Steered Molecular Dynamics Simulations on Human Beta Defensin Crossing Model Bacterial Lipid Membranes

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Milestone #1: Become familiar with Unity Linux system, NAMD simulation, and antimicrobial peptides by reading online documents and 1 to 2 related journal papers; launch presentation;

Milestone #2: Set up human beta defensin type 1 to 3 embedding in model Gram-negative bacterial lipid membranes using CHARMM-gui online program; then run NAMD simulations to equilibrate the systems built;

Milestone #3: Run steered MD simulations to pull hBD-1/hBD-2/hBD-3 out of model bacterial lipid membranes;

Milestone #4: Analyze structure, dynamics and free energy profiles from Steered MD simulations; compare results from different defensin simulations, and interpret the results;

Milestone #5: Write a project report, do a presentation based on research findings, wrap up the project, exit interview.

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- Goals
  - Learn background of antimicrobial peptides;
  - Learn basic knowledge of Linux system and simulation;
  - Learn how to set up simulations on protein in solvents using CHARMM-GUI online program;
  - Learn how to run NAMD simulations on UNITY;
  - Learn how to run Steered MD simulations;
  - Learn how to interpret simulation results;

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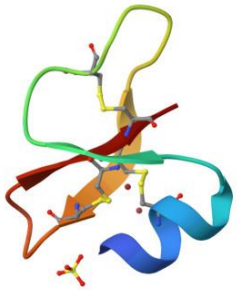
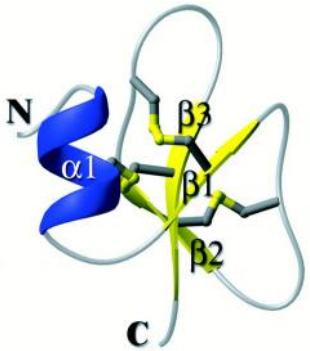
- ## Human Beta Defensin
  - Belongs to human innate immune system;
  - Peptides that are critical in providing antimicrobial barriers against viruses, fungi, gram-positive/negative bacteria, as well as antibiotic resistant bacteria;
  - They kill bacteria by breaking bacterial membranes;
  - How much is the energy barrier needed to overcome to break the membrane?
  - Working with 3 defensins: Human Beta Defensin type 1, Human Beta Defensin type 2, and Human Beta Defensin type 3;

Hoover et al. (2001) J Biol Chem **276**: 39021-39026



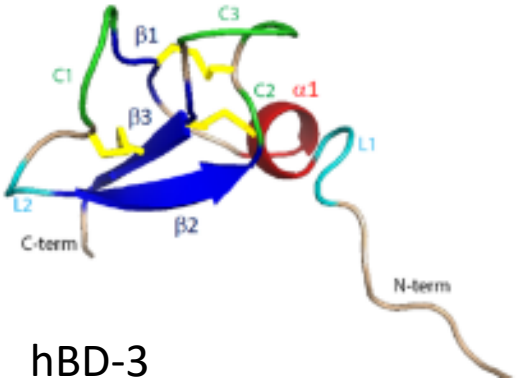
hBD-1

Hoover et al. (2000) J. Biol. Chem.,



hBD-2

Yeasmin et al. (**2018)** *J. Phys. Chem. B, 122* (50), 11883-11894



hBD-3

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- SMD (Steered Molecular Dynamics)
  - SMD simulations is used to apply external force onto one or more molecules, allowing the study of proteins under different media;
  - After creating the NAMD configuration file for the SMD simulation, one can run an SMD simulation;
  - After said simulations are complete, the simulation files can be uploaded to the VMD (visual molecular dynamics) software, which can generate images as well as data tables, aiding in the analysis of the simulation results;

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- VMD (Visual Molecular Dynamics)
  - Molecular modeling and visualization program;
  - Allows the viewing and analysis of the Steered Molecular Dynamics Simulation;
  - Enables us to view photos and videos of the human beta defensin's crossing model bacterial membranes;

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Finished work:

- Set up hBD-1 monomer/dimer, hBD-2 monomer/dimer, and hBD-3 monomer/dimer in POPC mixed with POPG membrane using CHARMM-GUI online program;
- Ran NAMD simulations on hBD-1 monomer/dimer, hBD-2 monomer/dimer, and hBD-3 monomer/dimer in model bacterial membrane on UNITY;
- Running Steered MD in order to pull hBD-1/-2/-3 monomer and dimer out of model bacteria membrane with the goal to find out the energy barrier for them to cross membranes;
- Analyze structure, dynamics and free energy profiles from Steered MD simulations; compare results from different defensin simulations, and interpret the results;
- Analyze Radius of Gyration, as well as the Root Mean Square Deviation of the hBD-1/-2/-3 monomer and dimer forms for both constant velocity and constant force pulling;

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Constant Force Pulling

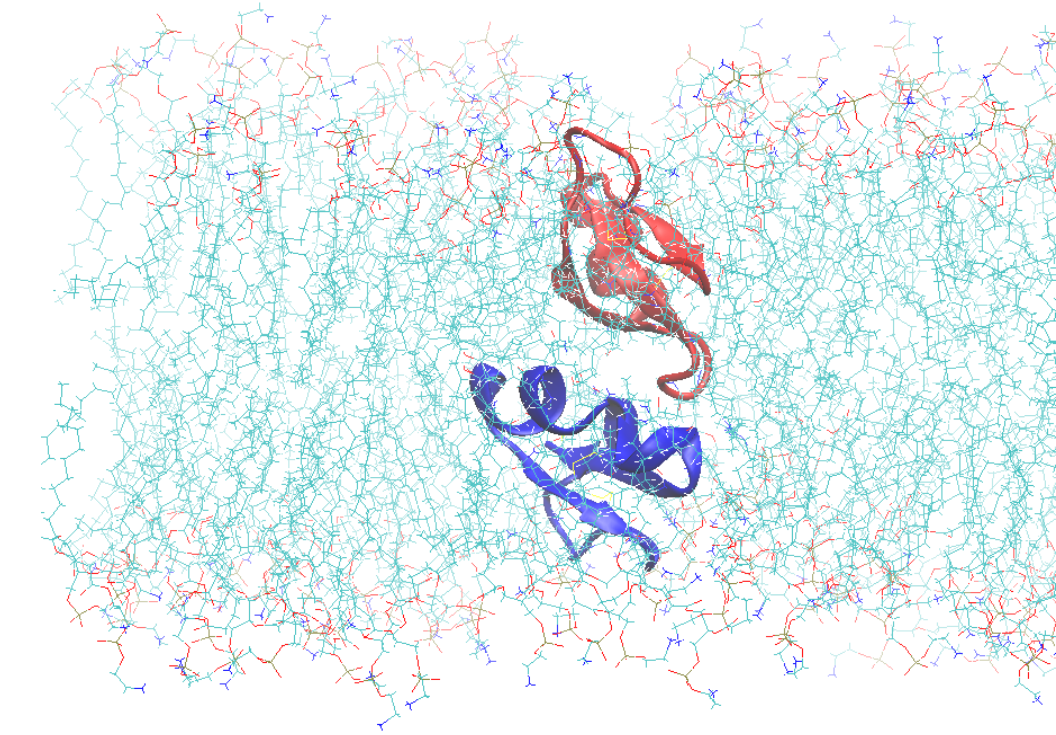
In SMD, atoms (center of mass of defensin monomer/dimer) experience a constant force in the Z direction to pull the protein out of the membrane using a constant force. One time was upward pulling and the other time was downward pulling.

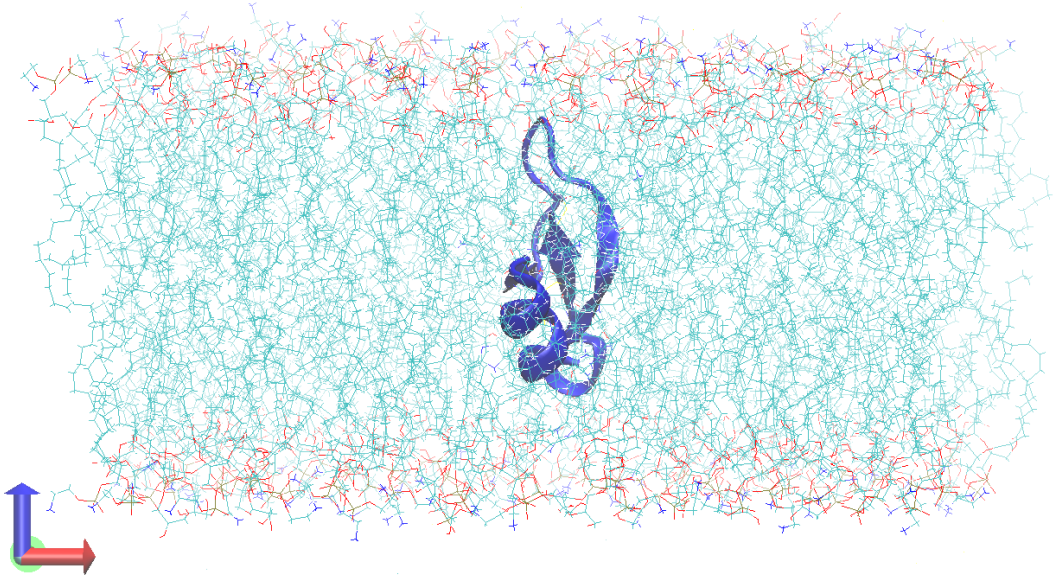
Constant Velocity Pulling

In SMD, atoms (center of mass of defensin monomer/dimer) experience a constant force in the Z direction to pull the protein out of the membrane using a constant force. One time was upward pulling and the other time was downward pulling.

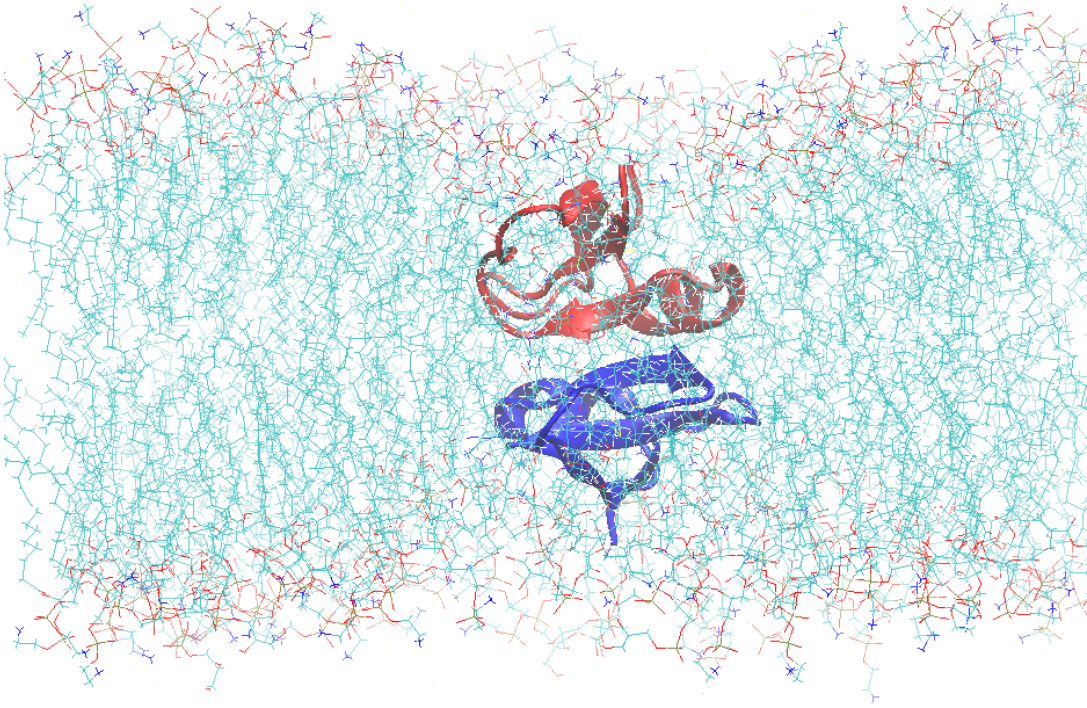
- After the simulations are complete on UNITY, results can be exported to VMD, in which results can be analyzed using tcl codes;

hBD-1 Dimer and Monomer Equilibration Structure Results (via VMD software)

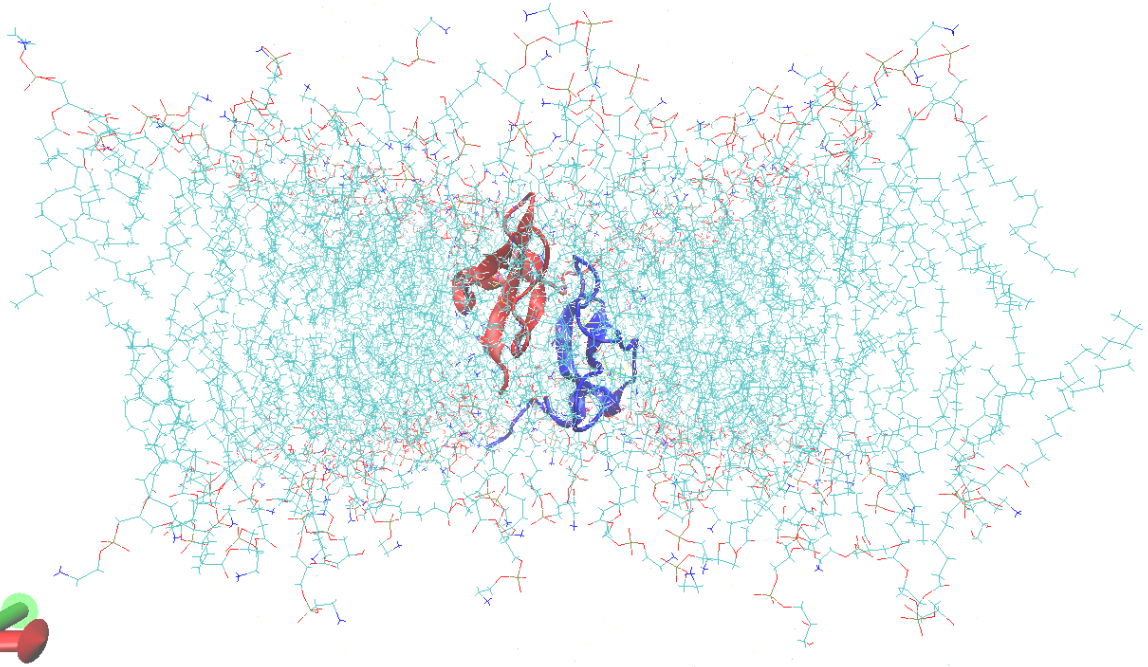


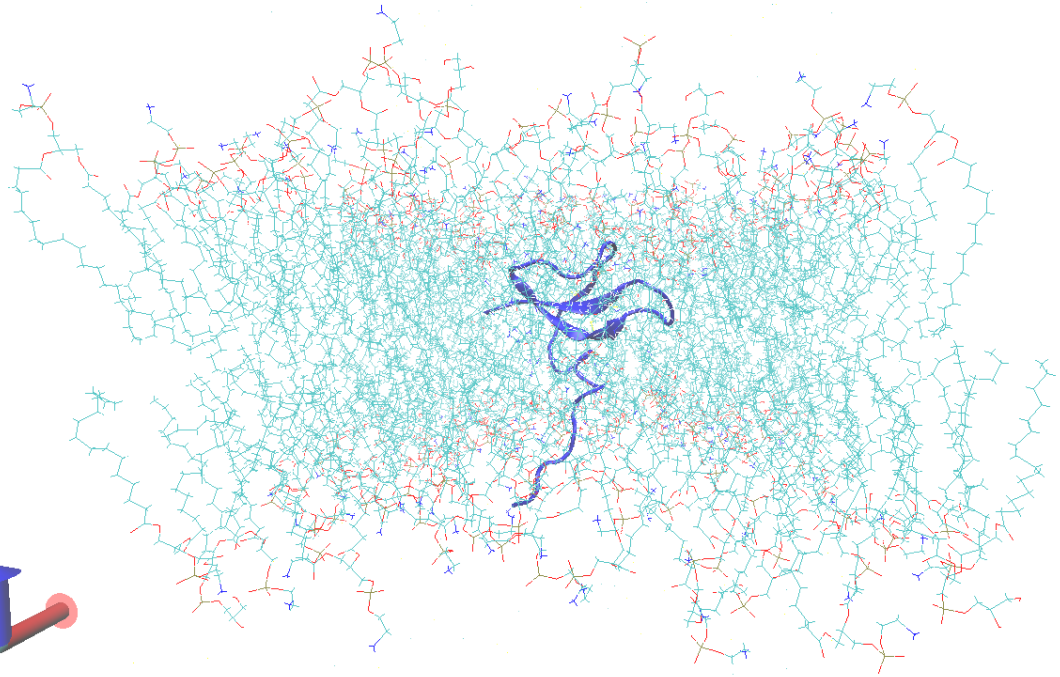


hBD-2 Dimer and Monomer Equilibration Structure Results (via VMD software)

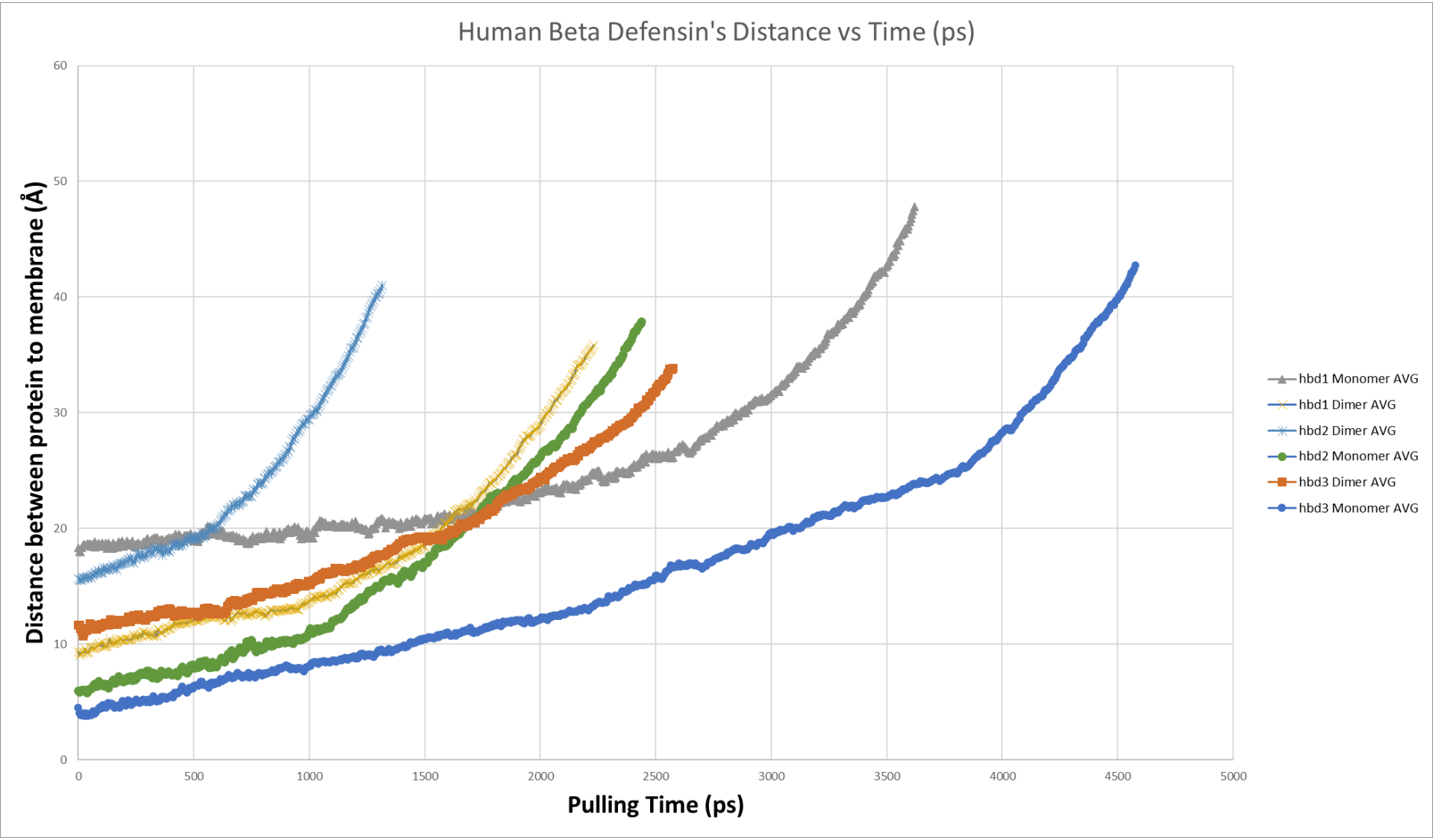


hBD-3 Dimer and Monomer Equilibration Structure Results (via VMD software)

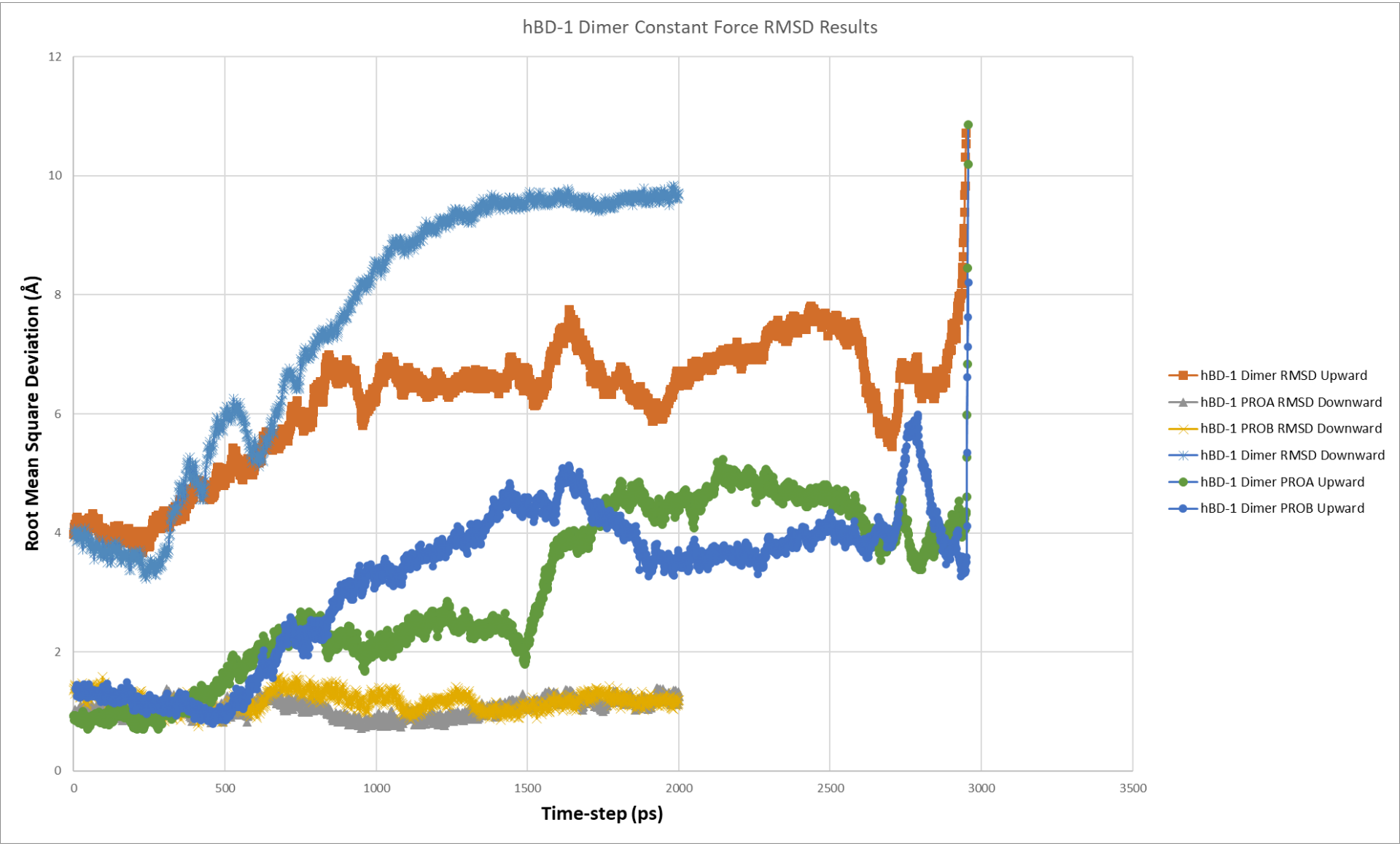




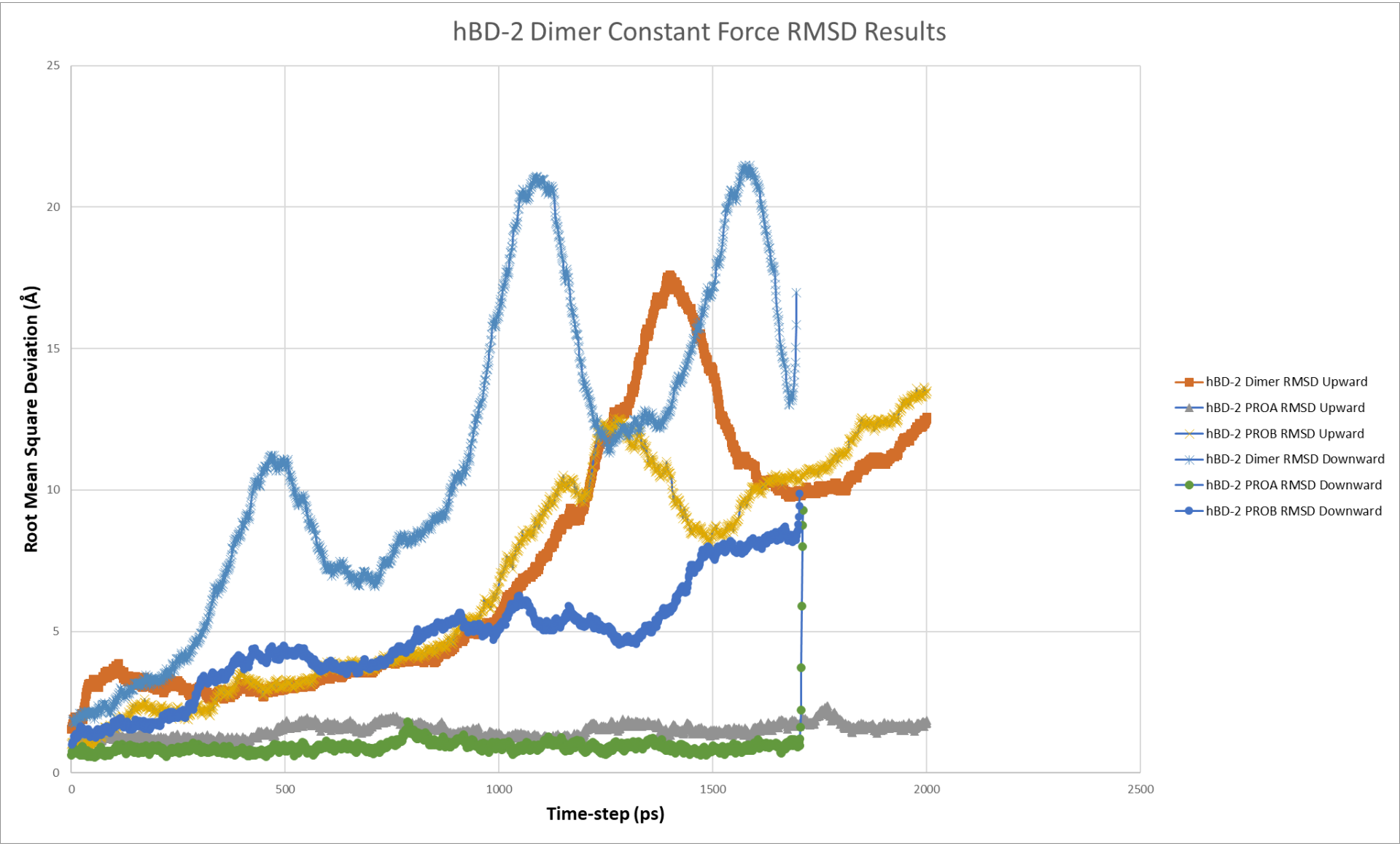
Constant Force Results



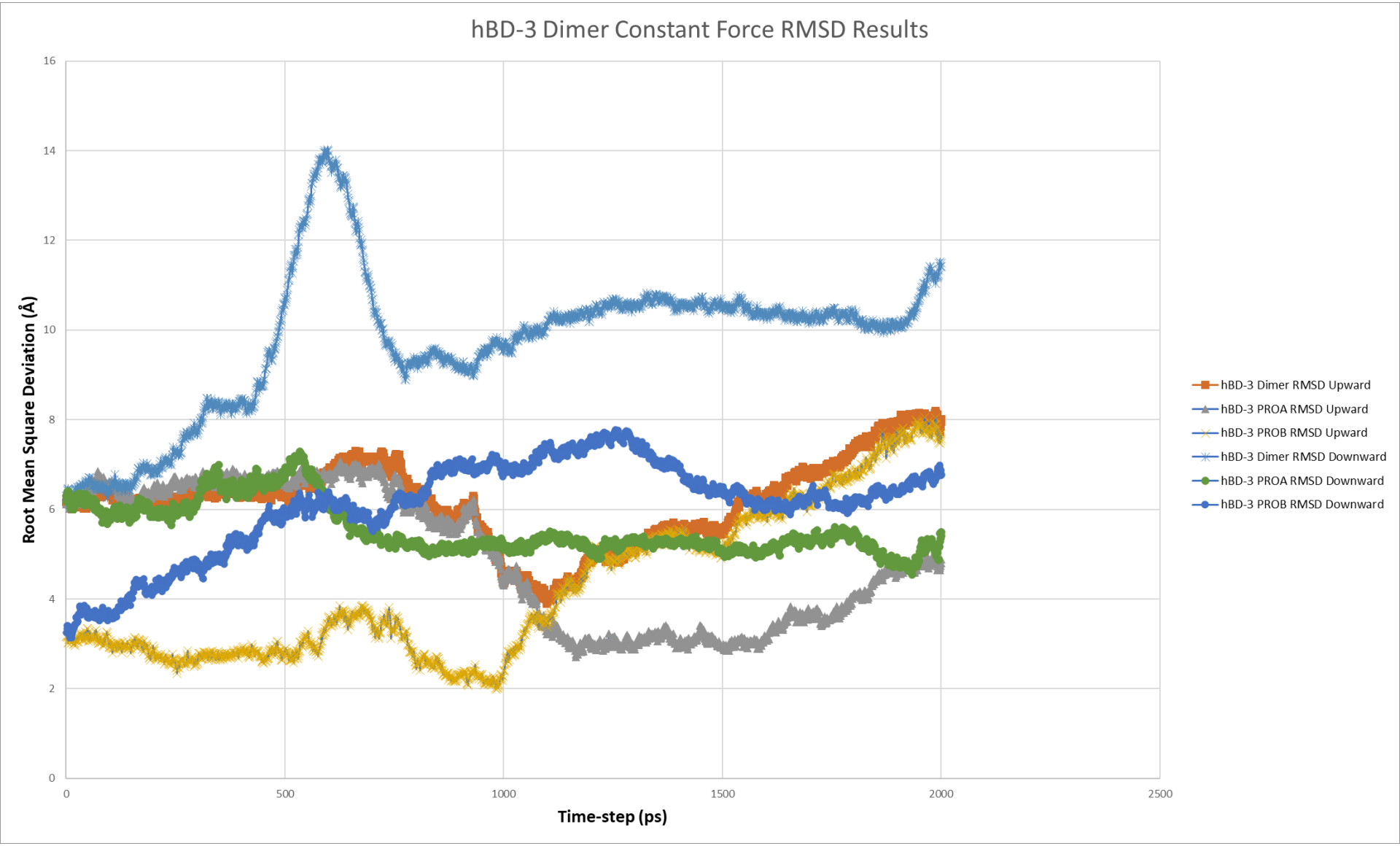
Constant Force RMSD Results



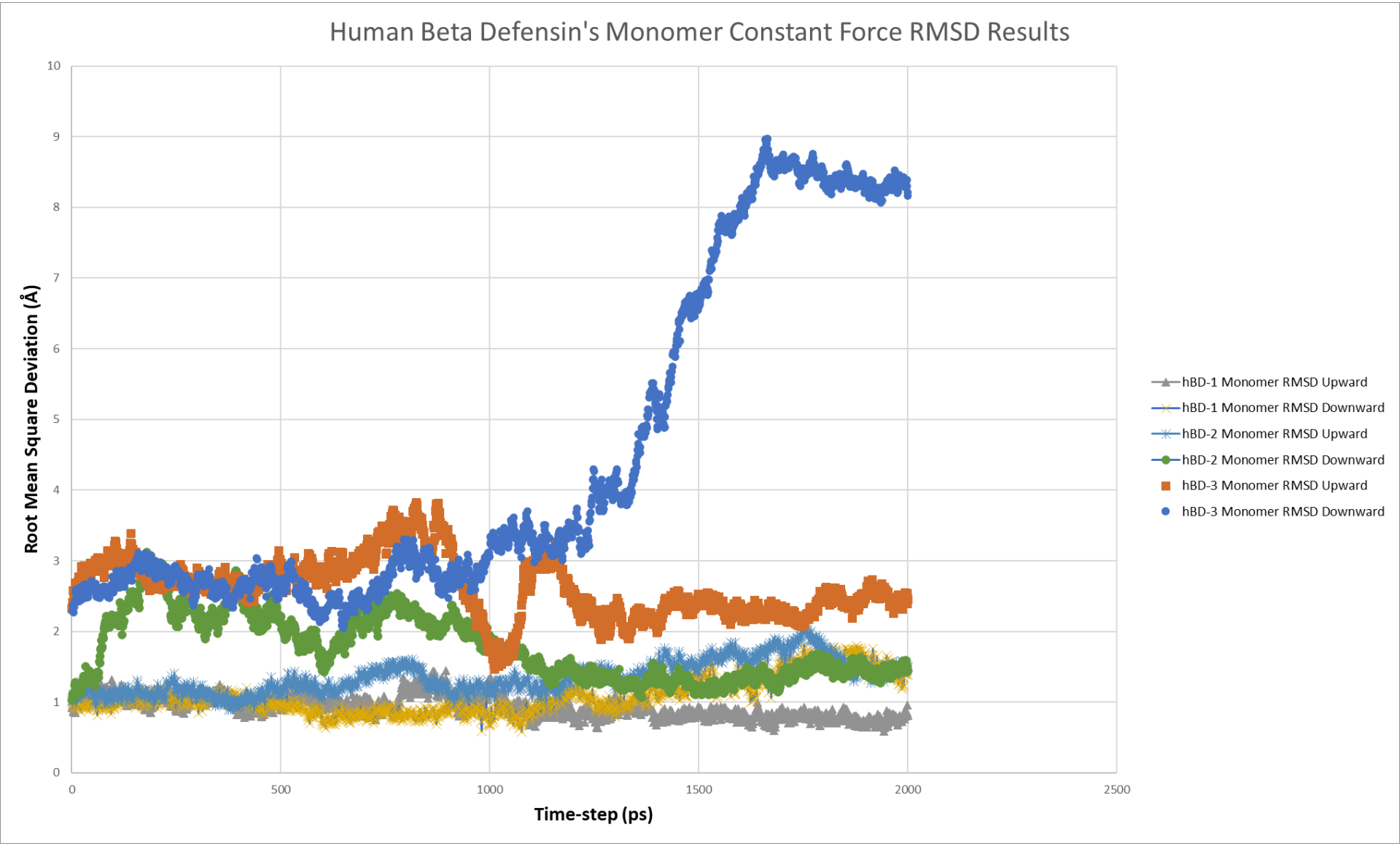
Constant Force RMSD Results



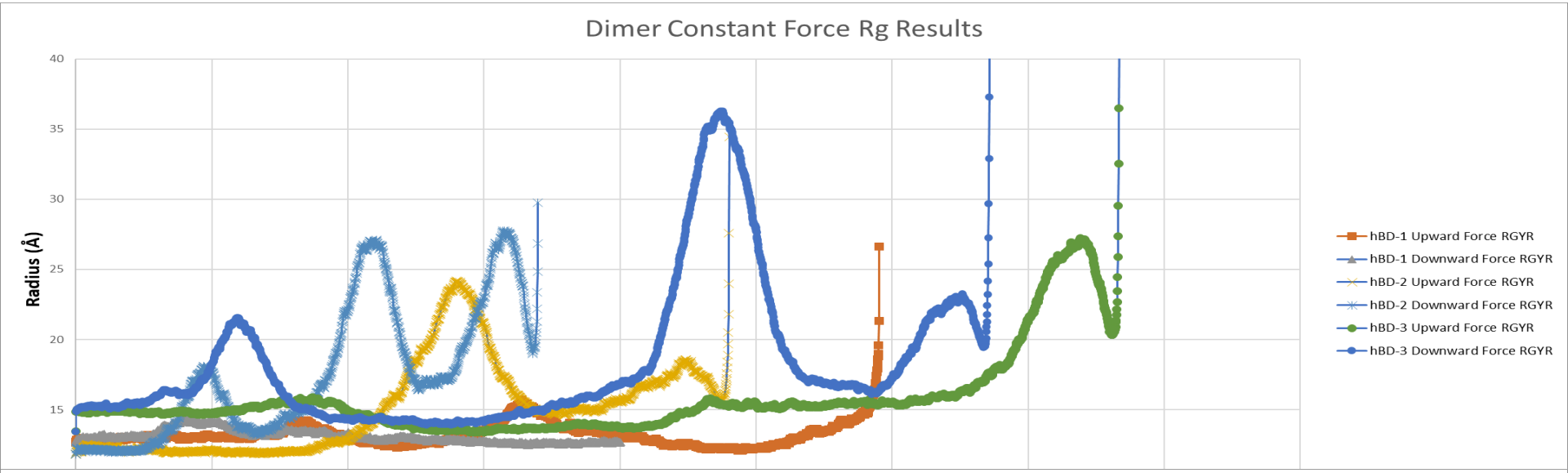
Constant Force RMSD Results

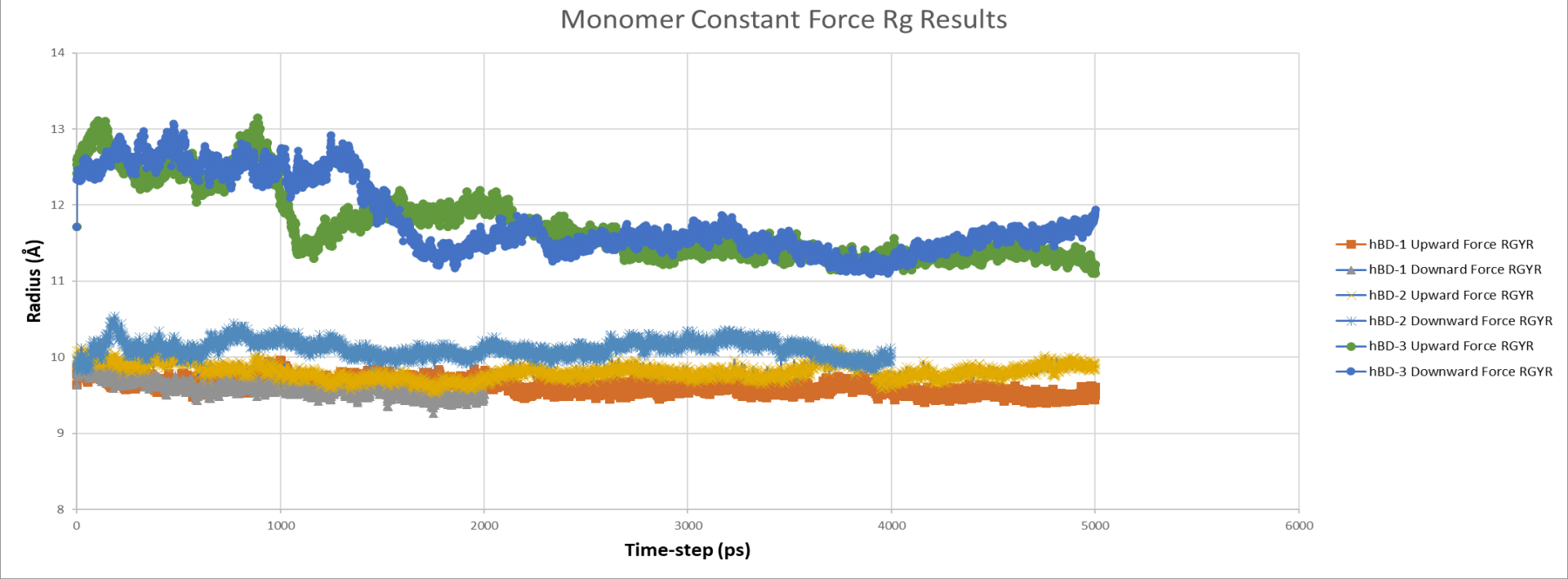


Constant Force RMSD Results



Constant Force Radius of Gyration Results

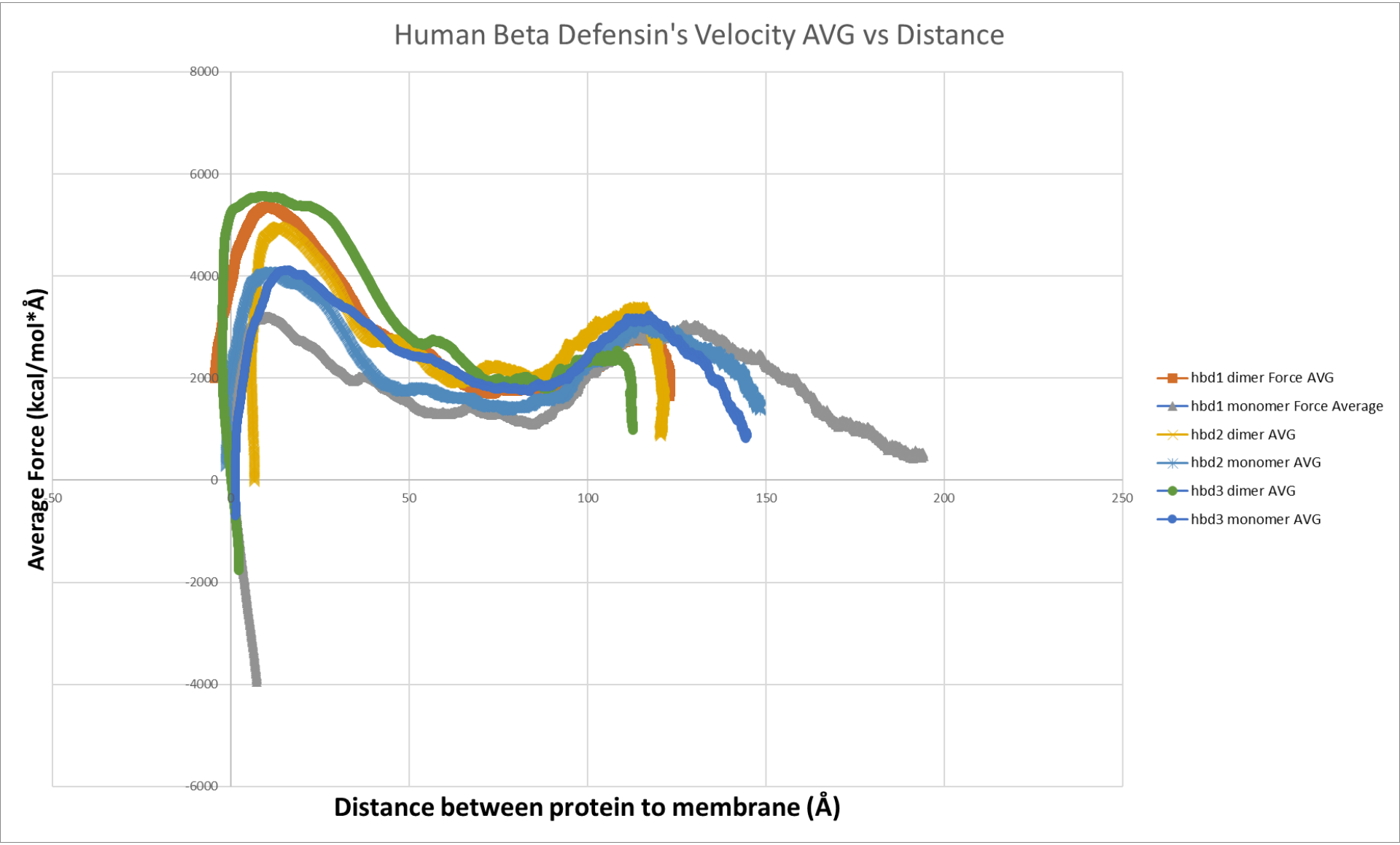




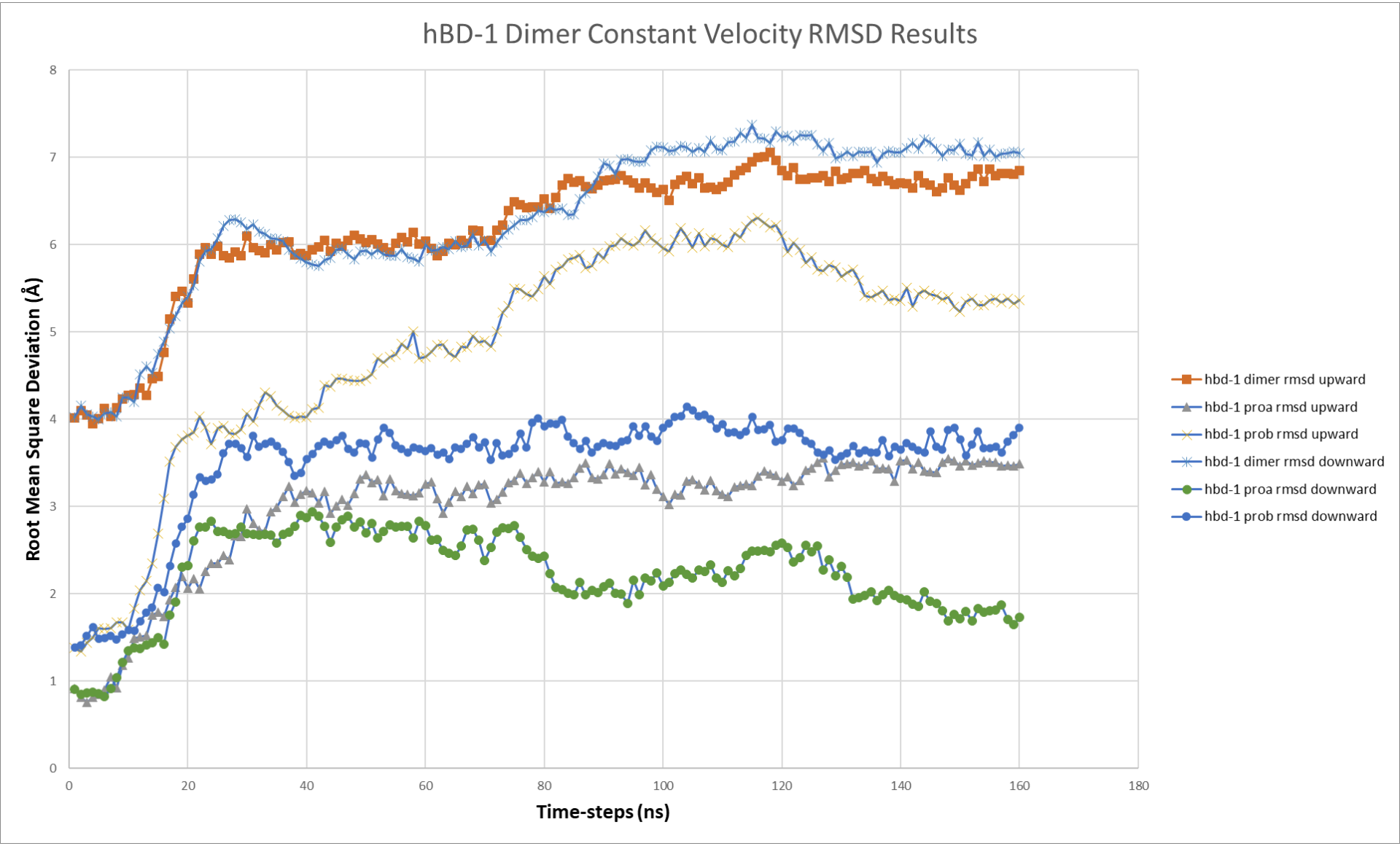
Based on Constant Force Pulling Results:

- Throughout each defensin comparison, averagely the monomer configuration underwent a longer pulling time to reach the same distance from the membrane as the dimer;
- The monomer and dimer form of hBD-2 pulling downward and hbd-3 pulling upward had a similar distance/time relation;
- Except for hBD-1, the dimer forms of hBD-2 and hBD-3 had a higher average distance from the membrane at the start of the pulling time;
- The possible reason for dimer moving out of the membrane faster than monomer could be that the defensin dimers are in a more globular shape than defensin monomers;
- The RMSD values for the dimers in downward pulling is higher than the other RMSD values;
- With the exception of the hBD-3 monomer, all other monomers have a Rg range between 9 and 11 Å;

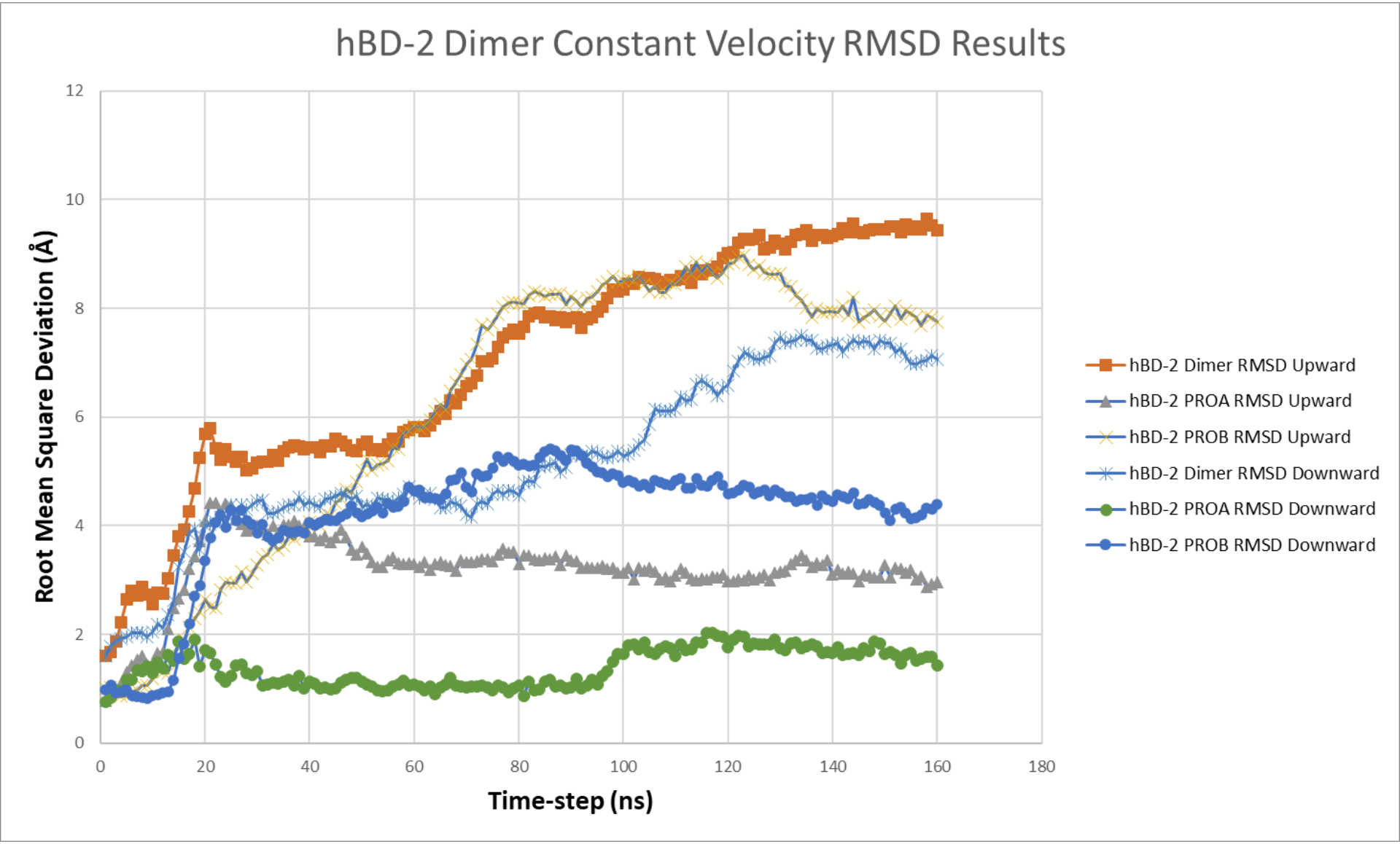
Constant Velocity Results



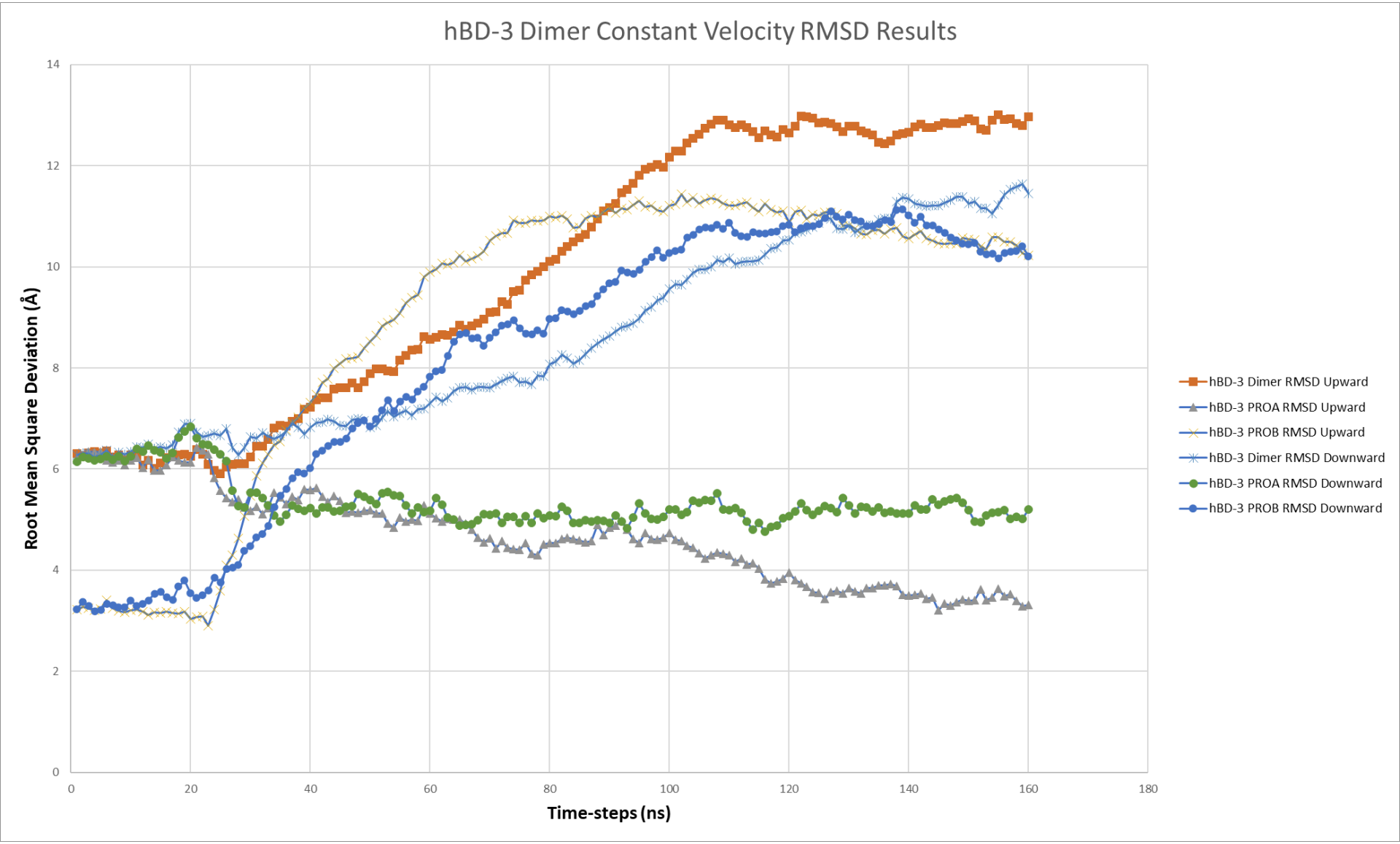
Constant Velocity RMSD Results



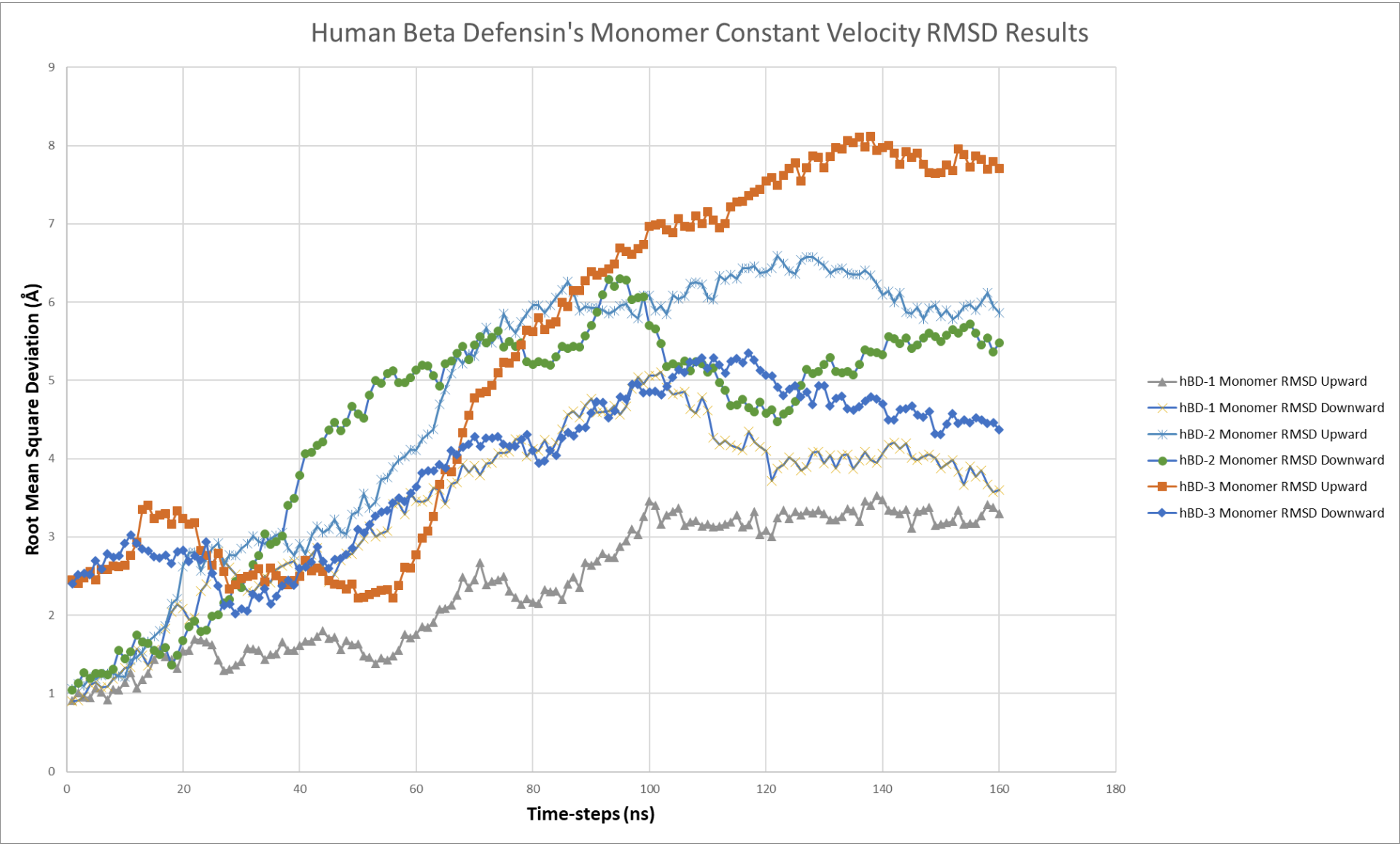
Constant Velocity RMSD Results



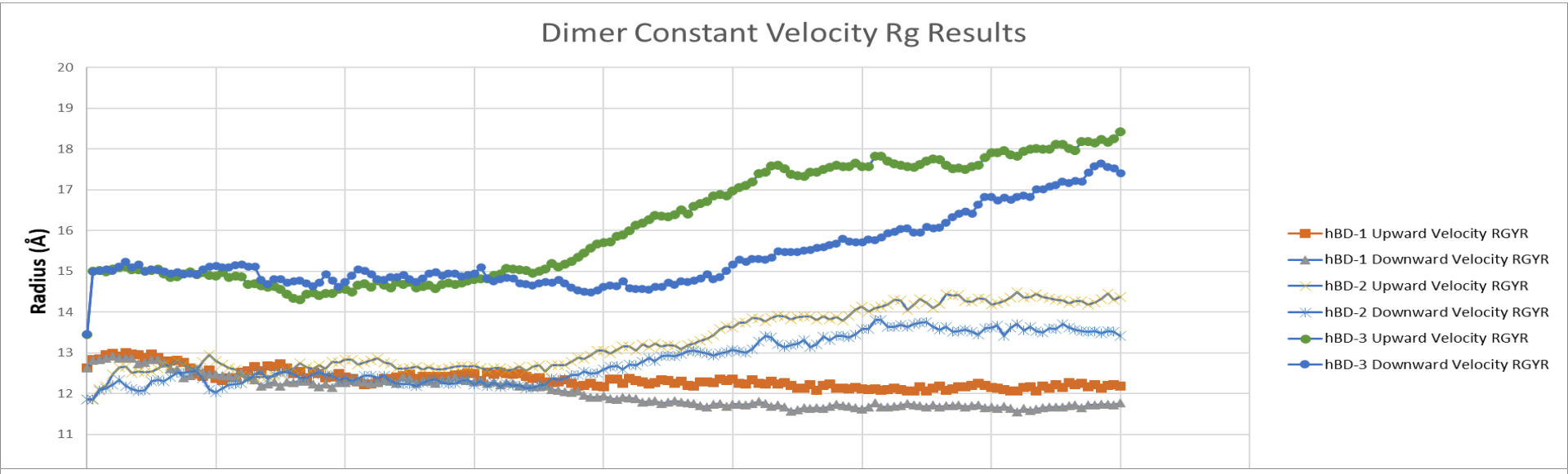
Constant Velocity RMSD Results

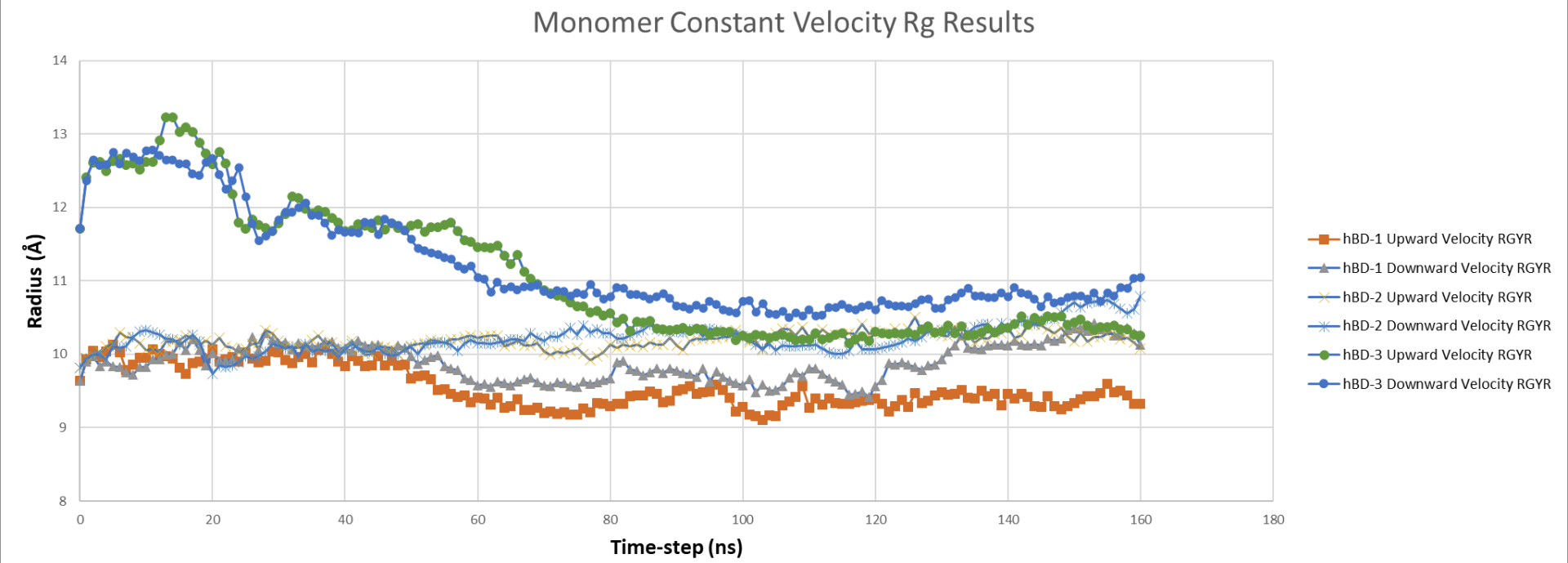


Constant Velocity RMSD Results



Constant Velocity Rg Results





Based on Constant Velocity Pulling Results:

- Similar to constant force results, averagely the monomer configuration were pulled a further distance from the membrane than their respective dimer form;
- Although the monomer configuration was pulled further, the dimer configurations had a higher average force;
- On average, the upward pulling of the dimer form had a higher RMSD value compared to other forms;
- It can also be seen that the PROA forms of the dimer during downward pulling have the lowest RMSD values (with the exception of hBD-3 where PROA downward is second lowest after PROA upward) in comparison to others;
- The monomer configurations have a smaller radius compared to their dimer counterparts;
- Apart from hBD-3 dimer, all of the other radii start between 12 and 13 Å, to which they then deviate from each other at approximately 80 ns;