NANO-MECHANICS OF CARTILAGE GLYCOSAMINOGLYCANS USING
MOLECULAR DYNAMICS METHODS

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ABSTRACT

Nano-mechanics of cartilage glycosaminoglycans using molecular dynamics methods

Kevin Neil Hendrickson

Articular Cartilage (AC) is the main load carrying material in synovial joints {Hamerman, 1962} and degeneration of AC can cause pain in the form of arthritis. Current work is centered on the method of replacing damaged cartilage inside the body (*in vivo*) with tissue engineered outside the body (*ex vivo*) {Temenoff, 2000}. In order to engineer tissue *ex vivo* similar to the native tissue in structure and function there must be a comprehensive understanding of the mechanical properties of AC. This work focuses on the study of glycosaminoglycans (GAGs), a molecule known to be primarily responsible for the compressive stiffness of AC, using molecular dynamics methods. First, a single chain simulation is run to establish a chain length to use for the rest of the study. Then two more simulations are run that mimic a possible physical scenario for changing GAG density. The first is a five chain simulation that mimics the situation where GAG chains are compressed and pushed together. Pressure and density relations are generated and compared to the micro-structural level Donnan model {Maroudas, 1979} and Poisson-Boltzmann unit cell (PB) model {Marcus, 1955}. The last simulation imitates the scenario of one GAG chain sliding between two adjacent GAG chains. The work to pull the central chain through the adjacent chains is calculated and plotted at different chain spacing. A 20 disaccharide-unit long chain is found to be the most stable chain length, but for the purpose of saving computational time without a large loss in stability a 10 unit chain is used for the rest of the simulations. The pressure-density relations found from the five chain simulation are of the same magnitude as the micro-structural level models. Observations made based on the graphical playback of the pulling simulation give insight into the importance of ion interaction with the GAG chains. It was found to take more work to pull the chain with more open space around because of the binding nature of the ions coming between the chains. The tighter spaced chains allowed fewer ions to fit between chains creating less binding force, therefore taking less work to pull. This work can be scaled up to the next level using coarse-graining methods which will be more comparable to experimental work, possibly leading to results that will help characterize AC for better implementation of engineered tissue.

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Table of Contents

List of Abbreviations.....	viii
List of Tables.....	x
List of Figures	xi
Chapter 1 - Introduction.....	1
Chapter 2 - Background.....	5
2.1 Articular Cartilage	5
2.2 Glycosaminoglycans.....	8
2.3 Biomechanical Properties.....	10
Chapter 3 - Molecular Dynamics – An Introduction	12
3.1 Theory	13
3.1.1 Molecular Dynamics	13
3.1.2 Potential Energy Function	15
3.1.3 Global MD Algorithm	23
3.1.4 Treatment of non-bonded energy terms	25
3.1.5 Treatment of solvent	27
3.1.6 Periodic Boundary Conditions	28

Chapter 4 - Methods	31
4.1 Aims.....	31
4.1.1 Single Chain Simulation	32
4.1.2 Five Chain Simulation	33
4.1.3 Pulling Simulation.....	34
4.2 Models	35
4.2.1 Force Field	35
4.2.2 Topologies.....	35
4.2.3 Water and Ions	36
4.3 Simulations	37
4.3.1 Single Chain Simulations.....	37
4.3.2 Five Chain Simulations.....	39
4.3.3 Pull Simulations	40
Chapter 5 - Results	43
5.1 Single Chain Simulation	43
5.2 Five Chain Simulation.....	47
5.3 Pulling Simulation.....	49
Chapter 6 - Conclusion	53
6.1 Discussion	53
6.3 Future Work.....	55
List of References	56
Appendix A - Derivation of Ionic Concentration.....	59
Appendix B - Sample simulation times.....	62
Appendix C - Steps to run a molecular dynamics simulation	63
Appendix D - Sample files.....	66

List of Abbreviations

Å	Angstrom
AC	Articular Cartilage
C4S	Chondroitin-4-Sulfate
C6S	Chondroitin-6-Sulfate
CGM	Cartilage Growth Model
CH	Chondroitin
CHARMM	Chemistry at HARvard Macromolecular Mechanics
COL	Collagen
CS	Chondroitin Sulfate
Da	Dalton (atomic mass unit)
ECM	Extra-Cellular Matrix
G1	Globular Domain 1
G2	Globular Domain 2
G3	Globular Domain 3
GAG	Glycosaminoglycan
Gal	Galactose
GalNAc	N-Acetyl-D-Galactosamine
GalNAc4S	N-Acetyl-D-Galactosamine-6-Sulfate
GalNAc6S	N-Acetyl-D-Galactosamine-4-Sulfate

GlcUA	D-Glucuronate
HA	Hyaluronan
KS	Keratin Sulfate
MD	Molecular Dynamics
nm	Nanometer
PB	Poisson-Boltzmann Unit Cell
PG	Proteoglycan

List of Tables

5.1	Average pressure data for 5 different GAG densities.	48
5.2	Swelling pressure data.	49
A.1	Ionic concentrations.....	60

List of Figures

2.1: Diagram showing the composite like nature of AC	6
2.2: One molecule of aggrecan bound to HA	7
2.2: Single disaccharide units of CH, KS, C4S and C6S.	9
2.3: GAG chain with 4 C6S disaccharide units.....	9
2.4: GAG spacing and length along aggrecan core protein.	10
3.1: Diagram of bond pairs.....	17
3.2: Diagram of bond stretching.	18
3.3: Diagram of bond angle bending.....	19
3.4: Diagram of bond twisting	20
3.5: Lennard Jones 12-6 potential curve.....	22
3.6: Three different methods for defining the cutoff region	26
3.7: Two-dimensional Periodic Boundary Condition.	29
4.1: Single chain simulation set up.	32
4.2: Physical relevance of the five chain simulations.	33

4.3: Physical relevance of pulling simulation	34
4.4: Set-up for the single chain simulations.	38
4.5: Five chain simulation set up	39
4.6: Schematic of pull simulation set up	41
5.1: Figure showing how end-to-end length is calculated	44
5.2: Single chain GAG end-to-end distance motion	45
5.3: Standard deviation of the normalized distance.	46
5.4: Pressure vs. density compared of 5-chain simulation	48
5.5: Snapshot of pull simulation at 1.5 nm spacing.....	51
5.6: Zoomed in view of Figure 5.5.....	51
5.7: Plot of the work required to pull the central chain	52

Chapter 1

Introduction

Articular Cartilage (AC) is the main load carrying material in synovial joints {Hamerman, 1962}. It also lubricates the joint and resists wear to provide a smooth sliding action between bones {Ateshian, 1998}. AC is subjected to intermittent contact pressures reaching 3-18 MPa as many as a million times per year {Hodge, 1986}. The extra-cellular matrix (ECM) of AC is composed of water (60-90%) and a solid matrix consisting mostly of a cross-linked collagen (COL) network and proteoglycans (PGs), a fixed charge polymer. AC behaves like a fiber-reinforced composite material, where the “fibers” correspond to the COL network and the “matrix” is made up of PGs. Because it behaves like a fiber reinforced composite material it will have different mechanical properties in tension and compression.

One major problem with AC is its avascularity, which prevents it from healing {Buckwalter, 1998}. Degeneration of the cartilage in joints can often lead to pain and discomfort in the form of arthritis {Sandell, 2001}. Arthritis is a common disability in the United States; 21% of adults are affected with some form of arthritis {Lethbridge, 2006}, however the medical techniques currently in place are inadequate for repairing or replacing damaged cartilage {Smith, 2005}. Current work is centered on the method of replacing damaged cartilage inside the body (*in vivo*) with tissue engineered outside the body (*ex vivo*) {Temenoff, 2000}.

In order to engineer tissue *ex vivo* similar to the native tissue in structure and function there must be a comprehensive understanding of the mechanical properties of AC. The molecule in AC most responsible for the mechanical stiffness in compression is glycosaminoglycan (GAG). GAGs are long un-branched polysaccharides consisting of repeating units of disaccharides. GAGs resist compression due to the repulsion of its fixed negative charges. GAG chains are on the order of 20 to 60 nm in length in AC {Bathe, 2005}, which makes them extremely difficult to analyze experimentally. Molecular dynamics methods have been used to analyze molecular behavior and gain insight into the relationship between the molecular structure, movement and function. With the increasing power and speed of computers, molecular dynamics has become a viable option for analyzing molecules.

The scope of this project is to model the C6S GAG in several configurations that may be related to changing GAG density. This will lead the way to using coarse graining methods, which will be discussed in more detail in chapter 6, to eventually predict pressure-density relations. However, pressure-density calculations will be made on these models in order to evaluate how nano-level models compare with data for GAGs in AC tissue. Three specific aims were considered to mimic physiological scenarios of GAG molecules in compression.

1. An experiment with single GAG chains will be studied in order to choose a length or number of C6S disaccharide units to use in the molecular dynamics simulations. This is important because the size of the system should be short enough to minimize the use of computing resources but long enough to avoid boundary conditions like end effects and chain curvature which will effect the results.
2. A simulation will be investigated to compare density and pressure calculations of a nano-level GAG system to that of AC cartilage. Molecular dynamics simulations will be performed on five GAG chains at different spacing in order to calculate the pressure in the system. This model represents one mechanism of changing GAG density. These results will be compared to the Donnan model {Maroudas, 1979} and Poisson-

Boltzmann unit cell (PB) model {Marcus, 1955} of AC GAG pressure and density.

3. The last experiment is modeled after another mechanism for changing GAG density where one GAG chain is pulled between two adjacent parallel chains. The work to pull the GAG chain will be calculated and compared among four simulations at different GAG spacing.

Chapter 2

Background

2.1 Articular Cartilage

Cartilage is made up of two main components, a cross-linked network of collagen (50-60 percent of tissue dry weight) and the large aggregating proteoglycan, aggrecan (30-35 percent of tissue dry weight) {Heinegard, 1989; Upholt, 1991}. These two constituents of the solid matrix work together as a fiber-reinforced composite which give the cartilage special characteristics. In general, the collagen network resists tensile and shear stresses while the proteoglycans resist fluid flow and compressive stresses {Mow, 1988}. There are no blood vessels in mature cartilage so it is fed through diffusion, propagated by the pumping action generated from the compression of the cartilage.

Collagen is a long fibrous protein. It provides most of the tensile strength and elasticity of cartilage. The molecule wraps itself up into organized bundles called fibrils and groups of fibrils make collagen fibers.

Figure 2.1: Diagram showing the composite like nature of AC. The tension in the collagen (red arrows) resists the swelling pressure provided by the proteoglycans (blue arrows).

Aggrecan is the predominant proteoglycan in articular cartilage. It is a modular proteoglycan with multiple functional domains: G1, G2, KS, CS and G3 which can be seen in Figure 2.2 {Roughley, 2006}. The globular domains G1, G2 and G3 are not of interest in this study because they are not known to contribute to the compressive properties of AC. However, the GAG region containing the keratin sulfate (KS) and chondroitin sulfate (CS) domains are of interest. A more detailed explanation of GAG's is provided in section 2.2.

Figure 2.2: One molecule of aggrecan bound to HA. Many molecules of aggrecan bound to HA produces a PG aggregate. Aggrecan structure: G1, G2, KS, CS, G3 and Core Protein.

Aggrecan is often aggregated into high molecular weight supra-molecule structures called PG aggregates. Aggregation is accomplished by the interaction of aggrecan with hyaluronan (HA) and a link-protein {Tsiganos, 1972}. The molecular weight of a single molecule of aggrecan in the tissue ranges from 1 to 3.5 MDa and the aggregates formed are of a molecular size up to 800 MDa {Heinegard, 1987}. The molecular weight of a single GAG molecule is 10-30 kDa corresponding to 20-60 disaccharide units with a contour length of 200-600 Å (20-60 nm) {Bathe, 2005}. The contour length is defined as the length at maximum physically possible extension for a linear polymer chain {IUPAC, 1997}.

2.2 Glycosaminoglycans

There are a few different forms of GAGs found in AC; chondroitin (CH), keratin sulfate (KS), chondroitin-4-sulfate (C4S), and chondroitin-6-sulfate (C6S) are the most common. GAGs are produced from the assembly of repeating disaccharide units seen in Figure 2.3. The negatively charged groups COO^- and OSO_3^- generate a high density of fixed negative charge. Large repulsive forces are created when GAGs are forced together due to compression of the negative charge groups. These negative charge groups are what cause the resistance to compression that we are interested in. Both C4S and C6S have these negative charge groups, but there are higher concentrations of C6S in AC and almost no C4S in adult AC {Mourao, 1988}. For these reasons only C6S will be investigated in this study.

Figure 2.3: Single disaccharide units of CH, KS, C4S and C6S. Note the only physical difference between C4S and C6S is the location of the OSO_3^- group.

Figure 2.4: GAG chain with 4 C6S disaccharide units. Light blue = Carbon; Dark Blue = Nitrogen; Red = Oxygen; Yellow = Sulfur; White = Hydrogen

2.3 Biomechanical Properties

The physiological spacing of the GAG chains along the core protein is important because it determines how the model will be set up. Data gathered using atomic force microscopy on bovine nasal cartilage shows GAG spacing in fetal aggrecan is 3.2 ± 0.8 nm and mature is 4.4 ± 1.2 nm {Ng, 2003}. Another important factor is the contour length or end-to-end length. Experimental studies show that the contour length of C6S is 20-60 nm {Ng, 2003; Bathe, 2005}

Figure 2.5: GAG spacing and length along aggrecan core protein.

Although GAG length is important another length property, called persistence length, is arguably more significant. The persistence length is defined as the length over which correlations in the direction of the tangent are lost. In other words a chain shorter than the persistence length will

behave much like a highly flexible elastic rod and the behavior of a chain much longer than the persistence length can only be described statistically like a random 3-dimensional path. The persistence length of GAG chains was found to be 21 nm for fetal and 14 nm for mature aggrecan. With a 95% confidence interval this becomes 17-25 nm and 10-21 nm, respectively {Ng, 2003}.

Typical PG concentrations in AC are 20-80 mg/ml {Buschmann, 1995}. Molecular Weight of one C6S unit is 442 Daltons (Da), which is related to kg via the conversion factor in Equation 2.1.

$$1Da = 1.6605 \times 10^{-27} kg \quad (2.1)$$

Chapter 3

Molecular Dynamics – An Introduction

The intent of this chapter is to educate those readers unfamiliar with molecular dynamics (MD) simulations. It is assumed that the reader has an understanding of classical mechanics, statistical methods and thermodynamics, but knows very little—if anything at all—on methods for atomistic computer simulations.

With the rapid increase in the speed of today's computers the computer experiment, or simulation, has inserted itself between experiment and theory as one of the chief methods of determining solutions. A model is still provided by theorists, but the calculations are carried out by the computer according to a given algorithm.

3.1 Theory

3.1.1 Molecular Dynamics

Molecular dynamics is a computer simulation technique where the time evolution of a set of interacting atoms is followed by integrating their equations of motion {Ercolessi, 1997}. MD follows the laws of classical mechanics, most notably Newton's law:

$$\mathbf{F}_i = m_i \mathbf{a}_i \quad (3.1)$$

for each atom i in a system constituted by N atoms. Here, m_i is the atom mass, $\mathbf{a}_i = d^2\mathbf{r}_i/dt^2$ is its acceleration, and $\mathbf{F}_i$ is the force acting upon it due to the interactions with other atoms. Given an initial set of positions and velocities the computer calculates a trajectory in a $6N$ -dimensional space ($3N$ positions and $3N$ momenta). However, such a trajectory is usually not very useful by itself. MD is a statistical mechanics method; it is a way to obtain a set of configurations distributed according to some statistical ensemble. A configuration is defined as one set of 3-dimensional position vectors for each atom at one moment in time. An ensemble is a collection of all possible configurations which have different microscopic states but have an identical

macroscopic or thermodynamic state. Different definitions of the thermodynamic state lead to different classifications for ensembles:

- Microcanonical Ensemble (NVE): The thermodynamic state characterized by a fixed number of atoms, N , a fixed volume, V and a fixed Energy, E .
- Canonical Ensemble (NVT): The thermodynamic state characterized by a fixed number of atoms, N , a fixed volume, V , and affixed temperature, T .
- Isobaric-Isothermal Ensemble (NPT): The thermodynamic state characterized by a fixed number of atoms, N , a fixed pressure, P , and fixed temperature, T .
- Grand Canonical Ensemble (μVT): The thermodynamic state characterized by a fixed chemical potential, μ , a fixed volume, V , and fixed temperature, T .

William Gibbs defined chemical potential by stating, “If to any homogeneous mass in a state of hydrostatic stress we suppose an infinitesimal quantity of any substance to be added, the mass remaining homogeneous and its entropy and volume remaining unchanged, the increase of the energy of the mass divided by the quantity of the substance added is the potential for that substance in the mass considered.” {Gibbs, 1906}

3.1.2 Potential Energy Function

In order to model the physical system a function for the potential must be chosen. That is, a function $\Phi(\mathbf{r}_1, \dots, \mathbf{r}_N)$ of the positions of the nuclei, representing the potential energy of the system when the atoms are arranged in that particular configuration. This function is usually constructed based on relative positions of the atoms with respect to each other, rather than absolute positions.

Forces are derived from the gradients of the potential function with respect to atomic displacements:

$$\mathbf{F}_i = -\nabla_{\mathbf{r}_i} \Phi(\mathbf{r}_1, \dots, \mathbf{r}_N) \quad (3.2)$$

This form implies that the total energy, $E = K + \Phi$, is conserved, where K is the instantaneous kinetic energy.

The simplest choice for Φ is a sum of pair-wise interactions:

$$\Phi(\mathbf{r}_1, \dots, \mathbf{r}_N) = \sum_{i=1}^N \sum_{j>1}^N \phi(|\mathbf{r}_i - \mathbf{r}_j|) \quad (3.3)$$

There are a few different potential energy functions. Some commonly used interaction models are the CHARMM (Chemistry at HARvard Macromolecular Mechanics) potential energy function {Brooks, 1983}, Amber and Gromos which is used in Gromacs. Potential energy functions consider the summation of the bonded, E_{bonded} and non-bonded energies, $E_{\text{non-bonded}}$, as a function of the atomic positions, $\mathbf{r}$. The bonded energies include bond stretching, bond angle bending, and bond rotation. The non-bonded energies account for the interactions between non-bonded atoms separated by 3 or more covalent bonds.

$$V(\mathbf{r}) = E_{\text{bonded}} + E_{\text{non-bonded}} \quad (3.4)$$

The E_{bonded} term is a sum of three terms:

$$E_{\text{bonded}} = E_{\text{bond-stretch}} + E_{\text{bond-bend}} + E_{\text{bond-twist}} \quad (3.5)$$

Figure 3.1: Diagram of bond pairs: bond stretching occurs between 1,2 type pairs, bond angle bend occurs between 1,3 type pairs and bond twist occur between 1,4 type pairs.

which correspond to three types of molecular motion. The first term in Equation 3.5 is a harmonic potential representing the interaction between atomic 1,2 pairs where atoms are separated by one covalent bond. The phrase harmonic potential is used to describe the oscillatory behavior of such a system where two masses are separated by a spring with an equilibrium position. The approximation of the energy of a bond as a function of displacement from the ideal bond length, b_0 , is shown in Equation 3.6. The force constant, K_b , depends on the atoms bonded and therefore determines the stiffness of the bond.

$$E_{bond-stretch} = \sum_{1,2 \text{ pairs}} K_b (b - b_0)^2 \quad (3.6)$$

Figure 3.2: Diagram of bond stretching.

The second term in the E_{bonded} equation is a harmonic potential associated with alteration of bond angles, θ , from the ideal value, θ_0 , where the values of θ_0 and K_θ depend on the chemical type of atoms constituting the angle. This potential occurs between atoms that are separated by 2 covalent bonds, also known as 1,3 pairs.

$$E_{\text{bond-bend}} = \sum_{1,3 \text{ pairs}} K_\theta (\theta - \theta_0)^2 \quad (3.7)$$

Figure 3.3: Diagram of bond angle bending.

The last term represents the torsion angle potential function between atoms separated by 3 covalent bonds also known as 1,4 pairs. The motion associated with this term is a rotation described by a dihedral angle, φ , and coefficient of rotational symmetry, $n=1, 2, 3, 4$ or 6 , where rotational symmetry of order n means a rotation by an angle of $360^\circ/n$ does not change the object.

$$E_{bond-twist} = \sum_{1,4\text{ pairs}} K_{\varphi} (1 - \cos(n\varphi)) \quad (3.8)$$

Figure 3.4: Diagram of bond twisting. View (b) is the perspective of looking down the 3,2 bond of view (a).

The non-bonded energy, $E_{\text{non-bonded}}$, is made up of two main components, the Van der Waals interaction energy and the electrostatic interaction energy. Some other potential energy functions also include a term accounting for hydrogen bonds, however in some potential energy functions these interactions are accounted for in the Van-der-Waals and electrostatic terms.

$$E_{\text{non-bonded}} = E_{\text{Van-der-Waals}} + E_{\text{electrostatic}} \quad (3.9)$$

The Van der Waals interaction between two atoms arises from a balance between repulsive and attractive forces. The repulsive forces occur when two atoms are near each other and the electron-electron interaction is strong. The attractive forces, sometimes known as dispersion forces, arise from the fluctuations in the charge distribution in the electron clouds. Fluctuations in one atom or molecule gives rise to an instantaneous dipole, which in turn induces a dipole in the second atom or molecule, therefore stimulating an attractive interaction. The Van der Waals interaction is most often modeled using the Lennard-Jones 12-6 potential which expresses the interaction energy using the atom-type dependant constants A and C {Stote, 1999}.

$$E_{Van-der-Waals} = \sum_{\substack{\text{nonbonded} \\ \text{pairs}}} \left[\frac{A}{\mathbf{r}^{12}} - \frac{C}{\mathbf{r}^6} \right] \quad (3.10)$$

The Lennard-Jones 12-6 potential function is characterized by attractive forces at long range, but as the distance becomes short the repulsive interaction becomes dominant. This give rise to a minimum in energy, E^* , where the positioning of the atoms at the optimal distance, r^* , stabilizes the system as seen in Figure 3.5.

Figure 3.5: Lennard Jones 12-6 potential curve

The last term in the potential energy function accounts for the electrostatic interactions between pairs of atoms represented by Coulomb potential.

$$E_{\text{electrostatic}} = \sum_{\text{nonbonded pairs}} \frac{q_1 q_2}{\epsilon_{\text{eff}} d} \quad (3.11)$$

Where ϵ_{eff} is the effective dielectric constant for the material separating the two atoms and d is the distance between two atoms having charges q_1 and q_2 .

The dielectric constant is sometimes known as the relative permittivity and is defined as the ratio of the amount of stored electrical energy when a potential is applied, relative to the permittivity of a vacuum. Permittivity relates to a material's ability to transmit an electric field.

3.1.3 Global MD Algorithm

To summarize the information given in section 3.1.1 and 3.1.2 the overall MD algorithm is shown below. In general the MD algorithm calculates the potential, positions and velocities of all the atoms in the system then repeats steps 2, 3 and 4 for the prescribed number of steps. Steps 2, 3 and 4 compute the forces, update the potential, positions and velocities and output the data.

THE GLOBAL MD ALGORITHM

1. Input initial conditions

Potential interaction Φ as a function of atom positions

Positions r of all atoms in the system

Velocities v of all atoms in the system

Repeat steps 2, 3, and 4 for the required number of steps:

2. Compute forces

The force on any atom

$$\mathbf{F}_i = -\frac{\partial \Phi}{\partial \mathbf{r}_i}$$

is computed by calculating the force between non-bonded atom pairs:

$$\mathbf{F}_i = \sum_j \mathbf{F}_{ij}$$

plus the forces due to bonded interactions (which may depend on 1, 2, 3 or 4 atoms), plus restraining and/or external forces.

The potential and kinetic energies and the pressure tensor are computed.

3. Update configuration

The movement of the atoms is simulated by numerically solving Newton's equations of motion

$$\frac{d^2 \mathbf{r}_i}{dt^2} = \frac{\mathbf{F}_i}{m_i}$$

or

$$\frac{d\mathbf{r}_i}{dt} = \mathbf{v}_i ; \quad \frac{d\mathbf{v}_i}{dt} = \frac{\mathbf{F}_i}{m_i}$$

4. Output step

Write positions, velocities, energies, temperature, pressure, etc.

3.1.4 Treatment of non-bonded energy terms

The calculation of the non-bonded energy terms is the most computationally expensive part of a molecular dynamics simulation. In theory, the interaction between every pair of atoms on the system should be accounted for, but the number of calculations increases as the square of the number of atoms for a pairwise model {Rosenberg, 1982}. In order to speed up the computation, the interactions between two atoms separated by a distance greater than a predetermined cutoff distance are ignored. There are a few ways to define how to terminate the interaction of a pair of atoms; they are plotted in Figure 3.6.

Figure 3.6: Three different methods for defining the cutoff region

In the *truncation* method all interactions are set to zero for interatomic distances greater than the cutoff distance. The *shift* cutoff method modifies the entire potential energy surface such that at the cutoff distance the

interaction potential is zero. The third technique is called the *switch* cutoff method. This method tapers the interaction potential over a predetermined range of distances. The potential takes its usual path up to the first cutoff distance and is then switched to zero between the first and last cutoff.

For the simulations in this study the switch method was used because it has been shown in previous studies to be stable and have a high correlation to experimental data {Garemyr, 1999}.

3.1.5 Treatment of solvent

Solvent, typically water, has a fundamental influence on the structure, dynamics and thermodynamics of biological molecules. One of the most important functions of the solvent is the screening of electrostatic interactions. Although water is a neutral molecule it's shape creates a dipole separating into a positive and negative end. This causes electrostatic interactions that should not be ignored. It is important to include solvent effects in molecular dynamics simulations of biological tissues, whether it's implicit or explicitly defined because many of the atomic interactions are with water molecules. Without water being at least implicitly included in the simulation the molecule is essentially simulated in a vacuum with no dielectric constant and therefore no electrostatic charge screening {Solmajer, 1991}.

The simplest method is to just include a dielectric screening constant in the electrostatic term of the potential energy function. In this *implicit* treatment the solvent water molecules are not included in the system but an effective dielectric constant is used. Often a distance dependant dielectric constant is used, ($\epsilon_{eff} = r_{ij}\epsilon$) where ϵ ranges from 4 to 20. Although this is a crude approximation it is still much better than using unscreened partial charges {Stote, 1999}. Many other implicit solvent models have been developed that range from relatively simple distance-dependant dielectric constants to models that base the screening on the solvent exposed surface area of the protein {Roux, 1999}.

In the *explicit* model the water molecules are explicitly included in the system, therefore the dielectric constant is set to one ($\epsilon = 1$) in the potential energy function. In this more detailed treatment of the solvent, boundary conditions must be imposed to prevent the water molecules from diffusing away from the system. One popular method for boundary treatment is called *periodic boundary conditions*.

3.1.6 Periodic Boundary Conditions

The periodic boundary condition enables a simulation to be run using a relatively small amount of particles in such a way that the particles

experience forces as if they were in a bulk solution. This is a classic way to minimize edge effects. For example see Figure 3.7, where eight two-dimensional boxes are assembled around the central box of interest. The particles in the neighboring boxes are called image particles and their coordinates are related to those in the primary box by simple translation.

Figure 3.7: Two-dimensional Periodic Boundary Condition. The blue tinted box represents the primary box which is surrounded by image boxes where x and y are the box vectors and r is the distance between an atom and its translated copy.

The simplest box is cubic but some other options include rhombic dodecahedron and truncated octahedron. Typically the image boxes are set

up in a three-dimensional format, so the primary box is surrounded by 26 neighboring boxes. Forces on the primary particles are calculated from particles within the primary box as well as the image boxes. Atomic interactions are calculated up to a distance called the cutoff distance which is prescribed by the user. The cutoff is chosen such that a particle or atom in the primary box does not see its image in the neighboring boxes. Therefore the cutoff distance must be less than each of the box vectors x , y and z .

Usually the box used is determined by the general shape of the molecule under study. In this study a rectangular brick like box is used because it supports the shape of the long chain like GAGs while not leaving too much empty space surrounding them.

Chapter 4

Methods

4.1 Aims

The objective of this study is to develop a molecular-based nanomechanical model of cartilage proteoglycans for several different mechanisms that may be related to changing GAG density. Molecular mechanics methods will be used to obtain pressure, work and density data for chondroitin sulfate GAGs for these mechanisms. A more descriptive explanation on these mechanisms will be given in sections 4.1.1-4.1.3. The long-term outcome of this aim is the development of molecular-based nanomechanical models of cartilage proteoglycan solutions and the refinement of the finite deformation constitutive equations used in a Cartilage Growth Model (CGM).

4.1.1 Single Chain Simulation

An experiment with single GAG chains will be studied in order to choose a length or number of disaccharide units to use in the molecular dynamics simulations. This is important because the size of the system should be short enough to minimize the computing resources but long enough to avoid boundary conditions like end effects and chain curvature. Figure 4.1 shows an example of the set up of one of these simulations.

Figure 4.1: Single chain simulation set up with the bottom of the chain fixed and the rest allowed to move and find equilibrium conformation.

4.1.2 Five Chain Simulation

Another simulation will be investigated to find the correlation between GAG density and pressure on the system. These results will be compared to the Donnan model {Maroudas, 1979} and Poisson-Boltzmann unit cell (PB) model {Marcus, 1955}. Five chains will be used in a 3-D pattern to imitate a physiological situation where aggrecan molecules are in close contact and many GAG chains are close together. Figure 4.2 shows the physical relevance to this study.

Figure 4.2: Physical relevance of the five chain simulations.

4.1.3 Pulling Simulation

The last experiment is designed to mimic a physiological scenario where one GAG chain is dragged between two adjacent parallel chains seen in Figure 4.3. This simulation will give insight into the forces involved to compress two aggregated groups of GAG chains together.

Figure 4.3: Physical situation where a GAG chain is pulled between adjacent GAG chains. Rate of pulling is 0.01 nm/pico-second.

4.2 Models

All models and simulations were created using the software GROMACS version 3.3. GROMACS is a program for performing molecular dynamics simulations and energy minimization using molecular modeling. Molecular modeling is described as the process of representing complex chemical systems in terms of a realistic atomic model with the goal of attaining macroscopic properties based on detailed knowledge on a nano-scale.

4.2.1 Force Field

The force field is what contains the sets of equations for the potential functions described in section 3.1.2. Also included in the force field are all the parameters used in these equations. Information on the atoms and atom combinations is pulled from the topology file and used in the potential functions to calculate potential energies and their derivatives, the forces.

4.2.2 Topologies

In order for GROMACS to use the potential functions in the force field it needs to know on which atoms and combinations of atoms to act. It must also

know what parameters to apply to the various functions. All this is defined in the topology file, which lists the constant attributes of each atom. Information such as atom type, mass, and charge are included. Atom combinations are also listed in the topology file, such as bond pairs, bond angle groups and bond dihedral groups. Depending on the atoms involved in the group different constants are used in the force field potential functions.

4.2.3 Water and Ions

The size of the periodic boundary box will determine how many explicitly defined water molecules will be in the system. Having too large of a box and therefore too many water molecules can slow a simulation considerably because Gromacs calculates all the bonded and non-bonded interactions of every atom in every water molecule. All simulations in this study use explicitly defined water in the form of the Single Point Charge (SCP) model {Berendsen, 1981}.

When studying molecules at the nano level one must decide how to include ions. For the simulations in this study a total net charge of zero is used because of the small scale. A hypothesis that could be explored in a future study is that over enough time positive and negative ions would flow in and out of a small area to create a zero net charge in any given location. The overall ionic concentration in the extra cellular matrix is 0.15 M NaCl

{Basser, 1998}, but concentrations from 0.01 to 1 M are often studied {Buschmann, 1995}. A derivation of the ionic concentration of the models used in this study is shown in Appendix A.

4.3 Simulations

Three simulations were considered to discover the compressive behavior of GAGs. These simulations were chosen because they mimic a physiological situation that would exist during compression of AC. There are many options when choosing a physical state of GAGs under compression. These particular states were chosen for their simplicity but also because they represent our hypothesis for several kinematic mechanisms for the compression of GAGs.

4.3.1 Single Chain Simulations

First a length of GAG, or number of CS units, must be chosen to use for subsequent simulations. A model of one chain is simulated by itself, only varying the length of the chain in each test. The goal behind this test is to find a length that is long enough to avoid unwanted boundary or end effects but short enough to remain computationally inexpensive. Four different lengths were tested: 5, 10, 15 and 20 CS units. A simple schematic of the simulation set up is shown in Figure 4.4.

Figure 4.4: Set-up for the single chain simulations. The bottom of the chain is fixed and the rest of the chain is allowed to move freely.

The general bending and stretching motion of the GAG chains is the result of interest. In order to quantify this, the distance between the distal and proximal chains ends will be calculated at each time step of the simulation. That data will be plotted and considered when choosing the optimal chain length. In general, the less bending and stretching a molecule undergoes during a molecular dynamics simulation the more stable it is. Therefore the standard deviation of the end to end length will be crucial for determining the most stable chain length.

4.3.2 Five Chain Simulations

Once a standard is set for the length of GAG to be used in the rest of the simulations the next step is to include multiple chains in a simulation. Five GAG chains were used because of the 3-dimensional arrangement that can be employed. A schematic showing the set up for this simulation is shown in Figure 4.5.

Figure 4.5: Five chain simulation set up. A top view is shown in the box on the right to reveal the 3-dimensional nature of the simulation arrangement.

In this simulation the only varied property is the distance between chains. As the distance between the chains change so does the periodic

boundary box size and as a result the density is varied. With the size of these systems and the computational power available a simulation of 1.5 nano-seconds was evaluated.

The goal for this simulation is to output the pressure inside the box for each density of GAGs and compare it to the Donnan {Maroudas, 1979} and PB cell model {Marcus, 1955}.

4.3.3 Pull Simulations

The last simulation in this study mimics a physical situation where two aggrecan molecules are being compressed together and the GAGs are forced into each other. This causes some of the GAG chains to slide in between the others. In order to mimic this situation a model was created with three chains in parallel. The middle chain is then pulled through the two outside chains using a theoretical spring which is a function GROMACS provides in their software. The spring is attached to the CS group at the end of the center chain. The spring constant, direction and velocity are prescribed in the parameter file. The only thing that changes for each case is the spacing between chains. A schematic of the simulation setup is shown in Figure 4.6.

Figure 4.6: Schematic of pull simulation setup including chain spacing, d .

The desired outcome is the work required to pull the center chain out at four different values of d . The output file for the simulation contains the location of pull group, pc , and the location of the spring end, sp . The spring is in equilibrium when the simulation starts and is stretched due to the resistance of the GAG chain being pulled through the others. The force is calculated using the equation for an ideal spring, where k is the spring constant and x_{sp} and x_{pc} are the locations of the spring and pull groups in the x -direction.

$$\mathbf{F} = k_{spring} (\mathbf{x}_{sp} - \mathbf{x}_{pc}) \quad (4.1)$$

The work done by the spring is:

$$W = \int \mathbf{F} \cdot d\mathbf{s} \quad (4.2)$$

The work is calculated numerically using a simplified version of Equation 4.2 for time-steps, $t = 1$ to N .

$$. \quad W = \sum_{t=1}^N \mathbf{F}_t(\mathbf{x}_t - \mathbf{x}_{t-1}) \quad (4.3)$$

Chapter 5

Results

5.1 Single Chain Simulation

Calculating the distance from the proximal end to the distal end of each chain length during a 2 nano-second simulation shows the relative bending and stretching movement of each chain. See Figure 5.1 for a visual description of the length calculation. The distance is normalized by the number of units in the chain and the motion is shown in Figure 5.2. The curves have different start values because each chain was simulated to a minimized state before running the single chain experiment.

Figure 5.1: This figure shows how the end-to-end length is calculated. It is the vector magnitude from the proximal end to the distal end.

Figure 5.2: Distance from one end of the chain to the other, normalized by the number of CS units. A value of one would represent the chain remaining at it's maximum contour length.

Figure 5.3 shows the standard deviation of the normalized length distance for each chain's motion. The standard deviation of the chain motion is a good method for judging each chain's stability. A lower standard deviation implies less bending and stretching of the chain during a simulation.

Figure 5.3: Standard deviation of the normalized distance for each chains motion.

The 20-unit chain is obviously the most stable, while there is not a large difference in the standard deviations of the other three chain lengths. The 5 unit chain should be discarded due to visualized end effects and the fact that the persistence length of chondroitin-6-sulphate is about 4 CS units {Bathe, 2005}. The 20-unit chain can justifiably be thrown out because it will create high computational costs when running the 5-chain and pull simulations. There is not a large difference between the standard deviation of the 10 and 15 unit chains so the most reasonable solution is to go with the shorter length to reduce computational costs. Therefore a 10-unit chain was selected to run the rest of the simulations.

5.2 Five Chain Simulation

The pressure data from the 5-chain simulations is presented in Table 5.1 and Figure 5.4. Figure 5.4 also includes data points from the PB cell model fit and the Ideal Donnan model {Buschmann, 1995}. The pressures observed in the 5-chain simulations are qualitatively similar to those in the PB cell and Donnan models except for the point at 179 mg/ml density. One possible reason why this data point does not fit well with the other models is that it has an ionic concentration of 0.25 M Na^+ when the overall ionic concentration in the extra cellular matrix is 0.15 M NaCl {Basser, 1998}.

Also, both the 1 and 2 nm spaced systems are not in equilibrium with the physiological GAG spacing. Recall that the physiological spacing of GAG chains is about 3-4 nm {Ng, 2003}. Therefore the systems at 3 and 4 nm spacing are already near equilibrium and therefore see pressures near atmospheric pressure (100 kPa). The systems starting with GAGs spaced at 1 and 2 nm are much closer to each than the physiological spacing. Therefore large pressures are exerted on the walls of the periodic boundary box to allow the molecules to separate. The simulation is set up to allow the volume of the box to grow to maintain atmospheric pressure but it can't do so instantaneously. Given a long enough simulation the systems would

equilibrate to atmospheric pressure. The pressure shown on Table 5.1 are the pressures exerted on the box by the system as it tries to expand.

Table 5.1: Average pressure data for 5 different GAG densities. Typical GAG spacing is 3-4 nm {Ng Grodzinsky, 2003}.

Spacing (nm)	Density (mg/ml)	Average Pressure (kPa)
1	179	269
2	87	257
3	55	48
4	36	91

Figure 5.4: Pressure vs. density compared with Donnan and PB cell models. The PB cell model was fit to the data of Williams and Comper. The simulation time was 1.5 nanoseconds.

Table 5.2: Swelling pressure data from the PB cell model and the Ideal Donnan model.

Density (mg/ml)	PB Cell (kPa)	Ideal Donnan (kPa)
20	2	22
30	12	50
40	31	85
50	63	130
60	112	181
70	172	-

5.3 Pulling Simulation

The work to pull the central chain out from between the two outer chains is plotted in Figure 5.7. Physiological AC GAGs are separated from each other on the main protein by 3-4 nm, which corresponds to a value of d equal to 1.5-2 nm {Ng, 2003}. Recall that d is the spacing between chains. The spacing at 4 nm represents infinite spacing because 4 nm is far enough that each molecule can not “see” the others. In other words, the molecules are spaced farther than the prescribed cut-off distance and therefore are not included in the calculations for potential.

In general, the data shown in Figure 5.7 suggests that it takes more work to pull chains that are spaced farther apart except in the case when the spacing is 1.5 and 2 nm. Those two values of GAG spacing represent the closest values to physiological AC. Observations from the visual

representation of the molecular dynamics simulations suggest that the positive ions added to the system are most responsible for how the charge groups on the GAGs react to each other. When there are positive ions between a negative charge group of the central chain and one of the outer chains, it causes an attractive force and as a result takes more work to pull the central chain out. When there are no positive ions between two charge groups they will have a repulsive force and take less work to pull out. In the simulations with a smaller space between GAGs there are less positive ions in between the GAGs and therefore cause less attraction between charge groups. In contrast, when there is more space between GAGs there is more room for the positive ions to get in between negative charge groups and cause attractions that make it more difficult to pull out. Figure 5.5 shows a snapshot of a pulling simulation where you can visually see the strain caused by a positive ion between two negative charge groups.

Figure 5.5: Snapshot of pull simulation at 1.5 nm spacing. See Figure 5.6 for zoomed view - A.

Figure 5.6: View - A: The dark blue atoms represent Na^+ ions. The yellow atoms represent Sulfur from the OSO^- charge groups. CCG = Central Charge Group; SCG = Side Charge Group

Figure 5.7: Plot of the work required to pull the central chain out from between the two outer chains. Rate of pulling is 0.01 nm/pico-second.

Using a different ion concentration could greatly affect these results. By altering the amount of ions in the system it will cause some of the attractive or repulsive interactions to change, therefore taking more or less work to pull the central chain.

Chapter 6

Conclusion

6.1 Discussion

There are several novel results that came from this study. One is that AC GAGs can be simulated using molecular dynamics simulations and the GAG pressure-density relations obtained are reasonably close to data of microstructural level models. Another significant finding is the role that the positive ions play in the interactions of GAGs.

Although the pressure-density values from the five chain simulations did not follow the exact trends of the PB cell and Donnan models, the values were of the same order. The pull simulations gave us insight into the ways that GAGs interact during a possibly physiological mechanism for changing GAG density. Through visual observations of the molecular dynamics pull

simulations we witnessed the effect that the positive ions have on GAGs. The positive ions act as a link to bind negative charge groups from one chain to another and make it difficult for the GAGs to slide along each other, thus attributing to the compressive stiffness observed in AC.

The spacing of the GAGs also affects compressive resistance. Interestingly the tighter spacing, 1 nm, did not take the most work. One hypothesis is that this is due to the fact that less positive ions could come between the negative charge groups and act as the linking between the charge groups of the GAGs. The spacing values that are more physiological, 1.5 nm and 2 nm, took more work to pull which only justifies real spacing of GAGs on aggrecan. In order to get results that will help us better understand the compressive mechanics of GAGs under compression this work must be taken to the next step.

6.3 Limitations

One limitation of this work is that *pulling* was simulated instead of *pushing*. The aim of this experiment was to study a mechanism that may occur during compression of cartilage. It would be more correct to simulate GAG chains being pushed together instead of pulled from each other. Pushing is much more complicated because of the buckling that would happen to the chains as they are pushed together. For this reason pulling

was used to get results while keeping the work within the scope of the project.

6.3 Future Work

The simulations of single GAG chains provided the selection of a 10 unit GAG chain as the best solution for this study. However, much of the justification was based on the current power and speed of computers and what they can handle in a reasonable amount of time. Computers continue to get faster and more powerful with time and therefore the computational cost of using a longer chain will cease to be a good reason for choosing a shorter one. Therefore it may be in the best interest of future investigators of this molecule to use a 20 unit GAG chain for its stability and proximity to a more physiological GAG chain.

It is very difficult to compare results from simulations with only a few GAG chains to experimental results on a macro scale, therefore the next step will be to scale up this work to the next level. The next level is often called coarse-graining and has already been studied by some authors {Bathe, 2005, Shelley, 2001}. Coarse graining is method in which not all atoms are explicitly defined in the simulation but “pseudo-atoms” are defined as groups of atoms. This allows for larger systems with more molecules and longer simulation time scales.

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Appendix A

Derivation of Ionic Concentration

The models used in the pulling simulations have 60 atoms of Na⁺ in 12,898 to 26,576 molecules of water.

$$60_atoms_Na^+\left(\frac{1_Mol_Na^+}{6.022\times 10^{23}_atoms_Na^+}\right)=9.963\times 10^{-23}_Mol_Na^+$$

$$12898_molecules_H_2O\left(\frac{1_Mol_H_2O}{6.022\times 10^{23}_molecules_H_2O}\right)=2.142\times 10^{-20}_Mol_H_2O$$

$$2.142\times 10^{-20}_Mol_H_2O\left(\frac{18.01528g}{1_Mol_H_2O}\right)\left(\frac{1ml}{1g}\right)\left(\frac{1Liter}{1000ml}\right)=3.858\times 10^{-22}_L_H_2O$$

The definition of a molar solution is how many moles of solute are contained in one liter of solution.

$$\left(\frac{9.963\times 10^{-23}_Mol_Na^+}{3.858\times 10^{-22}_L_H_2O}\right)=0.258_M_Na^+$$

Repeating this derivation for 26576 water molecules produces the results shown in Table A.1.

Table A.1: Ionic concentrations for the smallest and largest systems used in the pulling simulations

Spacing	Na+ atoms	H2O molecules	Ionic Concentration (M Na+)
1 nm	60	12898	0.25
4 nm	60	26576	0.13

Appendix B

Sample Simulation Times

Table B.1: This table shows simulation times for one sample for each simulation run.

	# Atoms	Simulation Time (ps)	# Steps	Real Time (hrs)	# CPU's
Single Chain (20 units)	12661	2000	1000000	14.7	2
5 Chain (2 nm spacing)	41984	200	100000	22.1	2
Pulling (1.5 nm spacing)	38709	1500	1500000	68.3	2

Appendix C

Steps to Run Simulation

Below are the general steps for running a simulation with Gromacs. Please refer to the manual for more information on file types. Help files can be obtained by typing the command then *-h* (i.e. *g_energy -h*). Some commands don't have help files but some will have manuals. To read the manual type *man* then the command (i.e. *man rm*)

1. Obtain a .pdb file of the molecule (These can be created or found using chemistry websites)
2. Convert .pdb file to a .gro structure file.

ex. *pdb2gmx -f molecule.pdb -p topol.top -o molecule.gro*

3. Select force field

There are now 4 files

molecule.pdb (original pdb structure file)
molecule.gro (gromacs structure file)
topol.top (generated topology file)
posre.itp (position restraint file)

4. Write or copy a .mdp file that contains the parameters for the simulation.

5. Create an index file (.ndx) to group atoms into categories.

ex. *make_ndx -f molecule.gro -o index.ndx*

6. Run grompp to pre-compile

ex. *grompp -f em_param.mdp -c molecule.gro -p topol.top
-o em_topol.tpr -po -n index.ndx*

These are the current files

molecule.pdb
molecule.gro
topol.top
posre.itp
em_param.mdp
index.ndx
mdout.mdp (complete parameter file output from grompp)
em_topol.tpr (binary topology file output from grompp)

7. Run energy minimization

ex. *mdrun -v -nice 0 -s topol.tpr -o em_traj.trr -c em_out.gro
-e em_energy.edr*

New files

<i>md.log</i>	(log of simulations)
<i>em_energy.edr</i>	(binary file containing energy data)
<i>em_out.gro</i>	(structure of last conformation)
<i>em_traj.trr</i>	(trajectory data)

8. Create a periodic boundary box

ex. *editconf -f em_out.gro -o box.gro -d 0.75 -bt cubic*

9. Fill the box with water

ex. *genbox -cp box.gro -o water.gro -p topol.top -cs*

10. Pre compile input files

ex. *grompp -f md_param.mdp -c water.gro -p topol.top -o md_topol.tpr*

11. Run molecular dynamics simulation

ex. *mdrun -nice 0 -v -s md_topol.tpr -o md_traj.trr -c md_out.gro
-e md_energy.edr*

Appendix D

Files

Single Chain Simulation – 20 unit chain

- Structure file sample

- Topology file sample

- Molecular dynamics parameter file

Five Chain Simulation – 2 nm spacing

- Structure file sample

- Topology file sample

- Molecular dynamics parameter file

Pulling Simulation – 1.5 nm spacing

- Structure file sample

- Topology file sample

- Molecular Dynamics parameter file

- Pulling parameter file

- Pulling output data file sample

Force Field Files

- Atom mass and descriptions

- Bonded interaction parameters

- Non-bonded interaction parameters

Single Chain Simulation – 20 unit chain Structure file sample

12661

```

1SCS  N  1  2.402  2.547  2.290  0.6026  0.0721  0.1487
1SCS  C8  2  2.316  2.445  2.068  0.3542  0.1278 -0.4196
1SCS  C7  3  2.427  2.509  2.150  0.1369 -0.2194  0.1462
1SCS  O7  4  2.499  2.589  2.056  0.2748 -0.3466  0.1424
1SCS  H4  5  2.435  2.487  2.362  0.3290  0.4235  0.5708
1SCS  C2  6  2.347  2.680  2.321  0.2415  0.0599 -0.4271
1SCS  C1  7  2.420  2.744  2.438 -0.0461  0.1589 -0.3015
1SCS  O5  8  2.351  2.718  2.560 -0.3646  0.8046 -0.3373
1SCS  C5  9  2.212  2.753  2.570 -0.5362  0.0132  0.1960
1SCS  C6 10  2.175  2.746  2.717  0.4786  0.2398  0.4716
1SCS  O6 11  2.190  2.614  2.768 -0.2745 -0.1196 -0.2207
1SCS  S 12  2.234  2.620  2.911  0.0559  0.0754 -0.3292
1SCS  O9 13  2.335  2.512  2.938  0.1891  0.3589  0.3173
1SCS  O8 14  2.344  2.519  2.930 -0.4115 -0.4047 -0.1319
1SCS  O10 15  2.349  2.716  2.916  0.3526 -0.2698 -0.4113
1SCS  C4 16  2.137  2.693  2.452  0.3178 -0.1160 -0.2949
1SCS  O4 17  2.065  2.573  2.485 -0.5289  0.4451 -0.0797
1SCS  H42 18  2.055  2.566  2.585 -0.6515 -1.4146 -0.1918
1SCS  C3 19  2.196  2.691  2.312  0.2427  0.1511 -0.3299
1SCS  O11 20  2.156  2.806  2.238 -0.0452  0.0188 -0.3818
1SCS  C11 21  2.059  2.754  2.146 -0.0934 -0.3560 -0.1177
1SCS  O15 22  1.927  2.778  2.194 -0.1889  0.1882 -0.6526
1SCS  C15 23  1.846  2.880  2.133 -0.1106  0.4515 -0.3182
1SCS  C16 24  1.724  2.941  2.201 -0.1741  0.7214 -0.6730
1SCS  O18 25  1.593  2.904  2.158 -0.0650 -0.5554  0.0465
1SCS  O17 26  1.692  3.047  2.290 -0.4979  0.0679 -0.0056
1SCS  C14 27  1.855  2.884  1.982 -0.0912  0.1061 -0.3272
1SCS  C13 28  2.001  2.919  1.958 -0.0256 -0.2940 -0.5326
1SCS  O13 29  2.030  3.051  2.008  0.1096 -0.5397  0.0475
1SCS  H85 30  1.980  3.113  1.948  0.0463 -0.2891  0.3589
1SCS  C12 31  2.100  2.819  2.015 -0.0999 -0.0503  0.0316
1SCS  O12 32  2.176  2.731  1.932  1.0005  0.0254  0.9316
1SCS  H86 33  2.200  2.780  1.849 -0.2880 -1.0095 -0.0976
1SCS  O 34  2.559  2.710  2.433  0.0363  0.4697 -0.1443
2CCS  N 35  3.056  2.480  2.386 -0.6269  0.0200  0.4099
2CCS  C8 36  2.895  2.296  2.433  0.4810  0.1509  0.5956
2CCS  C7 37  2.997  2.355  2.337 -0.0032 -0.0884 -0.0709
2CCS  O7 38  3.021  2.285  2.214 -0.3146 -0.7612  0.2441
2CCS  H4 39  3.034  2.505  2.481 -0.1991 -0.0438  0.5252
2CCS  C2 40  3.117  2.566  2.284  0.0549 -0.4198  0.4445
2CCS  C1 41  3.258  2.540  2.232 -0.0181  0.5365 -0.2589
2CCS  O5 42  3.355  2.616  2.303 -0.0527  0.0050  0.3698
2CCS  C5 43  3.339  2.755  2.272  0.1816  0.0282  0.3510
2CCS  C6 44  3.466  2.833  2.303 -0.0145  0.6549 -0.3982
2CCS  O6 45  3.543  2.861  2.186  0.0289  0.2537 -0.4669
2CCS  S 46  3.684  2.816  2.211 -0.0644  0.2259  0.0366
2CCS  O9 47  3.763  2.930  2.153  0.2872  0.0194  0.1077
2CCS  O8 48  3.771  2.932  2.172  0.2713 -0.1431 -0.3352
2CCS  O10 49  3.732  2.878  2.338 -0.2404 -0.4615  0.4405
2CCS  C4 50  3.211  2.812  2.331 -0.2751 -0.3766 -0.2418
2CCS  O4 51  3.239  2.832  2.470  0.0713  0.1073 -0.3777
2CCS  H42 52  3.227  2.927  2.498  1.8141  0.1874  0.1902
2CCS  C3 53  3.097  2.711  2.322 -0.3210 -0.3418 -0.0394
2CCS  O11 54  2.973  2.778  2.300  0.0063  0.3164  0.0705

```

Single Chain Simulation – 20 unit chain Topology file sample

```

;      File 'topol.top' was generated
;      By user: kevin (1000)
;      On host: kevin-laptop
;      At date: Wed Mar 26 14:37:01 2008
;
;      This is your topology file
;      chain
;
#define HEAVY_H

; Include forcefield parameters
#include "ffG53a6.itp"

[ moleculetype ]
; Name          nrexcl
Protein         3

[ atoms ]
;  nr      type resnr residue atom  cgnr  charge   mass typeB  chargeB  massB
;  1        N    1   SCS    N    1    0.141  10.9827 ; qtot 0.141
;  2       CH3    1   SCS    C8    2      0   15.035 ; qtot 0.141
;  3       CH1    1   SCS    C7    3    0.39   13.019 ; qtot 0.531
;  4        OA    1   SCS    O7    3   -0.65  15.9994 ; qtot -0.119
;  5         H    1   SCS    H4    3   -0.002   4.032 ; qtot -0.121

[ bonds ]
; ai  aj funct      c0      c1      c2      c3
;  1  3  2  gb_21
;  1  5  2  gb_2
;  1  6  2  gb_21
;  2  3  2  gb_26
;  3  4  2  gb_18
;  6  7  2  gb_26
;  6 19  2  gb_26
;  7  8  2  gb_19
;  7 34  2  gb_19
;  8  9  2  gb_19
;  9 10  2  gb_26
;  9 16  2  gb_26

[ pairs ]
; ai  aj funct      c0      c1      c2      c3
;  1  8  1
;  1 16  1
;  1 20  1
;  1 34  1
;  2  5  1
;  2  6  1
;  3  7  1
;  3 19  1

[ angles ]
; ai  aj  ak funct      c0      c1      c2      c3
;  3  1  5  2  ga_18
;  3  1  6  2  ga_13
;  5  1  6  2  ga_18
;  1  3  2  2  ga_13
;  1  3  4  2  ga_9
;  2  3  4  2  ga_9
;  1  6  7  2  ga_13
;  1  6 19  2  ga_13
;  7  6 19  2  ga_8
;  6  7  8  2  ga_13
;  6  7 34  2  ga_13
;  8  7 34  2  ga_9

[ dihedrals ]

```

```

; ai  aj  ak  al funct      c0      c1      c2      c3      c4      c5
  6  1  3  2  1  gd_41
  3  1  6 19  1  gd_41
  1  6  7  8  1  gd_4
  1  6 19 20  1  gd_4
  6  7  8  9  1  gd_1
  6  7 34 61  1  gd_1
  8  7 34 61  1  gd_2
  7  8  9 16  1  gd_1
  8  9 10 11  1  gd_18
[ dihedrals ]
; ai  aj  ak  al funct      c0      c1      c2      c3
  1  4  2  3  2  gi_1
  1  3  5  6  2  gi_1
 24 23 25 26  2  gi_1
 35 38 36 37  2  gi_1
 35 37 39 40  2  gi_1
 58 57 59 60  2  gi_1
 69 72 70 71  2  gi_1
 69 71 73 74  2  gi_1
 92 91 93 94  2  gi_1
; Include Position restraint file
#ifdef POSRES
#include "posre.itp"
#endif

; Include water topology
#include "spc.itp"

#ifdef POSRES_WATER
; Position restraint for each water oxygen
[ position_restraints ]
; i funct      fcx      fcy      fez
  1  1    1000    1000    1000
#endif

; Include generic topology for ions
#include "ions.itp"

[ system ]
; Name
chain in water

[ molecules ]
; Compound      #mols
Protein          1
SOL              3980
NA+              40

```


Single Chain Simulation – 20 unit chain MD parameter file

```
;
;      File 'out.mdp' was generated
;      By user: marco (1000)
;      On host: biogs26
;      At date: Fri Apr  4 03:51:00 2008
;

; VARIOUS PREPROCESSING OPTIONS
title          =
; Preprocessor - specify a full path if necessary.
cpp            = cpp
include        =
define         =

; RUN CONTROL PARAMETERS
integrator      = md
; Start time and timestep in ps
tinit          = 0
dt             = 0.002
nsteps         = 1000000
; For exact run continuation or redoing part of a run
init_step      = 0
; mode for center of mass motion removal
comm-mode      = Linear
; number of steps for center of mass motion removal
nstcomm        = 1
; group(s) for center of mass motion removal
comm-grps      = AggreCan

; LANGEVIN DYNAMICS OPTIONS
; Friction coefficient (amu/ps) and random seed
bd-fric        = 0
ld-seed        = 1993

; ENERGY MINIMIZATION OPTIONS
; Force tolerance and initial step-size
emtol          = 10
emstep         = 0.001
; Max number of iterations in relax_shells
niter          = 20
; Step size (ps^2) for minimization of flexible constraints
fcstep         = 0
; Frequency of steepest descents steps when doing CG
nstcgsteep     = 1000
nbgscorr       = 10

; OUTPUT CONTROL OPTIONS
; Output frequency for coords (x), velocities (v) and forces (f)
nstxout        = 1000
nstvout        = 1000
nstfout        = 0
; Checkpointing helps you continue after crashes
nstcheckpoint  = 1000
; Output frequency for energies to log file and energy file
nstlog         = 1000
nstenergy      = 100
; Output frequency and precision for xtc file
nstxtcout      = 0
xtc-precision  = 1000
; This selects the subset of atoms for the xtc file. You can
; select multiple groups. By default all atoms will be written.
xtc-grps       =
```

```

; Selection of energy groups
energygrps      =

; NEIGHBORSEARCHING PARAMETERS
; nblist update frequency
nstlist         = 10
; ns algorithm (simple or grid)
ns-type         = Grid
; Periodic boundary conditions: xyz (default), no (vacuum)
; or full (infinite systems only)
pbc             = xyz
; nblist cut-off
rlist           = 1.1
domain-decomposition = no

; OPTIONS FOR ELECTROSTATICS AND VDW
; Method for doing electrostatics
coulombtype     = pme
rcoulomb-switch = 0
rcoulomb        = 1.1
; Relative dielectric constant for the medium and the reaction field
epsilon_r       = 1
epsilon_rf      = 1
; Method for doing Van der Waals
vdw-type        = Switch
; cut-off lengths
rvdw-switch     = 0.9
rvdw            = 1.0
; Apply long range dispersion corrections for Energy and Pressure
DispCorr        = No
; Extension of the potential lookup tables beyond the cut-off
table-extension = 1
; Separate tables between energy group pairs
energygrp_table =
; Spacing for the PME/PPPM FFT grid
fourierspacing  = 0.12
; FFT grid size, when a value is 0 fourierspacing will be used
fourier_nx      = 0
fourier_ny      = 0
fourier_nz      = 0
; EWALD/PME/PPPM parameters
pme_order       = 4
ewald_rtol      = 1e-05
ewald_geometry  = 3d
epsilon_surface = 0
optimize_fft    = no

; GENERALIZED BORN ELECTROSTATICS
; Algorithm for calculating Born radii
gb_algorithm     = Still
; Frequency of calculating the Born radii inside rlist
nstgbradii      = 1
; Cutoff for Born radii calculation; the contribution from atoms
; between rlist and rgbradii is updated every nstlist steps
rgbradii        = 2
; Salt concentration in M for Generalized Born models
gb_saltconc     = 0

; IMPLICIT SOLVENT (for use with Generalized Born electrostatics)
implicit_solvent = No

; OPTIONS FOR WEAK COUPLING ALGORITHMS
; Temperature coupling
tcoupl          = Berendsen
; Groups to couple separately

```

```

tc-grps          = AggreCan SOL
; Time constant (ps) and reference temperature (K)
tau-t            = 0.1 0.1
ref-t            = 300 300
; Pressure coupling
Pcoupl           = No
Pcoupltype       = Isotropic
; Time constant (ps), compressibility (1/bar) and reference P (bar)
tau-p            = 1
compressibility   =
ref-p            =
; Random seed for Andersen thermostat
andersen_seed     = 815131

; OPTIONS FOR QMMM calculations
QMMM              = no
; Groups treated Quantum Mechanically
QMMM-grps         =
; QM method
QMmethod          =
; QMMM scheme
QMMMscheme        = normal
; QM basisset
QMbasis           =
; QM charge
QMcharge          =
; QM multiplicity
QMmult            =
; Surface Hopping
SH                =
; CAS space options
CASorbitals       =
CASelectrons      =
SAon              =
SAoff             =
SAsteps           =
; Scale factor for MM charges
MMChargeScaleFactor = 1
; Optimization of QM subsystem
bOPT              =
bTS               =

; SIMULATED ANNEALING
; Type of annealing for each temperature group (no/single/periodic)
annealing         =
; Number of time points to use for specifying annealing in each group
annealing_npoints =
; List of times at the annealing points for each group
annealing_time    =
; Temp. at each annealing point, for each group.
annealing_temp    =

; GENERATE VELOCITIES FOR STARTUP RUN
gen-vel           = yes
gen-temp          = 300
gen-seed          = 173529

; OPTIONS FOR BONDS
constraints        = all-bonds
; Type of constraint algorithm
constraint-algorithm = Lincs
; Do not constrain the start configuration
unconstrained-start = no
; Use successive overrelaxation to reduce the number of shake iterations
Shake-SOR         = no

```

```

; Relative tolerance of shake
shake-tol      = 1e-04
; Highest order in the expansion of the constraint coupling matrix
lincs-order    = 4
; Number of iterations in the final step of LINCS. 1 is fine for
; normal simulations, but use 2 to conserve energy in NVE runs.
; For energy minimization with constraints it should be 4 to 8.
lincs-iter     = 1
; Lincs will write a warning to the stderr if in one step a bond
; rotates over more degrees than
lincs-warnangle = 30
; Convert harmonic bonds to morse potentials
morse         = no

; ENERGY GROUP EXCLUSIONS
; Pairs of energy groups for which all non-bonded interactions are excluded
energygrp_excl =

; NMR refinement stuff
; Distance restraints type: No, Simple or Ensemble
disre         = No
; Force weighting of pairs in one distance restraint: Conservative or Equal
disre-weighting = Conservative
; Use sqrt of the time averaged times the instantaneous violation
disre-mixed    = no
disre-fc       = 1000
disre-tau      = 0
; Output frequency for pair distances to energy file
nstdisreout    = 100
; Orientation restraints: No or Yes
orire         = no
; Orientation restraints force constant and tau for time averaging
orire-fc       = 0
orire-tau      = 0
orire-fitgrp   =
; Output frequency for trace(SD) and S to energy file
nstorireout    = 100
; Dihedral angle restraints: No, Simple or Ensemble
dihre         = No
dihre-fc       = 1000
dihre-tau      = 0
; Output frequency for dihedral values to energy file
nstdihreout    = 100

; Free energy control stuff
free-energy     = no
init-lambda    = 0
delta-lambda   = 0
sc-alpha       = 0
sc-power       = 0
sc-sigma       = 0.3

; Non-equilibrium MD stuff
acc-grps       =
accelerate     =
freezegrps     =
freezedim      =
cos-acceleration = 0
deform         =

; Electric fields
; Format is number of terms (int) and for all terms an amplitude (real)
; and a phase angle (real)
E-x           =
E-xt          =

```

```

E·y      =
E·yt     =
E·z      =
E·zt     =

; User defined thingies
user1·grps =
user2·grps =
userint1   = 0
userint2   = 0
userint3   = 0
userint4   = 0
userreal1  = 0
userreal2  = 0
userreal3  = 0
userreal4  = 0

```

Five Chain Simulation – 2 nm spacing Structure file sample

41984

ISCS	N	1	1.413	3.519	3.219	0.3965	-0.0887	0.0632
ISCS	C8	2	1.274	3.739	3.179	-0.1775	0.0714	0.2293
ISCS	C7	3	1.361	3.648	3.265	-0.4066	-0.3821	-0.0154
ISCS	O7	4	1.337	3.664	3.405	-0.4116	-0.1537	-0.0426
ISCS	H4	5	1.437	3.507	3.123	-1.0068	-1.2726	-0.1853
ISCS	C2	6	1.424	3.411	3.319	0.1436	-0.0599	0.1229
ISCS	C1	7	1.536	3.316	3.278	0.2275	0.1443	-0.1228
ISCS	O5	8	1.547	3.198	3.358	-0.4576	0.2751	0.1707
ISCS	C5	9	1.431	3.115	3.366	0.0823	-0.5104	-0.0864
ISCS	C6	10	1.448	2.978	3.431	0.2374	-0.3306	0.2546
ISCS	O6	11	1.432	2.971	3.573	0.5084	0.0565	0.3056
ISCS	S	12	1.348	2.852	3.608	0.0072	0.2607	-0.1949
ISCS	O9	13	1.414	2.720	3.633	-0.2655	0.1896	0.1561
ISCS	O8	14	1.224	2.821	3.530	-0.3348	0.4133	0.2833
ISCS	O10	15	1.279	2.843	3.741	0.2192	-0.2983	-0.1219
ISCS	C4	16	1.310	3.198	3.408	0.5161	0.0241	0.1151
ISCS	O4	17	1.293	3.213	3.549	0.2132	-0.2720	0.1114
ISCS	H42	18	1.379	3.222	3.598	-0.2559	-1.7932	1.2663
ISCS	C3	19	1.302	3.320	3.318	0.2419	-0.1887	-0.1524
ISCS	O11	20	1.194	3.361	3.234	0.6417	-0.5871	-0.8762
ISCS	C11	21	1.091	3.278	3.181	-0.0452	-0.1513	-0.2546
ISCS	O15	22	0.967	3.315	3.242	0.0567	-0.3835	0.0941
ISCS	C15	23	0.850	3.253	3.189	-0.3157	0.4345	-0.0464
ISCS	C16	24	0.724	3.270	3.271	-0.0507	0.4616	0.3580
ISCS	O18	25	0.659	3.170	3.350	-0.0114	-0.4339	-0.7205
ISCS	O17	26	0.632	3.371	3.226	0.1735	0.5035	-0.0072
ISCS	C14	27	0.831	3.262	3.038	-0.0737	0.4523	-0.0759
ISCS	C13	28	0.964	3.224	2.974	-0.1021	-0.3983	0.3531
ISCS	O13	29	0.954	3.232	2.832	-0.5731	-0.3314	0.3883
ISCS	H85	30	0.862	3.201	2.808	-0.3069	-0.4852	-0.4642
ISCS	C12	31	1.089	3.291	3.029	-0.1672	0.2146	-0.2227
ISCS	O12	32	1.218	3.264	2.973	-0.1236	0.6092	-0.3163
ISCS	H86	33	1.252	3.177	3.011	-1.0177	0.4584	0.1915
ISCS	O	34	1.664	3.378	3.291	0.0889	0.3238	0.4479
2CCS	N	35	2.273	3.133	2.998	-0.1607	0.3835	0.0975
2CCS	C8	36	2.095	3.006	2.882	0.3251	0.3231	-0.6554
2CCS	C7	37	2.244	3.034	2.894	0.2760	0.2992	0.0533
2CCS	O7	38	2.339	2.965	2.812	-0.1056	0.0325	-0.1709
2CCS	H4	39	2.289	3.098	3.090	1.0127	-0.6851	-0.4732
2CCS	C2	40	2.281	3.274	2.956	0.3154	0.1549	-0.6061
2CCS	C1	41	2.424	3.324	2.940	0.3042	0.5304	0.3734
2CCS	O5	42	2.431	3.442	2.860	-0.0774	0.0234	-0.4237
2CCS	C5	43	2.360	3.550	2.921	-0.4636	-0.2466	-0.3967
2CCS	C6	44	2.363	3.672	2.831	-0.2481	-0.0568	-0.1333
2CCS	O6	45	2.496	3.722	2.839	-0.3773	0.1739	0.5881
2CCS	S	46	2.499	3.872	2.836	0.2670	0.1527	-0.0704
2CCS	O9	47	2.393	3.948	2.911	0.1926	0.3948	-0.4163
2CCS	O8	48	2.468	3.930	2.701	-0.1987	0.1699	0.0428
2CCS	O10	49	2.623	3.948	2.872	0.3556	-0.4198	0.8427
2CCS	C4	50	2.250	3.519	3.022	-0.0745	0.0470	0.1186
2CCS	O4	51	2.259	3.590	3.146	0.2487	-0.0810	0.1698
2CCS	H42	52	2.338	3.578	3.206	-1.7482	0.7548	3.2005
2CCS	C3	53	2.237	3.373	3.062	0.5060	-0.1171	-0.2682
2CCS	O11	54	2.097	3.352	3.082	0.5155	0.0391	-0.0270

Five Chain Simulation – 2 nm spacing Topology file sample

```

;      File 'topol_1.itp' was generated
;      By user: kevin (1000)
;      On host: kevin-laptop
;      At date: Mon Mar 10 15:24:47 2008
;
;      This is your include topology file
;      C6S-System
;
[ moleculetype ]
; Name          nrexcl
Protein_1       3

[ atoms ]
; nr      type resnr residue atom  cgnr  charge   mass typeB  chargeB   massB
1         N    1   SCS    N    1    0.141  14.0067 ; qtot 0.141
2         CH3  1   SCS    C8    2      0   15.035 ; qtot 0.141
3         CH1  1   SCS    C7    3    0.39   13.019 ; qtot 0.531
4         OA   1   SCS    O7    3   -0.65  15.9994 ; qtot -0.119
5         H    1   SCS    H4    3   -0.002  1.008 ; qtot -0.121
6         CH1  1   SCS    C2    3    0.163  13.019 ; qtot 0.042
7         CH1  1   SCS    C1    3    0.257  13.019 ; qtot 0.299

[ bonds ]
; ai  aj funct      c0      c1      c2      c3
1    3    2  gb_21
1    5    2  gb_2
1    6    2  gb_21
2    3    2  gb_26
3    4    2  gb_18
6    7    2  gb_26
6   19    2  gb_26
7    8    2  gb_19
7   34    2  gb_19

[ pairs ]
; ai  aj funct      c0      c1      c2      c3
1    8    1
1   16    1
1   20    1
1   34    1
2    5    1
2    6    1
3    7    1
3   19    1
4    5    1

[ angles ]
; ai  aj  ak funct      c0      c1      c2      c3
3    1  5    2  ga_18
3    1  6    2  ga_13
5    1  6    2  ga_18
1    3  2    2  ga_13
1    3  4    2  ga_9
2    3  4    2  ga_9
1    6  7    2  ga_13
1    6  19   2  ga_13
7    6  19   2  ga_8
6    7  8    2  ga_13

[ dihedrals ]
; ai  aj  ak  al funct      c0      c1      c2      c3      c4      c5
6    1  3    2    1  gd_41
3    1  6    19   1  gd_41
1    6  7    8    1  gd_4
1    6  19  20    1  gd_4
6    7  8    9    1  gd_1
6    7  34  61    1  gd_1

```

```

      8  7 34 61  1 gd_2
[ dihedrals ]
; ai  aj  ak  al funct      c0      c1      c2      c3
  1   4   2   3   2  gi_1
  1   3   5   6   2  gi_1
24  23  25  26   2  gi_1
35  38  36  37   2  gi_1
35  37  39  40   2  gi_1
58  57  59  60   2  gi_1
69  72  70  71   2  gi_1
69  71  73  74   2  gi_1
; Include Position restraint file
#ifdef POSRES
#include "posre_1.itp"
#endif

```


Five Chain Simulation – 2 nm spacing MD parameter file

```
;
;      File 'mdout.mdp' was generated
;      By user: kevin (1000)
;      On host: kevin-laptop
;      At date: Tue Jul  8 21:29:52 2008
;

; VARIOUS PREPROCESSING OPTIONS
title           =
; Preprocessor - specify a full path if necessary.
cpp             = cpp
include         =
define          =

; RUN CONTROL PARAMETERS
integrator      = md
; Start time and timestep in ps
tinit          = 0
dt              = 0.002
nsteps         = 100000
; For exact run continuation or redoing part of a run
init_step      = 0
; mode for center of mass motion removal
comm-mode       = Angular
; number of steps for center of mass motion removal
nstcomm        = 1
; group(s) for center of mass motion removal
comm-grps      = SCS

; LANGEVIN DYNAMICS OPTIONS
; Friction coefficient (amu/ps) and random seed
bd-fric        = 0
ld-seed        = 1993

; ENERGY MINIMIZATION OPTIONS
; Force tolerance and initial step-size
emtol          = 10
emstep         = 0.01
; Max number of iterations in relax_shells
niter          = 20
; Step size (ps^2) for minimization of flexible constraints
fcstep         = 0
; Frequency of steepest descents steps when doing CG
nstcgsteep     = 1000
nbgscorr       = 10

; OUTPUT CONTROL OPTIONS
; Output frequency for coords (x), velocities (v) and forces (f)
nstxout        = 1000
nstvout        = 1000
nstfout        = 0
; Checkpointing helps you continue after crashes
nstcheckpoint  = 1000
; Output frequency for energies to log file and energy file
nstlog         = 1000
nstenergy      = 100
; Output frequency and precision for xtc file
nstxtcout      = 0
xtc-precision  = 1000
; This selects the subset of atoms for the xtc file. You can
; select multiple groups. By default all atoms will be written.
xtc-grps       =
```

```

; Selection of energy groups
energygrps      = Aggrecan

; NEIGHBORSEARCHING PARAMETERS
; nblist update frequency
nstlist         = 10
; ns algorithm (simple or grid)
ns-type         = Grid
; Periodic boundary conditions: xyz (default), no (vacuum)
; or full (infinite systems only)
pbc             = xyz
; nblist cut-off
rlist           = 2.0
domain-decomposition = no

; OPTIONS FOR ELECTROSTATICS AND VDW
; Method for doing electrostatics
coulombtype     = pme
rcoulomb-switch = 0
rcoulomb        = 2.0
; Relative dielectric constant for the medium and the reaction field
epsilon_r       = 1
epsilon_rf      = 1
; Method for doing Van der Waals
vdw-type        = Switch
; cut-off lengths
rvdw-switch     = 1.9
rvdw            = 2.0
; Apply long range dispersion corrections for Energy and Pressure
DispCorr        = No
; Extension of the potential lookup tables beyond the cut-off
table-extension = 1
; Separate tables between energy group pairs
energygrp_table =
; Spacing for the PME/PPPM FFT grid
fourierspacing  = 0.12
; FFT grid size, when a value is 0 fourierspacing will be used
fourier_nx      = 0
fourier_ny      = 0
fourier_nz      = 0
; EWALD/PME/PPPM parameters
pme_order       = 4
ewald_rtol      = 1e-05
ewald_geometry  = 3d
epsilon_surface = 0
optimize_fft    = no

; GENERALIZED BORN ELECTROSTATICS
; Algorithm for calculating Born radii
gb_algorithm     = Still
; Frequency of calculating the Born radii inside rlist
nstgbradii      = 1
; Cutoff for Born radii calculation; the contribution from atoms
; between rlist and rgbradii is updated every nstlist steps
rgbradii        = 2
; Salt concentration in M for Generalized Born models
gb_saltconc     = 0

; IMPLICIT SOLVENT (for use with Generalized Born electrostatics)
implicit_solvent = No

; OPTIONS FOR WEAK COUPLING ALGORITHMS
; Temperature coupling
tcoupl          = Berendsen
; Groups to couple separately

```

```

tc-grps          = AggreCan SOL
; Time constant (ps) and reference temperature (K)
tau-t           = 0.1 0.1
ref-t           = 300 300
; Pressure coupling
Pcoupl          = berendsen
Pcoupltype      = Isotropic
; Time constant (ps), compressibility (1/bar) and reference P (bar)
tau_p           = 1.0
compressibility  = 4.5e-5
ref_p           = 1.0
; Random seed for Andersen thermostat
andersen_seed   = 815131

; OPTIONS FOR QMMM calculations
QMMM            = no
; Groups treated Quantum Mechanically
QMMM-grps       =
; QM method
QMmethod        =
; QMMM scheme
QMMMscheme      = normal
; QM basisset
QMbasis         =
; QM charge
QMcharge        =
; QM multiplicity
QMmult          =
; Surface Hopping
SH              =
; CAS space options
CASorbitals     =
CASelectrons    =
SAon            =
SAoff           =
SAsteps         =
; Scale factor for MM charges
MMChargeScaleFactor = 1
; Optimization of QM subsystem
bOPT            =
bTS             =

; SIMULATED ANNEALING
; Type of annealing for each temperature group (no/single/periodic)
annealing       =
; Number of time points to use for specifying annealing in each group
annealing_npoints =
; List of times at the annealing points for each group
annealing_time  =
; Temp. at each annealing point, for each group.
annealing_temp  =

; GENERATE VELOCITIES FOR STARTUP RUN
gen-vel         = no
gen-temp        = 300
gen-seed        = 173529

; OPTIONS FOR BONDS
constraints     = all-bonds
; Type of constraint algorithm
constraint-algorithm = Lincs
; Do not constrain the start configuration
unconstrained-start = no
; Use successive overrelaxation to reduce the number of shake iterations
Shake-SOR      = no

```

```

; Relative tolerance of shake
shake-tol      = 1e-04
; Highest order in the expansion of the constraint coupling matrix
lincs-order    = 4
; Number of iterations in the final step of LINCS. 1 is fine for
; normal simulations, but use 2 to conserve energy in NVE runs.
; For energy minimization with constraints it should be 4 to 8.
lincs-iter     = 1
; Lincs will write a warning to the stderr if in one step a bond
; rotates over more degrees than
lincs-warnangle = 30
; Convert harmonic bonds to morse potentials
morse         = no

; ENERGY GROUP EXCLUSIONS
; Pairs of energy groups for which all non-bonded interactions are excluded
energygrp_excl =

; NMR refinement stuff
; Distance restraints type: No, Simple or Ensemble
disre         = No
; Force weighting of pairs in one distance restraint: Conservative or Equal
disre-weighting = Conservative
; Use sqrt of the time averaged times the instantaneous violation
disre-mixed   = no
disre-fc      = 1000
disre-tau     = 0
; Output frequency for pair distances to energy file
nstdisreout   = 100
; Orientation restraints: No or Yes
orire         = no
; Orientation restraints force constant and tau for time averaging
orire-fc      = 0
orire-tau     = 0
orire-fitgrp  =
; Output frequency for trace(SD) and S to energy file
nstorireout   = 100
; Dihedral angle restraints: No, Simple or Ensemble
dihre        = No
dihre-fc     = 1000
dihre-tau    = 0
; Output frequency for dihedral values to energy file
nstdihreout   = 100

; Free energy control stuff
free-energy    = no
init-lambda   = 0
delta-lambda  = 0
sc-alpha      = 0
sc-power      = 0
sc-sigma      = 0.3

; Non-equilibrium MD stuff
acc-grps      =
accelerate    =
freezegrps    =
freezedim     =
cos-acceleration = 0
deform        =

; Electric fields
; Format is number of terms (int) and for all terms an amplitude (real)
; and a phase angle (real)
E-x           =
E-xt          =

```

```

E·y      =
E·yt     =
E·z      =
E·zt     =

; User defined thingies
user1·grps =
user2·grps =
userint1   = 0
userint2   = 0
userint3   = 0
userint4   = 0
userreal1  = 0
userreal2  = 0
userreal3  = 0
userreal4  = 0

```

Pulling Simulation – 1.5 nm spacing Structure file sample

39732

1SCS	N	1	15.412	2.963	1.191	0.3347	-0.1605	0.1097
1SCS	C8	2	15.424	3.177	1.057	0.0839	0.0596	-0.2096
1SCS	C7	3	15.463	3.100	1.181	0.4454	0.0343	-0.7402
1SCS	O7	4	15.507	3.184	1.295	-0.0143	0.0245	0.2985
1SCS	H4	5	15.373	2.919	1.117	0.9766	-2.8577	0.1823
1SCS	C2	6	15.389	2.918	1.326	-0.0305	0.5138	0.6651
1SCS	C1	7	15.414	2.766	1.338	0.1211	-0.1819	0.6838
1SCS	O5	8	15.301	2.684	1.291	0.1650	-0.9999	-0.1754
1SCS	C5	9	15.184	2.711	1.369	-0.2251	-0.3247	0.3930
1SCS	C6	10	15.064	2.610	1.360	-0.4072	0.0021	0.7124
1SCS	O6	11	14.997	2.614	1.232	0.4330	0.0391	-0.1826
1SCS	S	12	14.855	2.567	1.254	-0.2880	0.2866	-0.1577
1SCS	O9	13	14.764	2.609	1.134	0.1669	-0.0048	0.4436
1SCS	O8	14	14.772	2.613	1.130	0.2184	0.0622	0.1143
1SCS	O10	15	14.777	2.674	1.328	0.1335	-0.2618	-0.1071
1SCS	C4	16	15.142	2.854	1.346	-0.1402	0.0319	0.2575
1SCS	O4	17	15.120	2.855	1.206	-0.4730	0.1826	0.2529
1SCS	H42	18	15.154	2.771	1.172	0.8726	-0.5829	0.1846
1SCS	C3	19	15.253	2.947	1.399	0.5617	-0.7544	0.1246
1SCS	O11	20	15.210	3.082	1.425	0.1593	-0.3422	0.7278
1SCS	C11	21	15.227	3.104	1.568	0.3300	-0.4232	-0.0500
1SCS	O15	22	15.114	3.108	1.656	0.7820	0.1616	0.2059
1SCS	C15	23	15.026	3.218	1.635	-0.0935	-0.0787	0.3747
1SCS	C16	24	14.905	3.209	1.714	0.4330	0.5357	0.4889
1SCS	O18	25	14.770	3.230	1.667	-0.1183	-0.0398	0.7052
1SCS	O17	26	14.898	3.191	1.861	0.3366	-0.2330	-0.3464
1SCS	C14	27	15.098	3.347	1.658	-0.1392	-0.0317	-0.3605
1SCS	C13	28	15.224	3.358	1.575	0.1068	0.5810	-0.3147
1SCS	O13	29	15.185	3.387	1.441	0.3776	-0.0394	-0.5957
1SCS	H85	30	15.126	3.471	1.453	-0.5236	-1.0202	0.3959
1SCS	C12	31	15.314	3.229	1.589	-0.0429	0.1085	-0.4395
1SCS	O12	32	15.375	3.222	1.717	0.0544	-0.4266	-0.0107
1SCS	H86	33	15.312	3.242	1.792	-3.0565	1.4872	1.5694
1SCS	O	34	15.540	2.722	1.275	-0.0849	-0.6464	-1.0352
2CCS	N	35	16.205	2.507	1.372	-0.1907	-0.2082	0.8882
2CCS	C8	36	16.266	2.493	1.613	0.0419	-0.1455	0.9961
2CCS	C7	37	16.187	2.432	1.496	0.1774	0.6382	-0.2381
2CCS	O7	38	16.159	2.288	1.499	0.1602	0.0031	-0.1978
2CCS	H4	39	16.190	2.607	1.373	0.1640	0.7798	1.9383
2CCS	C2	40	16.210	2.419	1.254	0.0229	0.4923	0.2675
2CCS	C1	41	16.320	2.434	1.149	0.1869	-0.4614	0.1608
2CCS	O5	42	16.290	2.539	1.058	0.7229	0.2241	-0.1303
2CCS	C5	43	16.190	2.509	0.964	1.1577	-0.5703	-0.2243
2CCS	C6	44	16.169	2.624	0.868	-0.0878	-0.2594	-0.0097
2CCS	O6	45	16.139	2.745	0.938	-0.4120	-0.4766	-0.2532
2CCS	S	46	16.160	2.852	0.833	-0.1793	0.0675	0.1638
2CCS	O9	47	16.121	2.990	0.894	0.1583	0.0791	-0.2224
2CCS	O8	48	16.024	2.868	0.760	-0.1030	0.4268	0.6533
2CCS	O10	49	16.034	2.871	0.743	-0.3796	0.2730	-0.0572
2CCS	C4	50	16.064	2.442	1.026	0.1659	-0.6459	0.1986
2CCS	O4	51	16.050	2.311	0.973	-0.7324	-0.1798	0.2064
2CCS	H42	52	16.100	2.285	0.884	-1.5869	-1.3070	-2.7311
2CCS	C3	53	16.072	2.433	1.178	0.2136	0.4349	0.3590

Pulling Simulation – 1.5 nm spacing Topology file sample

```

;      File 'topol_1.itp' was generated
;      By user: kevin (1000)
;      On host: kevin-laptop
;      At date: Thu Apr 24 21:24:52 2008
;
;      This is your include topology file
;      C6S-System
;
[ moleculetype ]
; Name          nrexcl
Protein_1      3

[ atoms ]
; nr   type resnr residue atom  cgnr  charge   mass typeB  chargeB  massB
; 1     N    1   SCS   N    1    0.141  14.0067 ; qtot 0.141
; 2    CH3   1   SCS   C8    2     0    15.035 ; qtot 0.141
; 3    CH1   1   SCS   C7    3    0.39   13.019 ; qtot 0.531
; 4     OA   1   SCS   O7    3   -0.65  15.9994 ; qtot -0.119
; 5     H    1   SCS   H4    3   -0.002  1.008 ; qtot -0.121

[ bonds ]
; ai  aj funct      c0      c1      c2      c3
; 1  3  2  gb_21
; 1  5  2  gb_2
; 1  6  2  gb_21
; 2  3  2  gb_26
; 3  4  2  gb_18
; 6  7  2  gb_26
; 6 19  2  gb_26

[ pairs ]
; ai  aj funct      c0      c1      c2      c3
; 1  8  1
; 1 16  1
; 1 20  1
; 1 34  1
; 2  5  1
; 2  6  1
; 3  7  1

[ angles ]
; ai  aj  ak funct      c0      c1      c2      c3
; 3  1  5  2  ga_18
; 3  1  6  2  ga_13
; 5  1  6  2  ga_18
; 1  3  2  2  ga_13
; 1  3  4  2  ga_9
; 2  3  4  2  ga_9
; 1  6  7  2  ga_13

[ dihedrals ]
; ai  aj  ak  al funct      c0      c1      c2      c3      c4      c5
; 6  1  3  2  1  gd_41
; 3  1  6 19  1  gd_41
; 1  6  7  8  1  gd_4
; 1  6 19 20  1  gd_4
; 6  7  8  9  1  gd_1
; 6  7 34 61  1  gd_1
; 8  7 34 61  1  gd_2

[ dihedrals ]
; ai  aj  ak  al funct      c0      c1      c2      c3
; 1  4  2  3  2  gi_1
; 1  3  5  6  2  gi_1
; 24 23 25 26  2  gi_1
; 35 38 36 37  2  gi_1
; 35 37 39 40  2  gi_1
; 58 57 59 60  2  gi_1

```

```
69 72 70 71 2 gi_1  
; Include Position restraint file  
#ifdef POSRES  
#include "posre_1.itp"  
#endif
```


Pulling Simulation – 1.5 nm spacing MD parameter file

```
;
;      File 'gromppout.mdp' was generated
;      By user: onbekend (0)
;      On host: onbekend
;      At date: Fri Mar  7 01:08:28 2008
;

; VARIOUS PREPROCESSING OPTIONS
title                =
; Preprocessor - specify a full path if necessary.
cpp                  = cpp
include              =
define               =

; RUN CONTROL PARAMETERS
integrator           = md
; Start time and timestep in ps
tinit                = 0
dt                   = 0.001
nsteps               = 1500000
; For exact run continuation or redoing part of a run
init_step            = 0
; mode for center of mass motion removal

comm-mode            = Angular
; number of steps for center of mass motion removal
nstcomm              = 1
; group(s) for center of mass motion removal
comm-grps            = bs1 bs2

; LANGEVIN DYNAMICS OPTIONS
; Friction coefficient (amu/ps) and random seed
bd-fric              = 0
ld-seed              = 1993

; ENERGY MINIMIZATION OPTIONS
; Force tolerance and initial step-size
emtol                = 10
emstep               = 0.001
; Max number of iterations in relax_shells
niter                = 20
; Step size (ps^2) for minimization of flexible constraints
fcstep               = 0
; Frequency of steepest descents steps when doing CG
nstcgsteep           = 1000
nbgfscorr            = 10

; OUTPUT CONTROL OPTIONS
; Output frequency for coords (x), velocities (v) and forces (f)
nstxout              = 1000
nstvout              = 1000
nstfout              = 0
; Checkpointing helps you continue after crashes
nstcheckpoint         = 1000
; Output frequency for energies to log file and energy file
nstlog               = 1000
nstenergy            = 100
; Output frequency and precision for xtc file
nstxtcout            = 0
xtc-precision        = 1000
; This selects the subset of atoms for the xtc file. You can
; select multiple groups. By default all atoms will be written.
```

```

xtc-grps          =
; Selection of energy groups
energygrps        = s1 c s2

; NEIGHBORSEARCHING PARAMETERS
; nblist update frequency
nstlist           = 10
; ns algorithm (simple or grid)
ns-type           = Grid
; Periodic boundary conditions: xyz (default), no (vacuum)
; or full (infinite systems only)
pbc               = xyz
; nblist cut-off
rlist             = 1.1
domain-decomposition = no

; OPTIONS FOR ELECTROSTATICS AND VDW
; Method for doing electrostatics
coulombtype       = pme
rcoulomb-switch   = 0
rcoulomb          = 1.1
; Relative dielectric constant for the medium and the reaction field
epsilon_r         = 1
epsilon_rf        = 1
; Method for doing Van der Waals
vdw-type          = Switch
; cut-off lengths
rvdw-switch       = 0.9
rvdw              = 1.0
; Apply long range dispersion corrections for Energy and Pressure
DispCorr          = No
; Extension of the potential lookup tables beyond the cut-off
table-extension   = 1
; Seperate tables between energy group pairs
energygrp_table   =
; Spacing for the PME/PPPM FFT grid
fourierspacing    = 0.12
; FFT grid size, when a value is 0 fourierspacing will be used
fourier_nx        = 0
fourier_ny        = 0
fourier_nz        = 0
; EWALD/PME/PPPM parameters
pme_order         = 4
ewald_rtol        = 1e-05
ewald_geometry    = 3d
epsilon_surface   = 0
optimize_fft      = no

; GENERALIZED BORN ELECTROSTATICS
; Algorithm for calculating Born radii
gb_algorithm       = Still
; Frequency of calculating the Born radii inside rlist
nstgbradii        = 1
; Cutoff for Born radii calculation; the contribution from atoms
; between rlist and rgbradii is updated every nstlist steps
rgbradii          = 2
; Salt concentration in M for Generalized Born models
gb_saltconc       = 0

; IMPLICIT SOLVENT (for use with Generalized Born electrostatics)
implicit_solvent   = No

; OPTIONS FOR WEAK COUPLING ALGORITHMS
; Temperature coupling
tcoupl            = Berendsen

```

```

; Groups to couple separately
tc-grps          = AggreCan SOL
; Time constant (ps) and reference temperature (K)
tau-t            = 0.1 0.1
ref-t            = 300 300
; Pressure coupling
Pcoupl           = No
Pcoupltype       = Isotropic
; Time constant (ps), compressibility (1/bar) and reference P (bar)
tau-p            = 1
compressibility   =
ref-p            =
; Random seed for Andersen thermostat
andersen_seed    = 815131

; OPTIONS FOR QMMM calculations
QMMM             = no
; Groups treated Quantum Mechanically
QMMM-grps        =
; QM method
QMmethod         =
; QMMM scheme
QMMMscheme       = normal
; QM basisset
QMbasis          =
; QM charge
QMcharge         =
; QM multiplicity
QMmult           =
; Surface Hopping
SH               =
; CAS space options
CASorbitals      =
CASelectrons     =
SAon             =
SAoff            =
SAsteps          =
; Scale factor for MM charges
MMChargeScaleFactor = 1
; Optimization of QM subsystem
bOPT             =
bTS              =

; SIMULATED ANNEALING
; Type of annealing for each temperature group (no/single/periodic)
annealing        =
; Number of time points to use for specifying annealing in each group
annealing_npoints =
; List of times at the annealing points for each group
annealing_time   =
; Temp. at each annealing point, for each group.
annealing_temp   =

; GENERATE VELOCITIES FOR STARTUP RUN
gen-vel          = yes
gen-temp         = 300
gen-seed         = 173529

; OPTIONS FOR BONDS
constraints       = none
; Type of constraint algorithm
constraint-algorithm = Lincs
; Do not constrain the start configuration
unconstrained-start = no
; Use successive overrelaxation to reduce the number of shake iterations

```

```

Shake-SOR          = no
; Relative tolerance of shake
shake-tol          = 1e-04
; Highest order in the expansion of the constraint coupling matrix
lincs-order        = 4
; Number of iterations in the final step of LINCS. 1 is fine for
; normal simulations, but use 2 to conserve energy in NVE runs.
; For energy minimization with constraints it should be 4 to 8.
lincs-iter         = 1
; Lincs will write a warning to the stderr if in one step a bond
; rotates over more degrees than
lincs-warnangle    = 30
; Convert harmonic bonds to morse potentials
morse              = no

; ENERGY GROUP EXCLUSIONS
; Pairs of energy groups for which all non-bonded interactions are excluded
energygrp_excl     =

; NMR refinement stuff
; Distance restraints type: No, Simple or Ensemble
disre              = No
; Force weighting of pairs in one distance restraint: Conservative or Equal
disre-weighting    = Conservative
; Use sqrt of the time averaged times the instantaneous violation
disre-mixed        = no
disre-fc           = 1000
disre-tau          = 0
; Output frequency for pair distances to energy file
nstdisreout        = 100
; Orientation restraints: No or Yes
orire              = no
; Orientation restraints force constant and tau for time averaging
orire-fc           = 0
orire-tau          = 0
orire-fitgrp       =
; Output frequency for trace(SD) and S to energy file
nstorireout        = 100
; Dihedral angle restraints: No, Simple or Ensemble
dihre              = No
dihre-fc           = 1000
dihre-tau          = 0
; Output frequency for dihedral values to energy file
nstdihreout        = 100

; Free energy control stuff
free-energy         = no
init-lambda        = 0
delta-lambda       = 0
sc-alpha           = 0
sc-power           = 0
sc-sigma           = 0.3

; Non-equilibrium MD stuff
acc-grps           =
accelerate         =
freezegrps         =
freezedim          =
cos-acceleration   = 0
deform             =

; Electric fields
; Format is number of terms (int) and for all terms an amplitude (real)
; and a phase angle (real)
E-x               =

```

```

E·xt      =
E·y       =
E·yt      =
E·z       =
E·zt      =

; User defined thingies
user1-grps =
user2-grps =
userint1   = 0
userint2   = 0
userint3   = 0
userint4   = 0
userreal1  = 0
userreal2  = 0
userreal3  = 0
userreal4  = 0

```

Pulling Simulation – 1.5 nm spacing Pulling parameter file

```
;;FIRST read the chapter of manual where the afm-like simulation is explained
;
; you need to run a simulation in this way

; the grompp part is the same you know

; mdrun -s topol.tpr -pi pull.ppa -pn index.ndx -po pull.pdo

; pull.pdo is the output. the file contains all the coordinate of the reference group, pull group spring for each time
;
; time   Xref Yref Zref Xpull Xspring Ypull Yspring Zpull Zspring
;
;
;
; GENERAL
verbose          = yes
Skip steps       = 1
; Runtype: afm, constraint, umbrella
runtype          = afm
; Number of pull groups
ngroups          = 1
; Groups to be pulled
; it has to be defined in the index file!!

group_1          = pc

; The group for the reaction force ; if you want just move a molecule (your case!) do not choose a reference.

reference_group   =

; Select components for the pull vector. default: Y Y Y
pulldim          = Y Y Y

; AFM OPTIONS
; Pull rates in nm/ps
afm_rate1        = 0.01

; Force constants in kJ/(mol*nm^2)

afm_k1           = 3000

; Directions along X Y Z axes
afm_dir1         = 1 0 0

; Initial spring positions ; remember!! the spring initial position has to be equal to the pull group initial position (to
have starting force equal to zero), the spring position is referred to reference. Find the initial pull group position
with g_traj!
In particular don't choose a reference means that the spring position is referred to 0 0 0.

afm_init1        = 8.9172 3.03338 1.4222
```

Pulling Simulation – 1.5 nm spacing Pulling output data file sample

```
# AFM 3.0
# Component selection: 1 1 1
# nSkip 1
# Ref. Group "
# Nr. of pull groups 1
# Group 1 'pc' afmVec 1.000000 0.000000 0.000000 AfmRate 0.010000 AfmK 3000.000000
#####
0.000000 0.000000 0.000000 0.000000 8.917205 8.917200 3.033380
3.033380 1.422197 1.422200
0.001000 0.000000 0.000000 0.000000 8.917168 8.917210 3.033382
3.033380 1.422097 1.422200
0.002000 0.000000 0.000000 0.000000 8.917130 8.917220 3.033383
3.033380 1.421996 1.422200
0.003000 0.000000 0.000000 0.000000 8.917091 8.917230 3.033385
3.033380 1.421896 1.422200
0.004000 0.000000 0.000000 0.000000 8.917050 8.917240 3.033388
3.033380 1.421795 1.422200
0.005000 0.000000 0.000000 0.000000 8.917007 8.917250 3.033390
3.033380 1.421694 1.422200
0.006000 0.000000 0.000000 0.000000 8.916963 8.917260 3.033393
3.033380 1.421592 1.422200
0.007000 0.000000 0.000000 0.000000 8.916915 8.917270 3.033398
3.033380 1.421491 1.422200
0.008000 0.000000 0.000000 0.000000 8.916865 8.917280 3.033403
3.033380 1.421390 1.422200
0.009000 0.000000 0.000000 0.000000 8.916812 8.917290 3.033410
3.033380 1.421289 1.422200
0.010000 0.000000 0.000000 0.000000 8.916756 8.917300 3.033418
3.033380 1.421187 1.422200
0.011000 0.000000 0.000000 0.000000 8.916697 8.917310 3.033427
3.033380 1.421085 1.422200
0.012000 0.000000 0.000000 0.000000 8.916635 8.917320 3.033437
3.033380 1.420984 1.422200
0.013000 0.000000 0.000000 0.000000 8.916572 8.917330 3.033448
3.033380 1.420882 1.422200
0.014000 0.000000 0.000000 0.000000 8.916507 8.917340 3.033459
3.033380 1.420781 1.422200
0.015000 0.000000 0.000000 0.000000 8.916442 8.917350 3.033471
3.033380 1.420680 1.422200
0.016000 0.000000 0.000000 0.000000 8.916377 8.917360 3.033483
3.033380 1.420580 1.422200
0.017000 0.000000 0.000000 0.000000 8.916314 8.917370 3.033494
3.033380 1.420481 1.422200
0.018000 0.000000 0.000000 0.000000 8.916252 8.917380 3.033505
3.033380 1.420382 1.422200
0.019000 0.000000 0.000000 0.000000 8.916194 8.917390 3.033515
3.033380 1.420285 1.422200
0.020000 0.000000 0.000000 0.000000 8.916139 8.917400 3.033524
3.033380 1.420189 1.422200
0.021000 0.000000 0.000000 0.000000 8.916089 8.917410 3.033532
3.033380 1.420094 1.422200
0.022000 0.000000 0.000000 0.000000 8.916042 8.917420 3.033537
3.033380 1.420000 1.422200
0.023000 0.000000 0.000000 0.000000 8.916000 8.917430 3.033540
3.033380 1.419907 1.422200
0.024000 0.000000 0.000000 0.000000 8.915962 8.917440 3.033542
3.033380 1.419815 1.422200
0.025000 0.000000 0.000000 0.000000 8.915928 8.917450 3.033540
3.033380 1.419724 1.422200
```

Force Field Files Atom mass and descriptions (ffG53a6.atp)

O 15.99940 ; carbonyl oxygen (C=O)
 OM 15.99940 ; carboxyl oxygen (CO-)
 OA 15.99940 ; hydroxyl, sugar or ester oxygen
 OE 15.99940 ; ether or ester oxygen
 OW 15.99940 ; water oxygen
 N 14.00670 ; peptide nitrogen (N or NH)
 NT 14.00670 ; terminal nitrogen (NH2)
 NL 14.00670 ; terminal nitrogen (NH3)
 NR 14.00670 ; aromatic nitrogen
 NZ 14.00670 ; Arg NH (NH2)
 NE 14.00670 ; Arg NE (NH)
 C 12.01100 ; bare carbon
 CH0 12.0110 ; bare sp3 carbon, 4 bound heavy atoms
 CH1 13.01900 ; aliphatic or sugar CH-group
 CH 13.01900 ; aliphatic or sugar CH-group
 CH2 14.02700 ; aliphatic or sugar CH2-group
 CH3 15.03500 ; aliphatic CH3-group
 CH4 16.04300 ; methane
 CH2R 14.02700 ; CH2-group in a ring
 CR1 13.01900 ; aromatic CH-group
 HC 1.00800 ; hydrogen bound to carbon
 H 1.00800 ; hydrogen not bound to carbon
 DUM 0.00000 ; dummy atom, no idea what the mass should be. PT3-99
 S 32.06000 ; sulfur
 CU1+ 63.54600 ; copper (charge 1+)
 CU2+ 63.54600 ; copper (charge 2+)
 FE 55.84700 ; iron (heme)
 ZN2+ 65.37000 ; zinc (charge 2+)
 MG2+ 24.30500 ; magnesium (charge 2+)
 CA2+ 40.08000 ; calcium (charge 2+)
 P 30.97380 ; phosphor
 AR 39.94800 ; argon
 F 18.99840 ; fluor (non-ionic)
 CL 35.45300 ; chlorine (non-ionic)
 BR 79.90400 ; bromine (non-ionic)
 CMET 15.035 ; CH3-group in methanol (solvent)
 OMET 15.9994 ; oxygen in methanol (solvent)
 NA+ 22.9898 ; sodium (charge 1+)
 CL- 35.45300 ; chlorine (charge 1-)
 CCHL 12.011 ; carbon in chloroform (solvent)
 CLCHL 35.453 ; chloride in chloroform (solvent)
 HCHL 1.008 ; hydrogen in chloroform (solvent)
 SDMSO 32.06000 ; DMSO Sulphur (solvent)
 CDMSO 15.03500 ; DMSO Carbon (solvent)
 ODMSO 15.99940 ; DMSO Oxygen (solvent)
 CCL4 12.011 ; carbon in carbontetrachloride (solvent)
 CLCL4 35.453 ; chloride in carbontetrachloride (solvent)
 FTFE 18.99840 ; fluor in trifluoroethanol
 CTFE 12.01100 ; carbon in trifluoroethanol
 CHTFE 14.0270 ; CH2-group in trifluoroethanol
 OTFE 15.99940 ; oxygen in trifluoroethanol
 CUREA 12.01100 ; carbon in urea
 OUREA 15.99940 ; oxygen in urea
 NUREA 14.00670 ; nitrogen in urea
 SI 28.08 ; silicon
 MNH3 0 ; Dummy mass in rigid tetrahedral NH3 group
 MW 0 ; Dummy mass in rigid tyrosine rings

Force Field Files Bonded interaction parameter file sample (ffG53a6bon.itp)

```
; Table 2.5.2.1
;   GROMOS bond-stretching parameters
;
;
;   Bond type code
;   Force constant
;   Ideal bond length
;   Examples of usage in terms of non-bonded atom types
;
;
;   ICB(H)[N]  CB[N] B0[N]
;
#define gb_1      0.1000 1.5700e+07
; H - OA      750
;
#define gb_2      0.1000 1.8700e+07
; H - N (all) 895
;
#define gb_3      0.1090 1.2300e+07
; HC - C      700
;
#define gb_4      0.112 3.7000e+07
; C - O (CO in heme) 2220
;

;   Table 2.5.3.1.
;   GROMOS bond-angle bending parameters
;
;   Bond-angle type code
;   Force constant
;   Ideal bond angle
;   Example of usage in terms of non-bonded atom types
;
;
;   ICT(H)[N]  CT[N] (T0[N])
;
#define ga_1      90.00 380.00
; NR(heme) - FE - C 90
;
#define ga_2      90.00 420.00
; NR(heme) - FE - NR(heme) 100
;
#define ga_3      96.00 405.00
; H - S - CH2 95
;

;   Table 2.5.4.1
;   GROMOS improper (harmonic) dihedral angle parameters
;
;
;   Improper dihedral-angle type code
;   Force constant
;   Ideal improper dihedral angle
;   Example of usage
;
;
;   ICQ(H)[N]  CQ[N] (Q0[N])
;
#define gi_1      0.0 167.42309
; planar groups 40
;
#define gi_2      35.26439 334.84617
; tetrahedral centres 80
```

```

;
#define gi_3      0.0  669.69235
; heme iron      160
;
;   Table 2.5.5.1 (Note: changes with respect to the 43A1 table)
;   GROMOS (trigonometric) dihedral torsional angle parameters
;
;
; Dihedral-angle type code
; Force constant
; Phase shift
; Multiplicity
; Example of usage in terms of non-bonded atom types
;
;
; ICP(H)[N] CP[N] PD[N] NP[N]
;
#define gd_1  180.000   2.67   1
; CHn-CHn-CHn-OA (sugar) 0.6
;
#define gd_2  180.000   3.41   1
; OA-CHn-OA-CHn,H (beta sugar) 0.8
;
#define gd_3  180.000   4.97   1
; OA-CHn-CHn-OA (sugar)   1.2
;
#define gd_4  180.000   5.86   1
; N-CHn-CHn-OA (lipid)    1.4
; get the constraint distances for dummy atom constructions

#include "ff_dum.itp"

[ constrainttypes ]
; now the constraints for the rigid NH3 groups
MNH3 C 2 DC_MNC1
MNH3 CH1 2 DC_MNC2
MNH3 CH2 2 DC_MNC2
MNH3 MNH3 2 DC_MNMN
; and the angle-constraints for OH and SH groups in proteins:
CH2 H 2 DC_CO
CH1 H 2 DC_CO
C H 2 DC_CO
P H 2 DC_PO

; bond-, angle- and dihedraltypes for specbonds:
[ bondtypes ]
S S 2 gb_36
NR FE 2 gb_34

[ angletypes ]
CH1 CH2 S 2 ga_16
CH2 S S 2 ga_6
CR1 NR FE 2 ga_34
NR FE NR 2 ga_17

[ dihedraltypes ]
S S 1 gd_21
NR FE 1 gd_38
CH2 S 1 gd_26

```

Force Field Files Non-bonded interaction parameter file sample (ffG53a6nb.itp)

```
[ atomtypes ]
;name at.num mass charge ptype c6 c12
O 8 0.000 0.000 A 0.0022619536 1e-06
OM 8 0.000 0.000 A 0.0022619536 7.4149321e-07
OA 8 0.000 0.000 A 0.0022619536 1.505529e-06
OE 8 0.000 0.000 A 0.0022619536 1.21e-06
OW 8 0.000 0.000 A 0.0026173456 2.634129e-06
N 7 0.000 0.000 A 0.0024364096 2.319529e-06
NT 7 0.000 0.000 A 0.0024364096 5.0625e-06
NL 7 0.000 0.000 A 0.0024364096 2.319529e-06
NR 7 0.000 0.000 A 0.0024364096 3.389281e-06
NZ 7 0.000 0.000 A 0.0024364096 2.319529e-06

[ nonbond_params ]
; i j func c6 c12
OM O 1 0.0022619536 8.611e-07
OA O 1 0.0022619536 1.38651e-06
OA OM 1 0.0022619536 2.258907e-06
OE O 1 0.0022619536 1.1e-06
OE OM 1 0.0022619536 9.4721e-07
OE OA 1 0.0022619536 1.505529e-06

[ pairtypes ]
; i j func c6 c12
O O 1 0.0022619536 7.4149321e-07
OM O 1 0.0022619536 7.4149321e-07
OM OM 1 0.0022619536 7.4149321e-07
OA O 1 0.0022619536 9.687375e-07
OA OM 1 0.0022619536 9.687375e-07
OA OA 1 0.0022619536 1.265625e-06
OE O 1 0.0022619536 9.687375e-07
OE OM 1 0.0022619536 9.687375e-07
OE OA 1 0.0022619536 1.265625e-06
OE OE 1 0.0022619536 1.265625e-06
OW O 1 0.0024331696 1.3975653e-06
OW OM 1 0.0024331696 1.3975653e-06
```