UNDER THE LENS

Breaking free of labels

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This month's Under the Lens discusses the use of a novel label-free imaging method to study pilus dynamics at high spatial and temporal resolution.

Many pathogenic bacteria are able to crawl along surfaces by successively extending, tethering and retracting protein filaments called type IV pili (T4P). This crawling mechanism is termed twitching motility because it leads to small, intermittent jerks of the cell body. T4P are involved in many of the cellular processes associated with pathogenesis such as microcolony and biofilm formation, DNA uptake and protein secretion¹. Thus, understanding the dynamics, coordination and molecular mechanism of these nanomachines is of great interest.

Fluorescence-based microscopy has revolutionized our understanding of cellular processes in live bacteria. However, the finite photon budget of the fluorescent probe (that is, the number of photons emitted before photobleaching) prevents sub-second imaging over periods longer than about a minute. Furthermore, attached fluorophores often perturb the function of the molecules to which they are attached. In a recent paper, Talà et al.² show that it is possible to visualize T4P dynamics without fluorescence labelling by using interferometric scattering microscopy (iSCAT)³.

iSCAT collects both the light scattered by an object and a reference light field provided

by the reflection of the incident light source at the coverslip–water interface. The scattered and reflected light interfere at the detector, resulting in the object appearing as a dip in signal over a constant bright background. A coherent light source that emits photons of the same frequency further increases the interferometric contrast enabling imaging of nano-objects and even single proteins as small as 20 kDa (REF.⁴). Talà et al. took steps to minimize the phototoxicity of iSCAT to the bacterial cells by employing a relatively low energy (638 nm) laser that was shuttered to reduce the cell exposure. They also used a separate brightfield channel to allow initial localization of the cells before laser exposure.

iSCAT revealed obvious extracellular appendages on the bacterium *Pseudomonas aeruginosa* which were identified as flagella and T4P. iSCAT was able to determine the helical pitch of the flagellum. It also allowed T4P extension and retraction cycles to be tracked for one minute at 200 frames per second.

The spatial orientation of the T4P relative to the cell body could be visualized in three dimensions, revealing three distinctive pilus conformations. These were tensed, surface-attached states aligned either flat against the glass surface or at an angle to the cell body, or a state in which the pilus

is flexible. Deletion of the retraction motors prevented the formation of tensed states.

The high spatial and temporal resolution of iSCAT enabled insight into the coordination of attachment and retraction of the T4P. *P. aeruginosa* initially attaches the T4P tip to the surface. This interaction was strengthened by pilus retraction, an observation that is indicative of 'catch-bond' behaviour in which the dissociation lifetime increases with the applied

force. Approximately 130 ms after surface attachment the pilus retracts, suggesting that the bacterium senses surface attachment of the pilus through a currently unknown mechanism. Pilus retraction without a preceding attachment event was found to be extremely rare.

Talà et al. also applied iSCAT to identify the previously unknown functional differences between the two retraction motors PilT and PilU found in *P. aeruginosa*. When cells are sandwiched between glass and agar both *pilT* and *pilU*-deficient strains were immobile, although the parental strains were able to crawl in this environment. iSCAT revealed that when the cells were placed in liquid on glass, *pilU*-deficient strains could still retract their T4P and move the cell. These observations suggest that PilU might function to aid PilT in T4P retraction in higher friction environments when more force is required to displace the cell.

In summary, Talà et al. validate iSCAT as a powerful tool to decipher the dynamics of bacterial surface appendages without obstructing native functions with fluorescent probes.

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Competing interests

The author declares no competing interests.

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