PETER PAZMANY
CATHOLIC UNIVERSITY

SEMMELWEIS
UNIVERSITY

Development of Complex Curricula for Molecular Bionics and Infobionics Programs within a consortial* framework**

Consortium leader

PETER PAZMANY CATHOLIC UNIVERSITY

Consortium members

SEMMELWEIS UNIVERSITY, DIALOG CAMPUS PUBLISHER

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**Molekuláris bionika és Infobionika Szakok tananyagának komplex fejlesztése konzorciumi keretben

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Neurobiológia alapjai - Módszerek

BASICS OF NEUROBIOLOGY - Methods

By Imre Kalló

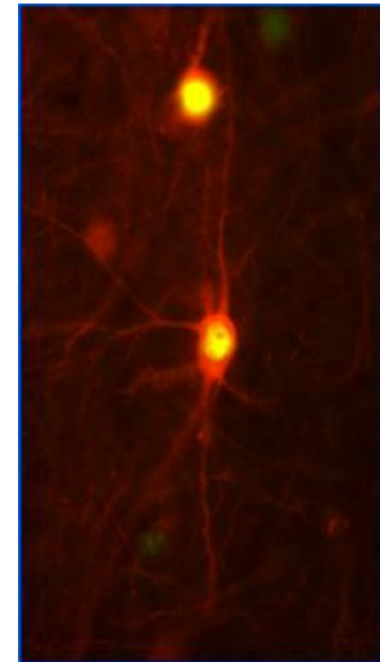
METHODS IN NEUROBIOLOGY II.

Histology techniques: electron microscopic studies

Imre Kalló

Pázmány Péter Catholic University, Faculty of Information Technology

I. Histology techniques: light microscopic studies
II. Applications using fluorescent dyes
III. Histology techniques: electron microscopic studies
IV. Techniques to map neuronal connections
V. Molecular biological techniques
VI. Living experimental models
VII. Electrophysiological approaches
VIII. Behavioral studies
IX. Dissection, virtual dissection, imaging techniques



PROPERTIES OF FLUORESCENT MOLECULES

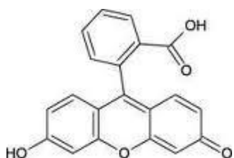
Fluorescent molecules absorb their own characteristic wave-length of light, which turn them into a higher energy state (excitation) and upon returning to the lower energy state, they emit light (emission), the wave-length of which characterizes the molecule again.

absorption maximum
emission maximum

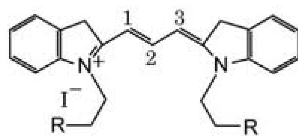
Organic fluorophores

Stokes-shift

Fluorescein

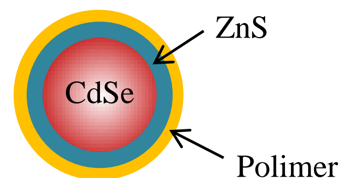


CY3



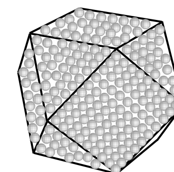
Inorganic fluorophores

Semiconductor nanocrystals
Quantum dots (QD)

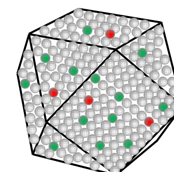


second harmonic generation
two-photon upconversion

Barium titanate nanocrystals



Lanthanide-doped nanocrystals



APPLICATIONS OF FLUORESCENT MOLECULES IN NEUROBIOLOGY

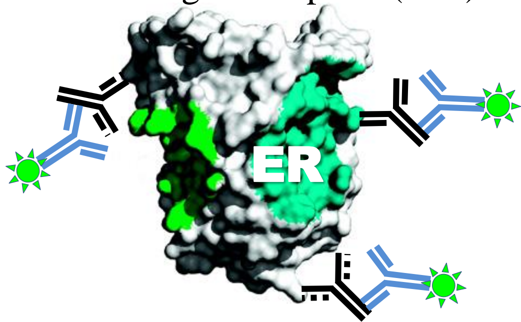
Fluorescent molecules used

- as **SELECTIVE MARKER** of cellular organelles and tissue components
- as labels of reagents and immunoglobulins in **IMMUNOCYTOCHEMISTRY**
- as labels of probes in ***IN SITU*** **HYBRIDIZATION HISTOCHEMISTRY**
- as **SENSORS** of intracellular calcium levels and potential changes
- as **REPORTER MOLECULES** expressed by genetically altered cell types of CNS

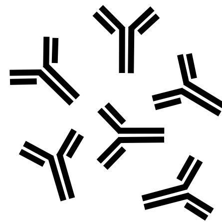
FLUORESCENT IMMUNOHISTOCHEMISTRY (FIHC) I.

Antigen (e.g. ER) detected | **Primary antibody (PAB)** | **Secundary antibody (SAB)**

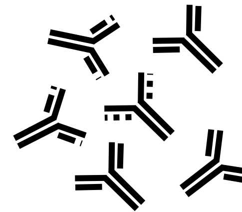
Immunofluorescent detection of estrogen receptors (ERs)



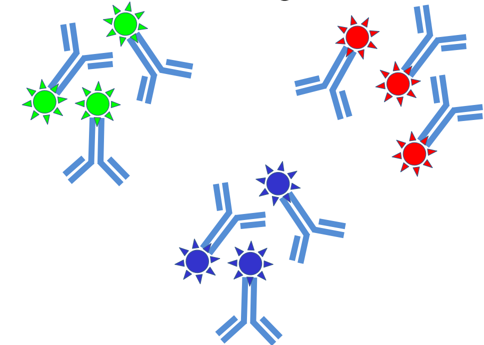
Using either monoclonal immunoglobulins



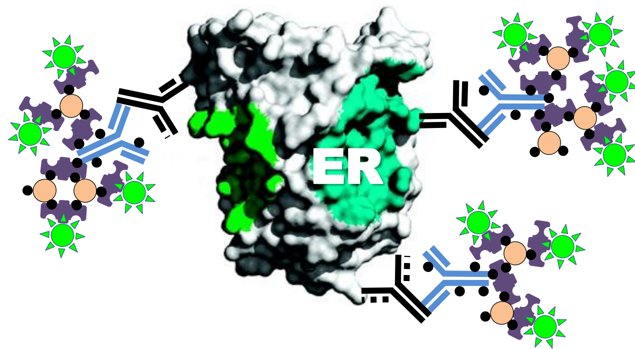
Or polyclonal immunoglobulins



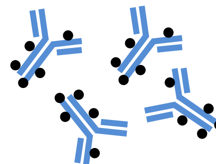
And either of the fluorescently-labelled immunoglobulins



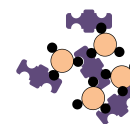
Signal amplification technique using the avidine-biotin system



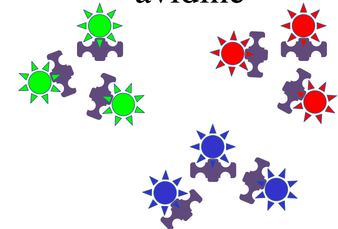
After PAB using biotinylated-immunoglobulins



And **A**vidine and **B**iotinylated-peroxidase enzym **C**omplex (**ABC**)

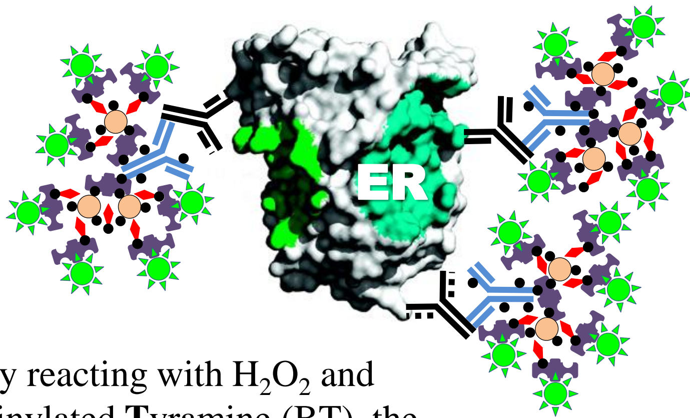


And either of the fluorescently-labelled avidine

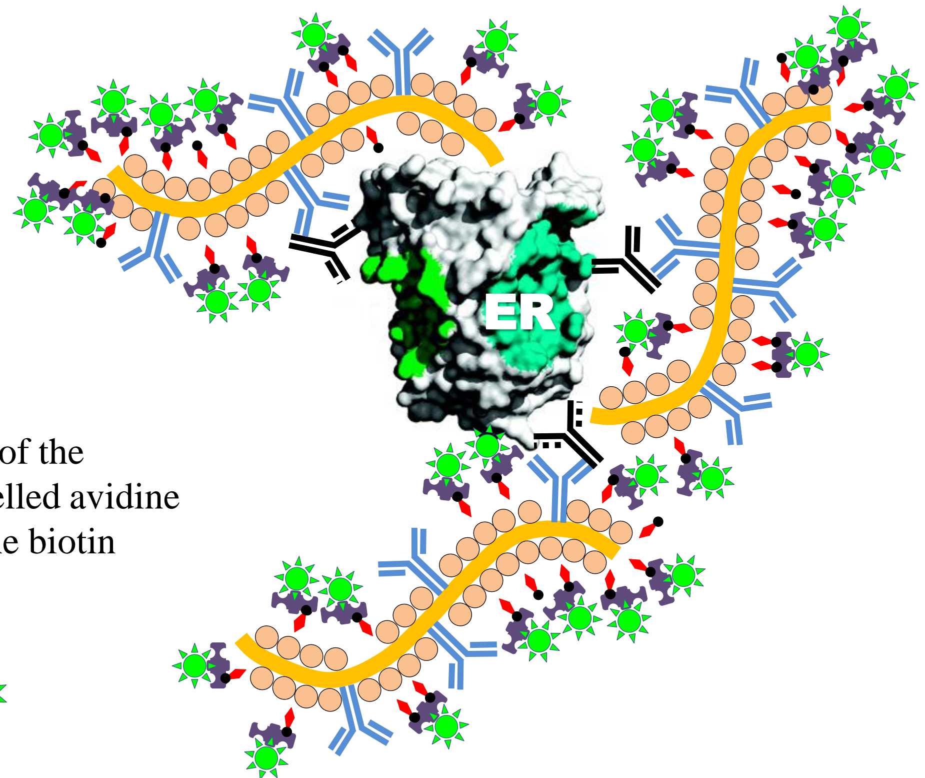


FLUORESCENT IMMUNOHISTOCHEMISTRY II.

Signal amplification technique using the biotinylated-tyramine system

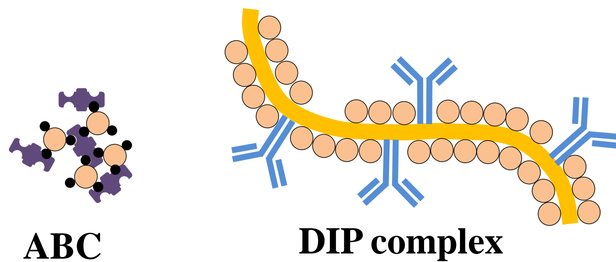


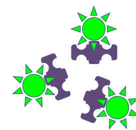
Signal amplification technique using the dextran-immunoglobulin-peroxidase (DIP) and the biotinylated-tyramine systems



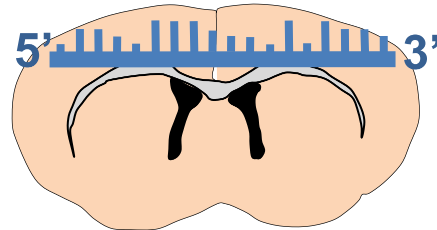
By reacting with H_2O_2 and **B**iotinylated **T**yramine (BT), the peroxidase enzyme of the ABC or the DIP complexes deposites BT.

And either of the fluorescently-labelled avidine is bound to the biotin



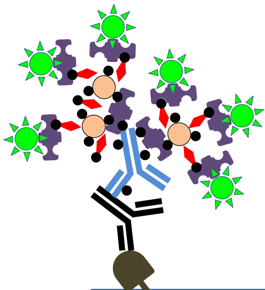


FLUORESCENT IN SITU HYBRIDIZATION (FISH)

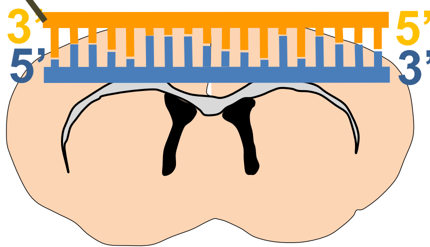


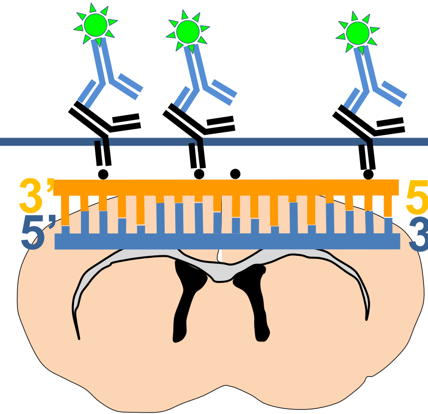
Single stranded mRNA in the tissue

Antisense RNA probes labelled either at the 3' end with digoxigenin/biotin or throughout with biotinylated nucleotids

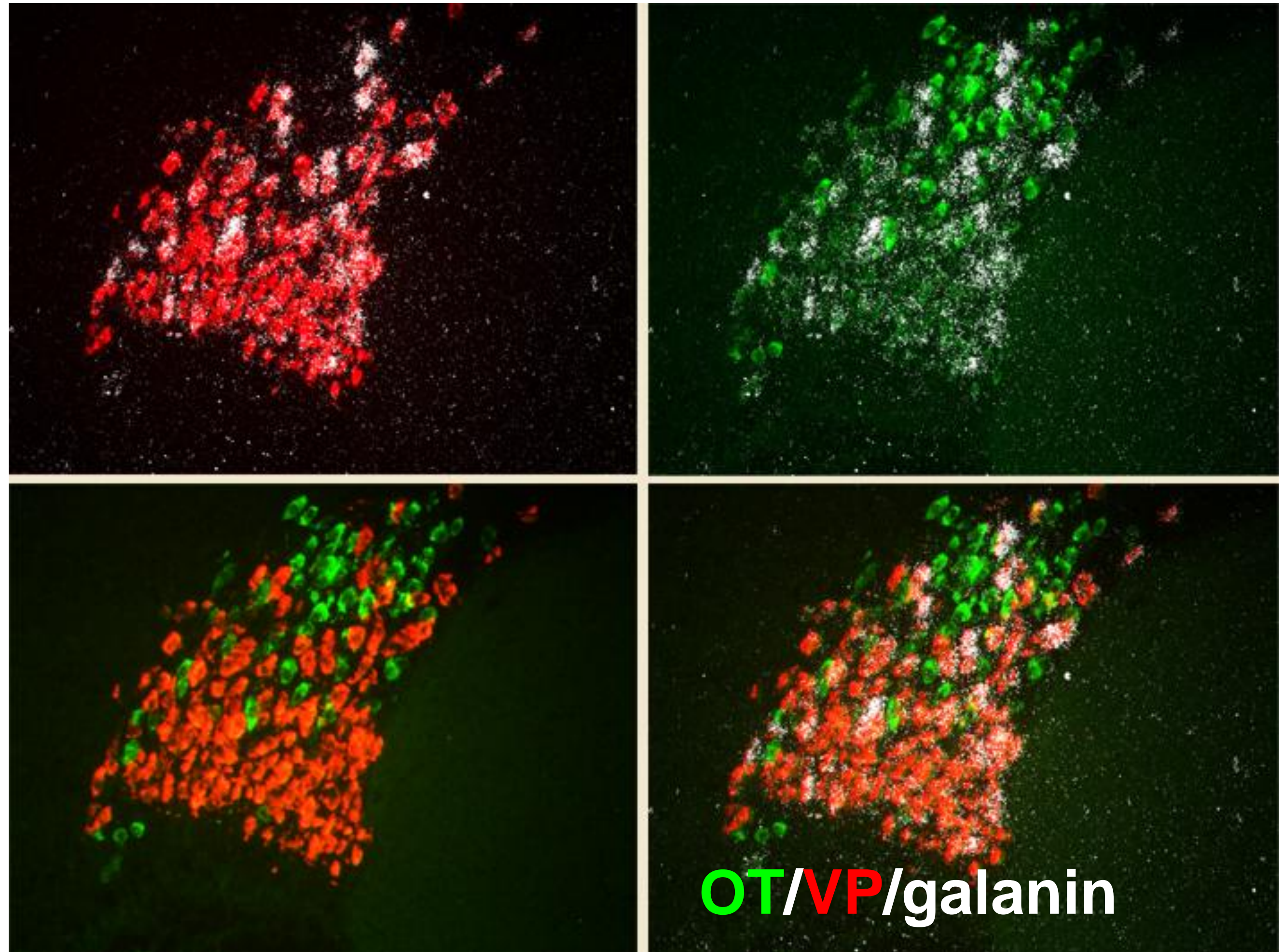


Digoxigenin and biotin are detected with specific antibodies, which then are revealed with simple or amplified fluorescence techniques





FLUORESCENT IN SITU HYBRIDIZATION (FISH)



By courtesy of Erik Hrabovszky, Institute of Experimental Medicine of the Hungarian Academy of Sciences, Budapest, Hungary

CALCIUM IMAGING

Physiology:

Calcium ions are kept intracellularly at nanomolar concentrations (100nM), elevations of which from the extracellular space (1.2 mM) and intracellular stores change the membrane potential, as well as activate calcium-dependent intracellular processes. – and ***can be investigated in fluorescent or two-photon confocal microscopy***
Slow, moderate and rapid changes can be distinguished

Calcium indicators:

- Chemical indicators (lipophilic molecules, which includes fura-2, indo-1, fluo-3, fluo-4 and Calcium Green-1) loaded in the cells
- Genetically encoded indicators (fluorescent proteins fused with calmodulin, which includes Pericams and Cameleons) expressed in specific subpopulations of cells

Usage:

- Stimulated cells either loaded with the indicator or expressing the indicator are wieved in a fluorescence microscope or a two-photon confocal microscope
- Images are captured by a CCD camera (data acquisition at rates 10 -100 ratios/sec; 30-200 msec/image required) and analysed according to intensity.

USING VOLTAGE SENSITIVE DYES

Physiology:

The membrane potential is a voltage difference generated by the altered ionic concentrations on the opposite sides of the cellular membrane. Profiles of propagating action potentials and subthreshold potentials can be monitored directly with voltage-sensitive dyes.

Voltage indicators:

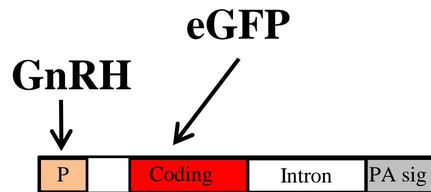
Voltage-sensitive dyes are organic molecules or proteins. They reside in a cell membrane and change their optical properties in response to a change in membrane potential. Slow dyes and fast dyes are distinguished for practical reasons. (e.g. ANEP dyes, ANNINE-6plus)

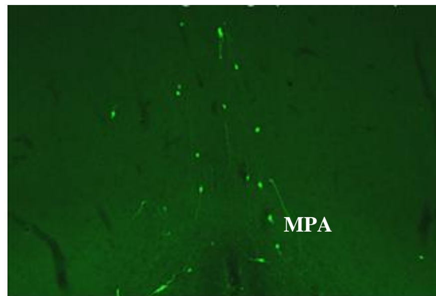
Usage:

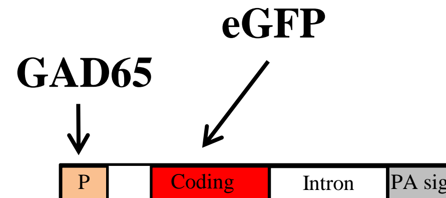
With fast (1 kfps frames rate) cameras voltage-senzitive dyes can monitor membrane potential in processes of individual neurons and from multiple cell bodies in localized brain regions.

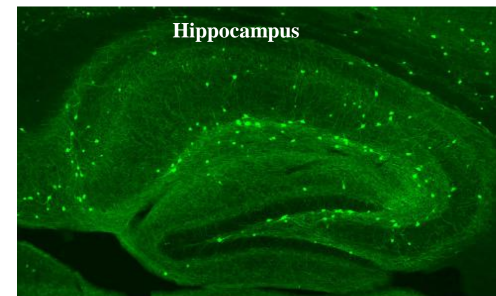
GENETIC ENGINEERING TO INTRODUCE FLUORESCENT MARKERS

Transfection: the introduction of gene sequences encoding GFP, YFP, CFP or BFP into eukaryotic cells using viral vectors, electroporation etc.



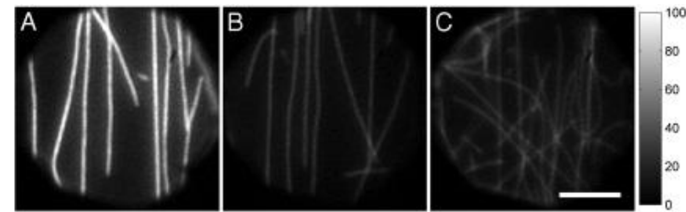






FÖRSTER (*fluorescence*) RESONANCE ENERGY TRANSFER

Mechanism: A donor chromophore transfers energy to an acceptor chromophore - if they close enough (typically less than 1 nm) to each other - through nonradiative dipole–dipole coupling.



Martin D S et al. PNAS 2010;107:5453-5458

FRET reporters are used to study:

- protein-protein interactions
- protein-DNA interactions
- protein conformational changes