Modeling dominant interactions that influence the pathogenesis of protein folding diseases

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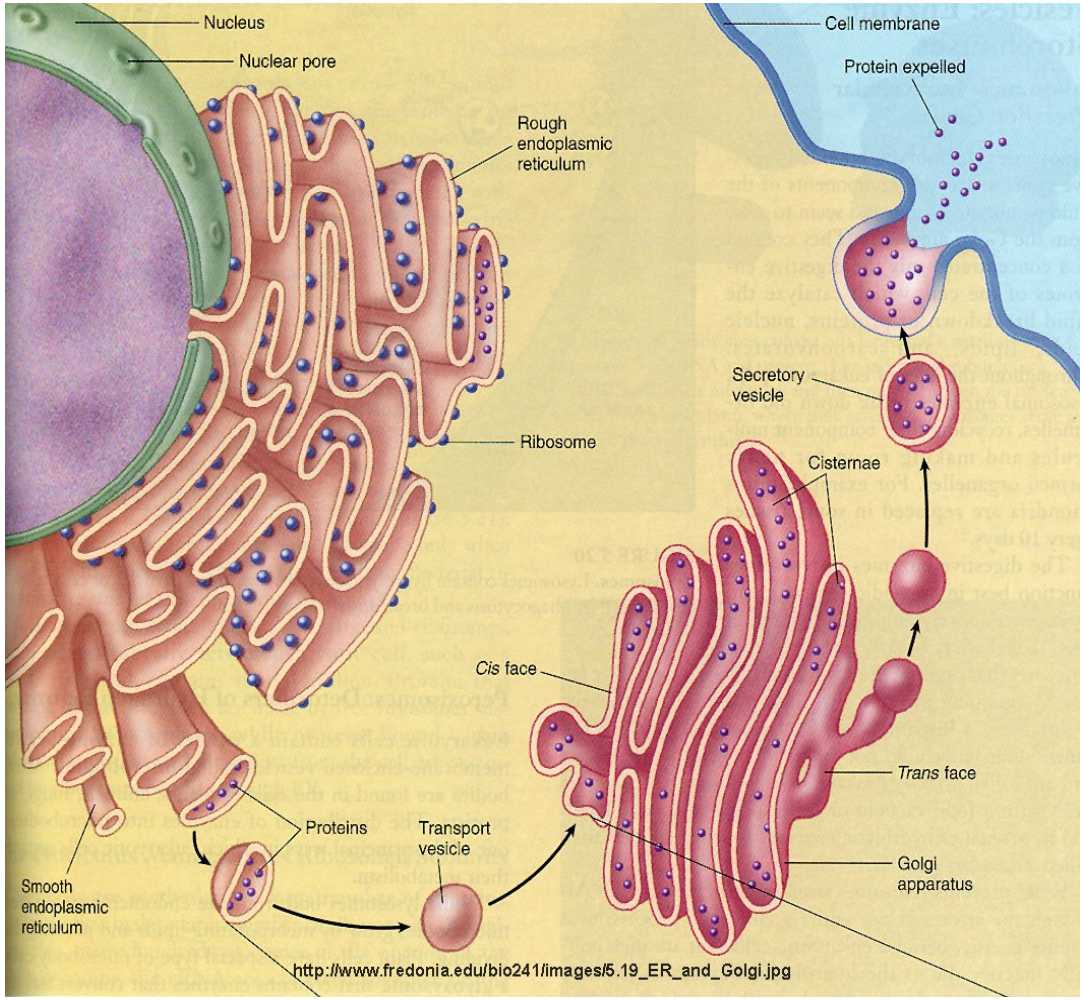
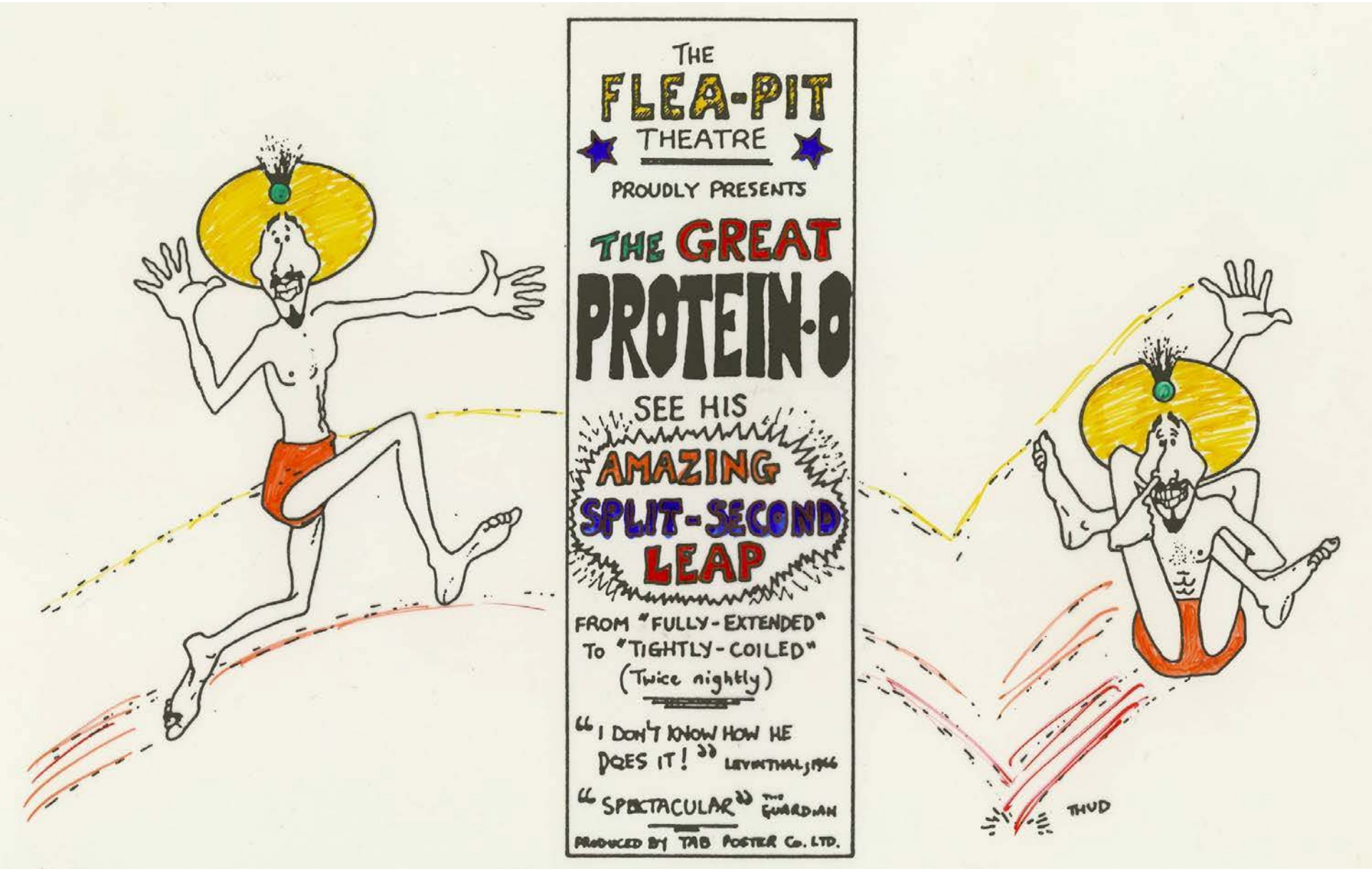
University of Michigan Health System

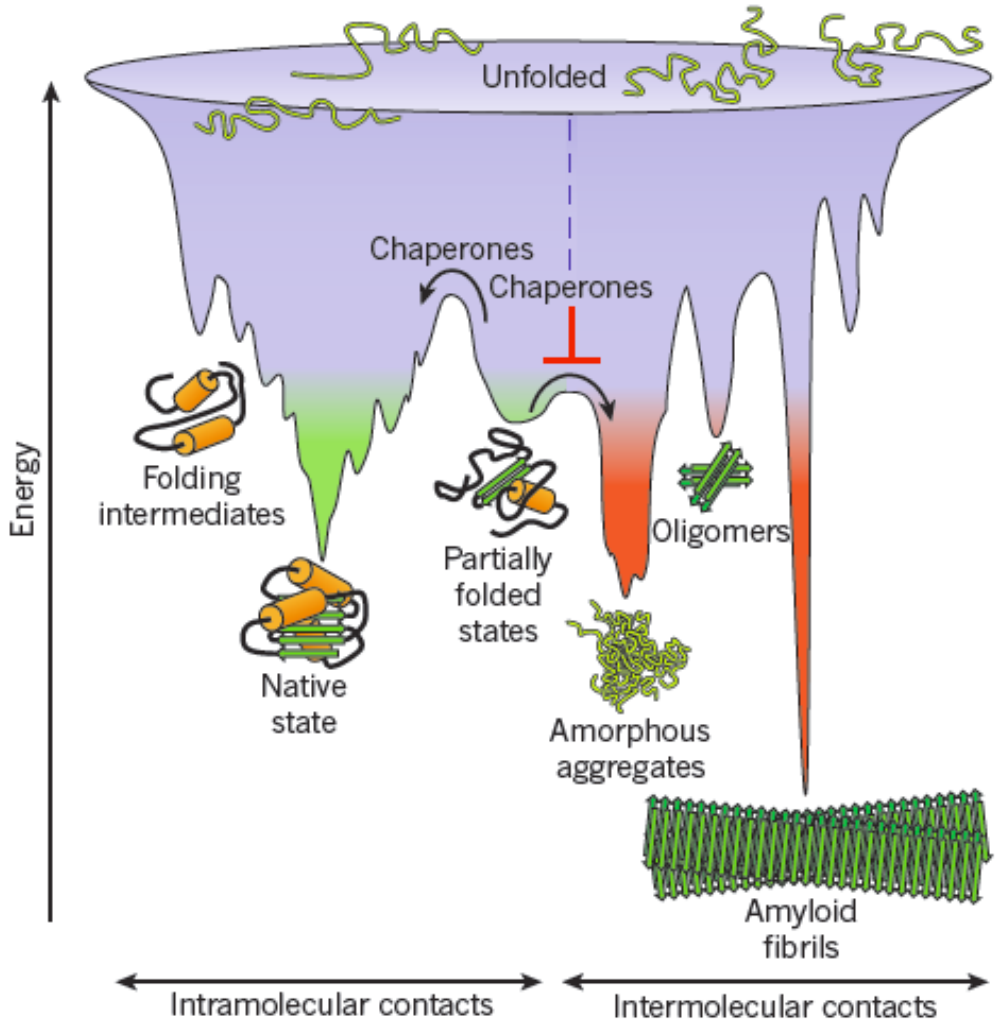
A gene is translated into a protein in the endoplasmic reticulum



There are scientists who believe that protein folding occur spontaneously



Protein folding is assisted through enzyme catalyzed chaperone/co-chaperone network



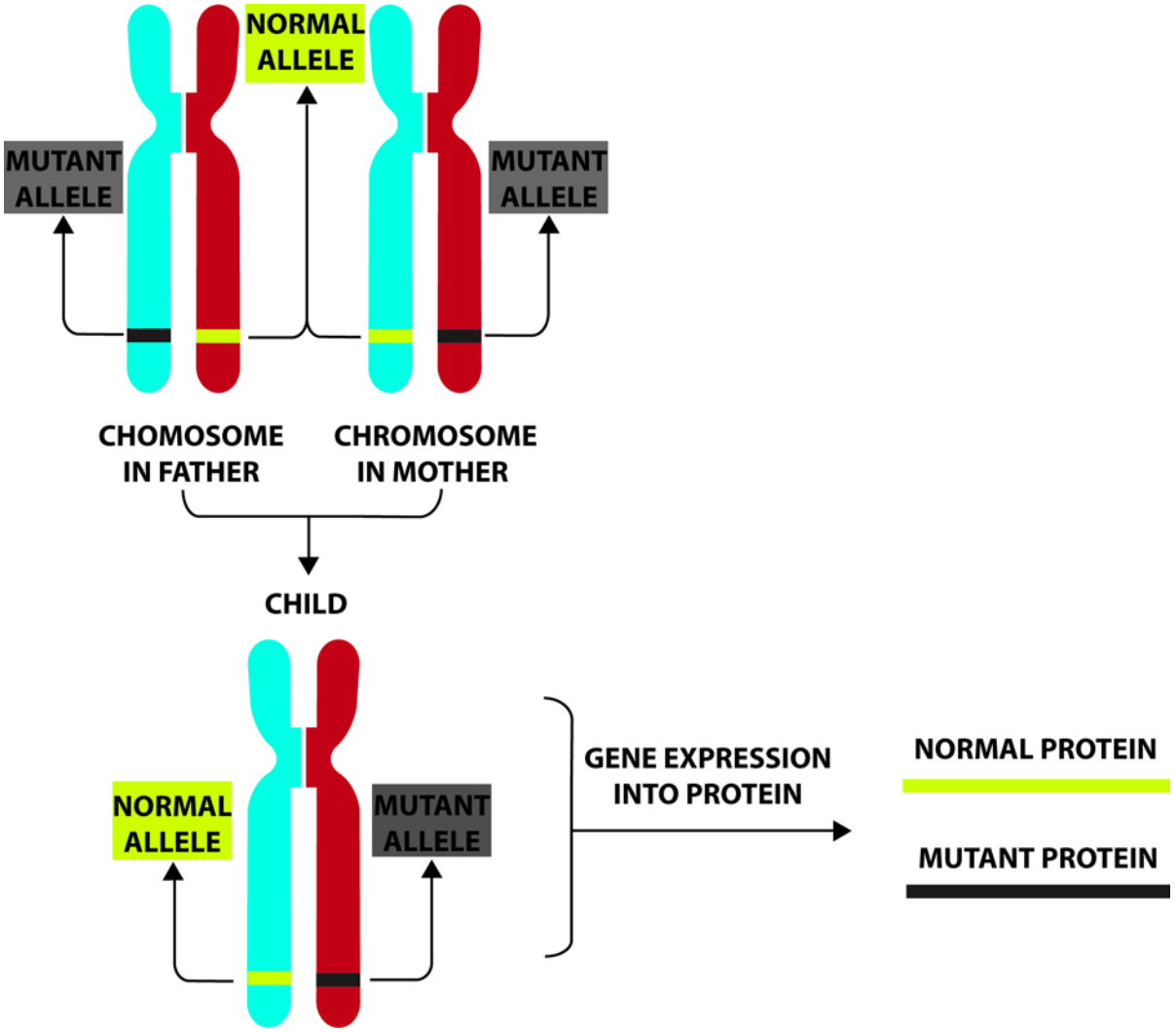
Hartl et al. *Science* 2011; **475**:324-332

Failure in protein folding is the leading cause of many diseases, particularly in old age

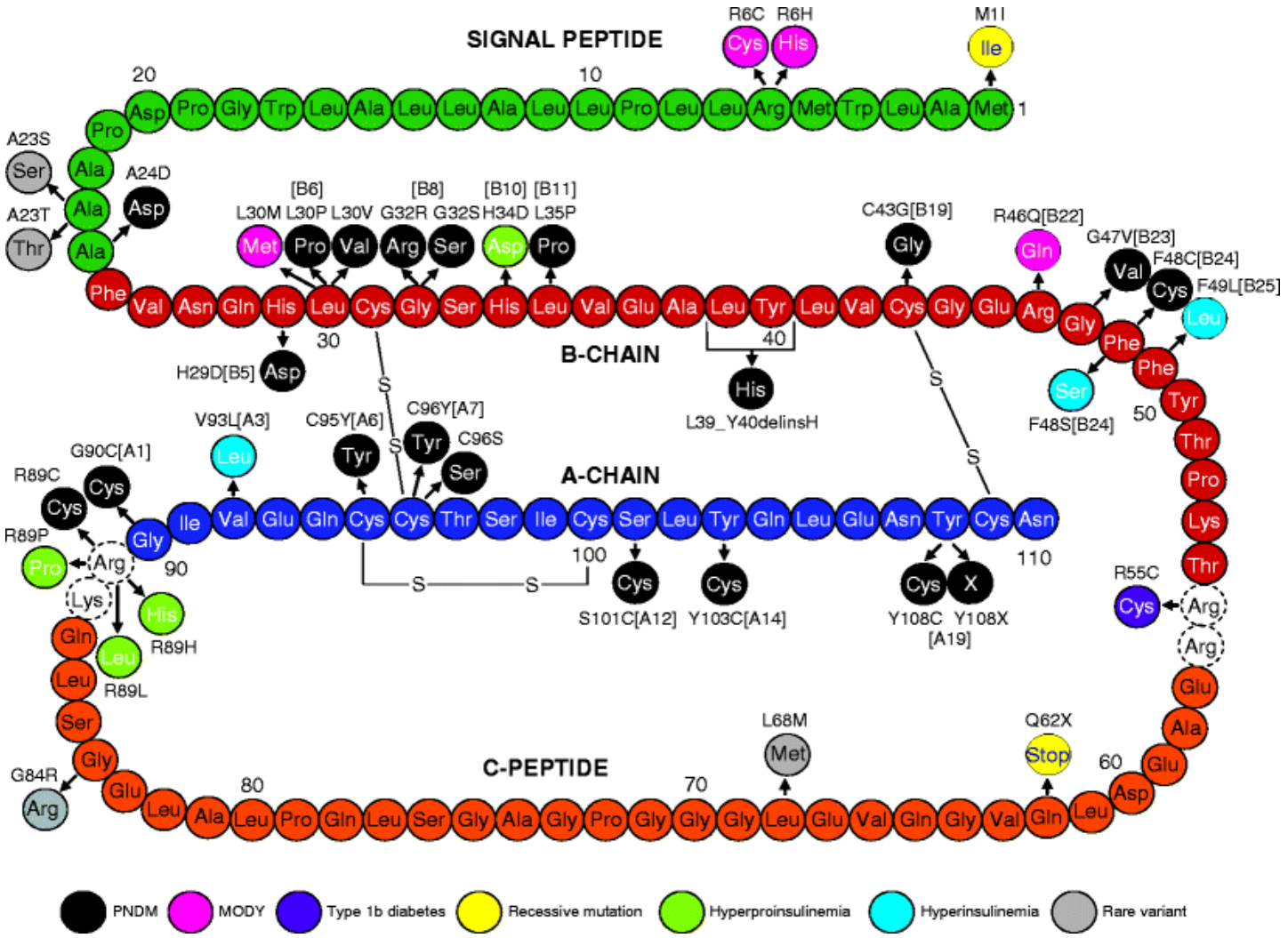
Protein	Location	Disease
Insulin	ER	Diabetes
Huntingtin	Nucleus and cytosol	Huntington
α-amyloid/presenilin	ER	Alzheimer's
p53	Cytosol	Cancer
Crystalins	Cytosol	Cataracts
Prion protein	ER	Creutzfeldt-Jakob
Fribillin	ER	Marfan syndrome
Cystic firbosis transmembrane regulator	ER	Cystic fibrosis
Collagen	ER	Osteogenesis imperfecta

In medicine, these diseases are known as protein folding diseases, conformational diseases or protein aggregation diseases.

A gene is composed of two alleles, which can be expressed as proteins at the same time



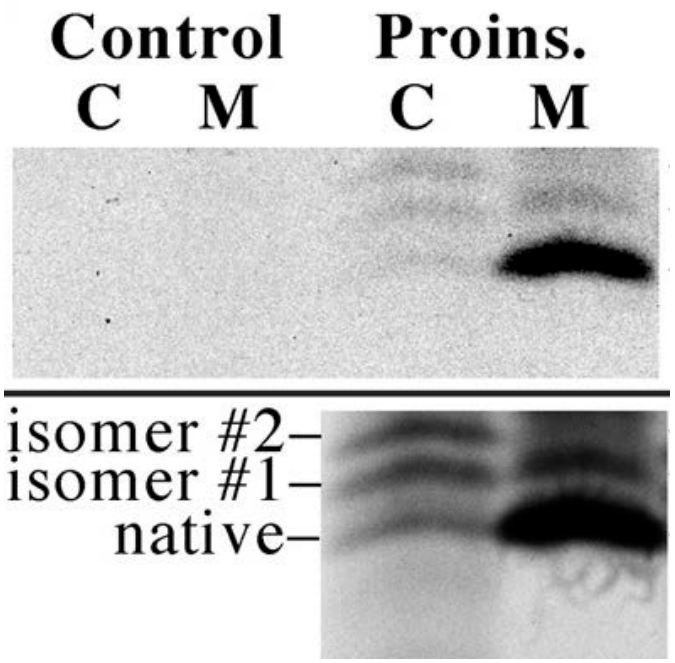
Single amino-acid substitution in proinsulin protein can cause protein misfolding and diabetes



Støy et al. *Reviews in Endocrine and Metabolic Disorders* 2010; **11**: 205–215.

Under normal physiological conditions, cells can produce misfolding protein without mutant allele

In the mouse, 3-8% of the proinsulin is misfolded under standard physiological conditions.



No glucose stimulation

30 minutes after glucose stimulation

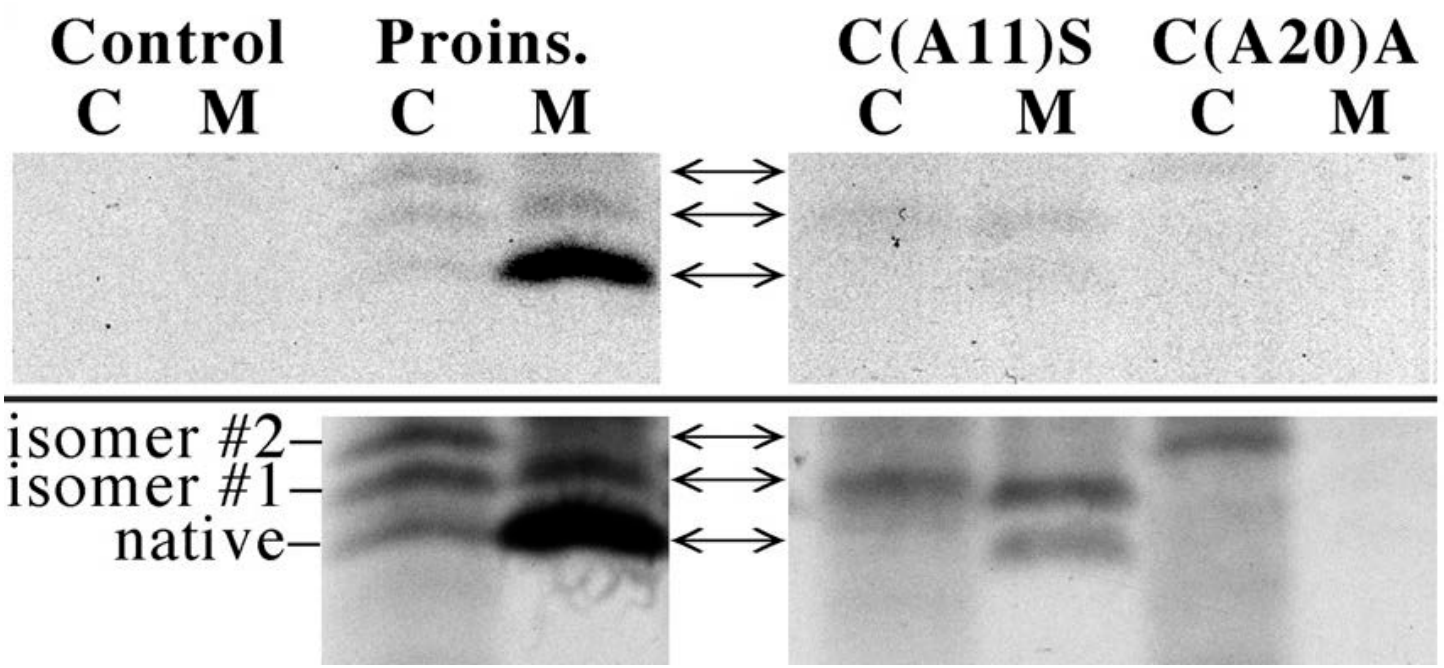
C = Cell
M = Medium

Liu et al. *J. Biol. Chem.* 2005; **280**:13209-13212

An increase in the expression of misfolded protein causes a loss-of-function disease

The mouse expresses four alleles of proinsulin. By changing one allele with a mutant misfolding proinsulin, native insulin is not secreted (loss-of-function)

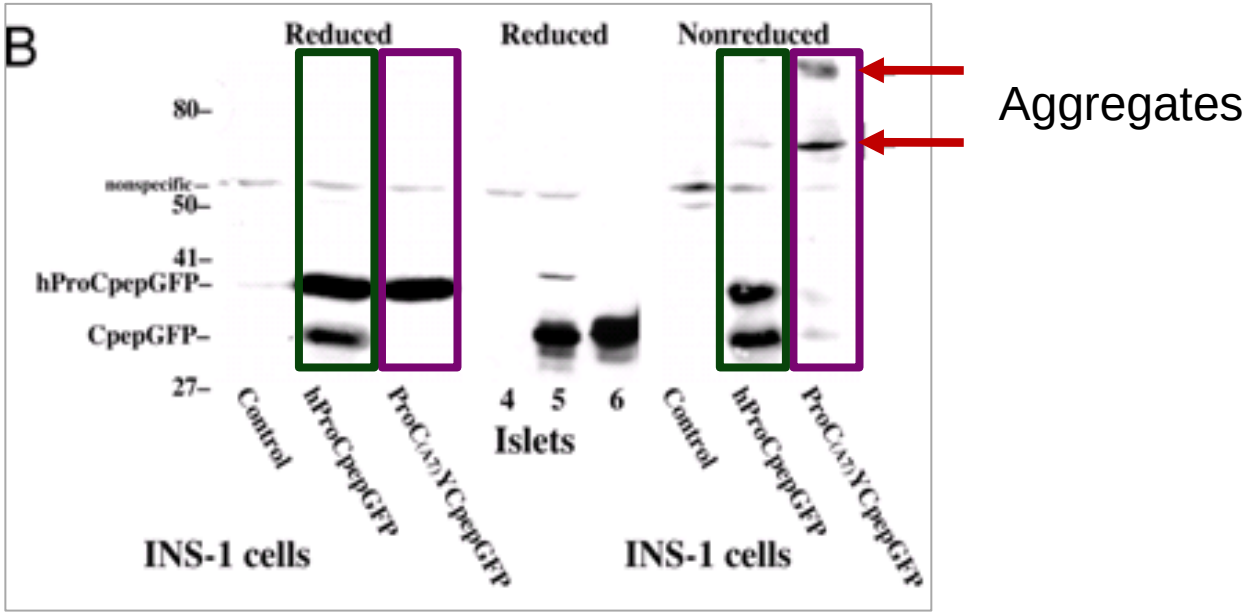
This causes a phenotype of diabetes known as Type 1b diabetes



Liu et al. *J. Biol. Chem.* 2005; **280**:13209-13212

Aggregation is a proximal event in conformation diseases and is characterized by the gain-of-function disease and lost-of-function disease

There is a threshold of misfolding protein expression, which triggers the sudden aggregation of proinsulin (gain-of-function) and decrease in insulin (lost-of-function).



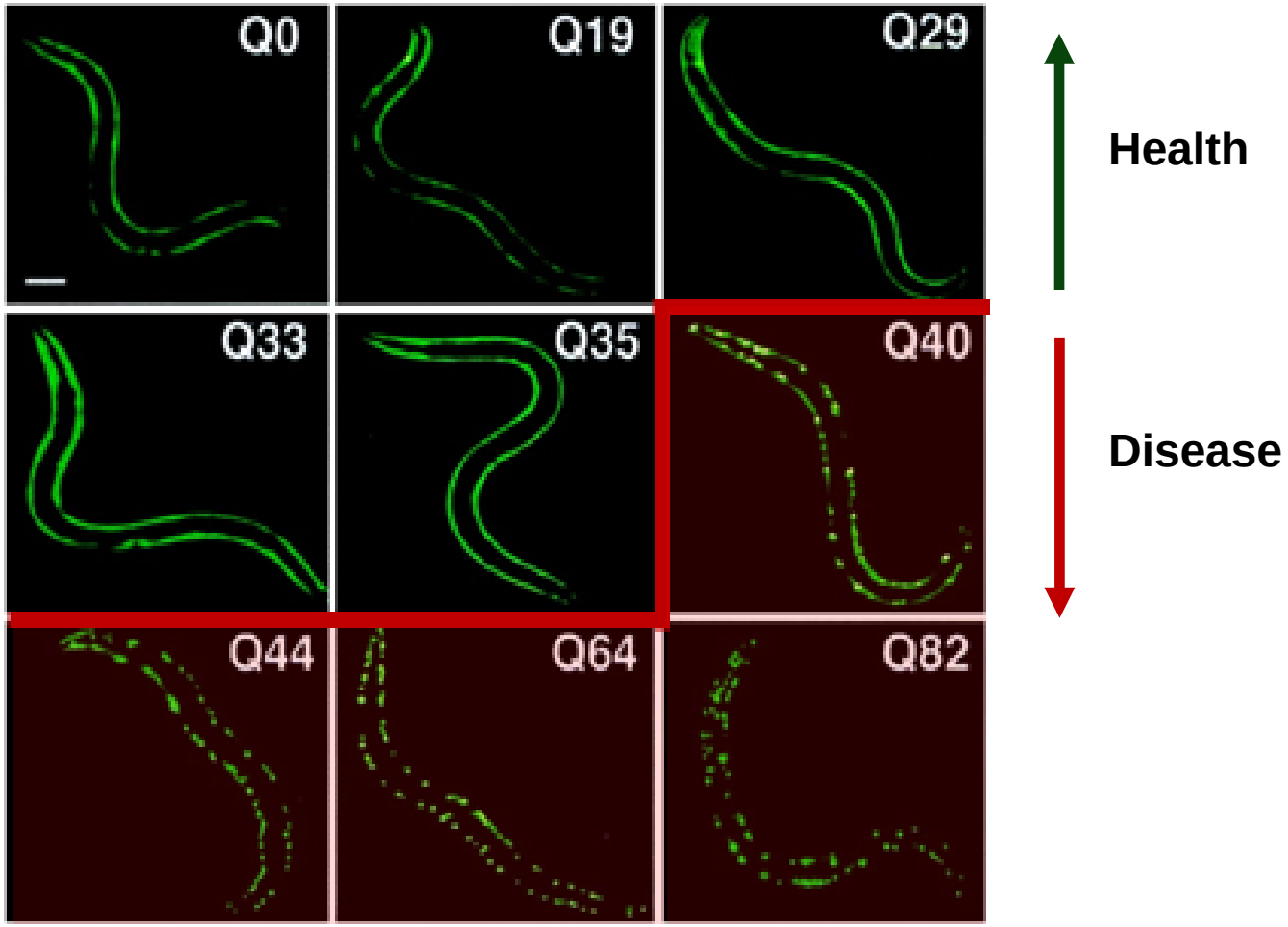
Liu et al. *Proc. Natl. Acad. Sci. USA* 2007; **204**: 15841-15846

The threshold behavior occurs when 8 to 25% of the total pro-insulin is in its misfolded configuration.

The expression of protein folding disease exhibit a threshold phenomenon

Huntington's disease

Polyglutamine (polyQ) expansion in the huntingtin protein



Morley et al. *PNAS* 2002; **99**: 10417-10422

The expression of protein folding disease exhibit a threshold phenomenon

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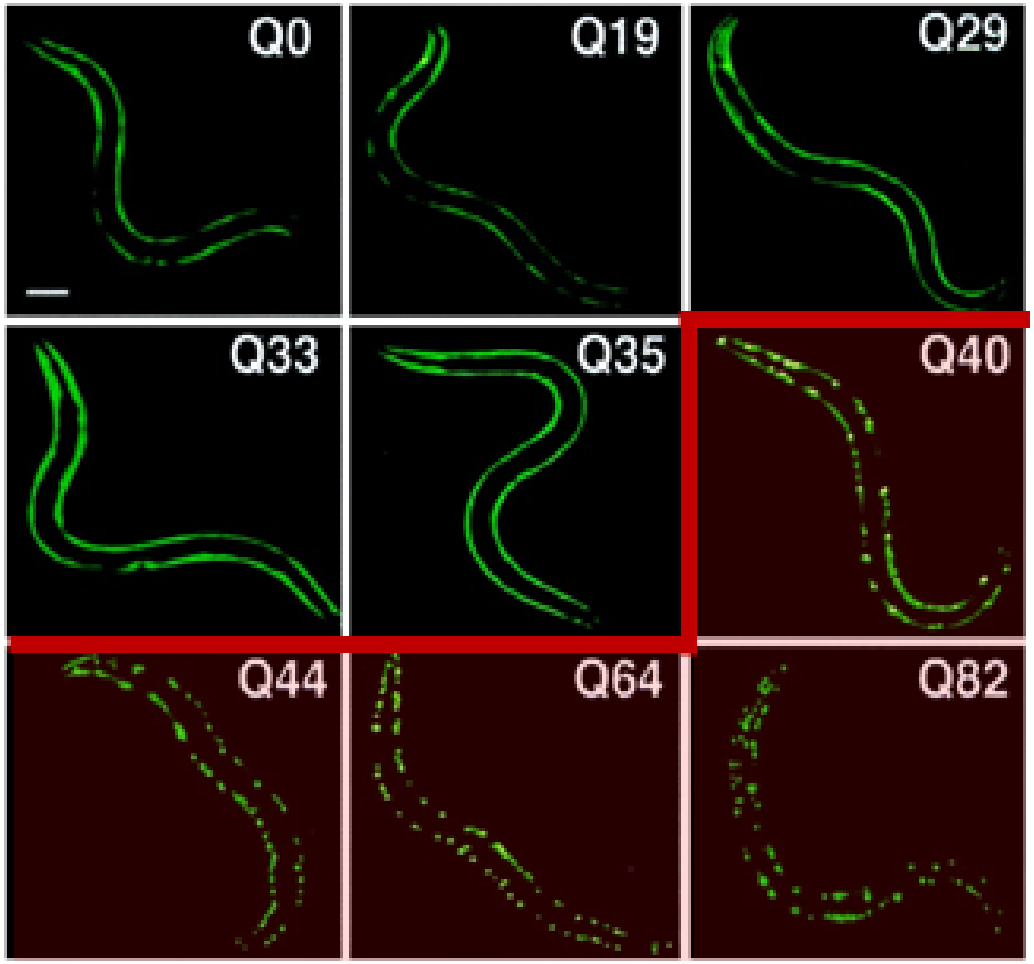
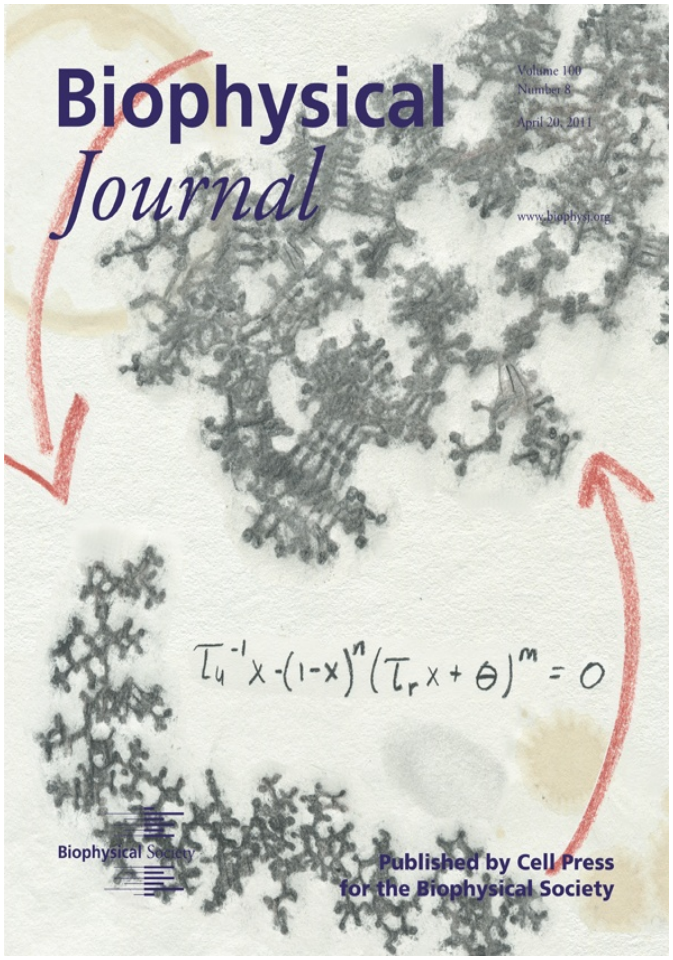


Health

Disease

Green body-wall muscle cells due to polyQ fluorescent tagged protein expression

Morley et al. *PNAS* 2002; **99**: 10417-10422



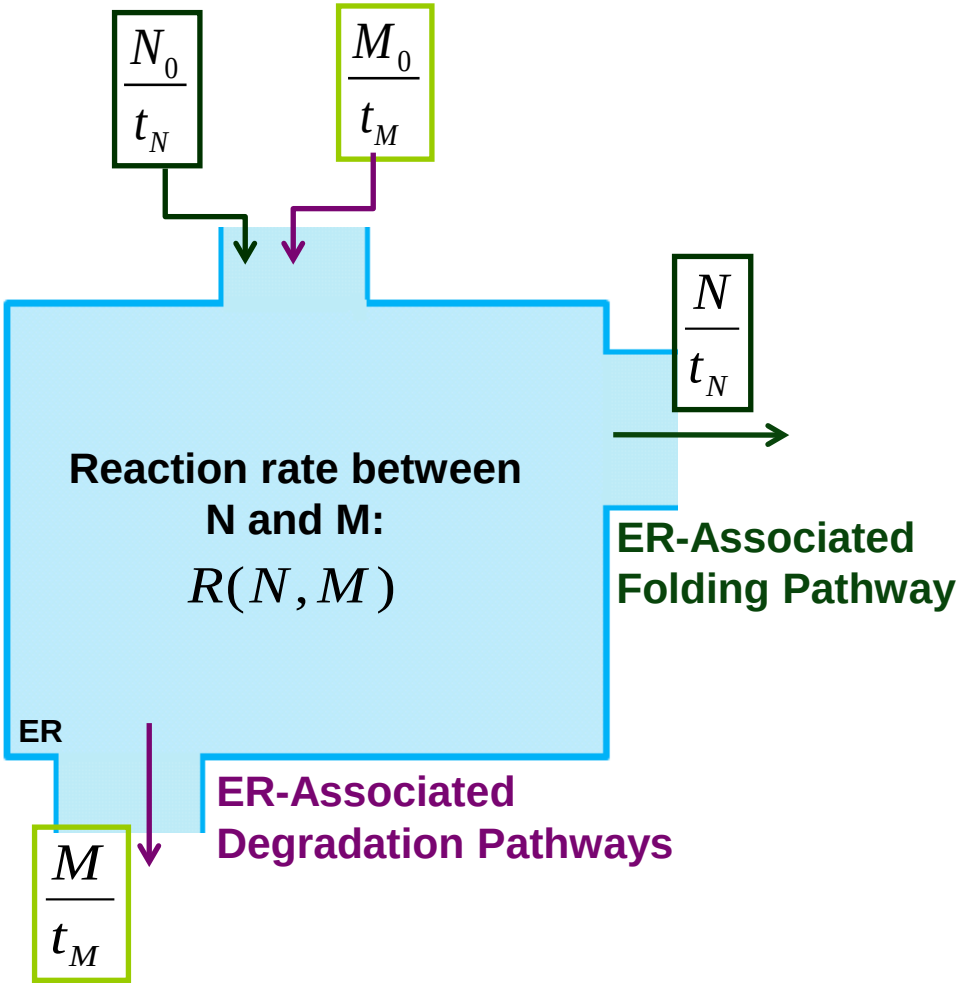
Artwork by Erin Shellman

I will now present a general model describing the wild-type (normal) protein disappearance (loss-of-function) through direct or indirect interaction with misfolded protein to explore potential treatments for protein folding diseases

Sandefur & Schnell *Biophysical Journal* 2011; **100**: 1864-1873

We model normal isomers disappearance and protein aggregation as a continuous flow reaction in the endoplasmic reticulum

1.



N: normal isomers (unfolded and folded proteins)

M: misfolded isomer

t_i: residence time in the ER

R(N,M) reaction rate

Sandefur & Schnell *Biophysical Journal* 2011; **100**: 1864-1873

The mathematical formulation of the continuous reactor model is

2. Applying mass action

$$\frac{dN}{dt} = \frac{N_0 - N}{t_N} - R(N, M)$$

$$\frac{dM}{dt} = \frac{M_0 - M}{t_M} + R(N, M)$$

(1)

At steady-state

$$\frac{N_0}{t_N} + \frac{M_0}{t_M} = \frac{N}{t_N} + \frac{M}{t_M}$$

(2)

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(2)

The problem now is to determine a rate function *R(N,M)* representative of misfolding and aggregation reactions

Sandefur & Schnell *Biophysical Journal* 2011; **100**: 1864-1873

We mined the literature for all reported protein misfolding and aggregation reaction mechanisms

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Aggmod – Repository of protein aggregation reaction
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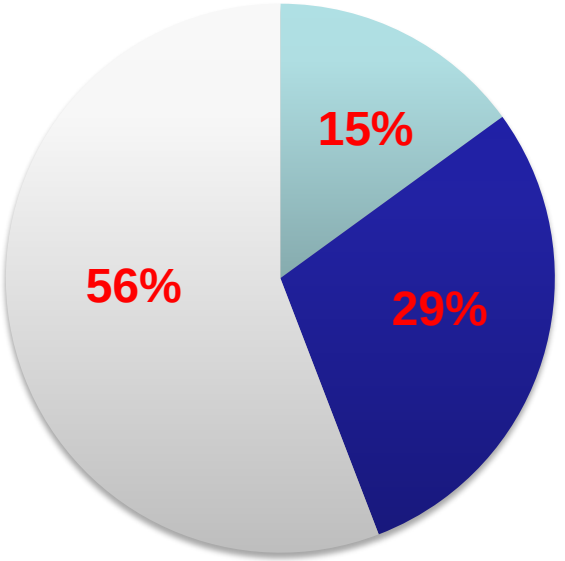


- Unique mechanisms (18/120)
- Duplicate mechanisms (35/120)
- No mechanism (67/120)

Aggmod – Repository of protein aggregation reaction
http://aggmod.ccmb.umich.edu

The main source of misfolded isomer is the conversion of normal isomers into misfolded isomers

In misfolding and aggregation reactions the misfolded isomer "sequesters" normal isomer in the endoplasmic reticulum to form aggregates

We find that majority of the mechanism can be described with the phenomenological overall reaction

$$N \xrightarrow{R(N,M)} M$$

$$R(N,M) = kN^n M^m \qquad \textbf{(3)}$$

http://aggmod.ccmb.umich.edu

The overall reaction can represent, for example, an associate-limited aggregation mechanism and other aggregation mechanisms

$$M + M \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} A$$

$$N + A \xrightarrow{k_2} M + A$$

$$N + 2M \xrightarrow{R(N,M)} 3M$$

$$R(N,M) \propto NM^2$$

if $k_{-1} \ll k_1$ and $k_{-1} \gg k_2$

Sandefur & Schnell *Biophysical Journal* 2011; **100**: 1864-1873

We reduce our mathematical description to the extend of the reaction

Substituting (3) in (1)-(2), the normal concentration at steady state is

$$\frac{N_0 - N}{t_N} - kN^n \left[\frac{t_M}{t_N}(N_0 - N) + M_0 \right]^m = 0$$

Defining the extend of the reaction, $x = 1 - \frac{N}{N_0}$

we obtained the nondimensional expression:

$$\underbrace{\tau_u^{-1} x}_{\text{Flow } y_f(x)} - \underbrace{(1-x)^n (\tau_r x + \theta)^m}_{\text{Reaction rate } y_r(x)} = 0$$

with

$$\tau_u^{-1} = \frac{1}{t_N k N_b^{n+m-1}}$$

Non-dimensional normal residence time

$$\tau_r = \frac{t_M}{t_N}$$

Ratio of residence times

$$\theta = \frac{M_0}{N_0}$$

Ratio of basal concentrations

Sandefur & Schnell *Biophysical Journal* 2011; **100**: 1864-1873

The parameters of our model can be controlled experimentally

$$\tau_u^{-1} = \frac{1}{t_N k N_b^{n+m-1}}$$

Non-dimensional normal residence time

$$\tau_r = \frac{t_M}{t_N}$$

Ratio of residence times

Introducing pharmacological chaperones accelerates residence time

$$\theta = \frac{M_0}{N_0}$$

Ratio of basal concentrations

Molecular engineering approaches allow differential expression of proteins

$$m$$

Stoichiometric coefficient of reaction

Protein misfolding and aggregation inhibitors reduce stoichiometry of reaction

Sandefur & Schnell *Biophysical Journal* 2011; **100**: 1864-1873

We can now investigate the steady state behavior by looking at the flow and reaction rates

Model equation at steady state:

$$\underbrace{\tau_u^{-1} x}_{\text{Flow } y_f(x)} \underbrace{-(1-x)^n(\tau_r x+\theta)^m}_{\text{Reaction rate } y_r(x)} = 0$$

Note that $y_r(x)$ is a polynomial of order $(n+m)$. We need to understand its shape for $x = [0,1]$.

We can estimate the critical points of $y_r'(x) = 0$ as

$$x = \left\{ -\frac{\theta}{\tau_r}, 1 - \frac{n(\tau_r+\theta)}{\tau_r(n+m)}, 1 \right\}$$

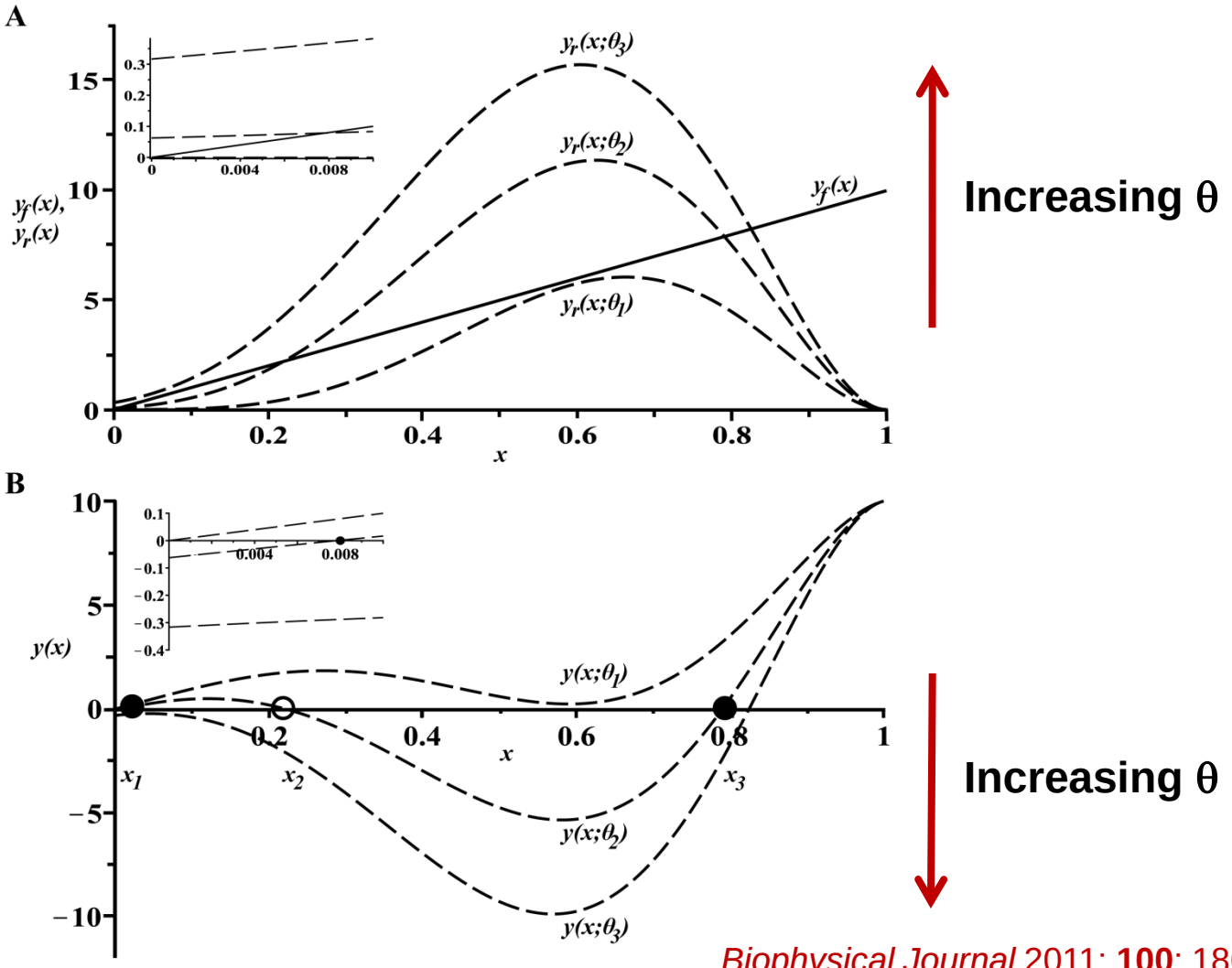
Using the second derivative test, we determine that $y_r(x)$ is concave downwards and has a maximum at the critical point:

$$x = 1 - \frac{n(\tau_r+\theta)}{\tau_r(n+m)}$$

Sandefur & Schnell *Biophysical Journal* 2011; **100**: 1864-1873

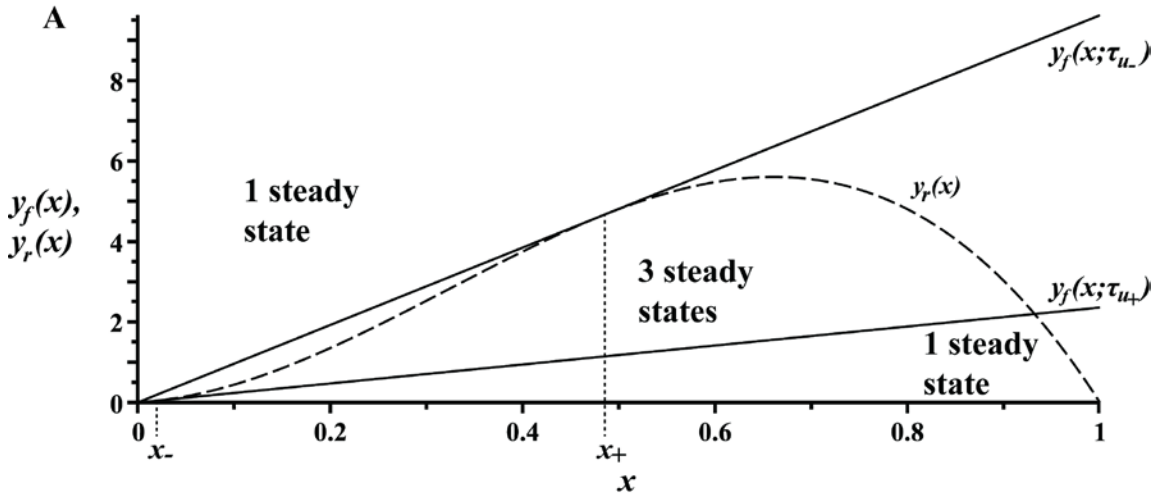
The model can exhibit bistability with changes in the ratio of basal isomers concentrations

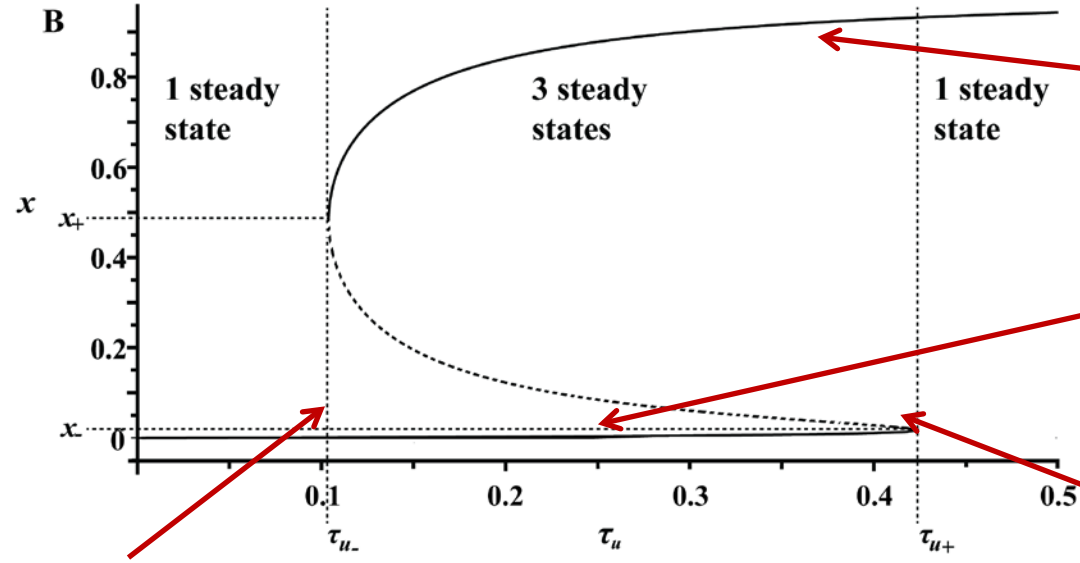
Although the reaction is of (n+m)th order, it can have up to 3 physically realistic steady-states.



Biophysical Journal 2011; **100**: 1864-1873

The steady state of the model changes with the normal protein residence time in the ER





Disease outbreak branch

Fast flow branch

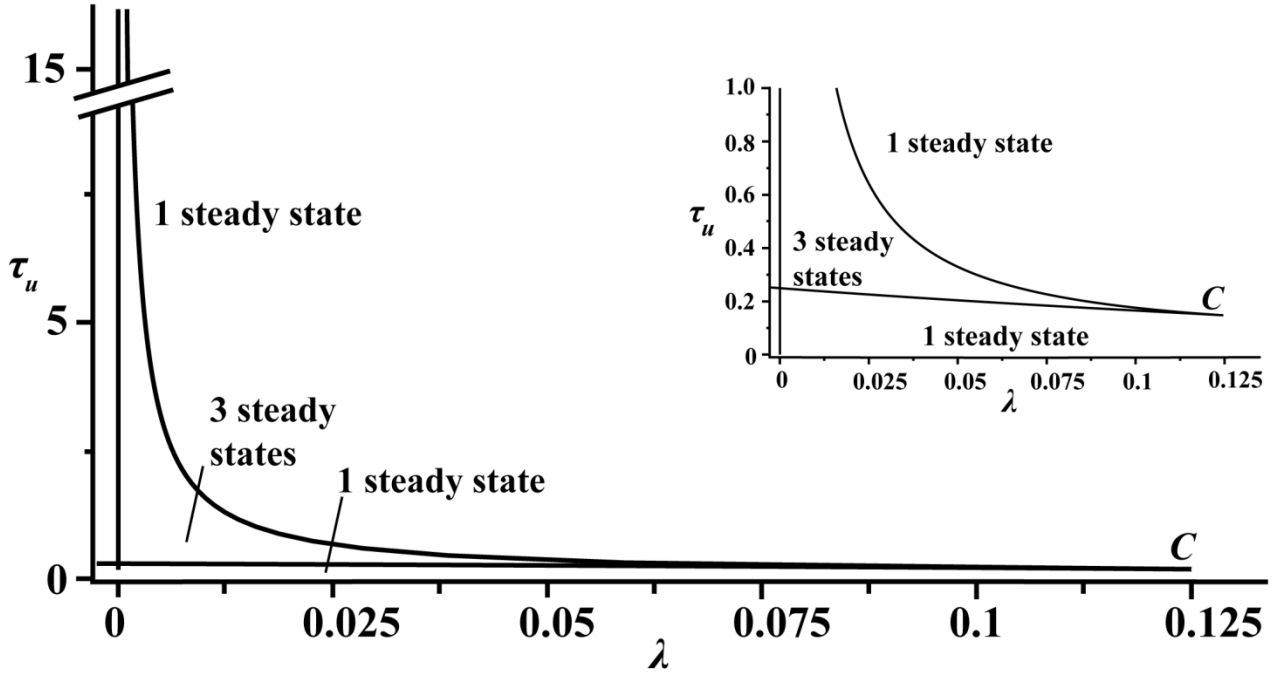
Rescue point

Outbreak point

Biophysical Journal 2011; **100**: 1864-1873

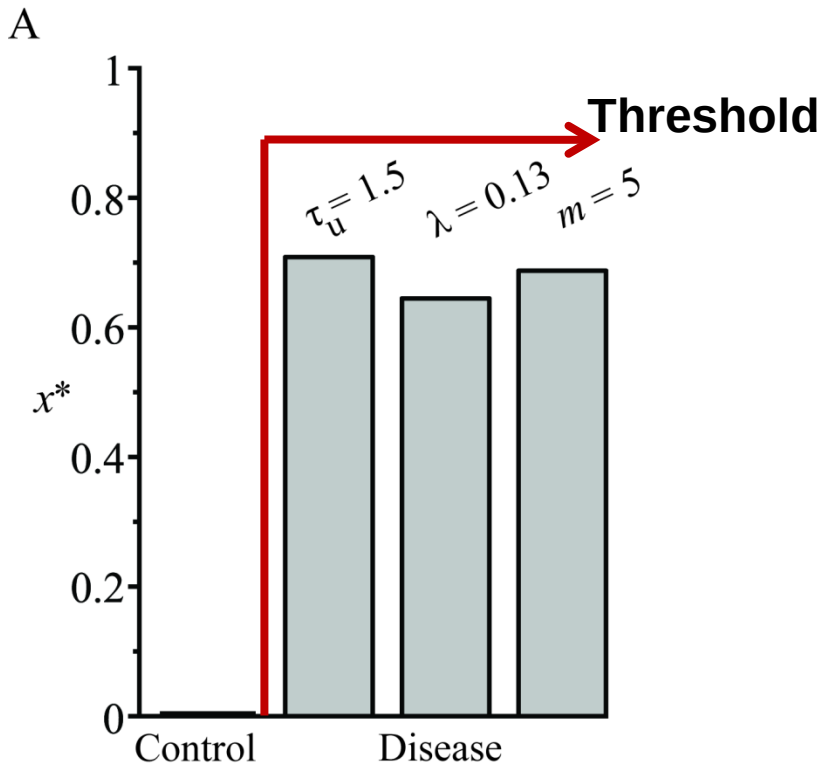
The threshold behavior depends on τ_u and basal isomer inflow rates

We define the basal isomer inflow rates as: $\lambda = \frac{\theta}{\tau_r} = \frac{[M_0]/t_M}{[N_0]/t_N}$



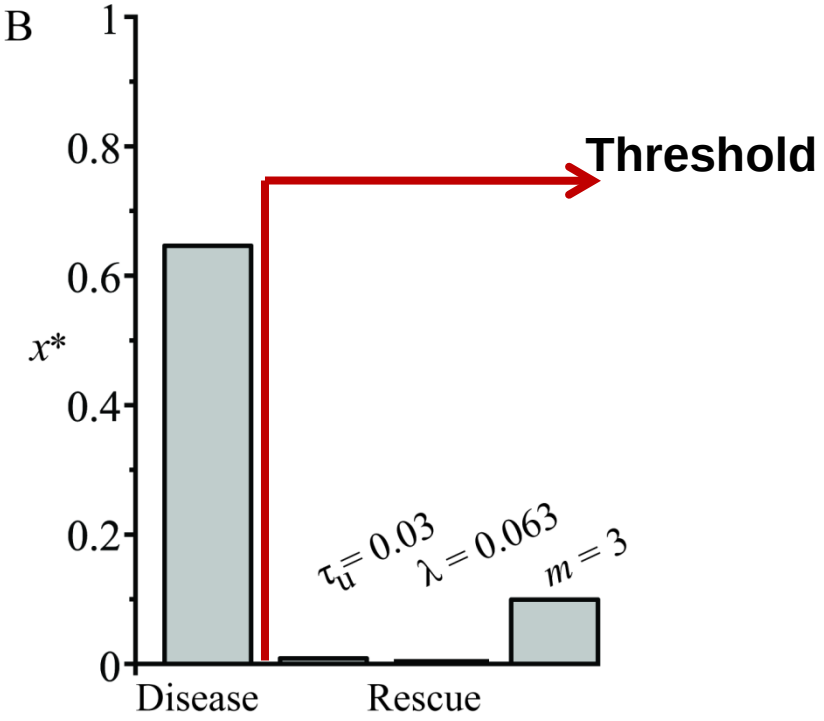
Sandefur & Schnell *Biophysical Journal* 2011; **100**: 1864-1873

We discovered that the onset and rescue from conformational diseases is controlled by three parameters



Control

$\tau_u = 0.45,\ \lambda = 0.025,\ n = 4, m = 4$



Disease

$\tau_u = 0.10,\ \lambda = 0.1875,\ n = 4, m = 5$

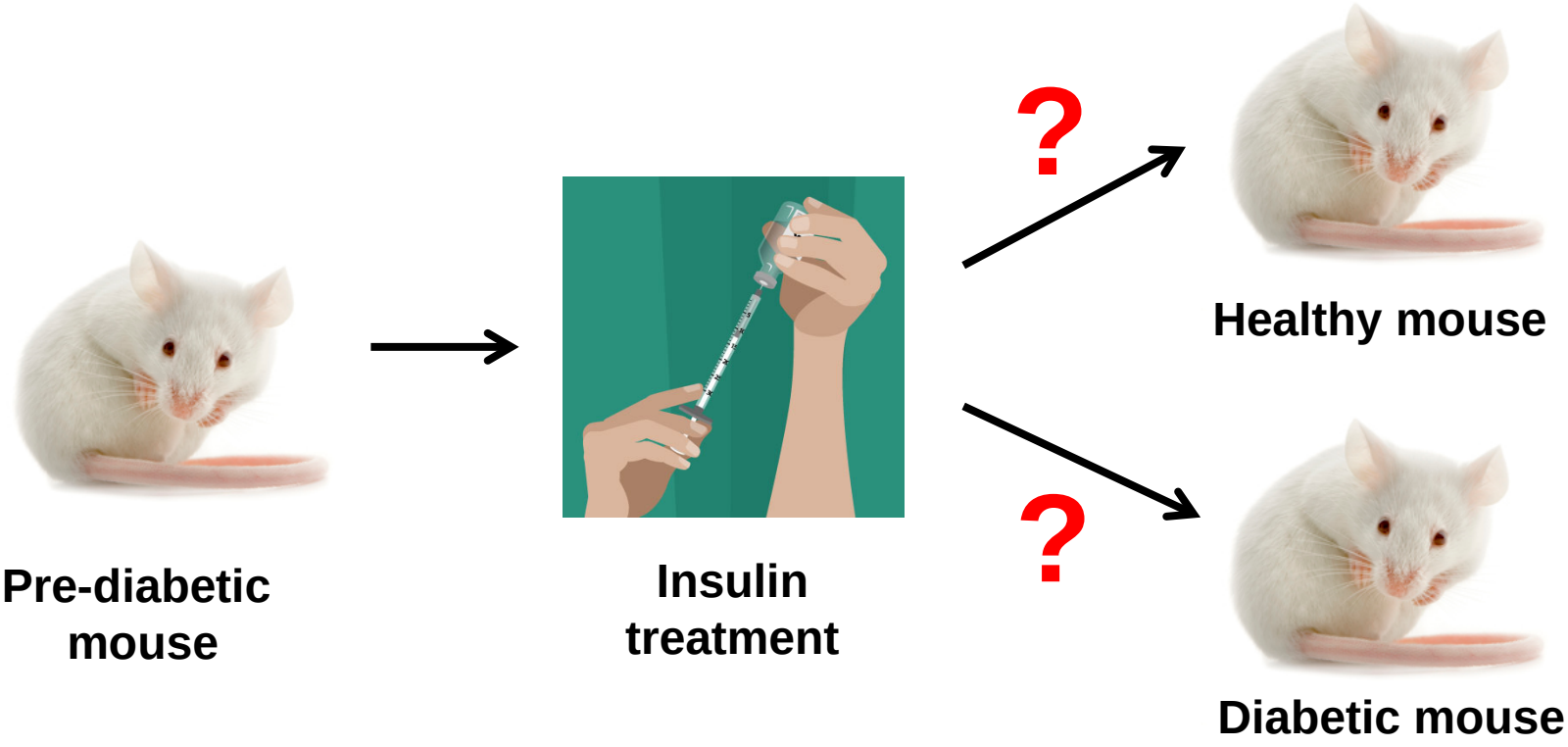
τ: Transition time in ER; λ: unfolded-misfolded ratio; *m:* stoichiometry of reaction

Sandefur & Schnell *Biophysical Journal* 2011; **100**: 1864-1873

Our model suggest that increasing the expression of normal insulin can rescue animals from diabetes

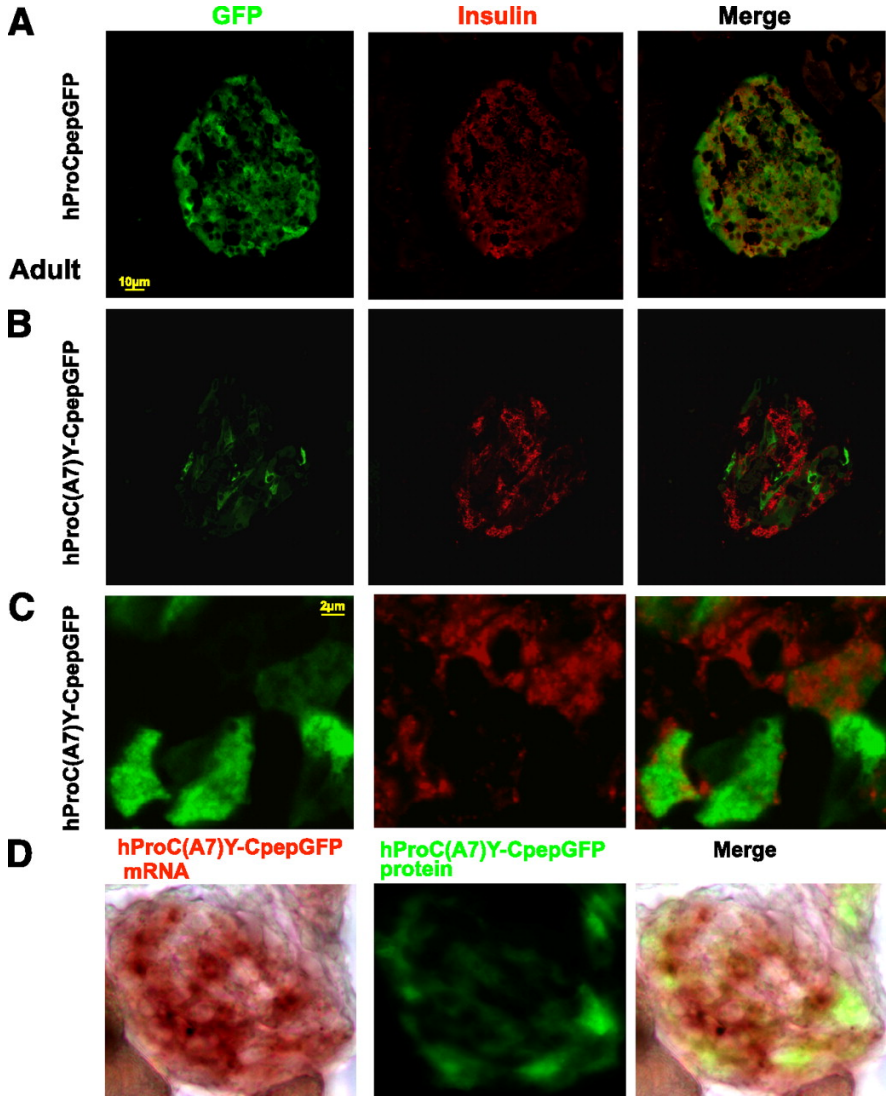
Experiment 1:

Rescuing pre-diabetic animals by preventive injection of insulin



Treatment of pre-diabetic animals with insulin shows a reduction in the amount of misfolding proinsulin

I. Hodish…, P. Arvan (2011) *Diabetes* 60, 2092-2101



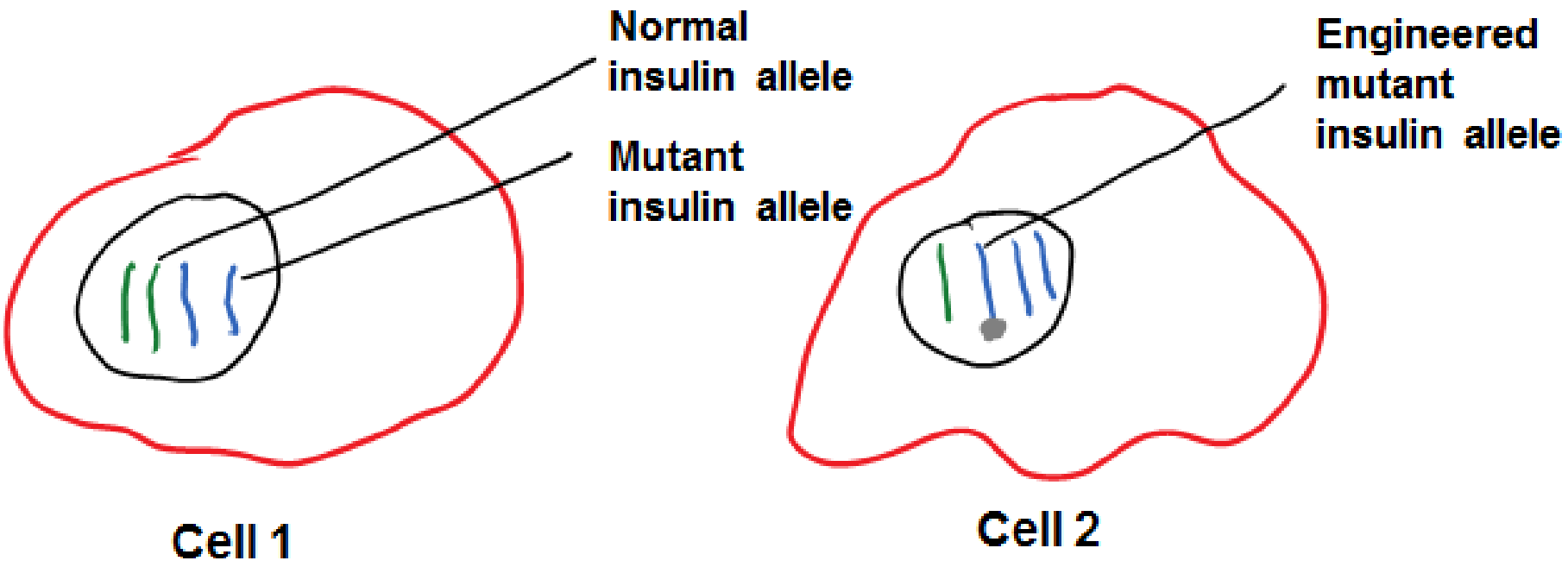
~40% of β-cell with insulin treatment show misfolding proinsulin accumulation with limited insulin staining.

~60% of β-cell with insulin treatment show limited misfolding proinsulin staining.

Our model suggest that increasing the expression of normal insulin can rescue animals from diabetes

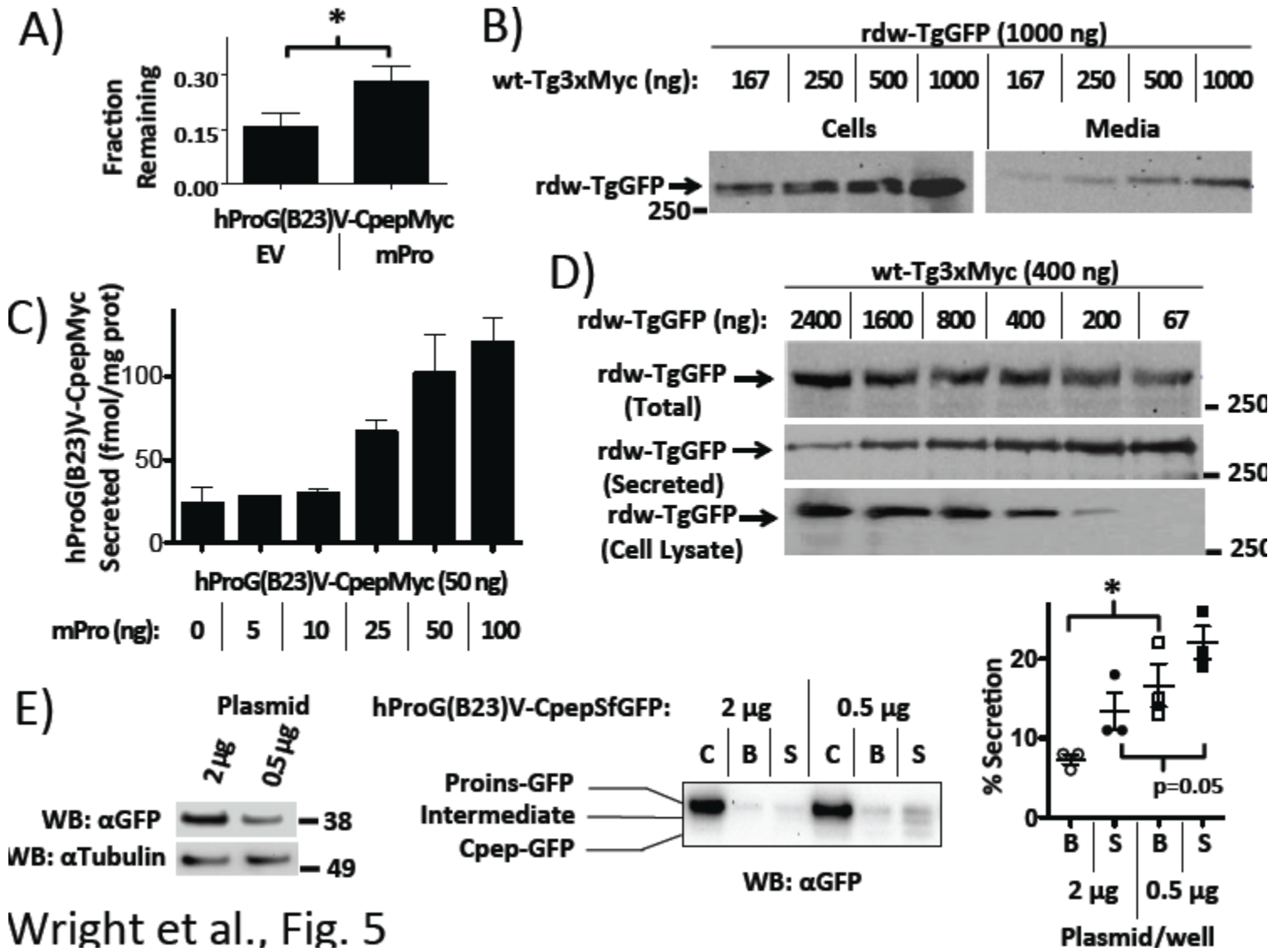
Experiment 2:

Genetic engineered 293T cell lines with wild-type and mutant proinsulin



Rescue of mutant proinsulin by wild-type proinsulin in tissue of animal model

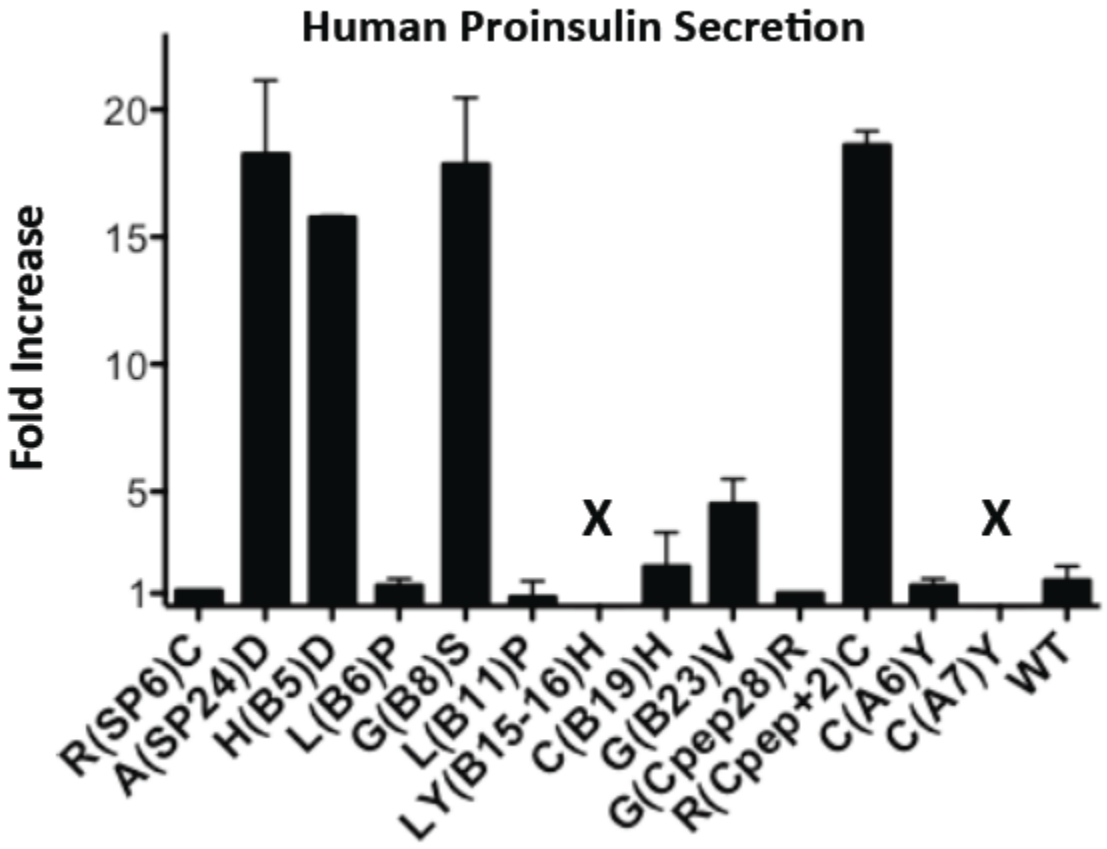
J. Wright…, P. Arvan (2013) *J. Clin. Invest*. **123**, 3124–3134



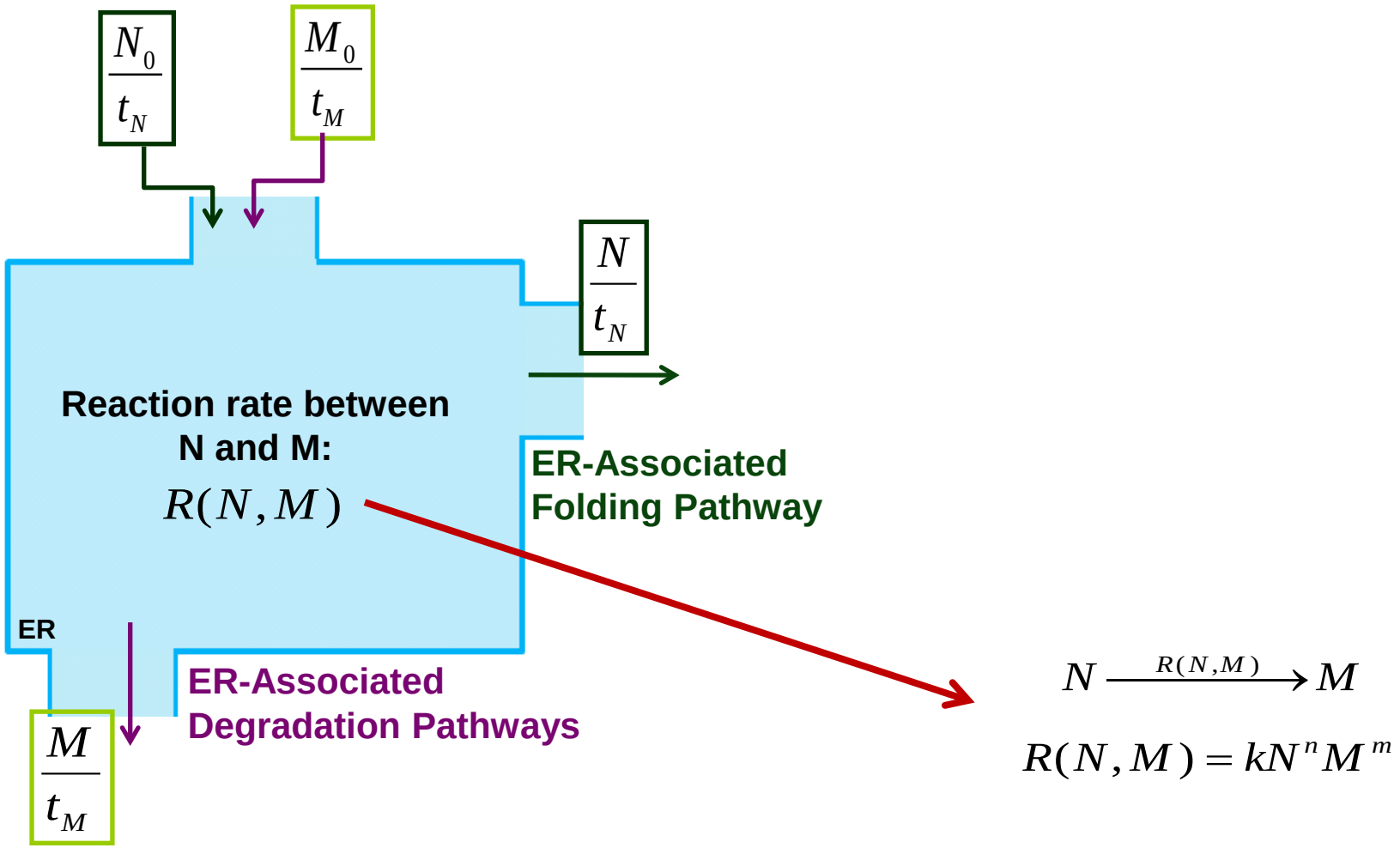
Wright et al., Fig. 5

Secretory rescue by wild type proinsulin is restricted to a subset of mutants for type 1b (MIDY)

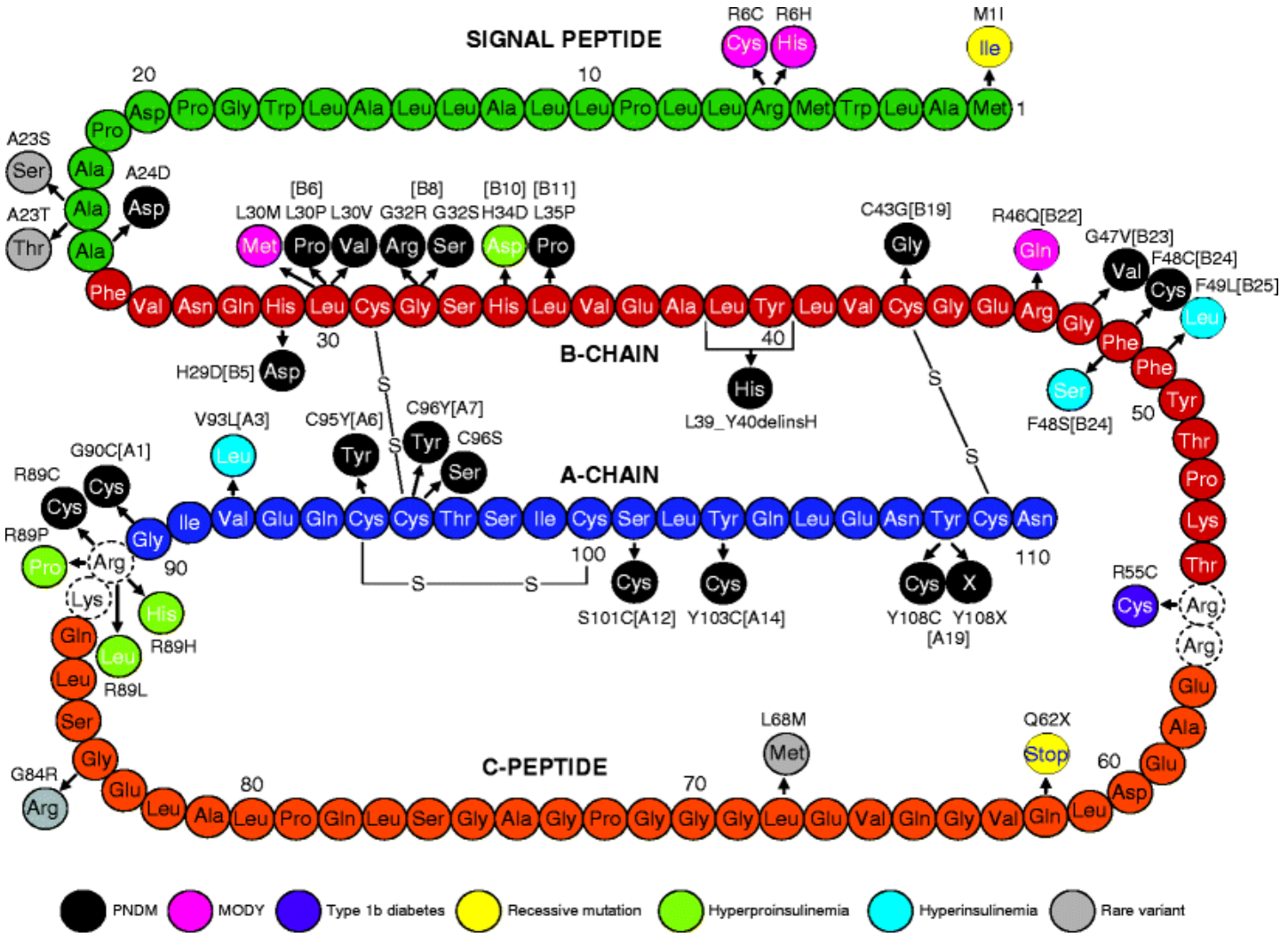
J. Wright…, P. Arvan (2013) *J. Clin. Invest*. **123**, 3124–3134



The limitation of our model is that we employed a generic and phenomenological reaction rate



Each mutant can have a different misfolding and aggregation pathway, so the universal rate is limited



Støy et al. *Reviews in Endocrine and Metabolic Disorders* 2010; **11**: 205–215.

Now we are characterizing proinsulin misfolding and aggregation reactions for each mutant to understand rescue mechanisms

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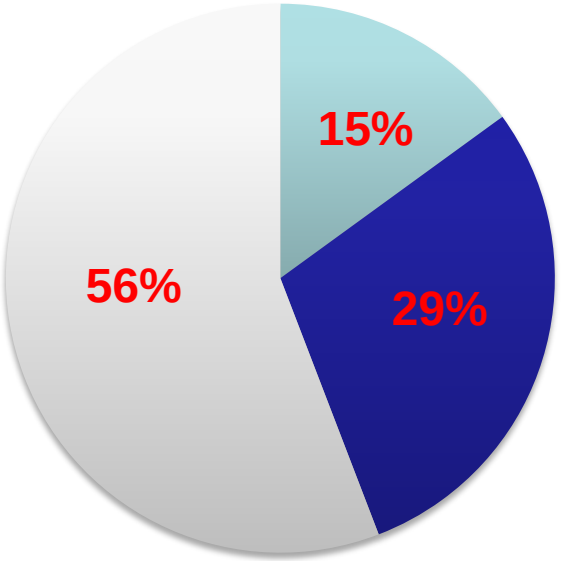

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Burant Lab

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