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Phosphorylated Sites on the Disordered Interface Signatures the Interacting Behavior of Proteins—A Comparative Mapping of Phosphorylation Propensities on Disordered Interfaces of Interactome and Negatome

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Abstract: Hub proteins in interaction networks involved in signaling pathways are known to have more disordered residues than non-hubs. Since the signaling mechanisms involving PPI are regulated by phosphorylation, disordered interfaces could be thought to be extremely phosphorylated. In the present study we sought to map the phosphorylated sites onto disordered regions on interacting proteins-Interactomes and non-interacting proteins-Negatomes. Dataset of non-interacting protein included 784 proteins retrieved from Negatome database 2.0. 2252 interacting proteins were retrieved from "GeneMania". Intrinsically disordered regions were predicted with "Disopred" program. The binding interfaces were defined by "PDBePISA" server, while, phosphorylation sites were derived from "NetPhos" program. All phosphorylation sites were mapped onto protein structures using alignments calculated by the MUSCLE program. As anticipated, the extent of phosphorylation in interactomes were significantly higher in disordered regions to its ordered counter parts ($p=0.04$). Insights revealed that the disordered regions in negatome were sparse in comparison to those in interactomes ($p<0.0024$). Declined phosphorylated sites were observed in negatomes. The widespread non-flexible and ordered regions in the negatomes confer the non interacting nature of the protein in turn makes it poor participant in signal transduction that involves phosphorylation. Our study sheds light on the importance of phosphorylated sites on disordered regions as a mark to decide whether protein would possibly interact or not.

1. Introduction

Protein–Protein interaction (PPI) forms the core of interactomics system and unsurprisingly, aberrant PPIs are the basis of diseases, such as Alzheimer's and cancers [1]. Growing body of evidence suggest that hub proteins in interaction networks in signaling pathways have more disordered residues than non-hubs [2, 3]. Intrinsically disordered proteins lack single well-defined structure and are characterized by specific amino acid composition, a propensity for post-translational modifications and the ability to bind to many different partners [4].The importance of disorder in protein–protein interactions is apparent from analysis of protein-protein interaction networks. Studies have shown that hub proteins in interaction networks have more disordered residues than non-hubs and that there may be a weak correlation between the disorder of a protein and the number of its partners [5 - 8]. The functional diversity of disordered proteins and their multi-binding properties, allow them to play a unique role in signaling networks [9]. Phosphorylations form the most important dynamic covalent modification involved in the signal transduction systems [10]. Therefore disordered interfaces which are involved in PPI could be thought to be extremely phosphorylated. Since the signaling mechanisms involving PPI are regulated by phosphorylation, disordered interfaces could be thought to be extremely phosphorylated. In the present study we sought to map the phosphorylation propensities of Ser,Tyr and Thr onto disordered regions on interacting proteins-"Interactomes" and non-interacting proteins-"Negatomes".

. METHODOLOGY

Dataset Compilation

For interactomes, we compiled a data set consisting of 2252 human protein complexes of known 3D structures from Protein Data bank. The interacting proteins were selected based on their interaction hubs; as provided from Genemania. 784 non-interacting proteins were selected from Negatome database, which provides the list of non-interacting proteins available in PDB. Further, redundant proteins were removed using BLAST, using a p-value threshold of 10e-07.

Identification of Phosphorylation Sites

Phosphorylation sites were derived from PhosphoSitePlus, Phospho.ELM, and PHOSIDA 26 web servers. All the phosphorylation sites were mapped onto protein structures in the PDB using alignments calculated by the MUSCLE program.

Prediction of Intrinsically Disordered Regions

Disordered regions were predicted using the support vector machine enabled Disopred programs. Amino acid propensities for Disorder was calculated using TopIDP program.

Statistical Analysis

The propensity of disorderness and phosphorylation in ordered and disordered regions was calculated by statistical functions like ANOVA, stepwise logistic regression analysis, Odds ratios and linear regression analysis using SPSS v17.0 suite

RESULTS AND DISCUSSION

Table 1 shows the distribution of disordered and ordered regions in Negatomes and

Interactomes. From the statistical analysis it is quite evident that the disordered regions in interactomes are 1.3 folds higher in comparison to negatomes (p value=0.033) (Figure 1). Given that disordered regions involves in PPI, the analysis reflects the non interacting nature of negatomes as evaluated through disordered prediction.

Table 1. Distribution of disordered and ordered regions in interactomes and negatomes.

	Ds %	Or %	$\chi 2$	OR (95% CI)	p Value	Ratio	Diff
Interactomes	62.3	37.7	3.367				
Negatomes	48.4	51.6		1.67 (1.2, 2.9)	0.033	1.3	13.90%
t-Test (95% CI)	F stats	df	p value				
Interactomes *vs.*Negatomes	5.6758	2491 7	<0.021				

Ds = Disordered regions , Or= Ordered Regions, OR= Odds Ratio, Diff= percentage difference

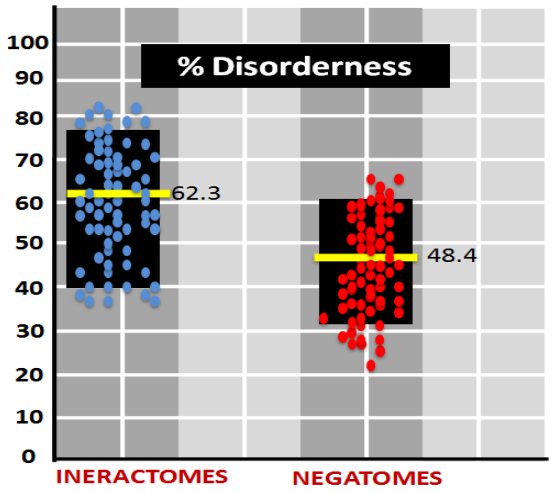

Figure 1. The plot shows the percentage distribution of disorderness in interactomes and negatomes. Each dot represents the disordered percentage of an individual protein falling in interactomes (blue dots) or negatomes (red dot). The bar represents the mean disorderness

We further analyzed the total phosphorylation propensities of Ser, thr and Tyr residues onto Interactomes and negatomes. We observed that percentage distribution of phosphorylation was 1.36 folds higher in the disordered region in both interactomes and negatomes combined (p value =0.0041) Table 2 and Figure 2.

Table 2. The distribution phosphorylation sites in ordered and disordered regions in interactomes and negatomes. The disordered regions have higher share of phosphorylated sites than ordered counterparts.

	Ds (I+N)%	Or (I+N)%	χ2	OR		p Value	Ratio	Diff
P	67	36						
NP	133	134	6.947	1.872	(1.13, 3.0)	0.0041	1.36	15.24 %
t-Test (95% CI)	F stats	df	p value					
P *vs.*NP	8.6758	2251,22	0.04					

P=Phophorylation percentage; NP= Non- Phophorylation percentage

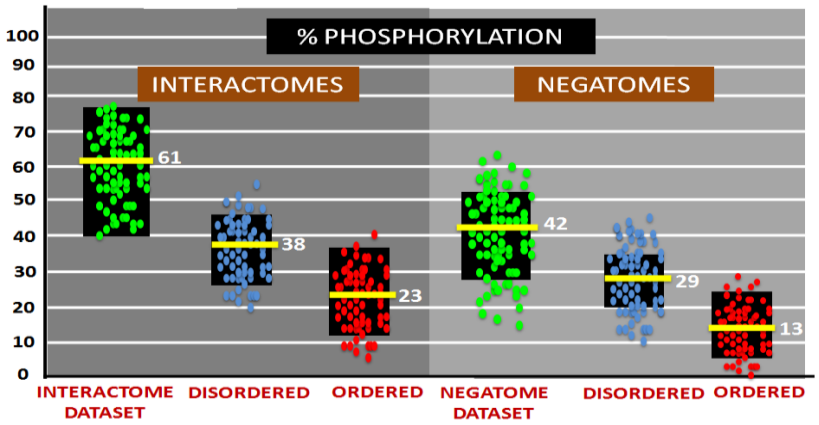

Figure 2.The plot shows the phosphorylation intensities in disordered and ordered regions in interactomes and negatomes. The phosphorylation dots being prominently intense in interactomes.

In the further perusal, we mapped for phosphorylation propensities on to ordered and disordered regions individually in interactomes and negatomes. From stepwise logistic regression analysis we found that disordered regions where 1.4 folds phosphorylated than ordered counter parts in interactomes while in case of negatomes phosphorylated regions were 24.1 folds higher disordered regions than ordered regions testifying the disordered regions are more prone to phosphorylation

TABLE 3. Table shows phosphorylated site with individualistic approach in interactomes and negatomes, In either cases (IN or N) disordered region has higher proportion of phosphorylation.

	Ds %	**Or %**	**χ2**	**OR**	**p Value**	**Ratio**	**Diff**
P (IN)	38	23	5.307				
NP (IN)	62	77		2.044 (1.1, 3.8)	0.01	1.4	19%
P(N)	29	13	7.715				
NP(N)	71	87		2.720 (1.3,5.7)	0.002	1.15	24.11%

P (IN) = Phosphorylated regions in Interactomes;
NP (IN) = Phosphorylated regions in Negatomes;
Ds = Disordered regions; Or= Ordered Regions; OR= Odds Ratio; Diff= percentage difference

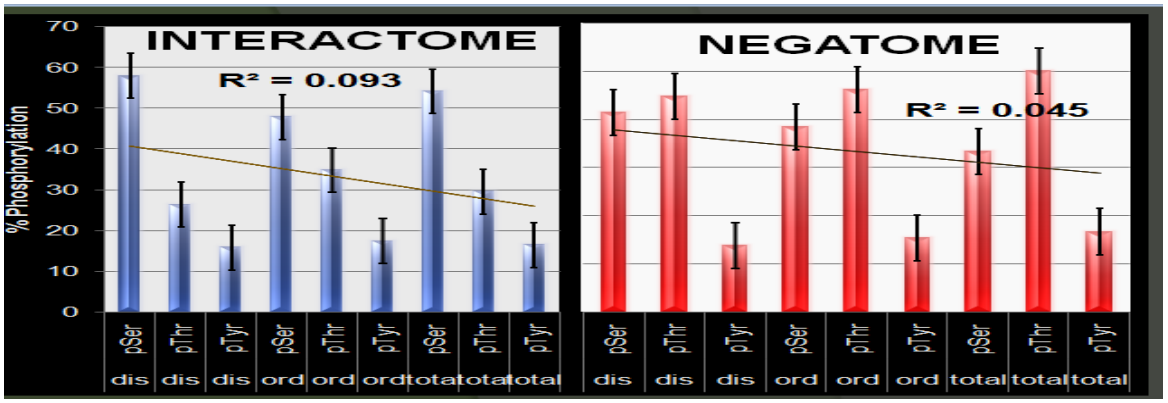

Figure 3. The above graph shows residue wise phosphorylation in interactome (blue bars) and in negatome (Red bars). In The interactome degree of phosphorylation follows the descending order of Ser>Thr>Tyr while no such specific order is present in Negatome.

Further, we performed linear regression analysis in order to confirm whether phopshoryation intensity actually depends on disordered state of the protein for which results were quite convincing (Figure 4). We found significant effect of disorderness on phosphorylation propensity of the protein (R^2= 0.79). The fitness of statistical results therefore confirms that positive correlation effects of diordeness to phosphorylation intensity of the protein.

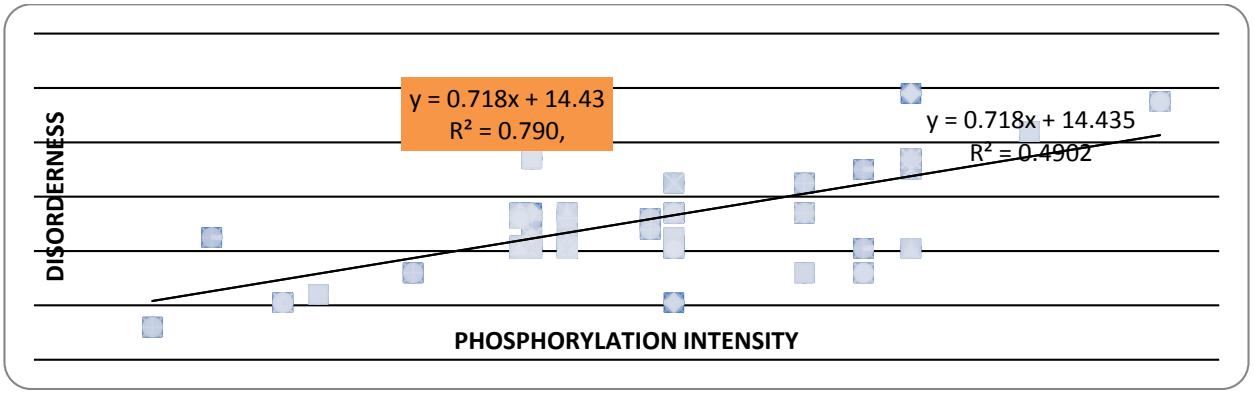

Figure 4. The graph shows the positive correlation (Pearson Corr. Coefficient 0.740) between disorderness and phosphorylation intensity. The graph plotted has the combined data from Interactomes and Negatomes.

4. Conclusions

In conclusion we report that the disordered regions are prominently higher in the interactomes while abruptly low in negatomes. We further observed that the phosphorylated sites which were prominent in disordered regions were significantly higher in interactome than negatome and this observation perhaps explains the interacting nature of proteins. The widespread non-flexible and ordered regions in the negatomes confer the non interacting nature of the protein in turn makes it poor participant in signal transduction that usually involves phosphorylation. Our study sheds light on the importance of phosphorylated sites on disordered regions as a mark to decide whether protein would possibly interact or not.

References and Notes

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