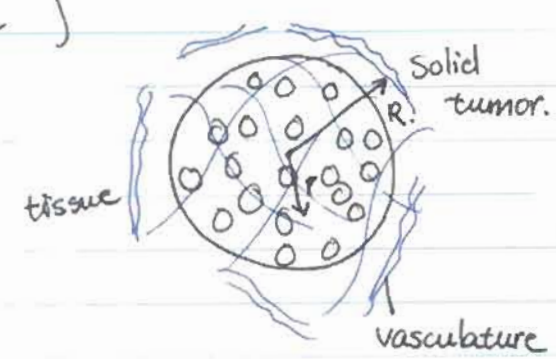


# Today - Wed. - chemical subsystem examples

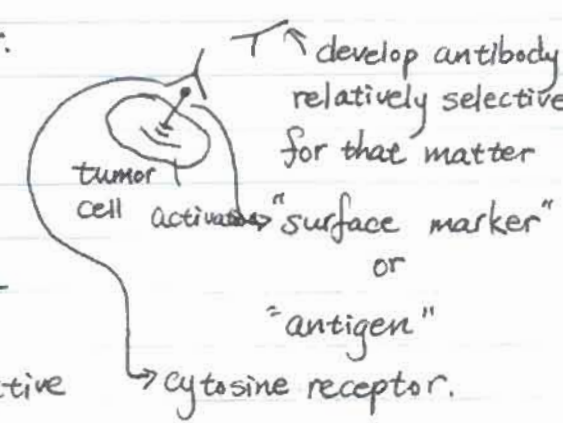
11/1/04

↳ Situation:

molecular therapeutic design } → Antibodies for diagnostics or therapeutics for solid tumor applications.



~ spheroid  $R \sim 50-300 \mu m$



## Diagnostics

- label Ab w/ fluorescent or radioactive tag for imaging

## Therapeutics

- conjugated with some toxic moiety.
  - radioactive
  - chemical
  - physical → heating.
- if marker is physiologically important, disrupt function.

Antibody creation capabilities? } rational, directed evolution or  
 - really good } combinational.

Key issue: What properties do we want Ab to have?

- small, uncharged ⇒ rapid diffusion
- binding properties
  - equilibrium
  - kinetics
  - other features

$a(r, t)$  = antibody concentration. unbound, freely diffusing in matrix  
 ↑  
 moles/vol. · time tissue/matrix.

Need to relate  $a(r,t)$  to  $b(r,t) \leftarrow \# \text{bound Ab/cell}$

Mass cons. on antibody on matrix

$$\underbrace{(1-\phi) \frac{\partial a}{\partial t}}_{\text{vol matrix/vol tissue}} = (1-\phi) D \nabla^2 a - \text{Ruptake by cells.} \quad \phi = \text{vol. fraction tumor cells in tissue}$$

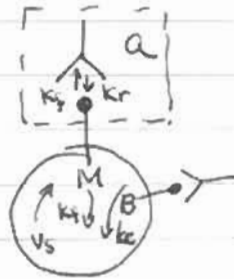
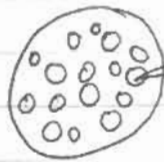
Spherical Coordinates

$$\nabla^2 = \frac{1}{r^2} \frac{\partial}{\partial r} \left( r^2 \frac{\partial}{\partial r} \right)$$

IC:  $a(r,0) = 0$       BC:  $a(R,t) = a_{\text{blood}}$

assume quasi-equilibrium  $\frac{\partial a}{\partial r}(0,t) = 0$

Ruptake by cells.



time<sup>-1</sup>  $\leftarrow \#/\text{cell}$   
 Ruptake =  $k_e B$   
 by cells.  
 $\#/\text{cell-time}$

M # of cell unbound surface markers.

B # of cell bound surface markers.

$M_T = M + B$  on fast time scale.

Solve for B in terms of a.

$$\frac{dB}{dt} = k_f M a - k_r B = 0$$

binding      dissociation

@ quasi-equilibrium @ all level:  $B = \frac{M_T a}{K_D + a}$

then  $k_e B = \left[ \frac{k_e M_T a}{K_D + a} \right] \frac{\gamma}{N_A V} \leftarrow \text{cell density}$   
 $\frac{\gamma}{N_A V} \leftarrow \text{cells/vol tissue}$   
 $K_D = \frac{k_r}{k_f} \equiv \text{dissociation equilibrium constant.}$   
 $\uparrow$   
 $\#/\text{mole.}$

need moles/vol-time matrix

$$\frac{\partial a}{\partial t} = \frac{D}{r^2} \frac{\partial}{\partial r} \left( r^2 \frac{\partial a}{\partial r} \right) - \frac{k_e M_T \gamma}{k_D + a} \frac{1}{N_A V (1-\phi)}$$

Design properties for Ab lie in parameters

Let's Analyze first before merely computing

scale variables -  $u = \frac{a}{K_D}$      $\rho = \frac{r}{R}$

$$\tau = \frac{t}{R^2/D}$$

All parameters are interdependently related.

$$\Rightarrow \frac{\partial u}{\partial \tau} = \frac{1}{\rho^2} \frac{\partial}{\partial \rho} \left( \rho^2 \frac{\partial u}{\partial \rho} \right) - \left( \alpha^2 \right) \left[ \frac{u}{1+u} \right]$$

Nonlinear

$\alpha^2 = \frac{k R^2}{D}$  ratio of rxn rate to diffusion rate

$\uparrow$   $\frac{D}{k R^2}$  Damkohler number = Da (chem)

$\alpha$  = Thiele Modulus (Eng.)

IC  $u(\rho, 0) = 0$

B.C.  $u(1, \tau) = u_{\text{blood}} = \frac{\alpha_{\text{blood}}}{K_D}$

$$\frac{\partial u}{\partial \rho}(0, \tau) = 0$$

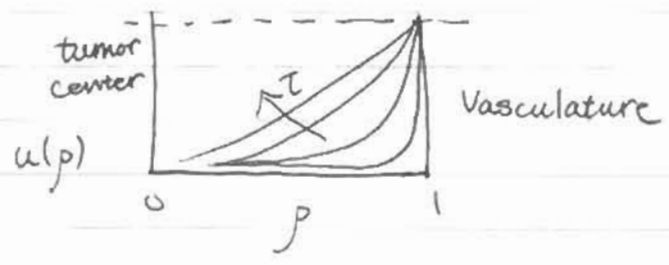
Could solve as is by:

- Numerical computation (finite element, finite difference)
- Analytically by perturbation method using Green's Function + iteration
- Analytically in approximation by linear simplification  
 → assume  $u_{\text{blood}} \ll 1 \Rightarrow -\alpha^2 u$

$$\frac{\partial u}{\partial \tau} = \frac{1}{\rho^2} \frac{\partial}{\partial \rho} \left( \rho^2 \frac{\partial u}{\partial \rho} \right) - \alpha^2 u \quad \begin{matrix} u(\rho, 0) = 0 \\ u(1, \tau) = u_{\text{blood}} \end{matrix} \quad \frac{\partial u}{\partial \rho}(0, \tau) = 0$$

linear approximation

We want to know how  $\alpha^2$  affects spatial-temporal antibody distribution



You can obtain solution to this problem

- separation of variables (Alan G.).
- Finite Fourier Transform (Deen, Chapter 4)
- Sturm-Liouville Linear operator methods.
  - K. Gustafson: Intro to PDEs + Hilbert Space Methods.
  - I. Stakgold - Green's Functions + BVPs.
  - P. Ritger & Rose - Diff. Eqns + Applications.

$$\text{Obtain: } \frac{u(p, \tau)}{u_{\text{blood}}} = \left[ 1 + \sum_{j=1}^{\infty} \left\{ \left[ \frac{2j\pi\alpha^2(-1)^j}{(j\pi)^2 + \alpha^2} \right] \frac{1}{p} \sin j\pi p \right\} \right] \\
 + \sum_{j=1}^{\infty} \left[ \frac{2j\pi(-1)^j}{(j\pi)^2 + \alpha^2} \right] \underbrace{\left[ \frac{1}{p} \sin j\pi p \right]}_{\text{eigenvectors}} e^{-\underbrace{[(j\pi)^2 + \alpha^2] \tau}_{\text{eigenvalues}}}$$

$j = 1, \dots, \infty$

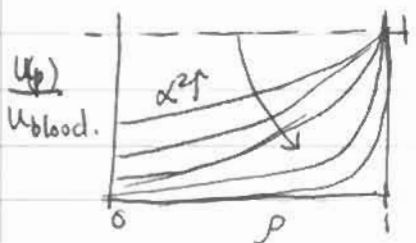
$\tau \rightarrow \infty, \frac{u(p, \tau)}{u_{\text{blood}}} \rightarrow u_{\text{ss}}(p) = \text{top 2 terms}$

Transient is 3<sup>rd</sup> term.

$$u_{\text{ss}}(p) = u_{\text{blood}} \cdot \frac{\sinh(\alpha p)}{p \sinh(\alpha)} = \text{same as the one extracted from entire solution.}$$

- eigenvector expansion of this simple function

Solution behavior: Steady-state ( $\tau \rightarrow \infty$ ),  $u(p)/u_{\text{blood}}$ .



So Ab penetrates further @ S.S. for smaller  $\alpha^2$ ;  $\alpha^2 = \frac{kR^2}{D}$  controls slope of ss profile

if  $\frac{k}{D/R^2} \gg 1, \alpha^2 \rightarrow \infty$  (sharp) = diffusion limited process

if  $\frac{k}{D/R^2} \ll 1, \alpha^2 \rightarrow 0$  (flat) = reaction limited process

What about transient solution?

How long does it take to get to SS?

Rate of approach is predominantly governed by smallest eigenvalue

$$e^{-[(j\pi)^2 + \alpha^2] \tau} \quad j=1, 2, \dots, \infty$$

$\uparrow$   $\uparrow$   
 $j=1$       smallest

so, rate of approach to SS is governed by  $e^{-(\pi^2 + \alpha^2)\tau}$

50% approach  $e^{-(\pi^2 + \alpha^2)\tau_{0.5}} = 0.5$

95% approach  $e^{-3}$

$\Rightarrow \tau_{0.5} \propto \frac{\ln 2 (0.7)}{\pi^2 + \alpha^2}$

$\tau_{0.95} \approx \frac{3}{\pi^2 + \alpha^2}$

in dimensional form  $\tau_{0.5} = \frac{\ln 2}{\frac{\pi^2 D}{R^2} + k}$

If  $\frac{k}{\pi^2 D/R^2} \gg 1 \Rightarrow \tau_{0.5} = \frac{\ln 2}{k + \frac{\pi^2 D}{R^2}}$  "diffusion-limited process"

Diffusion governs SS profile, reaction governs how you get there in diffusion limited process, vice versa.

If  $\frac{k}{\pi^2 D/R^2} \gg 1 \Rightarrow \tau_{0.5} = \frac{\ln 2}{k}$  "diffusion limited process"

If  $\frac{k}{\pi^2 D/R^2} \ll 1 \Rightarrow \tau_{0.5} = \frac{\ln 2}{\pi^2 D/R^2}$  "reaction limited process"

Example: #'s.  $\Rightarrow D \sim 1 \times 10^{-7} \text{ cm}^2/\text{s} \sim 5 \times 10^{-7} \text{ cm}^2/\text{s}$

IyG      scFv  
 MW ~ 180,000      MW ~ 30,000

$R \sim 50 - 300 \mu\text{m}$

$\eta = 10^7 - 10^8 \text{ cells/cm}^3$   
 $k_e = 10^2 - 10^1 \text{ min}^{-1}$

$M_T = 10^4 - 10^5 \text{ \# / cell}$   
 $k_D = 10^{-10} - 10^{-8} \text{ moles/l}$