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Structural Determinants of a Typical Leucine-Rich Repeat Protein

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Abstract. The structural and functional description of protein-protein complexes and their comprehension is a key concept, not only to increase the scientific knowledge in basic terms but also for the application to the biomedical and pharmaceutical industry. The binding association between proteins is nowadays attribute to a few key residues at the interface – the hot-spots. The complex between the RNase inhibitor (RI) and RNaseA protein provides an excellent system to study the role of the functional epitope as it is essential in various molecular recognition processes and constitute one of the tightest complexes known. An energetic pattern of the interface is accomplished by computational alanine scanning mutagenesis and a dynamical characterization is accomplished by a detailed study of the molecular dynamical simulations. A special emphasis is given to the role of solvation across the interface and the shielding of warm- and hot-spots from water.

AMS subject classifications: 92C05

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1 Introduction

The challenging process of drug development is time-consuming, labor intensive, and expensive, but has as a final goal finding, developing and marketing new useful chemical entities. These new drugs can be used against currently untreatable diseases, or as replacements to available but less effective compounds. In the last few years, we observed a change in the tendency for drug design, which not only focus on the binding of a small molecule to a biomolecular target but also to a crucial and popular target class: protein-protein interactions (PPIs). Its exploitation is still taking the first steps due to the

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exceptional complexity of these systems that makes them resistant as pharmaceutical targets. Therefore, it is essential to explore PPIs at an atomic level in order to understand the forces that drive their interaction. Since its initial application to human growth hormone and the growth hormone binding protein, alanine scanning mutagenesis continues to be a valuable procedure for both hot-spot detection and analysis of a wide range of protein-protein interfaces [1]. Although slow and labour-intensive, alanine-scanning mutagenesis is the most trendy method for mapping functional epitopes, as alanine substitutions remove side-chain atoms past the β-carbon without introducing additional conformational freedom [2]. Thus, the role of side-chain functional groups at specific positions and the energetic contributions of individual side-chains to protein binding can be inferred from alanine mutations. Clarkson and Wells demonstrate, via alanine scanning mutagenesis on the human growth hormone, that there is a highly uneven distribution of energetic contributions of individual residues across each protein [3]. Only a few key residues do contribute significantly to the binding free energy of protein-protein complexes: the hot-spots. Hot-spots have been defined as those sites where alanine mutation cause a significant increase in the binding free energy of at least 4.0 kcal/mol [3, 4]. Warm-spots were defined as residues that upon alanine mutation generate a binding free energy difference between 2.0 and 4.0 kcal/mol and null-spots lower than 2.0 kcal/mol [5]. Other values can be used for statistical purposes. This way, it is possible to differentiate the structural epitope, the amino-acids that interact at the tridimensional proteic complexes, and the functional epitope composed of the amino-acid important for protein-protein association [5]. The structural epitopes are normally large and composed of 10-40 residues from multiple discontinuous segments on each protein. In contrast, the functional epitopes are assigned by mutagenesis studies and composed by only a small number of residues, typically two to five on each protein. As said, detailed knowledge of the hot- and warm-spots on an interface and their importance and function has important implications for the design of small molecules that disrupt protein-protein interactions or to substitute one of the protein components. The complex between the RNase inhibitor (RI) and RNase A provides an excellent system to study the role of the functional epitope.

The RNase inhibitor (RI) is a leucine-rich repeat (LRR) protein that binds diverse proteins in the pancreatic RNase superfamily [6, 7]. The LRR motif is essential in various molecular recognition processes such as signal transduction, cell adhesion, cell development, DNA repair and RNA processing [8]. LRRs are present in over 2000 proteins and have been identified in viruses, bacteria, archaea and eukaryotes [9]. RNase A complexes comprises important and intriguing systems to study protein-protein association process [10]. RI complexes are some of the tightest complexes known with dissociation constants between 10^{-13} to 10^{-15} M [10]. RI adopts a "horseshoe" fold, formed by symmetrical arrangement of the 15 homologous tandem LRR units (alternately 28 and 29 residues long, that comprise nearly the entire molecule [6, 10]. LLRs are $\beta-\alpha$ hairpin units in which the -strand and the -helix are approximately parallel in individual $\beta-\alpha$ units and the units are all aligned roughly parallel to a common axis [8]. Only one of the proteic members of this complex was studied by experimental alanine scanning muta-