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Do Moonlighting Proteins Belong to the Intrinsically Disordered Protein Class?

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Abstract

Moonlighting is the capability of some proteins to execute two or more biological functions. According to some authors, there is a relationship between protein conformational fluctuations and promiscuous functions of proteins. This promiscuity would be due to the conformational properties of the structurally disordered regions. To check if moonlighting proteins belong to the Intrinsically Disordered Protein (IDP) class, we have predicted IDP/IDR (Intrinsically Disordered Regions) for a number of moonlighting proteins. Our results suggest that most moonlighting proteins do not belong to the IDP class.

Keywords: Moonlighting proteins; Multitasking proteins; Intrinsically disordered proteins; Intrinsically disordered regions

Moonlighting proteins refer to those proteins presenting two or more functions performed by a single polypeptide chain. They were initially reported by Wistow and Piatigorsky in the late 1980s when lens crystallins turned out to be previously known metabolic enzymes [1,2]. Moonlighting proteins present alternative functions which are mostly related to cellular localization, cell type, oligomeric state, cellular concentration of ligands, substrates, cofactors, products or post-translational modifications [3-11]. In many cases, a protein uses a combination of these mechanisms to switch between functions. Although some findings suggest involvement of a protein in extra functions, i.e., they can be found in different cellular locations or in amounts exceeding those required for their canonical function. Usually, moonlighting proteins are experimentally revealed by serendipity. Therefore, any alternative method to identify these proteins would be very valuable. In previous works, we have explored the possibility of identifying moonlighting proteins by bioinformatics [12] and protein interactomics-database mining [13].

Some authors have pointed out that there is a relationship between protein conformational fluctuations and promiscuous functions of proteins. This promiscuity would be possible due to the conformational properties of the structurally disordered regions. In solution, proteins exist in a range of conformations, and structurally disordered regions can alter their secondary-structure propensities as well as their conformational flexibility in response to different environments or to interacting partners [14-19].

To check if moonlighting proteins belong to the Intrinsically Disordered Protein (IDP) class, we have predicted IDP from their amino acid sequences for a number of well-known moonlighting proteins, mostly those used in our previous work in which we showed that moonlighting proteins can be indentified using protein interactomics databases (PPIs) [13]. In Figure 1a set of panels is shown containing IDP/IDR-predicted profiles for these proteins. Table 1 in Supplementary Material shows the list of proteins analyzed and their corresponding NCBI accession numbers. Figure 2 in Supplementary Material shows the panels containing IDP/IDR-predicted profiles for these proteins. Some of these proteins have a 3D structure, but their Intrinsically Disordered Regions (IDRs) are not found in PDB because the existence of a crystal implies a specific structure, not disorder. For this reason, programs aimed at predicting IDRs may be useful to disclose structural data that are hindered in crystallography. There are several programs for predicting IDPs/IDRs such as PrDos [20]; DisEMBL [21]; Disopred [22] and Iupred [23]. These programs can be found in the following web-servers:

PrDos: http://prdos.hgc.jp/cgi-bin/top.cgi

DisEMBL: http://dis.embl.de

Disopred: http://bioinf.cs.ucl.ac.uk/disopred/

Iupred: iupred.enzim.hu/

Prior to the IDR prediction of the above-referred-to set of moonlighting proteins, we checked two crucial questions: (a) Are proteins known to be disordered correctly predicted by these programs? (b) Do these programs yield similar results? Both questions have affirmative responses. As an example, as can be seen in Panels **a** and **b** from Figure 1, two well-known disordered proteins, calpastatin and Non-Histone High Mobility Group protein HMG17, are correctly predicted as completely disordered proteins. Panels c to f from figure 1 show that the predicted IDRs, using different prediction programs in two moonlighting proteins (human aconitase and ribosomal protein S9 from *E. coli*) show similar results (profiles). Panels g to i from Figure 1 show the IDP/IDR profiles for three top moonlighting proteins: GAPDH, p53 and chaperonin 60. GAPDH and chaperonin 60 practically lack disordered regions, whereas protein p53 has large stretches of predicted IDRs that allow the inclusion of this protein in the IDP class. So far, it can be concluded that most moonlighting proteins do not belong to the IDP class, since the disordered amino acid stretches are quite short. Only three out of 28 proteins show, to some extent, that they could belong to the IDP class. In fact, if we

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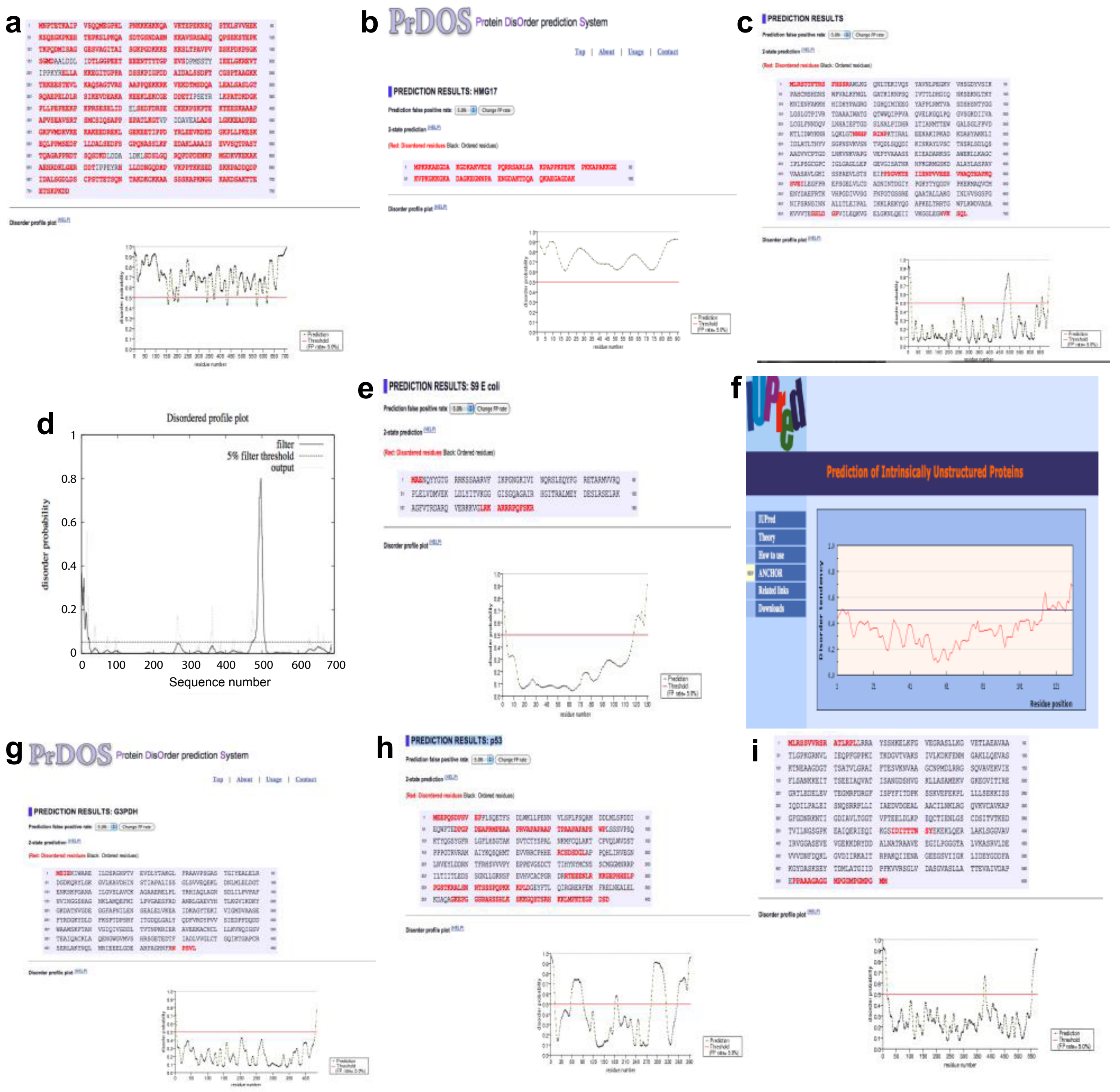

Figure 1: Prediction of Intrinsically Disordered Protein Regions. Results obtained for: (a) Calpastatin predicted by PrDos, (b) Non-histone protein HMG17 predicted by PrDos, (c) Aconitase predicted by PrDos, (d) Aconitase predicted by Disopred, (e) Ribosomal protein S9 predicted by PrDos, (f) Ribosomal protein S9 predicted by Iupred, (g) GADPH predicted by PrDos, (h) Protein p53 predicted by PrDos, (i) Chaperonin 60 predicted by Iupred.

consider that belonging to this class requires stretches of at least 40 amino acids in disordered regions [24], the number would be smaller, since the disordered amino acid stretches are quite short. In many cases, these structures are located in the N- and C-terminal regions of the polypeptide, which are known to be quite mobile regions. Indeed, many of the IDRs match loops and coil regions and, in fact, the IDP/IDR prediction program DisEMBL, which predicts both IDP and loop regions, shows that they usually coincide.

Alternative local conformations can be achieved without a great change in the structure of the protein. The analysis and mapping of X-ray structures of four moonlighting proteins indicate that they use different regions for each activity and that these regions correspond to quite complex domains or motifs, not to disordered amino acid stretches [6]. Of course, there are some examples of moonlighting proteins that are IDPs, such as the human chemokine lymphotactin [11,16,18,25], but we suggest that most moonlighting proteins do not belong to the IDP class. Indeed, a moonlighting protein, Ribosomal Protein S10, which was once considered an IDP, has recently been shown to adopt the same global fold in complex with NusB and in the ribosome. This fact excludes the possibility that its structure is extensively remodelled. Therefore, S10 binds to RNA and to NusB at different regions of the protein. RNA-binding is accomplished by a long loop, which is the only unfolded region [26].

The above results suggest that moonlighting proteins might not specially require fully disordered regions. Loops are flexible enough to allow for the adaptation to different interactions. And the capability to interact with new partners is probably the first step to achieve a new function. There are examples of multispecific proteins that interact with many partners through the same binding interface without being a disordered region [27]. Moreover, interactomics has shown that proteins – both IDPs or permanently structured - have the ability to interact with many partners, most of them other proteins. In fact, the cell has to solve the problem of choosing a specific partner by means of subcellular compartmentalization, expression phase, oligomeric state, etc. New functions could be related more to establishing additional interactions using existing sequences rather than incorporating new amino acid stretches or changing the local structure. This is suggested by the following analysis. We have multialigned, by CLUSTALW [28]), the sequences corresponding to different bacterial species of a number of the proteins used in this work in search of major differences in domains (shown as differences in length or even in amino acid sequence). The alignment shows that they are highly conserved (results not shown), thus new functions are not likely related to incorporating new amino acid stretches/conformations, but rather to new interactions. For example, a highly conserved group of moonlighting enzymes, those of glycolysis, interact with different host partners in different bacterial pathogens [29]).

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