Section 1

The authors state, “The forces are especially challenging since all particles interact via non-bonded interactions and share many different interactions based on their chemical bond configurations.” There may be a way to mention mechanics and other topics that must be approximated in simulations to provide clarity to the reader on the importance of forcefields without overwhelming detail.

Another sentence was inserted picking up the described topics:
"Furthermore, these interaction potentials can only be approximated mathematically, since electronic motion, mode-coupling and other effects are not explicitly treated in classical molecular dynamics simulations."

The authors state, “Software packages facilitating MD simulations then utilize positions, velocities, and forces to numerically integrate Newton’s equations of motion.” Perhaps a brief mention of various MD engines (e.g., NAMD and Amber) may be helpful to highlight the utility and advantages of GROMACS in comparison.

Although it would be beneficial for students to gain an overview over all possible MD engines we feel like this is not the scope of our tutorial. Furthermore, the MD engine is usually dictated by the work group a student joins or the project to be tackled and not so much by personal preference. Nevertheless, we included a few sentences hinting at that topic:
"A variety of molecular dynamics simulation software packages are available, with prominent examples including \texttt{GROMACS}, \texttt{NAMD}, \texttt{AMBER}, and \texttt{OpenMM}, each offering distinct advantages and limitations. Among them, \texttt{GROMACS} is known for its high performance, user-friendly interface, and extensive suite of analysis tools."

The authors state, “Limitations of MD simulations are electron motions, including charge transfer and chemical bond formation or breakage.” It may be helpful to (at some point) connect this to issues like modeling histidine.

We agree that a concrete example makes this problem more graspable for the students and especially newcomers. We have added the following sentences:
"These limitations effects the physical realism of MD simulations, especially in accurately capturing protonation state fluctuations that occur in biological environments. For example, histidine can adopt three protonation states, each affecting its hydrogen-bonding properties, but standard MD protocols generally fix it in one chosen form throughout the simulation."

Section 2

The authors mention, “Visualizing three dimensional structures is an essential part of molecular sciences, and a suitable program for that purpose is VMD.” Its worth considering VMD just released a new version, it might be worth checking that the tutorial works with the latest version, and weighing up the pros/cons of using the (advertised) more user-friendly GUI anyway.

We ensured compatibility with the new VMD version.

In “Program suites like Python or R can be used; however, due to simplicity, the command line tool (also known as xmgrace) is recommended.” To my understanding, xmgrace is relatively considered to be outdated. I would recommend ensuring readers understand that at one point it was the standard, ubiquitous tool, but now packages like mdanalysis or mdtraj are more widely used (and potentially easier to use with basic Python knowledge.)

The following sentences were added/edited to the manuscript:
"Due to its simple installation and command-line usage, the tool \texttt{grace} (also known as \texttt{xmgrace}) is primarily used in this tutorial. However, modern programming languages such as Python or R are recommended if the user has prior experience, as \texttt{xmgrace} is considered outdated by current standards."

Section 3

It may benefit the reader to include references to Slurm. Many users will likely eventually apply MD in a cluster environment.

While the reviewer's point is valid, managing SLURM-based systems is neither essential for completing the tutorial nor for understanding the principles of molecular dynamics simulations. Additionally, SLURM environments often differ in their specific configurations, and simply referencing them would likely raise more questions than it answers. For these reasons, we have chosen not to modify the content.

It may also help the reader to include the -v (verbose flag), especially for gromacs, to troubleshoot errors.

Sec. 3's Bash tutorial does not cover any GROMACS-specific commands. The -v flag is introduced in Section 4 and explained in detail in Table 2. As such, users are made aware of this option precisely at the point in the tutorial where it becomes relevant.

When mentioning chmod, a brief explanation of chmod commands would be helpful (e.g., 777) in case the user needs a command without deep permission knowledge

This is a good point, especially since this knowledge is required in Section 12. The following sentence was added:
"E.g., \code{chmod 777 program.sh} makes \code{program.sh} executable for the user."

Mentioning the find command could also be useful in the Basic Commands section

The find command was added to the list with the following sentences:
"find <path> -name <pattern>
Recursively searches for files and directories starting from \texttt{<path>} that match the given <pattern>. <pattern> can include wildcards like "*.txt". Combined with options like -type or -exec, find becomes a powerful tool for locating and manipulating files."

Section 4

It may be helpful to introduce trajectories to the reader as a series of pictures, where each snapshot is a propagation of Newton's laws.

While physics graduate students are likely familiar with the concept, students from other disciplines who are new to molecular dynamics may not be. To address this, we have added a figure and explanatory text in Section 4.1.

The authors mention integration errors in equations (3) and (4), but I believe there is a small technical issue with these equations:

Local and global errors may be conflated in their comparisons, see reference linked here:
https://math.libretexts.org/Courses/Monroe_Community_College/MTH_225_Differential_Equations/03%3A_Numerical_Methods/3.01%3A_Euler's_Method

The error was corrected accordingly

Local error of Eulers method is delta_t^2, where the global error is delta_t

The error was corrected accordingly.

For Leap-frog and Verlet integrators, the local error term is delta_t^4, yet the global error term is delta_t^2

The error was corrected accordingly.

For simplicity, it may be worth referencing the local terms but focusing on global errors for beginners.

The error was corrected accordingly.

Regarding timestep choice, it may be important to note that increasing the time step means better performance and reduces computational load relative to integrators that rely on shorter time steps.

We agree that timestep choice and the associated performance trade-offs between different integrators are important considerations in molecular simulations. However, in the context of this introductory tutorial, we deliberately chose not to go into detail on this topic.
The learning objective of this section is to understand that the timestep is primarily constrained by the fastest motion in the system (which depends on the particle mass in this case) — a concept which is reinforced through Task 2. While it is true that different integrators allow for different timestep limits and may offer varying levels of performance, these differences are relatively minor compared to the performance impact of non-bonded interaction algorithms, thermostats, barostats or using even slightly superior hardware.
We believe that including such a discussion at this stage would add complexity without directly supporting the core learning goals of the tutorial. That said, we have clarified the role of the timestep in performance and briefly noted that integrator choice can have subtle effects, for students who may wish to explore the topic further.

In Table 1, it might be helpful to include a footnote or something later that references the *.dcd file type used in other MD engines, which can be easily converted from *.xtc and vice versa

Trajectory, coordinate, and topology file conversion is an occasional requirement in computational biophysics, but it remains relatively rare for most researchers. This is mainly because scientists tend to work within the ecosystem of their preferred simulation software, which reduces the need for format conversion. That said, conversion becomes necessary when using external analysis tools that do not support a package's default file formats. Given the wide variety of formats in use — including DCD, DATA, MDCRD, NCDF, XTC, XYZ, and PDB, among others — it is important to be aware of this aspect of file handling. However, we do not see a compelling reason to emphasize a particular additional trajectory format in this tutorial as we strive to provide concepts and tools precisely where they directly support the user's understanding and problem-solving process. In this section, knowledge of file format conversion does not contribute meaningfully to those goals and is therefore not included.

Section 5

It would be helpful for the reader when first introduced to NVT ensembles (in Setup and Simulations) to be provided with a brief definition.

This was an oversight on our part. The NVT ensemble was not intended to be introduced explicitly in this section, as it is properly covered in Section 6. We have revised the sentence accordingly.
"In this exercise, a polymer consisting of 20 atoms should be simulated at \SI{300}{\kelvin} according to the downloadable \texttt{polymer.mdp} for the following cases:"

Additionally, the *.ndx file (which could be mentioned elsewhere) are valuable for extracting indices of protein structures from trajectory data

The .ndx files serve a wide range of purposes, and we believe it is best to either present a comprehensive overview or focus solely on functions relevant to the current task. Extracting indices of protein structures from trajectory data is not essential for this exercise, and for that reason, we do not consider it necessary to mention it explicitly.

Section 6

The exercise is clear and straightforward.

I believe a solution for the “flying ice cube” problem should be included. Something to the effect of “the center of mass translational velocity can be removed every 100 steps using this command…”

The solution to the flying ice cube problem is implicitly revealed through Task 5 of this exercise. A comparison of the two MDP files shows that the only difference lies in the choice of thermostat. This highlights that avoiding the Berendsen thermostat is key to preventing the flying ice cube effect.

Section 7

The exercise is clear and straightforward, no comments.

Section 8

The exercise is clear and straightforward, no comments.

Section 9

The exercise is clear and straightforward.

Given the exercise discusses the free energy related to the simple diffusion of the solute in solvent, the authors may find it beneficial to introduce statistical mechanics concepts (e.g., rates) as it may relate to future sections, providing a simple concept to build upon for beginners.

The introduction of statistical mechanics concepts and probability distributions is intentionally deferred to Section 10 for several reasons. First, understanding the BAR equations (Eqs. 42 and 43) in Sec. 9 does not require a prior understanding of probability distributions or rates. Second, Sec. 9 already introduces a substantial number of new and potentially overwhelming concepts, including thermodynamic cycles, alchemical transformations, the use of MD beyond conventional simulations, and several new functionalities of the MDP file. Adding statistical mechanical concepts at this stage would risk overloading the learner. In contrast, Sec. 10 involves the use of WHAM , which yields a PMF directly related to the unbiased probability distribution along a reaction coordinate. At this point, users are familiar with the idea of non-standard simulation pathways and advanced MDP file configurations, as introduced in Sec. 9. Only Sec. 10 is concerned with geometrical instead of alchemical transformations. Therefore, we believe Sec. 10 is more pedagogically appropriate for the introduction of statistical mechanics concepts related to probability rates.