

The course aims at providing the PhD students of the School in Molecular Science an up-to-date overview of the theoretical approach in studying drug-receptor interactions and of the chemical, biochemical and physical methods currently used for quantifying macromolecular interactions.

1) Generality on the structure and function of proteins as targets of drug activity: soluble receptors and nuclear receptors, membrane-bound receptors, G protein-coupled receptors, integrins, enzymes as key targets of drugs, DNA as a receptor for innovative drugs. 2) Theoretical aspects for describing drug-receptor interactions: rigid-body mechanism, adaptive mechanism, population-shift mechanism, implications of receptor and ligand conformational flexibility in drug discovery. Analysis of binding data: the Langmuir model of single binding, the tight- and slow-binding model, multiple equivalent and nonequivalent binding, model for allosteric interactions. Derivation of thermodynamic quantities of ligand binding using the van't Hoff treatment.

2) Experimental methods for monitoring and quantifying the strength of ligand-receptor interaction (a personal classification). • Molecular biology methods (two-hybrid systems, enzyme-linked immunosorbent assays, micro-chips and micro-arrays). • Chemical methods (affinity chromatography, size-exclusion chromatography; native electrophoresis, chemical cross-linking, "native" mass spectrometry techniques and Hydrogen-Deuterium Exchange mass spectrometry, HDX-MS) • Physical methods (equilibrium dialysis, ultracentrifugation at equilibrium, dynamic and static light scattering, surface plasmon resonance, calorimetric methods) • Spectroscopic methods (differential UV absorption and second-derivative UV spectroscopy, far- and near-UV circular dichroism, fluorescence spectroscopy, fluorescence anisotropy) • Biochemical methods (determination of the kinetic constants k_{cat} and K_m of enzyme activity, determination of the inhibition constant K_i of reversible competitive, noncompetitive and uncompetitive inhibitors).

3) Hands-on experience (4 hours): determination of binding constants by SPR, calorimetric and fluorescence techniques.

Teacher: DE FILIPPIS Vincenzo

Curriculum: Pharmaceutical Sciences

Duration: 24 hours