**In Vivo Characterization of Protein-Protein Interactions in the AP1 System with Fluorescence Correlation Spectroscopy (FCS)**

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**Experiments**

1. Construction of fusion proteins
2. Expression of fusion protein and FCS measurements

**Fig. 1** Schematic representation of the AP1-DNA complex

**Fig. 2** Vector pSV-API-XFP-His in HeLa cells stably transfected. The vector pSV-API-XFP-His contains the major components of AP1 as a coiled coil.

**Fig. 3** PHOTO BLEACHING in the nucleus of Fos-CFP cells to investigate the rate of dissociation of AP1. The bleaching curves measured in the nucleus show a fast component (t < 10 s) and a slow component (t > 10 s) of diffusion.

**Fig. 4** FCS in the nucleus of Jun-YFP cells: a fast (t < 10 s) and a slow component (t > 10 s) of diffusion are obtained in the ACF corresponding to free and bound component of AP1.

**Protein domains**
- group of transcription activator proteins
- subunits: c-Fos, c-Jun, JunB, JunD, FraI, FosII
- major components are c-Fos and c-Jun

**Dimerization and DNA-binding**
- All proteins have a basic region leucine zipper (bZIP) for dimerization
- The leucine zipper is formed as a coiled coil

**Region around an API-regulated promoter**
- transcription start
- TATA Box
- API
- RNA-polymerase II
- TBP
- DNA
- TPA responsive element
- DNA

**Conclusions**

The aim of these studies is the quantitative investigation of protein-protein interactions in the AP1 system in vivo.

First results of FCS measurements show an exchange in the nucleus of the proteins Fos-CFP and Jun-YFP in the stably transfected HeLa cells. This is also shown by fitting the bleaching curves measured in the nucleus with an appropriate model. We obtained dissociation times between 10 and 20 seconds in the nucleus.

In further studies doubly transfected cells with both proteins, Fos-CFP and Jun-YFP, were prepared. These cells will now be characterized with FCCS to investigate the protein-protein interactions.

The signals measured in the nucleus show a fast component (t < 10 s) and a slow component (t > 10 s) of diffusion.

In order to obtain the dissociation rates of the complex in the cell nucleus bleaching curves will be recorded on these cells.

We also overexpressed and purified Jun-YFP and Fos-CFP for in vitro studies.

**FCS and FCCS**

**One color FCS**

A laser is coupled into a microscope, illuminating a sub-femtoliter volume (Fig. 8a). Fluorescent molecules cross this focus by Brownian motion. Their fluorescence light is collected by the same optics and detected by a confocally arranged detector.

Single molecules can be detected due to the high sensitivity of the detectors (avalanche photodiodes).

**Two color FCCS**

In Fluorescence Cross-Correlation Spectroscopy (FCCS) two fluorophores with distinct spectra are detected simultaneously in two channels and their signals are cross-correlated (Fig. 9a–b).

Only particles which carry both fluorophores contribute to the cross-correlation signal. FCCS will enable us to measure for the first time protein-DNA and protein-protein interactions in living cells.
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**Abstract**

The aim of these studies is the quantitative investigation of protein-protein interactions in the AP1 system *in vivo*. First results of FCS measurements show an exchange in the nucleus of the proteins Fos-CFP and Jun-YFP in the stably mono-transfected HeLa-Cells. This is also shown by fitting the bleaching curves measured in the nucleus with an appropriate model. We obtained dissociation times between 10 and 20 seconds in the nucleus. In the autocorrelation function a free and an obstructed component of diffusion are shown. For further studies doubly transfected cells with both proteins, Fos-CFP and Jun-YFP, were prepared. These cells will now be characterized with FCCS to investigate the protein-protein interactions. In order to obtain the dissociation rates of the complex in the cell nucleus bleaching curves will be recorded on these cell lines. We also overexpressed and purified Jun-YFP and Fos-CFP for in vitro studies.

**Keywords:**

Genome, genomics, genome organization, genome architecture, structural sequencing, architectural sequencing, systems genomics, coevolution, holistic genetics, genome mechanics, genome function, genetics, gene regulation, replication, transcription, repair, homologous recombination, simultaneous co-transfection, cell division, mitosis, metaphase, interphase, cell nucleus, nuclear structure, nuclear organization, chromatin density distribution, nuclear morphology, chromosome territories, subchromosomal domains, chromatin loop aggregates, chromatin rosettes, chromatin loops, chromatin fibre, chromatin density, persistence length, spatial distance measurement, histones, H1.0, H2A, H2B, H3, H4, mH2A1.2, DNA sequence, complete sequenced genomes, molecular transport, obstructed diffusion, anomalous diffusion, percolation, long-range correlations, fractal analysis, scaling analysis, exact yard-stick dimension, box-counting dimension, lacunarity dimension, local nuclear dimension, nuclear diffuseness, parallel super computing, grid computing, volunteer computing, Brownian Dynamics, Monte Carlo, fluorescence in situ hybridization, confocal laser scanning microscopy, fluorescence correlation spectroscopy, super resolution microscopy, spatial precision distance microscopy, autofluorescent proteins, CFP, GFP, YFP, DsRed, fusion protein, in vivo labelling.

**Literature References**


