Integrative Network Analysis Reveals Organizational Principles of the Endocannabinoid System

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Abstract

Background: The endocannabinoid system (ECS) is a complex signaling network that regulates diverse physiological processes, including pain, mood, metabolism, and immune response, through coordinated interactions among receptors, enzymes, and lipid-derived ligands. Despite extensive research on individual ECS components, the systems-level organization and network resilience of the ECS remain underexplored. Here, we present a systems-level analysis of the ECS that integrates protein–protein and protein–chemical interactions into a unified network framework.

Methods: We constructed integrated ECS networks that combine protein–protein and protein–chemical interactions, utilizing data from multiple public databases. Network analyses were performed in Python using NetworkX to assess molecular connectivity and interaction topology. We utilized centrality measures to identify major hubs, employed community detection algorithms to examine the clustering of nodes, and performed targeted perturbations by sequentially removing the top-ranked nodes based on degree and betweenness centrality to assess network robustness.

Results: Centrality analyses identified the primary cannabinoid receptors, cannabinoid receptor 1 (CNR1) and cannabinoid receptor 2 (CNR2), as major hubs with extensive connectivity to endogenous and exogenous ligands. Non-canonical receptors, including transient receptor potential vanilloid 1 (TRPV1) and G-protein coupled receptor 55 (GPR55), also emerged as highly ranked nodes across multiple centrality measures, underscoring their integrative roles within the ECS signaling pathway. Community detection revealed biologically meaningful modules centered around receptor and metabolic clusters, with CNR1, CNR2, anandamide (AEA), 2-arachidonoylglycerol (2-AG), and major phytocannabinoids maintaining key network connectivity. Perturbation analyses demonstrated that removal of top hubs, particularly CNR1, caused pronounced losses in edge connectivity and disrupted signaling pathways among cannabinoids. However, the redistribution of influence toward CNR2 and GPR55 under multi-node removal conditions revealed compensatory plasticity and resilience within the ECS network.

Conclusion: This systems-level study highlights the hierarchical and robust architecture of the ECS. The identification of hub nodes, functional communities, and compensatory mechanisms provides insight into how the ECS maintains signaling integrity in the face of perturbation. These findings establish a network-based framework for studying cannabinoid biology and may inform future therapeutic strategies targeting the ECS and its interacting molecular pathways.

Keywords: Endocannabinoid system, biological network, cannabinoid receptors, cannabinoids, CNR1, CNR2, GPR55

Background

The endocannabinoid system (ECS) is a multifaceted regulatory network that maintains physiological homeostasis across diverse organ systems [1–3]. It is composed of endogenous ligands known as endocannabinoids, their cognate receptors, and the enzymes that mediate their synthesis and degradation [1,4]. Endocannabinoids are cannabinoids produced within the human body, in contrast to phytocannabinoids, which are derived from a number of plants, of which the best known is *Cannabis sativa* [5–7], and synthetic cannabinoids, which are laboratory-engineered compounds designed to mimic or modulate the effects of natural cannabinoids for research and therapeutic applications [1,4]. Through the coordinated activity of these molecular components, the ECS influences a broad spectrum of biological processes, including pain perception, inflammation, memory, and mood regulation [8–10].

Two primary receptors define much of the functional landscape of the ECS. Cannabinoid receptor 1 (CB1, also called CNR1) is predominantly expressed in the brain and central nervous system, where it regulates mood, appetite, pain perception, and cognitive processes [1,8–10]. Cannabinoid receptor 2 (CB2, also called CNR2) is primarily located in immune cells and peripheral tissues, where it regulates inflammatory and immune responses [1,8–10]. The ECS also interfaces with intracellular kinase signaling pathways that control cell survival, synaptic plasticity, and metabolic regulation. This extensive interconnectedness underscores how the ECS integrates with broader cellular signaling networks [1–4,8–12]. When the processes governing endocannabinoid synthesis and degradation become dysregulated, the resulting imbalance in "endocannabinoid tone" has been associated with a range of pathological conditions, including chronic pain, neurodegenerative disorders, metabolic syndromes, and cancer [11–15].

The growing acknowledgment of the ECS's role in human physiology has made it a key target for both basic and clinical research, particularly in understanding how endogenous and exogenous compounds can modulate it's signaling [16–19]. The active compounds delta-9-tetrahydrocannabinol (Δ9-THC) and cannabidiol (CBD), derived from medical cannabis, interact with and modulate the activity of cannabinoid receptors, specifically CNR1 and CNR2, influencing endocannabinoid signaling and therapeutic outcomes [20–22]. Specifically, CBD-rich formulations with minimal THC content are increasingly studied and prescribed for conditions such as epilepsy, anxiety, and inflammation [20–22]. Despite this rising interest, the ECS remains insufficiently characterized as a networked system, particularly in terms of how its molecular components interact and respond to changes [1]. A systems-level understanding of these interactions is critical for identifying novel therapeutic targets and developing strategies that more precisely modulate ECS function [23].

In this study, we employed a systems biology framework to characterize the ECS as an integrated molecular network. We constructed interaction networks encompassing protein–protein and protein–chemical relationships within the system. Through network analysis, we identified key nodes using multiple centrality measures and performed clustering analyses to delineate groups of molecules with shared functional characteristics. To further examine network dynamics, we introduced perturbations to assess robustness and to explore how the ECS responds to simulated stress. Collectively, this framework provides a systems-level representation of the ECS, offering insight into its organization, stability, and potential applications in therapeutic development.

Methods

Data Collection and Network Construction

To construct a comprehensive representation of the ECS, we compiled proteins, chemicals, protein–protein interactions (PPIs), and protein–chemical interactions (PCIs) from multiple databases (Figure 1). In the resulting networks, nodes represent ECS proteins or chemicals, and edges reflect experimentally supported interactions. All entities were annotated with structural and functional metadata to facilitate reproducibility and public access.

Proteins: A curated set of forty ECS-associated proteins, including receptors CNR1 and CNR2, enzymes fatty acid amide hydrolase (FAAH), monoacylglycerol lipase (MGLL), and N-acyl-phosphatidylethanolamine phospholipase D (NAPEPLD), and signaling partners, was compiled from prior literature [23] and expanded using UniProt and STRING database queries [24,25]. Core attributes, including amino acid sequence, sub-cellular localization, and complex membership, were retrieved from UniProt [24]. Structural mappings to the Protein Data Bank were obtained via SIFTS, and protein structures were extracted from RCSB PDB [26,27]. Protein–protein interactions were collected from STRING using a confidence threshold of 0.7 to reduce false positives [25]. STRING confidence scores integrate multiple evidence types to estimate the likelihood of a true interaction, with values approaching one indicating higher confidence. All protein entries and protein-protein interactions were derived from human (*Homo sapiens*) data.

Chemicals: Forty ECS-relevant chemicals, including endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG), phytocannabinoids Δ9-THC and CBD, and selected eicosanoids, were curated from prior literature [23] and expanded using PubChem and ChEMBL database queries [28,29]. Chemical metadata, including IUPAC names and SMILES strings, were retrieved from the PubChem database [28]. Molecular descriptors, including molecular weight, partial charges, and topological fingerprints, were calculated using RDKit to serve as metadata [30]. Protein–chemical interactions were obtained from ChEMBL and filtered to retain only interactions involving the predefined ECS proteins, ensuring network specificity [29].

Network Construction and Analysis

Three networks were constructed using NetworkX: a protein–protein only network, a protein–chemical only network, and a combined network containing all interactions [31]. Network topology was quantified using centrality metrics, including betweenness, closeness, degree, and eigenvector. These measures identify nodes that are highly connected, influential, or serve as critical bridges within the ECS, providing insight into the system's organizational hierarchy [32].

Network Clustering

Functional modules within the ECS network were identified using the Louvain algorithm, which maximizes modularity by clustering nodes with preferential interactions [33]. This approach highlights groups of proteins and chemicals that are likely to share biological functions or participate in coordinated processes.

Network Perturbations

Network robustness was evaluated by targeting the removal of specific nodes. Specifically, the nodes with the highest betweenness and degree centrality, namely the top one and top three nodes from each metric, were systematically removed to simulate the disruption of crucial, highly connected components. After each removal, the network was reassessed to determine the number of remaining interactions and connections and to evaluate changes in its overall structure. Centrality metrics were recalculated to identify shifts in the relative importance of the remaining nodes and to understand how the network reorganized in response to the loss of these critical elements [23,34,35].

Code and Data Availability

All data and code used in this study are available in a GitHub repository at https://github.com/aanyashridhar/ECS-Network.

Results

The combined ECS network, which integrates both protein–protein and protein–chemical interactions, comprised 80 nodes and 218 edges and served as the primary framework for subsequent analyses (Figure 2a, Tables S1-S4). In this network, proteins occupy the central core, while chemicals are positioned toward the periphery. This structural organization reflects the biological architecture of the ECS, where proteins act as molecular hubs mediating signaling and metabolic processes, and chemicals serve as ligands or substrates that connect through these central protein nodes. The resulting topology suggests that the ECS is organized around a dense protein interaction core that enables diverse chemical inputs to converge on shared molecular pathways.

For comparison, the protein–protein and protein–chemical subnetworks, analyzed separately, contained 40 nodes and 92 edges, and 80 nodes and 126 edges, respectively. These subnetworks

are shown in Figures S1–S4 and Tables S1–S4, and provide additional insight into the distinct interaction patterns that contribute to the overall connectivity of the ECS.

Network centrality analysis reveals the hierarchical organization of the ECS and provides insight into connectivity and influence patterns.

To understand the structural organization of the ECS network, we quantified key centrality measures that reflect node influence and connectivity (Figure 2b, Tables S11-S12). The analysis revealed distinct patterns in how proteins and chemicals occupy topological positions within the combined network.

The five nodes with the highest betweenness centrality [32] in the combined network were CNR1, transient potential vanilloid receptor 1 (TRPV1), peroxisome proliferator-activiated receptor alpha (PPARA), cyclooxygenase-2 (PTGS2), and G-protein coupled receptor 55 (GPR55), in order of rank (Figure 2b). High betweenness centrality in these proteins indicates that they act as major conduits through which information and signaling flow across the ECS network, underscoring their integrative roles across chemical and protein subnetworks. As one of the two primary cannabinoid receptors, CNR1 connects to numerous cannabinoids and signaling components, serving as a central bridge in the ECS [16,36]. The second-highest ranked node, TRPV1, though not a canonical cannabinoid receptor, is a ligand-gated ion channel that mediates pain perception, inflammation, and thermoregulation [37]. It is strongly activated by cannabinoids, positioning it as a key integrative node within the ECS. The third-ranked PPARA is a nuclear receptor linking cannabinoid signaling to metabolic regulation [38]. The fourth-ranked enzyme, PTGS2, converts endocannabinoids into prostaglandins, thereby integrating the ECS with inflammatory and pain pathways [39]. Finally, GPR55, ranked fifth, is a G-protein coupled receptor (GPCR) that functions as a major non-cannabinoid receptor, further expanding the ECS signaling landscape [40].

The five nodes with the highest closeness centrality [32] in the combined network were GPR55, CBD, tetrahydrocannabivarin (THCV), cannabidivarin (CBDV), and oleoylethanolamide (OEA), in descending order (Figure 2b). High closeness centrality in these compounds suggests that they can efficiently reach many nodes in the network through relatively few interactions, consistent with their broad influence on ECS signaling dynamics. GPR55 interacts with multiple cannabinoids and receptors, resulting in short path lengths across the network [40]. CBD, THCV, and CBDV follow closely, consistent with their established promiscuity across diverse ECS targets, including CNR1, CNR2, TRPV channels, and GPCRs [20]. The fifth-ranked OEA, an endocannabinoid-like lipid, shares biosynthetic and signaling pathways with canonical endocannabinoids but does not bind directly to the cannabinoid receptors CNR1 or CNR2 [41].

Nodes ranked highest by degree centrality [32] in the combined network were CNR1, CNR2, TRPV1, FAAH, and diacylglycerol lipase alpha (DAGLA), in order (Figure 2b). Nodes with high

degree centrality function as major hubs within the network, reflecting their broad engagement across ECS components and highlighting their central role in maintaining network connectivity. CNR1 and CNR2, the two canonical cannabinoid receptors, exhibit extensive connectivity across both protein and chemical domains [16,36]. TRPV1, again, emerges as a highly connected integrator of cannabinoid and non-cannabinoid signaling [37]. The fourth-ranked FAAH, a degradation enzyme [42], and the fifth-ranked DAGLA, a biosynthetic enzyme [43], emphasize the essential role of metabolic turnover in regulating ECS tone and signaling plasticity.

Finally, nodes ranked highest by eigenvector centrality [32] in the combined network were CBD, THCV, CBDV, AM251, and GPR55, in descending order (Figure 2b). High eigenvector centrality indicates that these nodes are not only well-connected themselves but also closely linked to other influential nodes, emphasizing their role in shaping highly interactive subnetworks within the ECS. The prominence of CBD, THCV, and CBDV highlights the influence of phytocannabinoids within the network, as they engage several central receptors and enzymes [20]. AM251, a synthetic CB1 antagonist, ranks fourth due to its strong association with the CNR1 receptor, reflecting its frequent use in receptor-targeting studies [44]. GPR55 reappears among the top five, reinforcing its status as a non-canonical yet highly connected receptor within the ECS [40].

Network clustering analysis uncovers the modular architecture of the ECS and identifies biologically meaningful groupings of receptors, enzymes, and ligands.

Clustering analysis identified thirteen distinct communities within the ECS network (Figure 2c, Tables S5-S6). The largest community consisted of six proteins and thirteen chemicals, while the smallest communities included only a single node (Tables S5-S6). Although these isolated nodes were formally classified as communities by the algorithm, they do not represent meaningful biological groupings. The overall modularity of 42.77% (Table S6) falls within the expected range for biological networks (typically 30–70%) [45], indicating a balance between connectivity and modular organization.

Among the identified clusters, two communities were particularly informative. The first and largest, designated community 0 (Table S5), captured the core signaling architecture of the ECS. This cluster included the two primary cannabinoid receptors (CNR1 [16,36] and CNR2 [36]), PLC enzymes (phospholipase C epsilon 1 (PLCE1) [46] and phospholipase C beta 1 (PLCB1) [46]), signaling partners (inhibitory Guanine nucleotide-binding protein G alpha subunits 1–3 (GNAI 1–3) [47]), numerous synthetic cannabinoids (WIN55,212-2 [48], WIN55,212-3 [48], JWH-018 [49], JWH-073 [49], JWH-133 [49], CP55,940 [50], SR141716A [51], SR144528 [51], and AM251 [44]), and endocannabinoid AEA [52,53]. The GNAIs represent canonical intracellular signaling mediators in the ECS, while synthetic cannabinoids and AEA illustrate the diversity of ligand–receptor interactions within the system. This cluster can be viewed as the cannabinoid receptor group, showing the cannabinoid receptors and their immediate effectors clustering strongly

together. This organization reinforces the biological validity of the network and demonstrates that its modular structure captures known ECS signaling relationships.

The second notable cluster, community 2 (Table S5), which is the fifth largest community, centered on enzymes and molecules involved in the metabolism of endocannabinoids. This community contained degradation enzymes (FAAH [42], MGLL [54], abhydrolase domain-containing protein 6 (ABHD6) [55,56], and abhydrolase domain-containing protein 12 (ABHD12) [55,56]), synthesis enzymes (NAPEPLD [57], DAGLA [43], and diacylglycerol lipase beta (DAGLB) [43]), non-canonical receptor GPR55 [40,58], transporter (fatty acid-binding protein 7 (FABP7) [59]), and endocannabinoid 2-AG [53]. Together, these nodes represent the metabolic group of the ECS, highlighting the tightly coupled nature of endocannabinoid synthesis, degradation, and transport. The inclusion of GPR55 within this community further underscores the functional integration of metabolic and signaling processes that maintain ECS homeostasis.

Network perturbation analysis illustrates how changes to key nodes reshape ECS connectivity, revealing dependencies within its signaling network.

A total of four perturbations were performed for each network, including the removal of the top one node by betweenness centrality, the top three nodes by betweenness centrality, the top one node by degree centrality, and the top three nodes by degree centrality. The protein–chemical only network was limited to two perturbations because betweenness centrality relies on shortest path calculations between nodes, which are not well-defined in networks where chemicals only connect to proteins, allowing only degree-based removals. Across all networks, this approach produced a total of ten perturbations (Table 1). After each perturbation, the change in the number of edges before and after node removal was calculated as a high-level indicator of network impact. Perturbation by betweenness centrality in the protein–protein only network produced the most pronounced effect, with a 40.22% reduction in edge count. In general, removing the top one node, regardless of metric, decreased edge count by approximately 14–16%, whereas removing the top three nodes caused a larger reduction of roughly 34–40%. Notably, CNR1 was removed at least once in all networks, regardless of whether it was identified as a top-ranked node based on betweenness or degree centrality, underscoring its structural importance within the ECS.

To evaluate how highly connected and influential nodes contribute to the stability of the ECS network, we performed targeted perturbations informed by network topology. Nodes were selected for removal based on their rankings in betweenness and degree centrality, which capture information flow and connectivity, respectively. For each perturbation, key centrality measures, including betweenness, closeness, degree, and eigenvector centrality, were recalculated to assess changes in network organization and influence distribution. The percent change in each metric was standardized relative to the distribution of changes across all nodes. Perturbations involving the removal of the top one or top three nodes by betweenness centrality (Figure 3) and degree centrality (Figure 4) were used to examine how hub disruption alters ECS structure and signaling balance.

Perturbation analysis revealed that the removal of highly connected or central nodes caused pronounced shifts in network organization, highlighting which nodes and interactions are most critical for ECS function. In particular, in the absence of CNR1 [16,36], receptors CNR2 [36] and GPR55 [40] increased in centrality, reflecting their compensatory relevance within the network (Figures 3a, 3b, 4a, and 4b). CNR2 and GPR55 are important receptors that can partially maintain connectivity and signaling when CNR1 is absent, indicating redundancy and resilience in the ECS. Conversely, degradation enzymes such as DAGLB [43] showed decreases in betweenness centrality by over two standard deviations, suggesting that key signaling or metabolic nodes lose influence when primary receptors are disrupted (Figures 3a and 4a). Similarly, the endocannabinoid-like lipid linoleoyl ethanolamide (LEA) [41] exhibited a drop in closeness centrality by nearly two standard deviations following the removal of the top-ranked node, indicating that its accessibility to other network nodes and its potential role in modulating signaling are reduced when primary hubs are lost (Figures 3e, 3f, 4d, 4e, and 4f). This observation highlights how perturbations to central nodes can directly affect both receptor-mediated and lipid-mediated pathways, with potential implications for ECS signaling, metabolic regulation, and therapeutic targeting.

Synthetic cannabinoids, including WIN55,212-2 [48], WIN55,212-3 [48], JWH-018 [49], JWH-073 [49], JWH-133 [49], HU-210 [60], HU-211 [60], CP55,940 [50], SR141716A [51], and SR144528 [51], exhibited significant decreases in network metrics following node removal. These effects were most pronounced in Figures 3e and 4e, which depict changes in degree centrality after removing the top one node by betweenness centrality and the top one node by degree centrality, respectively. Several synthetic cannabinoids dropped in degree centrality by nearly two standard deviations, with SR141716A decreasing by over four standard deviations (Figures 3e and 4e). This highlights the critical dependency of these chemicals on central nodes such as CNR1, underscoring how disruption of key receptors can dramatically reduce the functional influence of ligands within the ECS.

Finally, a comparison of eigenvector centrality changes following the removal of the top one versus the top three nodes by degree centrality revealed a dynamic redistribution of influence (Figures 3g, 3h, 4g, and 4h). Removal of a single top node led to substantial decreases in centrality across many nodes, indicating a loss of network cohesion and stability. In contrast, removing the top three nodes produced positive shifts in eigenvector centrality, reflecting a reweighting of influence toward the remaining highly connected and interlinked nodes. This pattern suggests that, in the absence of dominant hubs, the network reorganizes around secondary yet influential nodes, allowing new centers of connectivity to emerge. Such redistribution illustrates the inherent plasticity of the ECS network and its potential for adaptive reconfiguration, maintaining overall structural coherence even when key regulatory nodes are lost.

Discussion

Our network-based analysis reveals how centrality measures can pinpoint key molecular nodes that facilitate signaling and metabolic integration within the ECS. Across these centrality metrics, distinct patterns emerged. Betweenness and degree centrality highlighted proteins such as CNR1 and TRPV1, reflecting their structural and signaling roles, whereas closeness and eigenvector centrality emphasized chemicals such as CBD, THCV, and CBDV, underscoring their widespread interactions and influence (Figure 2b, Table S12). Together, these findings demonstrate that ECS organization is driven by a set of highly interconnected receptors, enzymes, and ligands that coordinate information flow across receptor-mediated, metabolic, and signaling pathways. The recurring prominence of both canonical receptors (CNR1 and CNR2) and non-canonical components such as GPR55 and TRPV1 underscores the distributed yet integrated nature of ECS signaling. Overall, these centrality-based insights provide a quantitative framework for identifying critical regulatory nodes and potential targets for pharmacological modulation.

Clustering analysis revealed a modular yet interconnected architecture of the ECS network (Figure 2c, Figures S3–S4, Tables S5–S10), in which distinct communities correspond to core biological functions such as receptor-mediated signaling and endocannabinoid metabolism. This modularity reflects the intrinsic organization of the ECS and highlights potential leverage points where pharmacological or genetic perturbations could exert system-wide effects. By linking functional clusters to specific biochemical roles, these findings provide a systems-level framework for investigating how modulation at key nodes might rebalance ECS activity under physiological and pathological conditions.

Our perturbation results also demonstrate that network integrity depends strongly on a subset of highly connected nodes, highlighting the critical influence of nodes in maintaining connectivity and signaling pathways within the ECS. These findings corroborate a previous study [23] that characterized ECS as a scale-free network, in which overall organization and robustness are governed by a relatively small number of highly connected nodes. Perturbation of a significantly important node such as CNR1 reshapes the relative importance of other receptors, enzymes, and ligands, revealing compensatory pathways, as reflected in the increased centrality of CNR2 and GPR55. Concurrently, decrease in network centrality metrics for endocannabinoid-like lipids, such as LEA, illustrate how node loss can reduce accessibility and signaling efficiency, thereby impacting metabolic and receptor-mediated pathways. Understanding the dependencies within these networks offers a framework for predicting how targeting specific receptors, enzymes, or ligands can influence endocannabinoid system signaling, ligand efficacy, or metabolic turnover. This knowledge can inform strategies for drug design, receptor modulation, and combination therapies, which leverage the resilience of the ECS to achieve specific physiological outcomes [23,34,35].

The consistent prominence of GPR55 across multiple network analyses underscores its potential as a key regulatory node within the ECS. In the combined network comprising 80 nodes, GPR55 ranked among the top five nodes for betweenness (5th), closeness (1st), degree (5th), and eigenvector centrality (5th), outperforming other non-canonical GPCRs such as GPR18 and GPR119, which were frequently unranked or placed substantially lower across all measures (Table S13). These trends were even more pronounced in the protein–protein network, which contained 40 nodes, where GPR55 achieved the highest closeness and eigenvector centrality values (both ranked first) as well as high degree centrality (3rd) (Table S13). Its normalized eigenvector centrality score of 0.99 was not only greater than those of GPR18 and GPR119 by 10^{15} and 10^{18} orders of magnitude, respectively, but also the highest observed across all three networks analyzed. High eigenvector centrality values indicate that GPR55 is strongly connected to other highly influential nodes within the ECS, reflecting its central role in maintaining network cohesion and stability. Consistent with this interpretation, perturbation analyses revealed that removal of canonical cannabinoid receptor CNR1 increased GPR55's relative centrality, suggesting a compensatory or adaptive role in preserving ECS connectivity and signaling integrity. Collectively, these findings position GPR55 as a structurally and functionally significant component of the ECS, supporting growing evidence that it functions as a cannabinoid-responsive receptor involved in modulating synaptic signaling, inflammatory responses, and other physiological processes [40,61,62].

In this study, we constructed a systems-level network of the ECS by integrating experimentally validated interactions between protein and chemical compounds. This network-based framework provides a structural perspective on how key nodes and interactions contribute to ECS signaling (Figures 2a and 2b), how molecular components organize into functional communities (Figure 2c), and how the removal of specific nodes can redirect signaling (Table 1, Figures 3 and 4), reflecting the intrinsic plasticity of the ECS. Although the network captures essential organizational and connectivity patterns, it does not incorporate information on directionality or dynamics. Metrics such as in-degree and out-degree centrality [32], which could reveal the directional flow of signaling, were not included because directional information is not readily available in the public databases used for this study (Figure 1). Similarly, dynamic features such as interaction strength [63–68], temporal responses [69–72], or context-dependent regulation [64,73–76] were not represented, meaning that the current model characterizes structural connectivity rather than functional activity. Despite these limitations, our analysis provides a robust foundation for understanding the ECS organization. Future studies could expand this network to incorporate kinetic parameters, tissue-specific interactions, or experimentally measured signaling dynamics, enabling predictive modeling of how endogenous ligands, phytocannabinoids, and synthetic compounds influence ECS signaling and therapeutic outcomes.

Conclusion

This study presents a comprehensive systems-level analysis of the ECS by integrating protein–protein and protein–chemical interactions into a combined network. Centrality analyses identified the primary cannabinoid receptors CNR1 and CNR2 as major hubs, reflecting their extensive connectivity with both endogenous and exogenous ligands. In addition to these canonical receptors, other key nodes, including TRPV1 and GPR55, ranked highly across multiple centrality measures, indicating their integrative roles in linking receptor-mediated signaling with metabolic and inflammatory pathways. The prominence of GPR55, in particular, supports emerging evidence [40,61,62] that it functions as a cannabinoid-responsive receptor with broad influence within ECS signaling. Clustering analyses further revealed that key nodes, including the primary cannabinoid receptors CNR1 and CNR2, as well as critical chemical ligands such as the endocannabinoids AEA and 2-AG and various phytocannabinoids, maintain network connectivity and organize into biologically meaningful communities, including receptor-centered and metabolic clusters. Perturbation analysis emphasized the significance of hub nodes in the network. The removal of CNR1 caused substantial disruptions in the network's organization, reducing the importance of dependent synthetic cannabinoids and shifting centrality to other receptors, such as CNR2 and GPR55, revealing compensatory pathways that support network resilience. These findings demonstrate that the ECS functions as a complex network orchestrating diverse physiological processes, including signaling and metabolic regulation. Characterizing the connectivity of its receptors, enzymes, ligands, and other components provides critical insights for the development of therapeutic strategies and drug design.

List of abbreviations

ECS: Endocannabinoid system
CNR1: Cannabinoid receptor 1
CNR2: Cannabinoid receptor 2
CB1: Cannabinoid receptor type 1 (alternate name for CNR1)
CB2: Cannabinoid receptor type 2 (alternate name for CNR2)
TRPV1: Transient receptor potential vanilloid 1
GPR55: G-protein coupled receptor 55
GPR18: G-protein coupled receptor 18
GPR119: G-protein coupled receptor 119
GPCR: G-protein coupled receptor
AEA: Anandamide
2-AG: 2-Arachidonoylglycerol
Δ9-THC: Delta-9-tetrahydrocannabinol
THC: Tetrahydrocannabinol
CBD: Cannabidiol
THCV: Tetrahydrocannabivarin
CBDV: Cannabidivarin

OEA: Oleoylethanolamide
LEA: Linoleoyl ethanolamide
FAAH: Fatty acid amide hydrolase
MGLL: Monoacylglycerol lipase
NAPEPLD: N-acyl-phosphatidylethanolamine phospholipase D
DAGLA: Diacylglycerol lipase alpha
DAGLB: Diacylglycerol lipase beta
ABHD6: Abhydrolase domain-containing protein 6
ABHD12: Abhydrolase domain-containing protein 12
FABP7: Fatty acid-binding protein 7
PPARA: Peroxisome proliferator-activated receptor alpha
PTGS2: Prostaglandin-endoperoxide synthase 2 (cyclooxygenase-2)
PLCE1: Phospholipase C epsilon 1
PLCB1: Phospholipase C beta 1
GNAI: Inhibitory Guanine nucleotide-binding protein G alpha subunit
GNAI1: Inhibitory Guanine nucleotide-binding protein G alpha subunit 1
GNAI2: Inhibitory Guanine nucleotide-binding protein G alpha subunit 2
GNAI3: Inhibitory Guanine nucleotide-binding protein G alpha subunit 3
PPI: Protein–protein interaction
PCI: Protein–chemical interaction
SMILES: Simplified Molecular-Input Line-Entry System
IUPAC: International Union of Pure and Applied Chemistry

Declarations

Availability of data and materials

All data and code used in this study are available in a GitHub repository at https://github.com/aanyashridhar/ECS-Network.

Competing Interests

The authors declare they have no competing interests.

Funding

Authors and the Cannabis Research Institute team would like to thank the Illinois Department of Human Services and the Illinois Cannabis Regulation and Oversight Office for funding support.

Author Contributions

RG, SMD, and AT conceived the project. SMD designed and supervised the project. AS conducted data curation, network construction, computational analyses, and visualizations. AS and SMD analyzed and interpreted the results and wrote and edited the manuscript. AT provided insights on

results and contributed to manuscript edits. RG provided insights on the results, contributed manuscript edits, and supported the research through funding.

Acknowledgements

Authors would like to thank Discovery Partners Institute and the SPARKS internship program for supporting the initiation of this project.

References

1. Lu H-C, Mackie K. Review of the Endocannabinoid System. Biol Psychiatry Cogn Neurosci Neuroimaging. 2021;6:607–15. https://doi.org/10.1016/j.bpsc.2020.07.016

2. Kunos G, Osei-Hyiaman D, Liu J, Godlewski G, Bátkai S. Endocannabinoids and the control of energy homeostasis. J Biol Chem. 2008;283:33021–5. https://doi.org/10.1074/jbc.R800012200

3. Sikyta M, Konin JG, Kutz MR. The Endocannabinoid System Signaling, a Precursor to Homeostasis: A Scoping Review. Internet J Allied Health Sci Pract. 2024;22:Article 10.

4. Schurman LD, Lu D, Kendall DA, Howlett AC, Lichtman AH. Molecular Mechanism and Cannabinoid Pharmacology. Handb Exp Pharmacol. 2020;258:323–53. https://doi.org/10.1007/164_2019_298

5. Gülck T, Møller BL. Phytocannabinoids: Origins and Biosynthesis. Trends Plant Sci. 2020;25:985–1004. https://doi.org/10.1016/j.tplants.2020.05.005

6. Ribeiro R, Da Silva YC, Finotti R, Carneiro GRA, Cardoso Dos Santos GR, Pereira HMG, et al. Trema micranthum (L.) Blume as a new source of cannabinoids. Sci Rep. 2024;14:29620. https://doi.org/10.1038/s41598-024-80857-6

7. Chavez BG, D'Auria JC. Turning a new leaf on cannabinoids. Nat Plants. 2023;9:687–8. https://doi.org/10.1038/s41477-023-01415-y

8. Barrie N, Manolios N. The endocannabinoid system in pain and inflammation: Its relevance to rheumatic disease. Eur J Rheumatol. 2017;4:210–8. https://doi.org/10.5152/eurjrheum.2017.17025

9. Campolongo P, Trezza V. The endocannabinoid system: a key modulator of emotions and cognition. Front Behav Neurosci. 2012;6:73. https://doi.org/10.3389/fnbeh.2012.00073

10. Simankowicz P, Stępniewska J. The Role of Endocannabinoids in Physiological Processes and Disease Pathology: A Comprehensive Review. J Clin Med. 2025;14:2851. https://doi.org/10.3390/jcm14082851

11. Dörnyei G, Vass Z, Juhász CB, Nádasy GL, Hunyady L, Szekeres M. Role of the Endocannabinoid System in Metabolic Control Processes and in the Pathogenesis of Metabolic Syndrome: An Update. Biomedicines. 2023;11. https://doi.org/10.3390/biomedicines11020306

12. Haspula D, Clark MA. Cannabinoid Receptors: An Update on Cell Signaling, Pathophysiological Roles and Therapeutic Opportunities in Neurological, Cardiovascular, and Inflammatory Diseases. Int J Mol Sci. 2020;21:7693. https://doi.org/10.3390/ijms21207693

13. Russo EB. Clinical Endocannabinoid Deficiency Reconsidered: Current Research Supports the Theory in Migraine, Fibromyalgia, Irritable Bowel, and Other Treatment-Resistant Syndromes. Cannabis Cannabinoid Res. 2016;1:154–65. https://doi.org/10.1089/can.2016.0009

14. Russo E. Hemp for Headache: An In-Depth Historical and Scientific Review of Cannabis in Migraine Treatment. J Cannabis Ther. 2001;1:21–92. https://doi.org/10.1300/J175v01n02_04

15. Ross EM. Handbook of Psychotropic Herbs: A Scientific Analysis of Herbal Remedies for Psychiatric Conditions. Nutr Clin Care. 2001;4:223–223. https://doi.org/10.1046/j.1523-5408.2001.00147.x

16. Howlett AC, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, et al. International Union of Pharmacology. XXVII. Classification of Cannabinoid Receptors. Pharmacol Rev. 2002;54:161–202. https://doi.org/10.1124/pr.54.2.161

17. Marzo VD. Targeting the endocannabinoid system: to enhance or reduce? Nat Rev Drug Discov. 2008;7:438–55. https://doi.org/10.1038/nrd2553

18. Pacher P, Bátkai S, Kunos G. The endocannabinoid system as an emerging target of pharmacotherapy. Pharmacol Rev. 2006;58:389–462. https://doi.org/10.1124/pr.58.3.2

19. Tudorancea IM, Ciorpac M, Stanciu GD, Caratașu C, Săcărescu A, Ignat B, et al. The Therapeutic Potential of the Endocannabinoid System in Age-Related Diseases. Biomedicines. 2022;10. https://doi.org/10.3390/biomedicines10102492

20. Blebea NM, Pricopie AI, Vlad R-A, Hancu G. Phytocannabinoids: Exploring Pharmacological Profiles and Their Impact on Therapeutical Use. Int J Mol Sci. 2024;25. https://doi.org/10.3390/ijms25084204

21. Mick G, Douek P. Clinical Benefits and Safety of Medical Cannabis Products: A Narrative Review on Natural Extracts. Pain Ther. 2024;13:1063–94. https://doi.org/10.1007/s40122-024-00643-0

22. Hoch E, Volkow ND, Friemel CM, Lorenzetti V, Freeman TP, Hall W. Cannabis, cannabinoids and health: a review of evidence on risks and medical benefits. Eur Arch Psychiatry Clin Neurosci. 2025;275:281–92. https://doi.org/10.1007/s00406-024-01880-2

23. Bernabò N, Barboni B, Maccarrone M. Systems Biology Analysis of the Endocannabinoid System Reveals a Scale-free Network with Distinct Roles for Anandamide and 2-Arachidonoylglycerol. OMICS J Integr Biol. 2013;17:646–54. https://doi.org/10.1089/omi.2013.0071

24. Ahmad S, Gonzales LJ da C, Bowler-Barnett EH, Rice DL, Kim M, Wijerathne S, et al. The UniProt website API: facilitating programmatic access to protein knowledge. Nucleic Acids Res. 2025;53:W547–53. https://doi.org/10.1093/nar/gkaf394

25. Szklarczyk D, Kirsch R, Koutrouli M, Nastou K, Mehryary F, Hachilif R, et al. The STRING database in 2023: protein–protein association networks and functional enrichment analyses for any sequenced genome of interest. Nucleic Acids Res. 2023;51:D638–46. https://doi.org/10.1093/nar/gkac1000

26. Dana JM, Gutmanas A, Tyagi N, Qi G, O'Donovan C, Martin M, et al. SIFTS: updated Structure Integration with Function, Taxonomy and Sequences resource allows 40-fold increase in coverage of structure-based annotations for proteins. Nucleic Acids Res. 2019;47:D482–9. https://doi.org/10.1093/nar/gky1114

27. Berman HM. The Protein Data Bank. Nucleic Acids Res. 2000;28:235–42. https://doi.org/10.1093/nar/28.1.235

28. Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, et al. PubChem 2025 update. Nucleic Acids Res. 2025;53:D1516–25. https://doi.org/10.1093/nar/gkae1059

29. Zdrazil B, Felix E, Hunter F, Manners EJ, Blackshaw J, Corbett S, et al. The ChEMBL Database in 2023: a drug discovery platform spanning multiple bioactivity data types and time periods. Nucleic Acids Res. 2024;52:D1180–92. https://doi.org/10.1093/nar/gkad1004

30. Landrum G, Tosco P, Kelley B, Rodriguez R, Cosgrove D, Vianello R, et al. rdkit/rdkit: 2025_09_1 (Q3 2025) Release [Internet]. Zenodo; 2025. https://doi.org/10.5281/zenodo.17232453

31. Hagberg AA, Schult DA, Swart PJ. Exploring Network Structure, Dynamics, and Function using NetworkX. In: Varoquaux G, Vaught T, Millman J, editors. Proc 7th Python Sci Conf. 2008. p. 11–5.

32. Gómez S. Centrality in Networks: Finding the Most Important Nodes. Bus Consum Anal New Ideas. Springer International Publishing; 2019. p. 401–33. https://doi.org/10.1007/978-3-030-06222-4_8

33. Li J, Lai S, Shuai Z, Tan Y, Jia Y, Yu M, et al. A Comprehensive Review of Community Detection in Graphs [Internet]. 2024. https://arxiv.org/abs/2309.11798

34. Pacher P, Kunos G. Modulating the endocannabinoid system in human health and disease–successes and failures. FEBS J. 2013;280:1918–43. https://doi.org/10.1111/febs.12260

35. Jaeger S, Aloy P. From protein interaction networks to novel therapeutic strategies. IUBMB Life. 2012;64:529–37. https://doi.org/10.1002/iub.1040

36. Mackie K. Cannabinoid receptors: where they are and what they do. J Neuroendocrinol. 2008;20 Suppl 1:10–4. https://doi.org/10.1111/j.1365-2826.2008.01671.x

37. Rosenbaum T, Simon SA. TRPV1 Receptors and Signal Transduction. 2007.

38. Iannotti FA, Vitale RM. The Endocannabinoid System and PPARs: Focus on Their Signalling Crosstalk, Action and Transcriptional Regulation. Cells. 2021;10. https://doi.org/10.3390/cells10030586

39. Fowler CJ. The contribution of cyclooxygenase-2 to endocannabinoid metabolism and action. Br J Pharmacol. 2007;152:594–601. https://doi.org/10.1038/sj.bjp.0707379

40. Ryberg E, Larsson N, Sjögren S, Hjorth S, Hermansson N-O, Leonova J, et al. The orphan receptor GPR55 is a novel cannabinoid receptor. Br J Pharmacol. 2007;152:1092–101. https://doi.org/10.1038/sj.bjp.0707460

41. Barrie N, Manolios N, Stuart J, Chew T, Arnold J, Sadsad R, et al. Design and function of targeted endocannabinoid nanoparticles. Sci Rep. 2022;12:17260. https://doi.org/10.1038/s41598-022-21715-1

42. Deutsch DG, Ueda N, Yamamoto S. The fatty acid amide hydrolase (FAAH). Prostaglandins Leukot Essent Fatty Acids. 2002;66:201–10. https://doi.org/10.1054/plef.2001.0358

43. Reisenberg M, Singh PK, Williams G, Doherty P. The diacylglycerol lipases: structure, regulation and roles in and beyond endocannabinoid signalling. Philos Trans R Soc Lond B Biol Sci. 2012;367:3264–75. https://doi.org/10.1098/rstb.2011.0387

44. Rodgers RJ, Evans PM, Murphy A. Anxiogenic profile of AM-251, a selective cannabinoid CB1 receptor antagonist, in plus-maze-na??ve and plus-maze-experienced mice. Behav Pharmacol. 2005;16:405–13. https://doi.org/10.1097/00008877-200509000-00013

45. Sah P, Singh LO, Clauset A, Bansal S. Exploring community structure in biological networks with random graphs. BMC Bioinformatics. 2014;15:220. https://doi.org/10.1186/1471-2105-15-220

46. Bunney TD, Katan M. PLC regulation: emerging pictures for molecular mechanisms. Trends Biochem Sci. 2011;36:88–96. https://doi.org/10.1016/j.tibs.2010.08.003

47. Villaseca S, Romero G, Ruiz MJ, Pérez C, Leal JI, Tovar LM, et al. Gαi protein subunit: A step toward understanding its non-canonical mechanisms. Front Cell Dev Biol. 2022;10:941870. https://doi.org/10.3389/fcell.2022.941870

48. Price DA, Martinez AA, Seillier A, Koek W, Acosta Y, Fernandez E, et al. WIN55,212-2, a cannabinoid receptor agonist, protects against nigrostriatal cell loss in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. Eur J Neurosci. 2009;29:2177–86. https://doi.org/10.1111/j.1460-9568.2009.06764.x

49. Huffman JW, Dai D, Martin BR, Compton DR. Design, Synthesis and Pharmacology of Cannabimimetic Indoles. Bioorg Med Chem Lett. 1994;4:563–6. https://doi.org/10.1016/S0960-894X(01)80155-4

50. Ortega A, Rangel-López E, Hidalgo-Miranda A, Morales A, Ruiz-García E, Meneses-García A, et al. On the effects of CP 55-940 and other cannabinoid receptor agonists in C6 and U373 cell lines. Toxicol Vitro Int J Publ Assoc BIBRA. 2015;29:1941–51. https://doi.org/10.1016/j.tiv.2015.08.003

51. Rinaldi-Carmona M, Barth F, Héaulme M, Shire D, Calandra B, Congy C, et al. SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. FEBS Lett. 1994;350:240–4. https://doi.org/10.1016/0014-5793(94)00773-x

52. Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G, et al. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science. 1992;258:1946–9. https://doi.org/10.1126/science.1470919

53. Lu H-C, Mackie K. An Introduction to the Endogenous Cannabinoid System. Biol Psychiatry. 2016;79:516–25. https://doi.org/10.1016/j.biopsych.2015.07.028

54. Dinh TP, Carpenter D, Leslie FM, Freund TF, Katona I, Sensi SL, et al. Brain monoglyceride lipase participating in endocannabinoid inactivation. Proc Natl Acad Sci U S A. 2002;99:10819–24. https://doi.org/10.1073/pnas.152334899

55. Marrs WR, Blankman JL, Horne EA, Thomazeau A, Lin YH, Coy J, et al. The serine hydrolase ABHD6 controls the accumulation and efficacy of 2-AG at cannabinoid receptors. Nat Neurosci. 2010;13:951–7. https://doi.org/10.1038/nn.2601

56. Savinainen JR, Saario SM, Laitinen JT. The serine hydrolases MAGL, ABHD6 and ABHD12 as guardians of 2-arachidonoylglycerol signalling through cannabinoid receptors. Acta Physiol Oxf Engl. 2012;204:267–76. https://doi.org/10.1111/j.1748-1716.2011.02280.x

57. Tevosian M, Todorov H, Lomazzo E, Bindila L, Ueda N, Bassetti D, et al. NAPE-PLD deletion in stress-TRAPed neurons results in an anxiogenic phenotype. Transl Psychiatry. 2023;13:152. https://doi.org/10.1038/s41398-023-02448-9

58. Tudurí E, Imbernon M, Hernández-Bautista RJ, Tojo M, Fernø J, Diéguez C, et al. GPR55: a new promising target for metabolism? J Mol Endocrinol. 2017;58:R191–202. https://doi.org/10.1530/JME-16-0253

59. Kaczocha M, Glaser ST, Deutsch DG. Identification of intracellular carriers for the endocannabinoid anandamide. Proc Natl Acad Sci U S A. 2009;106:6375–80. https://doi.org/10.1073/pnas.0901515106

60. Ottani A, Giuliani D. Hu 210: a potent tool for investigations of the cannabinoid system. CNS Drug Rev. 2001;7:131–45. https://doi.org/10.1111/j.1527-3458.2001.tb00192.x

61. Lauckner JE, Jensen JB, Chen H-Y, Lu H-C, Hille B, Mackie K. GPR55 is a cannabinoid receptor that increases intracellular calcium and inhibits M current. Proc Natl Acad Sci. 2008;105:2699–704. https://doi.org/10.1073/pnas.0711278105

62. Kargl J, Balenga N, Parzmair GP, Brown AJ, Heinemann A, Waldhoer M. The Cannabinoid Receptor CB1 Modulates the Signaling Properties of the Lysophosphatidylinositol Receptor GPR55. J Biol Chem. 2012;287:44234–48. https://doi.org/10.1074/jbc.M112.364109

63. Wu T, Zhou H-X, Tian J, Zhou R, Huangfu S, Jin B-K, et al. Enrichable cross-linkers for mapping direct protein interactions. Genome Biol. 2025;26:205. https://doi.org/10.1186/s13059-025-03669-5

64. Luo F, Liu J, Li J. Discovering conditional co-regulated protein complexes by integrating diverse data sources. BMC Syst Biol. 2010;4:S4. https://doi.org/10.1186/1752-0509-4-S2-S4

65. Vangone A, Bonvin AM. Contacts-based prediction of binding affinity in protein–protein complexes. eLife. 2015;4:e07454. https://doi.org/10.7554/eLife.07454

66. Tian B, Zhao C, Gu F, He Z. A two-step framework for inferring direct protein-protein interaction network from AP-MS data. BMC Syst Biol. 2017;11:82. https://doi.org/10.1186/s12918-017-0452-y

67. Hong Y, Ha J, Sim J, Lim CJ, Oh K-S, Chandrasekaran R, et al. Accurate prediction of protein–ligand interactions by combining physical energy functions and graph-neural networks. J Cheminformatics. 2024;16:121. https://doi.org/10.1186/s13321-024-00912-2

68. Yu J, Li Z, Chen G, Kong X, Hu J, Wang D, et al. Computing the relative binding affinity of ligands based on a pairwise binding comparison network. Nat Comput Sci. 2023;3:860–72. https://doi.org/10.1038/s43588-023-00529-9

69. Srihari S, Leong HW. Temporal dynamics of protein complexes in PPI Networks: a case study using yeast cell cycle dynamics. BMC Bioinformatics. 2012;13:S16. https://doi.org/10.1186/1471-2105-13-S17-S16

70. Kirouac DC, Saez-Rodriguez J, Swantek J, Burke JM, Lauffenburger DA, Sorger PK. Creating and analyzing pathway and protein interaction compendia for modelling signal transduction networks. BMC Syst Biol. 2012;6:29. https://doi.org/10.1186/1752-0509-6-29

71. Ou-Yang L, Dai D-Q, Li X-L, Wu M, Zhang X-F, Yang P. Detecting temporal protein complexes from dynamic protein-protein interaction networks. BMC Bioinformatics. 2014;15:335. https://doi.org/10.1186/1471-2105-15-335

72. Kim Y, Jang J-H, Choi S, Hwang D. TEMPI: probabilistic modeling time-evolving differential PPI networks with multiPle information. Bioinformatics. 2014;30:i453–60. https://doi.org/10.1093/bioinformatics/btu454

73. Nasser S, Cunliffe HE, Black MA, Kim S. Context-specific gene regulatory networks subdivide intrinsic subtypes of breast cancer. BMC Bioinformatics. 2011;12:S3. https://doi.org/10.1186/1471-2105-12-S2-S3

74. Rush STA, Repsilber D. Capturing context-specific regulation in molecular interaction networks. BMC Bioinformatics. 2018;19:539. https://doi.org/10.1186/s12859-018-2513-7

75. Chen C, Jiang L, Shen B, Wang M, Griffin CH, Chinchilli VM, et al. A Computational Atlas of Tissue-specific Regulatory Networks. Front Syst Biol. 2021;1:764161. https://doi.org/10.3389/fsysb.2021.764161

76. Li Z, Liu G, Yang X, Shu M, Jin W, Tong Y, et al. An atlas of cell-type-specific interactome networks across 44 human tumor types. Genome Med. 2024;16:30. https://doi.org/10.1186/s13073-024-01303-w

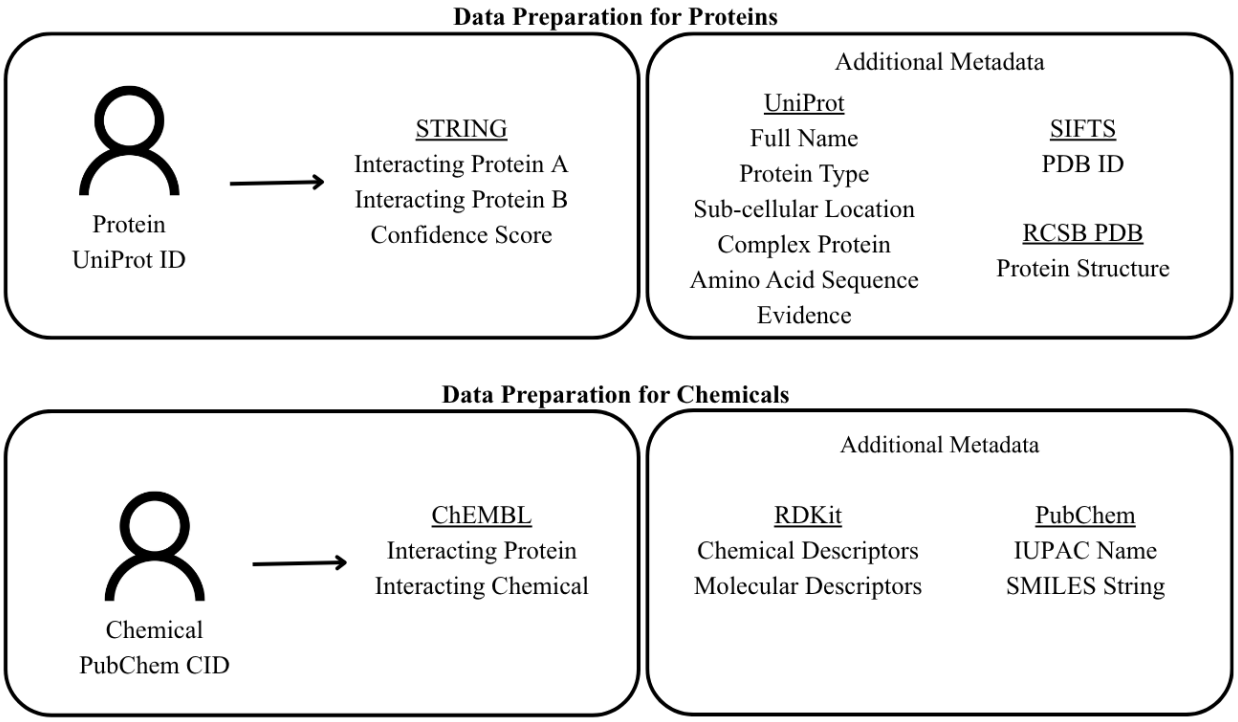
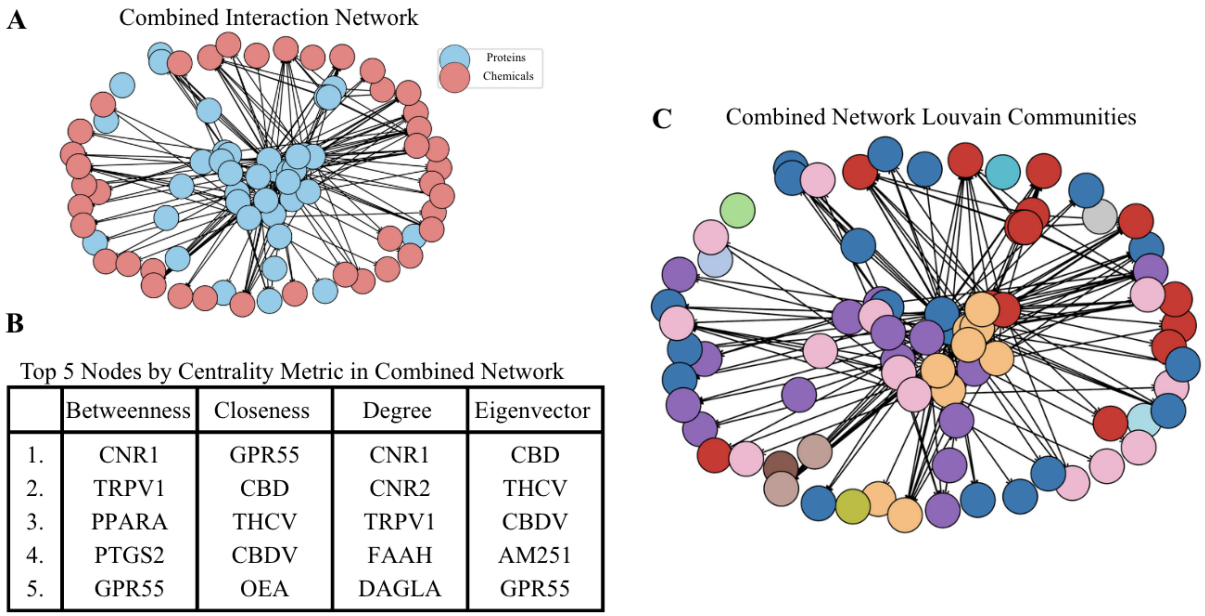


Figure 1: Data preparation pipeline for proteins and chemicals. Schematic depiction of the workflow used to compile protein–level and chemical–level data for network construction. Protein nodes (UniProt IDs) were annotated with interaction data from STRING, including interacting pairs and confidence scores. Additional metadata were integrated from UniProt, SIFTS, and RSCB PDB. Chemical nodes (PubChem CIDs) were annotated with interaction data from ChEMBL. Additional metadata were pulled from PubChem and calculated using RDKit.



B

Top 5 Nodes by Centrality Metric in Combined Network

	Betweenness	Closeness	Degree	Eigenvector
1.	CNR1	GPR55	CNR1	CBD
2.	TRPV1	CBD	CNR2	THCV
3.	PPARA	THCV	TRPV1	CBDV
4.	PTGS2	CBDV	FAAH	AM251
5.	GPR55	OEA	DAGLA	GPR55

Figure 2: Combined interaction network and node characterization. A) Visualization of the integrated network showing proteins (blue) and chemicals (red) connected through documented interactions. The network has 80 nodes and 218 edges. **B)** Top five nodes ranked by centrality measures in the combined network. Results highlight distinct sets of influential nodes across betweenness, closeness, degree, and eigenvector centrality metrics, underscoring multiple dimensions of node importance. Full rankings and centrality metric calculations can be found at https://github.com/aanyashridhar/ECS-Network. **C)** Louvain community detection applied to the combined network, partitioning the nodes (denoted by distinct colors) into modular clusters based on interaction density, which highlights potential functional or mechanistic groupings within the ECS network.

Table 1: Network perturbation analysis across protein–protein, protein–chemical, and combined ECS networks. Summary of robustness simulations following targeted removal of highly central nodes (top one or top three rankings in betweenness or degree centrality). For each perturbation, the table shows the number of original nodes and edges, number of edges after perturbation, and percent change in edge count.

Network	Perturbation Metric	Removal Type	Removed Node(s)	Original # Nodes	Original # Edges	Perturbed # Edges	% Change in Edges
Protein–Protein	Betweenness Centrality	Top 1	CNR1	40	92	77	16.30
Protein–Protein	Betweenness Centrality	Top 3	CNR1, PPARA, PTGS2	40	92	55	40.22
Protein–Protein	Degree Centrality	Top 1	FAAH	40	92	79	14.30
Protein–Protein	Degree Centrality	Top 3	FAAH, CNR1, GPR55	40	92	65	29.35
Protein–Chemical	Degree Centrality	Top 1	CNR1	80	126	105	16.67
Protein–Chemical	Degree Centrality	Top 3	CNR1, CNR2, TRPV1	80	126	82	34.92
Combined	Betweenness Centrality	Top 1	CNR1	80	218	184	15.60
Combined	Betweenness Centrality	Top 3	CNR1, TRPV1, PPARA	80	218	141	35.32
Combined	Degree Centrality	Top 1	CNR1	80	218	184	15.60
Combined	Degree Centrality	Top 3	CNR1, CNR2, TRPV1	80	218	153	29.82

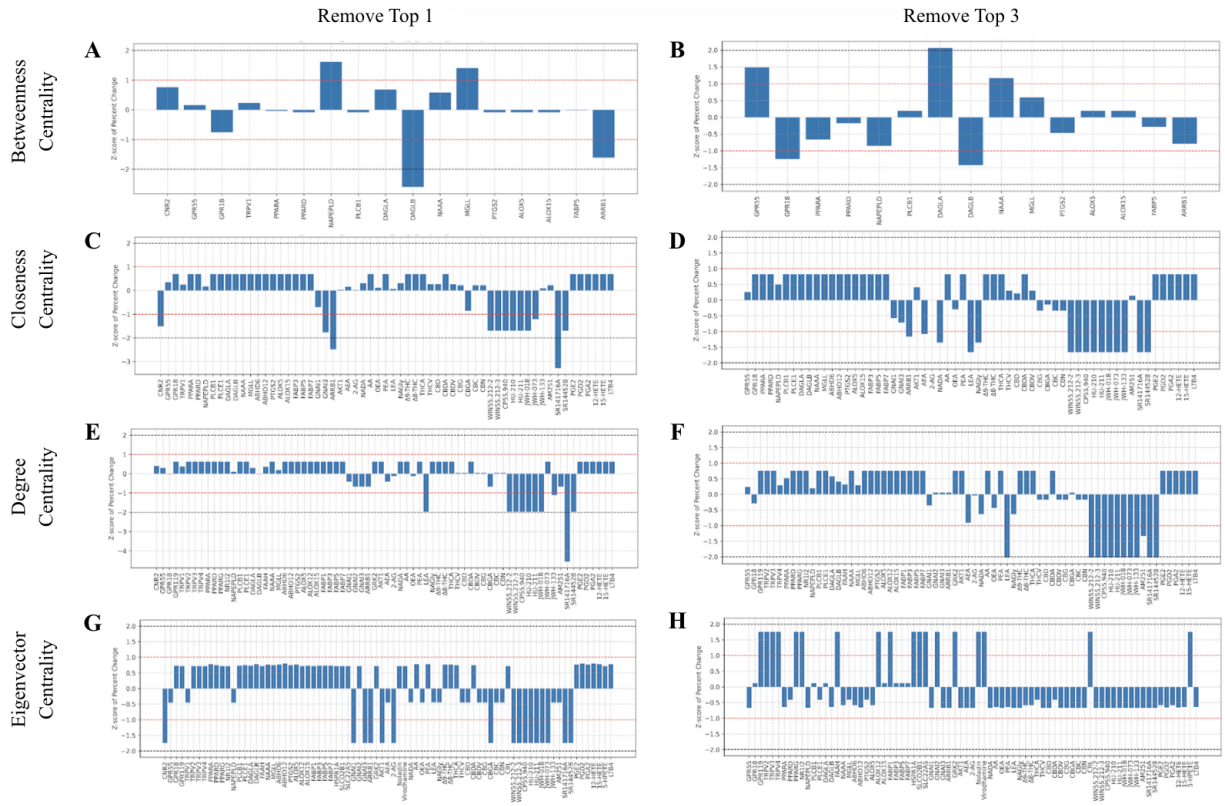


Figure 3: Network perturbation effects on node centrality following removal of high-betweenness nodes. Bar plots depict standardized changes (z-scores) in centrality metrics of the nodes after perturbation. The red and black horizontal dotted lines denote absolute z-scores of 1 and 2, respectively. **A-B)** Betweenness centrality recalculated after removal of the top one **(A)** and top three **(B)** nodes by betweenness. **C-D)** Closeness centrality recalculated after removal of the top one **(C)** and top three **(D)** nodes by betweenness. **E-F)** Degree centrality recalculated after removal of the top one **(E)** and top three **(F)** nodes by betweenness. **G-H)** Eigenvector centrality recalculated after removal of the top one **(G)** and top three **(H)** nodes by betweenness.

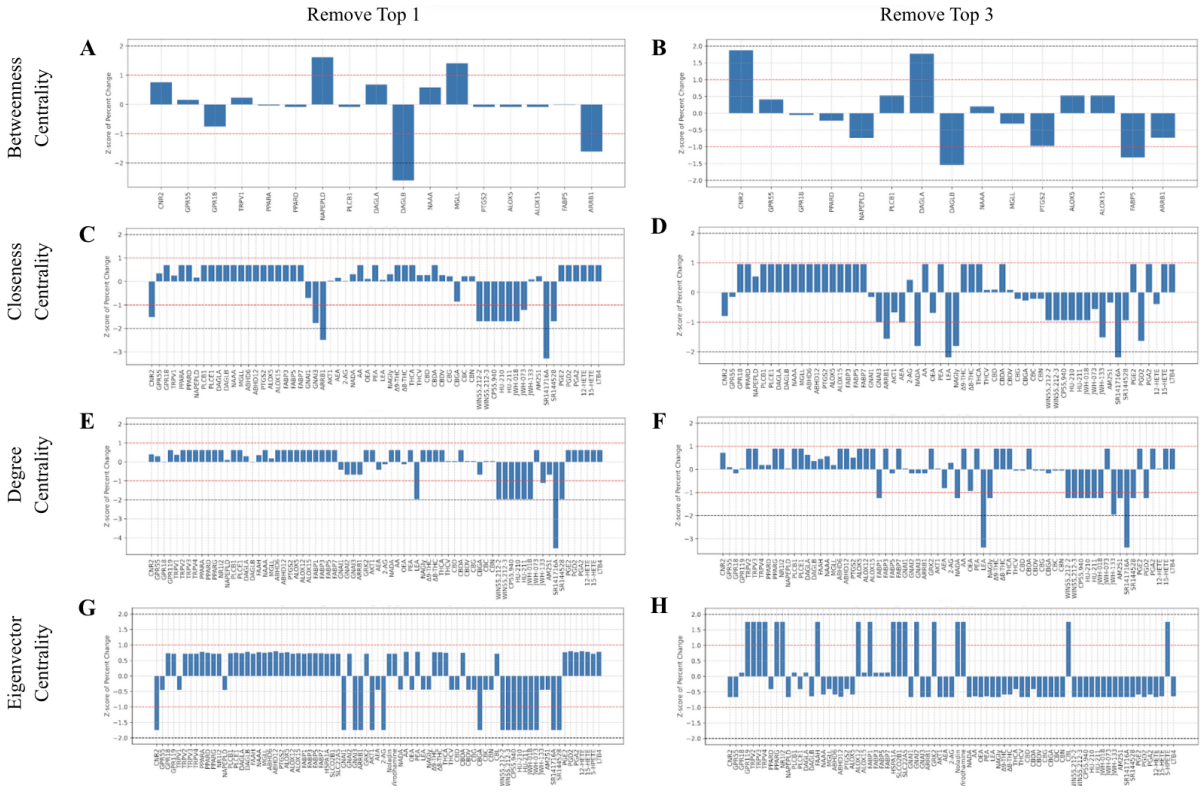


Figure 4: Network perturbation effects on node centrality following removal of high-degree nodes. Bar plots depict standardized changes (z-scores) in centrality metrics of the nodes after perturbation. The red and black horizontal dotted lines denote absolute z-scores of 1 and 2, respectively. **A-B)** Betweenness centrality recalculated after removal of the top one **(A)** and top three **(B)** nodes by degree. **C-D)** Closeness centrality recalculated after removal of the top one **(C)** and top three **(D)** nodes by degree. **E-F)** Degree centrality recalculated after removal of the top one **(E)** and top three **(F)** nodes by degree. **G-H)** Eigenvector centrality recalculated after removal of the top one **(G)** and top three **(H)** nodes by degree.