Multi-enzyme MOF nanoreactors transplant a drug biosynthesis pathway into cells

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Most proteins evolved to function as team players within multi-protein systems such as metabolic networks or signaling pathways. By contrast, most technical protein applications are built around one isolated protein, such as a therapeutic antibody or an industrial enzyme. Reliable methods for the stabilization, *in vitro* operation and intracellular delivery of multi-protein systems could unlock new applications in biotechnology, diagnostics, and in medical therapy. We are combining hierarchically engineered metal organic framework (MOF) nanoparticles with the *in vitro* reconstituted six-enzyme pathway for the biosynthesis of violacein. Pathway-MOF nanoreactors produced higher amounts of violacein than solute enzymes, enabled pathway reuse, lyophilisation and storage. Infiltration into MOFS modified pathway kinetics and side product formation and unlocks a long steady state production phase that could not be achieved with the free enzyme system. More importantly, MOF nanoreactors can deliver the entire multi-protein system into mammalian cells where it interfaces with the metabolic state of cancer cells leading to the enhanced production of the cytotoxic violacein as an *in-situ* therapeutic (Figure 1). To date, this represents the most complex protein system delivered into cells. We believe that violacein nanoreactors may pave the way towards a novel class of intracellular protein systems therapies.

Figures

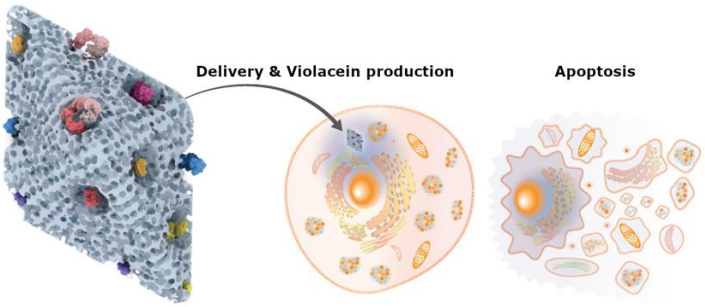

Figure 1: Illustration of multi-enzyme nanoreactors and their delivery into mammalian cells where the pathway produces the cytotoxic natural product violacein from intracellular substrates (tryptophan) and co-substrates ($NADPH+H^+$).