

El titulo de la tesi: In-silico methods to
drug discovery

El subtítulo de la tesi: Cancer

Autor: Francisco Martínez Jiménez

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DIRECTOR DE LA TESI

Director: Marc A. Martí-Renom Departament Departament:
Biomedicine



A mi madre.

Agradecimientos Agraexio....

Abstract

This is the abstract of the thesis in English. Please, use less than 150 words.

Resum

Vet aqui el resum de la tesi en catala.

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Summary

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Preamble

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Index

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CAPÍTOL 1

INTRODUCTION

1.1 Protein are essential molecules

The importance of proteins in biological chemistry is just reflected by their name, derived from the Greek word *proteios*, and that means "of the first rank"¹. Their presence is so essential that they constitute most of the cell dry mass [1]. They are not only the cell's building blocks, but also they perform nearly all the cell's functions. Some roles of proteins include serving as structural components of cells and tissues (e.g., *keratin* or *collagen*), transmission of information between cells by hormones such as the *insulin* or the *oxytocin*, facilitating the transport and storage of small molecules (e.g., the transport of oxygen by *hemoglobin*) or providing a defense against foreign invaders (e.g., antibodies). Other proteins such as the *actin* and the *myosin* are responsible of muscle contraction and therefore our movement. However, the most fundamental role of proteins is their ability to act as enzymes, which, catalyzes most of the chemical reactions in biological systems. In summary, proteins are crucial macromolecules present in most of the processes carried out by the cell and, in spite of being extensively studied for many years, they still have many unanswered questions.

1.1.1 Protein structure

A protein is a molecule made from a long chain of amino acids linked thorough a covalent peptide bond. Proteins are therefore also known as *polypeptides*. At-

¹The term protein was coined by Jons Jacob Berzelius in 1838. It was first used by Gerardus Johannes Mulder, advised by Berzelius, in its publication *Bulletin des Sciences Physiques et Naturelles en Néerlande* (1838). pg 104. *SUR LA COMPOSITION DE QUELQUES SUBSTANCES ANIMALES*, where he observed that all proteins seemed to have the same empirical formula and came out to the erroneous idea that they might be composed of a single type of very large molecule. Berzelius proposed the name because the material seemed to be the primitive substance of animal nutrition that plants prepare for herbivores.

tached to this repetitive chain are those portions of the amino acids that are not involved in the covalent bond, the **side chains**. Side chains confer the different physico-chemical properties of each of the 20 types of amino acids [2]. The composition of the amino acid sequence determines the function and the structure of a protein. That is because the unique sequence creates a specific pattern of attractive and repulsive forces between amino acids along the polypeptide that leads to a folding process resulting in a specific three-dimensional structure. These forces are usually non-covalent interactions between the side chains of the amino acids. Non-covalent interactions are weaker than covalent ones, allowing the folded structure to certain degree of conformation mobility i.e: to be dynamic. This phenomenon is really important to facilitate the interaction with other molecules as we will explore further in 1.1.3.

Protein structures are complex conformation of atoms organized in a hierarchical manner 1.1. The first level of this hierarchy, referred to as the **primary structure**, is the ordered sequence of amino acids of the polypeptide. Certain segments of these chains, tend to form simple shapes such as helices, strands, turns or loops. These folding patterns are referred to as secondary elements and collectively constitute the **secondary structure** of the protein. The two most frequent type of secondary elements are the α -helices and the β -sheets [3]. The overall chain tends to fold further into a three-dimensional **tertiary structure**. Contrary to the secondary structure, the tertiary structure folding is driven by interactions from amino acids far apart in the primary sequence. The tertiary structure, is generally the most stable form of the protein, that is, the one that minimizes its free energy [4]. Furthermore, the tertiary structure is also the biologically active form of the protein, and its unfolding usually leads towards partial or total inactivation of the protein. Finally, some proteins are composed by multiple folded chains. In such cases, each folded subunit folds independently and then joins the others forming a biologically active complex. This type of organization is considered as the **quaternary structure**.

This traditional paradigm of protein structure has been challenged by some exceptions of proteins that lack of a fixed or ordered three-dimensional structure. The intrinsically disordered proteins (IDPs) cover a wide spectrum of states from fully unstructured to partially structured including conformations such as *random coils* or *molten globules*. Moreover, some factors may lead to the permanent loss of structure of a protein, and when that occurs, they endanger the entire organism. How problematic protein misfolding can be for the organism is illustrated by examples such as cystic fibrosis, Alzheimer's, Parkinson's and Huntington's diseases.

Figure 1.2 from the seminal paper [5] shows the correlation degree to which protein structures changed as a function of sequence divergence. This work helped to set up the fundamentals of what is considered a central paradigm in protein evo-

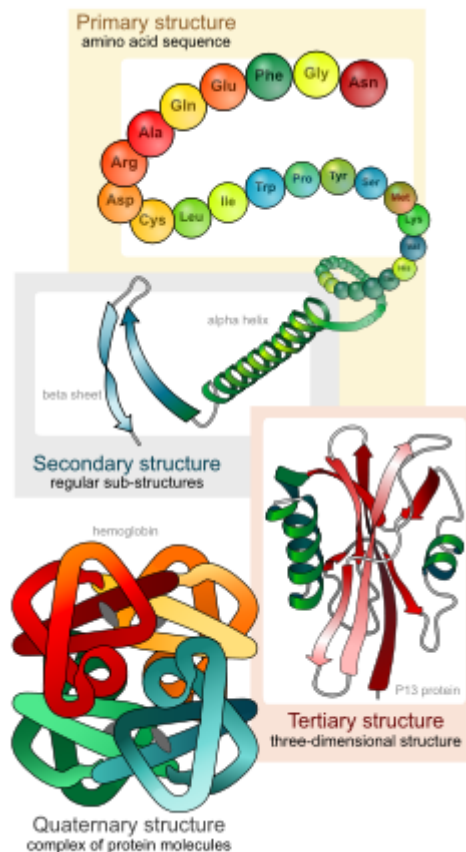


Figure 1.1: Hierarchical distribution of layers in protein structure

lution: protein structure is more conserved than sequence. However, not all the regions in a protein structure are equally conserved. It's been shown that functionally important amino acids, responsible of the interaction with other molecules, are more conserved than the rest of the protein structure [6]. Additionally, the structural core is more conserved than the surface [7]. The high conservation of the core enables the protein to maintain the global shape, while the surface is free to change (i.e. to mutate) some functional features [8]. These evolutionary mechanisms are in accordance with the central *sequence* \rightarrow *structure* \rightarrow *function* paradigm that prevails in the protein evolution field.

Protein Structure Determination

Since in 1960, the British biochemist John Kendrew determined the myoglobin structure [9], more than 37,000 different protein structures have been deposited in the Protein Data Bank (PDB) [10]. The PDB is a repository created in the

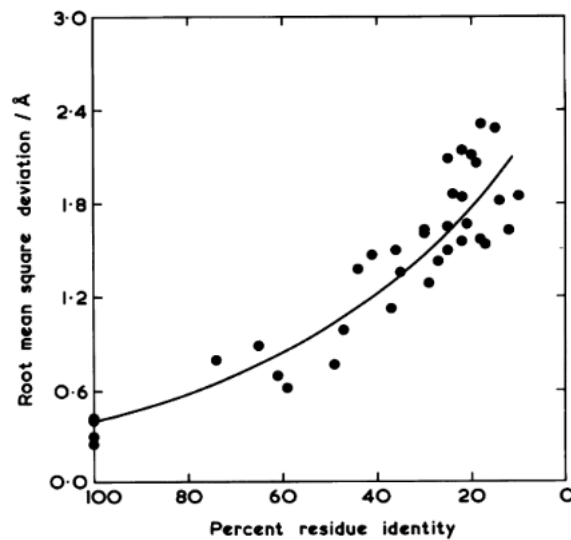


Figura 1.2: The original plot of the relation of residue identity and the r.m.s. deviation of the backbone atoms of the common cores of 32 pairs of homologous proteins. Figure extracted from [5]

1970s with the aim of storing all the 3D protein structures and unifying their format. Figure shows the variation of the number of deposited structures over the time. The number of PDB structures has significantly been increased over the last years thanks to initiatives such as the Protein Structure Initiative (PSI) [11] or the structural genomics [12]. The later, was born with the aim of determining the structure of all human proteins. However, soon after, they realized that the goal was unrealistic. Fortunately, the number of folds which represent the complete "fold space" observed in nature is much smaller than the number of proteins. Therefore, the current goal is to determine the structure of a representative set of proteins, that is, at least one protein per fold class. Once it is known the structure of one representative protein, and thanks to the *homology modeling* methods, it is usually feasible inferring the structure of other proteins belonging to the same fold class as we will explore in the next section 1.1.1.

Methods.

To talk about bias in globular vs. membrane.

Protein Structure Prediction

Plot proteins predictable with homology vs. only structure determination.

1.1.2 Protein function

The major question in the protein biology field has been to understand the protein sequence, structure, function relationship. It is known that structure of a protein determines its biological function. However, different *regions* of the structure can perform semi-independent functions from each other. These regions are referred to as **protein domains**. A domain is substructure produced by any part of polypeptide chain that can fold independently into a compact and stable structure [13, 14, 15]. Domains on average contain 80-250 residues [16]. Estimates of the number of domains per protein say that more than 70% of prokaryotic proteins and 80% of eukaryotic proteins include more than one domain [17, 18]. Among this multi-domain proteins, 95% of them contains only two to five protein domains [17]. Domains are not only the basic functional units of proteins, but also the evolutionary units of protein evolution. As proteins have evolved, domains have been modified and combined to build new proteins [19, 20]. Such is the importance of domains in protein evolution, that they have been included in current protein classification methods as one of the major classification parameters. Some of these domain classification methods such as SCOP [21] or CATH [22] are purely based on the structure, while others such as Pfam [23] or INTERPRO [24] include information about the function in their classification.

Domains, and consequently proteins, perform its biological activity by interacting with other molecules. Proteins can interact with other proteins, constructing a protein-protein complex, with ions or with small-molecules. The substance that is bound to the *target* protein is called the **ligand**, while the region of the protein where the ligand is binding is called ligand's *binding site*².

1.1.3 Protein-Ligand Interactions

The roles played by the ligands are diverse. Table X shows an example of the different functions that a small-molecule ligands can perform in a protein. Binding constants, allosteric and binding-site, induced fit model. Expandir. Importante.

²For simplicity, in this manuscript, unless otherwise indicated, the term ligand will only refer to small molecule ligands, while proteins ligands will be explicit named as protein-protein interactions

1.1.4 Protein-ligand prediction

1.2 Drug discovery

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Taula 1.1: Prova de taula

1.2.1 subsection

Subsection

1.3 Drug discovery

Second

1.3.1 In-silico methods in drug-discovery

Subsection

1.4 Mycobacterium tuberculosis

1.4.1 Tuberculosis treatments and PPcs

1.5 Drug resistance in cancer

1.5.1 Cancer Treatment and drugs

CAPÍTOL 2

OBJECTIVES

CAPÍTOL 3

NANNOLYZE

CAPÍTOL 4

PREDICTING TARGETS IN MTB

CAPÍTOL 5

DRUG RESISTANCE IN CANCER



Figura 5.1: Example

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