Gene Expression

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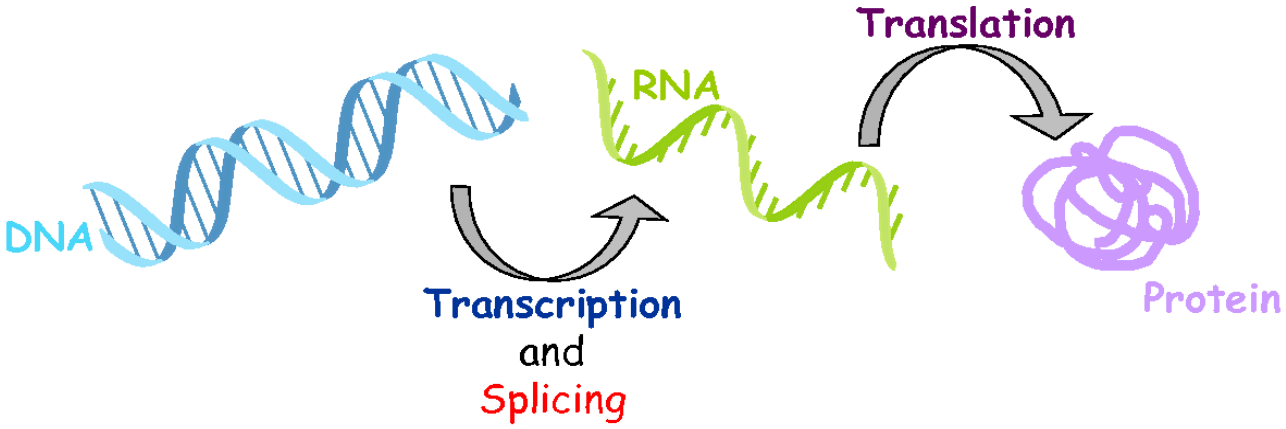
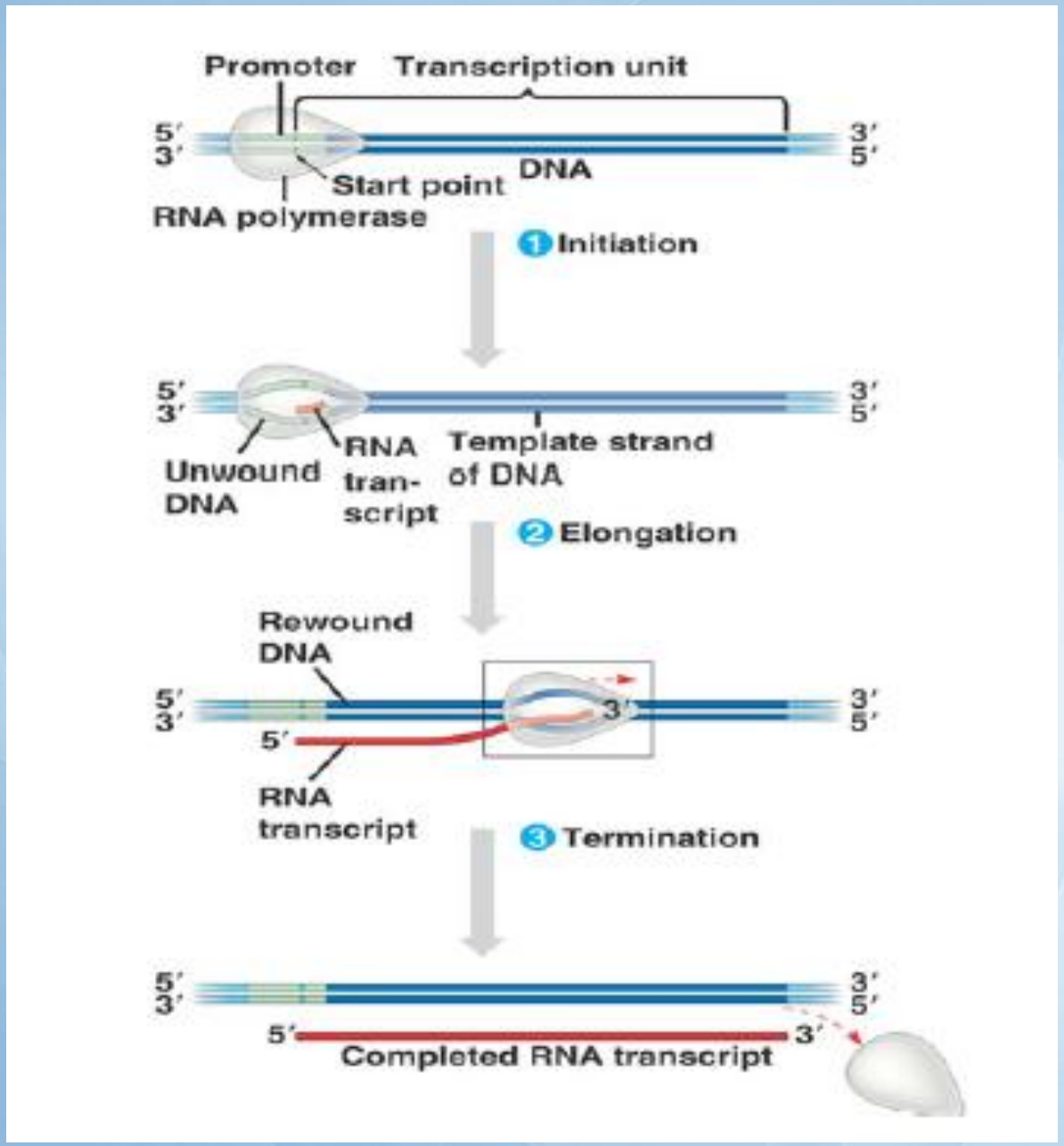
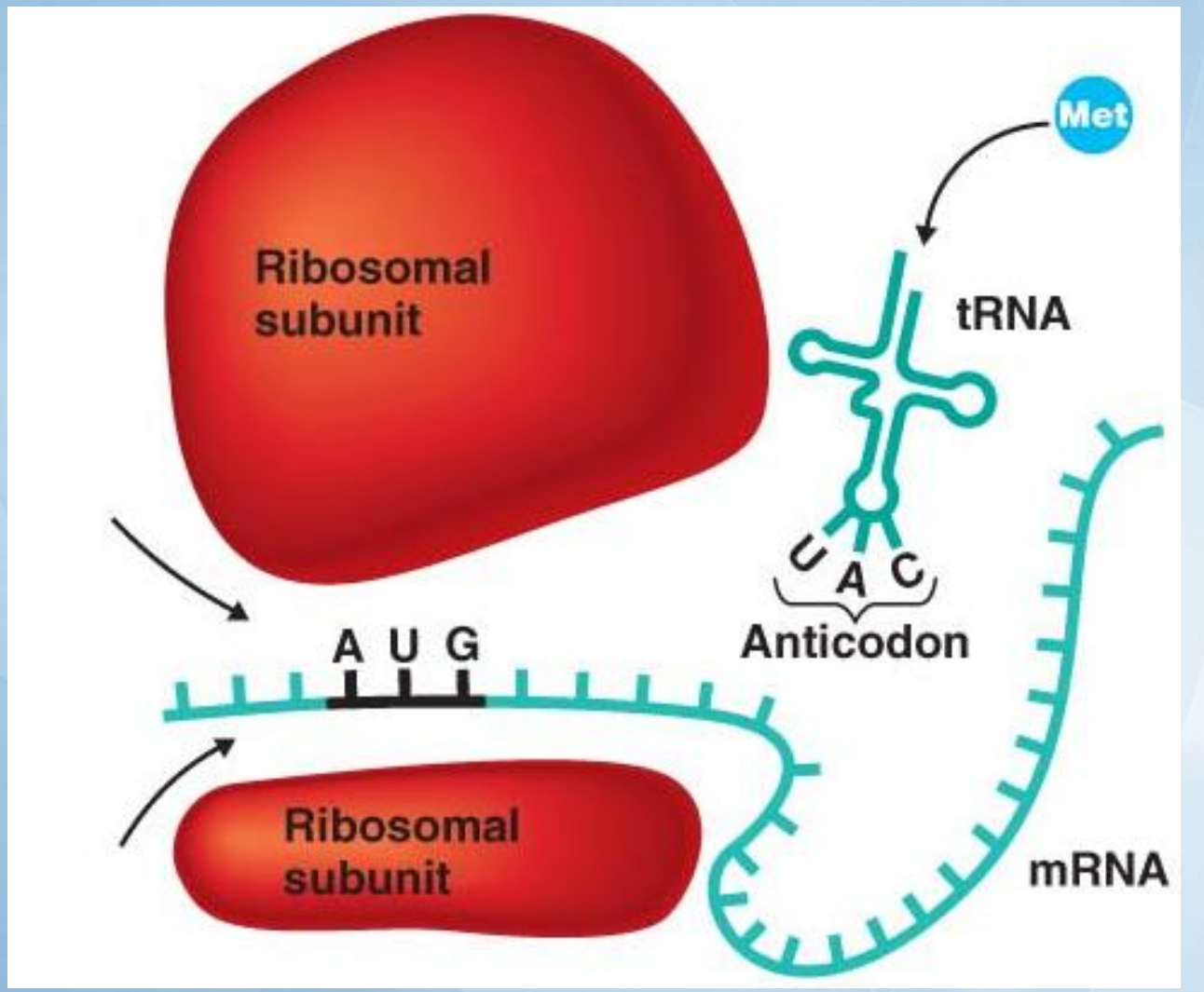
Gene expression

From Gene to Protein

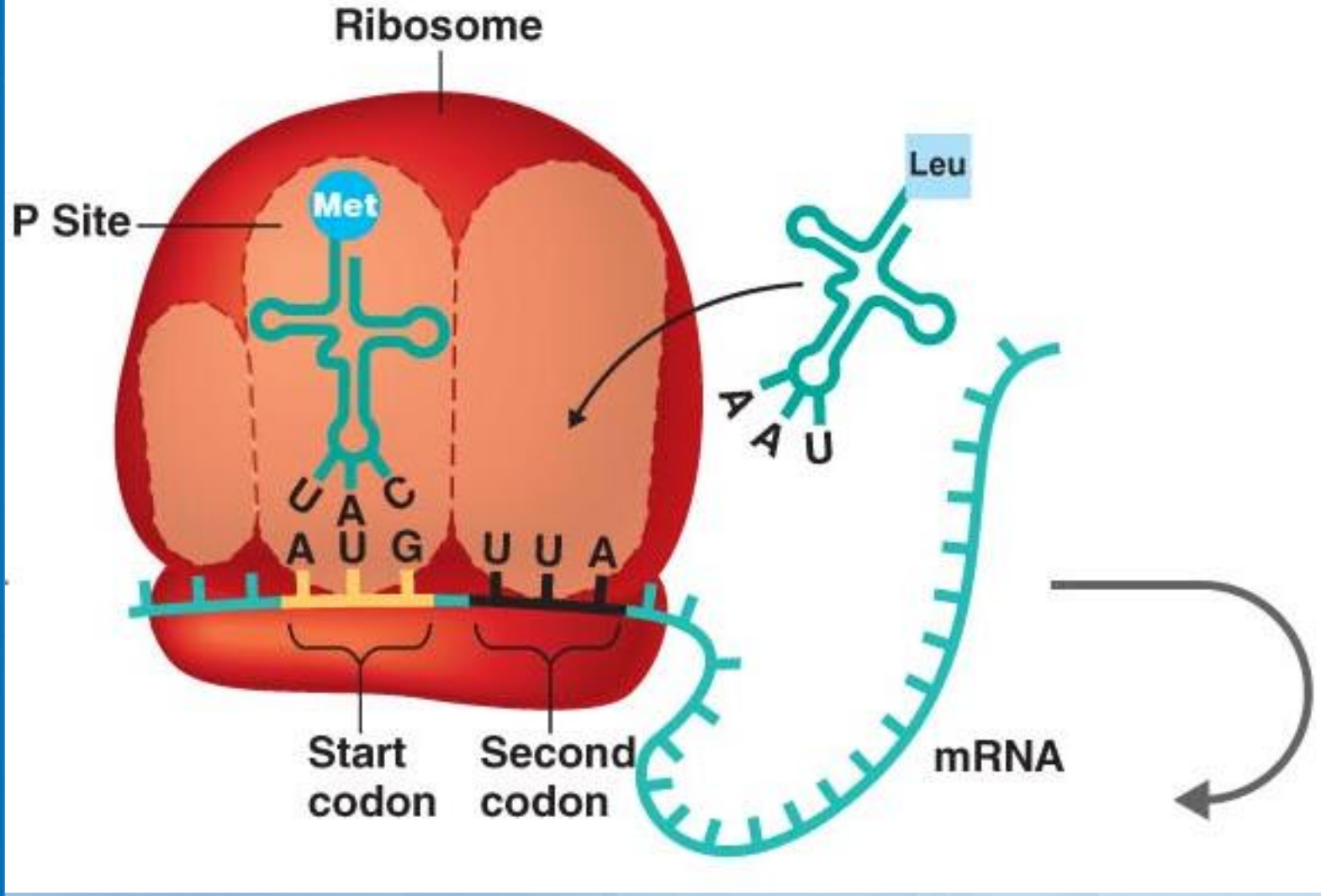



Promoter
Transcription unit
5′
3′
3′
5′
DNA
Start point
RNA polymerase
1 Initiation
5′
3′
3′
5′
Unwound DNA
RNA transcript
Template strand of DNA
2 Elongation
Rewound DNA
5′
3′
3′
5′
3′
5′
RNA transcript
3 Termination
5′
3′
3′
5′
5′
3′
Completed RNA transcript

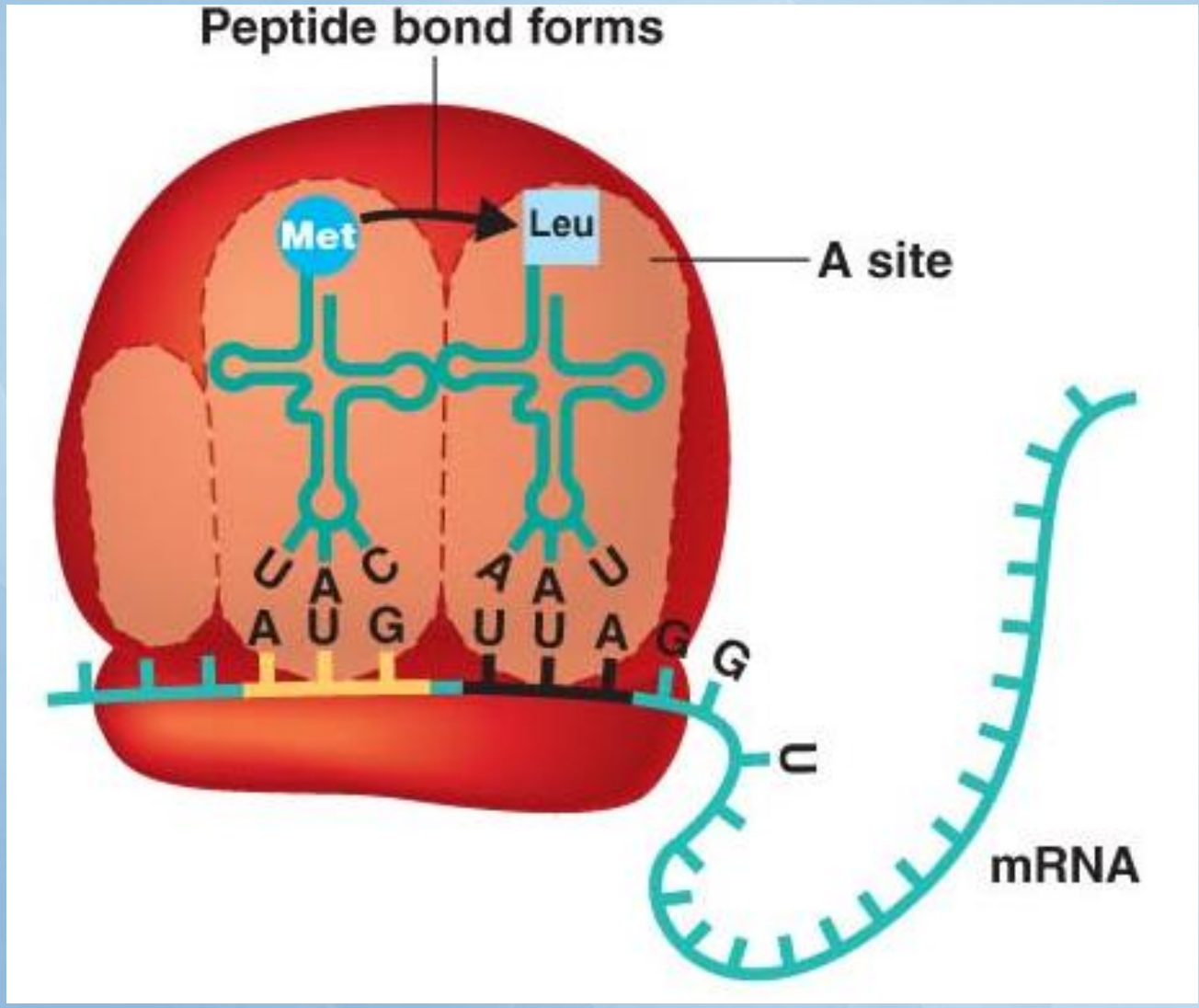
Initiation


Met
Ribosomal subunit
tRNA
U A C
Anticodon
A U G
Ribosomal subunit
mRNA

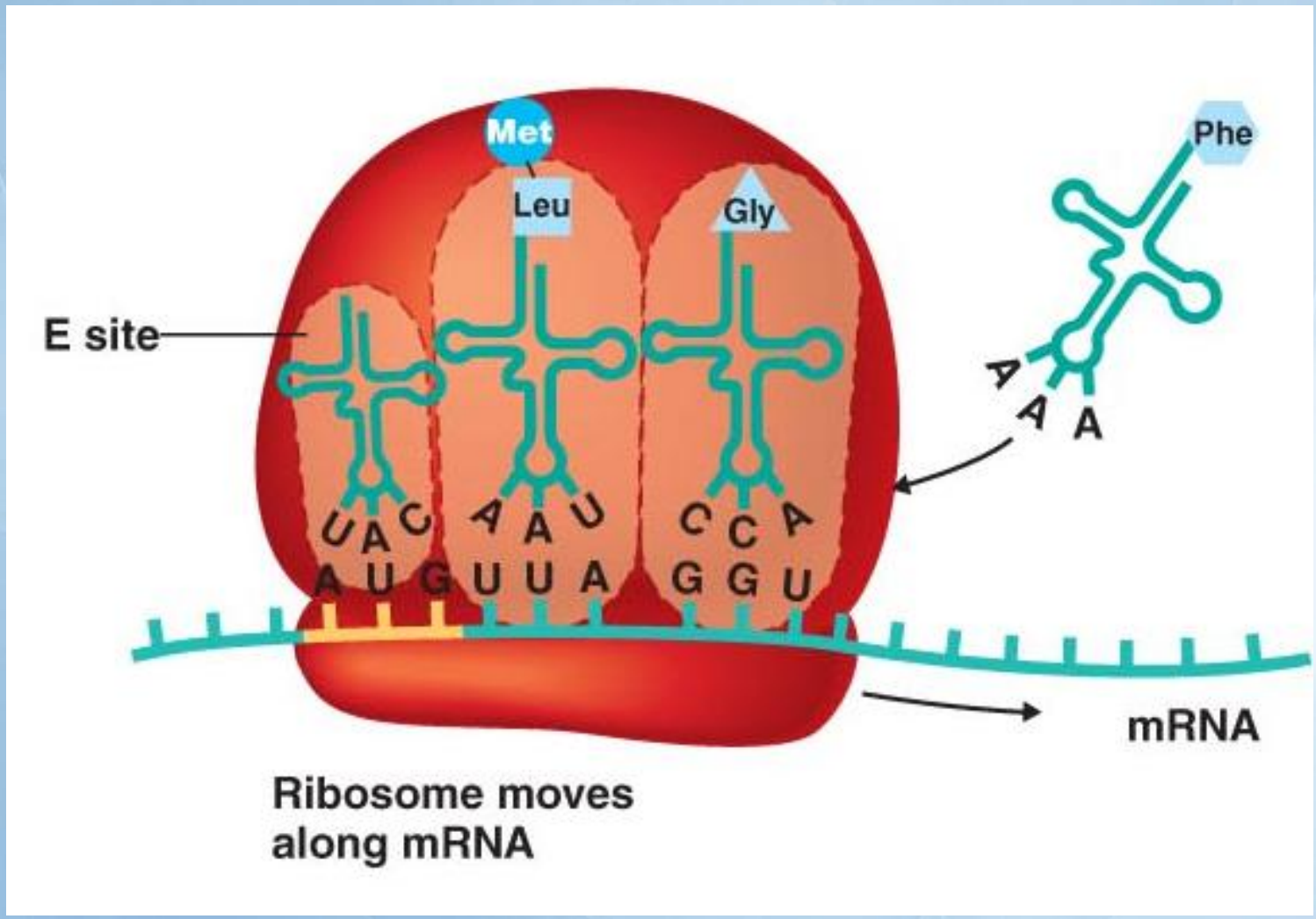
The Process of Translation



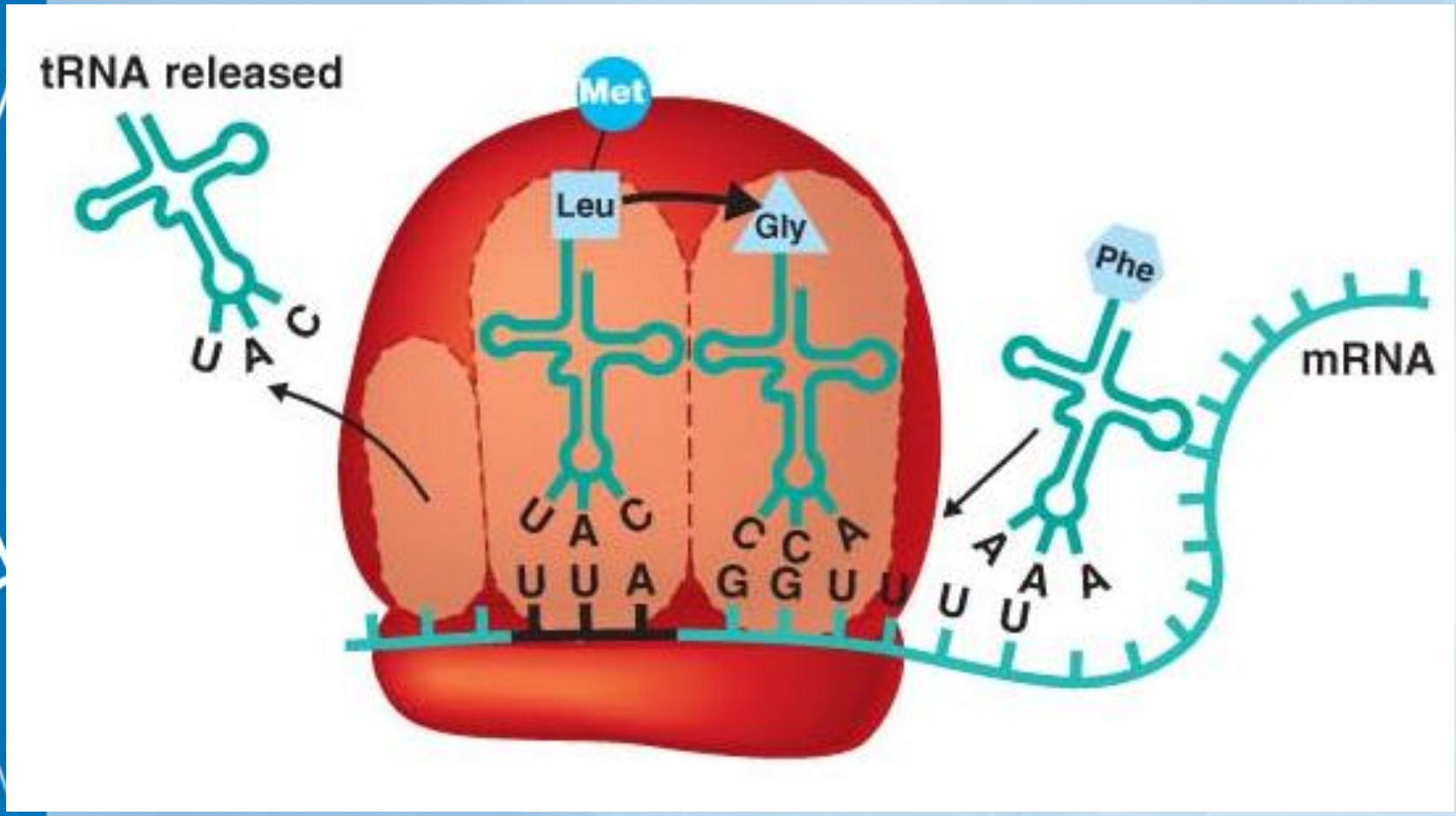
The Process of Translation



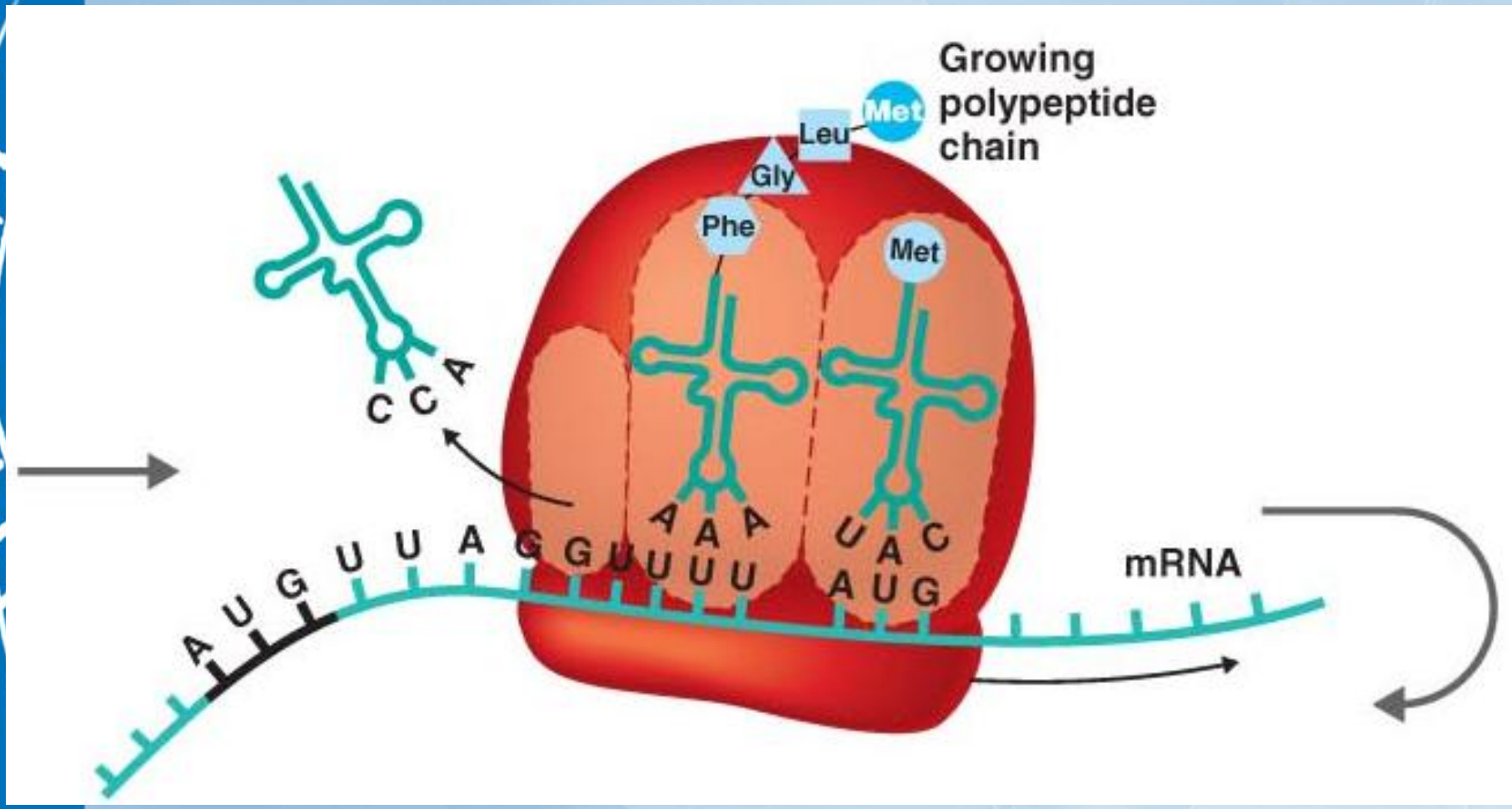
The Process of Translation



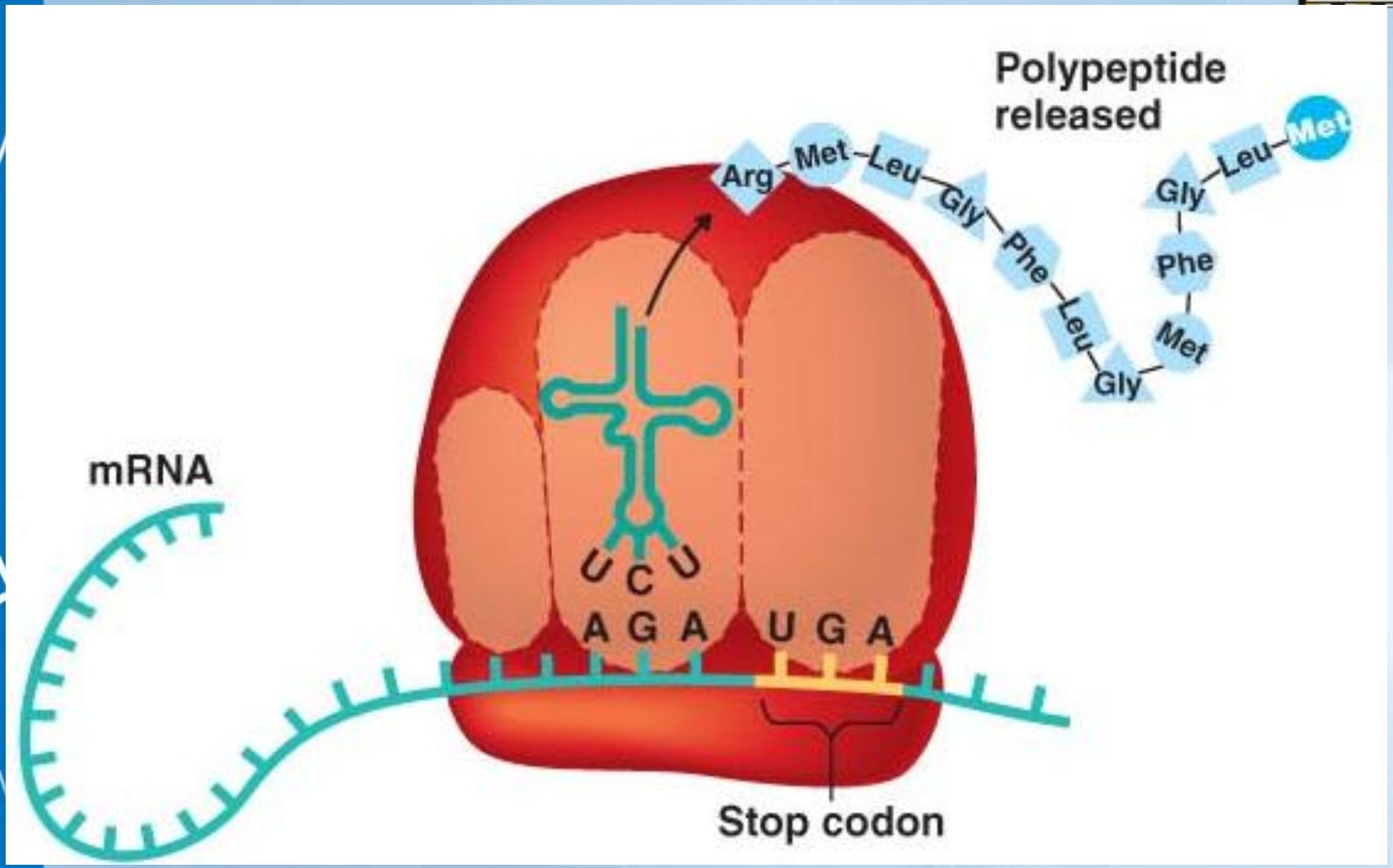
The Process of Translation



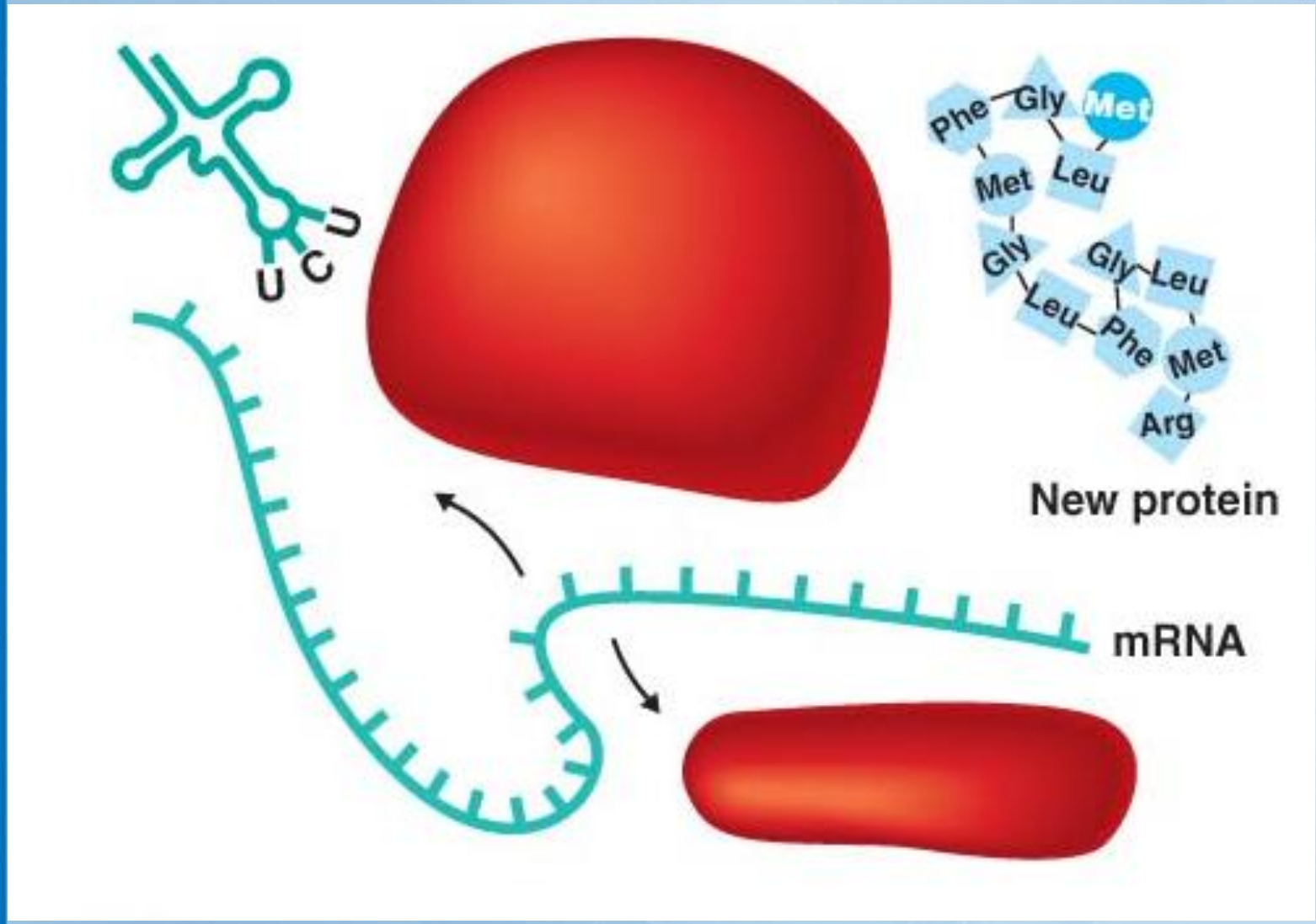
The Process of Translation



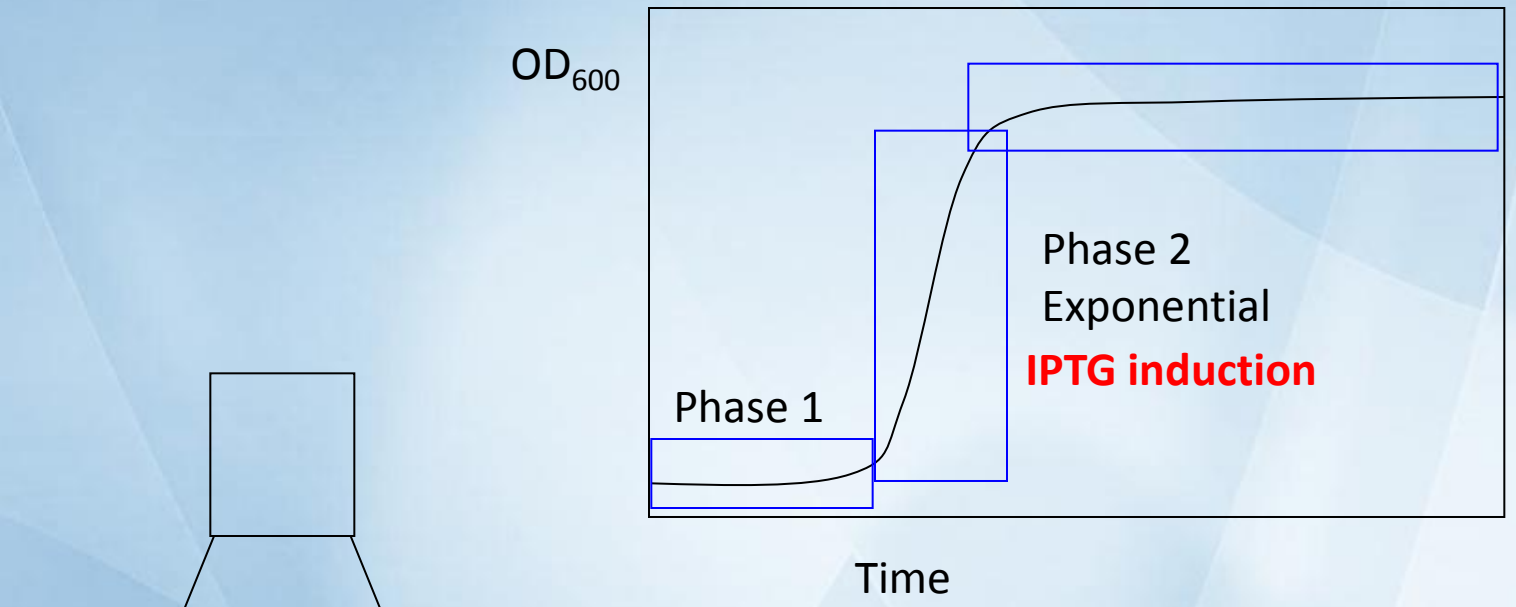
The Process of Translation

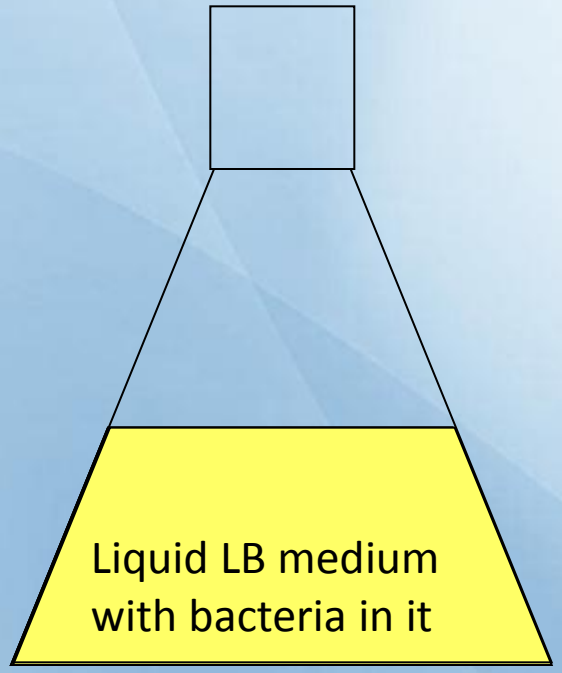


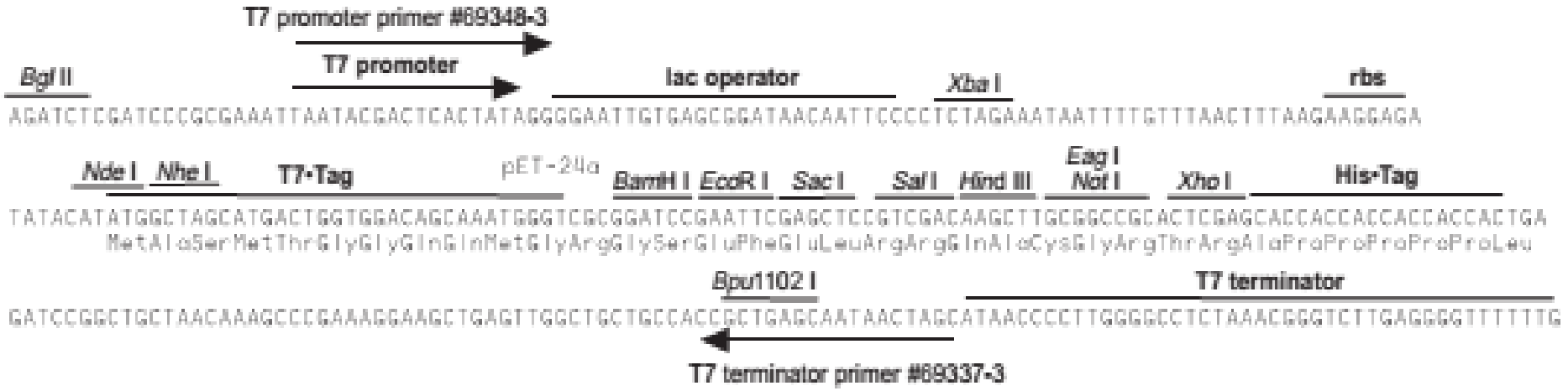
The Process of Translation



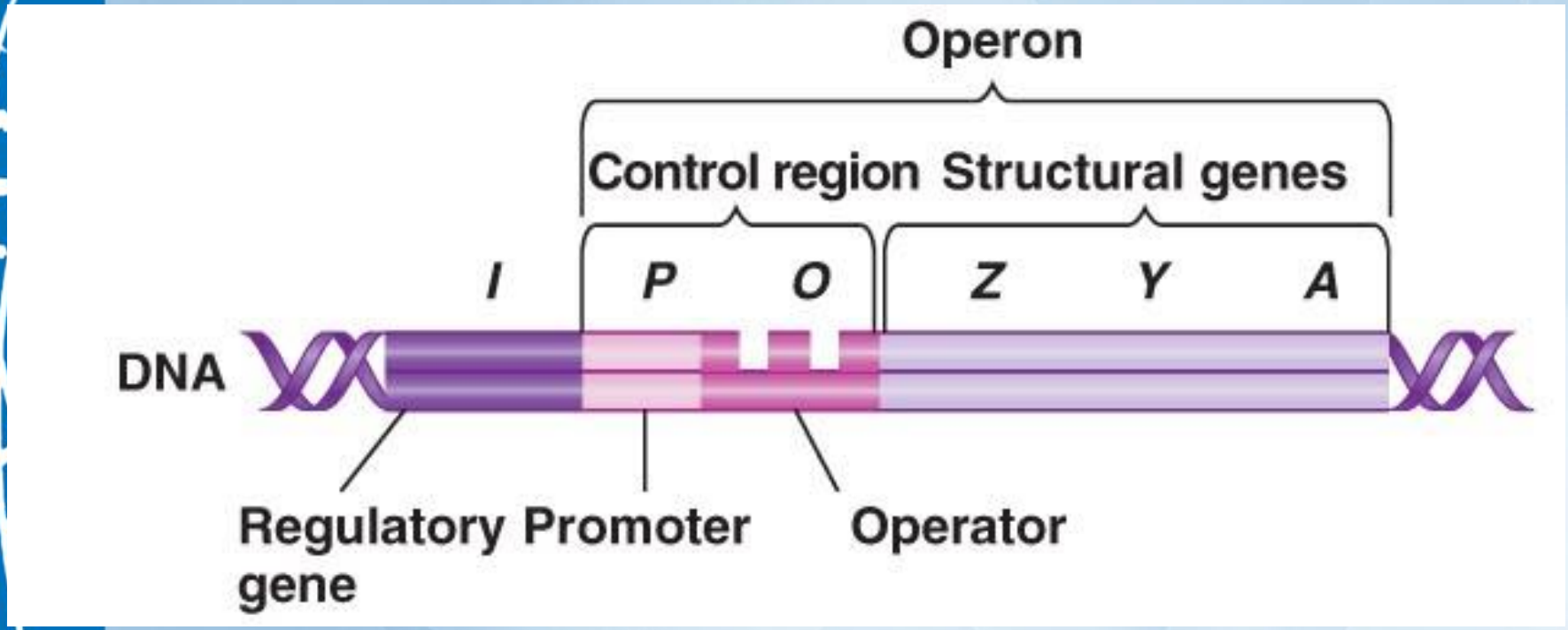
Protein Expression






T7 promoter primer #69348-3
T7 promoter
Bgl II
lac operator
Xba I
rbs
AGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGA
Nde I
Nhe I
T7•Tag
pET-24a
BamH I
EcoR I
Sac I
Sal I
Hind III
Eag I
Not I
Xho I
His•Tag
TATACATATGGCTAGCATGACTGGTGGACAGCAAATGGGTCGCGGATCCGAATTCGAGCTCCGTCGACAAGCTTGCGGCCGCACTCGAGCACCACCACCACCACCACTGA
MetAlaSerMetThrGlyGlyGlnGlnMetGlyArgGlySerGluPheGluLeuArgArgGlnAlaCysGlyArgThrArgAlaProProProProProLeu
Bpu1102 I
T7 terminator
GATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTG
T7 terminator primer #69337-3

Operon


Operon
Control region
Structural genes
I
P
O
Z
Y
A
DNA
Regulatory gene
Promoter
Operator

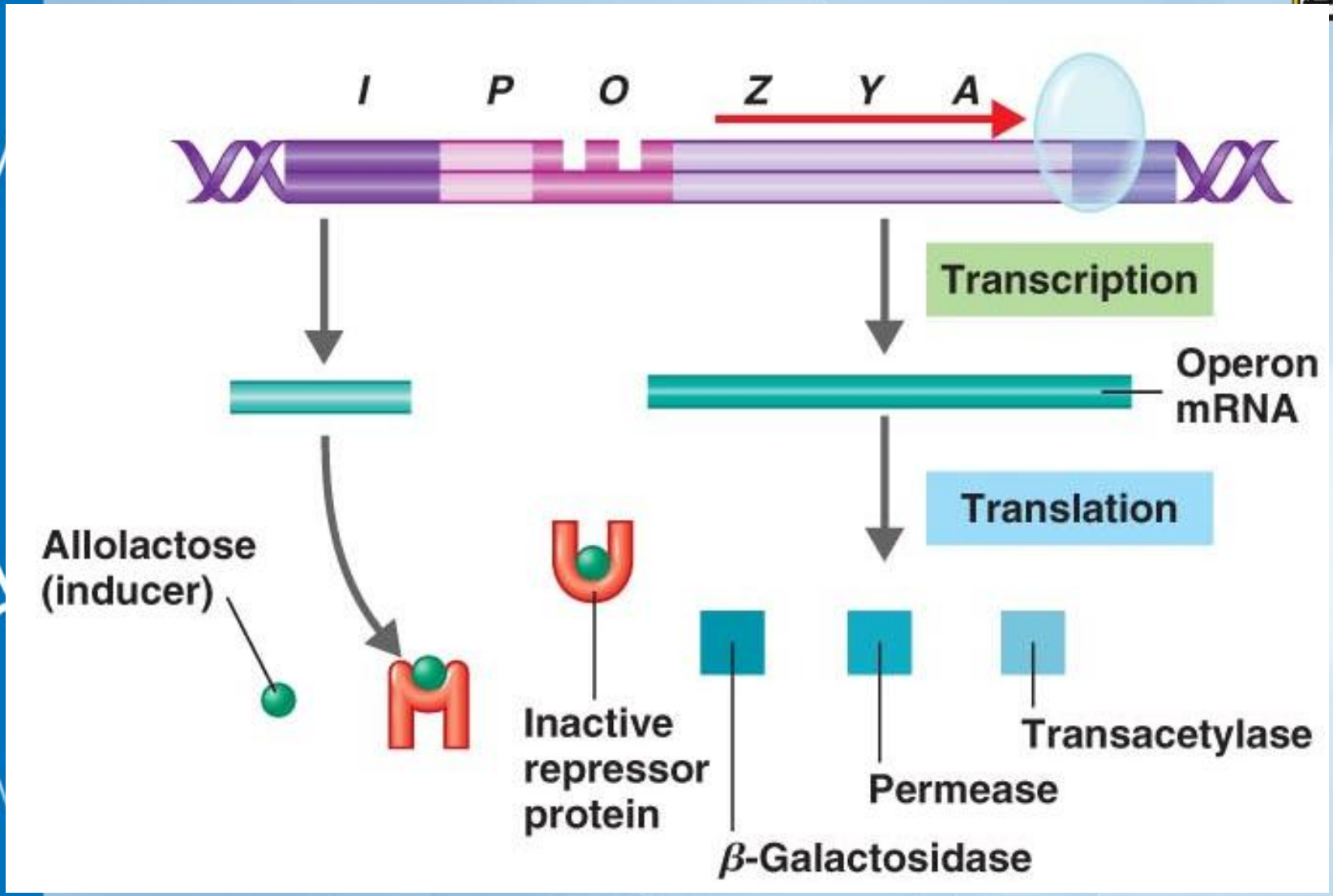
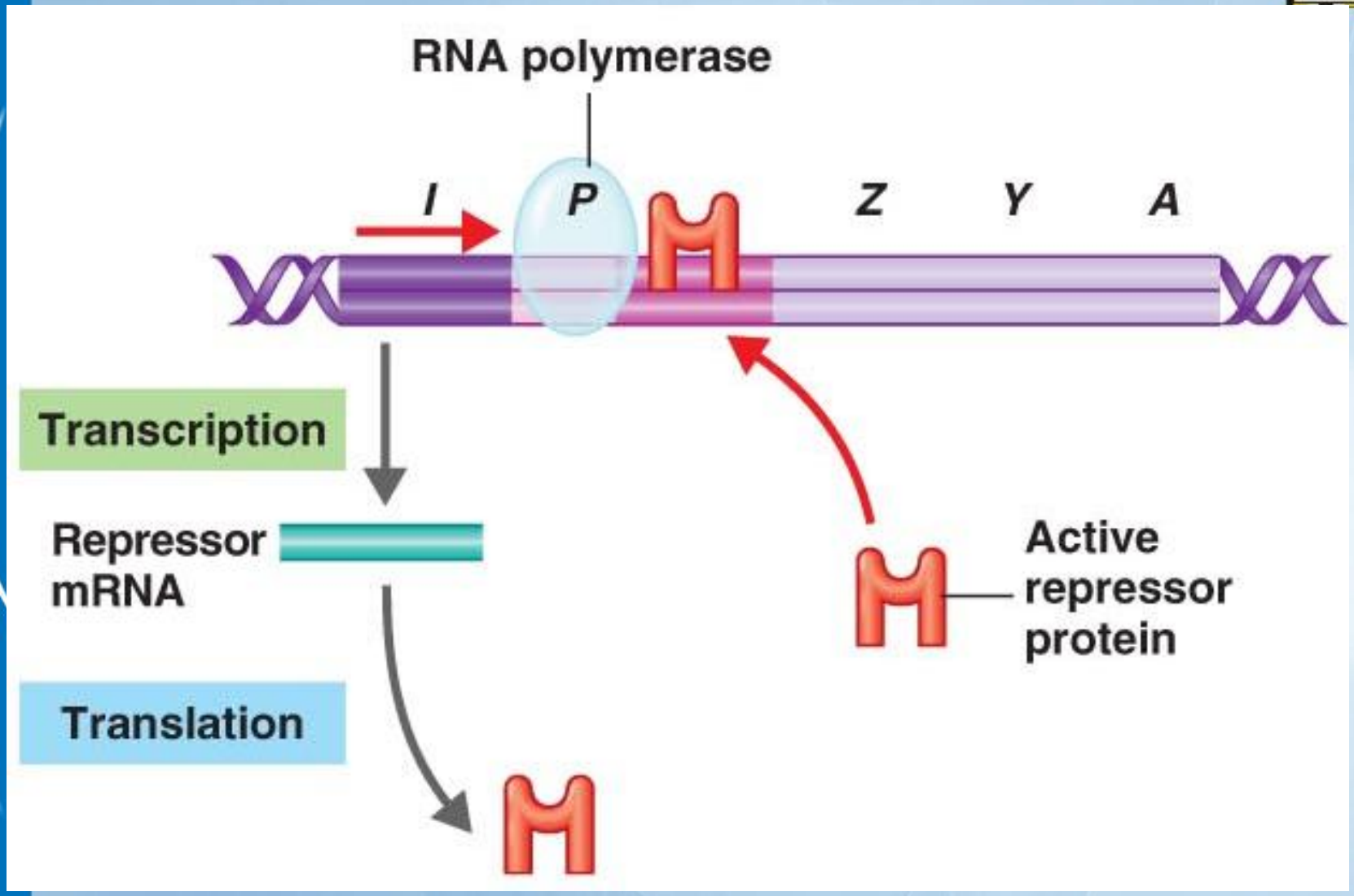
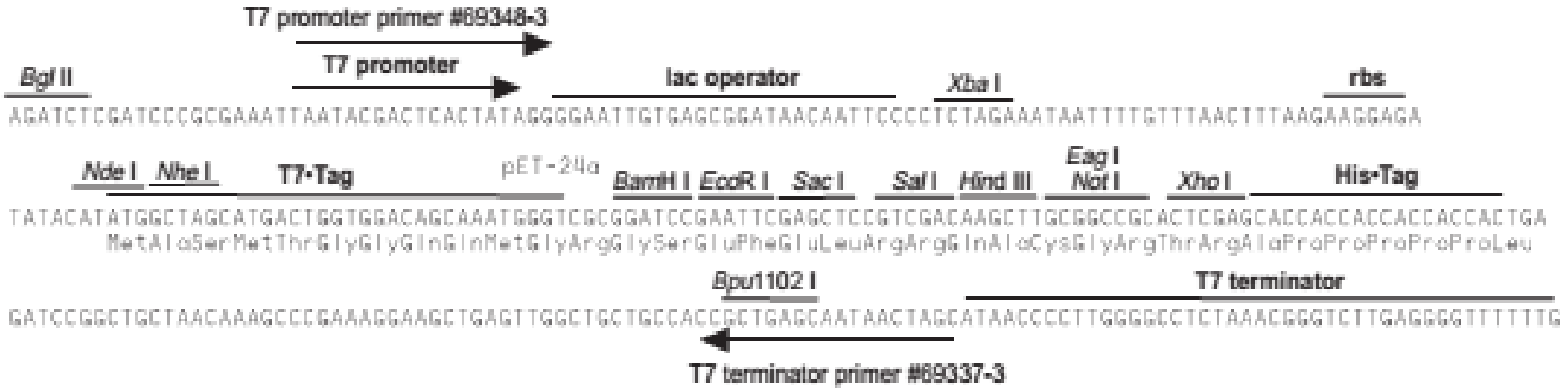

I
P
O
Z
Y
A
Transcription
Operon mRNA
Translation
Allolactose (inducer)
Inactive repressor protein
β-Galactosidase
Permease
Transacetylase

Figure 8.12

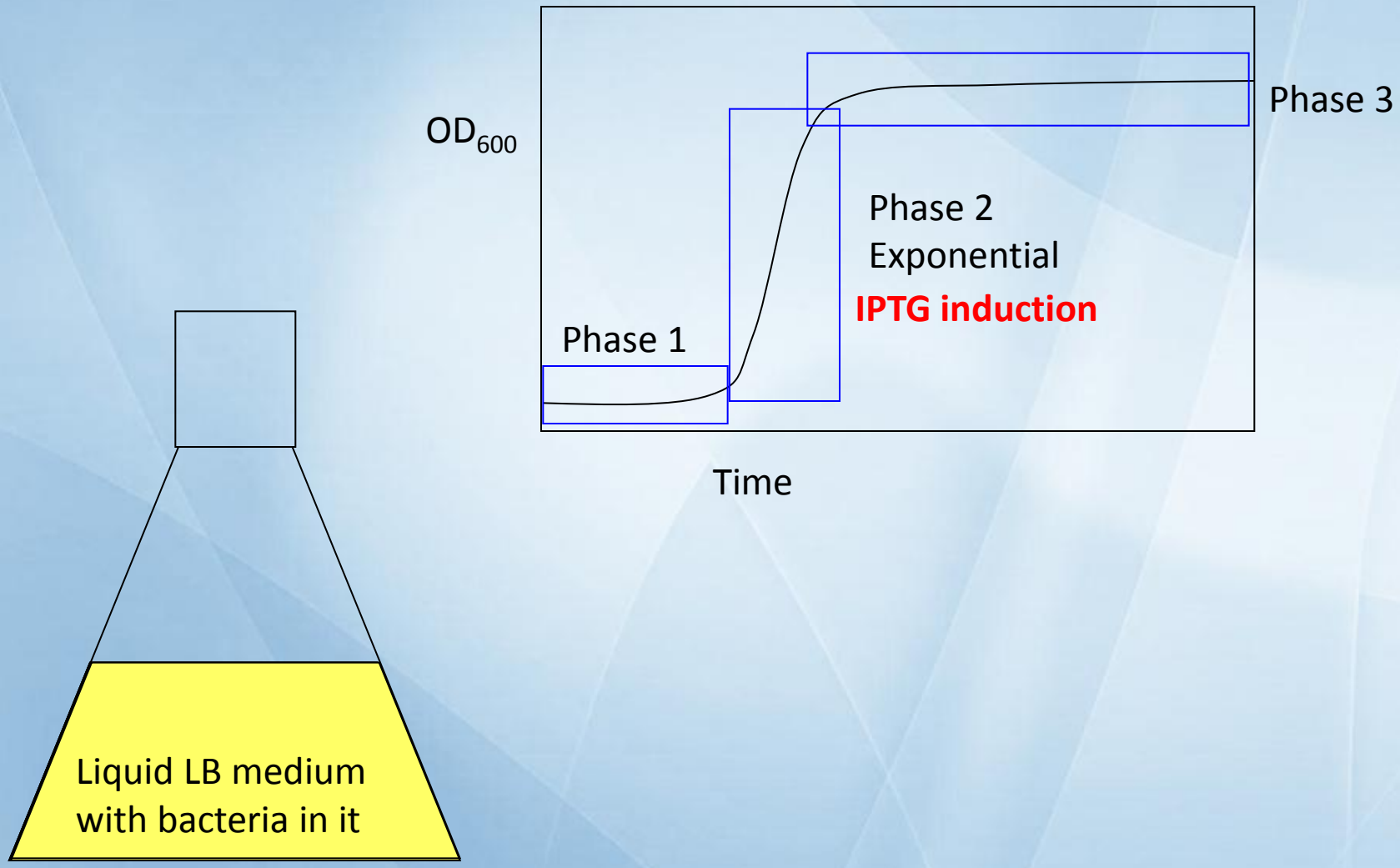

RNA polymerase
I
P
Z
Y
A
Transcription
Repressor mRNA
Translation
Active repressor protein


Assiut University
Biotechnology
Lab
Genetics Department

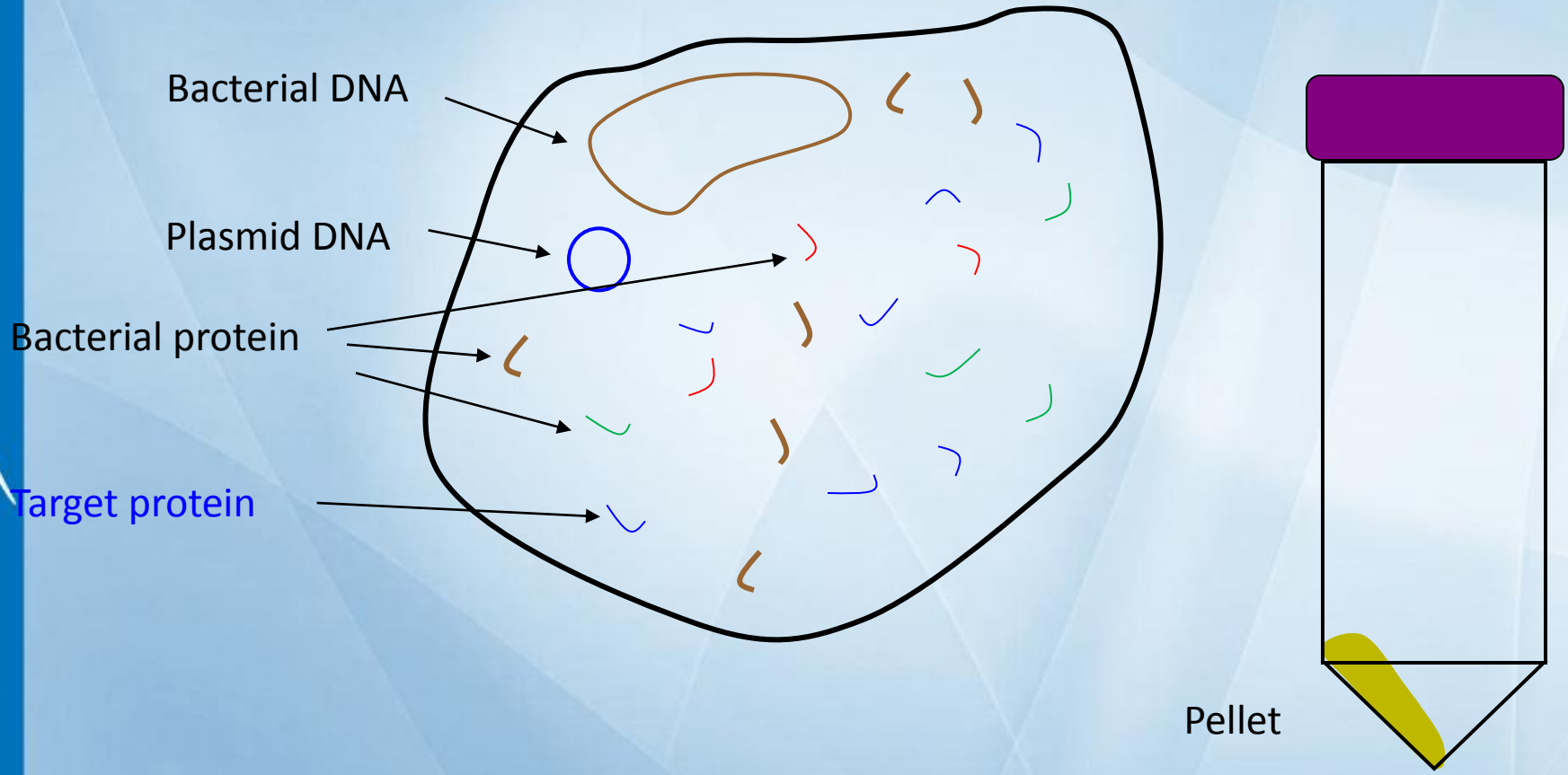
[animation]


T7 promoter primer #69348-3
T7 promoter
Bgl II
lac operator
Xba I
rbs
AGATCTCGATCCCGCGAAATTAATACGACTCACTATAGGGGAATTGTGAGCGGATAACAATTCCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAGA
Nde I
Nhe I
T7•Tag
pET-24a
BamH I
EcoR I
Sac I
Sal I
Hind III
Eag I
Not I
Xho I
His•Tag
TATACATATGGCTAGCATGACTGGTGGACAGCAAATGGGTCGCGGATCCGAATTCGAGCTCCGTCGACAAGCTTGCGGCCGCACTCGAGCACCACCACCACCACCACTGA
MetAlaSerMetThrGlyGlyGlnGlnMetGlyArgGlySerGluPheGluLeuArgArgGlnAlaCysGlyArgThrArgAlaProProProProProLeu
Bpu1102 I
T7 terminator
GATCCGGCTGCTAACAAAGCCCGAAAGGAAGCTGAGTTGGCTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTG
T7 terminator primer #69337-3

Protein Expression



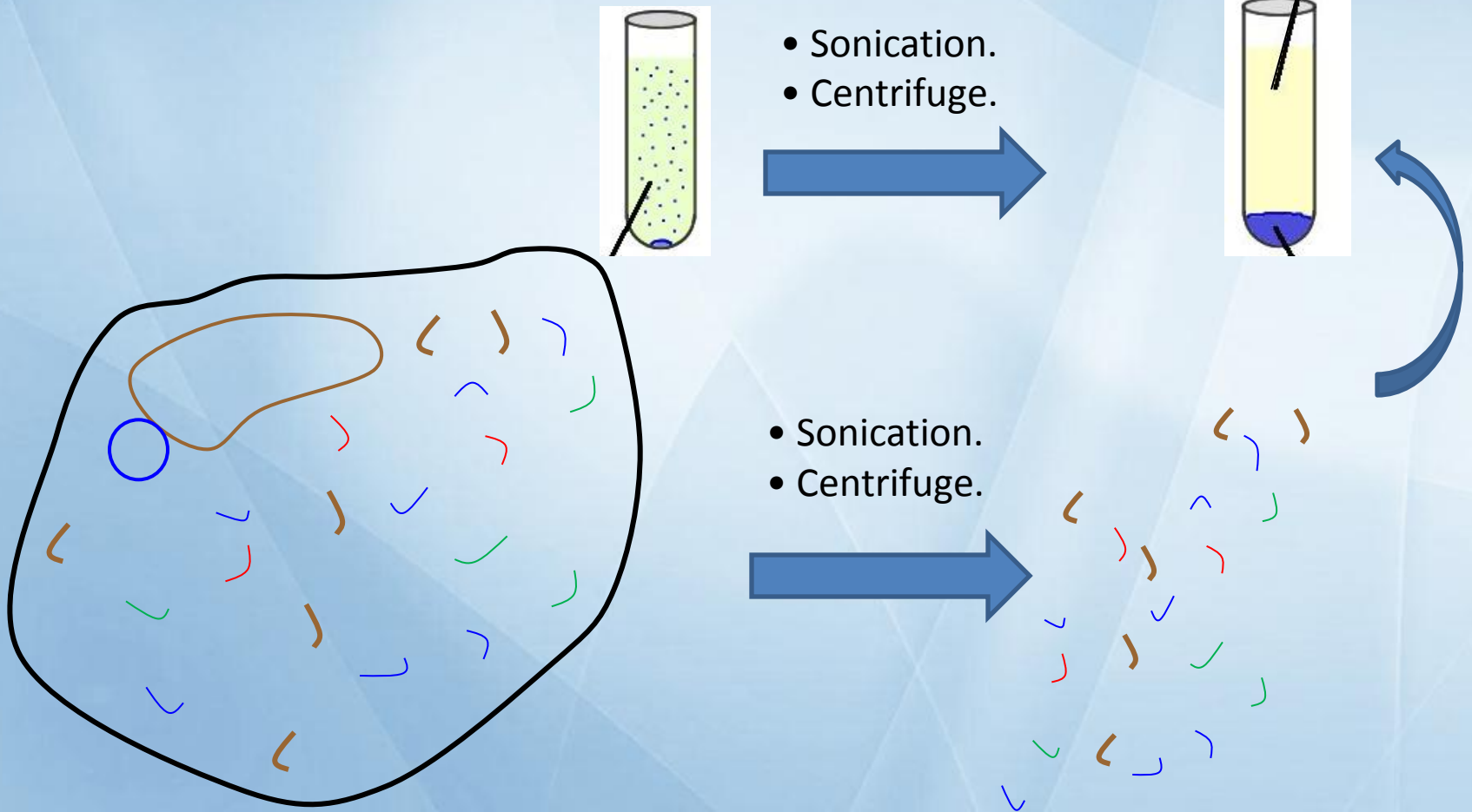
Bacterial Growth



Lysis

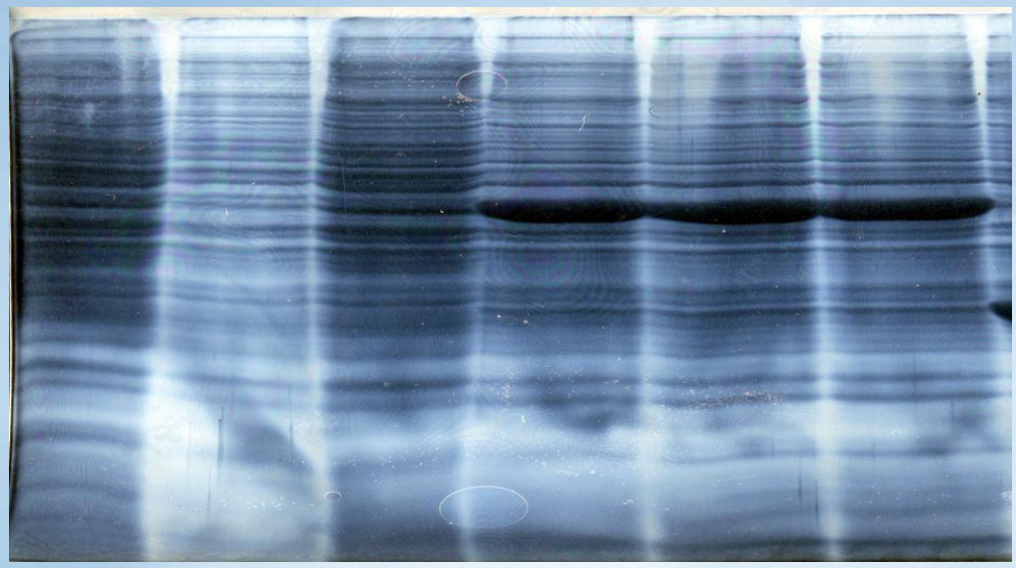
•Pellet is resuspended in the lysis buffer containing, and sonicated to further liberate the protein

• Spin down the denaturing lysis buffer, cell wall and debris will pellet at the bottom and our protein is in the soluble supernatant.



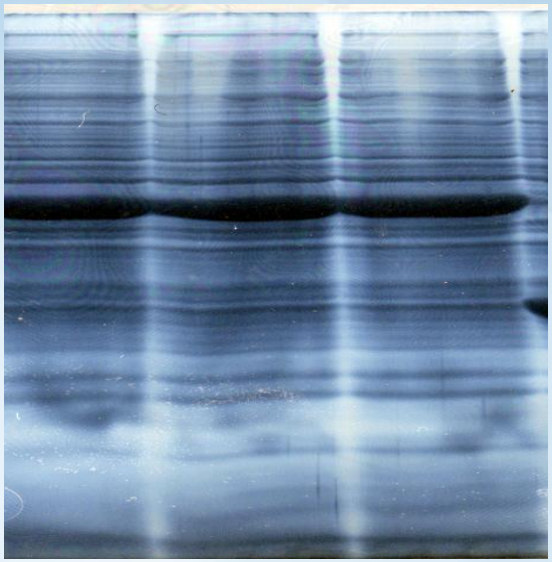
Expression of protein in *E. coli*

Uninduced | Induced Samples



Expression of protein in *E. coli*

Induced Samples



We want to work with pure proteins. How do we purify it from all the other *E. coli* proteins?

Why purify a protein?

- To study its function, Activity, Structure
- To analyze its physical properties
- To determine its sequence
- For industrial or therapeutic applications
- Use in assays
- Study protein regulation and protein interactions
- Produce Antibodies
- Perform structural analysis by X-Ray and Crystallography

Steps in Recombinant Protein Purification

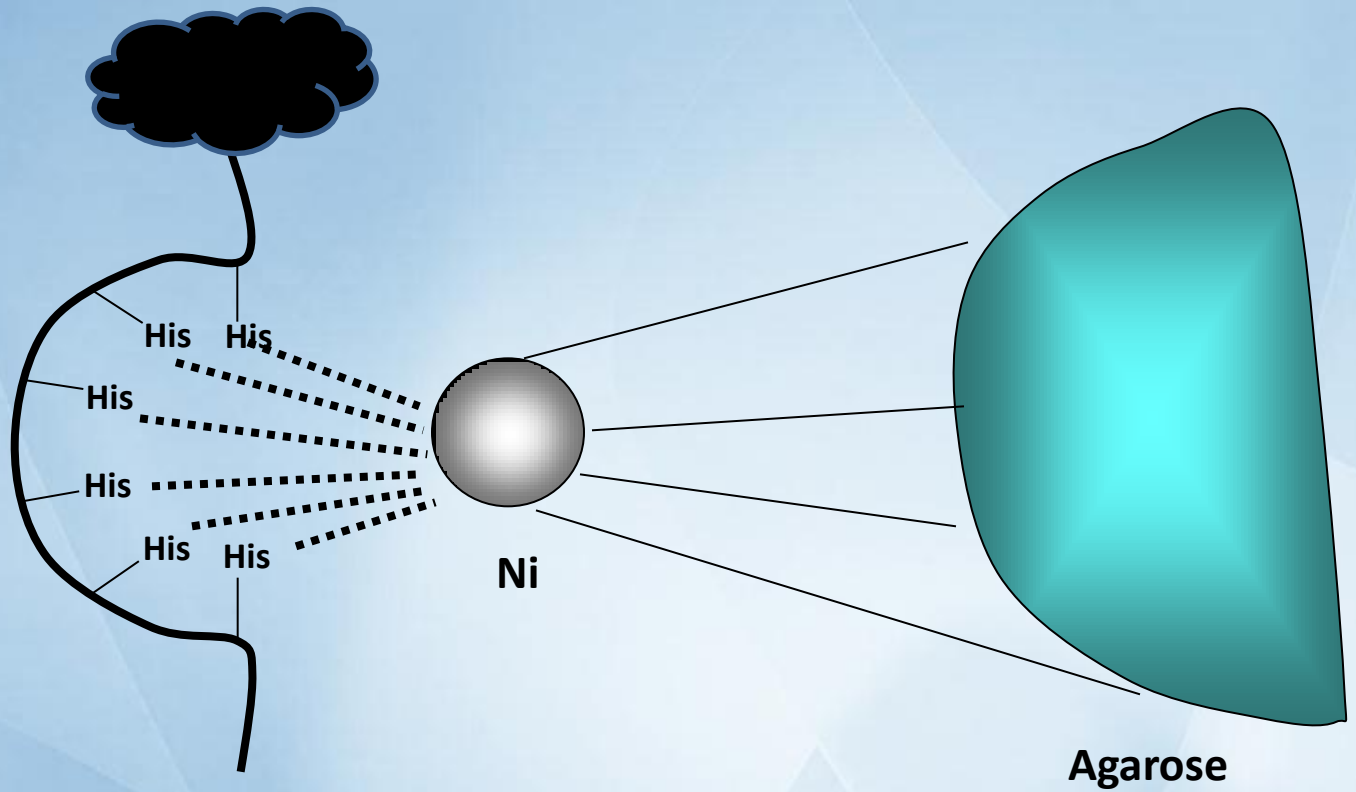
1. Grow culture of positive clone, induce expression
2. Lyse cells
3. Centrifuge to isolate protein-containing fraction
4. Column Chromatography—collect fractions
5. Assess purity on SDS-PAGE

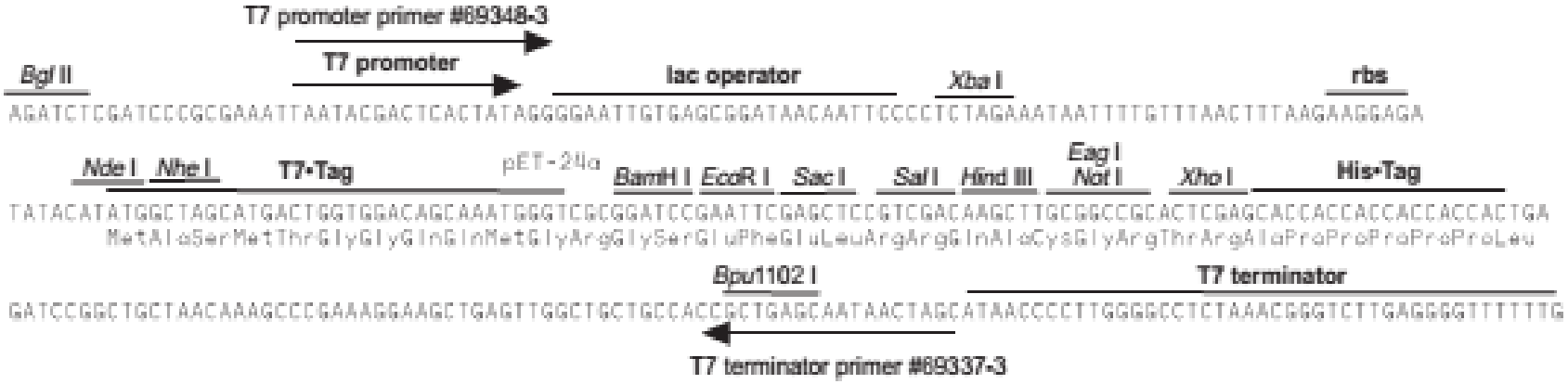
Affinity chromatography (AC)

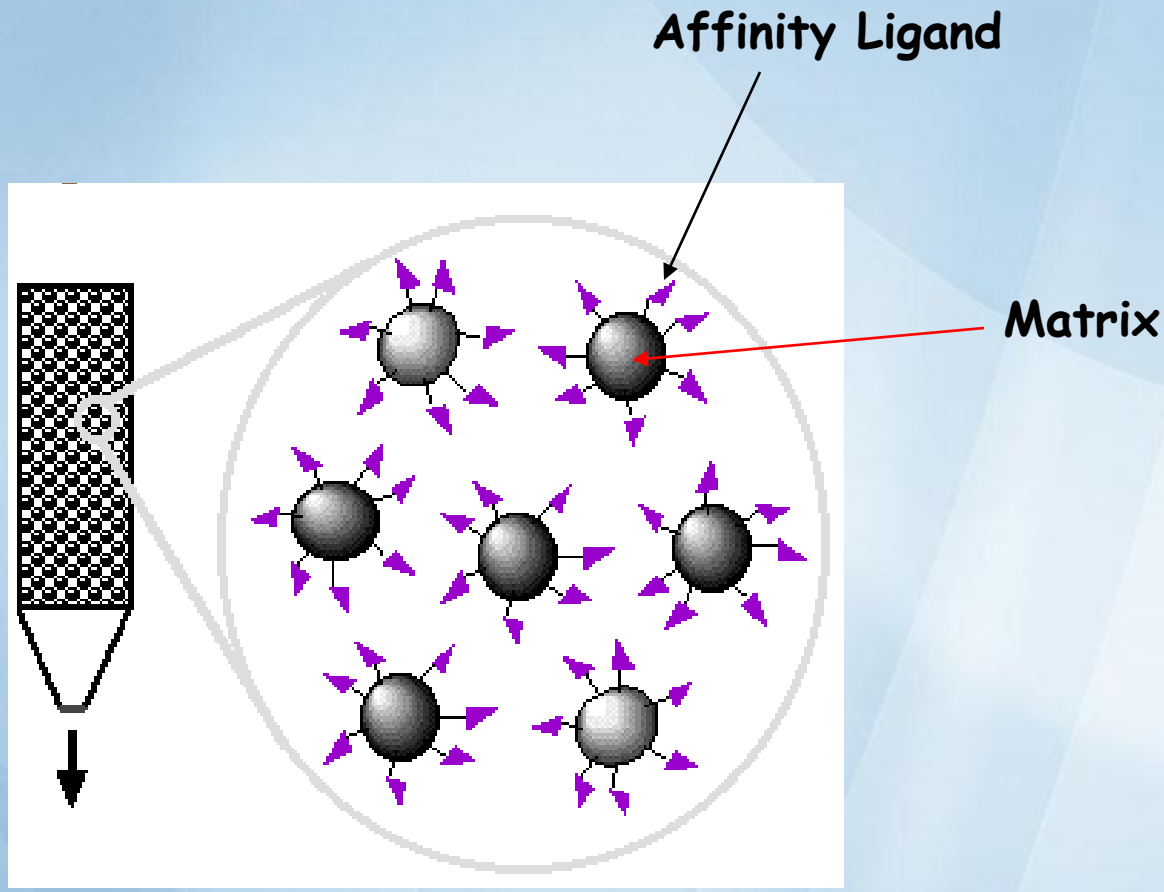
What is AC?

- **AC** is a technique enabling purification of a biomolecule with respect to biological function or individual chemical structure.
- AC is designed to purify a particular molecule from a mixed sample.

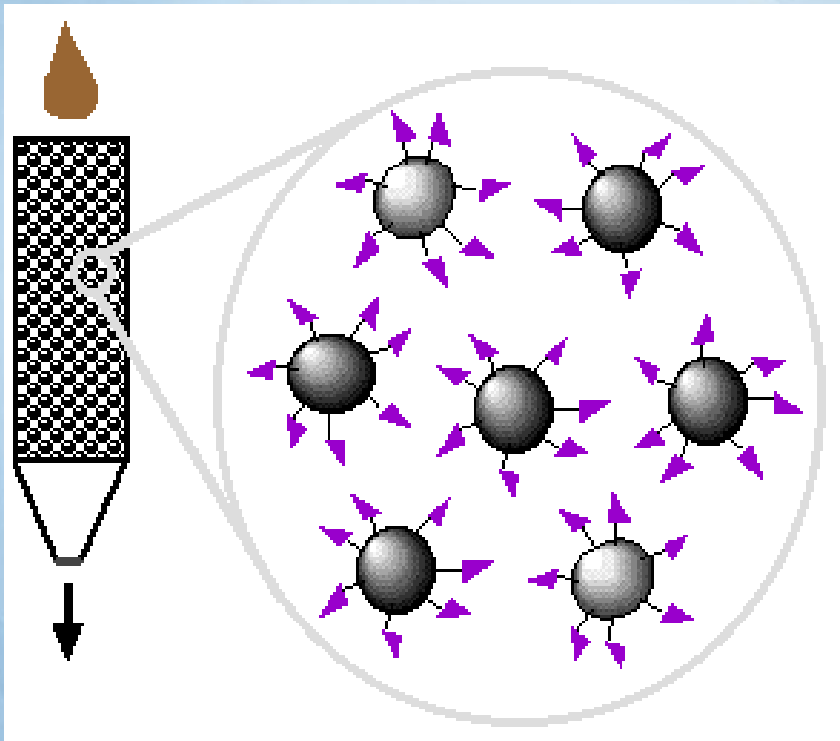
Affinity Chromatography



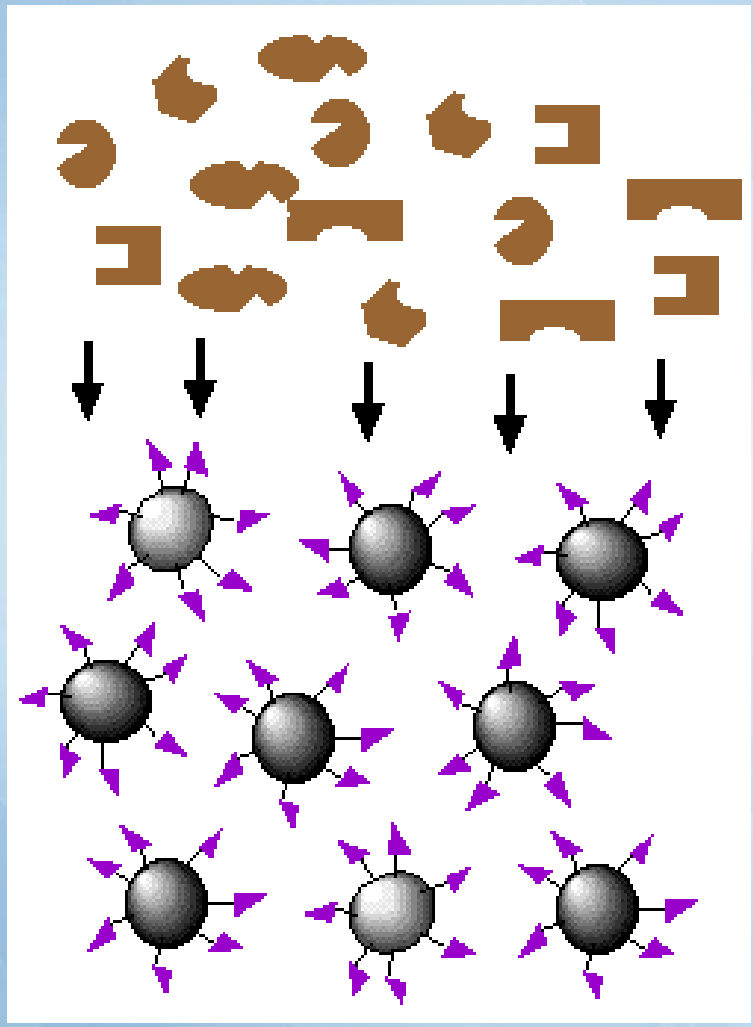



Affinity Ligand
Matrix

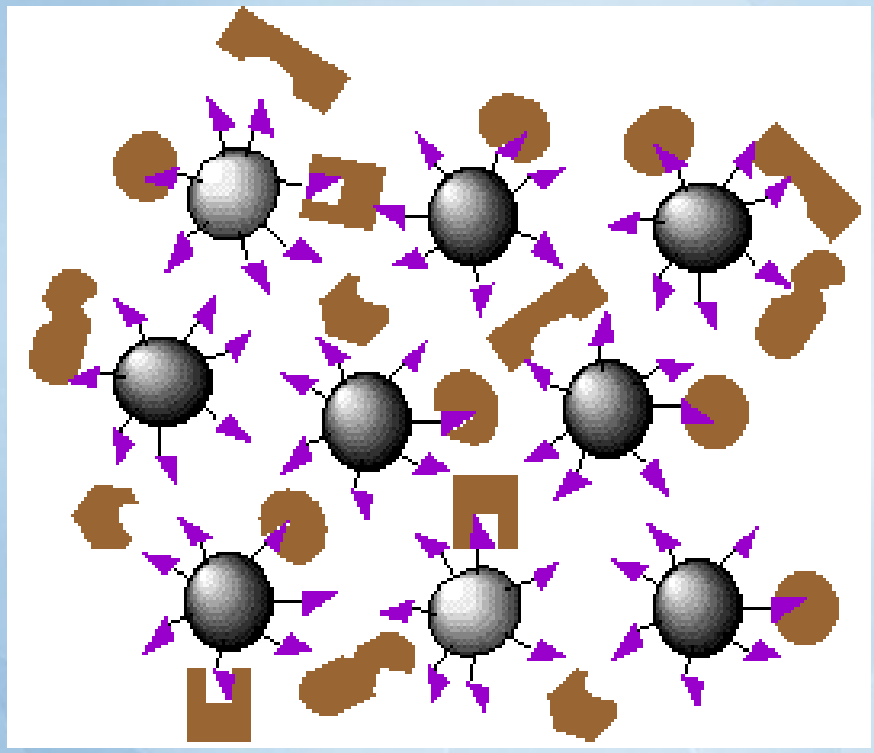
Step 1. Loading affinity column.



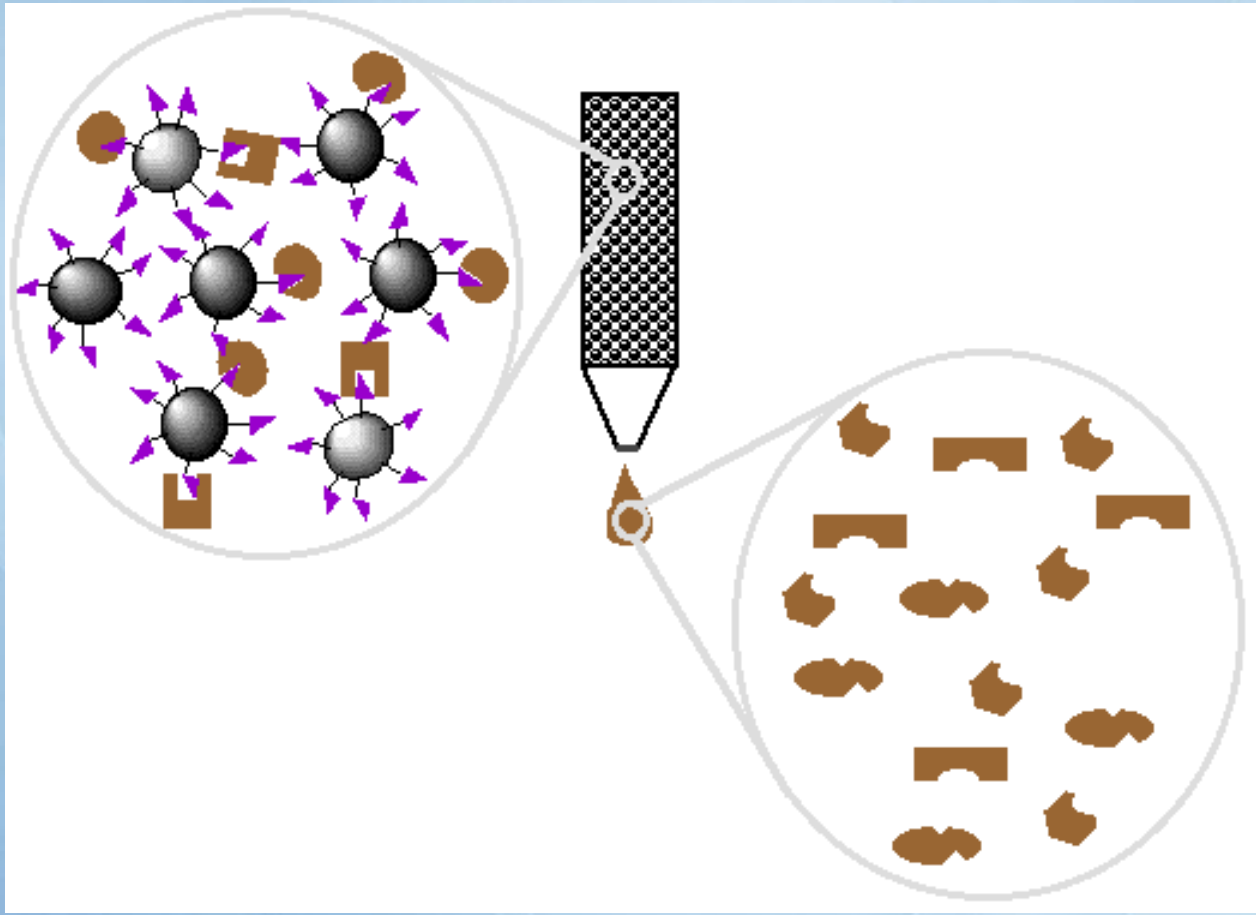
Step 2. Proteins sieve through matrix of affinity beads.



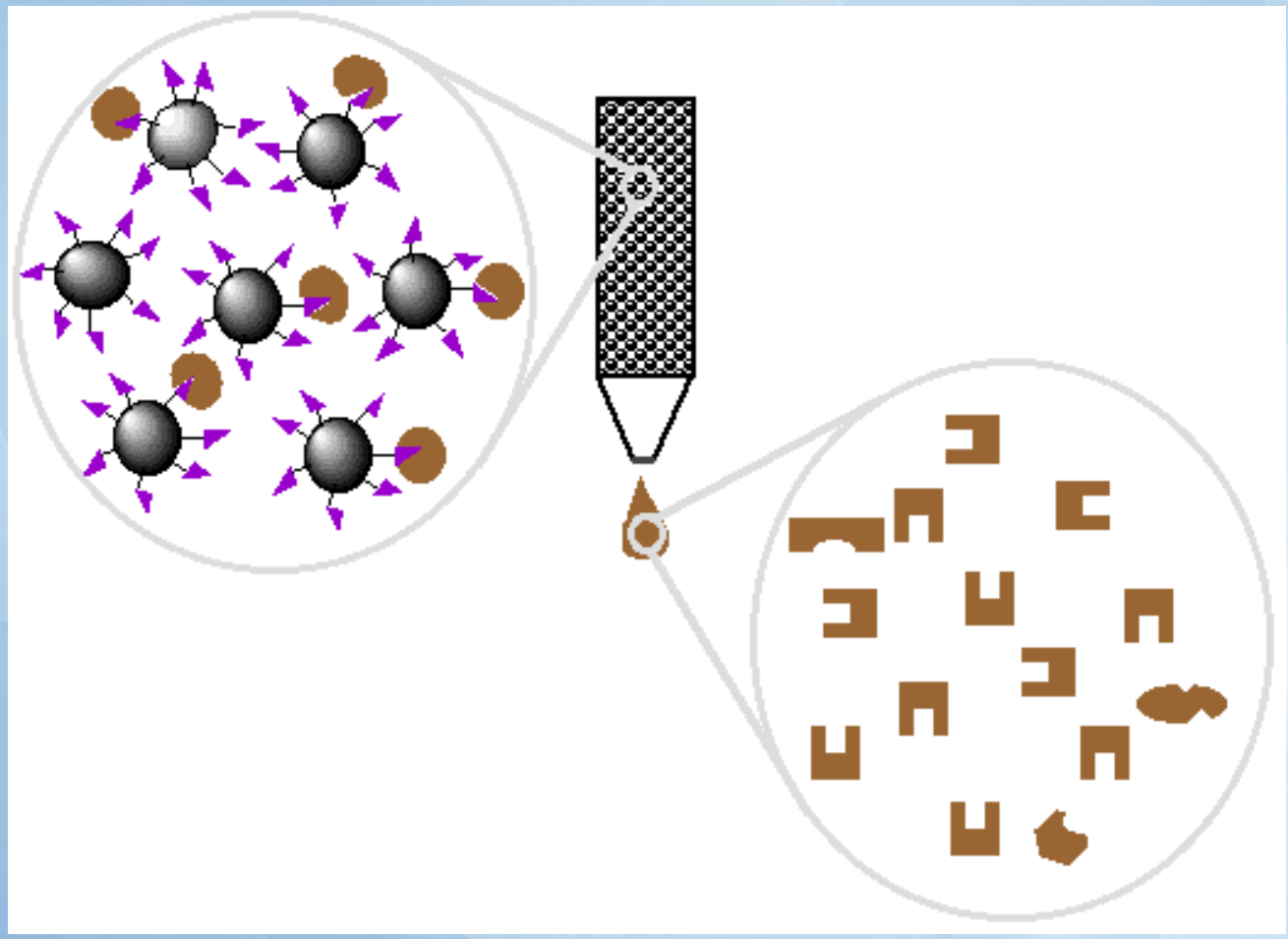
Step 3. Proteins interact with affinity ligand with some binding loosely and others tightly.



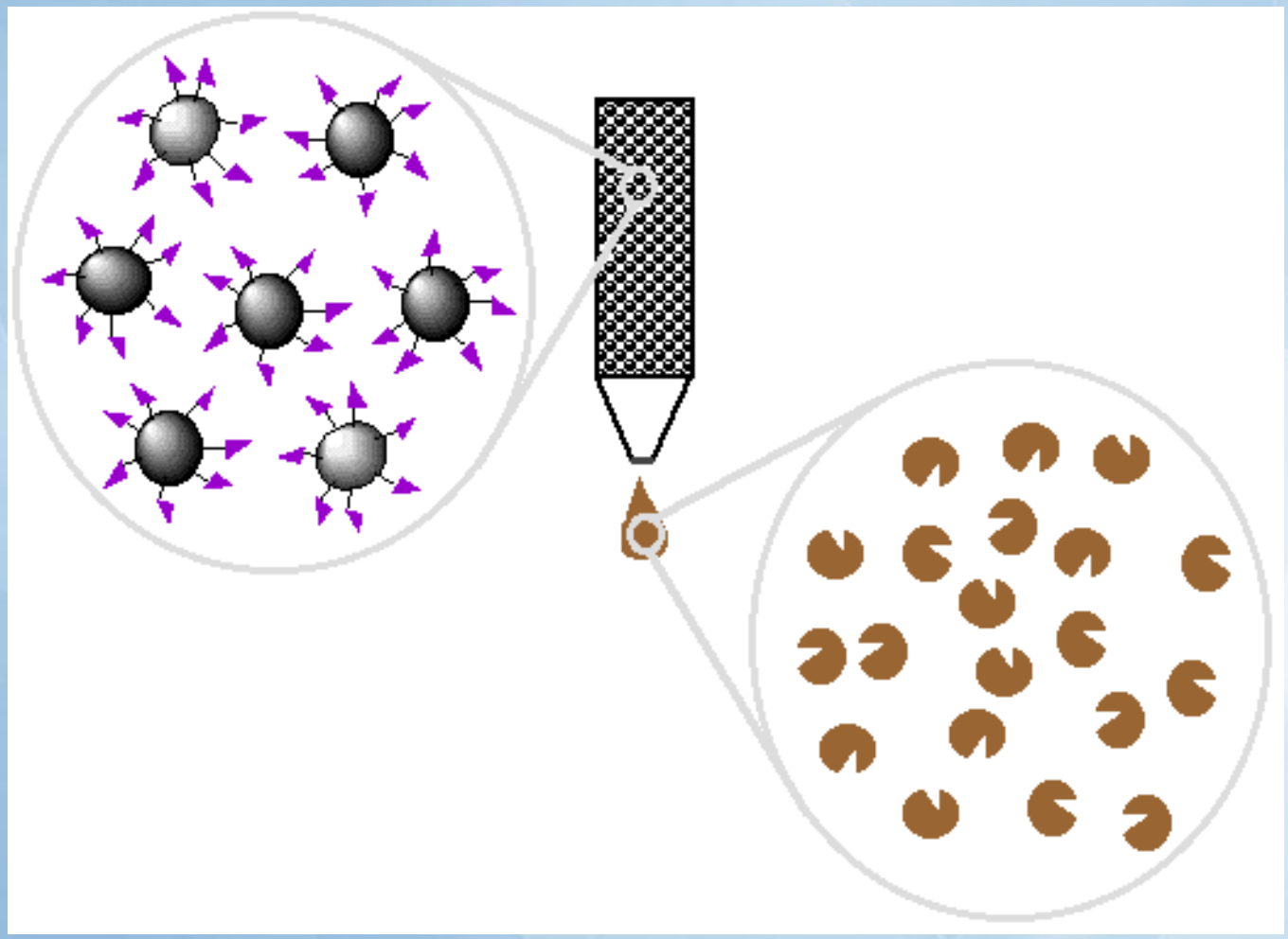
Step 4. Wash off proteins that do not bind.



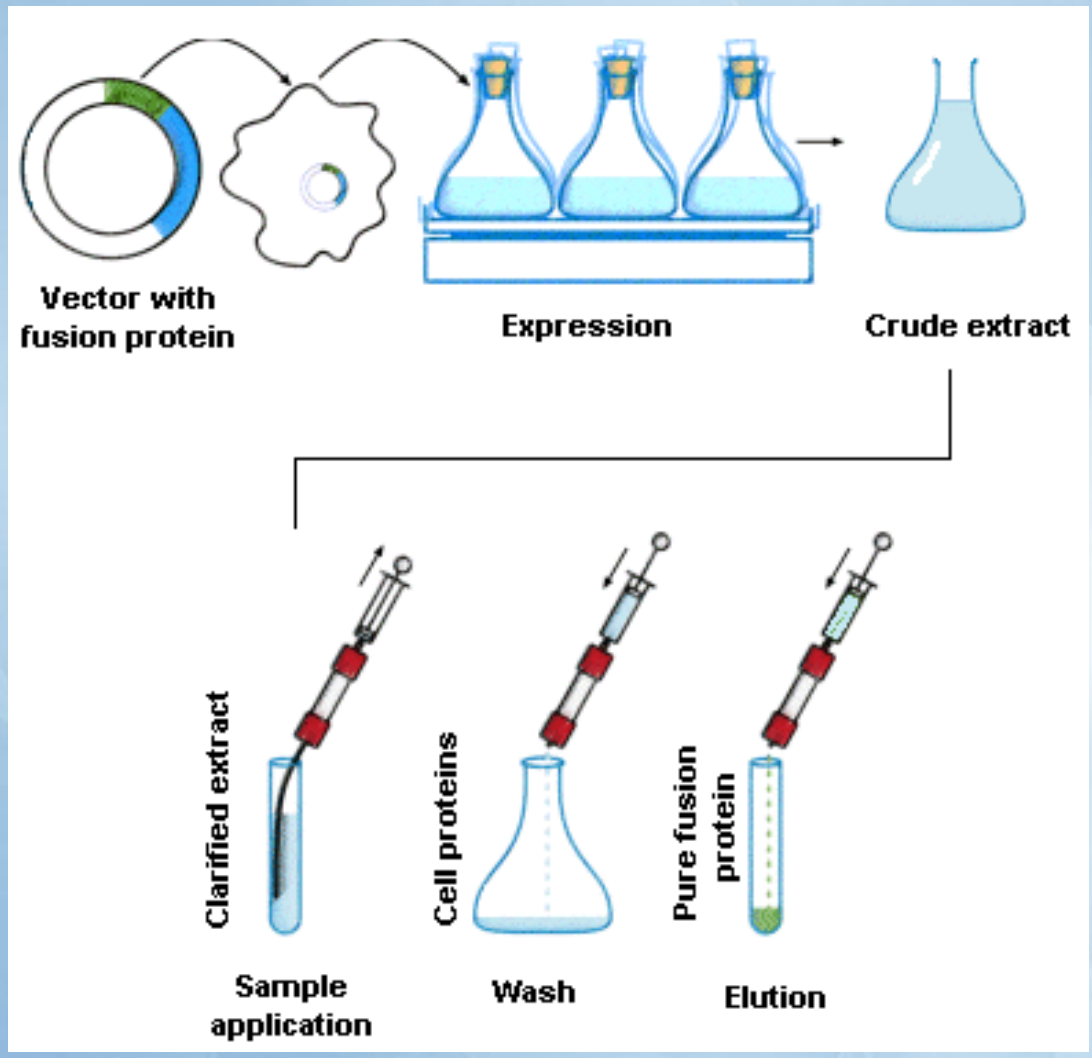
Step 5. Wash off proteins that bind loosely.



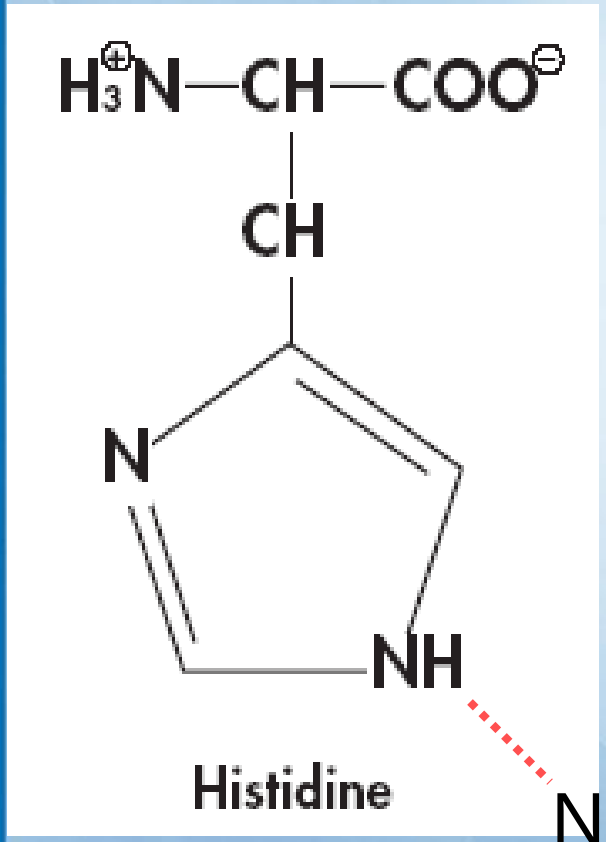
Step 6. Elute proteins that bind tightly to ligand and collect purified protein of interest.



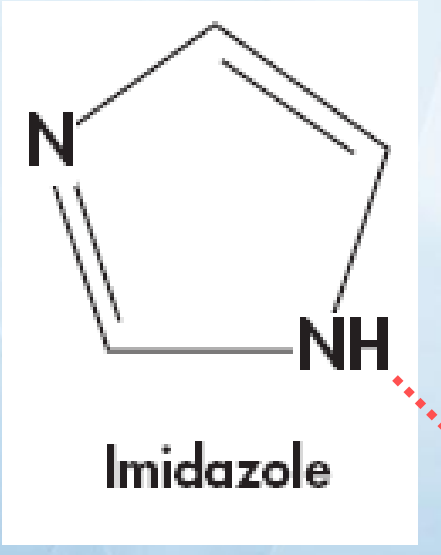
Affinity chromatography applied to recombinant proteins



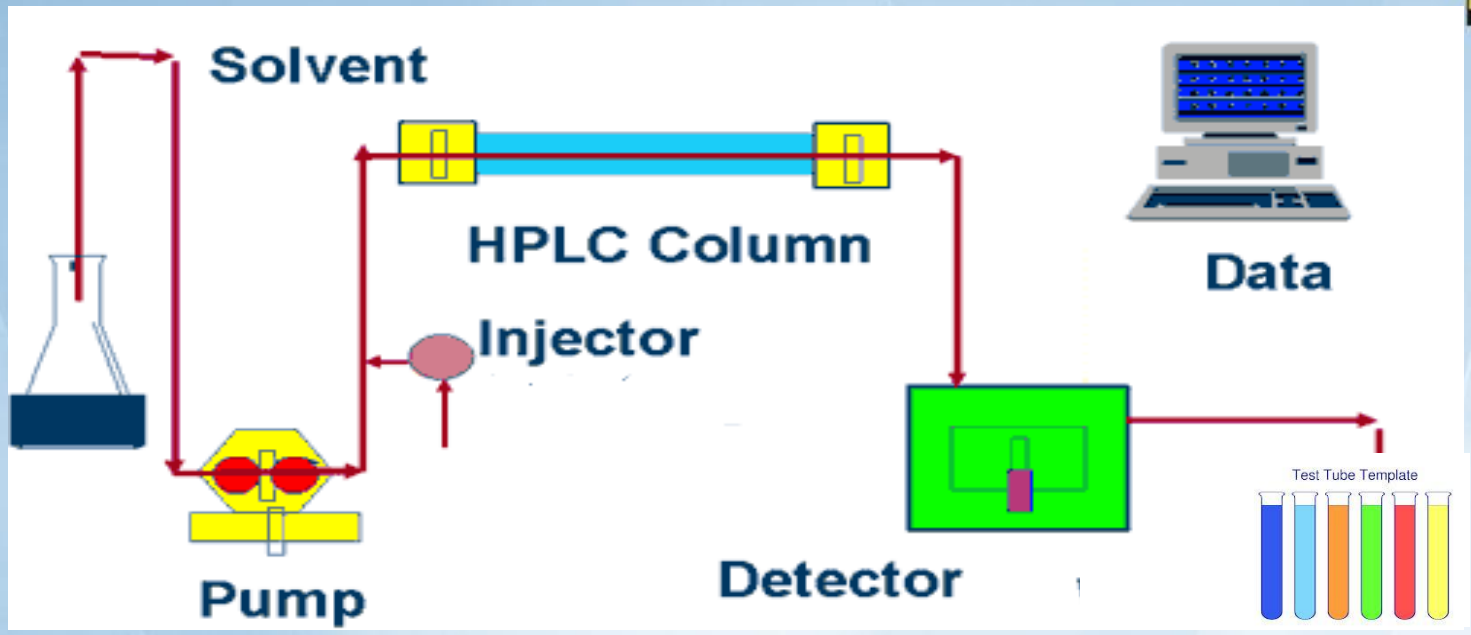
Elution with imidazole
Why imidazole?



The imidazole ring is part of the structure of histidine

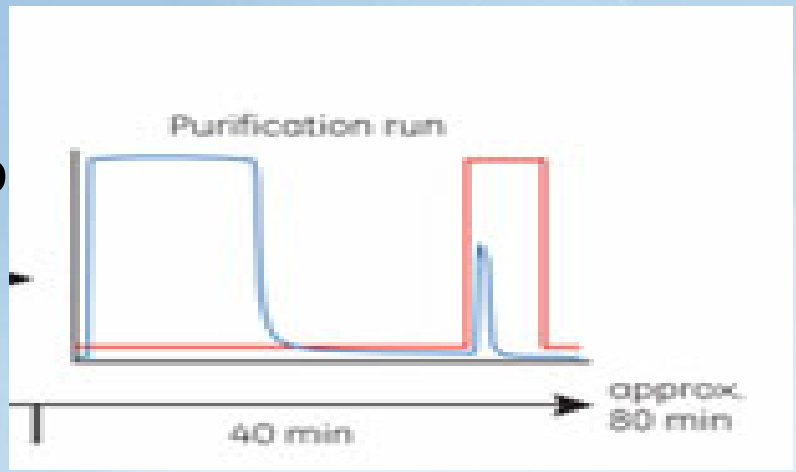


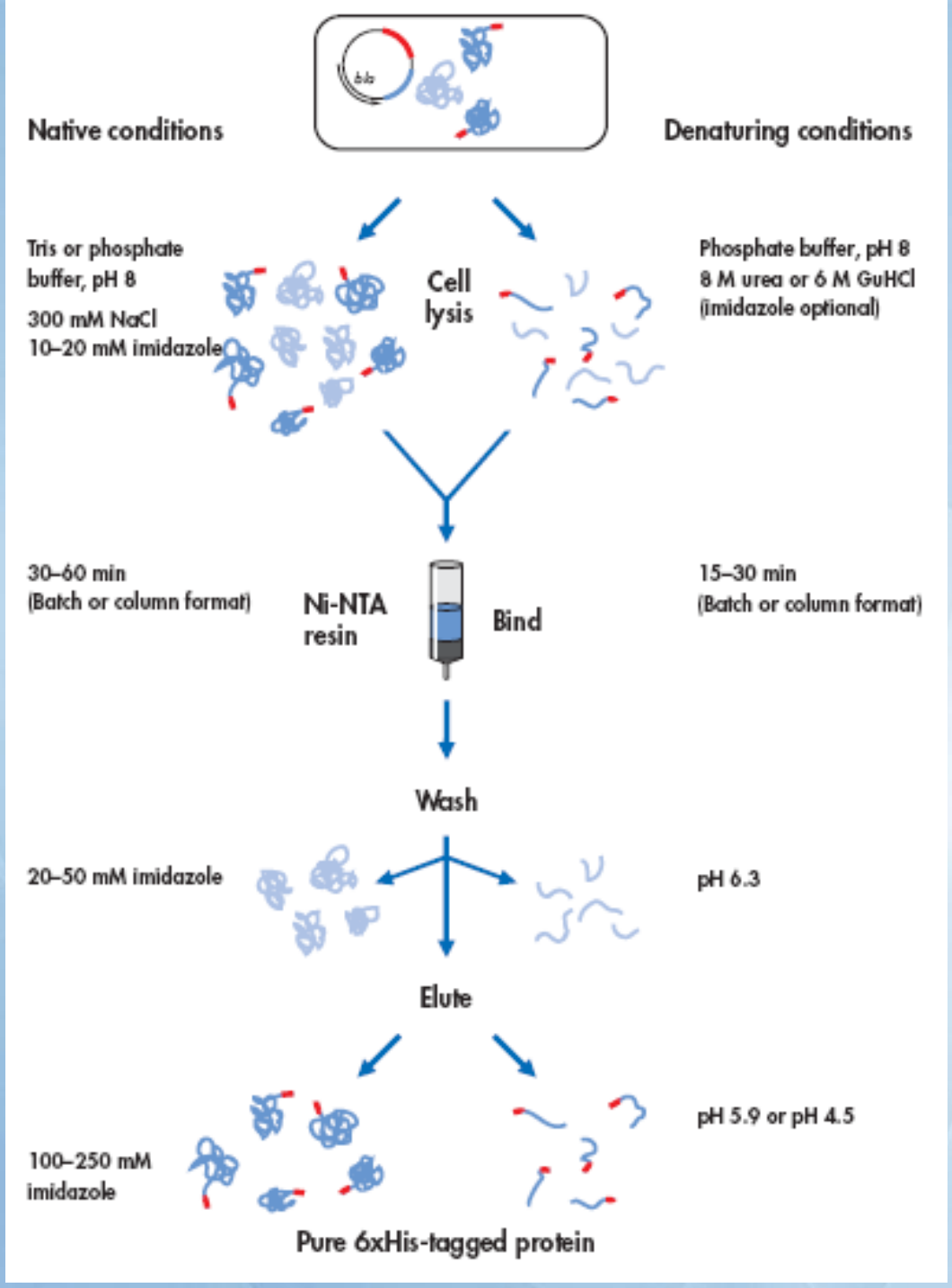
IMAC System


Solvent
HPLC Column
Data
Injector
Pump
Detector
Test Tube Template

Purity test

SD




Native conditions
Denaturing conditions
Tris or phosphate buffer, pH 8
300 mM NaCl
10–20 mM imidazole
Cell lysis
Phosphate buffer, pH 8
8 M urea or 6 M GuHCl
(imidazole optional)
30–60 min
(Batch or column format)
Ni-NTA resin
Bind
15–30 min
(Batch or column format)
Wash
20–50 mM imidazole
pH 6.3
Elute
100–250 mM imidazole
pH 5.9 or pH 4.5
Pure 6xHis-tagged protein

Protein dialysis

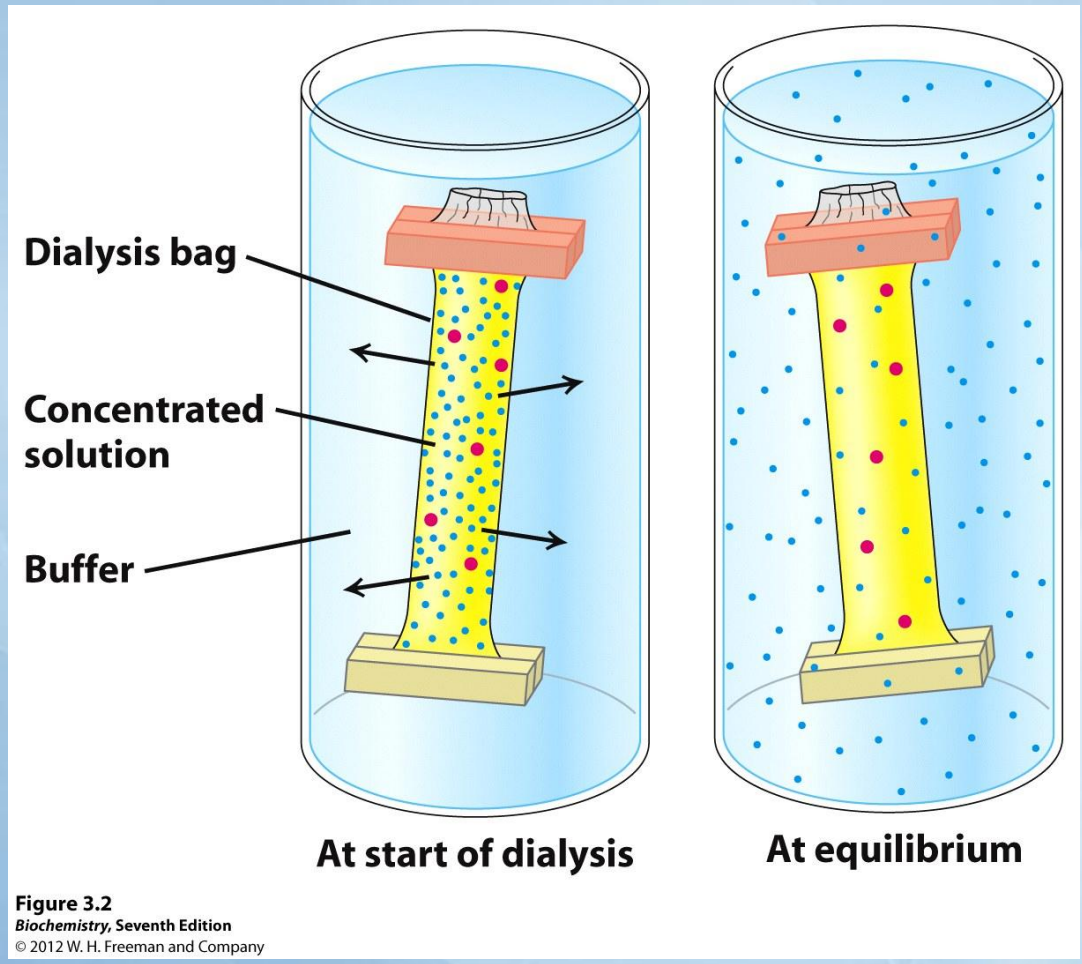


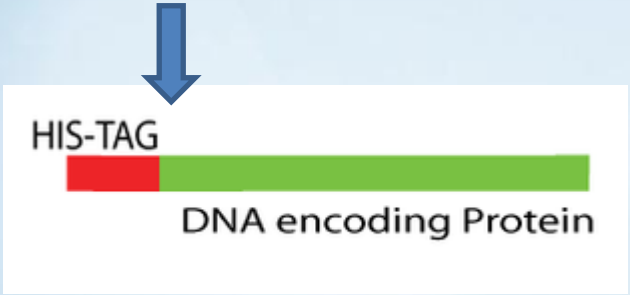
Figure 3.2
***Biochemistry*, Seventh Edition**

Protein Concentrators

Cleavage of His tag

His tag is not part of the protein. It needs to be removed in order to perform structural and biophysical studies on the protein.

- Thrombin is used to remove the His tag.



Thrombine:

It is a protease, an enzyme that cleaves of the protein at a specific recognition sequence.

When Thrombine is added in the solution it recognizes the cutting site, located just after the His tag and cleaves the his tag off.

Examples of tags and ligands

- His-tag
- FLAG™ peptide
- *Strep*-tag
- GST tag
- Maltose binding protein fusion
- Calmodulin binding protein fusion
- Transition metal ion
- Monoclonal antibody
- Biotin
- Glutathione
- Amylose
- Ca^{2+}

Vector	amp^R	kan^R	T7	T7*lac*	f1 ori	His•Tag	T7•Tag^{11}	T7•Tag^{260}	S•Tag	Trx•Tag	CBD•Tag™	KSI	HSV•Tag	PKA	Dsb•Tag	GST•Tag	protease	signal seq.	LIC available
pET-3a-c	●		●				N												
pET-5a-c	●		●				N												
pET-9a-d		●	●				N												
pET-11a-d	●			●			N												
pET-12a-c	●		●															●	
pET-14b	●		●			N											T		
pET-15b	●			●		N											T		
pET-16b	●			●		N											X		
pET-17b	●		●				N												
pET-17xb	●		●					N											
pET-19b	●			●		N											E		
pET-20b(+)	●		●		●	C												●	
pET-21a-d(+)	●			●	●	C	N												
pET-22b(+)	●			●	●	C												●	
pET-23a-d(+)	●		●		●	C	N												
pET-24a-d(+)		●		●	●	C	N												
pET-25b(+)	●			●	●	C							C					●	
pET-26b(+)		●		●	●	C												●	
pET-27b(+)		●		●	●	C							C					●	
pET-28a-c(+)		●		●	●	N,C	I										T		
pET-29a-c(+)		●		●	●	C			N								T		
pET-30a-c(+)		●		●	●	N,C			I								T,E		
pET-30 Ek/LIC		●		●	●	N,C			I								T,E		●
pET-30 Xa/LIC		●		●	●	N,C			I								T,X		●
pET-31b(+)	●			●	●	C						N							
pET-32a-c(+)	●			●	●	I,C			I	N							T,E		
pET-32 Ek/LIC	●			●	●	I,C			I	N							T,E		●
pET-32 Xa/LIC	●			●	●	I,C			I	N							T,X		●
pET-33b(+)		●		●	●	N,C	I							N			T		
pET-34b(+)		●		●	●	C			I		N						T,E		●
pET-35b(+)		●		●	●	C			I		N						T,X		●
pET-36b(+)		●		●	●	C			I		N						T,E	●	●
pET-37b(+)		●		●	●	C			I		N						T,X	●	●
pET-38b(+)		●		●	●	C			I		C						T	●	
pET-39b(+)		●		●	●	I,C			I						N		T,E	●	
pET-40b(+)		●		●	●	I,C			I						N		T,E	●	
pET-41a-c(+)		●		●	●	I,C			I							N	T,E		
pET-42a-c(+)		●		●	●	I,C			I							N	T,X		
pSCREEN-1b(+)	●		●		●	I		N	I								T,E		

Notes:
T7•Tag^{11} = 11 aa fusion tag T7•Tag^{260} = 260 aa fusion tag signal seq. = signal sequence for potential periplasmic localization
I = internal tag N = N-terminal tag C = optional C-terminal tag
protease cleavage sites: T = thrombin E = enterokinase X = Factor Xa
LIC = ligation independent cloning, vectors available separately as linearized DNA
pSCREEN-1b(+) carries the pUC origin of replication; all other pET vectors carry the pBR322 origin