




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

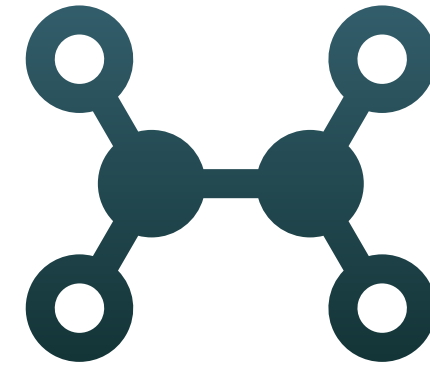
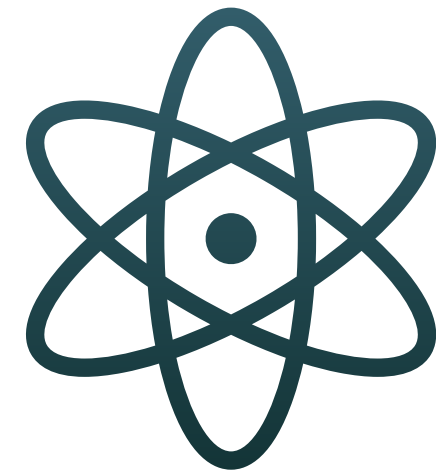
Physics



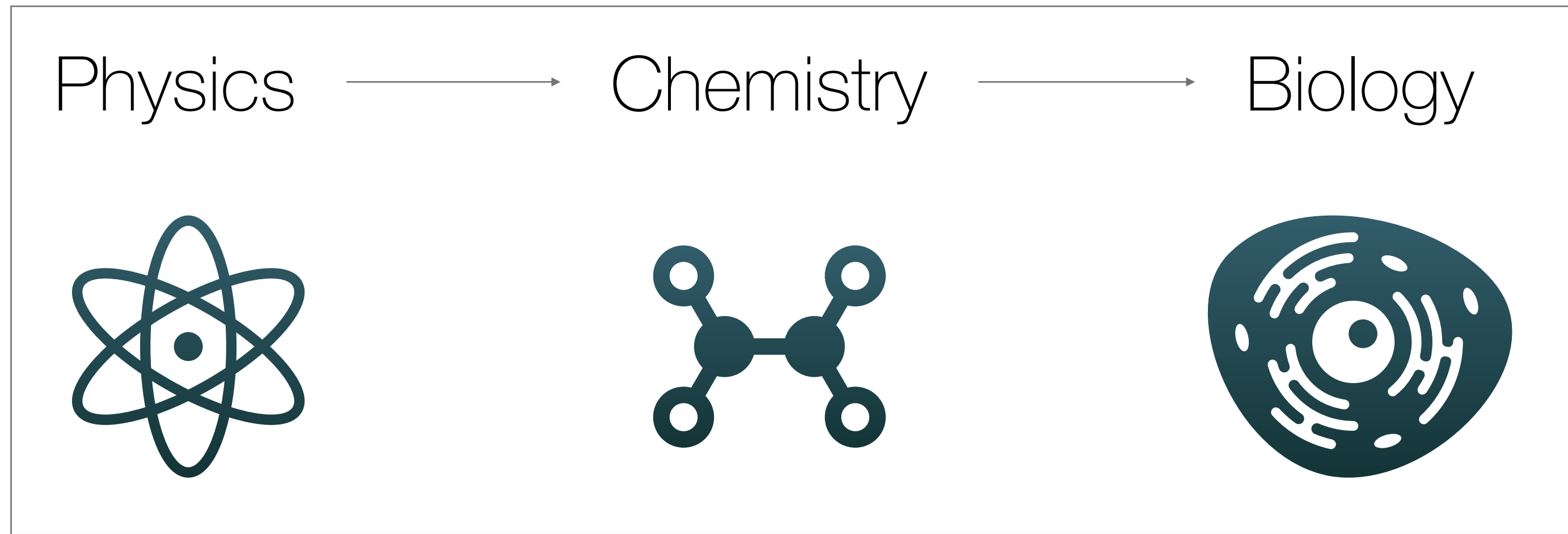
Chemistry



Biology



Life

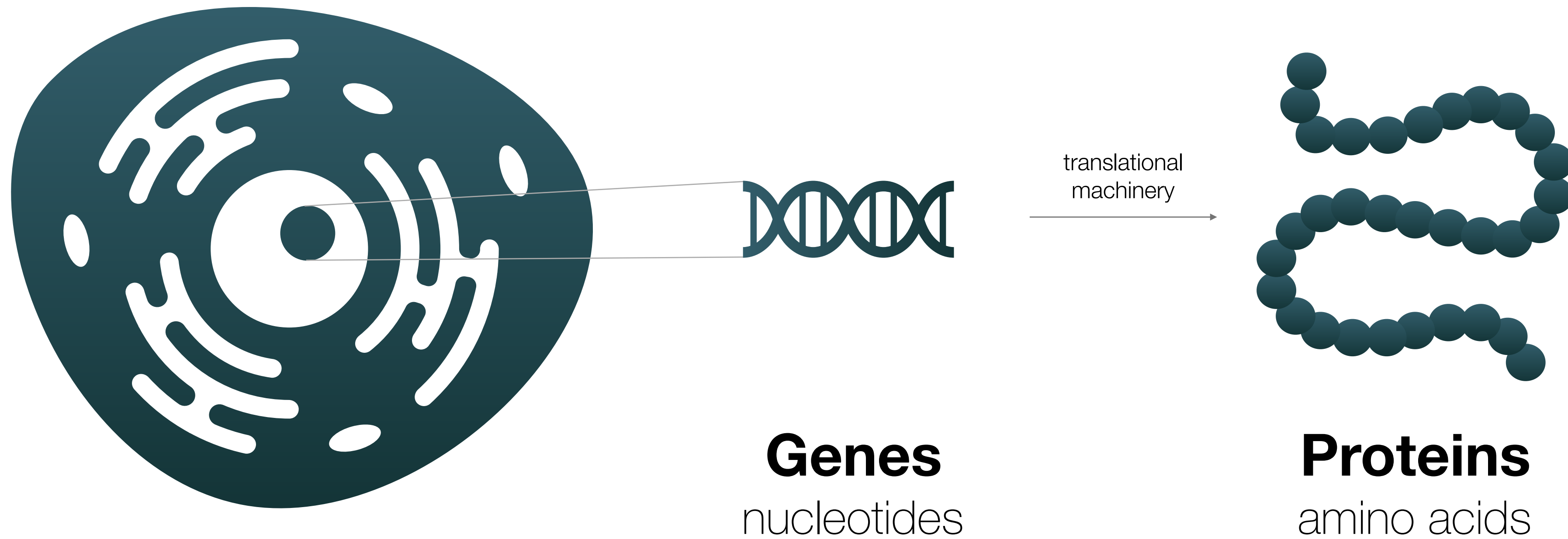


Biochemistry



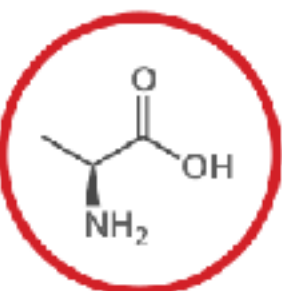
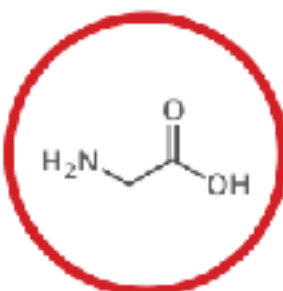
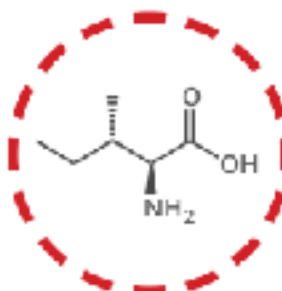
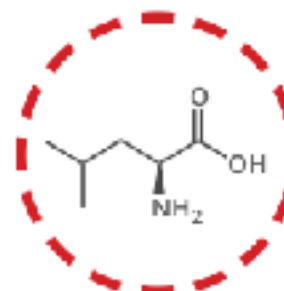
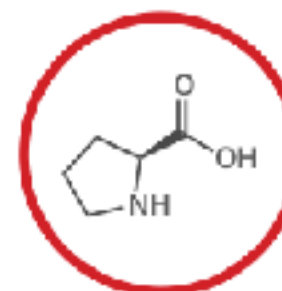
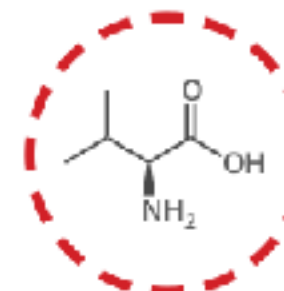
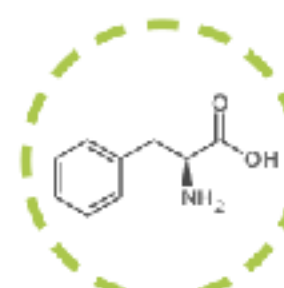
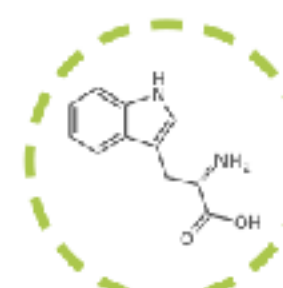
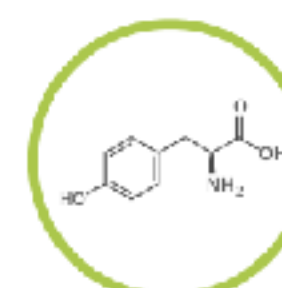
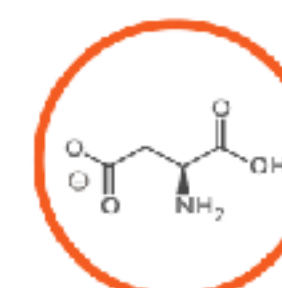
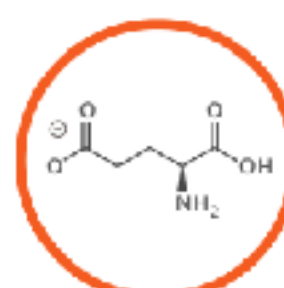
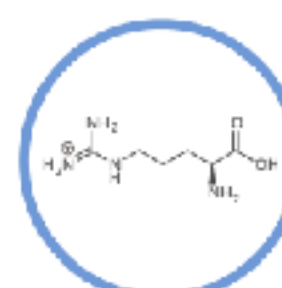
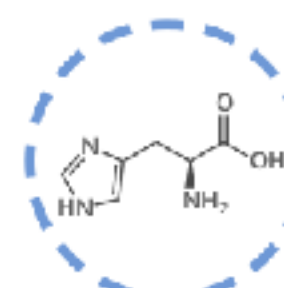
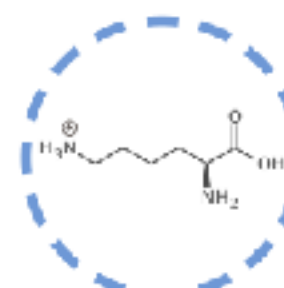
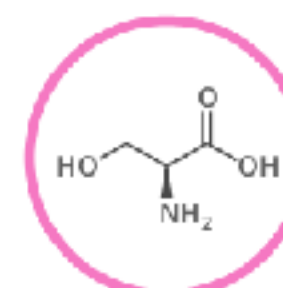
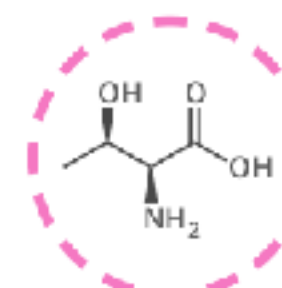
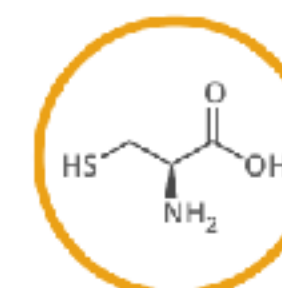
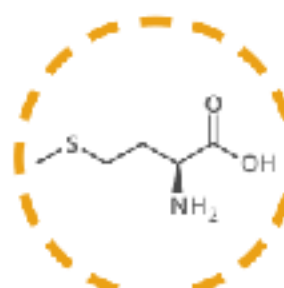
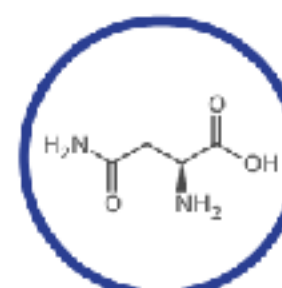
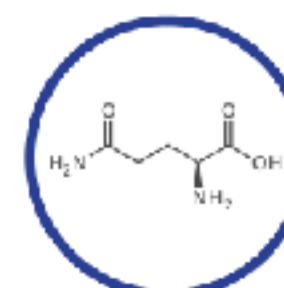
the branch of science concerned with the chemical and physico-chemical processes and substances which occur within living organisms.

Central dogma of molecular biology



Central dogma of molecular biology

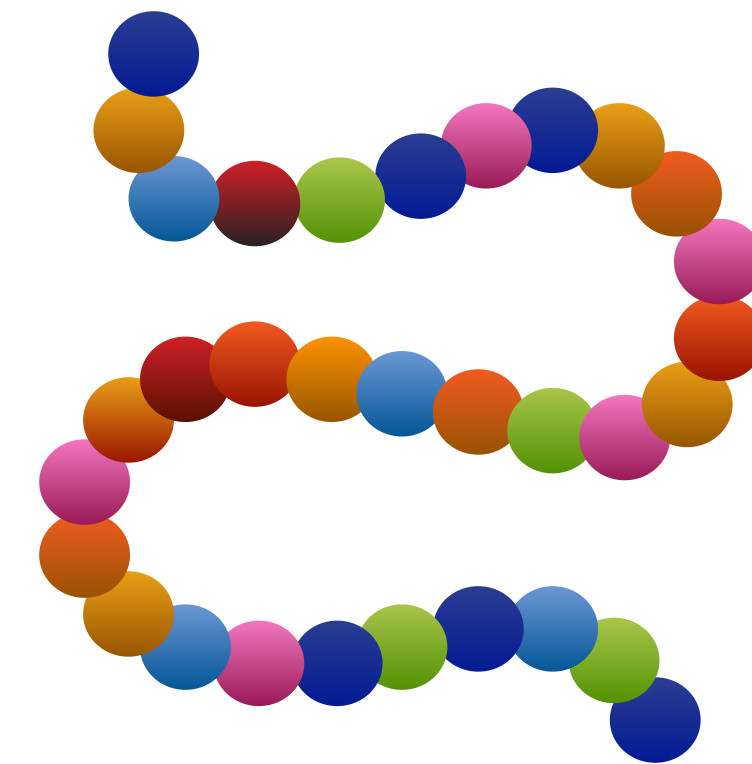
Chart Key: ● ALIPHATIC ● AROMATIC ● ACIDIC ● BASIC ● HYDROXYLIC ● SULFUR-CONTAINING ● AMIDIC ○ NON-ESSENTIAL ○ ESSENTIAL

Chemical Structure single letter code NAME A three letter code DNA codons	ALIPHATIC	AROMATIC	ACIDIC	BASIC	HYDROXYLIC	SULFUR-CONTAINING	AMIDIC	NON-ESSENTIAL	ESSENTIAL
 ALANINE A <i>Ala</i> GCT, GCC, GCA, GCG	●		●					○	○
 GLYCINE G <i>Gly</i> GGT, GGC, GGA, GGG	●		●					○	○
 ISOLEUCINE I <i>Ile</i> ATT, ATC, ATA	●		●					○	○
 LEUCINE L <i>Leu</i> CTT, CTC, CTA, CTG, TTA, TTG	●		●					○	○
 PROLINE P <i>Pro</i> CCT, CCC, CCA, CCG	●		●				●	○	○
 VALINE V <i>Val</i> GTT, GTC, GTA, GTG	●		●					○	○
 PHENYLALANINE F <i>Phe</i> TTT, TTC		●						○	○
 TRYPTOPHAN W <i>Trp</i> TGG		●						○	○
 TYROSINE Y <i>Tyr</i> TAT, TAC		●						○	○
 ASPARTIC ACID D <i>Asp</i> GAT, GAC			●					○	○
 GLUTAMIC ACID E <i>Glu</i> GAA, GAG			●					○	○
 ARGININE R <i>Arg</i> CGT, CGC, CGA, CCG, AGA, AGG				●			●	○	○
 HISTIDINE H <i>His</i> CAT, CAC					●			○	○
 LYSINE K <i>Lys</i> AAA, AAG								○	○
 SERINE S <i>Ser</i> TCT, TCC, TCA, TCG, AGT, AGC					●			○	○
 THREONINE T <i>Thr</i> ACT, ACC, ACA, ACG					●			○	○
 CYSTEINE C <i>Cys</i> TGT, TGC						●		○	○
 METHIONINE M <i>Met</i> ATG						●		○	○
 ASPARAGINE N <i>Asn</i> AAT, AAC							●	○	○
 GLUTAMINE Q <i>Gln</i> CAA, CAG								○	○



Proteins
amino acids

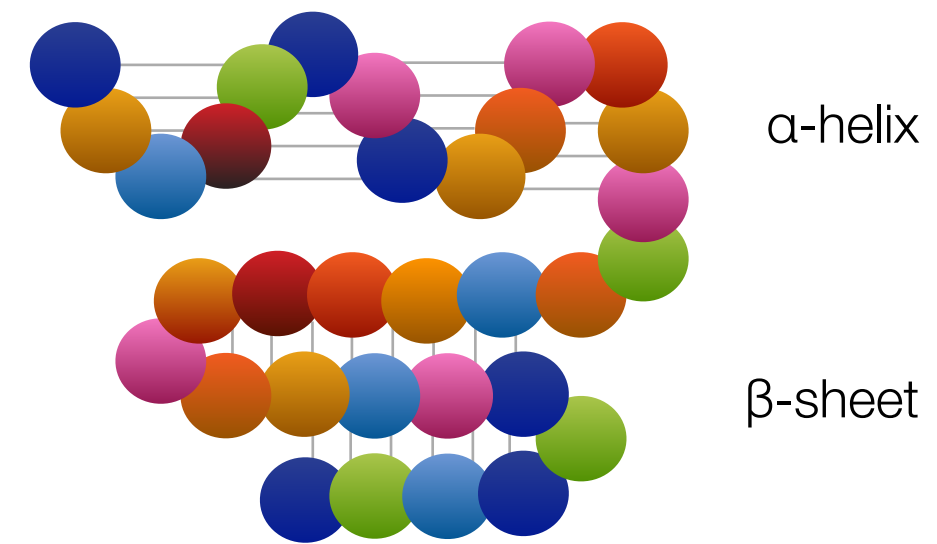
Orders of protein structures



**Primary
structure**

sequence

Orders of protein structures



Secondary structure

structural elements

Orders of protein structures



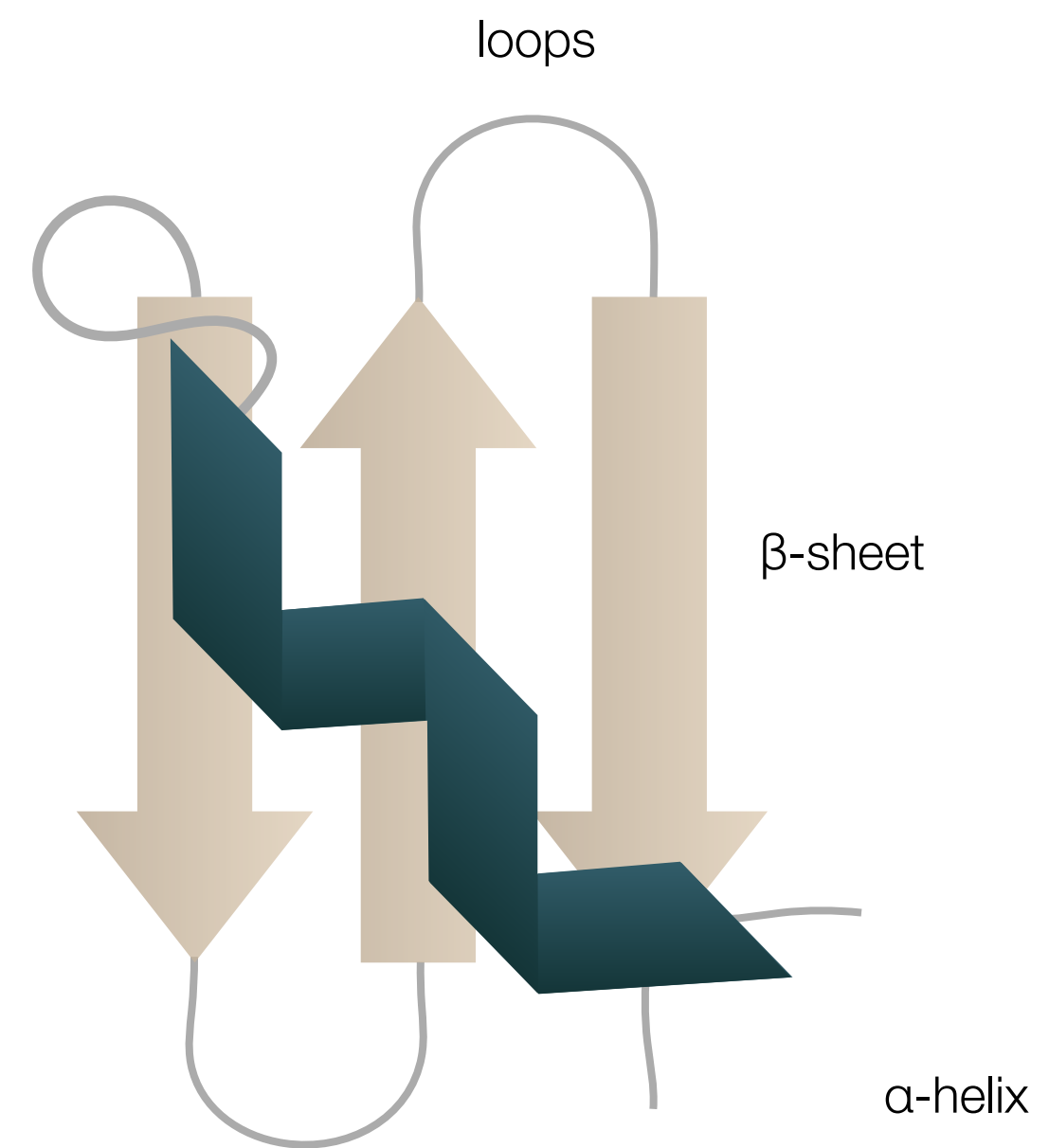
**Tertiary
structure**

3D fold

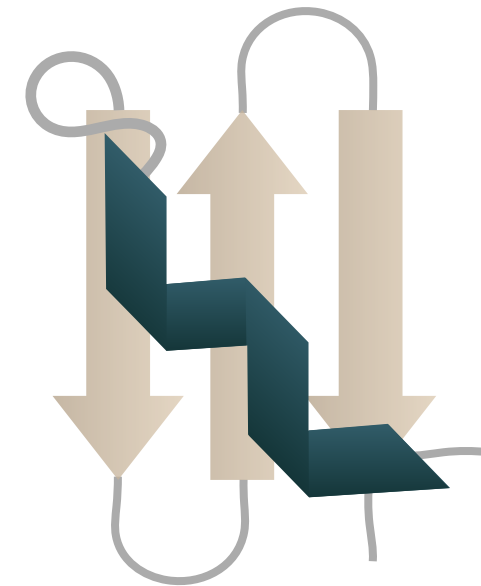
Presentation



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

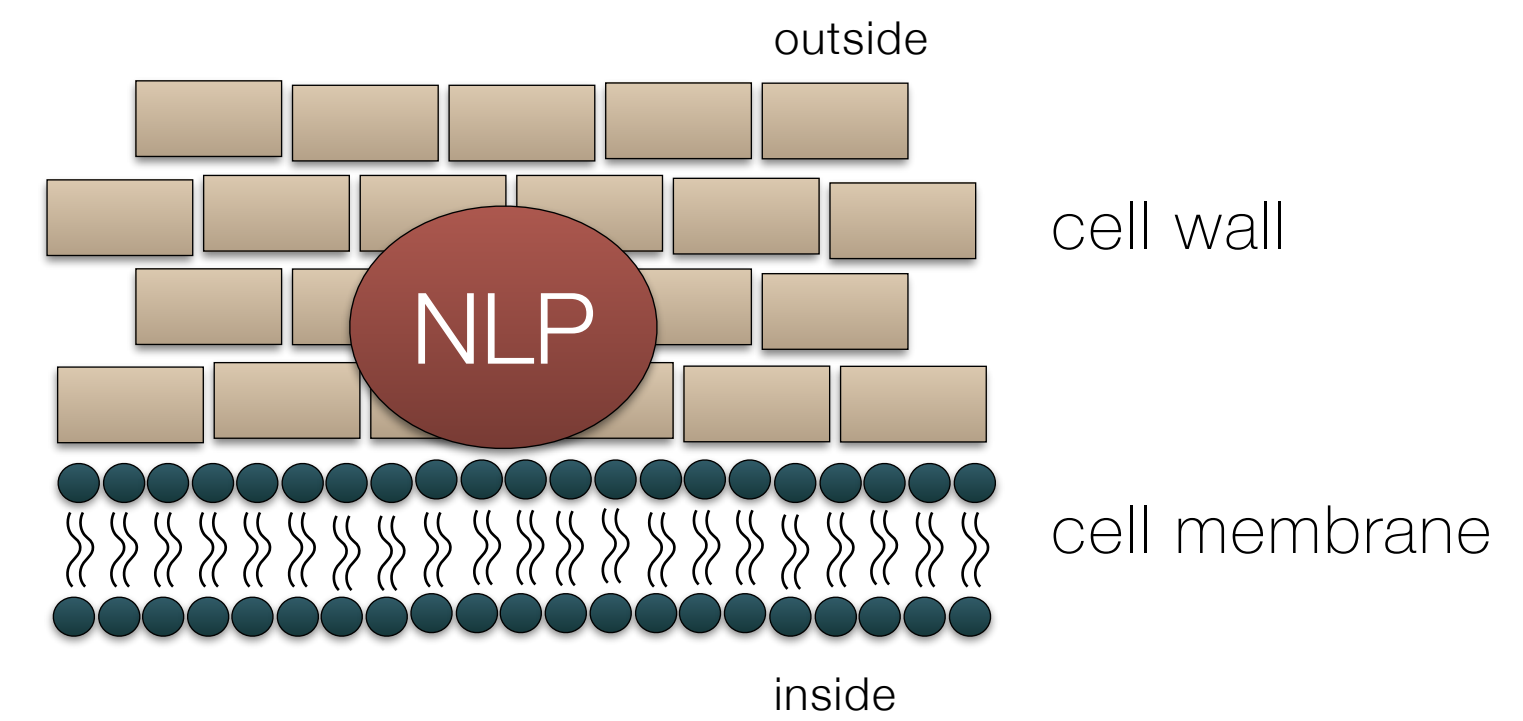
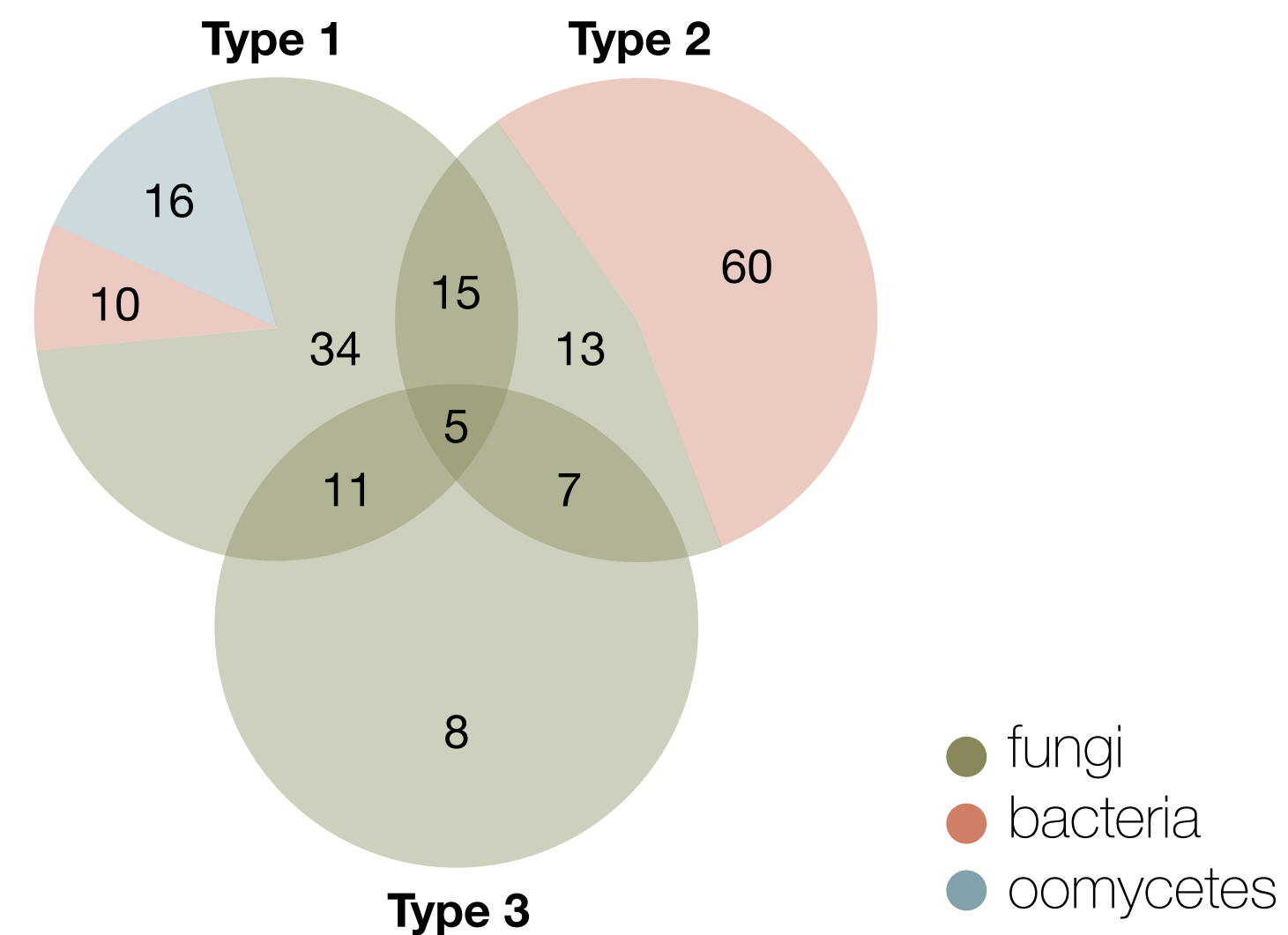
NLP toxins

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**



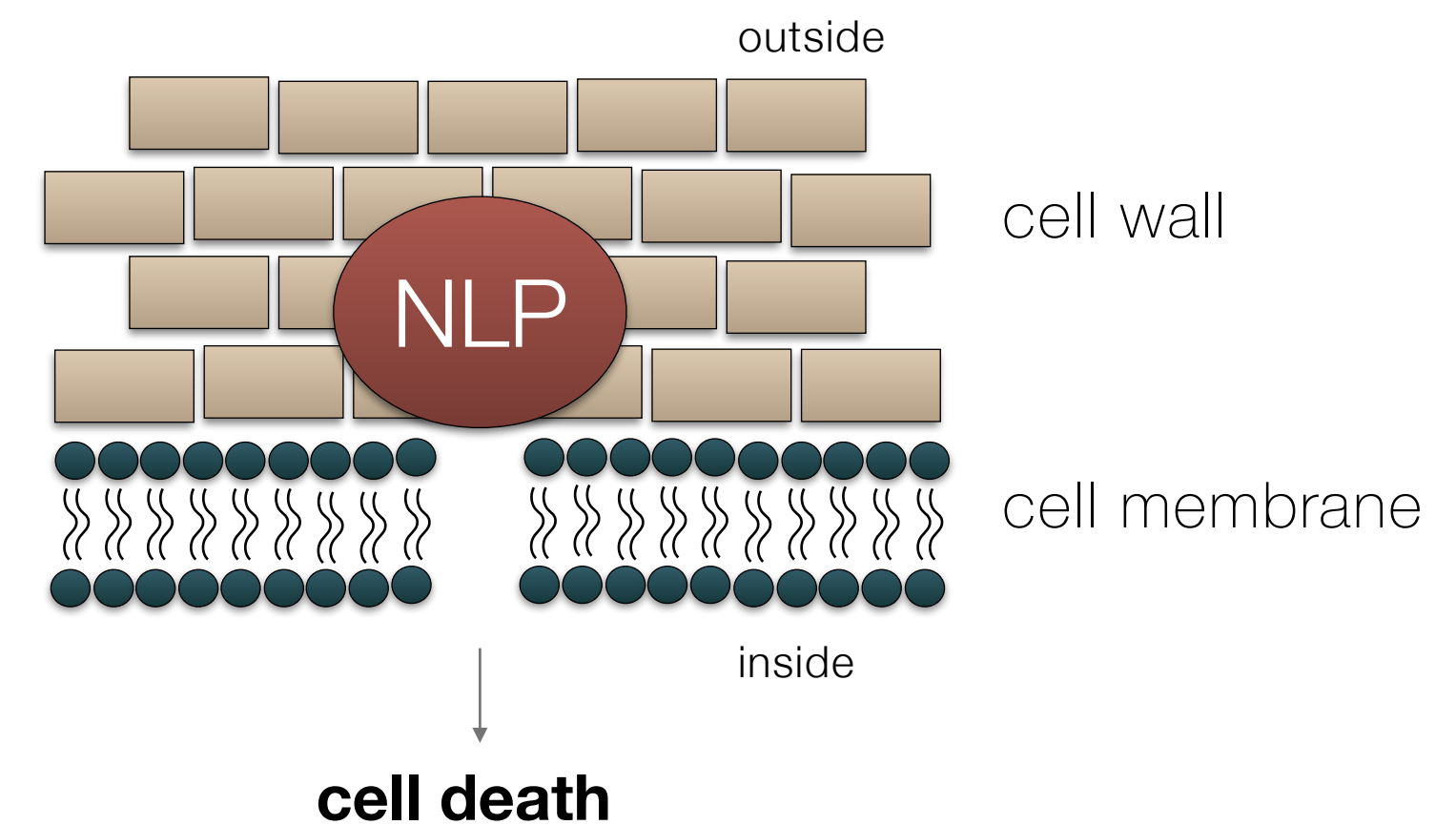
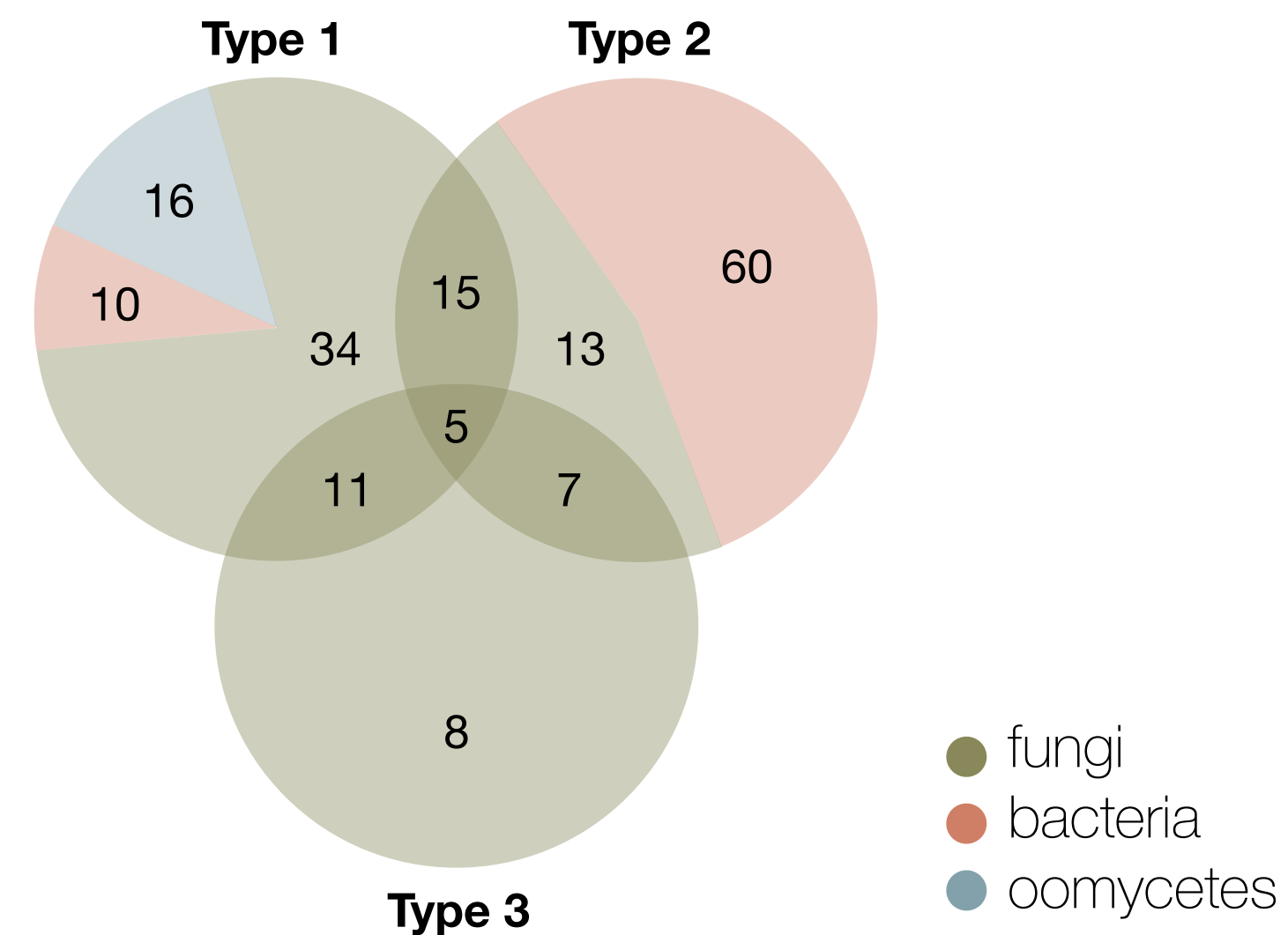
NLP toxins

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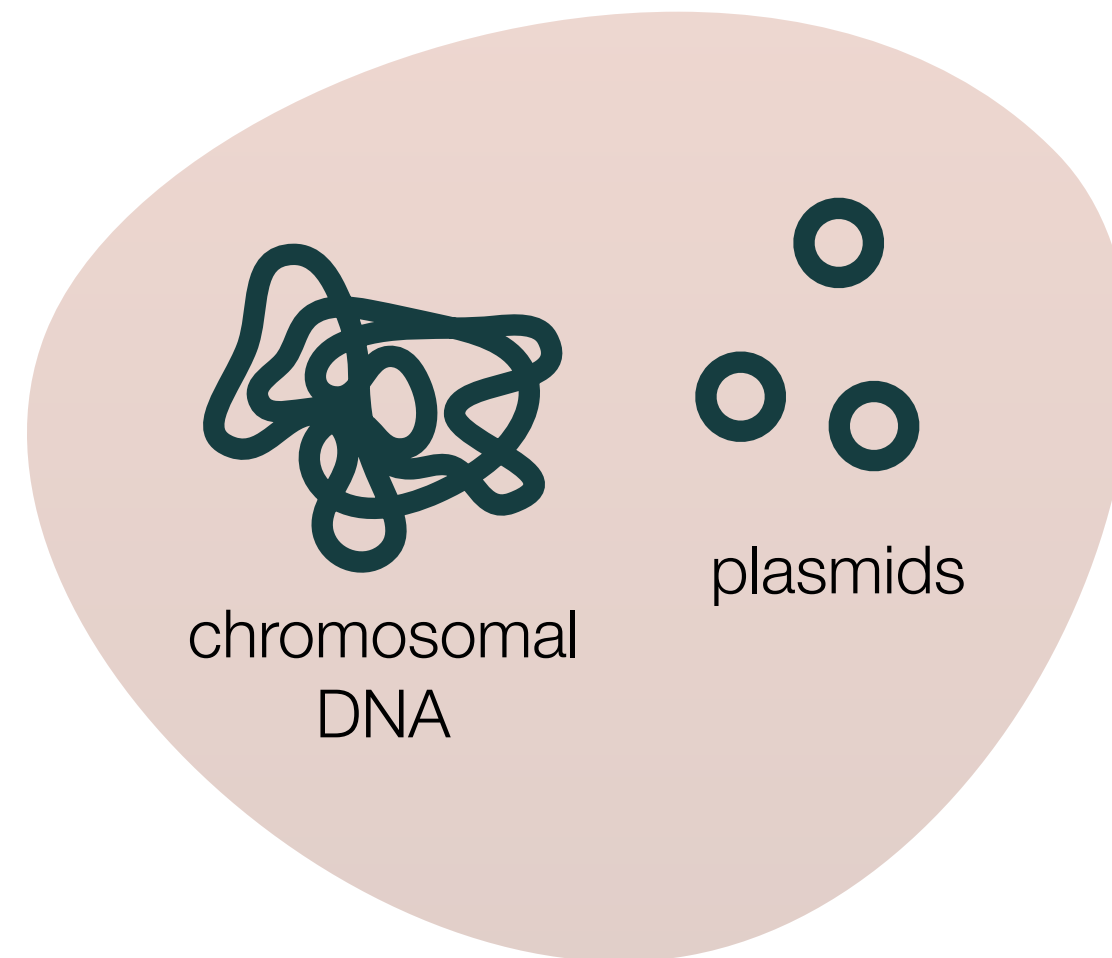
function **predicted to form pores in target membranes**



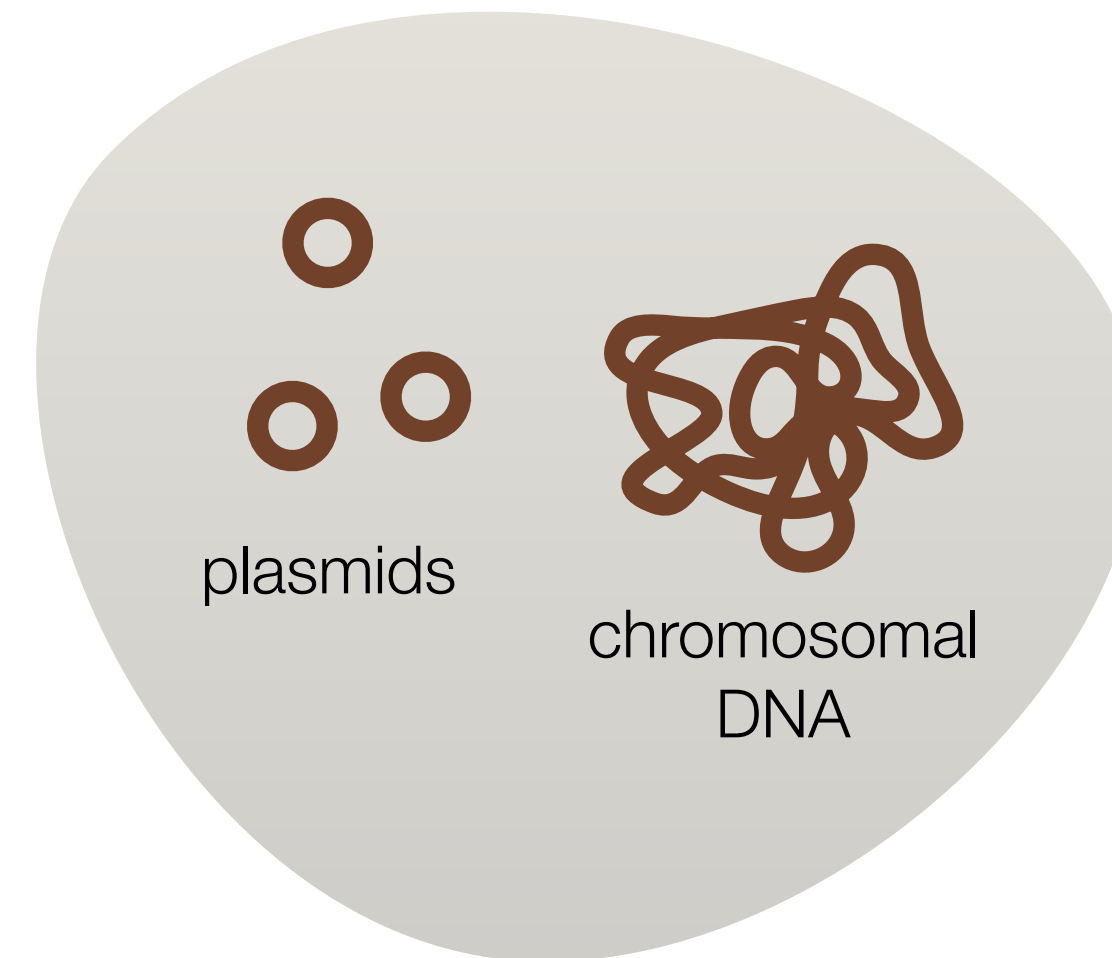
Biochemistry project

Sample preparation

Bacteria
#1

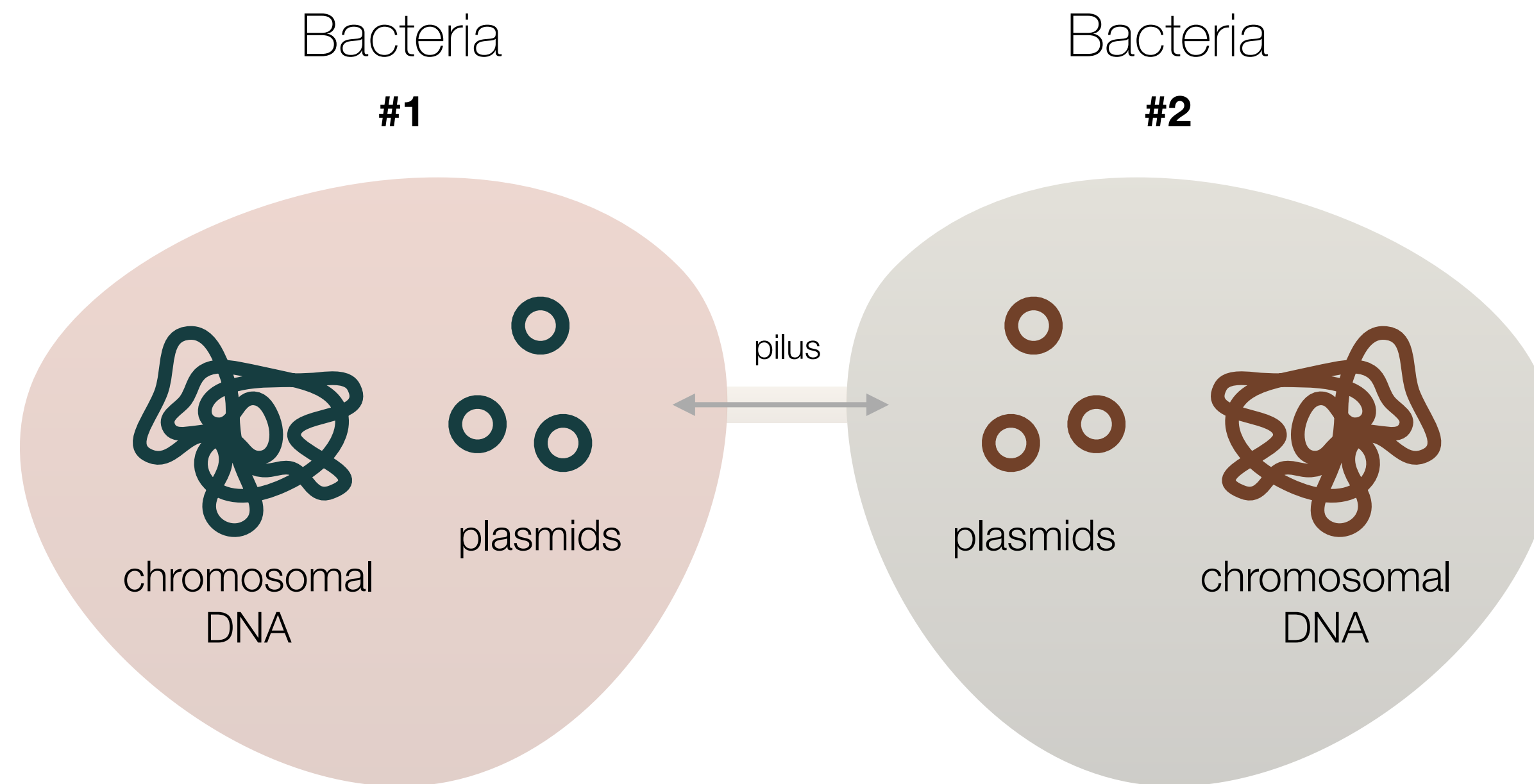


Bacteria
#2



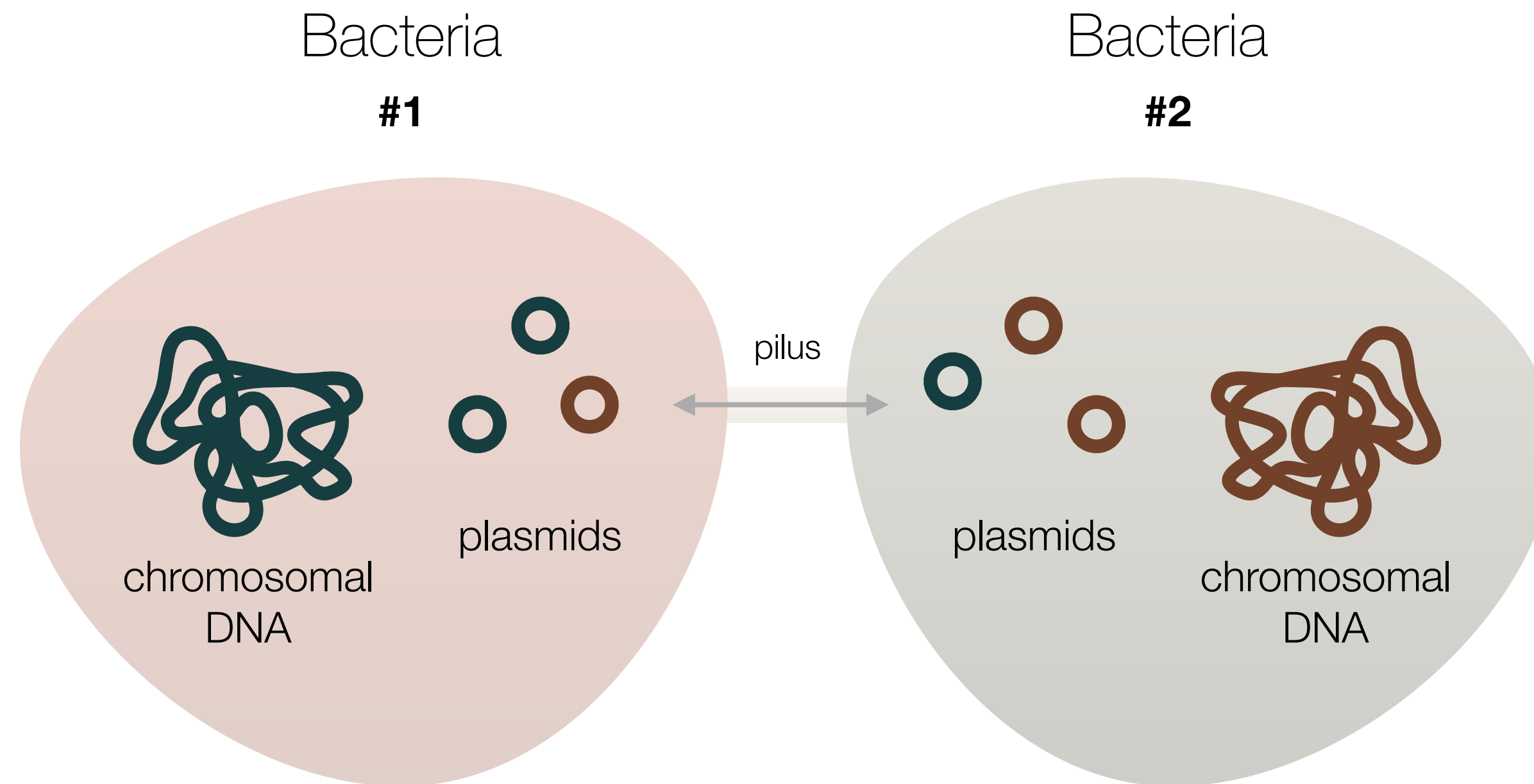
Biochemistry project

Sample preparation



Biochemistry project

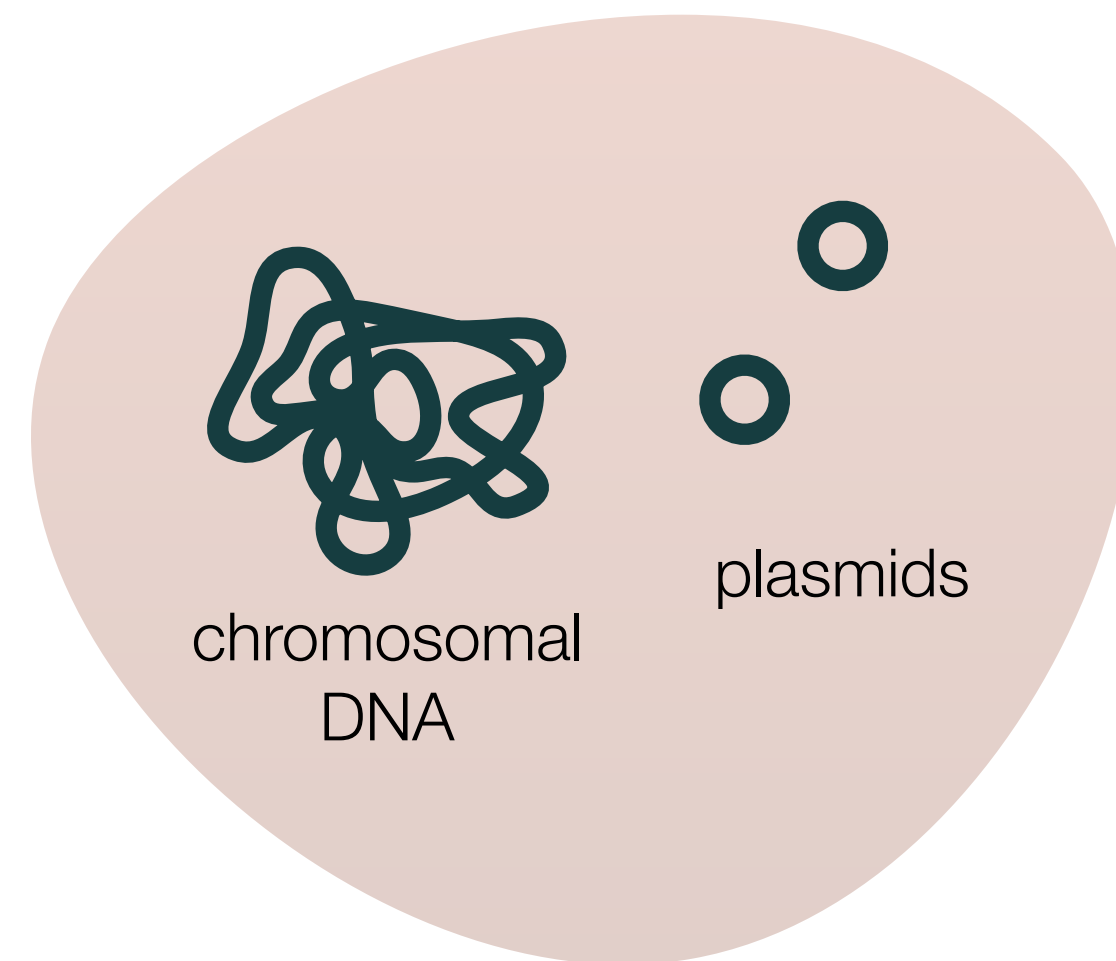
Sample preparation



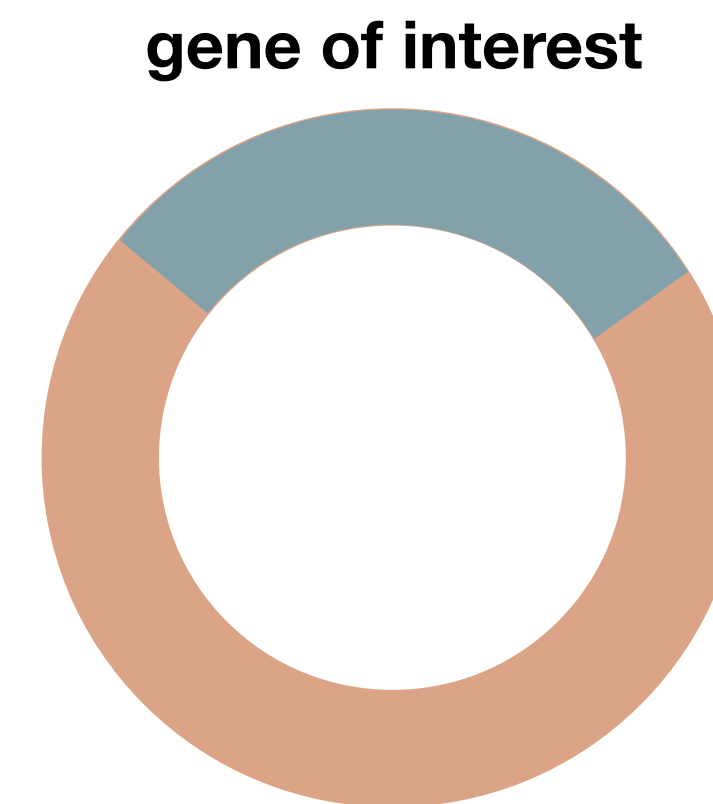
Biochemistry project

Sample preparation

Bacteria



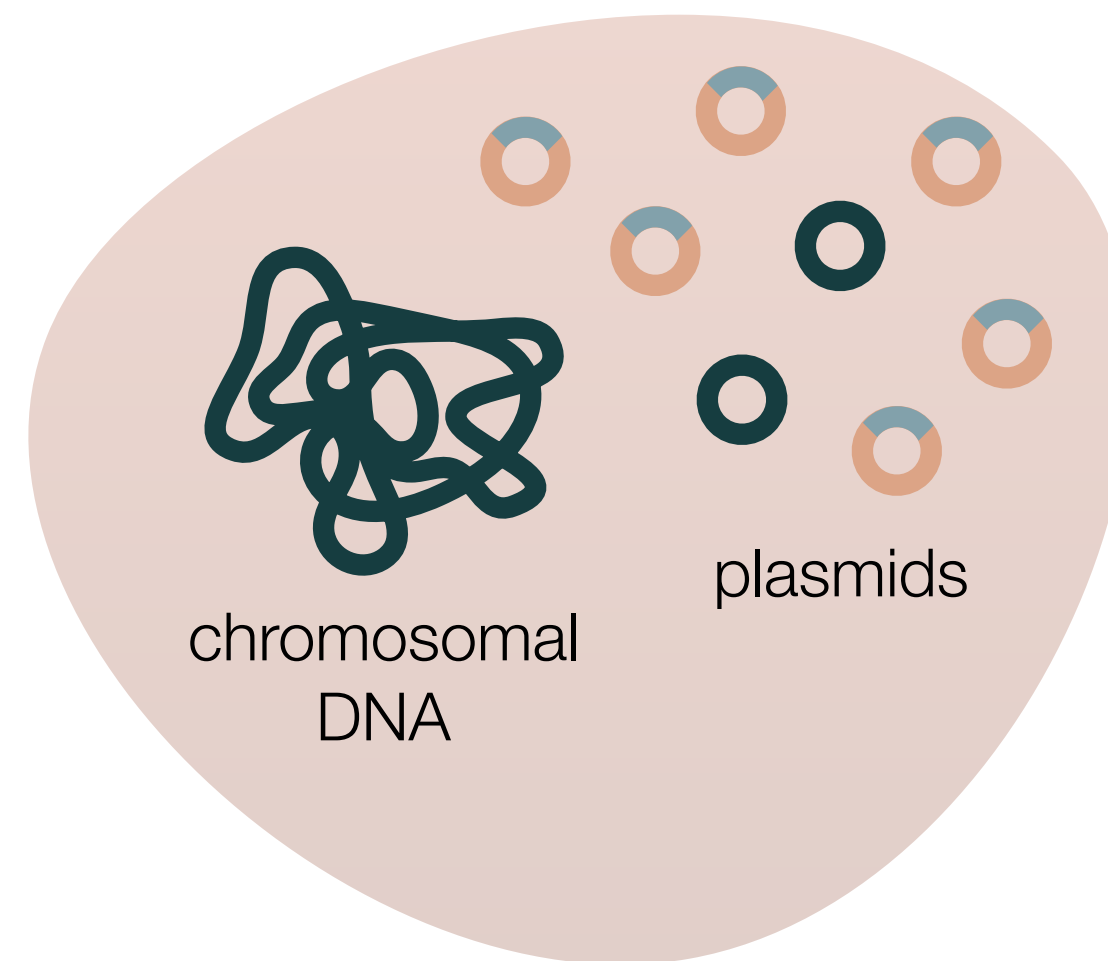
Vector



Biochemistry project

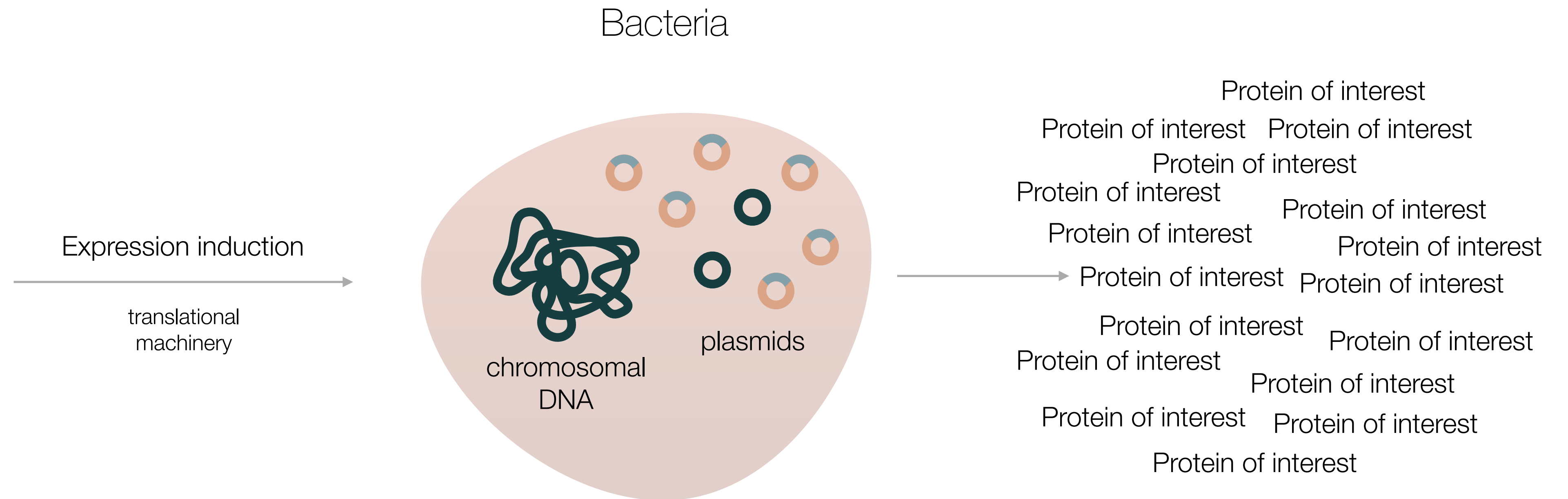
Sample preparation

Bacteria



Biochemistry project

Sample preparation



Biochemistry project

Protein purification

Bacterial protein E Bacterial protein A
Bacterial protein G Bacterial protein B Bacterial protein D
Bacterial protein C Bacterial protein H Bacterial protein G
Bacterial protein B Bacterial protein E Bacterial protein E
Bacterial protein F Bacterial protein D Bacterial protein H
Bacterial protein A Bacterial protein C Bacterial protein C
Bacterial protein D Bacterial protein G Bacterial protein E
Bacterial protein F Bacterial protein B Bacterial protein B
Bacterial protein C Bacterial protein A
Bacterial protein H Bacterial protein E Bacterial protein F

Protein of interest
Protein of interest Protein of interest
Protein of interest
Protein of interest Protein of interest
Protein of interest Protein of interest
Protein of interest Protein of interest
Protein of interest Protein of interest
Protein of interest
Protein of interest Protein of interest
Protein of interest
Protein of interest Protein of interest
Protein of interest

Biochemistry project

Protein purification

Protein of interest
Bacterial protein E Bacterial protein A Protein of interest
Protein of interest Bacterial protein G
Protein of interest Bacterial protein H Protein of interest Bacterial protein D
Protein of interest Bacterial protein E Bacterial protein B Bacterial protein C
Bacterial protein F Bacterial protein H Protein of interest Bacterial protein A
Bacterial protein A Protein of interest Bacterial protein C
Bacterial protein D Bacterial protein B Bacterial protein C
Protein of interest Bacterial protein G Protein of interest Bacterial protein G
Bacterial protein F Bacterial protein B Bacterial protein C Bacterial protein C Protein of interest
Protein of interest Protein of interest Bacterial protein B Bacterial protein F
Bacterial protein H Bacterial protein E Protein of interest
Protein of interest Bacterial protein D Protein of interest
Bacterial protein E Protein of interest

Biochemistry project

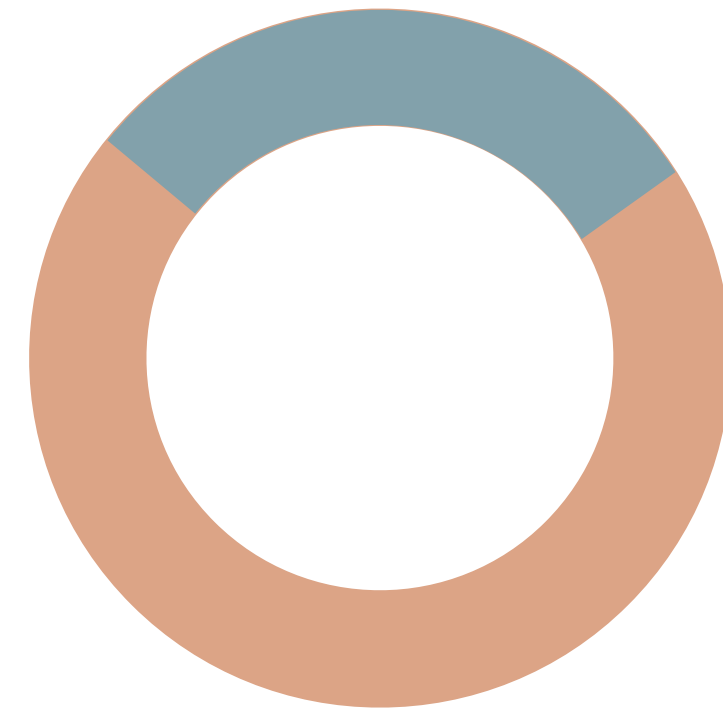
Protein purification

Protein of interest
Bacterial protein E Bacterial protein A **Protein of interest**
Protein of interest Bacterial protein G
Bacterial protein H **Protein of interest** Bacterial protein D
Protein of interest Bacterial protein E Bacterial protein B Bacterial protein C
Bacterial protein F Bacterial protein H **Protein of interest** Bacterial protein A
Bacterial protein A **Protein of interest** Bacterial protein B Bacterial protein C
Bacterial protein D Bacterial protein G **Protein of interest** Bacterial protein G
Protein of interest Bacterial protein B Bacterial protein C Bacterial protein C **Protein of interest**
Protein of interest **Protein of interest** Bacterial protein B Bacterial protein F
Bacterial protein H Bacterial protein E **Protein of interest**
Bacterial protein E **Protein of interest** Bacterial protein D **Protein of interest**
Protein of interest

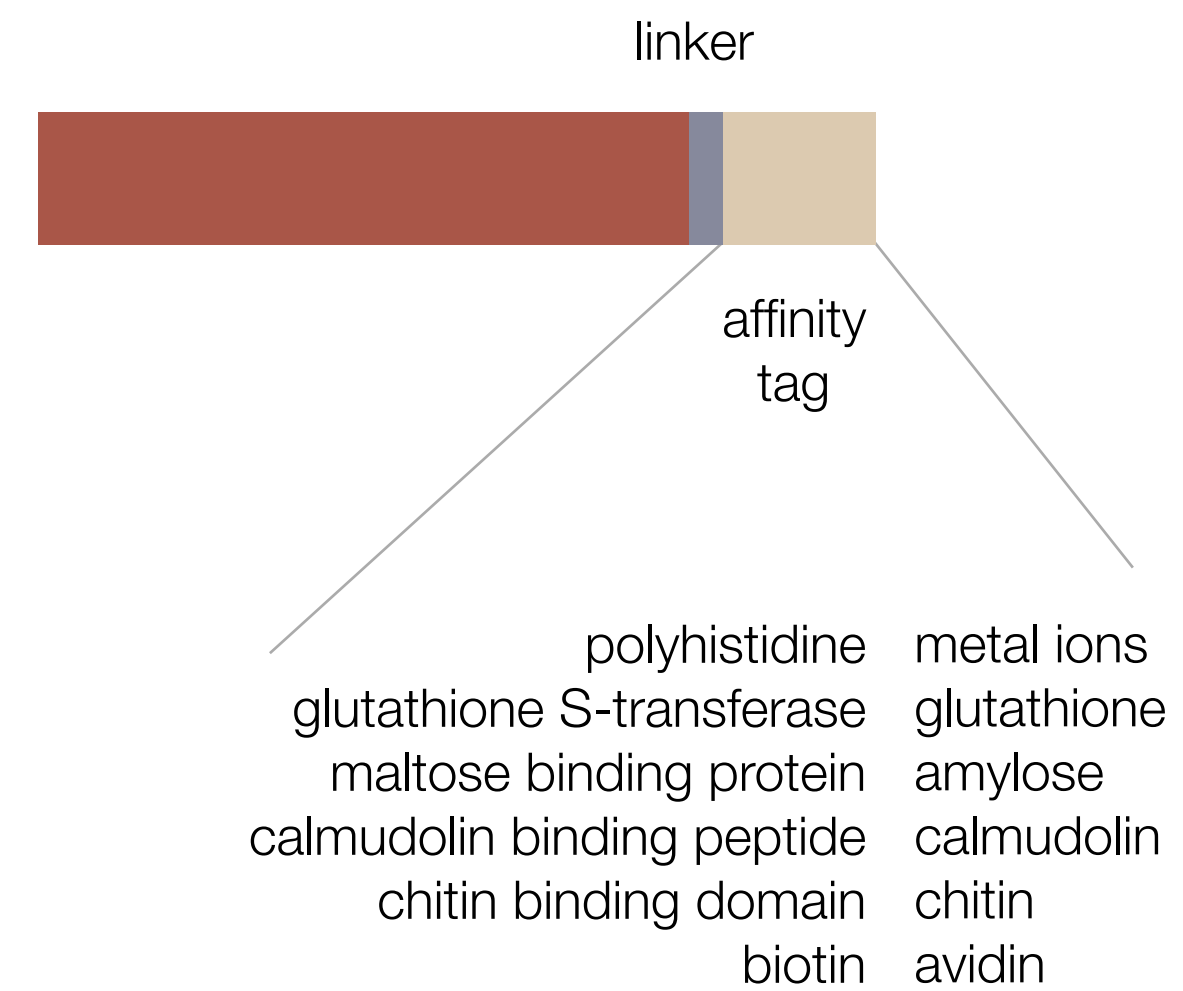
Biochemistry project

Protein purification

Gene of interest



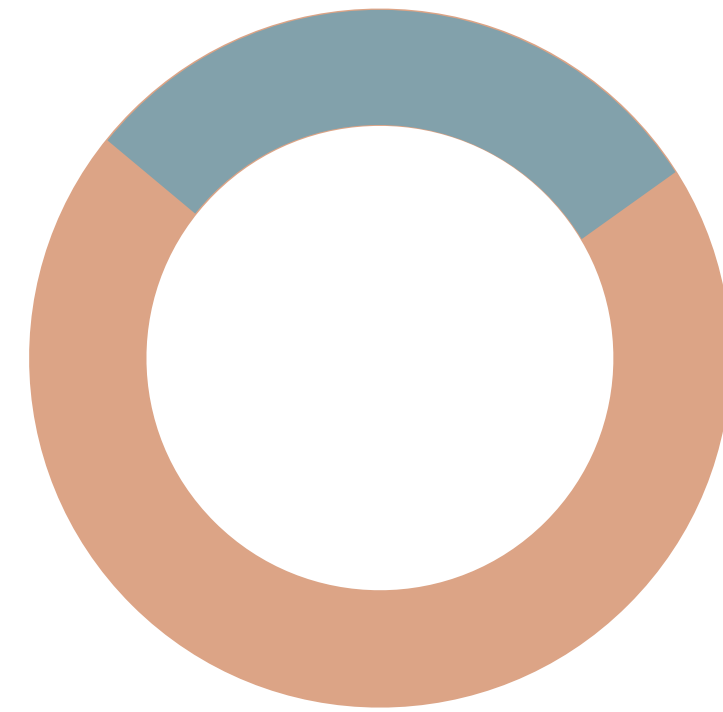
Protein of interest



Biochemistry project

Protein purification

Gene of interest



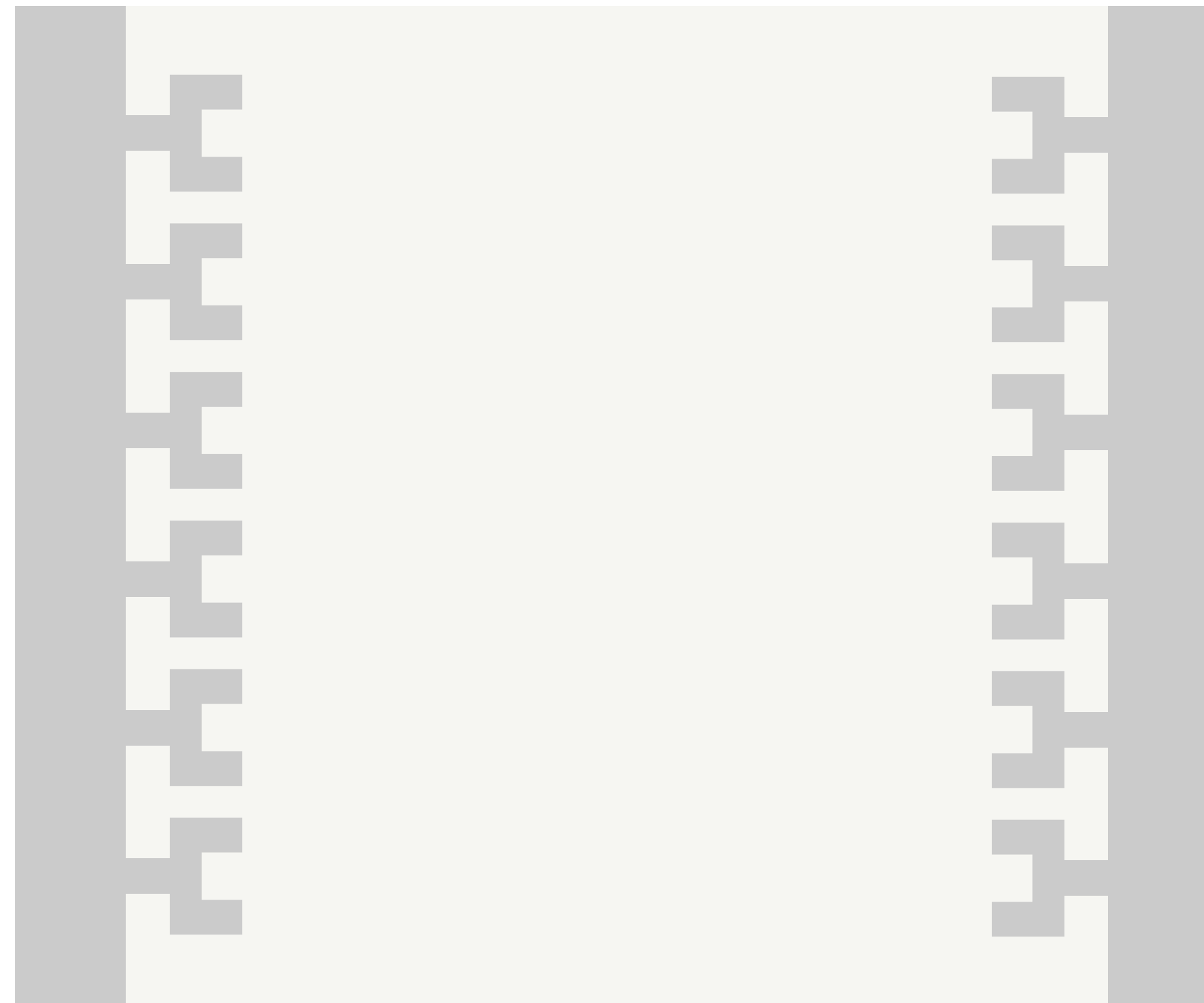
Protein of interest



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

Biochemistry project

Protein purification



affinity column

Biochemistry project

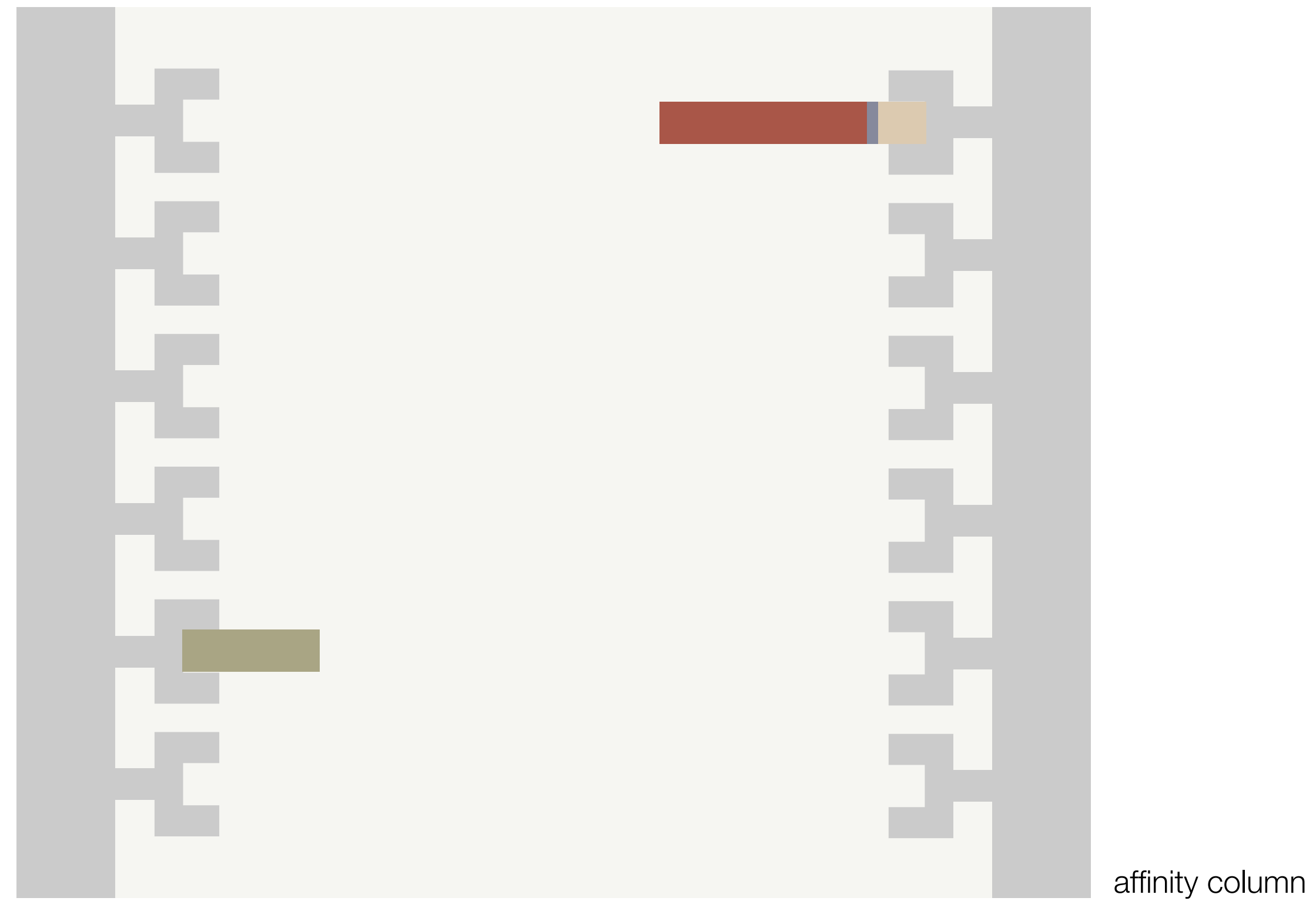
Protein purification



affinity column

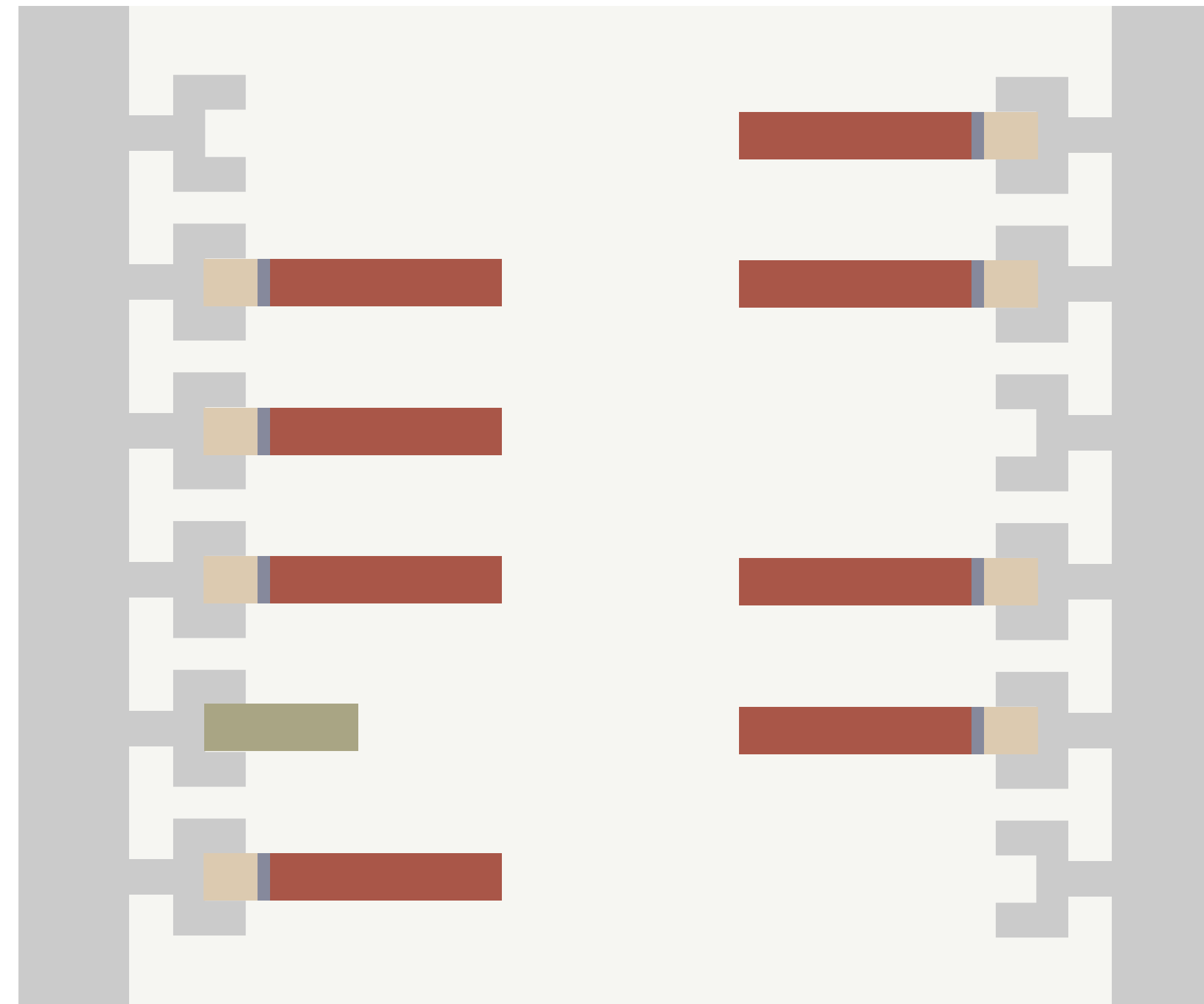
Biochemistry project

Protein purification



Biochemistry project

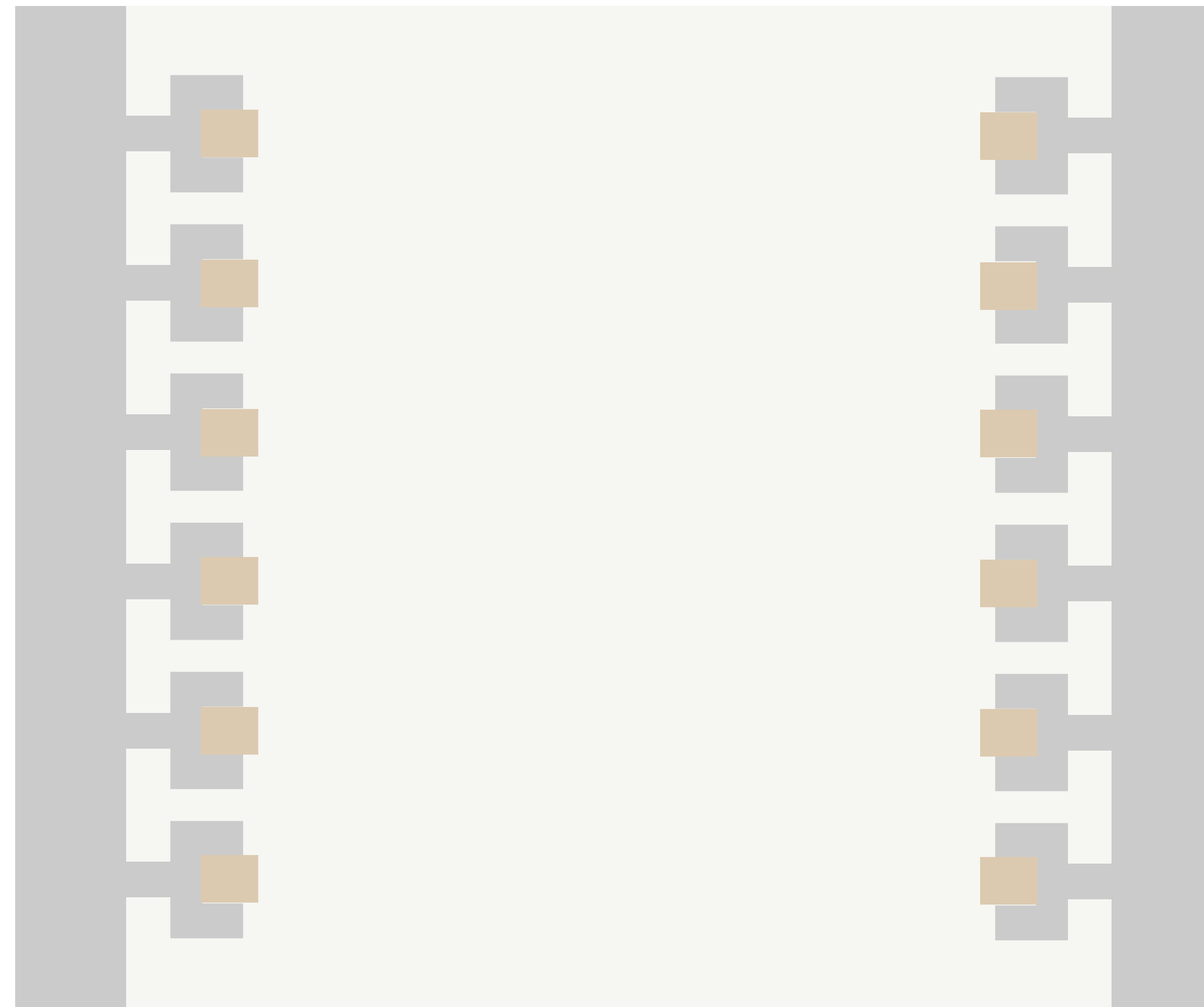
Protein purification



affinity column

Biochemistry project

Protein purification



affinity column

Biochemistry project

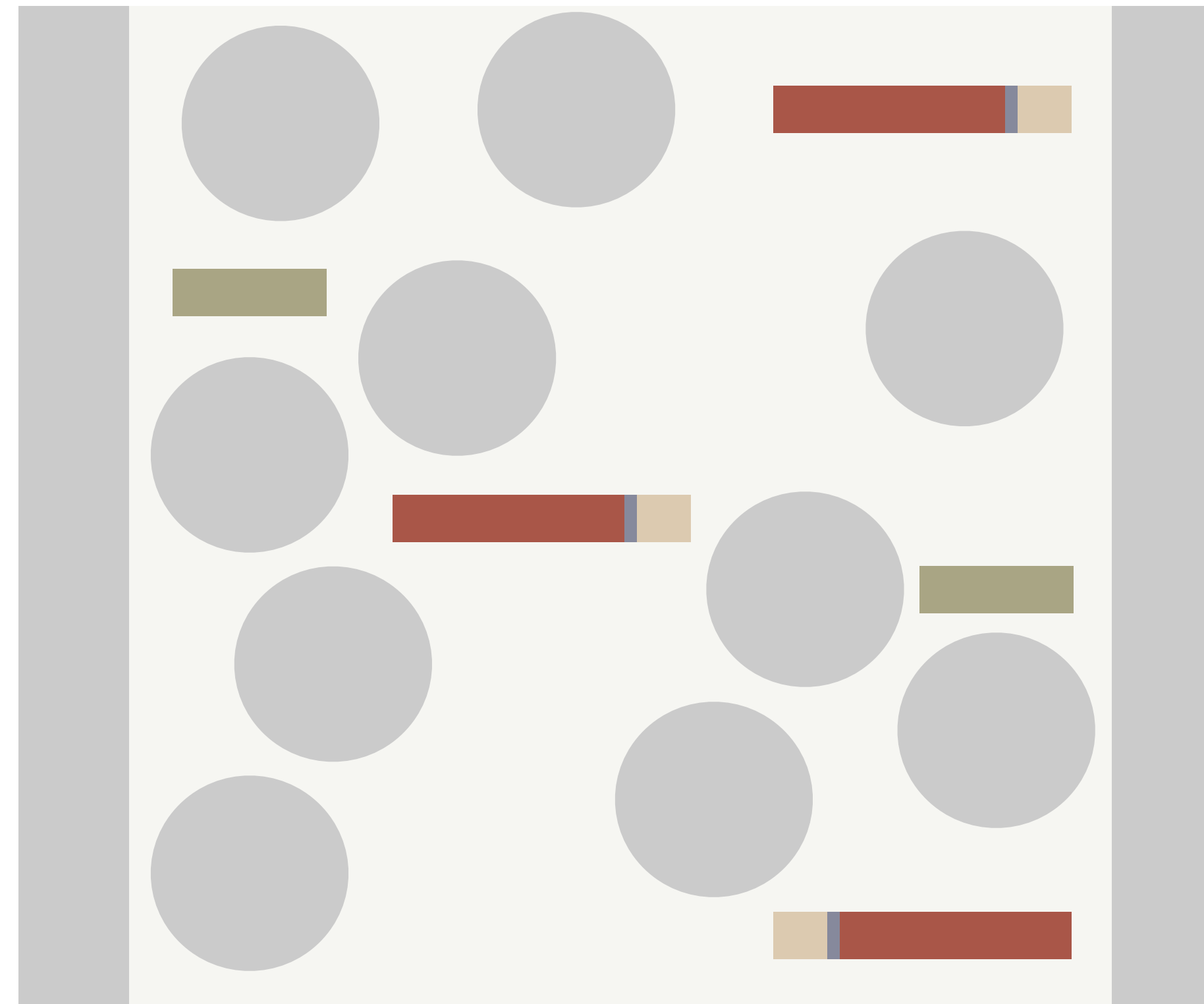
Protein purification



size-exclusion column

Biochemistry project

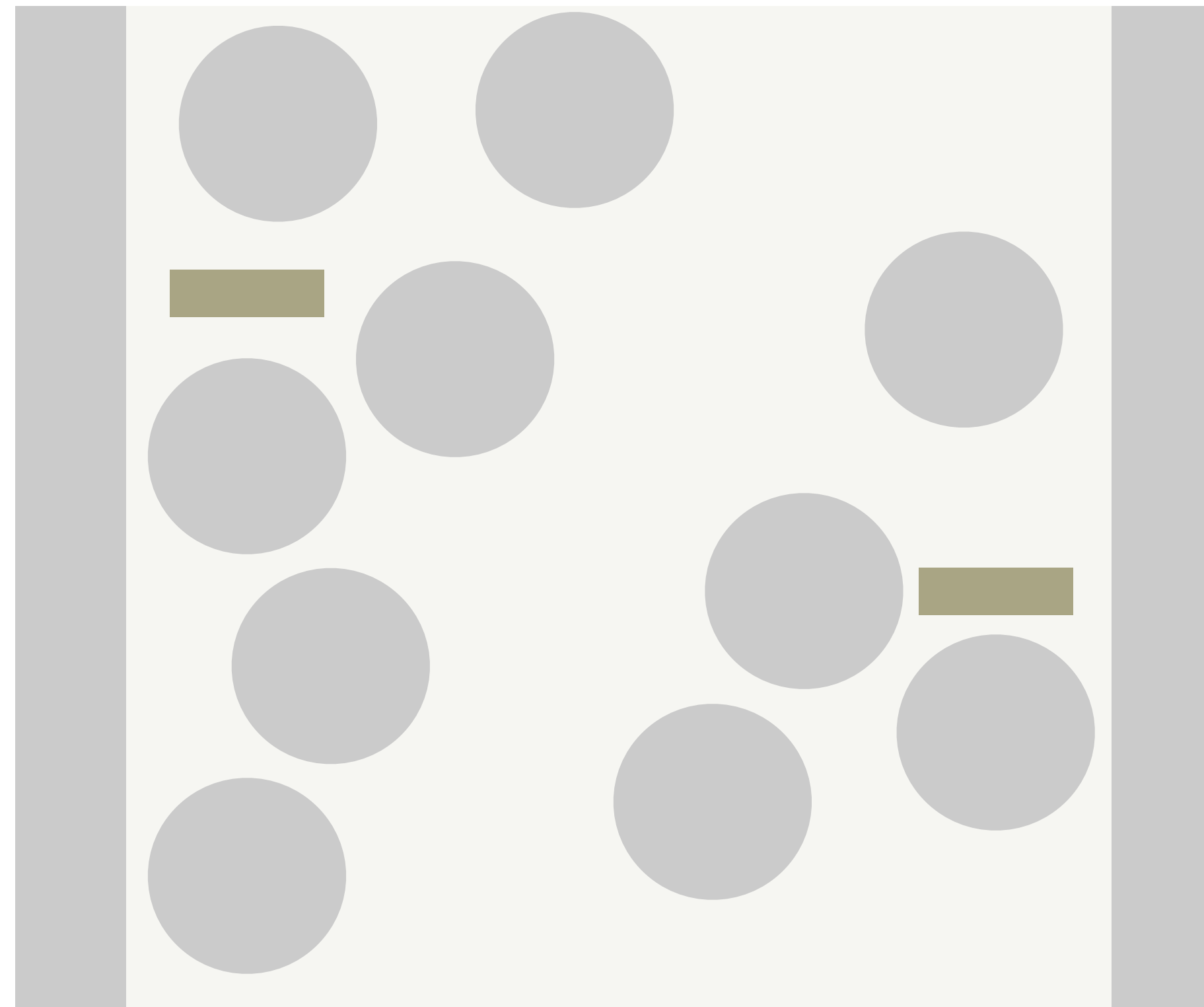
Protein purification



size-exclusion column

Biochemistry project

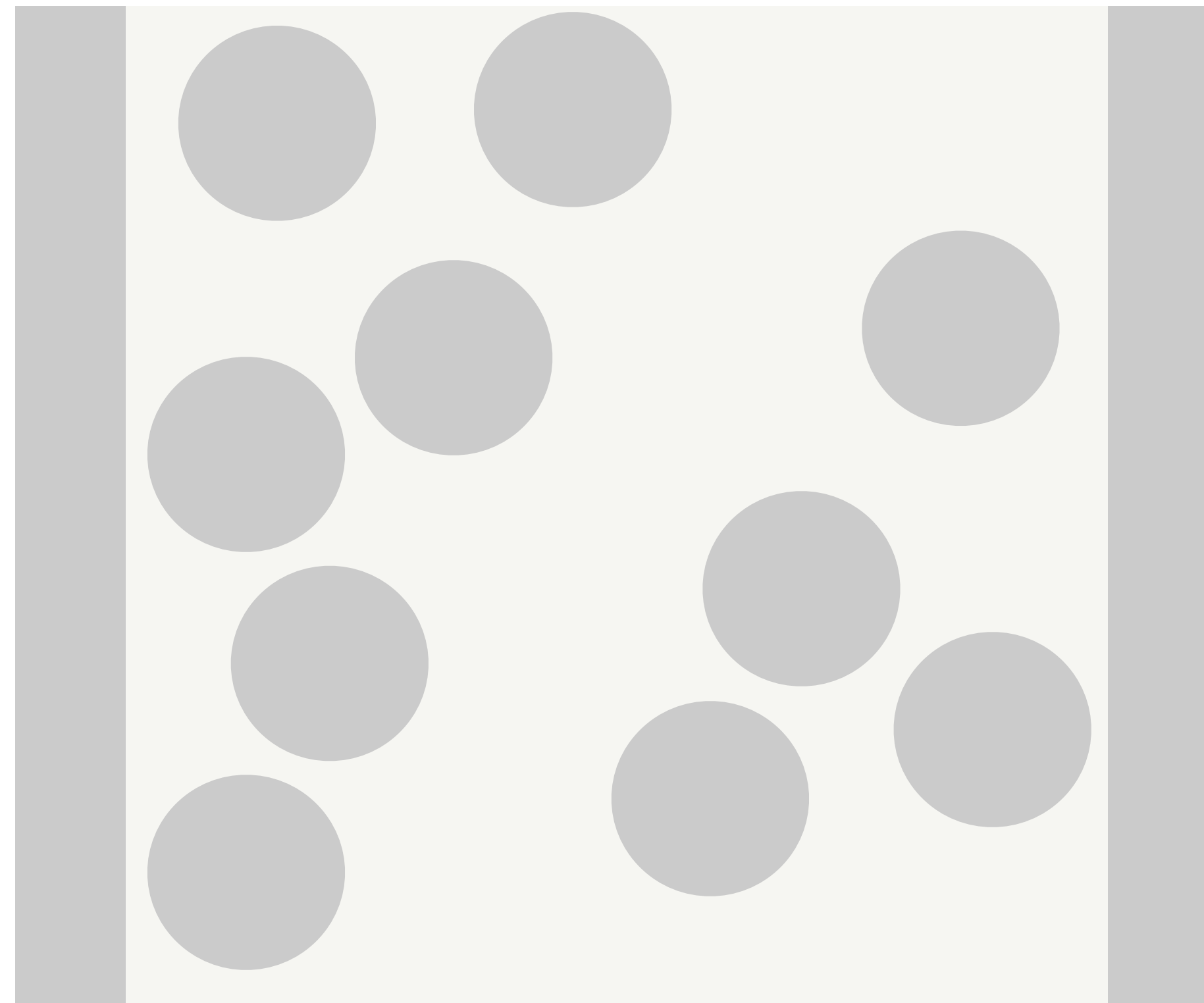
Protein purification



size-exclusion column

Biochemistry project

Protein purification



size-exclusion column

Biochemistry project

Protein purification

**pure fraction of your
protein of interest**

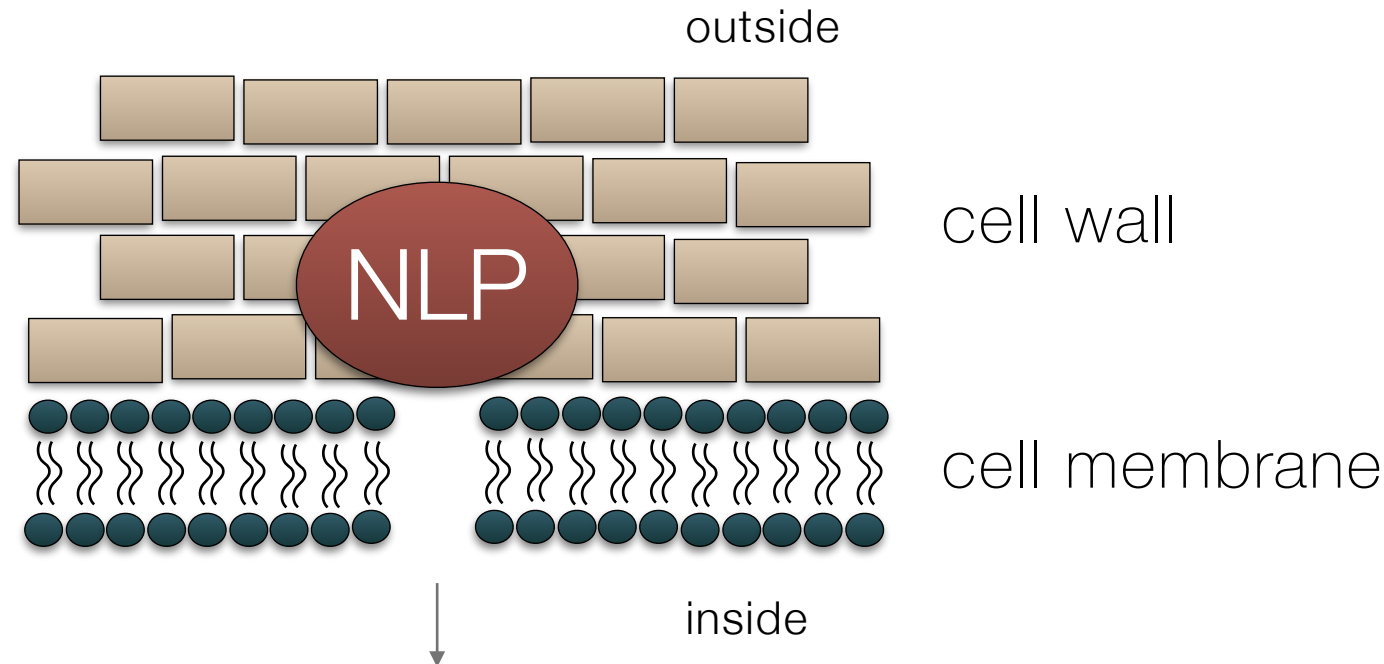
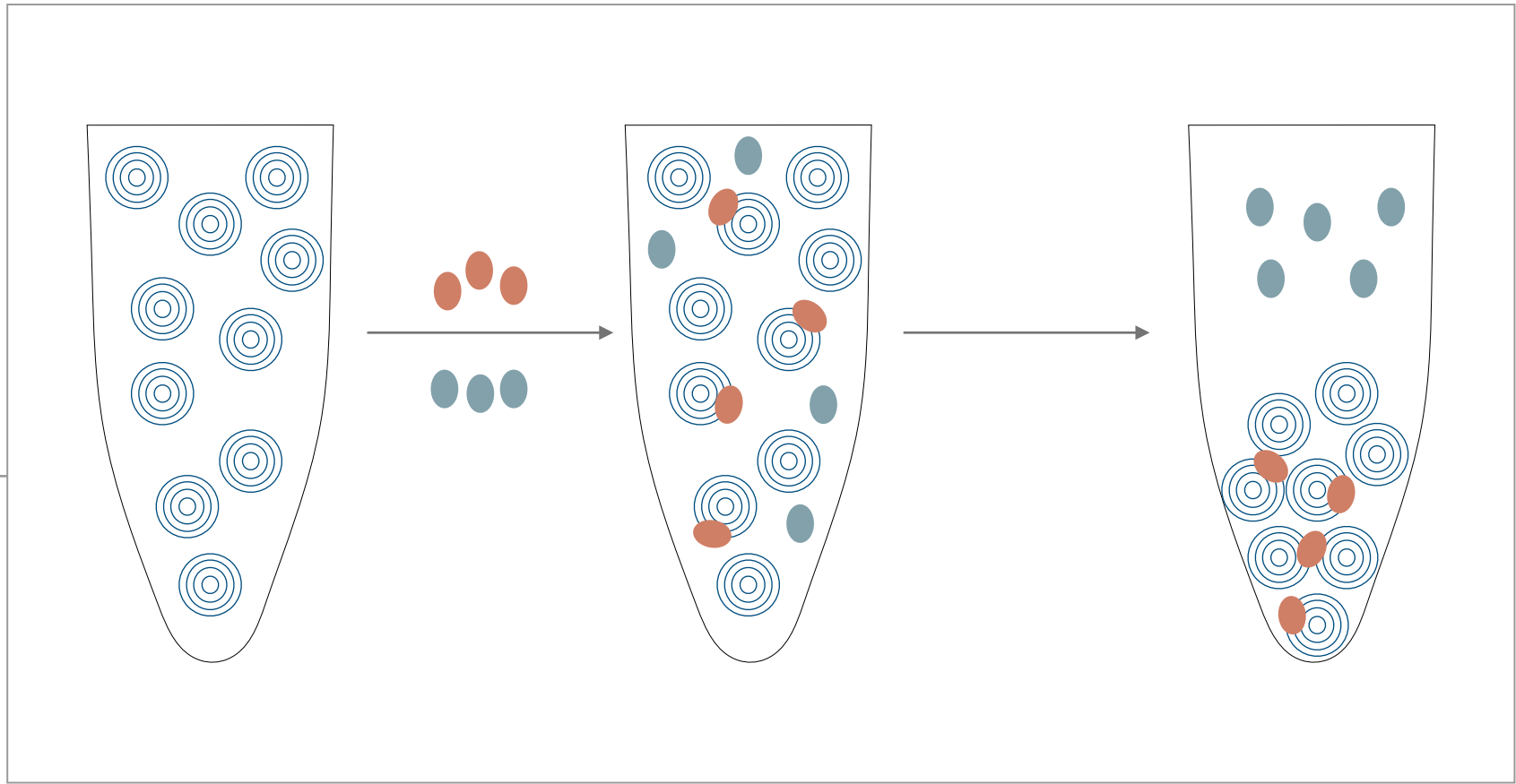


waste



NLP proteins

Structural biology



cell death

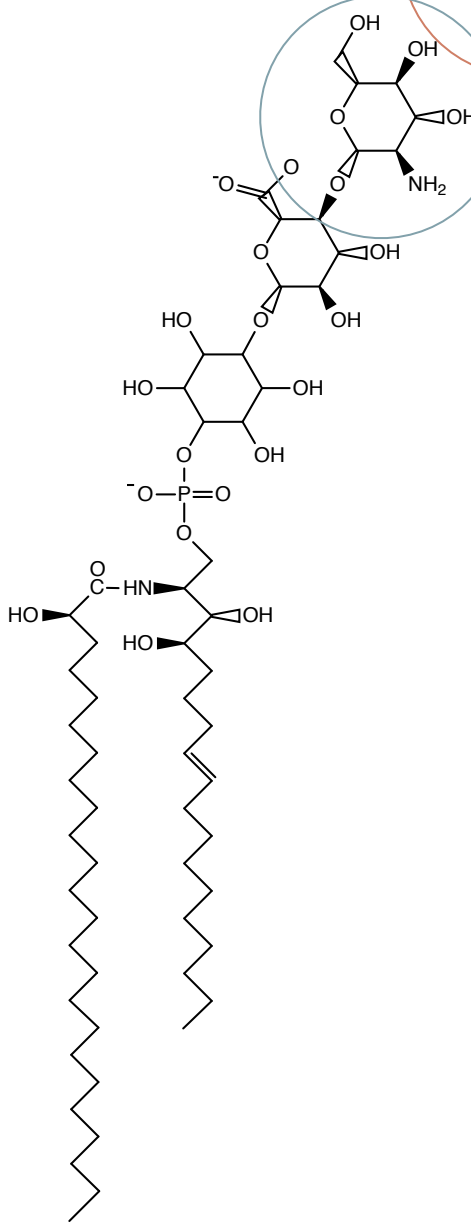
Lipid identification

Glycosylinositol Phosphoceramides

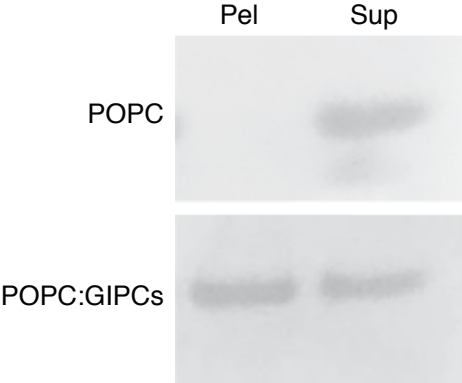
Eudicot
Monocot

- Series 0**
Cer-P-Ins-Hex
- Series A**
Cer-P-Ins-HexA-HexA-Hex(R1)
- Series B**
Cer-P-Ins-HexA-HexA-HexA-HexA-HexA
- Series C**
Cer-P-Ins-HexA-HexA-HexA-HexA-HexA-HexA
Cer-P-Ins-HexA-HexA-HexA-HexA-HexA-HexA
- Series C'**
Cer-P-Ins-HexA-HexA-HexA-HexA-HexA-HexA
Cer-P-Ins-HexA-HexA-HexA-HexA-HexA-HexA
- Series D**
Cer-P-Ins-HexA-HexA-HexA-HexA-HexA-HexA
Cer-P-Ins-HexA-HexA-HexA-HexA-HexA-HexA
- Series E**
Cer-P-Ins-HexA-HexA-HexA-HexA-HexA-HexA
Cer-P-Ins-HexA-HexA-HexA-HexA-HexA-HexA
Cer-P-Ins-HexA-HexA-HexA-HexA-HexA-HexA
- Series F**
Cer-P-Ins-HexA-HexA-HexA-HexA-HexA-HexA

Polar head
Ceramide

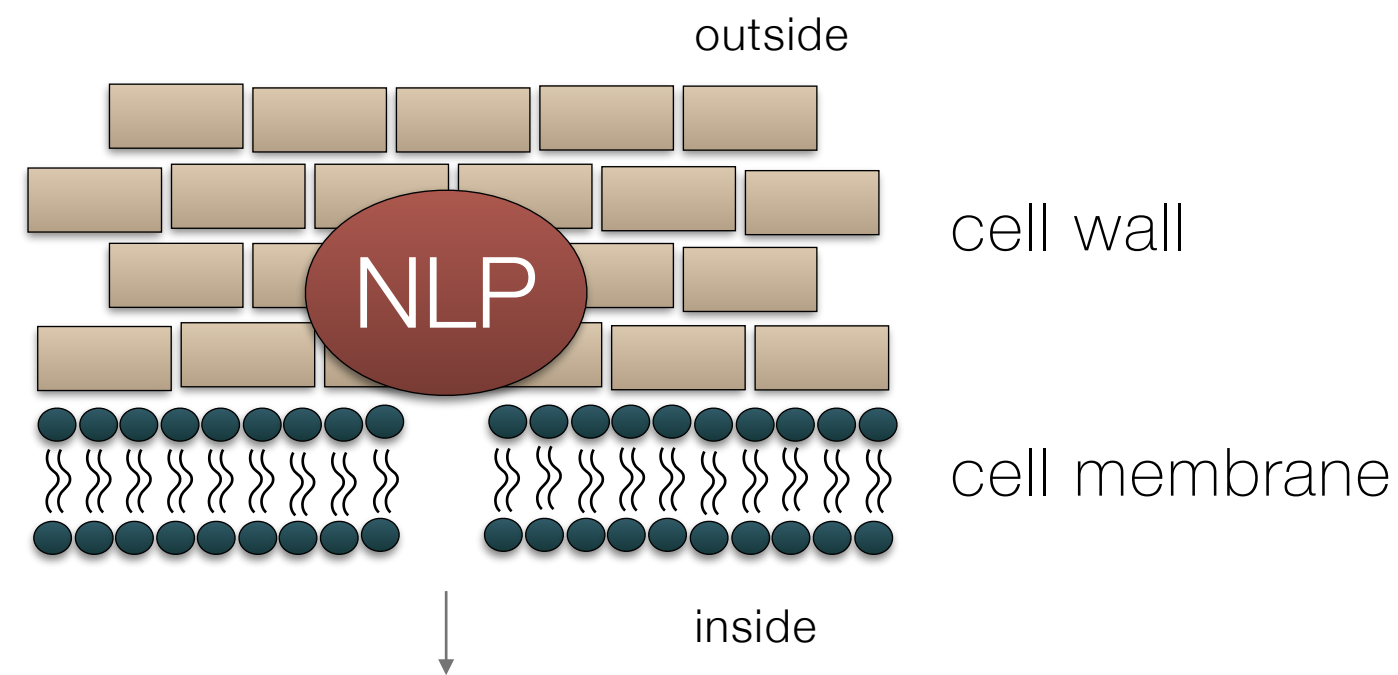
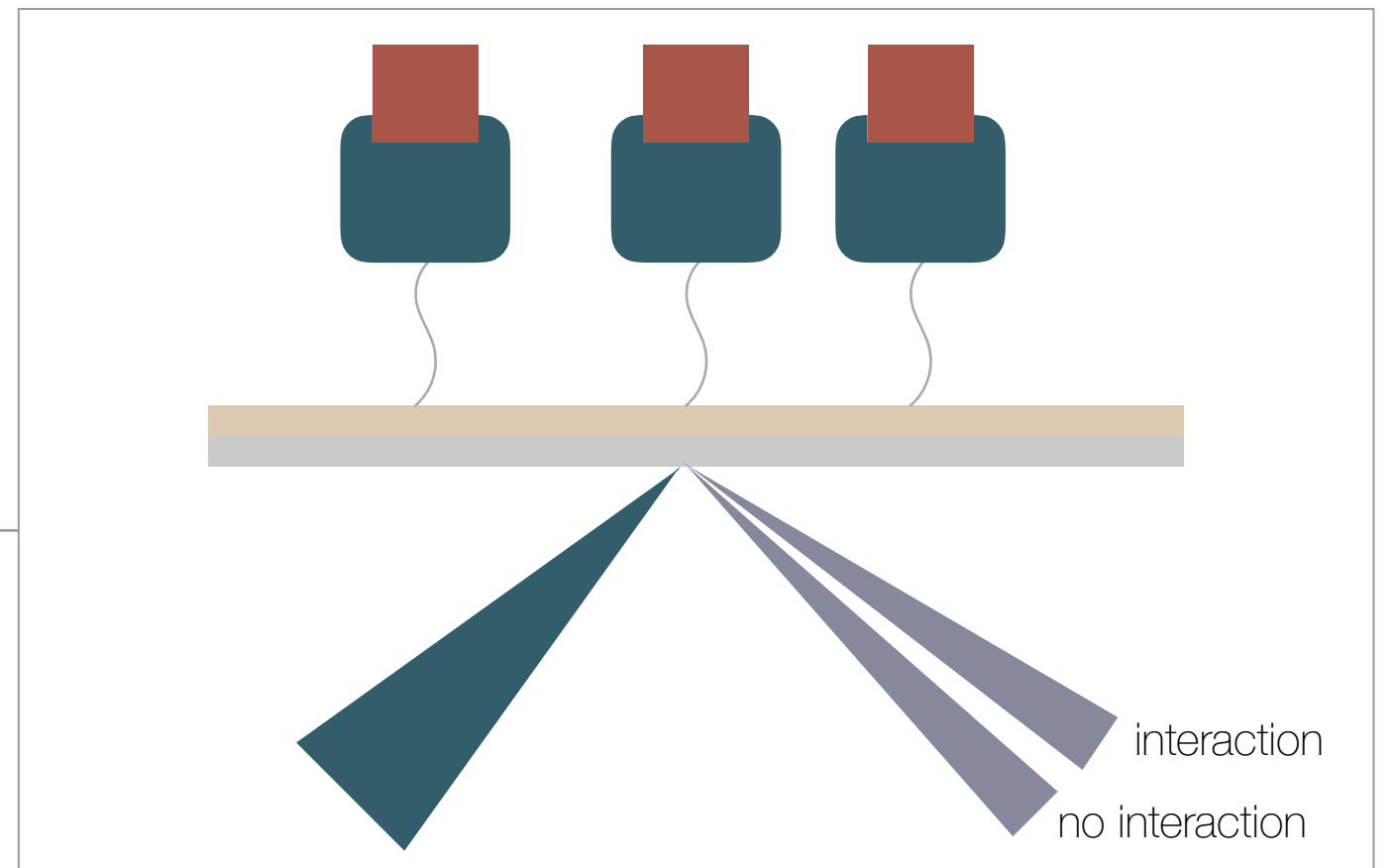


Liposome sedimentation assay



NLP proteins

Structural biology

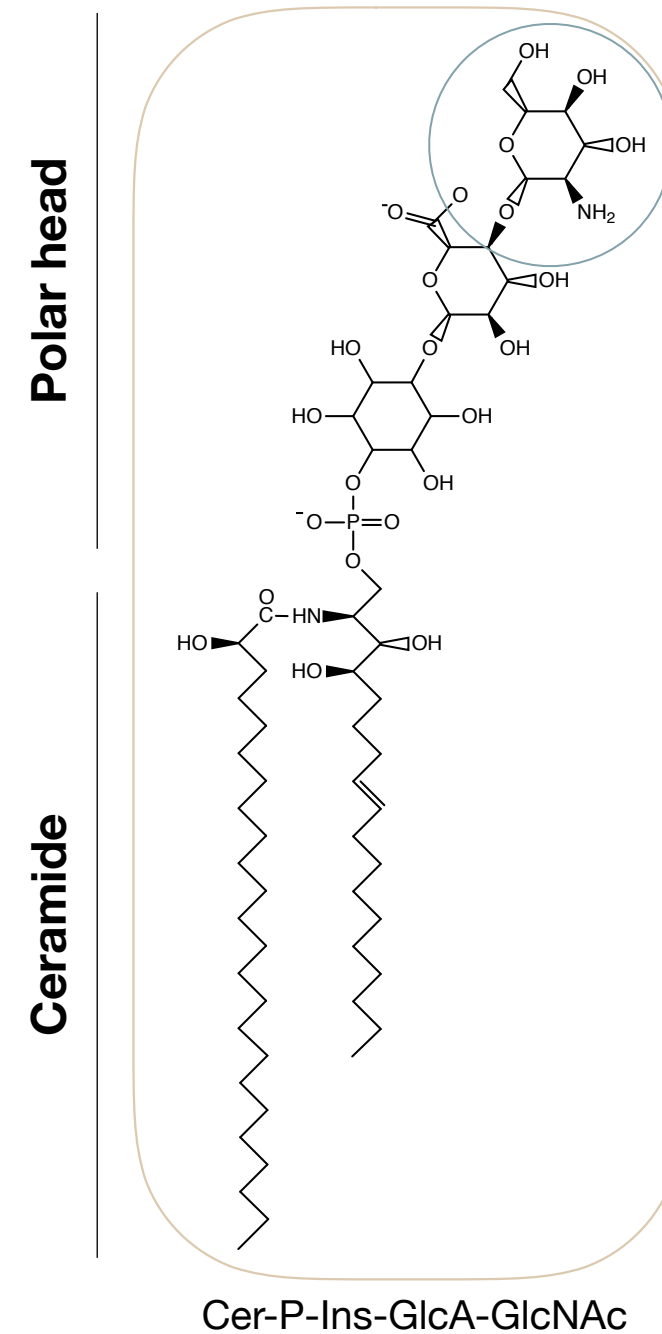


cell death

Lipid identification

Glycosylinositol Phosphoceramides

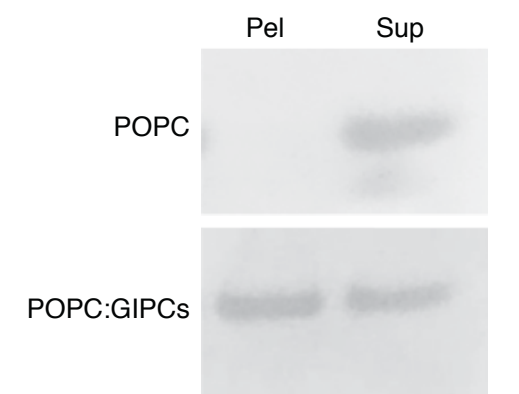
- Series 0**
Cer-P-Ins-Hex
- Eudicot** | **Series A**
Cer-P-Ins-HexA-HexA-Hex(R1)
- Monocot** | **Series B**
Cer-P-Ins-HexA-HexA-Hex(R1)-Hex
- Series C**
Cer-P-Ins-HexA-Hex(R1)-Hex-Pen
Cer-P-Ins-HexA-Hex(R1)-(Hex)₂
- Series C'**
Cer-P-Ins-HexA-HexA-HexA-Hex
Cer-P-Ins-HexA-HexA-HexA-HexA
- Series D**
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



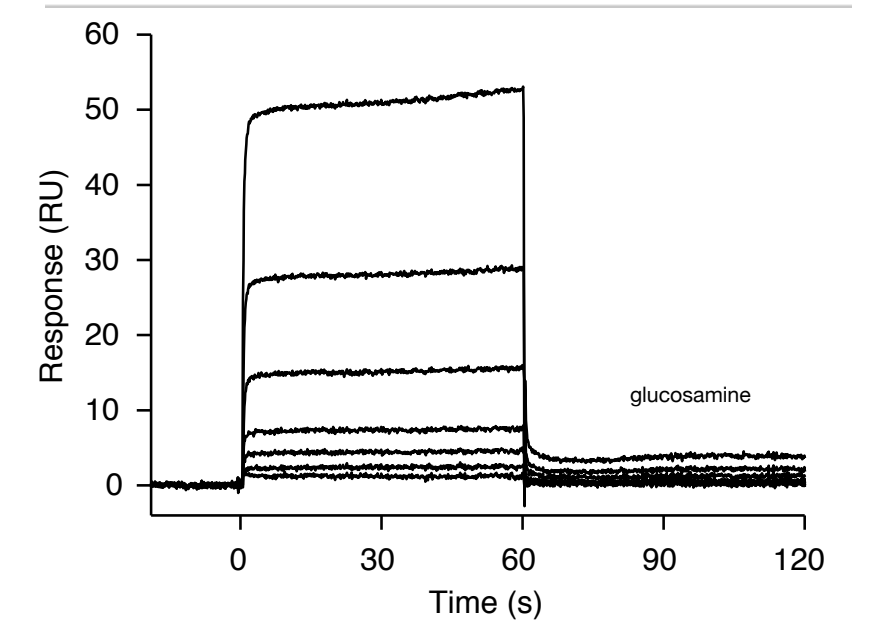
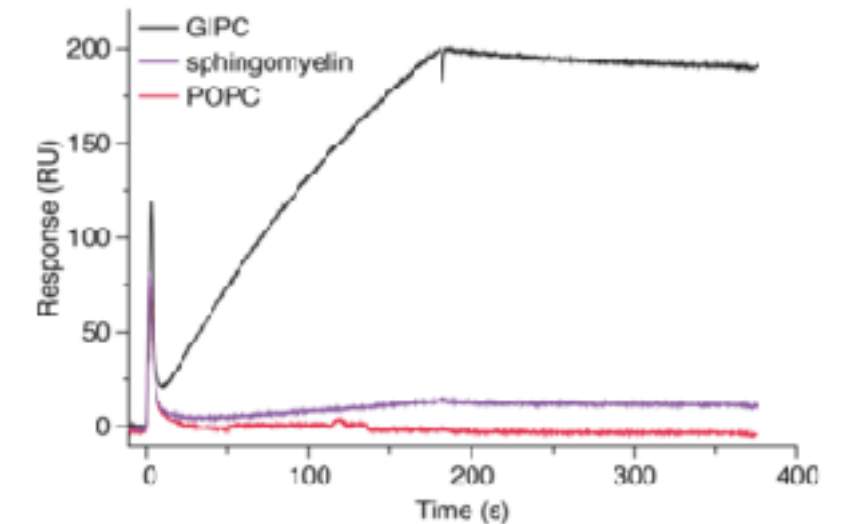
● micromolar affinity

● millimolar affinity

Liposome sedimentation assay

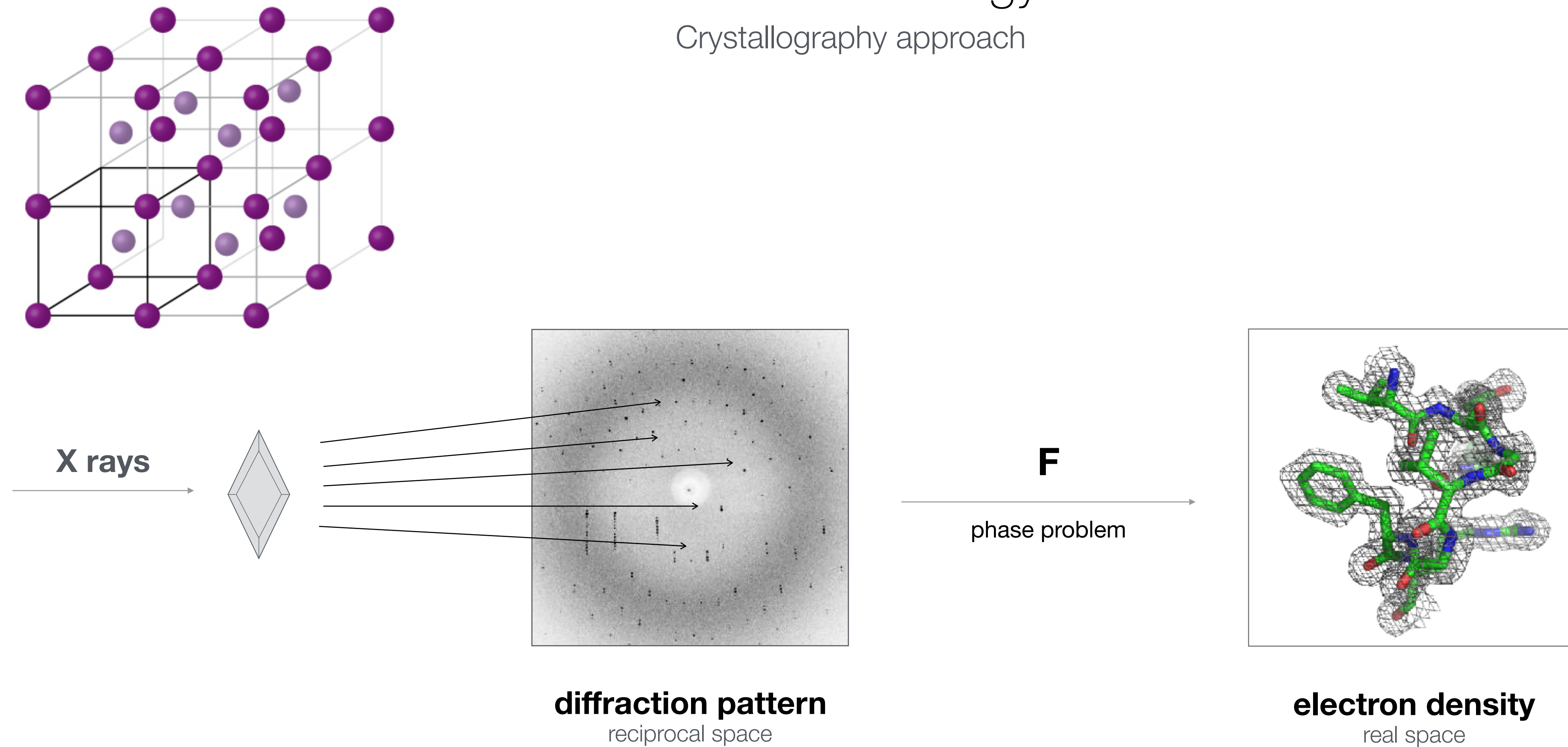


Surface plasmon resonance experiments



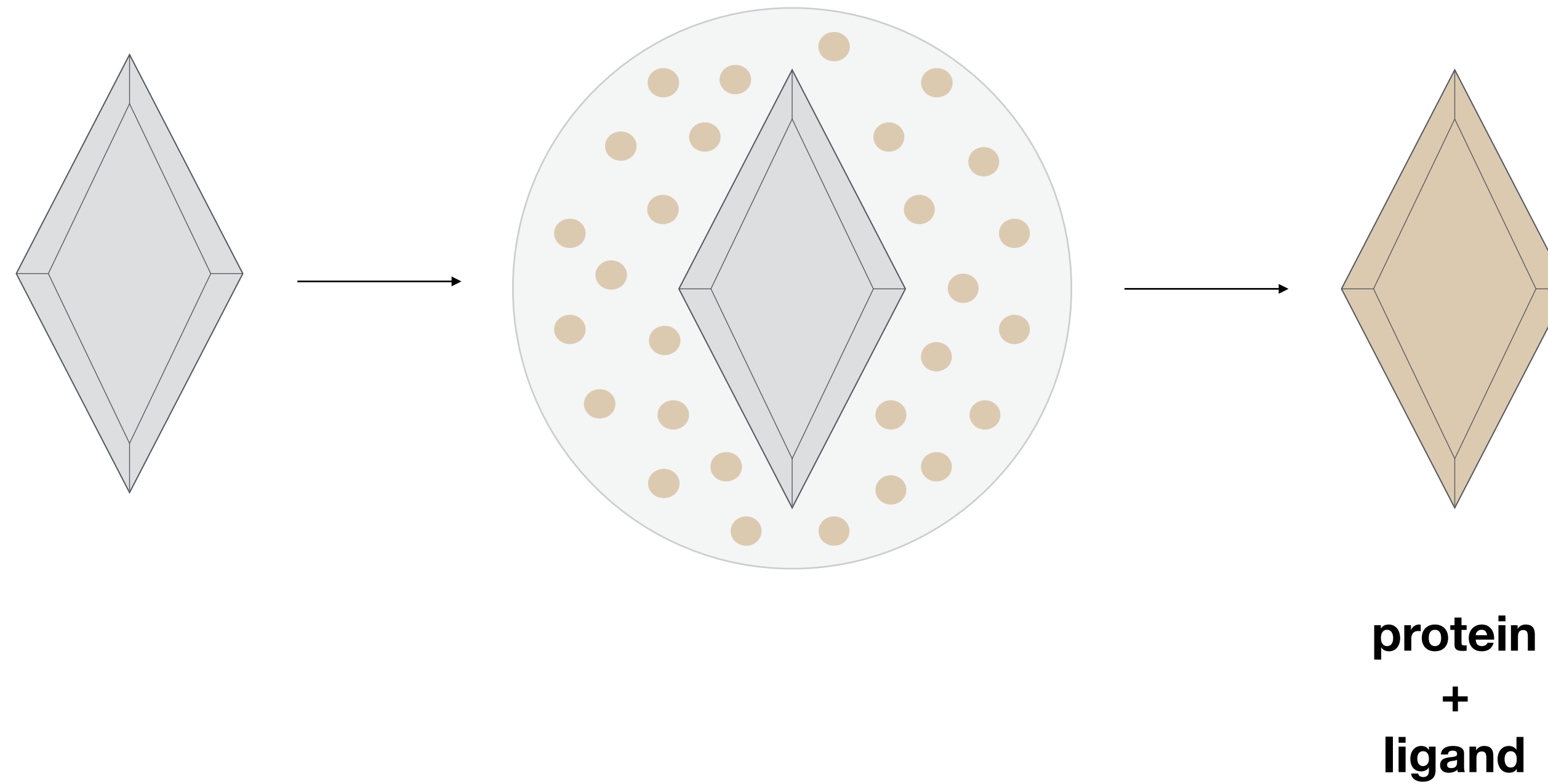
NLP proteins

Structural biology
Crystallography approach

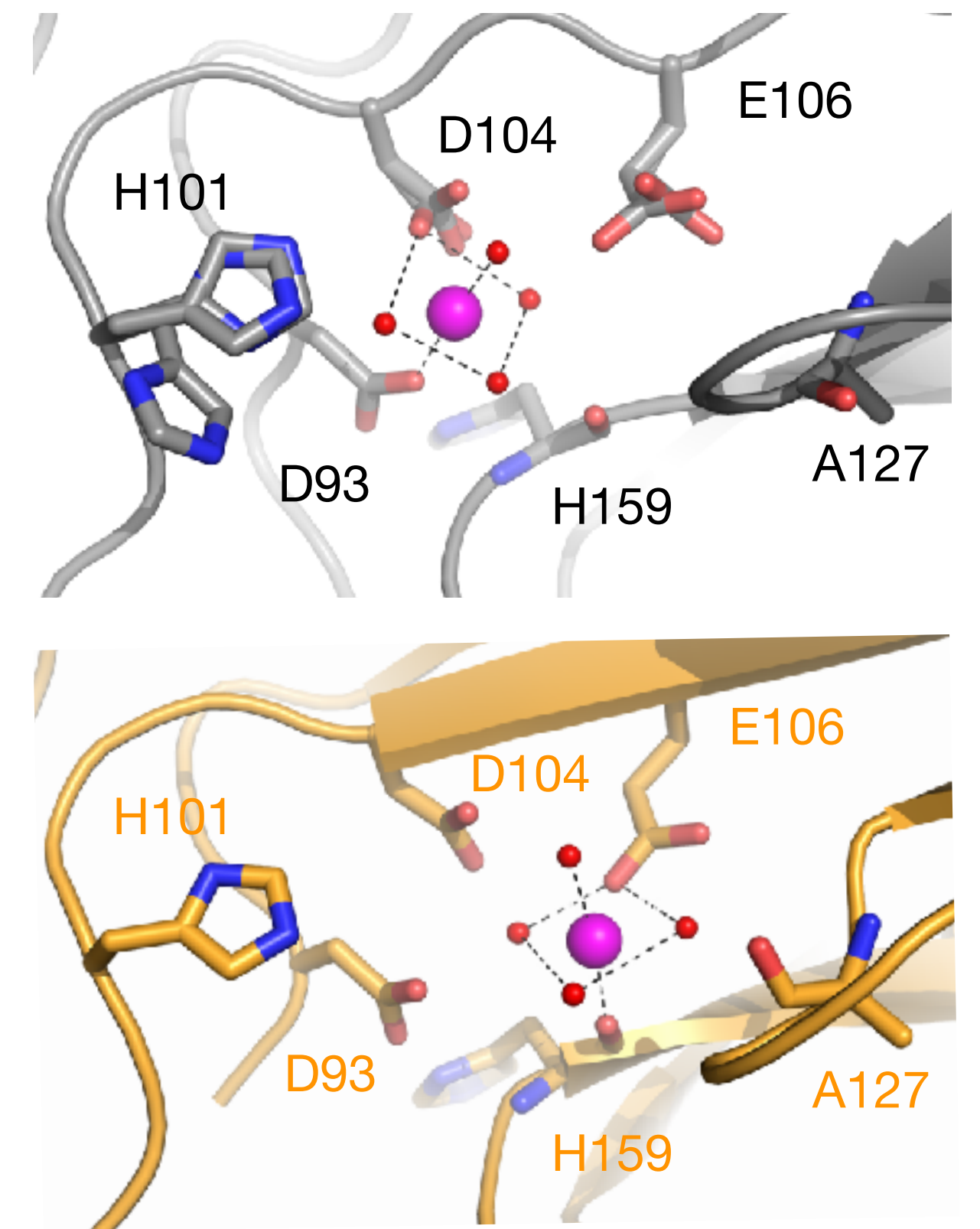
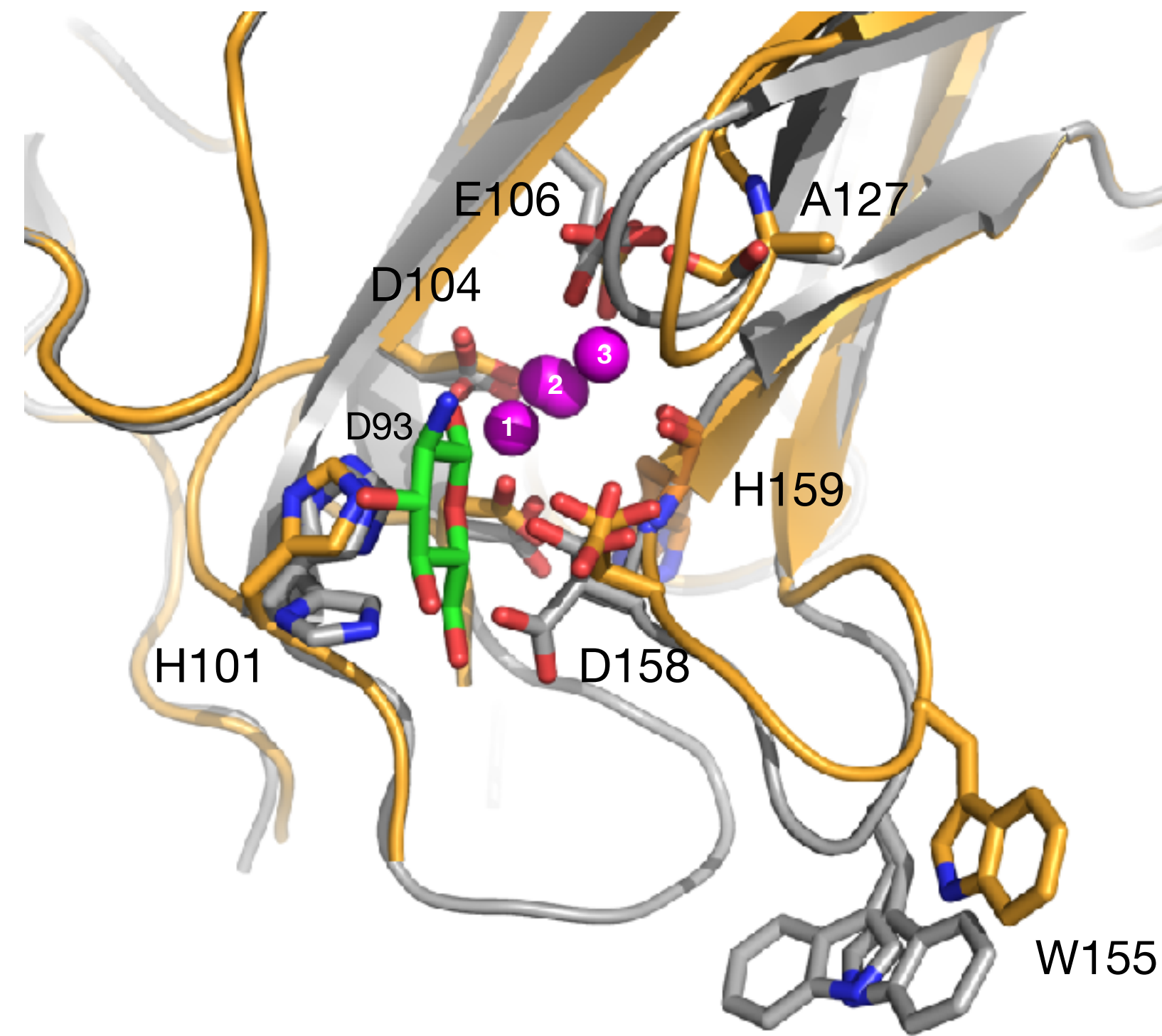
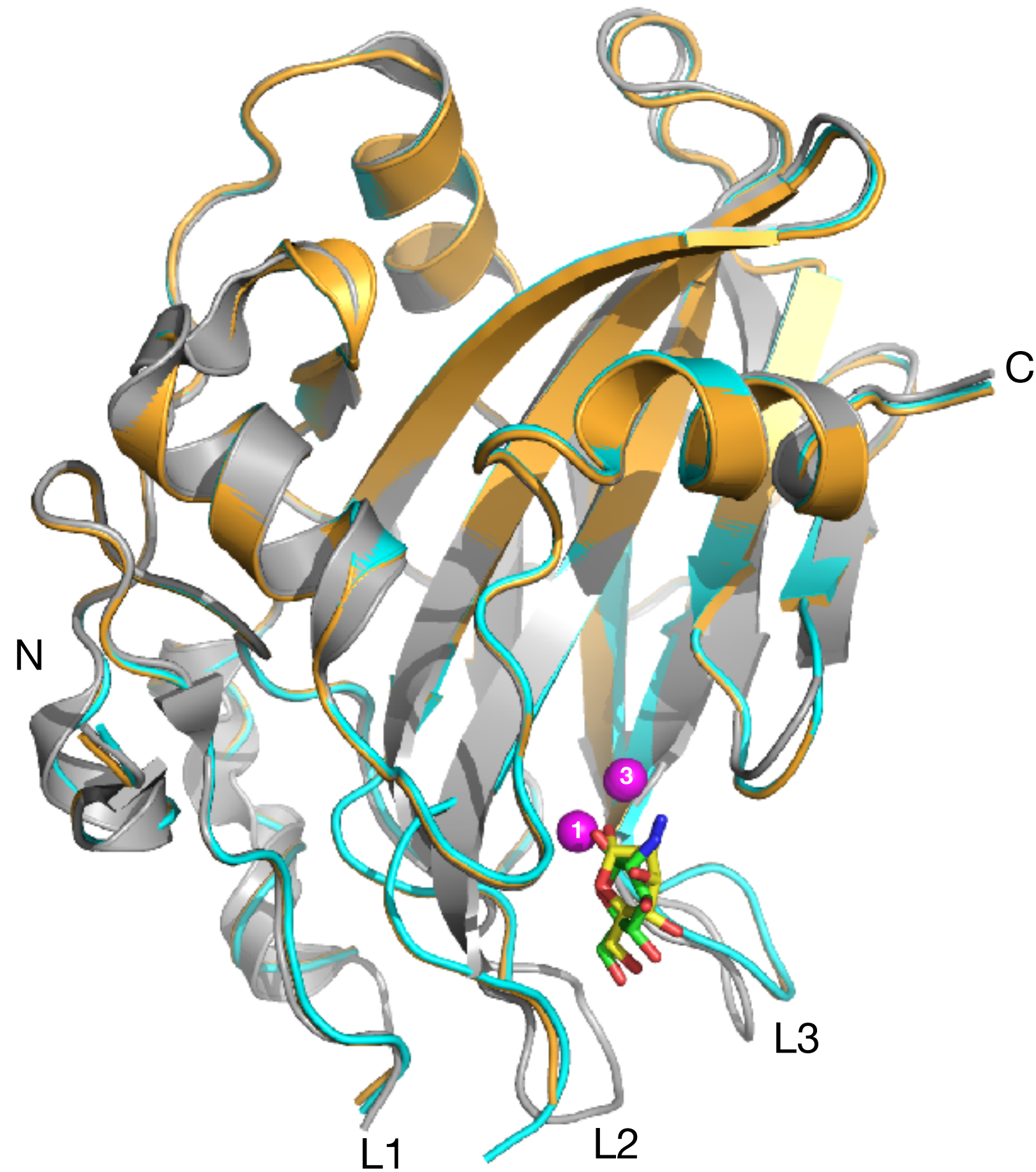


NLP proteins

Structural biology

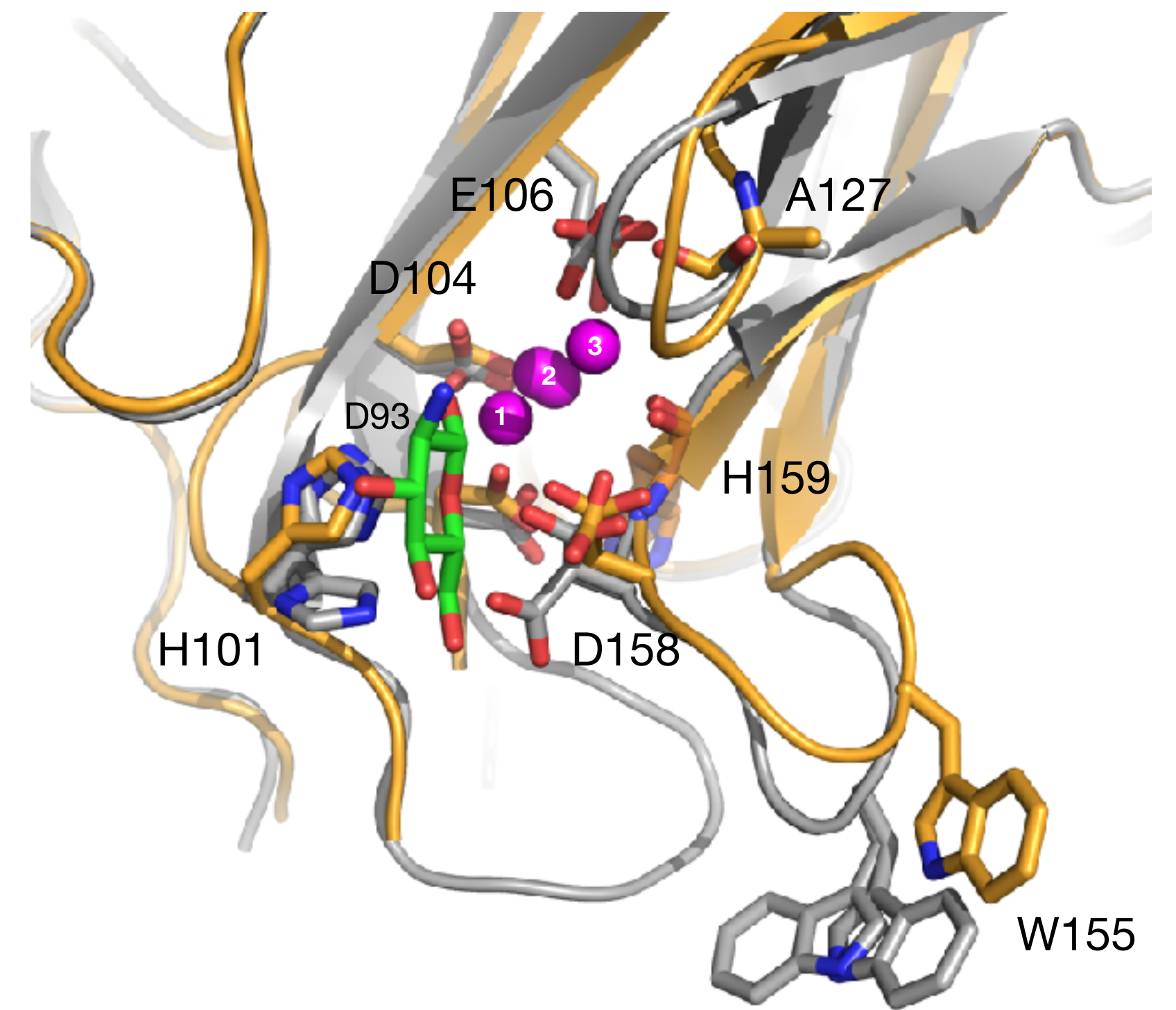
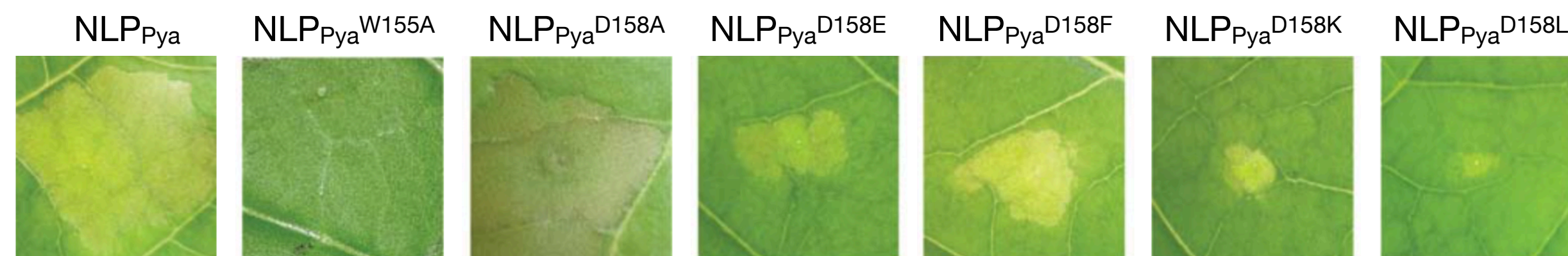


Structural analysis

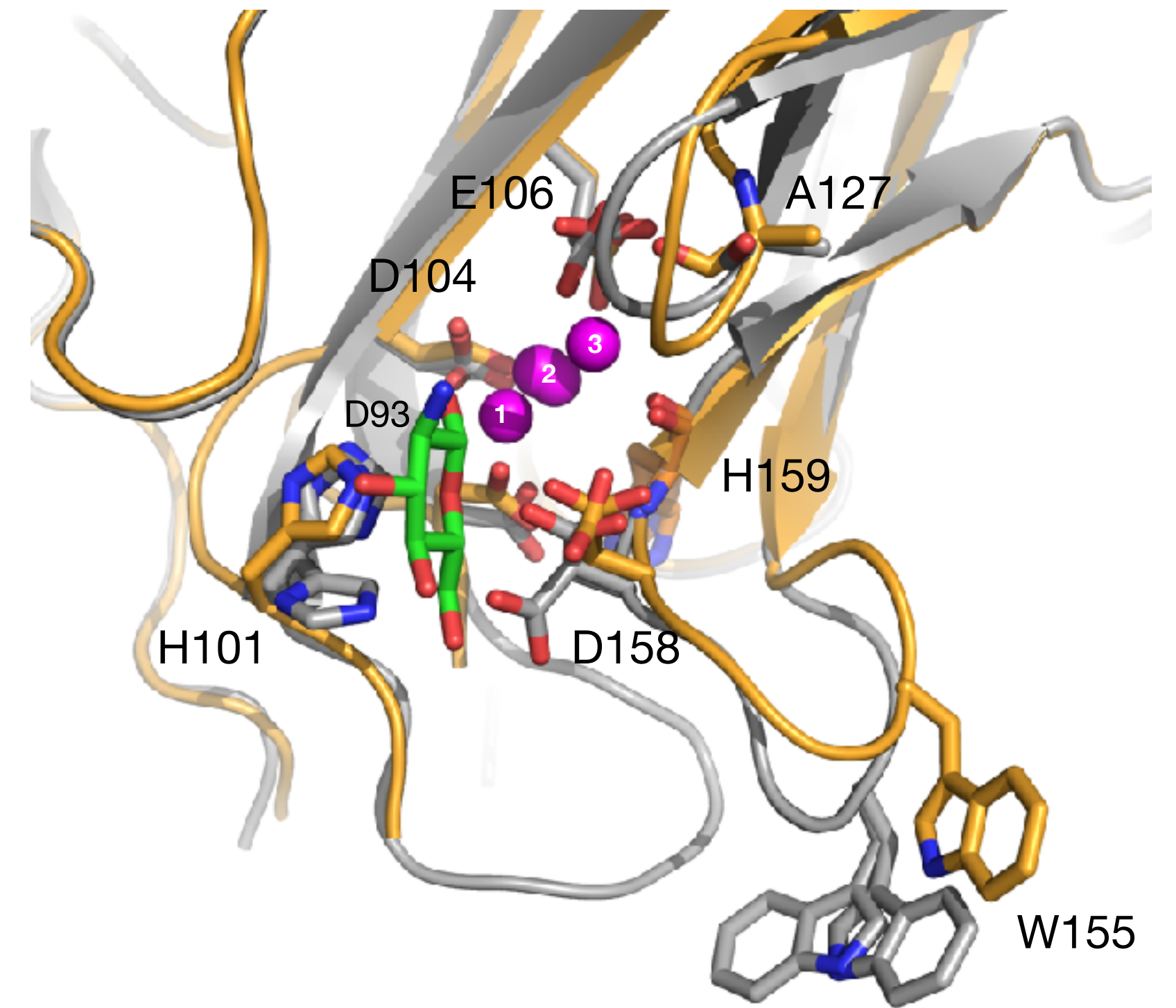
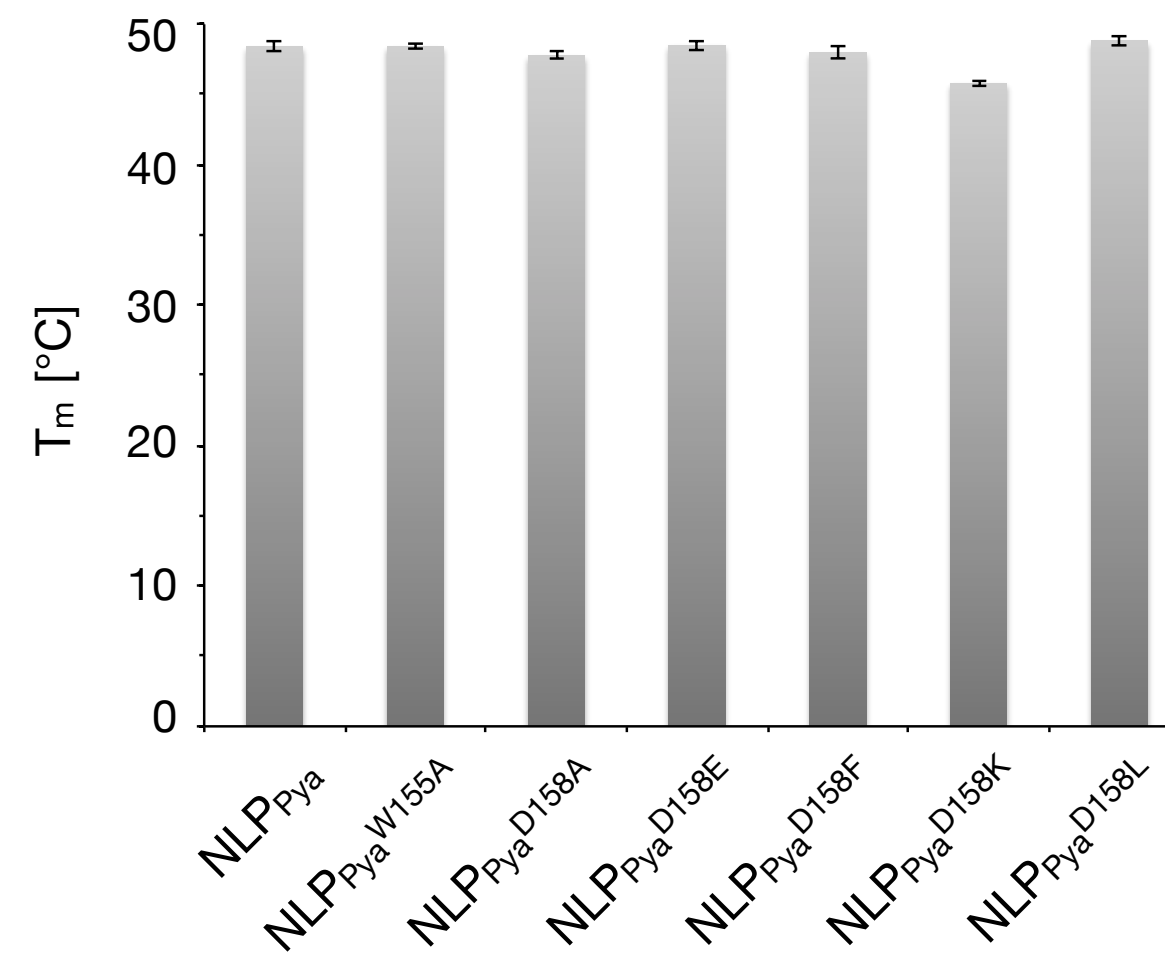
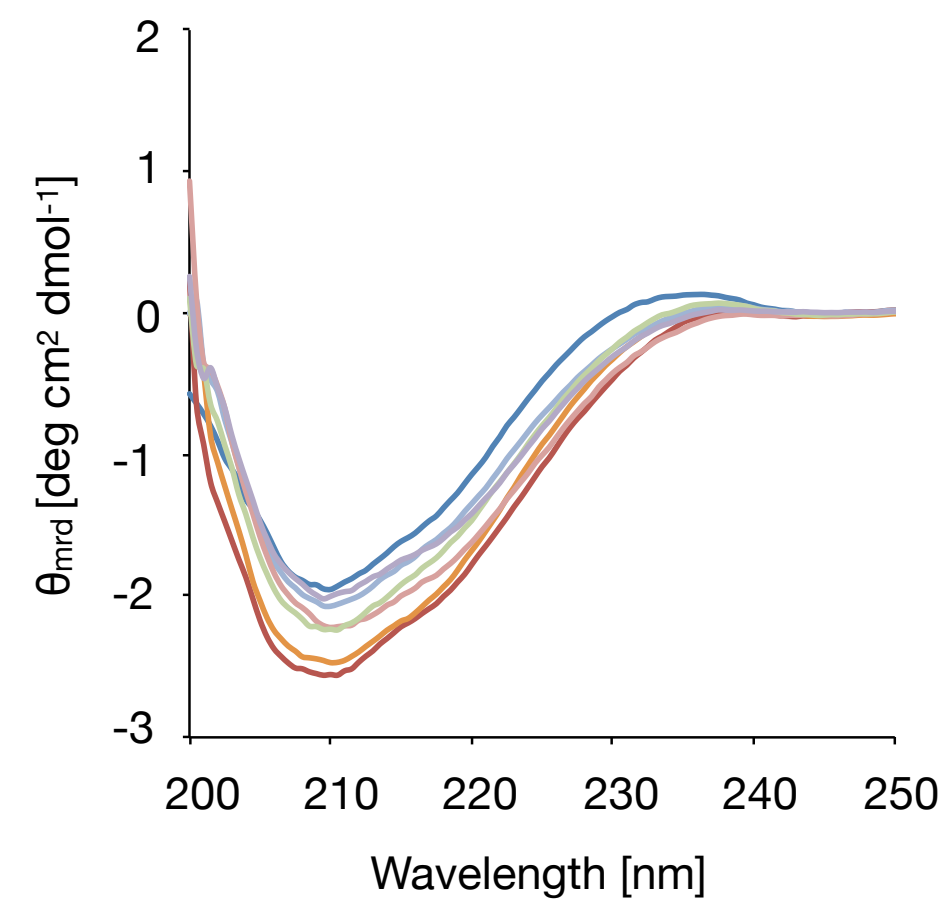
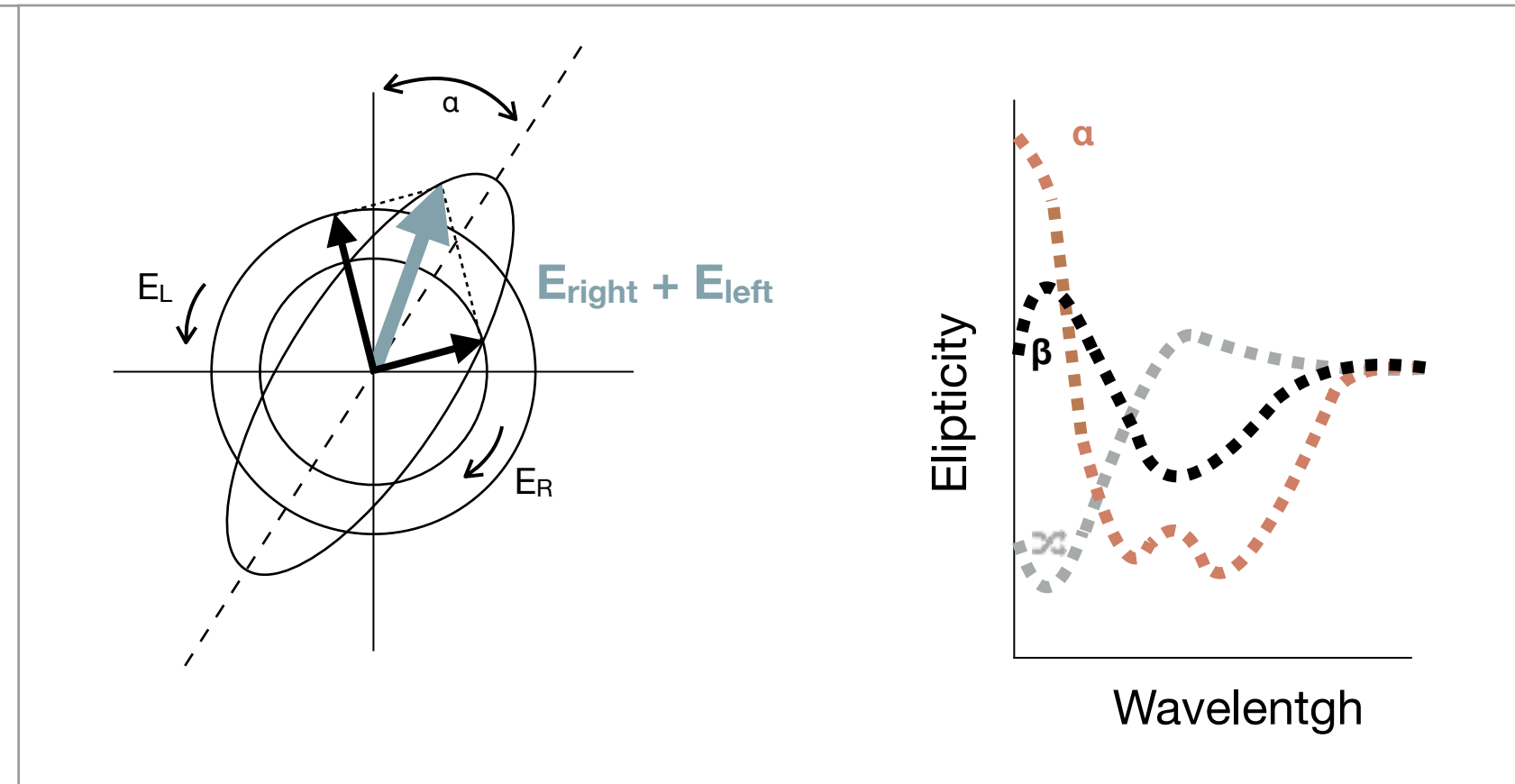


- apoNLP_{PyA}
- NLP_{PyA}-glucosamine
- NLP_{PyA}-mannosamine

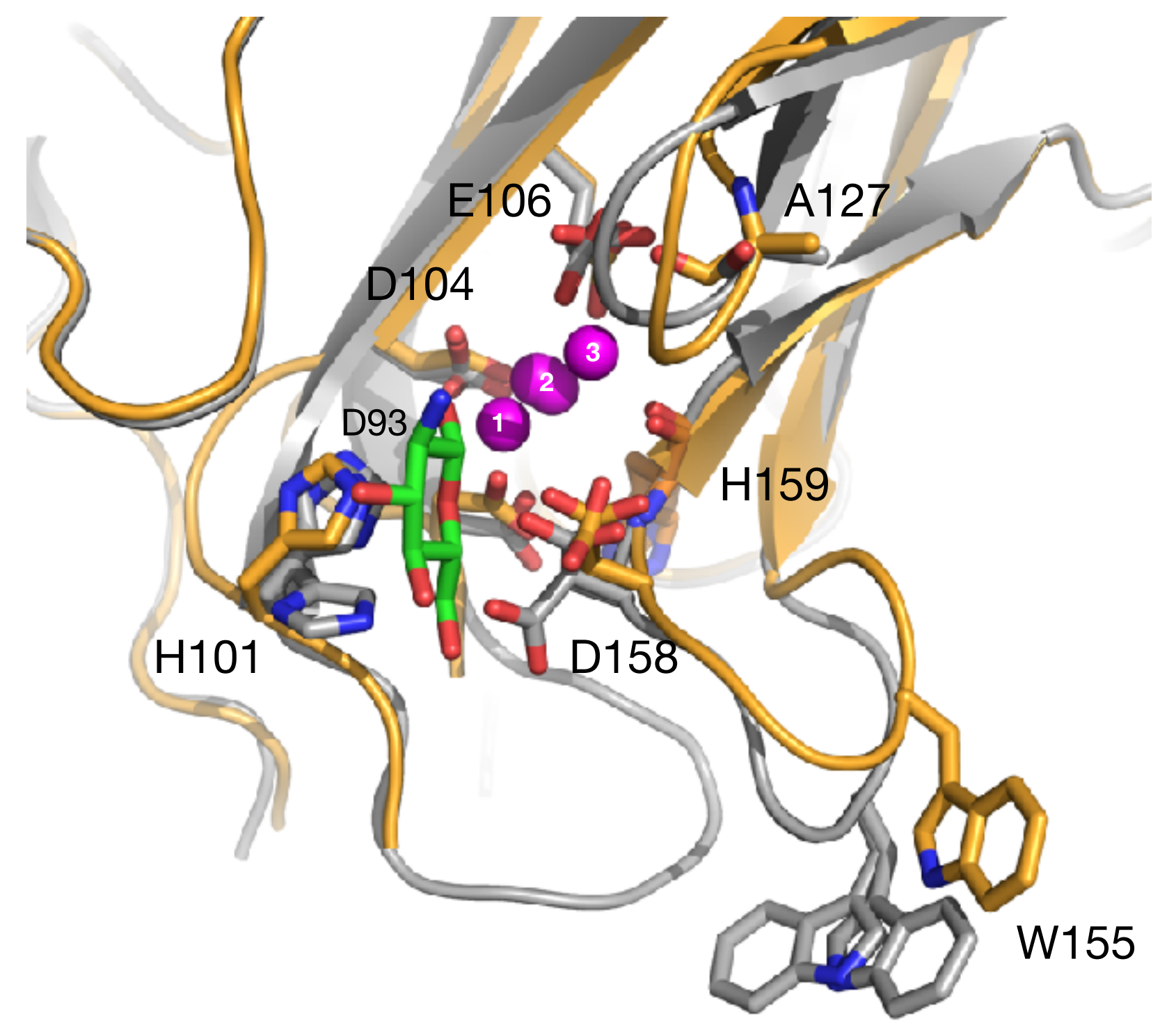
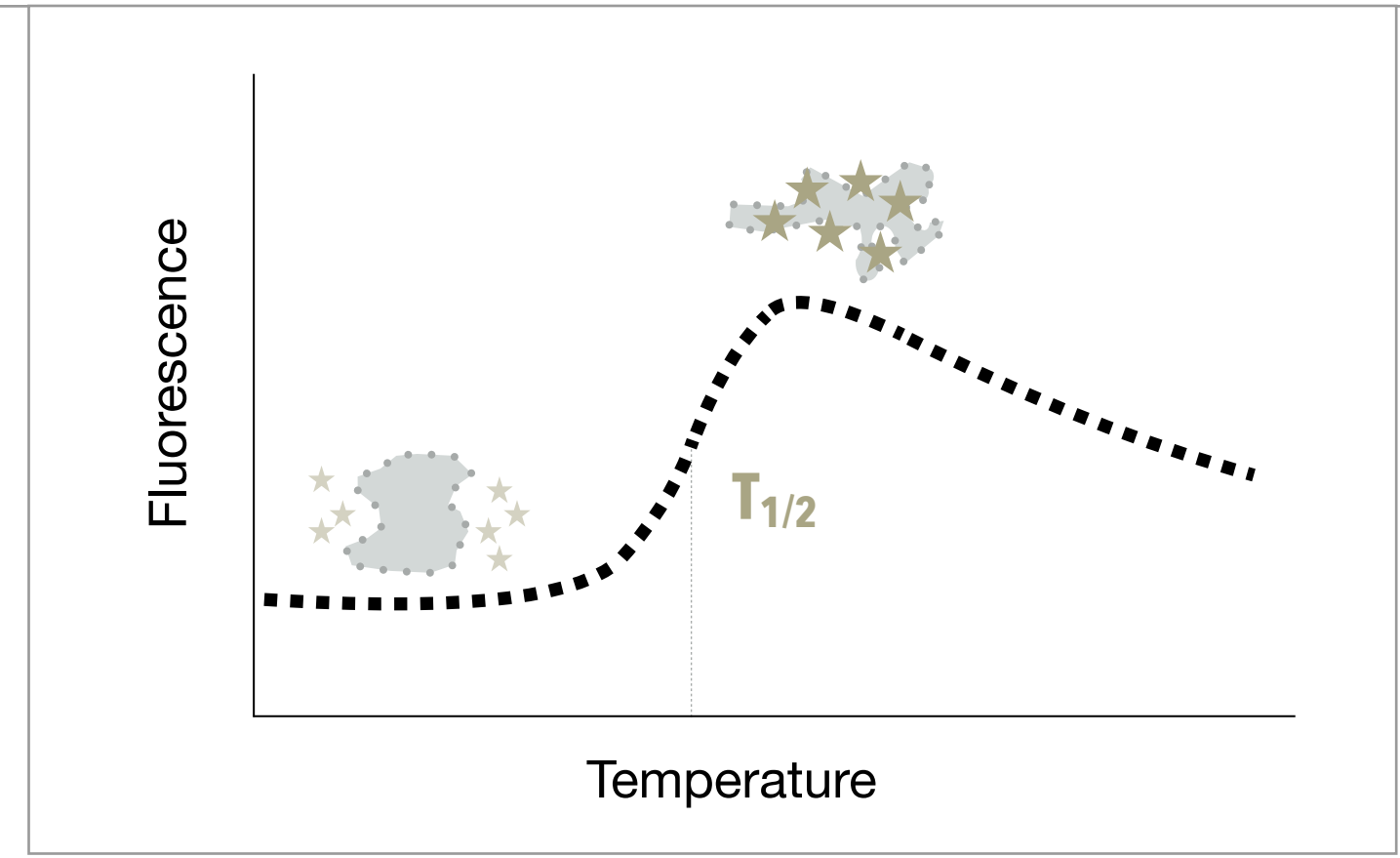
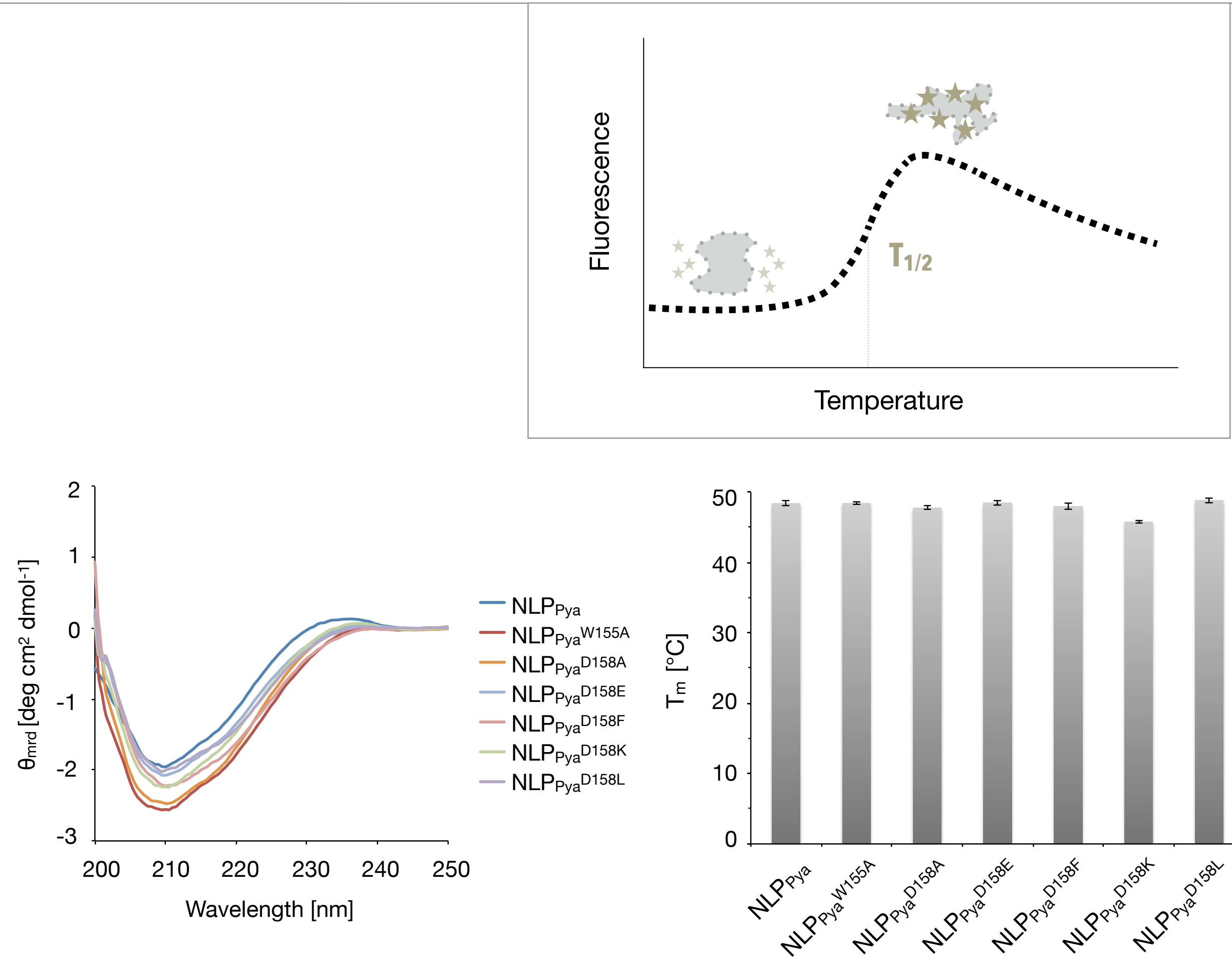
Structural analysis



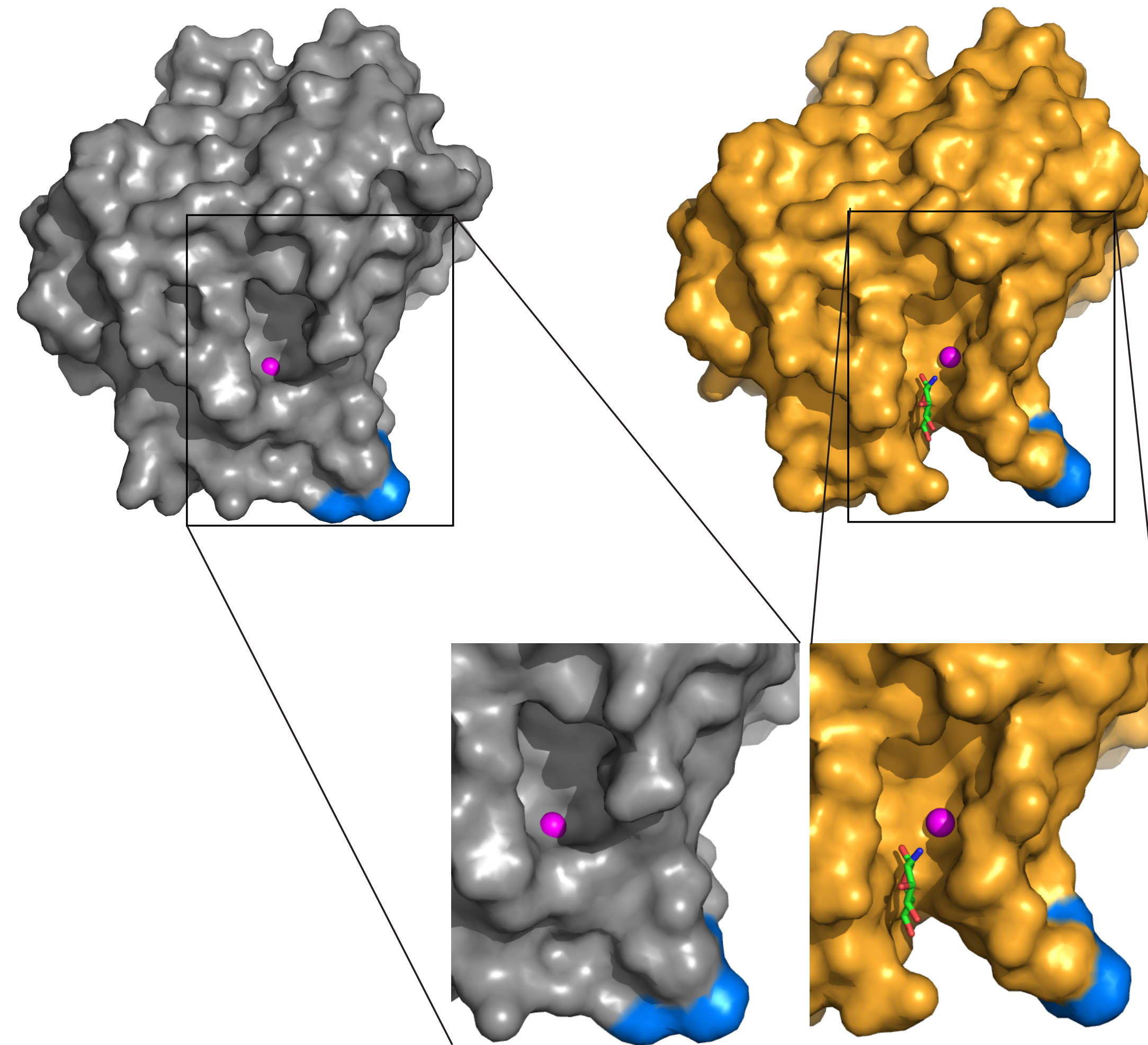
Structural analysis



Structural analysis



Structural analysis



BIOCHEMISTRY PROJECT

molecular cloning

BIOCHEMISTRY PROJECT molecular cloning

protein purification

BIOCHEMISTRY PROJECT

molecular cloning

protein purification

crystallography

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protein purification

crystallography

biophysical methods

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NLP PROJECT

NLP-plant membrane

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eudicot selectivity

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phytopharmaceuticals

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References

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