Biophysics Section

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Biophysics Section

Mission

- available technologies
- services provided

Contributions to NIDA projects

- published results
- current projects

Future directions

Mission: quantitative characterization of interactions between biomolecules using in solution biophysical methods

- determination of assembly states of each interacting partner
- measurement of binding affinity
- measurement of kinetics
- determination of stoichiometry
- characterization of thermodynamics of the interaction, i.e. enthalpic and entropic contributions

List of technologies:

- Size Exclusion Chromatography coupled with Light Scattering (SEC/LS)
- Dynamic Light Scattering (DLS)
- Isothermal MicroCalorimeter (ITC)
- Spectrofluorometer
- Stopped-Flow Spectrofluorometer
- Surface Plasmon Resonance (SPR) Sensor [BiaCore Biosensor; T100]
- Composition Gradient Static Light Scattering (CGSLS)
- Asymmetric flow Field-Flow Fractionation (AFFF)

Mission: quantitative characterization of interactions between biomolecules using in solution biophysical methods

- determination of assembly states of each interacting partner (light scattering: static LS and dynamic LS)
- measurement of binding affinity (ITC, SPR, LS, stopped-flow)
- measurement of kinetics (SPR and stopped-flow)
- determination of stoichiometry (SEC-LS; ITC-mass ratio)
- characterization of thermodynamics of the interaction, i.e. enthalpic and entropic contributions (ITC)

Technologies for affinity measurements

	Range of binding affinities			Highlights and Limitations
	k_{on} [$M^{-1}s^{-1}$]	k_{off}	K_d [M]	
ITC	N/A	N/A	10^{-2}-10^{-9} a)	▪no labeling needed ▪native reactant in solution ▪~1.8mL of microM concentration of protein (or macromolecule) for a single titration ▪provides full thermodynamic characterization- enthaplic and entropic contributions ▪provides information about stoichiometry ▪no restriction on molecular weight limit of reactant ▪multiple binding models can be tested , *i.e.* 1:1, 1:2 or sequential binding
SPR	10^{1}-10^{-6}	10^{-2}-10^{-6}	10^{-6}-10^{-11}	▪one reactant needs to be immobilized ▪provides k_{on} and k_{off} in a single experiment ▪does not provide information about stoichiometry ▪models only 1:1 binding mode ▪the molecular weight of the smaller reactant should be at least 200 Da
SEC/SLS/ DLS	N/A	N/A	10^{-3}-10^{-6}	▪~50 micrograms in volume <500 μL ▪provides information about stoichiometry ▪not an equilibrium method ▪provides information about shape from frictional ratio calculated from molecular weight (SLS) and Rh (DLS)
Stopped-flow	$<10^{3}$	Limited by stability of sample and signal	10^{-3}-10^{-8} (depends on the nature of spectroscopic signal)	▪requires spectroscopic signal (absorbance, fluorescence or scatter) ▪no information about stoichiometry ▪relatively large amounts of material needed (a few mLs, 1 mL minimum, of μM concentration for a single round of kinetics)

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- characterization of thermodynamics of the interaction, i.e. enthalpic and entropic contributions (ITC)

Two types of services:

“fee for service”
Submission of samples for analysis by the resource

“open access”
Training of users to operate instrumentation; reservation of instruments’ time via Web-based calendar

Project-specific selection of the appropriate technology, extensive help in: sample preparation, experimental design and execution, data processing, and manuscript submission

Contributions of Biophysics Section to NIDA projects:

Published results:

1. Lee, S.Y., Voronov, S., Letinic, K., Nairn, A.C., Di Paolo, G. and De Camilli, P. (2005) Regulation of the interaction between PIPKI gamma and talin by proline-directed protein kinases. *J Cell Biol.* **168,** 789-799 **(ITC)**

2. Folta-Stogniew, E. (2006) Oligomeric states of proteins determined by size-exclusion chromatography coupled with light scattering, absorbance, and refractive index detectors. In: New and Emerging Proteomics Techniques (Nedelkov, D. and Nelson, R., Eds.), Vol. 328, pp. 97-112. Humana Press, Totowa, NJ **(SEC/LS)**

3. Fogel, A.I., Akins, M.R., Krupp, A.J., Stagi, M., Stein, V. and Biederer, T. (2007) SynCAMs organize synapses through heterophilic adhesion. *J Neurosci.* **27**, 12516-12530. **(SEC/LS)**

4. Erdmann K S, Mao Y., McCrea H. J., Zoncu R., Lee S., Paradise S., Modregger J., Biemesderfer D., Toomre D., and De Camilli P. (2007) A Role of the Lowe Syndrome Protein OCRL in Early Steps of the Endocytic Pathway, *Developmental Cell* **13**: 377-390 **(ITC)**

5. Lauren,J.. David A. Gimbel, Haakon Nygaard, John Gilbert and Stephen M. Strittmatter, (2008) [XXX] mediates Impairment of Synaptic Plasticity by Amyloid-beta Oligomers. Submitted. **(SEC/LS)**

Current projects:

1. Mim, K., Unger, V., De Camilli, P. Fractionation and Characterization of Large Liposomes **(AFFF/LS)**

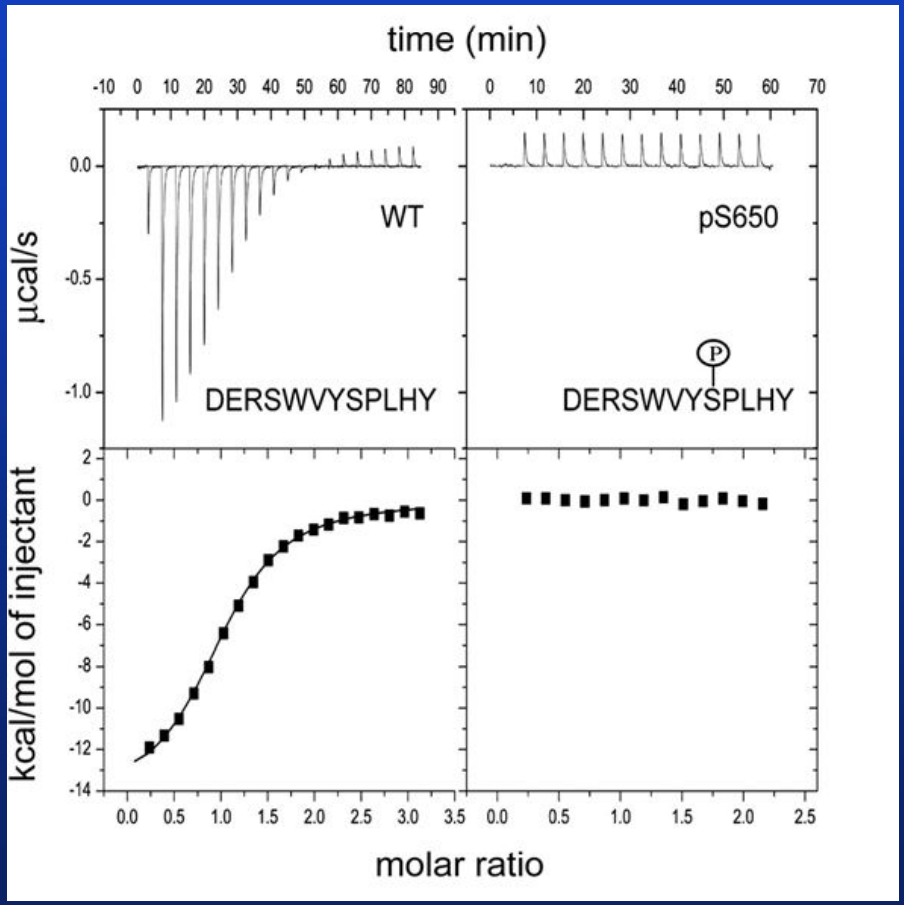
2. Pirruccello, M., De Camilli, P. Oligomeric state of Dynamin, Amphiphysin, Endophilin, dynamin/amphiphysin rings in solution **(SEC/LS; AFFF/LS)**

3. Andrade, E., Nairn, A: Regulation of protein phosphatase-2A (PP2A) by cAMP-regulated phosphoprotein-16/19kDa (ARPP-16/19) through direct interaction with the scaffolding A subunit (PR65) **(SPR)**

4. Brown, R., . Strittmatter, SM: Quantitative characterization of Interaction of Nogo Receptor with different myelin-derived ligands: Nogo, MAG and OMgp; 70 point-mutation mutants of NgR and 10 different ligands **(SPR)**

Effects of PIPKIγ90 Phosphorylation on Its Interaction with Talin; Measurement of Binding Affinity Using Isothermal Microcalorimetry (ITC)

NIDA Investigators: Pietro DeCamilli, M.D.; Angus Nairn , Ph.D.



Cdk5 phosphorylation of phosphatidylinositol-(4)-phosphate 5-kinase type Iγ (PIPKIγ90) inhibits its interaction with talin in vivo.

ITC analysis of the binding of 12-mer WT and pS650 peptides from the 28-aa tail of PIPKIγ90 to GST-talin head. Raw data as a function of time are shown in the top panels, and plots of the total heat released as a function of the molar ratio of each ligand are shown in the bottom panels. The continuous line in the bottom panels represents the nonlinear, least-squares best fits to the experimental data using a one-site model of binding. Note the roughly 1:1 stoichiometry indicated by the half-height point of the sigmoidal curve

Lee SY, Voronov S, Letinic K, Nairn AC, Di Paolo G, and De Camilli P. 2005. Regulation of the interaction between PIPKI(gamma) and talin by proline-directed protein kinases. J. Cell Biol. 168: 789-799.

Biophysical Characterization of Amyloid-beta Oligomers;

NIDA Investigator: Stephen M. Strittmatter, M.D.

Characterization of the oligomeric state of Amyloid-beta peptide in solution (SEC/LS)

Sample: A beta (Aβ; 1–42) , MW = 4.5 kDa, aged overnight in F12 media

Technology used: **SEC/LS**

Three size exclusion chromatography (SEC) columns in tandem:

Superdex S-200+Superdex-75+Superdex-peptide;
fractionation range: 100 Da to ~ 1 MDa

Detection: Static (multiangle) and dynamic LS
UV/Vis
Refractometry

Buffer condition: F12 media

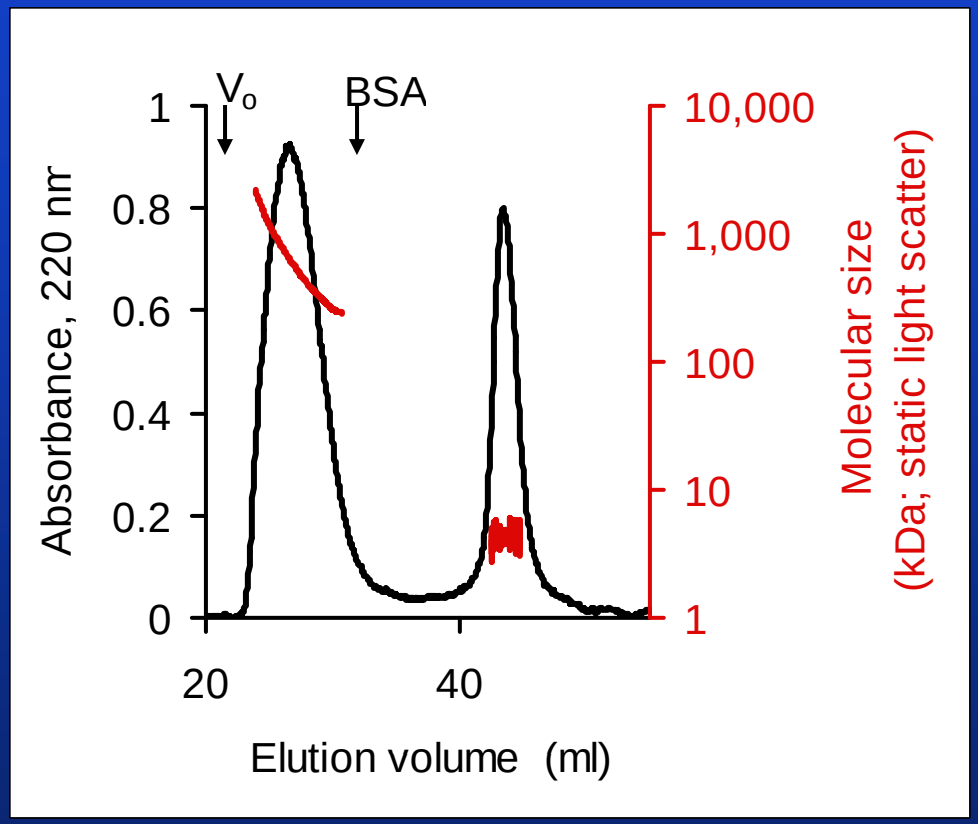
Amyloid-beta peptide: A beta (Aβ; 1–42) , MW = 4.5 kDa

Peak #1

High MW Oligomers

Peak #2

Low MW fraction

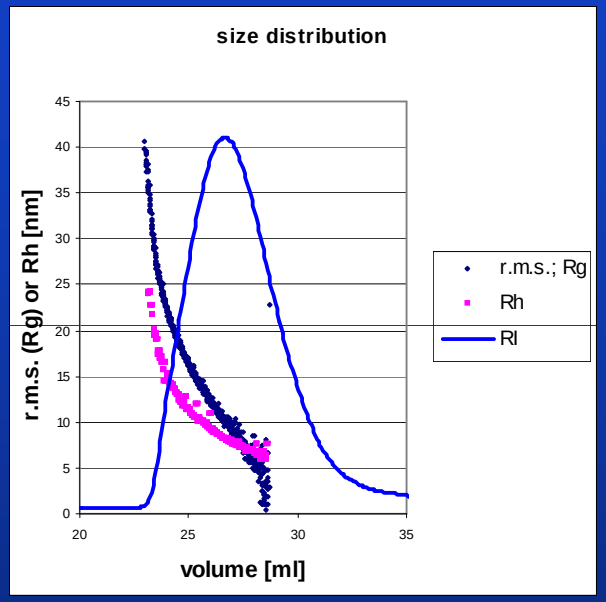


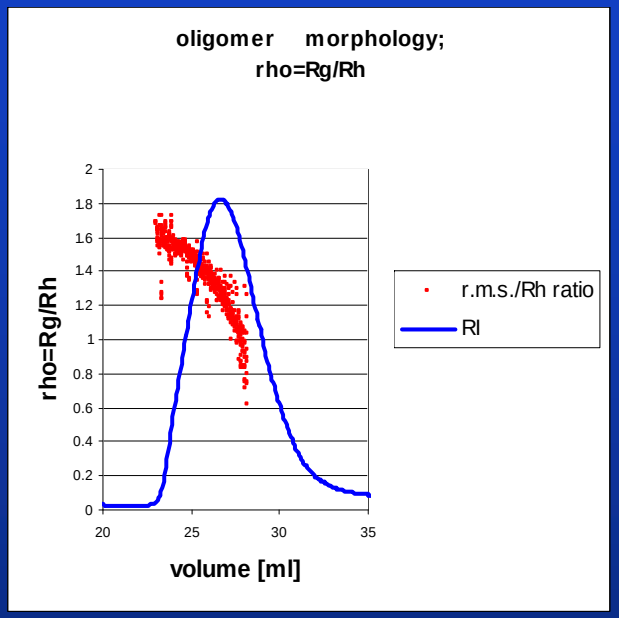
Population	**fraction of total mass**	**M_w (kDa) (weight-average)**	**range of M_w (kDa)**	**# of monomers**	**R_h (nm) DLS**	**r.m.s.; R_g (nm) SLS**
Peak #1	68%	690	250-2,000	~55-200	12 (6-25)	16 (10-40)
Intermediate	3%	180	4-250	1-55	N/D	N/D
Peak #2	29%	4.2	4-5	1	N/D	N/D

Amyloid-beta peptide: A beta (Aβ; 1–42) , MW = 4.5 kDa

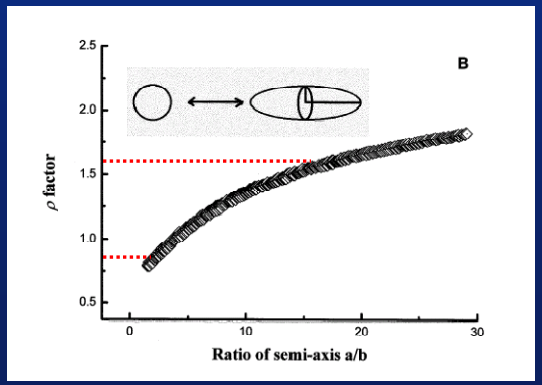
Peak #1

High MW Oligomers





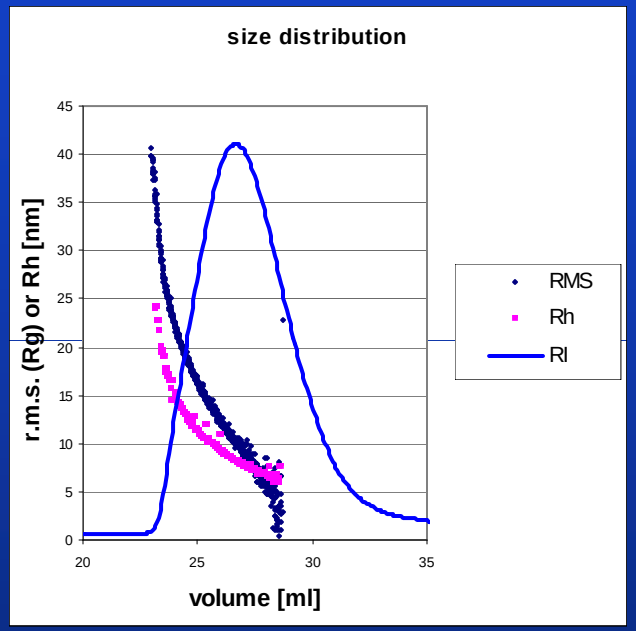
Population	range of M_w (kDa)	# of monomers	R_h (nm) DLS	r.m.s.; R_g (nm) SLS
Peak #1	250-2,000	~55-200	12 (6-25)	16 (10-40)

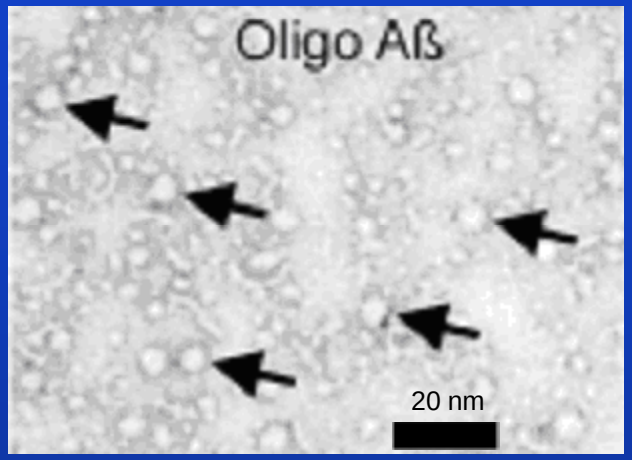


Amyloid-beta peptide: A beta (Aβ; 1–42) , MW = 4.5 kDa

Peak #1

High MW Oligomers

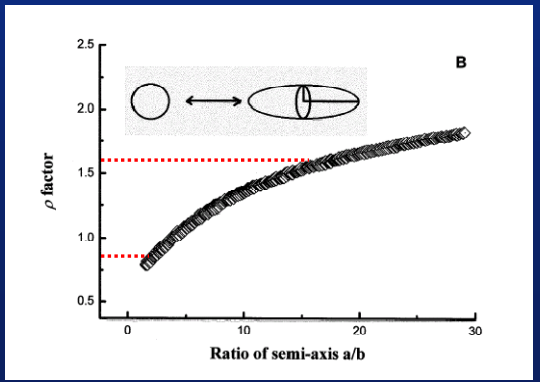




transmission electron microscopy with negative staining

spheres similar to those reported previously for ADDLs and short flexible fibrils of ~3-4 nm diameter and between ~10-20 nm in length.

Population	range of M_w (kDa)	# of monomers	R_h (nm) DLS	r.m.s.; R_g (nm) SLS
Peak #1	250-2,000	~55-200	6-25	10-40



Fractionation of Large Liposomes (diameter greater than 400 nm);

NIDA Investigator: Pietro DeCamilli, M.D.

Goal: get homogeneous population of 400 nm, 600 nm, ... liposomes

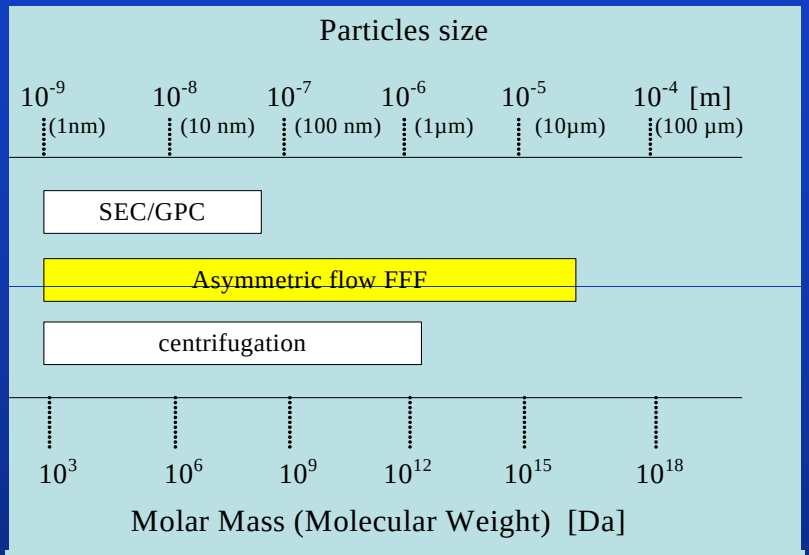
Technology used: **AFFF/LS**

Asymmetric Flow Field-Flow-Fractionation system

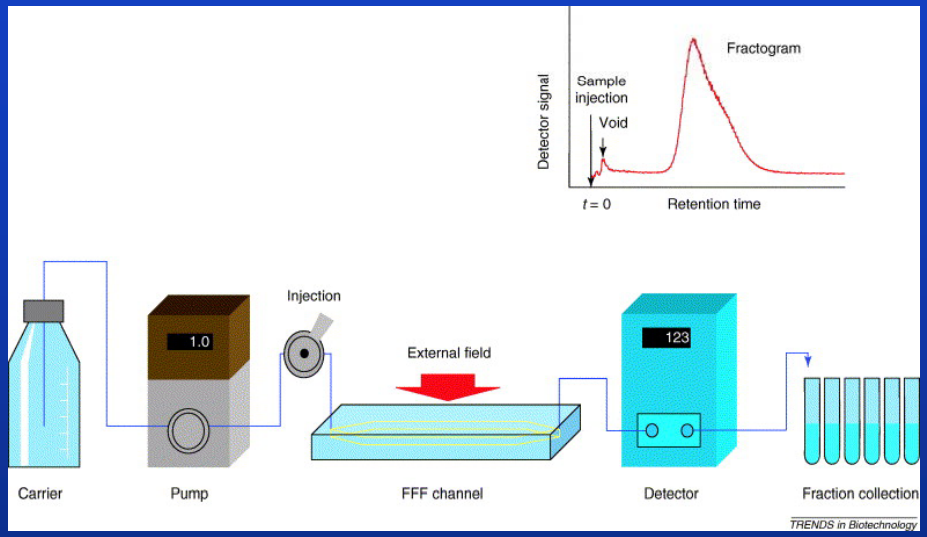
Detection:	Static (multiangle) and dynamic LS UV/Vis Refractometry
Buffer condition:	50 mM K-Aspartate, 10 mM Tris, 1 mM EGTA, pH 7.5

What is Asymmetric Flow Field-Flow-Fractionation (AFFF) ?

a flow-assisted, chromatography-like analytical technique that allows separation of polymers (biopolymers) and particles ranging in size from ~ 1nm to >20 μm



Separation range of different analytical methods compared to AFFF.



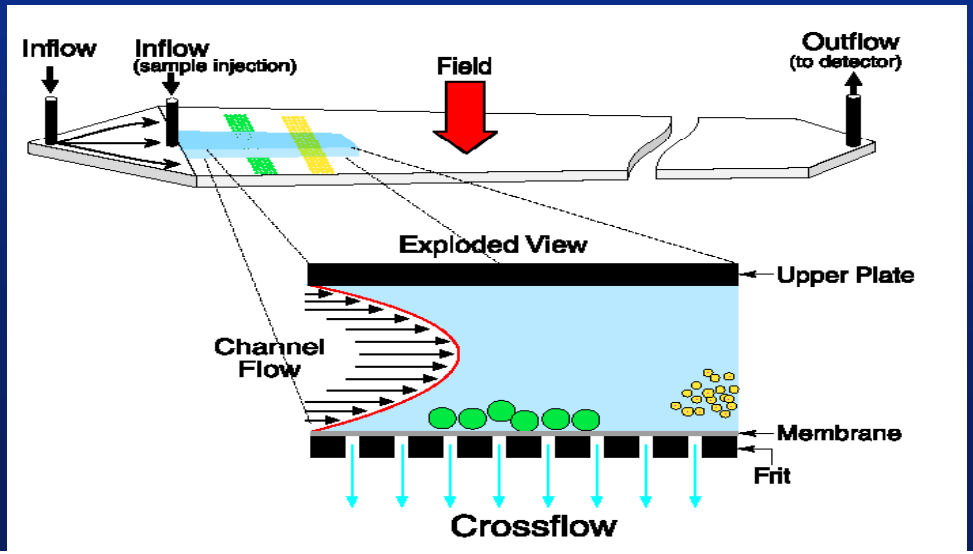
$$t_R = \frac{w^2}{6D} \cdot \frac{V_c}{V^o}$$

w is the channel height,

Vc is the cross-flow,

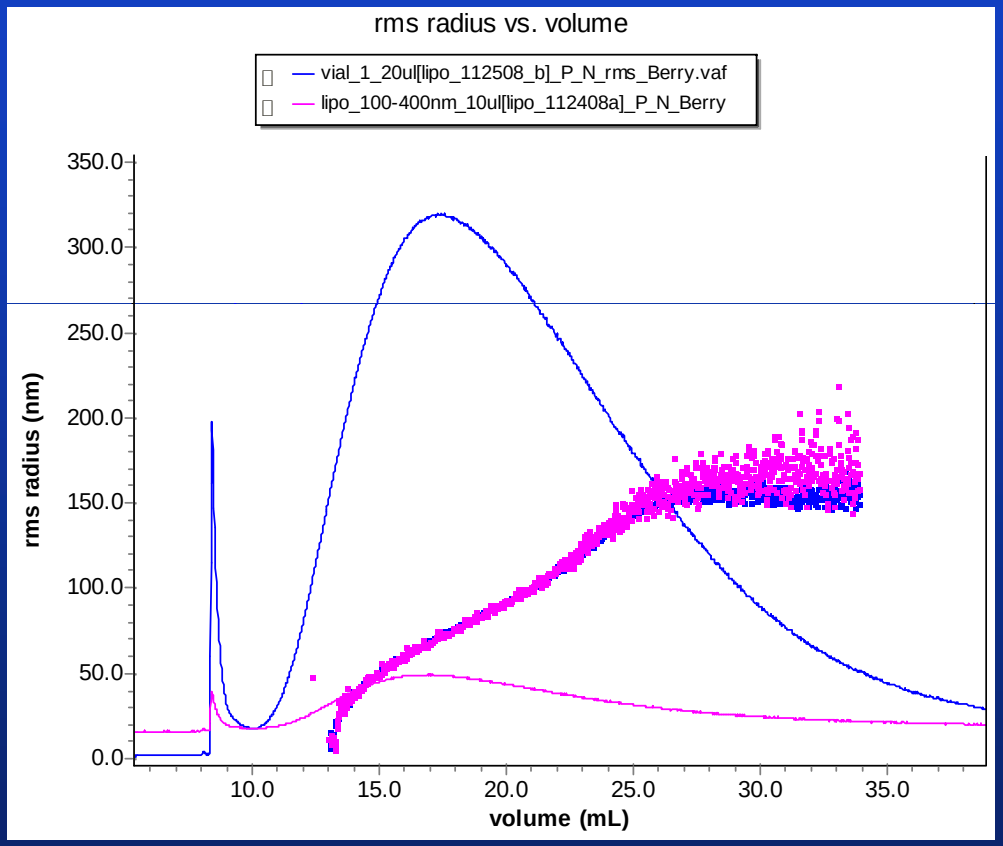
V^o is the channel outlet flow

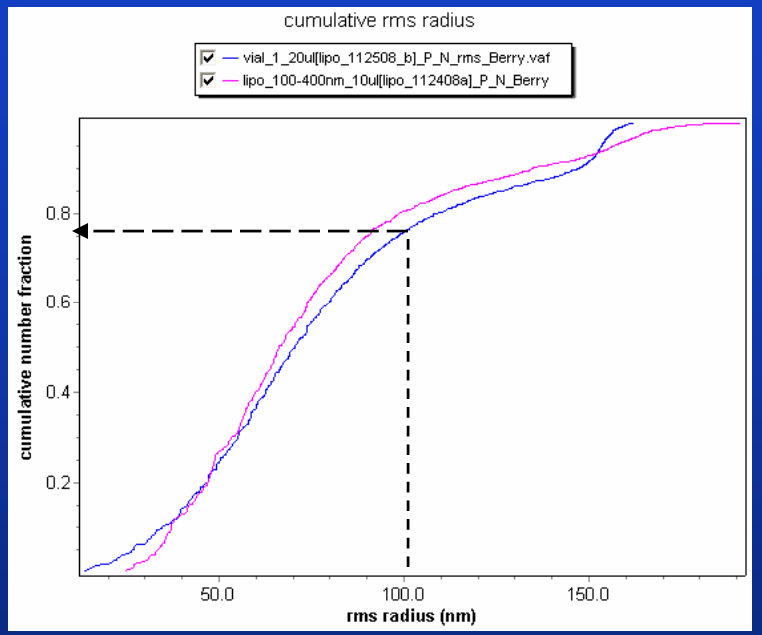
D is the diffusion coefficient



AFFF Fractionation of Large Liposomes

Sample 1: 200 nm extruded liposomes; relatively homogemeous preparation



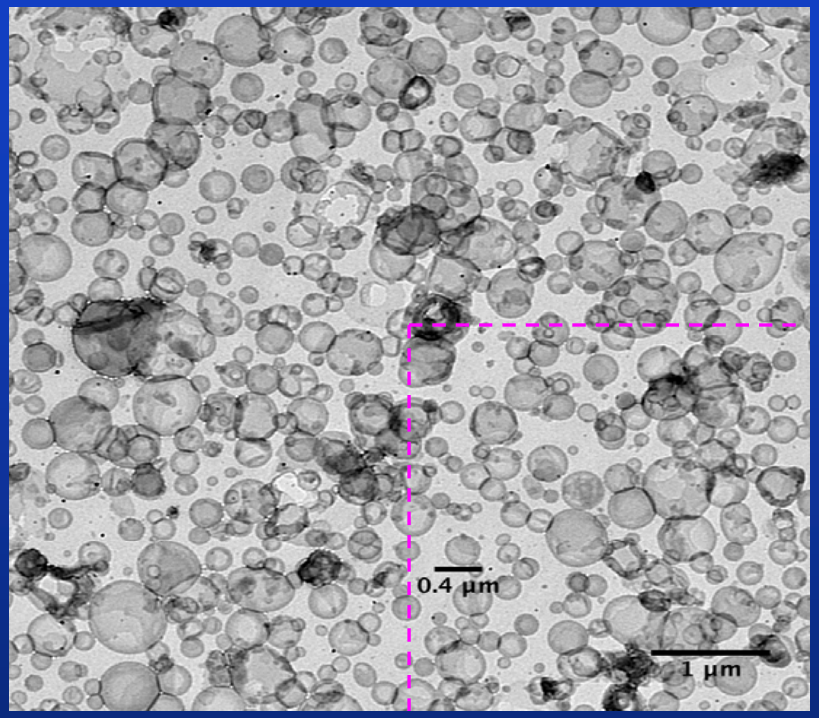


For hollow spheres (like liposomes)

r.m.s. (Rg) –derived from angular dependence of scattered light- corresponds to the geometric radius

AFFF Fractionation of Large Liposomes

Sample 1: 200 nm extruded liposomes; relatively homogemeous preparation



CryoEM image of Sample 1

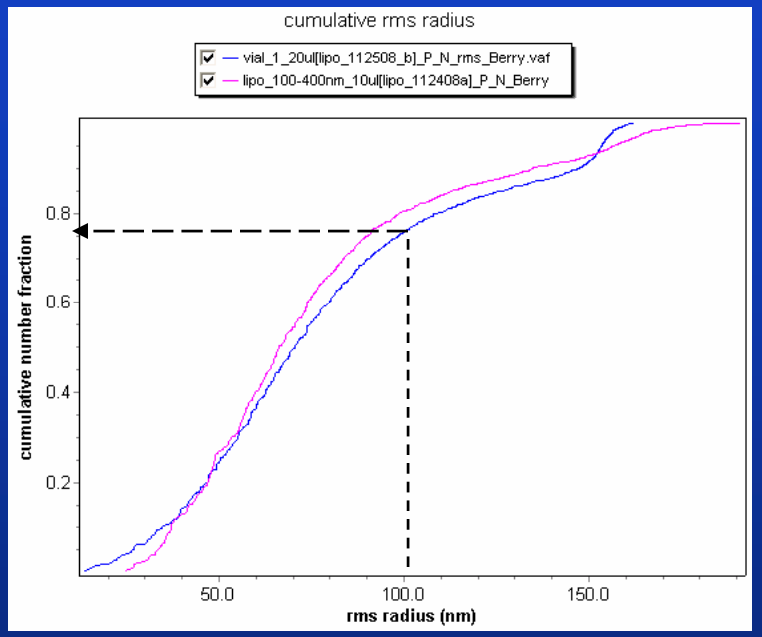
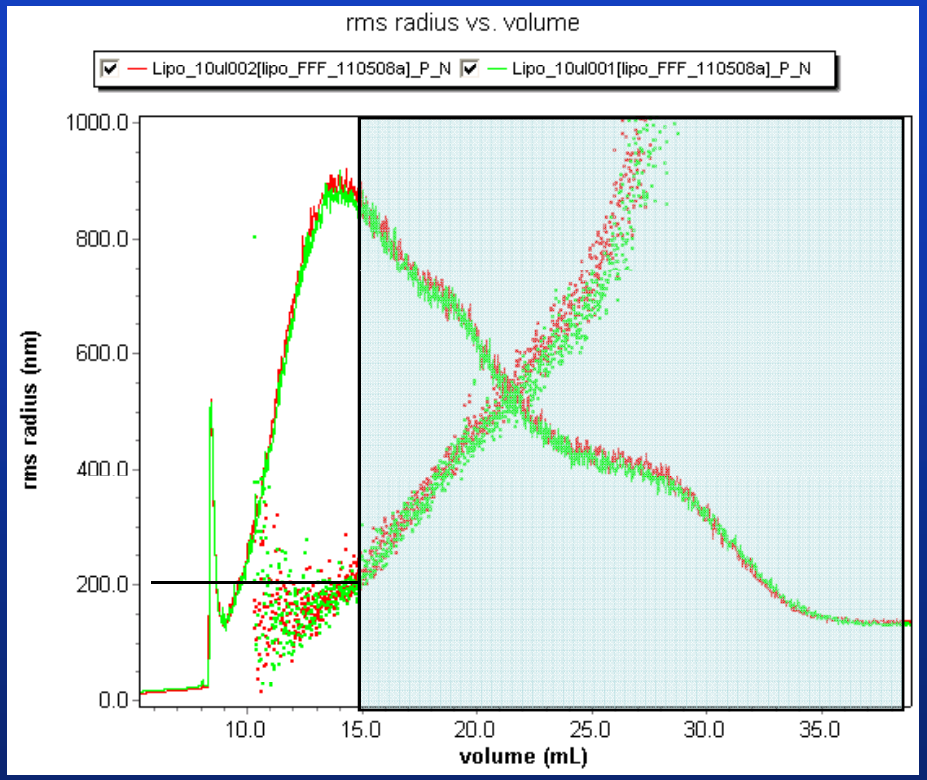


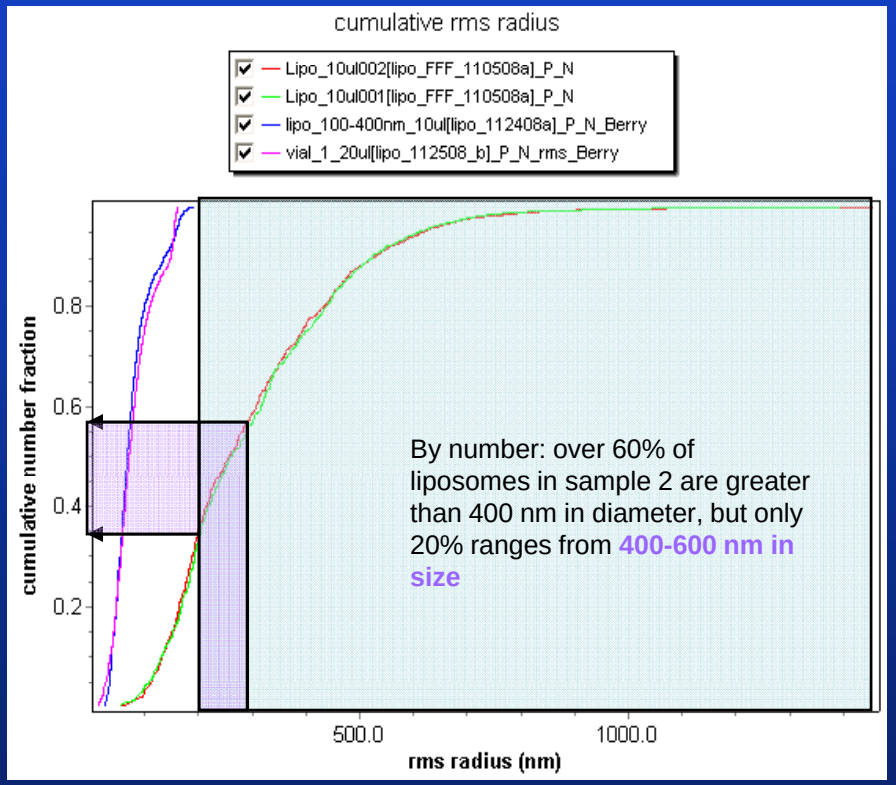
Image analyzed	liposomes larger than 100 nm radius (200 nm diameter)	number fraction
sector #1	54/180	23.1%
sector #2	33/111	22.9%

AFFF Fractionation of Large Liposomes

Sample 2: sonicated liposomes;

non-homogemeous preparation





Differences in Sample 1 and Sample 2

For hollow spheres (like liposomes)

r.m.s. (Rg) corresponds to the geometric radius

Contributions of Biophysics Section to NIDA projects:

Current projects:

1. *Mim, K., Unger, V., De Camilli, P. Fractionation and Characterization of Large Liposomes* ***(AFFF/LS)***
2. Pirruccello, M., De Camilli, P. Oligomeric state of Dynamin, Amphiphysin, Endophilin, dynamin/amphiphysin rings in solution ***(SEC/LS; AFFF/LS)***
3. Andrade, E., Nairn, A. : Regulation of protein phosphatase-2A (PP2A) by cAMP-regulated phosphoprotein-16/19kDa (ARPP-16/19) through direct interaction with the scaffolding A subunit (PR65) ***(SPR)***
4. Brown, R., . Strittmatter, SM: Quantitative characterization of Interaction of Nogo Receptor with different myelin-derived ligands: Nogo, MAG and OMgp; 70 point-mutation mutants of NgR and 10 different ligands ***(SPR)***

Future directions:

Implementation and method development for preparative mode AFFF for liposome fractionation (project #1)

Development of AFFF methods for proteins' analysis (project #2)

Implementation of the new BiaCore T100 system (project #3 and #4)

Thank you

Additional information at:

http://info.med.yale.edu/wmkeck/biophysics/