

Particle Size Analyses of Polydisperse Liposome Formulations with Multispectral Nanoparticle Tracking Analysis

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October 16, 2019



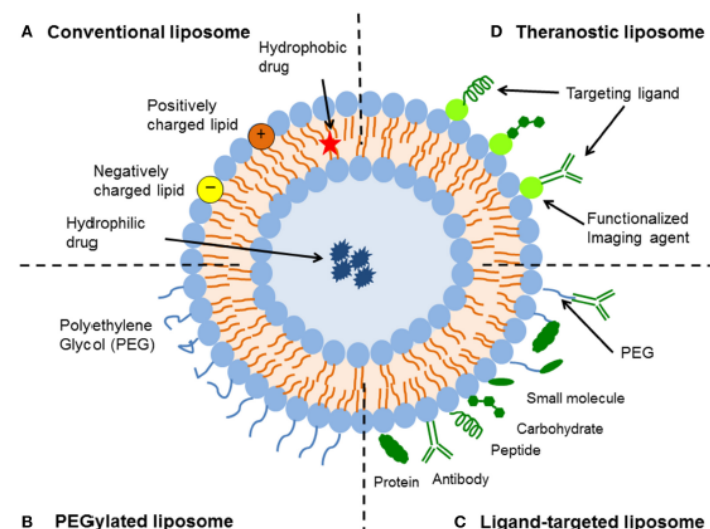
Outline

- Liposomes and their application in medicine
- Liposome manufacturing process
- Significance of particle size distribution for liposomes
- Challenges in particle size characterization

Liposomes in Medicine

TABLE 1 | Marketed liposomal-based therapeutics and products in clinical development.

Drug	Disease	Status	Type of liposomal-based delivery system	Source(s)
Paclitaxel LEP-ETU	Advanced triple-negative breast cancer	Phase I/II	siRNA	Zhang et al., 2005; Immordino et al., 2006
siRNA	Ovarian cancer	Phase I	DOPC neutral liposomes	Mangala et al., 2009
Paclitaxel EndoTAG-1	Advanced triple-negative breast cancer	Phase II	Cationic	Chang and Yeh, 2012; Awada et al., 2014
Paclitaxel EndoTAG-1	Pancreatic cancer	Phase II	Cationic	Löhr et al., 2012
Mitoxantrone LEM-ETU	Acute myeloid leukemia, multiple sclerosis, and prostate cancer	Phase I	Cationic	Immordino et al., 2006; Chang and Yeh, 2012
Verteporfin	Molecular degeneration	FDA Approved in 2000	Cationic	Chang and Yeh, 2012; Allen and Cullis, 2013; Gross et al., 2013
Amikacin	Lung infection	Phase II/III	Conventional	Chang and Yeh, 2012; Clancy et al., 2013; Olivier et al., 2014
Vincristine	Non-Hodgkin lymphoma	FDA Approved in 2012	Conventional	Allen and Cullis, 2013; Wang et al., 2015
Tretinoin	Acute promyelocytic leukemia and hormone-refractory prostate cancer	Phase II	Conventional	Ozpolat et al., 2003; Immordino et al., 2006
Irinotecan SN-38	Metastatic colorectal cancer	Phase I/II	Conventional	Zhang et al., 2004; Suenaga et al., 2015
Annamycin	Acute lymphoblastic leukemia	Phase I/II	Conventional	Wetzler et al., 2013
Amphotericin B	Anti-fungal prophylaxis	FDA approved in 1997	Conventional	Chandrasekar, 2008; Allen and Cullis, 2013
D Daunorubicin	Leukemia and solid tumors	FDA Approved in 1996	Conventional	Chang and Yeh, 2012; Allen and Cullis, 2013
Cytarabine or cytosine arabinoside	Neoplastic meningitis and lymphomatous meningitis	FDA Approved	Conventional	Chang and Yeh, 2012; Jahn et al., 2015
Morphine sulfate	Pain Management	FDA Approved in 2004	Conventional	Chang and Yeh, 2012; Allen and Cullis, 2013
Lurtotecan	Ovarian cancer, head, and neck cancer	Phase I/II	Conventional	Dark et al., 2005; Chang and Yeh, 2012
Vinorelbine	Newly diagnosed or relapsed solid tumors	Phase I	Conventional	Allen and Cullis, 2013
Topotecan	Advanced solid tumors	Phase I/II	Conventional	Seiden et al., 2004; Allen and Cullis, 2013
Nystatin	Fungal Infections	Phase I/II	Conventional	Offner et al., 2004
Doxorubicin	Leukemia, breast cancer, bone cancer, lung cancer, brain cancer	FDA Approved in 1995	PEGylated	Ning et al., 2007
Doxorubicin and bortezomib	Relapsed or refractory multiple myeloma	FDA Approved in 2007	PEGylated	Ning et al., 2007
Thermosensitive doxorubicin	Liver tumors	Phase III	PEGylated	Yarmolenko et al., 2010
Thermosensitive doxorubicin	Chest wall recurrences of breast cancer	Phase I	PEGylated	Yarmolenko et al., 2010
Irinotecan	Advanced refractory solid tumors and colorectal cancer	Phase I	PEGylated	Chang et al., 2015
Camptothecin analog	Ovarian cancer	Phase I	PEGylated	Zamboni et al., 2009



➤ Heavily being used in pharmaceutical industry as drug delivery system and vaccine

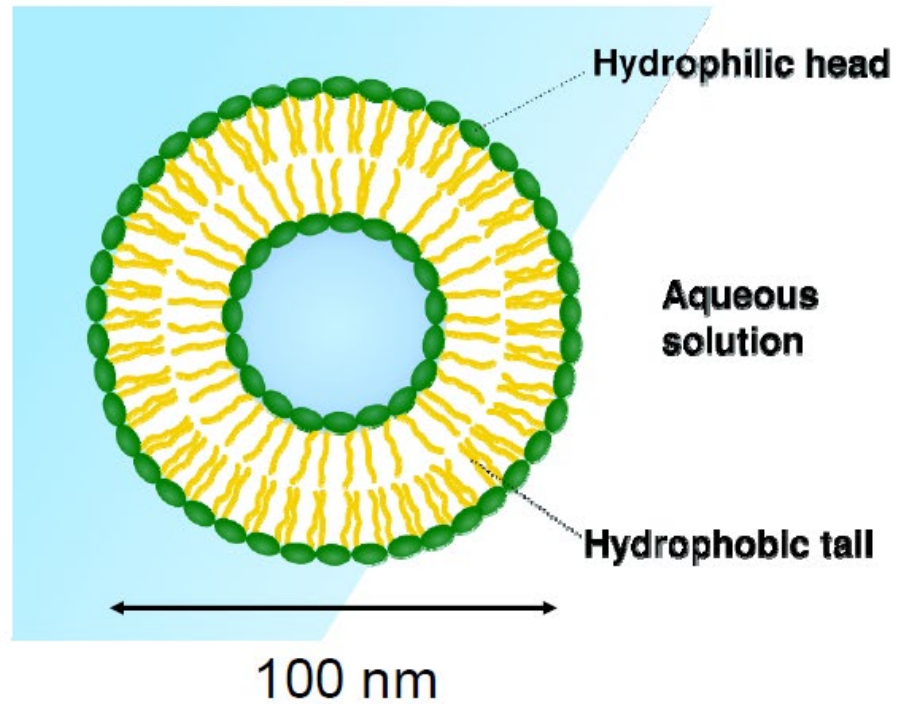
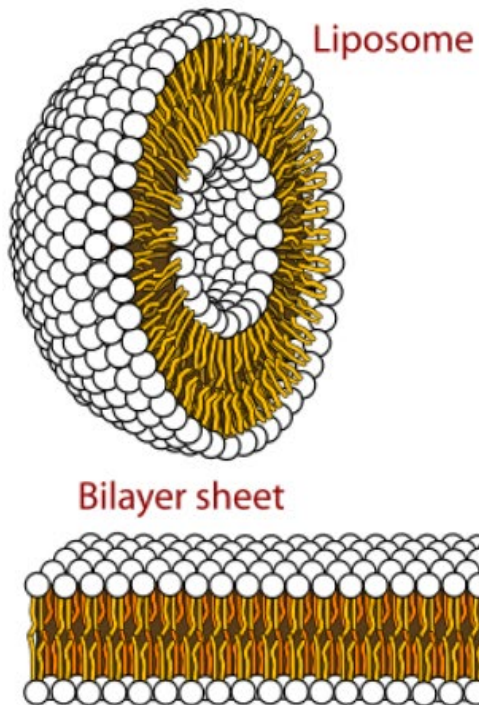
Sercombe et al., *Front. Pharmacol.* 2015, 6: 286

Liposomes in Medicine

- Specialized delivery vehicles that mimic biological membranes
- Shield a drug from degradation and increase its lifespan in circulation
- Possess the ability of encapsulation of hydrophilic drug as well as capability of solubilizing lipophilic drug

Modulate **pharmacokinetics and biodistribution** of drug to minimize side effects

Liposome



Liposome Manufacturing Process

- Drying of Lipids
- Lipid Hydration
- Downsizing of Lipid Vesicles

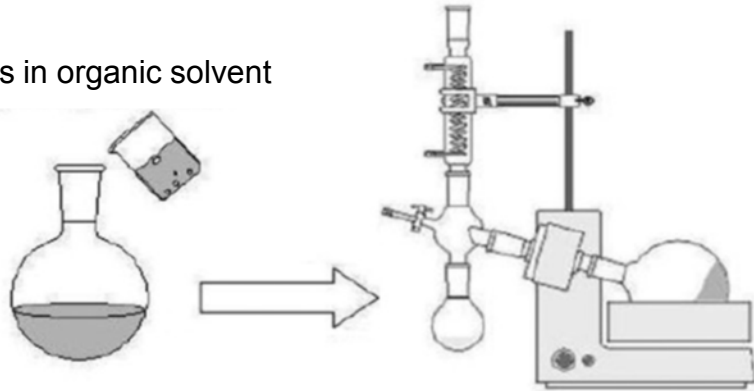
Liposome Manufacturing Process

Lipids in organic solvent



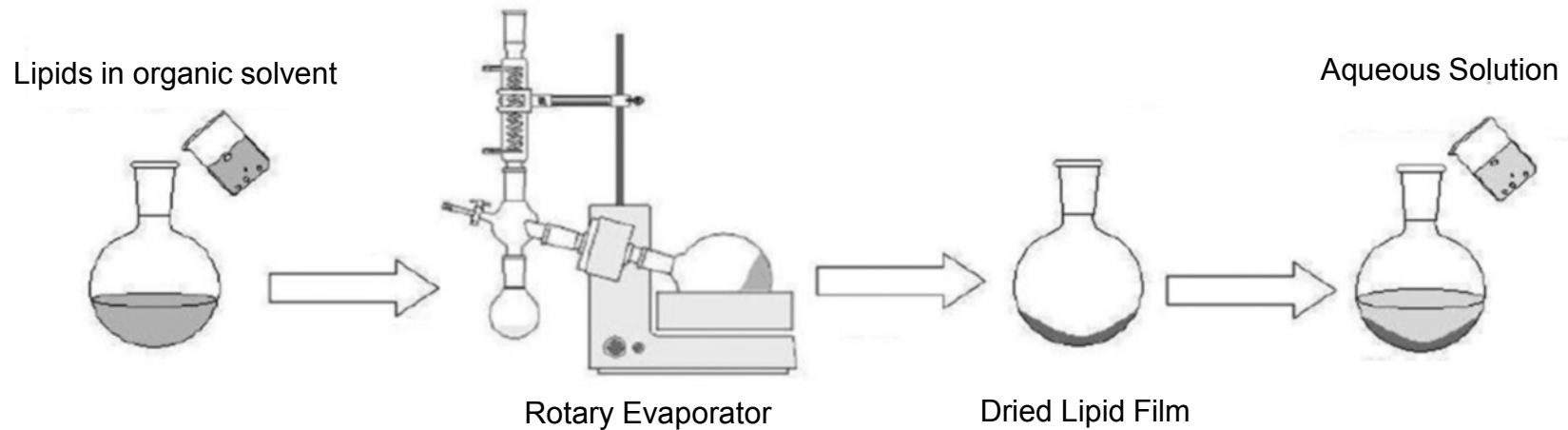
Liposome Manufacturing Process

Lipids in organic solvent

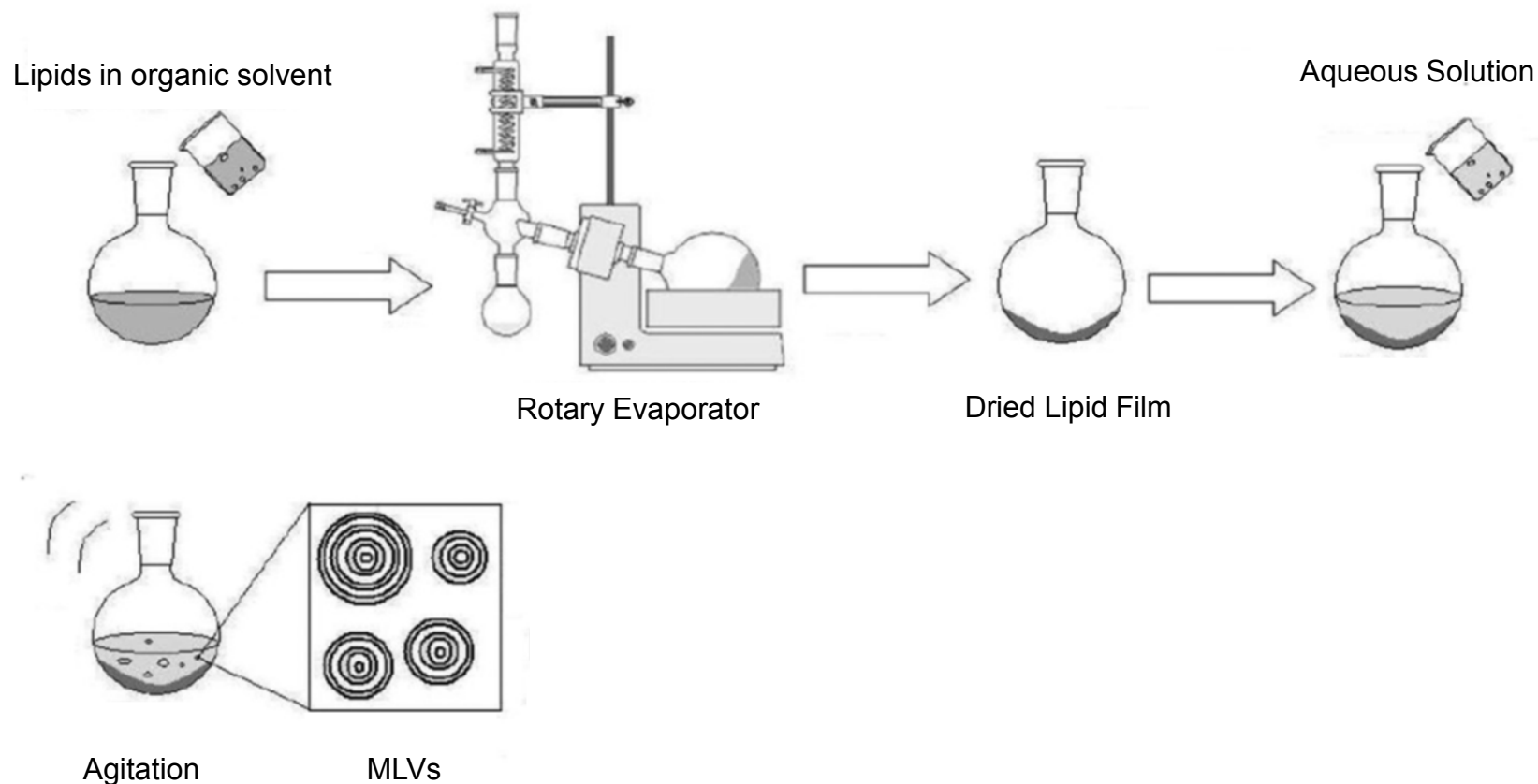


Rotary Evaporator

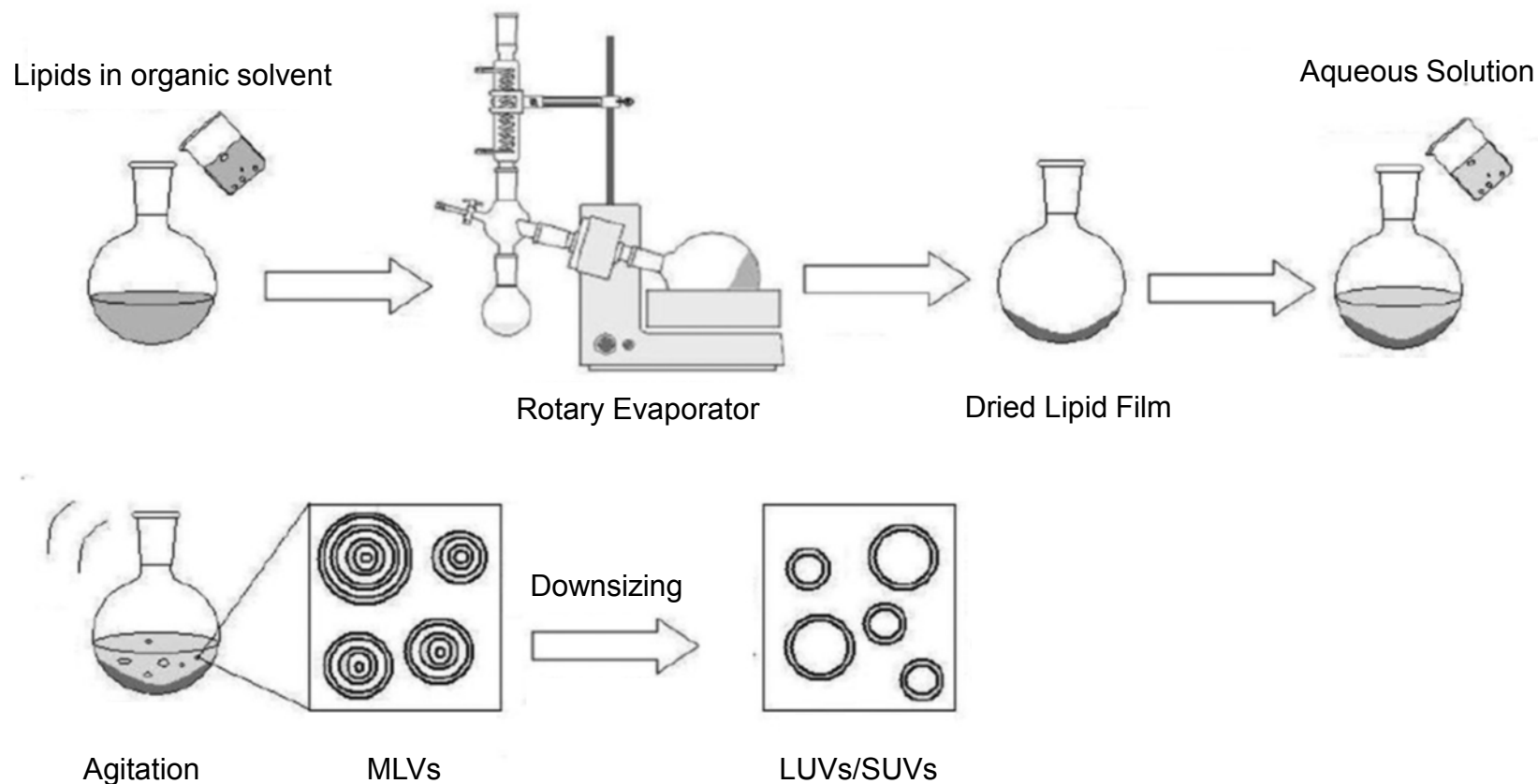
Liposome Manufacturing Process



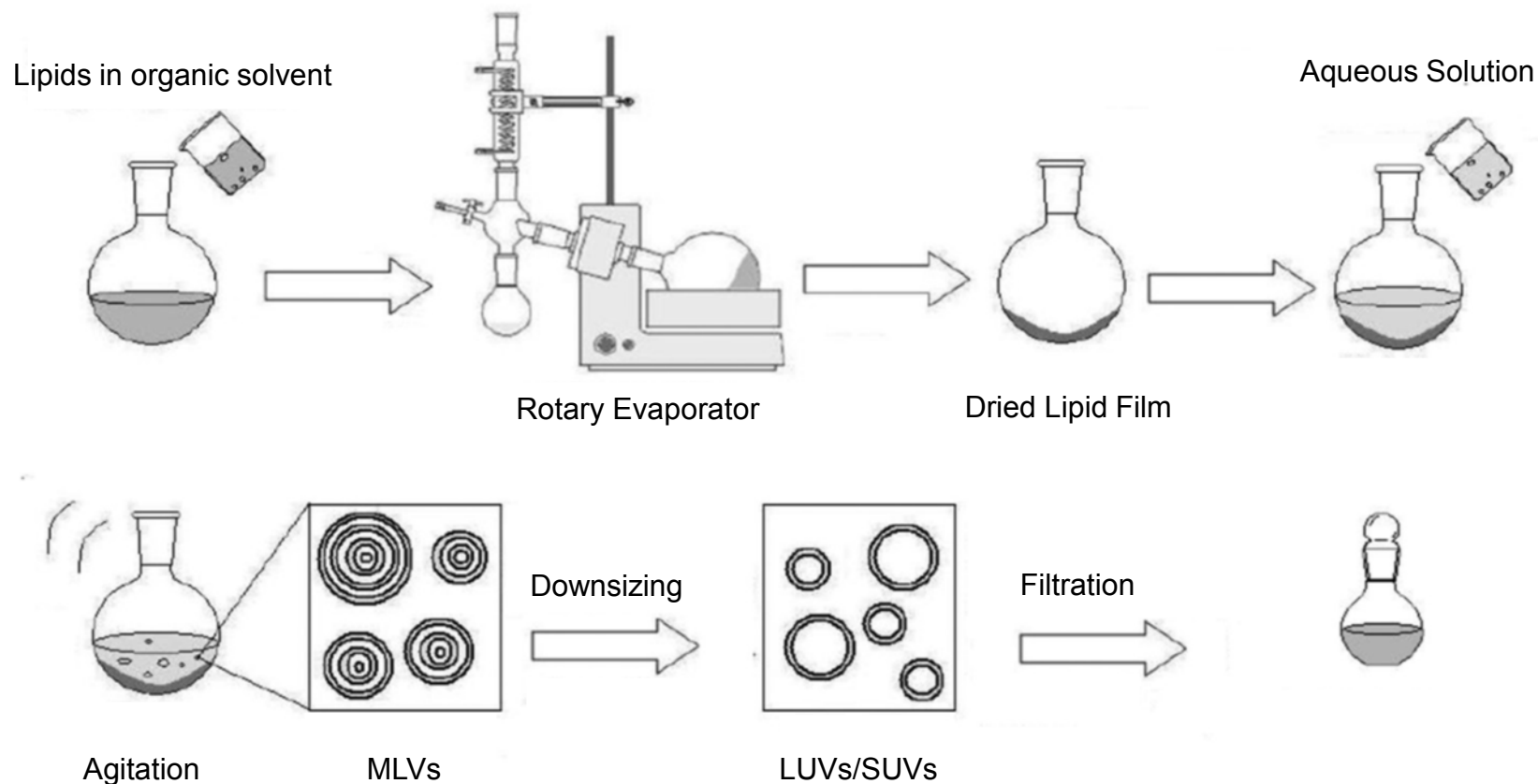
Liposome Manufacturing Process



Liposome Manufacturing Process



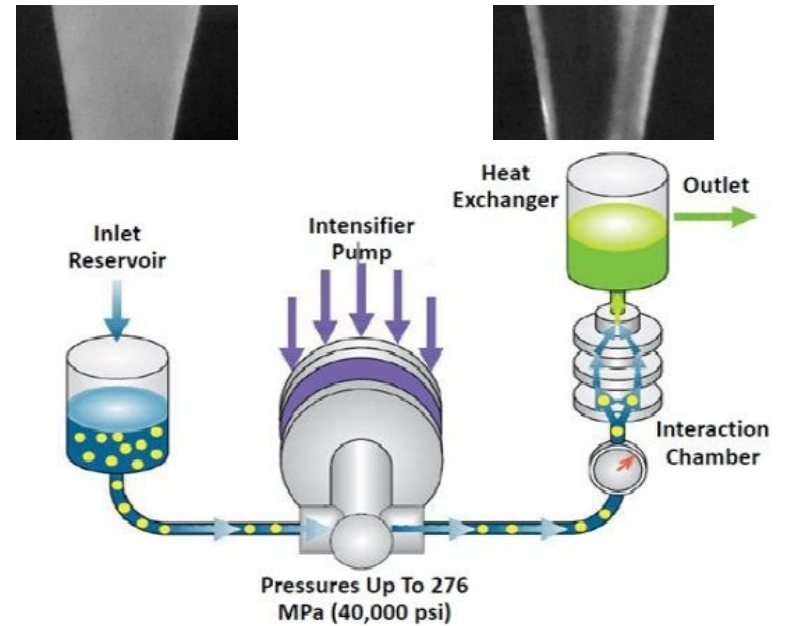
Liposome Manufacturing Process



Downsizing Methods

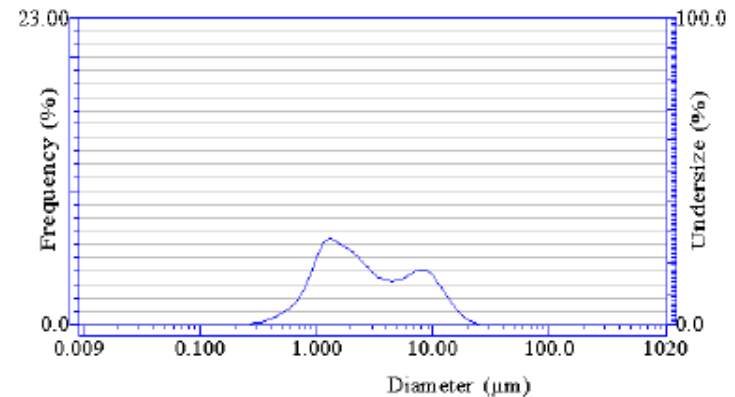
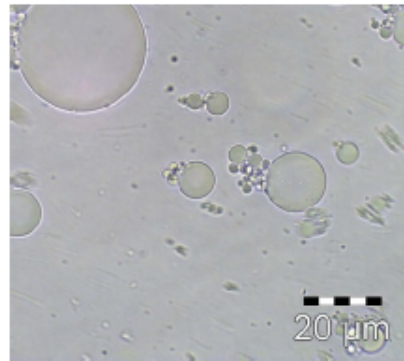
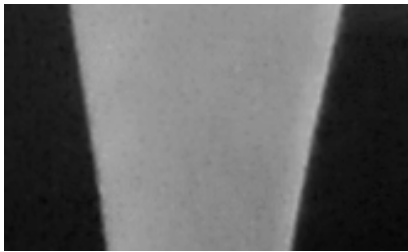
- Sonication
- Extrusion
- Microfluidization

Microfluidization

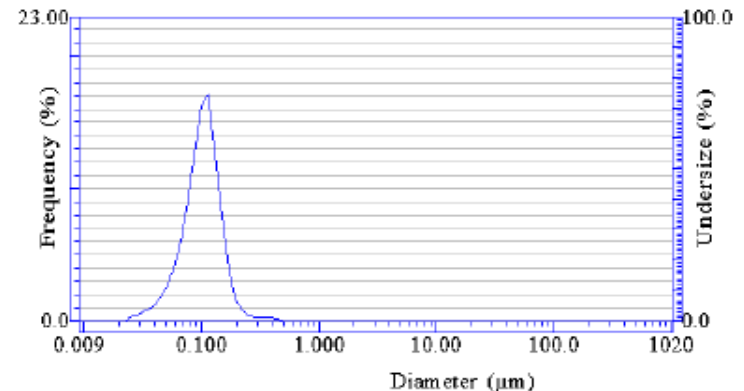
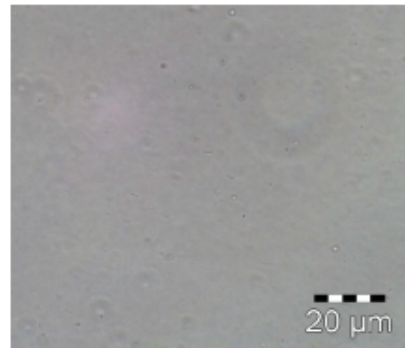
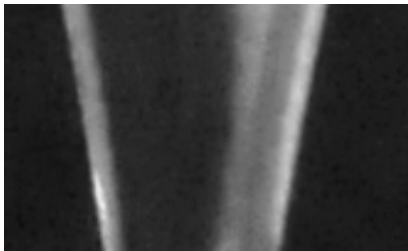


Liposome Size Distribution

Hydrated multilamellar vesicles



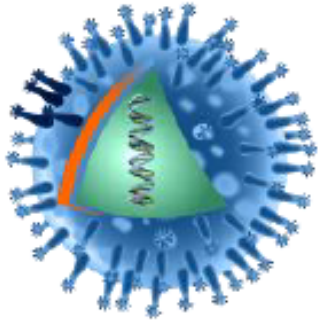
Small size liposomes



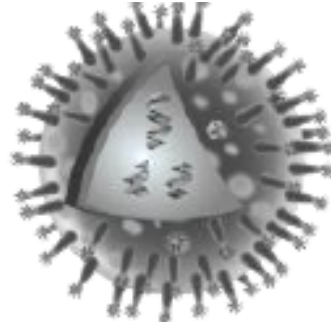
Liposomes in Vaccines

- **Adjuvant:** Enhance and direct the adaptative immune response to vaccine antigens
- Our lab created Army Liposome Formulation (ALF) that contains various immunostimulants (e.g., Lipid A, QS21 and Aluminum salt)

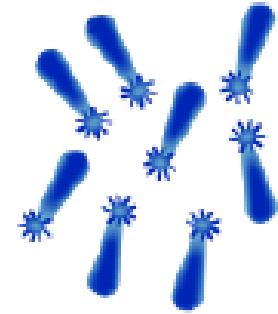
Development of vaccine design



Live Pathogen



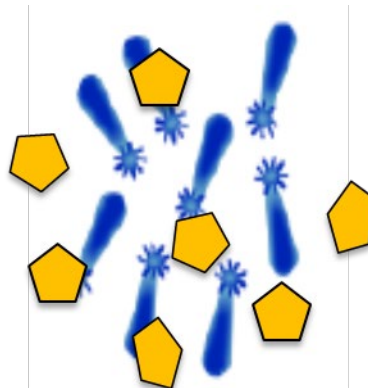
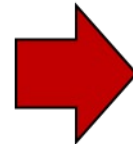
Attenuated Pathogen



Recombinant Immunogen

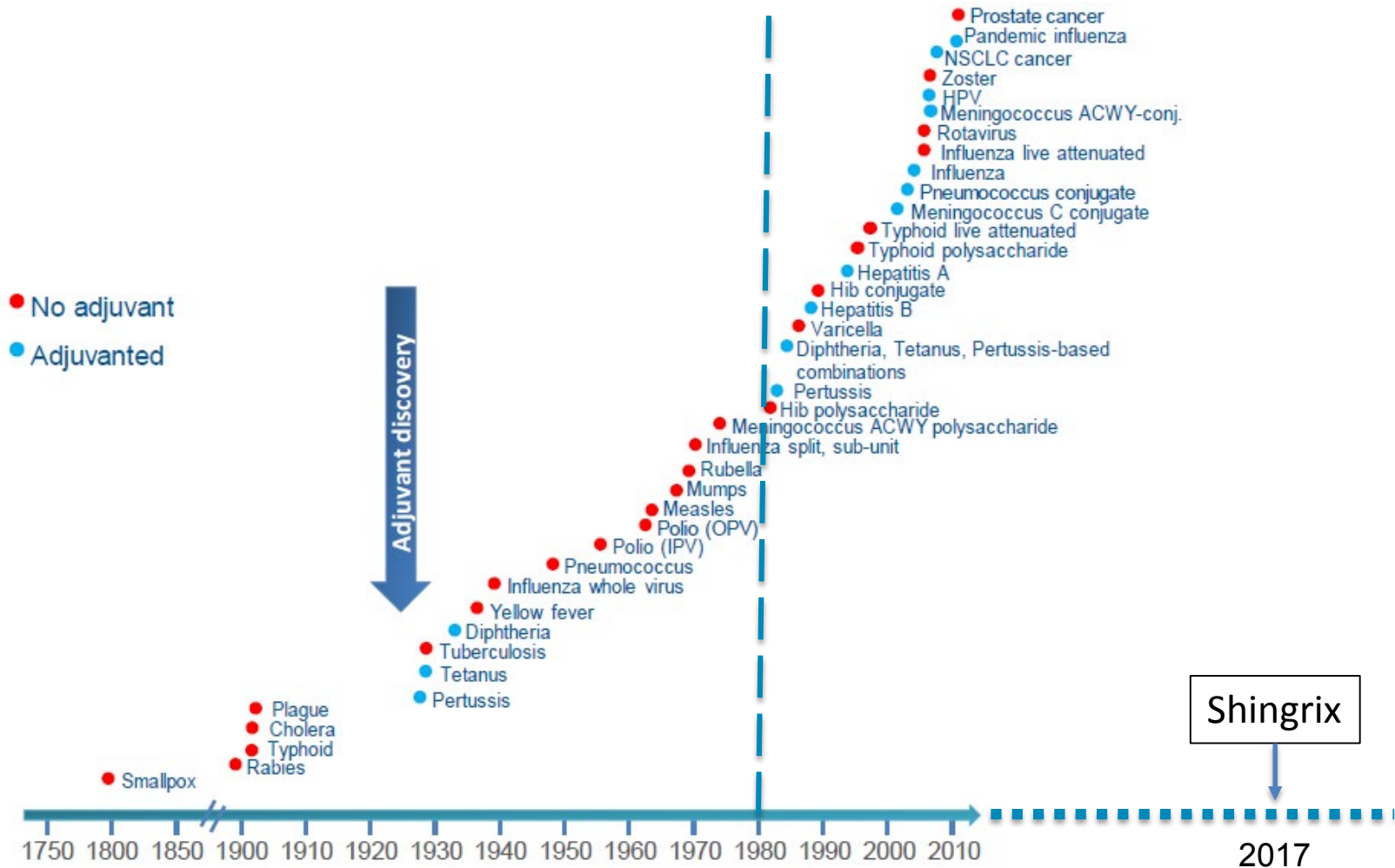
IMMUNOGENICITY

SAFETY



Adapted from Pasquale *et al.*, *Vaccine* 2015, 3: 320-343

Licensed Vaccines



Adapted from Pasquale *et al.*, *Vaccine* 2015, 3: 320-343

ALF in Vaccines

Experimental vaccine:

- Malaria
- Ricin
- Ebola
- Anthrax
- HIV
- Dengue
- Cancer

Human Studies Vaccines:

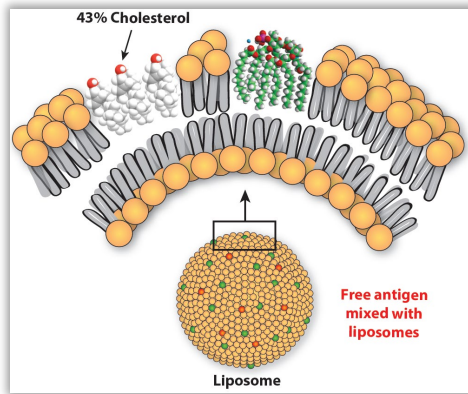
14 Phase I & 2 Phase II

- Malaria
- HIV
- Cancer
- Meningococcus

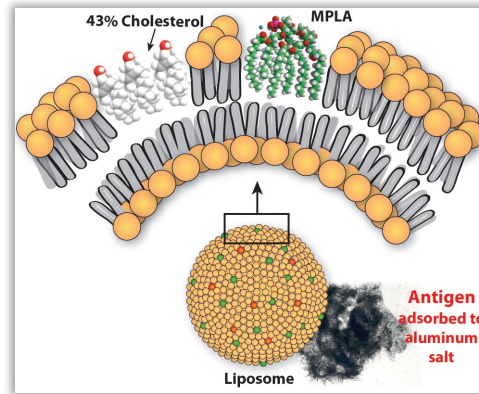
Core composition of ALF

- Dimyristoyl phosphatidylcholine (DMPC)
- Dimyristoyl phosphatidylglycerol (DMPG)
- Cholesterol
- Monophosphoryl lipid A (MPLA)

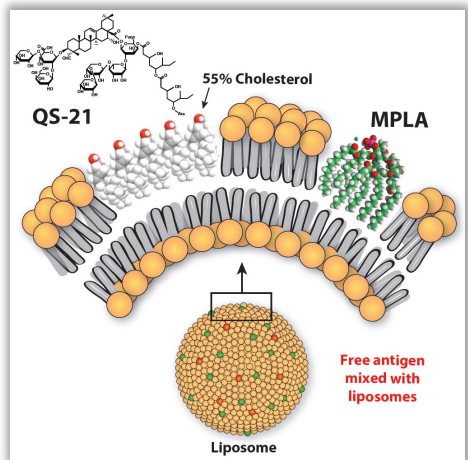
Types of ALF



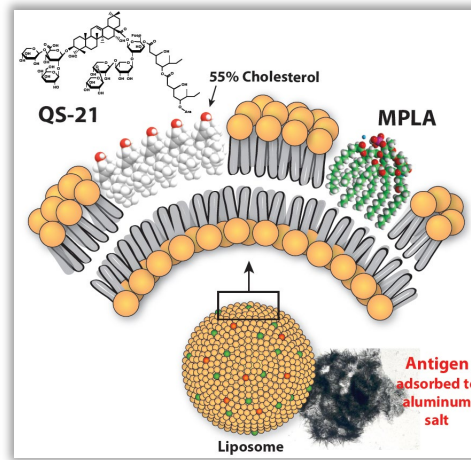
ALF: Liposomes with MPLA



ALFA: ALF + aluminum salt



ALFQ: ALF + QS21



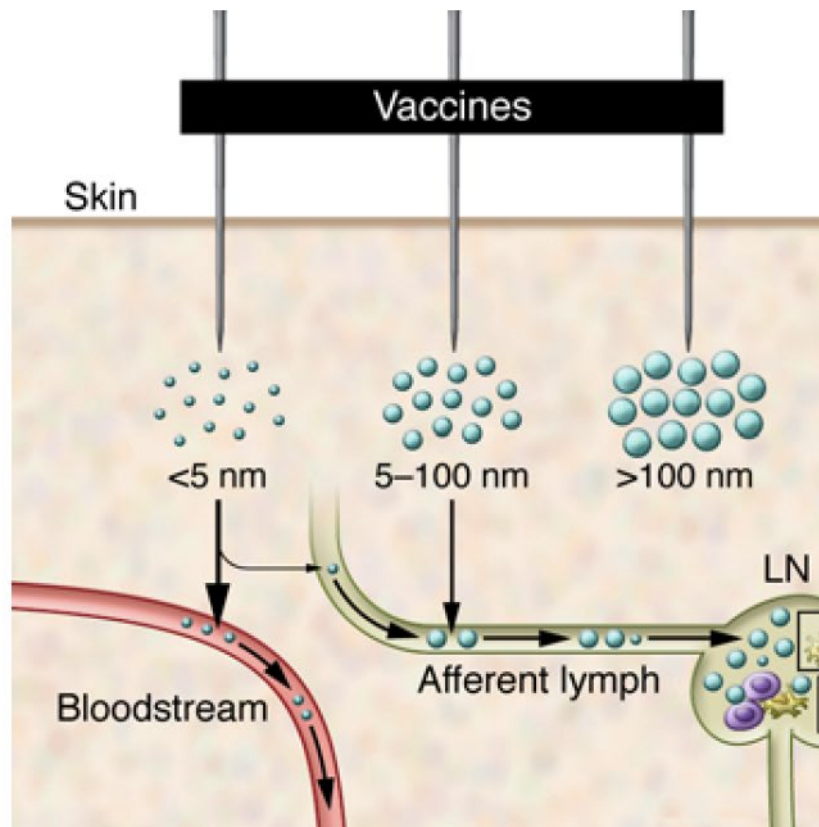
ALFQA: ALFQ + aluminum salt

Rationale

To establish a stand-alone method for particle size characterization of polydisperse liposomal adjuvants

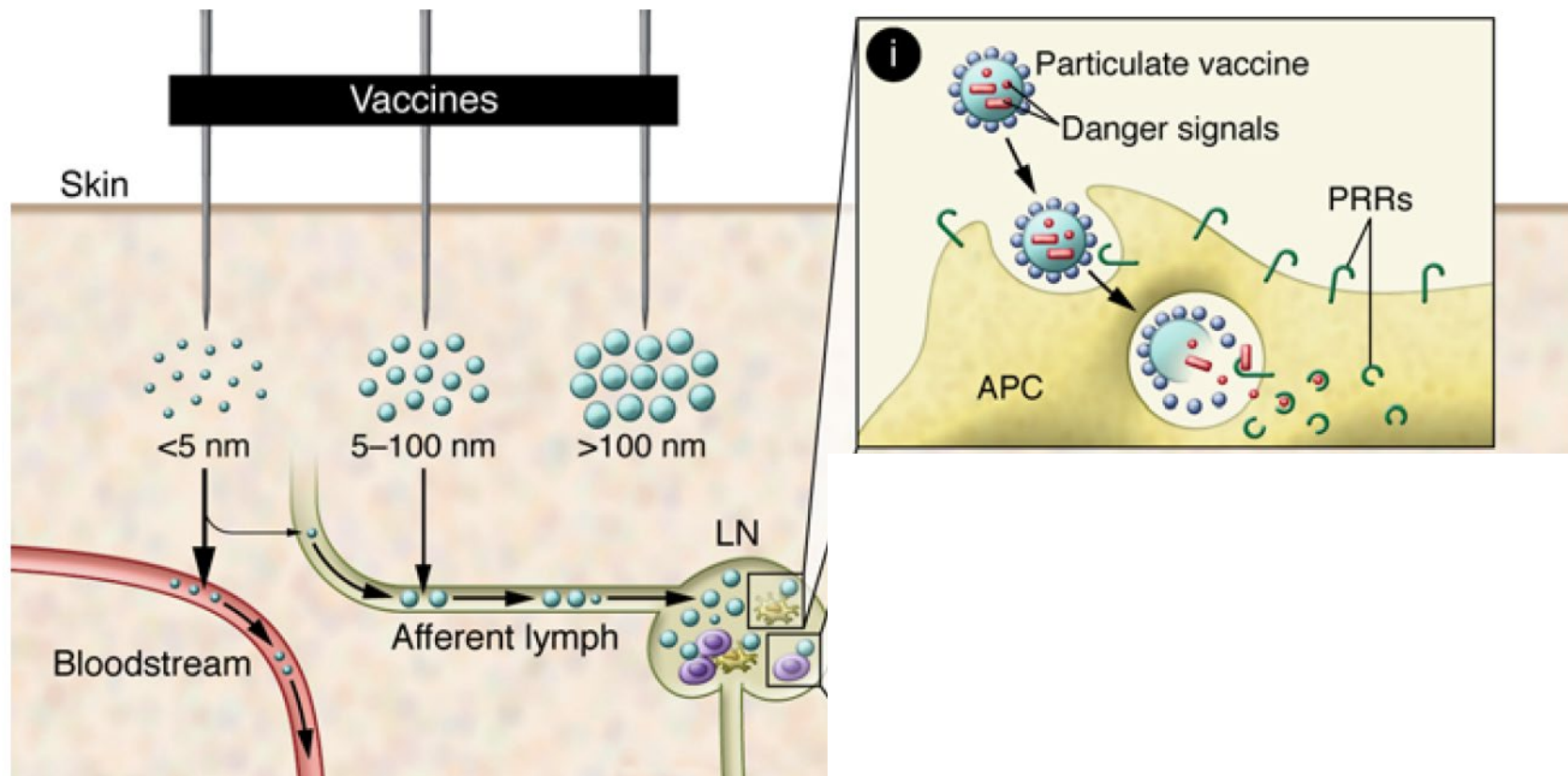
Why care about particle size?

Why care about particle size?



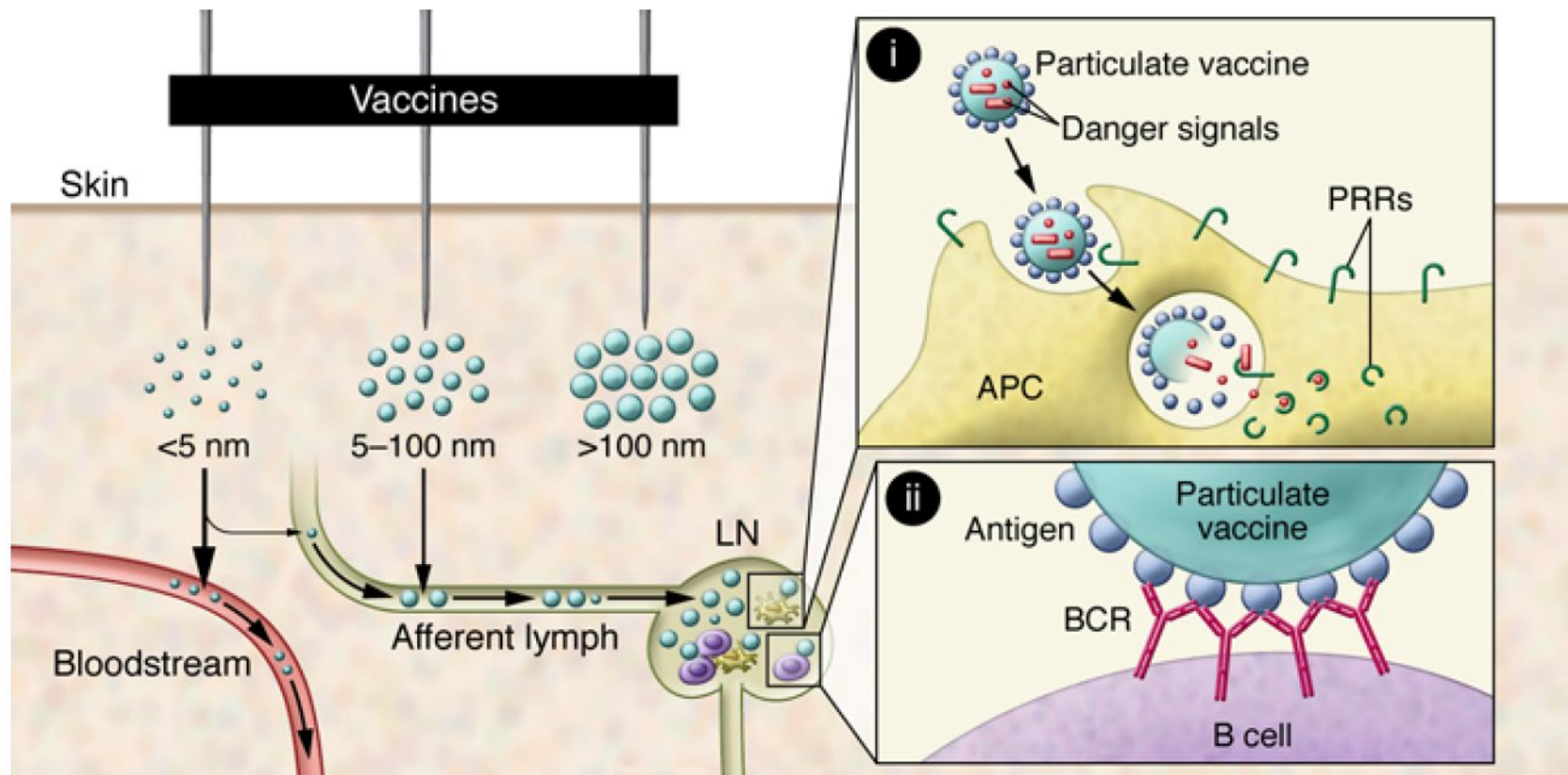
Biodistribution and Kinetics

Why care about particle size?



Biodistribution and Kinetics

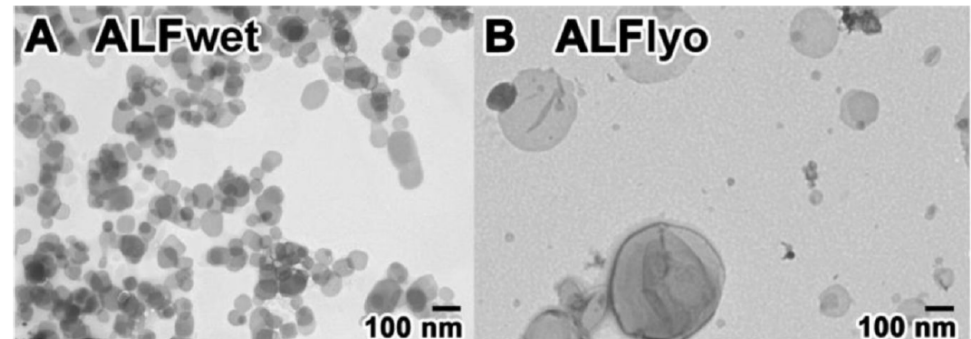
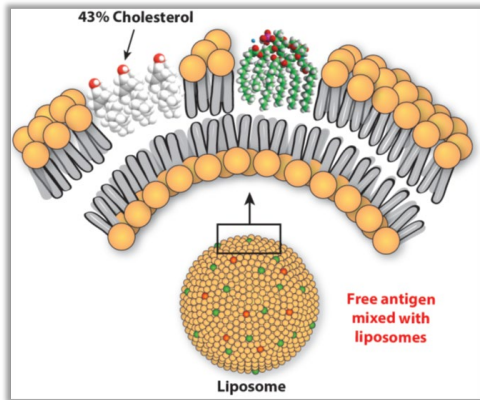
Why care about particle size?



Biodistribution and Kinetics

Processivity

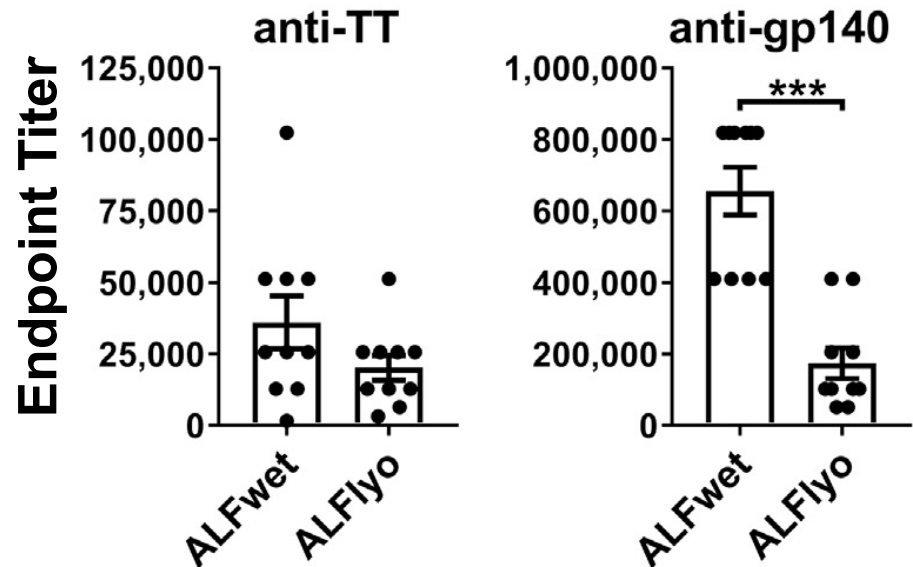
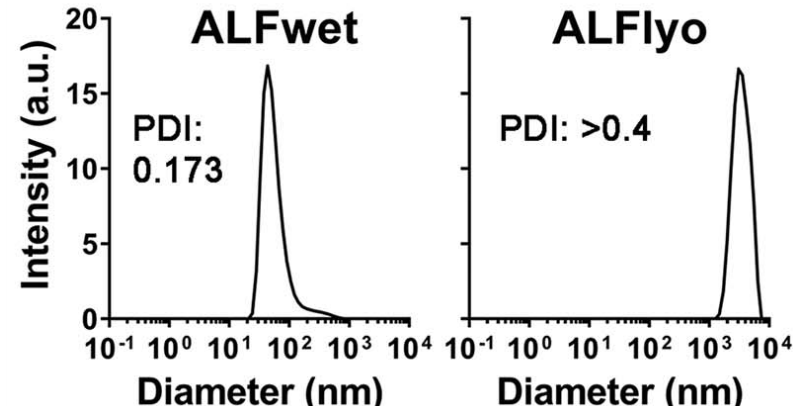
ALF: size vs. immune response



Monodisperse

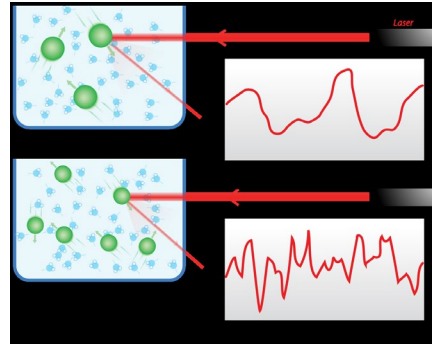
Polydisperse

ALF



Challenges of particle sizing methods

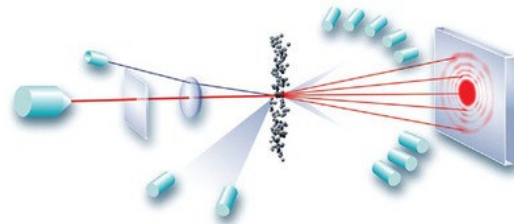
Dynamic Light Scattering (DLS)



Autocorrelation of scattered intensities to deduce **average** particle size

- Range 30 -10000 nm
- does not** work on polydisperse sample

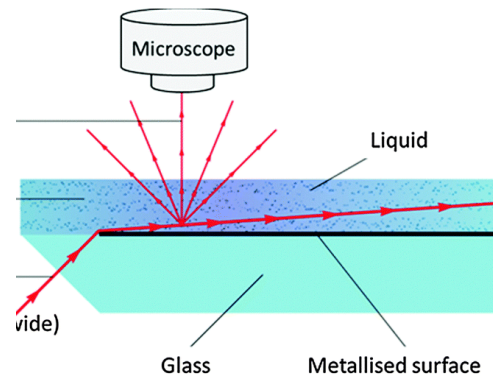
Laser Diffraction (LD)



Narrow and wide angle scattering to deduce **average** particle size

- Range 10 nm - 5 mm
- does not** work on polydisperse sample

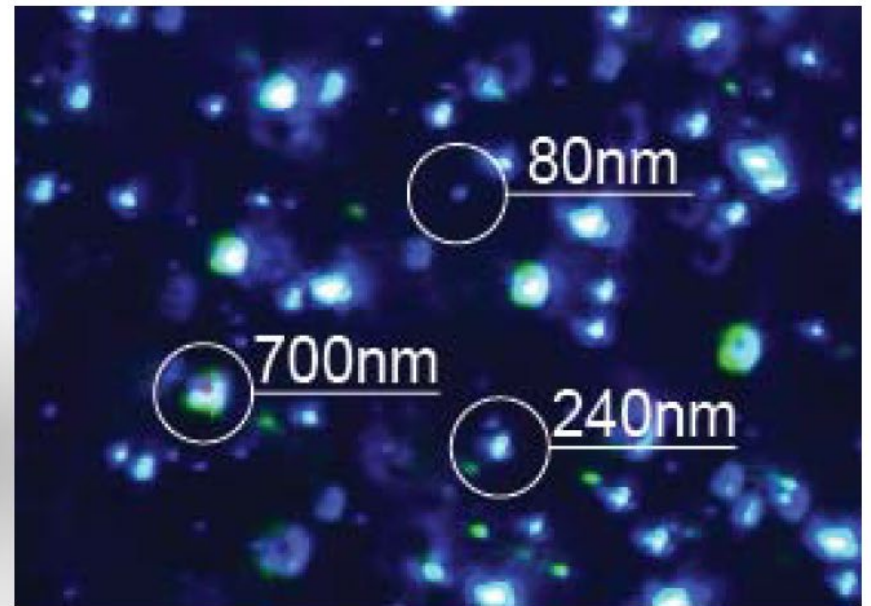
Nanoparticle Tracking Analysis (NTA)



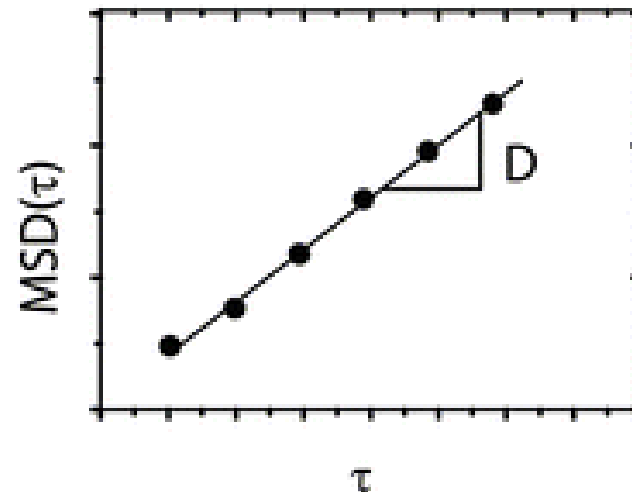
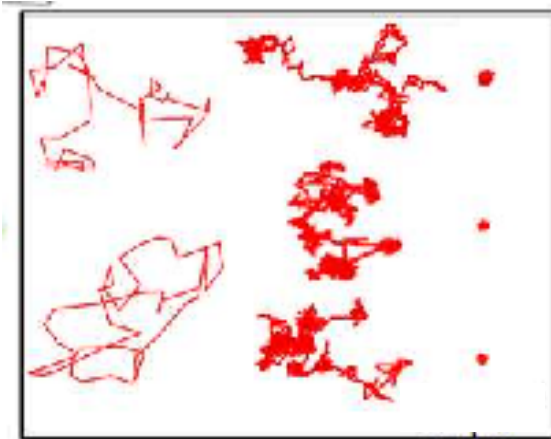
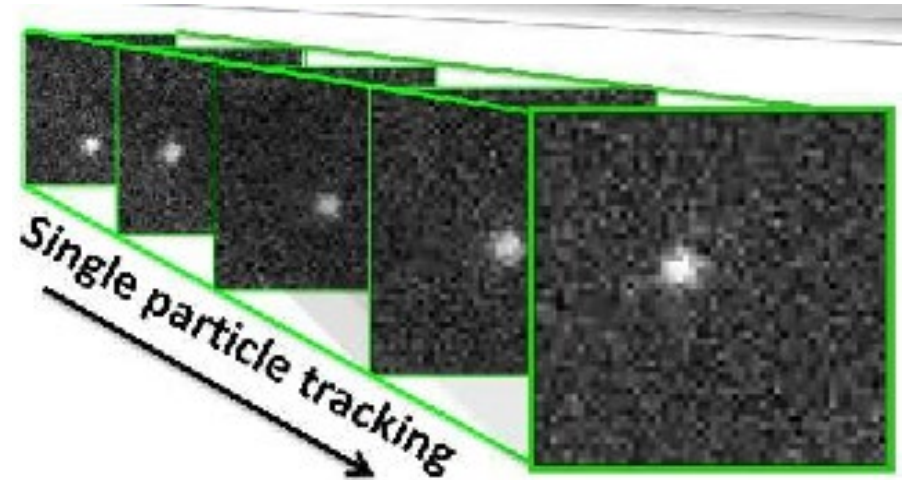
Tracking of each particle undergoing Brownian (random) motion to determine particle size

- Small size range **30 - 1000 nm**
- works on polydisperse sample

ViewSizer

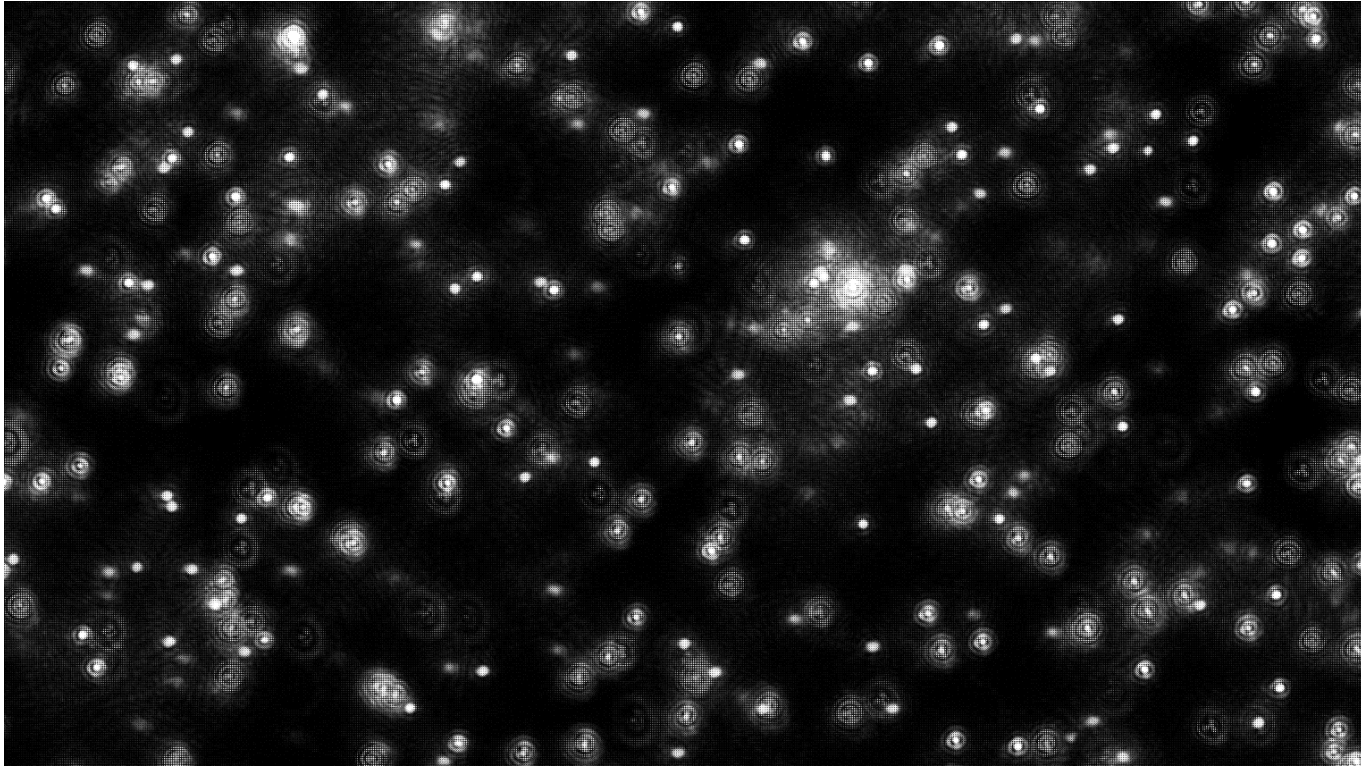


How does it work?

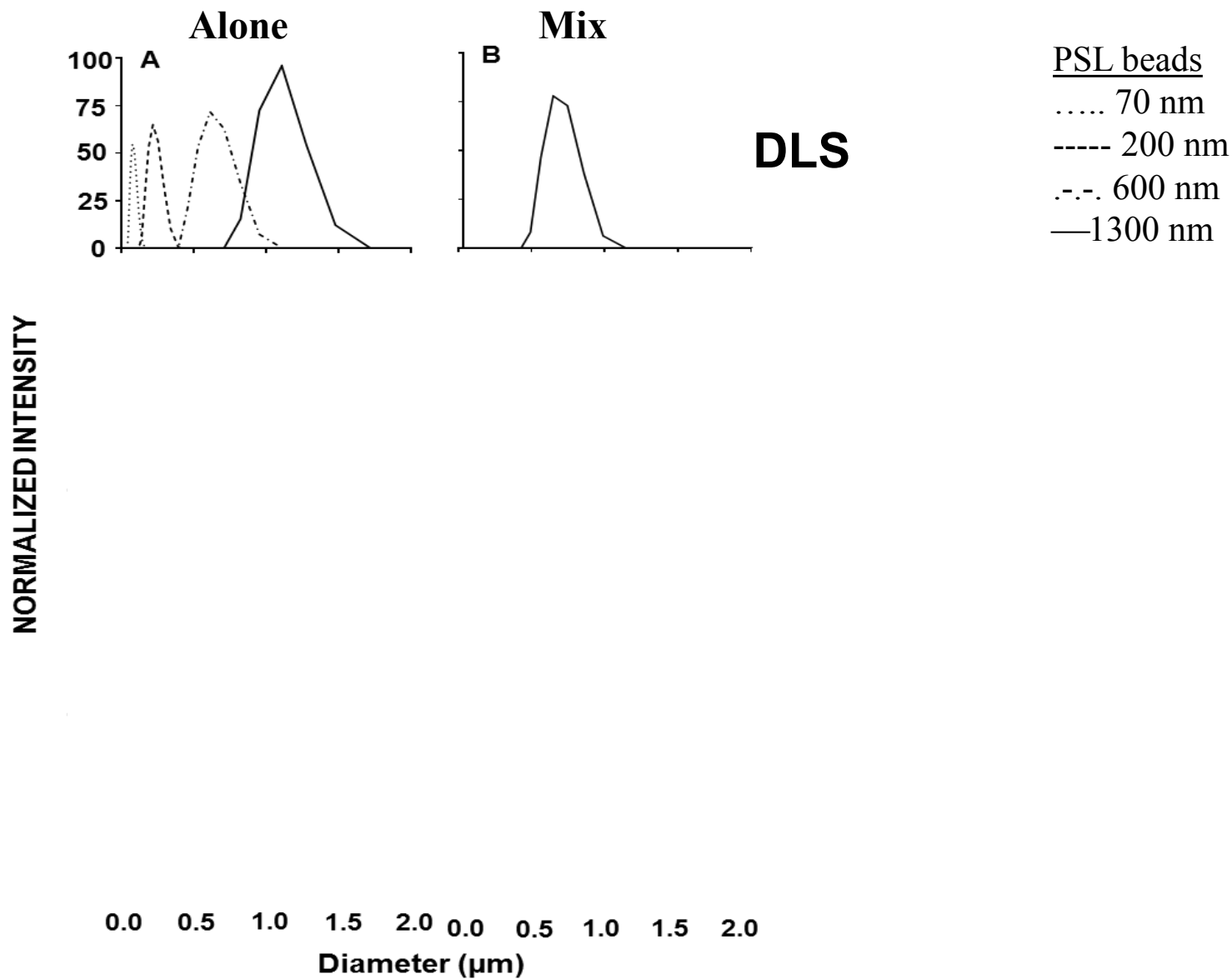


Manzo and Garcia-Parajo *Rep. Prog. Phys.* 2015, 78: 124601
Ernst and Kohler *Phys. Chem. Chem. Phys.*, 2013, 15: 845-849

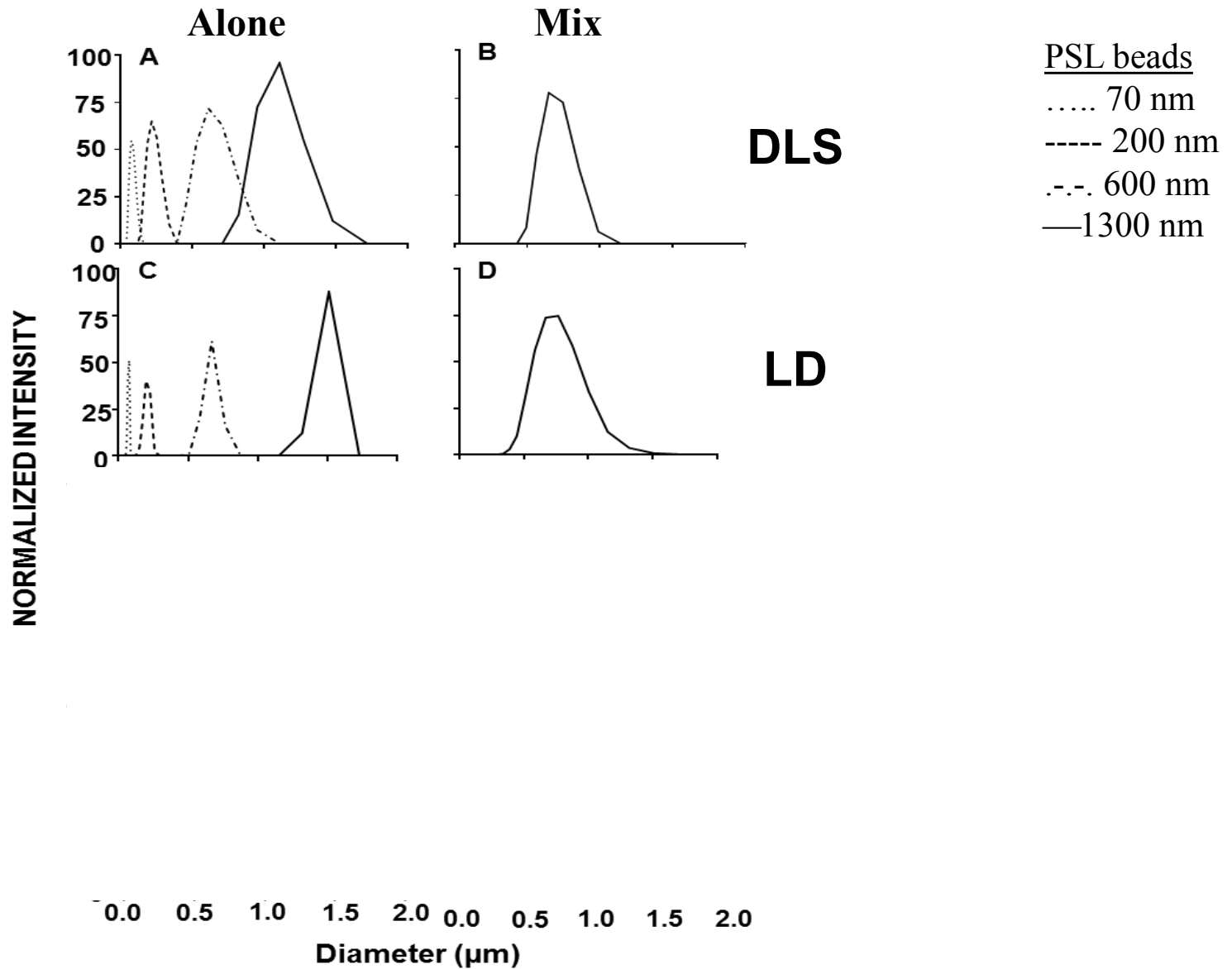
Particle size distribution by ViewSizer



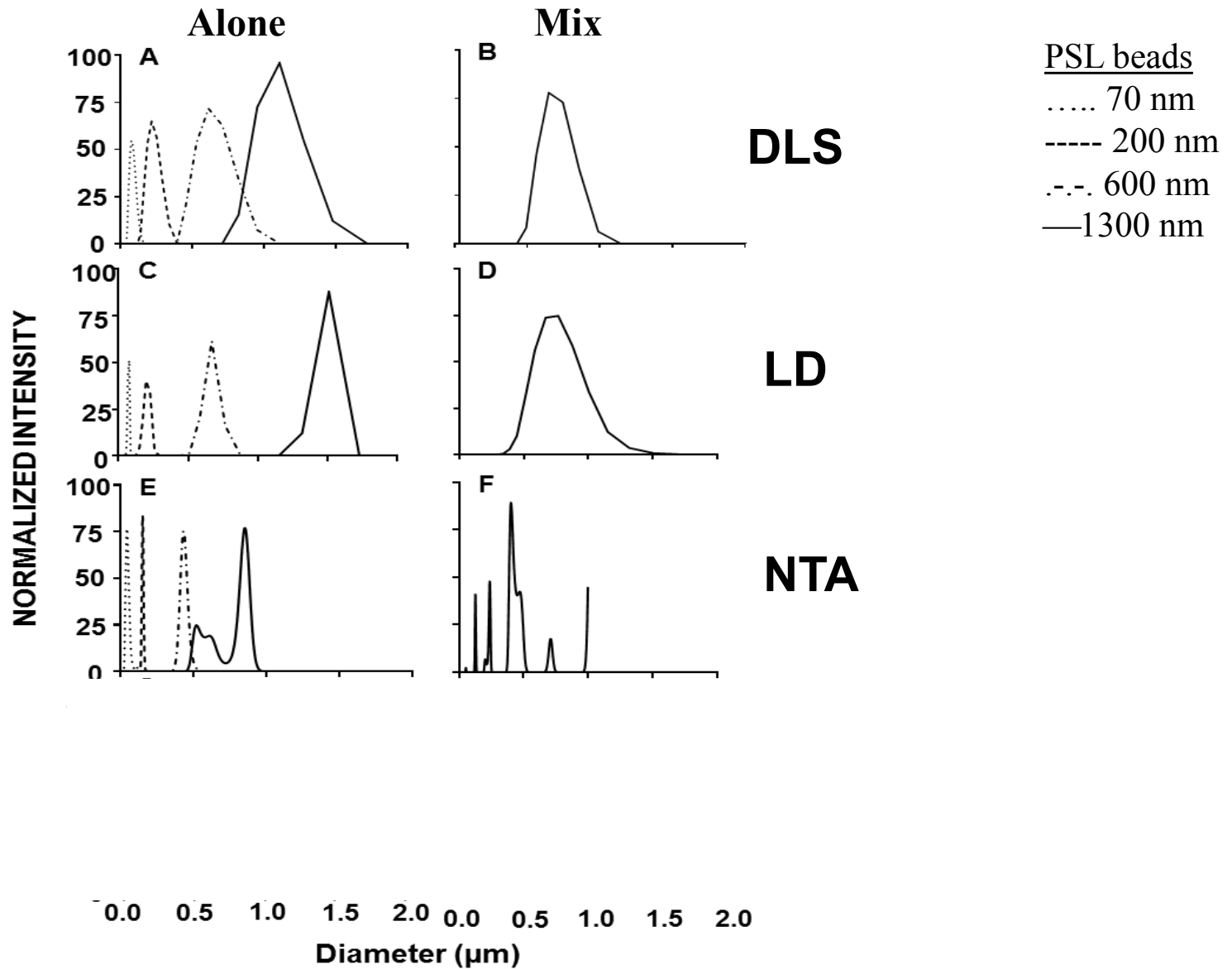
Size analysis of standards



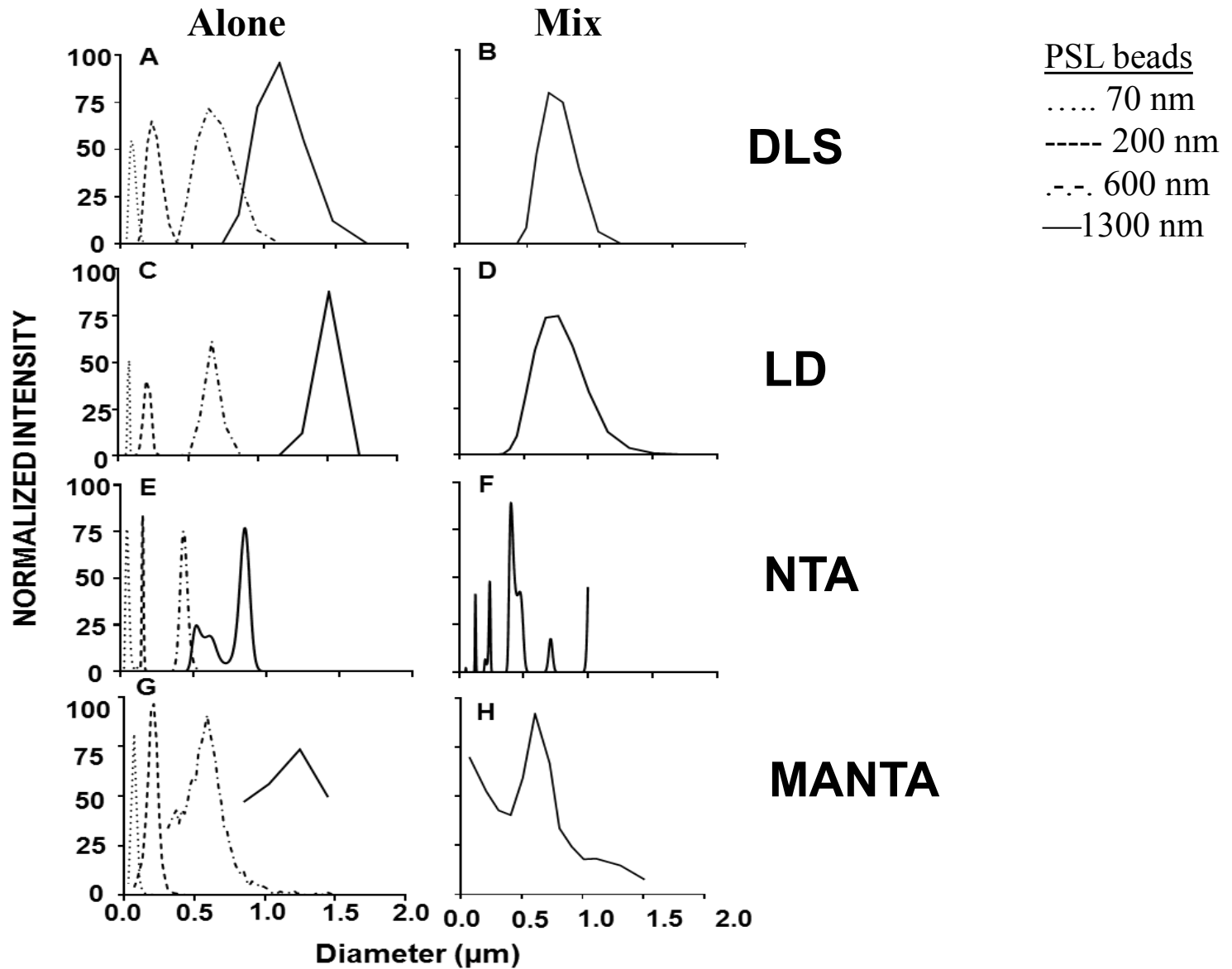
Size analysis of standards



Size analysis of standards



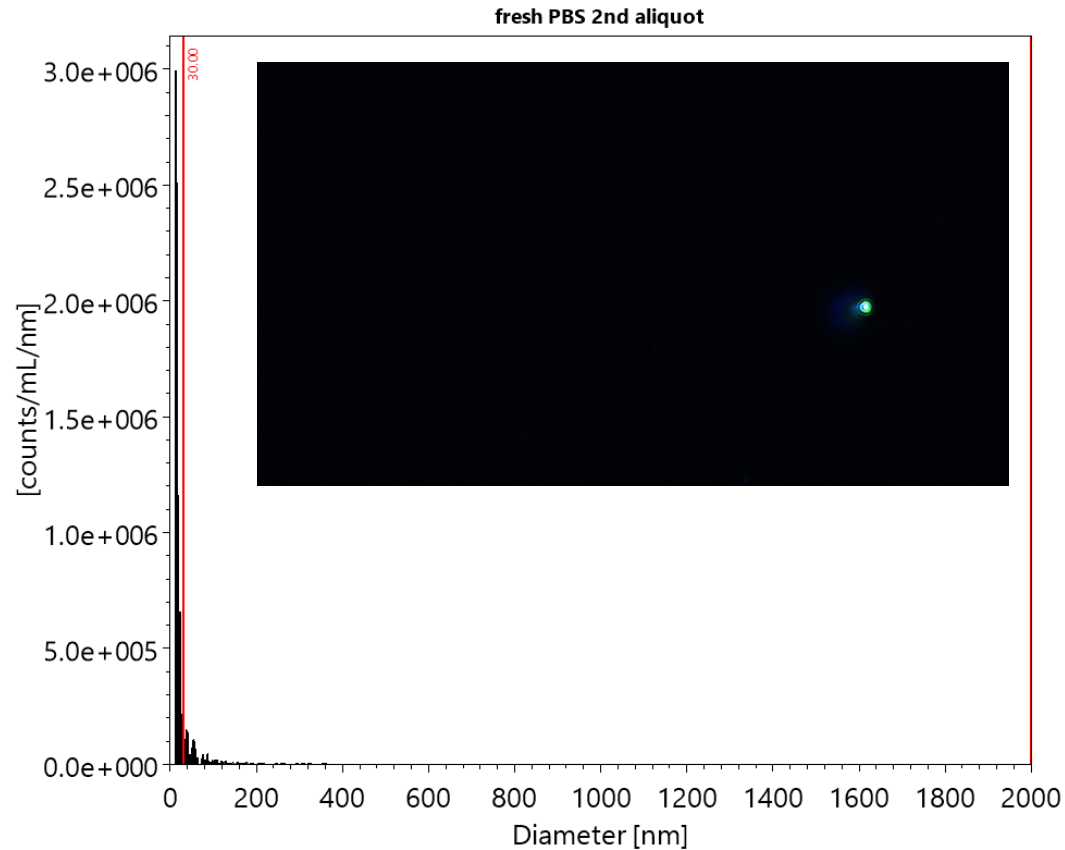
Size analysis of standards



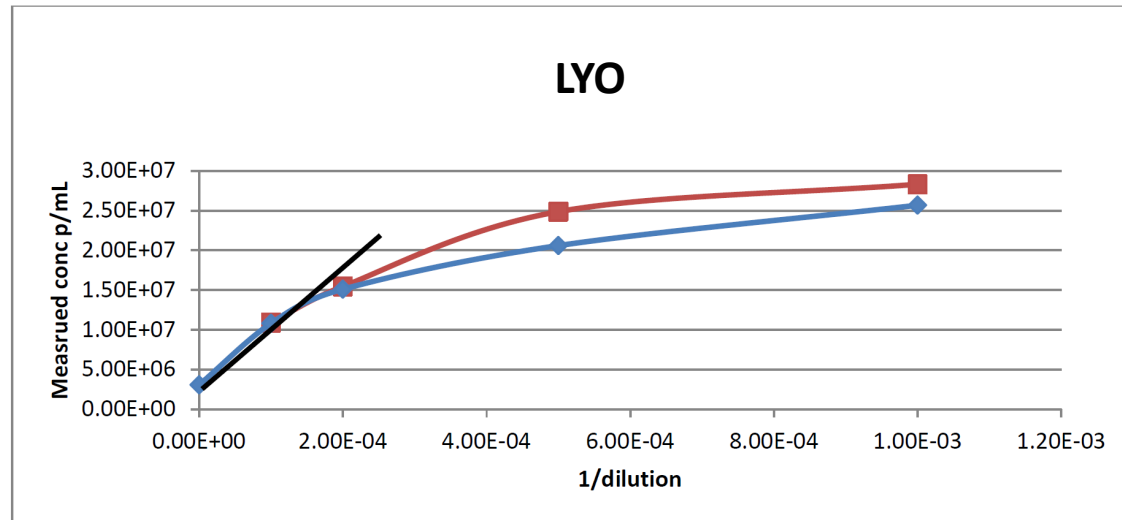
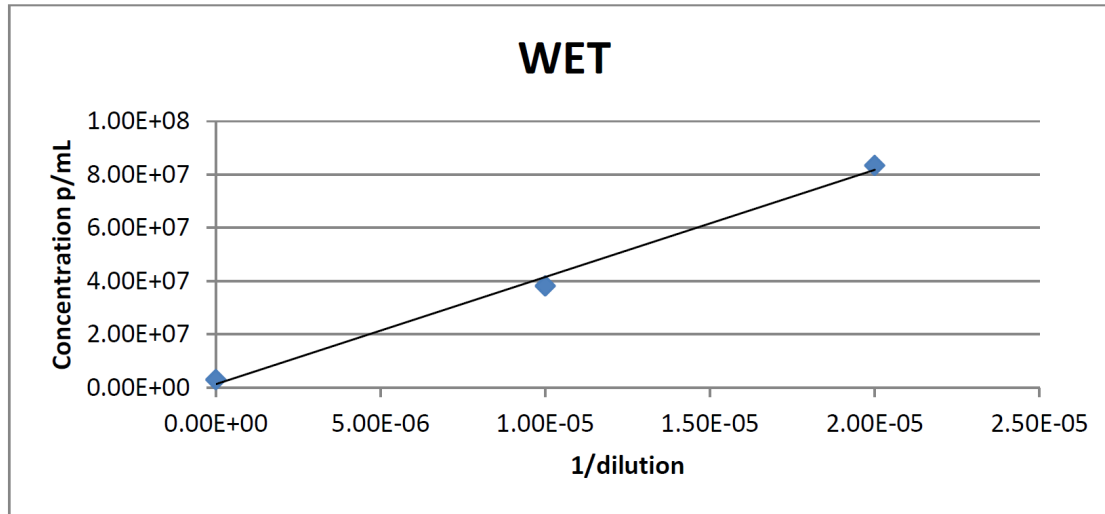
Liposomes

- **Lwet:** Uniform (monodisperse) small sized liposomes manufactured by microfluidization
- **Llyo:** Heterogenous (polydisperse) sized liposomes nano to micron size

Particle size distribution by ViewSizer



Linear range of sensitivity

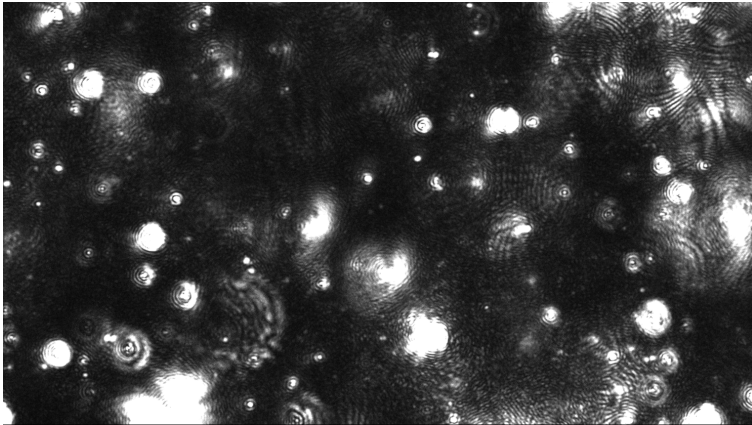


Snapshots



Lwet

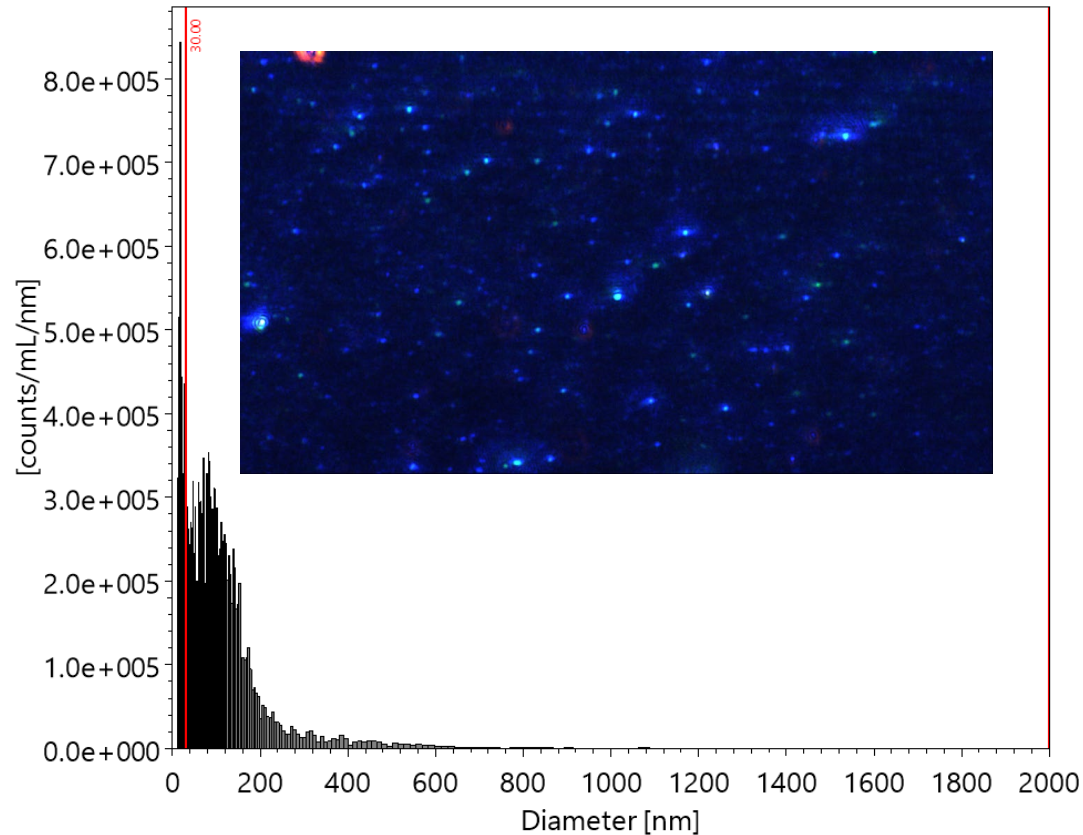
Monodisperse



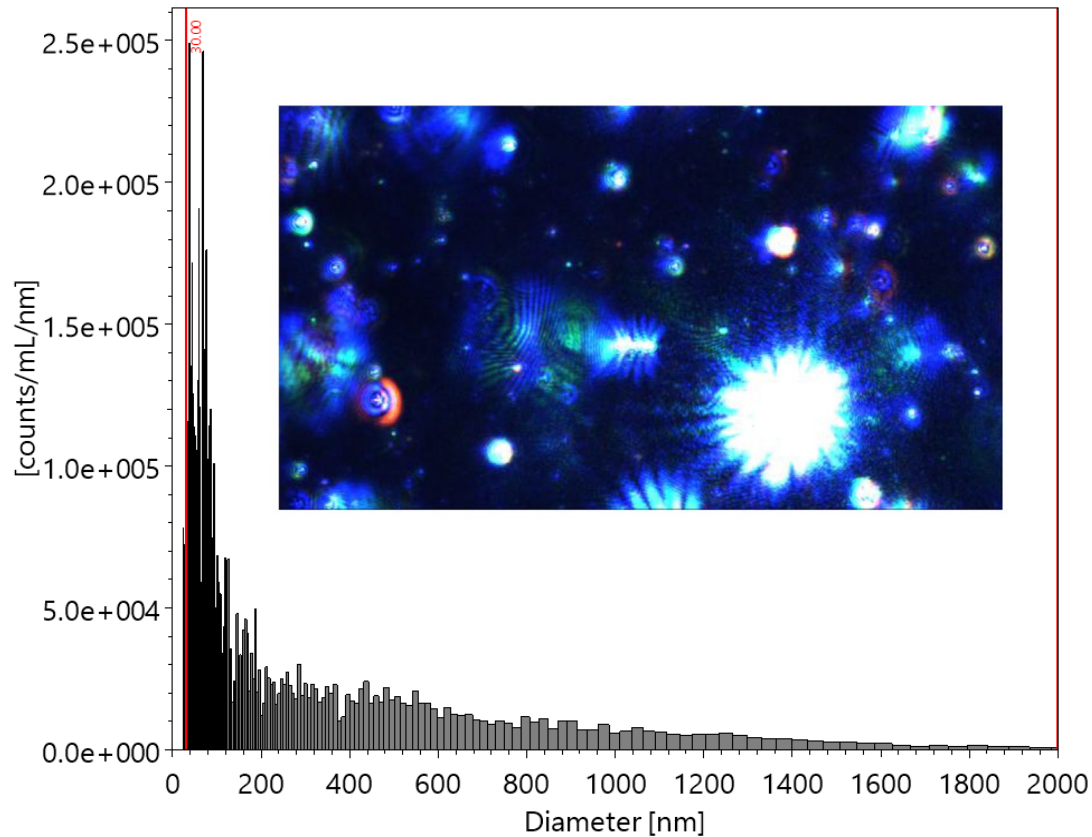
Llyo

Polydisperse

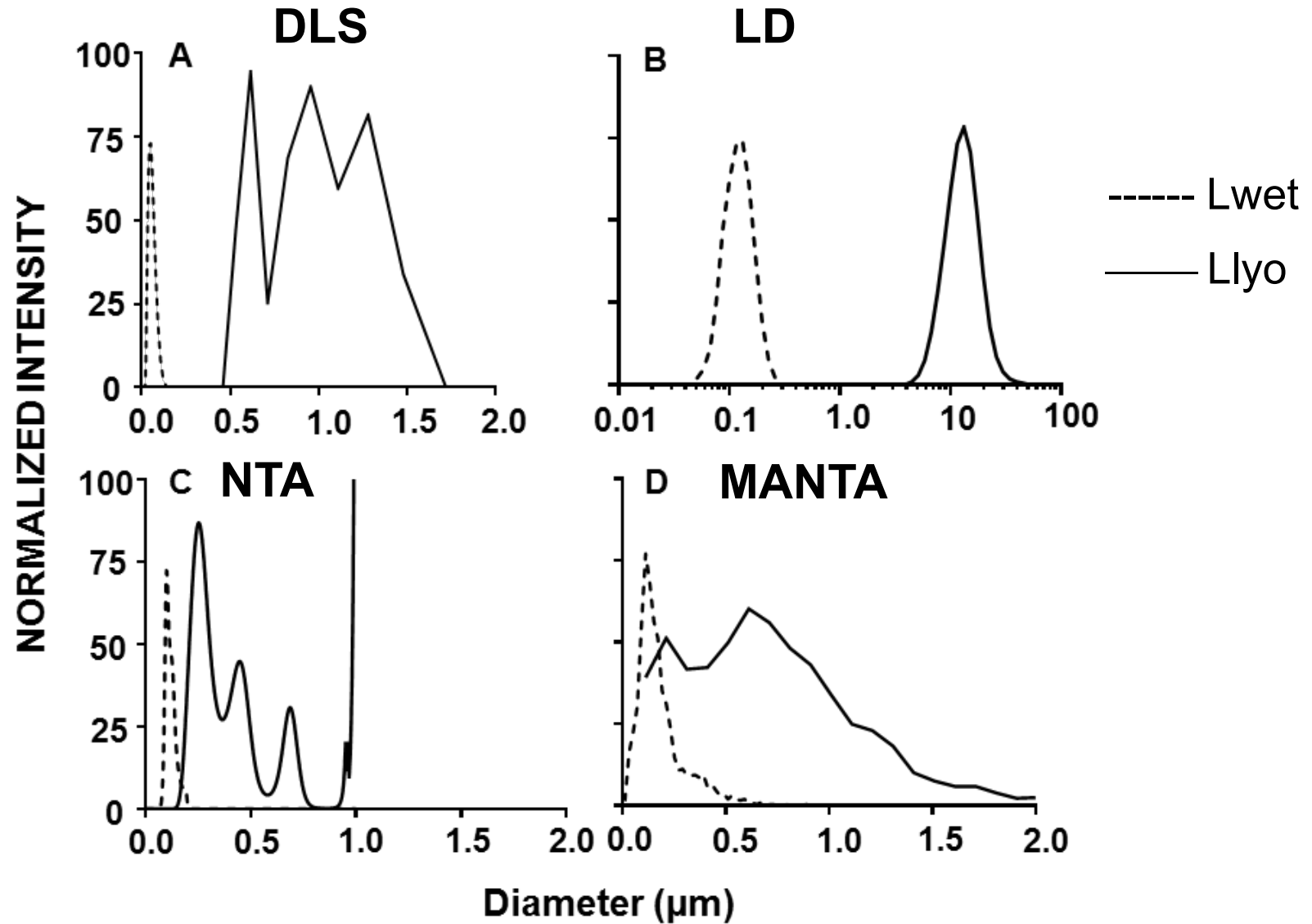
Liposome size distribution in Lwet



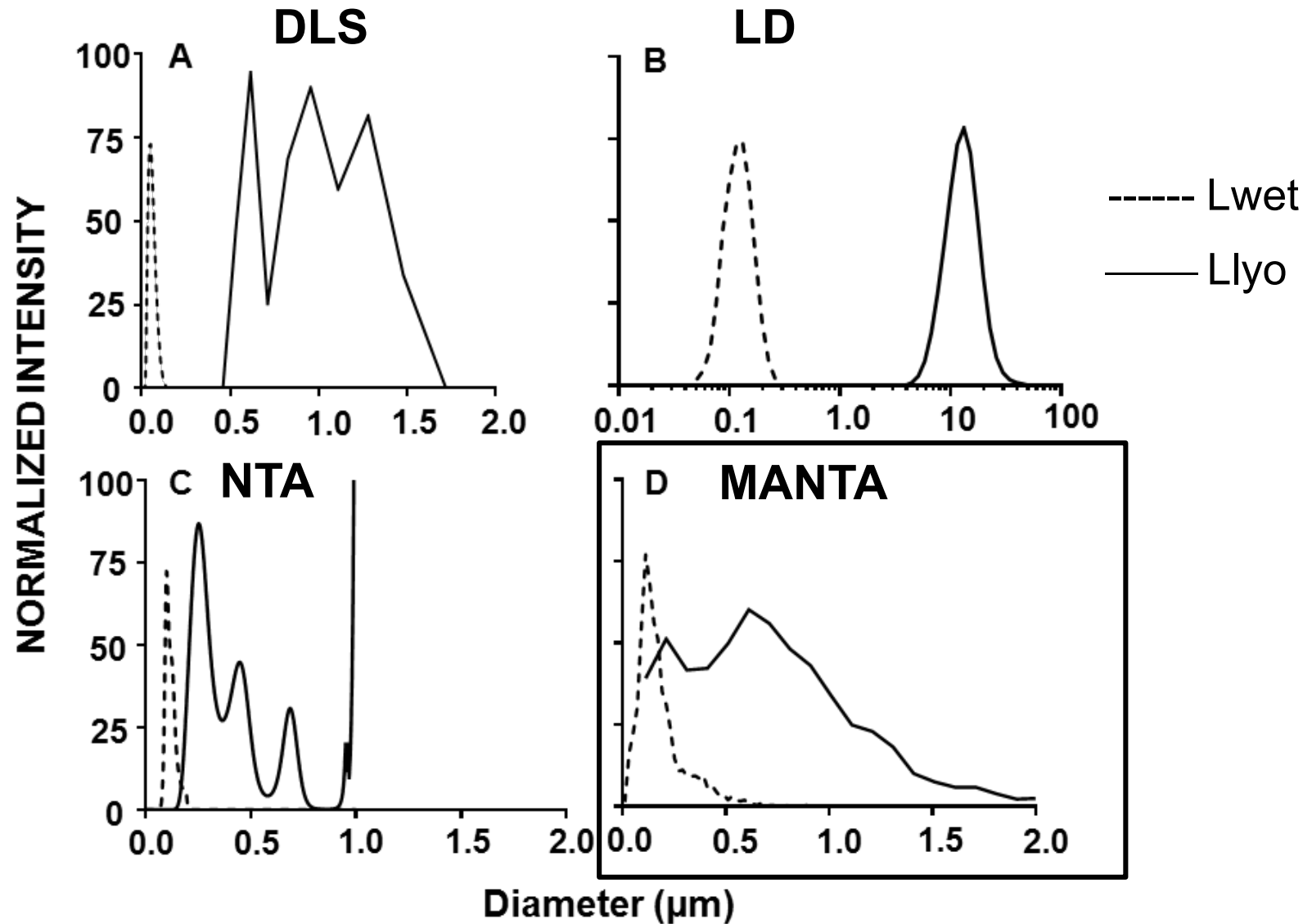
Liposome size distribution in Llyo



Size analysis of liposomes



Size analysis of liposomes



Summary

- Multispectral Nanoparticle Tracking Analysis can measure particle size 50 - 2000 nm in polydisperse liposomes
- Lyophilized liposome formulation contain >90% particles in the range of 500 - 1500 nm

Acknowledgements

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HJF

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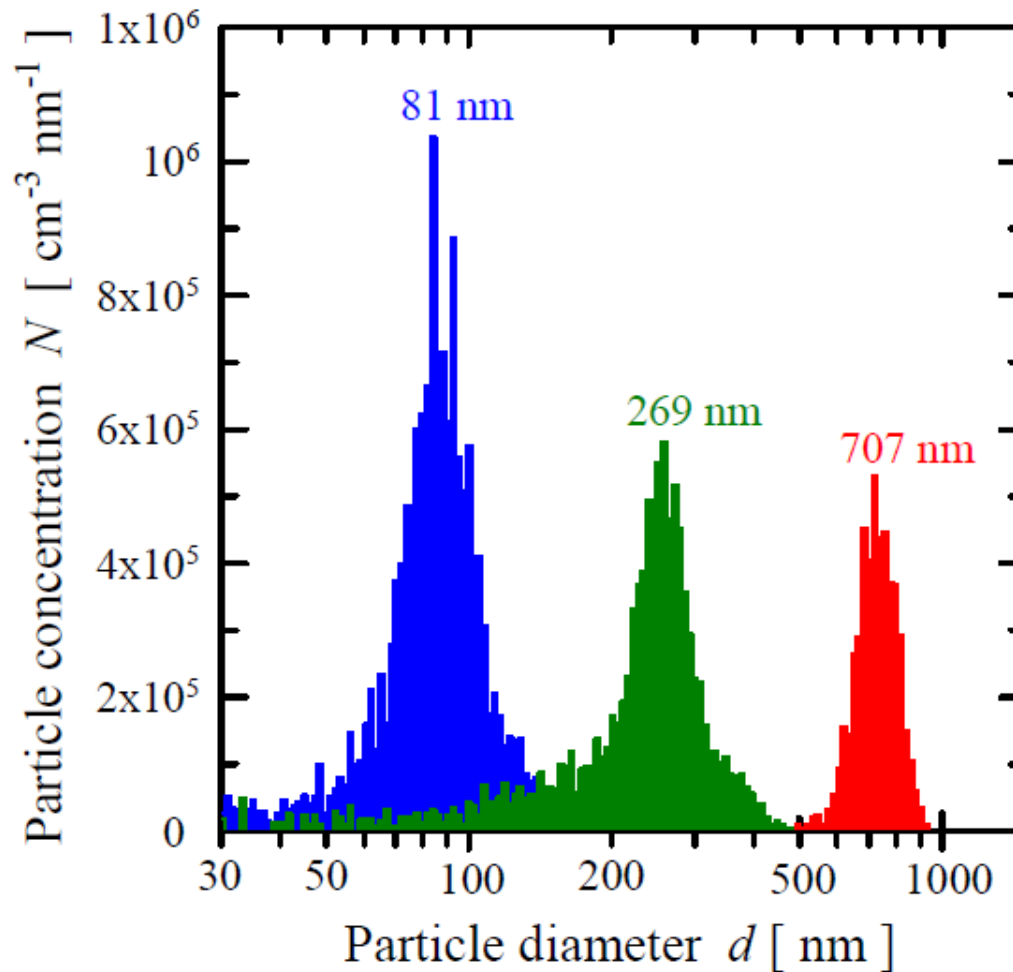
Publication

Singh, P., Matyas, G.R., Beck, Z. (2019)
Particle Size Analyses of Polydisperse
Liposome Formulations with a Novel
Advanced Nanoparticle Tracking Technology.
Int. J. Pharm. 566: 680-686.

doi: 10.1016/j.ijpharm.2019.06.013.

Thank You

Particle size distribution by ViewSizer



Particle size distribution by ViewSizer

