DIFFUSION AND MOLECULAR ASSOCIATION IN ARTIFICIAL PROTEIN HYDROGELS

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To Kara:

Some bonds are not reversible.

ACKNOWLEDGEMENTS

First and before anyone else, to my wife Kara: you are without equal. You handle all seasons, situations and stress levels of your husband with stability, grace, warmth, and encouragement. Without you I am nothing. Likening our marriage to the midblock of a telechelic polymer, I am the dangling chain end and you keep me tethered to the network called home, saving me from the perilous free state of bachelordom. An asymmetric distribution of labor permits me to still indulge in these itinerant musings.

I feel tremendously grateful for the opportunity to have studied at Caltech, a place that I believe stands as one of the best institutes dedicated to knowledge and scientific discovery of the most rigorous and fundamental kind. This institute is made truly remarkable by its people, many of whom have shaped me in meaningful ways that I did not expect. This short list of some of them does not sufficiently express the appreciation, admiration and respect that I have for them and many others who will go unmentioned.

David Tirrell has been an unforgettable advisor, scientific role model, and friend. He is the best kind of mentor and scientist there is: humble, creative, patient, easy to please and impossible to satisfy. He has always cheerfully supported my impulsive experimental undertakings, and has always been there when I needed him. None other than Jesus Himself said that “a student is not above his teacher, but everyone when he is fully trained will be like his teacher”. If the latter is at all applicable to my present situation then I am very lucky.

Among those who contributed directly to this work, Ahmad Omar has been a wonderful contributor, and I am indebted to him for his deep theoretical insights. It is thanks to Dave’s

patience, and Ahmad's rigor, that the novel theory presented in Chapter 2 was born. Bradley Silverman was an enthusiastic contributor to many experimental aspects of the FRAP work, and the data-sets we generated together were so large that I literally could not have examined them had he not also written several powerful scripts for high-throughput data processing. Maren Buck was my first post-doctoral mentor when I joined the lab, and without realizing it, she single-handedly defined the entire course of my Ph. D. by one day suggesting that I try labeling one of her proteins with fluorescein and photobleaching it. 5 plus years and 500 plus FRAP traces later, I remain grateful.

Each of my committee members has also played a central role in my scientific formation. Zhen-Gang Wang gave me a deep love for thermodynamics and polymer physics (maybe even statistical mechanics?), and has been a generous contributor both in terms of time and insight. Sarkis Mazmanian got me hooked on mucosal immunology, an addiction that I still have not yet managed to kick. I am thankful for the risk he took with me several years ago, allowing me to join his lab and collaborate so closely. None of our exciting and challenging work together has been reported here, but I am hopeful that some of it will be reported soon. Mark Davis has given valuable advice at many times, and his encouragement to explore the temperature dependence of the chain diffusivity led to the serendipitous discovery of the phase behavior described in Chapter 3.

So much more could said, but the key point is that one is born hungry for knowledge and is fed by others. Moreover, good men carry success well, for the most fruitful branches bow the lowest. Why then, I wonder, are we so seldom prostrate?

ABSTRACT

Artificial proteins may be programmed to reversibly self-assemble into water-soluble networks, or “hydrogels”, by encoding them with terminal coiled-coil forming domains. Such networks are model viscoelastic materials. The well-defined molecular structures adopted by proteins, combined with their facile preparation by recombinant synthesis, invite a careful exploration of the relationship between protein sequence and the resulting network properties.

This work explores the relationship between network reorganization and diffusion from the perspective of single chains, using artificial elastin-like proteins as a model system. We make use of fluorescence recovery after photobleaching (FRAP), a classic biophysical technique, to measure chain mobilities as a function of network structure and probe architecture. Reversible network association is demonstrated to control the effective diffusivity of network-bound chains, and a novel mechanism of chain transport is proposed: the chains naturally partition into various bound states, and move by “hopping” from site to site in between binding events.

A careful analysis of the equilibrium constants that control this partioning leads to the conclusion that the sequential binding of identical chain ends to the network is inherently asymmetric: the first association is always stronger than the second. This binding asymmetry is shown to arise from a strong entropic penalty for chain entry into the fully bound state due to local network structure. We derive a simple equation predicting the degree of binding asymmetry as a function of network geometry from equilibrium

statistical mechanics. A large set of self-diffusivity measurements on a series of model telechelic proteins finds good agreement with this new theory. Generalized binding asymmetry for chains with many associative domains also holds.

Finally, the inherent viscoelasticity of the elastin-like network is found to couple with an entropically driven phase separation above a critical temperature set point. Relaxation of the viscoelastic stress throughout the process of phase domain segregation is found to induce highly dynamic phase patterns. The time evolution of these patterns illustrates that a delicate balance of surface tension and viscoelastic stress controls pattern formation in viscoelastic materials.

PUBLISHED CONTENT AND CONTRIBUTIONS

CHAPTER 1 is published as an article in the Journal of the American Chemical Society, and may be cited as follows:

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Author contributions: P.B.R. and D.A.T. designed the experiments and wrote the chapter. P.B.R. performed the experiments and analyzed the data. A.K.O. and Z.G.W. designed and implemented the simulations. J.J.S. performed amino acid synthesis. M.E.B. cloned the proteins.

CHAPTER 2 was written in close collaboration with Ahmad K. Omar, who developed the statistical-mechanical framework for interpreting the experimental results. Bradley K. Silverman provided critical support in obtaining and analyzing the large data sets.

CHAPTER 3 describes the discovery of unusual phase behavior in a protein polymer originally designed by Maren E. Buck. Quantitative analysis of the phase patterns was performed by Bradley K. Silverman.

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