From Chaos to Function: Silk Fibroin as a Design Template for Intrinsically Disordered Multiscale Biomaterials

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Proteins, nature's molecular machines, play a pivotal role in a diverse range of essential biological functions spanning cell signalling, catalysis, and structural stabilisation of extracellular networks and biomaterials. Proteins are built from template-driven arrangement of up to twenty unique amino acid building blocks. The performance and functionality of proteins is directly related to the amino acid sequence and the subsequently formed three-dimensional conformation. Traditionally, protein function has been thought to depend on a single, precisely folded structure. However, a recent paradigm shift has revealed that many proteins depend on structural fluidity for function and/or assembly.

A feature that structural fluidity of "disordered" proteins often provides is the ability to phase separate, a process whereby solvated proteins separate into a dense proteinaceous phase and light buffer phase at both the nano and microscale. Understanding residues responsible for this phenomenon will pave the way for developing novel biomaterials with customisable properties [1]. We aim to understand and leverage these properties using silk proteins as a model [2]. Fibroin, silk produced by silkworms, is a large and repetitive intrinsically disordered protein, that primarily consists of glycine, alanine and tyrosine that assembles into a strong elastic fibre from a viscous phase separated dope. In essence this dope is a highly concentrated phase-separated solution of structurally disordered silk protein.

In this work, we identified tyrosine as a key amino acid governing intermolecular interactions that drive phase separation and aggregation dynamics in disordered proteins [1]. This was achieved through 1) bioinformatic analysis which highlighted conserved patterning of tyrosine residues, 2) coarse grained computational modelling showing a direct correlation between tyrosine content and phase thermal stability and 3) an array of *in vitro* biochemical assays that validate the *in-silico* work of tyrosine-tyrosine interactions driving self-assembly.

This knowledge was then applied to control multiscale material properties through the inhibition of tyrosine-tyrosine interactions via L-arginine titration, an excipient selected on the basis of the coarse-grained modelling. Introduction of L-arginine resulted in reversible nanocluster (100-300nm) and condensate formation (1-10 µm), formulation stability preventing microscale aggregation (2-Fold + Duration Increase) and regulation of dityrosine crosslinking dynamics in macroscale bulk hydrogels for tissue engineering purposes [3]. This work serves as a platform for designing modular tyrosine templated biomaterials suitable for multiscale applications.

References:

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