Suitability of the MARTINI Force Field For Use With Gas-Phase Protein Complexes

By Sarah K. Fegan and Mark Thachuk

Department of Chemistry
University of British Columbia
Vancouver, BC, Canada

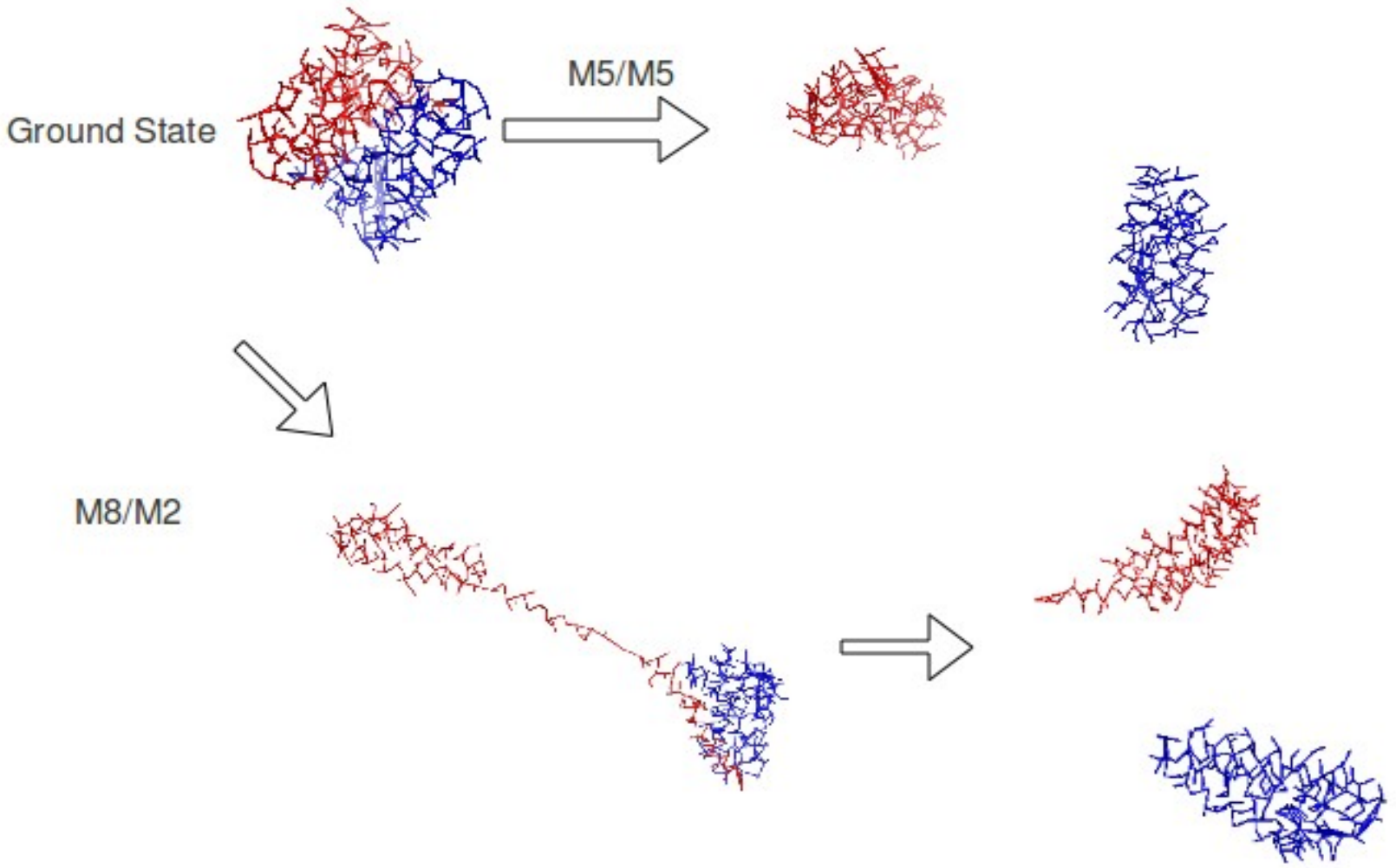
Introduction

- We would like to better understand the mechanism for the dissociation of non-covalently bound protein complexes in the gas phase.
- Several groups have performed mass spectrometry studies on the dissociation of protein complexes [1].
  - Collisionally Induced Dissociation produces asymmetric dissociation (the charge to mass ratio of the fragments differ).
  - For multimeric complexes, often only one monomer separates from the complex at a time.
  - The higher-charged monomer unfolds.
- Coarse-grained models treat groups of atoms as single interaction sites (beads), thus reducing the number of particles. They allow for molecular dynamics simulations of larger systems and/or longer time scales. [2]
- This work has been published in Fegan,S.K.; Thachuk, M. *J. Chem. Theory Comput.* **2012**, 8, 1304−1313.

Methods

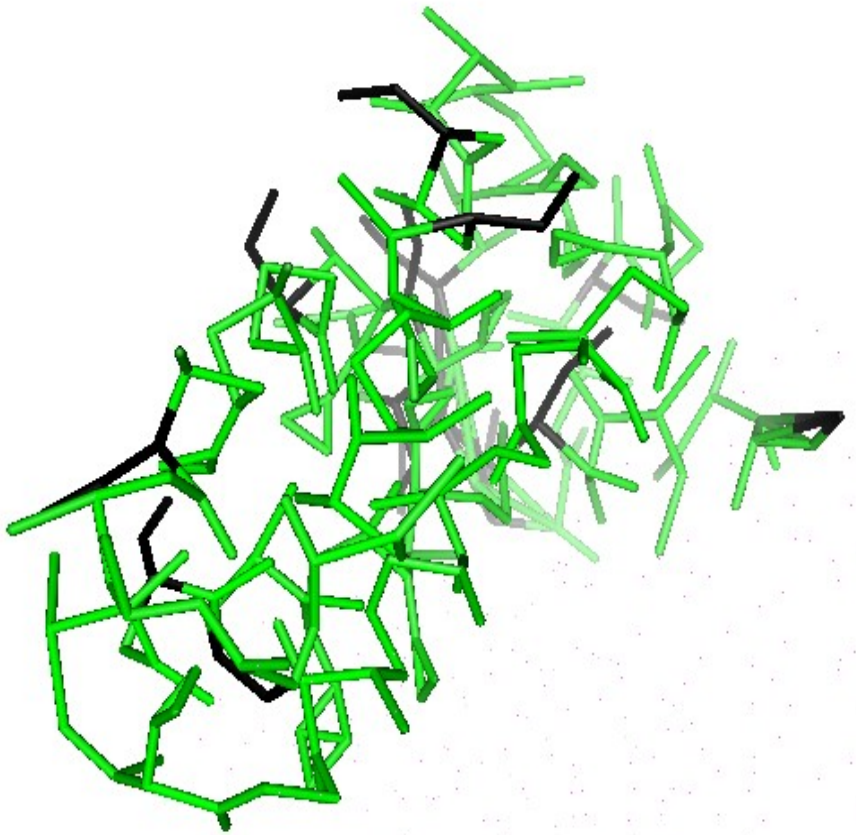
- The Gromacs molecular dynamics program was used with
  - Constant temperature of 300K with a Nose-Hoover thermostat
  - No periodic boundary conditions
  - Time step of 30 fs (with local elastic network bonds) and 2 fs (without local elastic network bonds)
- The MARTINI force field [3,4]
  - No electrostatic cut-offs
  - Relative dielectric constant = 1
- With a fixed total charge of 10+, M5/M5 and M8/M2 dimers of cytochrome c` (PDB ID 1bbh) were studied
  - With and without local elastic network bonds
  - With pulling at 0.00001 nm/ps and 0.001nm/ps, and stepwise pulling
  - With umbrella sampling (M5/M5)
- For each case, 100 trajectories were run and the results were then averaged.

Snapshot of Dissociation

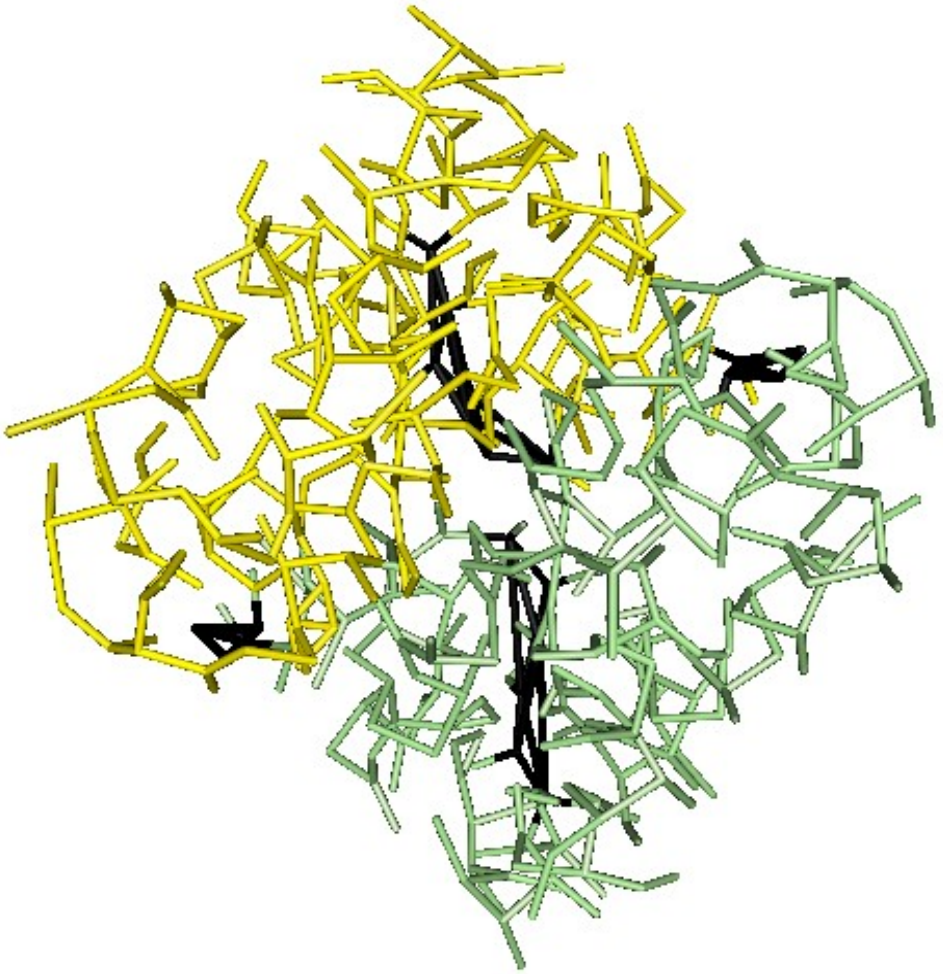


For the M8/M2 complex, the red monomer has +8 charges and the blue monomer has +2 charges.

Basic Sites (on monomer)

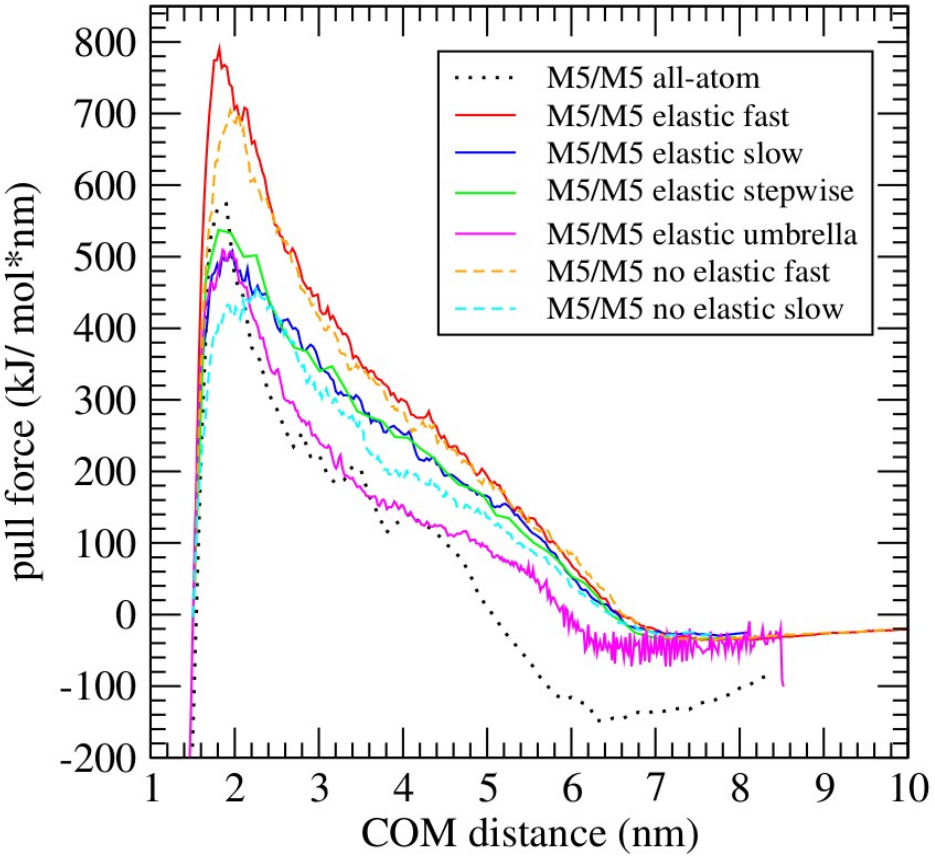


Local Elastic Nework Bonds (on dimer)



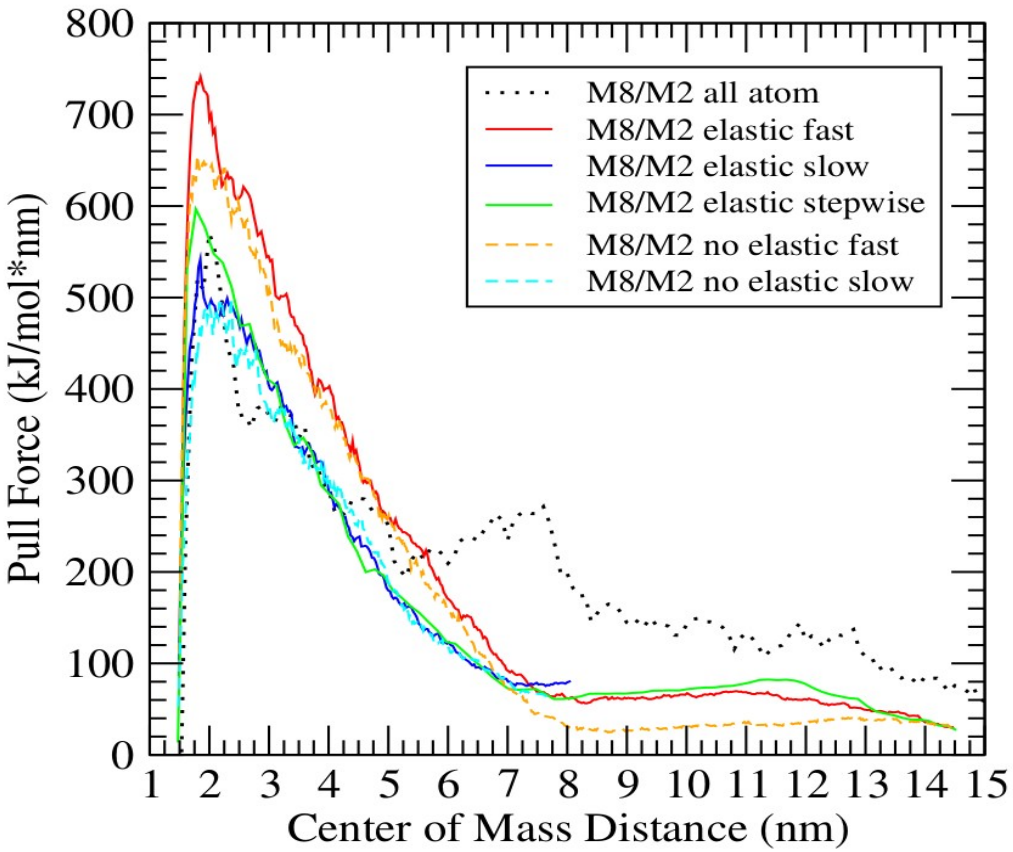
Results

M5/M5 Pull Force

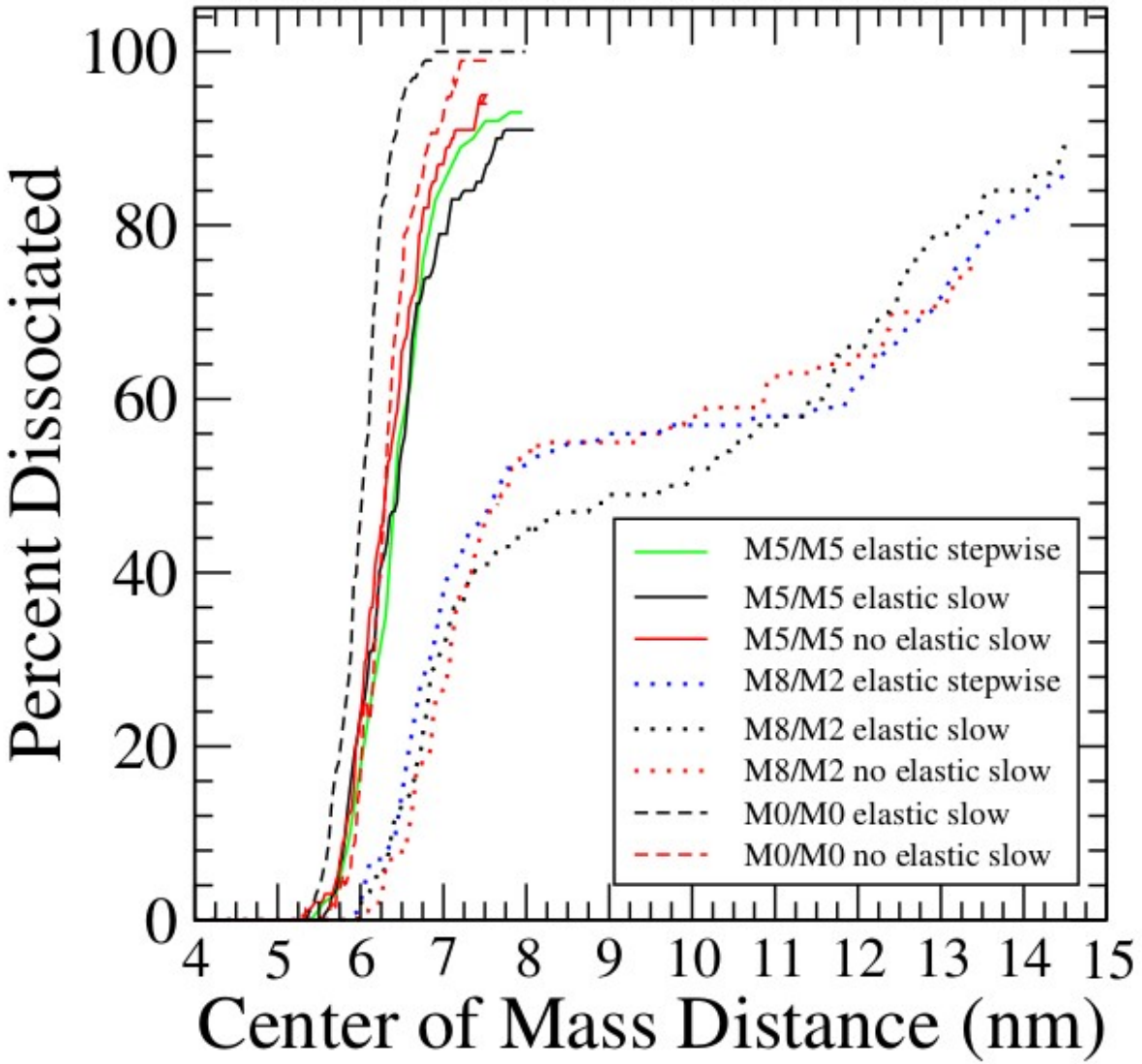


- At COM distances larger than 7 nm all the methods have a similar slightly negative pull force (from the Coulomb repulsion), but the all-atom pull force is more negative. The umbrella sampling and slow pull are closer to the all-atom results than the fast pull.

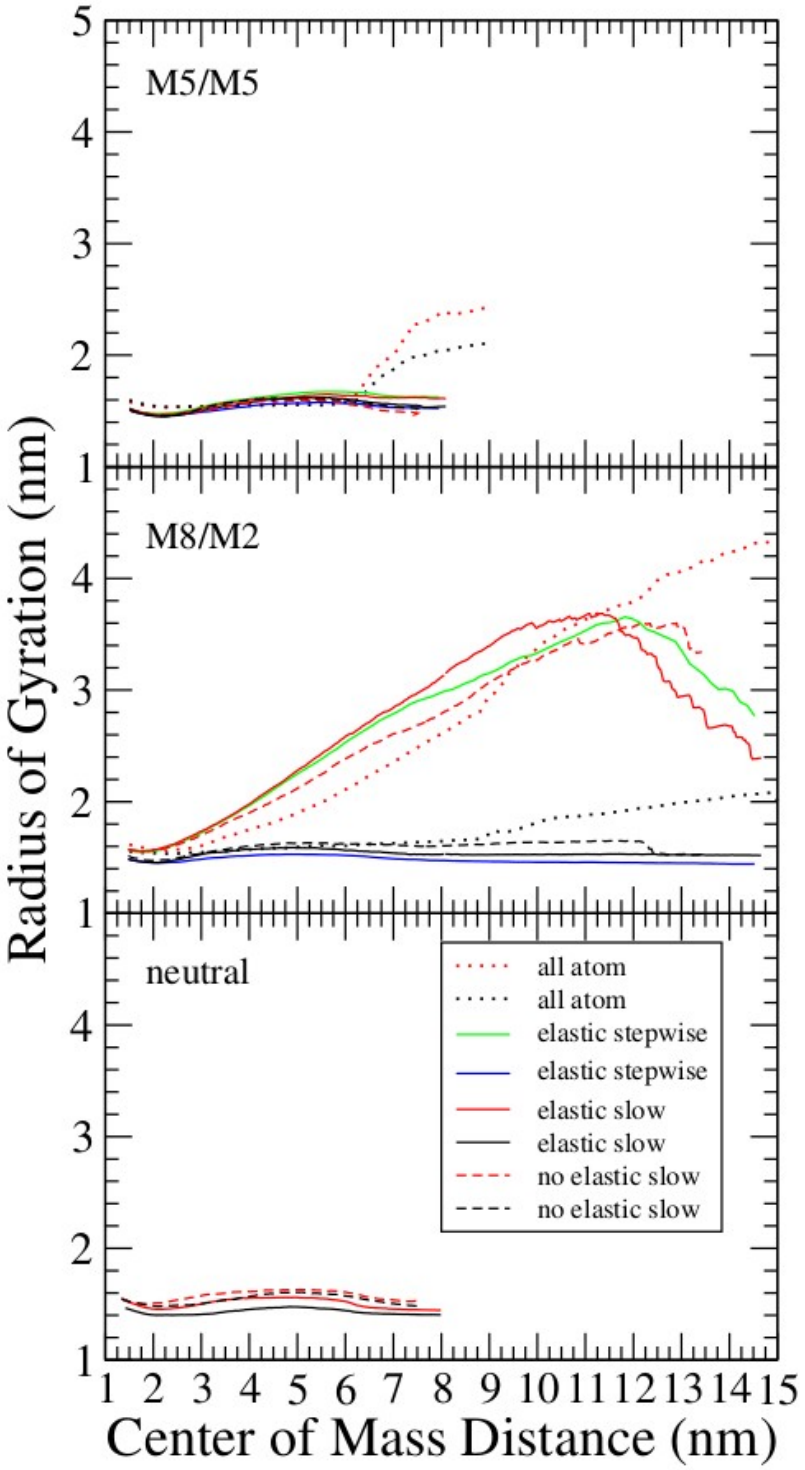
M8/M2 Pull Force



- The M8/M2 has similar results to the M5/M5, except at large COM distances (up to 15 nm) the pull forces remain positive.



- The neutral (dashed) and M5/M5 (solid) complexes dissociate quickly and with little structural changes.
- The M8/M2 (dotted) complexes dissociate at larger COM distances. About 50% have small structural changes and have dissociated by 8 nm, and the rest reach large COM distances before dissociation (unfolding behaviour).

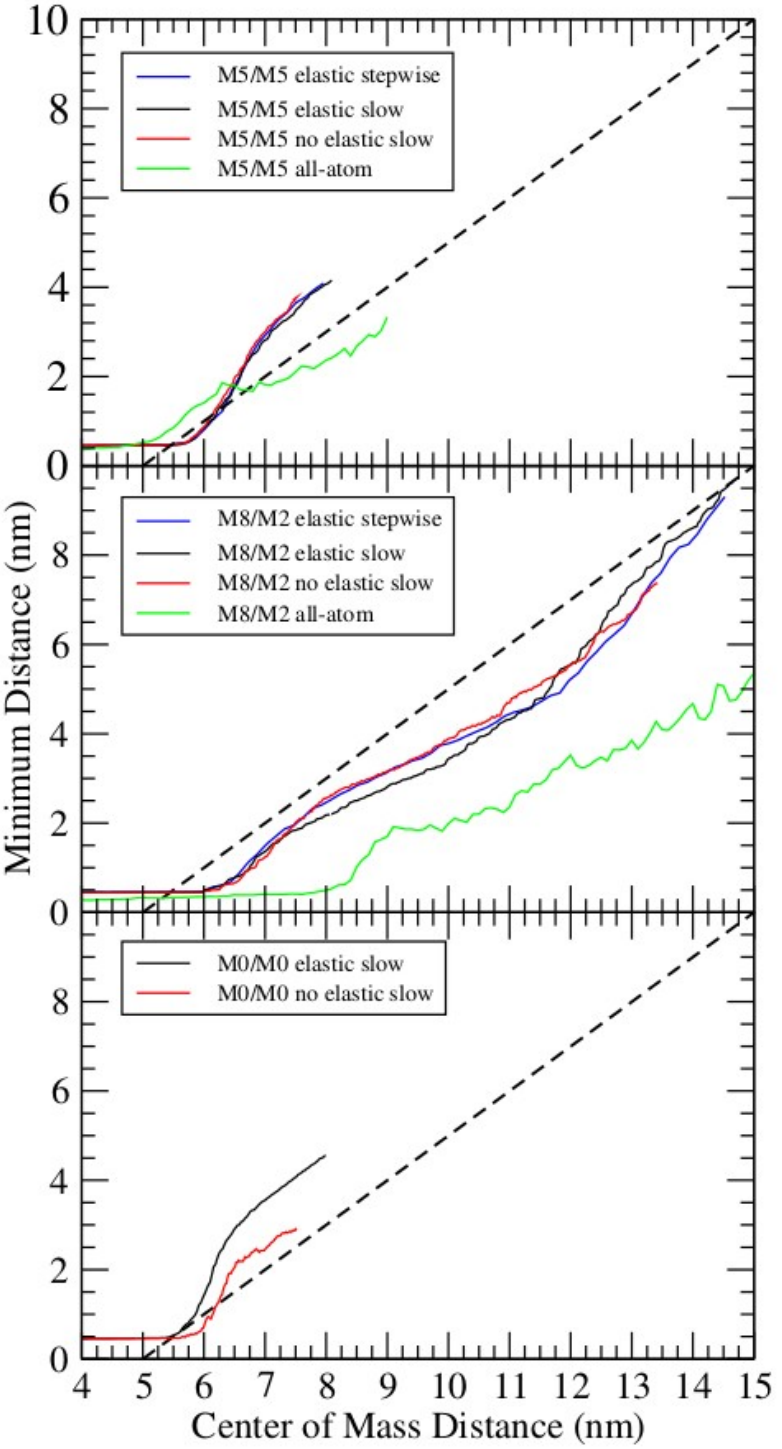


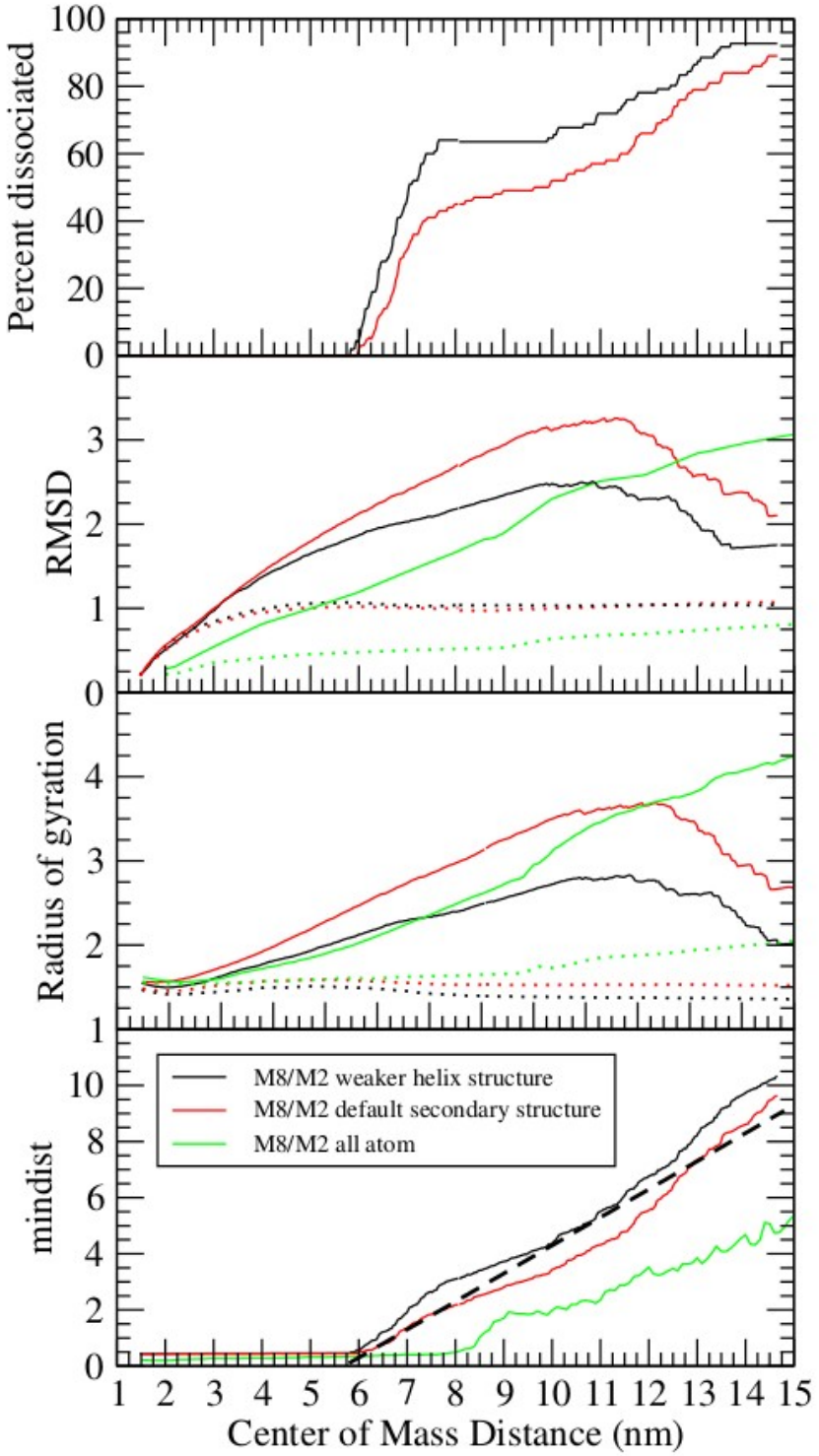
Radius of Gyration

- The M5/M5 complex shows very little change in size (compared to the all-atom which had an increase in radius of gyration after dissociation)
- In the M8/M2 complexes, the +8 charged monomer increased in size (unfolded) then decreased at large COM distances (unlike the all-atom which only increased). The +2 monomer remained compact.
- The neutral complexes also remained compact.

Minimum Separation of Monomers

- The dashed lines have a slope of 1.
- The minimum distance between the monomers remains small (and constant) until dissociation. After dissociation the minimum separation increases.
- At larger COM distances, the minimum separation lines have slopes of approximately 1, which indicates that the structures are no longer changing (i.e. the changes occur close to dissociation).





Effect of Secondary Structure

- In the MARTINI force field, secondary structure is imposed by fixing the force constants for the bonded interactions of the backbone beads. In particular, the force constants for helices are very high to hold those structures in place.
- We ran simulations with the default values (red) and with the force constants for helices at half the default values (black)
- The monomers with weaker helices are more compact (and unfold less), but the qualitative patterns are similar.

Conclusions

- The MARTINI force field reproduces the qualitative trends seen in the all-atom data [5].
  - The monomers with +8 charges show unfolding (while the lower charged monomers do not).
- The force field appears somewhat too attractive.
- The local elastic network bonds have a small effect on the results, but they stabilize the protein (allowing a larger time step).

Work in Progress

- Modifying the molecular dynamics code to allow for the movement of charges.
- Studying a tetramer (TTR).

References

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5. Wanasundara, S. N.; Thachuk, M. *J. Phys. Chem. A* **2009**, 113, 3814−3821. and Wanasundara, S. N.; Thachuk, M. *J. Phys. Chem. B* **2010**, 114, 11646−11653. (all-atom simulations)