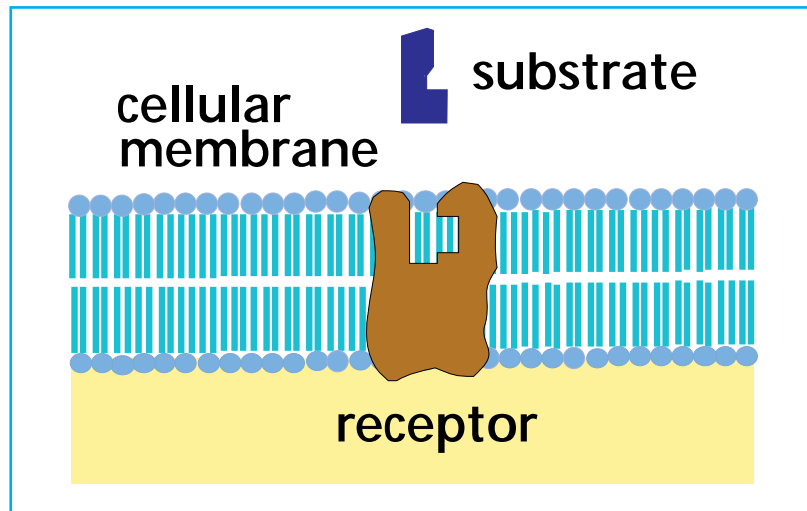


# EGF Binding to Specific Receptors on Membrane Vesicles



◀ Fig.1: Binding of a ligand to a cell membrane receptor.

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### INTRODUCTION

To study receptor/ ligand interactions using fluorescence correlation spectroscopy (FCS), the EGF-receptor, an integral cell membrane protein, was chosen as a model. Its extracellular domain binds EGF, a 6kD polypeptide, and consequently mediates the initial response of cells to EGF (proliferation, differentiation, etc).

The EGF receptor is overexpressed in more than a third of human epithelial cancers and, hence, many therapeutic approaches aim at inhibiting the receptor-associated tyrosine kinase by using receptor-specific antagonists.

Conventional receptor-binding studies require the use of radioactively labeled ligands and include several washing steps.

FCS allows the interaction of receptors with ligands to be observed on the molecular level

in their native environment on cell surfaces or cell-derived vesicles.

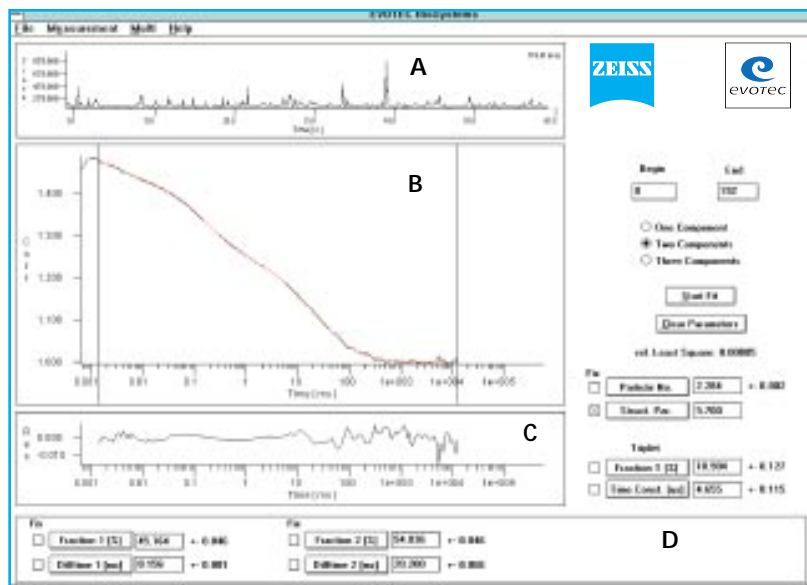
Binding and kinetic properties of a particular molecular interaction may be studied in a non-invasive manner using homogenous assays.

Also, nuclear receptors can be directly analyzed in nuclear extracts. Iso-receptors of the same family can be differentiated on the basis of binding constants and on/off rates. Binding and competition studies of the receptor/ligand interaction will be described here.

### MATERIALS AND METHODS

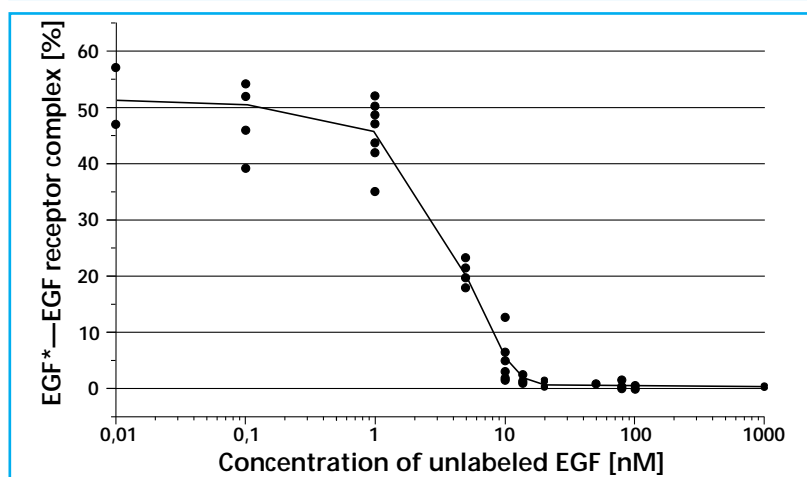
Substances: Membrane preparations of A431 cells, a carcinoma cell-line bearing approximately  $10^5$  receptors/cell; Membrane vesicles prepared according to standard protocols (modif. of Carraway et al., 1989); Tetramethyl-rhodamine-labeled EGF purchased from Molecular Probes; non-labeled EGF obtained from Sigma. Buffer: 140 mM NaCl, 20 mM Hepes (7.4), 5 mM  $MgCl_2$ , 1.8 mM  $CaCl_2$ , 0.35 g/l  $NaHCO_3$ , 1 g/l D-glucose. Equipment: ConfoCor with interference filter 580DF30 (Omega), helium neon laser (Uniphase, 1.5 mW, 543,5 nm), C-APOCHROMAT 40x/1.2 objective (Zeiss). Temperature: room temperature for incubation and measurement. Reaction: 10 nM labeled EGF with 250  $\mu$ g/ml membrane preparation; final volume of 20  $\mu$ l; incubation time 40 min. Measurement: Eight chamber coverglasses (Nunc); data acquisition time for all experiments 60 s. Competitor experiments: Non-labeled EGF concentrations of 10 pM to 100 nM in the reaction mixture (Fig. 3). Analysis: using the two-component model (FCS ACCESS software package).





◀ Fig.2: Typical screen image.

- A) Primary data: Large fluctuations of the fluorescence signal can be observed
- B) Autocorrelation of the primary data. Fit of the autocorrelated data (red curve)
- C) Excellent agreement of the fitted and the measured curve.
- D) Automatic calculation of diffusion times and functions:  $\tau_D$  for non-bound EGF = 166  $\mu$ s (45 %) and for EGF bound to receptor = 20 ms (55 %).



◀ Fig.3: Competition of the fluorescently labeled EGF (10 nM) and the non-labeled EGF (10 pM to 100 nM) for receptor binding sites on membrane vesicles. The fraction of the bound fluorescent EGF conjugate was plotted versus the concentration of the non-labeled competitor.

## RESULTS

Calculation of the diffusion times using the two component model of the FCS ACCESS software resulted in a  $\tau_D$  for non-bound EGF of 166  $\mu$ s and a  $\tau_D$  for receptor-bound EGF of 20 ms (see Fig. 2). The more than 100-fold increase in the diffusion time allows rapid and unequivocal quantification of the interaction. The distribution of the particle size of the membrane vesicles had its maximum at a radius of 160 nm. From this information an approximate number of receptors per vesicle of 200 has been calculated.

Relative affinities can be determined by competitive studies. Detailed kinetic analysis of these receptor/ligand interactions are currently under investigation.

### LITERATURE

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## SUMMARY

The successful analysis of the binding and competition of fluorescently-labeled EGF proves FCS to be a novel method for rapidly studying receptor/ligand interactions in homogenous assays without the need for radioactively-labeled ligands. Molecular interactions can be studied without interfering with the natural binding conditions, since only the reference substances in competition experiments, need to be fluorescently labeled. The analysis of the efficiency with which an unknown compound is able to compete a labeled substance from its target will open new perspectives in drug research.



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