

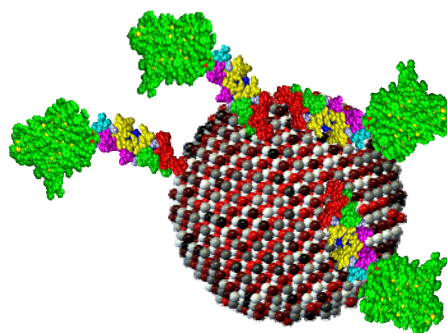
Grupo de Biotecnología de Proteínas

Instituto de Biología Molecular y Celular

Universidad Miguel Hernández

Elche (Alicante)

Specific immobilization and purification of recombinant proteins using diethylaminoethy (DEAE)-functionalized magnetic nanoparticles



Jesús Miguel Sanz

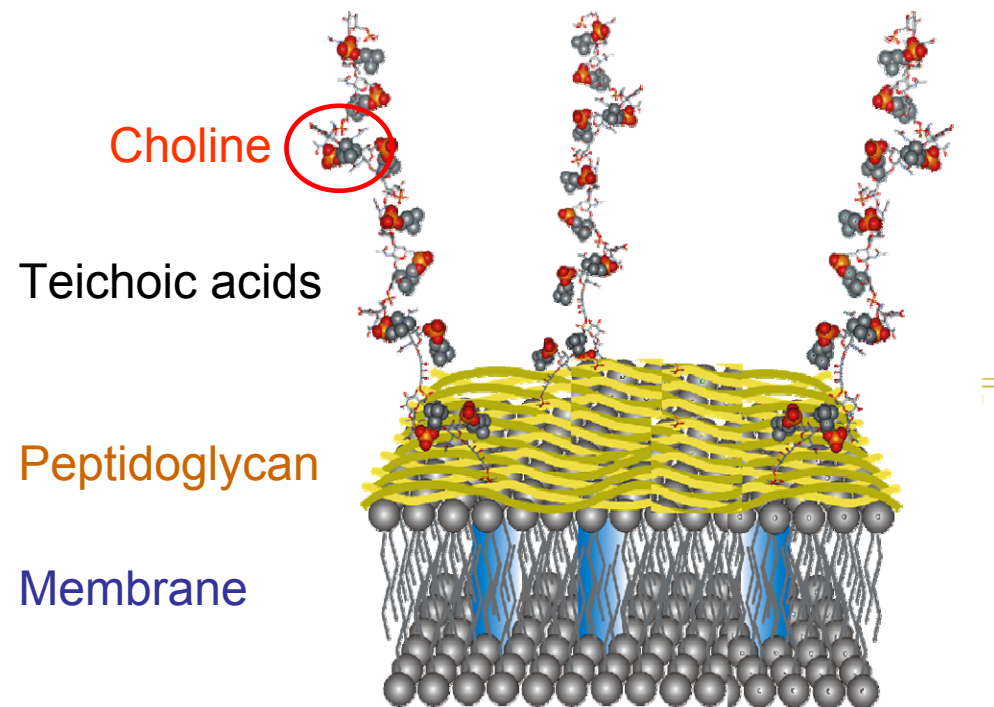
jmsanz@umh.es

<http://adan-embl.ibmc.umh.es/jmsanz/jmsanz.htm>

Imaginenano, Bilbao, 13-April-2011

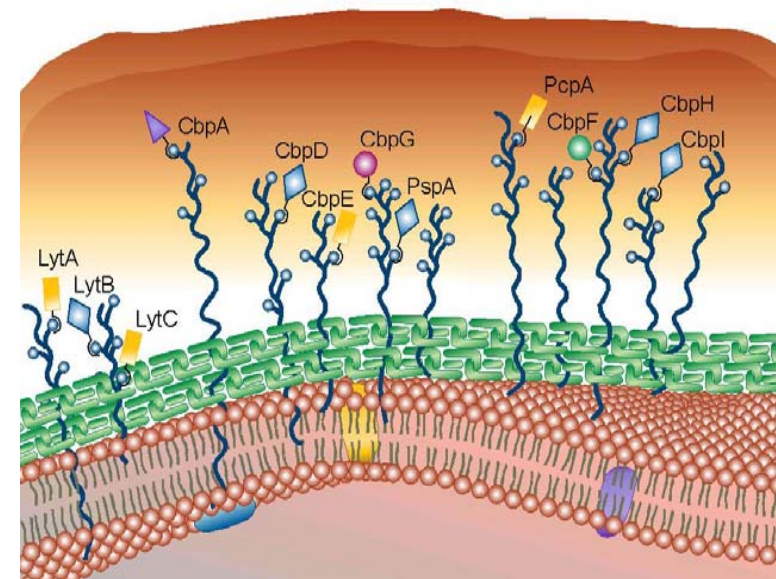


Streptococcus pneumoniae
(pneumococcus)



CBP relevance

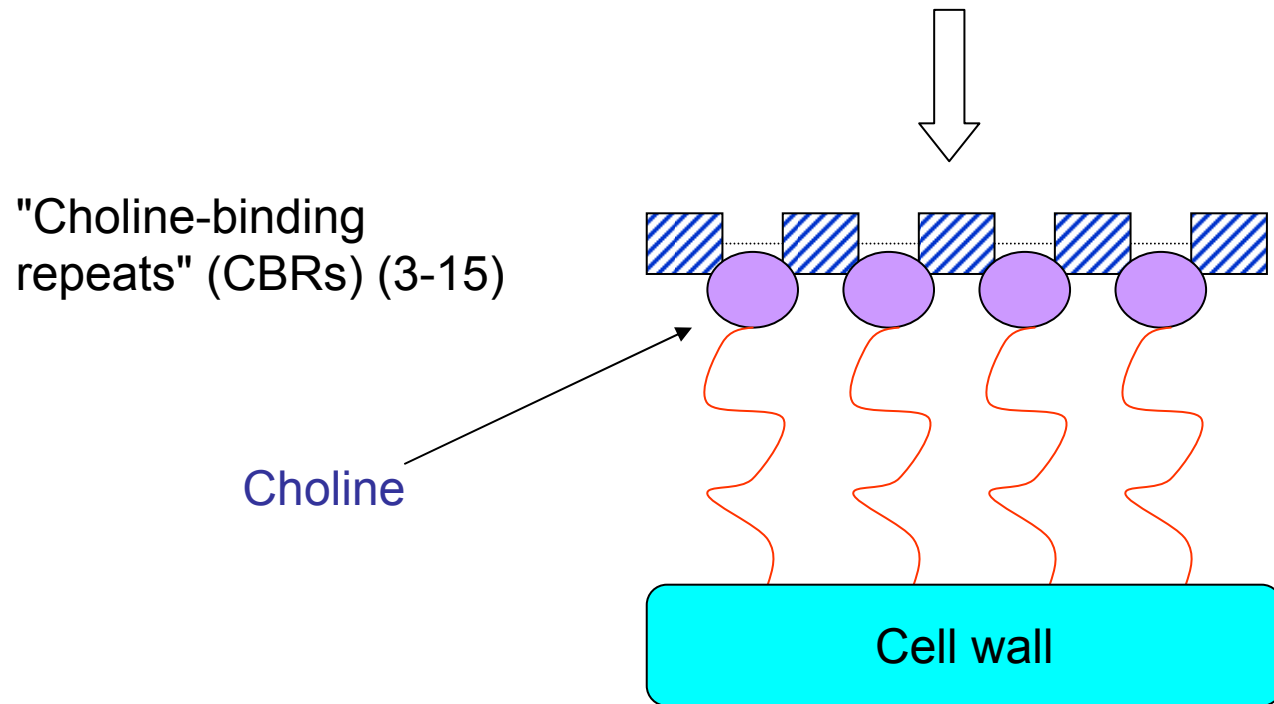
- Cell viability
- Infectivity
- Toxin release
- Lysis of cell wall (cell division)



Choline-binding proteins (CBPs)

Choline-binding proteins (CBPs)

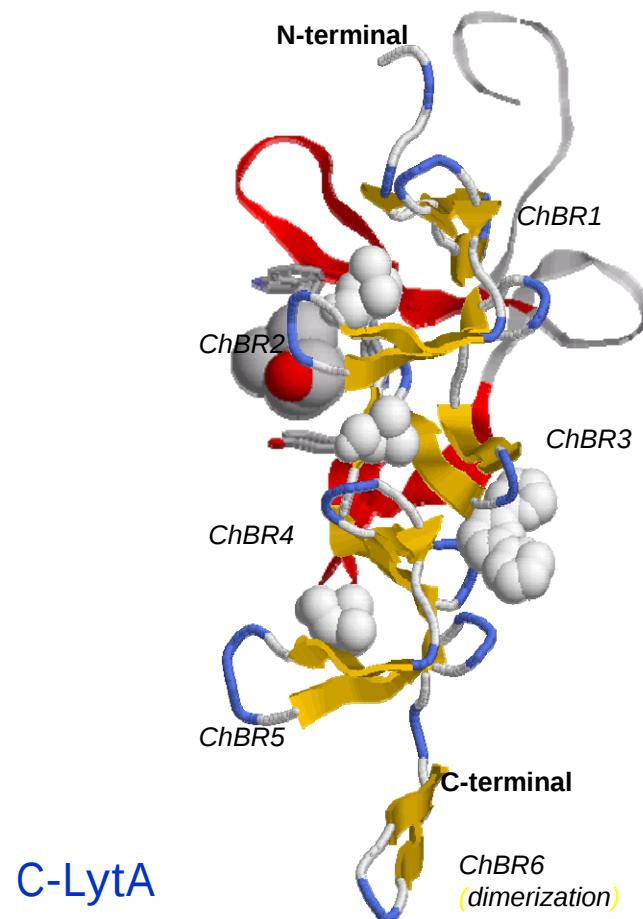
15 members described so far



C-LytA module

Structure of C-LytA (15 kDa)

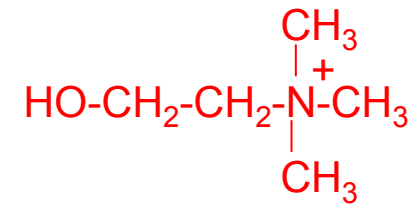
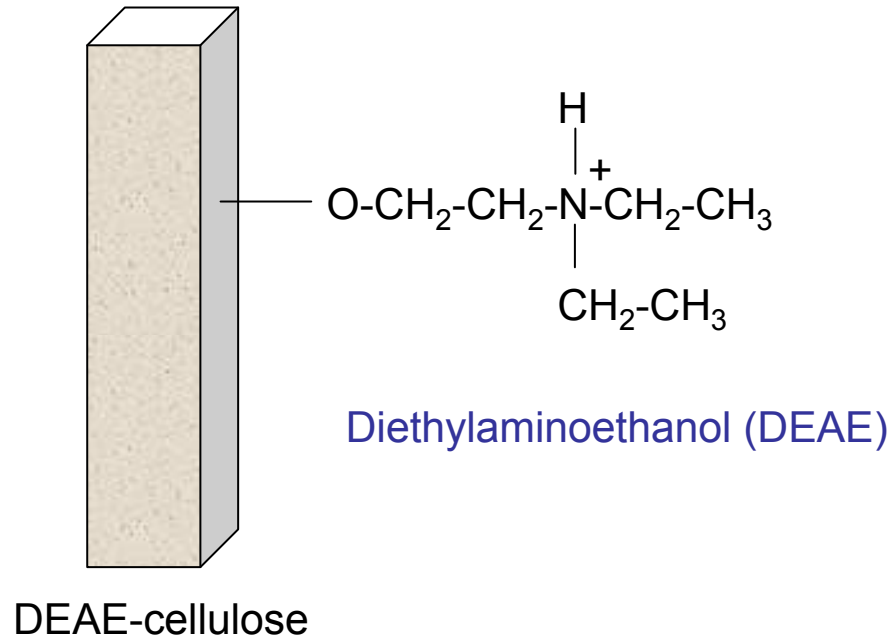
- The choline-binding repeats (CBRs) are β -hairpins
- Two consecutive CBRs configure a binding site
- The hairpins conform an extended superhelix - the $\beta\beta$ -3-solenoid



CBMs as affinity tags for protein purification/immobilization

Choline-binding proteins do not exclusively recognize choline

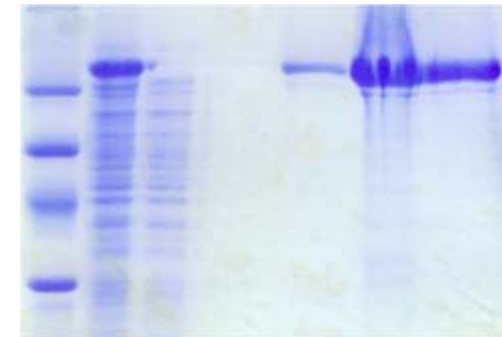
Sanz *et al.*, FEBS lett. 232, 308-312 (1988)



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

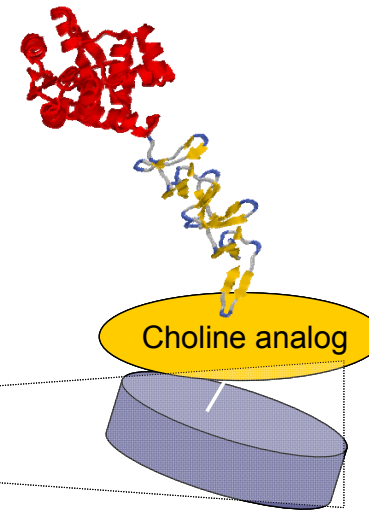
Proteins containing choline-binding domains are easily immobilized



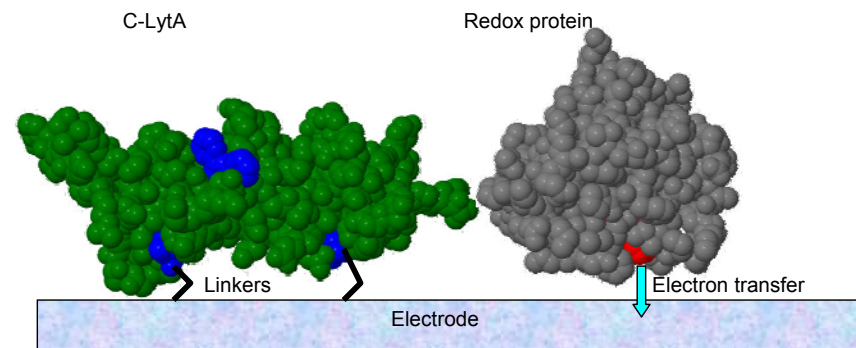
DEAE-Paper



Microcolumns



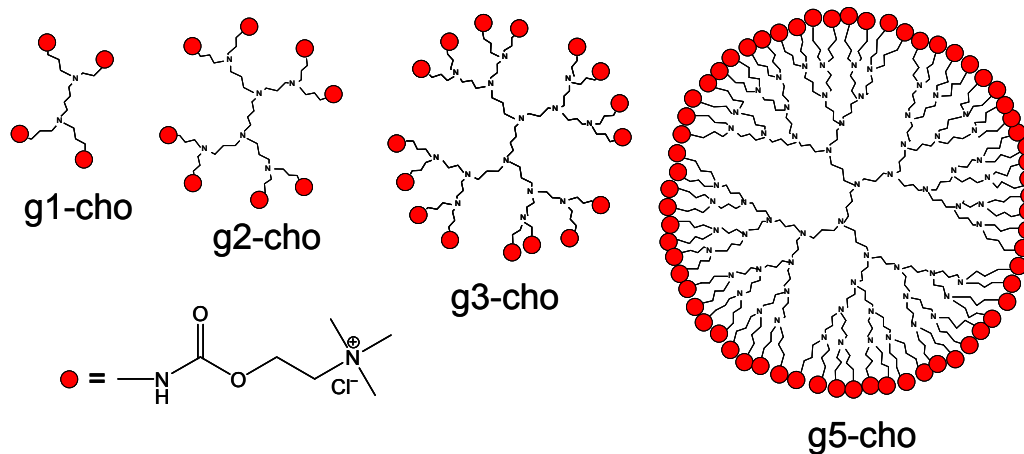
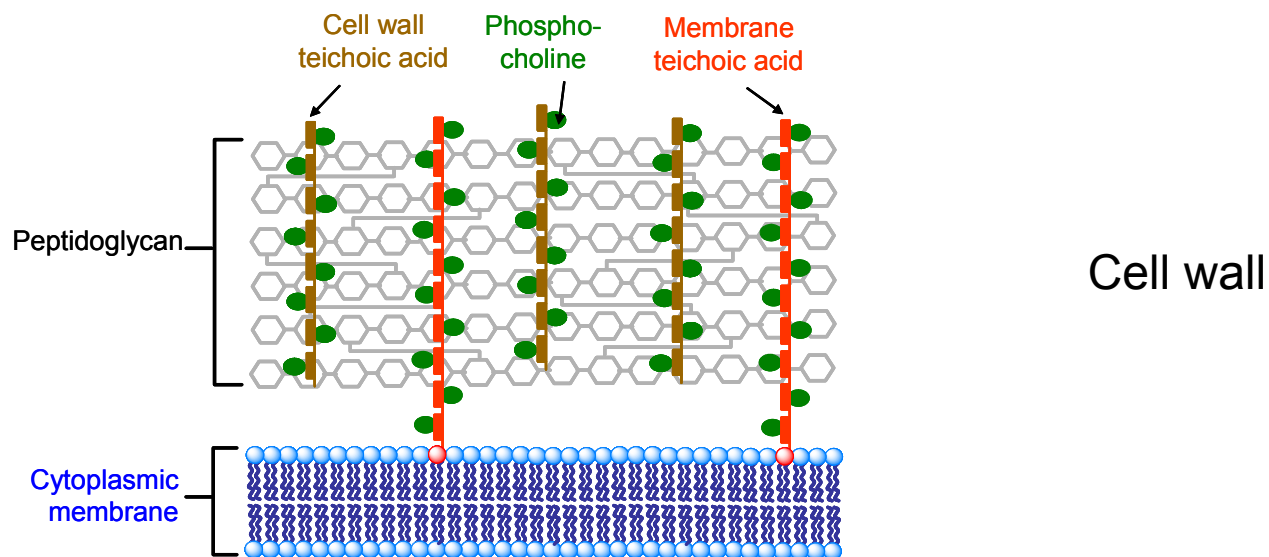
Protein arrays/biosensors



Enzymatic electrodes

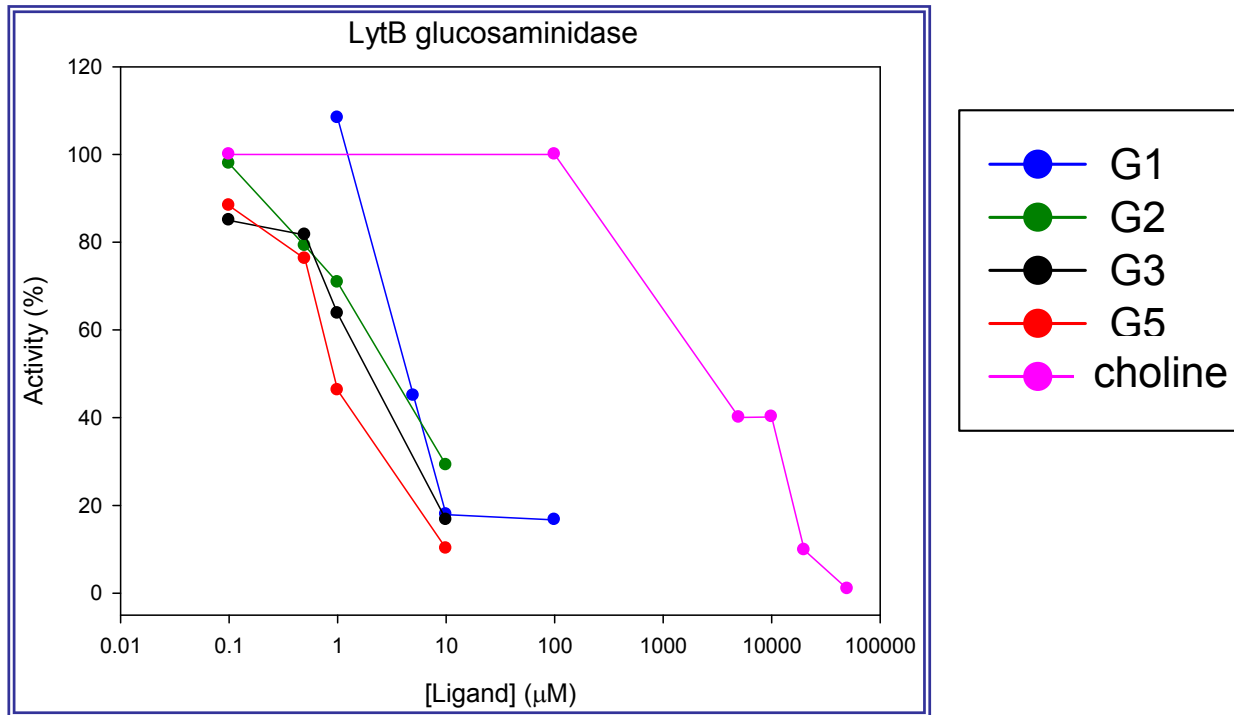
Bioinspired nanomaterials: Choline PPI dendrimers

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



Dendrimers as *mimics* of cell wall

Multivalent disposition of choline enhances CBP affinity

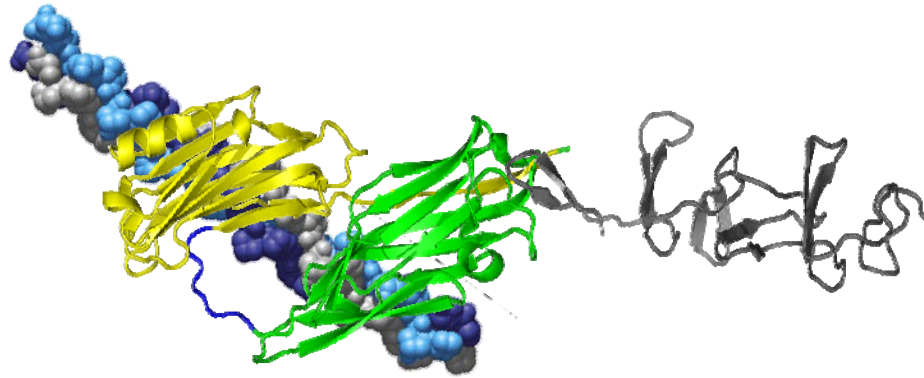


Dendrimer	Protein	Kd (μM)
g1-cho (4 cholines)	C-LytGFP	4.1 ± 1.0
g2-cho (8 cholines)	C-LytGFP	0.15 ± 0.05
g3-cho (16 cholines)	C-LytGFP	0.04 ± 0.01
g5-cho (64 cholines)	C-LytGFP	0.02 ± 0.01

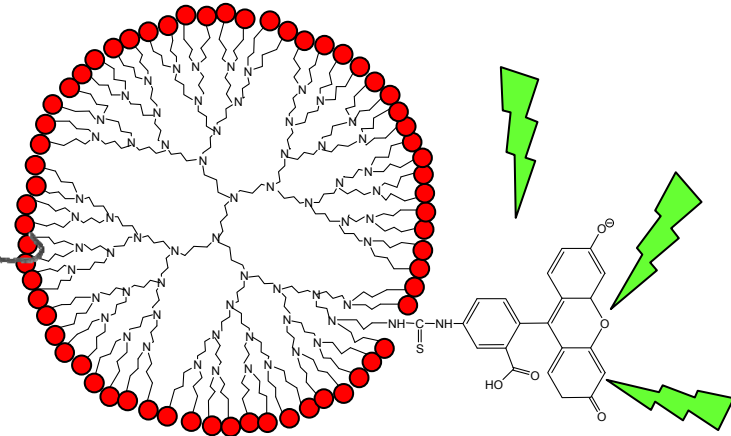
Tissue imaging using CBM-choline dendrimer cassettes

Hernández-Rocamora *et al.* Chem. Comm. in press (2011)

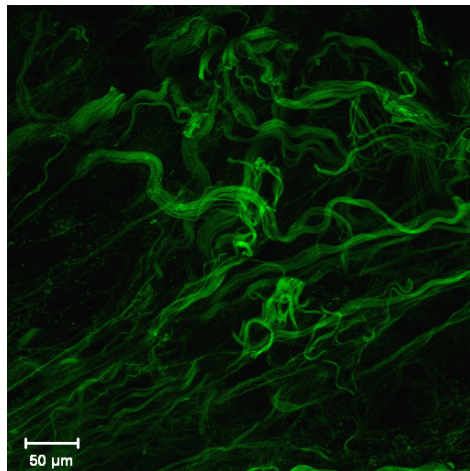
Collagen



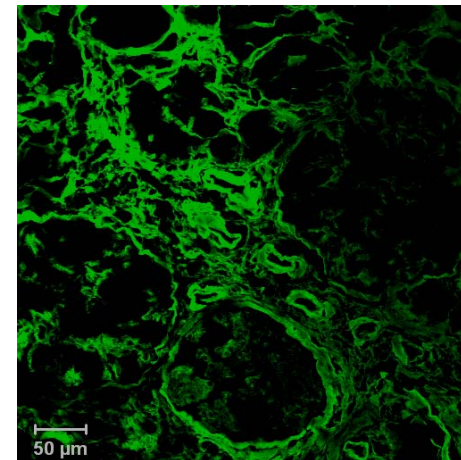
CNA35 (collagen binding protein)
fused to C-LytA



g5-cho/FITC

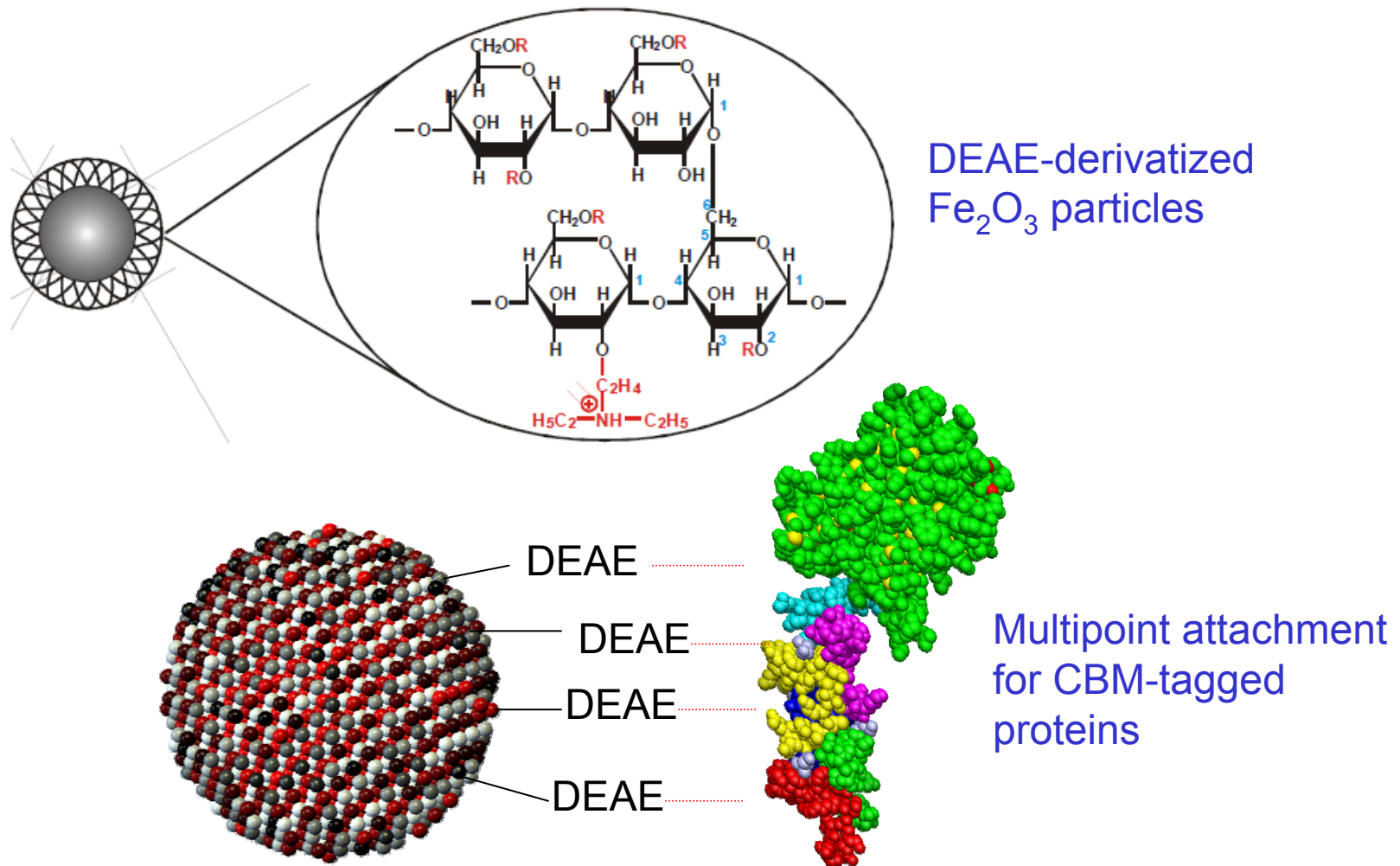


Porcine pericardium



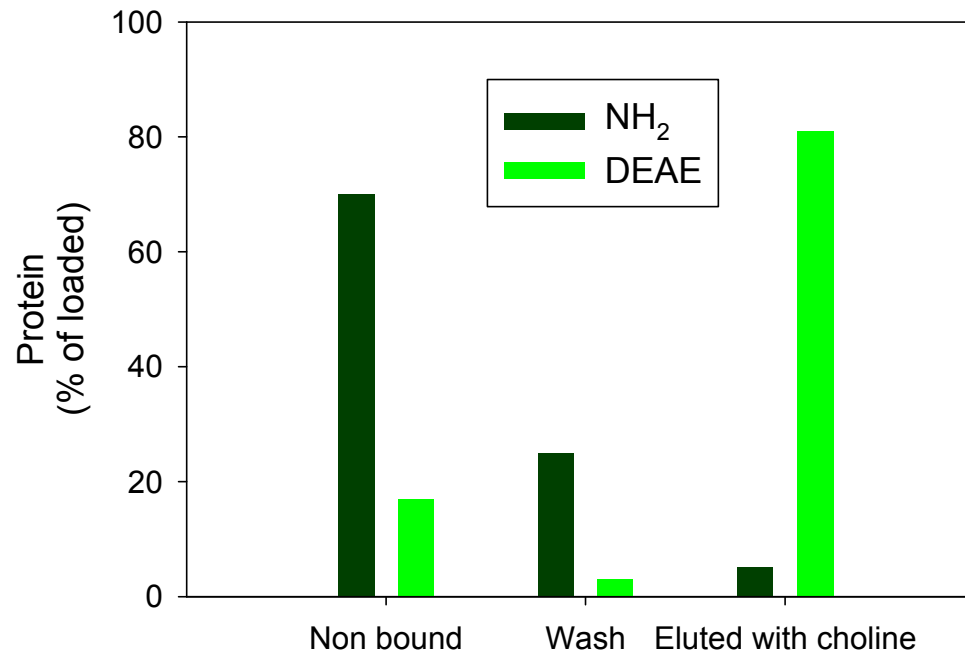
Human kidney

Biofunctionalization of magnetic nanoparticles



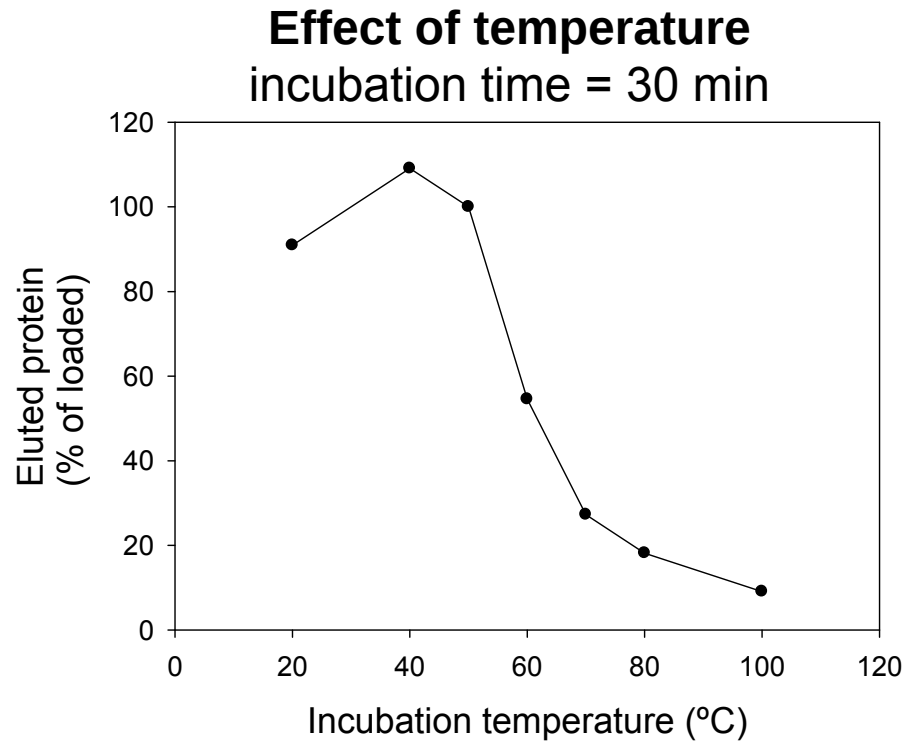
DEAE particles bind C-LytA with specificity and efficiency

Chemicell™ FluidMag
DEAE-starch or NH₂-starch
Ø = 200 nm



Yield: ≈ 50 mg protein/g of particles

Stability of the interaction



No effect of detergents on binding:
Sarkosyl 0.1%
CHAPS 0.1%

Other particles tested:

Chemicell™ SiMag

DEAE-silica $\varnothing$ = 750 nm

Obtained by coprecipitation

DEAE-dextran, $\varnothing$ = 10 nm

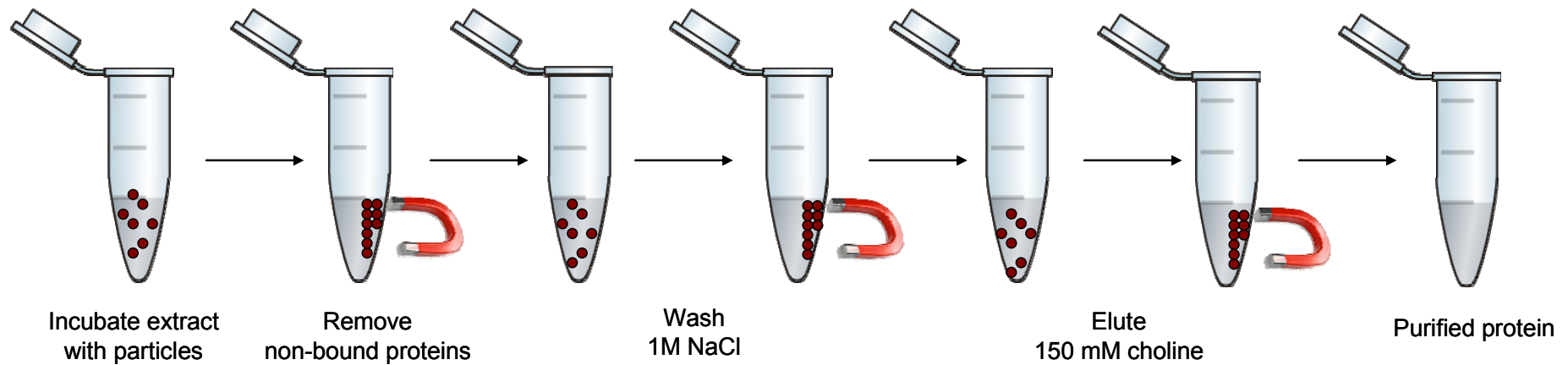
(Puerto Morales, ICMC, Madrid)

Magnetic nanoparticles as supports for CBM-tagged protein purification

Chemicell™ FluidMag

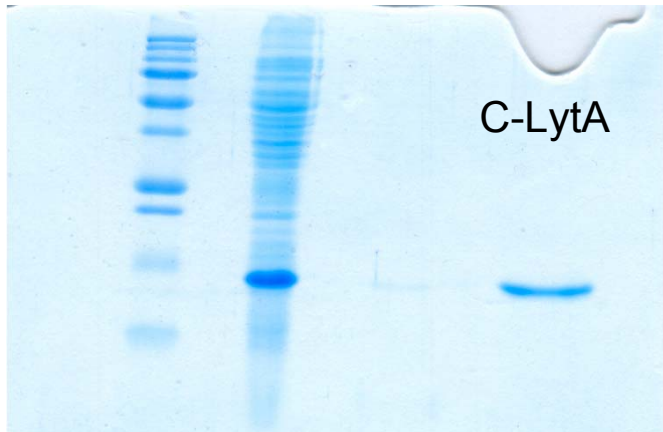
DEAE-starch

Ø = 200 nm

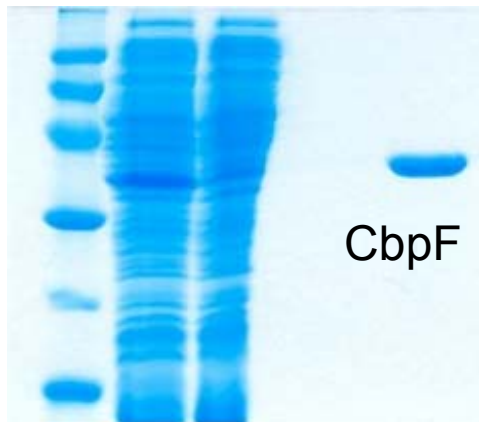


Purification from a cell extract

SDS-PAGE electrophoresis

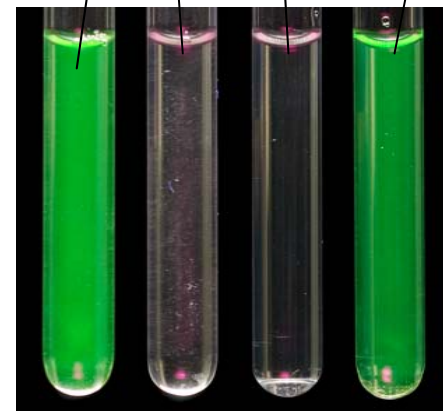
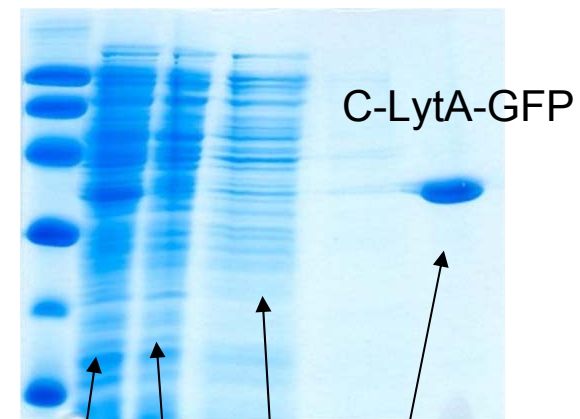


Isolated module



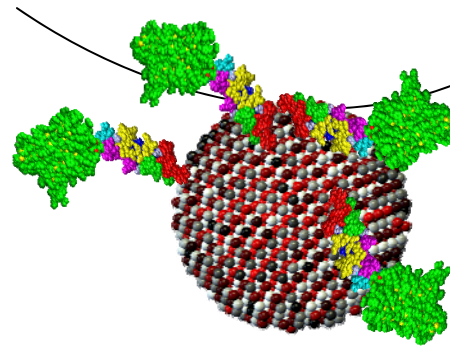
Full-length choline-binding protein

Fusion protein with GFP

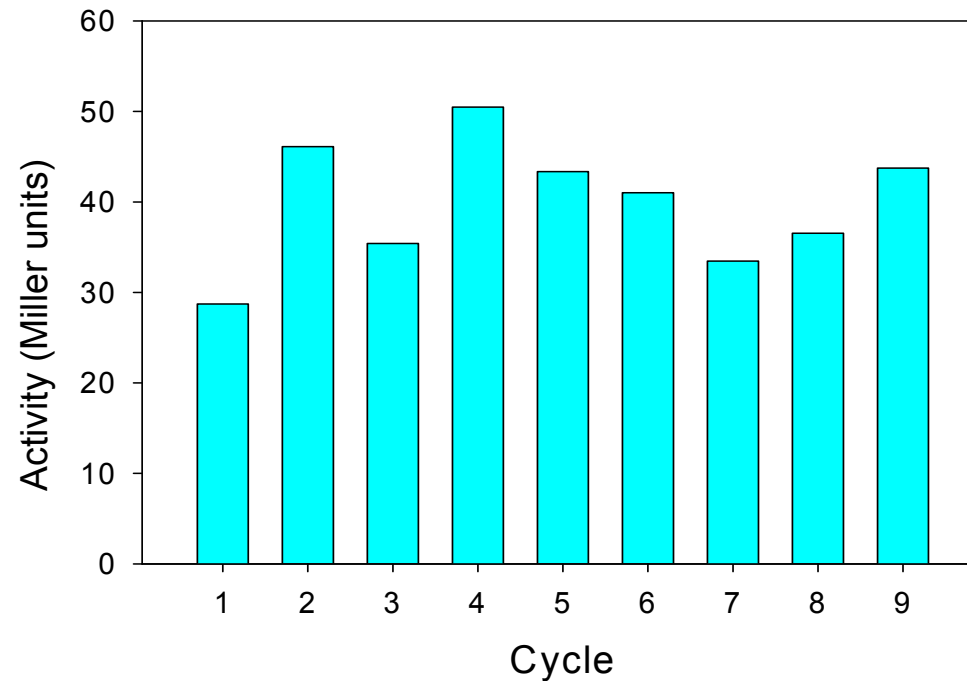


Bioinspired nanobioreactors: Immobilization of a functional C-LytA- β -galactosidase enzyme

o-nitrophenylgalactopiranoside

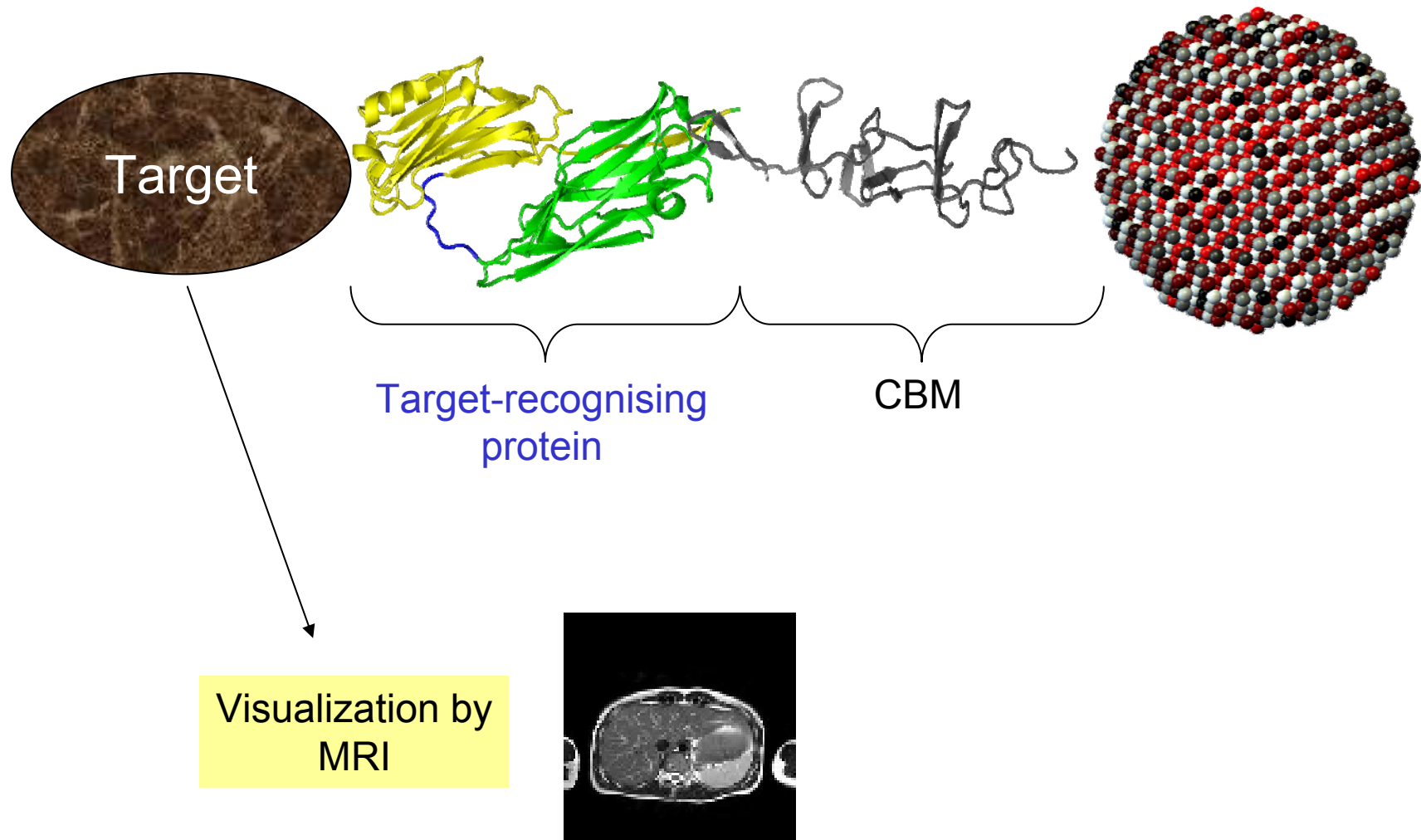


o-nitrophenol + galactose



Future developments

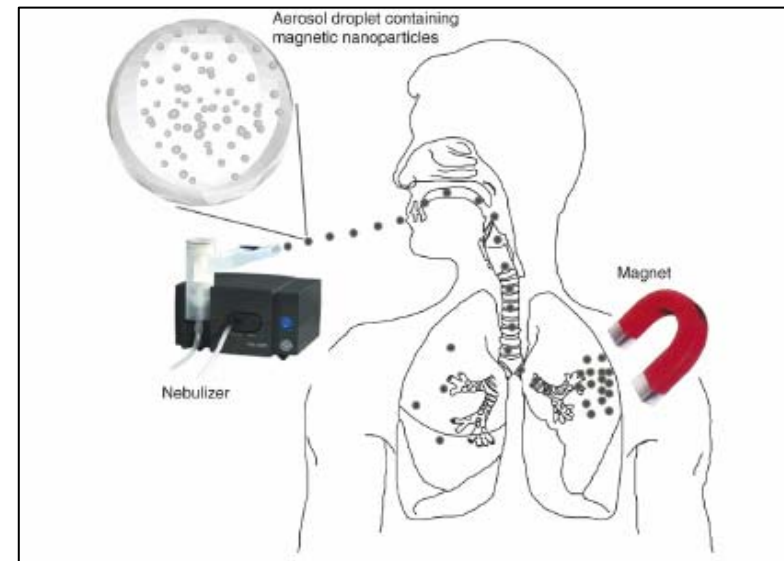
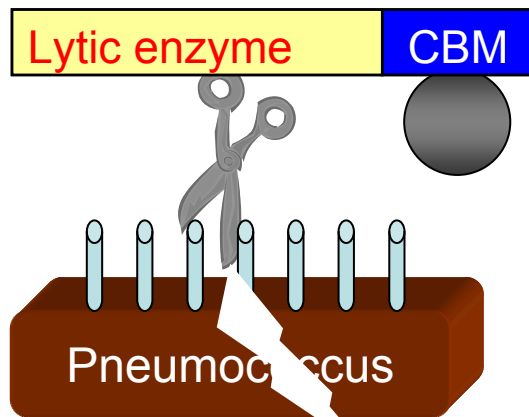
Directed targeting



Future developments

Magnet-guided targeting (nanomagnetoaerosols)

Transporters of pneumococcal lytic enzymes



Collaborations

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