Progetti di Tesi – Stefano CIURLI

Ambito: Chimica Bioinorganica, Spettroscopia NMR Biomolecolare

**Progetti da svolgere a Bologna (1 studente)**

Studi cinetici e strutturali di inibizione dell’ureasi un enzima nichel-dipendente e con un ruolo chiave nel metabolismo dell’azoto.

L’attività enzimatica dell’ureasi ha un impatto sia in patologie umane e animali che nella gestione eco-sostenibile della fertilizzazione azotata nell’ambito agro-ambientale. Lo studio implica metodologie di purificazione di proteine da fonti biologiche naturali o con tecniche di DNA ricombinante, studi della cinetica enzimatica in presenza e assenza di inibitori con metodi calorimetrici e spettrofotometrici, screening in silico ed in vitro di librerie di inibitori, cristallizzazione di complessi proteina-inibitore e risoluzione di strutture proteiche tramite tecniche di diffrazione di raggi X. Questo studio è completato con l’analisi biochimica e strutturale di proteine accessorie coinvolte nell’attivazione dell’ureasi, effettuata con tecniche cristallografiche, con spettroscopia NMR e con metodi computazionali.

**Progetti da svolgere in collaborazione con il Dr. Frans A.A. Mulder, Associate Professor of Biological NMR, Interdisciplinary Nanoscience Center (iNANO) and Department of Chemistry, Aarhus University (numero di studenti da concordare).**

1. Development of NMR methods

The study of protein chemistry strongly relies on the methods that scientists have developed over time. Just think of Bragg diffraction and protein 3D crystal structure, nuclear spin (NMR) and solution protein structure, van Leeuwenhoek’s lenses to the development of microscopes that can image structures down to nanometers. In my group we actively develop NMR spectroscopy as a tool to understand protein function through measurement. In this way we can understand the chemistry of enzyme catalysis (using NMR to determine pKa constants of active site residues) and are investigating protein entropy by studying the dynamics of side chains. In this project you will learn about protein biophysics, NMR software and hardware, and how to develop your own NMR pulse sequences for a chosen application.

1. NMR studies of cellular metabolism

Much of what we know about cellular metabolism can be found in biochemistry textbooks. But how do we know all that? In the past, much of the metabolic conversions were monitored by specific assays for specific compounds. Nowadays we possess techniques like mass spectrometry and NMR spectroscopy to identify and quantify chemical compounds from biological specimens. But how can we monitor metabolism in live cells? As we deal with small molecules we cannot modify or ‘tag’ them with fluorophors. Fortunately, 1H and 13C NMR spectroscopy present non-invasive ways to bring all molecules inside cells into view. In this project you will set up a system for making real-time NMR observations of cellular metabolism in various model systems, such as bacterial and yeast cells.

1. NMR studies of intrinsically disordered proteins and protein mis-folding

Proteins always fold into well-ordered 3D structures. At least, so we ‘believed’ until not so many years ago. The discovery of intrinsically disordered proteins (IDPs) makes us revise the dogma of structural biology. In fact, protein disorder and dynamics now appear very widespread and bring functional advantages for protein-protein interactions that are vital to function. In my group we therefore study IDPs in their own right. We use NMR spectroscopy to do this, since IDPs do not form crystals. In addition, we study protein disorder by NMR and bioinformatics since proteins can misfold and aggregate, a process that underlies diseases from systemic amyloidosis to Parkinson’s and Alzheimer’s disease. These diseases cannot currently be cured, as we still don’t understand their actual cause. In this project we aim to find out more about IDPs and protein self-association.