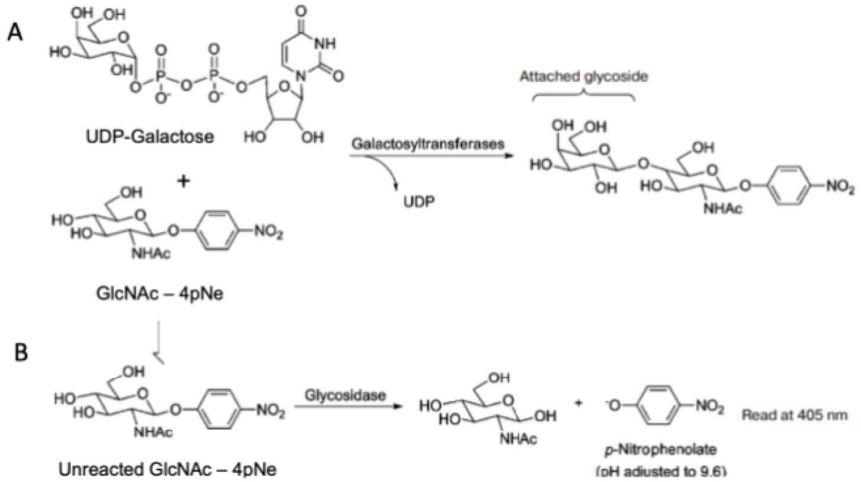
Mock Exam 2023

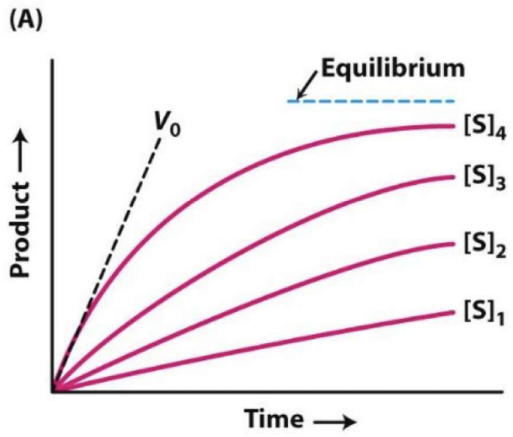
Question 1 a)

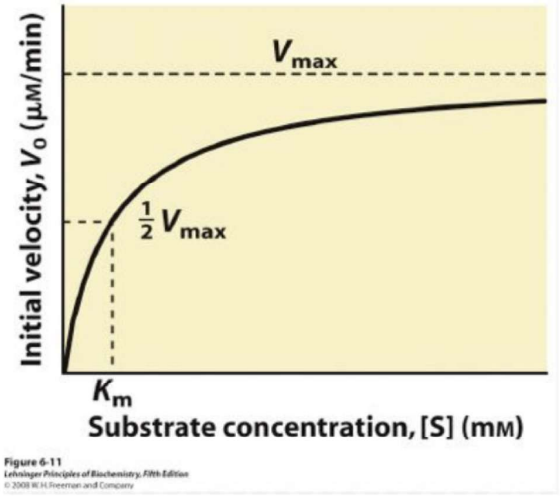
Galactosyltransferases are a class of enzymes which adds galactose to a sugar acceptor. You want to measure the activity, Km, and Vmax of the galactosyltransferase GalA, which uses UDP-Galactose as a substrate, and adds a galactose onto N-acetyl-glucosamine (GlcNAc). This reaction can be quenched by adding an excess of the enzyme exoglycosidase.

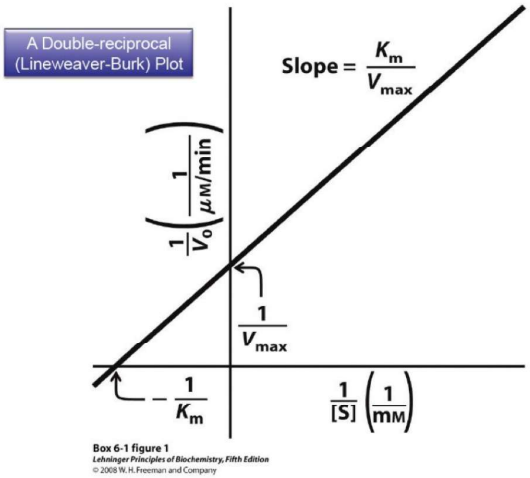
a) GlcNAc can be conjugated to the chemical group 4-nitrophenylene and 4-nitrophenylene conjugated MONO SUGARS are cleaved by glycosidase enzymes, which gives rise to an absorbance at 405 nm. Design an experiment for measuring the Km and Vmax of the galactosyltransferase GalA. Assume reaction time of both enzymes to be 30 min and we initially added 0.1 umol of the substrate GlcNAc-4pNe.



Michaelis-Menten and Lineweaver-Burk plots







Answer 1a)

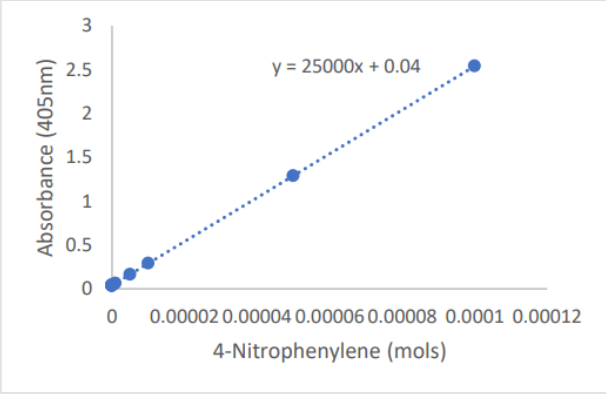
- Prepare a plate reader with a set amount of GalA and GlcNAc-PHe in each well
- Add varying amounts of UDP-Galactose
- Incubate for a set amount of time
- Add an excessive amount of exoglycosidase
- Incubate for a set amount of time
- Measure the absorbance at 405 nm which corresponds to free unreacted GlcNac-Phen being cleaved
- Plot the absorption at 405 nm vs UDP galactose
- Convert absorption at 405 in Gal-GlcNac [ ]
- Spot plateau and determine Vmax and half max, half max =km because of the excess of substrate (use double reciprocal plot and interpolation)

Question 1b

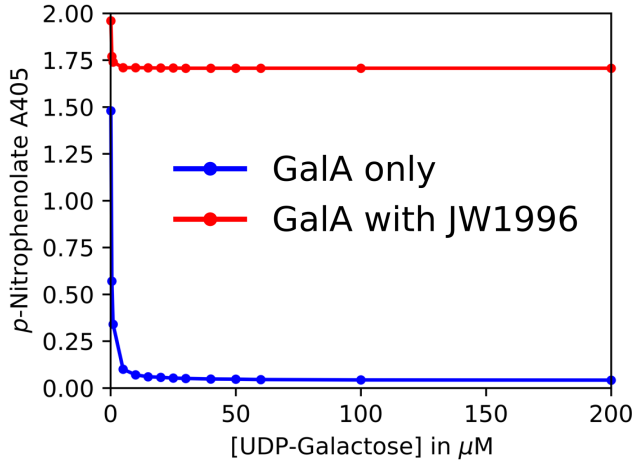
You are currently screening for potential drug candidates which inhibits the activity of GalA as it is an important protein in the virulence of a pathogenic bacteria. You identified a potential candidate JW1996. The kinetics data of GalA with and without JW1996 is shown below. What is the K_d, V_{max} of each respective case? Which type of competitor is JW1996? Assume reaction time of both enzymes to be 30 mins and we initially added 0.1 umol of the substrate GlcNAc-4pNe.

Concentration of UDP-Galactose	Abs 405 (GalA only)	Abs 405 (GalA with JW1996)
0.1 uM	1.48	1.96
0.5 uM	0.57	1.77
1 uM	0.34	1.74
5 uM	0.10	1.71
10 uM	0.07	1.71
15 uM	0.06	1.709
20 uM	0.057	1.708
25 uM	0.053	1.708
30 uM	0.051	1.707
40 uM	0.048	1.707
50 uM	0.047	1.707
60 uM	0.045	1.707
100 uM	0.043	1.707
200 uM	0.042	1.707

Given in the diagram is a standard curve of 4-nitrophenylene absorbance as a function of concentration:



A



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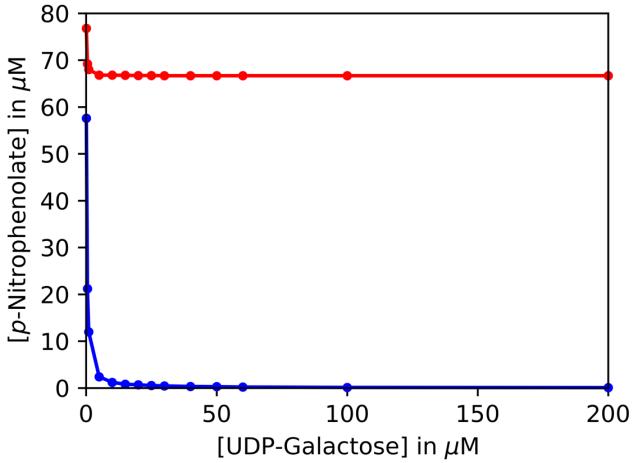

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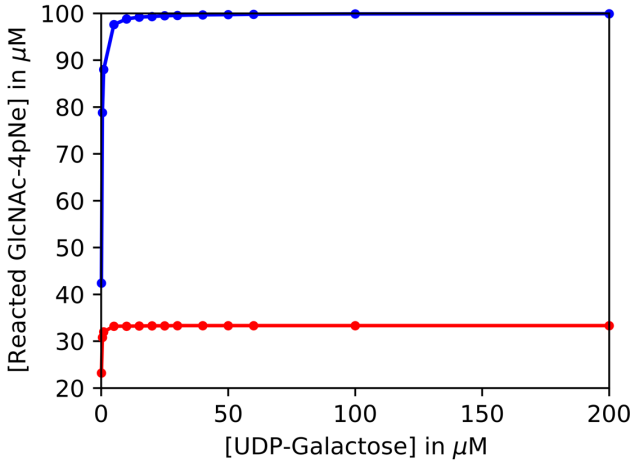

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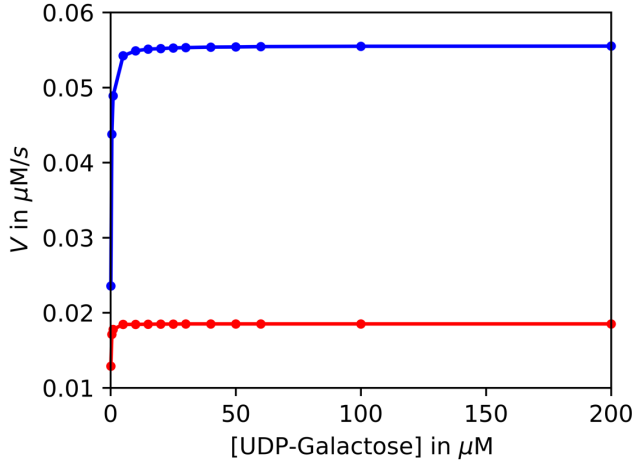
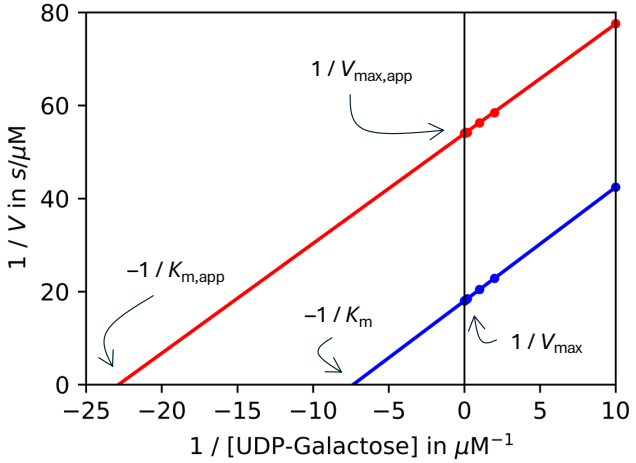


E



GalA only:

- $V_{max} \simeq 0.0556$ uM/s
- $K_m \simeq 0.136$ uM

GalA with JW1996:

- $V_{max,app} \simeq 0.0185$ uM/s
- $K_{m,app} \simeq 0.044$ uM

$\alpha' = V_{max} / V_{max,app} \simeq 3.01 > 1$

$\alpha = \alpha' K_{m,app} / K_m \simeq 0.97 \simeq 1$

⇒ Uncompetitive inhibition

A. First, we plot the raw *p*-Nitrophenolate absorbance at 405 nm (A405) vs the substrate concentration [UDP-galactose] (in uM).

B. Then, we convert the *p*-Nitrophenolate A405 to a concentration (in uM) and plot it vs [UDP-galactose] (in uM). For the conversion, we use the equation relating the A405 to the concentration of *p*-Nitrophenolate. Since each molecule of unreacted GlcNAc-4pNe yields one molecule of *p*-Nitrophenolate, the concentration of *p*-Nitrophenolate also corresponds to the concentration of unreacted GlcNAc-4pNe. We see that, as [UDP-galactose] increases, the concentration of *p*-Nitrophenolate decreases. This is because, as [UDP-galactose] increases, the first reaction gets faster (the galactosyl-transferase gets more efficient) and less GlcNAc-4pNe will remain after the first reaction. There will thus be less substrate for the second reaction, so less product (*p*-Nitrophenolate), which is exactly what we measure.

C. We can then calculate the amount of GlcNAc-4pNe used in the first reaction by subtracting the concentration of *p*-Nitrophenolate from the initial amount of GlcNAc-4pNe (0.1 mmol). This is justified because all the GlcNAc-4pNe that is not used in the first reaction (by the galactosyltransferase) will be degraded to GlcNAc + *p*-Nitrophenolate in the second reaction. The initial amount of GlcNAc thus corresponds to the amount of GlcNAc used in the first reaction (which we want to determine) + the unreacted GlcNAc (which we know from B). We then plot the amount of GlcNAc-4pNe used in the first reaction (in uM) vs [UDP-galactose] (in uM). Note that the term corresponding to the unreacted GlcNAc (calculated in B) is a concentration, while the initial amount of GlcNAc is an amount of substance (mol), so we can technically not subtract one from the other. However, since no volume was given in this exercise, we consider that amount and concentration are equivalent (we take a volume of 1 L, such that 0.1 mmol = 0.1 mM).

D. Recalling that the rate of a reaction is the concentration of product generated in a given time, or equivalently of substrate used in a given time, we can calculate the reaction rate of the first reaction (catalysed by the galactosyltransferase). For this, we divide the concentration of GlcNAc-4pNe used in the first reaction by the reaction time t (30 min = 1800 s). We then plot the reaction rate (in uM/s) vs [UDP-galactose] (in uM). This corresponds to a classical Michaelis-Menten plot (V vs [S]). During the lectures, you have derived all formulae for the *initial* reaction rate V_0. In all exercises (including this one), we consider this assumption to be verified, such that we use the terms "reaction rate (V)" and "initial reaction rate (V_0)" interchangeably.

E. The Michaelis-Menten plot shown in D can be used to determine the maximal reaction rate V_{max}, but it is very difficult to determine the Michaelis constant K_m from this plot. Therefore, we linearise the data (Lineweaver-Burk: 1 / V vs 1 / [S]) and use a linear regression to determine more precisely the kinetic parameters. Graphically, the x-intercept corresponds to –1 / K_m (or –1 / $K_{m,app}$) and the y-intercept corresponds to 1 / V_{max} (or 1 / $V_{max,app}$).

Analysis:

Graphically, we can see that the two regression lines on the Lineweaver-Burk plot are parallel, suggesting that the inhibitor JW1996 is an uncompetitive inhibitor. We can verify this by recalling that, for any inhibitor (competitive, uncompetitive, mixed, non-competitive), the following relations hold:

$$V_{max,app} = V_{max} / \alpha$$

$$K_{m,app} = \alpha K_m / \alpha'$$

where α and α' are larger or equal to 1. Since V_{max}, K_m, $V_{max,app}$, and $K_{m,app}$ can be determined graphically by looking at the x- and y-intercepts of the Lineweaver-Burk plot, we can easily determine the values of α and α'. We find that $\alpha \simeq 1$ and $\alpha' \simeq 3 \neq 1$, which is characteristic of an **uncompetitive inhibitor**.

NB: The data begin at a concentration of UDP-galactose of 0.1 uM. You could in fact have added a point for [UDP-galactose] = 0 uM. Indeed, you know that the initial concentration of GlcNAc-4pNe is 0.1 mM. If there was no UDP-galactose, the first reaction would not proceed, such that there would remain 0.1 mM of unreacted GlcNAc-4pNe which would then be degraded during the second reaction, yielding 0.1 mM of *p*-Nitrophenolate. This holds for both the reaction with and without JW1996. From the equation relating the A405 to [*p*-Nitrophenolate], you can calculate the A405 corresponding to a concentration of 0.1 mM:

$$\text{A405} = 25000 \cdot [p\text{-Nitrophenolate}] + 0.04 \quad \Rightarrow \quad \text{A405} = 25000 \cdot (10^{-4}\ \text{M}) + 0.04 = 2.54$$

You could thus have added the point (A405 = 2.54, [UDP-galacatose] = 0 uM) to the data. You should nonetheless have discarded it for the linearisation step (because 1 / *V* and 1 / [S] would have yielded divisions by zero), such that it wouldn't have affected the estimation of the kinetic parameters.

Question 2.c

Glycolysis and Oxidative phosphorylation are conserved metabolic pathways that allow living organisms to convert reduced carbon sources into energy. Most organisms ranging from bacteria to mammals use glycolysis and/ or oxidative phosphorylation to sustain their energetic needs.

(c) Escherichia coli is an example of a facultative anaerobe. Describe what the fate of glucose is when fed to Escherichia coli in a CO_2 chamber.

Lack of O_2 to use as final electron acceptor, can not use oxidative phosphorylation to make ATP, use glycolysis /fermentation instead to generate ATP

Question 2.d

(d) Calculate the energy yield (in terms of ATP molecules) of 5 molecules of glucose fed to Escherichia coli in the presence or absence of O2**.** Please depict the individual steps of the calculation (Assume four H+ ions must flow through ATP synthase to power the synthesis of one ATP molecule).

Presence - 30 ATP are generated per glucose molecule through aerobic respiration when 4x H+ ions flow through ATP synthase per ATP molecule.

Glycolysis = 2 ATP + 2 NADH + 2 pyruvate

Pyruvate =

Decarboxylation = 1 NADH

TCA = 3 NADH and 1x FADH2

NADH pumps 10 H+ across membrane

FADH2 pumps 6 H+ across membrane

4 NADH * 2 pyruvate = 8 NADH + 2 from glycolysis = 10 NADH

10 NADH * 10 H+ = 100 H+ / 4 per ATP = 25 ATP

1 FADH2 * 2 pyruvate = 2 FADH2

2 FADH2 * 6 H+ = 12 H+ / 4 per ATP = 3 ATP

25 ATP from NADH + 3 ATP from FADH2 + 2 ATP from glycolysis = 30 ATP per molecule of glucose. 30 *5 (molecules of glucose) = **150 ATP total**

Absence - Glycolysis **–** 2 ATP (4 produced , but 2 used)**.** So yield = 2 ATP**.**

2 * 5 (molecules) = **10 ATP total**

Question 2.e

(e) Uncoupling agents negatively affect oxidative phosphorylation. Some of these serve a specific biological purpose. The uncoupling agent thermogenin (a.k.a. UCP1) is a proton ion channel protein present in brown adipose tissue mitochondria. Thermogenin allows the passage of protons from the intermembrane space into the matrix disrupting the flow of protons. What is the effect of the action of Thermogenin and what is its physiological role?

Pumps protons back into matrix instead of inhibiting complexes from pumping protons to intermembrane space. Generates heat as more metabolic activity is needed to sustain ATP production, therefore more energy is dissipated as heat when proteins are uncoupled. Useful in situations such as hibernation or newborns that need more body heat.

Question 2.f

(f) Athletes try various approaches to try to maximize their physical performance potential. Some athletes train at high altitudes to increase their red blood cell levels, which naturally happens in response to the lower oxygen levels at high altitudes. What potential benefit would altitude training have on an athlete's performance, and why? (2pt)

high altitude training –> increases red blood cells -> increases capacity to carry oxygen (VO2 max), increases physical output capacity before anaerobic respiration kicks in.

(g) “Carb loading” is a nutritional strategy used by runners, where they eat a lot of carbohydrates for several days before a marathon and rest. Please describe the metabolic process leading to the potential benefits behind this concept? (2pt)

Days leading up to run -> ingest extra carbs

These carbs maximize the glycogen stores in the body (muscle and liver cells)

Rest to not use up glycogen stores.

These glycogen stores will then be full for endurance exercises/running to give cells a source of glucose to produce ATP whilst running.

Question 3

Inborn errors of sphingolipid catabolism lead to lysosomal storage diseases which are characterized by the toxic accumulation of metabolic intermediates in the lysosomes. Substrates reduction therapies are based on the idea that reducing sphingolipid production leads to decreased lysosomal accumulation and thus to mitigation of pathology. Gaucher, Fabry and Nieman-Pick Type A/B diseases are characterized by the accumulation of glucosylceramide (GlcCer), globotriaosylceramide (Gb3), and sphingomyelin (SM) respectively.

Question 3 a

You are asked to identify targets for inhibitors to be used in substrate reduction therapies for these diseases. Which are the factors that you might target for each of the cases? Articulate your answer.

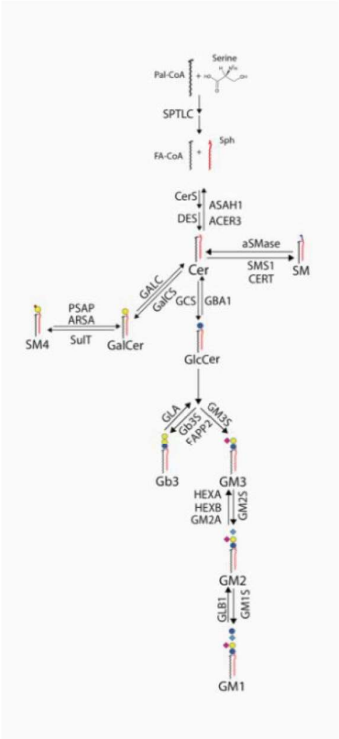


Figure 1: Schematic representation of sphingolipid metabolism

Question 3 a

Gaucher, Fabry and Nieman-Pick Type A/B diseases are characterized by the accumulation of glucosylceramide (GlcCer), globotriaosylceramide (Gb3), and sphingomyelin (SM) respectively.

Gaucher: GCS.

Nieman Pick: CERT/SMS.

Fabry: Gb3S, FAPP2

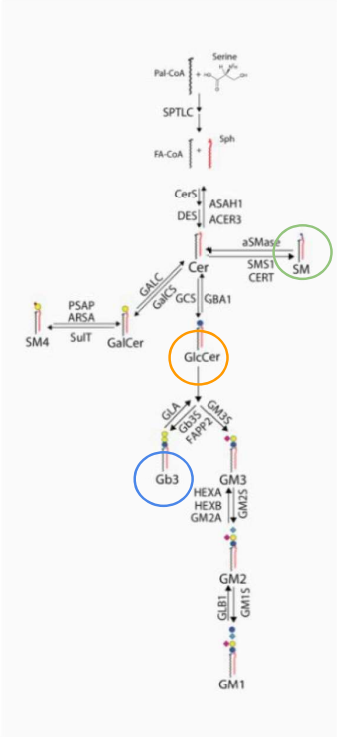


Figure 1: Schematic representation of sphingolipid metabolism

Question 3b

Sphingolipid synthesis occurs in different cellular compartments, however sphingolipid movement through the aqueous cytosol is unfavorable and lipid-transfer proteins are required to facilitate this process which are regulated by phosphoinositides. Explain the role of the lipid-transfer proteins and phosphoinositides regulation in the synthesis of sphingolipids. What is the pleckstrin homology domain, describe its fold, size and function?

→ Lipid-transfer proteins are essential to shuttle sphingolipid intermediates across organelles. Sphingolipid several transfer protein are targeted to the acceptor membranes by phosphoinositides. As a consequence, the concentration of phophoinositides regulates the activity of specific lipid transfer proteins. For example high levels of PIP4 lead to a effective CERT-mediated ceramide transfer and sphingomyelin synthesis.

Question 3b

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Pleckstrin homology domain:

Domain	Typical size (amino acids)	Structure	Preferred target*	Membrane insertion?	Ca^{2+} required?	Dimerization required?
PH	~125	β-sandwich	Phosphoinositides, quite diverse, some highly specific	Some reported[20]	No	Some examples

Lecture 8, slide 32

Question 3c

Activated isoprene units are synthesized starting from Acetyl-CoA. If 2-[14C]acetyl-CoA is added to a rat liver homogenate, where will the 14C label appear in Δ3-isopentenyl pyrophosphate (Figure 2)?

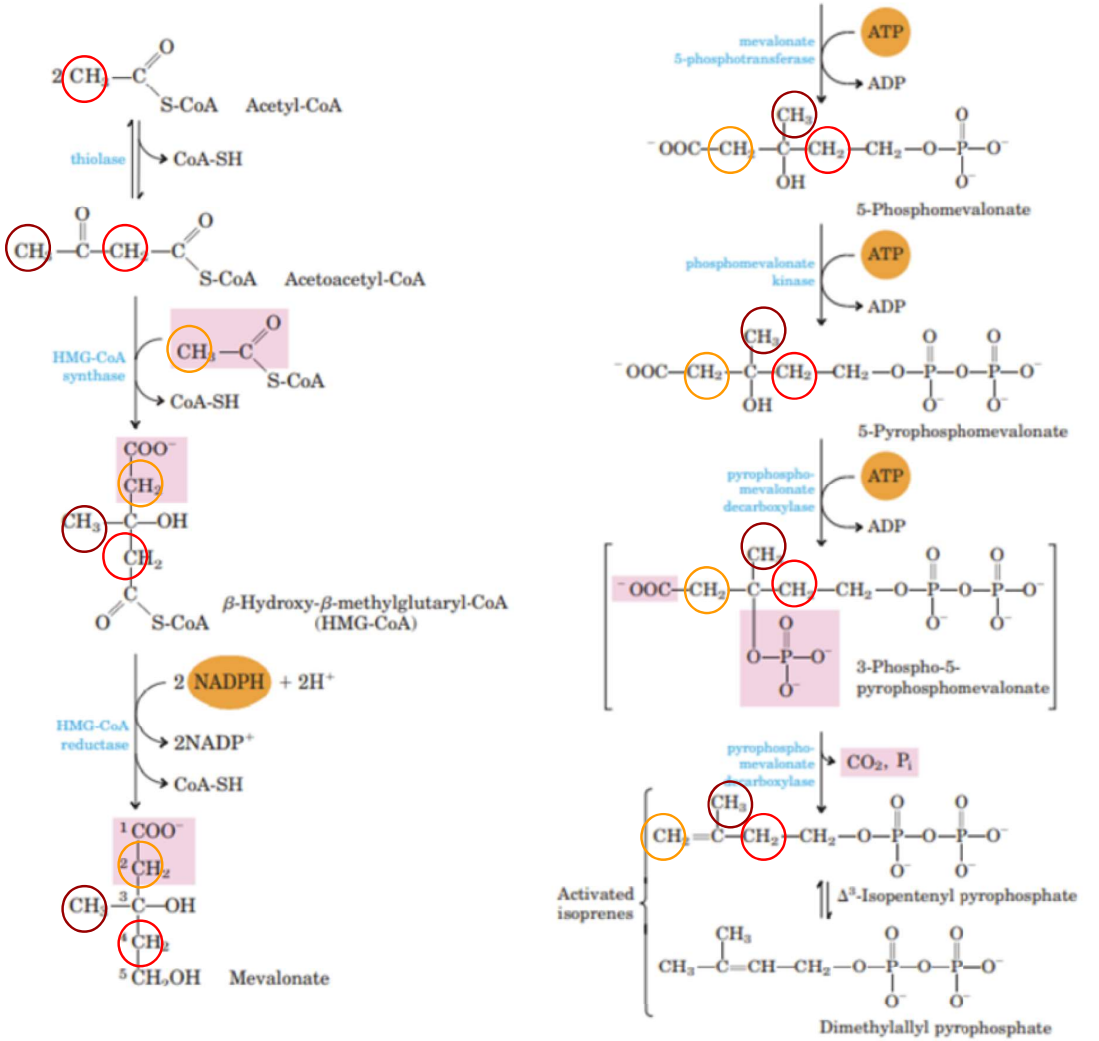
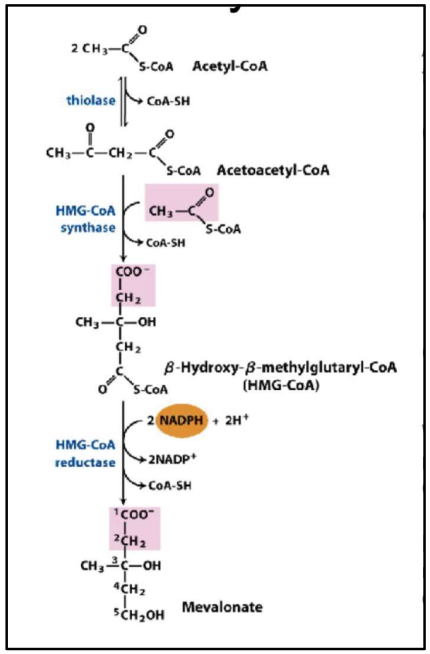


Figure 2: Schematic representation of the biosynthesis of activated isoprene units

Question 3d

HMG-CoA reductase is the rate-controlling enzyme in the mevalonate pathway. It is often targeted by inhibitors – statins. What is one of the most important downstream effects of statin treatment?

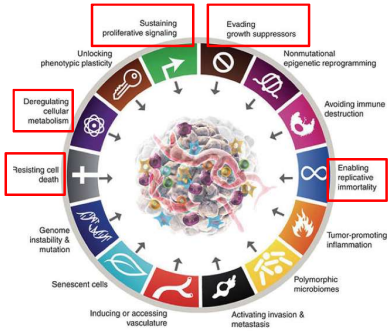
-> Inhibition of Cholesterol synthesis, drug is used to treat high cholesterol levels



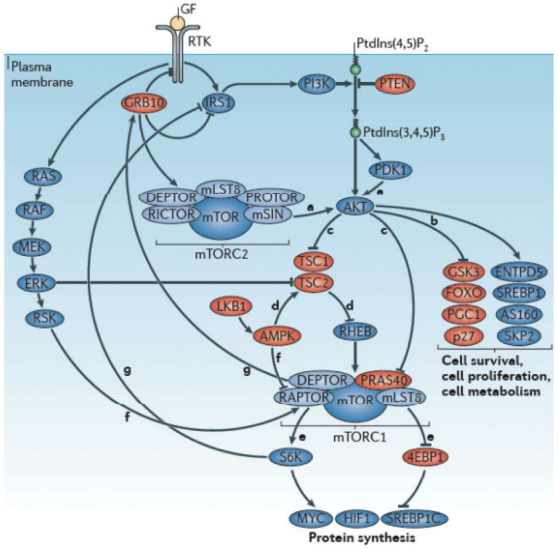
Question 3e

PI3K catalyzes the conversion of PtdIns(4,5)P2 to PtdIns(3,4,5)P3 while PTEN catalyzes the opposite reaction. Mutations rendering PI3K hyperactive and mutations disrupting PTEN activity are major cancer drivers. Can you explain the mechanism by which disturbed lipid metabolism result in malignant transformation?

PtdIns(3,4,5)P3 (PIP3) is a signalling lipid that can activate AKT. AKT is involved in upregulation of cell survival, proliferation and cellular metabolism. When the regulation of PIP3 and hence AKT signalling is over-active, it can lead to non-regulated cell proliferation and increased survival of the cell as well as altered metabolism, all being hallmarks of a cancerous cells.



https://www.aacr.org/blog/2022/01/21/new-dimensions-in-cancer-biology-updated-hallmarks-of-cancer-published/



Lecture 8 - slide 35

Answer 4a

Check Exercise 10 - Question 2!

When adapting to their environment, cells (as a consequence of transcriptional changes) change their metabolism.

Describe a workflow based on mass spectrometry to compare the metabolomes of a cell line grown under different conditions (please explain each step).

1) Grow the cells in the different conditions you would like to test. Feed one strain with isotopically labelled carbon source

2) Weigh samples, mix equal amounts

3) Organic extraction of metabolites to simplify sample (depending on what you want to study, analyze specific phase)

4) Liquid phase chromatography to separate metabolites (Reversed phase chromatography as most small molecule metabolites ar non-polar)

5) Mass spectrometry (MS1) to record initial ions followed by fragmentation (MS/MS)

6) Compound identification by MS/MS spectra – database match

7) Relative quantification via isotopic distribution (labelled vs unlabelled) of analytes of interest

*Note: step 2 is optional. You can also do relative quantification via comparing abundances of peaks which are not isotopically labelled of the same compound across the different conditions, but this is not as accurate. But answer is acceptable

Answer 4b

According to your metabolomics results intracellular glucose levels are increased when cells are cultured in the presence of compound A. In an attempt to evaluate the potency of your compound, you device an experiment where cells are incubated with increasing amounts of compound A. Which types of assay suit better the design of such experiment? (explain why)

- NMR: no good because sample is a mixture, not pure. Tough data analysis

- MS: not ideal for complex quantification. Doable, but a lot of work

- Colourimetric assay: this scenario is a targeted approach, we know we just want to detect glucose. If we can simply add something that binds glucose that generates a colour, we can simply quantify the compound based on this signal via standard curve and measure the amount of glucose secreted under different concentrations of our drug added!

- The answer: colourimetric assay.

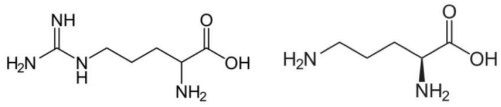
Answer 4c

When administered to mice, compound A induces: hypoglycaemia, increased glycogen synthesis in the liver and muscles, increased glycolysis, increased fatty acid and triglyceride synthesis. Based on these effects can you hypothesize a mode of action for compound A? explain your answer.

- Hypoglycemia: low blood sugar: insulin lowers blood sugar
- Increased glycogen synthesis: by insulin
- Increased glycolysis stimulated by insulin
- Insulin increases FA and triglyceride synthesis
- Mode of action: stimulate release of insulin and/or activation of insulin receptor

Question 4d

Arginase converts arginine into ornithine and urea. Below, you can find the structure and m/z of arginine and its metabolite ornithine:



Arginine
m/z 174.2

Ornithine
m/z 132.16

How can you measure arginine cellular uptake and its conversion into ornithine by mass spectrometry?

- Get a standard for Arginine and Ornithine, run those masses at different concentrations to obtain a standard curve.
- At different time points, take samples, Add isotope labelled arginine and Ornithine standard into your run, measure masses, compare to standard, abundance

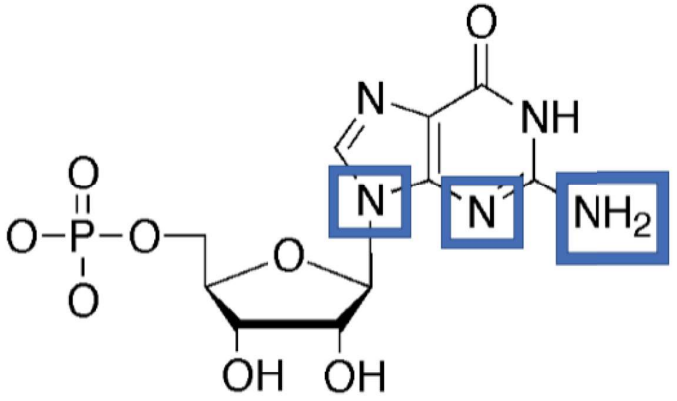
Question 5.a

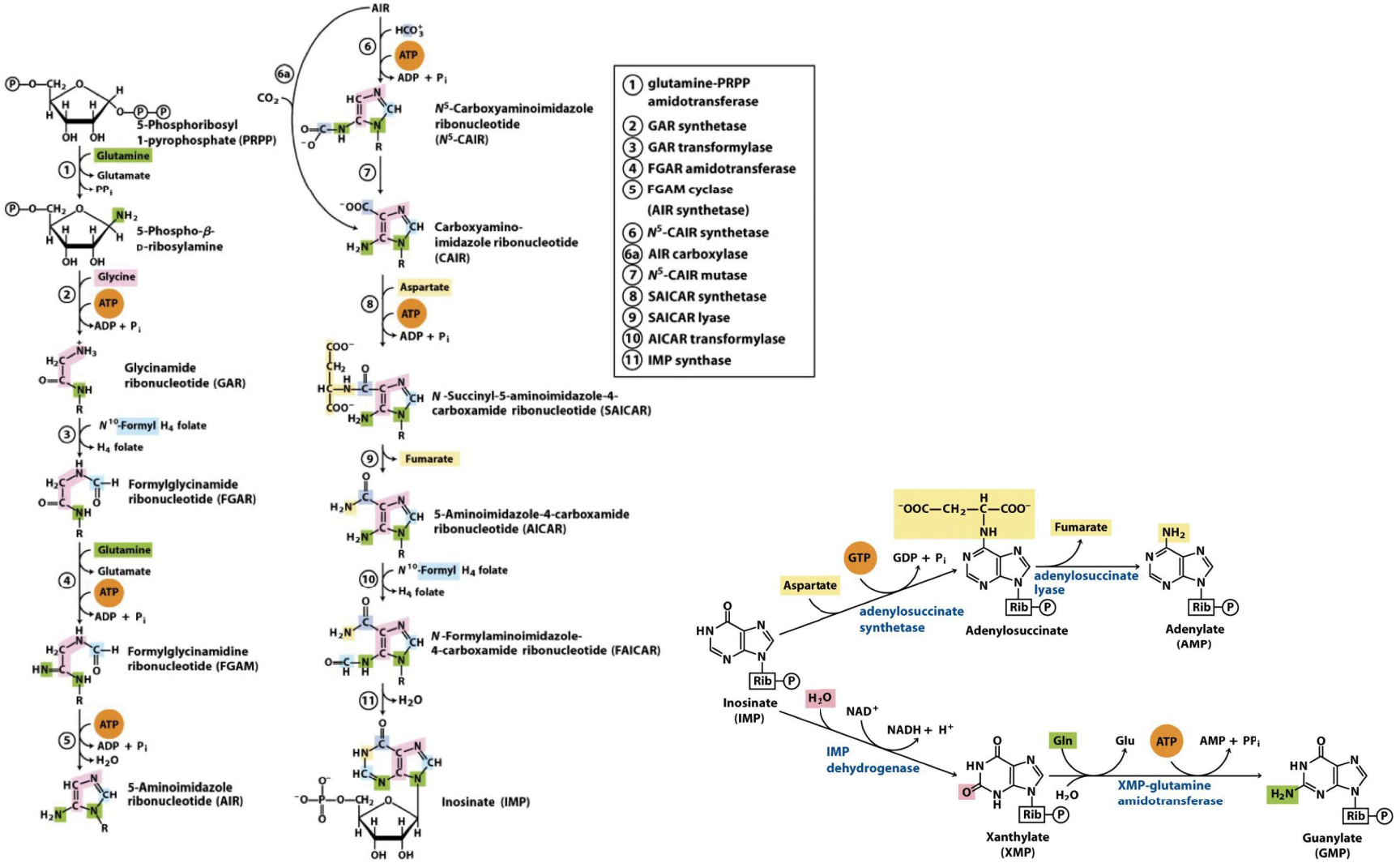
Amino acids are important precursors for the production and maintenance of the nucleotide pools in cells.

a) What is the 15N labelling of GMP (guanosine monophosphate) in cells grown in the presence of uniformly 15N labelled glutamine? Explain your answer.

The amide group of glutamine's side chain would be incorporated into GMP.

Follow slides 28 and 29 from lecture 9.




5-Phosphoribosyl 1-pyrophosphate (PRPP)
Glutamine
Glutamate
PPi
5-Phospho-β-D-ribosylamine
Glycine
ATP
ADP + Pi
Glycinamide ribonucleotide (GAR)
N10-Formyl H4 folate
H4 folate
Formylglycinamide ribonucleotide (FGAR)
Glutamine
Glutamate
ATP
ADP + Pi
Formylglycinamidine ribonucleotide (FGAM)
ATP
ADP + Pi
H2O
5-Aminoimidazole ribonucleotide (AIR)
AIR
HCO3+
ATP
ADP + Pi
CO2
N5-Carboxyaminoimidazole ribonucleotide (N5-CAIR)
Carboxyamino-imidazole ribonucleotide (CAIR)
Aspartate
ATP
ADP + Pi
N-Succinyl-5-aminoimidazole-4-carboxamide ribonucleotide (SAICAR)
Fumarate
5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR)
N10-Formyl H4 folate
H4 folate
N-Formylaminoimidazole-4-carboxamide ribonucleotide (FAICAR)
H2O
Inosinate (IMP)
1 glutamine-PRPP amidotransferase
2 GAR synthetase
3 GAR transformylase
4 FGAR amidotransferase
5 FGAM cyclase (AIR synthetase)
6 N5-CAIR synthetase
6a AIR carboxylase
7 N5-CAIR mutase
8 SAICAR synthetase
9 SAICAR lyase
10 AICAR transformylase
11 IMP synthase
Aspartate
GTP
GDP + Pi
adenylosuccinate synthetase
Adenylosuccinate
Fumarate
adenylosuccinate lyase
Adenylate (AMP)
Inosinate (IMP)
H2O
NAD+
NADH + H+
IMP dehydrogenase
Xanthylate (XMP)
Gln
Glu
ATP
AMP + PPi
H2O
XMP-glutamine amidotransferase
Guanylate (GMP)

Question 5.b

b) How does your answer change if glutamine is labelled as follows?

I. Exclusively on the N atom in the side chain (amide).

Nothing will change, as this is the N incorporated at each stage

II. Exclusively on the N atom in the amine group attached to the α-carbon atom.

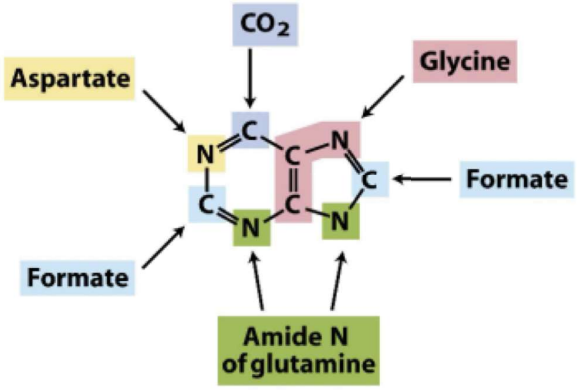
There will be no ^{15}N in the final GMP, as only the Amide ^{15}N is incorporated.

glutamine glutamate

Question 5.c

c) Name 2 other amino acids that can be labelled with ^{15}N and used to feed cells, so that the GMP synthesized in these cells will be labelled with at least one 15N atom.

Glycine and aspartate. Slide 26 lecture 9

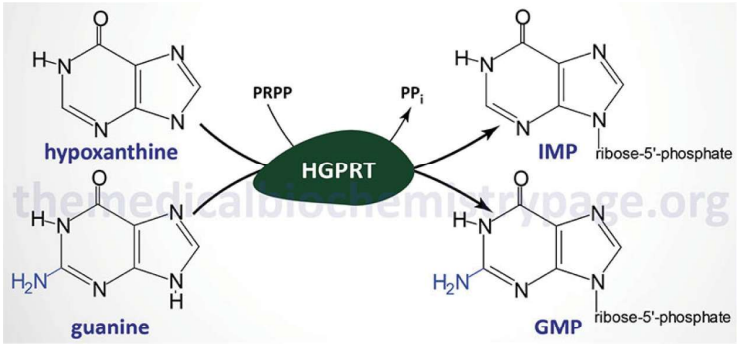


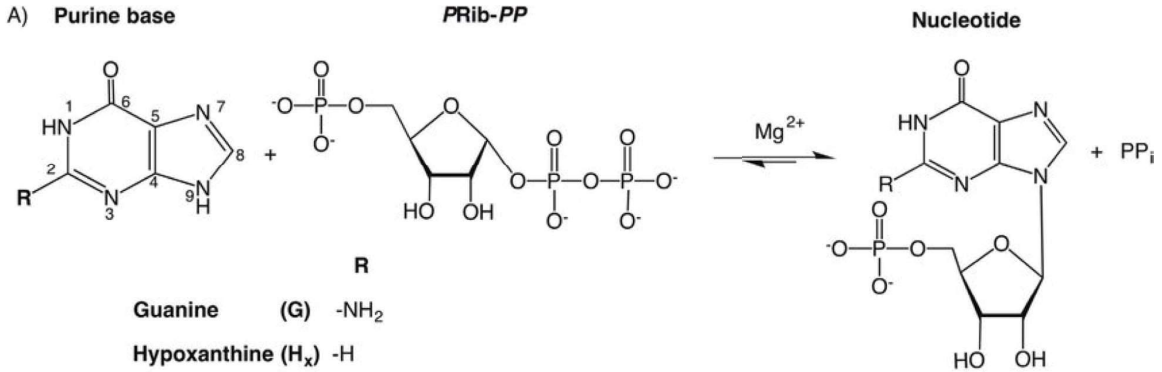
Question 5.d

d) Another way of synthetizing GMP is through its salvage pathway. HGPRT is a transferase that catalyzes conversion of hypoxanthine to inosine monophosphate and guanine to guanosine monophosphate as part of the salvage pathway. Lesch- Nyhan syndrome is a rare genetic disorder due to a deficiency of HGPRT. Such deficiency causes a build-up of uric acid in all body fluids.

I. Which molecule does HGPRT incorporate into the guanine when it converts it to guanosine?

Ribose (P Rib PP)





II. 5-amino 4-imidazolecarboxamide (AICA) is an inhibitor of enzyme guanine deaminase. Allopurinol, used to treat gout, is an inhibitor of xanthine oxidase. Which one of these two drugs would be best suited to alleviate symptoms of a patient suffering from Lesch-Nyhan syndrome and why?

Allopurinol, because it would prevent uric acid synthesis and accumulation from both GMP and AMP catabolic pathways.