

Title: In vitro self-replication and multicistronic expression of large synthetic genomes

The bottom-up generation of chemical systems with life-like properties is a key objective of synthetic biology and origin of life research. Our research focuses on the assembly of biomimetic systems capable of self-reproduction using building blocks derived from either extant biology or a hypothetical prebiotic chemistry. One part of our research focuses on the assembly of self-replicating systems from existing biological parts, in particular through the *in vitro* reconstitution of a minimal central dogma consisting of DNA replication, transcription and translation. Recently, we were able to design such a system that achieved *in vitro* self-replication and multicistronic expression of large synthetic plasmid ensembles with a combined length of 116,000 basepairs. This multipartite synthetic “genome” encodes the full set of *Escherichia coli* translation factors, all three ribosomal RNAs, an energy regeneration system, as well as RNA and DNA polymerases. Parallel to DNA replication, our system enables synthesis of at least 30 encoded translation factors, half of which are expressed in amounts equal to or greater than their respective input levels. Our optimized cell-free expression platform could provide a chassis for the generation of partially self-replicating *in vitro* translation systems that mimic the most essential features of existing living systems.