



Biological Database
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J Component Project Report

Title: A study of Alpha-synuclein and Visualization of it's Amino Acids

By

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Under the guidance of

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1. Problem statement

Alpha-synuclein is a protein that, in humans, is encoded by the SNCA gene. It is abundant in the brain, while smaller amounts are found in the heart, muscle and other tissues.

Synuclein family was discovered two decades ago, consisting of alpha-synuclein, beta-synuclein, and gamma-synuclein. Interest in the synuclein family began when alpha-synuclein was found to be mutated in several families with autosomal dominant Parkinson's disease.

Till this date there are many research being done regarding Alpha-synuclein protein.

2. Introduction

Synucleins are a family of soluble proteins common to vertebrates, primarily expressed in neural tissue and in certain tumors.

The screenshot displays the UniProtKB entry for P37840 (SYUA_HUMAN). The header shows the protein name and a 'Basket' button. Below the header, there are navigation links: Display, BLAST, Align, Format, Add to basket, History, Help video, Add a publication, and Feedback. The left sidebar contains links for Entry, Publications, Feature viewer, and Feature table. The main content area shows the protein details: Protein: Alpha-synuclein, Gene: SNCA, Organism: Homo sapiens (Human), and Status: Reviewed - Annotation score: 5/5 - Experimental evidence at protein level¹. Below this, the 'Function' section is expanded, showing a detailed description of the protein's role in synaptic activity and its association with neurodegenerative diseases. The function text is as follows: 'Neuronal protein that plays several roles in synaptic activity such as regulation of synaptic vesicle trafficking and subsequent neurotransmitter release. Participates as a monomer in synaptic vesicle exocytosis by enhancing vesicle priming, fusion and dilation of exocytotic fusion pores (PubMed:28288128, PubMed:30404828). Mechanistically, acts by increasing local Ca²⁺ release from microdomains which is essential for the enhancement of ATP-induced exocytosis (PubMed:30404828). Acts also as a molecular chaperone in its multimeric membrane-bound state, assisting in the folding of synaptic fusion components called SNAREs (Soluble NSF Attachment Protein REceptors) at presynaptic plasma membrane in conjunction with cysteine string protein-alpha/DNAJC5 (PubMed:20798282). This chaperone activity is important to sustain normal SNARE-complex assembly during aging (PubMed:20798282). Plays also a role in the regulation of the dopamine neurotransmission by associating with the dopamine transporter (DAT1) and thereby modulating its activity (PubMed:26442590). 4 Publications'. The left sidebar also has a 'None' button and a list of features: Function, Names & Taxonomy, Subcell. location, Pathol./Biotech, PTM / Processing, and Expression, all of which are checked.

Fig: Information from UniPort db

Alpha-synuclein is a protein that, in humans, is encoded by the SNCA gene. It is abundant in the brain, while smaller amounts are found in the heart, muscle and other tissues. In the brain, alpha-synuclein is found mainly at the tips of neurons in specialized structures called presynaptic terminals.

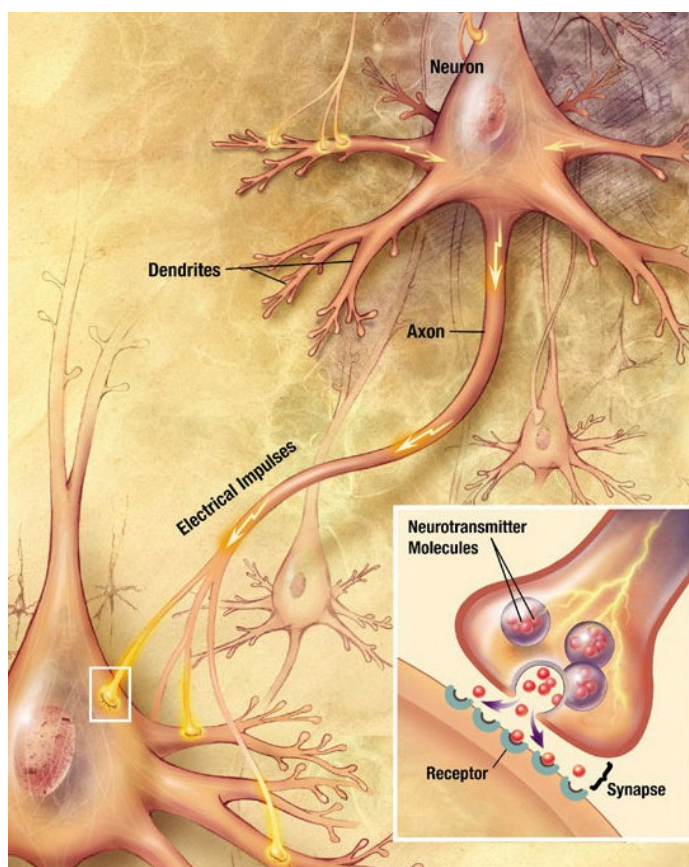


Fig: the white rectangle is the region where Alpha-synuclein is present

Family members

The synuclein family includes three known proteins: alpha-synuclein, beta-synuclein, and gamma-synuclein. Interest in the synuclein family began when alpha-synuclein was found to be mutated in several families with autosomal dominant Parkinson's disease.

Tissue Expression

Alpha-synuclein is a synuclein protein of unknown function primarily found in neural tissue, making up as much as 1% of all proteins in the cytosol of brain cells. It is predominantly expressed in the neocortex, hippocampus, substantia nigra, thalamus, and cerebellum. It is predominantly a neuronal protein, but can also be found in the neuroglial cells. In melanocytic cells, SNCA protein expression may be regulated by MITF.

It has been established that alpha-synuclein is extensively localized in the nucleus of mammalian brain neurons, suggesting a role of alpha-synuclein in the nucleus. Synuclein is however found predominantly in the presynaptic termini, in both free or membrane-bound forms, with roughly 15% of synuclein being membrane-bound in any moment in neurons.

It has also been shown that alpha-synuclein is localized in neuronal mitochondria. Alpha-synuclein is highly expressed in the mitochondria in olfactory bulb, hippocampus, striatum and thalamus, where the cytosolic alpha-synuclein is also rich. However, the cerebral cortex and cerebellum are two exceptions, which contain rich cytosolic alpha-synuclein but very low levels of mitochondrial alpha-synuclein. It has been shown that alpha-synuclein is localized in the inner membrane of mitochondria, and that the inhibitory effect of alpha-synuclein on complex I activity of mitochondrial respiratory chain is dose-dependent. Thus, it is suggested that alpha-synuclein in mitochondria is differentially expressed in different brain regions and the background levels of mitochondrial alpha-synuclein may be a potential factor affecting mitochondrial function and predisposing some neurons to degeneration.

Structure

Alpha-synuclein in solution is considered to be an intrinsically disordered protein, i.e. it lacks a single stable 3D structure

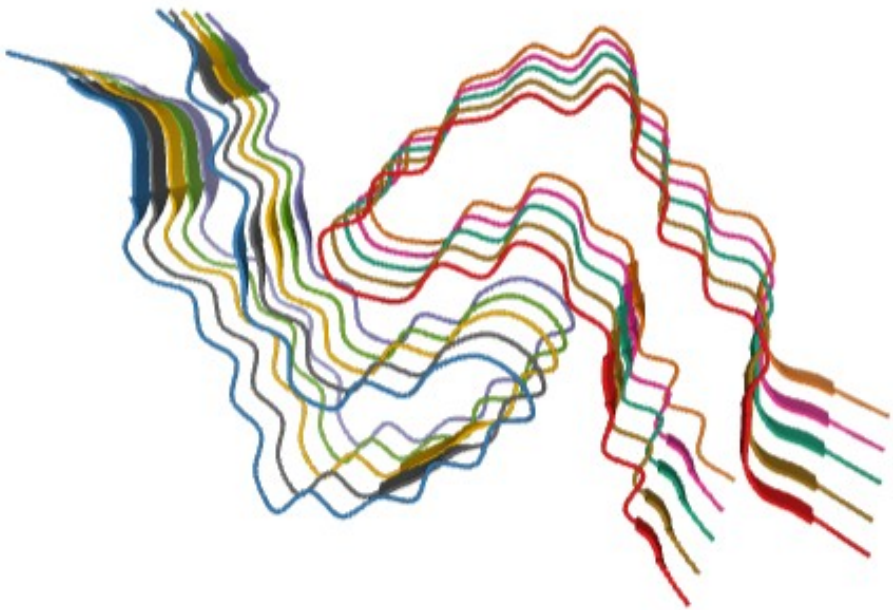


Fig: 3D view of Asymmetric Unit of a unique protein chain of Alpha-synuclein (6CU8)

Function

Alpha-synuclein modulates DNA repair processes, including repair of double-strand breaks (DSBs).[59] DNA damage response markers co-localize with alpha-synuclein to form discrete foci in human cells and mouse brain. Depletion of alpha-synuclein in human cells causes increased introduction of DNA DSBs after exposure to bleomycin and reduced ability to repair these DSBs. In addition, alpha-synuclein knockout mice display a higher level of DSBs, and this problem can be alleviated by transgenic reintroduction of human alpha-synuclein. Alpha-synuclein promotes the DSB repair pathway referred to as non-homologous end joining. The DNA repair function of alpha-synuclein appears to be compromised in Lewy body inclusion bearing neurons, and this may trigger cell death.

Sequence

Alpha-synuclein primary structure is usually divided in three distinct domains:

- Residues 1-60: An amphipathic N-terminal region dominated by four 11-residue repeats including the consensus sequence KTKEGV. This sequence has a structural alpha helix propensity similar to apolipoproteins-binding domains. It is a highly conserved terminal that interacts with acidic lipid membranes, and all the discovered point mutations of the SNCA gene are located within this terminal.
- Residues 61-95: A central hydrophobic region which includes the non-amyloid- β component (NAC) region, involved in protein aggregation. This domain is unique to alpha-synuclein among the synuclein family.
- Residues 96-140: a highly acidic and proline-rich region which has no distinct structural propensity. This domain plays an important role in the function, solubility and interaction of alpha-synuclein with other proteins.

Clinical significance

Classically considered an unstructured soluble protein, unmutated α -synuclein forms a stably folded tetramer that resists aggregation. This observation, though reproduced and extended by several labs, is still a matter of debate in the field due to conflicting reports. Nevertheless, alpha-synuclein aggregates to form insoluble fibrils in pathological conditions characterized by Lewy bodies, such as Parkinson's disease, dementia with Lewy bodies and multiple system atrophy. These disorders are known as synucleinopathies. In vitro models of synucleinopathies revealed that aggregation of alpha-synuclein may lead to various cellular disorders including microtubule impairment, synaptic and mitochondrial dysfunctions,

oxidative stress as well as dysregulation of Calcium signaling, proteasomal and lysosomal pathway. Alpha-synuclein is the primary structural component of Lewy body fibrils. Occasionally, Lewy bodies contain tau protein; however, alpha-synuclein and tau constitute two distinctive subsets of filaments in the same inclusion bodies. Alpha-synuclein pathology is also found in both sporadic and familial cases with Alzheimer's disease.

The aggregation mechanism of alpha-synuclein is uncertain. There is evidence of a structured intermediate rich in beta structure that can be the precursor of aggregation and, ultimately, Lewy bodies. A single molecule study in 2008 suggests alpha-synuclein exists as a mix of unstructured, alpha-helix, and beta-sheet-rich conformers in equilibrium. Mutations or buffer conditions known to improve aggregation strongly increase the population of the beta conformer, thus suggesting this could be a conformation related to pathogenic aggregation. One theory is that the majority of alpha-synuclein aggregates are located in the presynapse as smaller deposits which causes synaptic dysfunction. Among the strategies for treating synucleinopathies are compounds that inhibit aggregation of alpha-synuclein. It has been shown that the small molecule cuminaldehyde inhibits fibrillation of alpha-synuclein. The Epstein-Barr virus has been implicated in these disorders.

3. Literature review

[1] Alpha-synuclein and neurodegenerative diseases

Two developments have imparted a new direction to research on the aetiology and pathogenesis of Parkinson's disease. First, the discovery that a missense mutation in the α -synuclein gene is a rare genetic cause of Parkinson's disease. Second, the identification of the α -synuclein protein as the main component of Lewy bodies and Lewy neurites, the defining neuropathological characteristics of all cases of Parkinson's and several other diseases. The filamentous inclusions of multiple system atrophy are also made of α -synuclein. These findings have placed α -synuclein dysfunction at the centre of several common neurodegenerative diseases.

[2] Alpha-synuclein and Parkinson's disease

The involvement of α -synuclein in neurodegenerative diseases was first suspected after the isolation of an α -synuclein fragment (NAC) from amyloid plaques in Alzheimer's disease (AD). Later, two different α -synuclein mutations were shown to be associated with autosomal-dominant Parkinson's disease (PD), but only in a small number of families. However, the discovery that α -synuclein is a major component of Lewy bodies and Lewy neurites, the pathological hallmarks of PD, confirmed its role in PD pathogenesis. Pathological aggregation of the protein might be responsible for neurodegeneration.

[3] Alpha-synuclein in Lewy Body Disease and Alzheimer's Disease

Recent studies have shown that abnormal aggregation and accumulation of synaptic proteins, such as α -synuclein, might be associated with plaque formation in AD and Lewy body formation in LBD. Further reinforcing the hypothesis that α -synuclein plays a major role in the pathogenesis of these disorders, recent work has shown that mutations that alter the conformation of this molecule are associated with familial forms of Parkinson's disease. The mechanisms by which altered function or aggregation of α -synuclein might lead to neurodegeneration are not completely clear; however, new evidence points to a potential role for this molecule in synaptic damage and neurotoxicity via amyloid-like fibril formation and mitochondrial dysfunction. In this paper they review the data linking α -synuclein to the pathogenesis of AD and LBD.

[4] Therapeutic approaches to target alpha-synuclein pathology

In this paper they describe current challenges and possibilities with alpha-synuclein as a therapeutic target. They briefly highlight gaps in the knowledge of the role of alpha-synuclein in disease, and propose that a deeper understanding of the pathobiology of alpha-synuclein

can lead to improved therapeutic strategies. They describe several treatment approaches that are currently being tested in advanced animal experiments or already are in clinical trials. Finally, they briefly discuss challenges related to the clinical testing of alpha-synuclein therapies, for example difficulties in monitoring target engagement and the need for relatively large trials of long duration. They conclude that alpha-synuclein remains one of the most compelling therapeutic targets for Parkinson's disease, and related synucleinopathies, and that the multitude of approaches being tested provides hope for the future.

[5] α -synuclein toxicity in neurodegeneration: mechanism and therapeutic strategies


Alterations in α -synuclein dosage lead to familial Parkinson's disease (PD), and its accumulation results in synucleinopathies that include PD, dementia with Lewy bodies (DLB) and multiple system atrophy (MSA). Furthermore, α -synuclein contributes to the fibrilization of amyloid- β and tau, two key proteins in Alzheimer's disease, which suggests a central role for α -synuclein toxicity in neurodegeneration. Recent studies of factors contributing to α -synuclein toxicity and its disruption of downstream cellular pathways have expanded our understanding of disease pathogenesis in synucleinopathies. In this paper, they discuss these emerging themes, including the contributions of aging, selective vulnerability and non-cell-autonomous factors such as α -synuclein cell-to-cell propagation and neuroinflammation.

References


1. Goedert, "[Alpha-synuclein and neurodegenerative diseases](#)", Nat Rev Neurosci 2, 492–501 (2001).
2. Lücking, C., Brice*, "[A. Alpha-synuclein and Parkinson's disease](#)", CMLS, Cell. Mol. Life Sci. 57, 1894–1908 (2000).
3. Makoto Hashimoto, Eliezer Masliah, "[Alpha-synuclein in Lewy Body Disease and Alzheimer's Disease](#)", First published 05 April 2006
4. Patrik BrundinaKuldip, D. Dave Jeffrey, H. Kordower, "[Therapeutic approaches to target alpha-synuclein pathology](#)", Received 28 August 2017, Revised 26 September 2017, Accepted 3 October 2017.
5. Wong, Y., Krainc, D., " [\$\alpha\$ -synuclein toxicity in neurodegeneration: mechanism and therapeutic strategies](#)", Nat Med 23, 1–13 (2017).

4. Methodology and Implementation

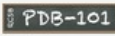




Visualizing network graphs of Amino acids for Alpha-synuclein







170597 Biological Macromolecular Structures
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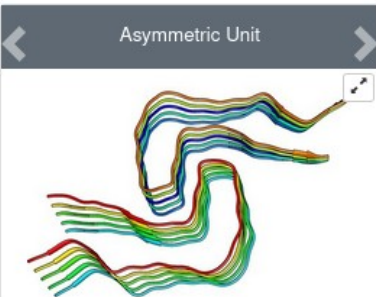


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[Genome](#)



Asymmetric Unit

3D View: [Structure](#)

Macromolecule Content

- Total Structure Weight: 144.76 kDa
- Atom Count: 2880
- Residue Count: 410
- Unique protein chains: 1

6CU8

Alpha Synuclein fibril formed by full length protein - Twister Polymorph

DOI: [10.2210/pdb6CU8/pdb](https://doi.org/10.2210/pdb6CU8/pdb) EMDDataResource: [EMD-7619](https://www.ebi.ac.uk/EMDDataResource/EMD-7619)

Classification: [PROTEIN FIBRIL](#)

Organism(s): [Homo sapiens](#)

Expression System: [Escherichia coli](#)

Mutation(s): No

Deposited: 2018-03-23 **Released:** 2018-09-12

Deposition Author(s): [Li, B.](#), [Hatami, A.](#), [Ge, P.](#), [Murray, K.A.](#), [Sheth, P.](#), [Zhang, M.](#), [Nair, G.](#), [Sawaya, M.R.](#), [Zhu, C.](#), [Broad, M.](#), [Shin, W.S.](#), [Ye, S.](#), [John, V.](#), [Eisenberg, D.S.](#), [Zhou, Z.H.](#), [Jiang, L.](#)

Funding Organization(s): XSEDE, National Institutes of Health/National Institute Of Allergy and Infectious Diseases (NIH/NIAD), National Institutes of Health/National Institute of General Medical Sciences (NIH/NIGMS)

Experimental Data Snapshot

Method: ELECTRON MICROSCOPY




Resolution: 3.60 Å



Aggregation State: FILAMENT

Reconstruction Method: HELICAL

wwPDB Validation

[3D Report](#) [Full Report](#)

Metric	Percentile Ranks	Value
Clashscore		7
Ramachandran outliers		0
Sidechain outliers		0

 Percentile relative to all structures
 Percentile relative to all EM structures

This is version 1.4 of the entry. See complete [history](#).

Fig: 6CU8 Apha Synuclein fibril

Configuration for 6CU8

```
{...} analysis_config.json × structure_analysis_tools.py ×  
{...} analysis_config.json > ...  
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3   "pdb_id": "6cu8",  
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20  "remove_hydrogen_atoms": true  
21 }
```

Fig: Configuration for the 6CU8 fibril

Node Data in CSV format

Columns:- Residue name, Position, Sequence position, Type of residue, Degree, Weight, Weight/Degree, Atomic number, Secondary structure, N. others, Neighbor position, Neighbor type, Pairwise weight, Relation

	C			D			E			F			G			H			I			J			K			L			M			N			O			P			Q			R			S			T			U			V			W			X			Y			Z			AA			AB			AC			AD			AE			AF			AG																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																									
1	Residue name	Position	Sequence position	Type of residue	Degree	Weight	Weight/Degree	Atomic number	Secondary structure	N. others	Neighbor position	Neighbor type	Pairwise weight	Relation																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																						

			C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC	AD	AE	AF		
356	pd6cu8D78	D78	78	ALA	7	44	6.28571428571429	5	loop18	13.666		VAL	3.40	B77 VAL	8.40	B78 ALA	12.40	B79 GLN	5.40	B80 LVS	1.40	C66 VAL												
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360	pd6cu8I78	I78	78	ALA	6	40	7	6	5	loop36	14.577		VAL	7.40	G78 ALA	12.40	G79 GLN	5.40	H66 VAL	3.40														
361	pd6cu8J78	J78	78	ALA	4	27	6.75	6.5	5	loop40	16.177		VAL	7.40	H78 ALA	12.40	H79 GLN	5.40	I66 VAL	3.40														
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391	pd6cu8I81	I81	81	THR	2	24	12	12	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
392	pd6cu8J81	J81	81	THR	2	24	12	12	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
393	pd6cu8A82	A82	82	VAL	6	66	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
394	pd6cu8B82	B82	82	VAL	3	31	10.3333333333333	10.3333333333333	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
395	pd6cu8C82	C82	82	VAL	3	29	9.66666666666667	9.66666666666667	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
396	pd6cu8D82	D82	82	VAL	6	61	10.1666666666667	10.1666666666667	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
397	pd6cu8E82	E82	82	VAL	5	58	9.83333333333333	9.83333333333333	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
398	pd6cu8F82	F82	82	VAL	6	57	9.5	9.5	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
399	pd6cu8G82	G82	82	VAL	6	63	10.5	10.5	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
400	pd6cu8H82	H82	82	VAL	6	61	10.1666666666667	10.1666666666667	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
401	pd6cu8I82	I82	82	VAL	6	61	10.1666666666667	10.1666666666667	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
402	pd6cu8J82	J82	82	VAL	3	30	10	10	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	
403	pd6cu8E83	E83	83	GLU	5	60	12	12	9	loop4	16.181		THR	16.40	H82 VAL	19.40	G83 GLU	5.40																

Legend for node

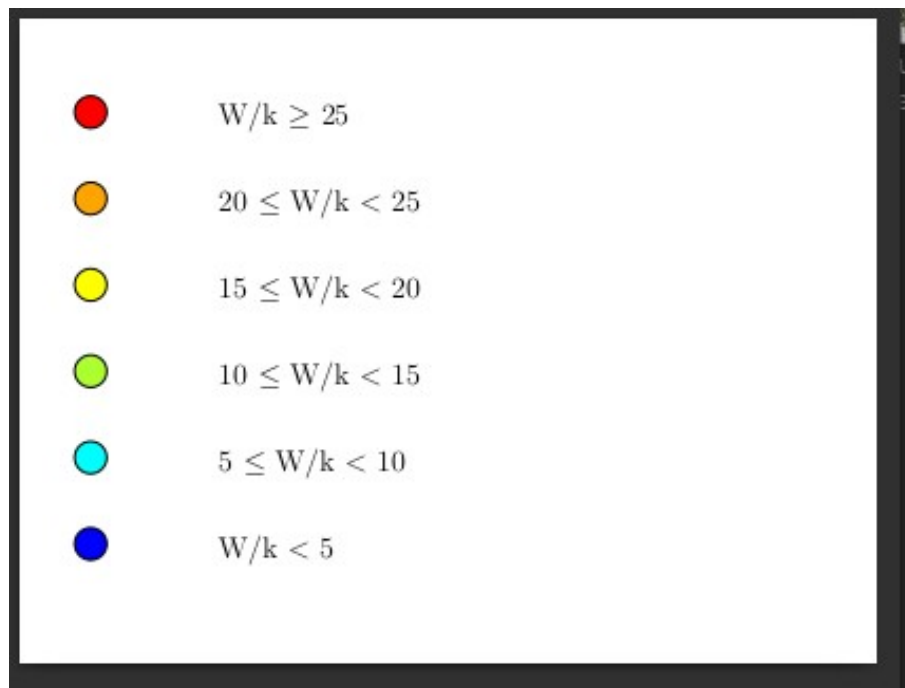


Fig: legend for node of the network graphs

4.1 Network Graphs

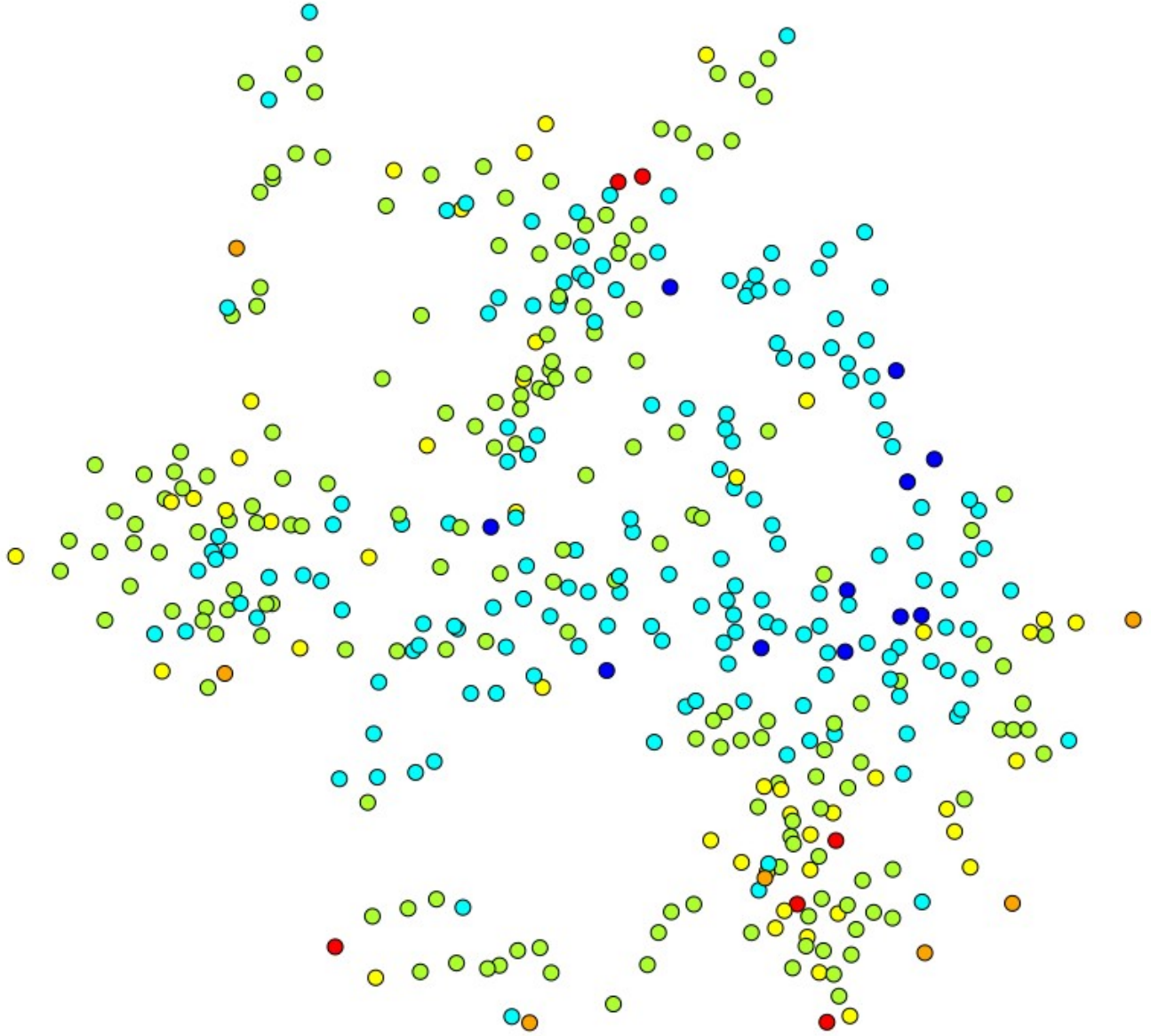


Fig: Nodes made according to the legend scheme

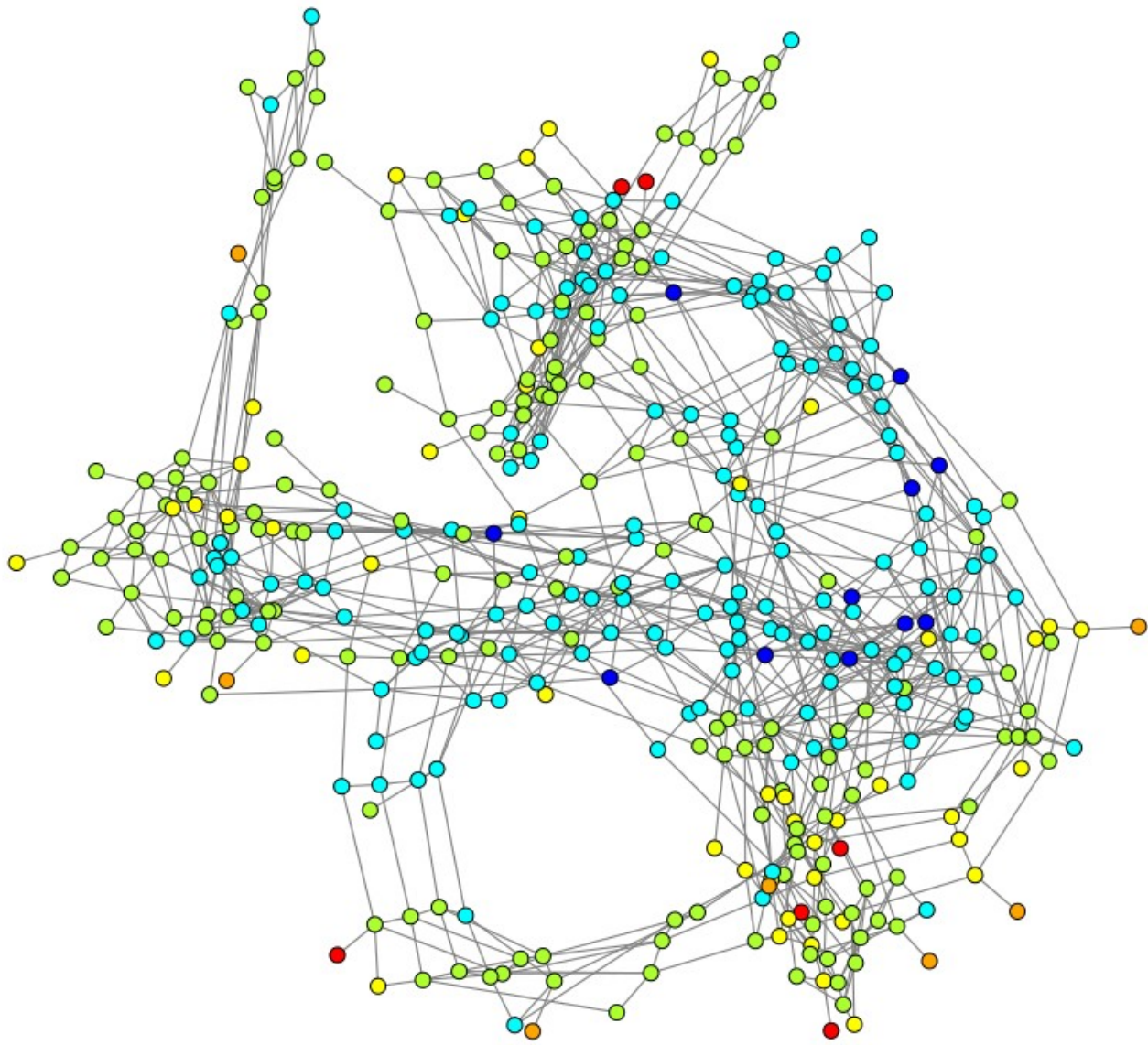


Fig: network graph without link weights

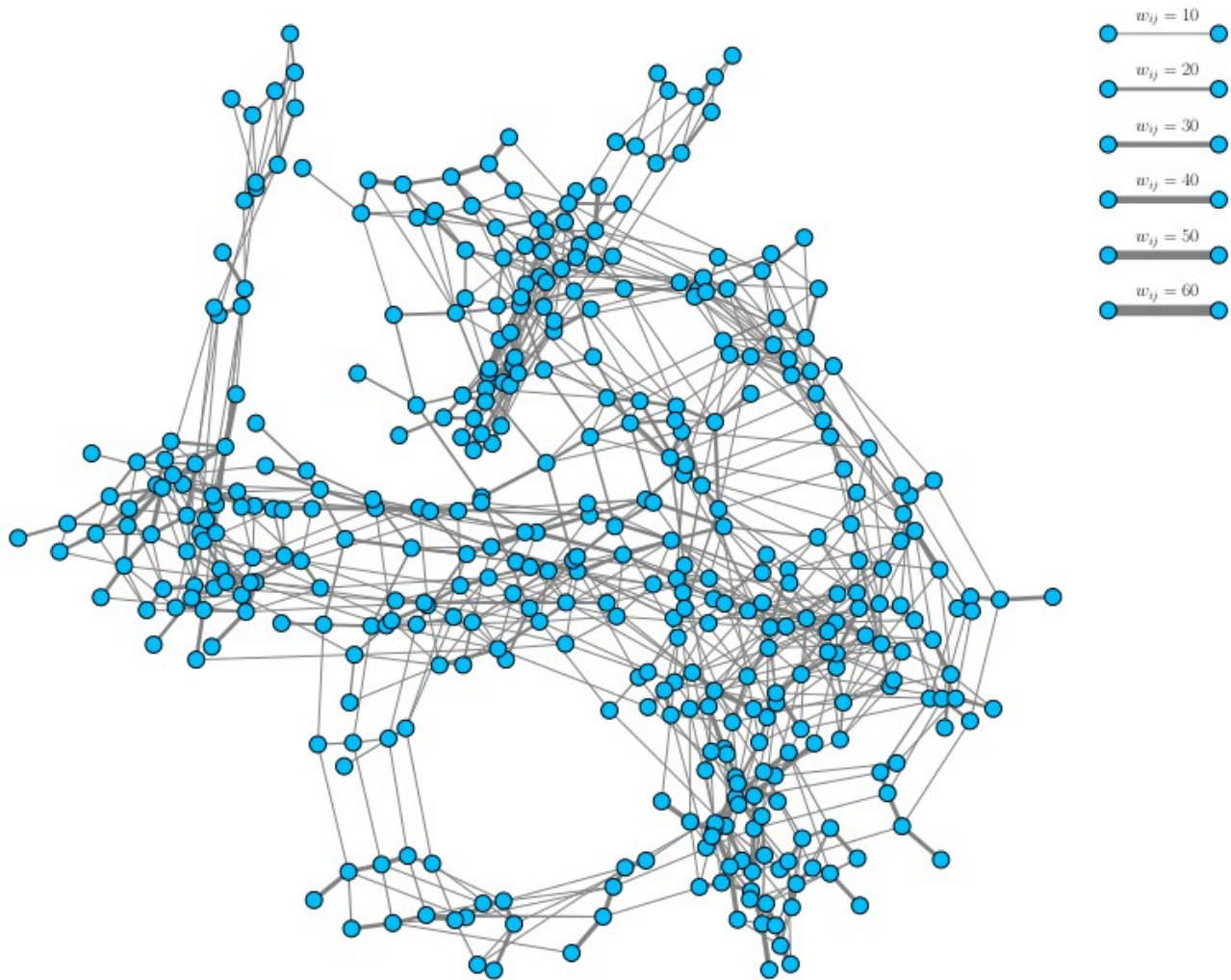


Fig: Network graph with weighted links