

LABORATORIO de DENDRIMEROS BIOMIMETICOS y FOTONICA

<http://www.ldbf.uma.es/>

Departamento de Química Orgánica
Facultad de Ciencias
Universidad de Málaga



UNIVERSIDAD
DE MÁLAGA



ibima

Instituto de Investigación
Biomédica de Málaga

*Grupo Consolidado de Investigación
de la Junta de Andalucía* **FQM017**

COMPOSICIÓN

Dr Ezequiel PEREZ-INESTROSA
CATEDRÁTICO DE UNIVERSIDAD
Investigador Principal

Miembros

Permanentes

Dr. Francisco Nájera
Profesor Titular de Universidad

Dr. Daniel Collado
Profesor Contratado Doctor

Dra. Yolanda Vida
Profesor Contratado Doctor

Contratados

Dr. Jose María Montenegro
Técnico Superior de Apoyo a la
Investigación

Dr. Nekane Barbero
Investigadora Post-Doctoral

Doctorandos

Noemí Molina Cabeza

Pablo Mesa

Anjara Morgado

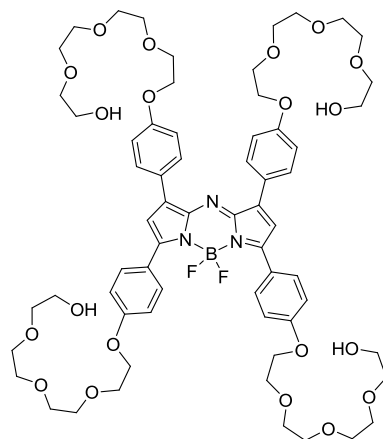
Estudiantes

Carlos Benitez

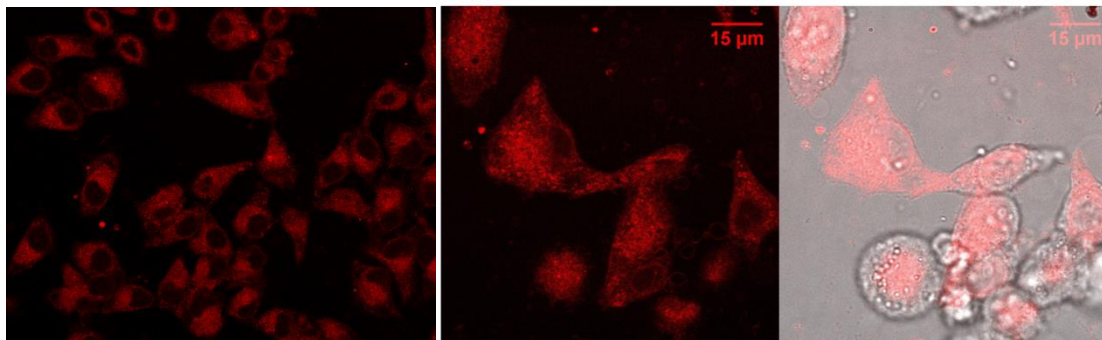
Líneas de Investigación

- **Síntesis de Estructuras Dendrimericas (Dendrimeros y Dendrones)**
 - Estructuras Dendrimericas que incorporan marcadores luminiscentes
 - Estructuras Dendrimericas para anclar a superficies.
 - Estudios Teóricos
- **Síntesis de marcadores fluorescentes**
 - Síntesis de marcadores con múltiples puntos de anclaje
 - Síntesis de marcadores con propiedades desplazadas al *Rojo*
 - Síntesis de marcadores para Two-Photon Absorption
 - Síntesis de marcadores para superresolución
- **Preparación de Partículas Sólidas adecuadas para el soporte de las estructuras Dendrimericas**
 - Partículas de Sílica
 - Partículas Metálicas
 - Zeolitas
 - Superficies de Oro
- **Fijación y Caracterización de estructuras Dendrimericas sobre superficies**
- ***Molecular Sensors and Molecular Logic Gates* para el procesamiento de información biomédica**
- **Estudio, caracterización y preparación de nuevos Haptenos derivados de fármacos**

✓ Aza - BODIPY



UV: $\lambda_{\max} = 704 \text{ nm}$
($\epsilon = 32300 \text{ M}^{-1}\text{cm}^{-1}$)
FLUO: $\lambda_{\max} = 727 \text{ nm}$ ($\Phi = 0.7$)



✓ BODIPY – Naphthalimide

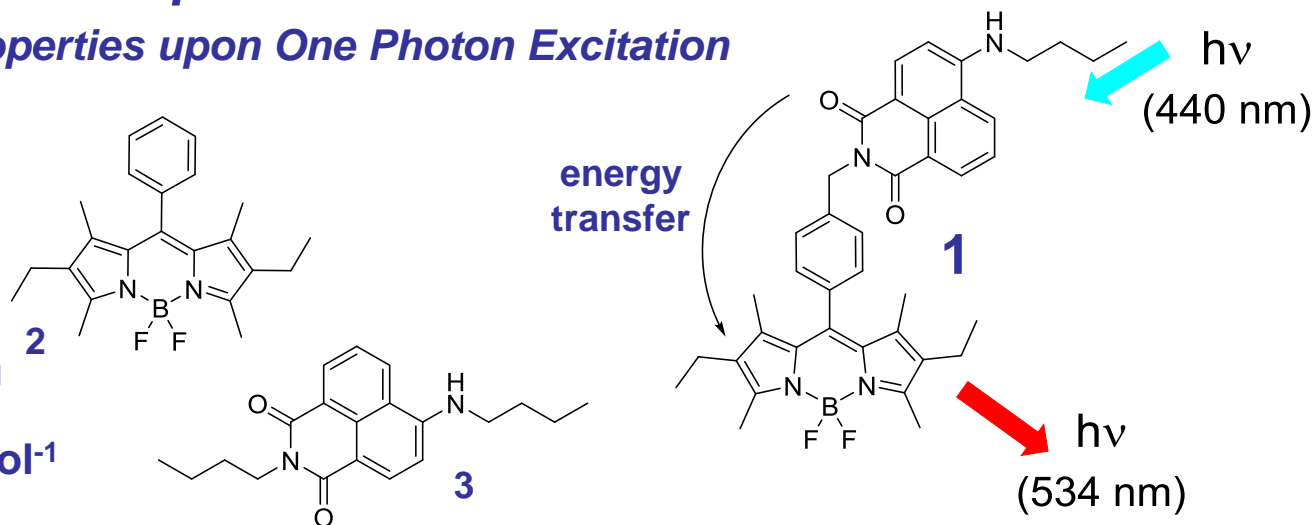
- Spectroscopic properties upon One Photon Excitation

$$\Phi_{\text{fluo}} = 0.7$$

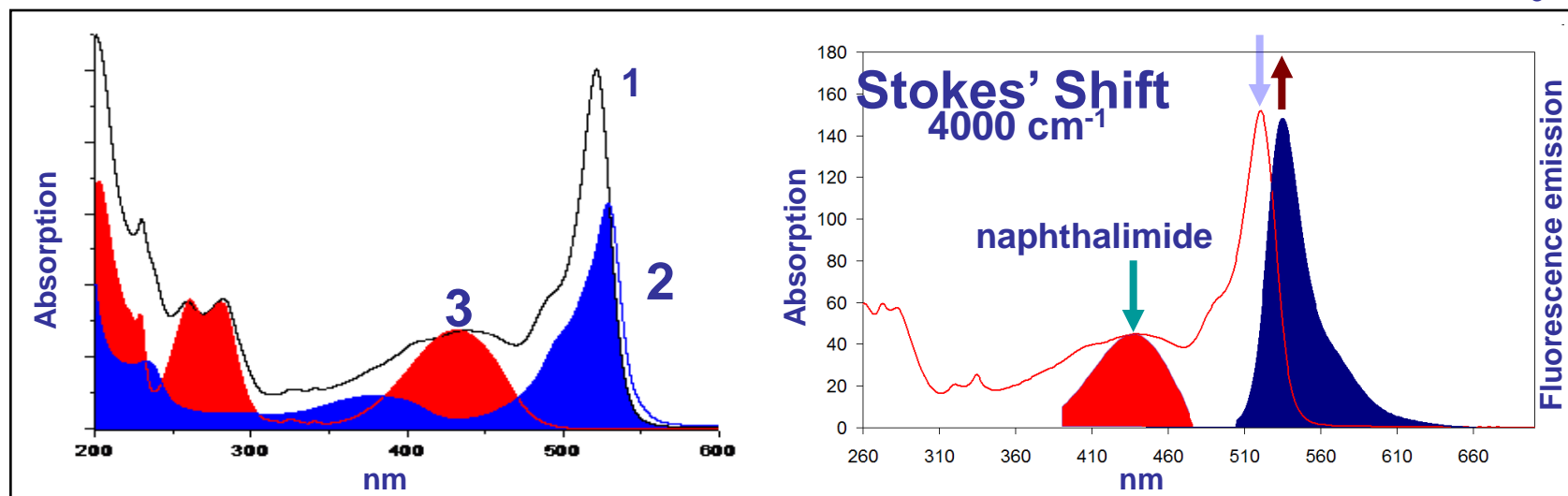
$$k_{\text{EET}} = 1.0 \times 10^{11} \text{ s}^{-1}$$

$$J = 1.6 \times 10^{-10} \text{ cm}^6 \text{ mol}^{-1}$$

$$\Phi_{\text{EET}} = 1.0$$

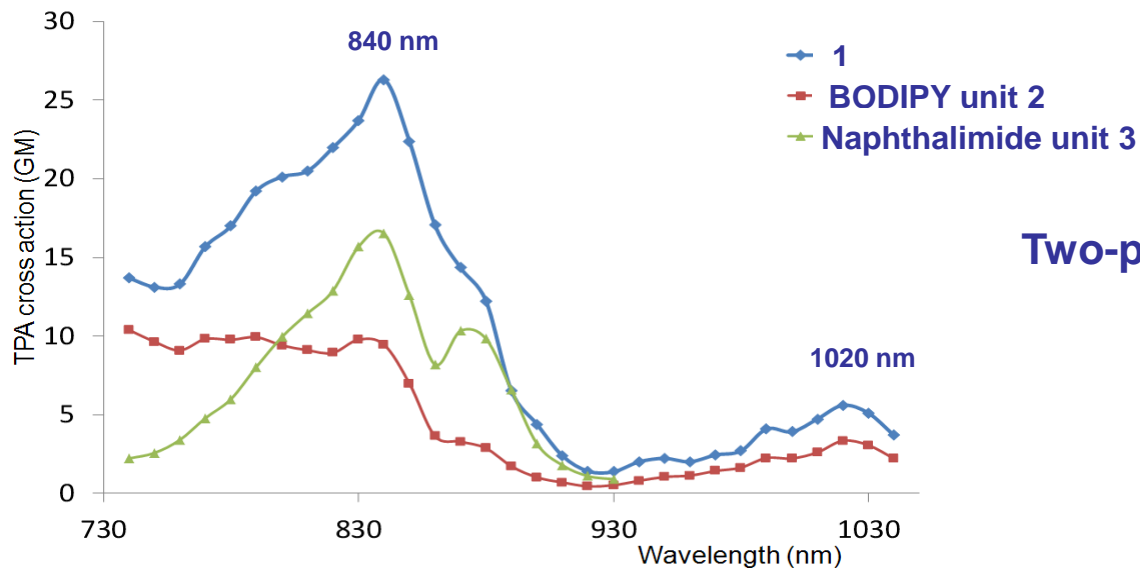
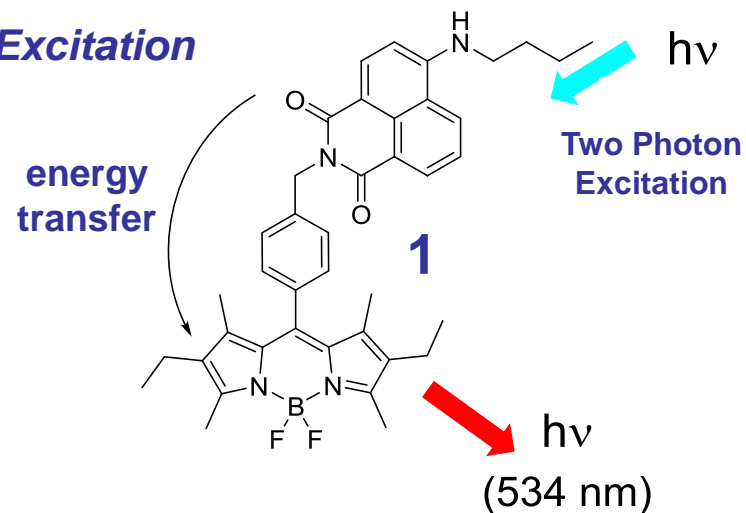
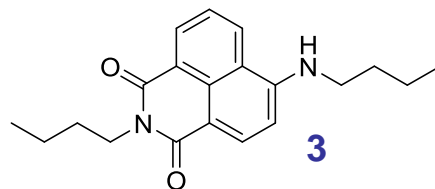
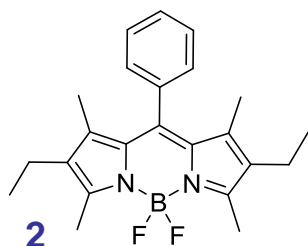


UV - Fluo spectra in CH_3CN



✓ BODIPY – Naphthalimide

- Spectroscopic properties upon Two Photon Excitation



Two-photon absorption spectra
TPA cross section

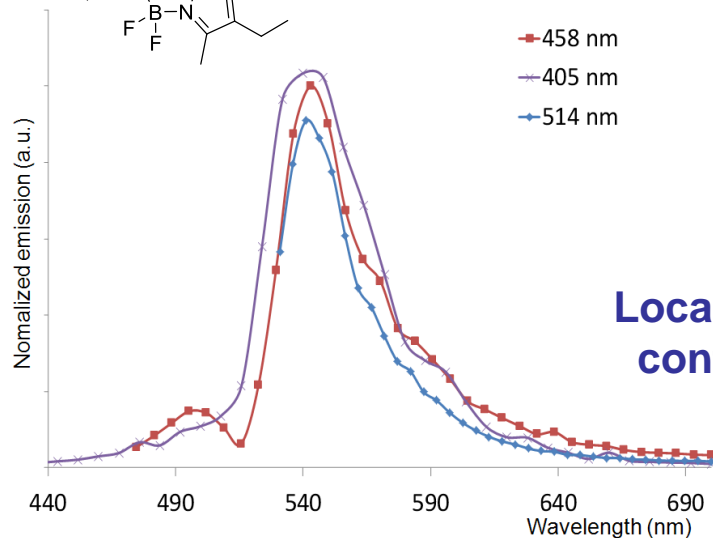
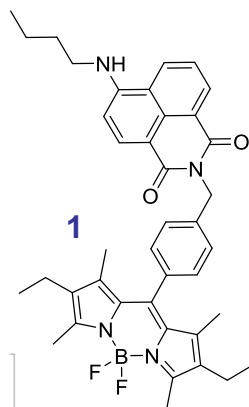
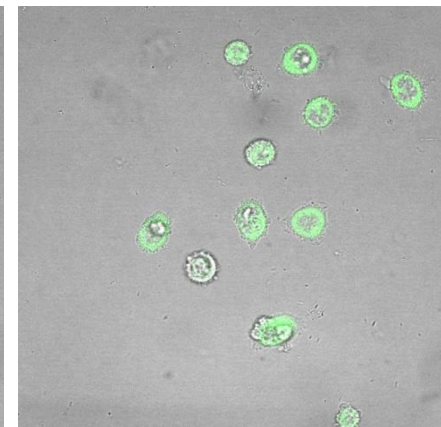
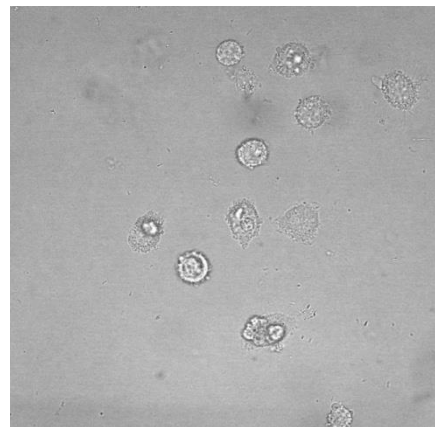
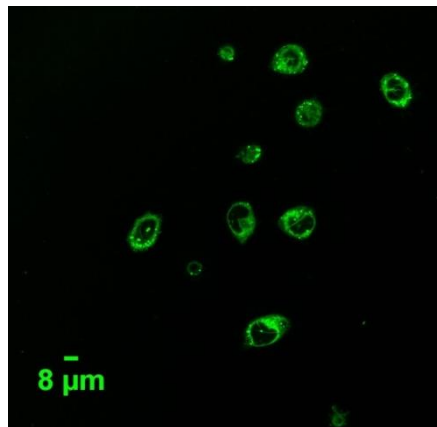
✓ **BODIPY – Naphthalimide**

- Cell Imaging

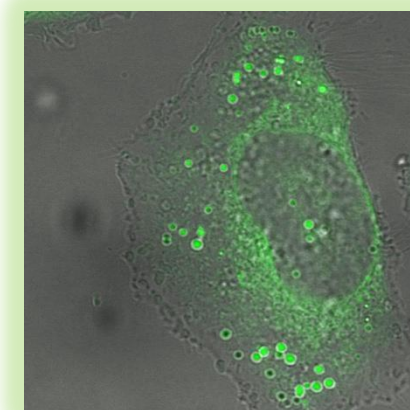
Confocal microscopy images of HeLa cells incubated with 1

One Photon Excitation conditions

$\lambda_{exc} = 514 \text{ nm}$



Localized spectra of the confocal microscopic images



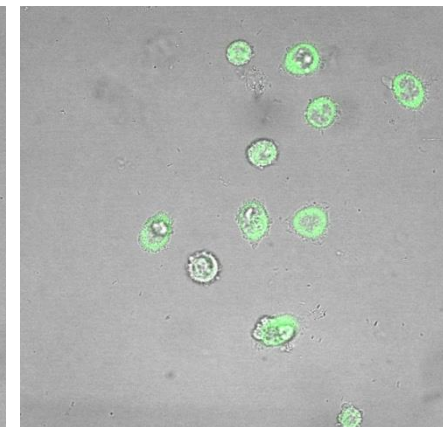
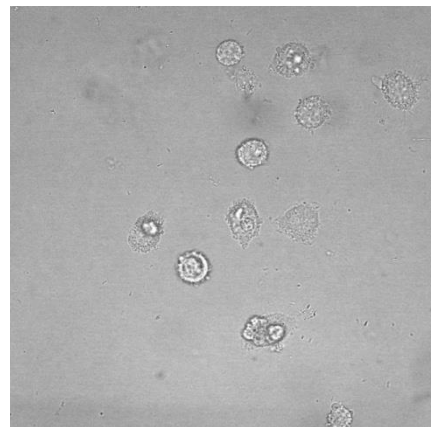
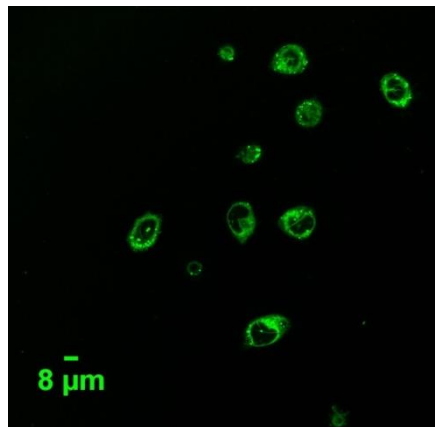
✓ BODIPY – Naphthalimide

- Cell Imaging

Confocal microscopy images of HeLa cells incubated with 1

$\lambda_{exc} = 514 \text{ nm}$

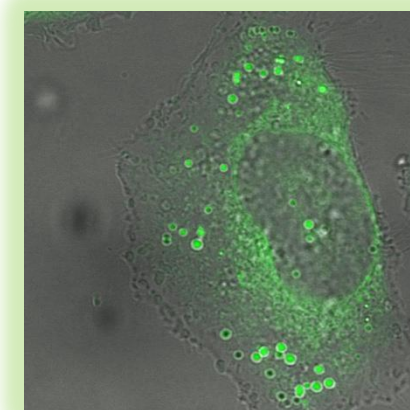
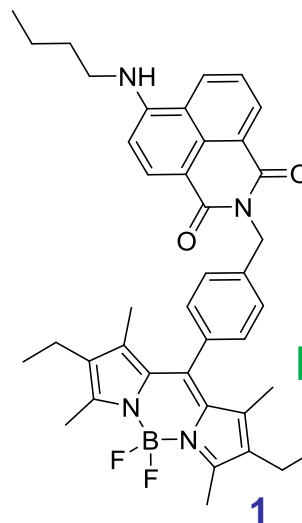
One Photon Excitation conditions



One Photon Excitation
440 nm



One Photon Excitation
514 nm

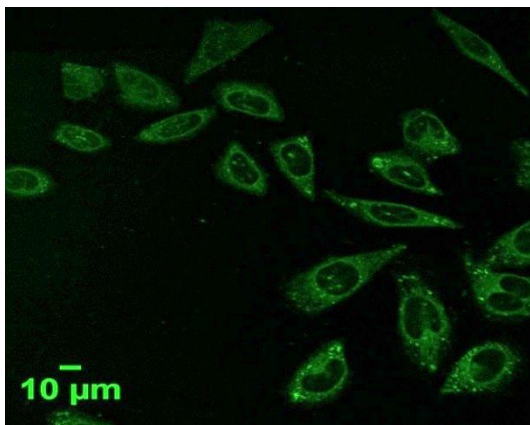


✓ BODIPY – Naphthalimide

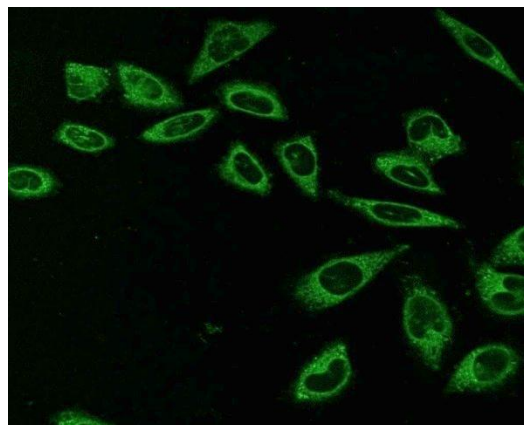
- Cell Imagin

Two-photon microscopy images of HeLa cells incubated

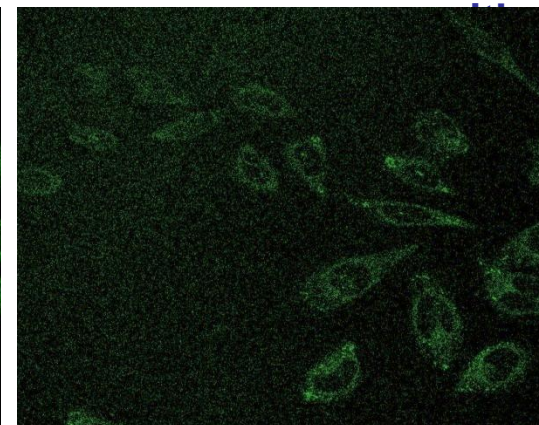
Two
Photon
Excitation
conditions



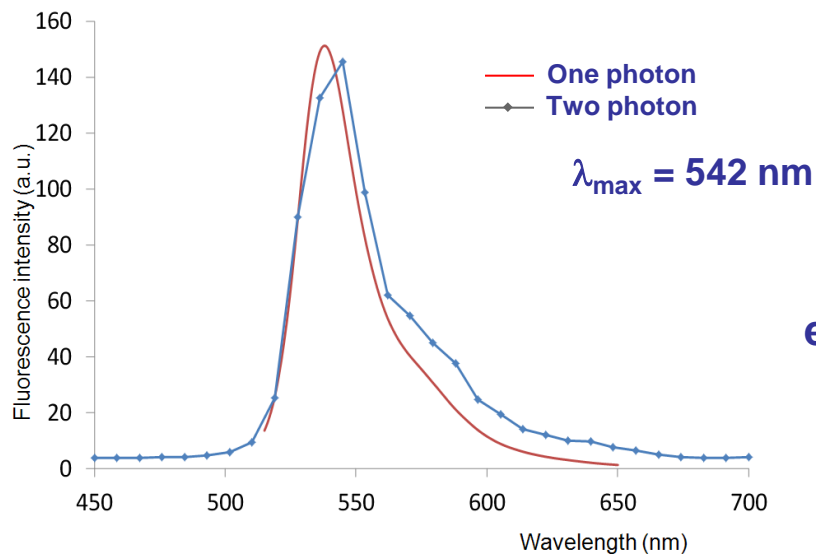
Laser excitation: 720 nm



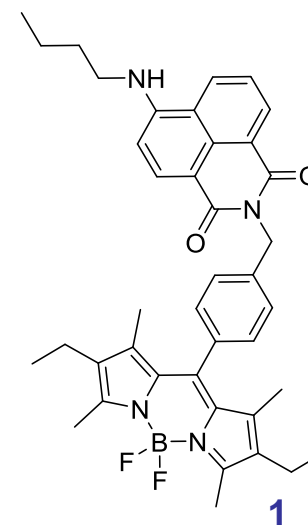
880 nm



1040 nm

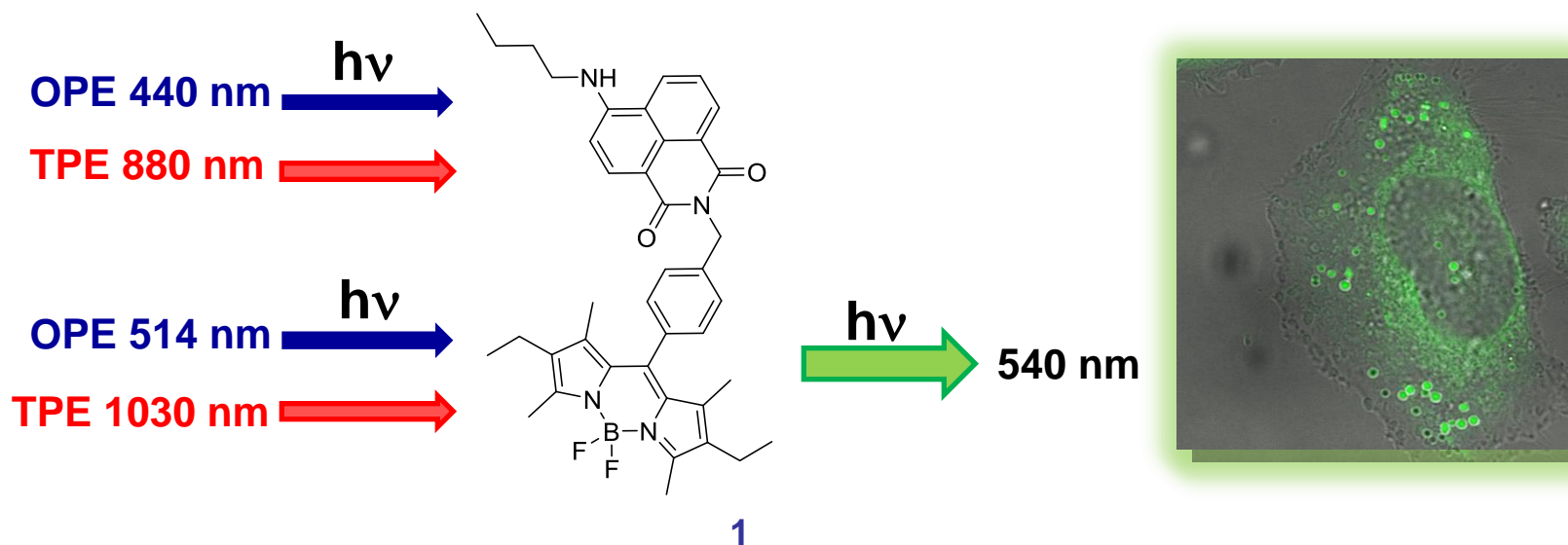


OPE and TPE
emission spectra of
1



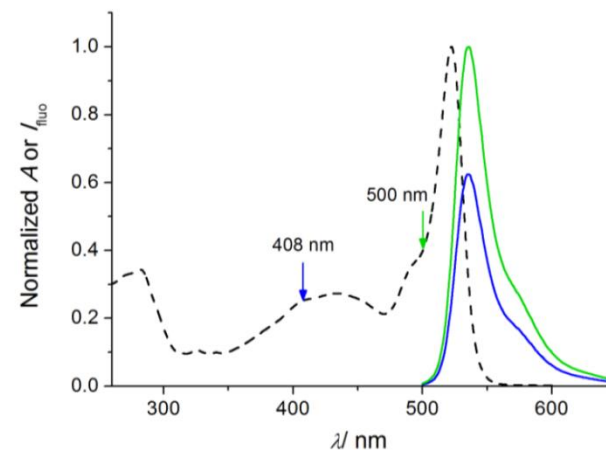
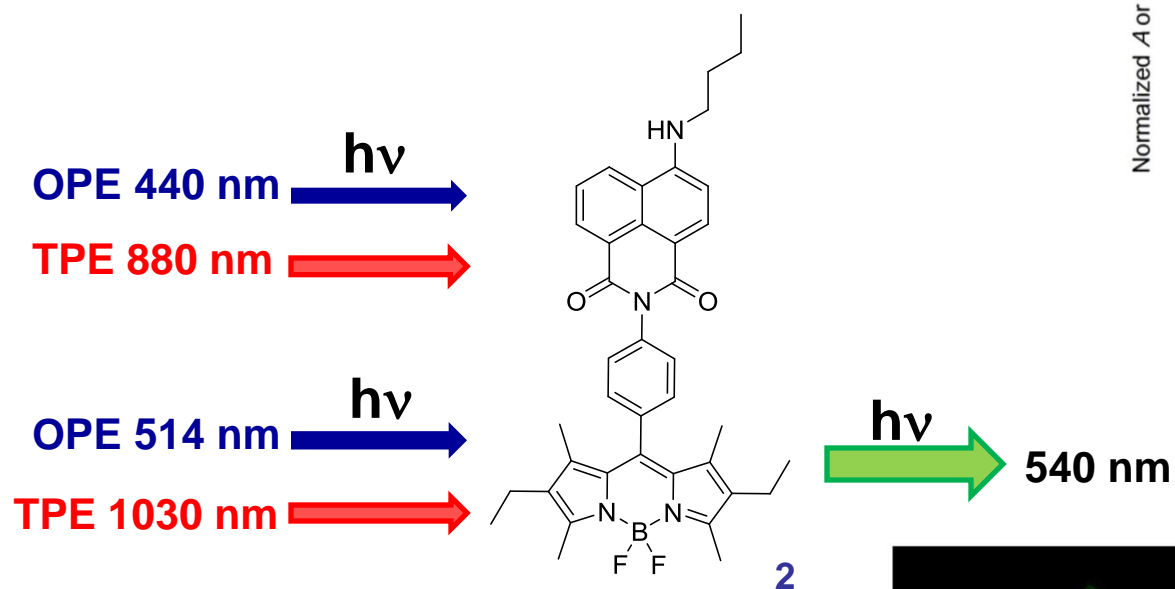
✓ *BODIPY – Naphthalimide*

- *Energy Transfer Cassettes*
- *Two Photon Excitation Fluorescent imaging*

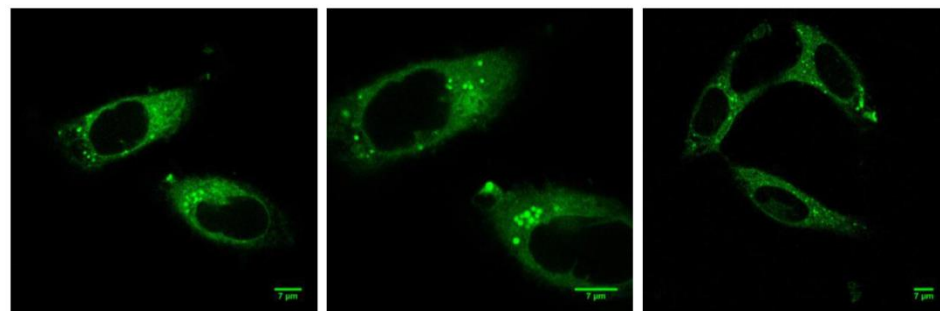


✓ BODIPY – Naphthalimide

- Energy Transfer Cassettes
- Two Photon Excitation Fluorescent imaging



One and Two-photon microscopy images of HeLa cells incubated with 2



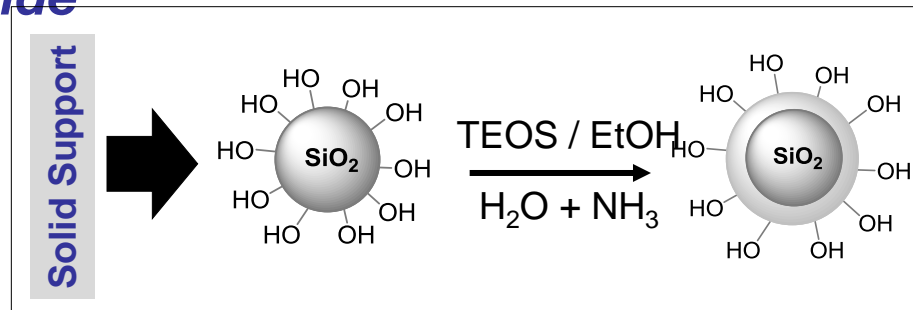
Laser excitation: 458 nm

514 nm

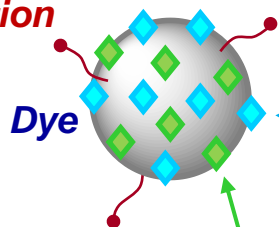
880 nm

✓ BODIPY – Naphthalimide

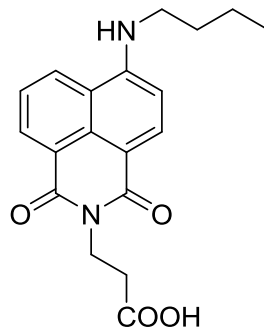
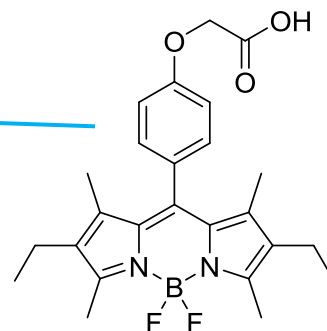
- Supported on Nps



Molecular Recognition



Dye

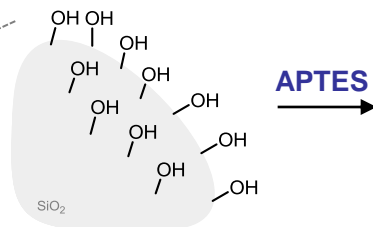
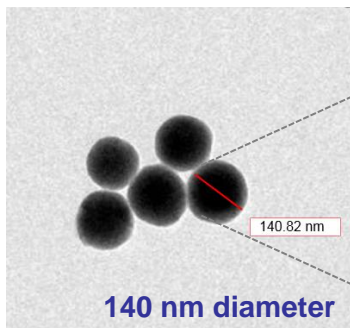


✓ BODIPY – Naphthalimide

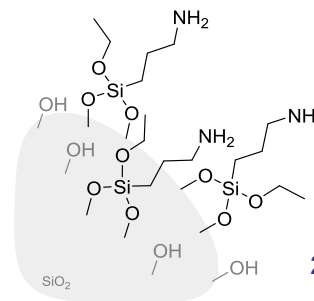
- SiO₂ surface modification

TEOS
NH₃
EtOH

r.t. 12h

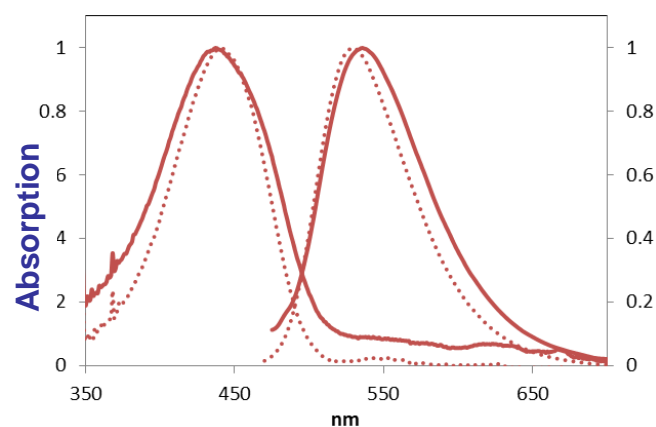


APTES

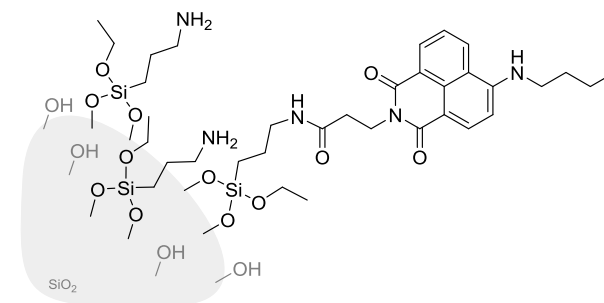
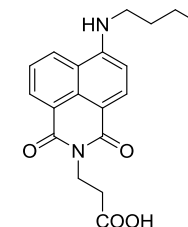


20 μmol -NH₂/g SiO₂ particles

UV - Fluo spectra in DMSO



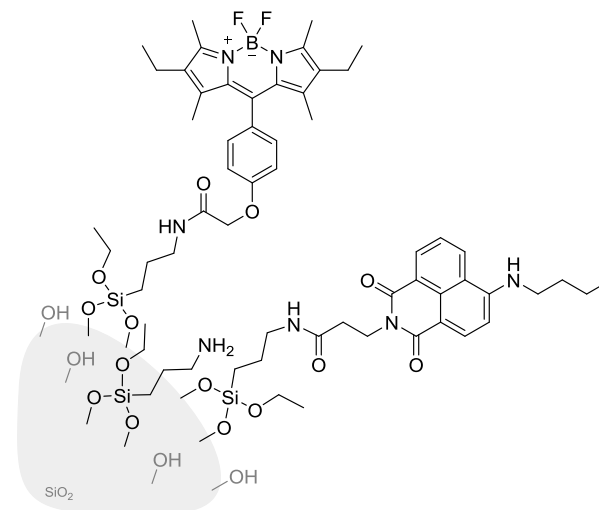
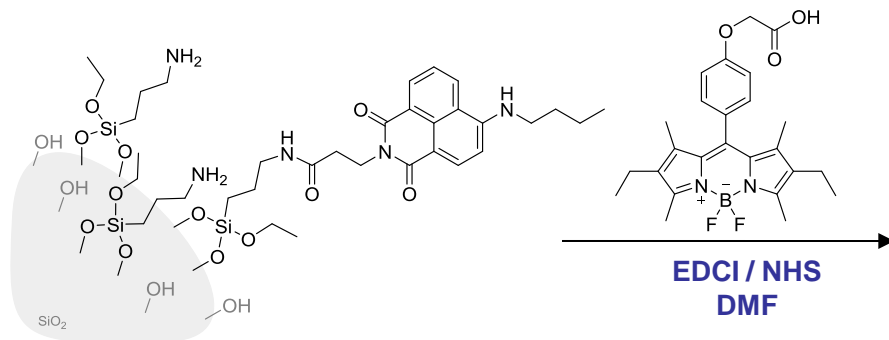
EDCI
NHS
DMF



2 μmol -Naphthalimide/ g SiO₂ particles

0.6 μmol -Naphthalimide/ g SiO₂ particles

✓ **BODIPY – Naphthalimide**
- **SiO₂ surface modification**



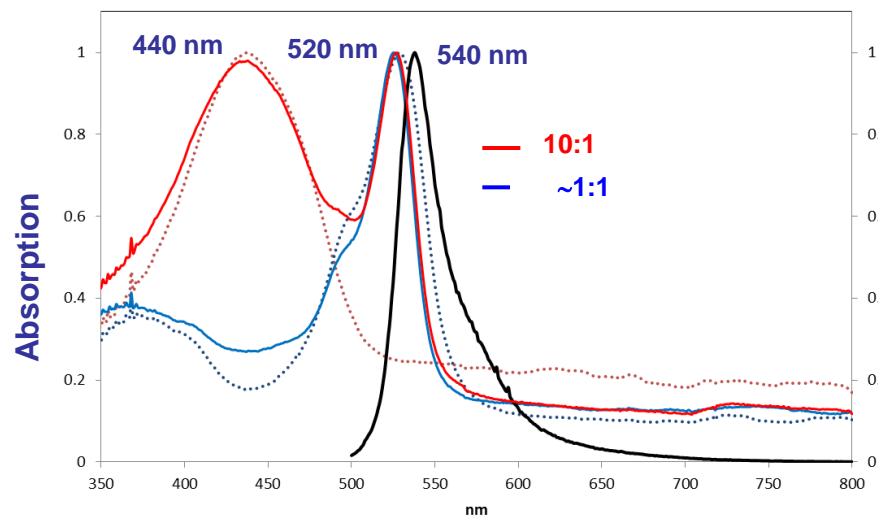
2 μmol –Naphthalimide/ g SiO₂ particles
0.6 μmol –Naphthalimide/ g SiO₂ particles

2 μmol –Naphthalimide/ g SiO₂ particles
0.2 μmol –BODIPY/ g SiO₂ particles

10 Naphthalimide / BODIPY

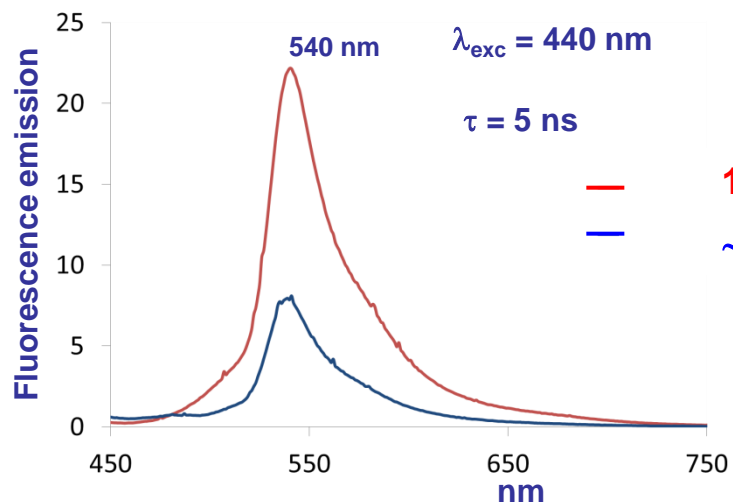
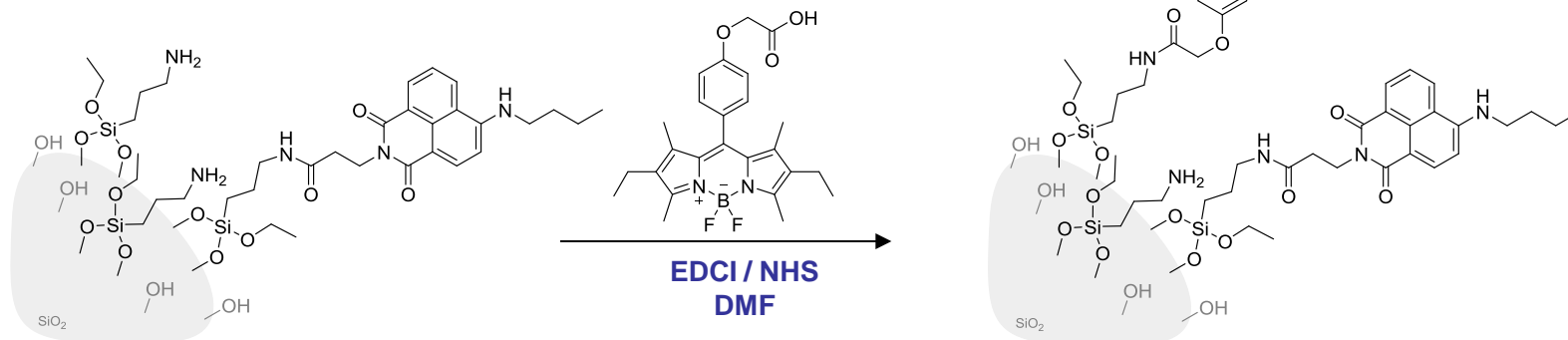
0.6 μmol –Naphthalimide/ g SiO₂ particles
0.2 μmol –BODIPY/ g SiO₂ particles

~1 Naphthalimide / BODIPY



$\lambda_{\text{exc}} = 440 \text{ nm}$
 $\lambda_{\text{exc}} = 490 \text{ nm}$ \rightarrow $\lambda_{\text{em}} = 540 \text{ nm}$

✓ **BODIPY – Naphthalimide**
- **SiO₂ surface modification**

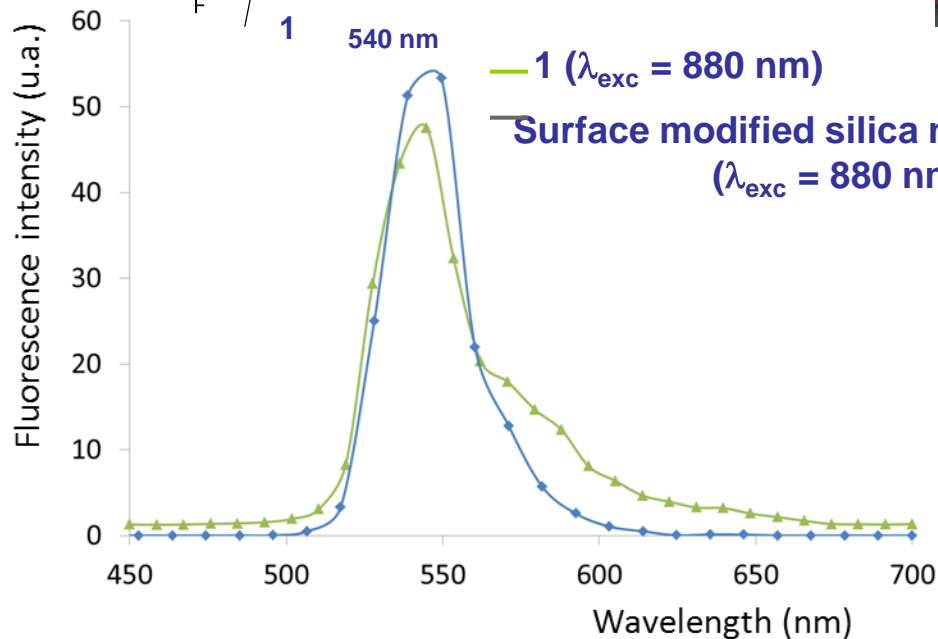
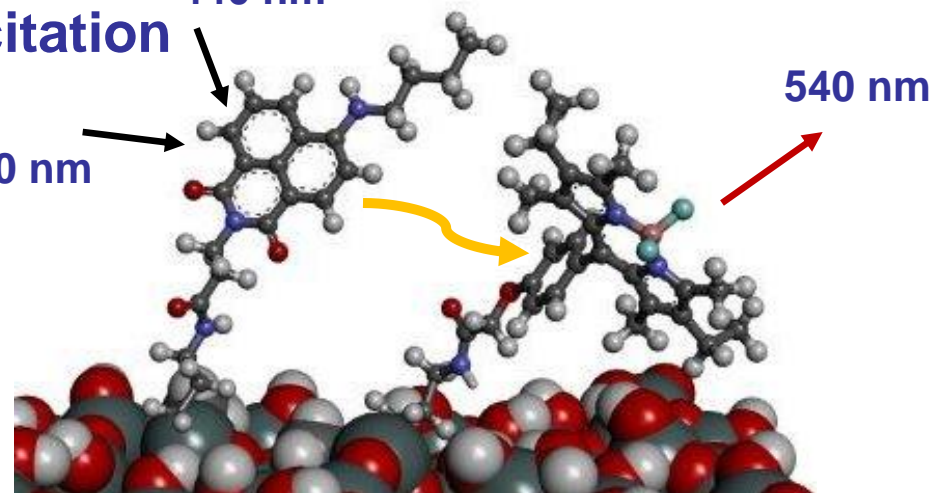


OPE emission spectra of surface modified naphthalimide-BODIPY silica nanoparticles in DMSO

✓ **BODIPY – Naphthalimide** **One Photon Excitation** 440 nm

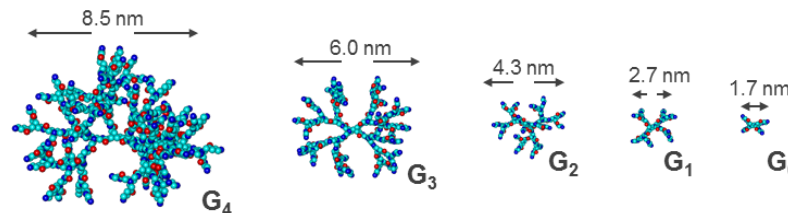
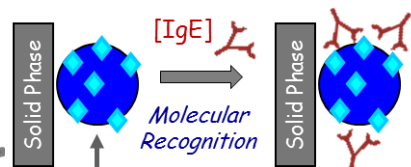
SiO₂ surface modification

Two Photon Excitation 880 nm



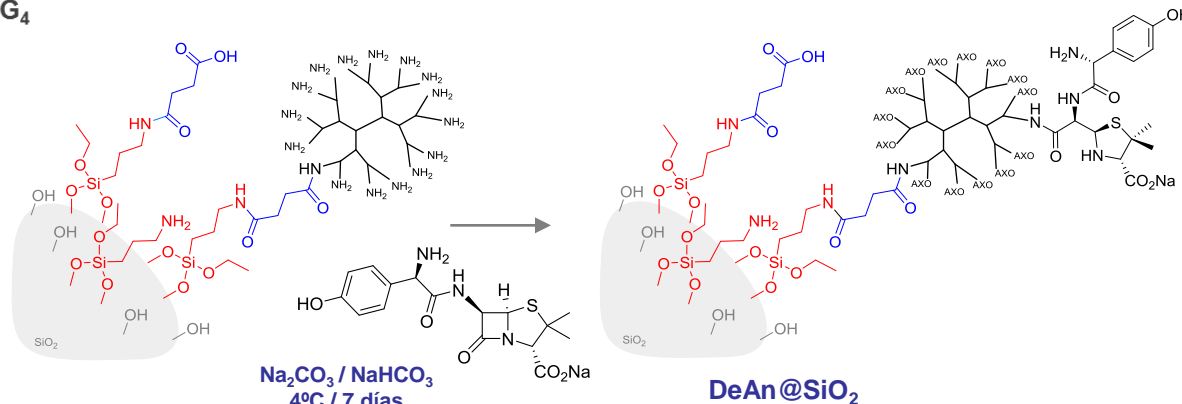
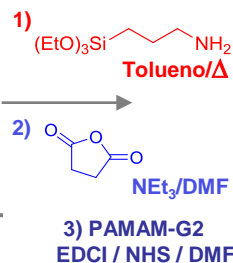
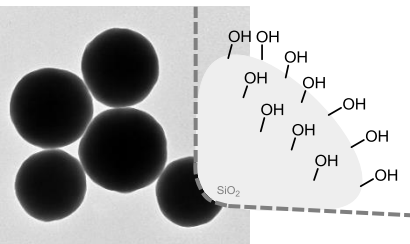
TPE emission spectra of 1 and naphthalimide-BODIPY silica nanoparticles in DMSO

✓ Nuevas fases sólidas para la detección de IgE mediante RAST .



Dendrimeros: PAMAM

Partículas de sílica

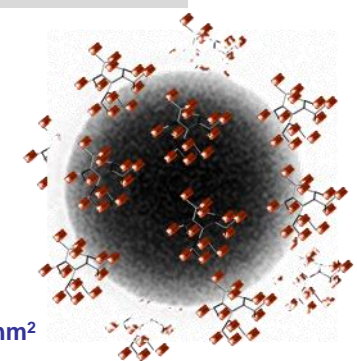


ξ (mV) en PBS:	-31	+17 (-NH ₂)	-41 (-COOH)	+13	-8
--------------------	-----	-------------------------	-------------	-----	----

1 esfera: Diámetro = 450 nm	Densidad SiO ₂ = 2 g/cm ³	SA /g Nps
Volumen = 47.7 10 ⁶ nm ³ Área superficial = 63.6 10 ⁴ nm ²	9.5 10 ¹² esferas/g of SiO ₂	6.1x10 ¹⁸ nm ²
DeAn@SiO₂		
30 $\mu\text{mol-NH}_2$ /g Nps	2 $\mu\text{mol PG2}$ /g Nps	12.0x10 ¹⁷ PG2/g Nps

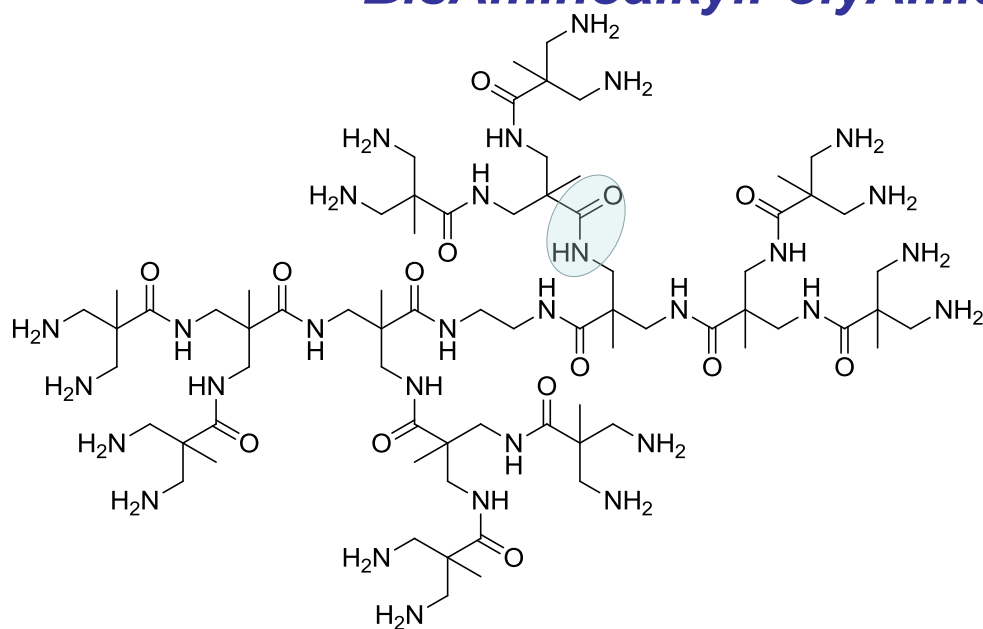
1 PAMAM-G2 per 5 nm²

PAMAM-G2: radio = 1.52 nm
Área teórica ocupada por DeAn = 7.3 nm²

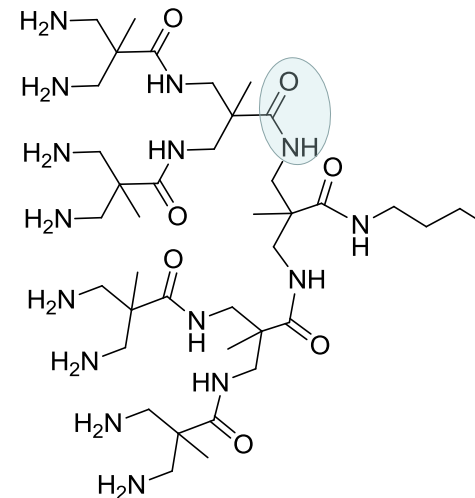


BAPAD Structures.

BAPAD =
BisAminoalkylPolyAmideDendrimers¹



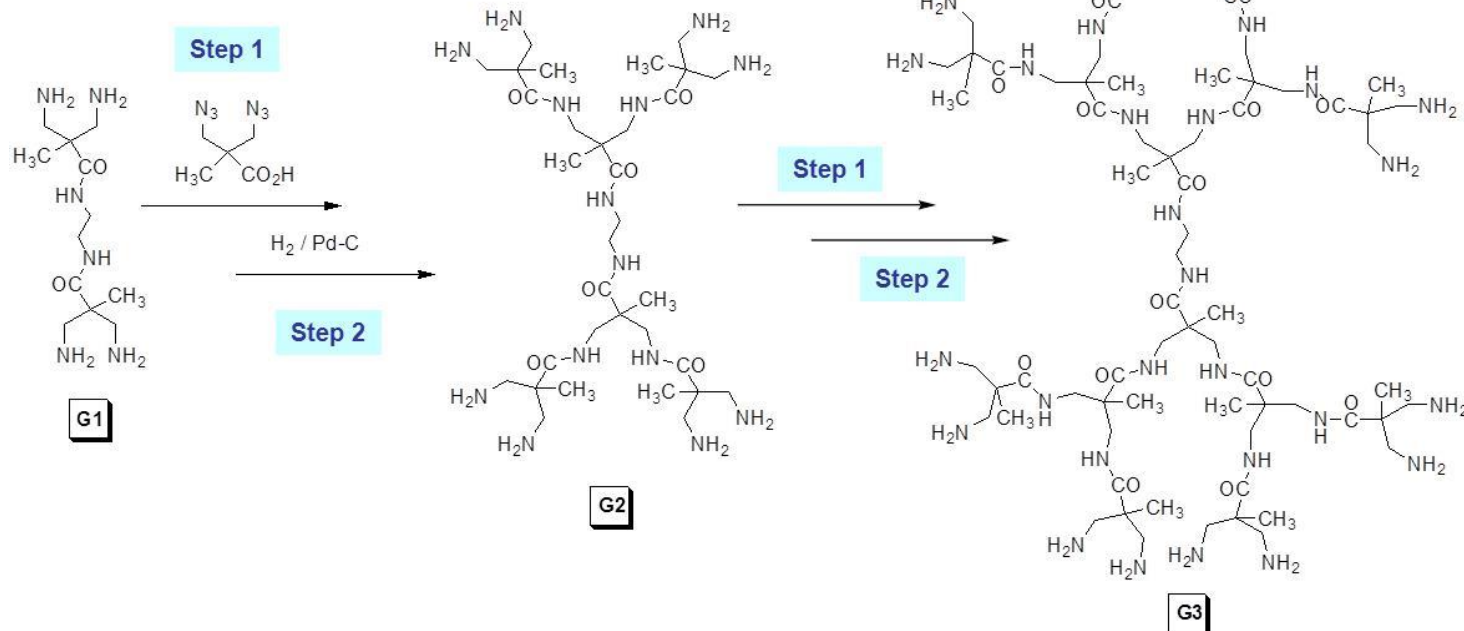
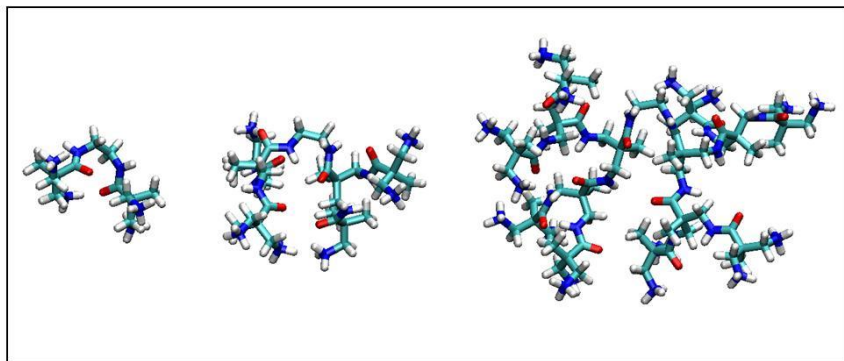
Dendrimer (DM)



Dendron

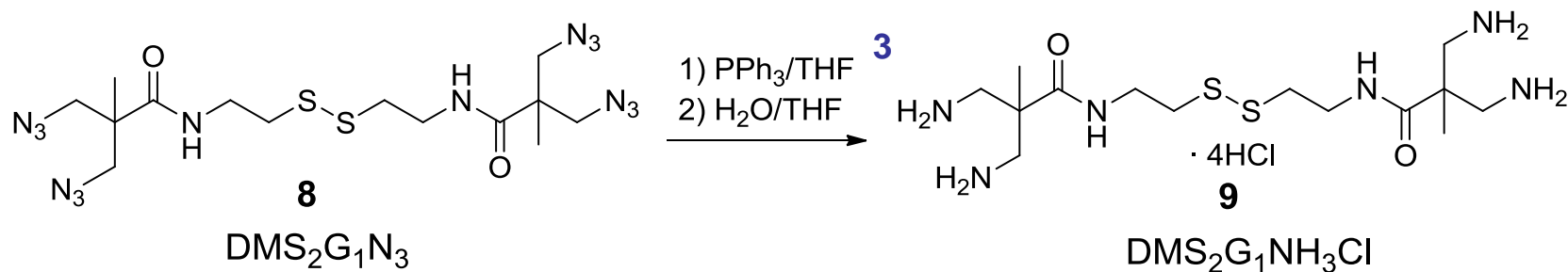
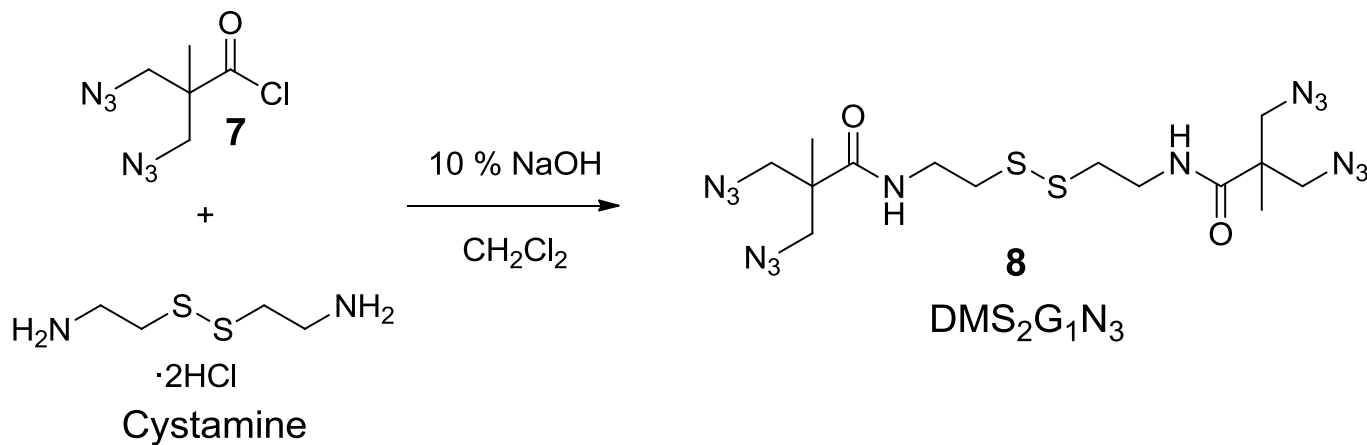
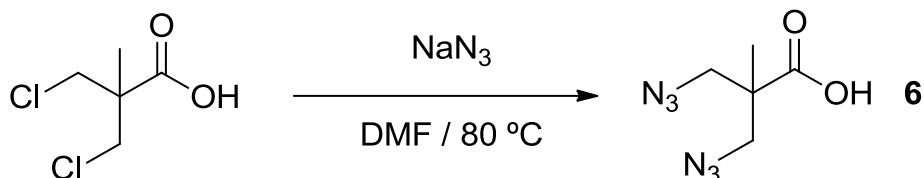
¹PCT/ES2012/000136 (dn)

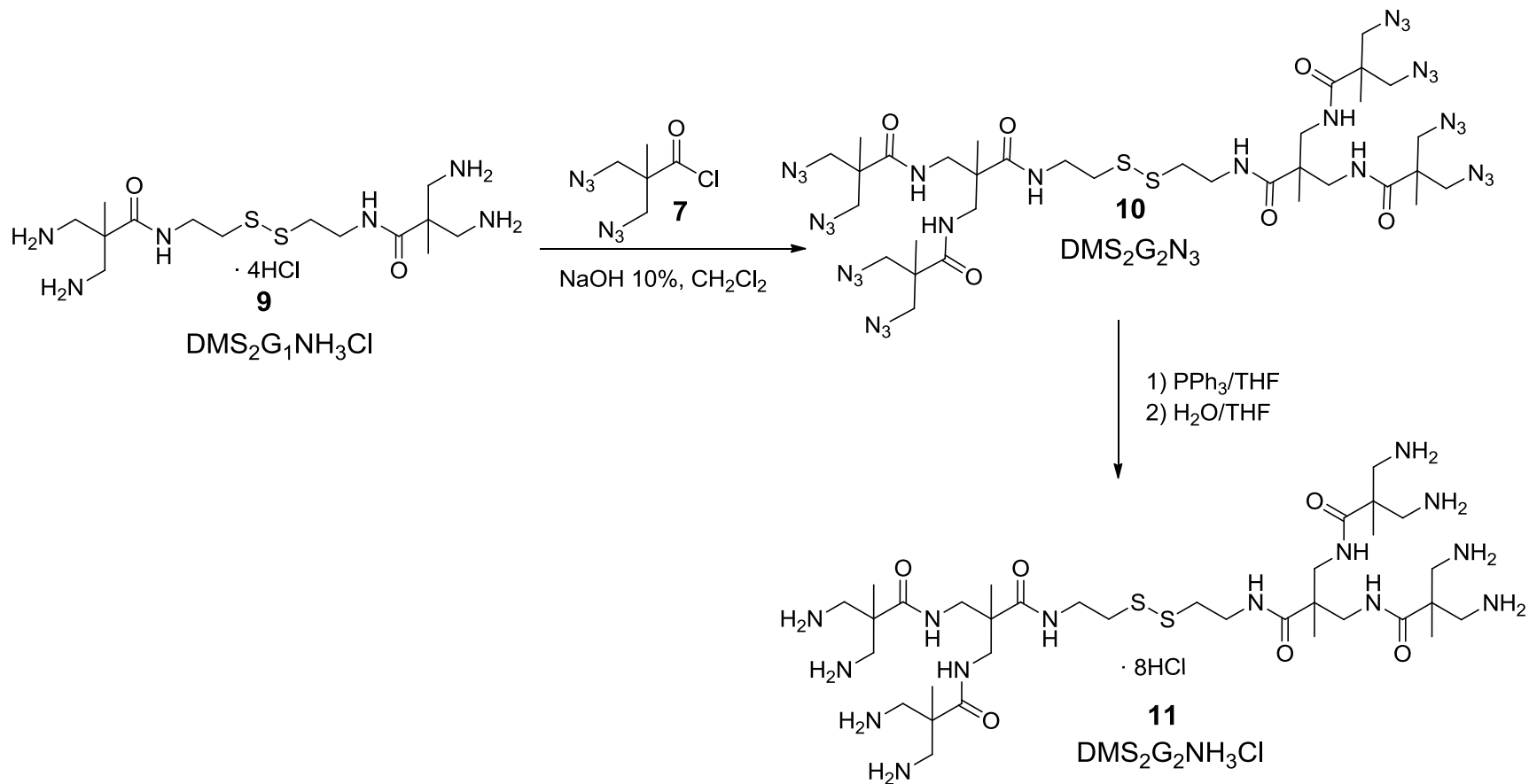
BAPAD	R_g (Å)	I_z/I_y	I_x/I_y	δ
G1	4.1	1.1	1.7	0.115
G2	6.4	1.2	2.6	0.068
G3	9.4	1.3	2.5	0.059



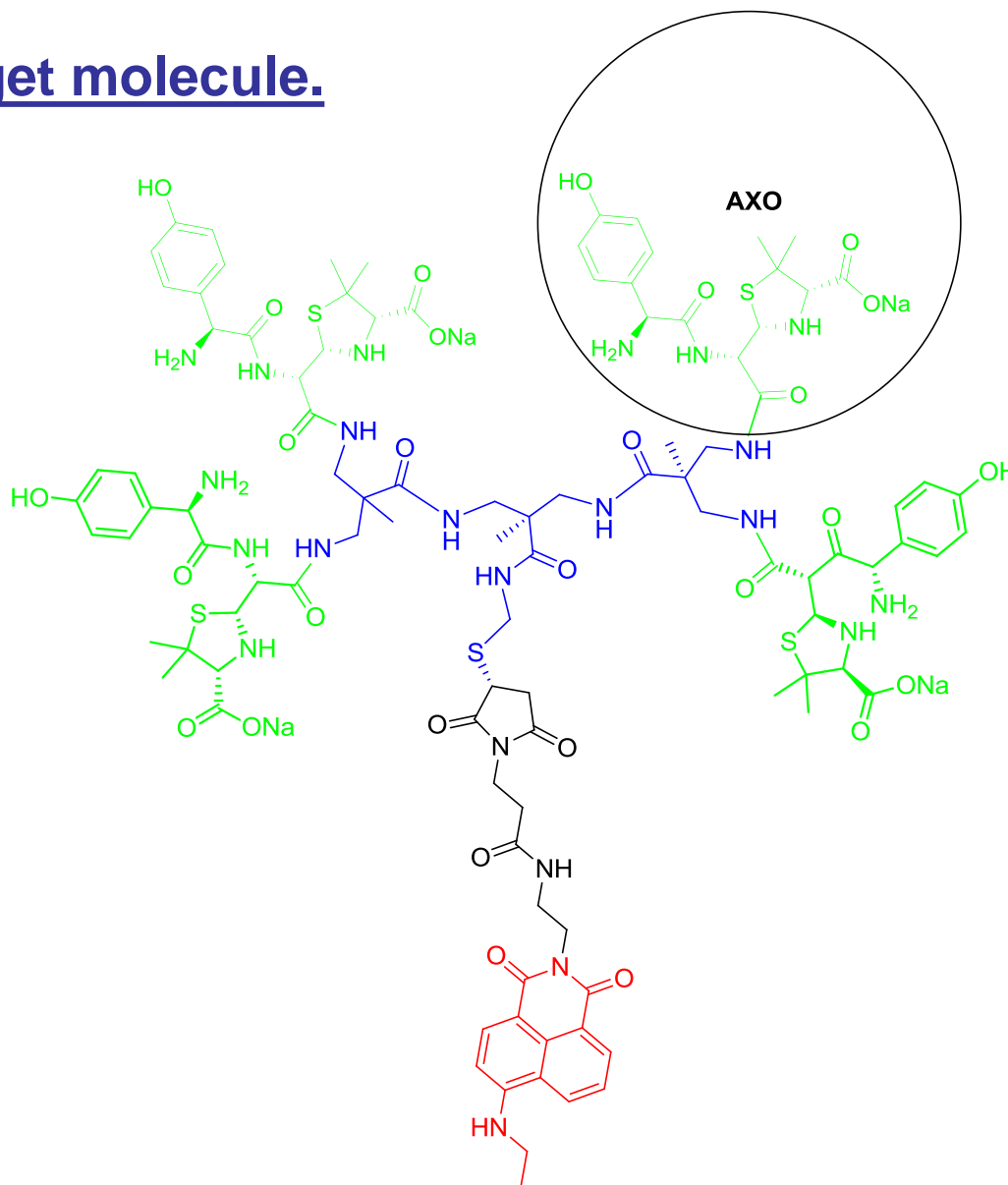
Dendrimer synthesis.

Monomer





Target molecule.



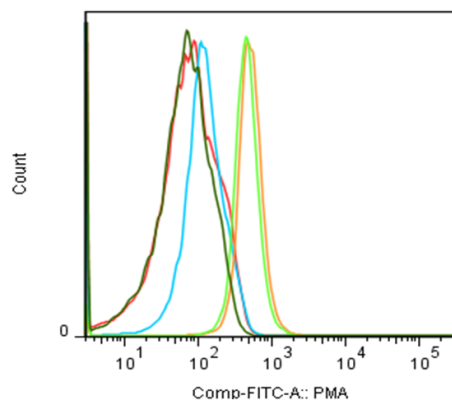
Biomedical considerations.

Flow cytometry study.

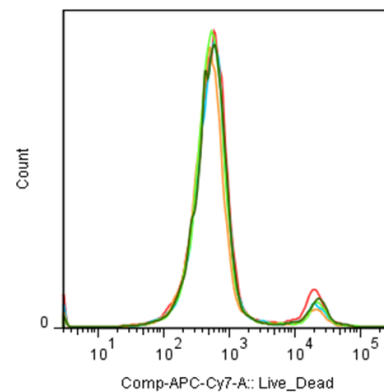
Compound 16

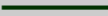




Compound 17

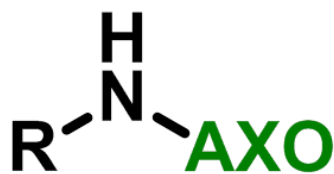
Uptake.



Live/Dead.



Sample	
	Positive Control (LPS)
	10 µg/mL
	1 µg/mL
	0,1 µg/mL
	Negative Control (DCi 1)

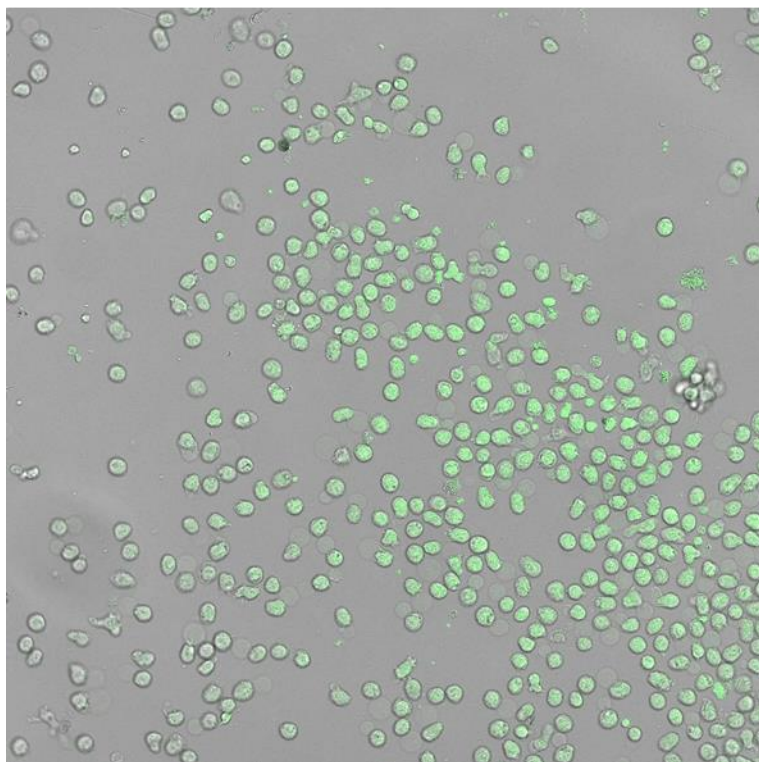


AXO = Amoxiciloilo

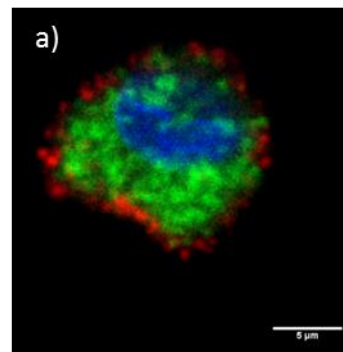


Confocal spectroscopy study.

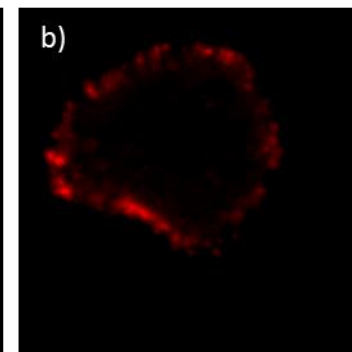
Compound 16



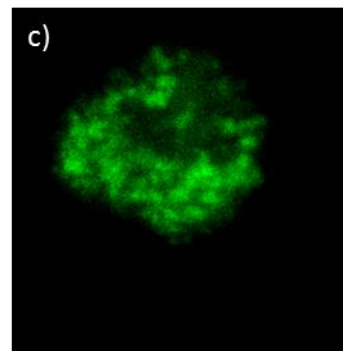
Combination



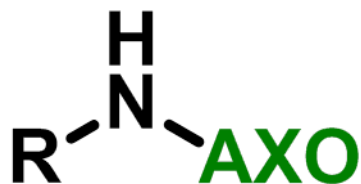
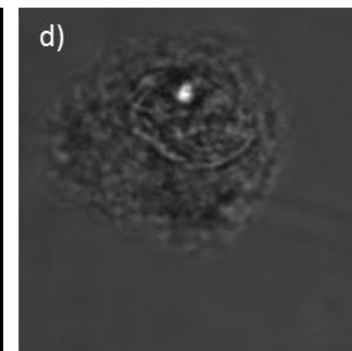
Faloidina-Alexafluor 647



Compound 17

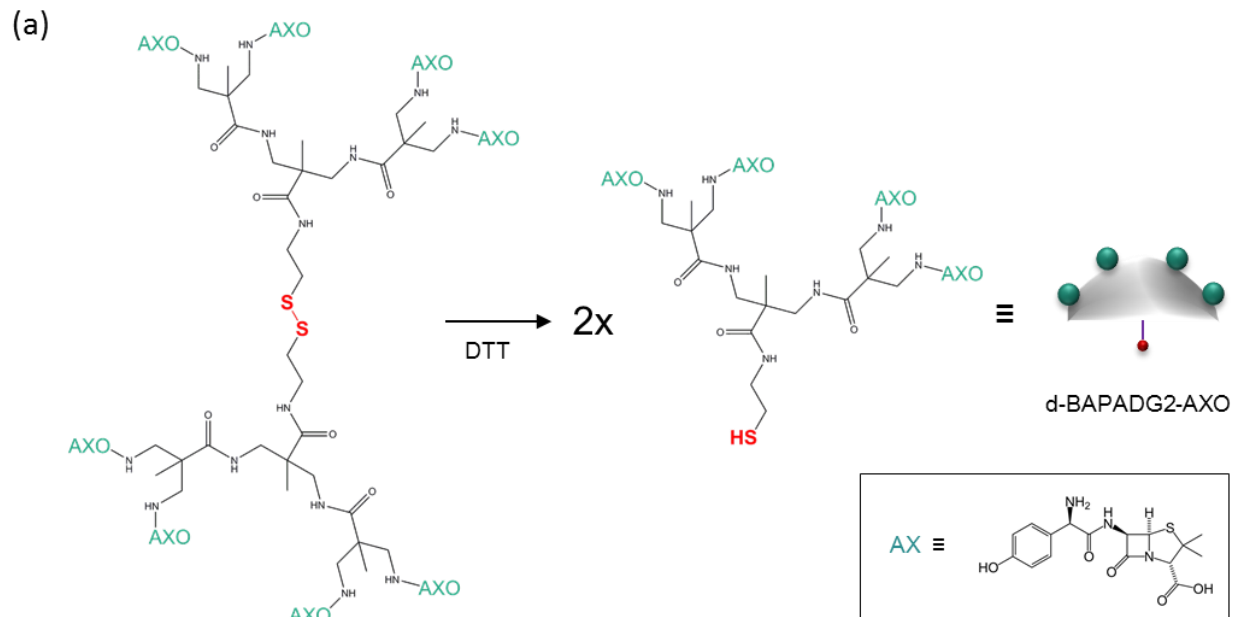


Transmitted

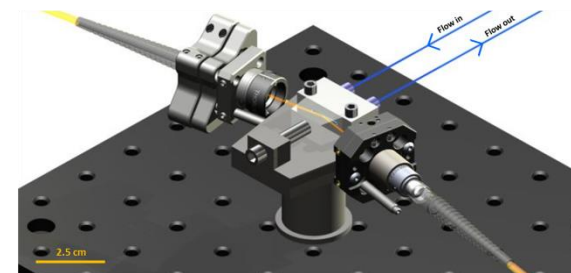
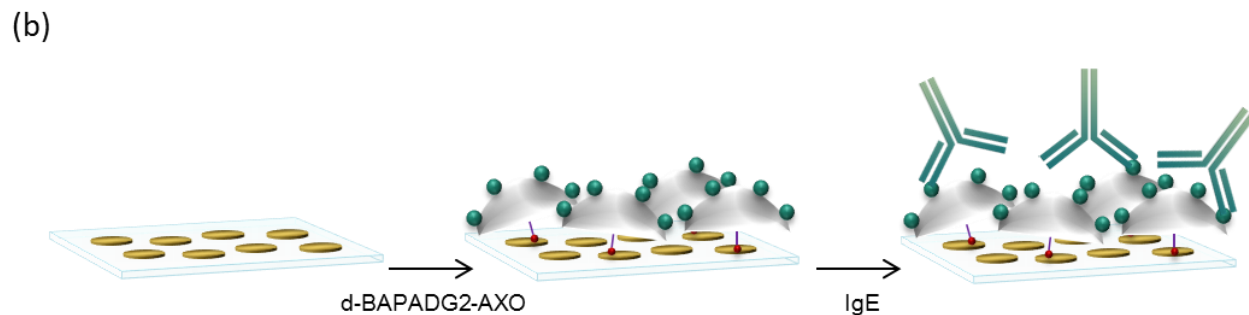


AXO = Amoxiciloilo

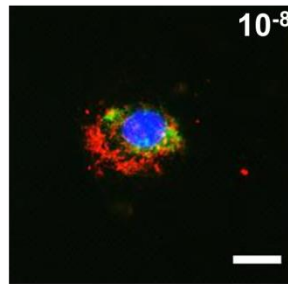
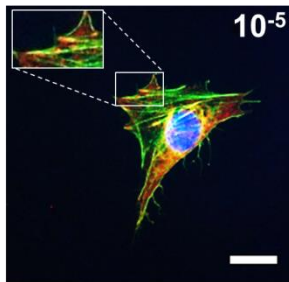
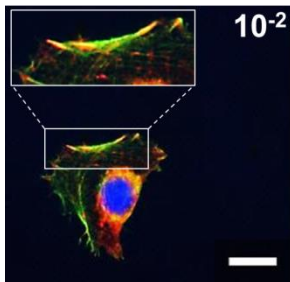
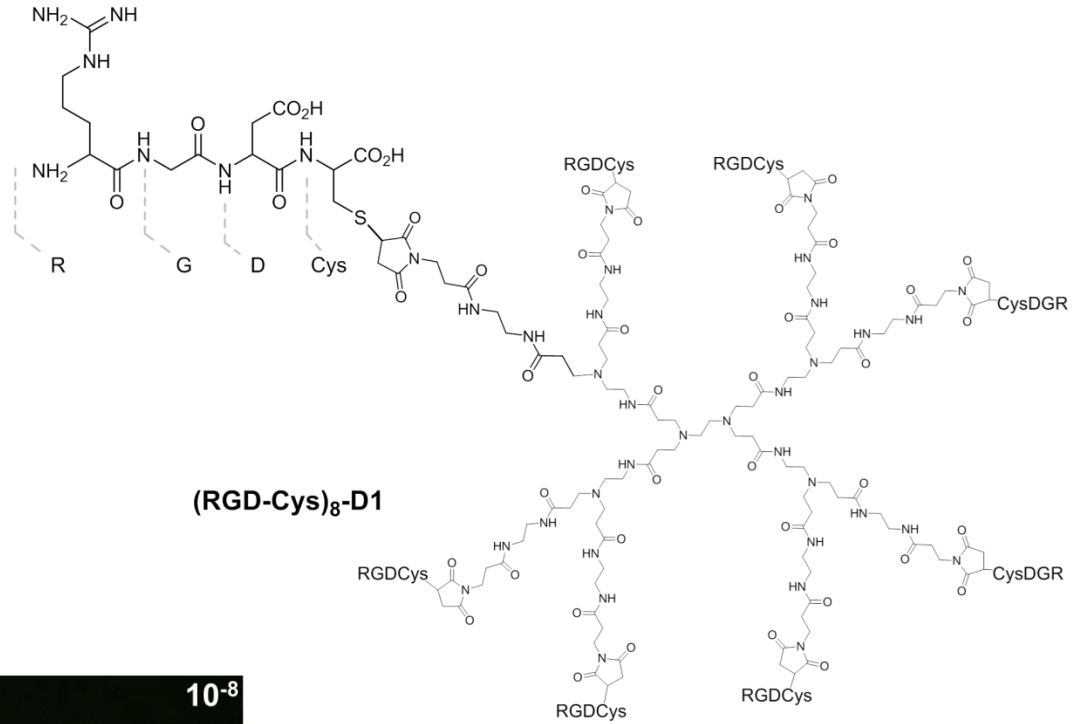
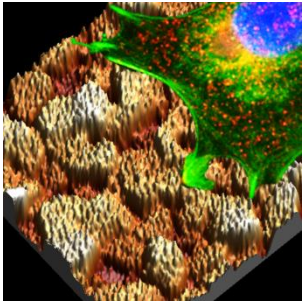




Dendrimer-Based Nanoplasmonic Biosensor for Drug Allergy Diagnosis



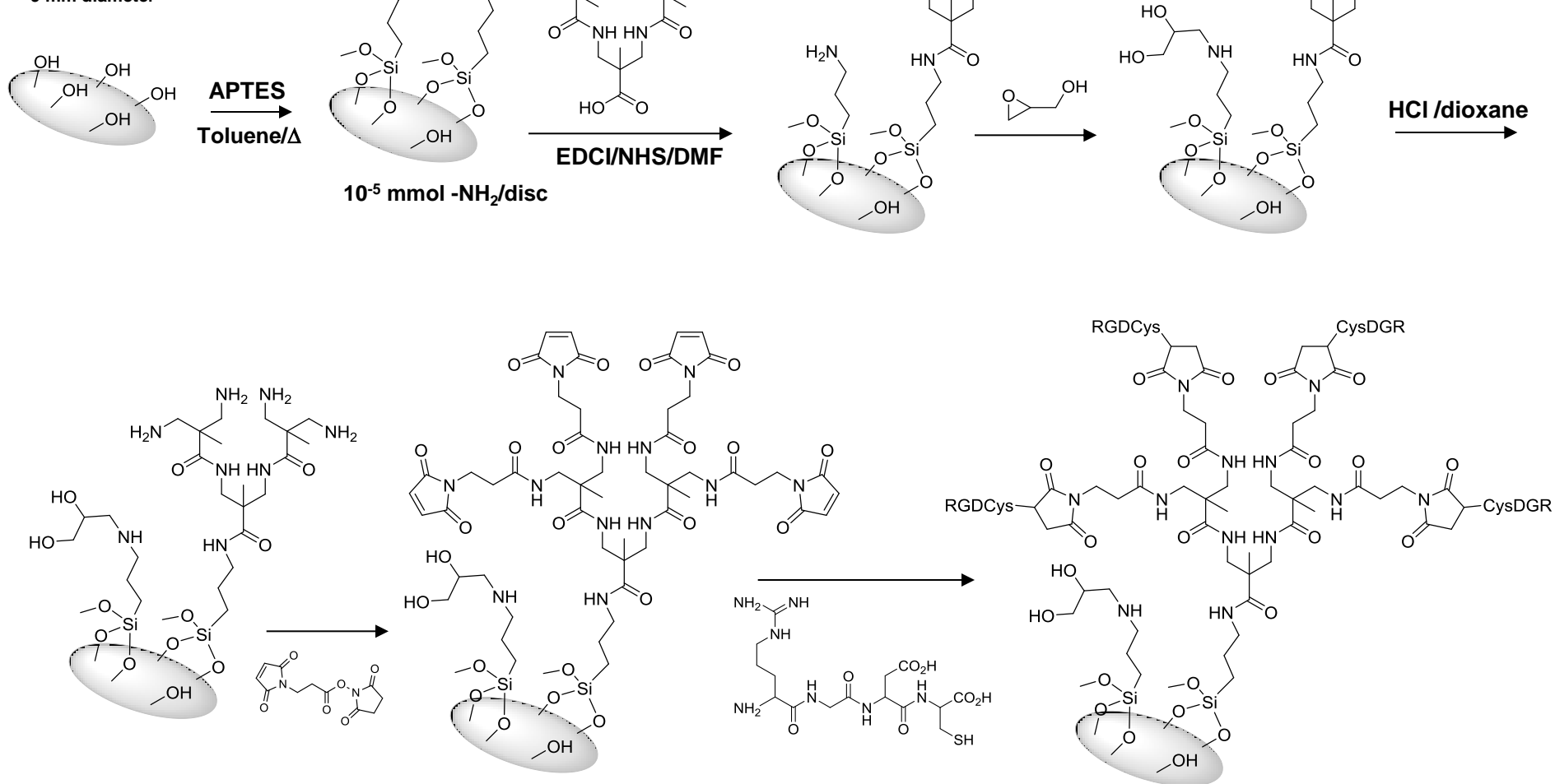
✓ Cell recognition and adhesion processes, with tissue regeneration applications.



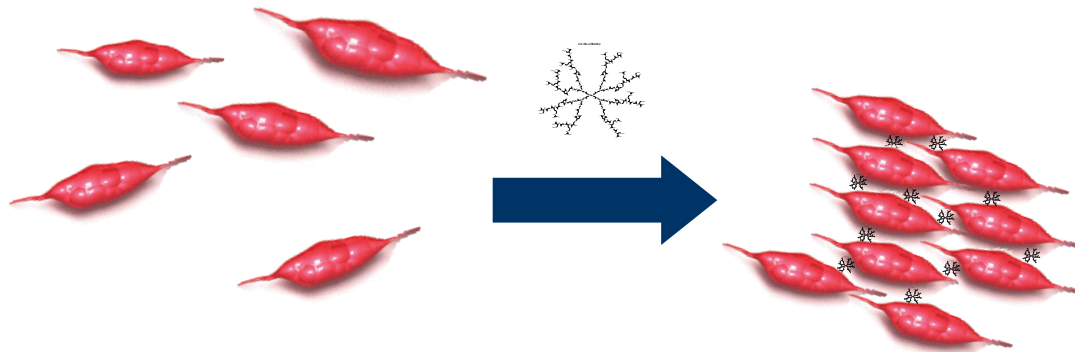
✓ Cell recognition and adhesion processes, with tissue regeneration applications.

Titanium disks

1 mm thick
9 mm diameter



✓ Cell recognition and adhesion processes, with tissue regeneration applications.



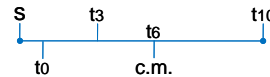
Diferenciación Celular



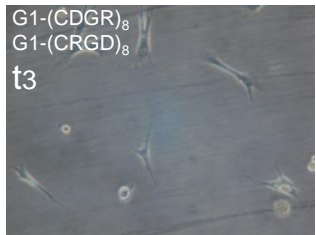
Vehicular el biomaterial al lugar de reparación

Placas convencionales
740.000 cfs/ml

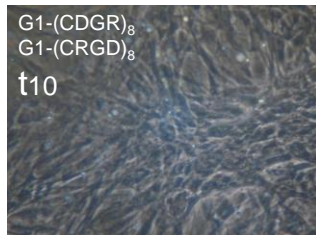
Placas NO tratadas



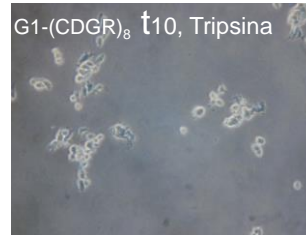
No se aprecia adhesión



Aspecto Fibroblástico
Algunas se adhieren



G1-(CDGR)₈ : 680.000 cfs/ml
G1-(CRGD)₈ : 460.000 cfs/ml



Áreas de Aplicación

Estudio de Reacciones Adversas a Medicamentos con base Inmunológica

- Diseño de nuevos test *In Vitro* e *In Vivo*.
- Desarrollo de metodologías para interaccionen con el sistema inmunológico: Inhibidores y Efectores.
- Dendrimeros como soportes multivalentes para interacciones entre células: activación de basófilos, proliferación celular.

Desarrollo de Nanobiosensores

- Desarrollo de nuevos marcadores luminiscentes para la visualización de interacciones con sistemas biológicos.
- *Lab on a Chip*
- *Lab on a Molecule*

Regeneración Tisular

- Cultivo in vitro de Células Madre Mesenquimales (MSC's)
- Empleo de Dendrimeros sobre Células Madres Humanas pluripotentes como vehículo para dirigir terapias a tumores cerebrales

LABORATORIO de DENDRIMEROS BIOMIMETICOS y FOTONICA

<http://www.ldbf.uma.es/>

Departamento de Química Orgánica
Facultad de Ciencias
Universidad de Málaga



UNIVERSIDAD
DE MÁLAGA



ibima

Instituto de Investigación
Biomédica de Málaga

*Grupo Consolidado de Investigación
de la Junta de Andalucía* **FQM017**