A short story about FRAP (fluourescence recovery after photobleaching)

Bob Pego (Carnegie Mellon)

work involving

team leader: James McNally (NCI Lab of Receptor Biology & Gene Expression)

- Biophysicists: Brian Sprague, Diane Stavreva, Carolyn Smith
- Math students and postdocs at NIH: Jonathan Pindrik, Florian Müller

Apologia — Why this topic?

Goals: Tell a story, about

- how one interdisciplinary collaboration worked, or not
- how mathematics aided biophysics in one project, cited/blamed 280+ times:

Biophysical Journal Volume 86 June 2004 3473–3495

Analysis of Binding Reactions by Fluorescence Recovery after Photobleaching

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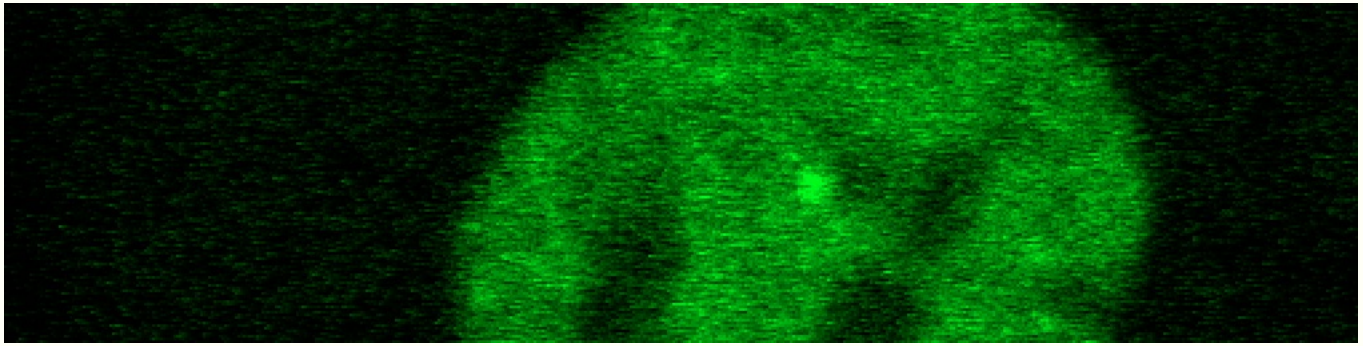
- how mathematics may help model diffusive transport in crowded and heterogeneous cellular environments ($in\ the\ future$)

The story starts with an email, out of the blue...

Basic Q: How do DNA transcription factors find binding sites?



Experimental techniques/ingredients:

- GR = glucocorticoid receptor (DNA transcription factor)
- GFP = Green Fluourescent Protein
- confocal microscopy

Schematic of a FRAP experiment

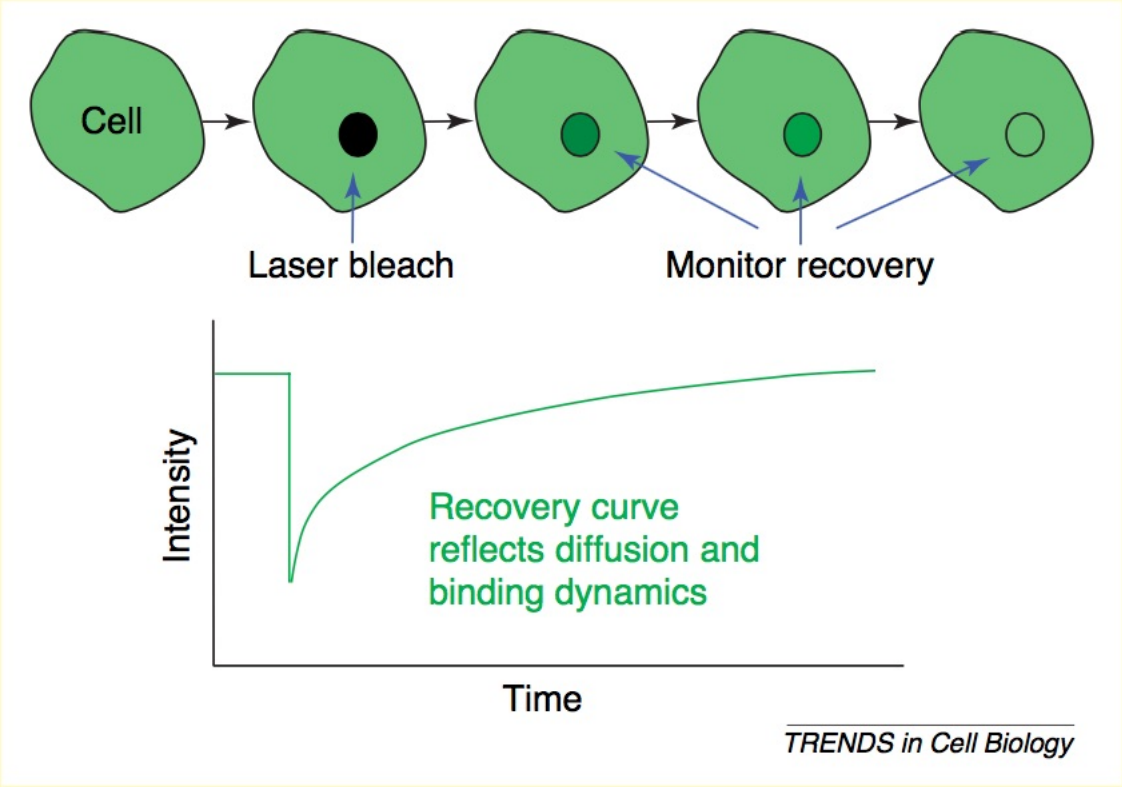
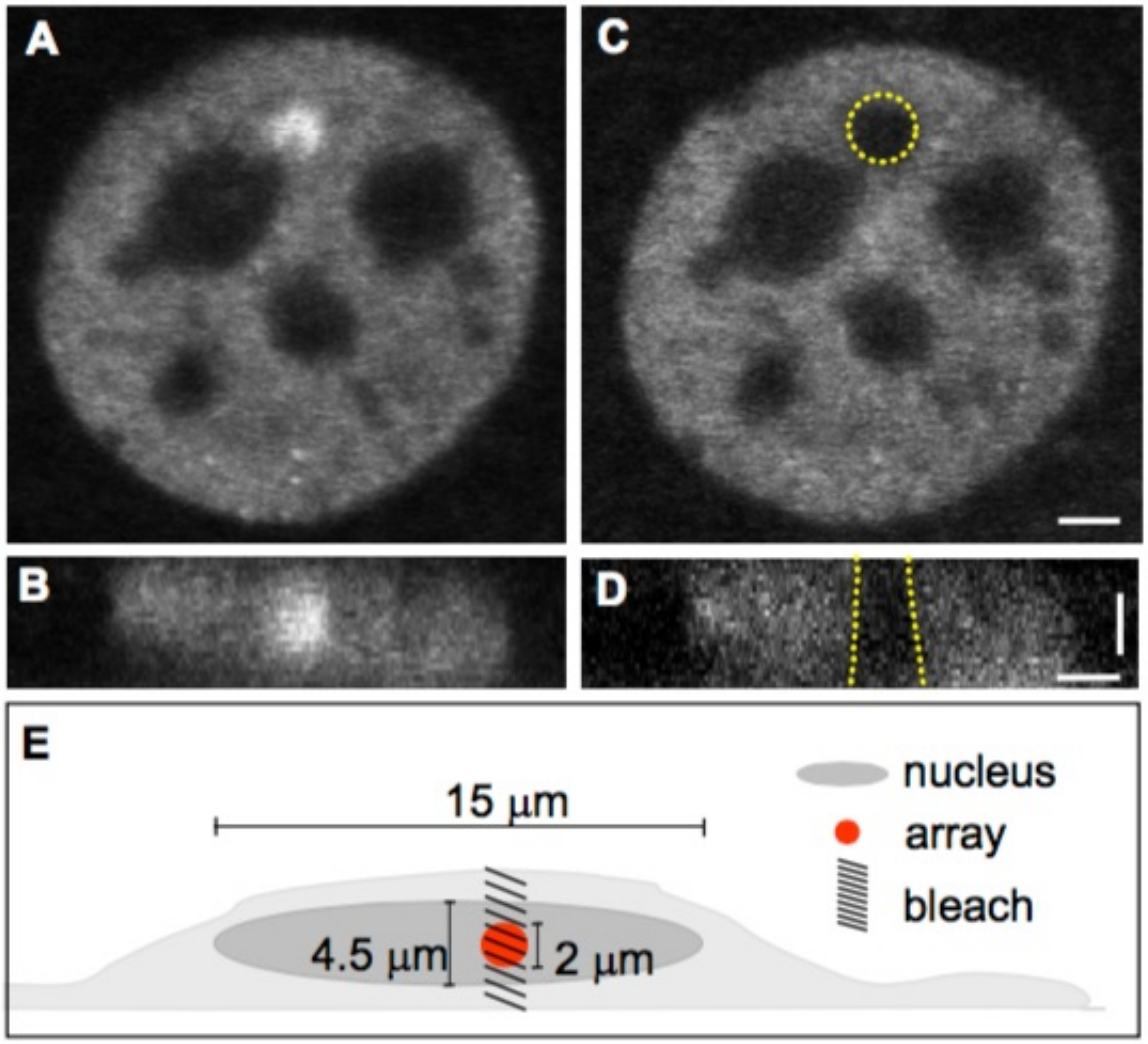
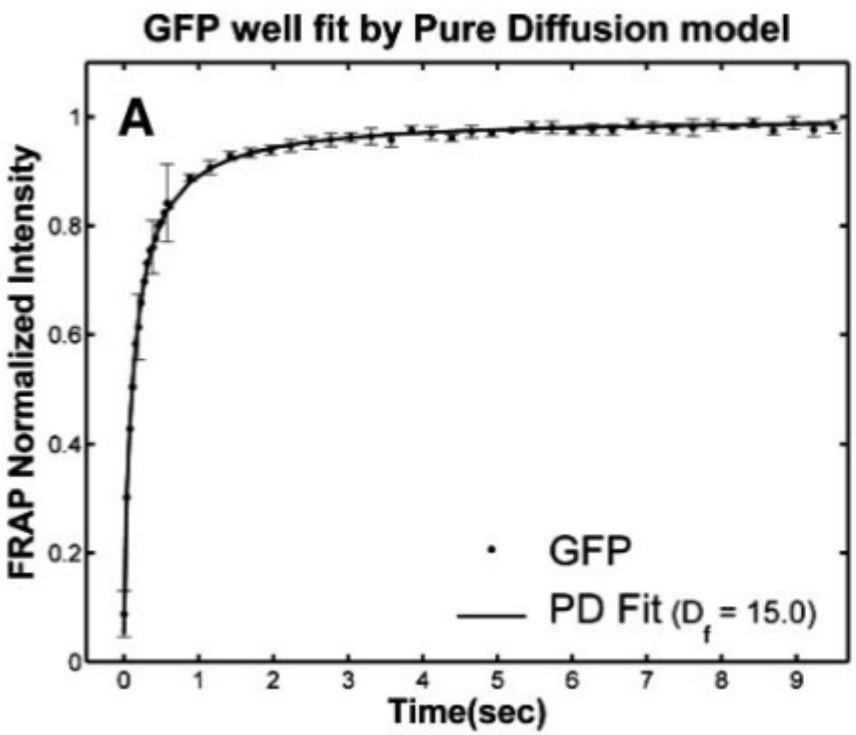


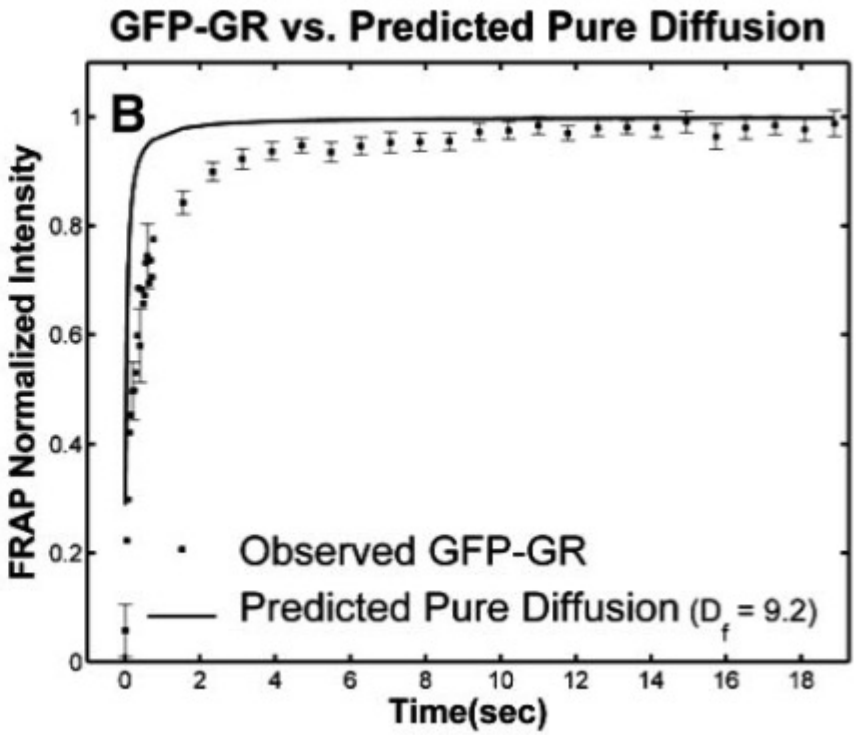
Figure I. Schematic illustrating the FRAP technique.

FRAP experiment geometry

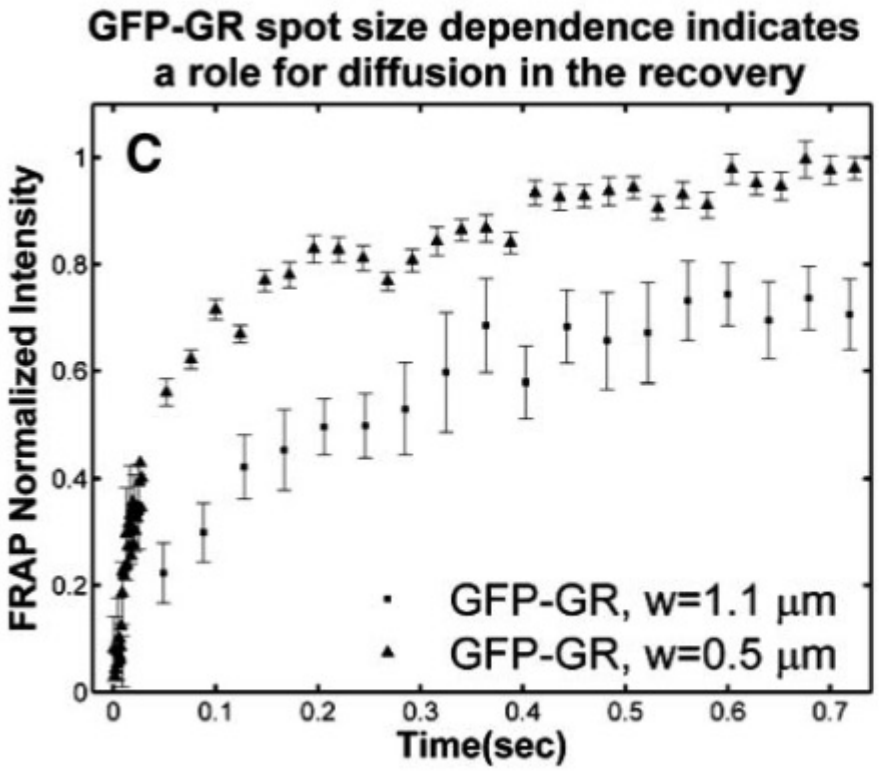


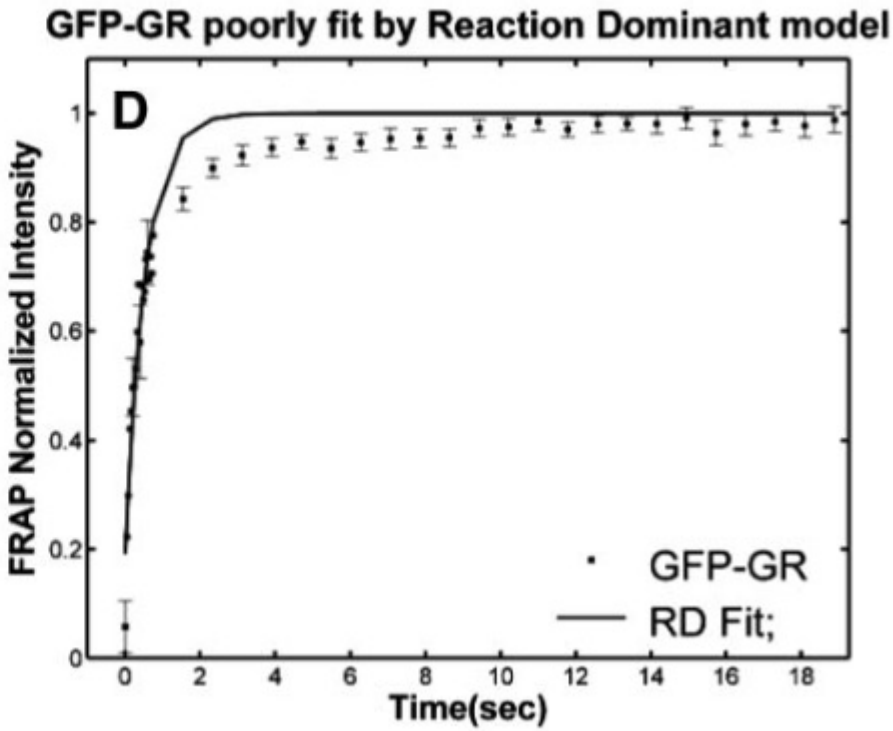
The problem: experimental data fail to fit





Failure to fit, part 2





Basic reaction-diffusion model

Free GFP-GR (F) reacts with free binding sites (S) to form a bound complex (C)

$$F + S \underset{k^*_{\mathrm{on}}}{\overset{k_{\mathrm{off}}}{\rightleftarrows}} C$$

Concentrations: $f = [F], \qquad s = [S], \qquad c = [C]$

$$\partial_t f = D_{\mathrm{f}} \nabla^2 f - k_{\mathrm{on}}\, f s + k_{\mathrm{off}}\, c$$
$$\partial_t s = D_{\mathrm{s}} \nabla^2 s - k_{\mathrm{on}}\, f s + k_{\mathrm{off}}\, c$$
$$\partial_t c = D_{\mathrm{c}} \nabla^2 c + k_{\mathrm{on}}\, f s - k_{\mathrm{off}}\, c$$

Simplifying assumptions

Pre-bleach, the system is at equilibrium: $s \equiv S_{\mathrm{eq}}, \quad k_{\mathrm{on}}^{*} = k_{\mathrm{on}} S_{\mathrm{eq}}$

Post-bleach, f, c correspond only to *fluorescing* GFP-GR molecules/complexes

- Assume sites and complexes do not diffuse: $D_{\mathrm{s}} = 0$, $D_{\mathrm{c}} = 0$
- Assume bleach profile is a perfect cylinder
- Neglect cell boundaries and presence of nucleoli
- Assume bleaching is instantaneous

These assumptions reduce the problem to 2D radial geometry.

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These assumptions reduce the problem to 2D radial geometry.

(All these assumptions are wrong, naturally!)

Reaction-diffusion model for FRAP w/binding

$$\partial_t f = D_{\mathrm{f}} \nabla^2 f - k^*_{\mathrm{on}}\, f + k_{\mathrm{off}}\, c \tag{1}$$
$$\partial_t c = k^*_{\mathrm{on}}\, f - k_{\mathrm{off}}\, c$$

Normalized equilibrium: $k^*_{\mathrm{on}}\, F_{\mathrm{eq}} = k_{\mathrm{off}} C_{\mathrm{eq}}, \quad F_{\mathrm{eq}} + C_{\mathrm{eq}} = 1.$

Initial value problem for modeling FRAP w/binding

With $u = F_{\text{eq}} - f, \quad v = C_{\text{eq}} - c,$

$$\partial_t u = D_{\text{f}} \nabla^2 u - k^*_{\text{on}}\, u + k_{\text{off}}\, v \qquad u(0) = \begin{cases} F_{\text{eq}} & r < w \\ 0 & r > w \end{cases}$$

$$\partial_t v = k^*_{\text{on}}\, u - k_{\text{off}}\, v \qquad v(0) = \begin{cases} C_{\text{eq}} & r < w \\ 0 & r > w \end{cases}$$

Measurable light intensity proportional to $f + c$. Average over the bleach spot:

$$frap(t) = \frac{1}{\pi w^2} \int_{r<w} (f + c)\, dx$$

Model solution by Laplace transform and numerical inversion

$$\bar{u}(r,p) = \int_0^\infty e^{-pt} u(r,t)\, dt \qquad \text{satisfies} \qquad -\nabla^2 \bar{u} + q^2 \bar{u} = V\, \mathbf{1}_{r<w}$$

$$q = \frac{p}{D_\mathrm{f}} \left(1 + \frac{k^*_\mathrm{on}}{p + k_\mathrm{off}} \right), \qquad V = \frac{F_\mathrm{eq}}{D_\mathrm{f}} \left(1 + \frac{k^*_\mathrm{on}}{p + k_\mathrm{off}} \right)$$

Averaging the solution $\bar{u}$ over $r < w$ yields the FRAP recovery transform:

$$\overline{frap}(p) = \frac{1}{p} - \frac{F_\mathrm{eq}}{p} \Big(1 - 2\, K_1(qw)\, I_1(qw) \Big) \left(1 + \frac{k^*_\mathrm{on}}{p + k_\mathrm{off}} \right) - \frac{C_\mathrm{eq}}{p + k_\mathrm{off}}$$

• Hollenbeck, K. J. (1998) INVLAP.M: A matlab function for numerical inversion of Laplace transforms by the de Hoog algorithm, http://www.isva.dtu.dk/staff/karl/invlap.htm

A useful Laplace transform pair lacking a proof

With $x > x' > 0$, $q = \sqrt{p/D}$,

$$F(p) = I_\nu(qx')K_\nu(qx) = \int_0^\infty e^{-pt} f(t)\, dt,$$

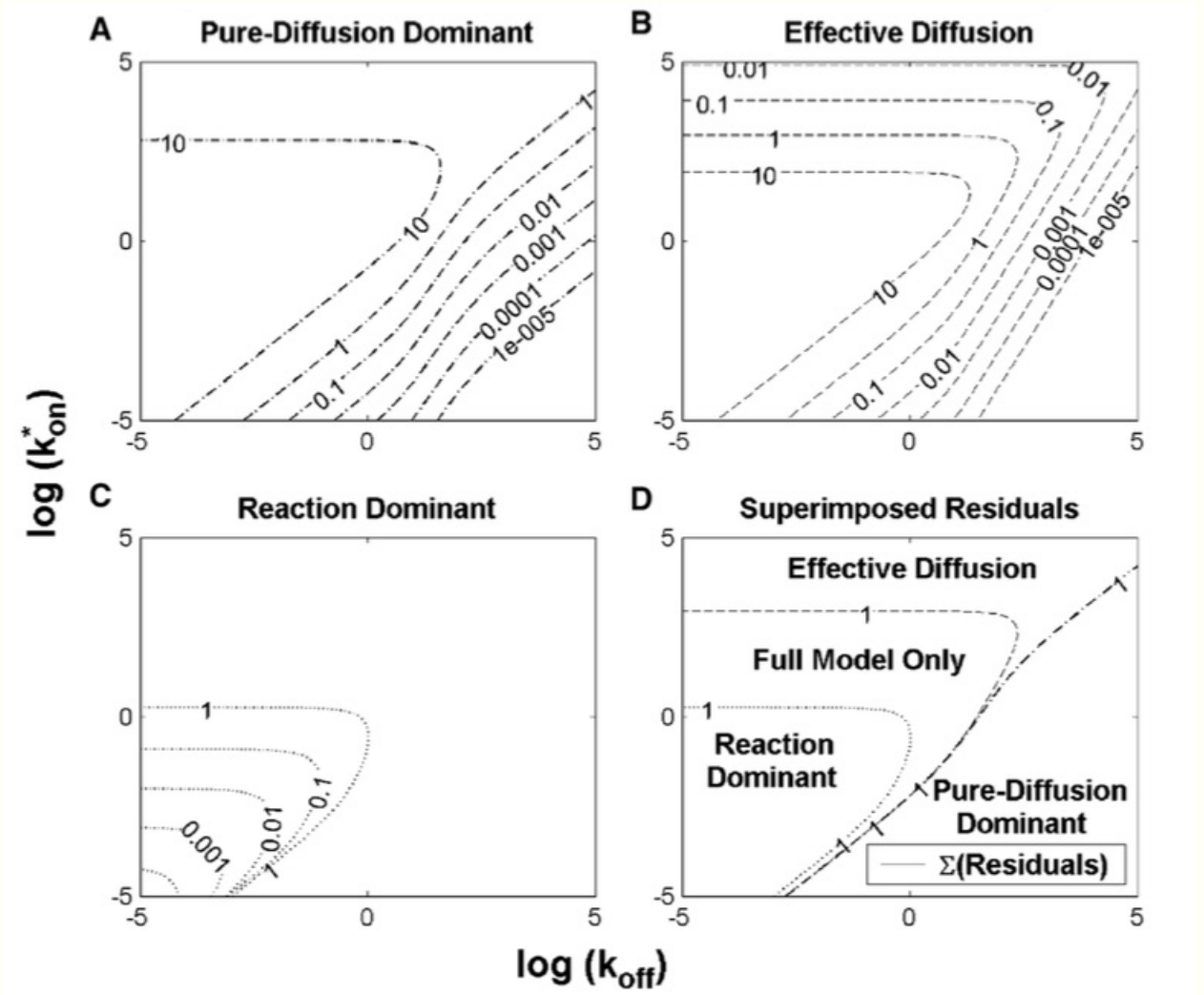
$$f(t) = \frac{1}{2t} \exp\left(-\frac{x^2 + x'^2}{4Dt}\right) I_\nu\left(\frac{xx'}{2Dt}\right)$$

Tables: Crank (1975, p378), Carslaw & Jaeger, Oberhetting & Badii 15.9

This yields the explicit pure diffusion FRAP recovery curve of Soumpasis 1983:

$$frap(t) = \exp\left(\frac{\tau_\mathrm{D}}{2t}\right)\left(I_0\left(\frac{\tau_\mathrm{D}}{2t}\right) + I_1\left(\frac{\tau_\mathrm{D}}{2t}\right)\right), \qquad \tau_\mathrm{D} = \frac{w^2}{D_\mathrm{f}}$$

Results I: What one can measure in practice



Results I: Classification of limiting regimes

Derived through nondimensionalization, scaling, and asymptotics

- Pure diffusion: $c \sim 0, \quad \partial_t f = D_{\mathrm{f}} \nabla^2 f.\ \tau_{\mathrm{D}} = \dfrac{w^2}{D_{\mathrm{f}}}.$

  Data fitting determines D_{f}. No measurable information about binding.

- Effective diffusion: fast reaction, slow diffusion.

  $D_{\mathrm{eff}} = \dfrac{D_{\mathrm{f}}}{1 + (k^*_{\mathrm{on}}/k_{\mathrm{off}})}, \quad \tau_{\mathrm{D}} = \dfrac{w^2}{D_{\mathrm{eff}}}.$ Can use to measure: $k^*_{\mathrm{on}}/k_{\mathrm{off}}$

- Reaction dominant: diffusion fast compared to reaction & time scale:

  $frap(t) = 1 - C_{\mathrm{eq}} e^{-k_{\mathrm{off}} t}$ Can use to measure both k^*_{on} and k_{off}.

- Full model

Plus: description of transition zones

Results I: Constraints/conditions for limiting regimes

Derived through nondimensionalization, scaling, and asymptotics

- Pure diffusion:

  $$k_{\mathrm{on}}^*/k_{\mathrm{off}} \ll 1, \quad \tau \sim \tau_{\mathrm{D}} = \frac{w^2}{D_{\mathrm{f}}}.$$

- Effective diffusion: fast reaction, slow diffusion.

  $$k_{\mathrm{on}}^* \frac{w^2}{D_{\mathrm{f}}} \gg 1, \quad \tau \sim \frac{w^2}{D_{\mathrm{eff}}}$$

- Reaction dominant: diffusion fast compared to reaction & time scale:

  $$k_{\mathrm{on}}^* \frac{w^2}{D_{\mathrm{f}}} \ll 1, \quad \frac{k_{\mathrm{off}}}{k_{\mathrm{on}}^*} \lesssim 1$$

- Full model

(Hiding here are convergence theorems. . .)

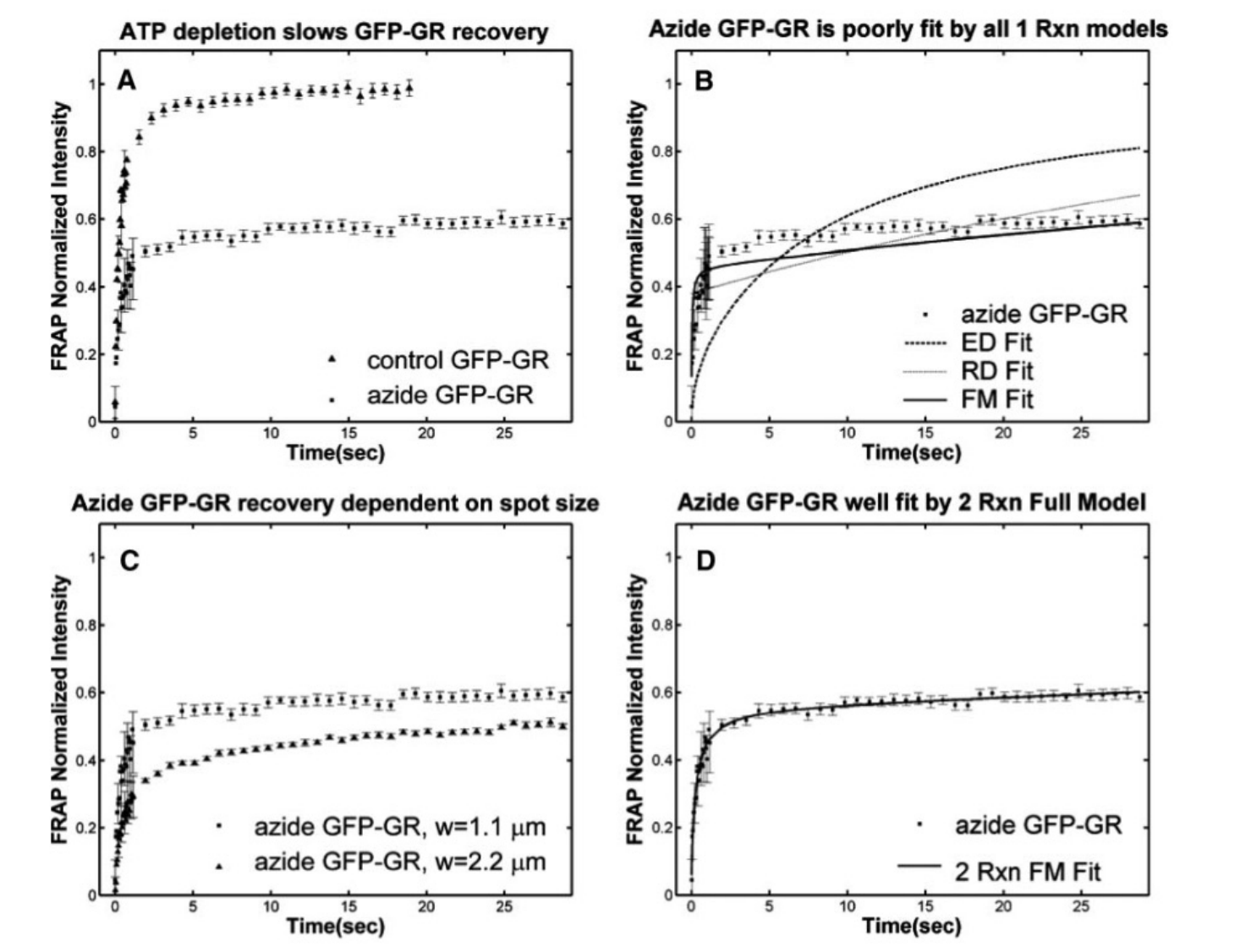
Results II: Physical conclusions

- Laid out a protocol for identifying roles of diffusion and reaction in FRAP experiments
- Predicted a new non-specific binding state of GR to DNA matrix, with average binding time

$$\frac{1}{k_{\text{off}}} \sim 12.7 \text{ ms}$$

- ATP-depleted GR binding may involve 2-species reactions

Results II: Physical conclusions



Summary

We clarify the roles of diffusion, binding, and number of binding states in FRAP recovery experiments.

Significant improvement on earlier styles of inference based on existence of fast/slow parts of recovery curves and failure to fit the pure diffusion model.

Subsequent work has improved the modeling of

- cellular geometry
- spatial bleaching intensity profile
- temporal bleaching profile
- site diffusion

A role for fractal geometric structures has been suggested.

Two later references regarding FRAP

Current Opinion in Cell Biology 2010, 22:403–411

FRAP and kinetic modeling in the analysis of nuclear protein dynamics: what do we really know?

Florian Mueller[1], Davide Mazza[1], Timothy J Stasevich[1] and James G McNally

Biophysical Journal Volume 99 November 2010 2737–2747

A Quantitative Approach to Analyze Binding Diffusion Kinetics by Confocal FRAP

Minchul Kang,[†] Charles A. Day,[†] Emmanuele DiBenedetto,[‡*] and Anne K. Kenworthy[‡§*]

[†]Department of Molecular Physiology and Biophysics, Vanderbilt University School of Medicine, Nashville, Tennessee; [‡]Department of Mathematics, Vanderbilt University, Nashville, Tennessee; and [§]Department of Cell and Developmental Biology, Vanderbilt University School of Medicine, Nashville, Tennessee

Mathematical models of anomalous diffusion

Definitions of anomalous diffusion differ, e.g.: $\langle x^2 \rangle \sim t^p$, $p \neq 1$.

Two basic types of models, associated with different kinds of random walks

- Superdiffusive behavior: Lévy flights, heavy-tailed jump distributions
- Subdiffusive behavior: random walks with waiting-time distributions

(Not discussed: nonlinear models, single-file models, random media,. . .)

Mathematical models of anomalous diffusion I: superdiffusion

- Lévy-Khintchine theory of diffusion processes & semigroups
(characterizes space- and time-stationary Markov processes)

- α-stable Lévy processes, Fractional heat equation: $\partial_t u + (-\Delta)^\alpha u = 0$

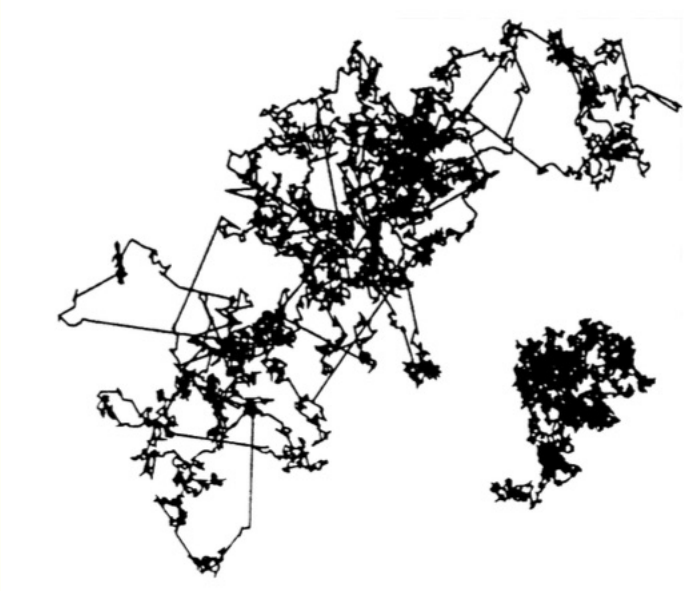


Fig. 2. Lévy trajectory with Lévy index 1.5 consisting of 7000 steps. The lines connect successive locations of the random walker, illustrating the clustering nature which gives rise to the fractal graph dimension (compare [16]). The small trajectory corresponds to a Gaussian random walk with the same number of steps. The space-filling character in this 2-D case contrasts the fractal structure of the Lévy trajectory.

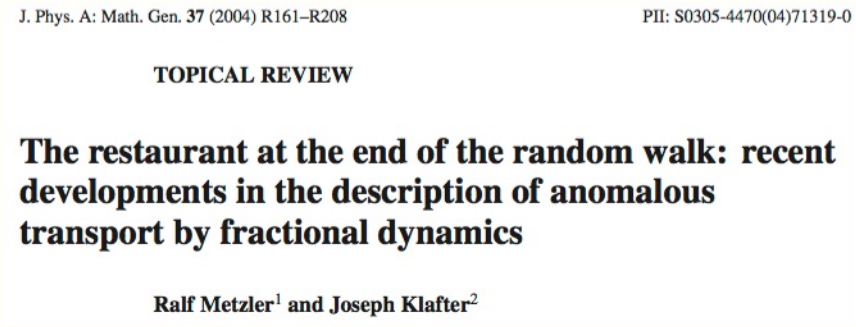
R. Metzler, T.F. Nonnenmacher / Chemical Physics 284 (2002) 67–90

Mathematical models of anomalous diffusion II: subdiffusion

- Subdiffusion: Fractional time derivatives, CTRW/Montroll-Weiss models

Mechanism: Continuous-time random walks with waiting-time distributions

A large physical literature:


J. Phys. A: Math. Gen. 37 (2004) R161–R208 PII: S0305-4470(04)71319-0

TOPICAL REVIEW

The restaurant at the end of the random walk: recent developments in the description of anomalous transport by fractional dynamics

Ralf Metzler[1] and Joseph Klafter[2]

$$u(x,t) = \Psi(t)u_0(x) + \int_0^t \phi(t-\tau) \int_{\mathbb{R}^n} w(x-y)u(y,\tau)\,dy\,d\tau$$

Gorenflo & Mainardi (2006) argue that a robust limit (analog of α-stable laws) involves *Mittag-Leffler* waiting time distributions:

$$\phi(t) = -\partial_t E_\beta(-t^\beta), \qquad E_\beta(z) = \sum_{n=0}^{\infty} \frac{z^n}{\Gamma(1+\beta n)}$$

Double porosity (subdiffusion) models by homogenization

Barenblatt, Zheltov, Kochina 1960: Reaction-diffusion model!

Derivation via two-scale homogenization theory/periodic unfolding:

- Arbogast, Douglas, Hornung SIAM J. Math. Anal. 21 (1990) 893,
- Hornung & Showalter, J. Math. Anal. Appl. 47 (1990) 69
- G. W. Clark, J. Math. Anal. Appl. 226 (1998) 364

Double porosity (subdiffusion) models by homogenization

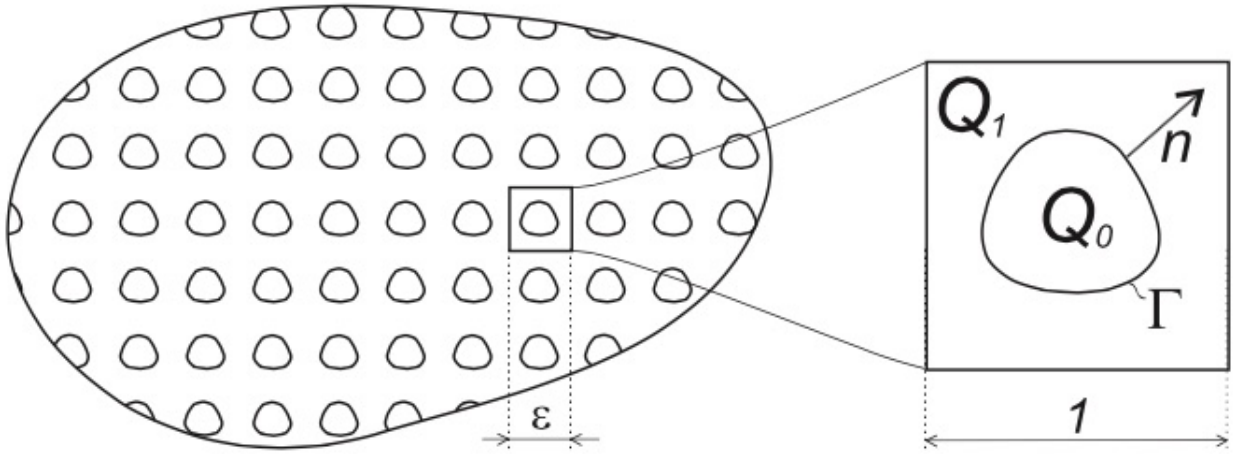


FIGURE 1. The geometry and the periodicity cell

$$\frac{\partial u}{\partial t} - \frac{\partial}{\partial x_i}\left(a_{ij}^h \frac{\partial u}{\partial x_j}\right) + \int_{\partial Y} A_{ij}\frac{\partial U}{\partial y_j}\cdot \nu_i \, dS = 0 \quad \text{in } \Omega \times (0,T)$$

$$\frac{\partial U}{\partial t} - \frac{\partial}{\partial y_i}\left(A_{ij}\frac{\partial U}{\partial y_j}\right) = 0 \quad \text{in } \Omega \times Y \times (0,T)$$

Outlook

Experimental technique, detail, data is amazing and fast evolving (FRAP, fluorescence correlation spectroscopy, single-molecule tracking)

Open questions abound! (And seem to be harder than they look)

- What kinds of CTRW/anomalous diffusion models arise naturally?
- Classification theorems are missing!

(By analogy to Markov processes/Levy processes: infinitely divisible laws, Levy-Khintchine representation formula for Markovian random walks.)

There is a great diversity of other types of transport phenomena in biology: microtubulues, filament networks, velocity-jump and velocity-diffusion processes. . .

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Thank you!