Novel Visualization and Analysis Tools to Elucidate RNA Nanostructure Dynamics

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Abstract

Anisotropic Network Modeling (ANM) is a method for computationally modeling structural dynamics in small molecules. This type of modeling boasts a significant advantage over Molecular Dynamics simulations in that it is far faster. Molecular Dynamics simulations are sluggish in comparison to ANM, spanning multiple days, whereas ANM can be run in as little as several hours for moderately large structures like the nano-ring and nano-cube.

Two different variants of ANM are cutoff ANM and distance dependent or power ANM. The difference lies in the mechanism used in generating a connectivity matrix for the simulation. If a cutoff is used, all atoms within a set radius (the cutoff) are connected, and none outside of this radius. If a power dependency is used, all nodes are connected to one another, and the strength of the connection varies as a function of the distance between the nodes. Using different cutoff values and powers has a noticeable effect on the normal modes predicted by ANM.

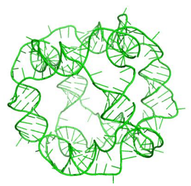
Multiple ANMs are presented for the nano-ring structure. Some of the ANMs use the cutoff method for generating the connectivity matrix, while others use the power method. Various cutoff values and powers are explored. These ANMs are compared to the principal components from a molecular dynamics trajectory for the nano-ring. The comparison involves a measure of the cumulative overlap of multiple modes spanning individual principal components, as well as pairwise overlaps between one normal mode and one principal component.

Several tools are implemented to fully automate the process of ANM simulation, a tedious process involving many separate scripts and command calls, which vary depending upon a collection of parameters assigned at the beginning of an ANM run. Included is a new mode visualizer. The automation of the ANM streamlines the process necessary to run ANM, and increases its speed advantage. This enables the extensive analysis of structures through ANM by removing the excessive time requirement for manually running ANM simulations and individually animating modes, saving variables, and managing large file structures.

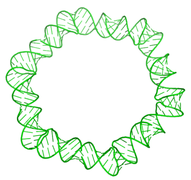
Tools are presented that were developed for enhanced visualization of normal modes and principal components. A program color codes PDB files atom by atom corresponding to b-factors calculated from eigenvectors and eigenvalues, creating a map of relative motion. Another program animates combinations of normal modes or principal components, with the option of weighing the contribution of individual eigenvectors manually, or with a list of eigenvalues. Tools for the analysis of eigenvectors are presented, including a program that measures the change in proximity of clusters of atoms across eigenvectors, and a program that searches for the transition to a target state in the eigenvectors. These programs are of potential interest for exploring structural assembly, or for identifying modes demonstrating certain interesting motifs of motion.

Modeling Nanostructure Dynamics

- Computationally modeling RNA structural dynamics is useful for predicting structural assembly potential and stability.
- Elastic Network Modeling (ENM) and Molecular Dynamics (MD) are approaches that are used for computational modeling of structural dynamics in small molecules such as protein and RNA.
- Molecular Dynamics simulations are computationally intensive, requiring extended amounts of time (measured in days or weeks) to generate results, and are currently impractical for large models, such as the nano-cube.
- Elastic Network Modeling is relatively fast, and can be completed in as little as a few hours for the nano-ring or nano-cube models.



Nano-cube

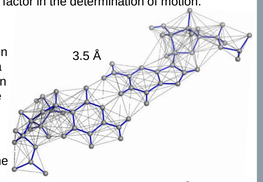


Nano-ring

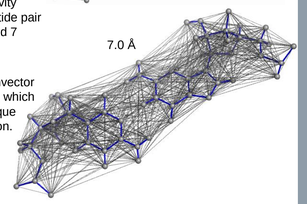
ANM and Power ANM

- Elastic Network Modeling Represents an molecule as a ball and spring network where nodes are connected to neighboring nodes by “springs” of uniform strength.
- The distance at which atoms are no longer considered to be in contact is a constant cutoff value. Applying spring connections between atoms within this cutoff radius produces a connectivity matrix.
- Because ENM is based upon a unique connectivity matrix, structural shape is the most important factor in the determination of motion.

- Created a tool for visualization of the connectivity matrix of a structure in PDB format, given an arbitrary cutoff value (see images of nucleotide pairs to the right).



- These are visualizations of the hypothetical connectivity matrices for a nucleotide pair with a cutoff of 3.5 and 7 Angstroms.

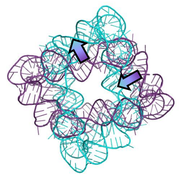


- ANM computes eigenvector and eigenvalue pairs, which each represent a unique normal mode of motion.

- Distance dependent ANM, or power ANM, is different from unaltered ANM in that it treats all nodes as connected, and varies the strength of springs between nodes as a power of the distance between them (1/distance^n).
- Power ANM runs do not use a cutoff, but instead can be performed with a varying power, thus altering the significance of distance in the weighting of spring strength.

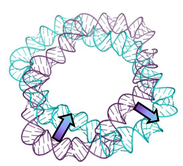
- Eigenvectors computed for an ANM can be interpreted as representing expected normal modes of motion of a structure.
- Normal modes can be visually represented by the linear transition between two structural configurations.

ANM Mode 1



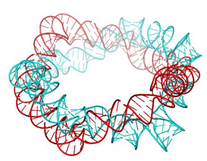
First nonzero normal mode of the nano-cube from ANM with a cutoff value of 7 Å

ANM Mode 3



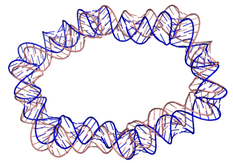
Third nonzero normal mode for nano-ring from ANM with a cutoff value of 7 Å

- Altering the cutoff of nodes connected in ANM, or altering the power used for scaling the strength of springs in power ANM, can result in significant changes in eigenvectors and eigenvalues.



Mode 7 from power ANM with powers of 2 and 3

Large differences are visible between the same normal modes with various powers.



Mode 4 from ANM with cutoffs of 6 and 8

Normal modes show some differences due to change of 2 Å in cutoff

- These differences suggest the possibility of finding an “optimal” cutoff value or power for a given model, as measured by agreement with Molecular Dynamics results, or agreement with experimental data.

Comparison of Molecular Dynamics results to ANM and Power ANM

- Principal Components calculated from a Molecular Dynamics trajectory contain eigenvectors and eigenvalues and can be compared to those from ANM simulations.
- Varying the type of ANM (cutoff-based ANM or power ANM) and the parameters of these simulations generates different eigenvectors and eigenvalues that will correlate differently to Principal Components from Molecular Dynamics.
- Cumulative overlap measures how well the first n modes can capture the motion of a single principal component, while pairwise overlaps measure how well individual modes span individual principal components.
- Presented below are cumulative and pairwise overlap comparisons between ANM , power ANM, and Principal Components from Molecular Dynamics. All of these simulations were done on the nano-ring.

Cumulative Overlaps

- Cutoff ANM shows much earlier high cumulative overlaps than power ANM. Some of the top 4 PCs require as many as 21 modes from power ANM for 90% overlap.

ANM, Cutoff 6 Å

Num. Modes	PC 1	PC 2	PC 3	PC 4
1	.7738	.3130	.2290	.4315
2	.8533	.9483	.2725	.4374
3	.9672	.9547	.2725	.9613
4	.9766	.9773	.9853	.9662
5	.9888	.9793	.9856	.9723
6	.9888	.9806	.9857	.9873

ANM, Cutoff 7 Å

Num. Modes	PC 1	PC 2	PC 3	PC 4
1	.7375	.2018	.2280	.5429
2	.7427	.9116	.4631	.5438
3	.9730	.9242	.6315	.8088
4	.9765	.9774	.9855	.9661
5	.9884	.9796	.9857	.9719
6	.9884	.9813	.9857	.9864

ANM, Cutoff 8 Å

Num. Modes	PC 1	PC 2	PC 3	PC 4
1	.1603	.8778	.4058	.0454
2	.7213	.8792	.4733	.6112
3	.9408	.9297	.6824	.7989
4	.9768	.9763	.9859	.9659
5	.9882	.9788	.9860	.9716
6	.9882	.9804	.9861	.9867

Power ANM, power 2

Num. Modes	PC 1	PC 2	PC 3	PC 4
1	.8291	.0396	.0035	.4710
2	.8359	.8971	.2690	.5288
3	.8360	.8982	.2720	.5288
4	.8360	.8993	.2730	.5542
5	.8374	.9043	.2731	.5542
6	.8472	.9048	.2731	.5576

Power ANM, power 2.5

Num. Modes	PC 1	PC 2	PC 3	PC 4
1	.8353	.1442	.0351	.4387
2	.8353	.8957	.2687	.5282
3	.8355	.8967	.2717	.5282
4	.8355	.8978	.2727	.5537
5	.8378	.9044	.2727	.5543
6	.8490	.9050	.2735	.5568

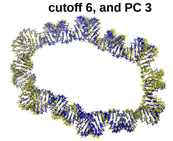
Power ANM, power 3

Num. Modes	PC 1	PC 2	PC 3	PC 4
1	.8240	.2962	.0811	.3790
2	.8354	.8947	.2675	.5269
3	.8356	.8957	.2704	.5269
4	.8356	.8967	.2714	.5528
5	.8484	.8996	.2718	.5624
6	.8764	.9299	.3137	.6381

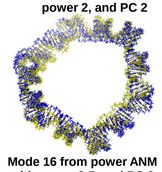
Pairwise Overlaps

- Visualizations of the best pairwise overlaps for individual normal modes and PCs. Normal modes are colored in blue, PCs in yellow.

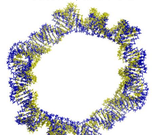
Mode 10 from ANM with cutoff 6, and PC 3



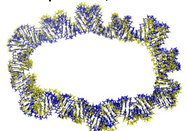
Mode 8 from power ANM with power 2, and PC 2



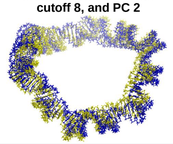
Mode 8 from ANM with cutoff 7, and PC 2



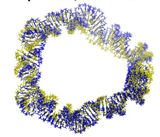
Mode 16 from power ANM with power 2.5, and PC 3



Mode 7 from ANM with cutoff 8, and PC 2

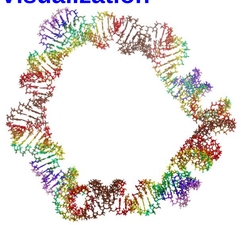


Mode 8 from power ANM with power 3, and PC 2



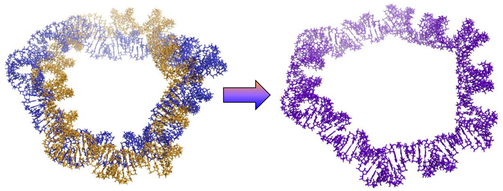
Enhanced Visualization

- B-factors, also known as Debye-Waller factors or temperature factors, are a measure of relative motion that can be calculated from the eigenvectors and eigenvalues produced by ANM, or by Principal Component Analysis of Molecular Dynamics.
- Created a tool to color the atoms of a PDB structure corresponding to a list of b-factors.
- The method in which colors are assigned can be selected. In one approach, the program attempts to create an equal number of atoms of each color (see right). Another alternative is to use a linear scale for coloration.



Mode 7 from ANM with cutoff of 7 Å

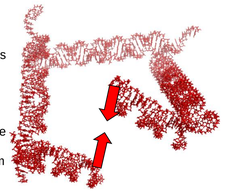
- Created a tool to visualize multiple eigenvectors. The program uses the animation method of the perl mode visualizer (see ANM Scripting).
- The vectors used for the animation can be either a simple mean of multiple eigenvectors, or eigenvectors can be weighted corresponding to their eigenvalues.
- The resulting animation will represent the motions of two or more normal modes or principal components.



Modes 8 and 9 from ANM with cutoff 7 Å | **Combined modes 8 and 9**

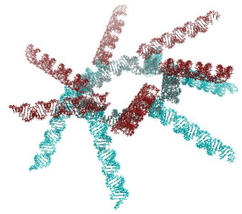
Tools for Analysis

- Created tool to measure proximity of clusters of atoms, and organize eigenvectors by how well they bring those atoms together or apart.
- Can be utilized to search for modes of motion or principal components that suggest structural assembly, such as the closing of the tectosquare (found in the second mode from ANM with cutoff 7 Å



Open Tectosquare

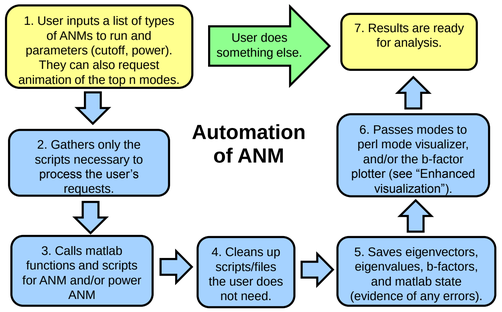
- Created a tool to look for transition between certain configurations or states of molecules in eigenvectors.
- One possible application includes looking for modes of motion or principal components showing transition between various arm orientations of the nano-rings with attached siRNA arms.



Overlay of two nano-ring models with different siRNA arm orientations.

Scripting of ANM and Improved Mode Visualizer

- The main attraction of Anisotropic Network Modeling is the significant increase in speed it offers when compared to Molecular Dynamics.
- Scripted the entire process of both ANM and power ANM from start to finish. This optimizes the speed advantage of these methods. The total autonomy allows the user to skip straight to analysis of results rather than manage the multiple steps of ANM and power ANM.



- Created a mode visualizer for animating eigenvectors. The new mode visualizer uses perl, and demonstrates a dramatic increase in efficiency over the modeAnimator function previously used, which is a MATLAB function.
- The new mode visualizer can animate a normal mode of the nano-ring in seconds, while the MATLAB version requires 5-10 minutes. This speed increase allows for the animation of a large number of modes without a significant addition to computation time.
- The animation of eigenvectors is performed by slowly transitioning a structure’s atoms to new locations along a vector, whose direction and relative magnitude is determined by the eigenvector. An arbitrary scaling factor is applied to the transition.
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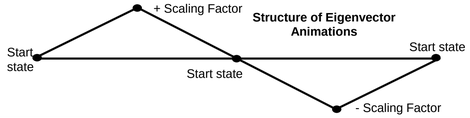

Conclusions

Anisotropic Network Modeling can produce eigenvectors with a significant level of agreement with principal components from molecular dynamics. The speed of ANM makes it an attractive alternative for representing structural dynamics.

The cutoff method of generating the ANM connectivity matrix generates eigenvectors with significantly more rapid cumulative overlaps than the distance dependent method. All types of ANM are capable of generating some normal modes that resemble principal components, as measured by pairwise overlaps.

Scripting and streamlining the process of ANM from a single starting state to analysis will result in increased readiness of ANM as a tool for rapid characterization of structural dynamics. Tools for analysis of eigenvectors that can look for visually defined motifs of motion will further increase the usefulness of ANM in evaluating structural assembly potential.

Effective and rapid visualization of normal modes is a critical issue in accelerating the process of analysis using ANM. Solutions for enhancing visualization are making the task of interpreting ANM data more accessible.

References

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