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PROTEIN ENGINEERING OF TERPENES SYNTHASE: AN OVERVIEW

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ABSTRACT

The significance protein engineering is the way toward creating helpful and beneficial proteins planning, proteins alongside pathways is underscored as an essential methodology for accomplishing biosynthesis of microorganisms and overproduction of pharmaceuticals and synthetic products. Here, two general methodologies for protein engineering, rational protein design and direct evolution. Other methodologies include joining data on precious crystal structure and protein science with counterfeit quality synthesis. Research have depicted a capable hunt strategy in light of the utilization of Hidden Markov Model (HMM) and Protein Family Database (Pfam) look which empowered the disclosure of bacterial determined monoterpene synthase. Analysts often apply these methodologies for protein engineering. Learning of protein structure and capacity has grown the limit of protein engineering. Proteins can be designed for modified substrate specificity/selectivity, expanded synergist action, decreased confinement of mass exchange because of particular protein restriction, and lessening of substrate/product restraint. The protein grouping information with structure of cyclized terpene product were rejected.

KEYWORDS: Protein engineering, Terpene synthesis, Diterpene synthase.

INTRODUCTION

The process by which novel proteins with desired properties are developed is known as protein engineering. The first example of protein mutagenesis was described over three decades ago. It has been grown by leaps and bounds. To specific industrial, medical, and research applications, protein engineering have successfully created a wide range of proteins tailored in last thirty years. yet challenge remain the prevent the engineering of complex protein functions on demand (Chica, 2015).

Eventually, even non-regular amino acids can be incorporated by means of more up to date strategies, for example, expended genetic code, which makes it conceivable to code novel amino acids in the genetic code (Turanli-Yildiz *et al*., 2012).

Approximately 76,000 discovered makes terpenoids the largest family of natural products in nature with widespread applications. The wide-spectrum of structural diversity of the terpenoids were largely due to the variable skeletons generated by terpene synthases. Here, we first demonstrated that the promiscuous synthases in vivo can produce more variable terpenoid products by converting precursors of different lengths

(C10, C15, C20, C25). This discovery was prompted by the development of an efficient in vivo platform by combining the two promiscuous terpene synthases and three prenyltransferases to generate 50 terpenoids, at least 3 ring systems of which were completely new. Furthermore, protein engineering was further integrated to 23 enhance product diversity. Clearly, the work is expected to dramatically reshape the terpenoid 24 research by widening the flexibility of the terpene synthases for the fresh discovery or creation of 25 the new terpenoid compounds by skeleton reframing.

It is additionally the product and services advertise with an idea of $ 168 billion of 2017 (Wells and Robinson, 2017). Since the principal case of protein mutagenesis has been depicted over 30 years, protein engineering, a procedure by which novel proteins with the coveted properties are produced, has grown significantly. In any case, there remain challenges that anticipate planning complex protein capacities as per necessities. To create a groups for the protein engineering group (Miller *et al*., 2013).

The Protein Engineering Canada Conference was held in Ottawa, Canada on June 20-22, 2014. At a two-day group, 115 protein researchers from more than 30 associations speaking to five nations shared thoughts, shaped a system with partners, and were engaged with enzyme engineering, computational protein plan, X-ray investigation, protein NMR, Molecular demonstrating, protein engineering, et cetera. This unique issue presents an extensive variety of research by including unpublished research papers not put together by speakers at Protein Engineering Canadian Conference and decisions specifically submitted to Protein Science (Kaushik *et al*., 2016).

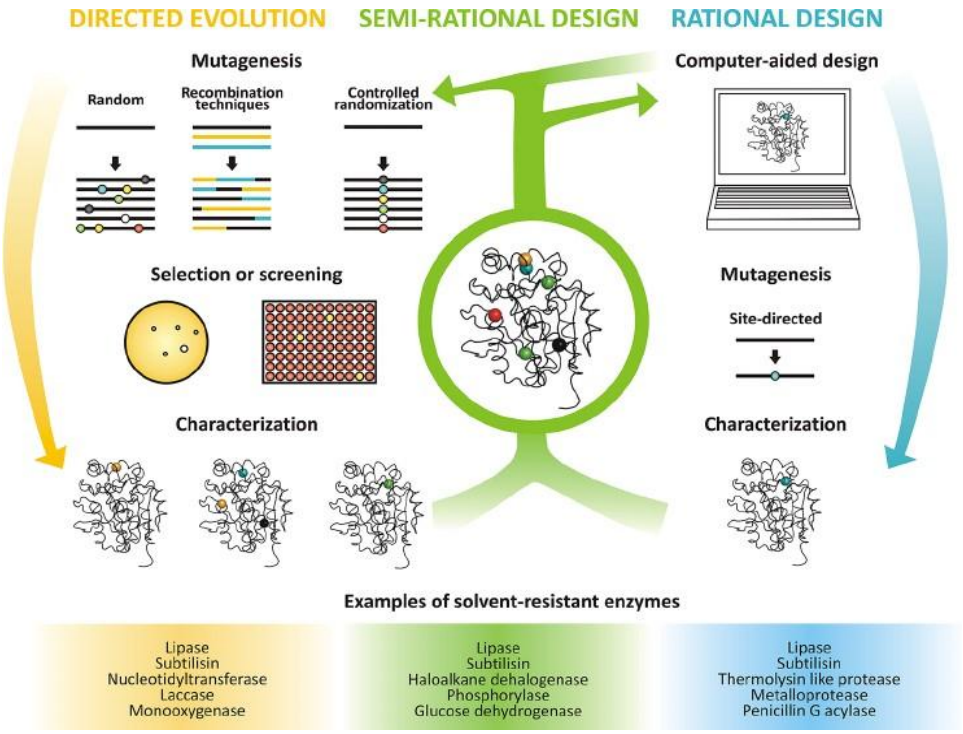

Fig. 1. Strategies for protein engineering (https://www.creative-biolabs.com).

Research talk about the possibilities of protein engineering including the part of X-ray crystallography, synthetic synthesis of DNA, PC displaying of protein structure and folding. By joining data on precious crystal structure and protein science with counterfeit quality synthesis it has turned out to be conceivable to alter a wide range of properties of proteins. Such methods give the likelihood to change protein structure and capacity in ways that are generally difficult (Benner *et al*., 1987; Longhi *et al*., 2007; Martin *et al*., 2003; Ulmer, 1983; Wang *et al*., 2012).

Even, the enhancement of the terpene structure for human utilize isn't just a carbon platform for terpenes, yet in addition an economy to implanted useful groups that takes into account particular alteration of the molecules by a sensible restorative science battle(Benner *et al*., 1987; Mazumdar *et al*., 2017). We have to get entrance. Further whether the crusade begins from a complex separate with an genetically in place platform (likewise called semi-synthesis) or a few little sections (now and then additionally alluded to as synthesis synthesis) It might be costly and tedious. In this article researcher has written about a few procedures to diminish the cost and time of creating terpene therapeutics(Liu *et al*., 2012).

Recognizing the issue: Organic parts and the therapeutic utilization of terpenes need to reveal to you how they are managing concoction synthesis issues. Bioactive terpenes are much of the time disengaged. Recognizing the last isn't really immediate before clinical confirmation (Kourist and Bornscheuer, 2011; Wu *et al*., 2006; Zhang *et al*., 2011).

Roughly 35,000 terpenes have been distinguished and most of the conceivable elements of these particles are obscure (Jansen and Shenvi, 2014). Therefore, the synthesis exertion at this "gullible" phase of terpene look into centers around raising generally little measures of materials or proclivity named substances for target ID (Drauz, 2012). An imbalanced number of terpenes is said to pull in consideration in synthesis writing since it has a known capacity of medicinal significance concentrating on either synthesis adaptability, or look for comparable structures. Compound synthesis should serve to address the real issues related with the objective structure, which predominantly relies upon the learning of the molecular component (Bartoli, 2014).

Different focuses of compound synthesis: It is important to hold up under at the top of the priority list that synthesis numbers are lessened keeping in mind the end goal to indicate how organically created particles (characteristic products) are orchestrated in the research center by chemical means. This is chiefly because of the weight of financing Further the first estimation of this absolutely logical exertion (O'Maille *et al*., 2004).

The legitimacy of these unions isn't really organic/pharmacological, however is fundamental/ synthetic, and often brings about a procedure to get to advancement or general structure of the response creation. It cannot be said that all complex sub-molecular synthesis is legitimized or adds to the advance of science. As opposed to finishing simply synthesis science practices themselves, the general natural science may decay and antagonistically influence the improvement of new medications (Tian *et al*., 2011).

This review examines some current cases of terpene synthesis from scholastic writing, clarify how they take care of issues related with target molecules, and at times talk about issues to be unraveled (Jansen and Shenvi, 2014).

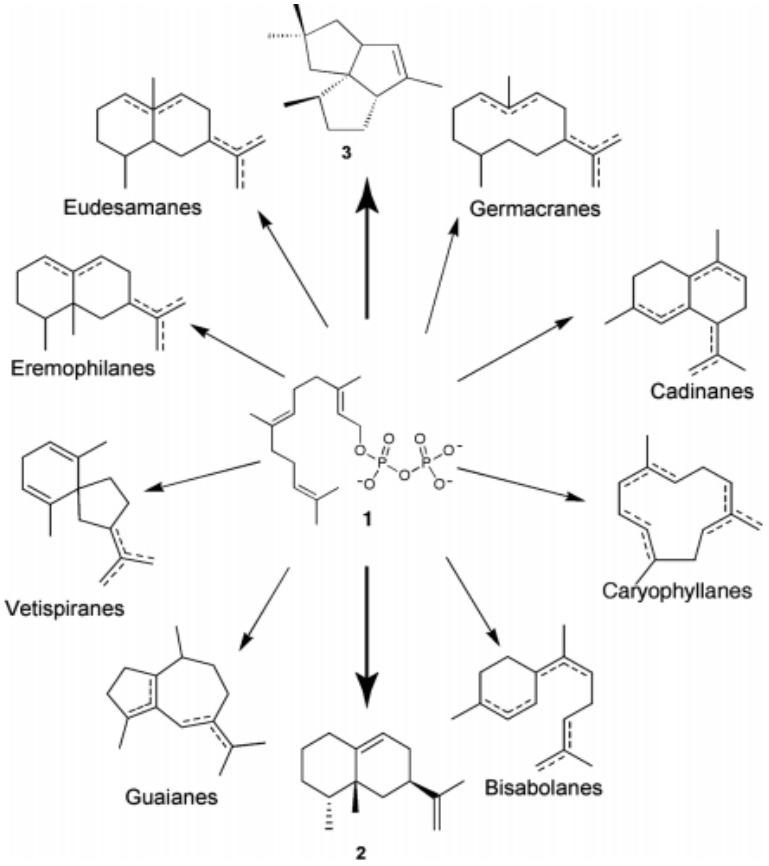

Fig. 2. Biosynthetic diversity of sesquiterpenes generated from a single linear FPP (1) substrate. (2) ¼ 5-epiaristolochene, (3) ¼ pentalenene. Figure adapted from Ignea *et al*. (2014).

Table 1. Set of yeast expression vectors used. All vectors carry a 2μ origin of replication and a cyc1 terminator sequence.

Vector	Auxotrophic selection	Promoter	N-terminal tag	Source
pYES2myc	URA3	PGAL1	myc-tag	Ignea *et al*. (2011)
pUTDH3	URA3	PTDH3	–	Ignea *et al*. (2012)
pUTDH3myc	URA3	PTDH3	myc-tag	Ignea *et al*. (2012)
pWTDH3	TRP1	PTDH3	–	Ignea *et al*. (2014)
pWTDH3myc	TRP1	PTDH3	myc-tag	Ignea *et al*. (2014)
pHTDH3	HIS3	PTDH3	–	Ignea *et al*. (2014)
pHTDH3myc	HIS3	PTDH3	myc-tag	Ignea *et al*. (2014)
pESC-Leu	LEU2	PGAL1/PGAL10	–	Agilent tech.

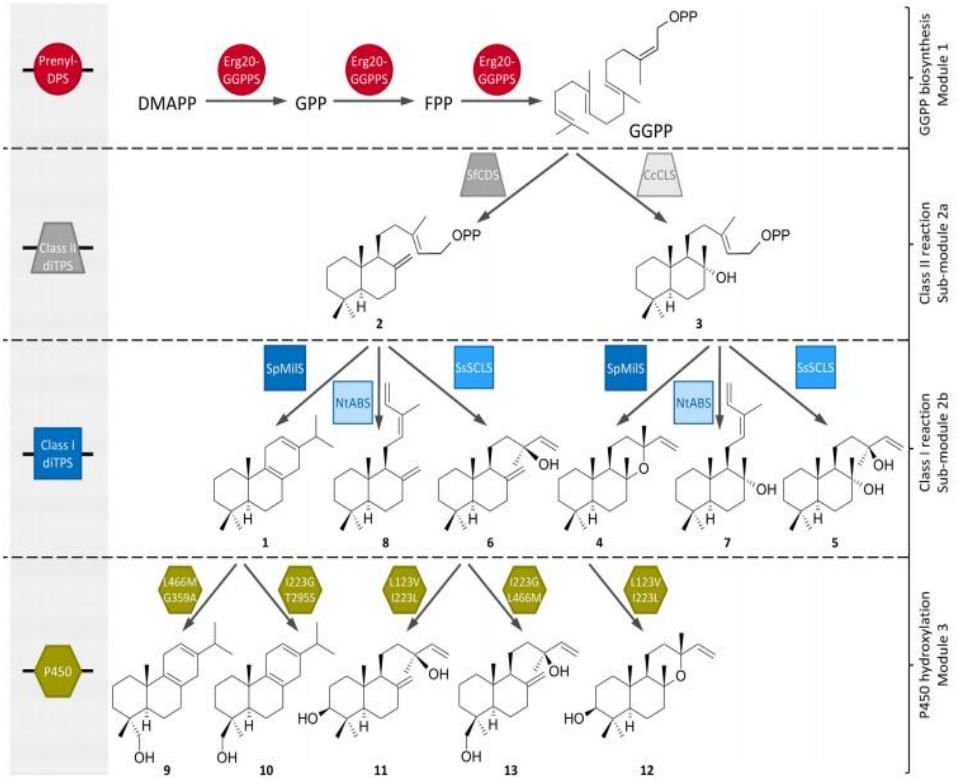

Fig. 3. Combinatorial biosynthesis for drug development (Nenzella & Reeves, 2007).

Summary of the combinatorial biosynthesis approach undertaken. The Cistus creticus GGPP synthase (CcGGPPS) fused with Erg20p was employed as the M1-specific part to support high level production of GGPP. In M2a module, two distinct enzymes *S. fruticosa* copalyl diphosphate synthase (SfCDS) and *C. creticus* 8-hydroxy copalyl diphosphate synthase (CcCLS), responsible for the synthesis of CPP (2) and 8-OH CPP (3), respectively, were exploited as interchangeable parts. Different class I diTPSs were evaluated for their ability to accept 2 and 3 as alternative substrates. *S. pomifera miltiradiene* synthase (SpMilS) uses 2 to synthesize miltiradiene (1), but also accepts 3 to produce manoyl oxide (4) with similar efficiency. Likewise, *S. sclarea* sclareol synthase (SsSCLS) catalyzes sclareol (5) biosynthesis in plants, using 3 as substrate, but efficiently synthesizes manool (6) when supplied with 2. Nicotiana tabacum abienol synthase produces cis-abienol (7) from 3 and (Z)-biformene (8) from 2. CYP720B1 from Pinus taeda (PtAO), was employed in M3 to oxidize the labdane-type scaffolds synthesized in M2. PtAO supports the biosynthesis of two new compounds, 18-hydroxymiltiradiene (9) and 19-hydroxy-miltiradiene (10). The same P450 enzyme catalyzes the stereoselective oxidation of 6 and 4 at position C-3 yielding 3β-hydroxy-manool (11) and 3β-hydroxy-manoyl oxide (12), respectively. Protein engineering of PtAO yielded dedicated synthases for each of these compounds (mutants indicated). Certain PtAO mutants produced 19-hydroxy-manool (13), a compound not synthesized by wild-type PtAO.

Table 2. Terpene titers obtained in AM102 cells carrying the indicated module-specific parts, together with Erg20p-CcGGPPS as the M1-specific part (Ignea *et al*., 2015).

M2a part	M2b part	M3 part	Main product	Titer (mg/L)
SfCDS	SpMilS	–	miltiradiene	26 ± 3.7
CcCLS	SpMilS	–	manoyl oxide	36 ± 5.4
SfCDS	SsSCLS	–	manool	96 ± 12.7
CcCLS	SsSCLS	–	sclareol	87 ± 10.9
SfCDS	NtABS	–	(*Z*)-biformene	1.3 ± 0.5
CcCLS	NtABS	–	*cis*-abienol	3.9 ± 1.3
SfCDS	SpMilS	PtAO	18-hydroxy-miltiradiene	19.1 ± 3.6 (89%)
			19-hydroxy-miltiradiene	2.5 ± 0.3 (11%)
SfCDS	SpMilS	PtAO(G359A)	18-hydroxy-miltiradiene	35.5 ± 5.6
SfCDS	SpMilS	PtAO(G359A-L466M)	18-hydroxy-miltiradiene	69.0 ± 8.3
SfCDS	SpMilS	PtAO(I223G-T295S)	18-hydroxy-miltiradiene	2.3 ± 0.5 (19%)
			19-hydroxy-miltiradiene	9.6 ± 2.3 (81%)
SfCDS	SsSCLS	PtAO	3*β*-hydroxy-manool	3.8 ± 1.2
SfCDS	SsSCLS	PtAO(L123V-I223L)	3*β*-hydroxy-manool	13.8 ± 3.4
SfCDS	SsSCLS	PtAO(I223G-L466M)	3*β*-hydroxy-manool	2.1 ± 0.7
			19-hydroxy-manool	1.2 ± 0.4
CcCLS	SpMilS	PtAO	3*β*-hydroxy-manoyl oxide	1.1 ± 0.3
CcCLS	SpMilS	PtAO(L123V-I223L)	3*β*-hydroxy-manoyl oxide	4.8 ± 1.2

The design and development of biochemical pathways builds the unpredictability of compound created by biosynthesis contrasted with single chemical bio-enzymes. In any case, to accomplish high titers and yields of the coveted aggravates, a progression of complex snags that can be overwhelmed by the coordination of various proteins can be presented (Huber *et al*., 2006). Hence, savvy protein configuration is vital for digestion and pathway engineering. This research portrays different systems and illustrations that apply protein configuration to way engineering to enhance the motion through the way (Eriksen *et al*., 2014).

Here research initially explore bacterial determined diterpenoid characteristic products and quickly look at their biosynthesis with accentuation on diterpene synthase (DTS), which sends geranylgeranyl diphosphate to different diterpenoid platforms. Stress the distinction amongst microorganisms and DTS of the inception of higher living beings and examine issues to find new bacterial DTS (Smanski *et al*., 2012).

The protein grouping information and the structure of the cyclized terpene product were were not popularized, even rejected on most of time. Hidden Markov Model (HMM) and Protein Family Database (Pfam) look empowered the disclosure of bacterial determined monoterpene synthase. Assessed 8,759,463 forecasts from open database and inside draft genomic information utilizing an improved arrangement of HMM parameters produced utilizing already recognized 140 arrangement of bacterial terpene synthase succession preparing sets Pfam inquiries of the bacterial proteins that were made uncovered a putative terpene synthase of 262 (Yamada *et al*., 2015).

The general system of metabolic engineering is to build the endogenous supply of forerunner metabolites to enhance the pathway profitability (Agger *et al*., 2008; Aharoni *et al*., 2004). The most beneficial pathway joining antecedent motion enhancement and mutant synthase brought about an around 2,600 overlap increment in levopimaradiene

levels. In this manner, a most extreme titer of around 700 mg/L was acquired by refined in a seat scale bioreactor (Leonard *et al*., 2010).

Advances in protein engineering, metabolic engineering, and synthesis science permit upgrade of the microbial cell system and tweaking of physiological ability, considering the creation of mechanically suitable A strain is produced. This audit portrays late advancements in outlining microbial plants for the synthesis of significant worth included products including alkaloids, terpenoids, flavonoids, polyketides, nonribosomal peptides, biofuels and chemicals (Du *et al*., 2011).

Terpene synthase has two distinctive metabolic pathways, a mevalonate-subordinate pathway situated in the cytosol and proposed to be associated with the synthesis of sesquiterpene (C15), and a substrate gave by two diverse metabolic pathways. It is in charge of the synthesis of various terpenes in plants. (C5), mono - (C10) and diterpene (C20). Late advances in the properties of qualities and proteins engaged with substrate and finished result biosynthesis and in addition endeavors in metabolic engineering have exhibited the presence of various substrate terpene synthases (Pazouki and Niinemets, 2016).

By specifically framing the bisulfite expansion product from the β-isomer, straightforward decontamination by filtration ends up plainly conceivable. Resulting in the recognized high-immaculateness β-touches, Arrival of free ketone when treated with Na_2 CO_3 was described utilizing IR, MS and NMR spectroscopy (French, 2011).

Terpenoids have numerous organic capacities and a far reaching application scope. Here, two monoterpene synthase qualities Tc-αpin/teo and Tc-teo got from Taiwaniacryptomerioides were cloned. The enzymes encoded by these qualities shared 97% amino corrosive succession closeness, yet had diverse terpene product profiles. Utilizing basic displaying. Researcher effectively distinguished three plastic deposits around (Hsu and Chu, 2015; Salmon *et al*., 2015).

Lauchli *et al*. (2013) create digestion and protein engineering of the terpenoid biosynthetic pathway for overproduction and selectivity control. In the previous decade, a huge inundation of research coordinated towards the making of maintainable and naturally determined fills was seen. A lot of exertion has been made to enhance the creation limit of regular has, for example, *Escherichia coli* and *Saccharomyces cerevisiae*, however inquire about on elective microorganisms is generally postponed.

To grow the spread of the described host for fuel generation, we mapped the terpene biosynthesis pathway of the model Actinobacterium Streptomyces venezuelae and additionally changed the optional digestion to frame progressed biofuel forerunner bisabolene (Leonard *et al*., 2010).

Over strain of basal generation to build bisayolin titer roughly five-crease is conceivable as research data received from the research of the first isoprenoid (Phelan *et al*., 2014).

Plants utilize terpenoid metabolites for different basic capacities in development and advancement however utilize the larger part of terpenoids for nonspecific compound cooperations and security in abiotic and organic situations (Tholl, 2015).

Synthesis science is spearheading new open doors for the creation of profitable chemicals that are reasonable and productive. It composed a microbial processing plant. Amassing into useful way, general system for product broadening, and new technique for enhancement. Enhance efficiency to monetarily suitable levels (Zebec *et al*., 2016).

REFERENCES

Agger, S.A., F. Lopez-Gallego, T.R. Hoye and C. Schmidt-Dannert. (2008). Identification of sesquiterpene synthases from Nostoc punctiforme PCC 73102 and Nostoc sp. strain PCC 7120. *Journal of Bacteriology*, 190(18): 6084-6096.

Aharoni, A., A.P. Giri, F.W. Verstappen, C.M. Bertea, R. Sevenier, Z. Sun and H.J. Bouwmeester. (2004). Gain and loss of fruit flavor compounds produced by wild and cultivated strawberry species. *The Plant Cell*, 16(11): 3110-3131.

Bartoli, G. (2014). Synthesis of bicyclo [3.2. 1] octane tetracyclic diterpenes.

Benner, S., R.K. Allemann, A. Ellington, L Ge, A. Glasfeld, G. Leanz and J. Piccirilli. (1987). *Natural selection, protein engineering, and the last riboorganism: rational model building in biochemistry.* Paper presented at the Cold Spring Harbor symposia on quantitative biology. Volume 52, Cold Spring Harbor Laboratories, Cold Spring Harbor**,** pp 53-63.

Chica, R.A. (2015). Protein engineering in the 21st century. *Protein Science*, 24(4): 431-433.

Drauz, K. (2012). *Enzyme catalysis in organic synthesis: a comprehensive handbook*: John Wiley & Sons.

Du, J., Z. Shao and H. Zhao. (2011). Engineering microbial factories for synthesis of value-added products. *Journal of Industrial Microbiology & Biotechnology*, 38(8): 873-890.

Eriksen, D.T., J. Lian and H. Zhao. (2014). Protein design for pathway engineering. *Journal of Structural Biology*, 185(2): 234-242.

French, L.G. (2011). Isolation and structure elucidation of the terpene β-thujone from cedar leaf oil. *Journal of Chemical Education*, 88(6): 829-831.

Hsu, L.-J. and F.-H. Chu. (2015). Plasticity residues involved in secondary cyclization of terpene synthesis in Taiwania cryptomerioides. *Tree Genetics & Genomes*, 11(1): 796.

Huber, G.W., S. Iborra and A. Corma. (2006). Synthesis of transportation fuels from biomass: chemistry, catalysts, and engineering. *Chemical Reviews*, 106(9): 4044-4098.

Ignea, C., Ivana Cvetkovic, Sofia Loupassaki, Panagiotis Kefalas, Christopher B Johnson, Sotirios C Kampranis, Antonios M Makris. (2011). Improving yeast strains using recyclable integration cassettes, for the production of plant terpenoids. *Microbial Cell Factories*, 10:4.

Ignea, C., Fotini A Trikka, Ioannis Kourtzelis, Anagnostis Argiriou, Angelos K Kanellis, Sotirios C Kampranis and Antonios M Makris. (2012). Positive genetic interactors of *HMG2* identify a new set of genetic perturbations for improving sesquiterpene production in *Saccharomyces cerevisiae*. *Microbial Cell Factories*, 11:162.

Ignea, C., E. Ioannou, P. Georgantea, S. Loupassaki, F.A. Trikka, A.K. Kanellis and S.C. Kampranis. (2015). Reconstructing the chemical diversity of labdane-type diterpene biosynthesis in yeast. *Metabolic Engineering*, 28: 91-103.

Ignea, C., M. Pontini, M.E. Maffei, A.M. Makris and S.C. Kampranis. (2014). Engineering monoterpene production in yeast using a synthetic dominant negative geranyl diphosphate synthase. *ACS Synthetic Biology*, 3(5): 298-306.

Jansen, D.J. and R.A. Shenvi. (2014). Synthesis of medicinally relevant terpenes: reducing the cost and time of drug discovery. *Future Medicinal Chemistry*, 6(10): 1127-1148.

Kaushik, M., P. Sinha, P. Jaiswal, S. Mahendru, K. Roy and S. Kukreti. (2016). Protein engineering and de novo designing of a biocatalyst. *Journal of Molecular Recognition*, 29(10): 499-503.

Kourist, R. and U.T. Bornscheuer. (2011). Biocatalytic synthesis of optically active tertiary alcohols. *Applied Microbiology and Biotechnology*, 91(3): 505-517.

Lauchli, R., K.S. Rabe, K.Z. Kalbarczyk, A. Tata, T. Heel, R.Z. Kitto and F.H. Arnold. (2013). Innenrücktitelbild: High-throughput screening for terpene-synthase-cyclization activity and directed evolution of a terpene synthase (Angew. Chem. 21/2013). *Angewandte Chemie*, 125(21): 5759-5759.

Leonard, E., P.K. Ajikumar, K. Thayer, W.-H. Xiao, J.D. Mo, B. Tidor and K.L. Prather. (2010). Combining metabolic and protein engineering of a terpenoid biosynthetic pathway for overproduction and selectivity control. *Proceedings of the National Academy of Sciences*, 107(31): 13654-13659.

Liu, S.-Q., X.-L. Ji, Y. Tao, D.-Y. Tan, K.-Q. Zhang and Y.-X. Fu. (2012). Protein folding, binding and energy landscape: A synthesis. In: *Protein engineering* (Edited Prof. Pravin Kaumaya). pp.344. Publisher In Tech. China.

Longhi, S., F. Ferron and M.-P. Egloff. (2007). Protein engineering. In: *Macromolecular Crystallography Protocols.* pp. 59-90. Springer Nature, Switzerland.

Martin, V.J., D.J. Pitera, S.T. Withers, J.D. Newman and J.D. Keasling. (2003). Engineering a mevalonate pathway in Escherichia coli for production of terpenoids. *Nature Biotechnology,* 21(7): 796.

Mazumdar, S., S. Wrigley and F. Ciravegna. (2017). Citizen science and crowdsourcing for earth observations: An analysis of stakeholder opinions on the present and future. *Remote Sensing,* 9(1): 87.

Miller, M.J., K.C. Foy P.T. Kaumaya. (2013). Cancer immunotherapy: present status, future perspective, and a new paradigm of peptide immunotherapeutics. *Discovery Medicine,* 15(82): 166-176.

O'Maille, P.E., M.-D. Tsai, B.T. Greenhagen, J. Chappell and J.P. Noel. (2004). Gene library synthesis by structure-based combinatorial protein engineering *Methods in Enzymology*, 388: pp. 75-91, Elsevier.

Pazouki, L. and Ü. Niinemets. (2016). Multi-substrate terpene synthases: their occurrence and physiological significance. *Frontiers in Plant Science,* 7: 1019.

Phelan, R.M., O.N. Sekurova, J.D. Keasling and S.B. Zotchev. (2014). Engineering terpene biosynthesis in Streptomyces for production of the advanced biofuel precursor bisabolene. *ACS Synthetic Biology,* 4(4): 393-399.

Salmon, M., C. Laurendon, M. Vardakou, J. Cheema, M. Defernez, S. Green and P.E. O'Maille. (2015). Emergence of terpene cyclization in Artemisia annua. *Nature Communications,* 6: 6143.

Smanski, M.J., R.M. Peterson, S.-X. Huang and B. Shen. (2012). Bacterial diterpene synthases: New opportunities for mechanistic enzymology and engineered biosynthesis. *Current Opinion in Chemical Biology,* 16(1-2): 132-141.

Tholl, D. (2015). Biosynthesis and biological functions of terpenoids in plants *Biotechnology of Isoprenoids* (pp. 63-106): Springer.

Tian, R., Z.-M. Liu, H.-W. Jin, L.-R. Zhang and W.-H. Lin. (2011). Target identification of isomalabaricane terpenes extracted from sponges. *Acta Physico-Chimica Sinica,* 27(5): 1214-1222.

Turanli-Yildiz, B., C. Alkim and Z.P. Cakar. (2012). Protein engineering methods and applications. In: *Protein Engineering* (Edited Prof. Pravin Kaumaya). pp.344. Publisher In Tech. China.

Ulmer, K.M. (1983). Protein engineering. *Science,* 219(4585): 666-671.

Wang, A., N.W. Nairn, M. Marelli and K. Grabstein. (2012). Protein engineering with non-natural amino acids. In: *Protein Engineering* (Edited Prof. Pravin Kaumaya). pp.344. Publisher In Tech. China.

Wells, E. and A.S. Robinson. (2017). Cellular engineering for therapeutic protein production: product quality, host modification, and process improvement. *Biotechnology Journal,* 12(1): doi: 10.1002/biot.201600105. Epub 2016 Dec 9.

Wu, S., M. Schalk, A. Clark, R.B. Miles, R. Coates and J. Chappell. (2006). Redirection of cytosolic or plastidic isoprenoid precursors elevates terpene production in plants. *Nature Biotechnology,* 24(11): 1441.

Yamada, Y., T. Kuzuyama, M. Komatsu, K. Shin-ya, S. Omura, D.E. Cane and H. Ikeda. (2015). Terpene synthases are widely distributed in bacteria. *Proceedings of the National Academy of Sciences,* 112(3): 857-862.

Zebec, Z., J. Wilkes, A.J. Jervis, N.S. Scrutton, E. Takano and R. Breitling. (2016). Towards synthesis of monoterpenes and derivatives using synthetic biology. *Current Opinion in Chemical Biology,* 34: 37-43.

Zhang, F., S. Rodriguez and J.D. Keasling. (2011). Metabolic engineering of microbial pathways for advanced biofuels production. *Current Opinion in Biotechnology,* 22(6): 775-783.

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