ENQUIRE RECONSTRUCTS AND EXPANDS GENE AND MESH CO-OCCURRENCE NETWORKS FROM CONTEXT-SPECIFIC LITERATURE

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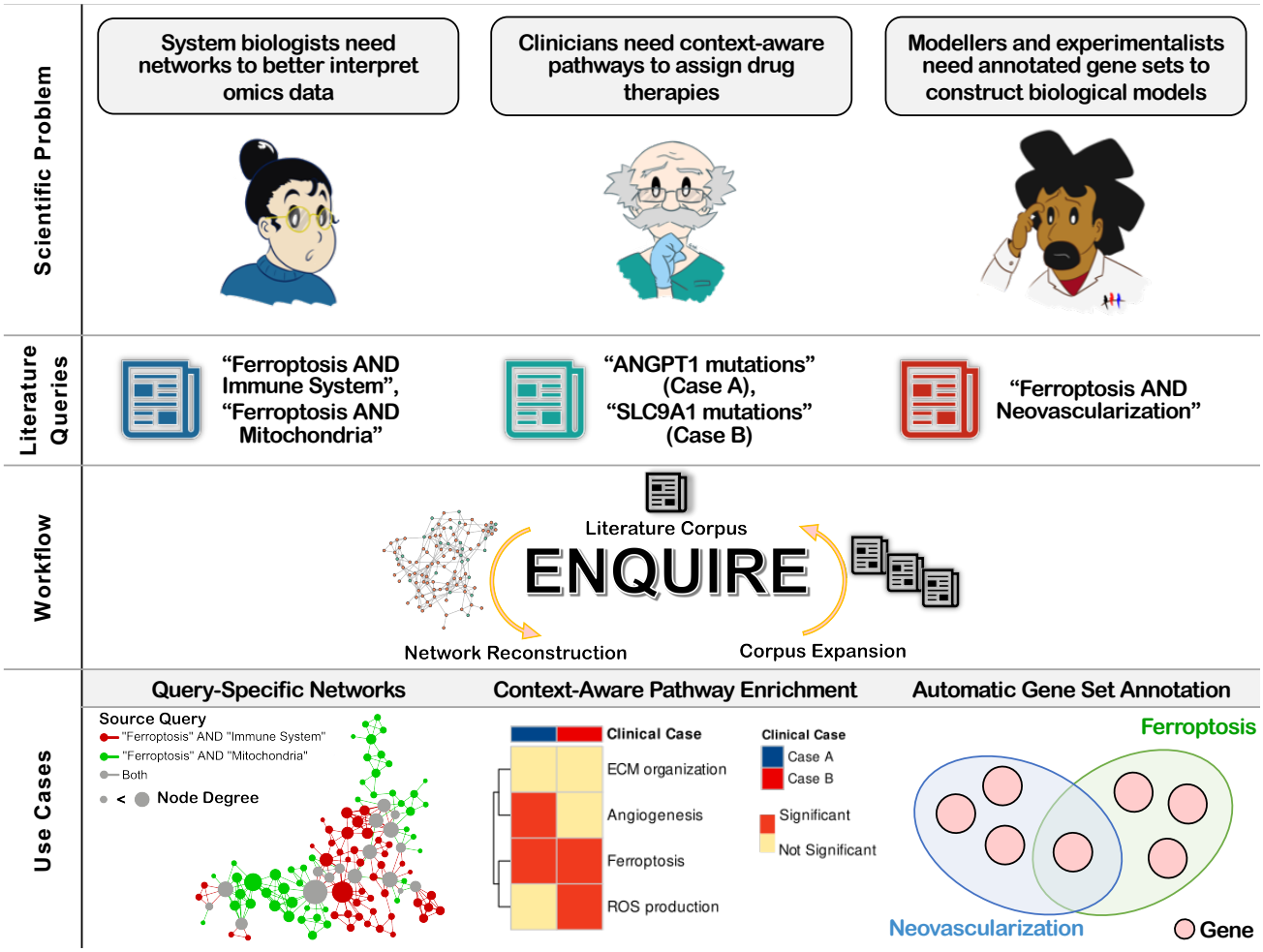
ABSTRACT

The accelerating growth in scientific literature is overwhelming our capacity to manually distil complex phenomena like molecular networks linked to diseases. Moreover, confirmation biases in search engines and databases influence the interpretation of facts and the generation of hypotheses. ENQUIRE (Expanding Networks by Querying Unexpectedly Inter-Related Entities) offers an alternative to manual literature curation and database mining to study complex biomedical phenomena. ENQUIRE generates a co-occurrence network of genes and biomedical ontologies (MeSH) using a corpus of publications as input. The algorithm iteratively reconstructs and expands the network by generating PubMed queries from significant interrelations, until it obtains an interconnected network of genes and MeSH relevant to the input corpus. We systematically evaluated ENQUIRE's versatility and found that it generates co-occurrence networks similar to those based on co-expression data and manually annotated databases with a fraction of the time and human resources. Using case studies spanning cancer, cell differentiation and immunity, ENQUIRE proved to identify interlinked genes and enriched pathways unique to each topic, thereby preserving their underlying diversity. ENQUIRE supports biomedical researchers by easing literature annotation, boosting hypothesis formulation and facilitating the identification of molecular targets for subsequent experimentation.

GRAPHICAL ABSTRACT



INTRODUCTION

Gene network annotation is of high interest to prime the analysis of biomedical omics data, identification of disease-specific regulatory modules, and therapy-oriented studies like drug repurposing (1–4). However, the reconstruction of such complex data takes time and manual effort. The growing biomedical literature corpus makes the annotation of biomolecular pathways and their function challenging to implement. Curating literature annotations requires domain expertise, but that same background knowledge can entail predispositions towards partial pictures of faceted biomedical problems (5). In contrast, information extraction from databases often omits the contextual information of gene interactions and can bias the results towards ubiquitously expressed, commonly investigated and richly annotated genes (6–8). This makes systematic comparisons across case studies and experiments unattractive from an expenditure perspective.

In recent years, there have been significant investments in the automatic annotation of scientific corpora. The knowledgebase immuneXpresso, constructed by systematic text mining of the PubMed database, indexes interactions among immune cells and cytokines(9). SimText provides a framework to interactively explore the content of a user-provided corpus of literature (10). These and other tools rely on natural language processing methods like named-entity recognition (NER) (11, 12), part-of-speech recognition parsers (13), algorithms to assign directionality (14), relationship detection, and scoring between entities (15, 16). These efforts in biomedical text mining aim at predicting meta-features and co-occurrences in literature corpora. However, assessing the significance of a text-mined interaction or the confidence level in dense, literature-based co-occurrence networks must be better addressed (17, 18). We find this striking considering the well-documented reproducibility crisis (19–21).

In this context, we designed ENQUIRE (Expanding Networks by Querying Unexpectedly Inter-Related Entities) to achieve context-specific, reproducible reconstruction and expansion of biomedical co-occurrence networks from primary literature indexed in PubMed. ENQUIRE processes scientific articles by recognizing genes cited in abstracts and extracting Medical Subject Headings (MeSH) to enrich gene-gene co-occurrences with contextual information. It applies state-of-the-art methods in statistical network analysis to reconstruct a network from an input corpus and generate relevant PubMed queries to contextually expand the underlying corpus and, in turn, the network. A distinctive element in our methodology is the use of a statistical framework that accounts for literature biases. In this study, we present the tool, discuss the features of its underlying algorithm, and assess ENQUIRE's applicability and effectiveness with three relevant test cases.

MATERIALS AND METHODS

A Tool to Generate Co-Occurrence Networks from Literature

ENQUIRE (Expanding Networks by Querying Unexpectedly Inter-Related Entities) is an algorithm that reconstructs and expands co-occurrence networks of human genes and biomedical ontologies (MeSH), using a corpus of publication abstracts as input. The method iteratively annotates MeSH and gene mentions from abstracts, statistically assesses their importance, and generates network-informed PubMed queries, until it obtains a connected network of genes and MeSH terms (or meets another exit condition). ENQUIRE's pipeline implements a loop consisting of serial modules with the following structure (**Figure 1**):

a) The user supplies an input literature corpus in the form of at least three PubMed identifiers (PMIDs).

b) The algorithm indexes the MeSH terms associated to the PMIDs listed. Next, their abstracts are parsed, and named entity recognition (NER) of genes is performed using a lookup table of gene aliases and abstract-specific blocklists of ambiguous terms.

c) ENQUIRE annotates and weights co-occurrences between gene and MeSH entities, giving higher significance to co-occurrences observed more frequently than average.
d) The method selects significant co-occurrences and generates an undirected, simple graph, using a test statistic based on a random graph null model generated from unbiased mining of the literature corpus.
e) Next, the algorithm selects "information-dense" maximal cliques in the network, i.e. clusters of high-weight nodes all connected to each other, and reconstructs larger-scale network communities.
f) ENQUIRE identifies optimal sets of community-connecting graphlets via an approximate solution to the "travelling salesman problem" (TSP).
g) Finally, the algorithm feeds the entity nodes corresponding to the identified community-connecting graphlets into PubMed queries to find additional, relevant articles. Should ENQUIRE find new articles, their PMIDs are joined with the previous ones and provided to module a), starting a new iteration.
ENQUIRE's goal is to generate a connected network and the respective gene- and MeSH-only subgraphs that individually consist of a single, connected component. The loop prematurely terminates if: i) the network is empty after module d); ii) no clique can be selected in step e); iii) the clique network consists of only one community; iv) all generated queries return empty results. With default parameters, ENQUIRE outputs node and edge lists of a gene/MeSH co-occurrence network and the respective gene- and MeSH-only subgraphs at each iteration. ENQUIRE outputs additional metrics, tabulated data, graphics, and links to collected resources for subsequent analyses and make the process reproducible. For instance, it is possible to extract subsets of the literature corpus that support a relation of interest and conveniently access the articles via hyperlinks redirecting to PubMed.

Description of the ENQUIRE algorithm

Recognition of MeSH and Gene Entities. ENQUIRE requires a corpus of PubMed identifiers (PMIDs) supplied by the user as input. For each PMID, the algorithm either collects gene mentions from the retrieved abstracts, the MeSH main headings ("descriptors") and subheadings ("qualifiers"), or both, depending on the entity scope set by the user. The retrieved MeSH terms can be pruned through a user-determined set of relevant categories, which exploits the tree-like, hierarchical structure of the MeSH vocabulary (see **Supp. Mat.**). As other authors have shown that the proportion of gene mentions does not significantly differ between abstracts and full-body texts (22), we only mine the abstracts for gene mentions. To perform named-entity recognition (NER) of genes in the abstracts, we designed a "Swiss cheese model", in which multiple methods complement each other to improve performance. Briefly, ENQUIRE employs a tokenization of the abstract into single-word tokens and interprets tokens as the corresponding reference gene symbol if they match unambiguously in an alias lookup table. Multiple mentions of the same gene within an abstract count as one. We customized ENQUIRE's lookup table to collect as aliases all *Homo sapiens* and *Mus musculus* gene names and descriptions recorded in the databases HGNC, ENSEMBL, Uniprot, miRBase, and MGI (23–27). We made use of MGI's mouse/human orthology table to reassign mouse genes under human, reference gene symbols. In addition, ENQUIRE applies two algorithms to each unprocessed abstract: i) the scispaCy model *en_ner_jnlpba_md* to identify words classified as "CELL_LINE" or "CELL_TYPE" (28); ii) the Schwartz-Hearst algorithm to detect single-word abbreviations and their respective definitions (29). This allows ENQUIRE to construct abstract-specific blocklists that discard i) potentially ambiguous mentions to cell entities, and ii) ambiguous abbreviations whose definitions are not similar to any lookup table alias. See further details in **Supp. Mat**.

Annotation and Weighting of Co-Occurrences. ENQUIRE records the occurrences of MeSH and gene entities within each input article. Then, it counts pairwise co-occurrences by enumerating the subset of PMIDs associated to both entities in each pair. For each pair of entities g_i and g_j that co-occur in at

least one article, we define the weights w and $\widetilde{w}$ accounting for the sheer co-occurrence $X(g_i, g_j)$ as follows:

$$w_{g_i,g_j} := \Psi(X(g_i, g_j), \bar{X}), \qquad w_{g_i,g_j} \in (0, 1]$$

$$\tilde{w}_{g_i,g_j} = 1 - w_{g_i,g_j} \tag{1}$$

$$X(g_i, g_j) = |\{P \mid g_i, g_j \in E^P\}_{P \in PMIDS}|$$

Where $\bar{X}$ is the mean co-occurrence between any two entities in the corpus, $\Psi(\cdot, \bar{X})$ is the zero-truncated, Poisson cumulative density function with a lambda of $\bar{X}$, and E^P is the set of all entities annotated within the PMID P that belongs to the submitted PMIDS corpus. This scoring system assigns higher relevance to co-occurrences that appear more often than average.

Reconstruction of a Weighted Network of Significant Co-Occurrences. ENQUIRE converts the recorded co-occurrences into an undirected multi-graph, where gene or MesH terms become nodes, and each recorded co-occurrence between two entities becomes an edge. Thus, the network has as many nodes as the number of unique MeSH and gene symbols, with as many edges between two nodes as the number of PMIDs in which they co-occur. ENQUIRE implements the Casiraghi-Nanumyan's soft-configuration model applied to pairwise, unweighted edge counts to select significant co-occurrences among entities, adjusted to 1% FDR (30). The test statistics follows a multivariate hypergeometric distribution, under the null hypothesis of observing a random graph whose expected degree sequence and number of edges correspond to the observed one. This allows us to condition the testing to the sheer, per-entity occurrence, which serves as a proxy for leveraging literature biases in the corpus. It is important to note that the null model does not assume independence of individual edges, but merely their equiprobability. This selection results in an undirected, single node-to-node edge co-occurrence graph (i.e. a simple graph). For each pair of adjacent entities g_i and g_j in the simple network, we assign the weights w_{g_i, g_j} and $\widetilde{w}_{g_i, g_j}$ to their mutual edge. Additionally, we prune poorly connected nodes by modularity-based, w-weighted Leiden clustering (31) and removal of singleton communities, that is, unconnected nodes. From the resulting gene/MeSH network, we also extract the respective gene- and MeSH-only subnetworks.

ENQUIRE-generated networks can consist of multiple connected components, i.e. subgraphs. To exclude unimportant components, a subgraph S is retained for subsequent analysis only if the fraction of corpus articles covered by S is higher than a threshold value, as formally defined in

$$T_S := \frac{|\{P \mid E^P \cap E^S \neq \emptyset\}_{P \in \mathrm{PMIDS}}|}{|\mathrm{PMIDS}|} \geqslant t, \quad T_S \in (0, 1] \tag{2}$$

where P denotes a PMID belonging to PMIDS, and E^P and E^S refer to the sets of gene or MeSH entities recorded in either P or S. T_S thus reflects the representativeness of S with respect to the entirety of the submitted corpus. The value of t can be set by the user. To avoid introducing irrelevant entities, ENQUIRE stops without further network expansion if the gene/MeSH network and the respective gene- and MeSH-only subnetworks individually consist of a single, connected component with $T_S \geq t$. We compute the weight of a node g in the connected graph S utilizing the composite function W,

which is the product of normalized metrics for betweenness centrality (b) and w-weighted degree strength (d):

$$W(g,S) := F_b(b(g,S)) \cdot F_d(d(g,S)), \qquad W \in (0,1] \tag{3}$$

Here, F_x denotes the empirical cumulative density function for the corresponding x parameter, calculated over S.

Construction of Communities from "Information-Dense" Cliques. To identify the most relevant parts of the gene/MeSH network, ENQUIRE first identifies the maximal cliques of order three or more. By definition, these are graphlets whose nodes are all adjacent to each other and not a true subset of a larger clique. Applying the KNet function from the SANTA R package (32), we retained cliques that form significant clusters of associated entities with p-values adjusted to 1% FDR. This process allows us to consider the network topology by adjusting each maximal clique's significance in case many of such graphlets exist in the network. Through KNet's assessment of node weights (W) and weighted distances (w), ENQUIRE finds a pruned network C containing only cliques. At this point, ENQUIRE stops if the gene/MeSH network contains less than two significant cliques according to KNet. Next, ENQUIRE identifies communities in the pruned network using modularity-based, w-weighted Leiden clustering. ENQUIRE stops if it detects a single community that encompasses all nodes in C.

Identification of Community-Connecting Entities. For any two communities C_i and C_j, we select the set of community-connecting, weighted graphlets $\Gamma_{C_i,C_j}(V_k, L_{k-1})$ satisfying the properties: i) all nodes g_i with weight W in the k-sized set V_k also belong to either C_i or C_j; ii) both intersections between V_k and C_i or C_j are non-empty; iii) the w-weighted, $k-1$ edges L_{k-1} are sufficient to obtain a single connected component; iv) there is only one edge l_{g_i,g_j} that connects nodes belonging to distinct communities. Here, k is a parameter chosen by the user.
This allows us to rank the set of community-connecting entities $V_k$in any graphlet Γ_{C_i,C_j} by means of the distance metric R:

$$\begin{aligned} R(\Gamma_{C_i,C_j}(V_k, L_{k-1})) &:= -\log\left(\prod\nolimits_{g_i \in V_k} W(g_i,\cdot) \prod\nolimits_{l_{g_i,g_j} \in L_{k-1}} w_{g_i,g_j}\right), \\ & R \in \mathbb{R}_{\geqslant 0} \\ & V_k \in C_i \cup C_j\,,\; V_k \cap C_i \neq \emptyset\,,\; V_k \cap C_j \neq \emptyset \\ & |\{l_{g_i,g_j} \mid g_i \in C_i, g_j \in C_j\}_{l_{g_i,g_j} \in L_{k-1}}| = 1 \end{aligned} \tag{4}$$

The smaller R, the closer two communities connected by V_k are.

Retrieval of New PMIDs via PubMed Queries Based on Optimal Connections. To evaluate which genes and MeSH terms are particularly suited for expansion queries, ENQUIRE constructs a multigraph M where network communities become nodes and all R-weighted connections between two communities become edges. R-weighted edges that does not fulfil the triangle inequality $R\left(\Gamma_{C_i,C_j}\right) \leq R(\Gamma_{C_i,C_z}) + R\left(\Gamma_{C_z,C_j}\right), \forall\, i,j,z$ are excluded. Then, we solve the travelling salesman problem (TSP) utilizing

Christofides' approximate solution as implemented in the Python package Networkx version 2.6 (33). Via the visited edges, this yields an optimal path across communities and a corresponding collection of V_k entity sets. Each selected k-sized set results in a PubMed query formulated via the NCBI's *esearch* utility (34). We condition the search terms representing gene aliases and MeSH with "[Title/Abstract]" and "[MeSH Terms]", respectively, and exclude review articles from the results. The constructed PubMed queries require a match for all the k entities in the optimal path – e.g. *"melanoma/immunology"[MeSH Terms] AND ("IL1B"[Title/Abstract] OR "interleukin 1-beta"[Title/Abstract] […]) AND […]*. If all queries that involve a community lead to an empty result, we prune all previously used edges from M, compute a new TSP solution, and submit newly generated queries, provided at least one entity per query belongs to a community whose previously queried entities yielded no PMIDs. This process is repeated A times, where A is a parameter specified by the user. If at least 1 new PMID matches any of the constructed queries, ENQUIRE starts a new analysis from the union of new and old PMIDs; otherwise, it stops. The rationale behind merging old and new PMIDs is to account for the original corpus when computing the statistics on new co-occurrences.

Network Expansion and User-Defined Parameters. Whenever ENQUIRE reconstructs a network from the union of old and new PMIDs, the previously reconstructed network is combined with the new one. The joined network has recomputed edge and node weights in accordance to the expanded literature corpus and connectivity. The rationale is to prioritize the original reconstruction, while also leveraging the expansion corpus. Users can tune five options to tailor the workflow, namely: 1) Restricting the target entities to annotate genes or MeSH only – default: both; 2) representativeness threshold t to discard subgraphs characterized by poor overlap with the literature corpus – default: 1% overlap; 3) query size k to control the number of entities that must be simultaneously used in a PubMed query – default: 4 entities; 4) trial attempts A to choose the number of attempts at reconstructing an optimal path between network communities and querying – default: 2 attempts; and 5) connectivity criterion K to exclude newly found entities not holding sufficient number of connections to K previously existing communities – default: 2 communities. See **Supp. Figure 1** for a comprehensive description of the algorithm.

Post-hoc **Analyses**

Context-Aware Gene Sets. To reconstruct contextual gene sets using gene/MeSH co-occurrence networks, adapted network-based relational data and the Non-Euclidean, Fuzzy Relational Clustering algorithm (NEFRC) to the method of Khan et al. (35, 36). To this end, we first construct the inverse log-weighted similarity matrix between the gene/MeSH network nodes (37). This metric prioritizes nodes sharing many lower degree neighbors rather than few higher degree ones. We convert the obtained matrix into a Manhattan distance matrix; then, we use the R package DynamicTreeCut and Ward's clustering to identify initial clusters and create an initial membership degree matrix (38, 39). Finally, we detect fuzzy clusters of genes and MeSH terms by applying NEFRC to the log-weighted similarity matrix, using the R package fclust (40). The resulting membership degree matrix allows annotating genes with desired cluster membership degrees and extracting the linked MeSH terms to characterize the gene set. We implement this context-aware gene set detection method in a standalone R script that uses ENQUIRE-generated gene/MeSH networks as inputs and outputs gene sets and associated MeSH metadata.

Context-Aware Pathway Enrichment Analysis. We designed a method to map any text-mined co-occurrence network G into a mechanistic reference network N and infer context-specific enrichment of molecular pathways. With this strategy, we attempt to mechanistically explain the indirect

relationships making up the co-occurrence network. To this end, we define the fitness score Q for every gene g in N with non-zero node degree d:

$$Q(g) := d(g,N)^{-1} \cdot \sum_{g_i \in V(G)} \sum_{g_j \in V(G)} e^{-\hat{\delta}_G(g_i,g_j)} \cdot \mathbf{1}_{\{\delta_N(g_i,g) + \delta_N(g,g_j) \leqslant 2,\ g_i \neq g_j\}} \tag{5}$$

$$Q \in \mathbb{R}_{\geqslant 0}$$

Here, $\hat{\delta}_G(g_i, g_j)$ and $\delta_N(g_i, g_j)$ are the $\widetilde{w}$-weighted and unweighted distances from g_i to g_j in the graphs G and N, respectively. The indicator function implies that non-text-mined genes without at least two text-mined nodes as neighbors have Q equal to zero. We normalize all scores to decorrelate Q from the node degree d, similarly to other approaches in network propagation (41, 42). As a mechanistic reference network, we chose STRING's (release 11.5) *H. sapiens* network of protein-coding, physically interacting genes (43). We exclusively combined the "experimental" and "database" channels to calculate STRING's confidence score, then pruned all edges with score below the 90th percentile and resulting isolated nodes, obtaining a reference network of 9482 nodes and 88333 edges. Then, we calculate Q scores for proteins in the reference STRING network using the ENQUIRE-generated gene network, and test for topology-based enrichment of gene sets using SANTA's KNet function. We used Neo4j 3.5 and the Reactome Graph database (44) to obtain gene sets from Reactome pathways. Significantly enriched pathways are the ones that are putatively functional in the ENQUIRE-generated gene-gene co-occurrence network. We implemented the resulting context-aware pathway enrichment analysis method in a standalone R script that uses ENQUIRE-generated gene/MeSH network and outputs enriched pathways.

Benchmarks

Reconstruction of Reactome Gene Sets from Referenced Literature. We extracted annotated genes and reference literature for all *Homo sapiens* Reactome pathways using the Neo4J 3.5 and the Reactome Graph database. We employed NCBI's *esearch* and *elink* utilities to retrieve primary research articles cited by included review articles (34). For each pathway literature corpus, ENQUIRE performed one network reconstruction set to only extract gene mentions from article abstracts. As a benchmark, we processed the same abstract sets using PubTator Central's implementation of GnormPlus (11, 45). For the latter, we used NCBI's lookup tables of *H. sapiens* and *M. musculus* to assign gene aliases to a reference *H. sapiens* symbol. After excluding pathways with less than three literature references or only one human gene annotated in either ENQUIRE's or NCBI's lookup tables, we obtained a set of 967 evaluable pathways. We evaluated ENQUIRE and GNormPlus performances by quantifying false positive rate (FPR), precision and recall of the annotations in the 952 pathways with at least one gene recognized by both ENQUIRE and PubTator Central. We also evaluated the effect of statistics-based pruning of multigraphs for 720 pathways, whose literature corpora generated a significant, pruned network.

Estimate of Molecular Interrelations. We automatically generated a list of case studies by crossing leaf nodes downstream of *Diseases* and *Genetic Phenomena* (G05) MeSH categories (MeSH tree 2021). We then constructed a PubMed query from each pair by "AND" concatenation. Examples of such queries are *"Stomach Neoplasm"[MeSH Terms] AND "Chromosomes, human, pair 18"[MeSH Terms]*, and *"Acquired immunodeficiency syndrome"[MeSH Terms] AND "Polymorphism, single nucleotide"[MeSH Terms]*.

For each query result with a size between 50 and 500 articles, we executed one network reconstruction. If obtaining a gene-gene co-occurrence network, we investigated whether its set of genes produced a network with more functional interactions than expected by chance. To obtain background distributions of edge counts for each gene set size observed with ENQUIRE, we sampled one million random gene sets and cumulated their interconnecting edges in STRING's v. 11.5 *H. sapiens* functional protein network. We only included functional associations from experiments, co-expression, and third-party databases with a cumulative score higher than 0.7 between proteins. The significance of each ENQUIRE-generated gene set's edge count was computed from the right-tailed probability of the empirical distribution.

Moreover, we compared the ENQUIRE-generated gene-gene wirings to STRING-derived associations using the DeltaCon similarity measure in a permutation test (46). To this end, we generated 10,000 random graphs for each observed ENQUIRE network. Each random graph was obtained through 300 random edge-swapping attempts while preserving the degree sequence of the original network. To obtain sensible probability densities, we focused on ENQUIRE-generated networks with degree sequences allowing at least ten different realizations of a graph. We followed the formula $\prod_i^n d_i!$, where d_i is the degree of the i-th node of a graph containing n nodes.

Assessment of Context Resolution by Topology-Based Enrichment of Molecular Pathways. To show that ENQUIRE preserves context-specific molecular signatures, we designed a broad panel of case studies (**Table 1**). Each corpus consisted of the union of references contained in three independent reviews accessible via NCBI's *elink* utility (34). We selected reviews from the results of PubMed search queries consisting of two or three MeSH terms (e.g. *"Melanoma"[MeSH Terms] AND "Signal Transduction"[MeSH Terms]*), favoring PubMed-ranked best matches when possible. We also included an unspecific positive control group consisting of the union of all context-specific corpora. This experimental design allowed us to construct a reference dendrogram that clusters the case studies only based on i) baseline biological knowledge and ii) expecting expanded networks of a case study to cluster together with the originally reconstructed one. Then, we applied ENQUIRE with default parameters to each case study and analyzed all resulting gene-gene networks, i.e., from original and expanded corpora. We applied the *post hoc*, context-aware pathway enrichment analysis described above to all generated networks. We tested the enrichment of Reactome pathways with sizes ranging from 3 to 100 genes, categorized as in the database's *Top-Level Pathways* and disease ontologies. We performed hierarchical clustering of the networks using Euclidean distance and Kendall's correlation based on network-specific, KNet-generated p-values. We compared the resulting dendrogram to the expected one by a permutation test of Baker's gamma correlation using one million permutations of the original dendrogram (47). We also compared the results to two alternative statistics: i) over-representation analysis of nodes from the ENQUIRE-generated networks (the collection of all genes observed in any case study was used as the "universe"); ii) KNet statistics, using Q scores based on STRING's high-confidence functional association network (described above) and ENQUIRE-derived gene nodes.

RESULTS

An Exemplary ENQUIRE Run

To showcase ENQUIRE, we looked for literature-based relationships between the immune system and ferroptosis, a recently described form of programmed cell death . First, we selected 27 papers associated with ferroptosis and the immune system (PubMed query: *("Ferroptosis"[MeSH terms] AND "Immune System"[MeSH terms]) NOT "review"[Publication Type]"*, as of 14.04.23). ENQUIRE's initial reconstruction based on the 27 input papers yielded six gene/MeSH subgraphs isolated from each other with 48 genes, 207 MeSH and 1395 gene/MeSH edges. ENQUIRE retrieved 64 additional PMIDs by PubMed querying. The network obtained after the first expansion included three gene/MeSH subgraphs, with 72 genes, 310 MeSH and 2499 edges. ENQUIRE further retrieve 50 additional PMIDs. The algorithm's second expansion produced a single, connected co-occurrence gene/MeSH network with 132 genes, 514 MeSH and 5243 edges. The algorithm stopped after the second expansion because obtaining a single, connected gene/MeSH network is one of the stop criteria. In total, for this case study the algorithm performed eleven unique queries that generated PMID matches. The benchmark on ENQUIRE's use of computational resources shows modest runtimes and RAM usage (**Supp. Figure 2**). The expansion process is depicted in **Figure 2A**. Similar to the full gene/MeSH networks, the respective subgraphs containing only "information-dense" cliques show a gradual reduction in the number of disconnected components as a well as a decrease in modularity (Figure 2B). We performed context-specific gene set annotation utilizing the gene/MeSH co-occurrence networks and our implementation of NEFRC, as described in Mat.Met.. We identified non-trivial contextual gene sets (**Figure 2C**-left), including ferroptosis-dependent inflammation supported by immune-related adaptor proteins (blue, top left), antineoplastic effects of the ferroptosis-inducer sulfasalazine acting on the amino acid transport system (magenta), and cross-talk between ferroptosis and autophagy (pink), as described in (48–50). We also performed context-aware pathway enrichment analysis using the respectively generated gene-only subgraphs and the approach described in Mat.Met.. We summarized the results in **Figure 2C**-right, which depicts the 30 Reactome pathways with highest p-value variances across expansions, sorted by Reactome category. We obtained associations with pathways linking metabolism and innate immune system, such as *Glutathione conjugation* (R-HSA-156590) and *TNFR1-induced NF-kappa-B signalling pathway* (R-HSA-5357956). Therefore, the ENQUIRE-generated output highlights a potential molecular axis between metabolism, ferroptotic cell death and immune response (51, 52).

ENQUIRE Networks Support the Annotation of Gene Sets from Literature.

To evaluate ENQUIRE's named-entity recognition (NER) method for annotation of gene sets from literature, we selected all *H. sapiens* Reactome pathways, obtained the genes in the pathways and retrieved their corresponding primary literature references. We used the lists of references as inputs and performed one gene entity-restricted NER and network reconstruction to gather genes for each pathway. To benchmark against existing methods, we analyzed the same literature corpus with PubTatorCentral's implementation of GNormPlus (see Mat.Met.). To guarantee that Reactome gene names match ENQUIRE and GNormPlus reference symbols, we respectively employed ours and NCBI lookup tables of reference gene symbols and respective aliases. In **Figure 3A**, we investigated how many genes in the Reactome pathways were correctly annotated by ENQUIRE's NER algorithm or GNormPlus. False positive rate (FPR), precision, and recall of ENQUIRE's NER appear comparable to those computed for GNormPlus, whose reported F1 score is 86.7% (45). ENQUIRE's FPR is 1.45 times that of GNormPlus (linear regression fit), although the maximum recorded value was below 10%. ENQUIRE'S NER shows, on average, 6% higher precision than GNormPlus, and the recall values of

the two methods are comparable. Nevertheless, both algorithms show modest precision and recall performances in the Reactome-derived corpora. A possible explanation is that the abstracts from Reactome reference literature contain sparse or unspecific mentions to genes defining the pathways, which naturally lowers the ceiling for recall and precision.

We also analyzed if ENQUIRE's performance in retrieving Reactome pathways' genes from their associated literature is conserved after network pruning, based on the test statistics for random graphs (**Figure 3B**). We then compared the non-isolated genes of the multigraphs to the remaining ones in the simple graph. **Figure 3B** shows that the test statistics has minimal effects on FPR and precision. However, we computed a 30% reduction in recall; a decrease is expected, as we set the FDR of the statistics to 1%, which lowers its power. We also evaluated the effect of input corpus size, pathway size and average entity co-occurrence per paper on the resulting pruned network (**Table 2**). Precision and recall show opposite Spearman's correlation trends concerning corpus and pathway sizes, which is expected, but average gene-gene co-occurrence per article seems uncorrelated. Taken together, these results show that ENQUIRE's NER algorithm is comparable to standard methods. In addition, ENQUIRE returns a statistical confidence for co-occurrences. The correlation between corpus size and FPR becomes relevant whenever multiple ENQUIRE searches are performed and compared. We suggest 1000 PMIDs as a reasonable upper boundary to keep FPR below 10% (**Figure 3A**).

Finally, we explored if the ENQUIRE-computed weight W, an aggregated measure of a node network centrality and robustness of its connections, is a useful measure of gene relevance regarding the input corpus. To this end, we analyzed the above-mentioned gene-scope co-occurrence networks. In **Figure 3C**, we compare the pan-pathway-aggregated distributions of true-positive (top panel) and false-positive (middle panel) gene recognitions against the computed node weight (x-axis). We subdivided the distribution into four evenly spaced intervals, performed a chi-square test of independence, which resulted to be significant, and extracted statistic standardized Pearson residuals for true positives and false positives (colored boxes beneath the distributions). True positives tend to have higher node weights than false positives. An over-representation of node weights higher than 0.75 is observed in the true-positive distribution, as indicated by the color gradient in Pearson residuals. This suggests one can use the node weights W to rank a set of ENQUIRE-derived genes based on their relevance for the literature corpus in question. Altogether, the results in **Figure 3** indicate that ENQUIRE provides ways to support gene annotation by recovering and ranking genes associated with a literature corpus.

ENQUIRE Recovers Genes with High Chances of Showing Biochemical Interrelations.

To check if the networks generated by the algorithm are also enriched in molecular gene-gene interactions we queried PubMed with all possible pairs of a *Diseases* and a *Genetic Phenomena* MeSH term. We further processed the 3307 queries that retrieved 50-500 matching PMIDs and extracted their gene-gene co-occurrence networks obtained after one network reconstruction. We then inspected whether their protein-coding genes can produce significant functional association networks based on STRING's protein-protein interaction (PPI) database (see Mat.Met.). **Table 3** indicates that for 1336 (40%) MeSH pairs, both ENQUIRE and STRING generated a minimal network with at least three genes and two edges. In a subset of suitable 733 networks (see Mat.Met.), we tested ENQUIRE's proficiency at recovering functional interactions by computing empirical probability distributions for STRING's edge count and DeltaCon similarity score. The gene sets from 730 networks (99%), respectively, produced a STRING network with a higher edge count than 95% of equal-sized random STRING networks. At the same time, 439 networks (60%) showed significant concordance with STRING-derived PPI networks based on DeltaCon. After p-value adjustment (1% FDR, **Table 3**), 722 (99%) and 344 (47%) ENQUIRE networks remained significantly representative of their expected physical interconnections, respectively.

To evaluate the effect of network size, we subdivided the set of 733 suitable networks into quartiles based on their node number and computed the distributions of the unadjusted p-values obtained in the two above tests. The edge count-associated p-values increased with network size (**Figure 4A**). In contrast, we did not find a clear correlation between network size and p-values derived from DeltaCon similarities (**Figure 4B**). DeltaCon similarity quantiles, however, monotonically decreased with network size, possibly due to the growing differences in edge counts between ENQUIRE and STRING when the networks get larger (**Table 4**).
Taken together, our benchmark suggests that ENQUIRE generates networks that frequently relate to established, high-confidence functional relations.

ENQUIRE's networks improve the context resolution of topology-based pathway enrichment analyses.

We also analyzed the ability of ENQUIRE to generate and expand co-occurrence networks with distinctive biological and biomedical signatures by literature querying. To this end, we applied the complete ENQUIRE pipeline with default parameters to a comprehensive set of case studies, spanning cancer, cell differentiation, innate immunity and autoimmune diseases (see description of case studies in **Table 1**). We then performed topology-based pathway enrichment analysis (see Mat.Met.). Figure 5A depicts the expected dendrogram of the different case studies and respective expansions. Figure 5B shows the observed clustering of ENQUIRE-generated graphs inferred from ENQUIRE-informed, topology-based pathway enrichment analysis (see Post Hoc Analyses in Mat.Met.). The 50 pathways with highest p-value variances across case studies are depicted.
The heat map suggests that the case studies cluster primarily based on the affinities between their major topics, in agreement with the expected clustering (**Figure 5A**). For example, pathways categorized under *Diseases of Metabolism*, *Diseases of Immune System*, and *Innate Immune System* are predominantly enriched in networks originating from the case study "Macrophage's signal transduction during M. tuberculosis infection" (MP-ST) and the major topic "Antigen Presentation in Autoimmune Diseases". Similarly, some of *Chromatin Organization* and *Developmental Biology* pathways are almost exclusively enriched in the networks corresponding to oligodendrocyte differentiation. Also, the proposed cancer-related case studies consistently enrich a small cluster of oncology-related pathways like *Aberrant regulations of the mitotic cell cycle* (R-HSA-9687139 and its sub-pathways). Interestingly, a set of pathways linked to cell cycle like *Cyclin D associated events in G1* (R-HSA-69231) are enriched in the oligodendrocyte case study and reported to be also relevant in glioblastoma (53–56). We included a positive control case study as we expected that a co-occurrence network derived from a highly heterogenous corpus should possess little context specificity. Its observed clustering position suggests that networks with large input corpora, expected to show higher false-positive rates, may result in a pathway enrichment biased towards a subset of Reactome's disease pathways associated with cancer (see also **Table 2**). All case studies appear constitutively enriched in a cluster of pathways annotated downstream of *Diseases of signal transduction by growth factor receptors and second messengers* (R-HSA-6802957). We hypothesize that this is a side-effect of how existing gene and pathway databases for *H. sapiens* have been curated, that is, consuming published experimental results biased towards oncology.
Finally, we quantitatively assess the context resolution of the ENQUIRE-informed enrichment (**Figure 5C**). To this end, we performed a permutation test on the observed Baker's gamma correlation value between dendrograms **(Figures 5A** and **5B**), which allows to statistically assess their similarity. We benchmarked its significance against two other methods, namely gene set over-representation analysis (ORA), and topology-based pathway enrichment analysis informed by STRING's high-confidence functional associations, instead of ENQUIRE-generated networks (see Mat.Met.). All methods generated a dendrogram significantly closer than expected to the reference. In our analysis, topology-

based enrichments outperform ORA, with the ENQUIRE-informed score moderately improving the performance over the STRING-informed equivalent (0.69 and 0.64, respectively).

Taken together, these results suggest that ENQUIRE-generated networks can accurately represent contextual, biological differences and similarities between case studies. While ENQUIRE-annotated genes are sufficient for context resolution, the use of topology-based methods that incorporate edge information strongly improves the performance.

DISCUSSION

ENQUIRE is a computational program for the automatic, quantitative, and reproducible annotation of scientific literature, offering an alternative to manual literature curation or database mining. ENQUIRE interrelates gene mentions and biomedical concepts through co-occurrence graphs and tabulated references. Its framework enables *post hoc* analyses that infer contextual gene sets and enriched molecular pathways. ENQUIRE can enhance the biological interpretation of omics data, suggest relevant processes and components for computational models, e.g. in systems biology, and motivate the selection of molecular targets for biological experiments and in scenarios like molecular tumor boards.

This work introduces ENQUIRE and systematically evaluates its performance using real-word case scenarios and large test samples. Since ENQUIRE's scope goes beyond gene normalization and named entity recognition, we did not benchmark against standardized literature corpora like JNLPBA (57). Still, our evaluation shows that ENQUIRE's NER capabilities for genes are on par with state-of-the-art algorithms like GNormPlus. The combination of a lookup table with abstract-specific blocklists prioritizes precision with little-to-no costs on recall, making ENQUIRE's NER adaptive to sentence context.

An added value of ENQUIRE is that the generated gene/MeSH co-occurrence network can prime further functional classification and data mining with reasonable computational costs and gold-standard-comparable performances. In line with this, our results indicate that ENQUIRE-generated gene networks correspond to high-confidence functional association networks in STRING. At the same time, ENQUIRE can also generate networks with previously unreported wirings that can complement database information and encourage novel explorative analyses (**Figure 4B**). We also analyzed the feasibility of corroborating ENQUIRE-suggested interactions by mapping co-occurrence information onto a mechanistic reference network. Since there is no generalizable method to project a network of indirect relations (e.g. co-occurrences) onto a mechanistic network (58–62), we designed a function to score a molecular interaction network using ENQUIRE-generated networks. This allowed us to verify that pathway enrichment analysis from original and expanded ENQUIRE networks preserves context specificity when comparing multiple case studies.

We designed ENQUIRE as a series of modular, open-source components that the user can freely combine and expand to tune its performance. For instance, one could insert a part-of-speech recognition parser upstream of the co-occurrence annotation step to strengthen this criterion (63). Similarly, one can implement a propensity matrix into the random graph model to further weight a co-occurrence with its textual context (15, 30). As NER and, in particular, gene normalization rely heavily on the utilized lookup table of reference gene symbols and aliases, ENQUIRE's accuracy depends on how comprehensive and free of ambiguities this table is. The current version of our algorithm only performs NER of human genes and corresponding mouse orthologs. Still, it can be adapted to perform NER of any other species by supplying an appropriate lookup table, such as those provided by the STRING database (43). Our main objective was to construct a reproducible network reconstruction pipeline accessible to bioinformaticians and systems biologists with affordable computational requirements (**Supp. Figure 2**). Since the standalone version of the algorithm requires some background in computer programming, we are working to provide a web version of ENQUIRE to ease its adoption among biomedical researchers.

CODE AVAILABILITY

Coming soon.

AUTHOR CONTRIBUTIONS

Initial idea and concept: LM and JV. Coding and benchmarking of the algorithm: LM and MW. Drafting of the manuscript: LM and JV.

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TABLES

Table 1. Selection of case studies for assessment of context resolution at the molecular pathway level. We obtained PubMed queries by "AND" concatenation of up to three MeSH terms and further filters to retrieve review articles only. The Corpus sizes refer to the non-redundant union of publications cited by three independent review articles, reported under the "References" column.

Major Topic	Case Study (abbreviation)	PubMed Query			Corpus size	References
		MeSH 1	MeSH 2	MeSH 3		
Signal transduction in solid tumors	**Melanoma (MM-ST)**	Signal transduction	Melanoma		944	(64–66)
	Uveal melanoma (UM-ST)	Signal transduction	Uveal neoplasms		218	(67–69)
	Colorectal cancer (COL-ST)	Signal transduction	Colorectal neoplasms		556	(70–72)
	Breast cancer (BRE-ST)	Signal transduction	Breast neoplasms		522	(73–75)
Macrophage's signal transduction in disease	**Macrophage signal transduction upon infection (MP-ST)**	Signal transduction	Macrophages	Mycobacterium tuberculosis	470	(76–78)
	Tumor-associated Macrophages (MP-TA)	Signal transduction	Tumor associated macrophages		386	(79–81)
Antigen presentation in autoimmune diseases	**Inflammatory bowel disease (IBD-AP)**	Antigen presentation	Inflammatory bowel diseases		445	(82–84)
	Rheumatoid arthritis (RA-AP)	Antigen presentation	Arthritis, rheumatoid		452	(85–87)
	Psoriasis (PSO-AP)	Antigen presentation	Psoriasis		435	(88–90)
Oligodendrocyte differentiation	**Oligodendrocyte (ODC)**	Cell differentiation	Oligodendroglia		355	(91–93)
Positive control	**All case studies**	All queries ("OR" concatenation)			3606	All of the above

Table 2. Effect of relevant covariates on quality indicators of ENQUIRE's gene entity recognition. We evaluated the effect of corpus size (input), Reactome's pathway size (number of genes to be retrieved) and average gene-gene co-occurrence per article, using Spearman's correlation, for each measure. FPR: false positive rate.

Metric	Corpus Size	Pathway Size	Average co-occurrence
FPR	0.83	0.25	0.37
Precision	-0.18	0.49	-0.06
Recall	0.46	-0.35	0.14

Table 3. Relevant quality indicators of ENQUIRE performance in a cohort of 3307 case studies. PPI: protein-protein interaction score, as number of observed edges over the STRING-inferred network. FDR: false discovery rate, expressed in percentage, calculated using Benjamini-Hochberg method**.** Percentages reported for PPI and DeltaCon significance independently refer to the set of 733 tested networks, i.e. those with 10 or more possible realizations with the same degree sequence as ENQUIRE-derived networks.

Property	Subset		Raw count	Percentage over the preceding step	Percentage over total (3307)
Network topology	**At least 3 genes and 2 edges in both ENQUIRE and STRING networks**		1336	90%	40%
	At least 10 possible realizations of the same degree sequence		733	55%	22%
Significance	**Edge count p-value**	**< 0.05**	730	99%	22%
		< 1% FDR	722	99%	21%
	DeltaCon p-value	**< 0.05**	439	60%	13%
		< 1% FDR	344	47%	10%

Table 4. Quantiles of DeltaCon similarities, ENQUIRE- and STRING-based edges counts, sorted by number of genes in the network. Median values with respect to each metric and range of gene counts are highlighted in bold.

Metric	Range of gene counts	Quantiles				
		0%	**25%**	**50%**	**75%**	**100%**
DeltaCon	4-9	0.75	0.83	**0.87**	0.94	1.00
	10-14	0.67	0.78	**0.81**	0.83	1.00
	15-23	0.65	0.74	**0.77**	0.79	0.87
	24-119	0.56	0.65	**0.69**	0.72	0.81
Edge count - ENQUIRE	4-9	4	6	**7**	8	16
	10-14	6	8	**10**	13	43
	15-23	8	13	**17**	22	66
	24-119	18	36	**49**	77	295
Edge count - STRING	4-9	4	6	**8**	10	23
	10-14	6	11	**15**	20	50
	15-23	10	21	**28**	37	94
	24-119	19	54	**89**	146	591

FIGURES

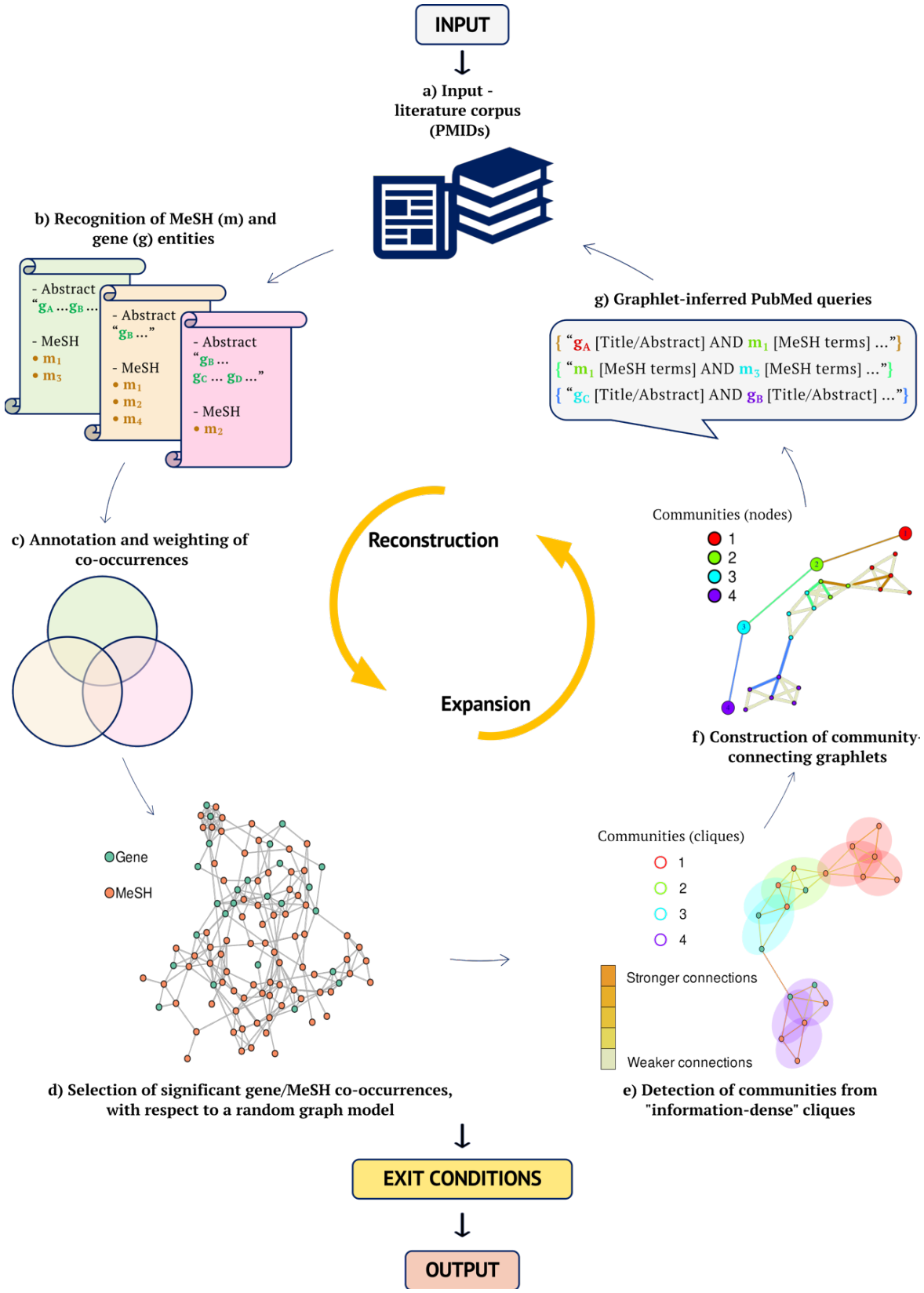

Figure 1. Overview of ENQUIRE methodology. ENQUIRE accepts a set of PubMed identifiers as input, together with optional, user-specified parameters. The pipeline iteratively orchestrates reconstruction and expansion of literature-derived co-occurrence networks, until an exit condition is fulfilled. Additional information about each alphabetically indexed module and output is provided in the Mat.Met. section. For a more detailed flowchart, see Supp. Figure 1.

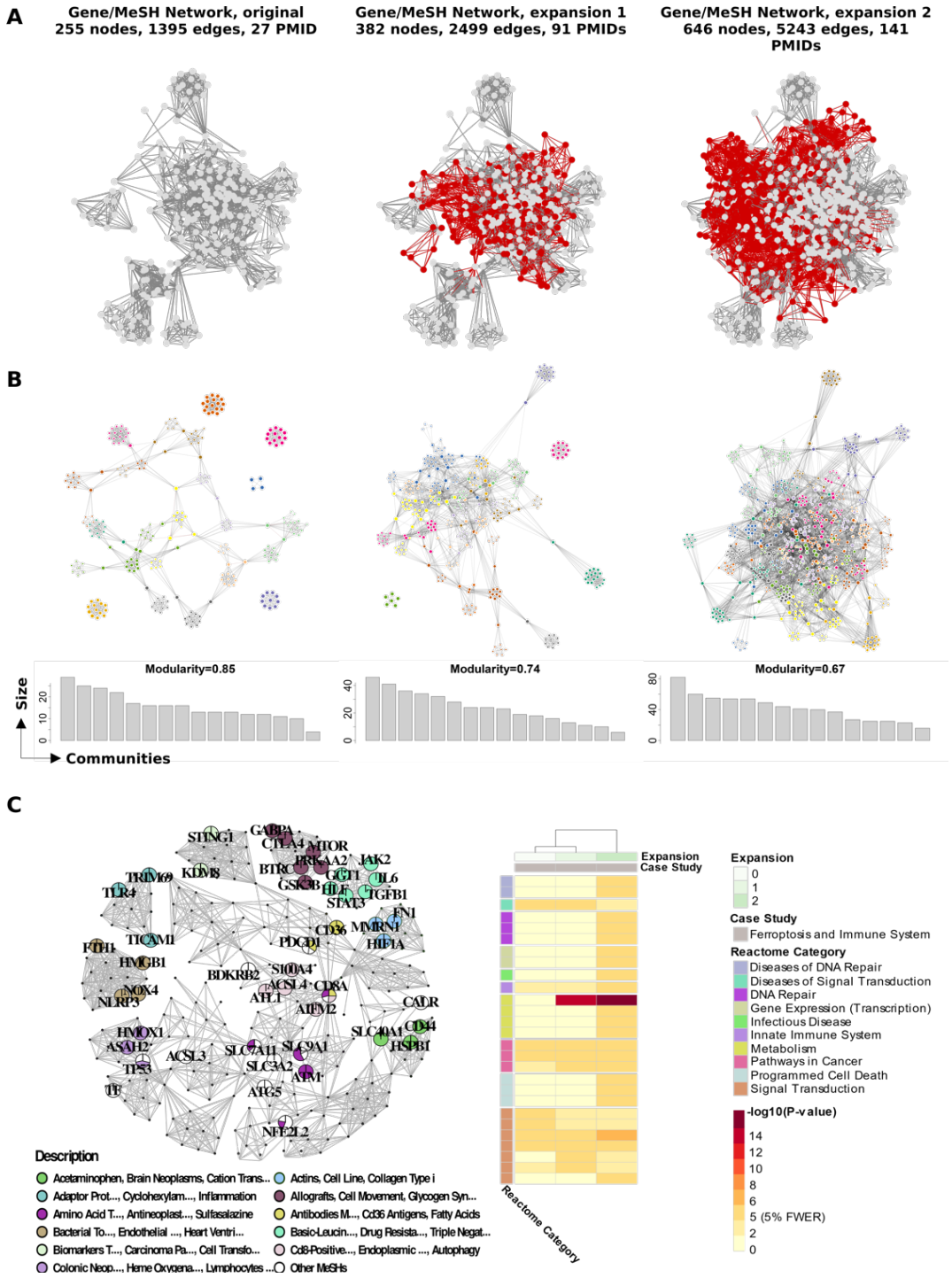

Figure 2. Example of ENQUIRE's network reconstruction, expansion and post-hoc analyses. We used the PubMed identifiers (PMIDs) obtained from the query *("Ferroptosis"[MeSH terms] AND "Immune System"[MeSH terms]) NOT "review"[Publication Type]* as input. A: visualization of ENQUIRE's network expansion process. Novel nodes and edges are indicated in red at each expansion. B: For each of the networks shown in A, the corresponding pruned network containing only significant cliques are depicted. Colors indicate distinct communities, and the network modularities as well as community sizes are reported below. C-left: output of the automatic gene set reconstruction, using the original Gene/MeSH network as input and a fuzzy clustering approach. For simplicity, only nodes referring to genes are enlarged and labelled, and a shortened description of computed gene sets of size 2 or bigger is provided. Sector sizes of the pie-chart-shaped nodes reflect their relative membership degree with respect to each cluster. C-right: topology-based enrichment analysis of Reactome pathways, using original and expanded networks, as

described in the Mat.Met. section. Reactome categories are constructed based on the database's *Top-level Pathways* and disease ontologies. FWER: Holm's family wise error rate.

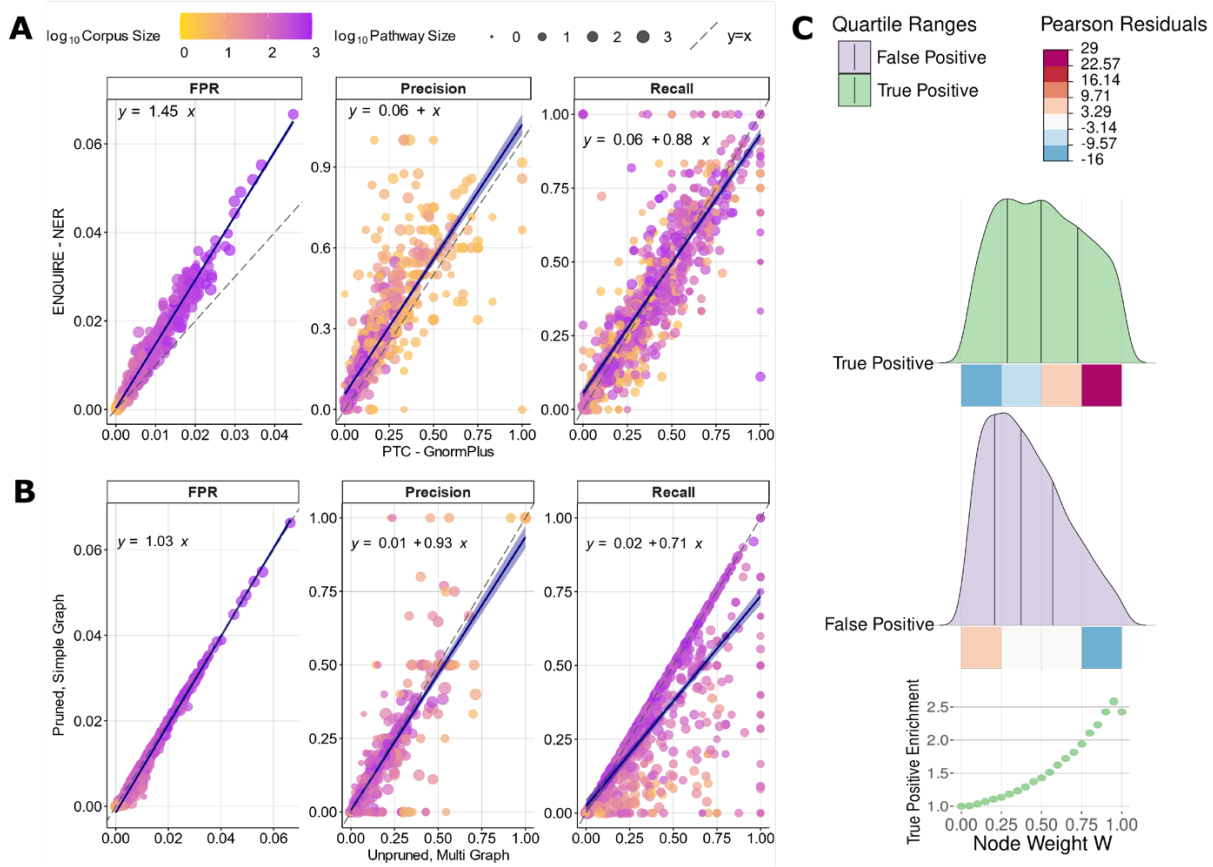

Figure 3. ENQUIRE-derived gene networks can support the manual annotation of gene sets. The scatter plots respectively compare ENQUIRE's named entity recognition (NER) to PubTatorCentral's (PTC) implementation of GNormPlus (**A**) and ENQUIRE-generated, unpruned multigraphs to pruned simple ones, obtained by applying the test statistics based on a random graph model (**B**). Each data point represents one reconstruction of a gene set from a Reactome pathway, using its reference literature abstracts. The data coordinates refer to false positive rate (FPR), precision and recall values independently computed for each tested methodology. We calculated linear regressions to compare the performances in both panels. We indicated input corpus and pathway sizes by adjusting color and dot size, respectively. **C**: aggregated distributions for true and false positive genes, segmented into quartiles. We defined four ranges of the node score W, indicated by squares, whose colors reflect Pearson standardized residuals resulting from a significant chi-square statistic. The lower chart depicts the enrichment of true positive genes, after pruning ENQUIRE-derived networks based on different values of W. Values are relative to the original proportion of true positives.

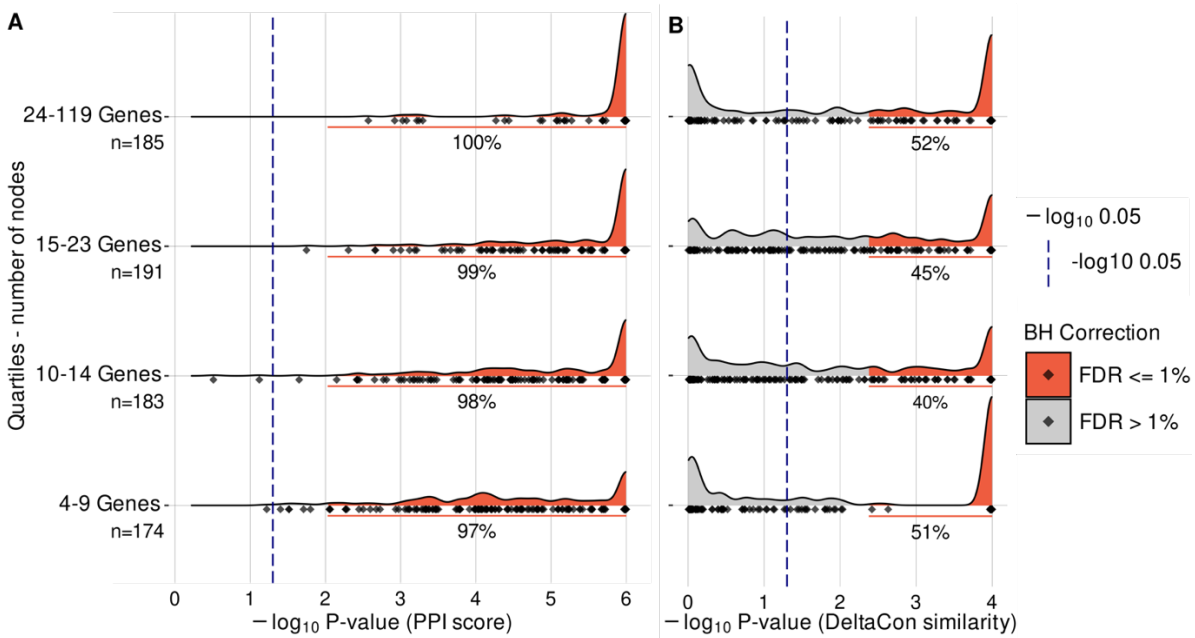

Figure 4. Protein-coding genes from ENQUIRE-generated graphs significantly share functional associations. Panels (**A**) and (**B**) respectively report the unadjusted p-value density distributions of STRING-informed edge counts and DeltaCon similarities, arranged by number of protein-coding genes (network size). We used the *H. sapiens* functional association network from STRING to generate networks from lists of ENQUIRE-derived, protein-coding genes. We tested 733 networks having 10 or more possible network realizations given the observed degree sequence. For each observed network size and degree sequence of ENQUIRE-generated gene networks, 1,000,000 and 10,000 samples were respectively generated to perform a test statistic on the observed edge counts and DeltaCon similarities. See Mat.Met. for additional information. Within each interval, grey and red areas respectively highlight insignificant and significant p-values after Benjamini-Hochberg correction (BH), and a percentage is indicated for those below 1% FDR. Diamonds indicate the observed data.

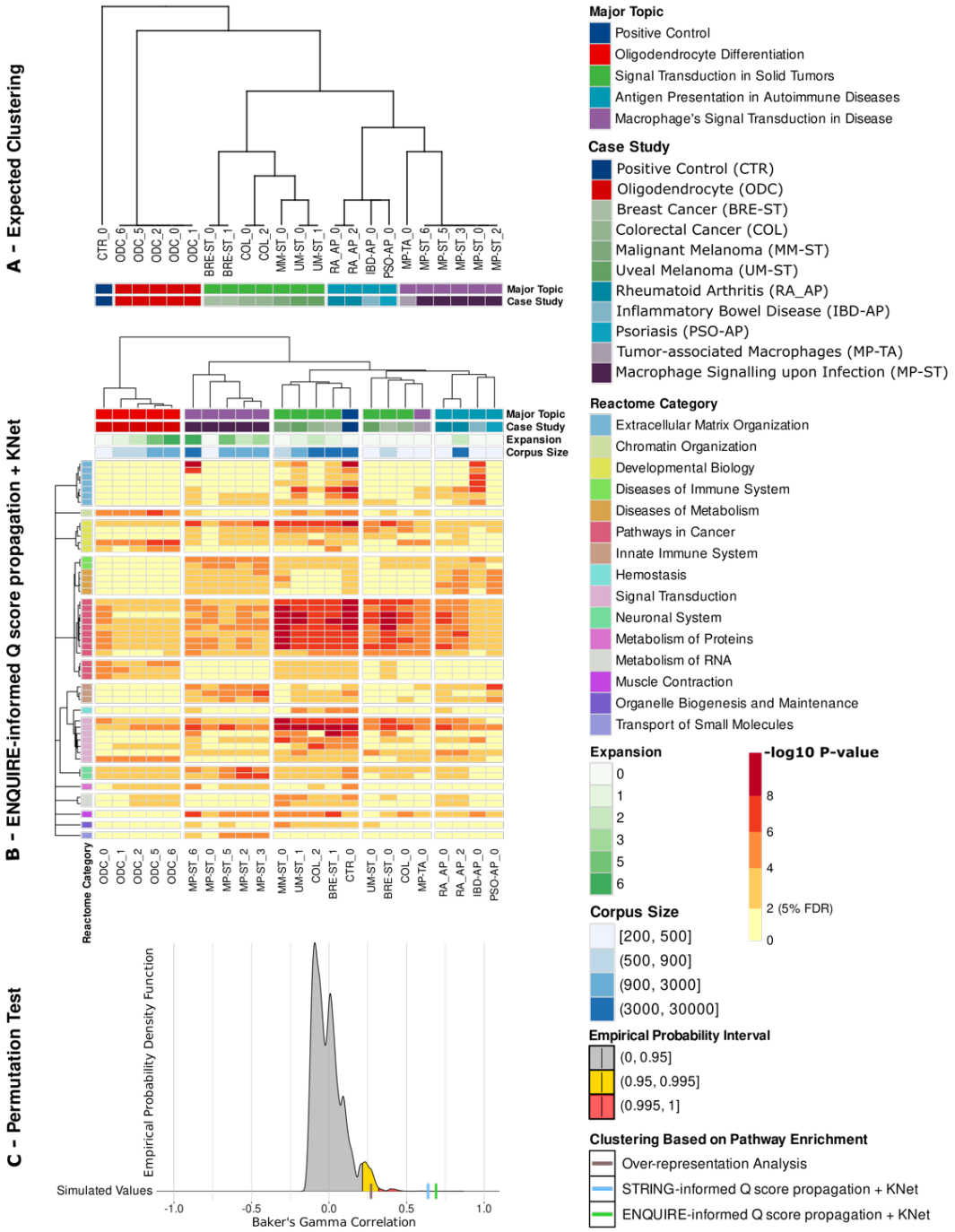

Figure 5. ENQUIRE-generated graphs enhance the context resolution of pathway enrichment analyses. A: reference dendrogram showcasing the expected categorization of the case studies described in **Table 1**. The number following a case study abbreviated name indicates the expansion counter. Network expansions that did not yield any new gene were excluded. **B**: Topology-based pathway enrichment, obtained by applying Q score propagation and SANTA's KNet function on ENQUIRE-informed gene-gene associations (see Post Hoc Analyses under Mat.Met.). The heatmap shows the unadjusted p-values for the 50 enriched Reactome pathways with highest variance across all case studies (the dendrogram was computed on the complete statistic). Pathways are clustered according to Reactome's internal hierarchy. We respectively apportioned the dendrograms into 5 and 15 partitions to visualize their coherence to Major Topic and Reactome Categories. Legends for expansions, rounded corpus size range and p-values with 5% FDR are provided. **C**: Permutation tests of Baker's gamma correlation between the reference dendrogram (**A**) and clustering obtained from alternative pathway enrichment analyses, as in **B**. Colored areas indicate different probability intervals obtained from the empirical probability density function of simulated correlations between reference and sampled dendrograms. See Mat.Met. for further details.