BE. 440
27 October 2004
Essigmann

Topic: Chemotaxis.

The Players

NON-MEMBRANE PROTEINS:

CheA: histidine protein kinase, captures signal from receptor and passes it along

CheW: partner of CheA and receptor (scaffold)

CheY: response regulator → carry signal through cytoplasm

CheZ: activate CheY by de-phosphorylation

CheR: methyl transferase, attenuate the passage of signal through regulator (adaptations)

CheB: methyl esterase/amidase activated by CheA-Ⓟ, demethylates receptor and makes receptor more sensitive to signals

MEMBRANE PROTEINS:

(MCPs: methyl-accepting chemotaxis proteins; ligand receptors)

Tsr $\left(2600 \frac{mol}{cell}\right)$ → serine receptor

Tar $\left(600 \frac{mol}{cell}\right)$ → Asp (D), Glu (E), Maltose*

Trg → ribose*, galactose*, glucose

Tap → dipeptides*

Air → O_2

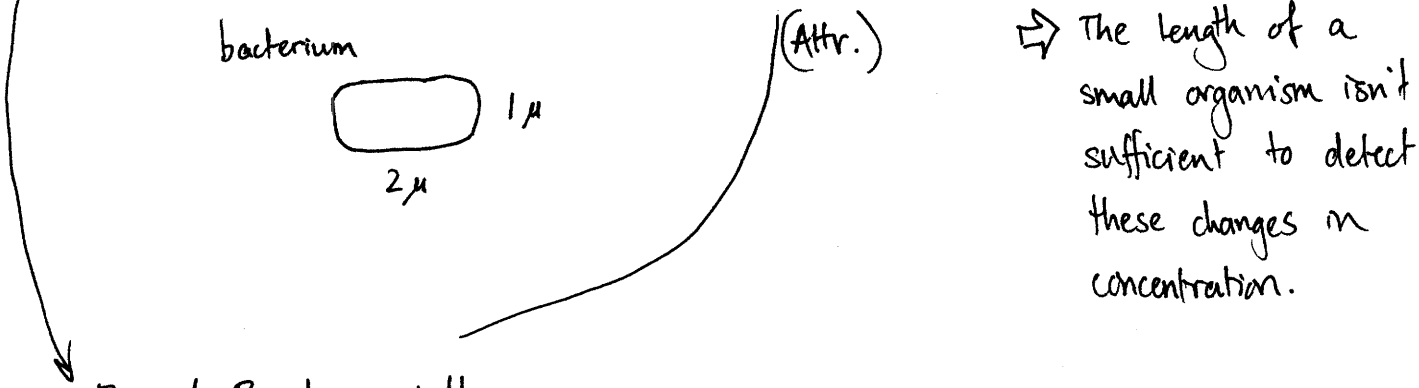
* = there is a partner protein

* CheZ: takes CheY-Ⓟ → CheY, causes straight (as opposed to tumbling) movement

Chemotaxis: A behavioral response involving the movement of an organism toward an attractant or away from a repellant.

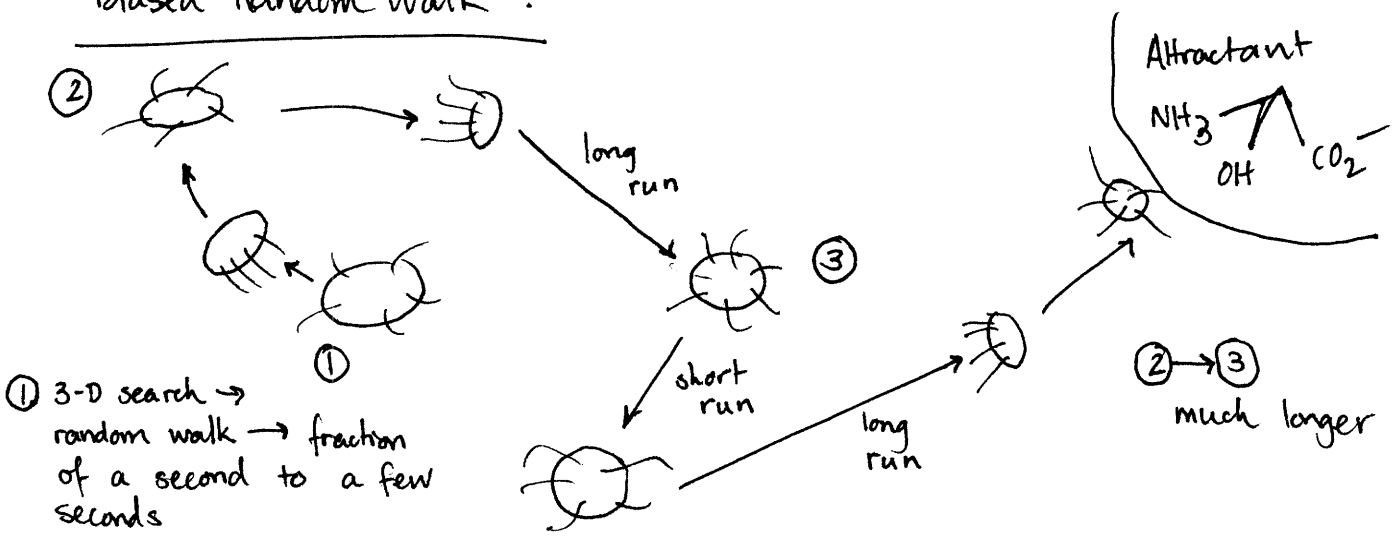
1. The Big Picture — look at core biochemical pathways to understand the chemotaxis ligands
2. Ligands $\rightleftharpoons$ trans. memb. receptors
3. Signal transduction at cytoplasm small molecule effector interface
4. How signal travels through the cytoplasm.
5. How signal affects a motor
6. Motor mechanics

Strategy nature uses for high sensitivity in high-gain systems



⇨ The length of a small organism isn't sufficient to detect these changes in concentration.

Biased Random Walk:



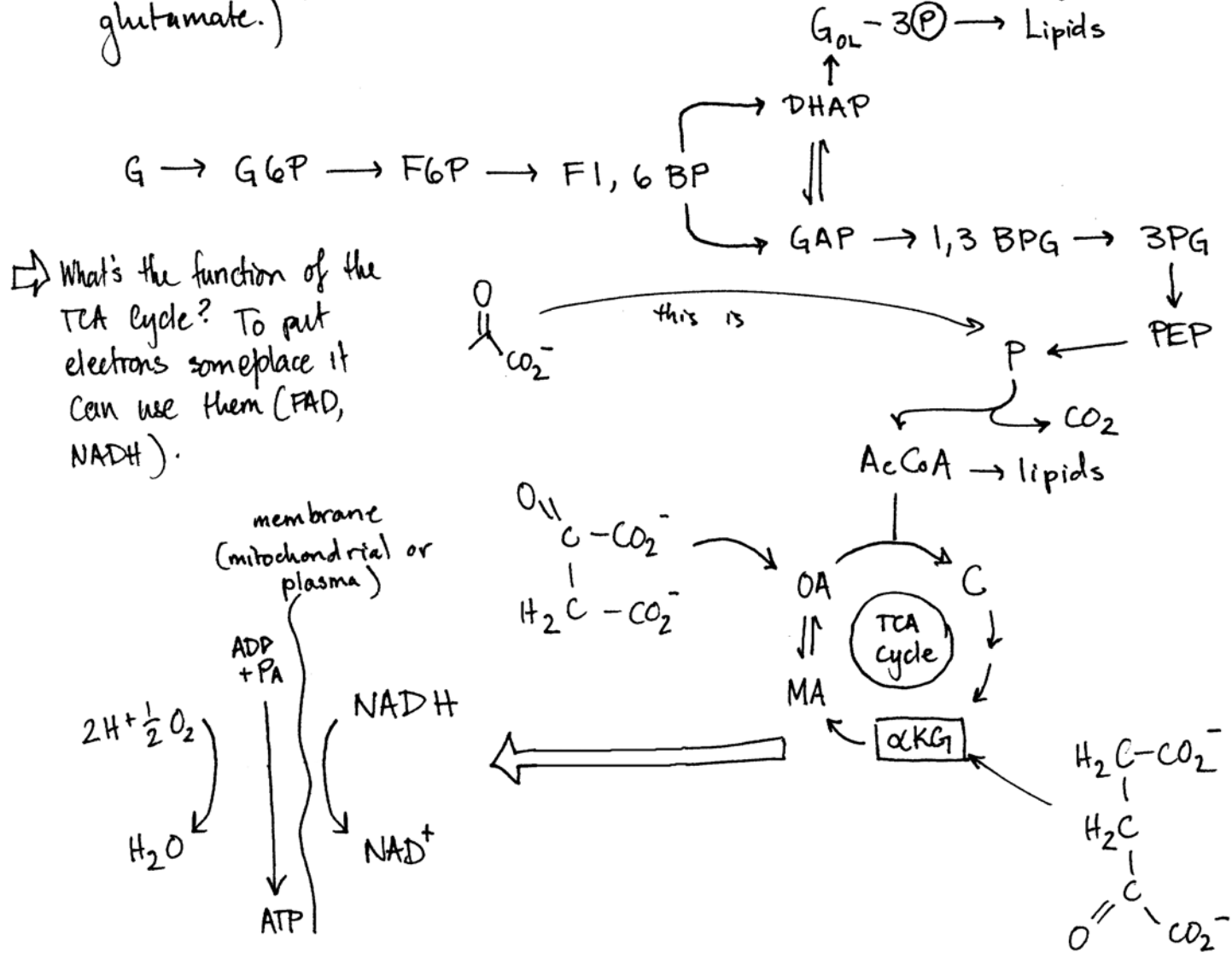
① 3-D search → random walk → fraction of a second to a few seconds

Targets:
- serine
- Asp
- Glu
- maltose
- Gal
- Glc (G)
- dipeptides
- O_2
- ribose

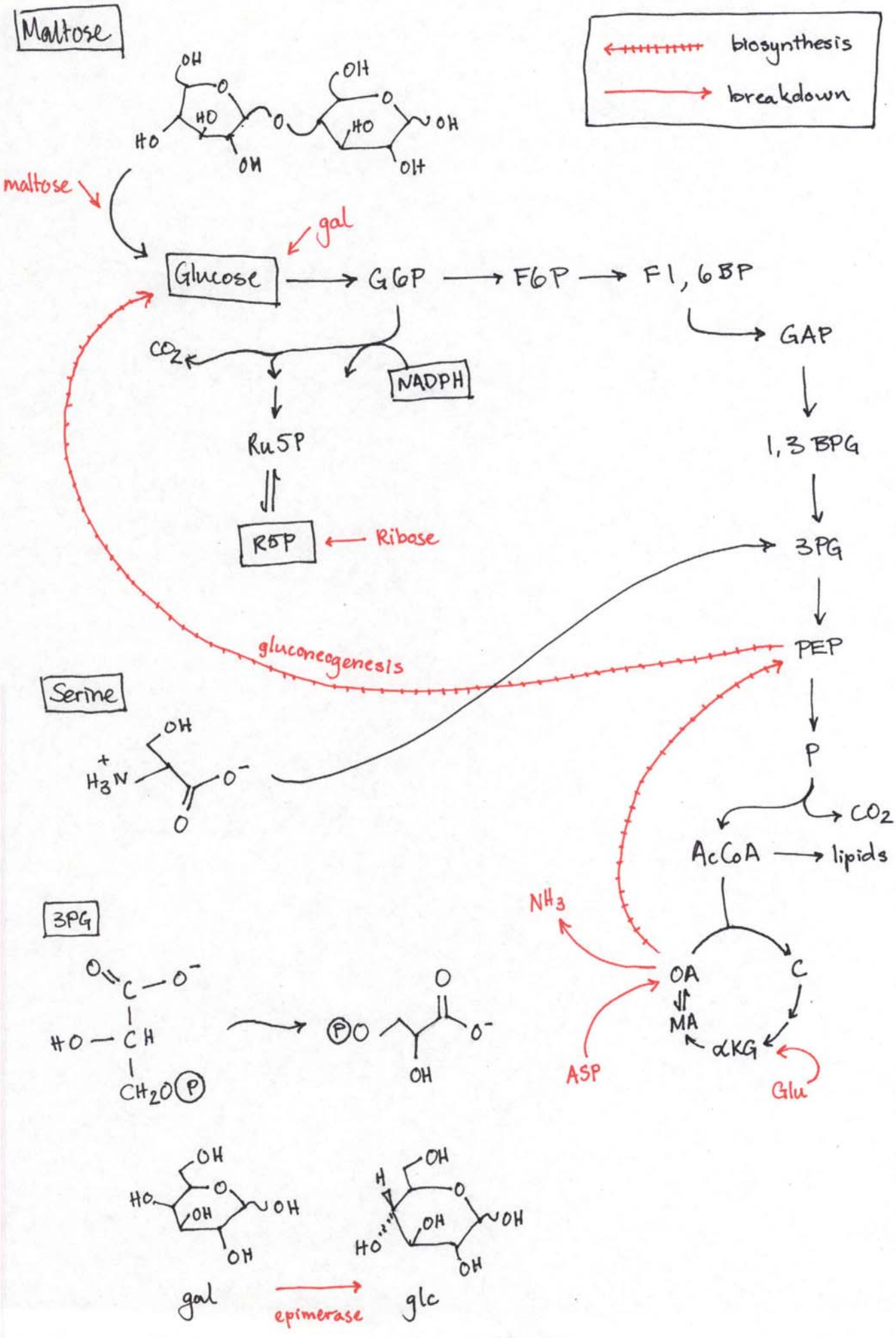
How the chemotactic targets get put into metabolic pathways ... All of them are 0 to 1 step away from a "core" pathway.

3PG = 3 phosphoglycerate

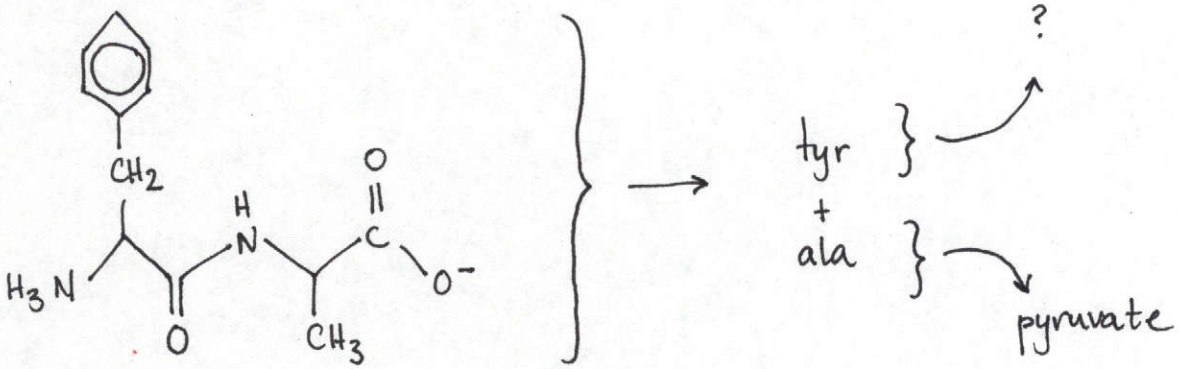
(Why glutamate? The nitrogen in amino acids comes from the air → nitrogen fixation → ammonia all gets into body via glutamate.)



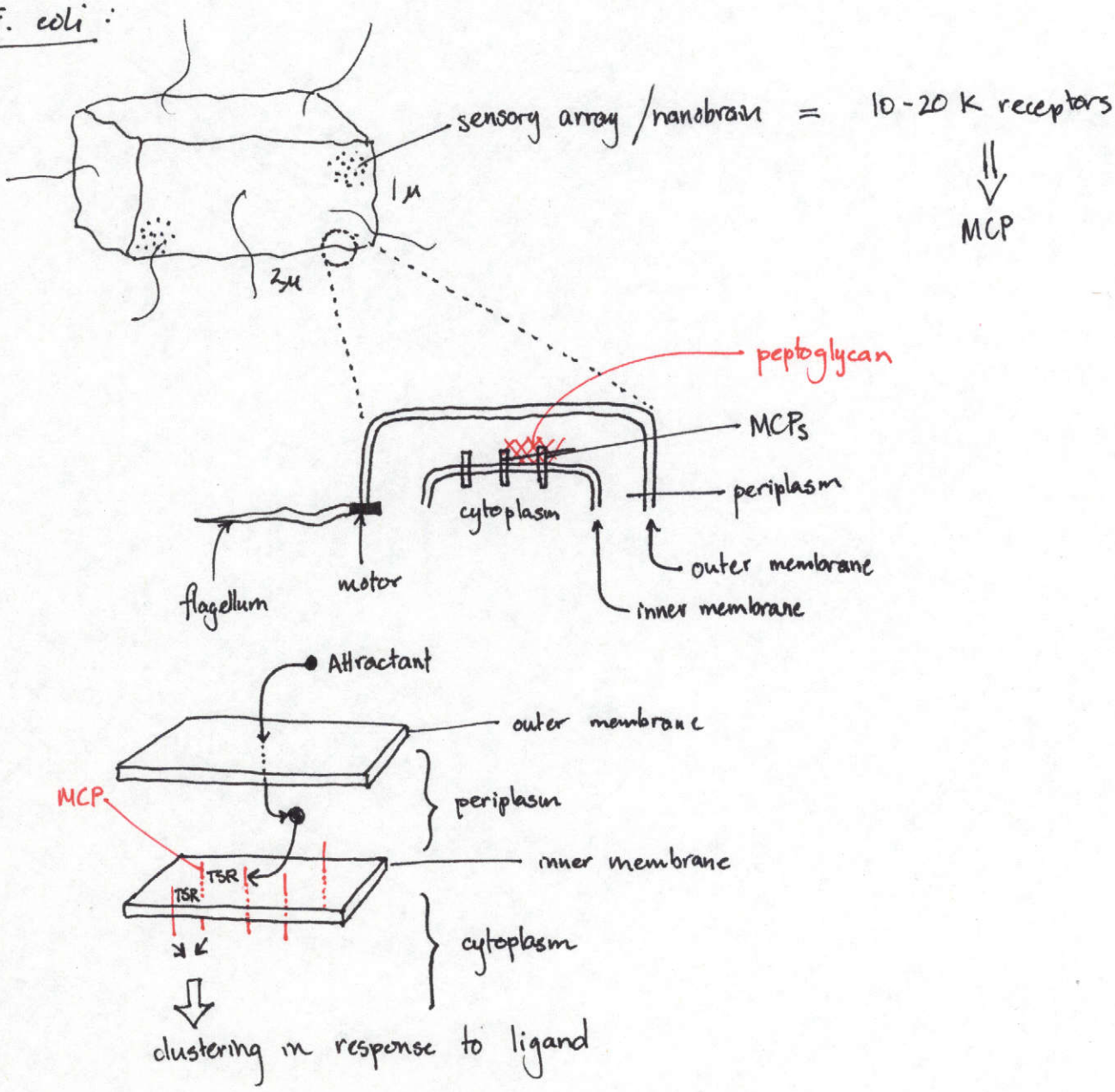
What's the function of the TCA cycle? To put electrons someplace it can use them (FAD, NADH).


Maltose
biosynthesis
breakdown
maltose
gal
Glucose
G6P
F6P
F1,6BP
GAP
CO2
NADPH
Ru5P
1,3BPG
R5P
Ribose
3PG
gluconeogenesis
PEP
Serine
P
CO2
AcCoA
lipids
NH3
3PG
OA
C
MA
αKG
ASP
Glu
gal
epimerase
glc

dipeptides

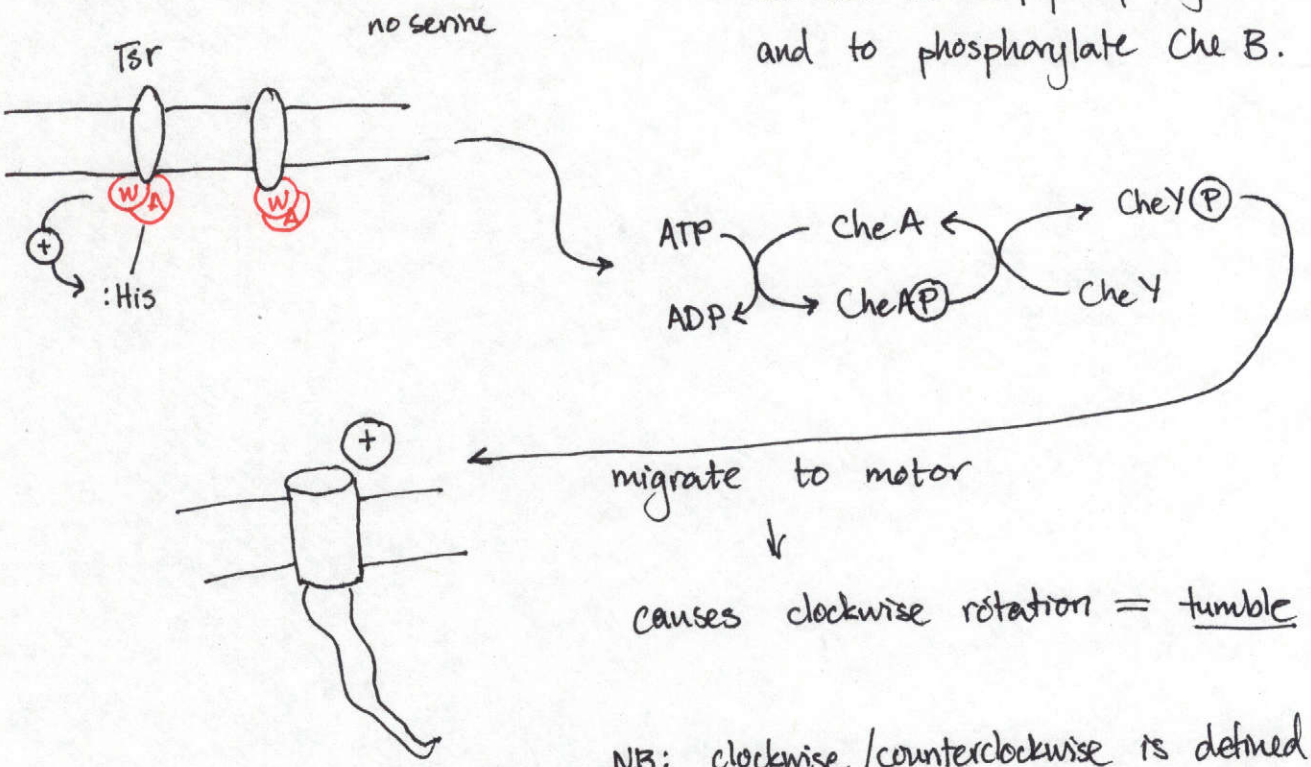


E. coli :

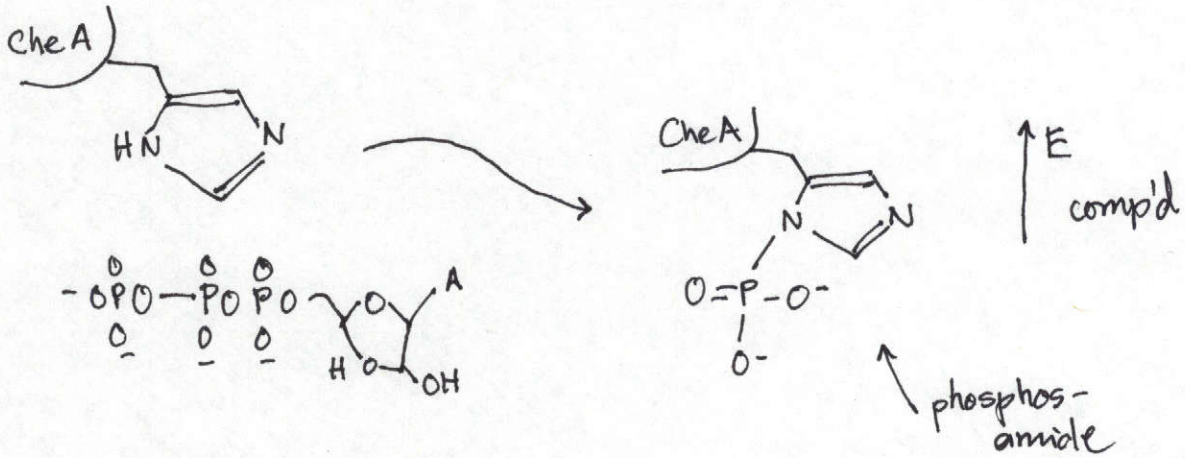


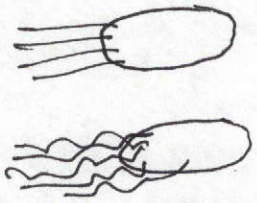
Case 1: No Attractant

⇨ Without a signal, CheA is activated to self-phosphorylate and to phosphorylate Che B.

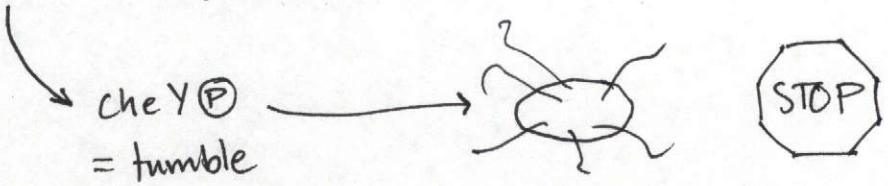


NB: clockwise/counterclockwise is defined as looking from flagellum to bacterium, i.e.

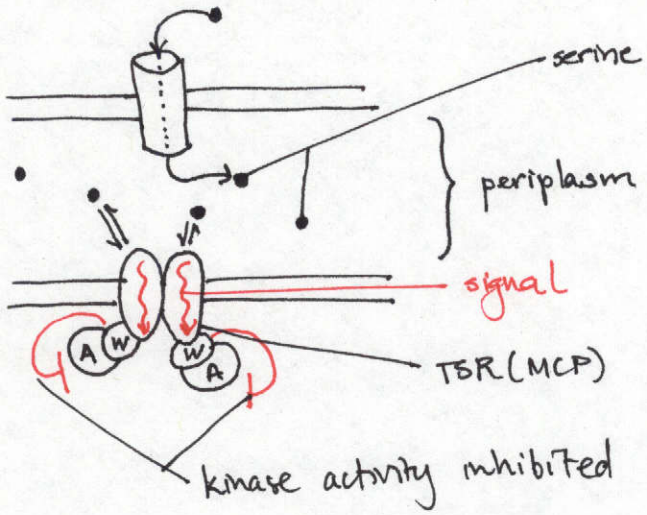




swimming in a run, all going CCW



Case 2: Sensing an Attractant / Repellant

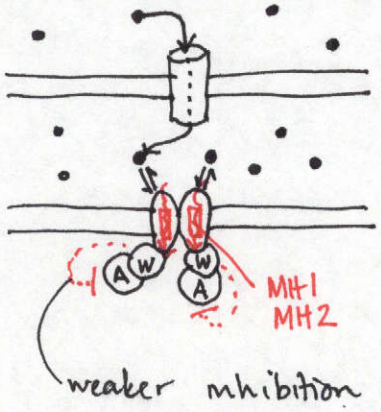


1. When A ⨯ AP, you cannot make Y–P.
2. Che Y ~P $t_{1/2}$ 0.1–0.2 sec
   Reason: Che Y–P $\xrightarrow[\text{CheZ}]{H_2O\ P}$ Che Y
3. Lack of Che Y P ⟶ no interaction w/ flagellum motor
4. Motor reverts to default CCW spinning state = Run.

⇨ Bacterium will go in longer runs as it moves up the concentration gradient. Direction is completely random, runs are just longer in the direction of the attractant.

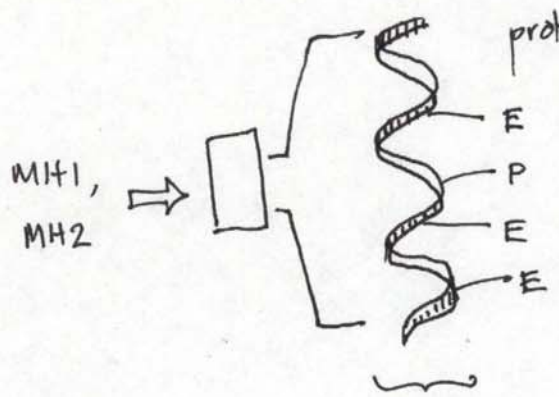
⇨ The number of MCPs in a cell indicate how desireable that receptor's target is to the cell. More receptors, more desireable. This is how bacteria make "decisions" between different attractors.

Case 3: Sustained Stimulus



1. Lots of attractant (signal) causes methylation of MH1 and MH2.
2. Methylation dampens signal.
3. Weaker inhibition of A HPK (histidine protein kinase) activity

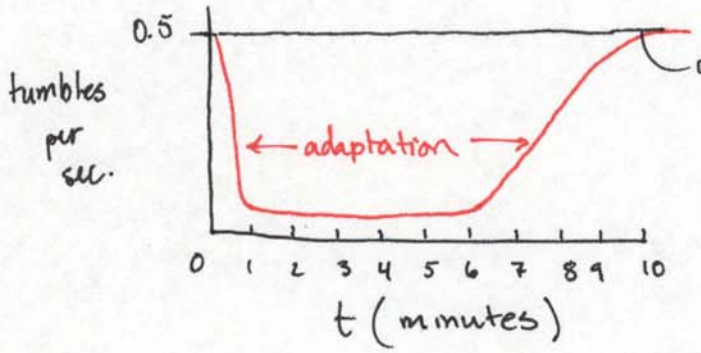
→ AP ⟶ Y P → flag. motor → CW rotation → tumble



4-6 methylation sites ⟶ P or E residues

Aron & Liebler (1999):

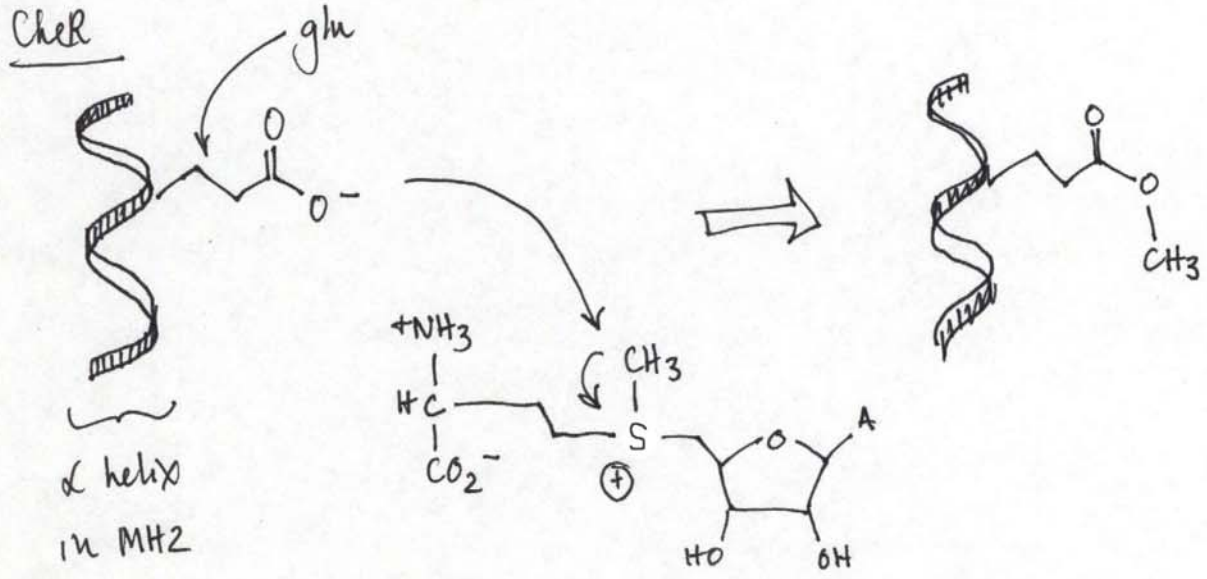
E. coli ± 1 mM Asp (D)



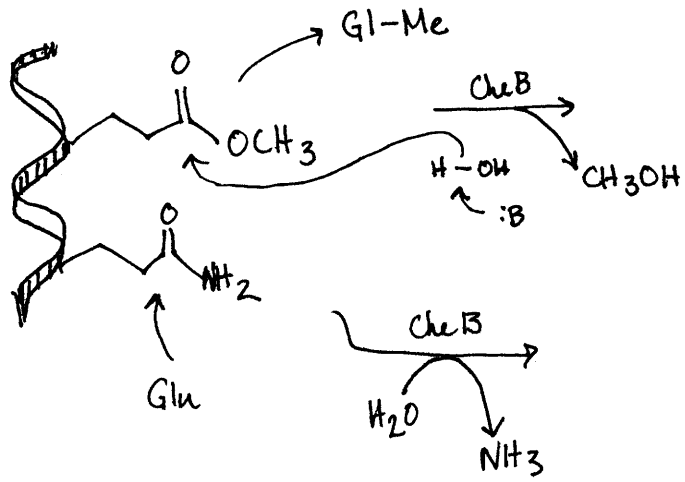
Exact Adaptation: system resetting itself = 6 minutes for system to adapt & turn off

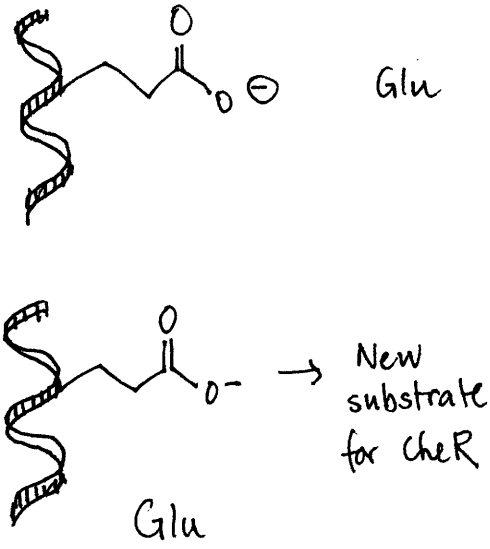
⇨ During period of adaptation, CheR (methyl transferase) is working. CheR makes methyl ester of MH2.

CheR



CheB undoes what was done by CheR; also deaminates glutamines



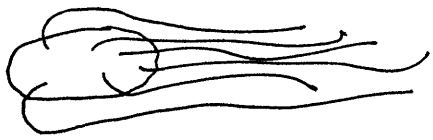


⇨ Keep in mind that there's a repellant system working in parallel with this...

Bacterial Flagellar Motor.

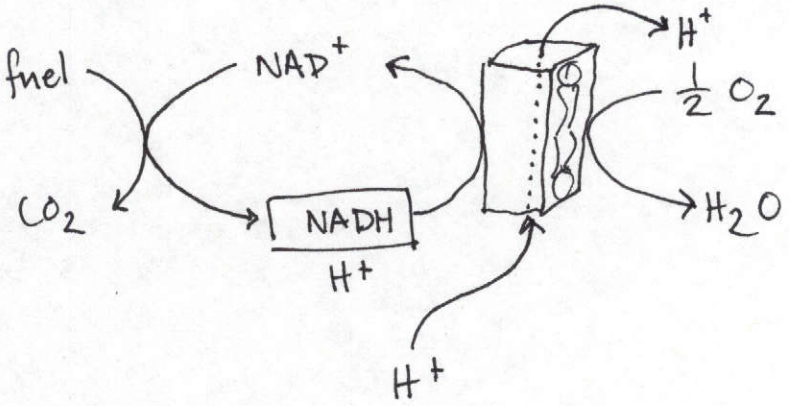
- chemical energy from metabolism ⟶ make something spin
- electrostatic interactions
- create gradient of protons; release of gradient drives motor

N.B.: flagella don't actually move around through the membrane:



- CheY(P) effects vast conformational changes in the protein

⇨ How do you generate a proton gradient?



ΔG (free energy) can be used to generate H^+ gradient

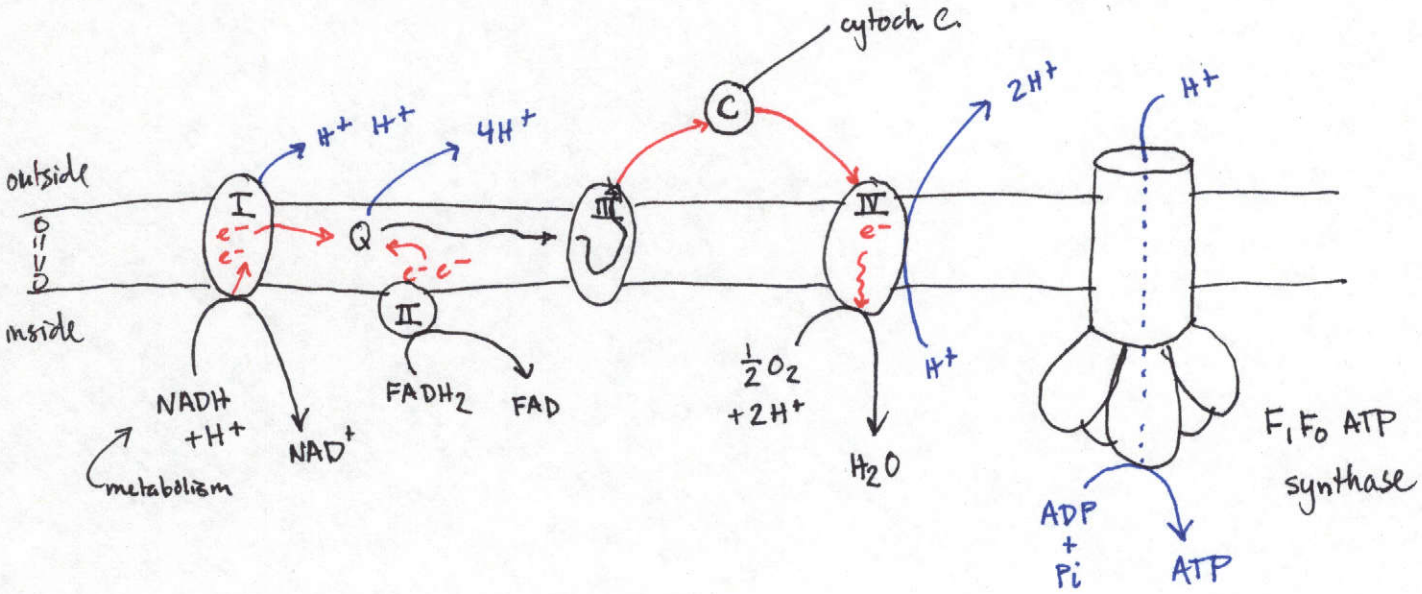
⇨ What is H^+ gradient used for?

1. $ADP + P_i \rightleftharpoons ATP$

$$\Delta G = 34 \frac{KJ}{mol}$$

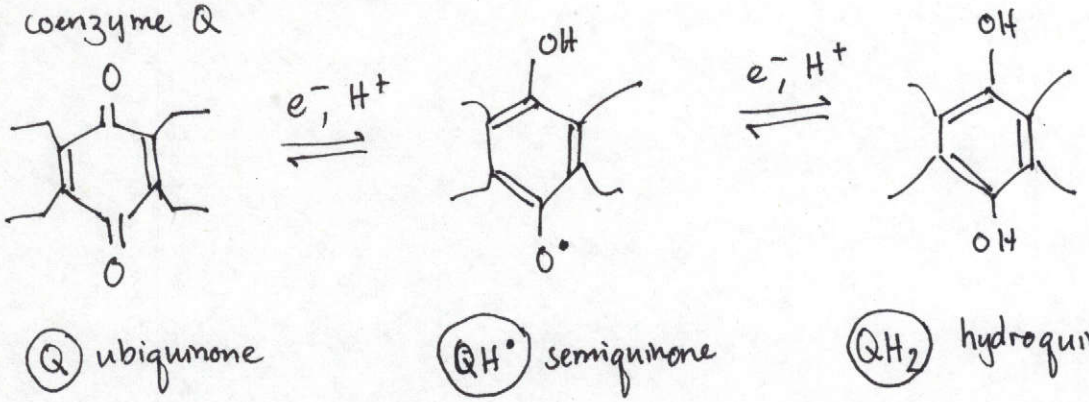
2. flagellar rotation

3. ion transporters

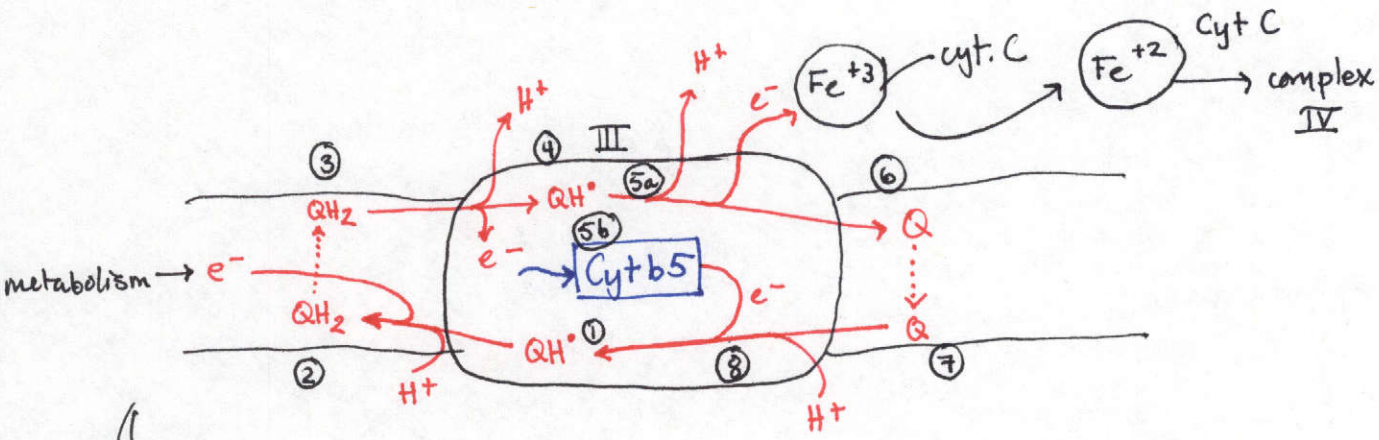


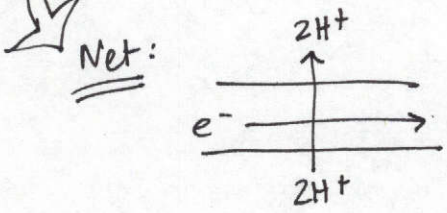
⇨ For each NADH oxidized, you transfer 8 to 10 H^+s.

⇨ How do pumps work?



(Q) ubiquinone (QH•) semiquinone (QH_2) hydroquinone





- One e^- flows across membrane
- Two protons get pumped
- e^- loses energy in the process

Second Pump Model: bacteriorhodopsin

$Fe^{3+} + e^- \rightarrow Fe^{2+}$ + conformational changes in protein complex