START COURSE

February 6, 2013

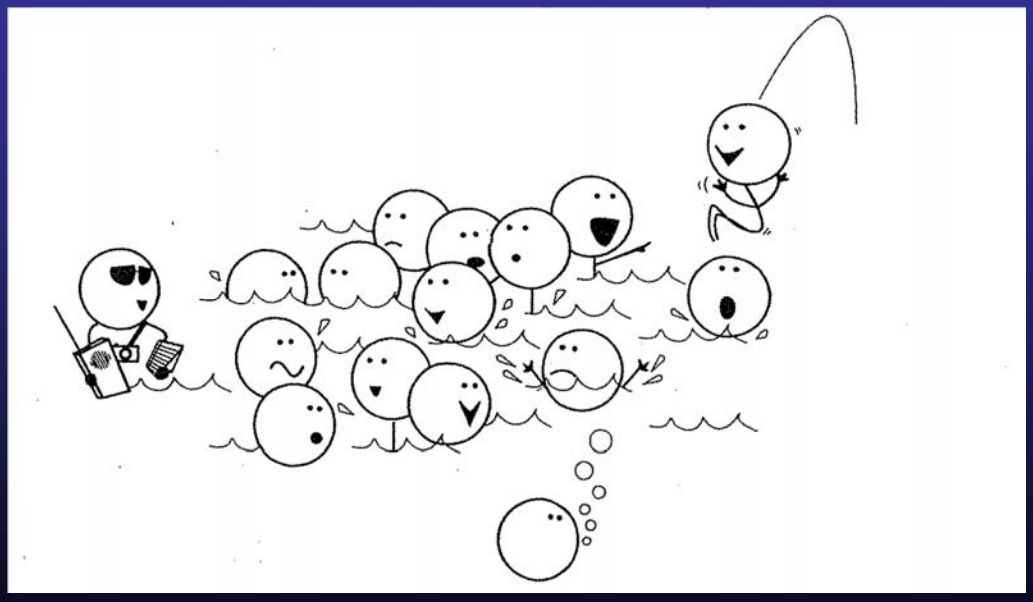
Introduction to PET Imaging and Biology

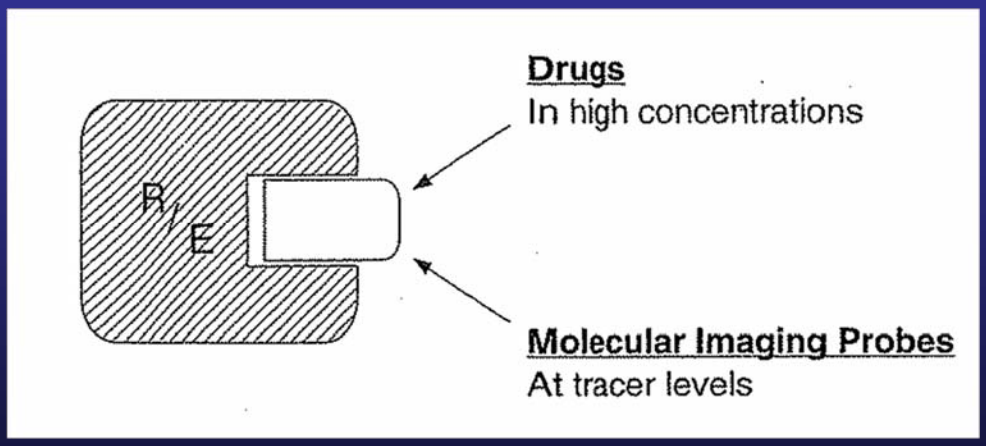
Probe Design and Biochemical Mechanisms

Jorge R. Barrio, PhD

- Definition of Disease
- Functional (Chemical) disturbances vs. Anatomical disturbances
- Drugs vs. Imaging Probes
- Molecular Imaging Probe Design

Imaging Probes as Reporters in in vivo Systems





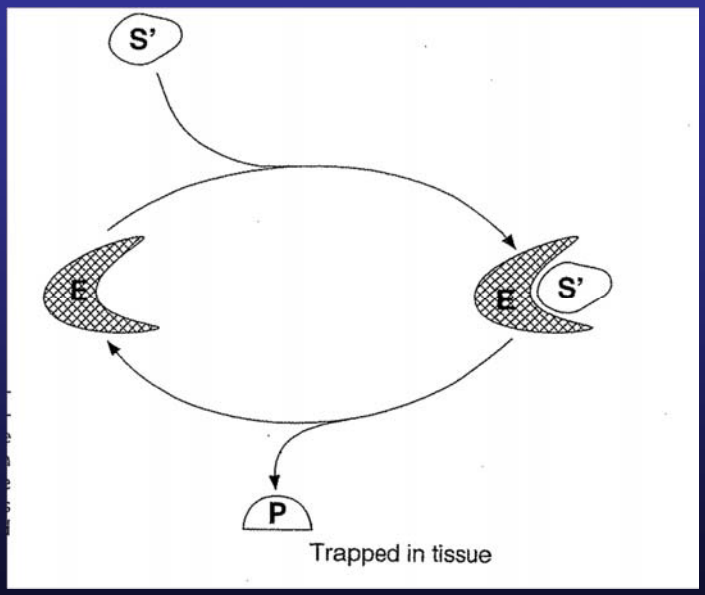
One has to know and study the nature of interaction a radioactive probe with its target before an accurate interpretation of images is possible

- "If one were to inject radioactive shoe-polish and image the radioactivity in the brain, one would almost certainly find patterns of distribution of radioactivity in the brain which might change with functional activation. One would not, however, obtain from the images alone any worthwhile information or useful knowledge about the nature of the processes involved that would allow one to design a model. Just injecting a radioactive compound and getting an image is not enough. It must be combined with basic fundamental research beyond the imaging in order to get meaningful information."

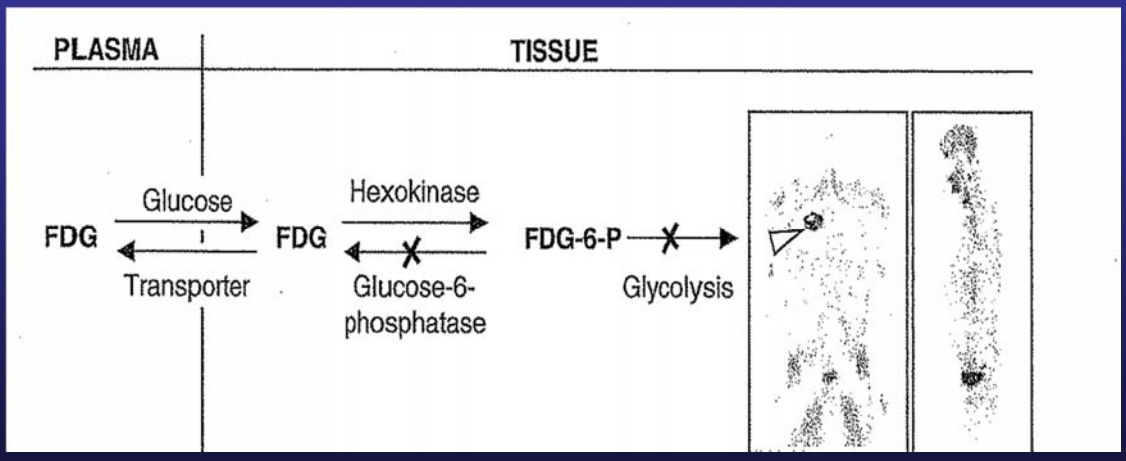
Lou Sokoloff (NIH)

1. Target specificity—ideally, the probe should be restricted to the target process.
2. High membrane permeability to reach target areas.
3. As a result of a specific interaction with a target molecule in tissue, trapping of the labeled molecule or labeled reaction product should occur in a slow turnover pool.
4. Use of analogs specific to one biochemical pathway to isolate one step or a few steps of the process—thus, the kinetics of only the administered compound is represented in the measured data.
5. Rapid turnover rates (small precursor pool) for the substrate precursor are desirable to allow reaction of the labeled molecule probe to proceed rapidly and, thus, reduce background signal rapidly. This implies high affinity of the molecular probe for its tissue target and rapid clearance of the probe from nonspecific areas.
6. Rapid blood pool clearance of the molecular imaging probe to reduce blood pool background at the tissue target (e.g., brain, heart, and tumor) and increase the rate of clearance of the probe from tissue as a result of the temporal decrease in probe concentration in blood.
7. No—or—slow peripheral metabolism of the probe to have the administered probe as the only—or—primary chemical entity in blood.
8. High-specific activity (low masses at the radioactivity concentrations used; Chapter 3) to trace the process under investigation without exerting mass effects on the target molecule.
9. Low nonspecific binding to increase target specificity and target-to-background ratios $>> 1$.
0. A small number of transport and biochemical reaction steps for the molecular imaging probe to allow tracer kinetic modeling to establish quantitative parameters for the imaging determination (Chapter 2).

Enzyme Mediated Processes



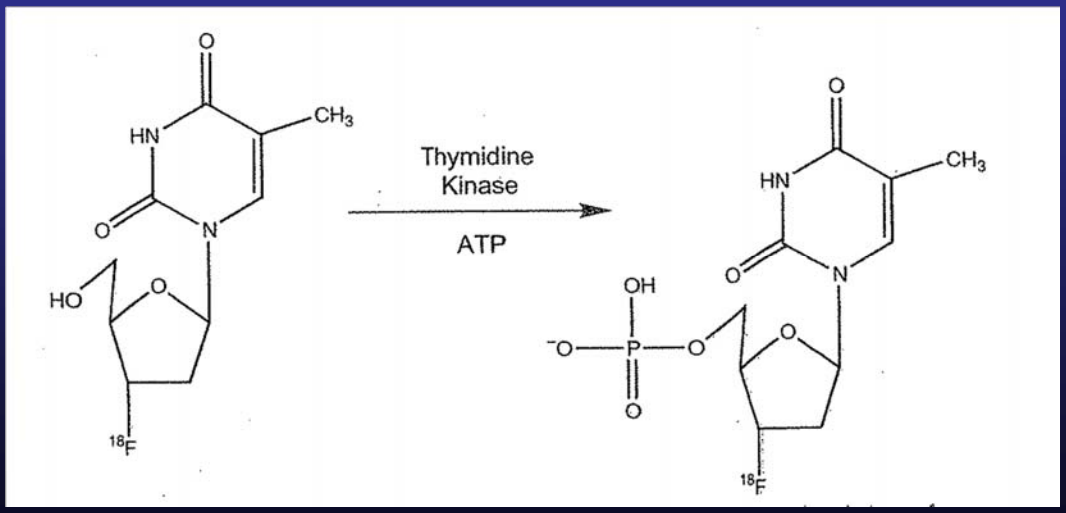
The Fluorodeoxyglucose (FDG) Model



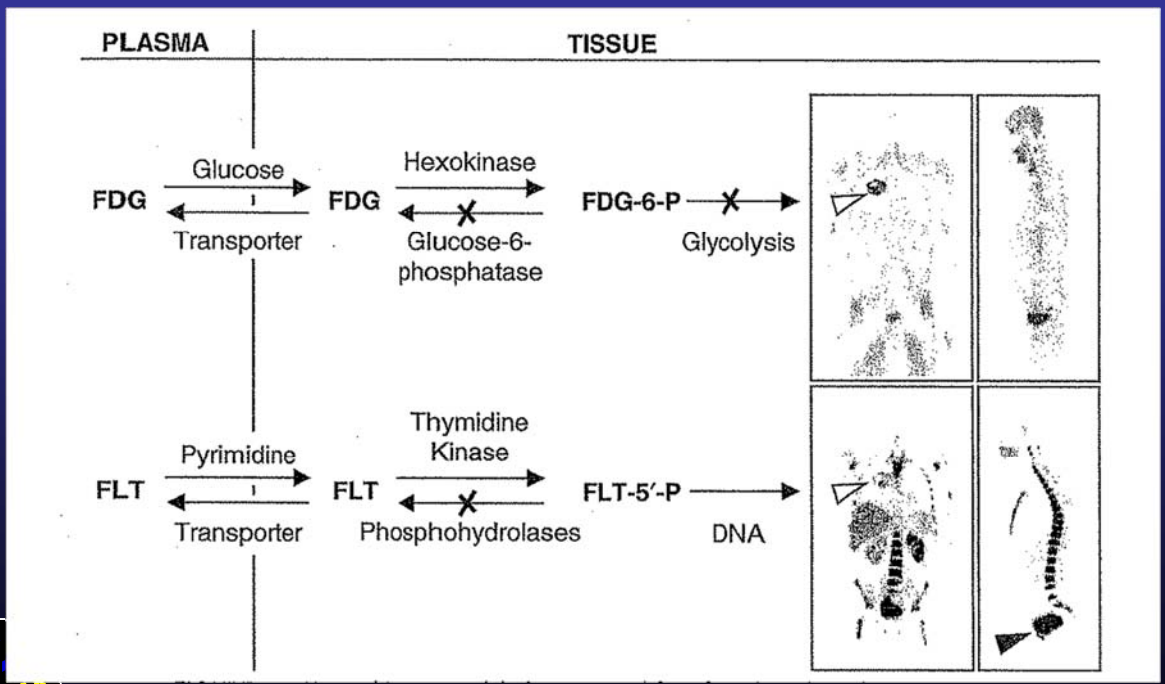
$$V = k_3'(PET)\frac{V_m'/K_m'}{V_m'/K_m}[S]$$

When the principle of competitive enzyme kinetics is used with PET, the molecular imaging probe (i.e., FDG) will compete with the endogenous substrate (i.e., glucose) for the same sites at the catalytic enzyme (e.g.; hexokinase). This competition between the newly designed imaging probe and the endogenous substrate for the same enzyme site immediately indicates that the imaging probe should have very favorable kinetic characteristics to trace the process under study. If the imaging probe has low Vm′ and high Km′ (low Vm′/Km′), it will compete unfavorably with the endogenous substrate, with two consequences: 1) a reduction in the probability of yielding a metabolic trapping product of the labeled analog and 2) as a result, a low PET signal. Therefore, favorable enzyme kinetic characteristics of the imaging probe permit competition with the endogenous substrate for successful formation of the radiolabeled trapping product leading to accumulation of the labeled product—an essential consideration in designing enzyme-mediated molecular imaging probes.

Targeting Tumor Enzymes: Thymidine Kinase with FLT



FLT and 2-FDG



Receptors as Tissue Targets

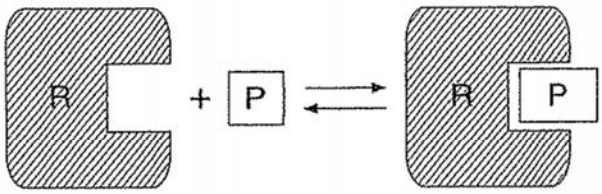
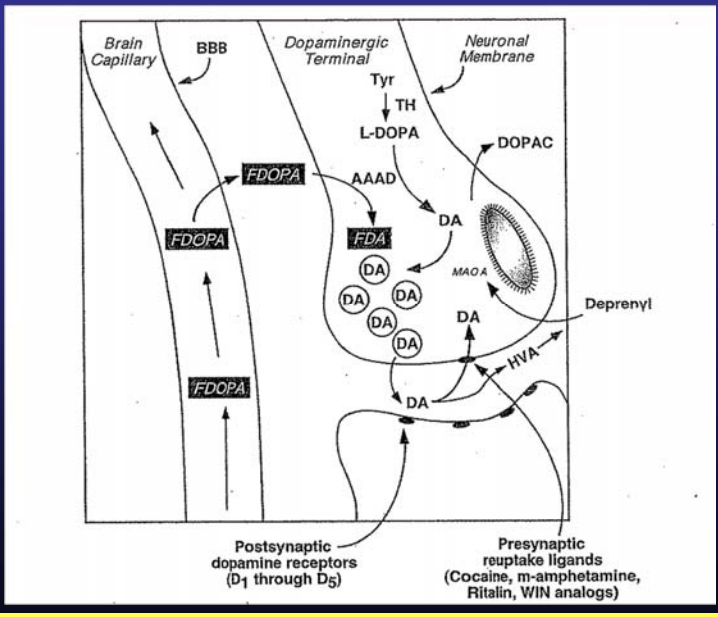
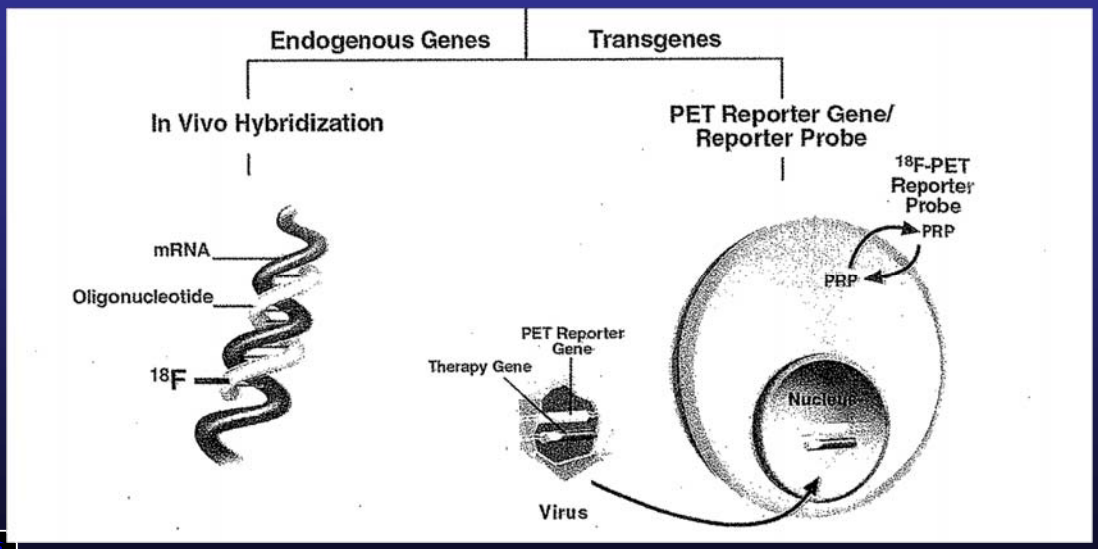


FIGURE 4-8. Schematic representation of the stoichiometric interaction of a molecular imaging probe (P) with a receptor (R). Similar interactions may exist with enzymes, mimicking enzyme inhibitor interactions commonly found with drugs.

Targeting Neurotransmitter Enzymes: 6-[F-18]FluoroDOPA

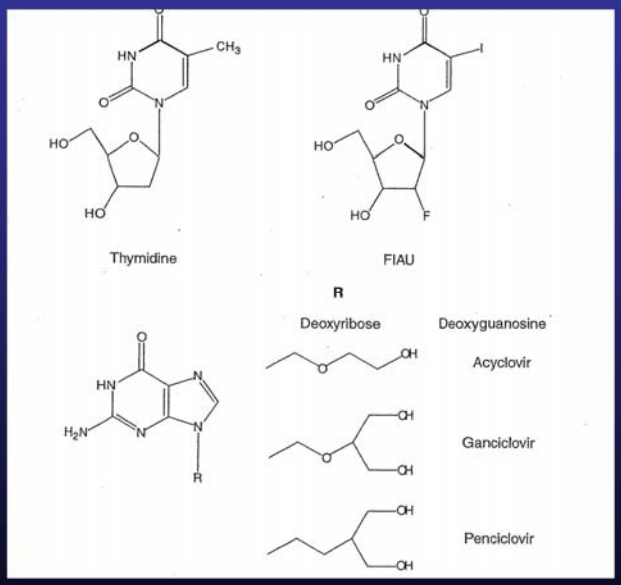


Targeting Transgenic Expression. Images approaches directed at gene expression involve either externally transferred genes into cells (transgenes) or endogenous genes

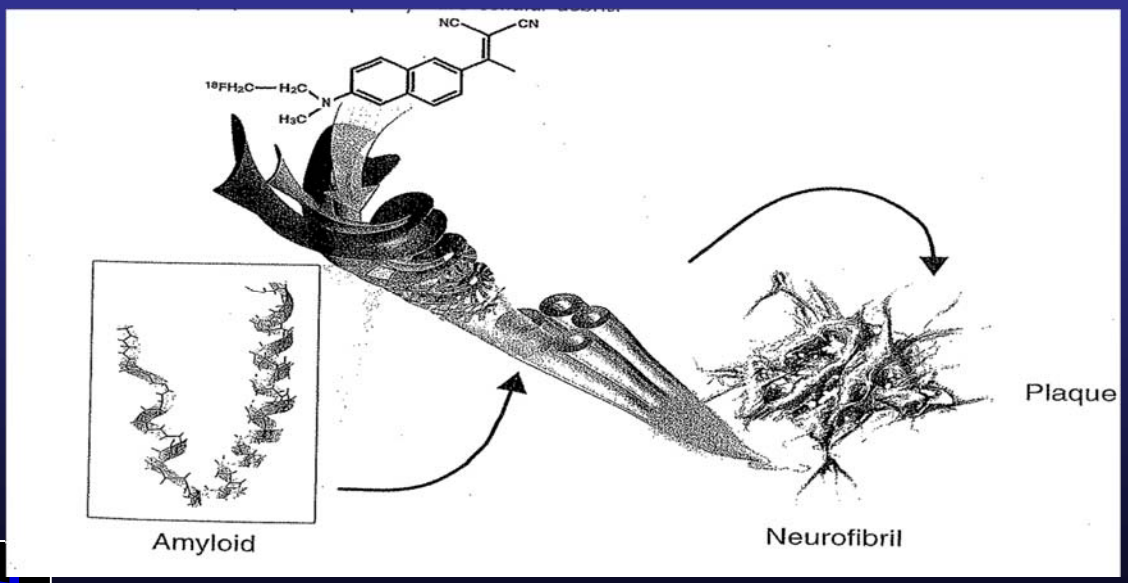


- *The imaging approach involves extending reporter gene techniques used in biology to PET using a PET reporter gene (PRG) and PET reporter probe (PRP)*
- *PRP is either an imaging probe that is a substrate of the PRG-enzyme or a probe that is a ligand that binds to the PRG-receptor*

Substrates for HSV1-tk Mediated Gene Expression

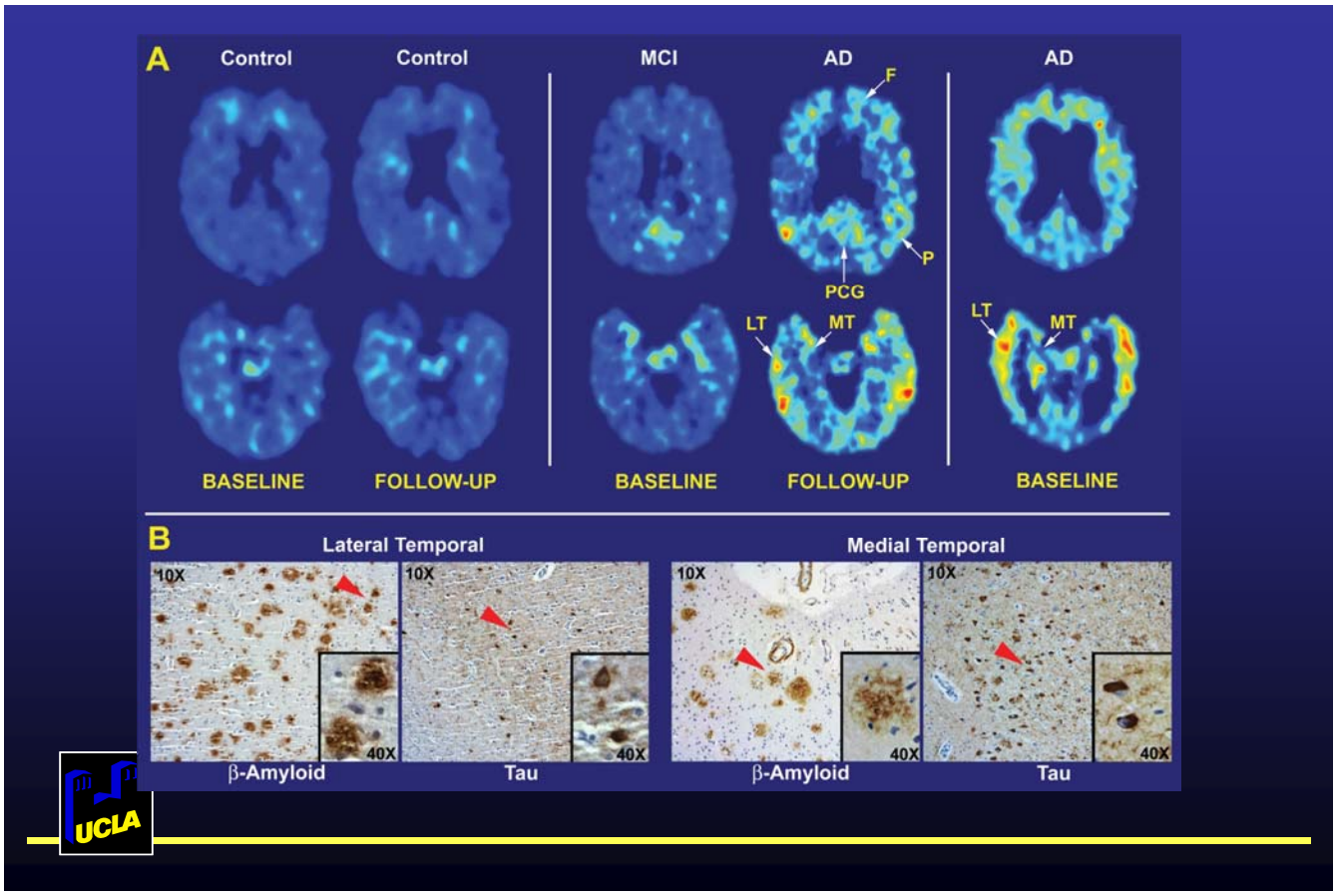


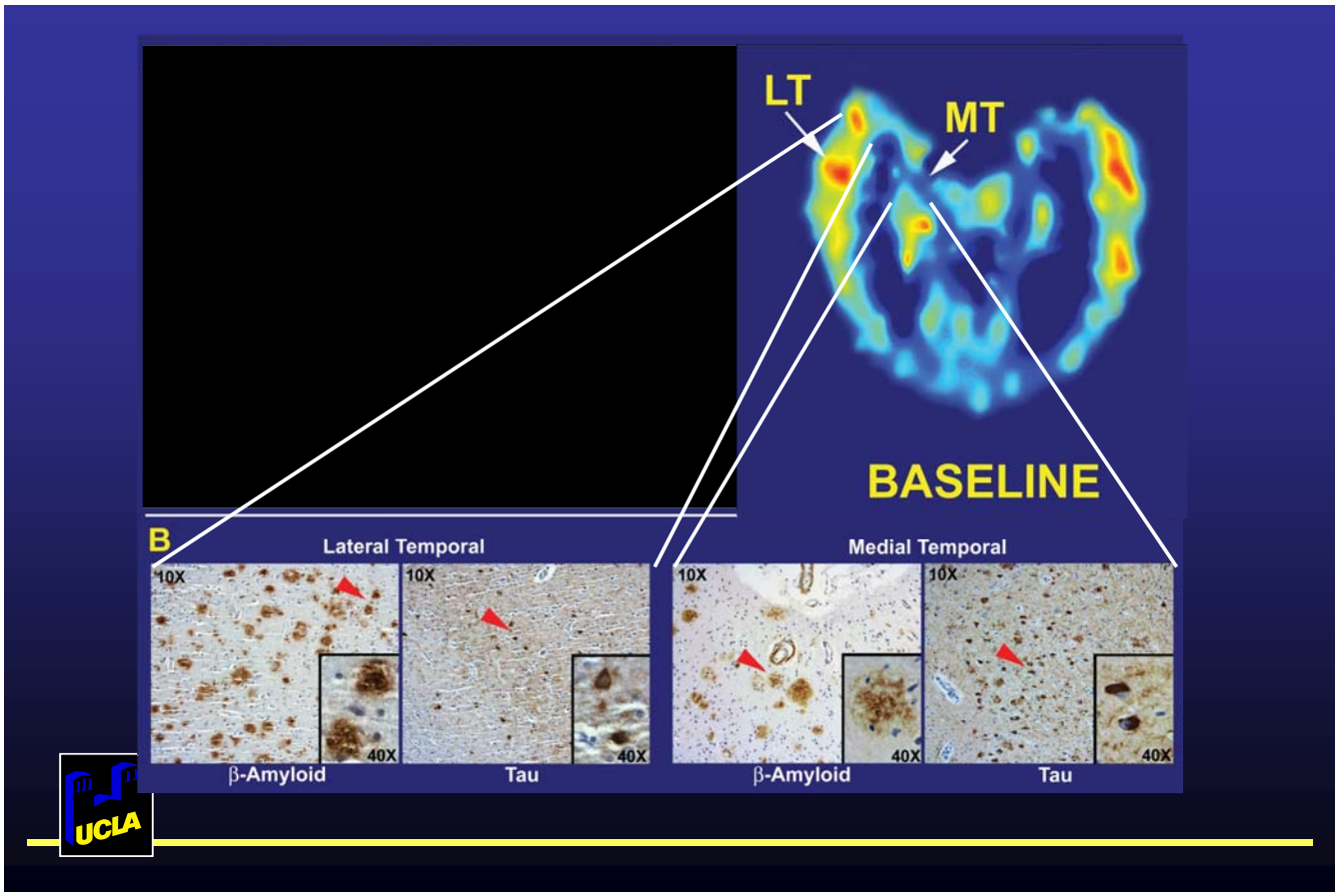
Amyloid Neuropathology Imaging



[F-18]FDDNP-PET and Autopsy Determinations in the Same Patient (AD, LBD, DS)

Small G, Barrio JR et al, New Engl J Med 2006


A
Control
Control
MCI
AD
AD
F
P
PCG
LT
MT
LT
MT
BASELINE
FOLLOW-UP
BASELINE
FOLLOW-UP
BASELINE
B
Lateral Temporal
Medial Temporal
10X
40X
β-Amyloid
Tau
β-Amyloid
Tau
UCLA


LT
MT
BASELINE
B
Lateral Temporal
Medial Temporal
10X
40X
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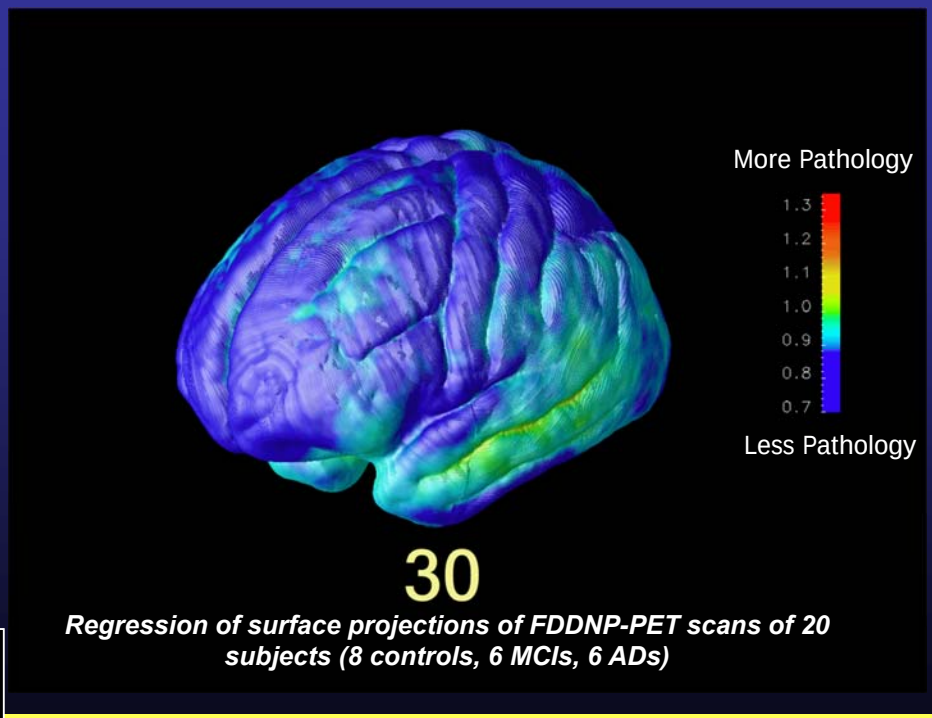
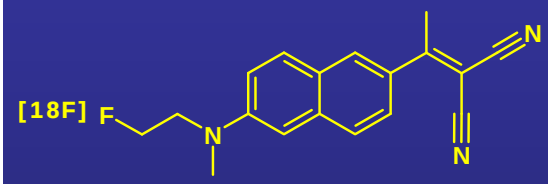
Tau
β-Amyloid
Tau
UCLA

[F-18]FDDNP Shows Full Picture of Progression of Neurodegeneration

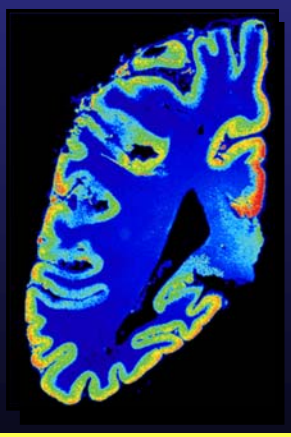


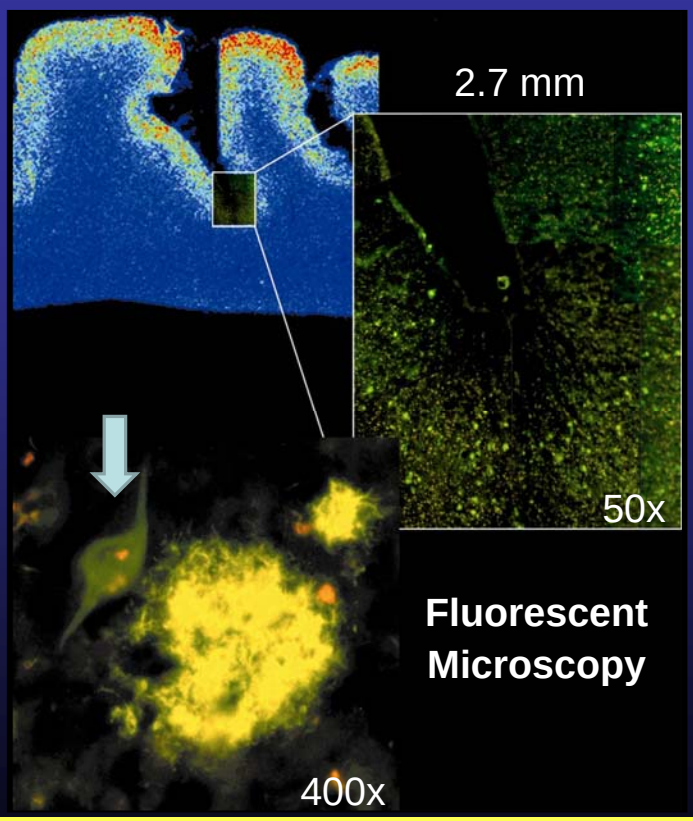
Regression of surface projections of FDDNP-PET scans of 20 subjects (8 controls, 6 MCIs, 6 ADs)

[^{18}F] FDDNP Labels β-Sheet Containing Aggregates



Autoradiography

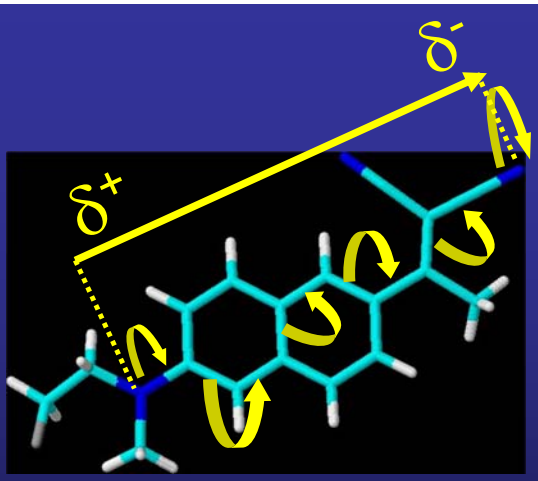




Fluorescent Microscopy

Development of a Predictive Model for β-Sheet Amyloid Aggregate Binding

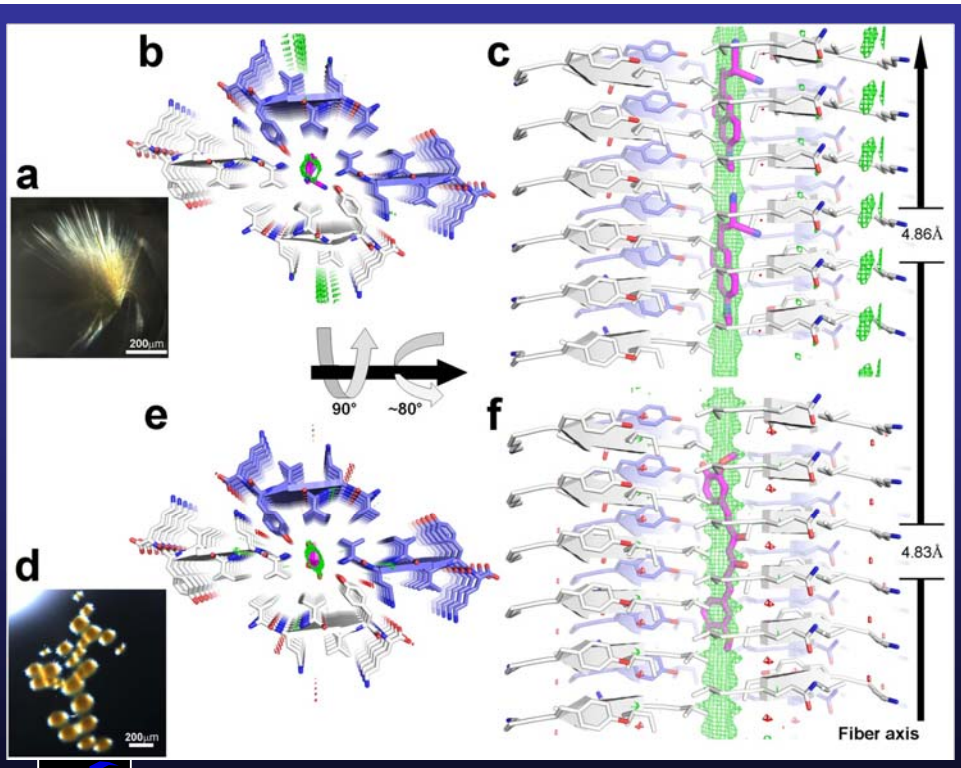
Molecular geometry and dipole moment calculations, as well as fluorescence Stokes shifts and H-5 and H-7 NMR chemical shifts appear predictive of the ability of these structures to bind tightly to aggregates.



In collaboration with Ken Houk, UCLA

Probing Tau Aggregates

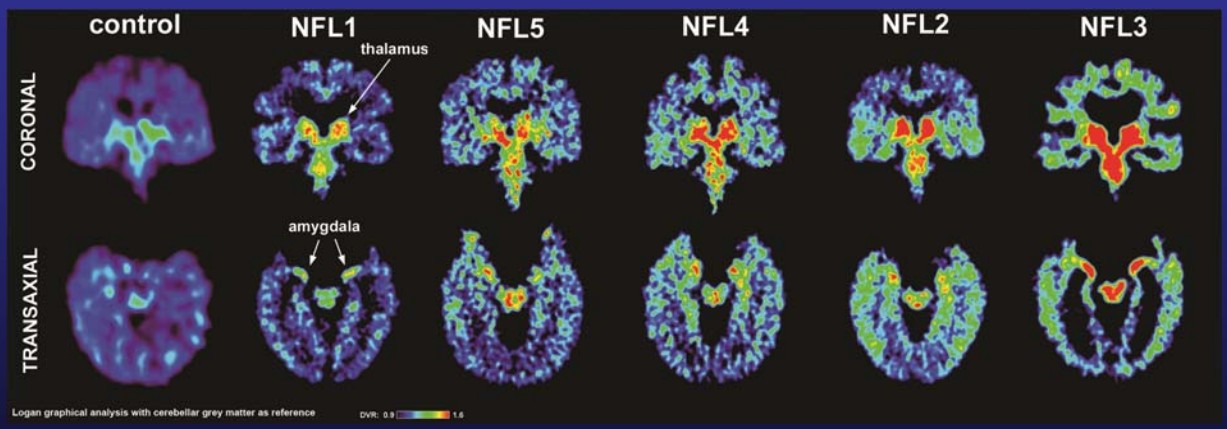
1. *In vitro Binding Affinities and Co-crystallization Experiments*
2. *Transfected Cell Lines*
3. *Transgenic Animal Models*
4. *Living Human Subjects*



DDNP Binding to Tau Segments

(Landau et al, 2011)

Brain FDDNP Scans in Football Players Reflects Degree of Brain Tau Deposition



Small, Barrio Am J Ger Psych, 2013

Regulations for the Use of PET Molecular Imaging Probes in Humans

- ***Research Use:***
  IRB and MRSC vs RDRC
  INDs

- ***Clinical Use:***
  NDA

- ***cGMP and USP Chapter 823***