

## **SECTION 1: Principles of biochemistry, cell biology, physics and microelectronics**

(Lecturers: Dr D.Mastellos, Prof. E.Eliopoulos, Dr S.Gardelis, Prof. Y.Shacham)

### **Lecture 1.1: Cell biology principles** (Instructor: **Dr Dimitris Mastellos**)

- Introduction to the cell and its organization (from prokaryotes to eukaryotic cells, distinct cellular compartments, main organelles and related functions)
- Cell membranes - structure and topography
- Protein biosynthesis and turnover-regulation and degradation
- Cell signaling: relaying the signal from the cell membrane to the nucleus
- The cell cycle - deregulated cell division and cancer
- The immune system-General overview :
  - Innate and acquired immunity, target cells and main immune mediators
  - Antibody structure and function - B and T- cell receptors
  - Monoclonal antibodies - applications
- Towards model biological systems-Divergent signal integration within the cell

### **Lecture 1.2: Structure of biological macromolecules** (Instructor: **Prof. Elias Eliopoulos**)

- Introduction to building blocks of biological macromolecules
- Principles of protein structure
- Principles of DNA structure
- Sequence / Structure relationships. Structural motifs. Examples
- Introduction to in silico prediction of 3D-structure
- Structure / function relationships. Examples

### **Lecture 1.3: Microelectronic Materials and Device Technology**

(Instructor: **Dr Spyros Gardelis**)

- Semiconductors (What is a semiconductor? energy gap, conduction and valence band, electrons and holes, p-type, n-type)
  - Basic Devices (p-n junction, bipolar transistor, MOS transistor, MOSFET, CMOS technology)
  - Optical properties of semiconductors (direct and indirect band gap semiconductors, laser, photodetector)
- Applications of microelectronics in every day life
- Integration - miniaturization revolutionize the way we live (Moore's law)
  - Examples of devices used in every day life (logic devices, memories, lasers, displays, detectors, sensors (chemical, gas, bio-sensors))

### **Lecture 1.4: Introduction to nanobiotechnology / Sensors for nanobiotechnology**

(Invited speaker from N2L: **Prof. Yossi Shacham-Diamand**, Tel-Aviv University)

- Systems concepts- sources, signals, noise, sensors, front-end units, signal processing, storage and display
- Optical signals- fluorescence, bioluminescence, optical path design, sensors, solid state sensors, photon counters, system modelling
- Electrical and electrochemical sensing- DC and impedance methods, passive electrodes based interfacing, nano-electrodes, active electrodes - Field effect transistors interfacing
- Other sensing methods - magnetic sensors, magneto-optics, and more
- Examples - "cell on chip" toxicity bio-sensors

## SECTION 2: Core Nanobiotechnology methods and practices

### UNIT 2.1: Micro and Nano-fabrication science and technology

(Lecturers: Dr E.Gogolides, Dr A.Tserepi, Dr P.Argitis, Dr E.Makarona)

#### **Lecture 2.1.1: Conventional patterning schemes for hard substrates for bioanalytic microdevices** (Instructor: **Dr Evangelos Gogolides**)

- The top-down approach in nano fabrication. History of microfluidics, and motivation for going micro and nano for life sciences and chemistry
- Lithography fundamentals: Light Sources, Lithography Systems, Photoresists, Lithographic Processes
- Wet etching and Silicon micromachining
- Plasma etching and its mechanisms. Isotropic and Anisotropic etching
- Deep etching processes for Si bioanalytic microdevices
- Glass micromachining
- Novel Deep etching processes for polymer bioanalytic microdevices

#### **Lecture 2.1.2: Microfabrication technologies for plastic analytical microfluidics** (Instructor: **Dr Angeliki Tserepi**)

- Why use plastic substrates?
- Master Fabrication for plastic patterning
- Injection molding
- Hot embossing
- Soft lithography and variations thereof
- Other/ emerging methods
- Surface modification and sealing
- Examples of analytical microfluidic devices / systems

#### **Lecture 2.1.3: Patterning of biomolecules and other biological substances** (Instructor: **Dr Panagiotis Argitis**)

- The necessity for the patterning of biomolecules and other biological substances on solid surfaces
  - Chemical /physical binding of biomolecules on surfaces
  - Patterning methods :
    - microspotting, mechanical methods for delivery and synthesis, dip-pen lithography
    - microcontact printing methods (soft lithography related methods)
    - photochemical modification of SAMs, light guided DNA and protein synthesis
    - photochemical modification of polymeric films, photoresist-based methods
    - other / emerging methodologies
- Potential applications and comparison

#### **Lecture 2.1.4: Molecular Bioelectronics** (Instructor: **Dr Eleni Makarona**)

- Definition of “Molecular Bioelectronics”
- Goals - Motivation
- Challenges
- Historical background
- Discussion of electronic transport mechanisms in molecular systems
- Recent Advances and Applications:
  - Molecular Electronics
  - Biosensors / Biochips
  - Neuronal Interfaces
- Future Perspectives - Discussion

## UNIT 2.2 : Nanomaterials for bio-applications, Characterization, Imaging

(Lecturers: **A.** Prof. S. Antimisiaris, Dr K.Yannakopoulou, Dr I.Mavridis  
**B.** Dr I.Rabias  
**C.** Dr M.Bennink)

### A. DRUG DELIVERY METHODS/ MATERIALS

#### **Lecture 2.2.1: Drug Delivery and Targeting Systems - Focus on Liposomes**

(Invited speaker: **Prof. Sophia Antimisiaris**, University of Patras)

Why controlled release? Therapeutic Background and Rationale for Time control and Place control requirements. Liposome Technology. Applications in Therapeutics.

- Pharmacokinetic / Therapeutic Based considerations for type of control required
- General Methods to control Drug Release Kinetics (time) from Nanosized DDS (Liposomes or PN). Kinetics of drug release. Membrane - Matrix - Swelling controlled systems - Environmentally sensitive hydrogels, etc.
- Liposomes (Technology, Drug Release mechanisms, In vivo fate - Routes of Administration, Targeting)
- Comparison of Lipid-based and Polymer based systems; Special constructions
- Examples of Therapeutic applications (Targeting cancer, Brain targeting possibilities, Development of Drug-eluting Biomaterials)

#### **Lecture 2.2.2: Drug Delivery and Targeting Systems – Focus on cyclodextrin delivery, studied by NMR and XRD)**

(Instructors: **Dr Konstantina Yannakopoulou, Dr Irene Mavridis**)

- The basis of cyclodextrins as pharmaceutical excipients
- Inclusion complexes of cyclodextrins with drugs - Pharmaceutical applications
- Basics in NMR spectroscopy and X-ray crystallography as methods to study
- Cyclodextrin inclusion complexes

### B. NANOSTRUCTURED MATERIALS FOR BIOAPPLICATIONS

#### **Lecture 2.2.3: Magnetic Nanoparticles for Bioapplications** (Instructor: **Dr Ioannis Rabias**)

Experimental techniques for magnetic characterization of ferrofluids and Applications of ferrofluids in medicine

- A study in ferrofluids and their uses in nanotechnology
  - A review on what is a ferrofluid?
  - The origin of their magnetism, their stability as magnetic colloids
  - Synthetic methods and applications
  - Nature, properties and their high technological value
- Synthetic Routes for Hydrosol and Organosol colloidal fluids
  - Differences and similarities in these two approaches
- Applications of ferrofluids in medicine. Two case studies:  
1) Hyperthermia and 2) Contrast Agents for Magnetic Resonance Imaging (MRI)

Two applications of ferrofluids in medicine will be discussed:

- Hyperthermia covers a wide variety of techniques in which elevation of temperature in ferrofluids is achieved using low-frequency electromagnetic radiation. In this way, hyperthermia is a promising approach for cancer therapy, by locally heating a tumor without damaging the healthy tissues in the tumor surrounding
- Magnetic Resonance Imaging (MRI) is one of the most powerful diagnostic techniques in medicine. The use of superparamagnetic contrast agents based on ferrofluids, which enhance image contrast, will be discussed

## C. IMAGING OF BIOMATERIALS

### **Lecture 2.2.4: Imaging with Scanning Probes (AFM, STM, SNOM)**

(Invited speaker from N2L: **Dr Martin Bennink**, University of Twente)

- Scanning probe techniques
- Scanning tunneling microscopy
- Atomic force microscopy
- Near-field optical microscopy
- Optical tweezers force spectroscopy

## **UNIT 2.3 : Molecular and Cellular biology and Applications**

(Lecturers: **A.** Dr A.Vlahou, Dr S.Garbis

**B.** Dr S.Kakabakos, Dr P.Petrou

## A. PROTEOMICS AND ANALYSIS

### **Lecture 2.3.1: Gel-based protein analysis methods** (Instructor: **Dr Antonia Vlahou**)

- Definition and significance of proteomics research
- Introduction to protein separation methodologies: Principle of two-dimensional electrophoresis (2DE) and liquid chromatography
- Steps of analysis by 2DE : isoelectric focusing , vertical polyacrylamide gel electrophoresis, protein spot detection and quantification
- Principles of matrix-assisted laser desorption ionization mass spectrometry (MALDI-MS)
- Identification by peptide mass fingerprinting
- Coupling of 2DE with MALDI
- Protein profiling with MALDI MS analysis
- Applications of 2DE-MALDI MS in biomedical research
- Limitations-Avenues for improvement

### **Lecture 2.3.2: Non-gel based protein analysis methods** (Instructor: **Dr Spiros D. Garbis**)

- Separation of proteins and peptides with high performance liquid chromatography (HPLC)
- Combining two or more chromatographic chemistries for peptide/protein separation (multi dimensional HPLC approaches)
- Introduction of principles of electrospray ionization (ESI)
- Coupling micro- and nano-bore LC with mass spectrometry ionization sources (MALDI; ESI; nano-ESI)
- Introduction to the principles of tandem mass spectrometry using ion-trap, time-of-flight, quadrupolar mass analyzer designs and their variants
- Protein identification by tandem mass spectrometry of their peptide products
- Protein quantification by LC-MS analysis of isotope-labeled peptide products
- Applications of LC-MS to biomedical research
- Limitations-Avenues for improvement

## B. ASSAYS AND ARRAYS

### **Lecture 2.3.3: Binding Assays and Immunosensors** (Instructor: **Dr Sotirios Kakabakos**)

- Binding assay principles
- Binder molecules
- Antibodies: structure and production
- Labels and labelling procedures
- Standard solutions for quantities determinations
- Surface immobilization of biomolecules
- Configurations of binding assays
- Signal amplification systems
  
- What is a (immuno)sensor?
- Main immunosensor configurations
- Principles of optical immunosensors
- Examples of optical immunosensors developed at NCSR “Demokritos”

### **Lecture 2.3.4: DNA and Protein arrays: fabrication, detection and applications** (Instructor: **Dr Panagiota Petrou**)

- What is a DNA array?
- The DNA molecule: structure and biological significance
- DNA analysis by conventional methods (Southern Blotting)
- DNA arrays versus DNA chip
- Fabrication of DNA arrays and chips
- Target DNA amplification and labelling by PCR
- Hybridisation and detection formats
- Main applications of DNA arrays
- Limitations of DNA arrays
- Perspectives
  
- What is a protein array?
- Protein structure & sources
- Formats and surfaces for protein arraysImmobilization of proteins onto solid surfaces
- Fabricationof protein arraysDetection schemes for protein chipsAntibody arrays: specificity and cross-reactivity Areas of application
- Challenges and bottlenecks
- Perspectives

## **SECTION 3: Towards Integrated Nanobiotechnology systems**

(Lecturer: Dr K.Misiakos)

### **Lecture 3.1: Principles of Integrated Biosensing Devices** (Instructor: **Dr Konstantinos Misiakos**)

- ISFETs
- Impedance Spectroscopy Devices
- SAW Devices
- Enzymatic Detection
- SPR Resonance
- Interferometric Devices

## HANDS ON EXPERIENCE - LABORATORIES

### SESSION 1

Choose 3 out of 6 laboratories

#### **Laboratory 2.3.8: State of the art fluorescence imaging & confocal microscopy of biological samples**

(Instructor: **Dr Stamatis Pagakis**, Bioacademy)

##### **Aim of the Lab:**

- Familiarize students with a fluorescence microscope
- Demonstrate the design differences which characterize a confocal microscope
- Demonstrate Image processing and 3D visualisation software

##### **Content:**

- Introduction to Fluorescence imaging and 3D image visualization using confocal microscopy
- Two microscope workstations (one confocal) will be used
- Observation of fluorescent proteins (e.g YFP, GFP) and other fluorophores common in fluorescence microscopy
- Hands-on experience and data acquisition on a digital camera fluorescence microscope
- Demonstration of 3D data acquisition using a confocal microscope
- Imaging methods for image deconvolution, 3D reconstruction and data analysis will be demonstrated on computer workstations with specialised software packages

#### **Laboratory 2.3.1: Protein separation by two-dimensional electrophoresis**

(Instructor: **Dr Antonia Vlahou**, Bioacademy)

##### **Aim of the lab:**

- Familiarize attendees with the isoelectric focusing technology and vertical polyacrylamide gel electrophoretic systems
- Familiarize attendees with automated technologies for spot picking and processing for peptide mass fingerprinting
- Demonstrate the processing of a cell extract by the 2DE methodology

##### **Content:**

- Processing of cell extract on IPG strips for first dimensional separation
- Application of IPG strips on PAGE gels
- Gel staining, scanning and processing for spot excision
- Tryptic digestion of excised spots

#### **Laboratory 2.3.2: Mass spectrometry**

(Instructor: **Dr Spiros D. Garbis**, Bioacademy)

##### **Aim of the lab:**

- Familiarize attendees with mass spectrometry technologies (MALDI; ESI QqTOF; ESI QIT)
- Demonstrate tryptic peptide analysis by MALDI-MS; LC-ESI-QqTOF MS; LC-ESI-QIT)
- Familiarize attendees with database searches for protein identification
- Familiarize attendees with the custom preparation of capillary LC columns to be used for the LC-ESI-MS based methods

##### **Content:**

- Tuning and calibration of mass spectrometers (MALDI; ESI QqTOF; ESI QIT)
- Application of tryptic peptide extracts and proteins and matrix on MALDI targets
- MALDI MS analysis of peptide and protein samples
- Sample preparation for LC-ESI-MS
- LC-ESI-MS-MS analysis of peptide extracts
- Database search methods

**Laboratory 2.3.3: Fabrication of protein microarrays using nanoplotter**

(Instructor: **Dr George Tsangaris**, Bioacademy)

- Microspotting methods for the fabrication of microarrays : presentation of nanoplotter and familiarization with its use

**Laboratory 2.3.6: Bioinformatics basic theory & laboratory**

(Instructor: **Dr Sophia Kossida**, Bioacademy)

**Aim of the lab:**

- Familiarize attendants with biological databases
- Familiarize attendees with software for genome analysis
- Familiarize attendees with software for proteome analysis

**Content:**

- Introduction into Bioinformatics
- Query and retrieve info from biological databases
- Analyze genes/ genomes with software packages
- Analyze peptides/ proteins with software packages

**Lecture 2.3.7: Structural Bioinformatics: Molecular Simulations and Visualization**

(Instructor: **Dr Georgios Spyrou**, Bioacademy)

- Applied Bioinformatics in BioNanoTechnology
- Bioinformatics tools for Molecular Simulation and Visualization

**SESSION 2**

Choose 3 out of 4 laboratories

**Laboratory 2.1.1: Fabrication of microfluidic devices on plastic substrates by soft lithography and deep polymer plasma etching**

**+2.1.2**

(Instructors: **Dr Angeliki Tseripi**, **Dr Evangelos Gogolides**, IMEL)

**Aim of the lab:**

- Familiarize attendants with conventional photolithography
- Familiarize attendees with replica molding / soft lithography
- Demonstrate the fabrication and sealing of a PDMS microfluidic device
- Familiarize attendees with the ICP plasma reactor, its operation and diagnostics, as a tool for microfluidics and surface modification
- Perform plasma etching of PMMA microchannels and observe results

**Content:**

- Fabricate mold on SU8
- Fabricate microfluidic channels by PDMS replica molding on SU8 mold
- Device Sealing
- Demonstration of the plasma reactor (ICP) and diagnostics
- Patterning of microchannels on PMMA using the ICP
- Sealing of microfluidic channels and measurement of flow properties of the microfluidic

**Laboratory 2.1.3: SPM Techniques for molecular devices**

(Instructors: **Dr Eleni Makarona**, **Dr Dimitrios Velessiotis**, IMEL)

**Aim of the lab:**

- Familiarize attendants with SPM Techniques (AFM, STM) for molecular systems characterization
- Discuss how the molecular properties can be connected to the device behavior and design

**Content:**

- Demonstration of AFM, STM measurements
- Analysis of experimental data and extraction of information for the demonstrated systems

#### **Laboratory 2.3.4: Fabrication of protein microarrays using lithography**

(Instructor: **Dr Antonis Douvas**, IMEL)

- Photoresist processing fundamentals : processing of chemically amplified photoresists
- Lift-off process : use of lift off process for the patterning of SAMs or proteins, and (optional)
- Patterning of different types of proteins on the same substrate by successive lithographic steps under biocompatible conditions

#### **Laboratory 2.3.5: Fluorescence detection of protein arrays**

(Instructor: **Dr Panagiota Petrou**, IRRP)

##### **Aim of the lab:**

- Familiarize the attendees with optical detection of protein arrays
- Demonstration of the epifluorescence microscope
- Image processing for quantitative fluorescence measurements

##### **Content:**

- Immunoreaction of protein in spots created by photolithography with fluorescently labeled molecules
- Observation of protein spots with the epifluorescence microscope
- Capture of images of the arrays
- Image processing to receive quantitative results

#### **Laboratory 3.1: Operation of a lab-on-a-chip optical device using model assays and real time measurements**

(Instructor: **Dr Konstantinos. Misiakos**, IMEL)

- Integrated biochip wafer alignment on wafer prober
- Protein/DNA spotting on integrated waveguides
- Washing and microfluidic device application on biochip
- Protein/DNA real time assays through photocurrent monitoring

### **SESSION 3**

**Choose 2 out of 3 laboratories**

#### **Laboratory 2.2.2: Drug inclusion in cyclodextrins: monitoring in situ by NMR spectroscopy X-ray diffraction characterisation of drug inclusion and 3-D visualisation**

(Instructors: **Dr Konstantina Yannakopoulou**, **Dr Anastasia Paulidou**, IPC)

- Determination of the formation of an inclusion complex of  $\beta$ -cyclodextrin and the drug piroxicam in situ by NMR spectroscopy
- Determination of the formation of the same inclusion complex ( $\beta$ -CD/piroxicam) in the crystalline state by powder X-ray diffraction
- Molecular docking of the drug inside the cyclodextrin cavity

#### **Laboratory 2.2.1: Liposomes: preparation and characterisation by dynamic light scattering and $\zeta$ -potential**

(Instructors: **Dr Dimitrios Tsiourvas**, **Dr Zili Sideratou**, IPC)

##### **Aim of the lab:**

- Familiarize attendees with liposome preparation
- Perform standart liposomal characterization techniques



**Content:**

- Preparation of anionic and cationic liposomes of 50 and 100 nm diameters employing the extrusion method
- Introduction to Dynamic Light Scattering (theory and applications)
- Dynamic light scattering experiments for the determination of the size and polydispersity of liposomal formulations
- Introduction to  $\zeta$ -potential (theory and applications)
- $\zeta$ -Potential measurements for the determination of the surface charge of liposomal formulations

**Laboratory 3.2: Demonstration of a capillary fluoroimmunosensor**

(Instructor: **Dr Sotirios Kakabakos**, IRRP)

**Aim of the lab:**

- Familiarize attendees with optical immunosensors
- Demonstration of multi-band capillary fluoroimmunosensor
- Real-time monitoring of the immunoreaction

**Content:**

- Creation of distinct reaction bands in a single capillary
- Performance of immunoreaction in the capillary
- Demonstration of fluorescent bands detection using the prototype optoelectronic set-up in real time or after assay completion
- Data processing and interpretation of the results