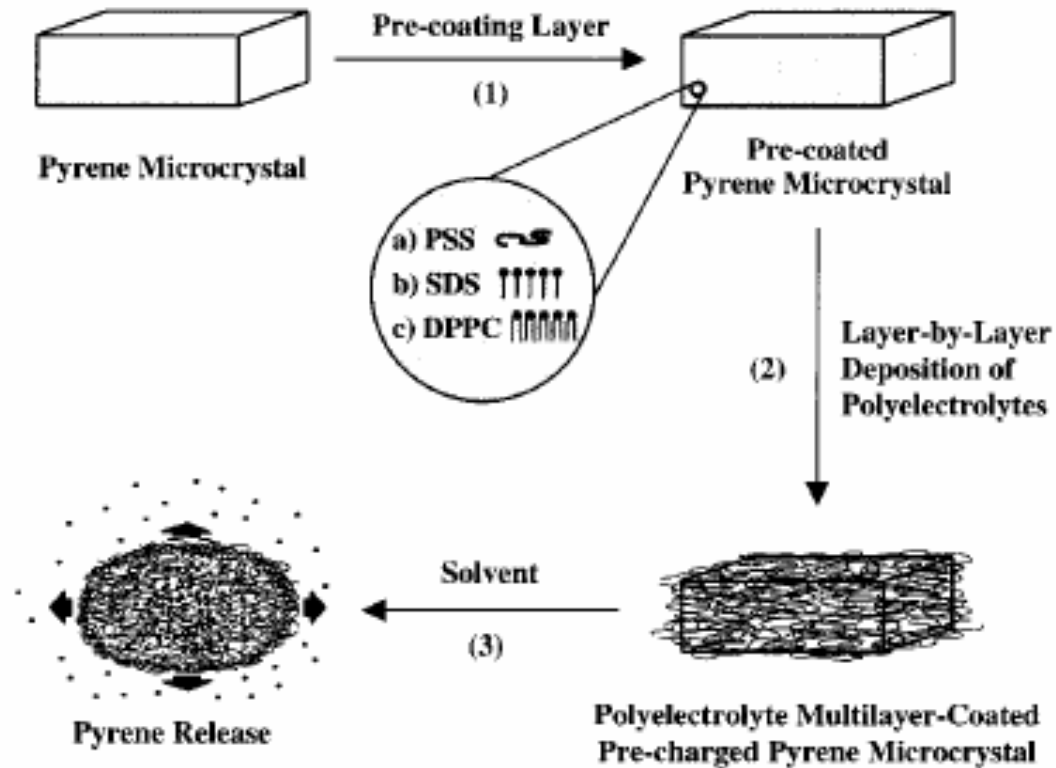
- Layer-by-layer self-assembly



Donath E, Sukhorukow GB,  
Caruso F, Davis SA,  
Möhwald H. *Angew Chem Int  
Ed* 1998;37:2201–5.

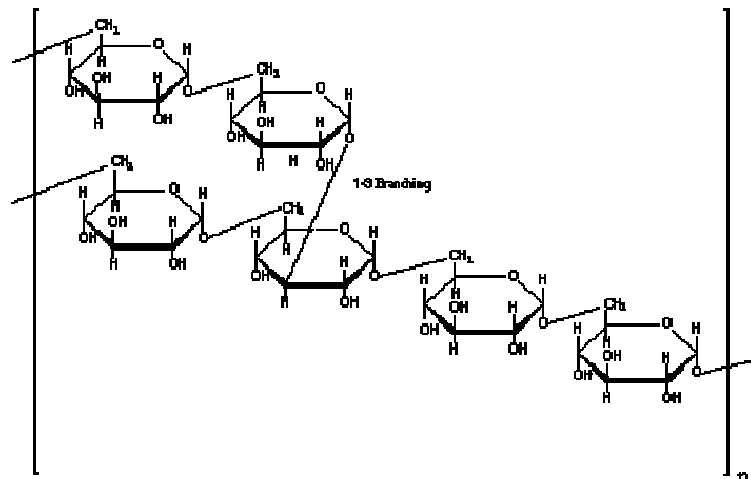
Decher G. *Science* 1997;277:1232–7.

# Self-Assembled Drug Capsules

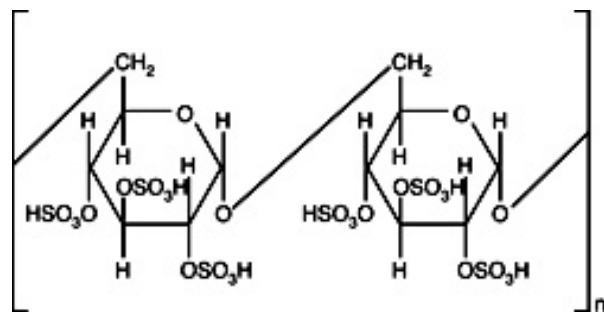


Shi, X.; Caruso, F. *Langmuir* **2001**, *17*, 2036-2042

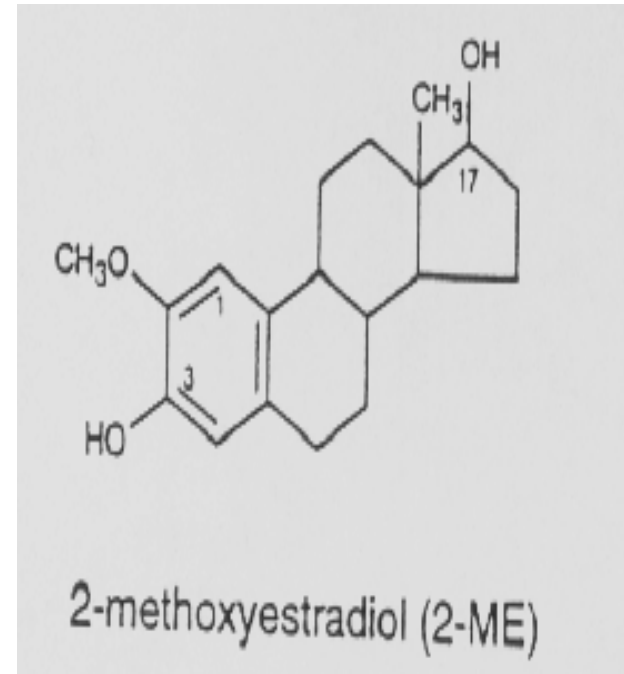
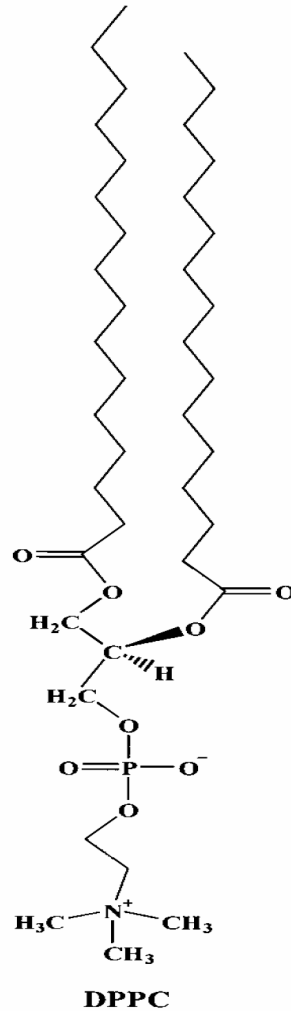




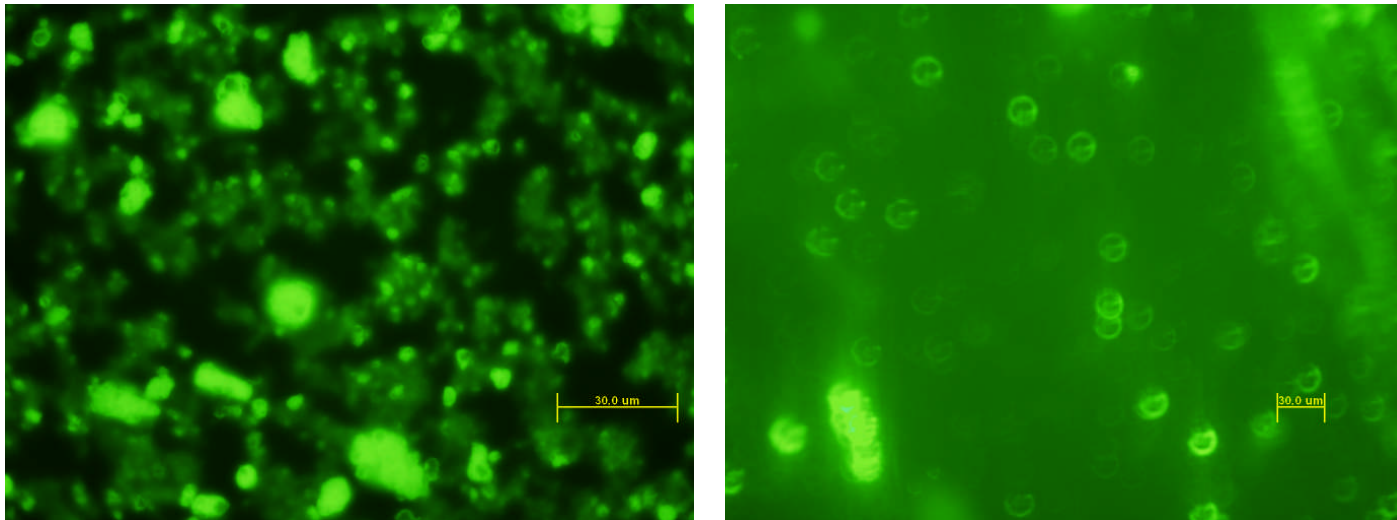
**Dextran**



**Dextran sulfate**

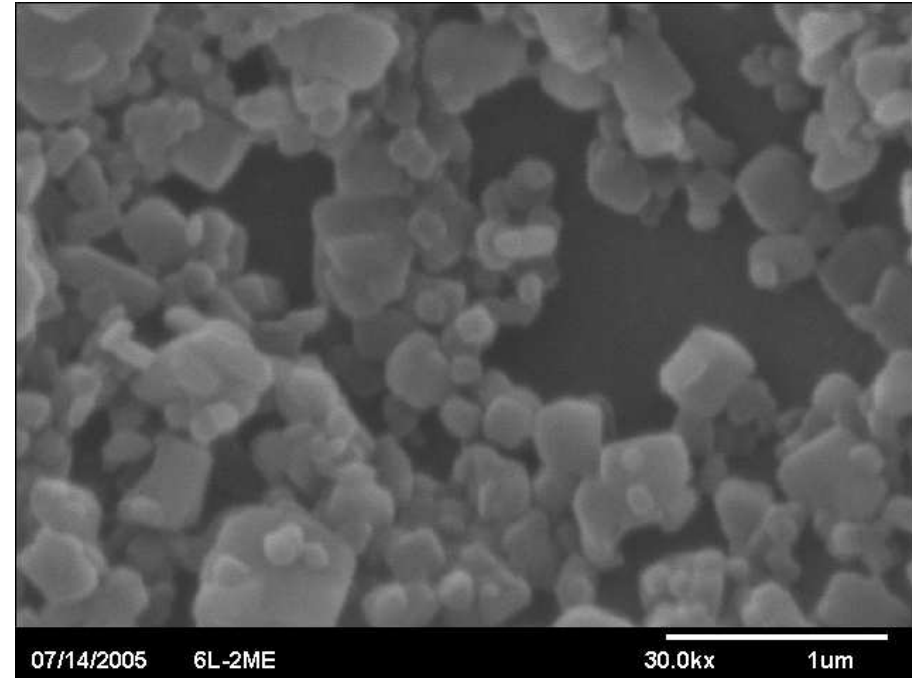
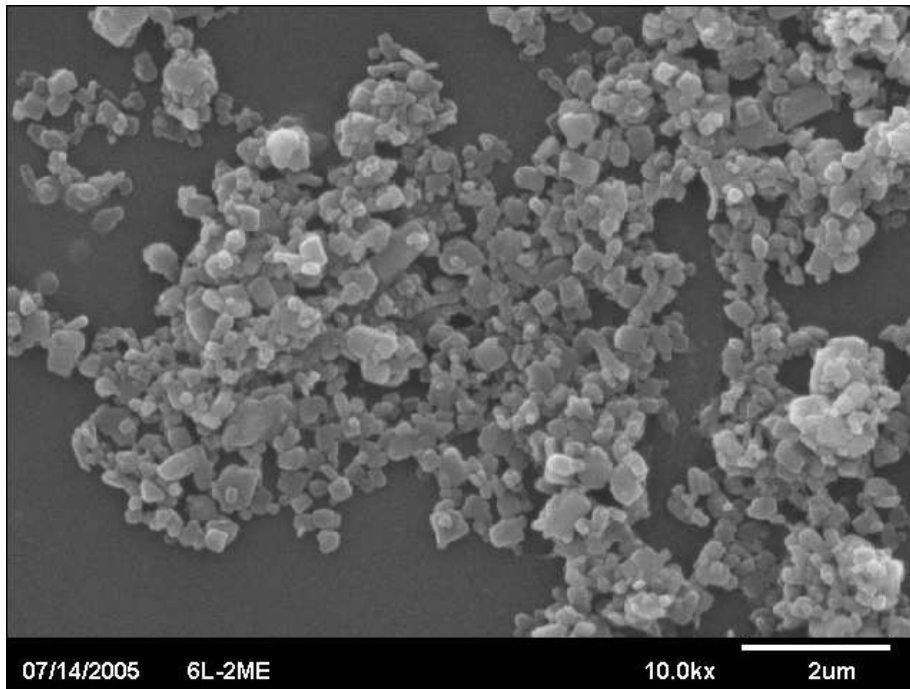


# Confocal Microscopy Imaging



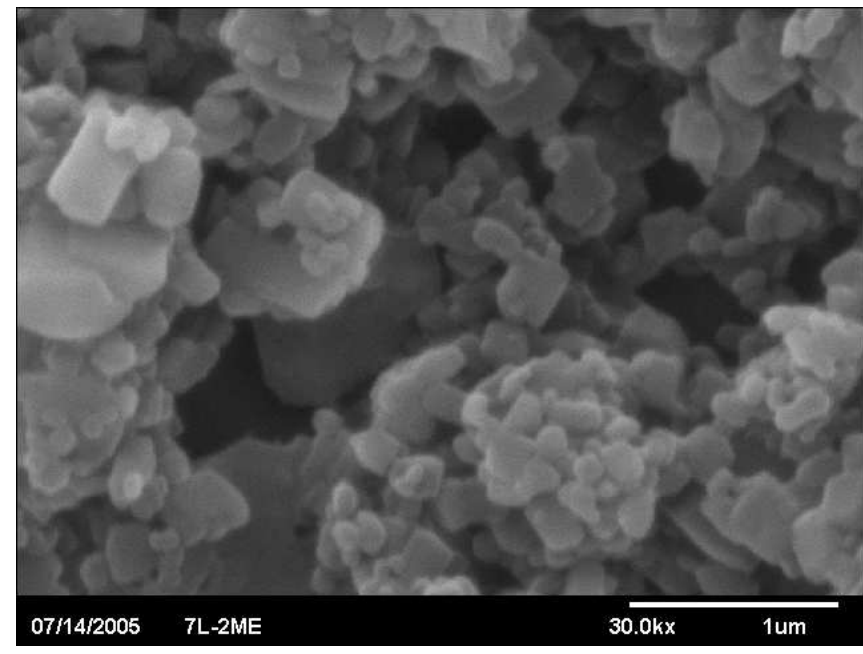
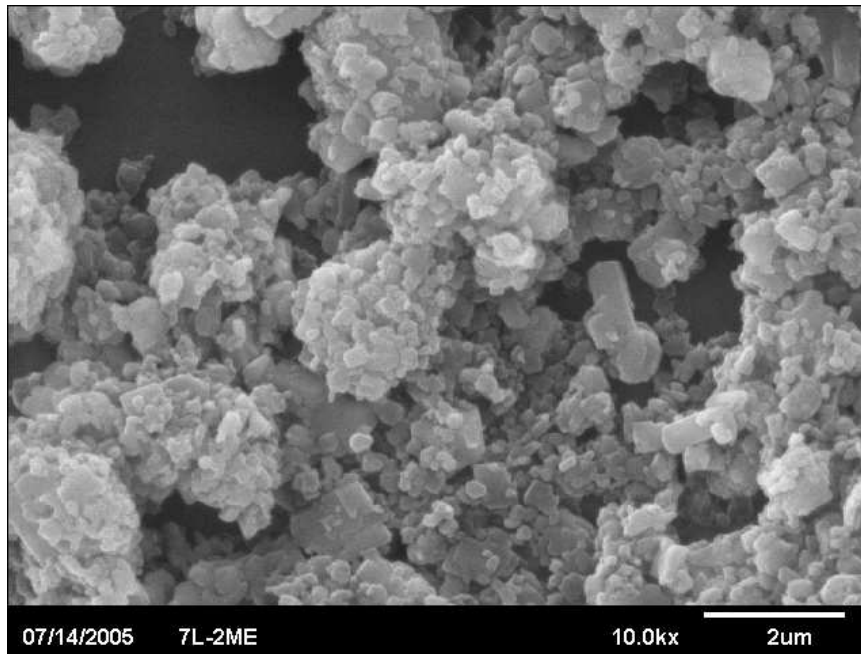
(2-ME)-DPPC-(DS/DN)<sub>3</sub>DS/DN-FI  
2-ME, 8Layer, outermost layer Dextran-FITC  
DS----Dextran Sulfate  
DN----Dextran  
DN-FI----Dextran-FITC

# SEM Imaging



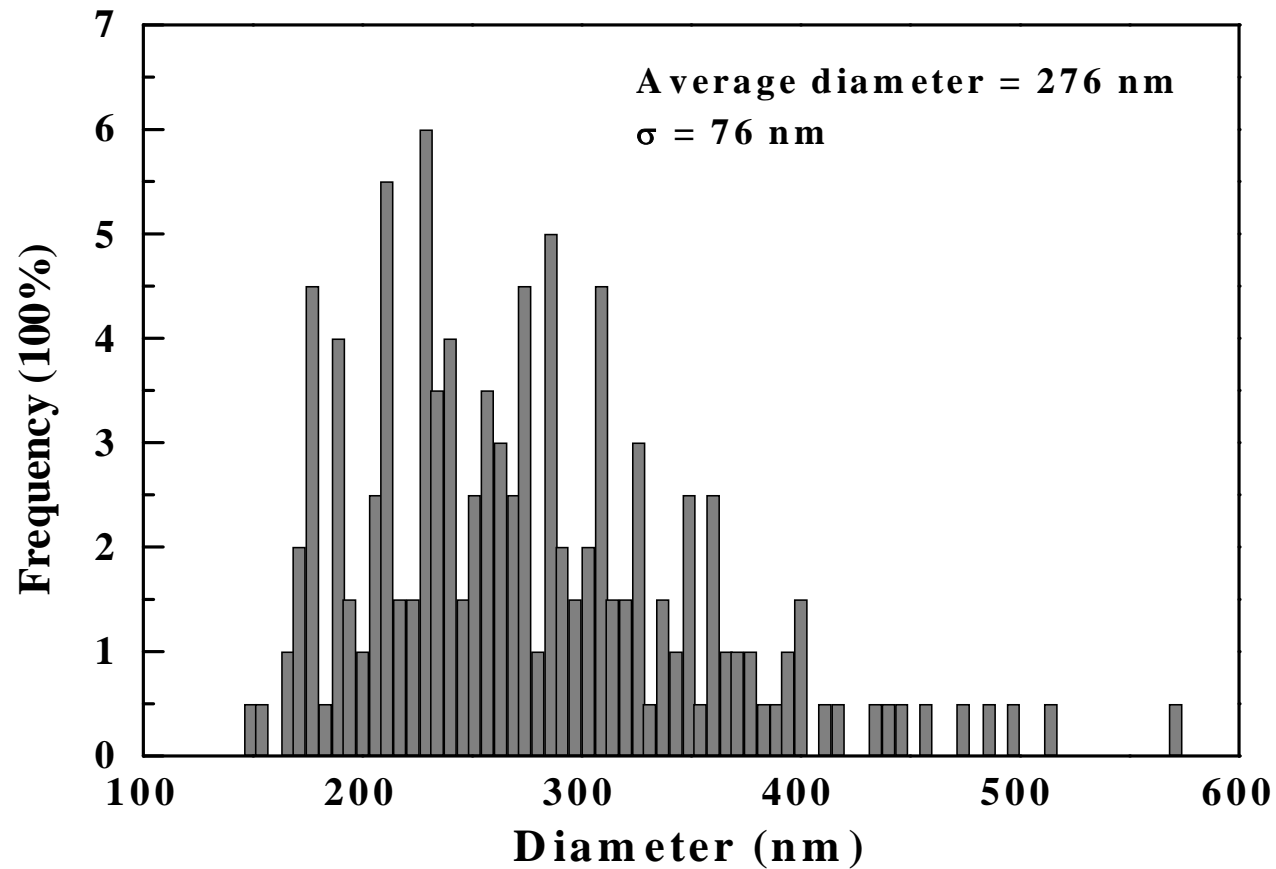
2-ME coated with  $(DS/DN)_3$

# SEM Imaging

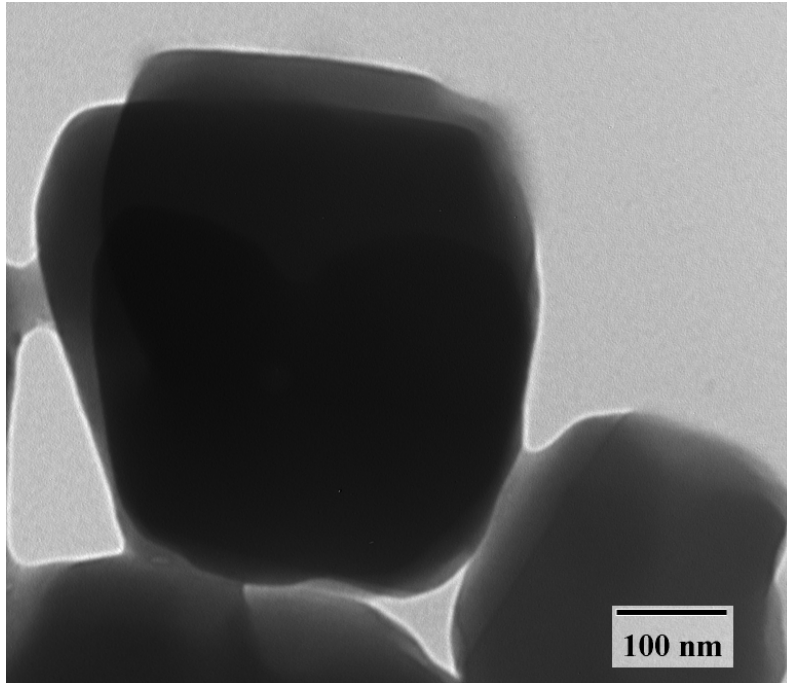


2-ME coated with (DS/DN)<sub>3</sub>DS multilayers

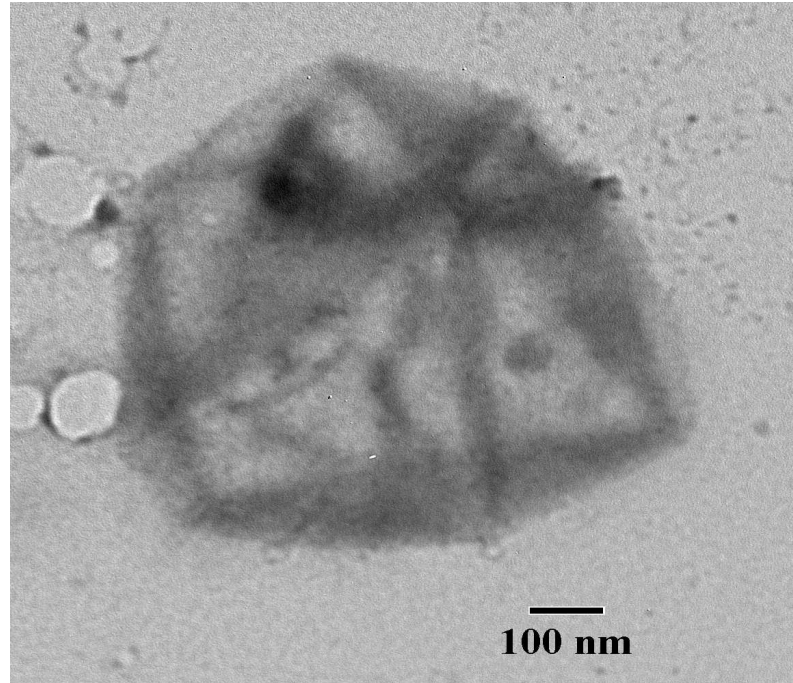
# Coated 2-ME Particle Size Distribution



# TEM Imaging



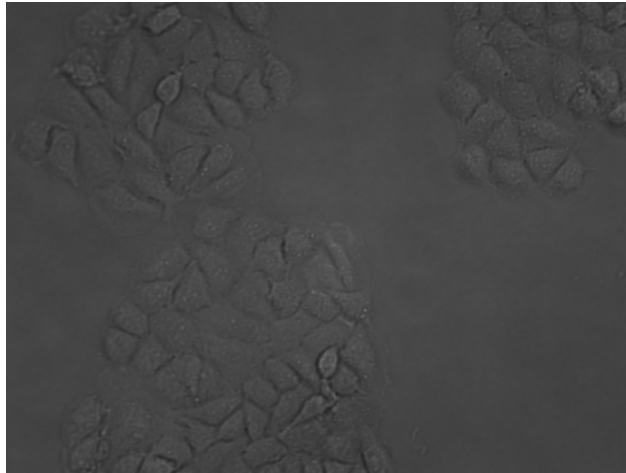
2-ME coated with  $(DS/DN)_4DS$



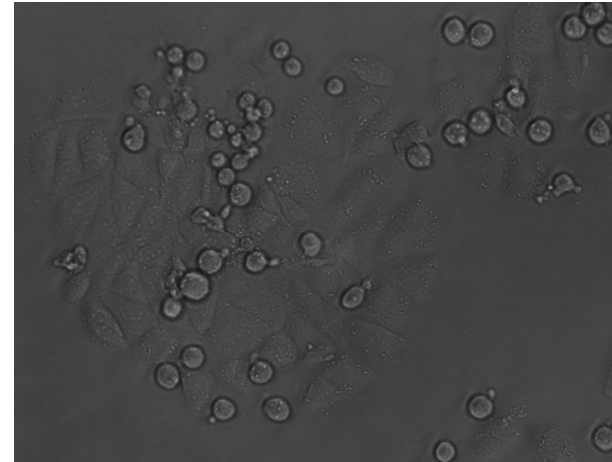
2-ME coated with  $(DS/DN)_3$  dissolved by Extraction of 2-ME using ethanol, leaving behind pure polymer shell structures.

# Bioactivity evaluation of 2-ME polymer capsules

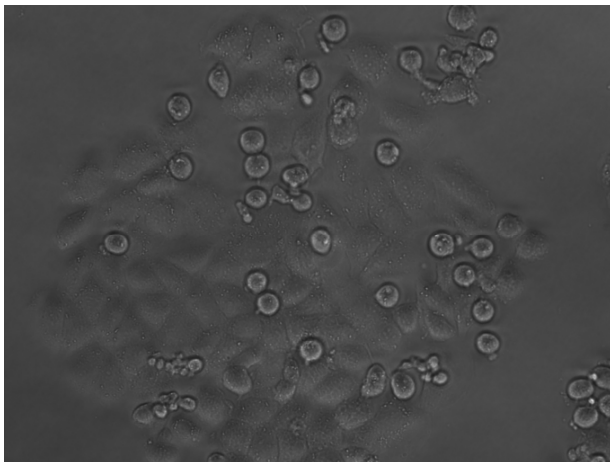
## 1. Phase contrast microscopy



Control cells

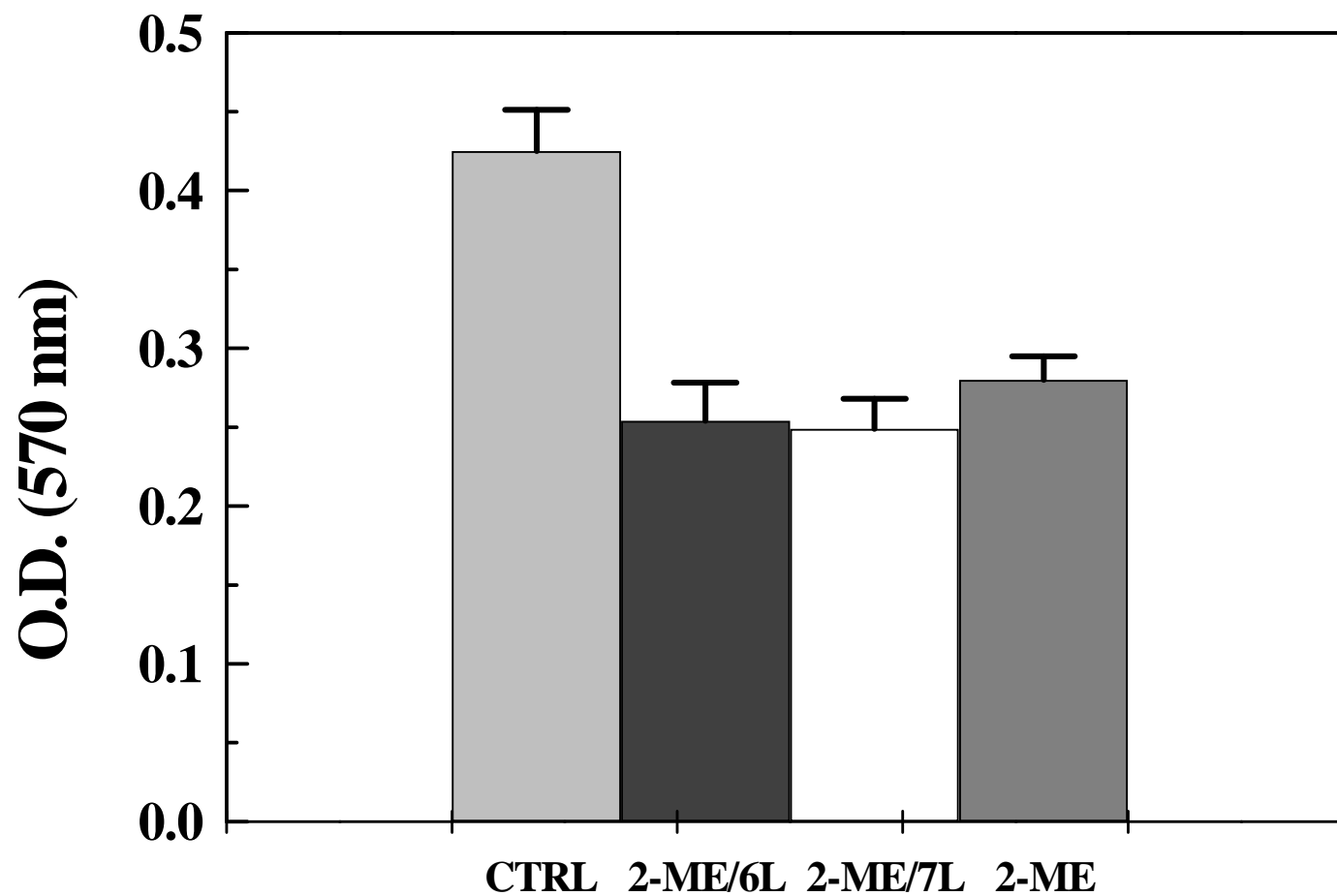


Cells treated with 2-ME ethanol solution



Cells treated with 2-ME capsules in PBS buffer

## Cell viability after treatment with free 2-ME, en encapsulated 2-ME particles

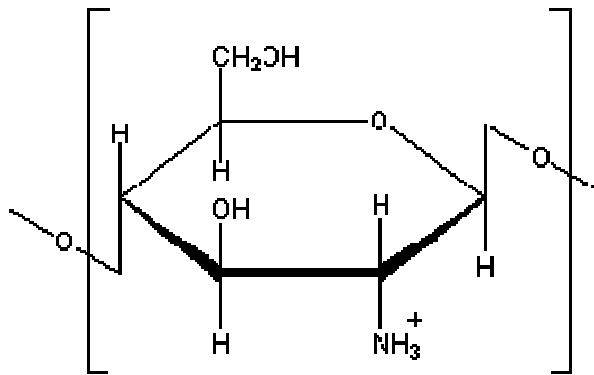




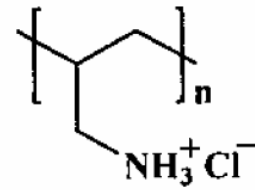
# Conclusion

- Submicrometer-sized 2-ME drug capsules were successfully fabricated and characterized using confocal, SEM, and TEM.
- The formed 2-ME capsules are bioactive and can induce thyroid cell apoptosis as verified by both phase contrast microscopy morphology studies and MTT assay.
- This approach of fabrication of 2-ME drug capsules opens a new pathway to encapsulate various drugs into polymeric multilayer capsules for a range of therapeutics applications.

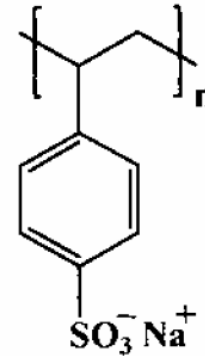
# Future work



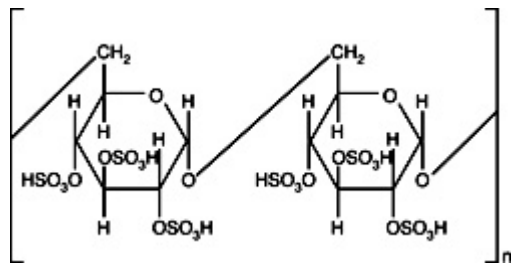
**Chitosan**



**PAH**



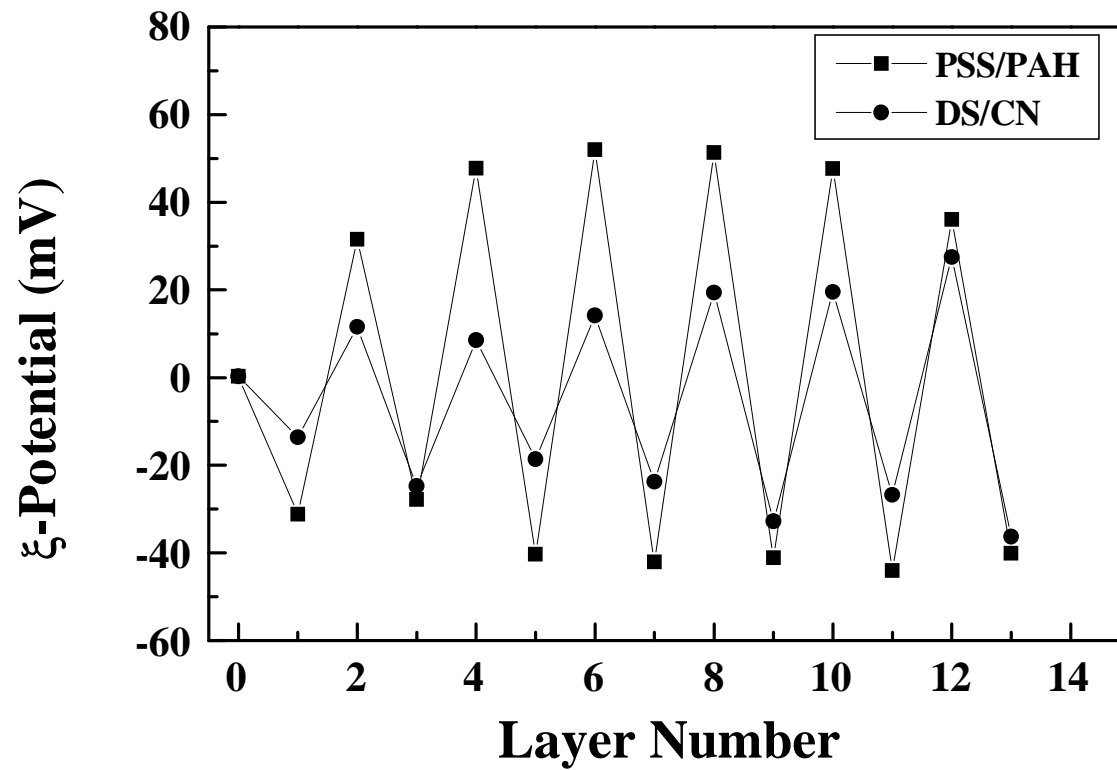
**PSS**



**Dextran sulfate**

poly(allylamine hydrochloride) (PAH)  
poly (sodium 4-styrenesulfonate) (PSS)

## Zeta Potential of polymer-coated 2-ME particles



# Acknowledgement

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NIH-NCI N01-CO-27173

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