Engineering amyloid assemblies through ligand-modulated protein conformational switches

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Over the last 10 years, we have studied the fundamentals of DNA-promoted conformational transactions in Winged-Helix (WH) domains involved in replication initiation of bacterial plasmids (RepA protein) and yeast chromosomes (Origin Recognition Complex, ORC) [1,2]. By combining genetic, biochemical and biophysical approaches, we have characterized the large structural switch experienced by RepA to be enabled as a replication initiator, establishing a paradigm in the plasmid biology and DNA replication fields [3-6]. We have recently discovered that a specific regulatory DNA sequence promotes the assembly of RepA-WH into amyloid fibres [7]. We have also found small molecules that inhibit amyloidogenesis by binding to the DNA recognition interface in the protein [8]. DNA-induced WHs amyloidogenesis mirrors the events leading to the amyloid pathogenic transformation ($PrP^{C} \rightarrow PrP^{Sc}$) underwent by the mammalian prion protein upon binding to nucleic acids [9]. We aim now to take advantage of the ligand-induced conformational switches in RepA and ORC WHs as the grounds for exploring their potencial in **Synthetic Biology**: i) the bottom-up design of protein devices to artificially control DNA replication and amyloid assembly; ii) engineering reliable protein arrays and microbial sensors for HTS of small molecule inhibitors/effectors of both processes; and iii) exploring their feasibility in building novel functional, self-assembling (orthogonal) microbial modules.

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