Nanoplasmonic optical antennas reveal the oscillatory enzyme activity of single bacteria

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Bacteria are the pathogens of many infectious diseases, and bacteria communicate with neighboring individuals and host cells via outer membrane vesicles (OMVs).[1] Such OMVs contain many important intracellular signal molecules such as nucleic acids, proteins, and enzymes. The secretion of OMVs allow bacterial enzymes to enter the extracellular environment as essential signaling molecules for signal perception, environmental adaption, information exchange, host infection, drug resistance, and biofilm formation. However, due to the low concentration of enzyme molecules carried by OMVs and the lack of technique for long-term, high-precision, and real-time monitoring of enzymes, many detailed enzymatic activities and rules via OMVs are always hidden.

Although fluorescence labelling has been widely used for monitoring molecular dynamics, it is of great challenge for the observation of enzymatic activities of OMVs in a long-term biological process due to the feature of photobleaching for fluorescent labels. Alternatively, nanoplasmonic optical antennas can be used as an excellent tool to solve this problem and have been widely used for molecular monitoring in vitro and in vivo.[2] Nanoplasmonic optical antennas are plasmonic nanostructures that receive resonant light signal and concentrate light fields into the deep sub-wavelength scale at the surface of plasmonic nanostructures owing to the collective oscillation and resonance of surface electrons, i.e., localized surface plasmonic resonance. These nanoantennas can also monitor quantum biological processes inside live cells.[3] Such plasmonic nanoantennas hold enormous potential to monitor bacteria's enzymatic activities via OMVs and reveal cell-cell communications.

Recently, Lu et al. designed a new nanoplasmonic optical antenna that can be used for long-term monitoring of enzymatic activities and bacterial communications via OMVs up to several hours.[4] Using this nanoplasmonic optical antenna, they revealed the oscillatory feature of enzyme activities via OMVs of single living bacteria in a periodic feature. The oscillation of enzymatic signal can couple with the signal from neighboring bacteria in communication. During this coupling, new oscillatory signals with heterogeneous features were captured, which can reveal the communication dynamics between neighboring bacteria.

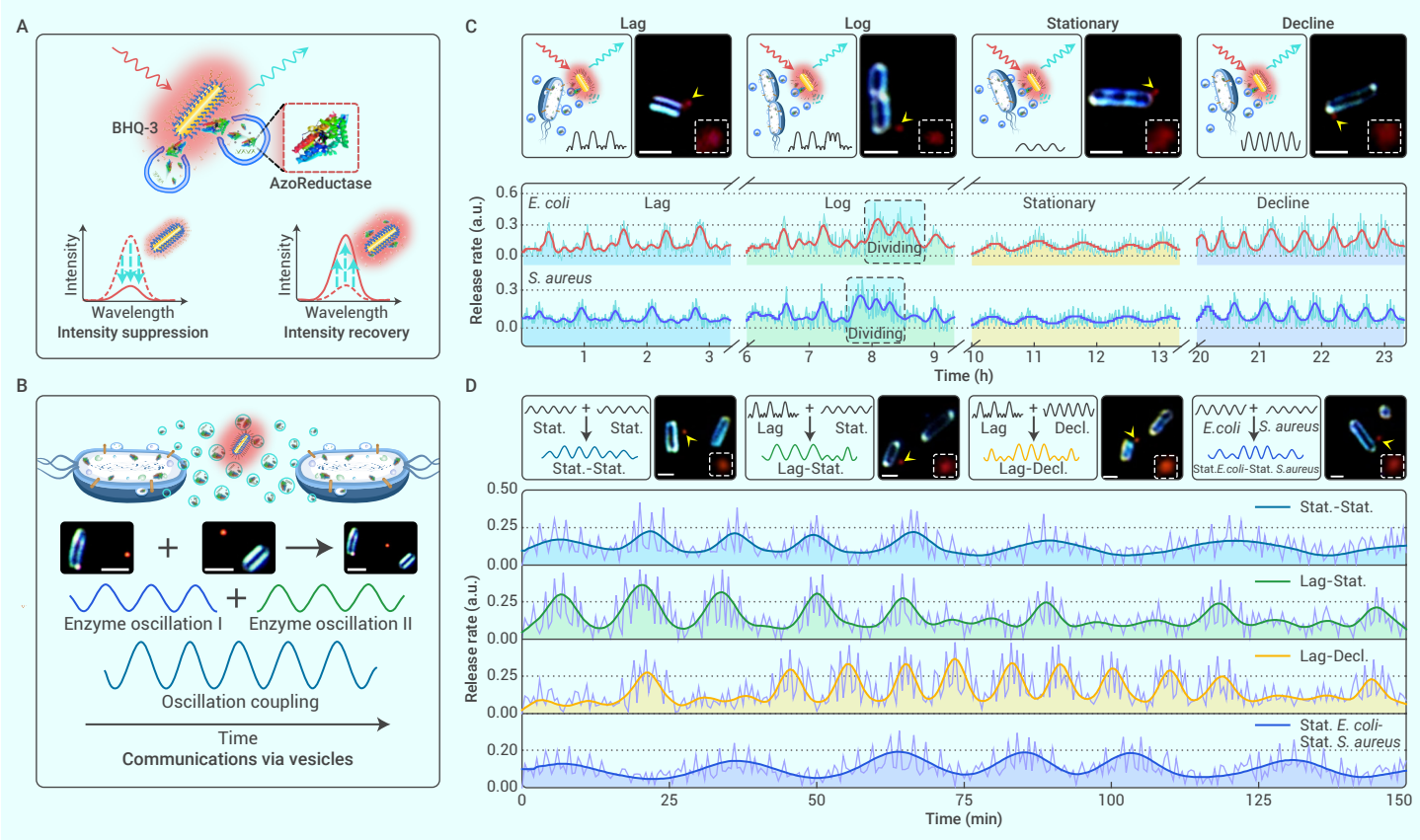

Figure 1. Dynamic monitoring of oscillatory enzymatic activity via nanoplasmonic optical antennas (A) Working principle of nanoplasmonic optical antenna for enzyme (AzoR) detection. (B) Illustration showing oscillatory enzymatic activity and oscillation coupling in neighboring bacteria during communication. (C) Monitoring of real-time enzyme release from a single bacterium at different living phases. (D) Monitoring of oscillation coupling of enzyme release during bacterial communication. Scale bars: 2 μm.

In their work,[4] the nanoplasmonic optical antennas were constructed by connecting gold nanorods (AuNRs) with black hole quencher (BHQ-3) molecules. As a most important factor of the AuNRs and BHQ-3 molecules that can be used to construct such optical nanoantennas, the AuNRs exhibit strong plasmonic resonance at around 670 nm which shows a large overlap with the absorption of BHQ-3 molecules. This large resonance overlap results in strong resonance coupling between AuNRs and BHQ-3 molecules when BHQ-3 molecules are covered onto the surface of the AuNRs, and the scattering cross-section of plasmonic AuNRs is thus reduced. Eventually, the initial scattering intensity of the nanoantenna is strongly suppressed (Figure 1A). Enzyme azoreductase (AzoR), an important flavoenzymes in bacteria, released from OMVs can break the azo bond of BHQ-3 molecules, which can change the absorption of BHQ-3 molecules. Therefore, in the presence of AzoR molecules, BHQ-3 molecules in the constructed nanoantenna are broken down, and the initial suppressed scattering intensity can then be effectively recovered. This sensitive recovery of scattering intensity enables the detection of AzoR molecules released from a single bacterium via OMVs. Different from fluorescent probes with the feature of photobleaching, this nanoplasmonic antenna can be used for real-time and long-term detection with detection time up to several hours, enabling new rules to be discovered during long-term bacteria communication (Figure 1B).

These optical nanoantennas enable the detection of enzyme release from single living bacteria via OMVs. From the captured optical signals, they derived signals of enzyme release rate. They find that the release rate exhibits various periodic oscillations for single bacteria at different living phases (Figure 1C). These oscillation features exist for both Gram-negative bacteria (*Escherichia coli*) and Gram-positive bacteria (*Staphylococcus aureus*). They also find that the oscillatory signal of enzyme release from single bacteria can couple with that of neighboring communicating bacteria, including across-species coupling (Figure 1D). Due to the different metabolism efficiency as well as signal transduction efficiency with surrounding microenvironment, this oscillation coupling exhibits distinctive features for neighboring communicating bacteria at different living phases.

These results demonstrated the real-time noninvasive detection of bacterial communication in living organisms, which co-exist in humans, animals, plants, soils, wastewaters, rivers, and oceans via nanoplasmonic antennas. This real-time observation method allows us to study why and how bacteria communicate. It will reveal a new biological understanding of how bacteria converse with each other by transmitting OMVs for enzymatic electrochemical signals in living biological systems. The observed oscillation features of enzymatic dynamics also widely exist in many other molecular dynamics and govern many important cellular processes in living systems,[5] which, however, are often unrevealed due to the weak signals. The real-time and long-term monitoring of enzyme activity with oscillation features in neighboring communicating cells will also provide new insights into across-species communication, host infection, disease transmission, and antibiotic-resistant bacteria, which can help us find a solution for healthy environmental sustainability.

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DECLARATION OF INTERESTS

The authors declare no competing interests.