
Interactome-scale comparison of co-immunoprecipitation and yeast two-hybrid assays for protein interaction prediction

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Abstract

Protein-protein interactions (PPIs) are fundamental to biological processes, and computational prediction of PPIs is important for supplementing gaps in experimental data coverage. However, the quality of PPI predictions critically relies on the distributional characteristics of the training data. Here we investigate how data obtained from two widely-used experimental methods for detecting PPIs, Co-Immunoprecipitation (Co-IP) and Yeast Two-Hybrid (Y2H), differ in graph-theoretic and functional aspects. We document substantial differences between the two modalities, and find each assay type to be significantly more predictive than the other at specific function and network-associated tasks. We accordingly provide concrete recommendations on assay choice for a range of downstream tasks. Our work emphasizes the need for careful curation of PPI data based on the downstream task and underscores the importance of accounting for subtle but critical variations within biological training data.

1. Introduction

Protein-Protein Interaction (PPI) Networks are known to capture the systems-level biological mechanisms that govern complex cellular activities. However, a pervasive concern in the field is whether the experimental protocols used to obtain the PPI can introduce assay-specific biases, which may affect not only the properties of interactions identified but also the connectivity patterns within the networks. Such biases may portray a distorted understanding of the underlying biological mechanisms, imposing both experimental and computational challenges. Especially in the context of deep learning-based predictive PPI models, poor curation

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of PPI training datasets that fails to account for the underlying detection protocols and their impacts on network-wide structures/functions risks severely compromising the generalizability and predictive power of the model.

Focusing on the two main assays for PPI detection, co-immunoprecipitation (Co-IP) (Iqbal et al., 2018) and yeast two-hybrid (Y2H) (Striebinger et al., 2013), we present a rigorous comparative analysis of the coverage, functional informativeness, and relative advantages of the human networks derived from Co-IP and Y2H protocols (Figure 1). We broadly sought to test the hypothesis that wide-ranging differences between Co-IP and Y2H protocols contribute to significant variations in the interpretability and applicability of PPI data. We began by curating high-confidence experimental reports of physically-binding PPIs and then deployed a broad array of statistical and computational tests. Our tests aimed to address a few fundamental concepts: network-structure similarities and differences between the two protocols, explicit and implicit overlap between their interactomes, functional consequences of variations in their coverage and connectivity, and lastly, guidance on protocol selection and appropriate computational techniques.

Our analysis systematically confirmed the field folklore that Co-IP assays are more likely to produce clique-rich networks. Going beyond such folklore, our analysis offers novel insights into the network-structural, functional and cross-functional differences between the Co-IP and Y2H networks. We believe our findings are of broad relevance to computational modeling (specifically, task-specific dataset curation) and could also guide biologists about the assay to choose for their specific investigation.

2. Results

Dataset creation We extracted high-confidence connected human PPI networks for both Co-IP and Y2H assays from the IntAct Molecular Interaction Database (Orchard et al., 2013). After a series of preprocessing steps that included thresholding (to obtain high-confidence PPI edges) and largest connected component extraction (to ensure global connectivity), the finalized product comprised of a Co-IP network with 8433 nodes and 29,233 edges, and a Y2H net-

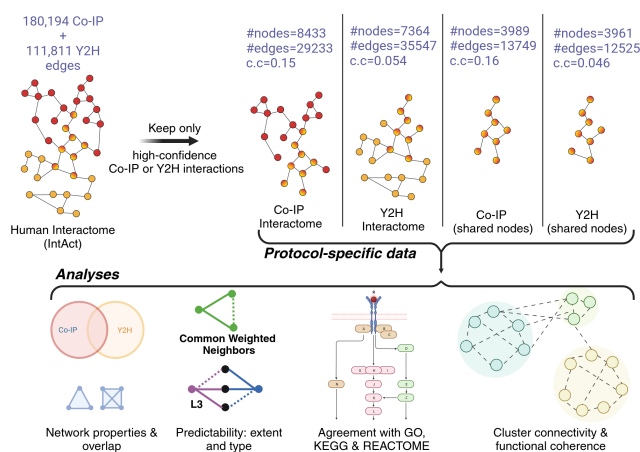


Figure 1. Schematic of different statistical analysis performed on the Co-IP and Y2H networks

work with 7364 nodes and 35,547 edges. We additionally constructed shared-node Co-IP and Y2H networks (which we called Co-IP-shared and Y2H-shared), containing only the proteins present in both the finalized networks. These networks consisted of 3989 nodes and 13749 edges for Co-IP-shared and 3961 nodes and 12525 edges for Y2H-shared (some of the nodes were removed from the shared networks to enforce network connectivity; Appendix A.1).

2.1. Graph-theoretic comparisons

We now describe the results from several graph-theoretic analysis we did on the Co-IP and Y2H networks.

Co-IP and Y2H networks have different local network properties. We found that the two graphs diverge significantly when considering clustering metrics, with the 3-clustering coefficient for the Co-IP network being approximately 2.75 times higher. This difference persists even in the shared networks, suggesting deeper differences in the underlying network structure. We tested this by checking if an unbiased sub-network sampling procedure causes significant divergence in the triangle-count between the two networks (Appendix A.2.1). We ensured that the resulting Co-IP and Y2H sub-networks sampled had fixed network sizes by controlling the number of nodes and edges. After conducting experiments on a broad range of node and edge combinations, we found that the triangle-counts from Co-IP sub-network samples were significantly larger, even after multiple testing correction. This shows that the two networks have substantial local differences, which is unlikely to have occurred through randomness.

Link prediction analysis shows that Co-IP and Y2H interactomes are connected in different patterns Link prediction, the task of identifying missing edges in a graph, can be repurposed as a test of graph structure: link prediction methods vary in their predictive scope (i.e., local vs. global), and the data-generating process they assume when predicting local links (Appendix A.2.2). By checking which link prediction method works best for a given network, we can thus decipher its governing network mechanism. For our analysis, we selected two local link-prediction methods with very distinct guiding principles, CWN and L3, and a global method called GLIDE (Appendix A.2.2). The link prediction results are provided in the Figure 2(A1). The results show that, while L3 outperformed CWN in both Co-IP and Y2H networks, it was especially true for Y2H edges: the ratio $\frac{AUPR(L3)}{AUPR(CWN)}$ is 1.82 (1.73) for the full (shared) Co-IP networks, but substantially higher at 3.31 (3.20) for the full (shared) Y2H networks. Biologically, this crucial finding allays the concern that Co-IP is optimized only for detecting protein complexes. Had this been true, CWN would have outperformed L3 for Co-IP, based on CWN’s governing principle (Appendix A.2.2). Notably, Y2H networks favor L3 over CWN even more, so L3 may still be the superior approach for identifying interactions spanning protein categories.

2.2. Functional associations in Co-IP and Y2H networks

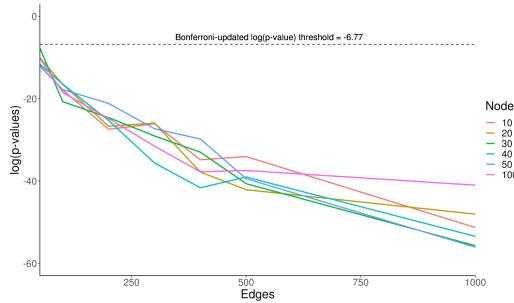
To check if these graph-theoretic differences induce significant changes in the functional relationships implied by the networks, we conducted a broad range of Gene Ontology (GO) based tests to quantify the functional differences between the Co-IP and Y2H networks. We utilized all three GO hierarchies: biological process (BP), molecular function (MF), and cellular component (CC) (Appendix A.3.1).

The edges of the Co-IP network link more functionally-similar proteins than Y2H. To assess per-edge functional relationship between nodes in Co-IP and Y2H networks, we used two variations of the Resnik score and the EI homophily index (for Resnik score, higher is better; for EI index, lower is better; Appendix A.3.2). These results show that the edge-wide similarities are substantially higher in the full and shared Co-IP networks. For the shared networks in BP and MF classes, Co-IP-shared’s Resnik scores were respectively 44% and 46% larger than Y2H-shared. The difference persisted, albeit weaker, even after we discarded nodes with no GO labels (17% and 5%, respectively). Co-IP edges are thus more functionally enriched and the local connectivity differences between the two networks are indeed reflected in the per-edge functional relationships.

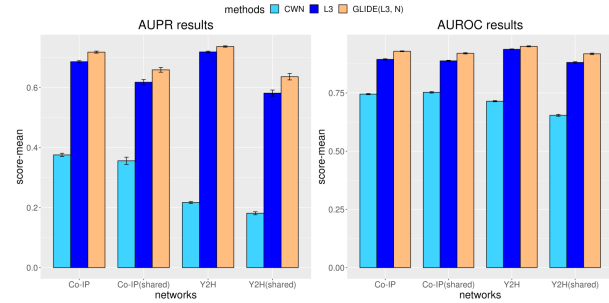
Going beyond per-edge measures, we employed two local-neighborhood assessments, majority-vote based WMV and

A. Network-Theoretic Analysis

1. one-tailed t-test results from sub-network sampling analysis, with different settings for the number of added nodes and edges.

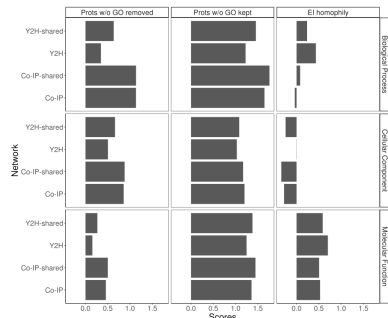


2. AUROC and AUPR link prediction results using two local (L3 and CWN) and one global (GLIDE) methods.

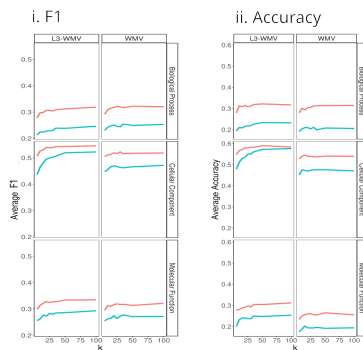


B. Function Based Analysis

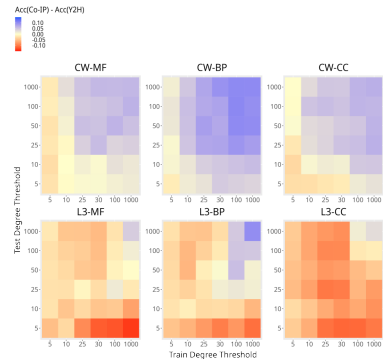
1. Edge-based Function Prediction Analysis using Resnik similarity and EI homophily indices.



2. Function prediction results: Average F1 and Accuracy.



3. Degree-stratified function prediction results.



C. Cross-Functional and Transient Analysis

1. Co-IP vs Y2H conductance plots for KEGG genesets.

2. Co-IP vs Y2H conductance plots for disease sets.

3. Transient interactions involving kinase and phosphatase proteins.

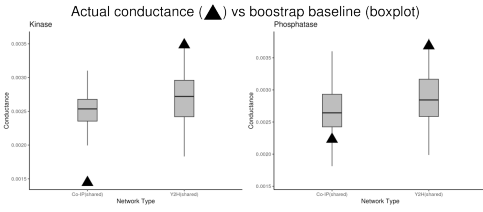
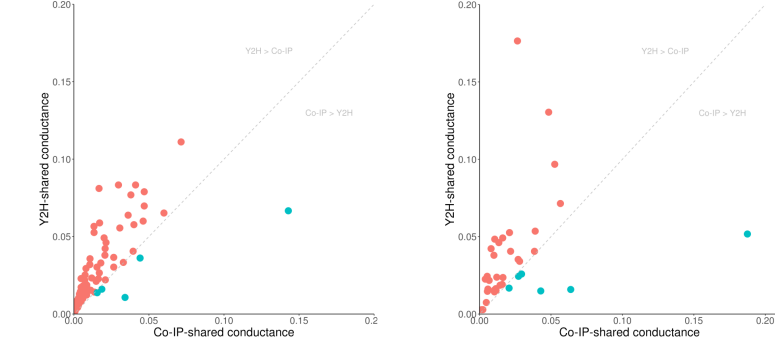


Figure 2. Network-based, function-based and cross-functional differences between Co-IP and Y2H networks

its L3-based variant, to predict GO labels for the Co-IP-shared and Y2H-shared networks (Appendix A.3.3). The results in Figure 2(B2{i-ii}) show that Co-IP-shared's top-1 accuracy and F1-max scores were noticeably larger for both WMV and L3-WMV based predictions across all the GO branches. The gain was the strongest in GO:BP (32% F1-max improvement with WMV; 29% with L3-WMV), and the weakest in GO:CC (12% F1-max improvement with WMV, 6% with L3-WMV).

Co-IP network's better function-prediction performance is largely due to the high-degree hub nodes. Both Co-IP

and Y2H networks have a scale-free characteristic, contain a limited number of high-degree hubs. However, the biological relevance and validity of these hubs has been debated. To explore this, we repeated our WMV and L3-WMV analysis on subsets of proteins that satisfy some degree constraints. Our majority-vote prediction framework consists of a training and a test set, and we used two degree-based thresholds, d_{tr} and d_{te} , respectively, to parameterize our degree-based analysis. The resulting scores for every (d_{te}, d_{tr}) pairs are presented as a heat-map in Figure 2(B3). Higher d_{tr} and d_{te} consistently result in greater Co-IP-shared outperformance over Y2H-shared. Conversely, discarding the contributions

from high-degree hub nodes resulted in Y2H-shared's L3 accuracy beating Co-IP-shared. This suggests strongly that the functional edge of Co-IP over Y2H is mainly due to the high degree hub proteins. That Y2H hubs are functionally less informative suggests they may be the consequence of promiscuous binding (Sontag et al., 2007).

2.3. Type of PPI coverage: transient PPIs and PPIs within functionally-related genesets

Disorders like Huntington's disease and autism affect the operation of many related biological pathways. For network-theoretic analyses (e.g., disease gene prioritization (Erten et al., 2011)), it is necessary that the networks have adequate connections between groups containing proteins with similar functions. The study of cross-functional relationships is also important in the analysis of transient PPIs, which regulate essential mechanisms involving signal transduction (Simon et al., 1991; Zapf et al., 2000), immune response (Srinivasan & Dunker, 2012), etc. In order to effectively measure this biologically-relevant property, we used the graph-theoretic measure called graph conductance. The graph conductance metric computes the probability that an edge originating from the set of nodes S terminates at a node outside the set ($V \setminus S$, where V is the set of all nodes). Therefore, a large graph conductance would imply that the set is well-connected with the rest of the network. We used this conductance metric to analyze the cross-connectivities of KEGG and disease functional genesets, and transient PPIs involving kinases and phosphatases for both Co-IP and Y2H networks (Appendix A.4.1).

Cross-functional connectivity is greater in the Y2H network. Limiting to the shared networks, we computed the Co-IP and Y2H conductances for all 107 KEGG genesets and present the results in a diagonal graph (Figure 2(C1-2)), where each geneset forms a data-point and the x - and y - axes correspond to the observed Co-IP and Y2H conductances respectively. We performed a similar analysis for 37 disease-related genesets. For both categories, Y2H-shared's conductance is almost always larger than Co-IP-shared. On average, the Y2H conductances were 132% and 117% larger than Co-IP for the KEGG (Kanehisa et al., 2007) and Jensen (Grissa et al., 2022) disease related genesets respectively. Additionally, only 4% KEGG genesets and 16% disease sets had larger Co-IP conductances. This strongly suggests that the functional groups in Co-IP networks are more locally concentrated and isolated from the rest of the network.

Y2H network has proportionally more transient kinase-substrate and phosphatase-substrate PPIs. Reasoning that phosphorylation is primarily a transient protein interaction, we created genesets consisting of kinases and phos-

phatases and assessed their connectivity to other proteins. The observed conductances for the kinase and phosphatase sets were 0.0014 and 0.0026, respectively, for the Co-IP-shared network. In contrast, the Y2H-shared results were substantially higher at 0.00348 and 0.00368, respectively. This strongly suggests that the Co-IP network contains a significantly fewer transient relationships involving phosphorylation and dephosphorylation phenomena in cells.

Additionally, we examined the statistical significance of the differences by comparing the observed conductances against their expected values (Appendix A.4.2). Here, the observed values being higher (or lower) than expected would imply that the kinase and phosphatase groups are even more connected (or isolated) than normal. As the box-plot in Figure 2(C) shows, the conductances of the Co-IP networks were significantly lower than expected for both the kinase (expected value = 0.0024 ± 0.0003) and phosphatase (expected value = 0.0026 ± 0.0003) sets. In contrast, observed Y2H conductances were higher than expected (expected for Y2H: kinase = 0.0027 ± 0.0003 , phosphatase = 0.0029 ± 0.0004).

3. Discussion

We have systematically probed some long-held assumptions about differences between Co-IP and Y2H networks. We found that substantial differences in the network-theoretic and functional relationships do indeed exist between these interactomes. We found that the Co-IP networks have a considerably larger number of triangles than Y2H, manifesting in greater common weighted (CWN) link prediction accuracy. However, a simple replacement of CWN with the more biologically-meaningful L3 heuristic mitigates this disparity, indicating that the changes in predictability caused by the differences in triangle motifs frequency can be easily avoided by changing the analytical approach.

The local functional relationships of the two networks were also substantially different from each other. Here we recommend the use of Co-IP networks: it significantly outperformed Y2H consistently, across a broad range of function prediction tests. Furthermore, we observed that the improvement was especially strong in its high degree nodes. Thus, if a deep study of a specific functional system is desired and substantial experimental coverage (of Co-IP or Y2H) can be obtained, we recommend the choice of Co-IP over Y2H.

On the other hand, for cross-functional analysis Co-IP networks may be less suitable, as functionally-related groups are more isolated in the Co-IP network. Moreover, transient PPIs associated with kinases and phosphatases were significantly under-represented in Co-IP data. The opposite was true for Y2H. Therefore, Y2H may be better suited for tasks where goal is a broad study of biological mechanisms that encompass more than one functional group.

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A. Results

A.1. Dataset Construction

We extracted human PPI networks for both Co-IP and Y2H assays from the IntAct Molecular Interaction Database (Orchard et al., 2013), which contains 180,194 unique Co-IP and 111,811 Y2H interactions; each interaction was associated with a confidence score between 0 and 1. We focused on human PPIs since they have the widest coverage under both protocols. In order to only allow for high-confidence edges from both networks and to control for network size differences, we selected edges with score ≥ 0.56 and ≥ 0.35 , for Co-IP and Y2H, respectively, as these thresholds corresponded to clear break-points in the distributions of the edge confidence scores.

Since many graph-based algorithms (e.g., random walk with restart) implicitly assume that the input graph is a single connected component, we next ensured graph connectedness by limiting ourselves to the largest connected component of both networks, which resulted in the final Co-IP network having 8433 nodes and 29,233 edges, and Y2H network having 7364 nodes and 35,547 edges. There were 1754 shared edges (i.e. PPIs) between these networks. While this overlap is statistically significant, as more than 5% of edges in one network is also present in another, it is nonetheless fairly modest and indicates that incomplete coverage as well as measurement biases remain a concern. Therefore, we defined overlap more leniently, focusing just on the nodes (i.e., proteins) that are present in both Y2H and Co-IP networks. For this shared subset, we extracted the largest connected sub-graph in each network, naming these “Co-IP-shared” (with 3989 nodes and 13749 edges) and “Y2H-shared” (with 3961 nodes and 12525 edges). We emphasize that only the nodes are identical in these “shared” networks, while the edges are sourced from the respective complete networks.

A.2. Link Prediction Analysis

A.2.1. SUB-NETWORK SAMPLING PROCEDURE TO FIND LOCAL 3-CLUSTERING DIFFERENCES BETWEEN CO-IP AND Y2H NETWORKS

To quantify the significance of greater triadic closure in Co-IP networks, we sampled sub-graphs from the interactomes and counted the number of triangular motifs in each. However, network attributes like the overall number of nodes and edges in the samples could induce biases in the computed differences. To mitigate these size-related effects, we fixed the number of nodes and edges within the Co-IP (or Y2H) samples. We first extracted the common sub-graph (comprising all nodes and edges present in both Co-IP and Y2H), and for various settings of m (number of new nodes) and n (number of new edges), we added exactly m new nodes and n new edges from the original Co-IP (or Y2H) network to this common graph. This measure effectively neutralized the size-related biases in assessing network attributes. After generating 50 samples for each (m, n) pair, we performed a two-sample one-tailed t-test on the closed triangle counts of Co-IP and Y2H subgraphs. For all possible (m, n) configurations appraised, where $m \in \{10, 20, 30, 40, 50, 100\}$ and $n \in \{50, 100, 200, 300, 400, 500, 1000\}$, all the observed p values were found to be less than 10^{-10} . Even after the application of multiple testing correction (using the Bonferroni method), all (m, n) parameters yielded corrected p -values well below the 0.05 threshold. These findings support our assertion that the differences in the local network organization of Co-IP and Y2H networks, particularly as defined by the presence of triangular motifs, are unlikely to occur due to randomness.

A.2.2. LINK PREDICTION METHODS EMPLOYED AND THEIR GOVERNING PRINCIPLES

We selected three link prediction methods that are commonly used and whose core concepts are seen also in other approaches. The first two, common weight normalized (CWN) and length-3 paths (L3), are localized techniques that predict an edge (p, q) based on the neighborhoods of nodes p and q . The CWN approach gives priority to edges between nodes, p and q , with numerous shared neighbors. Meanwhile, L3 employs a “lock-and-key” paradigm, emphasizing edge predictions that involve diverse protein categories. These approaches encapsulate distinct underlying mechanisms: CWN capitalizes on a “friends-of-friends” clustering model akin to triadic closure, while L3 focuses on interactions between “key” and “lock” protein categories. Notably, these lock and key categories are locally, not universally, applicable, i.e., there may be many types of keys as well as locks.

The third technique is a global link prediction method called GLIDE, which internally uses diffusion based distances (DSD) to characterize and evaluate prospective links. GLIDE scores are composed of both the global (informed by this DSD metric) and local components, where different choices of local heuristics can be selected based on the underlying network principles. For the link prediction results in the main paper, we chose the GLIDE variant which used the un-normalized version of DSD as the global and the L3 score as the local method, as this combination performed the best in our evaluations.

We used both full and shared-node versions of the Co-IP and Y2H networks in our experiments, and created the test set for each network by randomly choosing 25% of its edges (while ensuring network connectivity) as our hold-out set. The predictive performance on this set was assessed using the Area Under the Precision Recall Curve (AUPRC) and Area Under the Receiver Operator Characteristics (AUROC) scores.

A.3. Function Prediction Analysis

A.3.1. SELECTING FUNCTIONAL LABELS

We used Gene Ontology (GO) information to assess the functional similarity between adjacent nodes in each network. We assessed all three GO domains: Molecular Function (MF), Biological Process (BP) and Cellular Component (CC). To remove overly broad labels, we selected only those at level 5 or deeper in the GO hierarchy; to remove overly specific labels, we required that each label annotate at least 50 proteins in the network.

A.3.2. METRICS FOR EVALUATING FUNCTIONAL ENRICHMENT

Resnik similarity score: The hierarchical nature of GO as a Directed Acyclic Graph (DAG) allows distinct GO terms to be functionally related with each other based on the position of their shared ancestors. It is essential for an effective measure to exhibit the hierarchical nature of GO while evaluating protein functional similarities, which is why we used a popular function-based scoring method called Resnik similarity that preserves this property.

For a given GO-term ℓ , let $\mathcal{P}(\ell)$ represent the set of immediate ancestor of ℓ and let $\mathcal{A}(\ell)$ represent all its ancestors going back to the root of the GO hierarchy. The information content of ℓ (i.e. $i(\ell)$) is defined as

$$i(\ell) = -\log(\Pr(\mathcal{A}(\ell))) = -\log \prod_{v \in \mathcal{A}(\ell)} \Pr(v|\mathcal{P}(v)) \quad (1)$$

$$= -\sum_{v \in L} \log \Pr(v|\mathcal{P}(v)) \quad (2)$$

Where the probability $\Pr(v|\mathcal{P})$ denotes how likely it is to reach v from its immediate ancestors in $\mathcal{P}(v)$. Then, the Resnik similarity between two GO terms, x and y becomes:

$$res(x, y) = i(lca(x, y)) \quad (3)$$

Where $lca(x, y)$ is the lowest common ancestor of x and y .

As a protein can have more than one GO label, for proteins p and q , we compute the average of the pairwise Resnik scores between their GO labels as our final scoring metric $R(p, q)$; which can be written as:

$$R(p, q) = \frac{1}{|GO(x)||GO(y)|} \sum_{x \in GO(p), y \in GO(q)} res(x, q) \quad (4)$$

Where $GO(m)$ represents the set containing all the GO labels of a protein m .

EI homophily score: We additionally used the Resnik scores between protein neighborhoods to evaluate the degree of network homophily in a network. Network Homophily refers to the extent by which similar nodes (i.e. nodes with similar labels) form direct connections with each other, compared to the dissimilar nodes. We used a popular metric called the "EI homophily" to quantify this, which, given a node p and its neighbors N_p , can be described as:

$$EI(p) = \frac{|D_p| - |S_p|}{|N_p|} \quad (5)$$

Where D_p and S_p represents the set of dissimilar and similar neighbors of p respectively. Note that, the value of EI can range from -1 to 1 and the closer $EI(p)$ is to -1, the more similar p is to its neighborhood proteins.

For each protein p , we generated S_p by selecting all neighbors with non-zero R -scores; the remaining neighbors were added to D_p . The scores for all the proteins were averaged to return a network-wide average EI-score.

A.3.3. FUNCTION PREDICTION METHODS

We evaluated the neighborhood functional enrichment of the shared Co-IP and Y2H networks through the use of two simple function prediction algorithms; (a) Weighted Majority Vote (WMV), and (b) L3-based Weighted Majority Vote (L3-WMV), both of which are described below:

Weighted Majority Vote (WMV) Let p be a protein in a weighted graph $G = (V, E, w)$ and let N_p denote the set of its neighbors. We can rank the proteins in N_p by their edge weights with p in a descending order, and select the top k proteins from the ranked list to produce a new set $N_{p,k} \subset N_p$, for a positive integer $k < |N_p|$ (if $k \geq |N_p|$, set $N_{p,k} = N_p$). Then, we can let each protein $q \in N_{p,k}$ to give a weighted vote on functional labels of p , where the weight is decided by the edge weight $w(p, q)$. The label associated with the highest vote is then selected as the predicted label of p .

L3-based Weighted Majority Vote (L3-WMV) Given a network $G = (V, E, w)$, and its corresponding weighted adjacency matrix A , we use the L3 pairwise scores to produce a new adjacency matrix A' . In fact, A' can be conveniently represented by the matrix product $A' = AD^{-1/2}AD^{-1/2}A$, where D is the diagonalized degree matrix of A . Then, we repeat the same procedure for WMV to predict functional labels, while replacing the original edge weights with the edge-weights from the newly computed A' .

We evaluated both algorithms in a 5-fold cross validation setting, where we randomly separated the network's proteins into 5 uniform non-overlapping blocks. For each fold, a particular block has its GO labels masked (i.e. "testing block") and the remaining blocks are used to predict the masked labels (i.e. "training block"). We repeated this process 5 times, with the k values set to $k = \{5, 10, 15, 20, 25, 30, 35, 50, 100\}$, and reported the average Top-1 Accuracy and F1-max scores for the shared networks, and the results are available in the main document (Figure 2(B2)).

A.4. Cross-functional analysis

A.4.1. AGGREGATING FUNCTIONALLY RELATED GENESETS FROM MULTIPLE SOURCES

We gathered 186 pathway derived genesets from KEGG and filtered the sets with less than 10 nodes in common with the shared Co-IP and Y2H networks, leaving behind 107 genesets for conductance analysis.

Additionally, we extracted known kinase and phosphatase proteins from the HUGO Gene Nomenclature Committee (HGNC), and out of the 444 kinases and 437 phosphatases found, we selected only 178 kinases and 168 phosphatases that were also present as nodes in the shared Co-IP and Y2H networks, in order to observe the transient PPI relationships that occur in kinase-substrate and phosphatase-substrate interactions.

A.4.2. EVALUATING THE SIGNIFICANCE OF TRANSIENT KINASE-SUBSTRATE AND PHOSPHATASE-SUBSTRATE RELATIONSHIPS FOR CO-IP NETWORKS

For a kinase (or phosphatase) set, we measured its expected Co-IP (and Y2H) conductance by randomly sampling a set containing N_k nodes from the network, and measuring its conductance (where N_k is the size of the kinase set). Repeating this procedure 100 times, we get the estimated distribution of the conductance range for each network. Finally, to determine if the measured kinase (and phosphatase) conductance is statistically significant, we check if it is at the tail end of the estimated distribution.