Highly Efficient Immobilization of Bacteriophages on Magnetic Particles for the Capture, Separation, and Detection of Bacteria

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Immobilization of bacteriophages onto solid supports such as magnetic particles has demonstrated ultralow detection limits as biosensors for the separation and detection of their host bacteria. While the potential impact of magnetized bacteriophages is high, the current methods of immobilization are either weak, costly, inefficient, or laborious making them less viable for commercialization. In order to bridge this gap, we have developed a highly efficient, site-specific, and low-cost method to immobilize bacteriophages onto solid supports. While streptavidin-biotin represents an ideal conjugation method, the functionalization of magnetic particles with streptavidin requires square meters of coverage and therefor is not amenable to a low-cost assay. Here, we utilized a CRISPR/Cas9 system to genetically engineer bacteriophages to allow synthesis of a monomeric streptavidin (mSA) during infection of the bacterial host. The monomeric streptavidin was fused to a capsid protein (Hoc) to allow site-specific self-assembly of up to 155 fusion proteins per capsid. Biotin coated magnetic nanoparticles were functionalized with mSA-Hoc T4 bacteriophages which also contained a luciferase reporter. Bacteriophage functionalized nanoparticles were utilized in an *E. coli* detection assay achieving a limit of detection of <10 CFU in 100 mLs of water. This work highlights the creation of genetically modified bacteriophages with a novel capsid modification, expanding the potential for bacteriophage functionalized biotechnologies for targeting antimicrobial resistant bacteria.