

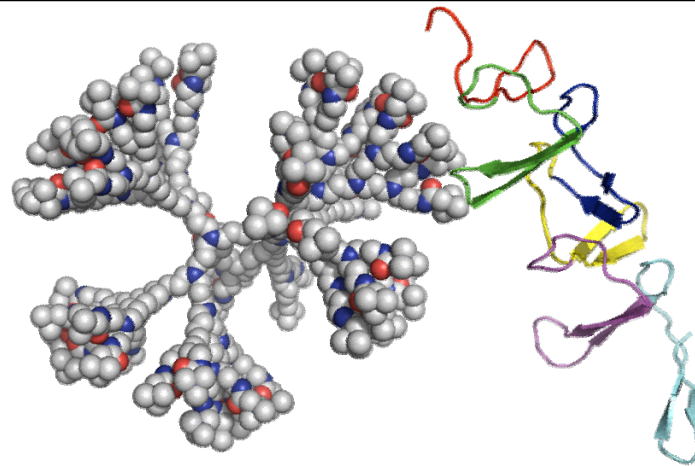
Protein Biotechnology Laboratory

Instituto de Biología Molecular y Celular

Universidad Miguel Hernández

Elche (Alicante)

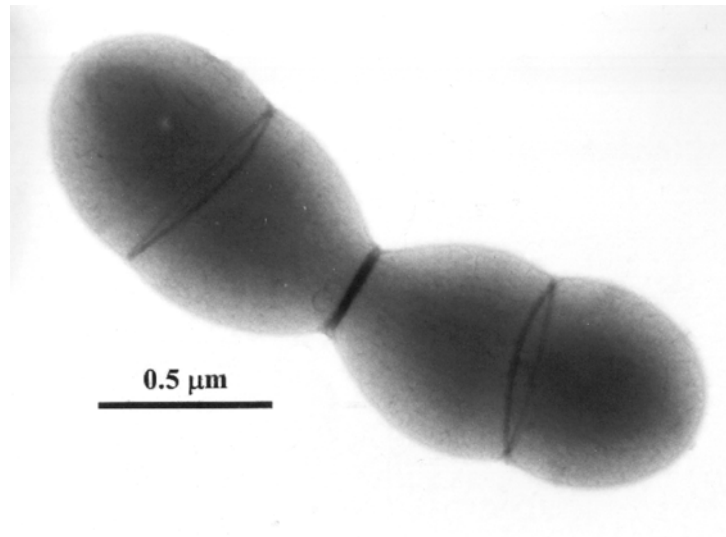
Choline dendrimers as versatile tools for the development of new antibiotics and tissue imaging



Jesús M. Sanz

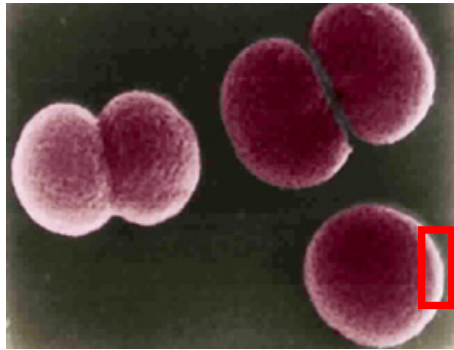
Málaga, March 26th, 2010

Streptococcus pneumoniae (pneumococcus)

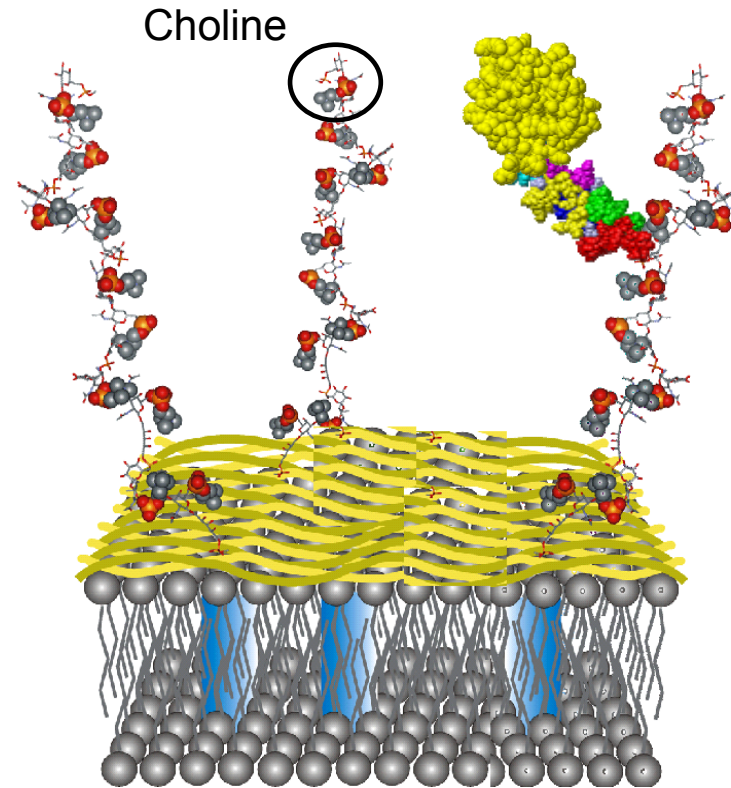


- 10-25% pneumonia cases in developed countries
- 25% mortality
- 4th cause of death in the world (World Health Organization)
- Responsible of bacterial child meningitis
- 4 million deaths in children under 5 (yearly)

Maestro & Sanz, Recent Patents Anti-Infect Drug Disc, 2007, 2, 188-196



Streptococcus pneumoniae



Choline-binding proteins (CBPs)



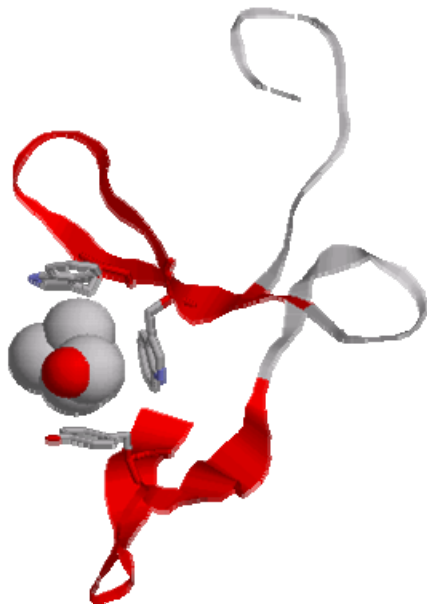
Functional domain

Choline-binding domain

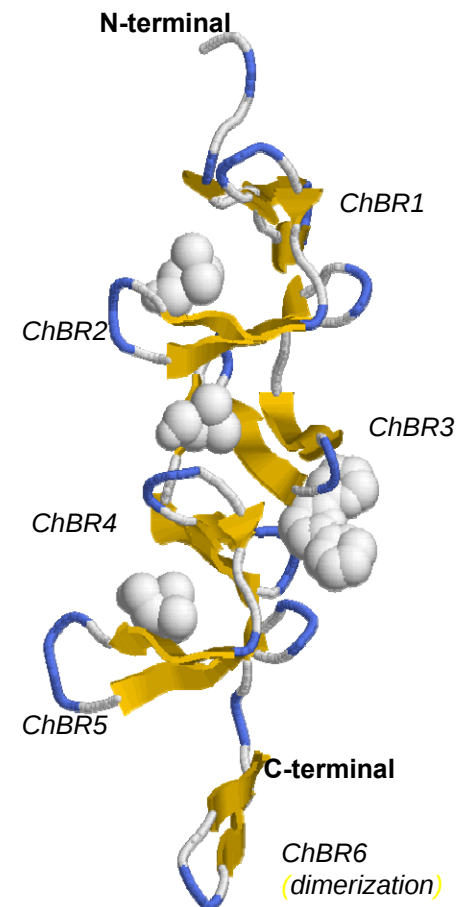
- Peptidoglycan hydrolases
- Phosphorylcholinesterases
- Adhesion to host
- Toxin release

Structure of C-LytA (15 kDa)

- The choline-binding repeats (CBRs) are β -hairpins
- Two consecutive CBRs configure a binding site
- The hairpins conform an extended superhelix - the $\beta\beta$ -3-solenoid



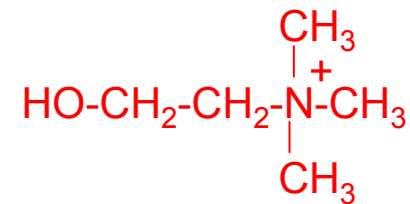
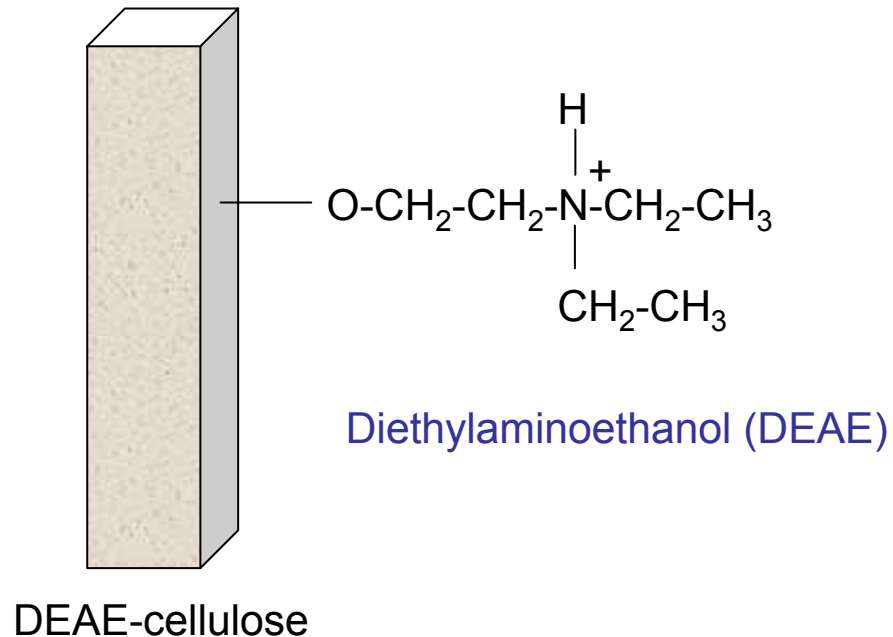
C-LytA



Protein purification/immobilization

Choline-binding proteins do not exclusively recognize choline

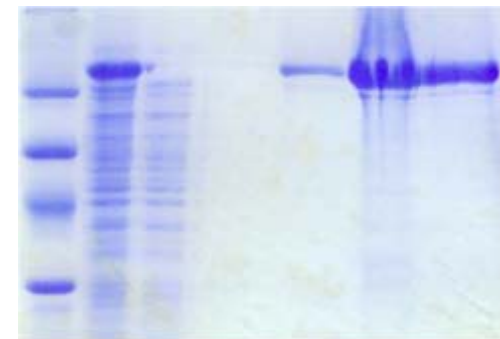
Sanz *et al.*, FEBS lett. 232, 308-312 (1988)



Choline

Purification of fusion proteins in DEAE

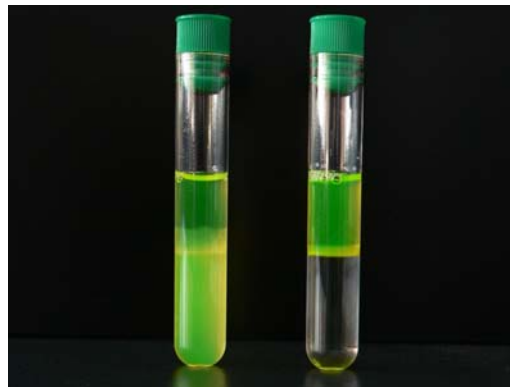
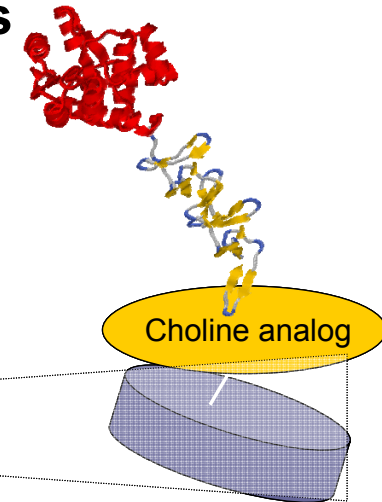
NH_2 - **Choline-binding module** **Protein** - COOH



Sanchez-Puelles *et al.*, Eur. J. Biochem. 203, 153-159 (1992)
Patents ES 2 032 717, ES 200700281

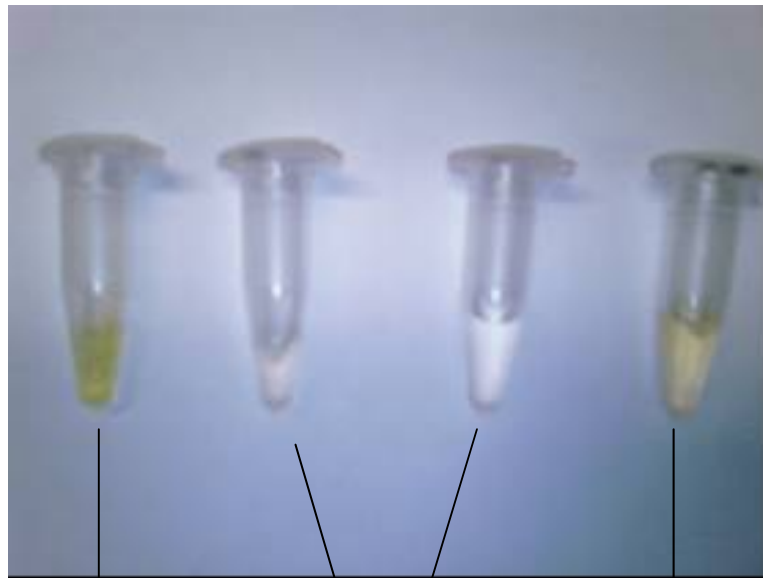
Proteins containing choline-binding domains are easily immobilized

Biosensors



Aqueous two-phase systems

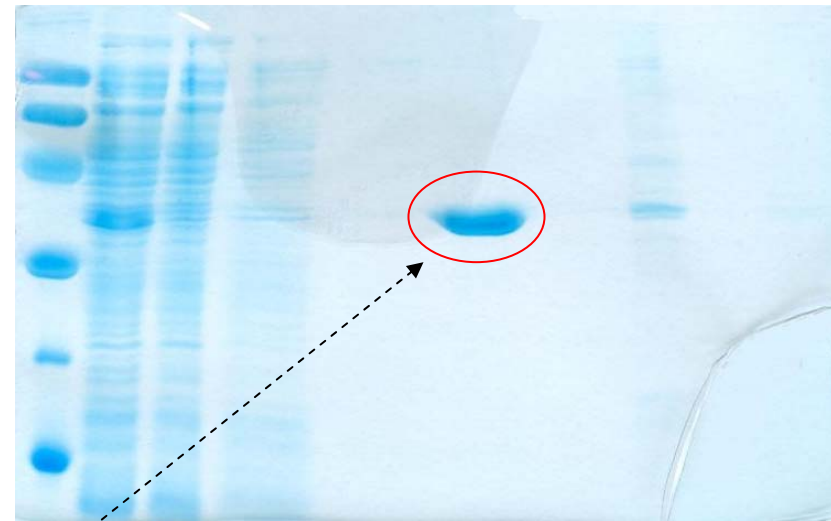
Binding of C-LytA-GFP to DEAE-functionalized
magnetic nanoparticles (≈ 750 nm and ≈ 200 nm)



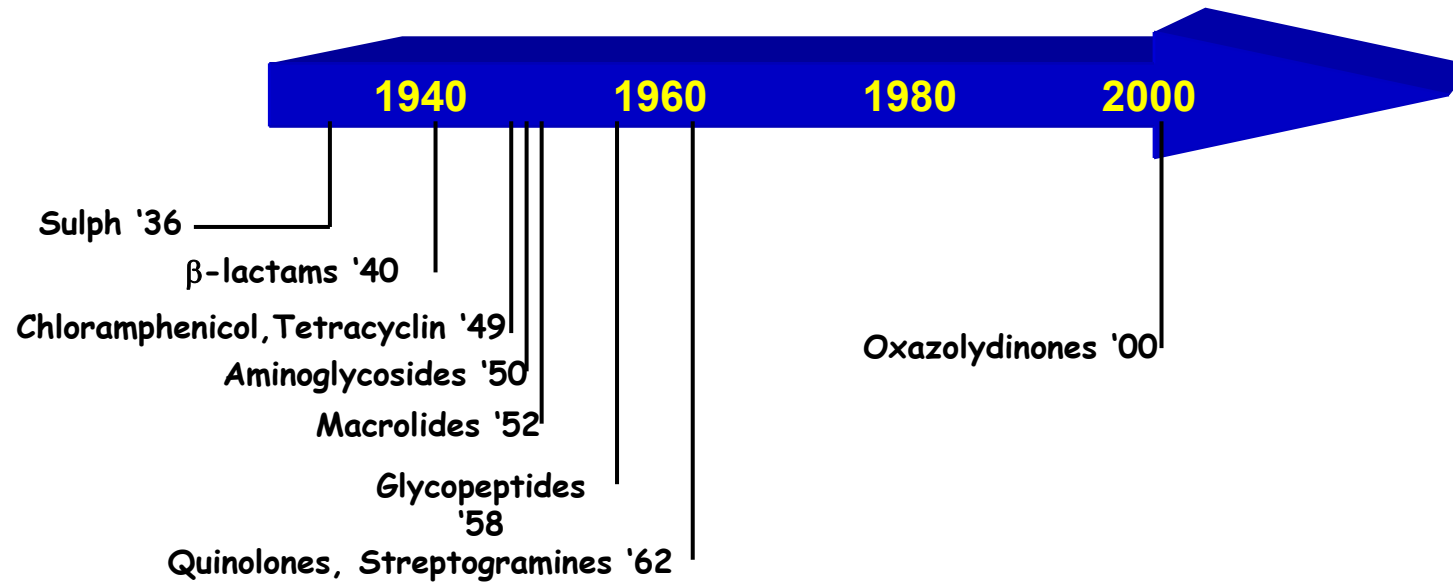
Protein +
particles

1.5M NaCl
washes

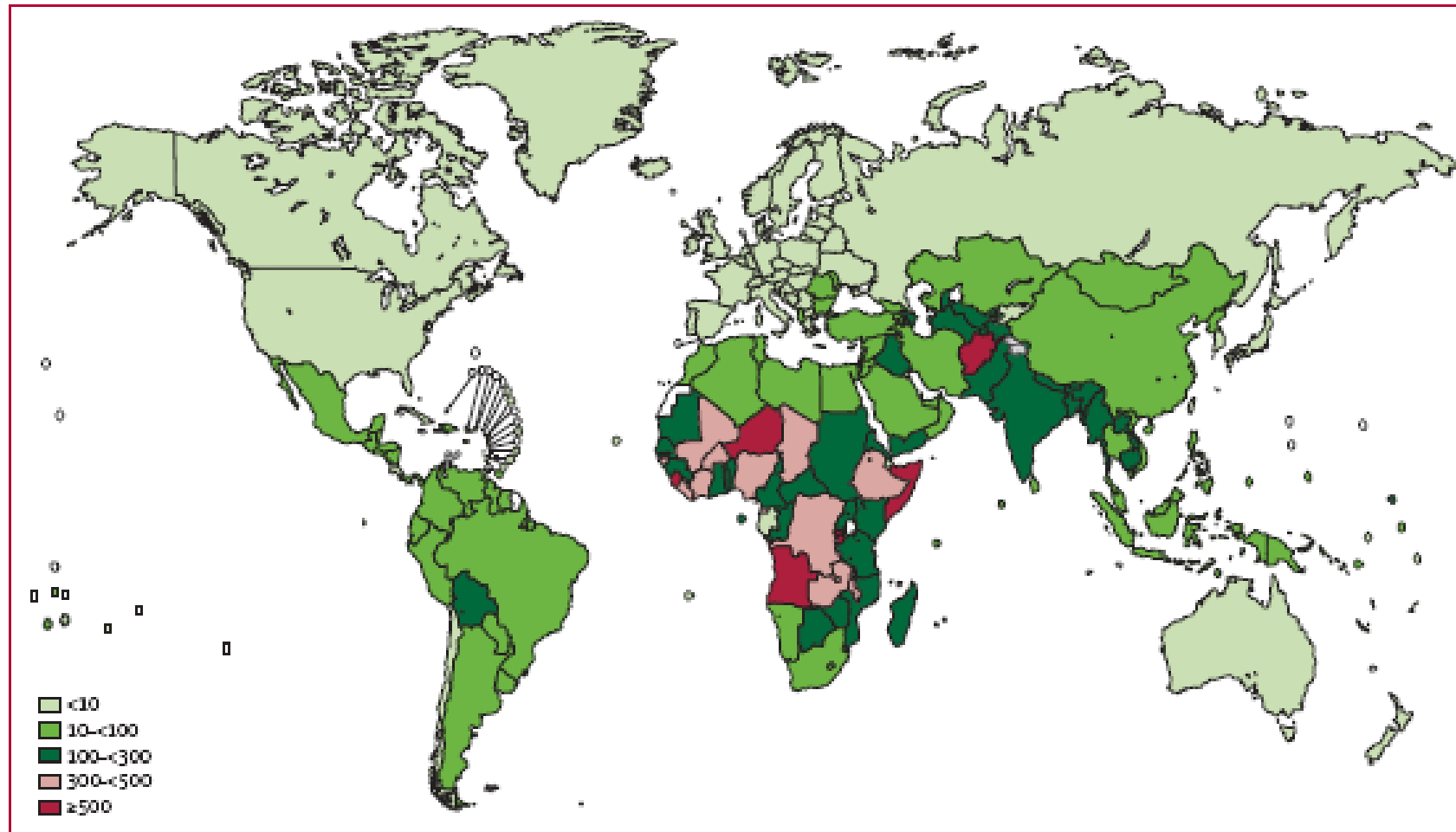
Choline
elution



Discovery of antibiotics

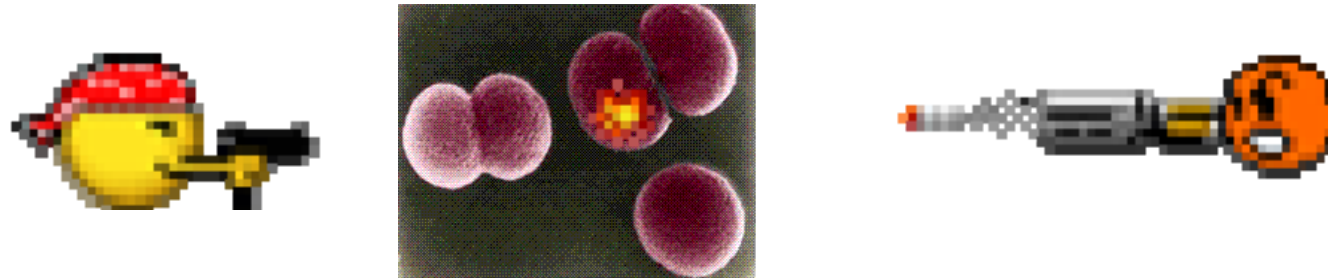


Mortality caused by *Streptococcus pneumoniae* in the world

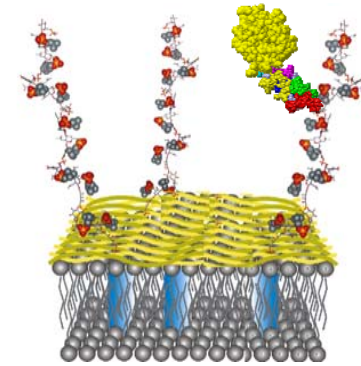


OMS, The Lancet, 374, 893-902 (2009)

Fighting pneumococcus the direct way



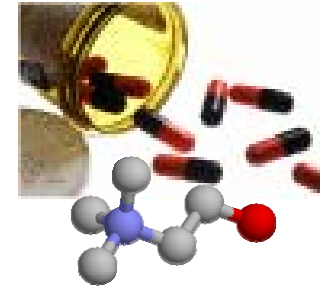
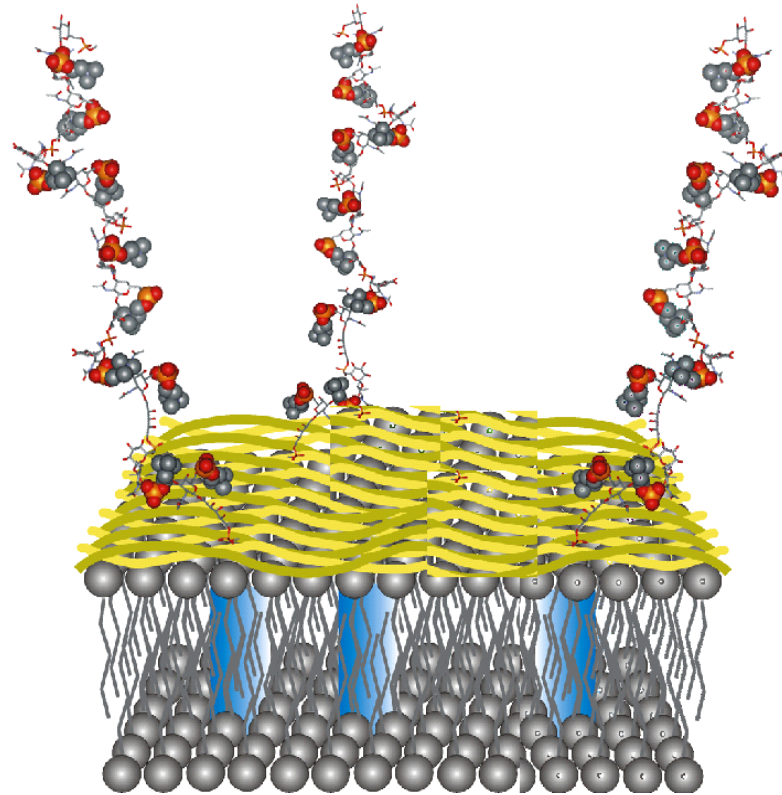
The subtle way: targeting choline-binding proteins



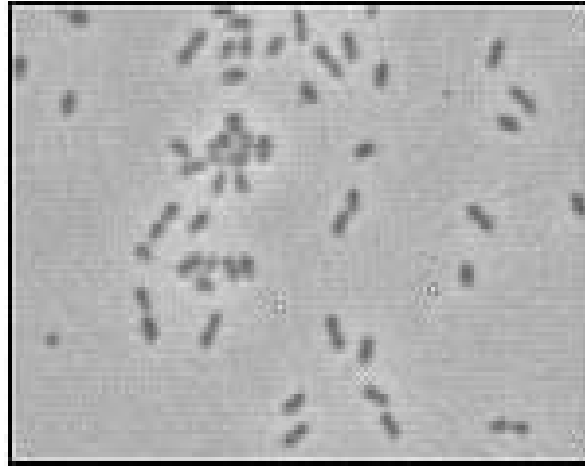
Why CBPs?

- Increasing levels of resistance to traditional antibiotics acting on the cell wall (penicillins) (Spain > 50%)
- Efficiency of current vaccines is lower than desired (90 serotypes to encompass)
- CBPs are common to all pneumococcal serotypes
- CBPs are involved in essential, physiological and virulence processes
- There are many CBPs: the same compound might inhibit all of them: Resistance becomes more difficult
- CBPs may recognize (and be inhibited by) many different molecules (choline analogs)

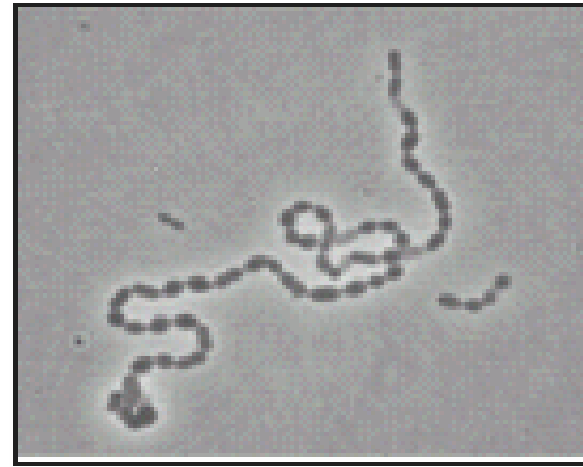
Searching for CBP inhibitors



Searching for CBP inhibitors



Control



+ 50 mM Choline

More easily fagocitable

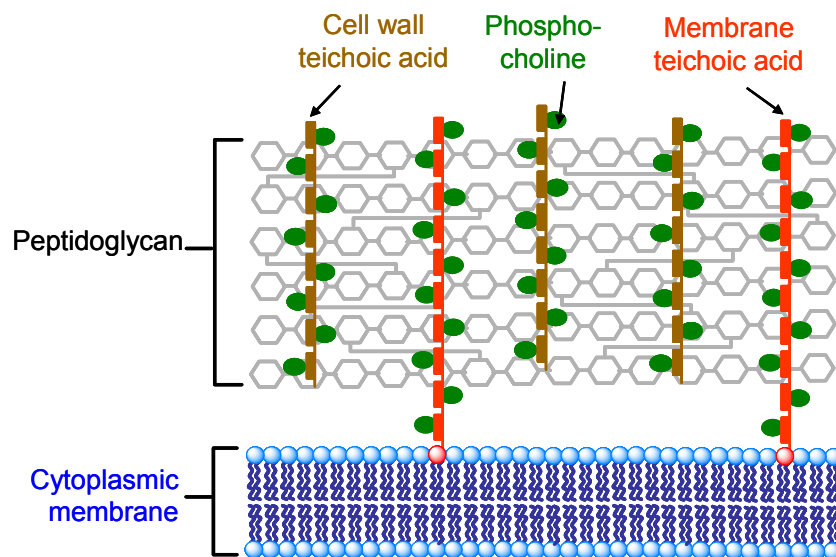
Less infective

Choline cannot be used as therapy:

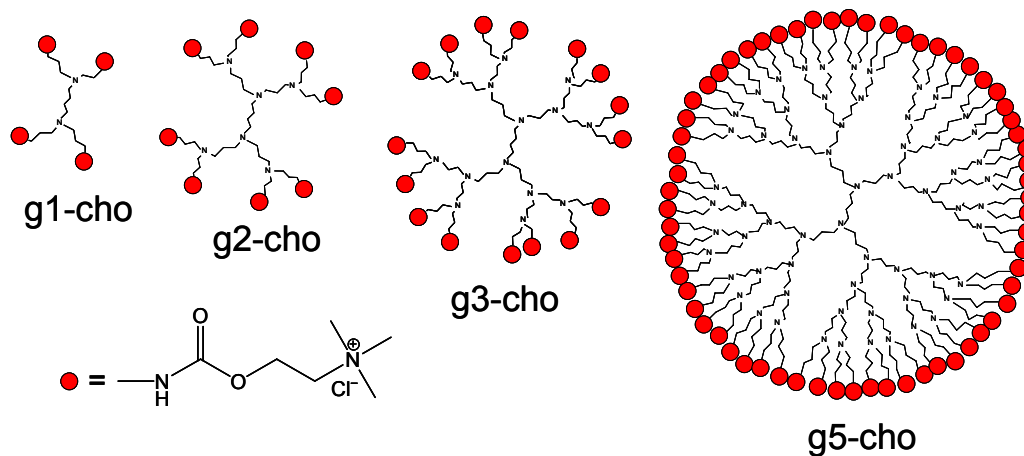
- High (millimolar) concentrations required
- Side interactions with muscarinic receptors

Searching for CBP inhibitors: Dendrimers

Hernández-Rocamora *et al.* Angew. Chem. Int. Ed. 48, 948-951 (2009)

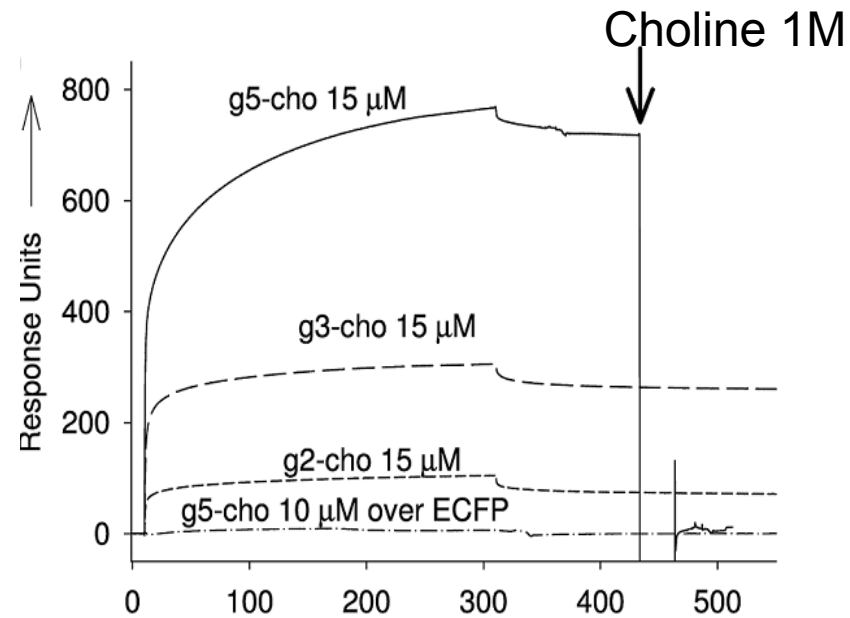


Cell wall



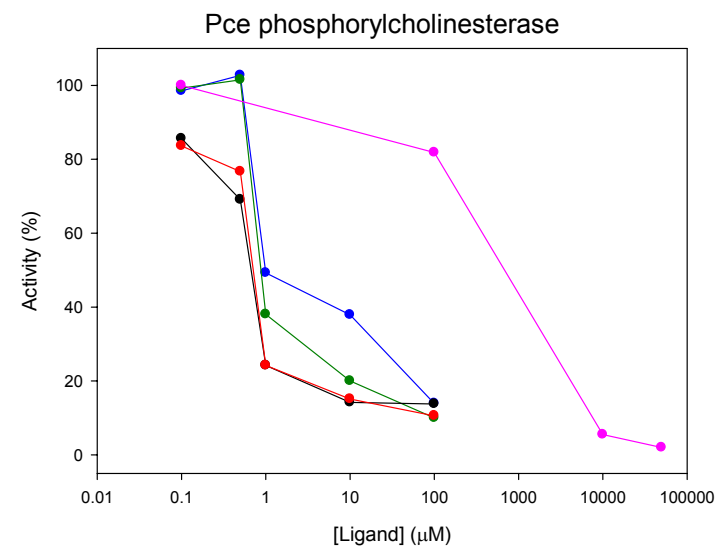
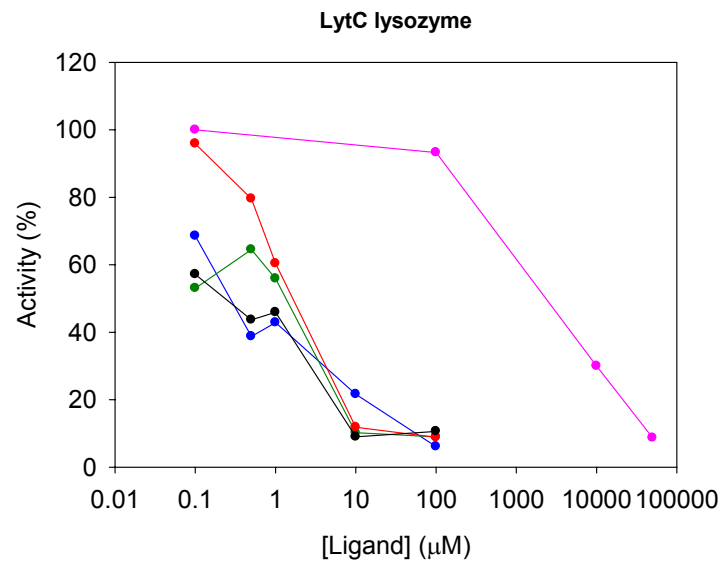
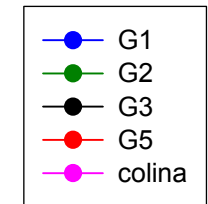
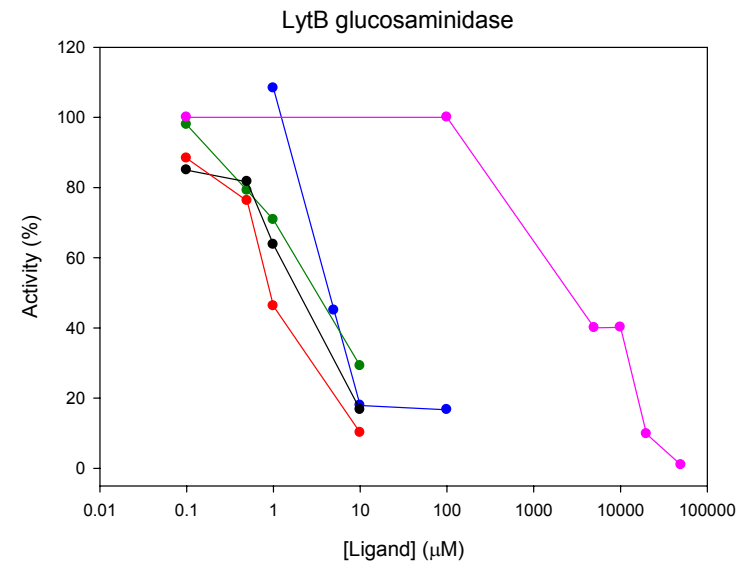
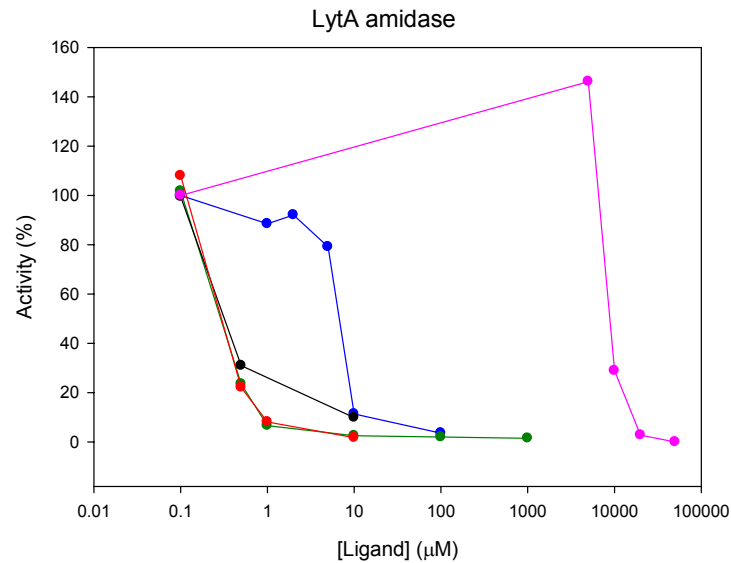
Dendrimers as *mimics* of cell wall

Surface plasmon resonance (immobilized C-LytA)

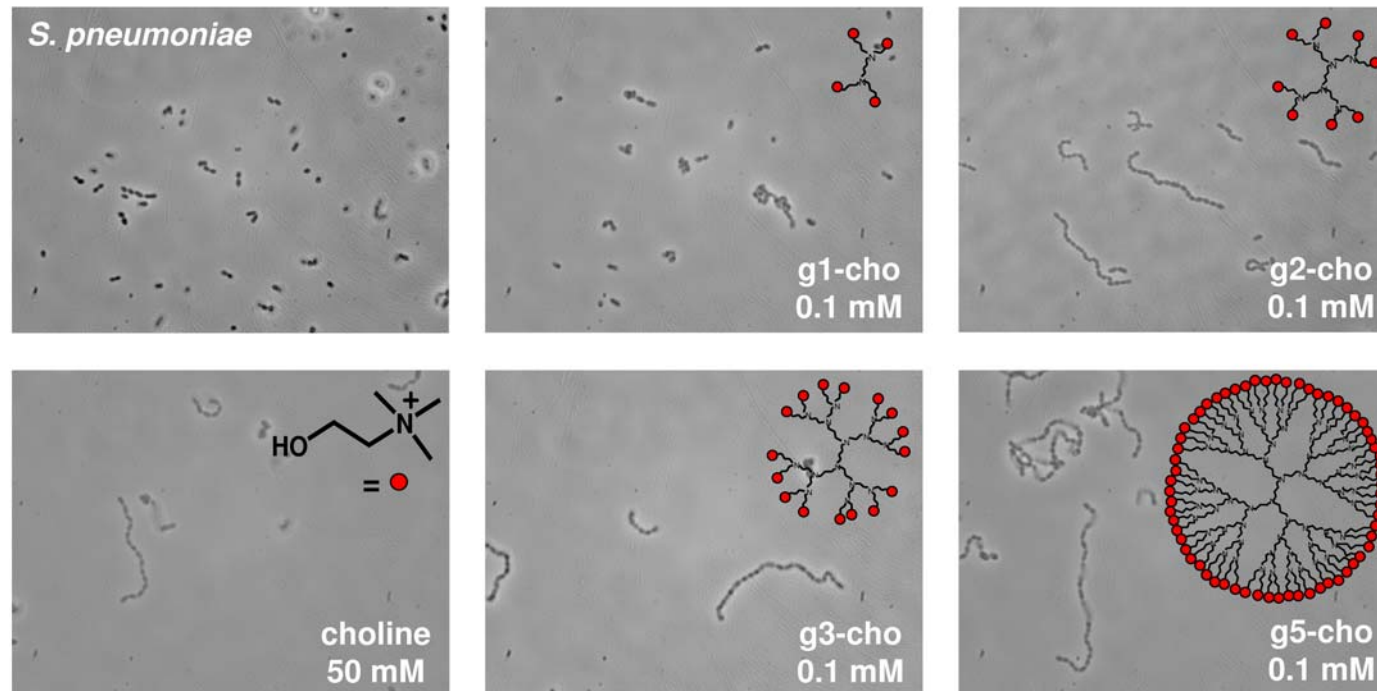


Analyte	Ligand	Kd_1 (μ M)	Kd_2 (μ M)	analyte : ligand
g1-cho	C-Lyt-GFP	4.1 ± 1.0	263 ± 52	2.3
g2-cho	C-Lyt-GFP	0.15 ± 0.05	23 ± 5	2.3
g3-cho	C-Lyt-GFP	0.05 ± 0.01	2.5 ± 0.8	1.7
g5-cho	C-Lyt-GFP	0.023 ± 0.004	3.0 ± 0.4	1.1

Searching for CBP inhibitors: **Dendrimers**



Searching for CBP inhibitors: Dendrimers



- Only **micromolar** concentrations required...
- Dendrimers increase the affinity of choline by **1000-10000 times**

Development of new dendrimers

Core:

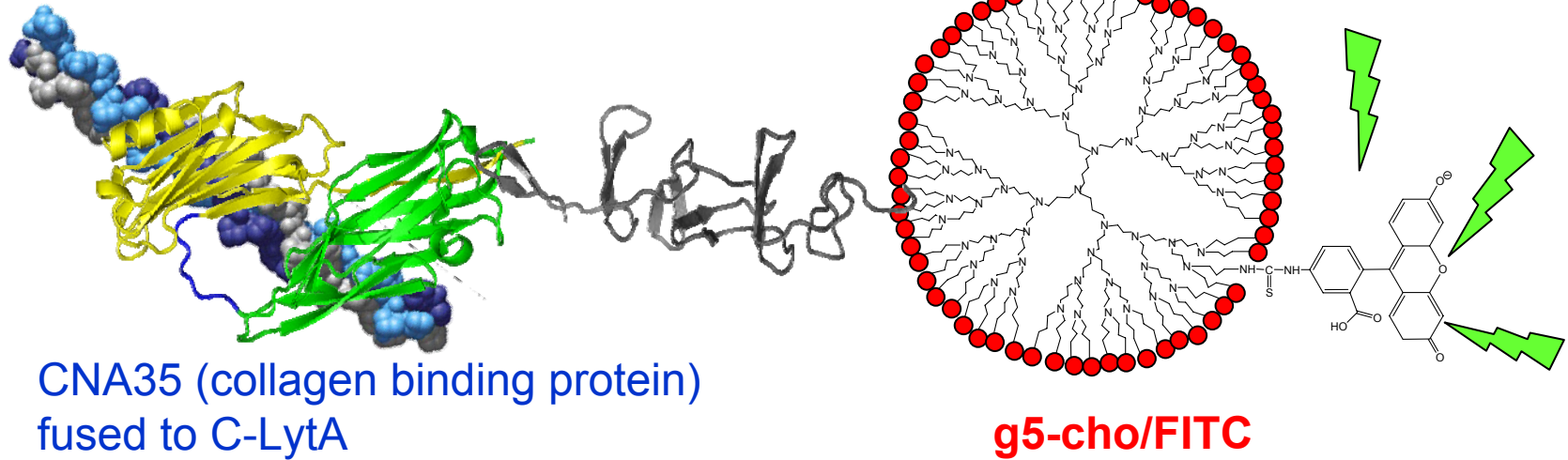
- PAMAM (Total positive charge is reduced)
- PEG (less toxicity)

Functionalization:

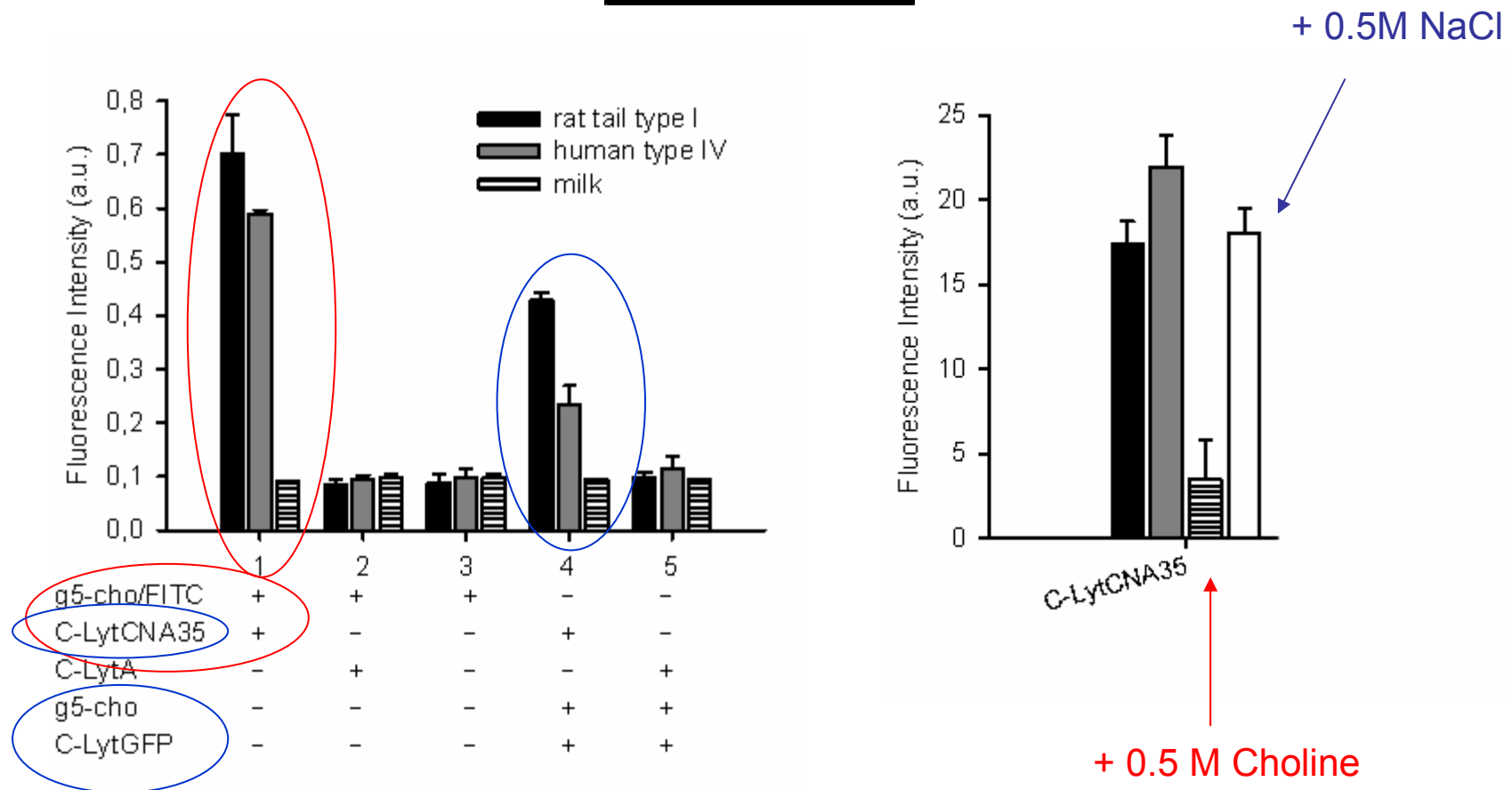
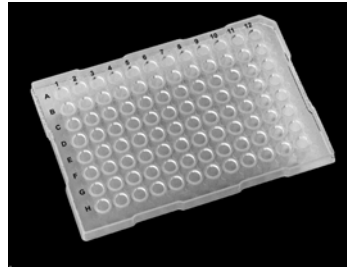
- Choline
- Tertiary amines (DEAE) (modulación of charge by pH)

Visualization of tissues: collagen fibers

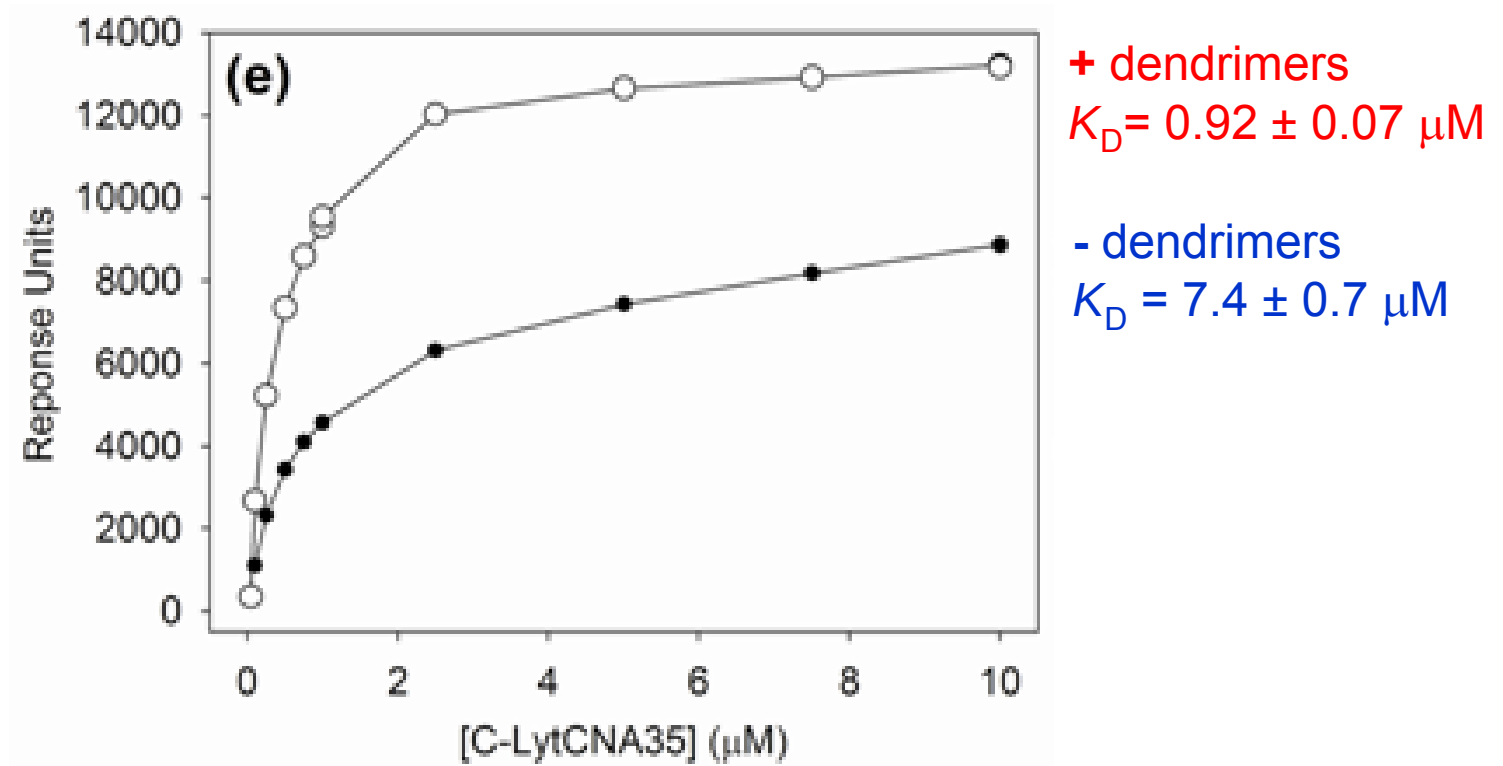
Collagen



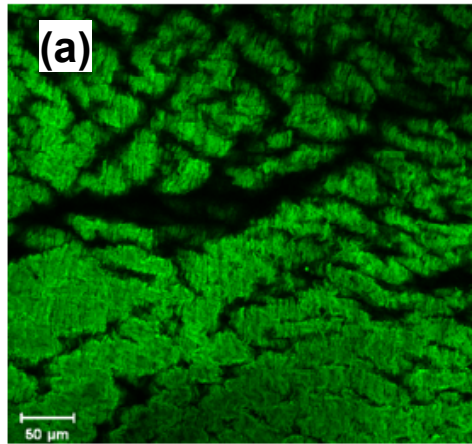
Solid-phase binding assays demonstrate specificity of interaction



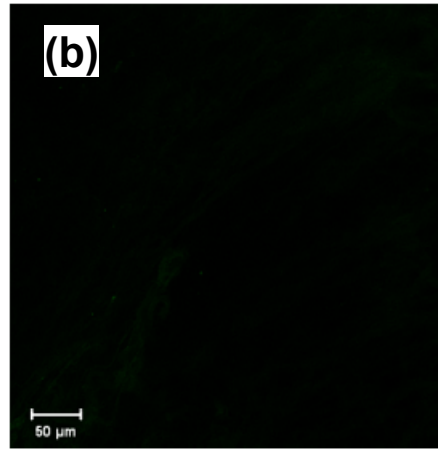
Surface plasmon resonance on immobilized collagen



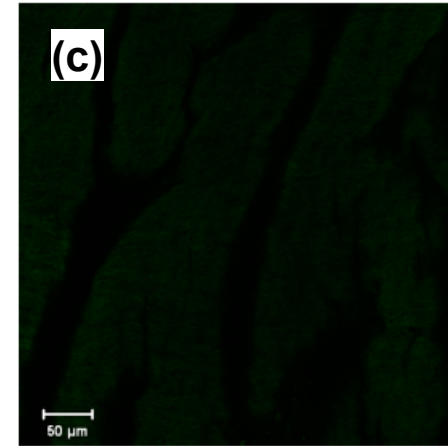
Visualization of collagen in porcine pericardium



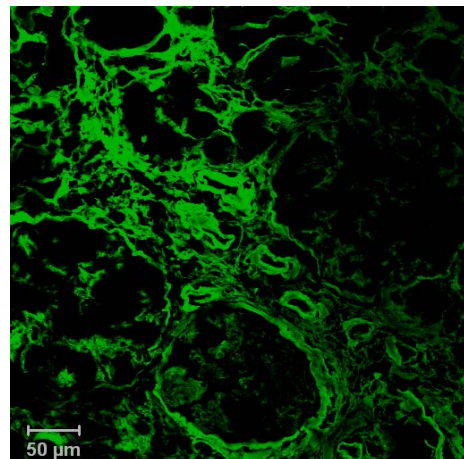
5 μM C-LytCNA35
0.5 μM g5-cho/FITC



5 μM C-LytA
0.5 μM g5-cho/FITC



No protein
0.5 μM g5-cho/FITC



Human kidney

External collaborations

Centro de Investigaciones Biológicas - CSIC (Madrid, Spain)

- Ernesto García
- Pedro García
 - *Microbiological effects of antimicrobials*

Eindhoven University of Technology (Eindhoven, The Netherlands)

- Maarten Merkx
 - *Synthesis of dendrimers*
 - *Collagen binding assays*

Unidad de Química Combinatoria (Parque Científico de Barcelona)

- Miriam Royo
- Fernando Albericio
 - *PEG dendrimers*