Teaching biologics formulation using molecular modeling and simulations

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Abstract

Teaching chemistry and chemical engineering students about biologics formulation remains challenging despite its increasing importance in pharmaceutical development. Monoclonal antibodies, commonly called mAbs, are the most popular biologics. They have been developed into drugs to treat various diseases in the past decades. Multiple challenges exist for designing proper formulations to stabilize mAbs, such as preventing aggregation and mitigating viscosity. Molecular modeling and simulations can improve pharmaceutical products by examining the interactions between mAbs and other compounds, such as excipients. To introduce students to biopharmaceuticals, eight chemical engineering students at the Stevens Institute of Technology participated in a semester-long course to learn the challenges of pharmaceutical development and different computational skills to study biologics formulation. The students started with a limited background in this field. Throughout one semester, they were introduced to various literature and software tools for modeling antibodies and studying their interactions with excipients. Positive learning outcomes were achieved through pre- and post-course assessments. This paper aims to develop a course structure to be replicated at other universities and institutions to teach biopharmaceutical development to students.

Keywords: Monoclonal Antibodies; Excipients; Formulations; Simulation tools; Computer-Based Learning

Abbreviations:

CDR: complementarity determining region

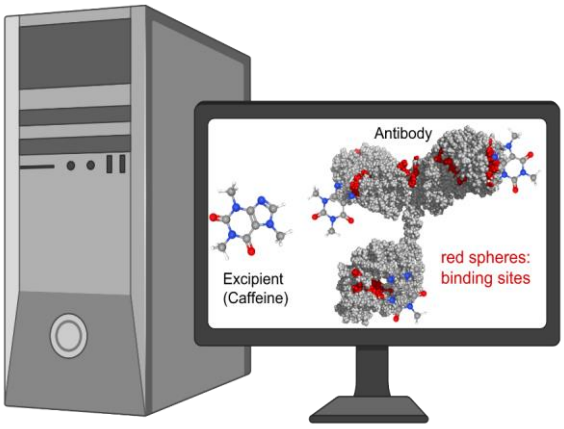
Fv: variable fragment

Fab: Fragment antigen-binding

IgG1: immunoglobulin G1

mAbs: monoclonal antibodies

MD: molecular dynamics



1. Introduction

Biopharmaceutical products, such as monoclonal antibodies (mAbs), demonstrate promise in treating many diseases and disorders (Kaplon et al., 2022). Developing antibody drugs need a proper formulation to stabilize the biopharmaceutical products, which requires knowledge of proteins (Roberts et al., 2014) and small molecules as excipients for formulation development (Hu et al., 2020). Because of the high cost of mAbs production, computer modeling, and simulation tools are desirable to facilitate formulation design (Blanco, 2022; Lai, 2022; Lai et al., 2021).

The current chemistry and chemical engineering curricula lack courses that teach the challenges and tools in biotherapeutics development. Biochemistry classes introduce students to protein structures, functions, and interactions. Organic chemistry requires students to understand the reaction mechanisms and behaviors of small molecules. Unfortunately, none of these courses, or the others in the curriculum, provide a comprehensive understanding of the interactions between small and large molecules. Incorporating such curricula would benefit students since such concepts are the pinnacle in large molecule drug applications, also known as biotherapeutics. Formulation design of biomolecular drugs cannot be done without understanding protein-protein interaction (Arora et al., 2016; Hung et al., 2019), excipient-protein interactions (Hribar-Lee, 2022; Hu et al., 2020), and more.

Some prior studies implemented computational tools for enhancing STEM student curricula. For example, computer simulations have been applied to teach cellular metabolism (Chaves et al., 2022) and dynamic mechanisms in signaling pathways (Sun and Zhao, 2023). Moreover, computational methods have been implemented to teach molecular modeling and drug design. Clauss and Nelson described the importance of integrating molecular modeling into introductory and intermediate-level organic

chemistry courses (Clauss and Nelsen, 2009). They proposed a curriculum for each unit, such as different types of organic reactions, and the plan to revise the curriculum each semester. The authors mentioned that this integration aims to use modeling more generally across the curriculum and help build skill and familiarity from the basics of organic chemistry. In another article, Bain et al. used custom-made 3D physical models such as α-helices and β-sheets, the zinc finger moiety, β-globin, and green fluorescent protein to help students understand the different structures of biomolecules (Bain et al., 2006). These models are advantageous because students could manipulate different backbones and side chains or recognize different features using different colors that they could not do with 2D images. The students were assessed before and after the activity on the same concepts, and the scores significantly improved on the post-test. This assessment shows the success of the training and the benefit of using 3D models. In addition, Tantillo et al. implemented computer-aided drug design to teach protein−ligand docking to undergraduates. Students have an increased appreciation of how chemical principles are applied to the drug discovery−development process (Tantillo et al., 2019). Finally, Yang et al. applied Rosetta software, a protein design tool, to address the challenge of improving the success rate of current computational methods for the de novo design of self-assembling protein complexes (Yang et al., 2022). Their research aimed to estimate the effect of point mutations at a particular position in a protein, through which students learned how to design computational experiments, maintain and analyze large amounts of data in collaboration, and develop scientific hypotheses from a fully remote environment. These studies demonstrated the advantage of incorporating molecular modeling in courses to enhance student learning. However, these works focused on molecular modeling for either large biomolecules or small organic molecules. There is a critical need to develop a curriculum that integrates

proteins and small molecules in computer modeling, which is essential for biopharmaceutical development and biologics design.

At Stevens Institute of Technology, the students undergo a rigorous, design-based curriculum that allows them to gain valuable experience applying knowledge before they reach the workforce. The research experience is organized with one instructor for two groups of four students each. These groups meet once per week with the instructor. The pedagogical methods include weekly assignments of literature review and in-class lectures on programming and modeling. The precedent of work is built around the instructor's prior experience with protein and antibody modeling. This research opportunity allows students to learn critical computational skills useful for protein modeling and important research skills necessary for academic journal writing. It has been shown that course-based undergraduate research experiences (CUREs) increase knowledge retention, academic performance, and the development of scientific process skills (Crisp and Cruz, 2009; Gershenfeld, 2014; Linn et al., 2015). Additionally, the non-traditionality of the research compared to classroom-set education allows students to experience learning in new and exciting ways, further developing their interest in academics and their advancement of knowledge.

2. Program Overview

Eight undergraduate students in two groups, mentored by one instructor, participated in the senior design course in the chemical engineering department at Stevens Institute of Technology. This course aims to teach the students the development and challenges of biopharmaceutical drugs through literature reviews and computational skills to facilitate biologics design. The learning objectives of the 10-module program are outlined in Table 1. There are several activities in the ten modules, each taking 1-2

weeks (2 hrs/week). Each module was allotted 2 hours, with the first 30 minutes for the literature review presentation and the following 1.5 hours for the lecture. The course was laid out so that every week the two groups would be assigned an article for homework regarding the development of antibody drugs and formulation design so that a member from each group would present their findings on the paper the following week. After the presentation, the class would participate in a training activity. The training activity includes molecular visualization software, PyMOL, and Visual Molecular Dynamics (VMD) (Humphrey et al., 1996). In addition, the students also learned how to use the Linux system on the high-performance computing (HPC) system to perform molecular dynamics (MD) simulations using the NAMD software (Phillips et al., 2020, 2005).

Table 1. Overview of the learning objectives in the 10-module program for understanding the development and challenges of biopharmaceutical drugs through literature reviews and computational skills to facilitate biologics design.

	Intended Learning Outcomes
Module 1	(ILO1) Understand the introductory concepts of the development and challenges of high-concentration monoclonal antibody drug development.
Module 2	(ILO2) Understand and present findings regarding the basics of biological drugs and the developmental challenges. (LO3) Develop an understanding of the complexity of antibodies in addition to improving teamwork and communication skills by constructing a physical antibody model.
Module 3	(ILO4) Develop an understanding of how different excipients reduce antibody viscosity by modulating protein-protein interactions. (ILO5) Implement PyMOL to present antibody structures in different graphical representations.
Module 4	(ILO6) Manipulate different fragments of antibody structures in PyMOL to construct a full-length antibody structure.
Module 5	(ILO7) Understand basic Linux commands and perform simulations on supercomputers.
Module 6	(ILO8) Communicate findings from the literature and gain insights on how to write a scientific paper. (ILO9) Adjust manipulated PyMOL files by aligning set template sequences with the target sequences
Module 7	(ILO10) Visualize molecular interactions from molecular dynamics simulations using Visual Molecular Dynamics (VMD).

Module 8	(ILO11) Construct a modeling system of an antibody and perform energy minimization, solvation, and molecular dynamics simulation.
Module 9	(ILO12) Incorporate excipients into the simulation environment to reach established experimental conditions with a desired concentration of the excipient.
Module 10	(ILO13) Conduct a simulation and analyze the excipient-antibody interactions to determine binding sites.

MODULE 1

During module 1, the main objective was to learn an overview of the development of antibody drugs (Jiskoot et al., 2021; Kollár et al., 2020). The instructor asked the students about biopharmaceutics and the challenges and formulation strategies associated with developing these drugs. From the lecture, the students learned that biopharmaceuticals are drugs made from living cells and organisms, the structures of these drugs, especially antibodies, and how they can be used to treat coronaviruses today (Chen et al., 2022). They also learned that high concentrations could lead to high viscosities and the downsides of high-viscosity formulations (Li et al., 2014; Neergaard et al., 2013). The students also learned formulation strategies, such as adding excipients to stabilize the biopharmaceuticals. This activity gave students a better understanding of why computational tools are helpful for antibody drug development (Agrawal et al., 2015; Kuroda and Tsumoto, 2020). The students were also tasked with finding a research paper that detailed biological drugs, their stability issues, and new formulation strategies to tackle biological drug stability.

MODULE 2

For the second module, both groups were asked to answer the stability and delivery issues of biological drugs, specifically mAbs. Instead of being assigned specific articles, the groups searched academic journals for relevant articles. This process forced the teams to comb through many articles on the subject matter to complete the assessment and get more information on mAbs that the first lecture did not cover. The students

learned that antibodies could exhibit aggregation, viscosity, solubility, and conformation instability due to their unique structures and interactions.

After the students presented their research, each team was asked to construct a mAb paper model. This activity is inspired by the Protein Data Bank educational resources (Zardecki et al., 2022). For the learning objectives of this course, manually cutting the materials and putting together the structure allowed them to see the structure of the antibody physically. In the following weeks, when the teams started using computer modeling software, they could better understand the structure and bonds present in the antibody. This activity also allowed the teams to practice communication and teamwork. While the two groups conducted the model, the exercise improved their comfortability with each other and created workflows to create the paper model efficiently.

MODULE 3

For the third module, the second literature review was the same for both groups. The literature review aimed for the students to answer what excipients are and how they affect biopharmaceutical products. The article by Zheng et al. (Zeng et al., 2021) was more accessible to the students. It discussed caffeine as an excipient to lower the viscosity of certain biological drugs, which sparked interest since caffeine is familiar to students. Students could quickly understand the topics discussed in the article while learning more about the topics that the other team highlighted. They learned that excipients are small compounds added to the drug solutions to improve stability. The excipients could interfere with the protein-protein interactions.

This module was also the first class in which PyMOL, a protein modeling software, was introduced. PyMOL has open-source software that is freely available to its users. PyMOL can load proteins to see their structures and sequences. The students were

asked to show antibody structures using different presentations using PyMOL. The group practiced utilizing the program with a guided tutorial from the instructor. PyMOL can zoom in and out, move the structure around, and better visualize parts of the structure. Users can select sequence fragments to change their style and color and export that specific part. They can also make each element a different color. Students learned how to model antibodies in different presentations. They were also introduced to the PDB protein data bank, where they will download any needed structures.

MODULE 4

For module four, the teams were assigned different academic articles for literature review focusing on the same topic, viscosity behavior for mAbs. The goal was for the students to answer the potential causes of high viscosity. Team 1 was given an article by Tilegenova et al. (Tilegenova et al., 2020) about a potential cause for high viscosity in some antibodies, while Team 2 was assigned an article by Wang et al. (Wang et al., 2015) about the effect of some excipients on lowering the viscosity of mAbs. Having the teams present two different articles allowed each team to fill in knowledge gaps and provide clarity to confusing subjects. These articles engaged in more complicated modeling, experimentation, and computations than previous literature review assignments, allowing students to understand the topic further. Students learned that the high viscosity of antibody drugs could be caused by attractive protein-protein interactions that can induce large cluster formation.

The second part of the PyMOL tutorial focused on teaching the students how to manipulate antibody structures in PyMOL. They learned how to properly align the fragment antigen-binding (Fab) structure with the human immunoglobulin G1 (IgG1) template. In addition, students learned how to select the sequences from the target

structure and the template to export two heavy chains and two light chains for building a full-length antibody.

MODULE 5

In module five, the two groups were asked to find examples of molecular modeling for excipient effects on antibodies in recent literature. This week focused on excipient-antibody modeling and simulations for increased stability. For team 1, the article by Jo et al. (Jo et al., 2020) was assigned, while for team 2, an article written by Saurabh et al. (Saurabh et al., 2022) was given. The articles had a higher level of complexity of terms, calculations, and experimentation that provided a more thorough examination and language relating to these areas of study. This practice was meant to challenge and develop students' understanding of antibody-excipient interactions. The students learned how computational tools could be implemented to create a model of a mAb, a model of an excipient, and using a theoretical knowledge of molecular interactions to predict the binding sites of excipients on antibodies.

The second part of module 5 aims to teach students what a Linux system is and how it can be used to process files for modeling. Basic usage of the Linux system and the Vi text editor was demonstrated to prepare students to perform MD simulations on the HPC system. The students were introduced to MobaXterm, an enhanced terminal for Windows that allows connecting to a server on HPC systems. In addition, the students can transfer files to the server through MobaXterm. The groups can access the EXPANSE supercomputers at the SAN DIEGO SUPERCOMPUTER CENTER (SDSC) under the instructor's supervision.

MODULE 6

In module six, the first objective is to learn how to write an educational paper to share their learning experience. Students read studies in peer-reviewed papers and

presented articles on how to write an educational paper (Johnson et al., 2017; Yang et al., 2022). One of the goals of this project was to document their learning. Students learned the layout of the paper, the information included, and the presentation of the results. Thus, the groups would understand what was expected when tasked to write their paper. Additionally, this assignment gave insight into other programs and allowed students to compare the techniques utilized. In the following weeks, the groups would present updates on the progression of the other groups' papers while also gaining insight from the instructor on revisions and additions. The literature reviews were completed in the first half of this program. In the remaining weeks, the students focused on learning how to perform molecular modeling and simulations to study antibody-excipient interactions.

The next objective is to learn how to make essential changes to the protein structures in PyMOL. The final product from the PyMOL activity of module five left the group with a protein with some missing residues and sequence mismatches in its amino acid sequence because it was aligned on a template. To correct this in the structure, the groups learned how to make mutations to the protein using VMD. In addition, the groups learned how to use EMBOSS Needle, a protein sequence alignment tool. With EMBOSS Needle, the groups noted the incorrect or missing residues and then mutated or added the target residues. Once all the residues were mutated to the correct ones, the groups connected the two heavy chains, two light chains, and two glycans by linking all disulfide and glycosidic bonds. Moreover, the students were able to apply the PDB2PQR tool (Dolinsky et al., 2007, 2004) to determine the titration state of the histidine residues on antibodies at a given pH value. At the end of this process, the groups learned to create one complete full-length antibody structure necessary for conducting MD simulations.

MODULE 7

During module seven, the students first learned how to load molecules into VMD and change different colors and graphical representations of the chains. They also learned how to use VMD to visualize molecular motion. In VMD, the students learned to change different drawing methods to help visualize the molecules. Moreover, the students also learned how to view the animation from MD simulations to visualize the dynamics of molecules. The instructor used a case study from Zeng et al. (Zeng et al., 2021) to investigate the interactions between infliximab (an antibody) and caffeine (excipients) that exhibit viscosity-lowering effects. Because MD simulations take a long time, the instructor provided an example output trajectory for students to practice before they started their simulations.

MODULE 8

In module eight, the students learned the concept of MD simulations. The instructor guided the students to use NAMD (Phillips et al., 2020, 2005), a parallel molecular dynamics code designed for high-performance simulation of large biomolecular systems, to conduct energy minimization, heat the system, equilibrate the system, and perform the production run by providing the students a script. In addition, the VMD tool was used to solvate the protein in water and add ions to neutralize system charges. The students learned to complete these tasks by submitting their jobs to the HPC system and using NVIDIA V100 graphics processing units (GPUs) to accelerate the simulations.

MODULE 9

For module nine, the groups learned how to add excipients, like caffeine, to the system using the Packmol software (Martínez et al., 2009), a tool for generating initial configurations for MD simulations by packing optimization. In addition, the students learned how to use the CGenFF tool to generate force field parameters for excipients.

During the second part of the NAMD tutorial, the students were able to calculate the number of caffeine molecules that needed to be added to the system to reach experimental conditions. After minimization, solvation, and heating, the students ran equilibrium and production simulations for further analysis.

MODULE 10

In module ten, the groups calculated the number of contacts between the caffeine molecules with each residue of infliximab using a VMD script provided by the instructor. The students implemented PyMOL to analyze the binding sites of the caffeine molecules on antibodies. This analysis helps the students to study the antibody-excipient interactions essential to their viscosity-modulating effects. They found that most of the binding sites are hydrophobic residues.

3. Software instruction and class exercises

In this work, we developed several tutorials to use standard molecular modeling and simulation software to demonstrate how to build antibody models and perform MD simulations for antibodies and antibody-excipient systems. After each tutorial, the students would answer several questions from class exercises to evaluate their understanding of each topic.

3.1. Building a full-length antibody model

This exercise demonstrates the basic structure building for antibody models and PyMOL usage, which complements the antibody paper model activity introduced in module 2.

Students will be able to (ILOs):

1. Implement PyMOL to present antibody structures in different graphical representations. (ILO #5)

2. Manipulate different fragments of antibody structures in PyMOL to construct a full-length antibody structure. (ILO #6)

PyMOL software is used to process and build the antibody structures. There is commercial and open-source PyMOL software. An instruction to install open-source PyMOL is provided in the Supplementary Material.

A full-length antibody model is not usually available. Therefore, we need to build one from existing Fab crystal structures or Fv homology models (Leem et al., 2016). The Fab crystal structures can be obtained from Protein Data Bank (PDB). After downloading the PDB file, we need to clean the PDB file to remove water, ions, ligands, and other proteins. We can use PyMOL to select antibody heavy chain and light chain sequences and export their structures. Figure 1 shows an example of the infliximab Fab structure (PDB code: 4G3Y) and how to use PyMOL to export the target structure. We can select the heavy and light chain residues using PyMOL and export the selected structure.

The clean Fab structure was saved as Infliximab_Fab.pdb. Because there are two Fab regions for a full-length antibody, an identical Fab structure was created and saved as Infliximab_Fab2.pdb. We aligned the two Fab structures on a full-length antibody template obtained from the KOL/Padlan structure (Boehm et al., 1999; Padlan, 1994). Figure 2 demonstrates the flowchart of the alignment process using PyMOL. First, the two Fab structures and the template structure (igg1_kappa.pdb) are loaded into PyMOL. Second, the Fab sequences of one heavy chain and one light chain on the template are selected, and the PyMOL *align* command was used to superimpose the Infliximab_Fab structure (green) on the template. This process was repeated for another Fab structure (cyan). Finally, one heavy chain from the infliximab Fab structure and the corresponding heavy chain Fc region was selected and saved as Infliximab_H1.pdb.

Another heavy chain was saved as Infliximab_H2.pdb. The two light chains from infliximab Fab structures were saved as Infliximab_L1.pdb and Infliximab_L2.pdb, respectively.

Figure 1. Search for antibody crystal structures on PDB and use PyMOL to clean and export the Fab structure.

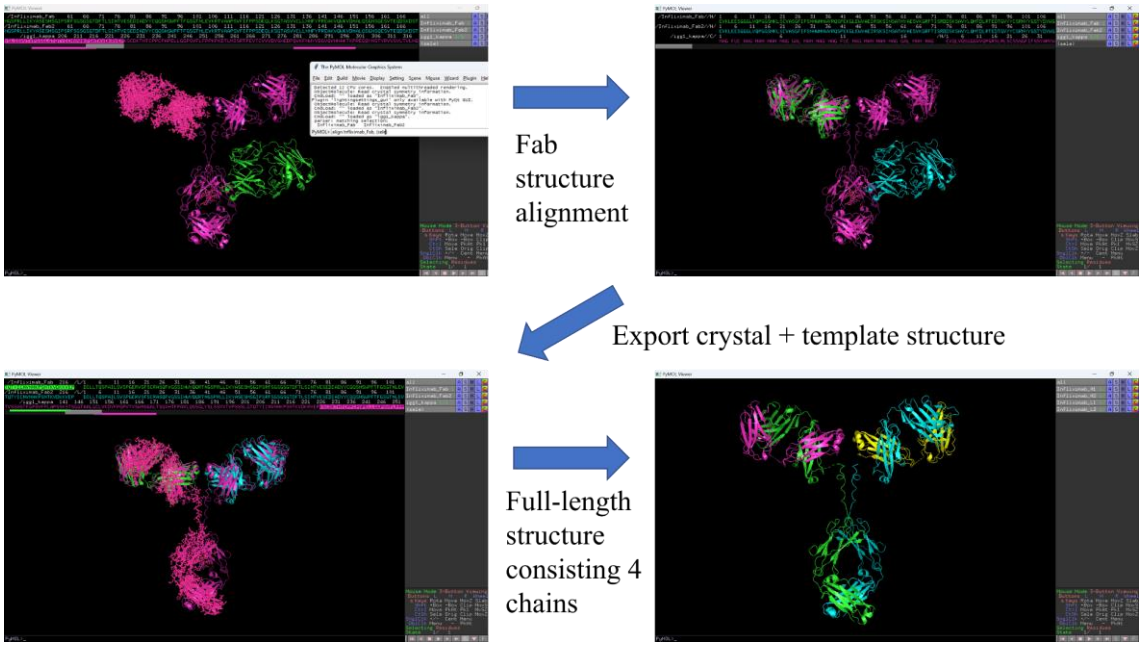


Figure 2. Flowchart of structural alignment of the Fab regions on an antibody template for building a full-length antibody model.

Questions:

1. Where are the variable regions and constant regions on the antibodies? Can you represent them in different colors using PyMOL?

2. Can you identify the complementarity-determining regions (CDRs) essential for antibody binding?

3. Can you find the Fab structure of another commercial antibody, such as adalimumab, from the PDB website and build a full-length antibody using a template?

3.2. Postprocessing of a full-length antibody model

There are several postprocessing steps for the full-length antibody structure. First, the four chains need to be connected by disulfide bonds. Second, the sequence of the full-length antibody model may not match exactly with the target sequence because of the inconsistency with the template structure or missing residues from the crystal structure. Third, the charge states for titratable residues need to match with experimental conditions. These postprocessing steps are required for running MD simulations to study molecular interactions. This section was conducted on a Linux system because of its powerful text-processing function. A short Linux tutorial was given to students to learn basic commands. There are plenty of resources for learning Linux commands; therefore, we only report antibody modeling tutorials.

Students will be able to (ILOs):

1. Understand basic Linux commands and perform simulations on supercomputers. (ILO #7)
2. Adjust manipulated PyMOL files by aligning set template sequences with the target sequences. (ILO #9)

3.2.1. Renumbering the sequences

The PDB files generated from the previous step were renumbered using a python program (reindex_pdb.py) with the following command.

```
python reindex_pdb.py 1 Infliximab_H1.pdb Infliximab_H1_renumber.pdb
```

The command for the other three chains is similar by replacing H1 with H2, L1, or L2. Before running the renumbering program, it is important to check that each PDB file has a consistent chain name (H for heavy chain and L for light chain).

3.2.2. Sequence alignment to identify incorrect sequences.

After renumbering, we can extract antibody sequences from PDB files using the following command:

```
cat Infliximab_H1_renumber.pdb | awk '/ATOM/ && $3 == "CA" && $5 == "H" {print $4}' | tr '\n' ' ' | sed 's/ALA/A/g;s/CYS/C/g;s/ASP/D/g;s/GLU/E/g;s/PHE/F/g;s/GLY/G/g;s/HIS/H/g;s/ILE/I/g;s/LYS/K/g;s/LEU/L/g;s/MET/M/g;s/ASN/N/g;s/PRO/P/g;s/GLN/Q/g;s/ARG/R/g;s/SER/S/g;s/THR/T/g;s/VAL/V/g;s/TRP/W/g;s/TYR/Y/g' | sed 's/ //g' | fold -w 60
```

After extracting the sequences, we use the EMBOSS NEEDLE Pairwise Sequence Alignment tool (https://www.ebi.ac.uk/Tools/psa/emboss_needle/) to compare the sequences from the PDB files and the target sequences to identify the incorrect or missing residues as shown in Figure 3.

Figure 3. Sequence alignment of the PDB file and the target sequence using the EMBOSS Needle Pairwise Sequence Alignment tool.

3.2.3. Sequence and structure modification

Visual Molecular Dynamics (VMD) software was used to modify antibody sequences and structures. VMD can process PDB files using tcl commands. We developed a tcl script (Infliximab.tcl) to mutate residues and connect the antibody chains. In addition to the four protein chains, there are two chains for glycans. Figure 4 exemplifies some sections in the tcl script. For the two heavy chains (one in segment H and one in segment I), we can modify the sequences by mutating or adding new residues to match the target sequences, as shown in Figure 3. Moreover, we can connect the chains by specifying the disulfide and glycosidic bonds. The tcl script is executed by running:

```
vmd -dispdev text -e Infliximab.tcl
```

This command will generate Infliximab_mod.pdb and Infliximab_mod.psf. The PDB file contains all antibody chains with correct sequences, and the PSF file stands for protein structure file containing information for bonds, angles, and dihedrals.

```
segment H {
first NTER
pdb H.pdb
mutate 275 GLU
mutate 286 GLU
mutate 297 GLU
mutate 315 ASP
mutate 318 ASN
mutate 359 ASP
mutate 361 LEU
residue 447 SER
residue 448 PRO
residue 449 GLY
residue 450 LYS
last CTER
}
coordpdb H.pdb H

segment I {
first NTER
pdb I.pdb
mutate 275 GLU
mutate 286 GLU
mutate 297 GLU
mutate 315 ASP
mutate 318 ASN
mutate 359 ASP
mutate 361 LEU
residue 447 SER
residue 448 PRO
residue 449 GLY
residue 450 LYS
last CTER
}
```

```
patch DISU H:22 H:98
patch DISU H:147 H:203
patch DISU H:264 H:324
patch DISU H:370 H:428
patch DISU I:22 I:98
patch DISU I:147 I:203
patch DISU I:264 I:324
patch DISU I:370 I:428
patch DISU L:23 L:88
patch DISU L:134 L:194
patch DISU M:23 M:88
patch DISU M:134 M:194
patch DISU H:229 I:229
patch DISU H:232 I:232
patch DISU H:223 L:214
patch DISU I:223 M:214

patch NGLB H:300 G:1
patch 14bb G:1 G:2
patch 14bb G:2 G:3
patch 16AT G:3 G:4
patch 12ba G:4 G:5
patch 13ab G:3 G:6
patch 12ba G:6 G:7
patch 16BT G:1 G:8

patch NGLB I:300 J:1
patch 14bb J:1 J:2
patch 14bb J:2 J:3
patch 16AT J:3 J:4
patch 12ba J:4 J:5
patch 13ab J:3 J:6
patch 12ba J:6 J:7
patch 16BT J:1 J:8
```

Figure 4. Sequence modification by mutation and addition and structure modification by connecting disulfide and glycosidic bonds.

3.2.4. Assigning charge state for titratable residues

The pH range in common formulation conditions for antibodies is from 5 to 6.5. Histidine (HIS) can be neutral or positively charged in this range. In this work, we used the CHARMM36m force fields. The titration states for HIS can be HSP (+1), HSD (+0), or HSE (+0). The default is HSP in the tcl script. We copy the Infliximab_mod.pdb to Infliximab_pqr.pdb and change the residue name of all histidine to HIS. Next, we used the pdb2pqr tool (Dolinsky et al., 2007, 2004) to determine the titration states by the following command.

```
pdb2pqr --with-ph=6.0 --ph-calc-method=propka --apbs-input --ff=charmm --ffout=charmm --verbose Infliximab_pqr.pdb Infliximab_pqr.pqr
```

After running this command, Infliximab_pqr.pqr file was generated, and the titration states of each histidine could be determined. Figure 5 shows that residue 32 on the heavy chain was predicted to be HSD. We can modify the Infliximab.tcl to add this mutation to change the titration state. After modifying all the histidine titration states, we can rerun Infliximab.tcl script to generate the final PDB and PSF files.

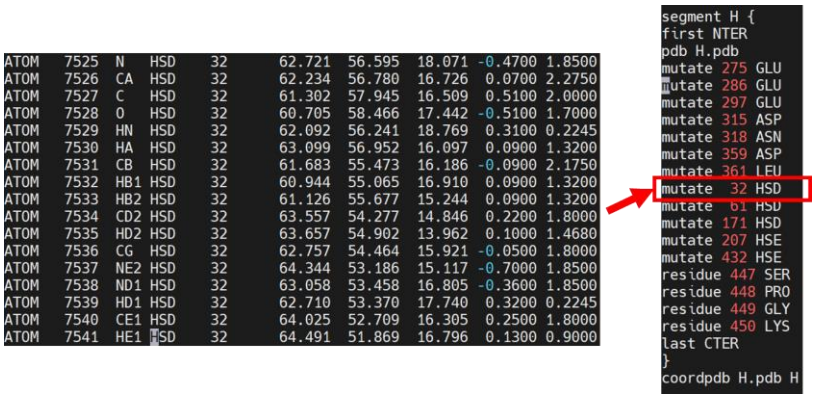


Figure 5. Determination of the histidine titration states using the PDB2PQR program and adjusting the titration state in the tcl script.

Questions:

1. Can you extract antibody sequences from a PDB file you generated previously for adalimumab using Linux commands?

2. How many amino acids are different between the adalimumab template and the target structure?

3. Can you mutate the amino acids in the adalimumab template to match the target structure?

4. Can you use VMD to assemble antibody chains by connecting disulfide and glycosidic bonds?

5. How many histidine residues are charged for adalimumab at pH=6.0 based on the prediction of PDB2PQR?

3.3. Molecular dynamics simulations of antibodies

This exercise instructs students how to perform MD simulations to study the structure change of antibody molecules. We also introduced the VMD software to visualize the dynamic movement of antibodies and their interactions.

Students will be able to (ILOs):

1. Visualize molecular interactions from molecular dynamics simulations using Visual Molecular Dynamics (VMD). (ILO #10)

2. Construct a modeling system of an antibody and perform energy minimization, solvation, and molecular dynamics simulation. (ILO #11)

After generating the tcl file, we need to run energy minimization and solvation before MD simulations. Energy minimization and MD simulations were performed using the NAMD program (Phillips et al., 2020), and the solvation was achieved by the VMD program. The execution commands depend on the specific computing system and

software version; therefore, we only give a general command for demonstrating the workflow.

For the MD simulations, we ran energy minimization in implicit solvent to stabilize the system using the following command:

```
namd2 Infliximab_min.conf
```

Next, we added water and ions to neutralize the system by running the following:

```
vmd -dispdev text -e Infliximab_solv.tcl
```

This command generated Infliximab_ion.pdb and Infliximab_ion.psf files. Next, we ran energy minimization for the solvated system.

```
namd2 Infliximab_ion_min.conf
```

Then we heated the system from 100K to 300K with position restraints on the antibody non-hydrogen atoms. The position restraint file was generated, and the heating was conducted by

```
vmd -dispdev text -e gen_restraints_heat.tcl
namd2 Infliximab_ion_heat.conf
```

Finally, we conducted equilibrium and production runs at 300 K and 1 atm for 10 ns and 50 ns, respectively.

```
namd2 Infliximab_ion_eq.conf
namd2 Infliximab_ion_prod.conf
```

Before students ran MD simulations, the instructor provided example outputs to guide students on using VMD to visualize the MD results. The NAMD program generates a binary trajectory file in the DCD format, and we need to load the PSF file first before loading the DCD file, as shown in Figure 6. The loaded system contains an antibody, water, and ions. VMD can change the molecules into different representations and colors. In addition, VMD can show the animation, five frames in this example, from the dynamic simulations, which allows students to visualize the structure change.

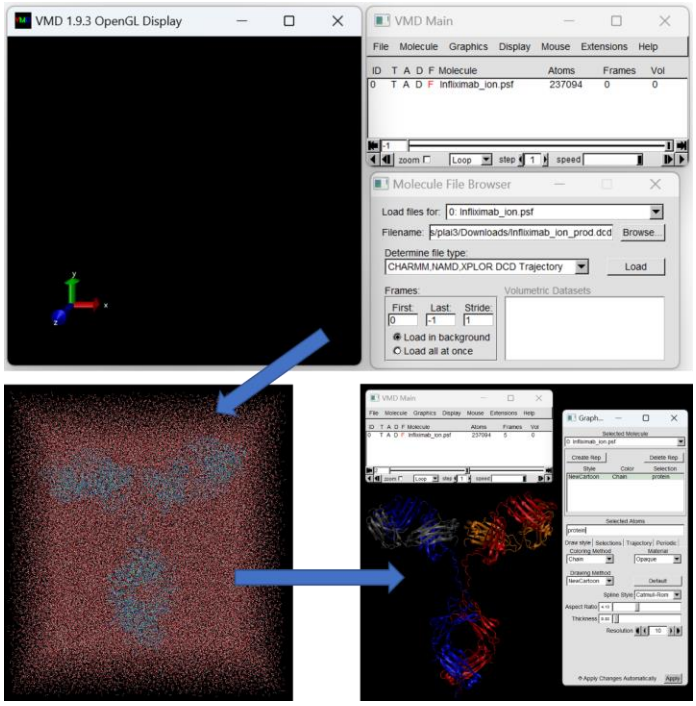


Figure 6. Flowchart of loading MD simulation results into VMD and change in a different representation.

Questions:

1. What is the potential energy of the antibody before and after the energy minimization step?

2. After the solvation step, how many water and ion molecules are in the system? What is the box size of the simulation system?

3. Can you set up MD simulations for the adalimumab?

4. What antibody conformational change do you observe during the MD simulations? Which regions are more flexible?

3.4. Adding excipients to study antibody-excipient interactions

Excipients are small molecule additives to biologics for stabilization. MD simulation is a great tool for studying the interactions between antibodies and excipients.

Students will be able to (ILOs):

1. Incorporate excipients into the simulation environment to reach established experimental conditions with a desired excipient concentration. (ILO #12)
2. Conduct a simulation and analyze the excipient-antibody interactions to determine binding sites. (ILO #13)

3.4.1. Generating excipient force fields

The force fields for proteins are well-documented and available for most molecular simulation software; however, the force fields for small molecules need to be generated separately. In this work, we used caffeine, a novel excipient, as an example to demonstrate how to include small molecule excipients in the MD simulations.

We used the CGenff tool (https://cgenff.umaryland.edu/) to generate force fields for caffeine, as demonstrated in Figure 7. The input molecule for CGenff can be in mol2, sdf, or pdb formats. We obtained the caffeine sdf file from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and uploaded it to the CGenff tool. The output file contains the information for caffeine structures and parameters. We split them into Caffeine.str and Caffeine.prm, which is the convention for the VMD and NAMD program. This flowchart applies to many small organic molecules.

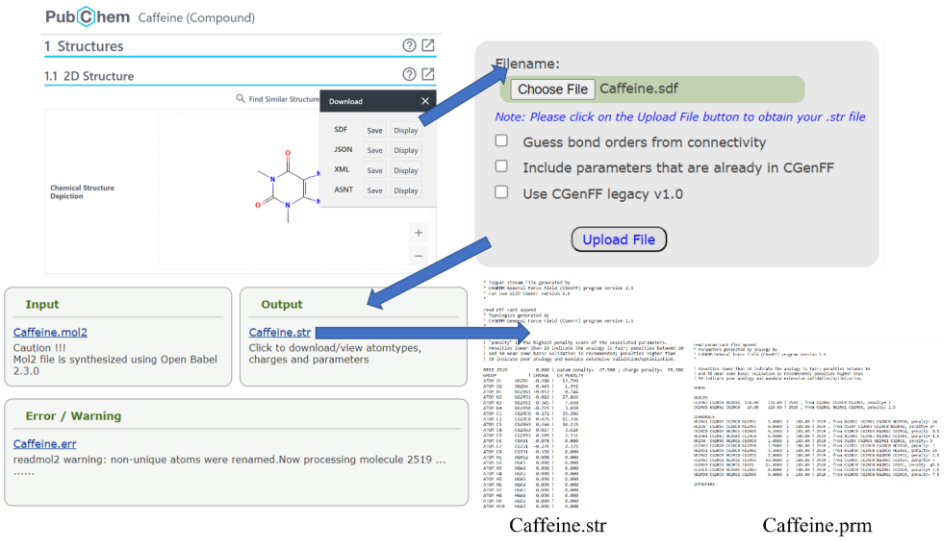


Figure 7. Flowchart of generating caffeine force fields using the CGenFF tool.

3.4.2. Generating initial configuration for antibody-excipient systems

Packmol is a tool for generating initial configurations for MD simulations (Martínez et al., 2009). We used Packmol to add infliximab and caffeine molecules to a target concentration. First, we used a tcl script to generate an input file for Packmol.

```
./solvate_PKL.tcl Infliximab_mAb.pdb -shell 10. -charge +16 -density 1.0 -o
packmol_solvated.pdb
```

Infliximab_mAb.pdb is the antibody structure generated from previous MD simulations. The shell is the water thickness on each side of the antibody. The charge is the net charge of the antibody (infliximab).

Figure 8 demonstrates a sample Packmol input file (packmol_input.inp). If we only want to add counterions to balance the net charge of infliximab (+16) without adding any salts, we can comment out the sodium section. We added a section for inserting caffeine molecules at the end of the file. The students were asked to calculate the number of caffeine molecules to be added to reach the target concentration based on the

chemical engineering principle. After modifying the file, we generated the initial configuration by running the following:

```
packmol < packmol_input.inp
```

```
tolerance 2.0
filetype pdb
output packmol_solvated.pdb

structure Infliximab_mAb.pdb
  number 1
  center
  fixed 0. 0. 0. 0. 0. 0.
end structure

structure WATER.pdb
  number   73628
  inside box -88.60650000000001 -69.9695 -49.705 88.60650000000001 69.9695 49.705
end structure

#structure SODIUM.pdb
#  number 196
#  inside box -88.60650000000001 -69.9695 -49.705 88.60650000000001 69.9695 49.705
#end structure


structure CHLORIDE.pdb
  number      16
  inside box -88.60650000000001 -69.9695 -49.705 88.60650000000001 69.9695 49.705
end structure

# 196 Na for 160 mM, how much Caffeine you need to reach 75 mM?  196/160*75 = 91.8

structure Caffeine.pdb
  number  92
  inside box -88.60650000000001 -69.9695 -49.705 88.60650000000001 69.9695 49.705
end structure
```

Figure 8. A sample Packmol input file.

3.4.3. MD simulations for antibody-excipient systems

First, we need to generate PDB and PSF files using VMD.

```
vmd -dispdev text -e Infliximab_EXP.tcl
```

The MD protocol is similar to the previous section without excipients, except we need to include the Caffeine.prm file in the configuration files.

```
namd2 Infliximab_EXC_min.conf
vmd -dispdev text -e gen_restraints_heat.tcl
namd2 Infliximab_EXC_heat.conf
namd2 Infliximab_EXC_eq.conf
namd2 Infliximab_EXC_prod.conf
```

Figure 9 shows an image from VMD showing a snapshot of infliximab and the caffeine molecules interacting with it. These interactions are weak, so we could observe several binding/unbinding events during the simulations.

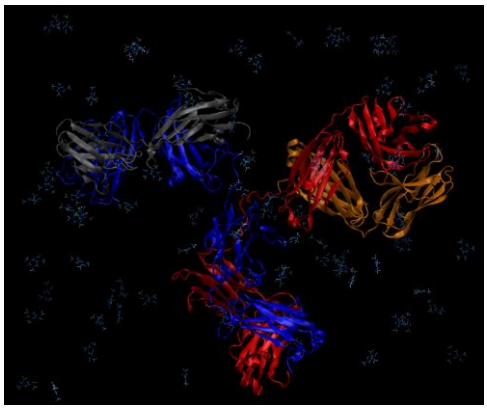


Figure 9. Infliximab with caffeine molecules in the MD simulations.

3.4.4. Analyzing antibody-excipient binding sites

After the simulation, we extracted only antibody and excipient molecules from MD simulations for further analysis.

```
vmd -dispdev text -psf Infliximab_EXC_mod.psf -dcd Infliximab_EXC_prod.dcd -e
extract_mAb_EXP.tcl -args Infliximab_EXC_only.dcd
```

We calculated the number of contacts between caffeine molecules with each residue of infliximab using a VMD script.

```
vmd -dispdev text -psf packmol/Infliximab_EXC.psf -dcd Infliximab_EXC_only.dcd -e
EXC_analysis.tcl -args contact_hist.txt
```

Figure 10 depicts the number of contacts, defined as any caffeine atoms within 4 Å of the respective residues, from MD simulations for heavy and light chains. High peaks indicate the excipient binding sites.

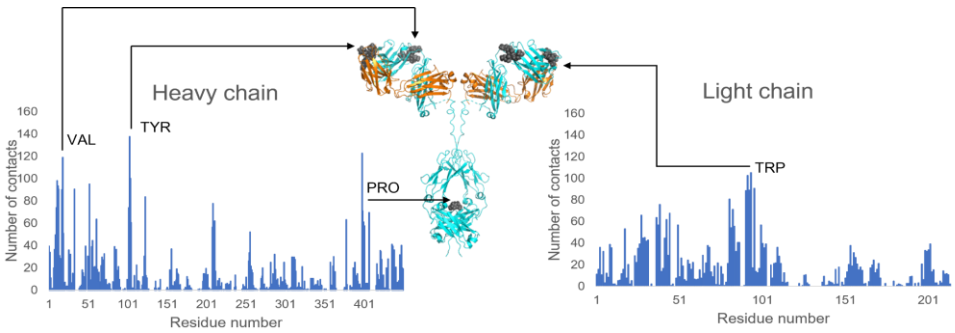


Figure 10. The number of contacts of the caffeine molecules with each residue on the heavy and light chains from MD simulations. The peaks and the corresponding spheres on the antibody model show the binding sites.

Questions:

1. Can you generate the force field parameters of pyridoxine, which is a novel excipient to enhance antibody drug stability?
2. How many pyridoxine molecules do you need to add to the antibody system to reach 150 mM? You can pick any antibody system for practice.
3. Can you list the top 5 binding residues of pyridoxine on the antibody system you choose?
4. What are the characteristics of these binding residues? Are they charged or hydrophobic? Are they in variable regions or constant regions?

4. Evaluation of the proposed methodology

Students were assessed before the course to gauge their background and level of experience related to this course (Table 2). After analyzing the pre-class survey, it was shown that most students had taken courses related to protein structures; however, less than half knew much about antibody structures and functions. Very few students in the class learned about the applications of antibodies in the biopharmaceutical industry. They also had little knowledge of challenges or formulation strategies in developing antibody drugs. In molecular modeling, most students knew what a supercomputer was; however, they did not know how to use a Linux system. None of the students understand the challenges of developing antibody drugs and computational methods for

modeling proteins and drugs. After this course, students were given the same survey (Table 2) to evaluate their understanding of biologics design and relevant computational tools. A significant improvement was found. The responses are available in the Supplementary Material.

Table 2. The questionnaire for the students to evaluate their understanding related to the course before and after the class.

Survey Questions
How much do you know about protein structures?
How much do you know about antibody structures and functions?
How much do you know about the applications of antibodies in the biopharmaceutical industry?
How much do you know about the challenges of developing antibody drugs?
How much do you know about the formulation strategies for drug development?
How much do you know about molecular modeling for proteins and drugs?
How much do you know about how to use a Linux system?
How much do you know what a supercomputer is?
How much do you know about molecular dynamics simulations?

In an anonymous survey conducted at the end of the course, students were asked to consider the course's learning outcomes and rank how strongly they agree with the statement on a scale of 1 to 5 (Table 3), 1 meaning strongly disagree, and 5 meaning fully agree. Levels 1 and 2 were grouped in a < 3 category, while levels 4 and 5 were grouped in a > 3 category. Overall, the > 3 category had many more responses than the other two categories, indicating that most of the students agreed or strongly agreed with the statements in the questionnaire.

Table 3. Summary of responses to the questionnaire used to assess the students' opinions of the intended learning outcomes (ILOs) to study biologics design from literature reviews and computational modeling.

ILO	<3	=3	>3

1	I understand the introductory concepts of the development and challenges high-concentration monoclonal antibody drug development faces	0	0	8
2	I understand and present findings regarding the basics of biological drugs, their functions, and the developmental challenges	0	0	8
3	I understand the complexity of antibodies in addition to improving teamwork and communication skills by constructing a physical antibody model	0	0	8
4	I understand how different excipients reduce antibody viscosity by interfering with protein-protein interactions	0	5	3
5	I can implement PyMOL to present antibody structures in different graphical representations.	0	1	7
6	I can manipulate different fragments of antibody structures in PyMOL to construct a full-length antibody structure	0	1	7
7	I can use basic Linux commands and perform simulations on supercomputers	0	1	7
8	I learned how to communicate findings from literature and gain insights on how to write a scientific paper	0	0	8
9	I can adjust manipulated PyMOL files and set target sequences to set template sequences	0	5	3
10	I can implement VMD to visualize antibody-excipient interactions from molecular dynamics simulations	0	0	8
11	I can construct a modeling system of an antibody and perform energy minimization, solvation, and molecular dynamics simulation	3	3	2
12	I know how to introduce excipients into simulation environment to reach established experimental conditions with a desired concentration of excipient	0	4	4
13	I can conduct a simulation and analyze the excipient-antibody interactions to determine binding sites	0	4	4

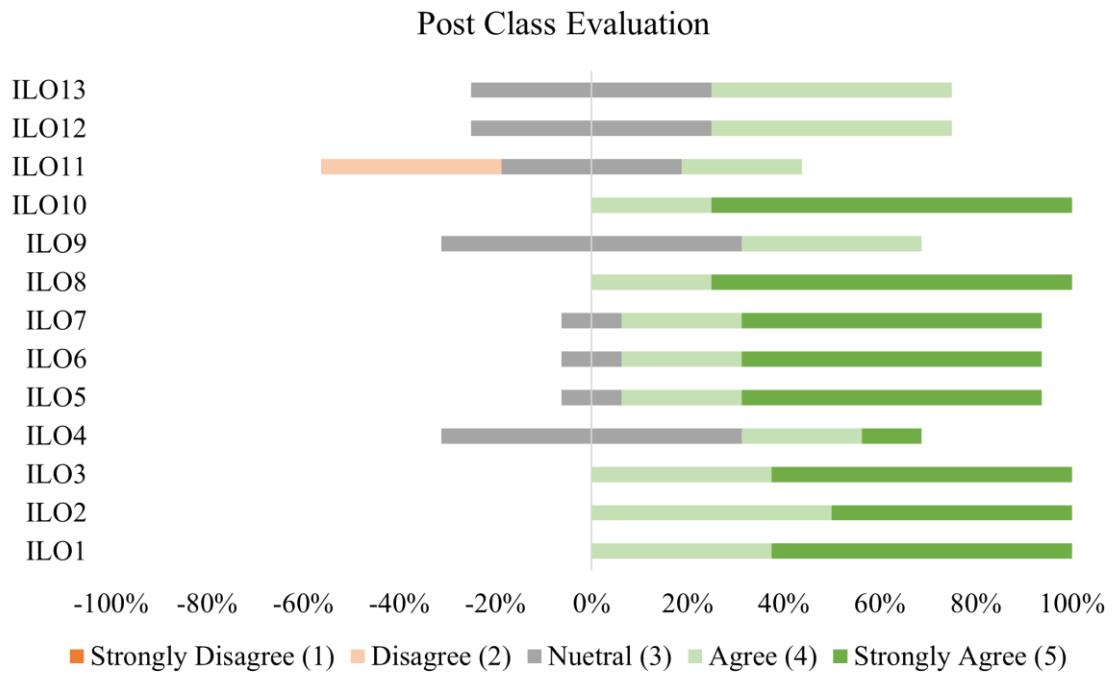


Figure 11. The responses to the questionnaire of intended learning outcomes (ILOs) using the five categories of the Likert scale.

Figure 11 shows the Likert scale (Likert, 1932) of the student's evaluation of the ILOs. For 13 expected learning objectives (Table 1), most students rated 4 and above for eight ILOs, meaning the students had a positive learning experience in those areas. The literature review and presentation remarkably improved students' understanding of the development and challenges of biopharmaceutical drugs and different formulation strategies (LO1-LO3). In terms of modeling skills, the students are confident in basic Linux usage (LO7), molecular visualization and manipulation using PyMOL (LO6), and trajectory analysis with VMD (LO10). The paper writing experience also enhanced students' communication skills and understanding of how to write a scientific paper (LO8), which none of the students had experienced.

There were also some obstacles that students encountered in this program. Performing MD simulations for antibodies and excipients and analyzing their binding

sites are challenging, receiving scores closer to 3.5, which is still a learning experience but not to the desired extent. MD simulations are usually covered in graduate statistical thermodynamics courses or as elective courses. Therefore, in this project, the instructor prepared a script for students to perform MD simulations and analysis. Designing another module to introduce the basic statistical thermodynamics and go through the simulation setup and the analysis more clearly could enhance students' learning.

5. Discussion

The Stevens Institute of Technology Design Spine is the heart of the core engineering and science curricua. The Design Spine teaches students to synthesize, analyze and optimize solutions for open-ended and societally impactful problems and culminates in Senior Design. These students utilized the format and materials presented in this article to develop their protein modeling skills and to understand the application of protein modeling in pharmaceuticals and research and development.

Literature reviews were the basis for this research experience, as participating students were unfamiliar with almost all topics relating to protein modeling, formulation of mAbs, and the excipients' effects on biological systems. Throughout the first five weeks of the research project, each group reviewed five different academic journals and articles. For each presentation, groups were required to touch upon the abstract, background information, challenges and knowledge gap, current development, research in this paper, materials and methods, results and discussion, and the conclusion.

The literature review of module two was the most helpful. The open-mindedness of the literature review, i.e., choosing articles and presenting the information as each group saw fit, allowed group members to grasp the theory before moving on to practical applications of that knowledge. Module three was slightly repetitive, as both groups had

the same article, so much of the information was the same. However, being able to see two different perspectives regarding one article was illuminating since no two people think the same. Module four was much like module two, although each group was teaching the other what their literature review was explaining, which made for efficient and exciting learning. For module five, the setup was the same as module three, although the computational basis of the articles made the articles stuffy, dry, and extremely difficult to dissect. Regardless, the computational side of biological drug formation was important, especially with the research project moving away from learning and towards application. Module six was also open-ended, where the goal was for each group to understand how educational papers are written and what ideas should be expressed in this type of literature. This review was less important for the overall research project but more aligned with the required deliverables for the 14-week training portion.

For the in-class demonstrations, PyMOL was the student's first introduction to creating a computer model. PyMOL was utilized to convert a template mAb into infliximab by accessing the Fab structure from Protein Data Bank. The learning experience provided students with a clear walkthrough of how to complete the following tasks: downloading the heavy and light chains of a specific mAb and replacing the template chains with the duplicated downloaded files to match the two heavy and two light chains. This understanding of the composition of the chains was later reinforced and utilized when verifying the chain content and order validity using EMBOSS Needle. The model also served as a critical step in students' ability to visualize the protein folding and 3D structure. This understanding created a strong foundation for students to navigate and understand the interactions between excipients and mAb binding sites that later occur using VMD.

A Linux system, connected through MobaXterm, was introduced after PyMOL to edit the protein molecules that were being imputed into the program. This step was important in the learning process as it incorporated the next software, VMD, to be used. Using VMD, the earlier examined protein in PyMOL could be edited to stitch the protein chains together and mutate to the target sequences. Finally, the NAMD program was introduced to perform MD simulations of the antibody systems and analyze their interactions with excipients. Through the program, students acquire the necessary skill to apply molecular modeling and simulations for biologics design. Overall, the students have positive feedback on the learning objectives of understanding biopharmaceutical development, formulation designs, and molecular modeling of antibodies. MD simulations are challenging for the students; therefore, the instructors can spend more time introducing the fundamental theory of statistical thermodynamics in the revised curriculum. Furthermore, the software implemented in this program is free, which is advantageous to classes with a limited budget.

6. Code availability

The code and scripts in this work can be accessed from http://tinyurl.com/mr45pcvj.

Acknowledgments

We thank the Advanced Cyberinfrastructure Coordination Ecosystem: Services & Support (Senior Design Projects: Formulation Design for Biological Drugs - BIO220114) for supporting computing resources.

References

Agrawal, N.J., Helk, B., Kumar, S., Mody, N., Sathish, H.A., Samra, H.S., Buck, P.M., Li, L., Trout, B.L., 2015. Computational tool for the early screening of

monoclonal antibodies for their viscosities. MAbs 8, 43–48. https://doi.org/10.1080/19420862.2015.1099773
Arora, J., Hu, Y., Esfandiary, R., Sathish, H.A., Bishop, S.M., Joshi, S.B., Middaugh, C.R., Volkin, D.B., Weis, D.D., 2016. Charge-mediated Fab-Fc interactions in an IgG1 antibody induce reversible self-association, cluster formation, and elevated viscosity. MAbs 8, 1561–1574. https://doi.org/10.1080/19420862.2016.1222342
Bain, G.A., Yi, J., Beikmohamadi, M., Herman, T.M., Patrick, M.A., 2006. Using Physical Models of Biomolecular Structures To Teach Concepts of Biochemical Structure and Structure Depiction in the Introductory Chemistry Laboratory. J. Chem. Educ. 83, 1322. https://doi.org/10.1021/ed083p1322
Blanco, M.A., 2022. Computational models for studying physical instabilities in high concentration biotherapeutic formulations. mAbs 14, 2044744. https://doi.org/10.1080/19420862.2022.2044744
Boehm, M.K., Woof, J.M., Kerr, M.A., Perkins, S.J., 1999. The Fab and Fc fragments of IgA1 exhibit a different arrangement from that in IgG: a study by X-ray and neutron solution scattering and homology modelling. J Mol Biol 286, 1421–1447. https://doi.org/10.1006/jmbi.1998.2556
Chaves, G.L., Batista, R.S., de Sousa Cunha, J., Altmann, D.L., da Silva, A.J., 2022. Teaching cellular metabolism using metabolic model simulations. Education for Chemical Engineers 38, 97–109. https://doi.org/10.1016/j.ece.2021.12.001
Chen, Y., Zhao, X., Zhou, H., Zhu, H., Jiang, S., Wang, P., 2022. Broadly neutralizing antibodies to SARS-CoV-2 and other human coronaviruses. Nat Rev Immunol 1–11. https://doi.org/10.1038/s41577-022-00784-3
Clauss, A.D., Nelsen, S.F., 2009. Integrating Computational Molecular Modeling into the Undergraduate Organic Chemistry Curriculum. J. Chem. Educ. 86, 955. https://doi.org/10.1021/ed086p955
Crisp, G., Cruz, I., 2009. Mentoring College Students: A Critical Review of the Literature Between 1990 and 2007. Research in Higher Education 50, 525–545.
Dolinsky, T.J., Czodrowski, P., Li, H., Nielsen, J.E., Jensen, J.H., Klebe, G., Baker, N.A., 2007. PDB2PQR: expanding and upgrading automated preparation of biomolecular structures for molecular simulations. Nucleic Acids Res 35, W522-525. https://doi.org/10.1093/nar/gkm276
Dolinsky, T.J., Nielsen, J.E., McCammon, J.A., Baker, N.A., 2004. PDB2PQR: an automated pipeline for the setup of Poisson–Boltzmann electrostatics calculations. Nucleic Acids Res 32, W665–W667. https://doi.org/10.1093/nar/gkh381
Gershenfeld, S., 2014. A Review of Undergraduate Mentoring Programs. Review of Educational Research 84, 365–391. https://doi.org/10.3102/0034654313520512
Hribar-Lee, B., 2022. The influence of excipients on the viscosity of monoclonal antibody solutions. Journal of Molecular Liquids 366, 120349. https://doi.org/10.1016/j.molliq.2022.120349
Hu, Y., Arora, J., Joshi, S.B., Esfandiary, R., Middaugh, C.R., Weis, D.D., Volkin, D.B., 2020. Characterization of Excipient Effects on Reversible Self-Association, Backbone Flexibility, and Solution Properties of an IgG1 Monoclonal Antibody at High Concentrations: Part 1. Journal of Pharmaceutical Sciences 109, 340–352. https://doi.org/10.1016/j.xphs.2019.06.005
Humphrey, W., Dalke, A., Schulten, K., 1996. VMD: visual molecular dynamics. J Mol Graph 14, 33–38. https://doi.org/10.1016/0263-7855(96)00018-5

Hung, J.J., Dear, B.J., Karouta, C.A., Chowdhury, A.A., Godfrin, P.D., Bollinger, J.A., Nieto, M.P., Wilks, L.R., Shay, T.Y., Ramachandran, K., Sharma, A., Cheung, J.K., Truskett, T.M., Johnston, K.P., 2019. Protein–Protein Interactions of Highly Concentrated Monoclonal Antibody Solutions via Static Light Scattering and Influence on the Viscosity. J. Phys. Chem. B 123, 739–755. https://doi.org/10.1021/acs.jpcb.8b09527

Jiskoot, W., Hawe, A., Menzen, T., Volkin, D.B., Crommelin, D.J.A., 2021. Ongoing Challenges to Develop High Concentration Monoclonal Antibody-based Formulations for Subcutaneous Administration: Quo Vadis? J Pharm Sci S0022-3549(21)00614–6. https://doi.org/10.1016/j.xphs.2021.11.008

Jo, S., Xu, A., Curtis, J.E., Somani, S., MacKerell, A.D., 2020. Computational Characterization of Antibody–Excipient Interactions for Rational Excipient Selection Using the Site Identification by Ligand Competitive Saturation-Biologics Approach. Mol. Pharmaceutics 17, 4323–4333. https://doi.org/10.1021/acs.molpharmaceut.0c00775

Johnson, S.M., Javner, C., Hackel, B.J., 2017. Development and Implementation of a Protein–Protein Binding Experiment To Teach Intermolecular Interactions in High School or Undergraduate Classrooms. J. Chem. Educ. 94, 367–374. https://doi.org/10.1021/acs.jchemed.6b00323

Kaplon, H., Chenoweth, A., Crescioli, S., Reichert, J.M., 2022. Antibodies to watch in 2022. mAbs 14, 2014296. https://doi.org/10.1080/19420862.2021.2014296

Kollár, É., Balázs, B., Tari, T., Siró, I., 2020. Development challenges of high concentration monoclonal antibody formulations. Drug Discovery Today: Technologies 37, 31–40. https://doi.org/10.1016/j.ddtec.2020.08.005

Kuroda, D., Tsumoto, K., 2020. Engineering Stability, Viscosity, and Immunogenicity of Antibodies by Computational Design. Journal of Pharmaceutical Sciences 109, 1631–1651. https://doi.org/10.1016/j.xphs.2020.01.011

Lai, P.-K., 2022. DeepSCM: An efficient convolutional neural network surrogate model for the screening of therapeutic antibody viscosity. Comput. Struct. Biotechnol. J. 20, 2143–2152. https://doi.org/10.1016/j.csbj.2022.04.035

Lai, P.-K., Ghag, G., Yu, Y., Juan, V., Fayadat-Dilman, L., Trout, B.L., 2021. Differences in human IgG1 and IgG4 S228P monoclonal antibodies viscosity and self-interactions: Experimental assessment and computational predictions of domain interactions. mAbs 13, 1991256. https://doi.org/10.1080/19420862.2021.1991256

Leem, J., Dunbar, J., Georges, G., Shi, J., Deane, C.M., 2016. ABodyBuilder: Automated antibody structure prediction with data–driven accuracy estimation. mAbs 8, 1259–1268. https://doi.org/10.1080/19420862.2016.1205773

Li, L., Kumar, S., Buck, P.M., Burns, C., Lavoie, J., Singh, S.K., Warne, N.W., Nichols, P., Luksha, N., Boardman, D., 2014. Concentration dependent viscosity of monoclonal antibody solutions: explaining experimental behavior in terms of molecular properties. Pharm Res 31, 3161–3178. https://doi.org/10.1007/s11095-014-1409-0

Likert, R., 1932. A technique for the measurement of attitudes. Archives of Psychology 22 140, 55–55.

Linn, M.C., Palmer, E., Baranger, A., Gerard, E., Stone, E., 2015. Undergraduate research experiences: Impacts and opportunities. Science 347, 1261757. https://doi.org/10.1126/science.1261757

Martínez, L., Andrade, R., Birgin, E.G., Martínez, J.M., 2009. PACKMOL: A package for building initial configurations for molecular dynamics simulations. Journal of Computational Chemistry 30, 2157–2164. https://doi.org/10.1002/jcc.21224

Neergaard, M.S., Kalonia, D.S., Parshad, H., Nielsen, A.D., Møller, E.H., van de Weert, M., 2013. Viscosity of high concentration protein formulations of monoclonal antibodies of the IgG1 and IgG4 subclass - prediction of viscosity through protein-protein interaction measurements. Eur J Pharm Sci 49, 400–410. https://doi.org/10.1016/j.ejps.2013.04.019

Padlan, E.A., 1994. Anatomy of the antibody molecule. Molecular Immunology 31, 169–217. https://doi.org/10.1016/0161-5890(94)90001-9

Phillips, J.C., Braun, R., Wang, W., Gumbart, J., Tajkhorshid, E., Villa, E., Chipot, C., Skeel, R.D., Kalé, L., Schulten, K., 2005. Scalable molecular dynamics with NAMD. J Comput Chem 26, 1781–1802. https://doi.org/10.1002/jcc.20289

Phillips, J.C., Hardy, D.J., Maia, J.D.C., Stone, J.E., Ribeiro, J.V., Bernardi, R.C., Buch, R., Fiorin, G., Hénin, J., Jiang, W., McGreevy, R., Melo, M.C.R., Radak, B.K., Skeel, R.D., Singharoy, A., Wang, Y., Roux, B., Aksimentiev, A., Luthey-Schulten, Z., Kalé, L.V., Schulten, K., Chipot, C., Tajkhorshid, E., 2020. Scalable molecular dynamics on CPU and GPU architectures with NAMD. J. Chem. Phys. 153, 044130. https://doi.org/10.1063/5.0014475

Roberts, D., Keeling, R., Tracka, M., van der Walle, C.F., Uddin, S., Warwicker, J., Curtis, R., 2014. The role of electrostatics in protein-protein interactions of a monoclonal antibody. Mol Pharm 11, 2475–2489. https://doi.org/10.1021/mp5002334

Saurabh, S., Kalonia, C., Li, Z., Hollowell, P., Waigh, T., Li, P., Webster, J., Seddon, J.M., Lu, J.R., Bresme, F., 2022. Understanding the Stabilizing Effect of Histidine on mAb Aggregation: A Molecular Dynamics Study. Mol. Pharmaceutics 19, 3288–3303. https://doi.org/10.1021/acs.molpharmaceut.2c00453

Sun, T., Zhao, Z., 2023. Teaching dynamic mechanisms in signaling pathways using computational simulations. Education for Chemical Engineers 42, 20–30. https://doi.org/10.1016/j.ece.2022.11.002

Tantillo, D.J., Siegel, J.B., Saunders, C.M., Palazzo, T.A., Painter, P.P., O'Brien, T.E., Nuñez, N.N., Nouri, D.H., Lodewyk, M.W., Hudson, B.M., Hare, S.R., Davis, R.L., 2019. Computer-Aided Drug Design for Undergraduates. J. Chem. Educ. 96, 920–925. https://doi.org/10.1021/acs.jchemed.8b00712

Tilegenova, C., Izadi, S., Yin, J., Huang, C.S., Wu, J., Ellerman, D., Hymowitz, S.G., Walters, B., Salisbury, C., Carter, P.J., 2020. Dissecting the molecular basis of high viscosity of monospecific and bispecific IgG antibodies. MAbs 12, 1692764. https://doi.org/10.1080/19420862.2019.1692764

Wang, S., Zhang, N., Hu, T., Dai, W., Feng, X., Zhang, X., Qian, F., 2015. Viscosity-Lowering Effect of Amino Acids and Salts on Highly Concentrated Solutions of Two IgG1 Monoclonal Antibodies. Mol Pharm 12, 4478–4487. https://doi.org/10.1021/acs.molpharmaceut.5b00643

Yang, E.C., Divine, R., Kang, C.S., Chan, S., Arenas, E., Subol, Z., Tinker, P., Manninen, H., Feichtenbiner, A., Mustafa, T., Hallowell, J., Orr, I., Haddox, H., Koepnick, B., O'Connor, J., Haydon, I.C., Herpoldt, K.-L., Wormer, K.V., Abell, C., Baker, D., Khmelinskaia, A., King, N.P., 2022. Increasing Computational Protein Design Literacy through Cohort-Based Learning for Undergraduate Students. J. Chem. Educ. 99, 3177–3186. https://doi.org/10.1021/acs.jchemed.2c00500

Zardecki, C., Dutta, S., Goodsell, D.S., Lowe, R., Voigt, M., Burley, S.K., 2022. PDB-101: Educational resources supporting molecular explorations through biology and medicine. Protein Sci 31, 129–140. https://doi.org/10.1002/pro.4200

Zeng, Y., Tran, T., Wuthrich, P., Naik, S., Davagnino, J., Greene, D.G., Mahoney, R.P., Soane, D.S., 2021. Caffeine as a Viscosity Reducer for Highly Concentrated Monoclonal Antibody Solutions. JPharmSci 110, 3594–3604. https://doi.org/10.1016/j.xphs.2021.06.030