PROTECTION - SCIENCE AND TECHNOLOGY ADVANCES FOR CHEMICAL AND BIOLOGICAL PROTECTION

Engineering Microbial Armor For Threat Detection

Behzad Rad Lawrence Berkeley National Labs

Cells can create complex assemblies from basic nutrients for sensing, motion, and protection. To harness the ability of cells to create new materials, our group researches bioinspired, biomimetic and biological materials for lithography, patterning of inorganic materials, sequestration of rare-earth metals in dilute solutions, biosensors and engineering hybrid living materials. These capabilities can greatly influence robust warfighter support materials, including microbial armors for real-time protection against chemical and biological threats.

I will present innovative work on engineered biomaterials using Surface (S) layer proteins. S-layers are the outermost protective layer of almost all Archaea and most Bacteria. These proteins also serve as virulence factors in both Bacillus anthracis and Clostridia difficile. When purified, these proteins from a two-dimensional, para-crystalline nanosheet without the need of a solid support. Using protein engineering, S-layers can display functional groups or fused proteins with nanometer spacing. The nanoscale tunability and folding stability of S-layers allow these biomolecules to seamlessly function as biomaterials. Our work, shown below, demonstrated that S-Layer proteins can be engineered for high density, modular display in solution or on living bacteria. S-layer proteins provide a modular scaffold, resistant to extreme temperature and pH, to engineer catalytic, regeneratable biomaterials in vitro and on the cell surface. Harnessing this ability would allow us to create deployable, reusable biomaterials for detection, catalysis and materials synthesis. In addition, such materials can be functionalized – with receptors and ligands, providing innate protection against threat agents.

I will highlight our work in understanding the assembly pathways of and engineer S-Layer proteins from several bacterial species. We analyzed SbpA self-assembly from Lysinibacillus sphaericus by correlating the formation of the nanosheets in solution with the nanosheet dimensions using Scanning Transmission Electron Microscopy. We developed a phase diagram for self-assembly using ionic strength and protein concentration as tunable parameters, leading to a computational framework to engineer materials more readily.

I will also show our research into engineering new functionality into S-later proteins using modular, split protein systems. For instance, in Geobacillus stearothermophilus, we engineered nanosheets with the SpyTag and SpyCatcher split protein system at various positions. Our engineered nanosheets were able to covalently bind to hybrid SpyCatcher proteins, generating novel 2D materials with emergent and modifiable properties.

Finally, I will show how we engineered the S-layer protein on the cell surface of Caulobacter crescentus, for high density display of materials on living cells. We engineered SpyTag at precise positions in RsaA by directly engineering the rsaA gene in the Caulobacter genome. Our work resulted in self-regenerating cellular based material that displayed a high density, modular tag that are able to be grown in low nutrient conditions. Using the SpyCatcher protein fused with a fluorescent protein, Elastin-like protein, or conjugated to fluorescent nanocrystals, we covalently attached and imaged these various materials to the cells while not affecting viability.

We propose that these engineered proteins with tensile strength, flexibility, modifiable properties, specific ligands and functionality can be used to develop robust microbial armor on warfighter uniforms, providing intrinsic protection in harsh terrains and duress.

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