Development of Photoswitchable, Phase Separable Biomaterials

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Abstract
Phase separation is a dynamic process that leads to the formation of biomolecular condensates. These condensates contain proteins with high concentrations of intrinsically disordered regions (IDRs), which lack a fixed 3D structure. We aim to control phase separation in human cells using the LOV domain, a protein sensor that homodimerizes under blue light. We hypothesize that extant LOV proteins may show nontrivial association with intrinsically disordered regions, and these proteins with IDR regions may induce phase separation under blue light. To determine IDR percentage, we processed sequences from a database of LOV proteins through an algorithm that provides scores for likely intrinsically disordered amino acids. The distribution of these LOV proteins as a function of IDR percentage was significantly different from that of random sequences. We expressed the highest scoring proteins in human cells and monitored their ability to form condensates under blue light. These novel proteins will provide a new tool for researchers to better understand the phenomenon of phase separation.

Results

As seen above in Figure 2, there is significant difference between LOV domain-containing proteins and randomly generated amino acid sequences in the distribution of frequencies of proteins as a function of IDR percentage. Whereas the distribution of randomly generated amino acid sequences (right) shows exponential decay as IDR percentage increases, the distribution of LOV-domain containing proteins (left) demonstrates clusters of normal distributions at certain IDR percentages.

Figure 2: Histograms of LOV-domain containing proteins (left) and randomly generated amino acids (right) as a function of IDR percentage.

In Figure 3 (above), a phylogenetic tree and heat map were generated using the sequences of the 100 LOV-domain containing proteins with the highest IDR percentages. The phylogenetic tree shows 21 distinct groups, from which one protein per group was selected for expression. The heat map, which represents the specific regions of each sequence as color-coded blocks, demonstrates a consistent pattern of non-IDR domains bordering the LOV domain region. Additionally, the placement of the LOV domain within certain closely-related sequences (top 3) is not necessarily similar, while the placement of the LOV domain within some more distantly related regions (bottom 10) shows much more similarity.

Figure 3: Heat Map of 100 LOV-domain containing proteins with Highest IDR%.

Conclusions
As shown in Figure 2 of the Results section, there is significant difference between LOV domain-containing proteins and randomly generated amino acid sequences in the distribution of frequencies of proteins as a function of IDR percentage. We conclude that there may be some nontrivial association between LOV domain-containing proteins and intrinsically disordered regions.

Future Directions
Following successful transfection of the plasmid, we intend to induce phase separation in the cells using blue light. After ensuring phase separation is successfully controlled, we will analyze the diffusion kinetics using fluorescence recovery after photobleaching. We also aim to express more sequences with higher IDR percentages and from different families as per our heat map. This will allow us to create a diverse array of cell lines to observe differences in the effects of different LOV domains.

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Literature cited

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