



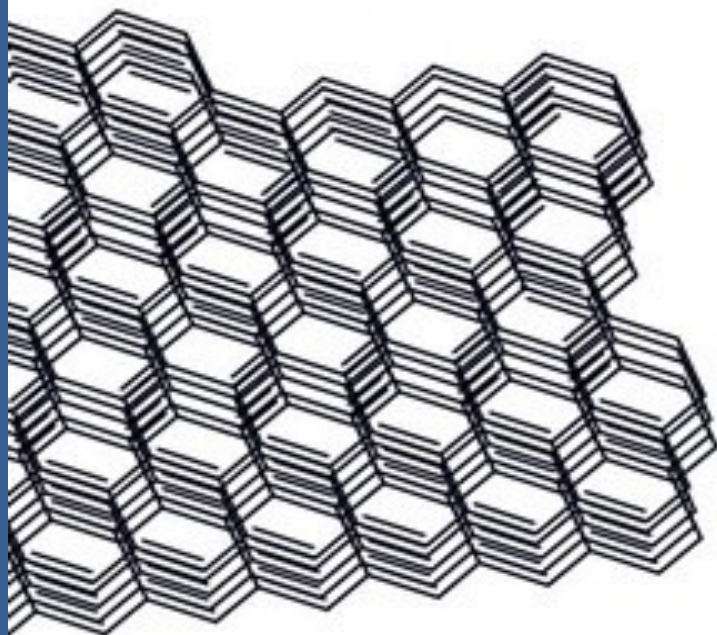
Biocompatibility and use of nanomaterials in medicine

Roberta Misasi

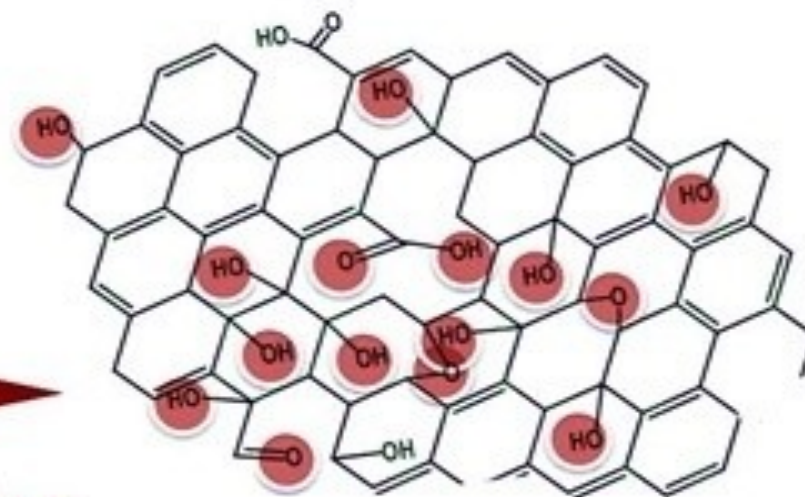
Department of Experimental
Medicine



Graphite



Graphene oxide



Oxidation



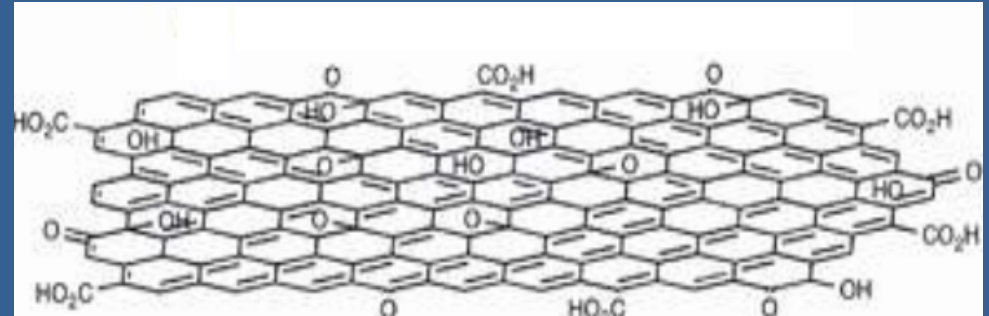
Graphene oxide (GO)

A drug delivery system should have a simple and untoxic design

Delivery to normal cells should be minimized

Graphene oxide:

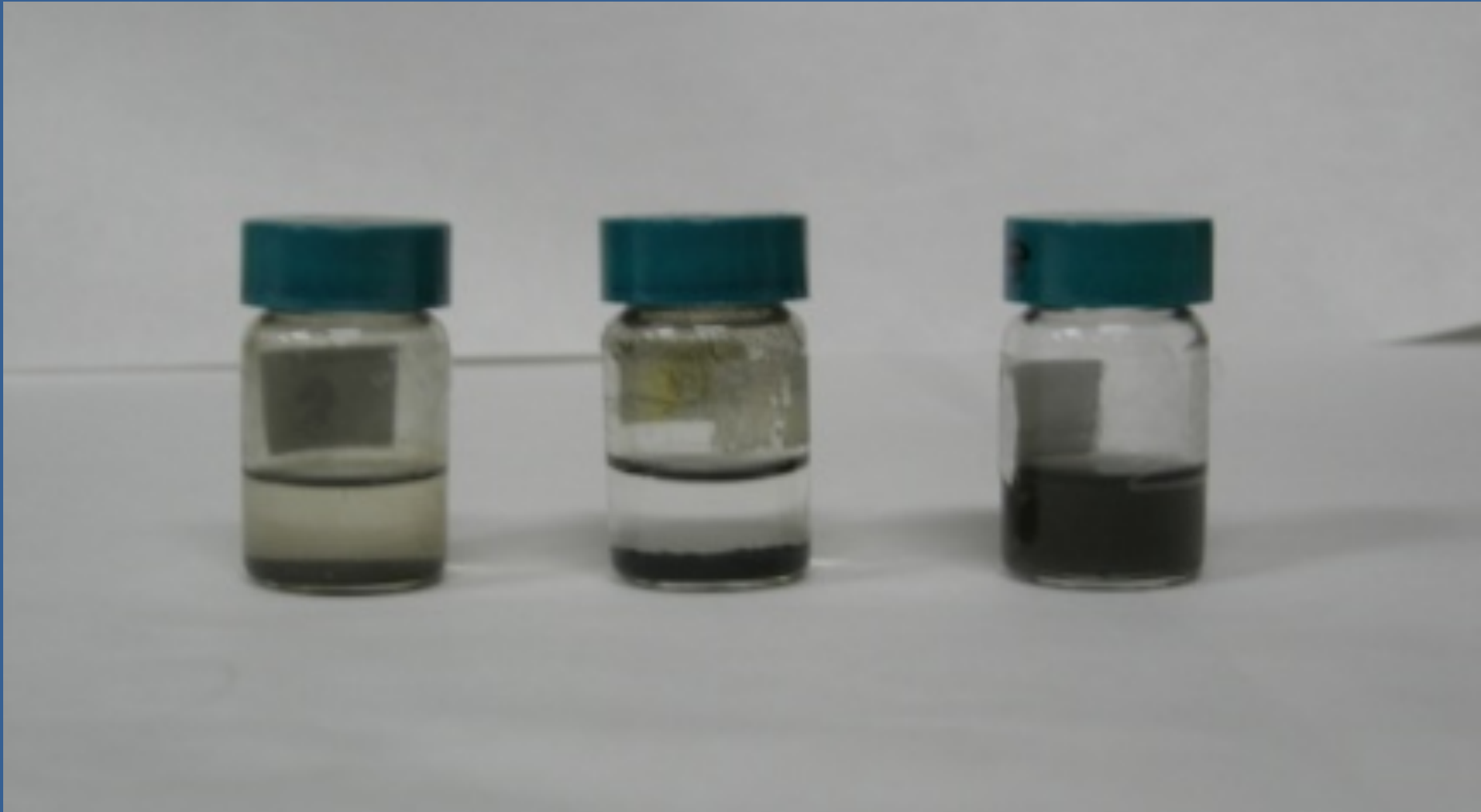
- ✓ High surface area to volume ratio
- ✓ High loading capacity of bioactive molecules.
- ✓ Different chemicals can be bound to same particles



GO can be functionalized in nano-ribbons, nano-platelets, nano-sheets, nano-tubes.



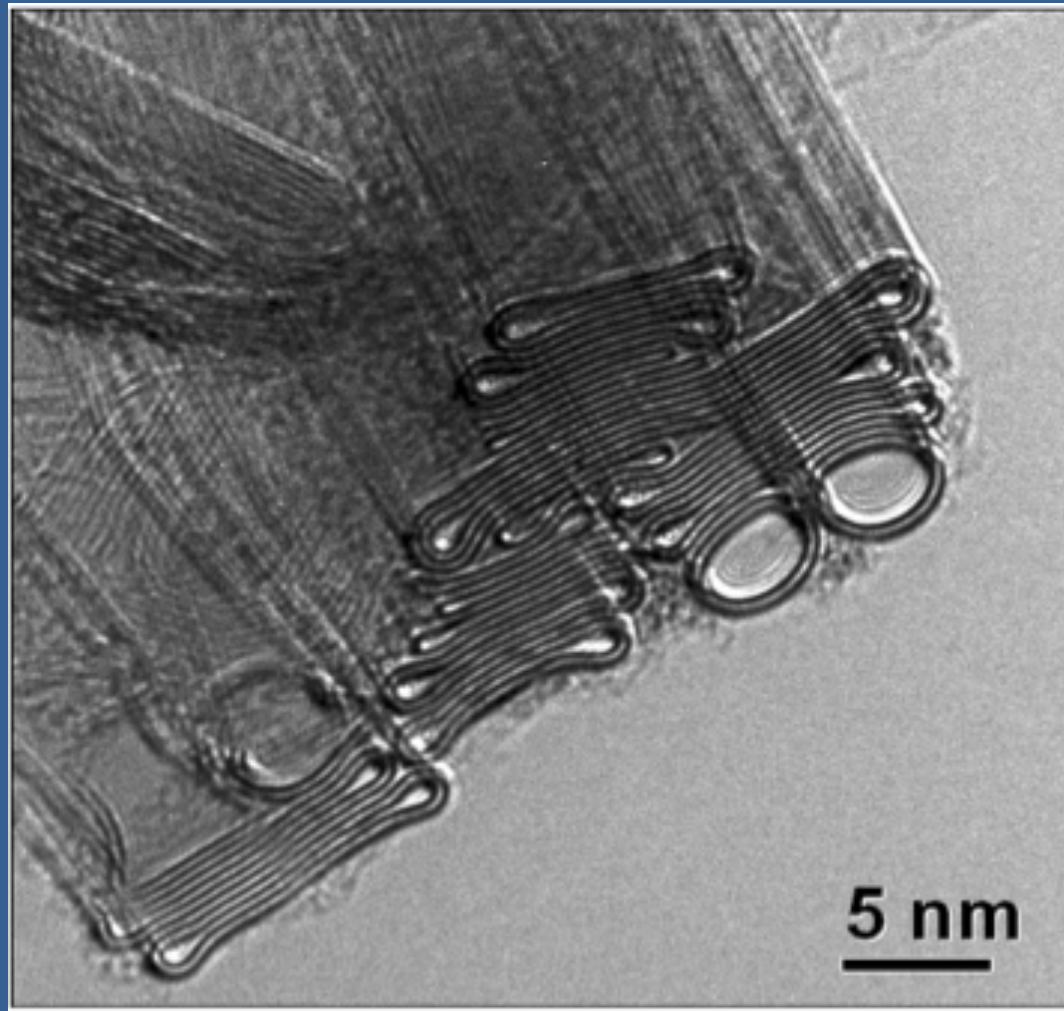
GO nanosheets, GO nanorods, GO nanotubes.





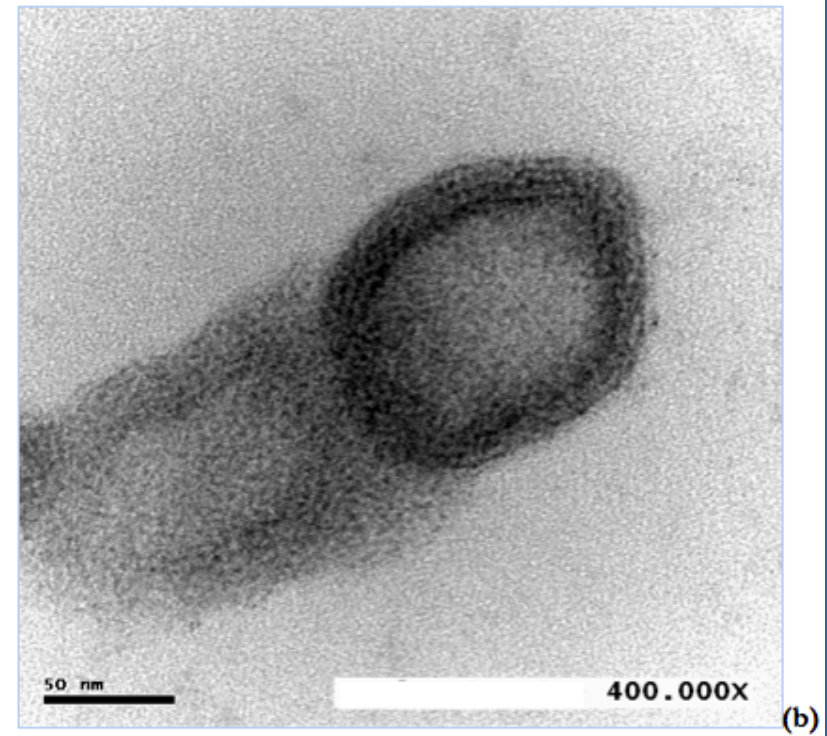
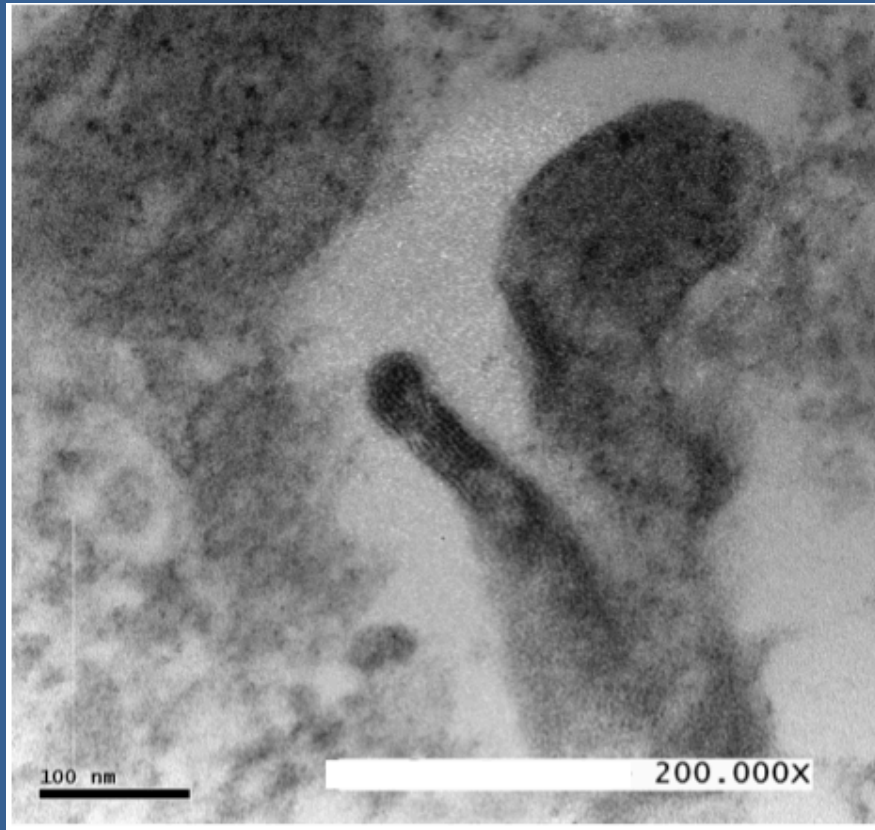
Unzipping of nanotubes

Single Wall Carbon NanoTubes (SWCNTs)



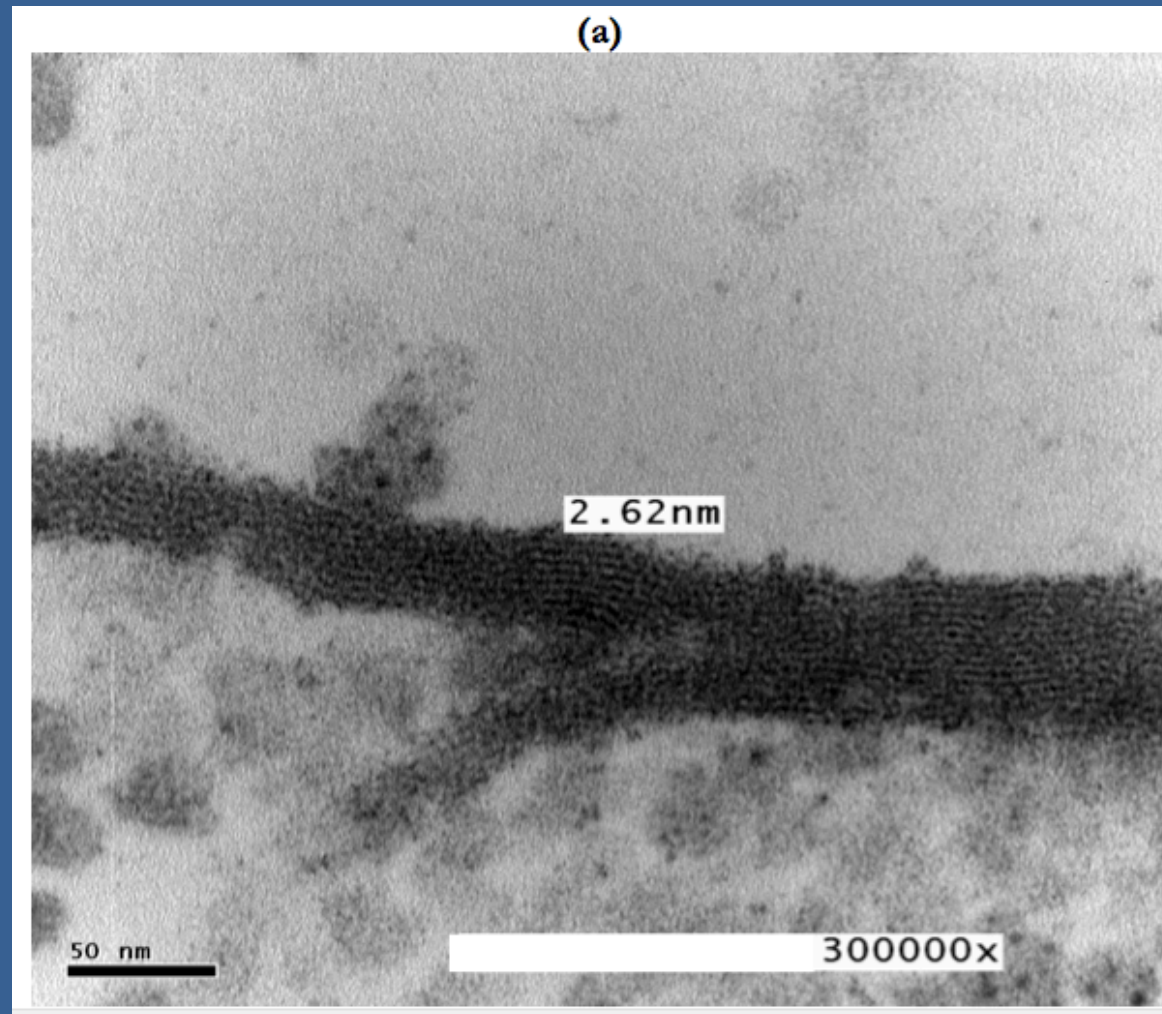


HR-TEM of carbon nanotubes



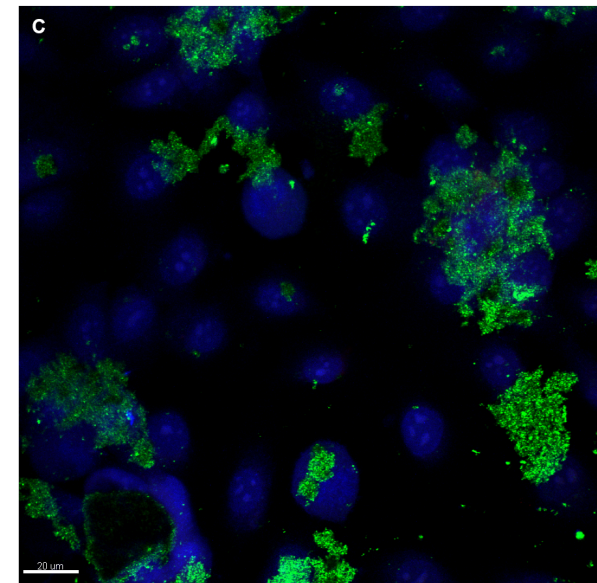
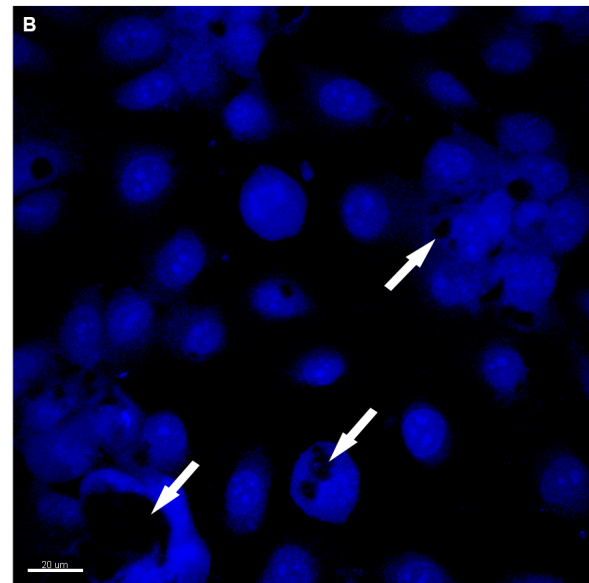
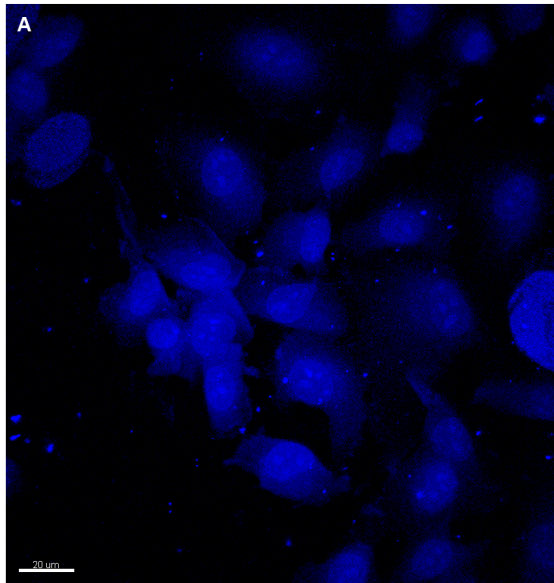


NANOTUBES in HR-TEM



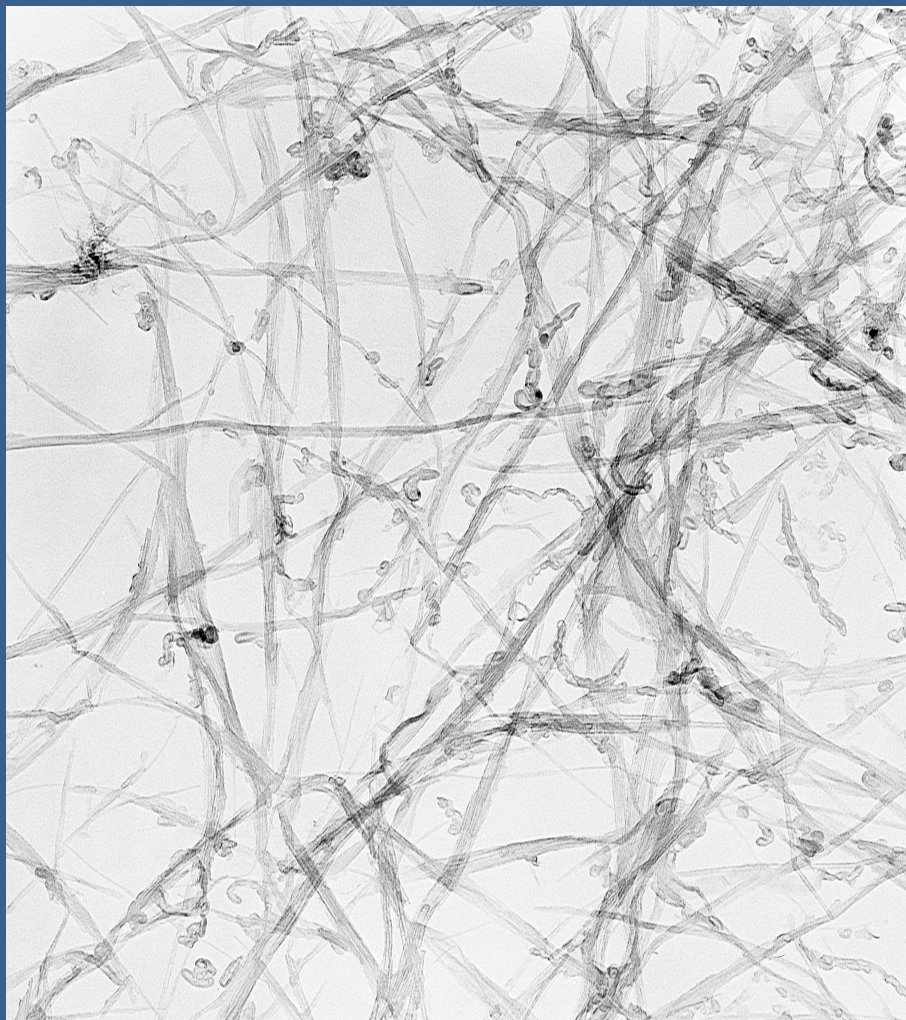


SWCNTs

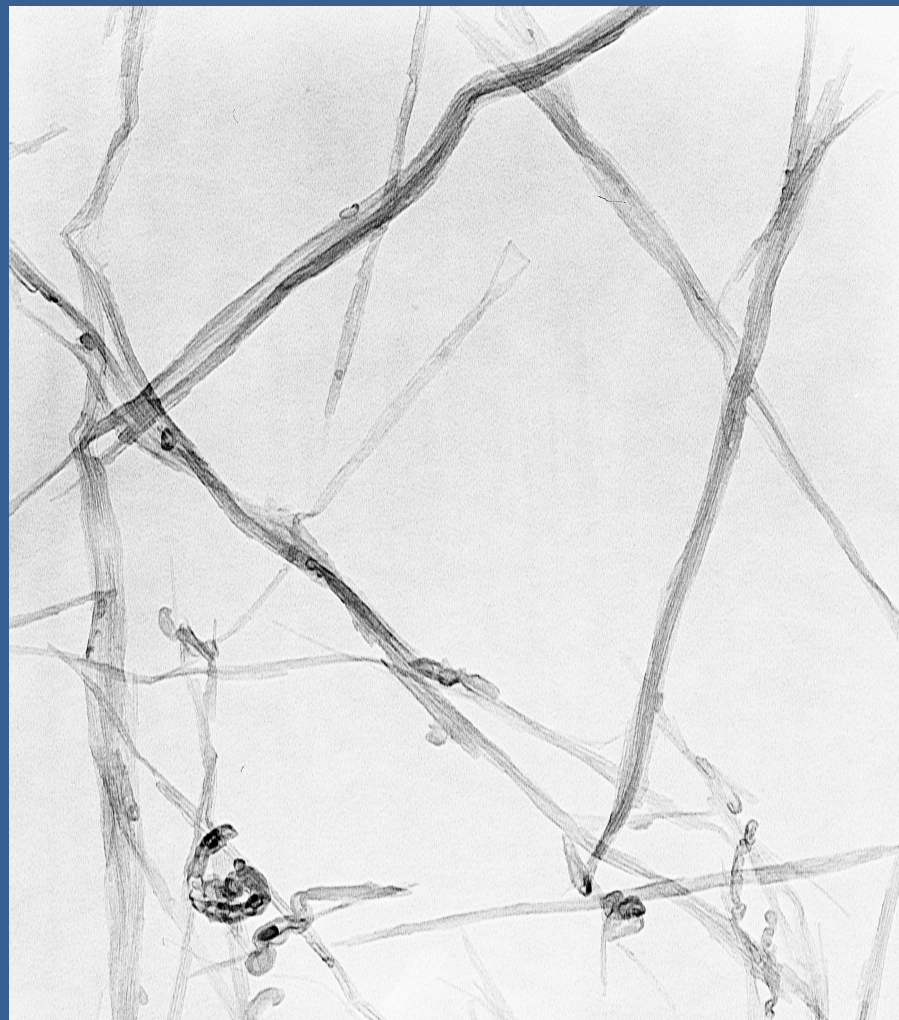




GO nanoribbons



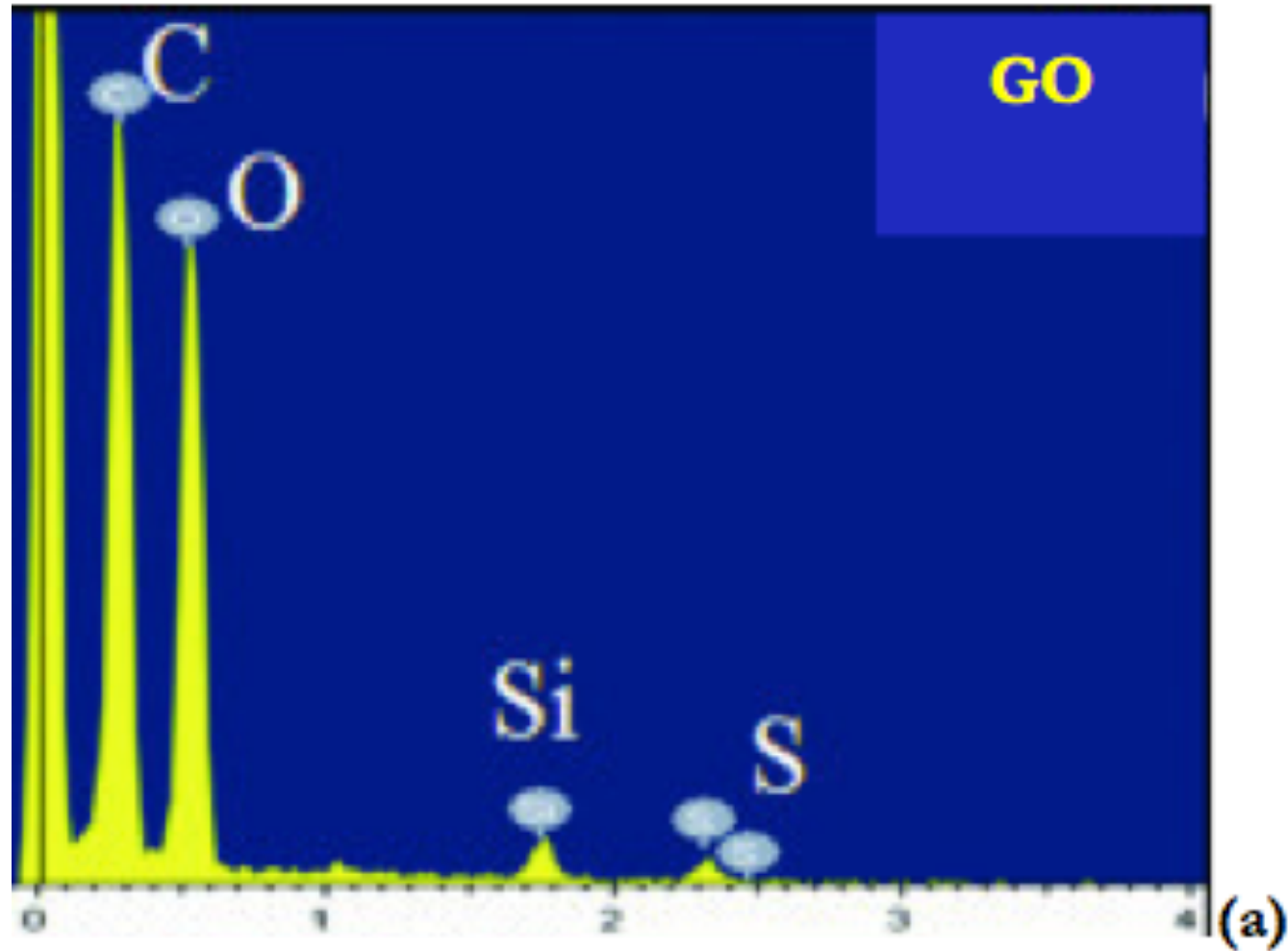
52000x



73000x

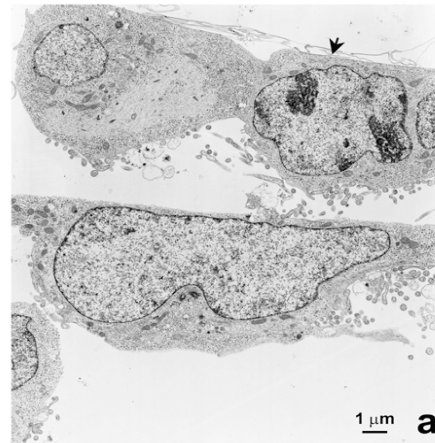


EDAX spectrum of graphene derivatives





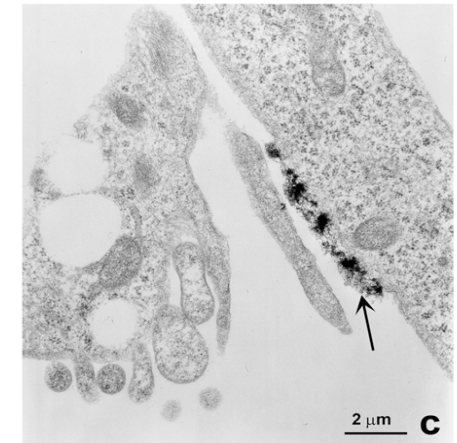
2 μg GO added
to
Neuroblastoma
Cell lines



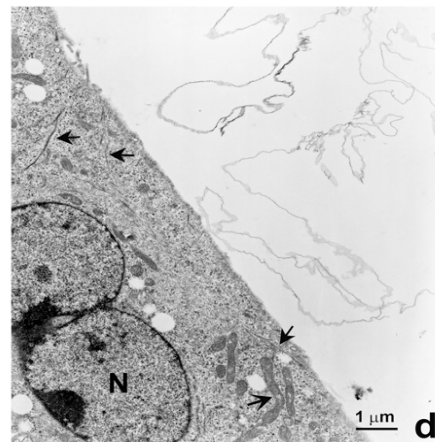
18h



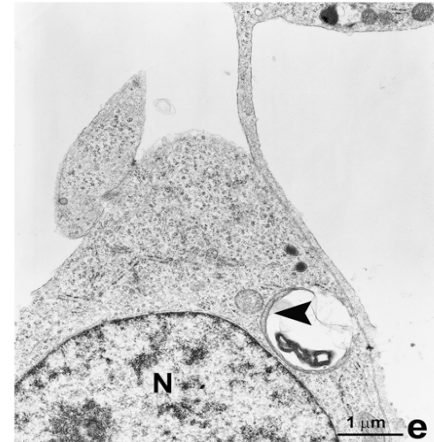
18h



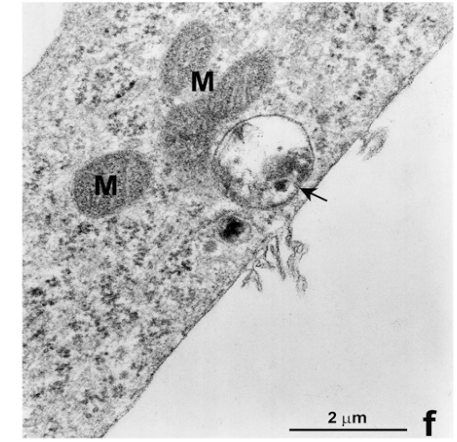
18h



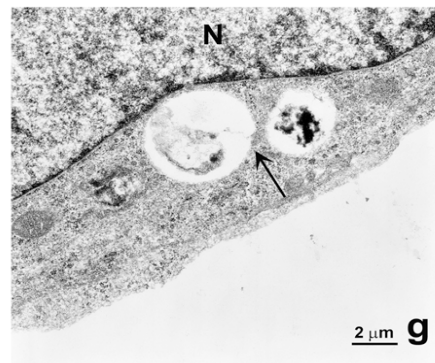
24h



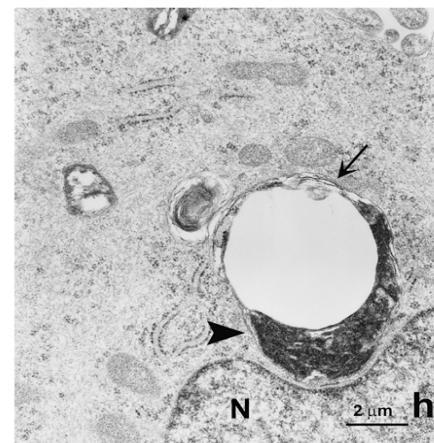
24h



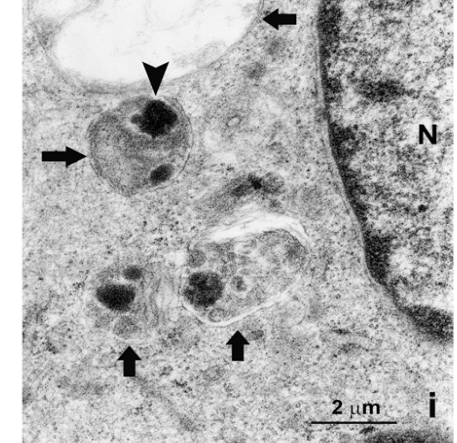
24h



48h



72h



72h



Swollen Mitochondria

Condensed Mitochondria

Mitophagy

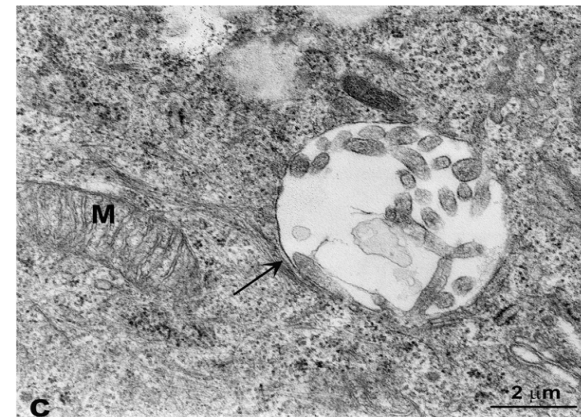
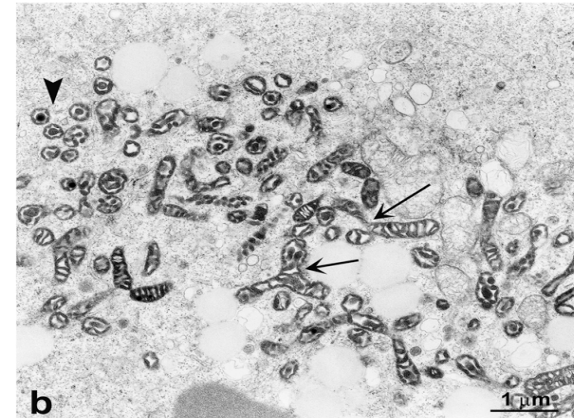
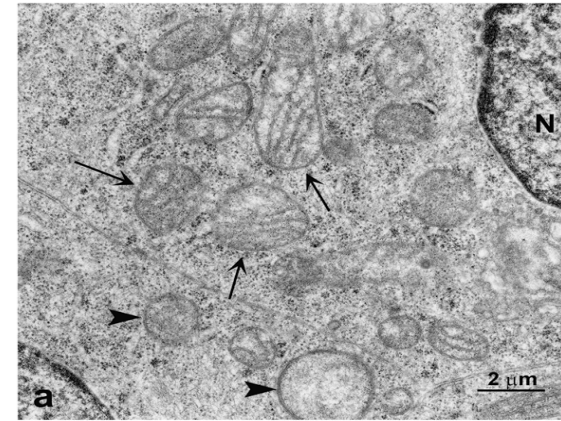
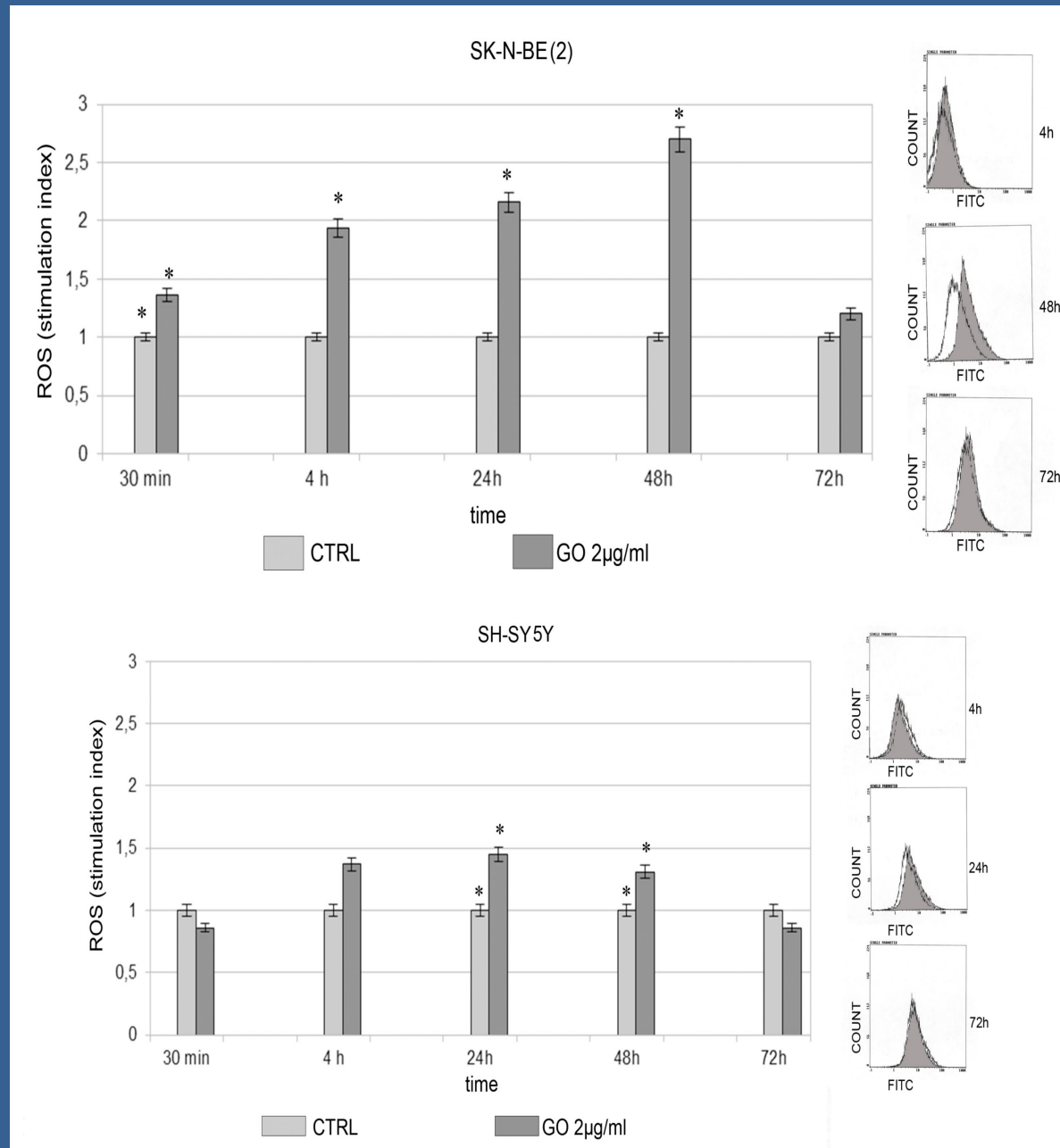


Figure 5

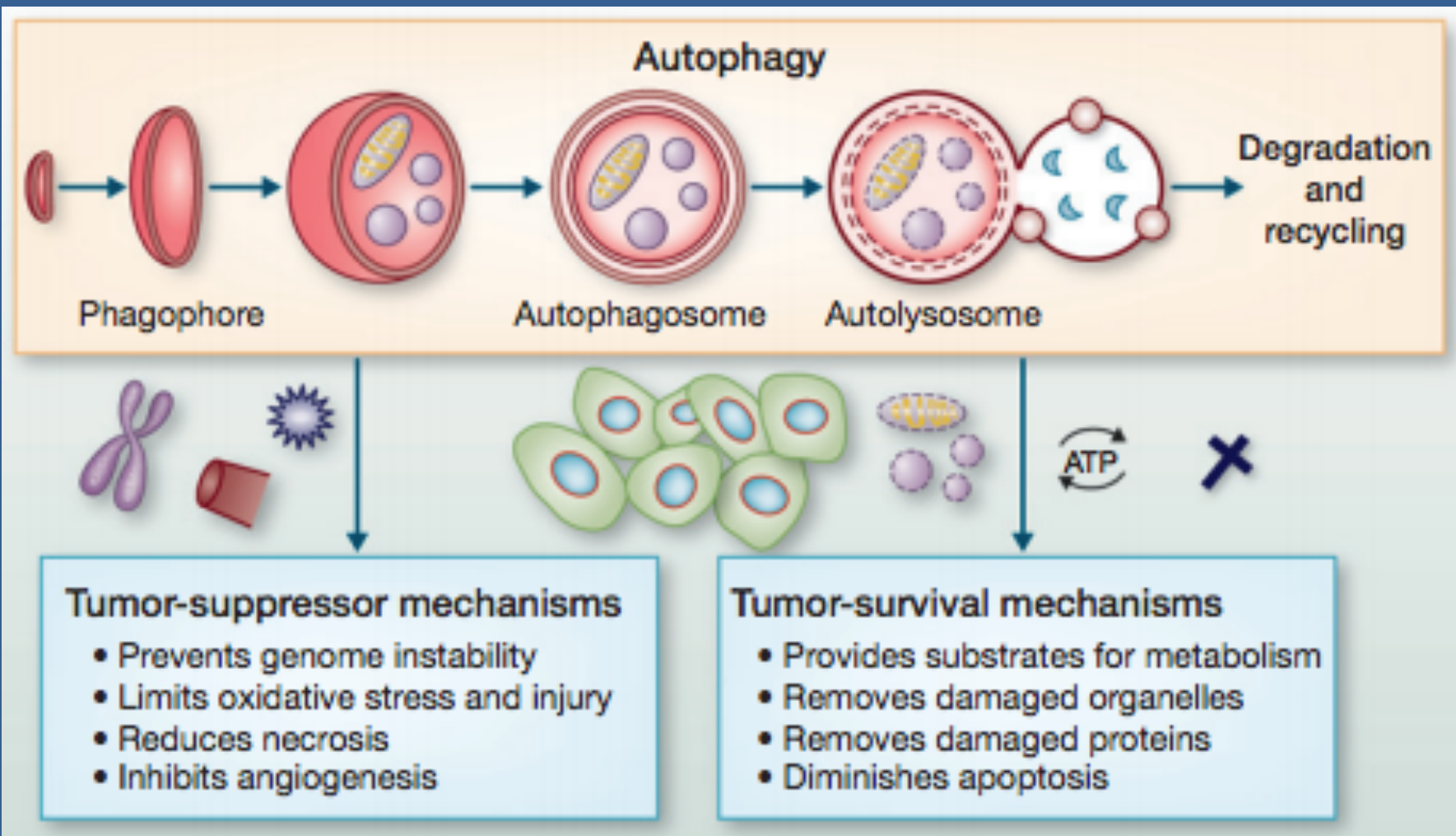


ROS production after addition of GO in cells





Autophagy





Autophagy Markers

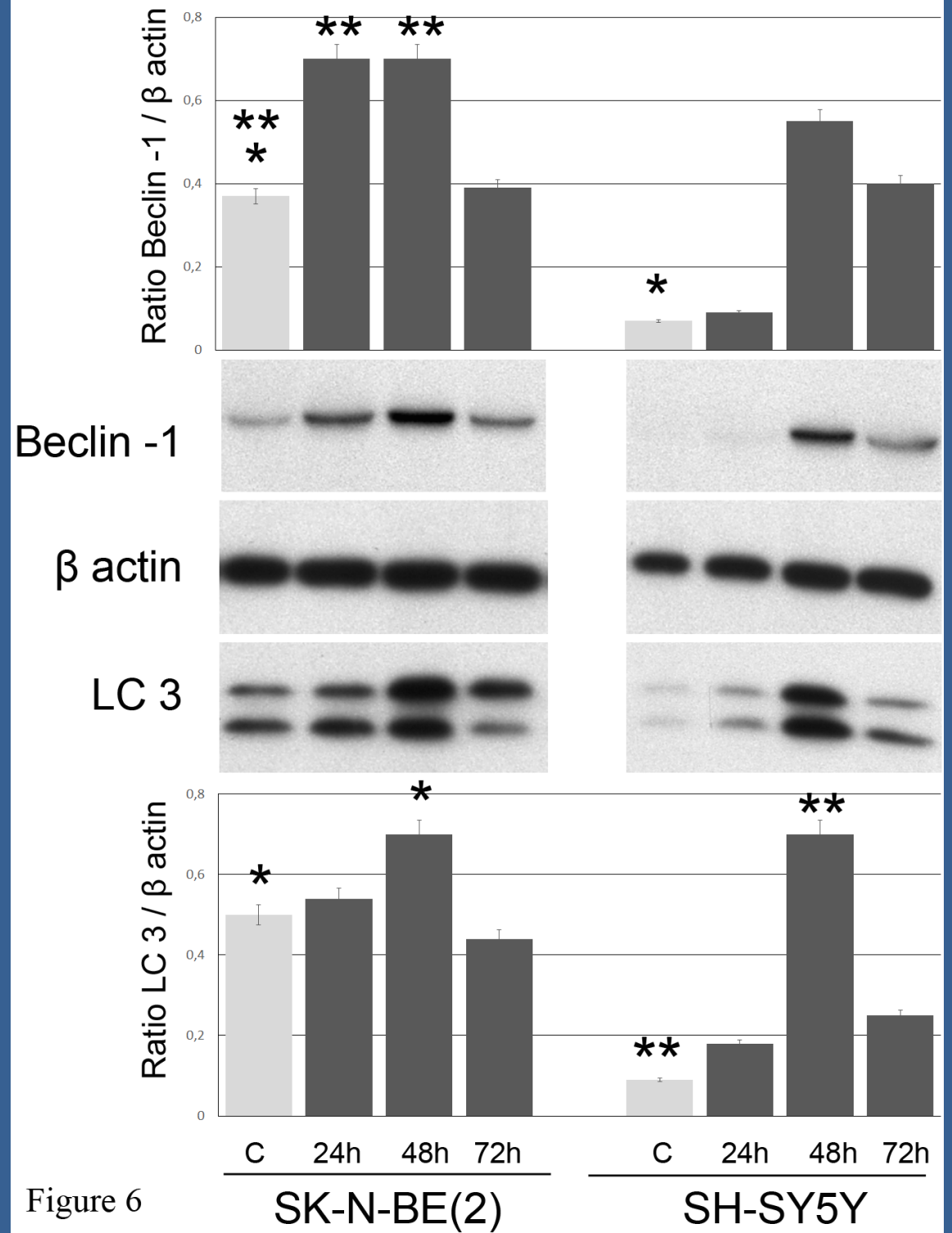
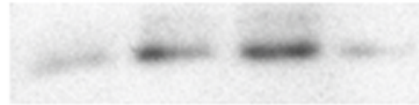


Figure 6



A)

BNIP-3



β -actin



Ctrl GO 24h GO 48h GO 72h

SK-N-BE(2)

B)

BNIP-3



β -actin



Ctrl GO 24h GO 48h GO 72h

SH-SY5Y

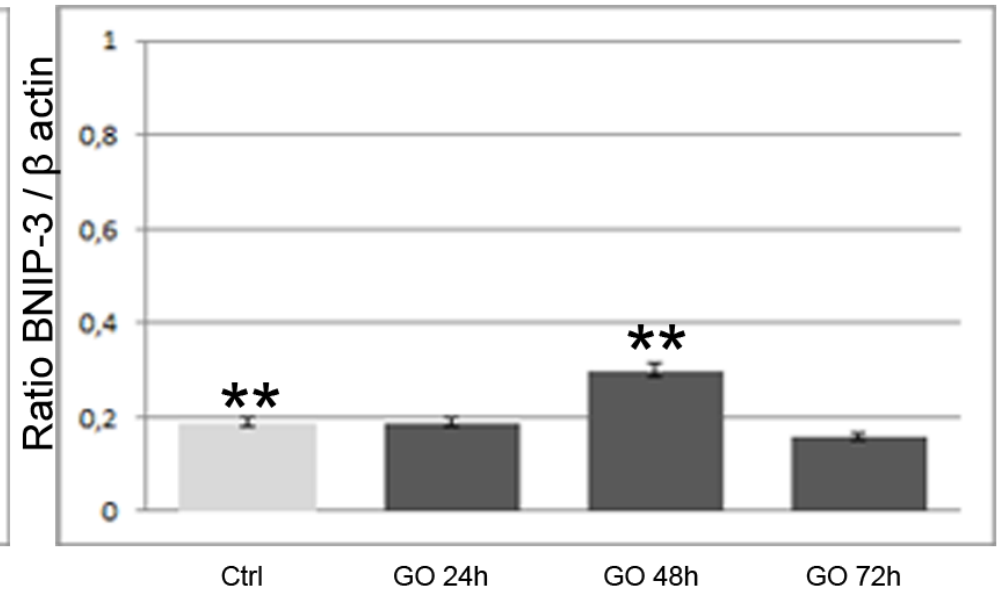
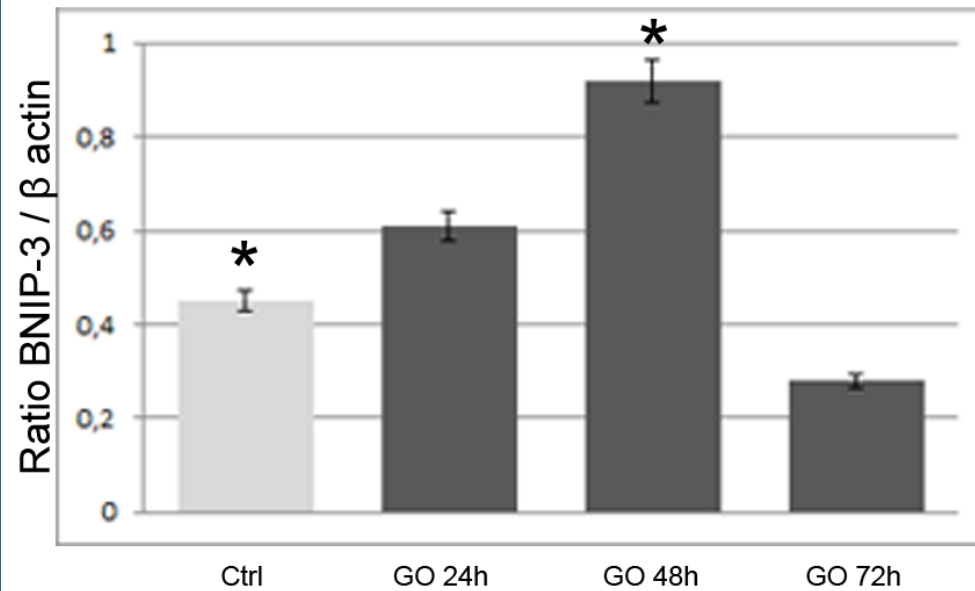
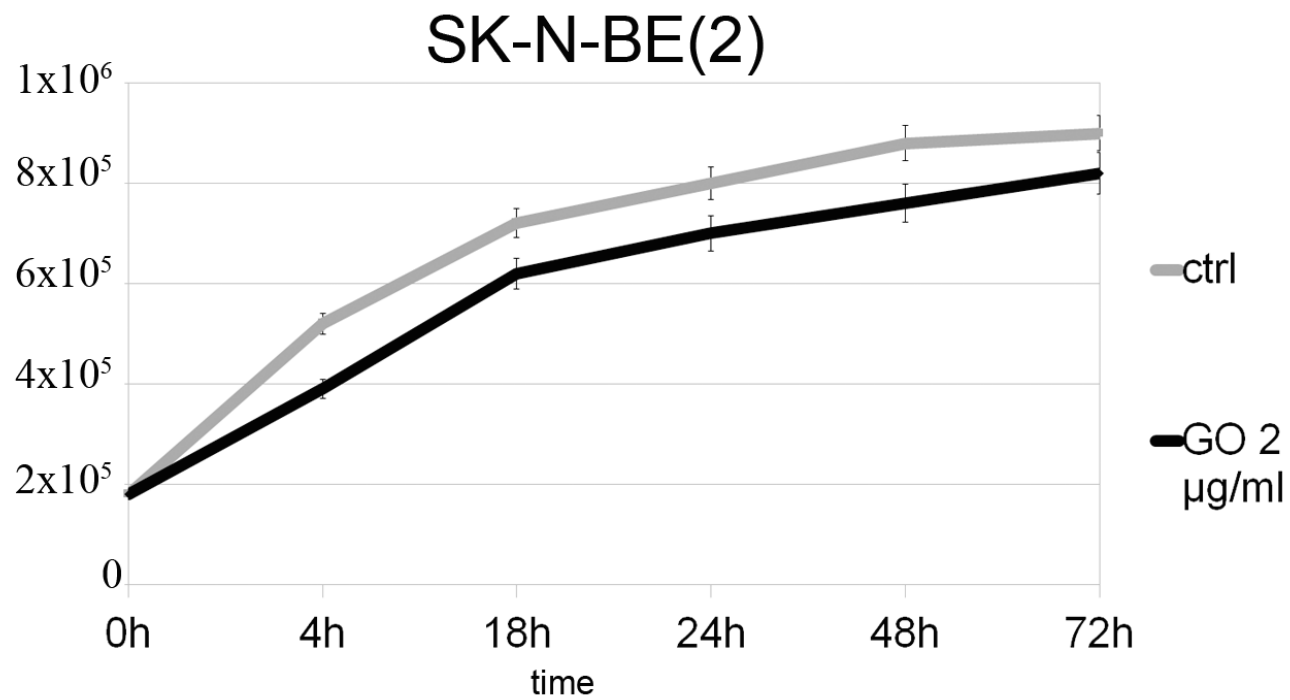


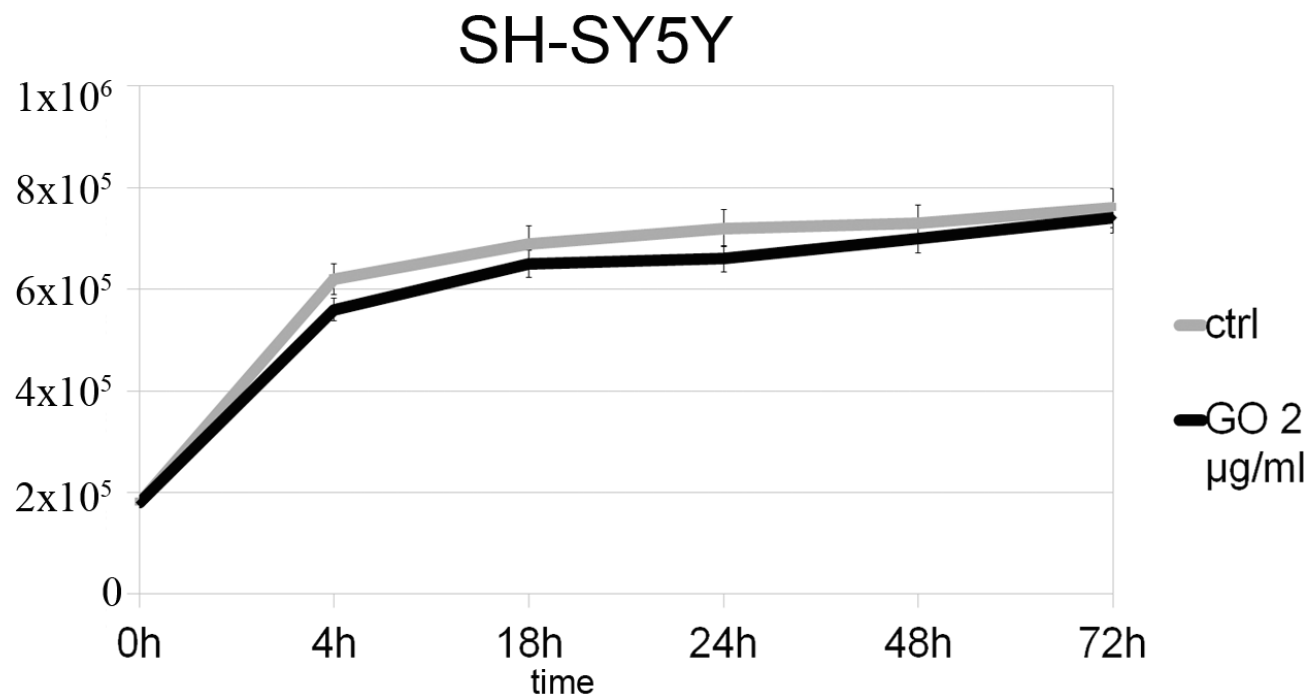
Figure 7



A)

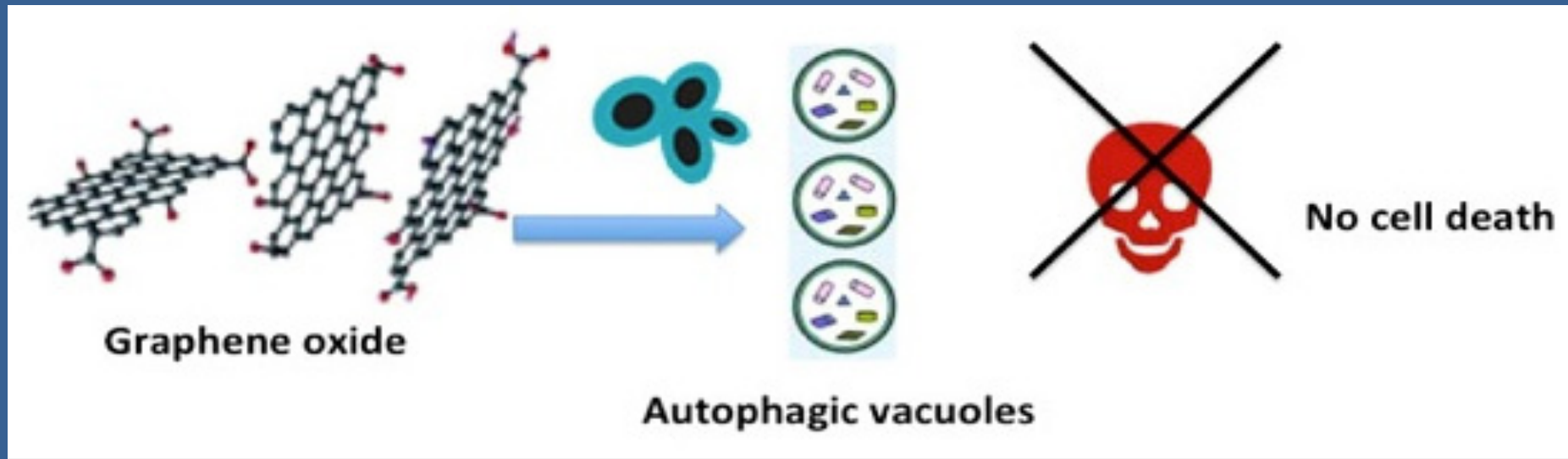


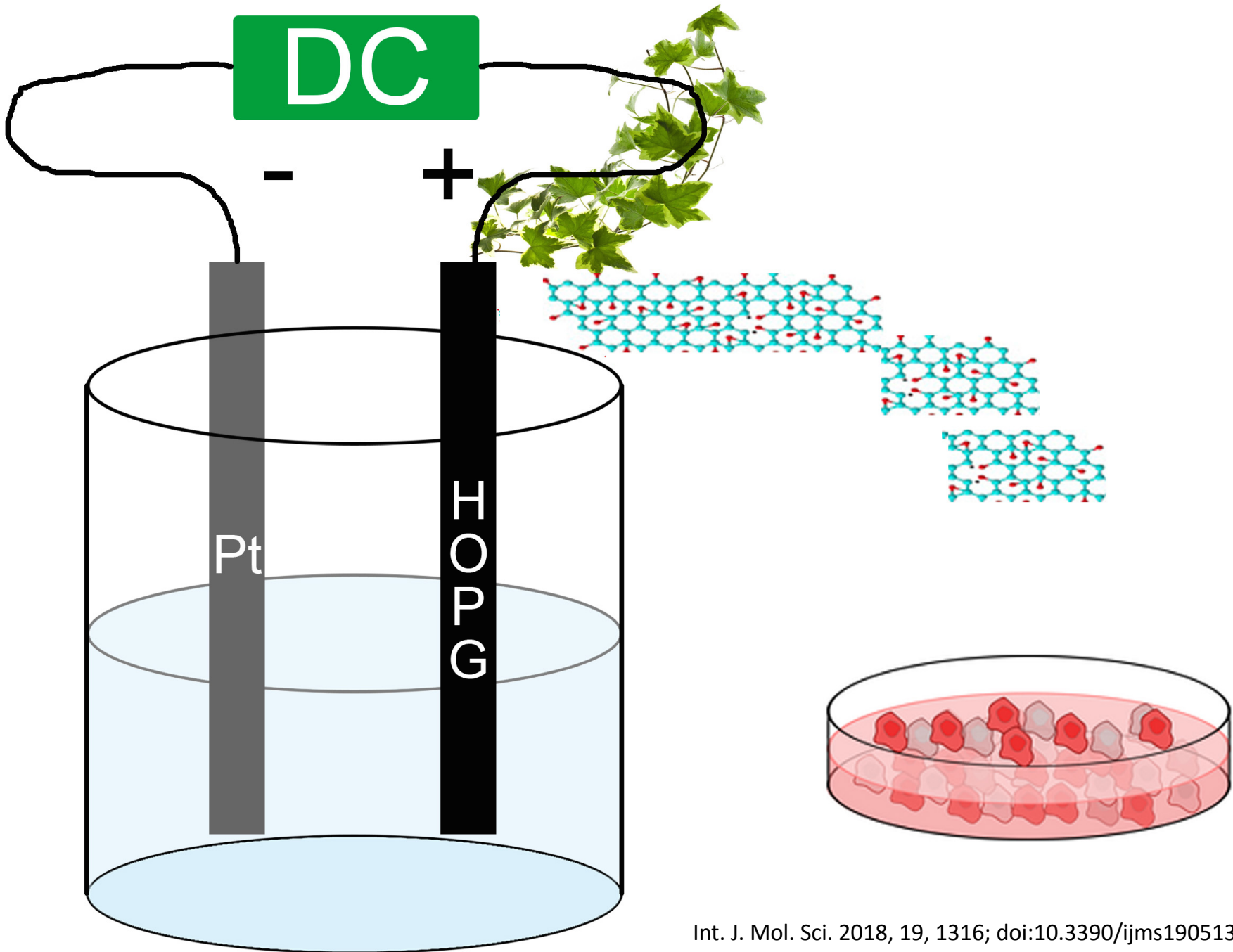
B)





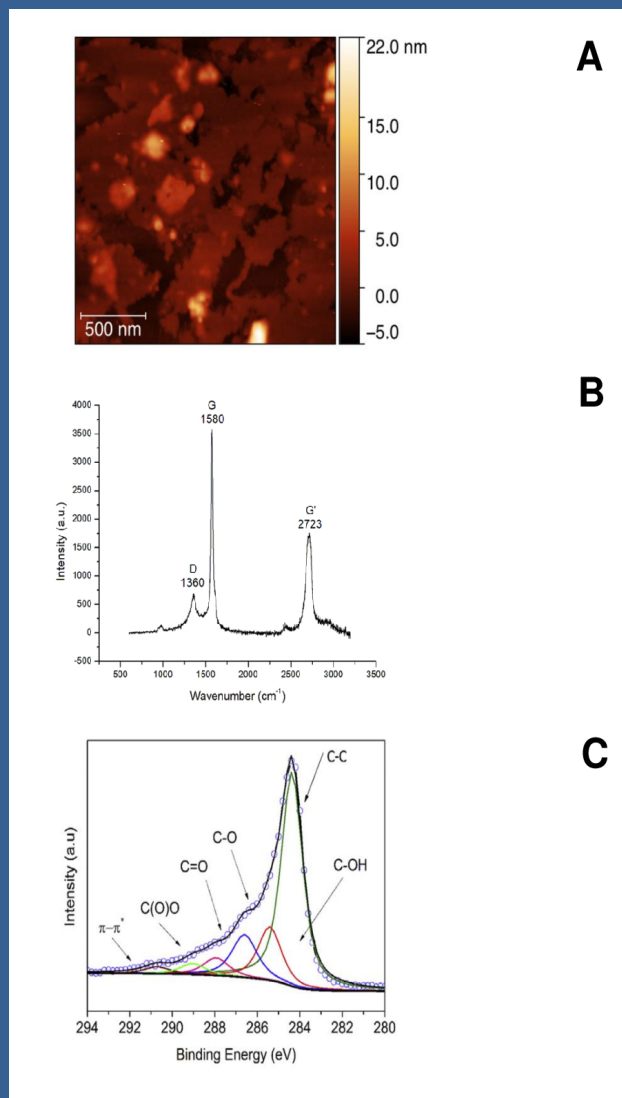
Graphene oxide and neuroblastoma cell lines







Physical-chemical properties of new GO



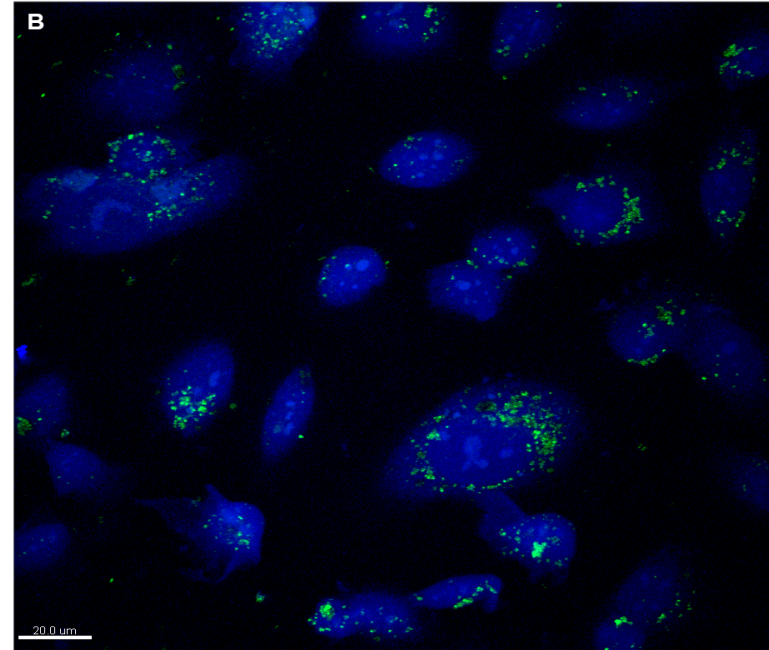
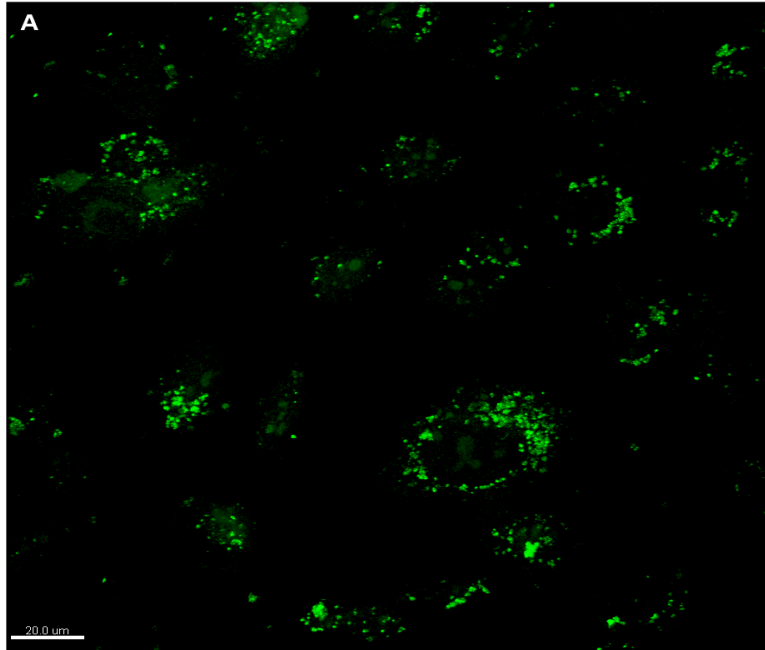
Chemical Properties	Metallic Elements
Elemental analysis ^a (% w/w)	Si (n.d)
	S (n.d)
	Ca (n.d.)
	Cr (n.d.)
	Fe (n.d.)
	Co (n.d.)
Physical Properties	Range Values
Surface Area (μm^2) ^b	0.1–3.0
Thickness (nm) ^b	1.2 ± 0.3
Number of Layer ^b	2 (bilayer)
Weight loss % (TGA) ^c	0.42 ± 0.40
Acidic sites (nmol/mg) ^d	4.02 ± 0.23
Extent of defects (I_D/I_G) ^e	0.10

Peak BE (eV)	C1s At. %	Functional Groups
284.4	59.0	C-C
285.4	16.0	C-OH
286.6	14.0	C-O
287.7	7.0	C=O
289.0	4.0	C(O)O
290.7	2.2	$\pi-\pi^*$

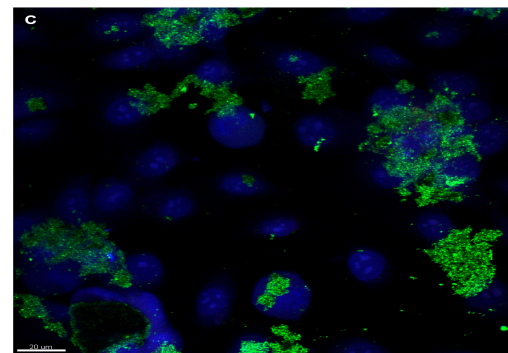
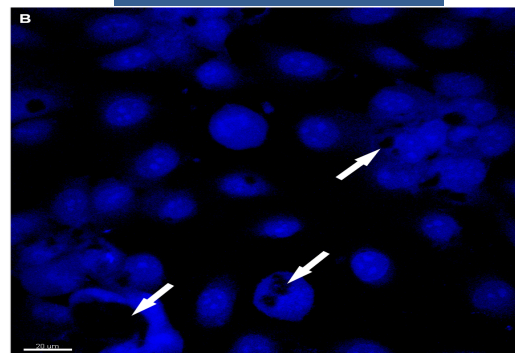
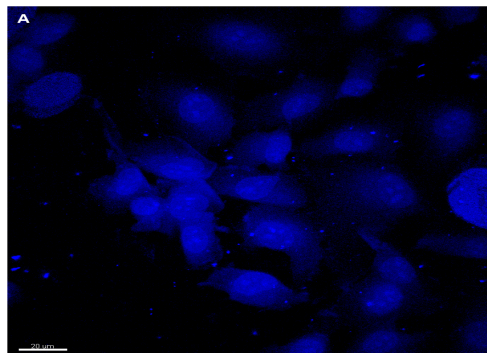
π^* refers to antibonding π molecular orbitals, with the highest energy level.



Green GO

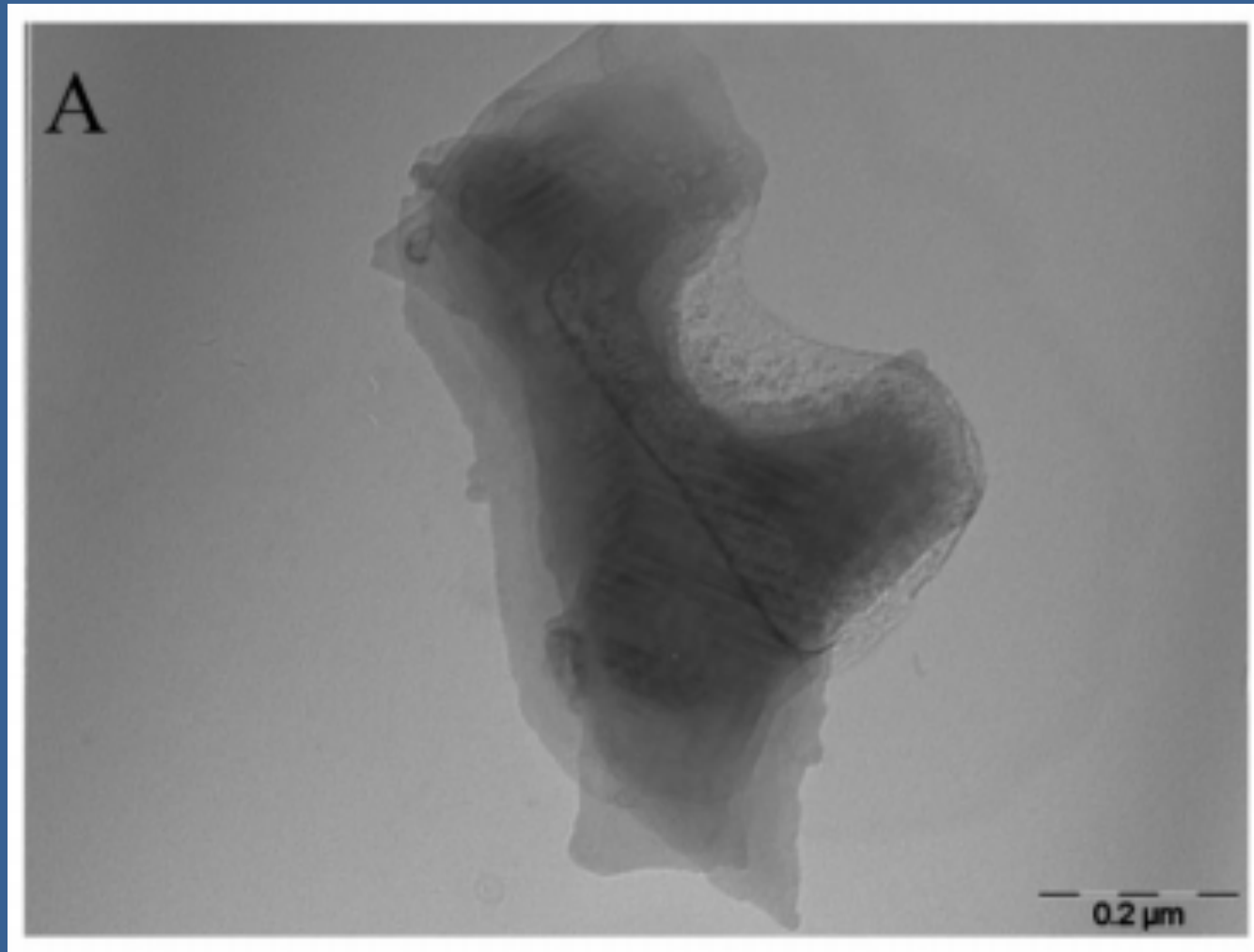


SWCNTs





Nanosheets



Patent (N°10201500023739)

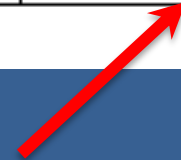


Chemical-physical parameters and microanalysis of the Graphene Oxide samples

Chemical-physical parameters and microanalysis of the graphene oxide samples

Sample	Shape	Thickness (nm)	Area (μm^2)	Micro Analysis % (w/w)
Electrochemically synthesized GO	rectangular nano sheets	1.2 \pm 0.3 N ^o layers: 2	0.1-3.0	Si, S, Ca, Cr, Fe, Co n.d

n.d.: not detectable



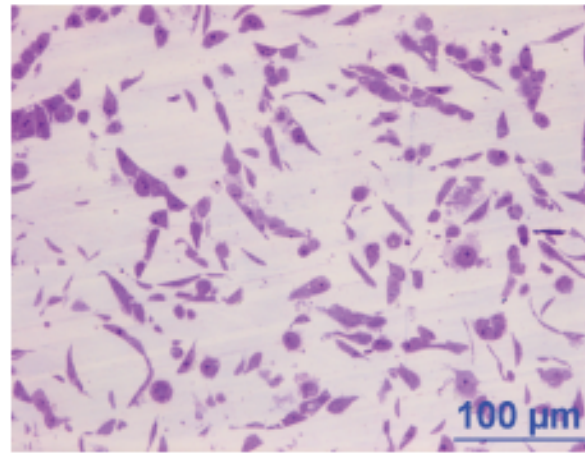


Many functional groups

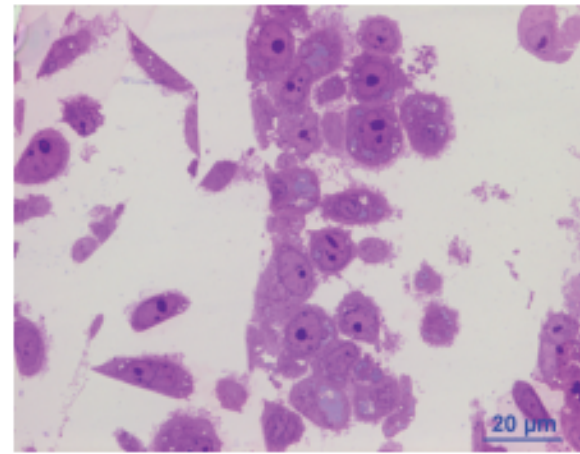
Table 2. Binding Energies (BE) and de-convoluted peaks (%) for C1s of GO

Peak BE (eV)	C1s At. %	Functional Groups
284.4	59.0	C-C
285.4	16.0	C-OH
286.6	14.0	C-O
287.7	7.0	C=O
289.0	4.0	C(O)O
290.7	2.2	π - π^*

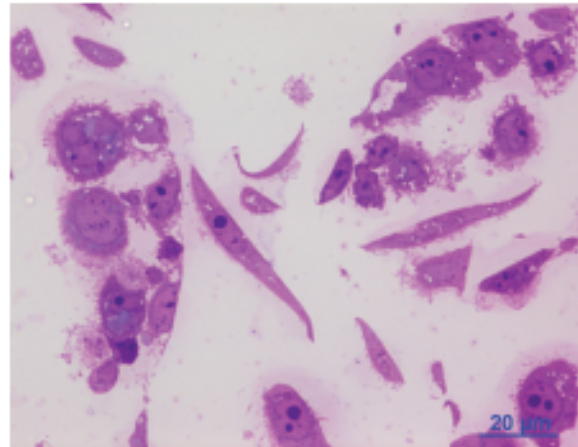
π - π^ : means the π -bonding orbital and the π^* -antibonding orbital*



(a)



(b)

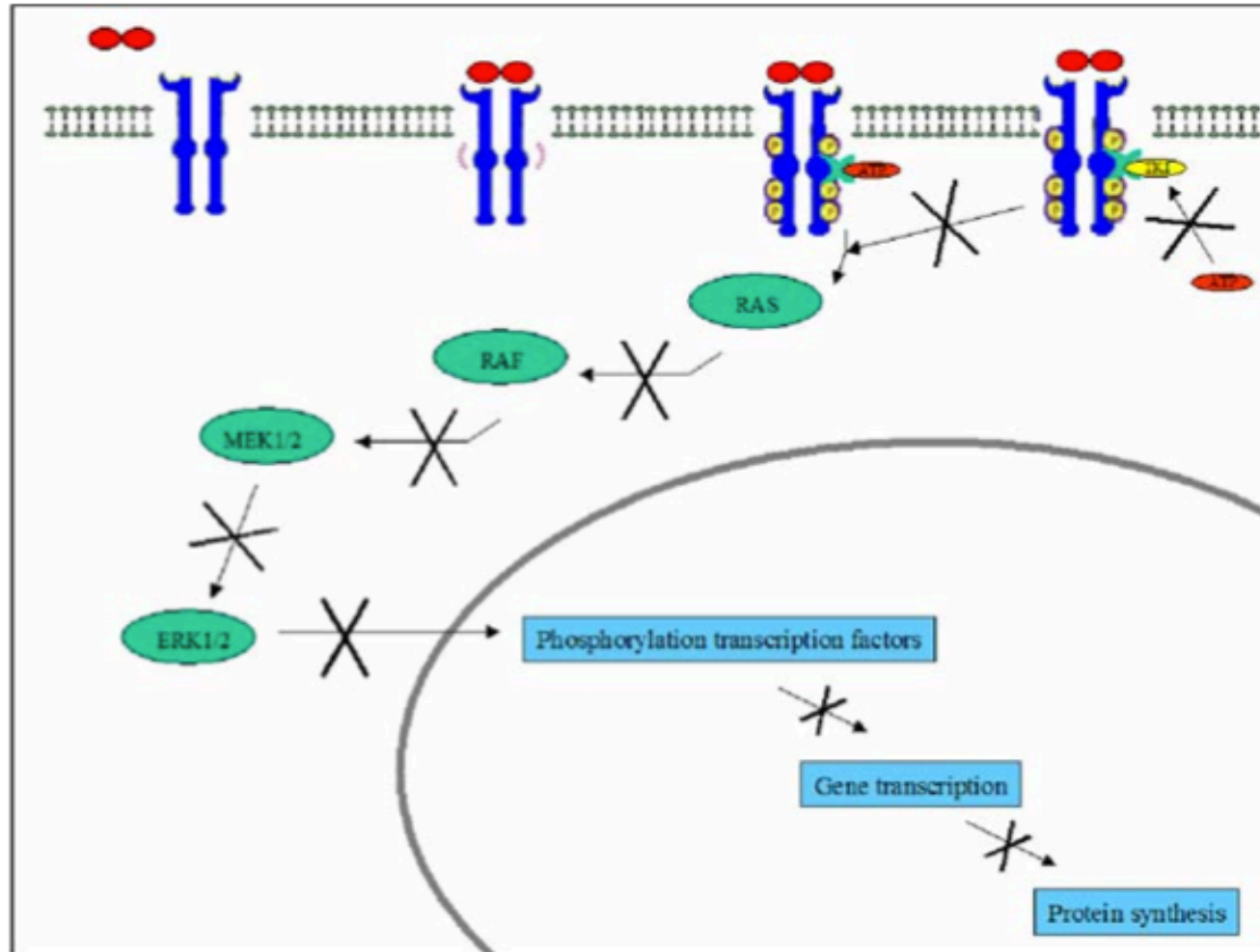


(c)

Optical microscopy of SK-N-BE(2)
treated with 2.0 µg/mL of GO at
(a) 24 h, magnification 20x;
(b) 24 h, magnification 60x;
(c) 48 h, magnification 60x
(May Grunwald Giemsa staining).



Small molecule Tyrosin Kinase inhibitors





S29 as TK Inhibitor

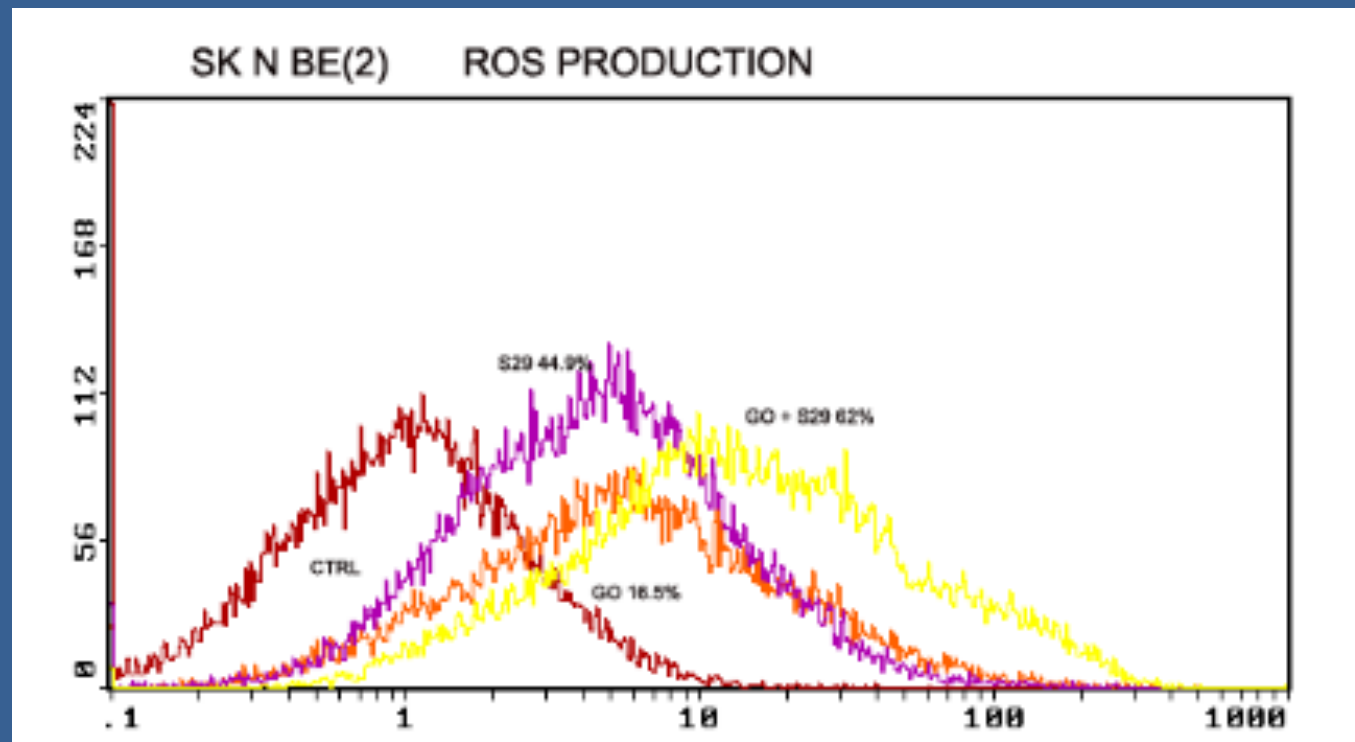
- **S29 (imatinib)**
S29 has been used
in combination
with radiotherapy
in Leukemias!!!!!!!!!!

...but has low
availability with
solid tumors



S29 driven by GO induces a higher amount of ROS when compared to S29 alone.

ROS formation was assayed by flow cytometry using the dye DCF

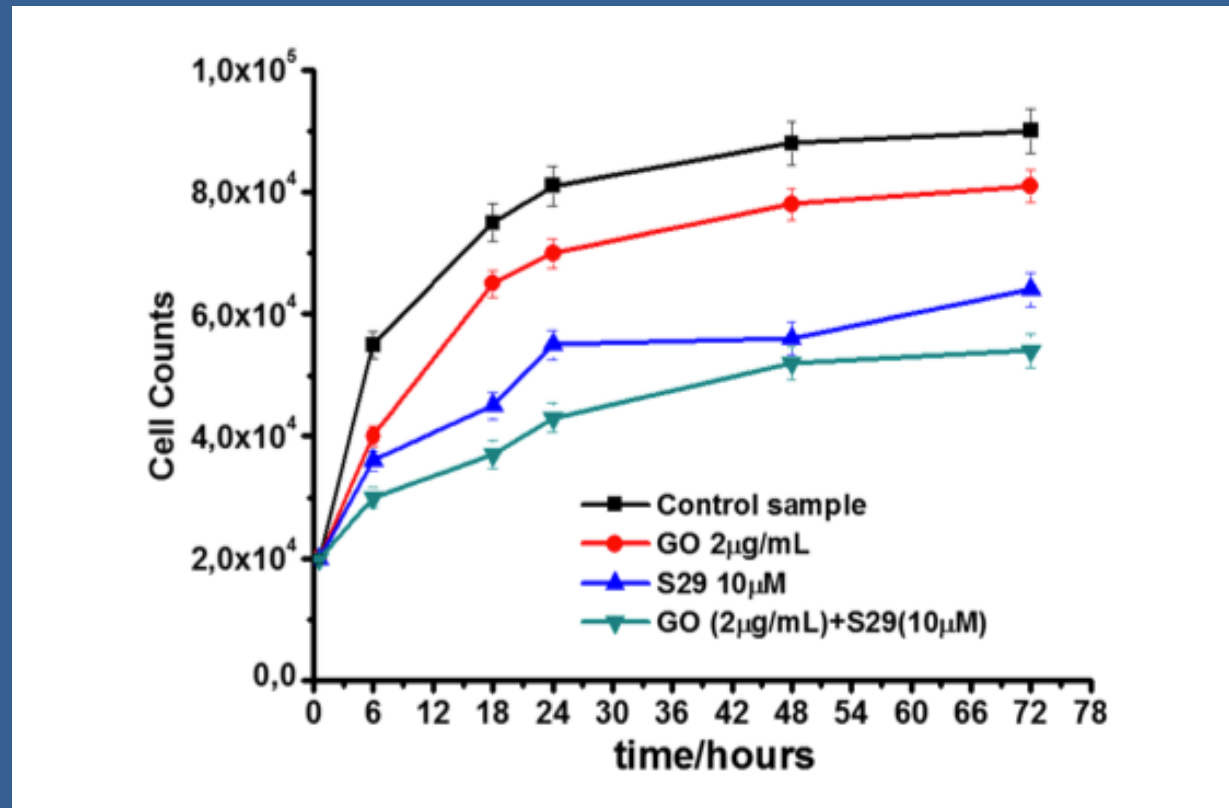




SK NBE cells treated with S29-GO

We tried to improve the S29 bioavailability

S29-GO increases growth inhibition!!!!!!!





Conclusions and future perspectives

- Low cytotoxicity of GO/possibility to use small doses with multiple drugs
- Improve uptake of GO in all cells and in 3D structures.
- What is the destiny of GO in cells in the long term?
- Is GO able to cross the blood-brain barrier?
- Is GO activating immune system?