Engineered Gene Circuits: From Clocks and Biopixels to Stealth Delivery

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Intracellular variability is a major obstacle to the fidelity required for a genetic circuit to execute a series of "pre-programmed" instructions. Over the past five years we have explored how determinism can arise from the synchronization of a large number of cells; in other words, synchronize genetic circuits between individual cells and view the colony as the primary design element. This approach led to three studies describing (i) how quorum sensing can be used to couple clocks between cells (http://biodynamics.ucsd.edu/Intercellular.mov), (ii) how redox signaling can synergistically combine with quorum sensing to couple *colonies* at centimeter length scales (http://biodynamics.ucsd.edu/Intercolony.mov), and
(iii) how intra- and inter-cellular genetic circuits can be rapidly coupled and used to encode information (http://biodynamics.ucsd.edu/Multiplexing.mp4). We are currently using our understanding of these processes to engineer bacteria for the safe production and delivery of anti-tumor toxins. The long-held monolithic view of bacteria as pathogens has given way to an appreciation of the widespread prevalence of functional microbes within the human body. Given this vast milieu, it is perhaps inevitable that certain bacteria would evolve to preferentially grow within tumors and thus provide a natural platform for the development of engineered therapies. We have engineered a clinically tested bacterium to lyse at a threshold population density and release a genetically en-coded anti-tumor therapeutic. Upon lysis, a small number of surviving bacteria reseed the population, thus leading to pulsatile lysis and delivery cycles with a stealth *in vivo* footprint. We have demonstrated the therapeutic potential of the bacteria in a syngeneic colorectal mouse model of cancer using luciferase to monitor the delivery dynamics. This work represents our early progress in transversing the scales of Synthetic Biology from the level of mathematically designed circuitry to therapeutically relevant animal models.