Synthetic biological circuits: digital or analog?

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When Max Planck postulated quanta of light as a measure of the emission or absorption of electromagnetic energy in discrete packets or quanta, it was for sure that the picture of physics was going to get altered altogether. However, what he did not expect at the time was that this or its analogous phenomenon may be of any purpose to biologists to apply it to the cellular processes occurring in a biological cell. Digital computation involving biological cells is assuming prominence as the novice field of synthetic biology makes descent amidst high expectations from the academics, industry and also among the general public regarding its immense capabilities to solve the current real-world challenges.

Most researchers right away started to chalk out the set of inputs that they would put into the biological cells to obtain a set of outputs as they do in digital computers, for example, give some input command and get the output signal, both being in the binary language of 0101010 binary bits that are discrete numbers comparable to Planck's discrete packets of energy. But what the workers in digital biological circuitry research also know is that biological entities are dynamic in nature, and they should not and cannot be easily correlated with the static electrical devices in which digital computation persists, for example, in your smart phone.

When it comes to adaptations of the electrical engineering models into cellular electronics by realizing and characterizing 'parts and devices' that could be essential in assembling functional systems *in vivo* cell environments in digital mode, perhaps currently is mostly dependent on a thought process that is essentially digital but not continuous!, by deciphering I gave this input and it resulted in this output. It is just like emulating the significance of analysing the light falling on a metal foil in terms of quanta of light over a conventional perception of continuous light hitting the metal foil. A biological cell does not just operate on a binary code; instead there occur life processes in it in unpredictable ways, sometimes when the binary code just does not work because a biological cell is not a static metal foil and is completely dynamic at any given point of time and space, i.e. the processes in a biological cell are spatio-temporally dynamic. Also the life-processes are in essentially thermodynamically non-equilibrium states which drive life. If you go to a cell and ask it to adapt to the binary software that you use in digital computer, it just may not accept your command at times, which itself is derogatory to the synthetic biology's presumable central dogma, that is, to harness cells to yield to what you instruct them to yield.

According to Daniel *et al.*[1], analog electronic circuitry methods may be handy in dealing with such ever dynamic systems, but again, they reinstate that there is a clear relation between the electronics and chemistry. According to Sarpeshkar[2], 'Chemical kinetics and electronics are analogous at several levels'[2]. It is true that chemical reactions involving the chemical potentials and molecular fluxes could provide background notes on the analog circuital approaches of electronics towards the life-processes in biological cell and the harnessing of these cells thereof. Thus, in its entirety chemistry along with its sibling chemical engineering could pave a suitable way for the synthetic biological advancements in the forthcoming days. After all synthetic biology is re-engineering by refactoring the constituents of the organisms to do chemistry[3].

Synthetic biology is dependent on chemistry. For example, to biologically show a gene on or off in an organism necessitates requirement of a fluorescent tag such as GFP (Green Fluorescent Protein). Currently this requires insertion of another gene that codes for the GFP synthesis in the organism which might result in greater strain such as metabolic strain in the organism. But with chemistry, a simple chemical fluorescent marker could be devised to get rid of this gene based brutality to a biological cell!

Persuading a bacterium to produce huge quantities of chemicals through synthetic biology is a far cry compared to the production of chemicals in commercial quantities in current industrial set-ups. Here would come to the aid, the scale-up dynamics and knowledge of reaction rates from chemical engineers. So in total, chemical science[4], the central science will be crucial to the success of synthetic biology.

International Genetically Engineered Machine (iGEM) competition is a latest phenomenon among synthetic biologists. But to some, iGEM resembles the cloning revolution that went raged in the 1970s which later subsided due to the over surmounting failures of the genetic systems as they could not yield to the so called 'predictable engineering of biology'. Just as Benner *et al.*[5] say, 'synthesis is a research strategy, not a field'. If the guiding principles underlying a synthetic strategy go wrong, the synthesis fails and fails inadvertently that it cannot be neglected. They also provide a hint about the fact that currently synthetic biology is in its 'tinker state' and if the aftermath of the failures that get into the way of the synthetic biological strategies are not dealt with, then there would come no true value and would again result in an another genetic engineering like outburst that might recede eventually.

It is hard to comprehend the fact that the synthetic strategies employed even for the most complex inorganic systems such as polyoxometalate clusters[6], and cages and cubes, etc. could not be correlated with the dynamic biological synthetic processes. In inorganic chemical synthesis, chemical equilibrium dictates the rate of reaction either towards uphill or downhill, and there are a set of well-defined rules and principles that underlie any given chemical reaction or process, be it inorganic or organic. Based on these rules and principles, it is possible to predict the end-product along with its quantity.

Similar, if not identical, guiding principles demand their obvious presence in the synthetic biology arena. For example, one should be able to train one's microorganism or any other organism for that matter to yield to the demand and this could be made possible by defining a set of rules that essentially underlie a synthetic pathway. Delving into the synthetic biology domain, the genetic expression of certain synthetic biological 'parts' in a system should be able to simply produce the desired function or product and also to stop at a predefined spatiotemporal point.

As is known, RNAPs (RNA polymerases) flowing through a DNA are compared to that of the electrons flowing in an electrical wire, where again an electrical wire being static, the count of electrons per second through a wire could be analysed, which just involves a simple knowledge of the material content of the wire, for example, copper, silver, etc. But the same cannot be possible (at least in the near future) to determine the number of RNAPs flowing through a point in the DNA, called as PoPs (polymerases per second)[7]. In the wake of all these challenges, analog circuitry models and systems thereof could get wider attention over digital circuitry models and systems because the analog circuitry is analogous to the chemical kinetic models and systems.

It could be possible to realize the full potential of a digital biological circuitry only after attaining the same in the analog biological circuitry, as was materialized in the discipline of physics from classical to the quantum world.

1. Daniel, R., Rubens, J. R., Sarpeshkar, R. and Lu, T. K., *Nature*, 2013, **497**, 619–623.
2. Sarpeshkar, R., *Philos. Trans. R. Soc. London Ser. A*, 2014, **372**, 20130110.
3. Bashor, C. J., Horwitz, A. A., Peisajovich, S. G. and Lim, W. A., *Annu. Rev. Biophys*., 2010, **39**, 515–537.
4. Yeh, B. J. and Lim, W. A., *Nature Chem. Biol*., 2007, **3**, 521–525.
5. Benner, S. A., Chen, F. and Yang, Z., In *Chemical Synthetic Biology* (eds Luisi, P. L. and Chiarabelli, C.), John Wiley & Sons, 2011, 1st edn.
6. Busche, C. *et al*., *Nature*, 2014, **515**, 545–549.
7. Endy, D., Deese, I. and Wadey, C., *Nature* (online), 2005; also available at http://mit.edu/endy/www/comic/

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Grains for ecosystem carbon management in North East India

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Preamble

North East India (NEI) comprises of eight states (Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Sikkim and Tripura) and covers 26.3 million hectare (M ha) or ~8% of total geographical area (TGA) of India (329 M ha)[1]. However, it has 17.2 M ha under forest cover, which is ~25% of India's total forest area[2]. Of the total forest cover, 1.5 M ha is currently managed by shifting cultivation in NEI[2]. Shifting cultivation, an integral part of culture and tradition of tribes of NEI, is presently unsustainable because of the population-driven reduction in the duration of the fallow cycle (3–5 years)[3]. Reduction in the fallow cycle has accelerated soil erosion and other ecosystem disservices across NEI. Deforestation and accelerated erosion have severely depleted the soil and ecosystem carbon (C) pools[4]. Ecosystem C management is a priority area of national and international programmes to decelerate climate change. Therefore, this note discusses the feasibility of introducing the 'grains for ecosystem carbon management' (GECM) in NEI through providing food grains to the shifting cultivator as an alternative to shifting cultivation. GECM can be a win-win-win option because it is envisaged to accomplish (i) land restoration: land degradation neutrality under the zero net land degradation (ZNLD) proposal of United Nations Convention to Combat Desertification (UNCCD); (ii) food security: through provisioning of grains under GECM and poverty alleviation under the Sustainable Development Goals (SDGs) of the United Nations through payment for ecosystem services (PES), and (iii) climate change mitigation: restoring soil organic carbon (SOC) for critical ecosystem functions and services.

Land degradation and shifting cultivation in NEI

Land degradation refers to the deterioration or total loss of the productive capacity of soils for present and future use[5]. It is caused by accelerated erosion by wind and water and decline in soil chemical and physical quality. Shifting cultivation (Jhum cultivation), the oldest farming system and intricately interwoven in the culture of the hill people[3], is now among the most severe anthropogenic perturbations exacerbating land and ecological degradation. Shifting cultivation and related bush fallow systems prevail in the tropics and are practised on ~30% of the world's uplands in Africa, Latin America, Oceania and Southeast Asia[6]. The system involves clearing of land by cutting the vegetation (tree or bushes), leaving biomass *in situ* for drying and finally burning the biomass for production of charred material for soil fertility enrichment. Subsequently, one or more crops are grown for 2–5 years until soil fertility is depleted and farmers move to a new forest patch and repeat the process[7]. The time difference between two subsequent cultivations in the same land, earlier extended to 20–30 years has now reduced to 3–5 years[6]. Such a dramatic shortening of the fallow cycle has raised concerns about the sustainability of shifting cultivation because of soil erosion, nutrient loss, decline in productivity and reduction in biodiversity which ultimately leads to ecosystem disservices[8]. As a consequence of shortening the fallow, crop yields are as low as 130 kg ha^{-1} year^{-1} under short fallow cycle in comparison to 2600 kg ha^{-1} year^{-1} under long fallow cycle jhum lands[9]. The vicious cycle can lead to irreversible degradation of the soil and disintegration of the ecosystem[6]. In NEI, shifting cultivation is practised in all the eight states between 22°05′–29°30′N lat. and 87°55′–97°24′E long. NEI covers ~8% of the TGA of India and represents around one-fourth of forest cover of the nation[1]. The region is situated at the