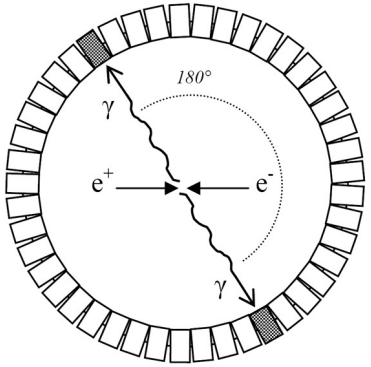
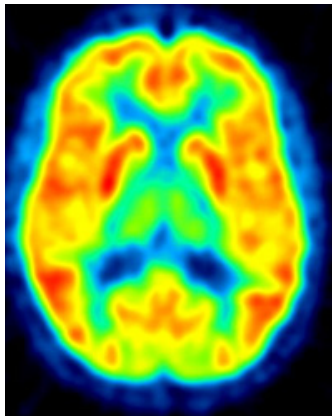
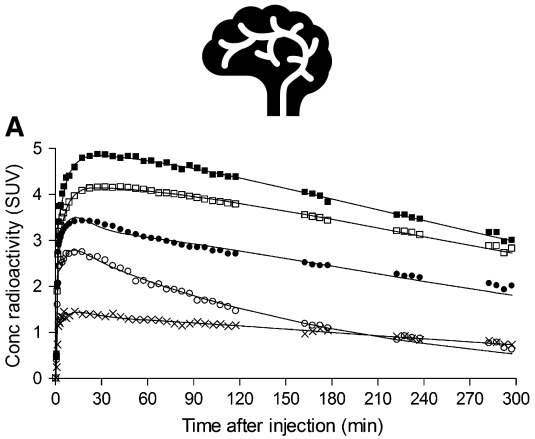
Pharmacokinetic modeling of PET neuroimaging data

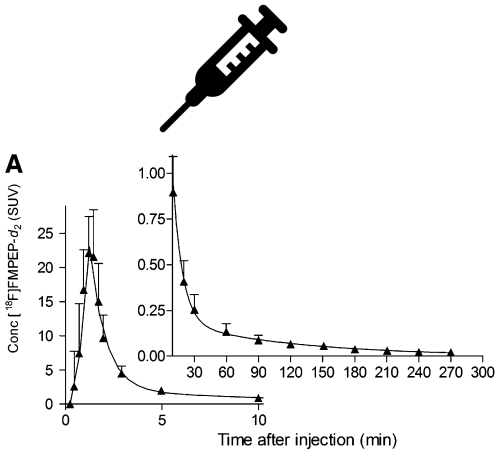
Turku PET Centre Brain Imaging Course 2025

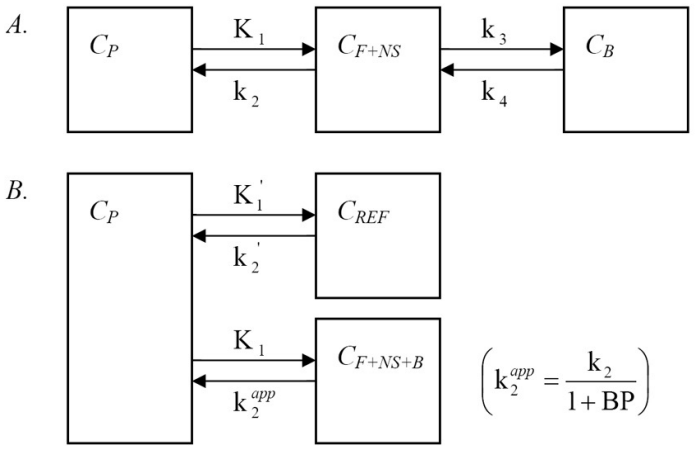
Jussi Hirvonen, Turku PET Centre











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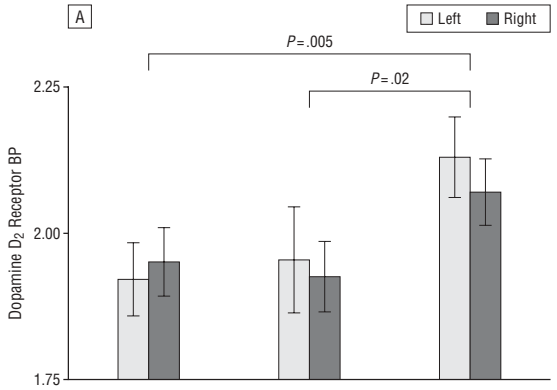


Increased Caudate Dopamine D_2 Receptor Availability as a Genetic Marker for Schizophrenia

Jussi Hirvonen, MD; Theo G. M. van Erp, MA; Jukka Huttunen, MD; Sargo Aalto, MSc; Kjell Någren, PhD; Matti Huttunen, MD, PhD; Jouko Lönnqvist, MD, PhD; Jaakko Kaprio, MD, PhD; Jarmo Hietala, MD, PhD; Tyrone D. Cannon, PhD

Journal of Cerebral Blood Flow & Metabolism (2007) 27, 1533–1539

www.jcbfm.com

Review Article

Consensus nomenclature for *in vivo* imaging of reversibly binding radioligands

Robert B Innis[1], Vincent J Cunningham[2], Jacques Delforge[3], Masahiro Fujita[1], Albert Gjedde[4], Roger N Gunn[5], James Holden[6], Sylvain Houle[7], Sung-Cheng Huang[8], Masanori Ichise[9], Hidehiro Iida[10], Hiroshi Ito[11], Yuichi Kimura[12], Robert A Koeppe[13], Gitte M Knudsen[14], Juhani Knuuti[15], Adriaan A Lammertsma[16], Marc Laruelle[2], Jean Logan[17], Ralph Paul Maguire[18], Mark A Mintun[19], Evan D Morris[20], Ramin Parsey[9], Julie C Price[21], Mark Slifstein[9], Vesna Sossi[22], Tetsuya Suhara[11], John R Votaw[23], Dean F Wong[24] and Richard E Carson[25]

http://www.turkupetcentre.net/petanalysis/

In vitro receptor binding concepts

B_{max} = concentration of receptor sites

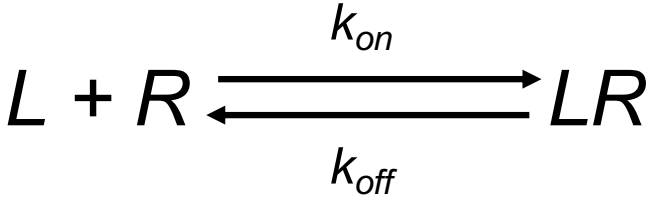
K_D = dissociation contast

(conversely, $1/K_D$ = affinity of each receptor)

$BP_F = B_{max}/ K_D$ = binding potential

The Law of Mass Action

”The rate of **association** is proportionate to the concentrations of the reactants, and the rate of **dissociation** is proportionate to the concentration of the complex.”



L = ligand
R = receptor
LR = ligand-receptor complex
k_{on} = the rate constant of association
= bimolecular association rate ($nM^{-1}min^{-1}$)
k_{off} = the rate constant of dissociation (min^{-1})

The Law of Mass Action

Thus, [LR] will increase in proportion to the product [L][R] and decrease in proportion to [LR]:

$$\frac{d[LR]}{dt} = k_{on}[L][R] - k_{off}[LR]$$

Dynamic equilibrium

At equilibrium, the rate of association equals the rate of dissociation:

$$\frac{d[LR]}{dt} = 0 \quad \text{, thus} \quad k_{on}[L][R] = k_{off}[LR]$$

rearrangement gives:

$$\frac{k_{off}}{k_{on}} = \frac{[L][R]}{[LR]} = K_D$$

Dissociation constant, units of concentration (nM)

”Michaelis-Menten” equation for receptor binding

- Redefine:

  B = [LR] = concentration of bound ligand

  F = [L] = concentration of free (unbound) ligand

- Total concentration of receptors:

  B_{max} = [LR] + [R]

- Concentration of available receptors:

  B_{max}' = B_{max} – B = [R]

”Michaelis-Menten” equation for receptor binding

Thus:

$$K_D = \frac{k_{off}}{k_{on}} = \frac{[L][R]}{[LR]} = \frac{FB_{\max}'}{B} = \frac{F(B_{\max} - B)}{B}$$

”Michaelis-Menten” equation for receptor binding

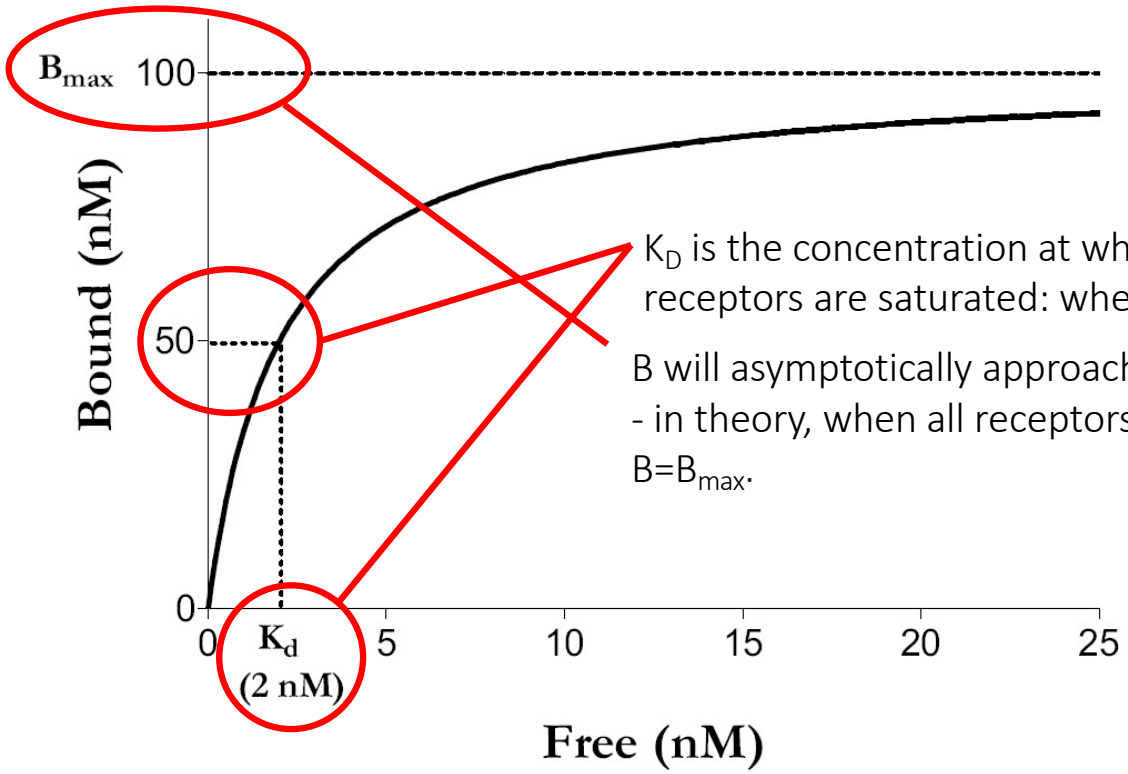
Solving for B:

$$B = \frac{B_{\max} F}{K_D + F}$$

The ”Michaelis-Menten” relationship

Saturation binding curve

$$B = \frac{B_{\max} F}{K_D + F}$$



K_D is the concentration at which half the receptors are saturated: when $B=B_{max}/2$, $F=K_D$.

B will asymptotically approach B_{max}
- in theory, when all receptors are saturated, $B=B_{max}$.

Image courtesy of Robert B. Innis (NIMH, USA)

Saturation binding curve

- Slope of the saturation binding curve:

$$\frac{B}{F} = \frac{B_{\max}}{K_D + F}$$

PET: tracer doses

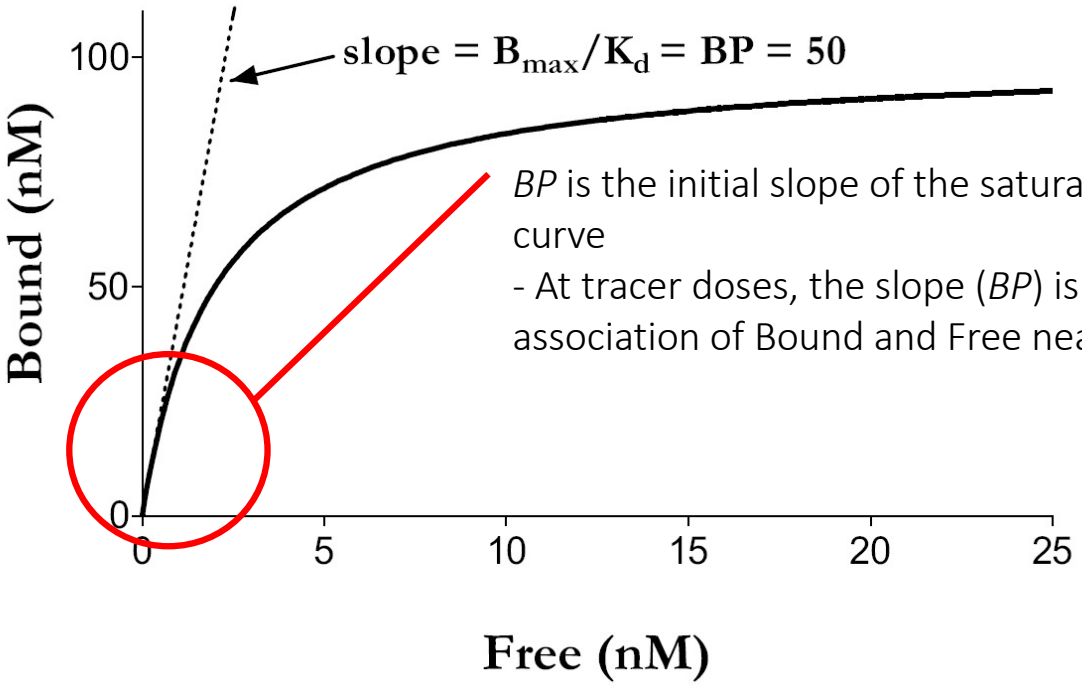
- In PET, minuscule amounts of the radiotracer are injected (”tracer” dose)
- Only <1% of the receptors are occupied (ideally)
- No pharmacological effects expected
- Molar activity (A_m, MBq/nmol): amount of labeled molecules relative to unlabeled (”cold”, ”carrier”) molecules
  - High A_m: tracer dose, <1% occupancy
  - Low A_m: significant occupancy at receptors!

PET: tracer doses

Thus, $F << K_D$ (the latter being the concentration at which 50 % of the receptors are occupied), and:

$$\frac{B}{F} = \frac{B_{\max}}{K_D} = B_{\max} * Affinity = BP$$

Saturation binding curve



BP is the initial slope of the saturation binding curve

- At tracer doses, the slope (*BP*) is constant and the association of Bound and Free nearly linear

Image courtesy of Robert B. Innis (NIMH, USA)

Scatchard linearization

Rearrangement of the ”Michaelis-Menten” equation gives:

$$\frac{B}{F} = \underbrace{\left(\frac{-1}{K_D}\right)}_{\text{Slope= } -1/K_D} B + \underbrace{\frac{B_{\max}}{K_D}}_{\text{Y-intercept= } B_{max}/K_D}$$

Scatchard linearization

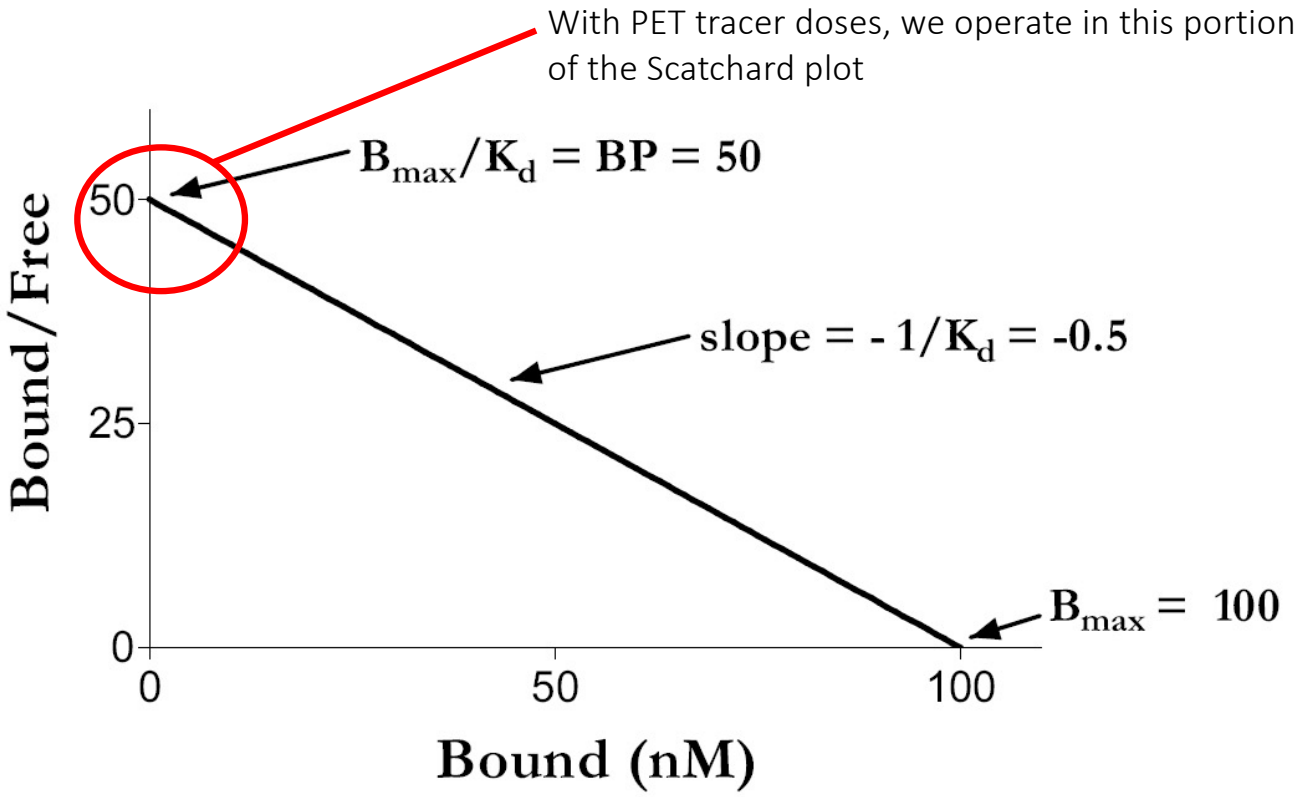


Image courtesy of Robert B. Innis (NIMH, USA)

Major differences between *in vitro* measurements and *in vivo* PET

- *In vivo* PET: usually, tracer doses are used ($F << K_D$)
- Thus, receptors are <u>not</u> occupied at all → B_{max} or K_D cannot be measured separately, only their ratio (BP_F)!
- *In vitro*, multiple levels of saturation is used to describe B_{max} and K_D
- *In vivo* PET: regional blood flow, extraction, binding to plasma proteins, non-specific binding, multiple populations of specific binding sites, internal milieu (pH, ion concentrations etc), radioactive metabolites of the radiotracer, endogenous neurotransmitters, factors related to PET instrumentation…

Interpretation of *in vivo* binding potential differences

From a pharmacological point of view, if BP_F differs between individuals, what is different?

- B_{max}: different individuals have different concentrations of receptors

- K_D: property of a single receptor: *eg*. conformational changes in the receptor protein structure may lead to differences in K_D

Receptor occupancy

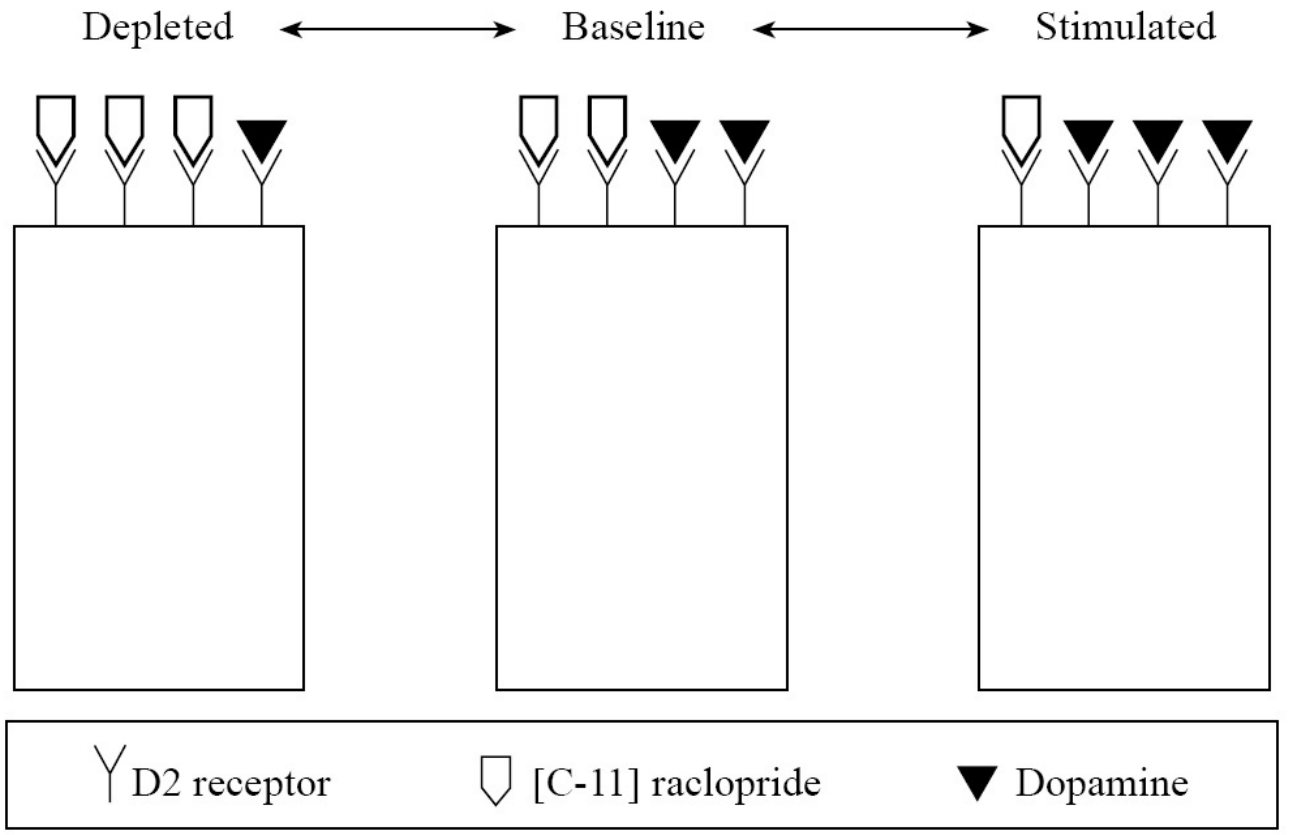


Image: Laruelle & Huang, Q J Nucl Med 2001;45:124-138

Competitive inhibition with PET

- Endogenous neurotransmitters or exogenous substances compete with the radioligand in binding to receptors
- Competitive inhibition can be studied with PET using two tracer dose scans: one in baseline, and another after pharmacological challenge
- Changes in *BP* are considered to reflect changes in the synaptic concentrations of endogenous neurotransmitters

Competitive inhibition with PET

- But what alters *in vivo* BP_F in competitive inhibition?
  - B_{max}: the total concentration of receptor cannot change, otherwise not competitive inhibition!
  - K_D: the affinity of each receptor cannot change in competitive inhibition!
- Introducing a new term: apparent affinity

$$\frac{1}{K_D^{app}} = \frac{1}{K_D\left(1+\sum\frac{F_i}{K_{D_i}}\right)}$$

Pharmacological interpretation of BP_F in vivo

$$BP = \frac{B_{\max}}{K_D^{app}} = \frac{B_{\max}}{K_D\left(1 + \sum \frac{F_i}{K_{D_i}}\right)}$$

K_D = equilibrium dissociation constant of the tracer
F_i = concentration of *i* competing substances
K_{Di} = equilibrium dissociation constant of *i* competing substances

Occupancy

For the measurement of occupancy of endogenous or exogenous ligands using two PET scans with tracer doses:

$$\text{Occupancy (\%)} = \frac{BP_{BEFORE} - BP_{AFTER}}{BP_{BEFORE}} * (100\%)$$

Scatchard analysis *in vivo* for the differentiation of B_{max} and K_D

- Multiple PET scans are needed with decreasing specific activities
  - Thus, gradually increasing the amount of unlabeled (”cold”) radioligand to yield significant occupancy at the receptors
- From multiple observations, pairs of B and B/F are calculated and plotted in the Scatchard plot
  - B can be measured at equilibrium as $C_B(t)/A_m$, where $C_B(t)=C_T(t)-C_{REF}(t)$
  - B/F can be measured as C_B/C_{REF}

Scatchard analysis in vivo for the differentiation of B_{max} and K_D

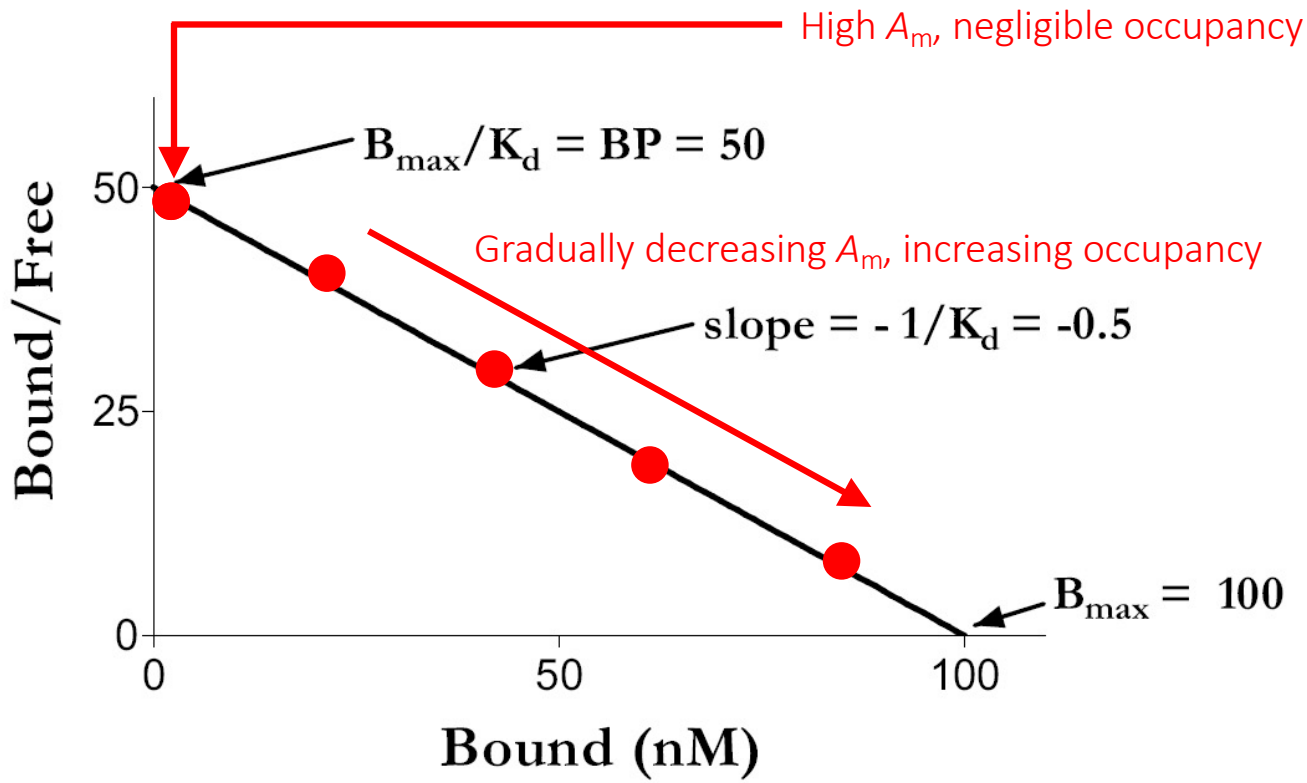
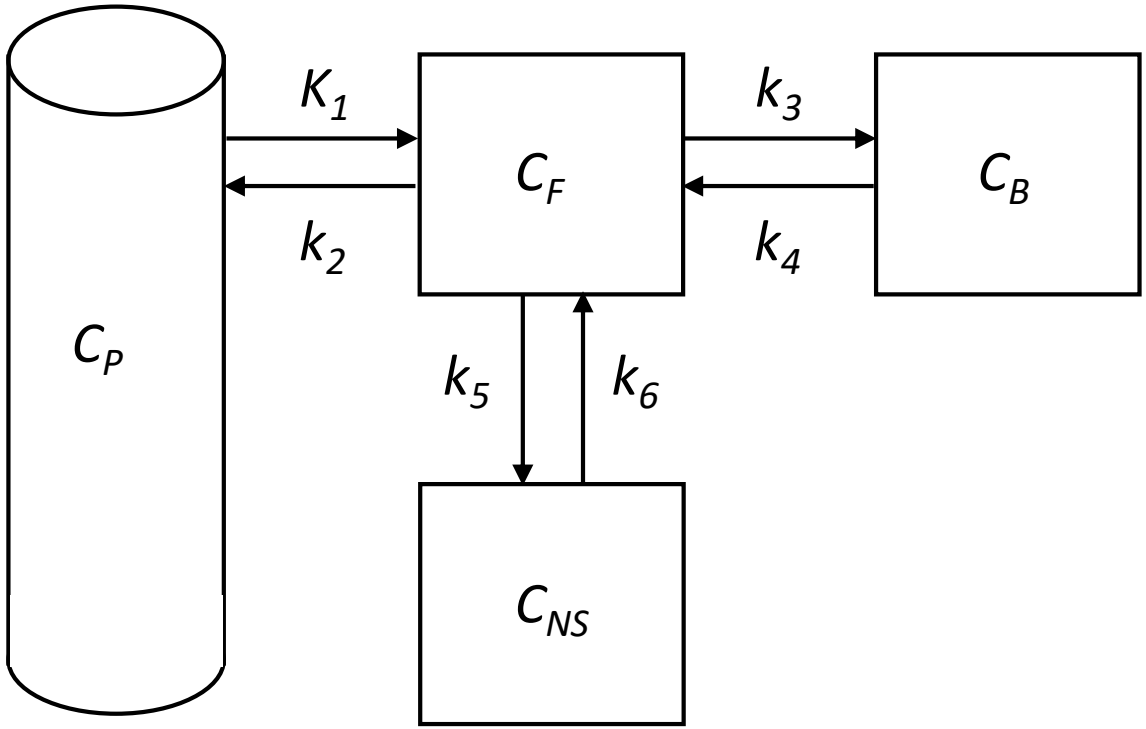


Image courtesy of Robert B. Innis (NIMH, USA)

Confounding factors and complications

- Properties of the radioligand
  - Target receptor population (affinity states etc.)
  - Physiological receptor variants
  - Is it comparable to the endogenous ligand?
- Receptor trafficking
  - Agonist-induced receptor internalization
  - How does is affect B_{max}?
  - Do PET radioligands bind to internalized receptors? How?
- Non-competitive inhibition, changes in receptor conformation

Full compartmental model



Practically, too many parameters to achieve reliable fits…

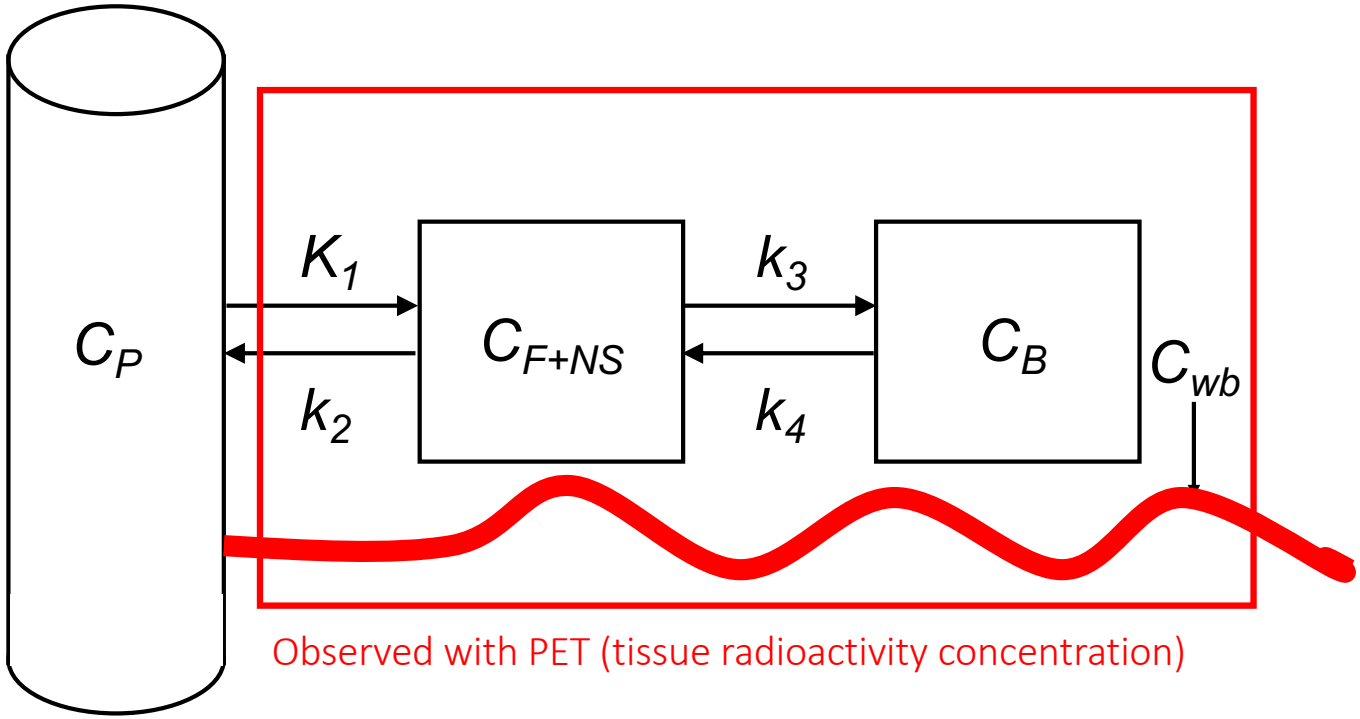
Full compartmental model

- C_P = radioactivity concentration in arterial plasma
- C_F = radioactivity concentration of free radioligand in tissue
- C_B = radioactivity concentration of specifically bound radioligand
- C_{NS} = radioactivity concentration of non-specifically bound radioligand
- K_1 = rate constant for transit between plasma and tissue (ml tissue)/(ml plasma)/min
- k_2 = rate constant for transit between tissue and plasma (min^{-1})
- k_3, k_4 = rate constants for transit between free and specifically bound compartments and vice versa (min^{-1})
- k_5, k_6 = rate constants for transit between free and non-specifically bound compartments and vice versa (min^{-1})

Assumption in all compartmental models

- Only free radioligand in arterial plasma in considered to pass the blood-brain barrier
- Free radioligand in plasma = not bound to proteins
- The fraction of total plasma radioactivity originating from free radioligand = f_P

Standard 3-compartmental model (2TC)



$$C_{PET} = (1 - V_b)C_T + V_b C_{wb}; \quad C_T = C_{F+NS} + C_B$$

Assumptions in the 3-compartmental model

- Free and non-specifically bound compartments are assumed to be at equilibrium rapidly
- Thus, these are treated as a single compartment
- The fraction of radioactivity in this combined compartment originating from free radioligand = f_{ND}

Volume of distribution (V_T)

The ratio of radioactivity concentration in a compartment and in plasma:

$$V_j = \frac{C_j}{f_P C_P}$$

V_j = the distribution volume of the *j*th compartment
C_j = radioactivity concentration in the *j*th compartment
f_P = plasma ”free fraction”
C_P = radioactivity concentration in arterial plasma

Derivation of V_T from rate constants:
Total V_T for 2-compartmental model (1TC)

$$\frac{dC_T}{dt} = K_1 C_P - k_2 C_T$$

Derivation of V_T from rate constants: Total V_T for 2-compartmental model

At equilibrium, no net transfer between plasma and tissue:

$$\frac{dC_T}{dt} = 0 \quad ; \quad K_1 C_P = k_2 C_T$$

and

$$V_T = \frac{C_T}{C_P} = \frac{K_1}{k_2}$$

Derivation of V_T from rate constants:
Total V_T for 3-compartmental model (2TC)

$$C_T = C_{F+NS} + C_B$$

$$\frac{dC_{F+NS}}{dt} = K_1 C_P - k_2 C_{F+NS} - k_3 C_{F+NS} + k_4 C_B$$

$$\frac{dC_B}{dt} = k_3 C_{F+NS} - k_4 C_B$$

Derivation of V_T from rate constants: Total V_T for 3-compartmental model

At equilibrium:

$$\frac{dC_B}{dt} = 0 \Rightarrow k_3 C_{F+NS} = k_4 C_B ; \quad C_B = \frac{k_3}{k_4} C_{F+NS}$$

thus

$$V_T = \frac{C_T}{C_P} = \frac{C_{F+NS} + C_B}{C_P} = \left(1 + \frac{k_3}{k_4}\right) \frac{C_{F+NS}}{C_P}$$

Derivation of V_T from rate constants: Total V_T for 3-compartmental model

At equilibrium:

$$C_{F+NS} = \frac{K_1}{k_2} C_P$$

thus:

$$V_T = \left(\frac{K_1}{k_2} \right) \left(1 + \frac{k_3}{k_4} \right)$$

How do rate constants relate to pharmacological binding parameters?

$$k_3 = k_{on} f_{ND} \left(B_{\max} - \frac{C_B(t)}{A_m} \right)$$

$$k_4 = k_{off}$$

How do rate constants relate to pharmacological binding parameters?

At tracer doses, A_m >> $C_P(t)$ (that is, negligible occupancy by the radiotracer), and k_3 formula reduces to:

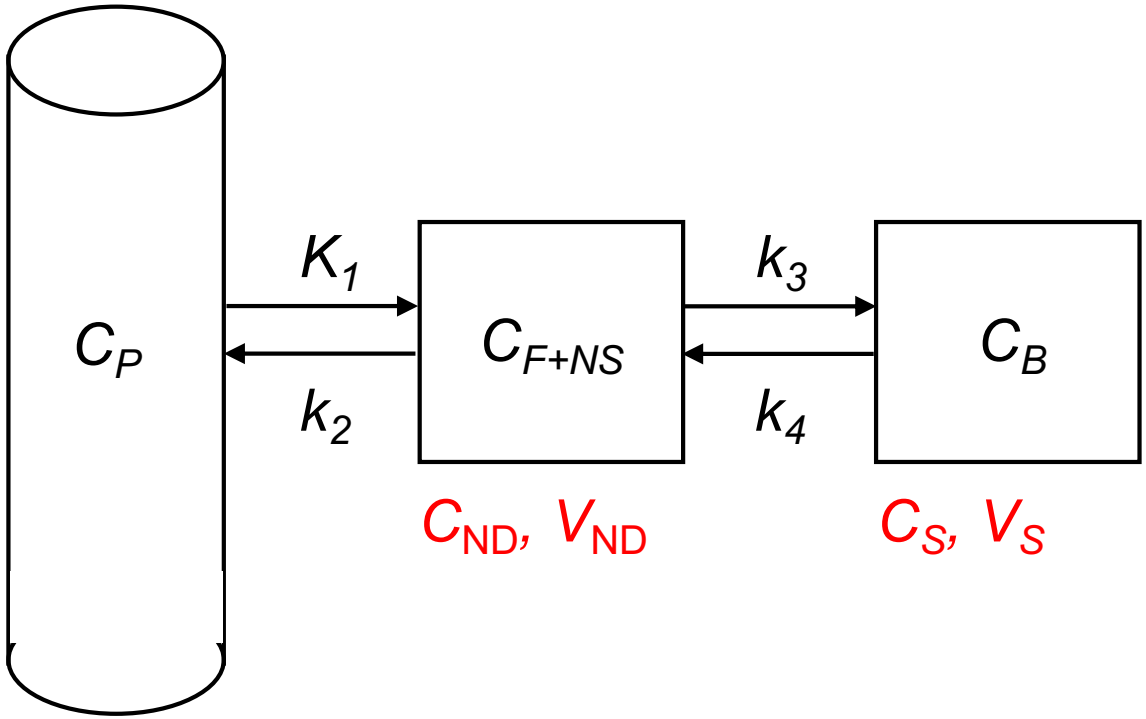
$$k_3 = k_{on} f_{ND} B_{\max}$$

Since

$$\frac{k_{off}}{k_{on}} = K_D \ ,$$

$$\frac{k_3}{k_4} = \frac{f_{ND} B_{\max}}{K_D} = BP_{ND}$$

Standard 3-compartmental model

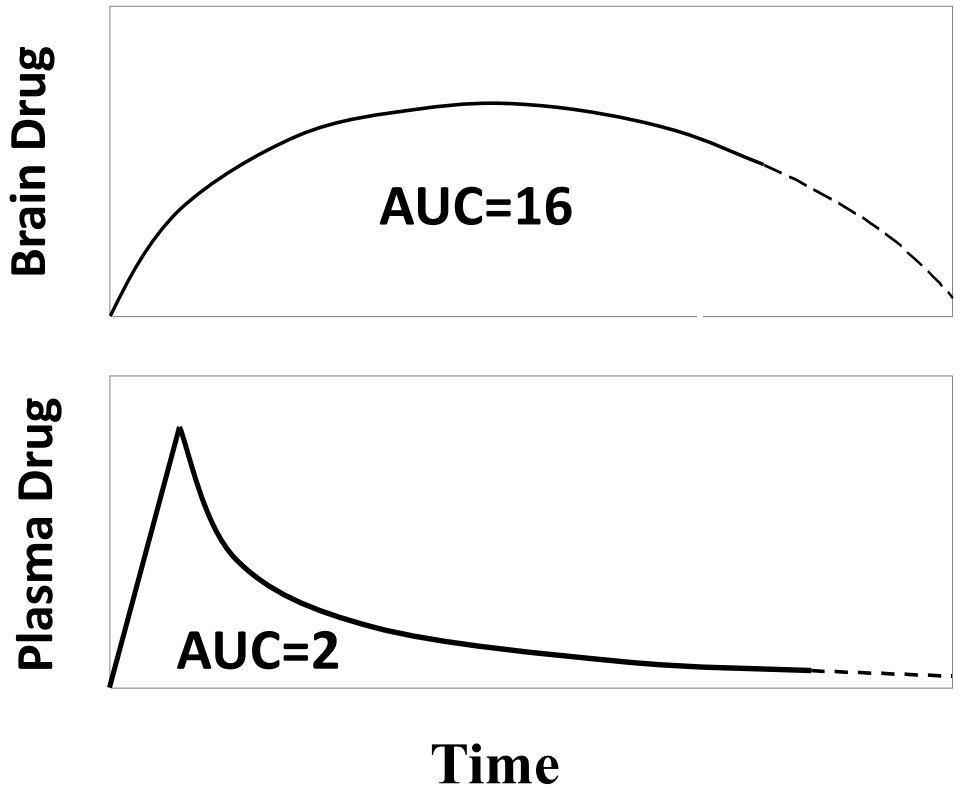


Nomenclature

BP notation	Pharmacological interpretation	Kinetic interpretation	V_T interpretation	f_P	f_{ND}
BP_F	$\frac{B_{max}}{K_D}$	$\frac{K_1 k_3}{f_P k_2 k_4}$	$\frac{V_T - V_{ND}}{f_P}$	No	No
BP_P	$\frac{f_P B_{max}}{K_D}$	$\frac{K_1 k_3}{k_2 k_4}$	$V_T - V_{ND}$	Yes	No
BP_{ND}	$\frac{f_{ND} B_{max}}{K_D}$	$\frac{k_3}{k_4}$	$\frac{V_T}{V_{ND}} - 1$	No	Yes

Distribution Volume (V_T)

V_T equals uptake in brain relative to how much activity is delivered in arterial plasma



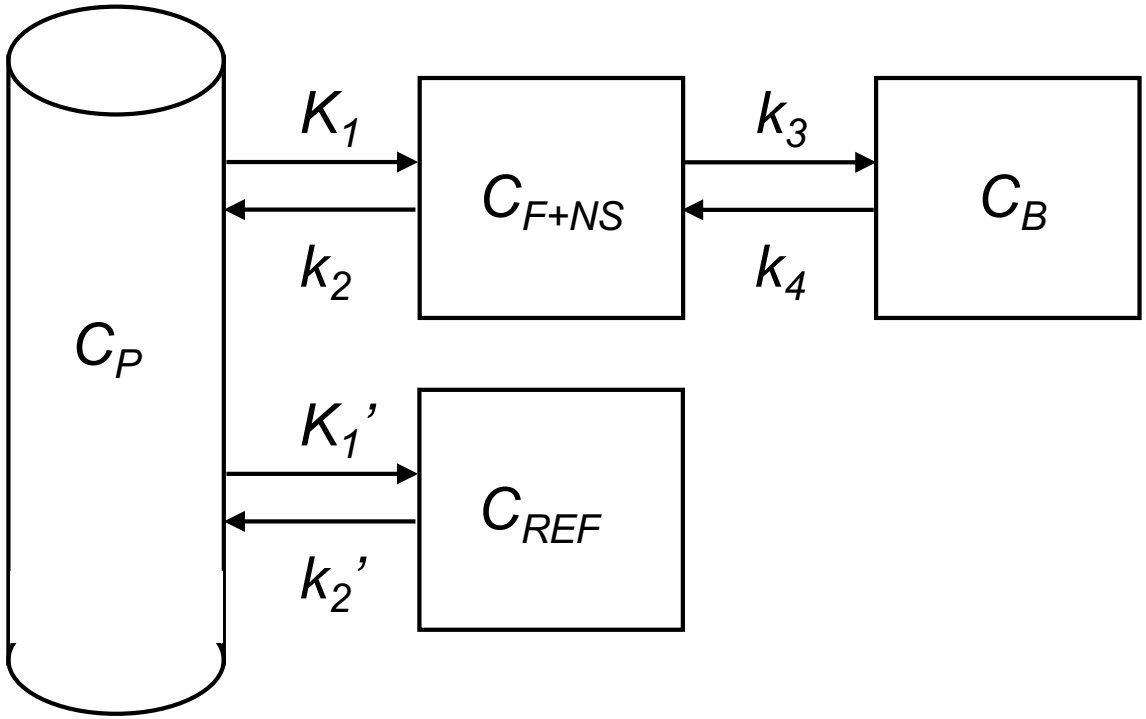
$$V_T = \frac{\text{Area Brain Curve}}{\text{Area Plasma Curve}}$$

$$V_T = \frac{16}{2} = 8$$

Methods for estimating BP in vivo

- Direct method
  - From rate constants: complicated
- Indirect method
  - Calculation from V_T values derived from target and reference regions using arterial plasma input: more robust
  - **Calculation using reference region models: robust, arterial blood sampling not required**
  - Caveat: critically dependent on the validity of the reference region to accurately estimate V_{ND}

Reference region methods



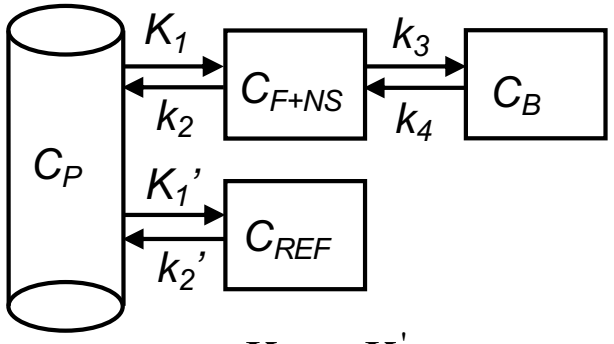
Reference region methods

- Estimation of the free and non-specific compartment (C_{F+NS}) from a reference region would obviate the need of arterial blood sampling
  - A major advantage in clinical studies!
- In a valid reference region, V_{ND} represents only free and non-specific radioligand – no specific binding to receptors
- Central assumption: free and non-specific binding is same between brain regions, *i.e.*:

$$\frac{K_1}{k_2} = \frac{K_1'}{k_2'}$$

Note that blood flow is not assumed to be equal across brain regions - only the ratio K_1/k_2.

Reference region methods: indirect BP estimation from V_T values



$$V_T = \left(\frac{K_1}{k_2}\right)\left(1+\frac{k_3}{k_4}\right)$$

Measured from the ROI

$$V_T = V_{REF} = \frac{K_1'}{k_2'}$$

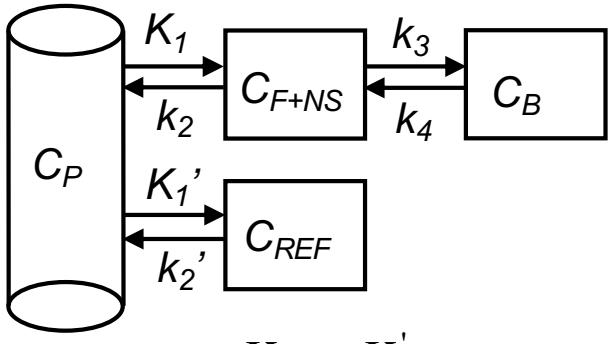
Measured from the reference region

Assumption: $\frac{K_1}{k_2} = \frac{K_1'}{k_2'}$

Thus:

$$\frac{V_T}{V_{REF}} - 1 = \frac{\left(\frac{K_1}{k_2}\right)\left(1+\frac{k_3}{k_4}\right)}{\left(\frac{K_1}{k_2}\right)} - 1 = \frac{k_3}{k_4} = \frac{f_{ND}B_{\max}}{K_D} \quad (BP_{ND})$$

Reference region methods: indirect BP estimation from V_T values



$$V_T = \left(\frac{K_1}{k_2}\right)\left(1+\frac{k_3}{k_4}\right)$$

Measured from the ROI

$$V_T = V_{REF} = \frac{K_1'}{k_2'}$$

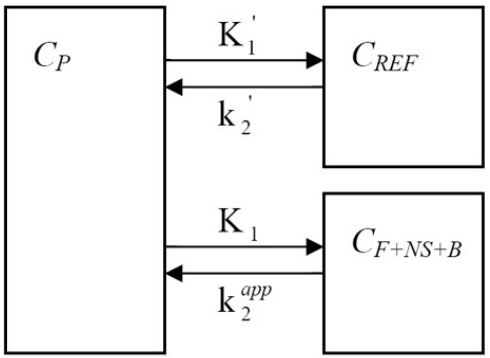
Measured from the reference region

Assumption: $\frac{K_1}{k_2} = \frac{K_1'}{k_2'}$

Accordingly:

$$V_T - V_{REF} = \left(\frac{K_1}{k_2}\right)\left(1+\frac{k_3}{k_4}\right) - \left(\frac{K_1}{k_2}\right) = \frac{K_1 k_3}{k_2 k_4} = \frac{f_P B_{max}}{K_D} \quad (BP_P)$$

Reference region methods: simplified reference tissue model (SRTM)



Further assumptions: bound and free+non-specific compartments reach equilibrium rapidly → they can be treated as a single compartment, C_{F+NS+B}

$$\left(k_2^{app} = \frac{k_2}{1+\mathrm{BP}}\right)$$

$$C_T(t) = R_1 C_{REF}(t) + \left(k_2 - \frac{R_1 k_2}{1+BP}\right) C_{REF}(t) \otimes e^{-\left(\frac{k_2 t}{1+BP_{ND}}\right)}$$

$C_T(t)$ = radioactivity concentration in the region of interest (=C_{F+NS}+C_B)
$C_{REF}(t)$ = radioactivity concentration in the reference region
R_1 = ratio of K_1 and K_1'
BP_{ND} = binding potential

Scenario 1.

- Radioligand 1 has no reference region, you choose:

V_T/f_P V_T BP_F

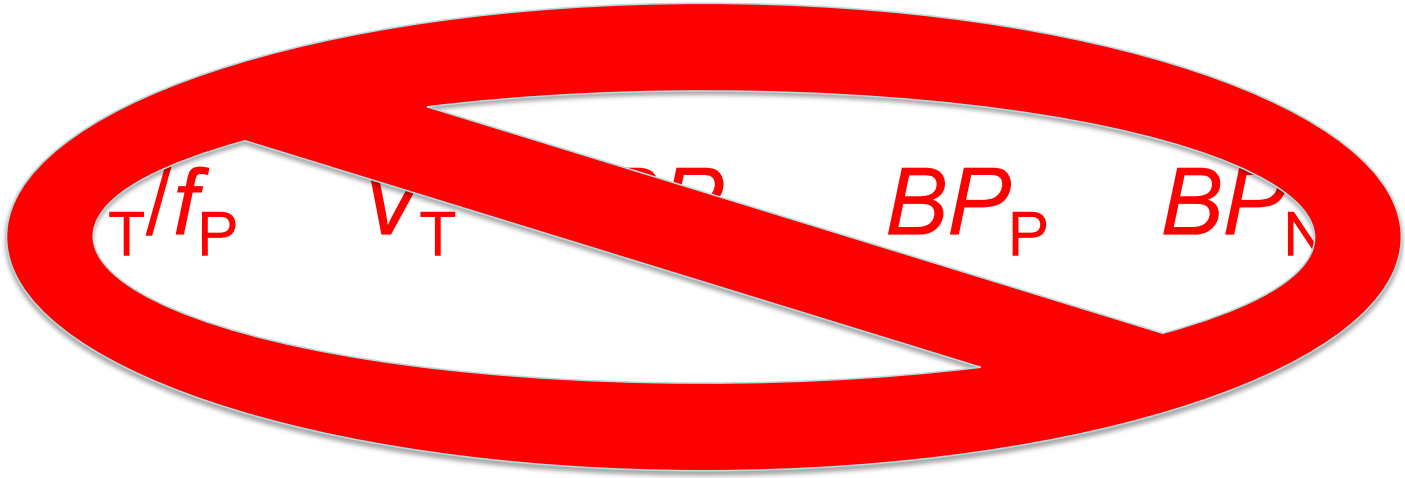
Scenario 2.

- Radioligand 2 may have different plasma protein binding (f_P) between subjects, difficult to measure… you choose:

BP_{ND}

Scenario 3.

- Radioligand 3 has a brain-penetrant radiometabolite, you choose:



Conclusions

- Nomenclature concerning the parameters estimates for specific binding may be confusing
- Always check what is really meant by ”BP”
- Always state explicitly in an article what you mean by ”BP”
- Keep in mind the limitation and vulnerabilities of each model
- Learn the model configurations and common formulas