Nanoalgosomes:

a drug delivery platform designed by nature

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Conferenza annuale del Dipartimento di Scienze Bio-Agroalimentari del CNR
16 dicembre 2021

Journal of EXTRACELLULAR VESICLES Open Access ISEV

Impact factor: 25.841

Received: 29 September 2020 | Revised: 26 February 2021 | Accepted: 9 March 2021

DOI: 10.1002/jev2.12081

ISEV

RESEARCH ARTICLE

Nanoalgosomes: Introducing extracellular vesicles produced by microalgae

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All the listed Authors are members of the VES4US consortium (financed by H2020 grant agreement 801338)

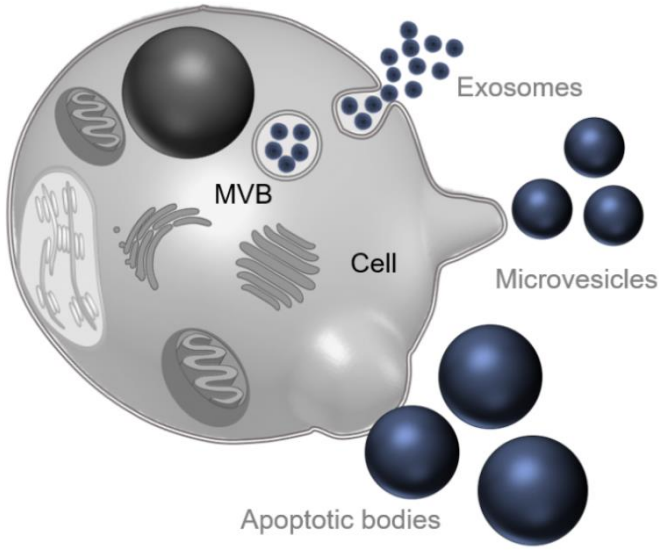
ves4US

The scope

Biophysical, biochemical, and biological characterization of the microalgal-derived small extracellular vesicles, which we named

nanoalgosomes

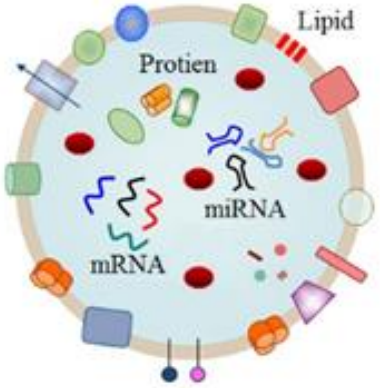
https://sc21.com/extracellular-vesicles-evs/



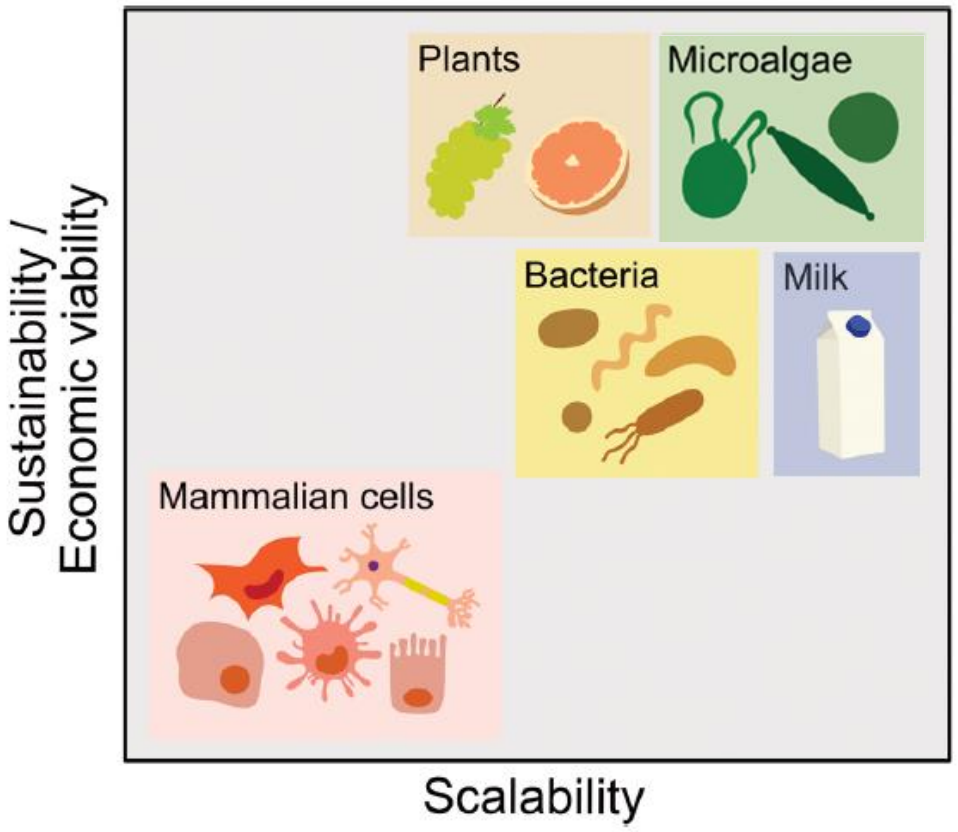
Le EV

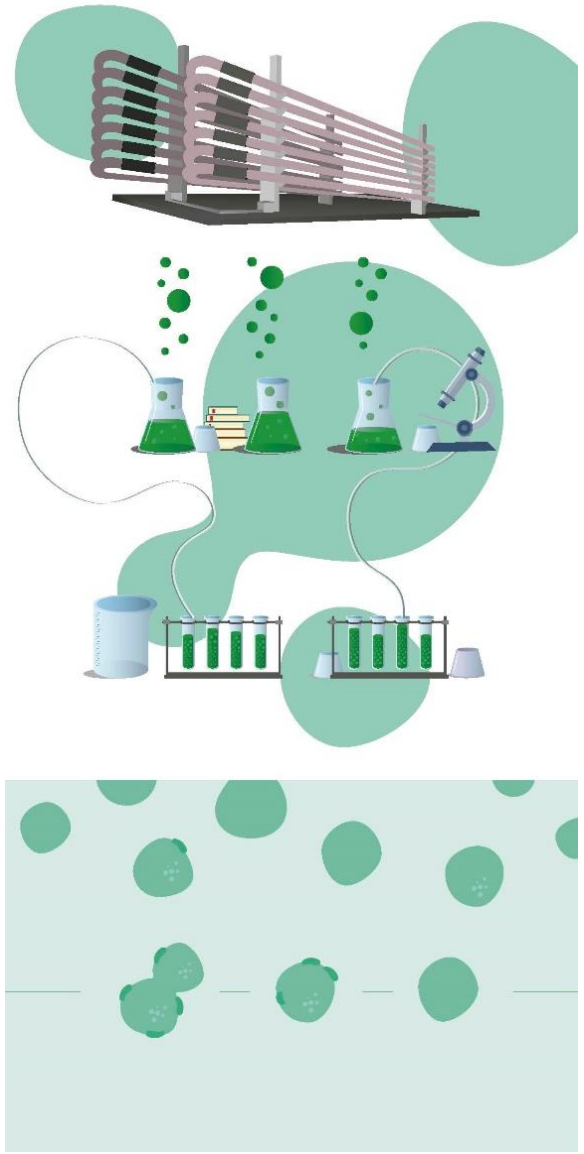
NUOVO CAMPO NELLA RICERCA BIOMEDICA

- le vescicole extracellulari (EV) sono particelle bilipidiche rilasciate naturalmente dalle cellule di tutti gli organismi viventi
- rivestono un ruolo chiave nella comunicazione intercellulare consentendo il trasferimento orizzontale di materiale genetico, proteico e lipidico
- complesse ed eterogenee
- rappresentano una nuova promettente soluzione nella “cell-free therapy“ e nelle biotecnologie come piattaforma per il “drug delivery“.



Microalgae a new green EV resource





Adamo et al., JEV 2021; Picciotto et al., Biomaterials Science 2021

Identification

of the most promising microalgae strains for the production of EVs

Strains selection criteria

- members from the main microalgae lineages
- seawater and fresh water inhabitants
- small and large sized cells
- colonial and single cells
- species with sequenced genomes

Prof. Nicolas Touzet

N.	strain	source
1	*LACW 34*	marine
2	*Phacodactylum Tricormutum*	marine
3	*LACW24*	marine
4	*Brachyomonas sp*	marine
5	*Nannochloropsis sp*	marine
6	*Rhodella sp*	marine
7	*Dunaliella tertiolecta*	marine
8	*Diacronema sp*	marine
9	*Ankistrodomius sp*	fresh water
10	*Pediastrum sp*	fresh water
11	*Cryptomonas Pyrenodofira*	fresh water
12	*Tetraselmis Chuii*	marine
13	*Rhodella Violacea*	marine
14	*Amphidinium sp*	marine
15	*Isochrysis Galbana*	marine
16	*Kirchneriella sp*	fresh water
17	*Cyanophora Paradoxa*	fresh water
18	*Chlamydomonas Reinhardtii*	fresh water

Biomaterials Science

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Cite this: *Biomater. Sci.*, 2021, **9**, 2917

Isolation of extracellular vesicles from microalgae: towards the production of sustainable and natural nanocarriers of bioactive compounds†‡

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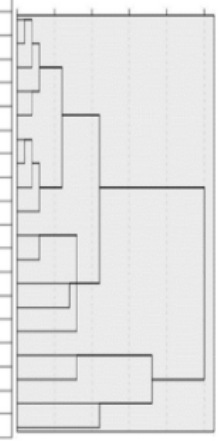
Fatty acid profiling : HPLC-MS/MS

Strain / Fatty acid	C14	C16	C16:1	C16:2	C16:3	C18	C16:4	C18:1	C18:2	C18:3	C20	C18:4	C20:4	C20:5	C22:6
Phaeothamnion sp. LACW34		+	+			+		+	+	+		+		+	+
Pediastrum sp. DMGFW31		+				+	+	+	+	+					
Stauroneis sp. LACW24	+	+	+	+	+	+							+	+	
Cryptomonas pyrenoidifera CCAP979/39		+				+		+	+	+		+		+	
Isochrysis galbana CCAP927/1	+	+				+		+	+	+		+			+
Brachiomonas sp. APSW11MA		+				+	+	+	+	+		+			
Chlamydomonas reinhardtii CCAP11/45		+				+	+	+	+						
Tetraselmis chuii CCAP66/21B		+	+			+	+	+	+	+		+		+	
Dunaliella tertiolecta CCAP19/22		+				+	+	+	+	+					
Rhodella violacea CCAP1388/5		+				+		+					+	+	
Cyanophora paradoxa CCAP981/1		+				+		+	+				+	+	
Phaeodactylum tricornutum CCAP1052/A	+	+	+		+	+		+						+	+
Diacronema sp. GMC45	+	+	+			+		+	+	+			+	+	+
Chloromonas sp. LAFW02		+				+	+			+			+	+	
Ankistrodesmus sp. DMGFW08		+		+		+	+	+	+	+		+			
Kirchneriella sp. DMGFW21		+	+	+	+	+	+	+	+	+		+			
Amphidinium sp. LACW42	+	+				+	+		+			+		+	+
Nannochloropsis sp. CCAP211/46	+	+	+			+		+	+				+	+	



Prof. Veronika Kralj-Iglič

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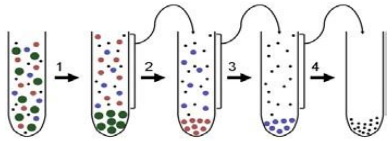
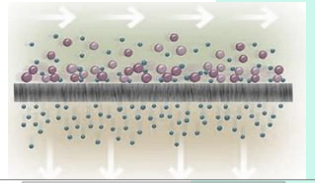
Isolation

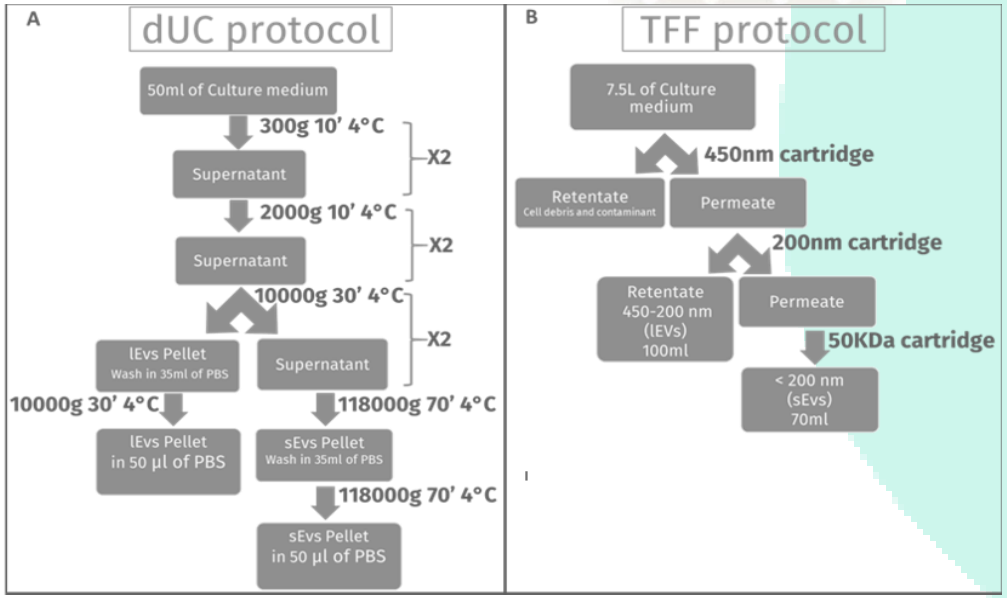
of extracellular vesicles (EVs) from microalgae

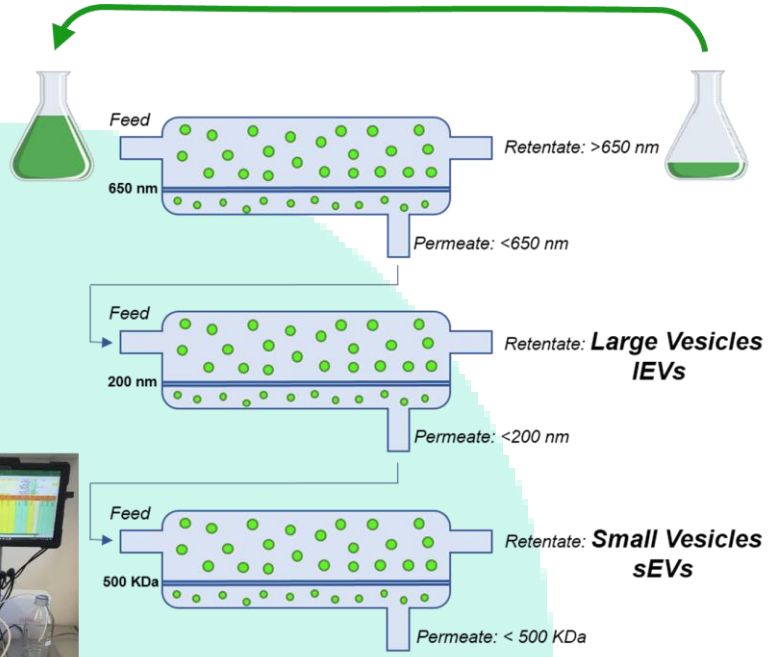
Differential ultracentrifugation



Tangential Flow Filtration







Yield of EVs *T. chuii*

	Isolation Method	Culture Volume	sEV pt µg/mg biomass pts	n. particle/mg biomass pts
sEV	dUC (118000g pellet)	50 ml	0.4±0.03	$2x10^9±8x10^7$
	TFF (<200 nm)	7500 ml	0.3±0.05	$10^9±2x10^8$

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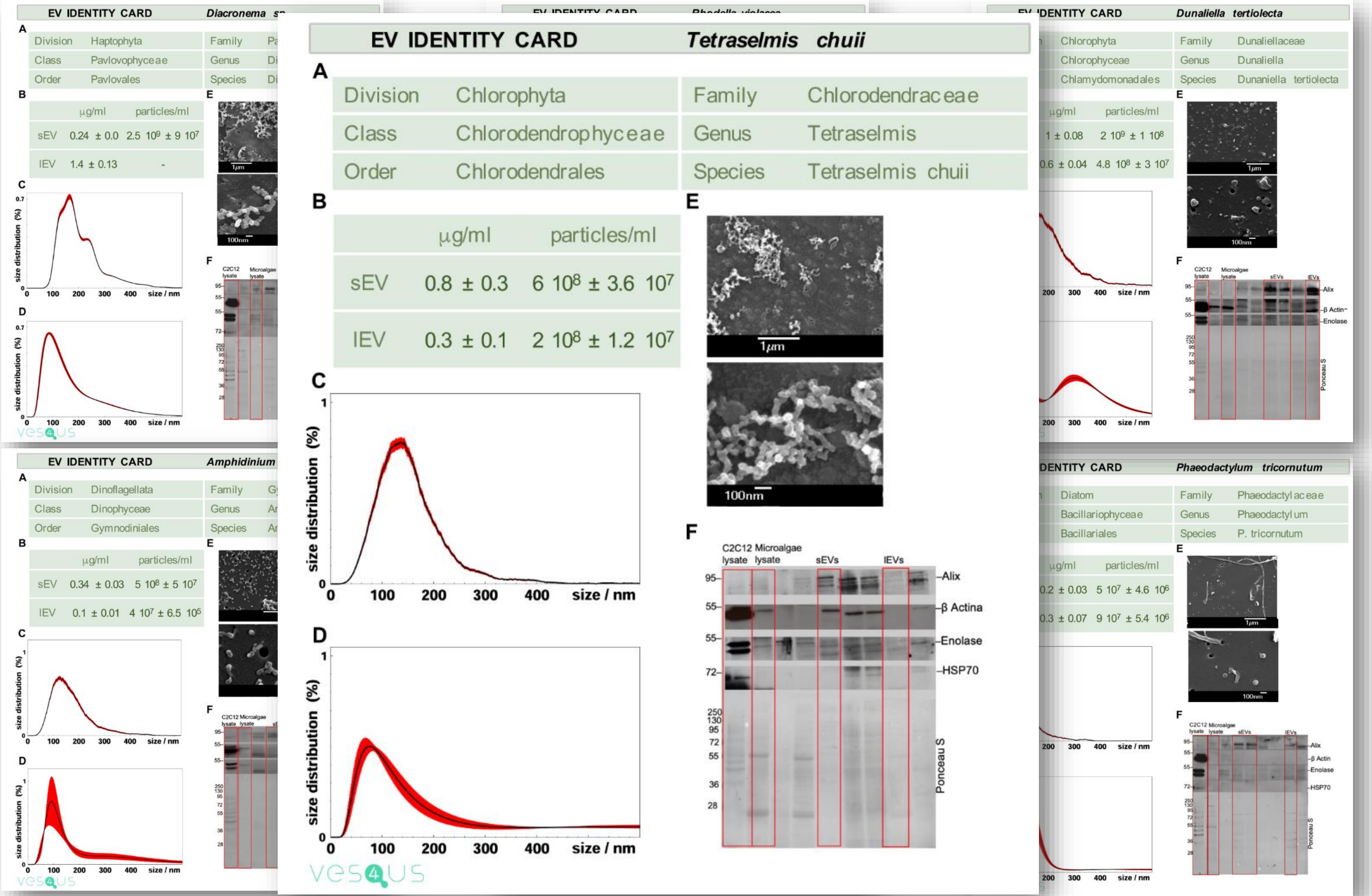

EV IDENTITY CARD
Tetraselmis chuii
A
Division Chlorophyta
Class Chlorodendrophyceae
Order Chlorodendrales
Family Chlorodendraceae
Genus Tetraselmis
Species Tetraselmis chuii
B
µg/ml
particles/ml
sEV 0.8 ± 0.3 6 10^8 ± 3.6 10^7
lEV 0.3 ± 0.1 2 10^8 ± 1.2 10^7
C
size distribution (%)
size / nm
D
size distribution (%)
size / nm
E
1µm
100nm
F
C2C12 lysate
Microalgae lysate
sEVs
lEVs
Alix
β Actina
Enolase
HSP70
Ponceau S
EV IDENTITY CARD Diacronema
EV IDENTITY CARD Amphidinium
Dunaliella tertiolecta
Phaeodactylum tricornutum
vesqus

Table 1 Ranking of the first seven EV-producing microalgal strains. Data refer to the sEVs isolated by dUC, starting from 50 ml of total culture volume

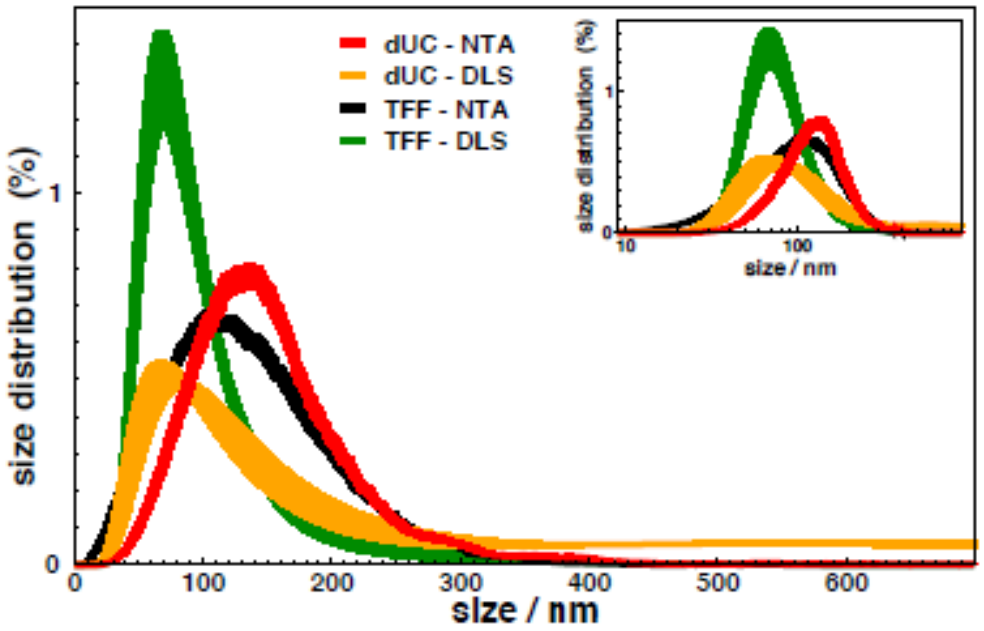
Rank	Species	Lineage	Score	µg sEV proteins per mg dry weight mass[a]	sEV particle numbers per mg dry weight mass[a]	Diameters (NTA mode)[a]
1	*Cyanophora paradoxa*	Glaucophyte	31	2.0 ± 0.2	$2.0 \times 10^9 \pm 3 \times 10^8$	130 ± 5
2	*Tetraselmis chuii*	Chlorophyte	28	0.4 ± 0.0	$2.6 \times 10^8 \pm 3 \times 10^7$	140 ± 5
3	*Amphidinium* sp.	Dinoflagellate	28	1.0 ± 0.1	$6.0 \times 10^8 \pm 2 \times 10^7$	120 ± 5
4	*Rhodella violacea*	Rhodophyte	28	0.4 ± 0.0	$8.0 \times 10^8 \pm 4 \times 10^7$	140 ± 5
5	*Diacronema* sp.	Haptophyte	25	0.3 ± 0.0	$1.0 \times 10^8 \pm 1 \times 10^7$	150 ± 10
6	*Dunaliella tertiolecta*	Chlorophyte	25	0.6 ± 0.0	$5.0 \times 10^9 \pm 9 \times 10^7$	160 ± 5
7	*Phaeodactylum tricornutum*	Diatom	25	0.2 ± 0.0	$2.4 \times 10^8 \pm 3 \times 10^7$	90 ± 5

[a] Technical replicates ($n = 6$).

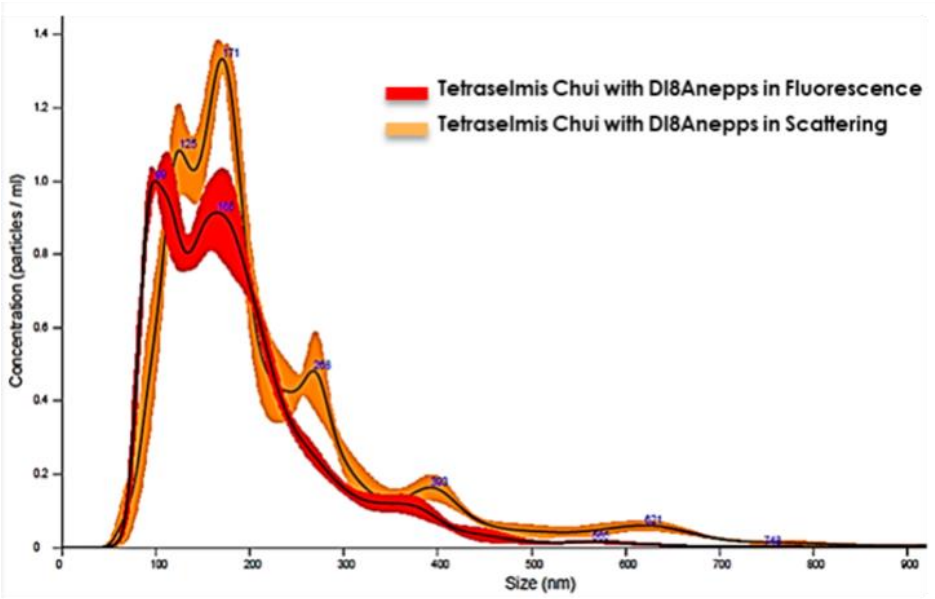
Picciotto et al., *Biomater. Sci*. 2021

nanoalgosomes identity:

Size distribution of EVs of *Tetraselmis chuii*



DLS and NTA analyses of dUC and TFF-isolated nanoalgosomes



NTA and f-NTA analyses

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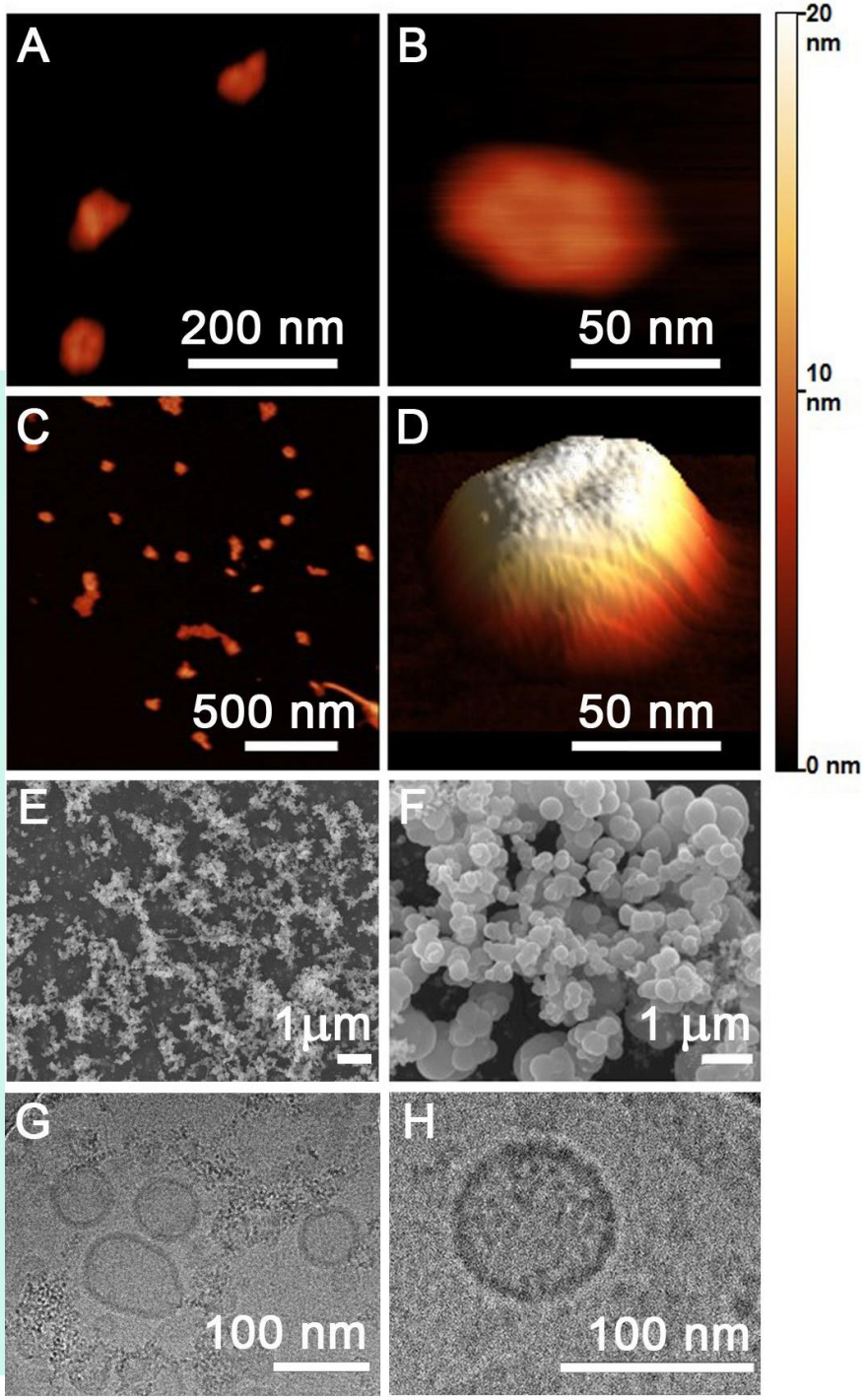
nanoalgosomes identity:

Morphology of EVs of *Tetraselmis chuii*

- **AFM** (Atomic Force Microscopy (AFM) mages were captured by using a Nanowizard III scanning probe microscope (IBF-CNR, Palermo, Dr. Mauro Manno)
- **SEM** (Scanning electronic microscopy, Univ. Ljubljana, Prof Kralj-Iglic)
- **cryo-TEM** (Cryo-transmission electron microscopy, Uni. LJU and Max Planck Inst, Mainz, Prof. Katharina Landfester)

globular particles, heterogeneous in size and shape, sometimes organized in clusters and has lipid bilayer structure



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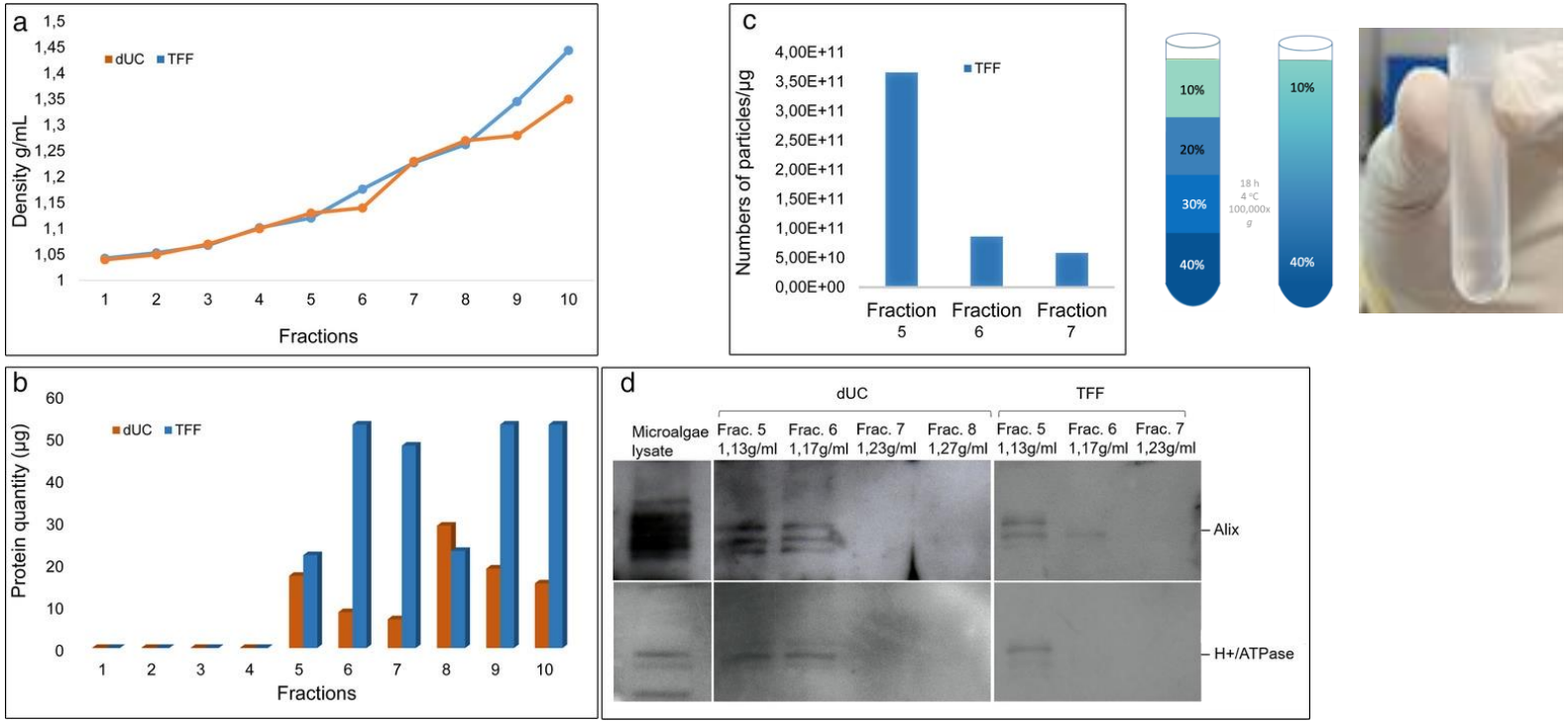
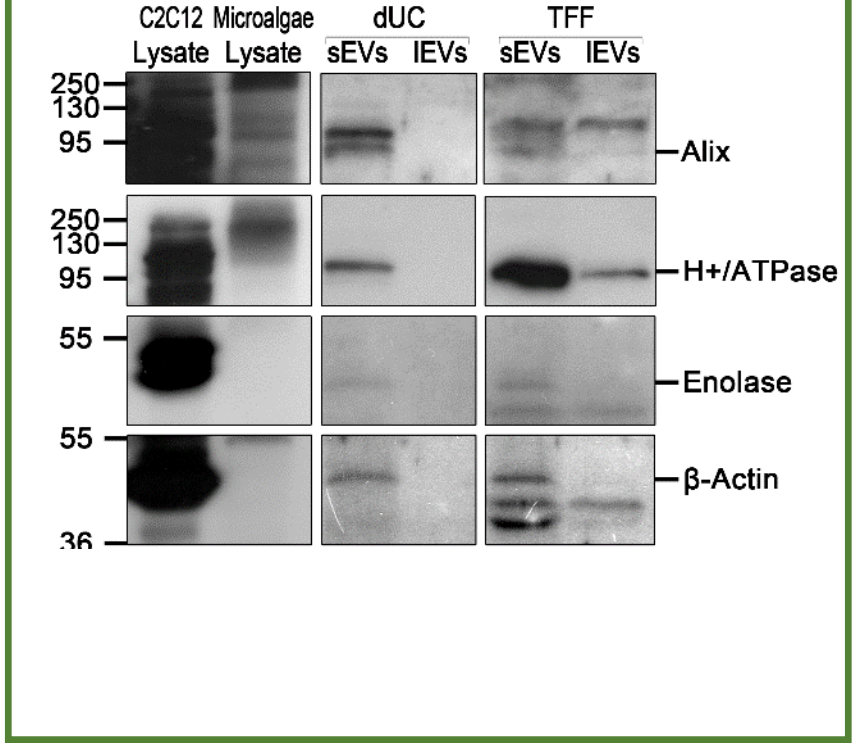
nanoalgosomes identity:

density determination and EV protein markers



Density determination by iodixanol gradient ultracentrifugation

EVs&MS, IBBR-CNR



Immunoblot analyses

A. Bongiovanni, IRIB-CNR

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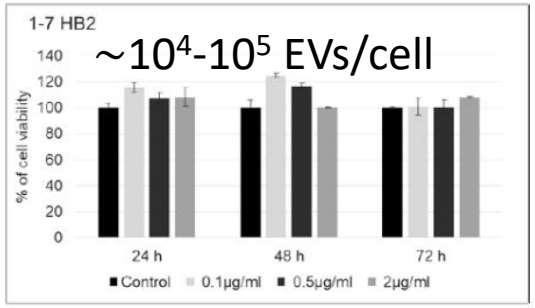
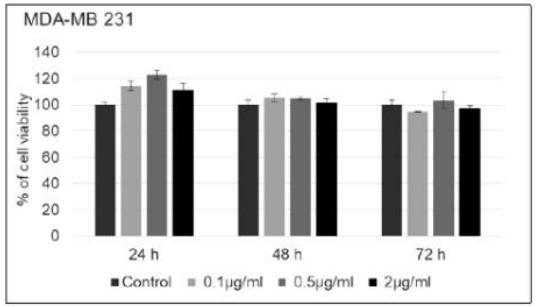
Stability and toxicity assays

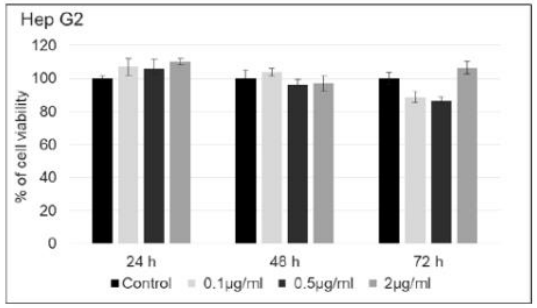
a

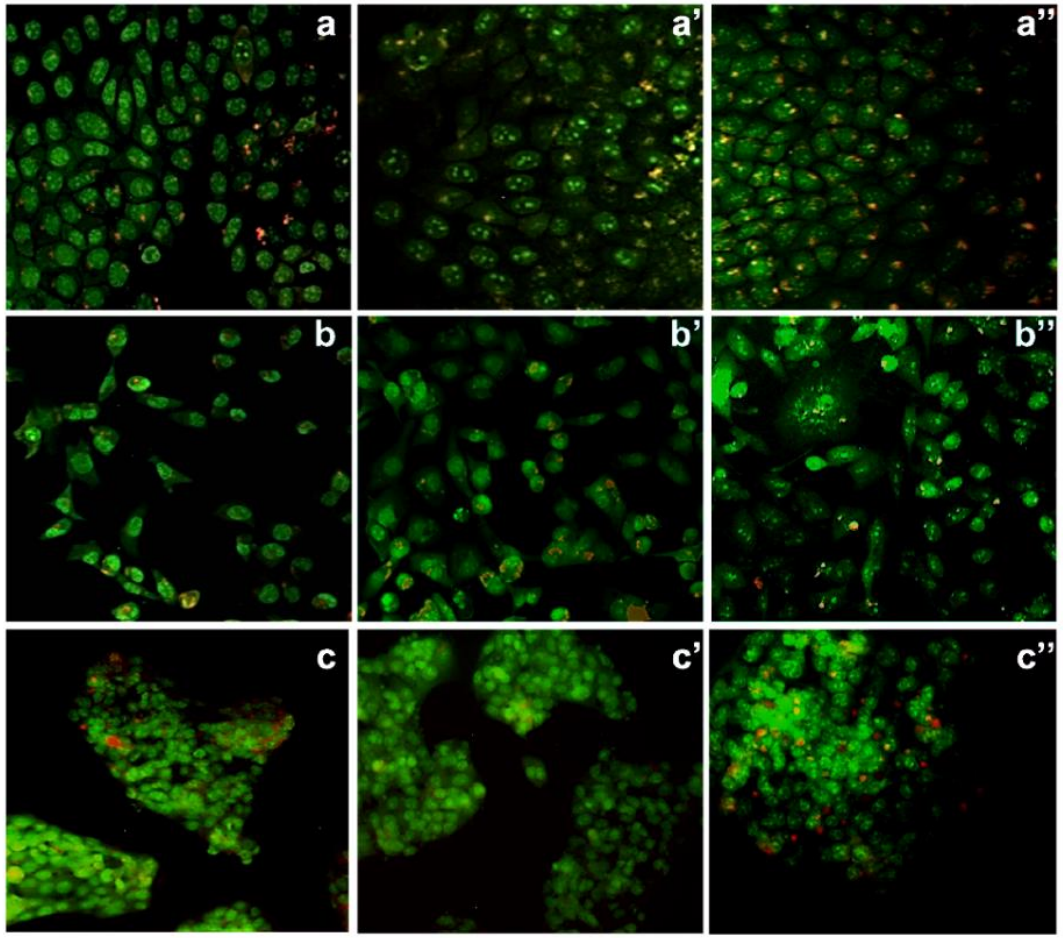


b



c





A. Bongiovanni *et al*, IRIB-CNR, Palermo

a 1–7 HB2, a normal mammary epithelial cell line

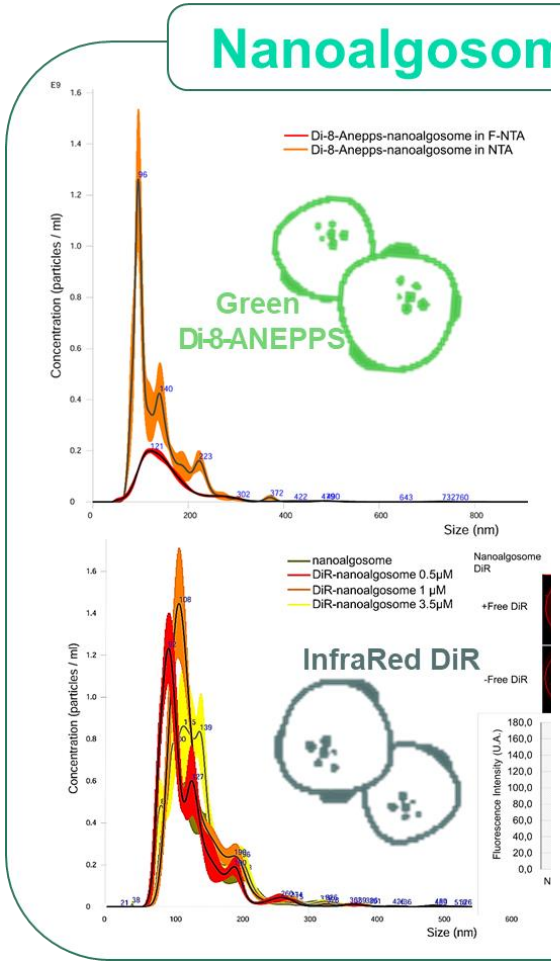
b MDA-MB 231, an epithelial human breast cancer

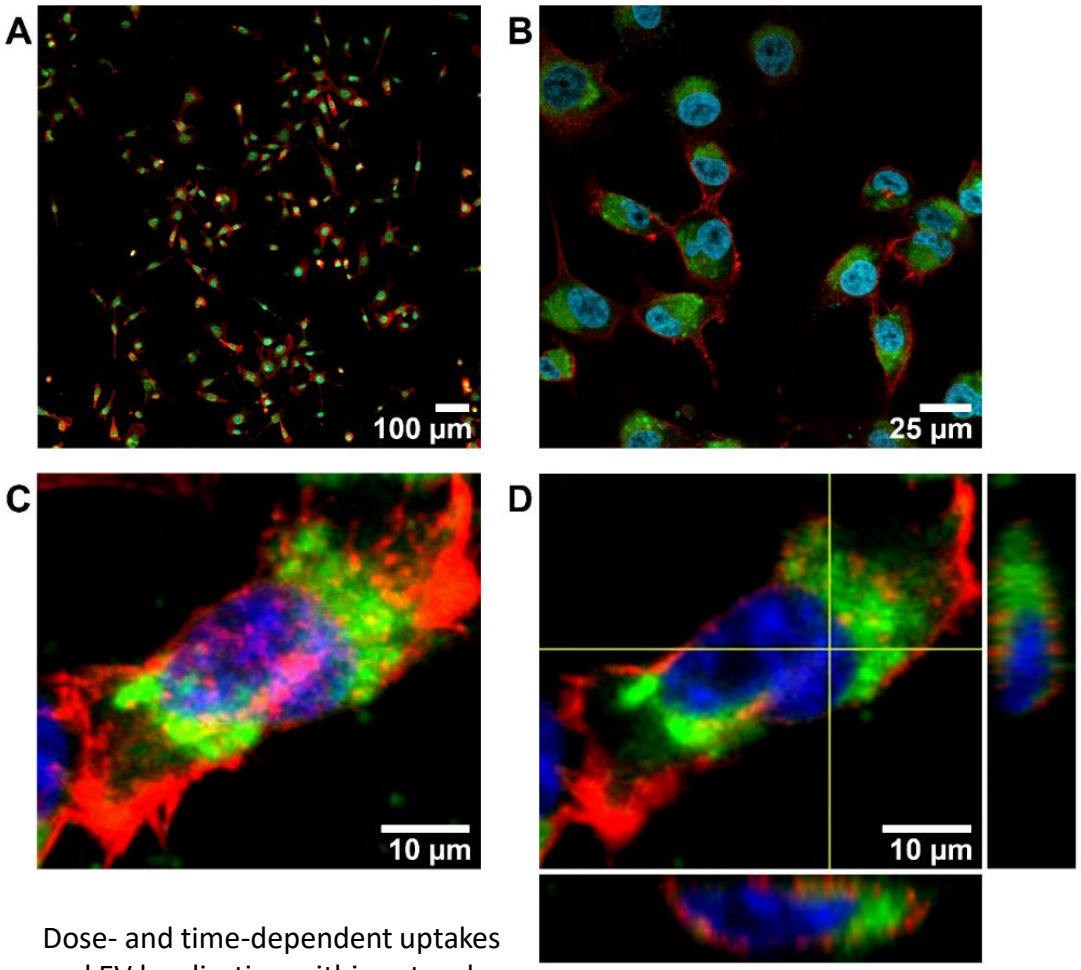
c HepG2 a human hepatocarcinoma

No cellular toxicity
No significant signs of DNA damage

nanoalgosome up-take study

Green Fluorescent Nanoalgosome





Dose- and time-dependent uptakes and EV localization within cytosol close to nucleus were observed

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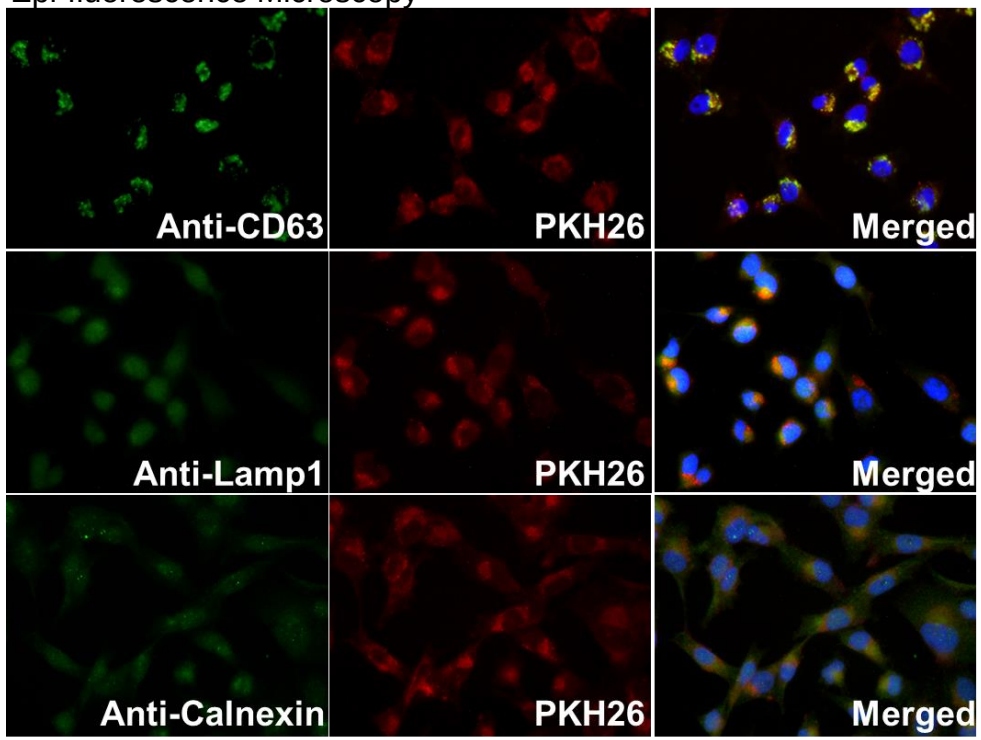
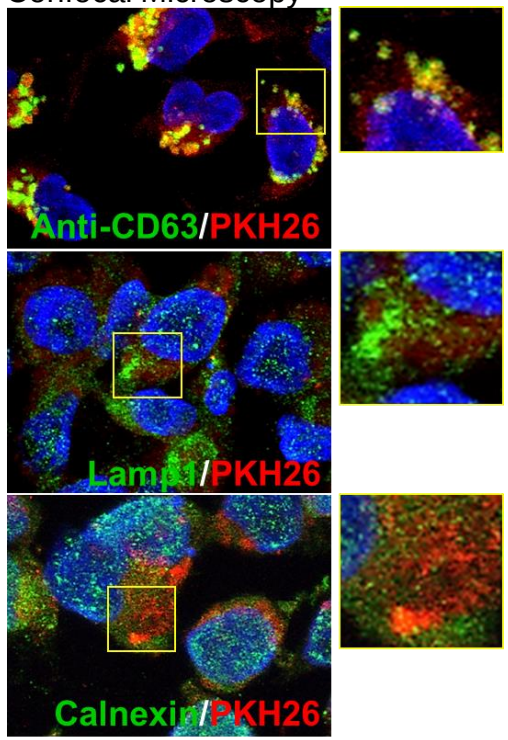
Subcellular localization

- **CD63** for Endosomal system
- **Lamp1** for Lysosomal system
- **Calnexin** for endoplasmic reticulum system

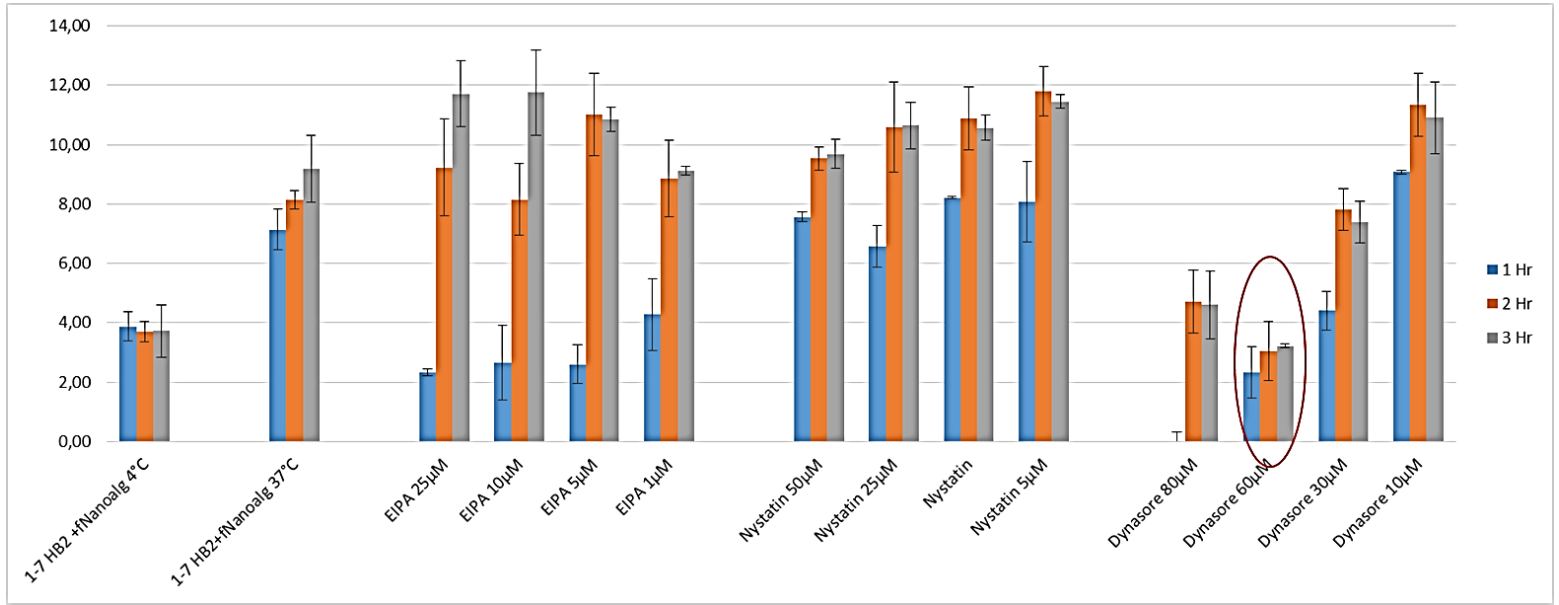
Epi-fluorescence Microscopy



Confocal Microscopy



Clatrin-dependent endocytosis



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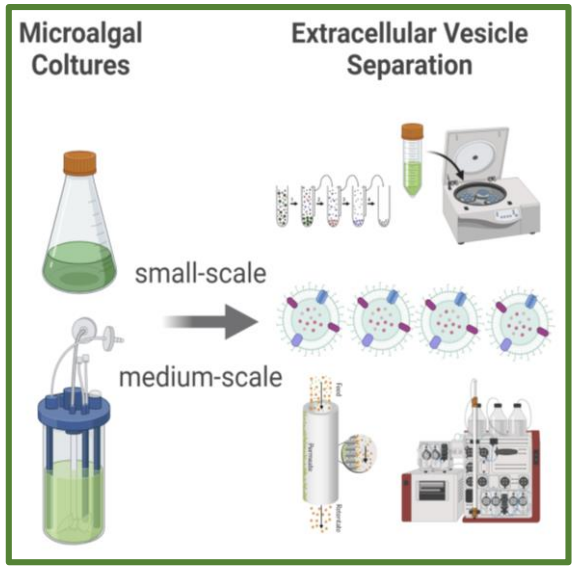
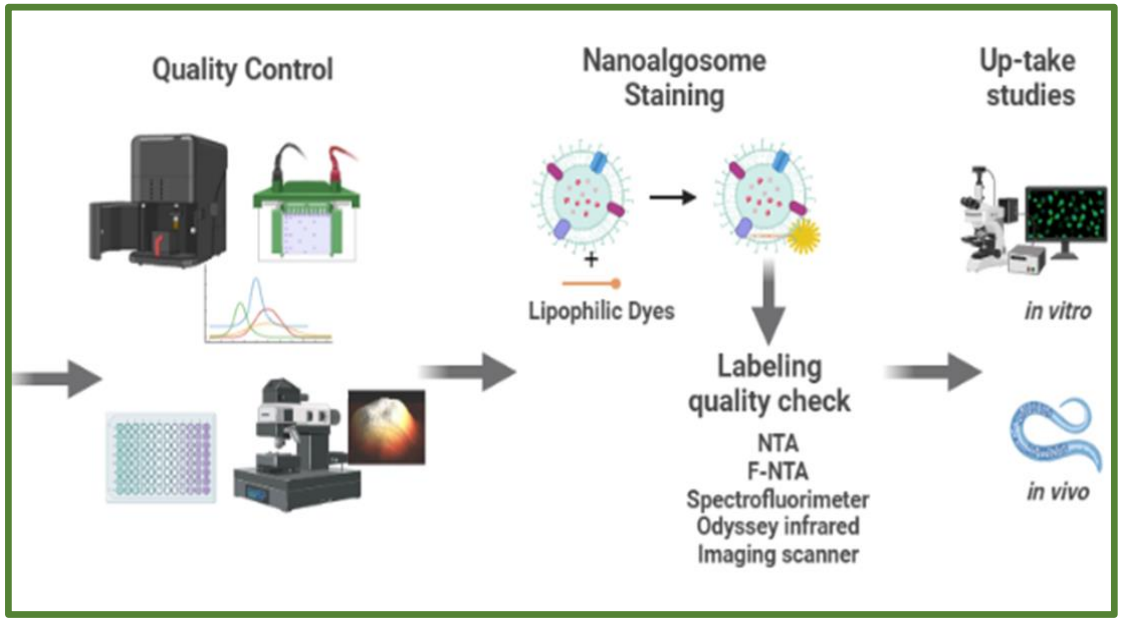
Summarizing…

- ✓ based on the **MISEV2018 guidelines**: nanoalgosomes are a novel extracellular vesicle type produced by microalgal cells;
- ✓ **dUC, TFF and gUC** methods allowed to isolate nanoalgosomes;
- ✓ nanoalgosome size is slightly above **100 nm**
- ✓ nanoalgosomes showed positivity to **EV protein markers and membrane staining** with lipofilic dyes
- ✓ nanoalgosomes are **not cytotoxic** and are efficiently **taken up by mammalian cells**





Ongoing…

- Proteomics and lipidomics
- Loading and functionalization with RNAs and small drugs
- *In vivo* studies *(C. elegans* and mice)

VES4US Team

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement 801338

Consiglio Nazionale delle Ricerche

EV-NET

- rete interdipartimentale del CNR per lo studio e l'applicazione delle vescicole extracellulari (CNR-EV-NET)
- Il gruppo proponente del CNR-EV-NET include ricercatori appartenenti a diversi dipartimenti del CNR (DSB, DSCTM, DISBA e DSFTM)
- **workshop a Roma a febbraio 2022** per lanciare CNR-EV-NET e presentare le attività previste

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Auguri di Buone Natale e Buone Feste