The DNA binding activity of p53 displays reaction–diffusion kinetics

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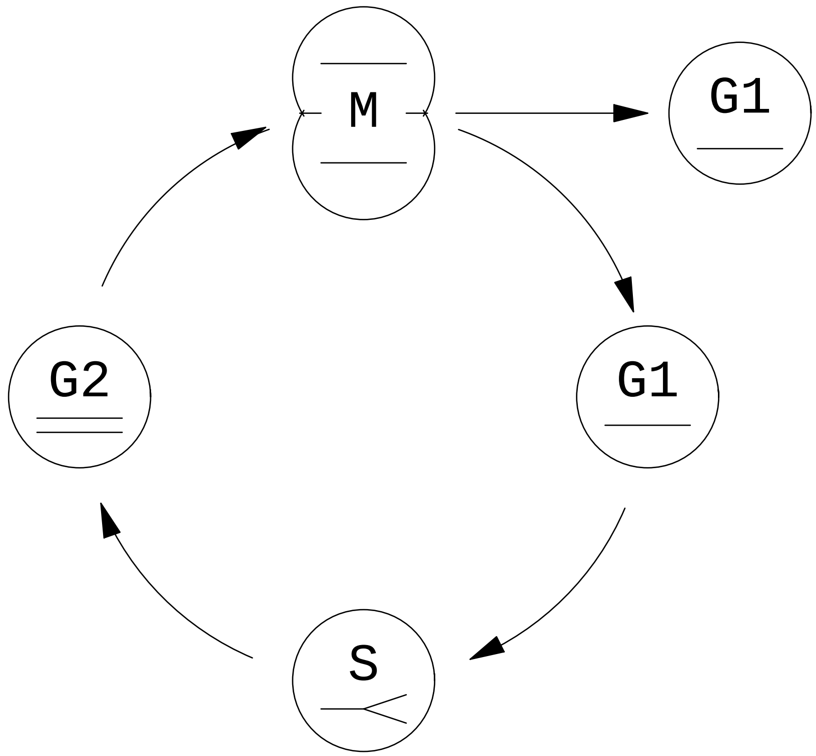
Outline of the talk

- the role of p53 in cell cycle control
- fluorescence recovery after photobleaching (FRAP)
- the mathematical model of simple diffusion
- the mathematical model of diffusion in the presence of an immobile structure
- experimental results for p53–GFP and GFP
- conclusions

Cell cycle



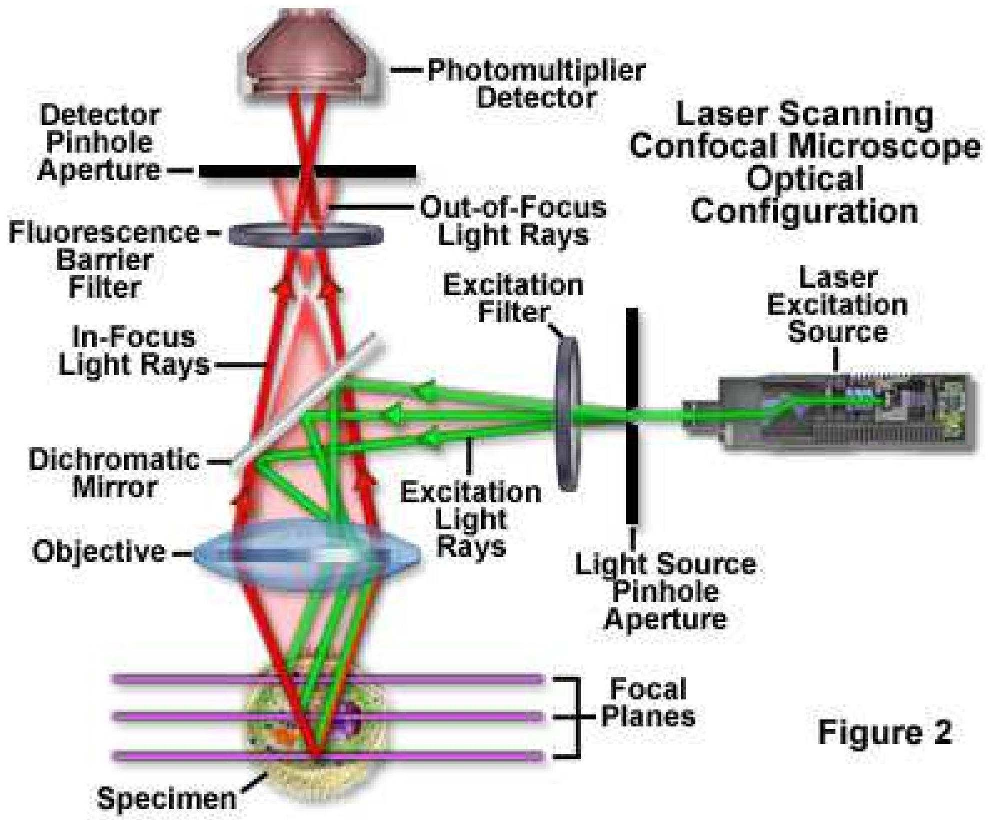
Several checkpoints control the healthy replication of the cells. Erroneous duplication of the cell's DNA content may lead to cancer.

The role of p53

- p53 is a protein that is able to block the cell cycle if DNA is damaged.
- p53 acts as a sequence–specific transcription factor. It localizes to the cell nucleus and initiates the transcription of target genes (DNA repair, apoptosis).
- The p53 gene is mutated in about 60% of all human cancers.

How does p53 move in the cell's nucleus? Are there processes competing with Brownian motion?

Confocal microscopy



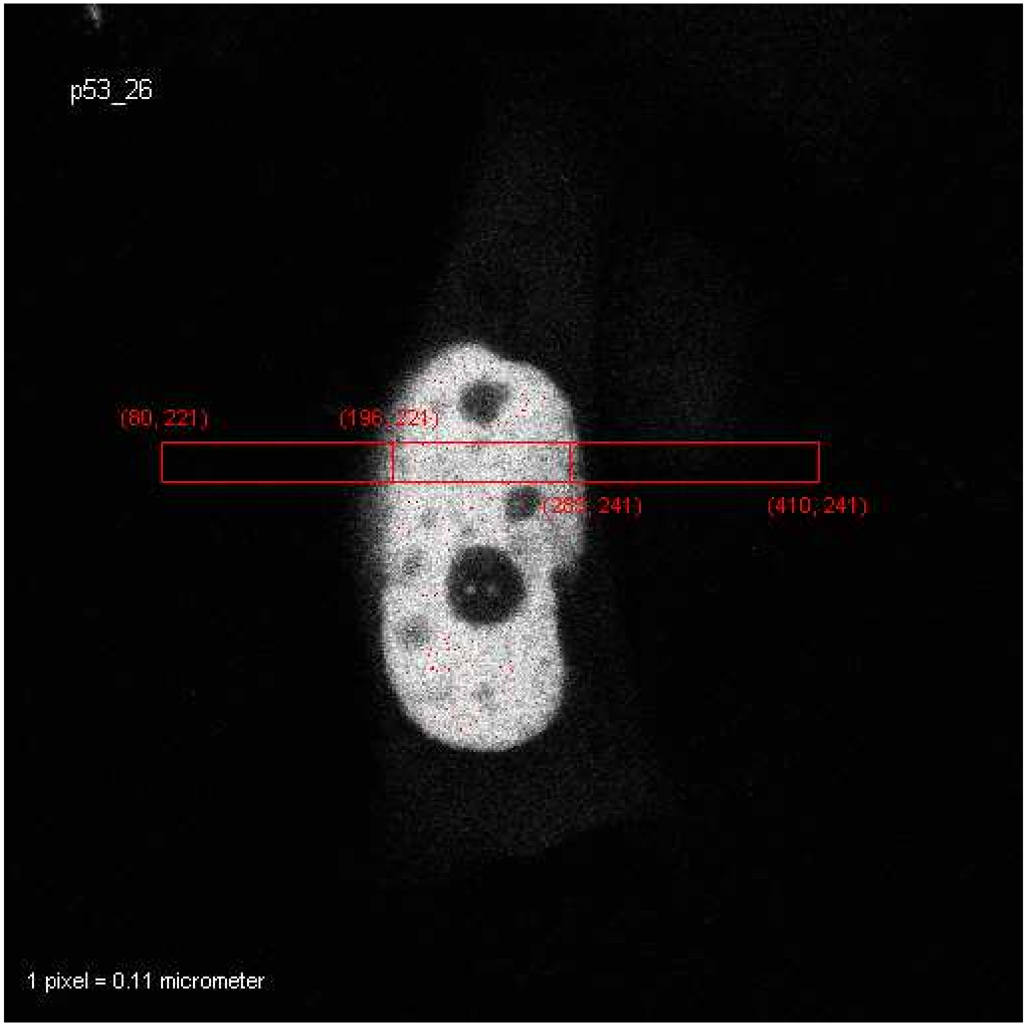
http://www.olympusconfocal.com

Fluorescence recovery after photobleaching

- Fluorescent molecules can be bleached by applying sufficiently strong laser radiation.
- The result is a non-equilibrium distribution of fluorescent molecules that will relax to an equilibrium distribution.
- The fluorescence recovery is observed with an attenuated laser beam.

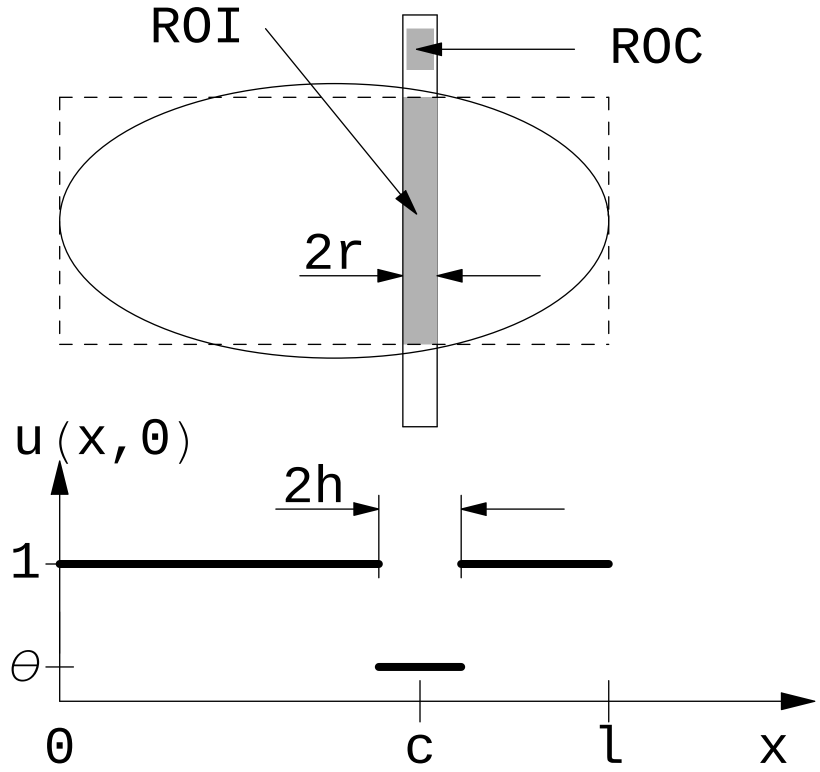
The goal is to determine physical parameters (such as diffusion constants or reaction rate constants) from the fluorescence recovery process.

Experimental setup



A cell nucleus expressing p53–GFP.

Geometrical model



The spatial domain becomes the interval $[0, \ell]$.

Fluorescence recovery after photobleaching

The fluorescence intensity at time t is modeled by

$$F(t) = \int_0^\ell I(x)u(x,t)\,dx,$$

where $I(x)$ is the intensity profile of the laser beam and $u(x,t)$ the concentration of fluorescent molecules at x at time t. We work with a uniform intensity profile

$$I(x) = \begin{cases} 1 & \text{if } |x-c| \leq r \\ 0 & \text{otherwise} \end{cases} \qquad x \in [0,\ell]$$

Mathematical model of simple diffusion

$$
\begin{aligned}
\frac{\partial}{\partial t}u(x,t) &= D\frac{\partial^2}{\partial x^2}u(x,t), \quad x \in (0,\ell),\ t \geq 0,\\
\frac{\partial}{\partial x}u(0,t) &= \frac{\partial}{\partial x}u(\ell,t) = 0, \quad t \geq 0,\\
u(x,0) &= \begin{cases} 1 & \text{if } |x-c| > h \\ \theta & \text{if } |x-c| \leq h \end{cases},
\end{aligned}
\tag{1}
$$

where ℓ is the length of the compartment, h is the half–width of the bleached region centered at c, and θ is the bleach depth ($0 < \theta < 1$).

Mathematical model of simple diffusion

It is solved with the help of a Fourier series.

$$F_1(t; D) = \frac{1}{2r} \int_{c-r}^{c+r} u(x,t) \mathrm{d}x.$$

This expression is used to determine the diffusion constant D by fitting the theoretical expression to experimental data.

Diffusion in the presence of binding

- Suppose the compartment is filled by a spatially homogeneous immobile structure to which fluorescent molecules can bind at rate k_1 and from which they are released at rate k_2 (Sprague et al. 2004, Carrero et al. 2004).
- There are always enough free binding sites so that saturation effects do not occur.
- Before the bleaching an equilibrium between free and bound molecules exists.

Let $u(x,t)$ denote the concentration of freely diffusing molecules and $v(x,t)$ denote the concentration of (temporarily) bound molecules.

Diffusion in the presence of binding

The equations of this model are

$$
\begin{aligned}
\frac{\partial}{\partial t}u(x,t) &= D\frac{\partial^2}{\partial x^2}u(x,t) - k_1 u(x,t) + k_2 v(x,t)\\
\frac{\partial}{\partial t}v(x,t) &= k_1 u(x,t) - k_2 v(x,t)\\
\frac{\partial}{\partial x}u(0,t) &= \frac{\partial}{\partial x}u(\ell,t) = 0\\
u(x,0) &= \frac{k_2}{k_1+k_2}\begin{cases} 1 & \text{if } |x-c| > h\\ \theta & \text{if } |x-c| \le h\end{cases}\\
v(x,0) &= \frac{k_1}{k_1+k_2}\begin{cases} 1 & \text{if } |x-c| > h\\ \theta & \text{if } |x-c| \le h\end{cases},
\end{aligned}
\tag{2}
$$

the geometrical parameters being the same as in the one parameter model.

Diffusion in the presence of binding

The model (2) is solved with help of Fourier and Laplace transforms. We obtain an expression for

$$F_2(t; D, k_1, k_2) = \frac{1}{2r} \int_{c-r}^{c+r} [u(x,t) + v(x,t)] \, \mathrm{d}x, \qquad (3)$$

which contains the parameters D, k_1 and k_2 that are to be determined. Observe that the models (1) and (2) are *nested* in the sense that if $k_1 = 0$ we have

$$F_2(t; D, 0, \cdot) = F_1(t; D).$$

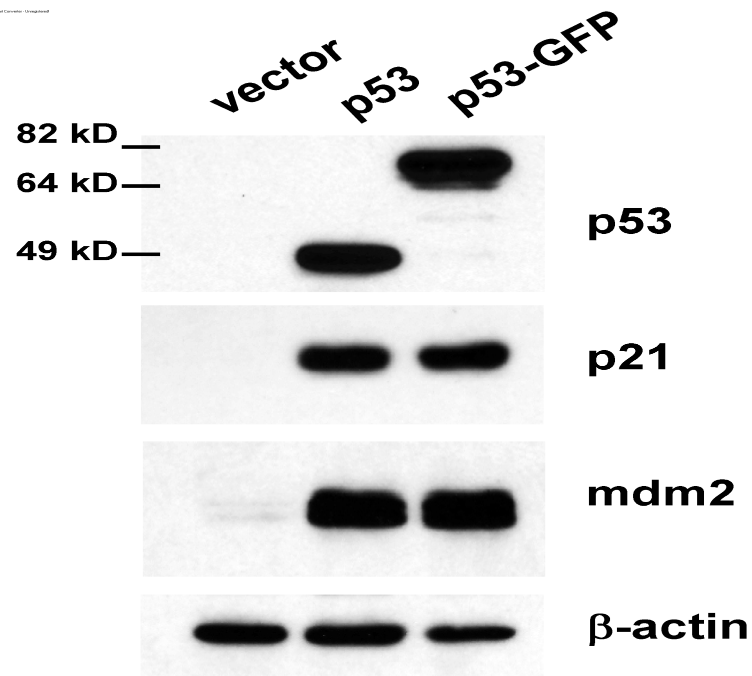
The least–square fit

We want to minimize the cost functional

$$J(q) = \sum_{i=1}^{n} (F(t_i; q) - F_{data}(t_i))^2 \rightarrow \min_{q \in \mathcal{Q}_{ad}} !,$$

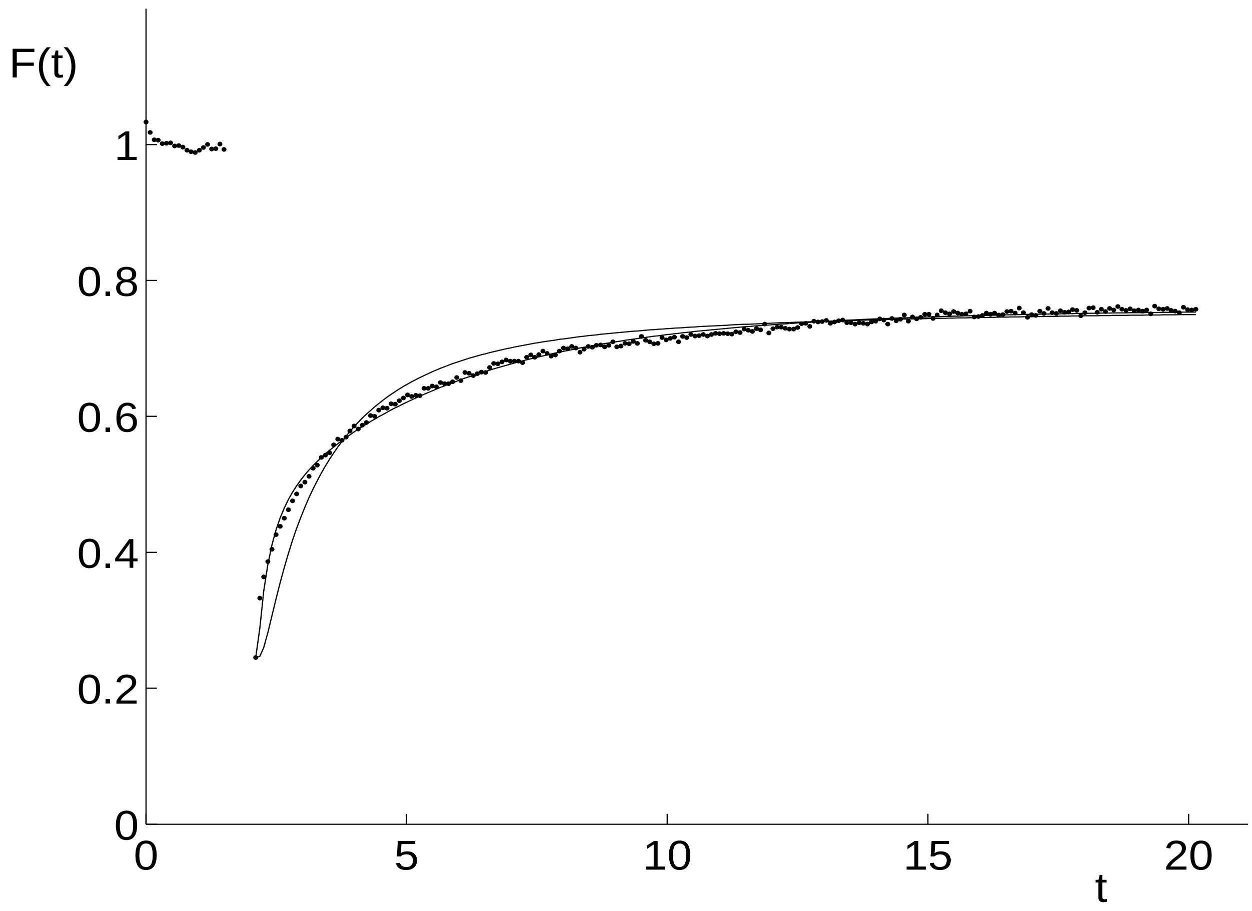
where n is the number of data points making up the recovery part of the experiment and $\mathcal{Q}_{ad}$ denotes the set of admissible parameters (1 resp. 3 parameters, positivity is the only constraint).

Experimental results



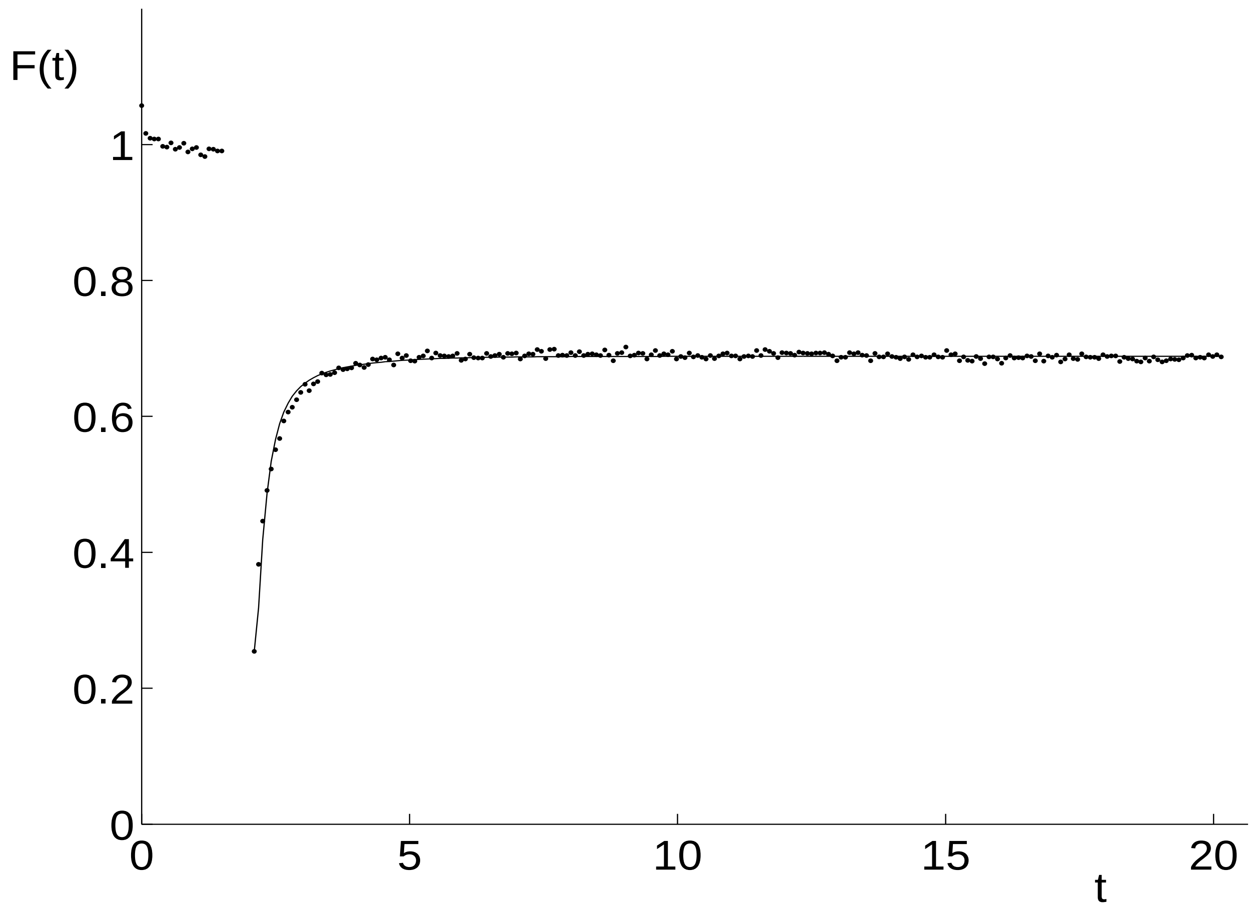
The p53–GFP fusion protein upregulates p53 target genes equivalent to unmodified p53. Our system is a valid probe for imaging of p53 nuclear dynamics.

Recovery curves



A representative fluorescence recovery curve for p53–GFP and the optimal fits with the one parameter model and the three parameter model.

Recovery curves



A representative fluorescence recovery curve for GFP (the control) and the optimal fit with the one parameter model.

The significance of a parameter in a nested model

Suppose the fluorescence measurements (3) arise in such a way that

$$F_{data}(t_i) = F_2(t_i; D^*, k_1^*, k_2^*) + \varepsilon_i,$$

where (D^*, k_1^*, k_2^*) is the "true" parameter and the ε_i are independent, identically distributed (iid) random variables with mean $\mathsf{E}(\varepsilon_i) = 0$ and variance $Var(\varepsilon_i) = \sigma^2 < \infty$. We want to test the hypothesis

$$H_0 \, : \, k_1^* = 0.$$

The significance of a parameter in a nested model

We make use of a result by Banks and Fitzpatrick.
Consider the statistic

$$U = \frac{J(\tilde{D}, 0, \cdot) - J(\hat{D}, \hat{k}_1, \hat{k}_2)}{J(\hat{D}, \hat{k}_1, \hat{k}_2)}, \tag{4}$$

where $\tilde{D}$ is the optimal parameter value obtained from the one parameter model and $\hat{D}$, $\hat{k}_1$ and $\hat{k}_2$ are the optimal parameter values obtained from the three parameter model.

The significance of a parameter in a nested model

Banks and Fitzpatrick *(J. Math. Biol.* **28**, *1990)* prove:
If H_0 is true, the random variable U converges in distribution to a chi–square distributed random variable with 1 degree of freedom, as the number n of data points goes to infinity (under certain assumptions on the noise process, the cost functional and the parameter space).

Results

- The one parameter model of simple diffusion for p53–GFP has to be rejected in almost all cases.
- The one parameter model is able to explain the diffusion of GFP.

protein	$D\,(\mu m^2 s^{-1})$	$k_1\,(s^{-1})$	$k_2\,(s^{-1})$
GFP	$41.6 \pm 13.6\,(10)$	-	-
p53–GFP	$15.4 \pm 5.6\,(58)$	0.31 ± 0.22	0.40 ± 0.13

Results

- The null–hypothesis $k_1 = 0$ can be rejected at high confidence levels in the majority of the individual runs.
- This indicates the significance of the binding rate constant k_1.

The observed diffusion constant

How does the observed diffusion constant of p53–GFP relate to its molecular mass?

Let two spherical proteins be of molecular masses m_1 and m_2, respectively. Their diffusion constants D_1 and D_2 should scale as

$$\frac{D_1}{D_2} = \left(\frac{m_2}{m_1}\right)^{\frac{1}{3}},$$

(Sprague et al. 2004). This is a straightforward consequence of the Einstein–Stokes relation (Einstein 1905).

The observed diffusion constant

Based on our measurements $D_{\text{p53–GFP}} = 15\,\mu m^2 s^{-1}$ and $D_{\text{GFP}} = 40\,\mu m^2 s^{-1}$ and on the known mass $m_{\text{GFP}} = 27\,kg/mole$ (Yang et al. 1996) we can estimate the mass of the p53–GFP fusion particle

$$m_{\text{p53–GFP}} = m_{\text{GFP}} \left(\frac{D_{\text{GFP}}}{D_{\text{p53–GFP}}} \right)^3 > 500\,kg/mole.$$

This is by far larger than the mass of a p53–GFP monomer $53 + 27 = 80\,kg/mole$.

Conclusions

- It is possible to discriminate between competing mathematical models with help of statistical methods.
- p53 binds to an immobile structure in the cell nucleus. This is in agreement with its role as a transcription factor.
- The average time a p53 molecule stays in the bound state is approximately $2.5\,s$. This time range has been termed “nonspecific” DNA binding.

Thank you for your attention

References

[1] P. Hinow, C. Rogers, C. E. Barbieri, J. A. Pietenpol, A. K. Kenworthy and E. DiBenedetto, The DNA binding activity of p53 displays reaction–diffusion kinetics, Biophys. J. **91**:330–342