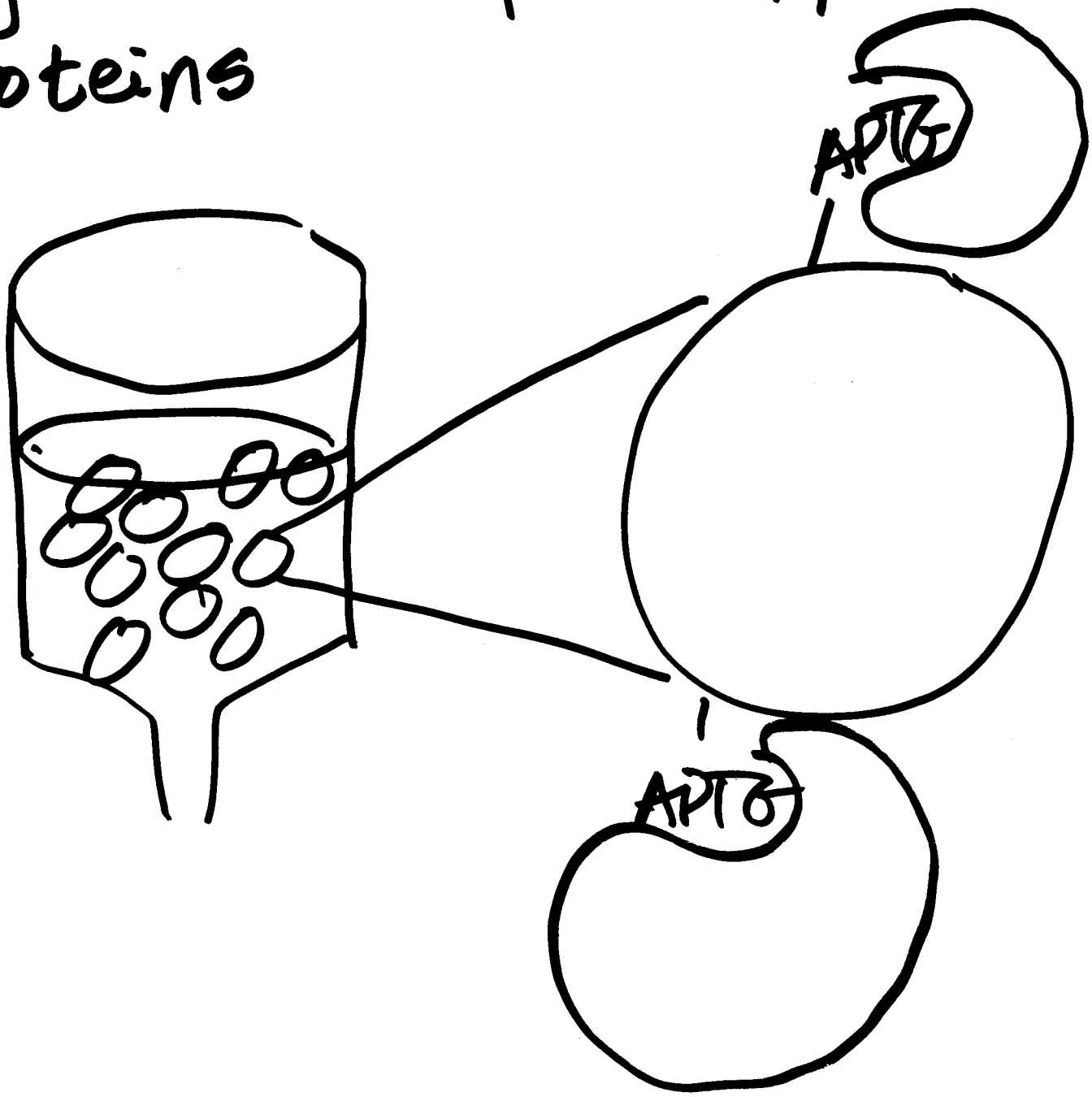
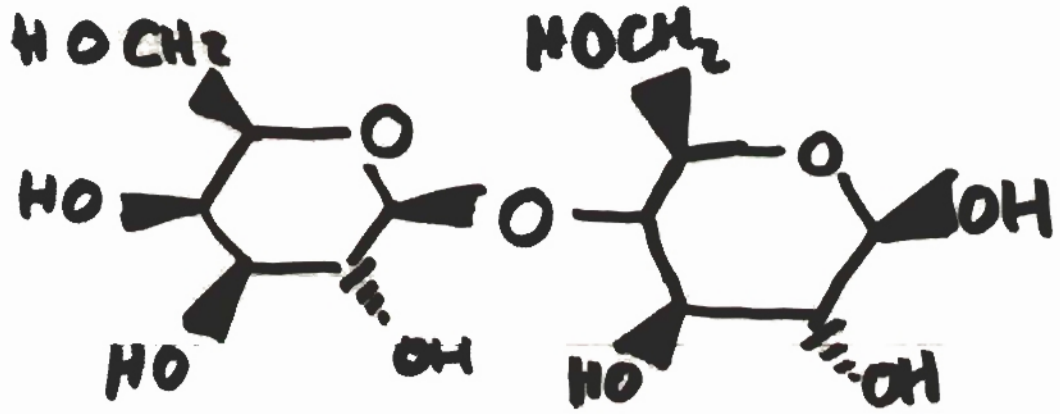
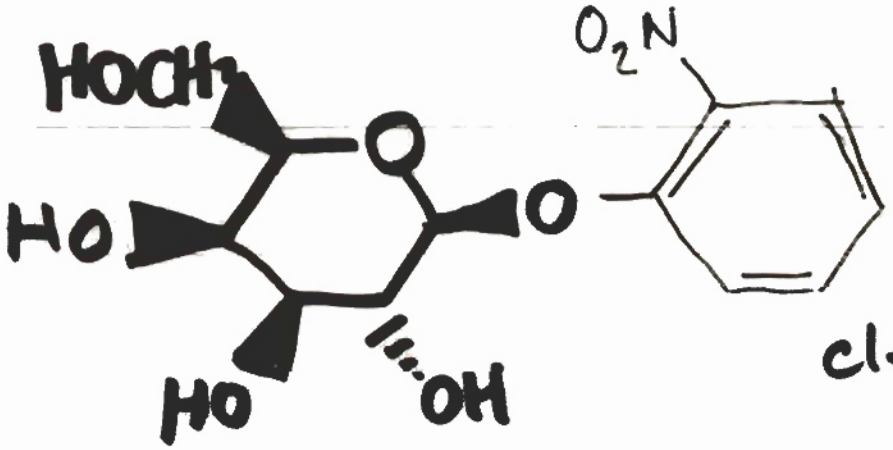
Affinity Chromatography

Use affinity to specific ligands to separate/purify proteins



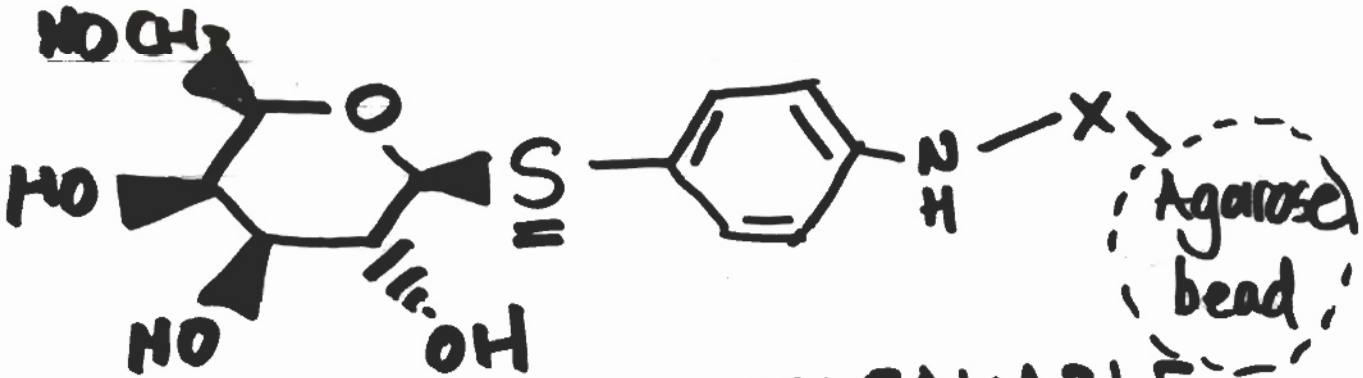


LACTOSE cleavable by β-gal



cleavable

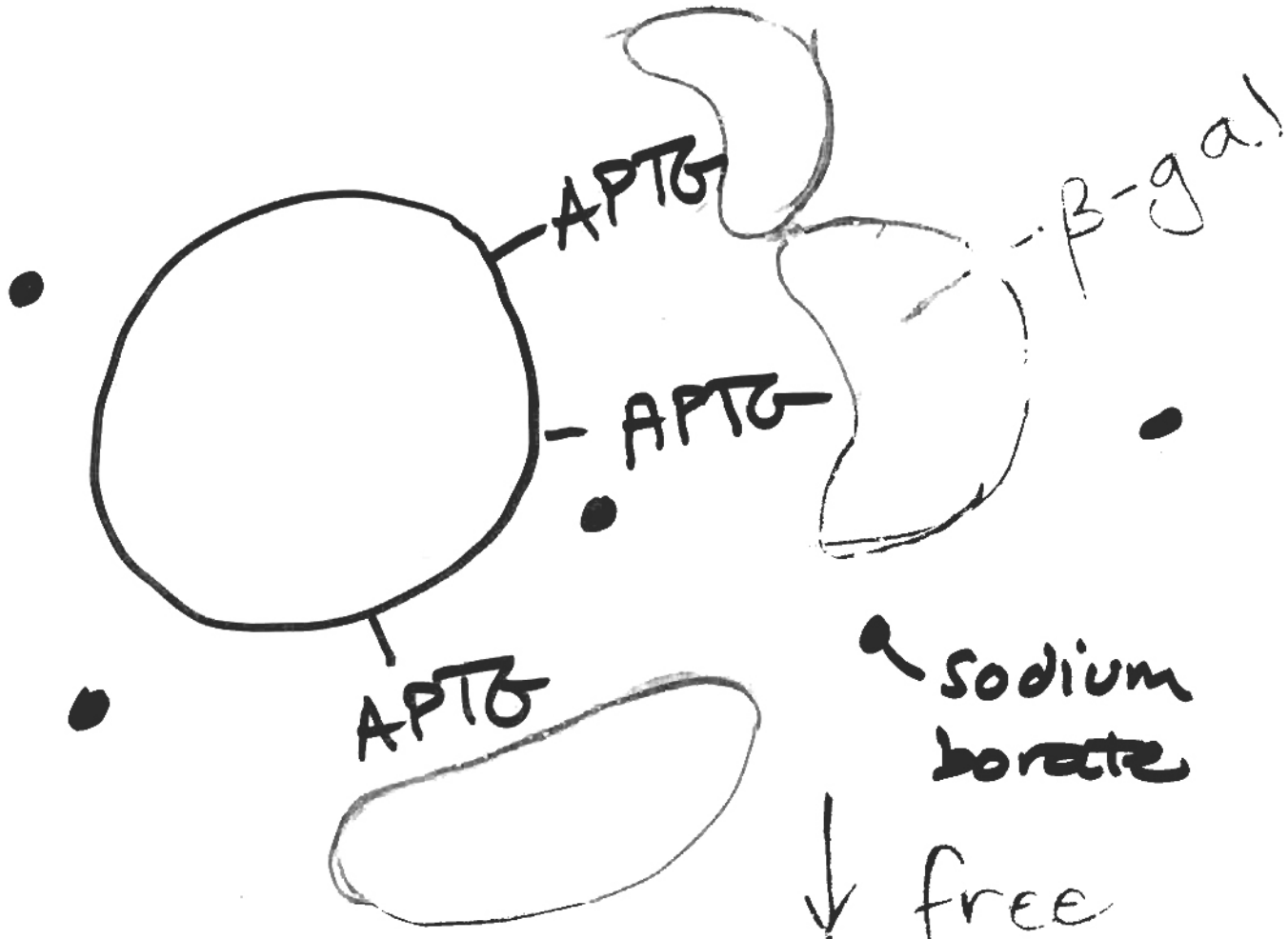
ONPG (2-Nitrophenyl-β-D-galactopyranoside)



Substrate analog - UNCLEAVABLE

4-Aminophenyl-β-D-thiogalactopyranoside (-Agarose)

APTG-agarose



Elution Buffer-
contains sodium borate
pH 10
mildly denaturing

$$\text{Yield} = \frac{\text{Activity after purification step (DEAE or APTG)} \times 100\%}{\text{Activity you started with (crude lysate)}}$$

DEAE ↑ Yield

- higher protein binding capacity
- No sodium borate involved in elution with DEAE

	DEAE column	Affinity column
Advantages	→ Higher yield → Need not know exact ligand/ sequence	→ Purer sample
Dis-advantages	⇒ Not as pure as affinity.	- less yield - Need to know specific ligand

	matrix	separation based on	wash?	Elution Buffer	order of elution
size exclusion	Porous beads	size!	N	COLUMN BUFFER	BIGGEST FIRST
DEAE	positively charged beads.	charge	Y LOW SALT	HIGH SALT	POSITIVE UNTIL NEUTRAL
APTG Affinity	APTG agarose	Affinity to APTG.	Y COLUMN BUFFER	SODIUM BORATE BUFFER	PROTEINS WITH NO AFFINITY TO LIGAND.