Supplemental Material

CBE—Life Sciences Education

Ngai and Sevian

Supplemental Materials

Practicing biochemist survey

(administered online via GoogleForms)

Introduction

Hello! The purpose of this survey is to collect information from biochemists about their work and how it relates to identification and differentiation of substances. By participating in this survey, you agree to let us use your responses in our research. All responses will be kept anonymous and will inform education research. We greatly appreciate your participation, and any questions may be directed to the PI of this work, Dr. Hannah Sevian (hannah.sevian@umb.edu), or the graduate student working on this project, Courtney Ngai (courtney.ngai001@umb.edu). You may withdraw from this study at any time.

Survey questions

1. Please describe briefly what your biochemistry-related work (e.g., research, applications, product design, regulatory) is about (e.g., characterization of a specific protein, purification of proteins).

2. We are exploring the extent to which the identification of chemical substances is relevant in biochemistry work. We consider the question of "chemical identity" to involve categorization of substances into classes of substances, and collection and analysis of relevant information to enable substance identification and differentiation. If you can, please give one example from your biochemistry-related work of a problem in which you answer questions of chemical identity. Describe the problem, why it is important to identify and/or differentiate the substance(s), and how you do so.

3. To what extent do you consider answering questions of chemical identity to be significant in your biochemistry work?

- o It is a major part of my biochemistry work.
- o It is essential to my biochemistry work, but not the majority of my biochemistry work.
- o It is sometimes relevant to my work, but not often a concern of my biochemistry work.
- o It is not very relevant to my biochemistry work.

If you wish to provide further explanation for your choice above, please include it here.

Demographics

We have included a few demographic questions to ensure a variety of fields are represented in the responses.

Please mark the answer(s) that best represents the sector(s) in which you work:

- o Academia
- o Pharmaceutical industry
- o Biotech/Biopharma industry

- Clinical or medical research
- Forensics
- Medical products and instruments
- Home/health products industry
- Food or agriculture industry
- Chemical products industry
- Government (federal, state, investigatory) sector
- Other

Are you affiliated with any professional societies related to your biochemistry work (e.g. American Chemical Society)? If so, please indicate the society, as well as the division or branch of that professional society (if relevant).
What are the top academic journals that are most relevant to you?

In what academic disciplines and specialties do you have terminal degrees and/or technical certifications?

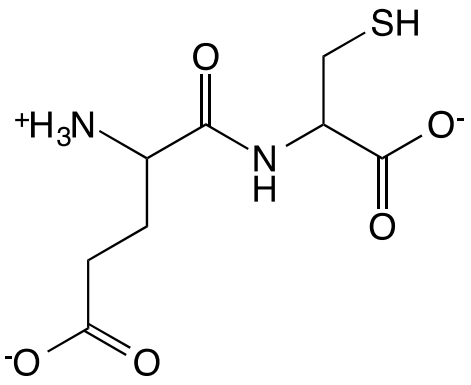
Where are you located?
- In the U.S.
- Outside the U.S.

Creative exercises

On the following pages, the creative exercises developed and implemented in this study are presented. The general rubrics associated with each creative exercise have also been included. These rubrics are not comprehensive of all the statements students might provide in response to the creative exercises, and are intended only to give an idea of the types of statements students might list. The statements in the rubrics have been organized into general categories. After each category, the types of CI themes that are likely to be present in the responses grouped under each category have been provided in parentheses. Other types of CI themes may be present; this listing is not exhaustive.

CE1 – Dipeptide (this heading was not included in student version)

Write down as many **correct, distinct, and relevant facts** you can about:



Ten (10) statements will get you full credit for the problem, which is worth a total of 5 points. The information you use should be information you learned in a chemistry course, including general chemistry, organic chemistry, and any biochemistry courses. All other outside information, combined, will only count as one distinct fact towards the correct responses.

CE1 general rubric

Molecule identification or classification (*class*)

- This is a dipeptide (this is two amino acids joined by a peptide bond between the carboxyl of the glutamate and the amino terminus of the cysteine)
- The two amino acids in this dipeptide are glutamate (Glu, E) and cysteine (Cys, C)
- The name of this dipeptide is glutamylcysteine

Composition or structure of molecule (*composition & structure*)

- Chemical formula: $C_8H_{13}N_2O_5S$
- Identification of the functional groups (carboxyl R group for Glu, thiol R group for Cys) and the terminal groups
- Mentioning of chiral center (2 chiral centers) and/or stereochemistry, physiologically this takes the L form
- Any indication of the charge (zwitterion or dipolar, overall negative charge at physiological pH)
- Mention of ionizable groups (terminal amino, terminal carboxyl, side chains) and current state of ionization and possible pH
- Calculation of molar mass or molecular weight (~250.3 g/mol, ~250 Da or amu)
- The peptide backbone is in the same plane, hybridization of the carbon atoms
- This dipeptide prefers to be in a trans position
- Position of amino acids: Cys at C-terminus, Glu at N-terminus
- The side chains of the amino acids are attached at the alpha carbon
- Description of bonds within the molecule (character, length, ability to rotate)

Properties or expected behavior of molecule (*structure-property relationships*)

- Any indication of polarity, resulting inability to pass through cell membrane
- Description of titration curve or buffer regions – should have two buffering zones
- Description of ionization steps, relating protonation/deprotonation to pKa and pH values
- Listing of generalpKa values – C-terminus is ~ 2, Carboxylic acid R group is ~ 4, thiol R group is ~ 8, N-terminus is ~ 10
- pI calculation
- Mention of cysteine's ability to crosslink with other cysteines through disulfide bonds/bridges, the reactivity of the thiol group in general
- Mention of hydrogen bond formation due to specific functional groups
- Water soluble
- Can act as an acid or a base, description of which specific groups act as an acid (amine) and base (thiol, carboxyl), nucleophile activity/strength
- Description of peptide bond formation, additional amino acids can be added to this dipeptide
- Mention of the resonance exhibited in the carboxyl groups and the peptide backbone, stability conferred by resonance

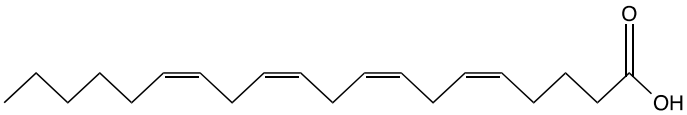
Biosynthesis/metabolism

- Peptide bonds are formed by a condensation reaction (water is produced), this reaction requires input of energy (ATP → ADP + P_i)
- The peptide bond can be hydrolyzed (addition of water molecule)
- The site of amino acid catabolism is the liver and/or the muscle

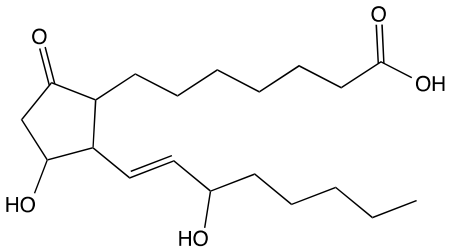
CE2 – Fatty acids (this heading was not included in student version)

Write down as many **correct, distinct, and relevant facts** as you can about **both molecules**:

Molecule A



Molecule B



Five (5) statements will get you full credit for the problem, which is worth 5 points. Recall the information you use should be information you learned in a chemistry course, including the general chemistry, organic chemistry, and biochemistry courses. All other outside information, combined, will only count as one distinct fact towards the correct responses. You may list more than five statements.

CE2 general rubric

Molecule identification or classification (*class*)

- Mention of type of molecule (Molecule A is a fatty acid and is arachidonic acid, Molecule B is a type of eicosanoid and is prostaglandin E1)

Composition or structure (*composition & structure*)

- The chemical formula for each molecule ($C_{20}H_{32}O_2$, $C_{20}H_{34}O_5$) or referencing the types and number of atoms
- The molecular weight of each molecule (~304 amu, ~354 amu)
- Degree of unsaturation: These are unsaturated molecules, Molecule A contains four cis double bonds (at 5, 8, 11 & 14 positions), Molecule B has one trans double bond
- Functional groups: Molecule A has a carboxylic acid group, Molecule B has a ketone, also has a terminal carboxylic acid group and two alcohol groups
- Other compositional features: Molecule A has a long hydrocarbon chain, Molecule B has two aliphatic chains attached to the 5-membered cyclic group
- At physiological pH, the carboxyl groups on both molecules will be deprotonated
- The long hydrocarbon chains in both molecules allow them to be flexible, B less so than A
- Mention of stereocenters: there are four chiral carbons in Molecule B, none in Molecule A

Biosynthesis (*composition & structure*)

- Both of these molecules were synthesized in the cytosol
- Precursors: Molecule A serves as the precursor for molecule B (eicosanoids are built from the 20-carbon unsaturated FAs), an essential fatty acid, linoleic acid, is needed to produce molecule A
- Additional double bonds were introduced by the fatty acyl-CoA desaturase in an oxidative reaction and linoleic acid was lengthened by an elongase
- Mention of type of enzyme responsible for synthesizing B or A (fatty acid synthase)

Catabolism (*function, composition & structure*)

- The catabolism of molecule A results in 10 Acetyl-CoA molecules, which can then be oxidized in the citric acid cycle or converted to a ketone body for further storage
- Molecule A can be used to produce energy or ATP, Molecule B is typically degraded and not used for energy production
- Isomerase is needed to isomerize the double bond so that it is between the alpha and beta carbons before the beta-oxidation cycle can occur to break the FAs into Acetyl-CoA units
- Since two of the cis, unsaturated bonds occur at odd-numbered carbons, a reductase is needed to convert the cis bond to trans, which the isomerase can then move to the appropriate location between the alpha and beta carbons

Properties or expected behavior (*structure-property relationships*)

- It is likely that molecule A has a lower melting point than molecule B due to the presence of four, cis unsaturated bonds that prohibit tight stacking

- Both molecules have large nonpolar regions and thus are largely hydrophobic
- Discussion of which regions will interact with water and the membrane, whether these molecules are membrane permeable
- Additional hydroxyl groups on molecule B make it slightly more polar and hydrophilic than A
- Molecule B has a higher pKa, ability of these molecules to possibly act as weak acids
- Mention of the reactivity or stability of parts of the molecules (functional groups, double bonds, nonreducing ends)

Function (*function*)

- Molecule A is used in phospholipids and in cell membranes, molecule B is a type of hormone used in signaling pathways
- Can be utilized to make diacylglycerol or triacylglycerol molecules

CE3 – DNA (this heading was not included in student version)

Write down as many **correct, distinct, and relevant facts** as you can about:

You have two solutions of DNA fragments purified from bacterial cells. In one solution, the DNA is purified directly from healthy bacterial cells. In the other solution, the purified DNA has been damaged by exposure to UV radiation for 10 minutes.

Five (5) statements will get you full credit for the problem, which is worth 5 points. Recall the information you use should be information you learned in a chemistry course, including general chemistry, organic chemistry, and biochemistry courses. All other outside information, combined, will only count as one distinct fact towards the correct responses. You may list more than five statements.

CE3 general rubric:

Normal and damaged DNA characterization (*class, composition & structure, tests & experimental values, structure-property relationships*)

- In DNA, the bases present are adenine, thymine, guanine, and cytosine
- In healthy DNA, adenine pairs with thymine and forms two hydrogen bonds, and guanine pairs with cytosine and forms three hydrogen bonds
- A-T base pairs are easier to separate than G-C base pairs
- Healthy DNA naturally assumes a double-stranded helical shape, and the strands run in opposite directions
- DNA is made up of nucleotides (base + pentose sugar + phosphate group), and these nucleotides are linked through phosphodiester bonds
- UV radiation can chemically alter nucleotides – UV-B (280-315 nm) is light wavelength primarily absorbed by DNA
- UV radiation can produce free radicals (e.g. hydroxyl radicals) which can disrupt DNA strands
- During one type of UV damage, pyrimidine (thymine & cytosine) base pairs that are stacked on top of each other dimerize into pyrimidine dimers (can form 6-4 photoproduct with a covalent bond formed, or can form cyclobutane linkages)
- The pyrimidine dimers are an example of intrastrand crosslinking
- The type of damage caused by UV radiation typically results in conformational changes and unpaired bases in the DNA strand, preventing DNA replication and transcription, but healthy DNA can replicate normally
- Damaged DNA results in unstable DNA, if it is not repaired can result in DNA degradation or cell death in functioning cells
- Thymine dimers (cyclobutane pyrimidine dimer – CPD) can be repaired successfully, whereas when cytosine forms a CPD it generally is deaminated, which cannot be repaired as easily and causes it to be turned into a uracil instead, which is later read as thymine

Cellular response to DNA damage (*composition & structure, structure-property relationships*)

- The conformational change or bulge in the DNA resulting from the damage is recognized by repair enzymes
- DNA photolyase is one type of repair enzyme, explanation of this type of repair
- A DNA repair complex consisting of a exonuclease, polymerase, and ligase can be used to cut the damaged portion of the DNA strand (exonuclease), synthesize a new complementary strand based on the remaining undamaged DNA strand (polymerase), and stitch the new strand back into the DNA (ligase) in nucleotide excision repair (also includes general statements about polymerase, exonuclease, etc.)
- There are two types of nucleotide excision repair: one occurs during transcription, and the other occurs when a distortion in the DNA helix is found by the repair complex

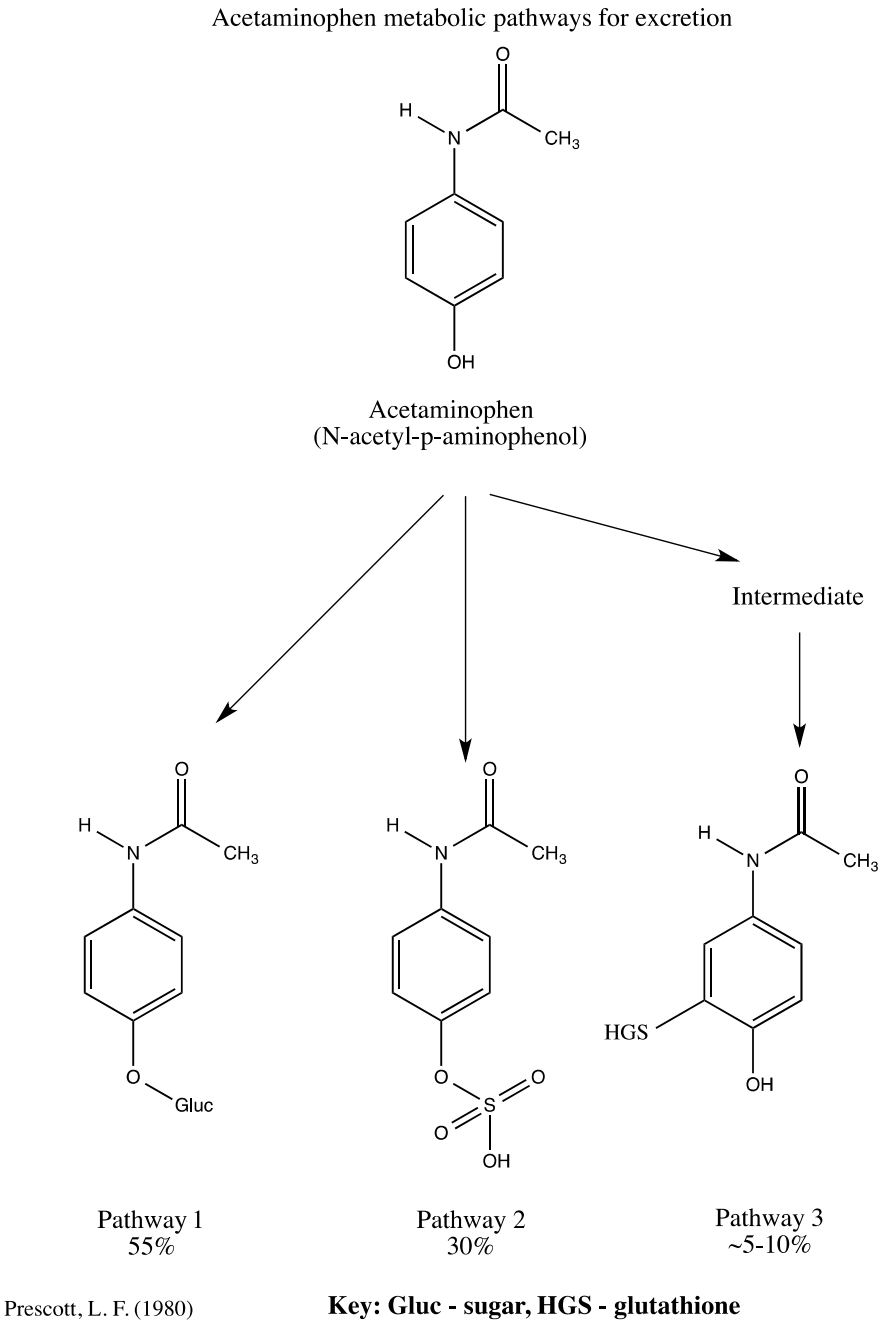
DNA detection or separation (*tests & experimental values, structure-property relationships*)

- Can use centrifugation to separate DNA fragments based on their SD values (sedimentation coefficients)

- Use gel electrophoresis to separate DNA fragments based on their negatively charged phosphate backbone, which is relative to their size and shape – compare to a standard to evaluate length of fragments
- Can use ion-exchange chromatography to separate DNA fragments
- PCR can be used to amplify selected DNA fragments in the solutions for further analysis

CE4 – Acetaminophen metabolism (this heading was not included in student version)

Write down as many **correct, distinct, and relevant facts** as you can about:



Five (5) statements will get you full credit for the problem, which is worth 5 points. Recall the information you use should be information you learned in a chemistry course, including general chemistry, organic chemistry, and biochemistry courses. All other outside information, combined, will only count as one distinct fact towards the correct responses. You may list more than five statements.

CE4 general rubric:

Characteristics of acetaminophen(*class, composition & structure, function*)

- Another name for acetaminophen is paracetamol
- The molecular formula of acetaminophen is $C_8H_9NO_2$
- Acetaminophen acts as a pain reliever and fever reducer
- Acetaminophen consists of a benzene ring with a hydroxyl group and an amino group attached in para (1,4) positions
- Acetaminophen can be considered a xenobiotic, or a substance foreign to the body that needs to be excreted
- The acetaminophen has polar groups

Reactivity (*composition & structure, structure-property relationships*)

- Acetaminophen as a whole is very conjugated due to the overlapping pi bonds in the benzene ring and the lone pairs of electrons on the nitrogen and oxygen atoms
- The conjugation draws electron density away from the H in the hydroxyl group, and thus its removal is stabilized by the rest of the compound making it slightly acidic, and the oxygen is able to act as a nucleophile
- The amide and hydroxyl functional groups of acetaminophen are ortho, para directors because they have lone pairs available for donation
- The benzene ring can act as a nucleophile
- The glutathione can form bonds through its thiol
- It is more favorable to attach substituents through the hydroxyl group than directly to the benzene

Final products (*composition & structure, structure-property relationships*)

- The product of each pathway has a different functional group attached: for pathways 1 and 2, the hydrogen is lost and replaced by a sugar (1) or a sulfate group (2), and in pathway 3, a glutathione group has been added in a meta position
- The metabolism of acetaminophen involves adding moieties to decrease its activity in the body and increase its solubility so it can be excreted via the kidneys, makes it difficult for the products to cross membranes
- Pathways 1 and 2 are the major routes of metabolism for acetaminophen, and are responsible for acting on ~90% of ingested acetaminophen, the added moieties are in abundance in the body
- If glutathione were to be used up, the intermediate formed is toxic and can negatively impact the liver
- In physiological conditions, it is likely that the sulfate and glutathione groups and possibly the hydroxyl group will be deprotonated
- In pathways 1 and 2, the substituent attaches to the hydroxyl group (two total substituents) while in pathway 3 the substituent attaches directly to the benzene ring (three total substituents)
- The added substituents all increase the polarity of acetaminophen

Comparison of pathways

- Pathway 1 is favored, since 55% of ingested acetaminophen is excreted through this pathways
- Pathway 3 might be less favorable because it requires an intermediate, or possibly because of steric hindrance from the GSH, a cofactor is needed
- Different enzymes are used in each pathway: the glucose group was added by a glucuronosyltransferase in pathway 1, the sulfate group was added by a sulfotransferase in pathway 2, and in pathway 3 the acetaminophen was activated by cytochrome P450 and a glutathione was subsequently added via a glutathione transferase

Student profiles

The following tables represent the total distribution of student responses to the CEs that were related to CI themes along with the distributions for two students in the course. The numbers represent the total number of statements provided in responses to a specific CE that had precursors of CI thinking related to one of the CI themes. The numbers do not reflect whether a student received credit for these statements, and thus are not reflective of their scores.These distributions are not intended to be viewed as a "goal," but can be used qualitatively by instructors to examine the types of responses students provide on the CEs. The total distribution of student responses can be used to understand what types of responses are more likely than others for each CE.

Key:

- C&S: composition & structure
- Tests & values: tests & experimental values
- SPR: structure-property relationships
- NR: not related to either CI or SPR

Total distribution of student responses

	Change	Class	Composition & Structure	Function	Organism effect	Sensory info	Source	Tests & exp. values	SPR	NR
CE2	0	25	78	15	0	0	0	0	51	10
CE3	5	8	47	2	0	0	0	12	15	45
CE4	0	1	60	5	1	0	5	0	29	36
Total	5	34	185	22	1	0	5	12	95	91

Profile 1: Riley

Riley received 100% on every CE. Riley's profile is shown in the table below. Additionally, Riley's responses to CE2 have been reproduced. Each numbered statement represents a statement made by Riley in response to CE2, as written by Riley. Each statement made by Riley was assigned to a rubric statement, which has been reproduced beneath each of Riley's statements. The rubric statements were coded for the presence of precursors to CI thinking, structure-property relationships, or coded as not relevant for either, and the coding for each rubric statement has been provided in italics.

Distribution of statements

	Source	Change	Organism	C&S	Class	Function	Tests	SPR	NR
CE2	0	0	0	3	1	2	0	4	0
CE3	0	0	0	2	0	1	1	0	5
CE4	1	0	0	1	0	1	0	1	3

CE2 responses

1. Molecule A is an unsaturated fatty acid
    a. Mention of type of molecule (Molecule A is a fatty acid and is arachidonic acid, Molecule B is a type of eicosanoid and is prostaglandin E1) - *class*
2. Molecule A has 20 carbon, so it's very hydrophobic in x area (student labeled the hydrocarbon tail of molecule A as "x")
    a. The chemical formula for each molecule ($C_{20}H_{32}O_2$, $C_{20}H_{34}O_5$) or referencing the types and number of atoms – *composition & structure*
3. The reactive portion of molecule A is the y area (student labeled the carboxyl group as "y")
    a. Mention of the reactivity or stability of parts of the molecules (functional groups, double bonds, nonreducing ends) – *structure-property relationships*
4. Molecule A has a carboxylic group, which can be used in H-bonding.
    a. Functional groups: Molecule A has a carboxylic acid group, Molecule B has a ketone, also has a terminal carboxylic acid group and two alcohol groups – *composition & structure*
5. This molecule can be added or combined with two other fatty acids to make TAG or triacyl glycerol
    a. Can be utilized to make diacylglycerol or triacylglycerol molecules - *function*
6. There are all cis double bonds in molecule A
    a. Degree of unsaturation: These are unsaturated molecules, Molecule A contains four cis double bonds (at 5, 8, 11 & 14 positions), Molecule B has one trans double bond – *composition & structure*
7. Molecule B also has a COOH group, but OH groups in all portions, so it's a polar molecule (first part of statement combined with statement 4 for credit and for analysis and coded as a, second part of statement coded as b)

    a. Functional groups: Molecule A has a carboxylic acid group, Molecule B has a ketone, also has a terminal carboxylic acid group and two alcohol groups – *composition & structure*
    b. Additional hydroxyl groups on molecule B make it slightly more polar and hydrophilic than A – *structure-property relationships*
8. Molecule B can make multiple hydrogen bonds
    a. Mention of the reactivity or stability of parts of the molecules (functional groups, double bonds, nonreducing ends) – *structure-property relationships*
9. This molecule (B) is more rigid in structure than molecule A
    a. The long hydrocarbon chains in both molecules allow them to be flexible, B less so than A – *structure-property relationships*
10. Molecule B is also capable of H-bonding (not unique compared to statement 8, combined for credit and analysis)
    a. Mention of the reactivity or stability of parts of the molecules (functional groups, double bonds, nonreducing ends) – *structure-property relationships*
11. The hydrophobic and hydrophic (sic) nature of molecule B makes it ideal for being embedded in phospholipid membrane (incorrect)
    a. Molecule A is used in phospholipids and in cell membranes, molecule B is a type of hormone used in signaling pathways – *function*
12. Molecule is capable of further unsaturation through z area (combined with statement 3 for credit and analysis)
    a. Mention of the reactivity or stability of parts of the molecules (functional groups, double bonds, nonreducing ends) – *structure-property relationships*

Profile 2: Harper

Harper received an 80% average across the CEs. Harper's profile is shown in the table below. Additionally, Harper's responses to CE2 have been reproduced. Each numbered statement represents a statement made by Harper in response to CE2, as written by Harper. Each statement made by Harper was assigned to a rubric statement, which has been reproduced beneath each of Harper's statements. The rubric statements were coded for the presence of precursors to CI thinking, structure-property relationships, or coded as not relevant for either, and the coding for each rubric statement has been provided in italics.

Distribution of statements

	Source	Change	Organism	C&S	Class	Function	Tests	SPR	NR
CE2	0	0	0	1	1	0	0	3	0
CE3	0	0	0	2	0	0	0	1	1
CE4	0	0	0	3	0	0	0	1	1

CE2 responses

1. The chemical formula of A is $C_{20}H_{32}O_2$ and the chemical formula of B is $C_{20}H_{34}O_5$
    a. The chemical formula for each molecule ($C_{20}H_{32}O_2$, $C_{20}H_{34}O_5$) or referencing the types and number of atoms – *composition & structure*
2. Molecule A is a saturated fatty acid (incorrect)
    a. Mention of type of molecule (Molecule A is a fatty acid and is arachidonic acid, Molecule B is a type of eicosanoid and is prostaglandin E1) – *class*
3. Both molecules A and B can form thioester bonds.
    a. Mention of the reactivity or stability of parts of the molecules (functional groups, double bonds, nonreducing ends) – *structure-property relationships*
4. Molecule B is a more polar molecule than A because it has more ketone and hydroxyl groups.
    a. Additional hydroxyl groups on molecule B make it slightly more polar and hydrophilic than A – *structure-property relationships*
5. Molecule B has more reactive portions than Molecule A (incorrect)
    a. Mention of the reactivity or stability of parts of the molecules (functional groups, double bonds, nonreducing ends) – *structure-property relationships*