Chem 570 Name_____

Midterm 2
November 3rd, 2016

1. Short Answer

   a. Cancer results from alterations to DNA. Name three types of DNA alterations that may play a role in cancer. (3 points)

      Point mutations, deletions, translocations (1 point each)

   b. What is VEGF and what is its role in cancer progression? (2 points)

      Vascular Endothelial growth factor (1 point)
      Binds to VEGFR, strongly promotes angiogenesis, which is essential for metastasized tumors in order for new cells to receive oxygen and other nutrients (1 point)

   c. List the six well-established hallmarks of cancer. (3 points)

      Self-sufficiency in growth signals, insensitivity to anti-growth signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, tissue invasion and metastasis (0.5 points each

   d. What are anti-mitotics? Name the two types of anti-mitotics and describe their mechanism of action. (4 points)

      Anti-mitotics are anti-cancer drugs that disrupt tubulin/microtubule equilibrium. (1 point) Two types: microtubule stabilizers (taxanes) and microtubule destabilizers (vinca alkaloids) (1 point) Destabilizers bind to tubulin, prevent assembly of tubulin into microtubules. (1 point) Stabilizers prevent disassembly of microtubules after they have formed.

   e. Name and define the three major classes of stem cells. (3 points)

      Totipotent: can differentiate into any cell type (1 point)
      Pluripotent: can differentiate into any of the three major tissue types (1 point)
      Multipotent: can differentiate into any type of cell within the major tissue type (1 point)

   f. Name two advantages and two disadvantages of virtual screening. (4 points)

      Answers may vary; 1 point for each disadvantage, 1 point for each advantage

Advantages: can screen millions of compounds, can screen compounds you don't actually posess then buy the ones that are hits, only a small subset will require actual testing (no need for high throughput biochemical assay), easier and cheaper than HTS

Disadvantages: sometimes difficult to address dynamics, certain considerations such as ADME no addressed, synthetic accessibility not always considered, certain programs work better for certain targets, little eorrelation between docking scores and experimentally determined K_i values

g. Name one challenge of developing clinically relevant phosphatase inhibitors. (1 point)

There is limited selectivity because phosphatases are very promiscuous. Also many phosphatase inhibitors have poor pharmacokinetics, limited membrane permeability. (1 point for one answer)

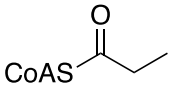
2. Polyketide synthases consist of successive modules that catalyze chain extension and keto-group processing reactions. Each module consists of multiple domains, and the overall structure of polyketide synthases are evolved to be highly ordered in structure and function. PikAIII, the polyketide synthase responsible for the biosynthesis of pikromycin, was evaluated using cryoelectron microscopy to determine its three-dimensional architecture. (*Nature*. **2014**. 510, 512-517)

a. What do each of the following PKS domain names stand for? Which are the three "optional" domains that may or may not be present in each module? (5 points)

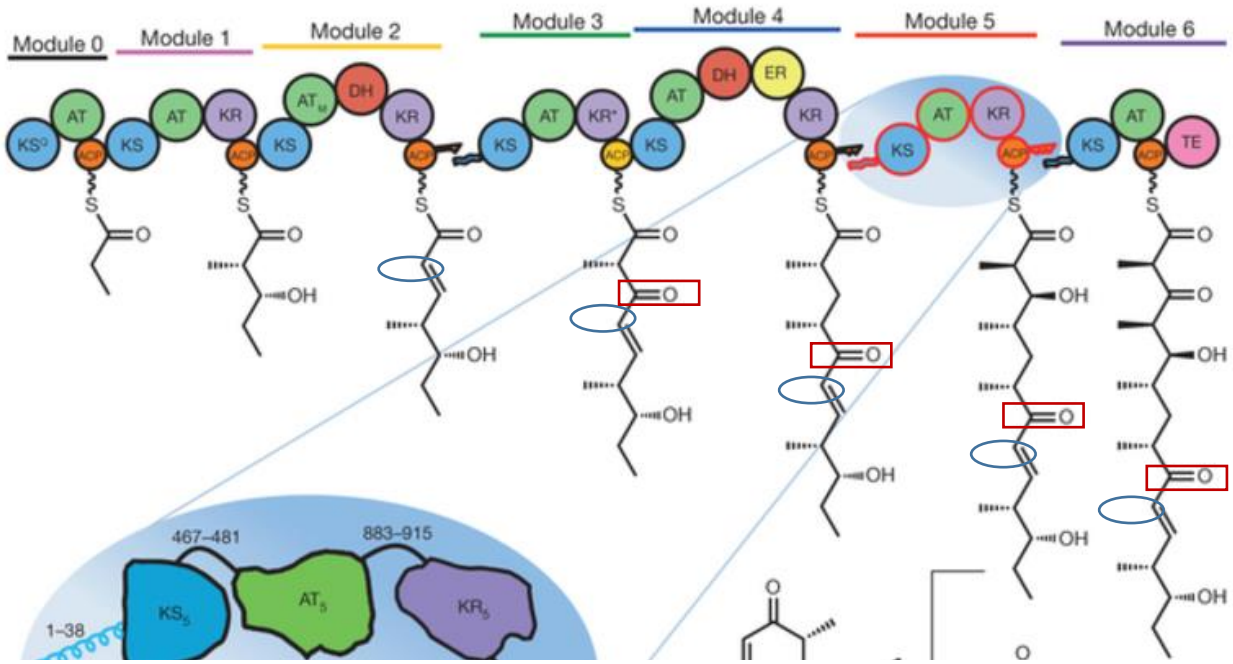
AT Acyl transferase
ACP Acyl carrier protein
KS Ketosynthase
KR Ketoreductase
DH Dehydratase
ER Enoyl reductase (0.5 point each)

Optional domains: ketoreductase, dehydratase, enoylreductase (2 points)

b. Starting from the loading of propionyl CoA in module 0, draw the intermediate present in each module of PikAIII (shown below), as well as the final product resulting from these 6 modules. (Don't worry about showing stereochemistry) (15 points)

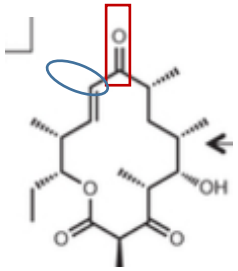


Propionyl CoA



1 point for module 0
2 points each for subsequent modules (1 point for adding unit in correct position, 1 point for proper transformation, total of 12 points)
2 points for final cyclized product (points were given regardless of ring size)

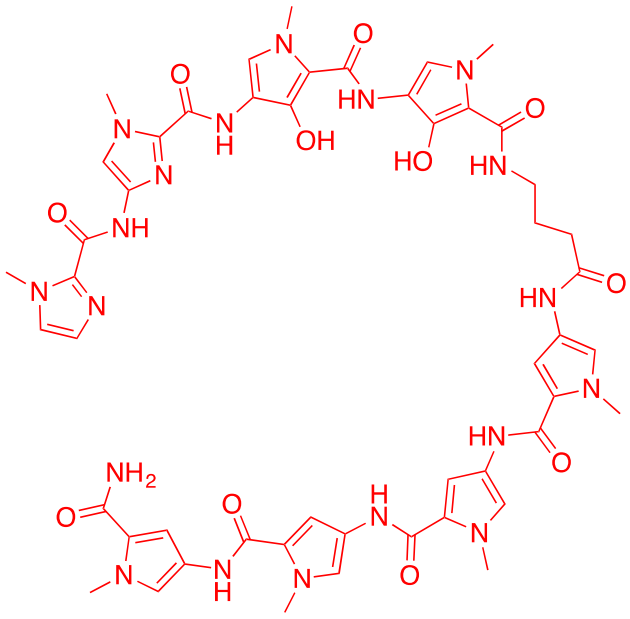
***Note: There should be a methyl where the blue circle is, and the ketone in the red box should be a hydroxyl.**



3. Sugiyama and coworkers recently demonstrated the use of pyrrole-polyamide sequence specific DNA binders for the use of detecting 24 bp repeats within telomeres using fluorescent imaging. To do this, they connected four hairpin units capable of binding selectively to smaller DNA sequences. (*J. Am. Chem. Soc.* **2016**. 138, 14100-14107).

   a. Shown below is the DNA sequence targeted by one of the hairpin units in the DNA binding construct mentioned above. Draw the structure of the polyamide that can selectively bind to this sequence. (10 points)

5’ GGTT 3’
3’ CCAA 5’



2 points for each amide unit, 2 points for linker between them

b. When targeting more than 4-5 base pairs with polyamides, a problem can arise known as overcurving. What is overcurving and how can it be prevented? (2 points)

Overcurving: after 4-5 rings are joined, polyamides no longer properly lineup with DNA because of the curvature in the polymer (1 points) This can be addressed by substitution of a flexible linker (b-alanine) for one of the py rings, which allows targeting of up 11-16 base pairs (1 point).

c. Introduction of a hydroxyl group onto a pyrrole in a polyamide unit allows for differentiation between which two base pairs? How does the hydroxyl group serve to increase the selectivity? (4 points)

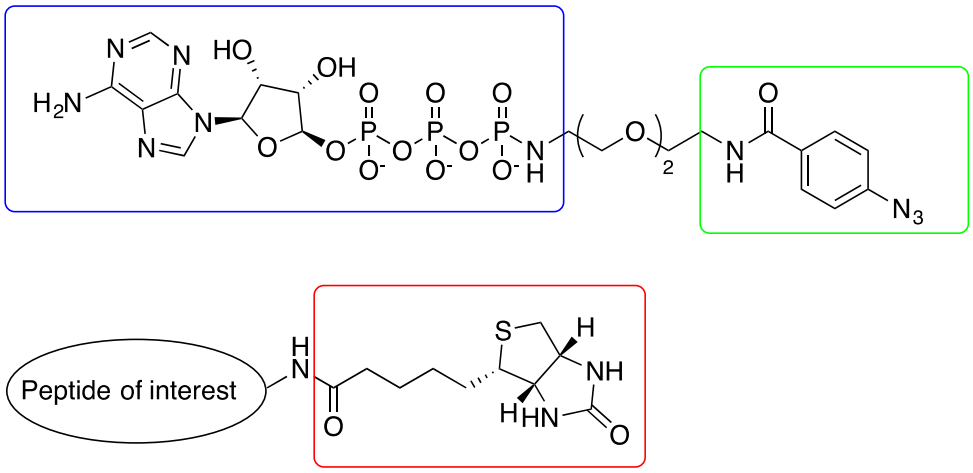
A and T (2 points)
The hydroxyl group will clash sterically with A but not with T (2 points)

d. Sugiyama and coworkers demonstrated the use of these sequence specific DNA binders for identification and visualization of specific DNA repeats in telomeres. Describe another potential application of sequence specific DNA binders. (4 points)

Answers may vary; most likely answer: inhibition of transcription of a gene target for disease treatment (4 points for reasonable answer)

4. Phosphorylation is an important post-translational modification that regulates many cellular signaling pathways. Phosphorylation is carried about by kinases, which catalyze the transfer of a phosphate group from ATP to a peptide substrate. Recently, a method was established for the identification of the particular kinase responsible for a certain phosphorylation. This method is known as K-CLASP, and has been demonstrated to be successful in identifying the kinase responsible for a phosphorylation event in cell lysates containing complex mixtures of other proteins and kinases. (ACS Chem. BIO. *In press*. DOI: 10.1021/acschembio.6b00289)

   a. The K-CLASP method involves the use of a biotinylated peptide substrate, and a modified ATP analog, ATP-arylazide. Describe in detail how these components can be used for kinase identification, including a description of the function of the components contained in the colored boxes. (6 points)



Blue: ATP mimic required for enzymatid reaction, intitiates interaction of the probe with the protein of interest. Green: **photoaffinity tag, covalently links probe to protein active site**. Red: biotin tag for purification on resin (1 point each)

Administer both components to cell lysates containing possible protein of interest. Peptide of interest and ATP-arylazide will interact within protein, peptide will be phosphorylated with phosphate-arylazide, then arylazide will be used for covalently linking phosphorylated peptide to protein. Biotin can then be used to purify protein/substrate complex. (3 points for description)

   b. What is one drawback to the use of large tags (such as the one shown in red above) when designing ABPP probes? Describe how you could modify the K-CLASP system

such that it uses a "tag-free" approach and draw the probe components you could use to carry this out. (4 points)

Drawback: large tags can alter normal protein activity (1 point)

Label peptide of interest with alkyne handle instead of with large biotin linker. Then have separate biotin-azide conjugate, attach biotin after peptide has already been phosphorylated and is covalently linked to protein (1 point for explanation, 1 point each for drawing components)

c. What information can ABPP give you that can't be determined from profiling mRNA or protein levels? (2 points)

ABPP gives you functional information; mRNA and protein levels only tell you how much is present, not the activity levels.

d. Name 4 possible mechanisms for regulation of enzyme function (4 points)

Expression as an inactive zymogen (proenzyme) or bound by endogenous inhibitors
Post-translational modifications
Cofactor requirements (NADPH, etc.)
Accessory proteins modulate function (1 point each)

e. The K-CLASP system demonstrates the use of ABPP for identifying a protein involved in a reaction of interest. Name two other potential functions of ABPP. (4 points)

Answers may vary. Examples include: identifying selective inhibitors, identifying conserved catalytic residues in active site, monitoring protein activity in response to other cellular factors/environmental changes, etc. (2 points for each potential function)