Molecular Model of Human Tropoelastin

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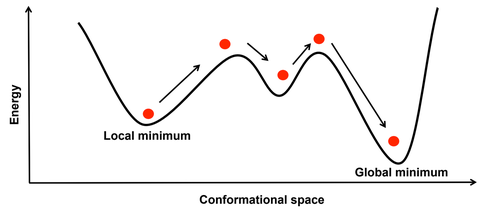
Abstract

Tropoelastin is the molecular precursor to the elastin polymer, a key structural component of elastic fibers, which confer elasticity and recoil to tissues. In recent years, numerous studies have focused on the structure and assembly of tropoelastin into elastic fibers. Yet, an atomistic structure of tropoelastin has evaded researchers because of the large size and dynamic nature of the molecule that has precluded more traditional approaches such as X-ray crystallography. Thus, an alternative path is needed to understand the molecule's detailed features. In this work, we predict a full-atomistic structure of the tropoelastin molecule, through one of the largest molecular dynamics protein-folding simulations to date, finding good agreement with the nanostructure determined by small angle X-ray scattering, secondary structure, and recent description of the molecule's dynamics. We consider two artificial tropoelastin mutations within key regions of the molecule, R515A and D72A, in comparison to the human wild-type isoform. We propose mechanisms to explain structural differences and develop an elastic network model based on the full-atomistic structure to predict the molecule's dynamics and explain differences in hierarchical assembly. This study and the molecular model serve as a template for studying elastin mechanics, assembly, and disease, yielding insight into molecular mechanisms behind these processes.

Methods

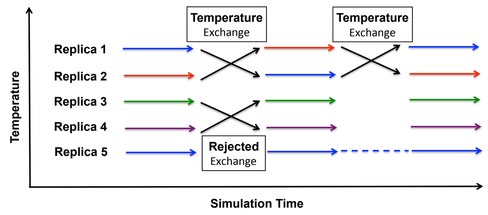
Limitations of classical molecular dynamics

- Molecular Dynamics (MD) methods are employed to study atomistic structure
- Classical MD is limiting as structures may remain in a local free energy minimum state, unable to overcome the energy barrier required to achieve the global minimum in conformational space



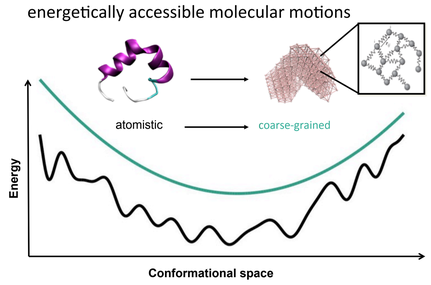
Replica exchange molecular dynamics (REMD)

- To mitigate these effects, REMD is used
- Each temperature replica travels in temperature space governed by the Metropolis criterion
- High temperatures allow for broad conformational space sampling
- Low temperatures provide structural stability and the ensemble for sampling



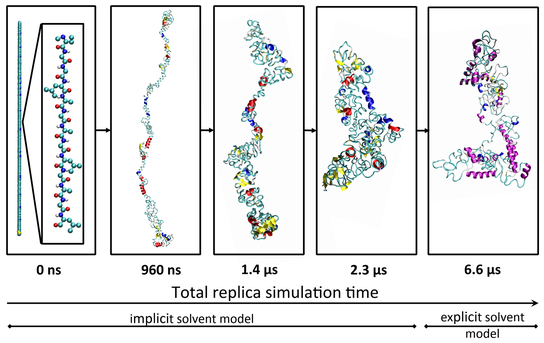
Normal mode analysis

- NMA is used to study motions of large molecular domains not accessible by MD
- The molecular system is simplified to a coarse-grained description
- Energy landscape is flattened to harmonic
- Normal modes represent oscillations around an energy minimum
- Lowest frequency modes correspond to the most energetically accessible molecular motions



Results

Structure Prediction

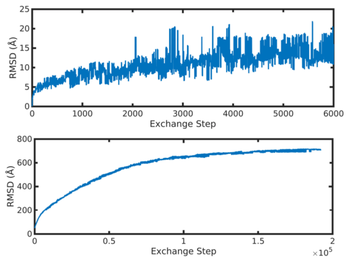


Implicit solvent phase:

- 24 replicas were exponentially distributed
- Temperature range 280 to 480 K
- Exchanges attempted every 0.5 ps to allow for system relaxation
- Each replica was simulated for 96 ns, for a total simulation time of 2.3 µs
- CHARMM19 all-atom energy function with the EEF1 force field was used

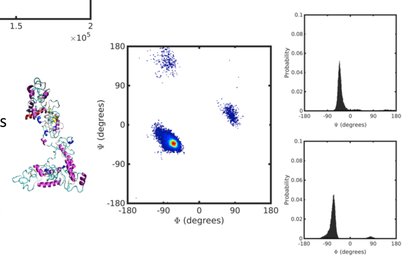
Explicit solvent phase:

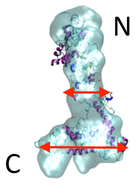
- 358 replicas
- Temperature range 280 to 500 K
- Exchanges attempted every 2 ps
- Each replica was simulated for 12 ns, for a total simulation time of 4.3 µs

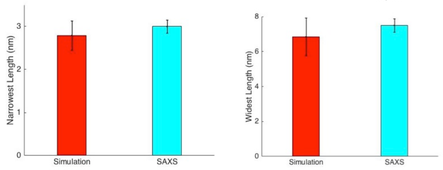


- Folding process is shown above
- Root mean square deviation (RMSD) from extended chain is shown for structures at 300 K for the implicit (left, top) and explicit (left, bottom) solvent simulation phases

- Dihedral angle distributions for each domain show matches with literature values
- Domain 15 is highlighted in red (right), showing a high content of alpha helix, polyproline II and coil regions

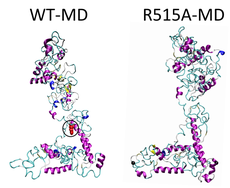


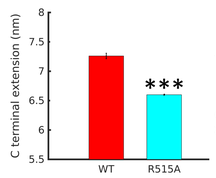


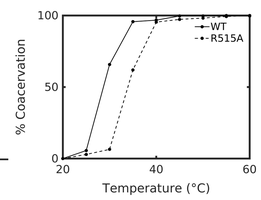


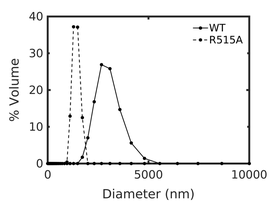
- There is a close dimensional match between SAXS and atomistic models
- Both show an extended molecular body, with two protruding legs

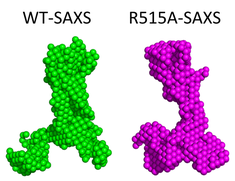
Mutation Studies

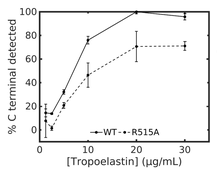


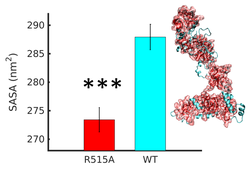


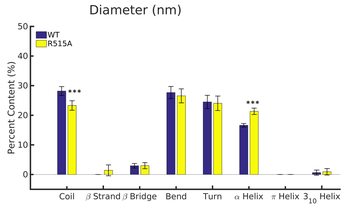




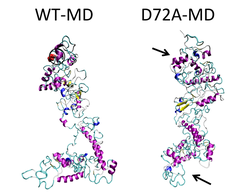


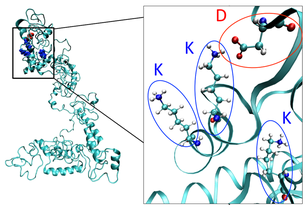


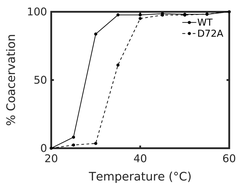


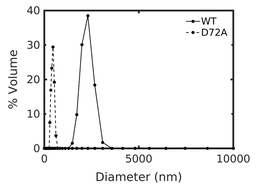


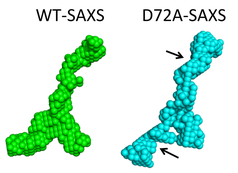
- Arginine 515 is highly conserved in mammalian species suggesting structural importance
- To explore this role, an alanine mutation is inserted to artificially mute the effect of the large positively charged side-chain
- SAXS and MD models show a qualitative match
- In experiment, reduction of C-terminal detection by antibody, coacervation and particle size in solution is observed for the mutant
- At the molecular scale there is a 9% decrease in the extension of the foot region, which can be explained by the formation of more compact alpha helices
- Hydrophobic solvent accessible surface area (SASA), driving assembly, is lower for the mutant

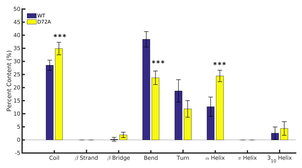


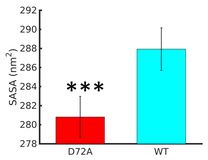






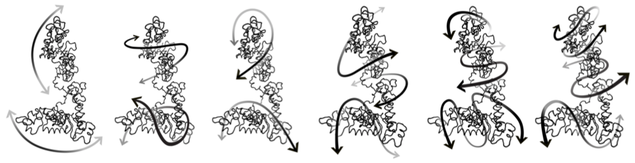




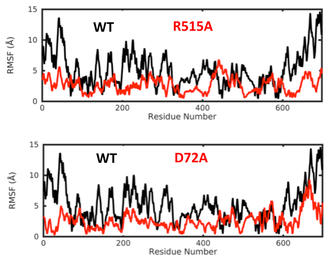


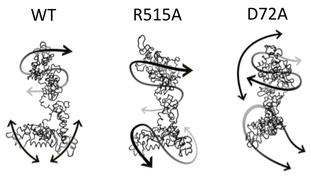
- Aspartate 72 is the only negatively charged residue in the upper half of the molecule
- Its effect is considered by inserting the neutral alanine residue
- In both SAXS and MD models a twist appears in the upper region of the molecule with a downward shift of the C terminal region
- Aspartate forms a salt bridge with three neighboring lysines over 50% of the time
- The absence of the stabilizing salt bridge in the mutant explains why the upper region of the molecule is free to twist
- The disrupted helix in the vicinity propagates the structural change down the molecule
- Coacervation and solution particle size reduction is again associated with reduced hydrophobic solvent accessible surface area (SASA)

Dynamics and Assembly



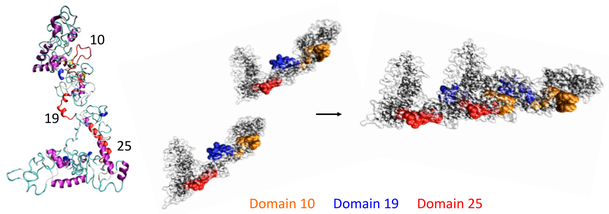
- Collective thermally-driven molecular motions are represented by low frequency normal modes intrinsically accessible to the molecule
- Six lowest-frequency modes are shown above (left to right).





- The root mean square fluctuation (RMSF) shows that the WT molecule is more dynamic than either mutant
- We propose that the elevated level of mobility in the WT tropoelastin predisposes it more strongly than either mutant to intermolecular assembly

- A linear combination of the first six lowest frequency modes, scaled by the modal amplitude, describes expected molecular motions dominating the molecule
- WT dynamics are characterized by a twist in the N-terminal region of the molecule and a scissors-like motion about the bridge region
- Both the R515A and D72A mutants display bending motions, foregoing the scissors-like movement, which may explain reduced propensity for assembly



- Interactions between domains 10, 19 and 25 are central to the aggregation and polymerization of tropoelastin
- The positioning of domains 10, 19 and 25 in WT tropoelastin enables head-to-tail assembly of tropoelastin molecules, as proposed by Baldock *et. al.*

References

G. C. Yeo, A. Tarakanova, C. Baldock, S. G. Wise, M. J. Buehler, and A. S. Weiss, "Subtle balance of tropoelastin molecular shape and flexibility regulates dynamics and hierarchical assembly," *Science Advances,* 2016.
G. C. Yeo, C. Baldock, S. G. Wise, and A. S. Weiss, "A negatively charged residue stabilizes the tropoelastin N-terminal region for elastic fiber assembly," *J Biol Chem,* 2014.
G. C. Yeo, C. Baldock, A. Tuukkanen, M. Roessle, L. B. Dyksterhuis, S. G. Wise, *et al.*, "Tropoelastin bridge region positions the cell-interactive C terminus and contributes to elastic fiber assembly," *PNAS*, 2012.
C. Baldock, A. F. Oberhauser, L. Ma, D. Lammie, V. Siegler, S. M. Mithieux, *et al.*, "Shape of tropoelastin, the highly extensible protein that controls human tissue elasticity," *PNAS,* 2011.

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