TNO report

ETP SB 2014
Annual report Enabling Technology Systems Biology 2014

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Author(s) Ivana Bobeldijk-Pastorova and Ben van Ommen
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1 Introduction

The Enabling Technology Systems Biology (ETSB) is the knowledge investment program which, during the period 2011-2014, provides the backbone for the biology oriented propositions of TNO. TNO has chosen to focus on all biology applications from a systems perspective. In addition to optimal facilitation of TNO propositions, execution of ETSB program aims to ensure a distinctive position for TNO in the Systems Biology arena.

As a simplification Systems Biology can be described as:
- measurement of complex biological processes at different levels (gene, protein, metabolite, morphology, physiology, behavior, etc.),
- modeling data into a mathematical description of the biological system in order to reduce its complexity,
- directing the system in the desired way, for instance by using it for novel therapies to reduce disease, new or more efficient microbial production processes, or development of improved food products.

Systems biology presents an enabling technology with potential applications in a broad range of domains: food, health, production processes, diagnostics, safety. The implementation of systems biology in these domains will result in major societal changes.

ETSB at TNO focuses on three themes, selected to cover the areas of interest of the related TNO propositions: systems biology of health (with key areas metabolism, inflammation and gut health), predictive toxicology and microbial production. Of these three themes, the health aspect is the predominant focus area and systems biology is exploited not only in the “classical” sense (combining genes, proteins, metabolites, etc.) but also from a “systems thinking” perspective, always approaching lifestyle related health and disease as systems. It’s content has been discussed with the internal stakeholders.
2 Signatures

The Hague, 3 March 2015
Dr. Ivana Bobeldijk-Pastorova
ETP Manager

The Hague, 3 March 2015
Drs. S. van Kooten
Managing Director ELSS
3 ETSB from 2011 to 2014

3.1 Transition 2011-2014 and contribution to the roadmaps

During the funding period 2011-14, Systems Biology at TNO has maturated from a multi-omics technology push in a number of fragmented areas (biomedical, food & health, microbiology) into a concerted strategy of systems approaches in human health(care). This is primarily due to the inclusion and elaboration of two concepts that have become drivers both for this integration and for the translation of systems biology into the TNO roadmap. The first concept is *systems flexibility* as a key component of health, i.e. the capacity of all relevant mechanisms and processes to absorb external challenges and perform a stress-response reaction leading to restoration of the resting condition (“homeostasis”). The second concept is the integrated need of four basic principles in health care, i.e. personalization, prevention, prediction and participation, coined as “P4health”. Together, these two concepts have been implemented in a strategy aiming at a number of changes in healthcare:

- All therapies focus on restoring flexibility;
- From a reductionist to a systems view on society and health;
- From disease management to health promotion;
- From medicine-focus only to include lifestyle, nutrition, psychology;
- From a generic to a personalized approach;
- From a passive patient to an optimally empowered and participating citizen.

Examples of each of these strategy aspects are found in chapter 3.7.

The initial elaboration of these aspects in the ETSB program has led to adaptation and implementation of P4Health in the roadmaps of Biomedical Innovation and Food & Health, The roadmap of Lifelong Healthy Living has focused on participation and self-empowerment in vitality, while the Work Health roadmap has collaborated in the aspect of self-empowerment. Sensor development for biomedical purposes has been stimulated, and ICT has gained a position in the development of Personal Health Portals within ETSB. Both developments are continued in 2015-18 Roadmaps. The infrastructural component of ETSB has led to the creation of a concerted health-related Big Data program from 2013 onwards, which is now continued in an ERP “Making Sense of Big Data” use case. The predictive health models initiated in ETSB have found applications in various aspects of health, including application in military healthcare and are now continued in the ERP “Complexity”. The concept of systems flexibility continues to be exploited in the ERP “Human Enhancement”.

In summary, ETSB has been instrumental in creating an unifying vision and strategy for TNO towards healthcare, has established the major technologies needed and has inspired numerous programs in the strategy period 2015-18.

3.2 Management summary progress in 2014

In the final year of ETSB, the focus of the program was two-fold:

- Finalize the technology portfolio which was constructed over the complete program period;
- Implement the program results in society, commercial collaborations and applied TNO programs in the next strategy period.

Both aspects were successfully executed and are summarized below.
The major developments of ETSB during its complete funding period 2011-2014 are summarized in the figure below. Aiming at a mid-term shift in healthcare and related commercial services from generic disease therapy to personal prevention and health promotion, we have initiated and developed two major transitions.

![Figure 1. summary of ETSB goals and their contribution to TNO's 2015-2018 Health strategy.](image)

Firstly, a “general population healthcare” shift towards personal health and wellness, based on an integrated view on health. This view is based on the concept of “systems flexibility”, i.e. the capacity to continuously adapt to changing external conditions. The practical translation of this concept was coined “P4 Health”, with P4 presenting Personalization, Prevention, Prediction and Participation. P4 Health has been adopted by TNO as the basis for its 2015-18 health strategy, see Figure 1. P4 Health is becoming the basis for many societal transitions in personal health applications, mostly initiated in ETSB. Major technology examples produced in 2014 in this area are:

- A new concept biomarkers for the nutrition and health arena and food industry (health claims), together with practical applications and interventions, allowing a continued effort of development of healthy foods.
- An integrated predictive model of health, combining physiological, psychological and sociological factors, with practical applications in general health, military health and work-related health.
- A push in personal and participative health applications by developing and integrating “do-it-yourself” health monitoring applications and ICT tools to present this information to the citizen, allowing them to guide their own lifestyle related health.
- The integration of gut health (the “microbiome”) with other aspects of lifestyle related health, both in scientific demonstrators and in practical applications.
- A start was made with integration of safety (toxicity, environmental exposure) aspects and the above mentioned examples, further integrating all relevant aspects of health. This was mainly done on a basic infrastructural level, preparing a further integration in the 2015-18 period.

The second transition focuses on healthcare practice, i.e. the patient care. TNO has focused on “lifestyle related diseases”, i.e. type 2 diabetes and resulting complications. Here, ETSB has introduced the same P4Health approach based on the systems flexibility concept, and developed a
series of applications personalized systems medicine. These all originate from a “systems view” on health, exploiting the concept that multiple connected processes collaborate in maintaining lifestyle related health, can differ between persons and from a therapeutic viewpoint all need to be diagnosed, and need to be corrected focusing on restoring systems flexibility. In 2014, taking the example of type 2 diabetes, a number of projects were finalized and the results implemented:

- The diagnosis of type 2 diabetes subgroups was optimized, taking into account all relevant processes, allowing for a tailored and efficient treatment.
- A predictive mathematical model was developed for type 2 diabetes interventions.
- Mechanistic insight into systems flexibility processes was developed, primarily using mouse studies and exploiting data and results from previously performed studies.
- A type 2 diabetes healthcare program based on these subgroups was implemented together with a group of General Practitioners in the Hillegom region.
- A pilot project with an integrated personalized lifestyle therapy (mental coaching, physical exercise and personalized food) with severe type 2 diabetes patients was established to demonstrate that even in advance diabetes disease states this approach is viable.

These two transitions were supported by a number of infrastructural projects, taking care of data flows.

In executing these programs, extensive collaborations were established both in the academic setting (national, European and global) and in the application (industrial, governmental/regulatory and societal).

The final year of ETSB was also characterized by extensive connections beyond the (systems) biology expertise groups within TNO, aiming to fully exploit all relevant disciplines within TNO in the two mentioned health(care) transitions. These included expertise groups in the area of the sensor development, ICT, motivation and behaviour, vitality, military health, labour health.

3.3 Description of the performed work in relation to the planning and goals of the program.

Within ETSB 2014 the projects were clustered into 6 technology clusters and an additional Management “Kennis Investerings Projects” (KIP) and Obligations KIP (see below).

In general, the goals planned for 2014 were achieved. In several cases the goals and the tasks of the projects were timely adjusted and redefined in order to maintain optimal contribution to the overall ETSB objectives. A summary of the changes (where applicable) and achieved results for each project is given in section 3.5. For selected projects highlights of the most important results are reported.

The pandemic of obesity is associated with a reduced metabolic flexibility, a growing incidence of metabolic diseases (e.g. T2M) and life-threatening complications such as non-alcoholic fatty liver disease (NAFLD/NASH) which often remain unrecognized. Currently used phenotypic parameters and conventional circulating risk factors, such as waist-hip ratio, body weight, HbA1c and glucose are insufficient to diagnose subjects at risk. Therefore, there is a growing need for identification of biomarkers of health as well as meaningful early diagnostic markers.

Furthermore, traditional interventions are mainly targeting late-stage disease mechanisms to control plasma glucose levels (examples are targeting gluconeogenesis in the liver by metformin, peripheral insulin sensitivity in muscle and adipose tissue by TZDs and pancreatic insulin production by sulfonylurea). Since normalizing glucose does not prevent pathologic diabetic complications to happen, novel strategies are currently sought. These novel interventions need to target underlying disease processes instead of conventional risk factors or epiphenomena like plasma glucose. The underlying disease processes may differ between specific sub-populations. For example, the major underlying disease processes for one sub-population may relate to a disturbance in glucose
metabolism, while for another sub-population the main underlying disease processes may relate to lipid handling or it may be strongly inflammatory driven. It has been shown in several studies that personalised lifestyle interventions can help prevent and in many cases also cure the disease. For adherence to lifestyle interventions it is of utmost importance that patients are empowered and coached during and after the intervention.

Most of the ETSB projects contributed to new knowledge and tool for personalized prevention and treatment of T2D.

**Cluster 1**

**Systems Diagnostics**

Goal: Provide a systems quantification toolbox of all aspects of health and disease related to health areas that are preventable by lifestyle.

Ideal markers would be those that are easy to measure and could serve as biomarkers for the health condition of the entire organism (‘systems markers’) reflecting ‘systems flexibility’ of an individual. Systems flexibility is defined as the capacity of the whole organism to cope with challenges such as metabolic overload. The current definition of health focuses on the ability to adapt to one’s environment. The most appropriate assessment of one’s health status in this sense is by performing challenge tests: the person is confronted with a challenge (e.g. exercise, consumption of high glucose or lipid) and the stress response time curve is measured by quantifying specific parameters in multiple time-points prior to and immediately following the challenge. The ability to efficiently re-establish parameter levels to a homeostatic (prior to challenge) levels is considered as a benchmark of good health status.

The specific projects in the KIP1 cluster focused on:

- **KIP1-1** Systems flexibility models: The key factors required for systems flexibility assessment (working towards a so called “health chip”) for glucose metabolism (subgroups for T2D) and lipid metabolism (non-alcoholic steato-hepatitis - NASH) based on existing data.
- **KIP1-2** Systems flexibility diagnostics: Identification of ‘systems flexibility markers’ which reflect the capacity of the adipose tissue to adapt to metabolic overload and identification of ‘systems flexibility markers’ that reflect the condition (health/disease) of the liver.
- **KIP1-3** Point-of-Care Diagnostics of corticosteroids and associated biomarkers: Development of technology for point-of-care diagnostics of corticoids and associated biomarkers.
- **KIP1-4** DiY methods and the Nutrition Researcher Cohort: Further profession-nalization of the Nutrition Researcher Cohort (NRC), a new generation open access cohort where each individual provides and owns her/his own health data from various tools or clinical analyses. This includes preparation of a larger sample and data collection study to prove the strength of this type of approach and validation of the Do-It-Yourself OGTT test in dry blood spots (that was developed in 2013) in a clinical setting.

**Cluster 2**

**Systems Interventions**

Goal: Derive optimal and personalized preventive and therapeutic interventions applicable in type 2 diabetes, its (cardio)vascular complications and optimal health maintenance. Develop a tool to model resilience that can be used to assess the applicability of interventions in different areas.

The two projects in the KIP2 cluster focused on:

- **KIP 2-1** Evaluation of previous interventions: Inventory of previous interventions for Type 2 diabetes (T2D) and nonalcoholic steatohapatitis (NASH) was made and evaluated for applicability in P4 field labs. The systems interventions should be applicable for disease
(sub)groups and may involve pharmaceutical, nutritional, lifestyle, and general health optimizing regimens or treatment strategies.

- KIP 2-2 Multidimensional resiliency assessment and optimization: Development of a multidimensional approach to modelling resilience, which provides useful tools for assessment and tracking resilience building practices for the general population and for service members (army) in particular.

**Cluster 3**

**Health data infrastructures**

Goal: Provide a functional infrastructure that exploits “big data” towards a series of relevant and “market driven” activities (biomarkers, drug targets, subgroup specific interventions, chemical safety, systems toxicology, etc).

In 2014, a (minor) further fine tuning of the data infrastructure for systems health was performed, focusing on “back-boning” of P4 health innovation: combining personal health data with the wealth of biological, physiological, biomedical, toxicological and mental research data to allow operation of optimal advice systems.

Activities were aligned with the Dutch Techcenter for Lifesciences (DTL) and the EU activities in EURO-DISH.

The specific projects in the KIP3 cluster focused on:

- KIP 3-1 The Phenotype database: Further professionalization of the database that facilitates the study data storage, connections to the Dutch Bioinformatics environment.
- KIP 3-2 SB Dashboard vouchers: General bioinformatics solutions and user interfaces developed based on running (theme) project needs that help data integration and processing.
- KIP 3-3 DIAMONDS: Development of advanced tools for retrieval and use of complex omics information for applications in toxicological evaluation of compounds (e.g. predictions of toxicity based on chemical similarities).
- KIP 3-4 Predimmune: Create a basis for a database and statistical modeling approach that generates a prediction of the immunogenic potential of a biological (a predictive algorithm).

**Cluster 4**

**Health Advice Systems**

Goal: Produce mathematical models that capture the complexity of (metabolic) health from a systems perspective, and thus provide practically applicable advice in health and healthcare based on all relevant input parameters (diagnostics, biomarkers).

Main objectives of the Cluster 4 KIP projects:

- KIP 4-1 Prototype Personal Health Infrastructure: develop a secure personal data store, and the basic applications for measuring, collecting, storing and accessing personal data on lifestyle & health in a secure way. Connect to external devices, apps, sensors using third party state of the art technology.
- KIP 4-2 Personal Health Portal: Specification, building, acquiring and assembling of a research P4-testbed to facilitate and accelerate the transfer of new P4-services, applications and tools into the Field Labs of TNO and its partners.
- KIP 4-3 Integrated prototype Diabetes Coach: The T2D e-coach is an extension of TNO’s efforts in metabolic disease diagnostics and interventions based on systems thinking and on mathematical models being built in ETSB and the EU FP7 projects NU-Age and MissionT2D. In 2013, the P4P Field Lab is being established where the deliverables will be implemented in a new healthcare approach.
- KIP 4-4 Integrated Health advice system: For the development of effective health advice systems two aspects are of great importance of great importance were addressed by this project. One is
the integration of mental and physical aspects of health, and eventually the entire health ecosystem. The other is the ability to subtype healthy subjects and target interventions to those subtypes.

- KIP 4-5: P4@TNO: Implement and test personalized work-related health improvement program
  The program will address physical, mental and social health goals of the individual and the population within TNO.

**Cluster 5**

**Microbiome functionality and human health**

Goal: establish functional relationships between aspects of the human microbiome (from oral to colon) and aspects of human health, disease and therapy, with emphasis on metabolic-inflammatory aspects, resulting in better understanding, diagnosis and treatment.

KIP5 focused on the microbiota as a major component of the human system. Overwhelming scientific evidence has been generated during recent years supporting the importance of our microbiota for our health. Most of this evidence until now is based on describing relationships with less emphasis on understanding and even less emphasis on influencing the microbiota effects on systems health. In the different subprojects of this KIP project we have addressed these issues with the aim of better understanding microbiota health effects and improving systems health:

- KIP 5-1 Improved microbiome resilience: Building a stable healthy microbiota of the upper respiratory tract and establish a host/microbe interaction system accessible to experimental analysis.
- KIP 5-2 Therapeutic use of personalized probiotics: Development of cultivation methods for the *ex vivo* enrichment of lactobacilli from human vaginal samples with the aim to apply the enriched culture as personalized probiotics.
- KIP 5-3: Microbiota in relation to metabolic disease: Link microbiota (composition) to host physiology (including probiotic effects).
- KIP 5-4: Synthetic microbial consortium: Investigate artificial human microbiota as a model for understanding interactions.

**Cluster 6**

**Biotechnology**

Goal: Support the development of a Strategic Research Program in microbial production, specifically by further developing the “parallel pathway production” technology developed within ETSB in 2011-2013.

In this project three highly related subproject are defined. All three subprojects are related to the use of systems biology approaches towards (fungal/yeast/bacterial) production platforms used in Industrial Biotechnology:

- KIP 6-1 The University chair Industrial Biotechnology: Provides the networking platform to exploit interaction with Leiden University and (inter)national research groups in the relevant research field.
- KIP 6-2 The PhD project Cell Factory 2.0: Focuses on production of new chemical building blocks via pathway engineering.
- KIP 6-3 Cell Factory 3.0: Aims the exploration of the use of a biotechnological production platform with one level of additional complexity using a (simple) microbial consortium.

**KIP Management**

Goal: to connect to other relevant programs and projects within TNO and with external parties, ensure adequate execution of ETSB projects and ensure communication and dissemination within and outside the TNO organization.
KIP Obligations
Goal: continuation of collaborations from the previous strategy period. This KIP was a collection of matching obligations for collaborations with academia or consortia which were not part of another ETSB KIP project.

- Between 2011 and 2014, the program financially supported many TNO collaborations with other EU, academic or industrial partners:
  - The FP7-project MISSION-T2D, on modelling of a virtual diabetic patient
    o Results of this projects were directly integrated in KIP 4-3 and 4-2.
  - The ZonMW project MKMD diabetic complications, a challenging and promising collaborative project focusing on delivering an early predictive signature on the development of various diabetic complications (including NASH, atherosclerosis, diabetic nephropathy and/or diabetic retinopathy) in on single mouse model that closely represents the human situation. Using a systems biology approach, this signature will be created by combining a large set of state-of-the-art technologies.
  - COSMOS and EURODISH, both ‘Coordination and support action’ EU projects. COSMOS aims at the development of an efficient e-infrastructure, standards and data-flow for metabolomics and its interface to biomedical and life science e-infrastructures in Europe and worldwide. EURODISH aims to provide advanced and feasible recommendations on the needs for Research Infrastructures (RIs) to ESFRI, the European Strategy Forum on Research Infrastructures, and other stakeholders. Both projects were directly aligned to the database and infrastructures KIP3 cluster.
  - ZonMW ASAT, on the development of the so-called ‘ASAT Knowledge Base’, a knowledge and data platform based on the ASAT principle (‘Assuring Safety Without Animal Testing’).
  - Six endowed chairs, at HU University of Applied Sciences Utrecht (Cyrille Krul), Leiden University (Jan van der Greef, Peter Punt), Leiden University Medical Center (Louis Havekes), Wageningen University & Research centre (Ruud Woutersen), and VU University Amsterdam (Remco Kort).

3.4 Contribution to the relevant TNO themes, Innovation areas and Top Sectors

The enabling technology program Systems Biology was prepared in discussion with the directors of the relevant TNO themes and Innovation areas as primary internal stakeholders. As a result a steering committee was formed that consists of Dr. N. Snoeij (Theme “Healthy Living”), Dr. J.-P. van der Lugt (Innovation Area “Nutrition”; member of the steering committee until September 1, 2014), Dr. P. van Dijken (Innovation Area “Biomedical Innovations” and from September Personalised food), Dr. A. Bronkhorst (representing Theme “Defense, Safety and Security”), A. van Berkel (Innovation Area “Sustainable Chemical Industry”), Dr. C. Krul (from September Innovation area Personalised health technologies) Dr. J.H. Brussaard (Director of Research, Life Sciences, TNO; member and chairman of the steering committee until October 1, 2014), Ir. P. Schulein (Director of Research, Life Sciences, TNO; member and chairman of the steering committee from October 1, 2014). The steering committee monitored the execution of the ETSB program in regular meetings.

The program directly contributes to the following Top Sector Life, Science and Health Roadmaps:
1. Molecular diagnostics: The development and application of new assays, possible diagnostics based on literature (Projects Cluster 1: Systems Diagnostics; KIP 5.1: Improved microbiome resilience; KIP 5.2: Personalized probiotics)
2. Homecare & self-management: Development, assessment and implementation of technologies, infrastructure and services that promote clients’ abilities to live
independently and manage their own care, adequately supported by healthcare professionals (Projects KIP 1.4: The Nutrition Researcher Cohort; Cluster 4: Health Advice Systems, Cluster 3: Data Infrastructures)

7. Specialized nutrition, health & disease: Researching specialized nutrition for nutritional intervention as part of integrated health solutions in terms of prevention, cure and care of chronic, acute and rare disease (Projects Cluster 1: Systems Diagnostics; KIP 2.1: Systems Interventions)

For the Top Sector Agro-food, the program contributes to the following program lines:
Weight Maintenance, Gastro-Intestinal Health (Projects Cluster 1: Systems Diagnostics; Cluster 5: Microbiome functionality and human health), Healthy Ageing (Projects Cluster 1: Systems Diagnostics; Cluster 5: Microbiome functionality and human health) and Valorization of Side Streams and Raw Materials (Projects Cluster 6: Biotechnology).

Table 1 gives an overview of the ETSB contribution to TNO relevant Innovation areas and Propositions.

Table 1  ETSB contribution to TNO relevant Innovation areas or Propositions

<table>
<thead>
<tr>
<th>project/cluster</th>
<th>Theme/Innovation area/Proposition *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster 1: Systems Diagnostics</td>
<td>BI, HF</td>
</tr>
<tr>
<td>Cluster 2: Systems Interventions</td>
<td>3R, BI, HF</td>
</tr>
<tr>
<td>Cluster 3: Health data infrastructures</td>
<td>3R, BI, FS, GH, HF</td>
</tr>
<tr>
<td>Cluster 4: Health Advice Systems</td>
<td>3R, BI, DR, FS, GH, HF</td>
</tr>
<tr>
<td>Cluster 5: Microbiome functionality</td>
<td>BI, FS, GH, HF</td>
</tr>
<tr>
<td>Cluster 6: Biotechnology</td>
<td>BBE, IF</td>
</tr>
</tbody>
</table>

* 3R: Refinement, Reduction and Replacement of animal testing  
BBE: Bio-Based Economy  
BI: Biomedical Innovations  
DR: Defense Research  
FS: Food safety  
GH: Gut Health  
HF: Healthy Food  
IF: Innovative Food Concepts

Specific examples of knowledge and technology developed in ETP Systems Biology applied in follow-up innovation projects either approved or submitted under the relevant themes and TOP Sectors in 2014:

- For the Samueli Institute project a model is in development that can map the main determinants for successful reintegration into society of military personnel in the U.S.; simulations with this model will contribute to determining optimal interventions to promote this challenging process
- An EU proposal, P4 Health Horizon 2020, was submitted, on the application of the different subtyping tools and personalized interventions in prevention or treatment of type 2 diabetes
- An EU twinning grant project on itaconic acid production was obtained (EU-LEVITA) as a start-up for joint project proposals and used to draft an project proposal on the role of transport and toxicity in the production of organic acids by fungi.
- The ERA-IB NOW proposal FILAZYME on bacterial enzyme production by filamentous organisms was submitted and accepted, scheduled to start in 2015 with a PhD position in Leiden and TNO as an associate participant.
- The 2nd phase of PPS Resilience was granted, on improving resilience with essential nutrients and whole wheat bread.
- The PPS project Proliver is in preparation, aiming to link animal health and the development of liver diseases by applying different dietary conditions while maintaining gut integrity.
• Furthermore, tools and knowledge developed from within ETSB between 2011 and 2014 are applied in many commercial collaborations (confidential).

More examples of project collaborations are given in ANNEX 1.

3.4.1 Overview of participation in (international) research programs and networks
Current collaborations with different partners are given in an overview in Table 2.

Table 2 Overview of current collaborations

<table>
<thead>
<tr>
<th>Open Innovation Networks</th>
<th>Countries</th>
<th>Focus on</th>
</tr>
</thead>
<tbody>
<tr>
<td>Center for Medical Systems Biology 2</td>
<td>Netherlands</td>
<td>Combination therapy in metabolic syndrome</td>
</tr>
<tr>
<td>DTL</td>
<td>Netherlands</td>
<td>data stewardship, databasing, data integration, technology</td>
</tr>
<tr>
<td>NBIC</td>
<td>Netherlands</td>
<td>Phenotype database</td>
</tr>
<tr>
<td>Netherlands Metabolomics Centre</td>
<td>Netherlands</td>
<td>Deconvolution metabolomics, bioinformatics tools data-study capture, synthetic biology, exometabolomics</td>
</tr>
<tr>
<td>Netherlands Toxicogenomics Centre</td>
<td>Netherlands</td>
<td>Toxicogenomics, bioinformatics</td>
</tr>
<tr>
<td>NGI</td>
<td>Netherlands</td>
<td>Metabolomics, phenotyping Type 2 diabetes</td>
</tr>
<tr>
<td>SB@NL</td>
<td>Netherlands</td>
<td>Systems biology, modelling, data integration</td>
</tr>
<tr>
<td>Sino Dutch PPM</td>
<td>Netherlands</td>
<td>Diagnosis diabetes, statistics, bioinformatics, metabolomics</td>
</tr>
<tr>
<td>Ti Coast</td>
<td>Netherlands</td>
<td>Breath analysis</td>
</tr>
<tr>
<td>TIFN</td>
<td>Netherlands</td>
<td>Oxylipids, endocannabinoids, food and metabolic syndrome</td>
</tr>
<tr>
<td>Top Institute Pharma</td>
<td>Netherlands</td>
<td>Metabolomics, Diabetes, CNS, Drug induced weight alterations</td>
</tr>
</tbody>
</table>

Table 2 continued

<table>
<thead>
<tr>
<th>Bilateral cooperation with (international) universities</th>
<th>Countries</th>
<th>Focus on</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dalian Institute of Chemical Physics (CAS)</td>
<td>China</td>
<td>Intervention studies, phenotyping diabetes, metabolomics</td>
</tr>
<tr>
<td>Eindhoven University of Technology / Centraalbureau voor Schimmelcultures</td>
<td>Netherlands</td>
<td>Biofilms</td>
</tr>
<tr>
<td>Erasmus University</td>
<td>Netherlands</td>
<td>Predicting response to a very low caloric diet in diabetes type 2 patients</td>
</tr>
<tr>
<td>Hospital Universitario Reina Sofia, Cordoba</td>
<td>Spain</td>
<td>Cohort study on subtyping in T2D</td>
</tr>
<tr>
<td>Karolinska Institutet</td>
<td>Sweden</td>
<td>Multivariate data analysis</td>
</tr>
<tr>
<td>TU Wien</td>
<td>Austria</td>
<td>Fungal Technology and metabolomics analysis</td>
</tr>
<tr>
<td>TUFTS University Boston</td>
<td>U.S.</td>
<td>Lipoprotein Modelling</td>
</tr>
<tr>
<td>Universiteit van Amsterdam</td>
<td>Netherlands</td>
<td>Fungal biotechnology, synthetic biology, metabolic engineering</td>
</tr>
<tr>
<td>University College Dublin</td>
<td>Ireland</td>
<td>Development alternative animal testing (cell based models), dbNP / in silico analysis</td>
</tr>
<tr>
<td>University of Debrecen</td>
<td>Hungary</td>
<td>Fungal Technology and metabolomics analysis</td>
</tr>
<tr>
<td>University of Groningen</td>
<td>Netherlands</td>
<td>Modelling</td>
</tr>
<tr>
<td>University of Leiden</td>
<td>Netherlands</td>
<td>Support for metabolomics analysis and supervision PhD projects, Fermentation Biotechnology</td>
</tr>
<tr>
<td>University of Leiden – LUMC</td>
<td>Netherlands</td>
<td>Vascular Medicine, metabolomics, chronobiology, identification</td>
</tr>
</tbody>
</table>
University of Maastricht | Netherlands | Toxicogenomics, network biology, T2D

University of Nijmegen | Netherlands | Biomarker Research

University of Utrecht | Netherlands | Innovative technologies in Exposure assessment, Fungal Biotechnology and metabolomics analysis

University of Wageningen | Netherlands | Food and Pharma, NMR, modelling, PBMC Biomarker research

University of Westminster | U.K. | Fatty liver / NASH biomarker research

Utrecht medical Center | Netherlands | Predicting response to biological treatment in rheumatoid arthritis patients

VU Amsterdam | Netherlands | Cardiovascular disease

VU, UvA Amsterdam | Netherlands | Bioinformatics, chronobiology

University of Lisbon | Portugal | Metabolic engineering

University of Salamanca | Spain | Enzyme technology

Table 2 continued

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3.5 Highlights 2014

Background
Type 2 Diabetes (T2D) and its life-threatening pathologic complications is a major health problem worldwide. The incidence of T2D has reached epidemic proportions and more than 350 million patients suffer from this disease. A rapidly growing number of subjects (>750 million) is at risk and will develop T2D and its life-threatening complications in the near future. Several projects within the ETSB 2014 program had goals to identify new intervention strategies in T2D, new diagnostic markers to stratify the patient and also new ways to empower the patients and citizens in improving their lifestyle and health.

The primary symptom is high plasma glucose and medical treatment focuses on lowering glucose by reducing glucose synthesis or increasing insulin excretion. Remarkably, the real problem (specific organ dysfunction) is hardly addressed except by lifestyle changes restoring the energy balance (“eat less and exercise more”). Increasing evidence of distinct subgroups within the T2D population exists, which may require tailored treatment approaches instead of a “one-size fits all” treatment. The most interesting results for 2014 are given in highlight 3.5. Several other are mentioned in section 3.7.

In prevention and treatment of Type 2 Diabetes, personal and participative health applications that develop and integrate “do-it-yourself” health monitoring applications and ICT tools to present this information to the citizen are essential for success in interventions. They allow citizens to guide their own lifestyle related health. ETSB achieved significant results in this area and provided directly applicable tools that are shown in highlights 3.5.2-3.5.5.

Other applications of Systems Biology: in addition to T2D and health monitoring applications and ICT tools, the ETSB program also contributed to toxicological applications such as predicting immunotoxicity and predicting toxicity based on integrating omics data from different databases and literature, highlight 3.5.6 and 3.5.9.

3.5.1 Circulating lipid signatures: a marker for limited metabolic flexibility (ETSB 2014, KIP 1.2, Systems flexibility diagnostics)

The pandemic of obesity is associated with a reduced metabolic flexibility and a growing incidence of metabolic diseases (e.g. T2M) leading to life-threatening complications such as non-alcoholic fatty liver disease (NAFLD/NASH). These disorders often remain unrecognized for a long time and are diagnosed once severe stages of the disease have already developed. Currently used phenotypic parameters and conventional circulating risk factors, such as waist-hip ratio, body weight, HbA1c and glucose are insufficient to diagnose subjects at risk. Therefore, there is a growing need for identification of meaningful early diagnostic markers which can mechanistically be linked to the disease process. It is assumed that the adipose tissue has a limited expandability and, once its maximal size has been reached, inflammatory mediators will be released in the circulation. Hence, the adipose tissue and factors released from this tissue may serve as a new type of ‘marker’ that indicates that the metabolic flexibility of the organism has been reached and that a subject is at high risk to develop disease.

Main results:
• It was observed that once healthy adipose tissue (fig. 2A) expands and becomes inflamed (fig. 2B), crown-like structures emerge in the tissue formed by inflammatory macrophages (fig. 2C).
• It has been shown that adipose tissue inflammation occurs once an adipose depot has reached its maximal storage capacity.
• This condition is associated with a change of the type and levels of circulating lipids (‘lipid signature’), which may be used as indicators for the limited metabolic flexibility.
• The project established the technologies that allow the detection and analysis of these markers in the context of diet-induced obesity and the metabolic syndrome.

Application:
• New types diagnostic markers (‘signatures’) that are linked to a disease process
• Tools that can predict risk of future disease (risk estimation of a subject).
• New targets for intervention in metabolic diseases (NAFLD, CVD) associated with obesity.

Figure 2. Perigonadal fat histology of control mice (A) and high fat diet (HFD) fed mice (B) with the presence of inflammatory cells (crown-like structures). Quantification of crown-like structures (C).

3.5.2 Resiliency based Systems Health Model: Towards a health ecosystem (ETSB 2014, KIP 2.2, Multidimensional resiliency assessment and optimization)
Resilience is a core characteristic required for healthy living, optimal performance and experiencing wellness. It is the ability to adapt, recover and even thrive after stress, burden, trauma and life changing events and adversity. Resilience is not just psychological, but requires optimal capacity in all dimensions of human functioning: mind, body, social and spiritual. However, recent studies have shown that there is not such a thing as an optimal set of variables driving resiliency, rather people tend to develop different styles in different situations. The main challenge therefore is how to get a comprehensive understanding of resiliency and the interaction between the determinants of resiliency for particular subgroups in particular contexts. The aim of this project is the development of a multidimensional approach to modelling resilience. Two specific use cases were further explored:
- metabolic syndrome and diabetes type 2
- military training and performance

Figure 3. Resilience based Systems Health Model
Main results:
- A semi-quantitative relational model of health and resilience, based on several group model building sessions with TNO experts active in various scientific disciplines and supported by an extensive literature study.

Application:
- Building shared understanding among multi-disciplinary experts groups
- Building shared understanding among stakeholders (e.g. health care or military settings)
- Exploring the relative effects of interventions on domains of health
- Starting point for the development of more dedicated (data driven) health and performance models
- Integrating markers of health (e-health app & e-coaching market)

3.5.3 Health data infrastructures (ETSB 2014, KIP 3 Cluster)
KIP3 delivered a data infrastructure that allows to integrate, align, and interpret available knowledge and data on (metabolic) health and safety, both for research studies and (personalized) healthcare. This activity is aligned with the Dutch Techcenter for Life Sciences (DTL) and the EU activities in EuroDISH and ELIXIR. DTL is a collaborative platform (public-private partnership) of life science and technology research groups in the Dutch clinical & health, nutrition, crop and livestock breeding and industrial microbiology sectors. Collectively, the DTL parties address the major ‘big science & big data’ challenges in biology R&D that no single organisation can address alone. DTL is the Dutch node of the research infrastructure ELIXIR. TNO is the coordinator of ENPADASI and heading the nutritional sector of DTL. Thereby we are seen as the central hub for nutritional data.

DIAMONDS (3.3) and PRED-IMMUNE (3.4) are two examples where the flow from data, to information, to knowledge is employed towards specific market applications. DIAMONDS integrates knowledge and data relating to the chemical structure, kinetics, metabolism, system biology and toxicity of substances. This allows a ‘new-style’ toxicological risk assessment method that makes it possible to determine the toxic properties of a chemical substance at an early stage, thereby reducing costs and preventing unnecessary testing on animals. PRED-IMMUNE is a database of biopharmaceuticals and encompassing physical chemical properties, preclinical in vivo toxicity data, clinical properties, and in vitro data on these substances mined from public sources. This has been the basis for a statistical modeling approach that generates a prediction of the immunogenic potential of a biological.

The Phenotype Database (3.1) and Infrastructure Apps (3.2) facilitate the study data storage and the integration of data in a broader sense. Where the Phenotype Database excels in a well-organized harmonized capturing of the biological experimental conditions as well storage the diverse pre-processed Omics data, the Infrastructure Apps provide complementary tools, such as the pre-processing and statistical analysis of Omics data as well as mining public knowledge resources and personalized health devices.

Applications:
- Efficient reuse of tools and existing data for the chemical and life-style related market
- Enable analysis on combination of studies including omics data, which makes them more affordable
- Faster evaluation of complex (large number of parameters) data
3.5.4 Type-2 diabetes e-coach (ETSB 2013, KIP 4.3, Prototype Type 2 Diabetes e-coach model)
Focusing on maintenance (prevention) and regain (preventive medicine) of optimal health, e-coaches can play an important role in personalization of health care and will allow implementation of better and more cost-effective prevention strategies. Type-2 diabetes (T2D) is an important application area.

A T2D e-coach should have the following functionalities:
1. Monitoring (body weight, physical activity, food intake, plasma glucose concentration, mood, appetite, and energy)
2. Prediction
3. Feedback
4. Goal setting
5. Social support
6. Problem Solving
7. Reminding

An existing e-coaching tool, PatientCoach, developed at the LUMC Leiden, provides an excellent platform for a T2D e-coach as it is already used by healthcare providers. PatientCoach was extended with a number of predictive models and functionalities that provide the support needed in dietary and lifestyle interventions applied in diabetes treatment. For example, the diabetes subtyping tool can be accessed via PatientCoach (see highlight in section 3.7) and thus help health care providers to determine the best intervention.
Main results:
- A prototype new application of PatientCoach for Type-2 diabetes
- A prediction module for body mass index, plasma fasting glucose and insulin sensitivity integrated in PatientCoach.

Application:
- Patient Coach is ready for application in Diabetes and Lifestyle intervention Field labs.

3.5.5 P4@TNO: TNO acting as a field lab for health measurements; effects on health and behavior (ETSB 2013, KIP 4.5, P4@TNO)
There is an increasing number of possibilities for individuals to map their own health status. Smartphone Apps, Quantified Self devices and self-tests give individuals the opportunity to measure several aspects of their health, like food intake, body weight, blood pressure, physical activity, blood sugar and cholesterol, with increasing accuracy. These measurements contribute to an individual’s awareness of their own health status and as such serve as a motivator to improve health. However, the usefulness of data resulting from self-monitoring devices for scientific purposes has not been investigated yet. Besides, it is not known to which extent increased awareness in an individual’s health parameters contributes to behaviour change and improved health status.
We studied and evaluated the potential of do-it-yourself devices for self-measuring health parameters by subjects in obtaining useful data and evaluated whether increased awareness of own health
status by self-monitoring health parameters also serves as motivational instrument for changing health behaviour.

Main results:
- 33 TNO employees from Delft and The Hague participated. Two subjects dropped out; one related to the burden of the study, the activities to monitor health were too time consuming. The study started in September and has finished in the first week of December 2014. With focus group discussions the user experiences were evaluated.
- It is interesting to note that self-monitoring led to more awareness and increase of physical activity. Registration of food intake lead to (temporary) decrease in calorie intake especially related to sweet treats. Further evaluation of results is still on-going.

Application:
The used tools were weight scale, blood pressure apparatus, blood glucose monitor, cholesterol measurement, activity tracker and the FatSecret app to measure energy intake. All personal data were transferred via the Nutritional Research Cohort Portal to a web-page for each subject showing his/her health parameters. The integration of data represents the health status of an individual and coaching tools help the subject to adapt life style changes for health improvement. The application of this research is therefore twofold:
- Use of do-it-yourself measurement of health parameters in (clinical) intervention studies, to lower the burden of participation for subjects.
- Self-monitoring of health by employees, to increase awareness of their own health status and thereby motivate healthy behavior. Coaching tools and advice can be integrated in the health platform to support employees in adapting to a healthy lifestyle.

Figure 6. Example of how different self-monitoring tool can help improve life style of TNO colleagues

3.5.6 TNO able to predict the harmfulness of chemicals (ETSB 2014, KIP 3.3 DIAMONDS)
The chemical industry is constantly on the lookout for new chemicals, for example as alternatives to chemical compounds that are harmful to humans or the environment, or for new applications. Any as yet unknown harmful effects of large numbers of existing chemicals also need to be identified. DIAMONDS is an ambitious research programme to help predict the harmfulness of new and existing chemicals.
The European REACH Directive requires the chemical industry to identify the harmful effects of existing chemicals swiftly. The expectation is that the EU will ban the use of economically valuable chemicals in future. The industry therefore needs to find far less harmful alternatives. ‘That faces the industry with a major challenge’, says TNO's Dinant Kroese. ‘When developing new chemicals too, an idea of their harmfulness is needed at an early stage, so that sensible choices can be made taking that into account – especially where certain complex harmful effects are concerned: effects due to chronic exposure, for instance, and effects on fertility and children’s development. Animal studies are time-consuming and very expensive, and there are no alternative methods available at
present. That’s why we have set up DIAMONDS. With it we think we can offer the industry an appropriate solution.’

Main Results:
- DIAMONDS pools all the publicly available knowledge on some thousands of chemicals that have already been studied. It is based on studies, scientific articles and everything that is known from other sources.
- Tools and infrastructures that enables us to detect links between chemical structure and toxicity. The big advantage is that this knowledge enables evaluation of the expected toxicity of chemicals that have not been studied to be predicted accurately.
- DIAMONDS infrastructure was presented at many relevant conferences to create many links to the chemical industry. Diamonds was also in several press releases in media relevant to the chemical industry, see Figure.

Application:
- DIAMONDS can also help to cut development costs.’ Using the database, TNO is able to advise on what chemicals are most suitable.
- Depending on the aim (registration or selection), we can carry out biological verification much more quickly, based on the prediction, thus speeding up time to market for production of chemicals from cosmetics, agrochemicals to food additive applications.
- The biological verification can be carried out using a targeted in vitro or in vivo test. In this way TNO helps to avoid extensive animal studies while still being able to provide biological substantiation for its predictions.

Figure 7. Example of publicity for the TNO Diamonds approach

3.5.7 TNO predicts unwanted immunogenicity of biologicals based on Integrated Analysis (ETSB 2014, KIP 3.4, PRED-IMMUNE)

“Immunogenicity is the ability of a particular substance, such as an antigen or epitope, to provoke an immune response in the body of a human or animal. Wanted immunogenicity is typically related with vaccines, where the injection of an antigen (the vaccine) provokes an immune response against the pathogen (virus, bacteria...) aiming at protecting the organism. However, unwanted immunogenicity is when the organism mounts an immune response against a therapeutic antigen (ex. recombinant protein, or monoclonal antibody). This reaction leads to production of anti-drug-antibodies (ADAs) inactivating the therapeutic effects of the treatment and, in rare cases, inducing adverse effects. The prediction of the immunogenic potential of novel protein therapeutics is a major challenge in biotherapy.’ (source Wikipedia)
This major challenge in biotherapy may be tackled by bringing together all relevant in vitro, in vivo and clinical measurements in combination with knowledge about the structural features of the biological and observed immunogenic prevalence in clinical studies. TNO had developed a data management and data integration tool that brings together these diverse types of information and combines this with a Bayesian immunogenicity prediction algorithm into a single platform. This platform is called PRED-IMMUNE.

While immunological experts continue to gather more and more relevant information and enter it in the PRED-IMMUNE platform, the Bayesian predictor is automatically updated and processes the newly found information to provide the best prediction possible given the available data. The predictor is able to integrate the diverse types of information and determines which unique combination of features provides the best prediction for novel biologicals. In addition, the prediction provides information about the uncertainty of the prediction.

With PRED-IMMUNE, TNO is able to help pharmaceutical companies in the development of novel protein therapeutics with reduced immunogenic potential. This can reduce the costs of drug development and avoid adverse effects in patients. The developed platform can easily be used for other disease areas where data integration provides the key to success and TNO is searching for alternative applications of this technology.

**Main results:**

- Prediction algorithm that processes available information (database) to provide predictions of immunotoxicity.

**Application:**

- With PRED-IMMUNE, TNO is able to help pharmaceutical companies in the development of novel protein therapeutics with reduced immunogenic potential.
- This can reduce the costs of drug development and avoid adverse effects in patients.
- The developed platform can easily be used for other disease areas where data integration provides the key to success and TNO is searching for alternative applications of this technology.
- Interest has been expressed by several companies that are going to provide in kind matching for further development by providing data than can be stored into the database and used for future predictions.

### 3.5.8 Metabolic interactions and co-occurrences in a synthetic human gut microbiota (ETSB 2014, KIP 5.4, Synthetic microbial consortium)

The human gut microbiome consists of $10^{13}$-$10^{14}$ bacterial cells, outnumbering the amount of human cells by at least a factor of ten. This vast microbial ecosystem plays an important role in human metabolism and energy homeostasis and is therefore a relevant factor in the assessment of metabolic health and flexibility. Understanding of these host-microbiome interactions aids the design of nutritional strategies that act via modulation of the microbiota. Nevertheless, relating gut microbiota composition to host health states remains challenging because of the sheer complexity of these ecosystems and the large degrees of inter-individual variation in human microbiota composition.

A simplified model of the human gut microbiome could provide insight into the important metabolic processes that occur within the human gut that are essential for human health. In this study 16 well characterized Bacterial species were selected to represent the synthetic gut microbiome.

In order to create a small synthetic gut microbiome that could functionally represent the complex human gut microbiome, we selected 16 bacteria according to the following criteria:

- The complete genome had to be available.
- The bacteria had to be a core member in the human gut microbiome. Meaning that it was present in the human gut microbiome of all individual humans.
• These bacteria had to be cultivatable in the lab with standard cell culture techniques.
• The bacteria had to be non-pathogenic to humans.

**Main result:**
A first version of the synthetic gut microbiome was created. The synthetic gut microbiome should have a similar metabolic potential as an average human gut microbiome. As seen in Figure 8, the abundance of the gene functions groups in the synthetic bacterial consortium resembled the abundance of the functional groups in the natural human gut microbiome.

**Application:**
This synthetic gut microbiome could be used to simulate the metabolic functionality of the human gut microbiome (Figure 9). However, some bacteria might antagonize the growth of other bacteria, resulting in a different metabolic function of the community.

**Figure 8** Barplot of the abundance of gene function groups present in the synthetic 16 species bacterial consortium and natural human gut samples. The red bars represent the abundance of the gene groups found in the complex human gut microbiomes. Blue bars represent the abundance of the gene groups found in the synthetic bacterial consortium.

**Figure 9** Illustrates the interactions between 5 important gut bacteria when grown in combinations of two species. The blue arrows indicate antagonizing interactions, e.g. E. coli antagonizes growth of all other bacteria.
3.5.9 Microbial Production (ETSB 2014, KIP 6, Microbial Production)

Towards greening of the chemical industry the efficient microbial production of enzymes and building block chemicals is very important. Enzymes contribute to development of cleaner processes in paper, textile and biofuel industry, while biobased production of building block chemicals contribute to the breadth of the chemical industry, as this compounds can be used as drop-in molecules in existing chemical production processes. One of the subtopics in the Microbial Production project was targeted to exploration of the use of synthetic ecosystem for microbial production. Microflora are traditionally already the basis of many food fermentation processes. However, until now only individual bacteria or fungi were considered for microbial production in the chemical industry, while also in natural ecological processes, such as degradation of wood, soil organic matter decomposition, but also in waste water treatment always microbial consortia are involved. Within the University Chair Industrial Biotechnology (Perter Punt) which is part of the KIP6 program a Postdoc project was granted dealing with the enzymatic repertoire of a simple soil consortium of a fungus (Aspergillus niger) and a bacterium (Streptomyces). Based on initial results of this project an ERA-IB project, FILAZYME, was granted to start in 2015. As a separate subproject at TNO, cocultivation experiments of this simple consortium revealed synergistic and mutualistic effects on growth and protein production. In conjunction with this project also in the TNO-Idea competition a project idea on fungal and algal cocultivation was selected for the TedEX-Amsterdam primaries. Unfortunately the project was not selected for the final TedEX presentation.

![Figure 10. Organic acid production in controlled cocultivation batch fermentations.](image)

**Main results:**
- Successful co-cultivation experiments
- ERA-IB project on *Streptomyces/Aspergillus* enzyme repertoire application granted
- TNO-Idea finalist

**Application:**
- Industrial interest in the production of building block chemicals from biomass and in the research towards increased yields of existing biobased products is growing, despite of difficulties to reach economic treshholds with increasing sugar prices and lowering oil prices.
- The final application of co-cultivation concepts in the fermentation industry are just starting to become accepted. The first SME companies addressing this topic are emerging.

3.6 Summary of other results

Several of the projects were large clusters of interconnected sub-projects and resulted in important knowledge and technology developments. In this section, results other than those highlighted in the previous section are described.
Several of the projects were large clusters of interconnected sub-projects and resulted in important knowledge and technology developments. In this section, results other than those highlighted in the previous section are described.

3.6.1 **KIP Cluster 1: Systems Diagnostics**

**Main results other than those highlighted in §3.5:**
- A proof of principle study was performed applying the ring-resonator technology for detection of cortisol in saliva. This analytical development is very important for developing an ambulant measurement method for cortisol, which is needed for stress detection and coaching as well as for other user group for example adrenal gland dysfunction patients
- an overview (in ppt) of the current status quo of biological mechanisms, biomarkers, knowledge and hypotheses related to the sequel of fatty liver – NASH – liver fibrosis;
- based on the mechanisms and in collaboration with KIP cluster 2, possible interventions to prevent or even cure NASH were mapped
- Biomarker (lipodomics) signature for the early prediction of NASH was found based on TNO mice studies
- This signature needs to be validated based on human data, which will be provided by the collaborations set-up within KIP cluster
- PhD student within KIP 1.1 started working on meta-analyses focusing on identification of translational diagnostic and predictive markers of liver health
- Collaborations were setup with external partners that will provide TNO with human data on NASH that will be used to translate the NASH biomarker profile found in mice to humans
- The diabetes subtyping tool (see highlight in 3.7) was further refined and validated using additional datasets from different relevant human cohorts

3.6.2 **KIP Cluster 2: Systems Interventions**

**Main results other than those highlighted in §3.5:**
- Main results: an inventory of intervention studies performed at TNO was made
- Based on the inventory, four main processes in development of NASH were identified
- For these processes, based on literature, intervention possibilities were identified that can be applied in future studies for testing human interventions in prevention of NASH

3.6.3 **KIP Cluster 3: Health data infrastructures**

- All developments are given in the highlights in §3.5.

3.6.4 **KIP Cluster 4: Health Advice Systems**

**Main results other than those highlighted in §3.5:**
- Collaboration was setup between PatientCoach (LUMC) and TNO in the area of Diabetes
- A personal health portal infrastructure was setup, that fulfills strict security and privacy requirements. The portal was setup in such a way that it can be extended with different services and tools that can be applied in personalized nutrition and lifestyle interventions or health monitoring
- A PhD student was supervised in modelling different processes involved in Diabetes T2D, this collaboration is with University of Groningen. First publications are expected in 2015
- Pragmatic definition of health set variables was chosen that can be used in the future for measurement of health status in different projects.
- A presentation on the set of variables was given in the TNO workshop Pragmatic health definition
- Pragmatic definition of health semi-quantitative model
- A manuscript was written (will be submitted in 2015) on the relationship between optimism and cortisol levels
• A prediction tool was developed for prediction of weight loss during very low calorie diet, based on multiple parameters, including mental health parameters, which makes this tool quite unique

3.6.5 KIP 5 Cluster- Microbiome functionality and human health:

Main results other than those highlighted in §3.5:
• A screening platform was established for the nasopharynx microbiota.
• Using an epithelial in vitro platform, the effects of microbiome composition were established with regard to epithelial integrity, cytokine production and mucin expression of the epithelium.
• Cultivation methods for the ex vivo enrichment of lactobacilli were established.
• Analysis of the microbiota composition in faecal samples from mice fed with either low fat diet or high fat diet was completed. The first remarkable finding was the large shift in microbiota composition when switching mice from chow to a HFD or LFD diet.

3.6.6 Cluster 6 Biotechnology

As described in highlight in §3.5.

3.7 Highlights of developments initiated and developed by ETSB during 2011-2014

Within the ETSB program, several developments were initiated before 2014 that continued in the last two years of the program and led to several products or technologies that are applied in academic or commercial collaborations through the theme (roadmap) projects. In this section a selection of these technologies is described as highlights indicating the development before and during 2014.

3.7.1 The Nutritional researcher Cohort (Initiated in 2013, continued in 2014 ETSB KIP 1-4).

In the near future, health measurements will shift out of the doctor’s office to measurements in the real world. Different types of consumers already monitor their sleep, activity, heart rate, and much more in a real life situation. In 2010, the Economist portrayed “a sea of sensors” gathering and transmitting information about the real world.

Furthermore, together with the development of concepts for personalized nutrition and personalized intervention approaches in T2D the concept of self-monitoring for personalized medicine emerged. Several studies have shown that self-monitoring can be used to identify health risks and show how by altering one’s lifestyle through e.g. diet and exercise certain diet-related illnesses can be reduced.

NUGO, the Nutrigenomics Organisation, launched the Nutrition Research Cohort (NRC) in 2013, which is an open access cohort where each individual provides and owns their own health data. TNO plays a crucial, coordinating role in this initiative and also exploits the NRC cohort as a testing environment of different tools and approaches developed for do-it-yourself testing and personalized lifestyle advice.

This novel approach taken by the NRC is a new way to merge health and dietary data to allow personal health empowerment and preventative healthcare with the goal to become a globally accepted standard (www.nugo.org/nrc). NRC took the first steps towards setting up participatory driven research in the area of nutrition and health monitoring.

Main results:
• In 2014, the NRC infrastructure, developed in 2013 was further professionalized in KIP 1-4 and in collaboration with one of the work packages in the Flagship Innovation in Healthcare. Security issues were improved and several visualization tools were developed for the collected data.
• A new dynamic website was setup, making it more attractive for researchers to actively participate and upload data from the tested tools and methods into the portal.
• Connection to P4Healthservices (KIP cluster 4) was set up; NRC participants have the opportunity to use their data to connect to the sub-typing tool and the diabetes marvel model.
• An authentication method was implemented, which makes it possible to connect existing personal databases from different self-monitoring tools to the NRC, see Figure 11.
• Manuscript was prepared describing the initiation study from 2013.
• Protocol was prepared and submitted for a new study, to be performed in collaboration with 12 other European research institutes, where beta version of different minimally invasive methods will be tested. In addition to the research institutes, two analysis providers will be participating in the study by providing different analyses for free.
• Two posters disseminating the results to the scientific community were prepared and presented at conferences to create awareness about participatory research.

Application:
• For TNO the applicability of DiY OGTT and the DiY metagenome sampling will be tested and further developed in the planned NRC study
• The collected data will directly be used in running EU project for modelling the development of T2 diabetes.
• The developed infrastructure can be offered to companies on a fee basis to test their tools and set-up different lifestyle advice services

Outlook 2015:
• The NRC researchers cohort initiative will be continued in 2015 within a KIP project under Predictive Health Technologies and within the EU project Qualify.
• Additional funding options are explored within EU consortia under Horizon 2020 programme.

Figure 11: Examples of collection of different self-monitoring and minimally invasive data through the NRC infrastructure.
3.7.2 Do it yourself – oral glucose tolerance test (DIY-OGTT) (ETSB 2011-2013, ETSB 2014 KIP 1-4, NRC and DiY diagnostics and KIP 1-1 Systems flexibility model)

An oral glucose tolerance test is routinely applied in the diagnosis of insulin resistance, T2D and gestational diabetes. Normally, this test is performed in hospitals and medical centres and takes about 2.5 hours to complete. During this period patients need to stay at the test location, and 2 blood samples are taken \( t=0 \), just before drinking 75g glucose in water, and two hours after the drink.

In 2012 and 2013 TNO developed a Diabetes subtyping tool using data from a 5-point OGTT. Currently, besides individuals with a poor \( \beta \)-cell function, 3 different clinical (pre)diabetic subtypes can be differentiated based on muscle insulin resistance (impaired glucose tolerant or IGT), on hepatic insulin resistance (impaired fasting glucose or IFG) or combination thereof (IGT and IFG).

In 2014, this tool was further validated using several datasets from different projects. The tool was transferred into a web application (restricted access) that can directly be consulted and used by participants of the NRC cohort and also from the Patient Coach application described in the highlight from KIP 4.3.

Considering the logistic and practical issues of this subtyping tool, there is great interest to modify the OGTT procedure in such a way that it can be easily performed in a point of care setting (physician’s office or even at home).

First version of the test that can be performed at home by collecting blood on simple sampling paper from a fingerprick was setup by TNO within ETSB in 2013. In 2014 we performed further validation of the test with respect to reproducibility, stability and increased sensitivity of the assay using healthy volunteers.

Main results:
- Validation of the subtyping tool in different cohorts
- Web application of the subtyping tool
- Sensitive assay and extraction procedure for analysis of insulin and C-peptide in dried blood spot samples, and its validation in healthy volunteers
- Collaboration setup with a provider/manufacturer of dosed glucose
- DiY OGTT manual and kits for participants of the NRC study

Application:
- The diabetes subtyping tool is already applied and used in the Theme project P4P (Diabetes fieldlab in Hillegom) and several other projects
- The diabetes subtyping tool will be setup as a service and will be presented to different companies and users, for example, it is accessible through the Patient Coach application developed in KIP 4.2, 4.3 and Flagship.

Outlook 2015:
- The DiY OGTT test will be applied in several TNO research projects (Pragmatische Definitie van Gezondheid, ERP Personalised Food program).

Figure 12: Replacement of venous blood collection by fingerprick blood in a OGTT test.
3.7.3 TNO as central hub for Nutritional study data in Europe (ETSB 2011-2013, ETSB 2014 KIP 3-1)

Performing animal and human studies is labor and cost intensive. Often the data of studies performed earlier can be useful to answer new research questions. For this purpose, a detailed, accessible and structured storage and retrieval of study data is essential, which is the Phenotype database. Phenotype database was developed within ETSB in 2011-2014 and currently stores 58 nutritional studies owned by TNO or one of TNOs collaborators.

Main results 2014:

- The Phenotype database is now part of the ENPADASI project and described as the central database for intervention studies. ENPADASI is a Joint Programming Initiative (JPI) project, which will develop an open access research infrastructure (RI) for all nutritional mechanistic, intervention and epidemiological studies. TNO is in the lead of this project.
- TNO has become member of the Dutch Tech centre for the Life Sciences (DTL). Jildau Bouwman is TNO representative and leading the nutritional sector within DTL. DTL data (earlier named DTL/DISC) is the Dutch node of ELIXIR. ENPADASI is seen as the nutritional use case of the data interoperability activities within ELIXIR.
- TNO is the coordinator of ENPADASI and heading the nutritional sector of DTL. Thereby the TNO Nutritional Database is seen as the central hub for nutritional data
- Training of personnel across Europe will be setup within the ENPADASI project

Application:

- The structured storage of data enables easy access and enables cross sections over studies with similar design, thus enabling efficient use of existing data
- The phenotype database is used as a data exchange platform for several collaborations

Outlook 2015

- The Phenotype database is included in several pre-competitive and commercial projects as study data analysis platform (e.g. project with PRA, EU project Nutritech, EU project Mission T2D)
3.7.4 Targeting inflammasome to reduce metabolic inflammation (Highlight ETSB 2011: ETSB I1& I4)

A novel strategy to treat DM2 involves the reduction of metabolic inflammation which is thought to be a major driver of disease development and closely linked to increase in body adiposity. There are currently no drugs available to treat or diminish metabolic inflammation. Treatment of metabolic inflammation in a setting of DM2 is thought to have great impact on several co-morbidities of the disease, all of which driven by inflammation. A recently identified multi-protein complex called the ‘inflammasome’ may be a sensor of metabolic overload. In this study, we used specific anti-inflammatory compounds, inhibitors, which allow to target the inflammasome in high fat-diet induced inflammation.

Main findings:

- High fat diet (HFD) feeding induced overweight and increase in body adiposity. The inhibitors did not affect body weight but showed a reduction in adipose mass. The effect was most pronounced for a specific caspase inhibitor. Interestingly, HFD feeding resulted in adipocyte hypertrophy (enlargement of the cells) while the adipocyte size remained small with the inflammasome inhibitor (see figure 14).
- The net effect of inflammasome inhibition on metabolic parameters (glucose and insulin) is beneficial (except for IL-1 receptor antagonist) and a reduction of fasting plasma glucose levels was observed.
Main results reported in 2011: This study is an important demonstrator. The results and insights gained in this project are relevant for companies that are interested in quenching the inflammatory component of cardiometabolic diseases.

Application planned in 2011: Companies that are working on chronic tissue specific inflammation already expressed interest in the concept to intervene in metabolic stress and thereby reduce tissue-specific inflammation that is resulting from metabolic overload of a particular tissue. New projects are being defined.

Update 2014:
This technology was further applied in an intervention study and we have shown that the development of liver pathology (non-alcoholic fatty liver disease (NAFLD) or non-alcoholic steatohepatitis; NASH) was markedly reduced by the intervention with the inflammasome inhibitor, see Figure 15. In a follow-up study we have used a different intervention targeting the farnesoid X receptor. This is a strategy that is currently under development in humans as well. This treatment was also very successful in our mouse model. Besides the inflammation, we have also demonstrated that liver fibrosis, which is the most severe characteristic of NASH and a strong risk for deterioration of the liver, was markedly reduced by the treatments. These two demonstrator studies have lead to three commercial studies and more opportunities. Furthermore these developments for the basis for multiple consortia that are currently set-up.
In 2012 and 2013 a number of ETSB newsletters were published sharing highlights from the different projects.

In 2014, a more active form of dissemination was pursued by organizing several sessions with business developers and Innovation directors (Roadmap directors) where new (possible collaborations) based on the developments within the KI clusters were discussed.

Two additional symposia were organized in 2014 and several smaller sessions were organized in order to ensure transfer of developed knowledge and tools to the themes and where relevant to the business developers.

**ETSB 2014: Market and impact took place March 14th 2014 in Woudenberg Conference Centre.** In addition to overview of the results from 2013 and presentation of the plans for 2014 flash presentations were given by young scientists that want to contribute to bringing the different technologies and tools developed within ETSB to the market through collaborations with the Theme Healthy Living. The discussions and input of the audience was very animated and resulted in a list of ideas that could further be used for market plans prepared by the scientists and different business developers.

The audience of the symposium was comprised of about 40 scientists, business developers, director of research and director of Healthy Living as well as a number of research managers.

**Enabling Technology Systems Biology Closing symposium took place in De Reehorst, Ede on December 4th 2014.** For this symposium we chose to focus on presenting ETSB results which were either achieved in collaboration with external partners of which were already adopted in theme projects with external collaborators or sponsors. Presentations were given by TNO scientists together with the external collaborators. External guests represented companies such as Friesland Campina, Mead Johnson, PON and Nestle. Other collaborations were represented by NI-Plan, Samueli institute from the US and CEFIC from Brussels. In addition to the external participants, the audience of the symposium was comprised of scientists, business developers, chief scientific officer of TNO, director of research and director of Healthy Living, Roadmap directors as well as a number of research managers. In total, about 60 participants were present.

### 3.8 Output 2014 (publications, presentations, posters, patents)

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1 including book chapters
2 includes interviews, press releases and websites

See ANNEX 2

### 3.9 Output 2011-2013 (publications)

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See ANNEX 3 for publications

Lists of output other than publications have been reported in the previous annual reports and are available upon request.
Annex 1: Examples of collaborative projects 2011-2014

Summary of collaborative project financially supported by ETSB between 2011-2014 that contributed to the general objectives of the ETSB program.

- FND project ADMIT on inflammation
- NMC microbial on improvements in microbial production processes
- NTC (Netherlands Toxicology Centre) project on predictive toxicology and Bayesain statistics
- BMM Desire, a collaborative project between UMCG, UMCU, TNO and a number of small companies focused on new material development. The aim of the project is to identify new methods for therapeutical intervention directed to kidney diseases
- The FP7-project MISSION-T2D, on modelling of a virtual diabetic patient
- ANTIRESDEV: an EU project on resistance to antibiotics
- TIP Designing QUAlity in PROduction processes
- CMSB II Postdoc project on improvement of the predictability of existing preclinical models for metabolic syndrome
- CTMM Predict AIO project on the development of improved mouse models to study the etiology and therapy of NASH in the context of IR/diabetes and atherosclerosis
- BMM iValve, a collaborative project between TUE, UMCU, UMM, Philips, TNO and some spin-outs. The aim of the project is the in vitro engineering of human heart valves for clinical application and the development of materials which are able to induce heart valve formation in vivo
- TIP OA, a TI Pharma project Osteoarthritis: models, mechanisms and markers for patient stratification

Specific examples of knowledge and technology developed in ETP Systems Biology applied in follow-up innovation projects either approved or submitted under the relevant themes and TOP Sectors in 2013/2014:

- ZonMW project “Systems toxicology supported data infrastructure for human risk assessment” on the further development of the ASAT (Assuring Safety without Animal Testing) Knowledge Base, and focusing on the integration of toxicological knowledge for development of alternatives to animal testing
- Bio-QED, a EU-project aimed at demonstrating the large scale production of the bio-based bulk chemicals BDO and IA, and targeting cost reduction and improved sustainability
- A Sino-Dutch Centre project for Preventive and Personalized Medicine (SDPPM) on systems health and modelling
- The PPS F4LS project will provide new insights in the effects of infant formulas and calf feed on the microbiota of newborns in relation to prevalence of airway infections
- The PPS PROBE project studies whether elderly, obese Diabetes Mellitus patients benefit more from a 13-week lifestyle intervention with energy restriction and exercise more alone, or when taking in addition to this lifestyle intervention a protein supplement
- For the Samueli Institute project a model will be developed that can map the main determinants for successful reintegration into society of military personnel in the U.S.; simulations with this model will contribute to determining optimal interventions to promote this challenging process
- The EU HEALS (Health and Environment-wide Associations based on Large population Surveys) project, bringing together in an innovative approach a comprehensive array of novel technologies, data analysis and modeling tools that support efficiently exposome studies
- The Cefic LRI project DECO: Data-integration for Endpoints, Chemo-informatics and Omics
- EU QUALIFY project with further development of self-monitoring tools is performed, together with numerous SMEs in this area.
- Eli-co project Red Ant Technology of single targets identification
- TIFN Denta
- EU project proposal Systems Medicine new concept for systems analysis
- EU project Nutritech: New methods for nutritional health (claims)
- EU project Bioconcept Optimization of production of organic acids
- EU project Abengoa Analytical methodology for the determination of oligosacharides
Annex 2 Output 2014 (publications, presentations, posters, patents)

Papers
7. K Smil...


23. DB van Schalkwijk, AA de Graaf, E Tsivtsivadze, LD Parnell, and others (2014). Lipoprotein metabolism indicators improve cardiovascular risk prediction PloS one 9 (3), e92840


Book chapters:


Oral presentations

2. Suzan Wopereis, Phenotypic flexibility as a new way to quantify effects of food and nutrition on health. Food Valley, Papendal (NL), 23 October 2014.


5. Suzan Wopereis, Phenotypic flexibility as a new way to quantify health effects of food and nutrition. Ilsi Functional Foods Taskforce meeting and Brussel (Belgium), 5 November 2014.


17. Rob Sterium: In vitro data combined with human disease data to improve toxicological hazard assessment: the ASAT Knowledge Base 9th World Congress on Alternatives and Animal Use in the Life Sciences, Prague, Czech Republic, August 24-28, 2014.


36. Dr. J. Bouwman, Work package 8: Case Study RI for innovative mechanistic nutritional studies and ‘D (Data),T (technologies), L (learning)’, EMBL-EBI Industry Programme Workshop Nutrition Information, Ontologies and Nutrigenomics, Hinxton (UK), April 2, 2014.

37. Dr. J. Bouwman, Nutritional Phenotype Data Sharing, JPI and Phenotype database (dbNP), EMBL-EBI Industry Programme Workshop Nutrition Information, Ontologies and Nutrigenomics, Hinxton (UK), April 2, 2014.

38. Dr. J. Bouwman, Nutritional Phenotype Data, JPI and Phenotype database (dbNP), JPI meeting, Rome, Italy, December, 2014.


40. Mariel van Stee, Interpolation of glucose-insulin dynamics model between healthy and disease state
(obese and type 2 diabetes). NVDO Jonge Onderzoekers bijeenkomst, Soesterberg (NL), January 31th and February 1st, 2014


42. Danyel Jennen, Data-integration for Endpoints, Chemoinformatics and Omics. Workshop Toxicogenomics: the emergence of a new research and regulatory paradigm, Curitiba Brazil, September 15th-16th, 2014


44. B. van Ommen. A systems view on health - ETSB symposium Zeist 14 March 2014


46. B. van Ommen. How can I use BIOCLAIMS to improve my own health? - Bioclaims Symposium, Grundlsee Austria, 28 March 2014

47. B. van Ommen. Nutrition research: where are we heading to and what do we need? - European Bioinformatics Institute Infrastructure Symposium, Hinxton UK, 1 April 2014


49. B. van Ommen. N=1 nutrition research - a revolution in science and healthcare - ISNN, Gold Coast Australia, 2 May 2014

50. B. van Ommen. N=1 nutrition research - a revolution in science and healthcare - Nutrition and Medicine Conference, Gold Coast Australia, 3 May 2014

51. B. van Ommen. Nutritional solutions and metabolomics diagnosis in type 2 diabetes - Nutrition and Medicine Conference, Gold Coast Australia, 4 May 2014


53. B. van Ommen. Do we need a nutritional (bioinformatics) research infrastructure(institute)? - NuGOweek 2014, Castellamare di Stabia, Italy 9 September 2014


55. B. van Ommen. Integrated vision of healthy ageing and systems biology - EU Symposium "Health for all: understanding the ageing process" Bologna 3 October 2014

56. B. van Ommen. Sistemi integrati Uomo e Ambiente. Flessibilità e plasticità - Symposium Terra e Cibo - Trani Italy, 4 October 2014

57. Why is food healthy (or not)? - EMBL Symposium Food are Us, Heidelberg 7 November 2014


59. B. van Ommen. Systems Biology at TNO - ETSB final symposium, Ede, 4 December 2014

Posters


4. P.Y. Wielinga, K. Salic, W. Liang, T. Kooistra, R. Kleemann. Intervention with Caspase-1 inhibitor attenuates the metabolic syndrome and prevents non-alcoholic steatohepatitis (NASH) in high fat diet fed LDLR−/-Leiden mice. Keystone meeting; Challenges and Opportunities in Diabetic Research and Treatment; Jan 12-17, 2014, Vancouver, Canada

5. Peter Y. Wielinga, Marieke Schoemaker, Robert Kleemann, Eric A.F. van Tol, Teake Kooistra. Novel predictive biomarkers for obesity identified a humanized mouse model. Keystone meeting; Challenges and Opportunities in Diabetic Research and Treatment; Jan 12-17, 2014, Vancouver, Canada


and K. E. Geillinger, Poster Nutrition Researcher cohort: Metabolomics in dry blood spot samples, NVMS spring meeting, April, Kerkrade, NL


Manuscripts


2. Ben van Ommen and Suzan Wopereis. Comparative effect of a Mediterranean diet versus a low fat diet on insulin sensitivity and β-cell function according to muscle or liver insulin resistance presence: from the CORDIOPREV study. Submitted.


8. Kirsten AC Berk, Monique T Mulder, Adrie J.M. Verhoeven, Herman van Wietmarschen, Ruud Boessen, Adriaan van t Spijker, Reinier Timman, Behiye Ozcan and Eric JG Sijbrands. Physiological and psychological predictors of weight loss in patients with type 2 diabetes following an 8 week very low calorie diet. Paper under internal review


Interviews and press releases:

1. Veerkraacht moet gezondheidsclaims volkoren onderbouwen. EVMI nr. 6, September 2014, p.28-29.

2. Subtiele gezondheidsverschillen tussen mensen op nieuwe manier aantonen. Voeding Nu nr. 9, September 2014.

Theses:

Patents:
1. WO/2014/178717: Novel organic acid pathway

Websites:
7. ETP systems Biology website
Annex 3 Compiled output ETSB papers 2011 - 2013

2011:

19. Melissa J Morine; Audrey C Tierney; Ben van Ommen; Hannelore Daniel; Sinead Toomey; Ingrid MF Gielstad; Pablo Pérez-Martinez; Christian A Drevon; Jose López-Miranda; Helen Roche (2011) Transcriptomic coordination in the human metabolic network reveals links between n-3 fat intake, adipose tissue gene expression and metabolic health. PLoS Computational Biology, 7(11): e1002223. doi:10.1371/journal.pcbi.1002223
2012:


9. S Krishnan, et al.- RCMS, Pre-processing liquid chromatography-high resolution-mass spectrometry data: extracting pure mass spectra by deconvolution from the invariance of isotopic distribution (Accepted for publication in Rapid Communications in Mass Spectrometry).


24. Lindeman et al. Statin pleiotropy: statins selectively and dose-dependently reduce vascular inflammation. PLOS One, in press.

2013:


28. Dick Wågsäter, Valentina Paloschi, Roeland Hanemaaijer, Kjell Hulteny, Ruud A. Bank, Anders Franco-Cereceda, Jan H.N. Lindeman, Per Eriksson (2013). Impaired collagen biosynthesis and
29. Marina A Aleksinskaya; Ernst E.H. van Faassen; Jelly Nelissen; Ben J.A. Janssen; Jo G. R. De Mey; Roeland Hanemaaijer; Ton Rabelink; Anton Jan van Zonneveld (2013). Identification of free nitric oxide radicals in rat bone marrow; implications for progenitor cell mobilization in hypertension. PLoS One, 8(3):e57761


33. Boelsma, E et al. (2013). Assessment and comparison of inflammatory responses upon challenges with glucose, fat, and a combination of glucose and fat. TNO report number V9248


36. F. Castiglione, P. Tieri, A. de Graaf, C. Franceschi, P. Liò, B. van Ommen, and others (2013). The Onset of Type 2 Diabetes: Proposal for a Multi-Scale Model. JMIR research protocols 2(2):e44
