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# MOLECULAR MODELING AS A VISUALIZATION TOOL IN DESIGN OF DNA CROSSLINKED POLYACRYLAMIDE

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## ABSTRACT

Polymers such as polyacrylamide form a diverse class of biomaterials in use today. The experimental research performed by our group has demonstrated how a critical concentration of crosslinking DNA strands can lead to gel formation in the polyacrylamide. The removal or addition of DNA strands can reverse or significantly increase the stiffness and strength of the gel. DNA is a versatile material for the exploration of nanoscale structures because its hybridization chemistry is very specific. DNA crosslinked gels use end-modified DNA oligonucleotides in the gels. The ability to choose the base sequence in the DNA crosslinks offers an opportunity to engineer the nanoscale structure of this material. However, it is extremely difficult to visualize the sequence of events that occurs when DNA is crosslinked with polyacrylamide. Computer modeling is a tool that enables the researchers to study the structural aspects of the newly engineered DNA crosslinkers. In this study, polyacrylamide gel crosslinked with DNA has been assayed with respect to energy and size using AMBER 7.0 software [1].

Since DNA-crosslinked gels are likely to find a range of applications it is important to know how to tailor the gel composition for a particular application. It is also of interest to know what the composition is that would induce the greatest change in stiffness. The molecular models generated in AMBER survey the mechanical properties of the gel as a function of crosslinker density, polyacrylamide density, and crosslinker length. The structure of an equilibrium state is computed using an explicitly solvated model. Visual inspection of the model determines other mechanical properties of the gel and helps predict chemical interactions. A long-term goal of this work is to use computer assisted modeling techniques to guide the experiments, to predict linker stiffness, and to examine other mechanical properties of the DNA crosslinker.

## MATERIALS

The mechanical properties of DNA crosslinked polyacrylamide gel with respect to energy and size are characterized using Assisted Model Building with Energy Refinement (AMBER). AMBER is one of the few molecular modeling programs that is commercially available, known, and well tested for DNA study [2]. Features of this software include: (i) SANDER (Simulated Annealing with NMR-derived energy restraints), which is the basic energy minimizer and molecular dynamics program; (ii) PMEMD (Particle Mesh Ewald Molecular Dynamics) is a version of SANDER with improved performance for Linux clusters; and (iii) CARNAL is the coordinate analysis program. The design tools SYBYL (Tripos, Inc.) and MOE (Molecular Operating Environment, CCG, Inc.) are molecule-building packages that are employed to build the unknown structure of the polyacrylamide as well as the known structure of DNA.

## METHODS

The polyacrylamide, as a polymer, has not been tested extensively and is not in the Cambridge Structural Database. Because the six-carbon chain connecting the DNA to the polyacrylamide is not normally found bonded to the polyacrylamide, there are no known structures with coordinates of the compound. The molecule, therefore, has to be made from scratch using SYBYL, MOE, and AMBER. The construction of these molecules is performed on a Silicon Graphics computer.

The file of the model made in SYBYL and MOE must be converted to AMBER file formats. The polyacrylamide fragments were parameterized manually using the Amber 99 parameter set [3] and RHF/6-31G* derived partial atomic charges computed using the Gaussian 98 program [4].

Sander is the basic minimizer and molecular dynamics program in AMBER. Sander determines the minimum energies of the models, the position-restraint molecular dynamics, and molecular dynamics calculations. In all these calculations, the pressure was held constant at 1 bar, the temperature was held constant at 300 K, and the volume was altered. Minimum energies were determined for both the long and short molecules in their unstiff and stiff states. The polyacrylamide gel model is solvated with approximately $10^4$ water molecules, and neutralized with sodium ions. All of these additions greatly increase the size of the model, which increase the calculation times. Because sander can take many hours, days, even weeks to run, PMEMD is used for faster calculations for the molecular dynamics calculations on a Linux cluster.

CARNAL is the coordinate analysis program in AMBER that allows graphs to be plotted, showing the locations of equilibrium structures and distances between polyacrylamide chains. The root mean square deviation (RMSD) plots of the atoms are used to create average structures from the equilibrium points determined from the graph. These models provide an idea of what the molecular structure looks like. The distance between polyacrylamide chains and a Fourier curve fit to the RMSD data is performed to compare the stability of various DNA crosslinked polyacrylamide gel structures.

To model our experimental research setup [5] unstiff and stiff models were developed, as seen in Figure 1.

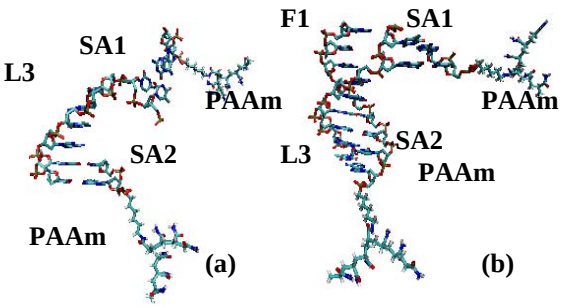


**Figure 1. (a) Polyacrylamide chains crosslinked by DNA strands. (b) The stiffening strand was added**

An unstiff and stiff short molecule was built with SA1 and SA2 lengths of 2 DNA bases, an L3 length of 8 bases, and an F1 length of 7 bases. Also an unstiff and stiff long molecule was built with SA1 and SA2 lengths of 4 DNA bases, an L3 length of 16 bases, and an F1 length of 11 bases. These simulations required run times of 5-7 days. Computations on this model are performed on several computers. Initially, the models are built and parametized on an SGI workstation at University of Medicine and Dentistry of New Jersey (UMDNJ). Then the minimizations and position-restraint molecular dynamics are calculated on a single Linux computer, also at UMDNJ. The molecular dynamics calculations are run on at least 4 Linux workstations at the School of Engineering, running in parallel to reduce the amount of time needed to perform the calculations.

## RESULTS

Average structures were created from the range of equilibrium points determined from the plot of root mean square deviations (RMSD) of the atoms.

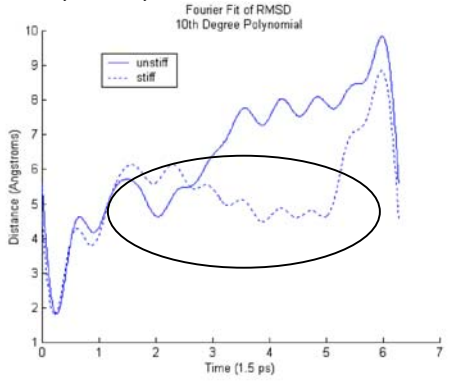


**Figure 2. Fourier analysis on unstiff and stiff molecule RMSD data**

The unstiff molecule in Figure 2 does not show any areas of stability. However, the stiff molecule shows some stability in the area within the circle. A Fourier curve fit to the RMSD is shown in Figure 2. The coefficients $a_0$, $a_1$, $a_2$, $a_3$, and $b_1$ and $b_2$ have significant differences in the oscillations. These coefficients quantify the differences between the stiff and unstiff molecules.

Distances between the polyacrylamide chains were also calculated and plotted as seen in the Figure 3 to indicate stiffness. The average distance for the long unstiff molecule was 55.7Å and 45.7Å for the long stiff molecule. This implies that the stiffness increased by 10%.

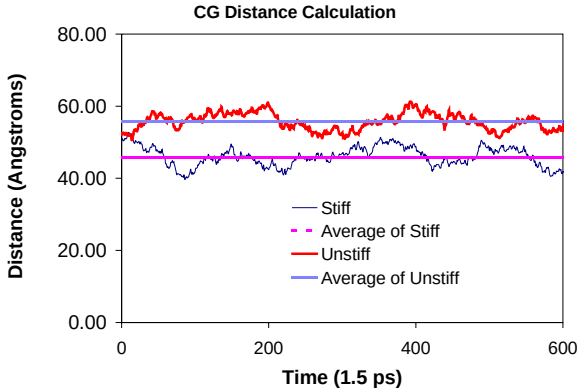


**Figure 3. Distances between Polyacrylamide Chains of the long Stiff and Unstiff Molecules.**

## CONCLUSION

For the first time polyacrylamide gel has been modeled on a molecular level. This modeling simulates the role DNA plays in the gel. The modeling provides an inexpensive design tool that allows for a convenient manipulation of the crosslinks to obtain optimum properties. The modeling saves both time and money. In the lab, the experiments take two days to run with more time needed for preparation. The modeling takes three to four days with little or no preparation. These results show that the simulations can provide a valid, predictive tool for future experiments.

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