Proteomics Course

LECTURE-38
Nanotechniques in proteomics

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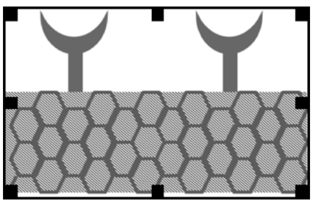
Previous lecture

- Detection techniques
  - Label-based vs. label-free
- Label-free techniques
  - Surface plasmon resonance
  - Ellipsometry
  - Interferometry

Lecture outline

- Nanotechniques for proteomic applications
  - Carbon nanotubes (CNT) and nanowires (CNW)
  - Carbon nanotube field effect transistors (CNT-FETs)
  - Quantum dots (QDs)
  - Gold nanoparticles and nanocages
  - Microfluidics
- Challenges of nanoproteomics

Carbon nanotubes (CNT) and nanowires (CNW)



Carbon nanotubes and nanowires

- CNTs are hollow, cylindrical graphite sheets which shows high chemical stability & mechanical strength
- Unique electrical, thermal and spectroscopic properties
- Unique features of CNTs and CNWs have opened up new perspectives for various proteomics applications

Carbon nanotubes

Single-walled (SWNT)

Composed of a single graphite layer, diameter 0.5-2 nm

Multi-walled (MWNT)

Composed of several concentrically arranged cylindrical layers

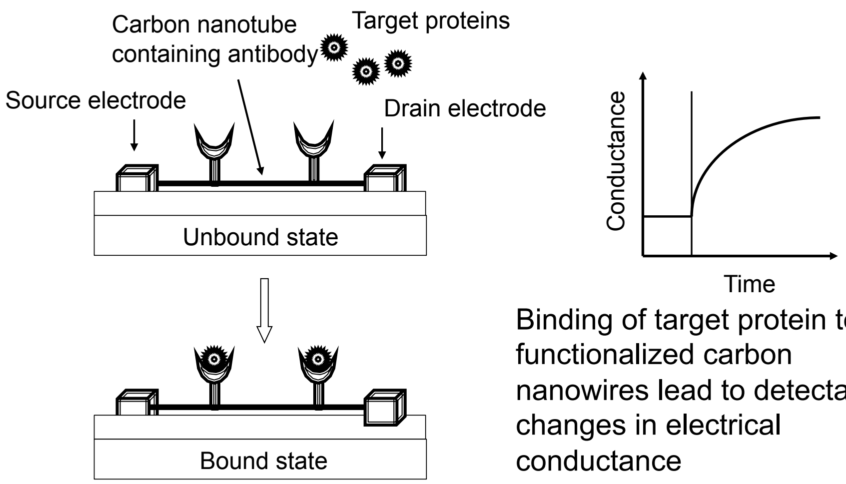
Carbon nanotubes

- SWNT - well-defined electrical and mechanical properties make them promising candidates for biosensors
- The application of SWNTs to proteomics relies on conductance change
  - binding of target protein to functionalized device shows changes in electrical conductance

Carbon nanoparticles: properties

- Very high potential for signal amplification
- Fast electron-transfer capabilities
- High surface area-to-weight ratio
- Selectively bind with biomolecules after functionalization
- High chemical stability and mechanical strength
- Change conductance upon binding of charged macromolecules

CNTs and CNWs



Binding of target protein to functionalized carbon nanowires lead to detectable changes in electrical conductance

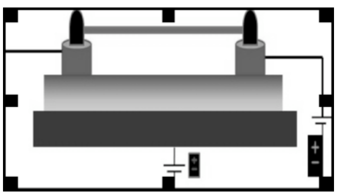
CNTs and CNWs

- Merits
  - Real-time analysis
  - Multiplexing
  - Miniaturization
- Demerits
  - Metallic impurities reduce activity
  - Difficult to determine degree of purity
  - Insoluble in biologically compatible buffers

CNTs and CNWs: applications

- Cancer markers detection
- Autoimmune disease detection
- Direct assay of human serum
- Toxin deactivation
- Biological detection and imaging

Carbon nanotube field effect transistors (CNT-FETs)



Carbon Nanotube Field Effect Transistors (CNT-FETs)

- SWCNTs which exhibit semiconductor properties are optimal to design CNT based electrical sensors
- Functionalization of CNT-FET with specific receptors brings about binding of the desired target biomolecules
- Conductance alteration of CNT-FET occurs due to charge modification of bound molecules

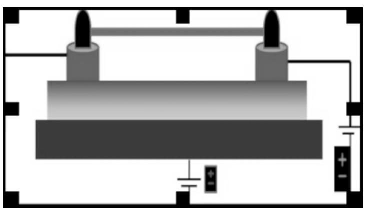
Carbon nanotube field effect transistors applications

- Detection of immunoglobulins
- Study of antigen and antibody reaction
- Detection of tumor markers
- Pathogen detection

Carbon nanotube field effect transistors

- Merits
  - Very high sensitivity
  - Real-time measurements
  - Label-free detection
  - Robust and cost effective
  - Extremely rapid
- Demerits
  - Lack of simple, flexible, well-established surface modification methods
  - Difficult to construct high density arrays

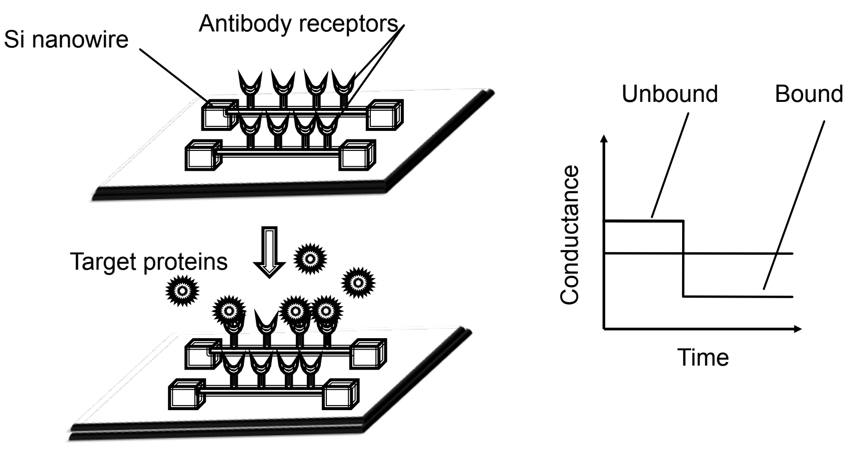
Silicon nanowire field effect transistors (SiNW-FETs)



Silicon nanowires: properties

- Excellent potential for signal amplification
- Fast electron-transfer capabilities
- Suitable for the immobilization of biological or chemical species
- Small size and large surface area-to-weight ratio
- Change conductance upon binding of charged macromolecules

Silicon Nanowire Field Effect Transistors (Si-NW FET)



Patolsky, et al. 2004. Proc. Natl. Acad. Sci. U.S.A. 101, 14017

Detection of low abundance proteins

- HT proteomic techniques such as protein microarrays provide high sensitivity
  - However, unable to detect very low concentration markers in small sample volumes
- Label-free detection of low abundance proteins is possible by carbon nanotubes and nanowires
- CNT based nanosensors are useful to target very low abundance protein analytes

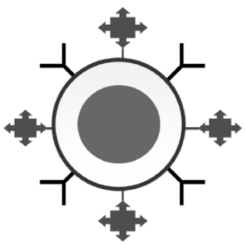
Silicon nanowire field effect transistors

- Merits
  - Real-time measurements
  - Multiplex analysis
  - Uniform and reproducible detection
  - High specificity
- Demerits
  - Lack of simple, flexible, well-established surface modification methods
  - Unsuitable for systematic studies

Silicon Nanowire Field Effect Transistor: applications

- Detection of cancer
- Detection of small molecule
- Study of small molecule interactions
- Virus particle detection
- Bio-sensing studies
- Bacterial toxin detection
- Therapeutics release technology

Quantum Dots



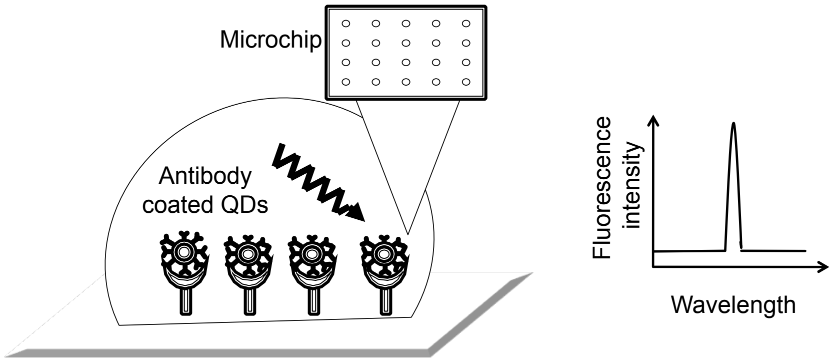
Quantum Dots (QDs)

- QDs are semiconductors whose excitons are confined in three spatial dimensions
- These inorganic fluorophores exhibit size-tunable emission, strong light absorbance, bright fluorescence, narrow symmetric emission bands, high photo stability
- Long life-time, high chemical and thermal stability, resistance to photo-bleaching, better fluorescence quantum yields of QDs overcomes the basic limitations of traditionally used organic fluorophores

QD properties

- Broad-range excitation
- Size-tunable narrow emission spectra
- Symmetric emission spectra
- Fluorescence lifetimes:10-100 ns
- Surface chemistry controls stability
- Suitable for labeling biomolecules
- Semiconductors

Quantum Dots



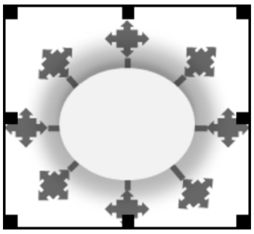
Quantum Dots

- Merits
  - High fluorescence quantum yields
  - Long life-time
  - High chemical and thermal stability
  - Resistant to photobleaching
  - Suitable for single molecule analysis
- Demerits
  - Toxic for cell
  - Mechanism incompletely known
  - Reproducibility of labels is limited

QDs: Applications

- Diagnostic imaging
- Cancer marker detection
- Study of DNA-protein interaction
- Study of motor protein motion
- Detection of antigen
- Tumor biopsy analysis
- MS studies
- Carbohydrate-protein interactions

Gold Nanoparticles and Nanocages



Gold Nanoparticles and Nanocages

- Gold nanoparticles (AuNPs) are considered as promising nanomaterials for cancer diagnosis due to their high signal enhancement capability
- Gold nanocages, are nanostructures with porous walls and hollow interiors
  - exhibit strong scattering and absorption peaks in near-infrared region

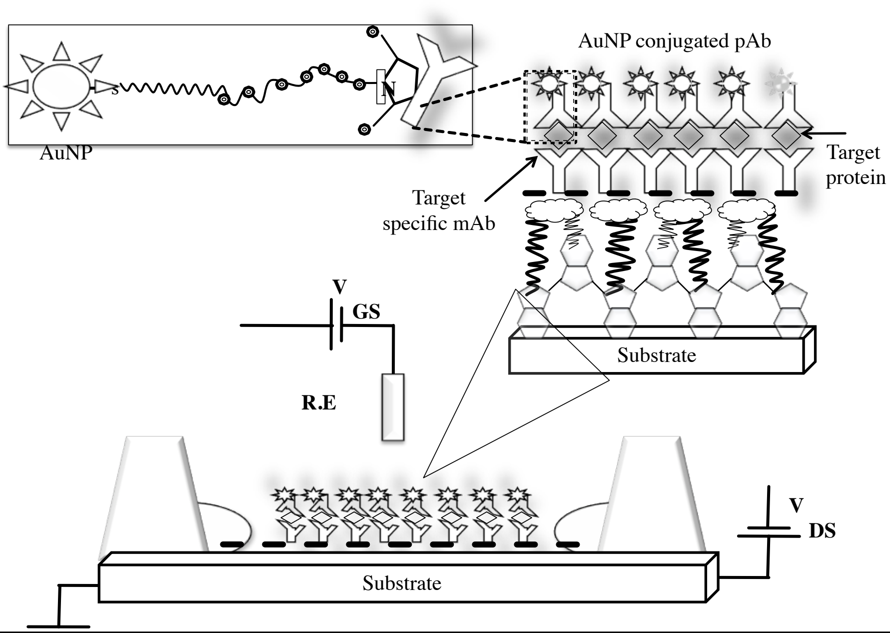
Gold nanoparticles and nanocages

- The surface modification of Au-NPs using different antibodies and molecular labels lead to the generation of surface-enhanced Raman scattering (SERS) response
- These modifications makes Au-NPs suitable for development of immunosensors for selective and ultrasensitive detection of protein biomarkers

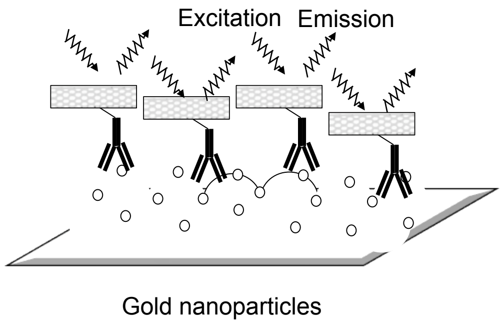
Gold nanoparticle and nanocages: properties

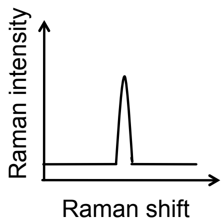
- Much smaller than the wavelength of light
- Strong scattering and absorption peaks in near-infrared region
- Narrow spectral bandwidth
- High potential for signal amplification
- Potential optical probes for reflectance-based optical imaging
- Change in spectra of scattered light on conjugation with biomolecules
- Easily conjugated to antibodies or peptides

Gold nanoparticles



Surface-enhanced Raman scattering (SERS)
Nanotubes functionalized with Raman labeled antibodies





Chen et al. 2008. Nat. Biotechnol. 26, 1285

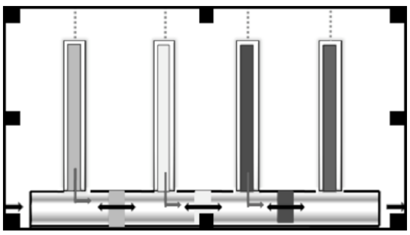
Gold nanoparticles and nanocages

- Merits
  - Narrow spectral bandwidth
  - Resistant to photobleaching and quenching
  - Simple detection systems
  - HT, multiplexed analysis
  - In vivo molecular imaging possible
- Demerits
  - Response highly dependent on shape and size of NP
  - Detection of molecules in complex solutions is difficult
  - Toxicity

Gold nanoparticles and nanocages applications

- Immunoassay studies
- Detection of cancer markers
- Detection of biomolecular interactions
- Photothermal destruction of breast cancer cells

Microfluidics



Microfluidics

- Microfluidics manipulates fluids at nano levels with help of channels having dimensions of tens to hundreds of micrometers.
- In addition to primary advantages of improved heat and mass transport, microfluidics offers other benefits like low reagent and sample consumption, multiplexed analysis, process automation and enhanced reproducibility

Microfluidics

- Microfluidic devices, coupled to MS, are emerging as an excellent tool for highly sensitive and simultaneous analysis of the complex proteome
- Another unique application of microfluidics is for printing protein microarrays

Microfluidics

- Merits
  - Improved heat and mass transport
  - Less reagent and sample consumption
  - Process automation
  - Improved reproducibility
  - Multiplexed analysis possible
- Demerits
  - High cost of chips
  - Non-specific interactions due to high surface to volume ratio
  - Highly sensitive detection devices required

Micofluidics applications

- Protein identification by MS
- Sample preparation for MALDI-MS or SPR analysis
- Immunological studies and protein interactions
- Cancer biomarker detection
- Enzymatic reactors
- Point-of-care diagnostics

Other promising nanotechniques for proteomic applications

Other nanotechniques

- Nanomechanical mass spectrometry
- Nanofluidics
- Microcantilivers
- Photonic microring resonators
- Detection methods employing inorganic and metal oxide nanoparticles
- Self-assembled cationic peptide nanoparticles and polymeric nanoparticles

Summary

- Nanotechniques
  - Carbon nanotubes (CNT) and nanowires (CNW)
  - Carbon nanotube field effect transistors (CNT-FETs)
  - Quantum dots (QDs)
  - Gold nanoparticles and nanocages
  - Microfluidics
- Diverse applications for proteomics

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