



UNIVERSITÀ  
DEGLI STUDI  
DI PADOVA

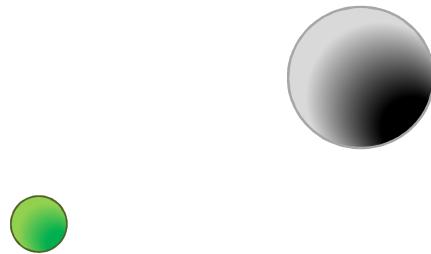
**CRIBI**  
Centro di Ricerca Interdipartimentale  
per le Biotecnologie Innovative

**DSB**  
Dipartimento Scienze Biomediche

**AMBITI DEI PROGETTI DI TESI:**  
Nanobiotecnologie, Nanomedicina,  
Nanotossicologia, proteine ricombinanti, farmaci  
biotecnologici

**SVOLGIMENTO:** CRIBI, DSB

**RELATORI:** Prof. Emanuele Papini, dr. Regina Tavano,  
Prof. Alessandro Negro

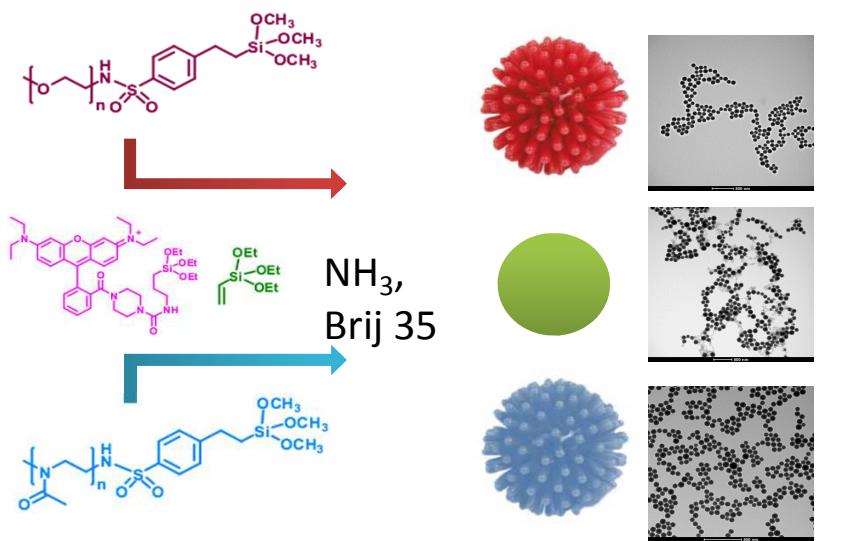


**PROGETTO 1.**  
INDIVIDUAZIONE E STUDIO DI  
RIVESTIMENTI MOLECOLARI INNOVATIVI  
DI **NANOSISTEMI “STEALTH”** PER  
MIGLIORARE IL LORO USO E RENDERLI  
**“INVISIBILI”** ALLE DIFESE IMMUNITARIE  
UMANE



*Supervisors: Prof. E.  
Papini; Prof. F. Mancin*

## Surface Passivation w/ Organic Polymers



PEG

no coating

Polyoxazolines

Macrophago

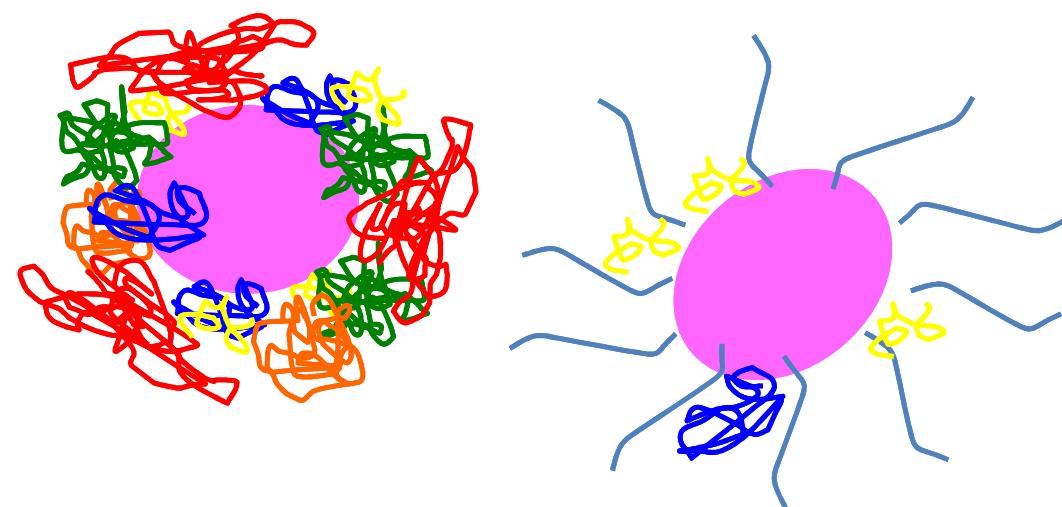
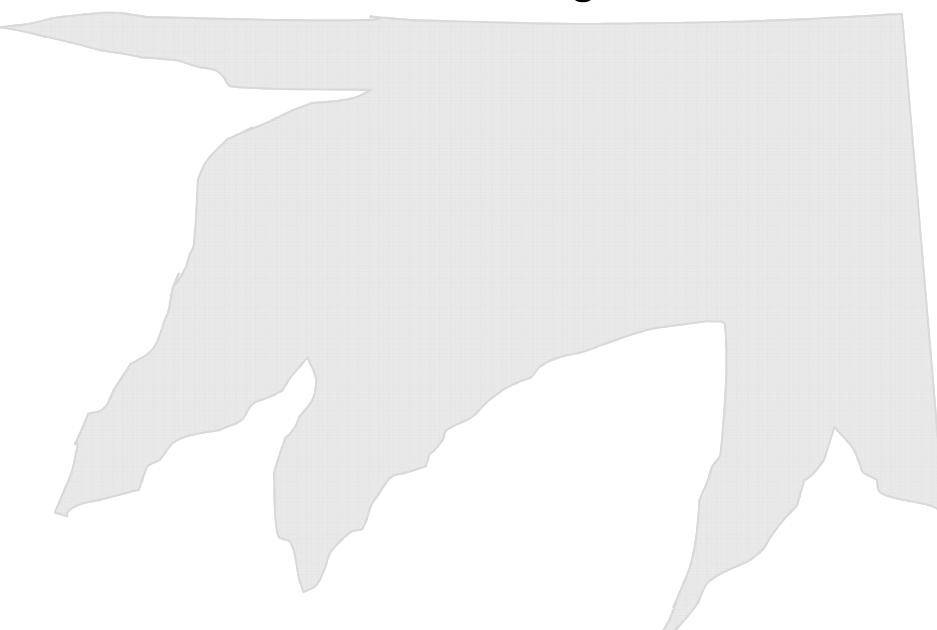
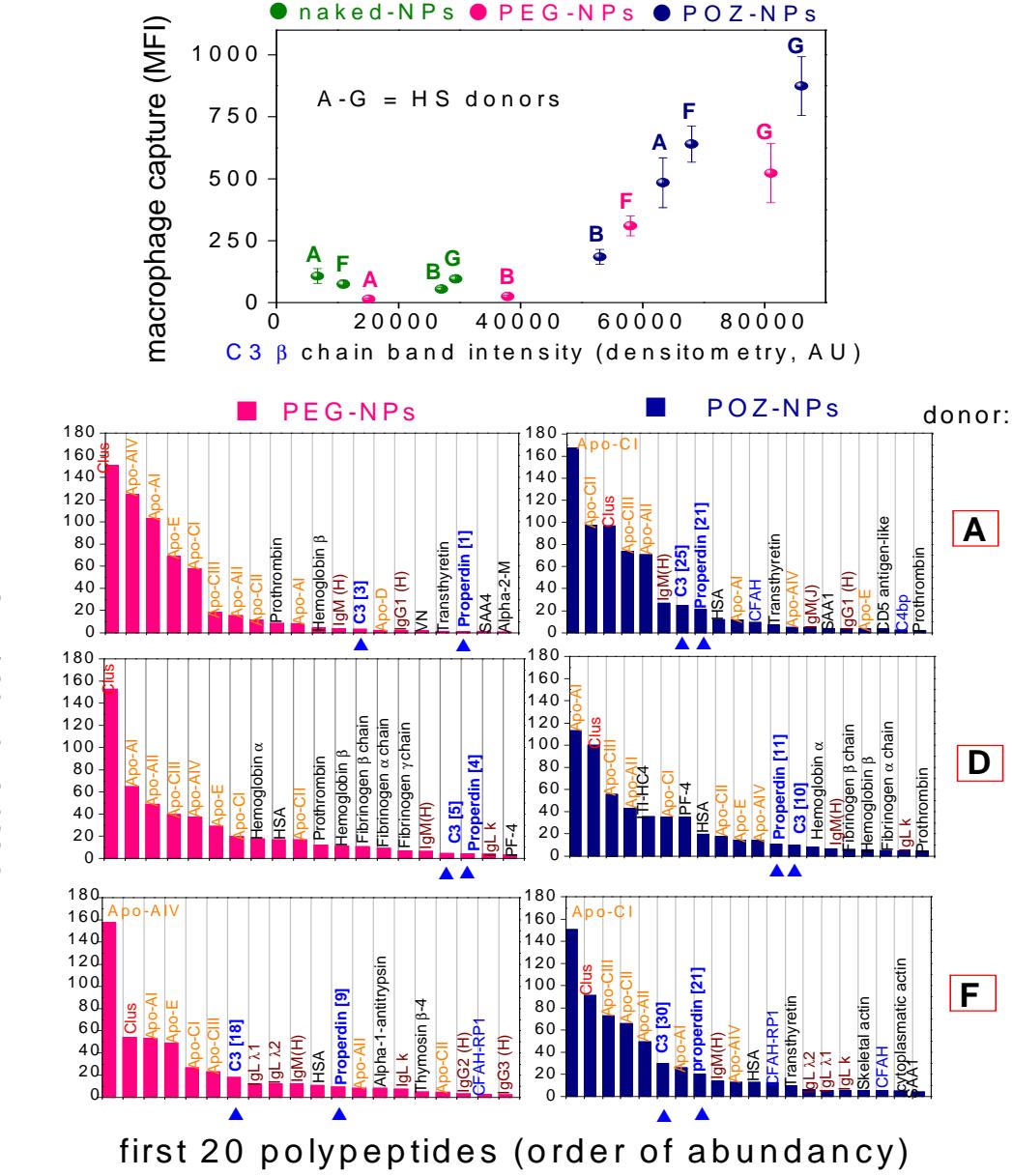
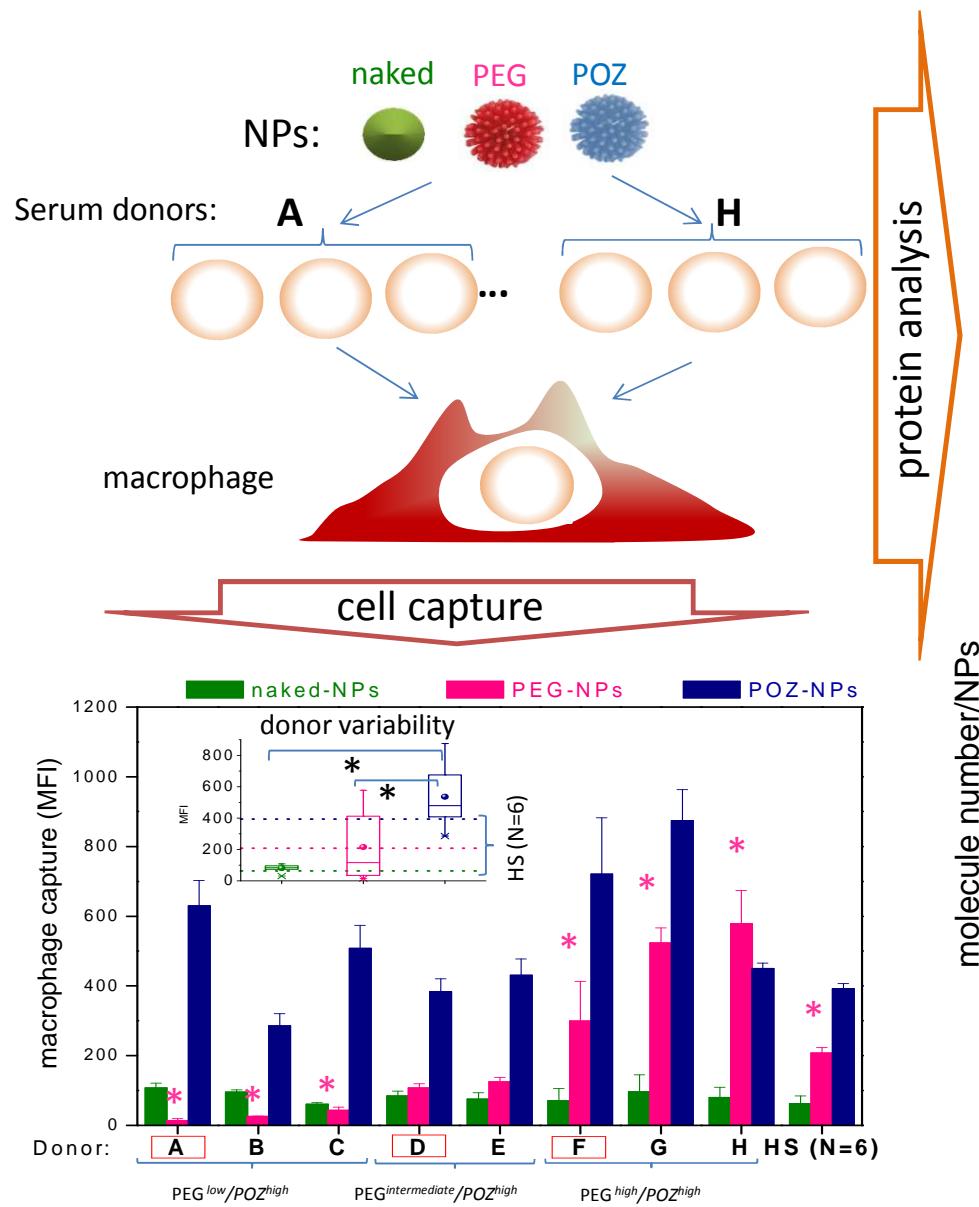
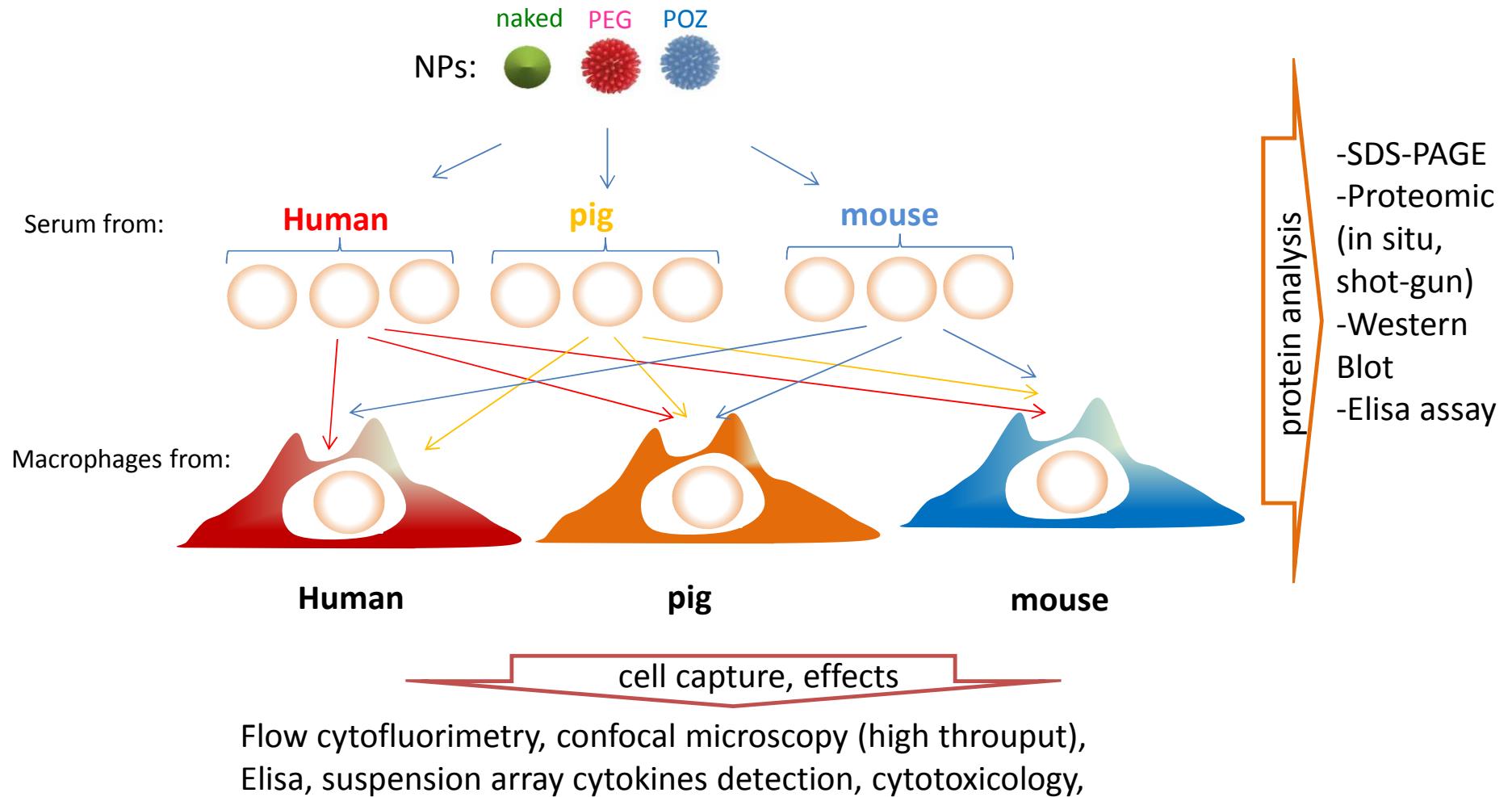


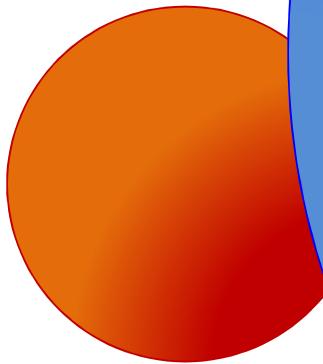
Fig. 6



Inter-species variability of nanomedicine-host interactions and the underlying biochemical mechanisms  
*Coll. dr. S. Nardelli Istituto Zooprofilattico Sperimentale delle Venezie*

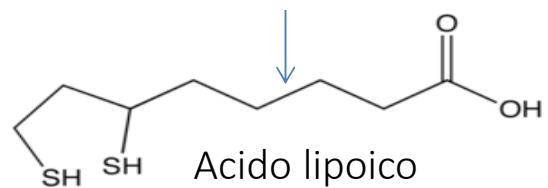


## PROGETTO 2. NANOFARMACI ANTISSIDANTI

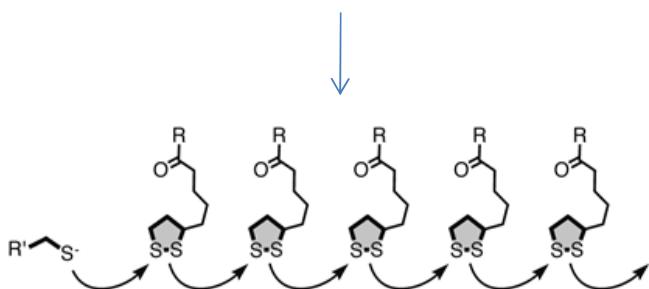


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Acido lipoico



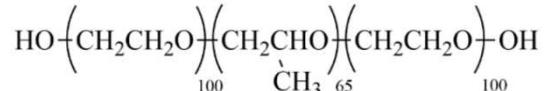
Ring Opening Thiol-Disulfide Exchange Polymerization performed in solution (RODEP)

NPs



# Experimental: NP synthesis

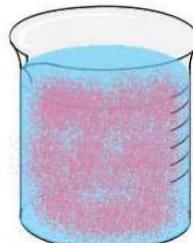
## Pluronic® F127



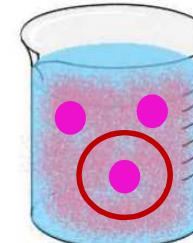
Monomer in acetone  
solution (5mg/ml)



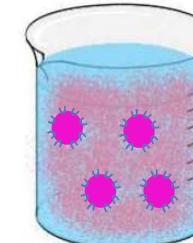
30 min



+ Octanethiol (5 mM)  
120 min  
Nucleation and growth



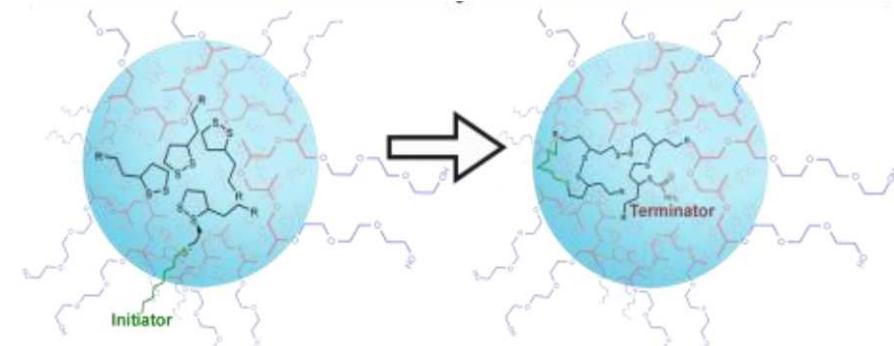
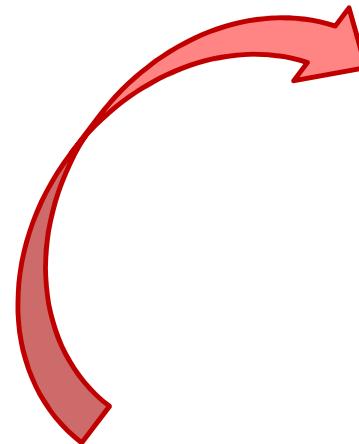
+ Iodoacetamide (5 mM)  
30 min  
Polymerization and final shaping



Purification



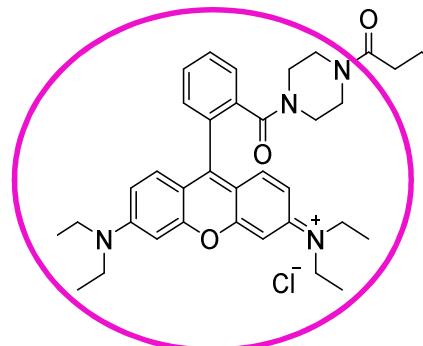
Aqueous phase



RODE polymerization

The nanoprecipitation method combined with RODEP: into the aqueous solution of 20 mM PBS (pH=7,4) and 2 mM F127 surfactant (*first beaker on the left*), the monomer in acetone solution was added (*second beaker*). Acetone diffuses and lipoic acid derivatives precipitate into F127 micelles, inside which the RODE polymerization occurred.

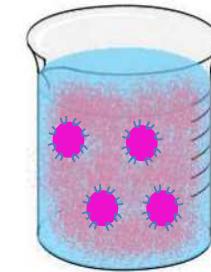
## Results: fluorescent-doped NP



1% v/v



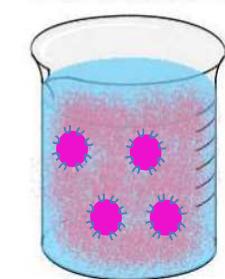
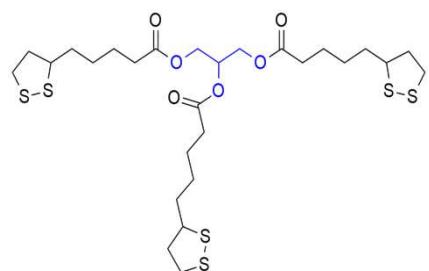
Monomer 1

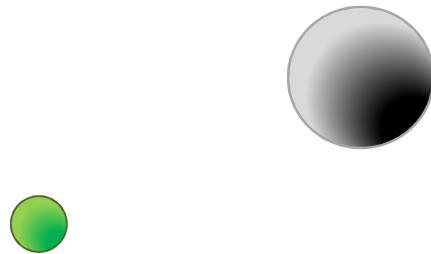


1% v/v



Monomer 3





## PROGETTO 3.

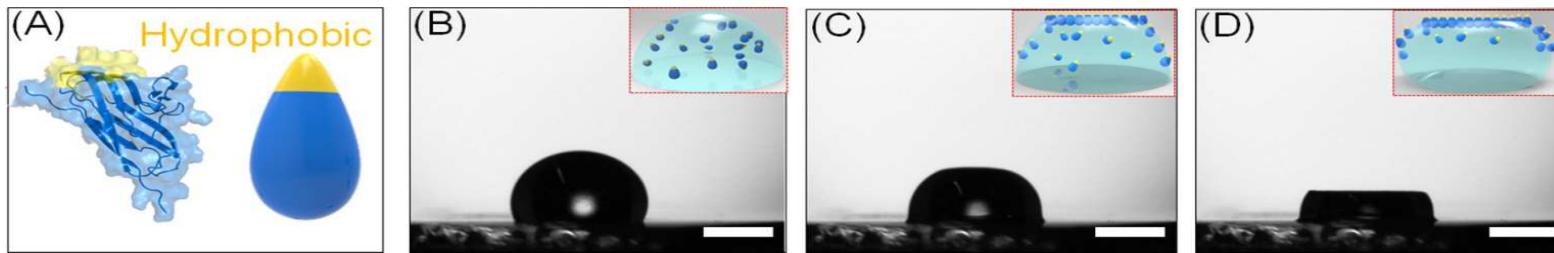
### PROTEINE RICOMBINANTI PER LE BIOTECNOLOGIE: Biofilm-surface layer protein A from *Bacillus subtilis* e tecnologie di ingegnerizzazione proteica innovative



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*Supervisors: Prof. A.  
Negro , Prof. e. Papini*

## Biofilm-surface layer protein A from *Bacillus subtilis*



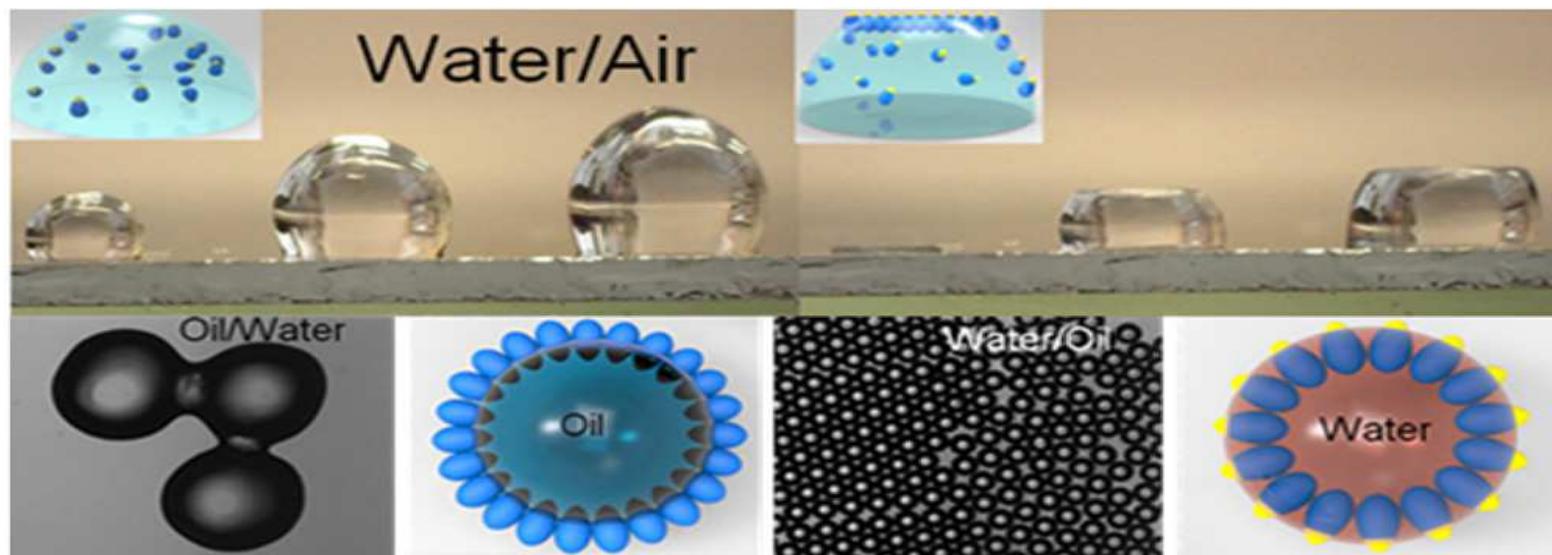
(A) Schematic representations of BsIA structure in ribbon and cartoon form.

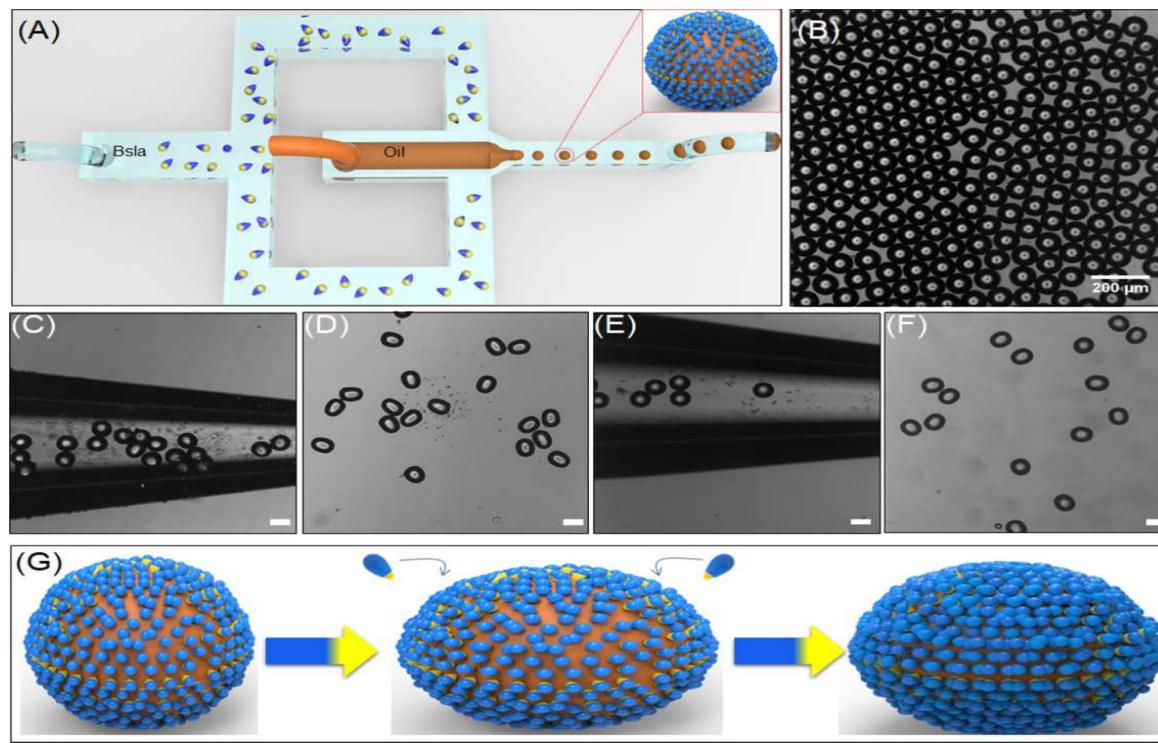
(B–D) Photographs and schematics (inset) showing time evolution of a droplet of an aqueous solution of BsIA (0.06 mg/mL) deposited onto a hydrophobized silicon wafer.

Immediately after placing the drop on the silicon surface the drop has a contact angle of roughly  $115^\circ$ ,  $t = 0$  min. (C)  $t = 5$  min. The contact angle decreased slightly, likely due to protein adsorption onto the hydrophobic substrate, and stabilized around roughly  $90^\circ$ . (D)  $t = 10$  min. The inset schematics show BsIA assembly into a flat film at the top of the droplet as this is where flattening was observed.

Scale bar is 0.5 mm.

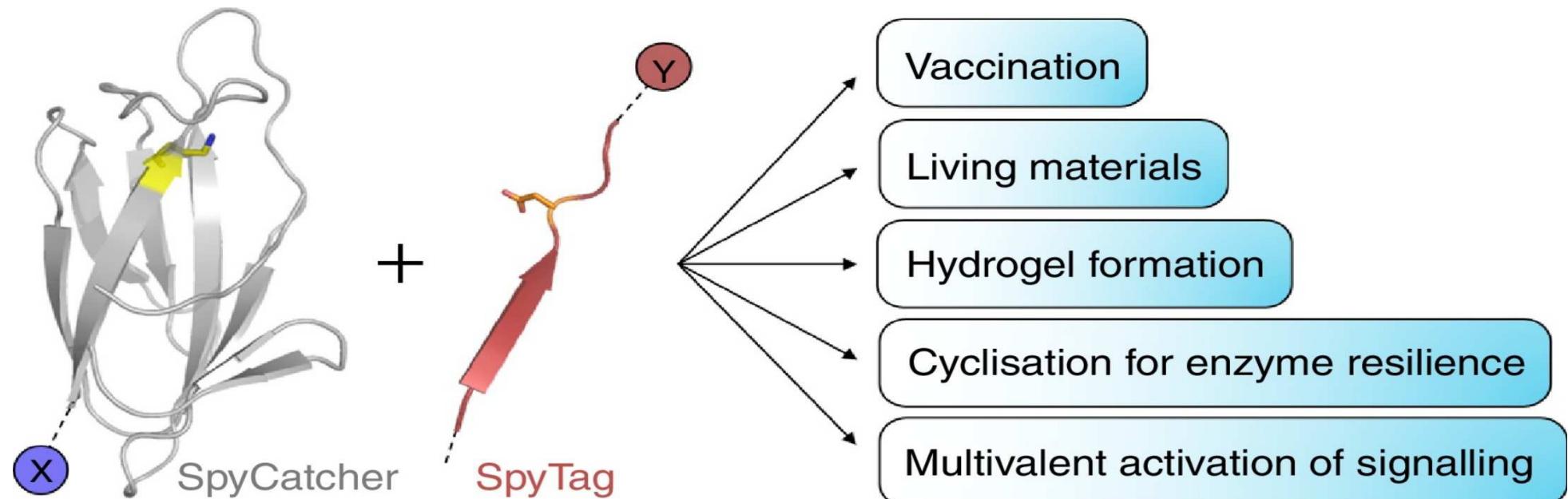
Optical microscopy images of arrested coalescence structures produced by water-in-oil BsIA droplets.



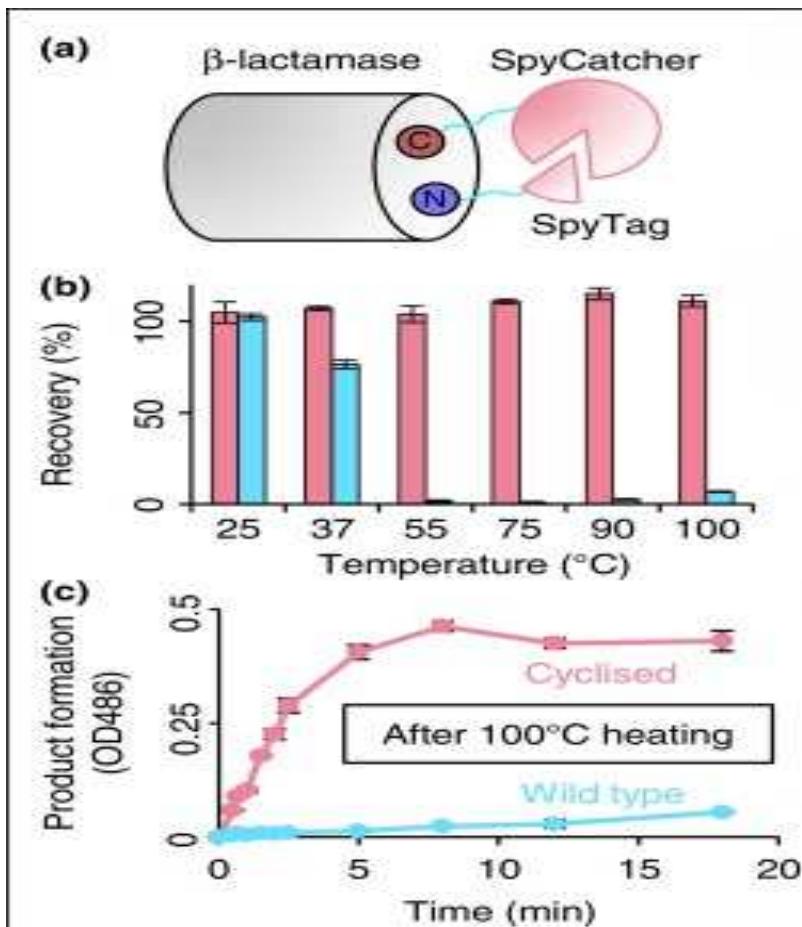


PDMS flow-focusing microfluidic device with an aqueous solution of BsIA as the outer phase and a mineral oil dispersed phase

# Secrets of a covalent interaction for biomaterials and biotechnology: SpyTag and SpyCatcher



## Cyclisation for enzyme resilience



SpyRing generation: the protein of interest is genetically fused with an N-terminal SpyTag and C-terminal SpyCatcher (red), which spontaneously lock together.

(b) SpyRing resilience to aggregation.  $\beta$ -lactamase were heated to the indicated temperature,

c) Preservation of enzyme activity of cyclised (red) compared to wild type  $\beta$ -lactamase (blue) at room temperature, following heating to 100 °C