UNICAM
Università di Camerino
1336

Università di Camerino

Gut dysbiosis leads to Parkinson Disease through misfolding of α-synuclein

Lorenzo Luzi
Federico Maria Cruciani

Scientific Background

Scientific background

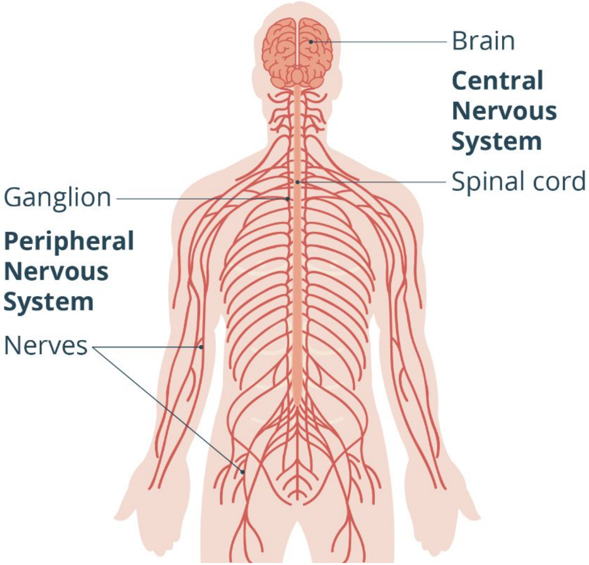
Parkinson’s Disease is a progressive neurological disorder that primarily affects movement.

Some potential causes:

- Neuroinflammation
- Mitochondrial Dysfunction
- α-synuclein misfolding

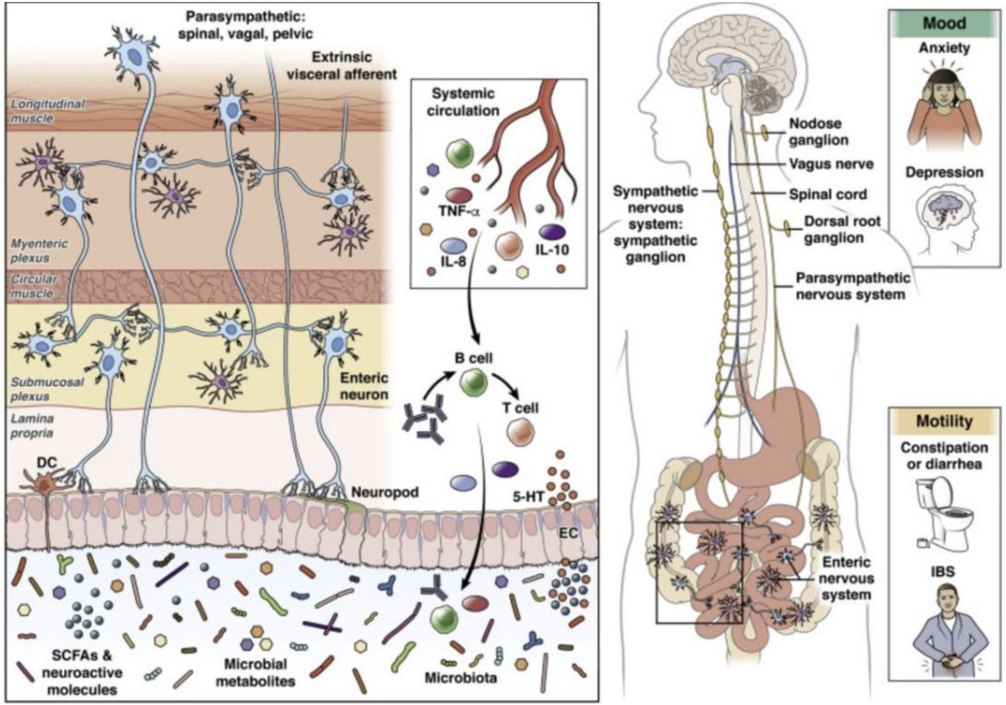
Scientific background

Central Nervous System is the master control of the body. It is the epicenter for processing sensory information, initiating response and orchestrating complex behaviours. It is composed by the brain and spinal cord. Together the brain and the spinal cord form a complex network of neurons that can communicate through electrical or chemical signals.



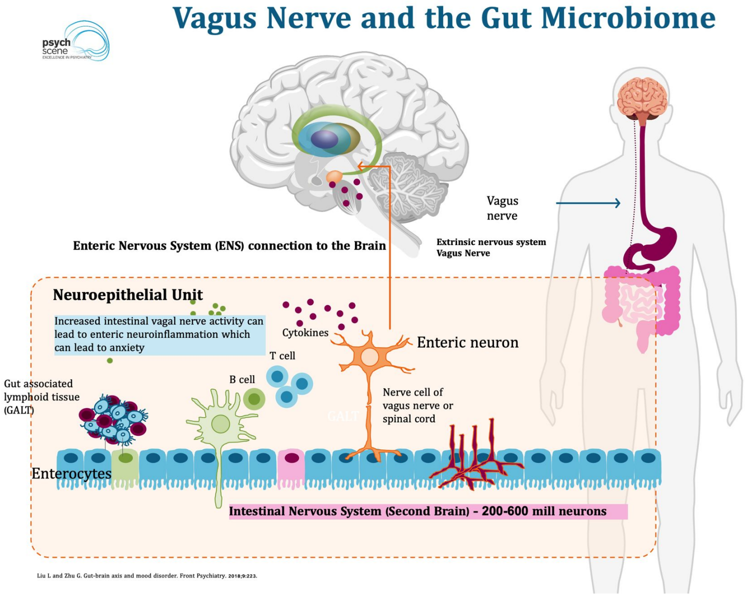
Scientific background

Enteric Nervous System is one of the three branches of the nervous system. It is kind of autonomous with respect to the nervous system. It regulates the motor and secretory functions of the gut.



Scientific Background

Vagus Nerve, also known as the tenth cranial nerve, is a major nerve in the body. Functions of this nerve include heart rate regulation, digestion control by influencing gastric secretion and motility and breathing patterns modulation.



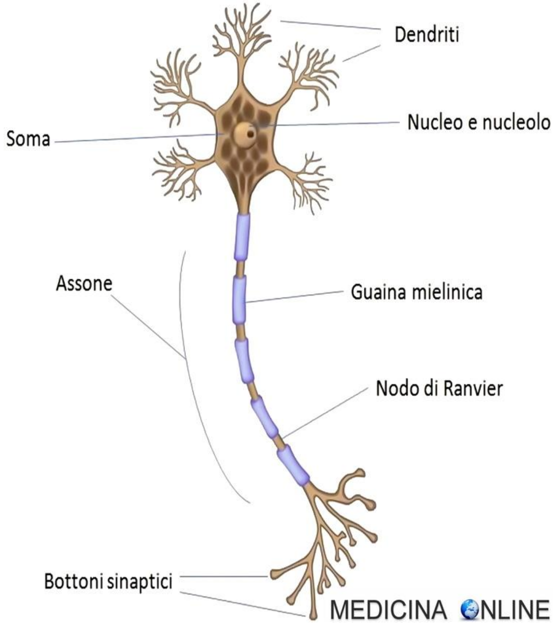
Vagus Nerve and the Gut Microbiome

psych scene
EXCELLENCE IN PSYCHIATRY
Vagus nerve
Enteric Nervous System (ENS) connection to the Brain
Extrinsic nervous system Vagus Nerve
Neuroepithelial Unit
Increased intestinal vagal nerve activity can lead to enteric neuroinflammation which can lead to anxiety
Cytokines
Enteric neuron
T cell
B cell
Gut associated lymphoid tissue (GALT)
GALT
Nerve cell of vagus nerve or spinal cord
Enterocytes
Intestinal Nervous System (Second Brain) - 200-600 mill neurons

Liu L and Zhu G. Gut-brain axis and mood disorder. Front Psychiatry. 2018;9:223.

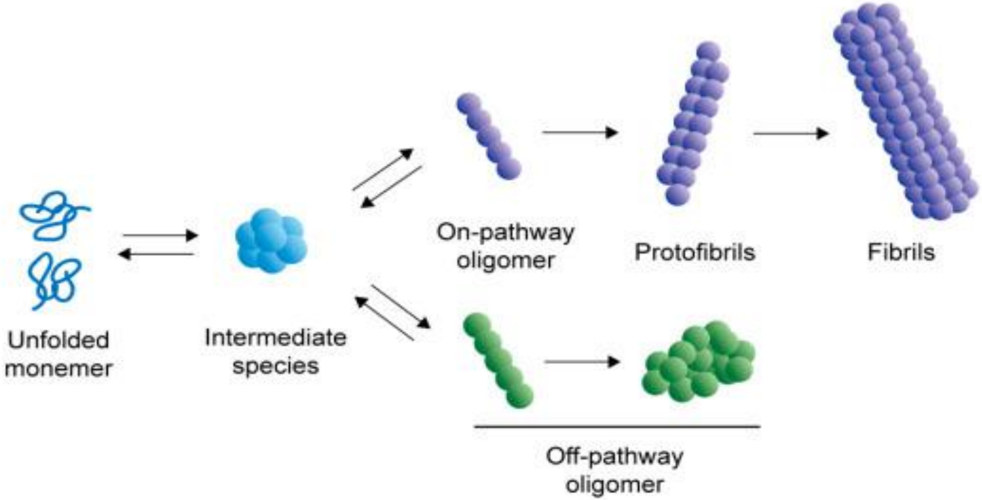
Scientific background

Neurons are the fundamental units of the nervous system. They are responsible for transmitting information throughout the body. Structurally, neurons consist of three main parts: the cell body, dendrites and axon.



Scientific background

α-synuclein is a protein abundant in the human brain, particularly in the neurons. In its normal form is soluble and exists as a monomer. In Parkinson's Disease (PD) it aggregates abnormally, forming insoluble clumps of proteins called Lewy Bodies. These aggregations are believed to contribute to the progressive degeneration of neurons.



Scientific background

Microbiota refers to the entire population of microorganisms that colonize a particular location including not just bacteria but also other microbes such as fungi, archaea, viruses and protozoans.

Gut dysbiosis is a condition of the gut characterised by a decrease in the diversity of the microbiota, a loss of beneficial microbiota and an overgrowth of harmful microorganisms.

Scientific Background

Lipopolysaccharide (LPS) is a molecule found in the outer membrane of **Gram-negative** bacteria. LPS in an endotoxin, it is released when Gram-negative bacteria die and their cell walls break down. The disruption of the gut barrier, which occurs in conditions like intestinal inflammation, increased permeability or dysbiosis, this can result in systemic exposure to LPS and activation of immune response, inflammation, and potential health problems.

Scientific Background

Bifidobacteria are a kind of beneficial bacteria commonly found in the gut microbiota, they have been shown to have several mechanisms through which they can counteract the effects of LPS:

- Reduction of LPS-producing bacteria
- Improvement of gut barrier function
- Modulation of immune response
- Binding and neutralization of LPS

System Description

System Description

The goal of our project is to describe how **the Gut dysbiosis can lead to PD focusing on the misfolding of proteins.**

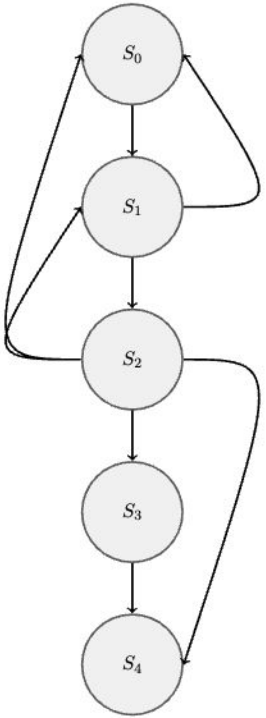
In our system we individuated **two different environments**: the Gut and the CNS.

System Description - CNS

The CNS environment has 5 different states:

- S_0 - Balanced state
- S_1 - State where we can find some aggregation in some neurons
- S_2 - Onset of the disease
- S_3 - Spread of the disease between neuron
- S_4- Death of the system

CNS Automata



The transition between the states depend on the level of misfolding, aggregation and Lewy bodies inside the neurons.

System Description - CNS

Agents

α-synuclein

Reactive agent. Its main action is to bind with a neuron.

Neuron

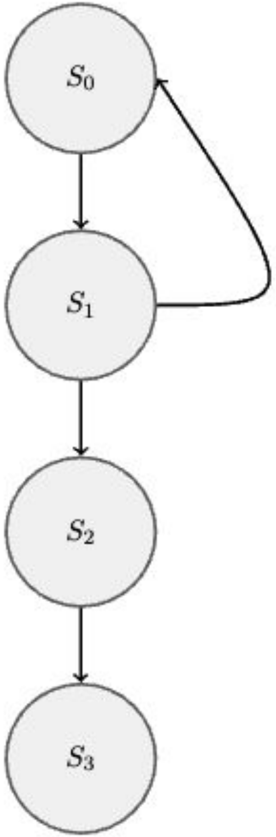
Adaptive agent, it modifies its internal state based on the level of proteins inside itself.

System Description - Gut

The Gut environment has 4 different states:

- S_0 - Balanced state
- S_1 - State onset of the disease
- S_2 - Dysbiosis
- S_3 - Spread of α-synuclein

Gut Automata



The transition between the states depend on the level of dysbiosis of the environment. This level is linked to the level of LPS present in the environment.

System Description - Gut

Agents

Gram-negative cells

Adaptive agents. They perceive a global value of LPS agents and can produce or release LPS agents.

LPS

Reactive agent, it can interact with Bifidobacteria agent. Its main role is to be a global variable for the environment.

Bifidobacteria

Reactive agent. Its main role is to kill the malicious agents like LPS and Gram-negatives.

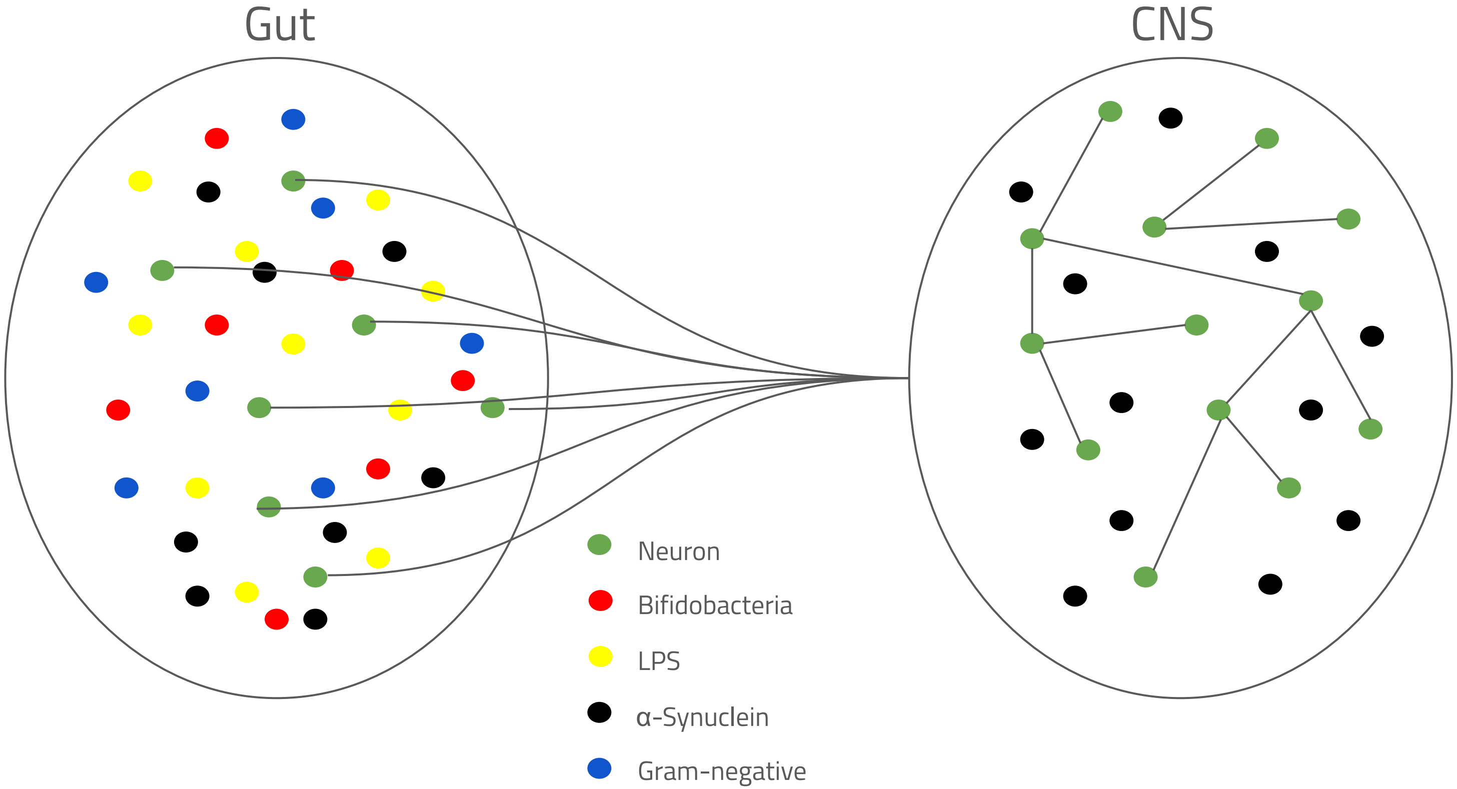
Gut Neuron

Reactive agent. It sends the α-synuclein from the Gut to the CNS.

α-synuclein

Reactive agent. Its main action is to bind with a neuron.

System Description



System Description

Transition function is the function that permits the movement of α-synuclein from the Gut to the CNS.

$$\mathrm{T} = \{\ S_{gut},\ S_{CNS},\ \text{alpha-synuclein, gutneuronagent}\ \}$$

- S_{gut}: is the S_4 state of the Gut so is the spread a–synuclein
- $S_{CNS(i)}$: is a subset of the CNS state composed by S_1, S_2, S_3

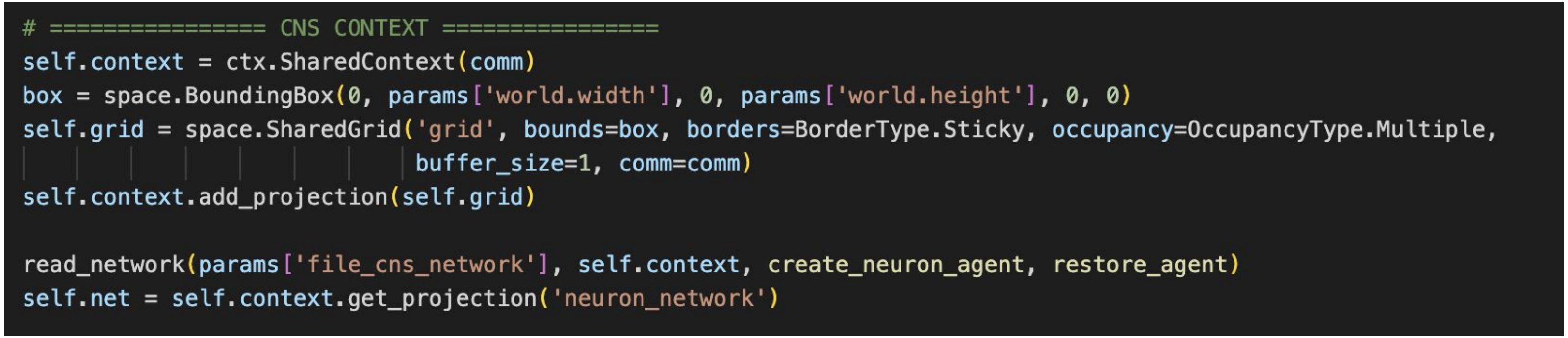
$$T : S_{gut} \times S_{CNS(i)} \times alpha\text{–}synuclein \times gutneuronagent \rightarrow S_{CNS(i)}$$
$$\mathrm{T}\text{: } \mathrm{S}_{gut} \times S_{CNS(i)} \times alpha\text{–}synuclein \times gutneuronagent \rightarrow S_{CNS(i+1)}$$

Model Implementation

Model implementation - CNS

We decided to represent the CNS with two different projections.

A network projection in order to represent the the neuron network and a grid projection in order to give physical space in which the agents can move and interact with each other.

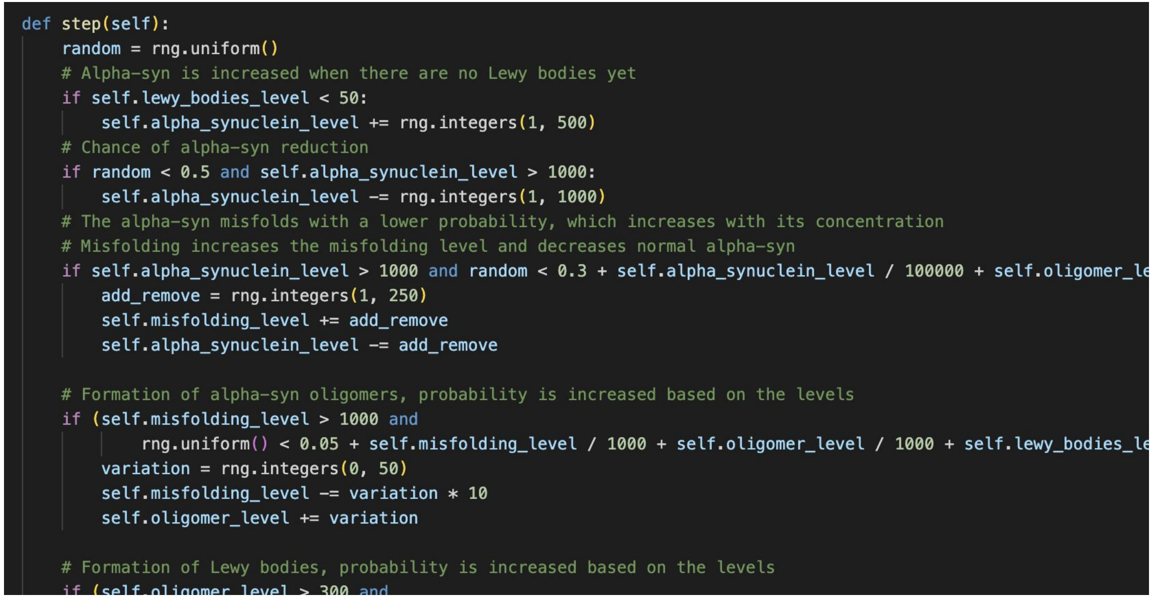


```
# ================ CNS CONTEXT ================
self.context = ctx.SharedContext(comm)
box = space.BoundingBox(0, params['world.width'], 0, params['world.height'], 0, 0)
self.grid = space.SharedGrid('grid', bounds=box, borders=BorderType.Sticky, occupancy=OccupancyType.Multiple,
                             buffer_size=1, comm=comm)
self.context.add_projection(self.grid)

read_network(params['file_cns_network'], self.context, create_neuron_agent, restore_agent)
self.net = self.context.get_projection('neuron_network')
```

Model implementation - CNS

For the **Neuron** we used the rng.uniform() method to generate a random value. This value is used to change the level of proteins inside a neuron.



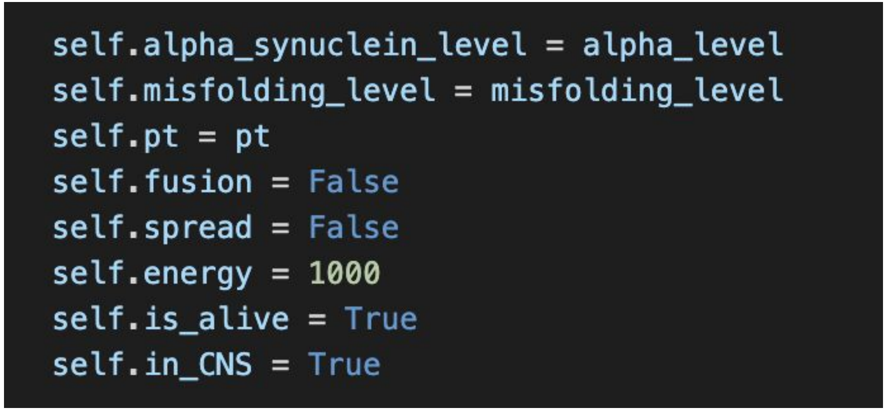
```
def step(self):
    random = rng.uniform()
    # Alpha-syn is increased when there are no Lewy bodies yet
    if self.lewy_bodies_level < 50:
        self.alpha_synuclein_level += rng.integers(1, 500)
    # Chance of alpha-syn reduction
    if random < 0.5 and self.alpha_synuclein_level > 1000:
        self.alpha_synuclein_level -= rng.integers(1, 1000)
    # The alpha-syn misfolds with a lower probability, which increases with its concentration
    # Misfolding increases the misfolding level and decreases normal alpha-syn
    if self.alpha_synuclein_level > 1000 and random < 0.3 + self.alpha_synuclein_level / 100000 + self.oligomer_le
        add_remove = rng.integers(1, 250)
        self.misfolding_level += add_remove
        self.alpha_synuclein_level -= add_remove

    # Formation of alpha-syn oligomers, probability is increased based on the levels
    if (self.misfolding_level > 1000 and
            rng.uniform() < 0.05 + self.misfolding_level / 1000 + self.oligomer_level / 1000 + self.lewy_bodies_le
        variation = rng.integers(0, 50)
        self.misfolding_level -= variation * 10
        self.oligomer_level += variation

    # Formation of Lewy bodies, probability is increased based on the levels
    if (self.oligomer_level > 300 and
```

Model implementation - CNS

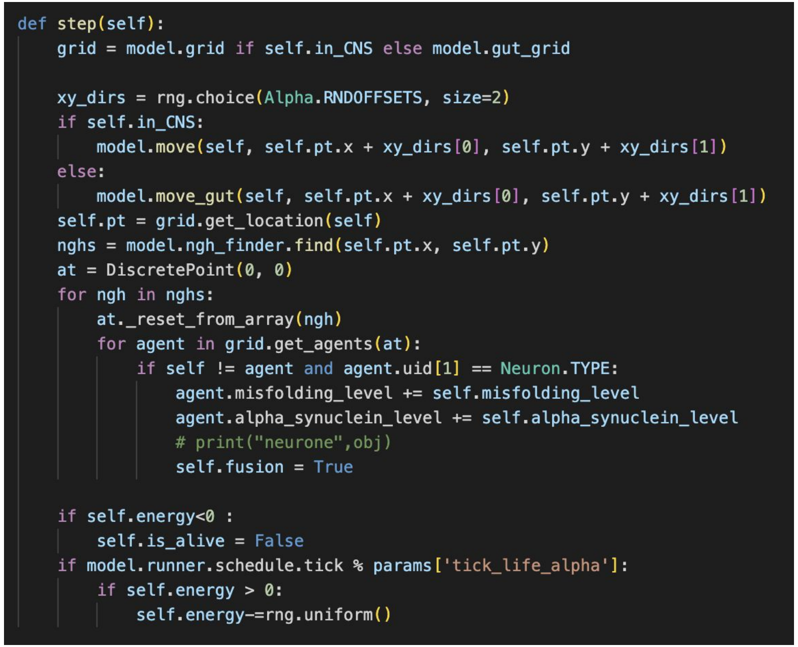
The **α-synuclein (Alpha)** has fields to represent the level of proteins inside it and its position. It also has flags to represent if the agent is alive, if it is in the CNS or in the Gut and if it has binded with a gut neuron.



```
self.alpha_synuclein_level = alpha_level
self.misfolding_level = misfolding_level
self.pt = pt
self.fusion = False
self.spread = False
self.energy = 1000
self.is_alive = True
self.in_CNS = True
```

Model implementation - CNS

The **α-synuclein step method** chooses a value between 1/-1 and adds it to its coordinates in order to move inside the grid. For an arbitrary amount of ticks its energy can decrease. Also if during its movement it meets a gut neuron it modifies its flag to move from gut to CNS.



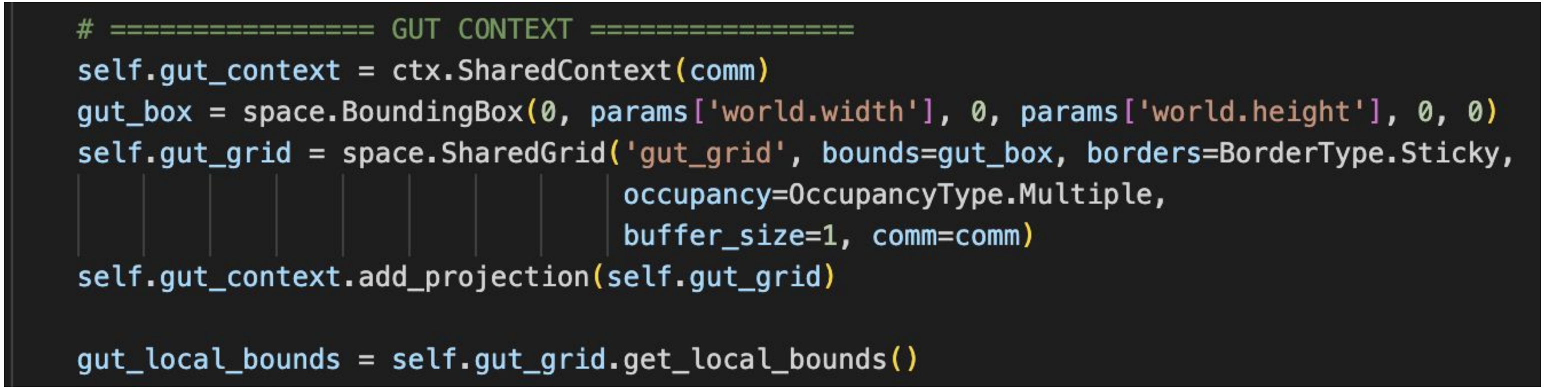
```
def step(self):
    grid = model.grid if self.in_CNS else model.gut_grid

    xy_dirs = rng.choice(Alpha.RNDOFFSETS, size=2)
    if self.in_CNS:
        model.move(self, self.pt.x + xy_dirs[0], self.pt.y + xy_dirs[1])
    else:
        model.move_gut(self, self.pt.x + xy_dirs[0], self.pt.y + xy_dirs[1])
    self.pt = grid.get_location(self)
    nghs = model.ngh_finder.find(self.pt.x, self.pt.y)
    at = DiscretePoint(0, 0)
    for ngh in nghs:
        at._reset_from_array(ngh)
        for agent in grid.get_agents(at):
            if self != agent and agent.uid[1] == Neuron.TYPE:
                agent.misfolding_level += self.misfolding_level
                agent.alpha_synuclein_level += self.alpha_synuclein_level
                # print("neurone",obj)
                self.fusion = True

    if self.energy<0 :
        self.is_alive = False
    if model.runner.schedule.tick % params['tick_life_alpha']:
        if self.energy > 0:
            self.energy-=rng.uniform()
```

Model implementation - Gut

In order to represent the Gut we use only a grid since we want to represent the physical interaction between agents.

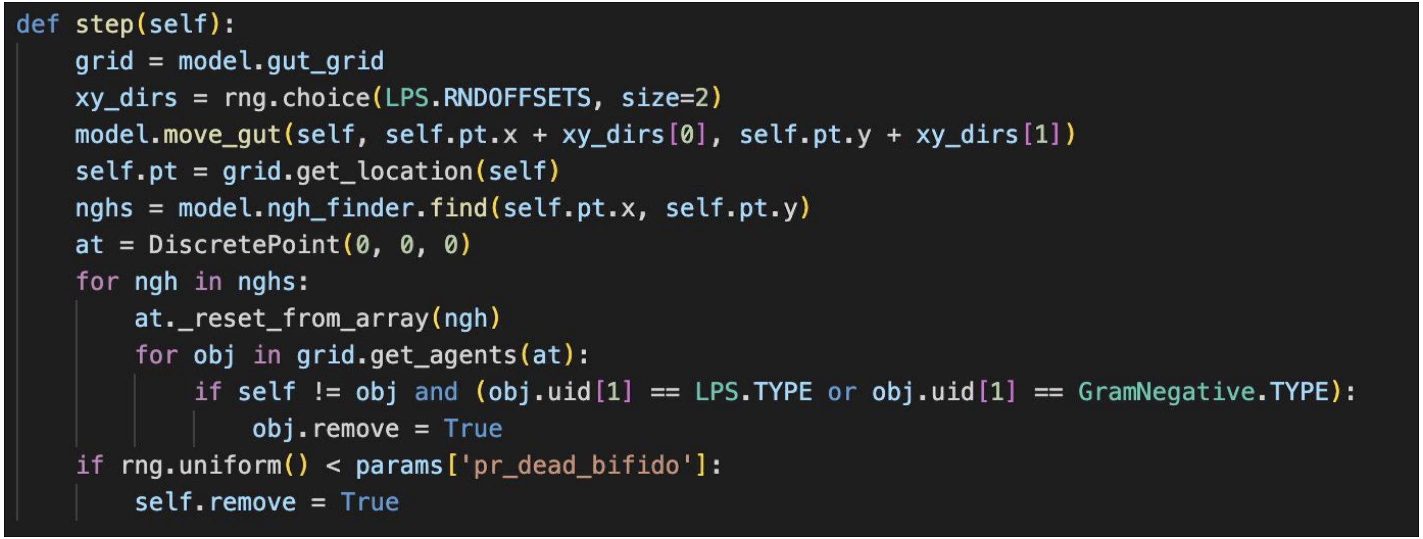


```
# ================ GUT CONTEXT ================
self.gut_context = ctx.SharedContext(comm)
gut_box = space.BoundingBox(0, params['world.width'], 0, params['world.height'], 0, 0)
self.gut_grid = space.SharedGrid('gut_grid', bounds=gut_box, borders=BorderType.Sticky,
                                 occupancy=OccupancyType.Multiple,
                                 buffer_size=1, comm=comm)
self.gut_context.add_projection(self.gut_grid)

gut_local_bounds = self.gut_grid.get_local_bounds()
```

Model implementation - Gut

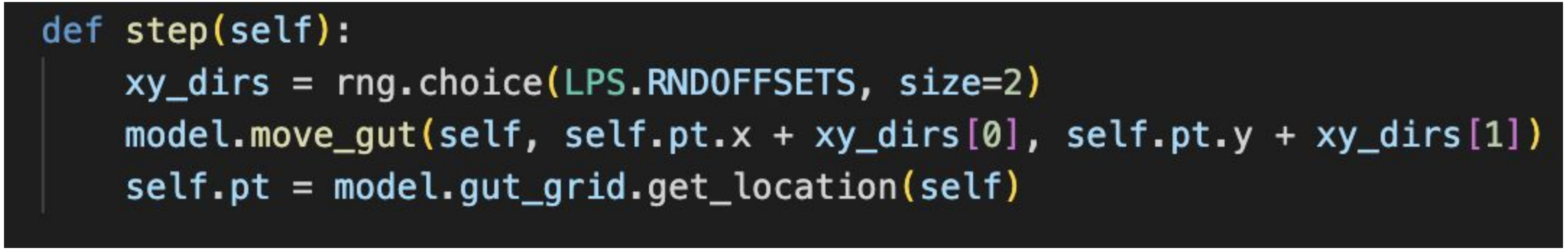
The **Bifidobacteria** can move randomically inside the Gut. When it finds an LPS or Gram-negative it changes its flag so in the next tick the model can delete the agent.



```python
def step(self):
    grid = model.gut_grid
    xy_dirs = rng.choice(LPS.RNDOFFSETS, size=2)
    model.move_gut(self, self.pt.x + xy_dirs[0], self.pt.y + xy_dirs[1])
    self.pt = grid.get_location(self)
    nghs = model.ngh_finder.find(self.pt.x, self.pt.y)
    at = DiscretePoint(0, 0, 0)
    for ngh in nghs:
        at._reset_from_array(ngh)
        for obj in grid.get_agents(at):
            if self != obj and (obj.uid[1] == LPS.TYPE or obj.uid[1] == GramNegative.TYPE):
                obj.remove = True
    if rng.uniform() < params['pr_dead_bifido']:
        self.remove = True
```

Model Implementation - Gut

The **LPS** agent just moves randomly inside the gut.

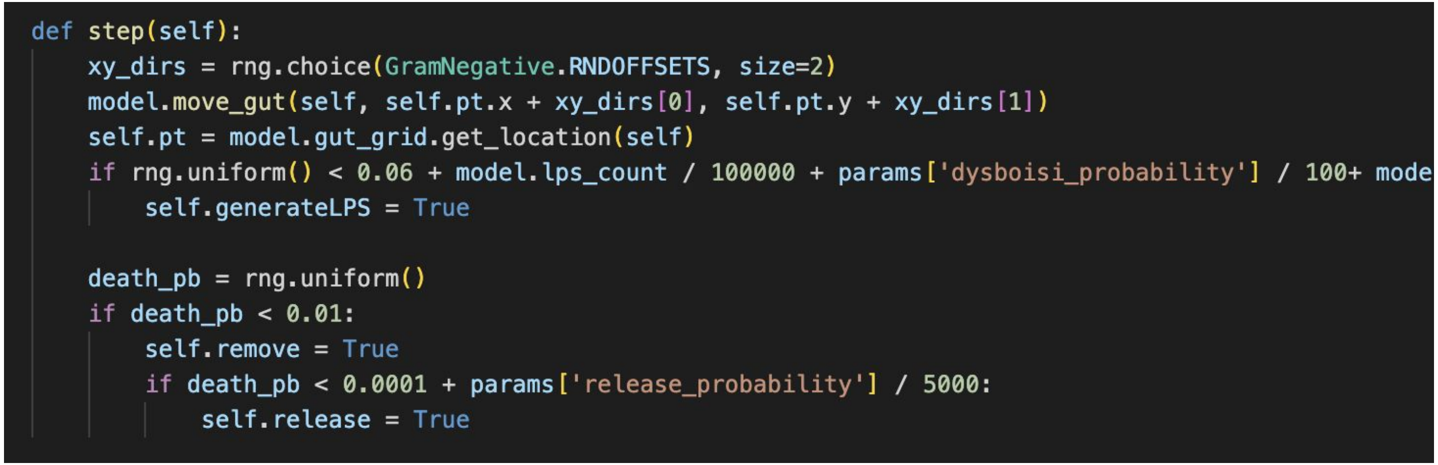


```
def step(self):
    xy_dirs = rng.choice(LPS.RNDOFFSETS, size=2)
    model.move_gut(self, self.pt.x + xy_dirs[0], self.pt.y + xy_dirs[1])
    self.pt = model.gut_grid.get_location(self)
```

Model Implementation - Gut

The **Gram-negative** can generate LPS agents based on a random threshold.

We also used another threshold in order to remove the agent from the context. If this value is enough small before removing the agent it can release a fixed amount of LPS.

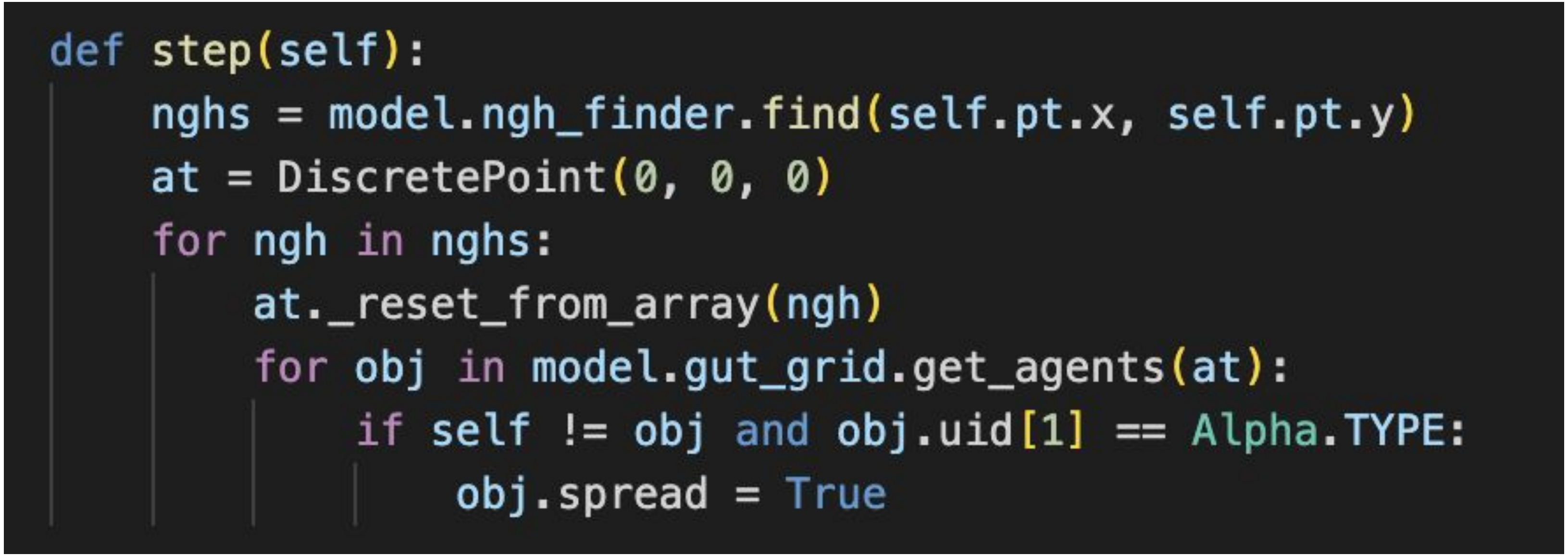


```
def step(self):
    xy_dirs = rng.choice(GramNegative.RNDOFFSETS, size=2)
    model.move_gut(self, self.pt.x + xy_dirs[0], self.pt.y + xy_dirs[1])
    self.pt = model.gut_grid.get_location(self)
    if rng.uniform() < 0.06 + model.lps_count / 100000 + params['dysboisi_probability'] / 100+ mode
        self.generateLPS = True

    death_pb = rng.uniform()
    if death_pb < 0.01:
        self.remove = True
        if death_pb < 0.0001 + params['release_probability'] / 5000:
            self.release = True
```

Model Implementation - Gut

The **Gut Neuron**’s main function is to check if there is an α-synuclein agent around it. If it founds one it changes the corresponding flag inside the agent.

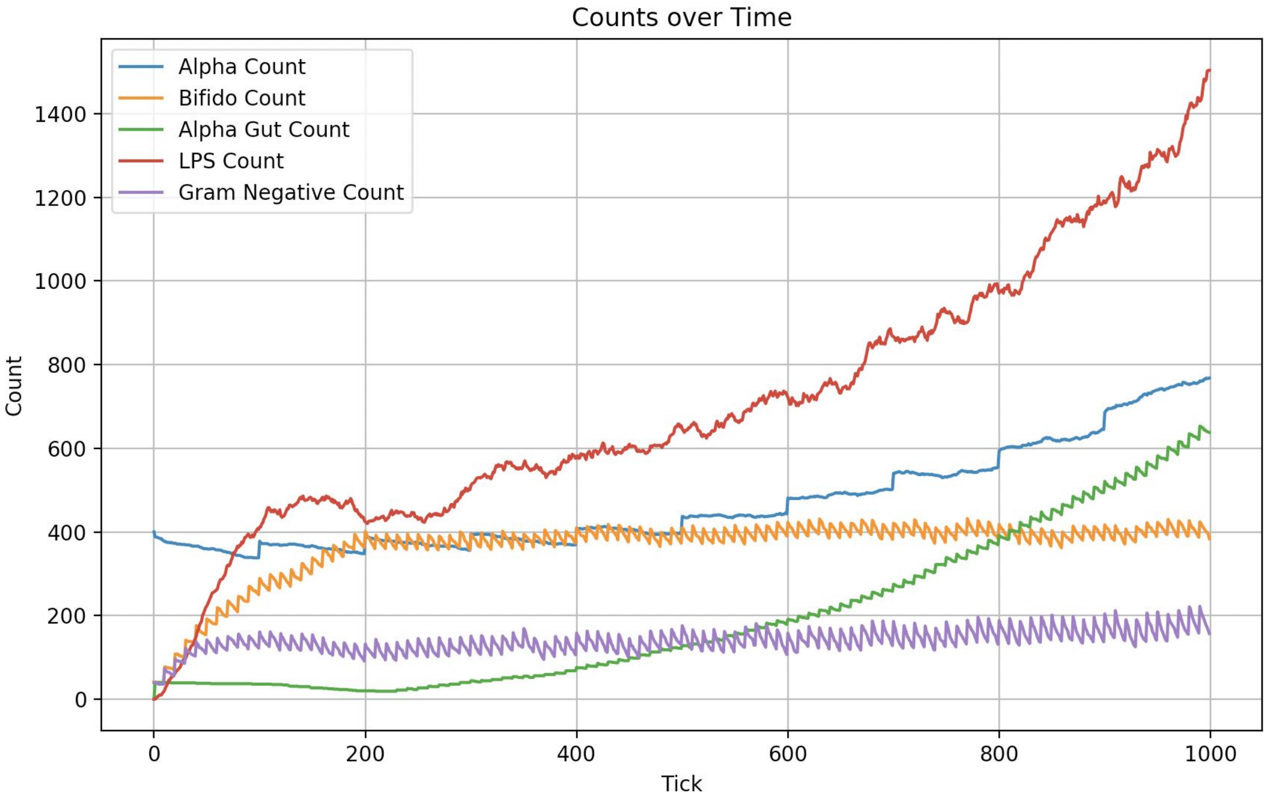


```
def step(self):
    nghs = model.ngh_finder.find(self.pt.x, self.pt.y)
    at = DiscretePoint(0, 0, 0)
    for ngh in nghs:
        at._reset_from_array(ngh)
        for obj in model.gut_grid.get_agents(at):
            if self != obj and obj.uid[1] == Alpha.TYPE:
                obj.spread = True
```

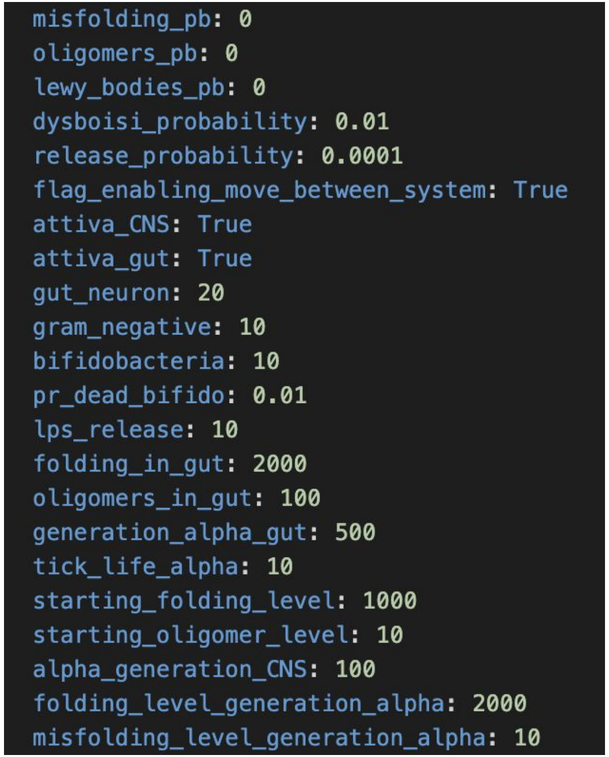
Model implementation - choices

In our implementation we decided to **not represent the vagus nerve,** since the goal of our project is to represent how gut dysbiosis can lead to PD. We are not interested in representing the physical movement of the protein from the Gut to the CNS so we simplified it in order to save computational cost.

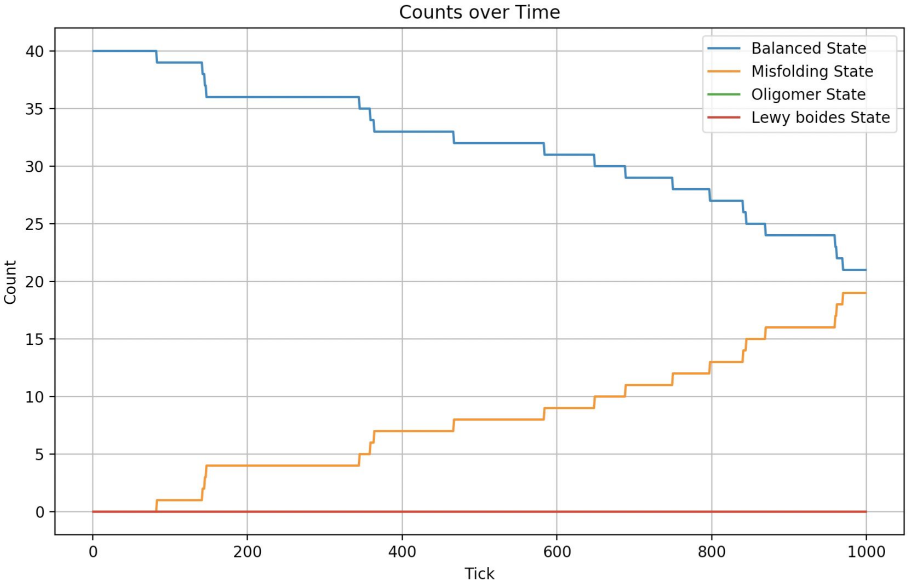
Simulation - normal condition



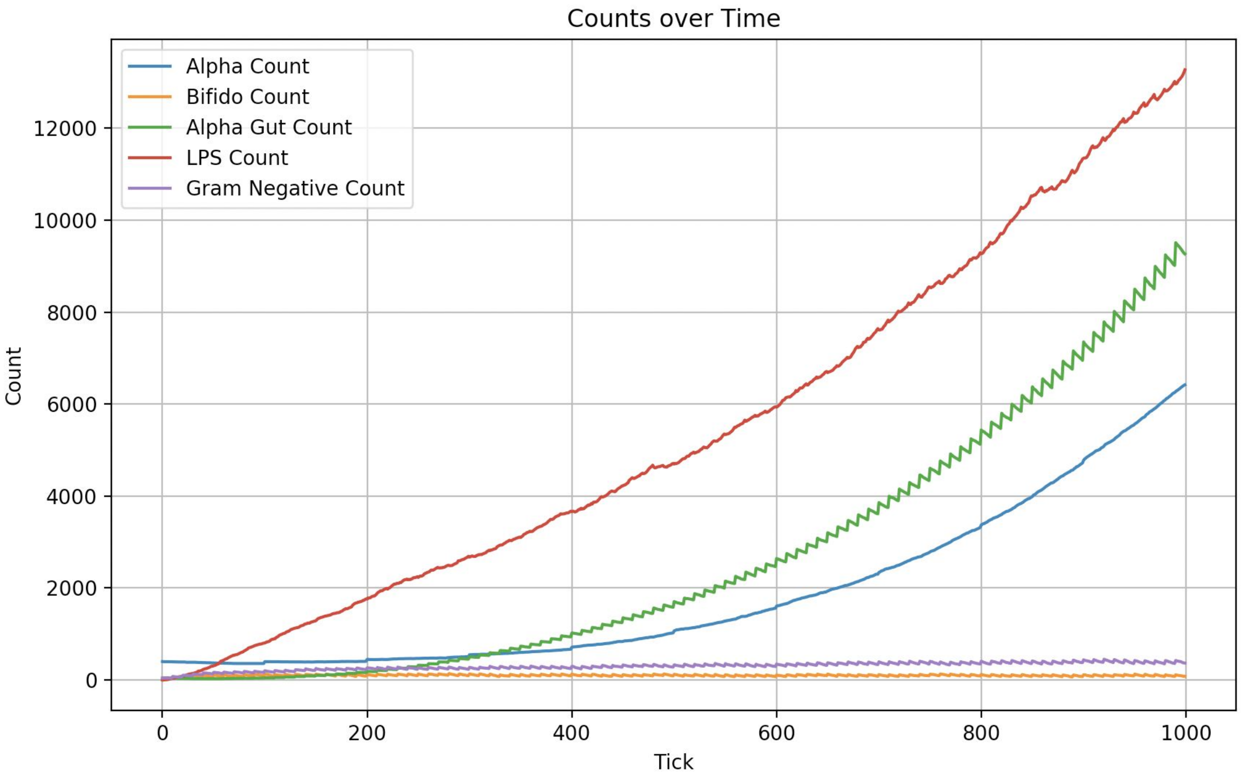
Simulation - normal condition



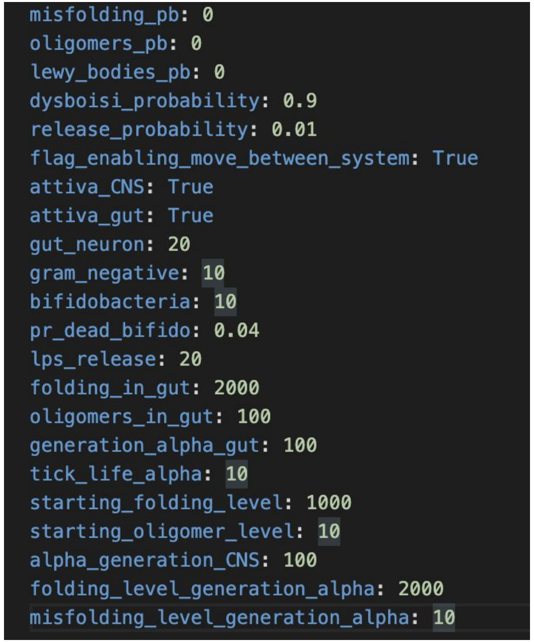
```
misfolding_pb: 0
oligomers_pb: 0
lewy_bodies_pb: 0
dysboisi_probability: 0.01
release_probability: 0.0001
flag_enabling_move_between_system: True
attiva_CNS: True
attiva_gut: True
gut_neuron: 20
gram_negative: 10
bifidobacteria: 10
pr_dead_bifido: 0.01
lps_release: 10
folding_in_gut: 2000
oligomers_in_gut: 100
generation_alpha_gut: 500
tick_life_alpha: 10
starting_folding_level: 1000
starting_oligomer_level: 10
alpha_generation_CNS: 100
folding_level_generation_alpha: 2000
misfolding_level_generation_alpha: 10
```



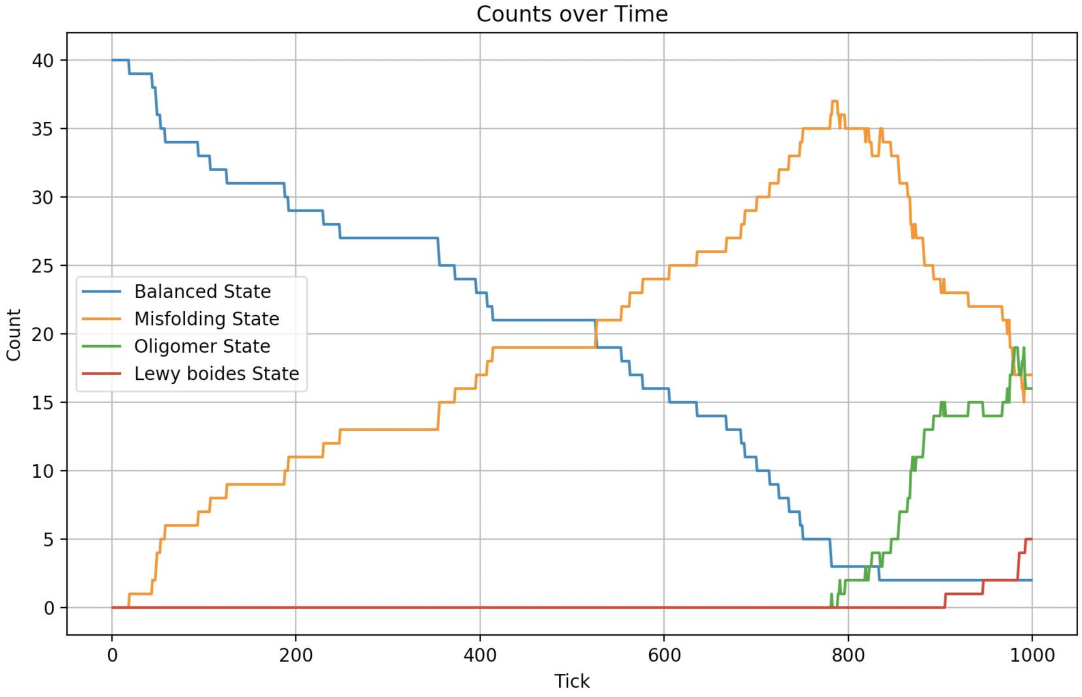
Simulation - weak gut



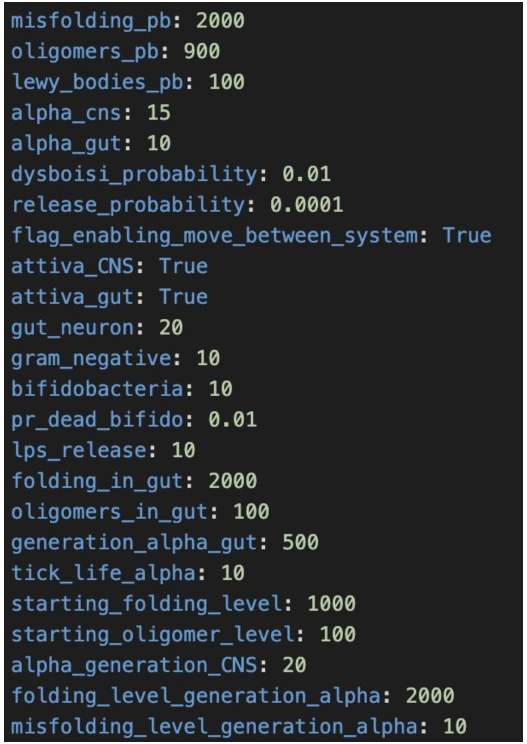
Simulation - weak gut



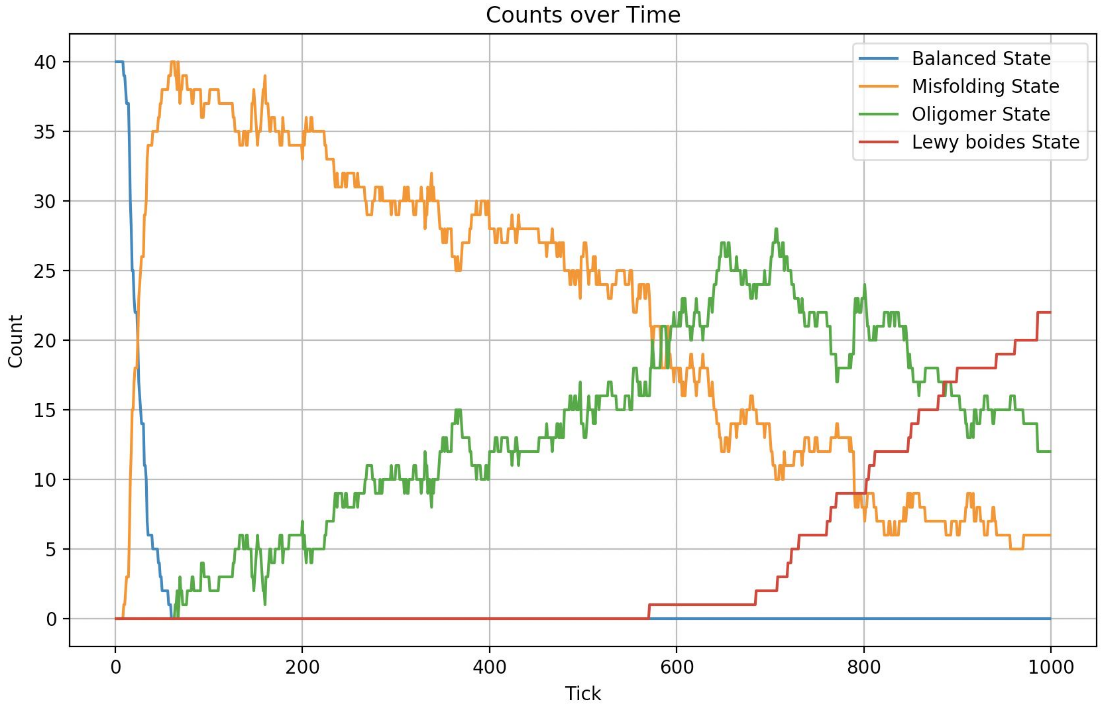
```
misfolding_pb: 0
oligomers_pb: 0
lewy_bodies_pb: 0
dysboisi_probability: 0.9
release_probability: 0.01
flag_enabling_move_between_system: True
attiva_CNS: True
attiva_gut: True
gut_neuron: 20
gram_negative: 10
bifidobacteria: 10
pr_dead_bifido: 0.04
lps_release: 20
folding_in_gut: 2000
oligomers_in_gut: 100
generation_alpha_gut: 100
tick_life_alpha: 10
starting_folding_level: 1000
starting_oligomer_level: 10
alpha_generation_CNS: 100
folding_level_generation_alpha: 2000
misfolding_level_generation_alpha: 10
```



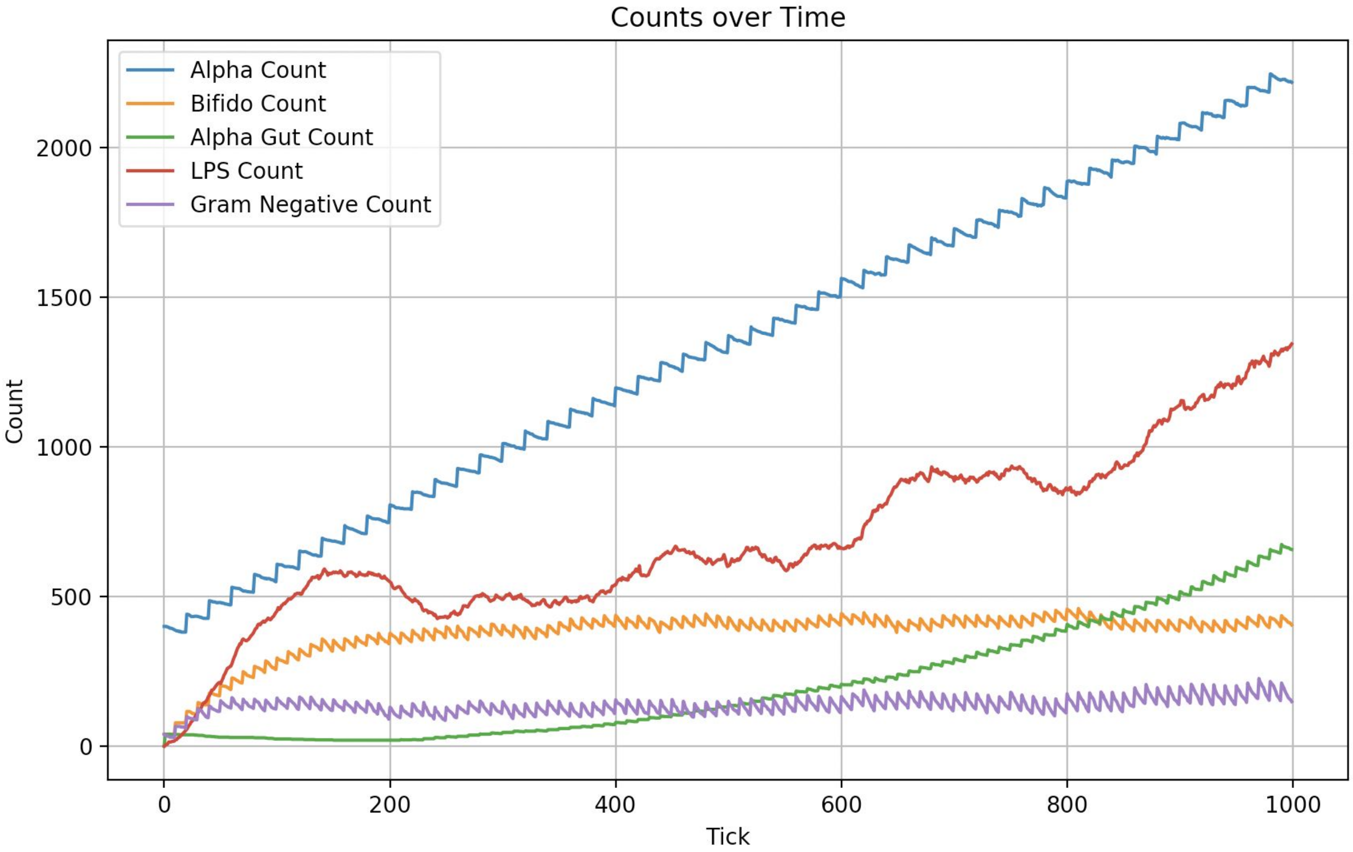
Simulation - onset of the disease in CNS



```
misfolding_pb: 2000
oligomers_pb: 900
lewy_bodies_pb: 100
alpha_cns: 15
alpha_gut: 10
dysboisi_probability: 0.01
release_probability: 0.0001
flag_enabling_move_between_system: True
attiva_CNS: True
attiva_gut: True
gut_neuron: 20
gram_negative: 10
bifidobacteria: 10
pr_dead_bifido: 0.01
lps_release: 10
folding_in_gut: 2000
oligomers_in_gut: 100
generation_alpha_gut: 500
tick_life_alpha: 10
starting_folding_level: 1000
starting_oligomer_level: 100
alpha_generation_CNS: 20
folding_level_generation_alpha: 2000
misfolding_level_generation_alpha: 10
```



Simulation - onset of the disease in CNS


Counts over Time
Alpha Count
Bifido Count
Alpha Gut Count
LPS Count
Gram Negative Count
Count
Tick