Deterministic Modeling

We have used sysBio[1] R package to simulate models using a differential equation solver. Modeling Protocol for each model started by Using *newModel* function to create a new model. Then we Created a model object list speceifieng information about model including; name, reactions, species, rates, parameters, rules, models, ODEs. Using the *addMAreaction* function, we added specefied reactions into the model - to be interpreted using the law of mass action. We used *addMAreactRate* and *addParameters* functions to specify information about the reaction rates and parameters involved in the model. Finally, we defined species using the *addSpecies*function. Consequently, we used *makeModel function* to create a mathematical representation of the model. This function transforms reactions into corresponding ODEs, and creates stochastic matrix and propensity function to perform stochastic modeling. we used *simulateModel* function to run simulation (solve ODEs). This function calls the *validateModel* function that checks if all components of the models have been defined. Finally, we used *plotResults* function to visualize simulation results.

ceRNA Deterministic Model

Our Model aims to describe the regulation of competing endogenous RNA (ceRNA) network using ordinary differential equations to get insights about the kinetics of molecular species inside the network [2]. The Model was constructed in Synthetic biology markup language SBML. SBML models were converted to SBOL (synthetic biology open language) to describe biological parts and their interactions including: transcription, degradation, association and dissociation of both the ceRNA and miRNA. The Model describes an inhibitory relationship, where the miRNA binding to ceRNA inhibits the miRNA action on its target mRNA. We can estimate that effect from the change in free miRNAs in the simulation run. Parameters have been estimated from the work of Bosia et al[3] and described as a system of ODEs.

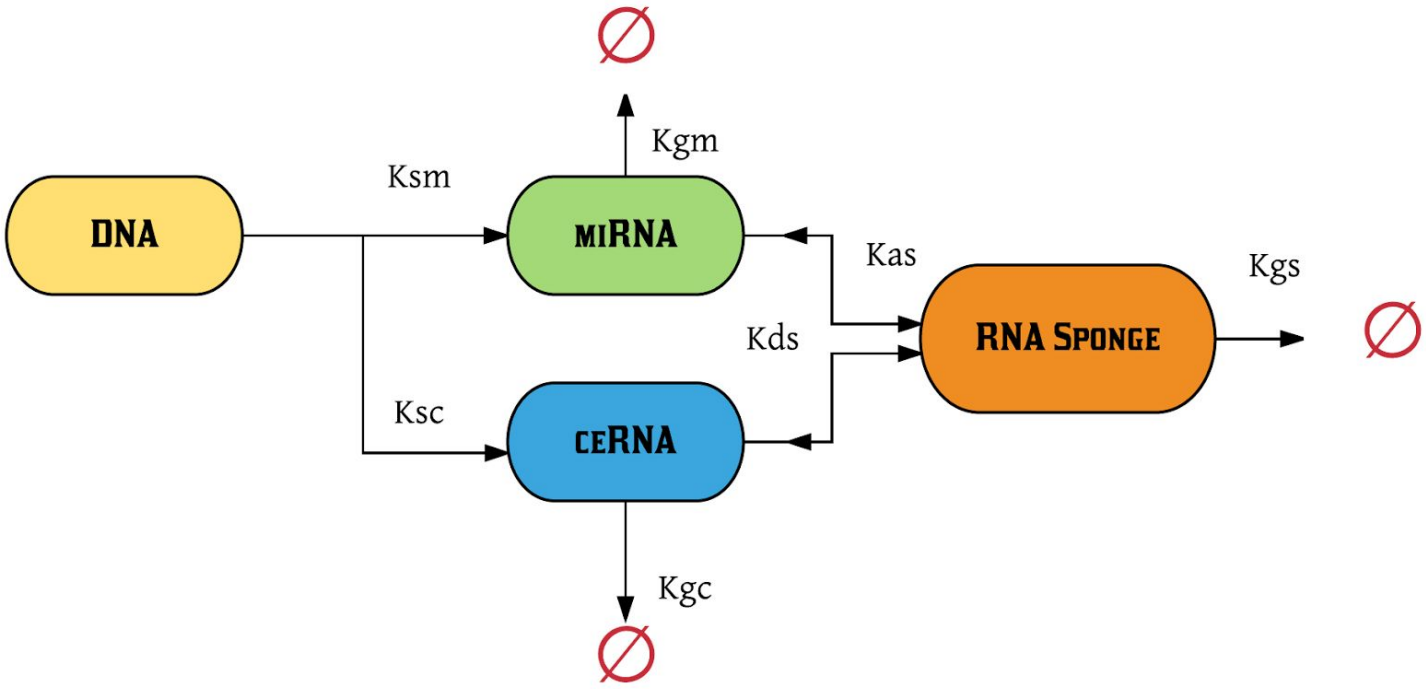

Figure-1 Graphical representation for ceRNA model

$$let\ [ceRNA] = C\ ,\ [miRNA] = Mi \text{ and } [RNA.Sponge] = CM$$

$$\frac{dC}{dt} = k_{sc} - k_{gc} \cdot C + \sum_{i=1}^{C} (-k_{as} \cdot Mi \cdot C + k_{gs} \cdot CM)$$

$$\frac{dC}{dt} = k_c - g_c + \sum_{i=1}^{C} -k_{as} \cdot Mi \cdot C + k_{ds} \cdot CM$$

$$\frac{dCM}{dt} = k_{as} \cdot Mi \cdot C - (k_{ds} + k_{gs}) \cdot CM$$

Symbol	Description	Value	Reference
K_{smi}	Rate of transcription of miRNA	$0.2s^{-1}$	Bosia et al
K_{sc}	Rate of transcription of ceRNA	$0.155s^{-1}$	Bosia et al
K_{gmi}	Rate of degradation of miRNA	$0.0003s^{-1}$	Bosia et al
K_{gc}	Rate of degradation of ceRNA	$0.0004s^{-1}$	Bosia et al
K_{as}	Rate of association of RNA Sponge Complex	$0.0005s^{-1}$	Bosia et al
K_{ds}	Rate of dissociation of RNA Sponge Complex	$0.0003s^{-1}$	Bosia et al
K_{gs}	Rate of degradation of RNA Sponge Complex	$0.00031s^{-1}$	Bosia et al
α	Catalytic Parameter	0.1	Bosia et al

Table-1 Parameter values of ceRNA network regulation

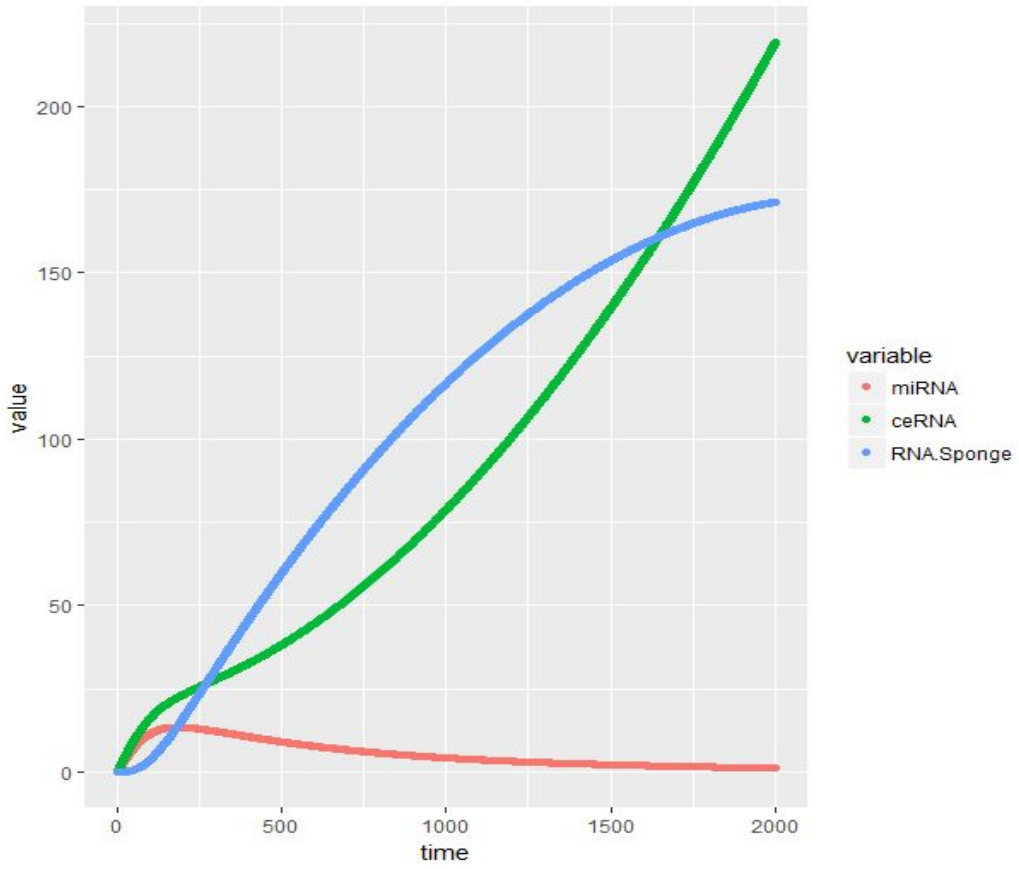

Figure-2 Simulation run for for ceRNA network model ODEs

The simulation in Figure-2 Shows inhibitory relationship along time axis to the quantity of free miRNA along the transcription of circular RNA as a competing endogenous RNA which may describe the sponge action, regarding the elevation of free miRNA action on target mRNAs.

CRISPR Deterministic Model

Our Model aims to describe the regulation of CRISPR network. The Model was constructed in Synthetic biology markup language SBML, including: transcription, degradation, and association of gRNA and cas9. The model describes the binding interaction between the gRNA and cas9 that is supposed to be informative to the cas9 about the cleavage site near the PAM. Parameters have been estimated from the work of R.moore et al.[5] and described as a system of ODEs.

$$\frac{\mathrm{d}cas9}{\mathrm{d}t} = k_{cas9} - \delta_{cas9} \cdot [cas9] - k_f \cdot [cas9:gRNA]$$

$$\frac{\mathrm{d}gRNA}{\mathrm{d}t} = k_{gRNA} - \delta_{gRNA} \cdot [gRNA] - k_f \cdot [cas9:gRNA]$$

$$\frac{\mathrm{d}[cas9:gRNA]}{\mathrm{d}t} = k_f \cdot [cas9:gRNA]$$

- CRISPR Circuit ODE system

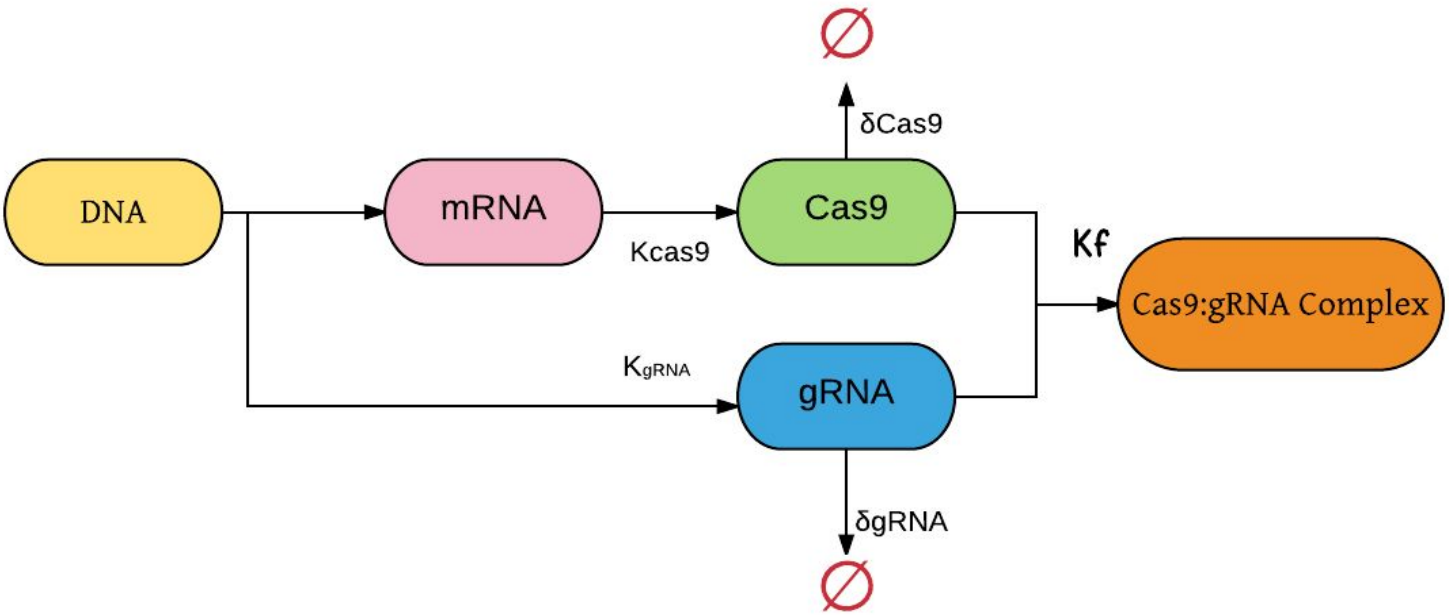

Figure-3 Graphical representation for cas9 model

Symbol	Description	Value	Reference
K_{cas9}	Rate of Cas9 Production	0.000374737	R.Moore et al
K_{gRNA}	Rate of gRNA Production	0.0025284	R.Moore et al
$\delta cas9$	Rate of Cas9 Degradation	0.0000552	R.Moore et al
$\delta gRNA$	Rate of gRNA Degradation	0.000252	R.Moore et al
K_f	Cas9+gRNA binding rate	0.00006	R.Moore et al

Table-2 Parameter values of cas9 network regulation

We are thankful to Valencia UPV iGEM 2016 Team[3] for providing cas9 modelling parameters[4] on their wiki

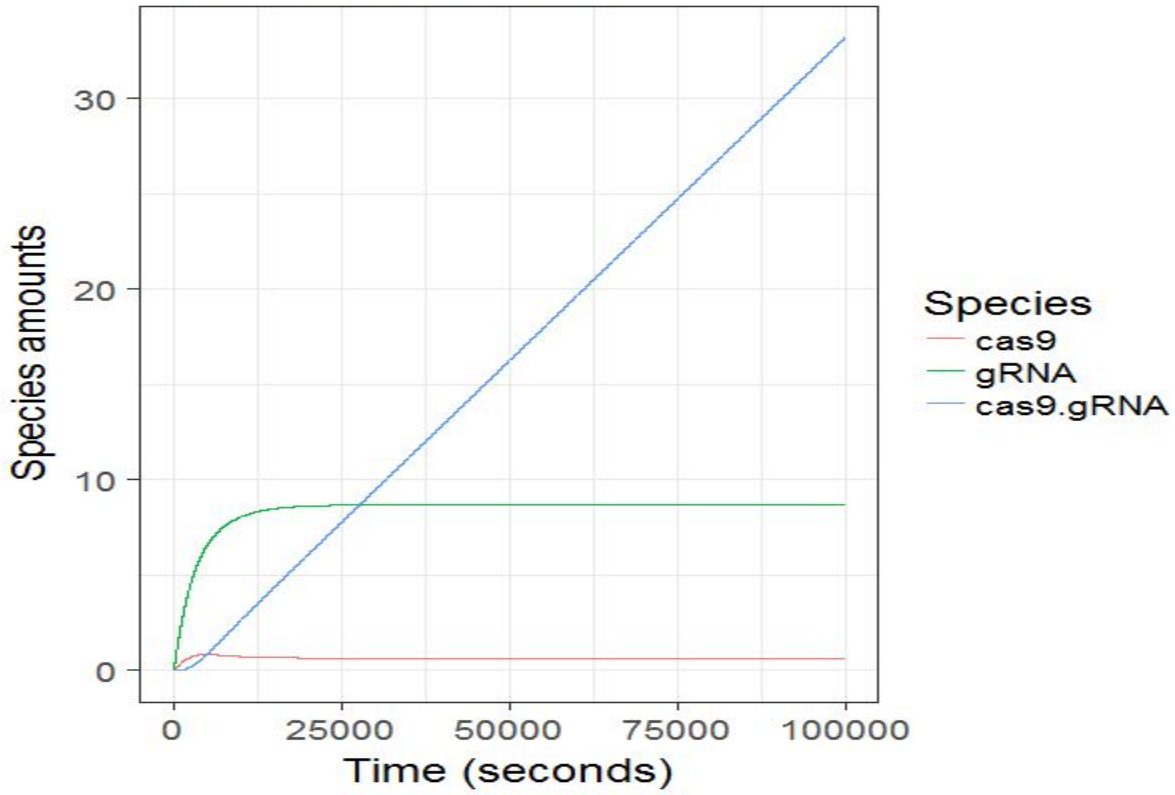

Figure-4 Simulation run for for cas9 network model ODEs to t = 10^5

The simulation in Figure-4 describes interaction association between gRNA and cas9 along time axis to the quantity of formed complexes cas9. gRNA along the transcription of gRNA as a directing RNA molecule which may describe the complex action regarding focusing cas9 cleavage action on target DNA.

All simulations have been run in R using deSolve[6] and sysBio[1] packages.

References

1- https://github.com/Vessy/sysBio

2- Mathematical Modelling in systems biology: An Introduction.

3- Bosia C, Pagnani A, Zecchina R (2013) Modelling Competing Endogenous RNA Networks. PLoS ONE 8(6): e66609.

4- 2016.igem.org/Team:Valencia_UPV

5- Moore R, Spinhirne A, Lai MJ, et al. CRISPR-based self-cleaving mechanism for controllable gene delivery in human cells. *Nucleic Acids Research*. 2015;43(2):1297-1303.

6- Karline Soetaert, Thomas Petzoldt, R. Woodrow Setzer (2010). Solving Differential Equations in R: Package deSolve. Journal of Statistical Software, 33(9), 1--25.