"Collaborative Research: In-vitro Diagnostics using a PBR-targeted Molecular Imaging Agent"

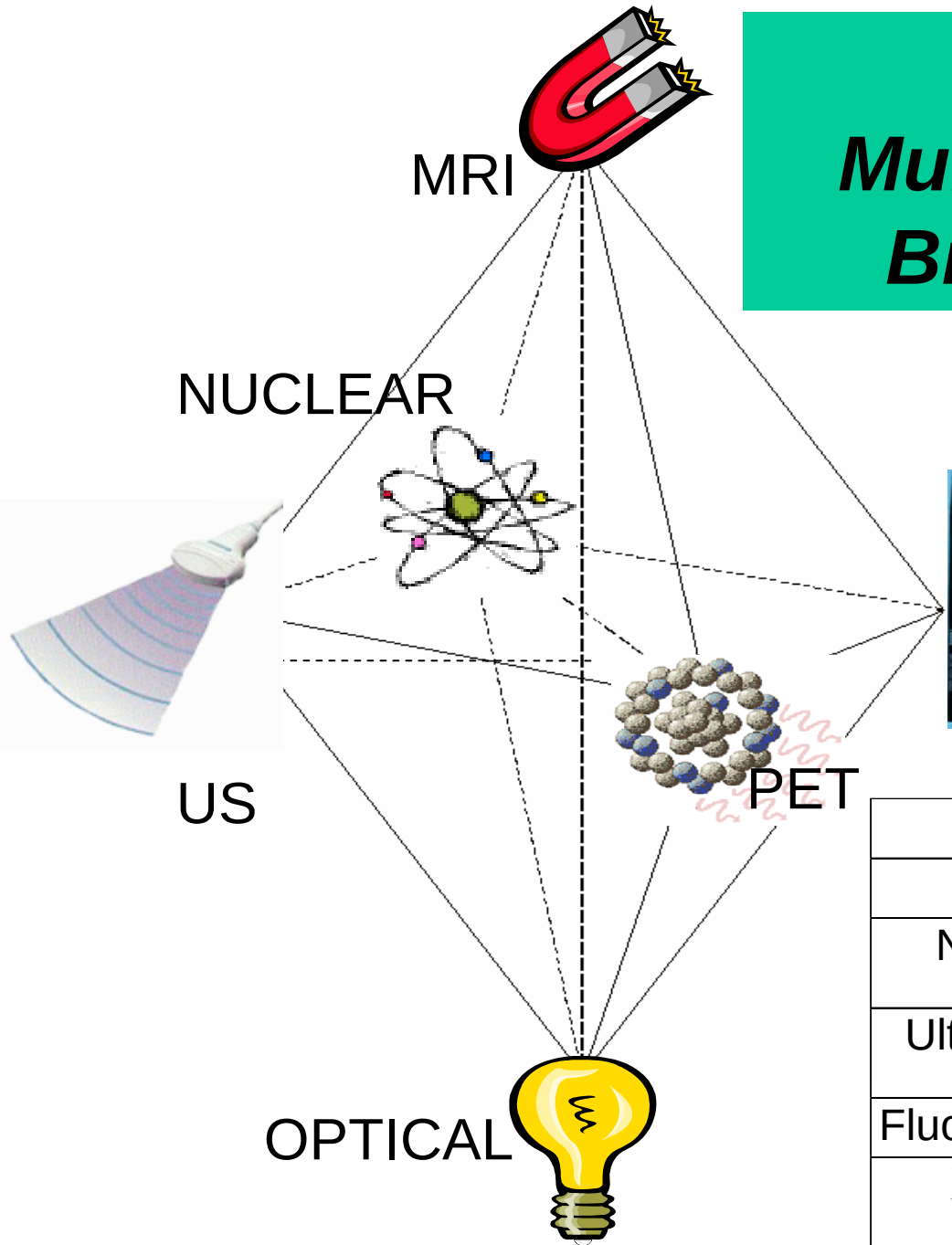
Professor Darryl J. Bornhop

Vanderbilt Department of Chemistry

darryl.bornhop@vanderbilt.edu

615-322-4226

Metal-Chelate Multidimensionality & Broad Applicability

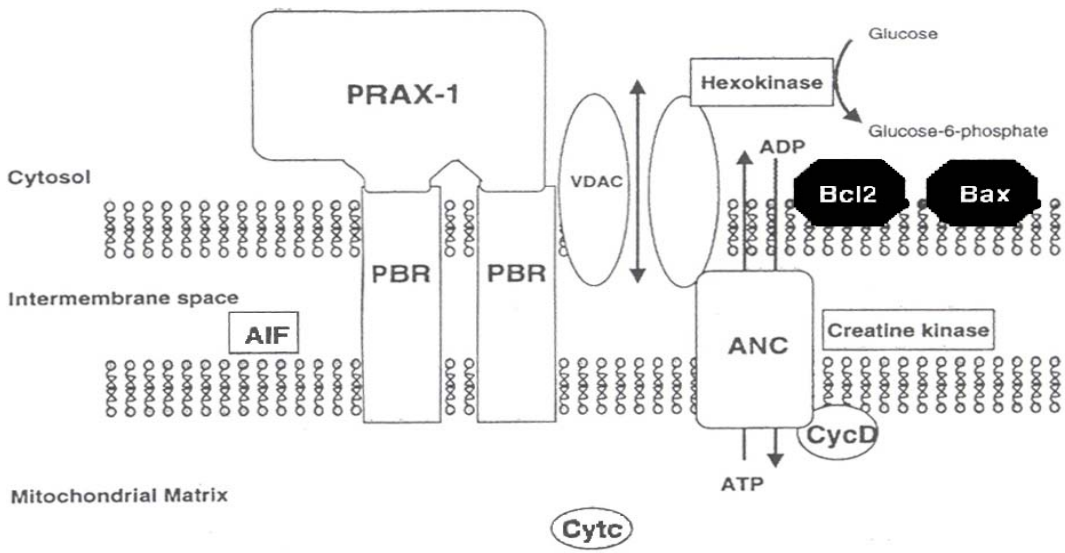


- ***Chemical behavior*** **mostly independent of lanthanide ion**
- ***Facile structural modifications***

Modality	Agent
MR (D)	Chelate
Nuclear (D,T)	Chelate, Metal Ion, Labeled Organics
Ultrasound (D,T)	Macro-organics, Complexes, Nanoparticles
Fluorescence (D,T)	Organics, Chelates
X-Ray (D,T)	Halogentated /iodated organics, Chelates
PET (D)	Special organics, Chelates

The Peripheral Benzodiazepine Receptor (PBR)

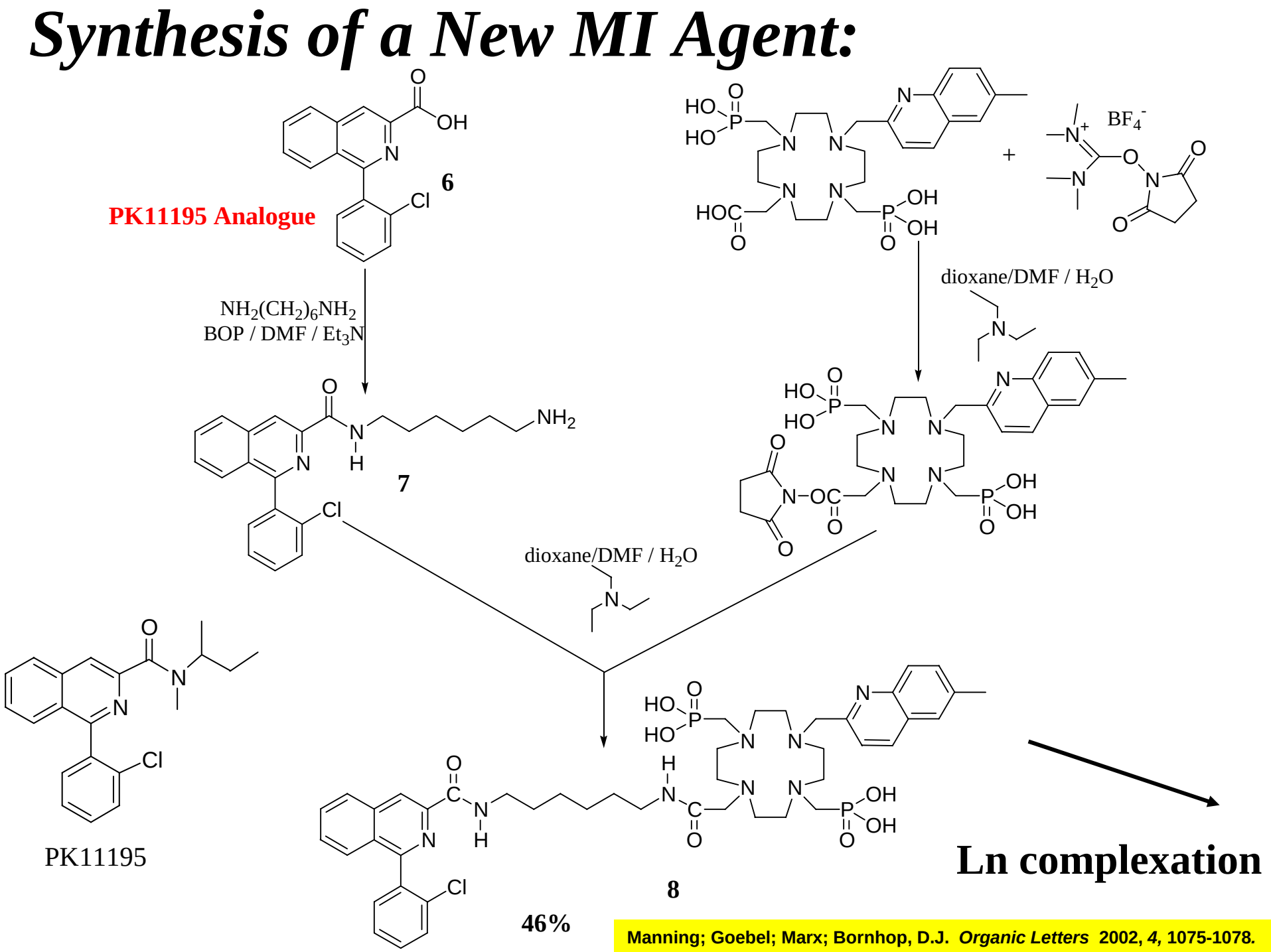
- PBR:18 kDa transmembrane protein whose function still somewhat of an “enigma”
  - Regulation of steroidogenesis
  - Functional role in ***apoptosis***
  - *Cytoprotective properties vs. Reactive Oxygen Species (ROS)*
  - Regulation of transmembrane potential: Mitochondrial Permeability Transition Pore (MPTP)
- ***Pathology***
  - Density correlates with metabolic status of cells
  - ***High expression levels in tumor cells***
  - ***Glioblasoma Detection***
  - Immunomodulatory roles: inflammatory response
  - Neurodegenerative disorders



PBR Ligands Are Known

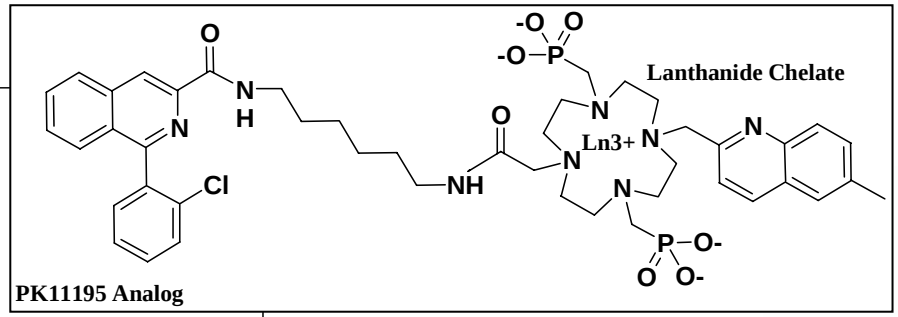
- Isoquinoline carboxamide (PK11195)
  - Binds with high affinity (3-8 pg/mg protein) to PBR in diseased cells
- [^{11}C]-PK11195 PET used clinically
  - Multiple sclerosis Alzheimer’s and Rasmussen’s encephalitis
- [^{1}H]-PK11195 for Human Gioblastoma Imaging*
- Controlled animal and clinical trials needed

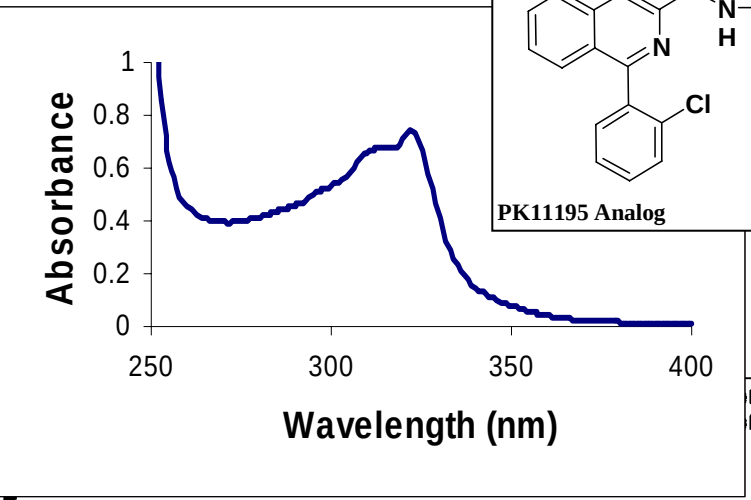
Synthesis of a New MI Agent:



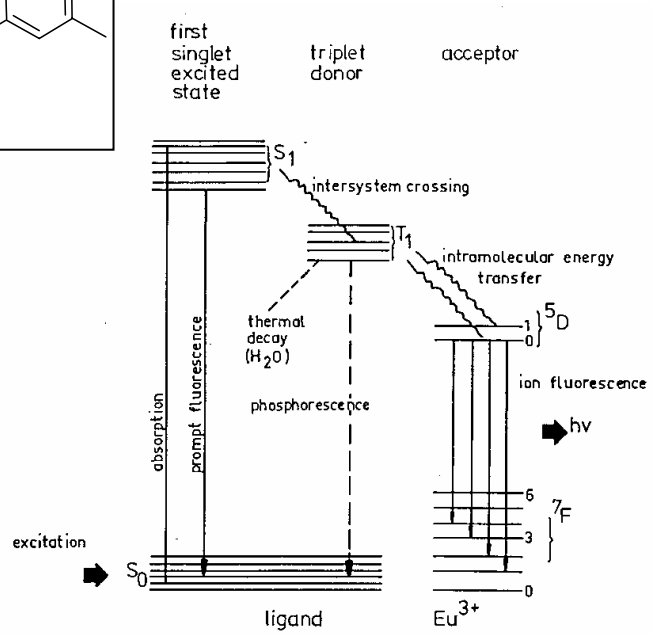
Manning; Goebel; Marx; Bornhop, D.J. *Organic Letters* 2002, *4,* 1075-1078.

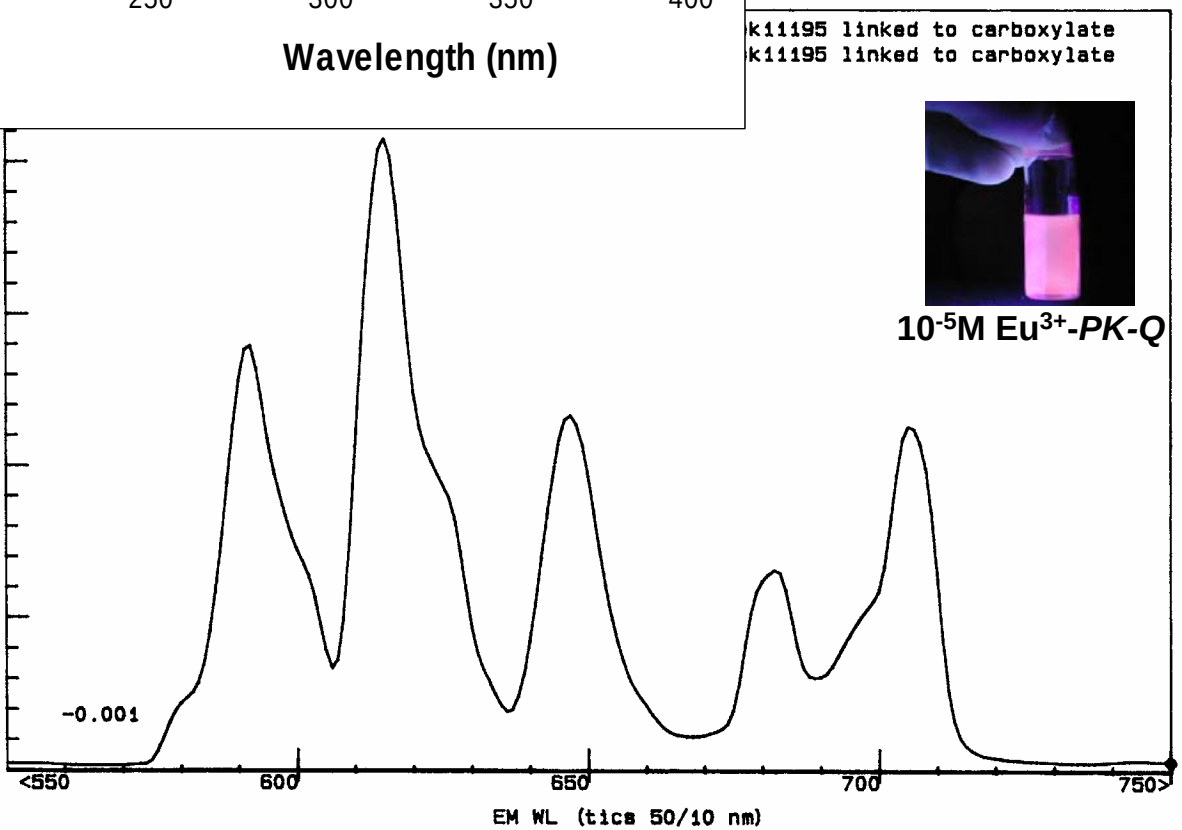
Spectral Properties of Eu-PK11195





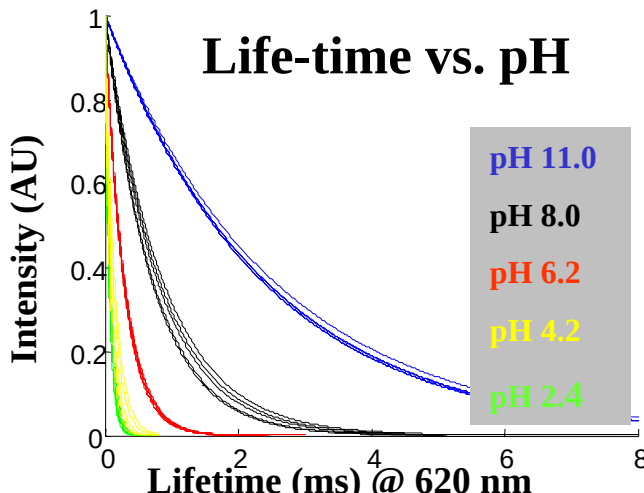
Eu-chelate Jablonski Diagram



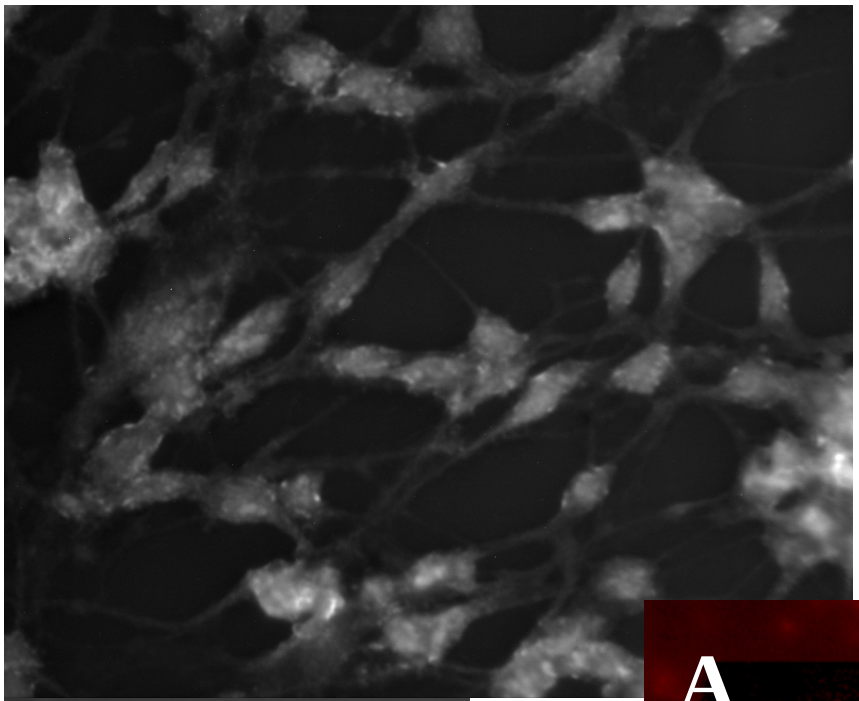


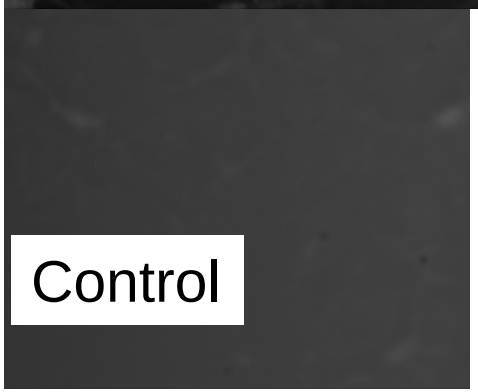
10^{-5}M Eu^{3+}-*PK-Q*

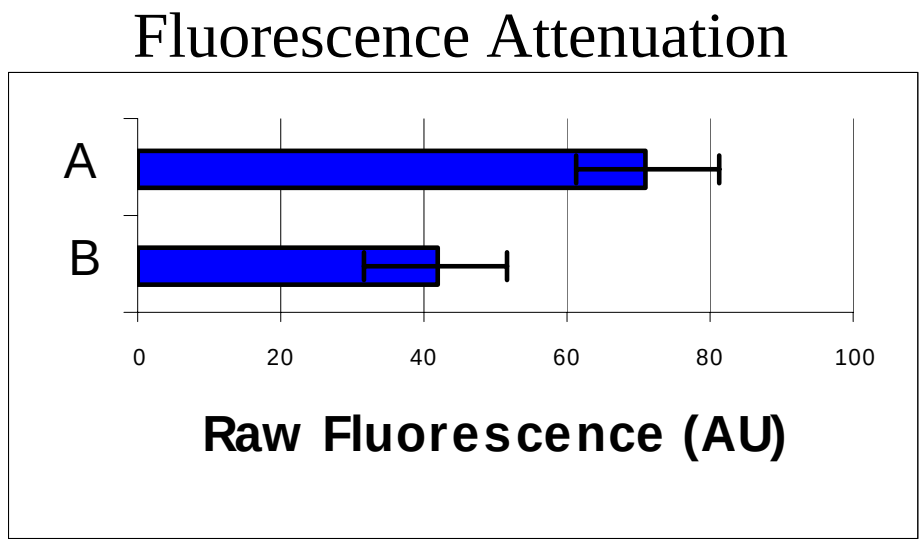
Life-time vs. pH

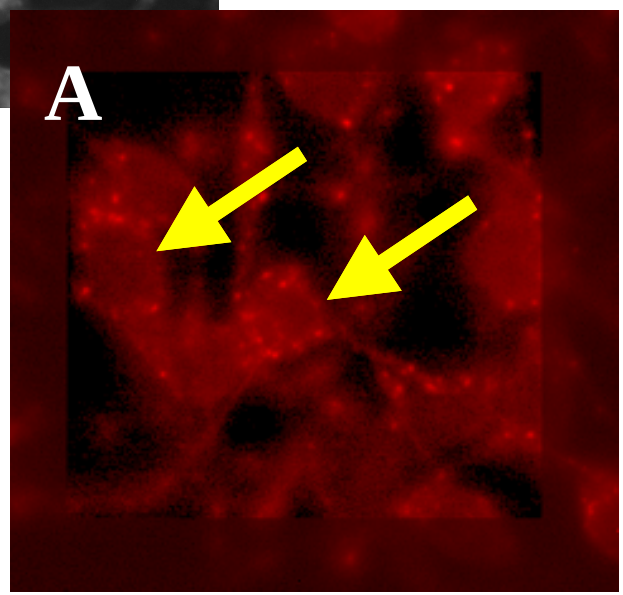


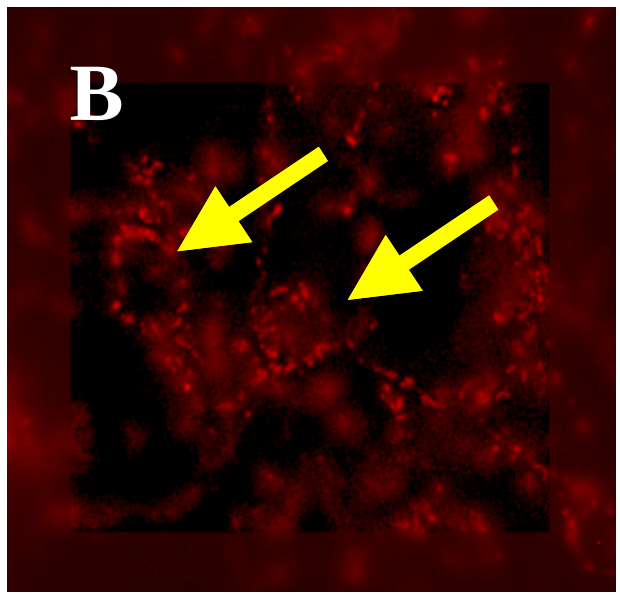
Uptake, Displacement & Co-registration Studies on Eu-PK and C-6 Glial Cells











A: JC1 **B: Eu-PK11195**

Examination of Human Glioblastoma *ex-vivo* with Fluorescence Microscopy

Procedure:

•Human brain tumor specimens were obtained during surgical resection.

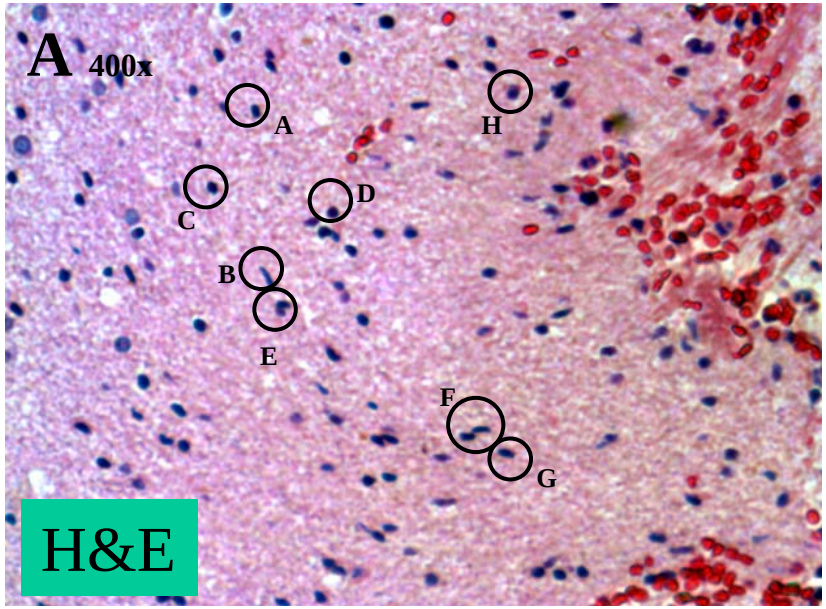
•Specimens were processed for fluorescence staining by de-paraffinization.

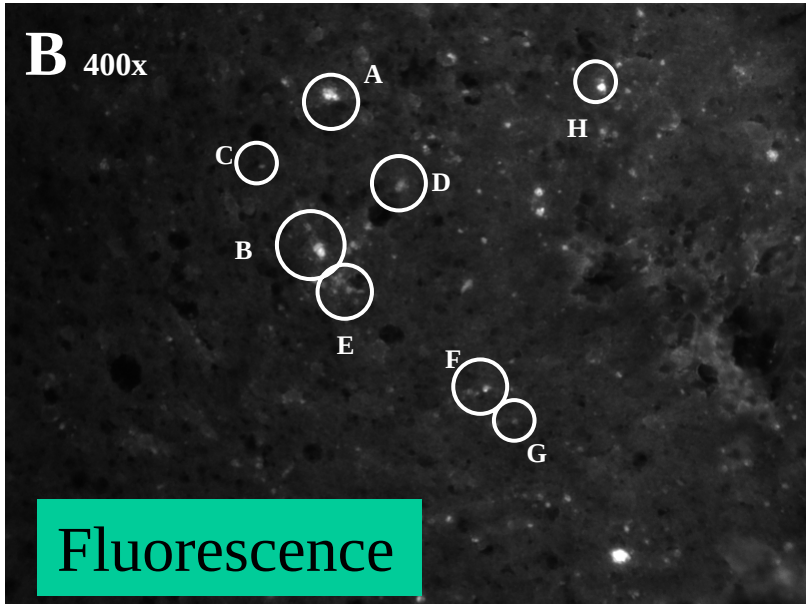
•Specimens were dosed with 2mM Eu-PK11195 in 50% water/50% DMSO, incubated for 15 min. and rinsed with de-ionized water.

•Specimens were examined with fluorescence microscopy.

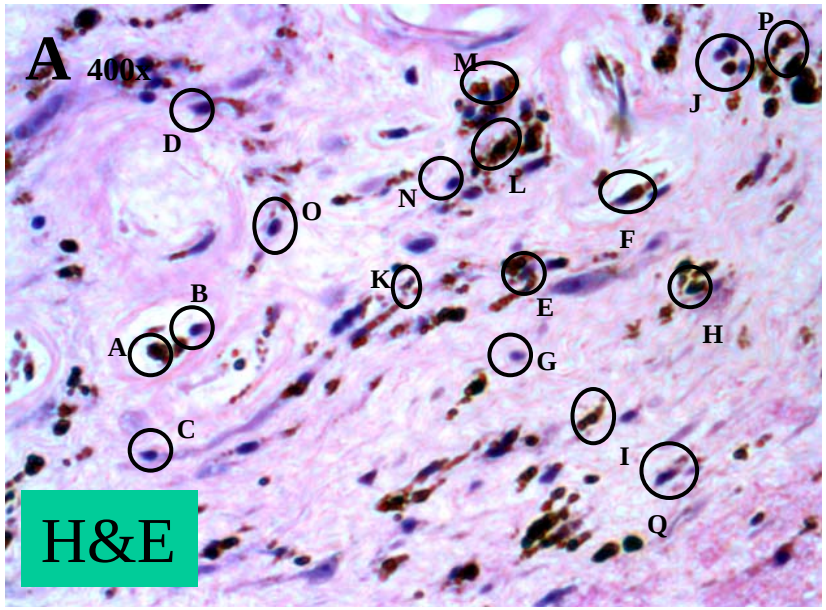
•Cellular fluorescence was correlated with H&E cell detection.

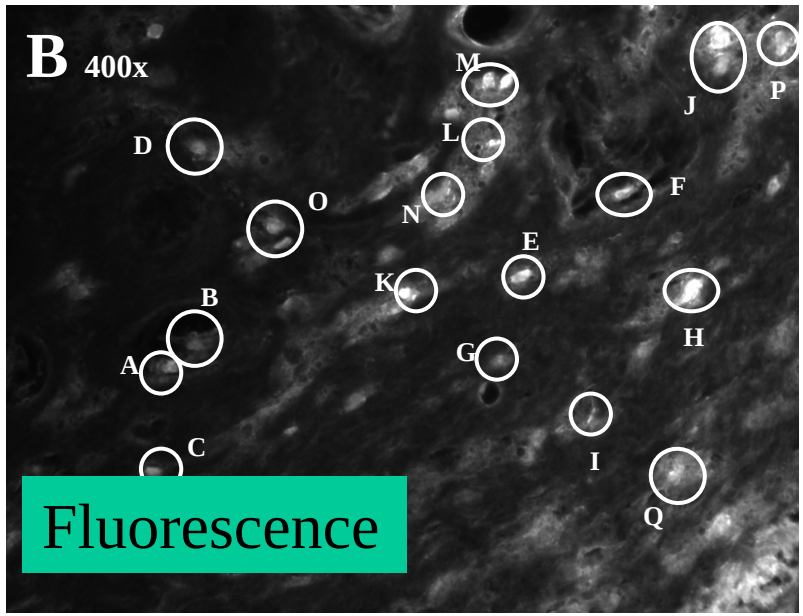
WHO grade II Oligodendriglioma



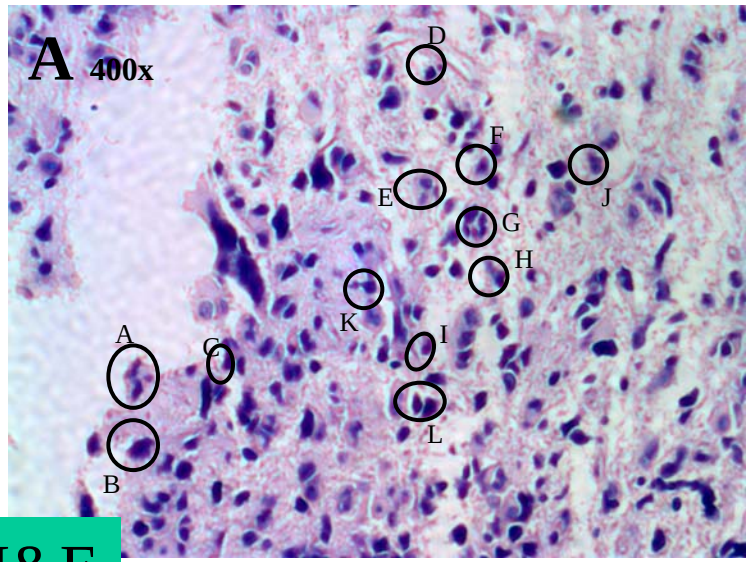


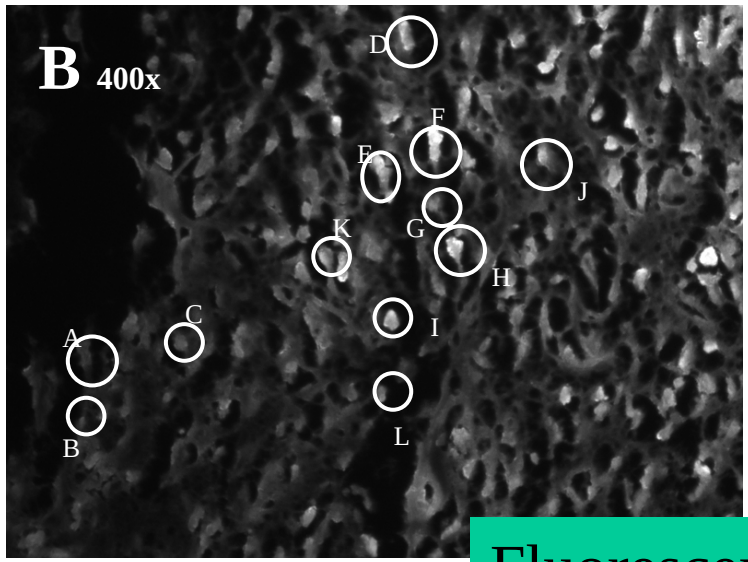
WHO grade II Oligodendriglioma Post Radiation





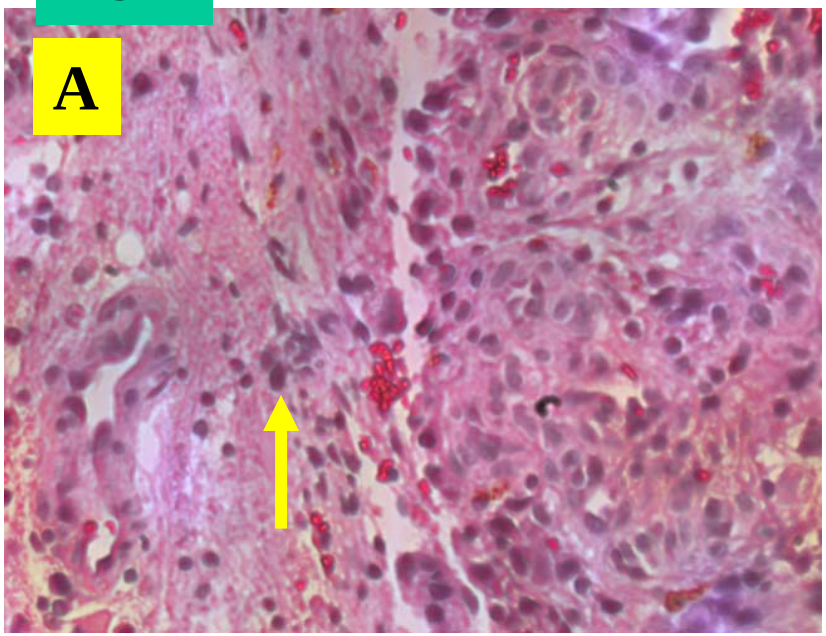
WHO grade III Anaplastic Astrocytoma



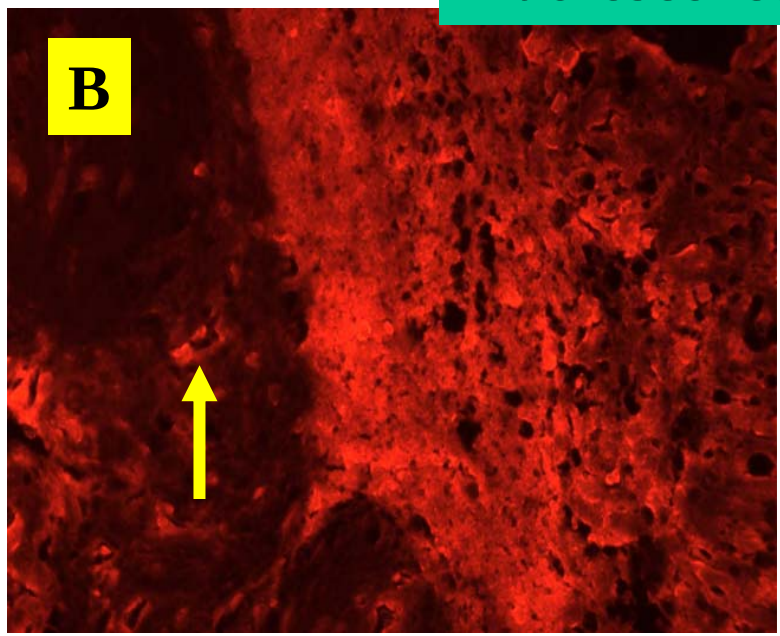


H&E

Fluorescence



HGB

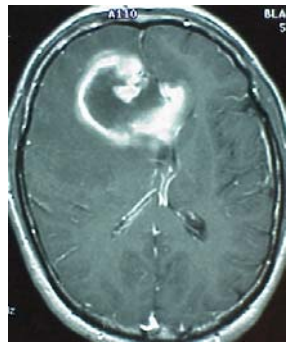


- Ex-vivo staining with Eu-PK11195 fluorescence may prove useful in defining infiltrating tumor margins in gliomas of low cellularity.

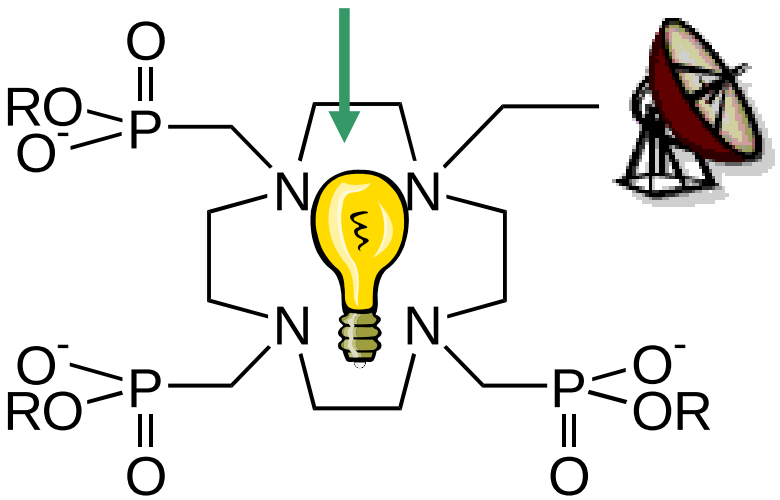
Multi-modal - IMAGING AGENTS

- The Cocktail Approach (*Same Ligand, Different Metal*)
  - Metal ion dictates signature and dose
  - ***Chelating agent*** or ***conjugate*** drives ***delivery***

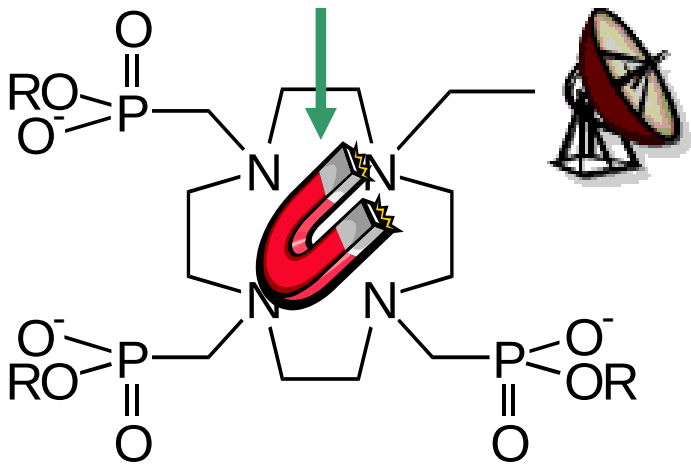
MRI Contrast of Gd-PK11195-Q
r_1 ~ 5.94 $mM^{-1}s^{-1}$
@ 37C° 20 MHz



Tb^{3+}, Eu^{3+}



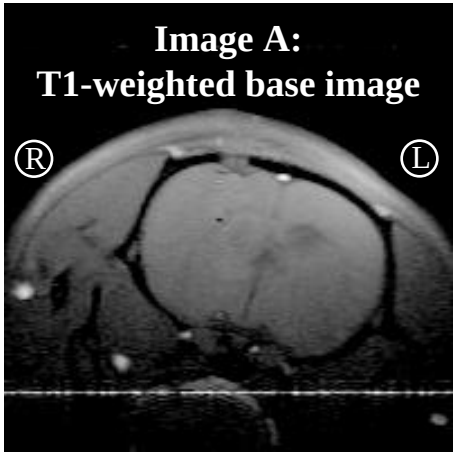
Gd^{3+}

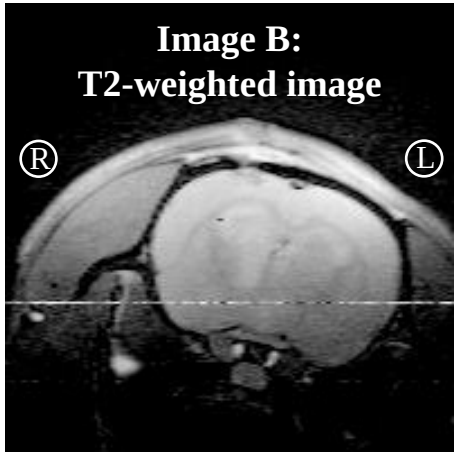


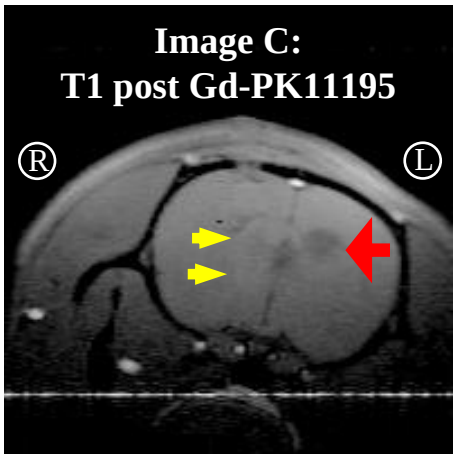
PET also be possible using Ga or Cu.

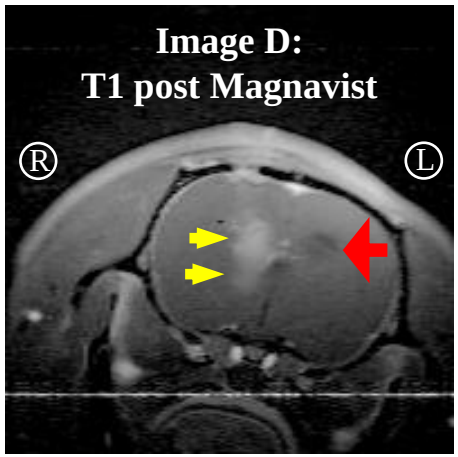
Wistar Rat *In Vivo* Glioblastoma Imaging Investigations I

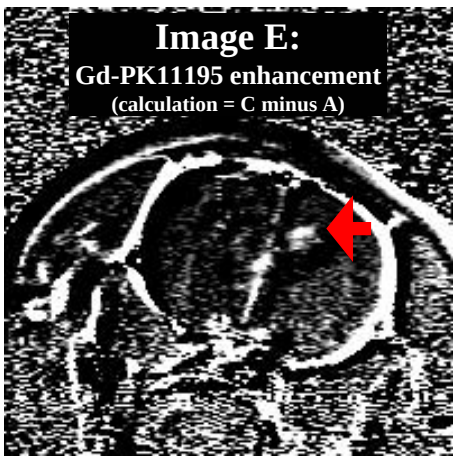
- Initial experiments on two animals
- Rats were injected with 1microliter=1million C6 glioma cells via right frontal craniectomy
- Tumors grew for 11-12 weeks, then injected with Four animals were given GdPK and two were given EuPK.
  - Inhalational anesthesia
  - Cut down to each rat's right femoral vein was accomplished.
  - Placement of a 26.5 guage catheter.
  - 1.0 mM injections of either Eu-PK11195 (fluorescence) or Gd-PK11195 (MRI) were administered as a bolus aqueous solution.
  - MR imaging performed on a 4.7 T small animal imager, fluorescence imaged at TTU after brain harvest

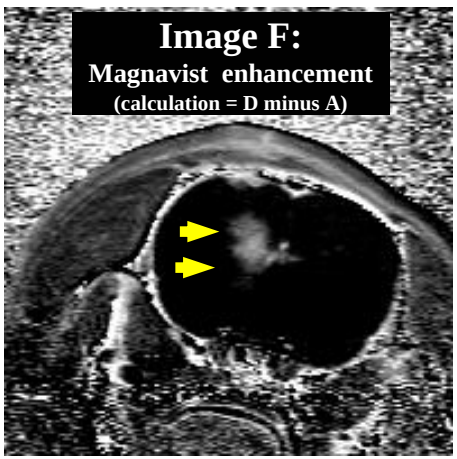












In vivo MRI of rat brain 14 days post injection of C6 glioma cells

Image A: Base T1 image without contrast. Gross morphology appears disrupted in both right and left hemispheres.

Image B: T2 image (no contrast). The brighter areas in this type of image indicate increased water content. Disrupted morphology seen in both hemispheres is in accordance with T1 image.

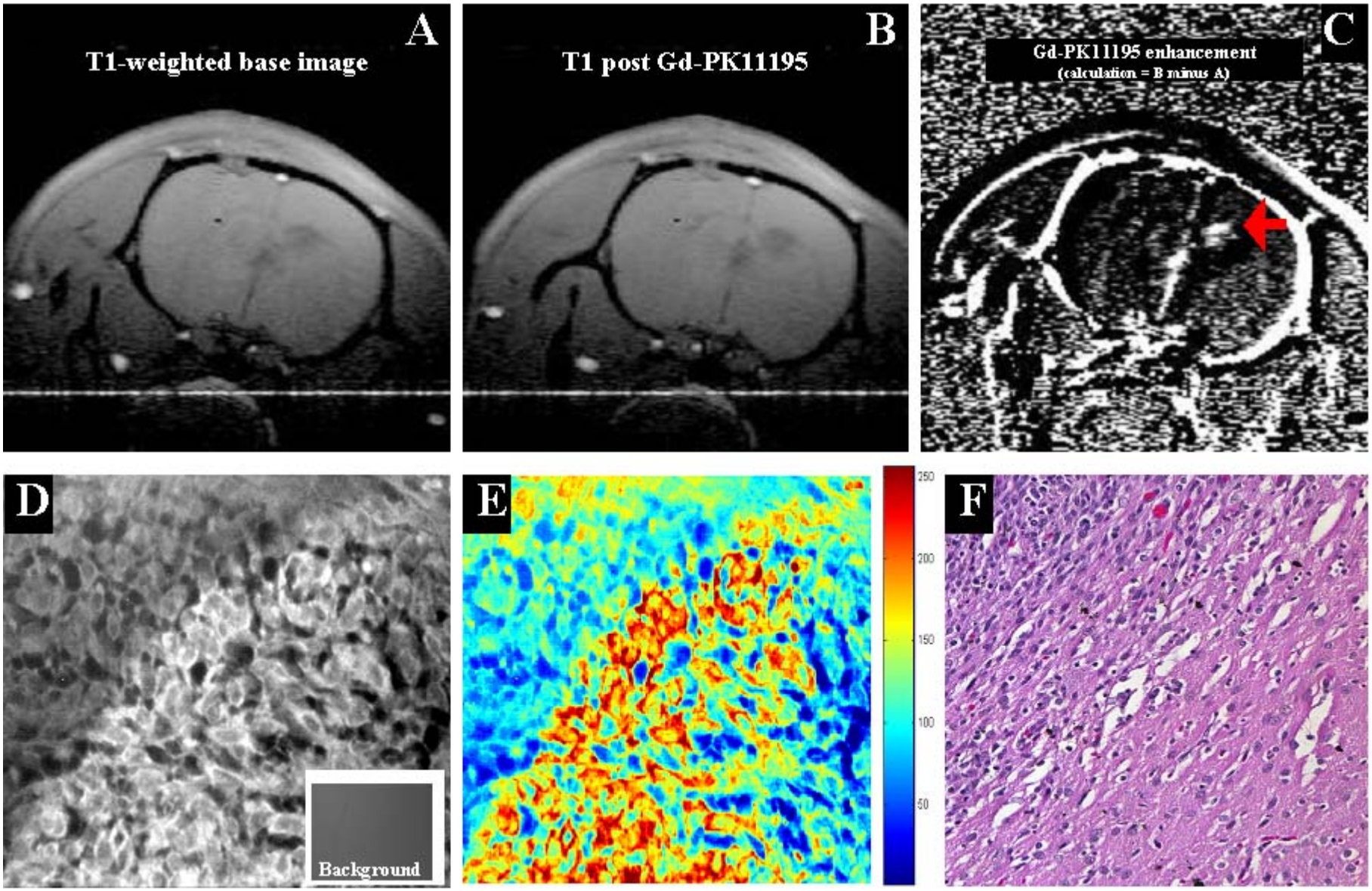
Image C: T1 image following Gd-PK11195 injection. This image appears grossly similar to the base image (Image A), with red and yellow arrows indicating the apparent lesions. To appreciate areas of subtle signal enhancement, a calculated image is required (see Image E).

Image D: T1 image following Magnavist injection. This shows brightness where the blood-brain-barrier has broken down. Refer to Image F to appreciate full enhancment.

Image E: Calculated Gd-PK11195 enhancement. This calculation reveals signal enhancement in the left hemisphere (RED ARROW). This enhancement may represent a migration of tumor cells from the right hemisphere to the left hemisphere. Note that the lack of enhancement in the right hemisphere may be the result of an older necrotic lesion rather than active glioma.

Image F: Calculated Magnavist enhancement. This calculation reveals significant enhancement in the right hemisphere (YELLOW ARROWS), but a ***paucity*** of enhancement in the left hemisphere. ***The lack of enhancement in the left hemisphere indicates that the blood-brain-barrier is intact in this area***.

In-vivo Bi-modal Imaging with Ln-PK-11195



Future Directions

- NIR Lanthanides, MRI, PET, Multi-modal Imaging
- New Molecular Targets
- ***DIAPEUTIC* / THERANOSTICS**
  - Combining diagnostics with therapeutics to facilitate ***integrated*** disease detection, therapy delivery and efficacy monitoring.

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- Vanderbilt Institute for Chemical Biology

Contributors

- **Graduate Students**
  - ***Tim Goebel***
  - ***Charles Manning***
  - ***John Griffin***
- **Undergraduate Students**
  - ***Michelle Sexton***
  - ***Sarah Smith***
  - ***Sarah Haviland***
- **Post Docs, Fellows & Research Associates**
  - Anna Skwierawska *(TTU)*
  - ***Jeffery Albea*** *(Vanderbilt)*
  - ***Ken Niermann*** *(Vanderbilt)*
  - ***Malgorzata A. Wegiel*** *(TTU)*
- ***Vanderbilt Collaborators***
  - ***Neurosurgery:*** Reid Thompson, M.D. and Moneeb Ehtesham, M.D.
  - ***Neuropathology:*** Dr. Mahlon Johnson, M.D.
  - ***Radiology and VUII:*** John Gore Ph.D., Martin Lepage Ph.D. and Ron Price, Ph.D.
  - ***Radiation Oncology:*** Dennis Hallahan, M.D.