Organic Dye Behavior in PEG Block Copolymer Nanoparticles

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Project objectives

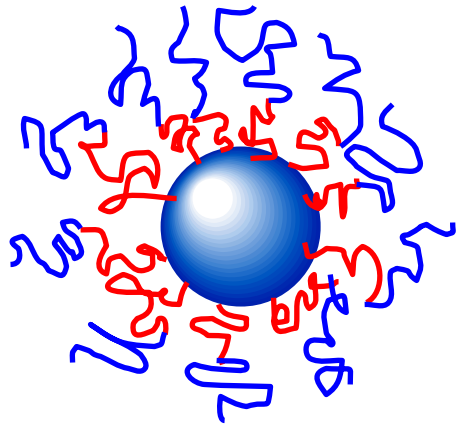
- To study the behavior and properties of nanoparticles made of block copolymers
- To find the optimal concentrations of fluorescent dyes in the nanoparticles
- To study the stability of various compounds encapsulated in nanoparticles

Why nanoparticles?

- Future uses in drug delivery
  - Many drugs are hydrophobic, thus hard to deliver in the body
    - Protect the drug from the body and most of the body from the drug
  - Timed drug release
  - Tissue-specific targeting

Block copolymers for nanoparticles

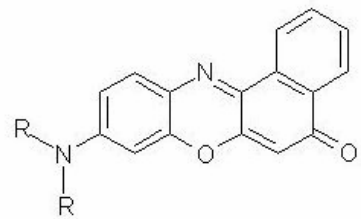
- Block copolymers have two components
  - Hydrophilic and hydrophobic
- During nanoparticle synthesis block copolymers form micelles
- Diblock copolymers used
  - Poly(ethylene glycol)-b-poly(styrene)
  - Poly(ethylene glycol)-b-poly(ε-caprolactone)



Fluorescent dyes

- Objective: Encapsulate fluorescent dyes in nanoparticle core
- Fluorescent dyes fluoresce more in organic environment
  - Nile red
  - 7-amino-4-methylcoumarin (AMC)

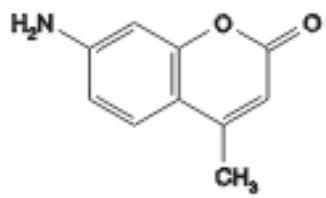
Nile Red



R=ethyl (C_2H_5)

http://www.indeco.jp/indeco_online/products/htm/structure/NileRed.jpg

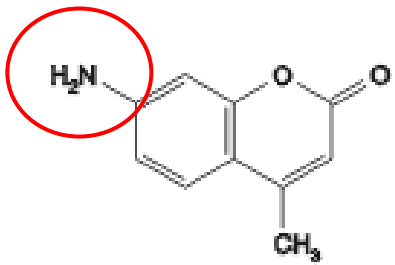
AMC



http://www.axxora.com/files/formula/610-028.gif

7-amino-4-methylcoumarin (AMC)

- Hydrophilic >> not compatible with nanoparticle micelle
- Solution: Conjugate AMC with vitamin E, a hydrophobic compound



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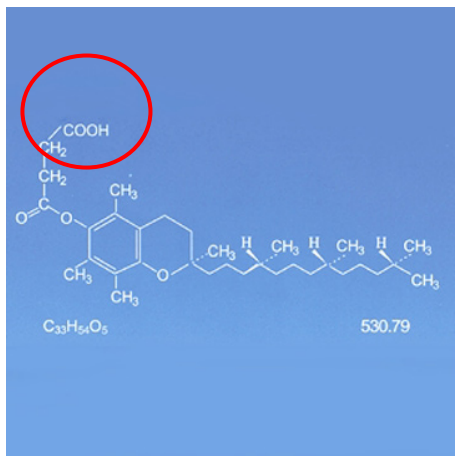


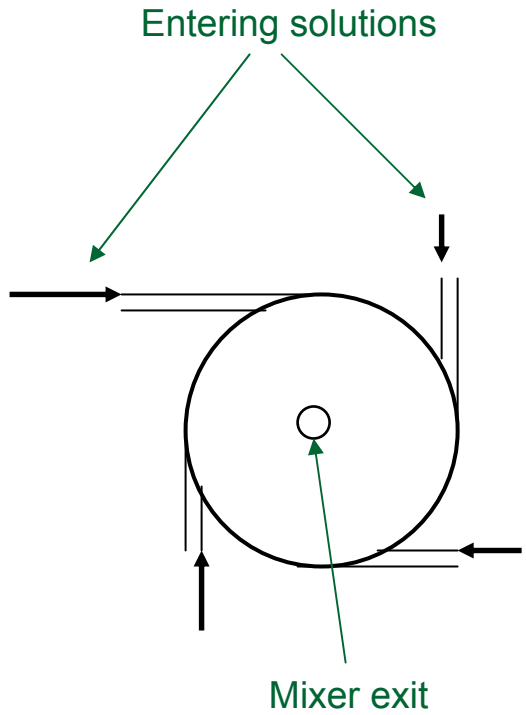
DCC / THF →

7-amino-4-methylcoumarin (AMC)- vitamin E succinate conjugate

http://img.alibaba.com/photo/50521181/Natural_Vitamin_E_Acid_Succinate.jpg

Making nanoparticles

- Confined impinging jet (CIJ) mixer
- Streams of solution mixed at high velocity in a small chamber
- 1 organic syringe, 3 water syringes for 1:9 tetrahydrofuran (THF):water



Cross section of CIJ mixer

Making nanoparticles (2)

- Why CIJ?
  - $T_{mixing} < T_{aggregation}$
    - Small nanoparticle micelles form before larger aggregates do
  - High drug (or dye) loading inside particles
  - It's quick!

B.K. Johnson and R.K. Prud'homme. Phys. Rev. Lett. **91**, 118302 (2003).

General procedure

Dissolve dye and block copolymer in THF → CIJ mixer → Analysis of nanoparticles

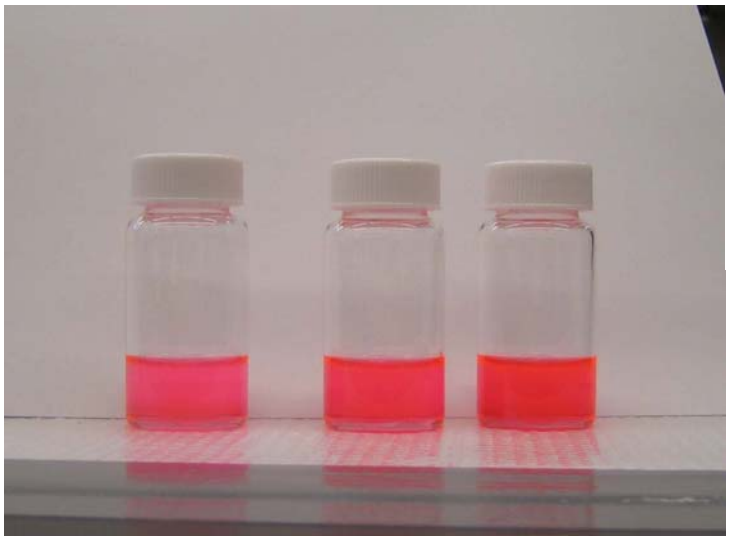
- Analytical methods
  - Dynamic light scattering (DLS)
  - Fluorescence spectroscopy

Fluorescence spectroscopy

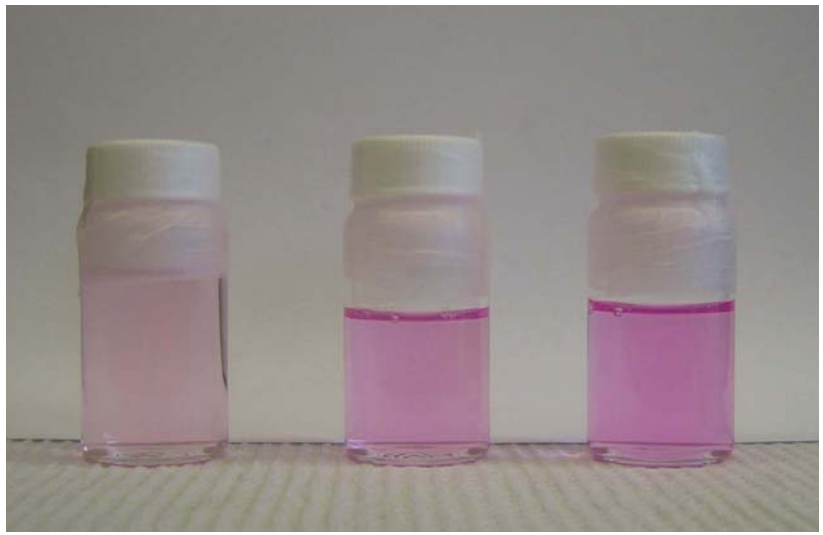
- Excite dye-loaded nanoparticles with a monochromatic wavelength of light
- Measure the emission spectrum of the sample to determine dye behavior in the particles
  - Nile red and AMC fluoresce more brightly in an organic environment than an aqueous one

Fluorescence spectroscopy (2)

- Visual evidence

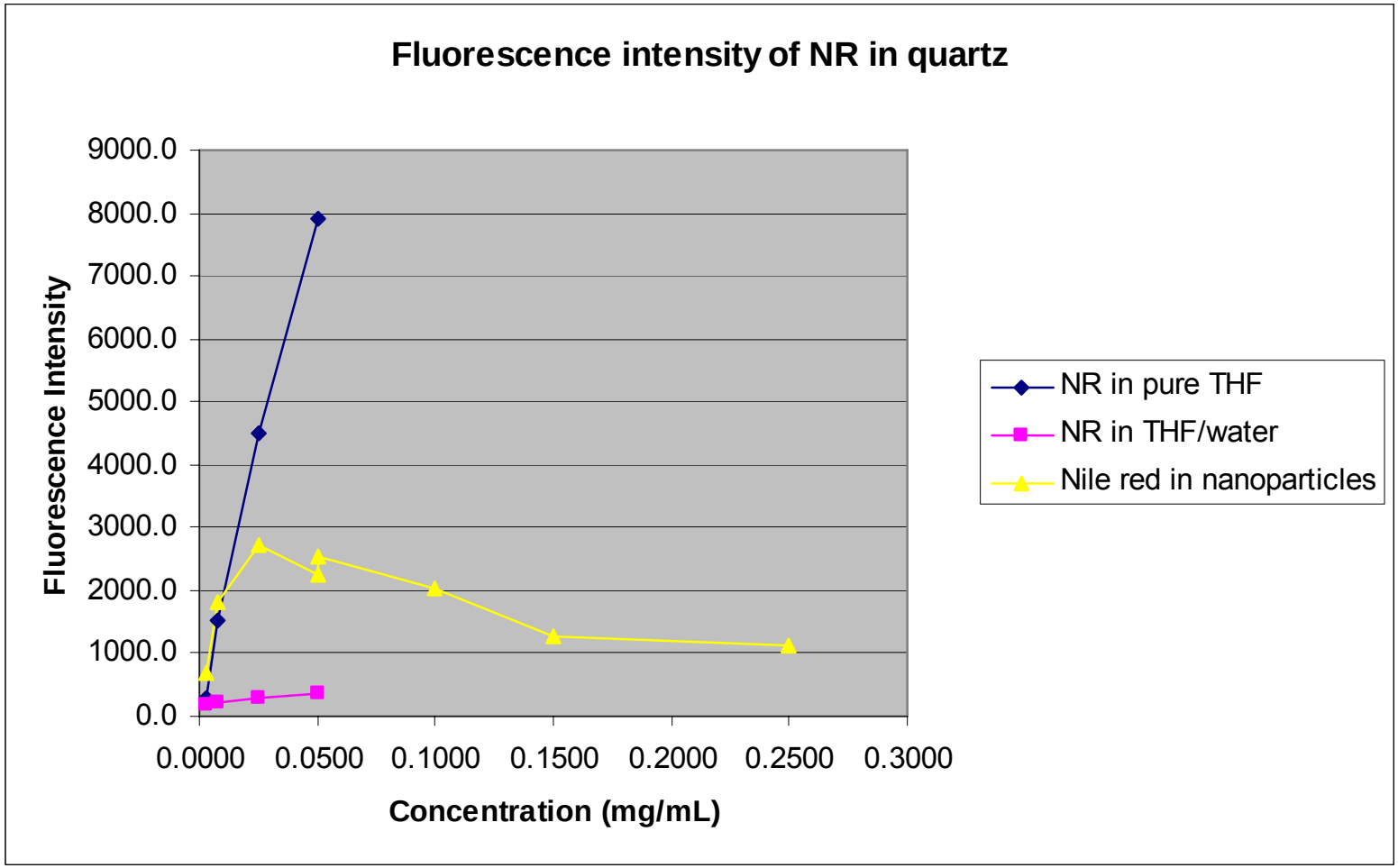


Nile red in pure THF

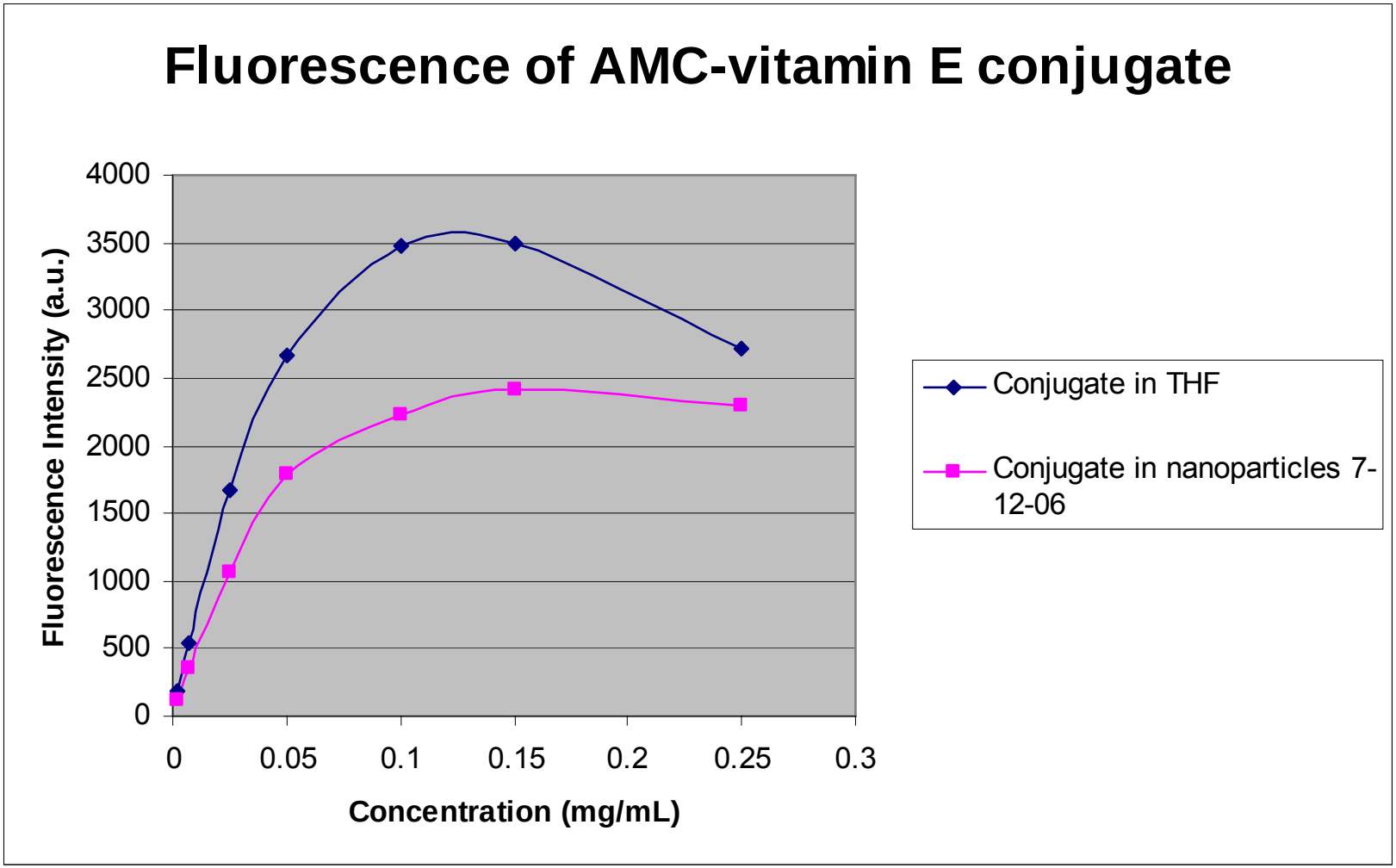


Nile red nanoparticles

Optimal dye concentration: Nile red



Optimal dye concentration: AMC conjugate



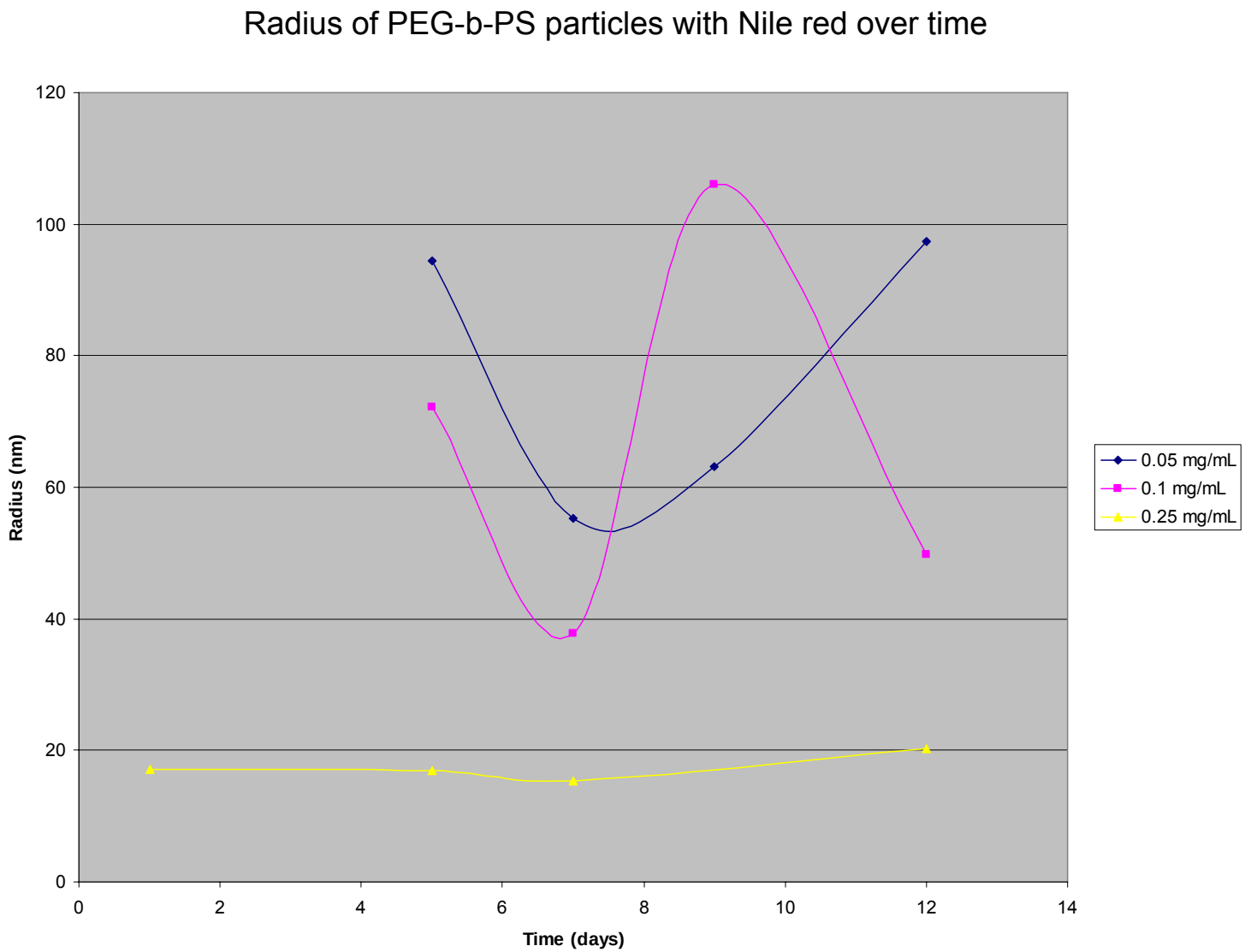
Dynamic light scattering (DLS)

Coherent laser light directed at particle solution → Doppler shift in the frequency of light due to Brownian motion → Frequency changes correlated to particle size

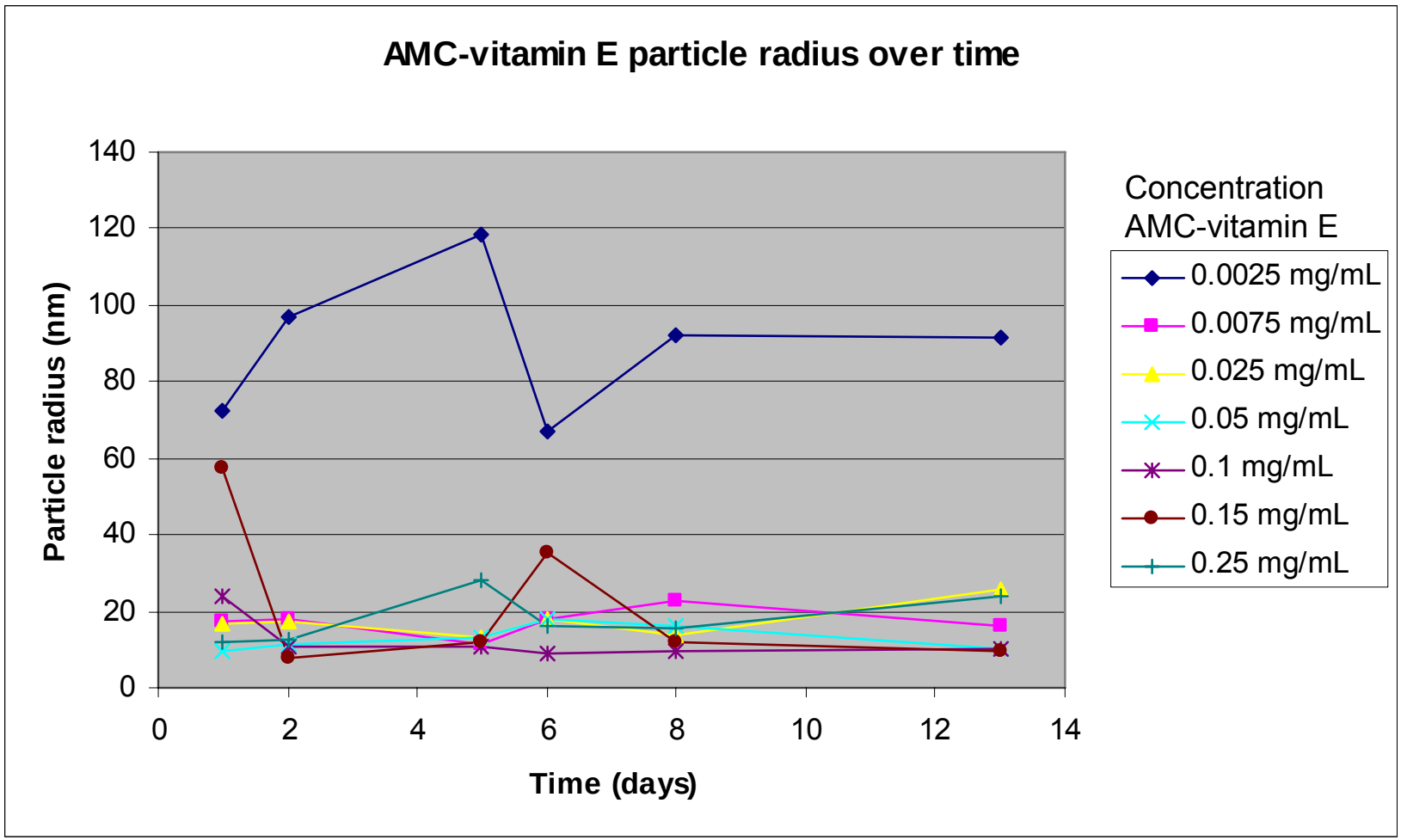
http://www.microtrac.com/dynamicscattering.cfm

- Objective: To determine whether nanoparticles are aggregating over time

Dynamic light scattering (2)



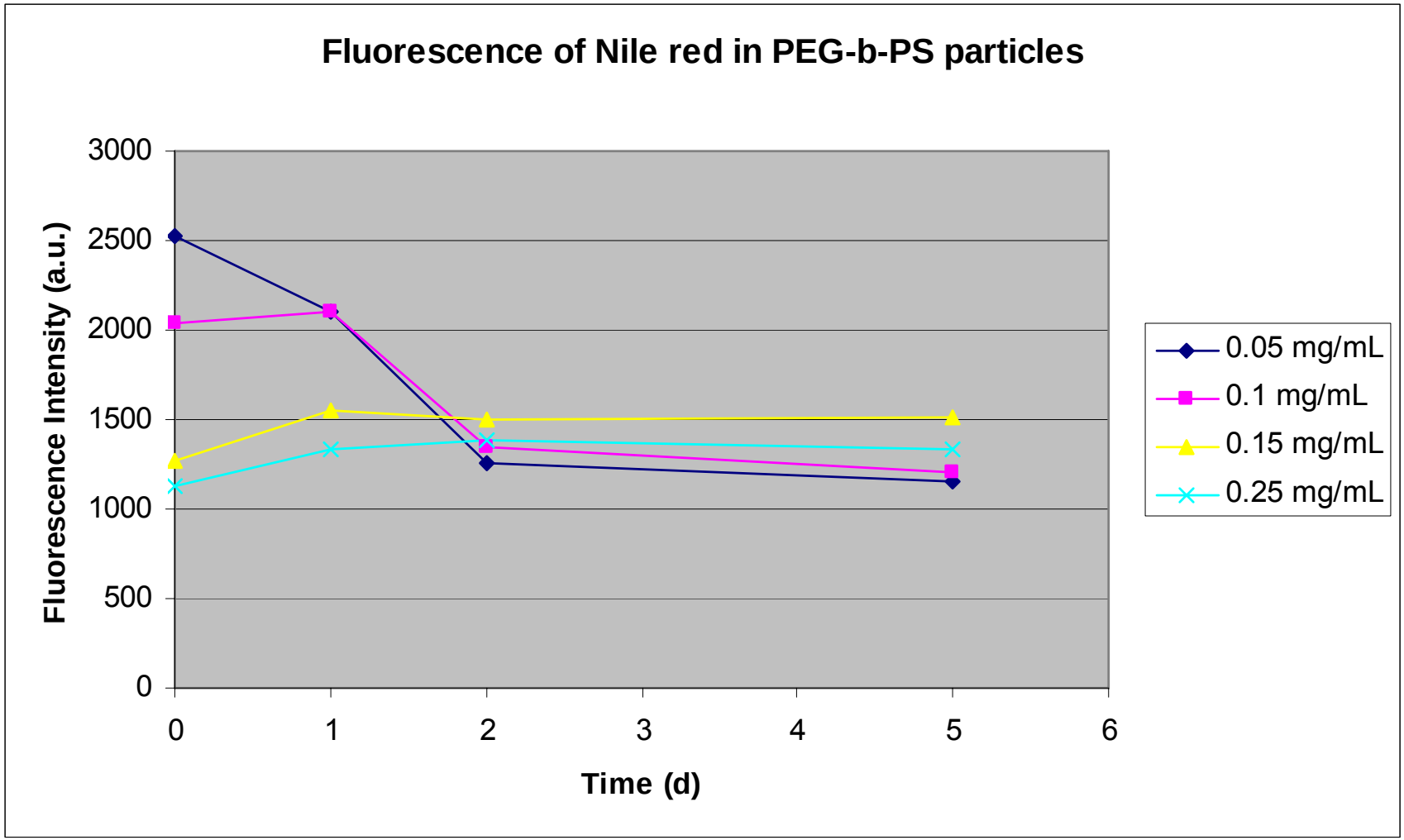
DLS (3)


AMC-vitamin E particle radius over time
Particle radius (nm)
140
120
100
80
60
40
20
0
0
2
4
6
8
10
12
14
Time (days)
Concentration AMC-vitamin E
0.0025 mg/mL
0.0075 mg/mL
0.025 mg/mL
0.05 mg/mL
0.1 mg/mL
0.15 mg/mL
0.25 mg/mL

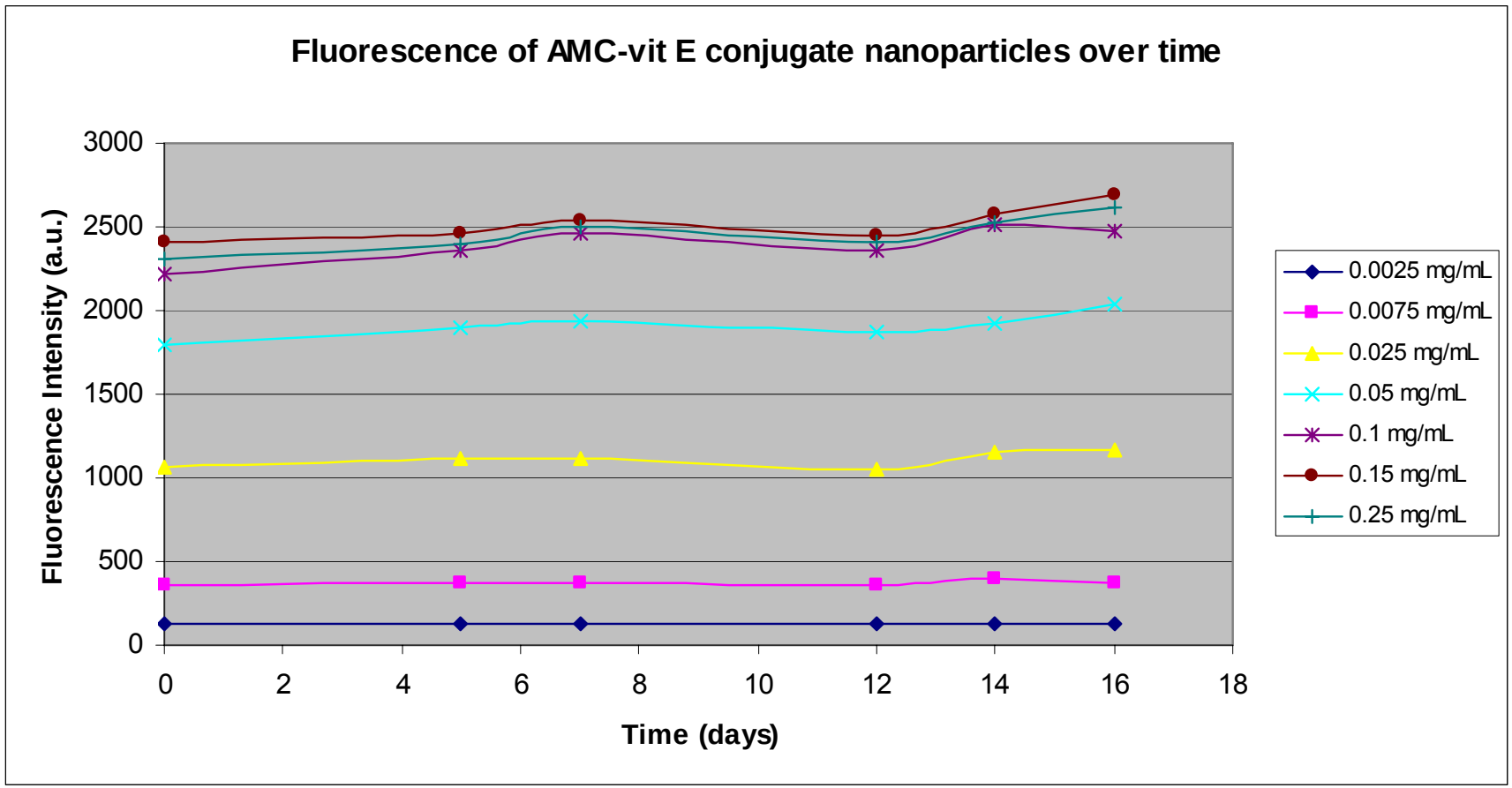
Particle core stability

- Dye movement changes fluorescence
  - Dye exits nanoparticle
    - At low concentrations, less fluorescence
    - At high concentrations, self-quenching effects cause higher fluorescence emission intensity once dye exits

PEG-b-PS particles (2)

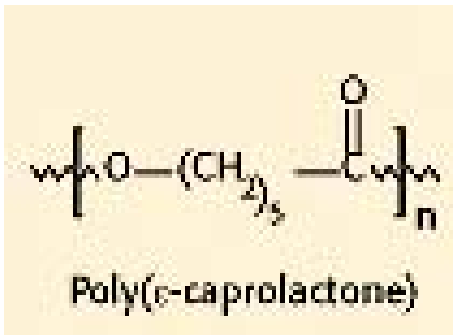


AMC-vitamin E nanoparticles



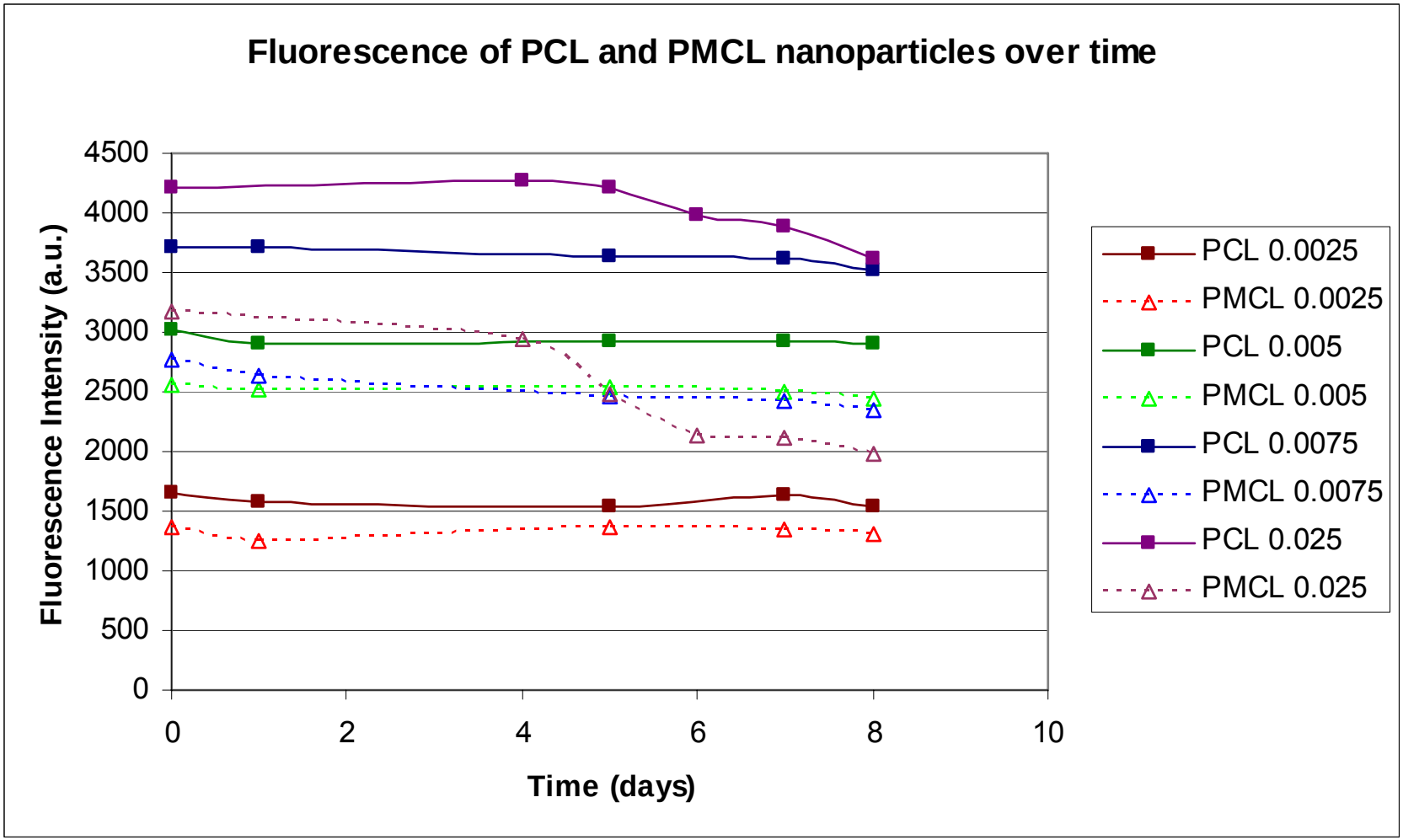
Stability of nanoparticles

- How does the hydrophobic block affect the nanoparticle core stability?
  - Poly(ethylene glycol)-b-poly(ε-caprolactone) (PEG-b-PCL)
  - Poly(ethylene glycol)-b-poly(methylcaprolactone) (PEG-b-PMCL

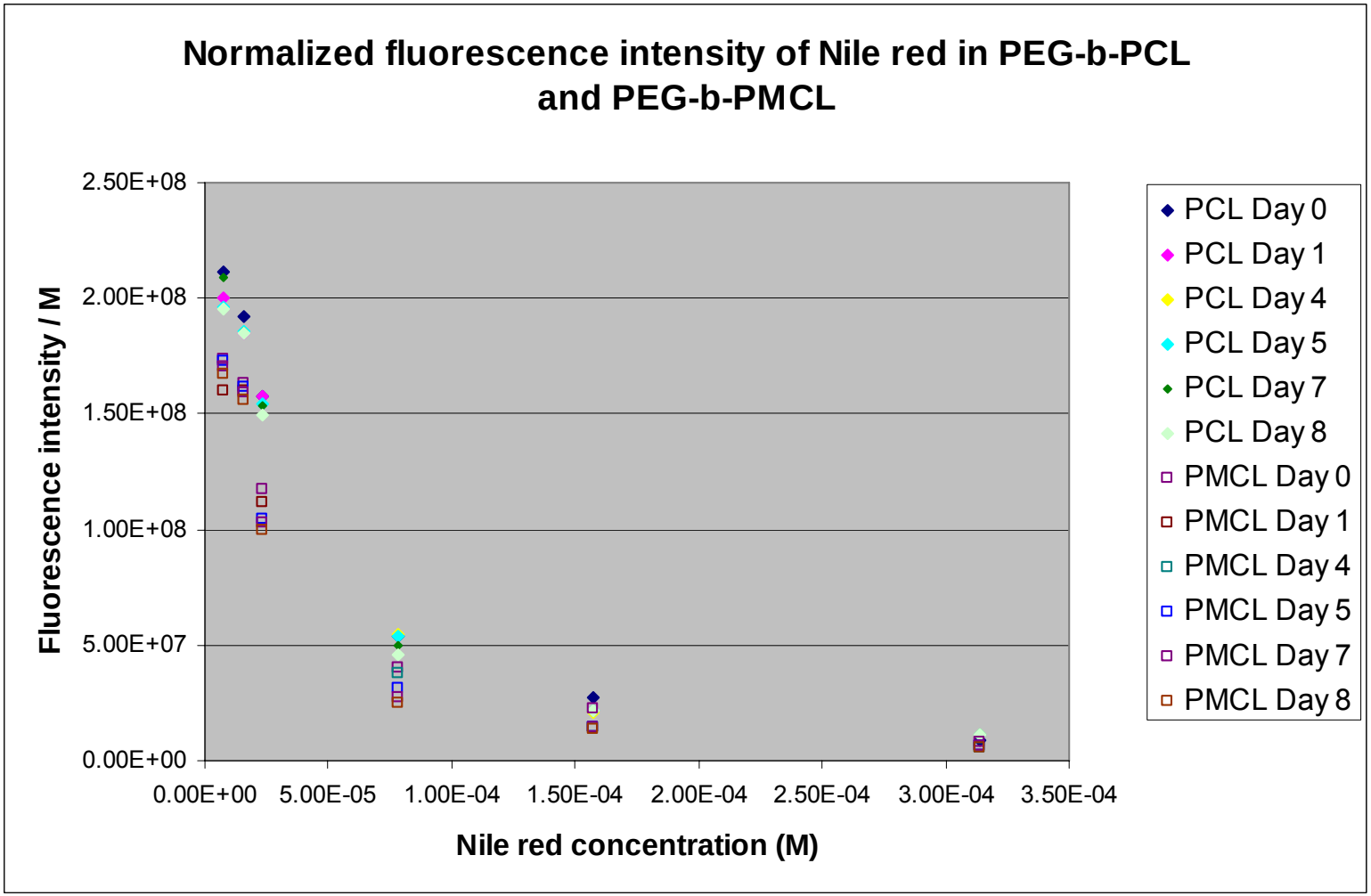


http://www.devicelink.com/mpb/archive/98/03/9803b30c.gif

PEG-b-PCL v. PEG-b-PMCL



PEG-b-PCL v. PEG-b-PMCL (2)

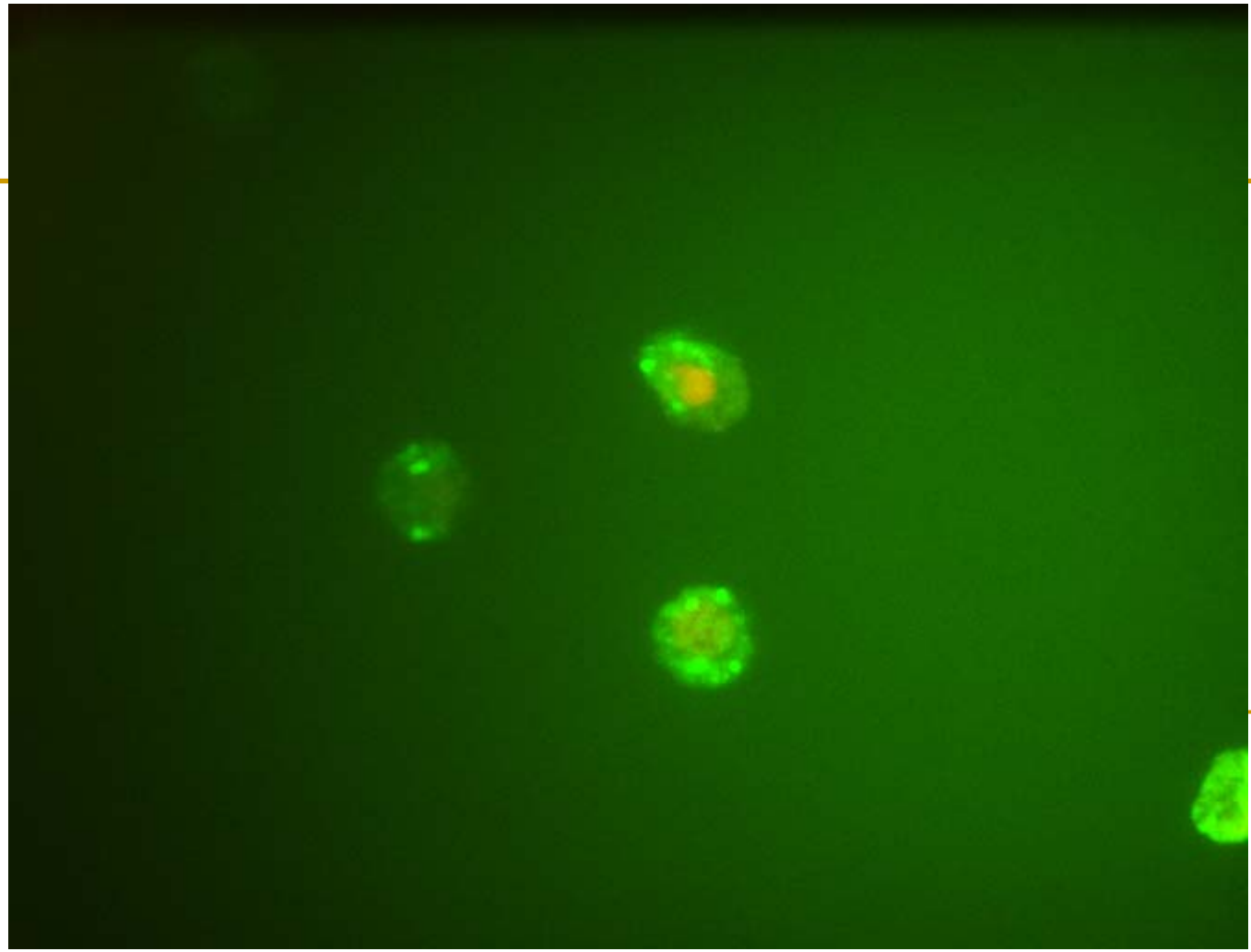


Conclusions

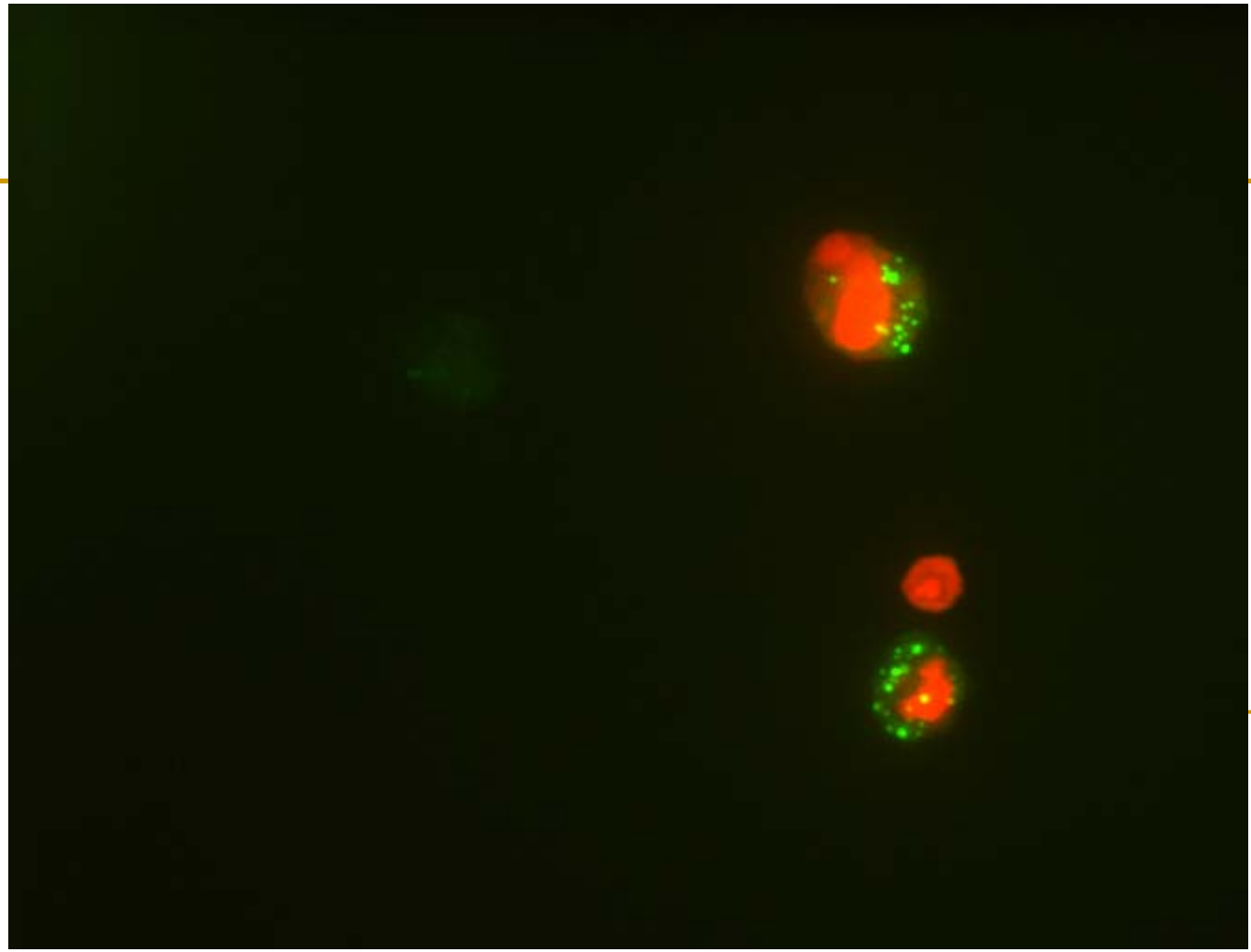
- Optimal concentrations
  - Nile red = 0.025-0.10 mg/mL
  - 7-amino-4-methylcoumarin = 0.10-0.25 mg/mL
- Nile red and the AMC-vitamin E conjugate are stable in nanoparticles.
- PCL appears to act more hydrophobic than PMCL.

The Future

- More PCL/PMCL experiments
  - X-ray diffraction
- Tag nanoparticles with proteins for receptor-specific intake
- Study nanoparticle uptake into cells
  - Where they go
  - How they degrade



Nanoparticle uptake of feline lymphocyte T-cell. Lymphocyte cells were incubated for 90 min in a solution containing PCL-PEG micelle nanoparticles of 70 nm diameter encapsulating Nile Red. Cells were then fixed and DNA was stained using Draq4. Image taken with Nikon Si confocal microscope and magnified 100x, Nile Red showed in green and Draq4 in red. P. Pawlowski, C. Thériault, M. Gindy, R. Prud'homme, W. Soboyejo



Nanoparticle uptake of feline lymphocyte T-cell. Lymphocyte cells were incubated for 90 min in a solution containing PCL-PEG micelle nanoparticles of 160 nm diameter encapsulating Nile Red. Cells were then fixed and DNA was stained using Draq4. Image taken with Nikon Si confocal microscope and magnified 100x, Nile Red showed in green and Draq4 in red. P. Pawlowski, C. Thériault, M. Gindy, R. Prud'homme, W. Soboyejo

Acknowledgments

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