L3.3: Protein Half Lives

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In this lecture

- Dig deeper into protein half lives
  - The gene product (protein) decay rate constant, α
  - From our hill function model of gene expression

- What does the half life of real proteins look like?

- Does this model hold up in real systems?

Return to our basic rate equation for gene expression

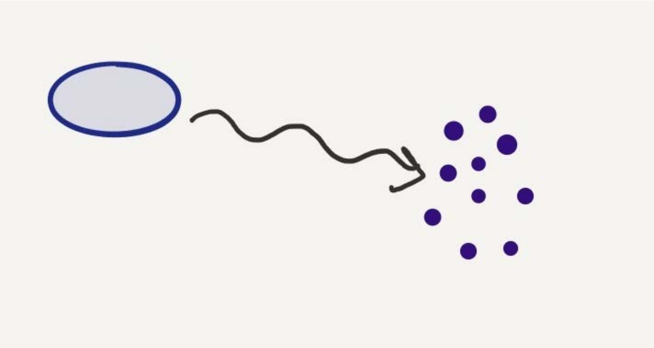
$$\frac{dy}{dt} = generation - consumption$$

$$\frac{dy}{dt} = \beta f(x) - (\alpha_{dilution} + \alpha_{degradation})y$$

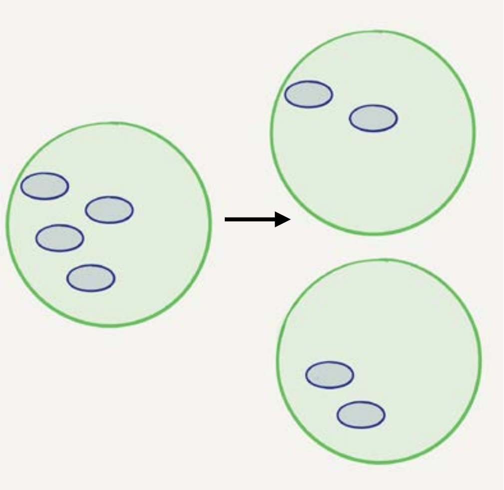
Where
y = conc. protein Y inside cell

Consumption

degradation: passive or active



dilution: cell division



$\alpha_{degradation}$ small for a stable protein
$\alpha_{degradation}$ large for rapidly degraded protein

$\alpha_{dilution}$ large for rapidly dividing cells
$\alpha_{dilution}$ large for slowly dividing cells

Rapidly Dividing Cells


Phys. Biology of the Cell

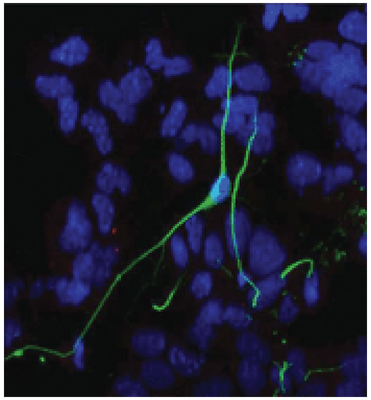
E.coli

$$\alpha = \alpha_{dilution} + \alpha_{degradation}$$

$$\alpha \approx \alpha_{dilution}$$

dominated by dilution

Slowly Dividing Cells


Neuron
Post-mitotic (no division)

$$\alpha = \alpha_{dilution} + \alpha_{degradation}$$

$$\alpha \approx \alpha_{degradation}$$

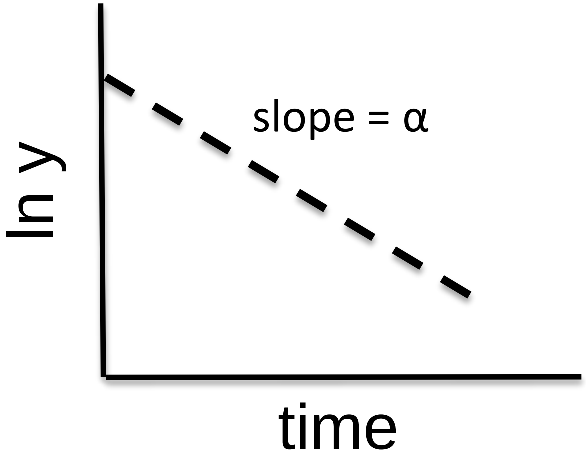
dominated by degradation

$$\frac{dy}{dt} = \beta f(x) - (\alpha_{dilution} + \alpha_{degradation})y$$

If we could prevent or eliminate production of protein

$$\frac{dy}{dt} = -\alpha y \qquad \alpha = \alpha_{dilution} + \alpha_{degradation}$$

Our model would predict simple exponential decay of protein levels with time



If we measured y levels over time we should be able to fit our data to a line with slope determined by the overall decay rate

testable predictions

Proteome Half-Life Dynamics in Living Human Cells

Eran Eden,*† Naama Geva-Zatorsky,* Irina Issaeva, Ariel Cohen, Erez Dekel, Tamar Danon, Lydia Cohen, Avi Mayo, Uri Alon†

Cells remove proteins by two processes: degradation and dilution due to cell growth. The balance between these basic processes is poorly understood. We addressed this by developing an accurate and noninvasive method for measuring protein half-lives, called "bleach-chase," that is applicable to fluorescently tagged proteins. Assaying 100 proteins in living human cancer cells showed half-lives that ranged between 45 minutes and 22.5 hours. A variety of stresses that stop cell division showed the same general effect: Long-lived proteins became longer-lived, whereas short-lived proteins remained largely unaffected. This effect is due to the relative strengths of degradation and dilution and suggests a mechanism for differential killing of rapidly growing cells by growth-arresting drugs. This approach opens a way to understand proteome half-life dynamics in living cells.

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How do you measure protein half life in living cells?
Bleach Chase Experiments

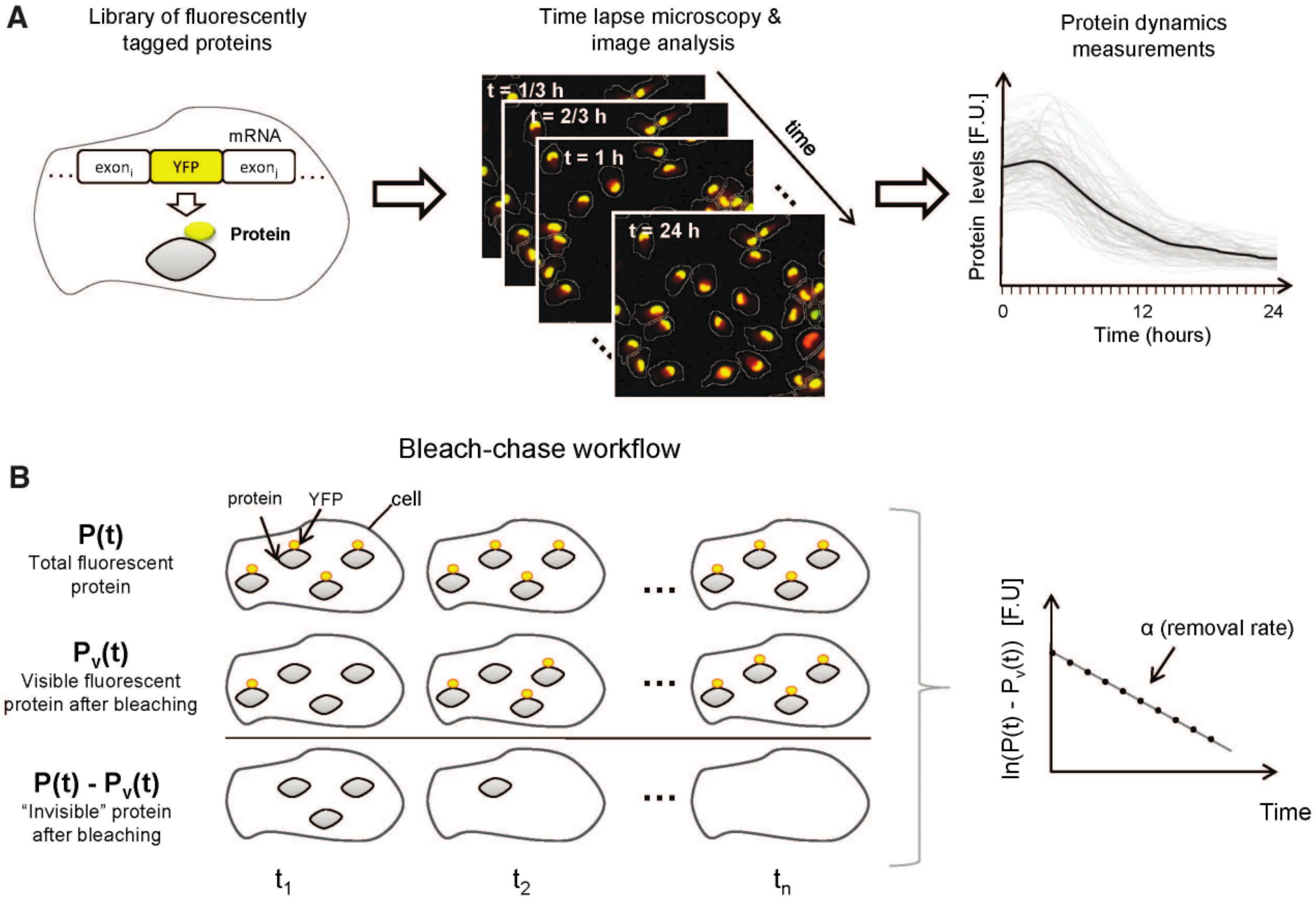

Fig. 1. Bleach-chase workflow. (**A**) Fluorescence of endogenously YFP-tagged proteins is automatically quantified from time-lapse movies (20-min resolution). Average dynamics (black) are means of ~500 individual cells (gray). (**B**) In bleach-chase, protein fluorescence dynamics is measured in bleached and unbleached cells (P_v and P, respectively). The difference between bleached and unbleached cells decays in time, with a slope on a semilogarithmic plot equal to the protein removal rate, α. Half-life is $T_{1/2} = \ln(2)/\alpha$. F.U., fluorescence units.

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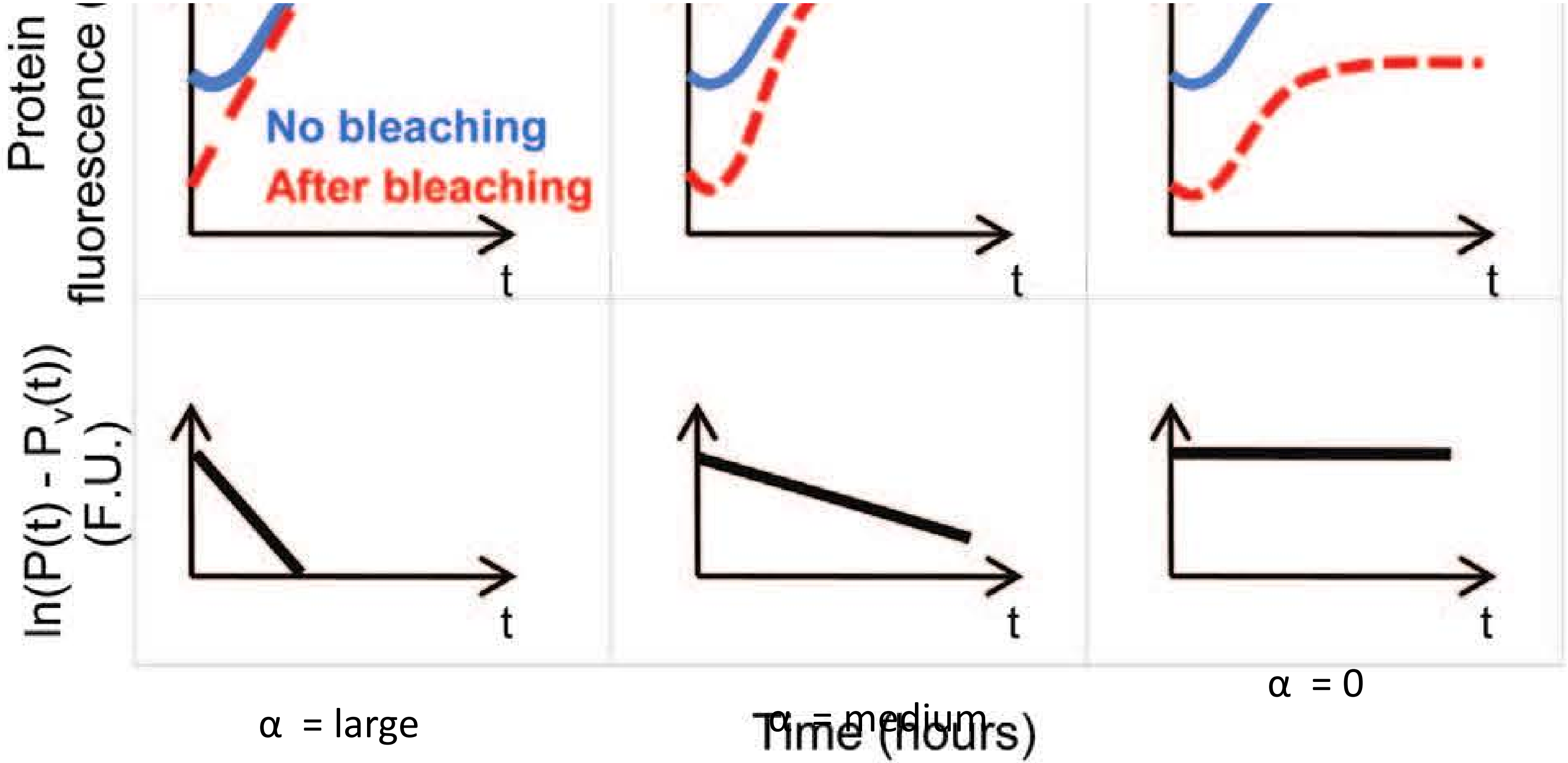
Expected curves



α = large

α = medium

α = 0

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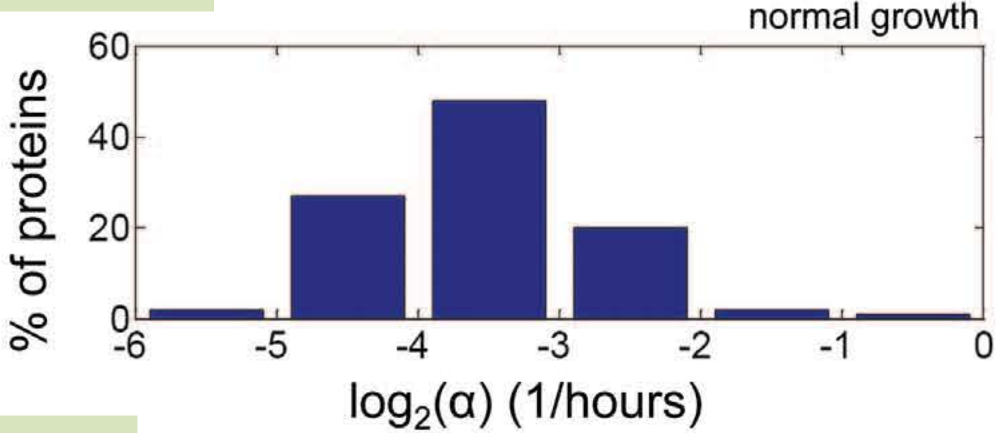
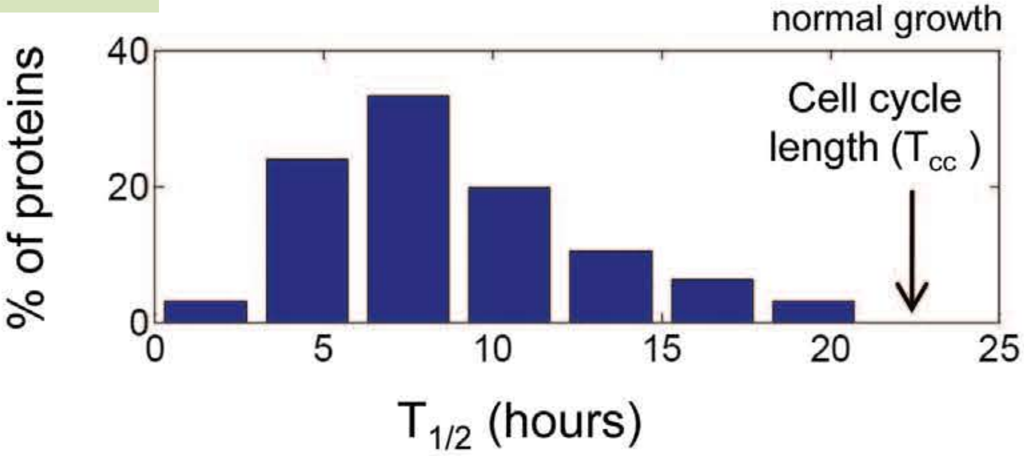
½ life of proteins in lung cancer cells

Decay Rate



Half Life



α = protein removal rate
units = [time^{-1}]
$\alpha = \alpha_{deg} + \alpha_{dil}$
$\alpha_{dil} = 0.03 \pm 0.004\ hr^{-1}$
(based on cell cycle time)

α range: 0.03 hr^{-1} - 0.82 hr^{-1}
ave. $\alpha = 0.1 hr^{-1}$

$$T_{1/2} = \frac{ln2}{\alpha}$$

- Wide range of $T_{1/2}$
- 45 min - 22.5 hours
- Mean = 9.0 hrs

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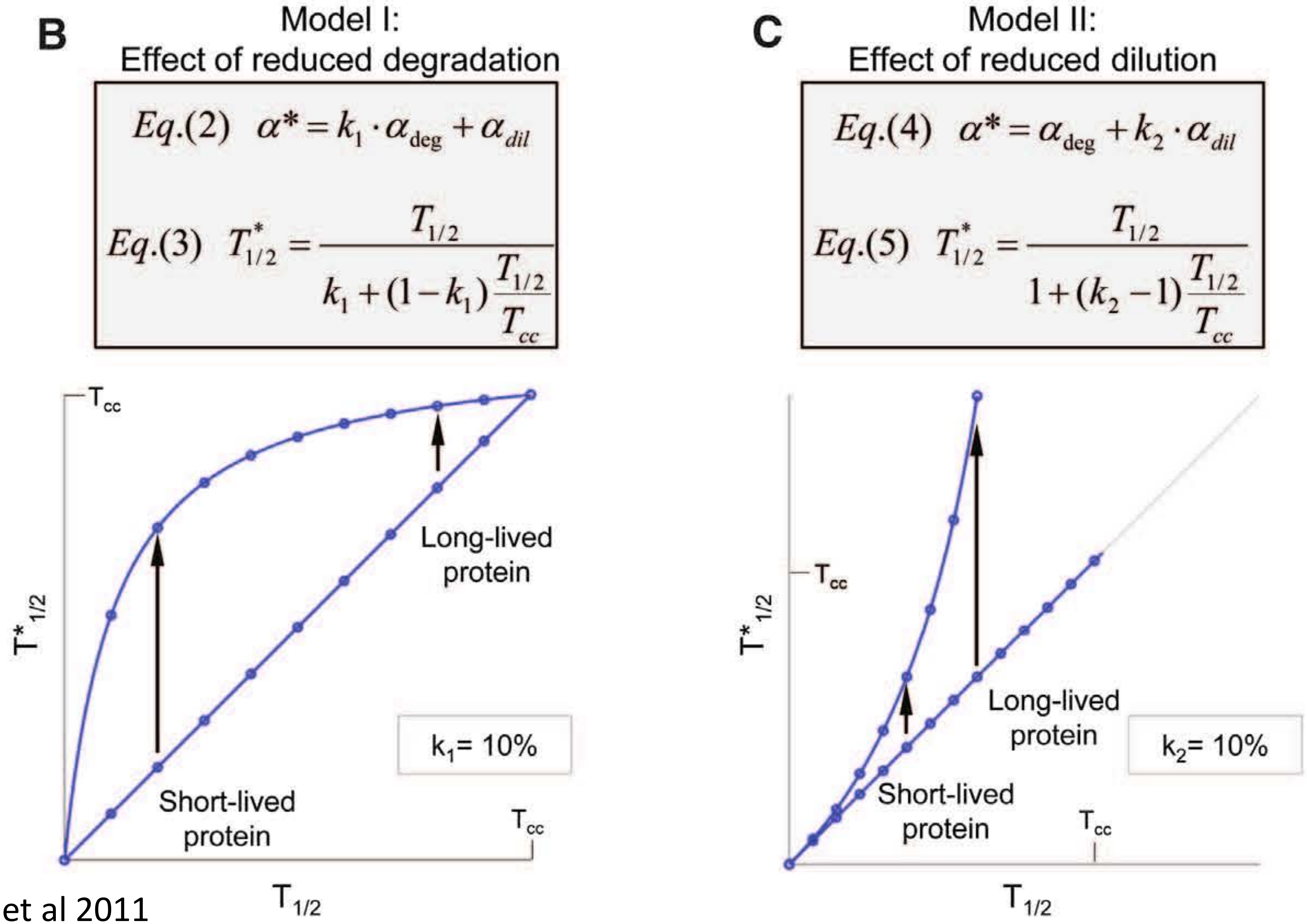
What if you add drug? Model Predictions

α^*, $T_{1/2}^*$ are altered α, $T_{1/2}$ after drug treatment



$$Eq.(2)\quad \alpha^* = k_1 \cdot \alpha_{\deg} + \alpha_{dil}$$

$$Eq.(3)\quad T^*_{1/2} = \frac{T_{1/2}}{k_1 + (1-k_1)\frac{T_{1/2}}{T_{cc}}}$$

$$Eq.(4)\quad \alpha^* = \alpha_{\deg} + k_2 \cdot \alpha_{dil}$$

$$Eq.(5)\quad T^*_{1/2} = \frac{T_{1/2}}{1 + (k_2-1)\frac{T_{1/2}}{T_{cc}}}$$

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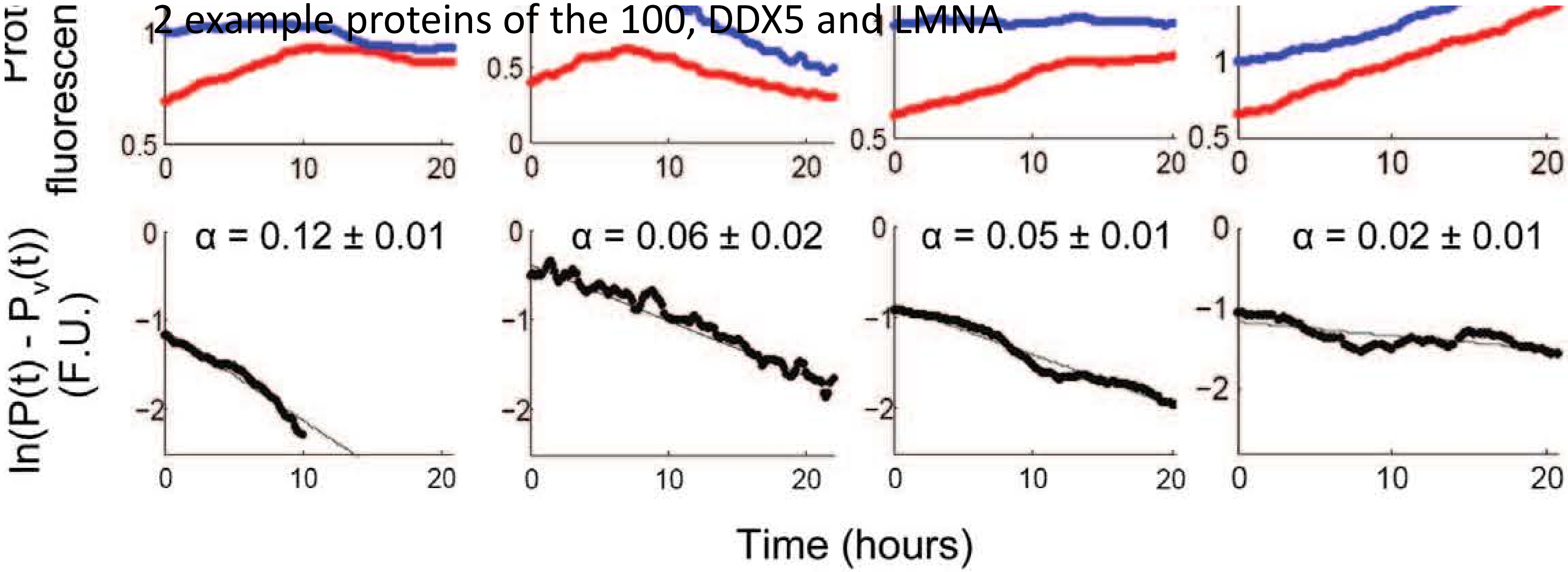
Apply anti-cancer drug, CPT, which arrests cell growth

Observed:
See global increase in protein half life (decrease in α, slope of bottom plot)
Is this dilution or degradation? CPT does change cell division rate.


2 example proteins of the 100, DDX5 and LMNA

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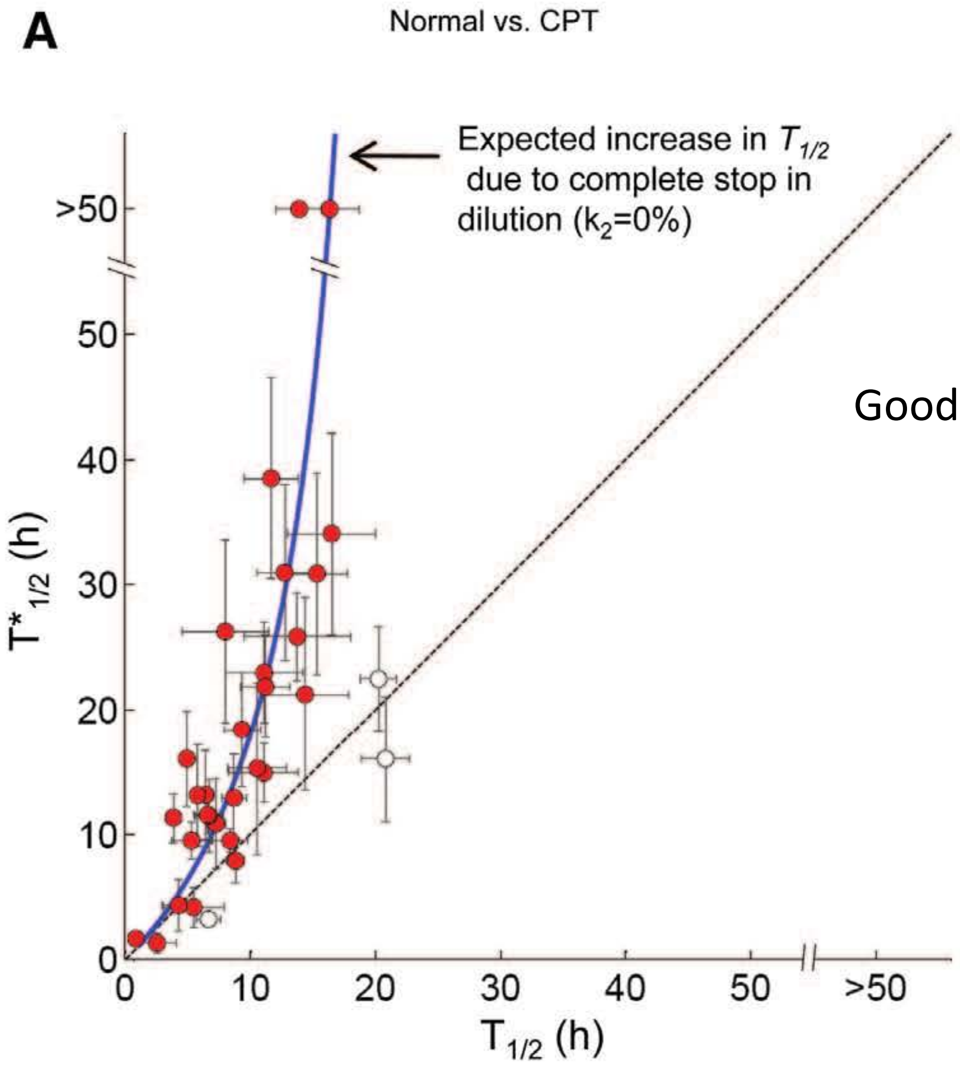
Observed: CPT arrests cell growth, so $k_2 = 0$



Good Fit of Experimental Data to Model

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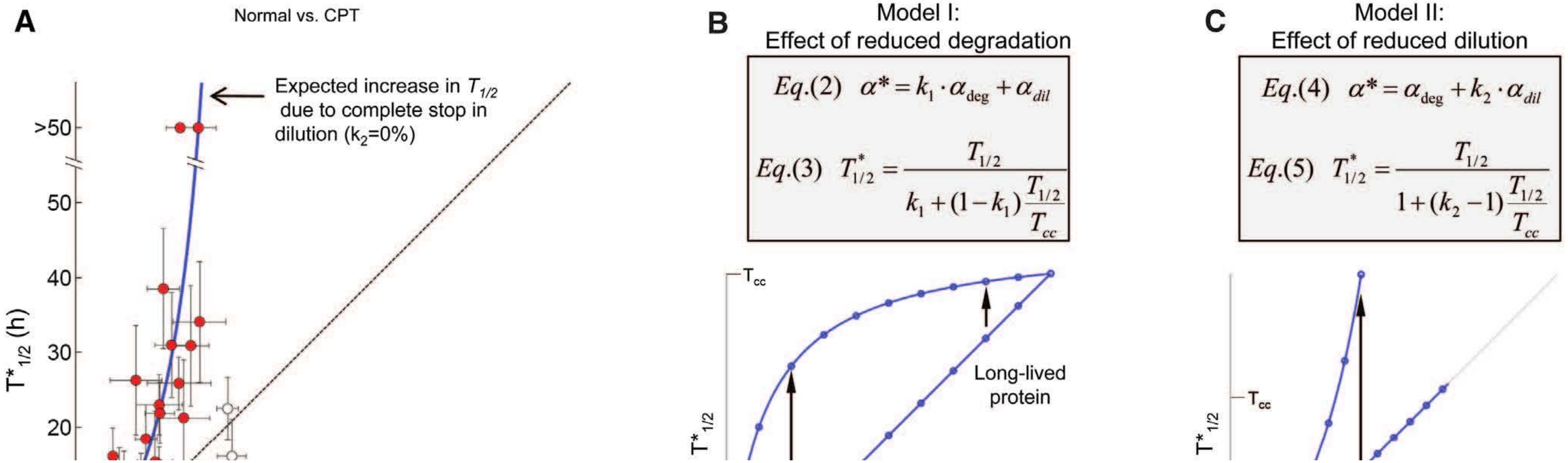
holds up quantitatively not just qualitatively

apply drugs that alter cell doubling time to varying degree, there by affecting k_2 to varying degrees

good fit of experimental to model



Design Curves: Can predict effects of altering cell cycle time on protein half lives

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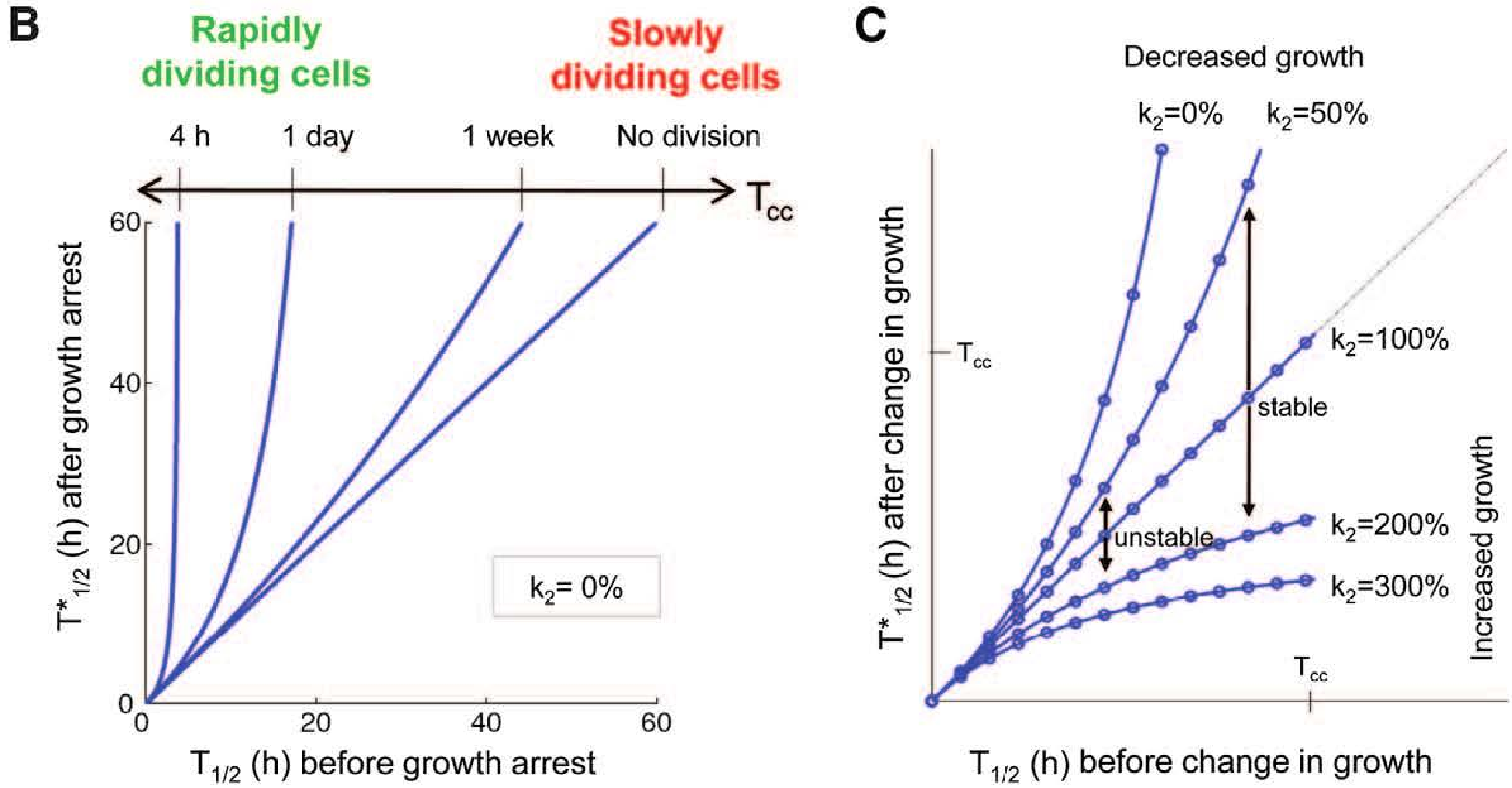
Design Curves

Functional consequence:

- Proteome of more rapidly dividing cells will get more out of whack when growth is arrested
- long half life proteins are more affected by growth arrest than short $T_{1/2}$ proteins



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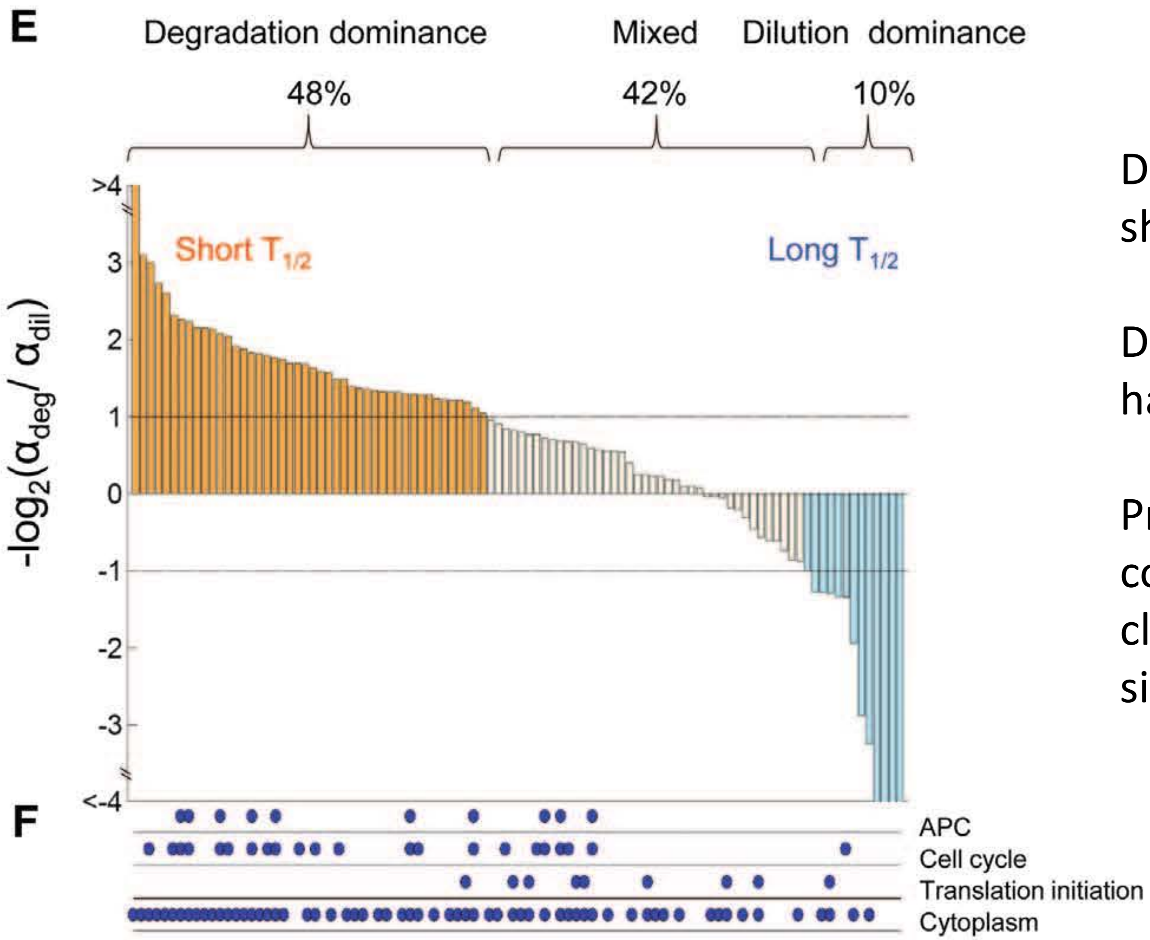
other implications of model



Degradation dominates short half life proteins

Dilution dominates long half life proteins

Proteins within a common functional cluster tend to have similar half lives.

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Coming up …

- Gene Circuit Motifs
  - Autoregulation
- Design features that are enabled
  - E.g. robustness