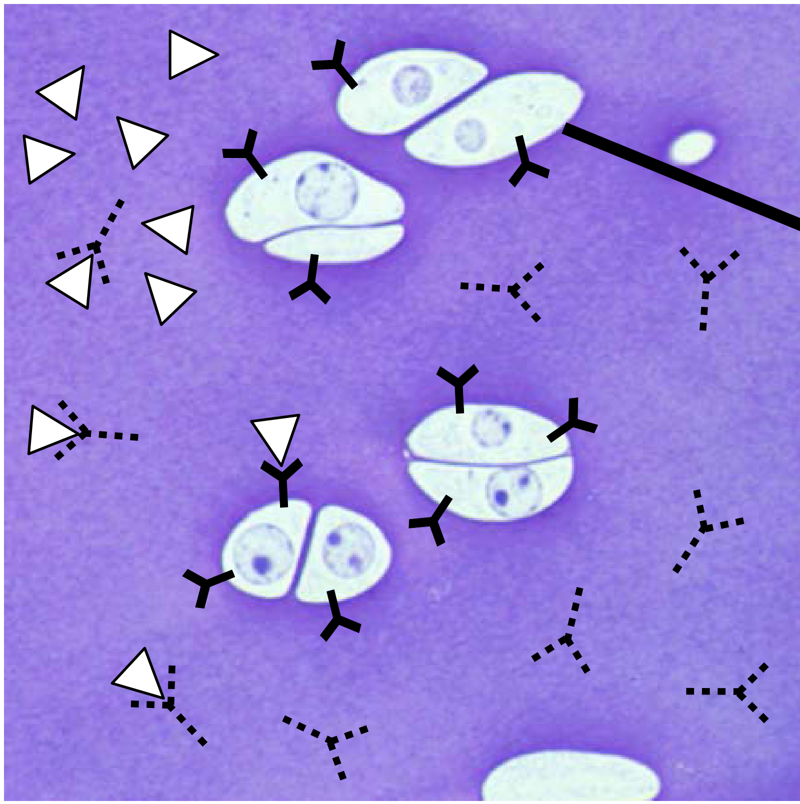
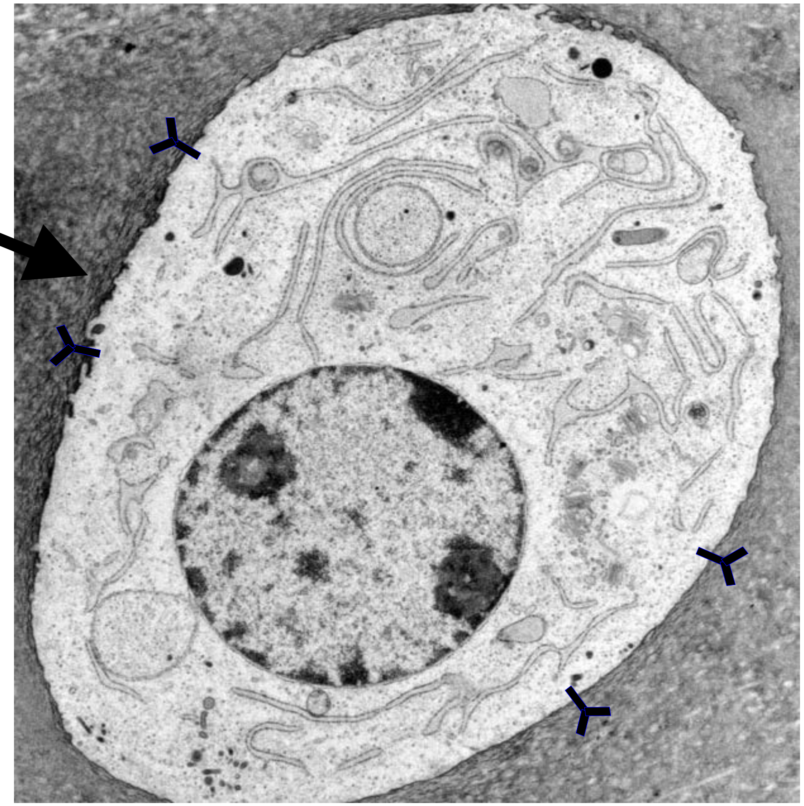
IGF-1 Transport: stimulate anabolic response

(1) Through dense **tissue Extracellular Matrix** (ECM) of **collagens, proteoglycans, glycoproteins**… **to cell**

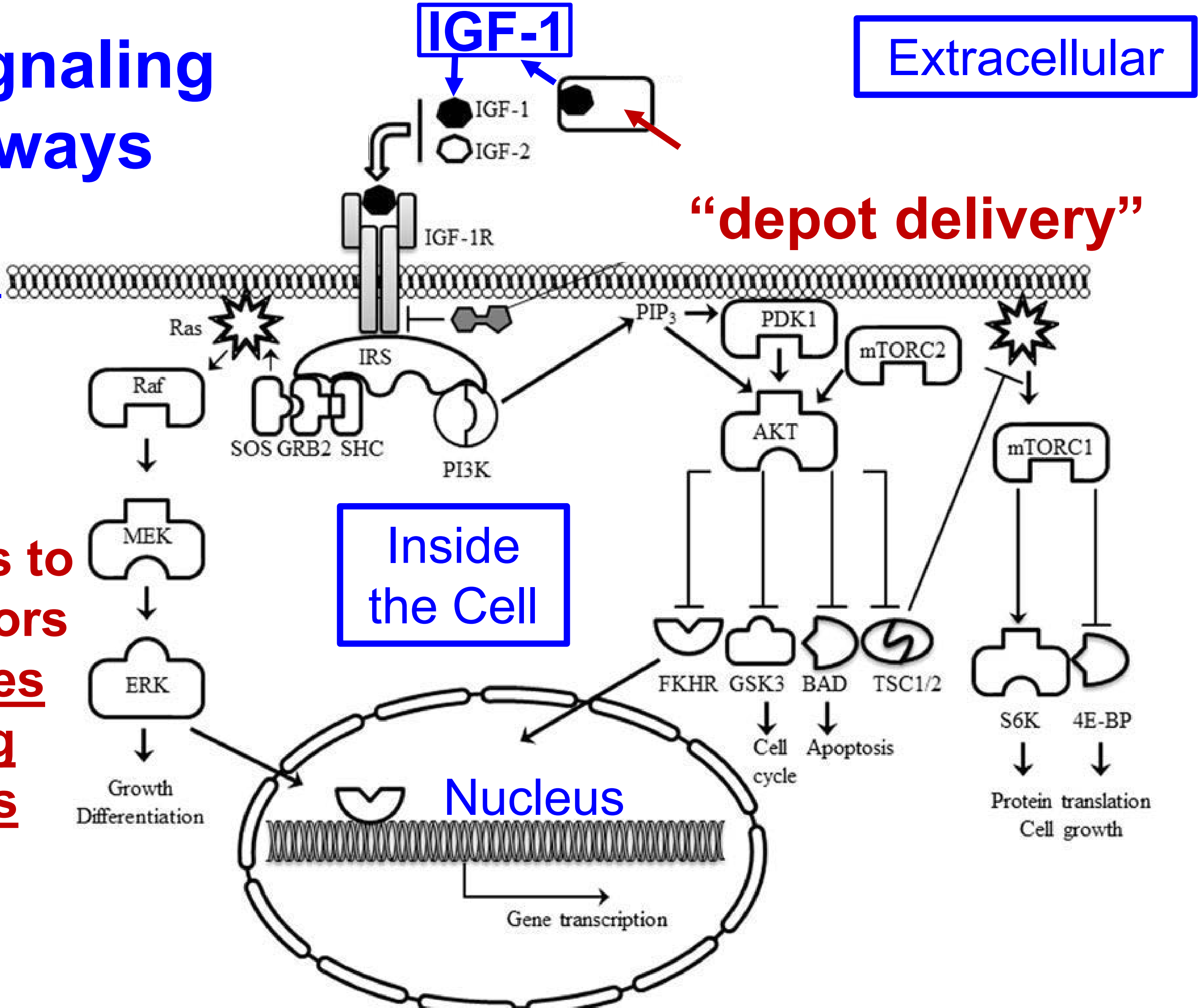




(2) Binding Sites:
- **Cell surface receptors (10,000 / cell!)**
- **IGF binding proteins**

IGF Signaling Pathways

Membrane….

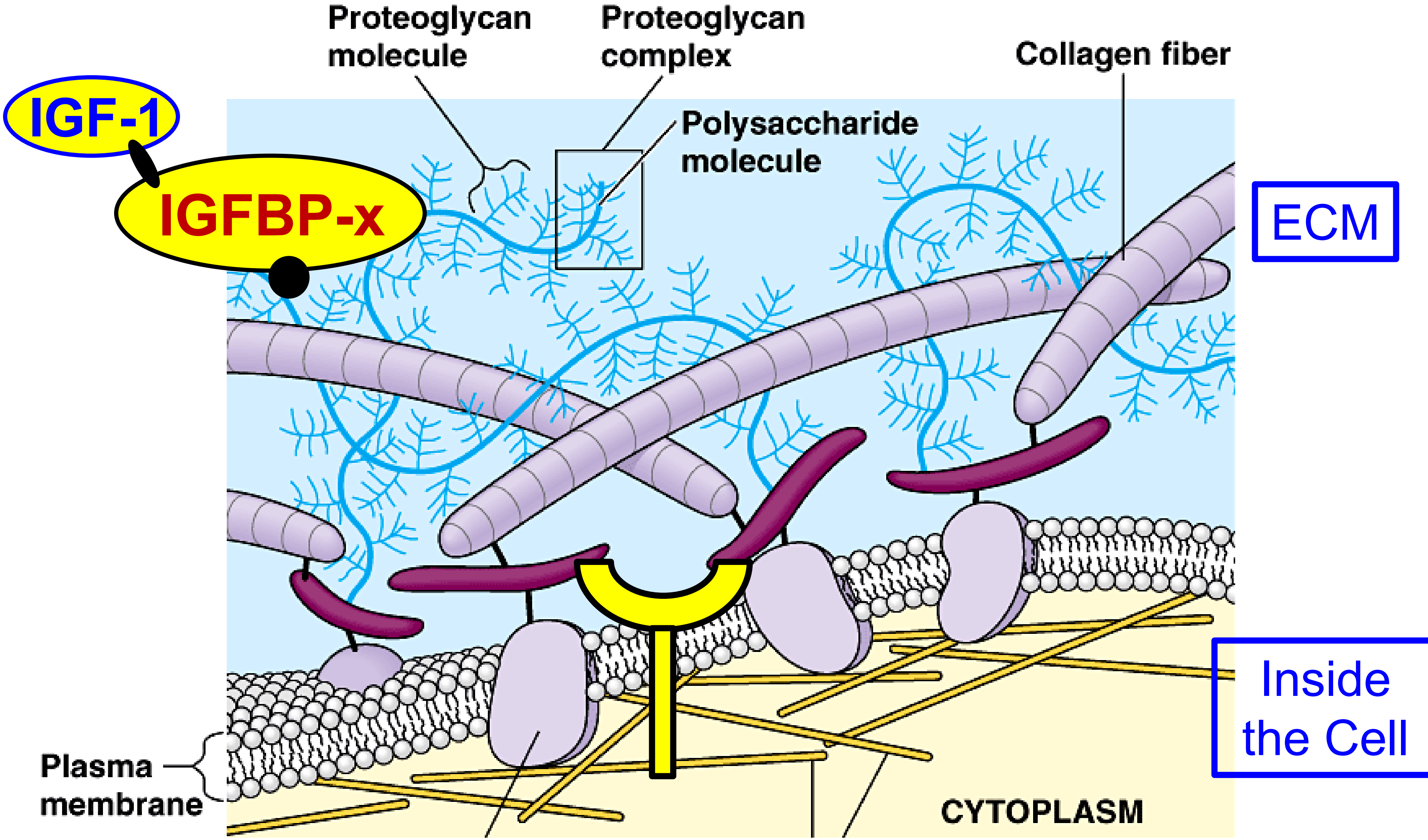


IGF-1 binds to cell receptors → activates signaling cascades

➡stimulates transcription, protein translation, cell cycle progression, cell proliferation and growth, and inhibition of apoptosis.

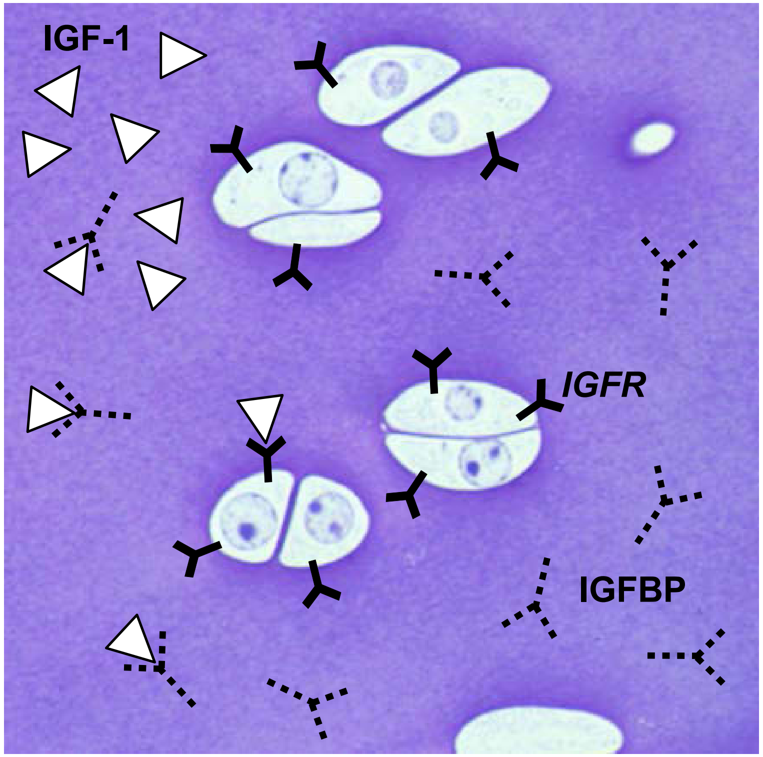
Source: Arcaro, Alexandre. "Targeting the insulin-like growth factor-1 receptor in human cancer." Front Pharmacol 4, no. 30.10 (2013): 3389.

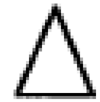
Can IGFBPs bind to ECM macromolecules and inhibit IGF-1 transport, uptake, & cell signaling



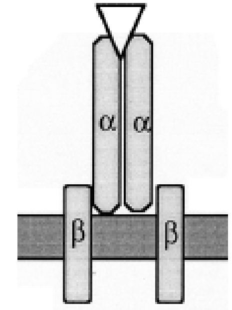
Implications for Drug Delivery: Dose and Timing

IGF-1 System

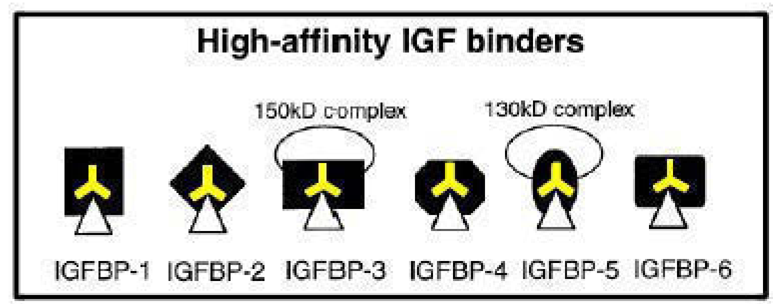


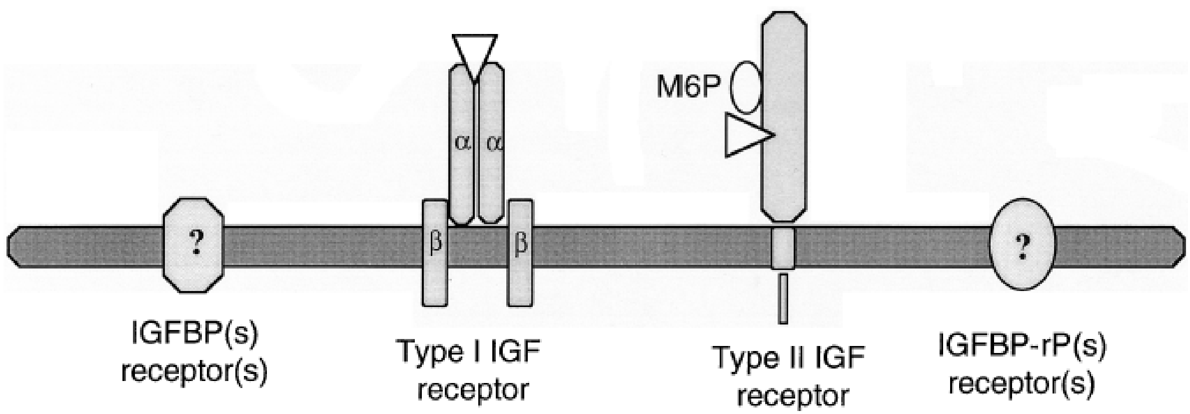


IGF-I/II



Type I IGF receptor

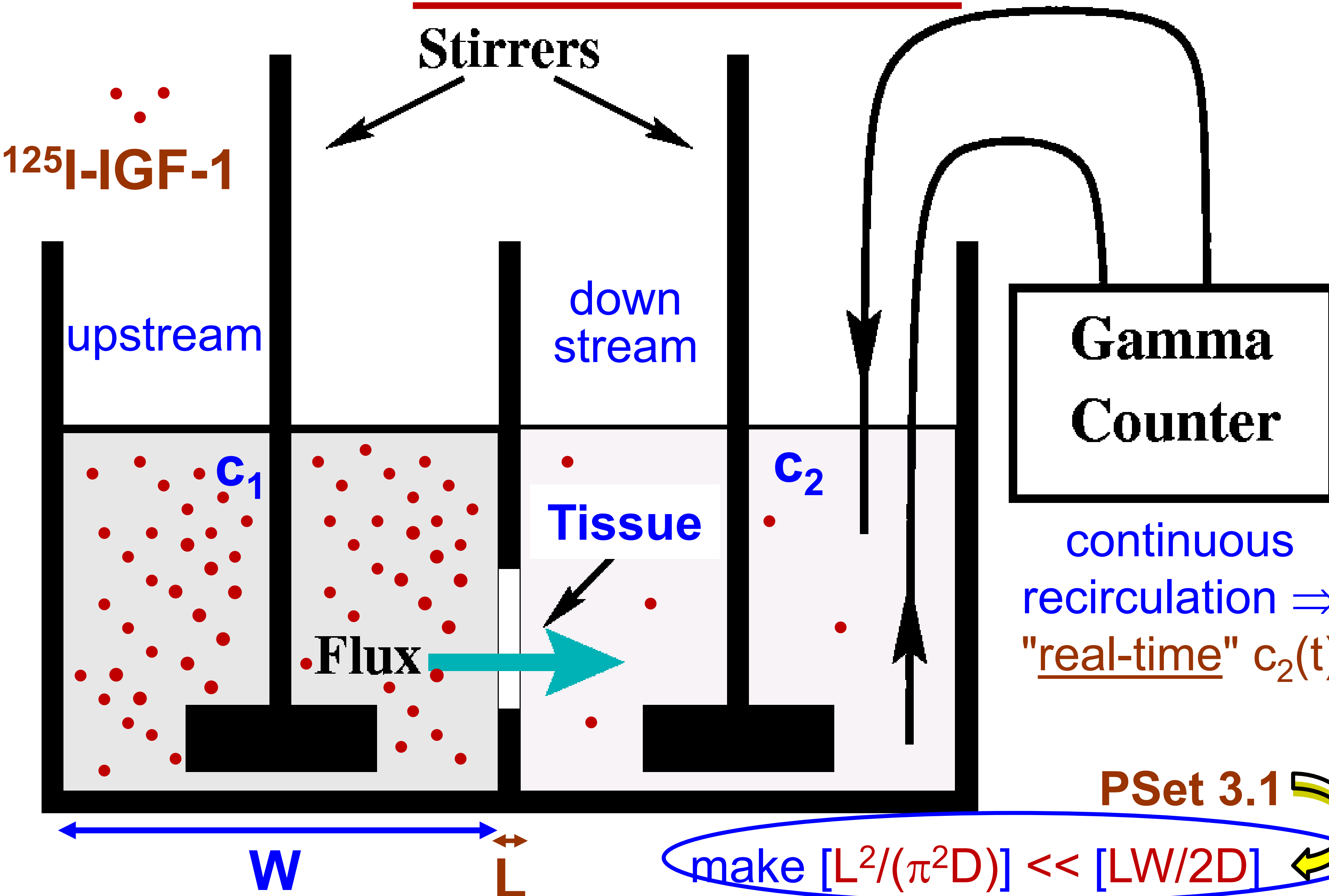




Outside the Cell

Inside the Cell

Transport & Binding within Native Tissue Microenvironment

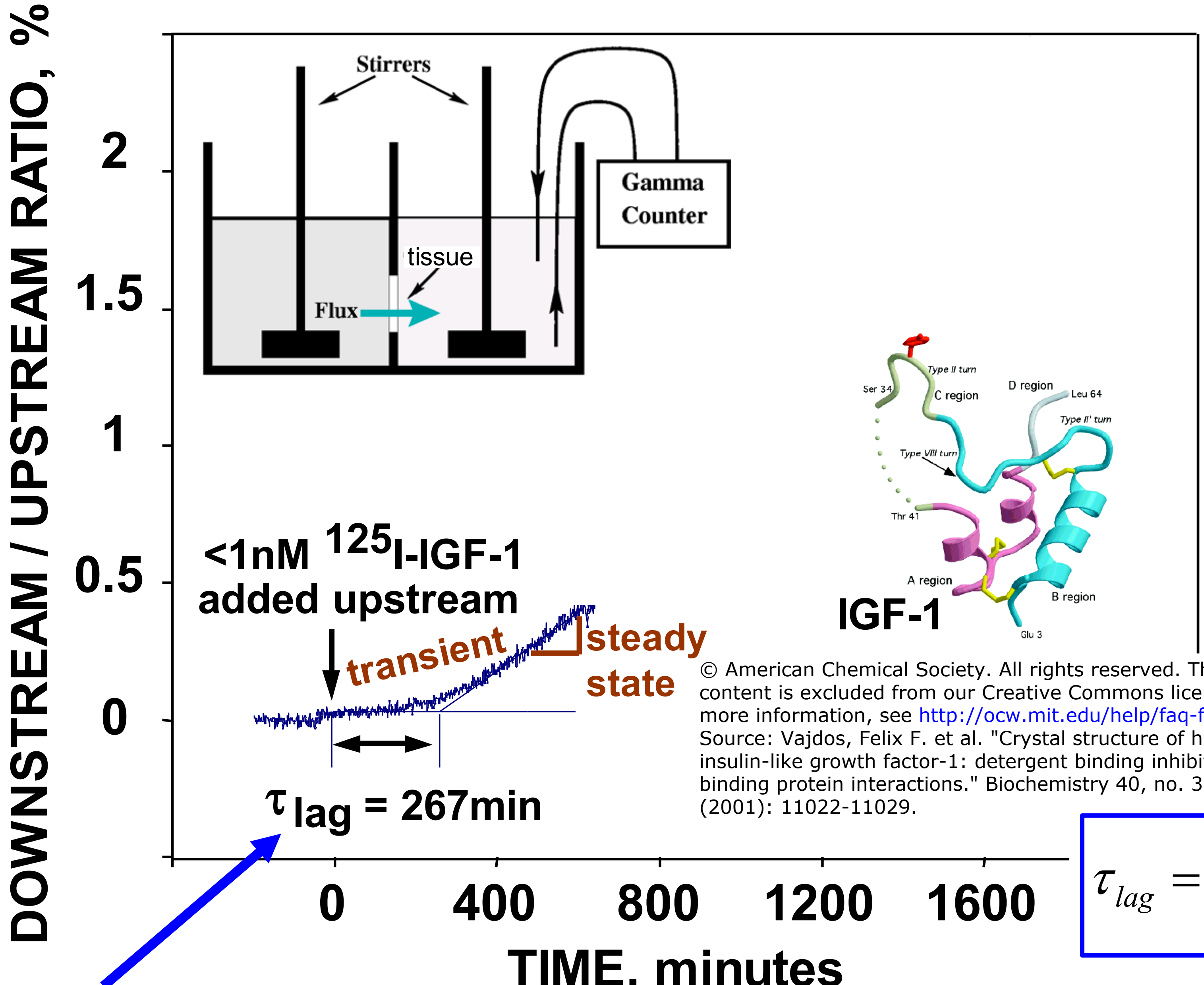


continuous recirculation ⇒ "real-time" $c_2(t)$

PSet 3.1

make $[L^2/(\pi^2 D)] \ll [LW/2D]$

IGFBP-3 Binding Slows entry of IGF-1 into Tissue!



Source: Vajdos, Felix F. et al. "Crystal structure of human insulin-like growth factor-1: detergent binding inhibits binding protein interactions." Biochemistry 40, no. 37 (2001): 11022-11029.

$$\tau_{lag} = \frac{L^2}{6D_{eff}}$$

slow reaction, or slow diffusion compared to reaction???

Last Time:

Reaction (1)

$$\underbrace{\overbrace{\left\{\underbrace{\text{IGF}}_{\text{bound}} * \text{site}\right\}}^{\text{unoccupied}}}_{c_B} \underset{k_{on}}{\overset{k_{off}}{\rightleftharpoons}} \underbrace{\text{IGF}}_{\text{free}\ (c_F)} + \overbrace{\text{site}}^{\text{occupied}}$$

Rate Eqn (2)

$$\frac{\partial c_B}{\partial t} = \left[k_{on} c_F [\text{sites}] - k_{off} c_B\right]$$

binding site density "*n*" (definition) (3)

$$n = \underbrace{\overbrace{[\text{IGF} * \text{site}]}^{c_B}}_{\text{occupied}} + \underbrace{[\text{site}]}_{\text{unoccupied}}$$

Binding "Isotherm" (4)

$$c_B = \frac{n\, c_F}{K_d + c_F}$$

IGF-1

Diffusion thru ECM

Multiple Reactions in ECM

$$\frac{\partial c_1^f}{\partial t} = D_{IGF}\nabla^2 c_1^f - k_{+11} c_1^f c_{BP1}^f + k_{-11} c_{SC11}^f + k_{-1BP} c_{SC11}^f - k_{+11R} c_1^f c_{IR} + k_{-11R} c_{IR1} - k_{+13R} c_1^f c_R + k_{-13R} c_{R1} - k_{-1IGF} c_1^f \qquad (1)$$

(Coupled PDEs)
(***x***,*t*)

IGFBPs

$$\frac{\partial c_2^f}{\partial t} = D_{IGF}\nabla^2 c_2^f - k_{+21} c_2^f c_{BP1}^f + k_{-21} c_{SC21}^f + k_{-1BP} c_{SC21}^f - k_{+22} c_2^f c_{BP2}^f + k_{-22} c_{SC22}^f + k_{-2BP} c_{SC22}^f - k_{+21R} c_2^f c_{IR} + k_{-21R} c_{IR2} - k_{+22R} c_2^f c_{IIR} + k_{-22R} c_{IIR2} - k_{-1IGF} c_2^f \qquad (2)$$

Last Time:

Reaction (1)

$$\underbrace{\{\overbrace{\underbrace{\mathrm{IGF}}_{\text{bound}} * \mathrm{site}}^{\text{unoccupied}}\}}_{c_B} \underset{k_{on}}{\overset{k_{off}}{\rightleftharpoons}} \underbrace{\underbrace{\mathrm{IGF}}_{\text{free}}}_{c_F} + \overbrace{\mathrm{site}}^{\text{occupied}}$$

Rate Eqn (2)

$$\frac{\partial c_B}{\partial t} = \left(k_{on} c_F [\mathrm{sites}] - k_{off} c_B\right) = 0 \text{ in equilibrium}$$

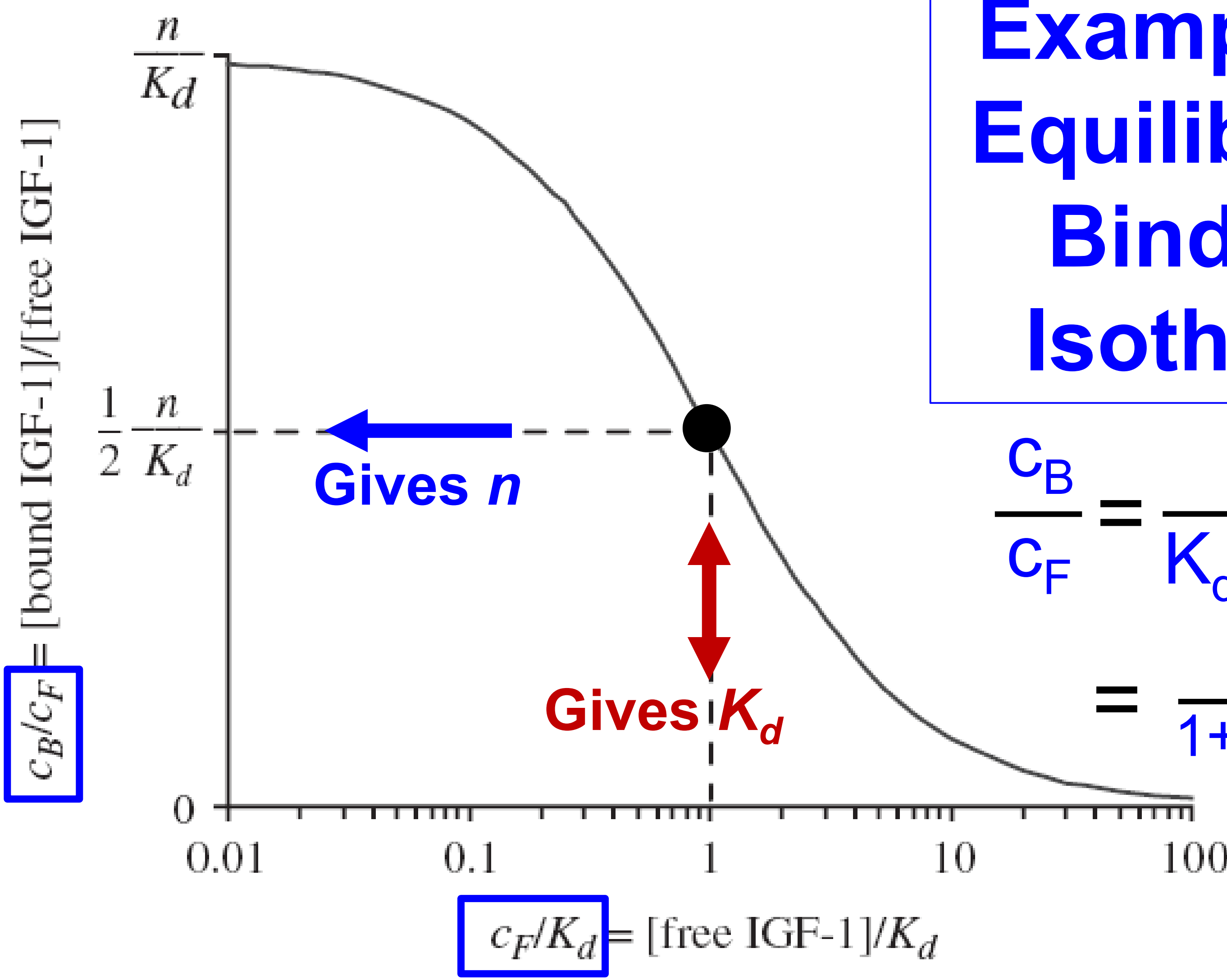
binding site density "n" (definition) (3)

$$n = \overbrace{\underbrace{[\mathrm{IGF} * \mathrm{site}]}_{\text{occupied}}}^{c_B} + \underbrace{[\mathrm{site}]}_{\text{unoccupied}}$$

Binding "Isotherm" (4)

$$c_B = \frac{n\, c_F}{K_d + c_F}$$

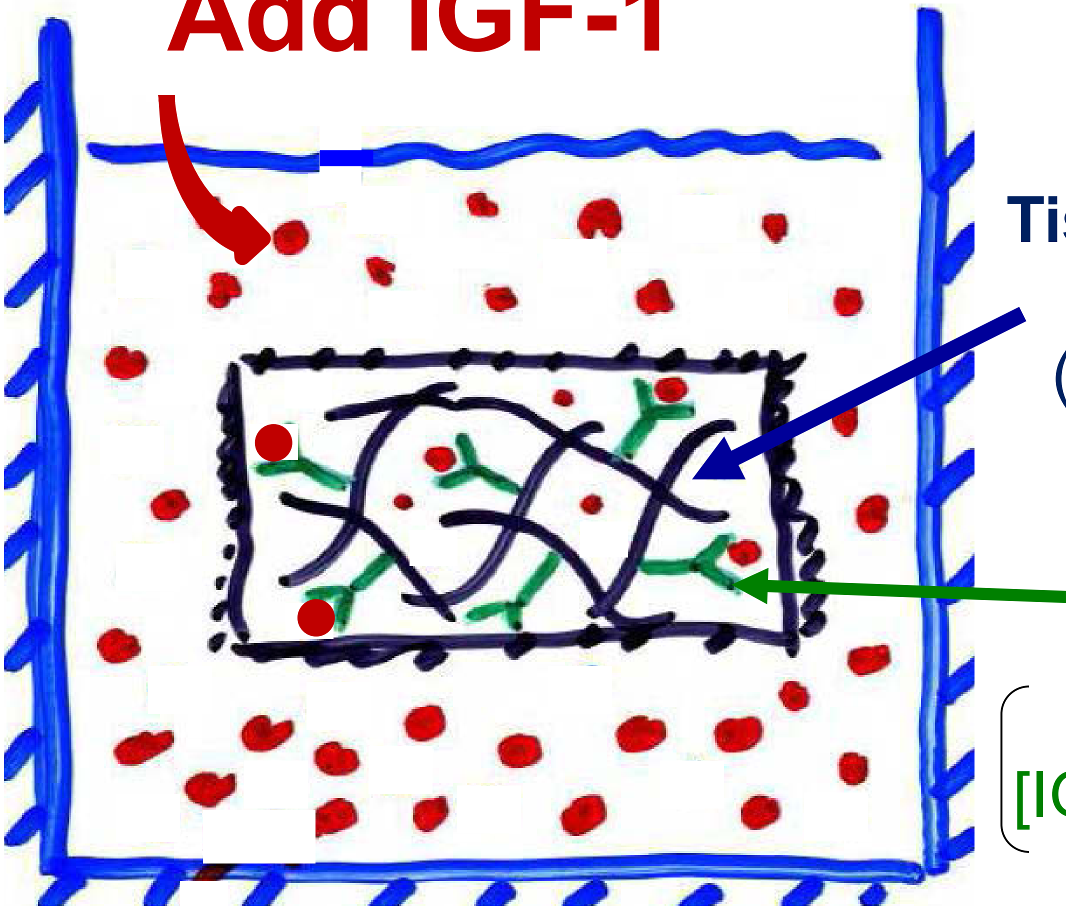
Example of Equilibrium Binding Isotherm



$$\frac{c_B}{c_F} = \frac{n}{K_d + c_F} = \frac{n/K_d}{1 + c_F/K_d}$$

(Bound IGF-1 / Free IGF-1) versus free IGF normalized to K_d

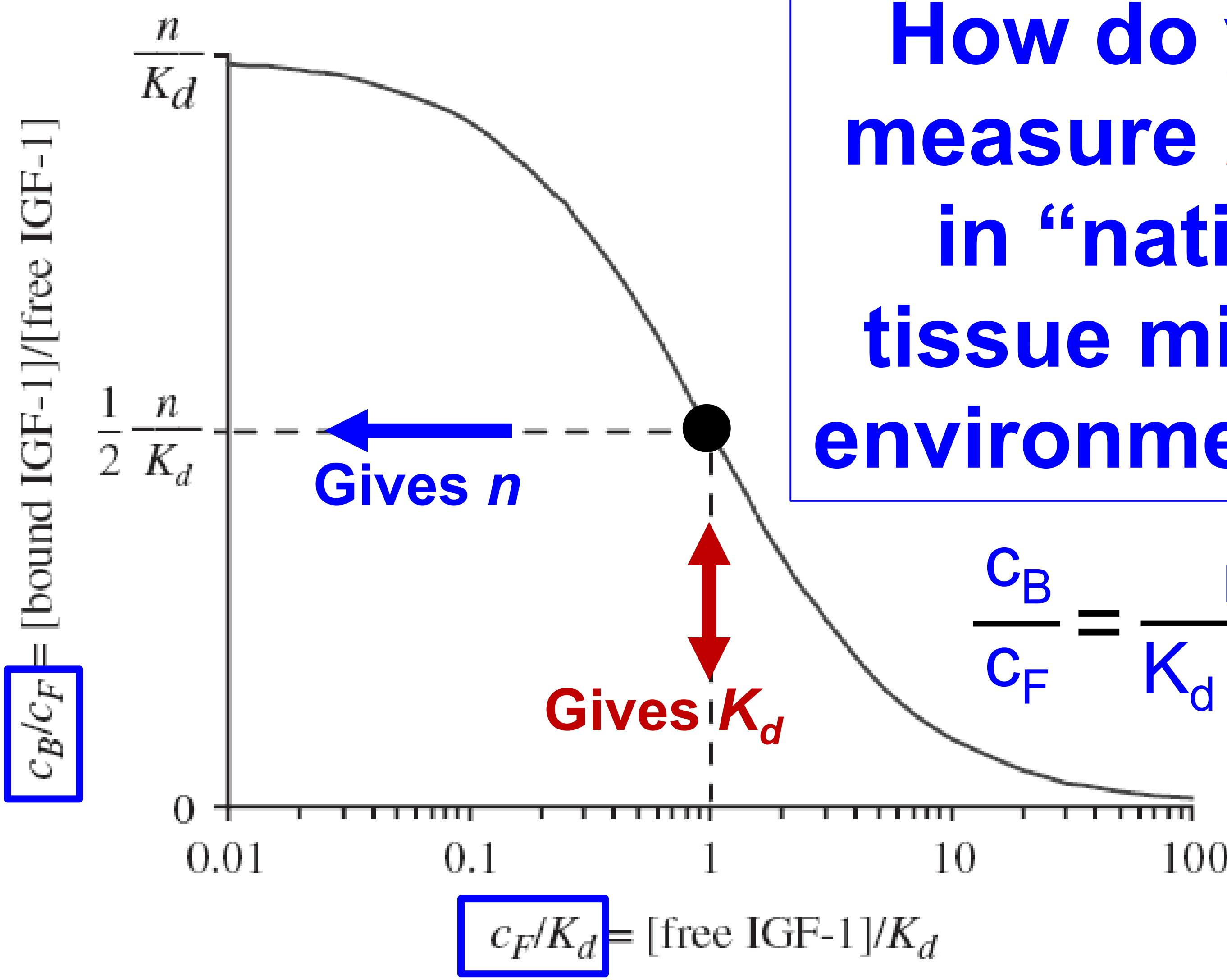
Add IGF-1



Tissue in medium at equilibrium (PBS or DMEM...)

IGFBP-"x"

<u>Assuming</u>:
[IGFBP-x] >> [IGF-R]

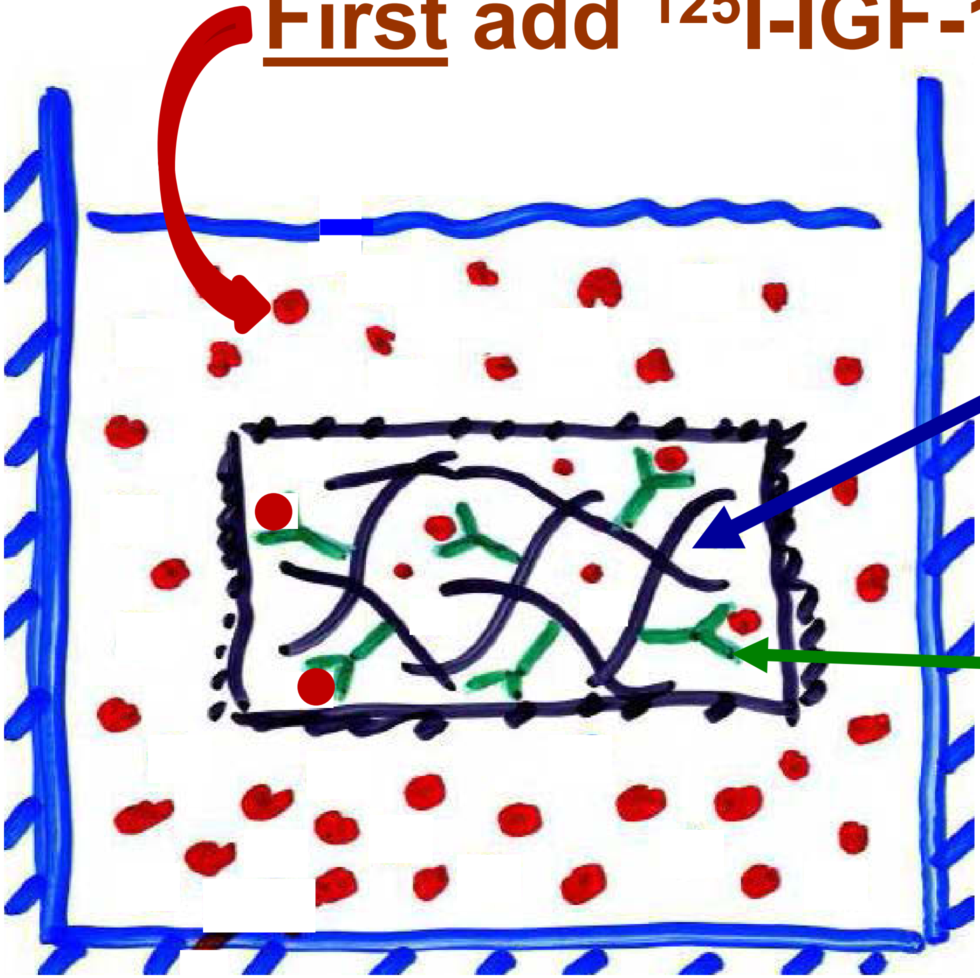


How do you measure n, K_d in "native tissue micro-environment" ?

$$\frac{c_B}{c_F} = \frac{n}{K_d + c_F}$$

(Bound IGF-1 / Free IGF-1) versus free IGF normalized to K_d

<u>First</u> add ^{125}I-IGF-1 (< 0.1 nM)

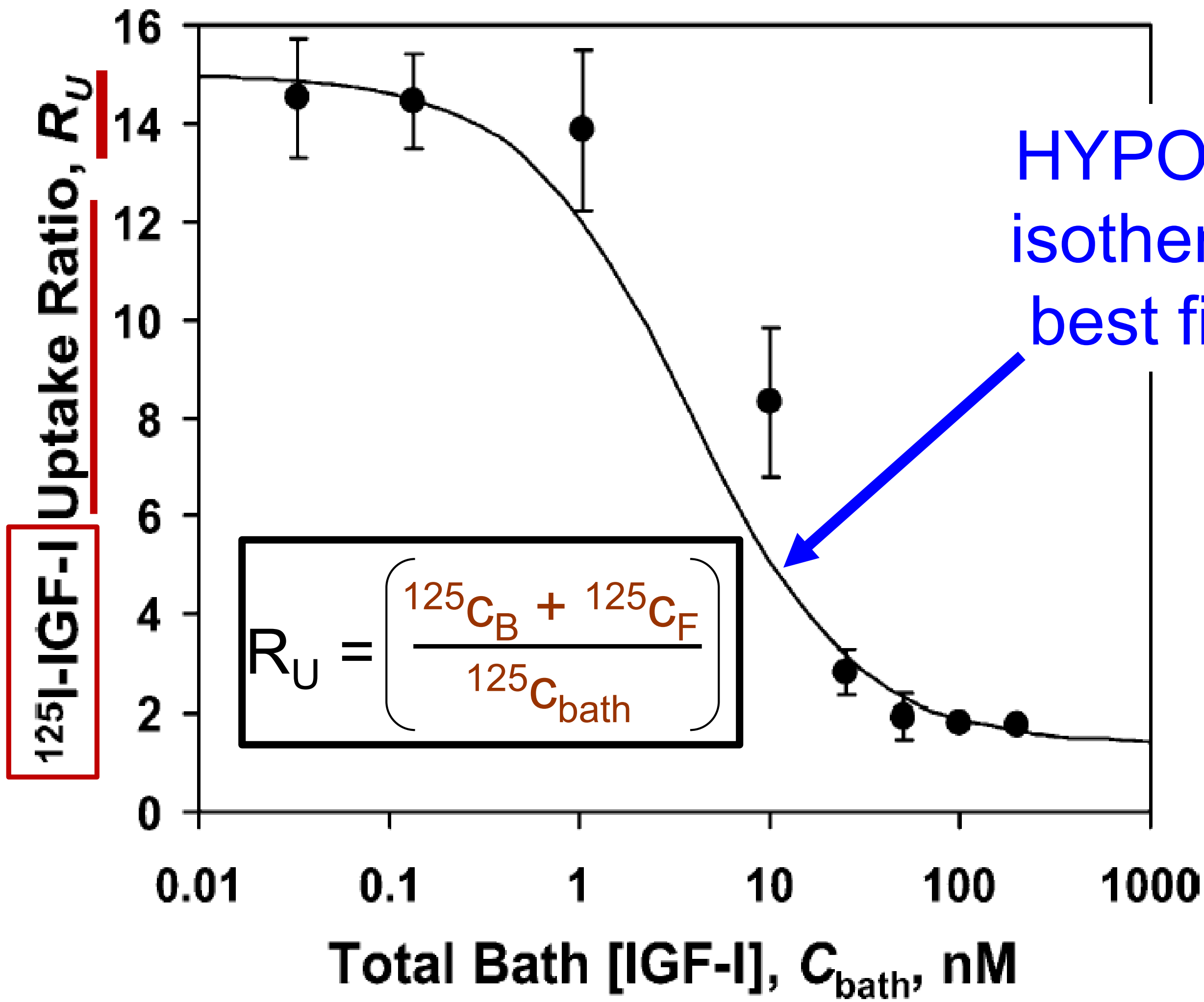


Tissue in medium at equilibrium (PBS or DMEM...)

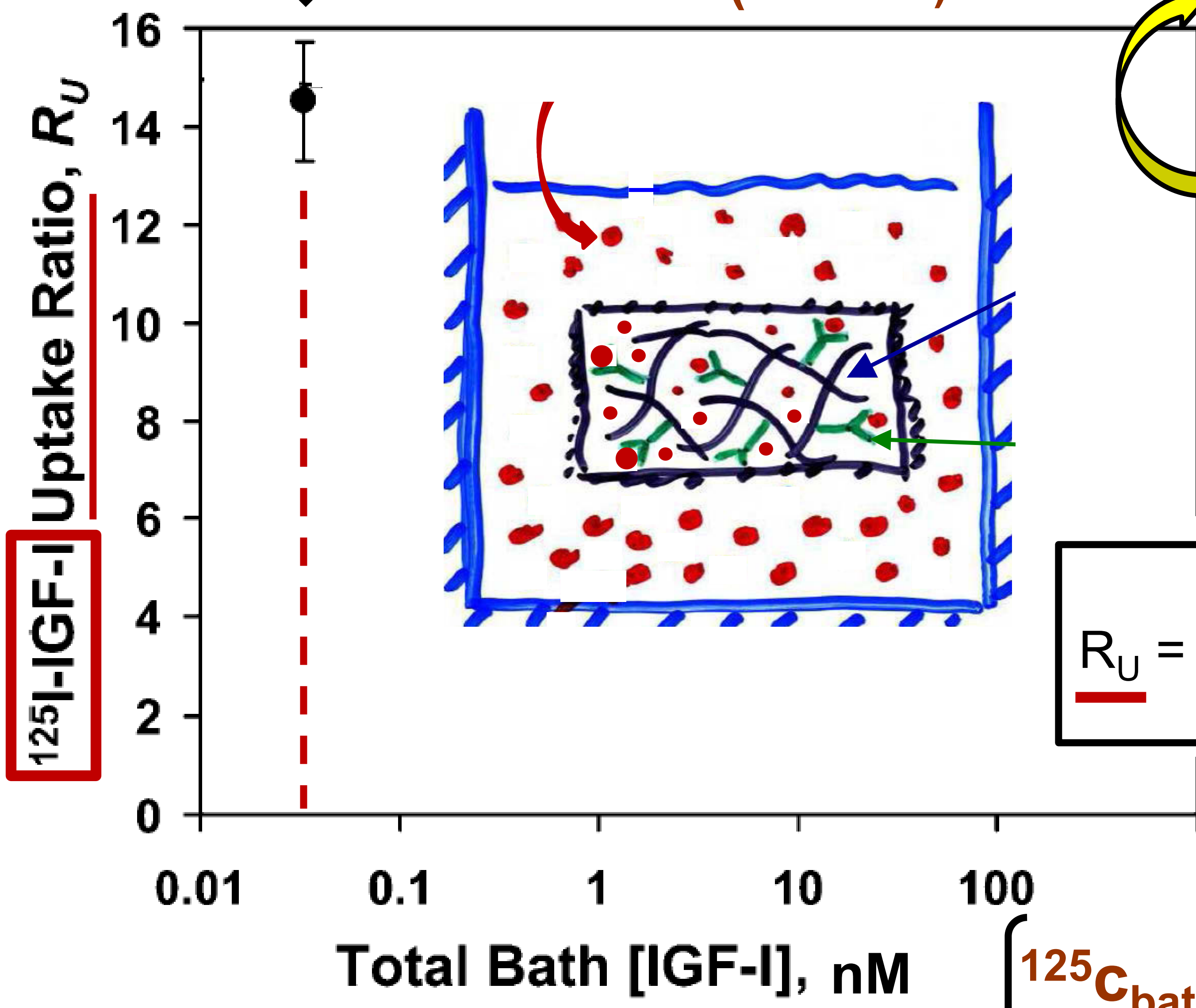
IGFBP-"x"

<u>Assuming</u>:
[IGFBP-x] >> [IGF-R]

Competitive Binding Experiment to Measure Equilibrium Binding Isotherm



1st addition (hot):
^{125}I-IGF-1 (< 0.1 nM)



$$R_U = \left(\frac{c_B^{tiss} + c_F^{tiss}}{c_{bath}} \right)$$

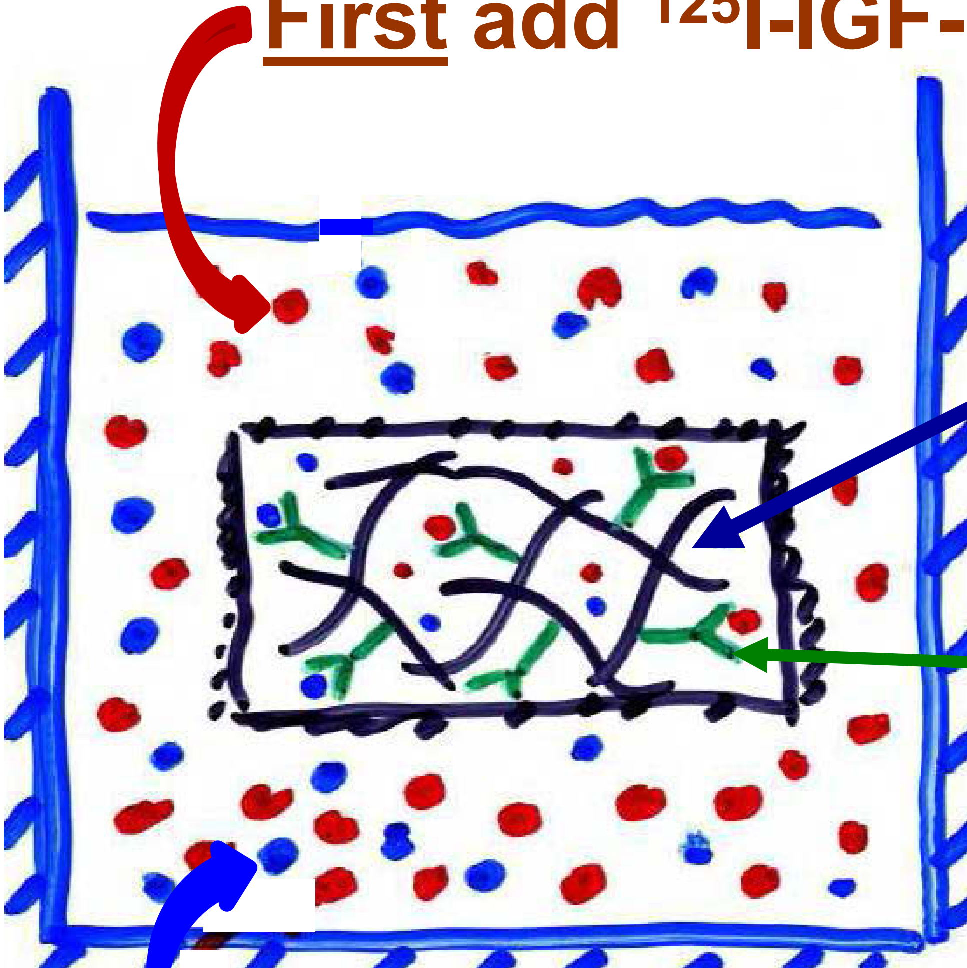
$$R_U = \left(\frac{^{125}c_B + {}^{125}c_F}{^{125}c_{bath}} \right)$$

since tracer distribution mimics "mother species"

$$R_U = K_{part}\left(1 + \frac{n}{K_d + c_F}\right)$$

$\left(^{125}c_{bath}\right)$

First add ^{125}I-IGF-1 (< 0.1 nM)

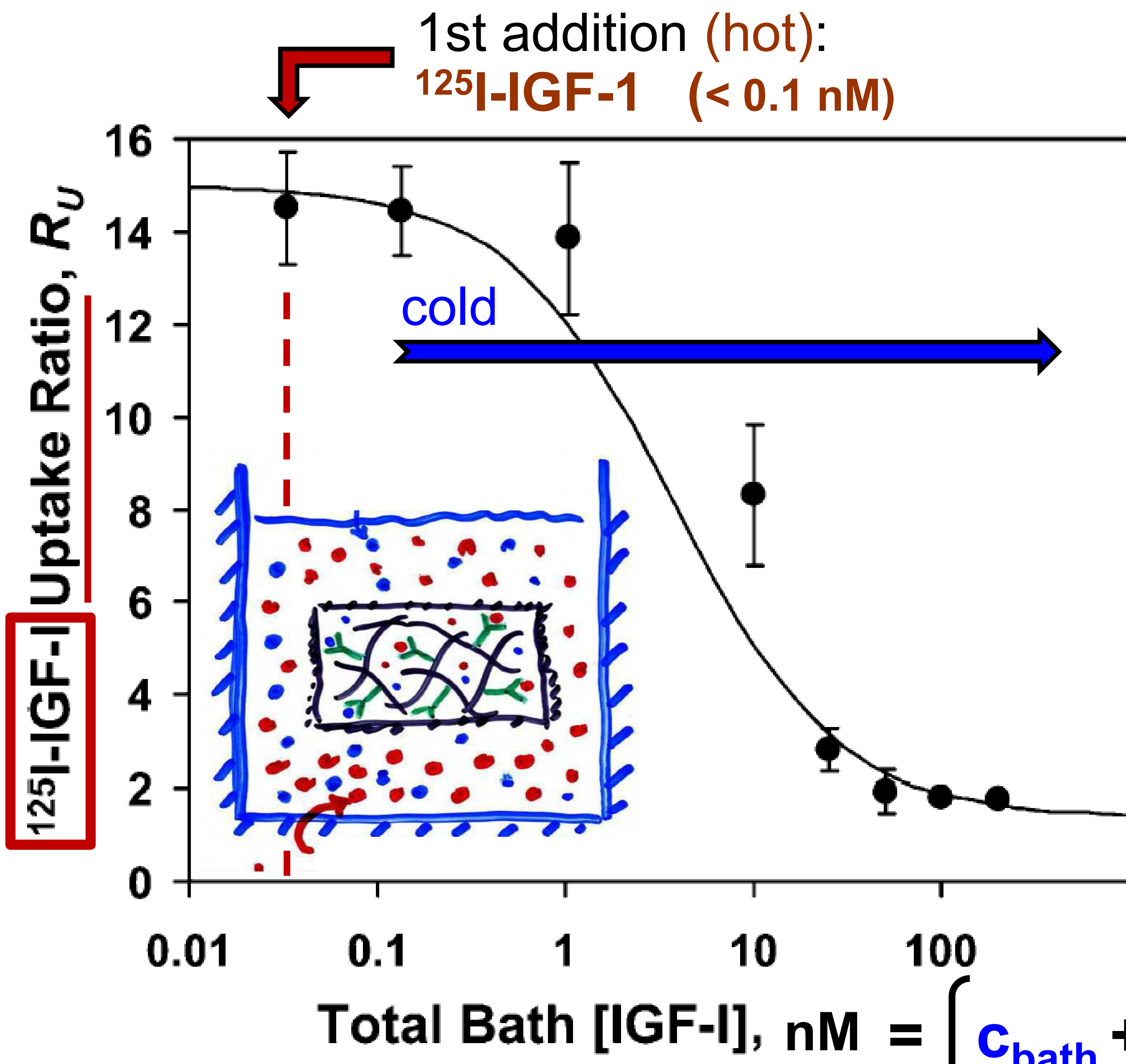


Tissue in medium at equilibrium (PBS; DMEM...)

IGFBP-"x"

Assuming:
[IGFBP-x] >> [IGF-R]

....then add • = **"cold" IGF-1**



$= \left(c_{bath} + {}^{125}c_{bath} \right)$

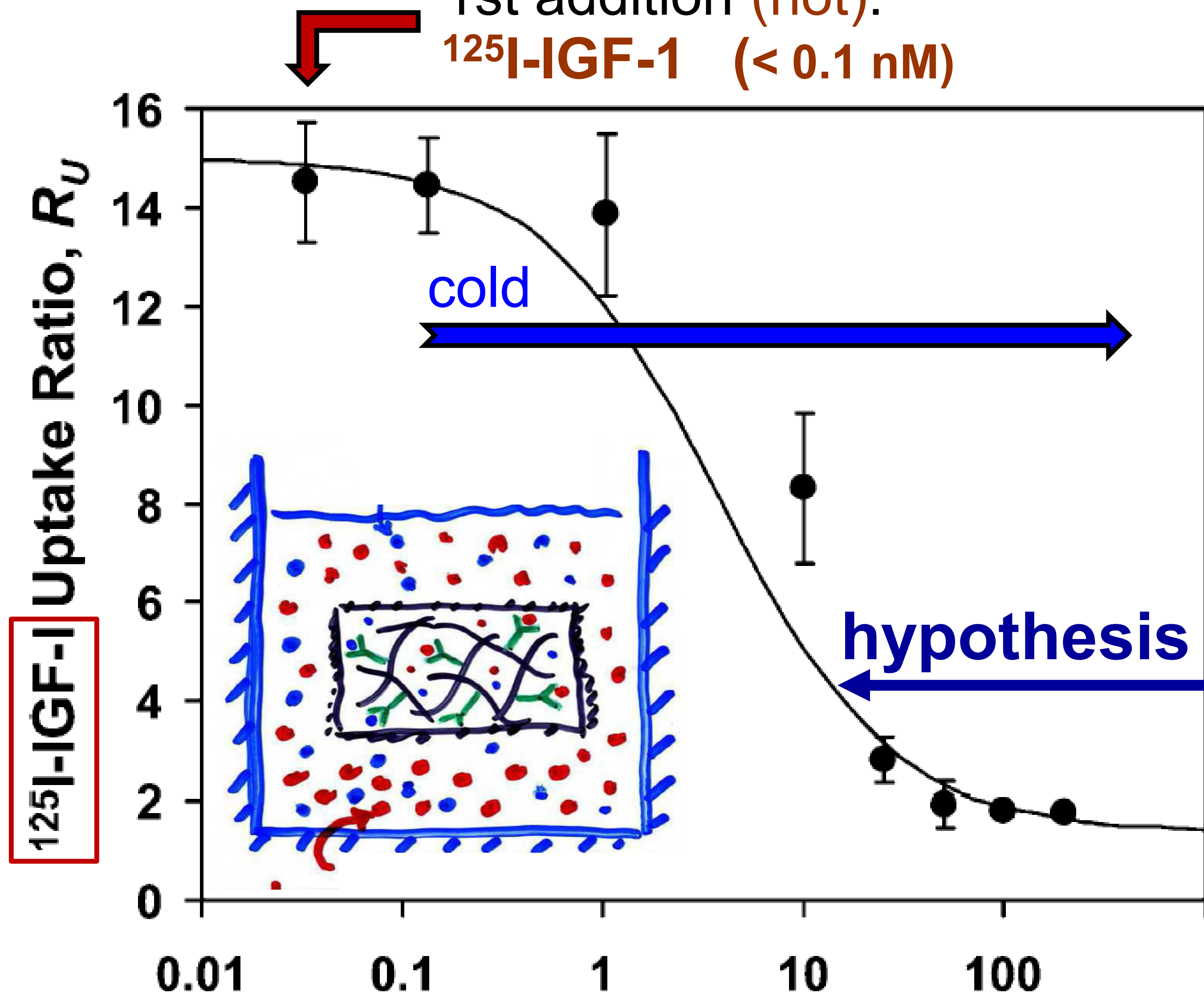
$$R_U = \left(\frac{c_B^{tiss} + c_F^{tiss}}{c_{bath}} \right)$$

$$R_U = \left(\frac{{}^{125}c_B + {}^{125}c_F}{{}^{125}c_{bath}} \right)$$

since tracer distribution mimics "mother species"

1st addition (hot):

^{125}I-IGF-1 (< 0.1 nM)



Total Bath [IGF-I], nM $= \left(c_{bath} + {}^{125}c_{bath} \right)$

$$R_U = \left(\frac{c_B^{tiss} + c_F^{tiss}}{c_{bath}} \right)$$

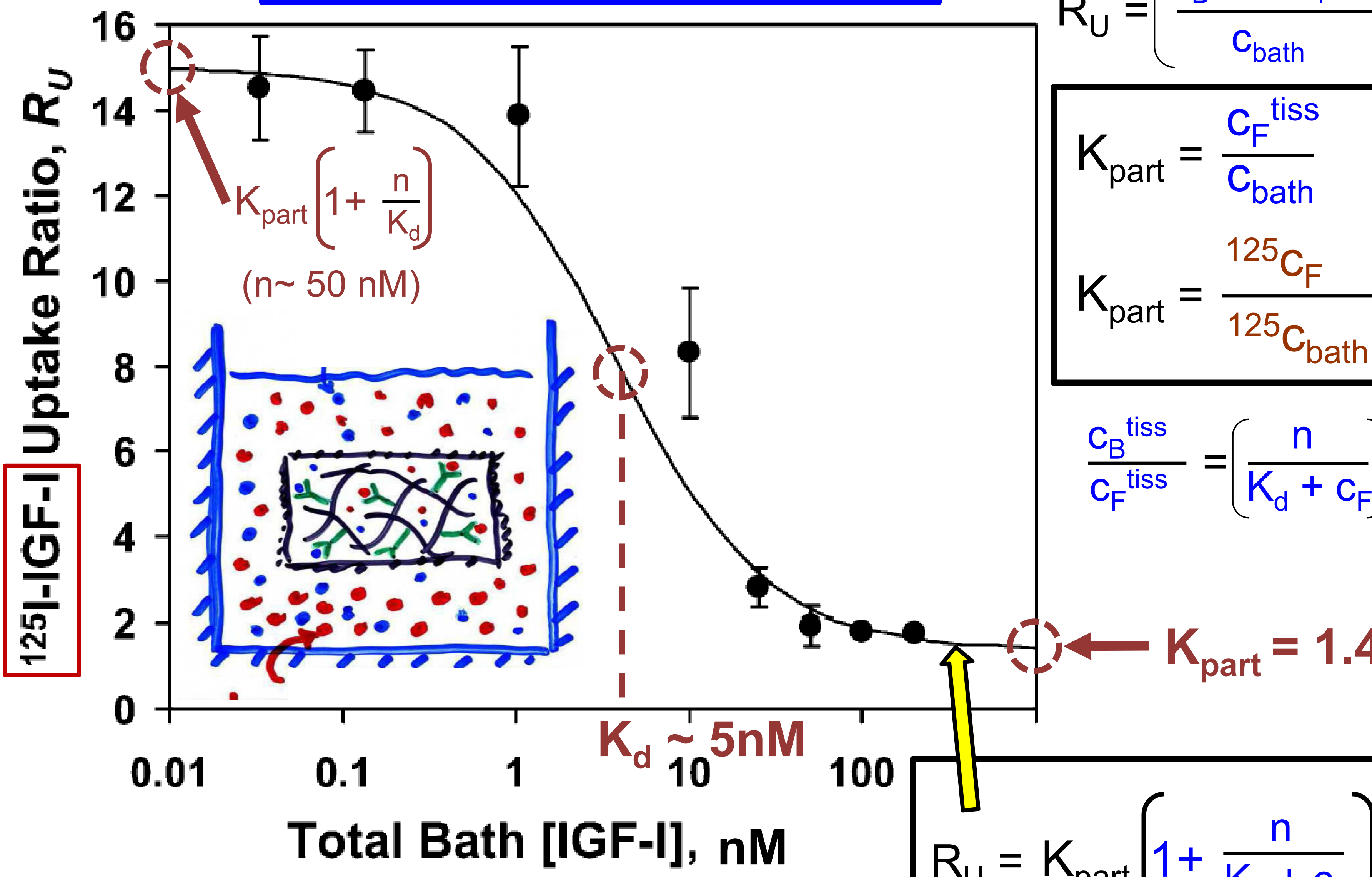
$$R_U = \left(\frac{{}^{125}c_B + {}^{125}c_F}{{}^{125}c_{bath}} \right)$$

since tracer distribution mimics "mother species"

$$\frac{c_B^{tiss}}{c_F^{tiss}} = \left(\frac{n}{K_d + c_F} \right)$$

"Binding Isotherm"

Equilibrium Binding



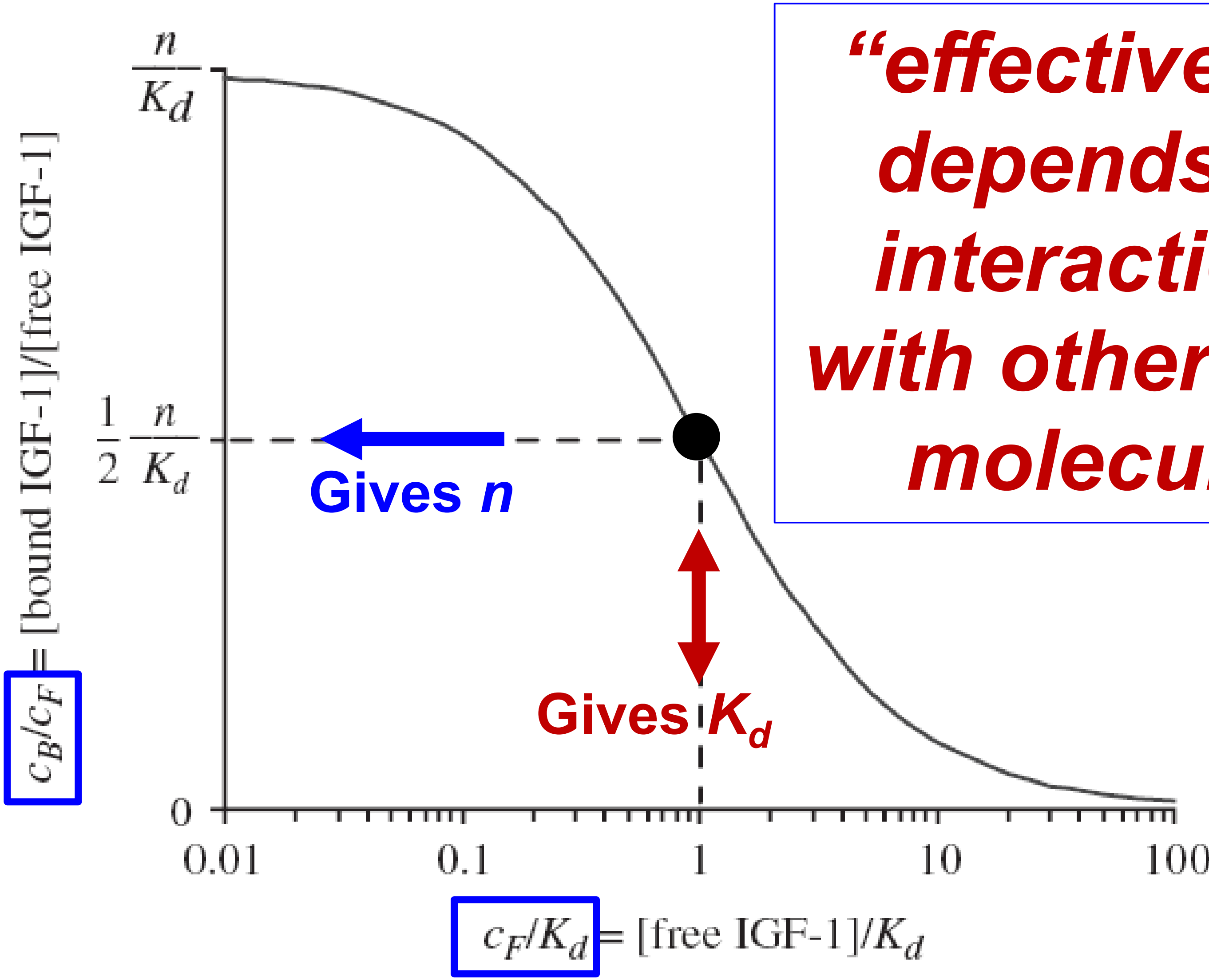
$$R_U = \left(\frac{c_B^{tiss} + c_F^{tiss}}{c_{bath}}\right)$$

$$K_{part} = \frac{c_F^{tiss}}{c_{bath}}$$

$$K_{part} = \frac{{}^{125}c_F}{{}^{125}c_{bath}}$$

$$\frac{c_B^{tiss}}{c_F^{tiss}} = \left(\frac{n}{K_d + c_F}\right)$$

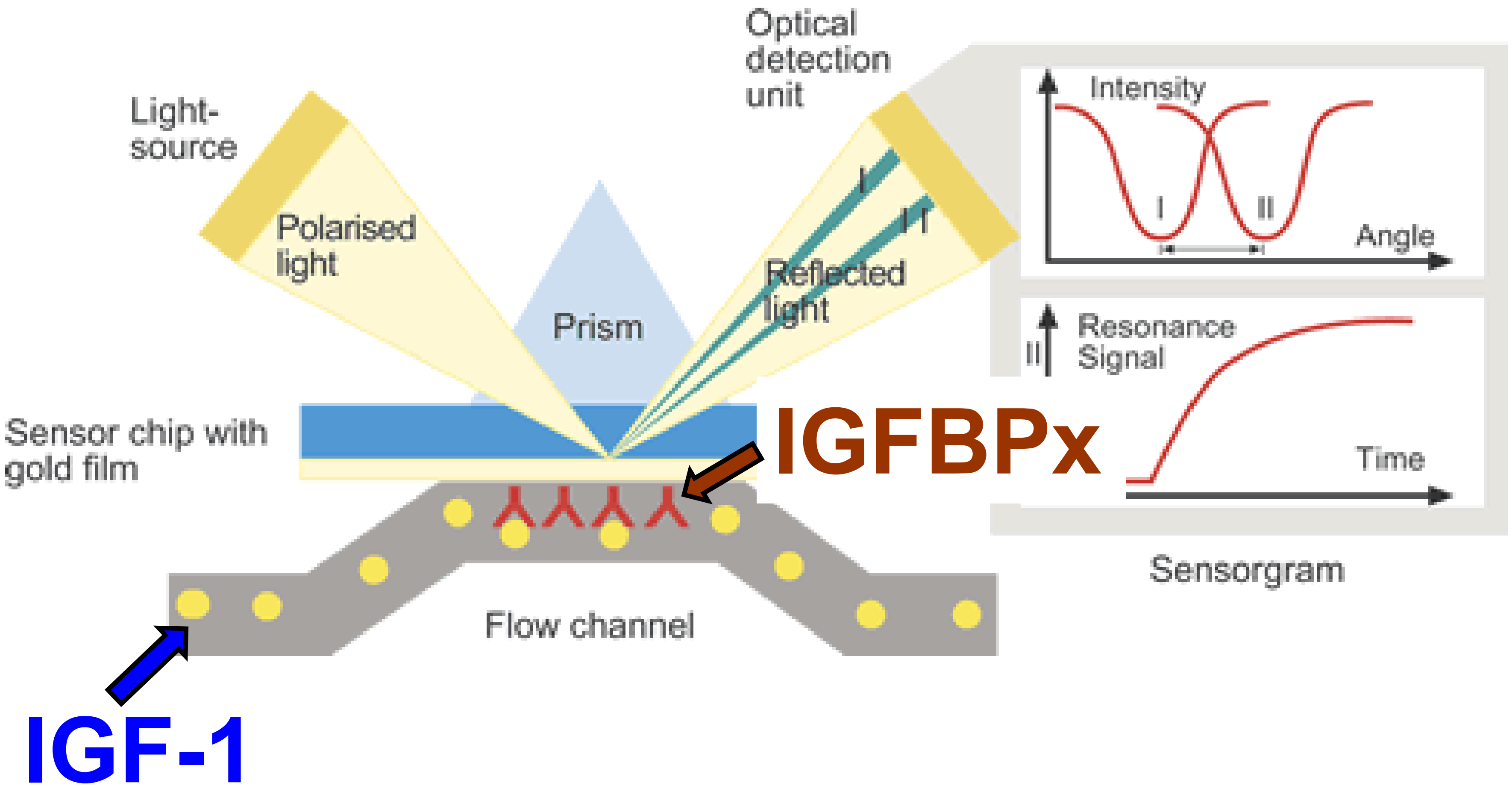
$$R_U = K_{part}\left(1 + \frac{n}{K_d + c_F}\right)$$



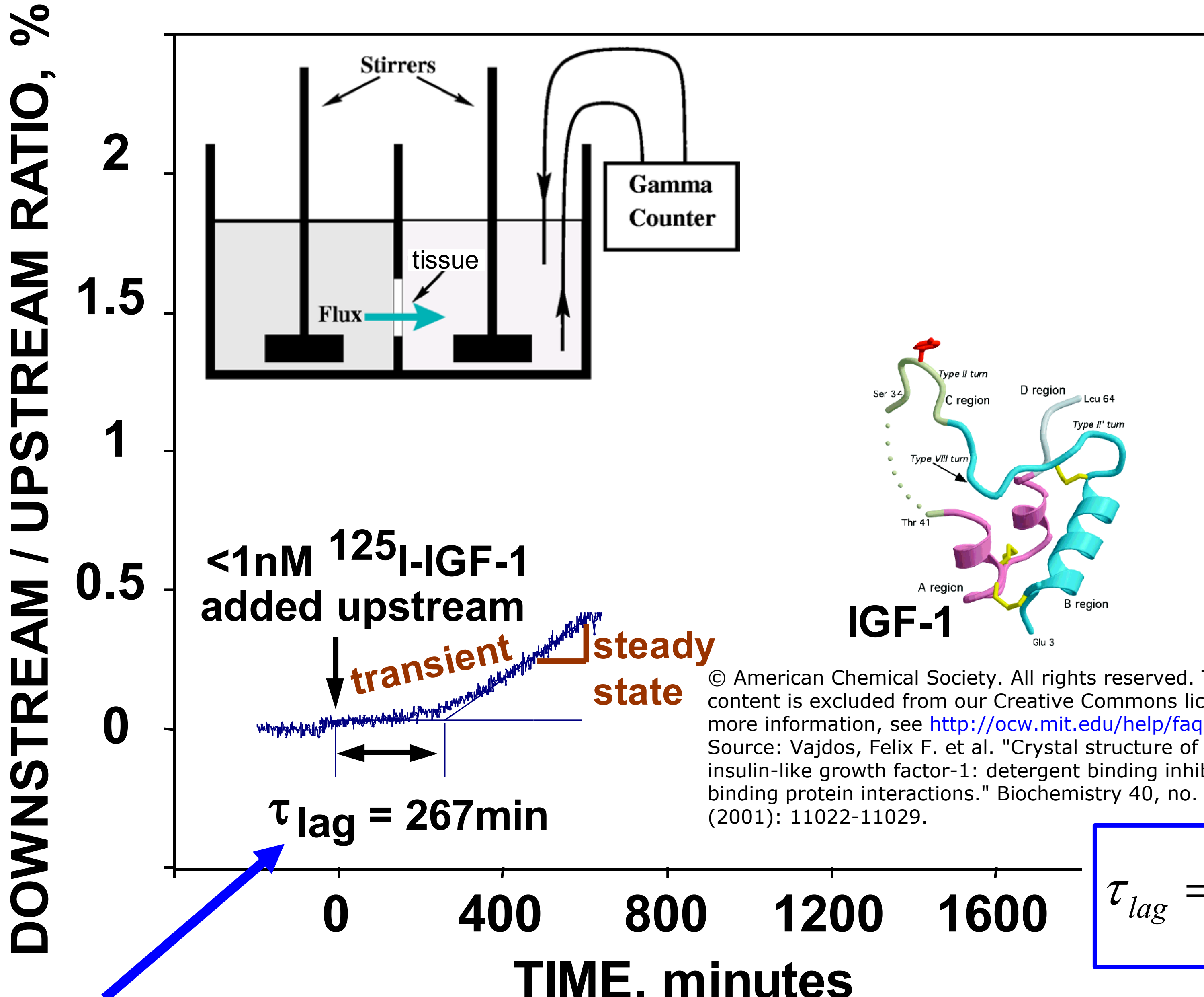
"effective" K_d depends on interactions with other ECM molecules

(Bound IGF-1 / Free IGF-1) versus free IGF normalized to K_d

Surface Plasmon Resonance Instrument ("BiaCore") to measure binding of ligand to specific target



IGFBP-3 Binding Slows entry of IGF-1 into Tissue!



Source: Vajdos, Felix F. et al. "Crystal structure of human insulin-like growth factor-1: detergent binding inhibits binding protein interactions." Biochemistry 40, no. 37 (2001): 11022-11029.

$$\tau_{lag} = \frac{L^2}{6D_{eff}}$$

slow reaction, or slow diffusion compared to reaction???

Last Time:

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$$\underbrace{\overbrace{\{\text{IGF} * \text{site}\}}^{\text{unoccupied}}}_{\text{bound} \rightarrow c_B} \underset{k_{on}}{\overset{k_{off}}{\rightleftharpoons}} \underbrace{\text{IGF}}_{\text{free} \rightarrow c_F} + \overbrace{\text{site}}^{\text{occupied}}$$

Rate Eqn (2)

$$\frac{\partial c_B}{\partial t} = \left[k_{on} c_F [\text{sites}] - k_{off} c_B\right]$$

= 0 in equilibrium

binding site density "*n*" (definition) (3)

$$n = \underbrace{\overbrace{[\text{IGF} * \text{site}]}^{c_B}}_{\text{occupied}} + \underbrace{[\text{site}]}_{\text{unoccupied}}$$

Binding "Isotherm" (4)

$$c_B = \frac{n\, c_F}{K_d + c_F}$$

HW Prob 3.1

The diffusion equation (1.65) then takes the form

$$\frac{\partial \bar{c}_F(x,t)}{\partial t} + \frac{\partial}{\partial t}\left(\frac{n\,\bar{c}_F(x,t)}{K_D + \bar{c}_F(x,t)}\right) = D_{\mathrm{IGF}}\frac{\partial^2 \bar{c}_F}{\partial x^2} \tag{1.68}$$

By using the chain rule for differentiation, (1.68) can then be written as

$$\frac{\partial \bar{c}_F}{\partial t}\left(1 + \frac{n K_d}{(K_d + \bar{c}_F)^2}\right) = D_{\mathrm{IGF}}\frac{\partial^2 \bar{c}_F}{\partial x^2} \tag{1.69}$$

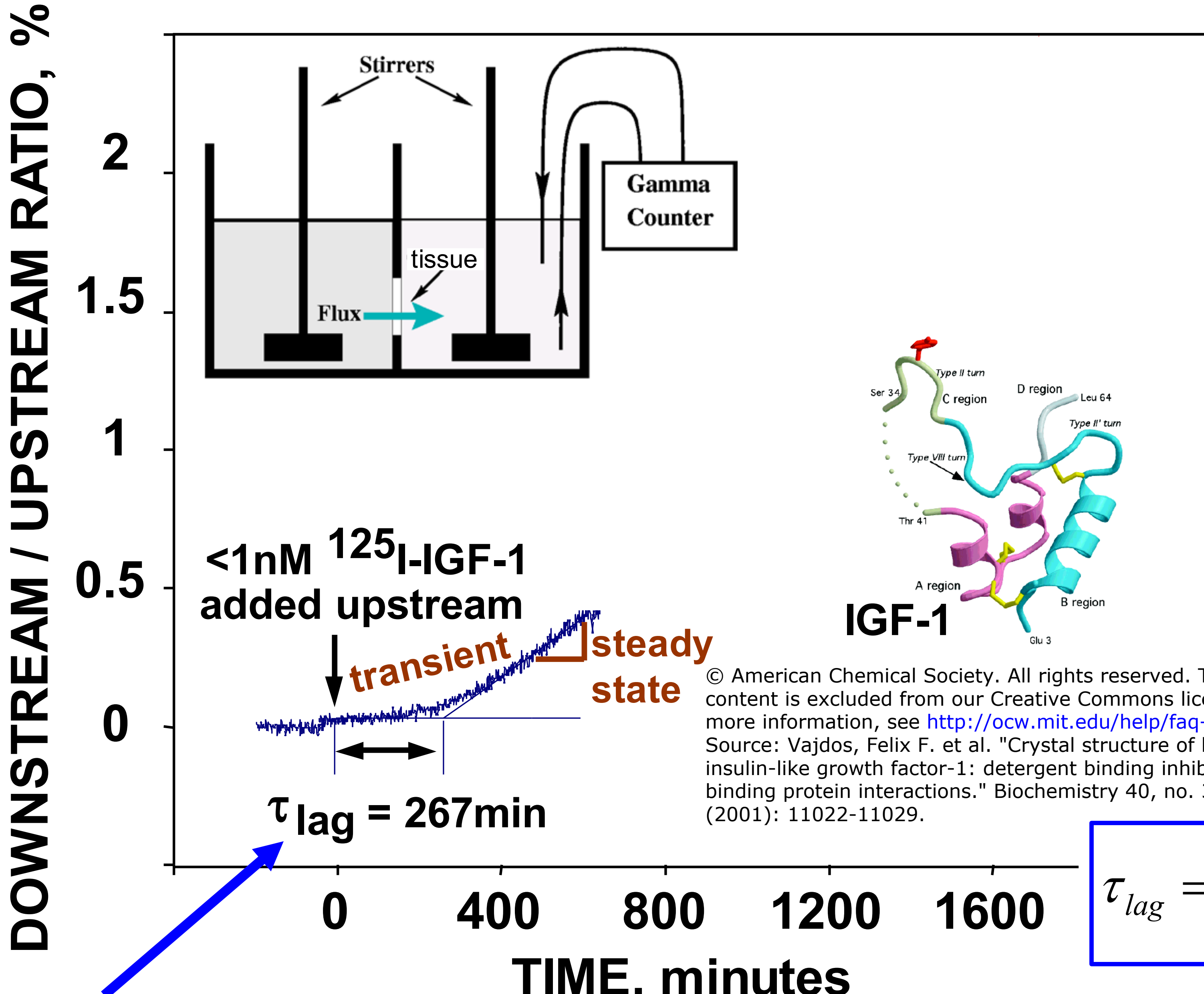
The diffusion–reaction equation (1.69) is nonlinear in c_F; however, for small enough concentrations $c_F \ll K_d$, it can be written in the form

Fast reaction, slow diffusion limit

$$\frac{\partial \bar{c}_F}{\partial t} = D_{\mathrm{eff}}\frac{\partial^2 \bar{c}_F}{\partial x^2} \tag{1.70}$$

$$D_{\mathrm{eff}} = \frac{D_{\mathrm{IGF}}}{1 + n/K_d} \tag{1.71}$$

IGFBP-3 Binding Slows entry of IGF-1 into Tissue!



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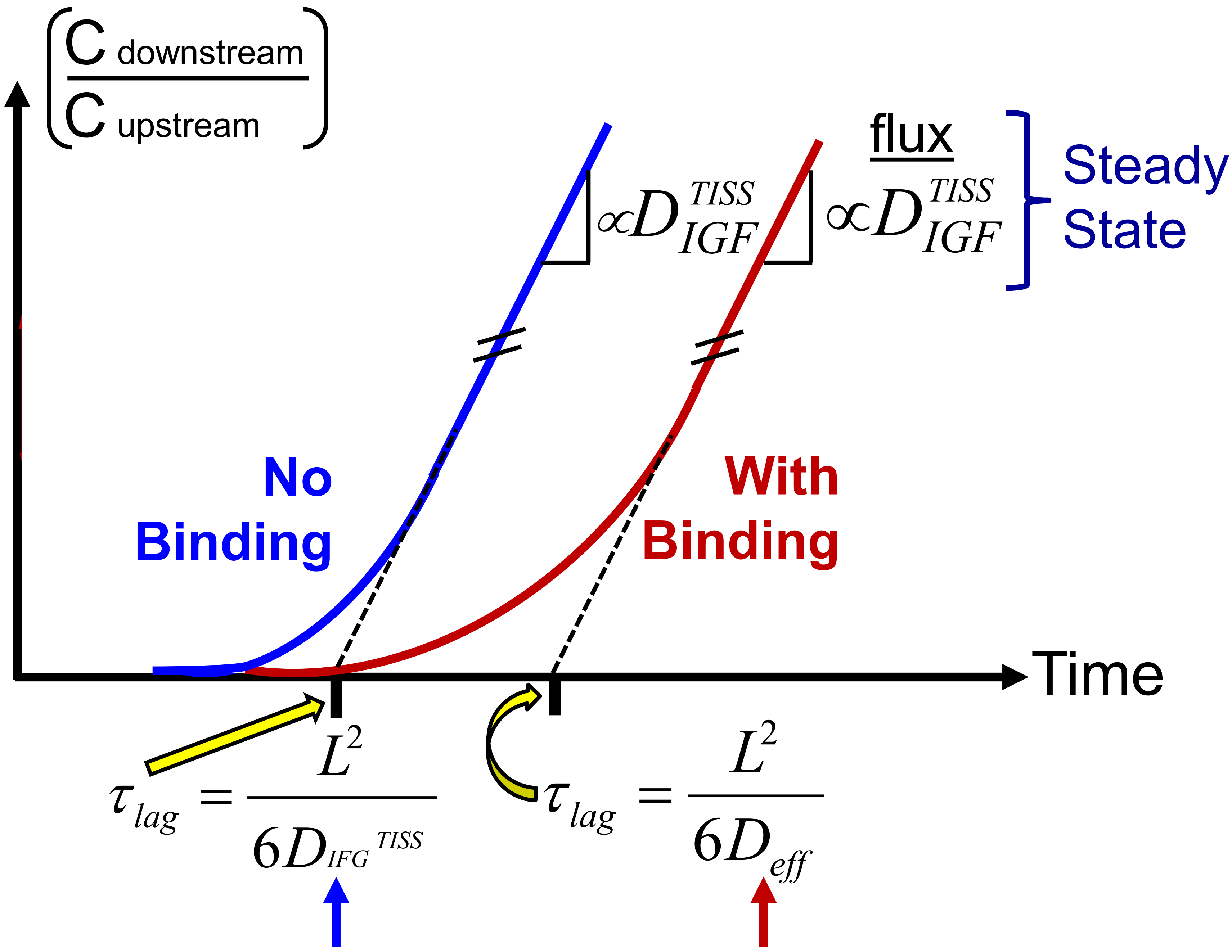
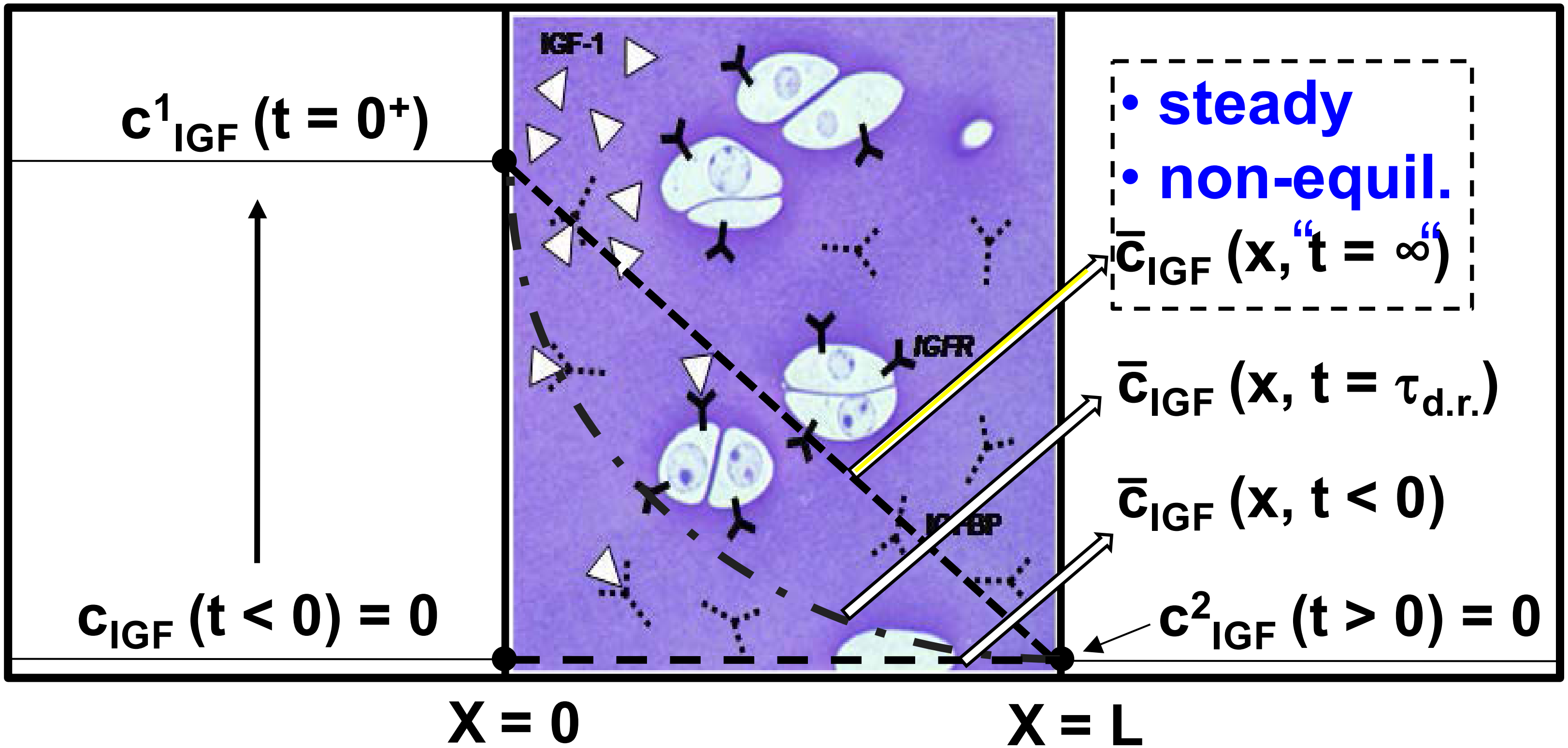
$$\tau_{lag} = \frac{L^2}{6D_{eff}}$$

slow reaction, or slow diffusion compared to reaction???



$\left(\frac{C_{downstream}}{C_{upstream}}\right)$
flux
$\propto D_{IGF}^{TISS}$
$\propto D_{IGF}^{TISS}$
Steady State
No Binding
With Binding
Time
$\tau_{lag} = \frac{L^2}{6D_{IFG}^{TISS}}$
$\tau_{lag} = \frac{L^2}{6D_{eff}}$

Find *c(x,t)* → Measure / Estimate D_{eff} ;
→ relate D_{eff} to D_{IGF} and binding parameters (n, K_d)



"Transient "

- **non-steady**
- **non-equil.**

$$\bar{c}_F(x,t) = c_1\left(1-\frac{x}{L}\right) - \frac{2Kc_1}{\pi}\sum_{n=1}^{\infty}\left[\frac{1}{n}\right]\sin\left(\frac{n\pi x}{L}\right)e^{-t/\tau_n} \qquad (1.72)$$

$$\tau_n = \frac{L^2}{n^2\pi^2 D_{eff}} \qquad \tau_1 = \frac{L^2}{\pi^2 D_{eff}} \qquad (1.73)$$

PSet 1.4