Hyper-dense single-molecule protein arrays for proteomics at scale

Introduction

The sheer scale of the proteome makes it difficult to comprehensively analyze with single-molecule resolution. Most emerging technologies aiming to analyze proteins at the single-molecule level cannot cover the wide dynamic range of the proteome, which limits the number of proteins that can be observed in a single sample. Low abundance proteins will be drowned-out by abundant proteins.

To solve this issue on the Nautilus Proteome Analysis Platform, we set out to create arrays with billions of landing pads that each isolate a single protein molecule. On these arrays, each individual protein can be analyzed in parallel and independently. The sheer amount of landing pads enables researchers to quantify both high and low abundance proteins across the dynamic range of the proteome. We cover this work briefly here and invite you to dive deeper in our preprint titled "High-density and scalable protein arrays for single-molecule proteomic studies."

Methodology

We create these arrays through the combination of a few key techniques:

- **Patterned chip design** – We use photolithography and vapor deposition to pattern a glass surface no larger than a standard microscope slide with billions of "landing pads." We then coat the landing pads with positively charged small molecules to attract negatively charged DNA nanoparticles that bind individual proteins.

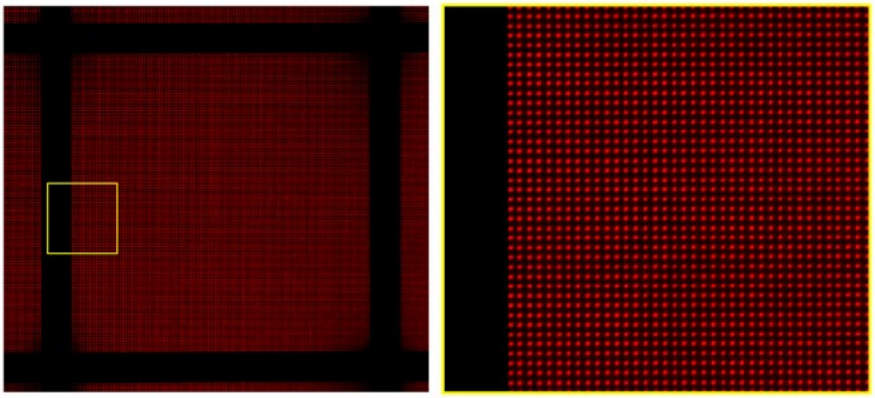

Figure 1. Grid pattern of landing pads on an array loaded with labeled DNA nanoparticles.

- **DNA nanoparticles** – We use DNA to create structured nucleic acid particles resembling nanoscale tiles. These act as scaffolds for single-protein display. After extending these tiles with a DNA synthesis technique, they fit precisely into the positively charged landing pads on our chips. These scaffolds are designed such that only one can fit on each landing pad and each scaffold can only bind one protein.

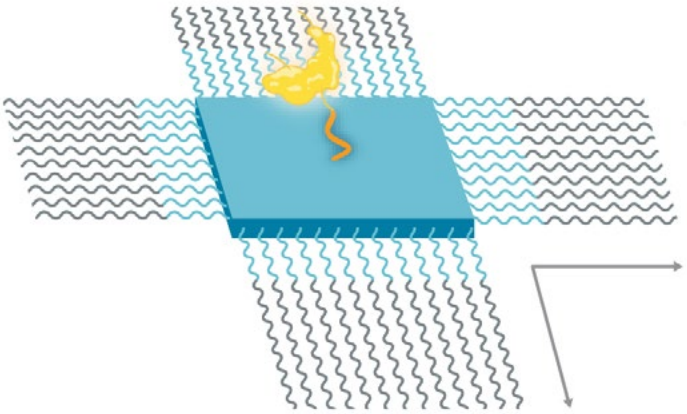

Figure 2. Schematic of the tiled structure of our DNA nanoparticle scaffolds. These tiles are extended with a DNA synthesis technique so that only one tile can fit on each landing pad of the array.

- **Click chemistry** – We modify the proteins in our samples so that single protein molecules can be attached to single scaffolds using standardized, scalable click chemistry techniques.

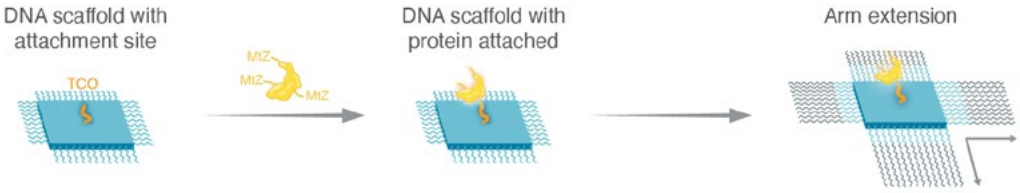

Figure 3. Proteins are attached to DNA scaffolds through standardized click chemistry and the DNA scaffolds are extended so that only one fits on each landing pad of the array.

To deposit proteins on an array, we first attach the proteins to the pre-assembled scaffolds and then load the protein-scaffold constructs on the array. Finally, the negatively charged scaffolds settle on the positively charged landing pads in the array.

Results

We checked whether we could successfully isolate single protein-DNA scaffold structures on each landing pad on an array by preparing a mixture of test protein-scaffold structures labeled with different fluorescent markers. If single protein-scaffold structures were isolated on each landing pad, we expected to see either one marker or the other on each landing pad of the loaded array. If there were multiple protein-scaffold structures on a landing pad, we would expect to see signals from both markers coming from that pad. As expected, we only rarely saw both markers on each landing pad.

To make sure multiple scaffolds with the same marker were not settling onto each landing pad, we measured the fluorescence intensity from each landing pad. One would expect to see increased signal intensity if there were multiple labeled protein-scaffold structures on a landing pad. Only at high protein-scaffold concentrations did we observe increased signal intensity from individual landing pads and we observed this less for less than two percent of the landing pads.

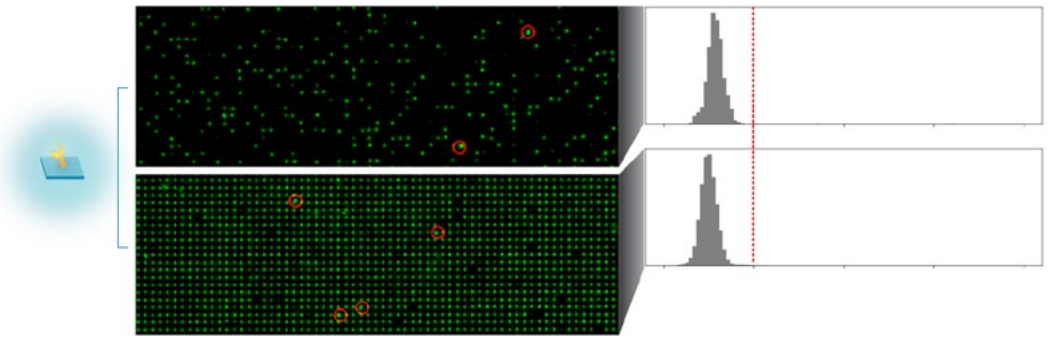

Figure 4. Protein-scaffold conjugates were labeled with a fluorescent marker and loaded onto an array. (Left) Circled sections reveal rare landing pads with increased signal intensity indicative of multiple scaffolds. (Right) Histogram of average signal intensity across pads showing it is very rare to observe increased intensity (which would create a multimodal distribution).

Conclusions

Excitingly, these results show we can confidently isolate billions of proteins on our hyper dense arrays and analyze them with single-molecule sensitivity. This brings us much closer to rigorously and comprehensively analyzing nearly all the proteins in the proteome and gives us confidence in the incredible potential of our platform.

For more information on our hyper dense arrays, see our preprint.

Learn more at:
nautilus.bio/technology

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