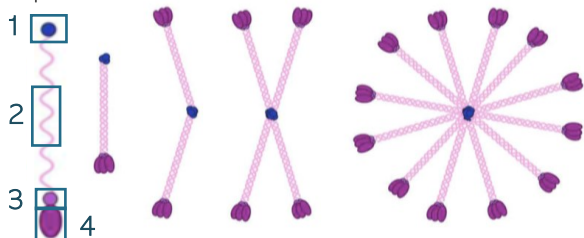


SURFACTANT PROTEIN D

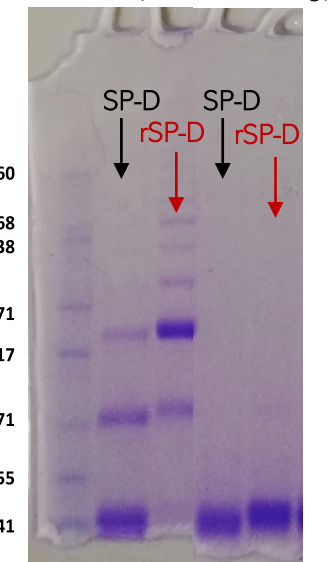
The protein is a **natural defence** against the airway's pathogens: it is described as the key player of the active regulation of immune response in the pulmonary environment.¹ The **structure** is characterized by four distinct domains and depending on its supramolecular assembly could elicit an anti/pro-inflammatory response.²



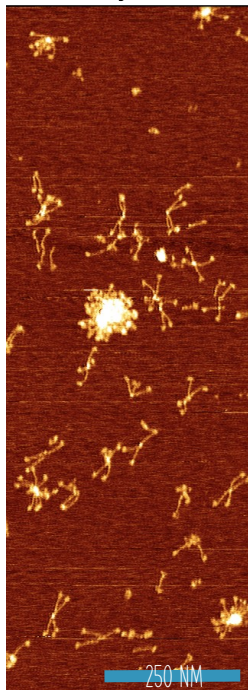
PROTEIN CHARACTERIZATION → (1) SP-D (2) rSP-D

SDS PAGE (Protein: 2.5ug)

AFM analysis (rSP-D)



+ reducing agent



(1) Front Immunol. 2019, 10, 2264
(2) Eur J Clin Invest 2006, 36, 423-435
(3) Mol. Imm. 2006, 43 (9), 1293-1315
(4) Journal of Bio Chem, 2012, 287, 39578 - 39588

PROTEIN NANO-CONJUGATION

Composition	% mol
DPPC	62
Chol	33
DSPE PEG 2000	4
DSPE PEG 2000 funzionalized	1

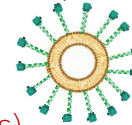
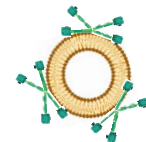
Liposome PEGilated post-insertion (Maleimide-liposomes)

→ TO LINK Cys 15/20 OF SP-D



Liposome PEGilated pre-insertion (COOH-liposomes)

→ TO LINK Lys OF rSP-D



NANOCONJUGATED CHARACTERIZATION

DINAMIC LIGHT SCATTERING ANALYSIS

APPROVED

Mean	Liposomes	Liposomes+rSP-D
Intensity (nm)	138.2 ± 7.72	169.4 ± 5.73
PDI	0.04 ± 0.02	0.09 ± 0.09
Z potential (mV)	-37.9 ± 0.78	-19.3 ± 0.40

SIZE EXCLUSION CHROMATOGRAPHY (Abs 300/280)

Fraction	PEG liposome (%)	rSP-D (%)
3	22.20	62.52
4	88.99	13.80
5	6.60	10.17

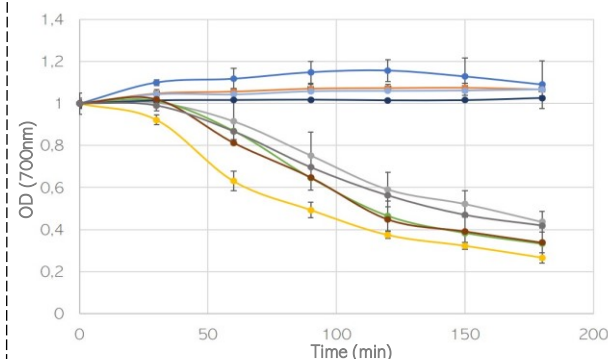
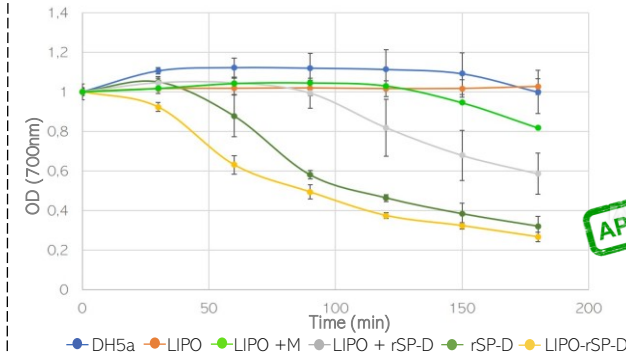
→ HOW TO PURIFY THE LIPOSOMES?
→ HOW TO VERIFY THE CONJUGATION?



IN VITRO EXPERIMENTS

AGGLUTINATION ASSAY

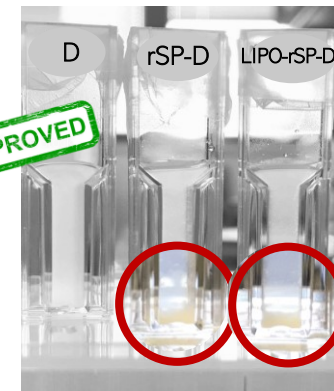
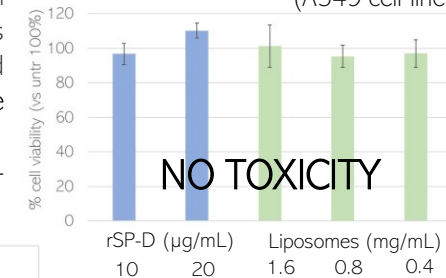
SP-D is "carbohydrate pattern recognition molecules" and interacts with glycoproteins on the surface of pathogens (e.g. virus and bacteria) through their carbohydrate recognition domain (CRDs).³ SP-D has been shown to increase calcium-dependent agglutination of E.coli(DH5α).⁴



CHECK POINT

- ✓ Characterization of SP-D and rSP-D
- ✓ Synthesis and characterization of Liposome protein conjugated
- ✓ Confirm the agglutination activity of protein and nanoconjugate

MTT ASSAY FOR CELLS VIABILITY (A549 cell line)



liposome with different size

APPROVED

NEXT STEPS

- Study a method of purification and characterization of liposome conjugated
- Verify the activity of protein and liposome vs SARS-CoV-2

