1 Introduction/Background/Motivation

Traditionally, studies of protein function have gone hand-in-hand with studies of protein structure. Proteins such as hemoglobin exhibit complicated behavior, such as cooperativity, by modifying their structure. The cooperative transition of hemoglobin from a closed to open state is well studied with the aid of crystallography and other structure elucidation tools. CITE

Of more recent interest is the behavior of molecules that cannot be crystallized. These proteins lack a defined structure and instead are capable of assuming many different conformations. Although examples of intrinsically disordered proteins (IDPs) have been reported since the 1970s, it was only in the past two decades that they became a focus of major research. [**Dunker2008**]

As our understanding of disordered proteins develops, so too will our understanding of a variety of cellular behaviors. These studies will elucidate aspects of signaling, cytoskeleton formation, clustered reactions, and really. I need a fourth function.. Investigations into how disordered proteins mediate each of these processes may lead to new drug targets or introduce new directions for synthetic biology.

1.1 Intrinsically Disordered Proteins

Studies have shown that disordered proteins or disordered domains are present in at least 40% of human proteins, including those involved with signal propagation.[**Tompa2012**] General functions of IDPs include as tethers between two globular domains cite, receptor subunits in signaling pathways cite, tethers to the membrane cite, and facilitators to actin polymerization cite - formin. Given the ubiquitous nature of IDPs, many questions arise: How does their existence influence cellular functions such as biochemical reactions, signaling networks, or cytoskeletal structure? Are there any benefits to being disordered over structured? and Can IDPs exhibit the same complicated behavior as structured proteins?

Paragraph focusing on disordered proteins in signaling pathways. E.g. CD28, CD3zeta, etc

1.2 T Cell Receptor Zeta Chain

One example of an intrinsically disordered protein is the CD3 ζ chain, one of six disordered chains composing the T Cell Receptor (TCR) intracellular region. This molecule facilitates signal propagation in the TCR network in the immune system. An antigen binding to the extracellular regions of the TCR creates a signal transmitted via a chain of events into the cell to the intracellular components of the TCR including the CD3ζ chain. CD3ζ undergoes multiple phosphorylation by kinase Lck before another molecule, ZAP-70 can attach and propagate the signal downstream. This pathway ultimately regulates T-cell cell fate decisions through cytokines production, (i.e. interleukin-2). Cite - Cell Signal.com?? Presumably I need to cite all of this somewhere?

Experiments with a reconstituted mouse CD3ζ dimer have shown that the number of tyrosines impacts the potency and maximum phosphorylation but not the switch-like response reword [**Mukhopadhyay2016**]. Initial differential equation models indicate to achieve these characteristics, there would need to be a phosphorylation-dependent enhancement of more than 100-fold. That is, the sixth phosphorylation event would be at least 100-fold faster than the first phosphorylation event. [**Mukhopadhyay2016**]

Questions:

Their ODE model is single chain, right?

Is a single zeta chain sufficient for signalling?

Mukhop. et al says a single ITAM phos is technically sufficient, but has it been shown that it will?

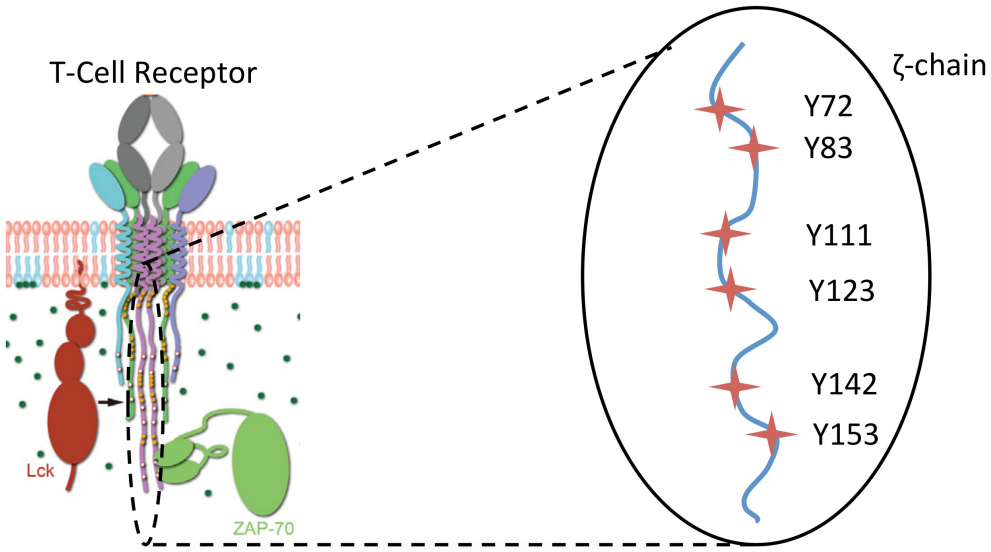

Figure 1: Cartoon of T Cell Receptor network (left) from [**Wu2015**]. Location of tyrosines in single ζ chain (right).

1.3 Multisite Phosphorylation

Stuff about switch-like responses

Multisite phosphorylation specifically is a well-studied post-translational modification. This phenomenon occurs on both structured and unstructured proteins in many cell systems. CITE CITE CITE In signaling pathways, multisite phosphorylation often creates ultrasensitivity. cite, anything else? Ultrasensitivity creating a strong response from intermediate signals while reducing the influence of noise. CD3ζ - does give ultrasensitivity and we know it or it doesn't and we know it or we don't know? We want to explore if and how multisite phosporylation of disordered proteins conveys similar signaling functions.

1.4 Previous Modeling Attempts - FJC as Model for IDPs

Disordered proteins are commonly represented with models from polymer physics.examples of where this is actually true cite{VanValen2009} The distribution of end-to-end distances for disordered domains matches a worm-like chain (WLC) model with persistence length 3.04Å. This suggests $l_k = 0.6$nm....which is not what we use...not sure how to explain that one.Seriously - even in cite{Zhou2001} Models of disordered proteins freely-jointed chain (FJC) and WLC converge in the thermodynamic limit, with the persistence length for the WLC as half the Kuhn length used for the FJC. cite Something

Could explain why those models were developed - what are they good at describing

Alternative models for multisite phosphorylation of IDPs include molecular dynamic, ordinary differential equations, or particle based models. Cite times when each of these has been used However, FJC or other 'mesoscale' approaches reach timescales on the order of microseconds to seconds, which are computationally out of reach for traditional atomic scale MD. Cite time scales? This approach also allows us to capture the steric effects of a disordered chain, which are missed by coarser models. Do I need to cite stuff here?

Representations of disordered proteins as freely-jointed chains have already been used to elucidate properties of IDPs. The disordered molecule, formin, captures profilin-actin monomers and delivers them to the growing

end of actin, increasing the actin polymerization rate. In experimental studies of formin, exerting force on the distal end of formin counterintuitively enhances the polymerization rate two-fold over relaxed formins. [**Jegou2013**] Models of formin as a freely-jointed chain offer an explanation for this phenomenon. A force exerted on a freely-jointed chain extends the polymer, increasing capture of profilin-actin by increasing the availabiltiy of binding sites. This increase of capture rate balances the reduction in delivery rate to have a net positive impact on the actin polymerization rate. [**Bryant2017**]

talk about initial models of TCR - aka your part in Mukhopadhyay 2016?

1.5 The Big Question

Since IDPs lack a consistent, rigid structure, they must fluctuate between multiple conformations. If we assume IDPs have no structure at all, then the protein may be in any conformation at any time and in fact would sample all of these conformations. This high degree of structure variability makes ligand binding to a specific binding site more challenging since it is now possible for the protein to transiently block its own binding site. That is, there will be conformations where the region around the binding site is occupied by other segments of the protein, preventing a ligand from occupying that space. In order to explore if and how disordered proteins can provide complex signaling behavior to a network, we create a model of a simple disordered protein using principles from polymer physics.