Mitochondria on guard of human life

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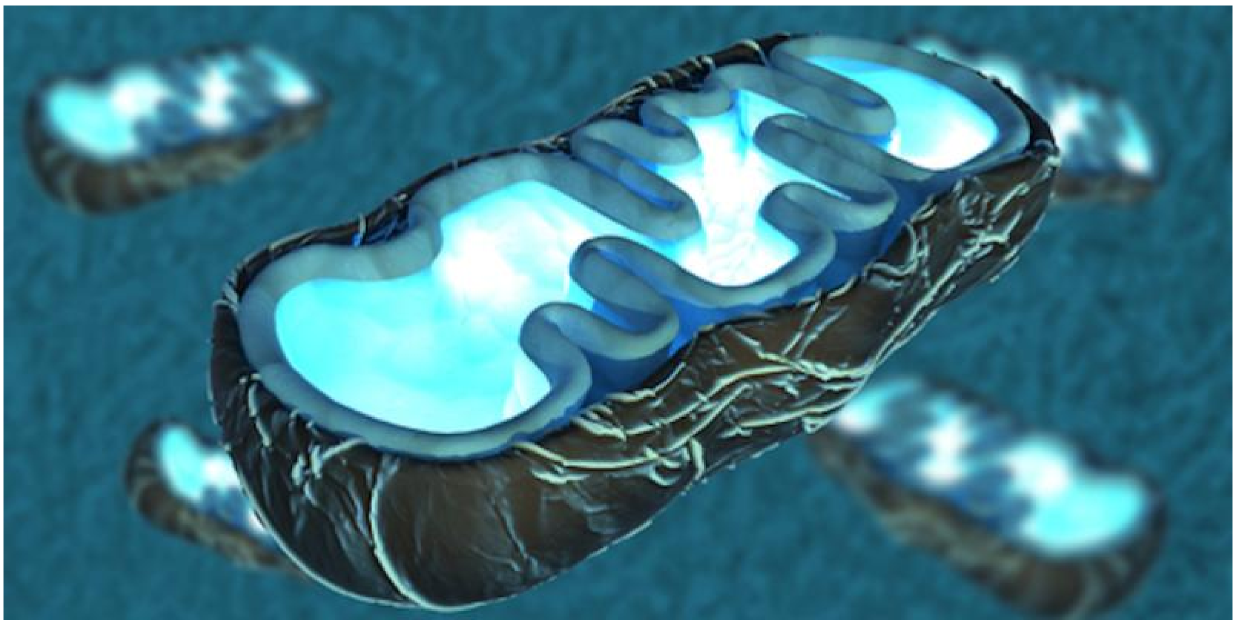

Living mitochondria will tell researchers about processes that occur inside of them grace to the work of scientists from Lomonosov Moscow State University and their colleagues from Denmark and Germany. The work will not only shed light on the intracellular life, but also will help to create new methods of disease diagnostics. Credit: Lomonosov Moscow State University

A group of researchers from Lomonosov Moscow State University in collaboration with Russian Science Foundation has developed a unique method for the selective study of electron transport chain in living mitochondria by using nondestructive analysis. The study was published in *Scientific Reports*.

Mitochondria are organelles of fundamental importance for cellular energy production and are often described as "the powerhouse of the cell." Mitochondria generate adenosine triphosphate (ATP), used as a universal source of chemical energy. The main role in the process of ATP synthesis belongs to the transport of electrons between special proteins in the inner mitochondrial membrane, one the most important of which is called cytochrome c.

Mitochondria are of special interest for scientists because these organelles contain mtDNA—molecules that carry maternally inherited genetic information. From this point of view mitochondria are a very interesting object of research for genetic and health scientists who study genetic disorders.

According to Nadezda Brazhe from the Department of Biophysics (Biological Faculty, Moscow State University), there are many methods of mitochondria study, but even the most advanced and sophisticated techniques cannot provide detailed information about the processes that occur inside and in between mitochondrial membranes during electron transport.

Researchers from Moscow State University suggested a novel and promising approach based on the surface-enhanced Raman spectroscopy (SERS). During last decade this method becomes more popular in studies of molecule properties in a tube and inside living cells.

There are two types of light scattering: Rayleigh scattering and Raman scattering. Rayleigh scattering (or elastic scattering) does not influence the state of a photon that falls off the obstacle without changing its frequency; during Raman scattering (or inelastic scattering), the photon interacts with molecules changing their energy level. As a result, the photon frequency also changes and the photon carries away some information about the encountered molecule. This information can potentially be used for the further study of the molecule.

Physicists developed a method that separates photons of Raman and Rayleigh scattering with the help of special spectrometers, but inelastic collisions occur extremely rarely. That is why combinational (Raman) scattering turns out to be weak and low intensity, which makes it almost undetectable.

The problem was solved in 1974 when an unusual effect was discovered: If the object that scattered Raman photons was located in close proximity to nanostructured metal surface, the scattering intensified a billionfold. Researchers still cannot give a full explanation for this phenomenon, but they suggest that this effect occurs due to plasmons—quasiparticles that represent surface electron oscillations relative to positively charged nuclei of nanostructure's metal. If the frequencies of plasmon and Raman photons coalesce, resonance is produced, which helps to make almost undetectable processes visible. This effect gave birth to a specific type of spectroscopy—SERS.

Biologists who study biomacromolecules understood that this type of spectroscopy could be a way to study the function of molecules inside living organelles or cells without destruction. Biologists tried to implement the method in practice, but not all attempts were successful.

According to Nadezda Brazhe, the main reasons for failure were related to the inefficiency of nanostructures. Thousands of nanostructured surfaces were invented, but some of them were not capable of creating resonance on the frequencies needed, and the others turned out to be toxic for mitochondria or degraded when added to physiological fluids.

"Biologists, chemists and physicists were involved in our work. This cross-disciplinary approach made our success possible. We were able to create nanostructured surfaces and a new methodological approach to study mitochondria. Success would be impossible without our colleagues from MSU Department of Material Science," says Nadezda Brazhe. Young researchers from the group of Prof. Eugene Goodilin after long and careful examinations found an appropriate and nontoxic nanostructure, which allowed us to complete the work."

The work lasted several years and was very difficult for the biologists and nanochemists. Sometimes, an achieved result seemed to be a miracle—but a miracle that can be explained from a scientific point of view.

The final result turned out to be surprisingly simple: A diluted mitochondria sample was placed on the nanostructured silver surface; laser light was focused on mitochondria aggregated on nanostructures and the SERS spectra of cytochrome c inside a living mitochondria was recorded. After that, researchers analyzed the SERS spectra. It turned out that only Raman scattering from cytochrome c experiences manifold enhancement. Therefore, the researchers had the chance to see in details the changes in cytochrome c structure that occur in the process of electron transport and ATP synthesis. With the use of different agents, scientists could initiate and stop electron transport and ATP synthesis in mitochondria, and these changes were easily detectable in recorded spectra.

No epoch-making discoveries were made during the work. Nevertheless, scientists developed a method that permits such observations. The method is now available for everybody, and Brazhe and her colleagues want to continue their work.

"Our next step will be to analyze mitochondria taken from heart and skeletal muscle of rats with cardiovascular diseases and diabetes. We hope that the results of the work will help to develop a method for the early diagnostics of pathologies. It will help to start the disease treatment earlier and to make medication more efficient," says Nadezda Brazhe.

More information: Nadezda A. Brazhe et al. Probing cytochrome c in living mitochondria with surface-enhanced Raman spectroscopy, *Scientific Reports* (2015). DOI: 10.1038/srep13793

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