# IR AND RAMAN SPECTROSCOPY AND MAPPING AT DAΦNE LUCE: ADVANCED TOOLS TO SUPPORT THE RATIONAL DESIGN OF FUNCTIONAL NANOSTRUCTURES

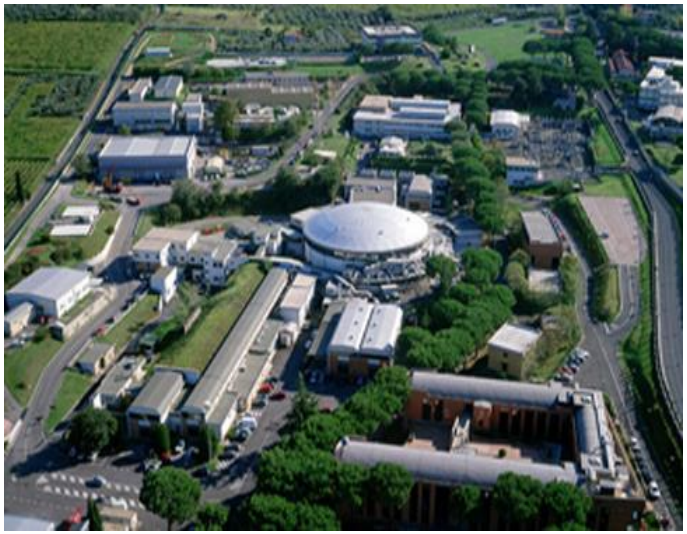

*Valentina Mussi*

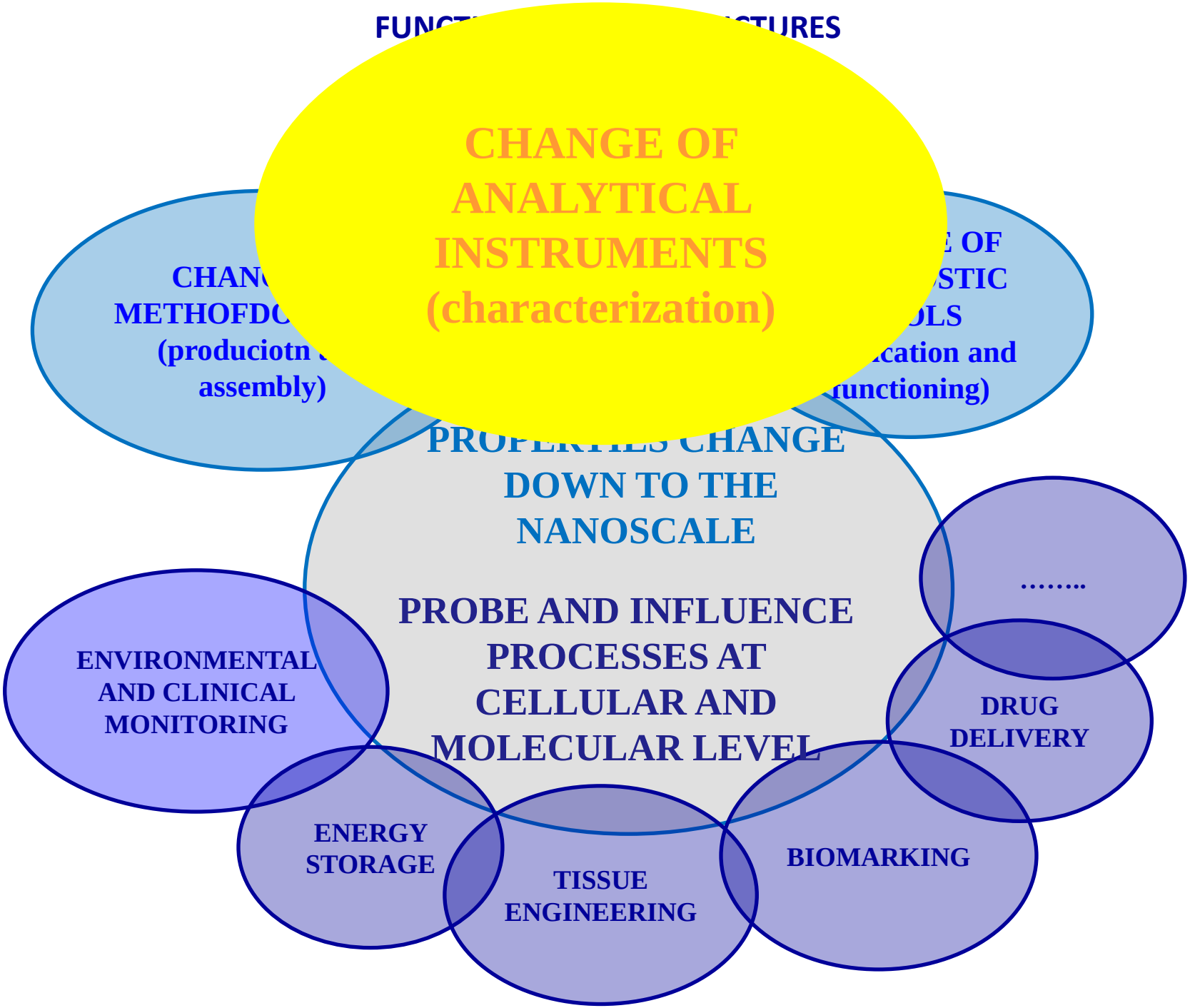
**Consiglio nazionale delle Ricerche**
**Istituto dei Sistemi Complessi**

**Area della Ricerca di Roma Tor Vergata**

*Mariangela Cestelli Guidi*

**Laboratori Nazionali di Frascati INFN**

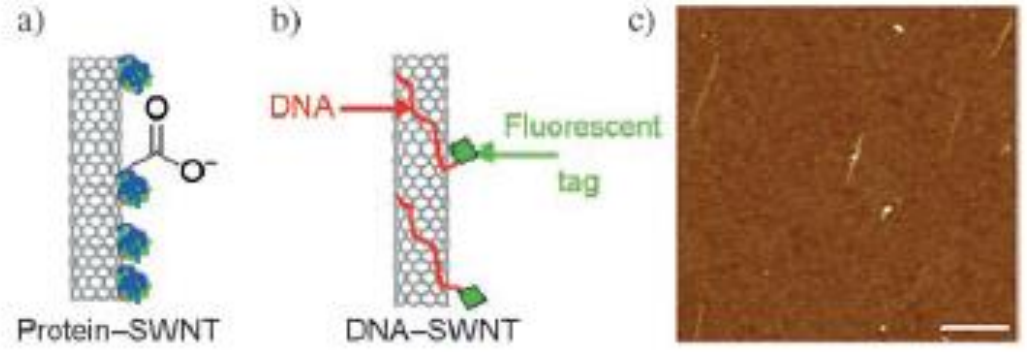



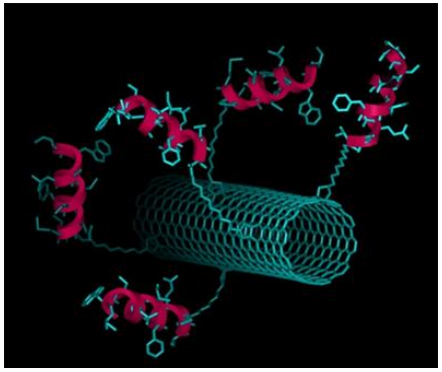

FUNCT CTURES
CHANGE OF ANALYTICAL INSTRUMENTS (characterization)
CHAN
METHOFDC
(produciotn
assembly)
E OF
STIC
OLS
ication and
unctioning)
PROPERTIES CHANGE DOWN TO THE NANOSCALE
PROBE AND INFLUENCE PROCESSES AT CELLULAR AND MOLECULAR LEVEL
ENVIRONMENTAL AND CLINICAL MONITORING
ENERGY STORAGE
TISSUE ENGINEERING
BIOMARKING
DRUG DELIVERY
……..

# CARBON NANOTUBES

***Carbon Nanotubes as Intracellular Transporters for Proteins and DNA: An Investigation of the Uptake Mechanism and Pathway, N. W. Shi Kam, Z. Liu, and H. Dai***

***Anaew. Chem. Int. Ed. 2005. 44. 1–6***

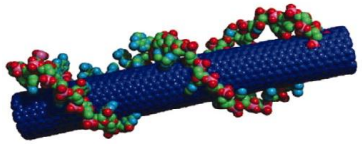


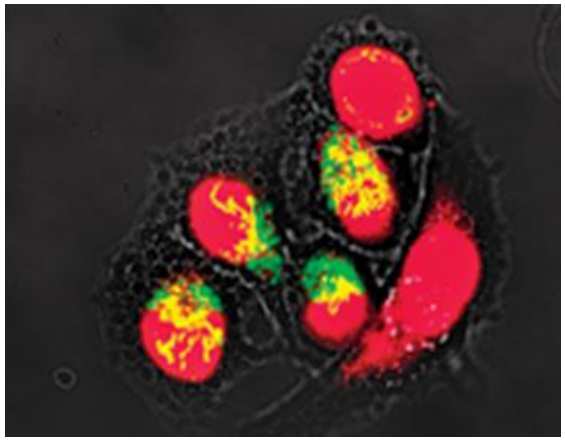



***Carbon nanotubes as multifunctional biological transporters and near-infrared agents for selective cancer cell destruction, N. W. Shi Kam, M. O'Connell, J. A. Wisdom and H. Dai***

***PNAS August 16, 2005 | vol. 102 | no. 33 | 11600-11605***

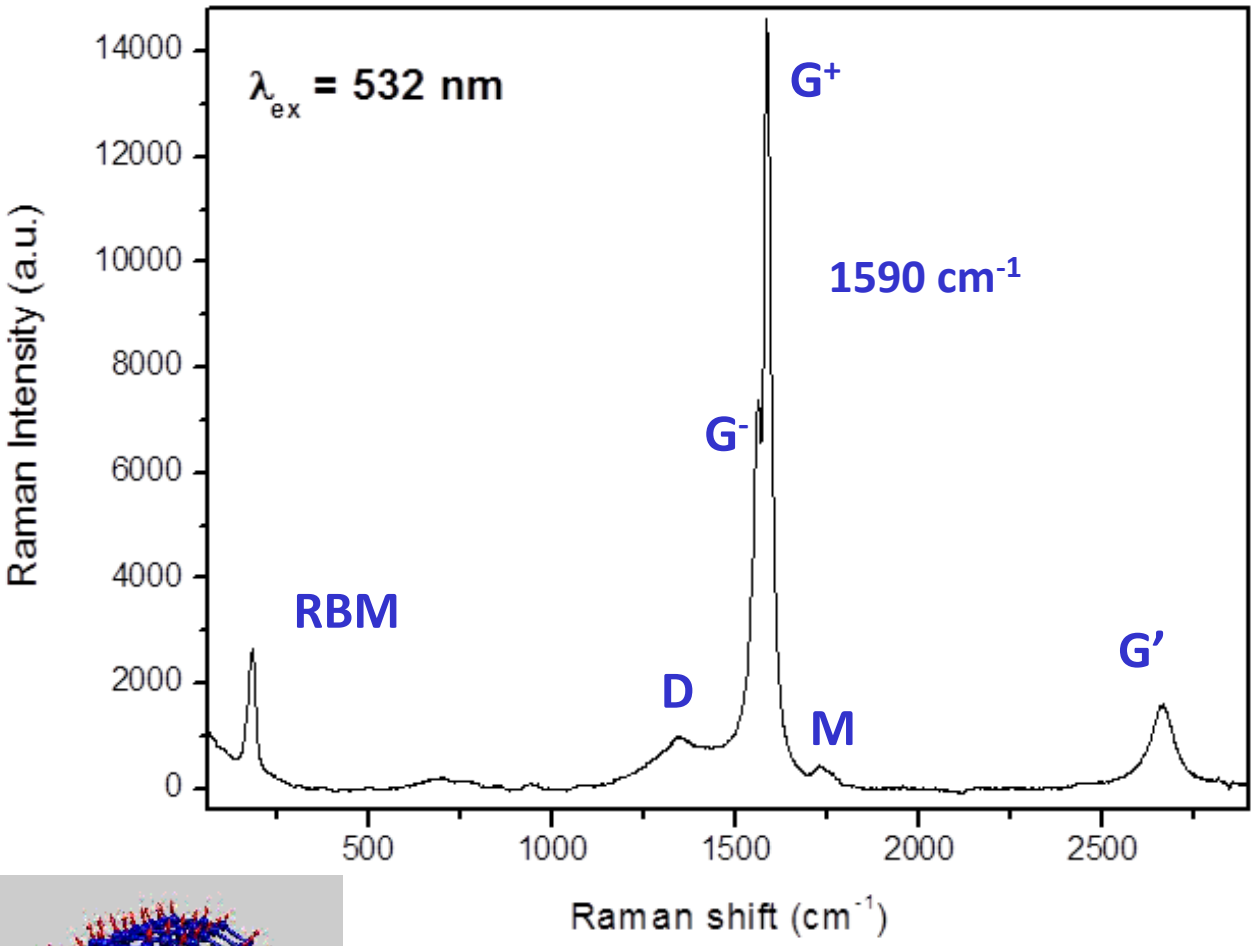
*"the transporting capabilities of carbon nanotubes combined with suitable functionalization chemistry and their intrinsic optical properties can lead to new classes of novel nanomaterials for drug delivery and cancer therapy"*

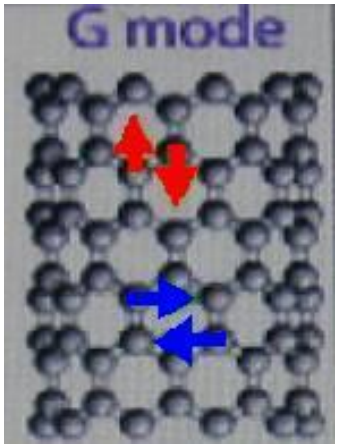




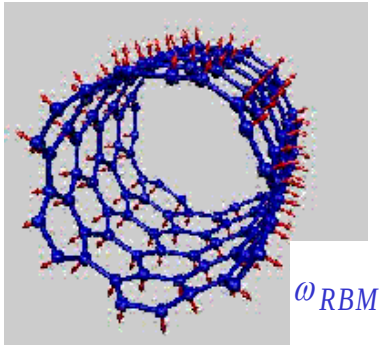
***Tiny Agents of Cancer Death***
**Carbon nanotubes (green)**
**take aim at cancer cell nuclei (red)**

# RAMAN SPECTROSCOPY




$$\omega_{G^-}(cm^{-1}) = \omega_{G^+}(cm^{-1}) - \frac{C_{s/m}}{d_t^2(nm^2)}$$



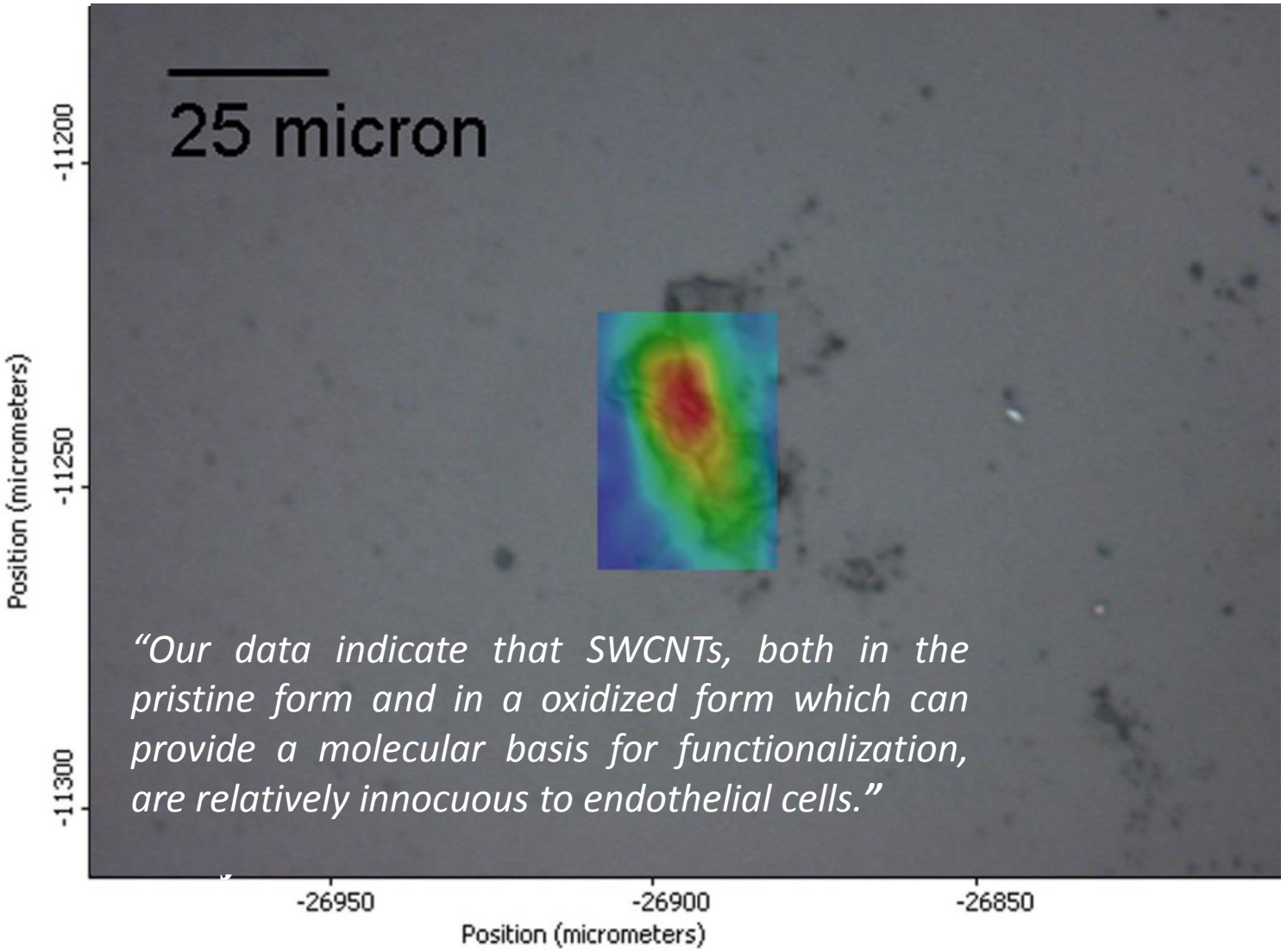
$$\omega_{RBM} = \frac{A}{d_t} + B$$

Graphitic nature

structural Disorder

# LOCALIZATION: CELL MAPPING *WITHOUT* LABELLING

**HUVE cells (Human Umbilical Vein Endothelial)** plated on **gelatin-coated glass coverslips** at **40,000 in 500µl** in a 24 well plate and grown in **complete M199 medium for 24 hours**. The cells were **treated with oxidized SWCNTs (50000 ng/ml)** for an additional **24 hours**, then **washed with Phosphate Buffer Saline** and **fixed in 0.25% gluteraldehyde.**



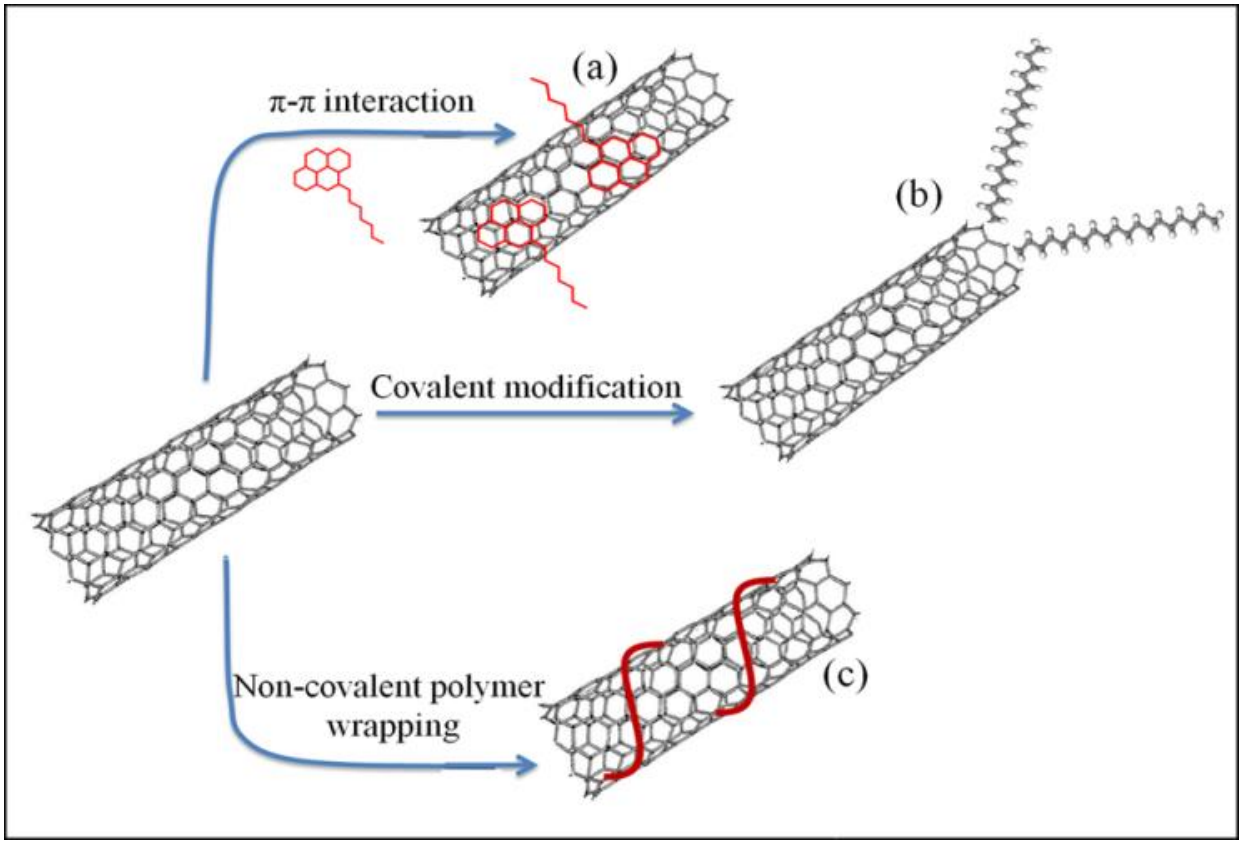
# SWCNT IN BIOMEDICINE

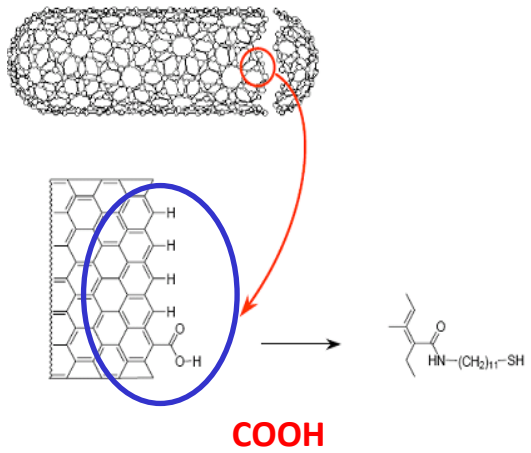
## NON-COVALENT COATING

**Sensing and Imaging**

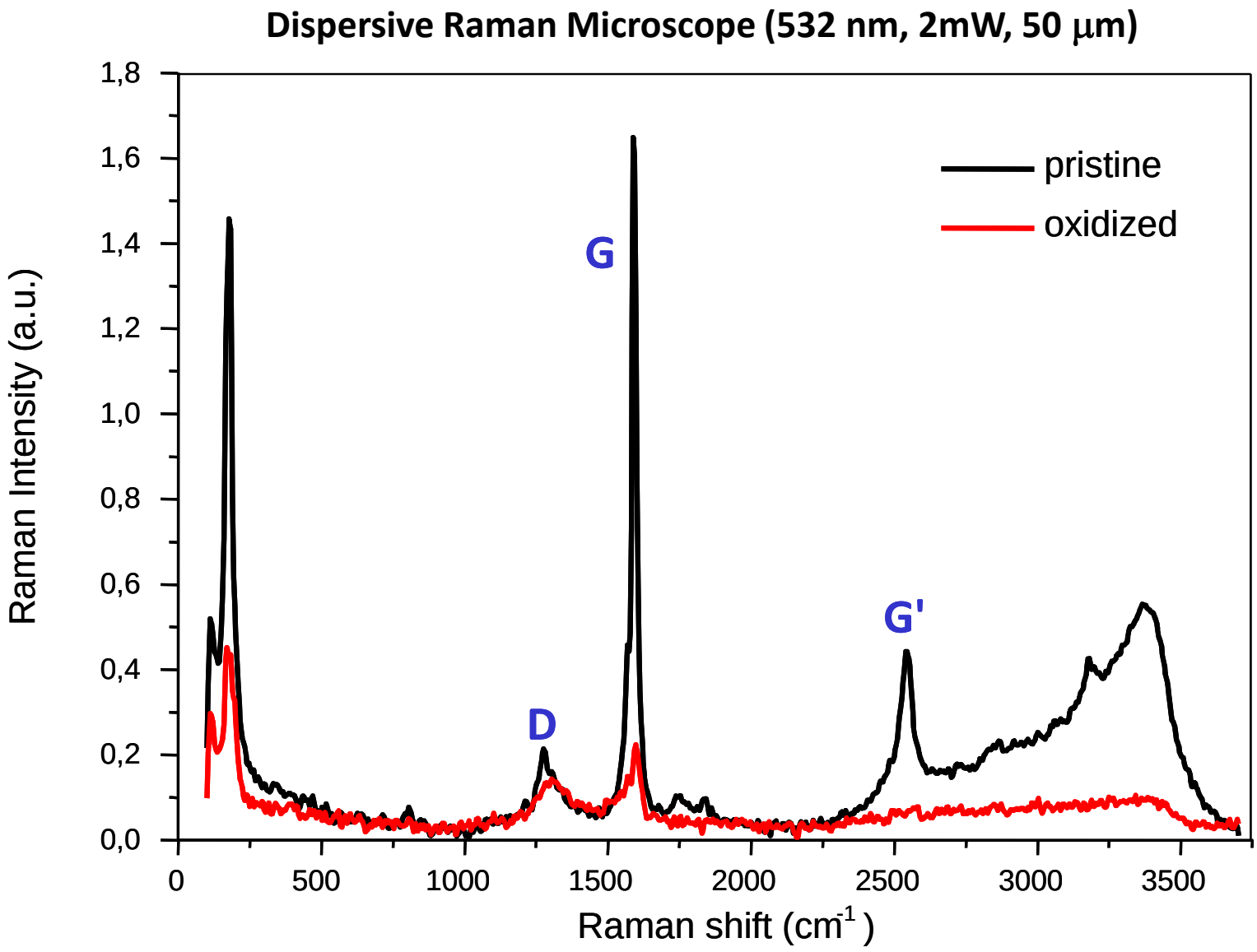
## COVALENT FUNCTIONALIZATION

**Therapy: Drug delivery and Targeting**



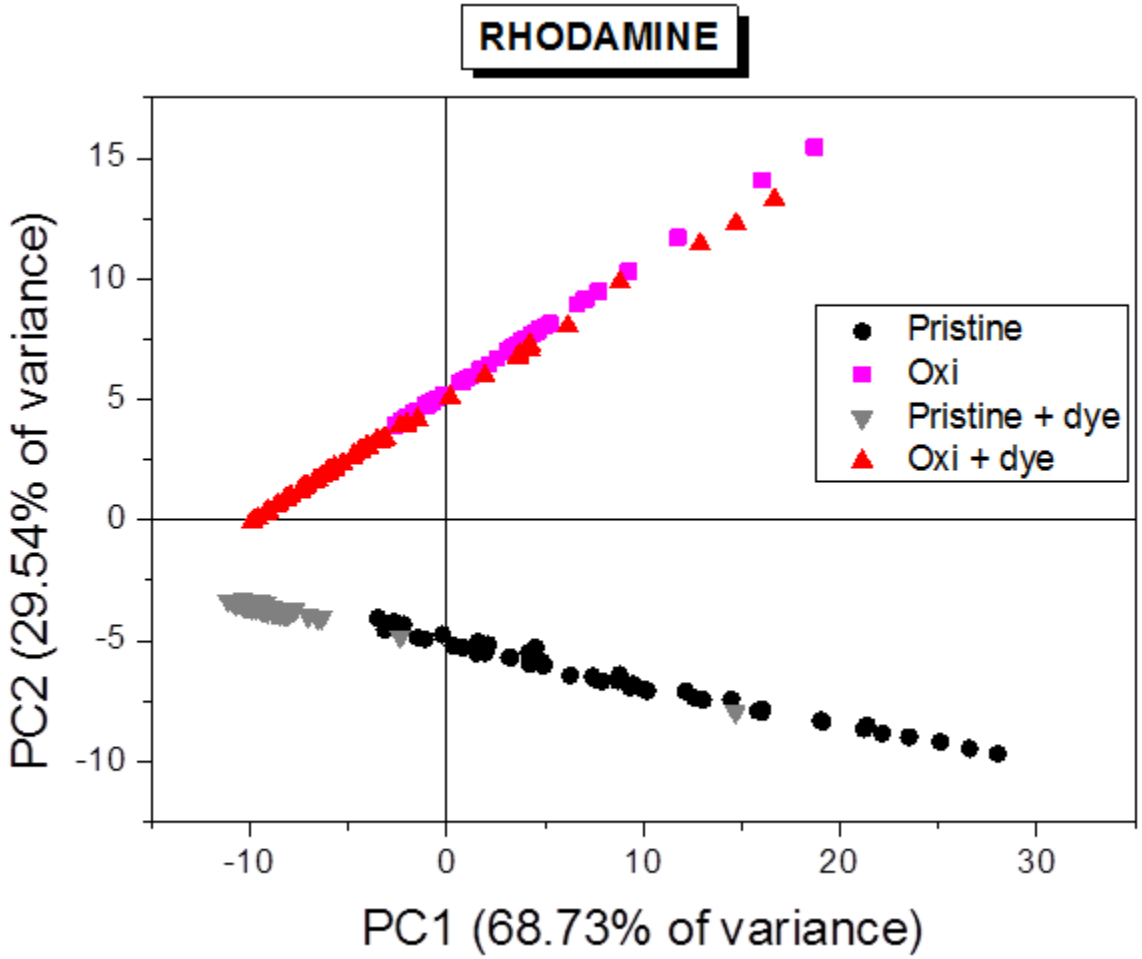
# RAMAN SPECTROSCOPY




SWCNT powder is ultrasonicated @40°C in a 3:1 mixture of $H_2SO_4$ and $HNO_3$ for 3 hours. The solution is neutralized by dilution and filtering. The powder deposited on the filter is washed in water, ethanol and acetone and dried in oven.

# MULTIVARIATE ANALYSIS APPLIED TO RAMAN MAPPING: «FUNCTIONALIZATION STATUS»

**PCA is a projection method to reduce the complexity of multidimensional data while retaining most of the information. The reduction is done by merely considering those between the new variables, obtained as linear combination of the original ones, that are the main for variance.**




**Raman Microscope XploRA, HORIBA Jobin Yvon.**

**$\lambda_{exc}$ @ 532 nm**

**Powder/flake samples directly deposited on a glass slide**

**49 spectra point-by-point on an area of 6x6 $\mu m^2$ with a step size of 1 $\mu m$.**

**Spectral range 74-2700 $cm^{-1}$**

**……..Is it possible to compare different functionalization molecules?.....**

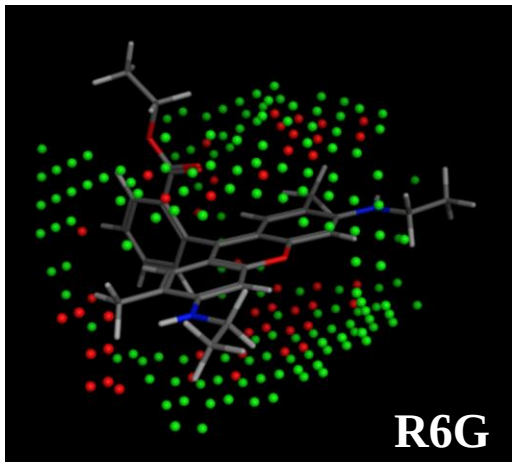
# COMPUTATIONAL RESULTS: GRID SOFTWARE

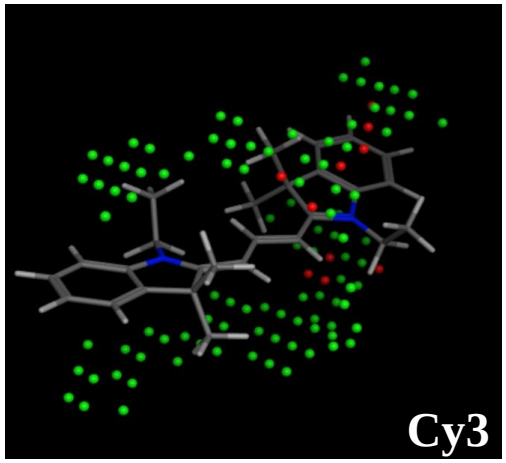
**The interaction energy between a small molecule or particle (called the probe) and a compound (the target, here THA, Cy3 and R6G) is calculated by positioning the probe in a 3D regular lattice of points surrounding the target. The set of interaction energy values generates a Molecular Interaction Field (MIFs) typical of the compound and the probe. The number of the points in the grid with energy less than a user–defined threshold gives an estimation of the propensity of the target to interact with a certain probe ("Interaction Volume").**

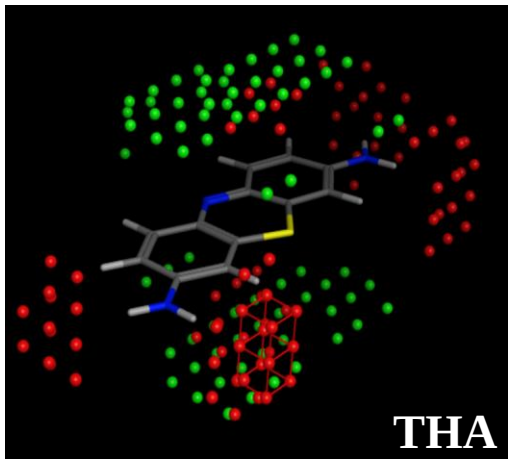
**CAR PROBE**: miming interaction with aromatic carbons **PRISTINE**

**O- PROBE**: miming carboxylate atoms **OSSIDATI**

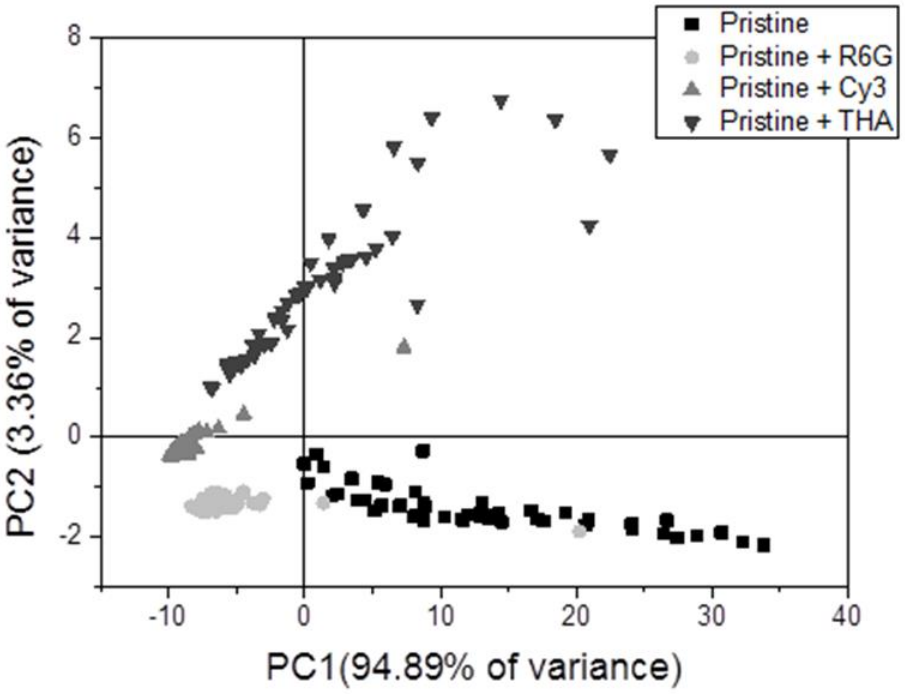
| | CAR (-1 Kcal/mol) | O (-3 Kcal/mol) |
|---|---|---|
| Cyanine | 98 | 9 |
| Rhodamine 6G | 185 | 11 |
| THA | 74 | 64 |

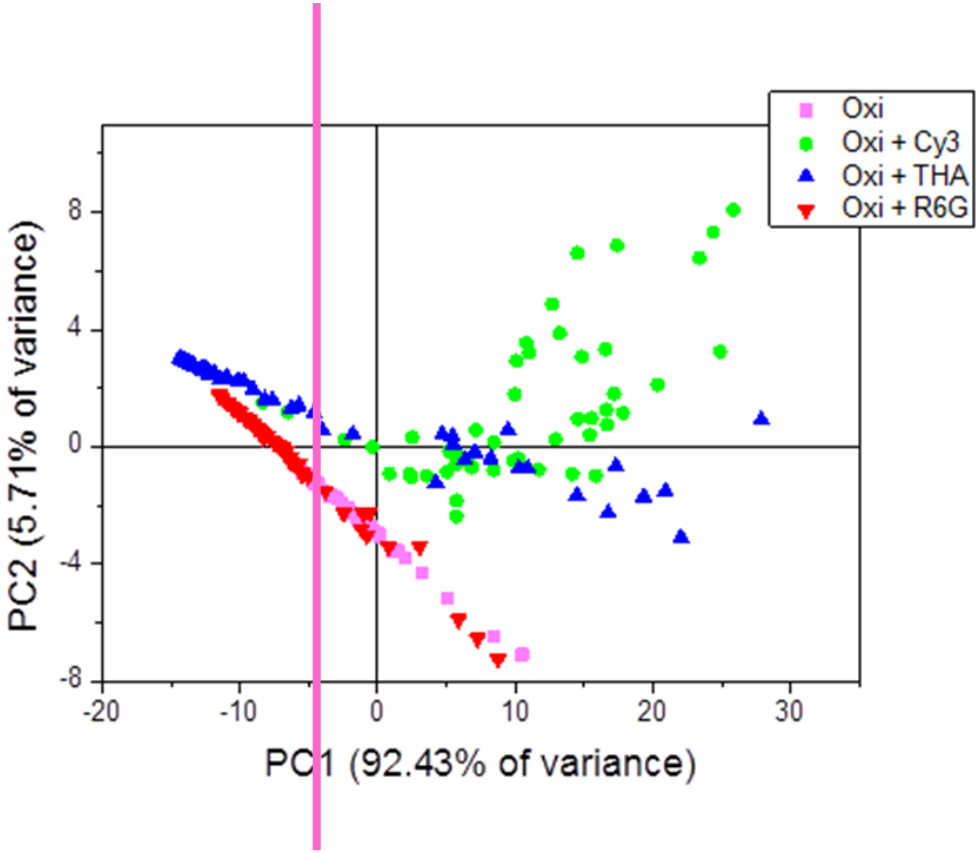


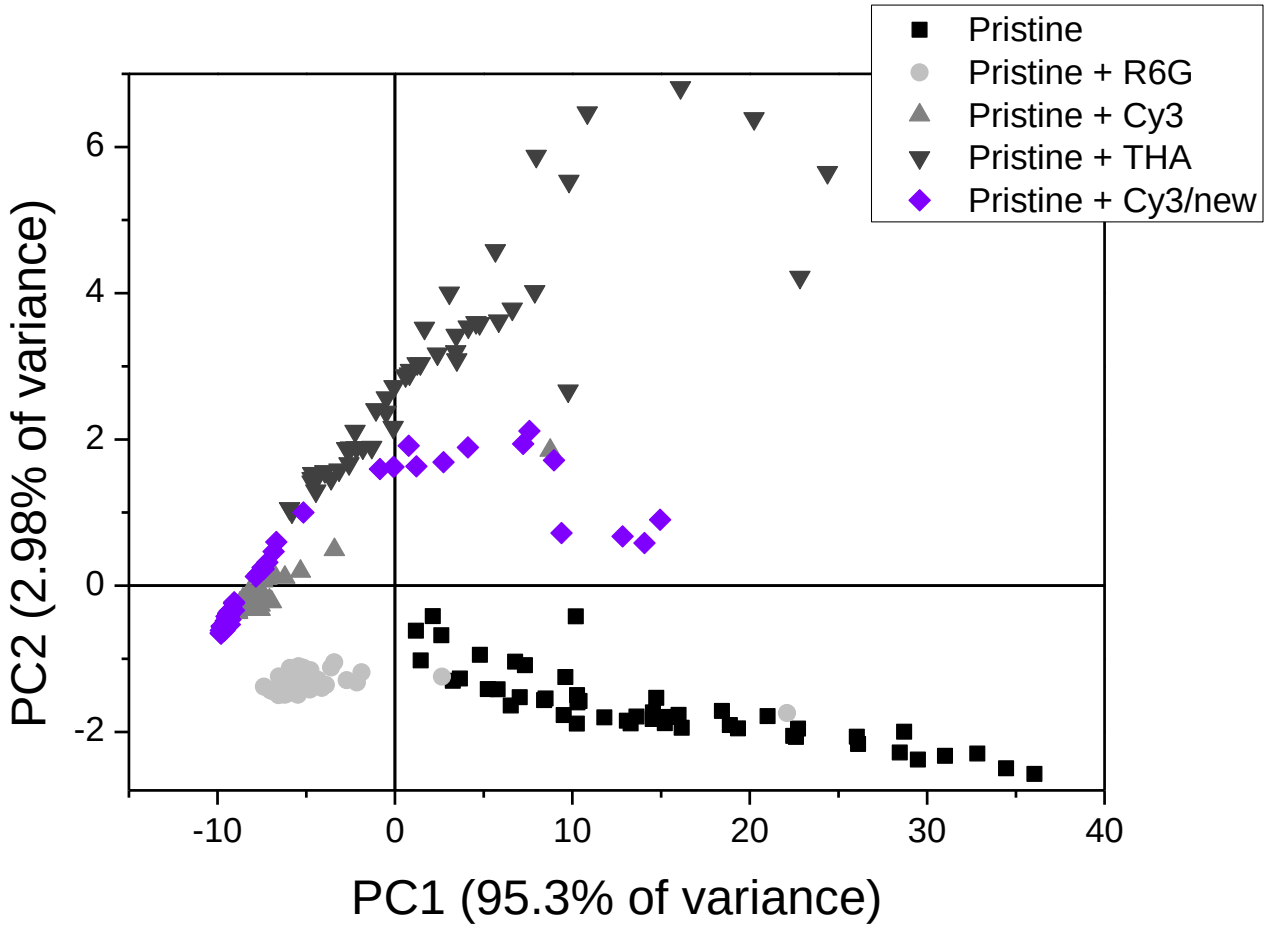
# FUNCTIONALIZATION EFFICIENCY




| | Car (-1.0 kcal/mol) | O- (-3.0 kcal/mol) |
|---|---|---|
| Cyanine | 98 | 9 |
| Rhodamine 6G | 185 | 11 |
| THA | 74 | 64 |

# METHOD RELIABILITY




**Multivariate Analysis Applied to Raman Mapping of Dye-Functionalized Carbon Nanotubes: a Novel Approach to Support the Rational Design of Functional Nanostructures**

***S. Visentin, N. Barbero, F. R. Bertani, M. Cestelli Guidi, G. Ermondi, G. Viscardi and V. Mussi***

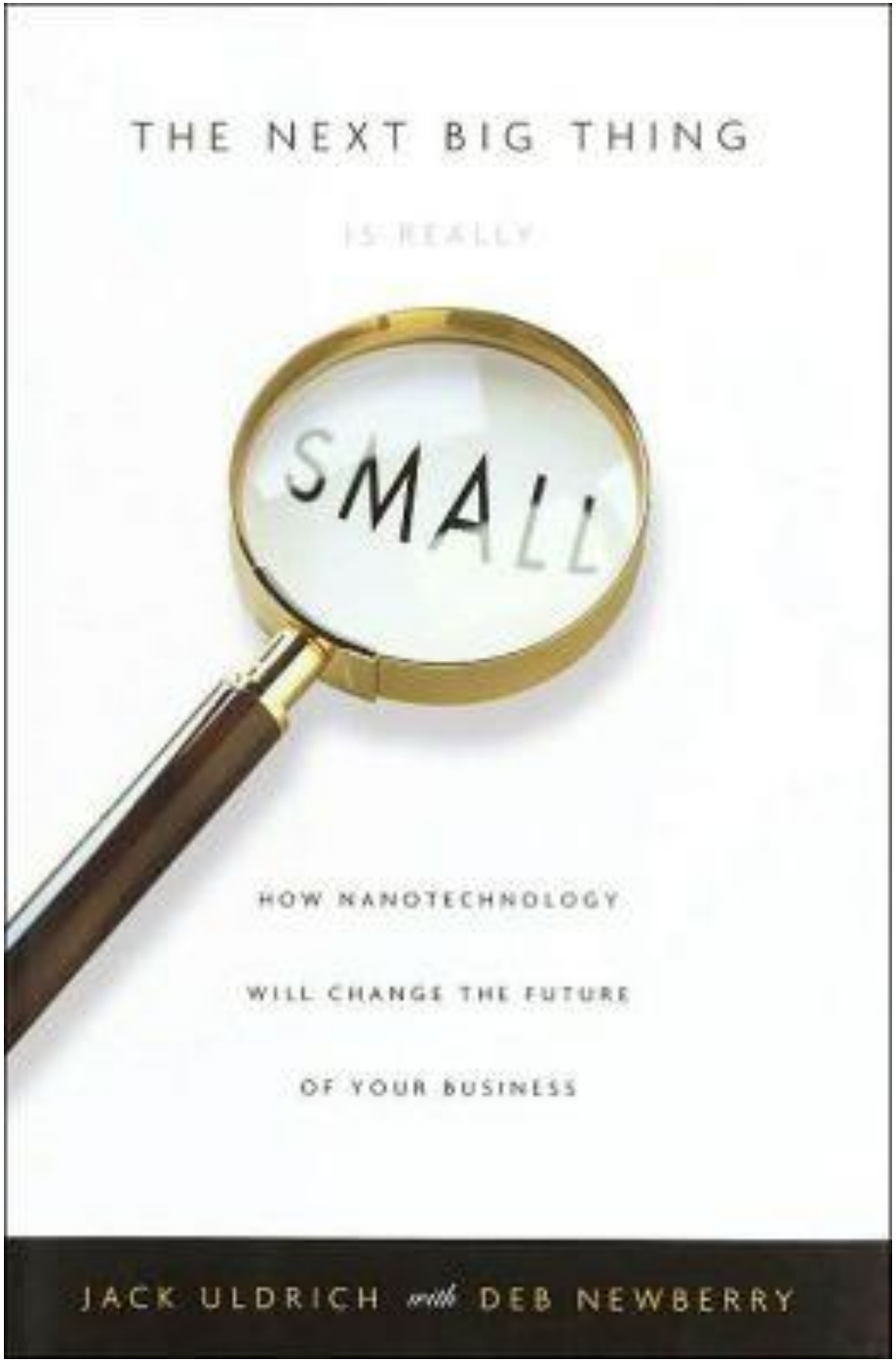
***SUBMITTED TO CARBON***

## FUTURE PROSPECTS: RAMAN ANALYSIS AND MAPPING FOR

- COOH groups determination (multi-functionalization);
- Study and Mapping of homogeneity of oxidation;
- Optimization of the oxidation procedure;
- Analysis of the efficiency of the functionalization procedure;
- Comparison of various labels/biomolecules/targeting moietis;
- Study of the homogeneity of the final sample;
- Dosing and toxicology studies for bio-imaging and delivery applications;
- Optimization of the design and synthesis of new nanoscale functional structures;
- Analysis of different functional/hybrid nanostructures (allosyte, metal nanoparticles..)

## MORE AMBITIOUS: DIAGNOSIS AND THERAPY

- Label-free carrier localization during *in-vitro* experiments;
- Label-free determination of carrier biodistribution for *in-vivo* experiments;




**..AND FUNCTIONAL(IZED)**

# FUTURE PROSPECTS

UNIVERSITÀ DEGLI STUDI DI TORINO
ALMA UNIVERSITAS TAURINENSIS


Dipartimento di Biotecnologie Molecolari e Scienze per la Salute

- Sonja Visentin
- Nadia Barbero
- Giuseppe Ermondi




http://www.biophysicsatrome.org


http://www.cost.eu/COST_Actions/bmbs/Actions/BM1401


ZZZ...
!SNORT!
Zzz...
THANK YOU FOR
YOUR ATTENTION!!