UNIVERSITY of MARYLAND
SCHOOL OF MEDICINE

# Exosomes: Exploiting the Diagnostic and Therapeutic Potential of Nature's Biological Nanoparticles

June 11, 2020
HORIBA Webinar Particle Characterization Series

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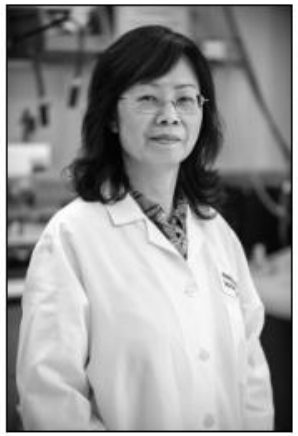
University of Maryland School of Medicine
Medical Scientist Training Program
Program in Neuroscience

# The Lab for the Study of Central Nervous System Injury


Dr. Alan Faden


Dr. Bogdan Stoica


Dr. Junfang Wu


Dr. Marta Lipinski


Dr. David Loane

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# Pathophysiology of CNS Injury

## ***Primary Injury***

## ***Secondary Injury***

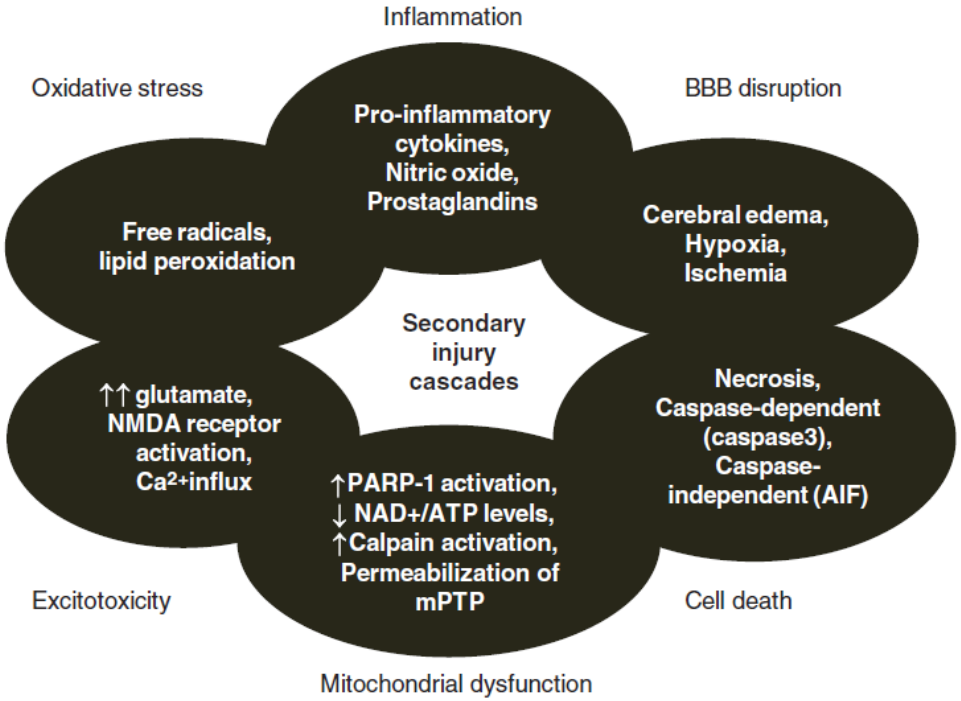
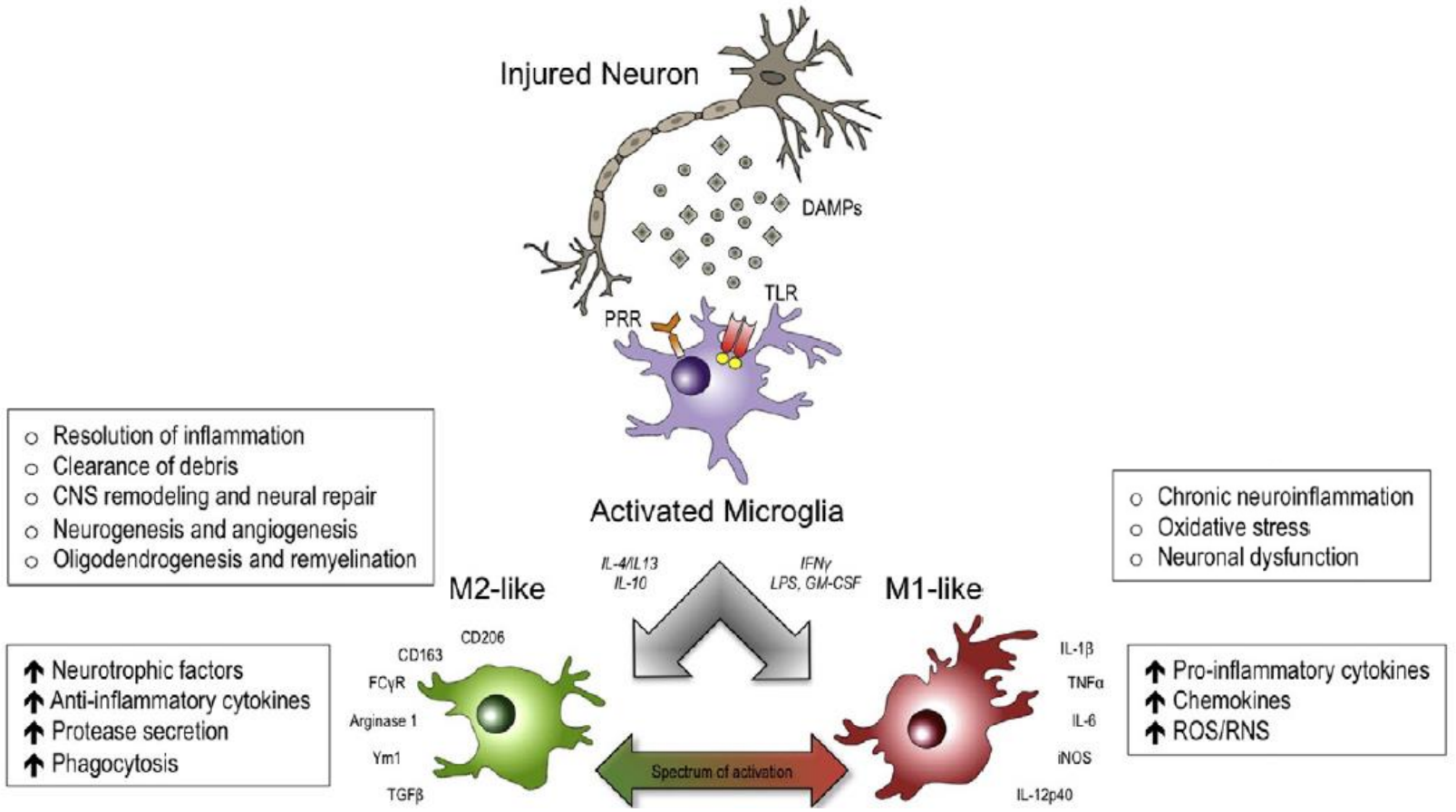



**Figure I.** Pathways of secondary injury in TBI.

Loane and Faden (2010), ***Trends Pharmacol Sci***

**Secondary injury contributes to progressive cell loss after neurotrauma.**

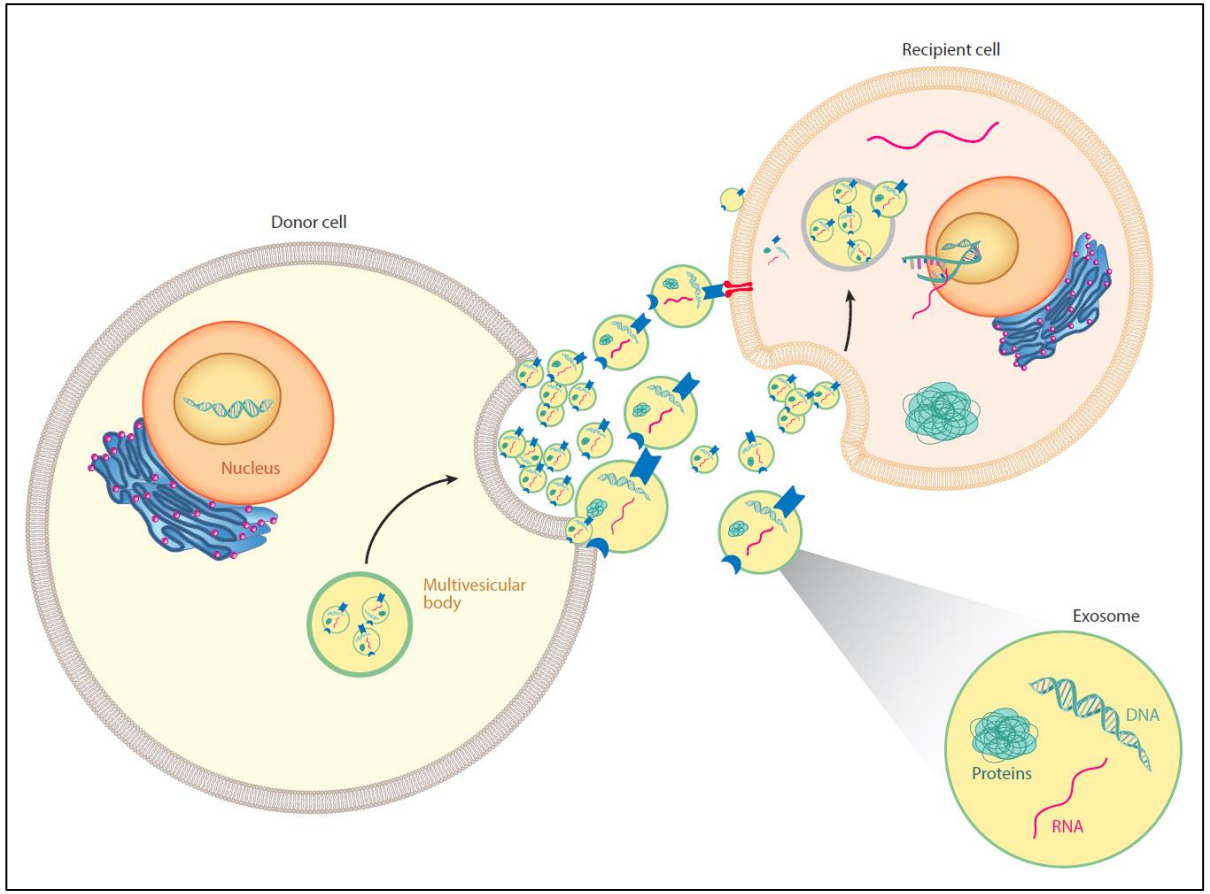
# Neuroinflammation after CNS Injury




Loane & Kumar (2015), ***Exp. Neurol.***

**Pro-inflammatory, microglial activation persists months after neurotrauma.**

# Extracellular Vesicles (EVs)




Kourembanas et al. (2015), ***Annu Rev Physiol***

**EVs are biological messengers that can transfer proteins, lipids, and nucleic acids.**

*Brit. J. Haemat.*, 1967, **13**, 269.

# The Nature and Significance of Platelet Products in Human Plasma

PETER WOLF

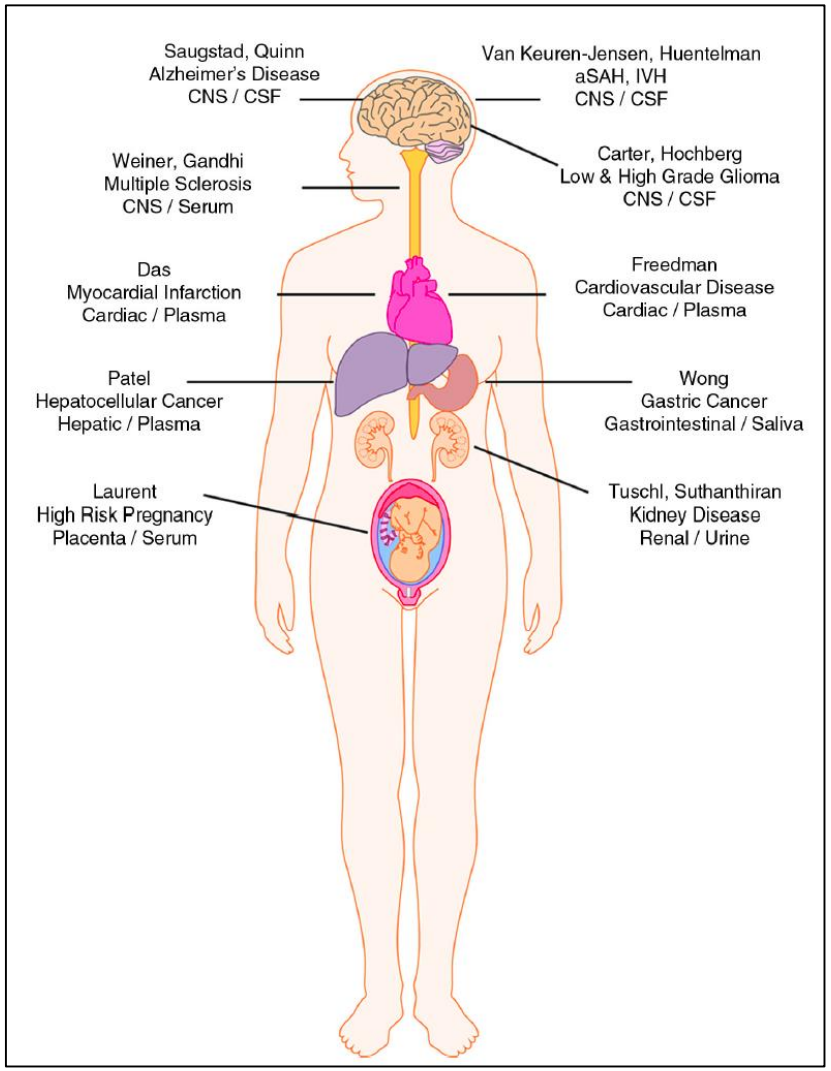
*External Scientific Staff of the Medical Research Council,*
*Department of Experimental Pathology, University of Birmingham*

It has been observed that the coagulant activity of citrated plasma increases progressively with storage over some hours. This process has been attributed to 'activation' of platelets (Hougie, 1955). On the other hand, it has been noted that plasma, freed from intact platelets, generates thrombin on recalcification and that the rate of this thrombin generation can be reduced by prior high-speed centrifugation of the plasma (Chargaff and West, 1946). Platelet-like activity has also been found in serum (O'Brien, 1955).

The purpose of the present communication is to provide evidence for the occurrence in normal plasma, serum and fractions derived therefrom of coagulant material in minute particulate form, sedimentable by high-speed centrifugation and originating from platelets, but distinguishable from intact platelets. It is suggested that this material, hereafter referred to as 'platelet-dust', is responsible for the phenomena referred to above. Observations on the

**EVs were once thought to be just "*dust*".**

# EVs in Biological Fluids




Quinn et al. (2015), ***J Extracell Vesicles***

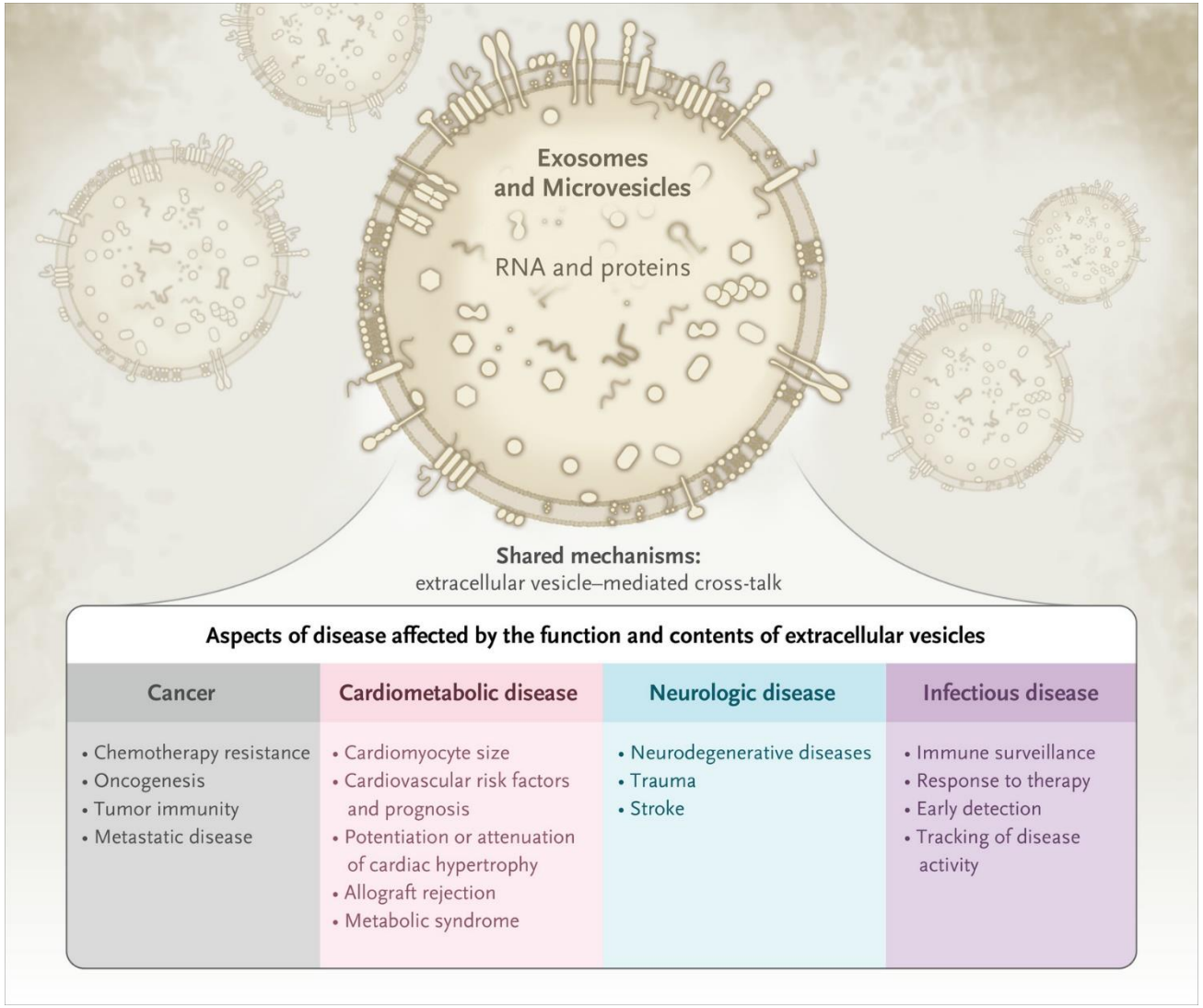
<u>Implications:</u>

(1) Biomarker Potential

(2) Long-distance communication between organ systems

(3) EVs as drug delivery carriers

# EVs as Biomarkers for Disease

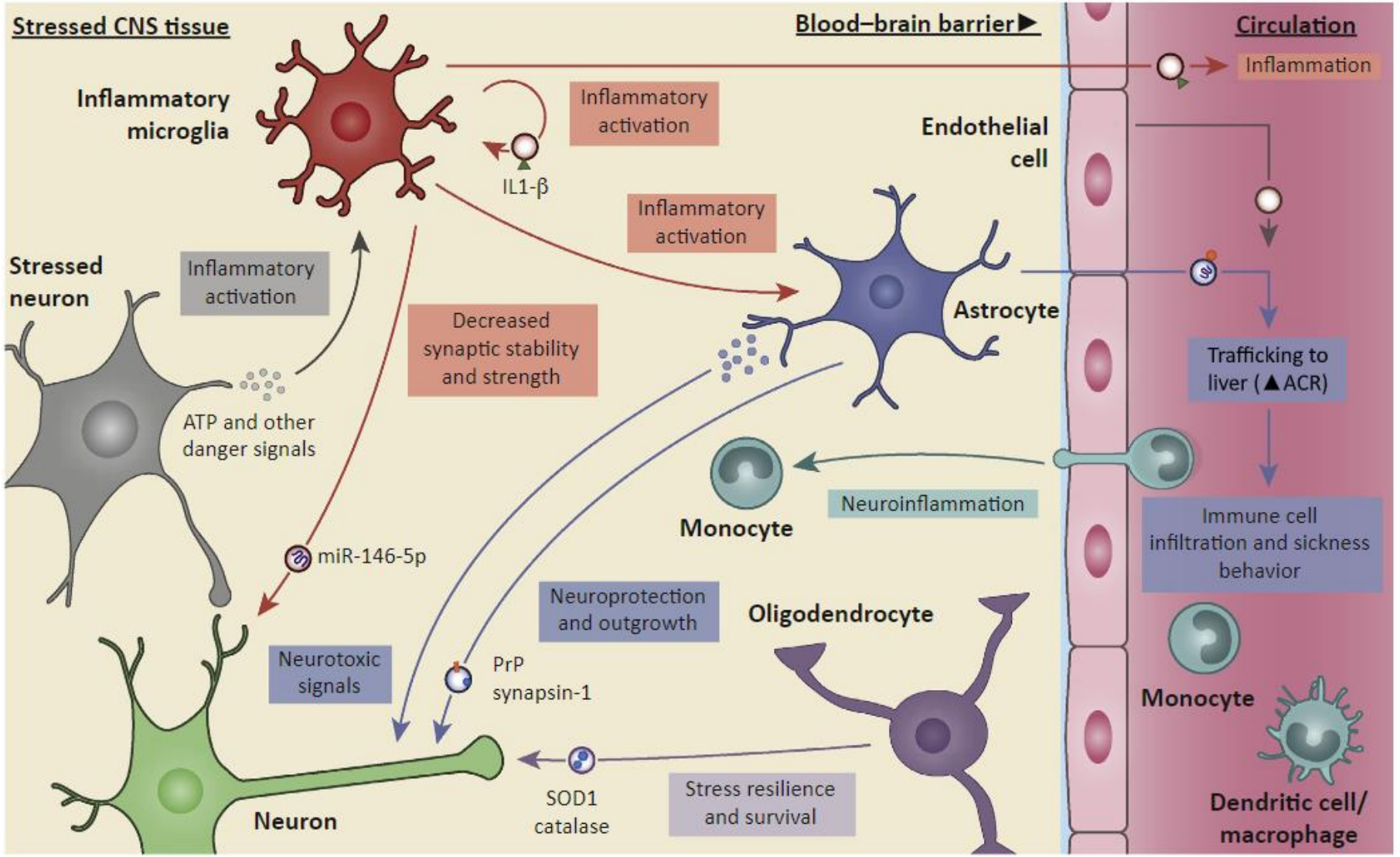



**Aspects of disease affected by the function and contents of extracellular vesicles**

| Cancer | Cardiometabolic disease | Neurologic disease | Infectious disease |
|---|---|---|---|
| • Chemotherapy resistance<br>• Oncogenesis<br>• Tumor immunity<br>• Metastatic disease | • Cardiomyocyte size<br>• Cardiovascular risk factors and prognosis<br>• Potentiation or attenuation of cardiac hypertrophy<br>• Allograft rejection<br>• Metabolic syndrome | • Neurodegenerative diseases<br>• Trauma<br>• Stroke | • Immune surveillance<br>• Response to therapy<br>• Early detection<br>• Tracking of disease activity |

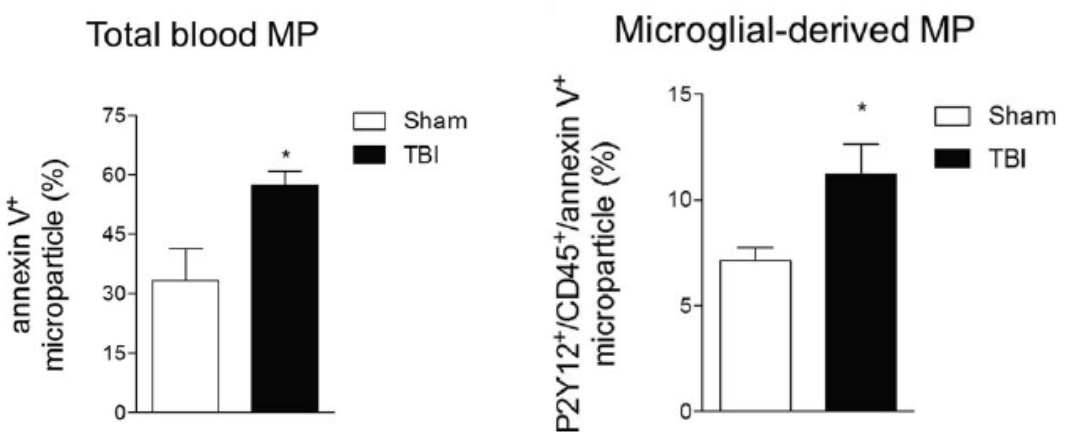
Shah et al. (2018), ***N Engl J Med***

# Bidirectional Cellular Crosstalk through EVs

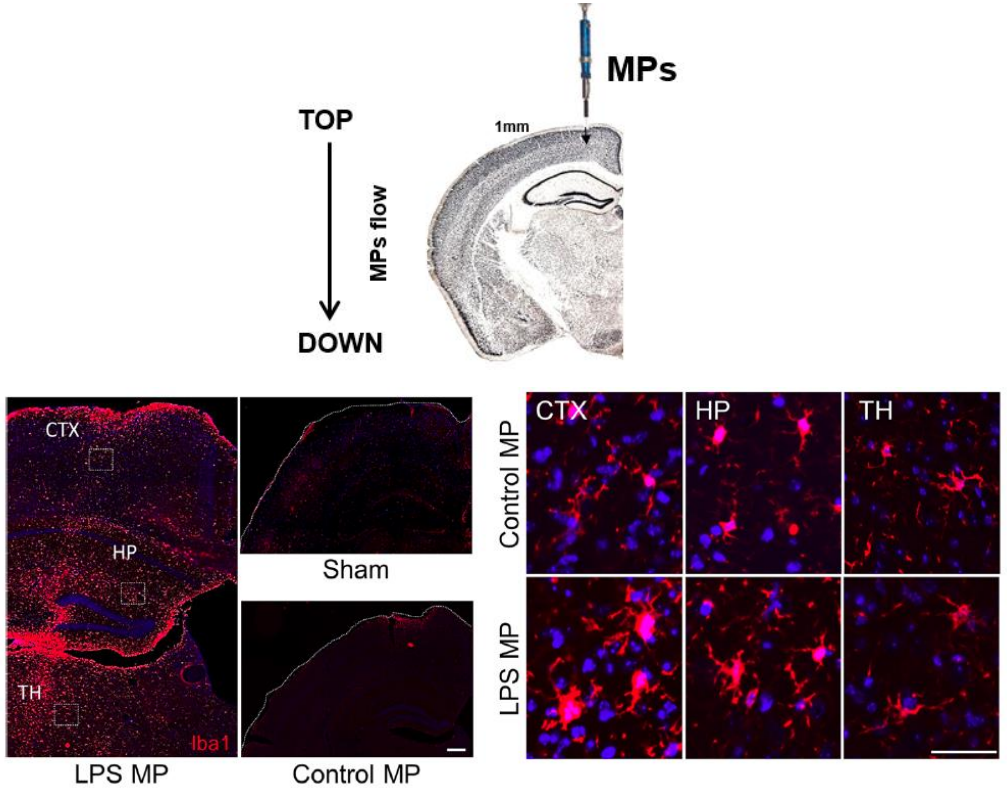



Holm et al. (2018), ***Trends Neurosci***

# EVs and Neuroinflammation after TBI




Kumar et al. (2017), ***J. Neuroinflammation***

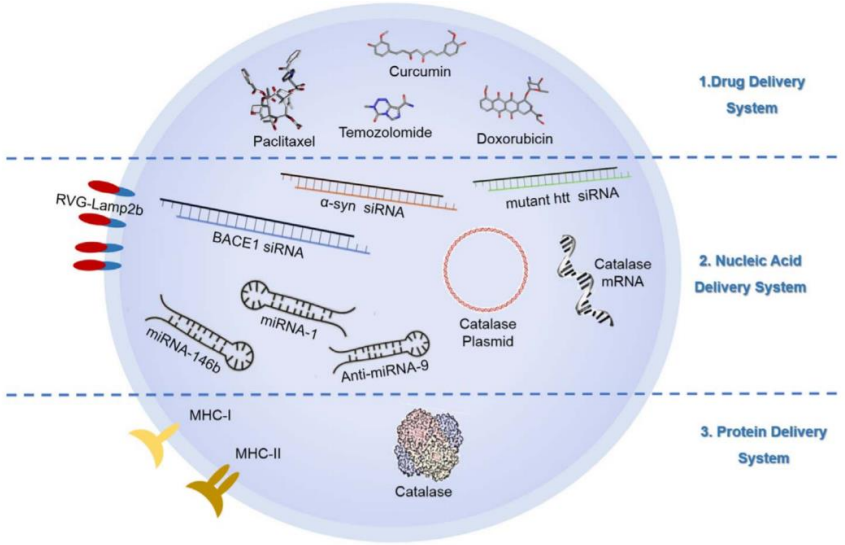


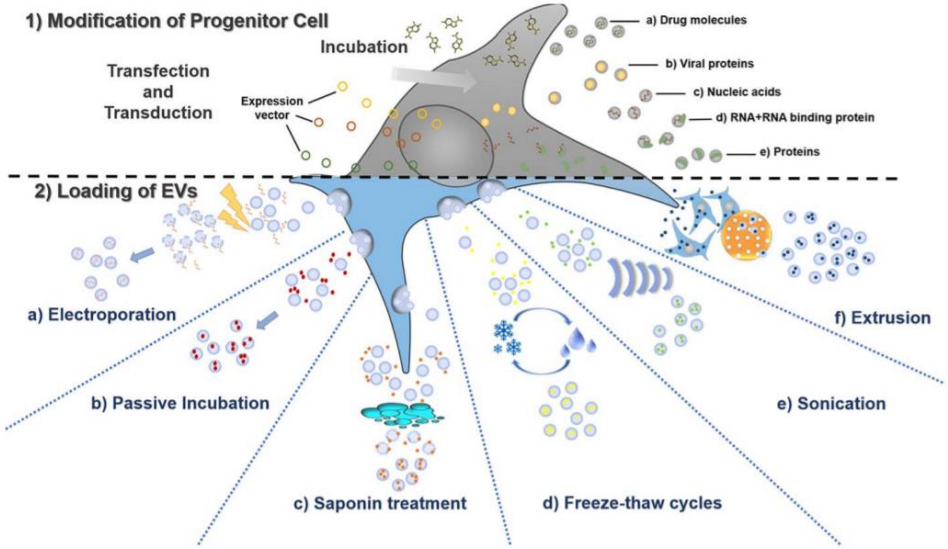

Kumar et al. (2017), ***J. Neuroinflammation***

**Blood microparticles (MPs) of microglial-origin analyzed by flow cytometry after TBI.**

**Pro-inflammatory microglia release MPs that can promote inflammatory activation.**

# EVs as Therapeutics

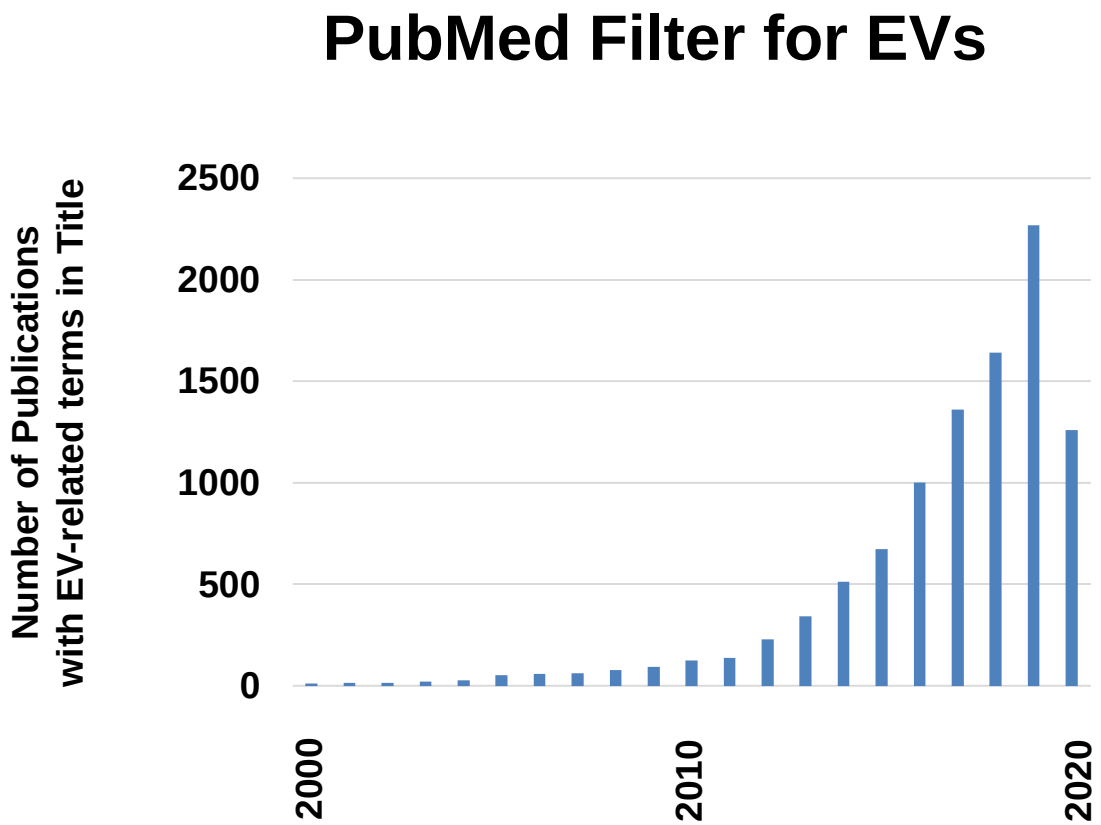



Rufino-Ramos et al. (2017), **J Controlled Release**

- Advantages over synthetic nanoparticle systems may include:
  - Can be Personalized
  - Long circulating half-life
  - Reduced immunogenicity
  - Inherent targeting capabilities
  - Ability to cross biological barriers such as the blood-brain barrier

# EV Research is Skyrocketing!



# Need for Standardization in EV Research

Journal of Extracellular Vesicles

COACTION

EDITORIAL

## Minimal experimental requirements for definition of extracellular vesicles and their functions: a position statement from the International Society for Extracellular Vesicles

Secreted membrane-enclosed vesicles, collectively called extracellular vesicles (EVs), which include exosomes, ectosomes, microvesicles, microparticles, apoptotic bodies and other EV subsets, encompass a very rapidly growing scientific field in biology and medicine. Importantly, it is currently technically challenging to obtain a totally pure EV fraction free from non-vesicular components for functional studies, and therefore there is a need to establish guidelines for analyses of these vesicles and reporting of scientific studies on EV biology. Here, the International Society for Extracellular Vesicles (ISEV) provides researchers with a minimal set of biochemical, biophysical and functional standards that should be used to attribute any specific biological cargo or functions to EVs.

Keywords: *extracellular vesicles*; *microvesicles*; *microparticles*; *exosomes*; *ectosomes*; *extracellular RNA*

JOURNAL OF EXTRACELLULAR VESICLES
2018, VOL. 7, 1535750
https://doi.org/10.1080/20013078.2018.1535750

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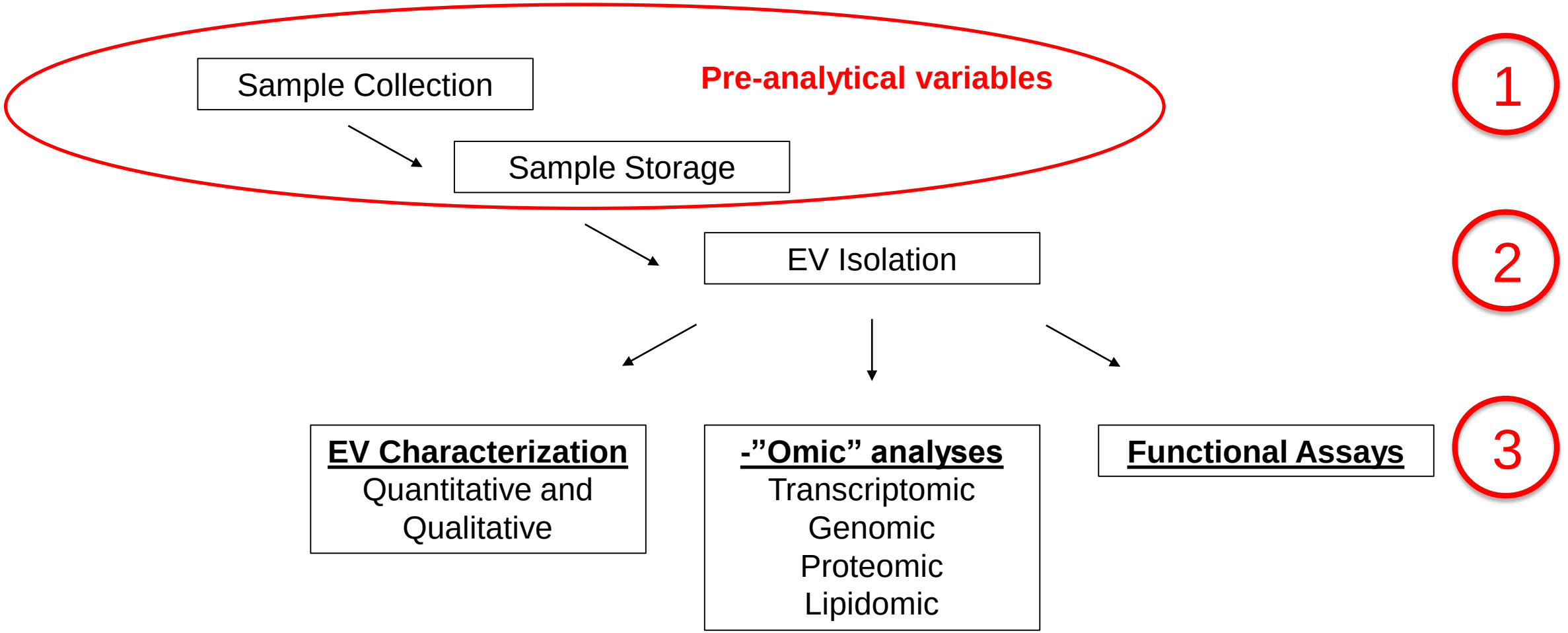
## Minimal information for studies of extracellular vesicles 2018 (MISEV2018): a position statement of the International Society for Extracellular Vesicles and update of the MISEV2014 guidelines

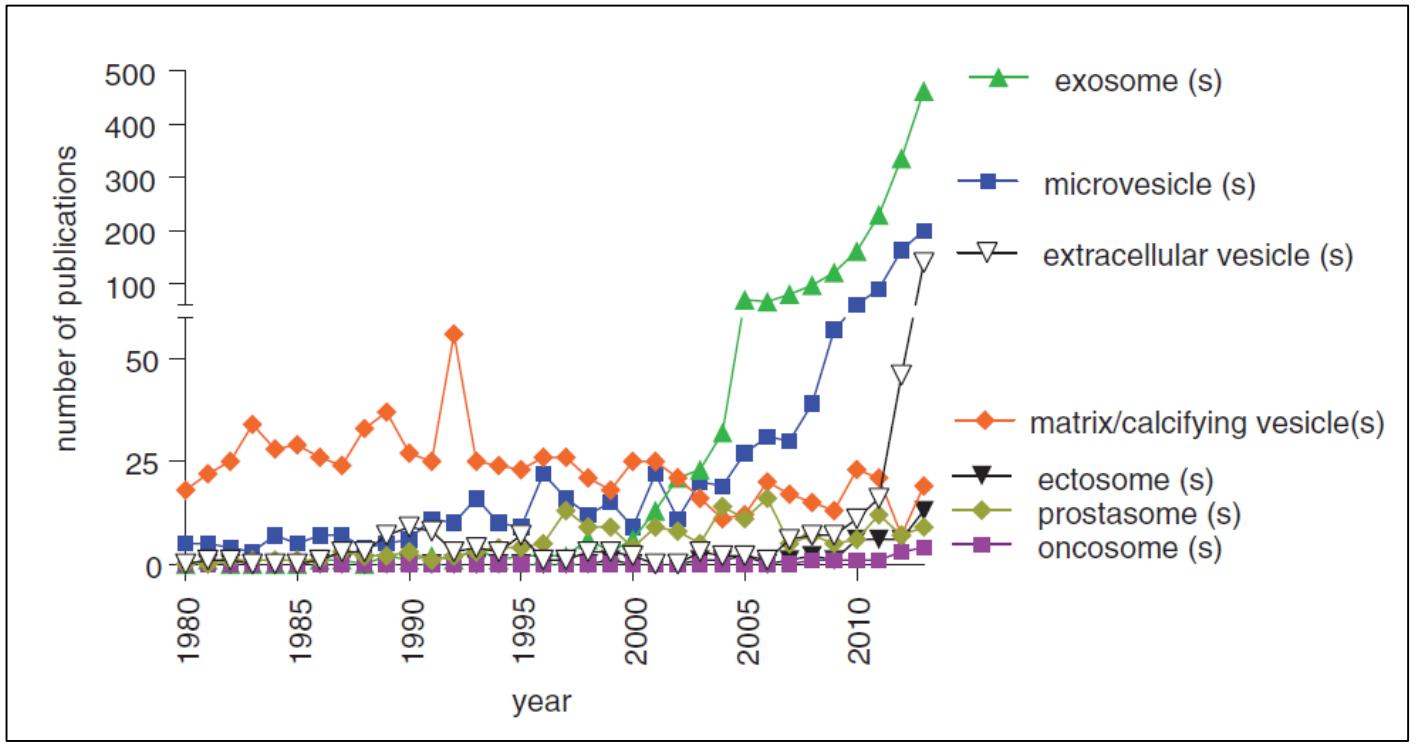
Clotilde Théry[103*£], Kenneth W Witwer[217,218*&£], Elena Aikawa[19,79£], Maria Jose Alcaraz[112], Johnathon D Anderson[288], Ramaroson Andriantsitohaina[97], Anna Antoniou[70,265], Tanina Arab[257], Fabienne Archer[318], Georgia K Atkin-Smith[131], D Craig Ayre[15,158], Jean-Marie Bach[254], Daniel Bachurski[301], Hossein Baharvand[195,353], Leonora Balaj[143], Shawn Baldacchino[321], Natalie N Bauer[354], Amy A Baxter[131], Mary Bebawy[357], Carla Beckham[350], Apolonija Bedina Zavec[165], Abderrahim Benmoussa[260], Anna C Berardi[179], Paolo Bergese[39,111,283], Ewa Bielska[282], Cherie Blenkiron[277&], Sylwia Bobis-Wozowicz[119], Eric Boilard[260], Wilfrid Boireau[58], Antonella Bongiovanni[106], Francesc E Borràs[72,73,250], Steffi Bosch[254], Chantal M Boulanger[100,261£], Xandra Breakefield[140], Andrew M Breglio[92,169], Meadhbh Á Brennan[82,144,258], David R Brigstock[174,221], Alain Brisson[238$], Marike LD Broekman[78,134,142], Jacqueline F Bromberg[155,379], Paulina Bryl-Górecka[136], Shilpa Buch[334], Amy H Buck[305], Dylan Burger[128,180,337], Sara Busatto[148,283], Dominik Buschmann[212], Benedetta Bussolati[360], Edit I Buzás[160,201&£], James Bryan Byrd[330], Giovanni Camussi[359£], David RF Carter[181], Sarah Caruso[131], Lawrence W Chamley[279], Yu-Ting Chang[170], Amrita Datta Chaudhuri[218], Chihchen Chen[171,172], Shuai Chen[133], Lesley Cheng[131], Andrew R Chin[25], Aled Clayton[23], Stefano P Clerici[239], Alex Cocks[23], Emanuele Cocucci[220,222£], Robert J Coffey[373], Anabela Cordeiro-da-Silva[346], Yvonne Couch[340], Frank AW Coumans[7$], Beth Coyle[227], Rossella Crescitelli[308], Miria Ferreira Criado[352], Crislyn D'Souza-Schorey[335], Saumya Das[141], Paola de Candia[116], Eliezer F De Santana Junior[225], Olivier De Wever[22,75], Hernando A del Portillo[101,104,117], Tanguy Demaret[256], Sarah Deville[262,377], Andrew Devitt[12], Bert Dhondt[22,74,75], Dolores Di Vizio[25&£], Lothar C Dieterich[49], Vincenza Dolo[315], Ana Paula Dominguez Rubio[243], Massimo Dominici[234,333#], Mauricio R Dourado[298,338], Tom AP Driedonks[369], Filipe V Duarte[53], Heather M Duncan[150,152], Ramon M Eichenberger[120], Karin Ekström[306], Samir EL Andaloussi[51,127], Celine Elie-Caille[58], Uta Erdbrügger[366&], Juan M Falcón-Pérez[32,94&], Farah Fatima[351], Jason E Fish[233,362], Miguel Flores-Bellver[302], András Försönits[201], Annie Frelet-Barrand[58], Fabia Fricke[68,267], Gregor Fuhrmann[86,87,197], Susanne Gabrielsson[126], Ana Gámez-Valero[72,251], Chris Gardiner[264&], Kathrin Gärtner[85], Raphael Gaudin[99,259], Yong Song Gho[187£], Bernd Giebel[266#], Caroline Gilbert[260], Mario Gimona[183], Ilaria Giusti[315], Deborah CI Goberdhan[339], André Görgens[51,123,266$], Sharon M Gorski[16,204], David W Greening[131], Julia Christina Gross[270,271], Alice Gualerzi[115], Gopal N Gupta[135], Dakota Gustafson[362], Aase Handberg[24], Reka A Haraszti[325], Paul Harrison[281], Hargita Hegyesi[201], An Hendrix[22,75], Andrew F Hill[131&£], Fred H Hochberg[200,293], Karl F Hoffmann[6], Beth Holder[95,159], Harry Holthofer[263£], Baharak Hosseinkhani[83], Guoku Hu[334], Yiyao Huang[162,217], Veronica Huber[61], Stuart Hunt[229], Ahmed Gamal-Eldin Ibrahim[26], Tsuneya Ikezu[18], Jameel M Inal[313], Mustafa Isin[118], Alena Ivanova[69], Hannah K Jackson[227], Soren Jacobsen[38,304], Steven M Jay[324], Muthuvel Jayachandran[145], Guido Jenster[47], Lanzhou Jiang[131], Suzanne M Johnson[322], Jennifer C Jones[166$], Ambrose Jong[30,355], Tijana Jovanovic-Talisman[34], Stephanie Jung[71], Raghu Kalluri[358&], Shin-ichi Kano[219], Sukhbir Kaur[167], Yumi Kawamura[164,365], Evan T Keller[327,331], Delaram Khamari[201],

CONTACT Clotilde Théry Clotilde.Thery@curie.fr Institut Curie/INSERM U932, 26 rue d'Ulm, 75005 Paris, France; Kenneth W Witwer kwitwer1@jhmi.edu 733 North Broadway, MRB 829 Baltimore, MD 21205, USA

# Workflow in EV Research



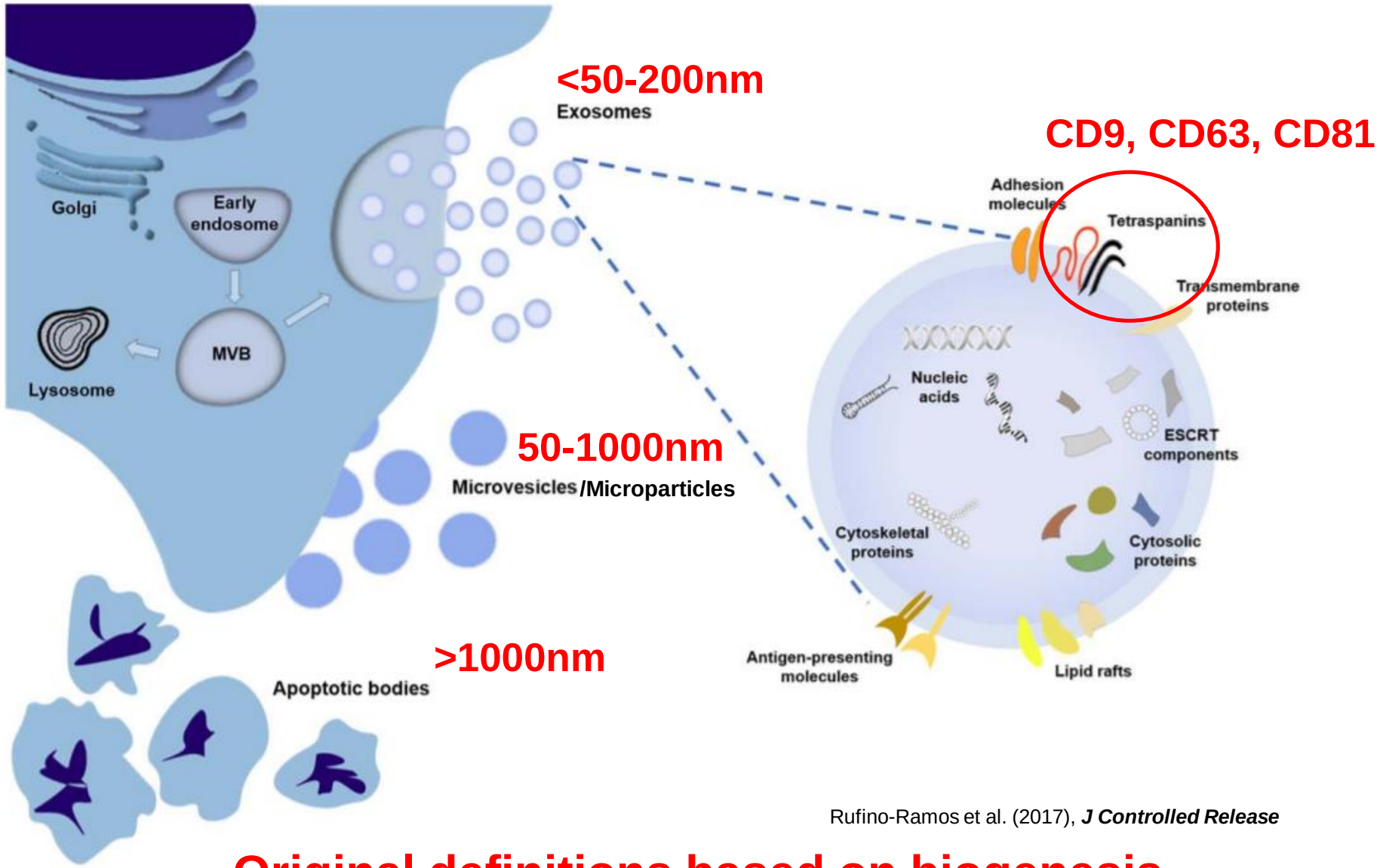
# EV Nomenclature




ISEV (2014), ***J Extracell Vesicles***

**Extracellular vesicle (EV) is the umbrella term endorsed by ISEV**

# Classification of EVs




Rufino-Ramos et al. (2017), ***J Controlled Release***

**Original definitions based on biogenesis**

# Classification of EVs

| Centrifugation Steps | | *Traditional definitions* |
|---|---|---|
| 1. 1000*g*, 10 min | → | *Cell Debris* |
| 2. 2000*g*, 20 min | → | *Apoptotic Bodies* |
| 3. 10,000*g*, 30 min | → | *Microvesicles* |
| 4. 100,000*g*, 2 hr | → | *Exosomes* |


Current ISEV recommendations

- No current isolation protocol can purify based on biogenetic origin
- Size is not an appropriate defining feature alone
- Describe EVs based on
  - Physical characteristics
    - Size: Large EVs, medium EVs, small EVs
  - Biochemical characteristics
  - Cell origin or stimulus condition

**Original definitions based on biogenesis and physical separation**

# EV Isolation

SCIENTIFIC REPORTS

OPEN

## Low-density lipoprotein mimics blood plasma-derived exosomes and microvesicles during isolation and detection

Received: 14 January 2016
Accepted: 21 March 2016
Published: 18 April 2016

Barbara W Sódar[1], Ágnes Kittel[2], Krisztina Pálóczi[1], Krisztina V Vukman[1], Xabier Osteikoetxea[1], Katalin Szabó-Taylor[1], Andrea Németh[1], Beáta Sperlágh[2], Tamás Baranyai[3], Zoltán Giricz[3], Zoltán Wiener[1], Lilla Turiák[4], László Drahos[4], Éva Pállinger[1], Károly Vékey[4], Péter Ferdinandy[3], András Falus[1] & Edit Irén Buzás[1]

Cell

Article

## Reassessment of Exosome Composition

Dennis K. Jeppesen,[1] Aidan M. Fenix,[2] Jeffrey L. Franklin,[1,2,8] James N. Higginbotham,[1] Qin Zhang,[1] Lisa J. Zimmerman,[3] Daniel C. Liebler,[3] Jie Ping,[4] Qi Liu,[4] Rachel Evans,[5] William H. Fissell,[5] James G. Patton,[6] Leonard H. Rome,[7] Dylan T. Burnette,[2] and Robert J. Coffey[1,2,8,9,*]

[1]Department of Medicine, Vanderbilt University Medical Center, Nashville, TN 37232, USA
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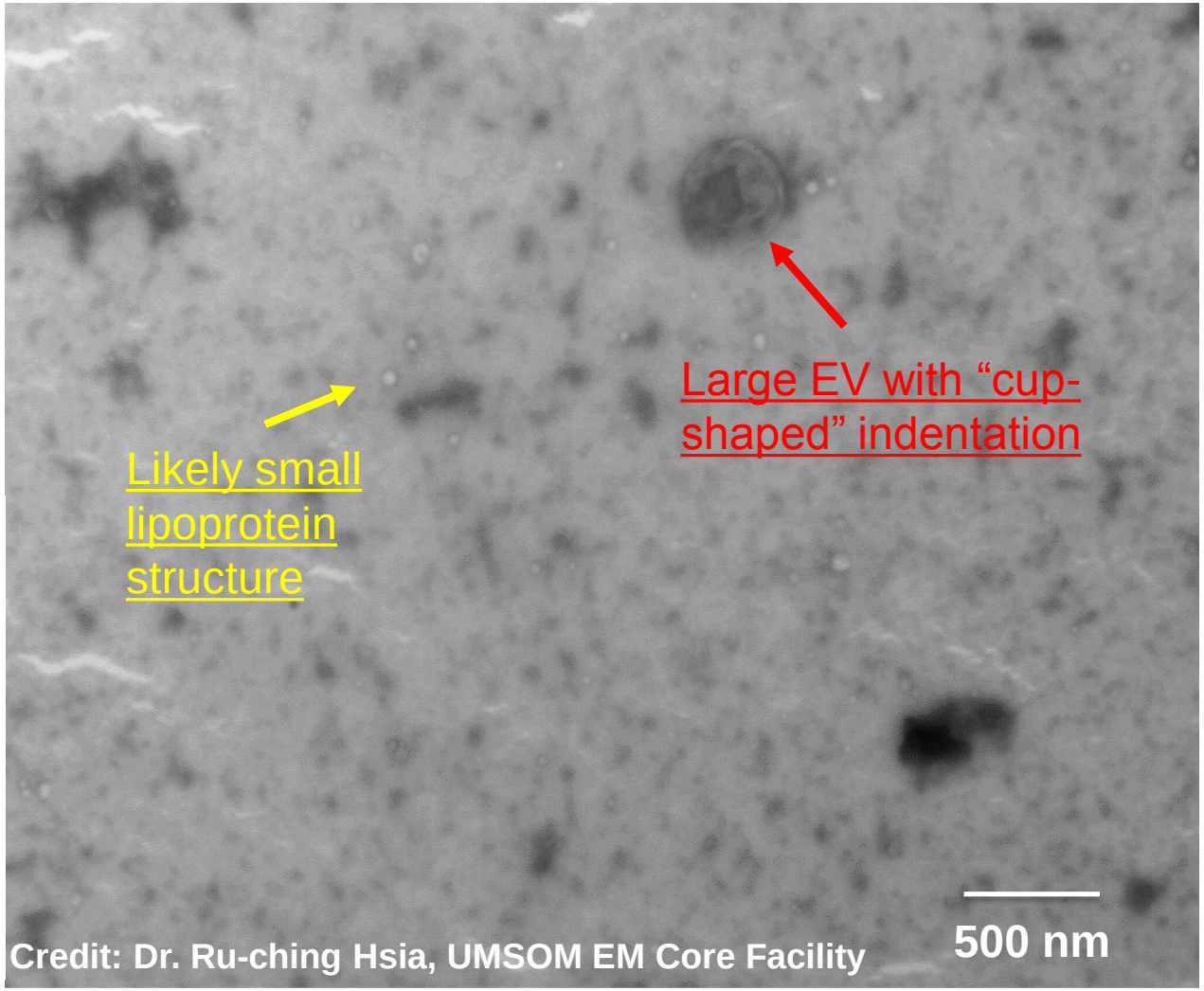
[5]Division of Nephrology and Hypertension, Vanderbilt University Medical Center, Nashville, TN 37232, USA
[6]Department of Biological Sciences, Vanderbilt University, Nashville, TN 37235, USA
[7]Department of Biological Chemistry, David Geffen School of Medicine and the California NanoSystems Institute, University of California Los Angeles, Los Angeles, CA 90095, USA
[8]Veterans Affairs Medical Center, Nashville, TN 37232, USA
[9]Lead Contact
*Correspondence: robert.coffey@vumc.org
https://doi.org/10.1016/j.cell.2019.02.029




**Ultracentrifugation has been the gold-standard procedure but lacks purity**

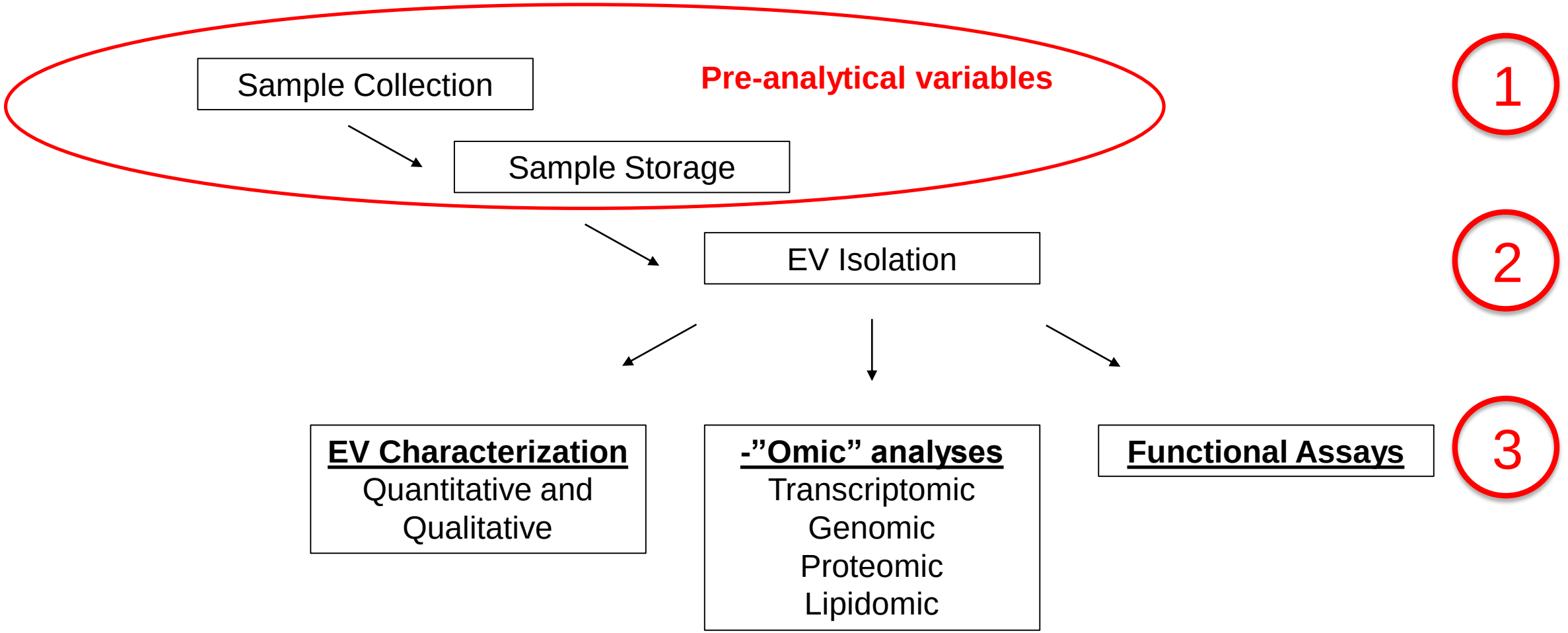
# EV Isolation

| | Differential Centrifugation (DC) | Density Gradient Centrifugation (DGC) | Size Exclusion Chromatography (SEC) | Ultrafiltration (UF) | Immuno Capture (IC) | Precipitation (P) |
|---|---|---|---|---|---|---|
| **CONTAMINANTS** | Lipoproteins, protein aggregates, viruses | Lipoproteins (HDLs) | Lipoproteins, protein, protein aggregates, viruses | Same size particles | Soluble proteins | Protein |
| **MAJOR ARTEFACTS** | EV-particle aggregates | | | EV-particle aggregates | | Protein complex, EV - particle aggregates |
| **EV RECOVERY %** | 2 to 80 | 10 | 40 to 90 | 10 to 80 | | 90 |
| **ASSAY TIME (h)** | 3 to 9 | 16 to 90 | 0.3 | 0.5 | 4 to 20 | 0.3 to 12 |
| **SAMPLE VOL** | mL-L | µL-mL | µL-mL | | µL-mL | µL-mL |
| **CLINICAL APPLICABILITY** | NO | NO | YES | NO | YES | YES |

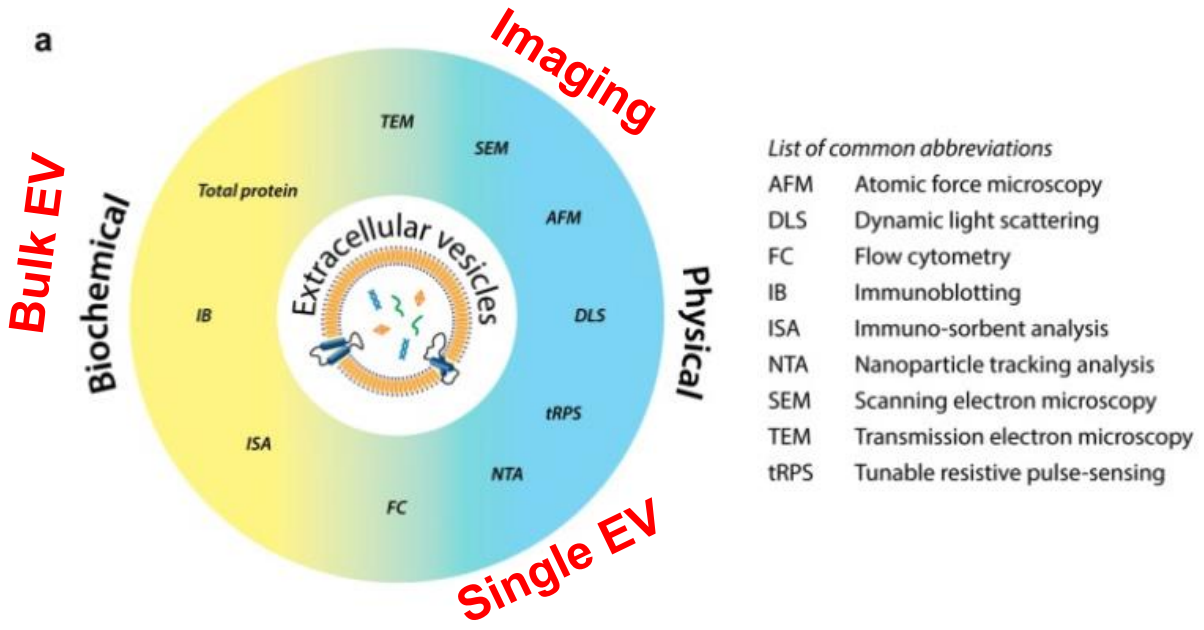
https://www.nanoviewbio.com/characterize

**Isolation methods have differing levels of recovery and purity**

# Workflow in EV Research

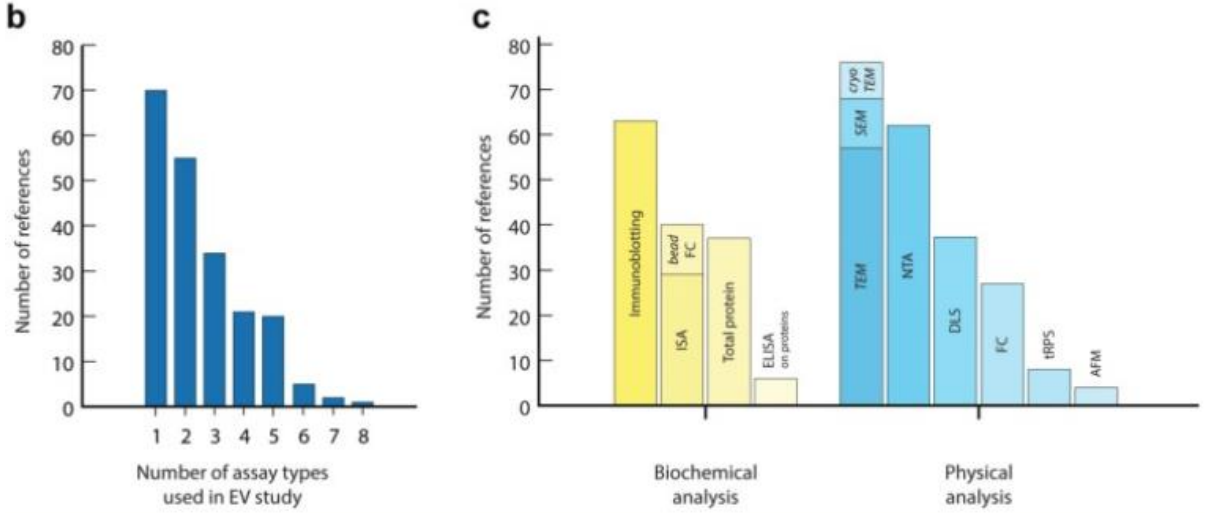


# EV Characterization Toolbox




*List of common abbreviations*

| | |
|---|---|
| AFM | Atomic force microscopy |
| DLS | Dynamic light scattering |
| FC | Flow cytometry |
| IB | Immunoblotting |
| ISA | Immuno-sorbent analysis |
| NTA | Nanoparticle tracking analysis |
| SEM | Scanning electron microscopy |
| TEM | Transmission electron microscopy |
| tRPS | Tunable resistive pulse-sensing |

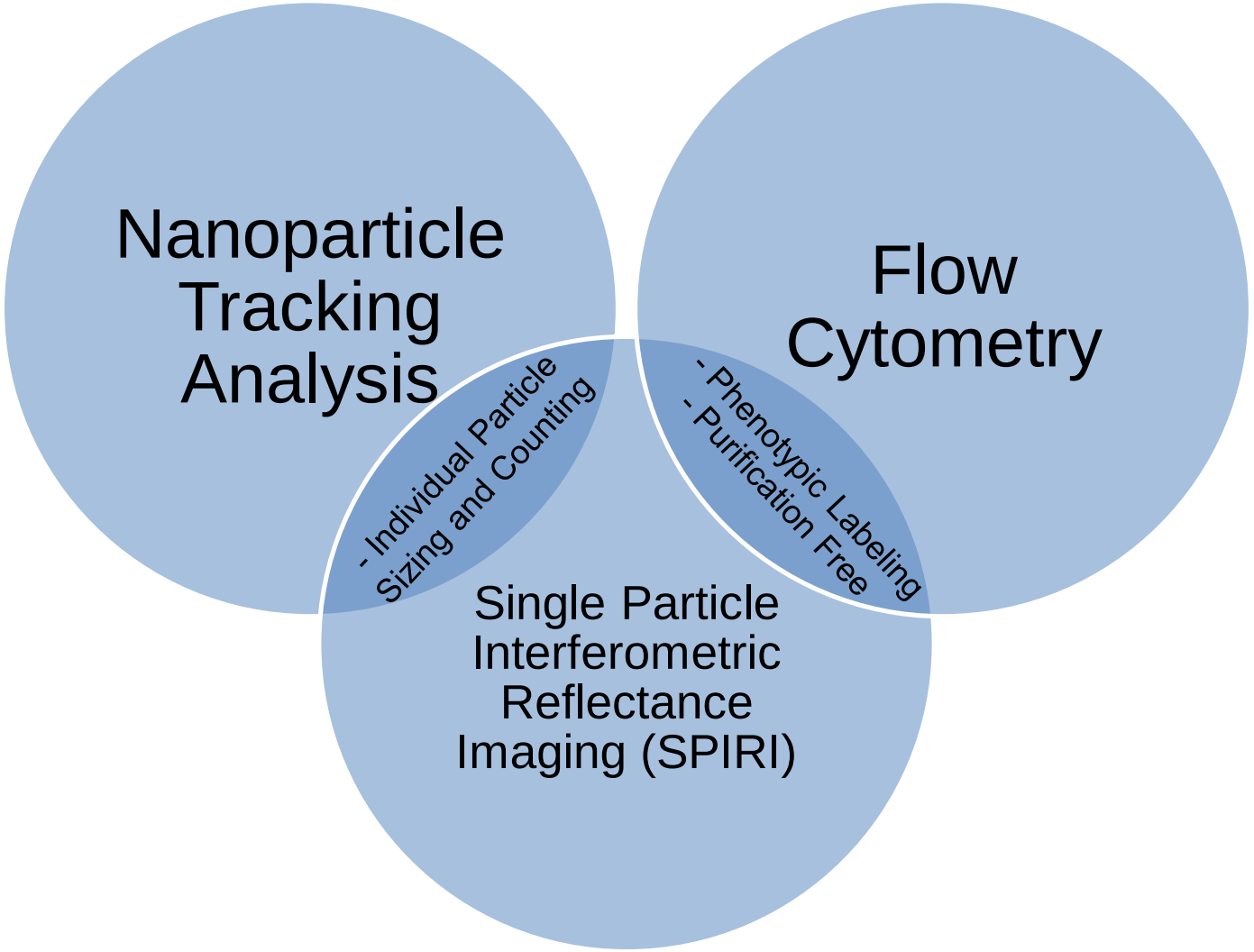



Hartjes et al. (2019), ***Bioengineering (Basel)***

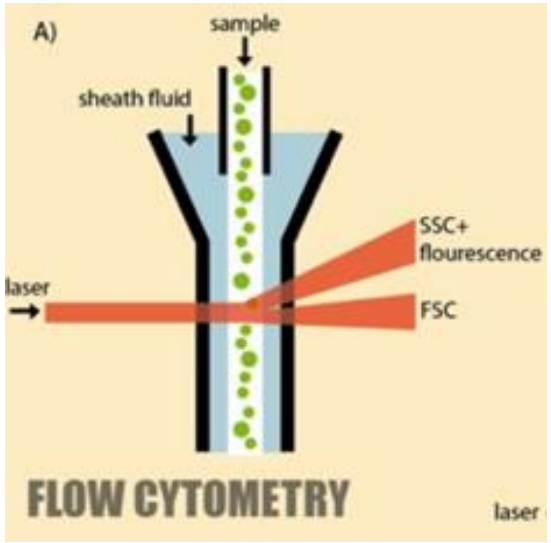
<u>Considerations in evaluating technology:</u>

- EV Size
- EV Count
- EV Phenotype
- EV Morphology/Visualization
- Single EV or Bulk analysis?
- Isolation or Direct detection?

# EV Characterization Toolbox

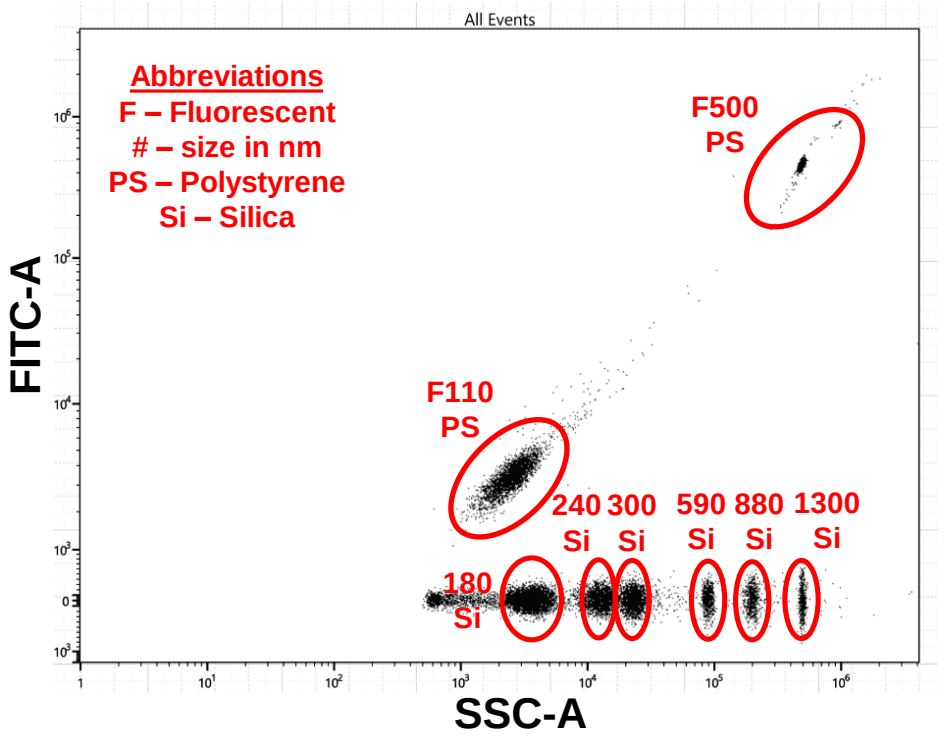


# Phenotyping EVs by Flow Cytometry



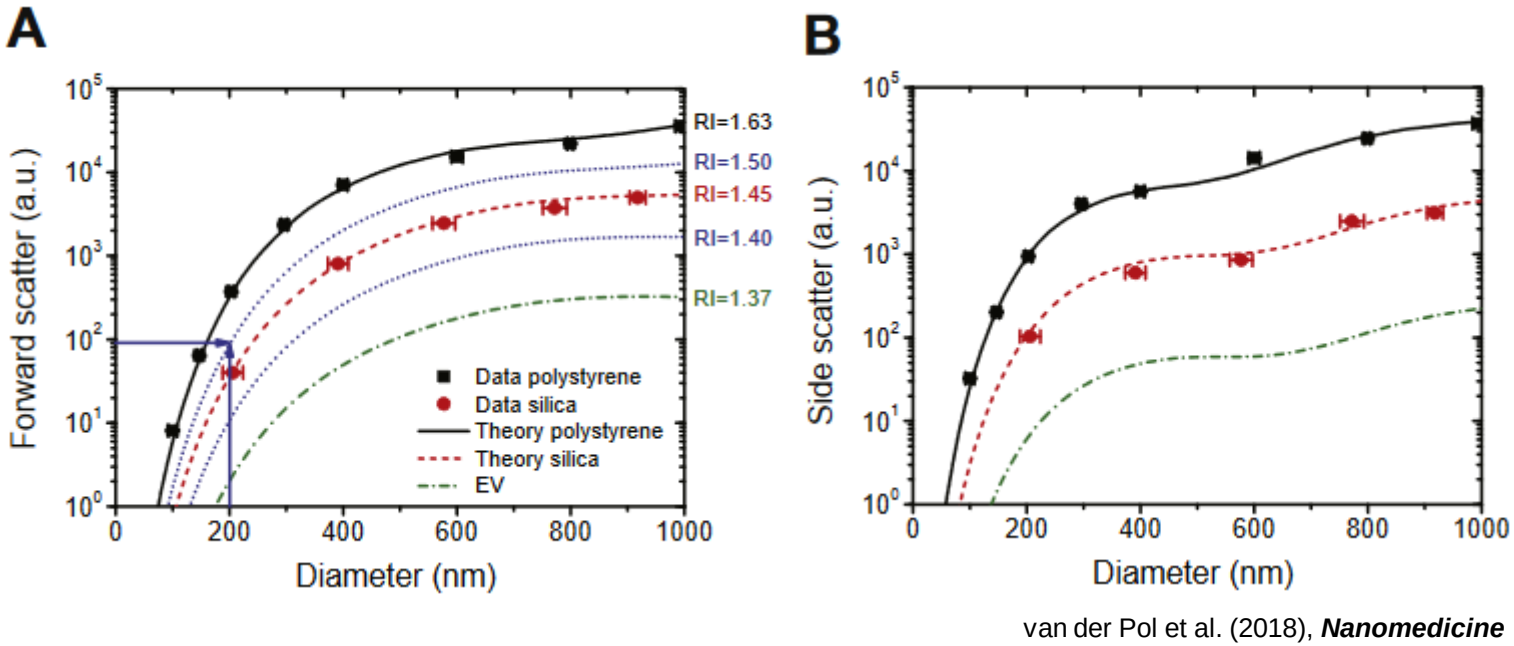

Erdbrügger et al. (2017), ***Cytometry A***


**Credit: Dr. Xiaoxuan Fan, UMSOM Flow Core Facility**

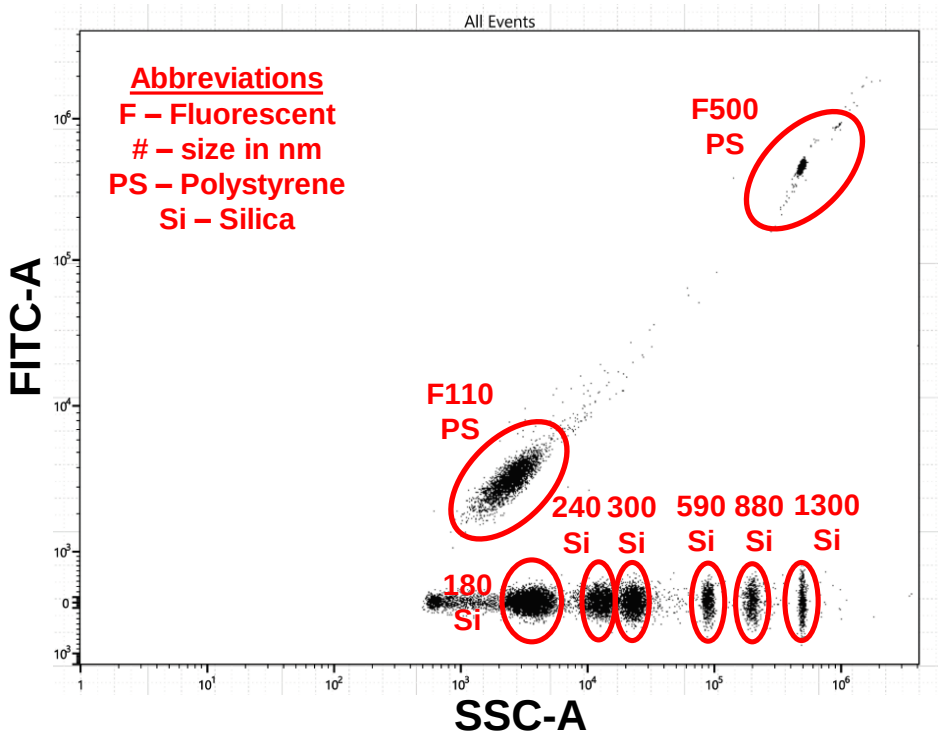



**Flow Cytometry provides excellent phenotyping capability but size resolution is a limitation, especially for small EVs**

# Phenotyping EVs by Flow Cytometry

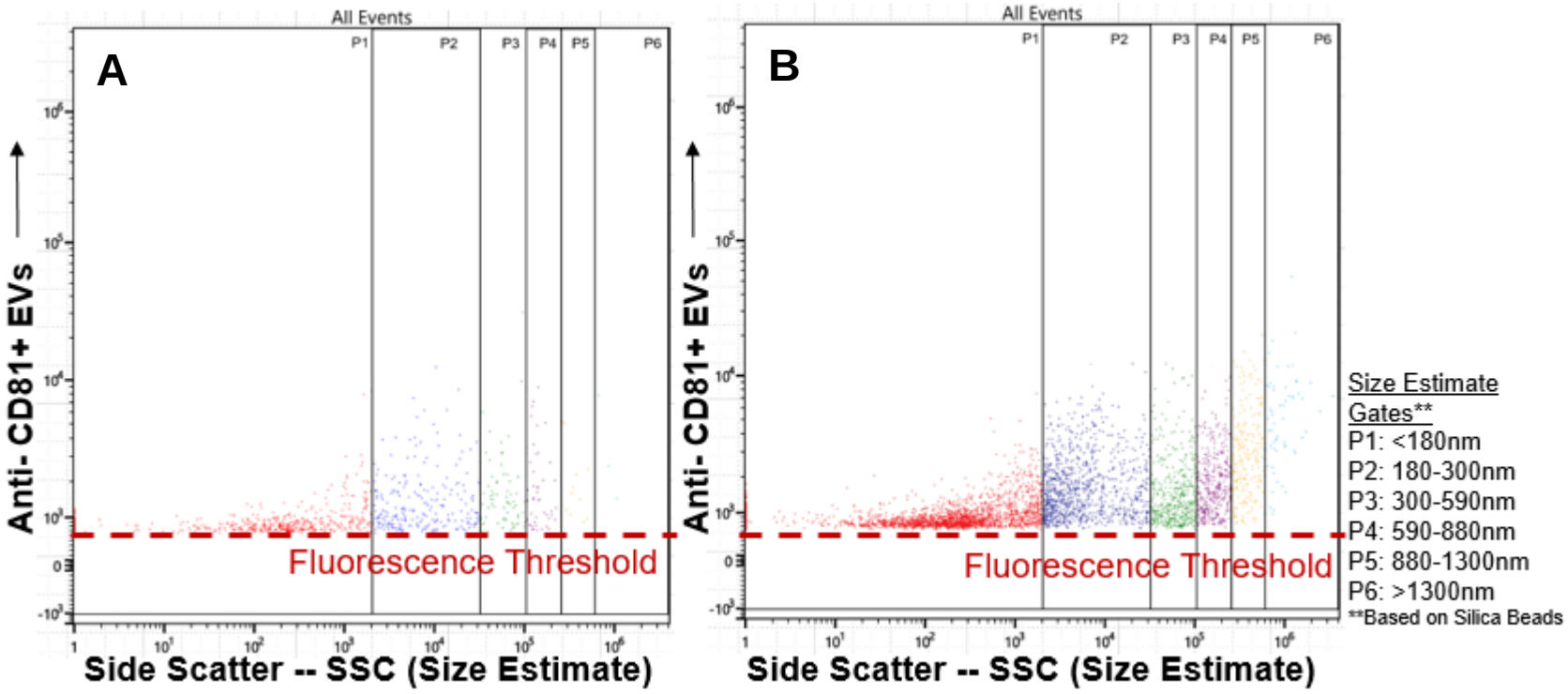


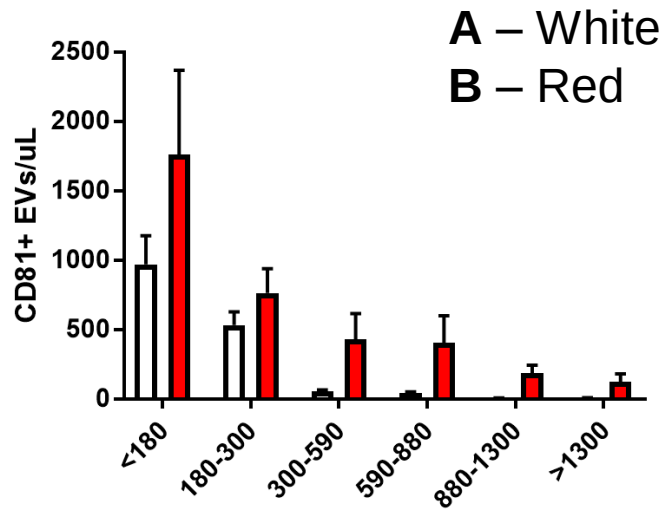

van der Pol et al. (2018), ***Nanomedicine***




**Sizing with beads to estimate EV size is inaccurate due to biophysical differences**

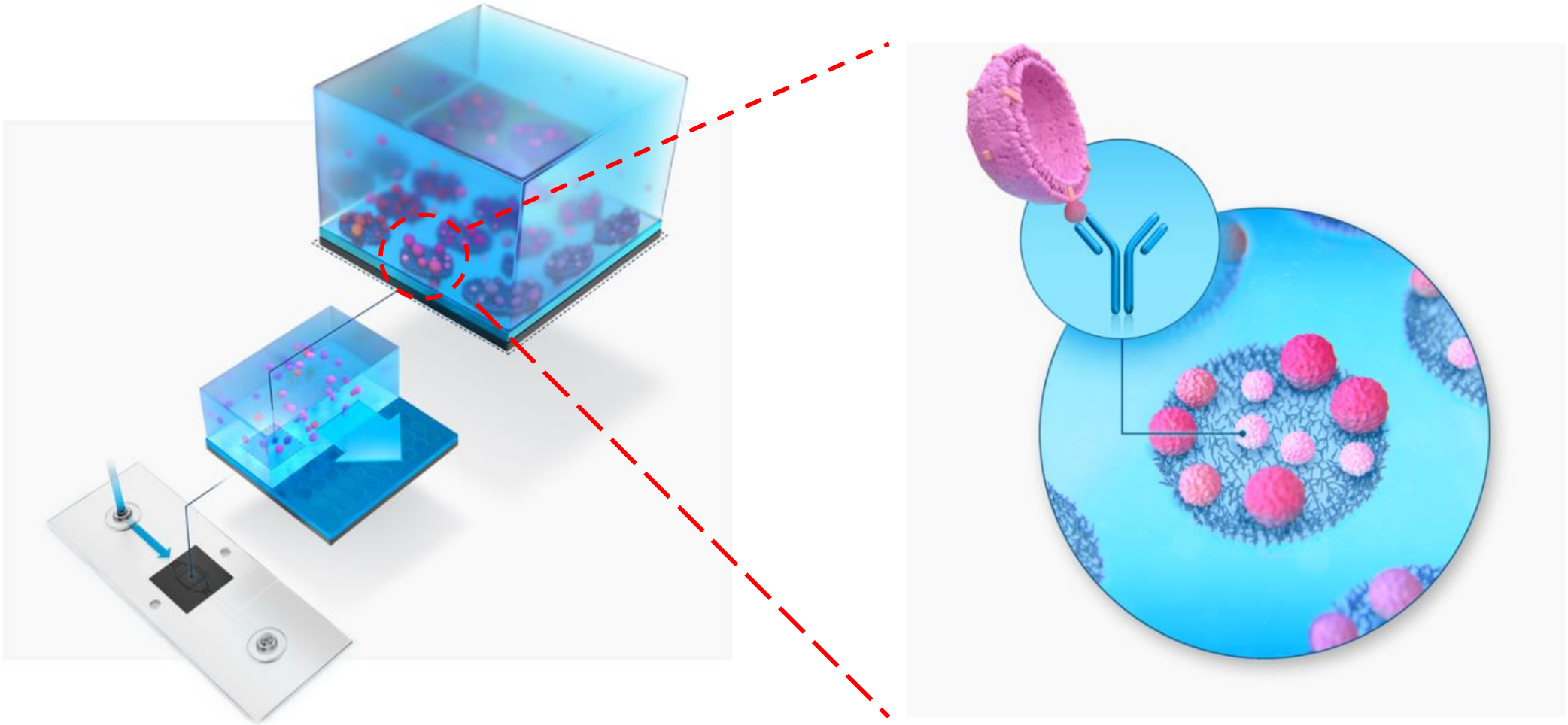
# Phenotyping EVs by Flow Cytometry



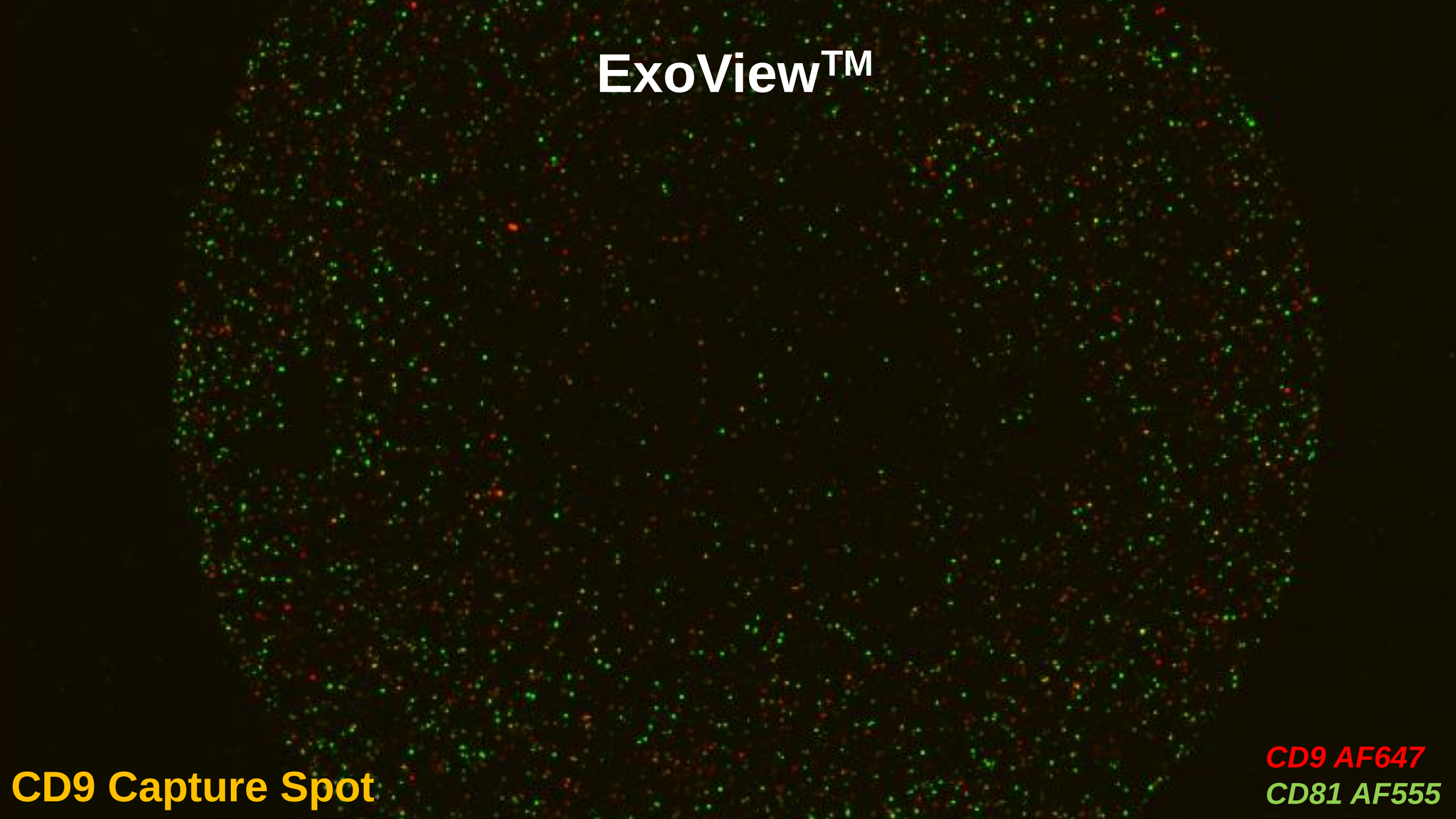




**Flow Cytometry provides excellent phenotyping capability but size resolution is a limitation, especially for small EVs**

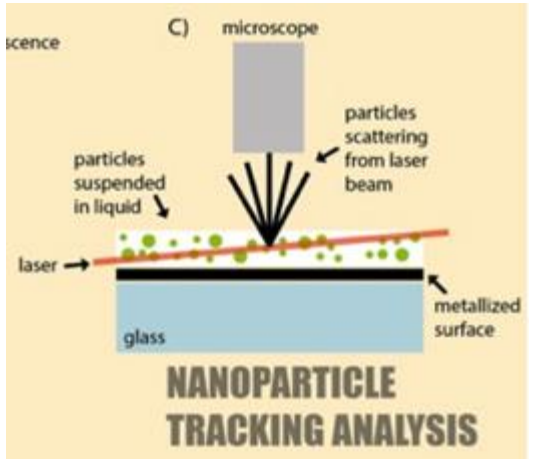
# ExoView® R100 Technology



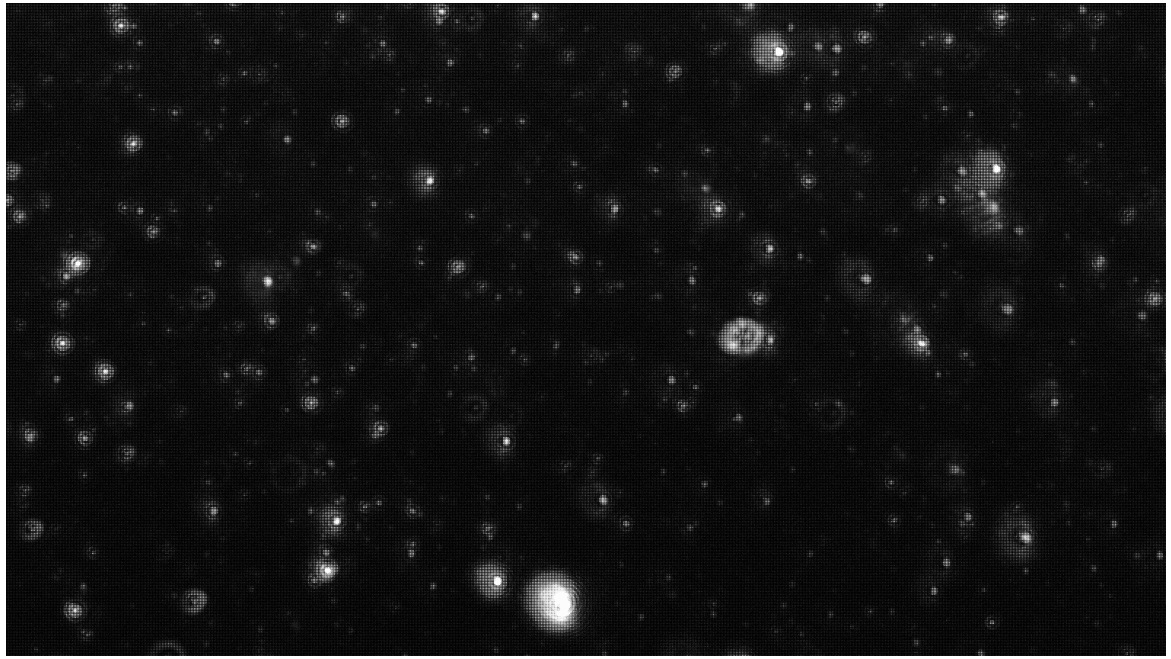
**Antibody capture and imaging of tetraspanin positive EVs -- CD9, CD63, CD81**


ExoView™
CD9 Capture Spot
CD9 AF647
CD81 AF555

# Nanoparticle Tracking Analysis for EVs




Erdbrügger et al. (2017), ***Cytometry A***



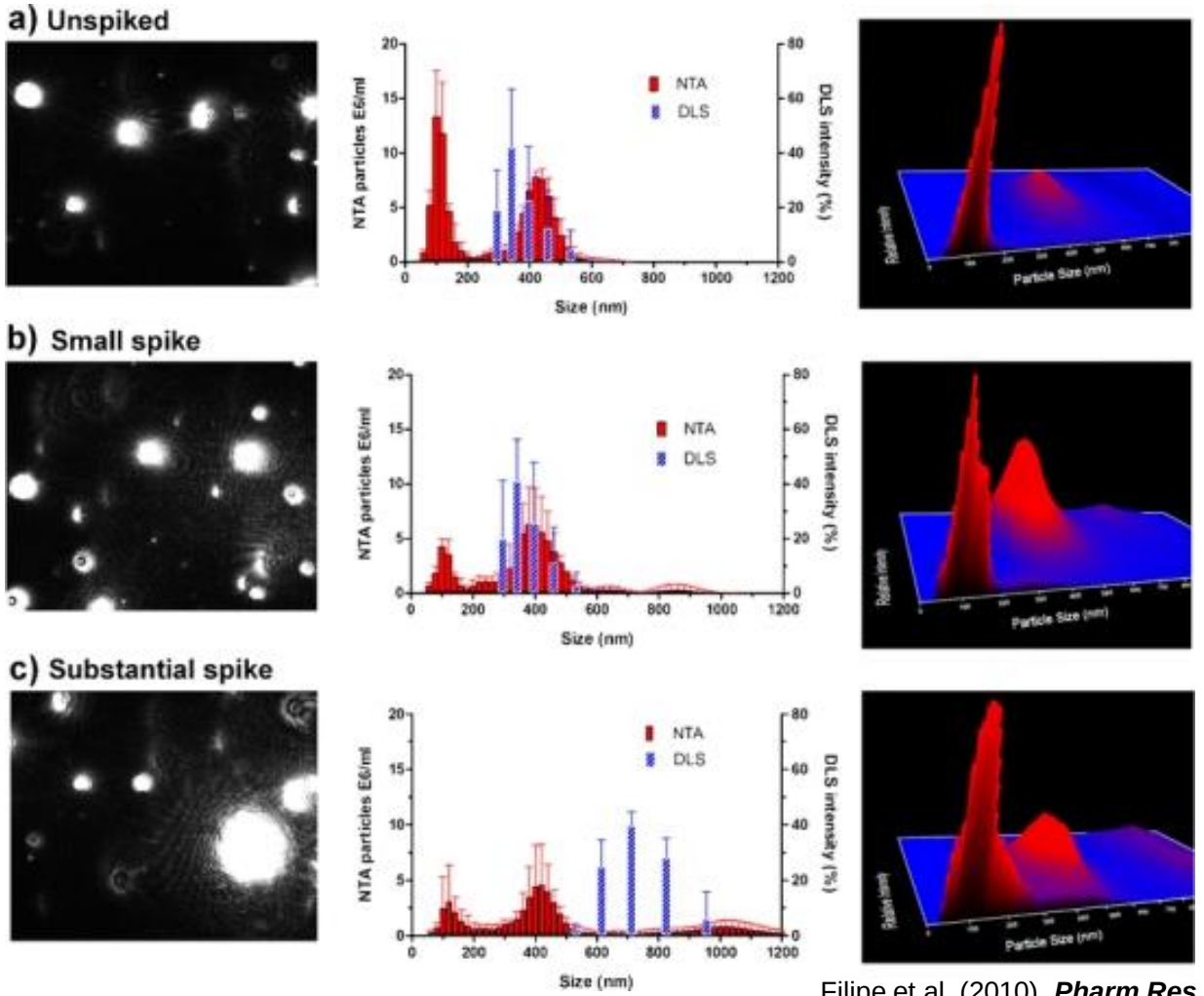
Stokes-Einstein equation

$$D = \frac{k_B T}{6\pi\mu R_0}$$

$D$ – diffusion coefficient
$\mu$ - solvent viscosity
$R_0$ – solute radius
$k_B$ – Boltzmann's constant
$T$ – temperature (K)

**NTA has been used extensively in EV research since the mid-2000s**

# Nanoparticle Tracking Analysis for EVs




Filipe et al. (2010), ***Pharm Res***

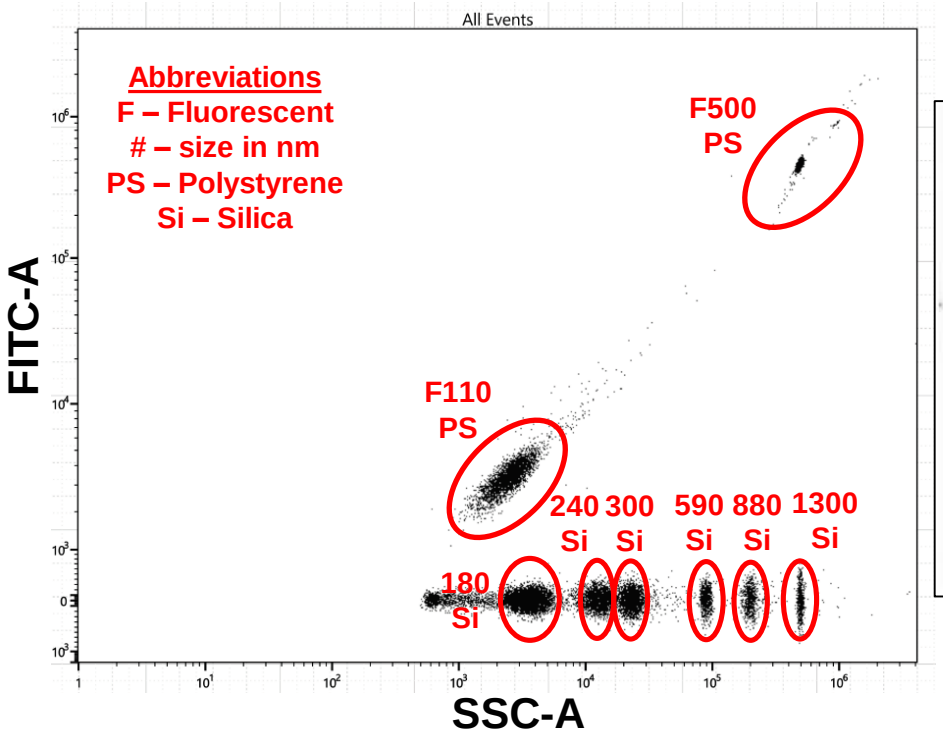
**NTA represented an important advance over DLS for polydisperse mixtures**

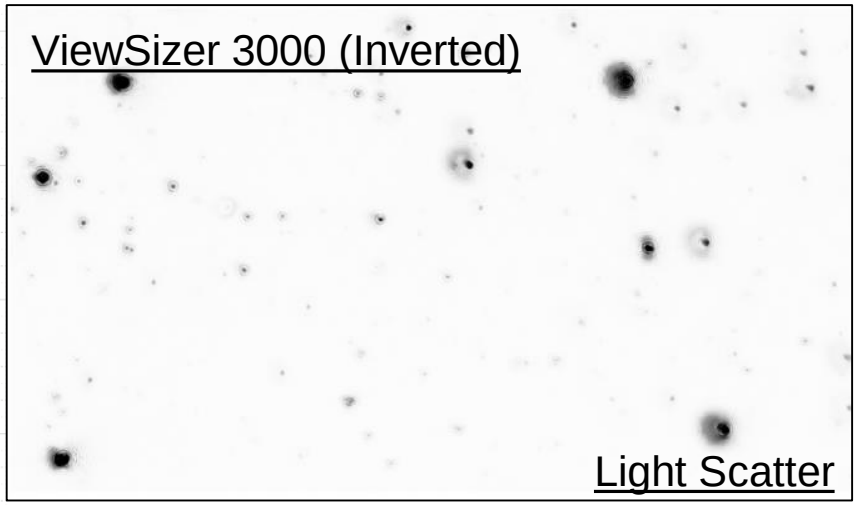
# Multi-Spectral Advanced NTA (MANTA) ViewSizer 3000

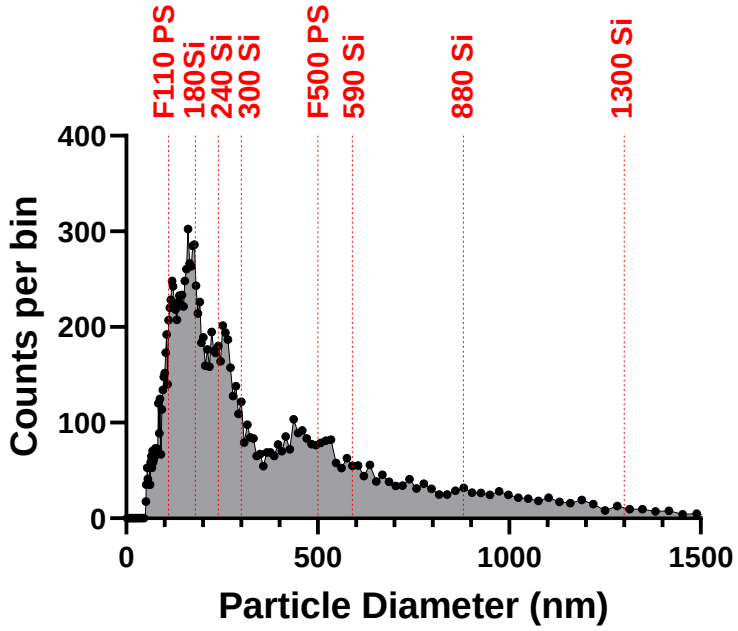

HORIBA Scientific

**ViewSizer can use three lasers simultaneously to visualize nanoparticle samples**

# ViewSizer 3000 Performance

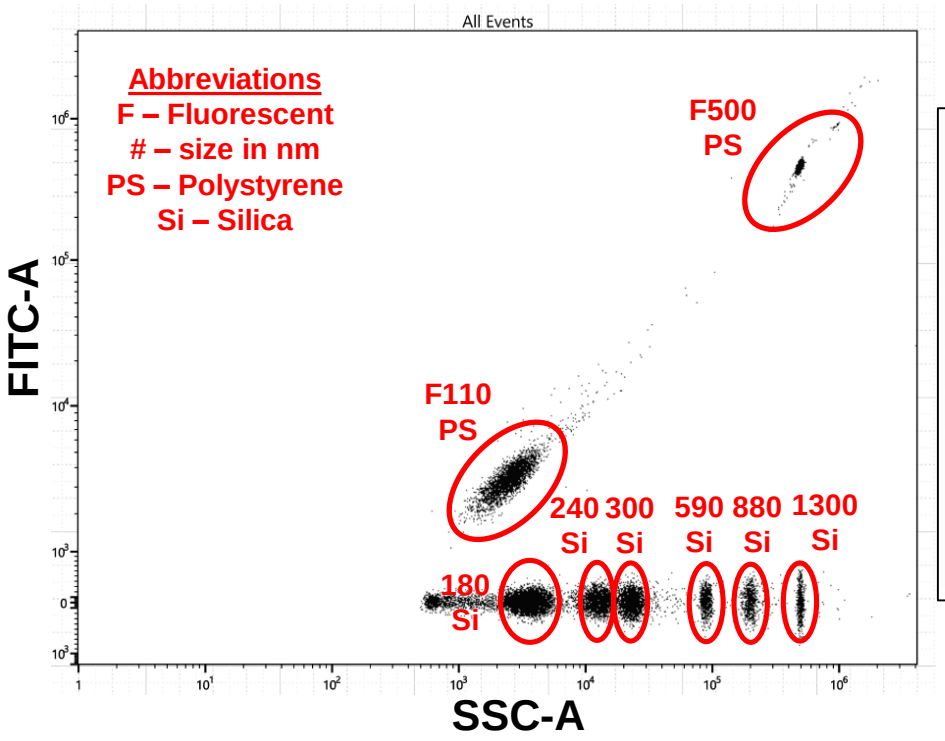


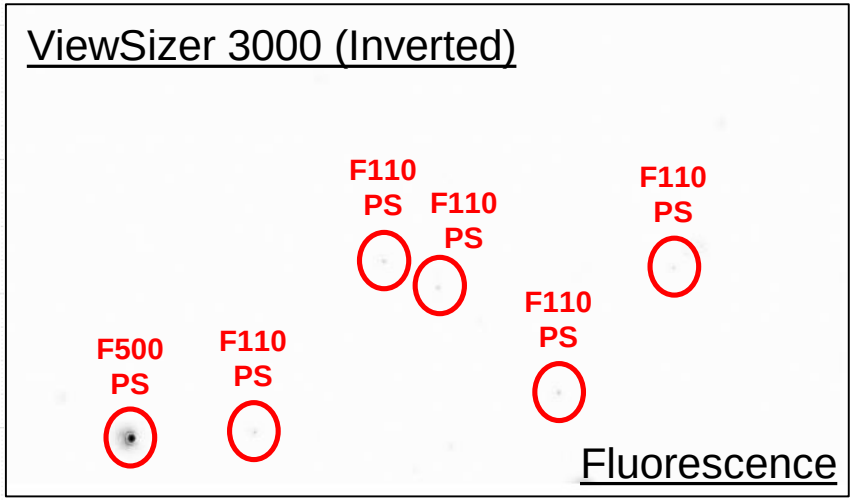



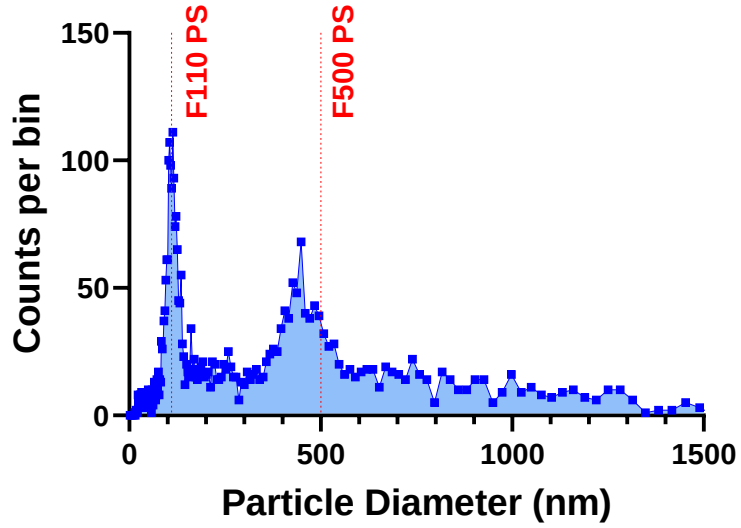




**ViewSizer can accurately resolve a complex, polydisperse bead mixture.**

# ViewSizer 3000 Performance

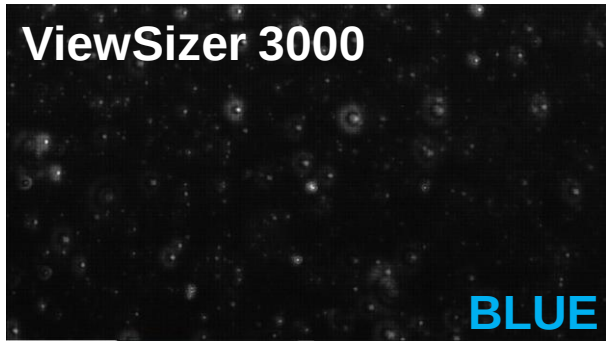


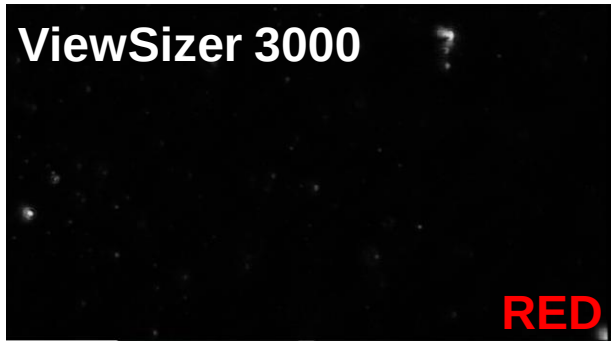



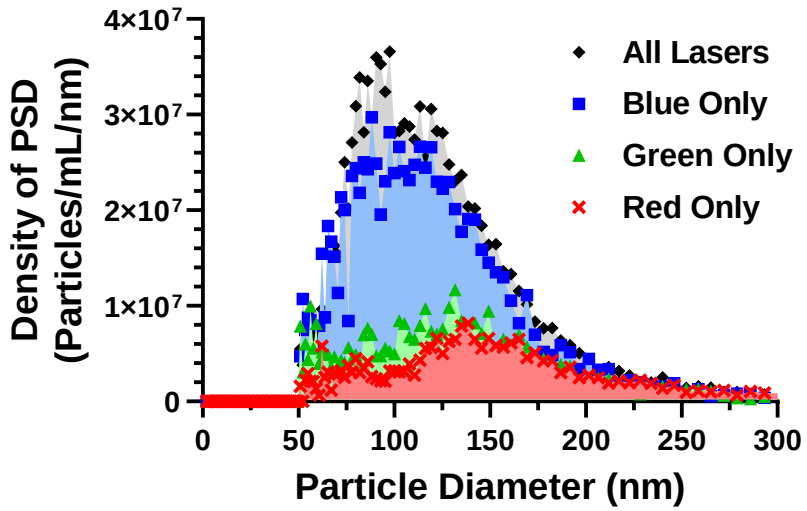

**ViewSizer can identify fluorescent particles uniquely out of a polydisperse mix.**

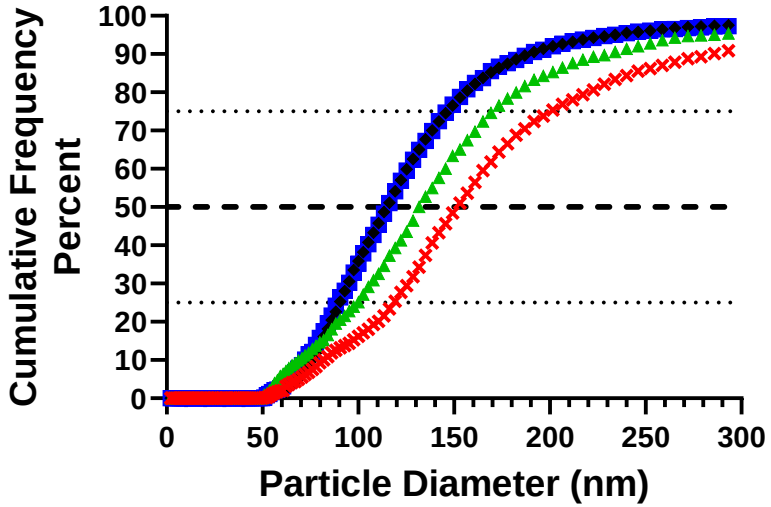
# Influence of Laser Wavelength on Particle Detection







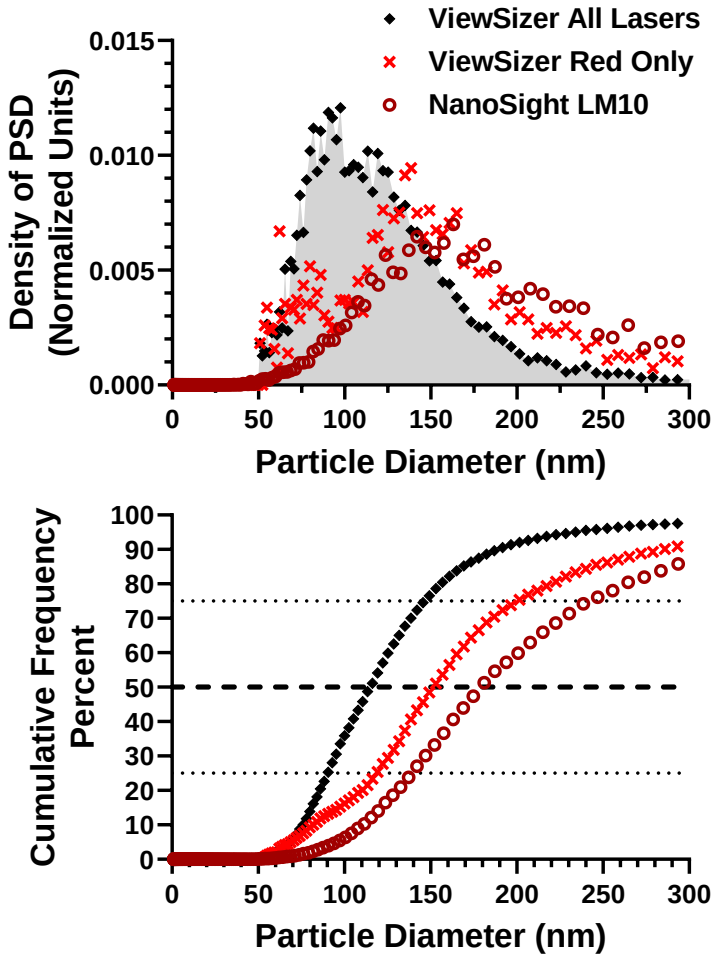



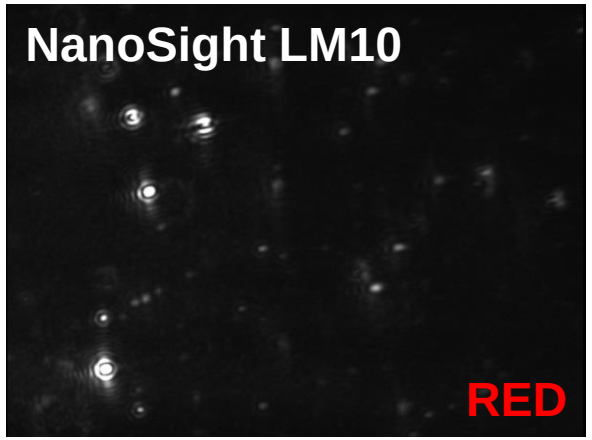

| | Average Size (nm) | Median Size (nm) | Particle Concentration (part./mL) |
|---|---|---|---|
| All Lasers | 131 | 116 | 3.03E+09 |
| Blue Only | 132 | 116 | 2.58E+09 |
| Green Only | 153 | 132 | 1.07E+09 |
| Red Only | 178 | 153 | 8.65E+08 |

**EVs isolated from plasma require higher energy wavelengths for accurate analysis**

# ViewSizer 3000 Comparison with NanoSight LM10








**Laser wavelength can significantly affect particle count and size distribution**

# Future Potential of NTA in EV Research

**Biggest Advantages**

Accurate counting and sizing of individual nanoparticles

Fluorescence NTA may help distinguish real EVs from contaminants

**Current Limitations**

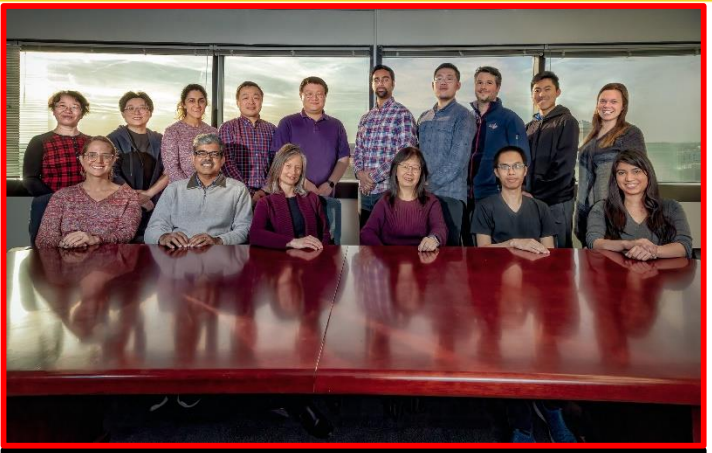
Conventional NTA requires a clean EV isolation procedure

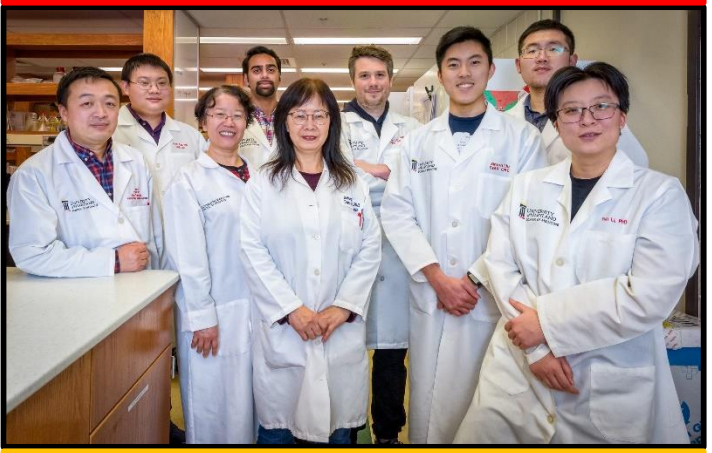
Minimum size detected for biologics – 50nm?

**Future Directions**

What design features can be added to improve lower detection limit?

Can instruments be designed for multiplex phenotyping like flow cytometry?





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Junfang Wu, M.D., Ph.D.

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…and the rest of the lab!

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HORIBA Scientific

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Kuba Tatarkiewicz, Ph.D.

# Resources to learn more about EVs/exosomes

- Latest MISEV guidelines (2018)

https://www.tandfonline.com/doi/full/10.1080/20013078.2018.1535750

- Original ISEV position statement (MISEV 2014)

https://www.tandfonline.com/doi/full/10.3402/jev.v3.26913

- Coursera Course “Basics on Extracellular Vesicles”

https://www.coursera.org/learn/extracellular-vesicles#about

- Extracellular Vesicle Club for latest advances in research

https://www.youtube.com/channel/UC0nhdTaTEUqpO8anXZqRdkQ