Pharmacokinetic modeling of PET neuroimaging data

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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.”

$$L + R \underset{k_{off}}{\overset{k_{on}}{\rightleftharpoons}} LR$$

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

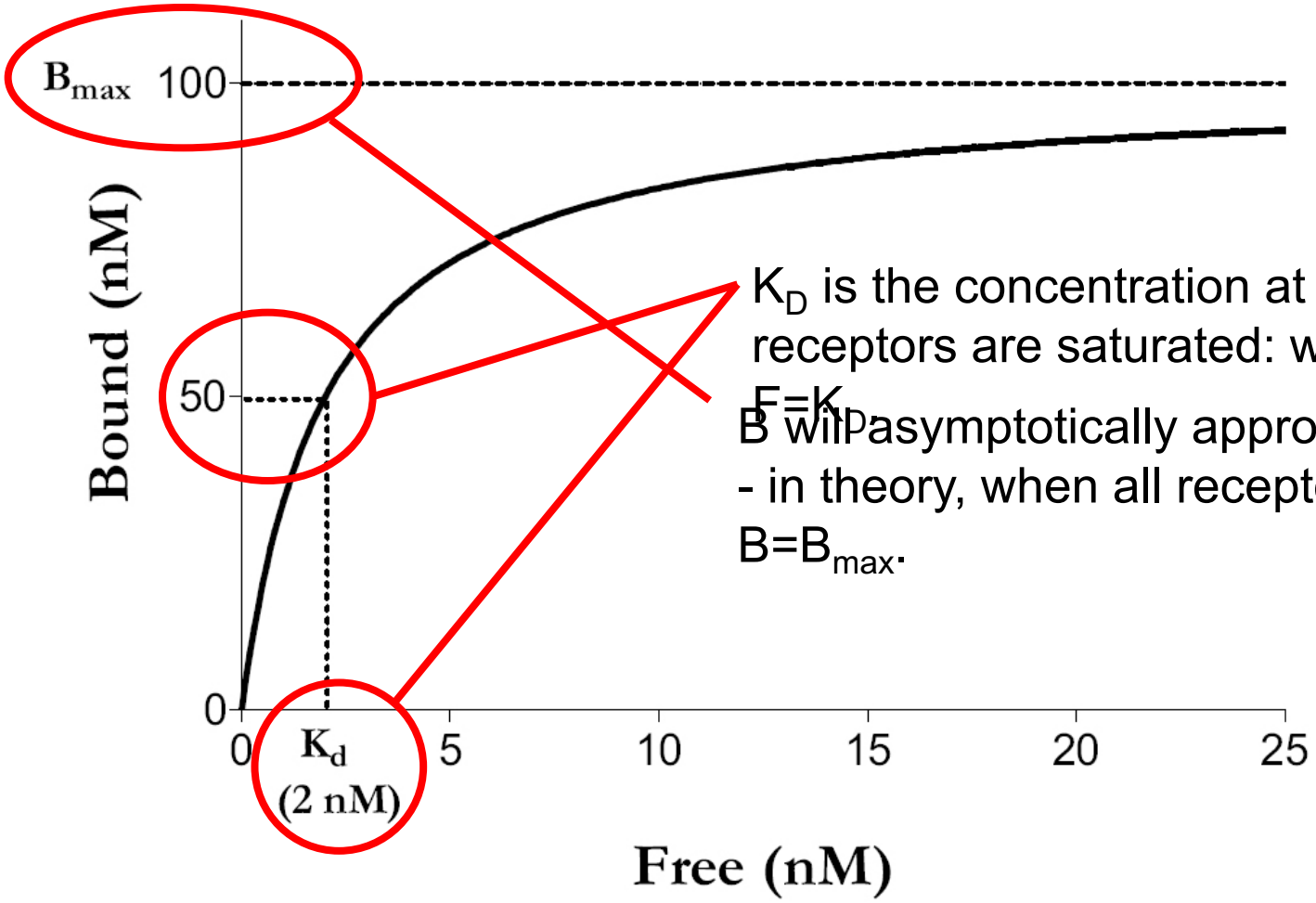
Solving for B:

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

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

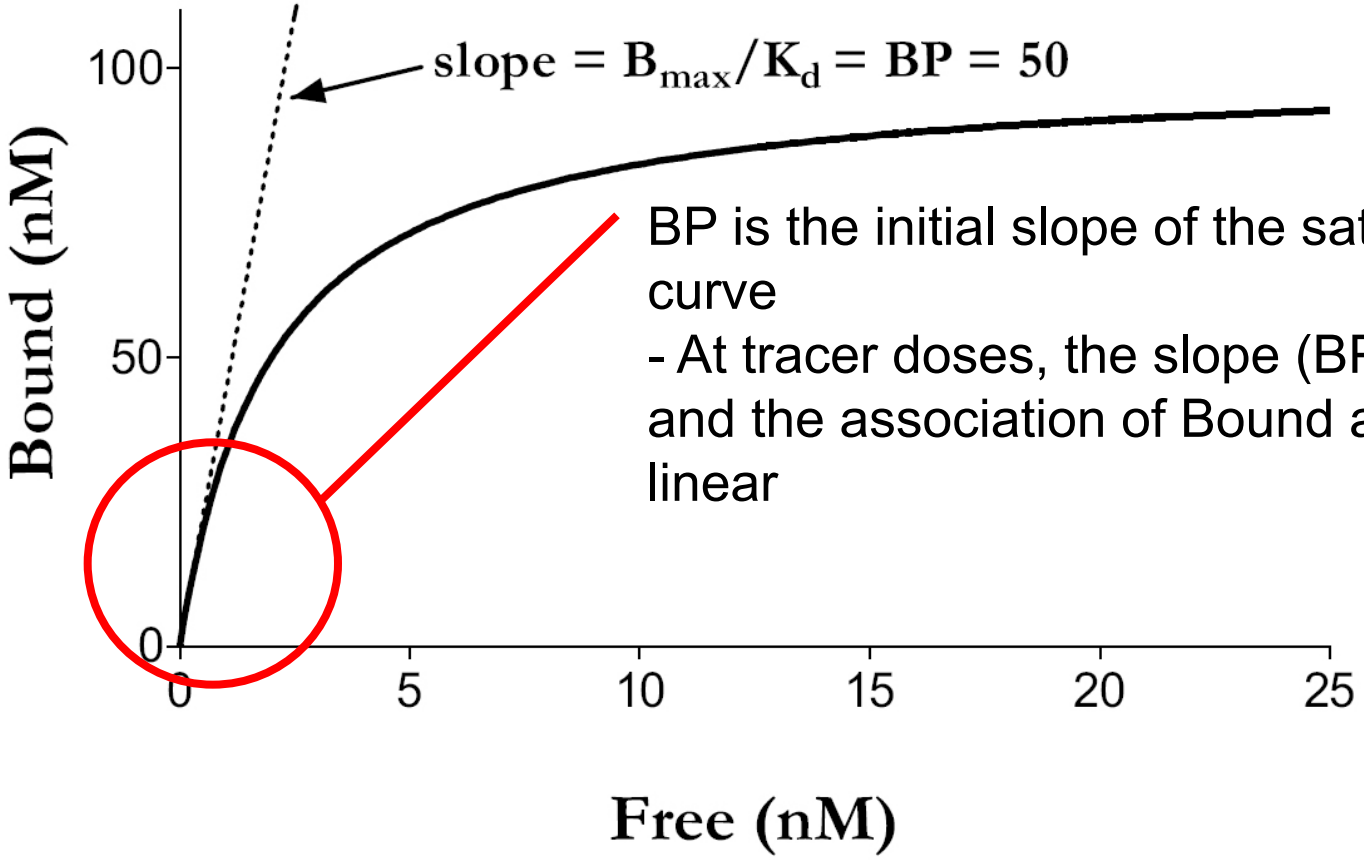
- In PET, minuscule amounts of the radiotracer are injected (”tracer” dose)
- Only <1% of the receptors are occupied (ideally)
- No pharmacological effects expected
- Specific activity (SA, MBq/nmol): amount of labeled molecules relative to unlabeled (”cold”, ”carrier”) molecules
  - High SA: tracer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

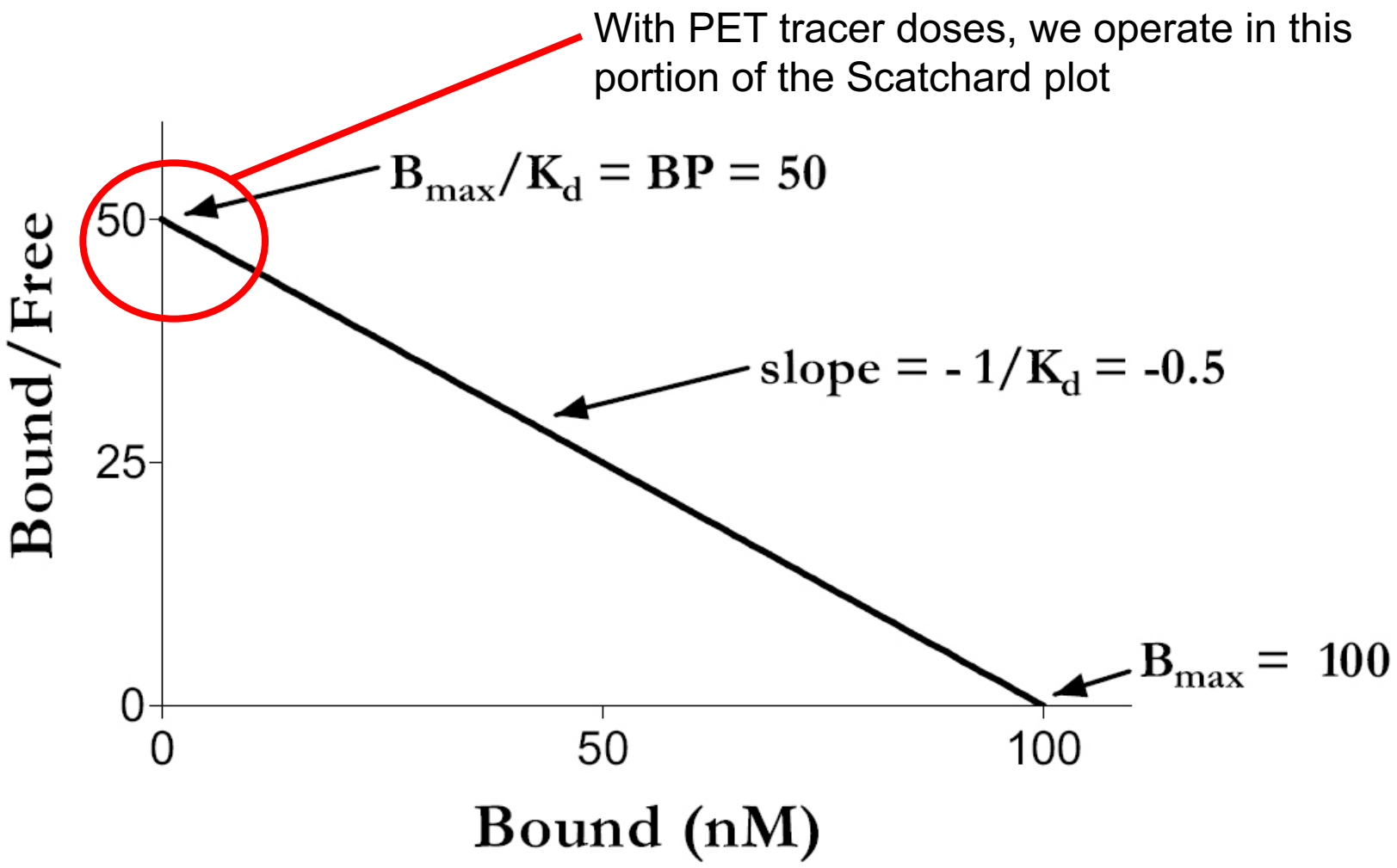


Image courtesy of Robert B. Innis (NIMH, USA)

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

- *In vivo* PET: usually, tracer doses are used ($F << K_D$)
- Thus, receptors are not 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* BP differences

- From a pharmacological point of view, if BP_F differs between individuals, what's 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

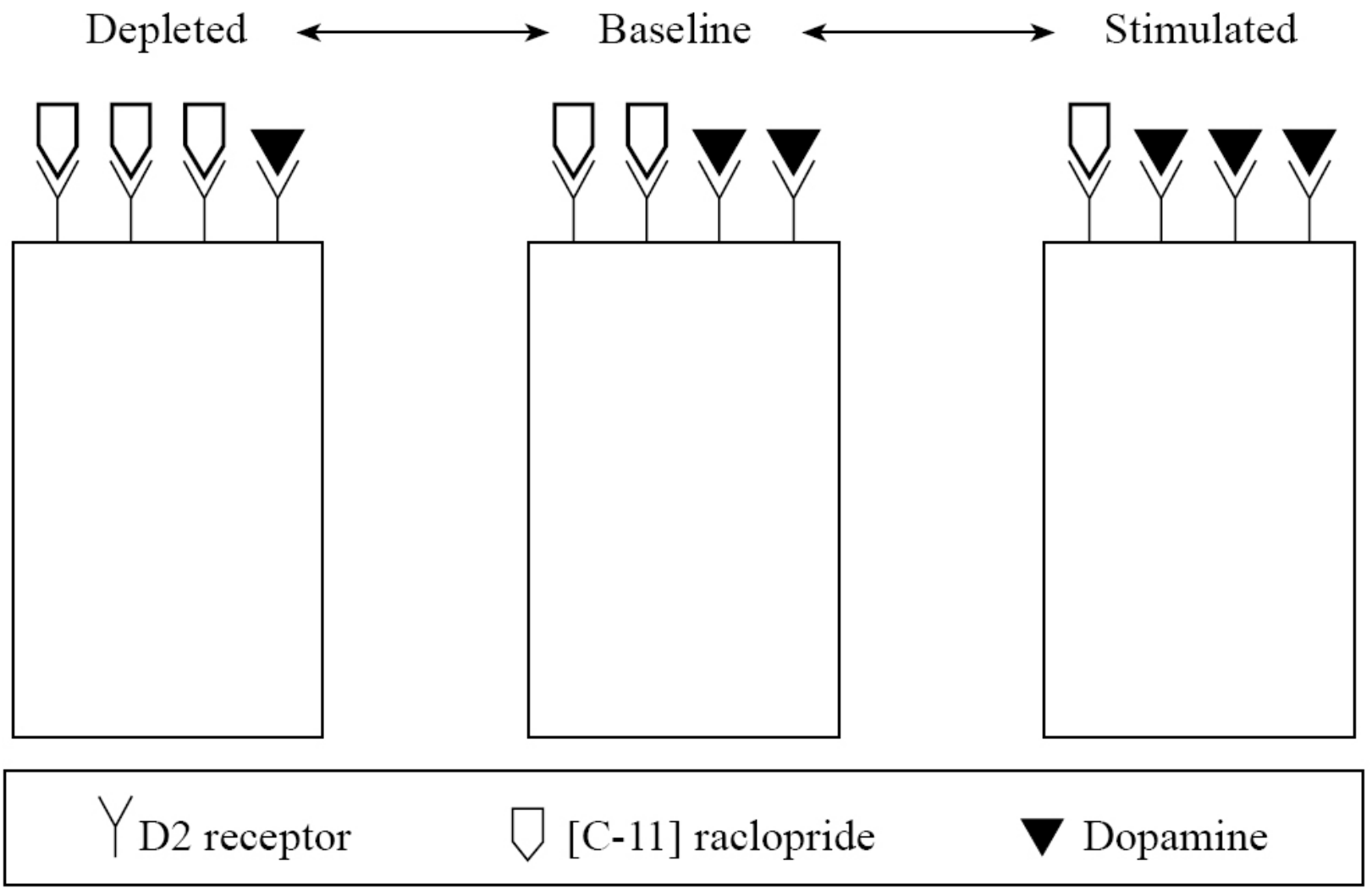


Image: Laruelle & Huang, Q J Nucl Med 2001;45:124-138

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 changes *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:

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

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 $C_B(t)$/SA, 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

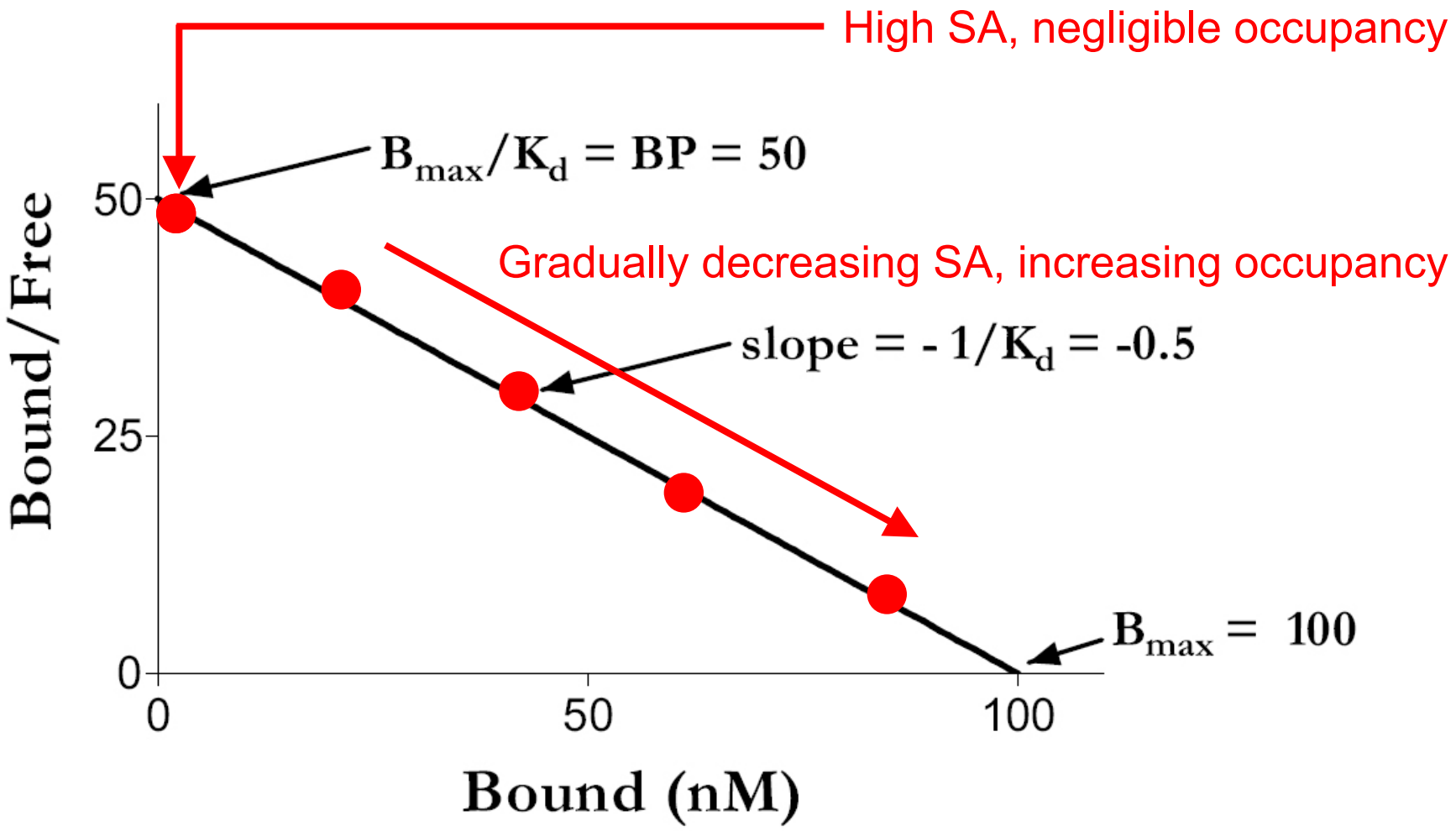
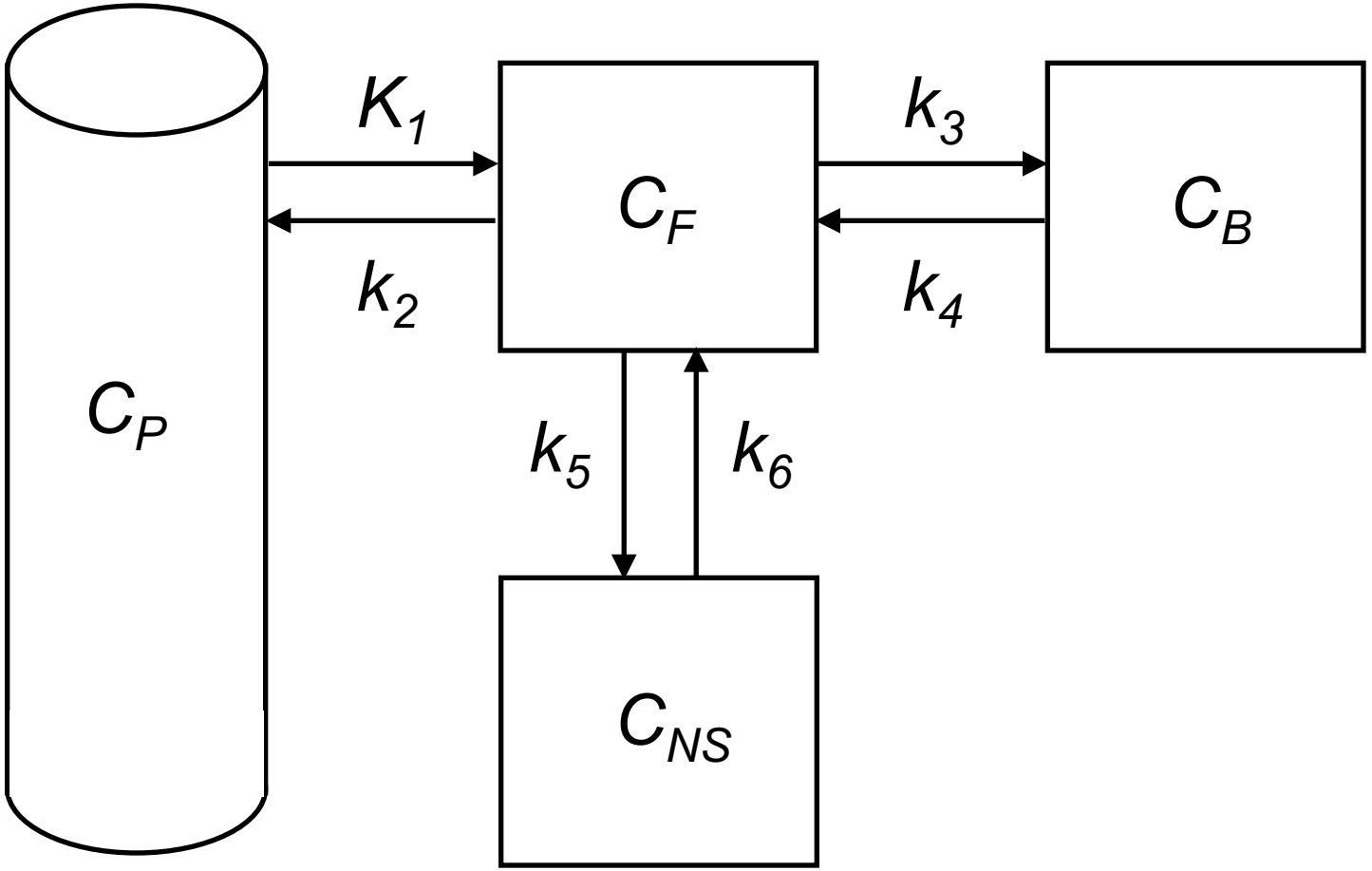


Image courtesy of Robert B. Innis (NIMH, USA)

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…

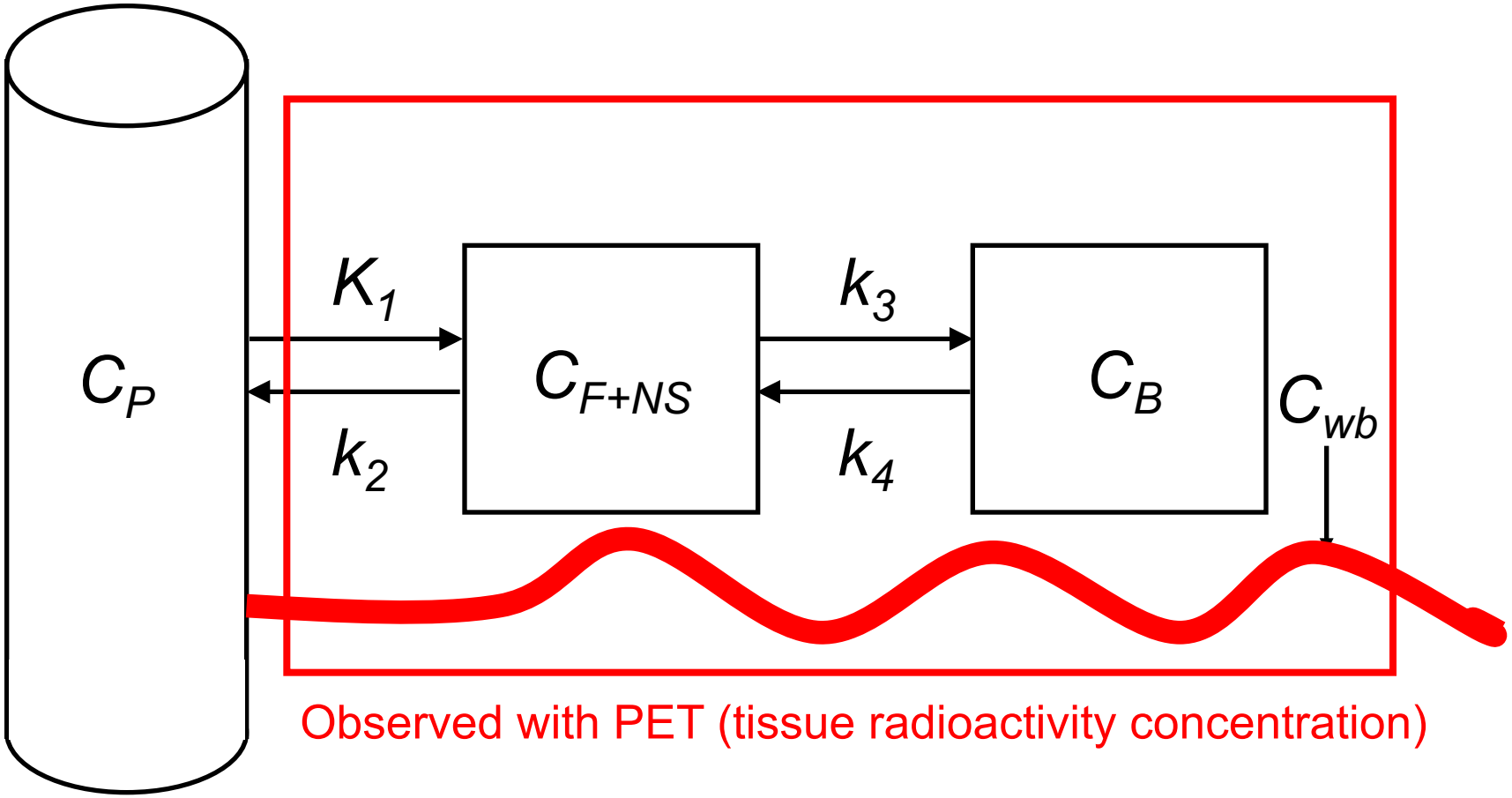
Full compartmental model

- 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



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

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, thus:

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

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

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

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

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

- At equilibrium, no net transfer between plasma and tissue, thus:

$$\frac{dC_T}{dt} = 0 \ ;$$

$$K_1 C_P = k_2 C_T$$

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

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

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

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

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?

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

$$k_4 = k_{off}$$

How do rate constants relate to pharmacological binding parameters?

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

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

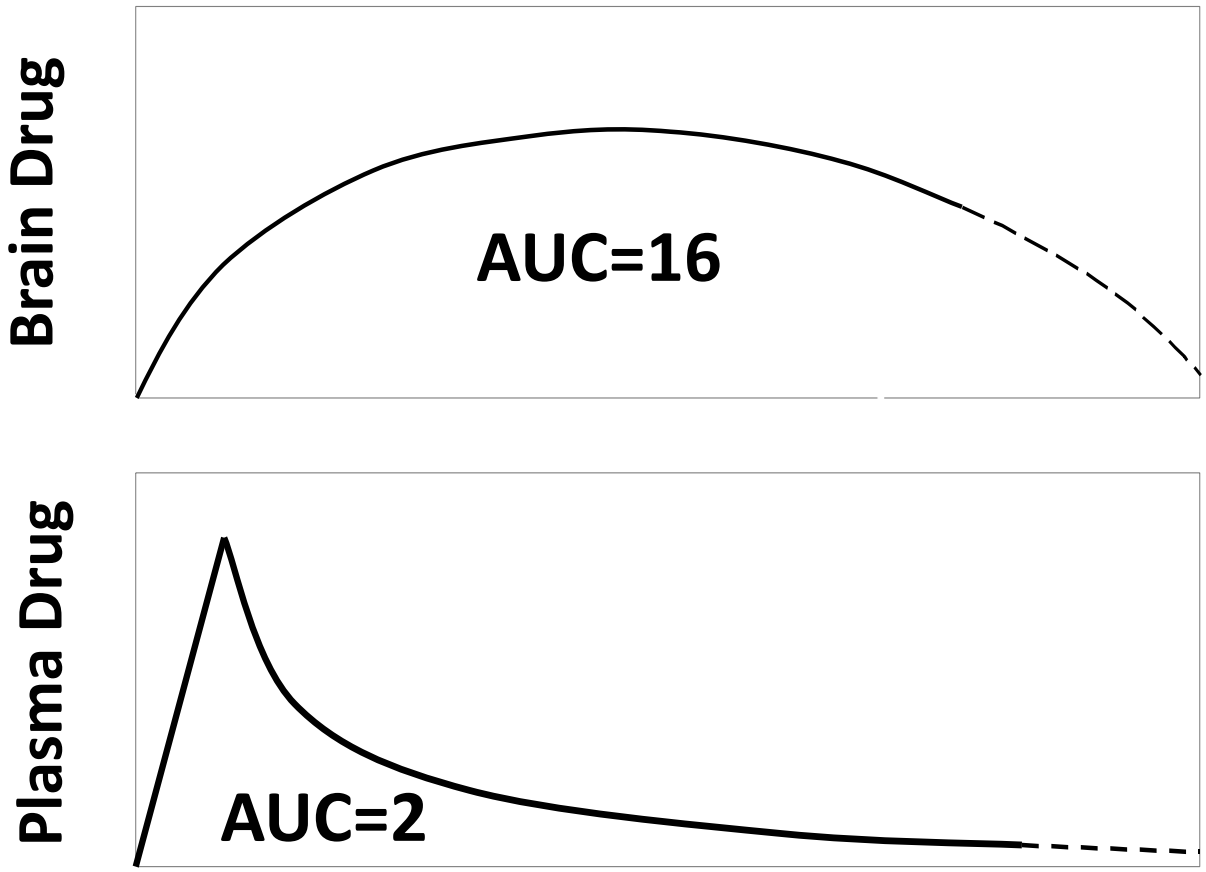
Since

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

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

Distribution Volume (V_T)

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

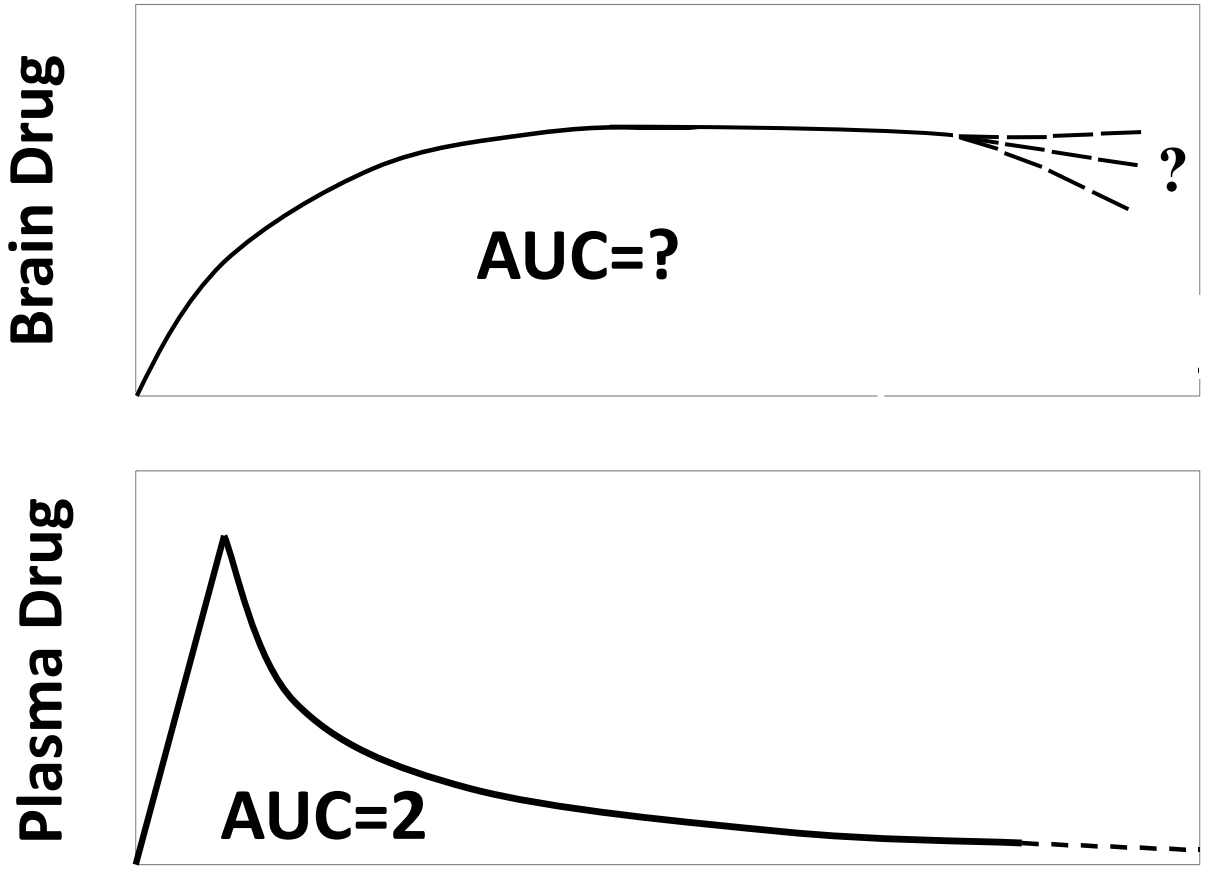


$$V_T = \frac{\text{Area Brain Curve}}{\text{Area Plasma Curve}}$$

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

Distribution Volume (V_T)

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

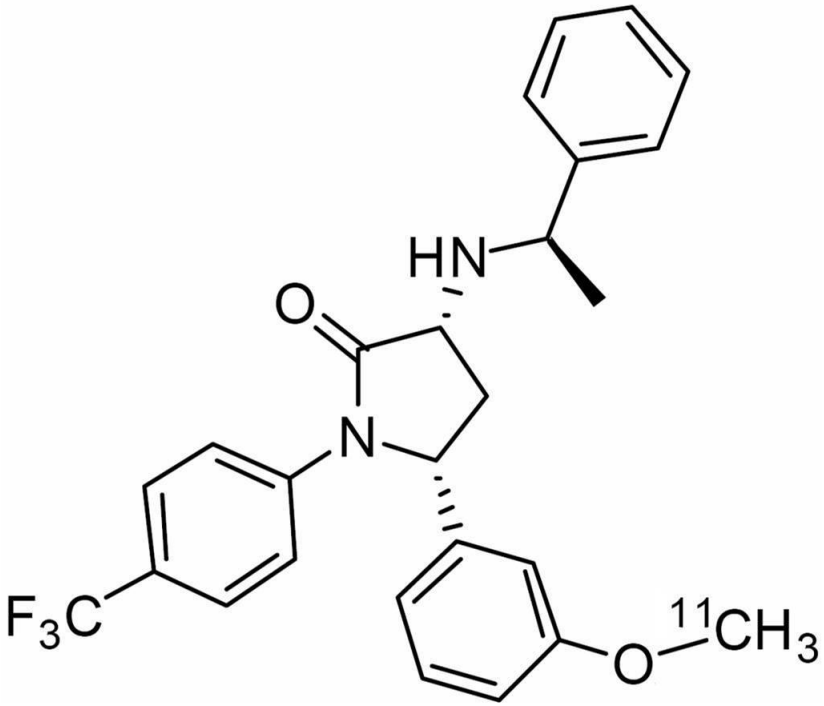


$$V_T = \frac{\text{Area Brain Curve}}{\text{Area Plasma Curve}}$$

$$V_T = \frac{?}{2} = ?$$

Two inverse agonist radioligands for cannabinoid CB_1 receptors

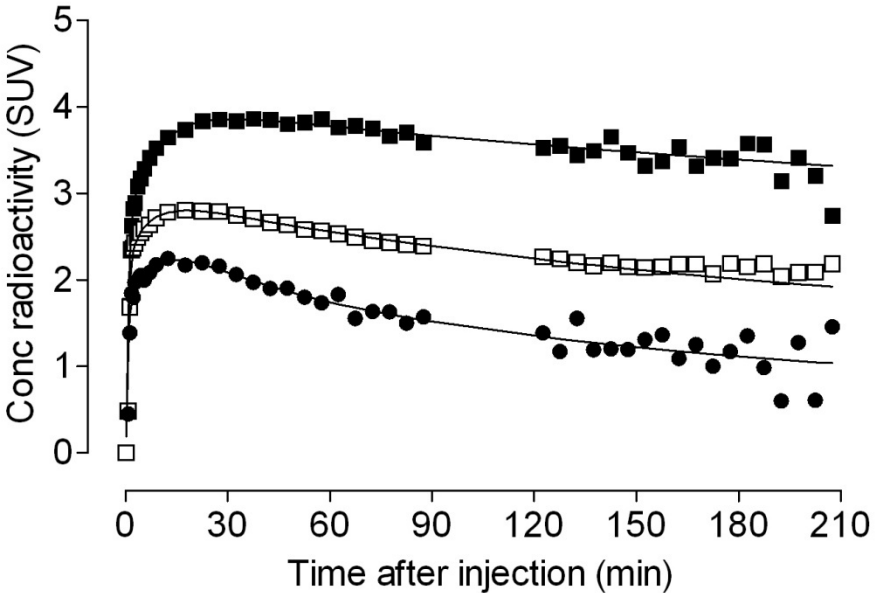
$[^{11}C]$MePPEP



$T_{1/2}$ = 20 min

Two inverse agonist radioligands for cannabinoid CB_1 receptors

[^{11}C]MePPEP



Comparison of ^{11}C-MePPEP and ^{18}F-FMPEP-d_2

	^{11}C-MePPEP	^{18}F-FMPEP-d_2
Radioactive half-life	20.4 min	109.7 min
Distribution volume		
V_T (mL • cm^{-3})	12 – 29	13 – 24
Intersubject variability	**> 50%**	**26%**
Retest variability	15%	14%
Intersubject variability	(*n* = 17)	(*n* = 9)
Retest variability	(*n* = 8)	(*n* = 8)

Terry et al. *J Nucl Med* 2010;51:112-120.

Two inverse agonist radioligands for cannabinoid CB_1 receptors

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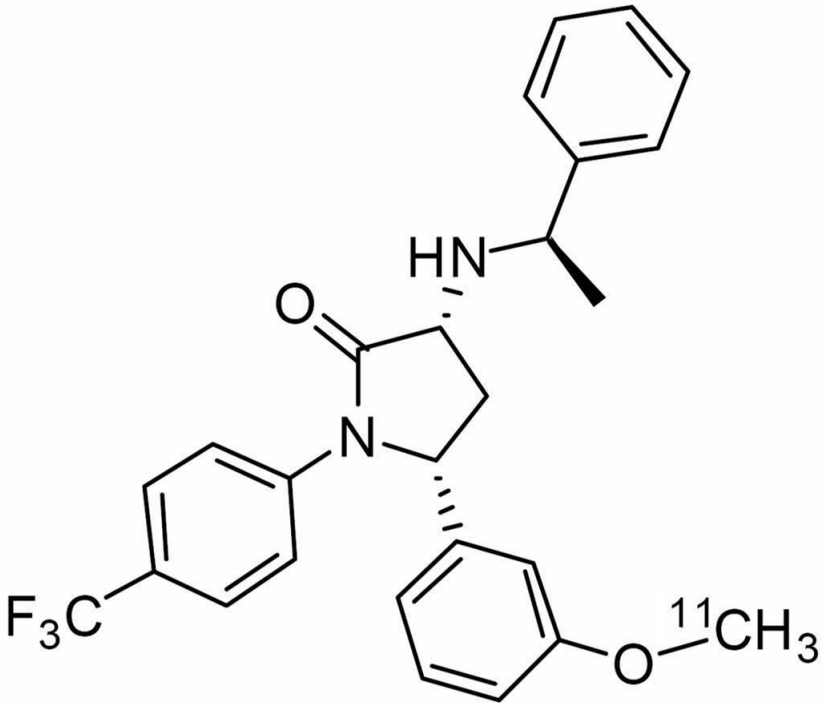

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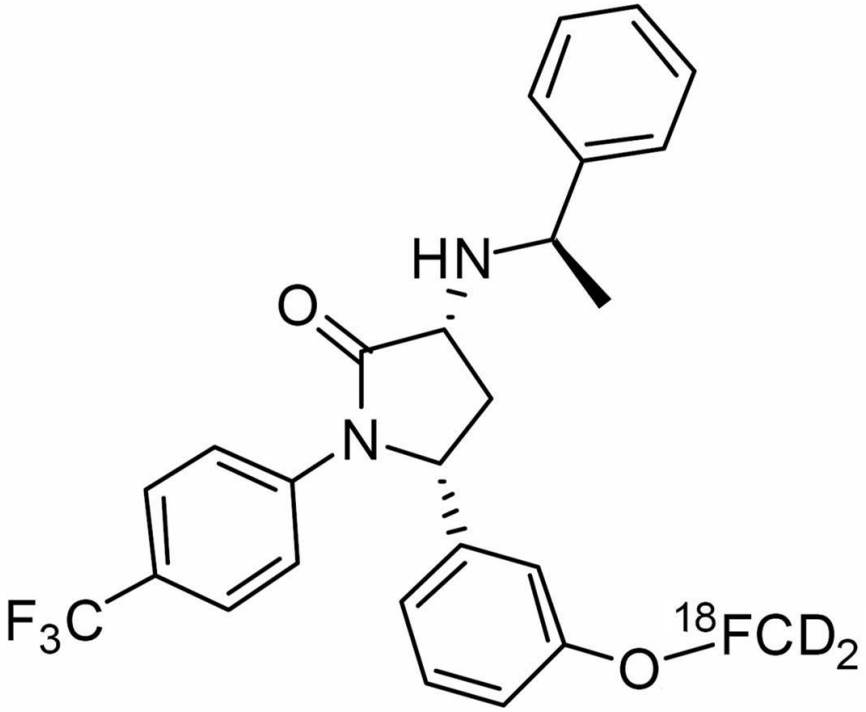
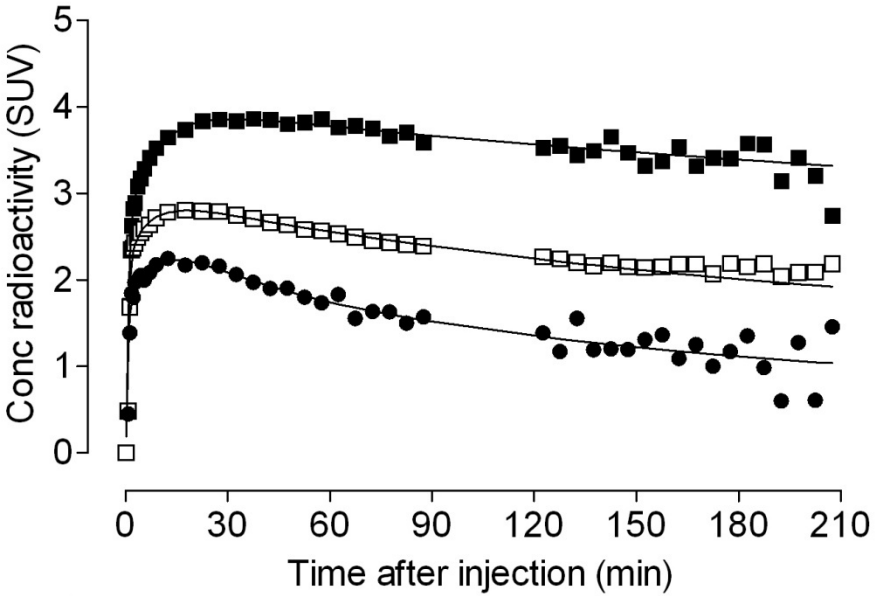
[^{18}F]FMPEP-d_2



$T_{1/2}$ = 110 min

Two inverse agonist radioligands for cannabinoid CB_1 receptors

[^{11}C]MePPEP



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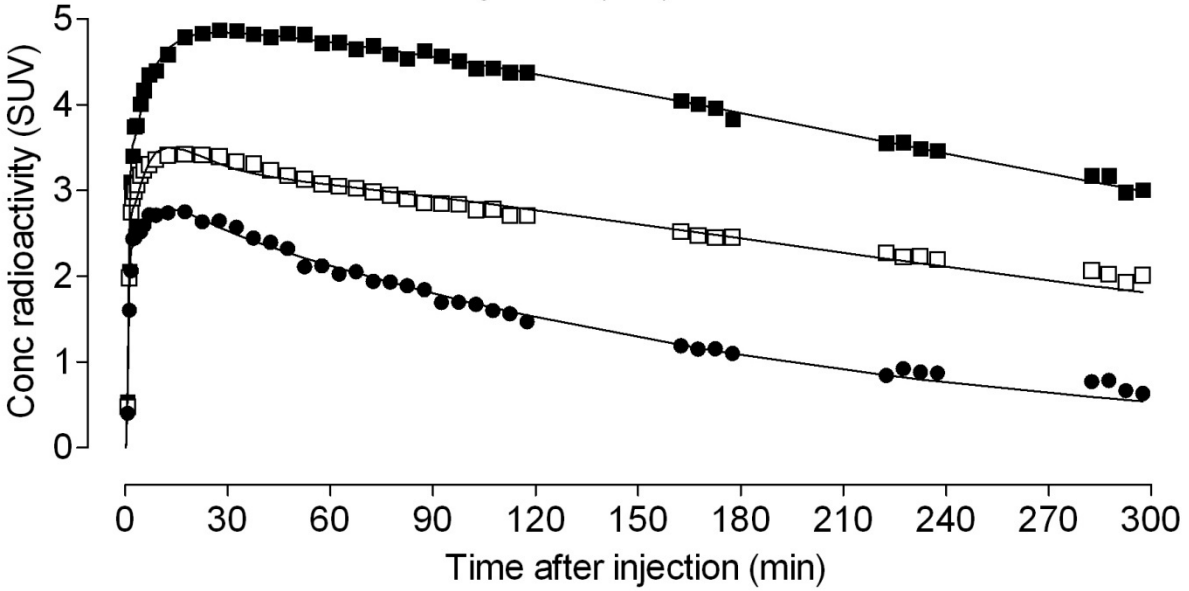

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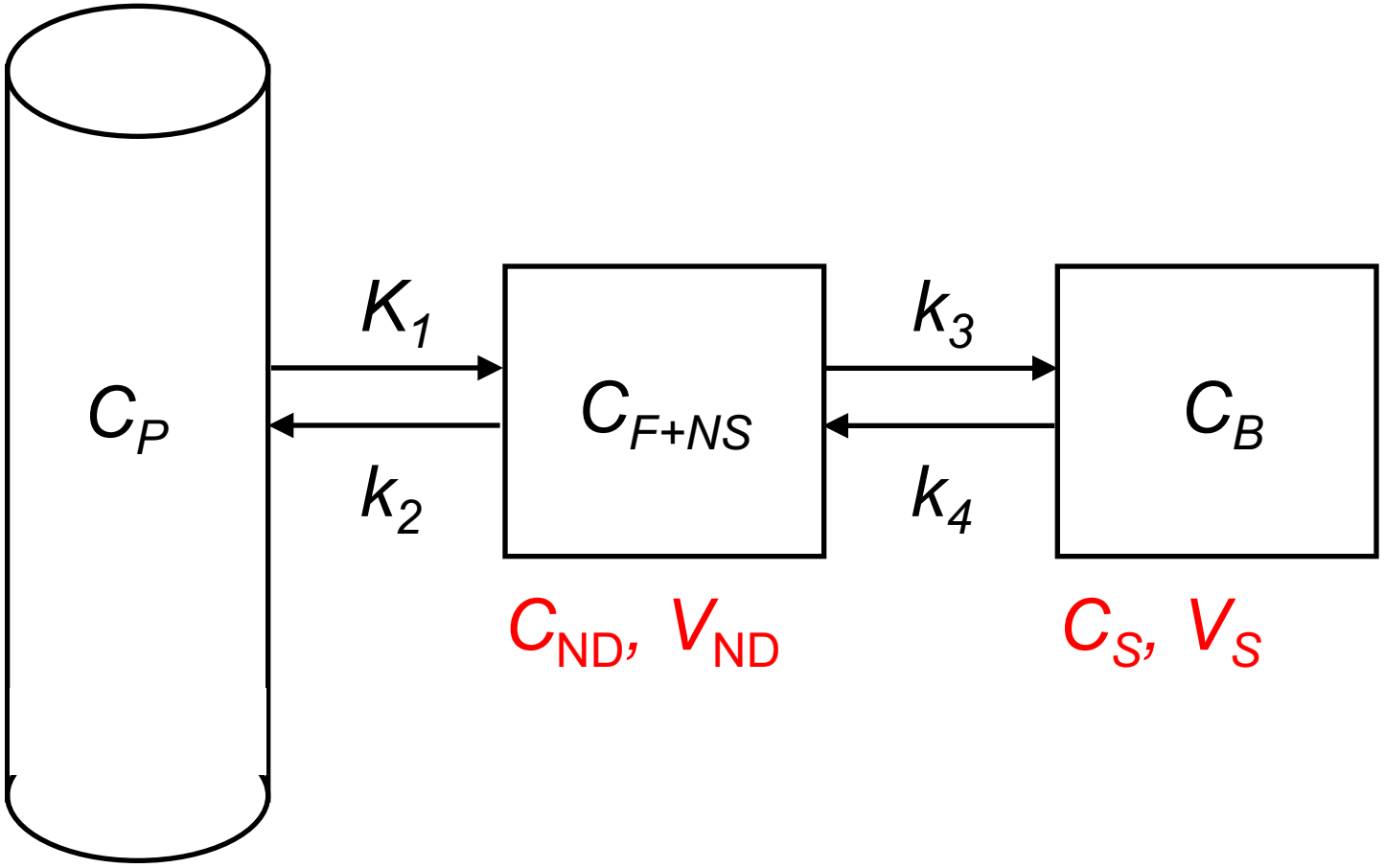


Nomenclature

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

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}

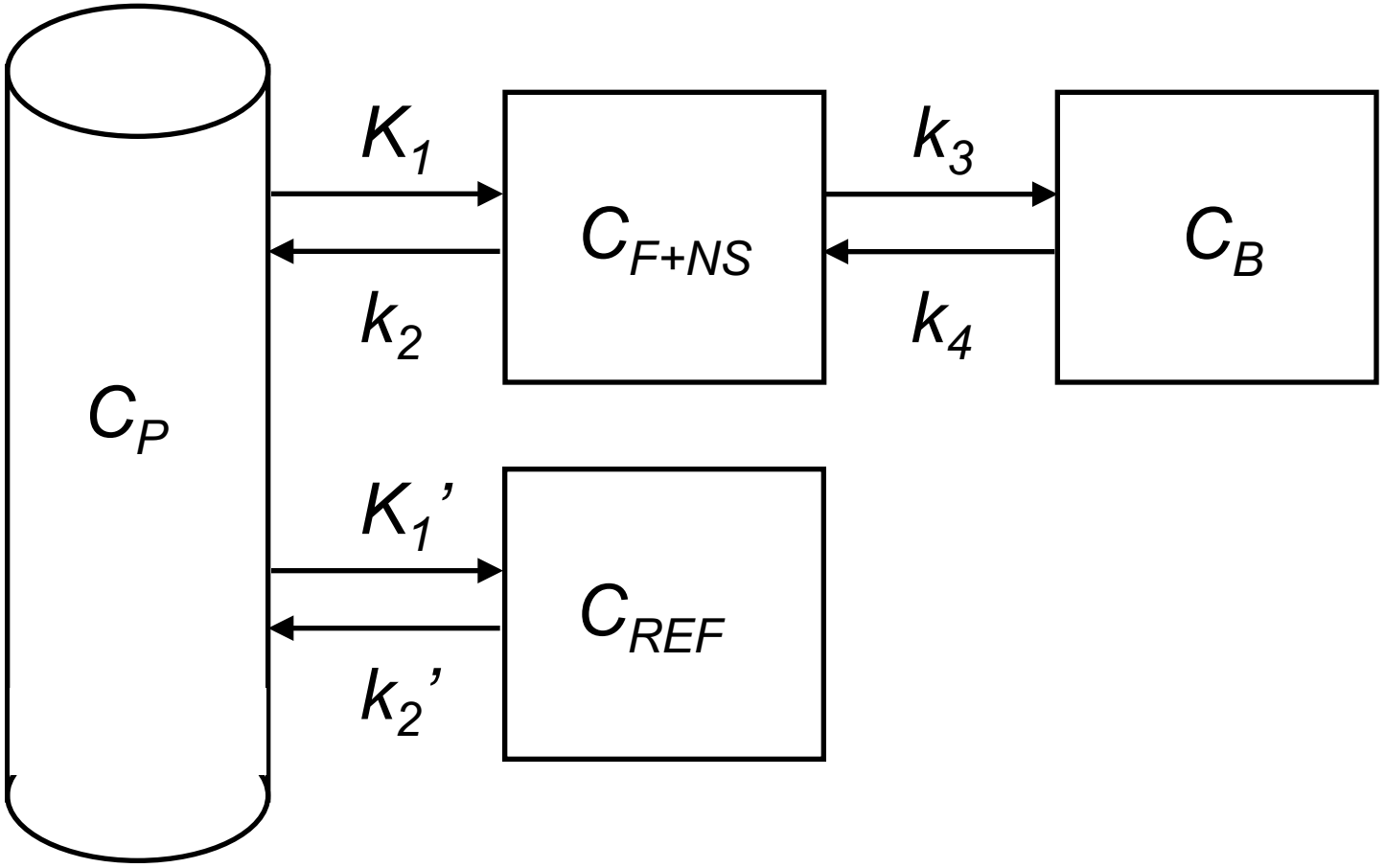
$$\frac{K_1 k_3}{f_P k_2 k_4} = \frac{B_{\max}}{K_D} = BP_F$$

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

$$V_T - V_{ND} = \frac{f_P B_{\max}}{K_D} = BP_P$$

$$\frac{V_T}{V_{ND}} - 1 = \frac{f_{ND} B_{\max}}{K_D} = BP_{ND}$$

Reference region methods



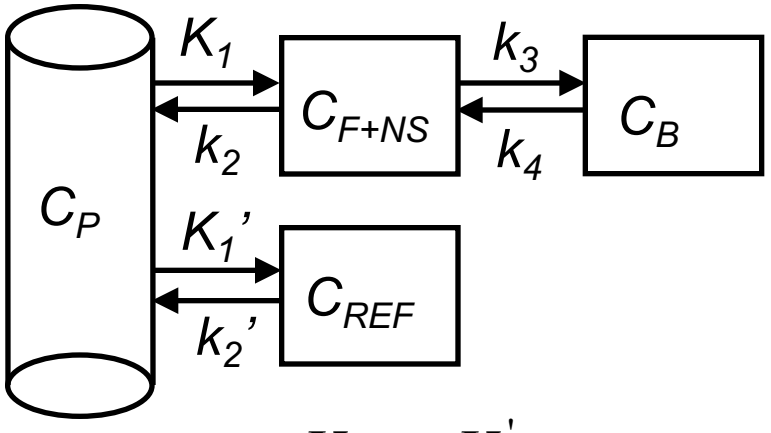
Reference region methods

- 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

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

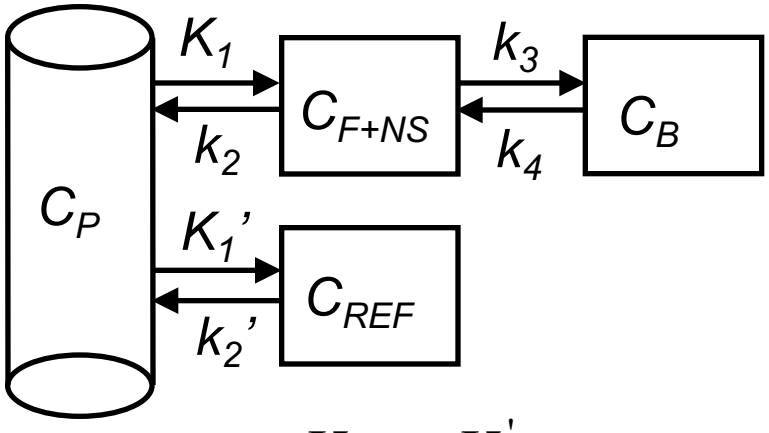
Measured from the reference region

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

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

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

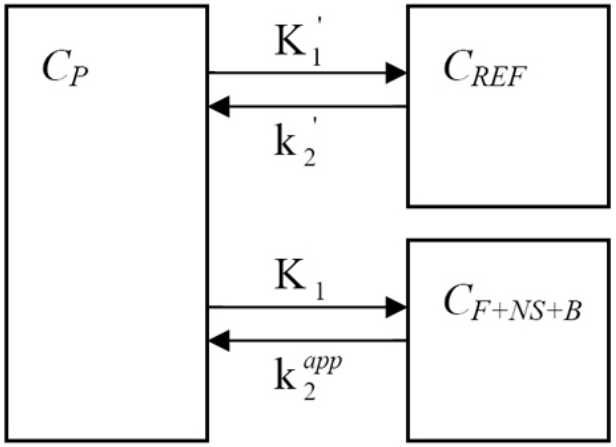
Measured from the reference region

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