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Dynamic imaging of single microvilli in opossum kidney cells

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Abstract

Reabsorption of inorganic phosphate (Pi) in the renal proximal tubule is fundamental to overall Pi homeostasis. Its regulation occurs via the apical brush border membrane (BBM) sodium gradient-dependent Pi (NaPi) cotransport proteins. Distinct families of NaPi cotransporters show differential regulation under dietary and hormonal stimuli, but how this is accomplished at a molecular level is not yet fully understood.

The application of modern fluorescence correlation spectroscopies to the BBM of living cultured Opossum Kidney (OK) cells expressing NaPi cotransporters with different GFP constructs could provide useful information on the dynamics of the proteins, including diffusion, aggregation and interaction with other proteins. However, BBM microvilli show a relatively fast motion that makes the use of fluctuation spectroscopy difficult.

We developed an optical imaging technique called Modulation Tracking (MT) in which we track the center of mass of the microvillus at an arbitrary point along its length via orbital scanning while the laser spot rapidly oscillates perpendicularly to the surface. Using this technique we obtained high resolution images of the microvilli and applied fluctuation correlation spectroscopies in order to study protein dynamics.

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