3D Multi-resolution visualization of the early stages of cellular uptake of peptide-coated nanoparticles

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Project Goals: The project, "Development of Quantum Dot Probes for Studies of Synergy between Components of the Wood-Degrading Fungal Enzymes," aims to develop quantum dot-based tagging and imaging technologies tailored for simultaneously monitoring, in real time and in the natural fungal / lignocellulose environment, the mode of action of several lignocellulosic enzymes at the single-molecule level. It is designed to be the first project of a long-term research program for which the overarching goal is to bridge the knowledge gap between the in vitro molecular biochemistry and the naturally occurring biodegradation pathways by a quantitative determination of the biochemical and biophysical properties of these fungal enzymes in realistic plant biomass-microbe milieus.

A major aim of the research program in this funding period is to develop a new way of directly visualizing the cellulase molecular actions (fast, nanometer scale) in the context of the biological environment (slower, micron to millimeter scale) in full three-dimensional (3D) fidelity. Indeed, biological processes, including the production of biofuels either by biodegradation or by biosynthesis, are inherently multiscale. In this breakout presentation, we will summarize the currently available methods, point out exciting opportunities for future developments, and describe a new visualization modality that will help to bridge the gap. As proof of concept, we applied the new instrument to visualizing the early stages of cellular uptake of Tat-peptide coated nanoparticles.[1] A detailed understanding of the cellular uptake process is essential to the development of cellular delivery strategies and to the study of viral trafficking; however, visualization of the entire process, encompassing the fast dynamics—local to the freely diffusing nanoparticle—as well the state of the larger-scale cellular environment, remains challenging. Using the new 3D multi-resolution apparatus, we were able to capture, in real time, the transient events leading to cellular binding and uptake of HIV1-Tat-modified nanoparticles. The direct observation of the nanoparticles landing on the cellular contour in 3D revealed long-ranged deceleration of the delivery particle possibly due to interactions with cellular receptors. Furthermore, by using the nanoparticle as a nanoscale "dynamics pen," we discovered an unexpected correlation between small membrane terrain structures and local nanoparticle dynamics. We are currently applying this new approach to unravel the hidden mechanistic steps in the molecular actions of cellulosic biodegradation.

Publications

1. Kevin Welsher and Haw Yang, *Nat. Nanotechnol.* (2014) in press

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PLENARY ABSTRACTS