Characterizing Nanoparticles Used in Bio Applications

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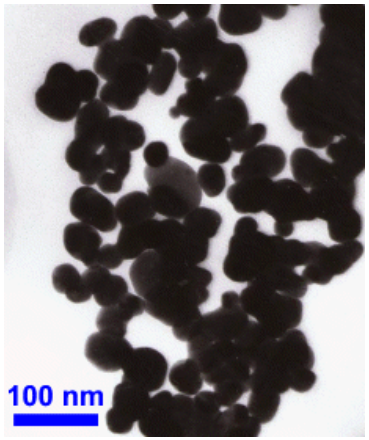
Outline

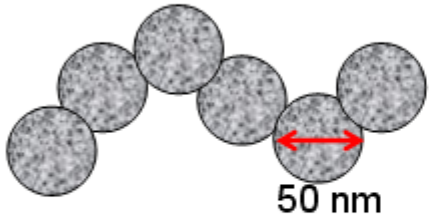
- Define nanoparticle
- Particle size analysis techniques
- Making nanoparticles
- Applications
  - Micelles, liposomes, engineered nanoparticles for drug delivery
- Other analytical techniques
  - Fluorescence
- Zeta potential

What is a Nanoparticle?

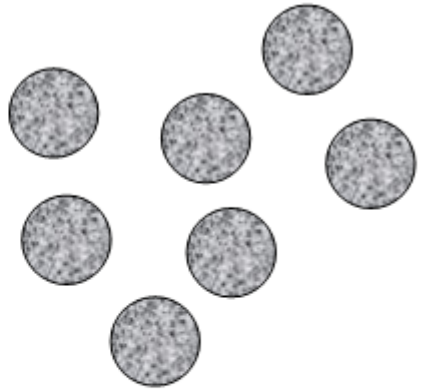
- Size range from approximately from 1- 100 nm



SSA = 6/ρD



ultrasound



D from SEM ~50 nm
D from SSA ~60-70 nm
D from DLS ~250 nm
D from diffraction ~ 230 nm

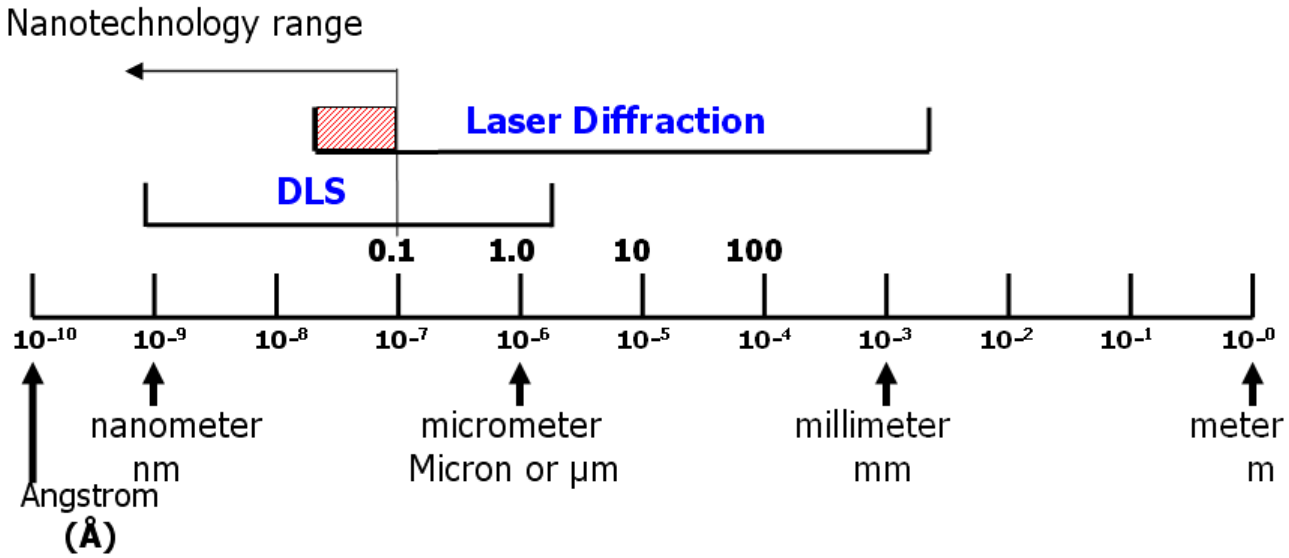
Used ultrasound to disperse to primary particles or use weak acid to break bonds
D from DLS ~50 nm

Particle size answer: “it depends..”

Size Measurements

- **Dynamic Light Scattering (DLS)**
- Particle size 0.3 nm – several µm
  - Suspensions only
- Zeta potential
- MW, A2

- **Laser diffraction**
- Particle size 30 nm – 3000 µm
  - Suspensions
  - Powders



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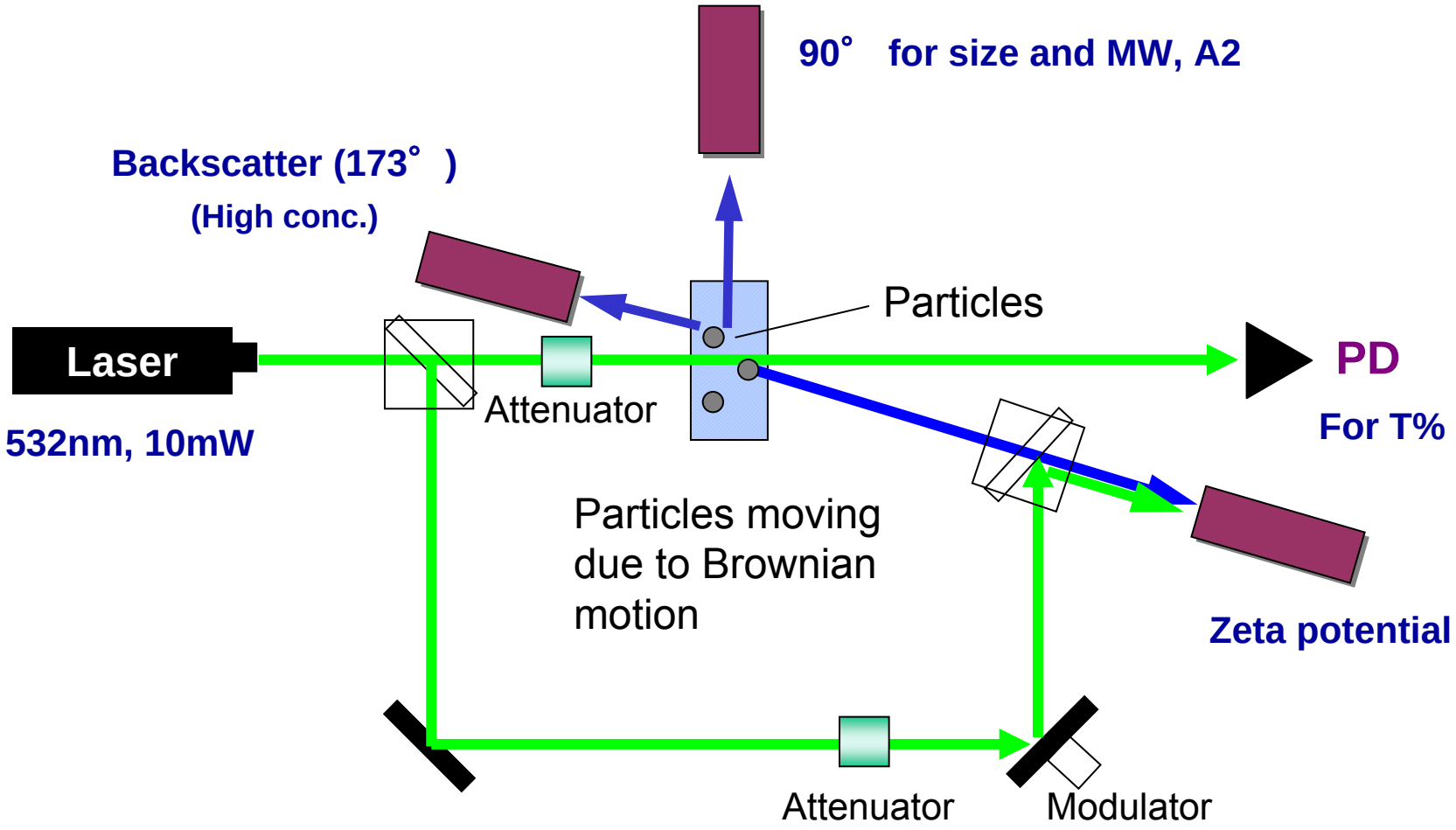
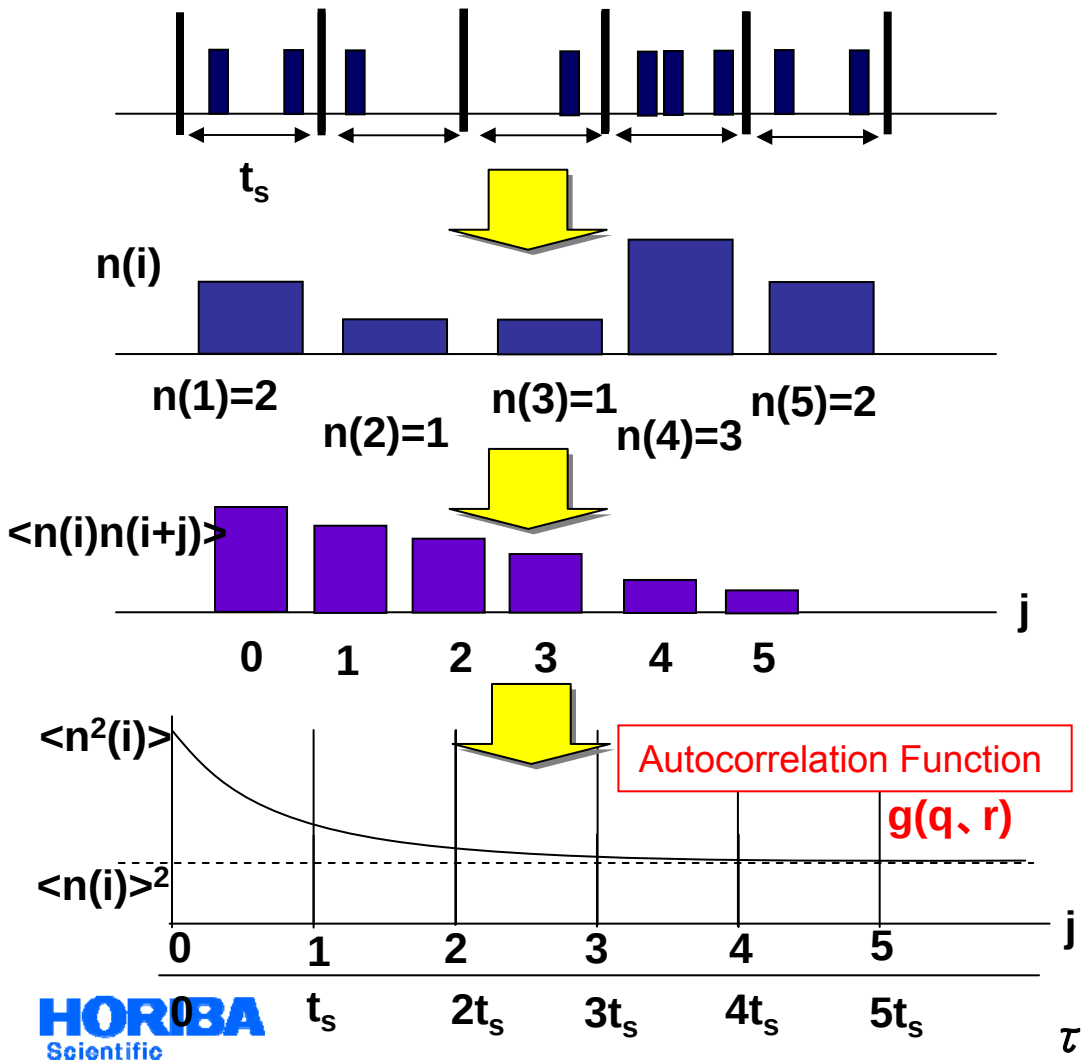
DLS Optics



DLS Measurement Principle



$$\frac{1}{\tau_R} = -\lim_{\tau \to 0}\left\{\frac{\partial \ln[g(q,r)]}{\partial \tau}\right\}$$

Relaxation time

$$\left\langle \left| \gamma(t) - \gamma(t+\tau)^2 \right| \right\rangle \cong 6D\tau$$

Particle's moving distance

Diffusion constant

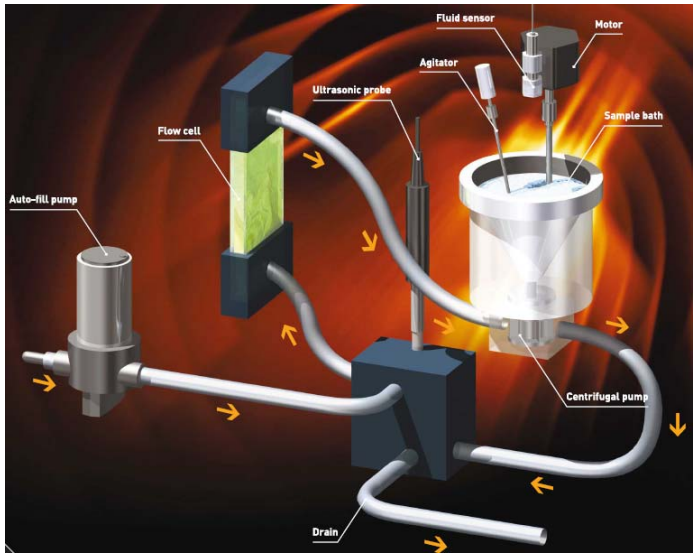
$$D = \frac{1}{q^2 \tau_R} = \frac{k_B T}{6\pi\eta a}$$

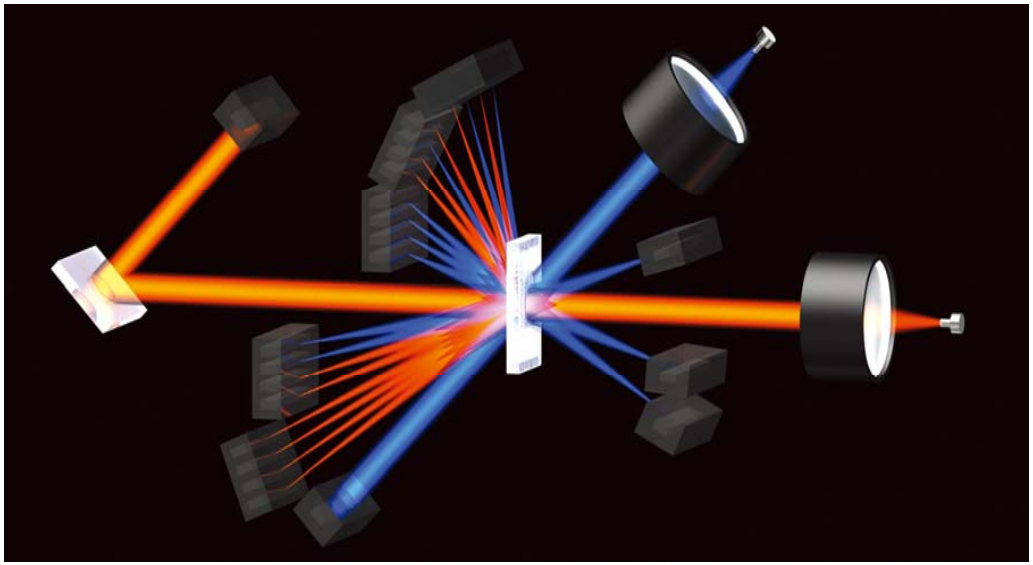
Relaxation time

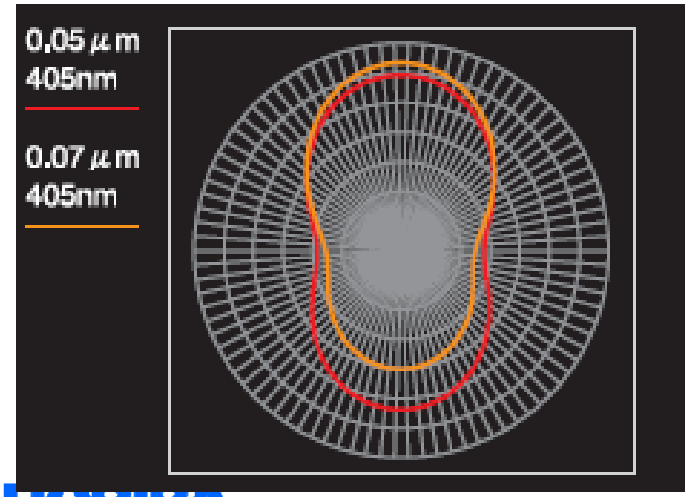
Particle radius

q: Scattering vector η : Viscosity
k_B: Boltzmann constant

Laser Diffraction







- Converts scattered light to particle size distribution
- Quick, repeatable
- Most common technique
- Low end: 30 nm

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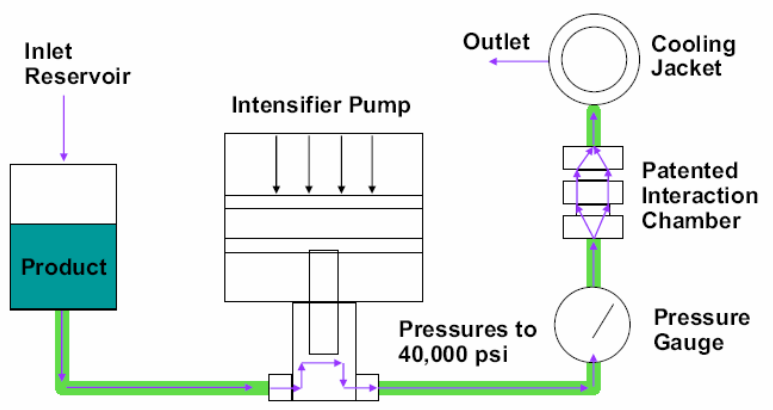
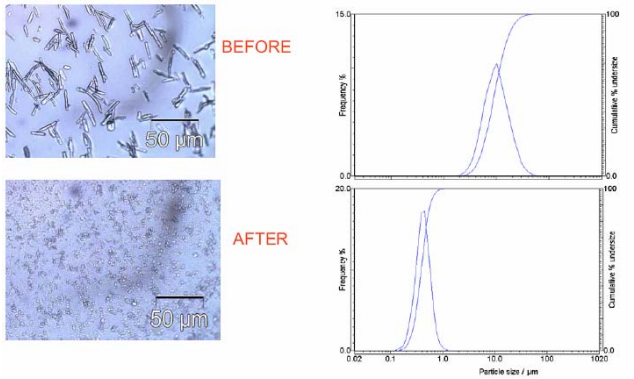
Making Nanoparticles

Top Down

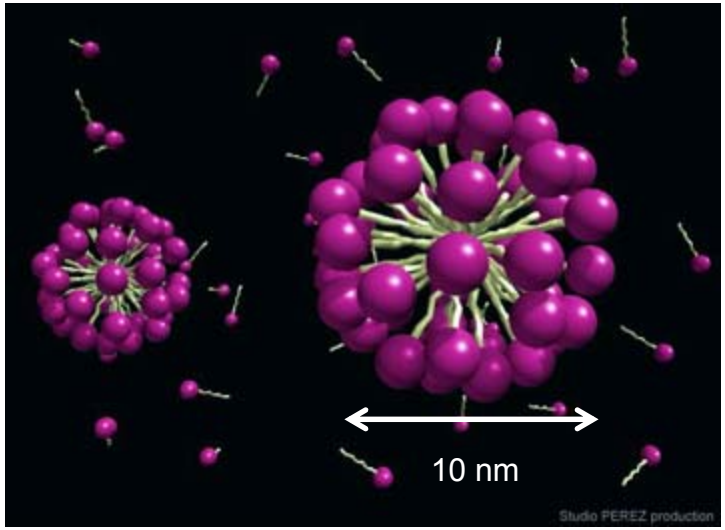
- Make particles smaller





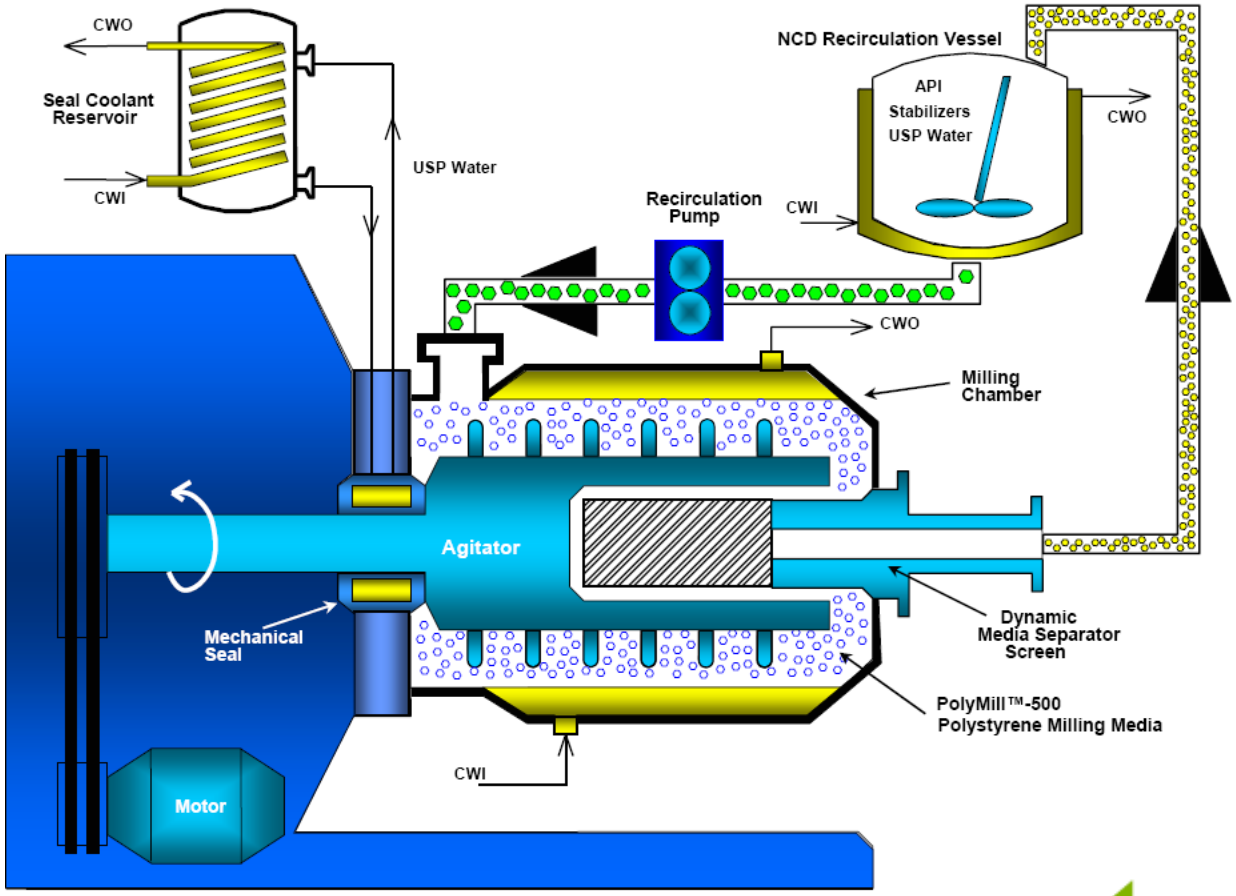
Bottom Up

- Build from atomic or molecular level up



Self assembly of micelles

Top Down: Elan NanoCrystal® Technology



Elan Drug Technologies

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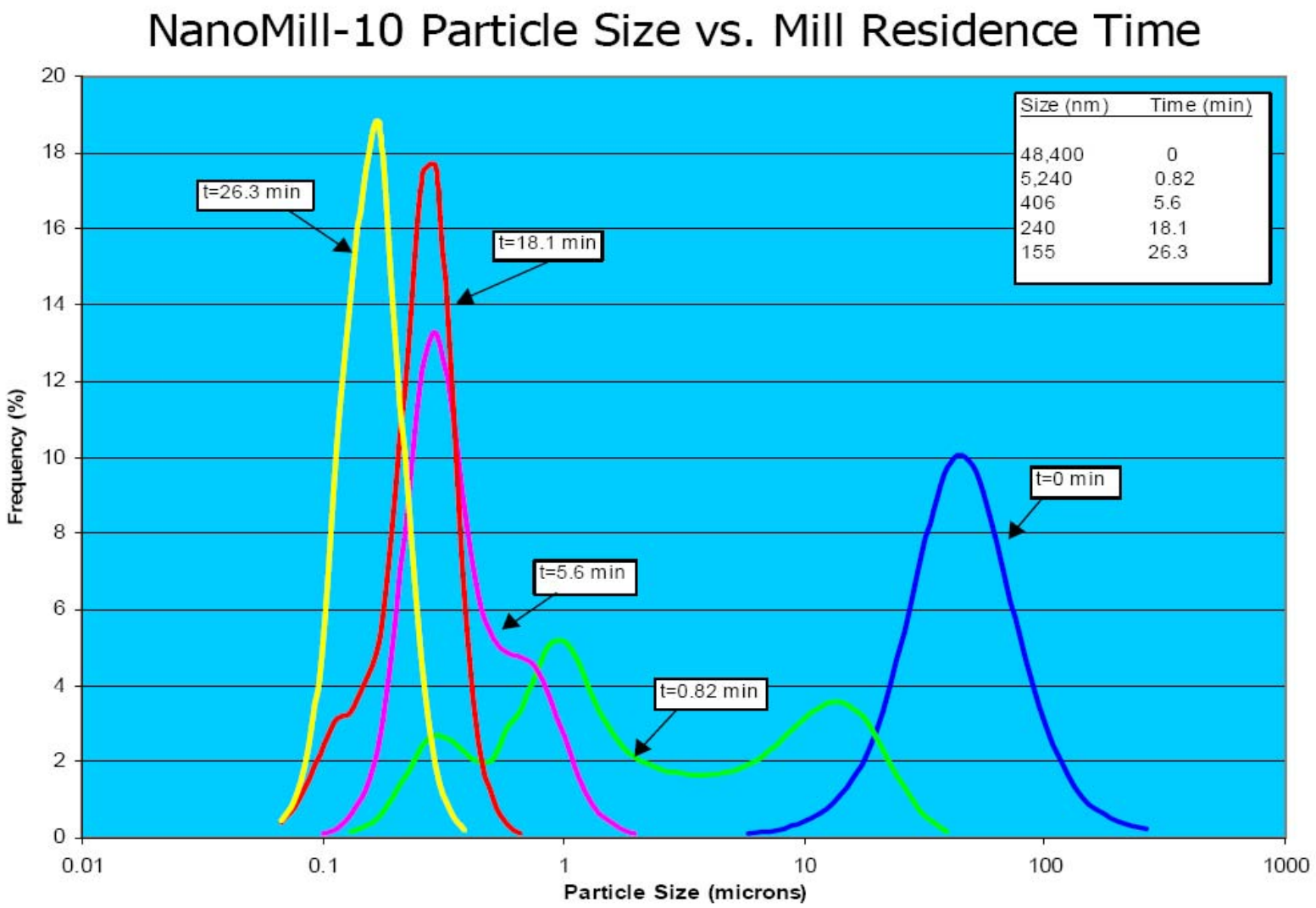
Top Down: Elan NanoMill

Size Reduction Measured on LA-950



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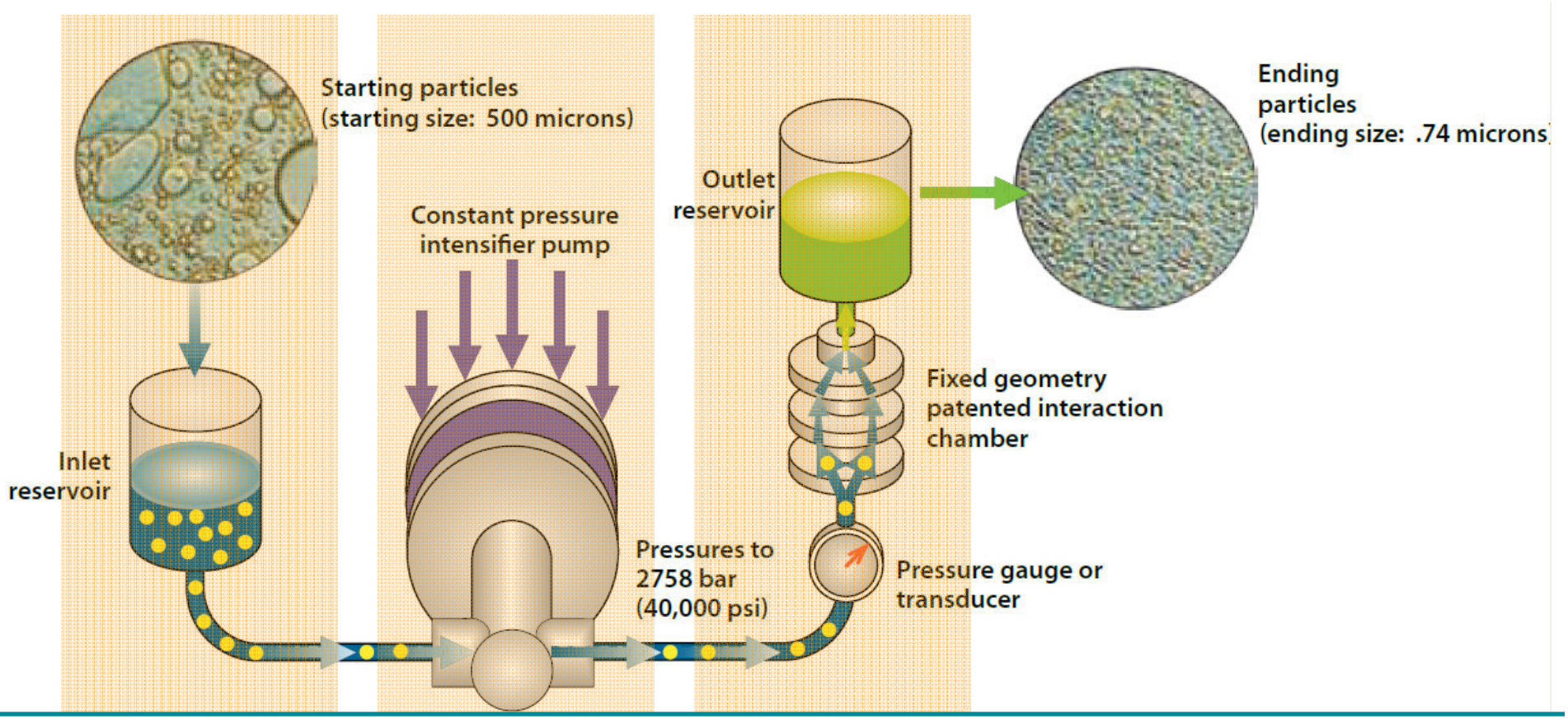
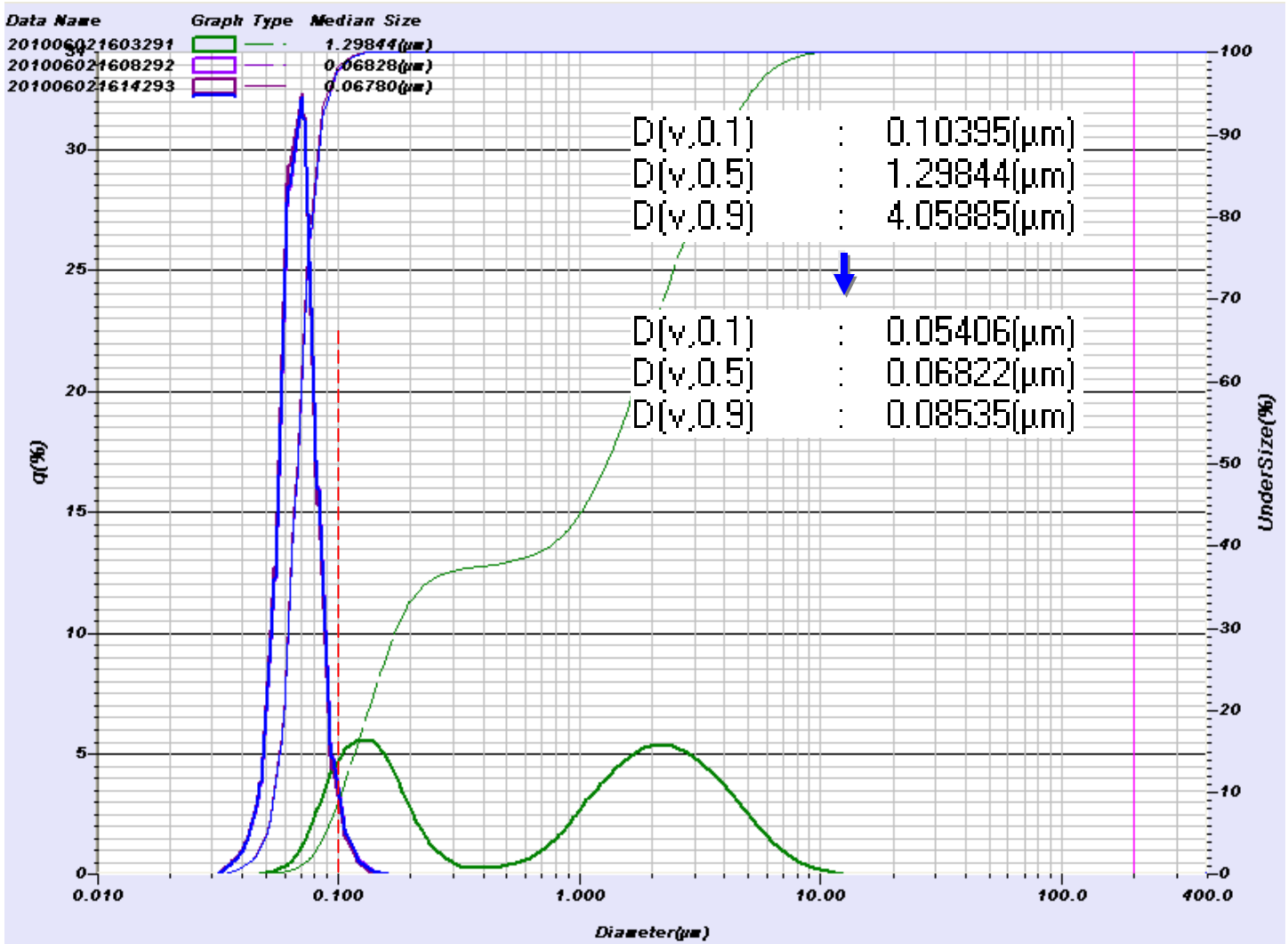
Top Down: Microfluidizer*



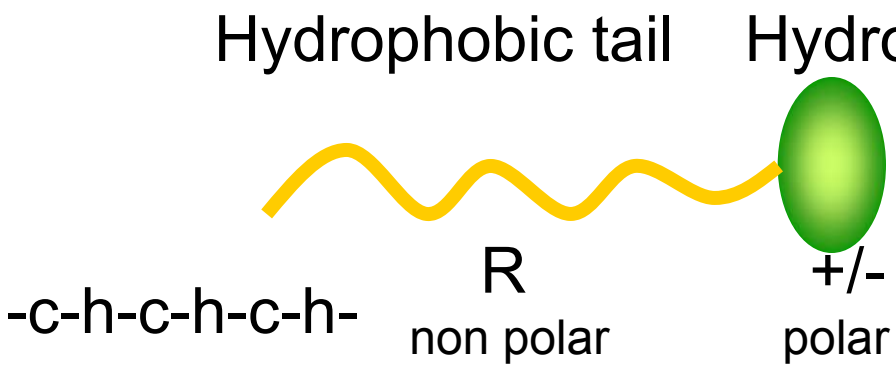
Microfluidics * See http://www.microfluidicscorp.com/

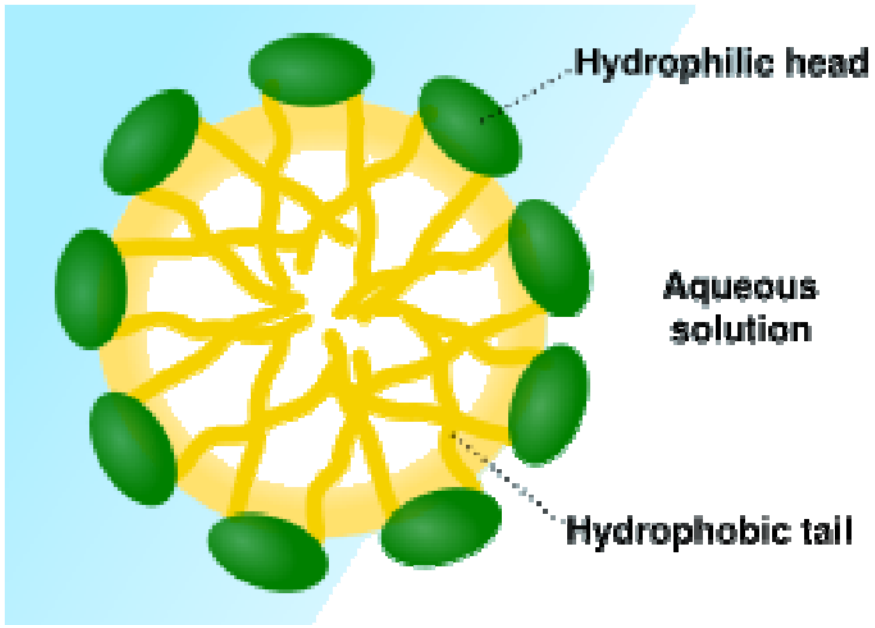
Ceria: Before, After Processing

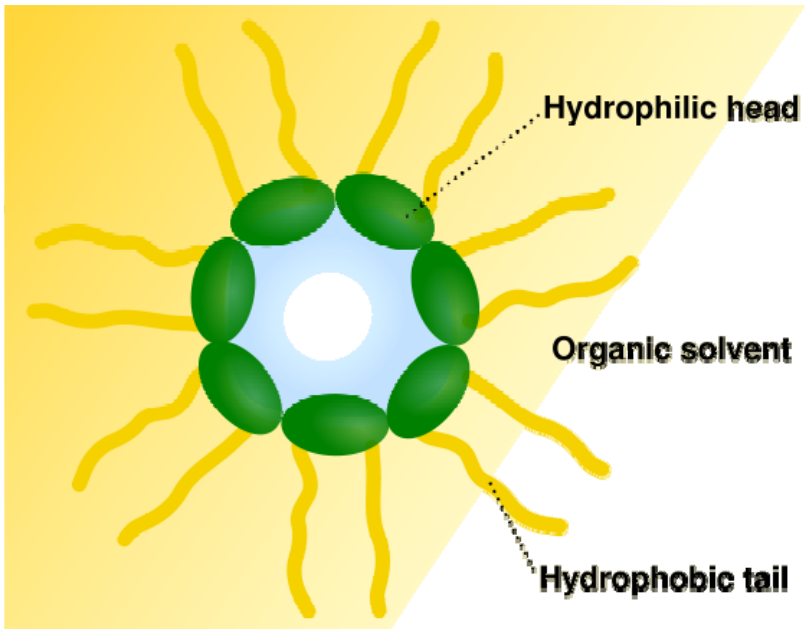


Laser diffraction required for before sample

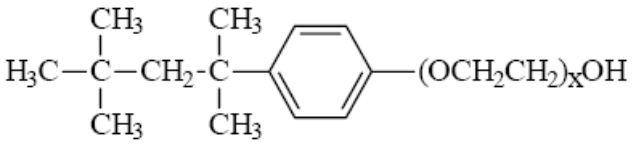
Bottom-up Self Assembly: Micelles







Critical Micelle Concentration



Triton X-100

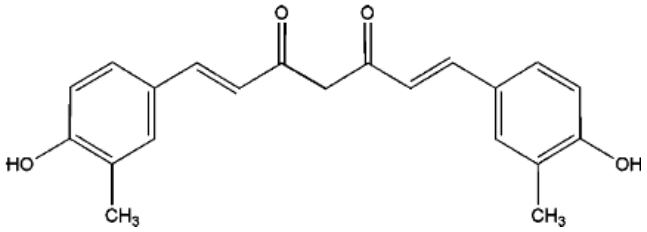
Experiment

1. Determine weight a drop from pipette
2. 10 mMol NaCl soln prepared in beaker w/stir bar
3. Drops Triton X-100 were added, mixed 10 minutes
4. Remove small amount, measure by DLS

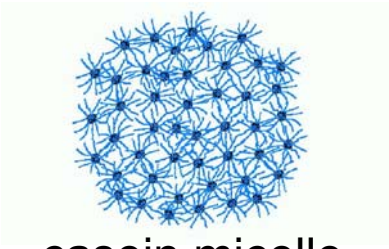
Triton x-100	Conc. wt%	Intensity	Size (nm)
10mMol NaCl	0	0.94	-
1 drop	0.0017	1.78	-
5 drops	0.0086	2.35	-
10 drops	0.0172	3.18	-
15 drops	**0.0255**	**4.78**	**9**

CMC →

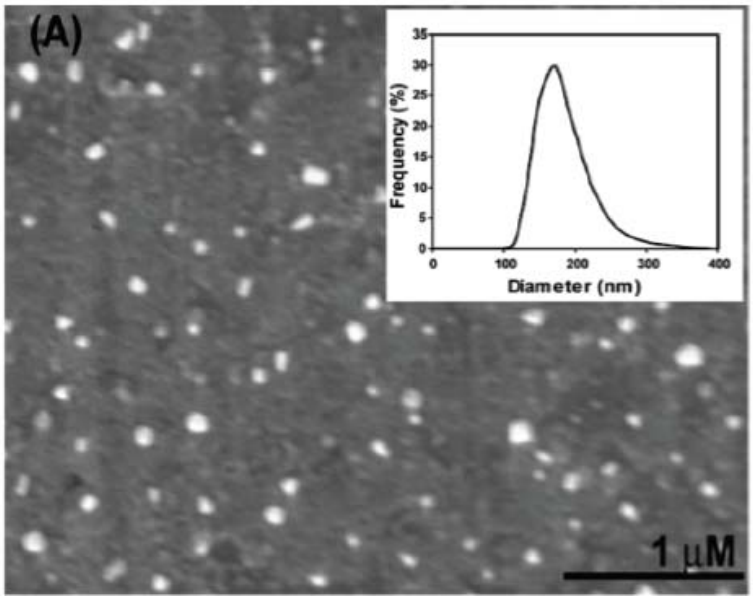
Curcumin- Casein Micelles*

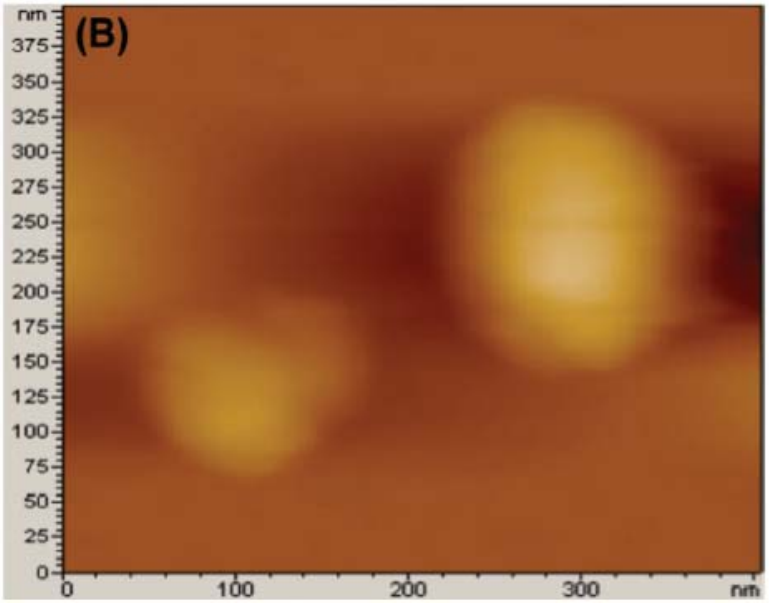


curcumin



casein micelle

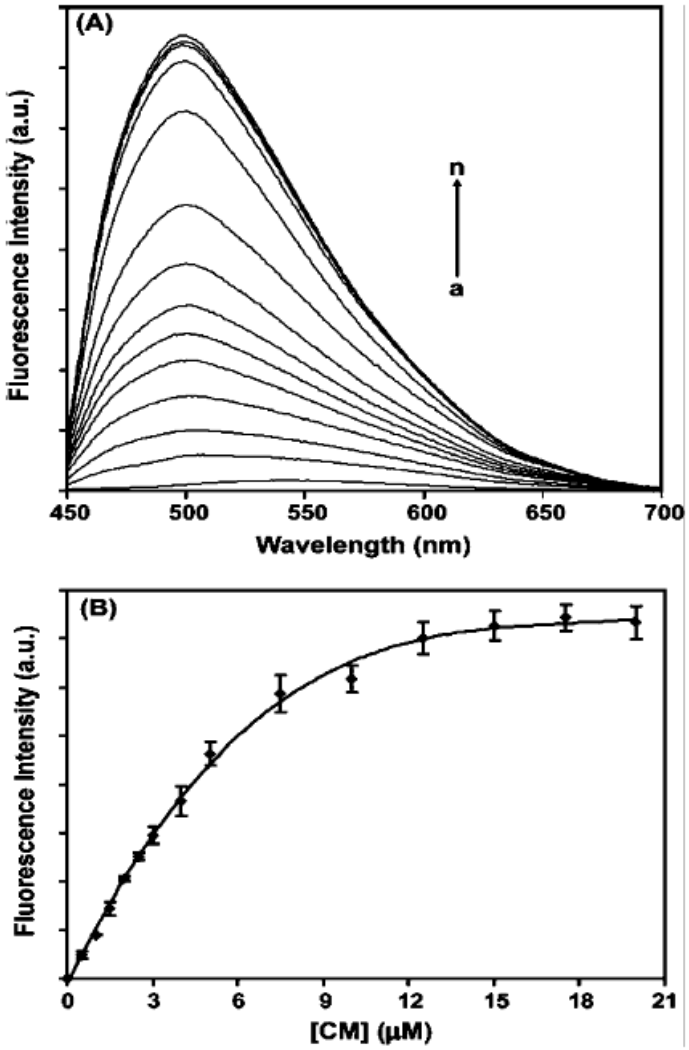


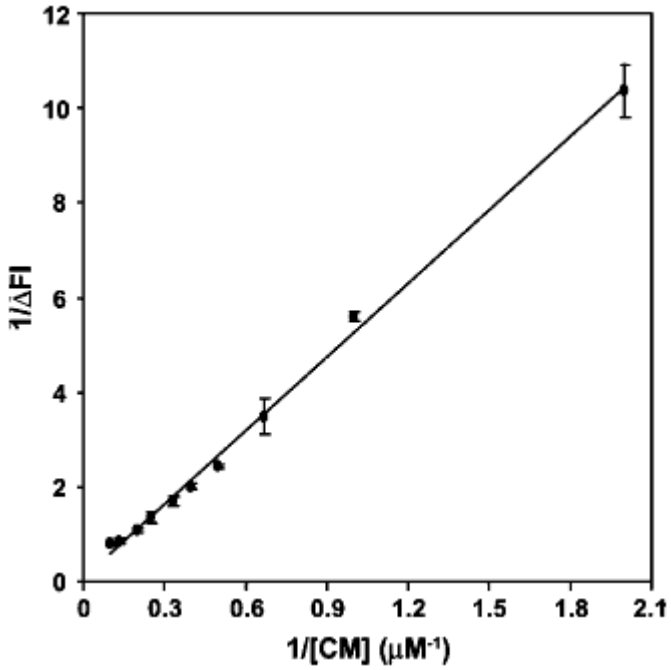


Particle size by DLS (insert), SEM (left), and AFM (right)

*Sahu,et el, Fluorescence Study of the Curcumin-Casein Micelle Complexation and Its Application as a Drug Nanocarrier To Cancer Cells, *Biomacromolecules* 2008, *9,* 2905–2912

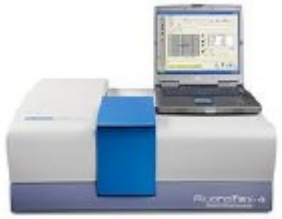
Curcumin- Casein Micelles*





Binding constant k_b = 1.48 x 10^4 M^{-1}

$$\frac{1}{\Delta FI} = \frac{1}{\Delta FI_{max}} + \frac{1}{K_b \Delta FI_{max}[CM]}$$



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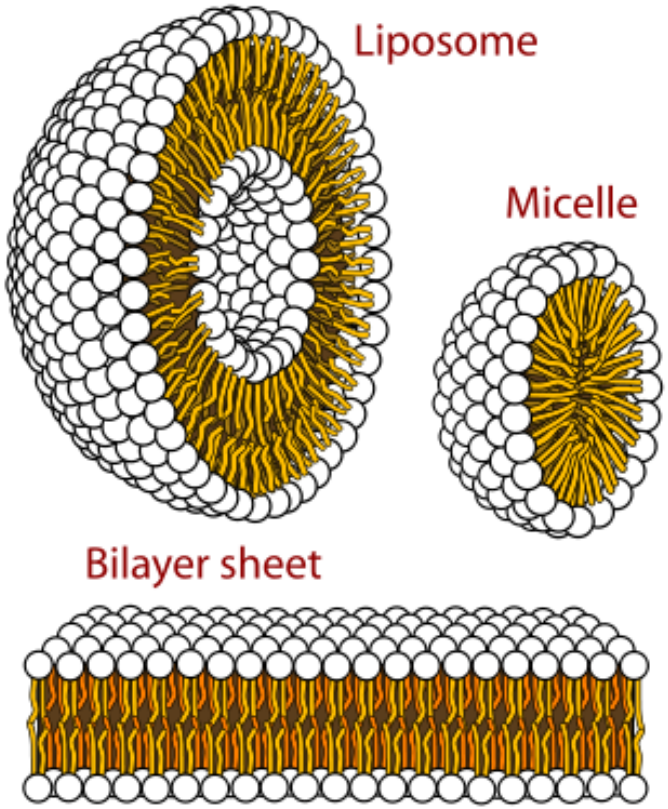
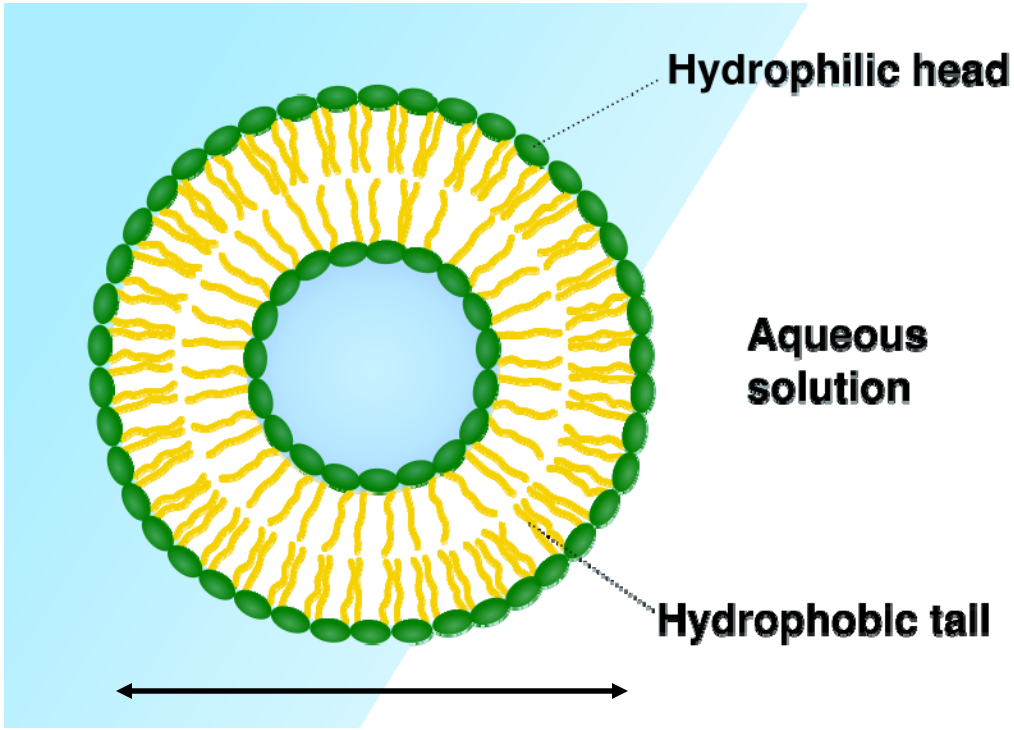
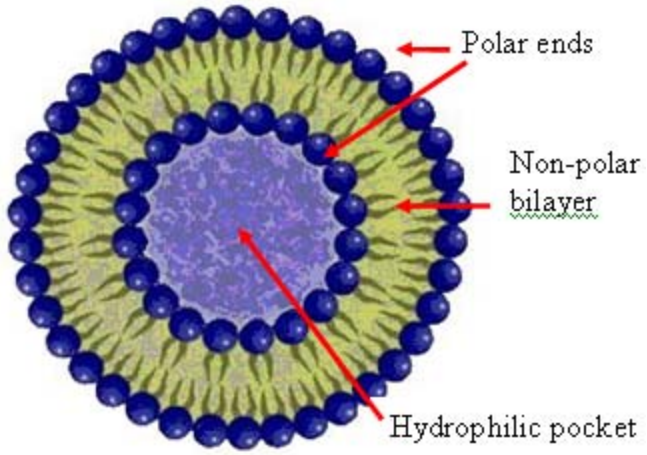
*Sahu,et el, Fluorescence Study of the Curcumin-Casein Micelle Complexation and Its Application as a Drug Nanocarrier To Cancer Cells, *Biomacromolecules* 2008, *9,* 2905–2912

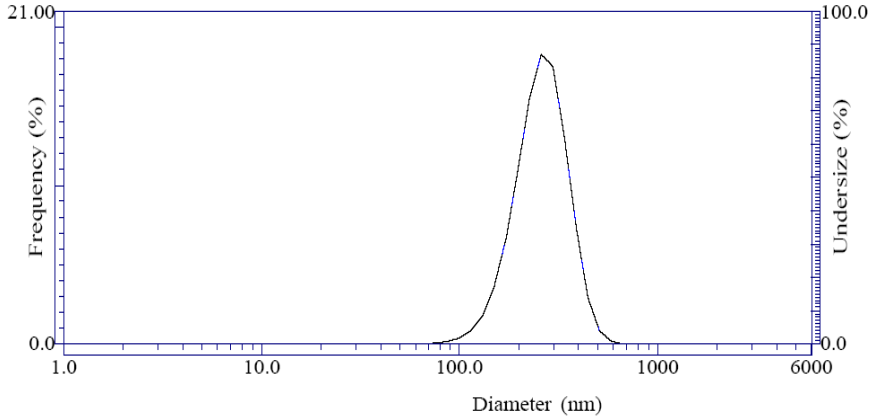
Liposomes





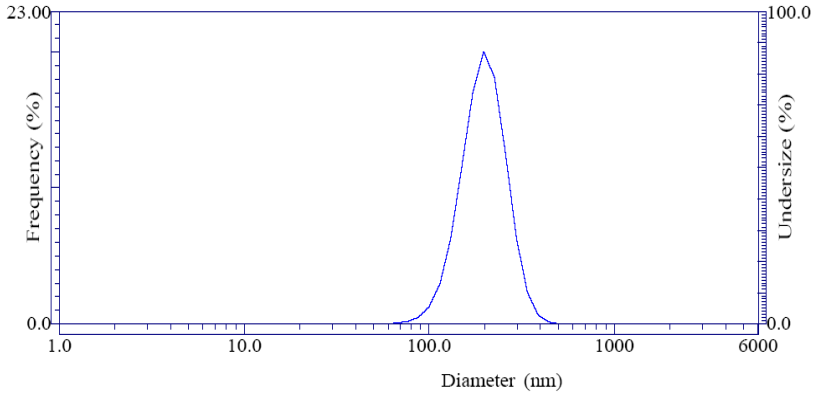
Liposome Size Reduction: Filter Membrane





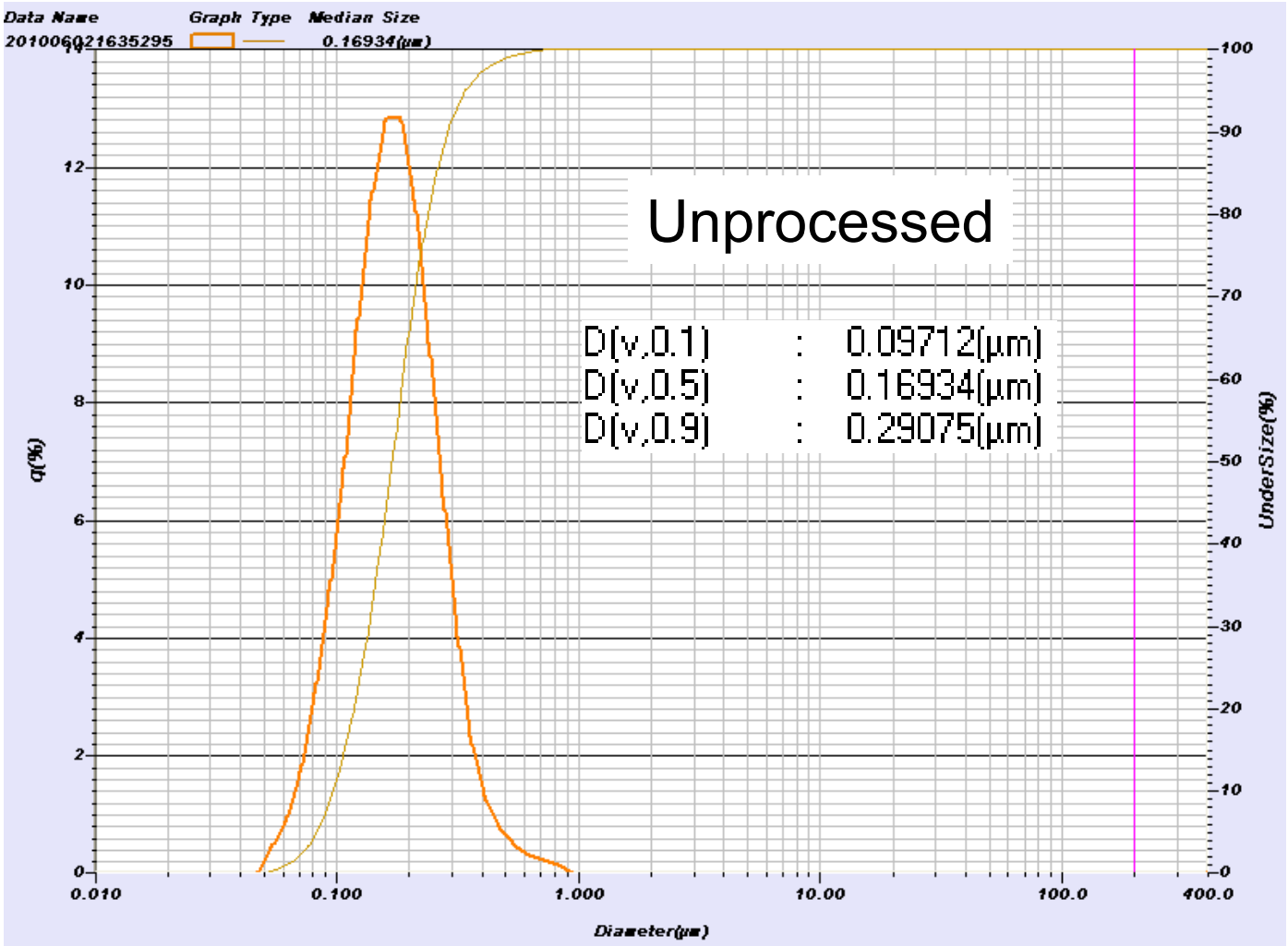
Liposome particle size after 5 passes through a 100 mm membrane ~ 250 nm

Size reduced by passing through 100 nm filter membrane
Measured by DLS



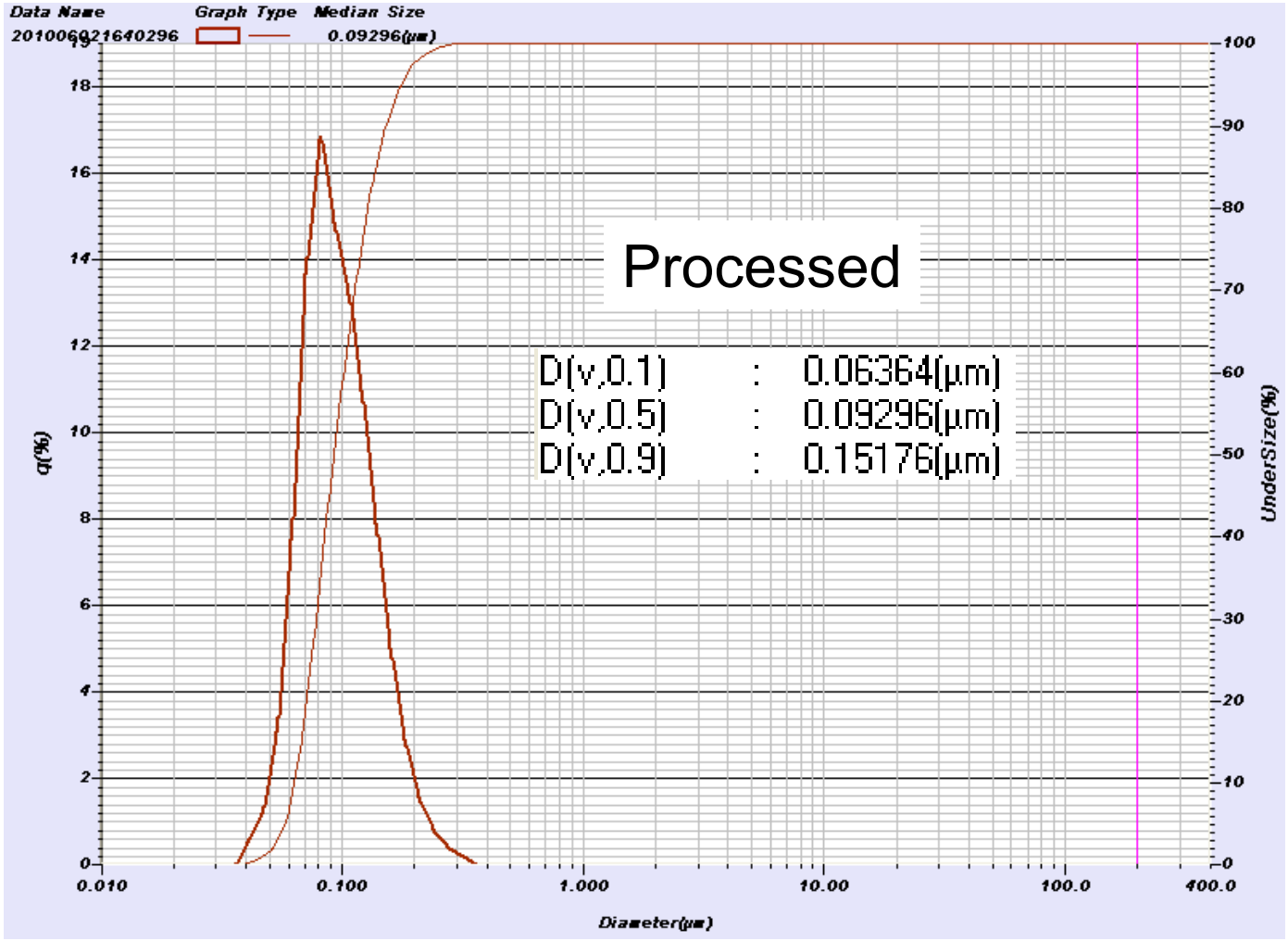
Liposome particle size after 20 passes through a 100 mm membrane ~ 150 nm

Liposome Size Reduction: Microfluidizer



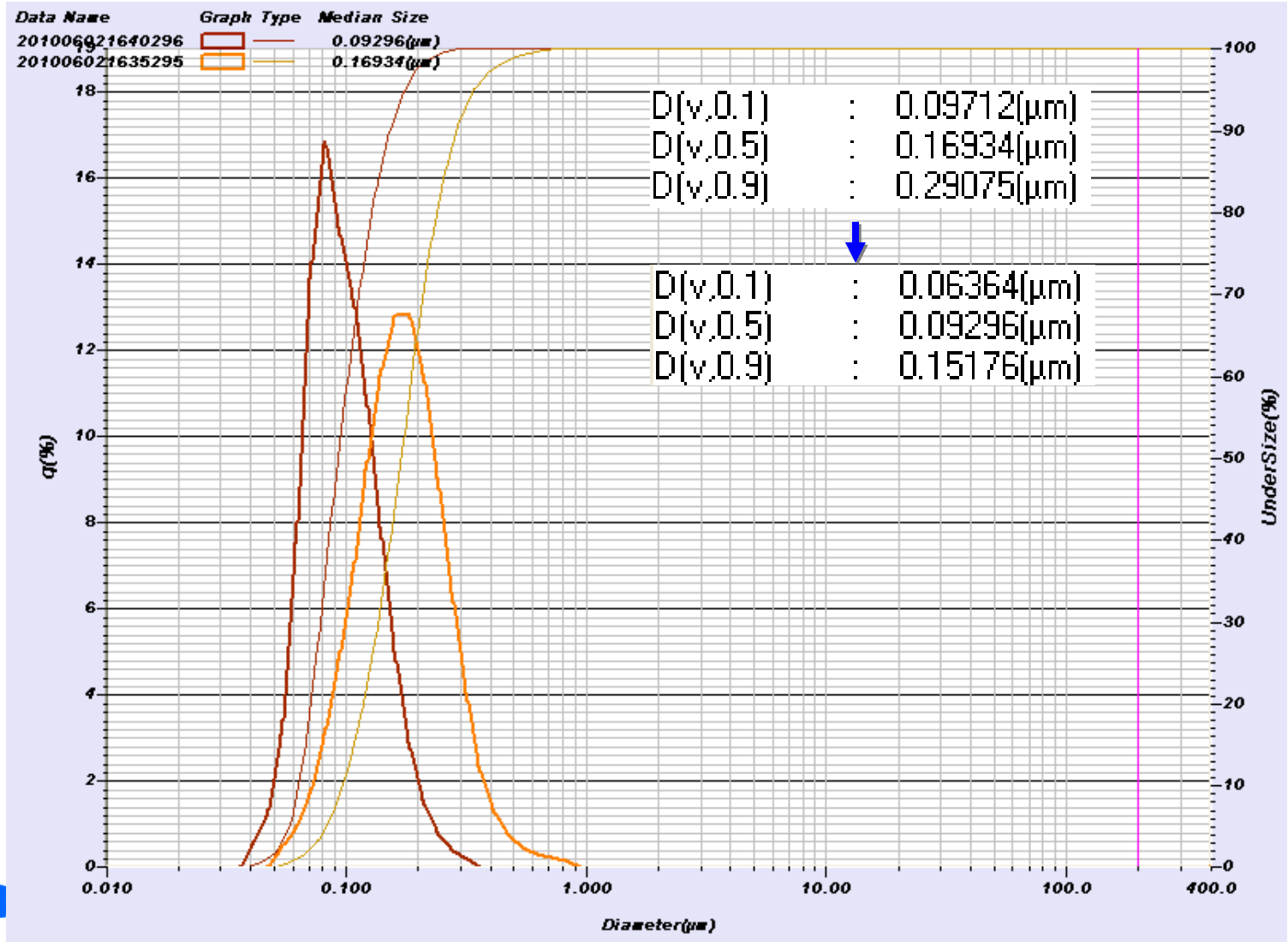
Laser diffraction

Liposome Size Reduction: Microfluidizer



Laser diffraction

Liposome: Before, After



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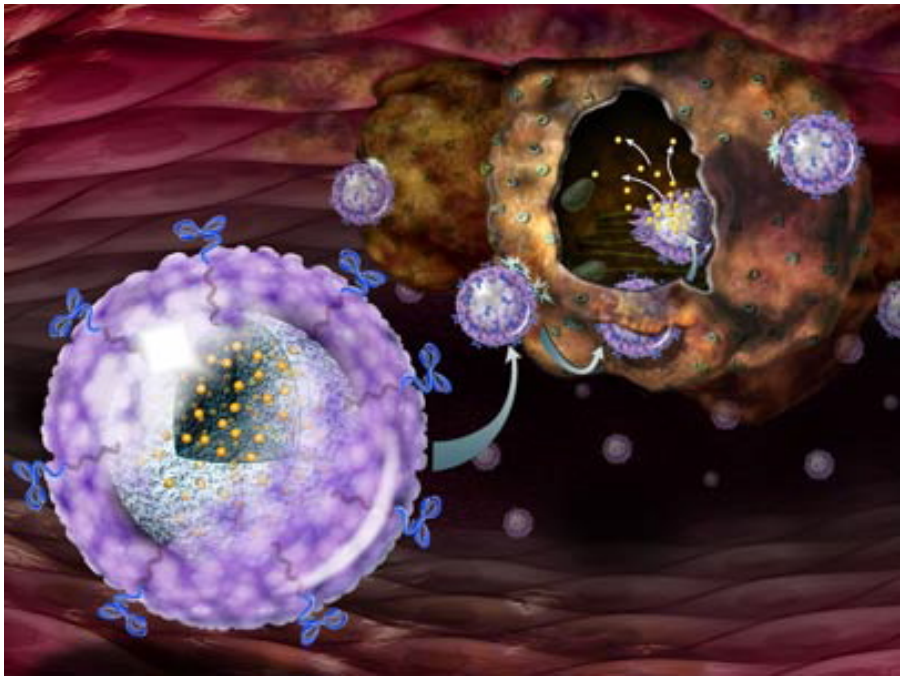
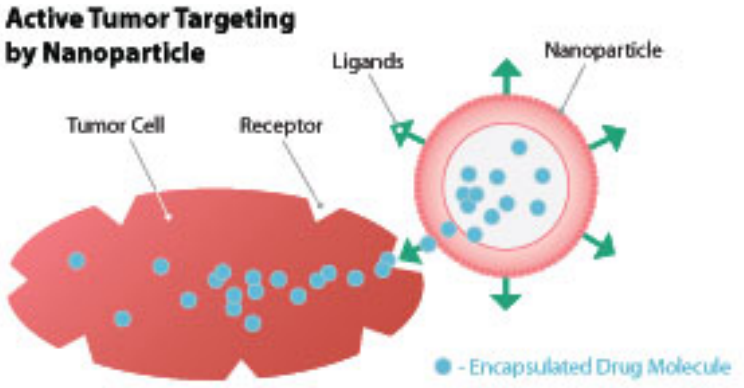
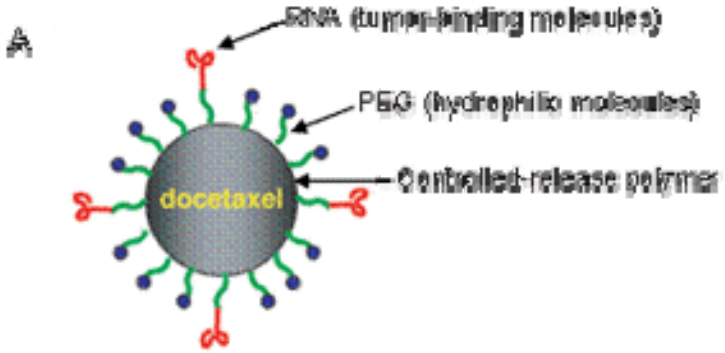
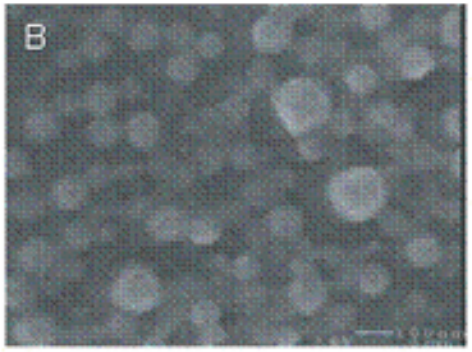
Nanoparticles for Drug Delivery: Bottom Up

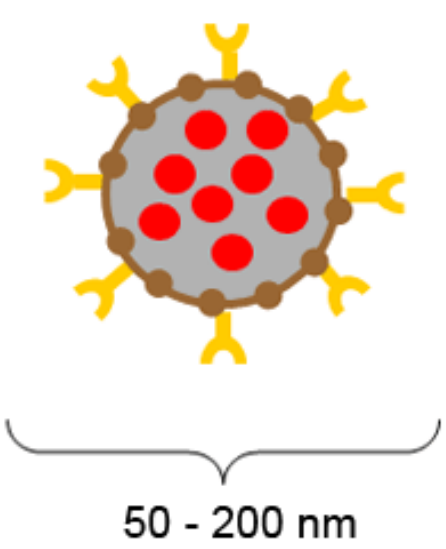


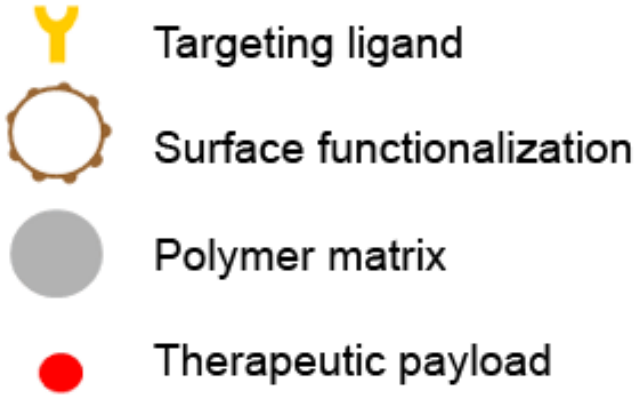


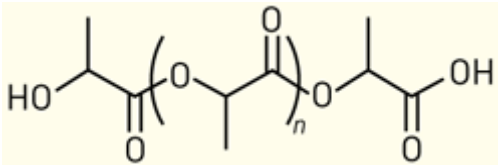


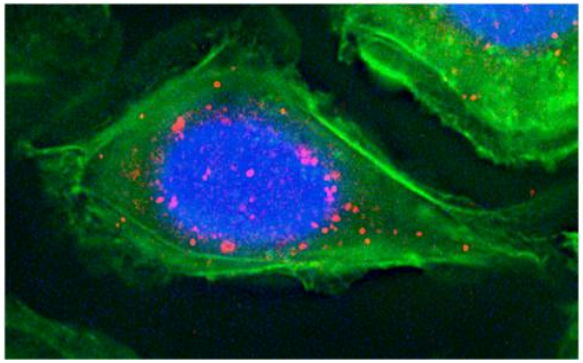


Nanoparticles for Drug Delivery



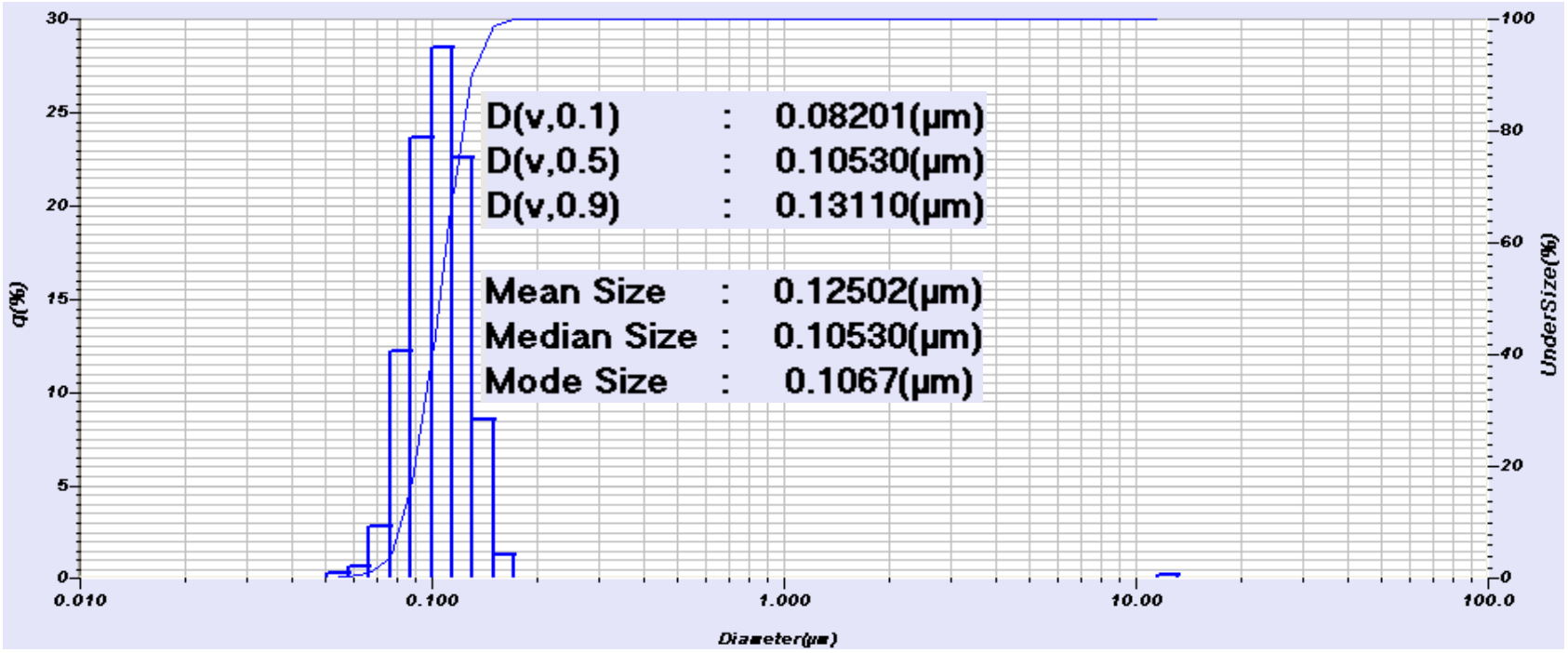




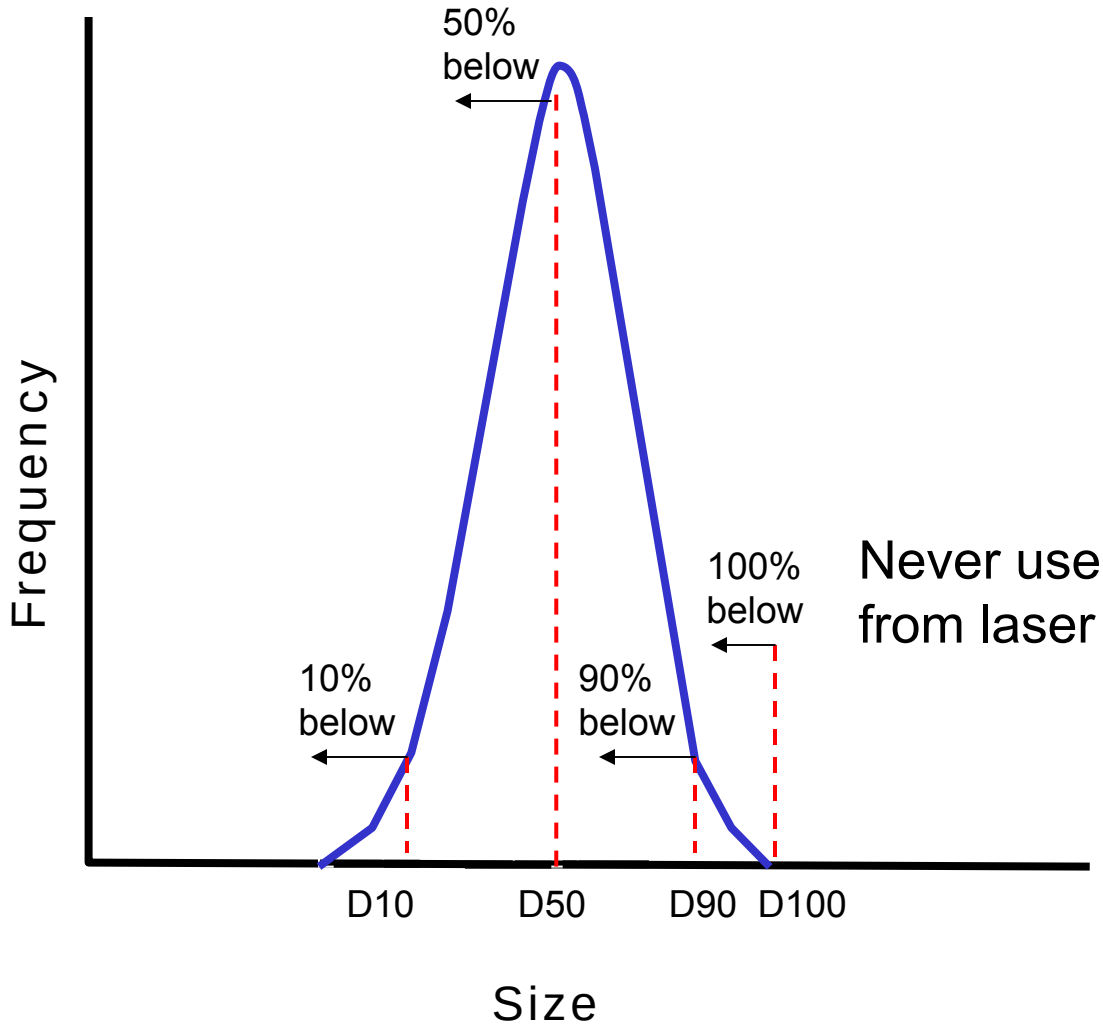


Targeting ligand provides recognition, enabling targeted nanoparticles to identify and bind to their intended target site.
Surface functionalization shields targeted nanoparticles from the immune system.
Polymer matrix encapsulates payload molecules in a matrix of biodegradable polymers .
Therapeutic payloads include small molecules, peptides, proteins, etc.

Nanoparticles for Drug Delivery



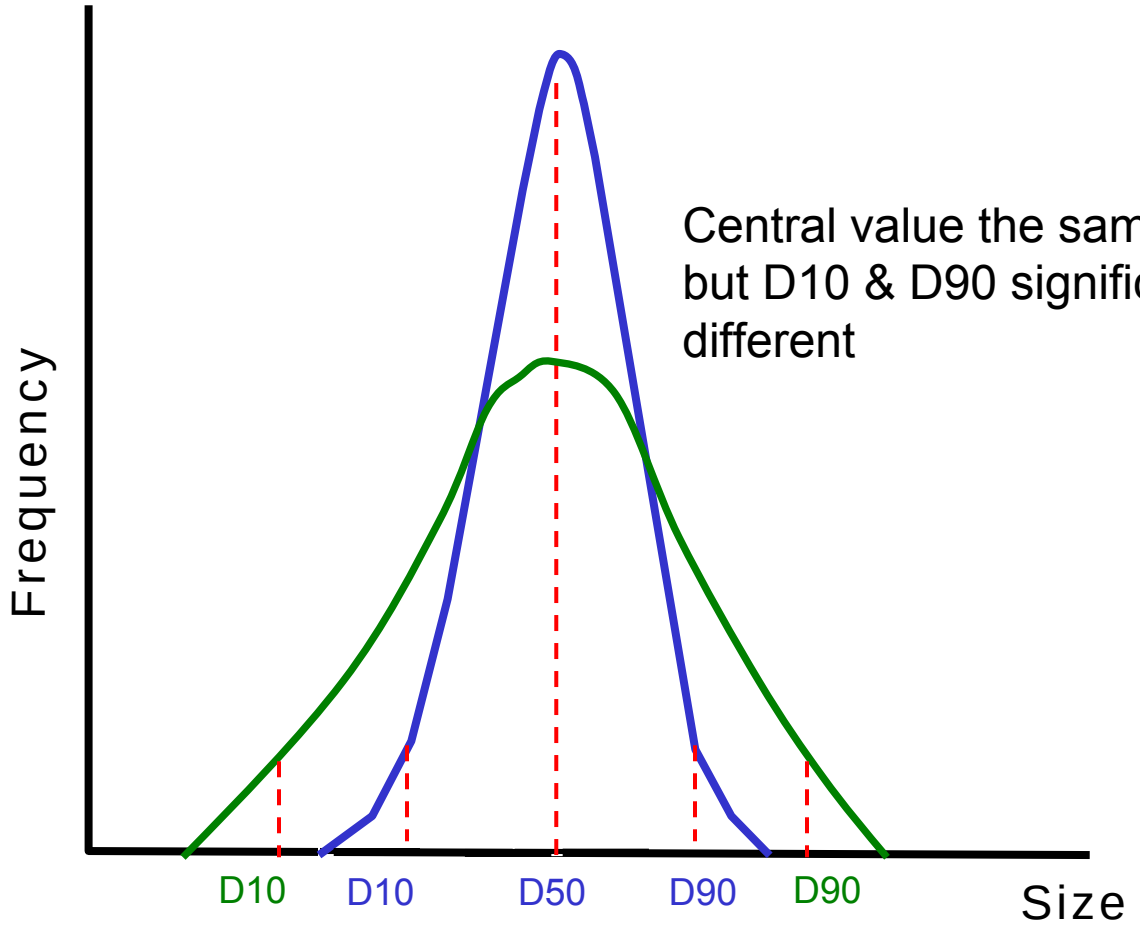
Laser Diffraction Results: D10, D50, D90



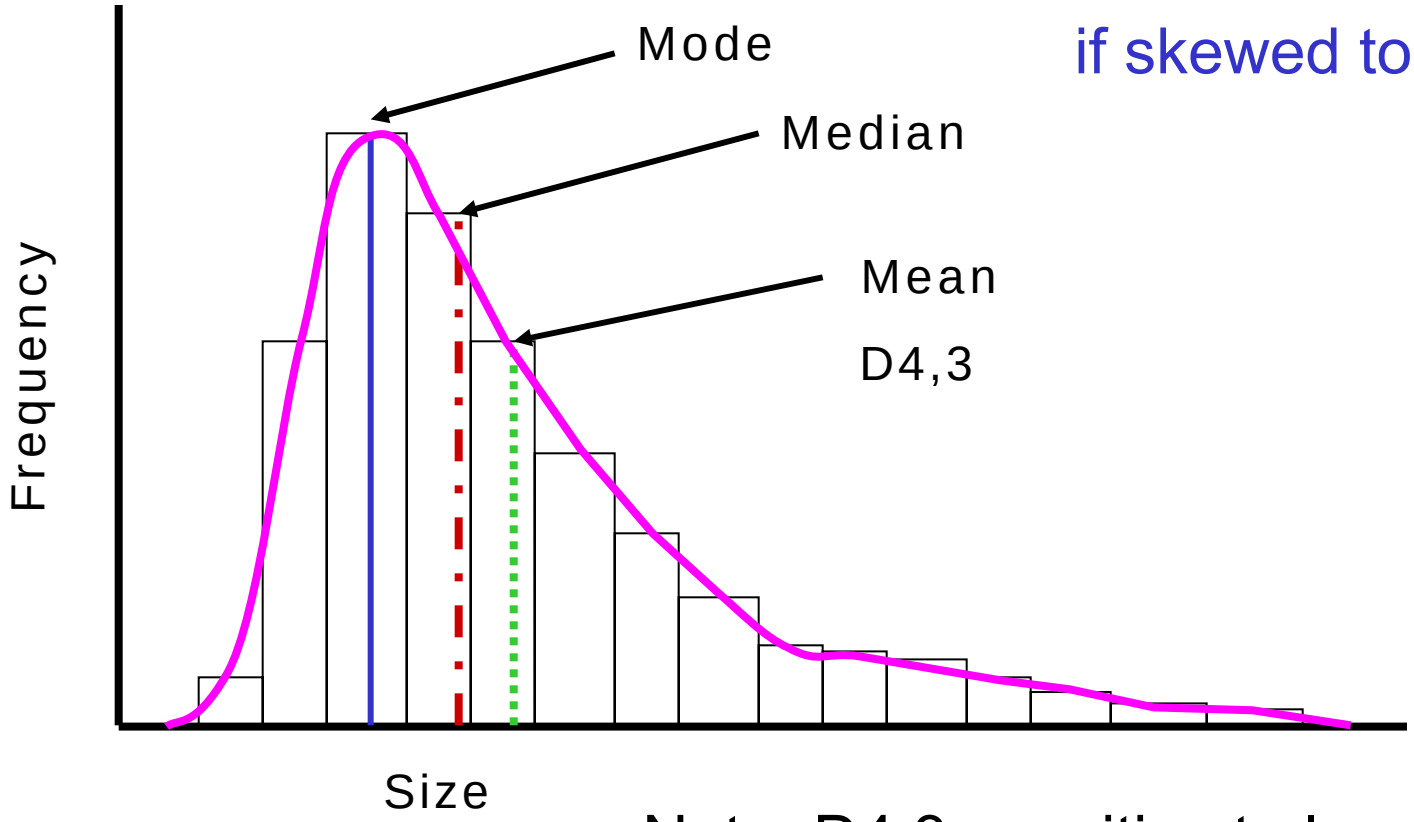
Never use D100
from laser diffraction

Diffraction Results: D10, D50, D90

Symmetric distribution: mean = median = mode



Asymmetric Distribution



Mode<Median<Mean
if skewed to larger sizes

Note: D4,3 sensitive to large particles

Volume Mean Diameter

- D[4,3] which is often referred to as the Volume Mean Diameter [VMD]

$$D[4,3] = \frac{\sum D_i^4 n_i}{\sum D_i^3 n_i}$$

Setting a D [4,3] specification will emphasize the presence of large particles

Mean Size

The frequency distribution is found using the arithmetical mean diameter, as shown in the formula below.

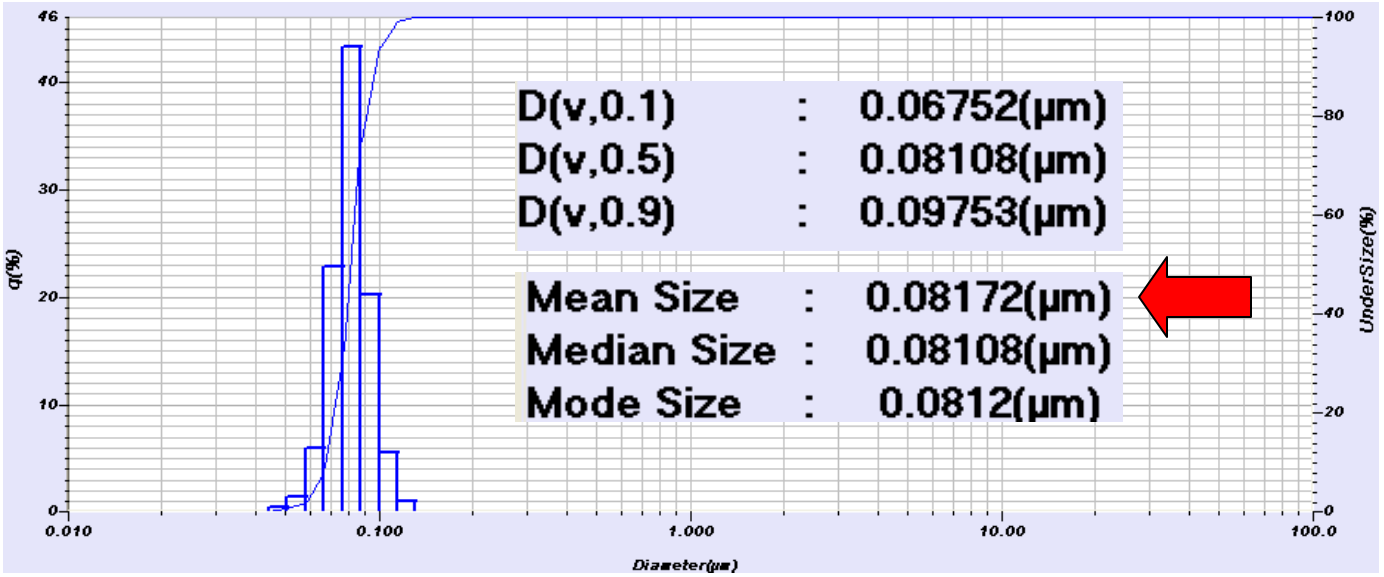
Mean Diameter = $\Sigma\{q(J) \times X(J)\} / \Sigma\{q(J)\}$

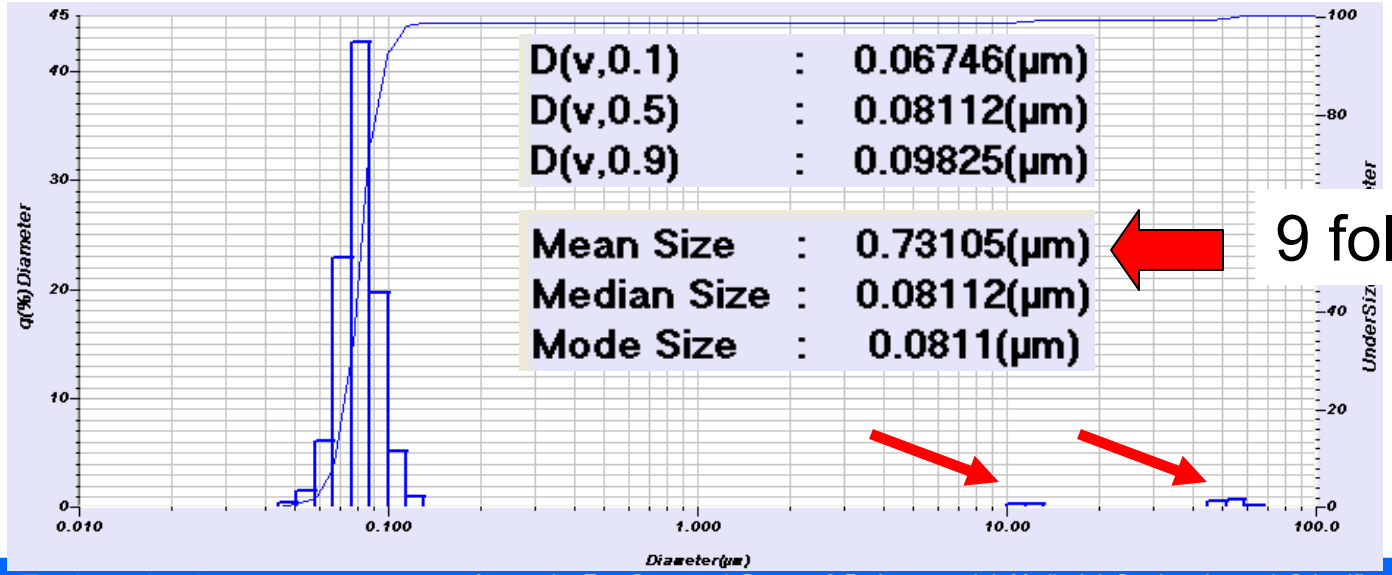
J : Particle Diameter Division Number

q(J) : Frequency Distribution Value (%)

X(J) : Jth Particle Diameter Range's Representative Diameter (μm).

PLA Nanoparticles for Drug Delivery

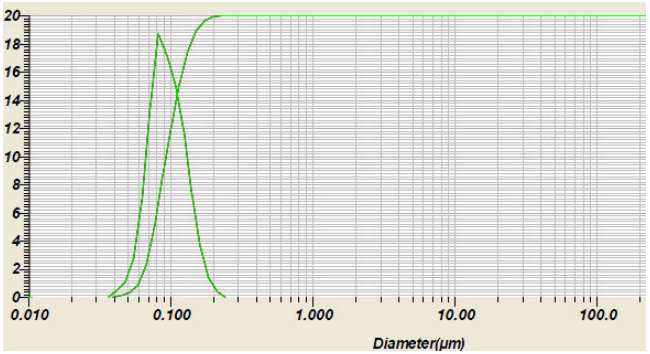




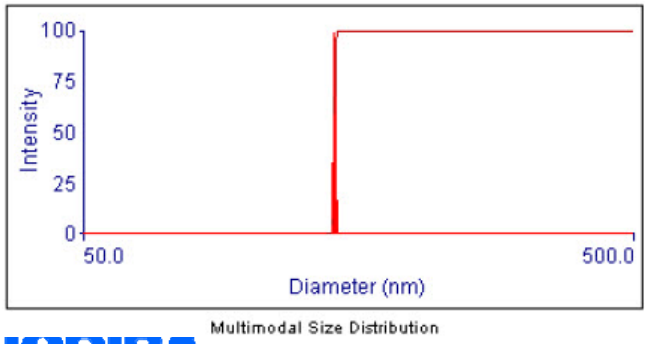
9 fold increase

PLA Nanoparticles for Drug Delivery

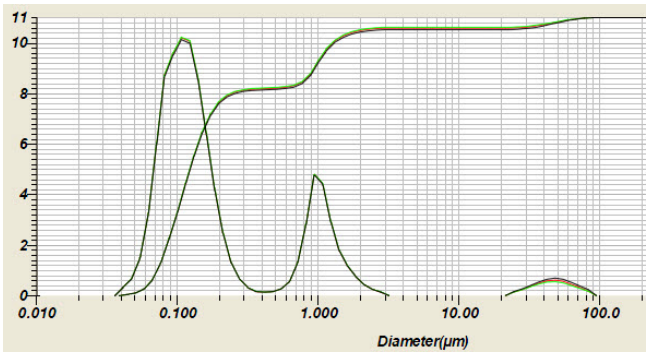
Pure



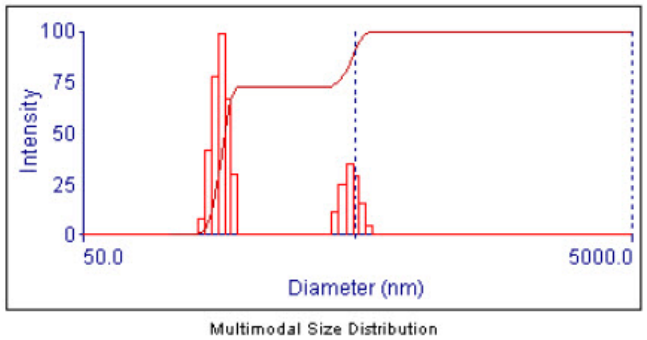
Sample Name	D(v,0.1)	D(v.0.5)	D(v.0.9)
50928-6-1	0.06541(µm)	0.09222(µm)	0.13789(µm)
50928-6-1	0.06541(µm)	0.09222(µm)	0.13788(µm)
50928-6-1	0.06540(µm)	0.09221(µm)	0.13787(µm)



Spiked with 1 µm PSL



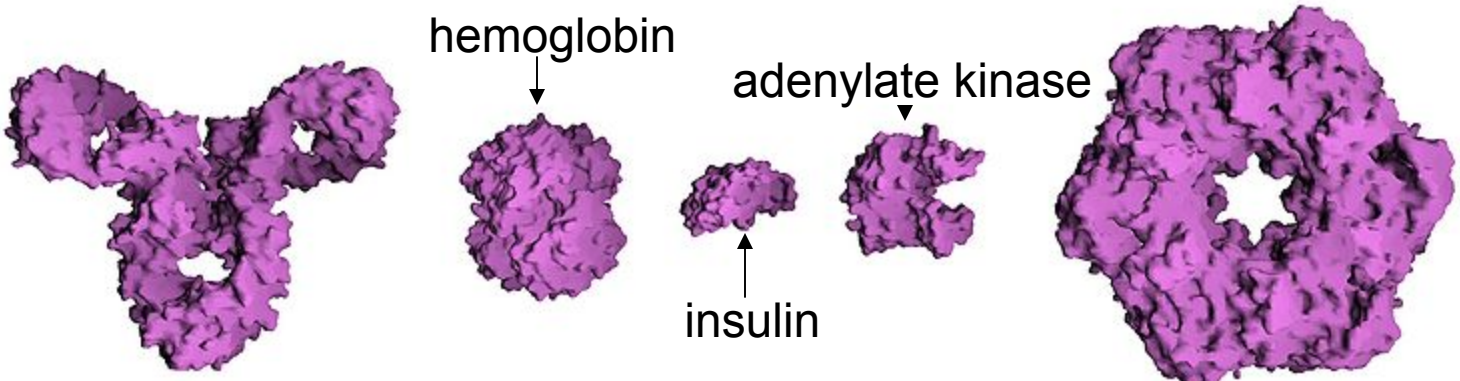
Sample Name	D(v,0.1)	D(v.0.5)	D(v.0.9)
50928-6-2	0.07348(µm)	0.13085(µm)	1.21951(µm)
50928-6-2	0.07345(µm)	0.13065(µm)	1.20702(µm)
50928-6-2	0.07360(µm)	0.13155(µm)	1.25225(µm)



HORIBA Scientific

Proteins

Molecular surface of several proteins showing their comparative sizes.



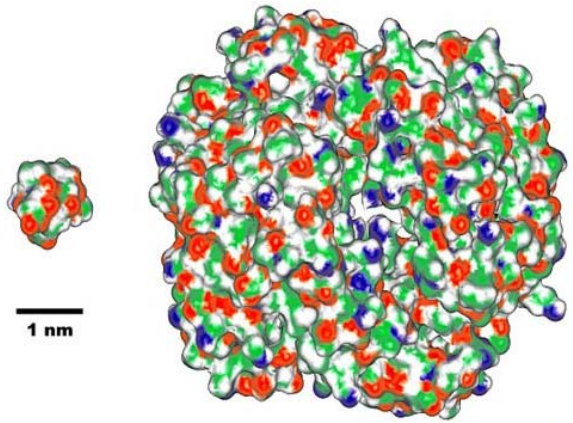


Fig. 2. Comparison of molecular size between chignolin (left, with 10 amino acid residues) and human hemoglobin, one of representative protein (right, with 574 residues). (1 nm = 1 / 1,000,000,000 m)

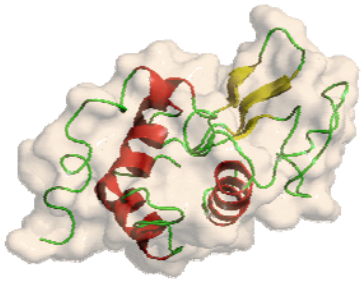
Protein: Lysozyme

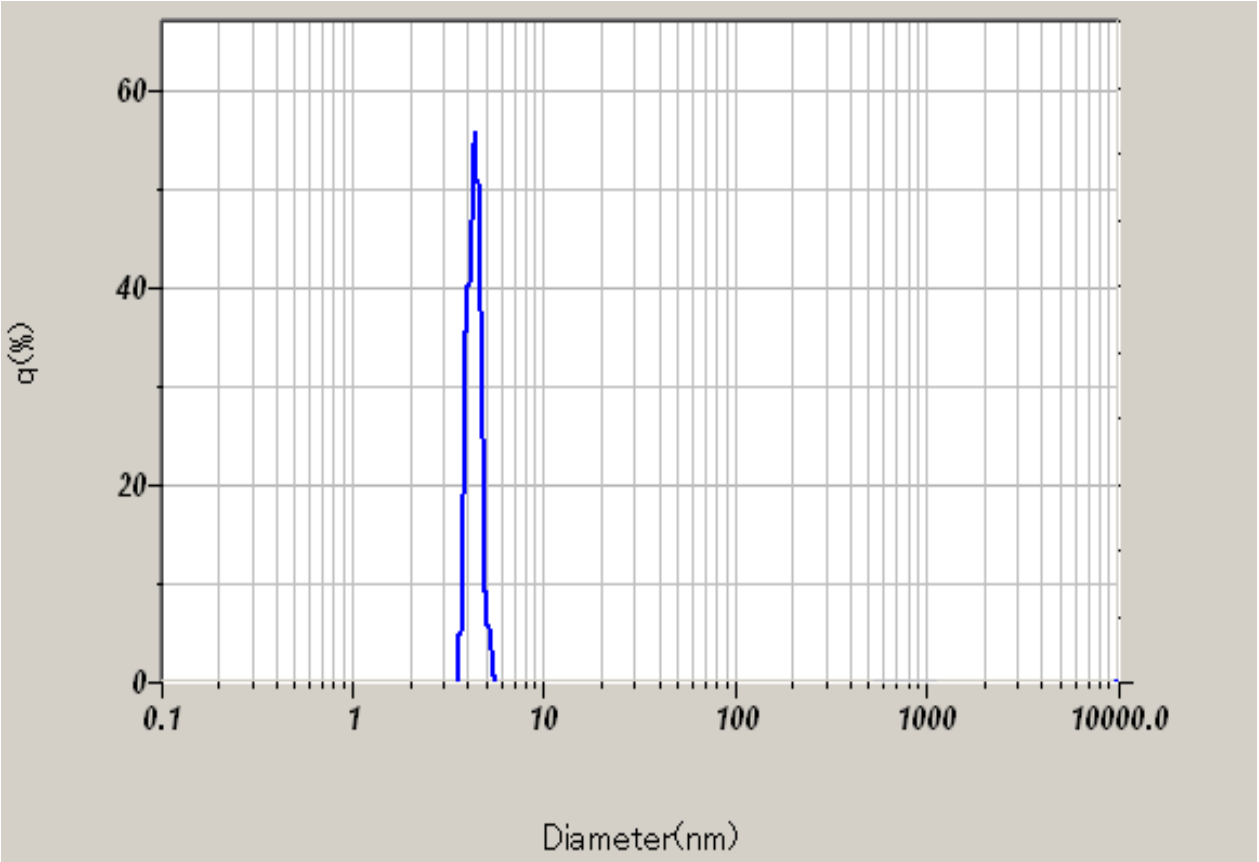
●Protein; Lysozyme

Lysozyme	from egg white, Molecular weight; 14,000
Sample Preparation	pH 4.3, 0.1 mg / mL, 0.1 M Sodium-Acetate buffer

Conditions
Temperature; 25 C degree
Solvent; Water
Refractive Index; 1.333
Distribution base; Mass

	Results
Mean Dia. (nm)	4.5





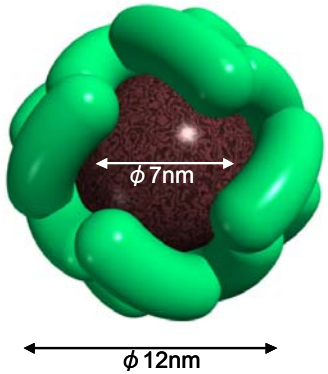
HORIBA Scientific

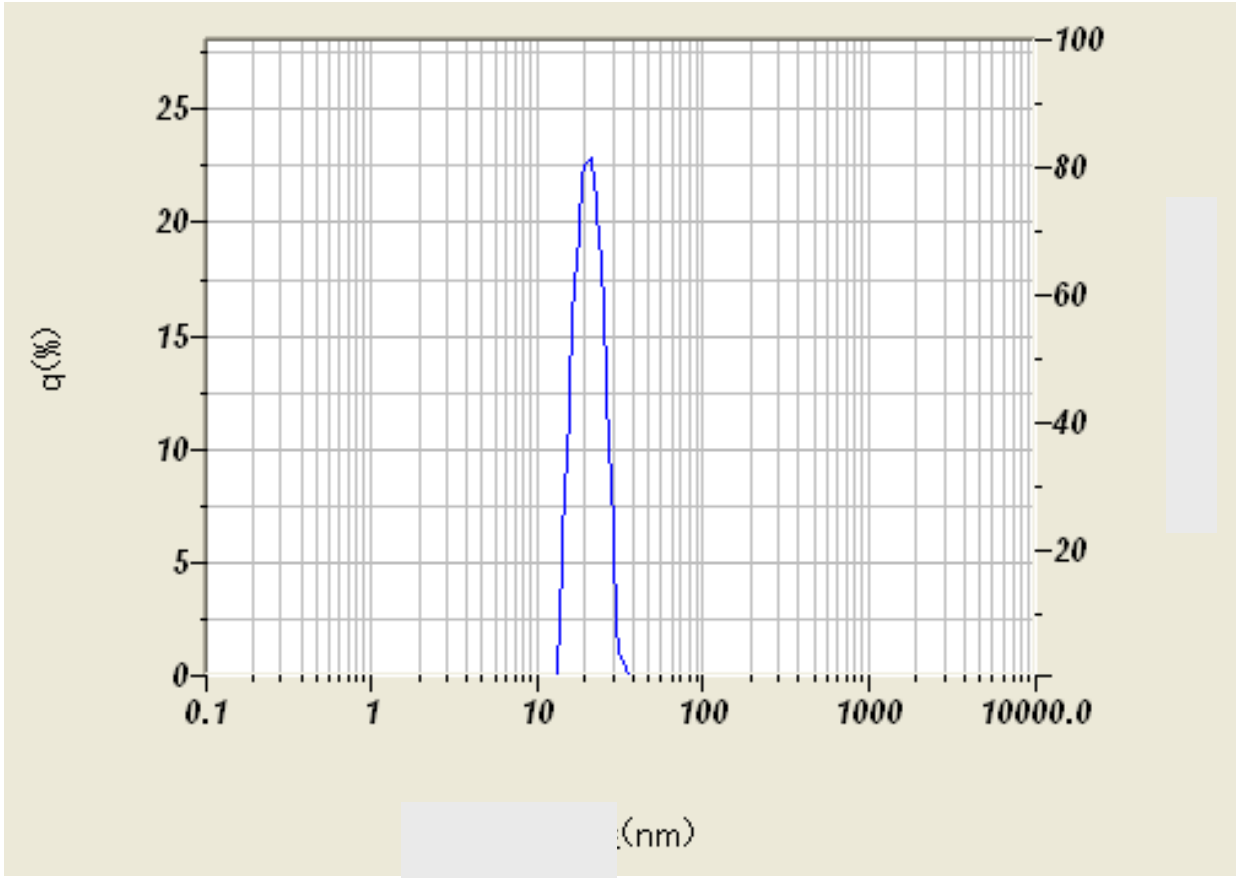
Ferritin

Protein	Ferritin
Sample Preparation	10 %, pH 6.0

Conditions
Temperature; 25 C degree
Solvent; Water
Refractive Index; 1.333
Distribution base; Mass

	Results
Z ave. (nm)	19.7

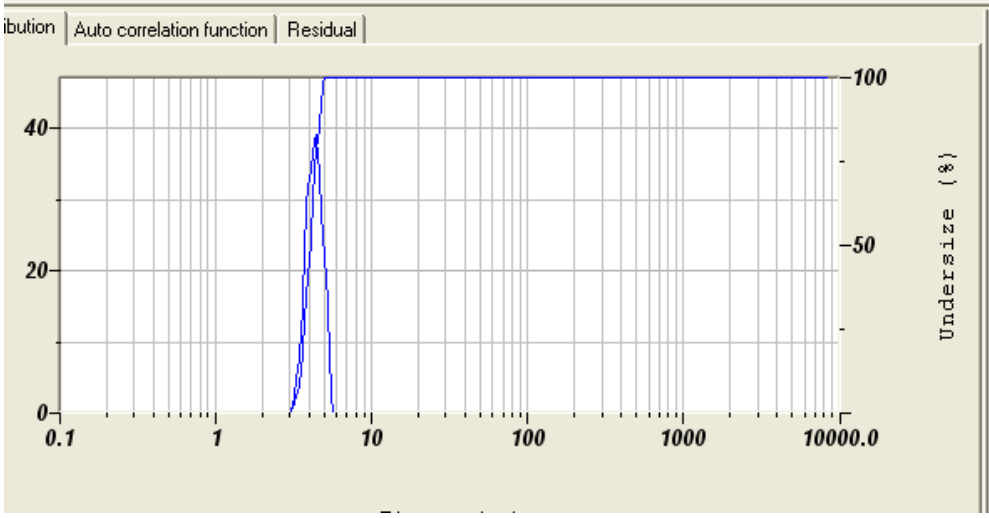




HORIBA Scientific

Protein Reproducibility

end	No.	D.Q.C	Date	Measurement Type	Sample Name	Scattering Angle	T% before meas.	T% after meas.	Z-Average(nm)
	75		mercredi 22 septembre	Particle Size	bsa protein	90	31831	31438	8.2
	76		mercredi 22 septembre	Particle Size	bsa protein	90	31831	31438	7.9
	77		mercredi 22 septembre	Particle Size	bsa protein dilute	90	32118	32086	8.1
	78		mercredi 22 septembre	Particle Size	bsa protein dilute	90	32118	32086	8.3
	79		mardi 12 octobre 2010	Particle Size	LYZ 10 F100nm NC AA StdM	90	32109	32293	4.0
	80		mardi 12 octobre 2010	Particle Size	LYZ 10 F100nm NC AA StdM	90	32109	32293	3.7
	81		mardi 12 octobre 2010	Particle Size	LYZ 10 F100nm NC AA StdM	90	32109	32293	3.7
Average							32109	32293	3.8
StdDev							0	0	0.1



Data | Condition | Table

Sample Name	: LYZ 10 F100nm NC AA StdM
Scattering Angle	: 90
Temperature of the holder	: 25.0 °C
T% before meas.	: 32109
Viscosity of the dispersion medium	: 0.896 mPa·s
Form Of Distribution	: Standard
Form of Distribution(Dispersity)	: ---
Representation of result	: Scattering Light Intensity
Count rate	: 397 kCPS

Calculation Results

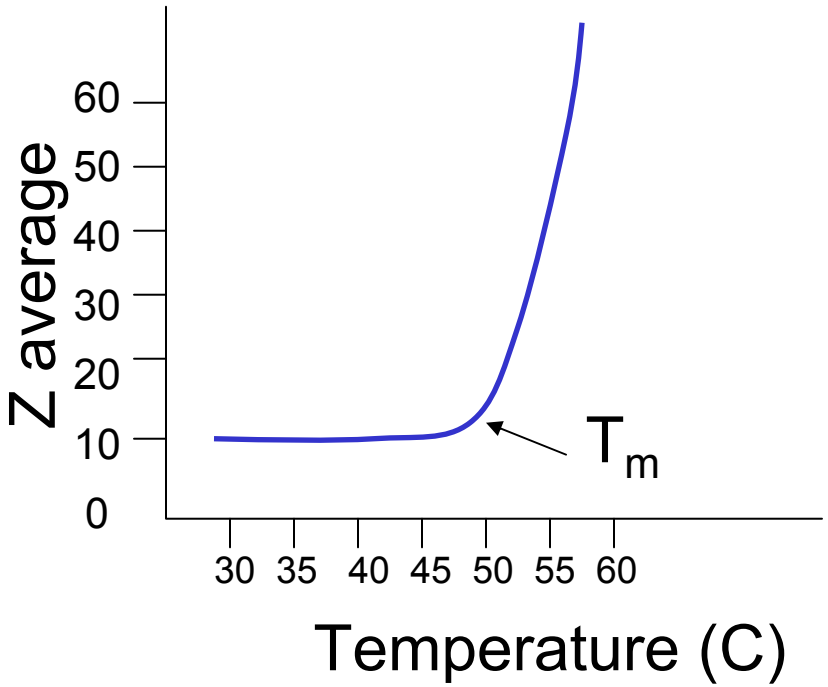
Peak No.	S.P.Area Ratio	Mean	S. D.	Mode
1	1.00	4.0 nm	0.4 nm	4.1 nm
2	---	--- nm	--- nm	--- nm
3	---	--- nm	--- nm	--- nm
Total	1.00	4.0 nm	0.4 nm	4.1 nm

Cumulant Operations

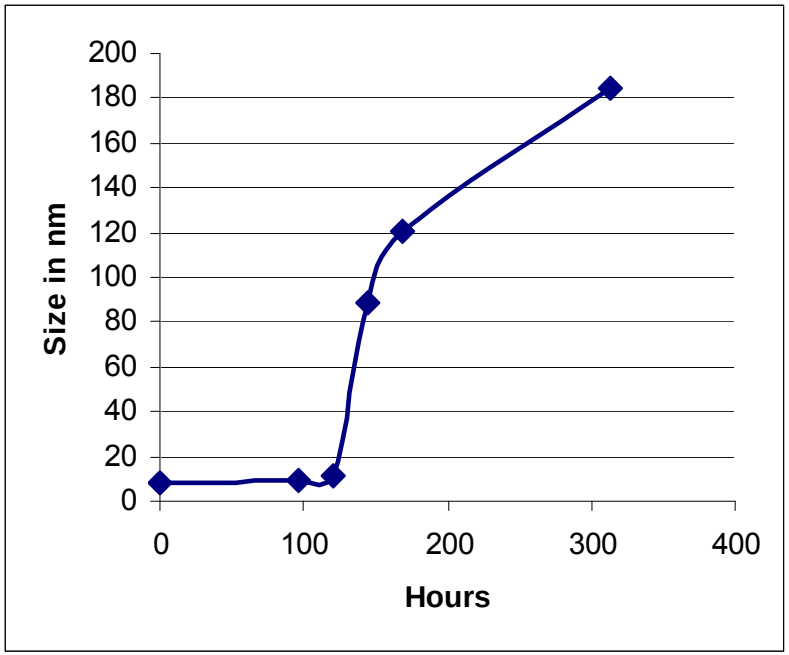
Z-Average : 4.0 nm

Protein Size

Melting Point



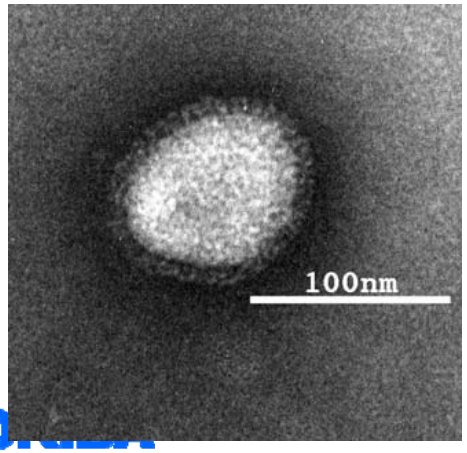
Lysozyme Aggregation

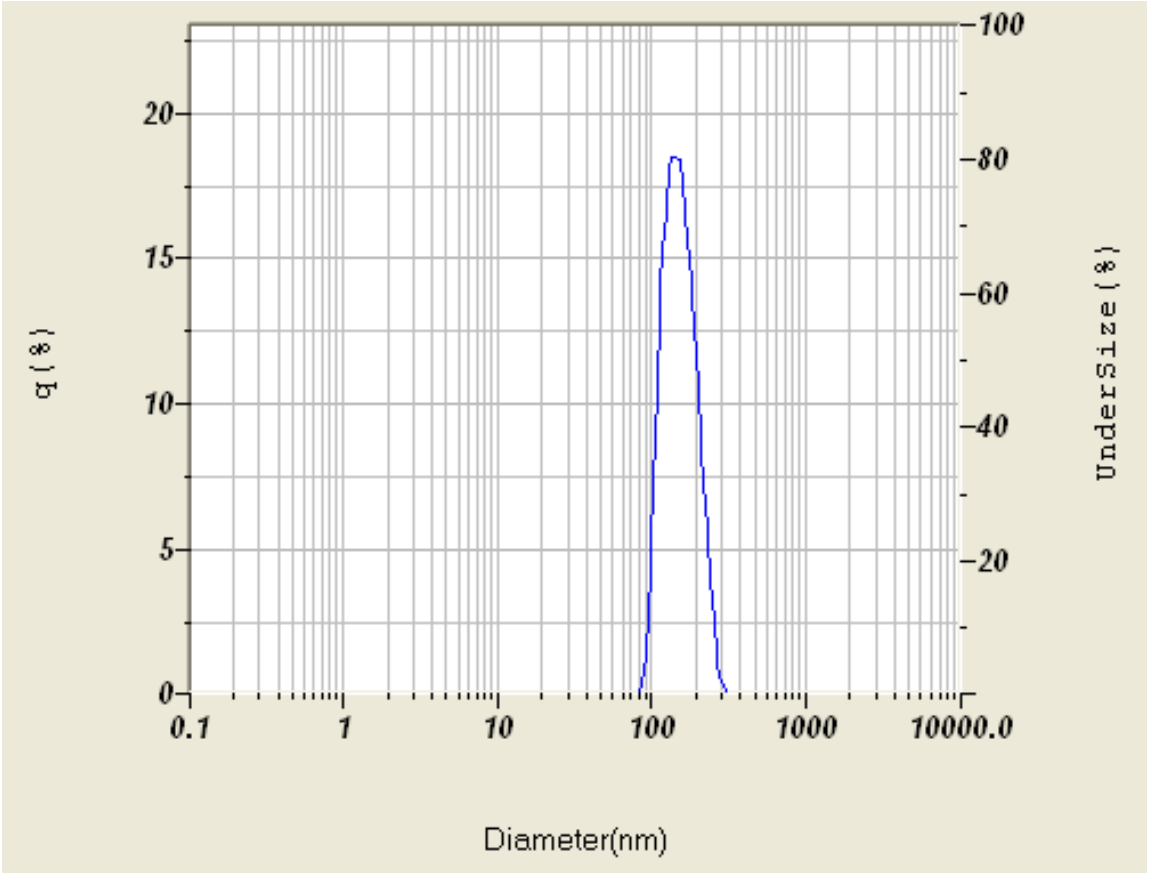


Virus

Virus	Influenzavirus
Sample Preparation	1%　Sodium-Acetate buffer

	Results
Z ave. (nm)	138.1



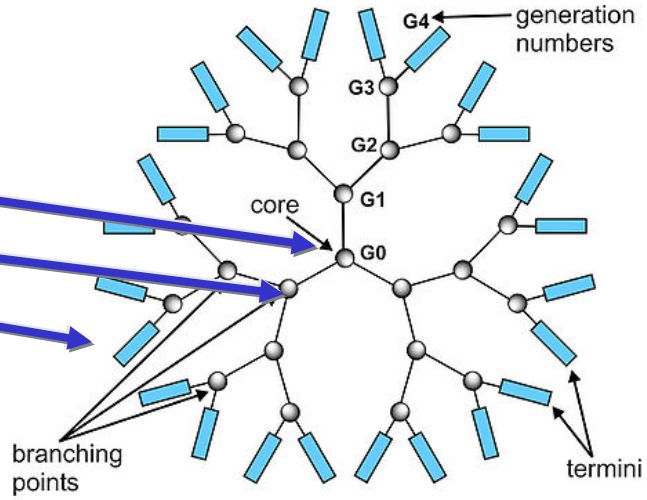


Scientific

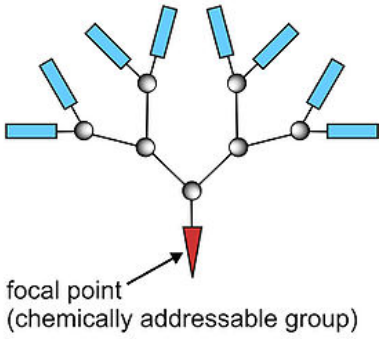
Dendrimers

Repeatedly branched, roughly spherical large molecules

- Core
- Inner shell
- Outer shell



DENDRIMER



DENDRON

Dendrimers

- Applications: typically involve conjugating other chemical species to the dendrimer surface that can function as detecting agents (such as a dye molecule), affinity ligands, targeting components, imaging agents, or pharmaceutically active compounds
  - Drug & gene delivery
  - Sensors

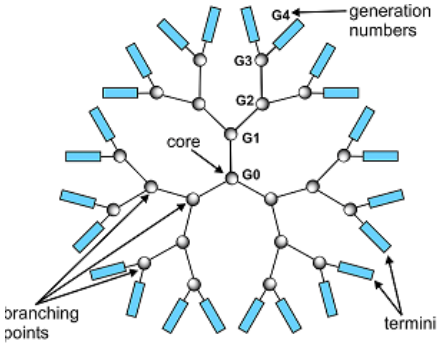
Dendrimers

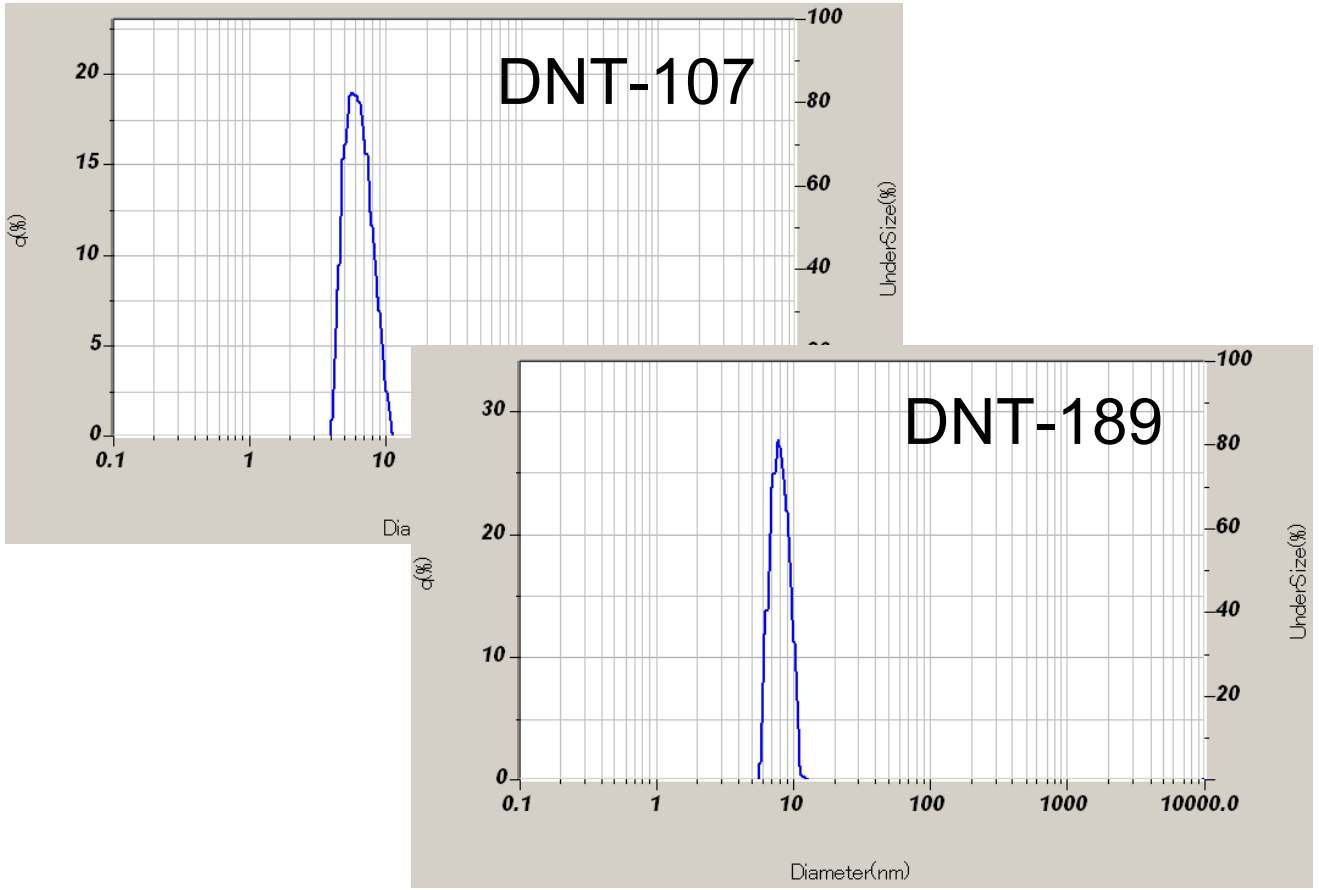
●DDS materials; Dendrimer

PAMAM Dendrimer In Methanol	DNT-107(7.2 nm); 1,4-Diaminobutan Core, Amidoamine surface, Gen 6.0,10% DNT-189 (9 nm) ; 1,4-Diaminobutan Core, Amidoethanol surface, Gen 6.0,10%

Conditions
Temperature; 25 C degree
Solvent; Methanol
Refractive Index; 1.329
Distribution base; Scattering light

	Z ave. (nm)
DNT-107	7.6
DNT-189	8.6





HORIBA Scientific

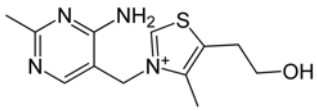
Detection Limit

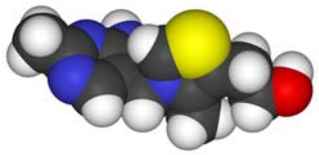
●Bio-sample; Vitamin B1

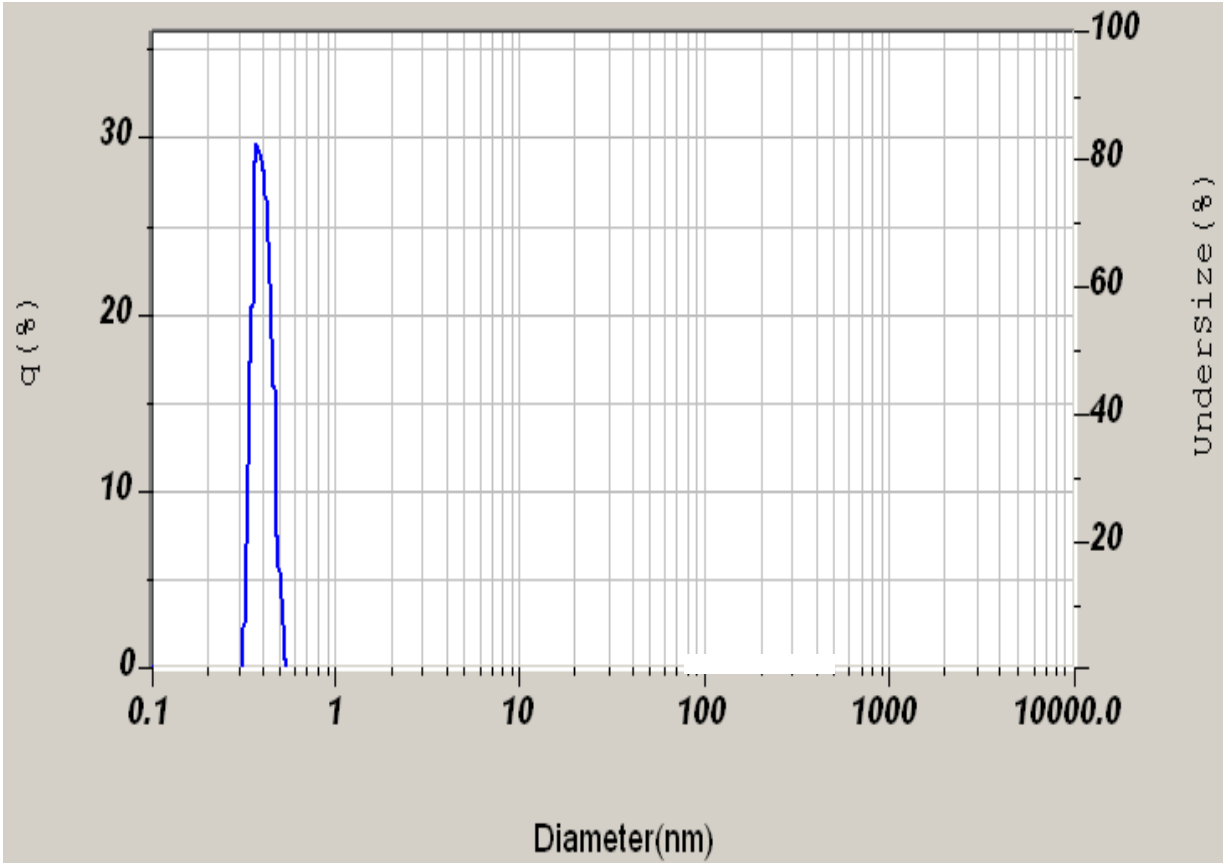
Thiamine	Vitamin B1 Molecular weight; 337.27
Sample Preparation	300 mg / mL

Conditions
Temperature; 25 C degree
Solvent; Water
Refractive Index; 1.333
Viscosity; 1.68
Distribution base; Mass

	Results
Mean Dia. (nm)	0.4



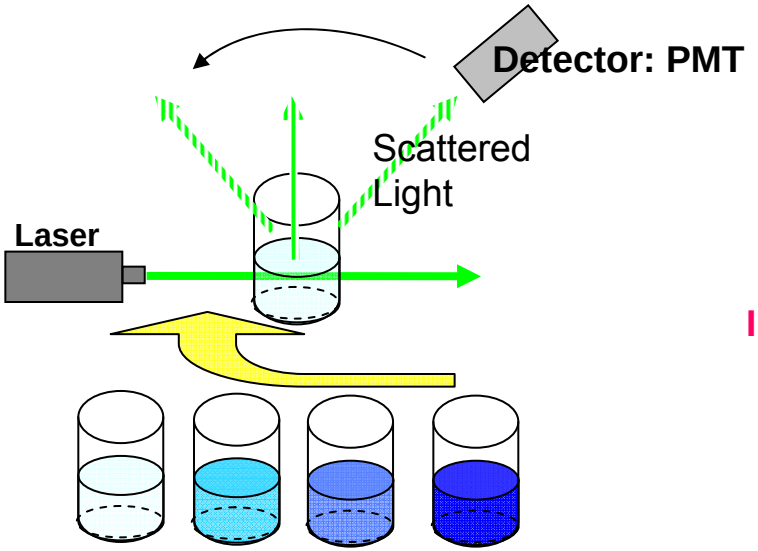


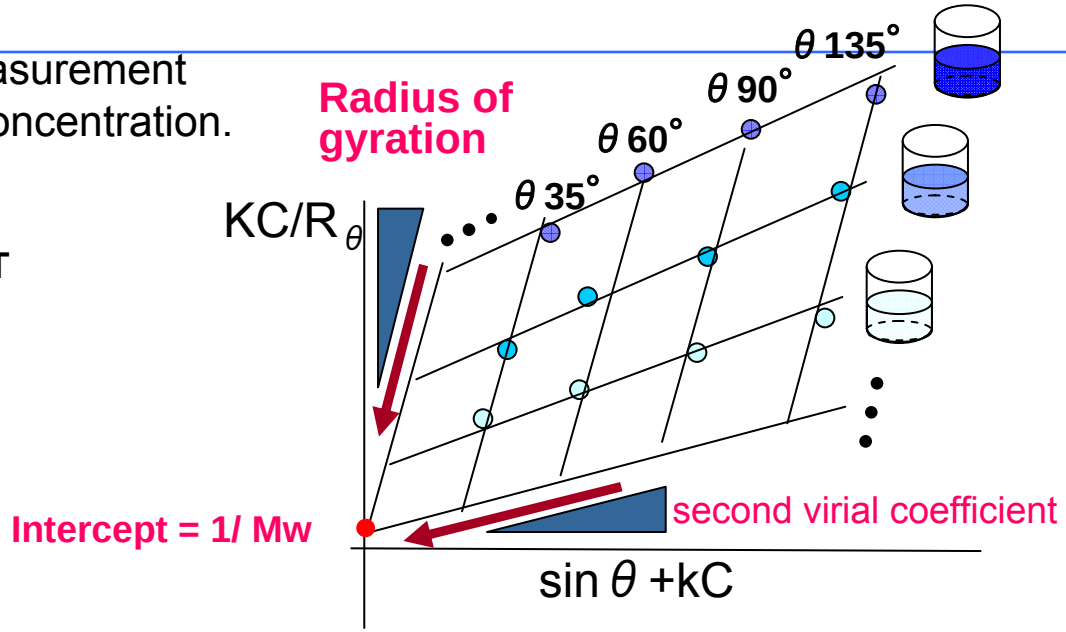


HORIBA Scientific

Molecular Weight Measurement Principle

Multi angle scattered light intensity measurement preparing diluted solutions of known concentration.





e.g. Polystyrene sample (Mw=5x10^5)

→ Mw =6.35 x10^5

$$\lim_{\theta \to 0} \frac{KC}{\Delta R_\theta} = \frac{1}{M} + 2A_2C + 3A_3C^2 \cdots$$

M: Molecular weight
A_2: Second virial coefficient
A_3: Third virial coefficient

KC/R_θ parameter is calculated by scattered light and sample concentration.

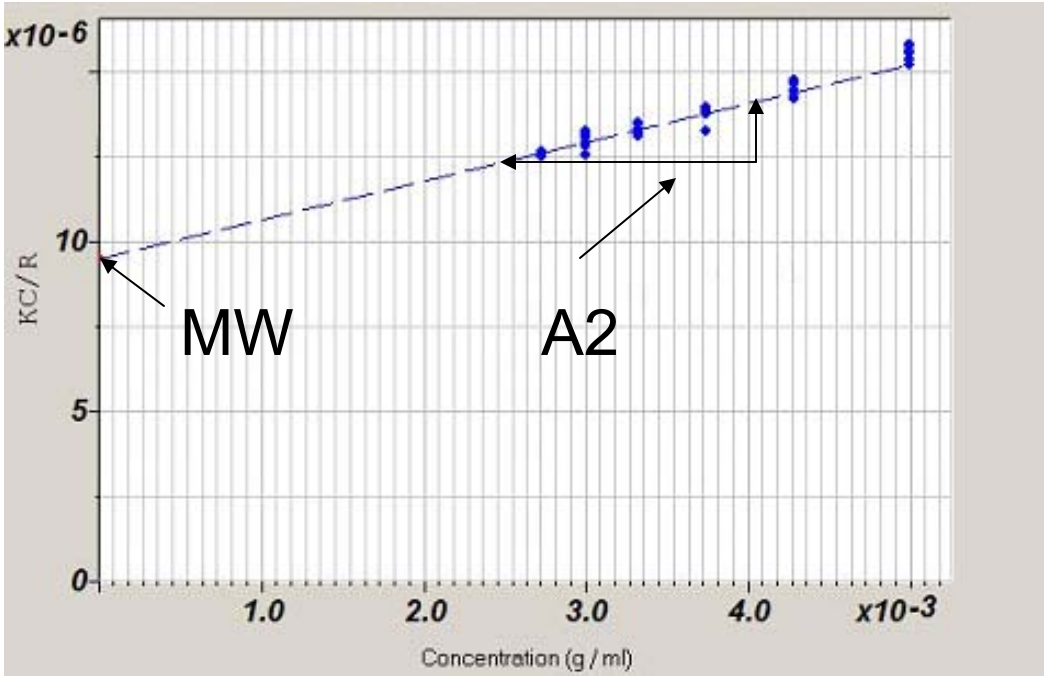
Optical Constant $K = \frac{4\pi^2 n^2}{N_A \lambda_0^4} \left(\frac{\partial n}{\partial c}\right)$

Reduced Scattered light Intensity $R_\theta = \frac{I_\theta r^2}{I_0 V}$

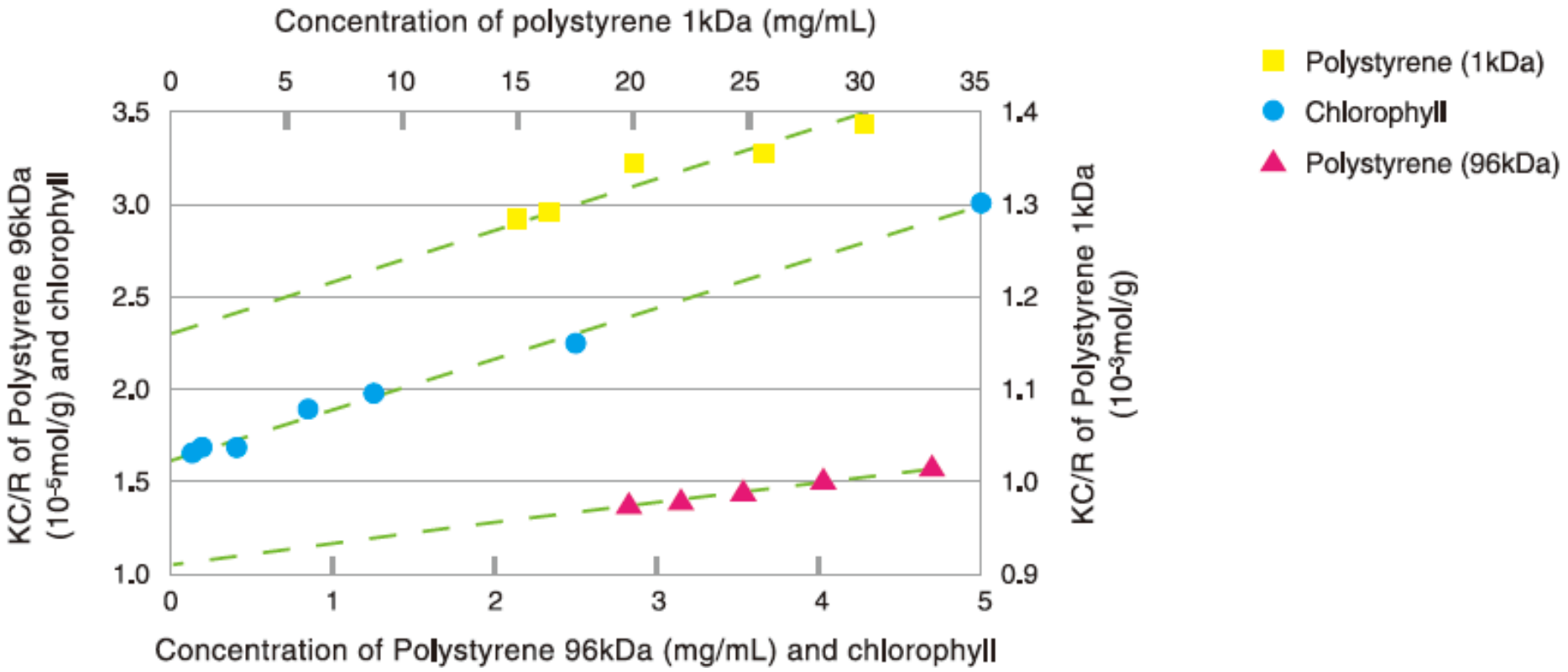
C = Concentration

MW and A2 using One Angle

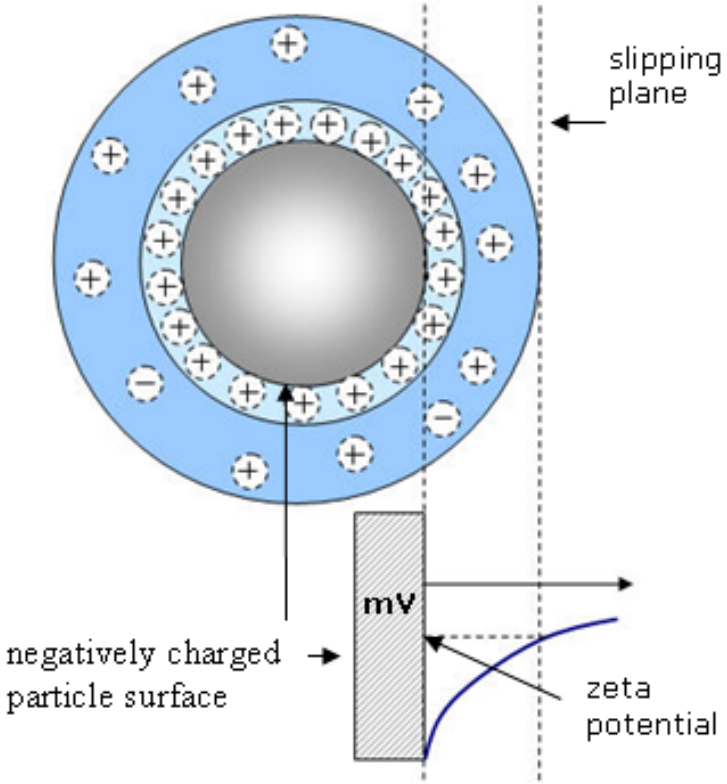
- Light scattering independent of angle at very small sizes (< 60-100 nm)
- Measure several concentrations at one angle to create Debye plot

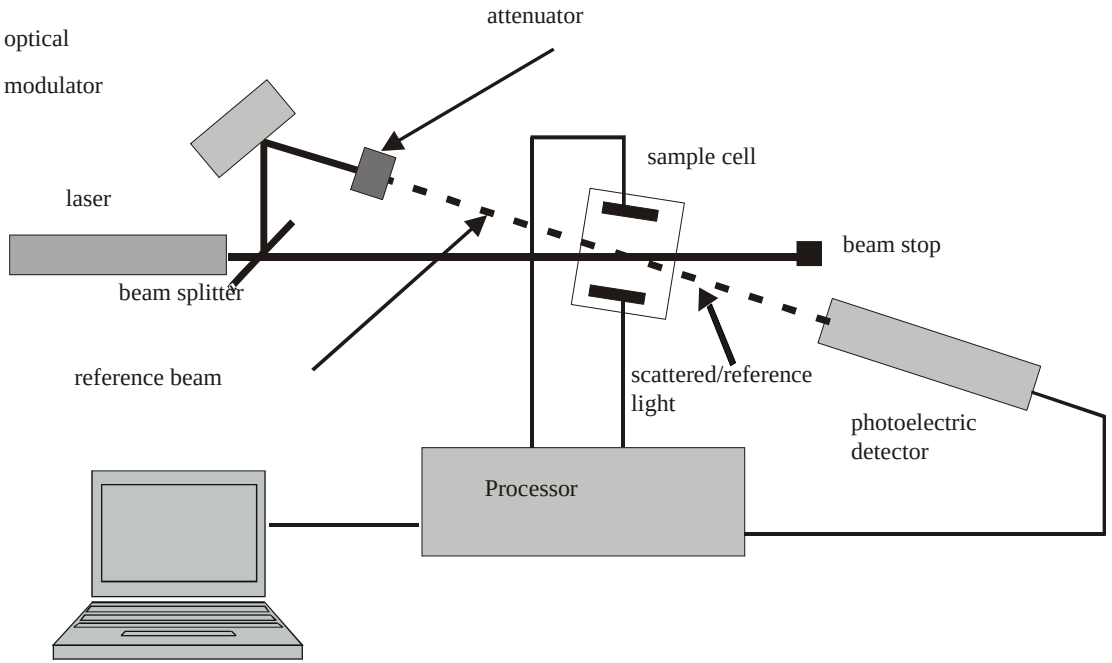


MW Data



Zeta Potential





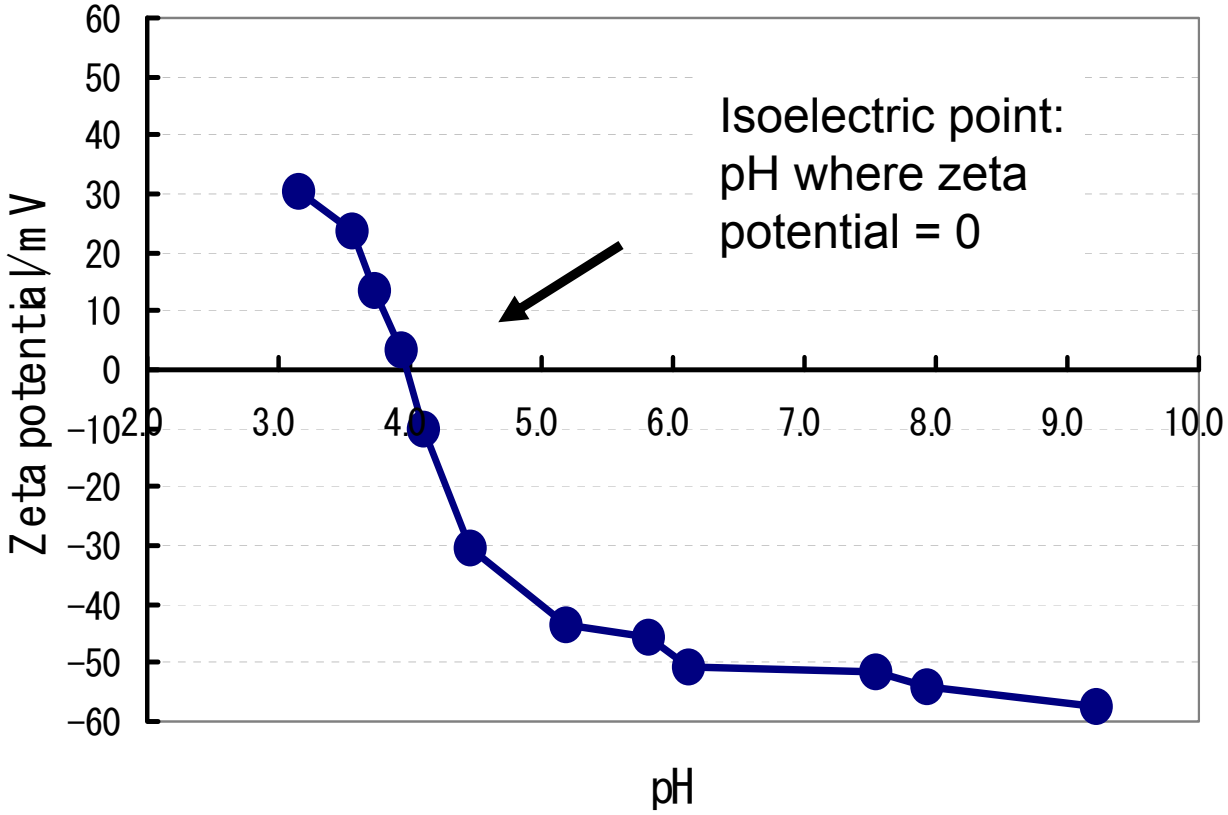
Measure mobility μ

Calculate zeta potential

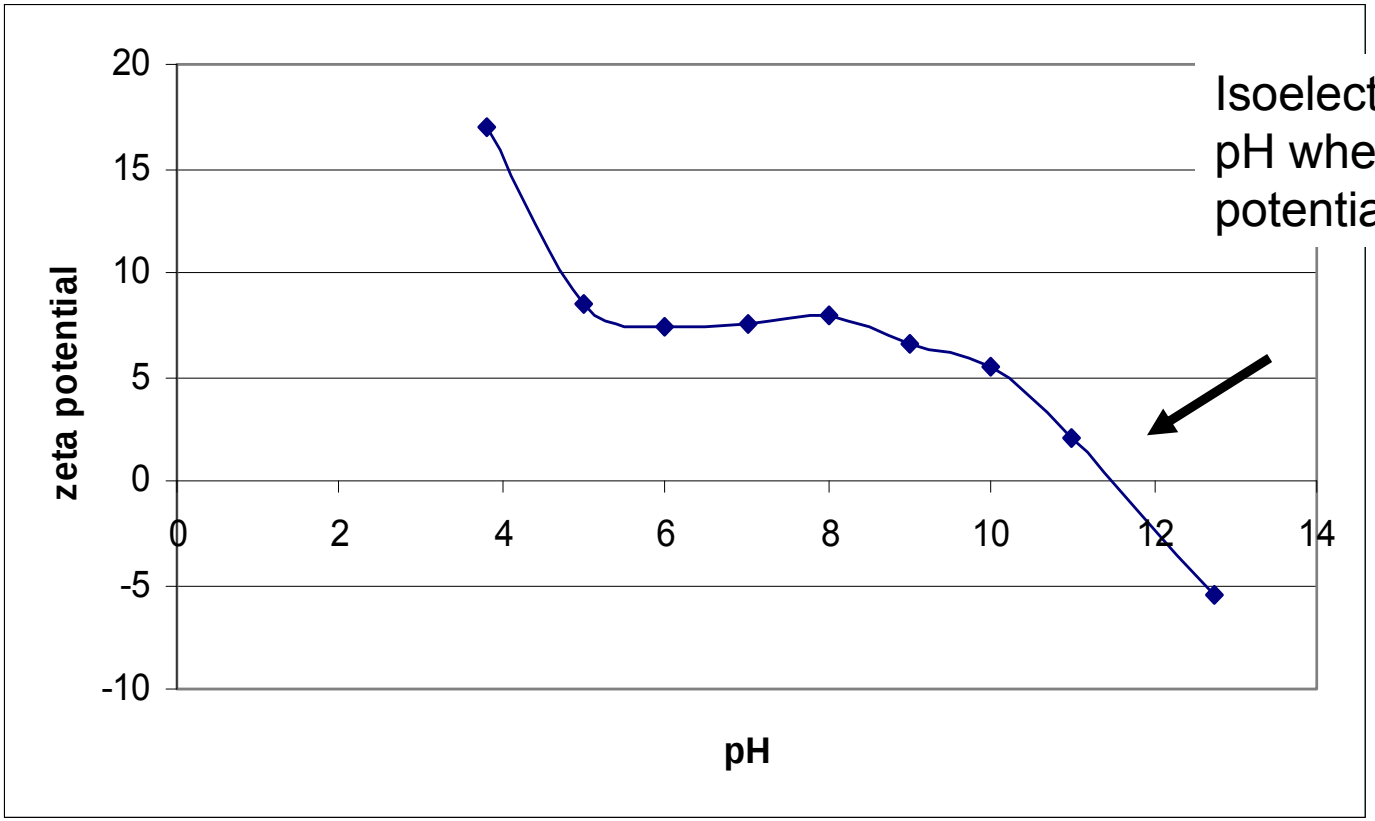
$$\mu = \frac{\Delta\omega\lambda_0}{4\pi nE\sin(\frac{\theta}{2})\sin(\frac{\theta}{2}+\xi)} \qquad \mu = \frac{2\zeta\varepsilon}{3\eta_o} f(\kappa r)$$

HORIBA Scientific

Zeta Potential: Emulsion



Zeta Potential: Lysozyme (4 nm, 10 mg/mL)



Isoelectric point: pH where zeta potential = 0

Summary

- Both laser diffraction (LA-950) and DLS can measure nanoparticles
- Use diffraction when some samples > 1 μm, but all > 30 nm
  - Check lower limit of system used
- Use DLS when all < 1 μm
- DLS + zeta potential + moleculer wt + A2

Acknowledgements

- Microfluidics 

- Elan 

- HORIBA Applications Lab Irvine, CA
- HORIBA Applications Lab, Kyoto, Japan
- HORIBA Applications Lab, Longjumeau, France
- Unnamed (at their request) customers

Tack

La ringrazio

Dank u

Obrigada

Gracias

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감사합니다

謝謝

A dank

谢谢

धन्यवाद

Ngiyabonga

Blagodaria

Thank you

Danke

شكرا

Merci

mark.bumiller@horiba.com

Trugéré

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Shukriya

Dziękuję

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Q&A

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Thank-you