EGF Receptor Trafficking and Viral Infection Resolved in Live Cells Using Synchrotron Radiation (SR) Microfluorimetry

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Cell proliferation is signaled by the binding of growth factors to their specific receptor tyrosine-kinases, which are embedded in the plasma membrane.[1] Upon binding EGF, the receptor's kinase becomes activated, allowing a signaling cascade that ultimately leads to gene transcription. Growth factor binding to the receptor also triggers rapid sequestration of active EGF-receptor complexes in the cell via a poorly understood, rapid, saturable internalisation pathway.[2] This may play an important role in the regulation of the mitogenic signal.

It is widely accepted that the molecular mechanism for activation of the receptor's tyrosine-kinase is EGF-induced receptor dimerisation. This model is supported by a corpus of evidence from molecular biology techniques, but thus far attempts to test this model in cells using electron microscopy and fluorescence methods have produced contradictory results. One of the major factors limiting the accuracy and time resolution of these data is the need to fix or freeze the specimens. This is done because the speed of data collection is generally much slower than the kinetics of the process under study. For fluorescence measurements, fixing may be also needed to alleviate photobleaching of dyes and cell photodamage because it allows the use of a different sample per data point.

We have developed a SR microfluorimeter capable of continuous measurement of the average inter-molecular distances and molecular motions from live cells.[3,4] The pulse structure, tunability, and stability of SR have been crucial for these measurements. The microfluorimeter sacrifices any spatial resolution within the area of illumination to rapidly accumulate fluorescence decays from as little as 10^3 fluorescent molecules at irradiance levels six orders of magnitude smaller than similar instruments. This substantially reduces fluorophore photobleaching and cell photodamage. Using time-resolved fluorescence resonance energy transfer (FRET)[5] the instrument can measure distances in the range of 5 to 100 Å in time slots of about 10 seconds. It is therefore capable of measuring the kinetics of molecular aggregation processes, such as receptor dimerisation. The microfluorimeter has also the option of recording time-resolved fluorescence anisotropy (TRFA) decays, which report on steric constraints of molecules and their modes of rotation.

We have used this instrument to determine the sequence of early EGF-receptor events that follow the binding of EGF to its receptor in the epithelial carcinoma A431 cell line. We are now applying these methods to study the infection of live, cultured Hep2 cells with adenovirus 2. The relevance of this work is that we have been able to follow the viral uncoating process at high time resolution (few seconds) while simultaneously maintaining conditions that mimic the native environment of cells and viruses.

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