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Researchers write protein nanoarrays using a fountain pen and electric fields

EVANSTON, Ill. --- Nanotechnology offers unique opportunities to advance the life sciences by facilitating the delivery, manipulation and observation of biological materials with unprecedented resolution. The ability to pattern nanoscale arrays of biological material assists studies of genomics, proteomics and cell adhesion, and may be applied to achieve increased sensitivity in drug screening and disease detection, even when sample volumes are severely limited.

Unfortunately, most tools capable of patterning with such tiny resolution were developed for the silicon microelectronics industry and cannot be used for soft and relatively sensitive biomaterials such as DNA and proteins.

Now a team of researchers at Northwestern University has demonstrated the ability to rapidly write nanoscale protein arrays using a tool they call the nanofountain probe (NFP).

"The NFP works much like a fountain pen, only on a much smaller scale, and in this case, the ink is the protein solution," said Horacio Espinosa, head of the research team and professor of mechanical engineering in the McCormick School of Engineering and Applied Science at Northwestern.

The results, which will be published online the week of Oct. 13 in the *Proceedings of the National Academy of Sciences* (PNAS), include demonstrations of sub-100-nanometer protein dots and sub-200-nanometer line arrays written using the NFP at rates as high as 80 microns/second.

Each nanofountain probe chip has a set of ink reservoirs that hold the solution to be patterned. Like a fountain pen, the ink is transported to sharp writing probes through a series of microchannels and deposited on the substrate in liquid form.

"This is important for a number of reasons," said Owen Loh, a graduate student at Northwestern who co-authored the paper with fellow student Andrea Ho. "By maintaining the sensitive proteins in a liquid buffer, their biological function is less likely to be affected. This also means we can write for extended periods over large areas without replenishing the ink."

Earlier demonstrations of the NFP by the Northwestern team included directly writing organic and inorganic materials on a number of different substrates. These included suspensions of gold nanoparticles, thiols and DNA patterned on metallic- and silicon-based substrates.

In the case of protein deposition, the team found that by applying an electrical field between the nanofountain probe and substrate, they could control the transport of protein to the substrate. Without the use of electric fields, protein deposition was relatively slow and sporadic. However, with proper electrical bias, protein dot and line arrays could be deposited at extremely high rates.

"The use of electric fields allows an additional degree of control," Espinosa said. "We were able to create dot and line arrays with a combination of speed and resolution not possible using other techniques."

Positively charged proteins can be maintained inside the fountain probe by applying a negative potential to the NFP reservoirs with respect to a substrate. Reversing the applied potential then allows protein molecules to be deposited at a desired site.

To maximize the patterning resolution and efficiency, the team relied on computational models of the deposition process. "By modeling the ink flow within the probe tip, we were able to get a sense of what conditions would yield optimal patterns," says Jee Rim, a postdoctoral researcher at Northwestern.

Espinosa collaborated closely with Neelesh Patankar, associate professor of mechanical engineering at Northwestern, and Punit Kohli, assistant professor of chemistry and biochemistry at Southern Illinois University, Carbondale.

"We are very excited by these results," said Espinosa. "This technique is very broadly applicable, and we are pursuing it on a number of fronts." These include single-cell biological studies and direct-write fabrication of large-scale arrays of nanoelectrical and nanoelectromechanical devices.

"The fact that we can batch fabricate large arrays of these fountain probes means we can directly write large numbers of features in parallel," added Espinosa. "The demonstration of rapid protein deposition rates further supports our efforts in producing a large-scale nanomanufacturing tool."

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The paper in the *Proceedings of the National Academy of Sciences* was authored by Loh, Ho, Rim, Patankar, Kohli and Espinosa.

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