

Large scale screening system for protein-protein interactions based on mRNA-display and Psoralen Analysis of RNA-Interactions and Structures (PARIS)

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A. Praznik¹

¹National institute of Chemistry - Department for synthetic biology and immunology, Ljubljana, Slovenia

Most proteins in eukaryotic cells do not exhibit their function individually, but mediate their action through interaction with other proteins, forming a complex protein-protein network interactome. Detecting and analyzing protein-protein interactions (PPI) is still a relatively cumbersome process, a big issue being that many methods rely on detecting PPI of previously known interacting proteins, failing to detect yet unknown interaction partners. On the other hand, methods to detect the interactions of DNA or RNA in vivo have been previously described. One such method is Psoralen Analysis of RNA-Interactions and Structures (PARIS), which locks the interacting RNA sequences inside of cells with the help of a crosslinking reagent AMT and UV light. The interacting RNAs are then ligated and sequenced with NGS to reveal all RNA—RNA interactions. We aim to develop a method for analyzing RNA-RNA interactions, similar to that utilized in PARIS, but instead of native RNA I will use mRNAs bound to their coded protein. The covalent binding of proteins to their coding mRNA will be achieved by using the mRNA-display, in which 3' modified RNA molecules are covalently linked to their coded polypeptide on the ribosome during protein translation in vitro. Interaction between the two polypeptides also brings into proximity their adjacent coding mRNAs. We expect that due to the proximity of the RNAs (which is achieved through PPI of their coded proteins) they will be more likely to interact with the mRNA coding for the interacting polypeptide, than with mRNAs of polypeptides, which do not form PPI. The initial work will be done for in vitro transcribed and translated polypeptides, which have been previously described to form strong PPI. The successful implementation of the method on proof of principle peptides will have strong implications for future development of modular bionanostructures.