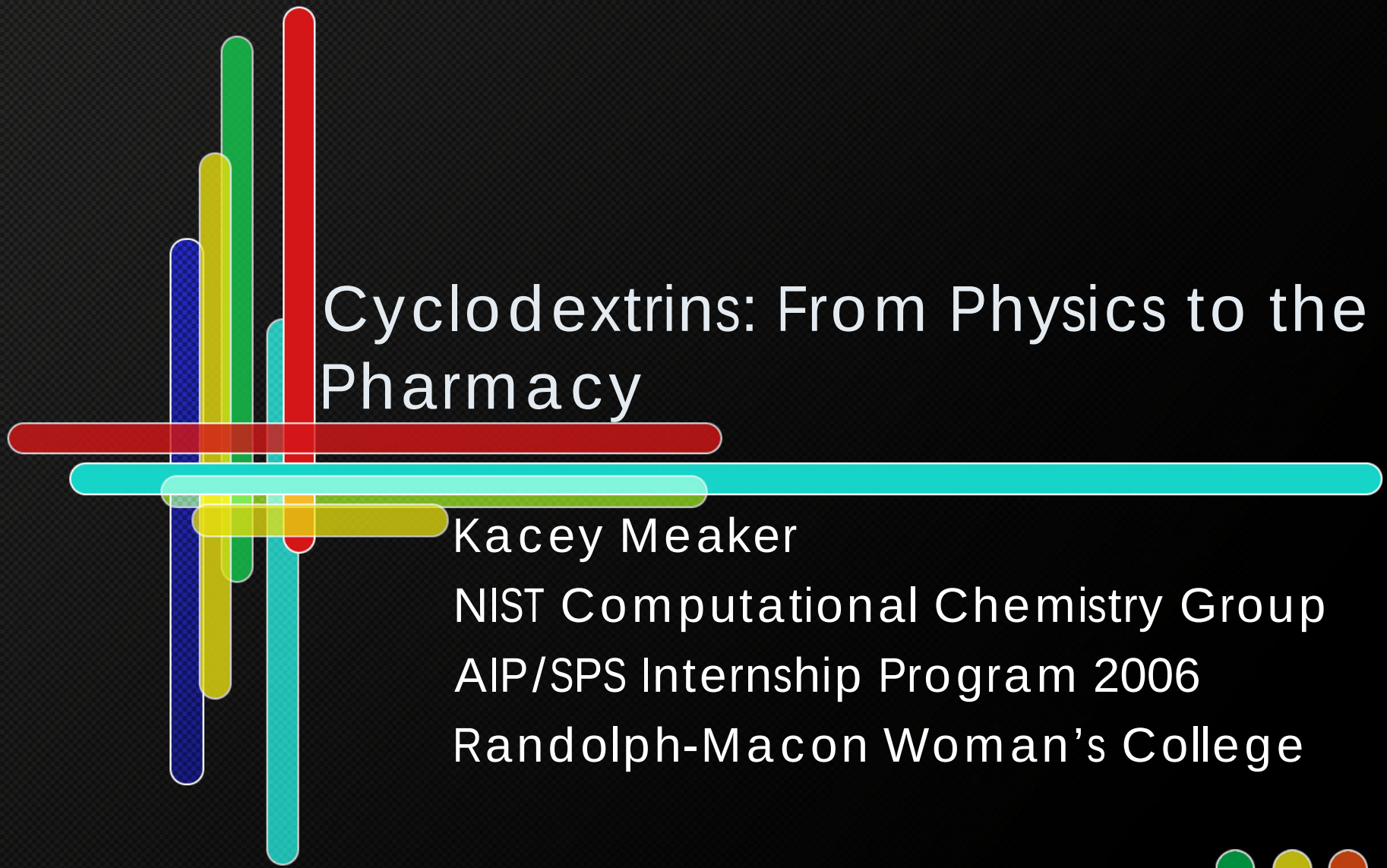


Cyclodextrins: From Physics to the Pharmacy

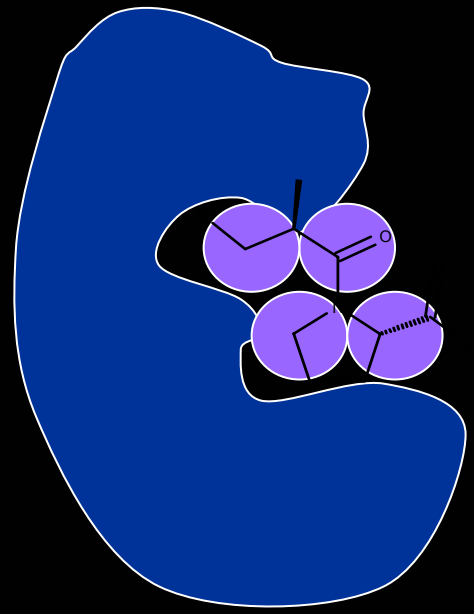
Kacey Meaker
NIST Computational Chemistry Group
AIP/SPS Internship Program 2006
Randolph-Macon Woman's College

Purpose

- Improve physical models for use in drug design
- Extend the medicinal applications of cyclodextrins

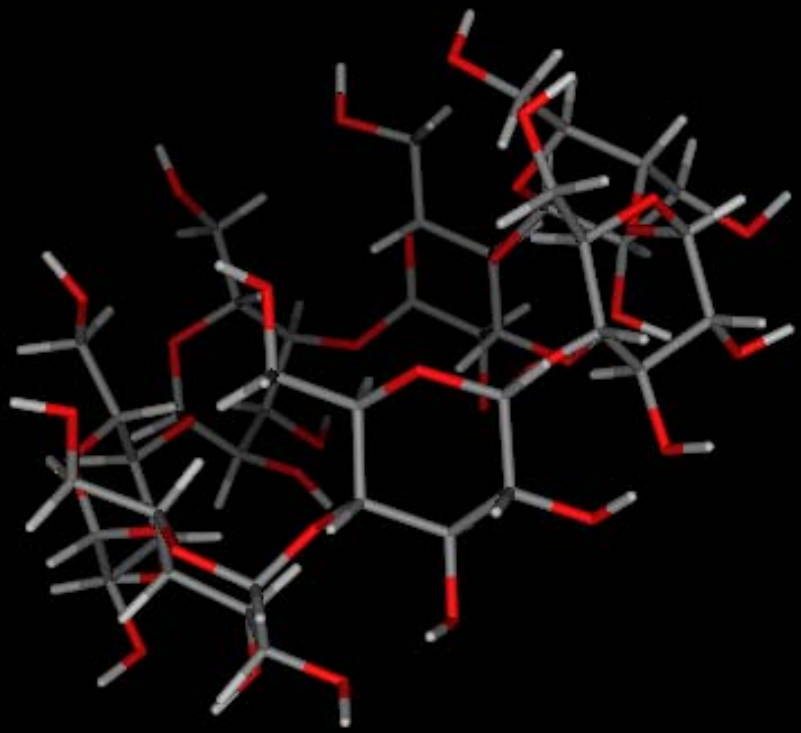
Computational Methods

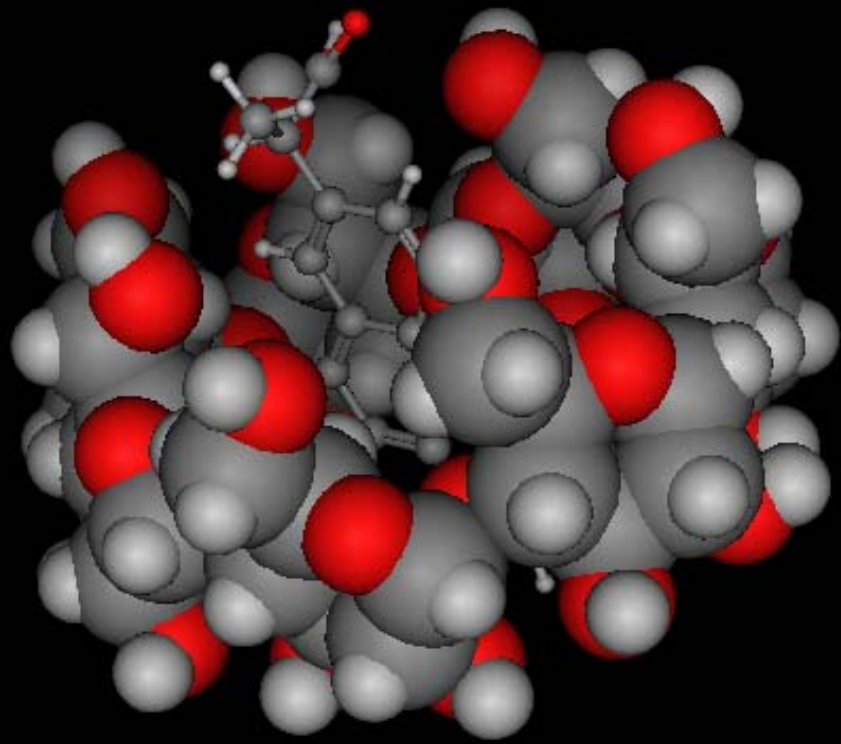
- Merck Molecular Force Field (MMFFs)
- Monte Carlo Simulations
- Cluster Analysis
- Docking and Scoring



Jensen, F. *Introduction to Computational Chemistry*. John Wiley & Sons (1999).
Halgren, T. A. *J. Comput. Chem.* **17**, 490-519 (1996).
Leach, A. R. *Molecular Modelling: Principles and Applications*. Addison Wesley Longman Limited (1996)
Shenkin, P.S. and McDonald, D. Q. *J. Comput. Chem.* **15**, 899-916 (1994).

Cyclodextrins 101





Loftsson, T. and Brewster, M. E. *J. Pharm. Sci.* **85**, 1017-1025 (1996).

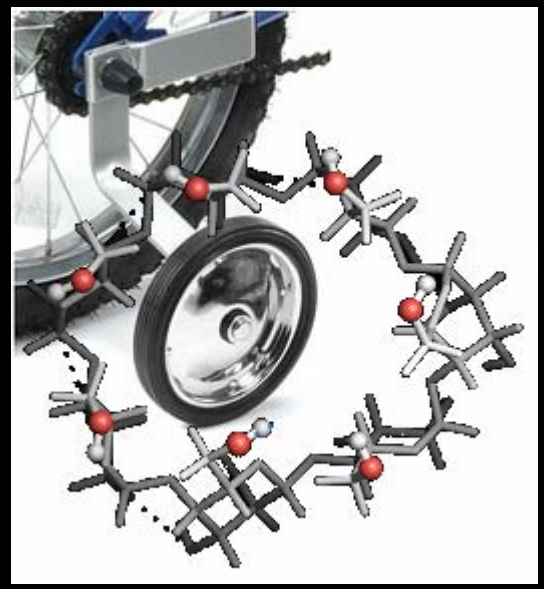
The Problem with Current Screening

- Current Screening: docking and scoring receptors and test ligands
- Problem: inconsistencies due to complexity of biological systems and incomplete physical models
- Solution: use a less complicated system like cyclodextrins that can be more easily understood

Leach, A. R. *Molecular Modelling: Principles and Applications*. Addison Wesley Longman Limited (1996)
Kapur, J. Presentation: From Sequence to Small Molecule: Drug Design Paradigms (2005).

Training Wheel Molecules

- Use cyclodextrins as training wheels to improve the model
- Calculate the free energy of binding with increasingly sophisticated methods
- Compare to experimental data to find where the errors are

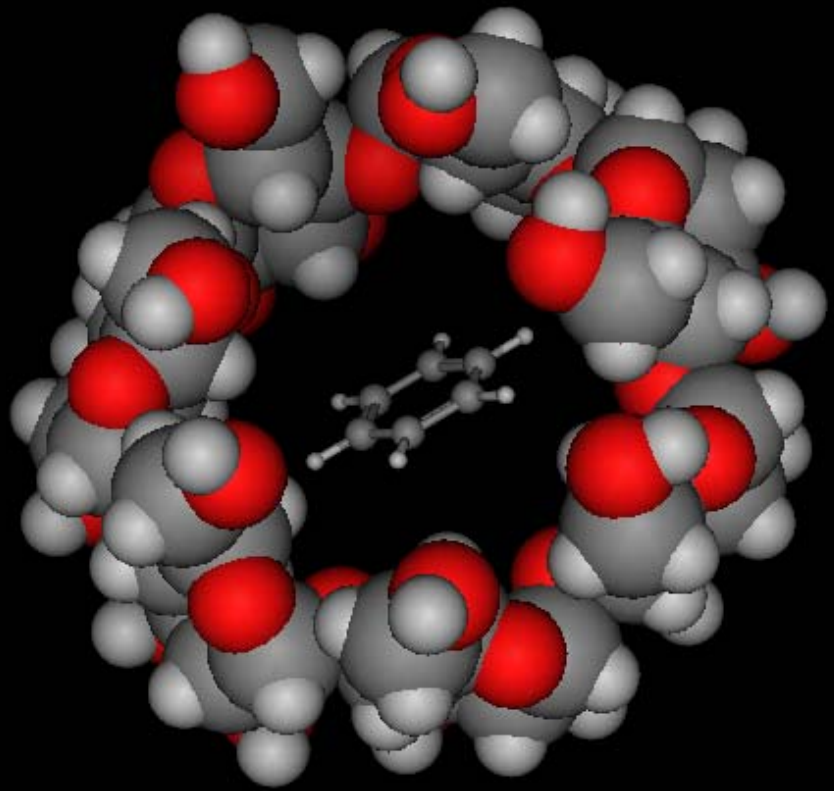


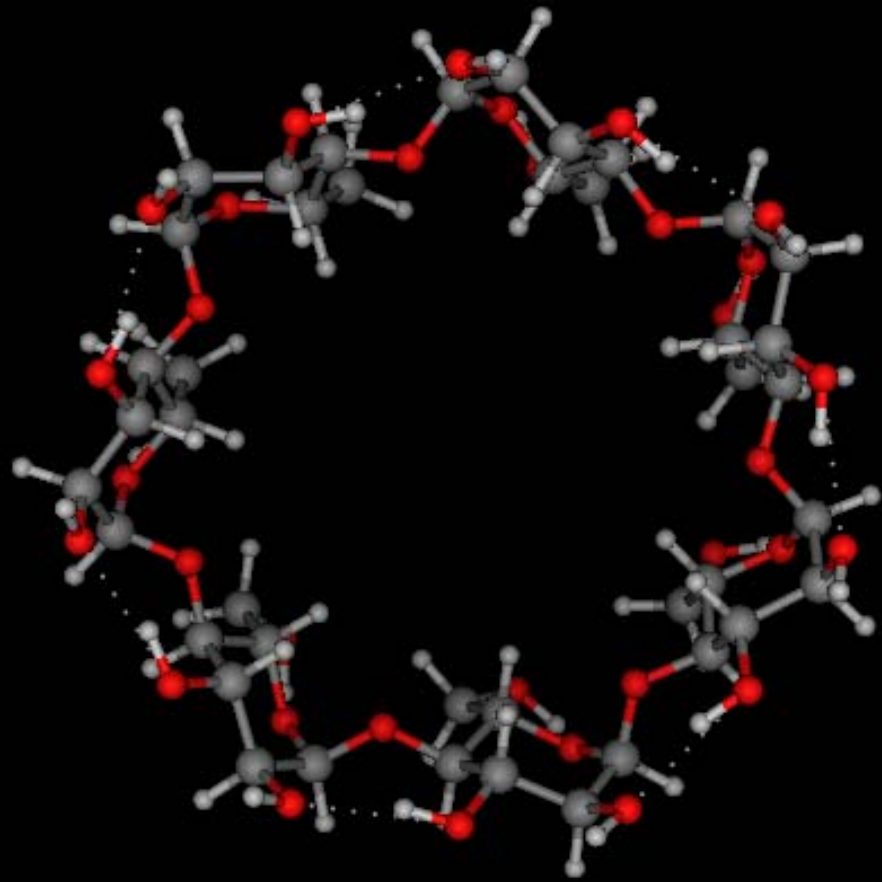
Kapur, J. Presentation: From Sequence to Small Molecule: Drug Design Paradigms (2005).

Progress toward Improving Models

- Calculated the free energy of binding for 22 derivatized β-cyclodextrins with 3 guest molecules for later experimental verification
- Possible future collaboration with University of Maryland to obtain experimental data

The Problem with β-cyclodextrin





Loftsson, T. and Brewster, M. E. *J. Pharm. Sci.* **85**, 1017-1025 (1996).

Heat of Solvation of Derivaterized β-CD's

Attached Group	Heat of Solvation (kcal/mol)	Attached Group	Heat of Solvation (kcal/mol)
Naked	-475.39	Ala-ala-cys-glu	-714.33
Hydroxypropyl	-500.81	Ala-ala-cys-arg	-403.88
Ala	-512.16	Ala-ala-cys-cys-glu	-695.62
Cys	-515.80	Ala-ala-cys-cys-arg	-475.91
Glu	-612.69	Bradykinin	-503.95
Arg	-349.32	Zonulin 1	-551.52
Ala-ala-glu	-646.51	Zonulin 3	-674.57
Ala-ala-arg	-375.20		

Amino Acids and Drug Interactions

- Hypothesis: Positive amino acids will cause negative drugs to bind more strongly and negative amino acids will cause negative drugs to bind less strongly
- This will allow us to choose different amino acids for different drugs and also be able to target drugs to different proteins

Docking: Benzene

Attached Group	Free Energy of Binding (kcal/mol)	Change from Derivaterizing (kcal/mol)	Attached Group	Free Energy of Binding (kcal/mol)	Change from Derivaterizing (kcal/mol)
Naked	-1.6868		Ala-ala-cys-glu	-1.6283	0.0585
Hydroxypropyl	-1.7324	-0.0456	Ala-ala-cys-arg	-1.7554	-0.0686
Ala	-1.7818	-0.0950	Ala-ala-cys-cys-glu	-1.7953	-0.1085
Cys	-1.7648	-0.0780	Ala-ala-cys-cys-arg	-1.8979	-0.2111
Glu	-1.0936	0.5932	Bradykinin	-1.6725	0.0143
Arg	-1.7815	-0.0947	Zonulin 1	-1.7458	-0.0590
Ala-ala-glu	-1.6963	-0.0095	Zonulin 3	-2.0955	-0.4087
Ala-ala-arg	-1.7457	-0.0589			

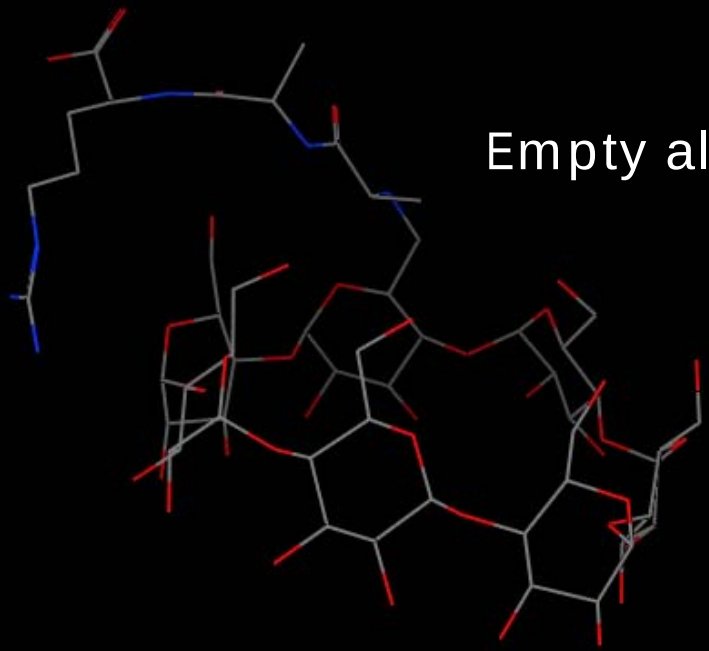
Docking: Naproxen

Attached Group	Free Energy of Binding (kcal/mol)	Change from Derivaterizing (kcal/mol)	Attached Group	Free Energy of Binding (kcal/mol)	Change from Derivaterizing (kcal/mol)
Naked	-4.3237		Ala-ala-cys-glu	-4.2644	0.0593
Hydroxypropyl	-5.2387	-0.9150	Ala-ala-cys-arg	-4.4746	-0.1509
Ala	-5.1545	-0.8308	Ala-ala-cys-cys-glu	-3.9905	0.3332
Cys	-4.3915	-0.0678	Ala-ala-cys-cys-arg	-4.4381	-0.1144
Glu	-4.7334	-0.4097	Bradykinin	-3.7227	0.6010
Arg	-4.3931	-0.0694	Zonulin 1	-2.8666	1.4571
Ala-ala-glu	-4.6726	-0.3489	Zonulin 3	-3.3238	0.9999
Ala-ala-arg	-4.7032	-0.3795			

Docking: Flurbiprofen

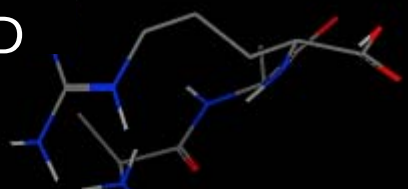
Attached Group	Free Energy of Binding (kcal/mol)	Change from Derivaterizing (kcal/mol)	Attached Group	Free Energy of Binding (kcal/mol)	Change from Derivaterizing (kcal/mol)
Naked	-4.1109		Ala-ala-cys-glu	-3.8418	0.2691
Hydroxypropyl	-3.9512	0.1597	Ala-ala-cys-arg	-3.5591	0.5518
Ala	-4.5858	-0.4749	Ala-ala-cys-cys-glu	-4.1750	-0.0641
Cys*	-2.2175	1.8934	Ala-ala-cys-cys-arg	-3.5683	0.5426
Glu	-4.4139	-0.3030	Bradykinin	-4.1060	0.0049
Arg	-4.3971	-0.2862	Zonulin 1	-5.5171	-1.4062
Ala-ala-glu	-4.4936	-0.3827	Zonulin 3	-5.2514	-1.1405
Ala-ala-arg	-4.3563	-0.2454			

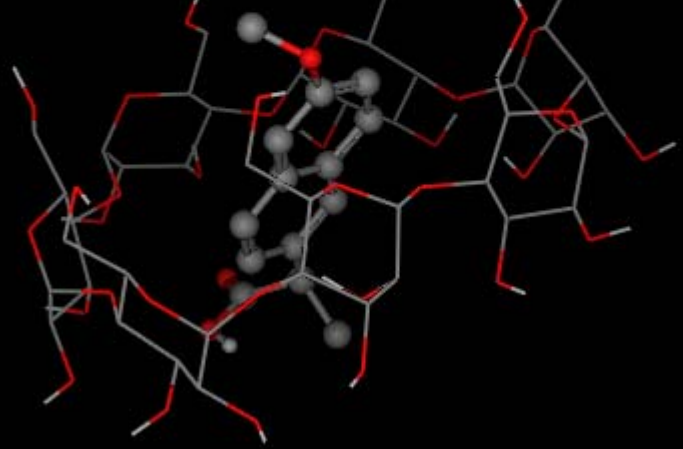
* Conformation of flurbiprofen is a false, very high energy conformation which leads to such a low binding energy. Calculation needs to be re-optimized and re-docked.

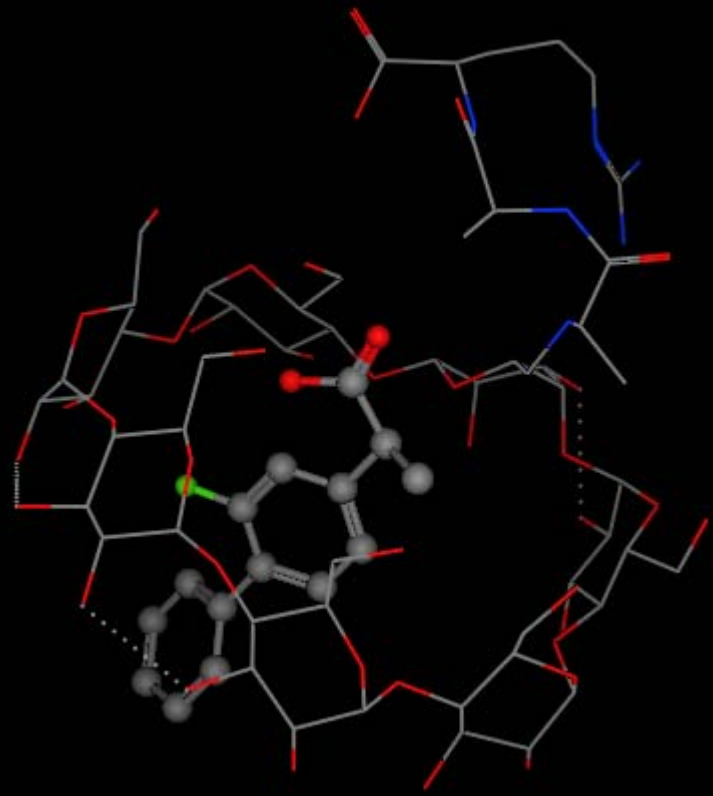


Empty ala-ala-arg-CD

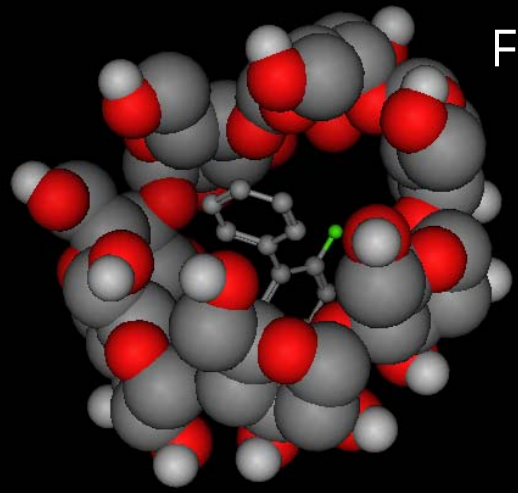
Naproxen in ala-ala-arg-CD
-4.7032 kcal/mol
Energy difference of
-0.3795 kcal/mol



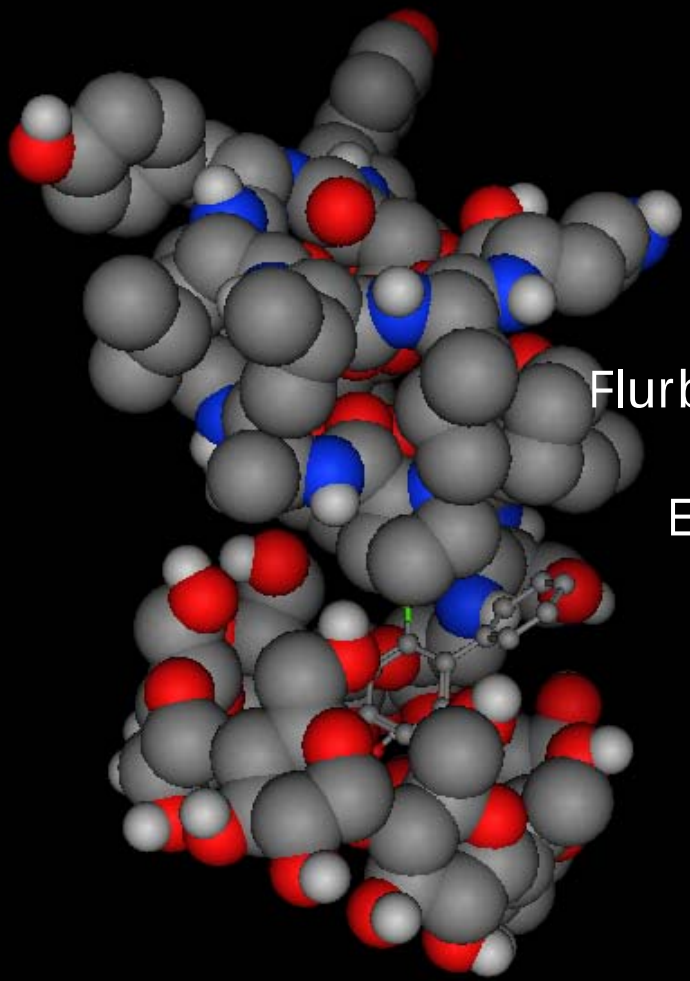




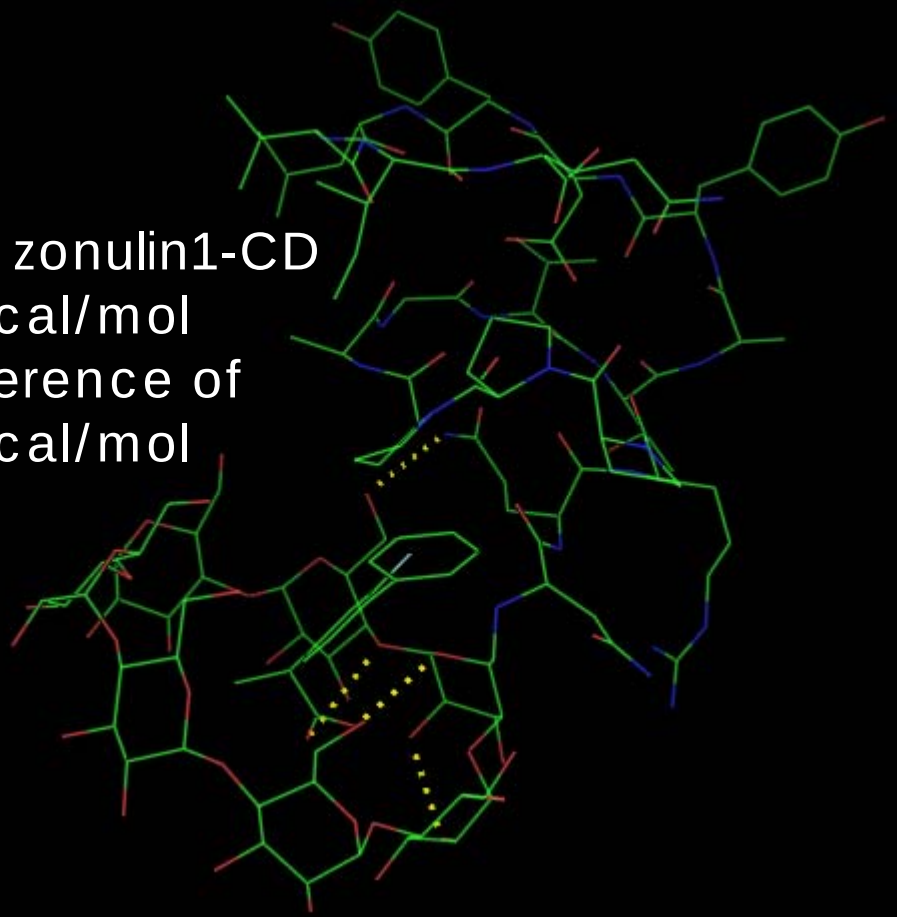
Flurbiprofen in ala-ala-arg-CD
-4.3563 kcal/mol
Energy difference of
-0.2454 kcal/mol



Flurbiprofen in Naked CD
-4.1109 kcal/mol

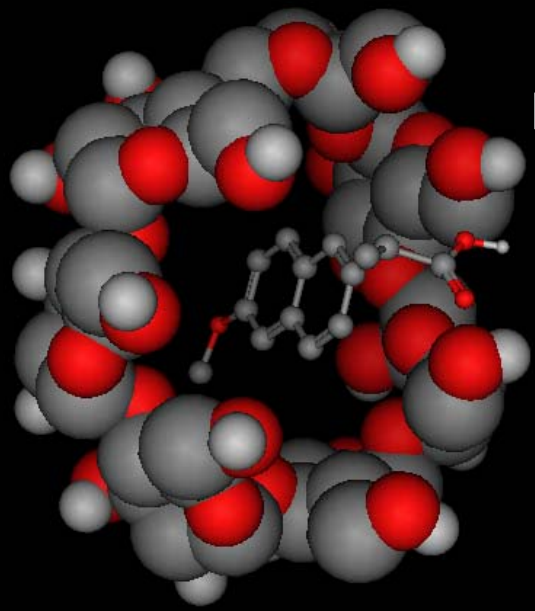


Flurbiprofen in zonulin1-CD
-5.5171 kcal/mol
Energy difference of
-1.4062 kcal/mol

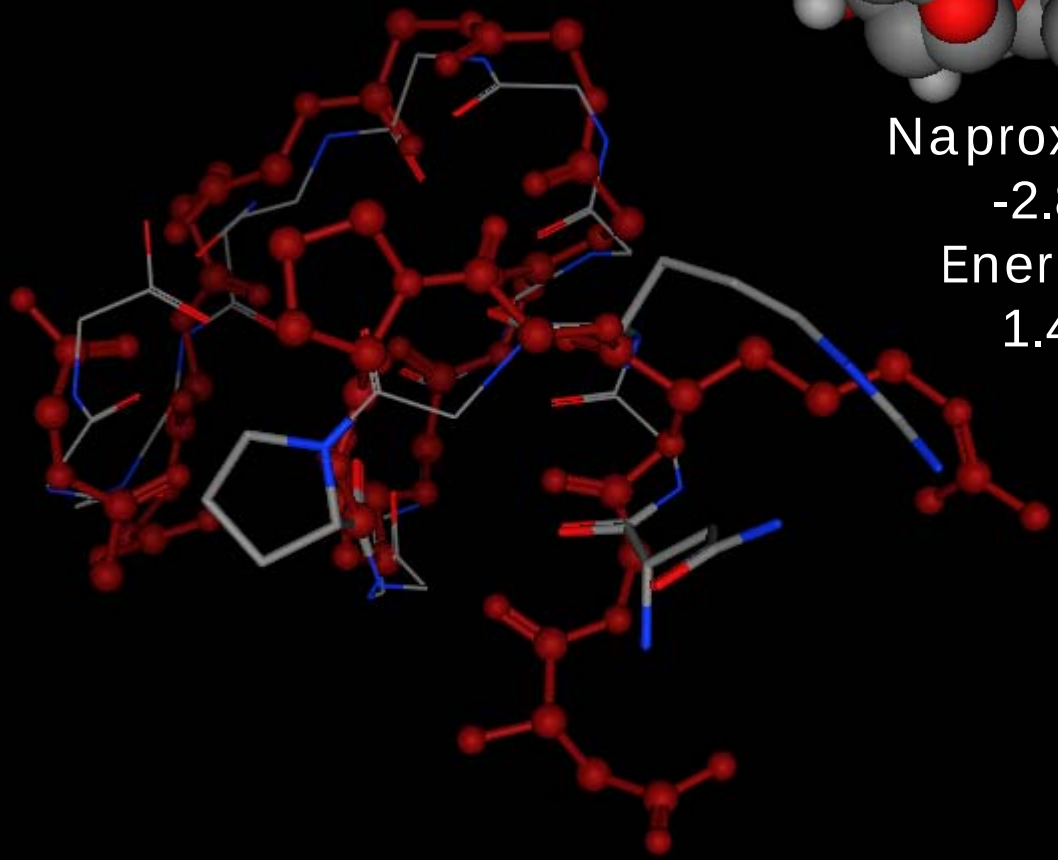


Auffinger, P. et al. *PNAS.* **101**, 16789-16796 (2004).

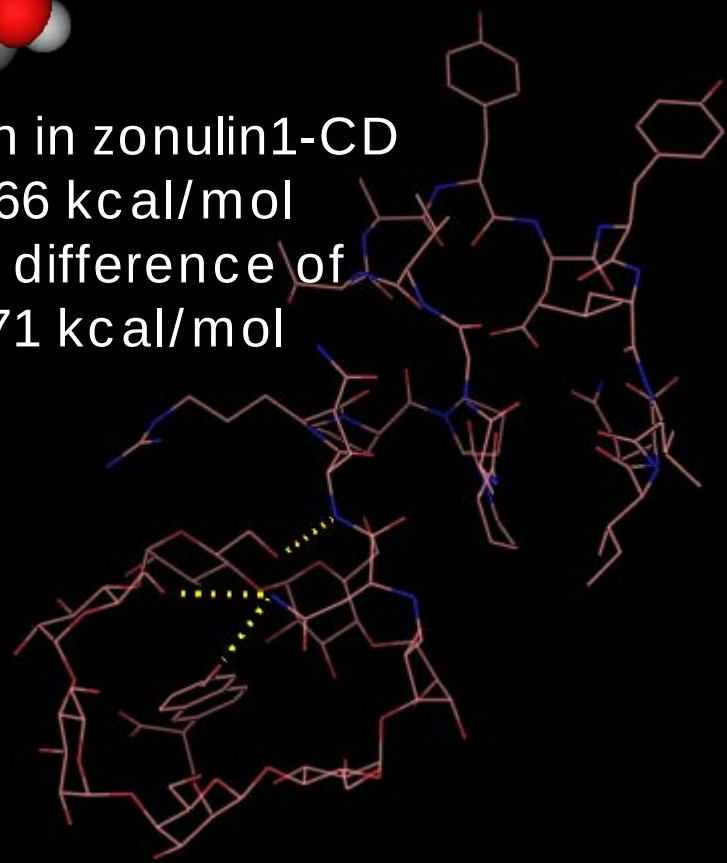
Zonulin1 peptide from naproxen (red)
Zonulin1 peptide from flurbiprofen (multi-colored)

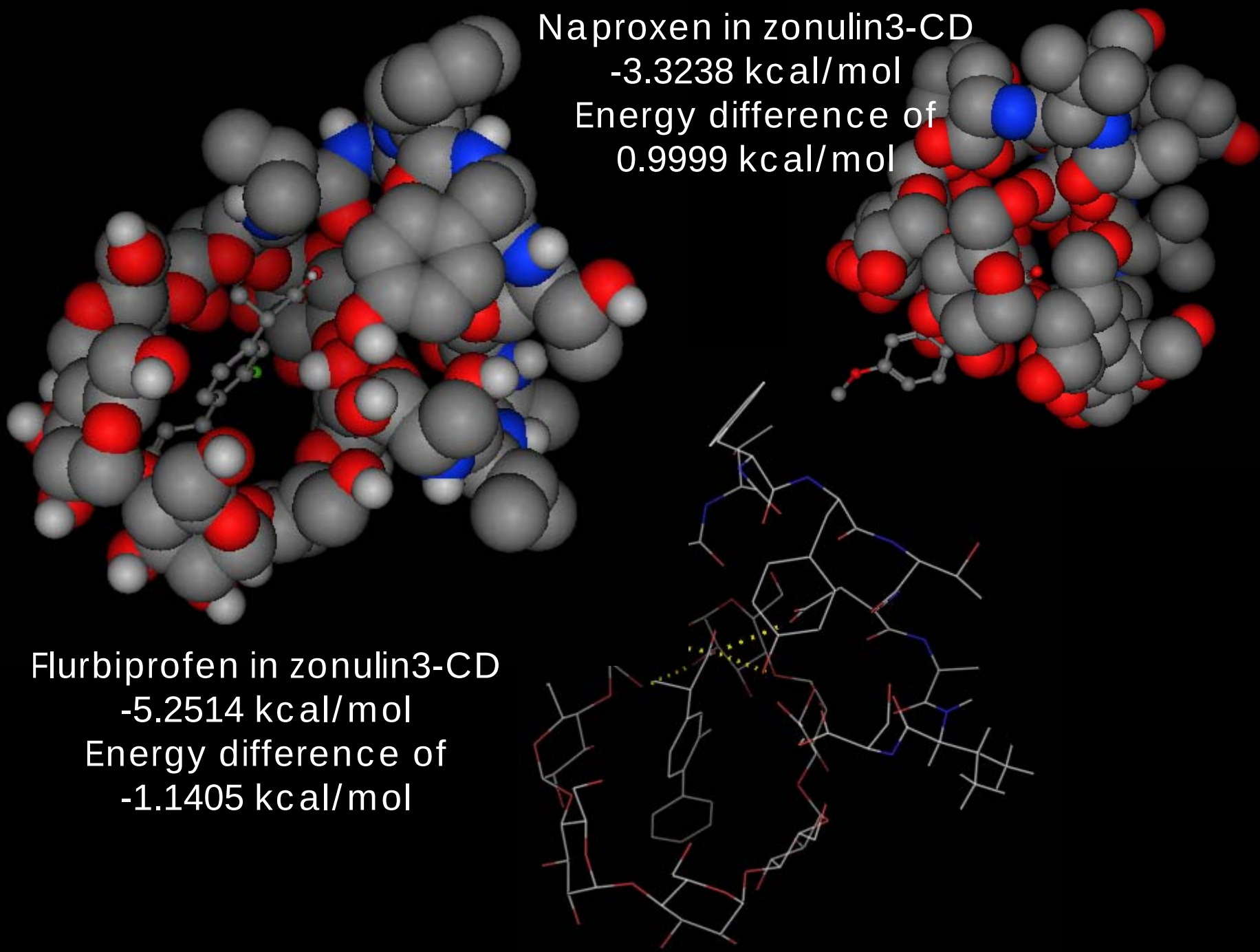


Naproxen in Naked CD
-4.3237 kcal/mol



Naproxen in zonulin1-CD
-2.8666 kcal/mol
Energy difference of
1.4571 kcal/mol




Naproxen in zonulin3-CD
-3.3238 kcal/mol
Energy difference of
0.9999 kcal/mol
Flurbiprofen in zonulin3-CD
-5.2514 kcal/mol
Energy difference of
-1.1405 kcal/mol

Results from Derivatization

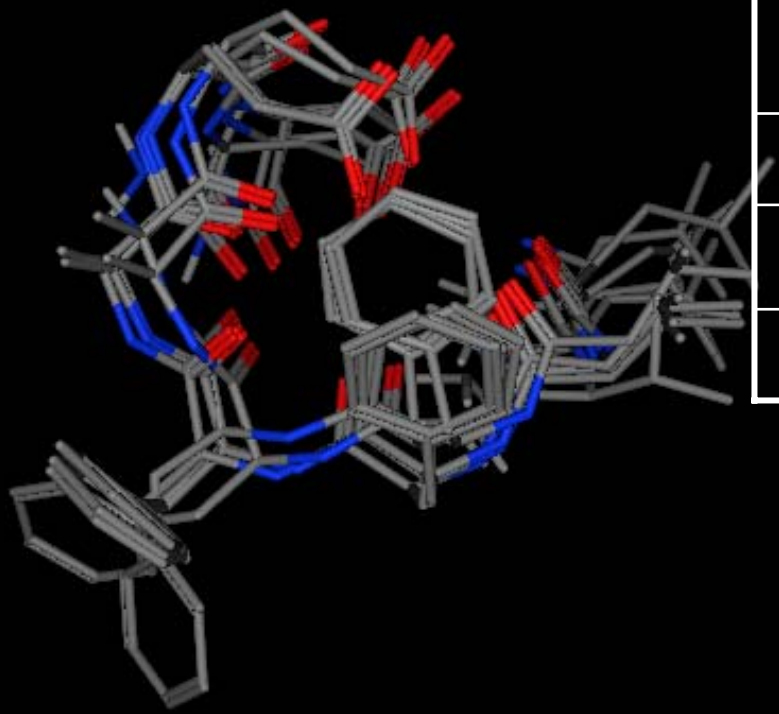
- Changes due to derivatization with amino acids have more to do with changes in pocket characteristics than a simple positive-negative interaction
- Need to do more analysis
- Derivatization with zonulin peptides results in a very stable docking with flurbiprofen

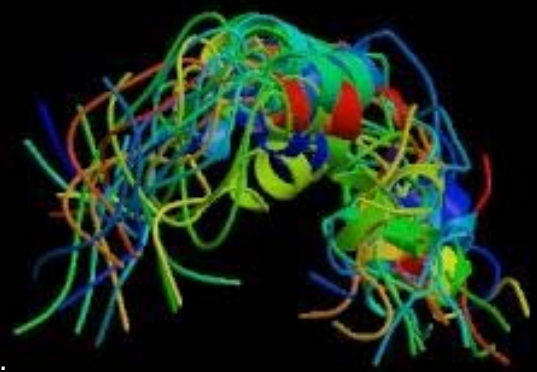
Karyekar, C. S. Fasano, A. et al. *J. Pharm. Sci.* **92**, 414-423 (2003).

Alzheimer's: A Real Life Example

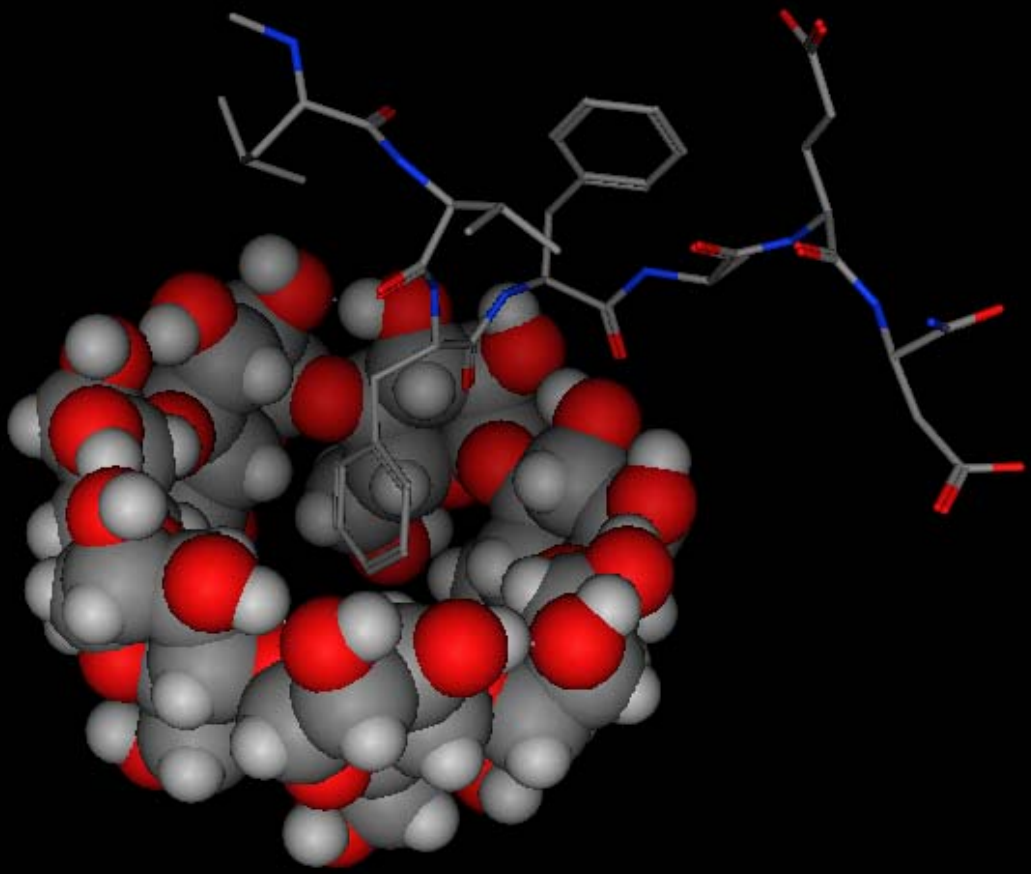
Conformational Analysis after 6000 steps

Peptide Residues	Minimum Energy (kcal/mol)	Conformations within 3 kcal/mol
16-23	-1373.73	90
12-28	-6318.39	56
1-40	-15341.57	30



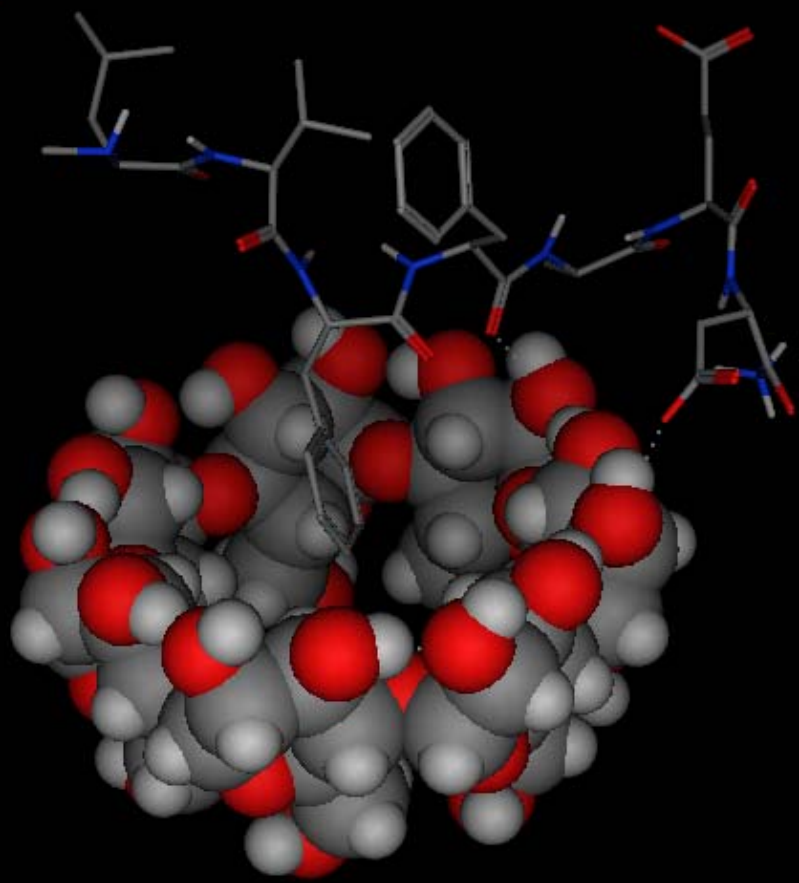


Sticht, H., Bayer, P. et al. *Eur. J. Biochem.* **233**, 293-298 (1995).
Danielson, J., Jarvet, J. et al. *Biochemistry.* **43**, 6261-6269 (2004).



Residues 16-23
Phe-19 docked into CD
0.60 kcal/mol

Residues 16-23
Phe-20 docked into CD
-2.37 kcal/mol



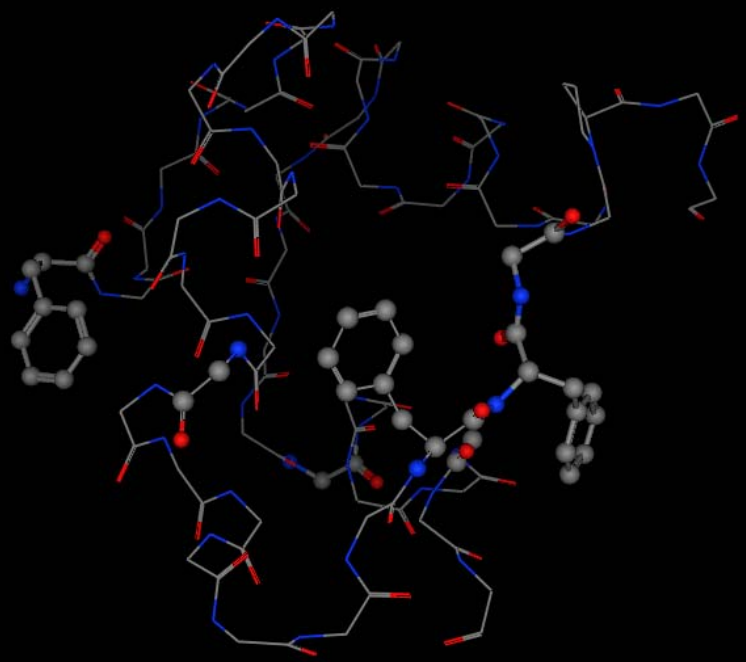
- Possible to derivatize a cyclodextrin so that it is an enzyme that acts on phenyl rings

- This would prevent aggregation in the brain

Breslow, R. *Acc. Chem. Res.* **28,** 146-153 (1995).
Danielson, J., Jarvet, J. et al. *Biochemistry.* **43**, 6261-6269 (2004).

Insulin

- Similar structure is found in insulin
- May also be possible to sequester insulin using cyclodextrins



Conclusion

- Constructed models of derivatized cyclodextrins and calculated energies with force fields
- Derivatization with zonulin peptides results in stable binding with flurbiprofen
- A cyclodextrin binds quite well to the Phe-20 residue of the amyloid beta protein

Acknowledgements

- Dr. Jayne Kapur
- NIST Computational Chemistry Group
- AIP/SPS Internship Program

Notes

- Solubilities
  - β-CD: 18 mg/mL
  - HP-β-CD: 500 mg/mL
- Bradykinin
  - arg-pro-pro-gly-phe-ser-pro-phe-arg
  - Dilates blood vessels in brain

www.cyclodex.com
http://users.ahsc.arizona.edu/davis/bbb.htm

- Zonulin peptides
  - Open tight junctions in brain
  - Closely related to zonula occludens toxin from cholera
  - Zonulin 1: asn-gln-arg-pro-pro-pro-ala-gly-val-thr-ala-tyr-asp-tyr-leu-val-ile-gln
  - Zonulin 3: val-thr-phe-tyr-thr-asp-ala-val-ser

Karyekar, C. S. Fasano, A. et al. *J. Pharm. Sci.* **92**, 414-423 (2003).
U.S. Patent 5945510

- Amyloid precursor protein (APP) → β-secretase → γ-secretase → Aβ(1-40) peptide
- Flurbiprofen
  - Interferes with γ-secretase to reduce Aβ peptide levels
  - In phase 3 of clinical trials

http://dev.sfn.org/content/AboutSFN1/NewsReleases/an2002_alzheimers.html