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Extended Abstract

Developing high sensitivity/specificity detection systems for proteomic studies using Adhirons coupled with fluorescence for studying protein interactions

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Abstract

Protein interactions are at the heart of nearly all disease states identifying and characterizing these interactions has led to the development of biological tools to expedite research in this area. This paper aims to present a review of the multiple types of interactions and the medley of biological tools employed in the study of proteomics. Established methodologies employed to monitor in-vivo and in-vitro protein interactions have identified multiple types of interactions ranging from heterocomplexes to homocomplexes, obligate and non-obligate to transient and permanent interactions. However the drawback with current studies is that they are restricted to a limited number of interactions and fail to elucidate networks of interactions which are important in understanding the basis of disease and in particular cancer, a disease caused by aberrant signalling along complex pathways. The diverse array of biological tools range from mass spectrometry, yeast-two hybrid to techniques such as: fluorescence resonance energy transfer (FRET) and bioluminescence resonance energy transfer (BRET). Biological fluorescence/luminescence approaches have been successful in providing spatiotemporal data of protein-protein interactions in live cells. In addition non-scaffold proteins such as Adhirons, DARPins and Repebodies have also been used with some success. The study aims to take the use of Adhirons a step further by adapting them for use with fluorescence tools such as FRET techniques and to identify not just single interactions but networks of protein interactions by exploiting DNA paint. The journey into proteomics has already begun and at the present juncture has reached a significant milestone

Spinal and bulbar muscular atrophy (SBMA) and amyotrophic A term which is used sparingly in protein-protein interactions studies is Interactome which denotes the entire number of protein interactions that occur in an organism. The last decade has witnessed resurgence in the study of protein-protein interactions (PPIs). Scientists are now becoming acutely aware of the pivotal role protein interactions play in the smooth regulation of virtually all biological processes in the cell Consequently researchers are now focused on building entire networks of protein interactions, and by finding out the identity of the interaction partners, ultimately leads to the identity of protein function. It has been suggested that the total number of protein interactions found in the human genome exceeds 100,000 and to date only a fractions of these interactions have been identified [3]. Further data into interaction studies suggest that a total of 130,000 binary interactions can occur in a human cell at any one time. At this present time, BioGRID (http://thebiogrid.org) a data base for the storage of protein interactions, has listed only 33,943 human protein interactions thus far Hakes et al.] estimates that in yeast approximately 50% of protein interactions have been identified which in contrast to human protein interactions which stands at just 10%. Although the exact number of protein interactions has not been deduced estimates suggest, it is anything from a hundred thousand to around a million..

The human genome project identified approximately 30,000 genes, and although this is a major feat for science however, an even greater challenge facing scientists will be to take it a step further, by mapping all the genes and protein interactions. Bonetta et al., Planas-Iglesias et al., Keskin et al. believes that the challenges of mapping the entirety of protein interactions in the human proteome will be a far greater challenge, than the human genome project, due to the temporal and spatial heterogeneity of the interactome. However, Bonnetta et al. states that exposing pathways, and understanding the role pathways have in disease states, and in the development of disease, is the next mile post in proteomics analysis. There are many reasons why a project of this type will be enormously challenging. One of the caveats presented is alternative splicing. It is estimated that in excess of 90% of all human genes produce alternatively spliced mRNA isoforms. In the human genome, there are approximately 20,000 protein coding genes, of which 196345 multiple transcripts have been released in Ensemble database (GrcH38, version 77), all of this adds to the repertoire of the variety of the human proteom. Another complexity is post translational modifications, that all proteins undergo, which include acetylation, phosphorylation, ubiquitination (Figure 1) [8], where a protein is localised in a cell as well as tissue specificity, all add to the complexity of the task [9-11]. A plethora of experimental approaches exist in protein function studies. Many of which have been avidly used in protein identification and protein interaction. These include yeast two-hybrid analysis, Mass spectroscopy and affinity purification to name but a few. In addition, complementary bioinformatic approaches have been successfully utlised to identify interactions involving gene clustering and pylogenetic profiling. However, issues around

the quality of data suggesting that high numbers of false positives and false negatives throw caution to the accurcy of the data produced]. Although the approaches can identify an interaction, they canot do so in the context of the complex from which the interaction occurs, a key component in understanding function Due in part to limitations in current diagnostic tools this critical area of interactomes, for the most part, remains in the dark. Herein lies the purpose of this study.

Adhirons will be adapted as biological cameras using fluorescence microscopy and with the aid of biological techniques such as FRET and BRET. It will bind to a target protein and follow its path along multiple interactions through the cell and with the aid of DNA Paint multiple protein interactions will be visualised in real time. The MAPK pathway is a highly understood pathway and for the purpose of the study, MAPK pathway will demonstrate proof of principle of the utility of Adhirons in protein interaction studies, and that these non-protein scaffolds can be adapted for use in multiple protein interaction studies.

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