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Promising Drug Design Strategies: Intrinsically Disordered Proteins

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Introduction

In contrast to the classical paradigm "one sequence - one structure - one function" that a given protein sequence corresponds to a well-defined three-dimensional (3D) structure and an associated function, it was discovered in the 1990s that an increasing number of proteins can be functional in the absence of a stable 3D-structure [1]. This new concept, the "disorder-function paradigm", assumes that an intrinsically flexible protein may have several structures and consequently various functions. Several terms were used to name these proteins: *e.g.* intrinsically disordered proteins (IDPs), natively unfolded proteins (NUPs), natively denatured proteins (NDPs) or intrinsically unstructured proteins (IUPs). Whereas some IDPs are predicted to be fully disordered, most of them are also known to have both structured domains and disordered regions.

Their native state is characterised as a dynamic ensemble of interconverting conformations under physiological conditions. The free energy landscape of IDPs can be described as a hilly plateau with numerous local energy minima, which is radically different from what is observed for ordered globular proteins, *i.e.* a well-defined global energy minimum [2]. This conformational plasticity is associated with specific sequence features including a low proportion of bulky hydrophobic amino acids and a high content of charged and hydrophilic residues [3-4]. Indeed, the combination of low mean hydrophobicity and high net charge is an important prerequisite for the absence of compact structure in a protein [5]. Depending on the environmental conditions and their binding partner, IDPs may exist in at least four conformations, the so-called protein quartet model: folded (ordered), molten globule, pre-molten globule, and random coil [6].

Disorder in proteins is highly prevalent in many organisms [7,8]. Indeed, more than one third of the eukaryotic proteins contain intrinsically disordered regions (IDRs) whereas for humans it is up to the half. Despite a lack of stable 3D-structure, IDPs play key roles in cellular processes, such as i) regulation of transcription, translation and cell cycle, ii) molecular recognition, and iii) chromatin organisation. Their functions usually operate by the binding of multiple protein partners [9]. Hence, most of IDPs are serving as hubs in protein-protein interactions (PPIs) networks [10,11]. IDPs are also regulated by extensive posttranslational modifications (PTMs), such as phosphorylation, acetylation and methylation, that affect their abundance, cellular distribution, fold ability and functions [12,13].

IDPs are overrepresented amongst proteins implicated in disease [14-16]. More than 79% of human cancer-associated proteins [17] and 57% of the identified cardiovascular disease-associated proteins [18] are predicted to contain intrinsically disordered regions. As they tend to misfold and aggregate under certain conditions, IDPs are associated with neurodegenerative disorders [19,20] such as Alzheimer's or Parkinson's diseases. Whilst IDPs are essential therapeutic targets [21], only a small number of molecules and peptides are able to inhibit their functions. Often, the conventional structure-based drug design strategies applicable to ordered proteins are not appropriate for IDPs, due to their

highly dynamic nature. Developing drugs targeting IDPs is therefore challenging. Currently, the most studied IDPs are: i) the nuclear protein NUPR1 [22] involved in pancreatic adenocarcinoma; ii) the transcription factor p53 [23] playing a role in cell cycle regulation, apoptosis and DNA repair; iii) the α-synuclein protein [24] that enters in the so-called synucleinopathies, a group of neurodegenerative disorders; iv) the transcription factor and oncoprotein c-Myc [25] which is involved in a broad range of human cancers.

IDPs as Druggable Targets

Currently, four drug design strategies are implemented to target IDPs and disrupt their biological functions [26-30]. The first one consists in developing small molecules that bind to the IDP ordered domains, causing the disordered region to become ordered. It is called an allosteric inhibition [29].

In the second approach, drugs are able to mimic the ordered (or disordered) binding partner of the IDP preventing its binding and consequently the associated PPIs. Several small molecules have already shown abilities to inhibit the disordered interface of PPIs based on the disordered regions [7]. For instance, by using the molecular recognition features (MoRFs) method [31], the p53-Mdm2 interactions [28] can be targeted by peptides or small molecules [26].

The third strategy is more challenging and considers blocking IDPs' disordered regions by stabilising the functionally misfolded structure. It allows for a conformational transition from a disordered to an ordered structure. That is, the ligand has to directly block all the IDP disordered states. Few rational drug design methods have been proposed in this context. A computational approach has recently been described to virtually screen compounds that can simultaneously bind to different conformations of the oncoprotein c-Myc. The latter contains a disordered basic helix-loop-helix-leucine zipper domain that adopts a helical conformation upon binding to Myc-associated factor X (Max) [27]. Furthermore, a methodology based on the synergy of biophysical, computational and biological methods have been set up to identify a drug against NUPR1 [32].

A fourth method is to prevent aggregation of IDPs, which form toxic amyloid oligomers or fibrils [30]. Several small molecules have been reported to interfere with the aggregation process of the Aβ peptide, an IDP associated with Alzheimer's disease [33], or the α-synuclein [34] involved in Parkinson's pathology.

Biophysical Characterisation of IDPs

In order to understand the IDPs functional mechanism and promote novel strategies for drug discovery, it is essential to first investigate their structural characteristics and their specific interactions with binding partners in their PPIs network. In this context, combining biophysical and computational methods turned out to be the most relevant strategy. Indeed, a serious bottleneck for this type of analyses is that no single method can provide access to the inherent flexibility and structural variability of IDPs; traditional structural biology method such as X-ray crystallography is not adapted.

Alternative techniques such as nuclear magnetic resonance (NMR), small-angle X-ray scattering (SAXS), circular dichroism (CD) or single-molecule fluorescence (particularly, the Förster resonance energy transfer method) can be used to attempt IDPs structural characterisation [35]. NMR provides local and long-range structural information while SAXS measures protein compactness and shape. Residual secondary structures and IDRs are revealed by CD, and fluorescence gives information about the structural heterogeneity and the intermediate structures.

Guided by experimental data, computational methods [36] may also help interpreting the results at the atomic scale and giving information about individual conformations in the ensemble. Molecular dynamics-based methods [37] are the most used, showing the atomic fluctuations of IDPs as a function of time. However, they still suffer some weaknesses such as the selection of an appropriate force-field or the need to generate uncorrelated atomistic microstructures. Several strategies are proposed to overcome these problems and produce a correct description of the IDP conformational ensemble [38,39].

Perspective

IDPs constitute a unique class of proteins with crucial biological functions. In recent years, multiple studies have highlighted their intrinsic properties and binding mechanisms. However, more efforts are still needed to fully understand these fuzzy systems and particularly the PPIs in which they are involved. Such insights are precious for the rational design of therapy targeting IDPs, which are present in human diseases.

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