



**PlantEngine**  
COST Action FA1006

**cost**  
EUROPEAN COOPERATION  
IN SCIENCE AND TECHNOLOGY

# Plant Metabolic Engineering for High Value Products



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**Strategy Meeting** September 16 – 17, 2013

Thon Bristol Stephanie Hotel, Brussels, Belgium

## Background

Within the European knowledge-based bioeconomy programs, significant efforts are being directed towards the sustainable use of resources. With respect to plants, a large proportion of research in the field has been allocated toward crop security, production of biofuels/bioenergy and biorefining of residual plant biomass. But plants also have a huge impact by serving as “chemical factories” providing a multitude of natural products. For centuries, plants have been utilized as renewable resources for the production of often complex chemicals, heavily used as fragrances, colorants, fine chemicals or medicines, fueling a whole branch of small- and large-scale industry.

With the scientific understanding of the biosynthetic capacity of plants and with the novel methodologies of metabolic engineering and synthetic biology, it might soon be possible to generate “designer plants” producing defined chemical components with minimal ecological impact and, therefore, serving as real “green factories”. To fully exploit the capacity of plants, the aim of COST Action FA1006, *PlantEngine* is to accumulate scientific expertise in the field, evaluate economic impact of the innovative technologies and pave the way for new products and production processes relevant to the industry. Moreover, the strong need for (i) expanding the fundamental knowledge in order to understand the underlying processes in plant metabolism and (ii) effective means of depositing and sharing data and resources across the community have been identified as vital objectives.

## Aim of the meeting

Within the frame of the COST Action FA1006, a number of advanced projects have been identified, mirroring the current state of the art in plant metabolic engineering and showing the potential of novel applications. As stated in the testimonial of the Horizon 2020 initiative, there is an urgent demand to translate basic research findings into a consumer-oriented product development to strengthen the European KBBE. Therefore, it is of utmost importance to communicate how basic research in this specific field can be coordinated, what the demands of potential industry partners are and how collaborative research efforts could suit the fundamental requirements of both sides.

## Program

**Monday, September 16**

### Perspectives of plant natural product research

#### Show-case projects:

How does curiosity-driven science lead to the development of novel products and technologies?

What infrastructure is needed to fuel research?

How does the current funding within the EU enable research and development?

- 14:00** Welcome and aims of the meeting  
*Heribert Warzecha, TU Darmstadt, Germany*
- PlantEngine: What have we achieved and where are we going?*  
*Paul Fraser, RHUL, United Kingdom*
- 14:15** Horizon 2020  
*Maria Fernandez Gutierrez, Research Program Officer Biotechnologies, EC*
- 14:45** Biomining South Africa's plant diversity  
*Bridget Crampton, University of Pretoria, South Africa*
- 15:30** Pharmaceutical industry and plant biotechnology: What does the future hold?  
*Yves Barbin, Pierre Fabre, France*
- 16:15** Programming for impact in KBBE  
*Peter Freeman, TiMet, United Kingdom*
- 17:00** **Coffee break**
- State of the art talks from top PNP labs in Europe**  
(each 7 min + 3 min discussion)
- 17:30** Mix & match: From plant chemical diversity to production of high-value diterpenoids  
*Björn Hamberger, University of Copenhagen, Denmark*
- 17:40** It's easy to get gene lists now, but how to get beyond?  
*Alain Goossens, VIB, Belgium*
- 17:50** A berry good idea®: Large-scale bioreactor cultivation of plant cells  
*Heiko Rischer, VTT, Finland*
- 18:00** Pathway and transport engineering – glucosinolates as a case study  
*Barbara Ann Halkier, University of Copenhagen, Denmark*
- 18:10** Tools for biofortification of foods by metabolic engineering  
*Cathie Martin, JIC, United Kingdom*
- 18:20** Roadmap for metabolic engineering of high-value terpenoids  
*Harro Bouwmeester, University of Wageningen, the Netherlands*
- 18:30** Discussion

**Tuesday, September 17****Future directions for PNP research and the COST Action**

- 9:00** Promoting plant science in Europe  
*Karin Metzlaf, EPSO, Belgium*
- 9:30** The National Contact Point (NCP) system in Germany and its services  
*Stefan Rauschen, NCP, Germany*

**Channeling different topics in plant science – COST Action presentations**

- 10:15** COST Action FA0806, *PlantiVax*  
*Andreas Voloudakis, Greece*
- 10:30** COST Action FA0907, *BioFlavor*  
*Gustavo M. de Billerbeck, France*
- 10:45** COST Action FA1106, *QualityFruit*  
*Mondher Bouzayen, France*
- 11:00** **Coffee break**
- 11:30** Final discussion
- 12:30** **Lunch** (end of the official meeting)
- 14:00 - 16:30** MC meeting (MC members only)

## Participants

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## Proceedings

**Monday, September 16**

### **Perspectives of plant natural product research**

The meeting was opened by the host and MC Chair, Heribert Warzecha, DE, who extended his welcome to the participants representing diverse domains.

The welcoming address was followed by a short introduction to the specific goals of COST Action FA1006, *PlantEngine*, presented by the Vice Chair and coordinator of METAPRO consortium, Paul Fraser, UK. After concisely recounting the achievements of the Action and the individual efforts of *PlantEngine* Working Groups (featuring especially training schools, workshops and recent WG1 publications in *Biotechnology Journal*), he focused on future activities and vital issues to be further addressed:

- training activities including not only ESRs but also experienced staff,
- adoption of modular cloning systems (*MoClo* & *GoldenBraid 2.0*),
- development and maintenance of databases,
- leveraging more funding (KBBE call and HORIZON 2020),
- understanding different types and needs of the potential industry partners,
- engagement with policy makers and representatives of industry,
- Systems Biology workshop(s),
- emergence of Synthetic Biology,
- final *PlantEngine* International Meeting (planning required).

Maria Fernandez Gutierrez, the EC Research Program Officer of Biotechnologies Unit, presented a concise overview of the relevant FP7 (2007-13), *Knowledge-Based Bio-Economy* (KBBE) EC initiative activities and further expounded upon its imminent successor, *Horizon 2020* (2014-20). As a financial instrument implementing the Innovation Union, a *Europe 2020* flagship initiative aimed at securing Europe's global competitiveness, *Horizon 2020* is to provide an adequate response to the persisting economic crisis. After introducing the new aspects of the initiative, as compared to its predecessor, the Officer highlighted its three priorities: excellent science, industrial leadership and societal challenges. Special focus was put upon issues relevant to the *PlantEngine* Action members, including the importance of industrial involvement and partnership building through coordinated development of Key Enabling Technologies (KETs) and Public-Private Partnerships (PPP) in the field of biotechnology and bio-based industries. For further details, The Officer referred the audience to appropriate internet resources:

- ✓ *Horizon2020*: [ec.europa.eu/research/horizon2020](https://ec.europa.eu/research/horizon2020),
- ✓ *Bioeconomy*: [ec.europa.eu/research/bioeconomy](https://ec.europa.eu/research/bioeconomy),
- ✓ *Participant Portal* (calls, experts, projects):  
[ec.europa.eu/research/participants/portal/page/home](https://ec.europa.eu/research/participants/portal/page/home),
- ✓ *Public-Private Partnership in bio-based industries*: [bridge2020.eu/about/](https://bridge2020.eu/about/).

Questions from the audience:

- Q:** How definitive is the proposed *Horizon 2020* framework?
- A:** While the financial aspect might be subject to changes (predicted budget reduction of 10%), no modifications are foreseen for the proposed activities.
- Q:** With the industrial partnership constituting the ultimate focus of the initiative, is it safe to be so dependent on the industry representatives?
- A:** Fundamental research is equally important and will be supported, as it is the driving force of long-term perspectives for innovation.
- Q:** Will the Marie Curie Program be subject to any modifications?
- A:** No. The Excellence in Science Pillar will continue its activities as in FP7.
- Q:** What are the budgetary details of the initiative (e.g. what % of means will be directed toward PPP development or addressing societal challenges, etc)?
- A:** Impossible to comment on the issue yet; the distribution of means will depend on the quality of submitted proposals.
- Q:** Who assumes direct responsibility for the activities of the formed PPPs?
- A:** The governance will be performed by the member states.

Biomining the diversity of South African flora was the featured topic of the following presentation by *Bridget Crampton, ZA*. After highlighting the driving aspects of the country's biodiversity, the speaker went on to emphasize the rich indigenous knowledge and traditional values of the native medicinal plants, specifically mentioning the therapeutic properties and constituents of *Sunderlandia frutescens* (antitumor), *Siphonochilus aethiopicus* (antimalarial), *Dichoma anomala* (antimalarial) and *Hoodia gordonii* (appetite suppressant). She then mentioned the political and legislative challenges concerning bioprospecting and benefit sharing. Finally, the speaker introduced the scientific goals of PNP research at the University of Petroia, putting a special emphasis on the investigation of *Helichrysum populifolium* and its anti-HIV constituents and the cannabinoid-containing *Helichrysum umbraculigerum*.

Questions from the audience:

- Q:** Was the observed anti-HIV activity of the *H. populifolium* extracts stronger than that reported previously, e.g. for cynarine from artichoke?

**A:** No comparisons were made yet.

**Q:** Was the *H. populifolium* virus population studied?

**A:** No. No apparent signs of a viral infection were observed.

Yves Barbin, FR, a representative of the *Pierre Fabre Corporation*, discussed the perspectives of plant biotechnology and PNP research in pharma industry. After highlighting the well-established role of natural products as drug leads and sources, he presented the current trends in R&D programs of major pharmaceutical companies. He concluded by pointing out that plant biotechnology applications are facing a global failure in drug discovery endeavors, citing their high cost, time inefficiency and low productivity as main detriments. He then implied that the platform for artemisinic acid production utilizing engineered yeast, recently initiated by *Sanofi*, might prove a turning point in re-establishing the importance of plant biotechnology in the industrial context. After enumerating the presumed advantages of biotech solutions over traditional extraction techniques, he discussed their prospective evolution directions converging with the needs of pharmaceutical companies, supporting his arguments with specific examples: efficient retrieval of antineoplastic agents from *Catharanthus roseus* (vincristine & vinblastine) and *Euphorbia peplus* (ingenol mebutate).

Questions from the audience:

**Q:** Is the highlighted platform for the production of artemisinic acid in engineered yeast the one proposed by Keasling *et al.*?

**A:** Yes.

**Q:** The findings of traditional Chinese medicine suggest that (in some cases) whole plant extracts boast stronger therapeutic activity than individual constituents. Is the company planning to channel any of its research efforts in that direction?

**A:** Some research attempts addressing the issue are underway, but it's too early to comment on their results.

After introducing TiMet, an EU-funded project contributing to KBBE by bringing together science, industry and other stakeholders to generate and exploit new knowledge related to plant and crop growth and productivity (<http://timing-metabolism.eu/>), Peter Freeman, UK focused on the role of project management applications in fulfilling its objectives. He further argued the universal pertinence of project management models and tools in establishing efficient communication channels facilitating alignment of priorities and coordination between all involved project partners (academia, industry, state/EC). He concluded that appropriate boundary organizations are the key to consolidated and successful oversight of scientific projects in the context of programmed impact on KBBE.

Questions from the audience:

**Q:** What exactly is the crux of 'crossing the boundaries'? How can it be achieved?

**A:** Through communication. Scientists should get more involved in the dialogue.

**Q:** Will the advent of *Horizon 2020* impact the project management models developed for the KBBE framework?

**A:** *Horizon 2020* will only enhance the validity of the proposed models.

### **State of the art talks from top PNP labs in Europe**

The concise exposés on innovative approaches in PNP research were initiated by *Björn Hamberger, DK*. Putting forth forskolin from *Coleus forskohlii* as a target high-value diterpenoid boasting anticancer and nerve-cell repairing properties, he highlighted the mix and match strategy encompassing *TPSs* and *P450s*. The proposed combinatorial biochemistry approach involved the investigation of alternative expression systems and production platforms (*E. coli*, yeast, tobacco, moss), development of new-to-nature pathways as well as novel strategies for intracellular storage of lipophilic specialised metabolites.

Suggestion:

Incorporation of the *Golden Braid 2.0* modular cloning system into the postulated mix and match approach (*Heribert Warzecha, DE*).

The challenges of efficient data integration and interpretation to fill in the persisting 'white spots' in PNP metabolic networks were addressed by *Alain Goossens, BE*. The aspects of possible gene clustering in plants and transcription co-regulation and transport were highlighted and supported by appropriate citations. Subsequently, the speaker accentuated the need for the development of novel or upgraded technological platforms for future gene discovery strategies, including increased spatiotemporal resolution of 'omics', bioinformatics pipelines for plant metabolic networks, mining of natural variation, advanced metagenomics and interactomics.

*Heiko Richer, FI* discussed the aspects of large-scale bioreactor cultivation of plant cells. He highlighted the cooperation between the *VTT Technical Research Center of Finland*, constituting the interface between basic and applied research, and *Lumene Finland*, the leading national manufacturer of cosmetics, aimed at efficient production and retrieval of high-value constituents from Nordic berries.

Facets of pathway and transport engineering were put forward by *Barbara Ann Halkier, DK*. Proposing glucosinolate biosynthesis investigation as an explanatory case study, she demonstrated the innovative platform for stable insertion of pathways into yeast and expounded upon functional genomics approach to identify secondary metabolite transporters. Of approx. 450 putative

*Arabidopsis* transporters, 240 were screened in *Xenopus* oocytes, resulting in identification of AtGTR1 responsible for channeling glucosinolates into the seeds. The *DynaMo* transporter library was further expanded and currently encompasses 800-1000 genes.

Cathie Martin, UK discussed metabolic engineering tools for biofortification of foods. Focusing on bioactive polyphenolics, she emphasized the role of transcriptional regulation elements as key factors driving the scale-up in production of relevant metabolites. To support her postulate, she presented the results of *AtMYB12* overexpression boosting both secondary and primary metabolism in tomato. She further discussed engineering strategies involving the aforementioned transcription factor to foster the enhanced biosynthesis of valuable phenolic compounds (resveratrol, genistein, wogonin and baicalein).

In the final talk of the day, Harro Bouwmeester, NL addressed the prospective challenges in metabolic engineering of high-value terpenoids. Highlighting the potential of plants as chemical factories and recent advances in PNP research pertaining to gene identification and characterization, pathway reconstitution and generation of new-to-nature metabolites, he identified major 'knowledge gaps' concerning metabolite flux, transport and sequestration. In conclusion, he articulated the need for increased support provided by the EU policy makers regarding genetic modification, to create the opportunities to develop high-value designer crops and safeguard Europe's competitiveness vs. USA and Asia.

## Tuesday, September 17

### Future directions for PNP research and the COST Action

Karin Metzloff, BE, a representative of the *European Plant Science Organization*, initiated the 2nd day of meeting proceedings by introducing the objectives of EPSO and its achievements in advancing and promoting plant science in the context of the FP7 initiative. She then highlighted the research opportunities for plant sector in the imminent *Horizon 2020* framework, specifying *Plants for healthy human & animal nutrition* and *Plants for non-food products* as especially relevant work programs. Emphasizing EPSO's involvement in European science policy, she drew the participant's attention to the required format of prospective research proposals encompassing all of the following elements:

- basic research,
- applied research,
- knowledge transfer,
- innovation.

After referring the audience to the EPSO website (<http://www.epsoweb.org/>) for the detailed information on the *Horizon 2020* strategic implementation plan, she invited its members to active participation in the upcoming *Fascination of Plants Day* and other EPSO initiatives.

*The National Contact Point* (NCP) system in Germany and its role as a mediator between the scientific community, Federal Ministry of Education & Research and the EU Commission was discussed by Stefan Rauschen, DE. After introducing the NCP structure and aims, he focused on the services provided to its clients, including individual assistance in addressing FP7 issues, critical review of proposals, organization of training seminars and workshops, networking, search for potential partners and communication of the clients' interests to the Commission. In conclusion, he invited the meeting participants to visit the NCP website for further relevant information (<http://www.nks-lebenswissenschaften.de/>).

#### **Channeling different topics in plant science – COST Action presentations**

The scientific context and objectives of the COST Action FA0806, *Plantivax* (<http://costfa0806.aua.gr>) were presented by Andreas Voloudakis, GR. The development of efficient and cost-effective methods for reactive and proactive response to viral diseases of plants for sustainable agriculture was addressed by high-throughput dsRNA production strategy targeted at the Cucumber mosaic virus (CMV) for exogenous application. The innovative delivery machinery (based on air-gun mechanism) was developed and implemented in the field. The speaker emphasized the non-transgenic character of the proposed approach to plant virus control employing RNA-based vaccines.

Gustavo M. de Billerbeck, FR introduced the general synthetic biology strategy of *Bioflavour* consortium (COST Action FA0907, <https://bioflavour.insa-toulouse.fr/>). He expounded upon its multidisciplinary and holistic approach for sustainable production of flavor and fragrance compounds in yeast, responding to both scientific and market demands. In conclusion, he highlighted the research funding outcomes of the *Bioflavour* initiative: EU Marie Curie ITN “YEASTCELL”, EU Research Project “RoBoYEAST” and EU Marie Curie ITN “CORNUCOPIA”.

The last presentation of the meeting, by Mondher Bouzayen, FR, featured the scientific background, incentives and challenges of the COST Action FA1106, *QualityFruit* (<http://qualityfruit.inp-toulouse.fr/en/home.html>). Its main research focus is placed on uncovering the mechanisms underlying the fruit ripening processes, encompassing the identification of regulatory genes as well as elucidation of the involvement of hormonal and environmental cues. The highlight achievement of the *QualityFruit* initiative was the re-annotation of the complete grape genome, made available for the scientific community worldwide.

## Final Discussion

In conclusion of the meeting, the participants discussed the future challenges of PNP research in general and *PlantEngine* in particular. The main focus was put on cooperation initiatives directed at other scientific consortia as well as small and large businesses. The industrial outreach was considered the main failing of the Action, remaining the aim to be further pursued, despite the seeming reluctance on the part of industry representatives.