

7.18 Day 7 Agenda

- □ introduction/background and significance

Introduction/Background & Significance

- □ provides context, focus, and justification of the project
- □ gives critical background information of the main elements of the project (e.g., disease, genes, protein) for the non-expert reader
- □ concisely reviews and stresses importance of past primary research papers most relevant to the project
- □ highlights deficiencies in current knowledge or understanding
- □ shows how references provide intellectual foundation of the project
- □ tell a story: start with the general area and questions, then narrow to the topic area

Example of an Introduction

Text of example introduction removed. Please see the Introduction for the following paper:

Chatelain, F. C., N. Alagem, Q. Xu, R. Pancaroglu, E. Reuveny, and D. L. Minor, Jr. “The pore helix dipole has a minor role in inward rectifier channel function.” *Neuron* 47, no. 6 (Sep 15, 2005): 833-43.

- This introduction provides background information about the state of knowledge about potassium channels, ion selectivity, role of pore helix in ion selectivity.
- Introduction does a nice job of going from the general to the specific, in about 2 double-spaced pages:
 - First paragraph: ion channels, role in electrical signaling
 - Second paragraph: potassium channels, ion selectivity
 - Third paragraph: pore helix
- The introduction also shows how references provide an intellectual foundation for their experiments.

Background and Significance

Briefly sketch the background leading to the present application, critically evaluate existing knowledge, and specifically identify the gaps that the project is intended to fill. State concisely the importance and health relevance of the research described in this application by relating the specific aims to the broad, long-term objectives. If the aims of the application are achieved, state how scientific knowledge or clinical practice will be advanced. Describe the effect of these studies on the concepts, methods, technologies, treatments, services or preventative interventions that drive this field. *Two to three pages are recommended.*

<http://grants2.nih.gov/grants/funding/phs398/instructions2/phs398instructions.htm>

- These are the instructions taken directly from PHS 398: Public Health Service Grant Application.
- The Office of Extramural Research (OER) is the focal point for NIH medical and behavioral research grant policies, guidelines and funding opportunities.

An example of Background & Significance

Our primary goal is to understand how the cell stores and uses energy by twisting and untwisting an actin filament. Using a combination of structural, biochemical, biophysical, and molecular biology approaches, we will continue our structural and biochemical studies on the acrosomal process of *Limulus* sperm and the energetics of the reaction. As background, this section will describe a poorly understood class of cellular engines, molecular springs, and then discuss how actin filaments power the extension of the *Limulus* sperm acrosome using a spring-type of mechanism.

Molecular Springs Among the types of cellular engines, molecular motors and ratchets are the best understood (17). Molecular motors include the cytoskeletal proteins: myosin, kinesin, and dynein and the membrane rotary motors such as the bacterial flagella rotor (4,39). Linear and rotary motors convert the energy derived from ATP or a membrane potential into movement—either a stepping along a filament or ATPase subunits. In the absence of an external energy source, there is no motion. Polymerization also performs work by pushing a membrane forward. According to the Brownian ratchet model, thermal energy causes a filament to fluctuate, permitting addition of a subunit to the end (35). The mass of unpolymerized subunits drives the reaction like the imbalance of ions forms a membrane potential (13).

Spring-like properties of individual proteins play widely diverse roles in proteins. A spring-loaded mechanism involving the formation of a coiled-coil segment may be responsible for positioning fusion peptides at the membrane (6). A major contribution to the elastic force in muscle is thought to be due to the spring-like titin molecule. Optical tweezer and AFM measurements show that titin extensibility arises from modular domains which unfold at moderate to high (20-300 pN) forces (21,38). In this case, the resting sarcomere length is restored by titin after contraction.

While individual proteins maybe elastic, they do not generate large movements like a crawling cell. However, the fastest and most powerful movements are caused by supramolecular springs made of bundles of a protein polymer. For example, the contractile stalk of *Vorticella* contracts several mm in several msec when the cell body is agitated (19). On a power-to-weight basis, the contractile stalk is ten-fold more efficient an engine than a gas-powered automobile engine and an ATP-fueled muscle and two orders-of-magnitude more efficient than the polymerization-drive Thyone acrosome reaction (17). In contrast to other types of molecular motors, contraction occur in the absence of nucleotide hydrolysis because glycerinated cells contract when exogenous calcium is added. Calcium binds a calmodulin-like centrin-homolog, spasmin, and the expanded lattice of filaments contract. Although the major protein in the stalk, spasmin does not form filaments and the identity of the filament protein is undetermined (20). Although a component of contractile fibers associated with the oral apparatus of protozoa and positioning the nucleus (40), centrin, like spasmin, has not been shown to polymerize. However, centrin is implicated in centriole reproduction (23).

- Example is from Professor Paul Matsudaira's NIH grant. (NIH grant proposal. Courtesy of P. Matsudaira. Used with permission.)
- Introduces the concept that elastic energy is used to cause motion.
- The three sections discuss the acrosome reaction which is the cellular event that we study, the structure of the actin bundle, and the structure of the scruin protein.
- The significance is that the project seeks to answer unsolved mechanisms of motility, describes a novel class of cellular engines, and generalizes the study to biological springs.

Assignments

Due Tuesday, 4 Oct:

- Preliminary figures and tables
- Critiques of Journal Club papers (Lincoln, Tiffany)

Due Thursday, 6 Oct:

- 2-page introduction/background & significance
 - full citation (author, year) & references
 - Annotated references: 20+ references, with a sentence or two highlighting the importance of the paper to the field