dr. Tea Lenarčič

Forum40

Structural insights into NLP binding to plant membranes

dr. Tea Lenarčič

Forum40

My academic path

Bachelor's degree programme ***Biochemistry***

FACULTY OF CHEMISTRY AND CHEMICAL TECHNOLOGY
University of Ljubljana

Doctoral degree programme ***Biomedicine***

FACULTY OF MEDICINE
University of Ljubljana

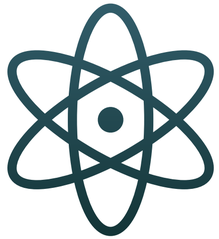
Department of ***Molecular biology and nanobiotechnology***

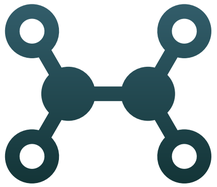
NATIONAL INSTITUTE OF CHEMISTRY
Ljubljana

prof. dr. *Gregor Anderluh* **mentor**
dr. *Marjetka Podobnik* **co-mentor**

Life

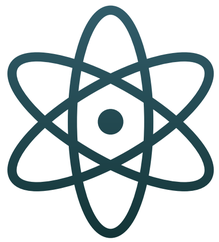
Physics → Chemistry → Biology

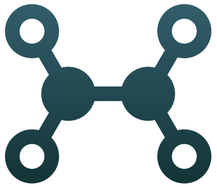




Life

Physics → Chemistry → Biology

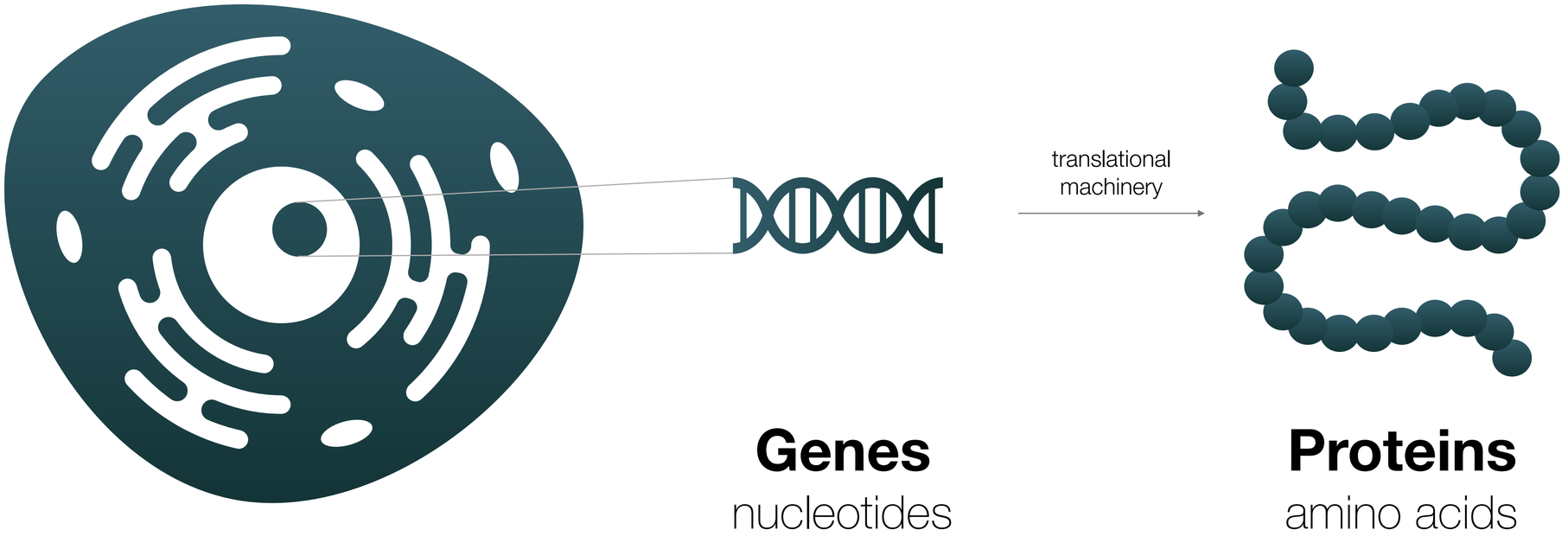




Biochemistry

the branch of science concerned with the chemical and physico-chemical processes and substances which occur within living organisms.

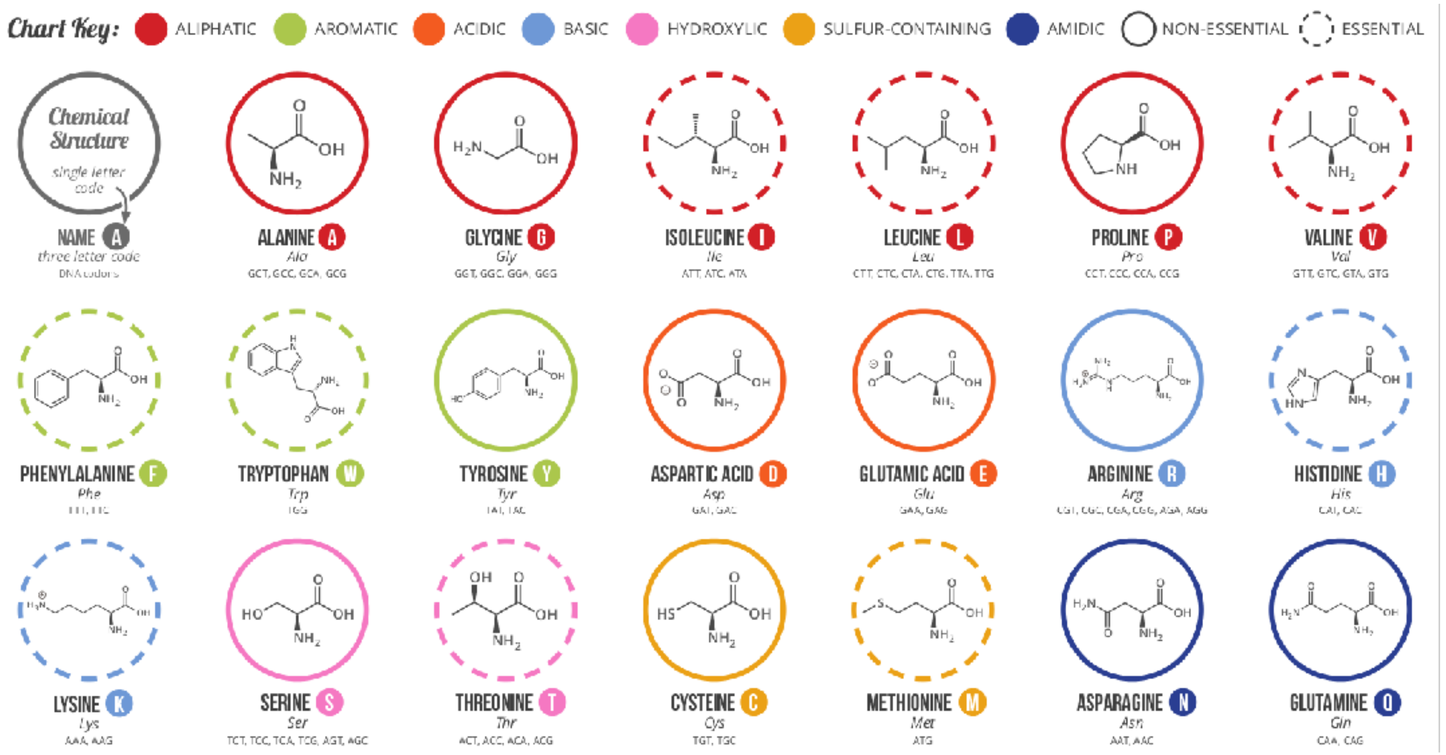
Central dogma of molecular biology



Genes
nucleotides

Proteins
amino acids

Central dogma of molecular biology



Proteins

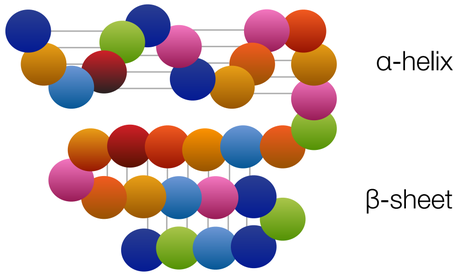
amino acids

Orders of protein structures

Primary structure

sequence

Orders of protein structures



Secondary structure

structural elements

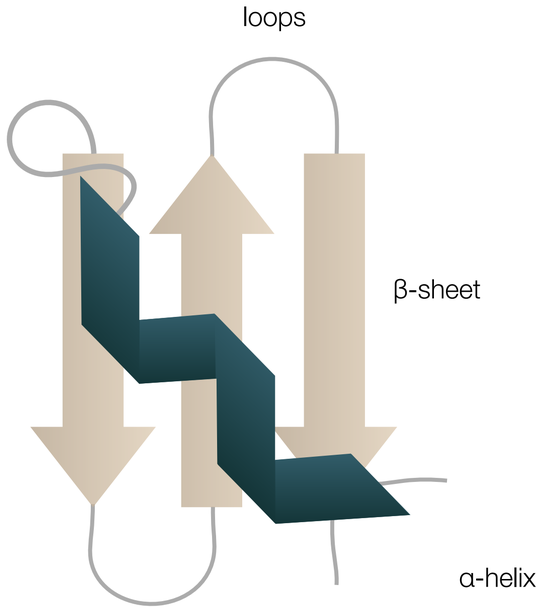
Orders of protein structures

Tertiary structure

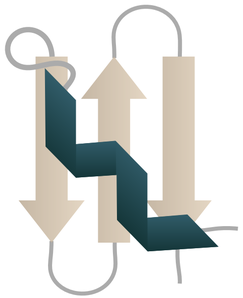
3D fold

Presentation

Presentation



Protein functions



3D structure

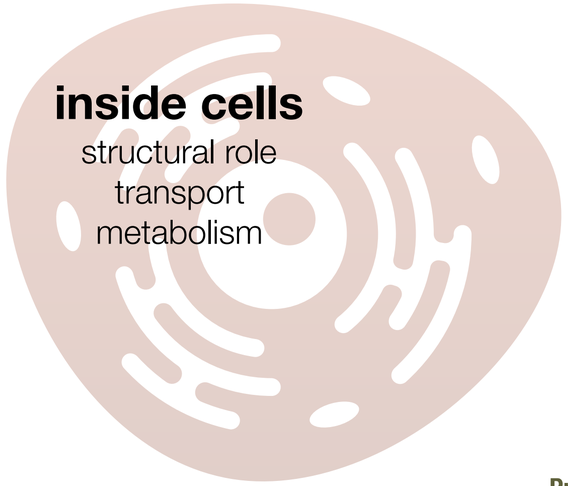
∀

functionality

(>55% cell mass)

Interactions

protein
DNA/RNA
lipids
substrates



inside cells

structural role
transport
metabolism

outside cells

hormons
receptors
antibodies
toxins

Primary research activity in D11

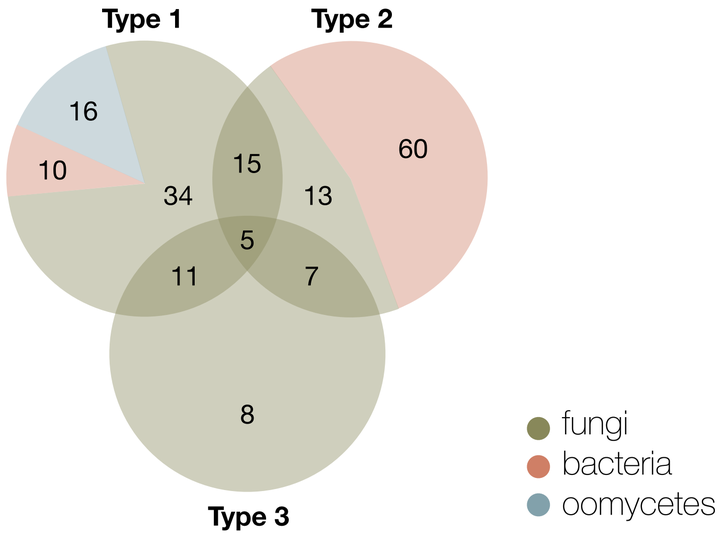
NLP toxins

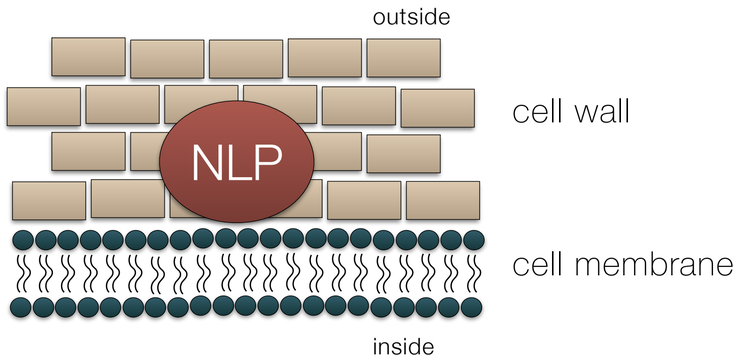
a protein family ***leaf necrosis in eudicot plants***

secretion ***plant associated microorganisms***

dual role ***activate plant immune response***

function ***predicted to form pores in target membranes***





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a protein family ***leaf necrosis in eudicot plants***

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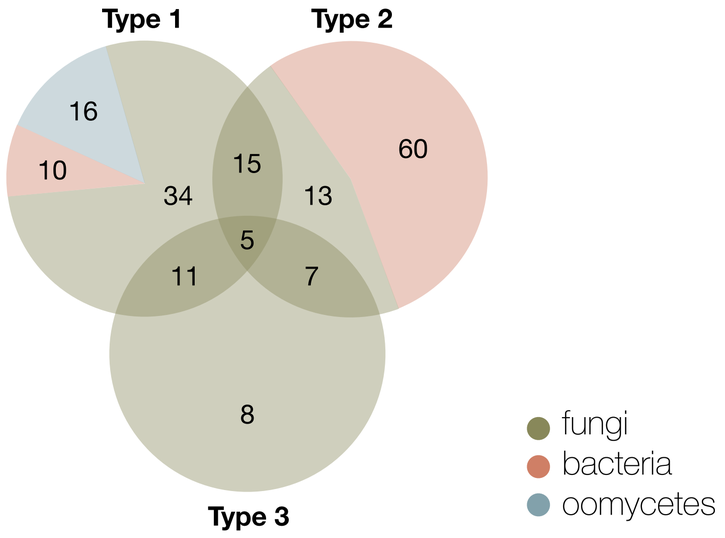
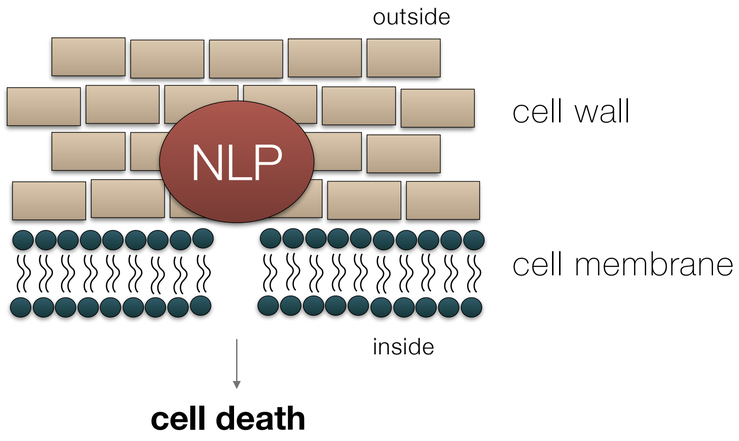
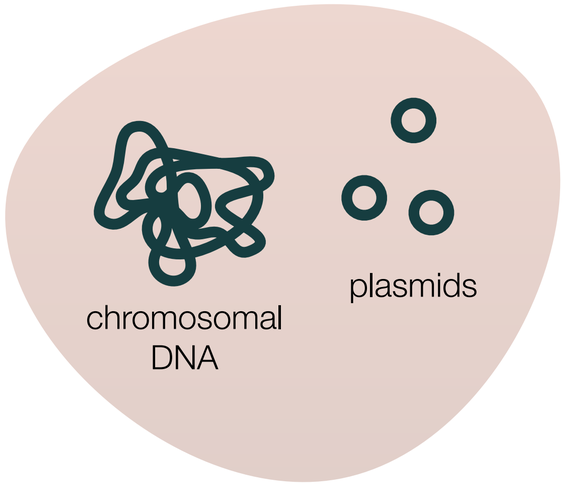




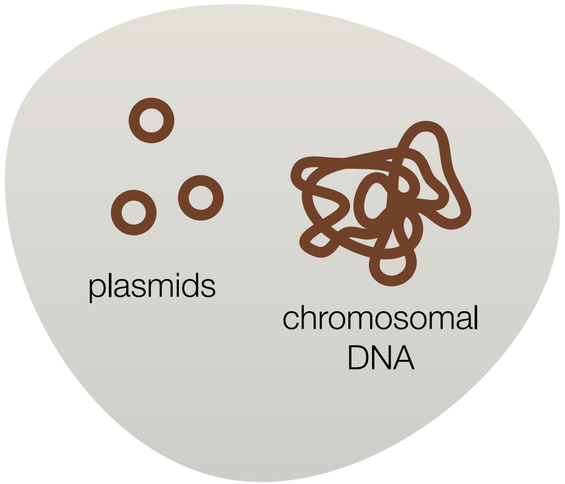
Biochemistry project

Sample preparation

Bacteria
#1

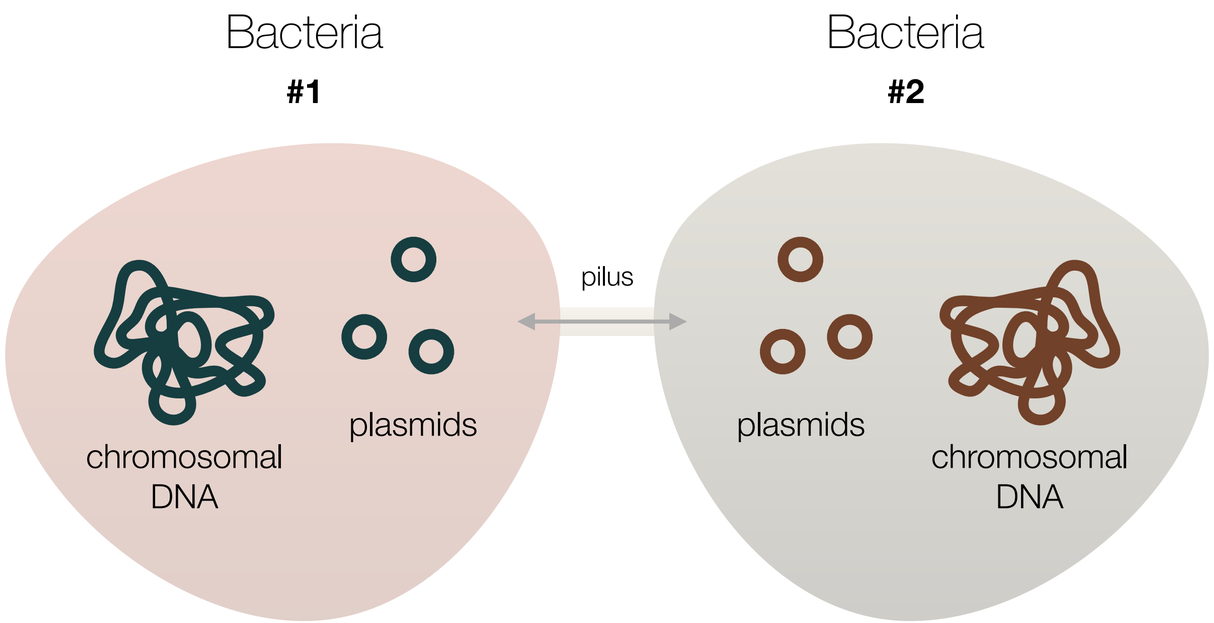


Bacteria
#2



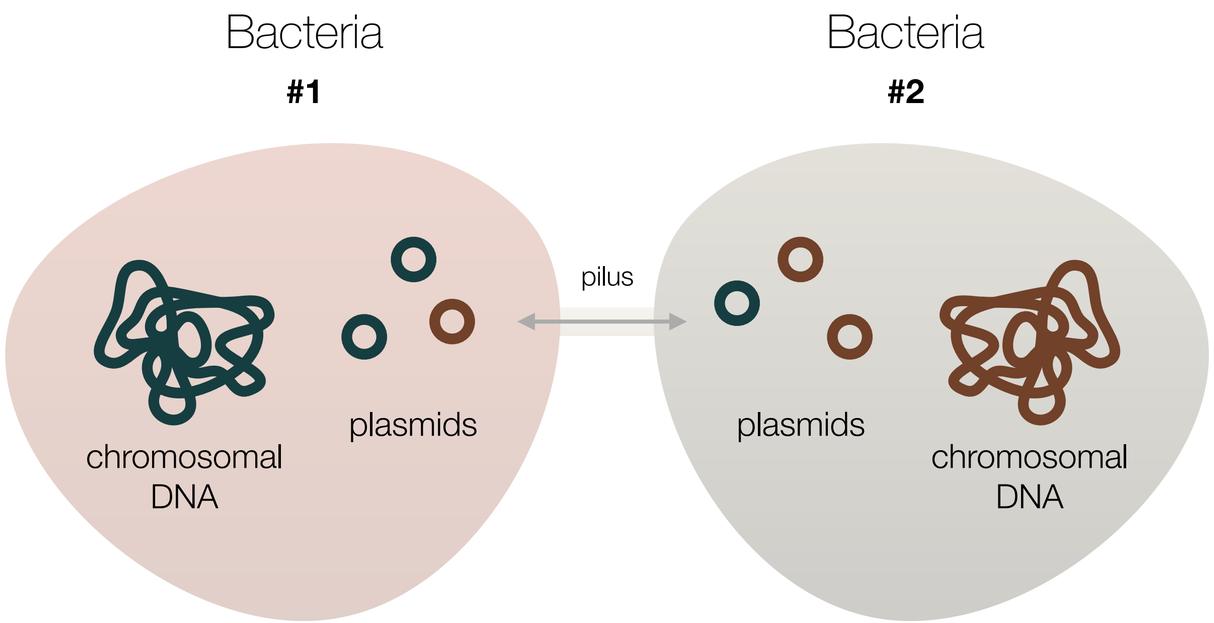
Biochemistry project

Sample preparation



Biochemistry project

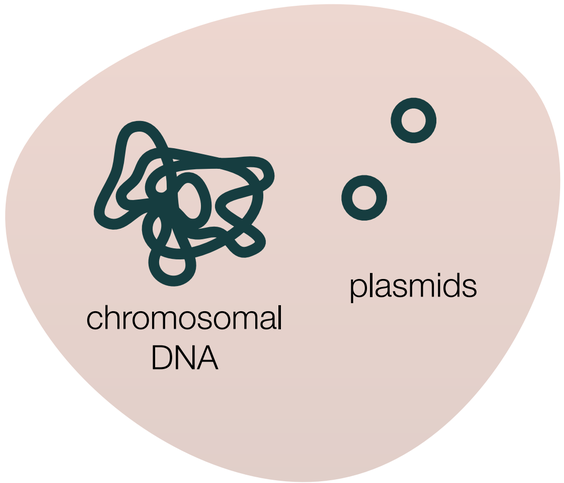
Sample preparation



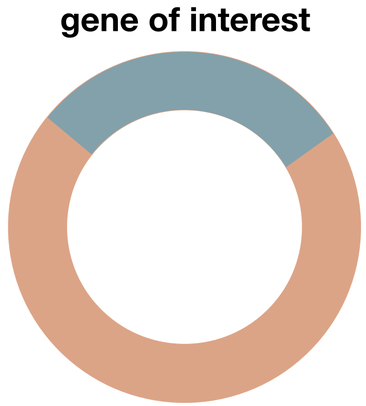
Biochemistry project

Sample preparation

Bacteria



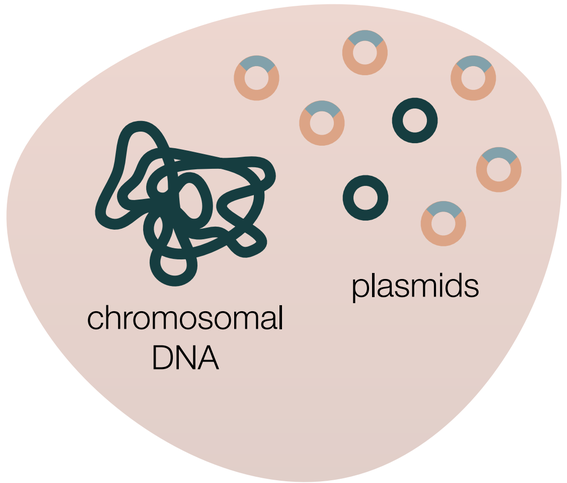
Vector



Biochemistry project

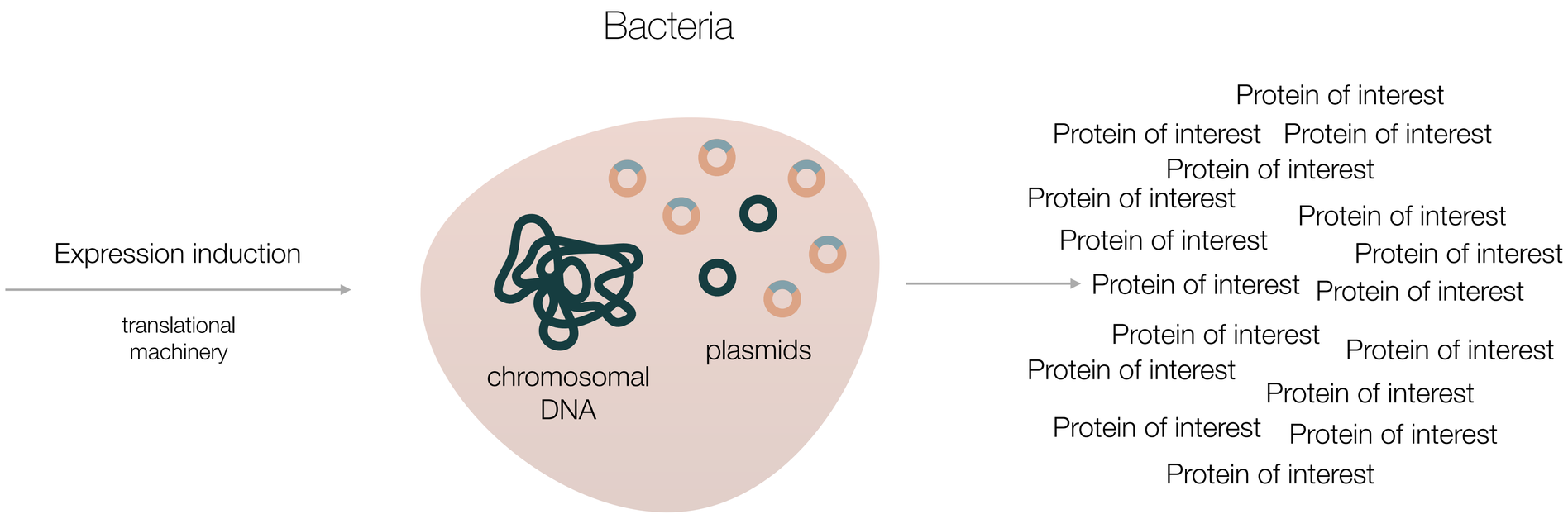
Sample preparation

Bacteria



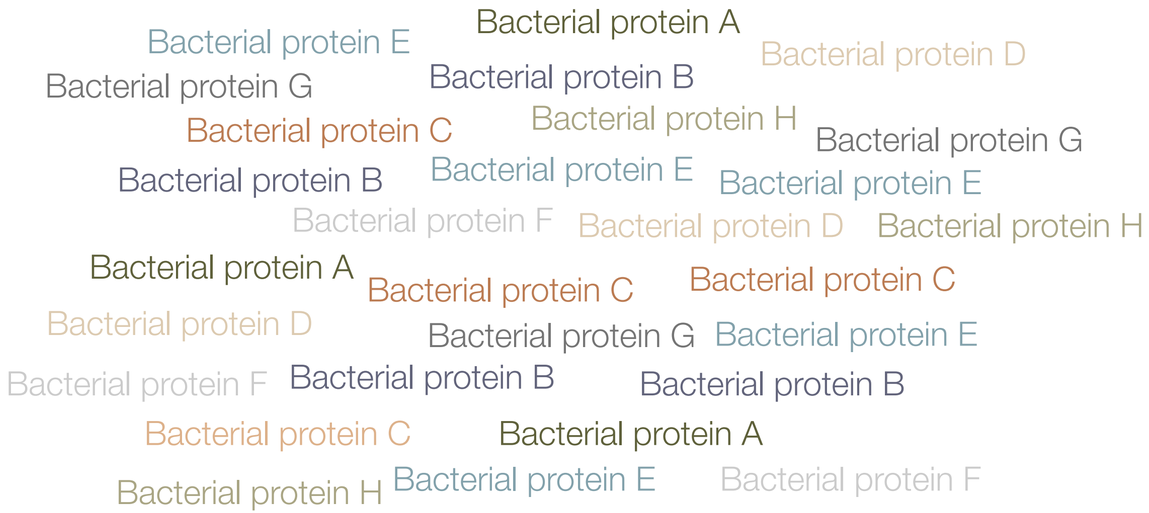
Biochemistry project

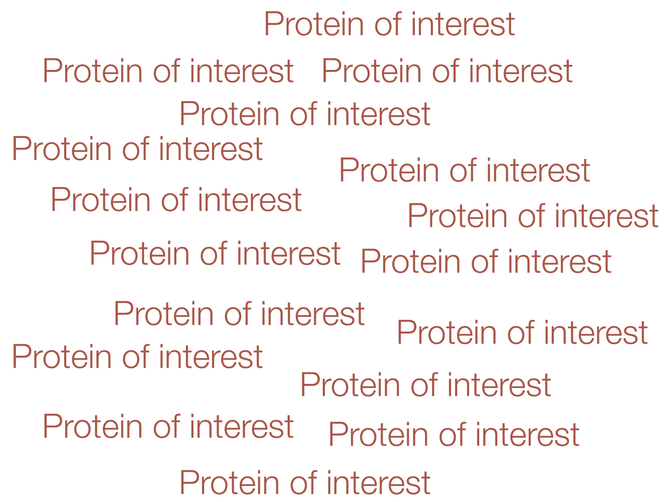
Sample preparation



Biochemistry project

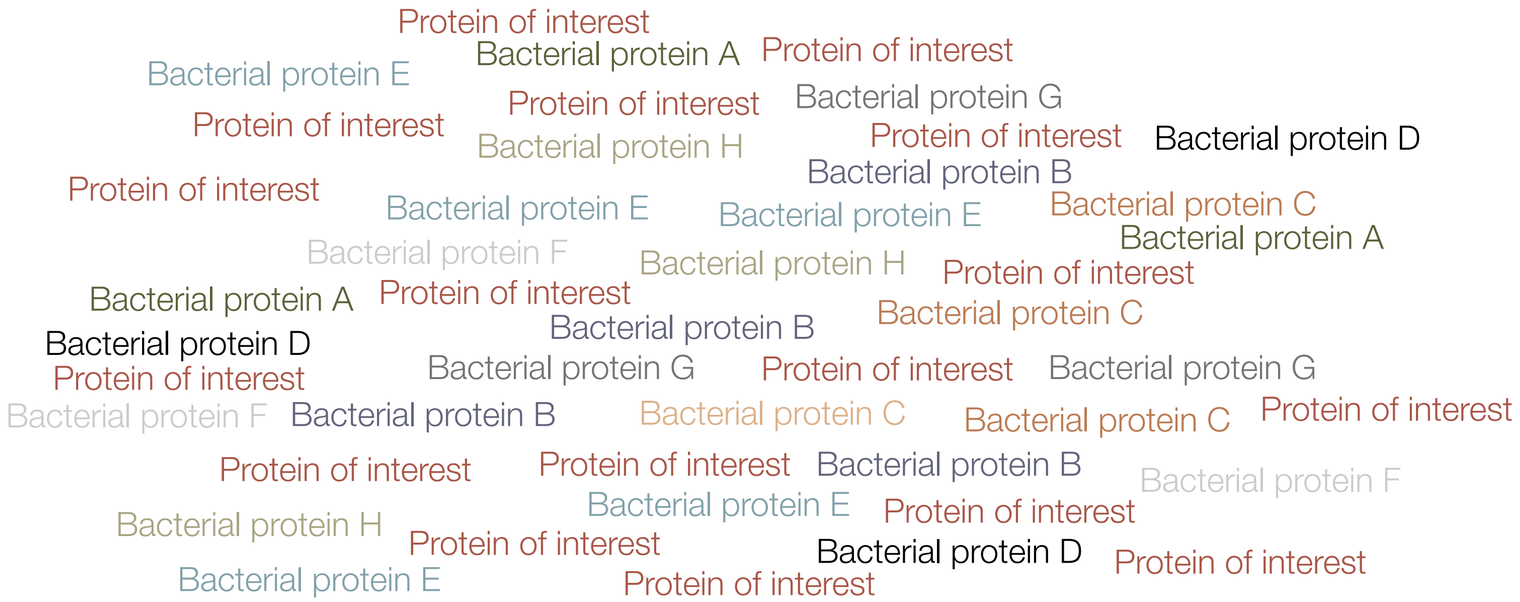
Protein purification





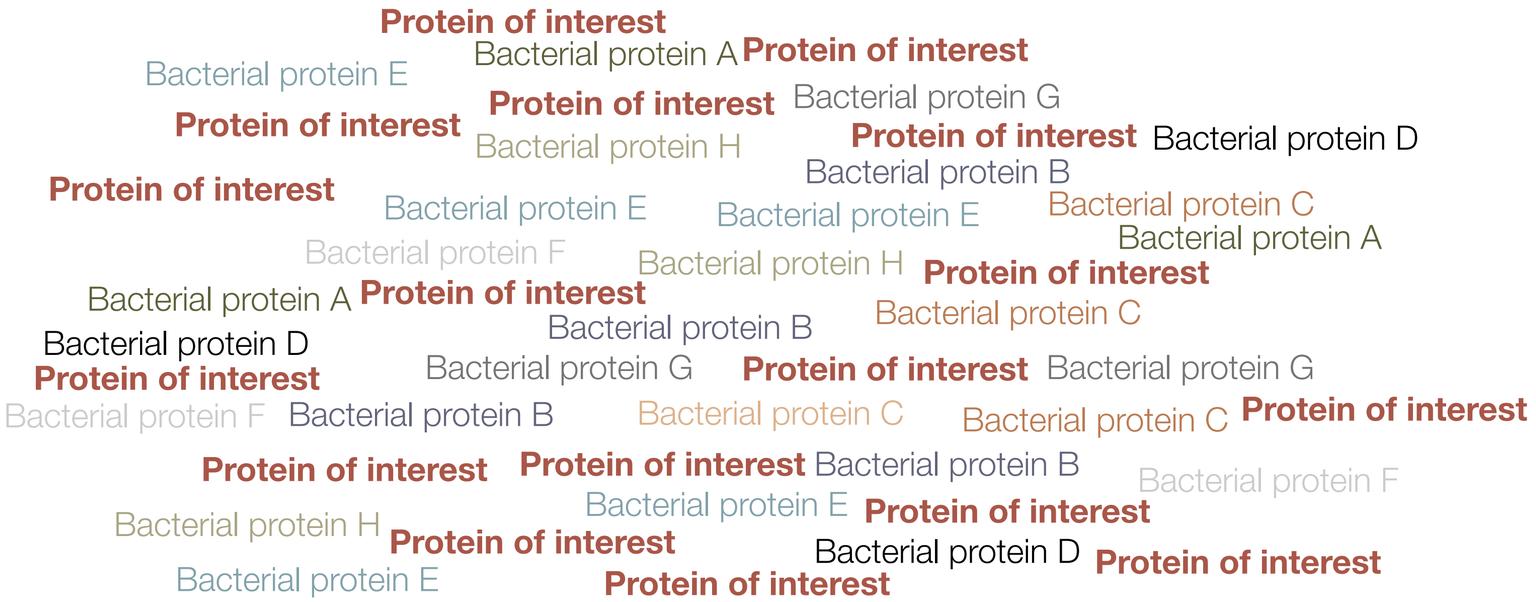
Biochemistry project

Protein purification



Biochemistry project

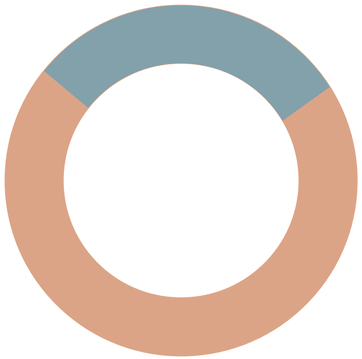
Protein purification



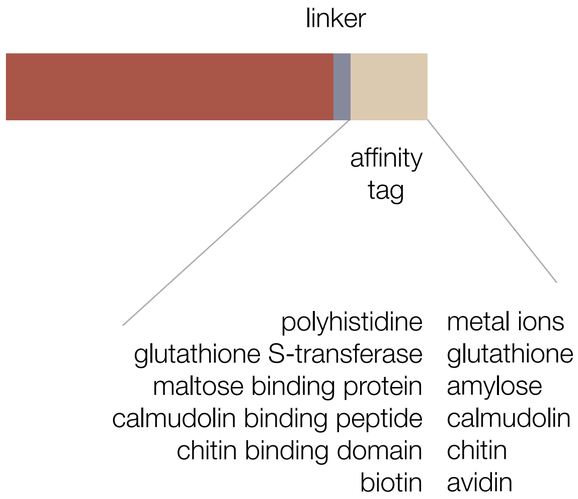
Biochemistry project

Protein purification

Gene of interest



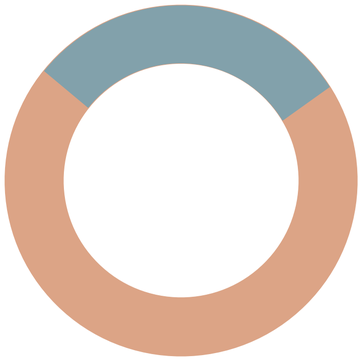
Protein of interest



Biochemistry project

Protein purification

Gene of interest

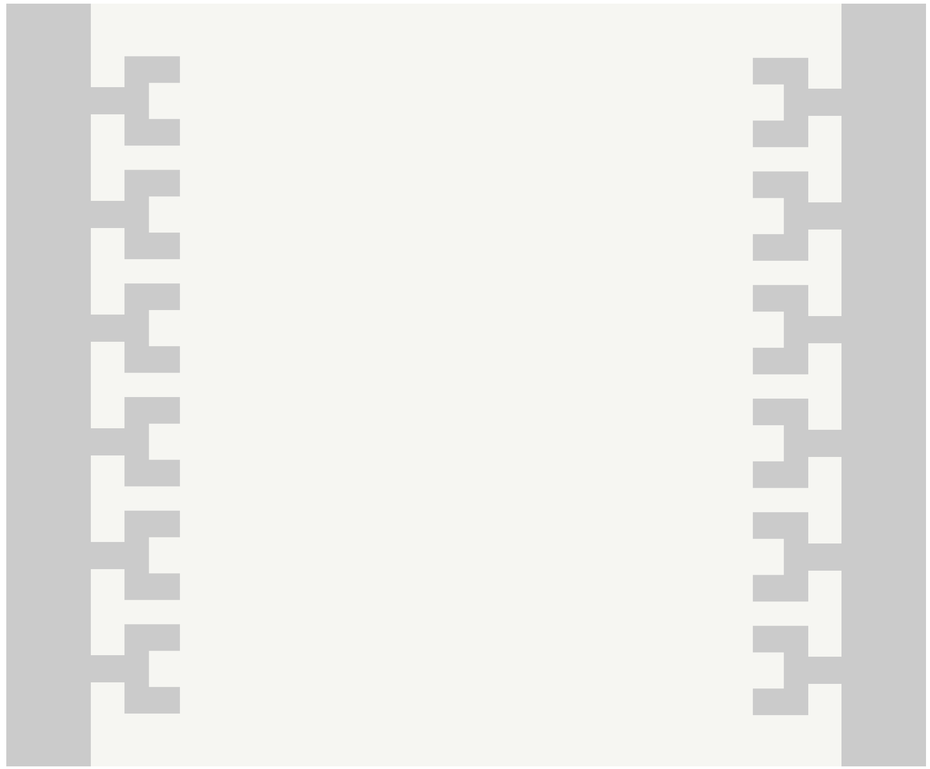


Protein of interest

polyhistidine	metal ions
glutathione S-transferase	glutathione
maltose binding protein	amylose
calmudolin binding peptide	calmudolin
chitin binding domain	chitin
biotin	avidin

Biochemistry project

Protein purification



affinity column

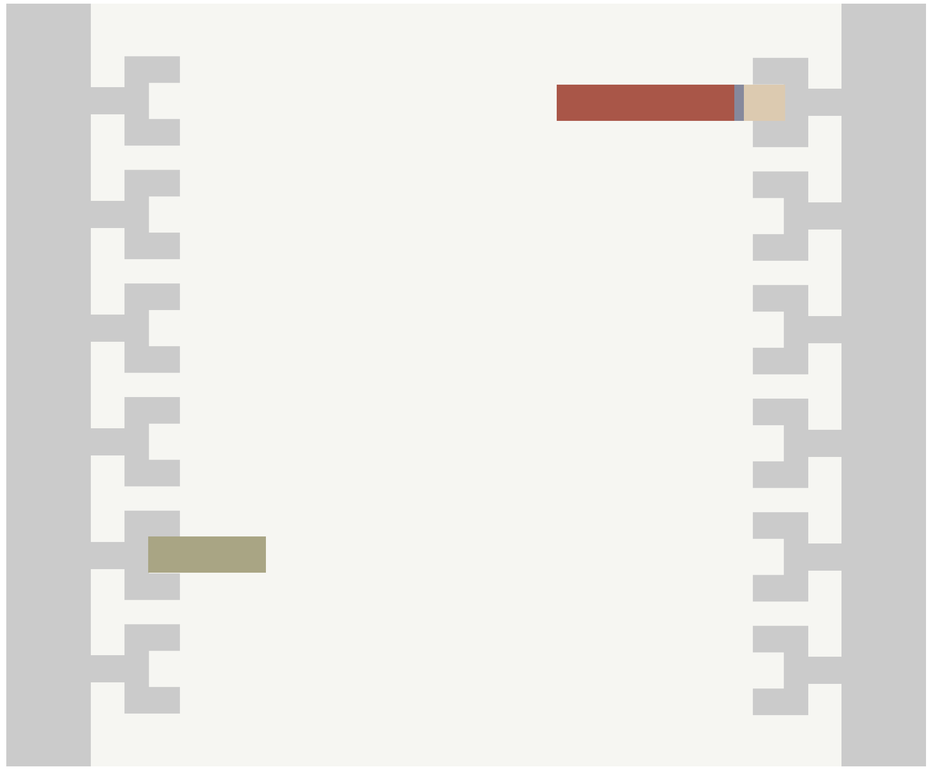
Biochemistry project

Protein purification

affinity column

Biochemistry project

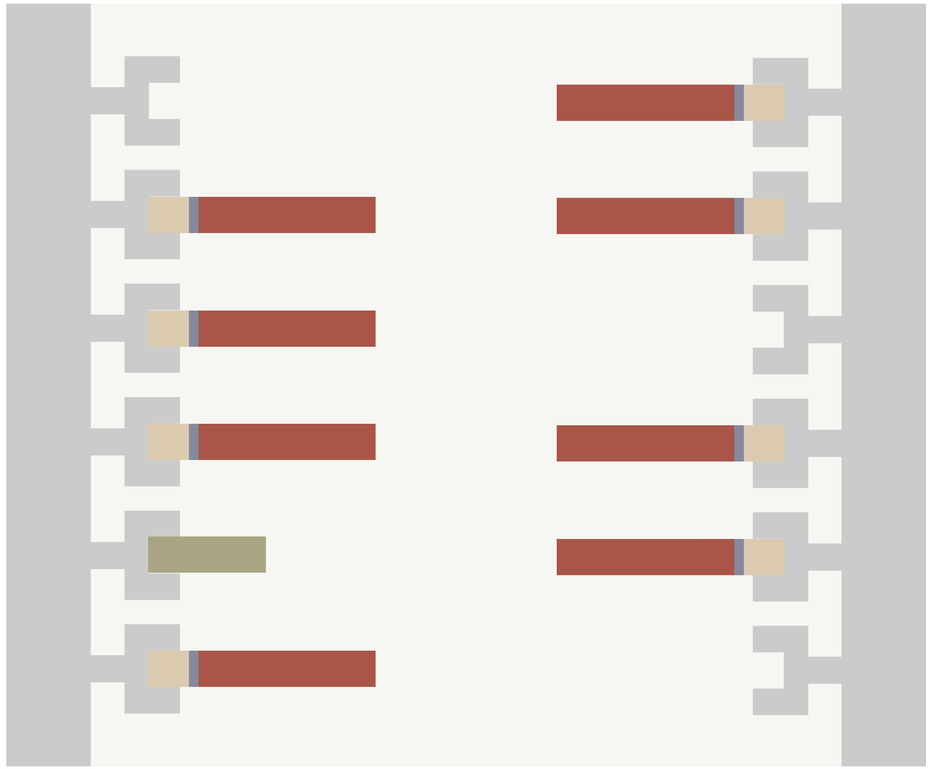
Protein purification



affinity column

Biochemistry project

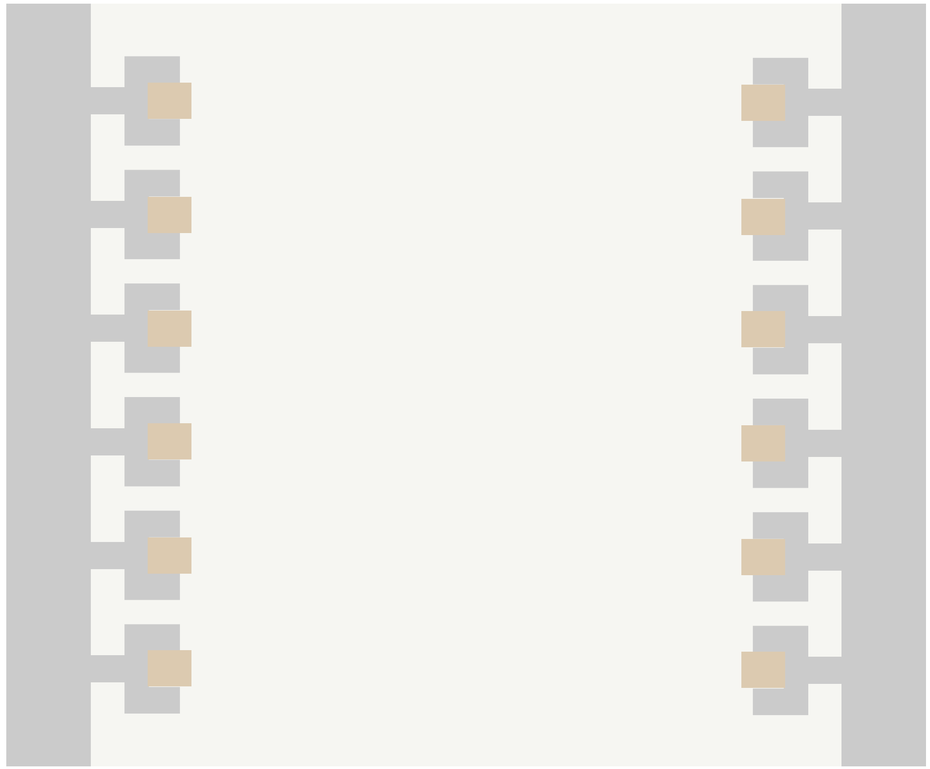
Protein purification



affinity column

Biochemistry project

Protein purification



affinity column

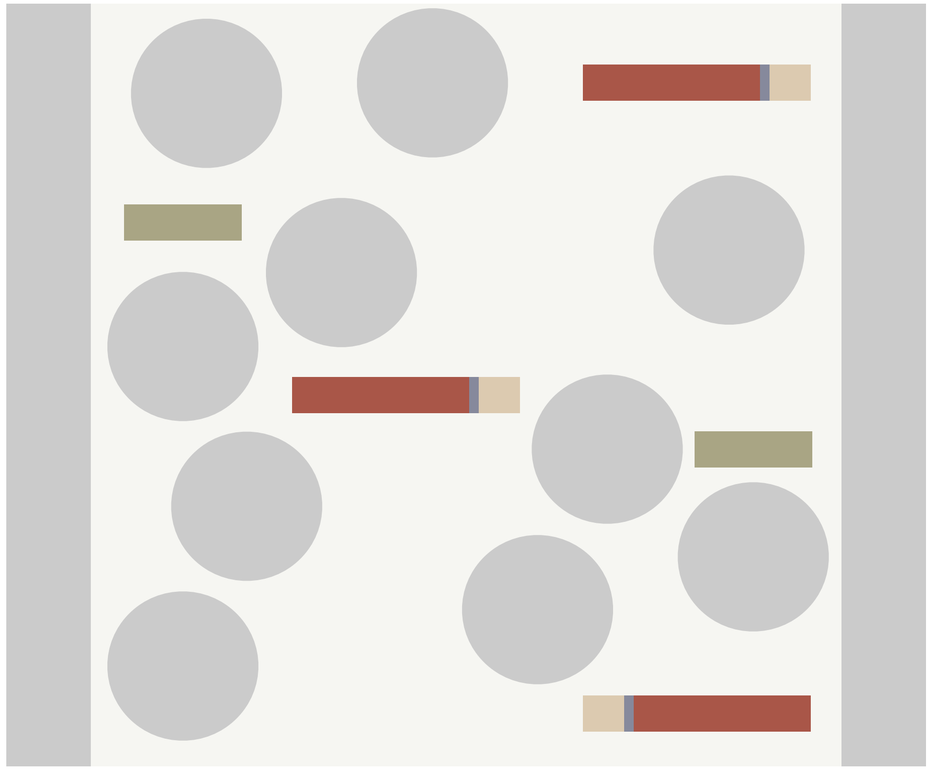
Biochemistry project

Protein purification

size-exclusion column

Biochemistry project

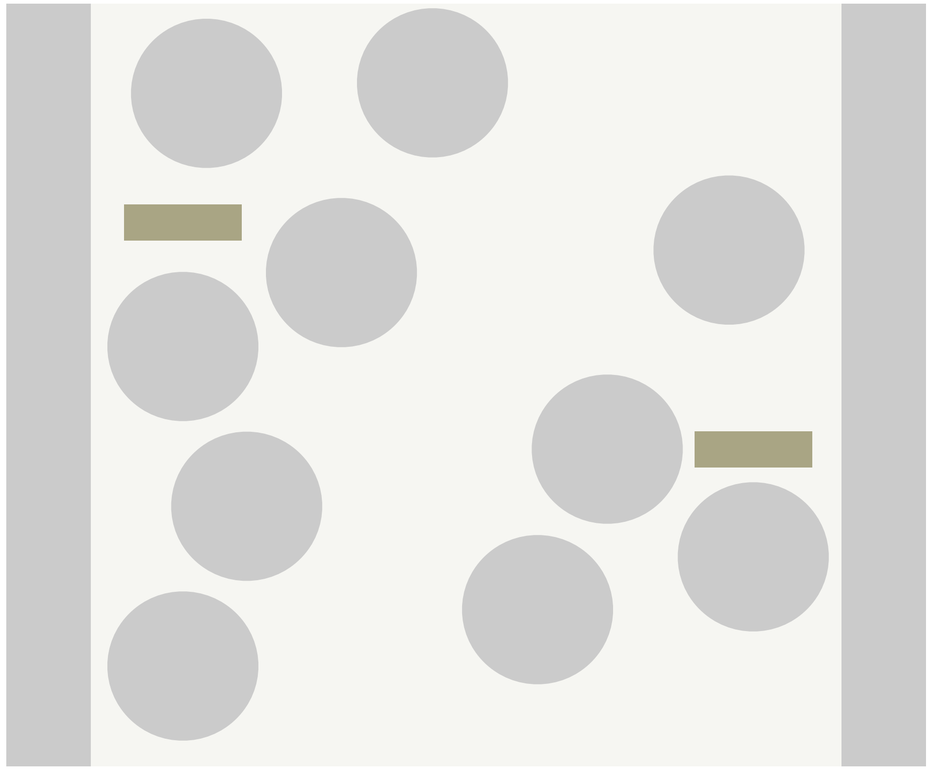
Protein purification



size-exclusion column

Biochemistry project

Protein purification



size-exclusion column

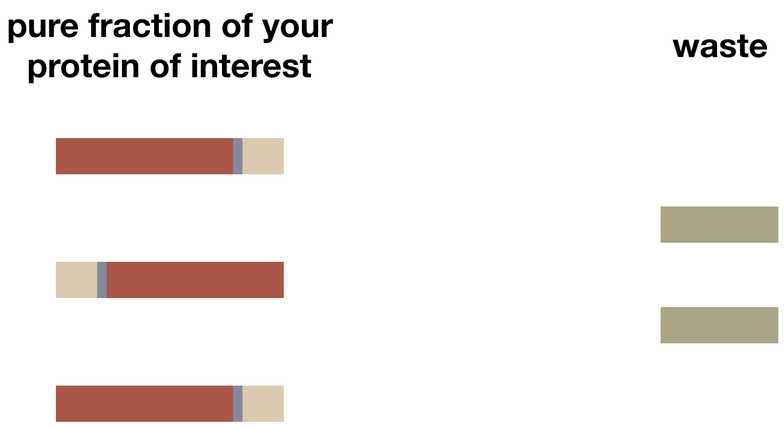
Biochemistry project

Protein purification

size-exclusion column

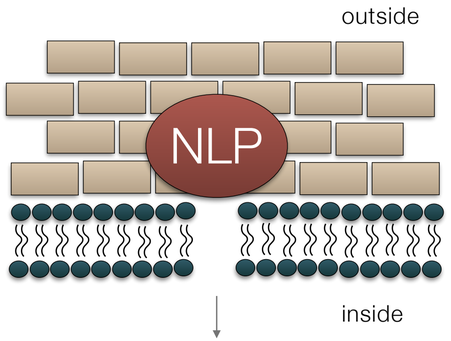
Biochemistry project

Protein purification



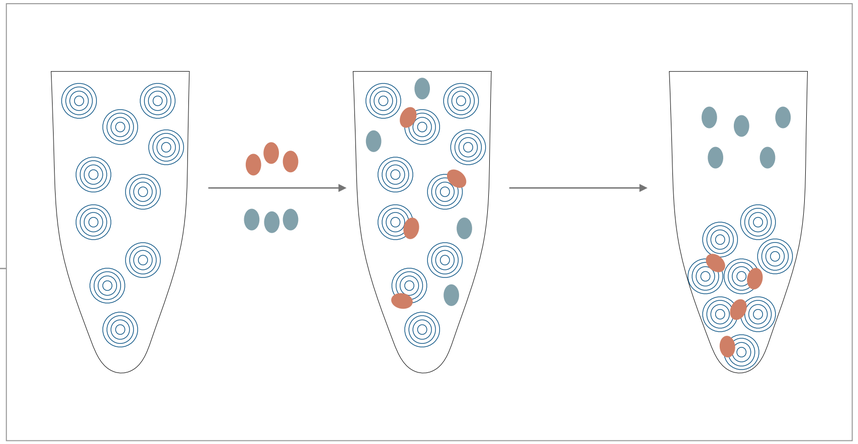
NLP proteins

Structural biology

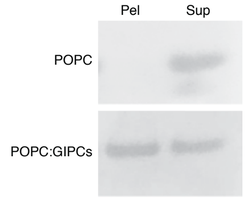


cell death

Lipid identification



Liposome sedimentation assay



Glycosylinositol Phosphoceramides

Series 0
Cer-P-Ins-Hex

Eudicot

Series A
Cer-P-Ins-HexA-Hex(R1)

Monocot

Series B
Cer-P-Ins-HexA-Hex(R1)-Hex

Series C
Cer-P-Ins-HexA-Hex(R1)-Hex-Pen
Cer-P-Ins-HexA-Hex(R1)-$(Hex)_2$

Series C'
Cer-P-Ins-HexA-HexA-HexA-Hex
Cer-P-Ins-HexA-HexA-HexA-HexA

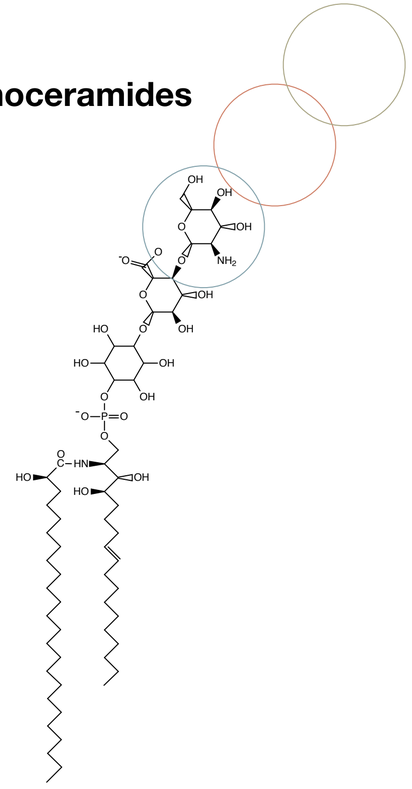
Series D
Cer-P-Ins-HexA-Hex(R1)-Hex-$(Pen)_2$
Cer-P-Ins-HexA-Hex(R1)-$(Hex)_2$-Pen

Series E
Cer-P-Ins-HexA-Hex(R1)-Hex-$(Pen)_3$
Cer-P-Ins-HexA-Hex(R1)-$(Hex)_3$-Pen
Cer-P-Ins-HexA-Hex(R1)-$(Hex)_2$-$(Pen)_2$

Series F
Cer-P-Ins-HexA-Hex(R1)-Hex-$(Pen)_4$

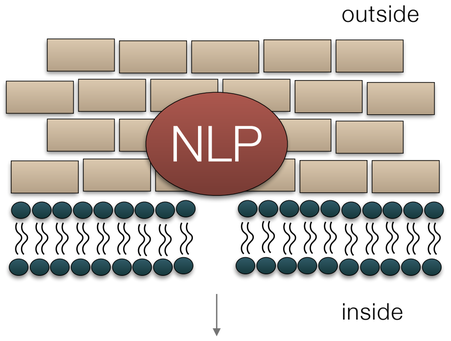
Polar head

Ceramide



Cer-P-Ins-GlcA-GlcNAc

NLP proteins



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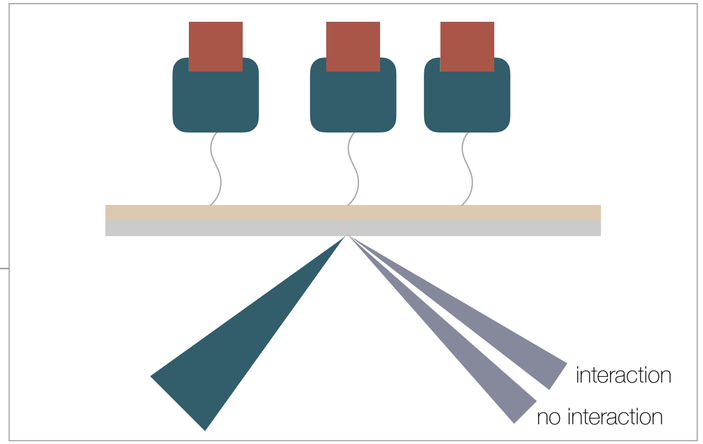

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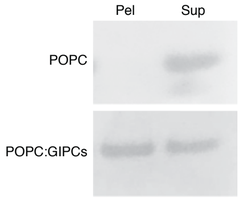
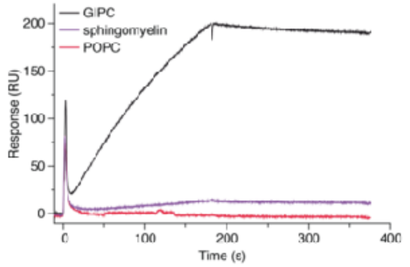
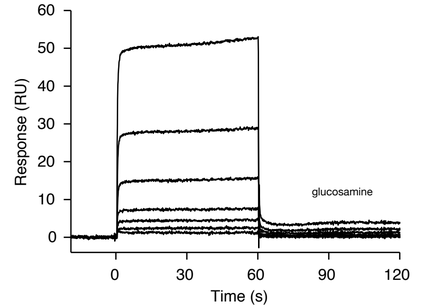


Surface plasmone resonance experiments

micromolar affinity



millimolar affinity



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Series C
Cer-P-Ins-HexA-Hex(R1)-Hex-Pen
Cer-P-Ins-HexA-Hex(R1)-$(Hex)_2$

Series C'
Cer-P-Ins-HexA-HexA-HexA-Hex
Cer-P-Ins-HexA-HexA-HexA-HexA

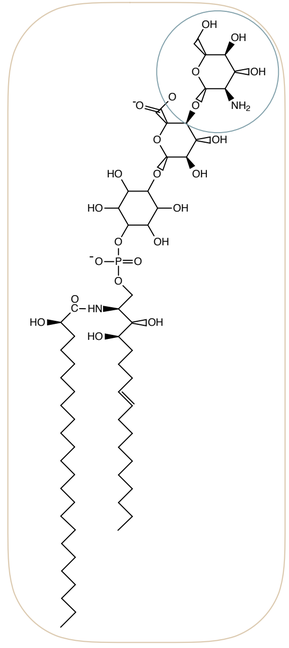
Series D
Cer-P-Ins-HexA-Hex(R1)-Hex-$(Pen)_2$
Cer-P-Ins-HexA-Hex(R1)-$(Hex)_2$-Pen

Series E
Cer-P-Ins-HexA-Hex(R1)-Hex-$(Pen)_3$
Cer-P-Ins-HexA-Hex(R1)-$(Hex)_3$-Pen
Cer-P-Ins-HexA-Hex(R1)-$(Hex)_2$-$(Pen)_2$

Series F
Cer-P-Ins-HexA-Hex(R1)-Hex-$(Pen)_4$

Polar head

Ceramide

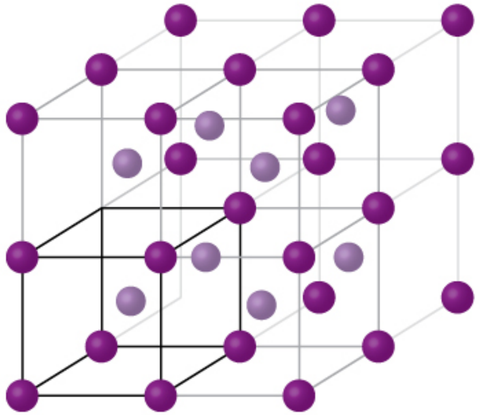


Cer-P-Ins-GlcA-GlcNAc

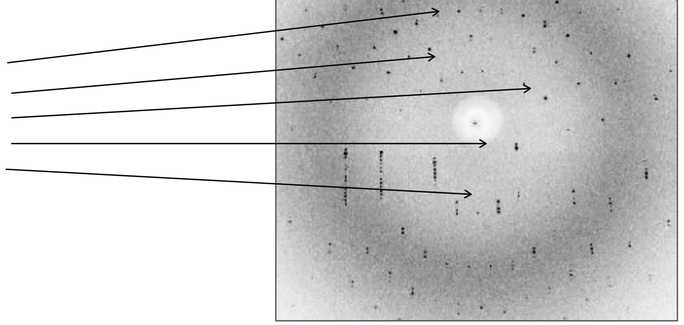
NLP proteins

Structural biology

Crystallography approach



X rays

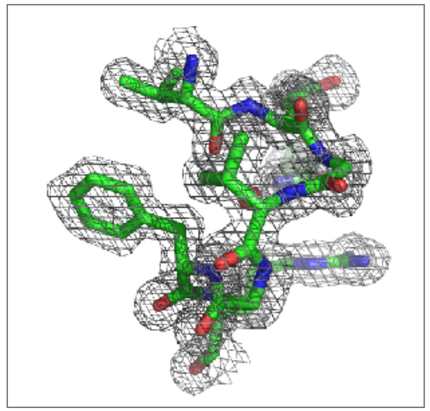


diffraction pattern

reciprocal space

F

phase problem

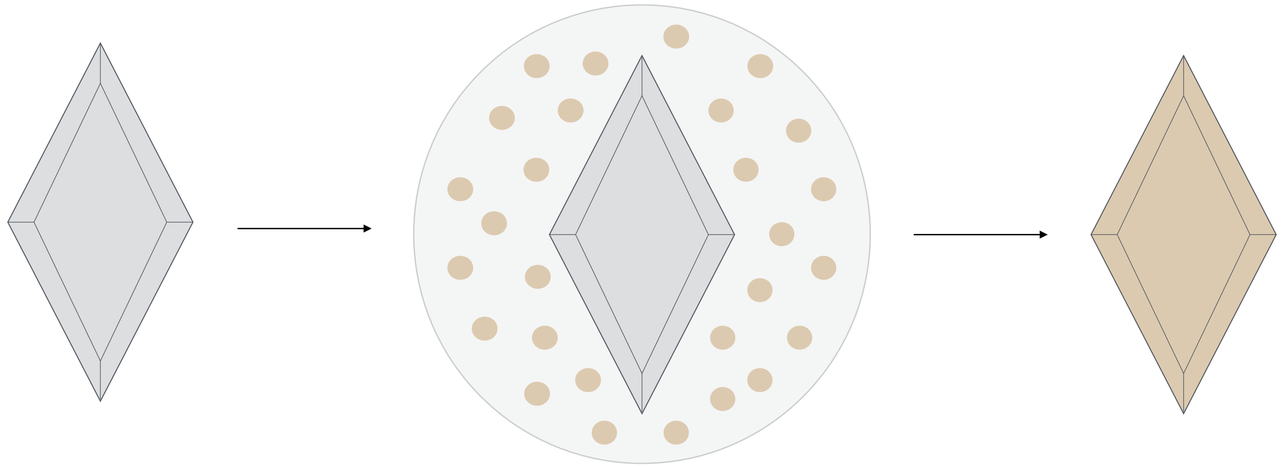


electron density

real space

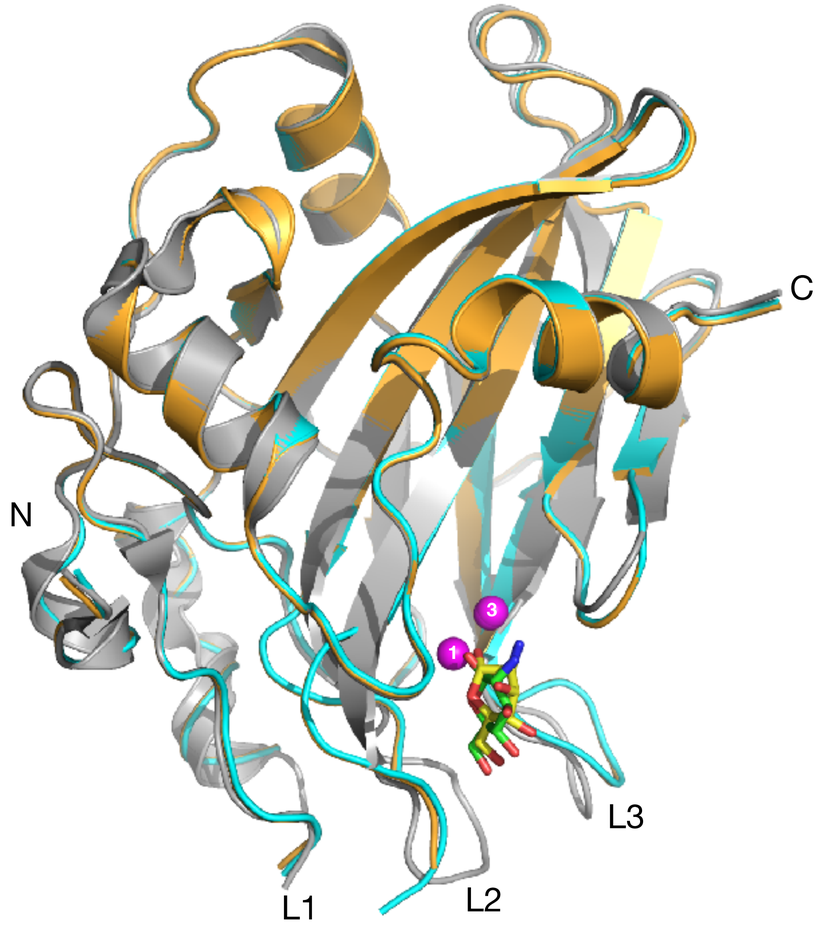
NLP proteins

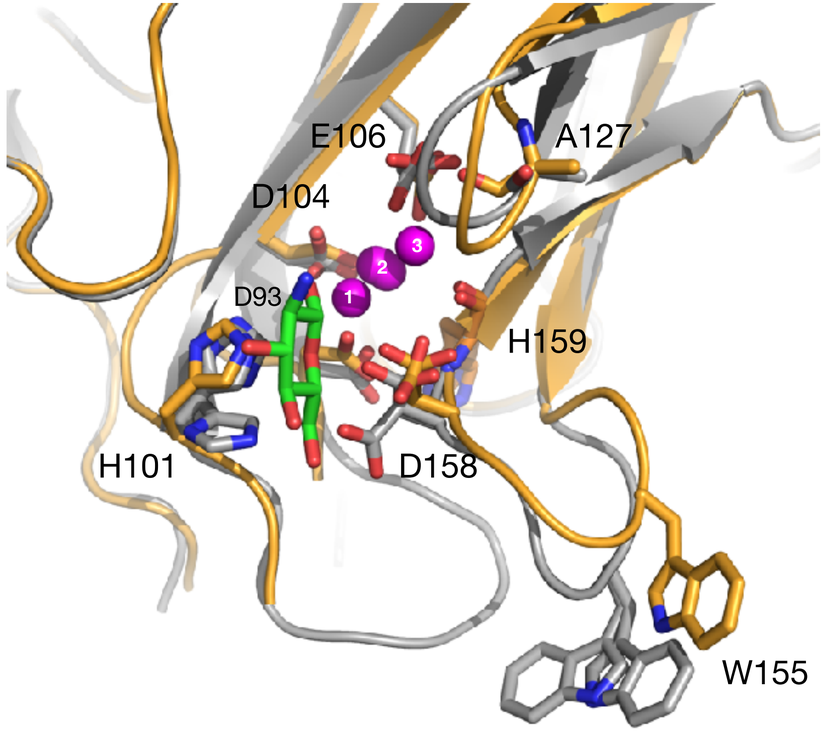
Structural biology

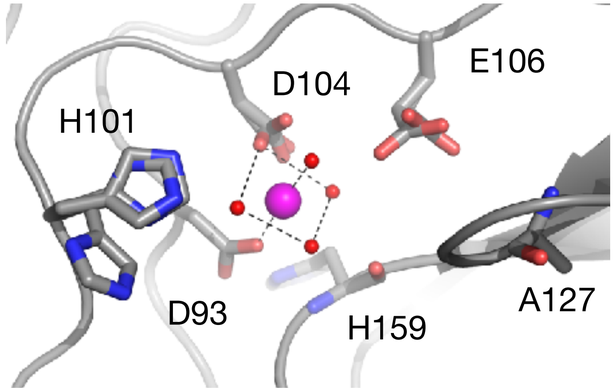


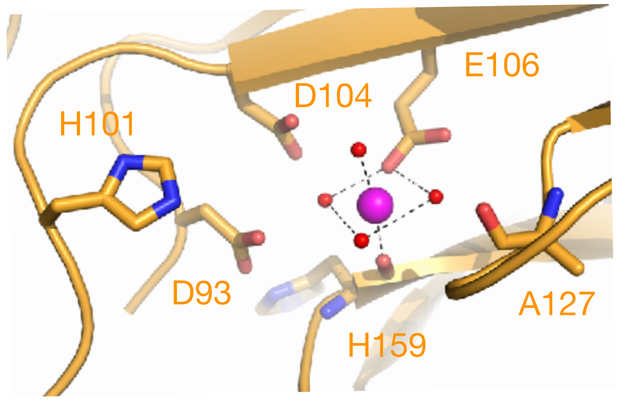
protein
+
ligand

Structural analysis



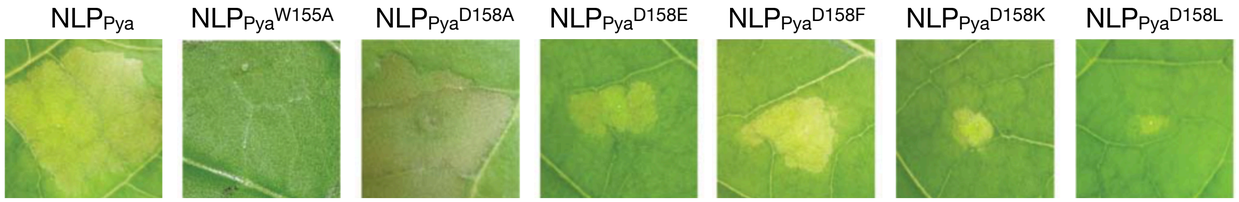


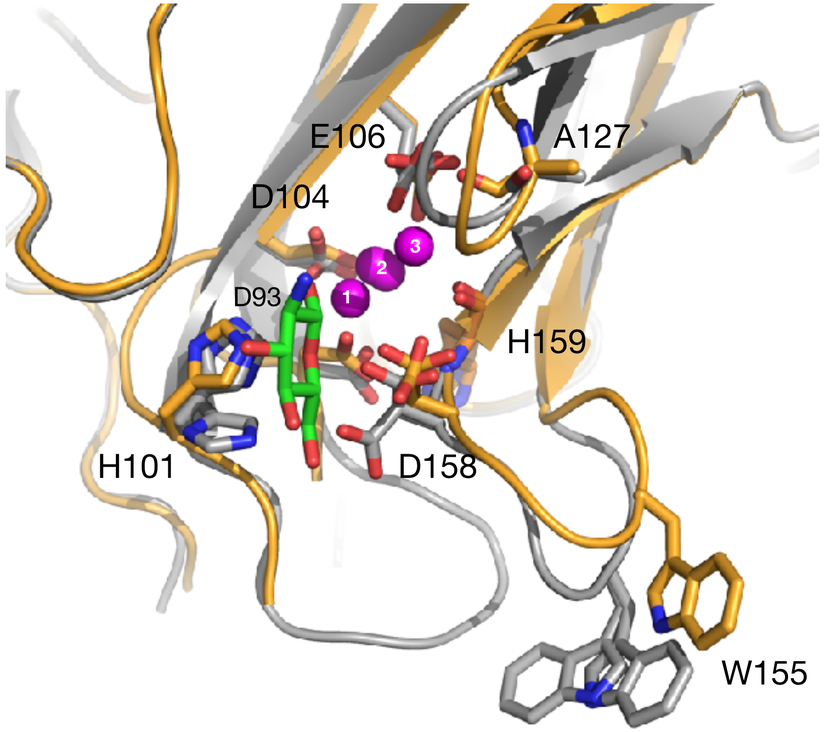




- apoNLP$_{Pya}$
- NLP$_{Pya}$-glucosamine
- NLP$_{Pya}$-mannosamine

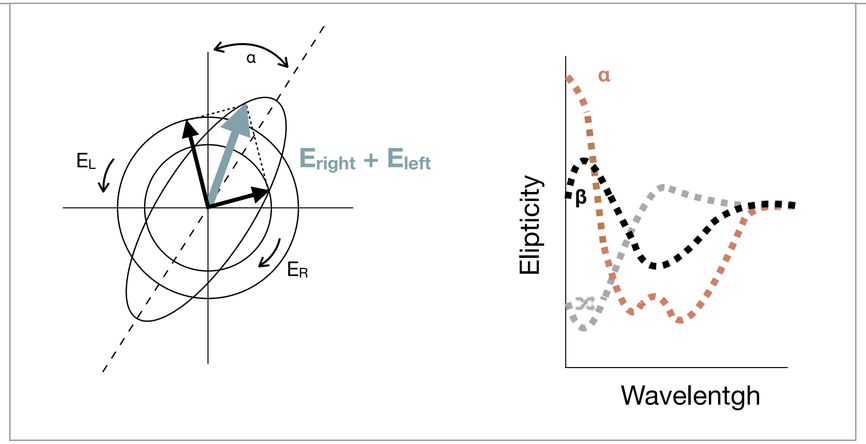
Structural analysis

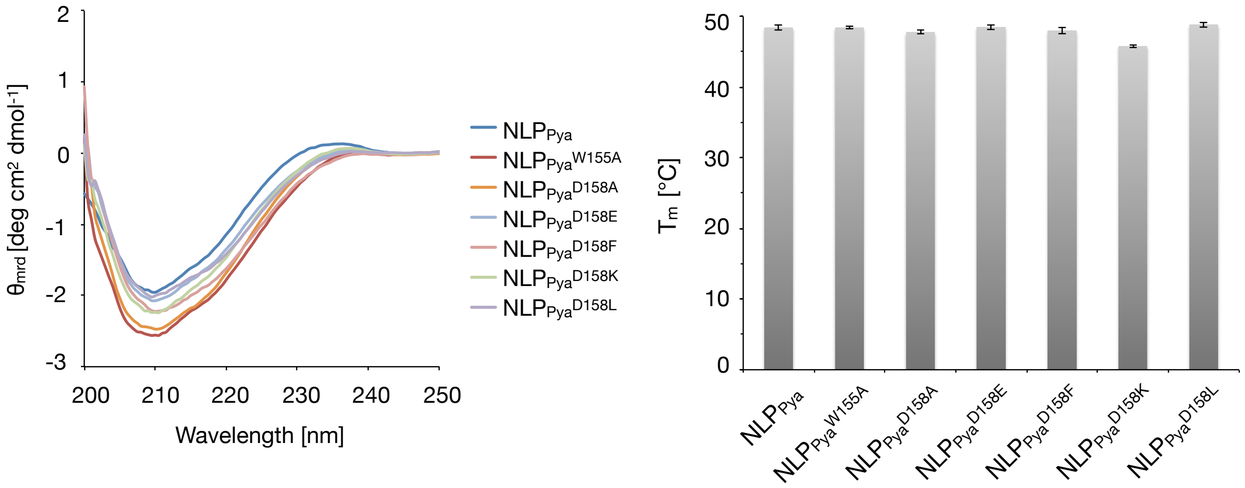


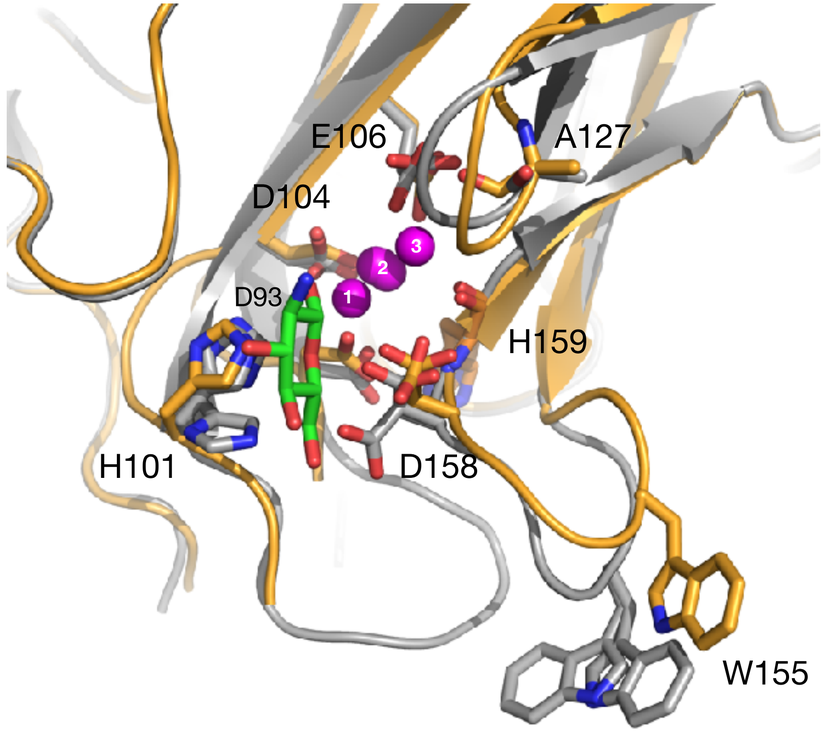


apoNLP$_{Pya}$
NLP$_{Pya}$-glucosamine

Structural analysis

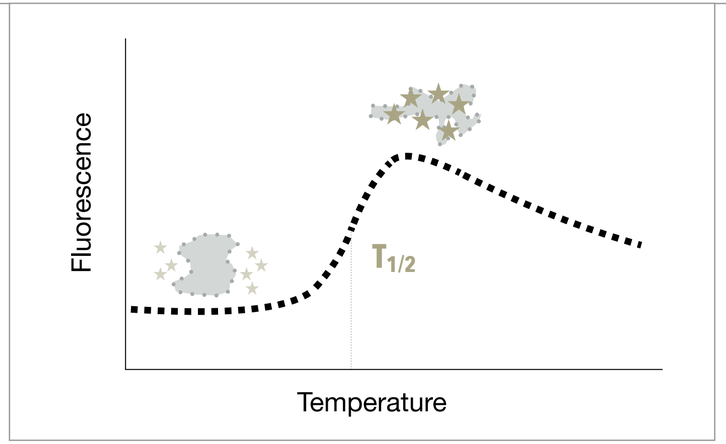


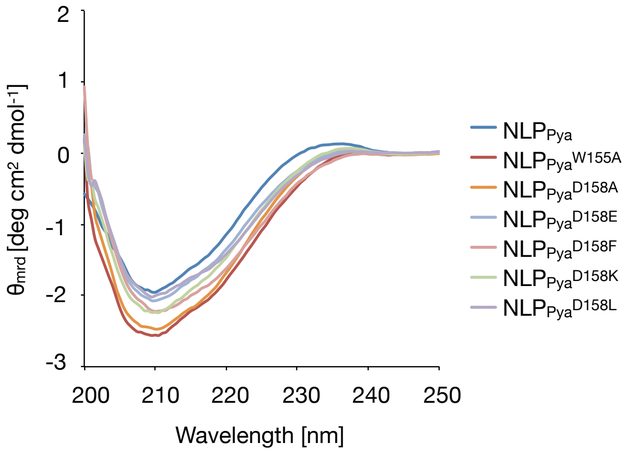


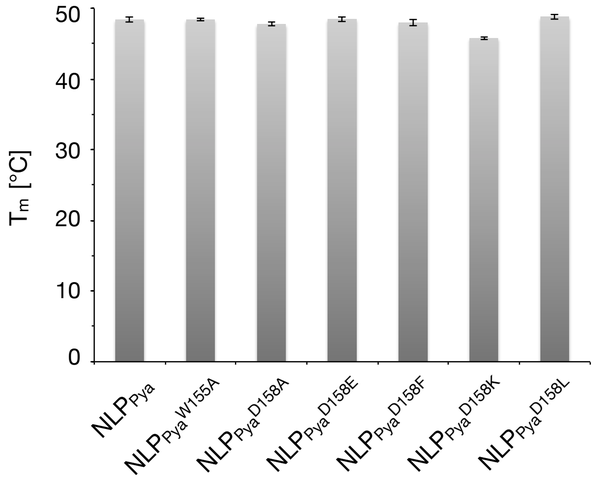


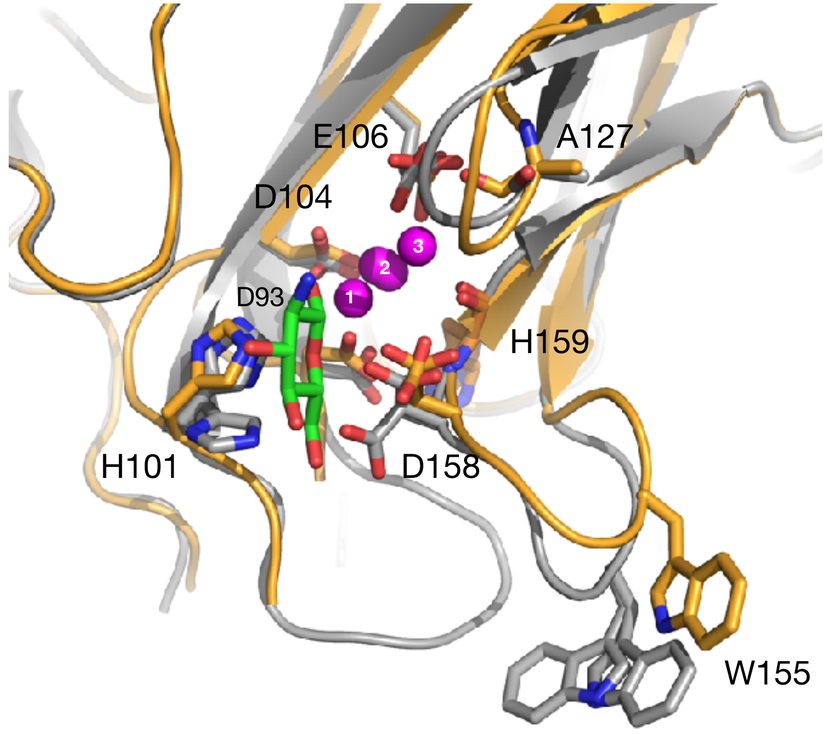
apoNLP$_{Pya}$
NLP$_{Pya}$-glucosamine

Structural analysis



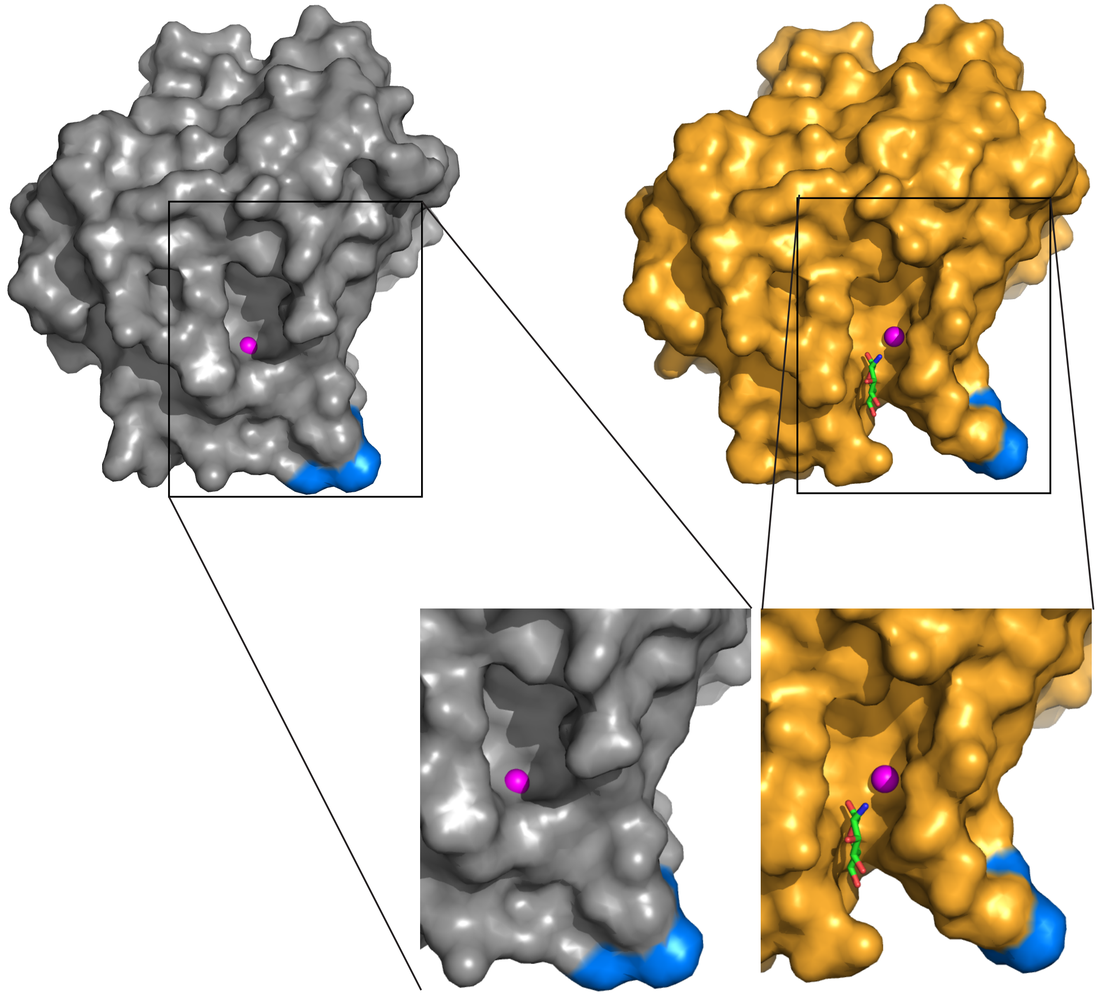






apoNLP_{Pya}
NLP_{Pya}-glucosamine

Structural analysis



BIOCHEMISTRY PROJECT molecular cloning

BIOCHEMISTRY PROJECT molecular cloning

protein purification

BIOCHEMISTRY PROJECT

molecular cloning

protein purification

crystallography

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molecular cloning

protein purification

crystallography

biophysical methods

BIOCHEMISTRY PROJECT molecular cloning

protein purification

crystallography

biophysical methods

NLP PROJECT NLP-plant membrane

BIOCHEMISTRY PROJECT molecular cloning

protein purification

crystallography

biophysical methods

NLP PROJECT NLP-plant membrane

eudicot selectivity

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biophysical methods

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eudicot selectivity

phytopharmaceutics

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eudicot selectivity

phytopharmaceutics

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