UNIVERSIDAD DE GRANADA

Departamento de
Química Física

Intereses de investigación

- Our group is interested in the thermodynamic and kinetic characterisation of protein folding-unfolding, protein stability, protein misfolding and aggregation and protein-ligand interactions.

Protein folding, misfolding and aggregation

- Protein folding is an essential step in the translation of genetic information into biological activity and understanding protein folding remains one of biology's grand challenges as important today as ever. It is still not possible to predict how an extended polypeptide sequence will fold. The majority of protein sequences are capable of folding spontaneously to their native conformation. However, some fraction of newly synthesised proteins in cells misfolds and is either degraded or aggregates. To understand essential aspects of the “code” that links a protein’s final tertiary structure to its amino-acid sequence it is critical to learn not only why certain sequences fold, but also why alternative sequences do not.

- The failure of proteins to fold, or to fold incorrectly can result in disease. Some 20 sporadic, genetic or infectious diseases in humans, including Alzheimer's disease, light-chain amyloidosis and the spongiform encephalopathies, originate when certain proteins misfold and aggregate into insoluble fibres or plaques, often know as amyloids. A prerequisite for the development of approaches to predict, prevent or to block amyloid formation is an understanding at an atomic level of the processes and interactions that lead to their formation. In particular we must understand the nature and structural features of the precursor states and the factors that promote aggregation.

- The main goal of our research is to understand in detail how interactions between amino acid residues in a polypeptide chain lead to the kinetic and thermodynamic control of protein folding. The usual approach is to characterize by a variety of biophysical techniques the folding and unfolding of proteins induced by changes in temperature or concentrations of denaturants. The aim of these experiments is to obtain as much thermodynamic and kinetic information as possible about these processes. These methodologies are supported by the use of protein engineering methods to design mutant and

chimeric proteins, which allow to examine the effects produced by the introduction of particular interactions of changes in the topology of the proteins under study.

- The techniques used in these studies are mainly calorimetric, including both differential scanning calorimetry (DSC) and isothermal titration calorimetry (ITC). Our group is pioneer in the development of mathematical models for the analysis of DSC data from denaturation of proteins involving reversible oligomerisation as well as irreversible aggregation processes.

- These calorimetries are complemented by a variety of spectroscopic techniques, such as circular dichroism (CD), fluorescence, Fourier Transform Infrarred spectroscopy (FT-IR) and high-resolution nuclear magnetic resonance (NMR). Amide hydrogen-deuterium exchange monitored by two-dimensional NMR is also being used to study the residue-specific stability of proteins.

Protein-ligand interactions

- Proteins work by interacting with specific ligands, which include other proteins. It is essential to investigate the various interactions that occur between the groups involved in the binding processes and to study the effects of these reactions on the co-operativity and dynamics of proteins in order to arrive at a full understanding of molecular recognition and the rational and efficient design of ligands for both therapeutic and biotechnological purposes. Undertanding the rules governing molecular recognition is of crutial importance for rational drug desing.

- The aim of our research is to increment our knowledge about the thermodynamic factors involved in protein-ligand interactions. Our approach is multidisciplinary, combining biophysical chemistry and molecular biology to study the protein-ligand binding.

- At present we are interested in acquiring a deep understanding of the folding properties and the determinants of binding affinity and specificity for the different families of proline-rich recognition modules (PRM) and PDZ domains. This research is focused in representative domains of different families of special biomedical relevance, such as: SH3 domains from c-Src, c-Yes, Fyn and Abl oncogenes, WW domains from Yap65 oncogen, WW and UEV domains from Nedd4 and Tsg101, and PDZ domains from PSD-95, ZO2 and nNOS proteins. These domains act as molecular recognition sites for other proteins and play an important role in pathological processes such as cancer and AIDS, among others. Their smallness (between 60 and 80 residues), stability, ease of folding, variety and function all contribute to making them particularly suitable

candidates for these studies.

- The methods we intend to use include calorimetry (scanning and isothermal), spectrophotometry (UV-visible, fluorescence, CD and FTIR) and H/D-exchange monitored by NMR, plus recombinant DNA techniques. Our prime intention is to make a precise thermodynamic analysis of the interactions between mutant chimeric SH3 domains and specifically designed ligands. In the long term we hope to obtain sufficient thermodynamic information to be able to design efficiently and rationally specific ligands and establish a versatile methodology capable of being applied to other protein-ligand interactions, including the de novo design of ligands.