P4SB - From Plastic waste to Plastic value using *Pseudomonas putida* Synthetic Biology

### Deliverable D8.4

Interim report on scientific dissemination and exploitation of results incl. risk assessment, ethical, societal, and intellectual property aspects

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1 Introduction
The objective of WP8 (Dissemination and exploitation) is to ensure the dissemination and exploitation of P4SB scientific results to relevant target audiences and to foster the scientific and public dialogue about project results and new applications in the field of synthetic biology, plastic recycling and biodegradable plastics with maximum visibility. P4SB contributes to the societal debate on the use of synthetic biology technology by addressing ethical, safety, discovery, intellectual property and innovation aspects. Within WP8, RWTH takes on the lead and is supported by all project partners to complete the tasks.

This report presents the dissemination activities of the first 18 months (first period) of the P4SB-project, as well as the objectives of its commercial exploitation. The dissemination activities during the first period focused in the launching of the project's website, the establishment and maintenance of social media resources, scientific publications and presentations, the collaboration and knowledge exchange with another ongoing H2020-project, and the issuing of press releases. In addition, several presentations and meetings were conducted where the P4SB-project and ideas were presented to various academic, industrial and public entities. With regards to the commercial exploitation of the project's results, the P4SB consortium has begun to analyse the pool of stakeholders that are already aware of the project and will be targeted in the following years for the purpose of exploitation.

This interim report will be re-written as final report at the end of the project (month 48), which will be adapted to the ongoing findings based on the project results.

A short summary of the project can be downloaded from the CORDIS-website as well: http://cordis.europa.eu/project/rcn/193263_en.html

2 Public communication and scientific dissemination
As universities, SMEs/industries, and research organisations are all operating on different levels and markets and thus representing different target groups, P4SB started at an early stage with public dissemination and outreach activities. The scientific results of the P4SB project are disseminated through various communication modalities to inform stakeholders in order to empower them to make rational decisions based on relevant data. Moreover, these communications are designed to engage stakeholders in active discussion regarding the pros and cons of synthetic biology and P4SB technologies and products a place in the public opinion.

An active communication with the general public is essential to foster social acceptance of P4SB technologies and synthetic biology in general. Main communication activities are handled by the coordinator, although each P4SB partner has the responsibility to actively communicate their research to society at large. This is reflected by the fact that each partner participates in the dissemination activities of WP8.

The scientific contributions are communicated via Open Access journals as well as well-known social media channels. The scientific results as well as publications of P4SB are collected continuously to
ensure that project results can be exploited and disseminated effectively. All consortium members are actively participating in scientific conferences or regional events to stimulate the scientific dialogue about P4SB and its results as well as to achieve maximum visibility of the project. All dissemination of results of P4SB via the project website, workshops, conferences, key scientific publications, etc. are described in detail below.

2.1 Project website

In order to spread information and to enhance target audience understanding, a project website was launched during the first months of the project (http://p4sb.eu/), which is accessible to the public and scientific community. The website is designed in such a way that the public project awareness is increased through an easy to understand overview of the P4SB goals, methods and achievements. The website contains all the necessary information regarding the project's objectives, impact, our partners and advisory board members, press material that has been or will be released, related publications and presentations, reporting of the project's results (if public), and news and contact information. The website is updated at least once a week and is also connected with the respective Facebook, LinkedIn and Twitter accounts of the project. The P4SB website is also advertised on the established websites of the consortium partners in order to ensure maximum traffic and visibility. The total number of visitors per day since the official launch of the website can be found in Figure 2.1. A screen shot from the main landing page of the official project's website is presented in Figure 2.3.

Deliverable D8.1 “P4SB project website” (submitted in month 3) provides more information about its structure and original content.

![Figure 2.1:](image-url) (Left) The number of visitors (>3000 total) to the P4SB project website since its official launch. (Right) 85 of the total visitors were directed to the P4SB project website from either Facebook or Twitter with a smaller number arriving via LinkedIn.
Figure 2.2: Graphical presentation of the countries reached by the P4SB project website. In descending order the top three visits came from Germany, France and the USA.

Figure 2.3: Landing page of the official P4SB project’s website (example from 21/08/2016)
2.2 Social media

In order to support public dissemination and outreach, P4SB is active in the social media websites Facebook, LinkedIn, Twitter and YouTube, as well as having a presence on television and radio. Social media is used to especially encourage young scientists (e.g., PhD and MSc students) who are actively performing P4SB research to share their thoughts and foster discussion on modern outreach channels.

2.2.1 Facebook

The Facebook page of P4SB can be reached via https://www.facebook.com/pseudomonasputida/. At present, 95 people or pages like this site.

![Figure 2.4: Statistics from Facebook showing the number of people reached with posts from project start until end of August 2016](image)

2.2.2 LinkedIn

The LinkedIn profile of P4SB can be reached via https://www.linkedin.com/groups/6972328. At present, this group has 31 followers. During the project, 20 posts have been launched related to P4SB. LinkedIn is not providing any information about the number of people reached via these posts.

2.2.3 Twitter

The P4SB twitter handle is @P4SB_Biotech and can be reached at https://twitter.com/P4SB_Biotech. The site currently has 50 followers on the site and is following 31 other twitter accounts. The twitter analytics suggest that the account averages at 900 impressions per month, however this number has been steadily growing month on month since the launch of the site in May 2015. Currently the P4SB
twitter account has had 45 tweets, which has resulted in an average of 32 profile visits per month, this too has been on the rise month on month since the launch of the site.

2.2.4 TV / YouTube
P4SB has been on German television on 17 February 2016. The local TV show “WDR Lokalzeit Aachen” reported about the P4SB research activities at the RWTH institute’s “Institute of Applied Microbiology”. As the original film was only stored in the WDR media centre for seven days, we were allowed to upload the video to our P4SB YouTube channel. Click here to watch the film about P4SB and activate English subtitles, if needed.

2.2.5 Radio
In March 2016 Lars Blank from RWTH was interviewed for the radio show “Logo – Das Wissenschaftsmagazin”. You can hear the full story by clicking here.

2.2.6 ResearchGate
P4SB is also active on the scientific platform Research Gate (www.researchgate.net). ResearchGate is the largest academic social networking site in terms active users for scientist and researchers to share papers, ask and answer questions, and find collaborators. Our scientists use this platform to spread as much information about P4SB as possible.
Figure 2.5: Activities of P4SB in ResearchGate
### 2.3 Scientific publications

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<tr>
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<th>Title of the scientific publication</th>
<th>DOI</th>
<th>ISSN or eSSN</th>
<th>Authors</th>
<th>Title of the journal or equivalent</th>
<th>Number, date</th>
<th>Publisher</th>
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<th>Year of publication</th>
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<th>Public &amp; private participation</th>
<th>Peer-review</th>
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<td>Tn7-Based Device for Calibrated Heterologous Gene Expression in Pseudomonas putida</td>
<td>10.1021/acssynbio.5b00058</td>
<td>21615063</td>
<td>Sebastian Zobel, Ilaria Benedetti, Lara Eisenbach, Victor de Lorenzo, Nick Wierckx, Lars M. Blank</td>
<td>ACS Synthetic Biology</td>
<td>4/12</td>
<td>American Chemical Society</td>
<td>United States</td>
<td>2015</td>
<td>1341-1351</td>
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<td>Article in journal</td>
<td>To be, or not to be biodegradable … that is the question for the bio-based plastics</td>
<td>10.1111/1751-7915.12393</td>
<td>17517915</td>
<td>M. Auxiliadora Prieto</td>
<td>Microbial Biotechnology</td>
<td>9/5</td>
<td>John Wiley &amp; Sons Ltd and Society for Applied Microbiology</td>
<td>United States</td>
<td>2016</td>
<td>652-657</td>
<td>NO</td>
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Besides these three foreground publications that deal with P4SB and its technologies, there is an existing call for papers to be edited by P4SB partners for the next special issue of Microbial Biotechnology with the title “From complex waste to valuable plastic”. The submission deadline is the 30th of November 2016. This Special Issue aims to present the most innovative advances in the field of waste bioconversion through mini-reviews, research articles, research notes, and opinion pieces. Therefore, possible subjects can be:

- Alternative feedstocks for bioplastic synthesis
- Metabolic engineering for the production of bioplastic
- Molecular basis for the synthesis and degradation of biopolymers
- Bioprocess design: pure and mixed cultures
- Novel applications of bioplastic

Click here for more information.

2.4 Presentations at conferences, symposia and workshops
The results of the P4SB project have been presented at a variety of national, European and international conferences, symposia and workshops.

2.4.1 Synthetic Biology UK 2015
The conference “Synthetic Biology UK 2015” took place from 1 until 3 September 2015 at Kingsway Hall Hotel, London, UK. Project partner USU gave a poster presentation with the title “Poster P026: Optimization of the cellular economy of Pseudomonas putida for ‘plastic recycling’.”
2.4.2 ESBP2015 Symposium Rome

The 8th European Symposium on Biopolymers took place from 16 until 18 September 2015 in Rome, Italy. Auxiliadora Prieto from CSIC and Kevin O’Connor from University College Dublin (UCD) presented the P4SB project to 150 scientists as a current trend in Europe involving synthetic biology. The presentation was held during the discussion “filling the gap from research/innovation to the market” and on “lesson learning” examples of cooperative public/private cooperation in European projects.
2.4.3 EC-workshop “Maximizing the Impact of KET Biotechnology”

Nick Wierckx from RWTH was invited to give a presentation at the workshop “Maximizing the Impact of KET Biotechnology”. This workshop was organised by the European Commission and took place in Brussels on 22 September 2015.

2.4.4 iGEM Giant Jamboree 2015

Lars Blank from RWTH attended the iGEM 2015 Giant Jamboree, which took place from 24 until 28 September 2015 at the Hynes Convention Center - Boston, MA (USA). The Giant Jamboree is the annual event where all of the collegiate and high school iGEM teams come together to present their synthetic biology projects. In 2015 more than 260 international, multidisciplinary teams were eager to share and celebrate their work.

The iGEM competition encourages university and high school student researchers to work in teams and solve real-world challenges by building genetically engineered biological systems with standard, interchangeable parts called BioBricks from the Registry of Standard Biological Parts. Each team manages their own projects, advocates for their research, and secures funding. Teams are also challenged to actively consider and address the safety, security and environmental implications of their work.

Involvement to iGEM 2015 resulted in an interview by James Fields about “Building with biobricks”, that has been published in Research Europe on 30 July 2015 (see Figure 2.8).
Building with biobricks

Researchers across Europe are jostling for early leadership in the nascent field of synthetic biology, reports James Field.

Now more than 400 million has already been allotted to synthetic biology projects under Horizon 2020. And national funding agencies are also queueing up to support groups that might eventually use the discipline to develop anything from enzymes that could polish cells to cells that can produce their own biofuels.

Synthetic biology is a phrase that might seem a mouthful to the French biophysicist Thierry Seluz, to describe the regulated reproduction of naturally occurring biological processes, yet it has only taken shape as a research discipline in the past decade. Whereas genetic modifications change organisms by cutting and pasting pieces of DNA that are already found in the natural world, synthetic biology seeks to give cells completely new properties—or even build the cells from scratch.

Biologists are divided as to whether this approach will prove to be—as some suggest—that a false underestimating of cells in need to be built, a handful of applications have already come to fruition. The French pharmaceutical company Sanofi, for example, is using the work of Jay Keasling, a biologist at the University of California, Berkeley, to synthetically produce antimalarial drugs in yeast, in volumes that have been defined 'vital interests'.

"Firms that on-going development is a synthetic function, make a business case and, if the cell has the resources, make it the way to a viable product," says Lars Blank, a microbiologist at RWTH Aachen in Germany. Blank recently coordinated a successful bid for more than €40m under the Horizon 2020 call 'Biobricks', to develop bacterial factories that convert plants into bio-renewable fuels.

Blank says the pace of progress in synthetic biology is increasing in the time the first synthetic version of L-3-propionylglycerol—a synthesized component used in ointments that was released by the US in 2007—took about 12 years to develop. Blank says, and Keasling's subsequent work on antimalarial drug derivatives also took a few years.

Nick Wierckx, a molecular biologist also at RWTH Aachen, says that the timelines required in synthetic biology are being compressed. "When I think of what I did 15 years ago, during my PhD dissertation, a semester could be subdivided in years now, or maybe six months."

The long-term goal is to develop standardised biological tools. Just as mechanical engineering tools are standardised and can be transferred seamlessly from workshop to workshop, synthetic biologists seek to create 'biobricks' that can be shared and combined to produce myriad biological products. "The aim is to create tools that can be shared and combined to produce myriad biological products.

On 28 October 2015 Lars Blank and Christine Kempchen gave a lecture at the RWTH to share their experience in applying and coordinating a H2020-project and used P4SB as a best practice example. P4SB was the first RWTH-coordinated project within the H2020-programme.

Figure 2.8: Interview of Lars Blank and Nick Wierckx from RWTH for Research Europe 2015

2.4.5 Workshop “EU-experience exchange” at RWTH

On 28 October 2015 Lars Blank and Christine Kempchen gave a lecture at the RWTH to share their experience in applying and coordinating a H2020-project and used P4SB as a best practice example. P4SB was the first RWTH-coordinated project within the H2020-programme.
2.4.6 European Networking Event “Successful R&I in Europe”

On 6 November 2015 Lars Blank from RWTH gave a presentation about P4SB in section “Success Stories in Europe based in NRW”. For more details, see Figure 2.10 for the flyer of this event.
2.4.7 3rd Applied Synthetic Biology in Europe meeting

The 3rd meeting about applied synthetic biology in Europe took place from 22 until 24 February 2016 in Lisbon, Portugal. Nick Wierckx and Sebastian Köbbing from RWTH gave presentations about synthetic promotor libraries as a powerful tool for tuning gene expression.
2.4.8 Forum BioChem 2016

Audrey Magnin from CNRS gave a poster presentation at the Forum BioChem 2016, which took place on 2 and 3 June 2016 at ESBS Parc d’Innovation in Strasbourg (France).

![Poster presentation at Forum BioChem 2016](image)

**Figure 2.12:** Audrey Magnin from CNRS proudly presents her poster “Development of a screening for the identification of polyurethane-degrading enzymes” at the Forum BioChem 2016

2.4.9 BIO.IBEROAMERICA 2016

Our project partner CSIC gave a P4SB presentation to 50 attendees with the title “Protein interactions drive the intracellular organization of bioplastics produced by *P. putida*” at the BIO.IBEROAMERICA 2016 conference, which took place from 5 to 8 June 2016 in Salamanca (Spain).
In this work we have focused on the study of some of these open questions, starting with the interaction of the cytosolic domain of the Wnt-11 receptor. We used a functional bacterial one-hybrid (Y1H) system to determine the interacting partner of the cytosolic domain. Our results indicate that the cytosolic domain interacts with a protein that we have named β-Catenin homologous protein (β-CatH). Further experiments are needed to identify the exact domain of β-CatH involved in the interaction. Finally, we have shown that the interaction between the cytosolic domain of the Wnt-11 receptor and β-CatH is mediated by the β-CatH N-terminus and is not required for the stability of β-CatH.

**Topics:** Molecular basis of bioproduct engineering

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**Figure 2.13:** Abstract and picture of the presentation by Natalia Tarazona from CSIC during the BIO.IBEROAMERICA 2016 conference

At the same event, Manuel Salvador from USU has been selected as best poster in the symposium “Bioenergia y Bioproductos”. We are very proud of his success.

**Figure 2.14:** Manuel Salvador from USU was selected as best poster at BIO.IBEROAMERICA 2016
2.4.10 Generation Plastik – Thementag Münster

Students from the University of Münster (Germany) invited Sebastian Köbbing from RWTH to give a presentation “From Plastic Waste to Plastic Value” during their info day “Generation Plastic”, which took place on 11 June 2016.

Figure 2.15: The talk about P4SB during the information day “Generation Plastic” was given by Sebastian Köbbing from RWTH.

2.4.11 UKSB 2016

CSIC gave a poster presentation with the title “Synthetic and Systems Biology Approaches Towards the Overproduction of Polyhydroxyalkanoates in Pseudomonas putida KT2440” to 100 participants at the UK Society for Biomaterials (UKSB) 2016 symposium, which took place on the 30 June and 01 July 2016 at the University of Westminster, United Kingdom.
2.4.12 7th European Congress on Biotechnology

Our project officer Mrs. Carmen de Vicente-Coll gave a presentation about P4SB during the 7th European Congress on Biotechnology, which took place in Krakow (Poland) from 3 July until 6 July 2016. Unfortunately there is no picture available of her during her talk, but her slides were approved in advance by the P4SB coordinator.

2.4.13 ISBP 2016

ISBP2016, the 15th International Symposium on Biopolymers taking place in Madrid (Spain) from 26 until 29 September 2016, is organised by CSIC, P4SB, Synpol, Sebiot, and CDTI and is especially geared towards connecting cutting edge technologies like systems biology and synthetic biology to thebiopolymer and bio-based polymer field (see flyer in Figure 2.17). From the P4SB-consortium, the following presentations will be given to 200 participants from either academia or industry:

- Lars Blank (RWTH) and Auxiliadora Prieto (CSIC): New feedstocks and bio-refineries
- Kevin O’Connor (UCD): Cutting-edge technologies for diversifying PHA and derivatives
- Luc Averous (UNISTRA): Applications and commercialization of biopolymers and bio-based polymers

Audrey Magnin from CNRS is presenting a poster with the title “Screening development for the identification of poly(ether)urethane degrading enzymes”.

Figure 2.16: Maria-Tsampilka Manoli from partner CSIC presented a poster at the UK Society for Biomaterials (UKSB) 2016 symposium.
Figure 2.17: Flyer of the ISBP 2016 symposium clearly showing that P4SB has a leading part in this event
2.5 Colloquium about Synthetic Biology

One objective of P4SB is to foster the scientific and public dialogue about project results and new applications in the field of synthetic biology and to achieve maximum visibility. Therefore Lars Blank, coordinator of P4SB and Principal Investigator at RWTH, contributed to a symposium about synthetic biology held on 24th of June 2015 at RWTH. 57 scientists from RWTH and Forschungszentrum Jülich (Germany) participated in this symposium.
2.6 Collaboration and knowledge exchange with EmPowerPutida

The scope of the topic BIOTEC-1-2014 clearly requires the liaison with other ongoing research projects. In order to comply with this requirement and to foster collaboration and communication between other international projects, we invited all other coordinators of projects that are funded by the Horizon2020 BIOTEC-1-2014 program to join the P4SB scientific advisory board (see chapter 2.10). In order to strengthen the collaboration between the two projects, P4SB and EmPowerPutida, Nick Wierckx (co-coordinator of P4SB) has been invited to attend the kick-off meeting of EmPowerPutida on 28th of May 2015. During this meeting both coordinators decided to have their first joint working meeting on 25 November 2015 in Cascais (Portugal), where both consortia held their General Assemblies. The next joint meeting between both projects is envisaged in autumn 2017.
2.7 Press releases and related publications

2.7.1 RWTH press release
On 2\textsuperscript{nd} of April 2015 RWTH published a press release (in German and English), which started to circulate immediately. Click here to download the press release (English version) as shown in Figure 2.21. It should be pointed out that P4SB project was the first RWTH-coordinated joint project under H2020 at Aachen University.
Bioplastic as a Natural Resource

02/04/2015

On April 1, 2015, the first RWTH-coordinated joint project will begin under Horizon 2020, the European Union’s framework program for research and innovation. The funding necessary for the project was raised by Prof. Lars Blank and Dr. Nick Wiercich at the Institute of Applied Microbiology, IAM. They were supported by EU project manager Christine Kempchen in RWTH’s Division of Research Funding.

Project P4SB – an abbreviation for From Plastic waste to Plastic value using Pseudomonas pulida Synthetic Biology – aims to transform plastic waste into bioplastic. Eleven partners – universities, research institutes, and industrial partners – from Germany, Spain, Ireland, the UK, and France, are involved in the project, which will run from April 2015 to March 2019 and has an entire volume of more than seven million Euros. Roughly 1.4 million Euros are reserved for RWTH alone.

With the help of concepts and methods from synthetic biology and through the use of the bacteria pseudomonas pulida, oil-based plastic waste will be transformed into completely biologically compostable material in Project P4SB. It will then be able to be used for the production of new products. Bioplastic made from oil-based plastic - the recycling chain is complete and closed.

P4SB is an answer to the widely-discussed debate about plastic as a natural resource and the associated environmental pollution. The project demonstrates that plastic does not equal plastic. The sustainable plastics of the second generation produced in the project are able to significantly reduce the detrimental environmental effects of plastic waste. Simultaneously, new markets arise based on plastic waste. This supports the European Union’s recycling goals till 2020.

Figure 2.21: English press release written by RWTH (coordinator of P4SB)

The press release was updated in January 2016, click here to read the full article.
Another update of the RWTH press release had been launched in March 2016, click [here](#) to read it in English.

**Figure 2.22:** The RWTH university’s newspaper RWTHInsight reports on P4SB in January 2016
Based on these press releases, to our knowledge P4SB has been cited in the following online newsletters and magazines:

3. [http://www.analytik-news.de/Presse/2015/208.html](http://www.analytik-news.de/Presse/2015/208.html)
6. [http://www1.wdr.de/wissen/natur/plastik-bakterium-recycling-100.html](http://www1.wdr.de/wissen/natur/plastik-bakterium-recycling-100.html)

Other publications in which P4SB has been cited:

1. [http://www.taz.de/Plastikfressendes-Bakterium-entdeckt/l5285954/](http://www.taz.de/Plastikfressendes-Bakterium-entdeckt/l5285954/)

### 2.7.2 ULEI press release

Based on the RWTH press release, ULEI started its own release as well. Click [here](http://www1.wdr.de/wissen/natur/plastik-bakterium-recycling-100.html) to download it.

![German press release of the Universität Leipzig (ULEI) on their university’s website](image-url)

**Figure 2.24:** German press release of the Universität Leipzig (ULEI) on their university’s website
2.7.3 CSIC press release


![Press release from CSIC in Spanish and English published on 1st of July 2015](image)

**Figure 2.25:** Press release from CSIC in Spanish and English published on 1st of July 2015

![Spanish typed text version of the article about P4SB published in the Braille magazine universo](image)

**Figure 2.26:** Spanish typed text version of the article about P4SB published in the Braille magazine *universo*
Besides this, Auxiliadora Prieto from partner CSIC was interviewed for the Spanish popular science magazine *universo*, which is published for the blind in Braille. They published a short article about the P4SB project (in Spanish only) that can be found on pages 10 to 12 (see Figure 2.26) of the full publication.

### 2.7.4 UCD press release

Kevin O’Connor, a leading green technologies researcher and innovator at UCD, has been honoured with the NovaUCD 2016 Innovation Award. The award was presented to Professor O’Connor, a professor at the UCD School of Biomolecular and Biomedical Science and a principal investigator at the UCD Earth Institute, in recognition of the quality and impact of his peer-reviewed research, his technological developments for the production of bio-based products, his industrial collaborations, and his successes in the commercialisation of intellectual property arising from his research carried out since joining UCD in 1999.

Kevin O’Connor has secured over €16 million in research funding to date and his research output includes the publication of over 85 international peer review articles and 150 international conference papers. His intellectual property portfolio currently consists of 11 invention disclosures; the filing of 9 priority patent applications and 6 licence agreements and the co-founding of BIOPLASTECH, a UCD spin-off company. Click [here](#) to read the full press release.

![Figure 2.27](#)

**Figure 2.27:** Kevin O’Connor was honoured with the NovaUCD 2016 Innovation Award

### 2.7.5 USU press release

On 25 July 2015 also our academic partner University of Surrey published a press release via their own website. Click [here](#) to read the full article.
2.7.6 **PROTEUS press release**

One of our French partners published an article about the P4SB project on their own website. Click [here](http://example.com) to read this article.
2.8 Training
Auxiliadora Prieto from partner CSIC presented a short course on "'Top-down' synthetic biology" in which she described the P4SB project to an academic audience consisting of approximately 50 university students. This was part of a symposium titled "Engineering Synthetic Biology of Biological Systems" at the Menéndez Pelayo International University in Santander (Spain) on 30 July 2015.

Figure 2.30: Programme of the training "'Top-down' synthetic biology" given by CSIC

2.9 Flyer
The first P4SB-flyer (1,000 copies) was printed in April 2016 and was distributed to all consortium partners for broad dissemination throughout their respective institutes.

Figure 2.31: The first P4SB flyer for public and scientific dissemination
2.10 External Advisory Board

The external scientific advisory board of P4SB is composed of outside experts that provide an external perspective of the project at large. These leaders in their field will also provide key contacts to engage scientific, commercial and societal stakeholders. The P4SB advisory board consists of five external members of considerable reputation in the scientific and dissemination activities related to the project. It meets at least once a year, adjoining in a bi-annual meeting upon the invitation of the coordinator. The role of the advisory board is to provide an independent assessment on the progress of the project in order to advise the consortium on new priorities or strategies to be implemented by the partners when required. The advisory board is composed of the following distinguished members:

- **Prof. Dr. Kenneth N. Timmis**, Technical University of Braunschweig. Prof. Timmis is a world-renowned member of the *Pseudomonas* community and has stood at the foundation of *Pseudomonas* biotechnology, which is reflected by the fact that the established paradigm organism (and P4SB chassis) *P. putida* KT2440 bears his initials. Besides being a leader in the scientific field (>600 publications, >30,000 citations), he also actively engages in commercial and societal debates and will thus provide a link to key scientific, commercial, social and policy maker stakeholders.

- **Prof. Dr. Sven Panke**, ETH Zürich. Prof. Panke is a leading scientist who has stood at the forefront of the implementation of synthetic biology in Europe and has published landmark papers in the field of synthetic biology, systems biology, and process engineering. His multidisciplinary background uniquely enables him to provide scientific guidance to the diverse P4SB consortium.

- **Dr. Hans-Christian Schaefer**, German Federal Environmental Foundation (DBU – Deutsche Bundesstiftung Umwelt). Dr Schaefer is renowned for exploiting biotechnology to improve the environmental impacts of production processes and ultimately for natural resource conservation and interdisciplinary research within the DBU. This organisation is one of Europe's largest foundations and has financially backed over 8,600 environmental projects with about €1.5 billion since 1991. He will provide communication channels with a wide variety of environmental stakeholders, initiate debate, and oversee the environmental impact of P4SB developments.

- **Prof. Dr. Vítor Martins dos Santos**, coordinator of another BIOTEC-1-2014 project called EmPowerPutida, accepted our invitation to join the P4SB scientific advisory board. This fosters collaboration and communication between the two H2020 projects that have much common ground, thus enabling coordinated development of synthetic biology in Europe while preventing needless competition.

- **Dr. Carmen de Vicente Coll**, Research Programme Officer DG for Research & Innovation at the European Commission (EC), also accepted our invitation to join the advisory board. During the 2nd General Assembly, Dr. de Vicente Coll was represented by the monitor expert Dr. Isabel Malcuit.

Prof. Luis Serrano Pubul, ICREA research Professor and group leader at the Center for Genomic Regulation in Barcelona (Spain) and coordinator of the BIOTEC-1-2014 project Mycosynvac.
unfortunately rejected his participation. Nevertheless, he was represented by his colleague Maria Lluch Senar to attend the P4SB kick-off meeting.

The joint advisory board has had two meetings:

1. Kick-off meeting in Aachen (Germany) from 28-29 April 2015.
2. 2nd General Assembly in Dublin (Ireland) from 26-27 April 2016.

### 3 Exploitation of results

Intellectual property rights as well as background and foreground IP are defined in the P4SB consortium agreement.

The commercial exploitation including exploring the market for P4SB applications and technologies is part of task 8.4. In this task a market analysis is carried out in order to develop an exploitation plan including an outline of the business plan.

Although a number of patents and licenses are expected to be achieved by P4SB, thus far there are no P4SB applications with regard to the following options:

- Patent
- Trademark
- Registered design
- Utility model
- Other

### 4 Risk assessment

As P4SB involves certain technical risks, a concrete risk management, mitigation and contingency plan has been developed by the consortium in order to mitigate risks and to ensure continuity of the innovative research work during the project lifetime. Therefore possible and occurring risks are proactively assessed during the implementation of the project. The risk register in the participant portal is continuously administrated and updated. When needed, changes to the project plan such as re-allocation of staff efforts or budgets will be implemented in collaboration with the involved partners. So far, the foreseen and unforeseen risks have not lead to such a re-allocation.
### 4.1 Foreseen risks

<table>
<thead>
<tr>
<th>Risk number</th>
<th>Description of risk</th>
<th>Work packages concerned</th>
<th>Proposed risk-mitigation measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>No agreement on cooperation contract</td>
<td>WP1</td>
<td>Partners had and have bi-/trilateral projects together and are experienced in working in multi partner projects. Management structure with a SC allows effective contract development.</td>
</tr>
<tr>
<td></td>
<td>• no joined work</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• low risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R2</td>
<td>Difficult to find co-workers with required skills</td>
<td>WP1, WP2, WP3, WP4, WP5, WP6, WP7, WP8</td>
<td>Partners have networks with people interested in industrial biotechnology and educate new co-workers. If difficulties occur, scientific job announcement platforms like Nature jobs will be used.</td>
</tr>
<tr>
<td></td>
<td>• possibility of delays</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• low risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R3</td>
<td>Underestimation of the effort required to reach the goals of the project</td>
<td>WP1, WP2, WP3, WP4, WP5, WP6, WP7, WP8</td>
<td>Monitoring and verifying the effort spent according to the scheduled work plan (deliverables and milestones), and adjustment of plans if needed. The Coordinator and the SC will be informed on a regular basis about progress and project difficulties.</td>
</tr>
<tr>
<td></td>
<td>• goals may not be reached</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• medium risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R4</td>
<td>PET hydrolytic activity too low</td>
<td>WP2</td>
<td>Parallel activities of enzyme optimisation and protein engineering to enhance activity are followed. As last resort, alternative physico-chemical methods are established at BIOPASTECH and UCD.</td>
</tr>
<tr>
<td></td>
<td>• enzymatic hydrolysis too slow for application</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• medium risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R5</td>
<td>Temperature of PET hydrolysis and PHA production differ substantially</td>
<td>WP2, WP6</td>
<td>If the temperature gap cannot be closed, a two-step process, in which PET hydrolysis with free enzymes is separated from PHA synthesis by the new cellular chassis, will be implemented.</td>
</tr>
<tr>
<td></td>
<td>• enzymatic hydrolysis too slow for application</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• medium risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R6</td>
<td>PU hydrolysis inefficient or incomplete</td>
<td>WP2, WP4</td>
<td>Multiple parallel activities of enzyme optimisation and alternative strategies for use of incompletely hydrolysed oligomers for production of second generation PUs.</td>
</tr>
<tr>
<td></td>
<td>• enzymatic PU hydrolysis too inefficient for application</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>• low risk</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### R7
Unable to metabolize TDI or MDI
- MDI and/or TDI not available as substrate for biocatalysis
- medium risk

WP3, WP4
Alternative strategy to purify MDI and TDI from hydrolysed PU for production of second generation PUs.

### R8
Multi-monomer utilisation in one strain inefficient
- final process too slow
- low risk

WP3, WP6
Dedicated tasks on dynamic control of monomer metabolism and development of process strategies. Alternative strategy of mixed-culture fermentation.

### R9
Synthetic biology tools for consolidation do not work
- consolidation not possible
- low risk

WP6
Existing, slower tools are available. Alternative strategy of mixed-culture fermentation.

### R10
Public perception that genetically modified bacteria would be released into the environment to degrade plastic
- no market acceptance of products
- low risk

WP8
Clear communication via several channels (scientific publications, press, and open dialogue event) that we only intend to apply the bacteria in closed contained settings.

### 4.2 Unforeseen risks

<table>
<thead>
<tr>
<th>Risk Number</th>
<th>Description of risk</th>
<th>Work packages concerned</th>
<th>Proposed risk-mitigation measures</th>
</tr>
</thead>
<tbody>
<tr>
<td>U1</td>
<td>difficulties in analysing the degree of PU hydrolysis</td>
<td>WP2</td>
<td>CNRS, UNISTRA &amp; PROTEUS: Use of colloidal PU</td>
</tr>
<tr>
<td>U2</td>
<td>difficulties in obtaining a HTS protocol for L-shuffling libraries screening</td>
<td>WP7</td>
<td>PROTEUS: Use of colloidal PU</td>
</tr>
<tr>
<td>U3</td>
<td>Unable to use depolymerised PU building blocks for new PU materials</td>
<td>WP4</td>
<td>CNRS, UNISTRA &amp; SOPREMA: Additional chemical modification of depolymerised PU building blocks; use of model molecules mimic of building blocks</td>
</tr>
<tr>
<td>U4</td>
<td>Unable to upscale depolymerisation technologies (technical and economic risks)</td>
<td>WP4, WP8</td>
<td>SOPREMA: Stay on the state of the art technologies</td>
</tr>
</tbody>
</table>
### States of the play for risk mitigation

<table>
<thead>
<tr>
<th>Risk number</th>
<th>Period</th>
<th>Did you apply risk mitigation measures?</th>
<th>Did your risk materialise?</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>R2</td>
<td>1st Period</td>
<td>Yes</td>
<td>No</td>
<td><strong>ULEI</strong>: Risk occurred, but did not materialise. No further comment.  <strong>UCD</strong>: Networks and job announcement platforms have been addressed and the issue has been solved for UCD.  <strong>BCM</strong>: Project is fully staffed with qualified personnel</td>
</tr>
</tbody>
</table>
| R3 | 1st Period | Yes | Yes | RWTH: Engineering of AA and BDO metabolism proceeds slower than planned. More person months have been planned on this activity. As a result, work on the diol dehydratase has been given a lower priority.  
ULEI: Risk occurred, but did not materialise. No further comment.  
BCM: No comment |
| R4 | 1st Period | Yes | No  | ULEI: Risk occurred, but did not materialise. No further comment. |
| R5 | 1st Period | Yes | Yes | ULEI: Effective enzymatic PET hydrolysis has to be performed at temperatures >60°C. Thus, the temperature gap for down-stream applications with Pseudomonas putida cannot be closed. A two-step process, in which PET hydrolysis with free enzymes is separated from PHA synthesis by the new cellular chassis will be implemented.  
BCM: Raising growth temperature for P. putida has shown promising results. |
| R6 | 1st Period | Yes | Yes | ULEI: Enzyme optimisation for obtaining higher PU hydrolysing activity will be performed.  
PROTEUS: Multiple parallel activities of enzyme optimisation.  
CNRS/UNISTRA: Additional enzymes screening and optimisation will be performed to obtain higher PU hydrolysing activity. |
| R7 | 1st Period | No  | Yes | UF2: Instead of TDI or MDI, a PU monomer synthesised by SOPREMA was successfully applied to isolate PU monomer-degrading bacteria. |
| R9 | 1st Period | No  | No  | BCM: Available tools for synthetic biology are working fine for the engineering of novel circuits. |
| U1 | 1st Period | Yes | Yes | CNRS, UNISTRA & PROTEUS: Colloidal PU synthesised by SOPREMA |
| U2 | 1st Period | Yes | Yes | PROTEUS: Colloidal PU synthesised by SOPREMA |
| U3 | 1st Period | Yes | Yes | CNRS & UNISTRA: Use of model molecules mimic of building blocks is envisaged.  
SOPREMA: Risk mitigation has not been applied, as task 4.7.3 did not start yet. |
| U4 | 1st Period | No  | No  | SOPREMA: Risk mitigation has not been applied, as task 4.7.3 did not start yet. |
| U5 | 1st Period | Yes | Yes | CNRS, UNISTRA & SOPREMA: Work with PU in fine dispersion. |
| U6 | 1st Period | Yes | Yes | UCD: No further comment |
| U7 | 1st Period | Yes | Yes | BCM: Measure of lytic activity in different growth stages |
| U8 | 1st Period | No  | Yes | BCM: Researching other expression systems |
| U9 | 1st Period | Yes | Yes | BCM: Implemented new method of measuring lytic activities |
5 Ethical and societal aspects
Currently no ethical or societal risks are foreseen for P4SB technologies. Nevertheless potential threats are monitored during the runtime of the project. So far no threats have been recorded. As already pointed out in the original proposal, in P4SB ethical and safety aspects are of minor concern with P4SB.

The P4SB consortium is aware that the dialogue surrounding the use of synthetic biology in Europe and beyond is ongoing. The interested public can participate in the results of the project, as P4SB has set up a very strong dissemination strategy as shown through this deliverable. Whenever needed or desired, society, including interested citizens and policy makers, are welcome to contribute to the educated societal debate on synthetic biology.