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Discovery of capsid-packaged phage proteins injected into bacteria

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The typical phage infection cycle begins with adsorption of a phage particle to a bacterial cell, followed by injection of the phage genome and production of new progeny phages. However, it is often overlooked that, in addition to their genomes, some phages also inject proteins into the host cell. Although a handful of such proteins have been described, their prevalence and diversity across phages remain largely unexplored.

Here, we developed a mass spectrometry-based proteomics approach to discover phage-injected proteins. Inspired by the classic Hershey–Chase "Waring blender" experiment (1952), we label all phage particle proteins with heavy isotope amino acids and infect unlabeled bacteria. This enables us to distinguish proteins originating from the infecting phage particle (heavy-labeled) from proteins synthesized *de novo* during infection (unlabeled). Following phage adsorption and DNA injection, empty phage particles are removed from the bacterial surface by the shearing force of a blender. As most phage structural proteins remain outside the cell, they are removed during this step, whereas proteins injected into the host cell are protected from blending and retained within infected cells.

Initial validation demonstrates that the method effectively distinguishes injected proteins from non-injected phage particle proteins. We are applying this approach to a diverse collection of phages to identify previously unknown phage-injected proteins and investigate their roles during infection.

Reference #1

Reference #2

Reference #3

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