Fluorescence labeling technology: from tube to animal.

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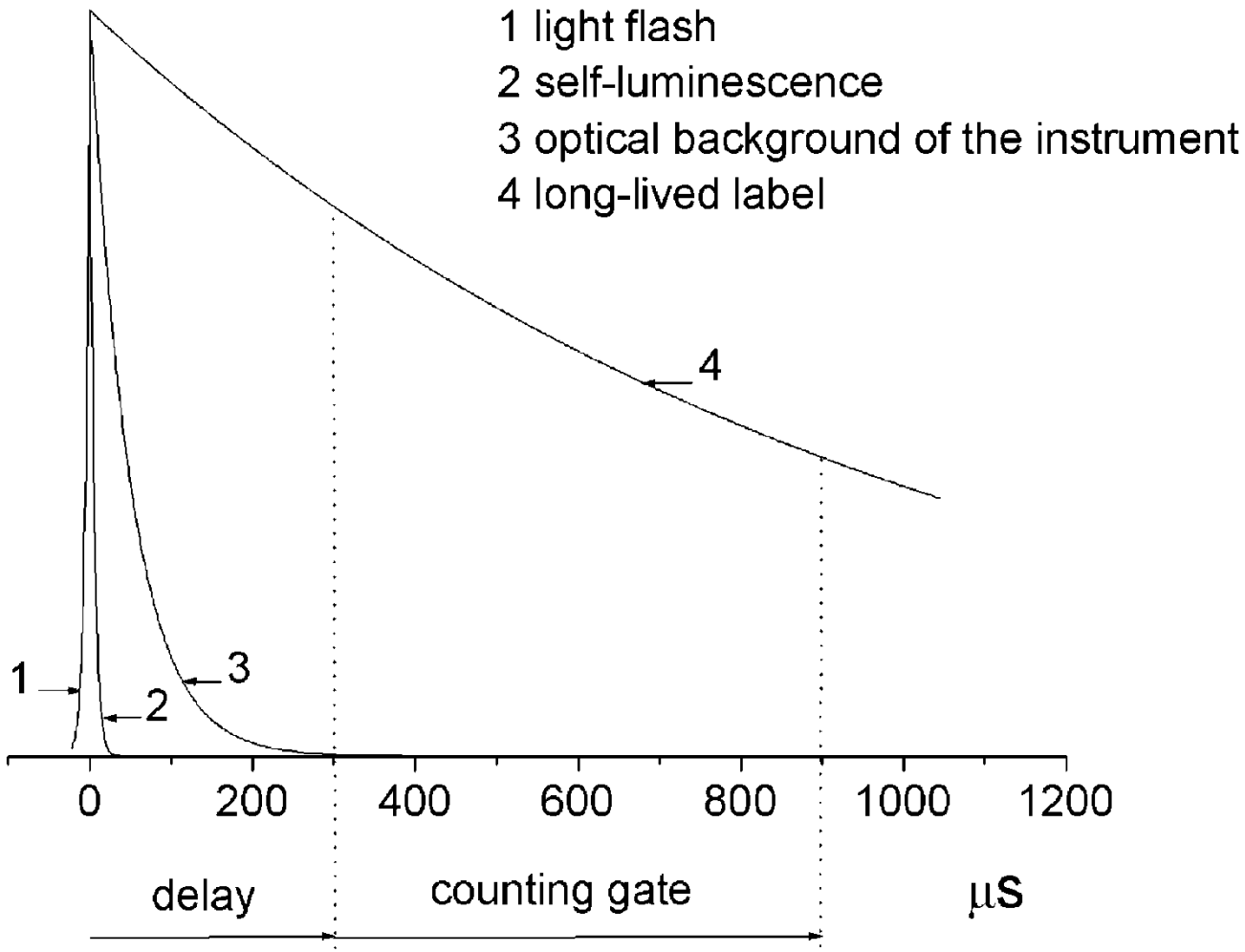
Fluorescent detection systems

- high sensitivity (up to 10^{-13}-10^{-15} M);
- real time measurements (milli or microseconds per sample or array);
- possibility of automatization;
- possibility to use micro and nanovolumes;
- long shelf-life of the labeled reagents;
- Genetical encoding;
- *in vivo* application, small animal whole body imaging.

Mode of measurement



Room Temperature Phosphorescence

RTP

Biomedical Applications

Oxygen detection

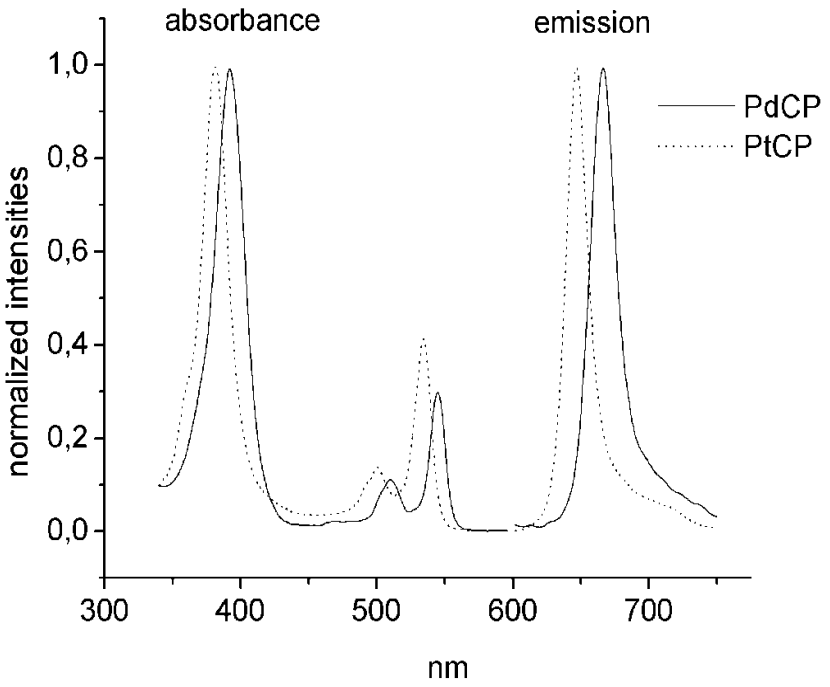
Oxygen imaging

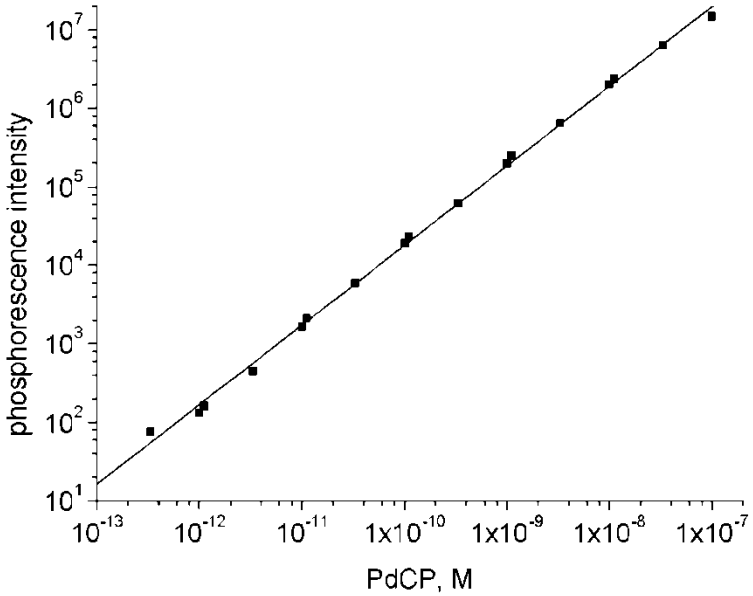
Immunoassay

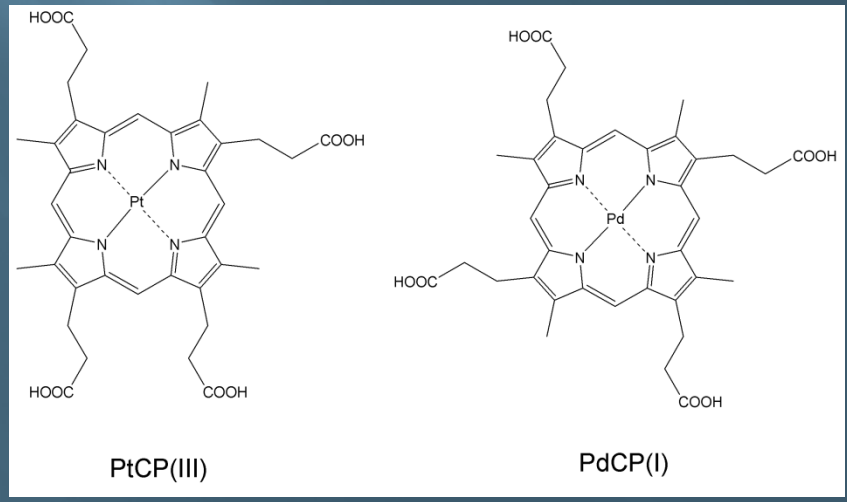
DNA biding assay

Microscopy

Excitation and emission spectra of PtCP, PdCP and detection limit in 2% met-β-cyclodextrin

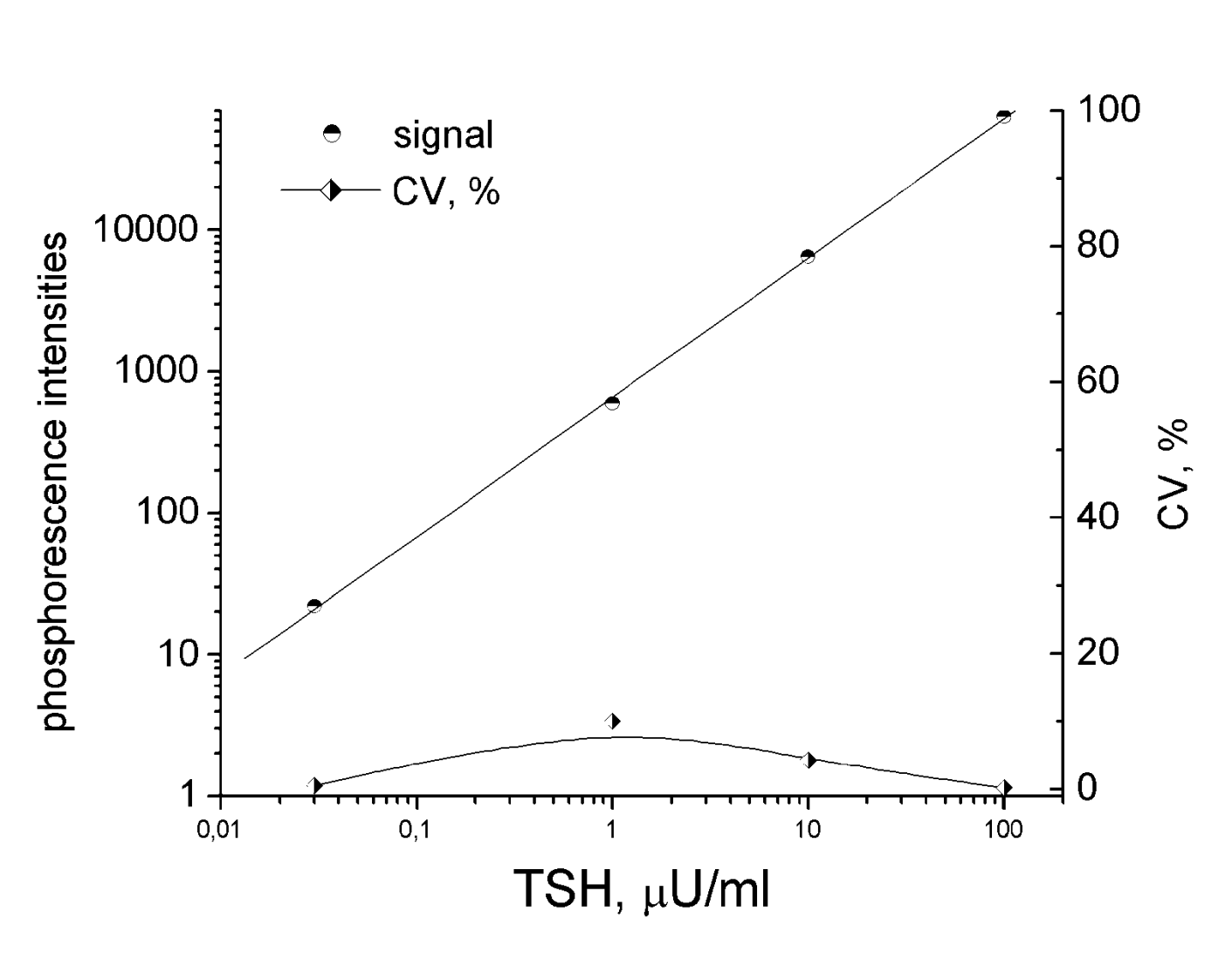






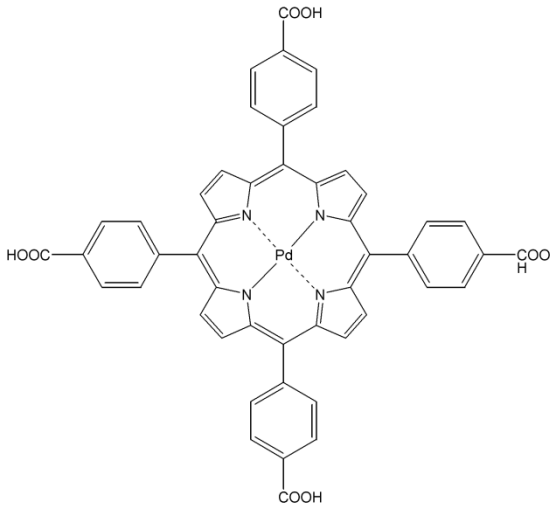
A.P.Savitsky. Subpicomolar Assay of antibodies and DNA using phosphorescence labels. In Fluorescence sensors and biosensors. R.B.Thompson Ed.CRC Taylor and Francis Group, London-N.Y., 2005

Calibration curve for thyrotropin assay obtained by MS-PhIA on an Arcus 1230 instrument (Wallac, Finland) with 400 nm excitation and 660 nm emission filters, delay time was 200 ms, measuring time was 800 ms, signal accumulation time was 1 s.

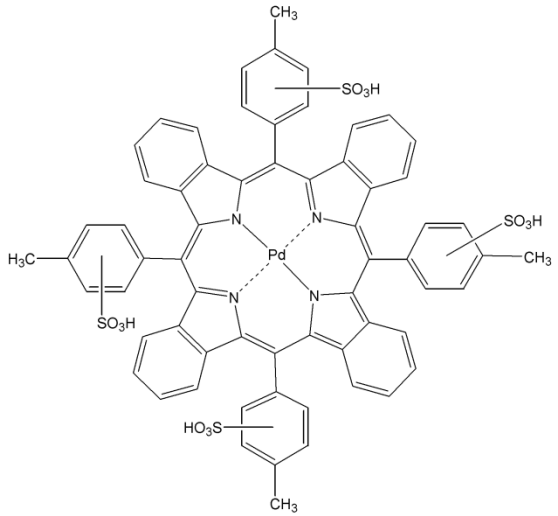


Normalized absorption and fluorescence spectra of NIR metalloporphyrins

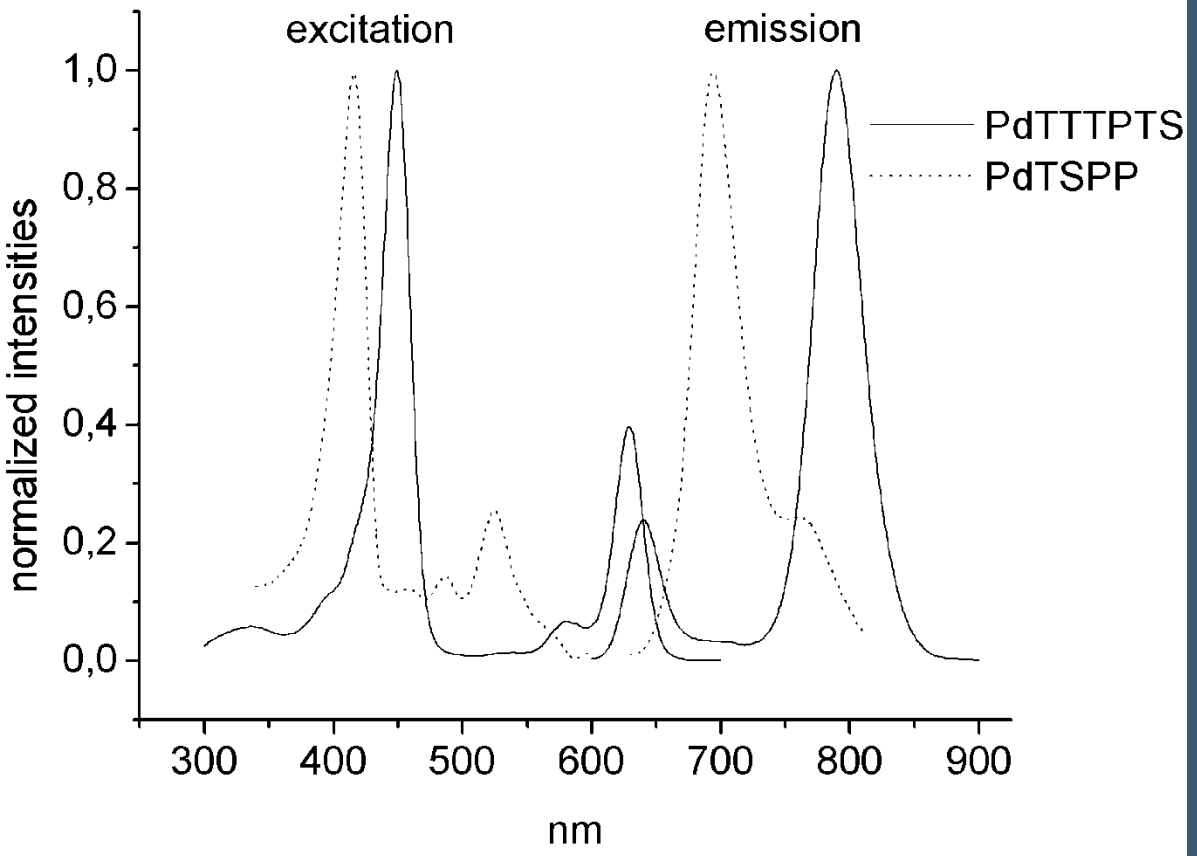
at room temperature in 2% Triton X-100, pH 7.2.



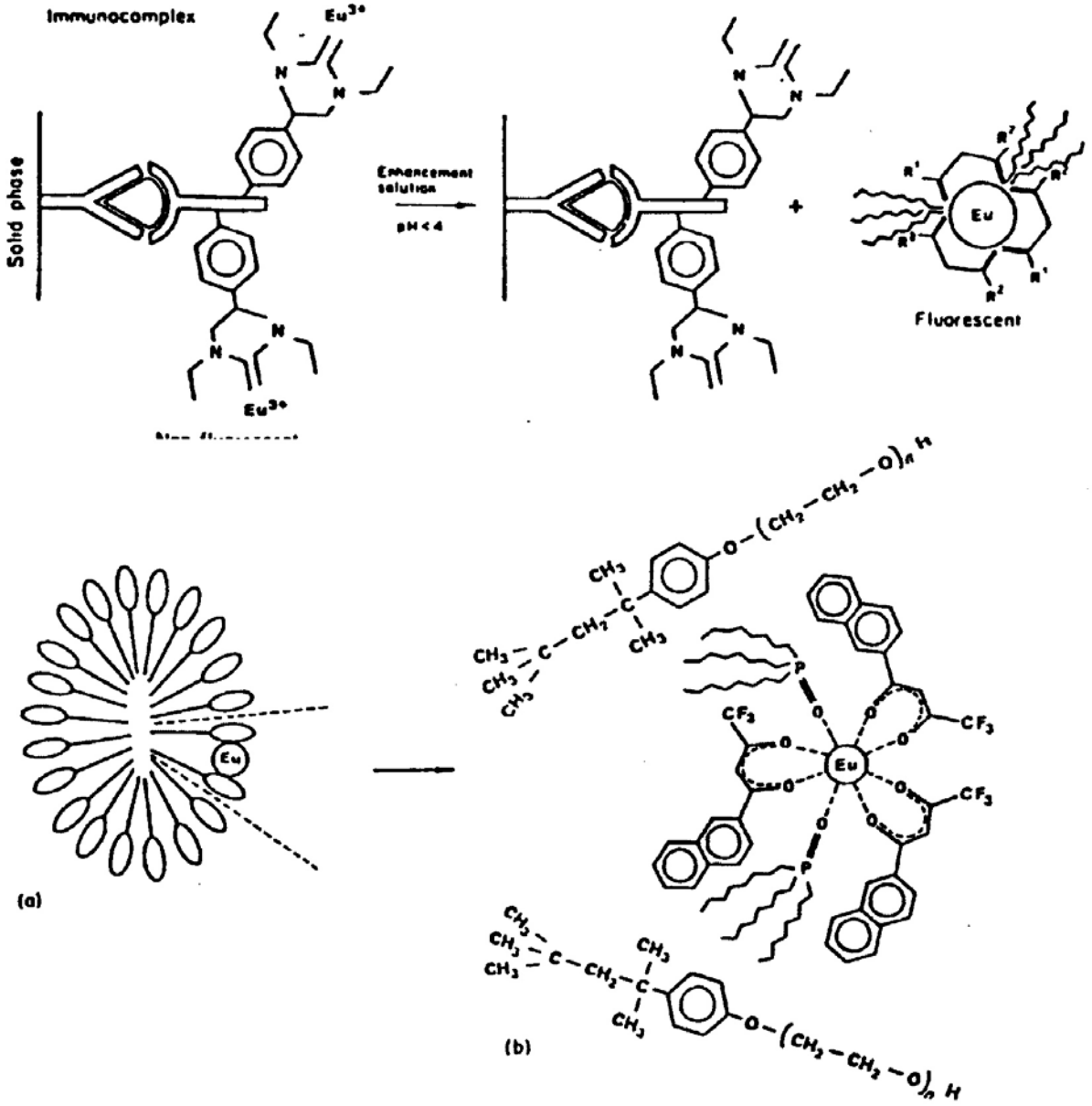
PdTCTP

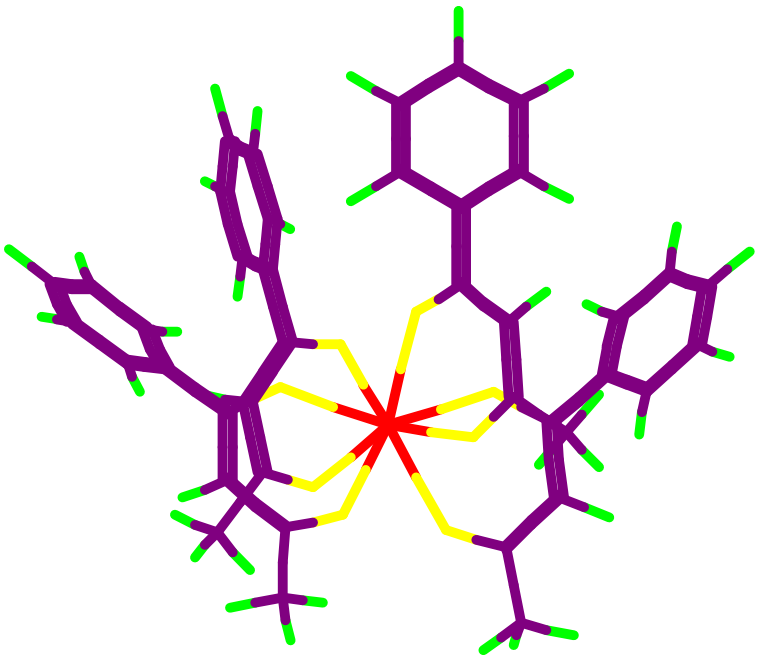


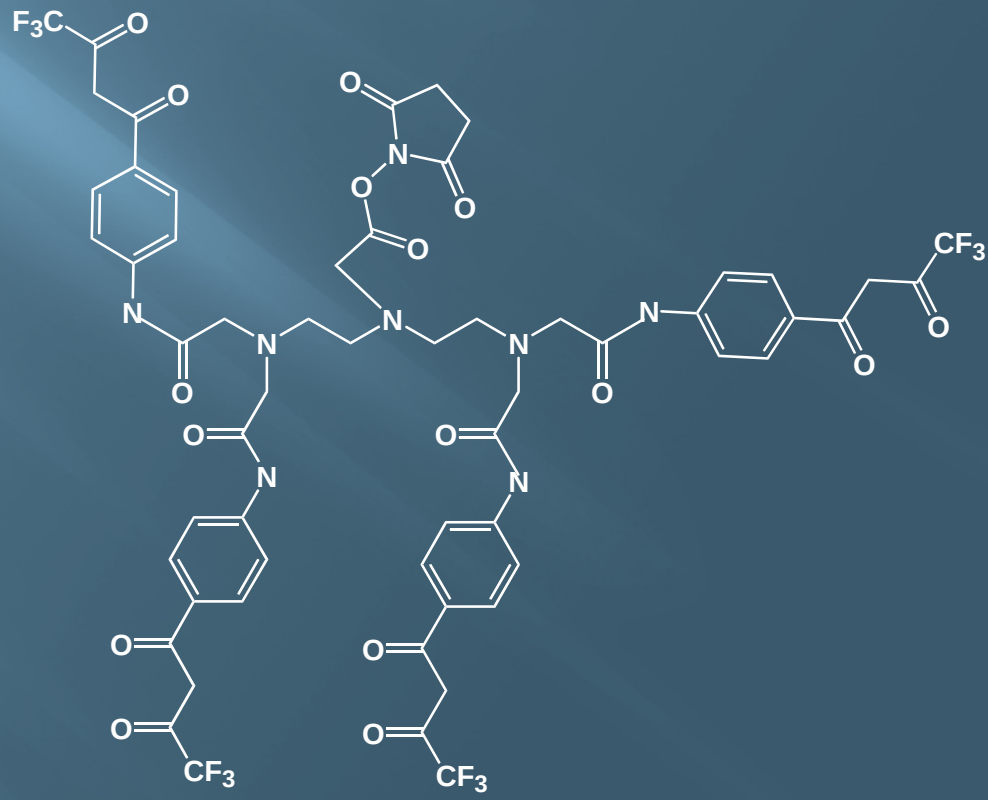
PdTTTPTS



DELFIA

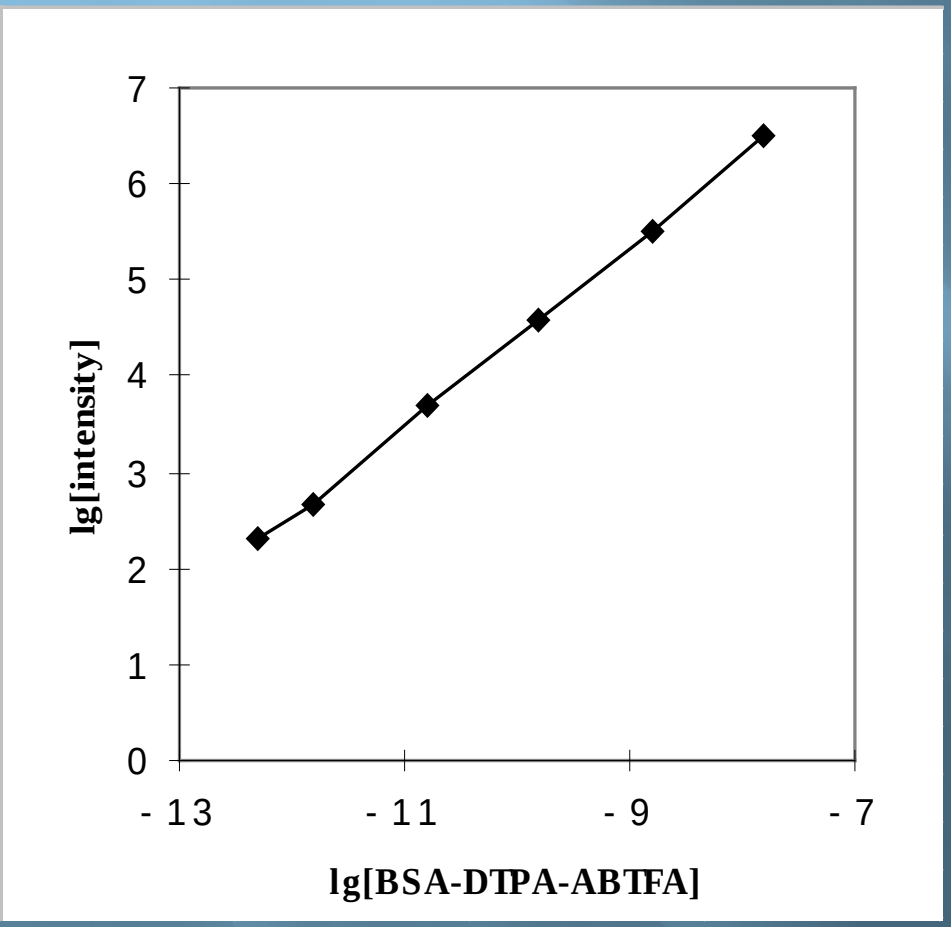

Immunocomplex
Eu3+
Solid phase
Enhancement solution
pH < 4
Eu
Fluorescent
(a)
(b)

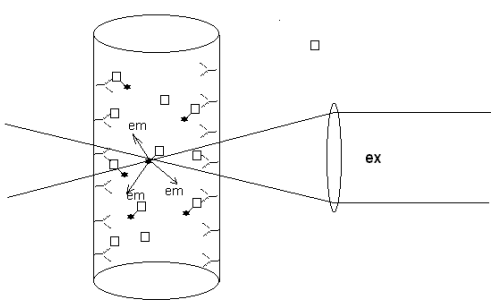


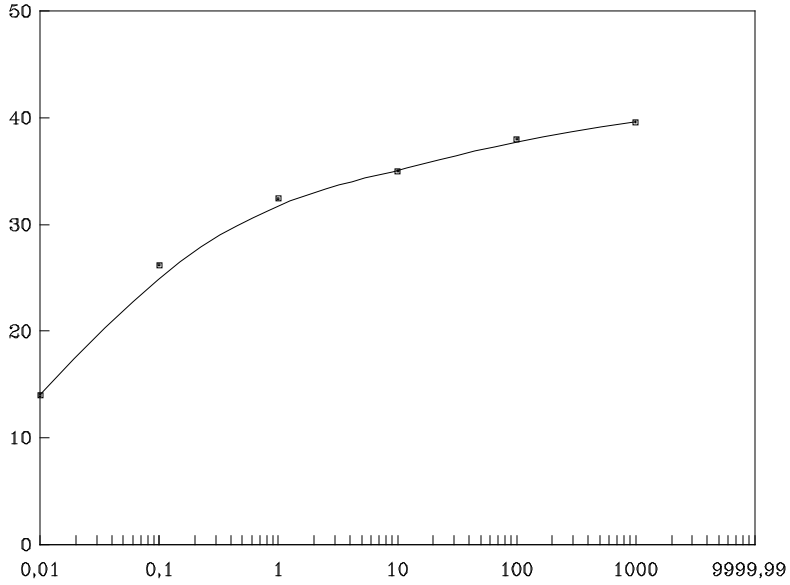


$C_{58}H_{50}O_{16}N_8F_{12}$

Nonseparation competitive assay of human IgG







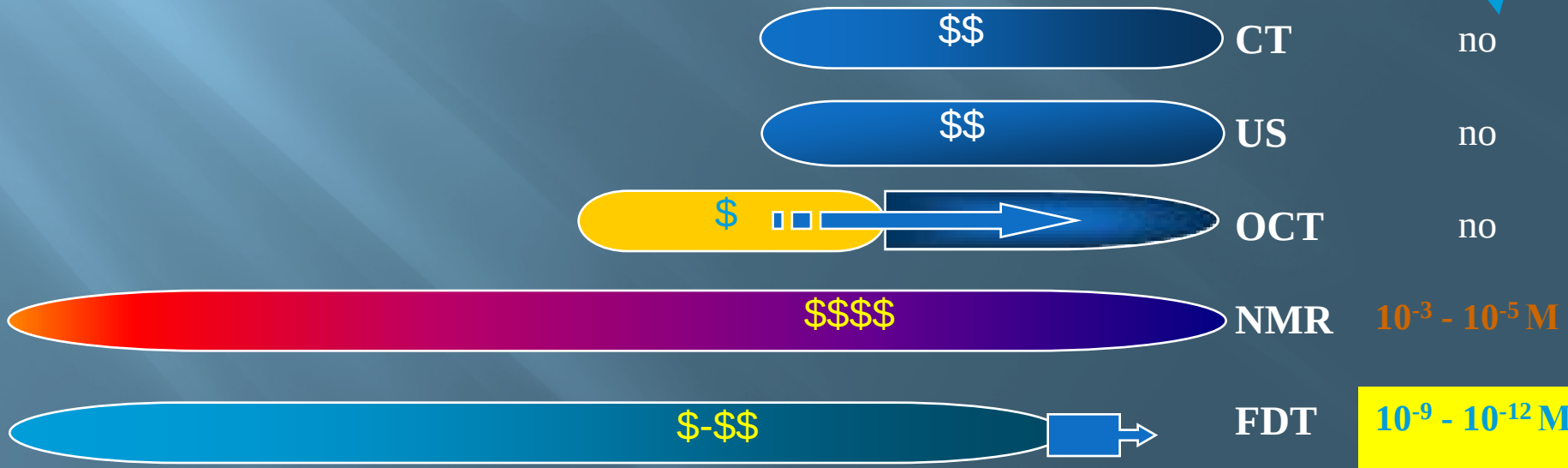
Savitsky et.al. Advances in Fluorescence Sensing Technology II, J.R.Lakowicz, Ed. Proc.SPIE Bellingham, Washington Vol.2388, pp.429-434, 1995

Tomography

Information level

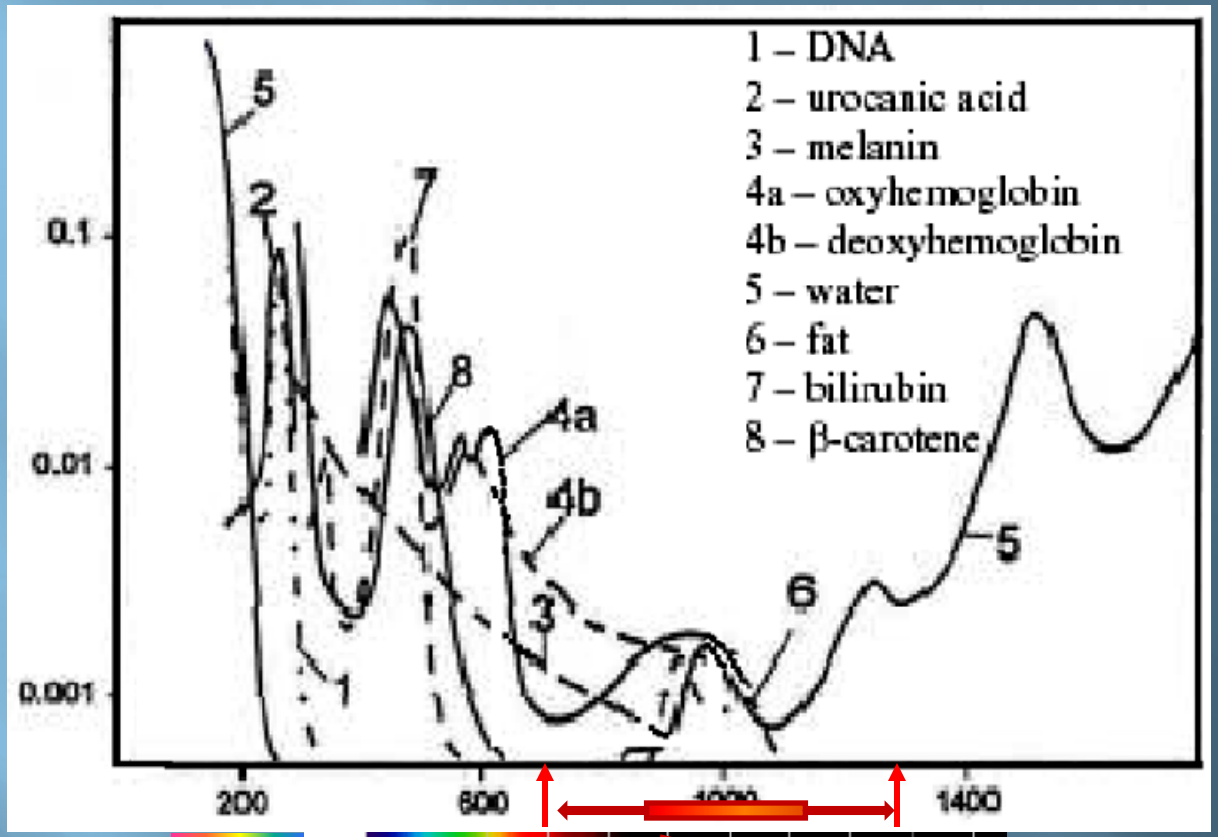
Sesitivity to malecular events

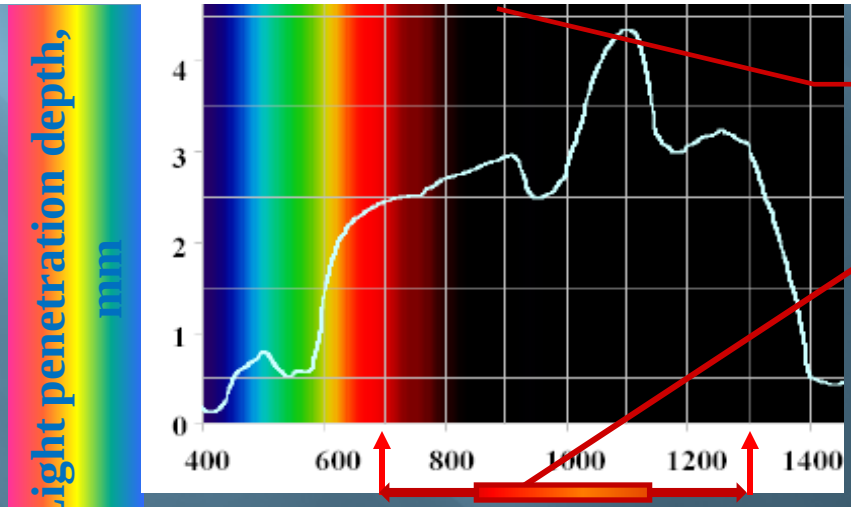
Molecular biology	Cell biology	Histology	Physiology	Medicine
DNA	Proteins	Cells	Tissue	Animal	Human
Genomics	Proteomics	Cytomics	Phenomic	Clinic



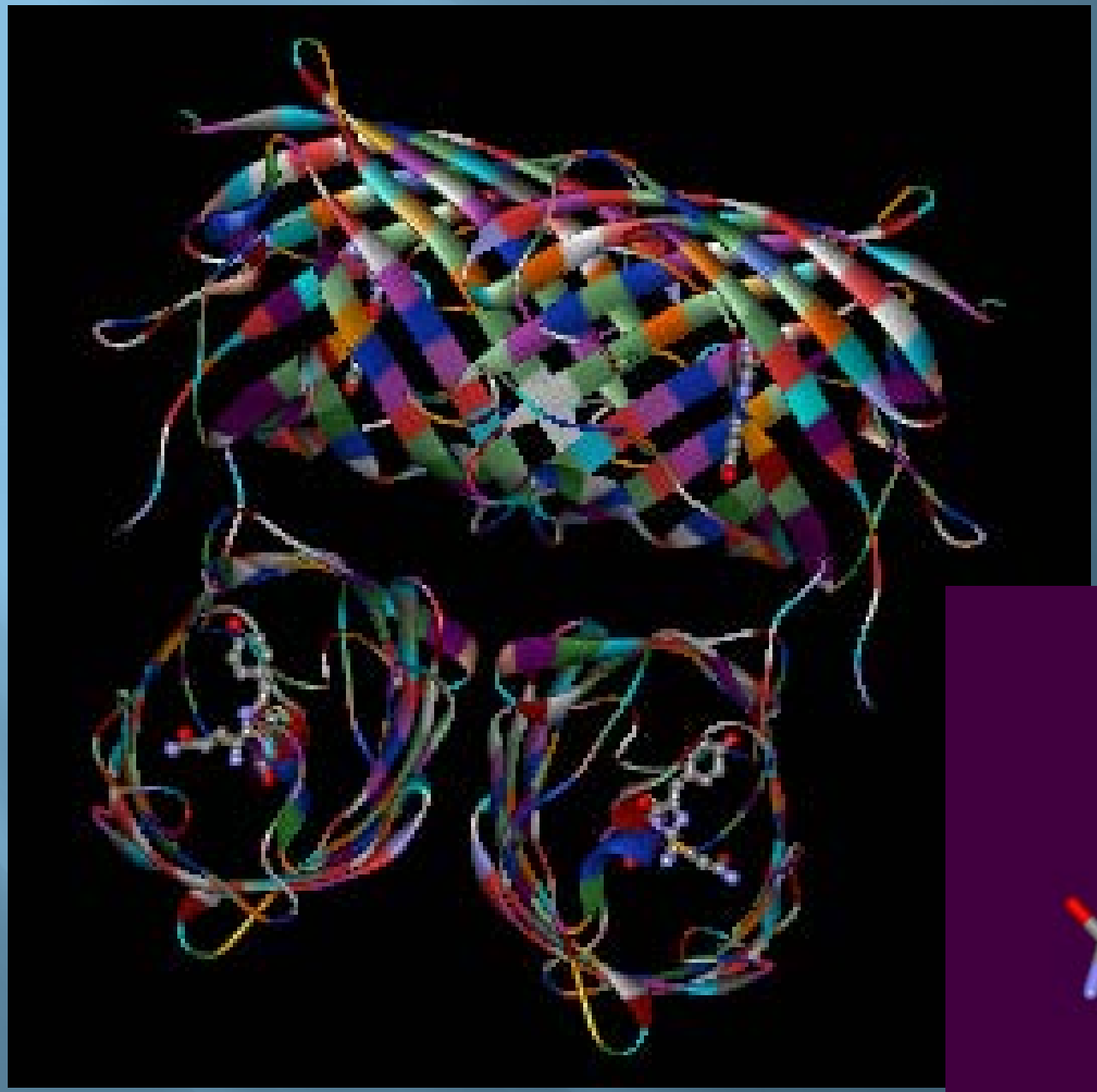
10^{-3} - 10^{-5} M

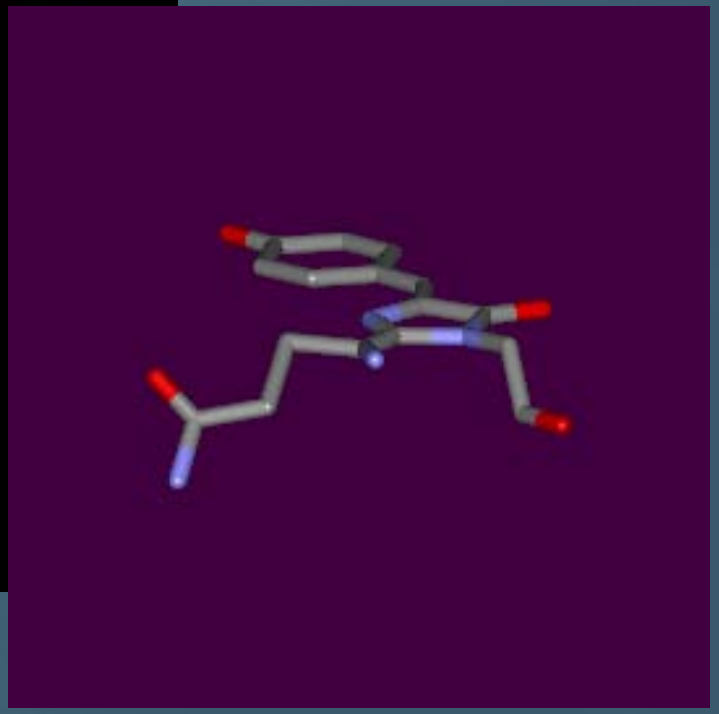
10^{-9} - 10^{-12} M





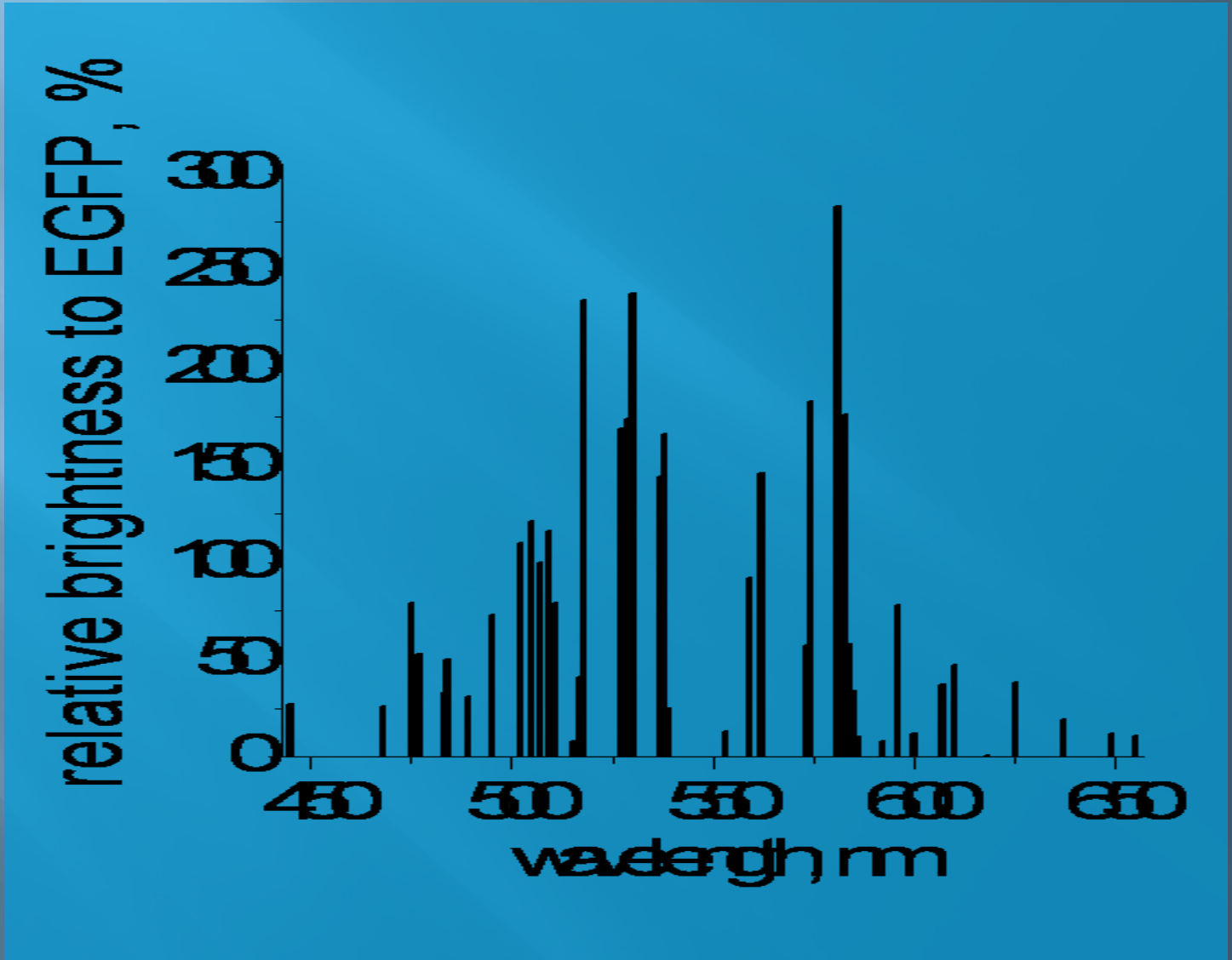
Therapeutic windows



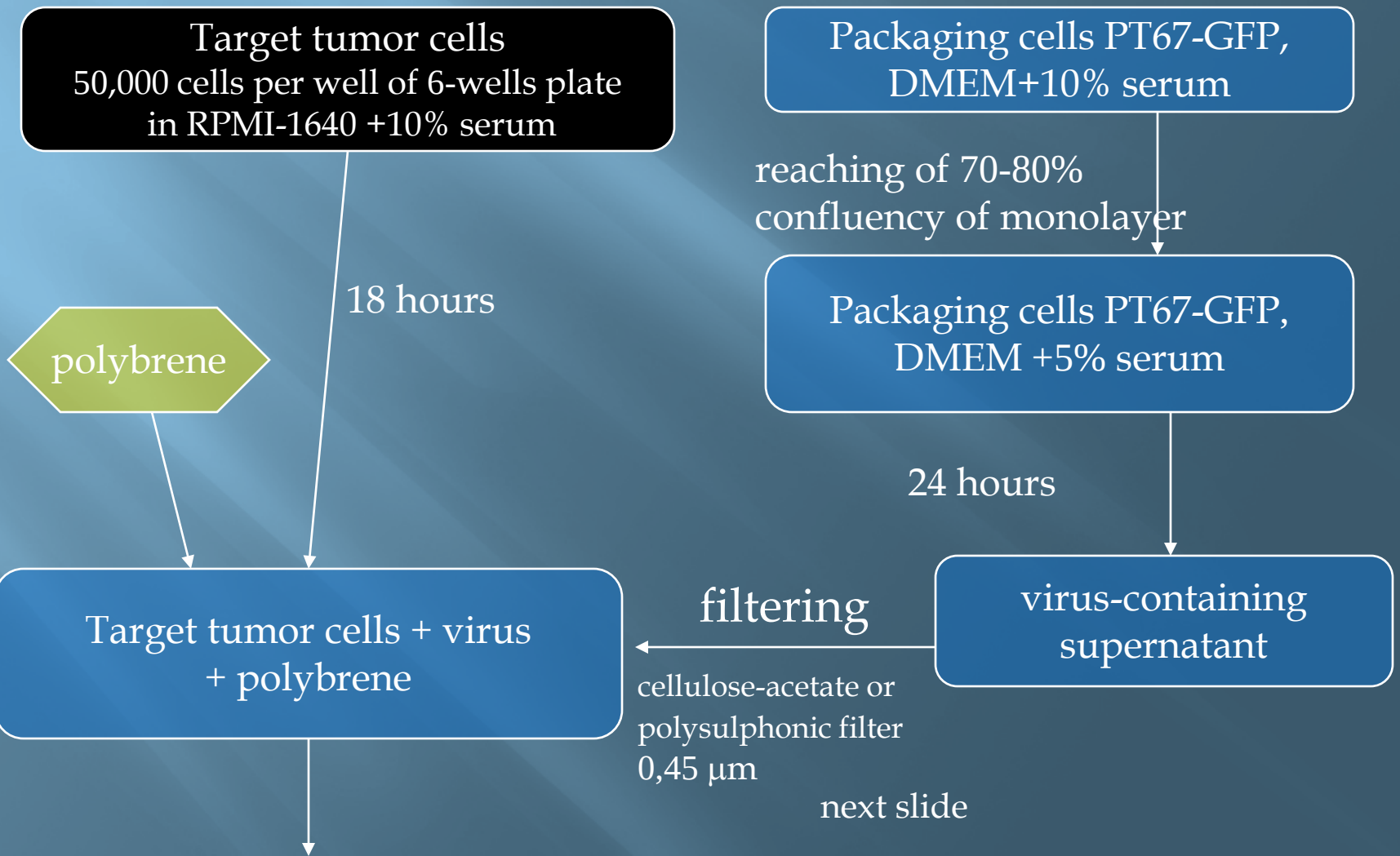


Spectral diversity and brightness of fluorescent proteins

brightness=$q \times \varepsilon$



Developing of FP–expressing tumor cell lines



24 hours

Changing of medium to RPMI-1640 + 10% serum

24 hours

Transfected target cells

selection

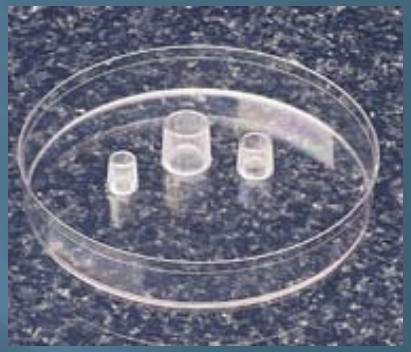
cloning

Individual clone of target cells expressing GFP

method of limited dilution

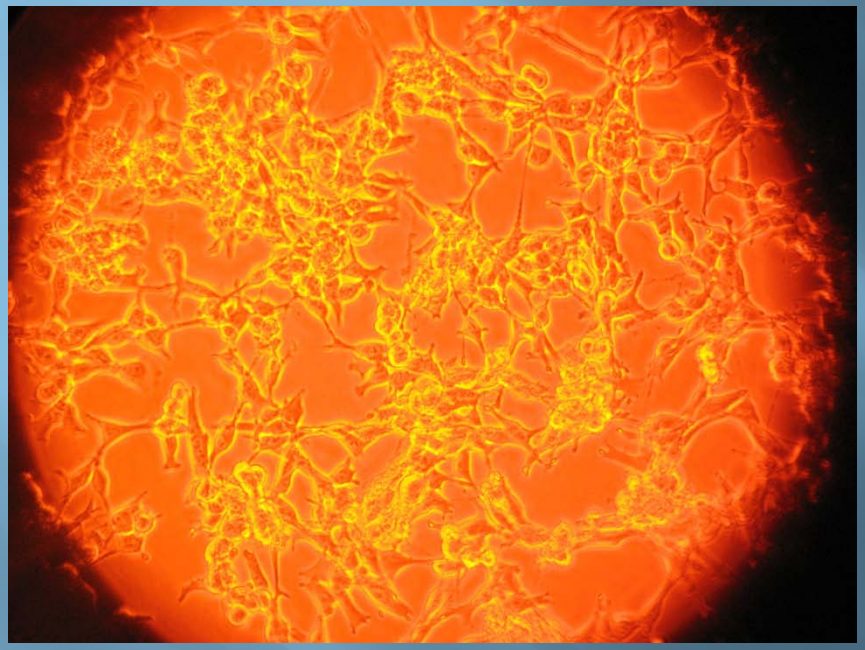
using of cloning cylinders

Individual clone of target cells expressing GFP

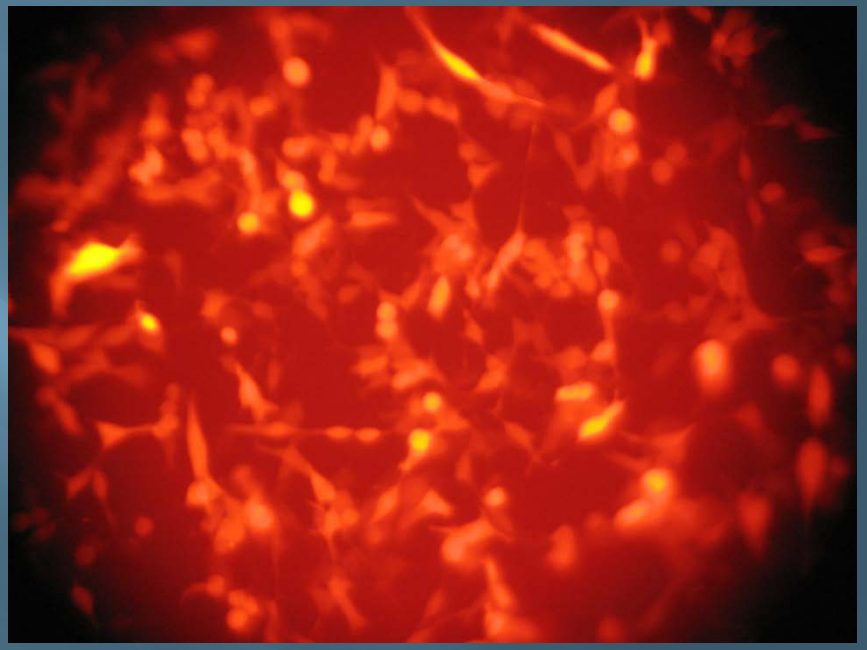


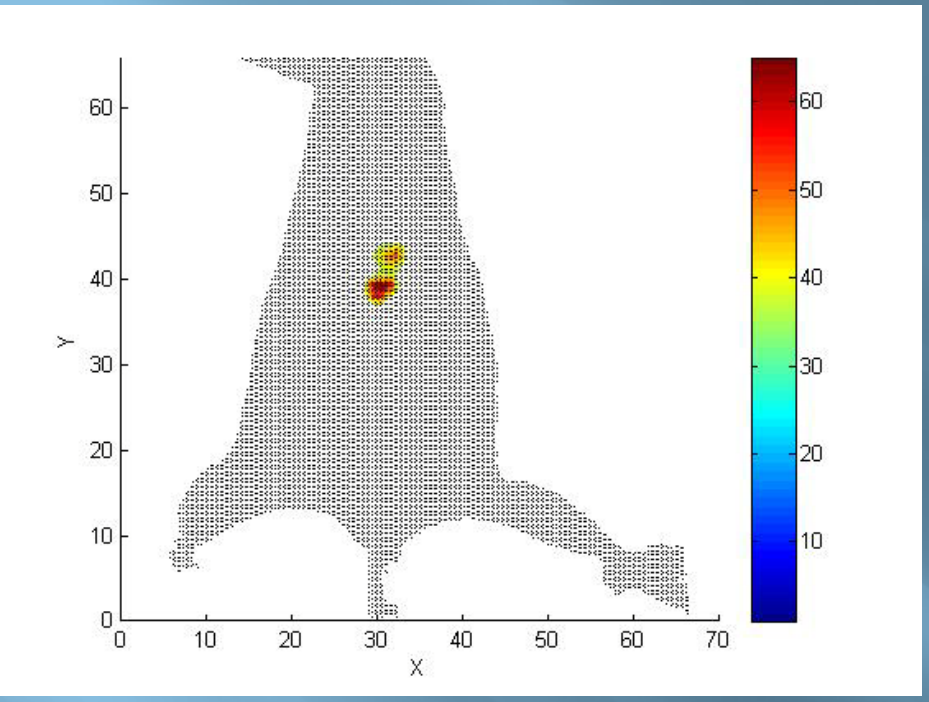
Mel-Kor-Turbo-RFP

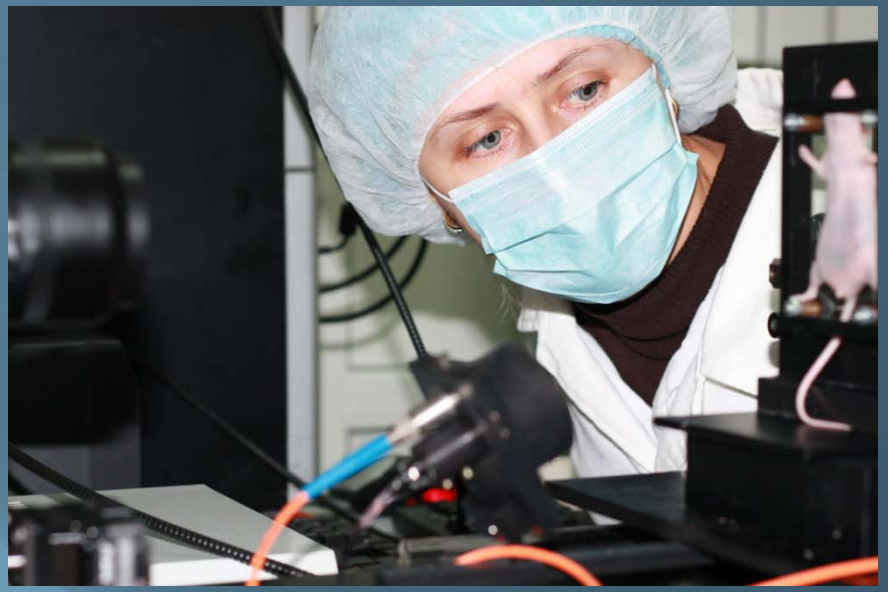
Phase contrast

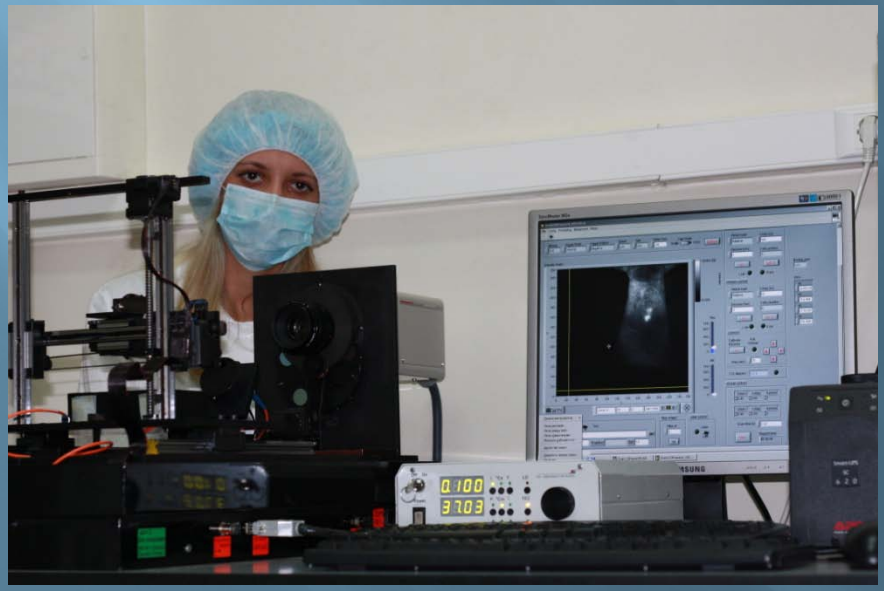


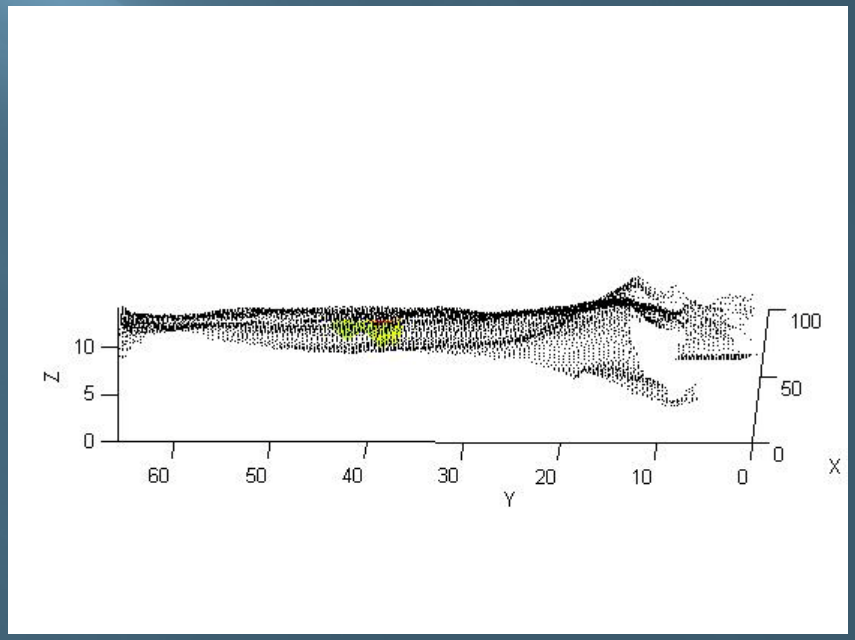
Fluorescence




Y
X
0
10
20
30
40
50
60
70






Z
Y
X
0
5
10
60
50
40
30
20
10
0
100
50
0

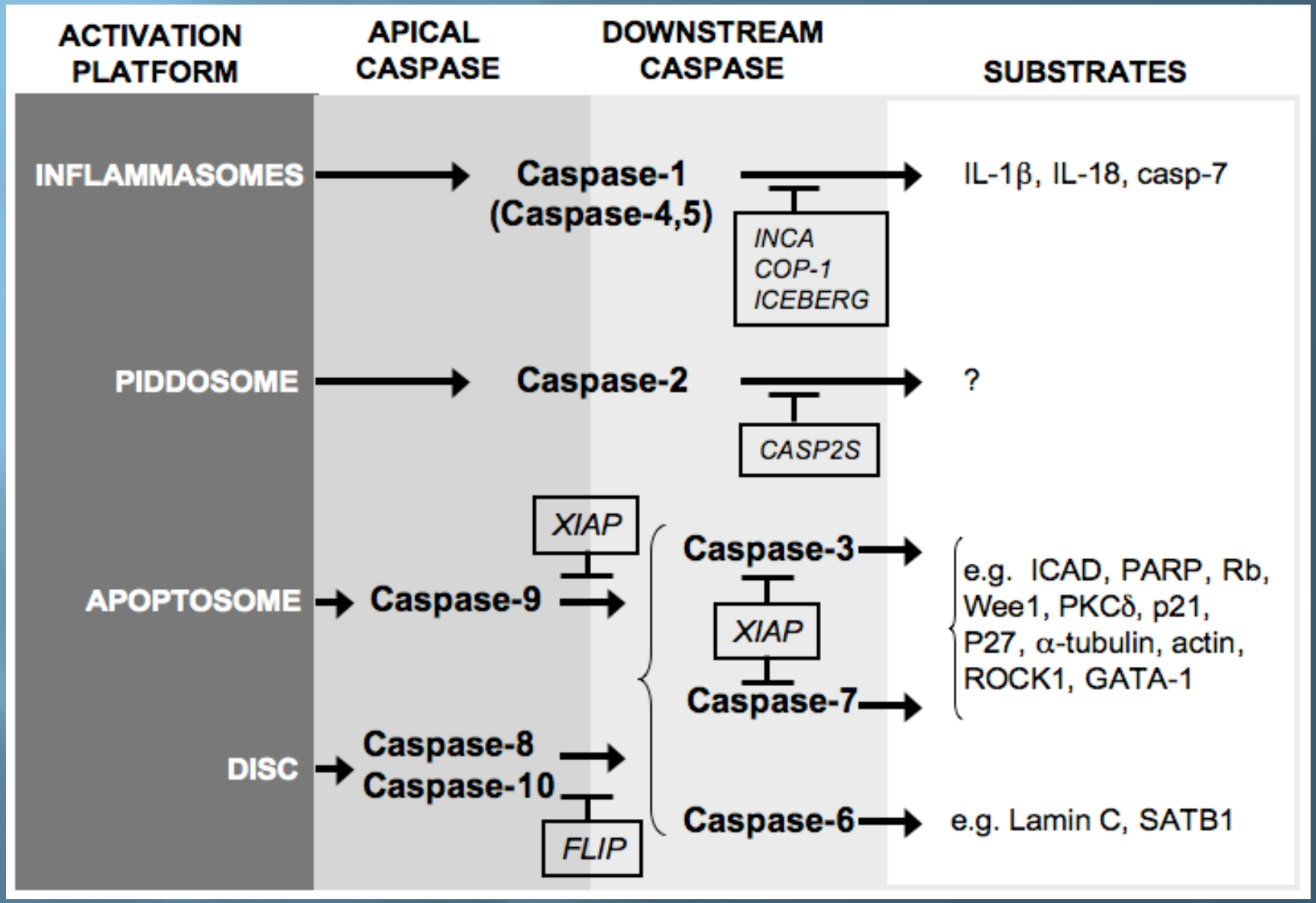
Molecular imaging in living subjects

Visual representation, characterization, and quantification of biological processes at the cellular and subcellular levels within intact living organisms.

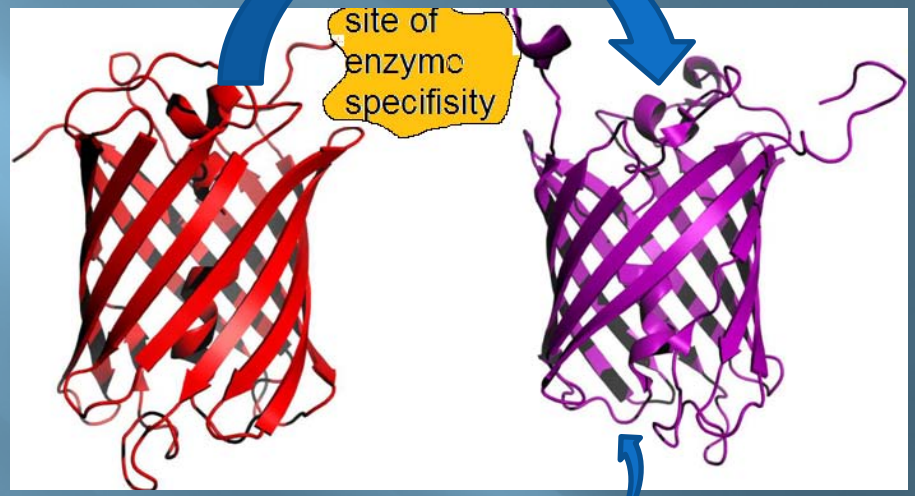
Each disease has molecular origin

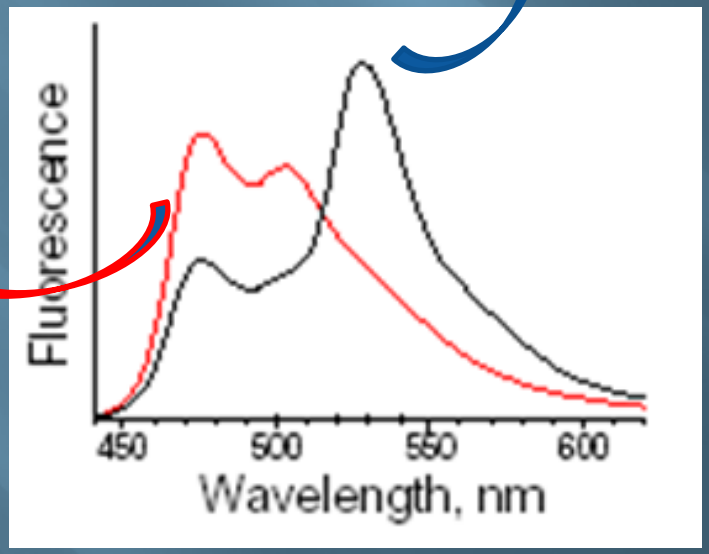
Diagnostic

New therapeutic methods targeted to molecular processes.


ACTIVATION PLATFORM
APICAL CASPASE
DOWNSTREAM CASPASE
SUBSTRATES
INFLAMMASOMES
Caspase-1
(Caspase-4,5)
INCA
COP-1
ICEBERG
IL-1β, IL-18, casp-7
PIDDOSOME
Caspase-2
CASP2S
?
XIAP
APOPTOSOME
Caspase-9
Caspase-3
XIAP
Caspase-7
e.g. ICAD, PARP, Rb, Wee1, PKCδ, p21, P27, α-tubulin, actin, ROCK1, GATA-1
DISC
Caspase-8
Caspase-10
FLIP
Caspase-6
e.g. Lamin C, SATB1

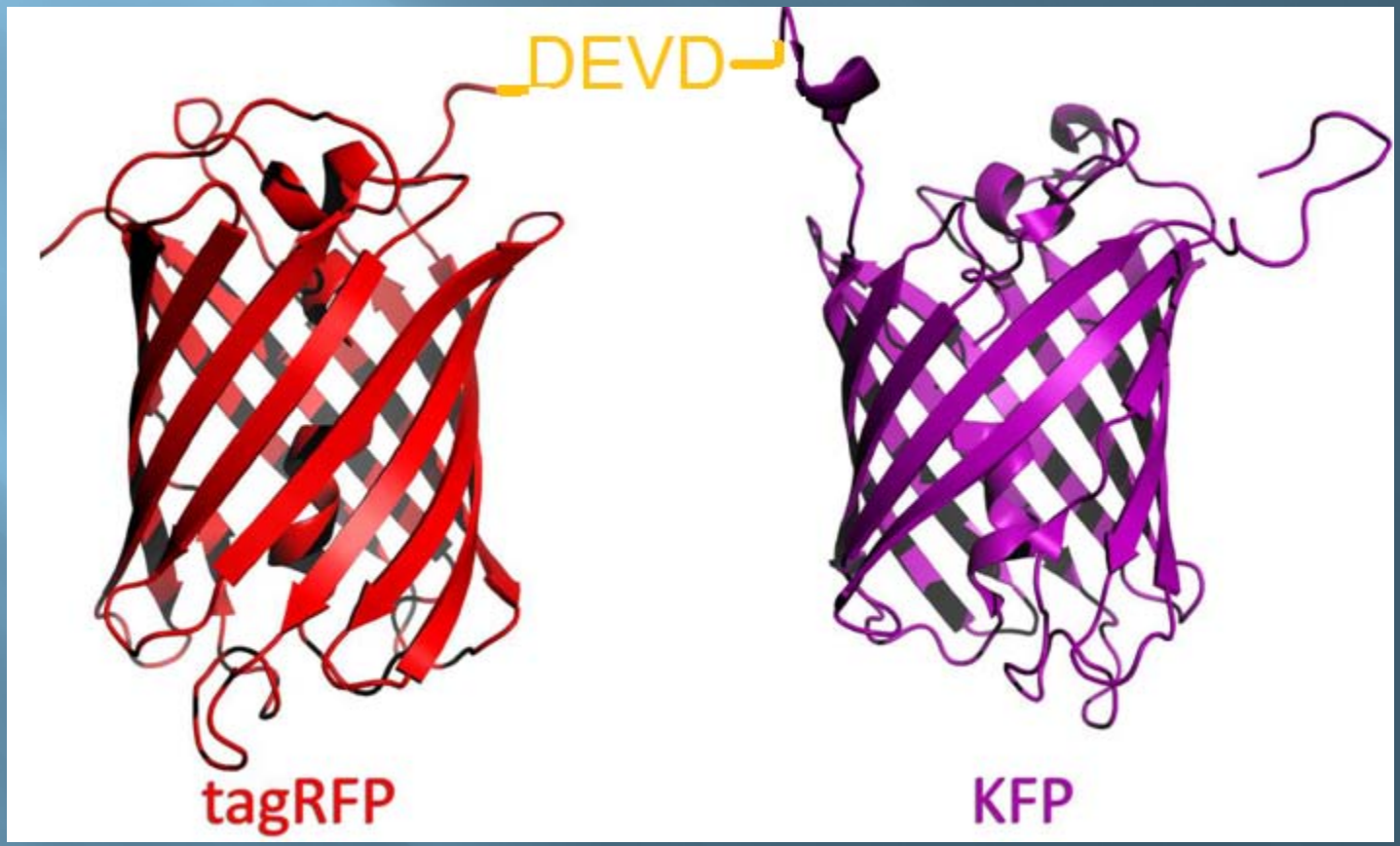
Fluorescence Resonance Energy Transfer (FRET)



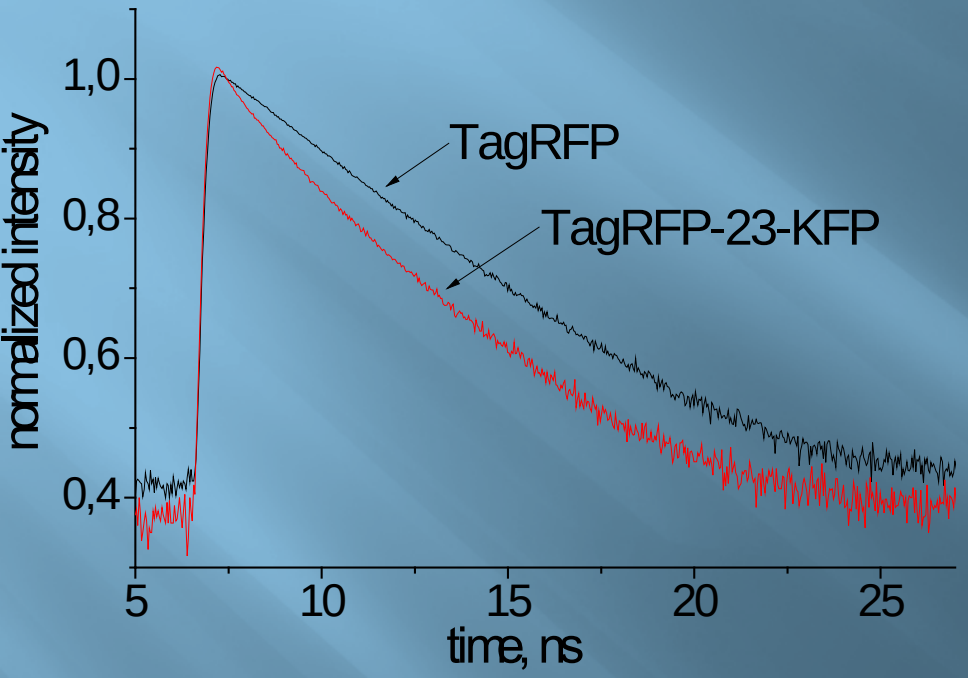


$R >> R_o$ No FRET


P CMV IE
TagRFP
GTGGSGGDEVDGTGGSGDPPVAT
KFP


DEVD
tagRFP
KFP

Time-resolved fluorescence decays of individual TagRFP and TagRFP-23-KFP



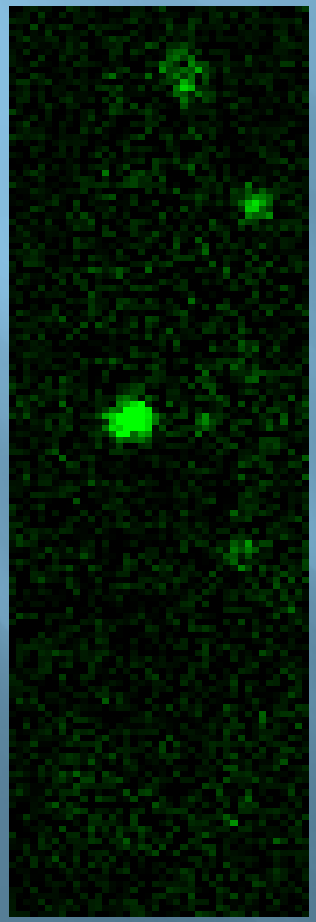
$$I(t) = \sum_{i=1}^{n} A_i \cdot e^{-t/\tau_i}$$

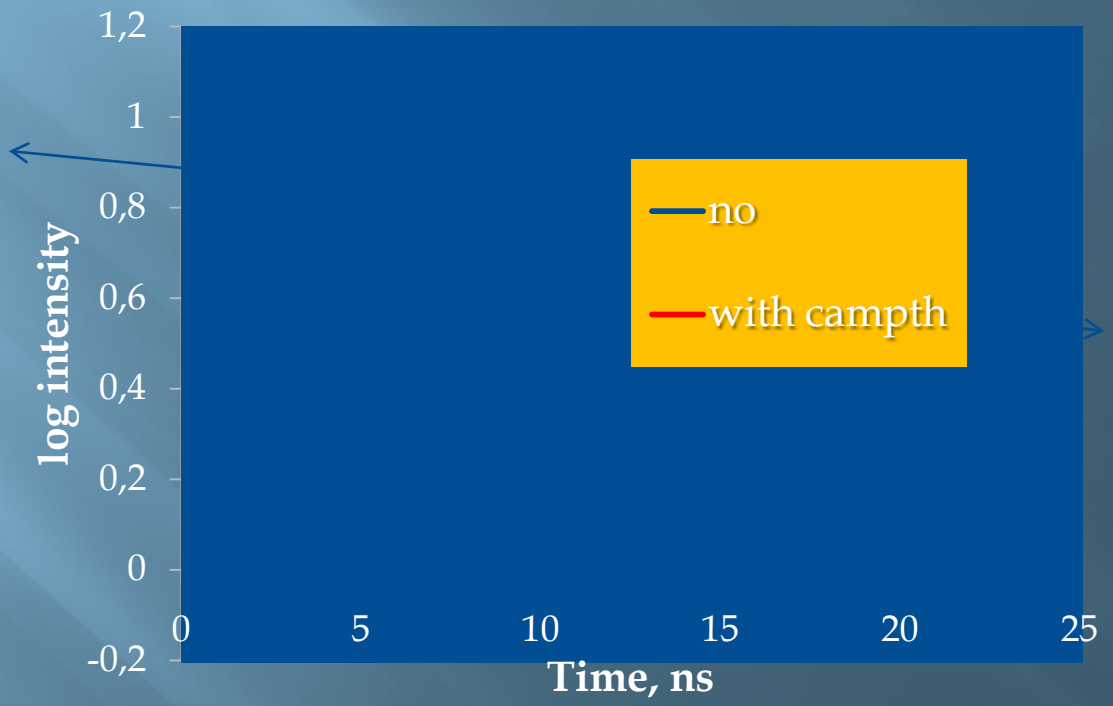
	τ_1 (No FRET)	τ_2 (FRET)
TagRFP	2.42	-
TagRFP-23-KFP	2.35	0.93

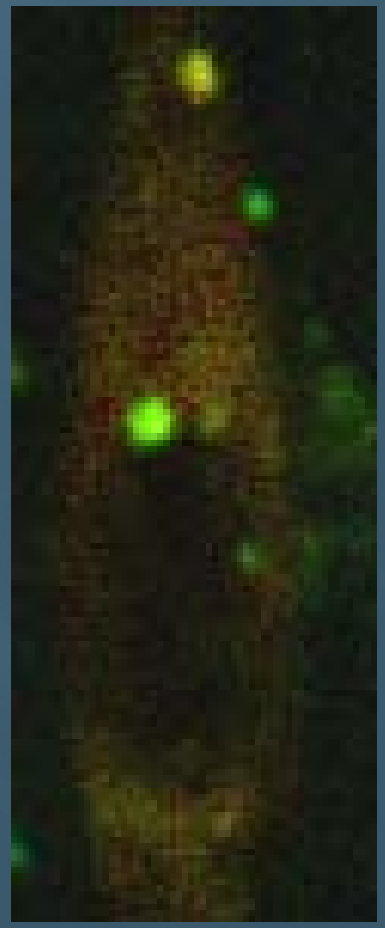
Campthotecin induced apoptosis

FLIM

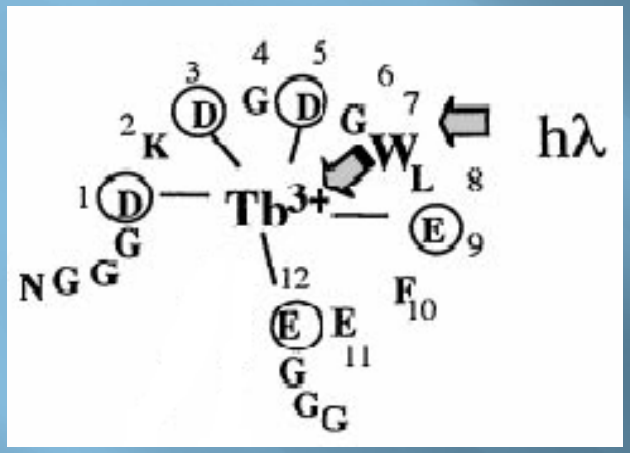
MicroTime 200, PicoQuant (Berlin)





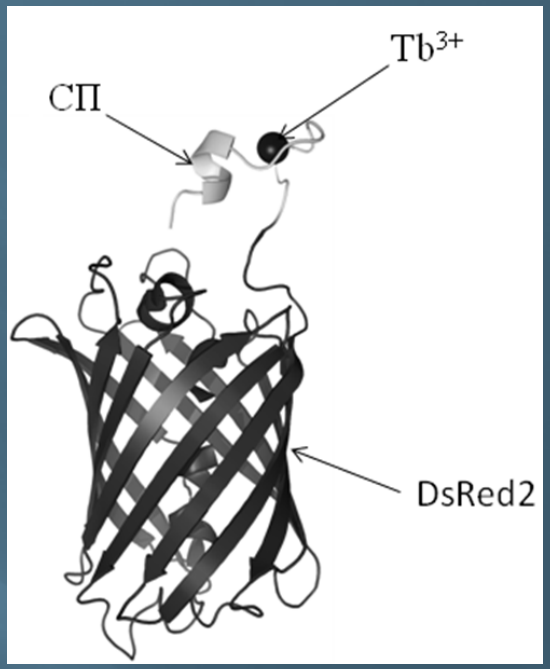


fluorescence resonance energy transfer for Tb – red fluorescent protein pair



FRET

DEVD



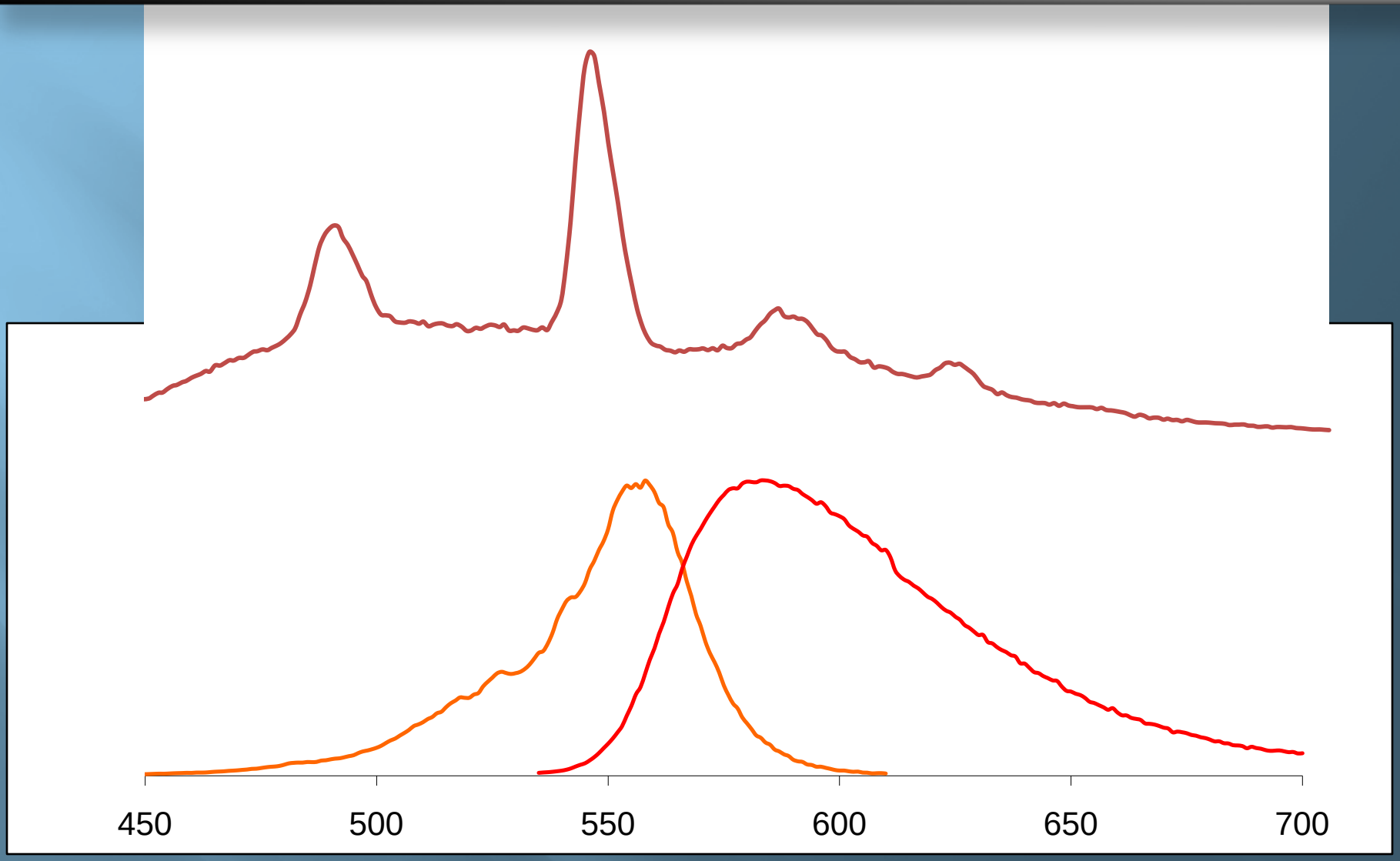
Donor –
Terbium chelate

Excitation max 280 nm
Emission max 545 nm

Acceptor – DsRed2 or
TagRFP

Excitation max 562 nm
Emission max 586 nm

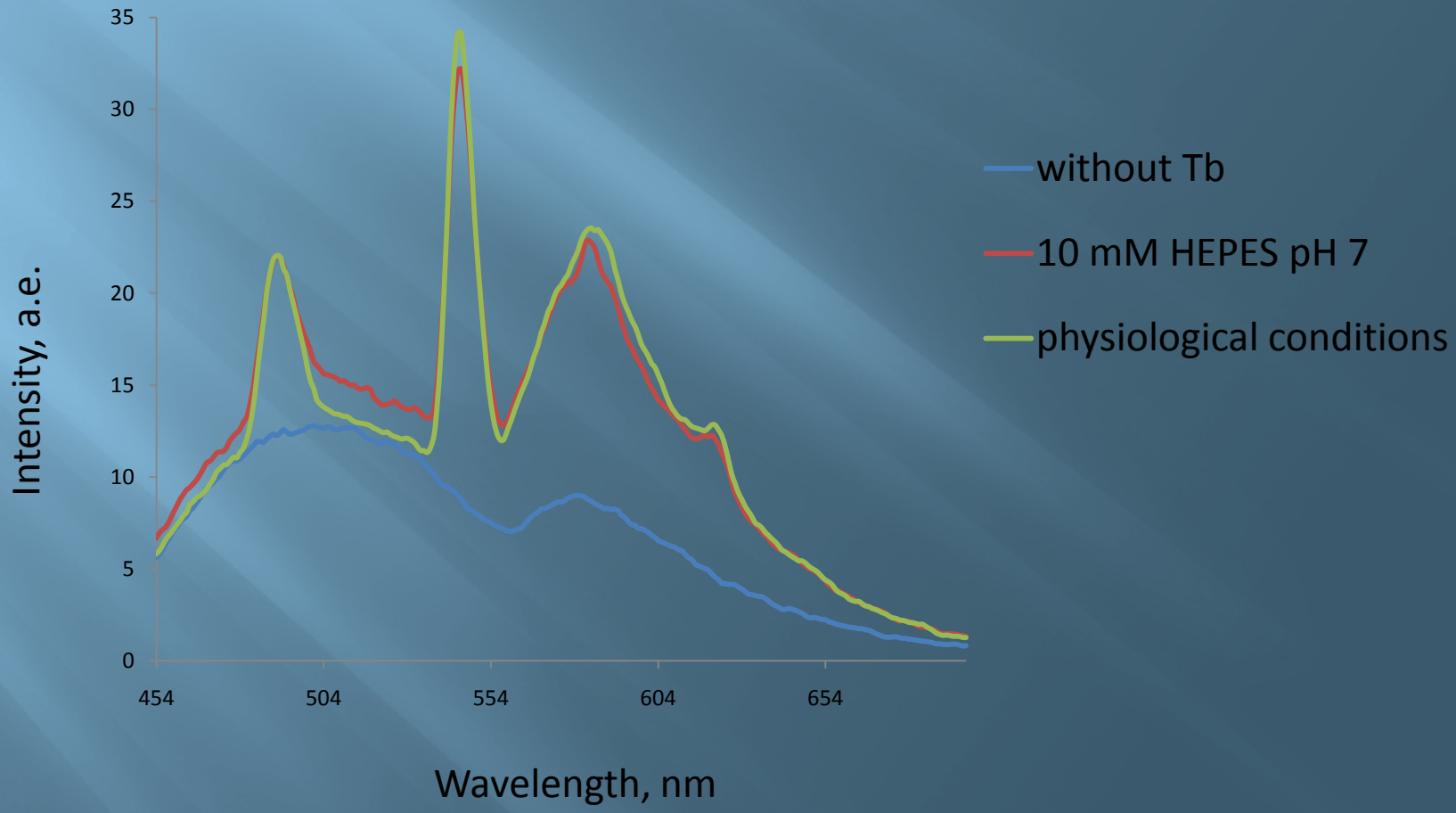
Overlapping of emission spectrum of terbium and excitation spectrum of DsRed2 or TagRFP



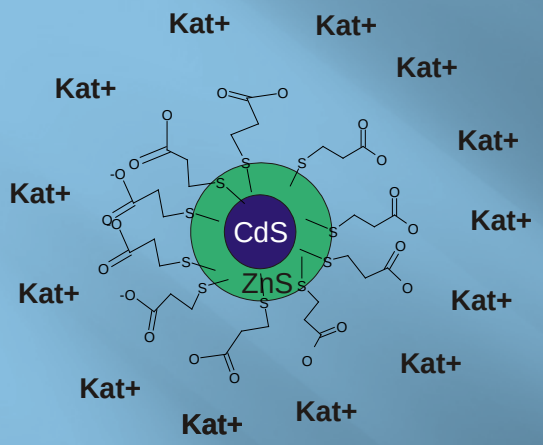
Emission of long-lived fluorescence of Tb-DEVD-TagRFP

concentration of protein - 10 mkM

concentration of Tb - 10 mkM

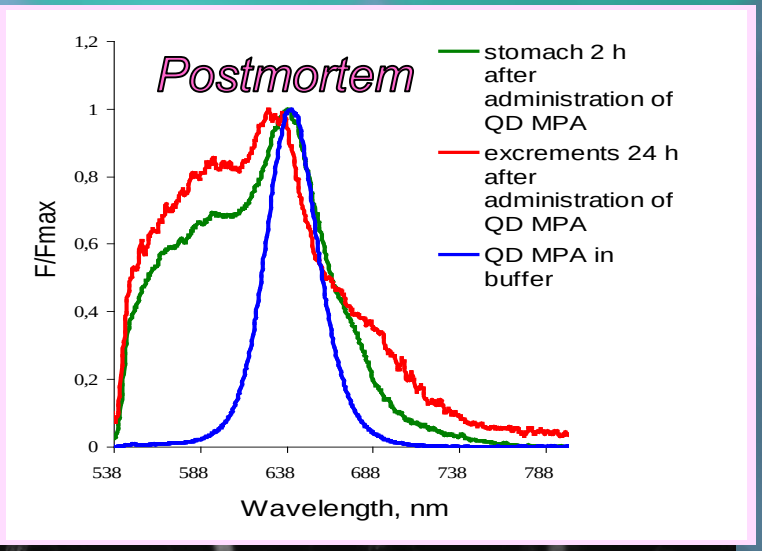


IN VIVO DETECTION OF QUANTUM DOTS IN *NUDE* MICE.



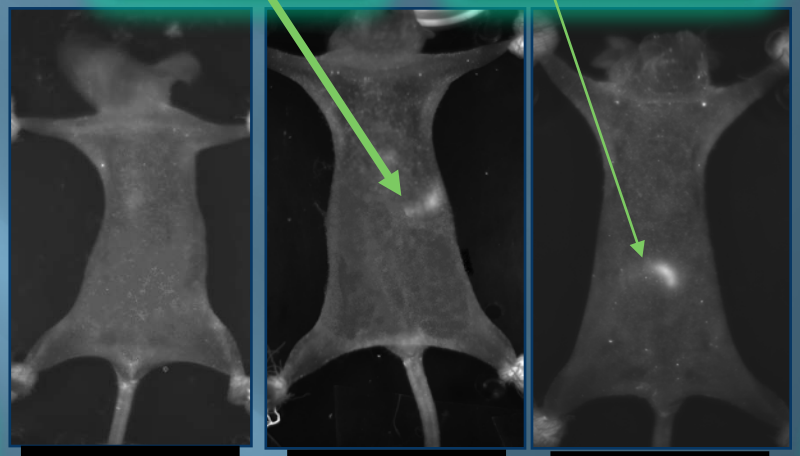
0 time

3 weeks



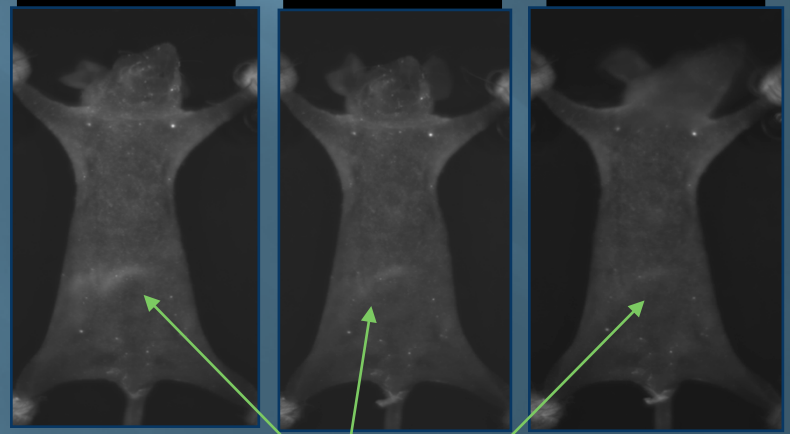
Stomach

Duodenum



Control | 10 min | 20 min

Time after administration



30 min | 1 h | 2 h

Intestine

Conclusion

Developed mouse model of the human fluorescent tumor (Mel-Kor)

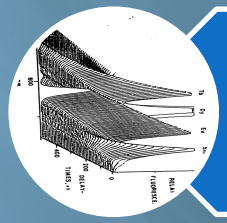
Life-time based FRET substrate for caspase 3 for FLIM

Lanthanide – red fluorescent protein FRET pare

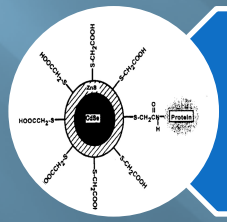
Nude mouse model for QD traffic and localization

Perspective

Fluorescence life-time tomography

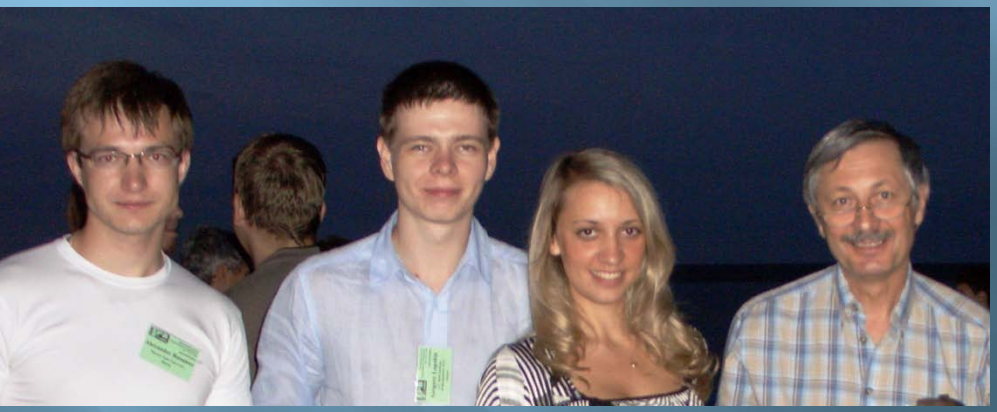


Lanthanide FRET substrate for multiphoton imaging



In vivo QD traffic and localization (skin, lung)

L.R. Arslanbaeva
T.V. Ivashina
V.V. Jerdeva
G. D. Lapshin
I.G. Meerovich
A.L. Rusanov



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