

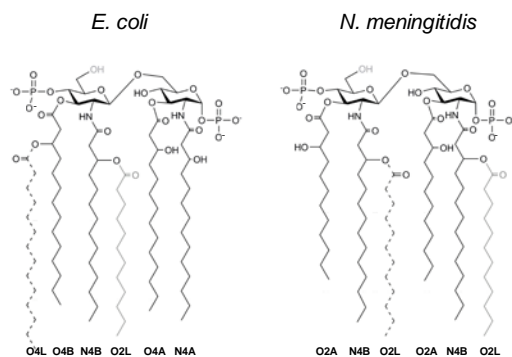
From Agonist to Antagonist: Structure and Dynamics of  
Innate Immune Glycoprotein MD-2 upon Recognition of  
Variably Acylated Bacterial Endotoxins

**Mari L. DeMarco & Robert J. Woods.**

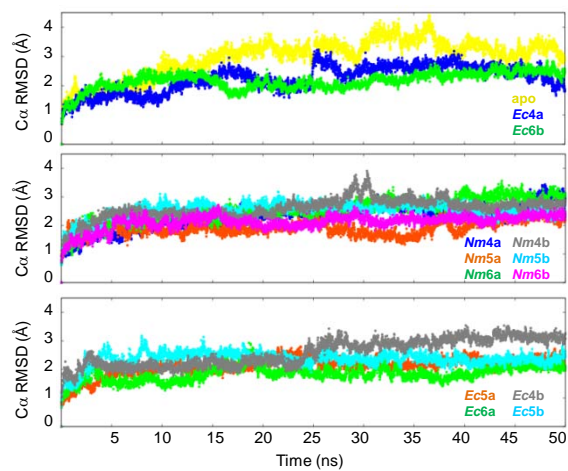
**Appendix.**

Supplementary Figures S1-S8

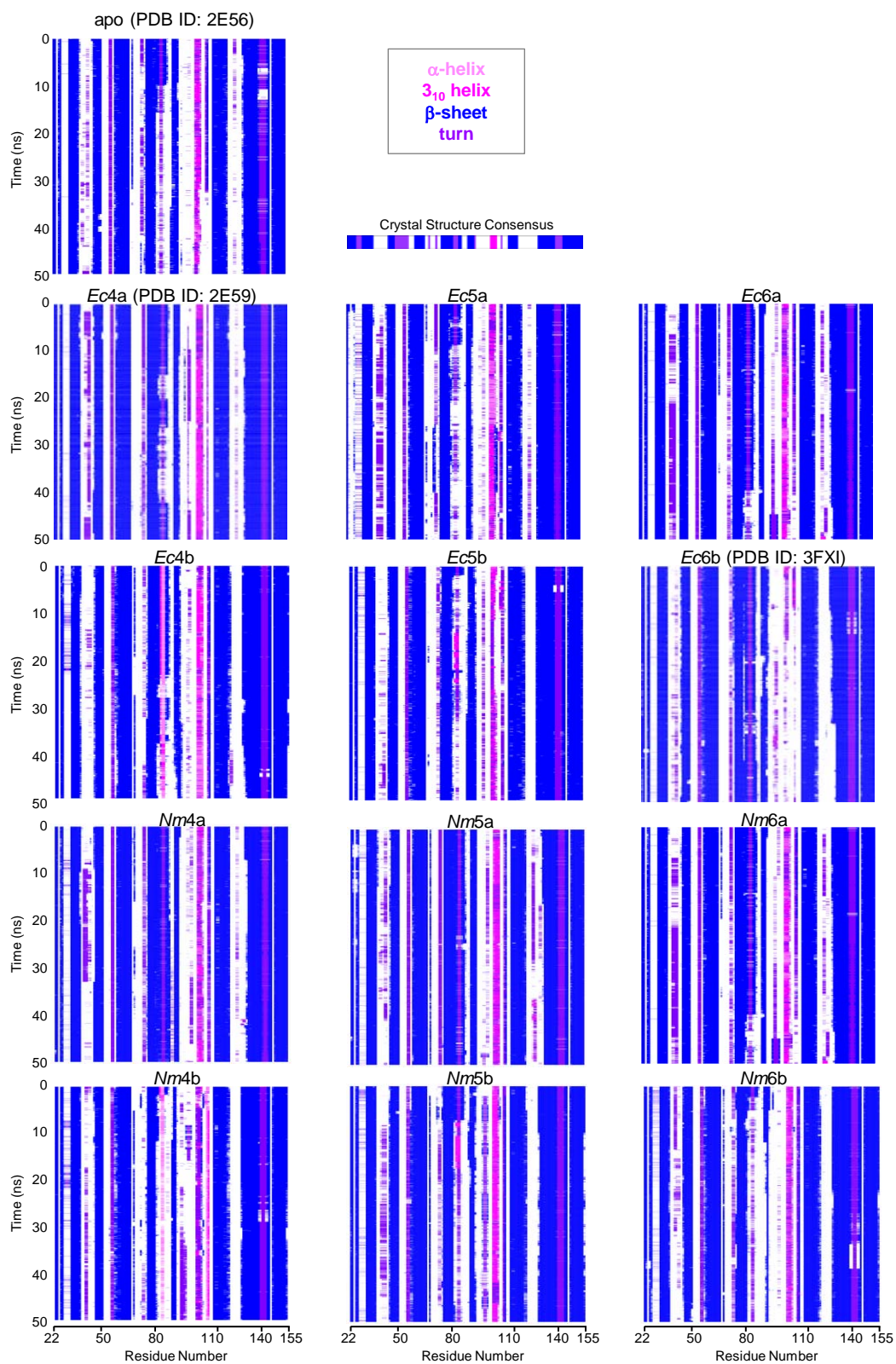
**Figure S1.** AMBER-formatted prep files for these acyl chains can be found at [www.glycam.org](http://www.glycam.org).



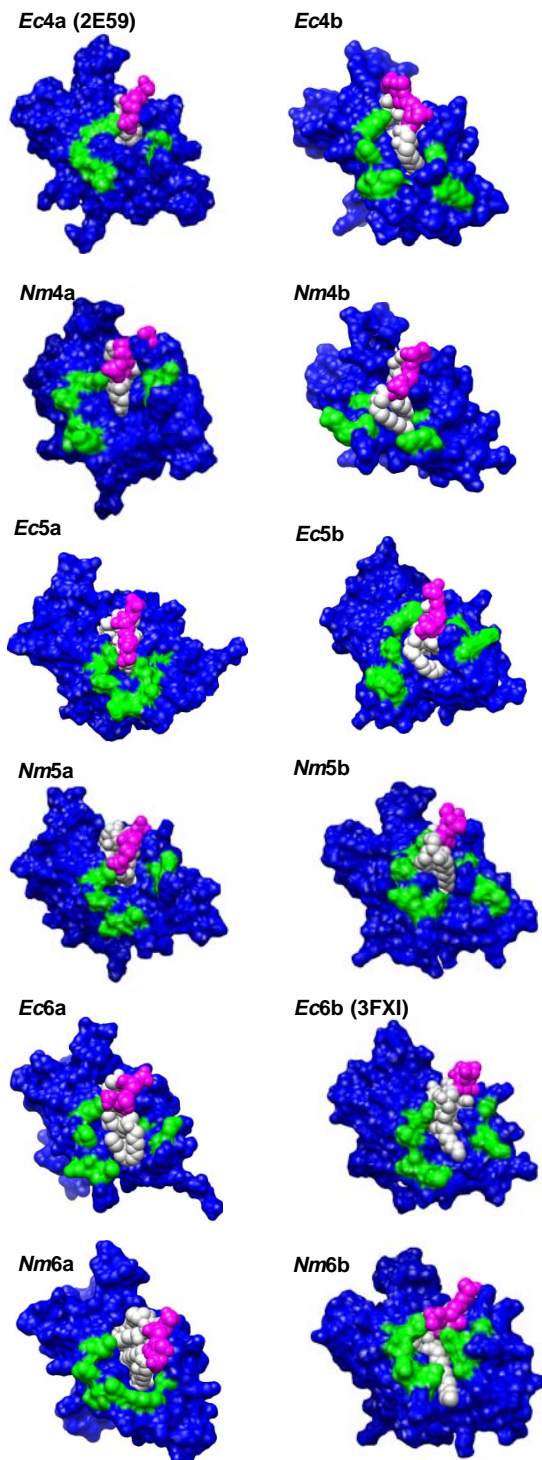
**Figure S2.** Global structural similarity measured via  $C\alpha$  RMSD.



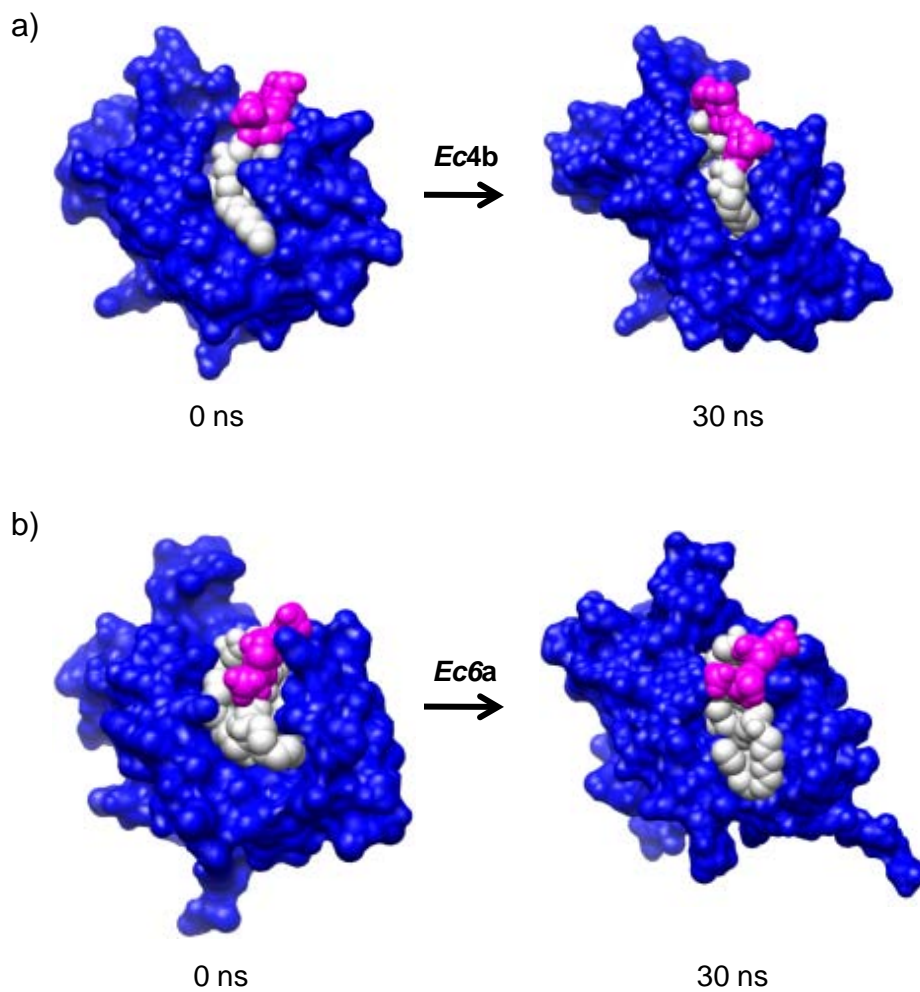
**Figure S3.** Secondary structure of MD-2 over the course of the simulations.



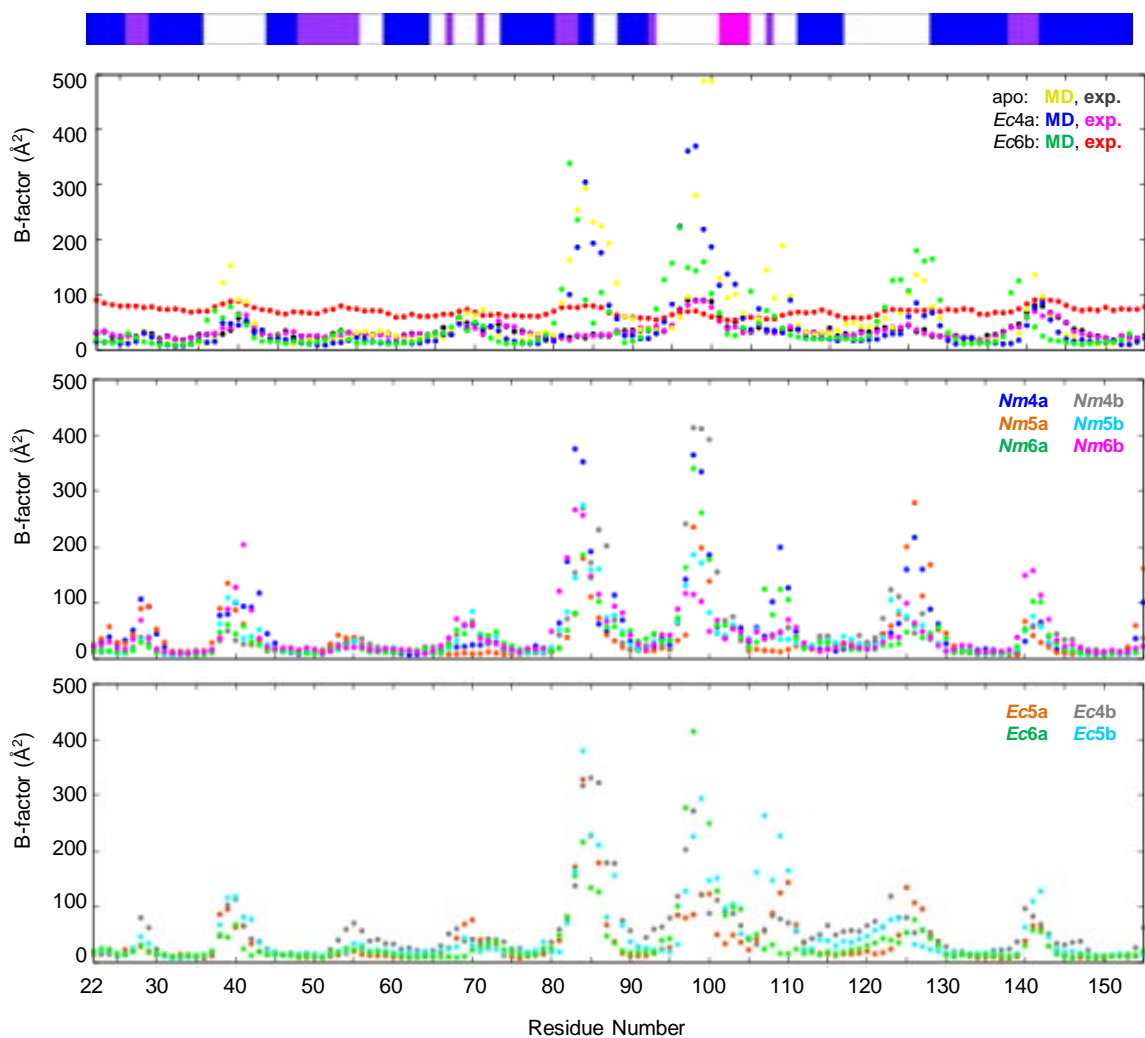
**Figure S4.** A change in the number of acyl chains of an endotoxin modifies the TLR4\* interaction face. For reference, the starting structures (based on crystal structures) of *Ec4a* and *Ec6b* are shown, whereas the remaining structures are the 30 ns snapshots from the simulations. MD-2 (blue surface) with residues within 3.5 Å of TLR4\* in the crystal structure 3FXI colored green, and the phosphoglycan domain and acyl chains of the endotoxin are colored magenta and grey, respectively.



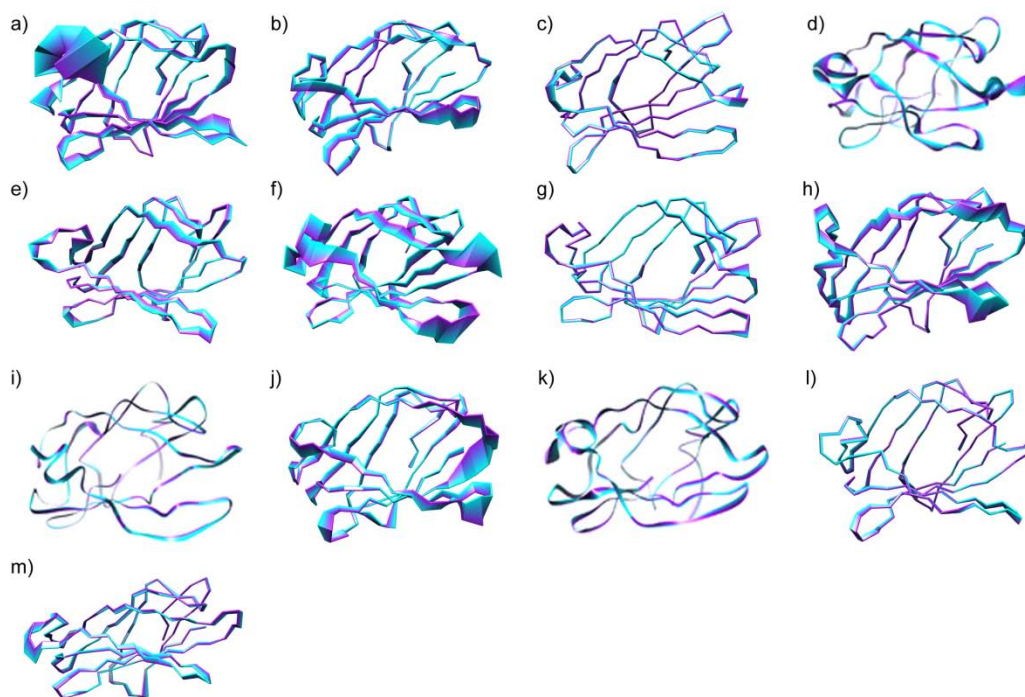
**Figure S5.** Location of the endotoxin within the binding cavity was independent the starting structure used for MD simulation. Initial and 30 ns snapshots from the simulation of complexes (a) *Ec4b*, and (b) *Ec6a*. MD-2, blue; endotoxins, magenta with acyl chains in grey.



**Figure S6.** Calculated and observed C $\alpha$  B-factors. Common secondary structure elements from the various crystal structures used as starting structures is noted at the top for reference.



**Figure S7.** Lowest frequency correlated backbone motions of MD-2 from each trajectory share a common pattern of motion. Viewed looking down at the binding pocket of MD-2, where the thickness of the ribbon representation of the backbone is proportional to the magnitude of the motion. (a) *Ec4a*, (b) *Ec4b*, (c) *Ec5a*, (d) *Ec5b*, (e) *Ec6a*, (f) *Ec6b*, (g) *Nm4a*, (h) *Nm4b*, (i) *Nm5a*, (j) *Nm5b*, (k) *Nm6a*, (l) *Nm6b*, (m) apo.



**Figure S8.** Evolution of apolar solvent accessible surface area of the endotoxin in the MD-2 complex: (a) *E. coli*, (b) *N. meningitidis*.

