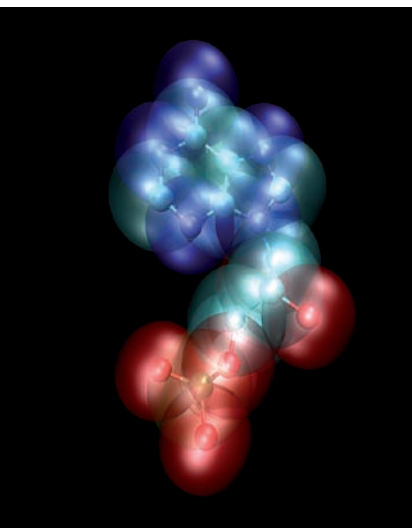


Protein-protein Interactions as Target for New Therapeutic Molecules

EU-project thera-cAMP



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Major diseases including cardiovascular and renal diseases, diabetes mellitus, obesity, diseases of the immune system, cancer, and neurological disorders are associated with dysregulation of compartmentalised cAMP (cyclic adenosine monophosphate) signalling pathways.

Cyclic AMP is formed from adenosine triphosphate (ATP) by adenylyl cyclases (ACs) in response to a plethora of extracellular signals (fig. 1). It is degraded by the action of phosphodiesterases (PDEs) that catalyse the hydrolysis of

Major human diseases are caused or are associated with disturbances of compartmentalised cyclic adenosine monophosphate (cAMP) signalling networks. The participants of the European research project "thera-cAMP" aim to identify lead compounds, which specifically modulate protein-protein interactions in such signalling cascades. The approach could lead to alternative strategies for the treatment for diseases that are currently not addressed effectively by conventional pharmacotherapy.

cAMP to adenosine monophosphate (AMP). The main effector of cAMP is protein kinase A (PKA), which upon activation by binding of four molecules of cAMP, catalyses the phosphorylation of a variety of target proteins and thereby regulates numerous metabolic processes. In order to warrant a specific cellular response to a particular stimulus, kinases including PKA and often further molecules of a signalling cascade are confined to defined cellular compartments by interaction with scaffolding proteins. A kinase anchoring proteins (AKAPs) are a family of scaffolding proteins that tether PKA to e.g. the plasma membrane, the outer mitochondrial membrane, the endoplasmic reticulum, or exocytic vesicles. This restricts the access of PKA to substrates in close proximity and facilitates spatially and timely coordinated cAMP-dependent signalling. According to their function all AKAPs possess a binding domain for PKA (for its regulatory subunits) and a targeting domain that interacts with cellular compartments. Many AKAPs have, in addition, docking domains for further signalling molecules such as phosphatases, kinases or phosphodiesterases, whereas only some AKAPs possess a catalytic domain. In contrast to the structurally

conserved PKA-binding domain, the targeting, docking and catalytic domains are specific for each AKAP (fig. 2).

EU-project thera-cAMP

The EU-project "Identification of therapeutic molecules to target compartmentalised cAMP signalling networks in human disease (thera-cAMP)" started in October 2006. It is coordinated by Dr. Enno Klußmann at the Leibniz-Institut für Molekulare Pharmakologie (FMP) in Berlin and unites nine project partners from Germany, Norway, Sweden, Scotland, and Italy – six academic groups and three enterprises.

In a multidisciplinary approach based on postgenomic research, established and novel cell lines representing different diseases will be used to identify small "druggable" therapeutic molecules de-

rived from small molecule libraries which affect the interactions of signalling proteins with anchoring proteins or the binding of anchoring proteins to cellular compartments. In particular, it will be attempted to identify molecules which

- disrupt protein-protein interactions of ACs, PDEs, AKAPs, and PKA and/or
- displace AKAPs, PKA and PDEs from their cognate intracellular location.

The disease models used for the experiments represent cardiovascular diseases, nephrogenic diabetes insipidus (NDI), asthma, chronic obstructive pulmonary disease (COPD), AIDS, obesity, and schizophrenia. Screening of compound libraries will be performed in living cells and *in vitro* with purified components of the cAMP signalling system.

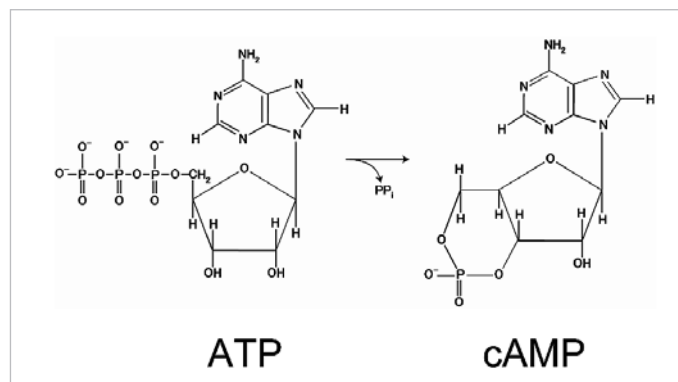


Fig. 1: Formation of cAMP from ATP

The targets of small molecule candidates will be identified using established and to be developed tools and bioassays. Cell signature responses to challenges with those compounds will be elucidated in order to gain mechanistic insight into the effects on the disease phenotypes and to anticipate side-effects of the identified substances. The small molecules will be valuable tools to investigate compartmentalised cAMP signalling. Moreover, due to the potentially high specificity of the lead compounds the consortium hopes to pave the way to alternative treatments with less side-effects. Conventional therapies typically target receptors, inhibit enzymes, or alter the permeability of ion channels, and thus inhibit protein functions throughout cells. In addition, many of the affected targets are present in all cells of the body. This most likely accounts for many undesirable side-effects of conventional medicine.

New trends in drug development

It is increasingly recognised that signal transduction events are confined to defined cellular compartments and are governed by protein-protein interactions. Both compartmentalisation and protein-protein interactions, therefore, offer attractive opportunities for therapeutic intervention.

Disruption of PKA-anchoring by AKAPs inside cells has already been achieved using membrane-permeable peptides derived from the PKA-binding domains of different AKAPs. Several of such high-affinity peptides have been developed by principal investigators within the consortium [1-3]. The peptides bind competitively to the AKAP interaction sites on regulatory subunits of PKA and have been found to inhibit cAMP signalling processes in disease-relevant cells. For example, it appears that the peptides protect cardiac myocytes from β -adreno-receptor-mediated increases in cardiac myocytes contractility. This effect resembles that of β -blockers. The results suggest that interference with PKA-anchoring is a suitable pharmacological concept for the treatment of diseases.



Project Details and Structures

thera-cAMP is a Specific Targeted Research Project (STREP) under the EU's Sixth Framework Programme. It is funded for three years within the thematic priority 1 "Life sciences, genomics and biotechnology for health" (contract no. 037189).

The project (fig. 3) is composed of 8 work packages (WPs), each of which is led by one responsible scientist. In WPs 2-5

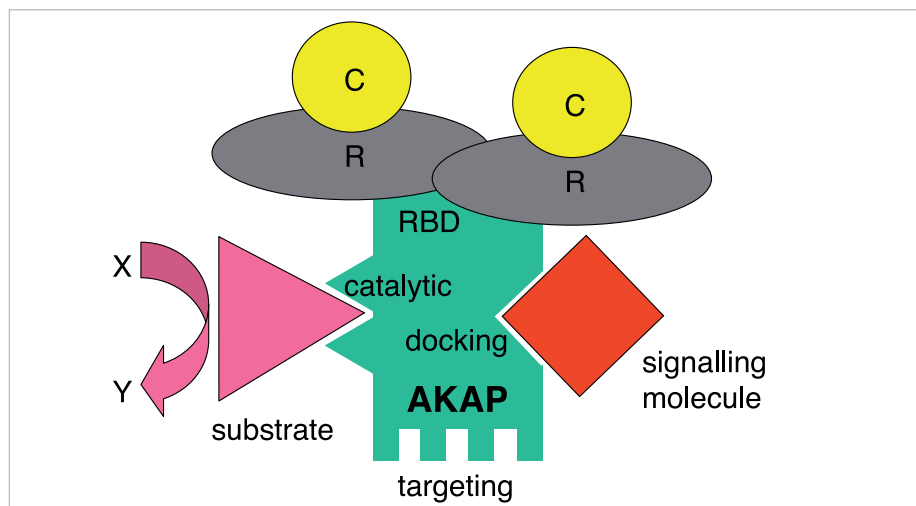


Fig. 2: Schematic illustration of an A kinase anchoring protein (AKAP) with different domains. The presence of a structurally conserved binding domain for the regulatory subunits of PKA (RBD, regulatory subunit binding domain) is the unifying characteristic of all AKAPs. The targeting domain, which tethers the AKAP to cellular compartments, and docking domains, which bind further signalling molecules (e. g. phosphatases, other kinases, or phosphodiesterases) are specific for individual AKAPs. Catalytic domains (with e.g. RhoGEF-activity) have been identified in only a few AKAPs.

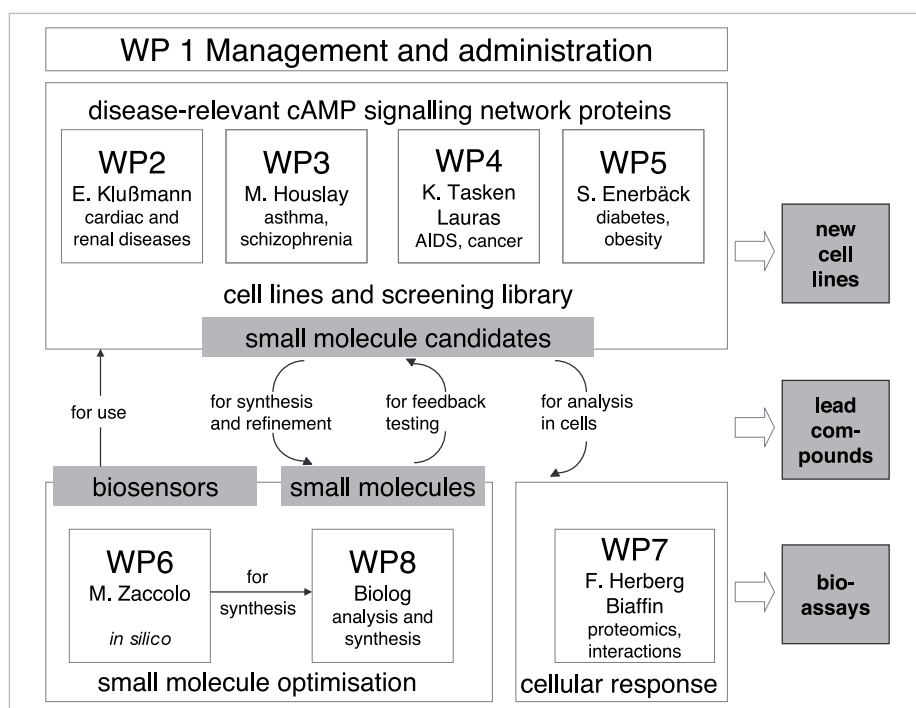


Fig. 3: Structure of the project

Table 1: Participants

1) FMP	Leibniz-Institut für Molekulare Pharmakologie im Forschungsverbund Berlin e.V.	Germany	Enno Klußmann
2) UNIK	Universität Kassel, Institut für Biochemie	Germany	Friedrich W. Herberg
3) UiO	University of Oslo, The Biotechnology Centre of Oslo	Norway	Kjetil Taskén
4) FTELE	Fondazione Telethon	Italy	Manuela Zaccolo
5) UGLA	University of Glasgow	United Kingdom	Miles D. Houslay
6) UGOT	Göteborg University, Institute of Biomedicine	Sweden	Sven Enerbäck
7) Biaffin	Biaffin GmbH & Co KG	Germany	Bastian Zimmermann
8) Laurus	Laurus AS	Norway	Vidar Hansson
9) Biolog	Biolog Life Science Institute, Forschungslabor und Biochemica Vertrieb GmbH	Germany	Hans-Gottfried Genieser

cell models for certain diseases will be utilized for the identification of small molecules that disrupt protein-protein interactions of ACs, PDEs, AKAPs, and PKA and/or displace PDEs, AKAPs, and PKA from their cognate intracellular location. WPs 6 and 8 will contribute to the discovery of small molecules by molecular modelling studies and small molecule synthesis. Novel biosensors based on FRET (fluorescence resonance energy transfer) and BRET (bioluminescence resonance energy transfer) techniques will be developed. The sensors will progressively enter the screening process. Once small molecules have been identified, their interactions with the assumed target protein and the interference with protein-protein interactions will be quantitatively analysed in WPs 7 and 8. In WP8 the small molecules will be chemically modified in order to enhance their

binding to the targets. Modified small molecules will be returned into WPs 2-5 for testing in cell models. The lead structures are transformed into selective high-affinity small therapeutic molecules. In WP7 the signature responses of cells to challenges with the small molecules will be characterised in order to monitor the specificity of the compounds and to anticipate side-effects. Thus all work packages are strongly interdependent, which leads to a quick transfer of project results within the consortium and thereby guarantees a speedy advancement of the planned work.

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[3] Carlson C.R. *et al.*: J. Biol. Chem. 281, 21535-21545 (2006)

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EVENTS

Nano2Life in Saarbrücken

The 3rd Annual Meeting of Nano2Life will take place 19-21 March 2007 in Saarbrücken, Germany, and is hosted by the regional NanoBioNet cluster.

N2L is a European Network of Excellence funded by the EC with the goal to establish itself as the reference centre in nanobiotechnology in Europe. The meeting will focus on activities towards this goal, common research projects and about new consortia and structures within the European Nanobio community. As technology transfer and cooperation are important points in the activities of the network, the participation of industry and SME's is very welcome.

Impact on the European Science Community

N2L scientists are very active in numerous European cooperation's which experts are often enquired by the EC or other political stakeholders. As for the FP7, the EC mentions the impact of such organisations in their work programme 2007: "In preparing this work programme, the Commission has relied on advice from a wide range of consulta-

tions, including from the European Technology Platforms and from specific advice of a series of advisory group" (Provisional Cooperation Work Programme 2007, European Commission: C(2006) 6839).

A strong participation in activities like the "ETP on Nanomedicine", the "ESF Forward look report on Nanomedicine" and the Nano roadmap Project (NRM) is therefore a strategic decision to help structuring the European research area.

Industry Relations: Building Tomorrow's Nanobiotechnology

The translation of science into economic benefits is fostered by facilitating the uptake of technology from cutting-edge academic institutions to key sectors such as health, environment, and security. Different talks, sessions, exhibition place and further activities are therefore planned to enforce the cooperation with industry. SME's are Nano2Life preferential partners as they serve as fast and flexible interfaces between the demand of academic research and the claim of industrial market pull.



Parc Científic de Barcelona

To date Nano2Life counts more than 40 industrial partners and the network has catalyzed over 20 joint projects between partners from academia and industry.

In short, by interfacing academic research with industrial needs, Nano2Life is the „one-stop-shop“ for nanobiotechnological solutions for challenges in medical, environmental and food industries.



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