Our composite part includes composite parts TALEsp-vioA-vioB-vioC-vioE (BBa_K3264024) and TALEsp2-tnaA-Fmo-UGT (BBa_K3264022). Our part contains deoxyviolacein expression genes and TALE stabilized promoter sp2.
We applied this the previous part TALE sp2(BBa_K2753019) of Greatbay_China to our expression of our deoxyviolacein and indigo pathway.
The complete pathway of biosynthesis of deoxyviolacein, a light purple pigment
Despite simply laying a complete database of spidroin, GreatBay_SZ this year also approached one major industry where spider silk held great potential: the cloth industry. Identifying the current chemical compound pollution during dying process as well as the damage brought by chemical fiber itself, we realized that the typically non-environmentally friendly material of cloth manufacture can be replaced by spider silk, as thread to weaved the cloth, and natural dyes, as pigments that granted cloth color.

Usage in biology

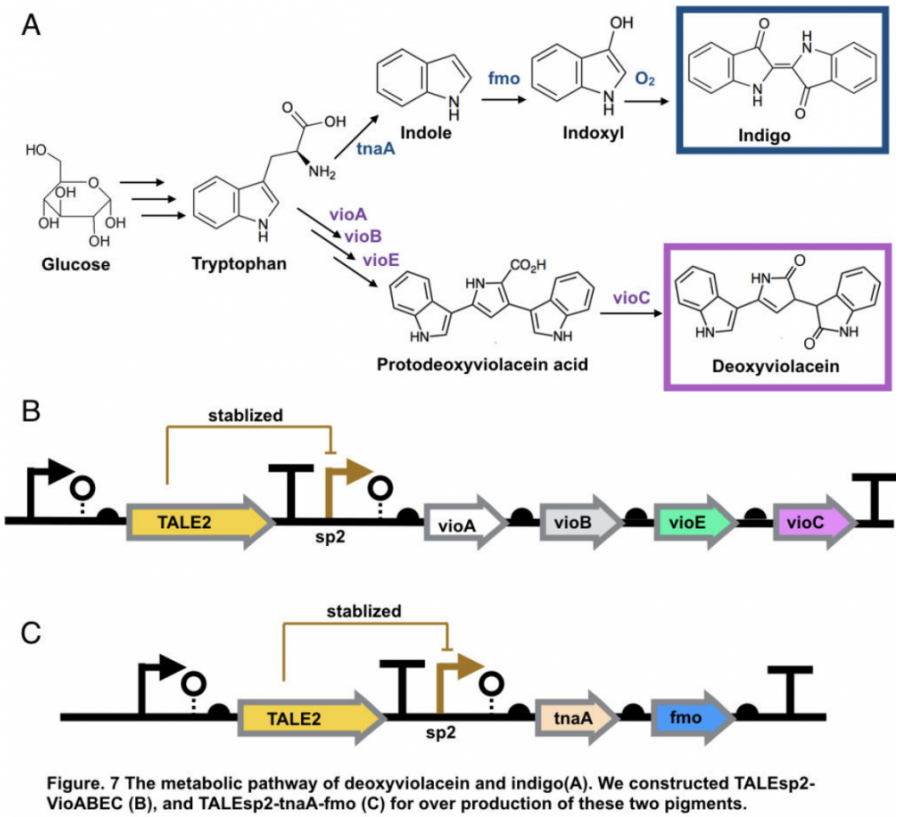

Figure. 7 The metabolic pathway of deoxyviolacein and indigo(A). We constructed TALEsp2-VioABEC (B), and TALEsp2-tnaA-fmo (C) for over production of these two pigments.

The pigment deoxyviolacein is produced from L-tryptophan in *E.coli* via a pathway involving four enzymes VioA, VioB, VioE, VioC, only vioD is excluded through the pathway. GreatBay_SZ 2019 borrow from team SHSBNU_China to acquire part thsR- BBa_K274003, which include one thiosulfate sensor and vioABDE which synthesize proviolacein. As shown in the graph below, different arrangement of VioA-E can synthesize pigment with deviate color. Our team wants to substitute VioD to vioC that gives as a light pink/purple pigment
Aiming for stable and high efficient production through deoxyviolacein pathway, using the original thiosulfate sensor cannot yield us high concentration of pigment. Instead, we look into GreatBay_China_2018's stabilized promoter BBa_K2753019, Talesp2 from the pTale family. Transcription-activator-like-effector (TALE) stabilised

promoters are a type of promoters able to untie gene expression level from gene copy number using an incoherent feed forward loop (iFFL) in which transcription-activator-like effectors (TALEs) function as a perfectly non-cooperative negative regulation. While copy number accretes gene expression, it also elevates the repression to the gene expression, thus has canceled out the effect of copy number on expression level. Thus using Tale sp2, we comprehend that it can yield high quantitative results

Characterization

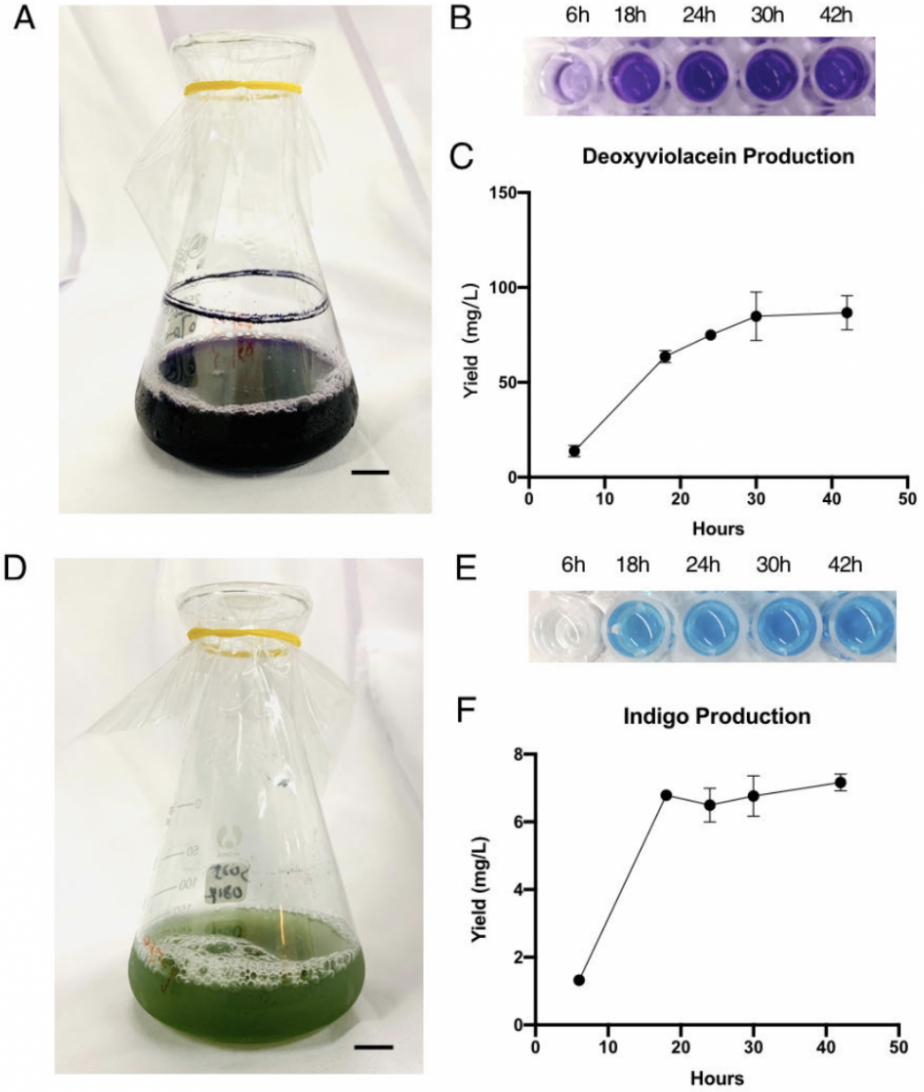

Figure. 8 The production of deoxyviolacein (A) and indigo (D). We constructed a yield versus time curve, extracting samples at the time of 0h, 6h, 18h, 24h, 30h, and 42h for OD measurement (B,E). The final production curve we obtained (C,F).

To achieve over production of both pigments, we utilized the stabilized promoter BBa_K2753019 from GreatBay_China(2018), transcription-activator-like-effector (TALE) stabilised promoters that untie gene expression level from gene copy number. We designed to place TALEsp2 promoter on standard pSC101 backbones. Combining the pathway with promoter, we hence characterized and obtained pigments by constructing part TALEsp2-VioABEC for deoxyviolacein through BBa_K2753019, BBa_K726015, and a new basic part BBa_K3264008, a gene-

transcript of VioC. Meanwhile, we also constructed a new composite part TALEsp2-tnaA-FMO for indigo.

Deoxyviolacein has long be identified as secondary metabolity actively against pathogenic bacteria like *Pseudomonas aeruginosa* and *Staphylococcus aureus*, and leukemia, lung cancer, human uveal melanoma, and lymphoma cells[10,11]. It also served other purpose like natural pigments. The importance of violacein urged us to search for over production of both metabolities. The hidden pathway for production is encoded by the VioABCDE operon. Bio-synthesis starts from L-tryptophan, converted into protodeoxyviolaceinic acid by VioA, VioB and VioE enzymes, and then into deoxyviolacein is therefore produced with the activation of VioC gene[10]. All promoters are placed on the standard biobrick assembly compatible backbone pSC101. We reassembled plasmid thsR- BBa_K274003 by PCR vioAB, VioE and talesp2, only VioC was synthesized de novo by Genescript. All parts were assembled using Gibson Assembly.

After we acquire pure extract deoxyviolacein pigment, we test it dying properties upon our spider silk sample. As documented as the process below, the pigment can be well soaked into fibers to give it light purple color.
The influence of talesp2 on the production and accumulation of deoxyviolacein is remarkable, with the highest yield round 90~100mg/L which nearly matched the standard of pure extracts deoxyviolacein. It can be concluded that talesp2 has positive influences on deoxyviolacein's metabolic reaction, which it stabilized towards an optimal level of gene expression that produce just enough enzyme to metabolize the substrate. After we acquire pure extract deoxyviolacein pigment, we test it dying properties upon our spider silk sample. As documented as the process below, the pigment can be well soaked into fibers to give it light purple color.
Pigments were obtained by extracting pigments after 42 hours of shake-flask incubation (without iptg) using solvent ethanol for violacein, and DMSO for indigo respectively. Through calculation based on standard indigo product and OD measurement of oh, 6h, 18h, 24h, 30h, 42h (peak of production), we are able to construct a yield versus time curve. Through that, we concluded our deoxyviolacein yields 85.81±9.09mg/L maximum and indigo yields 6.97±0.44mg/L maximum.

Testing Dying Properties on Spider silk Fibers

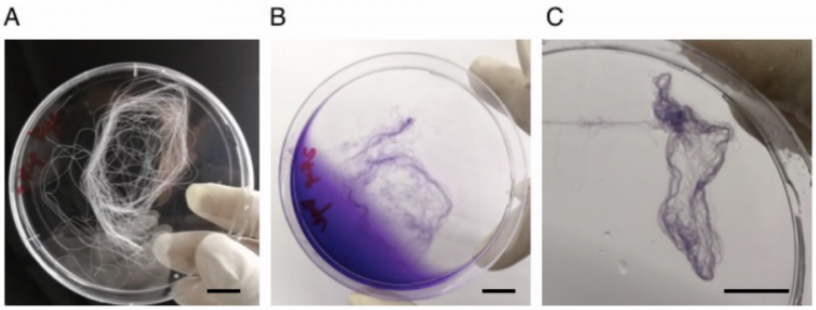

Fig.4 Testing Dying properties of Deoxyviolacein on spider silk fibers A) Sample of spider silk fibers B) Samples soaked in deoxyviolacein pigment to be colored C) Sample after dying

After we acquire pure extract deoxyviolacein pigment, we test it dying properties upon our spider silk sample. As documented as the process below, the pigment can be well soaked into fibers to give it light purple color.

Reference

[1]Rodrigues, André L., et al. “Systems Metabolic Engineering of Escherichia Coli fo r Production of the Antitumor Drugs Violacein and Deoxyviolacein.” Metabolic Engin eering, vol. 20, 2013, pp. 29–41., doi:10.1016/j.ymben.2013.08.004.

[2]Wen, Rui, et al. “Molecular Cloning and Analysis of the Full-Length Aciniform Spidroin Gene from Araneus Ventricosus.” International Journal of Biological Macromolecules, vol. 117, 2018, pp. 1352–1360., doi:10.1016/j.ijbiomac. 2017.12.090.