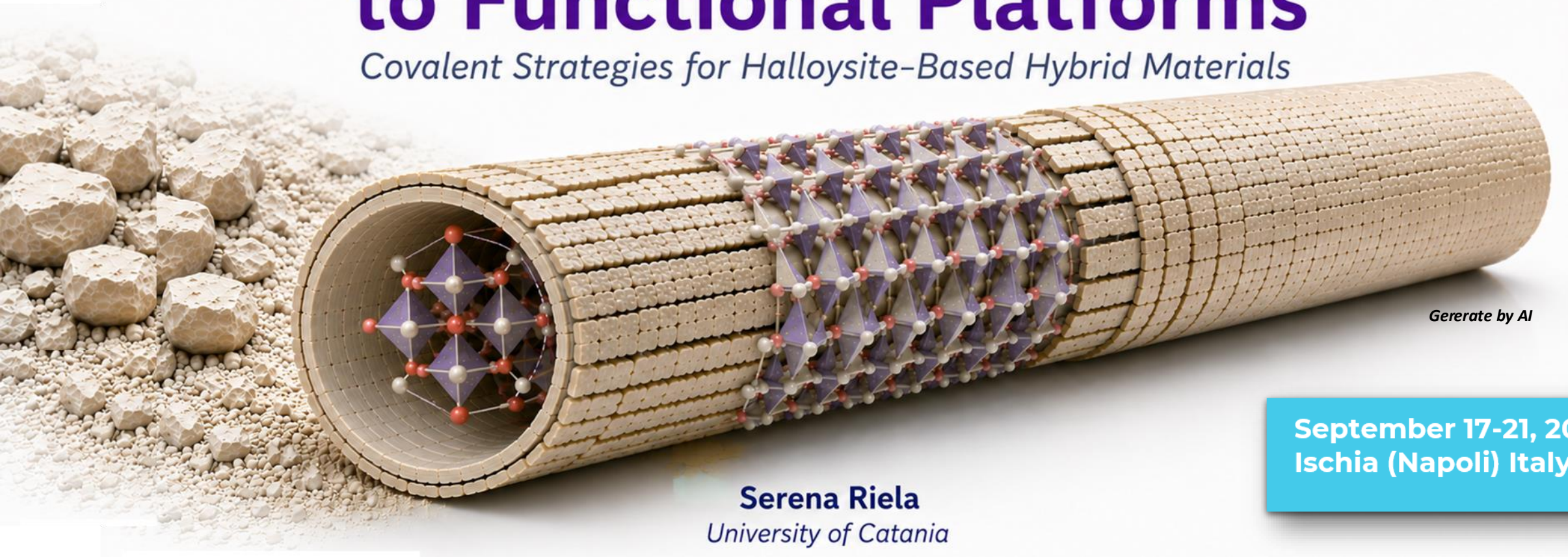
ISCHIA ADVANCED SCHOOL OF ORGANIC CHEMISTRY

IASOC 2026

From Natural Nanotubes to Functional Platforms

Covalent Strategies for Halloysite-Based Hybrid Materials

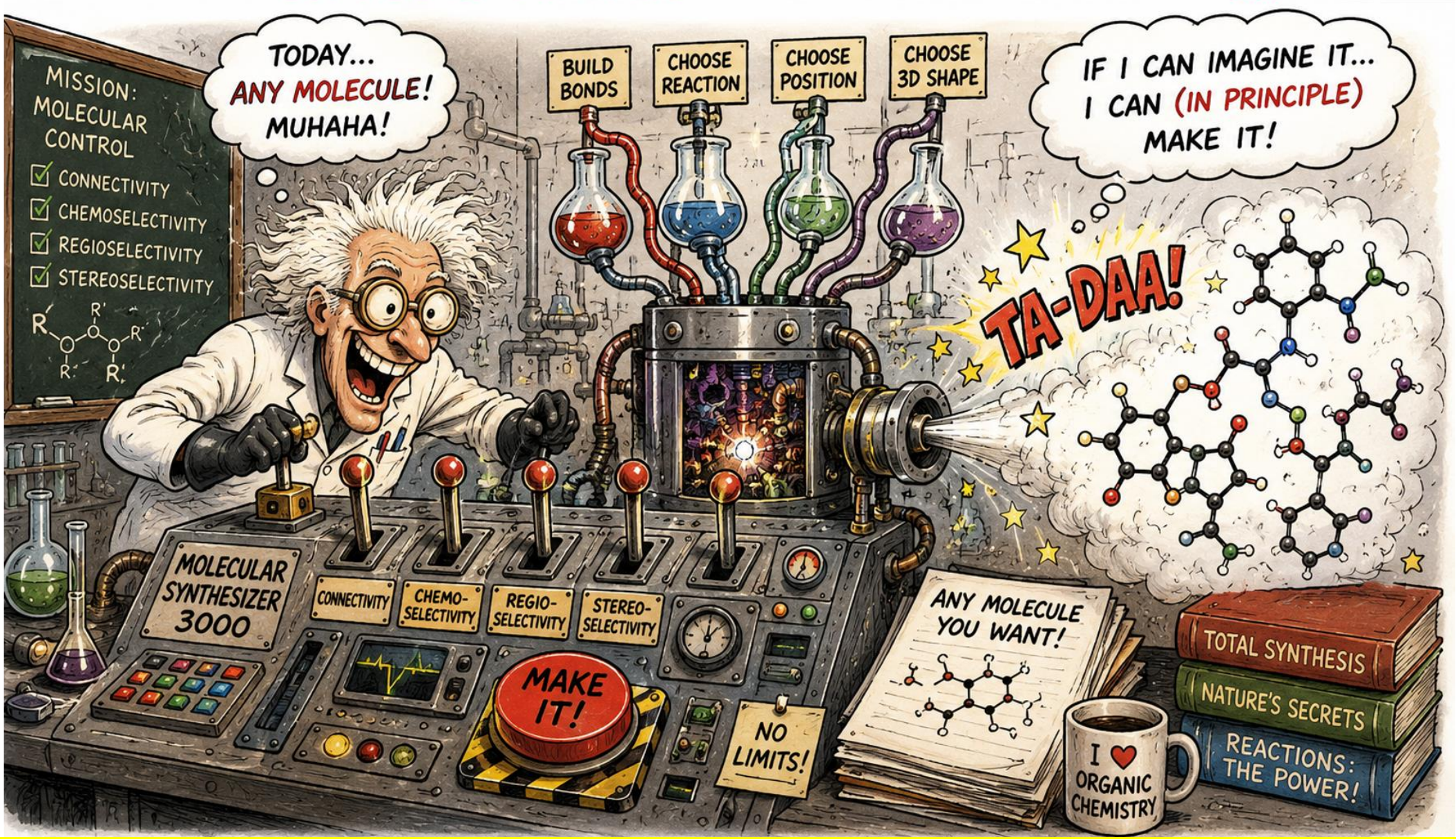


Gererate by AI

Serena Riela
University of Catania

September 17-21, 2026
Ischia (Napoli) Italy

TO GAIN COMPLETE CONTROL OVER MOLECULAR STRUCTURE ...



Connectivity, chemoselectivity, regioselectivity and stereoselectivity.

Gererate by AI

The *Holy Grail* of Organic Chemistry

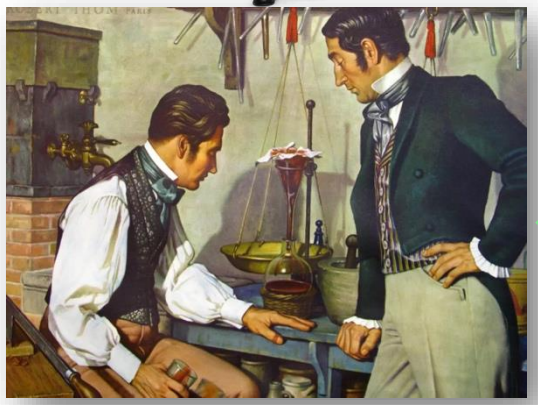


Can we make any molecule we want?

Gererate by AI

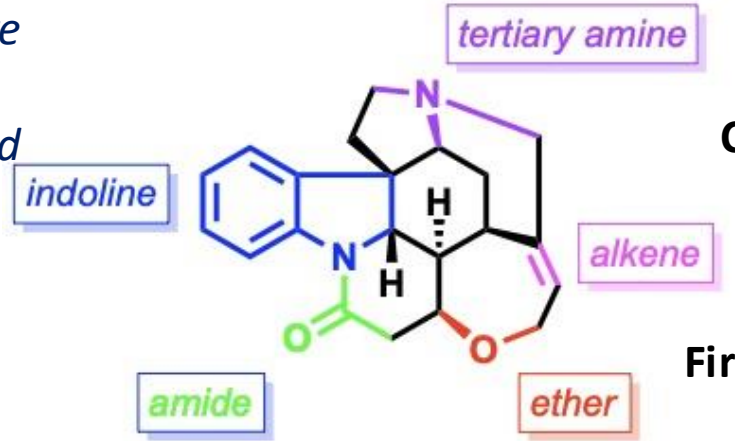
The Conquest of Molecular Complexity





Isolated in pure state in 1918 by Pelletier and Caventou

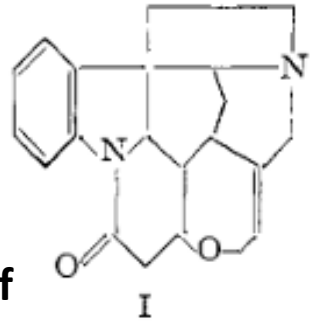
Strychnine: 70 years of evolution



Angew. Chem. Int. Ed. 2012, *51*, 4288-4311

Can we build it?

1954
Woodward *et al.*



I

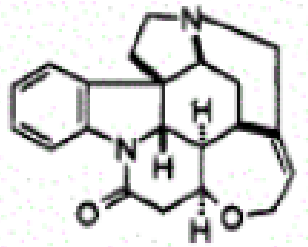
JACS 1954, *76*, 4749-4751

Constructing molecular complexity

First total synthesis of (±)-Strychnine

Can we control streochemistry?

1995
Overmann *et al.*



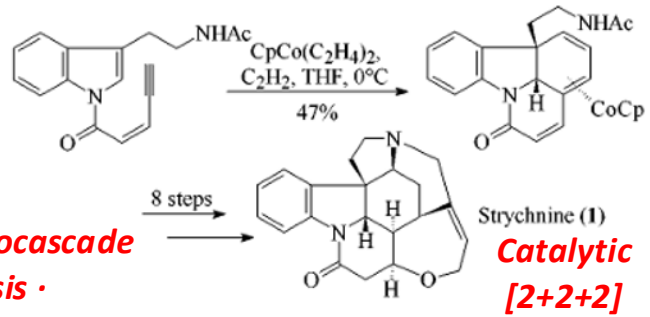
(–)-strychnine (1)

Asymmetric synthesis

JACS 1955, *117*, 5776-5788

Can we build complexity more strategically?

2000
Volhardt *et al.*

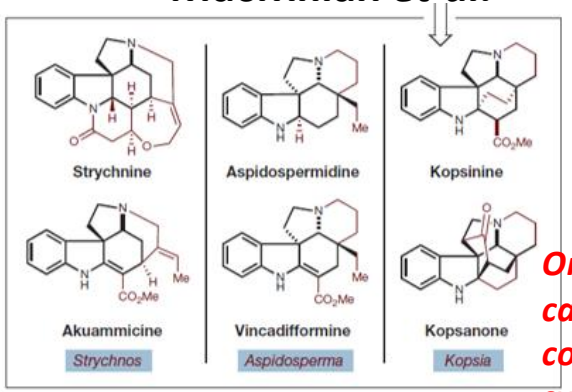


Catalytic [2+2+2] cycloaddition

Org. Lett. 2000, *2*, 2479-2481

Can we build it more efficiently?

2011
MacMillan *et al.*



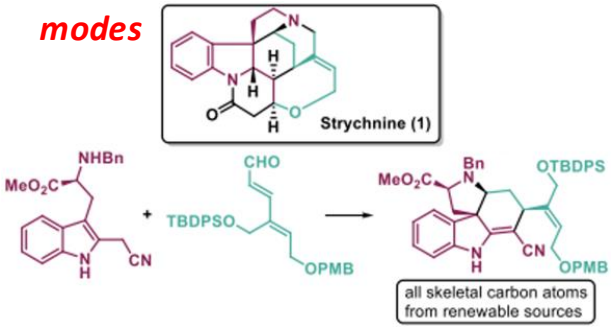
Organocascade catalysis · collective synthesis

Nature 2011, *475*, 183-188

Can light reshape the synthetic strategy?

2026
Opatz *et al.*

Photoredox catalysis · new activation modes



JACS Au 2026, *6*, 1822-1826

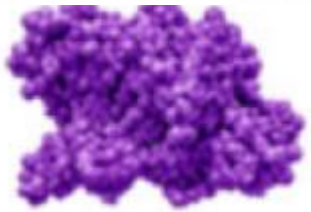


Does this control have to stop at the molecule?

…a molecular modification can ultimately generate a macroscopic function…

MOLECULE

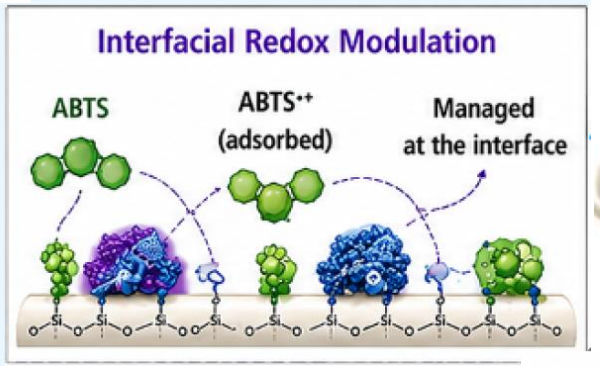
Precise control of structure and stereochemistry



Enzyme

INTERFACE

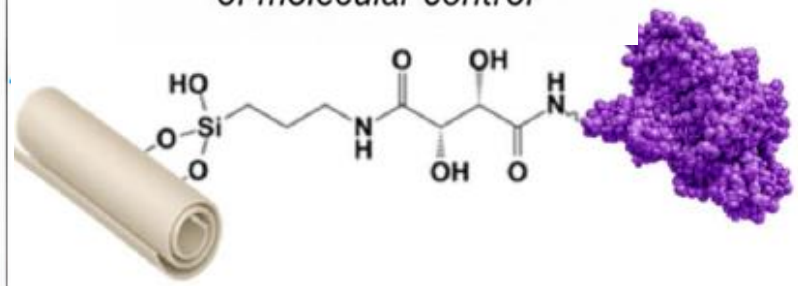
Molecules covalently anchored and oriented



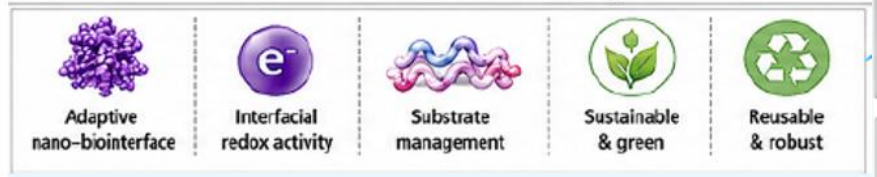
ACS Omega* 2026 *in press

MATERIAL

Organization and integration of molecular control



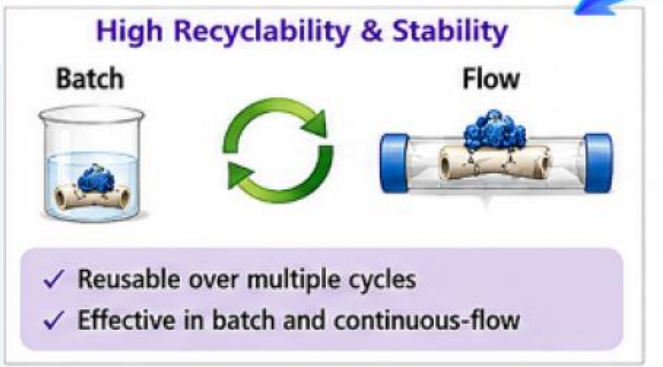
Covalent immobilization chemistry



FUNCTION

Controlled properties and targeted applications





From molecular control → *to materials control*

The same principles of organic chemistry can be extended beyond the molecule to create interfaces, build functional materials, and achieve designed functions.


What does Nature give us?

HALLOYSITE IS A CLAY MINERAL

But what comes to mind when we hear the word "clay"?



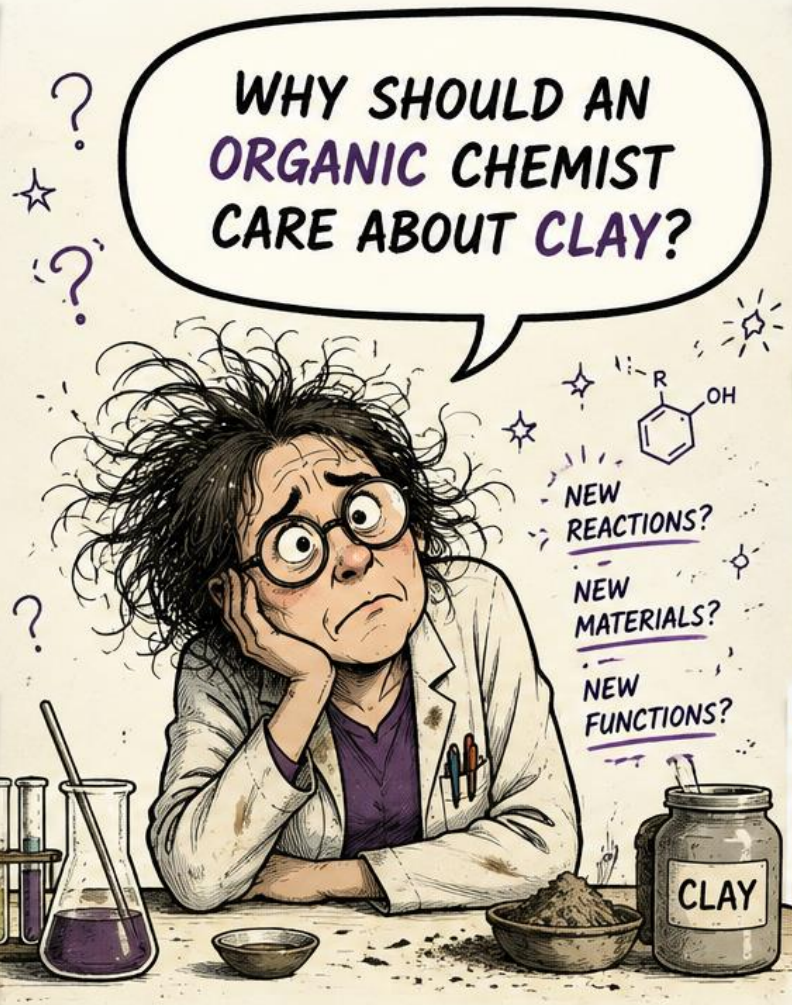
CAT LITTER



POTTERY

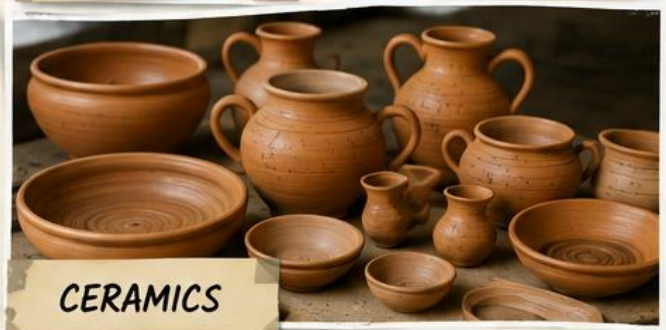


SOIL & EARTH





COSMETICS



CERAMICS



CONSTRUCTION

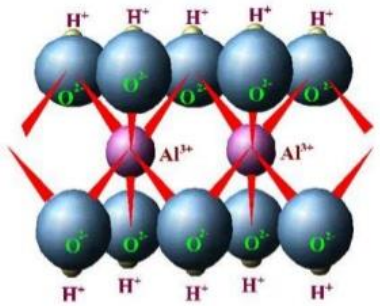
CLAY + COVALENT CHEMISTRY?

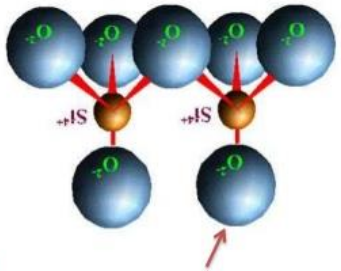
Gererate by AI

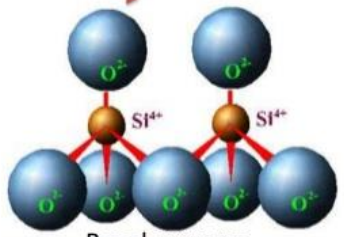
Structure

2 Tetrahedral sheets

+ 1 octahedral sheet

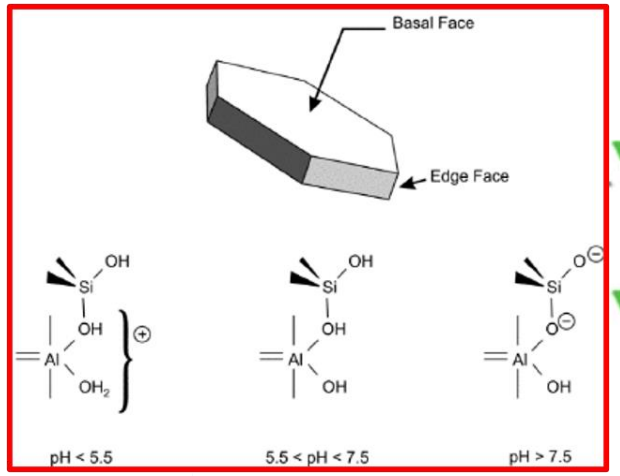




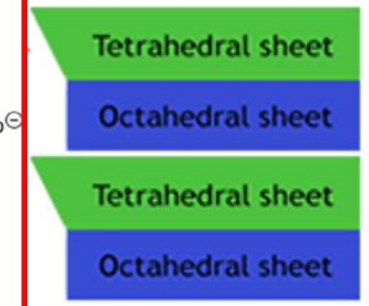


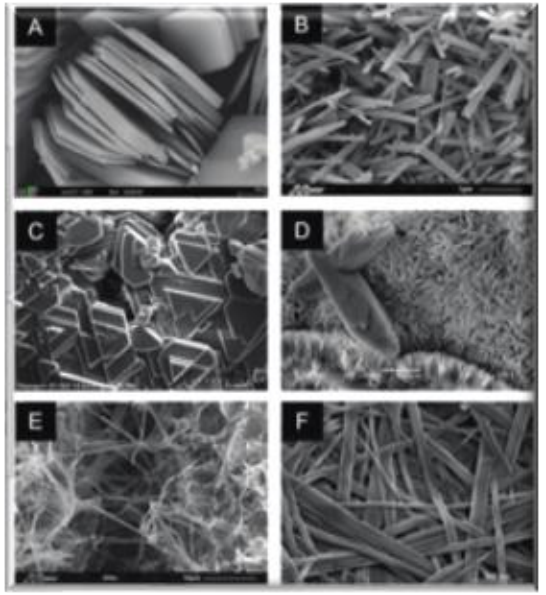
Makes one layer

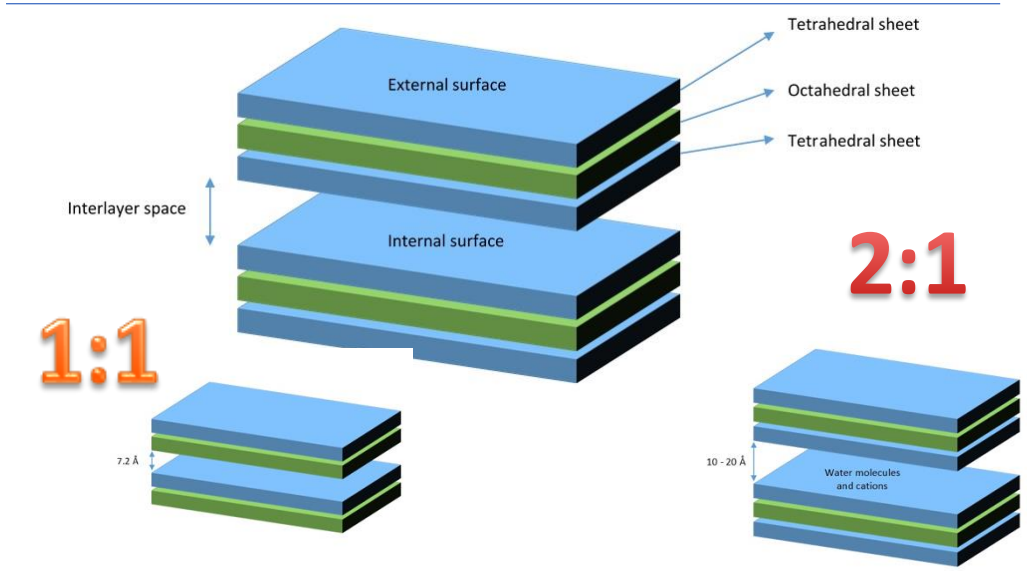
http://pubpages.unh.edu/~harter/crystal.htm#2:1%20MINERALS



Kaolinite (1:1)
Nonexpansive





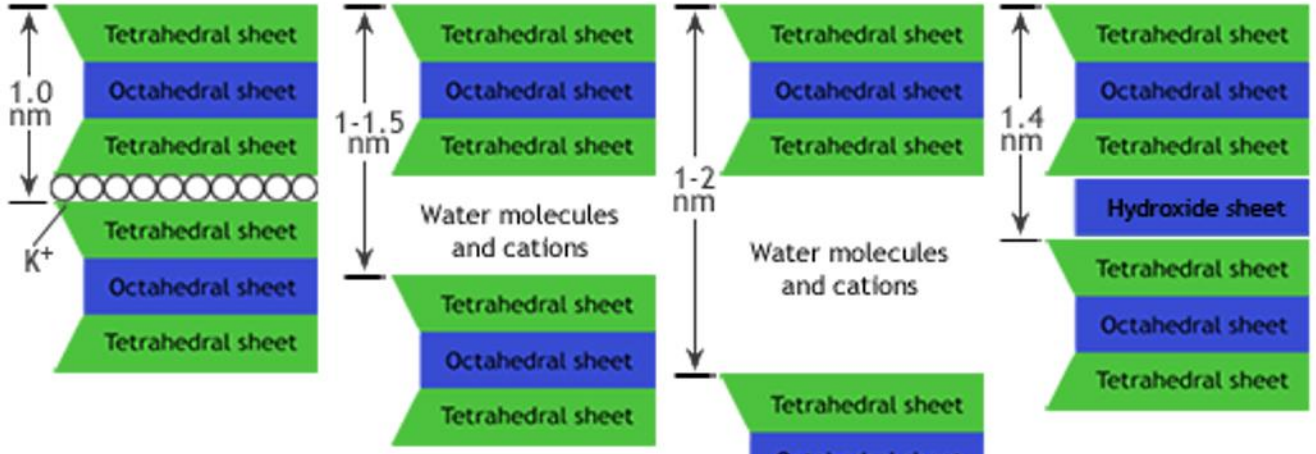


Illite (2:1)
Nonexpansive

Vermiculite (2:1)
Moderately Expansive

Smectite (2:1)
Highly Expansive

Chlorite (2:1)
Nonexpansive



Structure of Clays

Created by Josh Lory for www.soilsurvey.org

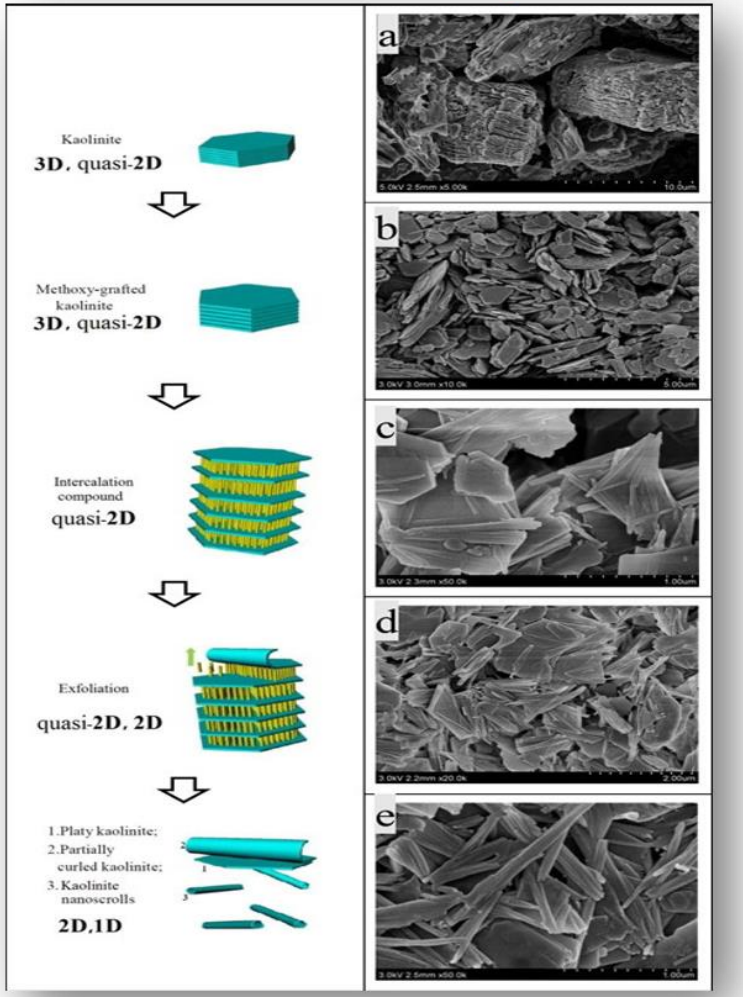
Their structure determines their surface chemistry. And for an organic chemist, this is where things become interesting!!

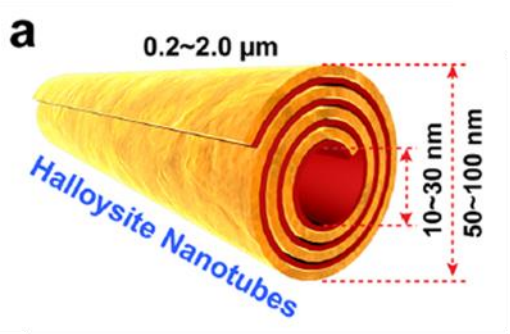
What is Halloysite?

- Naturally occurring aluminosilicate

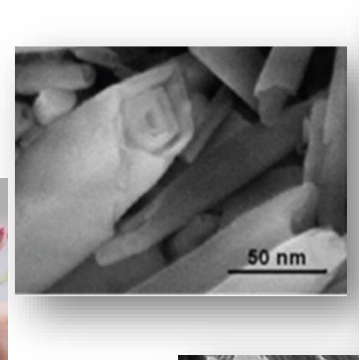


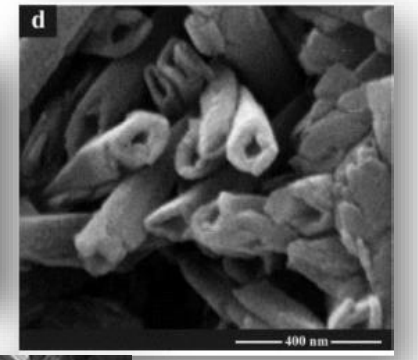
from Kaolinite to Halloysite

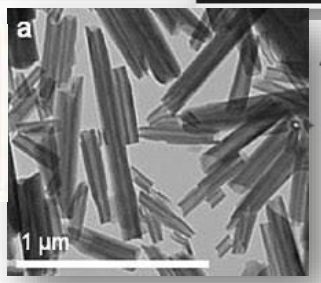






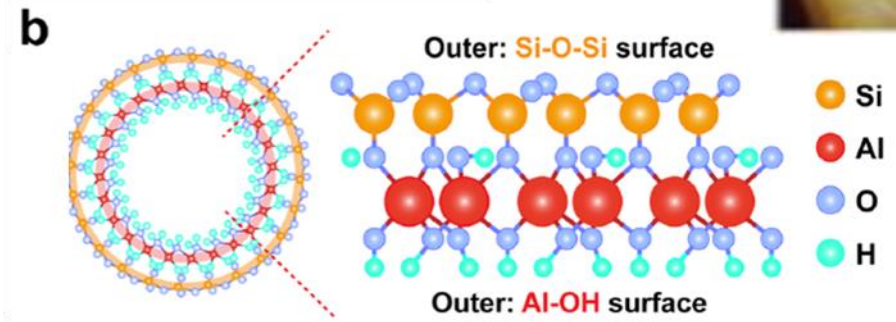


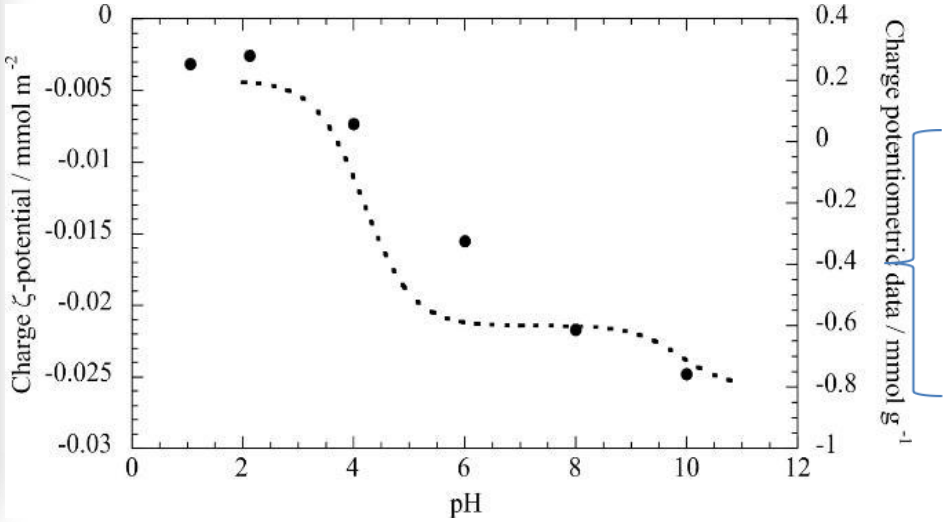


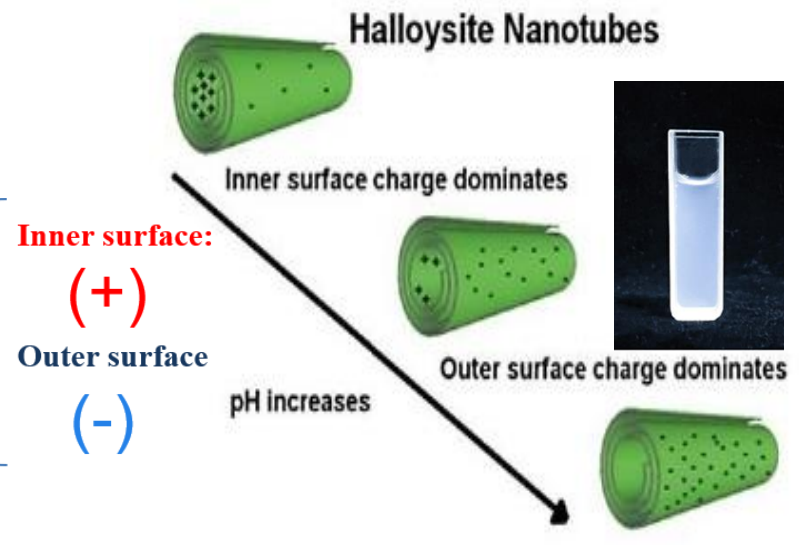




SEM, TEM and AFM images of Halloysite

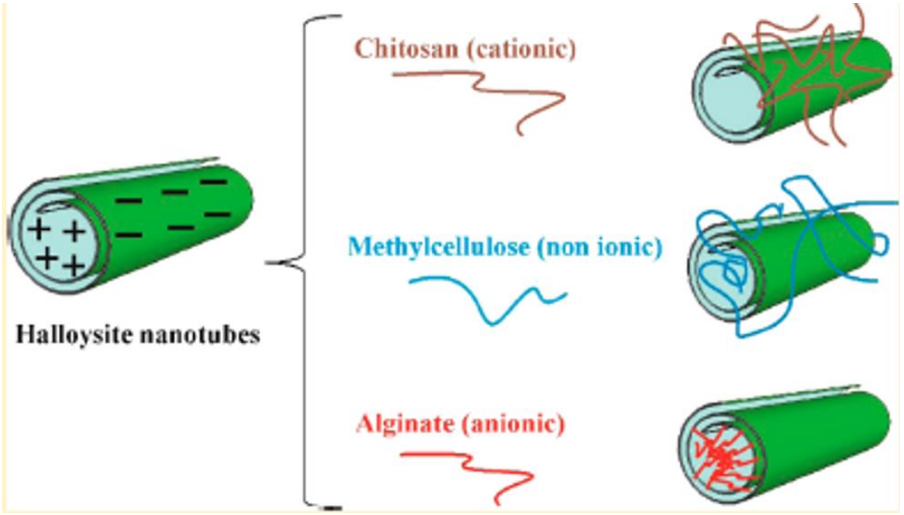






Pettignano A. *et al.* J. Phys. Chem. 2016, 120, 7849.

How does surface charge affect the way halloysite interacts with other species?



The mechanical properties of nanocomposites are strongly connected to the interactions between biopolymer and nanofiller. The alginate encapsulation into the nanotube cavity improved tensile properties of the nanocomposites.

G. Lazzara et al. *Ind. Eng. Chem. Res.* 2016, *55*, 7373.

Journal of Materials Chemistry B

ROYAL SOCIETY OF CHEMISTRY

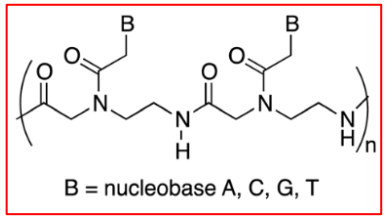
PAPER

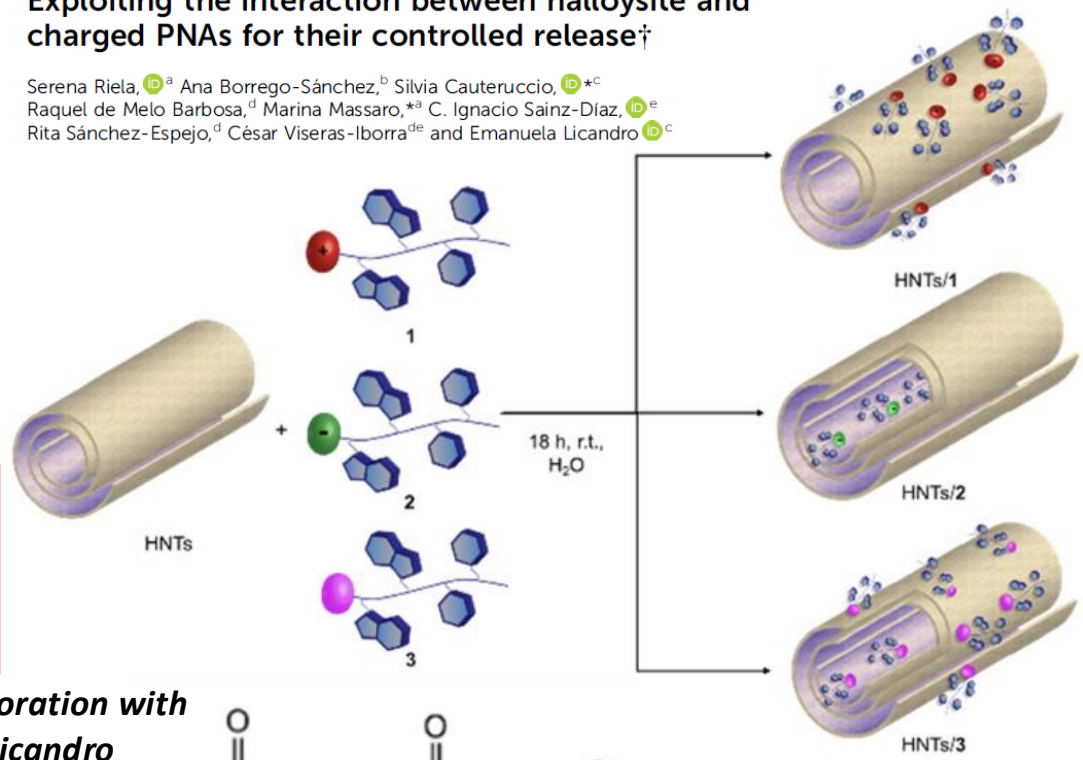
Check for updates

Cite this: *J. Mater. Chem. B*, 2023, 11, 6685

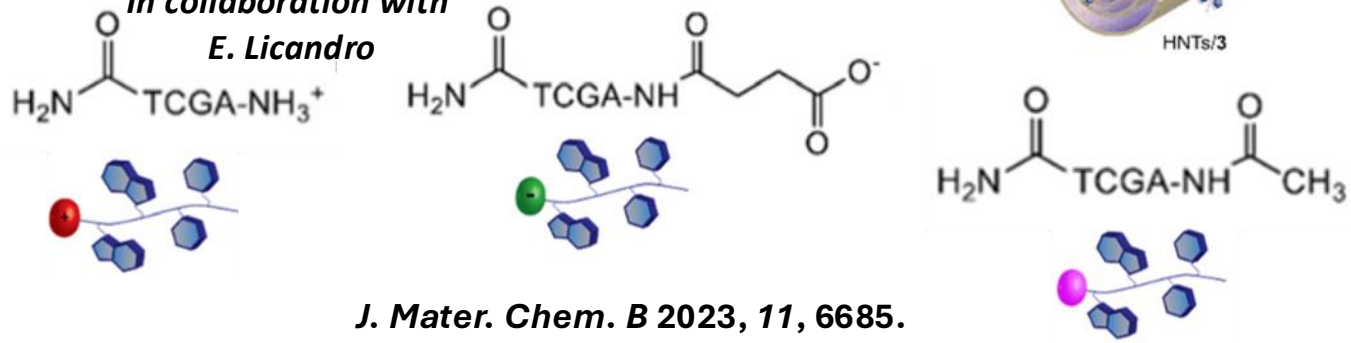
Exploiting the interaction between halloysite and charged PNAs for their controlled release†

Serena Riela,[a] Ana Borrego-Sánchez,[b] Silvia Cauteruccio,*[c] Raquel de Melo Barbosa,[d] Marina Massaro,*[a] C. Ignacio Sainz-Díaz,[e] Rita Sánchez-Espejo,[d] César Viseras-Iborra[de] and Emanuela Licandro[c]





In collaboration with E. Licandro



J. Mater. Chem. B 2023, *11*, 6685.

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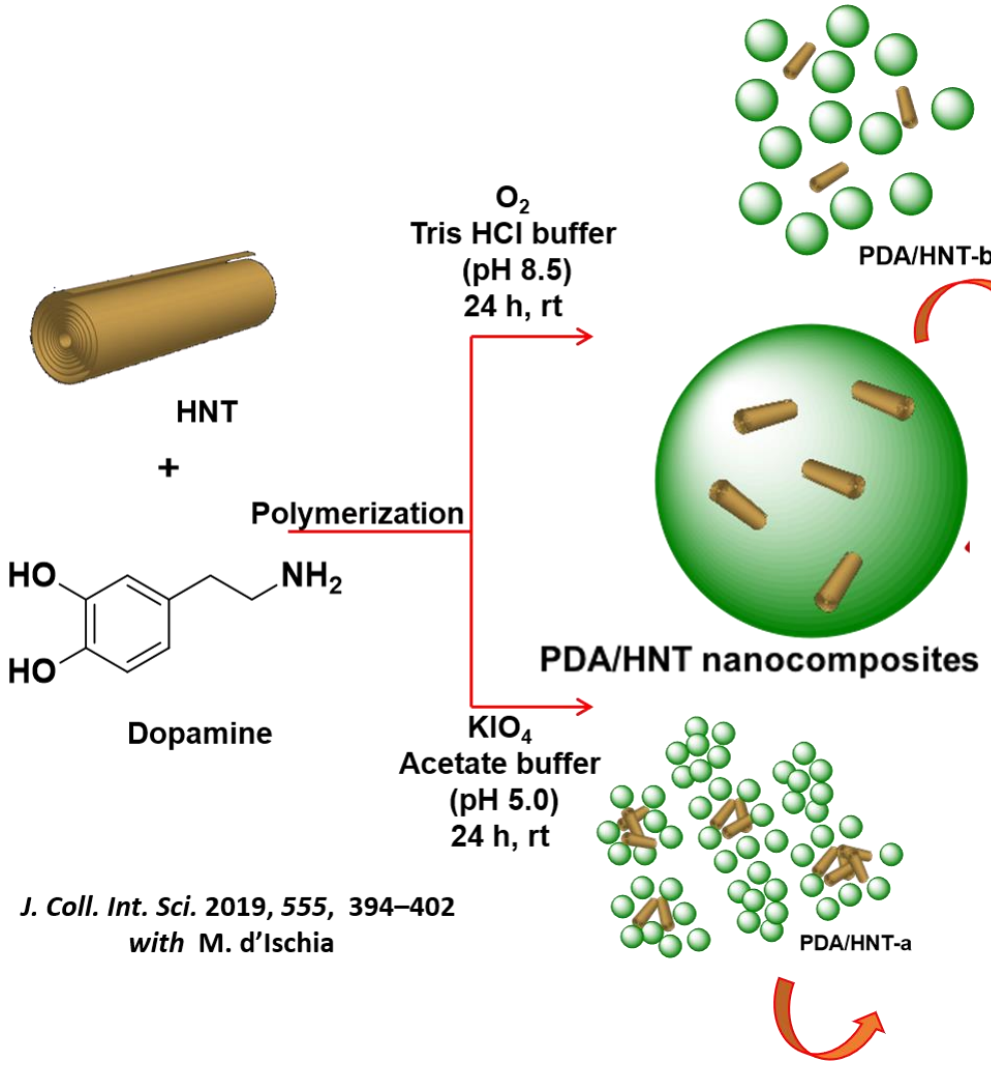
journal homepage: www.elsevier.com/locate/jcis

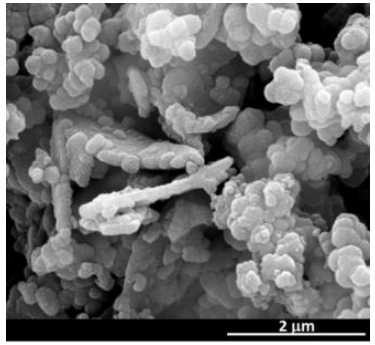


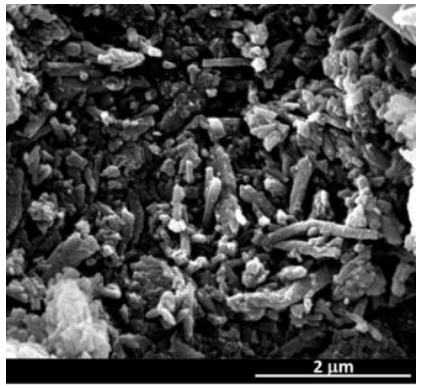
Regular Article

Effect of halloysite nanotubes filler on polydopamine properties

Marina Massaro [a], Francesco Armetta [a], Giuseppe Cavallaro [b], Delia F. Chillura Martino [b], Michelangelo Gruttadauria [a], Giuseppe Lazzara [b], Serena Riela [a,*], Marco d'Ischia [c]



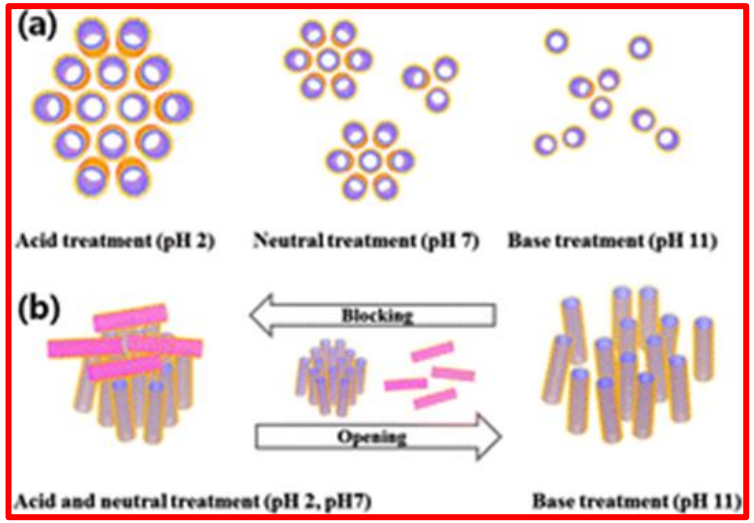




J. Coll. Int. Sci. 2019, *555*, 394–402
with M. d'Ischia

Can Halloysite Organization Influence a Chemical Process?

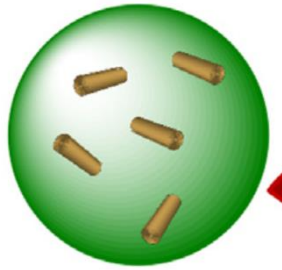
pH Controls Halloysite Organization



D. Sohn et al. *Chem. Commun.*, 2013,49, 4519.

PDA

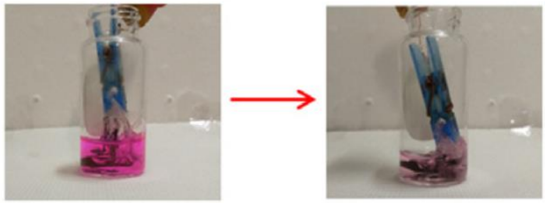
surface charge → organization → chemical process → function



PDA/HNT nanocomposites

From Organization to Function

Pollutant removal



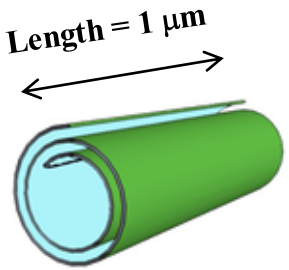

How Much Can Halloysite Load and Where?
Can We Control What Comes Out?
How Do We Know What Is Happening on the Nanotube?
Can We Extend the Release Even Further?
Is Halloysite Safe Enough to Become a Functional Platform?
What If We Need More Space?

Is Halloysite Safe Enough to Become a Functional Platform?



Halloysite can be classified a hazard class 4 material (low hazard substance).

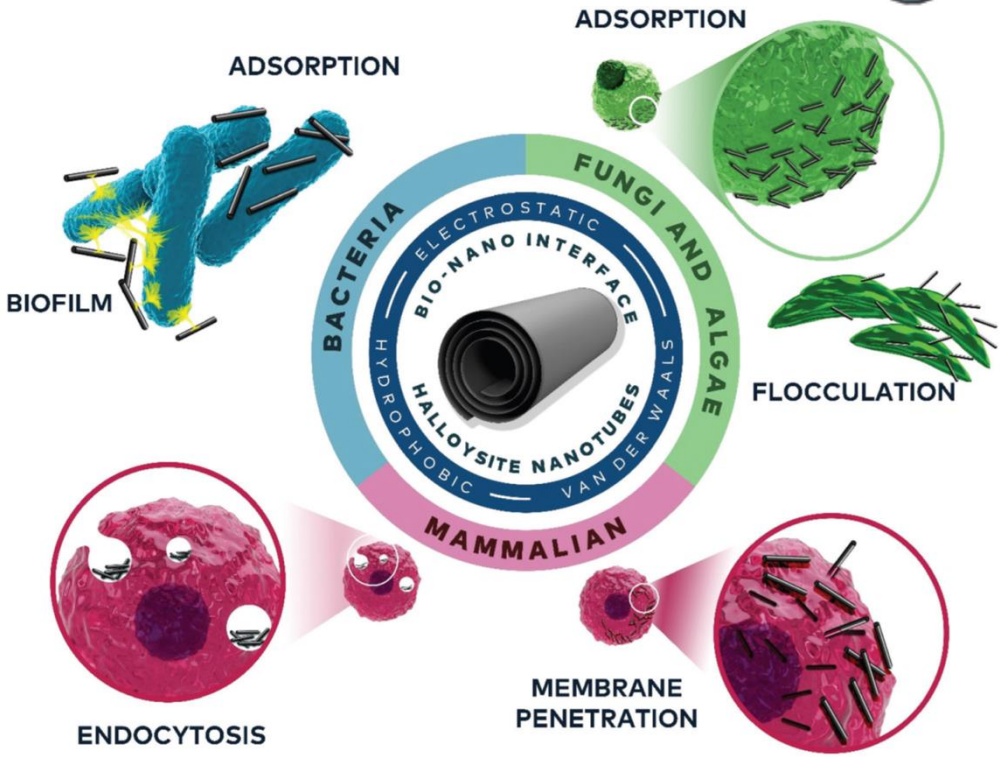
Toxicological Review, 2016, 137, 52.



in vitro studies

halloysite nanotubes exert relevant toxicity at 1000 mg/mL by blocking the passage of cell cycle!

F. R. Ahmed et al. *Colloid and Surf. B: Biointerface* 2015, *135*, 50.



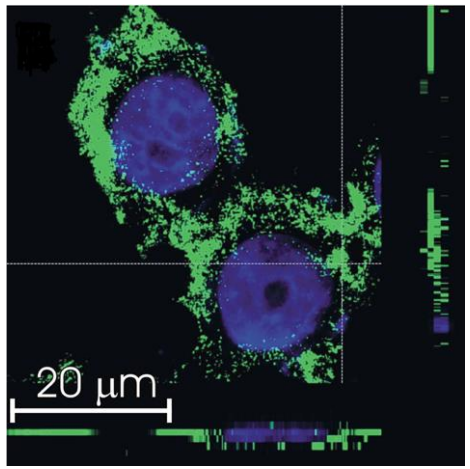
E. Segal et al. *Nanoscale* 2020,*12*, 23444.

in vivo by oral route

the oral administration of halloysite should be controlled ensuring the oral administration limit at ca. 20 mg /kg BW

X. Wang et al. *Environm. Tox.* 2018, *33*, 623.

If extrapolated for humans 3 g of HNTs may be safely consumed orally per day



HeLa cells

V. Vergaro *et al. Biomacromol.* 2010, 11, 820.

- "Ecompatibility"

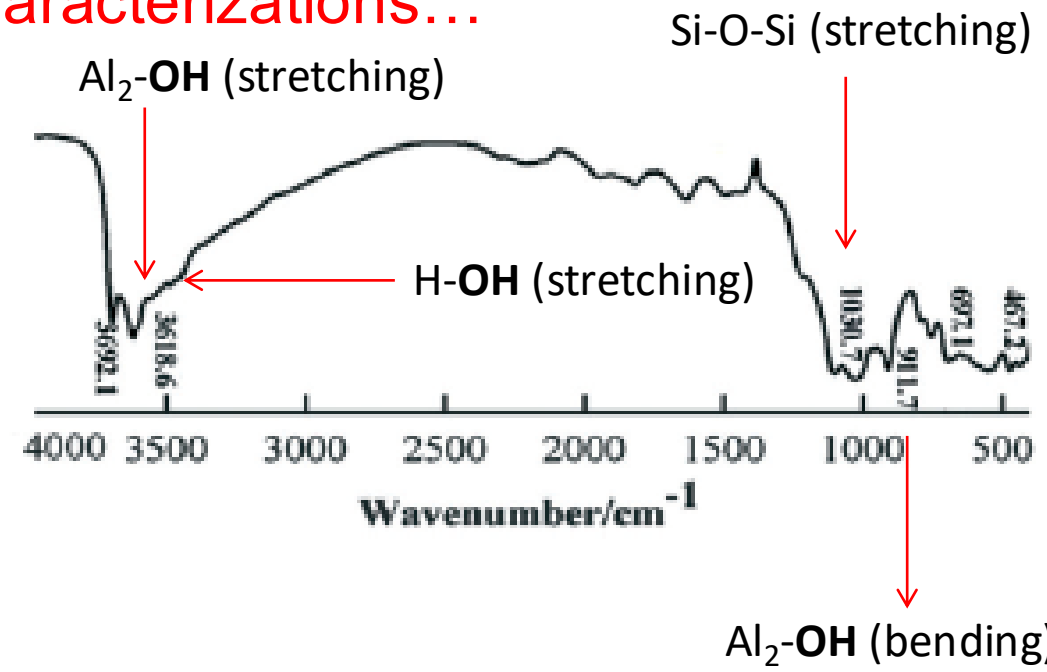


How Do We Know What Is Happening on the Nanotube?



Easy characterizations…

FT-IR



TGA

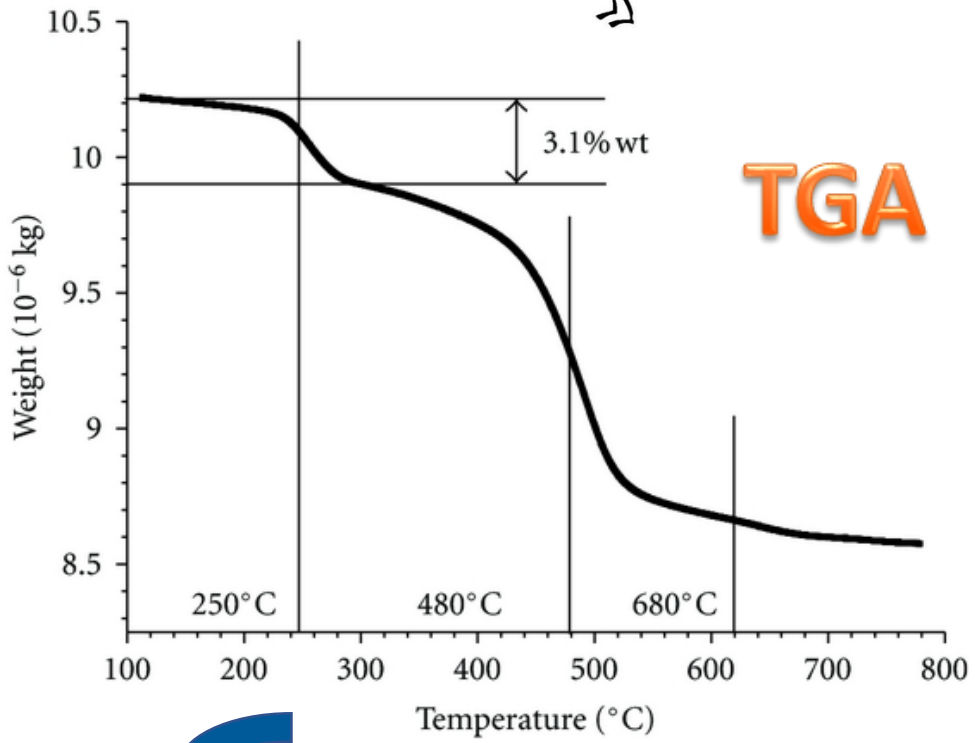


TABLE 3: Positions and assignments of the IR vibration bands

position (cm^{-1})	assignments	position (cm^{-1})	assignments
3696, 3656	O−H stretching of inner-surface hydroxyl groups	1266	deformation (scissoring) of C−H_3
3618	O−H stretching of inner hydroxyl groups	1112	perpendicular Si−O stretching
3450	O−H stretching of water	1088, 1025	in-plane Si−O stretching
3360	asymmetric stretching of N−H_2	940	O−H deformation of inner - surface hydroxyl groups
3300	symmetric stretching of N−H_2	912	O−H deformation of inner hydroxyl groups
2930	symmetric stretching of C−H_2	790	symmetric stretching of Si−O
1630	O−H deformation of water	755	perpendicular Si−O stretching
1556	deformation (scissoring) of N−H_2	697	perpendicular Si−O stretching
1490	deformation (scissoring) of C−H_2	536	deformation of Al−O−Si
1384	deformation (wagging) of C−H_2	470	deformation of Si−O−Si
1330	deformation (scissoring) of Si-CH	432	Deformation of Si−O

(250 °C) loss of crystallization water

(480 °C) interlayer water molecules

(680 °C) dehydroxylation of alumina

Experimental techniques for the characterization of HNTs hybrid materials

Technique	Property/insight
Thermogravimetry (TG)	HNT functionalization efficiency, surfactant loading, thermal stability
Fourier transform infrared spectroscopy (FTIR)	Specific interactions
X-ray diffraction (XRD)	HNT multilayer wall spacing, surfactant intercalation
X-ray photoelectron spectroscopy (XPS)	Surface chemical composition and surface bonds in the modified HNTs
Nuclear magnetic resonance (NMR)	Surface bonds in the modified HNTs
Dispersive X-ray spectrometry (EDX)	Local elemental analysis
Scanning electron microscopy (SEM)	Morphology and size distribution
Transmission electron microscopy (TEM)	Cavity loading
Atomic force microscopy (AFM)	Roughness
Water contact angle	External/internal hydrophobization
Dynamic light scattering (DLS)	Average translational diffusion dynamic, aggregation in solvent media
ζ-Potential	Particle charge
Turbidimetry	Sedimentation kinetics

Characterization Tools in Action

Contents lists available at ScienceDirect

Journal of Colloid And Interface Science

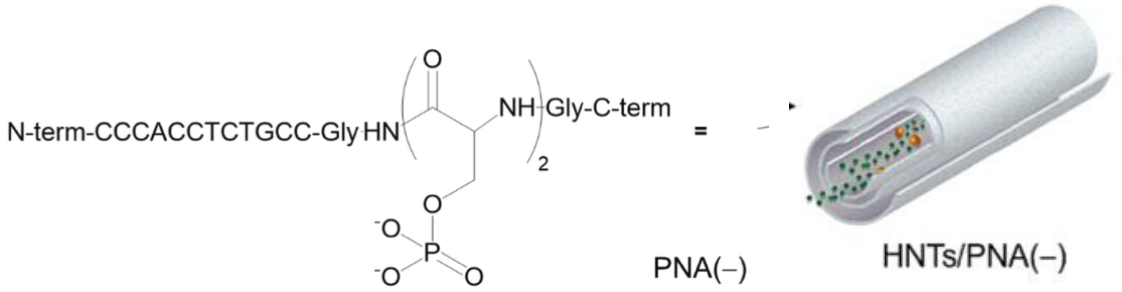
journal homepage: www.elsevier.com/locate/jcis

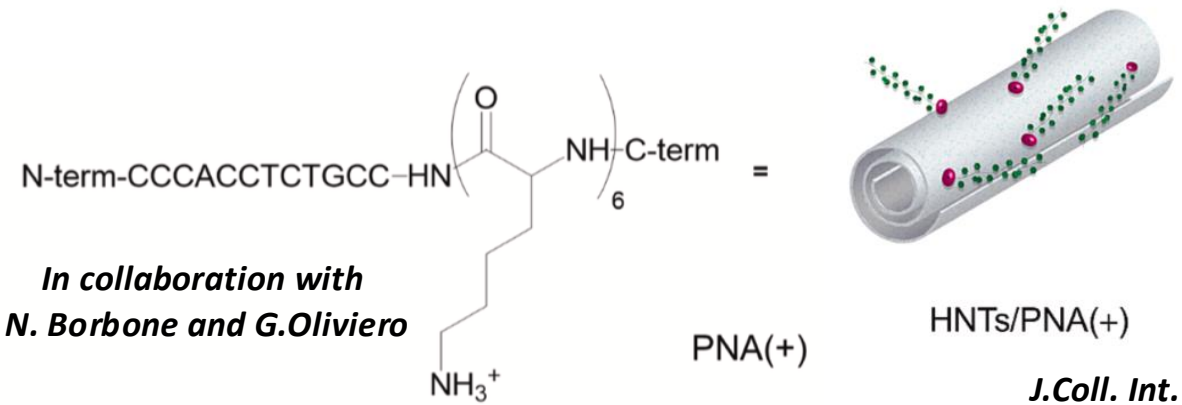


Regular Article

Carrier capability of halloysite nanotubes for the intracellular delivery of antisense PNA targeting mRNA of neuroglobin gene

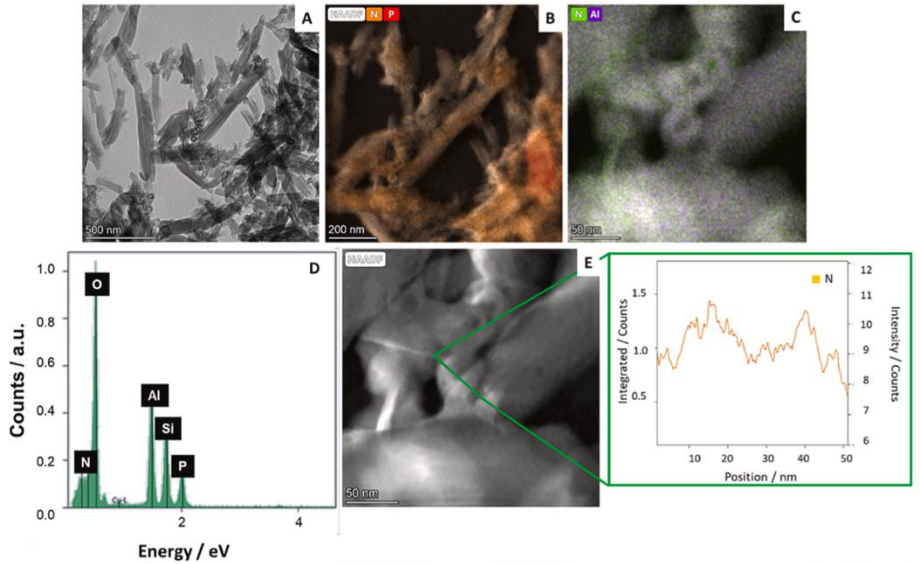
Andrea P. Falanga [a,1], Marina Massaro [b,1], Nicola Borbone [a,*], Monica Notarbartolo [b], Gennaro Piccialli [a], Leonarda F. Liotta [c], Rita Sanchez-Espejo [d], Cesar Viseras Iborra [d,e], Françisco M. Raymo [f], Giorgia Oliviero [g], Serena Riela [h,*]



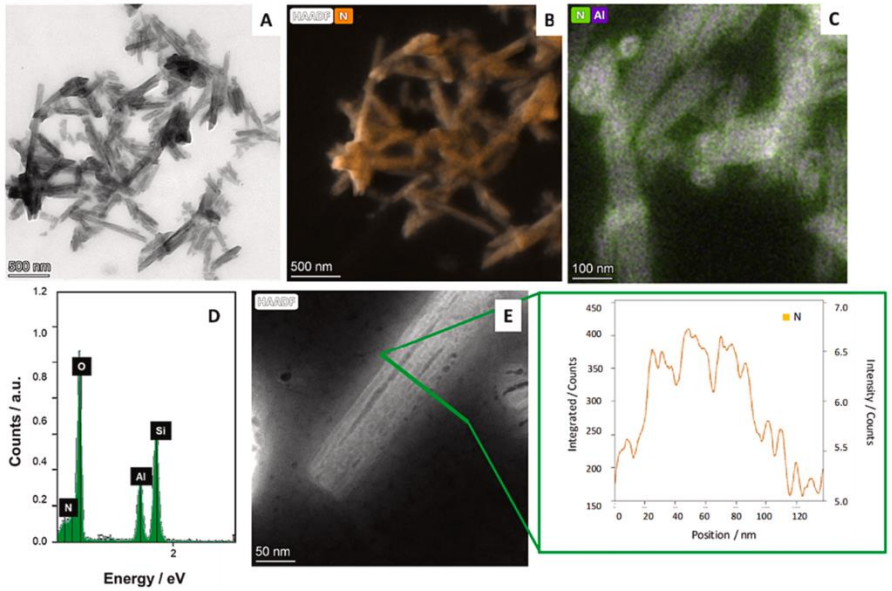


In collaboration with N. Borbone and G.Oliviero

TEM, EDX elemental mapping images, EDS analysis and EDS elemental mapping of Nitrogen



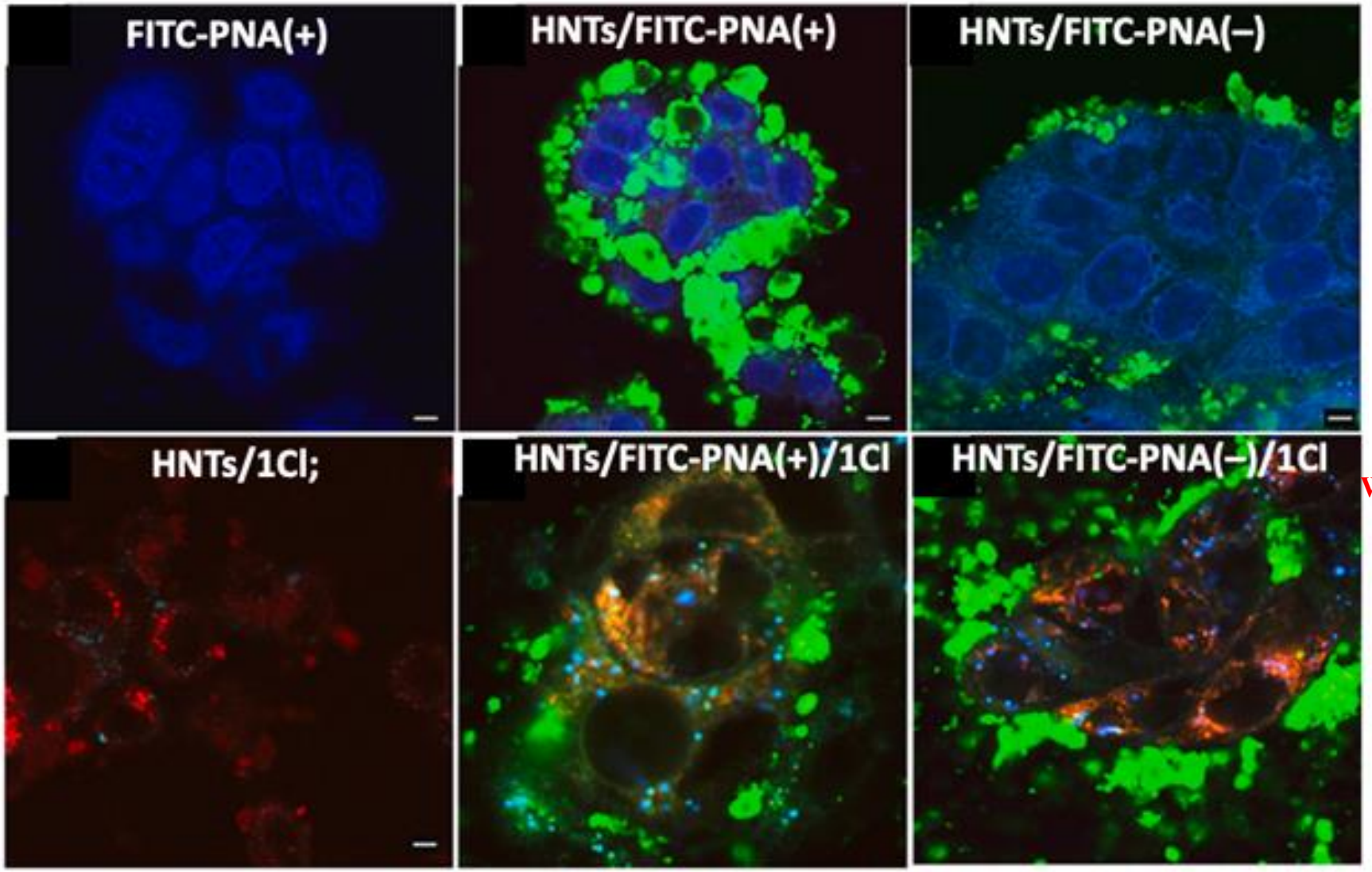
The 12-mer PNA molecules contain nucleobases capable of recognizing a 12-mer DNA molecule that mimics a purine-rich region of NGB mRNA



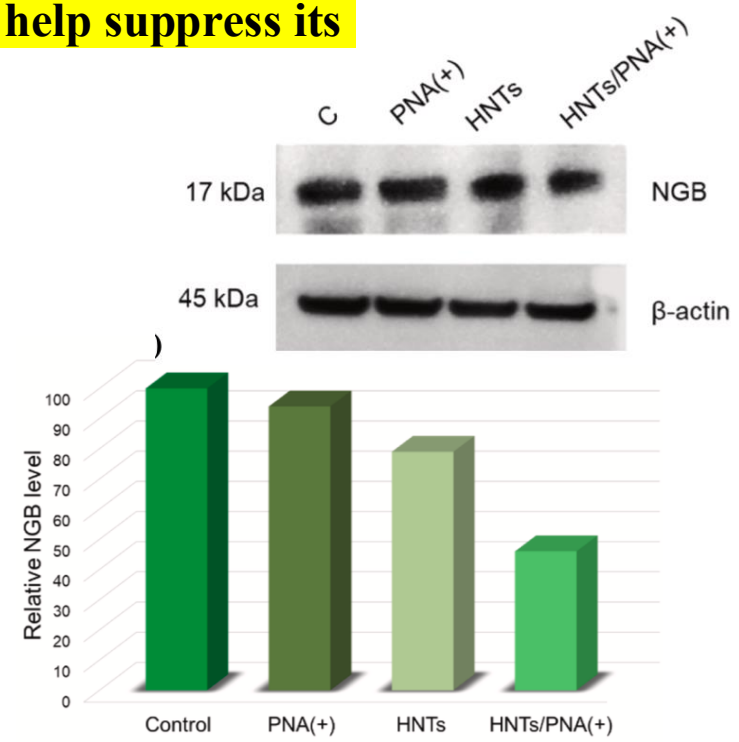
J.Coll. Int. Sci **2024, 663, 9-20**

NGB protein is overexpressed in breast cancer, and the antisense PNA molecules could help suppress its expression…

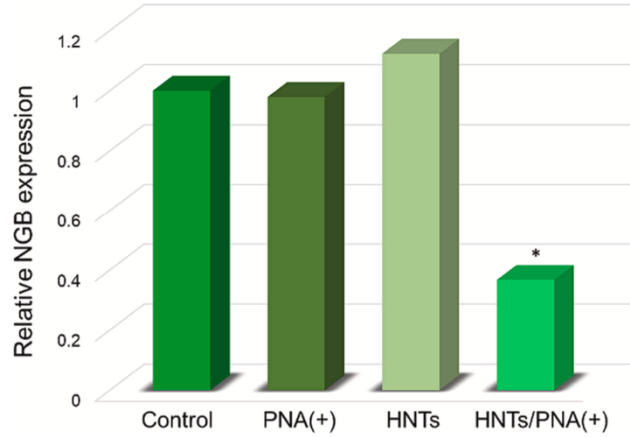
MCF-7 cell line



CLSM images of MCF-7 cells incubated for 24 h



Western blotting analysis of the neuroglobin level



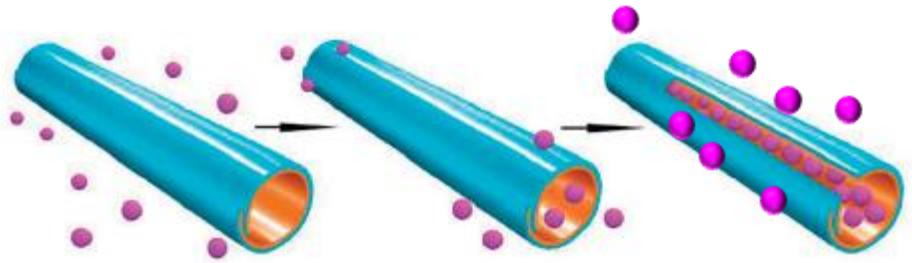
Evaluation of target mRNA expression levels

How Much Can Halloysite Load and Where?



Hydrogen bonding interactions,
Electrostatic attraction interactions and
Hydrophobic interactions

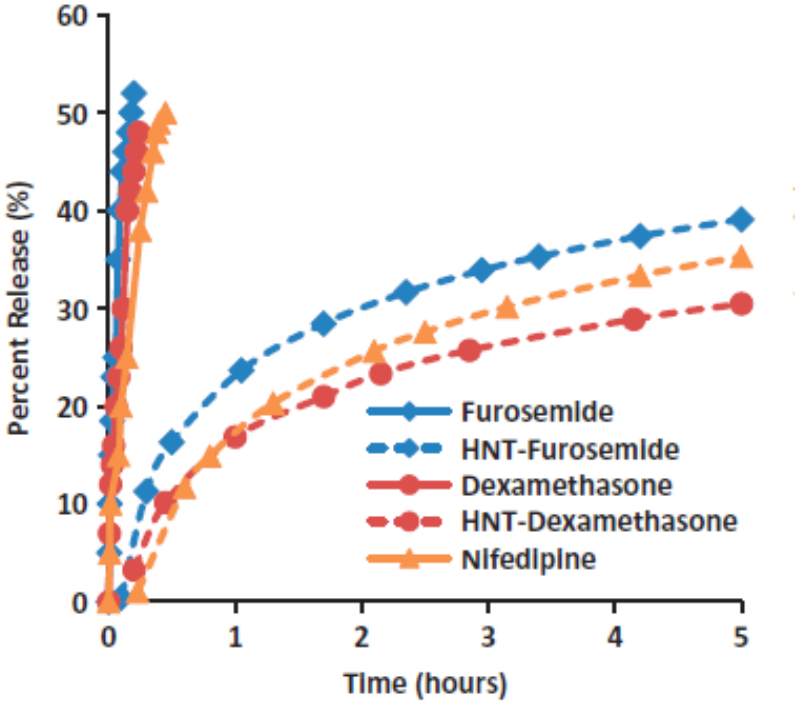
Absorbtion



Inner Lumen -15 nm-

LOADING: 1.5-10%

Can We Control What Comes Out?





Release

Isotherm adsorbtion equations

Hill $$Q_e = \frac{Q_m \cdot (K_H \cdot C_e) \cdot n}{1 + (K_H \cdot C_e \cdot n)}$$

Langmuir $$Q_e = \frac{K_L Q_m C_e}{1 + K_L C_e}$$

Freundlich $$Q_e = K_F C_e^{1/n}$$

Kinetic release equations

First-order kinetic	$F_t = M_\infty (1 - e^{-kt})$
Higuchi square kinetic	$F_t = kt^{1/2}$
Korsmeyer–Peppas model	$F_t = kt^n$
Double exponential model (DEM)	$F_t = F_e' \cdot (1 - e^{-k't}) + F_e'' \cdot (1 - e^{-k''t})$

Contents lists available at ScienceDirect

Journal of Colloid and Interface Science

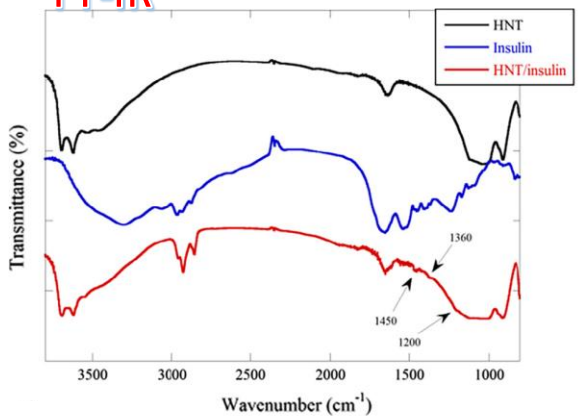
journal homepage: www.elsevier.com/locate/jcis

Halloysite nanotubes for efficient loading, stabilization and controlled release of insulin

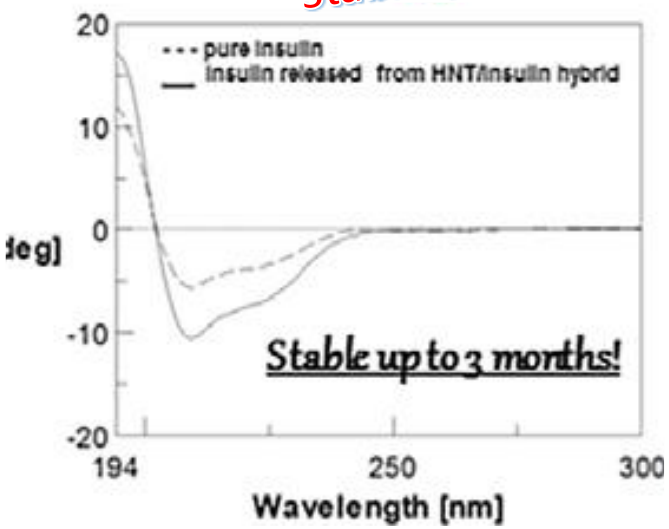
Marina Massaro [a], Giuseppe Cavallaro [b], Carmelo G. Colletti [a], Giuseppe D'Azzo [a], Susanna Guernelli [c], Giuseppe Lazzara [b], Silvia Pieraccini [c], Serena Riela [a,*]



FT-IR



Stability



RLS

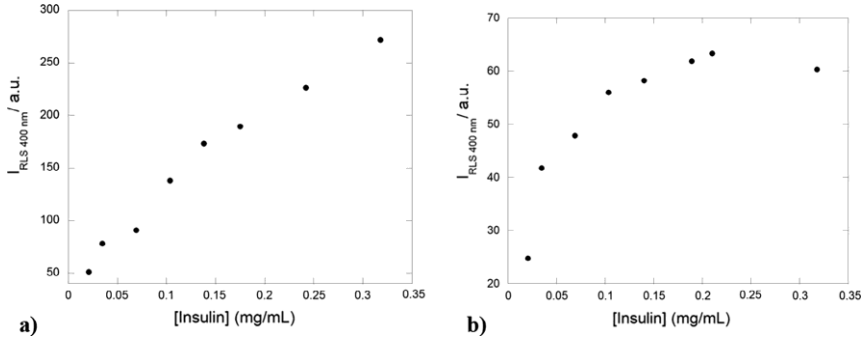
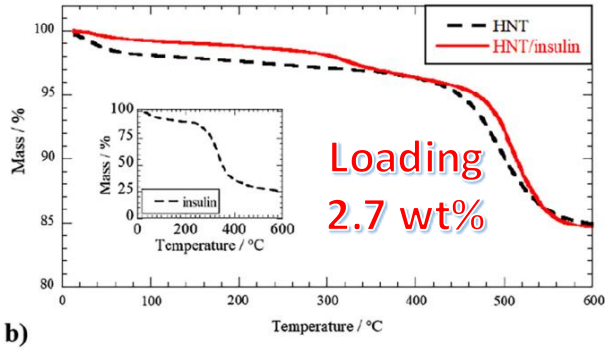


Fig. 1. Trend of I_{RLS} at 400 nm as a function of insulin concentration at (a) pH 4.54; (b) pH 8.0.

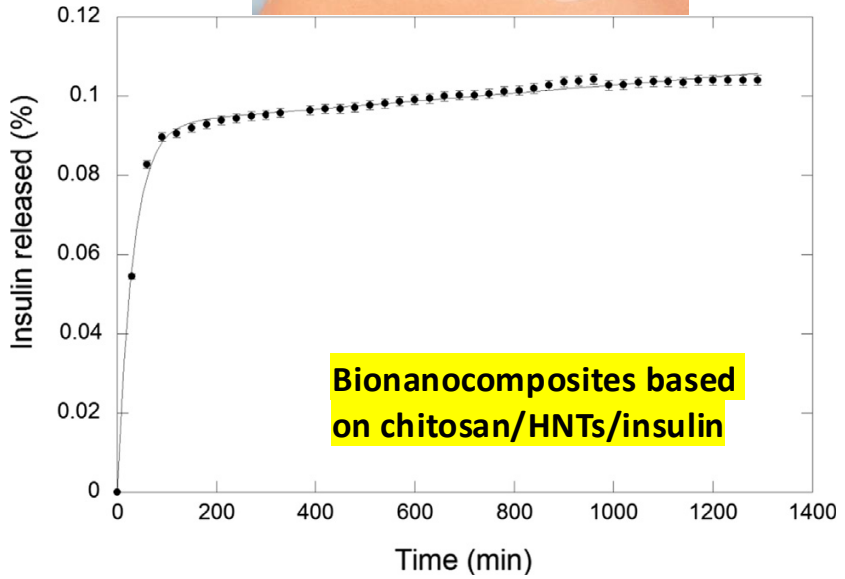
phosphate buffer pH 6.8

TGA

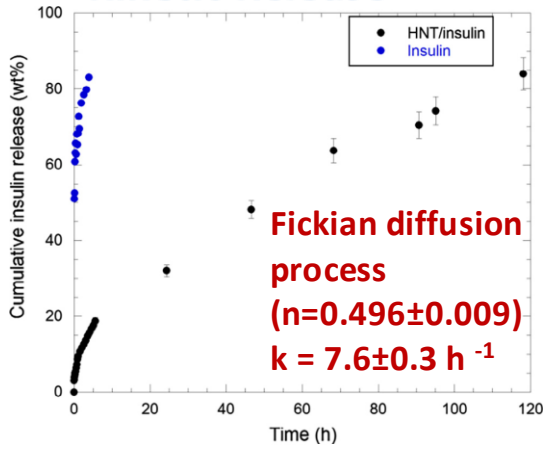


From Organization to Function





Kinetic Release



a) At pH 4.54 the outer surface of halloysite is negatively charged while insulin is positively charged (pI 5.2), which increases its interaction with the surface of the nanotube and minimizes interaction with the lumen.

b)At pH 8.0 the outer surface of the nanotube is strongly negative and the protein is also strongly negative, which would coerce it to adsorb in smaller amounts and mostly inside of the lumen of halloysite

J. Coll. Int. Sci. **2018, *524*, 156.**

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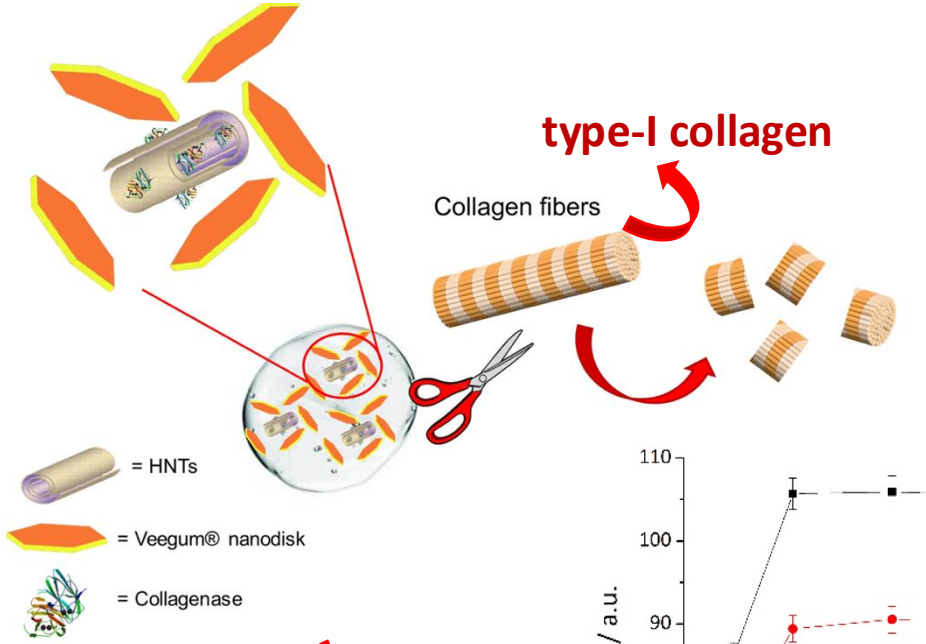
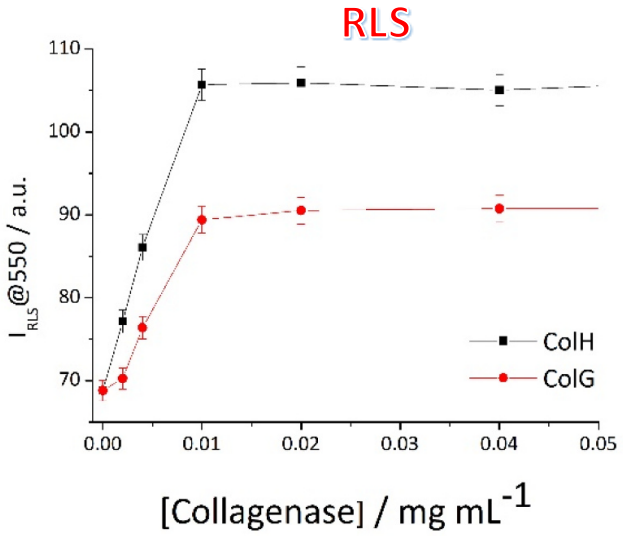
Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

Nanoformulations based on collagenases loaded into halloysite/Veegum® clay minerals for potential pharmaceutical applications

Marina Massaro [a], Giulio Ghersi [a,*], Raquel de Melo Barbosa [b], Simona Campora [a], Salvatrice Rigogliuso [a], Rita Sànchez-Espejo [b], César Viseras-Iborra [b,c], Serena Riela [a,*]



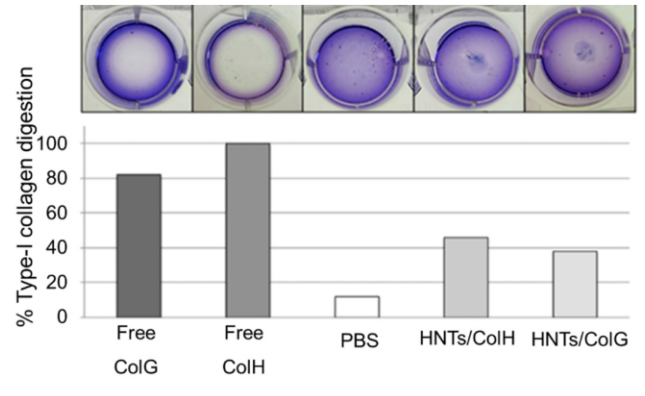


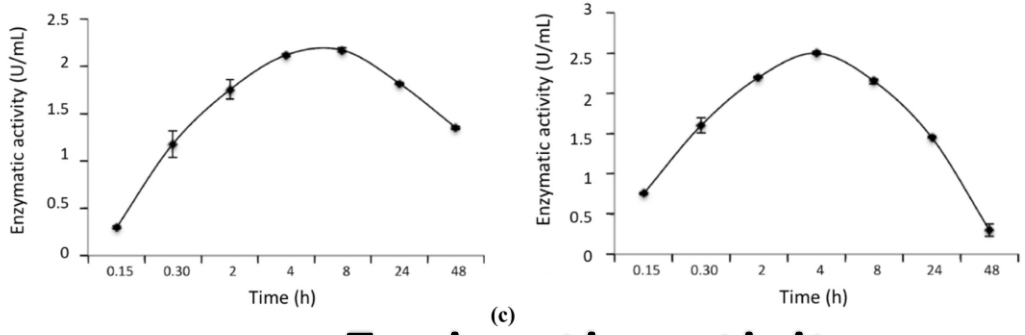
In collaboration with G.Ghersi

TGA DLS

Nanomaterial	Loading (%)	Z Average size (nm)	ζ-potential (mV)
HNTs	/	570±40	-16
HNTs/ColG	2.1	530±30	- 18.5
HNTs/ColH	4.5	1200±100	- 19.2

From Organization to Function

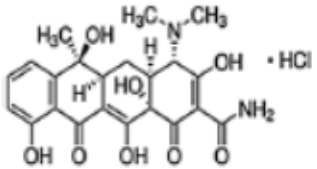
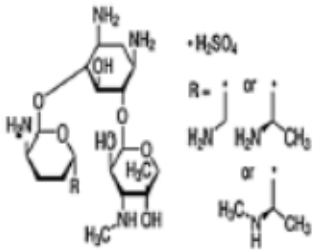
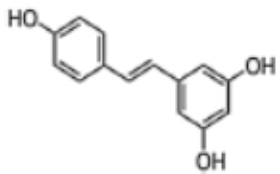
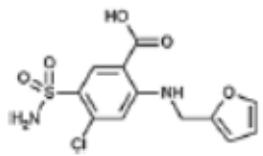
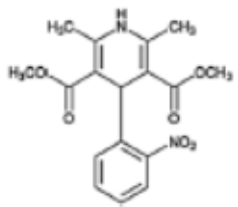
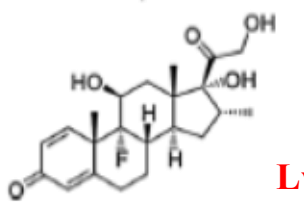




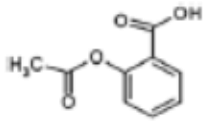
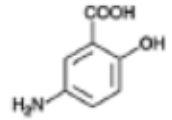
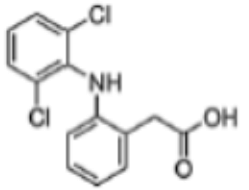
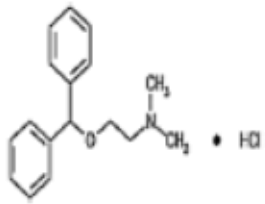
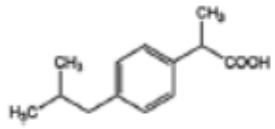
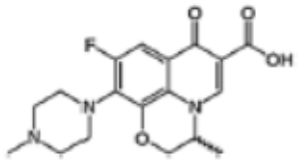
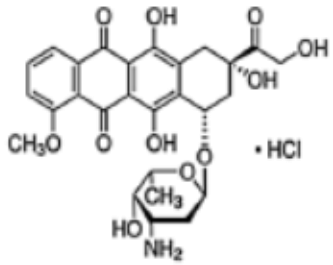
Coll. Surf. B 2023, *230*, *113511*.

Enzimatic activity

Parameters essential for loading and release studies of drugs loaded in halloysite.

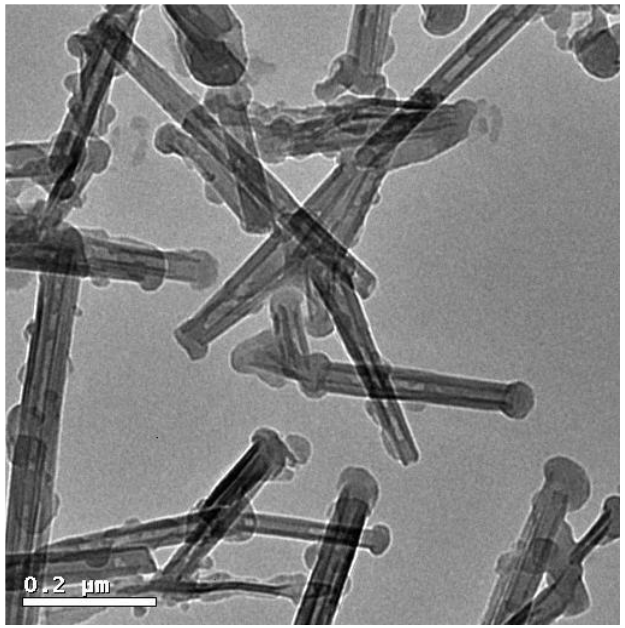
Drug name	Structure	Solubility in water[b] (mg mL^{-1})	pK_a[b]	Adsorption from media	Release
Tetracycline hydrochloride		10	3.3	Water and ethanol vacuum[2] Hot water[6]	Water[2] pH 7.4[6]
Gentamicin		100	12.55[a] 10.18[a]	Aqueous solution 50 mg mL^{-1}	Water
Resveratrol		0.03	8.99 9.63 10.64	Ethanol	pH 7.4
Furosemide		73.1 mg L^{-1}	3.8 7.5	Ethanol	pH 7.4
Nifedipine		0.0177	5.33	Ethanol	pH 7.4
Dexamethasone		0.0505	12.42	Ethanol	pH 7.4

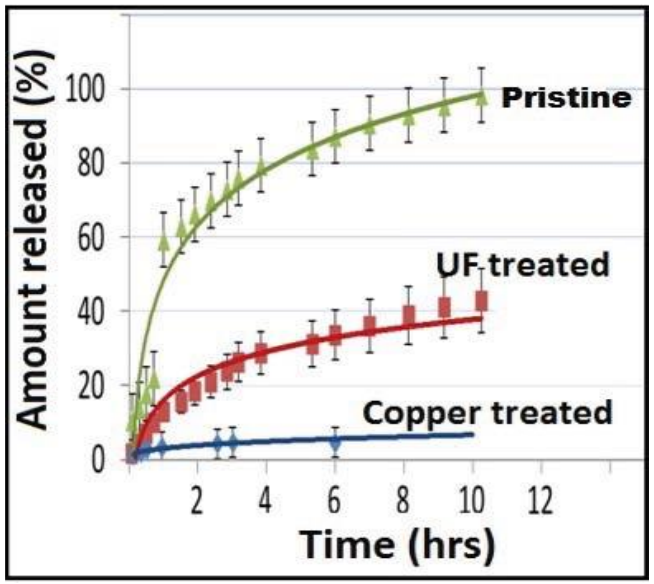
Lvov, Y.; Guo, B.; Fakhrullin, R. F. RSC Smart Materials 2017

Drug name	Structure	Solubility in water[b] (mg mL^{-1})	pK_a[b]	Adsorption from media	Release
Aspirin		3	3.49	Ethanol 0.03 g mL^{-1} of aspirin + 2 g of HNTs; stirring 12 h	Aqueous, pH 6.8
5-Aminosalicylic acid		0.84	2.09, 5.26, 13.64	Aqueous 0.225 g L^{-1} at 40 ± 0.1 °C for 24 h, stirring	Water, 0.1 M HCl, pH 6.8 buffer
Diclofenac, sodium salt		50	4.15	25 mg mL^{-1} (30 mL) + 0.5 g HNTs, stirring	pH 1.2 pH 7.4
Diphenhydramine hydrochloride		1000	8.98	29 mg mL^{-1} (30 mL) + 0.5 g HNTs, stirring	pH 1.2 pH 7.4
Ibuprofen		<1	4.91	0.1 g mL^{-1} in ethanol + 2 g of halloysite; stirring	—
Ofloxacin		28.3	5.97 9.28	0.002 g mL^{-1} in pH 3 acetic acid + 1 g of halloysite, shaker at 35 °C	pH 1.2
Doxorubicin		2.6	7.34 8.46 9.46	1 mL of 2 mg mL^{-1} doxorubicin solution (in water) mixed with halloysite and DNA; mixture dissolved in 30 mL water.	pH 7.4

End-Stoppers …

Can We Extend the Release Even Further?



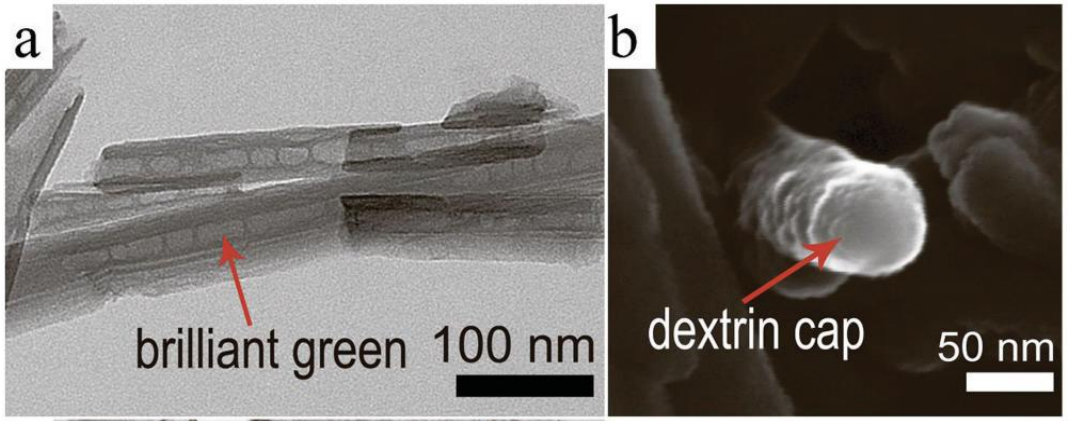


1) urea–formaldehyde (UF) coating on the halloysite surface

Y. Lvov et. al. *Langmuir* 2013, *29*, 7439

2) benzotriazole and 0.1 M $CuSO_4$ solutions for about 30 s for the formation of the stoppers.

Y. Lvov, et al. *J. Mater. Chem.* 2010, *20*, 6681.



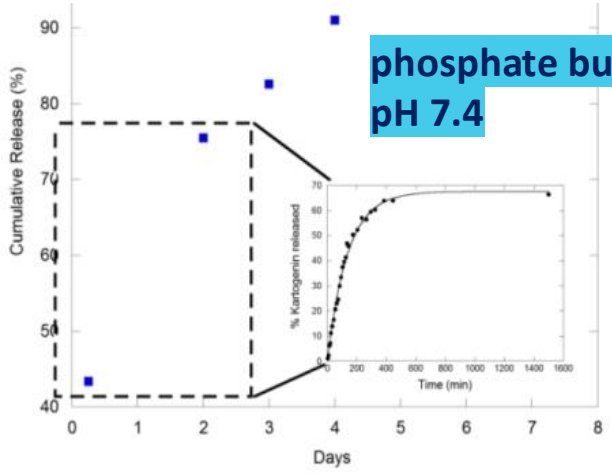
a) TEM image of brilliant-green-loaded halloysite.
b) SEM image of a dextrin cap on the end of the functionalized nanotube.

D. Mills, Y. Lvov *RCS Adv.* 2014, *4*, 488.

ACS Medicinal Chemistry Letters
ACS Med. Chem Lett. 2019, *10*, 419
Letter
Cite This: ACS Med. Chem. Lett. 2019, 10, 419−424
pubs.acs.org/acsmedchemlett

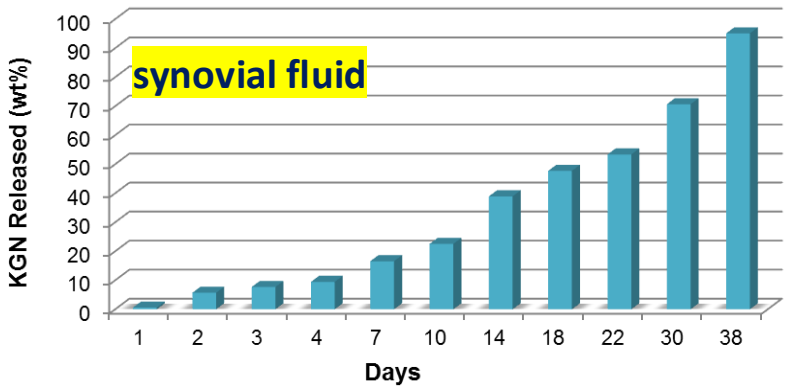
Multifunctional Carrier Based on Halloysite/Laponite Hybrid Hydrogel for Kartogenin Delivery

Marina Massaro,[†] Gabriella Buscemi,[†] Luca Arista,[⊥] Giuseppa Biddeci,[†,§] Giuseppe Cavallaro,[‡] Francesca D'Anna,[†] Francesco Di Blasi,[§] Angelo Ferrante,[∥] Giuseppe Lazzara,[‡] Carla Rizzo,[†] Gaetano Spinelli,[§] Thomas Ullrich,[⊥] and Serena Riela*[,†]

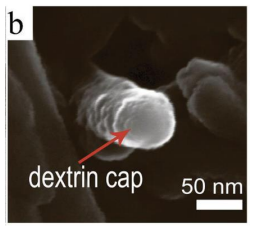


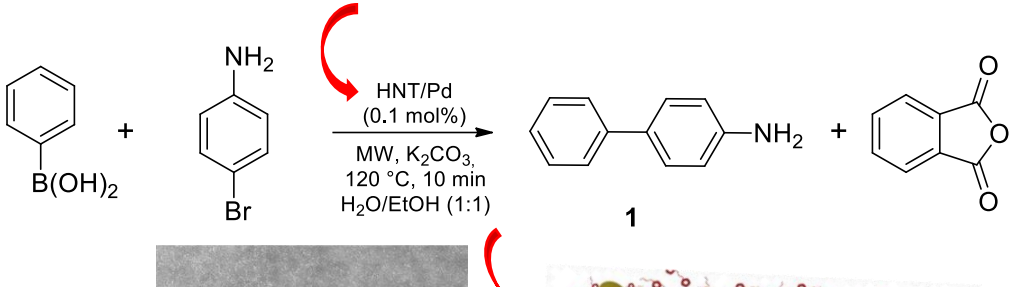
phosphate buffer, pH 7.4

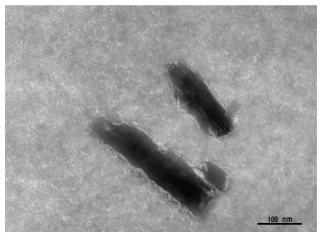
Release



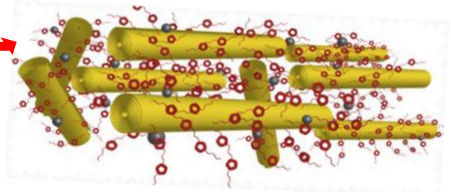
synovial fluid





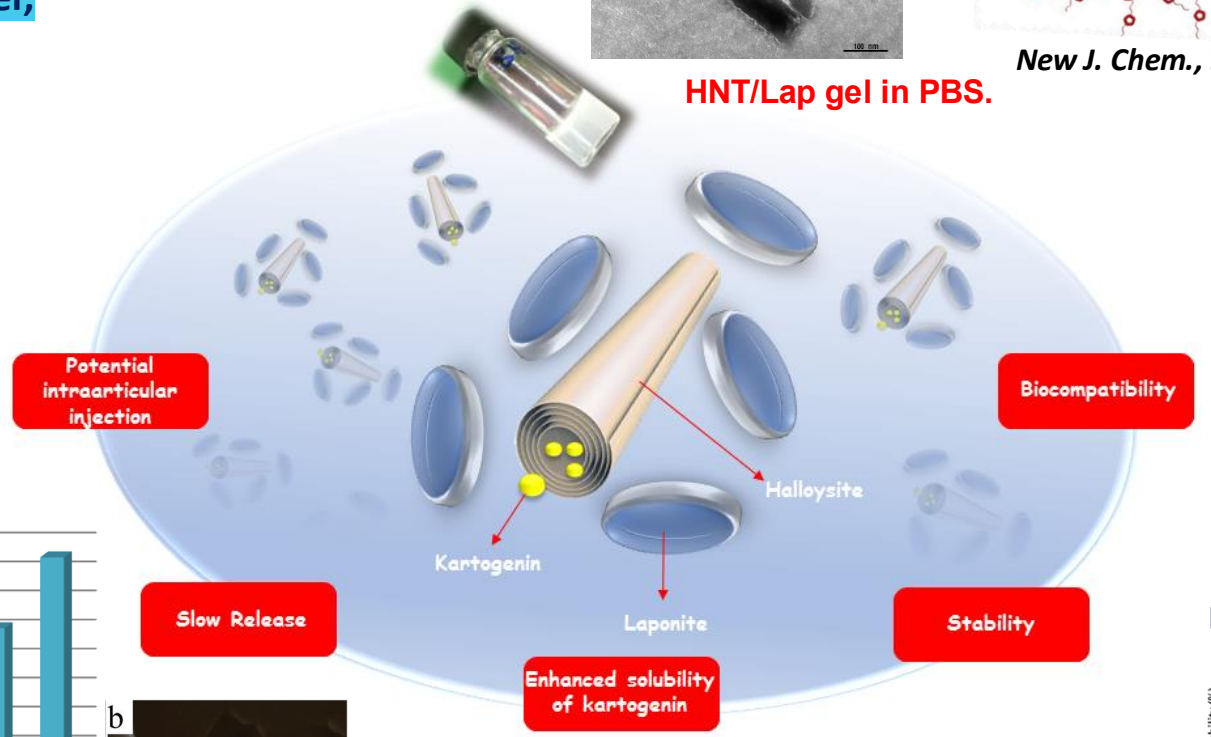


HNT/Lap gel in PBS.

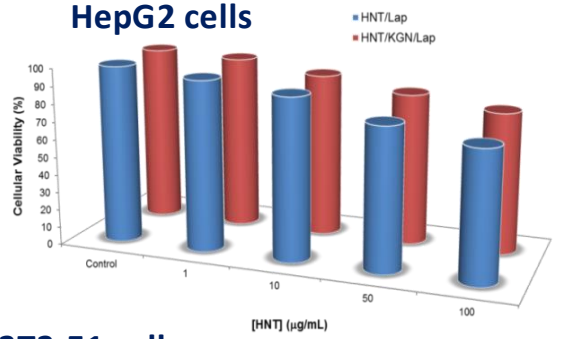


New J. Chem., 2018, 42, 13938.

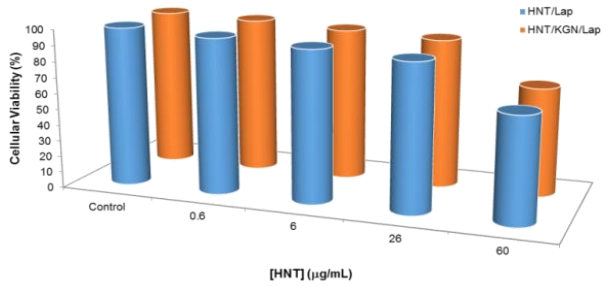
loading 7.2 wt% *by TGA*



HepG2 cells



MC3T3-E1 cells



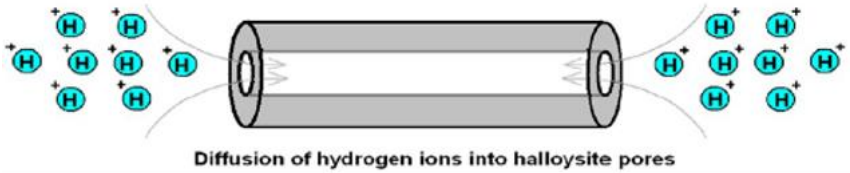
The major difference between synovial fluid and other body fluids derived from plasma is the high content of hyaluronic acid in synovial fluid

Enlargement of Halloysite Clay Nanotube Lumen by Selective Etching of Aluminum Oxide

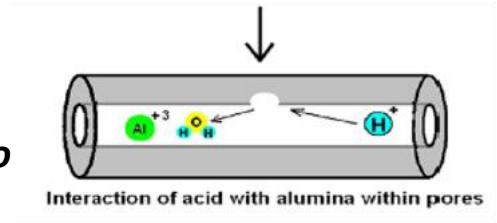
ARTICLE

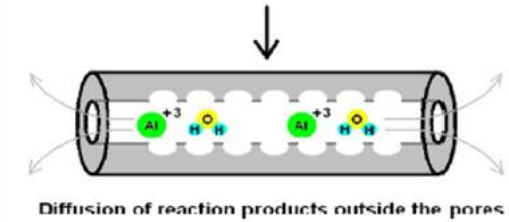
Elshad Abdullayev, Anupam Joshi, Wenbo Wei, Yafei Zhao, and Yuri Lvov*

Lvov Y. et al. ACS Nano, 2012, *6*, 7216–7226



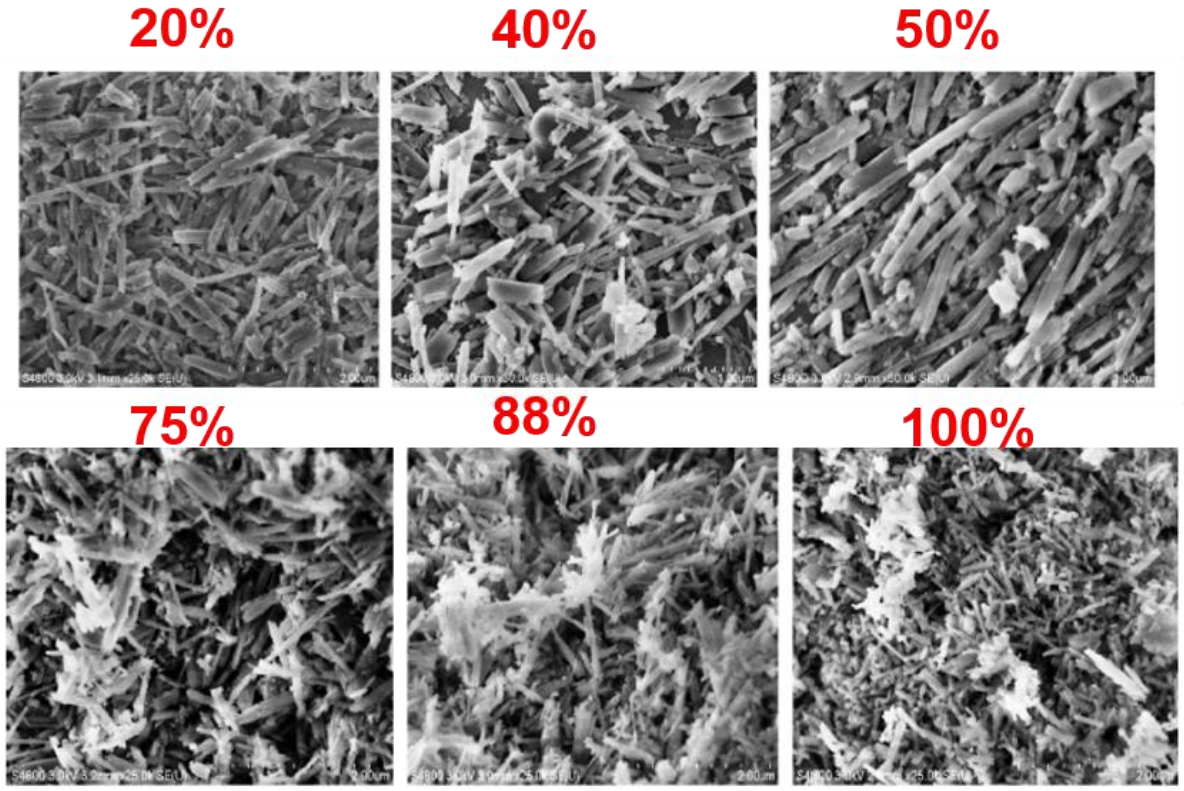
Etching starts by diffusion of hydrogen ions into HNT pores





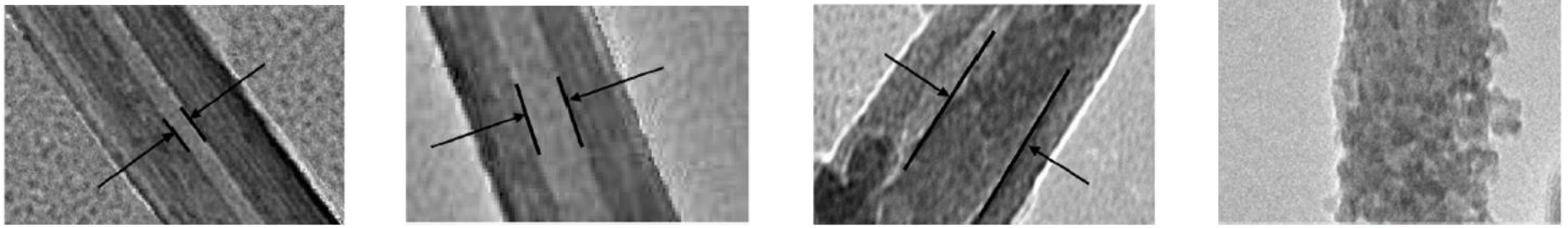
What If We Need More Space?

SEM



The morphology obtained depending on % of removed alumina

TEM




What if we want to decide where a molecule is placed, make its interaction more stable, or introduce a completely new function?

Covalent chemistry comes into play!



"Covalent modification"

Bertier first described HNT

1826

For ca. two centuries HNTs were mainly used for ceramics

HNT was used for the first time as nanocontainer for drugs

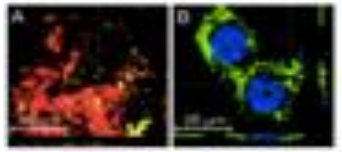
2001

HNTs as filler for polymeric matrices

First example of covalent modification of HNTs

2008

HNTs for pollutant removal



HNT was found to be no toxic and able to penetrate cell membrane

2010

HNTs as su for met nanopartic catalyst of o reactio

2012

Covalent modification of the lumen



Site-Specific Functionalization

2022

J. Coll. Int. Sci 2022, 606, 1779

J. Mater. Chem. B, 2018,6, 3415

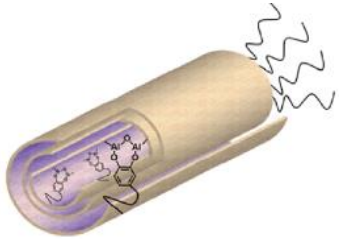
Contents lists available at ScienceDirect

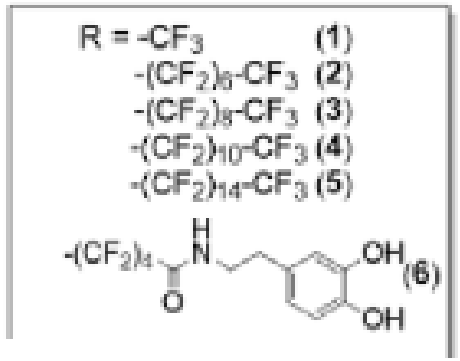
Journal of Colloid And Interface Science

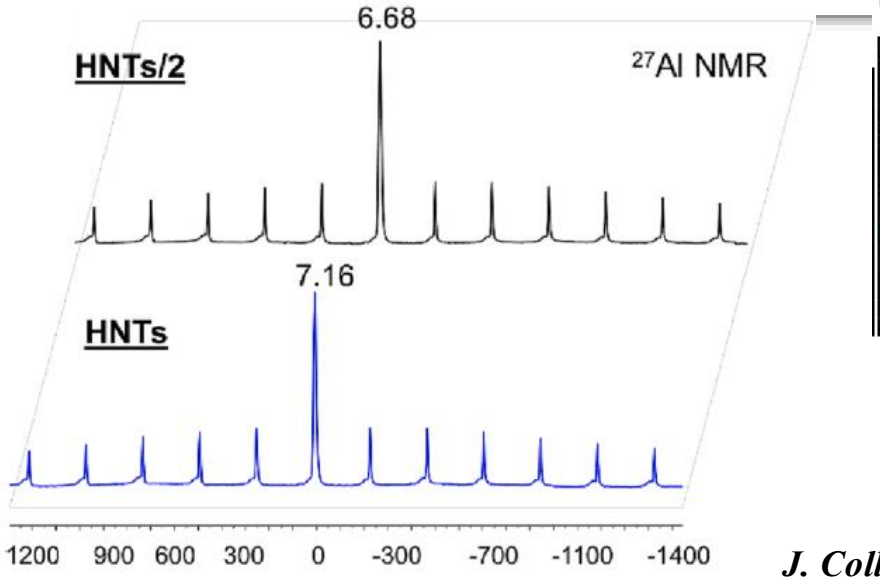
journal homepage: www.elsevier.com/locate/jcis

Modification of halloysite lumen with dopamine derivatives as filler for antibiofilm coating☆

Marina Massaro [a,1], Maria Laura Alfieri [b,1], Giorgio Rizzo [c], Francesco Babudri [c], Raquel Barbosa de Melo [d], Teresa Faddetta [a], Giuseppe Gallo [a], Alessandra Napolitano [b], Rita Sanchèz-Espejo [d], César Viseras Iborra [d,e], Serena Riela [a,*]



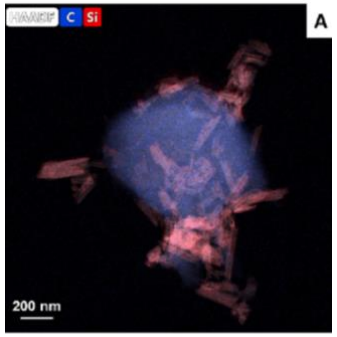




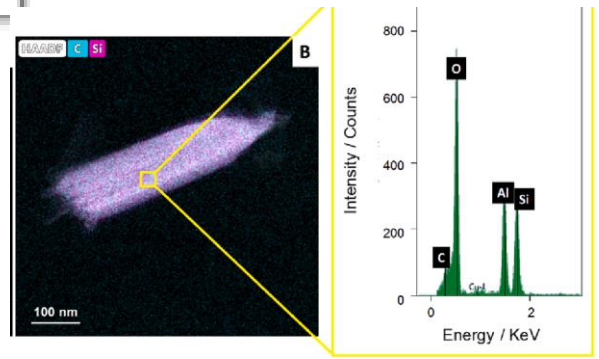
J. Coll. Int. Sci. 2023, *646*, 910.

-inner surface

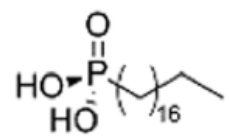
***E. coli* biofilm treated with:**

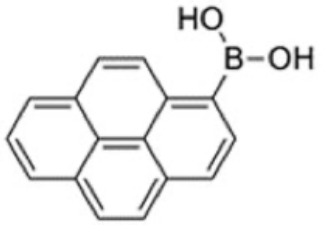


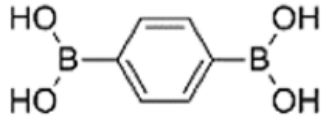
TEM **HNTs/5**

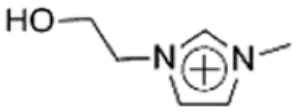


HNTs/2

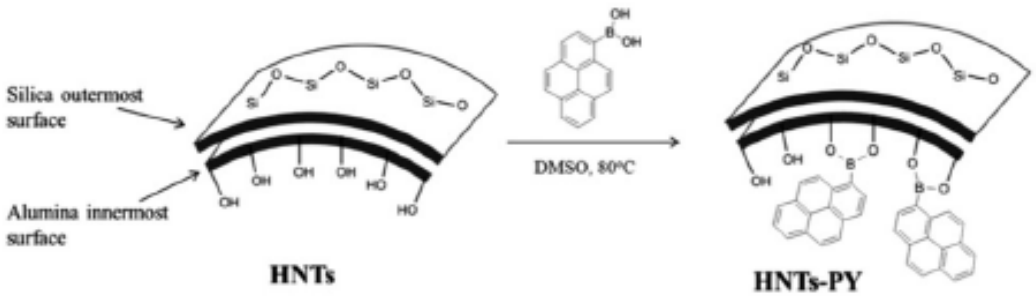






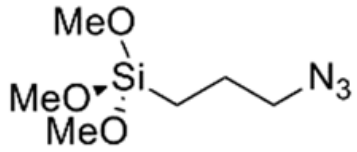


Lvov Y. *et al.*
J. Am. Chem Soc. 2012, *134*, 1853-1859.



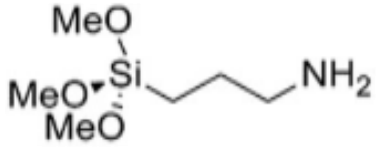
H. Zhang *et. al. ACS Appl. Mater. Interf.* 2015, 7, 23805.

-external surface

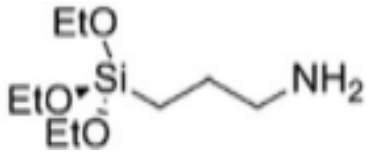


3-Azidopropyltrimethoxysilane[24]

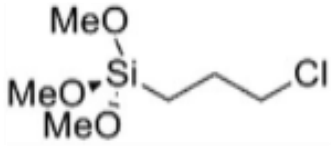
Int. J. Pharm. 2014, *475*, 613-623



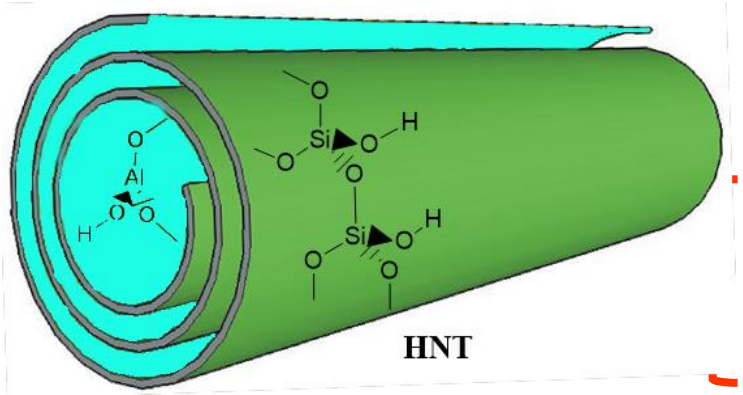
3-Aminomethyltrimethoxysilane[38–40]

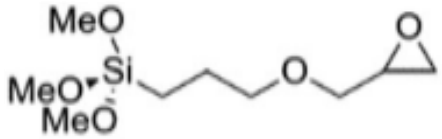


3-Aminopropyltriethoxysilane[26,32,33]

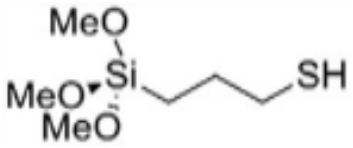


3-Chloropropyl-trimethoxysilane[49]





γ-Glycidoxypropyltrimethoxysilane[52,53]



3-Mercaptopropyltrimethoxysilane[44,45]

J. Organomet. Chem. 2014, *749*, 410-415

J. Mater. Chem. B, 2018,6, 3415.

Surface area (BET)

SiONPs 400 m^2/g

HNT 65-120 m^2/g

±3 wt% loading

Contents lists available at ScienceDirect

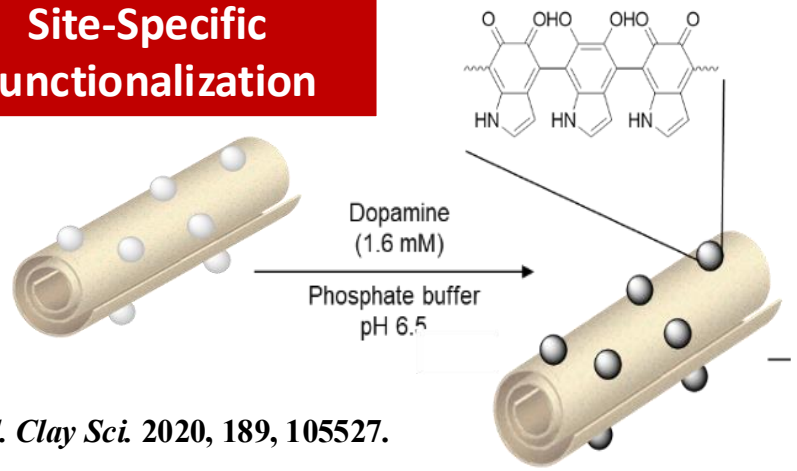
Journal of Colloid and Interface Science

journal homepage: www.elsevier.com/locate/jcis

Site-specific halloysite functionalization by polydopamine: A new synthetic route for potential near infrared-activated delivery system

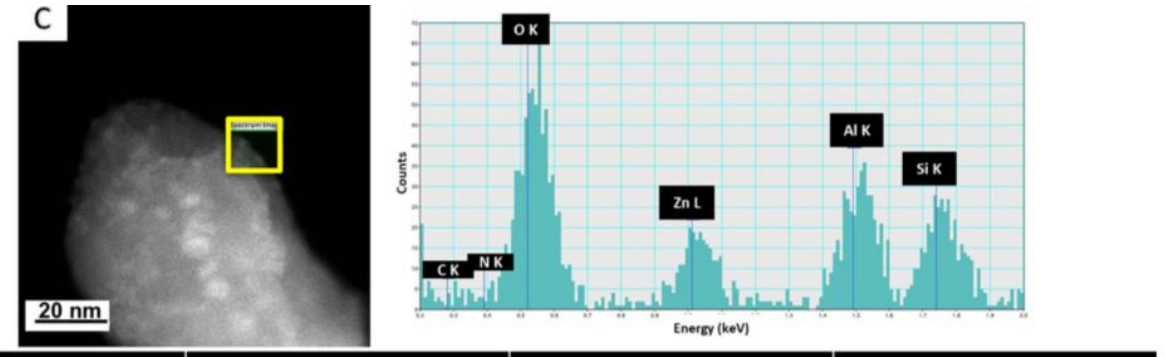
Maria Laura Alfieri [a,1], Marina Massaro [b,1], Marco d'Ischia [a,*], Gerardino D'Errico [a], Noemi Gallucci [a], Michelangelo Gruttadauria [b], Mariano Licciardi [c], Leonarda F. Liotta [d], Giuseppe Nicotra [e], Gianfranco Sfuncia [e], Serena Riela [b,*]

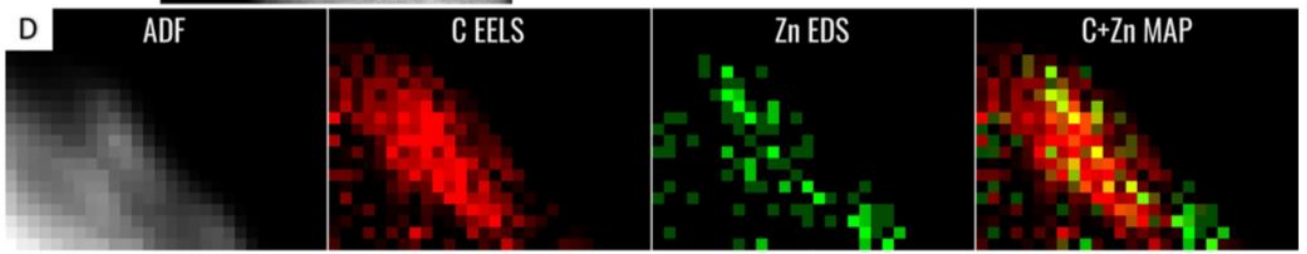
Site-Specific Functionalization

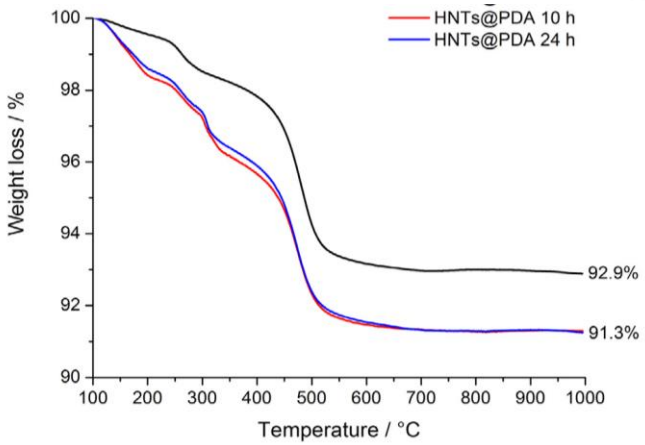


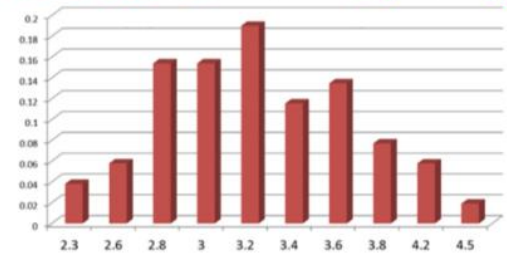
Appl. Clay Sci. **2020, 189, 105527.**

In collaboration with M. d'Ischia and M.L. Alfieri

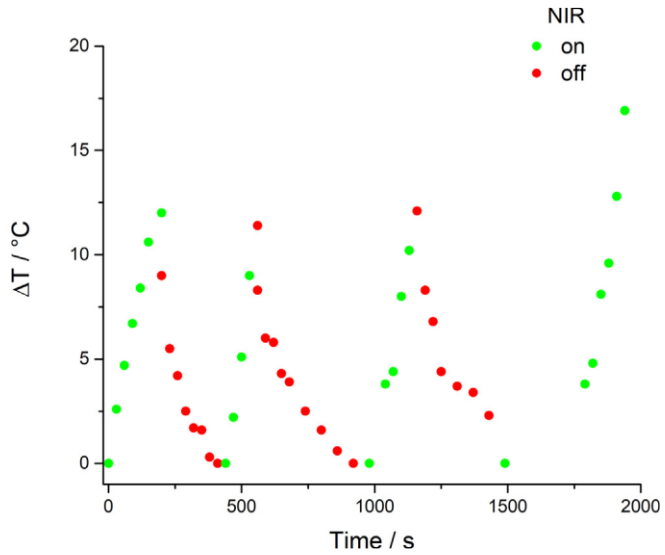






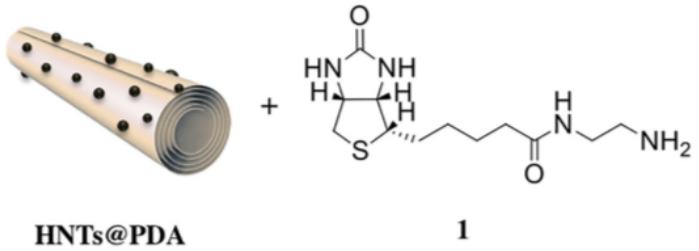


Sample	Z-average size (nm)	PI	ζ-potential (mV)
HNTs	570 ± 70	0.60	−34 ± 4
HNTs@ZnO	750 ± 160	0.36	−38 ± 8
HNTs@PDA	1320 ± 110	0.57	−26 ± 6

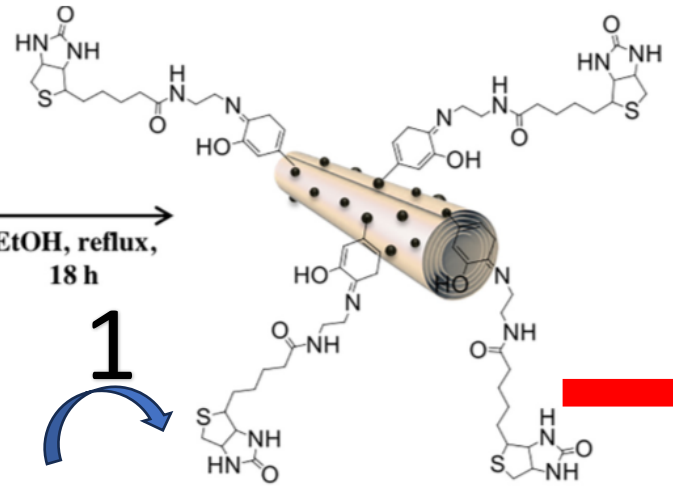


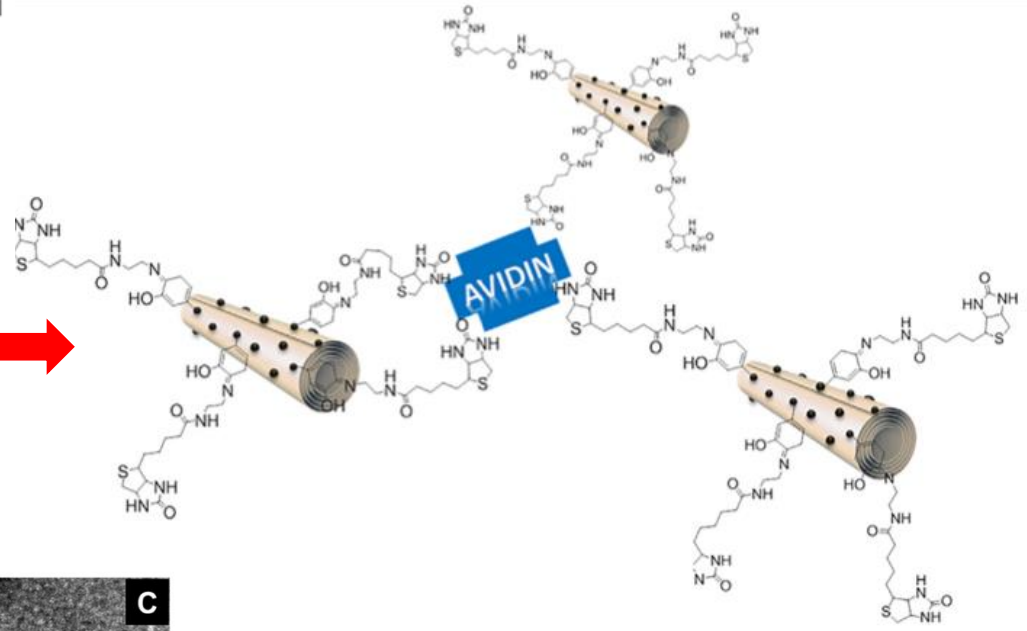
***J. Coll. Int. Sci* 2022, 606, 1779.**

From Organization to Function



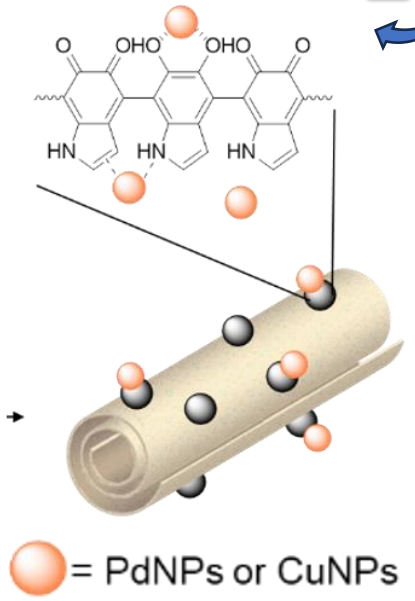
J. Coll. Int. Sci 2022, 606, 1779.





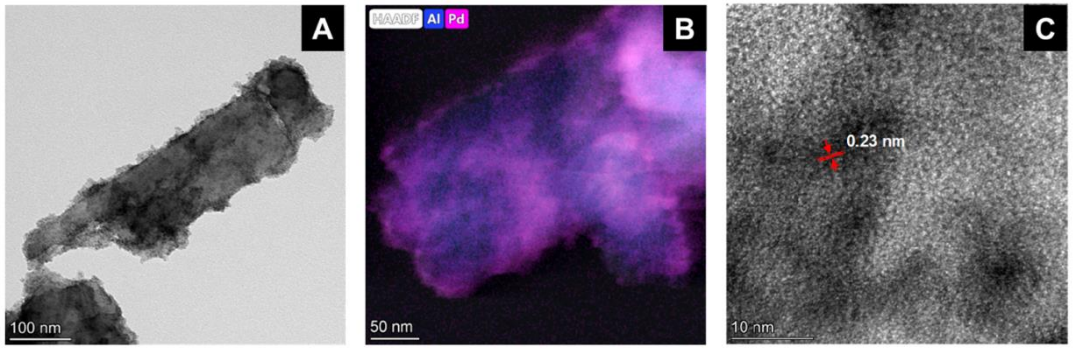
site-selective post-modification

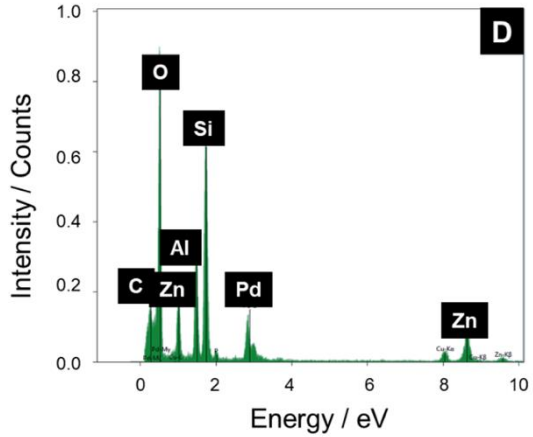
2

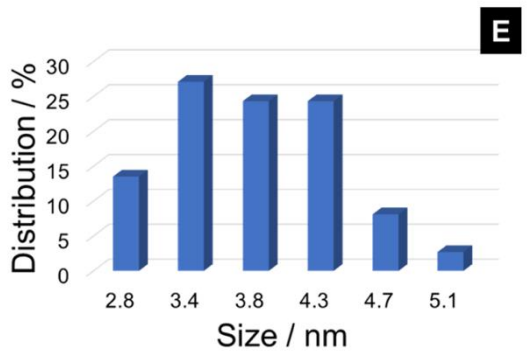


Catalysts 2025, 15, 1029

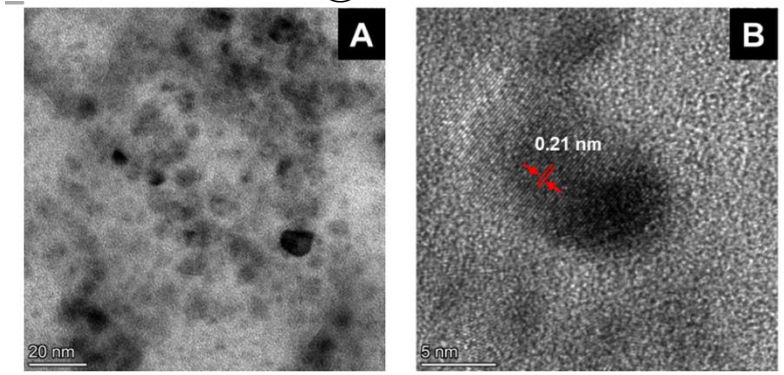
Hal@PDA/PdNPs

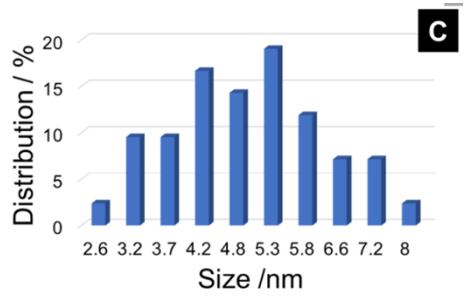






Hal@PDA/CuNPs







From covalent bonds...

Covalent chemistry can transform **natural** *materials into advanced functional platforms*



Organic molecule detected
≠
covalent functionalization



Adsorbed? Entrapped? Electrostatic interaction? Truly covalently bound?

true covalent bond

Prove the bond, not just the molecule.

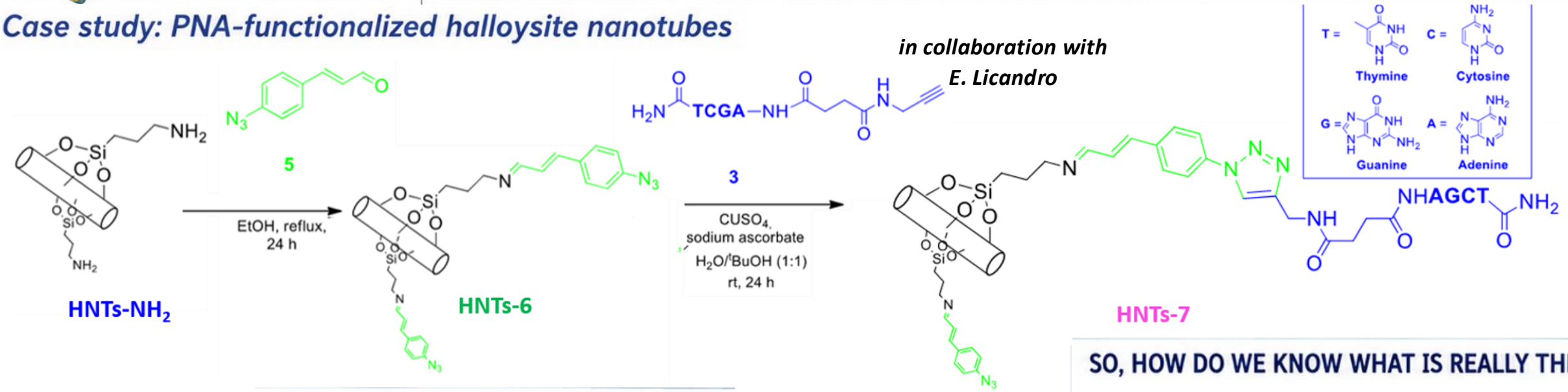
PROVE IT. QUANTIFY IT. REPRODUCE IT.

And always check the reproducibility of the functionalization!

Can we track a covalent functionalization step by step?

Case study: PNA-functionalized halloysite nanotubes

in collaboration with
E. Licandro



WHAT DID WE ADD?

HOW MUCH? DID IT REMAIN ATTACHED?

HNTs-NH_2	HNTs-6	HNTs-6	HNTs-7
Loading (wt%)	Loading (wt%)	Loading (wt%)	Loading (wt%)
3.5	9.5	6.3	23.5
Degree of functionalization (mmol g^{-1})	Degree of functionalization (mmol g^{-1})	Degree of functionalization (mmol g^{-1})	Degree of functionalization (mmol g^{-1})
0.63	0.61	0.36	0.18



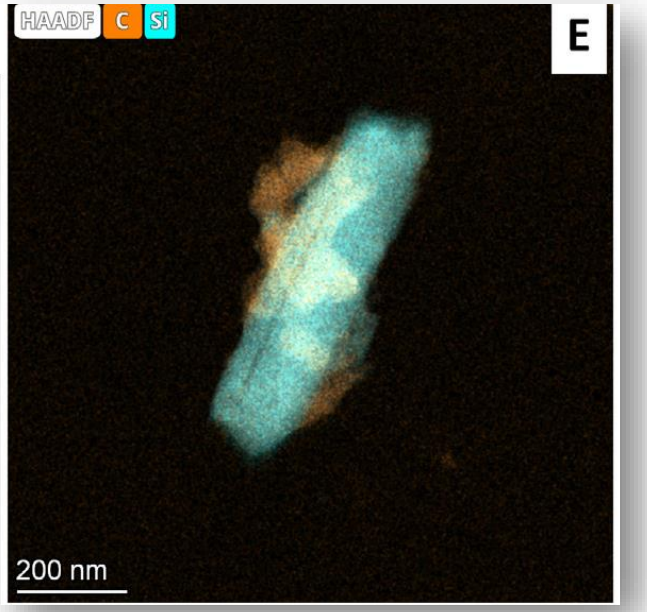
3.2 wt% of the total amount of linker grafted was **LOST** during the reaction

Every synthetic step can change what was already on the surface.

PROVE IT. QUANTIFY IT. REPRODUCE IT.

SO, HOW DO WE KNOW WHAT IS REALLY THERE?

HAADF-STEM



J. Coll. Int. Sci. **2022, 620, 221.**



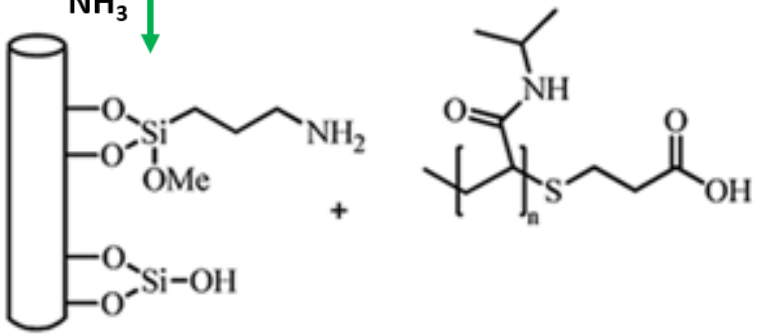
Can covalent chemistry give us control not only over WHERE, but also WHEN?

thermo-responsive materials



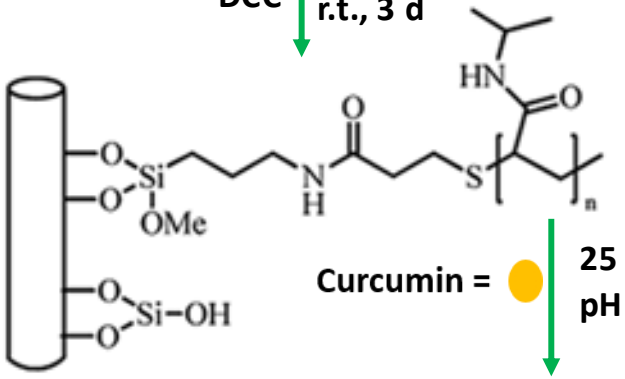
HNT-N_3 Covalent grafting of PNIPAAM on HNTs

PPh_3, NH_3 | DMF



HNT-NH_2 + PNIPAAM-COOH

DCC | CH_2Cl_2, r.t., 3 d



HNT-PNIPAAM

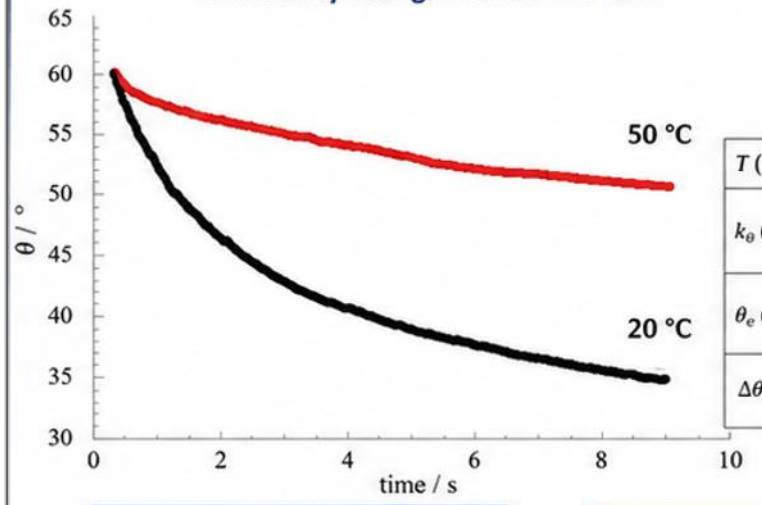
Curcumin = | 25 °C, pH 7.4

HNT-PNA/Curc

Loadind 14.2 wt % by TGA

Loadind 3.6 wt % by TGA

Wettability changes across the LCST



Exponential decay fit

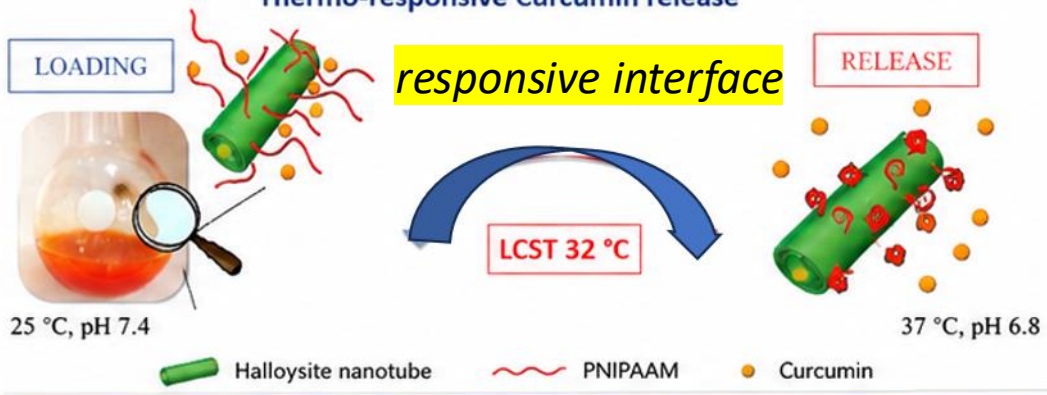
$\theta = \theta_e + \Delta\theta \exp(-k_\theta t)$

T (°C)	20 °C	50 °C
k_θ (s^{-1})	0.416 ± 0.006	0.149 ± 0.002
θ_e (°)	34.7 ± 0.3	50.5 ± 0.3
$\Delta\theta$ (°)	25.3 ± 0.3	9.8 ± 0.3

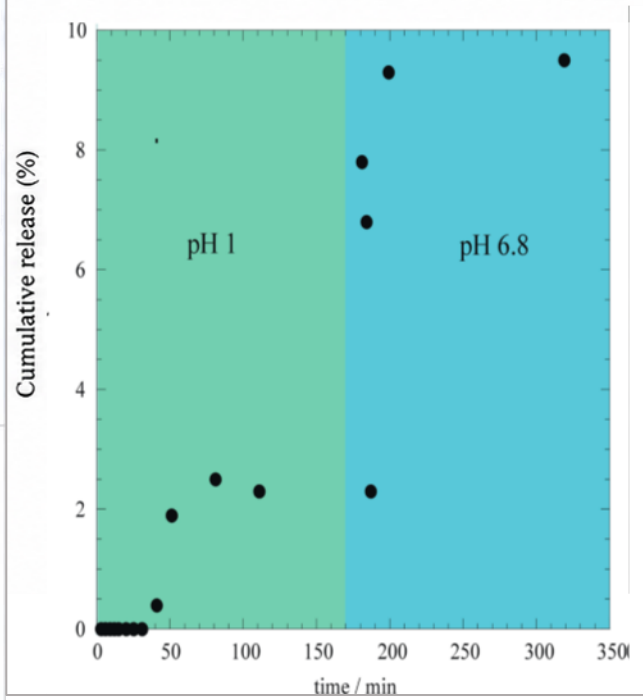
20 °C < LCST
hydrated PNIPAAM
fast spreading, hydrophilic

50 °C > LCST
collapsed PNIPAAM
slow spreading, more hydrophobic

Thermo-responsive Curcumin release



pH- and temperature-dependent Curcumin release

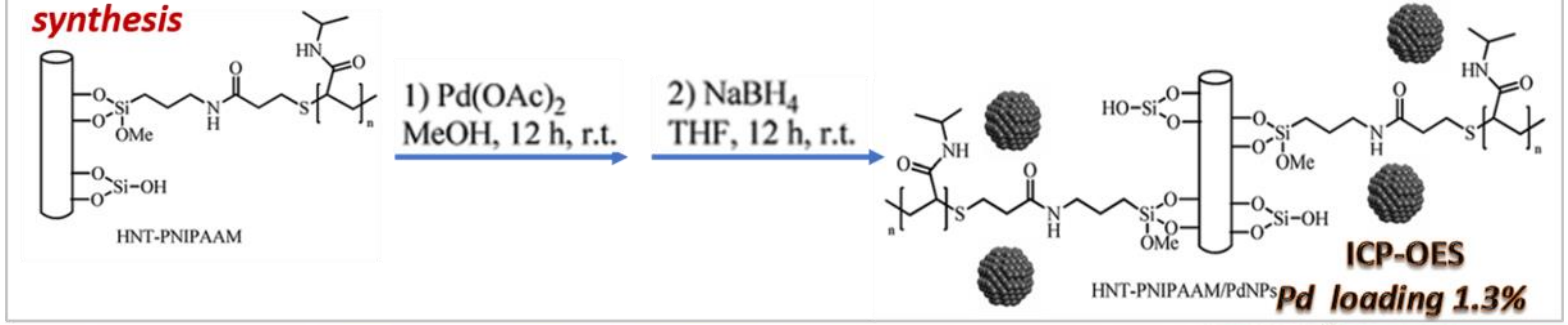


J. Phys. Chem. C 2015, *119*, 8944−8951.

Can the same responsive interface host a completely different function?

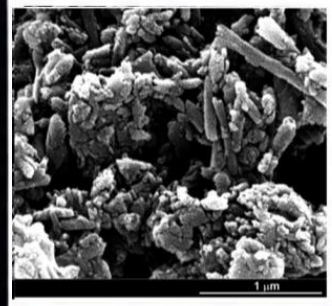
thermo-responsive catalysis

synthesis



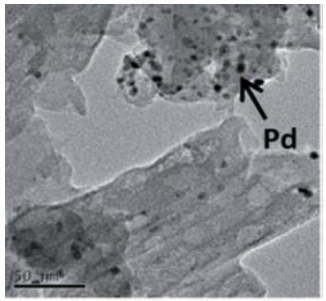
CHARACTERIZATION OF HNT–PNIPAAM/PdNPs

SEM



Aggregated nanotubes with rough surface

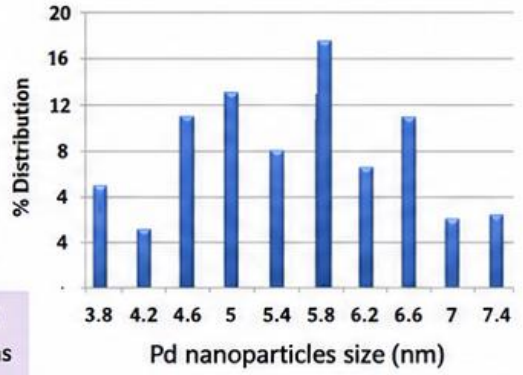
TEM



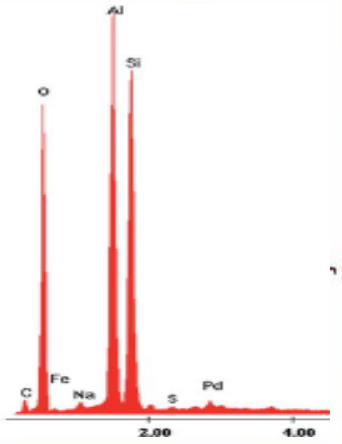
PdNPs mainly on the external surface and in interlayer regions

d_{mean} = 5.5 ± 1.0 nm

Pd nanoparticles size distribution



EDS

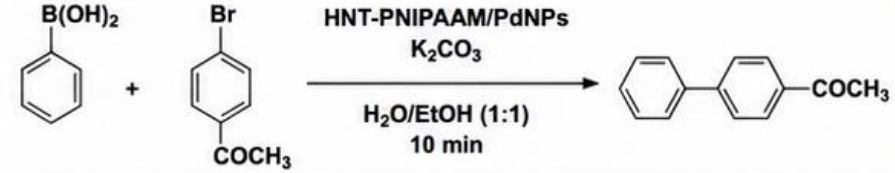


RECYCLABILITY OF HNT–PNIPAAM/PdNPs

5 consecutive runs (120 °C)

	1	2	3	4	5
Conversion (%)	99%	97%	92%	90%	

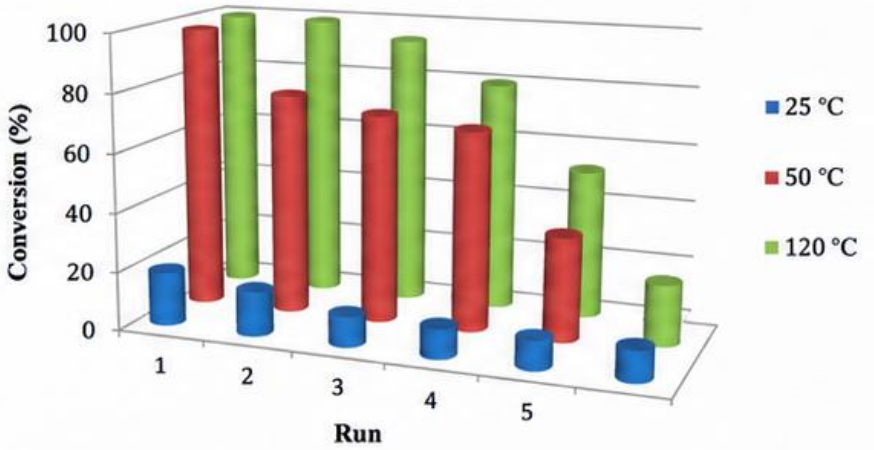
- ✓ Recovered by centrifugation, washed and reused
- ✓ Small drop in activity after five cycles



Entry	T (°C)	Conv.[a] (%)
1	25	17
2	50	95
3	120	>99
4[b]	120	>99

[a] Determined by ^{1}H-NMR (Fig. S1–S4). [b] Reaction conditions: H_2O/EtOH (1 : 1) 1.2 mL, catalyst 0.016 mol%, 2 mg.

STRONG TEMPERATURE DEPENDENCE OF THE CATALYTIC ACTIVITY



RSC Adv., 2016, 6, 55312–55318

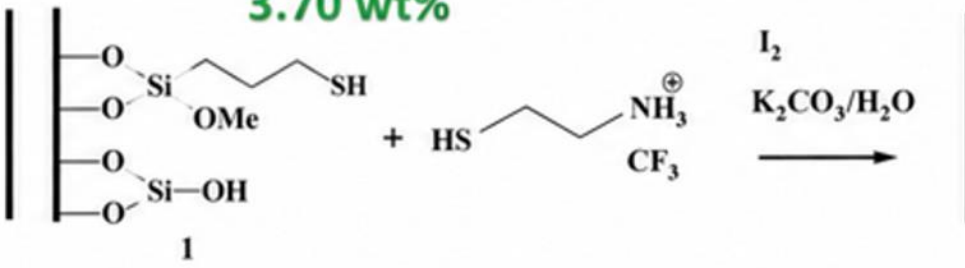


Can we make the covalent bond itself responsive?

dual-responsive covalent design

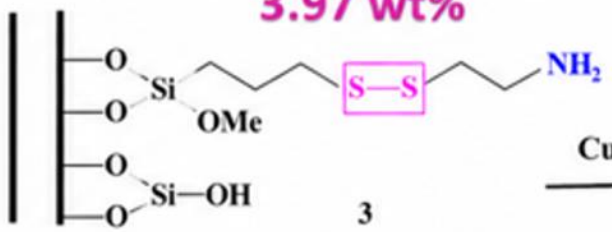
Synthetic Covalent Approach

3.70 wt%



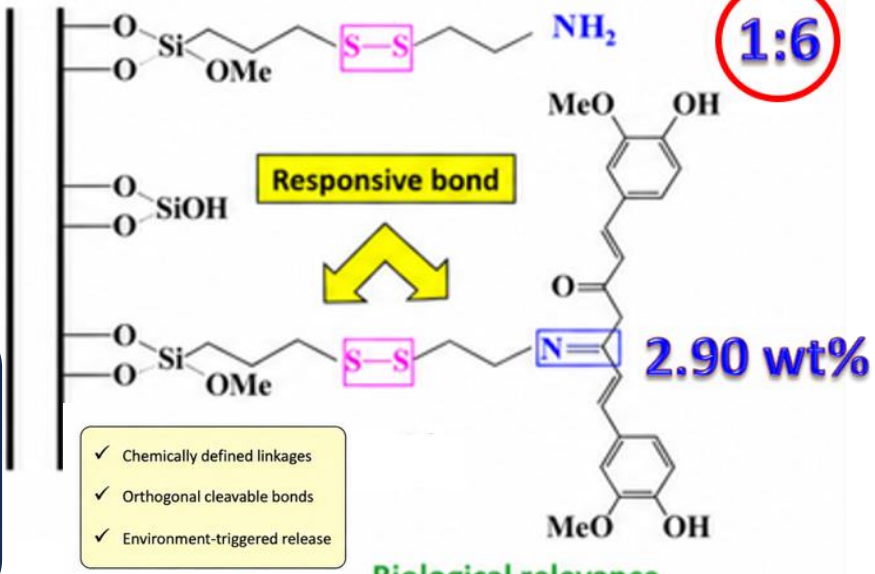
1

3.97 wt%



3

Curc



1:6

2.90 wt%

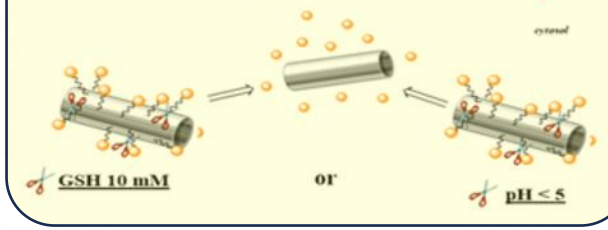
- ✓ Chemically defined linkages
- ✓ Orthogonal cleavable bonds
- ✓ Environment-triggered release

Compound	Heating	Solvent	Time (h)	Tan (δ)	Temp. (°C)	Loading (%)
3	Trad.	MeOH	12	0.659	25	3.70
3	MW	MeOH	1	0.659	50	3.57
3	MW	/	1	/	50	4.00
4	Trad.	EtOH	24	0.941	78	0.88
4	MW	/	1	/	78	0.80
4	MW	H_2O	1	0.123	78	/
4	MW	H_2O/EtOH	1	/	78	0.40
4	MW	EtOH	1	0.941	78	2.90
4	MW	THF	1	0.047	78	/

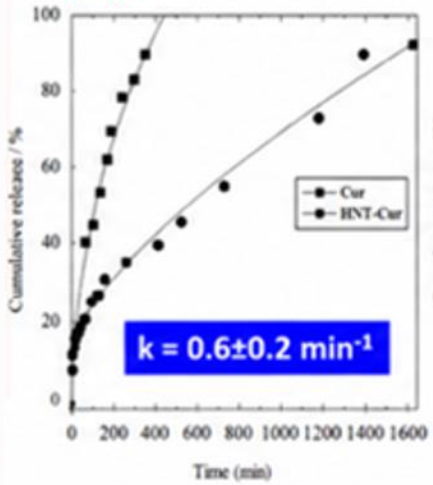
Coll. and Surf. B 2016, 140, 505–513

K I N E T I C

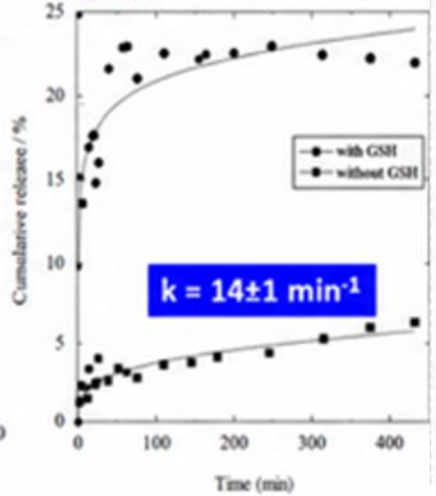
Dual-responsive ability



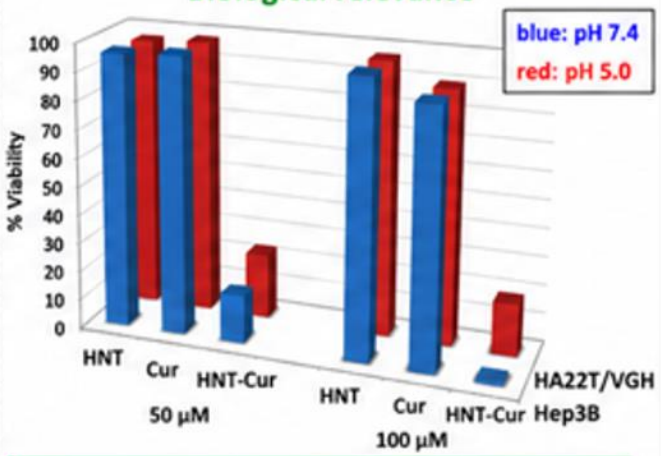
pH 1.0



pH 7.4



Biological relevance



Higher toxicity of HNT-Cur at pH 5.0 confirms pH-triggered curcumin release.



Can we obtain a pH/temperature–responsive system without covalent modification?

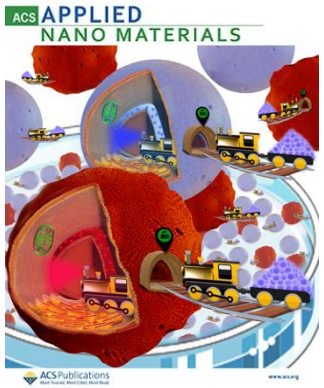
Supramolecular loading of a halochromic probe into HNTs

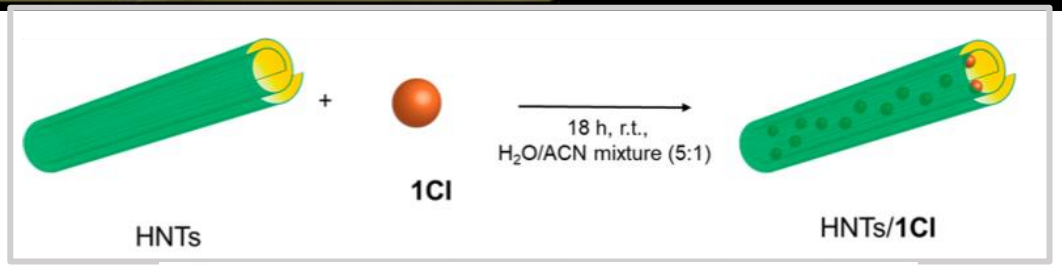
ACS APPLIED NANO MATERIALS

www.acsanm.org Forum Article

Supramolecular Association of Halochromic Switches and Halloysite Nanotubes in Fluorescent Nanoprobes for Tumor Detection

Marina Massaro,[†] Monica Notarbartolo,[†] Françisco M. Raymo,[*] Giuseppe Cavallaro, Giuseppe Lazzara, Mercedes M. A. Mazza, Cesar Viseras-Iborra, and Serena Riela[*]



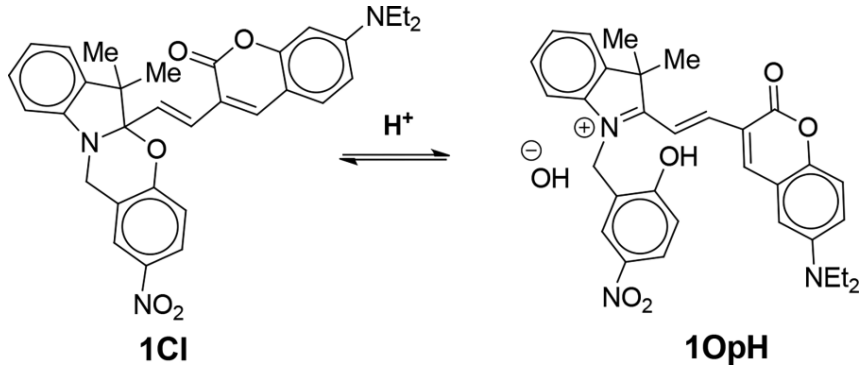


Successful loading confirmed by TGA and FT-IR

- ✓ **Loading of 1Cl:** ~ 6 wt%
- ✓ **Entrapment efficiency:** ~95%

pH-dependent halochromic probe: 1Cl

hydrophobic and water-insoluble compound



1Cl — *closed (oxazine)*

1OpH — *protonated open form*

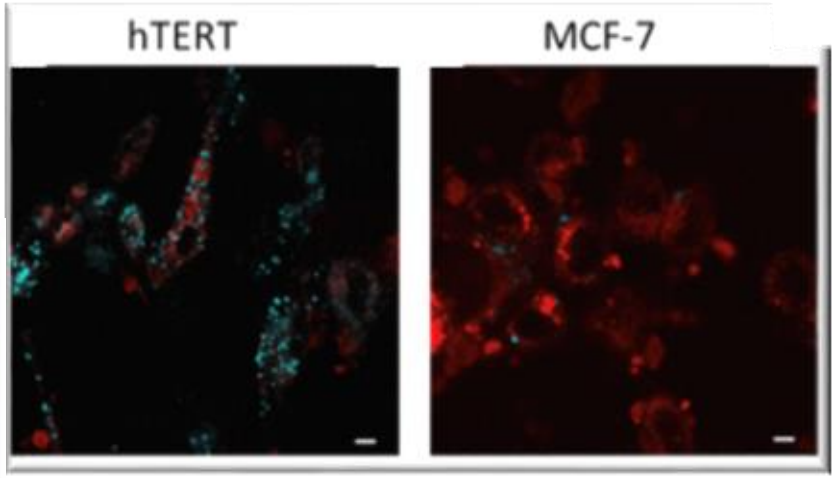
Acidic conditions → ring opening → bathochromic shift and red fluorescence

In collaboration with F. Raymo

We can bring the probe into the nanotubes and still retain its pH/temperature responsiveness.

But…do we know where the probe is located?

- ✓ Efficient internalization in tumor cell lines (MCF-7 and HL-60R).
- ✓ Bright red fluorescence activates in the intracellular acidic environment.



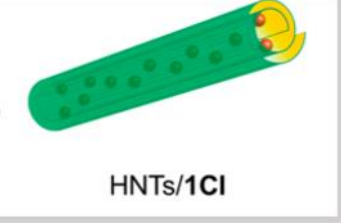
…after 6 h of incubation…

ACS Appl. Nano Mater. **2022, 5, 13729.**

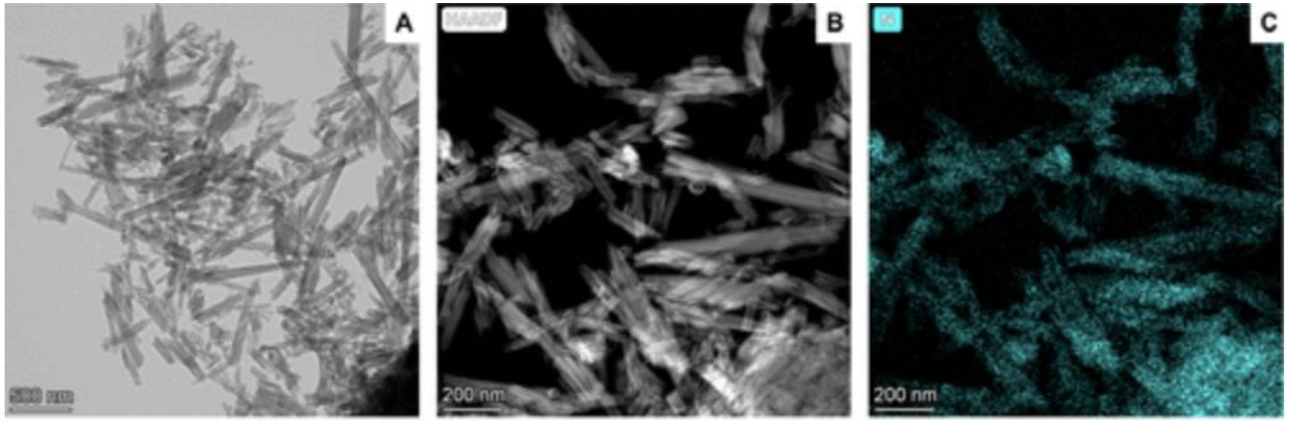


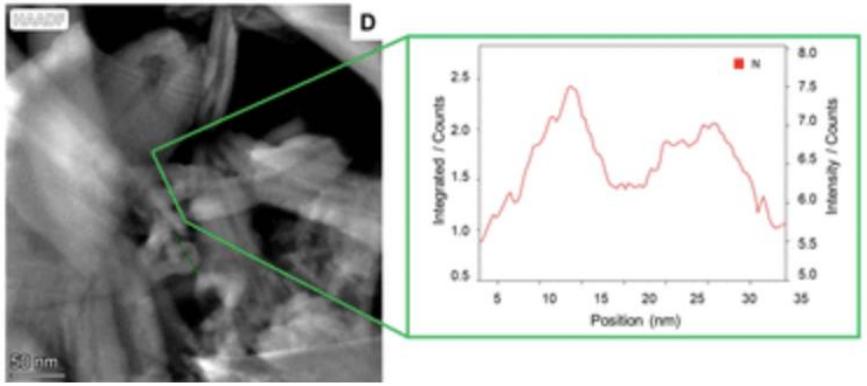
But where is the probe located?

Supramolecular association → probe inside AND outside



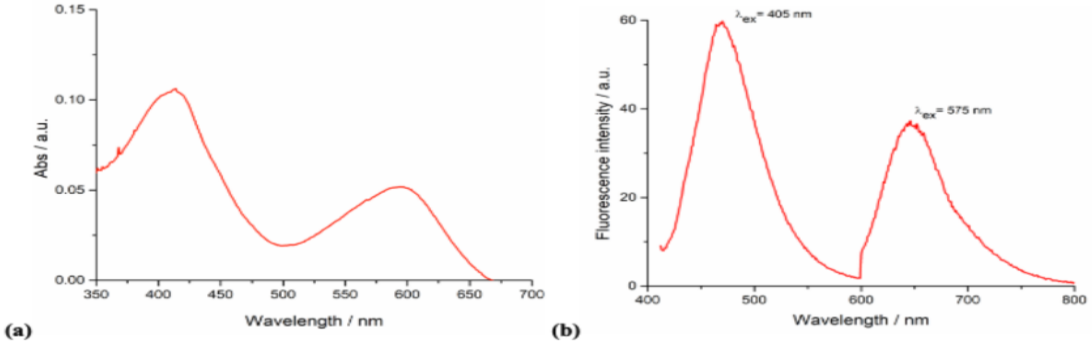
Evidences from TEM-EDX mapping



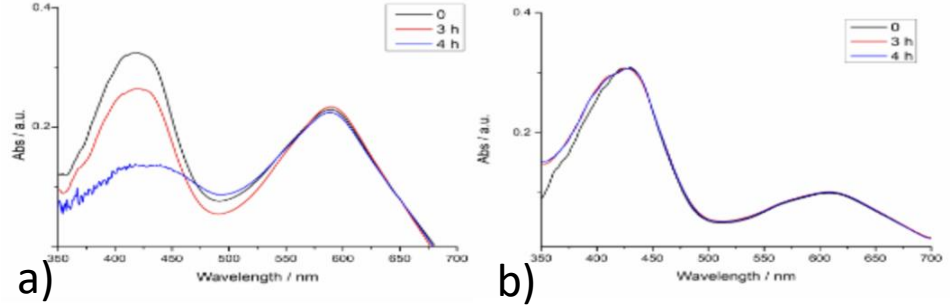


- ✓ Carbon and nitrogen signals from the probe are detected along the tubes.
- → Probe is associated with both the lumen and the external surface/outer Environment.

Optical response



(a) UV-vis and (b) fluorescence spectra of 1 in a water/ACN mixture



UV-vis spectra of 1 solution in a mixture H_2O/ACN 4:1 (10^{-4} M) in (a) the presence or (b) without 5 mg of HNTs over the time

Limitations of the supramolecular approach

- ✗ Probe location is not chemically defined.
- ✗ Acidic environment of halloysite can induce partial ring opening even without external pH stimulus → coexistence of closed and open forms.
- ✗ Heterogeneous distribution may affect reproducibility and control over functionality.

Responsive? Yes. Spatially controlled? Not really. We need a strategy to control where the probe is placed.



What changes when we make it covalent?

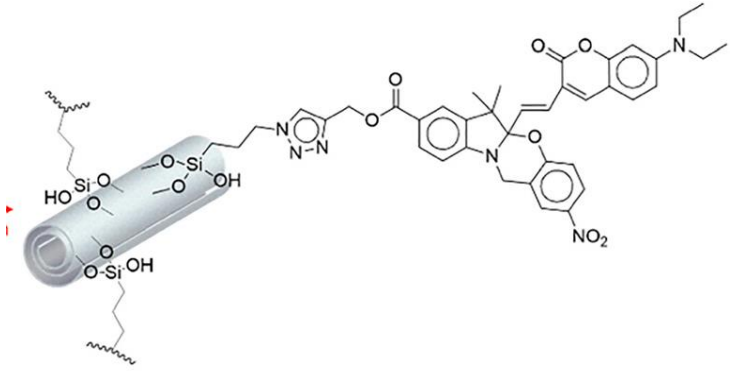
Covalent functionalization → selective placement on the external surface



This work is dedicated to the memory of Prof. D. Spinelli and Prof. sir F. Stoddart, exceptional scientists and inspiring mentors. Their vision and humanity deeply influenced the scientific and personal growth of several of us, and we are grateful to carry forward a small part of their vision and spirit.

Strategy 1: top down

Strategy 2: bottom up

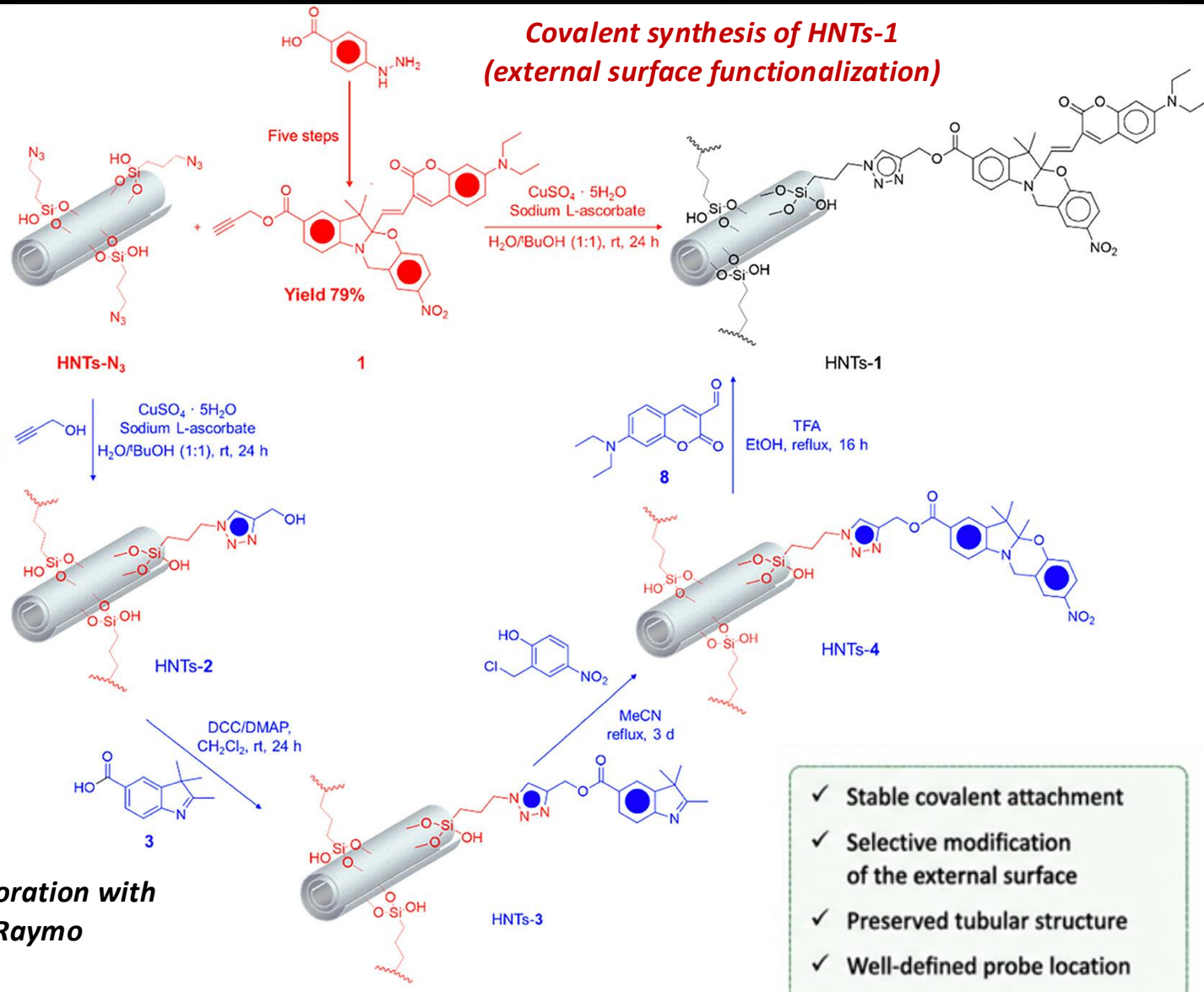


HNTs-1

	Nanomaterial	Loading[a] (wt%)	Degree of functionalization (mmol g^{-1})
Top-down	$HNTs-N_3$	2.3	0.27 ± 0.02
	HNTs-1	7.2	0.16 ± 0.01
Bottom-up	HNTs-2	1.5	0.26 ± 0.01
	HNTs-3	4.7	0.25 ± 0.01
	HNTs-4	3	0.20 ± 0.01
	HNTs-1	<1	<0.04

[a] Calculated using TGA.

Covalent synthesis of HNTs-1 (external surface functionalization)



- ✓ Stable covalent attachment
- ✓ Selective modification of the external surface
- ✓ Preserved tubular structure
- ✓ Well-defined probe location

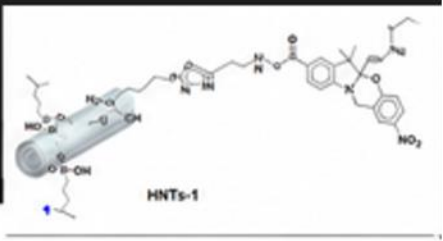
In collaboration with F. Raymo

J. Mater Chem B 2025, 13, 9407

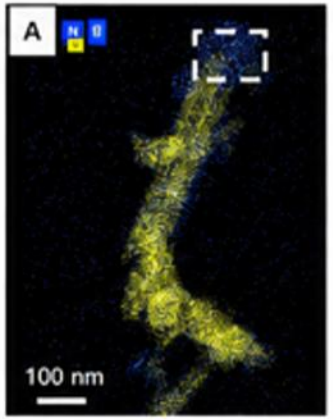


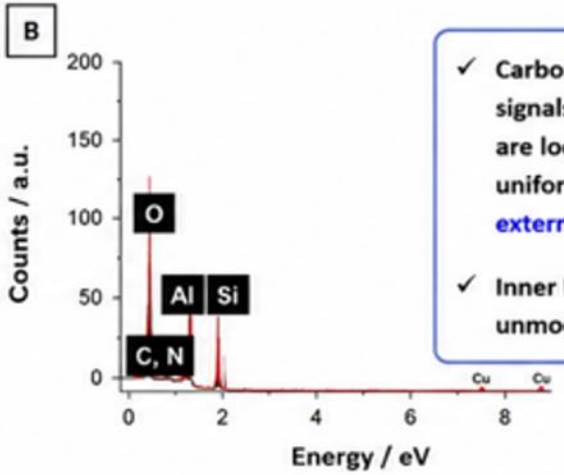
What changes when we make it covalent?

Covalent functionalization → selective placement on the external surface



Selective localization of the probe on the external surface

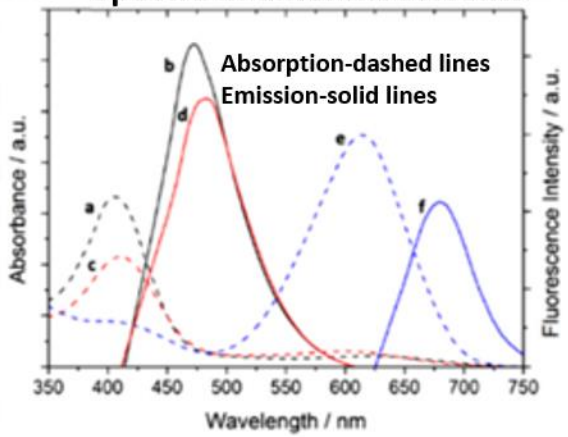




- ✓ Carbon and nitrogen signals (from probe) are localized uniformly on the external surface.
- ✓ Inner lumen remains unmodified.

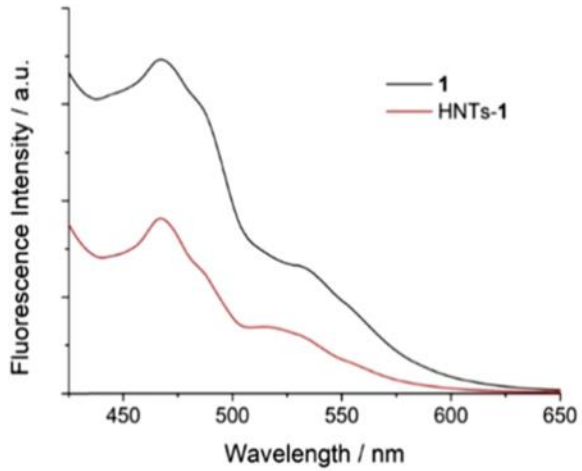
Photophysical properties

Spectra in different solvents

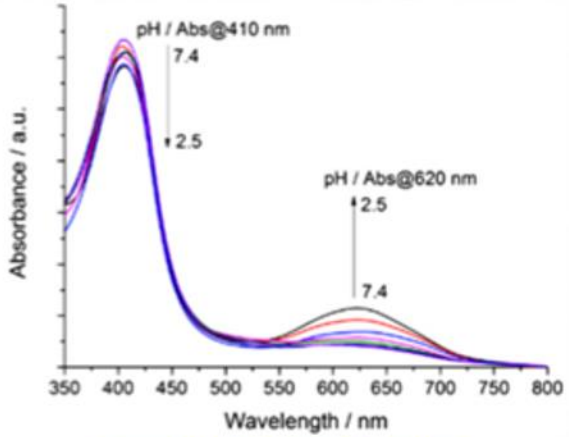


MeCN (a and b), H_2O/MeCN (1 : 1) (c and d)

Solid-state fluorescence spectra

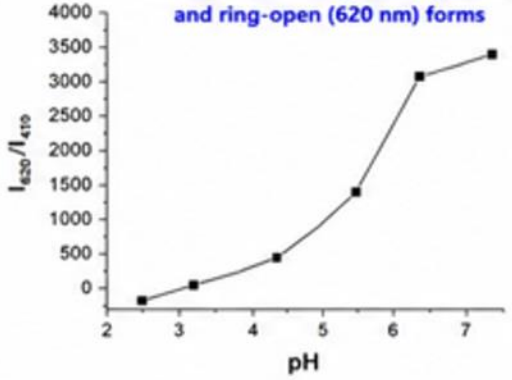


pH–responsiveness is preserved after covalent attachment



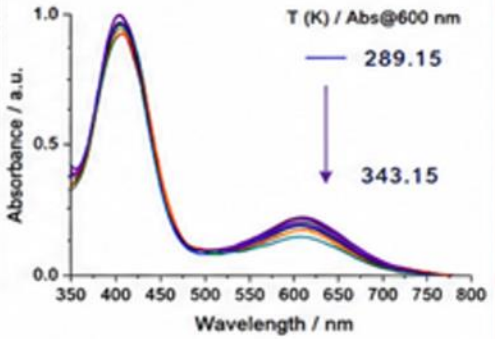
Decrease of closed form (~410 nm) and increase of protonated open form (~620 nm) at lower pH

Trend of the ratio between the emission intensities of the ring-closed (410 nm) and ring-open (620 nm) forms



The probe responds to pH while remaining covalently anchored.

Temperature–responsive (absorption band @ ~600 nm)



Spectral changes are maintained with increasing temperature.

Supramolecular loading (previous slide)

- X Probe associated with lumen AND external environment
- X Halloysite acidity can induce partial ring opening (even without external acid)
- X Probe location not chemically defined → less control

Covalent strategy

Covalent functionalization

- ✓ Probe placed ONLY on the EXTERNAL surface
- ✓ Halloysite internal acidity no longer affects the probe
- ✓ Better control over localization and function

Covalent chemistry gives us control over placement → *preserved responsiveness* and *improved reliability*.

Can covalent and supramolecular strategies work together?

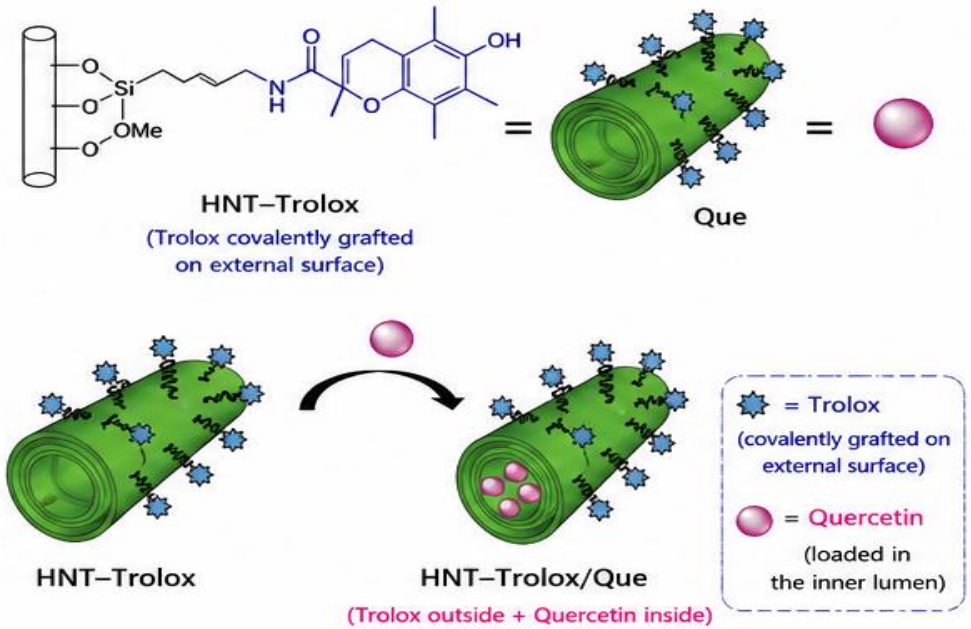
Covalent on the outside + Supramolecular on the inside → synergic nanoantioxidant



Synergistic nanoantioxidant

J. Mat Chem B 2016, *4*, 2229.

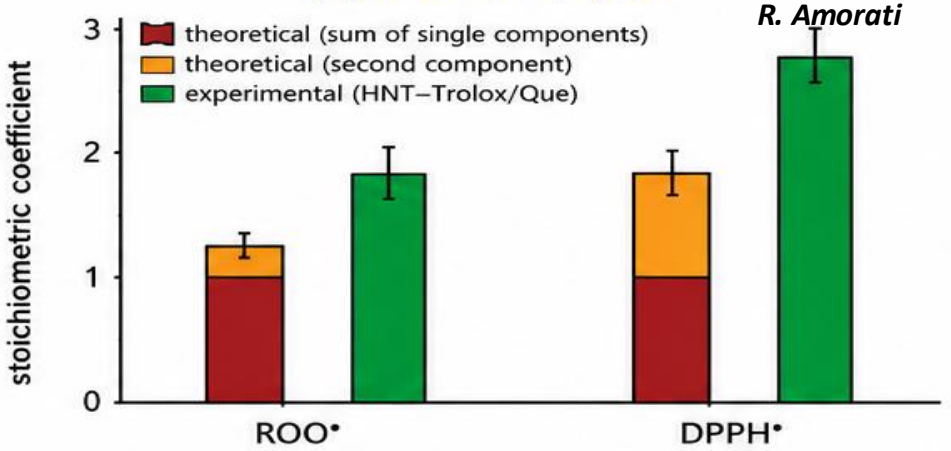
Dual functionalization of halloysite nanotubes



Synergy in radical trapping activity

(Fig. 10 from the paper)

in collaboration with R. Amorati



Peroxyl radicals (ROO•)
n_{theor} = 1.24 (MeCN)
n_{theor} = 0.91 (PhCl)
n_{exp} = 1.9 (MeCN)
n_{exp} = 1.5 (PhCl)

DPPH• radicals
n_{theor} = 1.9 (MeCN)
n_{exp} = 2.8 (MeCN)

Experimental > theoretical → SYNERGY

Fig. 10 Stoichiometric coefficients (*n*) for the reaction of antioxidants with (a) peroxyl radicals and (b) DPPH• radicals in MeCN and PhCl at 30 °C.

Synergic antioxidant mechanism (Scheme 4 from the paper)

External surface (Trolox) fast response

Trolox–OH + ROO• → Trolox–O• + ROOH

fast radical trapping (main antioxidant function)

Inner lumen (Quercetin) controlled release

Quercetin (release) → Trolox–O• + Que–OH → Trolox–OH + Que–O•

regenerates Trolox (co-antioxidant function)

regeneration Trolox

regeneration of Trolox prolongs antioxidant activity

Scheme 4 Synergic effects of Trolox and quercetin.

Different locations. Different interactions. One cooperative function. → Covalent control + Supramolecular loading → Synergy

Does it matter where we place the drug?

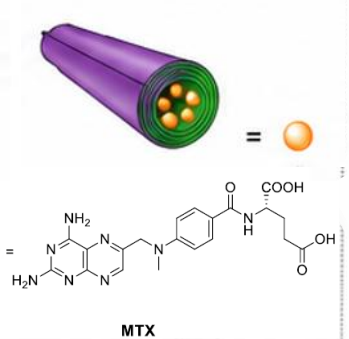
Same drug. Different placement. Different release.



Coll Surf. B 2022, 213, 112385.

Three nanostrategies with methotrexate (MTX)

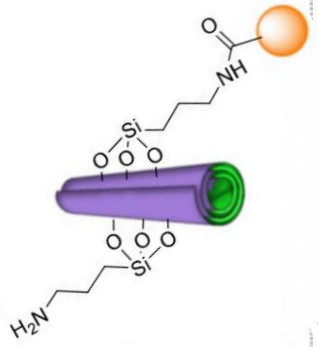
A) Supramolecular loading in the lumen



HNTs-1

MTX supramolecularly loaded in the lumen

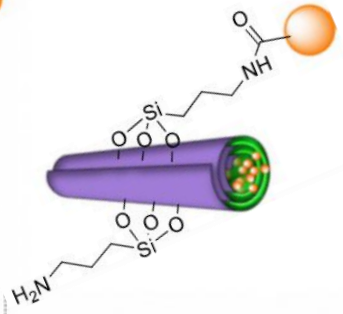
B) Covalent grafting on the external surface



HNTs-3

MTX covalently grafted on the external surface

C) Combined approach (lumen + external surface)

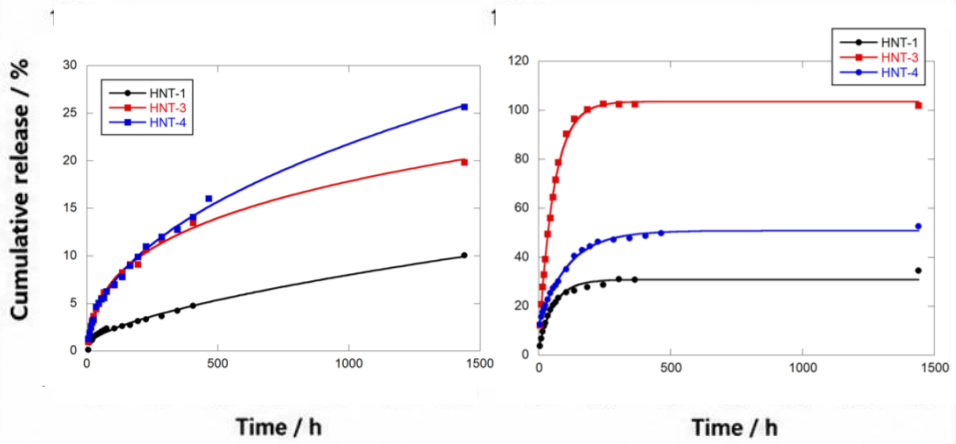


HNTs-4

MTX in the lumen + MTX covalently grafted outside

Kinetic release of MTX
(Fig. 4 from the paper)

(a) pH 3.0 (HCl $1 \cdot 10^{-3}$ M) (b) pH 7.4 (phosphate buffer 0.05 M)

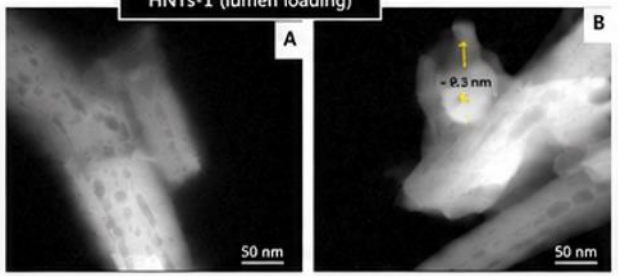


At pH 7.4: HNTs-1 ~30% << HNTs-3 ~80% < HNTs-4 ~100%

Covalent grafting on the external surface
strongly accelerates drug release at physiological pH.

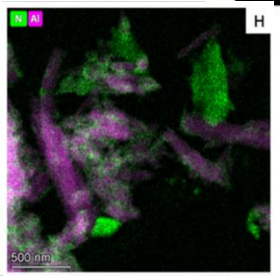
HAADF-STEM images

HNTs-1 (lumen loading)



Lumen shrinks from ~15 nm (pristine HNTs) to ~9.3 nm → MTX is loaded inside the lumen.

HNTs-3 (external grafting)



Tubular lumen remains visible; N-rich clusters (from MTX) are localized on the external surface → covalent MTX coating outside the tubes.

Morphology confirms the design

- HNTs-1 (supramolecular): MTX inside the lumen → reduced inner diameter
- HNTs-3 (covalent): MTX covalently attached on the external surface → C/N-rich clusters on the outside; lumen preserved
- HNTs-4 (combined): MTX in the lumen AND covalently on the surface → both locations contribute to the fastest release

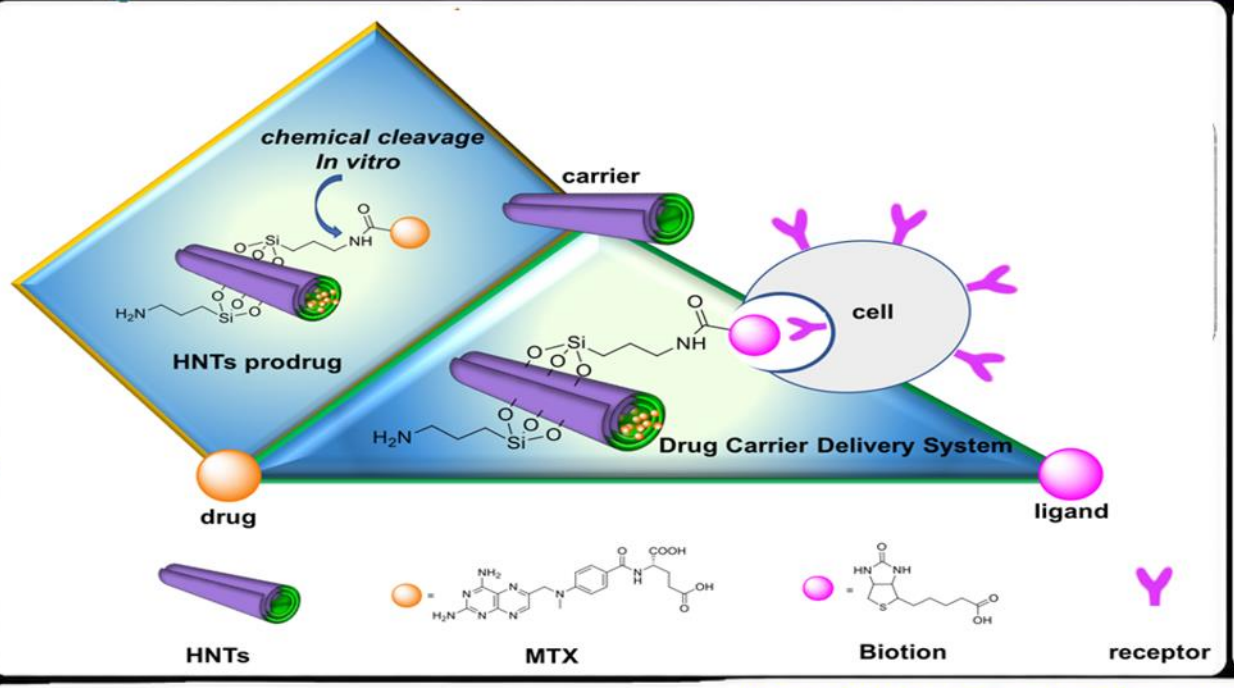
✓ TEM/HAADF-STEM provides direct evidence of spatially different localization of MTX.
✓ The location of the drug determines how fast it is released.

	First-order		
	M_∞ (wt%)	k (min^{-1})	R^2
HNTs-1	12.2 ± 1.4	$(9 \pm 2) \cdot 10^{-4}$	0.9762
HNTs-3	17.5 ± 0.8	$(2.8 \pm 0.2) \cdot 10^{-3}$	0.9829
HNTs-4	25.0 ± 1.0	$(1.7 \pm 0.2) \cdot 10^{-3}$	0.9875
HNTs-1	27 ± 1	$(1.72 \pm 0.02) \cdot 10^{-2}$	0.9789
HNTs-3	99 ± 1	$(1.78 \pm 0.05) \cdot 10^{-2}$	0.9984
HNTs-4	107 ± 4	$(3.60 \pm 0.02) \cdot 10^{-2}$	0.9907

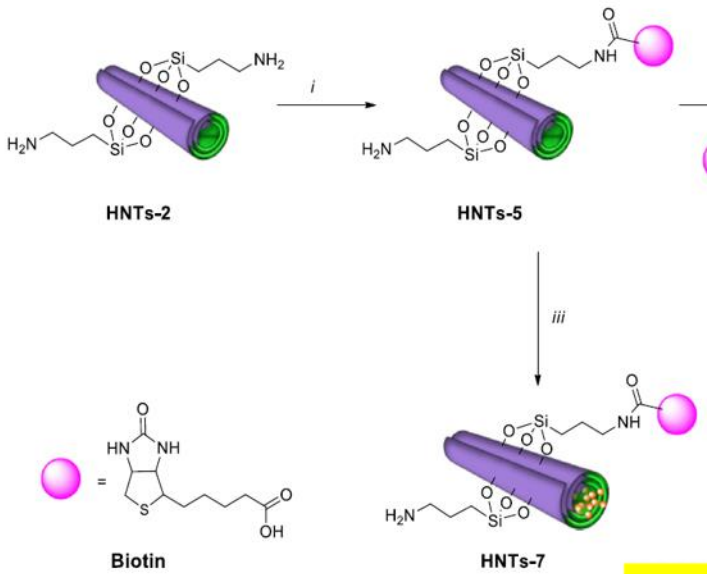
Where the drug is placed determines how it is released. → Supramolecular loading vs Covalent grafting vs Combined approach

Can we also control where the carrier goes?

Covalent surface targeting → selective cellular uptake



Synthesis of biotin-targeted HNTs-7 *(MTX in the lumen)*



⚠ **HNTs-6: when more surface chemistry becomes too much**

- × Biotin + MTX both covalently exposed
- × Larger dimensions → poorer cellular uptake

DLS: 712 ± 117 nm

ζ-potential: −25.3 ± 0.8 mV

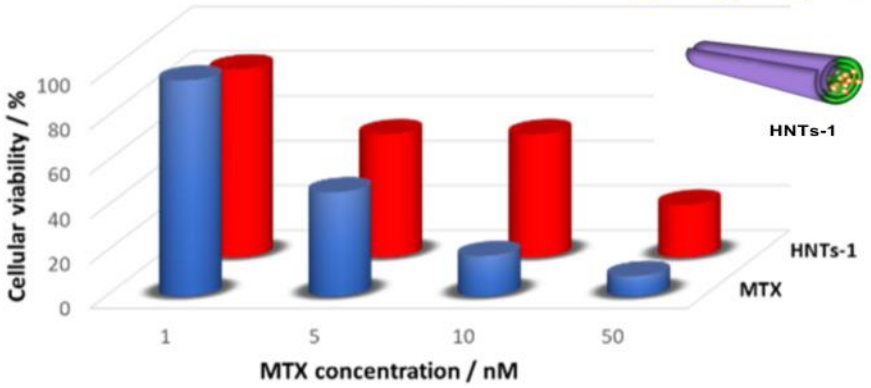
Targeting requires an accessible ligand

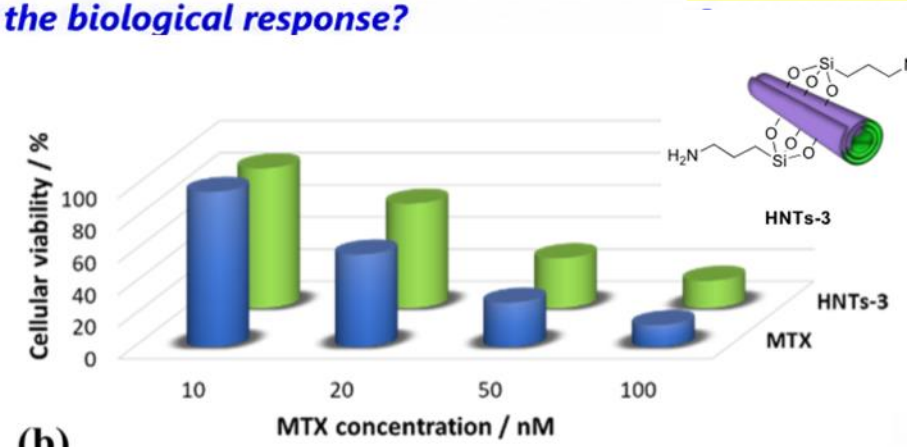
Does biotin targeting improve the biological response?

HL60 (sensitive)

HNTs-7
IC_{50} = 6.0 ± 0.1 *nM*

vs HNTs-1
≥3× lower





HL60-R (MDR)

HNTs-7
IC_{50} = 29.8 + 10.0 *nM*

vs HNTs-3
≈7× lower

From controlling **WHERE the drug is** → to controlling **WHERE the carrier goes**



Can we turn a non-emissive natural nanotube into a photoluminescent platform?

Surface-confined carbon-dot growth on halloysite

Journal of Materials Chemistry C

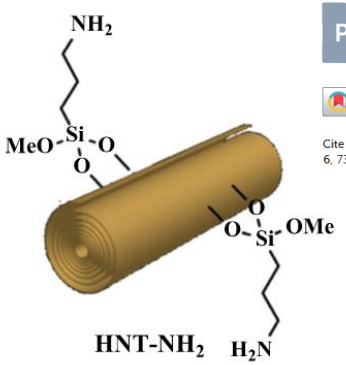
PAPER

Check for updates

Cite this: *J. Mater. Chem. C*, 2018, 6, 7377

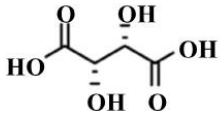
Photoluminescent hybrid nanomaterials from modified halloysite nanotubes†

M. Massaro,[a] C. G. Colletti,[a] S. Guernelli,[b] G. Lazzara,[c] M. Liu,[d] G. Nicotra,[e] R. Noto,[a] F. Parisi,[c] I. Pibiri,[a] C. Spinella[e] and Serena Riela*[a]

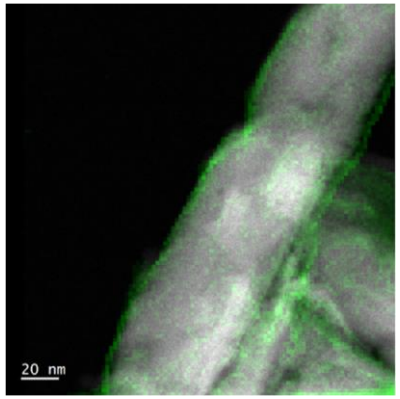


2.0 wt% loading

CH_2Cl_2, r.t.
4 d

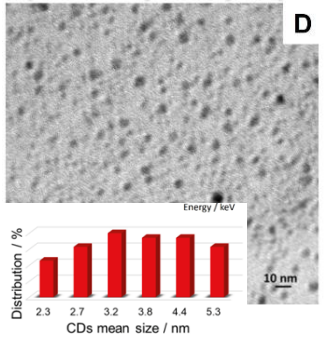


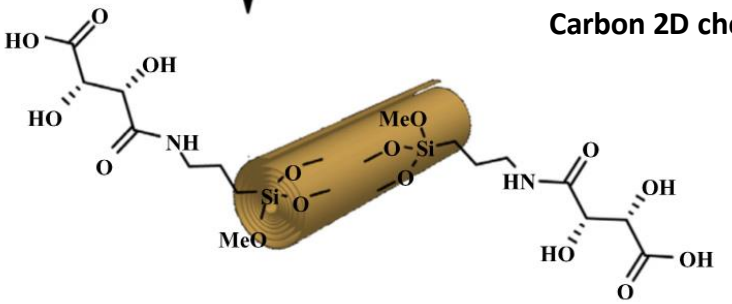
DCC



Carbon 2D chemical map

Released CDs (after Hydrolysis)

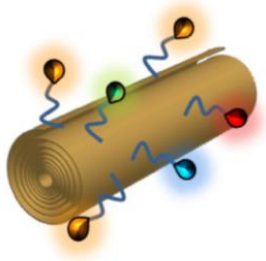




1

13.8 wt% loading

H_2N NH_2
MW
H_2O, 240 °C,
3 min



HNT-CDs

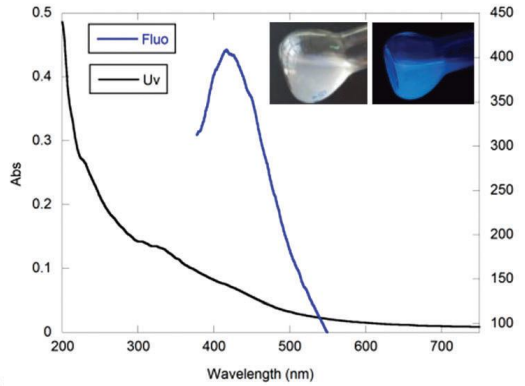
3.1 wt% loading

J. Mater. Chem. C 2018, 6, 7377.

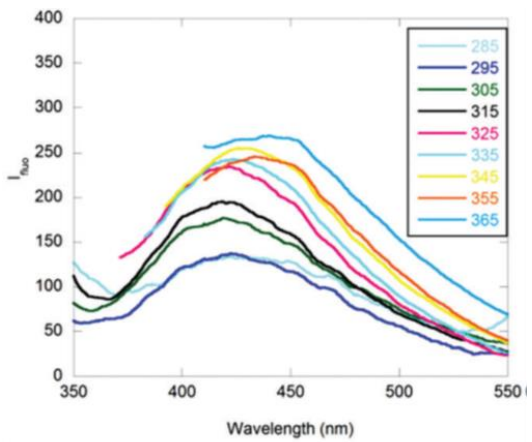
A new optical property emerges!

Photoluminescence of HNT–CDs in water

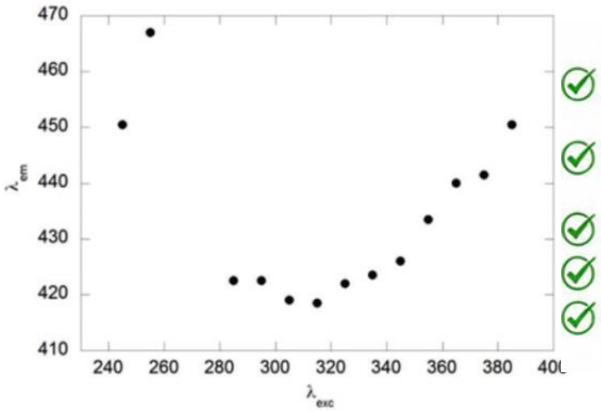
irradiated at 325 nm



Absorption and PL emission spectra of HNT-CDs ([CDs] = 0.04 mg mL



Excitation-dependent photoluminescence emission spectra



Excitation-dependent emission wavelength

Why is this approach advantageuus?

- Carbon dots are generated directly on the HNT surface (no pre-synthesis or post-coupling)
- Covalent linkers act as spacers → well-separated nucleation sites → minimal aggregation
- No purification needed: all CDs are retained on the nanotube
- Small, relatively uniform carbon dots (~4 nm)
- Halloysite is not only a carrier, but a template for nanodot formation

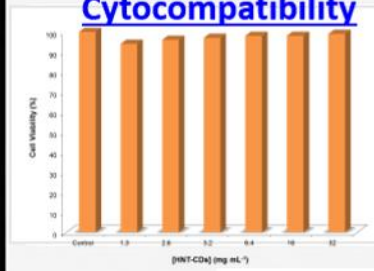
Can the same photoluminescent platform address different challenges?

From non-viral gene delivery to sensing and active food packaging

1 GENE DELIVERY & SELF-TRACKING

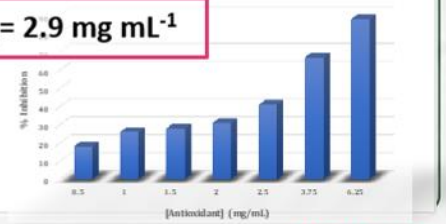
HNT–CDs as a trackable non-viral vector

Cytocompatibility



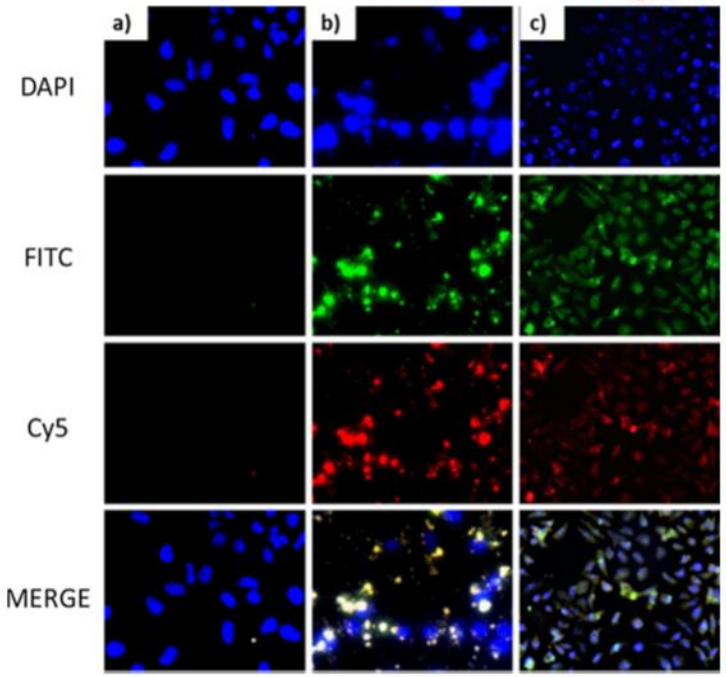
Antioxidant properties

IC_{50}= 2.9 mg mL^{-1}



DPPH test

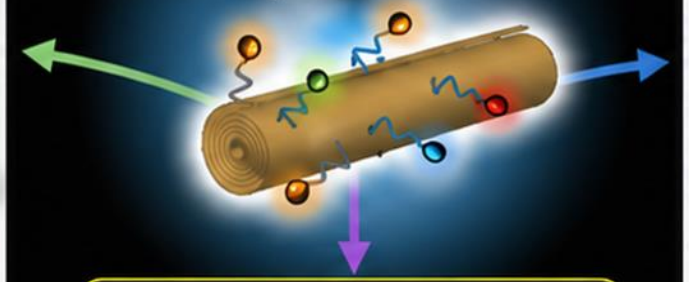
Fluorescence imaging in HeLa cells



J. Coll. Interf. Sci. 2019, *552*, 236.

HNT–CDs

photoluminescent platform



One covalent strategy

⬇

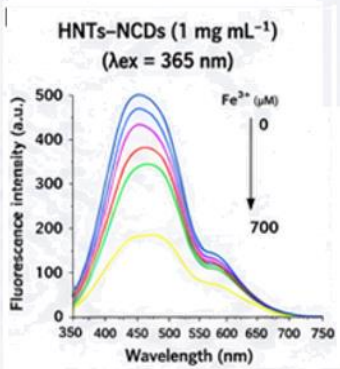
One photoluminescent platform

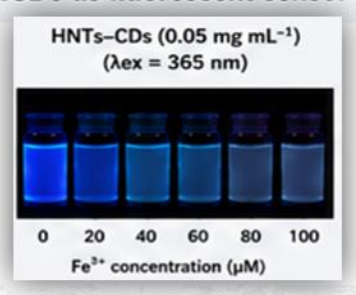
⬇

Multiple functions

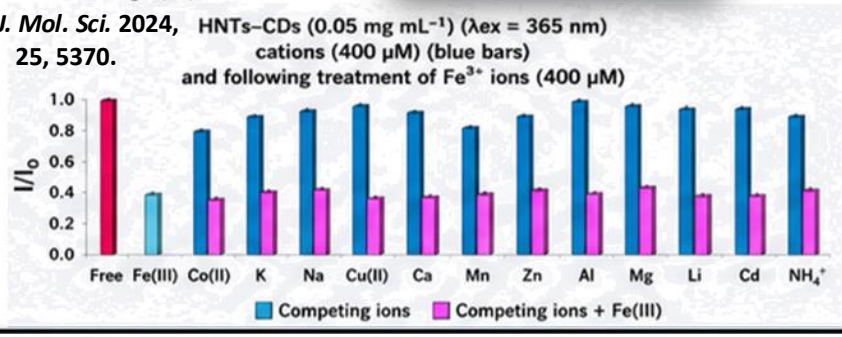
2 BIOLOGICAL SENSING

HNT–NCDs as fluorescent sensor for Fe^{3+}





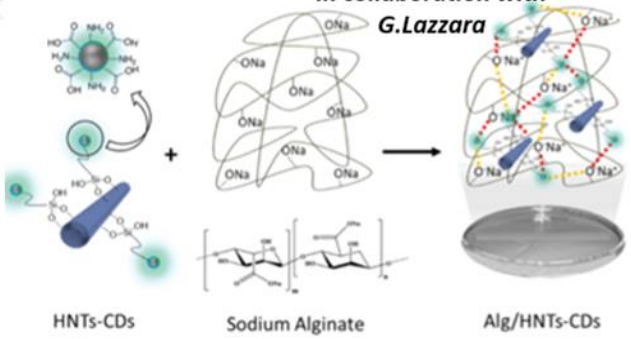
Int. J. Mol. Sci. 2024, 25, 5370.

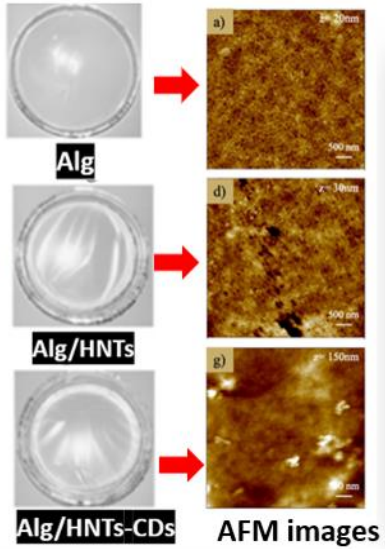


3 ACTIVE FOOD PACKAGING

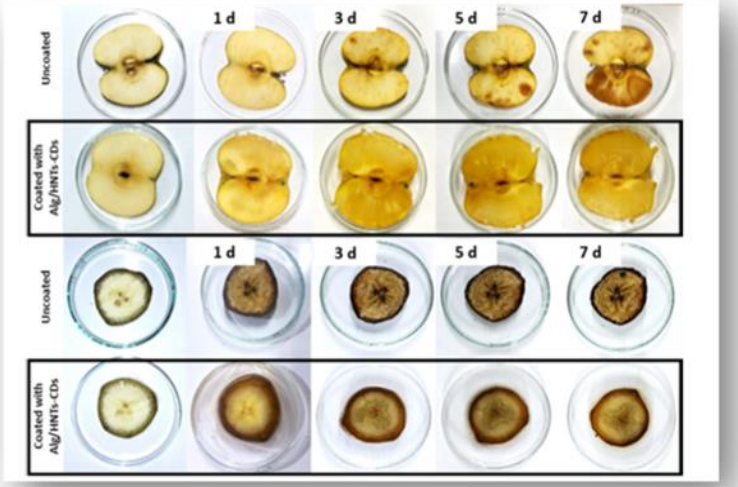
Alginate/HNTs–CDs films as active coatings

in collaboration with G.Lazzara





AFM images



Int. J. Biol. Macromol. 2024, 277, 1343.



Can we create multiple cavities on a single nanotubes?

From surface functionalization to multicavity drug.delivery systems

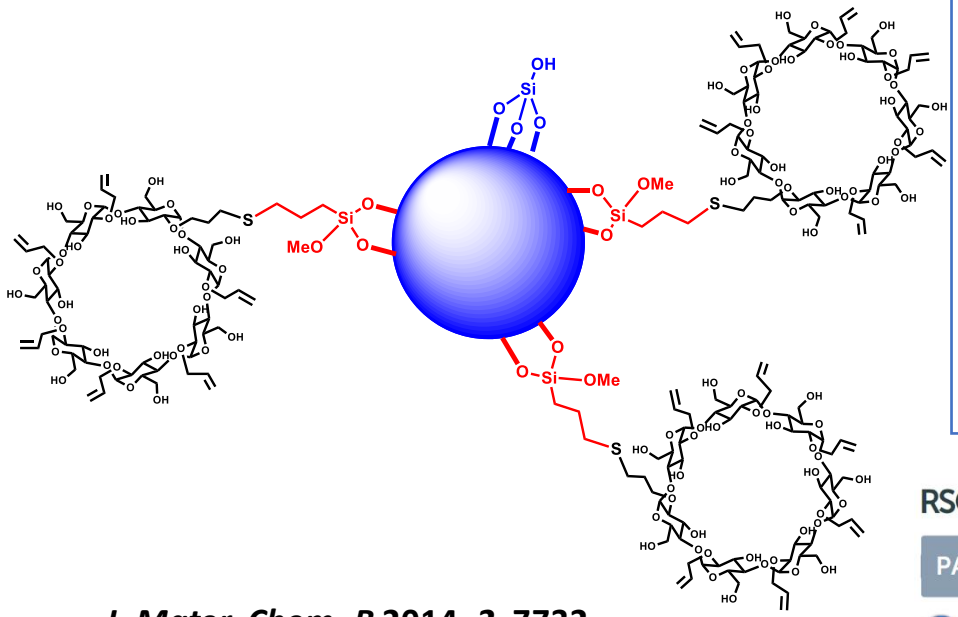
Journal of Materials Chemistry B

PAPER

Functionalized halloysite multivalent glycocluster as a new drug delivery system†

Cite this: J. Mater. Chem. B, 2014, 2, 7732

M. Massaro,[a] S. Riela,*[a] P. Lo Meo,[a] R. Noto,[a] G. Cavallaro,[b] S. Milioto[b] and G. Lazzara*[a]

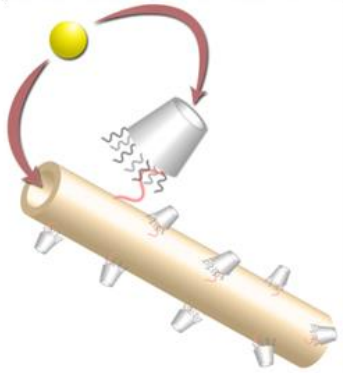


J. Mater. Chem. B **2014, *2*, 7732.**

Concept:

Introduce **multivalent recognition sites** on the HNT outer surface

Add a new **supramolecular function** without losing the **HNT lumen**



RSC Advances

PAPER

Dual drug-loaded halloysite hybrid-based glycocluster for sustained release of hydrophobic molecules†

Cite this: RSC Adv., 2016, 6, 87935

M. Massaro,[a] S. Riela,*[a] C. Baiamonte,[b] J. L. J. Blanco,[c] C. Giordano,[b] P. Lo Meo,[a] S. Milioto,[d] R. Noto,[a] F. Parisi,[d] G. Pizzolanti[b] and G. Lazzara*[d]

RSC Adv. 2016, 6, 87935.

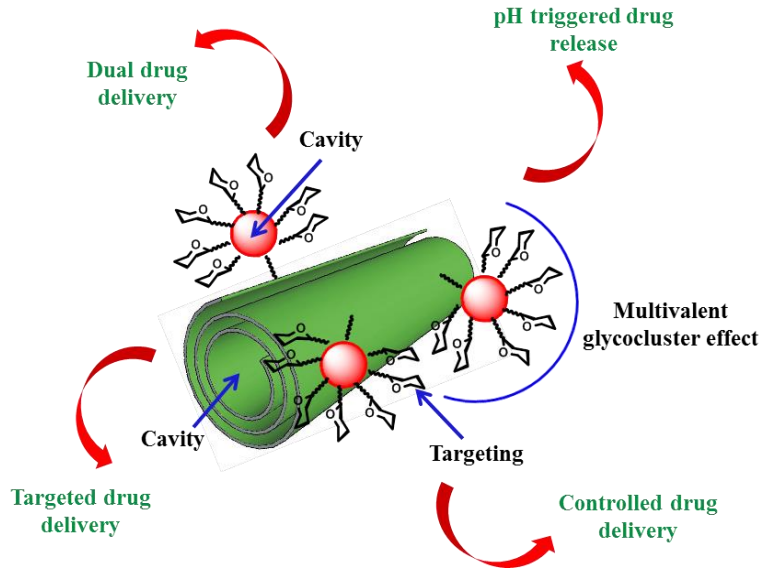
Journal of Materials Chemistry B

PAPER

Multicavity halloysite–amphiphilic cyclodextrin hybrids for co-delivery of natural drugs into thyroid cancer cells†

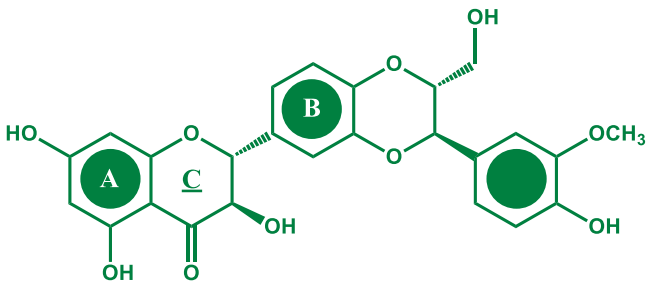
Cite this: J. Mater. Chem. B, 2015, 3, 4074

M. Massaro,[a] S. Piana,[a] C. G. Colletti,[a] R. Noto,[a] S. Riela,*[a] C. Baiamonte,[b] C. Giordano,[b] G. Pizzolanti,[b] G. Cavallaro,[c] S. Milioto[c] and G. Lazzara*[c]

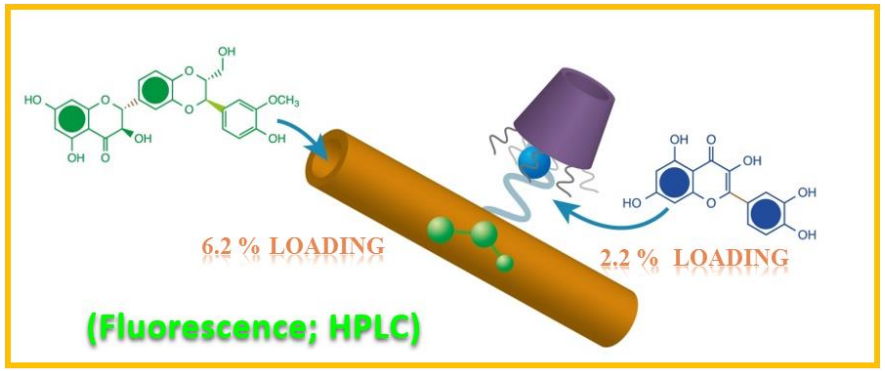


J. Mater. Chem. B **2015, *3*, 4074.**

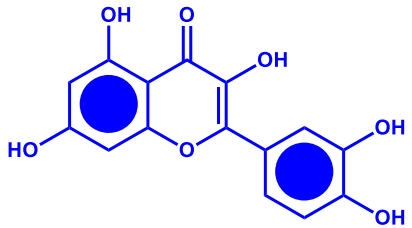
Can different cavities control different guests?



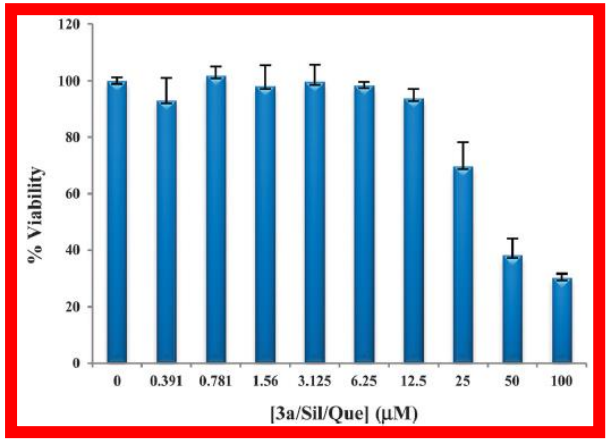
Silibinin



Affinity only with HNT cavities (Uv; HPLC)

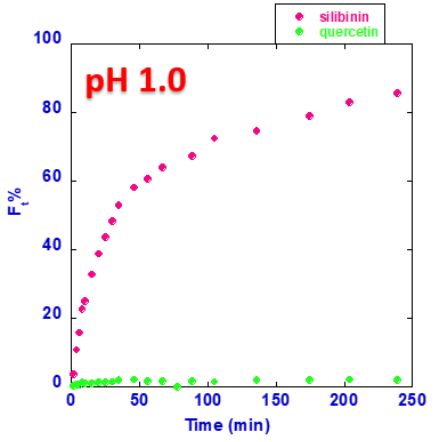


Quercetin



IC_{50}= 27.6±6.1 μM

MTS test for the cell viability of 8505C cells cultured for 72 h

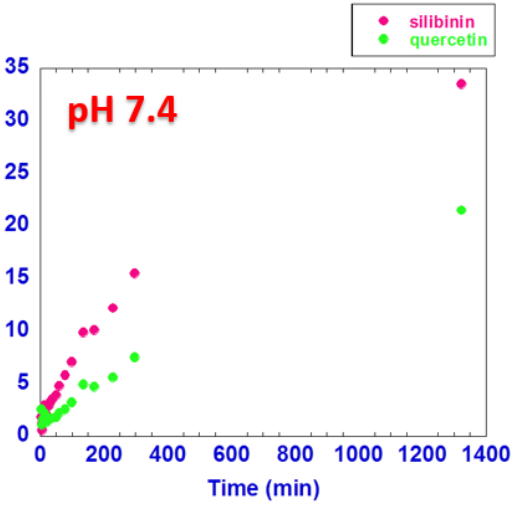


Quercetin

0.0007 ± 0.0001 min^{-1},

Silibinin

***k* = 0.00173 ± 0.00009 min^{-1}**



quercetin

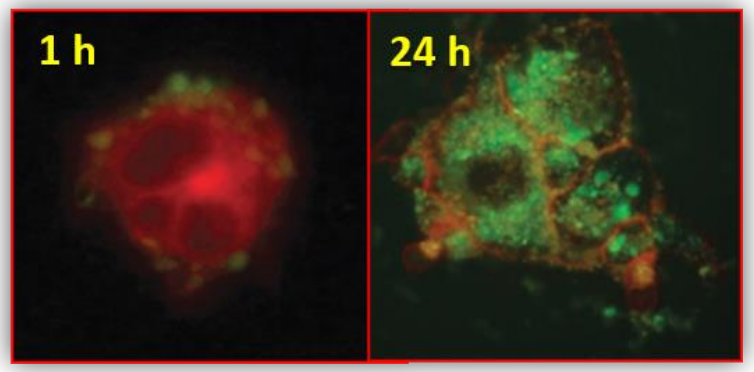
Synergic effect

silibinin

$CDs + Que \underset{}{\overset{K_1}{\rightleftharpoons}} CDs\text{-}Que$ (1:1) $\quad \beta = K_1 x K_2 = (6.3 \pm 0.3)*10^5\ M^{-2}$ (ITC)

$CDs\text{-}Que + CDs \underset{}{\overset{K_2}{\rightleftharpoons}} CDs\text{-}Que\text{-}CDs$ (2:1) $\quad \beta = K_1 x K_2 = (9 \pm 2)*10^5\ M^{-2}$ (UV-*vis*)

Affinity with both the cavities



Fluorescence Microscopy

J. Mater. Chem. B 2015, *3*, 4074.

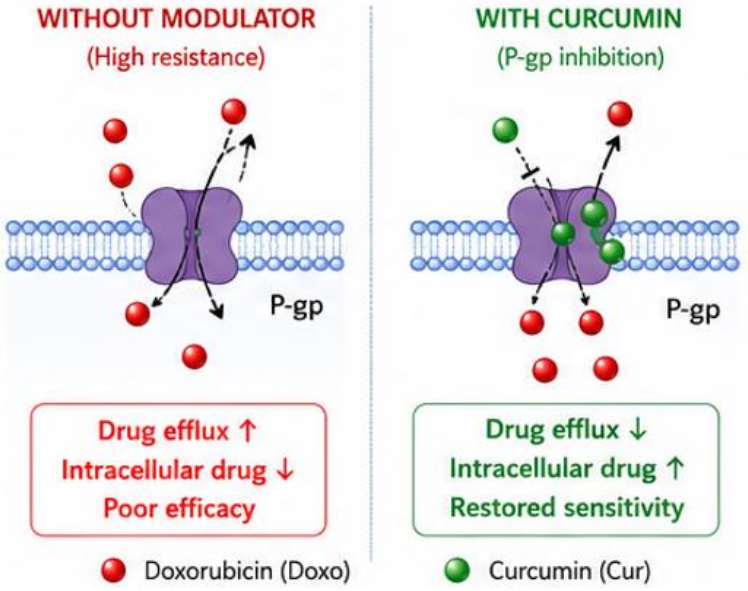


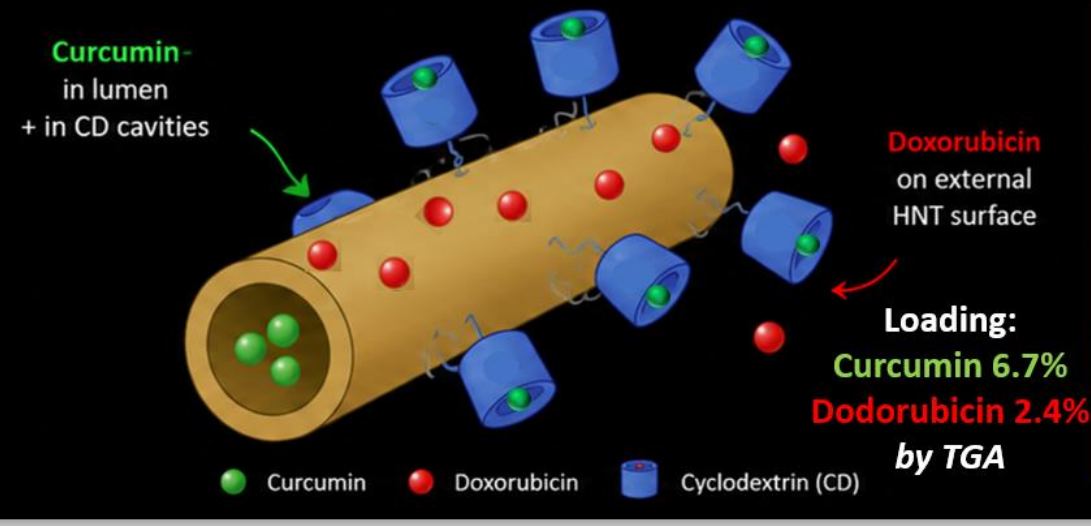
Can different cavities coordinate drugs with different roles?

Different spaces → different drugs → complementary functions

work in progress…

RATIONALE: OVERCOMING P-gp MEDIATED EFFLUX



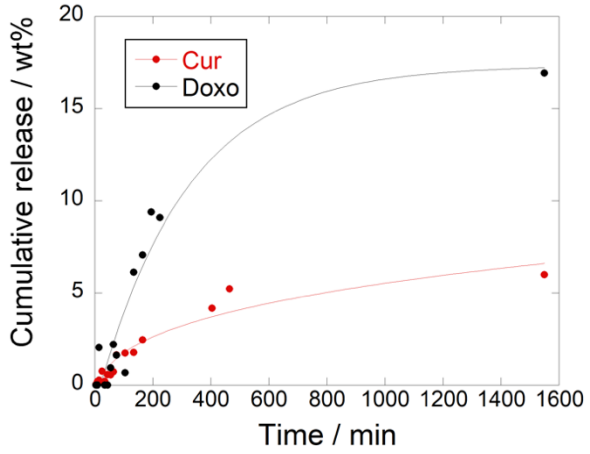


TWO DRUGS, TWO ROLES

Our design ensures:

- ✓ Curcumin retained within lumen/CD → sustained effect on P-gp inhibition
- ✓ Doxorubicin anchored on external surface → available for cellular uptake
- ✓ Co-delivery → synergistic action against MDR

SUSTAINED & DIFFERENTIAL RELEASE (pH 7.4)



Curcumin (lumen + CD cavities): slower release (~20% / 24 h)

Doxorubicin (external surface): faster release (~35% / 24 h)

KINETIC MODELS OF RELEASE

Drug	First order		Power Fit		
	k (min^{-1})	R^2	k (min^{-1})	n	R^2
Cur	0.0033 ± 0.0003	0.97	0.8 ± 0.6	0.31 ± 0.09	0.9353
Doxo	0.0010 ± 0.0002	0.9822	0.27 ± 0.05	**0.70 ± 0.03**	**0.9887**

Curcumin: best described by first order model → release consistent with diffusion/desorption from confined host environments.

Doxorubicin: best described by Power Fit model n = 0.70 (> 0.45) → non-Fickian diffusion consistent with release from external surface.

Different location → distinct release kinetics → complementary therapeutic roles

Does this molecular design traslate into biological efficacy?

From molecular design → 3D tumor model → in vivo response

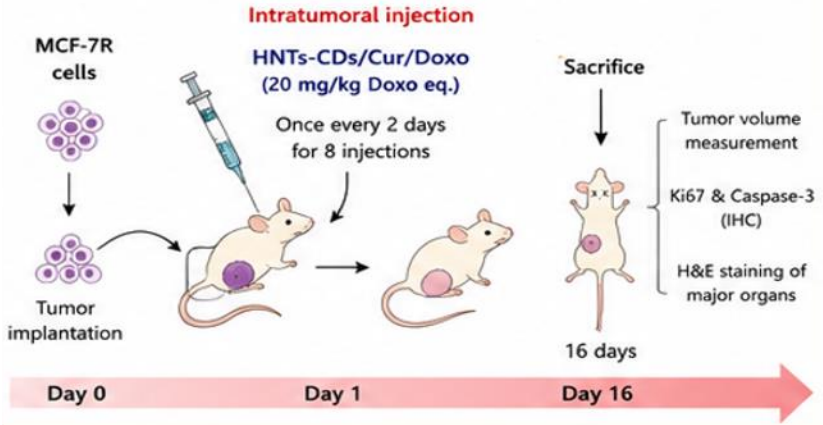
work in progress…

1 3D MCF-7R SPHEROID MODEL

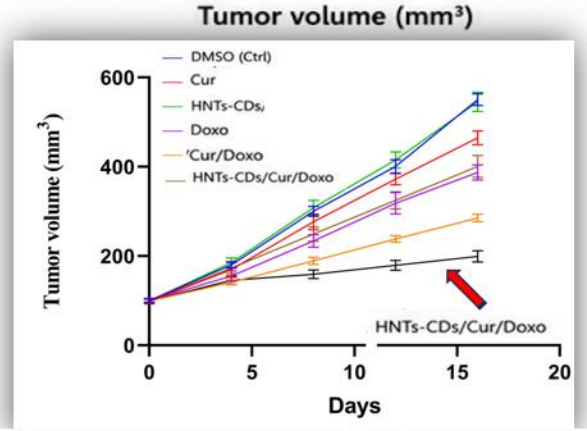
HNTs-CDs/Cur/Doxo in 3D resistant tumor model

- ✓ HNTs-CDs/Cur/Doxo is the **most active formulation** in the 3D MCF-7R spheroid model.
- ✓ The anti-proliferative activity is maintained in the 3D resistant tumor model.
- ✓ IC_{50} in 3D is **~10-fold higher** than in 2D MCF-7R cells.
- ✓ Confirms penetration and efficacy of the multicavity HNTs-CDs carrier in a more complex 3D environment.

2 IN VIVO STUDY DESIGN (MCF-7R XENOGRAFT)



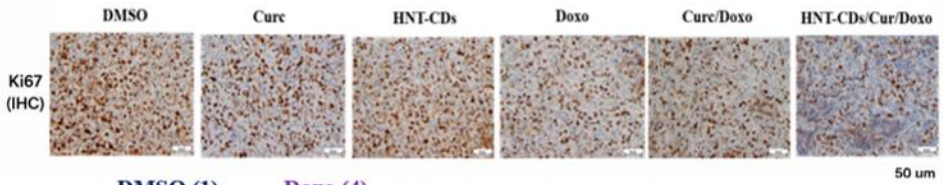
3 IN VIVO ANTITUMOR EFFICACY

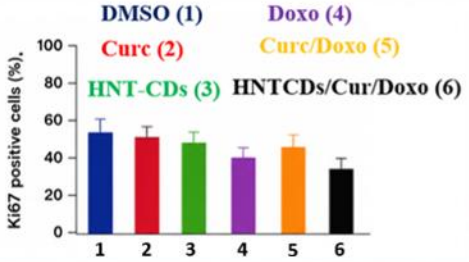


HNTs-CDs/Cur/Doxo significantly inhibits tumor growth vs DMSO and vs Empty carrier

4 Ki67 EXPRESSION (PROLIFERATION)

Ki67 is a proliferation marker: high in control tumors and reduced by treatments





Ki67 immunostaining and quantification of Ki67-positive cells demonstrate that HNTs-CDs/Cur/Doxo (Arg 748) significantly reduces tumor-cell proliferation.

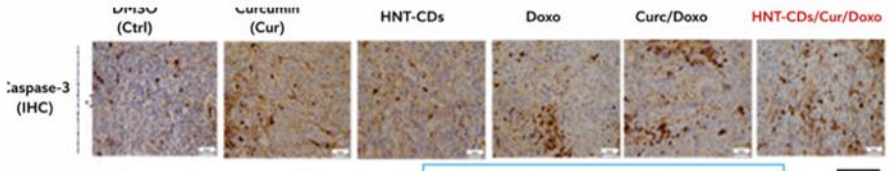
Compared to DMSO (control), Ki67 expression is markedly lower in the nanoformulation group.

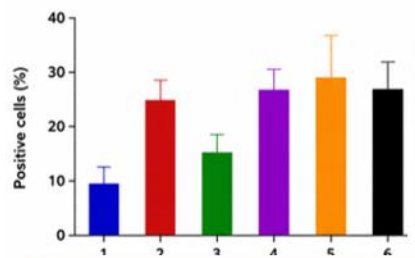
Importantly, HNTs-CDs/Cur/Doxo **outperforms both doxorubicin alone and the free combination of curcumin + doxorubicin**, indicating a superior anti-proliferative effect of the nanoformulated triple therapy.

✓ HNTs-CDs/Cur/Doxo significantly reduces Ki67 expression, indicating a marked inhibition of tumor-cell proliferation.

The nanoformulation outperforms the free drug combination in suppressing tumor-cell proliferation.

Caspase-3 EXPRESSION (APOPTOSIS)





Caspase-3 is an executioner protein in apoptosis.

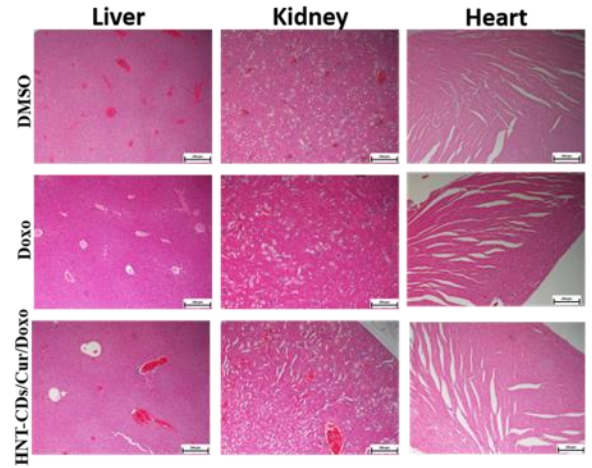
Immunostaining and quantification show that HNTs-CDs/Cur/Doxo significantly increases Caspase-3 expression compared to the control (DMSO).

This indicates that the nanoformulated combination

HNTs-CDs/Cur/Doxo significantly increases apoptosis (Caspase-3) vs DMSO (Ctrl)

Statistical analysis: One-way ANOVA with Tukey's multiple comparisons test.

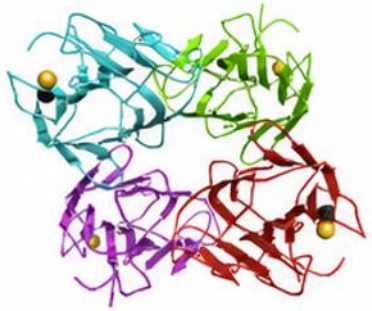
5 SAFETY: MAJOR ORGANS (H&E STAINING)



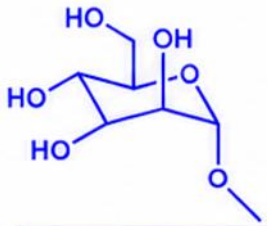
No evident histological damage, necrosis or inflammatory infiltrates in major organs

Can we make our multicavity system recognize a specific target?

TARGET: Concanavalin A (ConA)

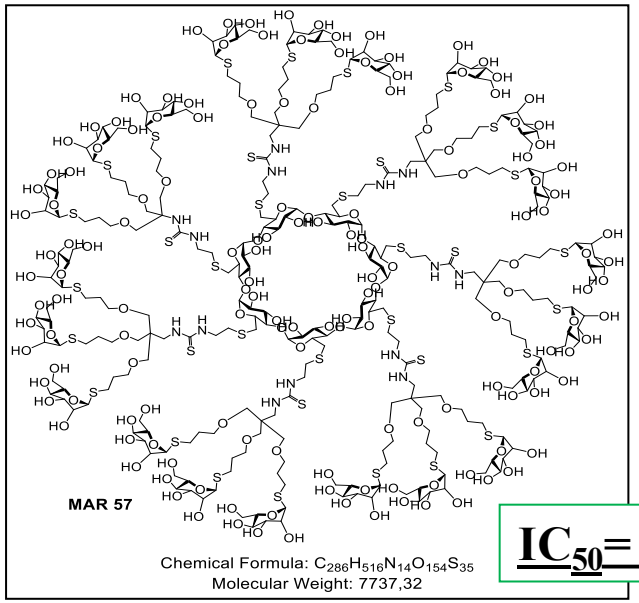


Me-α-mannopyranoside

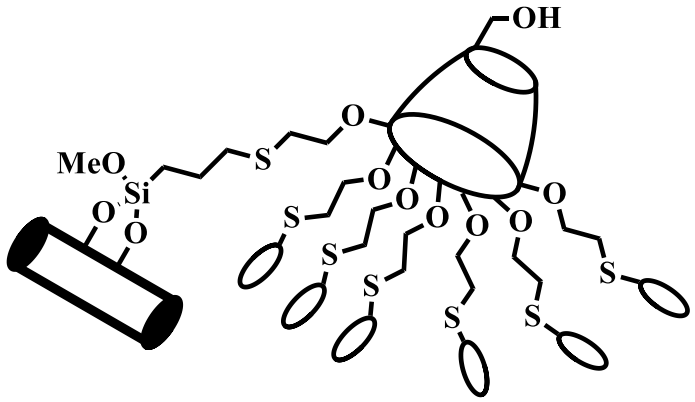


IC_{50} = 1300 µM

ELLA Tests



IC_{50}= 100 µM

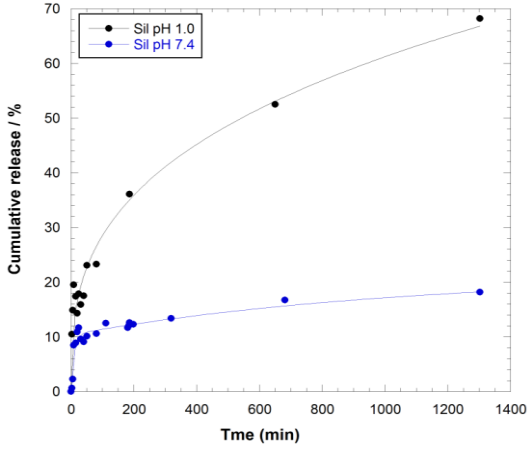


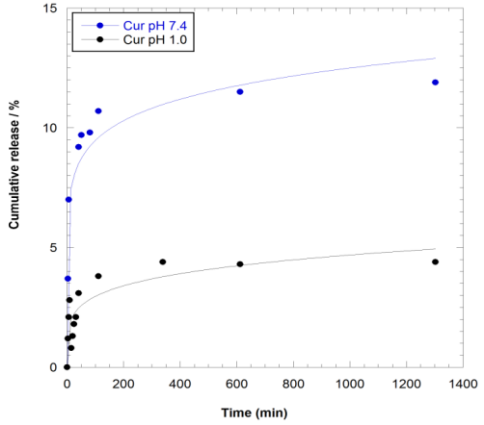
HNT-CD glycocluster hybrids

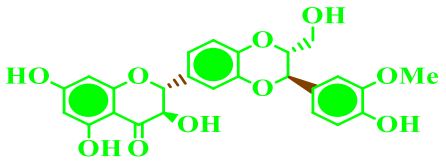
IC_{50}= 1.9 µM

a: α-mannose
b: β-galactose
c: β-lactose

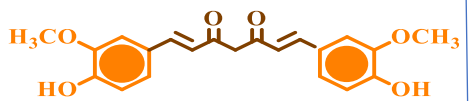
Kinetic Release







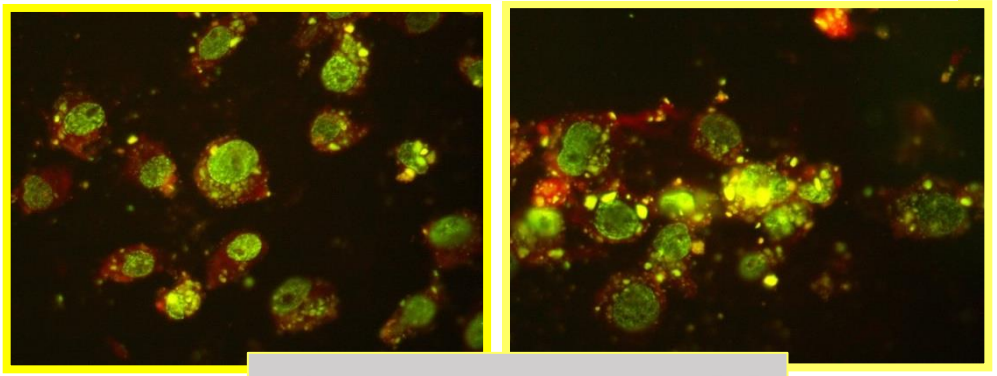
Sil



Cur

RSC Adv. 2016, 6, 87935.

TARGETED DELIVERY: sugars on CD-HNTs



IC_{50}= 11.3±3.1 µM

Can we increase the number of recognition sites on a new nanotube architecture?

ACS Sustainable Chemistry & Engineering

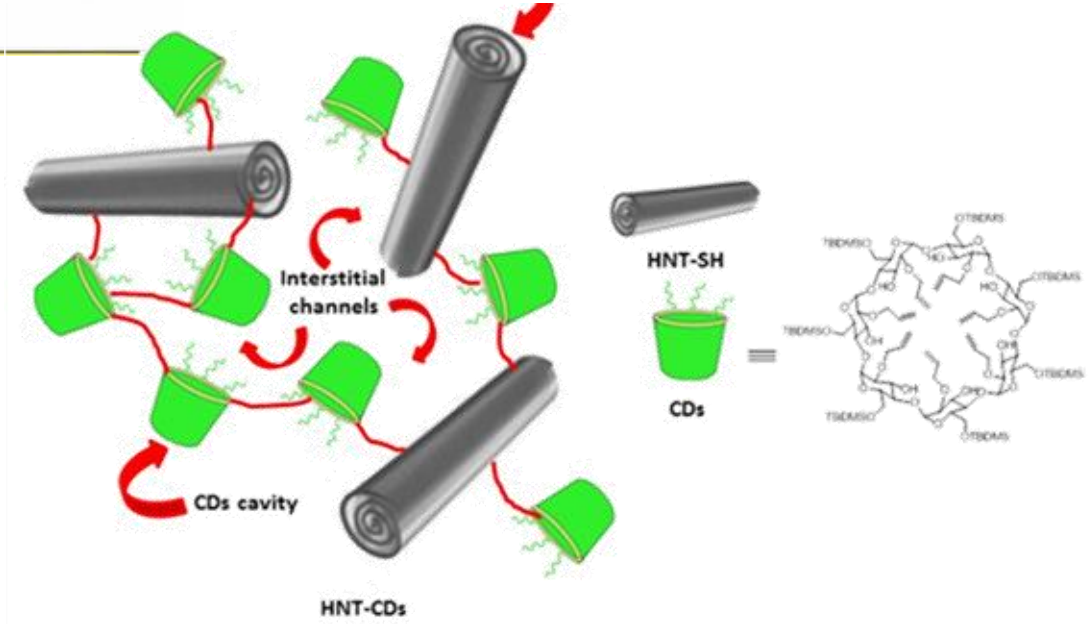
Research Article

pubs.acs.org/journal/ascecg

Synthesis and Characterization of Halloysite–Cyclodextrin Nanosponges for Enhanced Dyes Adsorption

Marina Massaro,[†] Carmelo G. Colletti,[†] Giuseppe Lazzara,[*,‡] Susanna Guernelli,[§] Renato Noto,[†] and Serena Riela[*,†]

ACS Sustain. Chem. Eng. 2017, 5, 3346–3352



Contents lists available at ScienceDirect

Applied Clay Science

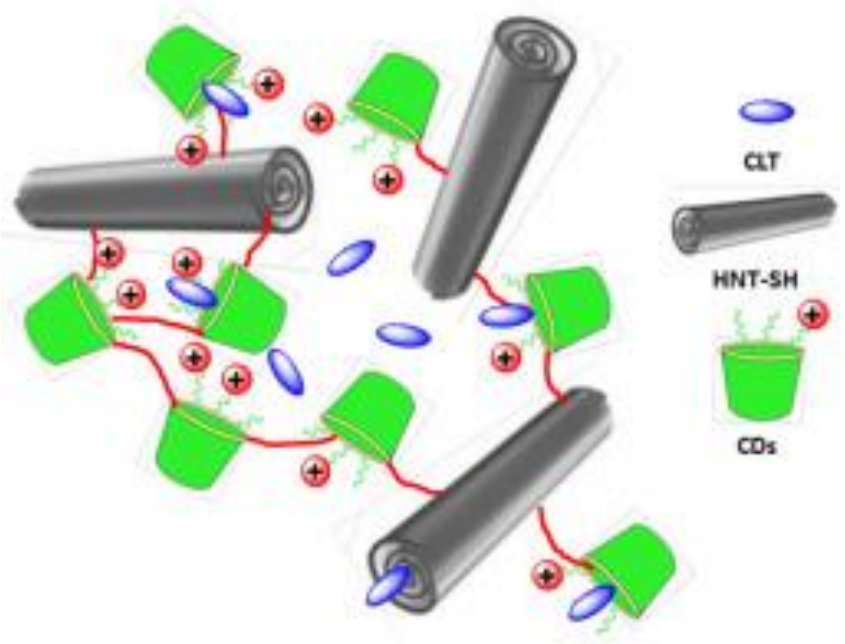
journal homepage: www.elsevier.com/locate/clay

Research paper

Functionalized halloysite nanotubes: Efficient carrier systems for antifungine drugs

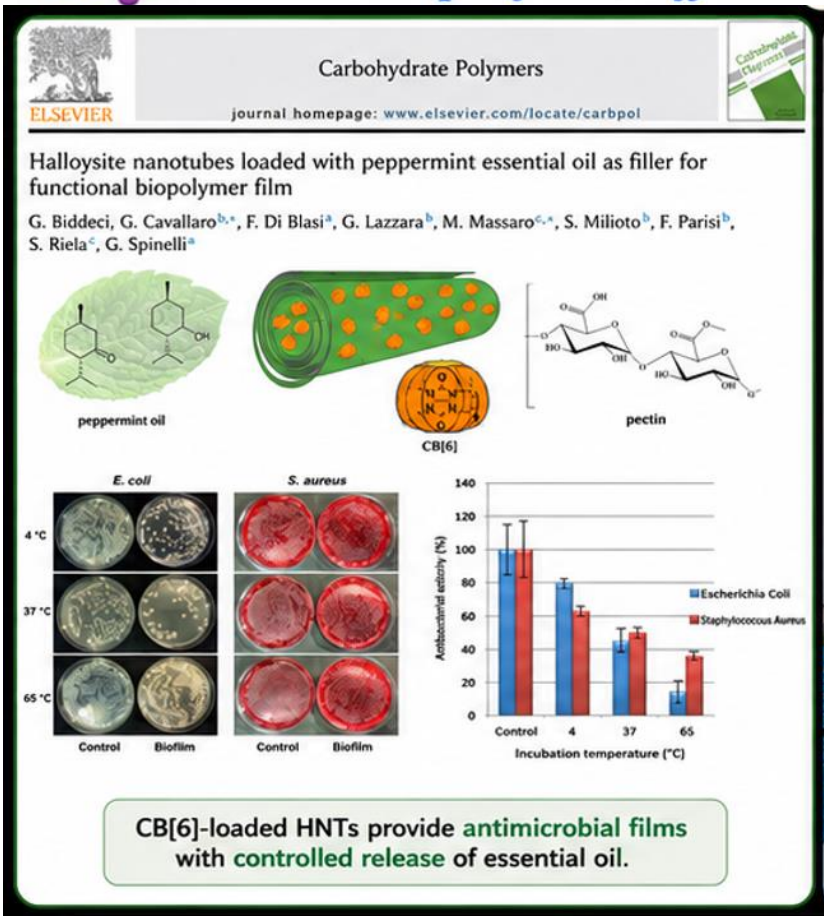
Marina Massaro[a], Ambra Campofelice[a,b], Carmelo Giuseppe Colletti[a], Giuseppe Lazzara[b], Renato Noto[a], Serena Riela[a,*]

Appl. Clay Sci. 2018, 160, 186–192

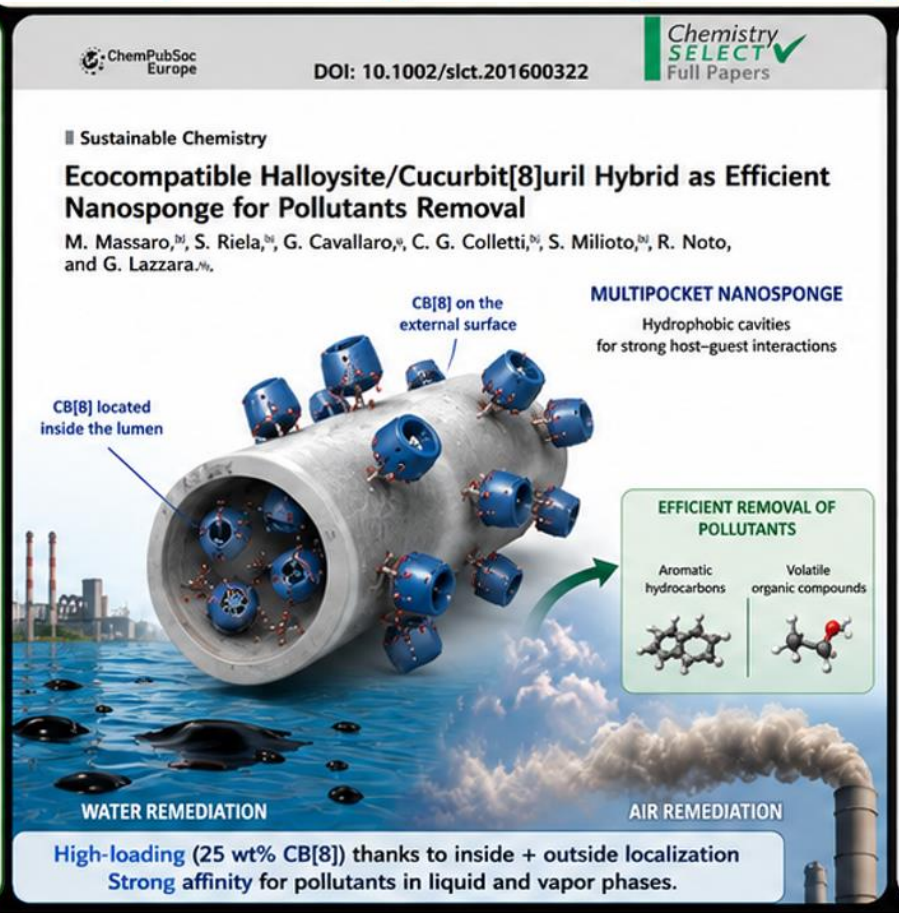


Can we expand the multicavity concept beyond cyclodextrins?

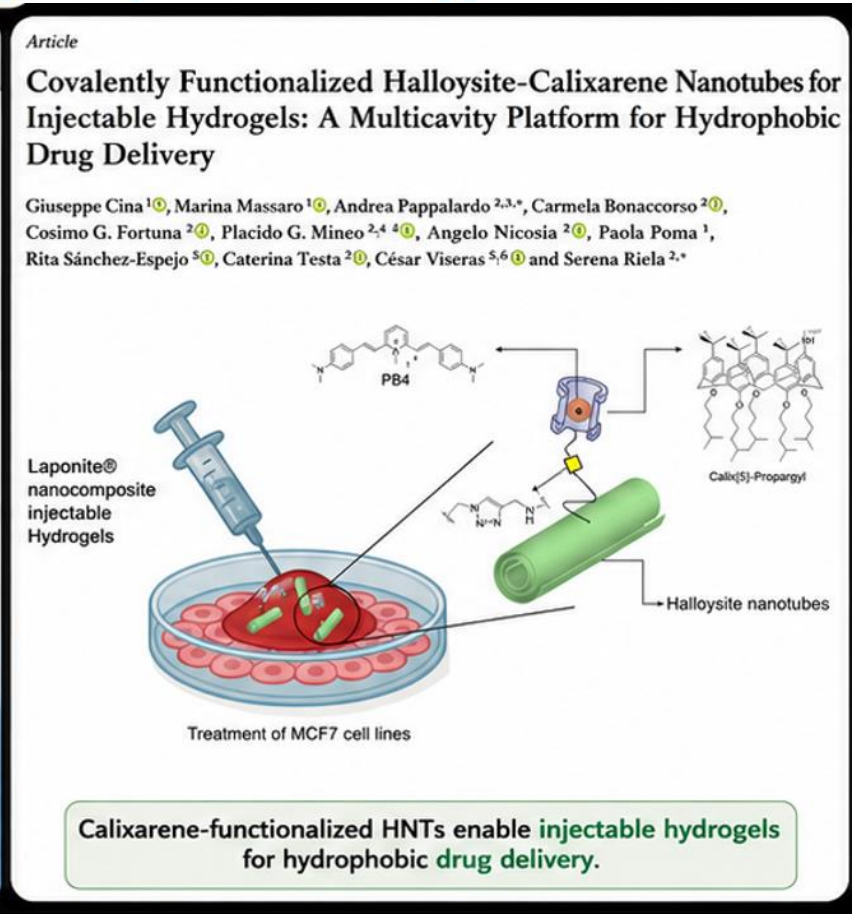
Same platform, different macrocycles → different recognition → different functions and applications



Carboh. Polym. 2016, *152*, 548.



Chemistry Select 2016, *1*, 1773.



Pharm. 2025, *18*, 1356.

Different macrocycles → different recognition → different loading → different release → different functions & applications

Change the host → change the function

Our journey with Halloysite Nanotubes

1826 — Bertier first described HNT



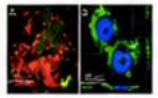
2001 — HNT used for the first time as nanocontainer for drugs



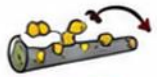
2008 — First example of covalent modification of HNTs



2010 — HNT was found to be non toxic and able to penetrate cell membrane



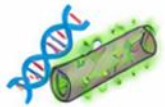
2012 — HNTs as support for metal nanoparticles as catalyst of organic reactions



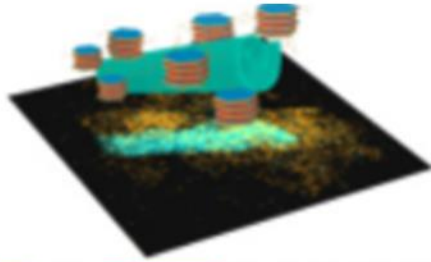
2015 — Evaluation of HNTs maximum concentration for oral use



2018 — Fluorescent HNT as potential carrier for gene therapy



2019 — HNTs covalently linked to other



What's next?

For ca. two centuries HNTs were mainly used for ceramics



HNTs as filler for polymeric matrices



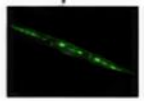
HNTs for pollutant removal

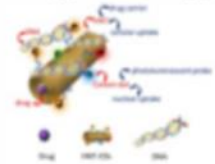


Covalent modification of the lumen



First *in vivo* study on Caenorhabditis







WHAT SHOULD COME NEXT?

More precise chemistry?

Site-selective and controlled functionalization

More complex functions?

Multifunctional, responsive and smart systems

Smarter biological interfaces?

Targeting, cellular uptake and biological outcomes

Translation to real applications?

From design to therapies, devices and beyond

The next challenge is not simply to add more functions, but to control how they work together.



IASOC 2026

AMOS Clay Lab

Advanced Materials and Organic Synthesis Clay Laboratory

Conclusions

WHAT HAVE WE LEARNED?



1 Covalent chemistry gives CONTROL



We can decide **where**, **how much** and **how stably** a function is introduced.

2 Supramolecular chemistry adds DYNAMISM



Loading, recognition and release can be **programmed** through non-covalent interactions.

3 The real power comes from COOPERATION



Covalent and supramolecular interactions can **work together** within the same nanotube.

4 FUNCTION emerges from molecular design



The same natural nanotube can become a **carrier**, **catalyst**, **sensor**, **responsive system** or **biological platform**.

Take a message

Halloysite is not just a support.
It is a **molecularly addressable** platform.





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