UNDERSTANDING FIBRIN PROTOFIBRILS AT THE MOLECULAR LEVEL:

Formation and Flexibility Through a New Coarse-Grained Protein Modelling Approach

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Introduction

The process of fibrin clot formation, together with the mechanical properties that arise from its topology are subjects of great biomedical interest. Despite recent experimental advances, the limitations in our knowledge of the behaviour of these structures at the molecular level prevents us from fully understanding clot formation and from designing drug treatments for associated diseases.

Computer simulations using atomistic molecular dynamics are well established as a theoretical tool for structural characterisation which are complementary to experimental studies, but the high computational expense of the calculations means that simulations of fibrin aggregation are unfeasible, even with modern supercomputers.

To overcome this limitation, we have developed a new coarse-grained model for proteins known as **FFEA** (Fluctuating Finite Element Analysis) which treats proteins as a non-rigid continuum subjected to thermal fluctuations.[1]

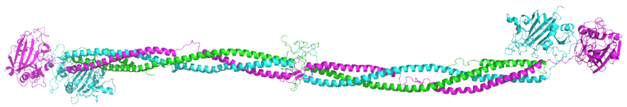
Objectives

This project aims to simulate the early stages of fibrin clot formation while developing new coarse-grained models of general applicability. So far, we have worked on:

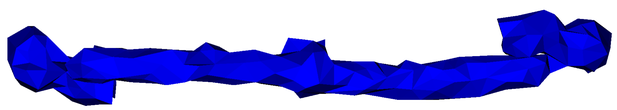
- Parametrisation the stiffness of the monomer.
- Parametrisation the interaction between the β nodule and the coiled-coil-D domain.
- Checks that the methodology lead to experimentally consistent results.

A Tetrahedral Mesh

Within the FFEA model, molecules are described using a tetrahedral mesh. In our case, we started with the known atomic crystal structure description of the fibrin monomer:

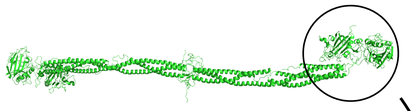


We calculated its Van der Waals volume, and prepared a mesh paying special care to allow the movement of the β/γ-nodules with respect to the coiled-coil connector:

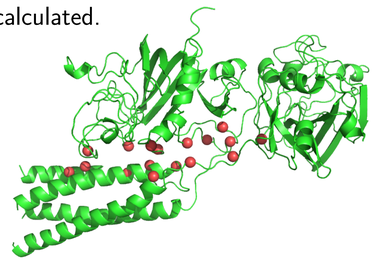


Bottom-up parameterisation of the interactions

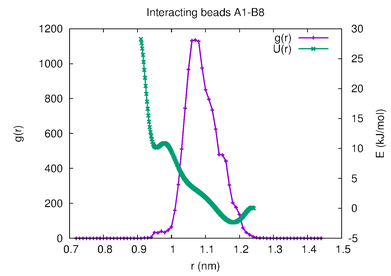
We are currently focusing on describing the interaction between the β-nodule and the coiled-coil-D domain.



After getting an all-atom molecular dynamics trajectory, the interface residues are grouped into beads, and the forces recalculated.

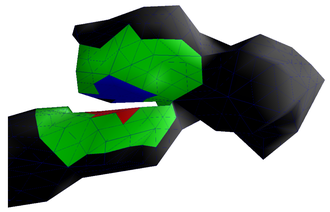


A Force-Match method[2] allows us to calculate the pair CG force profile for each bead pair. Then, potential energy functions can be obtained through numeric integration.



The potential between two beads, one in the β-nodule the other in the coiled-coil domain shows a small attraction well.

Analogous to the "atom types" in standard molecular dynamics, the FFEA model uses "face types". Hence, bead-bead pair potentials are translated into surface potentials.



Different colours standing for different interaction types, in the β-nodule and coiled-coil-D domains.

The low computational cost of the FFEA description allows the simulation of large systems for long times.

Our approach still requires the addition of restraints between monomers to simulate protofibrils. Soon, these will be replaced with sequence-dependent interactions, opening the door to study pathological mutations.

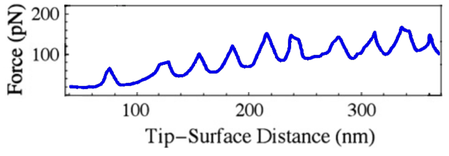
A protofibril in the FFEA framework

Molecular Flexibility

The stiffness of the molecules in the FFEA model is defined through the **Young's modulus**:

$$E = \frac{L_o dF}{A_o dL}$$

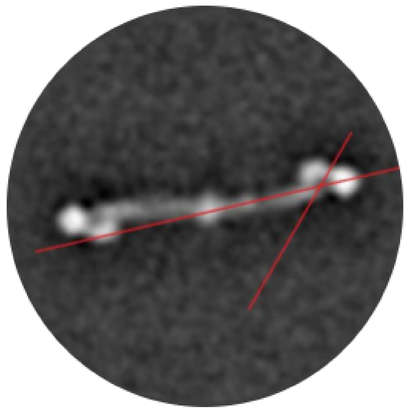
where L_o is the original length, A_o is the cross-sectional area through which the force is applied and F and L are force and length. Experimental data coming from forced unfolding of coiled-coil regions through AFM single-molecule experiments[3] allows us to estimate dF/dL:



Forced unfolding of a fibrin pentamer through AFM[3]

while A_o was calculated as the cross-sectional area of a cylinder of the same volume and height as the tubular VdW volume of the coiled coil region.

Experimental Information



Negative-stain electron microscopy experiments reveal different orientations of the β/γ nodules with respect to the coiled-coil region.

Constant communication between theory and experiments allows us to ensure that the coarse-grain methodology under development will describe precisely the dynamics of the fibrin monomer. Once this milestone is accomplished, we will work on modelling the effects of flow.

References

[1]R. C. Oliver et al., J. Comput. Phys. **239**, 147 (2013).

[2]W. G. Noid et al., J. Chem. Phys. **128**, 244114 (2008).

[3]A. E. X. Brown et al., Biophys. J. **92**, L39 (2007).

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