Syst Synth Biol (2015) 9:191–195
DOI 10.1007/s11693-015-9181-y

COMMENTARY

Translational synthetic biology

Mamta Singh[1] · **Anuradha Vaidya**[1]

Received: 11 August 2015 / Accepted: 12 August 2015 / Published online: 18 August 2015

Abstract Synthetic biology is a recent scientific approach towards engineering biological systems from both pre-existing and novel parts. The aim is to introduce computational aided design approach in biology leading to rapid delivery of useful applications. Though the term reprogramming has been frequently used in the synthetic biology community, currently the technological sophistication only allows for a probabilistic approach instead of a precise engineering approach. Recently, several human health applications have emerged that suggest increased usage of synthetic biology approach in developing novel drugs. This mini review discusses recent translational developments in the field and tries to identify some of the upcoming future developments.

Keywords Synthetic biology · Translational · Malaria · Cancer · Tuberculosis · Neurodegenerative

What is synthetic biology?

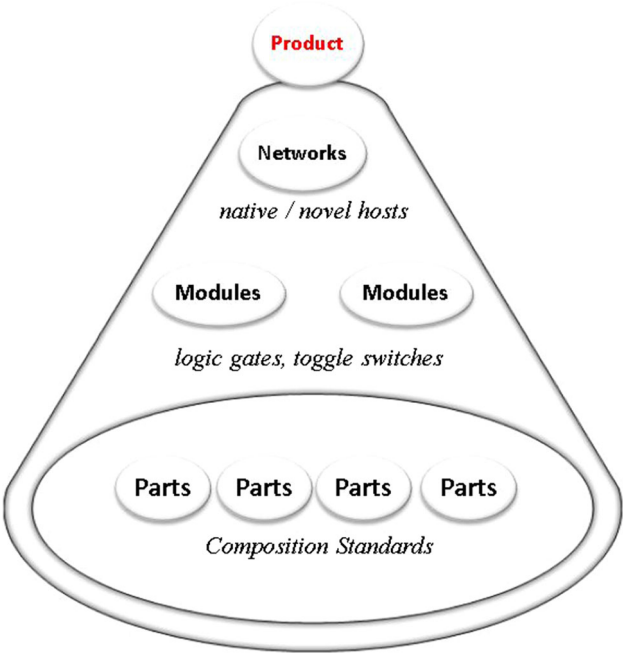
At a first look the term synthetic biology might give an impression of 'chemical biology'. However, the intended application of the term is 'engineering inspired' construction of biological components ranging from parts to networks. The term synthetic biology was used in 1980 to describe genetically engineered bacteria (Hobom 1980). However, the term largely remained synonymous with bioengineering. In 2000, the term "synthetic biology" was reused to describe molecules that are synthesized and can work in living systems (Benner and Sismour 2005).

The engineering flavour of a new discipline appeared when this term was formally proposed and accepted by popular vote in the first annual meeting of synthetic biology at MIT (June 2004) to indicate the beginning of construction approach in biology as against the classic reductionist approach. The intended meaning of "synthetic" was 'non-native' or 'novel', not "chemical".

The intent was to create novel combinations by assembling existing cellular components or design brand new components towards practical applications. Currently, the term 'biological engineering' is used to indicate synthetic approach in biology.

✉ Anuradha Vaidya
anuradha.vaidya@ssbs.edu.in

[1] Symbiosis School of Biomedical Sciences (SSBS), Symbiosis International University (SIU), Symbiosis Knowledge Village, Lavale, Mulshi, Pune 412115, India



The de novo design of biological systems is achieved by combining basic input/output units. From these sets of standard biological parts, modules and networks are constructed. Modules are a set of biochemical reactions like translation, transcription, allosteric regulation, enzymatic reactions and so on. Networks are a combination of modules to translate genome level information into a higher level behaviour.

The application of synthetic biology approach has evolved over time. Currently, the term indicates design and installation of biological components in a novel host, making a non-AGTC genome, synthesizing proteins from not-coding DNA, installing small networks as standalone applets, whole genome cloning, engineering inter-cellular signal transmission, long DNA synthesis, developing standards for data exchange and so on. It is encouraging that variants of the original approach indicate evolution of the field and its future prospects.

Over time, the design aspect of synthetic biology has found applications in a wide range of human health conditions. Here we cover some of the key successful translational stories and present upcoming developments in this rapidly evolving field.

Translating synthetic biology research into applications

Malaria

Malaria continues to be a major global health problem threatening a large section of population on this planet. It is a leading cause of death and disease in many developing countries especially affecting the vulnerable groups (http://www.cdc.gov/malaria/malaria_worldwide/impact.html). In addition to the immense challenges in preventing the occurrence of the disease, the control is severely hampered by the emergence of multi-drug-resistant strains of the malaria parasite *Plasmodium falciparum*. To address this serious situation, synthetic antimalarial drugs and malarial vaccines are currently being discovered and developed for clinical testing (Ro et al. 2006).

Artemisinin is currently considered one of the most potent drugs against all forms of malarial parasites. The WHO has recommended Artemisinin based combination therapies (ACTs) for reliable treatment of malaria. The compound is derived from the sweet wormwood plant, *Artemisia annua* and is made of three isoprene units linked to cyclic organic esters. Traditionally Artemisinin has been extracted from sweet wormwood leaves or flower clusters of *A. annua* by distillation process. Though the process is fairly standardized, a huge unmet demand has led to drastic reduction in the African and South American tree population. Furthermore, due to its high cost the drug has remained out of reach of many patients suffering from this life threatening disease.

Artemisinin is a sesquiterpene lactone endoperoxide made up of three isoprene units linked to cyclic organic esters. To meet a huge global demand, scientists have tried a direct chemical synthesis route (Covello and Patrick 2008). Unfortunately, total synthesis of artemisinin is difficult and expensive. To address this grand challenge, a team of scientists led by Dr. Jay Keasling at UC Berkeley implanted three new plant genes in *Escherichia coli*, and successfully synthesized a precursor to artemisinin. Following this the group engineered yeast to construct the entire pathway leading to synthesis of artemisinic acid, a precursor form of artemisinin (Hale et al. 2007).This project received a support of 45 million USD from Bill and Melinda Gates Foundation (http://www.gatesfoundation.org/Media-Center/Press-Releases/2008/03/Malaria-Partners-Letter).

Though the team was successful in meeting the goals of the project, they faced problems in large scale manufacture of artemisinin in the laboratory conditions. This led to spinning off ‘Amyris’ company to mass produce the drug. Unable to meet the demands of the industrial scale up, they turned to Sanofi for large scale production and distribution of the drug on ‘no profit, no loss basis’. Since 2007, Sanofi has been producing close to 60 tonnes a year of artemsinin to meet 1/3 of the global demand (Hale et al. 2007).

Recently, Berkeley group developed a semi-synthetic form of artemisinin which would provide a consistent supply and stabilize market prices (Paddon and Keasling 2014). In the coming years, the translational, environmental and socio-economic impact of microbial synthetic

biology to meet global demand of antimalarial compounds would be better understood.

Tuberculosis

Tuberculosis (TB) is one of the deadliest communicable diseases in the world with an estimated global annual incidence of 9 million affected people (WHO 2014). India alone accounts for one quarter of the worldwide incidence. Billions of dollars worldwide are spent on diagnostics, drugs and vaccines research. Still the challenge is enormous and mostly unsolved.

The Bacillus Calmette-Guerin (BCG) vaccine prepared from the virulence-reduced *Mycobacterium bovis*, provides some degree of immunity against human tuberculosis. The BCG vaccine is partially effective based on geographical and genetic factors and does not confer lifelong immunity. Although several new vaccine candidates are now undergoing clinical trials, there is a need for more effective and long lasting vaccination against TB. The progress and need for novel treatment strategies using synthetic biology has been highlighted in a recent paper (Saxena et al. 2014).

Mycobacterium tuberculosis is mostly resistant to antibiotics Isoniazid and Rifampicin. Ethionamid, currently the most effective drug against TB, is structurally analogous to Isoniazid. High doses of Ethionamid are required to fight *M. tuberculosis*. However, high doses of this drug results in increased fatality in the patients. The key therefore is to make the microbe more sensitive to Ethionamid.

At ETH Zurich, scientists took the challenge making tuberculosis bacteria more sensitive to antibiotics. The existing literature revealed that Mycobacterium blocks antibiotic action by producing EthR which is synthesized by gene ethR (Rv3855) of *M.tb*, leading to the suppression of the production of EthA, which is synthesized by gene ethA (Rv3854c) of *M.tb* (Baulard et al. 2000). The EthA enzyme is responsible for converting inactive precursor of Ethionamid to an active compound that kills the microbe (Engohang-Ndong et al. 2004).

One of the strategies to handle this situation would be to take EthR out of circulation. The team led by Martin Fussenegger at ETH, Switzerland, relocated the EthR blocking genetic circuitry of *M. tuberculosis* into mammalian cell for testing a large number of chemical compounds that bind EthR. They quickly found 2-phenylethylbutyrate (a food additive) as the most effective EthR blocker (Weber et al. 2008).

Synthetic vaccines based on synthetic long peptides (SLPs) have been used in the development of vaccines to prevent cancers. In a recent study (Rosendahl Huber et al. 2015), synthetic long peptides derived from Rv1733c, a major *Mtb* latency antigen (highly expressed by dormant form of *M. tuberculosis*) was administrated to HLA-DR3 transgenic mice leading to significant reduction in the pleural bacterial load of *Mtb* challenged mice. Similarly, another report demonstrates use of synthetic long peptides in protecting people against tuberculosis (Coppola et al. 2015). Clearly, the field is beginning to open up its translational potential in several unexpected ways.

Cancer

Cancer is a disease caused by uncontrolled proliferation of genetically abnormal cells in the body. The global health burden caused by cancer stands at $\sim$15 million per year (Siegel et al. 2015).

Scientists are beginning to use synthetic biology toolkits for drug target identification, drug discovery and therapeutic treatment in cancer (Shankar and Pillai, 2011). Use of artificial ligands leading to selective inhibition of the Chemokine C-X-C motif receptor 4-Gα (13)-Rho axis has been found to prevent metastatic spread of basal-like breast cancer cells (Yagi et al. 2011).

A recent review has discussed the development of synthetic receptors, switches, and circuits to regulate T cell activity against tumours (Chakravarti and Wong 2015). Furthermore, authors also discuss the cellular engineering and genome editing of host cells to improve the efficacy of cell-based cancer therapy. Ziopharm Oncology uses a proprietary technology called Ad-RTS-IL-12 to deliver synthetic DNA into cancer cells via the immune system. The key is to design drugs that work with different programmable switches to control the effect of IL-12 cytokine, both in vitro and in vivo.

Currently we are making anti-breast cancer peptides originating from junk DNA of *E. coli* (unpublished observations). This is based on a previous report where the feasibility of making novel therapeutic molecules from never-expressing DNA was demonstrated (Dhar et al. 2009).

Neurodegenerative diseases

Neurodegeneration is a general term to describe progressive loss of function and death of neurons leading to diseases like ALS, Parkinson's, Alzheimer's, and Huntington's. The disease has a huge impact on the quality of life for affected people and health care providers and because it places massive demands on the health care system (Hebert et al. 2003). Currently, these diseases are incurable. Brain cells of such patients often show extra glutamine residue leading to toxic effects, misfolding of proteins like alpha synclein, tau and beta amyloid. Making early therapeutic intervention in neurodegenerative disorders is important as the condition is irreversible and results in lethal outcome. Added to that is the difficulty of drugs to cross blood brain barrier. Using synthetic biology methods one hopes to design better vectors for gene delivery and more effective drugs.

Synthetic biology is an emerging discipline that is being used in the field of neurodegenrative disorders to design and rewire biological components so as to achieve novel functions in a robust and predicatble manner (Agustín-Pavón and Isalan 2014). Alzheimer's and Parkinson's are the two most common neurodegenerative diseases whose multifactorial etiology has been well established. Researchers have used synthetic nucleases to create brain disease models. For example, using zinc finger nucleases, cell lines expressing Alzheimer's disease (AD) and Parkinson's disease (PD) specific mutations have been created. In a study conducted by Soldner et al. (2011), it was seen that by combining zinc finger nuclease (ZFN)-mediated genome editing and patient specific induced pluripotent cell (iPSC) technology, the point mutations in α-synuclein gene in Parkinson's patients was genetically rectified. This robust potential of n patient-derived hiPSCs represents substantial development for basic biomedical research and a procession toward hiPSC-based cell replacement therapies (Fong et al. 2013). In a similar study, scientists have designed a long poly zinc finger protein that binds CAG repeats, which are characteristic of Huntington's disease (Garriga-Canut et al. 2012), thereby repressing mutant huntingtin in human patient cell lines. Likewise in a rat model of Parkinson Disease, a synthetic zinc finger targeting the glial derived neurotrophic factor (GDNF) was found to be neuroprotective (Laganiere et al. 2010). A recent study has demonstrated that several peptide inhibitors against Beta-secretase 1 (Raj et al. 2015) is a prime therapeutic target for reducing cerebral amyloid beta concentrations in Alzhemier's patients (Yan and Vassar 2014).

Conclusion

Synthetic Biology is an emerging scientific discipline that integrates several mature and evolving technologies with an aim towards introducing rational engineering concept in biology. To ensure that computational designs meet translational criteria, it is important to build an inventory of well characterized biological components, rules of composition and exchange standards. In the coming years, people are bound to move from 'standard parts' to 'standard pathways' for rapid and efficient installation and expression of well-behaved genetic modules on demand. Currently, the way genetic engineering is practiced; it looks more of a probability than a precision science. Synthetic Biology offers the first glimpse of introducing engineering back into genetics. In future, the community will witness construction of more complex systems using rational approach leading to practical applications.

Acknowledgments We are thankful to Symbiosis Centre for Research and Innovation (SCRI), Symbiosis International University (SIU), Lavale, Pune, India for providing Ph.D. fellowship.

References

Agustín-Pavón C, Isalan M (2014) Synthetic biology and therapeutic strategies for the degenerating brain: synthetic biology approaches can transform classical cell and gene therapies, to provide new cures for neurodegenerative diseases. Bioessays 36:979–990

Baulard AR, Betts JC, Engohang-Ndong J, Quan S, McAdam RA, Brennan PJ, … Besra GS (2000) Activation of the pro-drug ethionamide is regulated in mycobacteria. J Biol Chem 275(36):28326–28331

Benner SA, Sismour AM (2005) Synthetic biology. Nat Rev Genet 6(7):533–543

Chakravarti D, Wong WW (2015) Synthetic biology in cell-based cancer immunotherapy. Trends Biotechnol 33(8):449–461

Coppola M, van den Eeden SJ, Wilson L, Franken KL, Ottenhoff TH, Geluk A (2015) Synthetic long peptide derived from *M. tuberculosis* latency antigen Rv1733c protects against tuberculosis. Clin Vaccine Immunol. 2015 Jul 22. pii:CVI.00271-15

Covello, Patrick S (2008) Making artemisinin. Phytochemistry 69(17):2881–2885

Dhar PK, Thwin CS, Tun K, Tsumoto Y, Maurer-Stroh S, Eisenhaber F, Surana U (2009) Synthesizing non-natural parts from natural genomic template. J Biol Eng 3(1):2

Engohang-Ndong J, Baillat D, Aumercier M, Bellefontaine F, Besra GS, Locht C, Baulard AR (2004) EthR, a repressor of the TetR/CamR family implicated in ethionamide resistance in mycobacteria, octamerizes cooperatively on its operator. Mol Microbiol 51(1):175–188

Fong H, Wang C, Knoferle J, Walker D (2013) Genetic correction of tauopathy phenotypes in neurons derived from human induced pluripotent stem cells. Stem Cell Rep 1:226–234

Garriga-Canut M, Agustin-Pavon C, Herrmann F, Sanchez A (2012) Synthetic zinc finger repressors reduce mutant huntingtin expression in the brain of R6/2 mice. Proc Natl Acad Sci USA 109:E3136–E3145

Global Tuberculosis Report, World Health Organization, 2014. ISBN 9789241564809

Hale V, Keasling JD, Renninger N, Diagana TT (2007) Microbially derived artemisinin: a biotechnology solution to the global problem of access to affordable antimalarial drugs. Am J Trop Med Hyg 77(6 Suppl):198–202

Hebert LE, Scherr PA, Bienias JL, Bennett DA, Evans DA (2003) Alzheimer disease in the US population: prevalence estimates using the 2000 census. Arch Neurol 60(8):1119–1122

Hobom B (1980) Gene surgery: on the threshold of synthetic biology. Medizin Klinik 75(24):834–841

http://www.cdc.gov/malaria/malaria_worldwide/impact.html

http://www.ziopharm.com/

Laganiere J, Kells AP, Lai JT, Guschin D (2010) An engineered zinc finger protein activator of the endogenous glial cell line-derived neurotrophic factor gene provides functional neuroprotection in a rat model of Parkinson's disease. J Neurosci 30:16469–16474

Paddon CJ, Keasling JD (2014) Semi-synthetic artemisinin: a model for the use of synthetic biology in pharmaceutical development. Nat Rev Microbiol 12(5):355–367

Raj N, Helen A, Manoj N, Harish G, Thomas V, Singh S, Sehrawat S, Seth S, Nair As, Grover A, Dhar PK (2015) In silico study of peptide inhibitors against BACE 1. Syst Synth Biol 9:67–72

Ro DK, Paradise EM, Ouellet M, Fisher KJ, Newman KL, Ndungu JM, … Keasling JD (2006) Production of the antimalarial drug precursor artemisinic acid in engineered yeast. Nature 440(7086):940–943

Rosendahl Huber SK, Camps MG, Jacobi RH, Mouthaan J, van Dijken H, van Beek J, Ossendorp F, de Jonge J (2015) Synthetic

long peptide influenza vaccine containing conserved T and B cell epitopes reduces viral load in lungs of mice and ferrets. PLoS One 10(6):e0127969

Saxena A, Mukherjee U, Kumari R, Singh P, Lal R (2014) Synthetic biology in action: developing a Drug against MDR-TB. Indian J Microbiol 54:369–375

Shankar S, Pillai MR (2011) Translating cancer research by synthetic biology. Mol Biosyst 7(6):1802–1810

Siegel RL, Miller KD, Jemal A (2015) Cancer statistics, 2015. CA Cancer J Clin 65(1):5–29

Soldner F, Laganiere J, Cheng AW, Hockemeyer D (2011) Generation of isogenic pluripotent stem cells differing exclusively at two early onset Parkinson point mutations. Cell 146:318–331

Weber W, Schoenmakers R, Keller B, Gitzinger M, Grau T, Daoud-El Baba M, Sander P, Fussenegger M (2008) A synthetic mammalian gene circuit reveals antituberculosis compounds. PNAS 105(29):9994–9998

www.gatesfoundation.org/Media-Center/Press-Releases/2008/03/Malaria-Partners-Letter

Yagi H, Tan W, Dillenburg-Pilla P, Armando S, Amornphimoltham P, Simaan M, Weigert R, Molinolo AA, Bouvier M, Gutkind JS (2011) A synthetic biology approach reveals a CXCR4-G13-Rho signaling axis driving transendothelial migration of metastatic breast cancer cells. Sci Signal 4(191):ra60

Yan R, Vassar R (2014) Targeting the β secretase BACE1 for Alzheimer's disease therapy. Lancet Neurol 13(3):319–329