Ubiquitination mathematical modelling.

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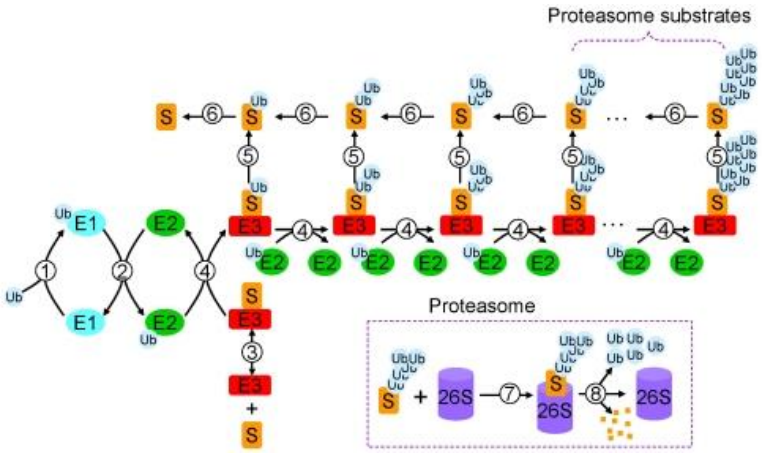
Introduction:

The ubiquitin–proteasome system (UPS) is the major intracellular protein quality control system in eukaryotic cells [1]. We depend on UPS pathway for the degradation of our target protein; microtubule associated protein tau (MAPT) abbreviated as tau which is the substrate in our model. This model simulates the degradation process of UPS including all previously demonstrated steps using ODE model. The model was built based on the previous work of (Xu & Qu, 2012).



Aim and outline:

The model is built to determine the time and the initial concentration of involved enzymes needed for complete degradation of the substrate with known initial concentration or determining the amount of degraded substrate given a specific concentration of ubiquitin, E1, E2, E3 and 26S proteasome.

Assumptions and parameters:

The model assumes that there is no deubiquitinase (dub) enzyme which removes ubiquitin from substrate. Therefore, the ubiquitins bonded to the substrate in any number (ub_nS) will not be dissociated. This assumption is based on the wet lab assay that does not utilize dub in the process. The model also assumes that our E3 ligase, which is truncated, tTrim21 will behave as normal E3 from the paper, since we cannot identify its effect until the wet lab experiment. The model also neglects the protein half-life, assuming that proteins are persistent in the reaction medium. Finally, the model assume that ATP concentration is in excess in the medium and will not interfere with the rate of the reactions or the concentrations of the intermediates.

Parameter	**Symbol**	**Value**	**Symbol in the code**	**Unit**
Rate of association of E1 with ub	**k_1**	**10^{-5}**	**k1f**	**$nM^{-1}.s^{-1}$**
Rate of dissociation of E1 with ub	**k_{-1}**	**0.55**	**k1b**	**s^{-1}**
Rate of association of E2 with ub	**k_2**	**10^{-5}**	**k2f**	**$nM^{-1}.s^{-1}$**
Rate of dissociation of E2 with ub	**k_{-2}**	**$19x10^{-5}$**	**k2b**	**$nM^{-1}.s^{-1}$**
Rate of association of E3 with S	**k_3**	**10^{-3}**	**k3f**	**$nM^{-1}.s^{-1}$**
Rate of dissociation of E3 with S	**k_{-3}**	**0.37**	**k3b**	**s^{-1}**
Rate of association of ub with S	**$k_{4.1-8}$**	**0.00034, 0.0078, 0.002, 0.0011, 0.00062, 0.00082, 0.0008, 0.0005**	**k4**	**$nM^{-1}.s^{-1}$**
Rate of dissociation of ubiquitinated substrate ub_n from E3	**$k_{5.1-8}$**	**0.4, 0.29, 0.27, 0.29, 0.89, 0.8, 0.5, 0.2**	**k5**	**s^{-1}**
Rate of association of ubiquitinated substrate with 26S proteasome	**$k_{6.4-8}$**	**0.01, 0.02, 0.04, 0.06, 0.08**	**k6**	**$nM^{-1}.s^{-1}$**
Rate of degradation of ubiquitinated substrate by 26S proteasome	**$k_{7.4-8}$**	**0.1, 0.2, 0.4, 0.6, 0.8**	**k7**	**$nM^{-1}.s^{-1}$**

All parameters obtained from (Xu & Qu, 2012).

Model explanation:

The first step in the model is the activation of the ubiquitin (ub) by ubiquitin activating enzyme (E1) which is an ATP dependent step for the formation thioester bond between the cysteine in the active site of E1 and the carboxyl terminus of the ubiquitin.

$$E_1 + ub \underset{k_{-1}}{\overset{k_1}{\longleftrightarrow}} E_1\, ub$$

The next step is the transfer of ub from E1 to ubiquitin conjugating enzyme (E2) which is also an ATP dependent step.

$$E_2 + E_1 ub \underset{k_{-2}}{\overset{k_2}{\longleftrightarrow}} E_2\, ub + E_1$$

In parallel the ubiquitin ligase enzyme (E3) binds to the substrate molecule, which is going to be tagged and degraded, in a reversible binding.

$$E_3 + S \underset{k_{-3}}{\overset{k_3}{\longleftrightarrow}} E_3\, S$$

Afterwards the E2-ub complex binds to the E3 enzyme carrying substrate, which is either free, monoubiquitinated or polyubiquitinated.

$$E_2 ub + E_3 ub_n S \xrightarrow{k_{4.n+1}} E_3\, ub_{n+1} S + E_2 \qquad where\ n \in [0:7]$$

Then the ubiquitinated substrate may be separated from the E3 at any instant, the rate of separation $k_{5.n}$ increases when ubiquitin chain (n) increases.

$$E_3 ub_n S \xrightarrow{k_{5.n}} E_3 + ub_n S \qquad where\ n \in [1:8]$$

After separation from E3 the ubiquitinated substrate that carries at least 4 ubiquitins, which is the minimum number needed for degradation by 26S proteasome (26S), binds to the 26S proteasome and become one step away from degradation.

$$ub_n S + 26S_p \xrightarrow{k_{6.n}} ub_n S26S_p \qquad where\ n \in [4:8]$$

After binding, 26S proteasome degrades the substrate releasing the ubiquitins (n.ub) and peptides byproducts (P) of the substrate.

$$ub_n S26S_p \xrightarrow{k_{7.n}} n.ub + 26S_p + P \qquad where\ n \in [4:8]$$

From the above equations we can conclude the rate laws of each reaction as the following:

$R_1 = k_1.[E_1].[ub]$

$R_{-1} = k_{-1}.[E_1 ub]$

$R_2 = k_2.[E_2].[E_1 ub]$

$R_{-2} = k_{-2}.[E_1].[E_2 ub]$

$R_3 = k_3.[E_3].[S]$

$R_{-3} = k_{-3}.[E_3 S]$

$R_{4.n+1} = k_{4.n+1}.[E_2 ub].[E_3 ub_n S] \qquad where\ n\ \in [0:7]$

$R_{5.n} = k_{5.n}.[E_3 ub_n S] \quad where\ n\ \in [1:8]$

$R_{6.n} = k_{6.n}.[ub_n S].[26S_p] \quad where\ n\ \in [4:8]$

$R_{7.n} = k_{7.n}.[ub_n S26S_p] \quad where\ n\ \in [4:8]$

From the rate laws we can write the ODE equations for each compound as follows:

$$\frac{dub}{dt} = R_{-1} - R_1 + \sum_{n=4}^{8} n.R_{7.n}$$

$$\frac{dE_1}{dt} = R_{-1} - R_1 + R_2 - R_{-2}$$

$$\frac{dE_2}{dt} = R2b - R2f + \sum_{n=1}^{8} R_{4.n}$$

$$\frac{dE_3}{dt} = R_{-1} - R_1 + R_2 - R_{-2}$$

$$\frac{d26S_p}{dt} = -\sum_{n=4}^{8} R_{7.n} + \sum_{n=4}^{8} R_{8.n}$$

$$\frac{dS}{dt} = -R_3 + R_{-3}$$

$$\frac{dubE_1}{dt} = -\frac{dE_1}{dt}$$

$$\frac{dubE_2}{dt} = -\frac{dE_2}{dt}$$

$$\frac{dSE_3}{dt} = R_3 - R_{-3} - R_{4.1}$$

$$\frac{dub_nSE_3}{dt} = R_{4.n} - R_{4.n+1} + R_{5.n} \quad where\ n \in [1:7]$$

$$\frac{dub_8SE_3}{dt} = R_{4.8} - R_{5\_8}$$

$$\frac{dub_nS}{dt} = R_{5.n} \quad where\ n \in [1:3]$$

$$\frac{dub_nS}{dt} = R_{5.n} - R_{7.n} \quad where\ n \in [4:8]$$

$$\frac{dub_nS26S_p}{dt} = R_{7.n} - R_{8.n} \quad where\ n \in [4:8]$$

Results and discussion:

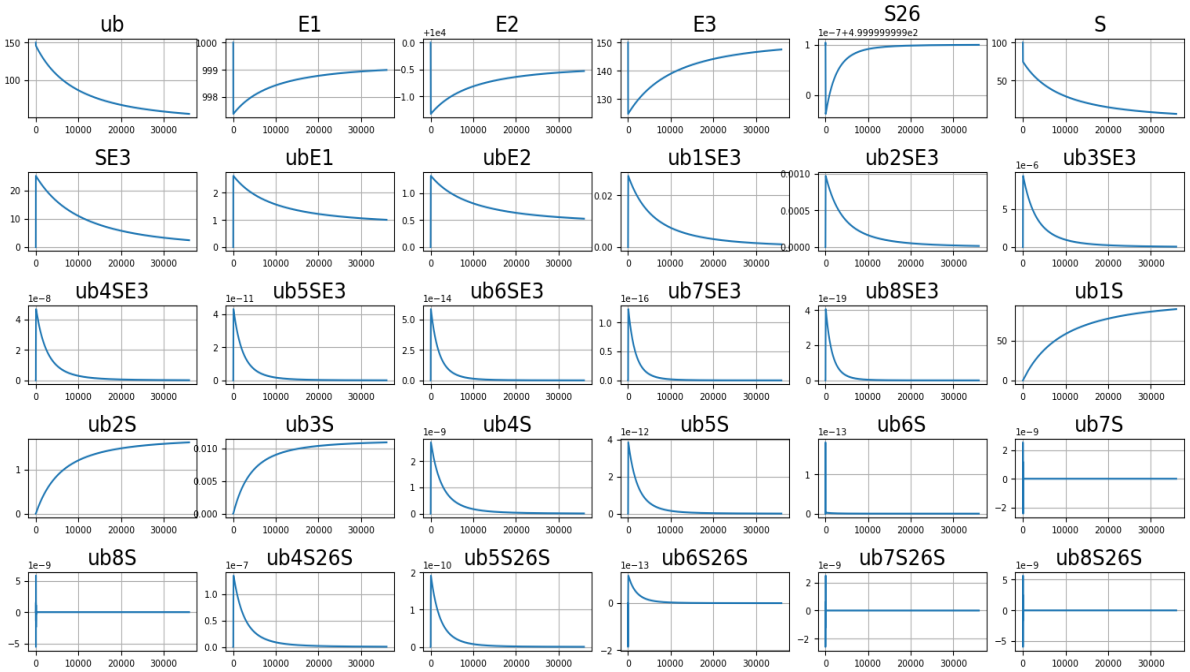


Figure 1: graphical representations of the variation of concentration, in nanomolar. of all compounds and enzymes, free or in complex, involved in the UPS system with time of 10 hours (36000 sec).

The model works efficiently in simulating the whole process of UPS, the change in the concentration of every compound with time is almost as expected. However, ub_8S26S, ub_7S26S, ub_8S and ub_7S complexes graphs show anomalous behaviour as the concentration becomes of negative value, this may be due to the high rate of degradation in reactions 7.7 and 7.8 and high rate of binding in reactions 6.7 and 6.8 where at the point of highest concentration the calculated decrease in concentration becomes higher than the concentration itself, we hope to optimize this problem in the future versions of the model.

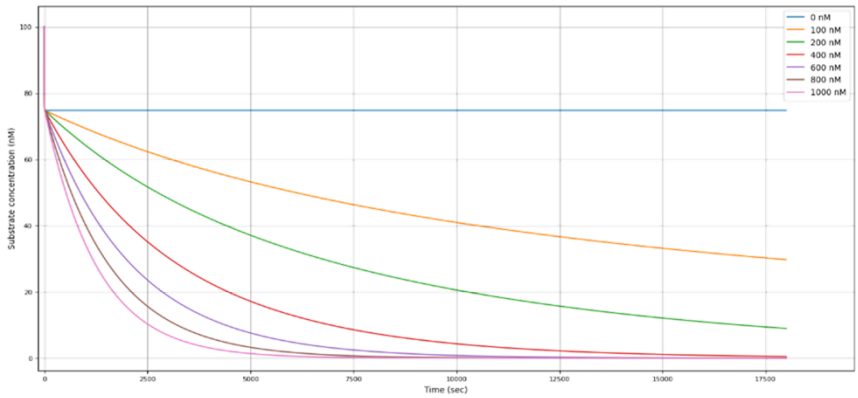


Figure 2: Graphical representation shows the effect of different initial ubiquitin concentrations on the change of the substrate concentration

We wanted to see how different concentrations of ubiquitin will affect the change in concentration of the substrate with time. It seems that as the concentration of initial ubiquitin increases the rate of degradation of the substrate increases.

Even though there is no initial concentration of ubiquitin in the blue curve the substrate concentration shows the same initial decline as other curves, so we conclude that this decline is not due to the ubiquitination, but due to the formation of E_3S complex, the graph showing the change in concentration of substrate and E_3S complex at zero initial concentration of ubiquitin demonstrate this.

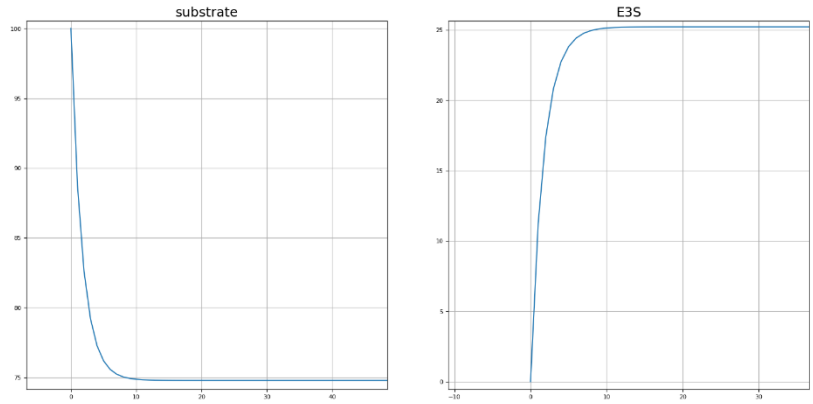


Figure 3: Graphical representation shows the change in substrate concentration with time when the initial ubiquitin concentration is set to zero, concentration is plotted on the y-axis in nM and time is plotted on the x-axis in sec.

Another observation is that ub_1S, ub_2S and ub_3S are formed and remained since in the original model of (Xu & Qu, 2012). These complexes are consumed due to the effect of deubiquitinase enzyme while it is absent in our experiment which means that they will not be consumed or go through further processing in this model.

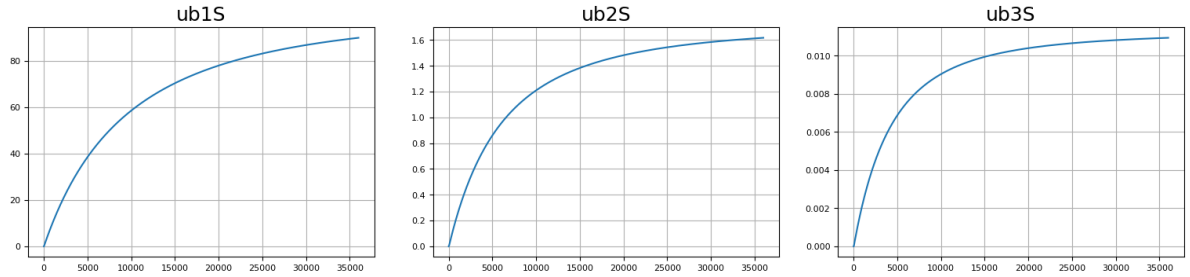


Figure 4: graphical representation shows the change in concentration of ub1S, ub2S and ub3S complex with time when the initial concentration of ub, E1, E2, E3 and substrate concentrations are set to 150, 1000, 10000, 150 and 100 nM respectively, concentration is plotted on the y-axis in nM and time is plotted on the x-axis in sec.

Code review:

Code can be found here.

At the beginning, the required packages: NumPy, SciPy and matplotlib are imported, then the function that solve the ODEs is defined.

Inside the function, the rate constants are defined, then each compound is indexed to the concentrations list to which the initial concentration will be inputted, and results will be stored.

After that, the rate laws are defined by the equations mentioned in the model explanation, then these equations are used to solve the ODEs.

The list of initial conditions and the time array is defined, then the outputs are stored in new list and each compound concentration array is taken from this list by the same index given inside the function.

References:

1- Kleiger, G., Saha, A., Lewis, S., Kuhlman, B., & Deshaies, R. J. (2009). Rapid E2-E3 assembly and disassembly enable processive ubiquitylation of cullin-RING ubiquitin ligase substrates. Cell, 139(5), 957-968.

2- Xu, L., & Qu, Z. (2012). Roles of protein ubiquitination and degradation kinetics in biological oscillations. PloS one, 7(4), e34616.