PROTEOMICS

Capturing dynamic protein interactions

A method based on heat denaturation reveals how proteins interact in different cells

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Protein-protein interactions form the molecular basis for organismal development and function (*1*, *2*). In cells, protein interactions are dynamic and subject to spatiotemporal regulations that are specific to the cell type and cell cycle phase. Mutations that abolish or rewire protein-protein interaction networks (the interactome) are often detrimental and manifest in developmental anomalies and diseases (*3*, *4*). Recent advances in quantitative proteomics offer snapshots of cell type–specific proteomes, but scientific understanding of how protein-protein interactions vary between physiological and disease conditions is limited. On page 1170 of this issue, Tan *et al.* report a technique for inferring dynamics of protein interactions by characterizing protein thermal stability upon heat denaturation (*5*).

Thermal stability is a key molecular fingerprint of a protein and can be represented by the melting curve generated from its stepwise heat denaturation (*6*). Interactions between proteins and their ligands can influence protein conformation and, hence, thermal stability. The magnitude of change in thermal stability correlates with interaction affinity and can thus be used to quantify the interactions between proteins and other molecules. This principle forms the basis of the cellular thermal shift assay (CETSA), in which cells are treated with a compound of interest, the cell lysates are denatured by heating to different temperatures, aggregates are separated from the soluble fraction, and the proteins in the soluble fraction are quantified (*7*, *8*). The shifts in melting curves of proteins in the soluble fraction upon adding a drug represent the changes in thermal stability as a result of protein-drug interaction.

Coupling this technique with mass spectrometry enables high-throughput characterization of the melting curves for thousands of proteins (*9*, *10*). In this way, protein-drug interactions can be characterized on a systems level, revealing unknown targets stabilized by particular drugs and providing insights into drug efficacy in normal and diseased tissues.

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Tan *et al.* adapt CETSA to observe the dynamic changes in the interactome. Their technique, called thermal proximity coaggregation (TPCA), is based on the assumption that proteins that interact with each other tend to coaggregate during denaturation and will exhibit similar solubility at a given temperature; they will hence display similar melting curves (TPCA signature; see the figure). TPCA can be performed on intact cells without specific treatment, allowing proteome-wide detection of interactions.

By obtaining melting curves for 7693 human proteins and investigating 111,776 published interactions between these proteins, the authors show that, as hypothesized, interacting protein pairs tend to have more similar melting curves than noninteracting proteins. The use of intact cells circumvents postlysis loss or gain of interactions and minimizes false discovery rates. For instance, the authors demonstrate the existence of two distinct subcomplexes of the kinetochore protein NDC80, which were not observed in cell lysates. These results highlight the importance of preserving the integrity of the cellular environment to reveal basic functional states of protein complexes, which dynamically change under different conditions.

How can TPCA advance our understanding of the dynamic interactome? TPCA can be used to track the dynamics of protein complexes in different cell cycle phases and cell states to discover new core protein subcomplexes. By comparing differential TPCA signatures of K562 cells (human leukemia cell line) that were synchronized to the DNA replication phase (S phase) of the cell cycle to those of K562 cells that were not synchronized, the authors identified 18 protein complexes implicated in S phase; three of these had not been previously reported.

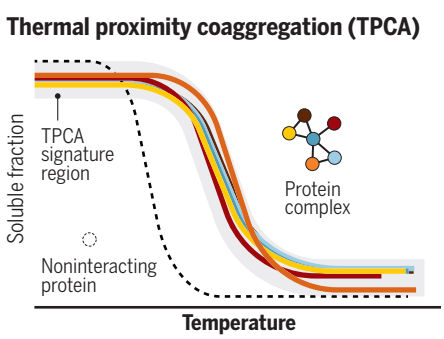
TPCA can also be used to monitor interactome differences across different cell lines and tissues. For example, Tan *et al.* show that across six different cell lines, there was only 70% overlap of TPCA signatures among the detected protein complexes, indicative of cell type–specific interactions. Notably, protein complex stoichiometry and composition vary even in fundamental and abundant complexes, such as the eukaryotic initiation factor 3 (eIF3) core complex. Furthermore, using these data, the authors generated a TPCA-weighted interaction network and elucidated dynamic interactions in different pathways. Thus, use of TPCA signatures could contribute to the discovery of cell-specific interactions and signaling pathways that have been rewired, thereby revealing new biology.

Finally, TPCA signatures can be used to infer the stoichiometry and/or abundance of subunits in a complex in a tissue-specific manner. With the convenience of using intact cells, the authors show that mouse liver cells could be used as a source for comparing TPCA signatures.

Although interacting proteins tend to show similar melting curves, similarity in melting curves cannot be interpreted directly as protein interactions. However, it should be possible to develop algorithms that use TPCA signatures to predict protein-protein interactions in different tissues or cell lines. It should also be possible to adapt TPCA to

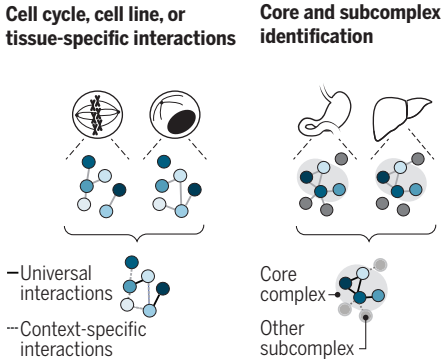
How to monitor protein interactions

Interacting proteins tend to show similar melting profiles when compared to noninteracting proteins.



Applications

TPCA can be used to monitor protein-interaction dynamics in a range of different contexts.



discover proteins that interact with DNA and RNA in different cellular contexts. This could provide an integrated description of how DNA-, RNA-, and protein-protein interactions govern cell physiology.

In a recent study, Leuenberger *et al.* used proteome-wide heat denaturation to measure protein stability from cell lysates using a different method called limited proteolysis–coupled mass spectrometry (*11*). They found that half of the detected proteins that were computationally predicted to lack stable tertiary structures (that is, intrinsically disordered) exhibited a two-state denaturation profile, which is indicative of a stable structure. This seeming contradiction may now be interpreted in light of Tan *et al.*'s findings. Because intrinsically disordered proteins (IDPs) interact with other structural partners, this may result in a melting curve similar to that seen for structured proteins.

Because of their lack of stable tertiary structure and their promiscuous interactions, IDPs are referred to as the dark proteome (*12*, *13*). Techniques such as TPCA could provide much-needed insights into protein-protein interactions involving IDPs in a cellular context, and on a proteome-wide scale. This would be especially useful considering the role of IDPs in modulating protein interaction networks. By offering the possibility to decipher and interpret the dynamic interactome, techniques such as TPCA may be the key to determining how cellular function emerges from dynamic changes in protein interaction networks. ■

"TPCA [thermal proximity coaggregation] can be performed on intact cells... allowing proteome-wide detection of interactions."

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