Evaluating the flexibility of the formin FH1 domain

Background and Significance: Actin cytoskeletal networks are necessary for various essential cell processes, such as cytokinesis, cell migration, cell adhesion, filopodia formation, yeast bud polarity, and endocytosis.[1] The formin family of proteins are critical for actin filament assembly and remodeling,[2] and mutations in the formin proteins can lead to the development and metastasis of cancer cells, among other human diseases such as focal segmental glomerulosclerosis and Charcot-Marie-Tooth disease.[3,4]

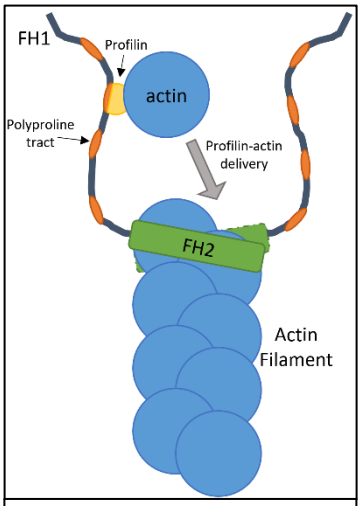

Figure 1. Mechanism of formin-mediated actin polymerization.

Formins contain two domains, Formin Homology 1 and 2 (FH1 and FH2), which nucleate and elongate actin filaments by delivering profilin-actin to the barbed "growing" end while remaining processively attached to the filament.[2] The FH2 domain is a homodimeric complex that associates with the barbed end of the actin filament, while the FH1 domain is flexible and contains two or more stretches of polyproline residues and lies N-terminal to the FH2 domain.[2,5,6] The polyproline tracts in the FH1 domain bind profilin, an actin-binding protein, and facilitate elongation of the actin filament by bringing the profilin-actin complex to the barbed end (Fig. 1).[2,5,6] While the structure of the FH2 domain has been determined by x-ray crystallography,[7] the FH1 domain contains a low-complexity sequence and is thought to be disordered, aside from the polyproline tracts, and thus remains difficult to study via traditional methods.[8-10]

However, the FH1 domain is key to actin filament elongation because barbed ends associated with only the FH2 domain elongate more slowly than completely unbound filaments.[6] Paul and Pollard[6] proposed that each polyproline tract independently binds profilin-actin, and the

elongation rate increases with the number of polyproline tracts in the FH1 domain. Characterizing the structure of the FH1 domain and elucidating the effect of profilin binding are key to both generating rigorous models of formin-mediated actin filament elongation and better understanding how formins regulate a multitude of integral cell processes. Specifically, it will be useful to determine the effect of profilin binding on the overall flexibility of the FH1 domain as the flexibility of the domain may modulate delivery of profilin-actin.

Hypothesis: I expect that binding of profilin causes the FH1 domain to become more rigid and less compact, thus, I hypothesize that the end-to-end distance of the FH1 domain will increase in the presence of profilin compared to the distance without profilin.

Specific Aims: *Aim 1*. Measure the end-to-end distance of variants of the FH1 domain of Bni1. I will measure the fluorescence emission spectra from donor alone and donor plus acceptor constructs using fluorescent protein Főrster Resonance Energy Transfer (FRET), and determine the distance spanned by the FH1 domain using the Főrster equation.

Aim 2. Measure the changes in the end-to-end distance of Bni1 FH1 variants in the presence of profilin. I will compare the distance measurements of the FH1 domain with profilin present to the measurements obtained in Aim 1.

Research Plan: *Aim 1*. Bni1 is a well-characterized formin from budding yeast, a model organism.[11] Furthermore, it has an average polymerization rate compared to other formins,[12] and the FH1 domain specifically contains four polyproline tracts, making it particularly suited for our study.[9] I will purify a series of recombinant deletion constructs of the FH1 domain of Bni1 expressed in BL21 *E. coli* that contain terminal fluorescent reporter proteins which will enable me to measure the end-to-end distance of the FH1 domain using FRET.[13] The "donor alone" construct consists of mCerulean on the C-terminus of the FH1 domain, and a non-fluorescent

molecule, SUMO, on the N-terminus, while the experimental constructs contain mCerulean on the C-terminus of the FH1 domain, and mCitrine on the N-terminus (Fig. 2).

FRET measures the distance between two fluorescent proteins, a donor and an acceptor, based on the principles of resonance energy transfer and fluorescence quenching.[13] Using FRET and fluorescence spectroscopy, I will measure the fluorescence emission spectra of the donor plus acceptor and donor alone constructs. The distance between the fluorescent probes, *r*, will be calculated from the difference between the emission spectra using the Förster equation.[13]

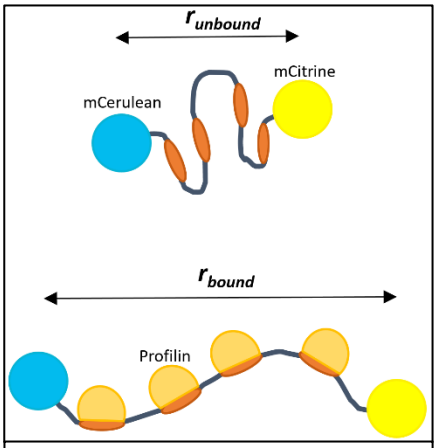

Figure 2. Schematic of FH1 domain constructs for FRET measurements, and expected change upon profilin binding.

Aim 2. I will then measure the fluorescence emission spectra and calculate the end-to-end distance of the constructs in the presence of profilin with the same techniques, and compare the data with measurements from Aim 1. We will use the distances to evaluate changes in the length of the FH1 domain with and without profilin present, and the results can be compared to general predictions of disordered protein structures.[14]

Expected Outcomes: If my hypothesis is correct, the calculated distance *r* will be larger with profilin present compared to the no profilin condition, indicating that binding of profilin decreases the flexibility of the FH1 domain. Ultimately, this research will aid in greater understanding and more accurate modelling of formin-mediated actin filament elongation, a critical process in myriad cell structures and processes. The results of this project will be presented at a lab meeting, at the Undergraduate Research Symposium on a poster, or written in a full-length scientific paper. My results will be compared to data obtained using different methodologies in the future, including time-resolved fluorescence and dynamic light scattering.

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