**Teaching Notes: Maria vs Malaria**

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5. **About the Molecular Case Study:**
6. **Case Title**: Maria vs Malaria
7. **Discipline(s):** Biochemistry
8. **Keywords**:Lactate Dehydrogenase (LDH), malaria, competitive inhibitor, Azole
9. **Abstract**:

The case discusses an environmental scientist Maria’s search for an antimalarial drug treatment after she contracts Malaria during her research trip to the Amazon Rainforest. The case begins with her confirming that her symptoms were from Malaria via rapid diagnostic and then her doctors identifying the specific Malaria parasite to be *Plasmodium falciparum.* Through information she received from her colleague at the Center for Disease Control (CDC) and her own research, she starts focusing on a newly identified Lactate Dehydrogenase inhibitor to determine if it could be used as a safe and effective treatment for Malaria. The case explores the structure-function relationship of the critical metabolic enzyme Lactate Dehydrogenase and exemplifies how small molecular differences between the active sites of the same enzyme from two different organisms (in this case *Plasmodium Falciparum*and human)could be leveraged to develop selective competitive inhibitors of the enzyme to provide safe and effective drug treatments.

1. **Molecules explored**:

The primary molecule studied in this case is Lactate Dehydrogenase. Visualization and exploration of both the parasitic and human structures of this enzyme are carried out throughout the case. The other two important molecules visualized are the coenzyme NADH (Nicotinamide adenine dinucleotide) and the competitive inhibitor

1. **About the Course:**

*Use this information for planning the implementation of this molecular case study.*

1. **Department**: Chemistry
2. **Course Name**: Principles of Biochemistry
3. **Audience**: Undergraduate
4. **Level**: Upper Division, STEM Majors

Note: The audience for this course was juniors and seniors but the course was an introductory biochemistry course.

1. **How does this case study and molecular visualization fit into the course curriculum?**

The course covers protein structure, function, with a dedicated module on enzyme kinetics and function, including a detailed coverage of active site structure, substrate binding, catalytic mechanisms, and different types of enzyme inhibition, followed by carbohydrate metabolism. This case is used at the beginning of the carbohydrate metabolism unit right after the completion of the enzymes unit to reinforce concepts on enzyme structure function through an important enzyme in carbohydrate metabolism.

**4. Societal Relevance:**

The main character is a woman researcher who contracts a neglected tropical disease during her trip for work. Using suggestions and pointers from her colleague in the CDC (a national information site) she sets out to do her own research to participate in her own treatment.

1. **LEARNING GOALS**

**\*A1. Main biology-related learning goals (1–3 major goals) related to:** refer to the [ASBMB Foundational concepts](https://www.asbmb.org/education/core-concept-teaching-strategies/foundational-concepts) and [Biocore Guide](https://www.lifescied.org/doi/10.1187/cbe.13-12-0233#F3a)

* Learning Goal 1: ASBMB Energy and Metabolism-
	+ 2. Catalysis; Students should be able to explain what a substrate is in terms of being a reactant and how a molecule resembling a substrate can act as a covalent inhibitor.
* Learning Goal 2: ASBMB Structure and Function-
	+ 2. Structure is determined by several factors:
		- Students should be able to compare and contrast the primary, secondary, tertiary, and quaternary structures of proteins and nucleic acids
		- Students should be able to use various bioinformatics and computational approaches to compare primary sequences and identify the impact of conservation and/or evolutionary change on the structure and function of macromolecules.
		- Students should be able to predict the effects of mutations on the activity, structure, or stability of a protein

**\*A2. Main chemistry-related learning goals (1–3 major goals):** refer to the [Macromolecular, Supramolecular, and Nanoscale (MSN) Systems in the Curriculum](https://www.acs.org/content/dam/acsorg/about/governance/committees/training/acsapproved/degreeprogram/macromolecular-supramolecular-nanoscale-supplement.pdf)

* Learning Goal 1: Physical Properties of MSN Systems: Students should be able to describe intramolecular interactions and relationship of structure and function
* Learning Goal 2: Biochemistry: Students should be able to explain how the intermolecular interactions of biopolymers and influence of polymer primary, secondary, and tertiary structure

**\*A3. Molecular Modeling/Visualization Learning Goals**: refer to the [BioMolViz Framework](https://biomolviz.org/framework/)

* Learning Goal 1: MI2.06: Using molecular visualization tools, students can determine which intermolecular force is most critical to stabilizing a given interaction
* Learning Goal 2: LM2.03: Students can use molecular visualization tools to predict how a specified ligand or modified building block contributes to the function of a given protein.

**\*A4. Bioinformatics Learning Goals and Skills used in the case**: refer to [NIBLSE Bioinformatics Core Competencies](https://doi.org/10.1371/journal.pone.0196878.t002)

* Learning Goal/Skill 2: NIBLSE: C4: Students will be able to use bioinformatic tools to examine complex biological problems.
* Learning Goal/Skill 3: NIBLSE: C5: Students will be able to find, retrieve, and organize biological data from protein databases.
* Learning Goal/Skill 4: NIBLSE; C6. Explore and/or model biological interactions, networks, and data integration using bioinformatics.

**A5. Other sub-disciplinary learning goals:** *Please specify the sub-discipline and specific learning goals framework, if any.*

* Subdiscipline: Parasitology
* Learning Goal 1: Students will be able to identify a common metabolic step between a parasite and a human and explain how this commonality can be leveraged to develop selective treatments against the parasite
1. **BACKGROUND MATERIALS**

|  |  |
| --- | --- |
| **Type of Background Material** | **Link to the Material** |
| Review/ Journal article | Malaria in Brazil: an overview. *Malar J* 9, 115 (2010). <https://doi.org/10.1186/1475-2875-9-115>Optional:The proliferating cell hypothesis: a metabolic framework for *Plasmodium* growth and development. <https://doi.org/10.1016/j.pt.2014.02.001> Modeling of the Glycolysis Pathway in *Plasmodium falciparum* using Petri Nets. <https://doi.org/10.4137/BBI.S37296>  |
| Video or other educational resource  | Malarial Parasite Detection by Rapid Card Method: <https://www.youtube.com/watch?v=hft79S__C_8>Malaria management by community health workers: <https://vimeo.com/88041399>Malaria and Life Cycle of Plasmodium: <https://www.youtube.com/watch?v=BVRnNbb9cLU>  |
| A reliable website  | Malaria Facts: who.int Parasites: <https://www.cdc.gov/parasites/about.html>  |

1. **BIOINFORMATICS RESOURCES AND TOOLS USED**

*Educators: Use this information to select the case study or sections of the case study for planning implementation in your course.*

|  |  |  |
| --- | --- | --- |
| Bioinformatics Resource | Used (mark with x) | Task accomplished in the MCS  |
|  UniProt | X | To find specific sequences for Plasmodium falciparum and humanLDHTo link to Protparam Tool to retrieve basic physicochemical information about the sequencesTo link to Align Tool to align the two sequences and to compare and contrast them using the built in criteria (Physical Properties, Chemical Properties, and sequence specific properties derived from annotations) |
| KEGG |  |  |
| Binding DB | X | To obtain the KI of the inhibitor drug (OXQ) |
| DrugBank  | X | The development and uses of Chloroquine (discussed in the assessment section) |
| Gene Ontology |  |  |
| Other  |  |  |

|  |  |
| --- | --- |
| Name of Bioinformatics or Molecular Visualization Tool | Task accomplished in the MCS  |
| Pairwise Structure Alignment tool | Align Tool within UniProt to align sequences. |
| Mol\*  | To visualize the structures of pfLDH and hLDH-B with and without different ligands. Students also use this tool to measure distances and display non-covalent interactions. |

1. **SUGGESTED IMPLEMENTATION**

*This section can help teachers select, prepare, and implement the case study.*

**D1. Prerequisites (if any) –** *e.g., skills, concepts, and resources that instructors and students should know about in order to participate in the case study*.

This case uses Mol\* as the molecular visualization tool to explore structures. Even though it does not assume that the students have used the tool before, familiarity with the platform using smaller activities would certainly decrease the cognitive load on the students and play a role in how much time it will take for students to complete each question.

The bioinformatics section of the case is written with explicit instructions with no prior familiarity assumed. However, if students are familiar with the idea of sequence alignment and the impact of amino acid conservation or changes at the sequence level on function more in depth exploration and discussion can be carried out.

In terms of concepts, it is written with the intention to give students who have covered enzyme structure and kinetics a real case example to practice what they have learned and not to teach enzyme kinetics. So, if students have never done enzyme kinetics either a mini lecture would be necessary. Similarly, the students are expected to be familiar with the main ideas and major steps of carbohydrate metabolism, especially the difference between the fates of pyruvate in aerobic vs anaerobic respiration.

**D2.a. Suggested Instructor Preparation** – *e.g., training in a specific molecular visualization program, reading a specific review article on the topic, preparing a lecture on topic/theme discussed in the case* -

Note: The level of required instructor preparation would depend on the level of students’ comfort level in each of the mentioned areas. The case does not assume prior knowledge, however, to facilitate productive discussions the instructor should have more familiarity with the concept and the skill s/he plans to focus on than the students.

* Instructor preparation Step 1: Review of the metabolic step involving Lactate Dehydrogenase enzyme as well as a basic understanding of LDH structure and function including the idea of isozymes
* Instructor preparation Step 2: Familiarity with Mol\*
* Instructor preparation Step 3: Familiarity with UniProt
* Instructor preparation Step 4: Familiarity with the basic similarities and differences between parasites and higher eukaryotes.

**D2.b. Suggested time for instructor preparations**:

# Please see the note for B2.a**.** The time will greatly depend on at what level the instructor would like to implement the case. For the bare minimum the instructor should invest time to fully complete the case by themselves and reviewing the background materials which is expected to take a minimum of 2-4 hrs

**D3.a. Suggested Student Preparation** - *these could include providing basic experience with molecular visualization, reviewing background knowledge about the case theme and molecule(s)*

Note: In my implementation, this is the second molecular case study students do. The first is a case study on Hemoglobin (Nicholas’s Story) where some of the explorations are done using premade scenes. Any similar small activities would be good preparations. For navigating PDB using the exercise “Interrogating the PDB” is recommended – see <https://pdb101.rcsb.org/teach/box-of-lessons/topics/biological-macromolecules>

* Student preparation Step 1: Introduction and small exercises using the databases UniProt and PDB
* Student preparation Step 2: Simple exercises of loading in and visualizing any PDB ID

**D3.b. Suggested time for student preparation**:
If integrated well into the curriculum (both in terms of content and skills) students do not need any additional preparation for the case.

# Note: To save class time, I assign the earlier part as the “preparatory work” to be able to focus on the 3D exploration section in class.

**D4. Suggested implementation details**

* Resources

All required links are included in the case and a few additional resources about the background are included in the instructor version of the case

* Implementation timeline suggestions
	+ Time required for students to read/view the case related material prior to start of the molecular case study: I assign the part until the end of Q13 as pre-class work where the students can enter many of the answers to the questions via an online platform (I use TopHat but can also be done in any LMS). The students are expected to complete this pre-work in ~ 1 hr.
	+ Minimum in-class time required:

The ideal time to complete the rest of the case (without the assessment) would be 1.5- 2 hrs:

1st hour: Explore the higher structure LDH, the individual subunit, and the active site within the subunit with its cofactor NADH. (Q14-Q20)

2nd hour: Complete the “Connecting Structure to Function” section Q21-Q30.

Author comments:

“However, since I have my students complete Nicholas's Story (a MCS on Hb) earlier in the semester during a 1.5 hr session, I use a 50 min discussion time slot to complete as much of this case in class and assign the rest of it as post-class work.

Field implementation in S23 showed that most students were able to get to “Connecting Structure to Function” section in the 50 min period. Whether or not they were able to start the next section was highly dependent on how much of the last part of the pre-work (Q11-13) they had completed before coming in as those questions required them to remember and practice some of the most common commands in Mol\* that they had first learned during the Hb MCS.

To help students progress as far as they could without losing on content or skills, I have students work through the in-class part in pairs, one student carrying out the instructions using the parasite LDH PDB structure and the other the human LDH structure. To ensure that both students are actively participating in the exploration and answering the questions, one student puts a copy of the MCS in their google folder and shares it with their partner. Then both students place their figures for the structure they are exploring on this shared google doc side by side and answer the questions that require comparison of the findings together. This approach significantly shortens the in-class time needed for implementation of the case.

Having teaching assistants and former undergraduate students of this course who have done the case previously come in to help during implementation by being facilitators to a few groups at a time, also helps a lot in ensuring that students don’t get stuck too long in certain areas due to lack of experience with the tools (esp. Mol\*)”

* + Time outside class needed to complete the case:

See above. I assign the first part as a flipped activity prior to in-class time with an expectation that students will spend ~ 1-1.5 hrs to complete it.

* If the case study needs to be implemented in multiple sessions, suggest in-class activities for each session
	+ Session 1: Case context, Getting to the Structure, and Exploring the structure (Exploring the primary Structure of *Plasmodium falciparum* LDH (*pf*LDH) and *human* LDH (*h*LDH) section) This part can be assigned as flipped.
	+ Session 2: Exploring the Structure (starting from exploring the higher order structure of LDH using PDB and Mol\* section),
	+ Session 3: Connecting Structure to Function section
* (Expected/ observed) Bottlenecks and Suggestions:
	+ Students need time to get used to some functions requiring the selection mode “on” and some “off”. This confusion is a significant time sink when students are asked to change representation or color a single component and then asked to create a focus area around it.
	+ If you are considering assigning the last section “Connecting Structure to function”, I recommend that you have students do a “measurement” exercise once they visualize NADH in the active site. How to make a measurement is described for measuring distances between the inhibitor and the enzyme in steps 23-25.

**D5. Provide suggestions for how this case study may be used in other courses/settings:** *list if any of the sections of the case study need to be modified for a different course setting*

* Other course/settings 1: This case has been used in a parasitology course by Dr. Swati Agrawal.
* Suggested changes: More emphasis was given to the background on the parasite life cycle and the implications of inhibition of this enzyme in the survival of the parasite as well as understanding malaria.