Justin Barry

Title: Pet Enzyme kinetics lesson plan- POGIL for Pet Enzyme: Methyl-coenzyme M reductase (MCR)

Audience: 11th and 12th grade High School Chemistry or Biology (advanced students)

Goals:
To discuss how anaerobic bacteria use enzymes to produce methane gas.

Student Objectives: At the end of the lesson, students will be able to:
1. Discuss what enzymes kinetics means
2. Illustrate how methyl coenzyme M reductase produces methane in a chemical reaction
3. Discuss what regulates enzymes.

Purpose: To relate the carbon cycle to a particular enzyme that produces methane in methane clathrates.

Materials/ Resources: Computer with Internet access; POGIL handout.

Time required: One class period of block scheduling (65 minutes). Depending on class, this might take two days.

Procedure: Students will read the information provided and use the web resources to answer the questions in the following handout.

Assessment: The POGIL answers as well as the exercises, which includes a concept map will be the assessment.

Methanogen Bacteria POGIL

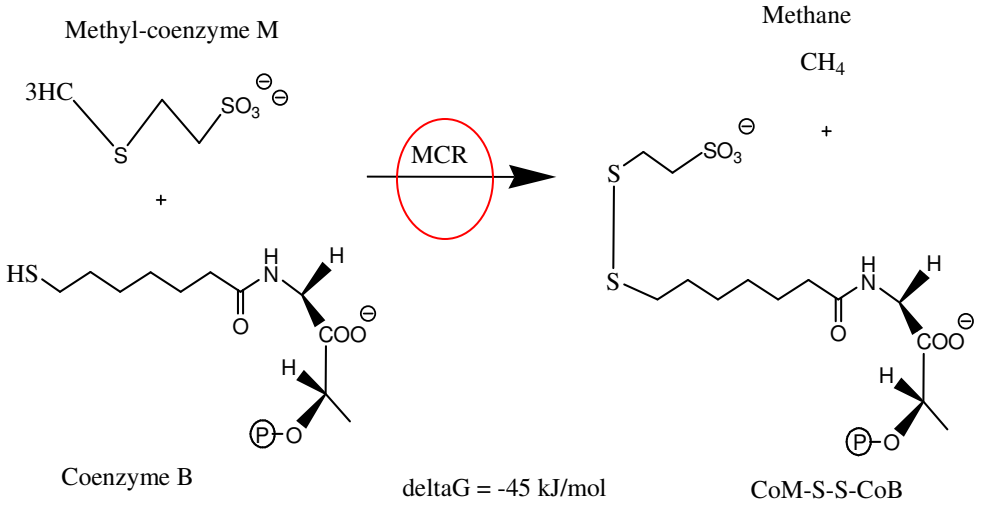
Name:_____ Date:_____

INFORMATION:
In the past we have discussed about the carbon cycle and how carbon is transferred through various biological systems. We have also discussed what methanogens are and what they produce; methane gas. Methane gas produced by bacteria is actually a bit more complicated.

Enzymes are chemical substances which speed up a chemical reaction. Enzymes are necessary to lower the energy needed for a reaction to proceed, called activation energy. The reactants within enzyme-catalyzed reactions are called substrates. Enzymes bind one or more of the substrates to produce new products.

One such enzyme within the methanogen bacteria is called methyl coenzyme M reductase (MCR). MCR catalyzes the final reaction of the "energy conserving pathway" of methanogenic bacteria. Methyl-coenzyme M (coM) and coenzyme B (coB) are converted to methane and CoM-S-S-CoB.

MODEL 1: Proposed Reaction Catalyzed by MCR



According to Model 1 above, answer the following questions

1. List the reactants in the reaction from Model 1.

Methyl-coenzyme M, Coenzyme B

2. List the products in the reaction from Model 1.

Methane and CoM-S-S-CoB

3. Circle the enzyme used in the reaction from Model 1.

4. What type of linkage is created in the products?

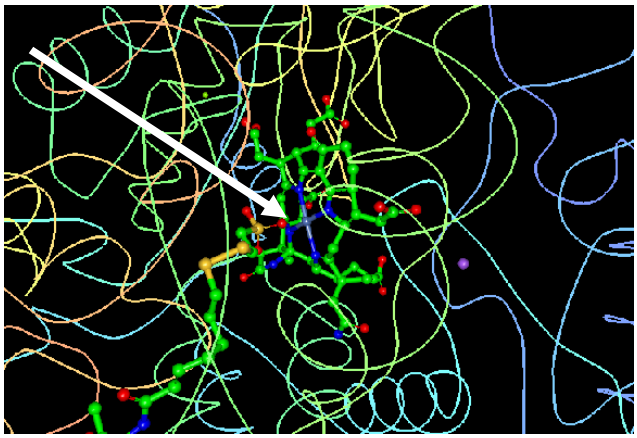
A disulfide linkage.

INFORMATION:
Many enzymes can only function under certain conditions. One such condition is the existence of a cofactor. A cofactor, which can be a coenzyme or metal ion work by changing the shape of an enzyme or by actually participating in the enzymatic reaction. MCR uses nickel porphinoid (F_{430}) as the cofactor. Other cofactors associated with MCR include chloride ion, glycerol, magnesium ion, sodium ion, zinc ion.

(Of the 19 magnesium ions, half are coordinated to protein atoms. Of the 11 sodium ions, all are coordinated to the oxygens of the protein located in MCR. A single zinc ion was found along the symmetry axis of MCR. Two chloride ions are also found attached to the beta subunits.)

The most notable cofactor present is the nickel porphinoid F_{430}. Here is an image of the cofactor located within the methyl-coenzyme M reductase (shown in blue and green with arrow pointing towards it).

MODEL 2: Cofactor F_{430}



According to Model 2 and the information above, answer the following questions

5. What is a cofactor and why is it necessary?

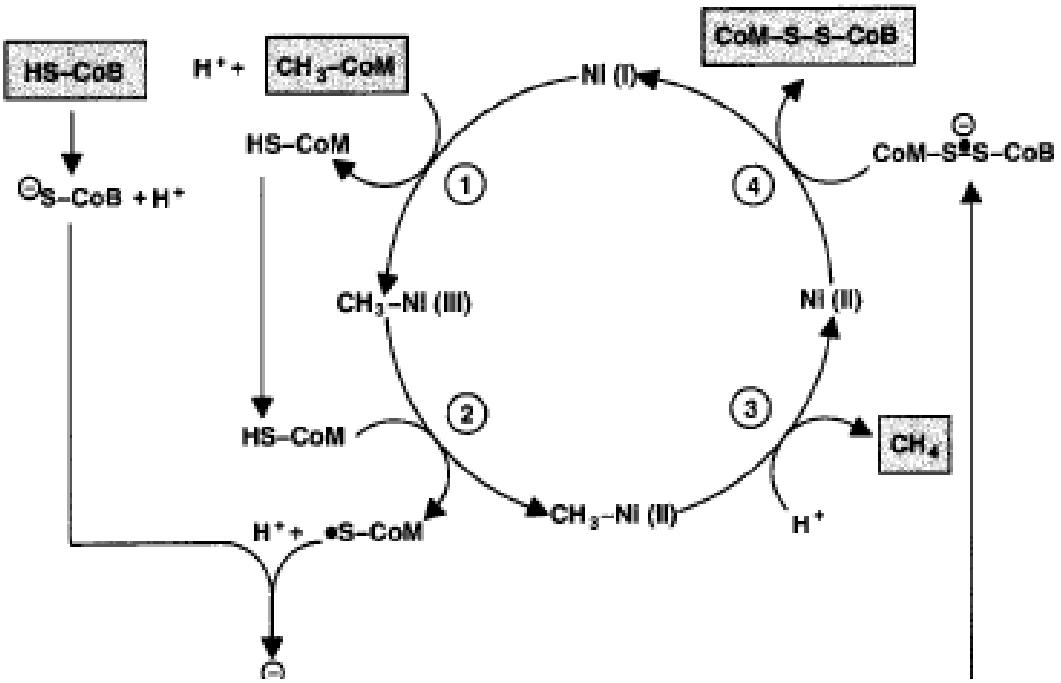
A cofactor is a coenzyme or metal ion that works by changing the shape of an enzyme or participating enzymatic reaction.

6. Describe F_{430} and its function.

F430 is a nickel based compound that binds to the coenzymes.

INFORMATION:
A more detailed mechanism is found in the literature for how methane is produced in methanogenic bacteria (according to the reaction in model 3 below). The substrates (coM and coB) bind to the cofactor (F_{430}), methyl-Ni (III) in step one. F_{430}, is bound to histadine gamma-156. In step two, methyl-Ni (III) is reduced to methyl-Ni (II). Upon further reduction, methane gas is formed. This reaction above occurs in anaerobic conditions, such as deep ocean sediment. Upon methane production in deep ocean sediment, methane clathrate hydrates can form.

MODEL 3: Hypothetical catalytic reaction mechanism of methyl-coenzyme M.



According to Model 3 and the information above, answer the following questions.

7. How many main steps are there in this proposed reaction mechanism?

Four

8. What transition metal (the cofactor) is used to bind to the methyl (CH_3) group in step 1?

Nickel

9. What is the charge on the nickel between each of the 4 steps? Is the nickel being oxidized or reduced?

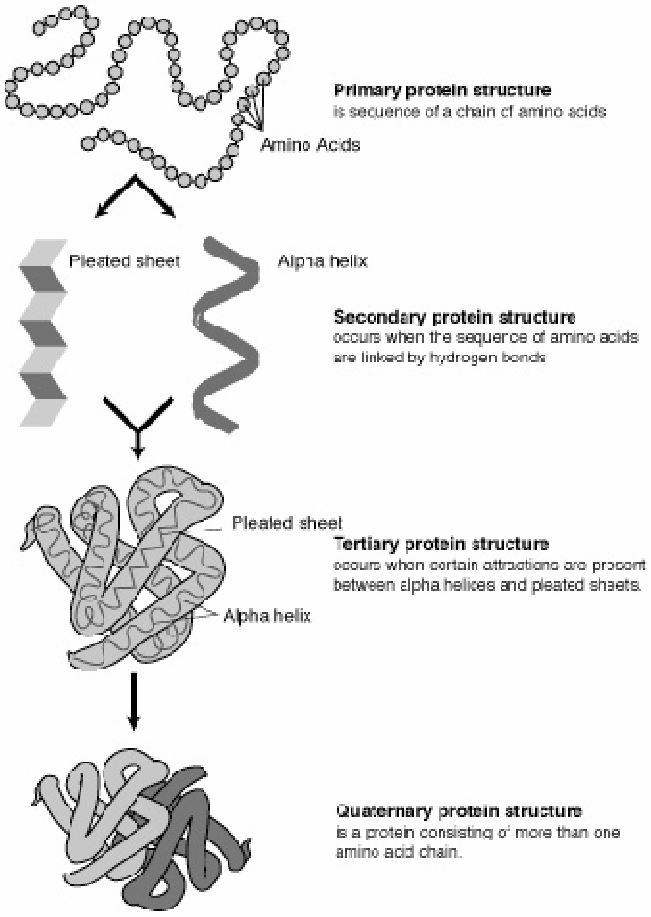
1-2 step = +3 charge, 2-3 step = +2 charge, 3-4 step = +2 charge

10. Describe using your own words how methane (CH_4) is being produced.

Methane is produced through the reduction of the Ni cofactor.

INFORMATION:
Enzymes, such as MCR act upon proteins within methanogen bacteria. Protein structure is classified into four different levels.

MODEL 4: Four different structures of proteins.



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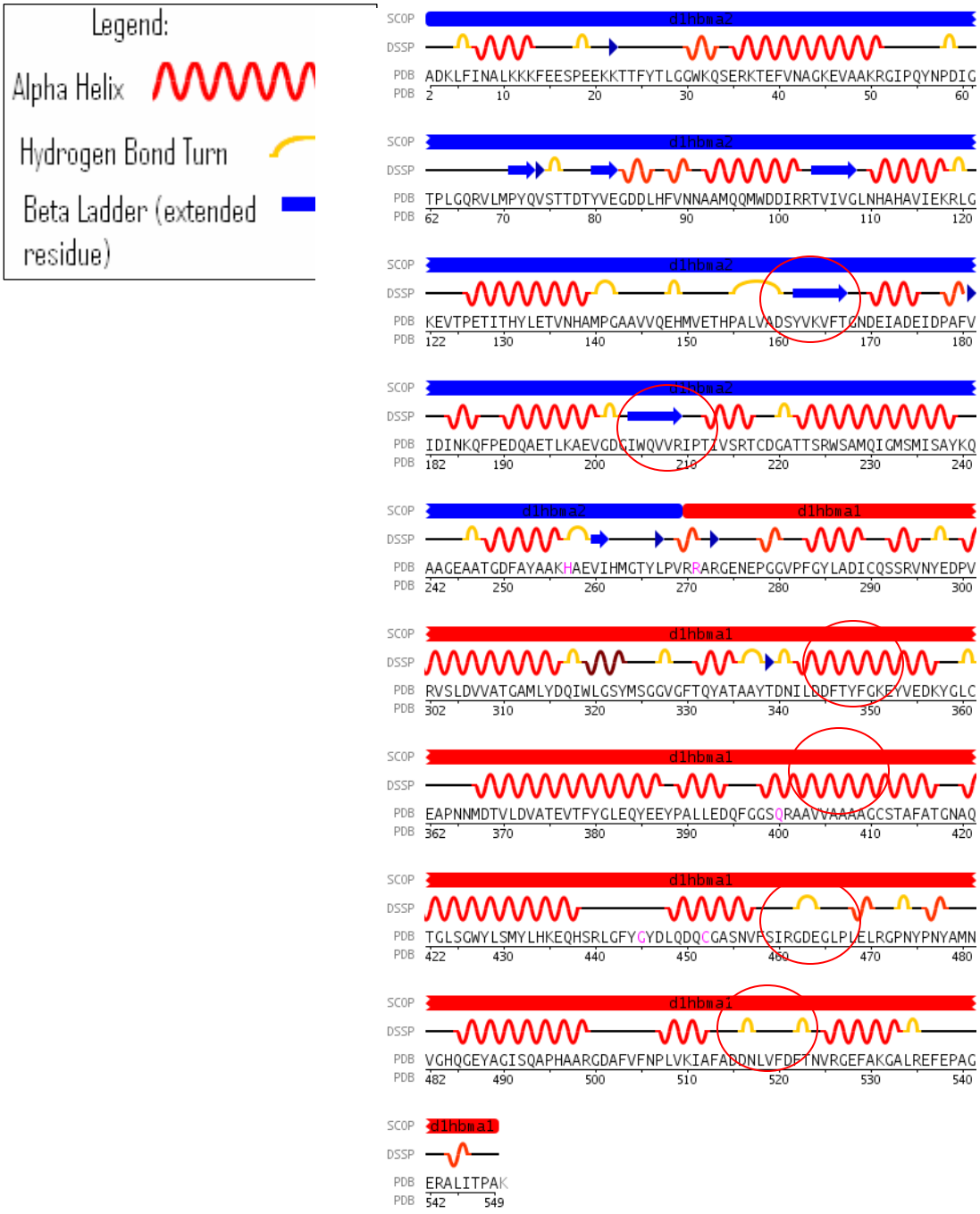
INFORMATION:
Below is the Primary structure sequence for one of the subunits of the protein. This sequence can be found through published sources. This sequence of amino acid chains has polar and nonpolar groups, which affects its function in biological systems. You will use this sequence to see the polar and nonpolar groups later in an exercise.

Primary structure sequence for the alpha subunit:

1 mnkknkklfl ealekkfkge speekkttfy cfggwkqser krefveyakk lakkrgipfy
61 npdigvplgq rklmayrisg tdayvegddl hfvnnaaiqq mvddikrtvi vgmdtahavl
121 ekrlgvevtp etineymeti nhalpggavv qehmvevhpg lvddcyakif tgndeladel
181 dkrvlidink efpeeqaeml kkyignrtyq vnrvptivvr ccdggtvsrw samqigmsfi
241 sayklcagea aiadfsfaak hadviemgti lpgrrargpn epggipfgvf adiiqtsrvs
301 ddparislev igaaatlydq vwlgsymsgg vgftqyasat ytddilddfv yygaeyvedk
361 ygfcgvkpsm evvkdiatev tlygleqyee yptlledhfg gsqraavvaa aagcstafat
421 gnsnaginaw ylsqilhkeg hsrlgfygyd lqdqcgasns lsirsdeglv helrgpnypn
481 yamnvghqpe yagiaqapha argdafvvnp likvafadnd lsfdfrwprk eiargalref
541 mpdgertlii pask

INFORMATION:
Below is the secondary structure for the alpha 1 subunit. This subunit consists of an amino acid sequence with alpha helices and beta pleated sheets.

Model 5: Secondary Structure. (for the alpha 1 subunit)

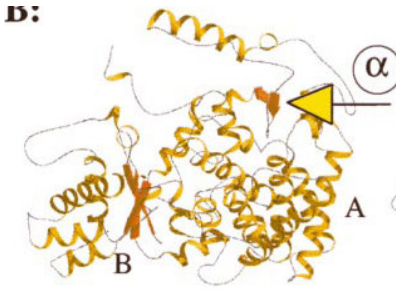


According to Model 5 and the information above, answer the following questions.

11. Circle 2 alpha helices, 2 hydrgen bond turns, and 2 beta ladders in the secondary structure above.

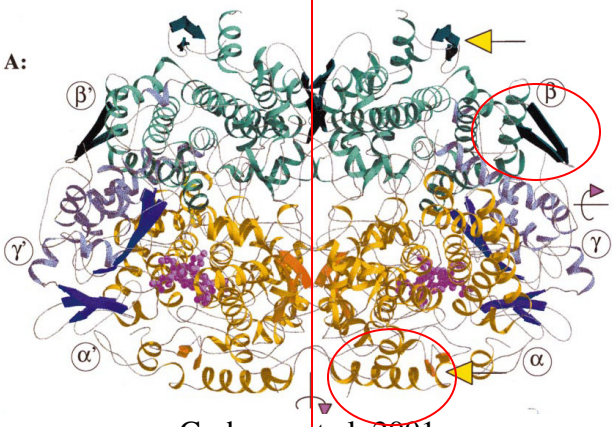
INFORMATION:
Below is the tertiary structure for the alpha 1 subunit. This subunit consists of interactions between the alpha helices and the beta sheets. Within the alpha subunit, there are two sections A and B that are labeled. In addition, the yellow arrow indicates an activation site where molecules typically bind.

Model 6: Tertiary Structure for the Alpha Subunit:



INFORMATION:
A quaternary structure for a protein consists of several amino acid chains, which can be described as subunits. MCR is a heterohexamer with two copies of the subunits alpha, beta, and gamma. The alpha subunit contains two domains, A and B. Alpha subunit A has many parallel helices, while the alpha B subunit has a four-fold anti-parallel beta sheet. The alpha and beta subunits are perpendicular to the symmetry axis running down the center of the hexamer and are remarkably similar in their three dimensional structures. The last subunit, gamma, contains an alpha/beta fold with two antiparallel beta sheets which are perpendicular to each other.

Model 7: Quaternary Structure of MCR



Grabarse et al, 2001.

According to Model 7 and the information above, answer the following questions.

12. Draw a line for the symmetry axis in the quaternary structure above.

13. Identify the 6 subunits for the quaternary structure.

Alpha, alpha prime, beta, beta prime, gamma, gamma prime

14. Circle an alpha helix and beta sheet in the structure above.

INFORMATION:
There are many catalytic parameters for the enzyme MCR. The catalytic parameter of Km is an estimate of the dissociation between the enzyme (MCR) from the substrate (coM and coB). A small Km means that the enzyme is tightly bound, while a high Km means that the enzyme is weakly bound. The substrate coenzyme B has a Km value of 0.000023-0.000075 (M) (depending on the literature sources).[2] The substrate methyl coenzyme M has a Km value 0.002-0.004 (M).[2] Therefore, coenzyme B binds more tightly to the substrate while methyl coenzyme M does not bind as tightly.

15. How would the Km value affect the production of methane in the enzyme MCR?

If the Km value was small then the enzyme would tightly bind to the substrate and then reduce the production of methane.

INFORMATION:
The enzyme MCR is also regulated in several different ways. As indicated earlier, the most important aspect of the enzymatic regulation is the necessity for anaerobic conditions. (The reaction is typically coupled with ATP synthesis via the formation of an electrochemical proton gradient). Also, depending on the electronic state of the biological system, the MCR is either in the reductive or oxidative state. Furthermore, the enzyme is regulated by pH and temperature. The optimum pH is around 7.[3] The optimum temperature is around 65 ºC.[3] The greater the temperature, the greater effect MCR reductive state has on the biological system.

16. What is significant about the optimum pH for the MCR enzyme.

The optimum pH is close to the pH of water.

17. Why would a greater temperature favor the enzyme and hence the production of methane?

A great temperature would reduce the activation energy for the reaction and help in the production of methane.

Exercises:

1. A ***Hydropathicity Plot*** is a plot of the primary sequence of an amino acid sequence. The information taken from the primary sequence gives you information about the possible structure of a protein. Each amino acid is given a hydrophobicity score between 4.6 and -4.6. A score of 4.6 indicates that the amino acid is the most hydrophobic and a score or -4.6 indicates that it is the most hydrophilic. Copy the primary sequence from the information in POGIL above in to the hydrophobicity plot at http://gcat.davidson.edu/rakarnik/KD.html. Give a brief interpretation of what you observe.

See Answer below.

2. Create a flow chart of the principles discussed in this POGIL. Start with Protein in the middle and include the terms listed below (not limited to). Write on the conection lines how the words relate to each other.

   Some words to use: enzyme, substrate, MCR, methane, structure, primary, secondary, tertiary, quaternary, subunits, temperature, pH, regulated.

Answers may vary.

References:

1. Grabarse, W., Mahlert, F, Duin, E.C., Goubeaud M., Shima, S., Tauer, R.K., Lamzin, V., & Ermler, U. 2001. On the Mechanism of Biological Methane Formation: Structural Evidence for Conformational Changes in Methyl-coenzyme M Reductase upon Substrate Binding. *J. Mol. Biol.,* 309, 315-330.

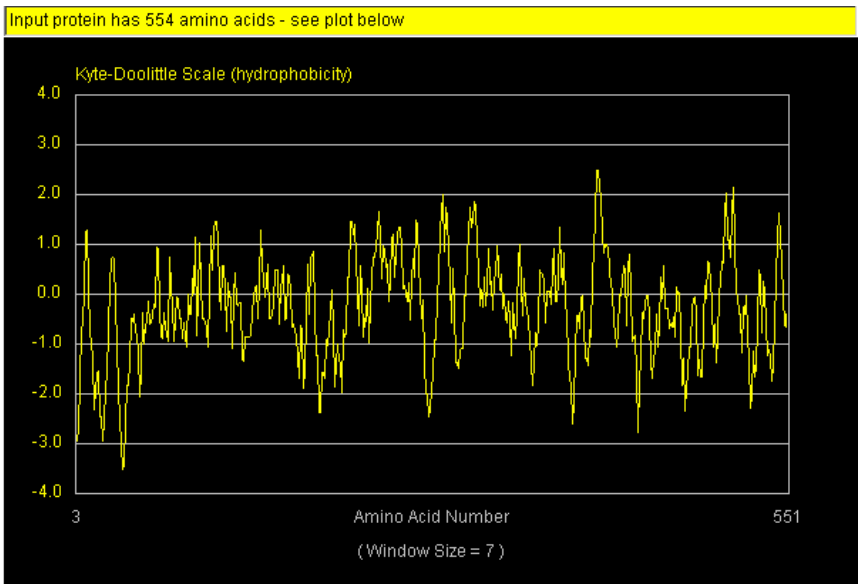
2. http://www.brenda-enzymes.info/php/result_flat.php4?ecno=2.8.4.1

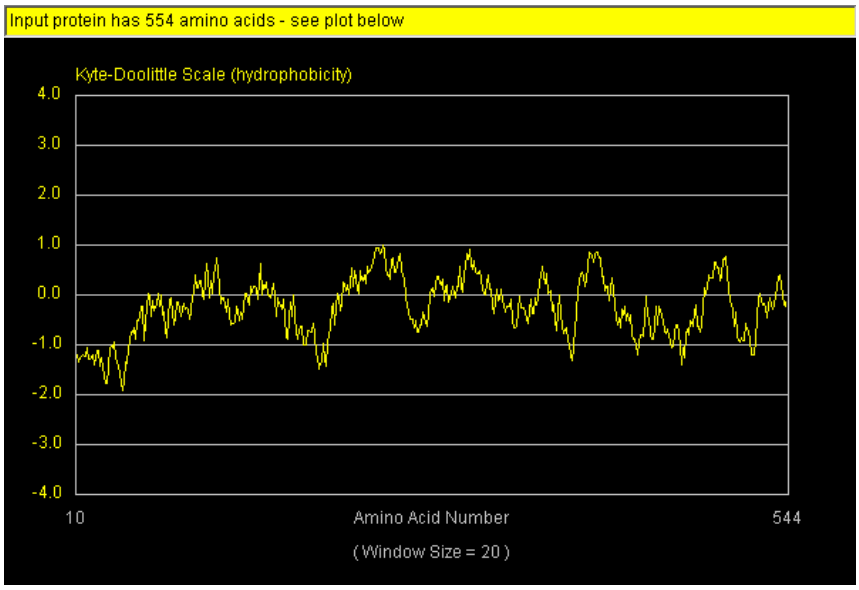
3. Bonacker, L.G.; Baudner, S.; Morschel, E.; Bocher, R.; Thauer, R.K.; (1993). Properties of the two isoenzymes of methyl-coenzyme M reductase in Methanobacterium thermoautotrophicum. *Eur. J. Biochem*. 217, 587-595.

4. http://matcmadison.edu/biotech/resources/proteins/labManual/chapter_2.htm

Answers to exercise #1.

Hydrophobicity Plots:





Hydrophobicity Plots:

According to the plots above, the alpha subunit does not appear to be membrane bound. None of the amino acids are close to the 1.9 threshold, which would indicate a trans-membrane region. The alpha subunit appears to be globular because of the equal amounts of hydrophilic and hydrophobic regions. Amino acids 38 and 388 have large negative values, which correspond to alpha helices in the secondary structure on the outside of the protein (hydrophilic regions). Hydrophobic residues are probably on the inside of the protein where the subunits bind.

Hydrophobic AA (Large y axis values):
negative values for the amino acids of: 25, 38, 190, 198, 388, 439, 474, 529
positive values for the amino acids of: 111, 146, 241, 309, 365, 408, 508