

Stochastic Modeling of Gene Regulatory Networks in Escherichia coli

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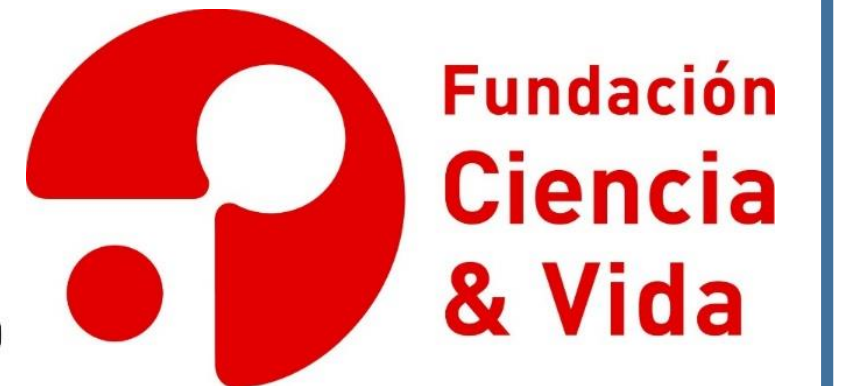
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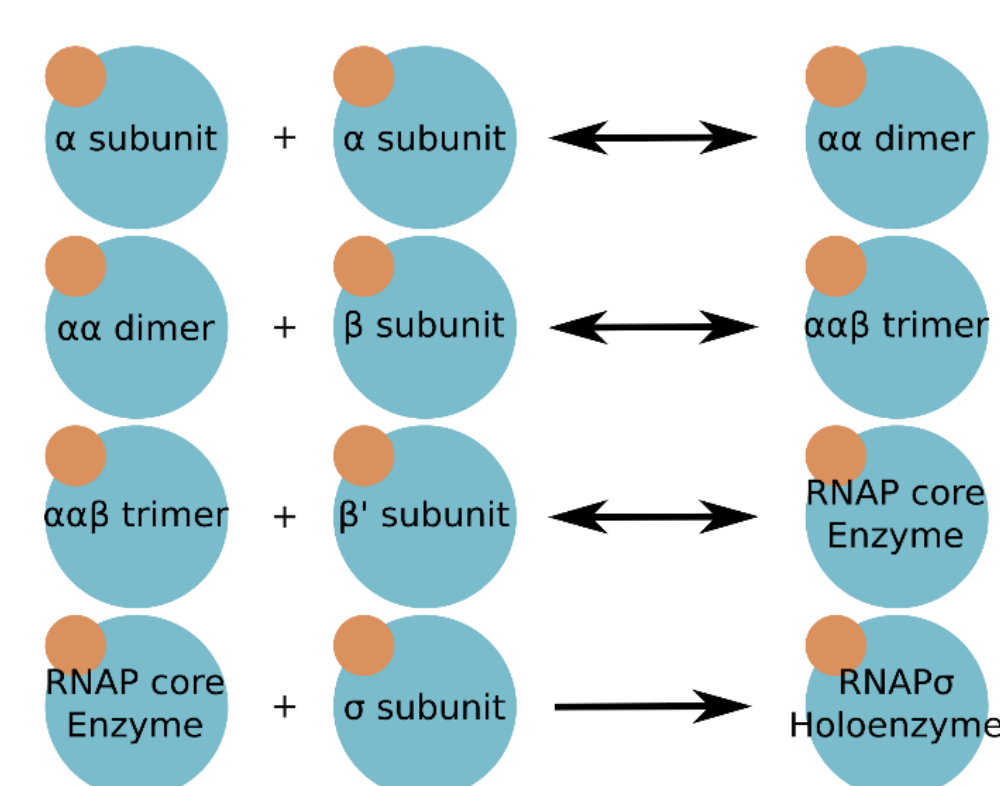
Introduction.

Synthetic Biology has the ultimate objective of designing cells, tissues, organisms and communities to produce predictable responses [1]. Interestingly, mathematical and computational modeling has impacted prominently Synthetic Biology, where the manipulation of biological systems is cost-intensive, and computational resources could leverage experimental procedures [2–4]. Traditionally, differential equations (ODEs) have been employed, but their assumptions are not as realistic as those made by other approaches. Particularly, it has been known that biological systems are stochastic, discrete and structurally complex, hampering ODEs to fit these properties [5]. To further resolve a connection between modeling and designing organisms, we present a Rule-Based Model of Gene Regulatory Networks (GRNs) in *Escherichia coli* simulated using the Gillespie's Stochastic Simulation Algorithm [6,7]. Under this approach, rules are macroscopic chemical reactions between entities that recapitulate one or several patterns necessary for a transformation [8].

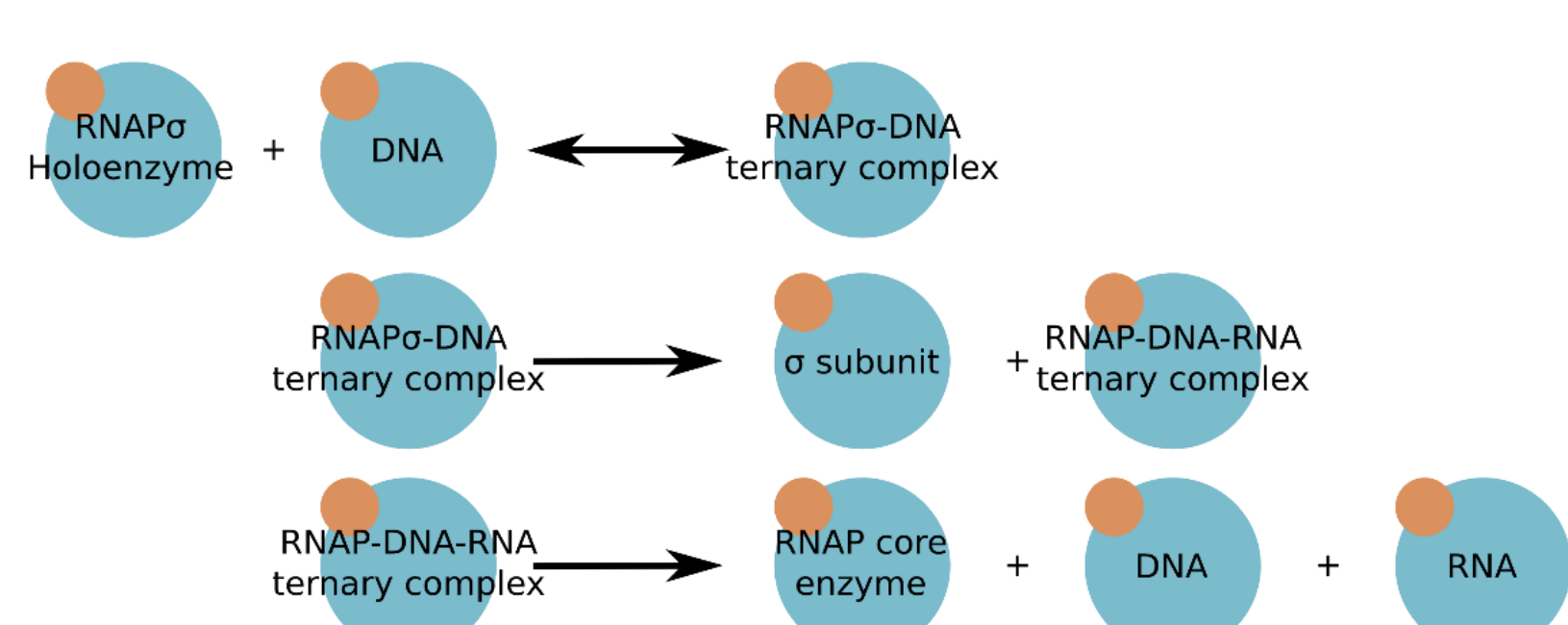
Methodology. Models were developed from literature GRNs [9,10] within the *Kappa BioBrick Framework* [11], exported to *kappa* with PySB [12] and simulated 1000 Arbitrary Time Units with PISKaS v1.3 [13]. Model rules are depicted in following figure.

- The **Core GRN Model** recapitulates the interaction of RNAP with each of the 7 *E. coli* Sigma Factors (σ 's) and the control of its transcription [9]. The GRN has 10 nodes and 28 edges. Three settings were explored: one RNAP and 7 σ 's (blue); 7 RNAPs and 7 σ 's (green); and 28 RNAPs and 28 σ 's (red) distributed as many regulations each σ 's has in the GRN. Results are shown in percentage of the total RNAP.
- The **Plasmid Copy Regulation Model** recapitulates the expression and regulatory interaction of RNA I and RNA primer from plasmid ColEI [14], which transcription are controlled solely by $\sigma 70$. Two settings were simulated: no interaction between the non-coding RNAs (blue) and interaction between them at an arbitrary rate of 0.5 (green).
- A **Combined Model** of both models was developed as shown in the Figure 1. Simulations were performed with null transport rates of σ 's, RNAP protein subunits, and proteins complexes.
- A *script* that employ a **Genetic Algorithm (GA)** was developed to read a *kappa* model and construct new models with modified parameter values in an arbitrary range selected by the user. The *script* calls KaSim [15] or PISKaS [13] to run simulations inside a SLURM *sbatch* task. Finally, all simulations are ranked using a distance-based function and an arbitrary number of models are selected to generate the subsequent model population. The GA was tested using the Core GRN Model and data from [16] with *default* options: 100 individuals, 100 iterations, 10 best models, 0.3 mutation rate [17].

RNAP Holoenzyme assembly to each of 7 Sigma Factors



Transcription: RNAP-Promoter binding, initiation and termination



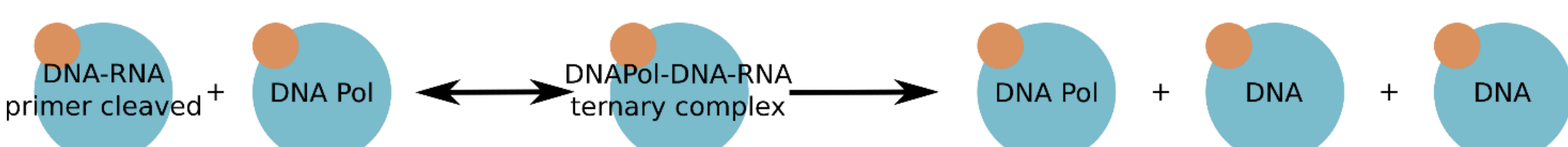
Transcription of RNA primer coupled to DNA-RNA hybrid formation



RNA primer processing by an RNase



DNA Polymerase binding and plasmid replication coupled to RNA primer degradation



Negative feedback by RNA-RNA interaction

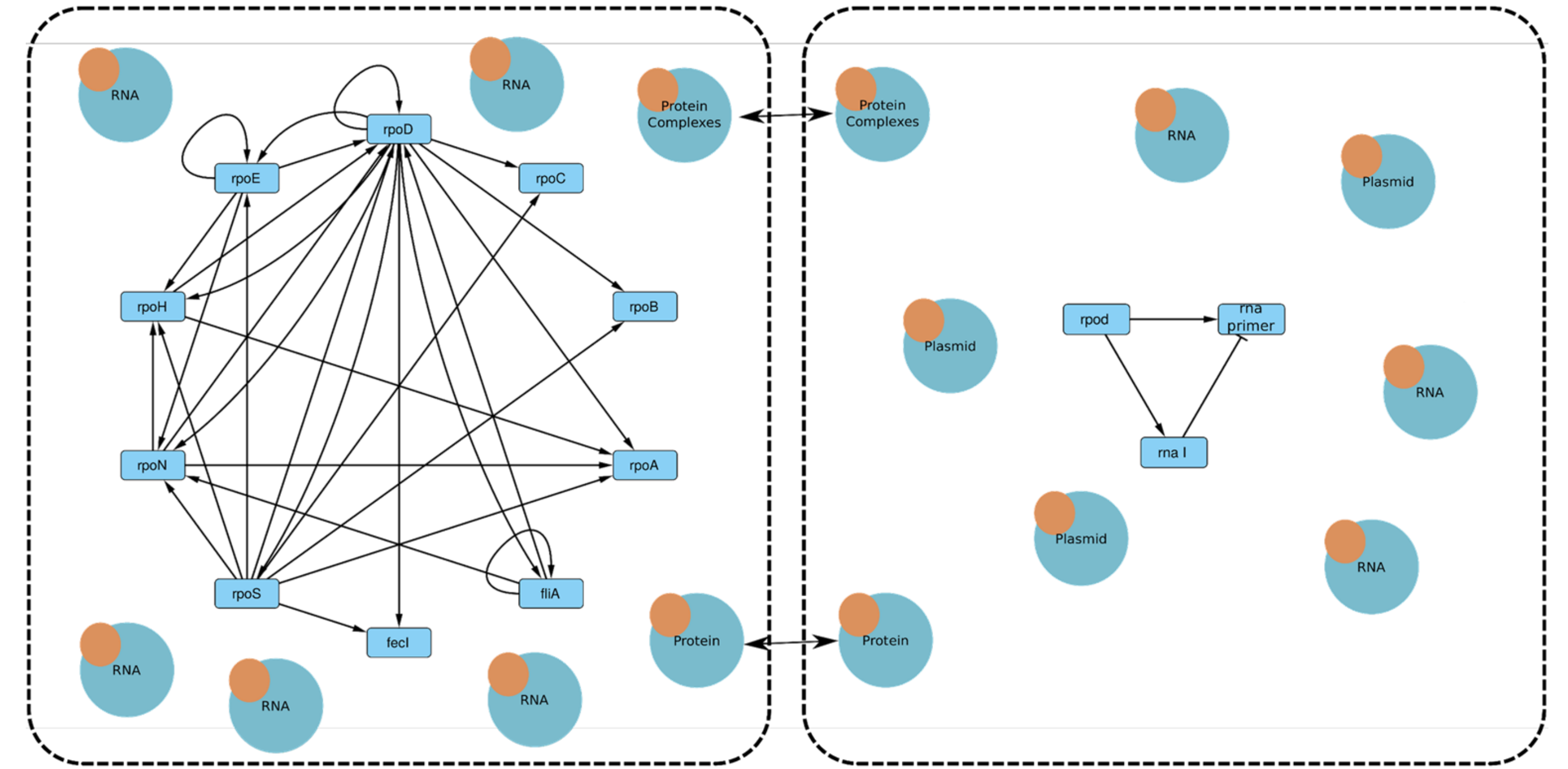
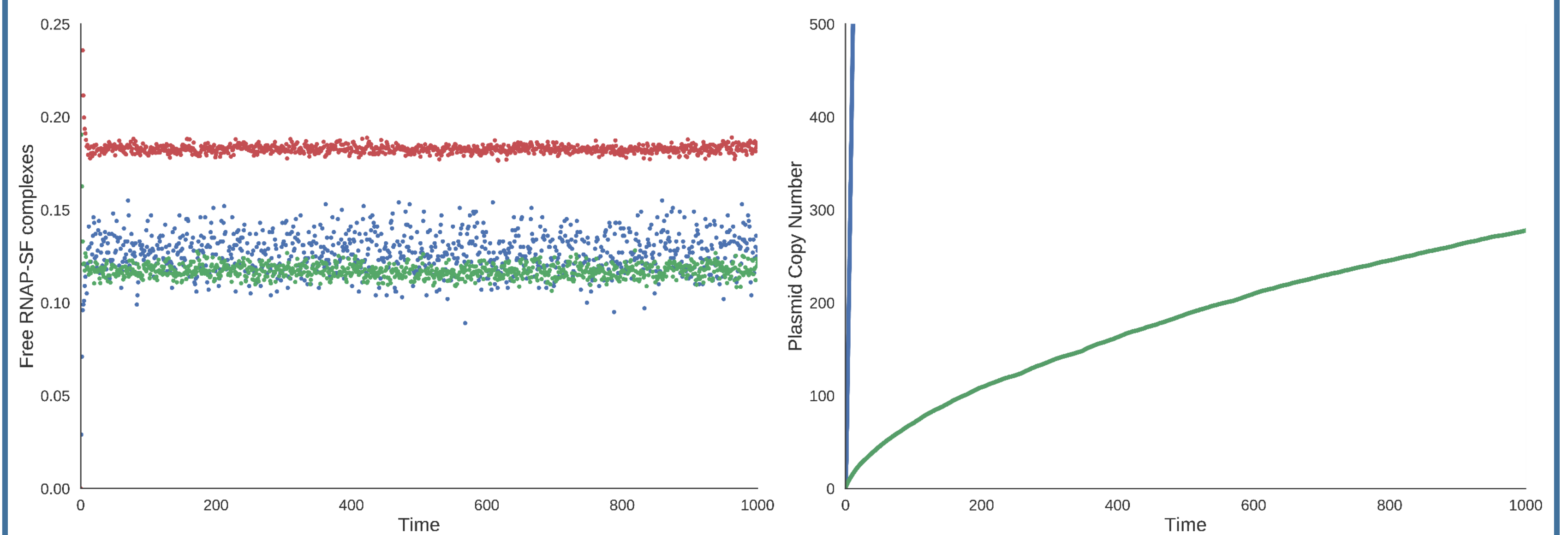
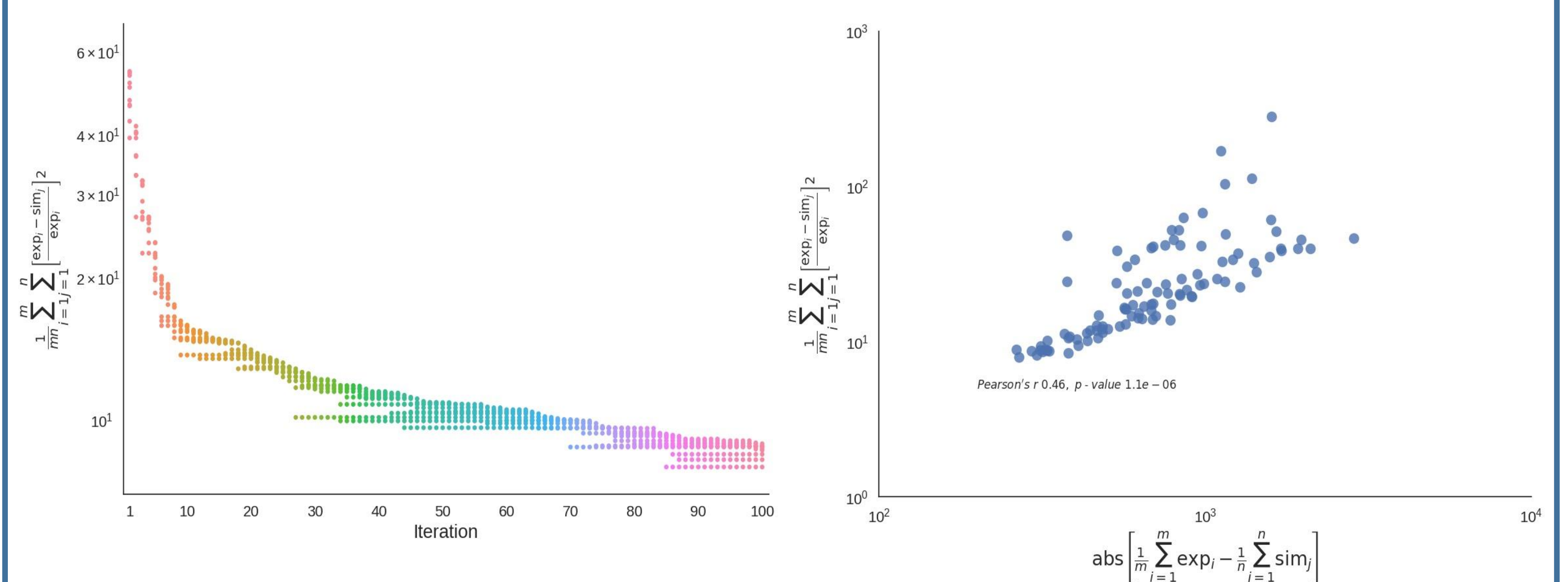


Figure 1. Representation of the Core GRN Model (Left) and the Plasmid Copy Regulation Model (Right) as a two-compartment model. Only agents that represent free proteins and protein complexes are able to move between models as depicted by the solid double arrows.



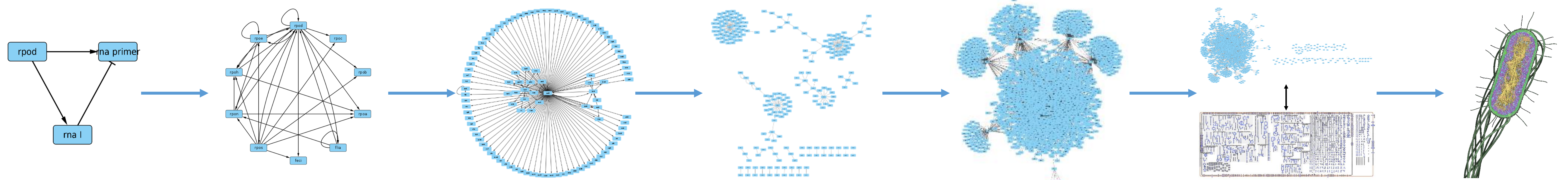
Results. Left, the Core GRN Model simulates free RNAP- σ complexes with good agreement with reported values [18]; right, the Plasmid Copy Regulation Model predicts a saturation dynamic when there is explicit interaction between the non-coding RNAs (green).



Results. Left, the error of the ten best models per iteration is reduced effectively using a common distance-based function; right, comparison between two implemented functions to score goodness of fit and select better models.

Conclusions and Further Work.

Rule-Based Models are a useful modeling framework to reconstruct known interactions between biological components and to scale-up efficiently the number of agents and processes considered. An ongoing effort to model every known gene, metabolism and processes that encompass the Molecular Biology Central Dogma would lead to a better understanding of cell behavior and novel exploratory methods in Bioengineering, Synthetic Biology and Metabolic Engineering.



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