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Multi-Hierarchical Dynamics of Antimicrobial Resistance Simulated in a Nested Membrane Computing Model

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Abbreviations: AMP, aminopenicillins, CTX, cefotaxime, FLQ, fluoroquinolones, EC: *Escherichia coli*; KP: *Klebsiella pneumoniae*; EF: *Enterococcus faecium*; PA: *Pseudomonas aeruginosa*; H: Hospital; C; Community; AbR, resistance to antibiotic Ab; PL, plasmid; CO: conjugative element; MTP: model-targeted populations; BCM: basic colonic microbiota

Abstract

The membrane-computing model in this work reproduces complex biological landscapes in the computer world. It uses nested “membrane-surrounded entities” able to divide, propagate and die, be transfer into other membranes, exchange informative material according to flexible rules, mutate and being selected by external agents. This allows the exploration of multi-hierarchical interactive dynamics resulting from the probabilistic interaction along time of genes (phenotypes), clones, species, hosts, environments, and antibiotic challenges in the real world. Our model facilitates the simultaneous analysis of several features of interest in the prediction of the rules governing the multi-level evolutionary biology of antibiotic resistance. These include the overall integrated dynamics of species, populations with resistance phenotypes, and mobile genetic elements in different environments (hospital and community) and experimental landscapes. In the examples included here, we predict the effects of different rates of patient’s flow from hospital to the community and *viceversa*, cross-transmission rates between patients with bacterial propagules of different sizes, the proportion of patients treated with antibiotics, antibiotics and dosing in opening spaces in the microbiota where resistant phenotypes multiply. We can also predict the selective strength of some drugs and the influence of the time-0 resistance composition of the species and bacterial lineages in the evolution of resistance phenotypes. However, many other analyses are possible to implement. In summary, we provide a bunch of examples about the multi-hierarchical dynamics of antibiotic resistance using a novel computable model with reciprocity within and between levels of biological organization, a type of approach that can be easily expanded to fulfil the needs of risk-analysis and evolutionary predictions in a multiplicity of complex ecological landscapes.

Significance Statement *(120 words)*.

Antibiotic resistance is recognized as a priority problem in Public Health. The design of interventions able to bend the rampant spread of resistant bacterial populations should be based on deeper knowledge of the eco- and population biology of antibiotic resistance. However, biology of resistance has a highly complex structure, because of the nested nature of the biological and genetic elements involved. Resistance genes are in mobile-genetic-elements, bacterial genomes, populations, species, microbiomes, hospitalized and healthy humans, and at each of these levels there are vertical and horizontal interactions shaping the fate of resistance in changing landscapes. Because of its structural resemblance with biological systems, membrane computing offers an opportunity to model and predict these processes in an unprecedented way.

Antibiotic resistance is the result of the complex interaction of a number of discrete evolutionary entities placed in different hierarchical levels of biological organization, encompassing resistance genes, mobile genetic elements, clones, species, genetic exchange communities, microbiomes, and hosts of these bacterial ensembles placed in particular biological environments (1,2,3). Under the influence of external environmental variation (as exposure to antibiotics) each one of these evolutionary entities might have independent rates of variation and selection, but as they are hierarchically-linked, the changes in each one of them should influence, in bottom-up or top-down dynamics, all other entities (4). If at large this organization constitutes a global "nested biological system" (5), it can be better defined here as a "mobile nested system" in which one entity "a_x" exists *inside* another one of the superior level "*b*", but it can move across a network of individuals in this level "$b_1..b_n$", exchanging information with other entities of its own level, "$Ab1_{...}a_n$". Each one of the combinations between entities of "*a*" and "*b*" levels implies changes, thus influencing selective events on both levels. In a simple example, the fate of a resistance gene might depend on his capture by a particular plasmid, and the fate of this plasmid depends on its capture by a particular clone, and the fate of this clone in its ability to colonize particular hosts, treated or nor with antibiotics. Because of the asymmetries of the transfers and transmission processes, the use of antibiotics might exert asymmetrical effects on the gene, plasmid, clones, and host populations.

Biological organization in nested (embedded) systems has been one of the main ingredients for membrane computing, a natural computing model inspired by the living cell (6). Membrane computing has been successfully applied in both systems biology and synthetic biology (7). In this work, we apply a membrane-computing model to simulate multi-level dynamics of antibiotic resistance. This model is based on our first

published prototype (8). Membrane-computing consists in placing objects (in our case biological entities) into virtual cell-like membrane structures (constituting also biological or ecological entities), so that one membrane represents a hierarchical level, a region of the embedded system. But, each one of the entities can also be a region, a compartment surrounded by a membrane, containing other (nested) compartments limited by membranes and containing objects. For instance, each bacterial cell is a membrane containing plasmids (as objects), and a plasmid is a membrane containing genes (as objects). The mobility of entities, objects, across membranes is possible according to pre-established rules, and the ensemble of multisets of entities will evolve in a synchronous, parallel, and non-deterministic (stochastic) manner. The entities are formally described by pre-defined symbols, or strings of symbols (these strings are also treated at symbols themselves). The objects can not only pass through membranes, the compartments containing objects within membranes can be moved from one place to another (to mimic intracellular or intercellular transmission (9), dissolved (to mimic elimination), and divided (to mimic replication), and more processes can also be represented.

This computational model facilitates a task which is hard to accomplish or simply impossible to address experimentally. Our work allows the estimation and evaluation of global and specific effects on the frequency of each one of the biological entities involved in antibiotic resistance occurring because of changes taking place (as following antibiotic exposure) in one or (simultaneously) in several of them. From the computational perspective, if the model is a probabilistic one, it instrumentally acquires a “heuristic search” value. This reveals the possibility of hidden complex interactive patterns and suggests values for processes of almost impossible experimental investigation in hospitals or the community, such as the rate of between-host contagion

or the effects of therapy in opening exploitable colonization spaces for resistant bacteria. Those could well play a role in the epidemiology, ecology, and evolution of antibiotic resistance.

Of course, antibiotic resistance is a major problem in Public and Global Health and constituted a priority for the application of our model. But antibiotic resistance is only a particular example of “evolution in action”, and our model can be easily applied to many other complex evolutionary landscapes, involving other genes, phenotypes, cells, populations, communities and ecosystems.

Results

In this section, we illustrate some of the possibilities of this innovative computable model to study the multi-hierarchical dynamics of antibiotic resistance. We are representing the results obtained for the basic scenario, and then include a number of variant scenarios to show their influence in the observed dynamics. Note that the model allows a number of probabilistic steps, so that the results obtained are not entirely identical in consecutive runs of the program. However, they are extremely close (see Supplementary Information, Fig SI1). Of course, the parameters of the model can be changed by the investigators and the results of these new scenarios will change accordingly. Along the next paragraphs, the antibiotics and the corresponding resistances are named in a general way as Ab1, Ab2 and Ab3, and Ab1R, Ab2R, and Ab3R respectively; however, to facilitate the understanding of medical and public health oriented readers, we suggest the identification of Ab1 as the aminopenicillins (AMP), Ab2 as cefotaxime (and/ or ceftriaxone or ceftazidime), and Ab3 as fluoroquinolones, three of the major groups of antibiotics used in clinical practice.

The basic scenario in the hospital and community compartments

Dynamics of bacterial resistance phenotypes in *E. coli*. The waves of successive replacements of resistance phenotypes in hospital-based *E. coli* along 20,000 steps are illustrated in Fig 1. The main features of this evolutionary process, mimicking clonal interference, are: 1) sharp decrease in the density of the fully susceptible phenotype, but this phenotype does not disappear (pink); 2) rapid increase towards high densities of the phenotype Ab1R, aminopenicillin (AP) resistance, not only well represented at the baseline (t_0), but resulting from the transfer of the plasmid with Ab1R to the susceptible population, and consequent selection (red); 3) increase by selection, and marginally by acquisition of mutational resistance, of the phenotype including Ab3R, fluoroquinolone (FLQ) resistance (violet) 4) increase in the size of double resistances Ab1R and Ab3R (AMP, FLQ), by acquisition of an Ab3R mutation with the organisms Ab1R-only phenotype, and more probably by the transfer of the plasmid encoding Ab1-R from the Ab1-R-only phenotype to the Ab3R-only phenotype (brown); 5) increase in size of the phenotype with double resistances Ab1R and Ab2R by capture by the Ab1R-only (AP) predominant phenotype of a plasmid containing Ab2R (CTX) resistance originated in *K. pneumoniae*) (light blue); 6) almost simultaneous emergence but later predominance of the multi-resistant organisms with phenotype Ab1R, Ab2R, and Ab3R (AMP, CTX, FLQ) by mutational acquisition of Ab3R by the double-resistant phenotype Ab1R-Ab2R and, more probably, of the plasmid-mediated Ab2R by the Ab1R-Ab3R phenotype (dark blue); 7) close in time, there is an emergence, but with low density, of the phenotype Ab2R-only, due to the acquisition of the plasmid encoding Ab2R by the fully-susceptible phenotype, and also the acquisition of this plasmid by the population with an Ab1R phenotype, and loss of this plasmid-mediated Ab1R by incompatibility with the incoming plasmid (light green); 8) the acquisition of the Ab3R mutation by the

Ab2R-only phenotype, or more probably, by plasmid-reception of an Ab2R trait from *K. pneumoniae* in Ab3R, giving rise to the phenotype Ab2R-Ab3R or , with low representation but increasing slowly in time, eventually because the use of AP slightly contributes to its selection (olive green).

In the community, where the antibiotic exposure occurs in a much lesser degree, a similar dynamic sequence occurs, but at a much slower rate (fig. 2): 1) there is a constant decrease in the density of the fully-susceptible phenotype, without reaching extinction (pink); 2) the *E. coli* Ab1R (AMP) phenotype reaches strong predominance, which is essentially maintained along the experimental time (red); 3) the acquisition of Ab3R (FLQ) mutation by the fully susceptible results in the phenotype Ab3R (violet); 4) the emergence of this mutation in the Ab1R phenotype and the transfer of the plasmid Ab1R to Ab3R-only populations produces the emergence and selection of Ab3R (AMP, FLQ); and 5) only very late, the phenotypes including Ab2R emerge, but certainly increase in time .

Dynamics of bacterial species. Antibiotic use and antibiotic resistance influences the long-term dynamics of bacterial populations in the hospital environment (Fig. 2). In the conditions of our basic scenario, *E. coli* populations (red) tend to prevail, steadily increasing and doubling their absolute number from the 5,000 to 10,000 steps. As will be discussed later, the evolution of resistance favors the dominant populations at the baseline. At the start of this period Ab1R *E. faecium* and *K. pneumoniae* reached a density which is maintained along the experiment, maybe with a mild ascent in the case of *K. pneumoniae* (olive green) and a mild decrease in the case of Ab1S, ampicillin-susceptible, *E. faecium* (dark blue) However, the overall species landscape remains relatively stable. In the community, there is a stronger dominance of the density of *E.*

coli over that of the other species, and similar dynamics occur as in the hospital, but at much slower rates.

Klebsiella pneumoniae (Fig SM3) is intrinsically resistant to Ab1 (Ab1R, AMP) and, in our case, it harbors a plasmid encoding Ab2R (CTX), and is Ab3R (FLQ). In the hospital, the phenotype population containing Ab2R is readily selected (olive green). However, because of the high density of *E. coli* with the plasmid-mediated Ab1R, several *Klebsiella* strains receive this plasmid. As they are already (intrinsically, chromosomal trait) Ab1R, these strains have no benefit in this acquisition, but an effect of incompatibility with the plasmid determining Ab2R might occur, eliminating Ab2R and giving rise to the phenotype Ab1R (plasmid-mediated)-Ab3R (purple). That might contribute to the decline in Ab2R-containing phenotypes (olive green). In any case, the dominance of *E. coli* prevents a significant growth of *K. pneumoniae*.

Enterococcus faecium (Fig SM3) is intrinsically resistant to Ab2 (Ab2R, CTX), but there are two variants, one Ab1 (AMP) susceptible (Ab1S), and the other resistant (Ab1R), this last one has also Ab3R. However, the Ab1S susceptible variant can acquire the Ab1R trait from the resistant one by (infrequent) horizontal genetic transfer. Once the Ab1R trait is acquired it can be disseminated by new transfers to cells with the AbS phenotype. Therefore, there is replacement dynamics of the susceptible by the resistant phenotype. We do not detail the dynamics of *Pseudomonas aeruginosa*, as under our model conditions its number always remains extremely low, probably because of low replication rates in the anaerobic colonic microbiota.

Influence of baseline resistance composition on the dynamics of bacterial species. The local evolution of antibiotic resistance should be dependent on the baseline composition of susceptible and resistant bacterial populations. In this section, we compare how the same array of selective and epidemiological conditions will influence

two scenarios with a different resistance baseline (Fig 3). Considering a density of 8,600 h-cells of *E. coli*, under the basic conditions of our model, we have 5,000 susceptible, 2,500 with plasmid-mediated aminopenicillin-resistance (PL1-AR1), 1,000 with fluoroquinolone resistance (AR3), and 100 combining both resistances. To mimic a "more susceptible scenario" we changed these values to 8,000 susceptible, 500 with PL1-Ab1R, 50 with Ab3R, and 50 with PL1-Ab1R and Ab3R.Note how the increase in susceptible *E. coli* facilitates the quantitative advantage of more resistant organisms, as *K. pneumoniae* or Ab1R *E. faecium*. Because of the selection of *K. pneumoniae* (olive green) harboring cefotaxime-resistance (PL1-Ab2R), and the ability of transfer of the PL1 plasmid to *E. coli*, the proportion of *E. coli* with cefotaxime-resistance (mainly light and dark blue) increases in the scenario with a lower resistance baseline for *E. coli*. This computer example illustrates that resistance in *E. coli* might reduce the frequency of other resistant organisms. This effect should be considered at the time of designing possible interventions directed to restore susceptibility in particular species (10, 11)

Single lineage *E. coli* dynamics: influence of baseline resistances. Note that in the former analysis it was presented as discrete populations those composed by *E. coli* organisms sharing an identical antibiotic-resistance phenotype (phenotype populations). However, in our model we can follow the evolution in time of several independent lineages (EC0 to EC3) that are tagged as such at time 0, as starting with specific resistance traits (Fig 4). How does the original resistance phenotype influence the evolution of a particular lineage within a species (in our case, *E. coli*)? Four lineages, with different resistance phenotypes at the origin, are followed along the simulation experiment (Fig 4). The fully susceptible *E. coli* lineage (EC0) first acquires Ab1R (red, AMP), and Ab2R (green, CTX). The increase in number of Ab1R phenotype facilitates the capture by lateral gene transfer of Ab2R (CTX), giving rise to the double Ab1R and

Ab2R phenotype (light blue). The incorporation of Ab3-R (violet, FLQ) in the fully susceptible lineage occurs early, but later in the Ab1R population and remaining at very low numbers. Because of this low density the rise of the multi-resistant phenotype (dark blue) occurs late and again at low numbers. The presence of the Ab1R trait in the lineage at time 0 (EC1) increases the success of the clone, including the acquisition of Ab3R, and consequently the multi-resistant phenotype. Interestingly, the presence of Ab3R (FLQ) at the origin, with or without Ab1-R, was critical to enhance the numbers of double-resistant and multi-resistant phenotypes. Note that if Ab3R is not present at the origin, its emergence depends on mutational events requiring high cell densities. If frequent, the Ab3R phenotype population acquires other resistances in an easier way by lateral gene transfer. A summary of these lineage trajectories is presented in Fig SM2, showing the advantage of the lineages with Ab3R at the origin (violet and brown curves). However, the more susceptible clones at the origin, including the fully susceptible clone (red and pink-fuchsia), remain relatively stable in numbers at the hospital scenario, suggesting that clonal composition tends to level-off along the continued challenges under antibiotic exposure.

Dynamics of mobile genetic elements and resistance traits. We consider *E. coli*, *K. pneumoniae*, and *P. aeruginosa* as members of a "genetic exchange community" (12,13) for the plasmid PL1, as they are able to transfer and receive this mobile genetic element carrying resistance mechanisms (Ab1R or Ab2R, AMP and CTX). In Fig. 5, we can compare the evolutionary advantage of the same resistance phenotypic trait (Ab1R) when harbored in a plasmid, as in *E. coli* (bright red line) or in the chromosome, as in *K. pneumoniae* (brown-red). The overall success of the plasmid PL1 (blue line) benefits from the fact that this mobile element is selected by two different antibiotics (Ab1 and Ab2, red and green lines respectively). Interestingly, the trait Ab3R (FLQ) (violet) is

selected from early stages of the experiment, and after 4,000 steps it converges with the Ab2R, a plasmid-mediated trait. This probably means that this plasmid is maintained almost exclusively in strains harboring Ab3R gene, coincidentally with what is found in the clinical field (14,15) (*Fuzi*). If the conjugation rate of PL1 was increased, the main effect observed was the reduction in selection of *K. pneumoniae*, as the predominance of the PL1-Ab1R plasmid from the more abundant populations of *E. coli* tended to dislodge PL1-Ab2R from *K. pneumoniae* (results not shown).

Dynamics under changing scenarios in the hospital and community compartments

Frequency of patients' flow between hospital and community. The frequency of exchange of individuals between the hospital and the community, two compartments with different rates of cross-colonization and antibiotic use, determines the evolution of antibiotic resistance. The influence of this flow (hospital admission and discharge rates) has been previously analyzed by mathematical modeling. This occurs because sensitive bacteria enter the hospital with newly admitted patients from the community (where resistance rates are low), and this ''immigration'' allows sensitive bacteria to ''wash out'' resistant bacteria (16). This is illustrated in Fig 6. Note how multi-resistant *E. coli* strains emerge much earlier with decreased flow rates, as resistant bacteria with fewer resistances have more time to coexist and thus exchange resistances by gene flow, and because the length of frequent exposure to different antibiotics increases, and consequently selection (17). In the subtractive representation it is patent that in the hospital compartment the multi-resistant phenotype (Ab1R-Ab2R-Ab3R, -dark blue) and the double (Ab1R-Ab2R -light blue), have long-term advantage when flow is

decreased from 2 h to 4 h, meaning a longer stay of patients in the hospital. The effect of this decrease in flow of patients to the community is a late reduction in multi-resistance (Ab1R-Ab2R-Ab3R, dark blue) and more precocious double resistances (Ab1R-Ab3R, brown) and (Ab1R-Ab2R, light blue). In the hospital compartment, an even slower discharge of patients to the community (from 4 to 8 h) clearly increases the multi-resistant phenotype (dark blue), On the contrary, in the community compartment, multi-resistance increases when the flow from the hospital is more frequent (4 h), and the more susceptible phenotype (Ab1R only) is preserved and increases if the contact with hospital patients is minimized. Interestingly, lowering the hospital discharge rates from one patient every 2 to 4 h has a much lesser effect on selection of multi-resistant phenotypes than reducing from 4 to 8 h. This effect of infrequent exchange between hospital and community, typically expressed as reduced discharge rates, benefits essentially *E. coli* over the other species considered in the model; even though aminopenicillin-resistant *E. faecium* (dark blue) and cefotaxime-resistant *K. pneumoniae* (olive green) are intensively selected in earlier periods, the *E. coli* overgrowth tends to reduce such selection; in any case, they are in higher proportion in the scenarios of low patients' exchange.

Frequency of patients treated with antibiotics. The proportion of patients exposed to antibiotics in the hospital increases the selective effect for resistance and has been considered as a major factor in the emergence and spread of antibiotic resistance (16). We analyzed this effect in our model considering proportions of 5%, 10%, or 20% of treated patients. The original pattern of antibiotic use is maintained but reduced proportionally in the model. The effect in changes in the frequency of antibiotic use (prescription) is illustrated in Figure 7. A low proportion of patients treated (5%) reduces the amount of resistant *E. coli* cells and delays the emergence of multi-resistant

bacteria. If the number of treated patients increases to 10%, and particularly to 20%, multi-resistance is efficiently selected. In that concerning the structure of MTP species, increased proportions of treated patients contribute to the selection of resistant *K. pneumoniae* and *E. faecium*. However, the evolution of *E. coli* towards more multi-resistance restricts the growth of these species, even in the scenario of high density of treated patients.

Frequency of bacterial cross-transmission rates in the Hospital. Cross-transmission of bacteria among individuals in the hospital should influence the spread of antibiotic resistance. This effect is presented in Figure 8, which shows the effect of transmission rates of 5%, 10% and 20% per hour (percentage of individuals that acquire bacteria from another individual per hour). Note that these proportions do not necessarily correlate with the published data for “hospital cross-infection rates”, as we are considering only cross colonization rates, both of susceptible and resistant bacteria, commensals or pathogens, and not infection rates. Cross-colonization rates which have rarely been measured, probably require a complex metagenomic approach (18). The effect of the number of cells transmitted per event is analyzed in the following paragraph. Unexpectedly, in the representation of the evolution of *E. coli* phenotypes comparing 5-10-20% of inter-host transmission rates, the difference between 10% and 20% of colonization rates it is not easy appreciate. The subtractive representation allows discernment of a global advantage for the multi-resistant phenotypes (Ab1R-Ab2R-Ab3R, dark blue) when the proportion of inter-host transmission rises from 5 to 20%. On the contrary, the mono-resistant Ab1R phenotype (red) tends to be maintained longer under low contagion rates. However multi-resistant phenotype "bursts" occur (dark blue spikes) also with low contagion rates (5% box in fig. 8), and "bursts" of less-resistant bacteria (red spikes) also occur in high contagion rates (20% box). Some double-

resistance phenotypes (as Ab1R-Ab3R, brown) have "bursts" particularly in the 5%, but the double-resistance (Ab2R-Ab3R, olive) tends to prevail in the 20% transmission rates. It is to be noticed that the increase in cross-colonization rates favors not only the transmission of resistant populations, but also of the more susceptible ones, in a certain extent compensating the spread of the resistant phenotypes populations. In fact, if the number of multi-resistant bacteria increases, the transmission of multi-resistance is also enhanced.

Size of transmitted bacterial load. The absolute number of intestinal bacteria that are transmitted from one host to another one, the intestinal propagule, is certainly a factor influencing the acquisition of resistant (or susceptible) bacteria by the recipient. However, this number is extremely difficult to determine, as it depends not only on the mechanism of transmission (19,20), but essentially because the recipient might harbor bacterial organisms indistinguishable from those that are transmitted (21). On the other hand, efficient transmission able to influence colonic microbiota depends on the number of bacteria in the donor host, and the colonizing ability of different bacteria, not only in the intestine, but also in intermediate locations in the body, as probably the mouth or upper intestine (22). In any case, to show the potential effect of different bacterial loads acting as inocula, in our model we used different population densities, considering a final inoculum reaching the colonic compartment equivalent to 0.1%, 0.3%, 0.5% and 1% of the donor microbiota. As in previous cases, the evolution of multi-resistance favors *E. coli* (Fig SI4). Multi-resistant *E. coli* emerges earlier and reaches higher counts in higher-counts inocula, but there is a maintenance of less resistant strains because the higher-counts inocula might also contain more susceptible bacteria.

Intensity of the effect of antibiotics on model targeted populations (MTPs). The question of the relation of the "potency" (intensity of antibacterial activity) of

antibiotics in relation with the selection of resistance has been a matter of recent discussions (23, 24, 25, 26). To illustrate the point, we changed the bactericidal effect of the antibiotics used in the model. Model-target species were killed at rates of 30%-15% the first and second hour of exposure respectively, and these rates were decreased to 7.5-3.75%. As illustrated in figure 9, the more susceptible *E. coli* phenotypes (red, violet lines) are maintained for longer when the killing intensity of antibiotics is lower; on the contrary, the multi-resistant phenotype (dark blue) emerges earlier and reaches higher numbers when the intensity of antibiotic action increases. Under high antibiotic intensity, there is also a (small) increase in the resistant *K. pneumoniae* and *E. faecium* phenotypes. This experiment shows that a high rate of elimination of the more susceptible bacteria favors the colonization by the more resistant ones, the typical selective process.

Intensity of the antibiotic effect on colonic microbiota (BCM). Increase in the colonization space triggered by antibiotic exposure, acting on BCM populations, constitutes an important factor in the multiplication of potentially pathogenic MTP bacteria, and hence facilitates acquisition (mutational or plasmid-mediated) of resistance, and increases transmission to other hosts. In the basic model, reduction of BCM is 25% for Ab1, 20% for Ab2, and 10% for Ab3; in our computational experiment these proportions were modified to 10%, 5% or 2% respectively. The result of this change is impressive (Figure 10): not only the amount of MTP bacteria are reduced but the evolution towards antibiotic resistance (EC) occurs at a slower rate, and even if the proportion of resistance phenotypes steadily increases along time, its absolute number does not grow, thus limiting host-to-host transmission. Note the smoother profile of the evolution of resistance under low intensity of antibiotics.

Strength of antibiotic selection on particular resistance traits. Strength of antibiotic selection is an important parameter in evolutionary biology of antibiotic resistance (27). Our computable model allows decision about the strength of selection of an antibiotic for a particular resistance trait, considering how the resulting trend is (or not) compatible with the observed reality. An example case is the question: -do plasmid-mediated cefotaxime-resistance (Ab2-R) also provide protection against aminopenicillins (Ab1R)? This point has not been addressed in the literature. Hypothetically, strains harboring TEM- or SHV-extended-spectrum beta-lactamases hydrolyzing cefotaxime retain sufficient levels of aminopenicillin hydrolysis to be selected by aminopenicillins. However, the phenotype cefotaxime-resistant/aminopenicillin-susceptible is rare in hospital isolates. In our model, this was investigated providing different strengths of ampicillin (Ab1) selection for a cefotaxime-resistant phenotype (Ab2-R): no selection (0%), selection only in 10% of the cases (10%), and full selection (100%). The results of the model (Fig SM5) show that if cefotaxime-resistance (Ab2-R) was fully effective to protect against aminopenicillins, the phenotype aminopenicillin-susceptible and cefotaxime-resistant (light and olive green in the figure) should be prevalent from relatively early stages. This is not what is observed in the natural hospital environment. These data suggest that ampicillin provides only a small protection (10% or less) against cefotaxime challenge.

Discussion

The rate of antibiotic resistance among bacterial species in a given space is the result of the interaction of elements in a complex parametric field (28, 29, 30). There is a need to consider in an integrated way how changes in these parameters might influence the

evolution of resistant organisms. This endeavor requires the application of new computational tools that should take into account the nested structure of the microbial ecosystems, where mechanisms of resistance (genes) can circulate in mobile genetic elements among bacterial clones and species belonging to genetic exchange communities (12, 13) located in different compartments (as the hospital, or the community). A number of different factors critically influence the evolution of this complex system, such as antibiotic exposure (frequency of treated patients, drug dosages), the strength of antibiotic effects on commensal bacterial communities, the replication rate of the microbial organisms, as well as the fitness costs imposed by antibiotic resistance, the rate of exchange of colonized hosts between compartments with different levels of antibiotic exposure (hospital and community), or the rates of cross-transmission of bacterial organisms among these compartments. The challenge that we were addressing in this work was to simultaneously combine for the first time in a single computational model all these (and potentially more) factors to understand the selective and ecological processes leading to the selection and spread of antibiotic resistance. In comparison with available classic mathematical models that have been applied to the study of evolution of antibiotic resistance (31), the one we are discussing in this work is far more comprehensive in terms of the level of capture of the multi-level parametric complexity of the phenomenon. Note that our model includes random exchanges between elements, and therefore results are to a certain extent of probabilistic nature, and not as rigid as a fully deterministic model. Results obtained with the model presented in this work correspond only to a very limited number of possible “computational experiments”. Our choice tends to show the possibilities of the model, rather than to address particular “real” problems. Indeed, a virtually unlimited number of other experiments, with different combinations of parameters, are feasible in a

relatively simple way. The practical usefulness of our model will depend on the possibility of entering own data in a user-friendly interface, facilitating programs" *à la carte*".

It is of note that one of the main values of our model is its instrumental heuristic nature (32). As is presented here, its heuristic role is to illustrate principles, generate hypothesis and guide and facilitate the interpretation of empirical studies, not to provide numerically precise analogs of the dynamics of antibiotic resistance (33). In our work, we have examples of heuristic predictions as the possible minor role of aminopenicillins (AMPs) in the selection of extended-spectrum beta-lactamases (Ab2R), or the possibility of the presence of plasmids containing aminopenicillin-resistance in *K. pneumoniae*, phenotypically "invisible" as this organism has chromosomal resistance to the drug. Heuristic models of this sort are most useful when they detect discrepancies in a qualitative way in parameters able to explain observational facts. In such a way the parameters can be modified and re-tested in the model, ideally pointing to approach values and processes that can account for the deviation between observations and theory, following a classic knowledge path (33).

We have included in the architecture of the membrane computing model designed for this work several new concepts that have recently emerged in the field of evolution of eco-biology of antibiotic resistance. Results are presented in terms of the ensemble of biological entities contained in the whole landscape (ecosystem, for instance the hospital), and not referred to the "small world" of individual hosts. This a more ecological approach for the analysis of resistance, resembling "pooling" techniques (34), and tends to reflect environments (as the hospital) as single "big world" units colonized by "broader populations" than those of the individual host. In this context, transmission of microbiota between hosts, and the resulting colonization events apply

not only to resistant, but also susceptible microorganisms, in this sense “promoting health” (35), and probably assuring the long-term survival of susceptible populations. In relation with this view, how might antibiotics modify the available colonization space in the host? Competition for colonization space has been previously considered for couples of variants of pathogenic bacteria sharing the same niche, as in MRSA/M, favoring MSSA (36, 37) but not to our knowledge considering the intestinal space occupied by normal microbiota. Also, recent studies indicate that strong antibiotic exposure, not only significantly increases the number of bacteria formerly in minority, as Proteobacteria (represented in our model by *E. coli*, *Klebsiella*, and *Pseudomonas)*, but that the reduction of the BCM bacteria in the colonic space remains relatively limited. Our model includes the elimination of part of the colonic microbiota with antibiotic use, favoring the colonization of resistant organisms. In fact, the increase in the bactericidal intensity of antibiotics on the model-target bacteria also favors an earlier and denser colonization by multi-resistant phenotypes.

In our computational experiments we can reproduce the successive “waves” of increasingly resistant phenotypes, corresponding to succeeding events of selection (and habitat occupation) of intermediate resistant populations, giving rise to multi-resistant phenotypes, mimicking the clonal interference phenomenon (38) that indeed occur in the real world. The speed and intensity of this process depends on the global resistance landscape and the density and phenotype of the bacterial subpopulations. For instance, our model predicts that ciprofloxacin-resistance (a chromosomal-mediated trait arising by mutation) clearly enhances the possibility of evolving faster towards multi-resistance, by acquisition of the resistant organism of resistance genes by horizontal gene transfer (14, 15). We show that indeed this process towards multi-resistance might also develop in the community, but at a much slower rate. Moreover, we also show that

the long-term dissemination of chromosomally-encoded genes is by far less effective than the spread of traits encoded in transferable plasmids, even though some limitations are detectable because of plasmid incompatibility. A frequently overlooked aspect of antibiotic resistance suggested by our membrane computing experiments, is that probably the evolution of multi-resistance favors at long term some predominant species, as *E. coli*. Also, because of the overall limitation of spaces for multiplication in the intestine, other multi-resistant organisms, as *K. pneumoniae* or *E. faecium*, will remain at comparatively lower densities. Inside a single species (*E. coli*), there is also an increasing benefit for the more resistant lineages. However, plasmid-mediated resistance spread in all of them, resulting in a certain lineage equilibrium at long term. In fact, it is non-transmissible resistance (as ciprofloxacin) which finally create efficient fitness differences among lineages under antibiotic exposure.

The consequences of changes in the ecological (inter-hosts transmission) and selective (therapy) conditions of the hospital and the community were also explored in our computational model. For reasons of space, combinations of conditions (easy to perform) were not presented here. Several mathematical models have also investigated these changes (16, 37, 38, 39, 40, 41, 42, 43, 44, 45). Certainly, the effect of reducing patient discharges and admissions in the hospital increases the local rates of antibiotic resistance, as was previously suggested in former models, but not following a linear relation. In our model, the proportion of antibiotic treated patients in the hospital has the stronger effect on the rates of antibiotic resistance, stressing the importance of a precision-medicine based restrictive approach for prescribing antibiotic therapy. That has also been pointed out in previous mathematical modeling exercises (44). The role of increasing rates of inter-host transmission of bacteria (hospital cross-colonization) also influences the rise of resistance, but this effect seems lower than expected, probably

because higher transmission rates also assures transmission of the more susceptible antibiotic populations, a kind of “washing out” process of resistance, similar to the one that occurs when the community-hospital flow increases (16). The model also predicts that the “amount” of bacteria transmitted between hosts favors the ascent of antibiotic resistance, which is of interest when examining the ecological conditions surrounding host-to-host transmission in underdeveloped countries. As stated in the previous paragraph, in our model we consider another frequently overlooked factor: the “intensity” (aggressiveness) of the antibiotic therapy, because of frequent dosage and particularly in terms of its intrinsic ability to reduce the commensal colonic microbiota, and therefore “colonization resistance” for resistant opportunistic pathogens (47). Finally, we presented an example of the usefulness of our model in casting light on questions of almost impossible experimental investigation in the field, as the possible selection of cefotaxime resistance by aminopenicillins.

We should advert that our results do not refer to any particular (objective) situation, and that they cannot be taken as data necessarily reflecting the complex reality of antibiotic resistance. Parameters in our basic model have been chosen to illustrate the possibilities of our computational approach. Of course, precise data are not always easy to obtain, and the type of model should influence the results (48), However, because of the functional analogy of membrane computing with the biological world, we trust that the trends revealed in our computational model reflect general processes in the evolutionary biology of antibiotic resistance. If the model were fed with objective data extracted from a real landscape (which will be possible with a user-friendly interface), it could provide a reasonable expectation of the potential evolutionary trends in this particular environment and could support the adoption of corrective interventions (49). Finally, we would like to stress that the type of membrane-computing model that was applied in this

work can be easily escalated or adapted to a variety of applications in systems biology, and particularly to understand complex ecological systems with nested hierarchical structures and involving microorganisms. This new tool might provide useful predictions for risk analysis, including risk assessment and risk management, in critical problems of Global Health, as for anthropogenic-driven changes of the microbiosphere.

Material and Methods

Computational model. In this article, we applied to the analysis of the dynamics of antibiotic resistance an adapted version of the ARES (Antibiotic Resistance Evolution Simulator) software device that we have recently developed (8). ARES uses embedded membrane-model scenarios that in our case includes several types of nested computing membranes oriented to simulate a hierarchy of biological compartments, and emulating entities such as: i) resistance genes, able to detoxify antibiotics, located in the plasmid, other conjugative elements or in the chromosome; ii) plasmids and conjugative elements transferring genes between bacterial cells; iii) bacterial cells; iv) microbiotas where different bacterial species and subspecies (clones) can meet; v) hosts containing the microbiotic ensembles; vi) environment(s) where the hosts are contained. Other computational objects can be introduced, either to tag particular membranes, or to interact with the embedded membranes, for instance mimicking antibiotics, according to a set of pre-established rules and specifications. A global “skin” membrane surrounds the system. The software implementation 2.0 of ARES can be downloaded at https://sourceforge.net/projects/ares-simulator/. Although ARES can be run in any computer it is highly recommendable to install it in at least a 4× 6 Core Server and 128 GB of RAM because the performance of this simulator may have significant requirements in both processor and RAM depending on the complexity of the simulation’ scenario and the number of iterations.

Anatomy of the model application. The current application of the model was structured accordingly with the following composition: 1) There are two compartments containing individual hosts at particular densities (humans or animals), mimicking a hospital environment (H) and a community environment (C); flux of individuals between both compartments occurs at variable rates, mimicking admission or discharge from the hospital. 2) There are four clinically relevant bacterial species (populations) colonizing these hosts, that we designate "Model Targeted Populations" (MTPs): EC, representing *Escherichia coli*; EF, *Enterococcus faecium*; KP, *Klebsiella pneumoniae*), and PA**,** *Pseudomonas aeruginosa*. These populations might diversify from their initial phenotype by acquisition of mutations and/or be recipients for mobile genetic elements, plasmids PL1 and for EC, KP, PA circulating in these species, or, in EF, integrative-conjugative, ICE elements (only for EF). Populations might differ by harboring resistance genes inactivating three antibiotics, Ab1, Ab2, Ab3, respectively mimicking amino-penicillins (ampicillin-amoxicillin, AMP), third-generation cephalosporins (cefotaxime-ceftriaxone, ceftazidime, CTX), and fluoroquinolones (ciprofloxacin, levofloxacin, FLQ), and in the location of these genes (chromosome or plasmid). Acquired resistance (not intrinsic) to Ab1 (Ab1R) is mediated by the acquisition of PL1 (or CO1), resistance to AB2 (Ab2R, also providing Ab1R) by acquisition of PL1 containing the Ab2R resistance determinant, and resistance to Ab3 (Ab3R) by mutation. Note that our representations, for example, when EC0 (susceptible) receives PL1 with Ab1R it becomes EC1, if it receives PL1 with Ab2R it becomes EC2, and when EC0, EC1 or EC2 mutate to Ab3R they are converted in EC3, EC13, and EC23. The genetic acquisition of PL1 with Ab1R by EC23 or PL1 with Ab2R by EC13 produces the multi-resistant strain EC123. Of course, the order of acquisition of antibiotic traits could be any other one. In some cases, we follow the evolution of resistance of a single clone (for

instance EC0), so that this clone is the only substrate for the emergence of all other resistance variants.

The cell can maintain two copies of the plasmid PL1 (containing resistance to Ab1 (PL1-Ab1R) or Ab2 (PL1-Ab2R) but not more, so that when a third plasmid PL1 enters into the cell, one of the three is stochastically removed. The plasmid PL1-Ab2R is able to produce resistance to both Ab2 and, in 10% of the cases, also to Ab1. Between EFs there is a “plasmid-like” (conjugative-element) mechanism of transfer of chromosomal gene AB1R (CO1-Ab1R). Only a single copy of CO1-Ab1R is accepted in the receiving host.

Quantitative structure of the basic model application.

Hospitalized hosts in the population. The number of hosts in the H and C environments reflects an optimal proportion of 10 hospital beds per 1,000 individuals in the community (https://data.oecd.org/healtheqt/hospital-beds.htm). In our model, the basic H compartment has 100 occupied beds, and corresponds to a population of 10,000 individuals in the C compartment.

The admission and discharge from hospitals are equivalent, 3-10 individuals/10,000 population/day (http://www.cdc.gov/nchs/data/nhds/ 1general/). In the basic model, 6 individuals from the community are admitted to the hospital and 6 are discharged from the hospital to the community per day (approximately at 4 hour-intervals). The patients are stochastically admitted or discharged, meaning that about 75% of the patients stay in the hospital between 6 and 9 days.

The bacterial colonization space of the model-targeted clones-species populations (MTPs) and other basic colonic microbiota (BCM) populations is defined as the physical space (volume) occupied by these bacterial populations. In natural conditions,

the sum of all CSPs considered has **10^8 cells per ml** (cm^3), of the colonic content. But the number of cells in 1 ml are not only of MTPs. These constitute only 1% of the cells in each ml (cm^3), as the other are BCMs, typically members of the healthy microbiota of the colon (BCM, see below). Consequently, the bacterial populations of our interest (MTPs) have (per ml) a basal colonization space of 1ml/100 = 0.01 ml.

The ensemble of other BCMs populations is considered in our basic study model as an ensemble surrounded by a single membrane. The space occupied by these BCM populations can change because of antibiotic exposure. In our model, we consider that along a treatment course (7 days) the antibiotics Ab1 (AMP), Ab2 (CTX) and Ab3 (FLQ) reduce the intestinal microbiota 25%, 20% and 10% respectively. As an example, if we consider for instance that 10% of the BCM populations were eliminated by antibiotic exposure, their now empty space (0.1 ml), will be occupied by antibiotic resistant MTPs, and by the BCM populations that have survived the challenge. Therefore, the colonization space available for resistant strains increases with antibiotic exposure, as does the total number of MTP populations. In the absence of antibiotic exposure, the BCM populations are naturally restored in their original population size, and this process has been modeled to occur in two months**.** MTP populations are comparatively faster in colonizing the empty space.

MTPs operative packages and counts. To facilitate the process of model running, we consider that 10^8 cells in nature is equivalent to 1,000,000 cells in the model. In other words, one “hecto-cell” (h-cell) in the model is an “operative package” of 100 cells in the real world. Because of the very high effective population sizes in bacteria, these 100 cells are considered as a uniform population of a single cell type. Similarly, for making affordable the computing process, and to allow considering a “pooled-superpopulation" constituted by the sum of MTPs of all hosts in a compartment, we considered that each

patient (in hospital) or individual (in the community compartment) is represented in the model by 1 ml of its colonized colonic space (about 3,000 ml) and is referred as a “host-ml”. Consequently, in most of the figures we represent our results as “number of h-cells in all hosts-ml".

Quantitative distribution of species and clones in MTPs. In the basal scenario, we consider that the distribution of MTPs in these 1,000,000 cells, contained in 1 ml (see former paragraph) is the following: For EC, 860,000 cells, including 500,000 susceptible cells, 250,000 containing PL1-Ab1R, 100,000 with the AbR3 mutation, and 10,000 with both PL1-Ab1R and AbR3 mutation; for EF, 99,500 Ab1 susceptible (EF1) and 20,000 Ab1R (EF2), resistant to Ab1. For KP, 20,000, with chromosomal Ab1R, PL1-Ab2R and Ab3R; and PA, 500 containing PL1-Ab2R. At time 0, this distribution is identical in hospitalized and community patients. This distribution changes under the influence of factors as antibiotic exposure, antibiotic resistance, the flow of the patients between community, and hospital, and plasmid transfer rates.

Tagging lineage populations in *E. coli*. The intention is to be able to follow the evolution of particular lineages (clones) inside *E. coli.* For this purpose, four clones were used, differing in the original resistance phenotype, EC0 as a fully susceptible clone, EC1 harboring PL1 determining Ab1R, EC2 harboring Ab3R, and with EC3 with PL1-Ab1R and Ab3R. At time 0 each one of these clones is tagged with a particular object (ec0, ec1, ec2, ec3) which remains fixed to the membrane, multiplies with the membrane, and is never lost. Each one of the daughter membranes along the progeny can alter its phenotype by mutation or lateral gene acquisition, but the original clone will remain detectable.

Multiplication rates. We consider the basal multiplication rate the one corresponding to EC0, where each bacterial cell gives rise to two daughter cells every hour. Note that

the number cell replications will be limited by the available space (see above, point 3). This “time for duplication” of EC0 is considered as 1. In the basic model (of course these parameters can be modified) other species have lower multiplication rates; EF=0.85, KP=0.9, and PA=0.15 (the lower multiplication rate of PA is due to the difficulties in multiplying in the colonic anaerobic environment). The acquisition of a plasmid of a mobile element imposes an extra cost of 0.03, and also the acquisition of mutational resistance Ab3R. Therefore, we have the following rates: EC0=1, EC1=0.97 (because of the cost of PL1-Ab1R), EC2=0.97 (cost of Ab3R), EC3=0.94 (PL1-Ab1R and Ab3R3), EF1=0.85, EF2=0.79 (CO1-Ab1R and Ab3R), KP=0.84 (PL1-Ab1R y Ab3R), and PA with PL1-AR2 =0.12 (PL1-AR2).

Transfer of bacterial organisms from one host to another one is expressed by the proportion of individuals that can stochastically produce an effective transfer of susceptible or resistant MTP bacteria to another one (contagion index, CI). If contagion is 5% that means that from 100 patients, 5 “donors” transmit bacteria to 5 other “recipients” per hour. In the case of the basic scenario, CI=5 in the hospital and CI= 0.01 in the community. Lack of appropriate sanitation, crowding, hospital hygiene deficiencies, and failure in interventions to prevent cross-infection increase this index. In the basic scenario, donors contribute to the colonic MTP colonic microbiota of recipient individuals with 0.1, 0.3, 0.5 and 1% of their own bacteria. Note that this inoculum does not necessarily result from the *propagulum* size, but considers the addition of the endogenous multiplication, as proposed in other models (50).

Frequency of plasmid transfer between bacteria occurs randomly and reciprocally at an equivalent high frequency among EC and KP; in the basic model, the rate is 0.0001, one effective transfer occurring in 1 of 10,000 potentially recipient natural cells. Plasmid transfer occurs at a lower rate, of 0.000000001 in the interactions of EC and KP

with PA. Plasmid (or integrative-conjugative-elements) mediated transfer of resistance among EF occurs at a frequency of 0.0001, but EF are unable to receive or donate resistance genes to any of the other bacteria considered. In the case of EC and KP plasmids we consider plasmid limitation in the number of accepted plasmids, so that if a bacterial cell with two plasmids receives a third plasmid, there is a stochastic loss of one of the resident or the incoming plasmid, but all three cannot coexist in the same cell.

Mutational resistance is only considered in the present version of the model for resistance to AB3, which might correspond to fluoroquinolones in real life. Bacterial organisms of the model-targeted populations mutate to Ab3 at the same rate, 1 mutant every 10^{-8} bacterial cells.

Antibiotic exposure occurs at different rates in the H and C compartments. In the basic model, 5%-10%-20% of the individuals in the H compartment are under antibiotic exposure each day, each individual being exposed (treated) for 7 days. In the C compartment 1.3 % of individuals are under treatment (based on data on DDDs/1000 inhabitants/day, REF.), also are exposed each of them to antibiotics for 7 days. The proportion of antibiotics used in H and C are also different; in H, Ab1-Ab2-Ab3 are used at a proportion (percentage) of 30-40-30; in the C compartment, of 75-5-20 respectively. In the basic scenario a single patient treated with only one antibiotic. Antibiotics are administered every 6 hours along 7 consecutive days. Note that the starting time of using antibiotics in the model is not simultaneous, but randomly scattered along the scheduled time.

Intensity of the effect of antibiotics on susceptible model-targeted clones-species populations (MTPs). Antibiotics might differ in their killing kinetics of target bacterial populations. In our basic model we consider that, after each dose administered, all three (bactericidal) antibiotics induce after a decrease of 30% in the susceptible population

after the first hour after of dose exposure, and 15% in the second hour. The program assures that stochastically the antibiotic stochastically penetrates in these percentages of bacterial cells, and those that are susceptible are removed (killed). Therapy is maintained in the treated individual along 7 consecutive days.

Intensity of the effect of antibiotics on colonic microbiota (BCM). Antibiotics exert an effect reducing the density of the colonic commensal microbiota, resulting in free-space and nutrients that can benefit MTPs. In the basic model, such reduction is 25% for Ab1, 20% for Ab2, and 10% for Ab3.

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Figure Legends

Figure 1. Dynamics of bacterial resistance phenotypes in *E. coli*. Pink, susceptible; red, Ab1R (AMP); violet, Ab3R (FLQ); brown, Ab1R and Ab3R; light blue, Ab1R and Ab2R; dark blue, Ab1R, Ab2R and Ab3R; light green, Ab2R; olive green, Ab2R and Ab3R. In ordinates, number of hecto-cells (h-cells, packages of 100 identical cells) in all hosts-ml (each host represented by 1 ml of colonic content); in abscissa, time (1000 steps, roughly equivalent to 42 days).

Figure 2. Comparative dynamics of *E. coli* phenotypes in the hospital (left, up), and the community (left, down). Axes and color code, as in Fig 1. On the right, species dynamics in the hospital (up) and the community (down): E. coli (red), *K. pneumoniae* (olive green), *E. faecium* Ab1S (light blue), and *E. faecium Ab1R* (dark blue). *P. aeruginosa* is not visible in this representation (low numbers).

Figure 3. Influence of baseline *E. coli* resistance phenotypes composition on the dynamics of bacterial species. On the left, comparative dynamics of *E. coli* phenotypes in the basic hospital scenario (up) and with reduced numbers of resistant phenotypes (down). Colors and axes, as in Fig. 1. On the right, comparative dynamics of bacterial species in the basic model (up), and the reduced basal resistances (down); colors as in Fig 2.

Figure 4. Single lineage *E. coli* dynamics in the hospital: influence of baseline resistances. In pink, lineage starting with full susceptibility, in red, with Ab1R (AMP); in violet, with Ab3R (FLQ); in brown, with Ab1R and Ab3R.

Figure 5. Dynamics of a plasmid and resistance traits in the hospital environment. The species *E. coli*, *K. pneumoniae* and *P. aeruginosa* are included as a genetic exchange

community. In blue, total number of the plasmid PL1; in bright red, plasmid PL1 with the gene Ab1R (AMP); in green, PL1 with Ab2R (CTX); in violet, chromosomal Ab3R (FLQ) gene; in red-brown, chromosomal Ab1R (as in *K. pneumoniae*). In ordinates, number of plasmids or resistance traits in h-cells (packages of 100 identical cells) in all hosts-ml (each host represented by 1 ml of colonic content).

Figure 6. Influence of patients' flow between hospital and community. On the left, influence on *E. coli* resistance phenotypes in the hospital when one patient is admitted at/discharged from the hospital every 2 (up), 4 (mid), or 8 hours (down). On the right, subtractive representation of *E. coli* phenotypes in the hospital: 2h versus 4h (first panel); 4h vs. 8 h (second panel). Below, *E. coli* phenotypes in the community with a flow of 2h versus 4h (third panel); 4h vs. 8 h (fourth panel).

Figure 7. Influence of the frequency of patients treated with antibiotics. On the left, *E. coli* phenotypes when 20% (up), 10% (mid) or 5% (down) of patients receive antibiotics. In the right part, effect on bacterial species. Colors as in Figs 1 and 2.

Figure 8. Influence of the frequency of bacterial cross-transmission rates in the hospital. On the left, dynamics of *E. coli* phenotypes when bacterial exchanges between patients occur in 5% (up) or 20% (down) per hour. A subtractive representation is provided below (5 vs. 20%). On the right, influence on the species composition: 5% (up), and 20% (down). Colors as in Figs 1 and 2.

Figure 9. Influence of the activity of the antibiotic on *E. coli* phenotypes (left) and the species composition (right). Upper panels, susceptible bacteria are eliminated 30% after the first hour of exposure and 15% after the second hour; in the lower panels, the elimination is lower, 7.5% the first hour and 3.75% the second hour. Colors as in Figs 1 and 2.

Figure 10. Influence of the intensity of the antibiotic effect on colonic microbiota of patients in the hospital. On the left, effects on *E. coli* phenotype of a reduction in microbiota of 25% for Ab1, 20% for Ab2, and 10% for Ab3 (upper panel) ; these values were reduced to 10%, 5% or 2% respectively (lower panel). The effects on the species composition is shown at the right side.

Figures (Supplementary material)

Figure SM1. Three consecutive model iterations, in the three panels of the figure, representing the dynamics of *E. coli* resistance phenotypes in the hospital compartment. As the model include several stochastic and probabilistic steps, the results obtained are not entirely identical in replicated runs of the program. However, there are extremely close.

Figure SM2. Dynamics of *E. coli* lineages starting with different resistance phenotypes in the hospital compartment. On the left half, upper part, lineage EC0 starting without resistance and EC1 starting with Ab1R and Ab3R. On the right half of the figure, the same in logarithmic representation, allowing to perceive minority phenotypes.

Figure SM3. Dynamics of *K. pneumoniae* (left column), Ab1S (middle) and Ab2R (right column) *E. faecium* in the hospital environment. Colors code: In *K. pneumoniae*, plasmid mediated Ab2R plus Ab3R, without plasmid mediated Ab1R (green), plasmid-mediated Ab1R-Ab3R phenotype (purple), and plasmid-mediated Ab1R, plus plasmid-mediated Ab2R-Ab3R (blue). In *E. faecium*, starting Ab1S population (pink), Ab1R received by conjugation (red); in the third column, starting Ab1R population (brown). Upper and lower rows, arithmetic and logarithmic representations respectively.

Figure SM4. Influence of the size of transmitted bacterial load. On the left half of the figure, *E. coli* phenotypes dynamics in the hospital, when the mean transmitted bacterial load is equivalent to 0.1% (up), 0.5% (mid) or 1% (bottom) of the colonic microbiota. On the right side, evolution of the different species with these transmission loads. Color codes as in Fig 1 and 2.

Figure SM5. Expected dynamics of hospital-based *E. coli* under the hypothesis that Ab2R might provide: 0% of resistance to Ab1 (upper panel), 10% (mid panel), or 100% (lower panel). Colors as in Fig 1.

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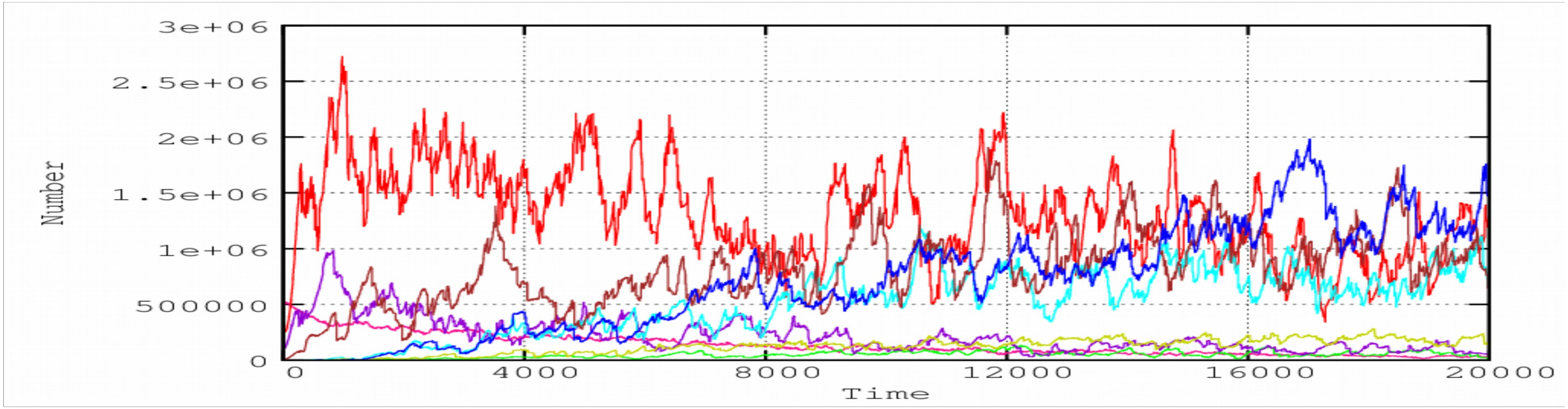
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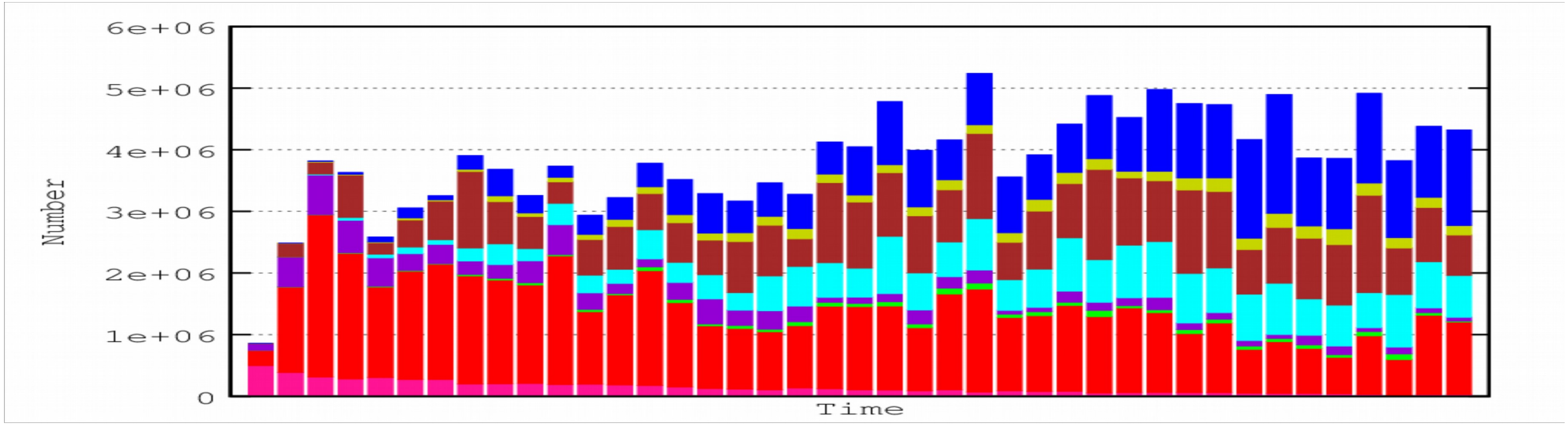
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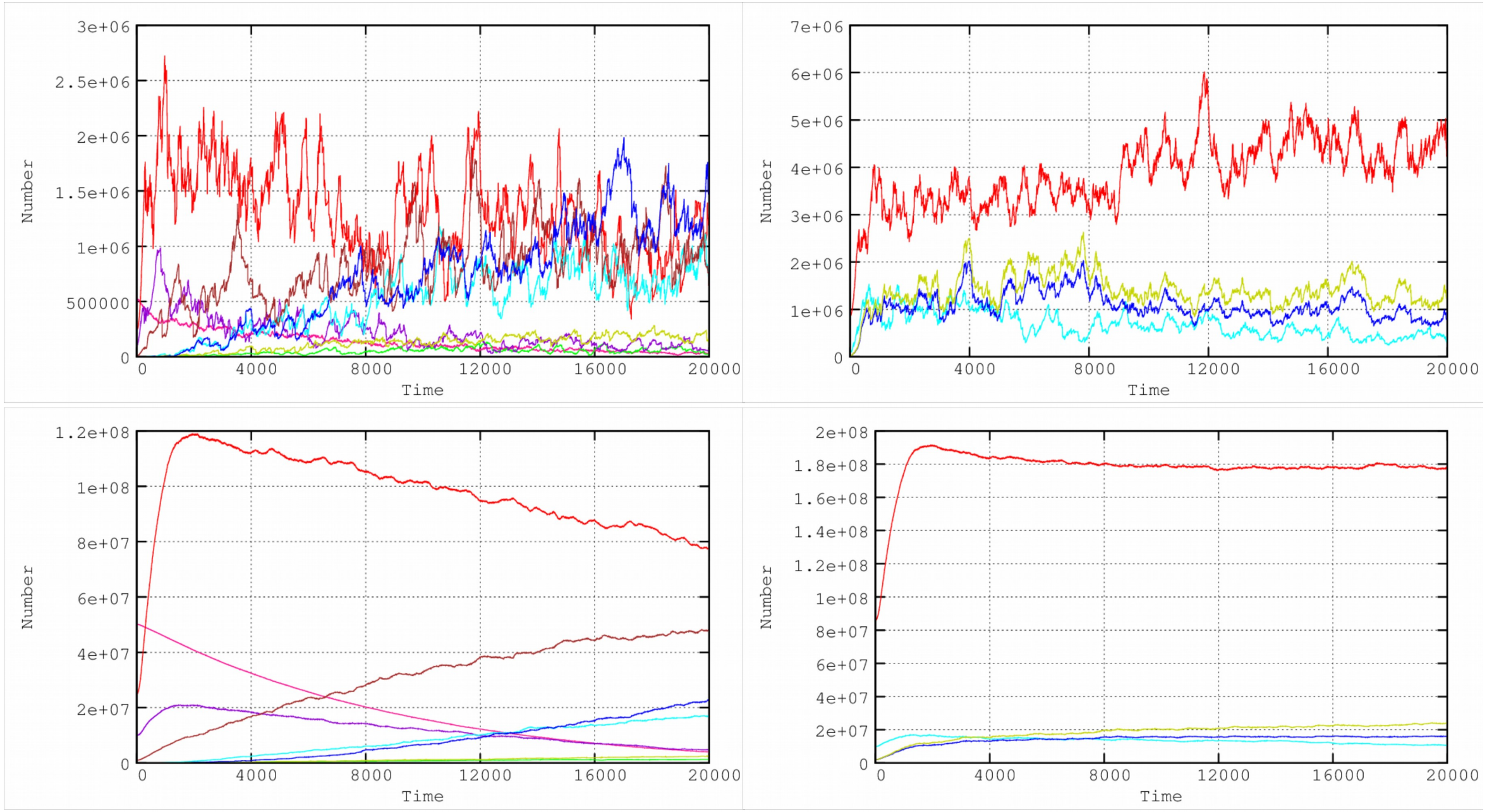
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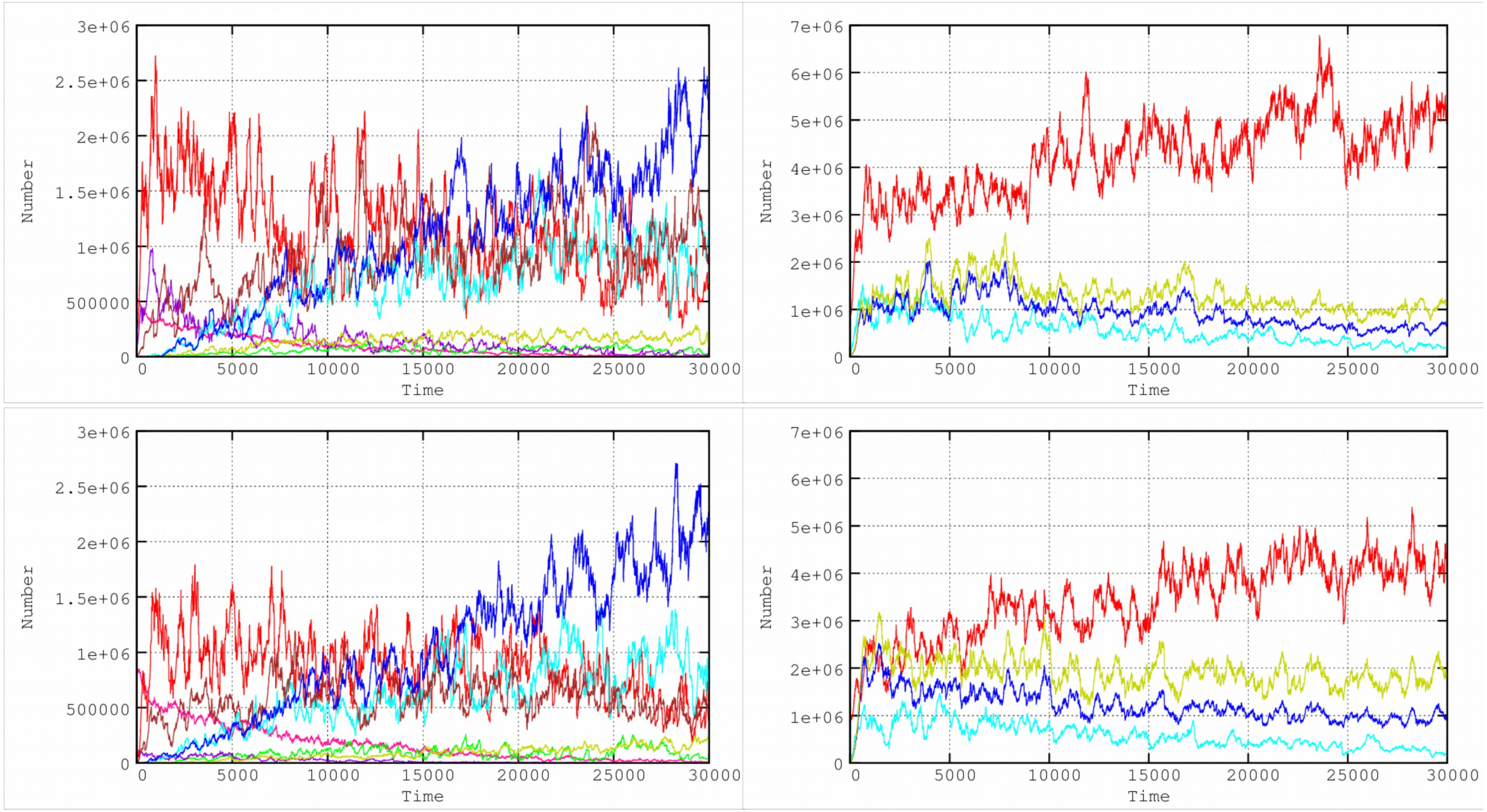
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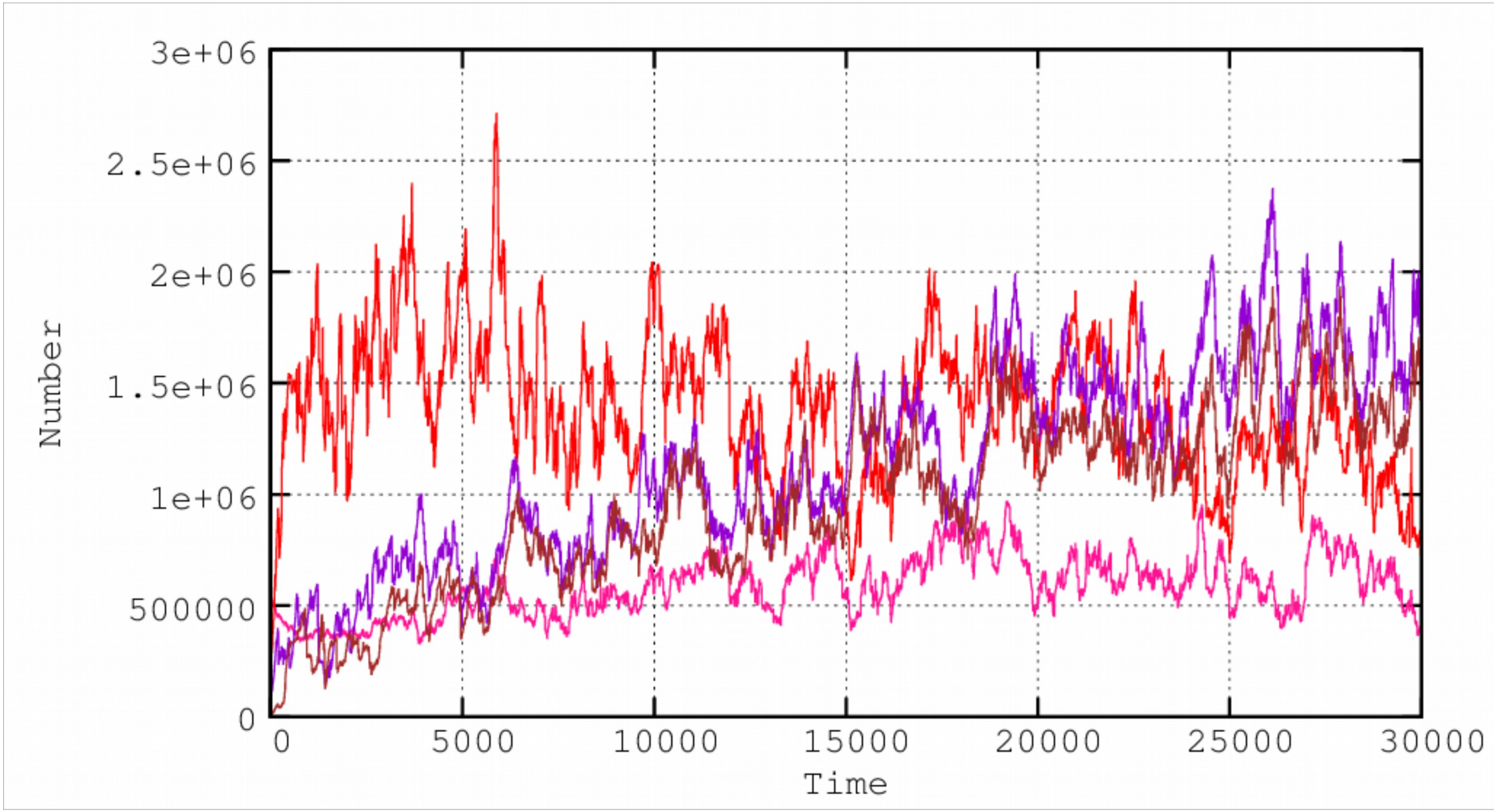
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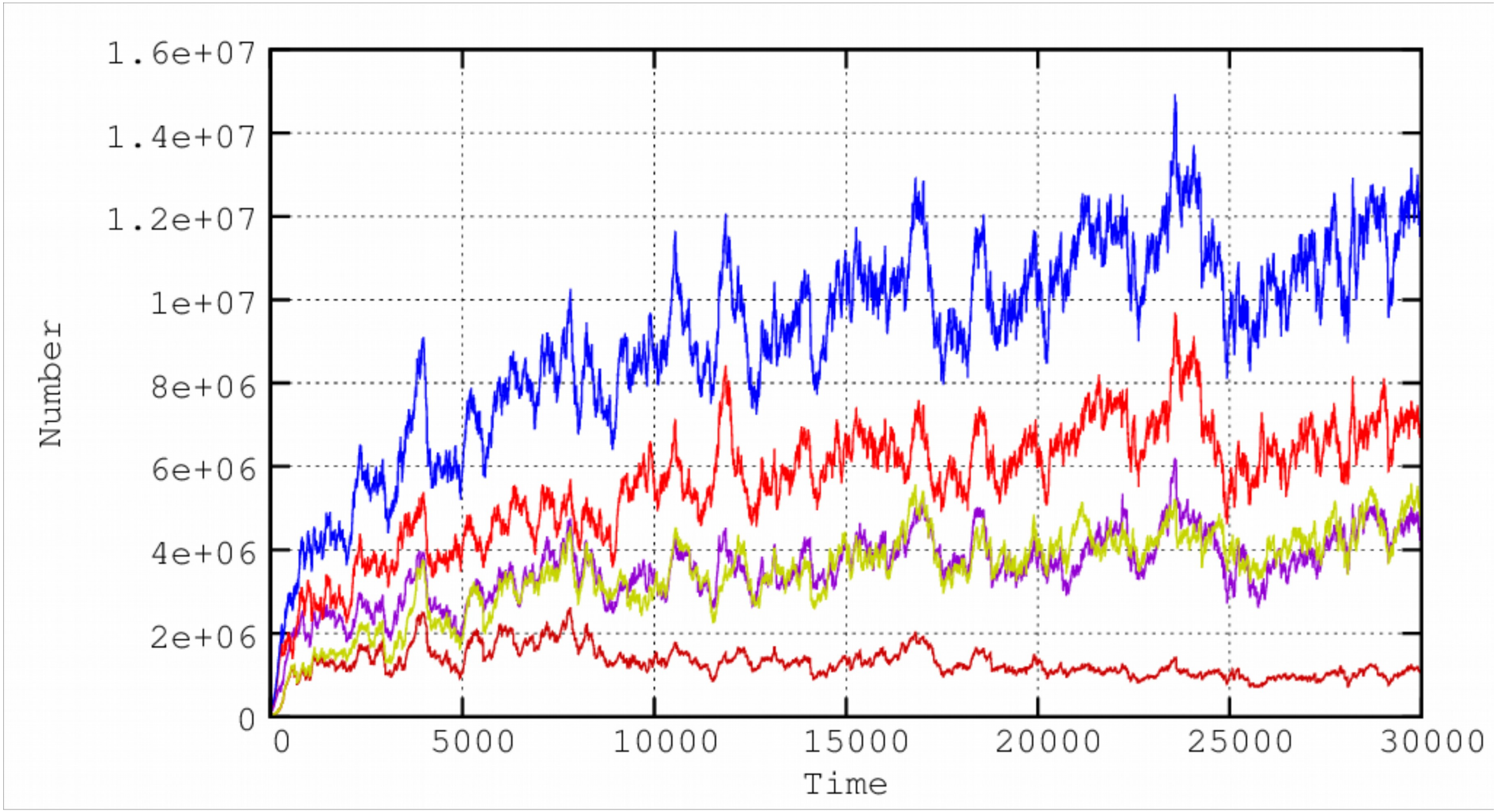
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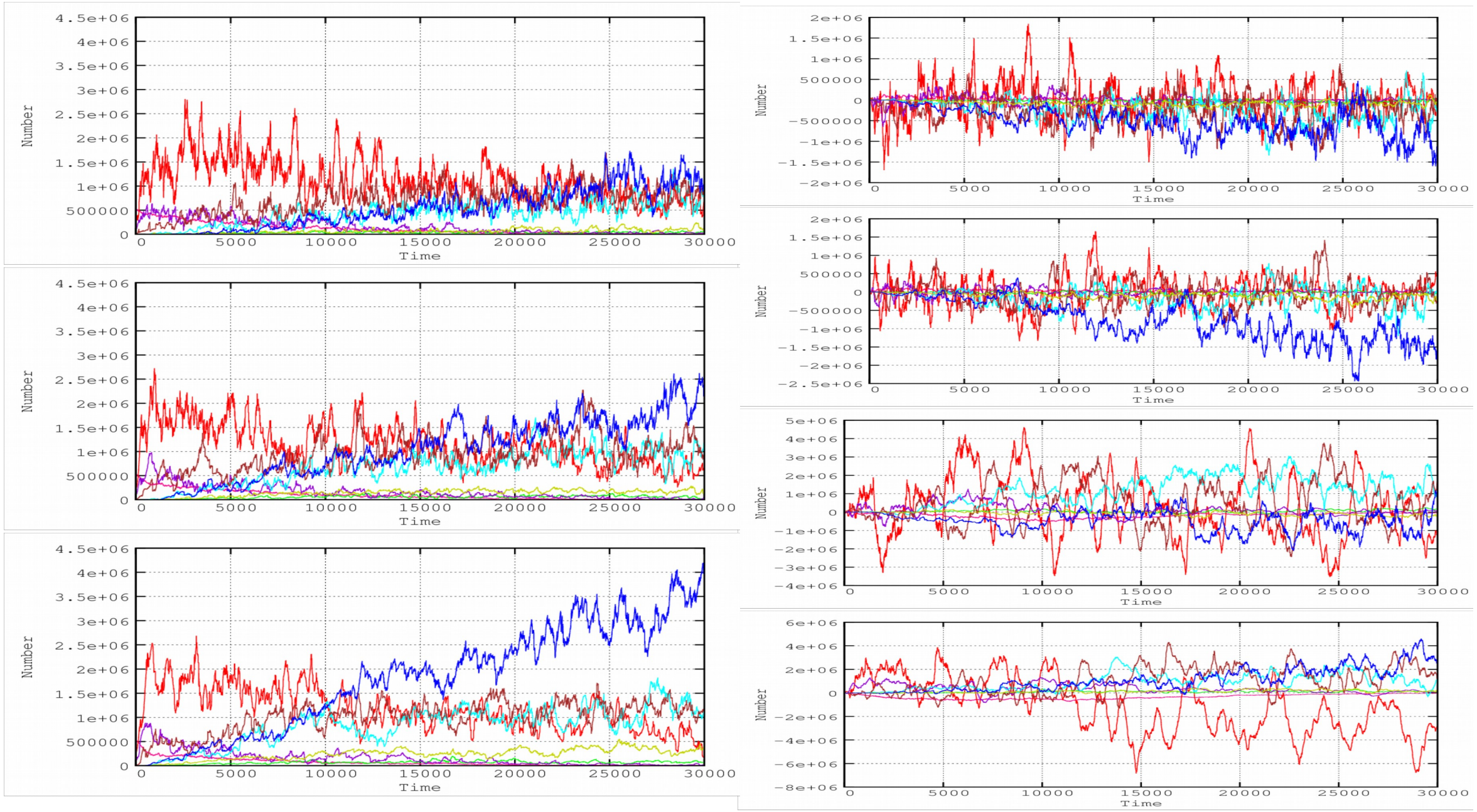
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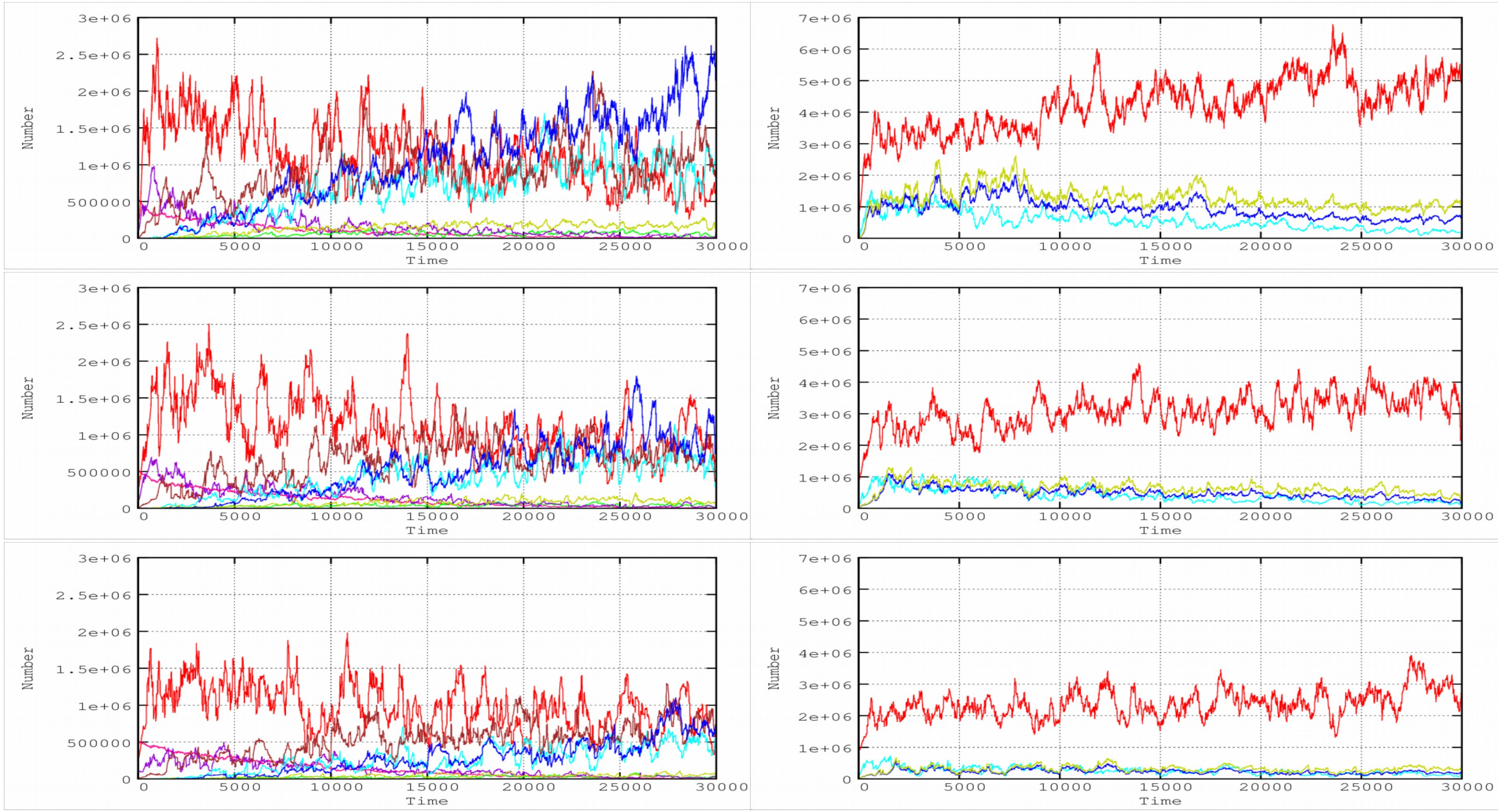
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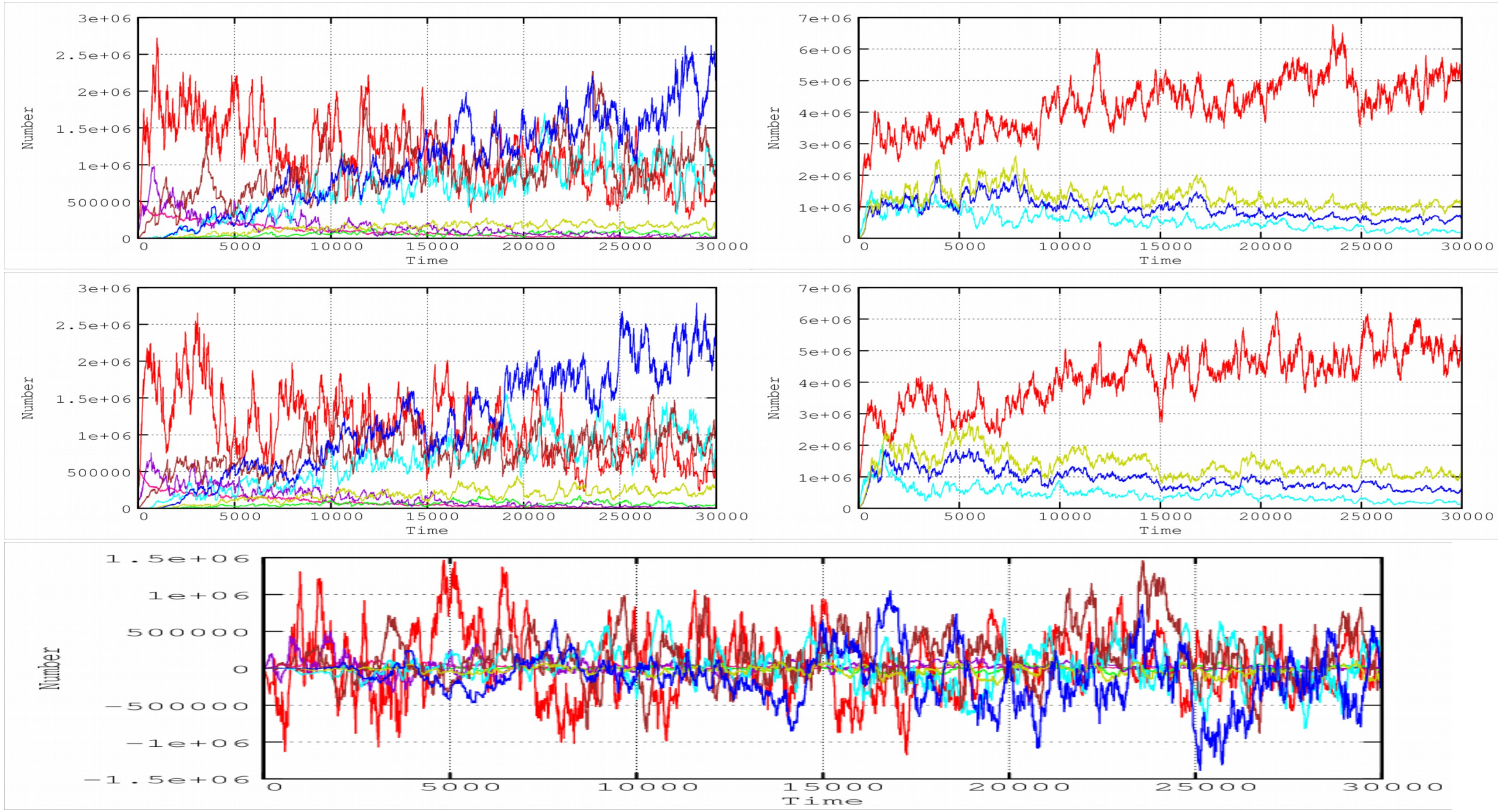
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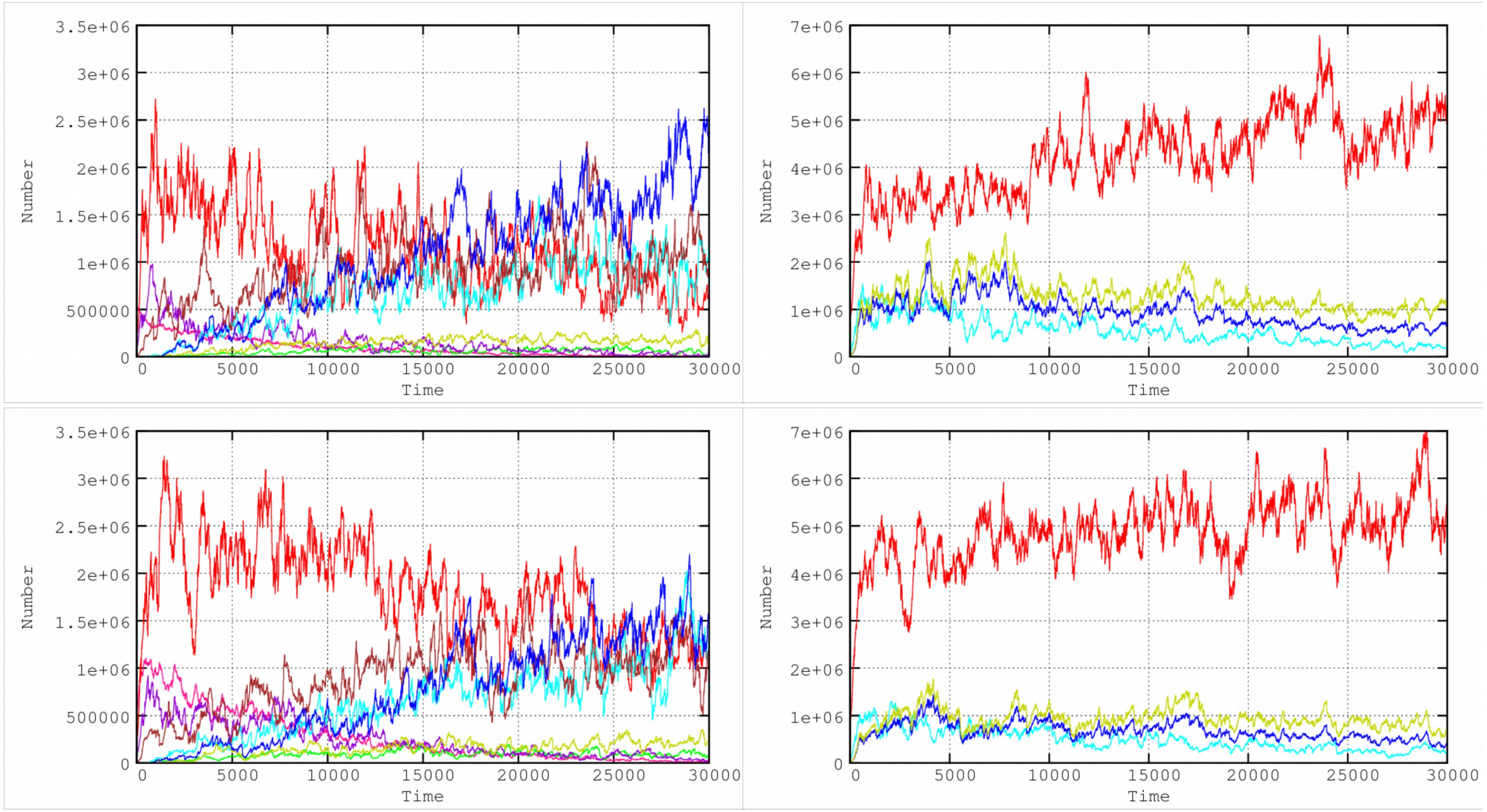
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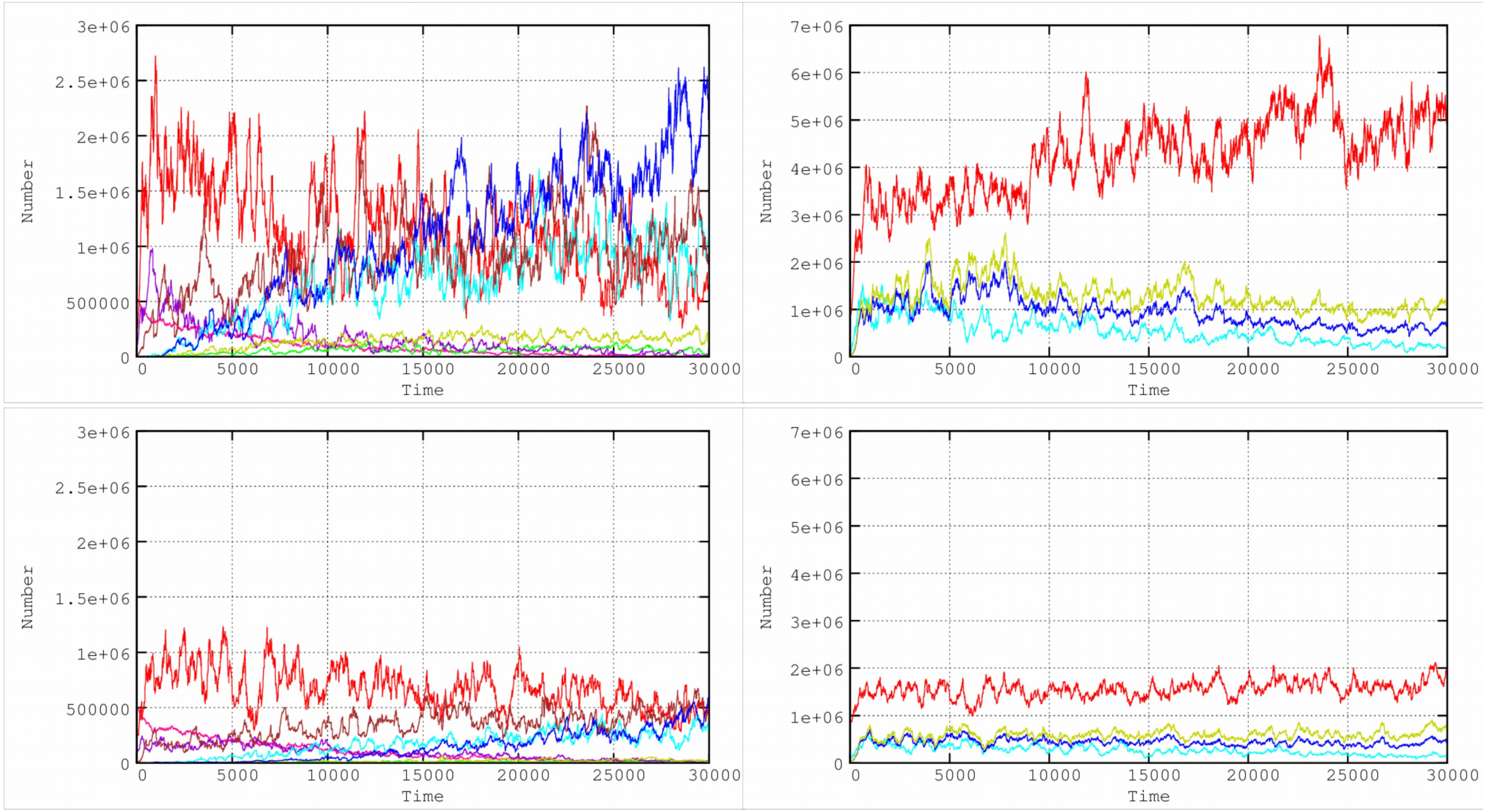
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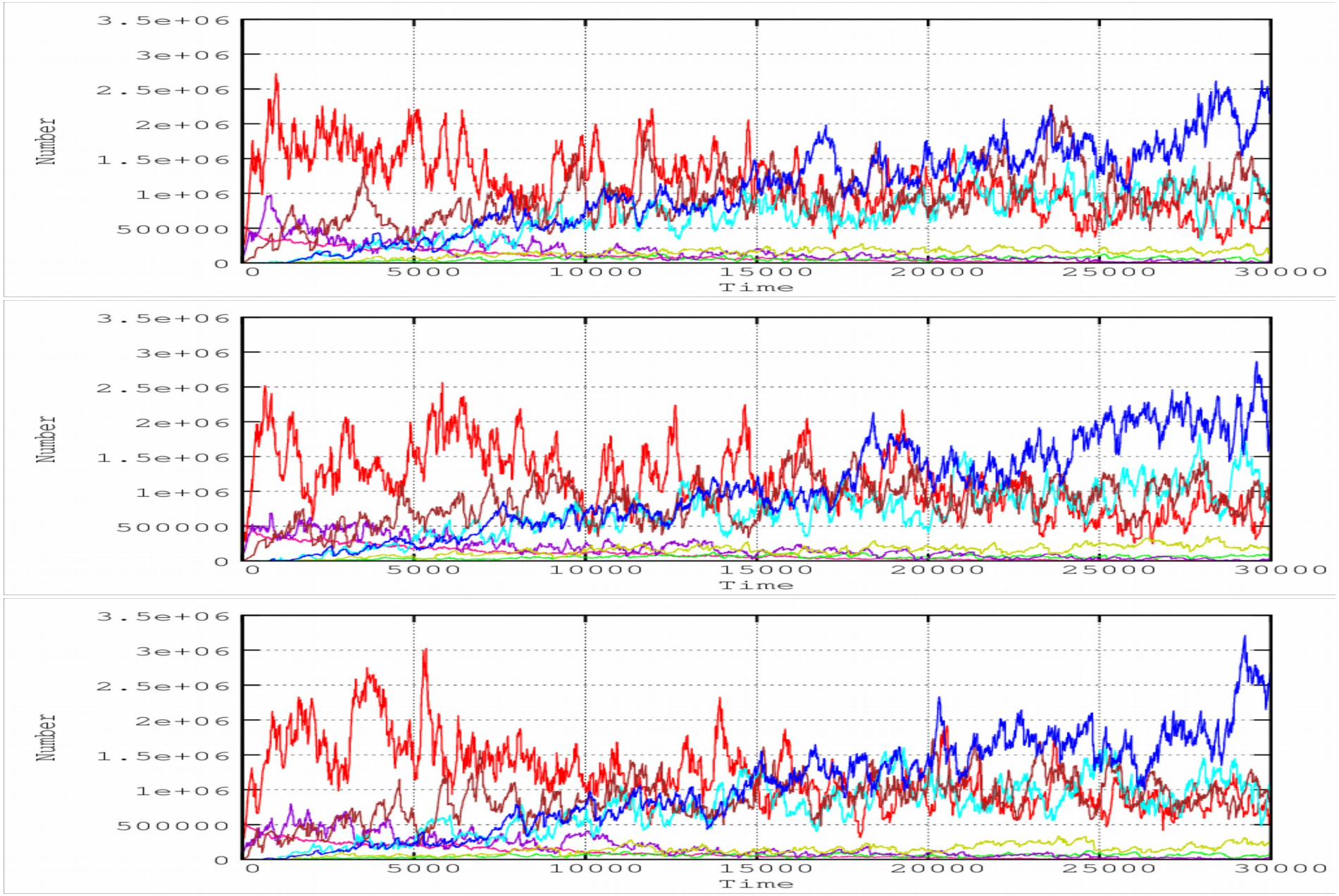
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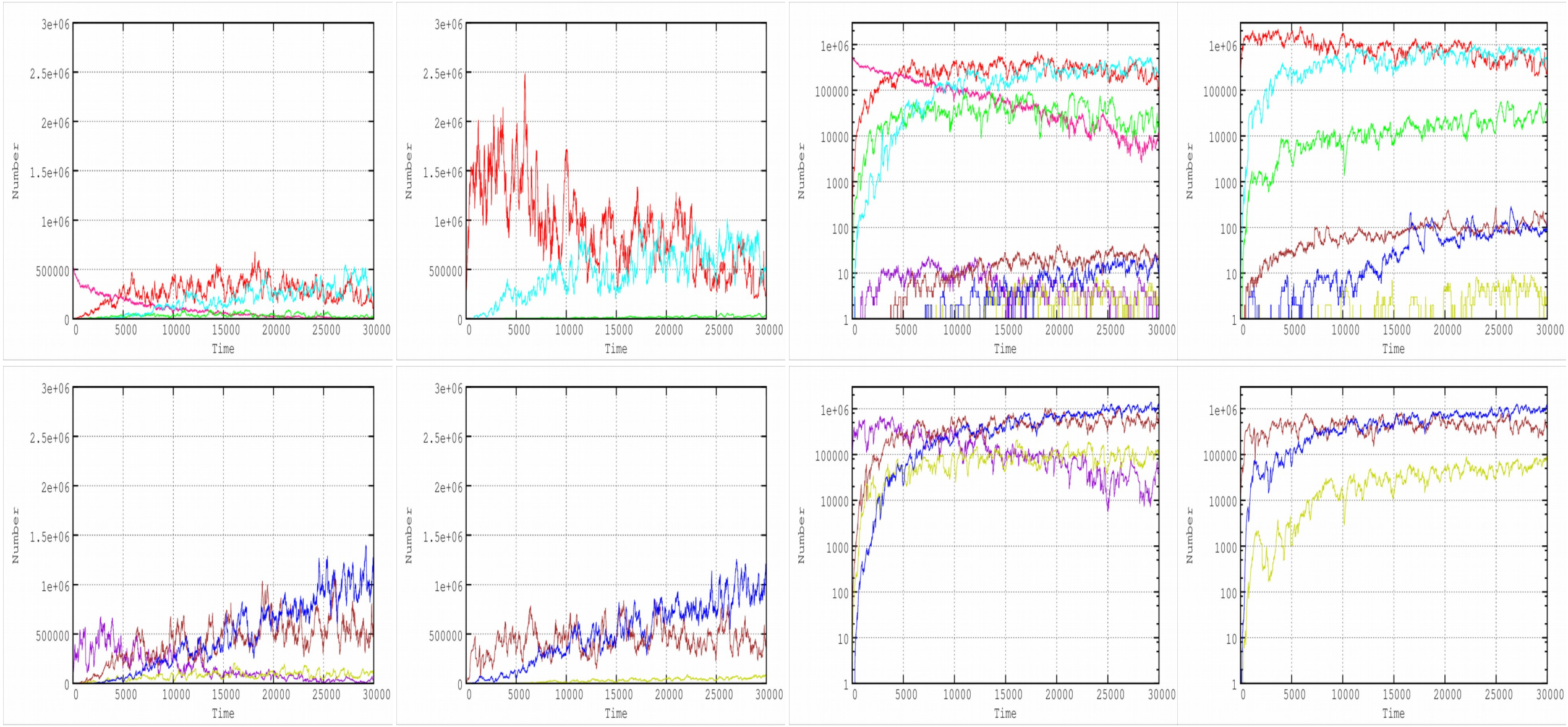
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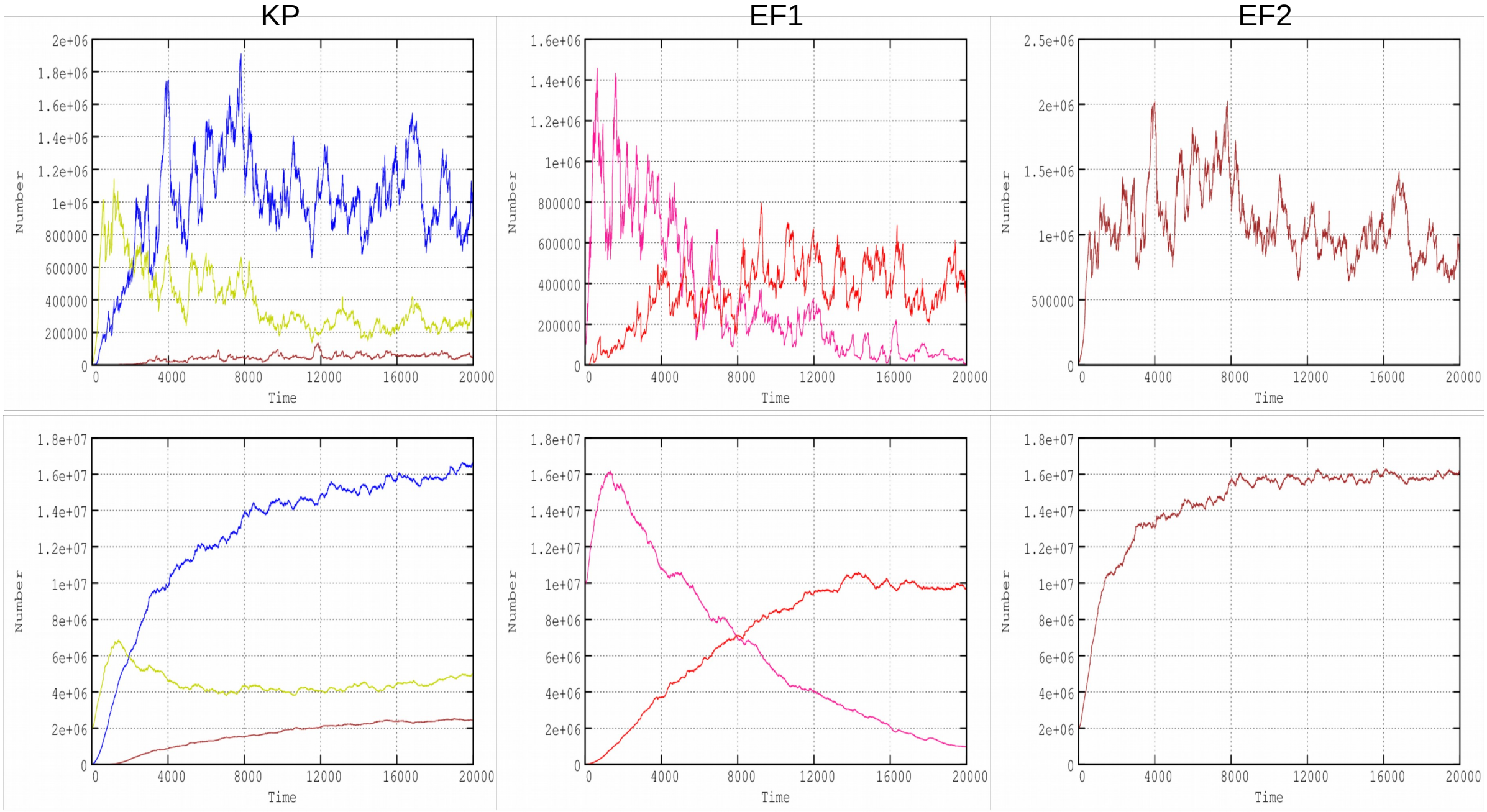
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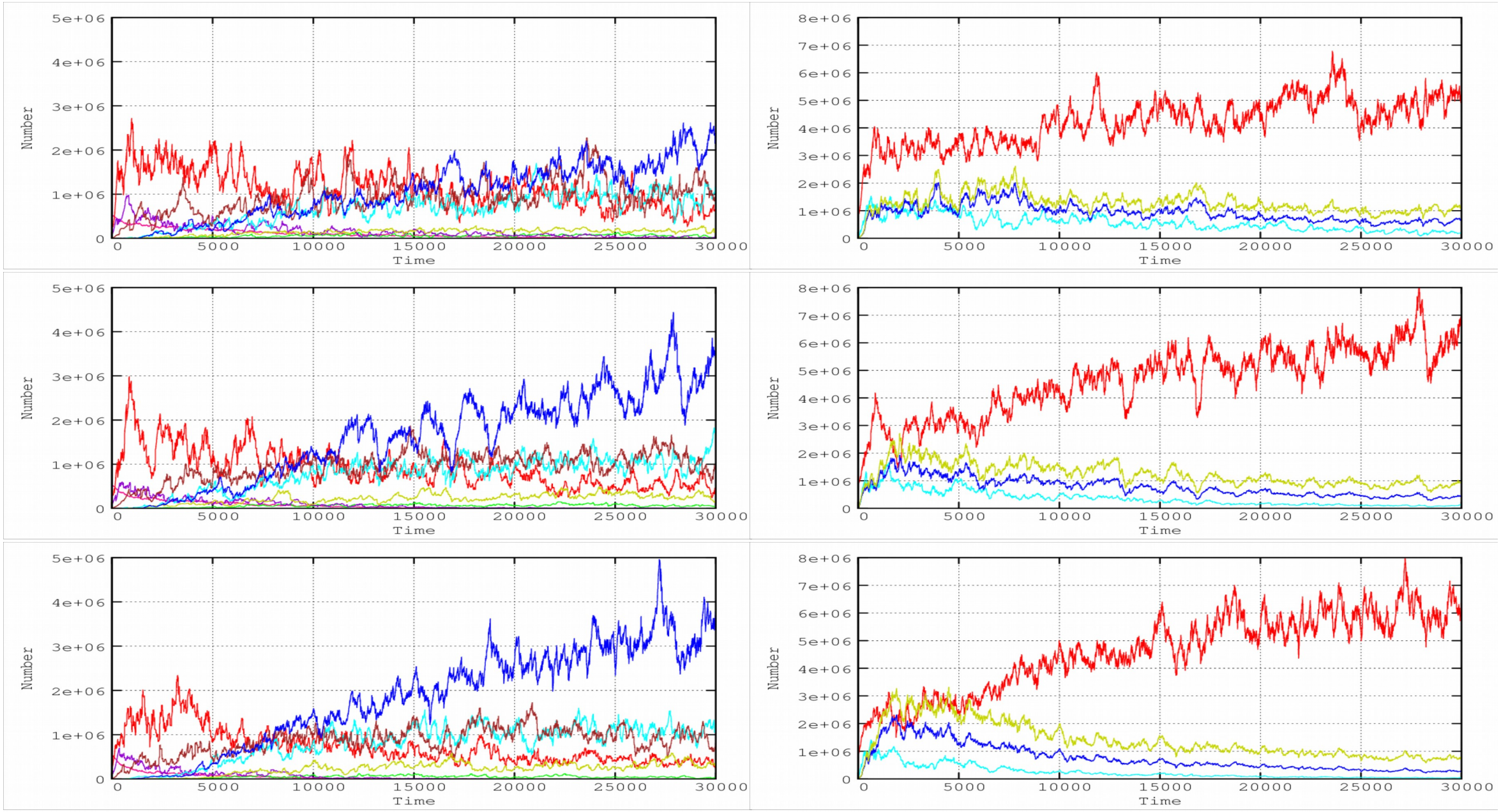
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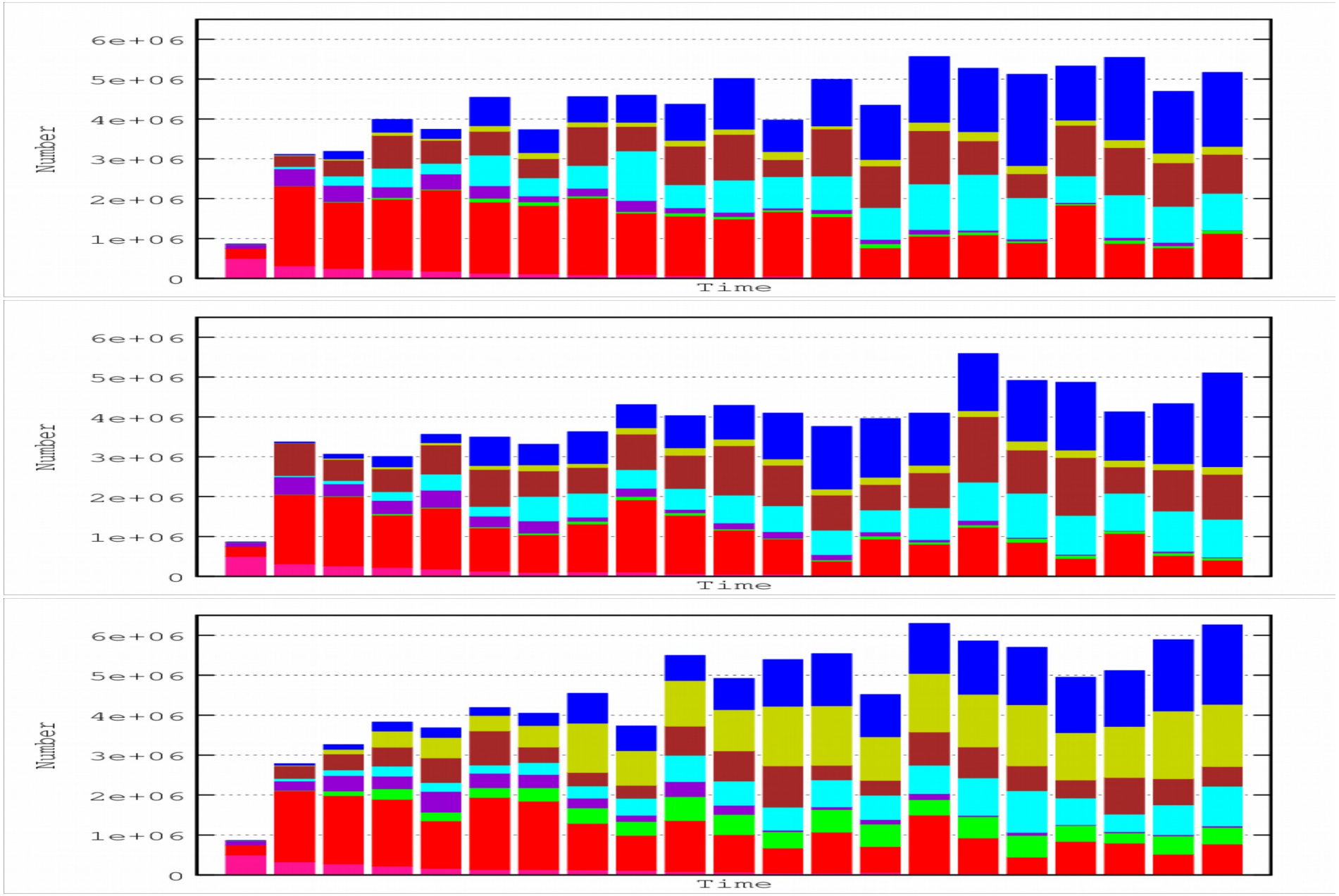
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