Réunions scientifiques

Séminaires

Séminaire SOLEIL

Single molecule fluorescence imaging of molecular interactions and conformation in mammalian cells

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Invitée par P. DUMAS et M. REFREGIERS

Single molecule fluorescence imaging has achieved much in the last decade; to cite some good examples, it is now possible to study in detail interactions, stoichiometry and conformational changes for immobilised proteins and DNA (1), for different types of immobilized proteins (2) and in very complex molecular motor structures in bacteria (3). It is also possible to determine the position of immobilised proteins and protein filaments with nanometer accuracy, leading to a detailed understanding of their mode of interaction (4). However, extension of single molecule fluorescence imaging to investigations in mammalian cells is challenging because of the inherently poor signal-to-noise resulting from these samples. This is the result of the presence of cell autofluorescence background, which is reduced but not eliminated through the use of total-internal-reflection fluorescence (TIRF) excitation and a side effect of TIRF, by which fluorophores located at varied axial distances, as with proteins in the plasma membrane, experience different excitation field strengths. The fluorescence from single-molecules can therefore be much fainter and have a wider spread of signal-to-noise ratios than biomolecules immobilized on the same 2D glass surface exposed to the same incident intensity. I will review in the talk the progress made in the last three years towards extending single molecule methods to investigations of protein interactions and changes in conformation in these challenging cell samples.

1. Rahul Roy, Alexander G. Kozlov, Timothy M. Lohman, Taekjip Ha. (2009). SSB protein diffusion on single-stranded DNA stimulates RecA filament formation. Nature, 461:1092-1097.
2. Yasushi Okada, Hideo Higuchi, Nobutaka Hirokawa. (2003). Processivity of the single-headed kinesin KIF1A through biased binding to tubulin. Nature, 424:574-577.
3. Leake MC, Chandler JH, Wadhams GH, Bai F, Berry RM, Armitage JP. (2006). Stoichiometry and turnover in single, functioning membrane protein complexes. Nature, 443:355-358.
4. Ahmet Yildiz, Michio Tomishige, Ronald D. Vale, and Paul R. Selvin. (2004). Kinesin Walks Hand-Over-Hand. Science, 303:676-678.

Formalités d'entrée *: accès libre dans l'amphi du Pavillon d'Accueil. Si la manifestation a lieu dans le Grand Amphi Soleil du Bâtiment Central, merci de vous munir d'une pièce d'identité (à échanger à l'accueil contre un badge d'accès).*

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