The problem
Concrete is the main material used in the construction industry. Cracks frequently form in concrete structures, due to earthquakes, excessive weight, freeze–thaw effects, wind-loading and traffic. Cracks allow water into the steel reinforcements, causing them to corrode, weakening the structure.

Our solution is BacillaFilla: *Bacillus subtilis* engineered to swarm down cracks and fill them with a mixture of glue and reinforcing fibres.

Low oxygen concentration at the end of the crack triggers the subtilin quorum sensing system inducing cells to precipitate CaCO₃ crystals (with similar properties to concrete) or become filamentous (acting as reinforcing fibres). All cells produce Levan glue which acts as a binding agent. The resulting mixture of filamentous fibres, crystals and glue forms a strong matrix, preventing water from entering and damaging the steel reinforcements.

Safety & ethics
*B. subtilis* 168 is a lab strain and has disabling tryptophan auxotrophic that makes it less likely to survive in the environment. We also designed a kill switch to prevent growth outside the crack. We used the mazEF toxin-anti-toxin system. mazE encodes a stable toxin and mazF the labile antitoxin to MazF. Under stress MazF is degraded faster than MazE, and MazF kills the cell.

Filamentous cells
We designed this part to overexpress yneA in the presence of IPTG, leading to the filamentous cell phenotype. This helps to strengthen the CaCO₃ repair like fibre-reinforced concrete.

Levan glue
sdcB encodes the enzyme levansucrase which converts extracellular sucrrose into the long chain polysaccharide levan, which acts as a glue. Below, *B. subtilis* has grown and produced levan glue which is holding together sand to form the letters.

Quorum sensing
The subtilin production BioBrick contains the spaBCTS gene cluster. It has an oxygen limitation sensitive promoter which responds to low oxygen conditions at the end of a crack. Subtilin is a lantibiotic as well as a quorum sensing molecule. Therefore BacillaFilla must be resistant to subtilin. The immunity BioBrick contains the spoIFEG gene cluster. When subtilin concentration reaches a critical level it will trigger BacillaFilla to become either filamentous or to produce calcium carbonate.

Swarming
*B. subtilis* 168 contains mutations in the sfp and swrA genes. 168 cells grown on 0.7% w/v agar cannot swarm. However *B. subtilis* 3610 retains its wild type swarming ability. Our BioBrick reintroduces the wild type genes into *B. subtilis* 168.

CaCO₃ precipitation
CaCO₃ production is regulated by an antisense RNA, SR1, which binds to the mRNA for the regulator arhc, regulating arginine synthesis. Arginine is broken down by arginase (RocF), producing ornithine and urea. Increasing urea enhances urease production, breaking down urea into NH₃ and CO₂. Both ions leave the cell to form ionic bonds with Ca²⁺ ions in the medium. We designed a construct encoding antisense SR1 to relieve Arhc based repression of arginine production. We also included rocf to enhance the production of arginine.

References