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COMMENTARY

Crossing the arterial wall with CARS

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Regulation of the flow and movement of water is essential to the health and well-being of our bodies. Involving a diverse array of structures that range from molecular gatekeepers to tissue barriers, water flow and concentration are maintained at precise levels. However, in many instances we do not have a complete picture of how this works. A case in point is the arterial wall, which separates the luminal space of the artery, where water flow is highly directional and at high pressure, from the surrounding tissue, where the movement of water is diffusive and pressures are lower. To maintain the pressure difference across the arterial wall, the permeability of water through this barrier is necessarily low. Although the limited water permeability of the arterial wall has been measured extensively, it has remained unclear how this dedicated tissue structure is able to limit the flow of water or even which layer of the wall is responsible for the decrease in permeability. To solve this puzzle, a closer look at the water barrier is needed to bring the location of the water-blocking component into sharper view. In PNAS, Lucotte et al. accomplish this feat by using coherent anti-Stokes Raman scattering (CARS) microscopy (1). By creating water concentration maps with submicrometer resolution, the authors are able to pinpoint the endothelial basolateral membrane as the key player in limiting water permeation across the arterial wall.

CARS Enables the Visualization of Water Distributions in Cells and Tissues

Given the essential role of water to sustain biological life, it is perhaps surprising that it requires an advanced technique like CARS microscopy to identify cellular and tissue structures associated with the water movement in our bodies. After all, visualizing water concentration and mobility in biological organisms can be achieved in a myriad of ways. A prime example is MRI, an NMR technique that can be used to measure the mobility of water diffusion in tissues. NMR techniques have been used extensively for measuring and mapping water diffusion in biological systems (2), yet the resolution of MRI is insufficient to identify the structures that control water mobility on the (sub)micrometer scale. To investigate water movement at these smaller dimensions, an imaging technique with a high spatial resolution and chemical selectivity in complex materials is needed. More than other imaging methods, CARS microscopy is well suited for this task.

The CARS technique is a nonlinear optical alternative to Raman spectroscopy. CARS is sensitive to the same vibrational modes probed in spontaneous Raman scattering methods, enabling selective detection of molecular species based on their vibrational spectroscopic signatures. Compared with its linear predecessor, however, CARS offers much higher signals. In CARS, molecular oscillators are coherently set in motion, producing a strong signal in the direction in which their radiation constructively interferes. These stronger signals make it possible to acquire images at much higher rates. The O-H stretching vibrational motions of water give rise to a broad and rather strong band in the Raman vibrational spectrum, which facilitates the detection of water when tuned to this resonance. The excellent sensitivity of the nonlinear Raman technique to water distributions in biological materials was already evident in the very first demonstration of CARS microscopy in 1982 (3). In this early work, Duncan et al. soaked onion skin in heavy water and produced CARS images of cell boundaries with contrast produced by the O-D stretching mode of D_2O. Subsequent improvements in this nascent imaging technology, mostly spearheaded by Xie and coworkers (4), enabled rapid imaging of water distributions in lipid membrane vesicles and live cells with submicron resolution (5, 6).

Vibrational Isotope Effect Highlights Diffusion and Flow of Tissue Water

Although the CARS sensitivity to O-H bonds gives rise to intuitive images of water concentration levels in tissue samples, this ability does not automatically translate into clear recordings of water diffusion and flow. To visualize such dynamics, H_2O/D_2O exchange measurements have proven indispensable. Because of the higher mass of deuterium, the vibrational energy of the O-D and O-H stretching modes are substantially different. This vibrational isotope effect makes it possible

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to discriminate H_2O from D_2O, and follow the motion of water distributions as they are replaced with their deuterated analogs in perfusion-type measurements. This principle has been used to extract intracellular diffusion and membrane permeability parameters from single living cells, allowing, for example, a direct view of the permeability-enhancing effects of aquaporin channels in the plasma membrane of various cell types (5, 7). Recent improvements have made it possible to perform similar dynamic H_2O/D_2O exchange measurements in 3D tissues. An excellent example of the latter was given by Yu et al., who were able to characterize luminal and basolateral water diffusion pathways in a 3D epithelial cyst model (8).

In their latest work, Lucotte et al. (1) push CARS water measurements beyond controlled model systems to intact arteries. Through carefully executed imaging experiments in pressurized arteries, the authors record water concentration profiles across the arterial wall after perfusion with D_2O. By comparing the relative concentration profiles with immunofluorescence microscopy of relevant structural components of the arterial wall, Lucotte et al. are able to single out the endothelial basolateral membrane as the main barrier to water permeation. In doing so, the authors not only connect specific tissue structures of the cardiovascular system to their respective biophysical function, but also underline the role that advanced techniques like CARS microscopy can play in uncovering such structure/function relationships.

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