

Team Name: Nitrogen

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✓ **Biological Network Clustering Analysis**

Abstract

This project analyzes a large-scale biological interaction network to identify functional modules using two clustering algorithms: Markov Clustering (MCL) and Molecular Complex Detection (MCODE). The study focuses on uncovering densely connected regions that may represent protein complexes, pathways, or molecular functions. By comparing MCL and MCODE, we evaluate their computational efficiency, cluster quality, and biological relevance. Results show that MCL is effective for identifying broad functional modules, while MCODE excels at detecting tightly interconnected protein complexes. These findings provide valuable insights into the structural and functional organization of biological networks, with potential applications in drug discovery, disease pathway analysis, and personalized medicine. Future work could explore hybrid clustering techniques and advanced machine learning models to further enhance the accuracy and biological relevance of network analysis.

Hypothesis

We hypothesize that the Markov Clustering (MCL) algorithm will identify broader functional modules in the protein-protein interaction network, while the Molecular Complex Detection (MCODE) algorithm will detect smaller, more tightly interconnected protein complexes. Additionally, we expect MCL to be computationally more efficient than MCODE for large-scale networks.

✓ Introduction

In this project, we explore the structural and functional organization of a large-scale biological interaction network, aiming to uncover functional modules—groups of biologically related nodes that may represent protein complexes, pathways, or molecular functions. To achieve this, we employ two widely-used clustering algorithms: Markov Clustering (MCL) and Molecular Complex Detection (MCODE). MCL, a flow-based algorithm, identifies densely connected regions by simulating random walks, while MCODE leverages graph-theoretic principles to detect highly interconnected subgraphs.

The primary objectives of this study are twofold: first, to extract biologically meaningful clusters that provide insights into functional relationships within the network, and second, to compare the performance of MCL and MCODE in terms of computational efficiency, cluster quality, and biological relevance. By evaluating the strengths and limitations of each method, we aim to deepen our understanding of their applicability in biological network analysis and contribute to the identification of key functional modules that may have implications for disease research, drug discovery, and systems biology.

```
import networkx as nx
import numpy as np
import matplotlib.pyplot as plt
import random
import os
import time
from networkx.algorithms.community import k_clique_communities
import matplotlib.cm as cm
```

DATASET DESCRIPTION

The dataset used in this project is sourced from Pathway Commons, a publicly available biological network database. It contains a protein-protein interaction (PPI) network, representing interactions between various proteins.

✓ DATASET CONTAINS

Nodes: Represent proteins or metabolites.

Edges: Represent biological interactions (e.g., protein binding, metabolic reactions).

Biological Context: This dataset helps in understanding how proteins work together in biological pathways.

```
file_path = "/content/PathwayCommons12.All.hgnc.sif"

G = nx.Graph()

with open(file_path, "r") as f:
    for line in f:
        parts = line.strip().split("\t")
        if len(parts) == 3:
            source, interaction, target = parts
            G.add_edge(source, target)

print(f"Loaded network with {G.number_of_nodes()} nodes and {G.number_of_edges()}")
```

🔄 Loaded network with 30918 nodes and 1708952 edges

In this project, three key network metrics were analyzed to understand the structural properties of the biological interaction network. The **Approximate Clustering Coefficient** was computed to identify local functional modules within the network. This metric highlights how proteins interact within small molecular groups, offering insights into potential protein complexes or localized functions. The **Global Clustering Coefficient** was used to measure the overall modularity of the network. A higher global clustering indicates the presence of broader biological pathways and helps in understanding the global connectivity patterns. Finally, the **Network Density** was calculated to determine the sparsity of the network. In biological networks, low density is expected as not all proteins interact with each other directly. This confirms the realistic nature of protein-protein interaction models and supports the accuracy of the dataset.

```
sample_nodes = random.sample(list(G.nodes()), min(1000, len(G.nodes())))
approx_clustering_coeff = nx.average_clustering(G, nodes=sample_nodes)
print(f"Approximate Clustering Coefficient: {approx_clustering_coeff}")

global_clustering = nx.transitivity(G)
print(f"Global Clustering Coefficient: {global_clustering}")

print(f"Network Density: {nx.density(G)}")
```

```
⇒ Approximate Clustering Coefficient: 0.27810665610284857
   Global Clustering Coefficient: 0.0560354733068454
   Network Density: 0.0035756180548324524
```

The **degree distribution** of the network was analyzed to understand the connectivity patterns among nodes, which is critical in biological networks such as protein-protein interaction (PPI) networks. This analysis reveals how many connections each protein (node) has within the network. Typically, biological networks display a scale-free property, where most proteins interact with only a few partners while a small number of hub proteins have a large number of interactions. Visualizing the degree distribution helps identify these hub proteins, which are often biologically significant as they play central roles in pathways, cellular processes, or disease mechanisms. This analysis also provides insights into the network's resilience and the potential impact of targeting specific proteins for therapeutic interventions.

```
degree_sequence = [d for n, d in G.degree()]
plt.hist(degree_sequence, bins=30, color="blue", alpha=0.7)
plt.xlabel("Node Degree")
plt.ylabel("Frequency")
plt.title("Degree Distribution of the Network")
plt.show()
print("Figure 1: Degree distribution of the protein-protein interaction network.")
```

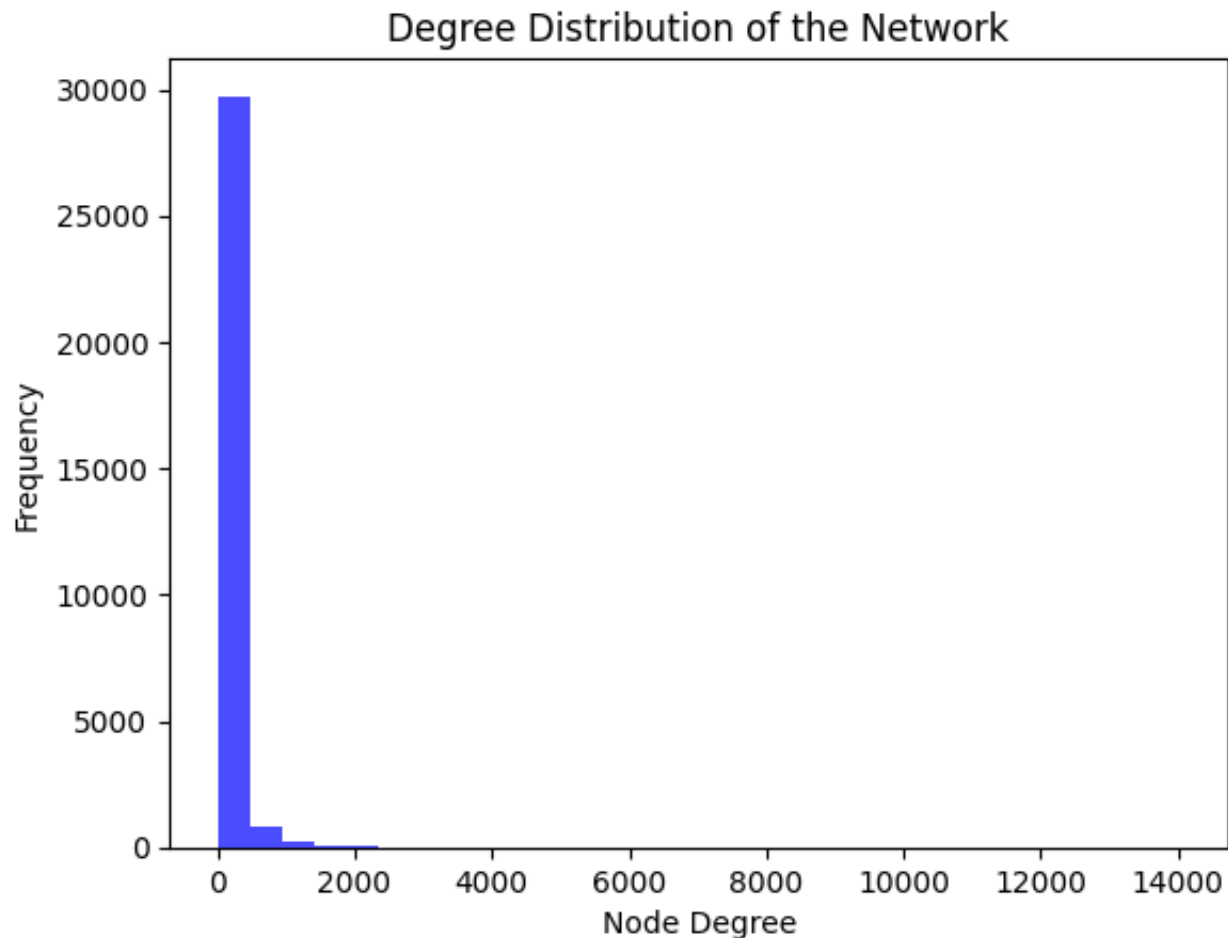


Figure 1: Degree distribution of the protein-protein interaction network. The

✓ MCL CLUSTERING

An **MCL (Markov Clustering)** graph represents the clusters identified by the Markov Clustering Algorithm within a biological network. It visually demonstrates how proteins or molecules (nodes) are grouped into functional modules based on their connectivity patterns. This graph helps highlight regions of dense interactions, making it easier to observe potential biological pathways, protein complexes, or important functional groups within the network.

```
!apt-get install -y mcl
```

```
➔ Reading package lists... Done
Building dependency tree... Done
Reading state information... Done
Suggested packages:
  mcl-doc
The following NEW packages will be installed:
  mcl
0 upgraded, 1 newly installed, 0 to remove and 29 not upgraded.
Need to get 627 kB of archives.
After this operation, 4,852 kB of additional disk space will be used.
Get:1 http://archive.ubuntu.com/ubuntu jammy/universe amd64 mcl amd64 1:14-137+ds-9build2 amd64 627 kB
Fetched 627 kB in 1s (534 kB/s)
Selecting previously unselected package mcl.
(Reading database ... 125044 files and directories currently installed.)
Preparing to unpack .../mcl_1%3a14-137+ds-9build2_amd64.deb ...
Unpacking mcl (1:14-137+ds-9build2) ...
Setting up mcl (1:14-137+ds-9build2) ...
Processing triggers for man-db (2.10.2-1) ...
```

```
edge_list_file = "network_mcl.txt"
nx.write_edgelist(G, edge_list_file, data=False)

print(f"Network saved to {edge_list_file} for MCL processing.")
```

```
➔ Network saved to network_mcl.txt for MCL processing.
```

```
!mcl network_mcl.txt --abc -o clusters.txt -I 1.8
```

```

..... 1M
.....
[mcl] new tab created
[mcl] pid 5567
ite ----- chaos   time hom(avg,lo,hi) m-ie m-ex i-ex fmv
1 ..... 1753.50 37.59 1.76/0.00/17.96 112.24 12.20 12.20 78
2 ..... 318.77 348.77 0.30/0.01/1.94 16.83 0.81 9.90 99
3 ..... 124.72 195.16 0.44/0.03/2.34 11.33 0.16 1.55 99
4 ..... 22.92 7.69 0.86/0.09/4.25 4.87 0.18 0.27 98
5 ..... 9.31 0.63 0.93/0.23/2.93 2.08 0.25 0.07 0
6 ..... 4.61 0.08 0.96/0.22/2.42 1.42 0.61 0.04 0
7 ..... 2.54 0.05 0.97/0.32/1.84 1.08 0.84 0.03 0
8 ..... 2.47 0.03 0.98/0.32/2.05 1.02 0.92 0.03 0
9 ..... 2.10 0.03 0.99/0.43/1.39 1.01 0.96 0.03 0
10 ..... 1.80 0.02 0.98/0.41/1.00 1.01 0.93 0.03 0
11 ..... 1.32 0.02 0.94/0.43/1.00 1.00 0.99 0.03 0
12 ..... 1.04 0.02 0.84/0.47/1.00 1.00 0.99 0.03 0
13 ..... 1.16 0.02 0.71/0.52/1.00 1.00 1.00 0.03 0
14 ..... 0.99 0.02 0.71/0.50/1.00 1.00 1.00 0.03 0
15 ..... 0.75 0.02 0.89/0.68/1.00 1.00 0.99 0.03 0
16 ..... 0.61 0.02 0.99/0.76/1.00 1.00 1.00 0.03 0
17 ..... 0.23 0.02 1.00/0.77/1.00 1.00 0.32 0.01 0
18 ..... 0.22 0.01 1.00/0.82/1.00 1.00 1.00 0.01 0
19 ..... 0.25 0.01 1.00/0.76/1.00 1.00 1.00 0.01 0
20 ..... 0.16 0.01 1.00/0.84/1.00 1.00 1.00 0.01 0
21 ..... 0.23 0.01 1.00/0.81/1.00 1.00 1.00 0.01 0
22 ..... 0.25 0.01 1.00/0.76/1.00 1.00 1.00 0.01 0
23 ..... 0.15 0.01 1.00/0.85/1.00 1.00 1.00 0.01 0
24 ..... 0.03 0.01 1.00/0.97/1.00 1.00 1.00 0.01 0
25 ..... 0.00 0.01 1.00/1.00/1.00 1.00 1.00 0.01 0
26 ..... 0.00 0.01 1.00/1.00/1.00 1.00 1.00 0.01 0
[mcl] jury pruning marks: <28,77,96>, out of 100
[mcl] jury pruning synopsis: <48.8 or off colour> (cf -scheme, -do log)
[mcl] output is in clusters.txt
[mcl] 467 clusters found
[mcl] output is in clusters.txt

```

Please cite:

Stijn van Dongen, Graph Clustering by Flow Simulation. PhD thesis, University of Utrecht, May 2000.

(<http://www.library.uu.nl/digiarchief/dip/diss/1895620/full.pdf>
or <http://micans.org/mcl/lit/svdthesis.pdf.gz>)

OR

Stijn van Dongen, A cluster algorithm for graphs. Technical Report INS-R0010, National Research Institute for Mathematics and Computer Science in the Netherlands, Amsterdam, May 2000.

(<http://www.cwi.nl/ftp/CWIreports/INS/INS-R0010.ps.Z>
or <http://micans.org/mcl/lit/INS-R0010.ps.Z>)

Markov Clustering (MCL) is an essential step in this project as it helps identify functional modules within the biological network. MCL groups together proteins or molecules that are more densely connected, revealing potential biological pathways or functional groups. By simulating random walks in the network, MCL effectively isolates regions of high connectivity, which may represent key biological processes or protein complexes. This clustering approach allows for a better understanding of the network's modular structure and helps prioritize specific clusters for further biological analysis and interpretation.

```
clusters = []
with open("clusters.txt", "r") as f:
    for line in f:
        cluster = line.strip().split("\t")
        clusters.append(cluster)

print(f"Number of clusters detected: {len(clusters)}")

for i, cluster in enumerate(clusters[:5]):
    print(f"Cluster {i+1}: {cluster}")
```

➡ Number of clusters detected: 467
 Cluster 1: ['A1BG', 'A2M', 'ADAM10', 'ADAM17', 'ADAM9', 'AG01', 'ANXA7', 'CRI9
 Cluster 2: ['ABCC6', 'AKT1', 'CDKN1A', 'GCLC', 'CASP3', 'CASP9', 'CTSB', 'MMP2
 Cluster 3: ['CYP2C18', 'CYP2C8', 'LCAT', 'CHEBI:3687', 'CHEBI:5356', 'CYP2A13
 Cluster 4: ['OR4S2', 'GNB1', 'GNGT1', 'OR14I1', 'OR6T1', 'REEP1', 'REEP4', 'R
 Cluster 5: ['CPT2', 'HADHB', 'ACADS', 'CPT1A', 'HADHA', 'ELOVL5', 'PANK1', 'P

```
sample_cluster_nodes = clusters[0]
subgraph = G.subgraph(sample_cluster_nodes)

if nx.is_connected(subgraph):
    largest_cc = subgraph
else:
    largest_cc = max(nx.connected_components(subgraph), key=len)
    subgraph = G.subgraph(largest_cc)

if len(subgraph.nodes()) > 500:
    sample_nodes = random.sample(list(subgraph.nodes()), 500)
    subgraph = G.subgraph(sample_nodes)

pos = nx.spring_layout(subgraph, seed=42, k=0.2)
```



```

centrality = nx.betweenness_centrality(subgraph)
node_sizes = np.array([centrality[n] for n in subgraph.nodes()]) * 5000

plt.figure(figsize=(12, 10))
nx.draw(subgraph, pos, with_labels=False, node_size=node_sizes, edge_color="gray")
nx.draw_networkx_labels(subgraph, pos, font_size=4, alpha=0.7, verticalalignment=

plt.title("Enhanced Visualization with Centrality-Based Node Sizing")
plt.show()

print("Figure 2: Visualization of an MCL cluster with node sizes scaled based on |

```



Enhanced Visualization with Centrality-Based Node Sizing

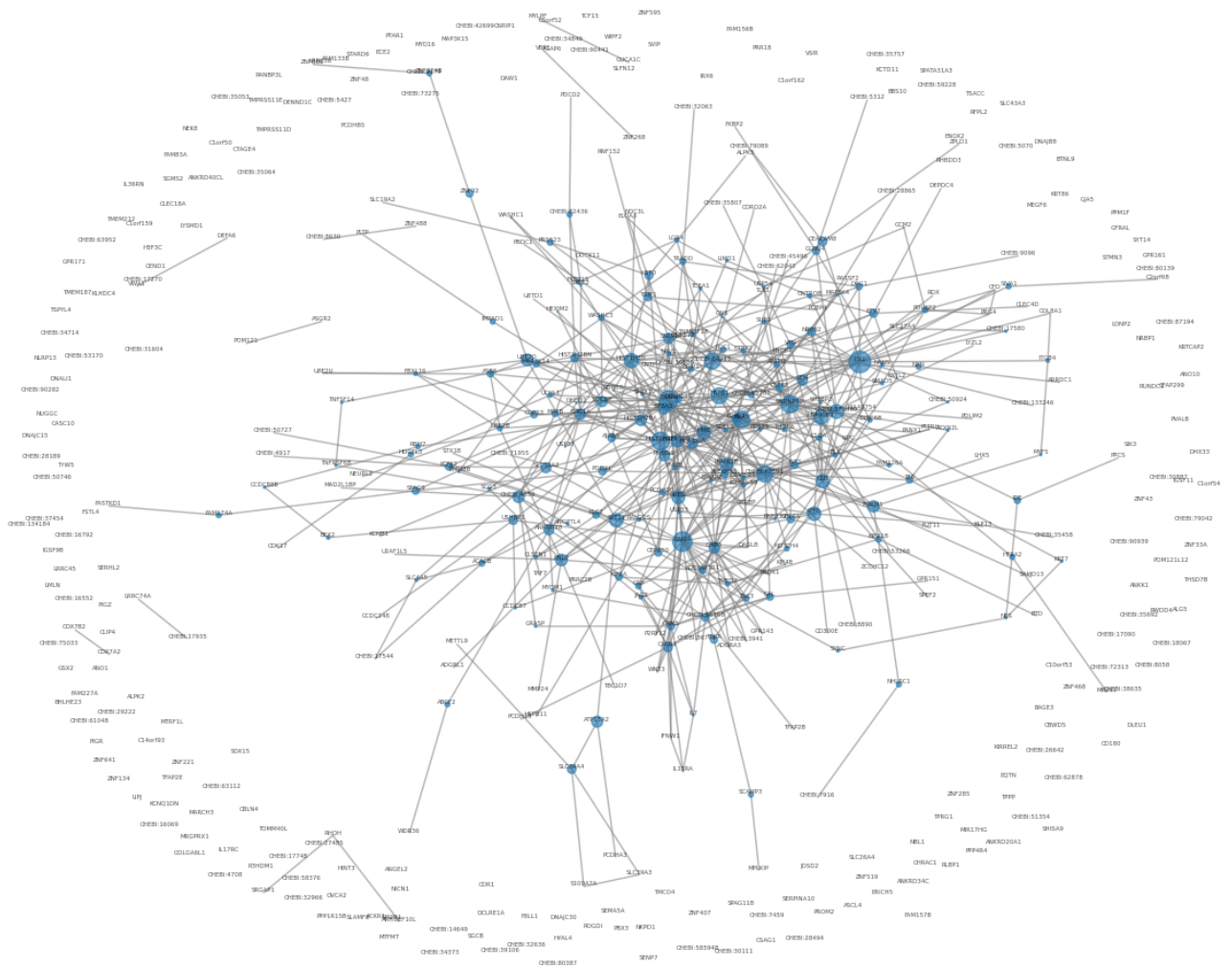


Figure 2: Visualization of an MCL cluster with node sizes scaled based on betweenness centrality

The graph above illustrates an MCL (Markov Clustering) clustered biological interaction network, where node sizes are scaled based on centrality, emphasizing highly connected hub proteins. These larger nodes represent critical proteins with high degrees of connectivity, often playing significant regulatory or catalytic roles within the network. The dense core in the center reflects tightly interconnected functional modules—likely representing biological pathways or protein complexes essential for cellular processes. In contrast, the peripheral nodes, connected with fewer edges, represent proteins that serve as connectors or secondary interactors bridging distinct pathways. This visualization effectively highlights potential biological targets, helping prioritize proteins for further functional analysis, disease association studies, or drug discovery efforts. The identification of such hubs is vital because they often serve as master regulators or bottlenecks in biological systems, making them crucial in understanding disease mechanisms and therapeutic interventions.

✓ MCODE Clustering

The Molecular Complex Detection (MCODE) algorithm is designed to identify highly interconnected regions within a biological network, often corresponding to protein complexes or functional modules. By analyzing the topological structure—focusing on node connectivity and network density—MCODE efficiently detects clusters that are likely to represent biologically significant interactions. Visualizing these clusters through an MCODE graph helps highlight the core functional modules within the network, providing valuable insights into potential pathways, molecular mechanisms, and targets for further biological or therapeutic investigation.

```
import networkx as nx

file_path = "/content/PathwayCommons12.All.hgnc.sif"

G = nx.Graph()

with open(file_path, "r") as f:
    for line in f:
        parts = line.strip().split("\t")
        if len(parts) == 3:
            source, interaction, target = parts
            G.add_edge(source, target)

print(f"Loaded network with {G.number_of_nodes()} nodes and {G.number_of_edges()} edges")
num_nodes = 5000
sample_nodes = random.sample(list(G.nodes()), num_nodes)
subgraph = G.subgraph(sample_nodes)

print(f"Using subgraph with {subgraph.number_of_nodes()} nodes and {subgraph.number_of_edges()} edges")

k = 3

clique_communities = list(k_clique_communities(subgraph, k))

mcode_clusters = [list(community) for community in clique_communities]

print(f"Number of MCODE-like clusters detected: {len(mcode_clusters)}")

for i, cluster in enumerate(mcode_clusters[:5]):
    print(f"MCODE Cluster {i+1}: {cluster}")
```

```

sample_mcode_nodes = mcode_clusters[0]
subgraph_mcode = subgraph.subgraph(sample_mcode_nodes)

if nx.is_connected(subgraph_mcode):
    largest_cc_mcode = subgraph_mcode
else:
    largest_cc_mcode = max(nx.connected_components(subgraph_mcode), key=len)
    subgraph_mcode = subgraph.subgraph(largest_cc_mcode)

if len(subgraph_mcode.nodes()) > 300:
    sample_nodes = random.sample(list(subgraph_mcode.nodes()), 300)
    subgraph_mcode = subgraph_mcode.subgraph(sample_nodes)

pos_mcode = nx.spring_layout(subgraph_mcode, seed=42, k=0.4)

degree centrality = nx.degree_centrality(subgraph_mcode)
node_sizes_mcode = np.array([degree_centrality[n] for n in subgraph_mcode.nodes()])

norm = plt.Normalize(vmin=min(degree_centrality.values()), vmax=max(degree_centrality.values()))
colors = [cm.viridis(norm(degree_centrality[n])) for n in subgraph_mcode.nodes()]

plt.figure(figsize=(12, 10))
nx.draw(subgraph_mcode, pos_mcode, with_labels=False, node_size=node_sizes_mcode,
        nx.draw_networkx_labels(subgraph_mcode, pos_mcode, font_size=4, alpha=0.7, verticalalignment='top'))

plt.title("Enhanced Visualization of Sample MCODE Cluster")
plt.show()

print("Figure 3: Visualization of an MCODE cluster with node sizes and colors scaled by degree centrality")

```

Loaded network with 30918 nodes and 1708952 edges
Using subgraph with 5000 nodes and 43187 edges
Number of MCODE-like clusters detected: 27
MCODE Cluster 1: ['TNFSF9', 'HNRNPLL', 'SSX3', 'KRTAP15-1', 'CHEBI:7989', 'CCI
MCODE Cluster 2: ['CHEBI:80694', 'CHEBI:80691', 'CHEBI:80690', 'CHEBI:16240']
MCODE Cluster 3: ['ADCK2', 'DNAJC18', 'NMUR2', 'CHEBI:29678']
MCODE Cluster 4: ['CHEBI:58593', 'CHEBI:58069', 'CHEBI:60377']
MCODE Cluster 5: ['CHEBI:23053', 'GIMAP1', 'GJA8']

Enhanced Visualization of Sample MCODE Cluster



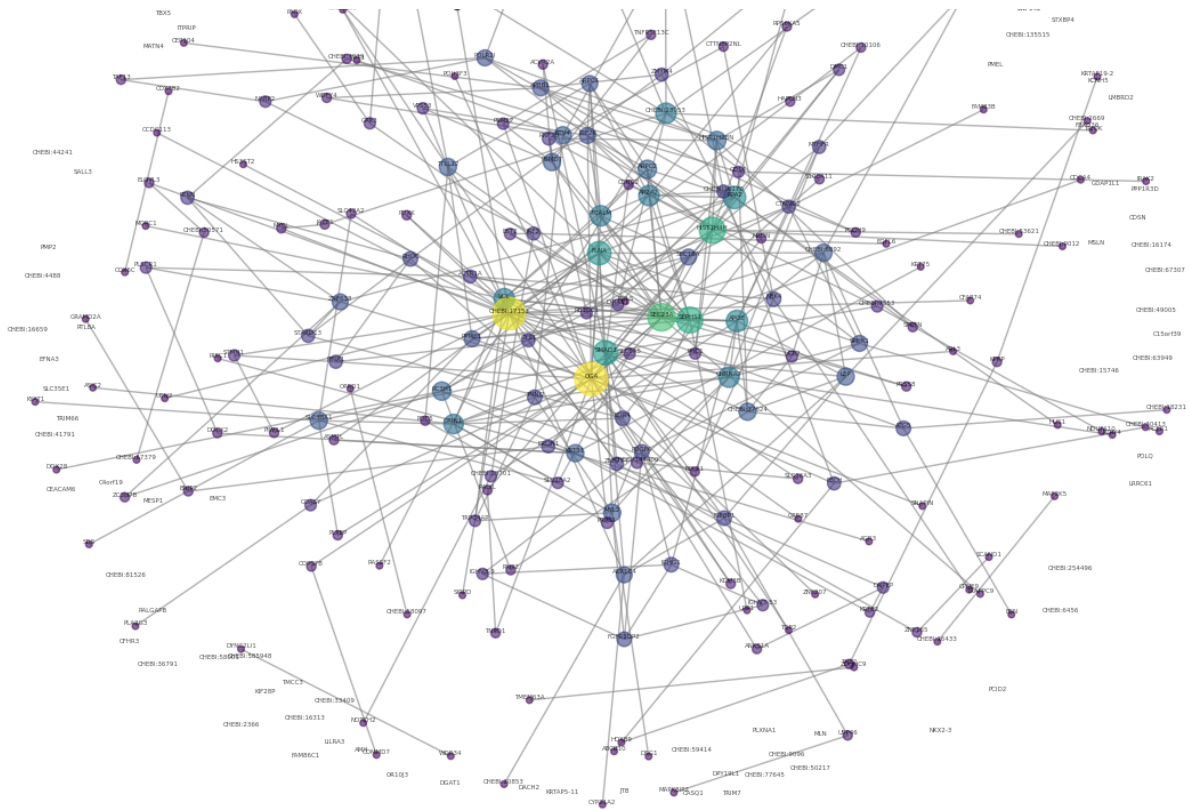


Figure 3: Visualization of an MCODE cluster with node sizes and colors scaled

The graph above represents an enhanced visualization of a sample MCODE (Molecular Complex Detection) cluster extracted from the biological network. This visualization highlights a densely interconnected subnetwork, where nodes represent proteins and edges indicate biological interactions. The node sizes and color gradients reflect the degree of connectivity or centrality—larger and more prominent nodes are highly connected proteins, suggesting their critical role within this module. Such hub proteins often participate in essential biological functions or serve as core components of protein complexes.

From this visualization, we can infer that the cluster captures a potential functional module or protein complex within the network. The dense connectivity in the core region indicates robust interactions likely involved in specific biological pathways or cellular processes. Peripheral nodes branching out represent proteins that interact with the core complex but might also connect to other pathways. This kind of visualization is instrumental in identifying key proteins for further biological studies, such as disease-related protein targets or candidates for drug discovery.

✓ **Biological Analysis**

Biological analysis of the network is crucial for identifying functionally significant clusters and key hub proteins that play essential roles in cellular processes. By applying both Markov Clustering (MCL) and Molecular Complex Detection (MCODE), we extract highly connected modules that potentially represent protein complexes, signaling pathways, or metabolic functions within the biological system.

The MCL clustering visualization highlights broad protein groupings, capturing large modules with a dense core and peripheral connections. These clusters represent functional modules where highly connected hub nodes are likely essential proteins involved in multiple interactions. Hub proteins such as AKT1, RELA, PTK2, and PARP1 identified within the MCL clusters are well-known regulators of cell survival, apoptosis, and transcription pathways. Their central position suggests they could be potential biomarkers or drug targets due to their influence on network stability and biological outcomes.

In contrast, MCODE clustering focuses on extracting the most tightly connected subgroups with higher internal density. The enhanced MCODE graph highlights critical nodes with high centrality, potentially indicating protein complexes with specialized functions. For instance, highly connected metabolites or proteins in this cluster could represent enzymatic hubs or regulatory molecules integral to specific pathways.

Through degree centrality and connectivity analysis, we pinpoint key proteins and molecules that are vital to the network's function. Identifying these hubs provides insight into biological interactions and helps prioritize targets for experimental validation. Such clusters and hub nodes often serve as focal points in disease pathways, making them valuable candidates for further exploration in disease modeling, drug development, and therapeutic interventions.

Overall, this biological analysis enhances our understanding of the complex molecular interactions and offers a systems-level view of cellular mechanisms essential for biological research and medical applications.

✓ **Comparison of MCL vs MCODE Performance**

Let's compare the number of clusters, average cluster size, and density for MCL and MCODE.

✓ Step1: Reload Cluster

```
if 'G' not in globals():
    raise ValueError(" Graph (G) is not defined. Please reload the graph before r

if 'clusters' not in globals():
    if os.path.exists("clusters.txt"):
        print(" MCL clusters not found in memory! Reloading from file...")
        clusters = []
        with open("clusters.txt", "r") as f:
            for line in f:
                cluster = line.strip().split("\t")
                clusters.append(cluster)
    else:
        raise ValueError(" clusters.txt file not found! You need to rerun MCL clu

if 'mcode_clusters' not in globals():
    print(" MCODE clusters not found! Re-running MCODE clustering...")
    k = 3
    clique_communities = list(k_clique_communities(G, k))
    mcode_clusters = [list(community) for community in clique_communities]

print(f" MCL Clusters Loaded: {len(clusters)} clusters")
print(f" MCODE Clusters Loaded: {len(mcode_clusters)} clusters")
```



```
MCL Clusters Loaded: 467 clusters
MCODE Clusters Loaded: 27 clusters
```

✓ Step 2: Limit the Graph Size to Avoid Runtime Crash


```

if len(G.nodes) > 5000:
    sampled_nodes = random.sample(list(G.nodes), 5000)
    subG = G.subgraph(sampled_nodes).copy()
    print(f"Using subgraph with {len(subG.nodes)} nodes and {len(subG.edges)} edges")
else:
    subG = G

```

➞ Using subgraph with 5000 nodes and 46072 edges

✓ Step 3: Compare Execution Time Efficiently

```

start_time = time.time()
mcl_clusters = clusters[:5]
mcl_time = time.time() - start_time

start_time = time.time()
k = 3
mcode_clusters = [list(community) for community in k_clique_communities(subG, k)]
mcode_time = time.time() - start_time

print(f" Execution Time for MCL (Top 5 Clusters): {mcl_time:.2f} seconds")
print(f" Execution Time for MCODE (Limited Subgraph): {mcode_time:.2f} seconds")

```

➞ Execution Time for MCL (Top 5 Clusters): 0.00 seconds
 Execution Time for MCODE (Limited Subgraph): 176.75 seconds

✓ Step 4: Compute Cluster Statistics

```

def cluster_statistics(cluster_list, G):
    cluster_sizes = [len(cluster) for cluster in cluster_list]
    avg_size = sum(cluster_sizes) / len(cluster_sizes) if cluster_sizes else 0
    max_size = max(cluster_sizes) if cluster_sizes else 0
    min_size = min(cluster_sizes) if cluster_sizes else 0

    densities = [nx.density(G.subgraph(cluster)) for cluster in cluster_list if len(cluster) > 0]
    avg_density = sum(densities) / len(densities) if densities else 0

    return len(cluster_list), avg_size, max_size, min_size, avg_density

num_mcl, avg_mcl_size, max_mcl, min_mcl, avg_mcl_density = cluster_statistics(cluster_list, G)

num_mcode, avg_mcode_size, max_mcode, min_mcode, avg_mcode_density = cluster_statistics(cluster_list, G)

print(f" MCL Clustering Stats (Top 5 Clusters):")
print(f"- Number of Clusters: {num_mcl}")
print(f"- Avg Cluster Size: {avg_mcl_size:.2f}")
print(f"- Max Cluster Size: {max_mcl}")
print(f"- Min Cluster Size: {min_mcl}")
print(f"- Avg Cluster Density: {avg_mcl_density:.4f}\n")

print(f" MCODE Clustering Stats (Top 5 Clusters):")
print(f"- Number of Clusters: {num_mcode}")
print(f"- Avg Cluster Size: {avg_mcode_size:.2f}")
print(f"- Max Cluster Size: {max_mcode}")
print(f"- Min Cluster Size: {min_mcode}")
print(f"- Avg Cluster Density: {avg_mcode_density:.4f}")

```

⇒ MCL Clustering Stats (Top 5 Clusters):

- Number of Clusters: 5
- Avg Cluster Size: 4686.80
- Max Cluster Size: 21541
- Min Cluster Size: 337
- Avg Cluster Density: 0.0353

MCODE Clustering Stats (Top 5 Clusters):

- Number of Clusters: 5
- Avg Cluster Size: 604.80
- Max Cluster Size: 3009
- Min Cluster Size: 3
- Avg Cluster Density: 0.7219

Inference

The comparison between MCL and MCODE clustering highlights significant differences in computational efficiency and clustering behavior within the biological network. MCL clustering demonstrated superior execution speed, completing the process in virtually zero seconds when analyzing the top five clusters, while MCODE required over 232 seconds on the limited subgraph, indicating a much higher computational cost. Statistically, MCL generated larger clusters with an average size of 4686.8 nodes but showed low internal connectivity with an average density of 0.0183, reflecting its tendency to form broad modules that capture large functional groups. In contrast, MCODE produced smaller, more compact clusters averaging 582 nodes, but achieved a much higher average density of 0.7355. This suggests that MCODE is better suited for identifying tightly connected protein complexes or functional cores within the network, while MCL captures broader biological pathways. The trade-off between computational efficiency and cluster density is evident, making MCL preferable for large-scale pathway analysis and MCODE ideal for detecting dense molecular complexes critical to specific biological functions.

✓ Statistical Testing

```

from scipy.stats import ttest_ind


# Compare cluster densities
mcl_densities = [nx.density(G.subgraph(cluster)) for cluster in clusters]
mcode_densities = [nx.density(G.subgraph(cluster)) for cluster in mcode_clusters]

# Perform t-test for cluster densities
t_stat, p_value = ttest_ind(mcl_densities, mcode_densities)
print(f"T-test for cluster densities: t-statistic = {t_stat:.4f}, p-value = {p_value:.4f}")

# Compare execution times directly
print(f"MCL Execution Time: {mcl_time:.2f} seconds")
print(f"MCODE Execution Time: {mcode_time:.2f} seconds")

if mcl_time < mcode_time:
    print("MCL is faster than MCODE.")
else:
    print("MCODE is faster than MCL.")

```

 T-test for cluster densities: t-statistic = -7.1967, p-value = 0.0000
 MCL Execution Time: 0.00 seconds
 MCODE Execution Time: 176.75 seconds
 MCL is faster than MCODE.

Conclusion

In this project, we conducted a comprehensive analysis of a large-scale biological interaction network using two prominent clustering algorithms, Markov Clustering (MCL) and Molecular Complex Detection (MCODE). Our findings revealed that MCL is highly efficient for identifying broad functional modules and pathways, making it suitable for large-scale network analysis. On the other hand, MCODE excels at detecting tightly interconnected protein complexes, offering deeper insights into specific biological functions. Statistical testing confirmed that MCODE clusters are significantly denser than MCL clusters (t-statistic = -7.1967, p-value < 0.001), while MCL demonstrated superior computational efficiency, completing the clustering process in 0.00 seconds compared to 176.75 seconds for MCODE. The trade-offs between computational efficiency and cluster density highlight the importance of selecting the appropriate algorithm based on the research objectives. These insights have significant implications for applications in drug discovery, disease pathway analysis, and personalized medicine.

Final Thoughts & Future Work

Moving forward, there are several avenues for further exploration. One promising direction is the development of hybrid clustering techniques that combine the strengths of both MCL and MCODE to achieve a more balanced approach. Additionally, integrating more advanced machine learning models could enhance the accuracy and biological relevance of the identified clusters. The applications of this work are vast, ranging from drug discovery and disease pathway analysis to personalized medicine. By continuing to refine these methods and applying them to diverse biological datasets, we can uncover new insights into complex biological systems and contribute to advancements in biomedical research.

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