

Chapter 1

Introduction

1.1 Protein Analysis and Proteomics

The last decade has witnessed substantial advancements in technologies developed for faster and more comprehensive protein sequencing¹⁻³, analysis of protein structure and function^{4,5}, and profiling of proteins involved in human diseases such as cancer⁶⁻⁸. Efforts to determine increasingly complex sequences and structures of proteins within a rapid timeframe have been led by improvements in mass spectrometry^{6,9}, a technology that has proven far superior to the days when the Edman Degradation was the only method for sequencing peptides. To help gain insight into the molecular basis of diseases, technologies such as the enzyme-linked immunosorbent assay (ELISA)¹⁰, and gel chromatography¹¹, as well as mass spectrometry, have allowed for the analysis of large amounts of protein data, proving indispensable in detecting protein expression from human tissue and blood. Indeed, the field of proteomics owes its existence to these technologies.

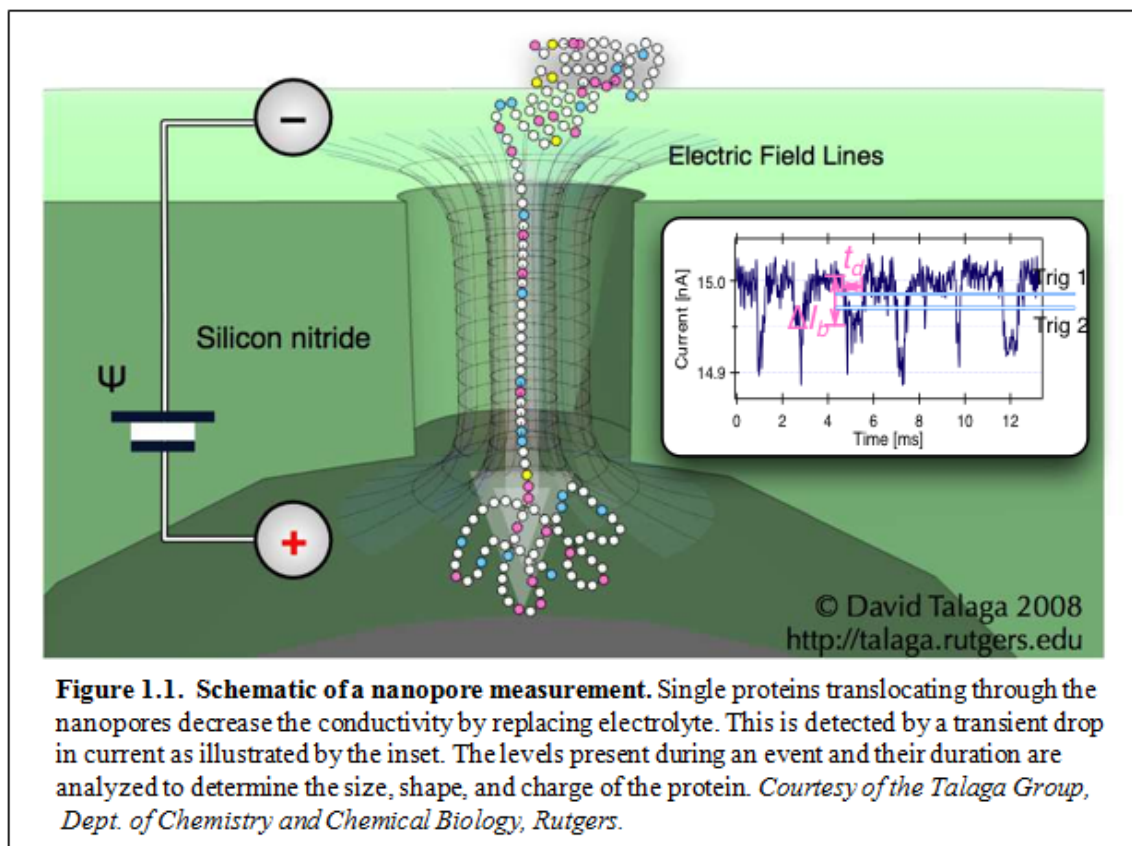
However, the shortcomings of these analytical devices are beginning to be realized as the need for measuring ever increasing amounts of biological information will require higher throughput and lower cost processes. As such, the field has pushed toward miniaturization of these technologies to the micro- and nanoscale^{7,12}. Using inexpensive, yet robust materials to build biochips will allow information on protein sequences, structures, and functions to increase by orders of magnitude. At the same time, these analyses would occur a great deal faster than current methods allow.

These microchip technologies would also boost large scale proteomic studies, which would be especially useful in treating cancer, since gaining information about the disease depends on understanding interactions between many proteins^{13,14}. For example, the rapid detection of the levels of thousands of human blood proteins at low cost could be realized in a minimally invasive at home diagnostic device for the early detection of cancer. By the same token, a related device could help diagnose a multitude of diseases in the third world, where access to healthcare is limited.

In both the academic setting and the biotechnology industry, processes that require entire laboratories could be scaled down to the size of a chip. At the same time, the extensive parallelization enabled by such small devices would vastly increase throughput.

1.2 Nanoscale Protein Sequencing Devices

As researchers have worked on elucidating the structures and functions of proteins, simple nanoscale devices have been designed toward sequencing these proteins faster and more efficiently, without the need for fluorescent labels and expensive optical devices for imaging. Some examples of these contraptions include fabricated nanopores¹⁵⁻¹⁷ (**Figure 1.1**) and nanofluidic channels¹⁸⁻²⁰ (**Figure 1.2**), as well as synthetic and naturally occurring nanoporous materials integrated into new designs²¹ (**Figure 1.3**).



Metal electrodes are usually attached to either side of the nanopores or nanochannels, and voltage biases between the electrodes modulate the electric field within. As these technologies are refined, it is anticipated that the interaction of the

electric field with charges along an unknown biomolecule could result in an electrical readout that would identify the analyte. Multiple efforts are currently underway on using these devices to sequence DNA molecules rapidly and in real time, with the expectation that entire genomes could be sequenced at low cost. The personal genetic codes obtained could advance personalized medicine by the creation of new drugs capable of treating far more specific illnesses, and they could help determine a person's predisposition to particular diseases.

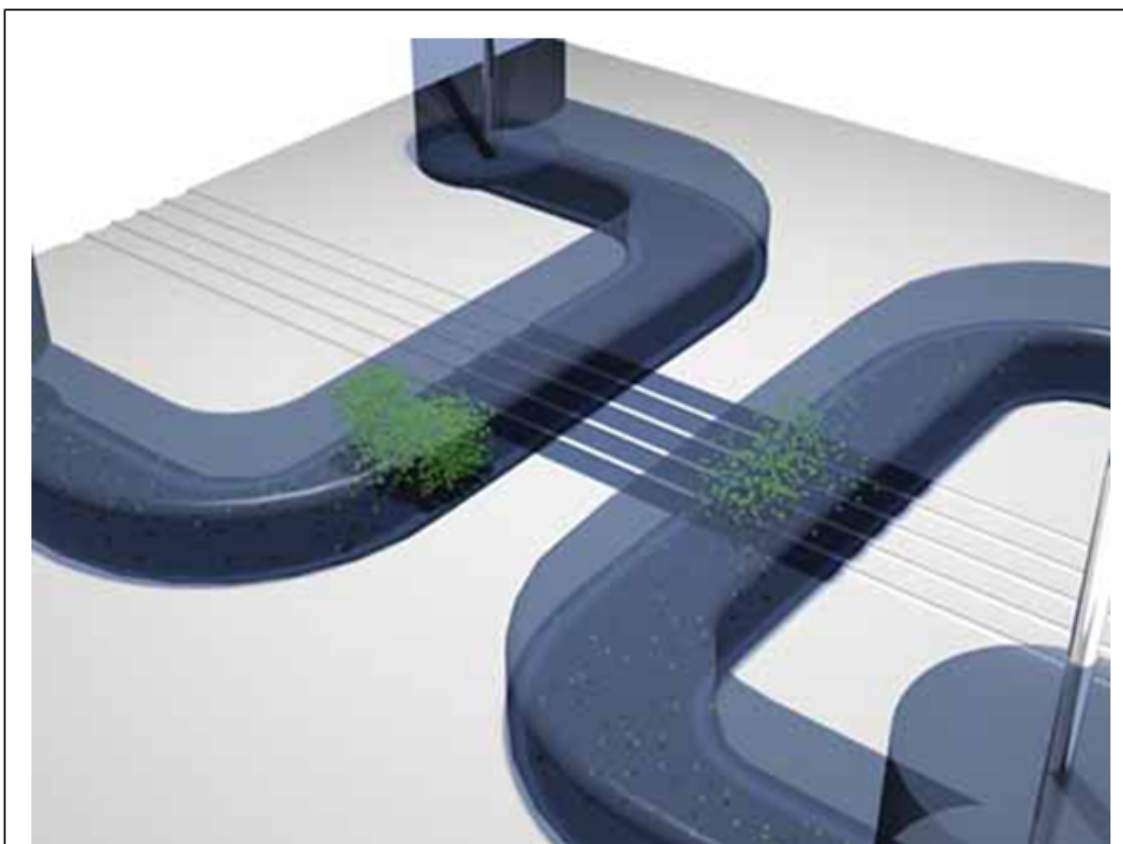


Figure 1.2. Schematic of an array of nanofluidic channels. Multiple nanochannels are positioned between two microfluidic channels. By inserting an electrically charged analyte into the fluidic system, current can be read across the nanochannels when a voltage is applied. Adapted from *Adv. Mater.* **2008**, *20*, 3011–3016.

However, as research into personalized medicine has progressed, it has moved toward identifying protein signatures to diagnose human diseases. Accordingly, in the near future, protein sequencing devices will have to follow suit and find applications in the analysis of proteins rather than DNA.

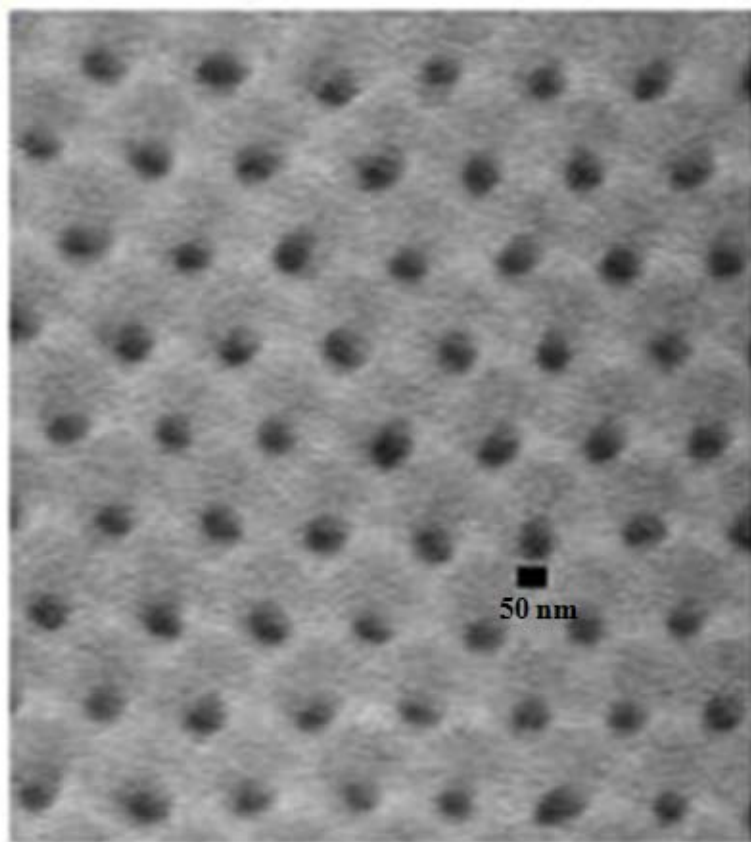


Figure 1.3. Nanoporous material sample. A dense matrix of well oriented porous channels in aluminum oxide is shown. *Courtesy of the Shpyrko Group, Dept. of Physics, UCSD.*

1.3 Plasma Protein Profiling for the Detection of Cancer

The rapid expansion of the field of proteomics has made it necessary to develop technologies capable of analyzing complex proteomes, specifically the levels and interactions of differentially expressed proteins in the human body. In particular, the ability to measure these on a systemic level in a noninvasive and cost-effective manner has brought great interest to the detection of cancer biomarkers found in the blood²²⁻²⁵.

The current gold standard for the high sensitivity identification and measurement of proteins found in plasma, the blood component in which proteins are dissolved, is the sandwich ELISA²⁶. In this assay, a layer of capture antibodies specific to the antigen of interest adheres to a solid support, usually contained within a 96 well microtiter plate. Another layer of detection antibodies conjugated to an enzyme “sandwiches” the antigen, and the addition of an enzyme substrate quantifies the level of antigen. Although the standard ELISA exhibits great sensitivity, its drawback is that it can only measure a relatively small number of analytes, as each well is devoted to one protein. Therefore, its limitations in cancer diagnostics are becoming more apparent as most cancers are now known to affect large panels of proteins rather than any single marker.

The recent advent of multiplexed platforms for protein detection, such as antibody microarrays (**Figure 1.4**), has shown great promise for large scale plasma protein profiling^{6,26-28}. The method of detection is similar to the ELISA, as antigens are sandwiched between two layers of antibodies. However, the capture antibodies are immobilized on-chip, using either microarray spotters or microfluidic flow patterning, to create spots a few hundred microns in diameter. Thus, it is possible to create hundreds of separate protein detection regions in an area equal to that of a typical ELISA well. Furthermore, the detection antibodies can be conjugated to fluorescent molecules or covalently linked to nanoparticles, resulting in even higher sensitivity. Streamlined variations of such multiplexed devices will prove vital in the early stage detection of multiple types of cancer.

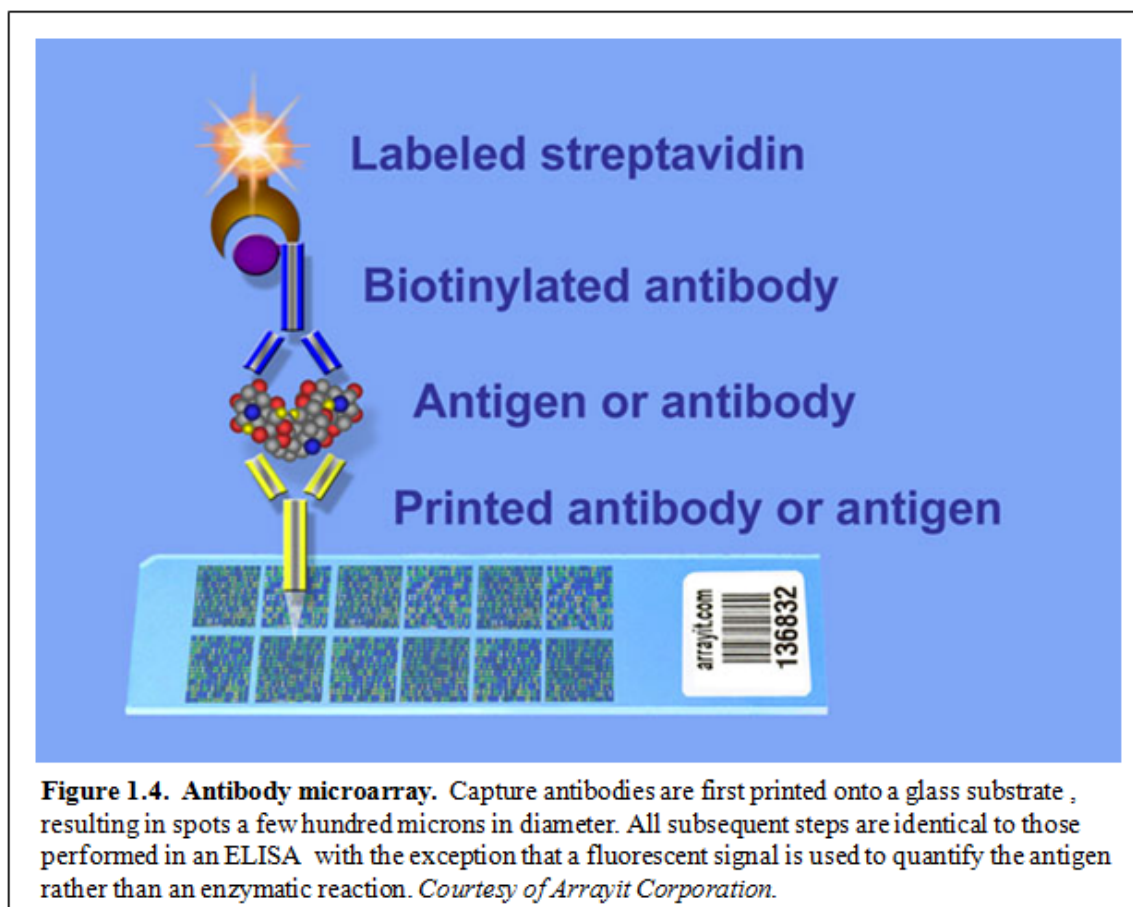


Figure 1.4. Antibody microarray. Capture antibodies are first printed onto a glass substrate, resulting in spots a few hundred microns in diameter. All subsequent steps are identical to those performed in an ELISA with the exception that a fluorescent signal is used to quantify the antigen rather than an enzymatic reaction. *Courtesy of Arrayit Corporation.*

1.4 Thesis Synopsis

The overall theme of this thesis centers on the development of new technologies for high throughput analysis of proteins. Chapter 2 begins with a description of the construction of silica nanofluidic channels using semiconductor fabrication methods and concludes with their applications to peptide sequencing. The entire nanochannel device consists of an array of 20 nm-wide, 20 μm -long channels in close proximity to a gate electrode along with microfluidic channels that cross perpendicularly at the nanochannel ends. Ionic solutions, such as potassium chloride (KCl), and peptide solutions, such as polylysine and polyaspartate, are flowed into the device, and source-drain biases are

applied across the nanochannels to study the characteristics of the current produced by the particular charged species. For KCl, at sufficiently low ionic strength, the Debye screening length exceeds the channel width, and ion transport is limited by the negatively charged silica channel surfaces so that only positively charged species can flow through the channels. At source-drain biases > 5 V, the current exhibits a sharp, nonlinear increase, with a 20 – 50-fold conductance enhancement. This behavior is attributed to a breakdown of the zero-slip condition. This phenomenon results in amino acid specific I-V traces when aspartate and lysine monomer solutions are added separately, which shows great promise for the use of nanofluidic channel devices for high throughput protein sequencing and structure determination. Chapter 2 has been taken in part from © *Nano Lett.*, **2009**, 9(4), 1315-1319²⁹.

Chapter 3 delves into the field of proteomics and presents a diagnostic device for the detection of differentially expressed proteins in the blood of patients with glioblastoma multiforme (GBM), the most common and aggressive class of brain cancer in humans. The device architecture improves on recently developed antibody microarrays in terms of device stability and longevity and consists of DNA encoded antibody libraries (DEAL), whereby orthogonal sets of anchor single-stranded DNA oligomers are spotted onto polylysine borosilicate slides and their complementary oligomers are conjugated to antibodies and hybridized to the anchor strands. Plasma samples from both GBM patients and healthy controls are positioned onto the array, followed by the addition of secondary antibodies and fluorescent markers to allow detection of the levels of cancer biomarkers. The chapter then discusses investigations into the effects of the vascular endothelial growth factor (VEGF) inhibitor Avastin on the

plasma protein profile of glioblastoma patients with actively growing tumors vs. non-growing tumors.

Lastly, Chapter 4 presents a variation on the DEAL technology for the creation of ultra-high density oligonucleotide arrays for the assembly of tissue engineered structures with single cell resolution. The creation of oligonucleotide spots $10\ \mu\text{m} \times 10\ \mu\text{m}$ in size and $30\ \mu\text{m}$ in pitch is achieved using a microfluidic flow patterning technique to arrange single-stranded bridge oligo sequences onto complementary anchor sequences. Cells are then coded with single-stranded oligos complementary to specific bridge sequences and allowed to hybridize. Single cell resolution patterns of human neurons and astrocytes and, separately, mouse pancreatic islet cells are illustrated and shown to be viable. The advantage of such a technology is that it has the potential to allow for the assembly of any tissue from scratch in virtually any pattern imaginable.

1.5 References

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