Meetings

Systems biology of mammalian cells: A report from the Freiburg conference

Background to the conference

The third international conference on 'Systems Biology of Mammalian Cells' (SBMC 2010) was held in Freiburg, Germany, on 3–5 June 2010. The conference, which took place under the auspices of Annette Schavan, the German Federal Minister for Education and Research, was attended by 330 scientists from 18 countries (Fig. 1); there were 36 speakers and 175 posters were presented and actively discussed.

The conference was organised by HepatoSys, the German Competence Network for Systems Biology of hepatocytes and its successor the German Virtual Liver Network. In 2004, HepatoSys was launched by the German Federal Ministry of Education and Research (BMBF) to investigate intracellular processes in hepatocytes. Since April 2010, the German Virtual Liver Network has been striving to understand these processes at the next level. Building on the results of HepatoSys, the German Virtual Liver Network has started to examine processes in cell assemblies up to the whole organ.

In his opening speech the parliamentary state secretary of the BMBF, Helge Braun, emphasised the importance of Systems Biology for the future of medical research. To support this innovative field the BMBF has up to now invested €300 million for past, present and future Systems Biology projects.

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The chair of the organising committee, Jens Timmer, University of Freiburg, welcomed the international participants with an overview of the history of the meeting venue.

Afterwards, Adriano Henney, Program Director of the German Virtual Liver Network, introduced the ambitious goals of the network. The project started in April 2010 and is financed by the BMBF with €42 million for 5 years.

Integrating the multi-level properties of systems

Systems biology is frequently referred to in terms of its propensity to reveal so-called 'emergent' properties, *i.e.* characteristic behaviours of a system that cannot be predicted simply by combining a knowledge of the behaviours of the individual components. However, what is the source of such emergent properties? In an example presented by keynote speaker Denis Noble (Fig. 2), University of Oxford, UK, the rhythmicity of the beating heart is not encoded at a subcellular level – so looking for a genetic basis for this behaviour, for example, is futile – but emerges first at the level of the individual cell. It is modulated as the other layers of the system are combined: from an individual cell, the electrophysiology of its encapsulating membrane, up to the tissue level and finally whole organ.

Dennis Noble started his work on the virtual heart in the 1960s and is one of the pioneers on whole organ systems biology worldwide. In exemplifying the sheer mathematical problem of identifying gene interactions that might produce a given effect (assuming 2 genes per property, over 300 million knockout mutants would have to be made to systematically scan all interactions), he emphasised the importance of recognising the 'feed-downs' from high-level properties to more reductionist ones, as well as working bottom-up, assuming 'feed-up' effects. The extent to which extrapolation can be performed up through the levels from gene to organ is very limited without incorporating the feed-down effects.

As Noble remarked, in the heart, it is now possible to model a large number of ion channels, intracellular calcium signalling mechanisms, the contractile machinery, substrate transporters and some of the receptors, hence producing up to 100 differential equations. Arrhythmia of the heart is thus recognised as not emerging at the level of the individual cell (in contrast to rhythmicity), but first at the level of the whole organ.

He emphasised the importance of not assuming a particular percentage contribution of a given gene on the system, based on the effect seen upon knocking it out. Genetic buffering often minimises the effect of a knockout, explaining why the majority of knockouts reveal no function. To address the problem of the genetic differential effect, it is necessary to reverse engineer to ask what quantitative contribution a particular gene product makes to the whole system. This approach has now been used for the first time to produce a successful beta blocker.

Noble critically discussed the central dogma of molecular biology 'DNA makes RNA, RNA makes protein', dating back to Crick and Watson, by arguing that it is oversimplified. For example, feedback regulation and epigenetics are not considered. Therefore, the

Figure 1. Picture of the participants of the Conference on Systems Biology of Mammalian Cells, Freiburg, 3–5 June 2010.

situation is much more complicated and each level of regulation and complexity has to be fully understood and then integrated into a model of the whole. 'Top' and 'bottom' (with respect to hierarchy), he noted, are human metaphors that are not recognised by the nature of evolution.

Denis Noble ended his discourse by noting the importance of the relativity theory in biology, and asserting in particular the importance of scale-dependent effects. He also relativised the challenge of understanding and modelling the liver, which has many more biochemical pathways to consider than the 100 component model of the heart. That said, it was clear that significant progress is being made in understanding and modelling the liver and other mammalian systems at their individual levels of organisation. The most recent progress is reported here according to the six sessions of the conference, at which internationally renowned invited speakers, and young scientists chosen from their poster abstracts, presented their work. The ground has now been laid to tackle the challenge of integrating these insights into multi-level models, among which is the 'Virtual Liver'.

Figure 2. Denis Noble, University of Oxford, the keynote speaker of the conference

New theoretical approaches and cutting-edge technologies

In the session 'New theoretical approaches and cutting-edge technologies', Denis Thieffry, TAGC-INSERM, Marseille, applied logical modelling to investigate the diversity and plasticity of T helper cells. In contrast to the classic point of view of the differentiation potential of T cells in terms of a branching tree, the computational analysis points to a reticulate network of alternative, environment-dependent, differentiation and reprogramming events. Forest M. White, MIT, reported a mass spectrometry-based methodology to monitor phosphorylation of specific residues of dozens of key signalling proteins at multiple time points under a variety of perturbations. Based on these data, he demonstrated that a computational approach is capable of describing glioblastoma cell growth based on the analysis of just 13 phosphoproteins. Andre Levchenko, Johns Hopkins University, introduced the use of microfluidic devices to address how signalling pathways cope with noise. To demonstrate the limits of information processing in signalling pathways through a combination of modelling and experimentation, he reported examples from signalling in the innate immune system response and survival pathways. Andrius Serva, BioQuant Heidelberg, reported on experimental and modelling approaches for the functional analysis of microRNA as regulators of secretory membrane trafficking. Jan Hasenauer, University of Stuttgart, presented a mathematical modelling method that is able to deal with cell-to-cell variability. As an example, he applied his method to the data from TRAIL-induced apoptosis and inferred markers for survival and death of individual cells. In the final talk of the session, Martin Fussenegger, ETH Zurich, provided a perspective towards Synthetic Biology and demonstrated how mathematical modelling and mammalian synthetic biology can lead 'From tools to therapies'.

Signalling

In the session 'Signalling', Steven Wiley, Pacific Northwest National Laboratories, Washington, reported on mathematical modelling for epidermal growth factor receptor-induced signalling based on quantitative mass spectrometry data, gene expression profiles and protein-protein interaction data. This has resulted in the discovery of numerous positive and negative feedbacks that operate over a wide range of temporal scales and that are sensitive to different levels of stimulation. Joseph Zhou, Technical University of Dresden, showed how a hierarchical multi-attractor model is able to predict decisions of pancreatic cells and facilitate insights into which set of transcription factors need to be modulated for cell fate reprogramming. Jason Haugh, North Carolina State University, investigated the dynamic regulation of growth factor receptor signalling. His data-based mathematical modelling provided new insights into the ERK signalling network in terms of cross-talk effects and its regulation by feedback loops [1]. Tilo Beyer, University of Magdeburg, demonstrated by merging logical models how signals from the T cell receptor and the interleukin 2 receptor could be integrated. Michael Yaffe, MIT, introduced a systems biology approach to gain insights into regulation of DNA damage response and demonstrated the implications for a rational design of cancer therapy. In particular, he showed the potential for improving existing combinations of chemotherapeutics, and for the development of novel personalised therapies. Michael White, University of Liverpool, showed by live cell imaging, in combination with deterministic and stochastic modelling, how the NF-κB signalling system encodes information by spatial and temporal means. In particular, he discussed the robustness and heterogeneity of the system [2]. One highlight was the short talk by Verena Becker. She demonstrated by a combination of experimental and modelling work how the erythropoietin receptor can cope with huge differences of its stimulating hormone. Three biologically plausible mechanisms were formulated as mathematical models that were calibrated by experimental data. Statistical analysis of the resulting models demonstrated that only a fast ligand-independent receptor turnover explained the experimental data. Furthermore, the model is able to successfully predict the outcome of an experiment interfering with the turnover process (Fig. 3). These results appeared in Science shortly after the conference [3].

Finally, Carsten Carlberg, University of Luxembourg, analysed nuclear receptors by a systems biology approach and elucidated the impact of transcriptional cycling [4].

Metabolism

In the session 'Metabolism', Hans-Georg Holzhütter, Charité, a member of HepatoSys and the German Virtual Liver Network, talked about 'Mathematical modelling of liver metabolism'. He presented the first stoichiometric model of hepatocyte metabolism. The network includes 740 metabolites in eight compartments and encompasses 2440 reactions, including 1435 transport reactions between the compartments. Using the example of hepatic detoxification of ammonia, he showed how the availability of nutrients and oxygen potentially modulates the interplay of various metabolic pathways to allow efficient responses of the liver to perturbations of the homeostasis of blood compounds. Christian Tiemann, University of Eindhoven, used a data-based computational model to identify persistent behaviour of hepatic lipid metabolism. The model is able to describe the physiological situation as well as data from a transgenic mouse that exhibits severe hepatic steatosis. Damjana Rozman, University of Ljubljana, investigated the cross-talk of hepatic cholesterol and the drug metabolism transcriptome of mouse and human hepatocytes. The analysis revealed the mechanisms of drug effects and pinpointed to novel side effects. Gunnar Cedersund, University of Linköping, Sweden, demonstrated a combined top-down and bottom-up modelling approach that provided internally consistent explanations of whole-body glucose homeostasis through the identification and elimination of data inconsistencies and missing regulation. The conceptual approach was exemplified by combining a hierarchically formulated whole-body model with a detailed adipose tissue model. In his talk 'Networking metabolites, diseases, and drugs' Deok-Su Lee, Inha University, South Korea, showed that our current understanding of the topology of the human metabolic network provides insights into potential relationships among distinct disease phenotypes and drugs.

Biomedicine

In the session 'Biomedicine', Walter Kolch, University College Dublin, discussed systems properties of the MAP kinase pathway as a negative feedback amplifier and demonstrated the consequences for the robustness of the pathway. Niels Grabe, University of Heidelberg, reported on the quantification of cell streams in epidermal wound healing, using a three-dimensional *in vitro* culture model of human skin and whole slide imaging as a first step towards a multi-scale model that can be used to explore pathologies or potential therapies in epidermal wound healing. In his talk 'How do cancer cells escape from targeted interventions ?', Nils Blüthgen, Charité, Berlin, once more addressed the MAP kinase system and discussed possible strategies on how tumour cells circumvent the effects of therapeutic kinase inhibitors. Supported by experimental data and modelling studies, he suggested a relation between fluctuations of protein levels in single cells and their robustness against kinase inhibitors. Ritsert C. Jansen, University of Groningen, talked about 'The road ahead to systems genetics' by discussing the promises, statistical methods, pitfalls and results of network reconstruction and causal inference using system-wide data on chromatin modification, gene expression, proteins and metabolites.

Whole body

In the session 'Whole Body', Claudio Cobelli presented 'Minimal and maximal models of glucose metabolism in health and diabetes'. The mathematical model [5], see Figure 4, was approved by the Federal Drug

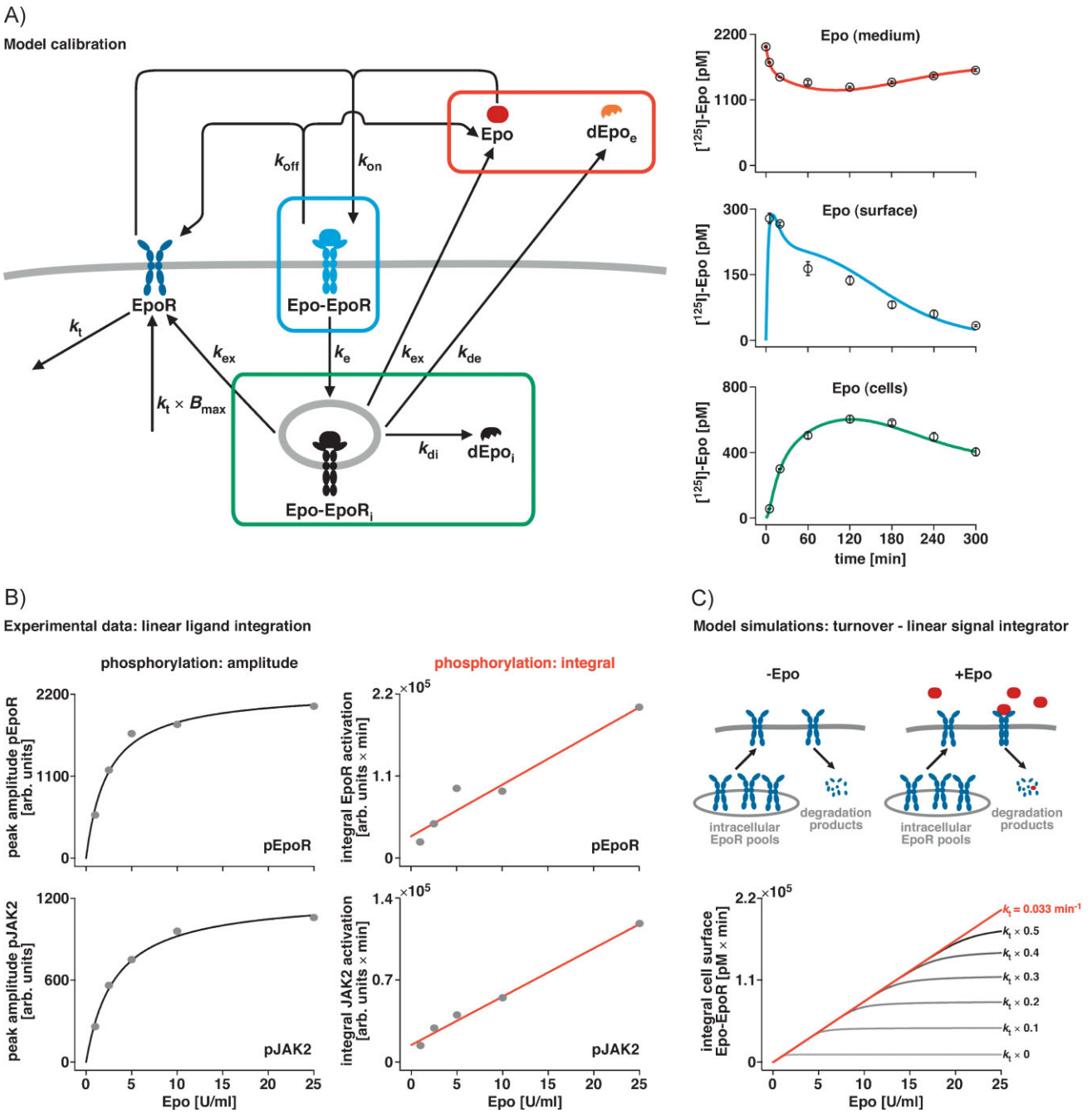

Figure 3. Linear signal integration of extracellular erythropoietin (Epo) on the receptor level is dependent on constitutive Epo receptor (EpoR) turnover. **A**: Schematic representation of an ordinary differential equation-based model for Epo and EpoR interaction as well as trafficking kinetics (left panel). Model calibration with experimental time course data (right panel) results in a fully identifiable model. **B**: Quantitative immunoblot analysis of EpoR and Janus kinase 2 (JAK2) phosphorylation (circles) reveals a linear relation between Epo input and activated species integrated over time (right panels) despite saturating signals for the peak amplitude (left panels). **C**: Model simulations show that a linear conversion of extracellular Epo levels into the integral amount of cell surface Epo-EpoR complexes is strictly dependent on rapid EpoR turnover.

Administration of the USA to be applied in clinical studies for the development of drugs to treat diabetes. This represents one of the first examples of how mathematical modelling can accelerate drug development and reduce animal experiments.

In a selected short talk, Stefan Hoehme, University of Leipzig, demonstrated by the combination of imaging data and three-dimensional tissue modelling that cell alignment along microvessels is an order principle to restore

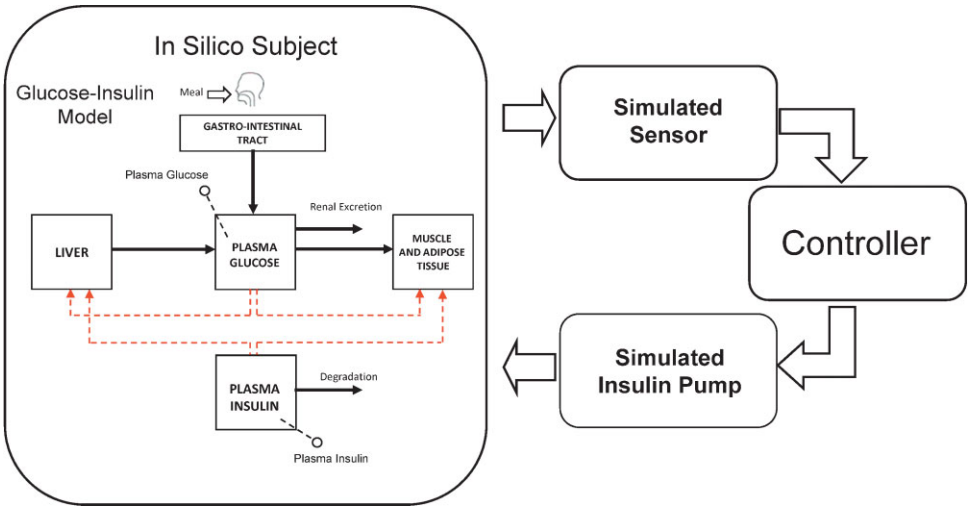

Figure 4. Computer simulator of Type 1 diabetes accepted by FDA as an *in silico* substitute to animal trials for the preclinical testing of control strategies in artificial pancreas studies (adapted from ref. 5).

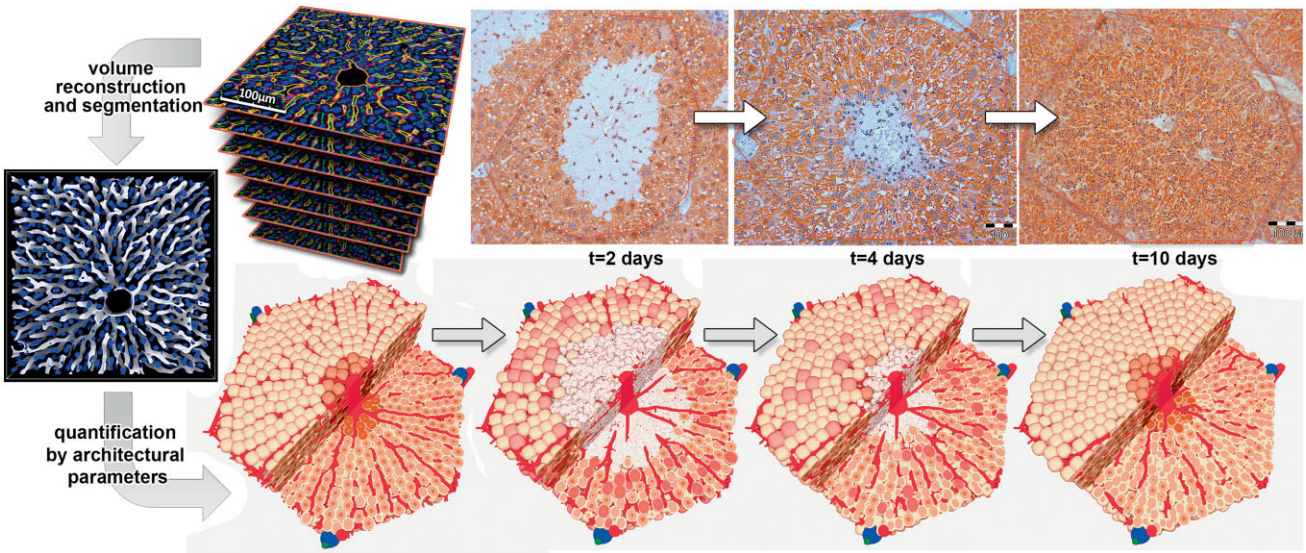

Figure 5. The three-dimensional architecture of liver lobules is quantified from stacks of confocal micrographs by volume reconstruction and segmentation (upper left) and this information is merged with data on the dynamics of the regeneration process after intoxication with CCl_4 (upper right). This allows a three-dimensional single-cell-based model of the regeneration process (lower images) to be set up. Predictions of this model led to the elucidation and verification of a yet-unknown key mechanism of liver regeneration-hepatocyte-sinusoid alignment.

tissue architecture during regeneration of hepatocytes (Fig. 5). This work was published in PNAS briefly after the conference [6].

Jörg Lippert, Bayer Technology Services GmbH, showed how computational Systems Biology is successfully applied in pharmaceutical research and development. In particular, he discussed how integrative modelling facilitates decisions during the process of drug development concerning the identification of promising candidates and design of the next project steps. Jens Reich, Max Delbrück Centre, reported a whole-body model of iron regulation and provided a kinetic description of the pathway network.

Industry

In the session 'Industry', Birgit Schoeberl, Merrimack Pharmaceuticals, demonstrated in her talk on 'Applying engineering principles to the development of novel cancer therapies' how mathematical modelling, combined with quantitative biological data, leads to insights that can be translated into practice by engineering and testing novel antibody therapies in the context of computer simulations. Christopher Taylor from AstraZeneca, extended the topic of Jörg Lippert and discussed how Systems Biology can support the decision-making process in drug discovery. Richard Ho from Entelos, talked about a mathematical model of liver homeostasis and perturbation, which has been developed in collaboration with the US Federal Drug Administration. The model allows drug-induced liver injury to be examined in mouse, rat and human

Box 1

The MTZ Award for best PhD thesis

During the conference, the MTZ award, named after its donors Monika and Thomas Zimmermann, for the best PhD theses in medical Systems Biology was presented to the three award winners who also introduced their work. Stefan Legewie, German Cancer Research Centre, talked about 'Transcription feedback regulation of signal transduction', Thomas Maiwald (by live transfer from Harvard Medical School) presented 'Dynamical modelling of biological systems' and, last but not least, Edda G. Schulz, Institute Curie, presented her work on 'Two positive feedback loops control effector function and memory induction in type 1 T helper lymphocytes'.

to support preclinical and clinical research with the aim of reducing the risk of drug-induced liver injury. Last, but not least, Sean Ekins, Collaborations in Chemistry, talked about 'Toxicity pathways and models: mining for potential off-target effects' and showed how computational techniques help to prospectively identify molecules as early as possible that might fail in the clinic due to toxicity, so, again, supporting the process of decision-making in drug development.

Conclusions and challenges

The conference has demonstrated that Systems Biology of mammalian cells is maturing. There are substantial insights in basic research as well perspectives for clinical and pharmaceutical applications that are based on the tight interplay of model-based experimentation and data-based mathematical modelling. The largest challenge lies in integrating the models at each level of the hierarchy (from genes, through metabolism, cells and tissue, up to the whole organs and systems) into predictive models that can serve basic research and medical application. Attendees of the conference in Freiburg can look forward to the next chapter in this story at the fourth instalment of the conference Systems Biology of Mammalian Cells, which will take place in 2012 in Berlin.

For more information about the SBMC 2010 including live video-streams of the talks, see www.sbms2010.de.

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