Question 1:

a) Using estimates for transcription and translation energetics in bacteria, calc the minimum time required to produce a day's worth of insulin for a single patient. 68 units/day where 1u = 34.7ug. Assume ATP availibilit limiting reaction and 1 ATP synthse makes 100ATP/s

Need 68(34.7*10^-6)g = 2.36mg @ atomic weight 51 AA
or 2.36(86/51) = 3.98mg of proinsulin

ATP rate from vessel:

ATP rate from lecture = 5x10^9 cells (OD of 1 is 5x10^8, so OD of 10 is 5x10^9) and then * 5000 mL to get 2.5x10^13 cells/ second

Converting 3.98mg to moles/1mg/1ml

3.98g/(8696*22.4*1000*1000) = 2*10^-11

>>> (2*10**-11)* (38.7*10**-3)
7.74e-13

>>> (5.5*10**13)* (7.7*10**-13)
42.35 seconds

b) From a biotech standpoint does this make sense? Do you think it is reasonable to assume ATP availability alone limits protein synthesis? Why?

Yes, as long as you don't see something unreasonable like 1y or something where you know the reaction won't complete.
ATP availability is one metric above the generation of proteins. Another example is using Met synthesis rates which correlate

with start codons as a bottleneck and as a correlation tool to correlate growth media with Met availability.

Translation ATP:

mRNA lives 8 minutes
each cell has 60 copies of insulin producing plasmid
Translation @48n/second with 120N between each ribosome/second
Transcription @50n per gene/second

C:
Need 2*10^16 insulin molecules.
There are (5*10^8)(5000) cells = 25*10^11
We need 2*10^16/(25*10^11 cells) transcipts=8000 transcripts.

How many mRNAs/cell?
http://book.bionumbers.org/how-many-mrnas-are-in-a-cell/
10^3, 10^4 mRNA per bacterial cell (milo),

This is order of magnitude ~ 8k

How many mRNAs/cell/gene?
http://bionumbers.hms.harvard.edu/bionumber.aspx?id=111919
.4 typical range .2-3.

8000 transcripts/4600 genes ~ 1.7. within range of Milo.

D. Translation rate at 120nt/48nt/s = 2.5s per ribosome init. 8 minute mRNA half life; 480s/2.5s/init=192 initiations or 192AA codons.

Insulin=192 AA or 192*3=576NT. 576nt/50n/s ~11.52s per transcript (mRNA creation).

There are (5*10^*8 cells/ml)(5000ml) = 25*10^11 cells.
60plasmids(25*10^11)/11.52 seconds = 13*10^12 mRNA/second

We need 2*10^16 insulin; 2*10^16/(13*10**12)=1539 s or 25 minutes.

Question 2:

a) atp-foreignAA, atp-synthase-foreignAA. There are 2 places to do the modificatin for the synthase, modify the ecoli DNA or add the gene into the plasmid. The tRNA(foreignAA) has to be modfied to accept the foreignAA. There is no guarantee the shape of the foreignAA+ribosome will work and not interfere with the rest of the function of the cell such as the stop codon functionality.

b) 2 key challenges for making enzyme-foreignAA starting from a native variant of the same enzyme. Taking enzyme-stopcodon and modifying it to enzyme-foreignAA may stop the stop codon from working. Introducing a new functionaliy my modifiying the enzyme may interfere with the functioning of other translation enzymes preventing elongation at the last step, peptide formation between existing AA chain to the foreignAA.

Additional risk: adding protein purification tags to either the N or C terminals change the folding structure of the protein. Unknown with the foreign AA if this will be an issue.

c) interference with over ~200(source Weismann paper) Post Translational Modifications in ecoli. Increasing the translation rate, by replacing wobble codons with perfect matching codons, results in errors in folding (P. S. Spencer et al, J. Mol. Biol., 422:328, 2012)

Question 3:

a) how long to produce 1000 new ATP synthases:

MOPS is millions of molecules per generation. Assuming a 20 minute generation(doubling time):

	MOPS Coplete	Time/1k ATPx in 1 generation	
atpA	30696	39.1s	
atpB	10508		
atpC	12695		
atpD	30603		
atpE	112959	10.6s	
atpF	17866		
atpG	9832		
atpH	9335		

	MOPS Coplete	Time/1k ATPx in 1 generation	
	234494		

Verify there really is an atpI. thought it wasnt there earlier.

234494 ATP/total per generation

234494 ATP per generation/20 minutes per generation
= 11725 ATP per minute
for 1k ATP need ~5 seconds

b) how long does ATP synthase need to operate to produce enough ATP to pay for cost of the protein translation that generated it? Assume ATP synthase turnover rate of 100ATP/second

ADP: 427g/mol.

$$ADP + P_i + H^+_{out} \rightleftharpoons ATP + H_2O + H^+_{in}$$

~38m mM ATP/1mg/1ml cost per AA.

TBD

c) In E coli; ATP synthase is composed of several subunits. The subunits are all produced from a single mRNA transcript but are not needed in a 1:1 ratio. From Fig. 2 of the paper which subunit is the most abundant in ATP synthase?

atpE

Are the subunits synthesized in a 1:1 ratio? **No** or does the protein synthesis more accurately reflec the sto. of the subunits in the ATP synthase complex? **Proportional synthesis; reflects**

the stoichimetry of the subunits. Rates are independent, proportion of genes is about same as porportion of protein! What mechanism? Proportional synthesis could be because of translational autoregulation, coupling or RNA seconday structures. Ribosome affinity mentioned in lecture along with the effect of flanking sequences as possible contributors to proportional synthesis or fine tuning of protein synthesis rates.