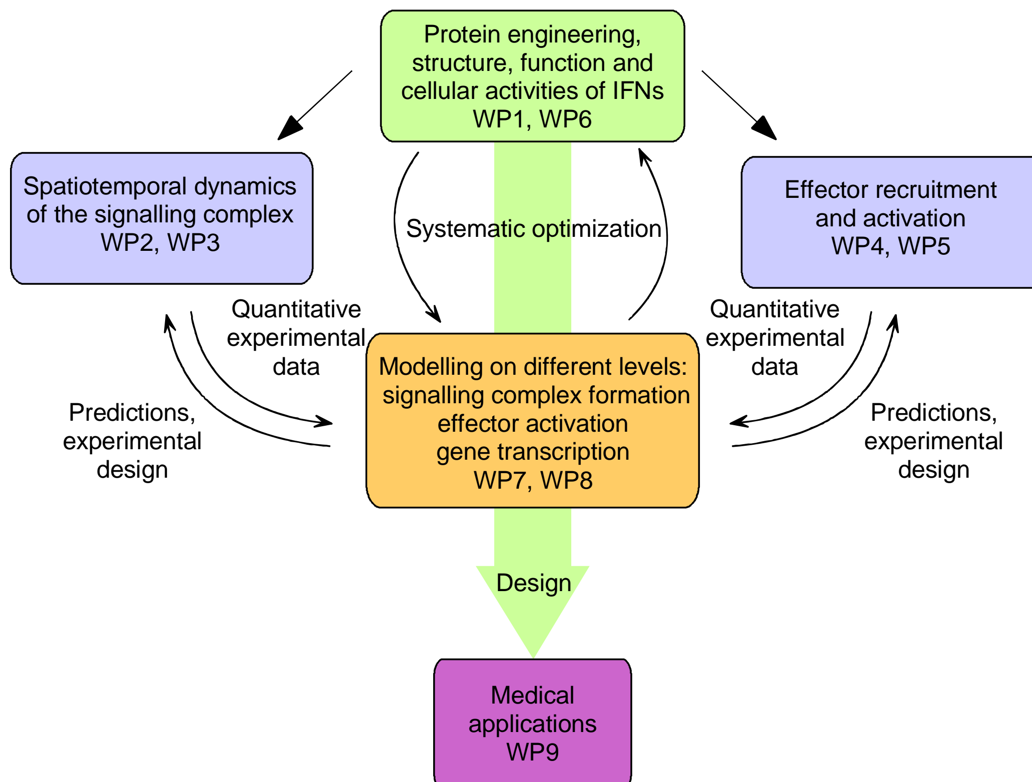
1 PUBLISHABLE SUMMARY

Project objectives

Type I interferons (IFNs) form a restricted network of highly related immune cytokines that elicit differential biological responses through a single cell surface receptor comprised of the subunits IFNAR1 and IFNAR2. We have shown that differential signal activation correlates with differential interaction and conformational dynamics of the receptor induced by binding of different members of the IFN family. The goal of this project is to employ a systems biology approach to identify the molecular and cellular mechanisms responsible for translating receptor dynamics into differential cellular responses by combining biochemical, biophysical and genetic analysis of the signaling outputs. To this end, we will collect quantitative data describing type I interferon signaling from ligand recognition to phenomenological cellular responses in a number of well defined cell lines. Using these data sets, input and output signals are correlated on different levels by various mathematical approaches to understand how the processing of differential input signals is translated within the cell to produce different responses to binding the same surface receptors. As a proof-of-concept for this approach, we design IFNs with optimized potencies for medical application, such as the ex vivo differentiation of monocytes into dendritic cells for application as cancer vaccines.



IFNaction project at a glance

Summary of results since the beginning of the project

In order to achieve these long-term goals, the following important objectives have been addressed within the first 18 months of the project:

1. Detailed structure-function analysis of the IFN-receptor complex (WP1)

In collaboration with Prof. Garcia the structure of the ternary interferon receptor complex was solved for IFNα2 and IFNω, and a detailed picture of the structure-function relationship for different IFNs and the receptor subunits was established. These studies confirm small differences in the overall architecture of the ternary complex, with the differences in affinity being attributed to small changes in the binding site.

2. Design of IFNs with pronounced response patterns (WP1, WP6, WP9)

Based on these studies, novel interferon variants were engineered. These were tested for differential function on different cell lines. Some of these mutants with increased binding affinities reach the maximum level of activity. Differential gene expression upon stimulation with different IFNs was demonstrated in several cell lines. Moreover, a differential effect on differentiation of dendritic cells by IFNs was demonstrated by gene chip analysis, expression of markers on the cell surface and the release of cytokines and chemokines.

3. Development of tools for elucidating the spatiotemporal dynamics of the IFN-receptor complex in context of the membrane and in living cells (WP2, WP3)

Reconstitution of transmembrane IFNAR1 and IFNAR2 into polymer-supported membranes was established for probing interactions in context of the membrane. Interactions between the transmembrane helices were not detectable. Diffusion and interactions of the receptor subunits in living cells was explored by single molecule localization techniques. Organization of IFNAR into microdomains and a key role of the cytoskeleton for the diffusion properties of IFNAR1 and IFNAR2 were demonstrated. Strong transient confinement of IFNAR after stimulating with IFN suggests specialized zones, which may be required for efficient signalling. Moreover, methods for purification and proteomic analysis of endosomes containing the signalling complex were established (deliverable D3.1). Three fibroblast-type clones have been established and finely characterized for receptor density, ligand binding, signal outputs and biological read-outs in response to two IFN subtypes. Moreover, IFNAR1-deficient cell lines were stably transfected with IFNAR1 tagged for posttranslational labelling and the functional properties of these cells were established in detail. These cell-based reagents are made available to all partners as common reference for further studies (deliverable D2.1).

4. Establishing methods for probing trafficking and interactions of IFN effector proteins in a quantitative manner (WP4, WP5)

Proximity ligation as a generic tool for probing and localizing endogeneous protein complexes revealed formation of a STAT1/STAT2 heterodimer only after IFNAR stimulation and STAT phosphorylation. This finding was confirmed by fluorescence cross-correlation in life cells. A live cell activation assay for fluorescence-tagged STAT1 and STAT2 was

established and the kinetics of STAT1/STAT2 translocation into the nucleus was determined. Moreover, receptor-independent translocation of STAT1 and STAT2 to the membrane guided by the actin membrane skeleton was identified.

5. Setting up models describing (i) the receptor assembly and (ii) signal transduction and gene transcription (WP7, WP8)

Competitive IFN binding experiments carried out with reference cell lines expressing different surface levels of IFNAR1 and IFNAR2 were modelled by a set of ordinary differential equations based on kinetic rate constants determined in *vitro*. These experiments suggest that either addition interaction between the receptor subunits stabilize the complex or that local pre-organization of the receptor subunits increase the effective concentration on the cell surface. Moreover, a core model structure has been established for the Jak-STAT pathway. The proposed mechanism shows a pivotal role of STAT2 in IFN signalling.

Potential impact and use

Our project is contributing to the objectives of the call on different levels. In the first step, we are combining established approaches with innovative methodologies for quantitatively describing ligand recognition (WP1) and receptor assembly (WP1, WP2), as well as signal activation of the type I interferon receptor (WP3-6). Lack of quantitative data is a key limitation for tackling signal transduction by systems biology. Thus, establishing and validating novel approaches for quantitative assessment of diffusion, interactions and translocation within the cells is a key step for promoting systems biological approaches. Based on highly controlled input parameters, which we can vary systematically by protein engineering, we are correlating the output on different levels. In systems biology, ideally an iterative cycle of experimentation and theory identifies gaps in our current understanding, specifies new hypotheses and experiments, and validates or falsifies the predictions experimentally. The modeling approaches further developed in this project is helping in the realization of this paradigm, even with uncertain biological mechanisms and not sufficient quantitative experimental data (WP7, WP8). The expected results on early IFN signaling are likely to underline the importance of model structures, a possibly general phenomenon due to widespread robustness in biology. The understanding of how IFNs can promote differential activations, such as antiviral, growth arrest and cell differentiation will make it feasible to design IFNs that are more specific for one condition or another and thus improve their efficiency. Particularly, understanding the relation between receptor binding and differential system activation will allow the engineering of IFNs with more specific actions and reduced side effects. As a proof of concept, we are systematically designing IFNs for medical application. Here, we have chosen the important ability of IFN (and many other cytokines) to induce differentiation of hematopoietic stem cells into immune cells. With an input-output model in hand, we are working on improving *ex vivo* dendritic cell maturation for generating DCs for anti cancer vaccination (WP9). Reaching this ambitious goal will only be possible by integrating molecular and systems understanding of the signaling processes. We envision that the understanding of the differential responses generated by IFNs will lead to improved medical applications for several other diseases. Moreover, we believe that our approach will prove a generic paradigm how to improve cytokine function for medical application.

Project Website

http://www.biologie.uni-osnabrueck.de/Biophysik/ifnaction