Quantitative Central Dogma I:

Reference: http://book.bionumbers.org

Initiation Transcription: **RNA polymerase** protein and **Transcription Factor** (TF) proteins binds to **promoter** region of **DNA**	**Transcription**: Messenger RNA, **mRNA**, is produced and **transported** to **Ribosomes**	**Translation**: **mRNA** read by **Ribosomes** to produce proteins

Basic Size and Geometry
http://book.bionumbers.org/what-is-the-range-of-cell-sizes-and-shapes/

Volume: one litre = $1L = 10^{-3}m^3$; E.Coli size is about $1\mu m$, and the volume is estimated to be $V_{Ecoli} \simeq 1\mu m^3 = 10^{-18}m^3$ $(1\mu m^3 \times \left(10^{-6}m \bullet \mu m^{-1}\right)^3 = 10^{-18}m^3)$.

Concentration: $1M = 1mol \bullet L^{-1}$, but $1mol = 6.023\times10^{23}$, so $1M = 6.023\times10^{26}m^{-3}$, In assignment 4, you will show that $1nM \sim$ one molecule in a volume of $1\mu m^3$.

Mass: One Dalton = Mass of Hydrogen = $1Da = 1.66\times10^{-27}kg = 1.66\times10^{-24}g$, and **one mole** (Avogadro's number $N_A = 6.023\times10^{23}$) of **hydrogen** weighs 1 gram = 1g $(\left(1Da\right)^{-1} = \left(1.66\times10^{-24}g\right)^{-1} = 6.022\times10^{23}g^{-1})$: $1g = 6.023\times10^{23}Da$.

One amino acid (aa) is $\sim 100Da = 1.66\times10^{-25}kg$: $1aa \sim 100Da$.
An average protein (gene) has about 300 amino acids: 1 protein $\sim$ 300 aa.
One base pair (bp) is $\sim 650Da$: 1 bp $\sim$ 650 Da

Protein Concentration:
http://book.bionumbers.org/how-many-proteins-are-in-a-cell/

Data shows that the protein mass per unit volume is about $0.2\frac{g}{mL}$, but

$1mL = 10^{-6}m^3 = 10^{-6}m^3 \times \left(10^6 \frac{\mu m}{m}\right)^3 = 10^{12}\mu m^3$, so **Average Mass concentration** of **proteins** in cell is $\left(\frac{M}{V}\right) = 0.20\frac{g}{ml} \times 6.023\times10^{23}\frac{Da}{g} \times 10^{-12}\frac{ml}{\mu m^3} = 1.2\times10^{11}\frac{Da}{\mu m^3}$

Average Number concentration of **proteins** in cell is found by:
$\left(\frac{N}{V}\right) = \left(\frac{M}{V}\right)\frac{1}{100Da \bullet aa^{-1} \times 300aa \bullet \text{protein}^{-1}} = 4\times10^6\frac{\text{protein}}{\mu m^3}$. Note that the answer in the webpage is wrong! There will be a similar question on the assignment.

mRNA in Cells: http://book.bionumbers.org/how-many-mrnas-are-in-a-cell/
Experimentally, it is known that a bacterium has about $N_{mRna}^{bacteria} \approx 10^3$ mRNA, and a mammalian cell has about $N_{mRna}^{mamalian} \approx 10^5$. It is known that the number of mRNA in a cell remains relatively **constant**. It seems that mRNA concentration scales with size.

Bacteria: Let's assume that the protein concentration in a cell is $\sim 4\times10^6 \frac{\text{proteins}}{\mu m^3}$, which means that a bacterium of volume $1\mu m^3$ has about 4×10^6 proteins. It is known that, on average, bacteria divide every hour. This means that the number of proteins must double every hour, so a cell must produce 4×10^6 new proteins every hour, giving a rate $\frac{dN_{protein}^{bacteria}}{dt} = \frac{4\times10^6 \text{proteins}}{3600s} \sim 10^3 \frac{\text{proteins}}{s}$. Assume now that one mRNA can be **translated** to about 1 protein per second, or $r_{mRNA\to protein} \sim 1 \frac{\text{proteins}}{\text{mRNA}\bullet\text{s}}$, which gives number of mRNA $N_{mRNA}^{bacteria} \sim \left(dN_{protein}^{bacteria}/dt\right)/\left(r_{mRNA\to proteins}\right) = 10^3$.

Mammalian Cell: Assume a volume of $3000\mu m^3$ or 1.2×10^{10} proteins. Mammalian cells divides every 24 hr, $\frac{dN_{protein}^{mammalian}}{dt} = \frac{1.2\times10^{10}\text{proteins}}{24hr\times3600s\bullet hr^{-1}} \sim 1.4\times10^5 \frac{\text{proteins}}{s}$, and with $r_{mRNA\to protein} \sim 1 \frac{\text{proteins}}{\text{mRNA}\bullet\text{s}}$, $N_{mRNA}^{mammalian} \sim \left(dN_{protein}^{mammalian}/dt\right)/\left(r_{mRNA\to proteins}\right) = 1.4\times10^5$.

Protein and mRNA degradation:
http://book.bionumbers.org/how-fast-do-rnas-and-proteins-degrade/

Preamble: As mentioned **mRNA** number **remains**, more or less, **constant**, and described by the equation, $\frac{dN_{mRNA}}{dt} = r_{mRNA} - k_{mRNA}^{decay} N_{mRNA}$, where N_{mRNA} is the number of mRNA (in a cell) $\frac{dN_{mRNA}}{dt}$ is the rate of increase (or decrease) of mRNA in units of $mRNA\bullet s^{-1}$, r_{mRNA} is the rate of mRNA production, in $mRNA\bullet s^{-1}$, and k_{mRNA}^{decay} is the rate of mRNA decay in unit of s^{-1}.

How are **mRNA degraded**? In living cells, mRNAs are degraded by enzymes (proteins), such as **Ribonucleases** (**RNase**). High concentration of these enzymes increase the mRNA decay rate, k_{mRNA}^{decay}. The decay rate is the inverse of the half-life of mRNA, $k_{mRNA}^{decay} = \ln 2/\tau_{1/2}^{mRNA}$. For mRNA in living cells $\tau_{1/2}^{mRNA} = 3\text{min}$ to 10Hr
NOTE: **DNA** are **degraded** by proteins called **deoxyribonuclease** (**DNase**)

Steady-State (SS) Solution After a sufficiently long period, the system reaches a steady state (SS) of constant mRNA number, $N_{mRNA} = N_{mRNA}^{SS}$:

$$\frac{dN_{mRNA}}{dt} = 0 \rightarrow 0 = r_{mRNA} - k_{mRNA}^{decay} N_{mRNA}^{SS} \rightarrow N_{mRNA}^{ss} = r_{mRNA} / k_{mRNA}^{decay}$$

Exact Solution for mRNA: The solution of $\frac{dN_{mRNA}}{dt} = r_{mRNA} - k_{mRNA}^{decay} N_{mRNA}$ is $N_{mRNA} = N_{mRNA}^{SS}\left(1 - \exp\left(-k_{mRNA}^{decay} t\right)\right)$. After a long period, $\exp\left(-k_{mRNA}^{decay} t\right) \sim 0 \rightarrow$ $N_{mRNA} = N_{mRNA}^{ss} = r_{mRNA} / k_{mRNA}^{decay}$.

Proteins in living cells are produced by Ribosomes that "reads" the mRNA code. As such, the protein production rate is proportional to the number of mRNA, N_{mRNA} .

The rate of increase (or decrease) of proteins is $\frac{dN_{protein}}{dt} = r_{protein} N_{mRNA} - k_{protein}^{decay} N_{protein}$, where $N_{protein}$ is the number of proteins, $r_{protein}$ is the rate of protein produced by translation per mRNA, and has unit of s^{-1} , $k_{protein}^{decay}$ is the rate of decay.

How are **proteins degraded**? In living cells, proteins are degraded by enzymes (proteins), such as **proteases**. High concentration of these enzymes increases the protein decay rate, $k_{protein}^{decay}$. The decay rate is the inverse of the of proteins, $k_{protein}^{decay} = \ln 2 / \tau_{1/2}^{protein}$. For mRNA in living cells $\tau_{1/2}^{protein} = 30\text{min}$ to days .

Steady-State (SS) Solution After a sufficiently long period, the system reaches a steady state (SS) of constant protein number, $N_{protein} = N_{protein}^{SS}$:

$$\frac{dN_{protein}}{dt} = 0 \rightarrow 0 = r_{protein} N_{mRNA} - k_{protein}^{decay} N_{protein}^{SS} \rightarrow N_{protein}^{ss} = r_{protein} N_{mRNA} / k_{protein}^{decay}$$

Exact Solution for mRNA: The solution of $\frac{dN_{protein}}{dt} = r_{protein} N_{mRNA} - k_{protein}^{decay} N_{protein}$ is $N_{protein} = N_{protein}^{SS}\left(1 - \exp\left(-k_{protein}^{decay} t\right)\right)$. After a long period, $\exp\left(-k_{protein}^{decay} t\right) \sim 0 \rightarrow$ $N_{protein} = N_{protein}^{ss} = r_{protein} N_{mRNA} / k_{protein}^{decay}$.

WHAT IS THE PROTEIN TO mRNA RATIO?

http://book.bionumbers.org/how-many-proteins-are-made-per-mrna-molecule/

From the last section we showed that at steady state $N_{protein}^{ss} = r_{protein} N_{mRNA} / k_{protein}^{decay}$, ans the ratio of Proteins to mRNA in a cell is $N_{protein} / N_{mRNA} = r_{protein} / k_{decay}^{protein}$. For E. Coli $r_{protein}$ is the translation rate per mRNA which can be argued to be $r_{protein} \sim 1 s^{-1}$, and

$k_{decay}^{protein} = 1/\tau_{protein}^{decay}$, where the average protein decay time is $\tau_{protein}^{decay} \sim 1000s$, which gives $N_{protein}/N_{mRNA} \sim 1000$, and indeed, experiments usually observe that the number of proteins is about 1000 times of the number of mRNA.

HOW FAST DO PROTEASOMES DEGRADE PROTEINS?

http://book.bionumbers.org/how-fast-do-proteasomes-degrade-proteins/

Proteasomes (or proteases) comprised about 1% of total bulk proteins. One proteasome degrades $\sim 0.05-5$ proteins/min, which is equivalent to about $1aa \bullet s^{-1}$. However, this depends on protein concentration and temperature

In-Vitro Proteins

In vitro (test tube) protein solutions usually contain protease contaminants that will degrade proteins. But unlike in living cells, there are no ribosomes to produce new proteins to replace the degraded proteins. The rate equation becomes:

$\frac{dN_{protein}}{dt} = -k_{protein}^{decay} N_{protein}$, with solution $N_{protein} = N_0 \exp\left(-k_{protein}^{decay} t\right)$, where N_0 is the number of proteins at t = 0. We can calculate the half-life, $\tau_{1/2}^{protein}$, of proteins, which is the time it takes for the population to decrease by 1/2,

$$N = N_0/2 = \exp\left(-k_{protein}^{decay}\tau_{1/2}^{protein}\right) \rightarrow \tau_{1/2}^{protein} = \frac{\ln 2}{k_{protein}^{decay}} = \frac{0.693}{k_{protein}^{decay}}$$

Example 1: For a typical *in vivo* system, the protein concentration is $4\times10^6 \frac{\text{protein}}{\mu m^3}$, and the half-life is about $\tau_{1/2}^{protein} = 40\text{min}$, or $k_{protein}^{decay} = \frac{0.693}{\tau_{1/2}^{protein}}$, $k_{protein}^{decay} = 0.0173\text{min}^{-1}$. The time it takes for the concentration to decrease to 10% is found by $N = 0.1N_0 = N_0 \exp\left(-k_{protein}^{decay} t\right)$, which gives

$t = -\ln(0.1)/k_{proein}^{decay} = -\ln(0.1)/0.0173\text{min}^{-1} = 133\text{min}$.

The above calculation is done for a typical *in vivo* protein concentration of 4 mM, at room temperature T = 300K, and protease concentration of about 1% of proteins, or $4\times10^{-2} mM = 40\mu M$. To **estimate** the **rate** at **different temperatures** and **concentrations**, we use Arrhenius Equation:

Arrhenius equation for reaction rate reaction rate $k_{protein}^{decay} = A\exp\left(-\Delta E/k_B T\right)$, where $A = A\left(c_{protein}, D\right)$ depends on the collision frequency, which in turns depend on the protein concentration, $c_{protein}$ and the diffusion coefficient, D. In class, it is shown

that we can expressed $A = \frac{A_0 T}{\overline{\ell}^2}$, where A_0 is a constant, T is the **temperature**, $\overline{\ell}$ is the mean, or average, **separation distance** between **2 adjacent** proteins. In the equation, ΔE is the reaction free-energy barrier, which we will make an educated guess equals to about the energy released by a single ATP hydrolysis, $\Delta E = 4.8 \times 10^{-20} J$

Diversion on ATP hydrolysis

$ATP + H_2O \rightarrow ADP +$ phosphate + energy , energy released is $\Delta H_{ATP} = 29 \frac{kJ}{mol}$

$$29 \frac{kJ}{mol} \times \frac{1000 J / kJ}{6.023 \times 10^{23} ATP \bullet mol^{-1}} \rightarrow 4.8 \times 10^{-20} \frac{J}{ATP}$$

Example 2: Suppose the temperature is lowered from room temperature, T_i = 300 K (27 C), to just above freezing T_F = 277 K (4 C). We can calculate the low temperature (Final, F) rate $k^{decay}_{protein,F}$ from the initial (i) room temperature rate,

$k^{decay}_{protein,i} = 0.0173 \text{min}^{-1}$, by writing:

$k^{decay}_{protein,i} = \frac{A_0 T_i}{\overline{\ell}^2} \exp\left(-\frac{\Delta E}{k_B T_i} \right)$ and $k^{decay}_{protein,F} = \frac{A_0 T_F}{\overline{\ell}^2} \exp\left(-\frac{\Delta E}{k_B T_F} \right)$, and taking the ratio:

$$\frac{k^{decay}_{protein,F}}{k^{decay}_{protein,i}} = \frac{T_F}{T_i} \frac{\exp\left(-\frac{\Delta E}{k_B T_F} \right)}{\exp\left(-\frac{\Delta E}{k_B T_i} \right)} = \frac{T_F}{T_i} \exp\left(-\frac{\Delta E}{k_B} \left(\frac{1}{T_F} - \frac{1}{T_i} \right) \right)$$, we have assumed that $\overline{\ell}$ remains the same. This gives $k^{decay}_{protein,F} = k^{decay}_{protein,i} \frac{T_F}{T_i} \exp\left(-\frac{\Delta E}{k_B} \left(\frac{1}{T_F} - \frac{1}{T_i} \right) \right)$

$$k^{decay}_{protein,F} = 0.0173 \text{min}^{-1} \frac{277K}{300K} \exp\left(-\frac{4.8 \times 10^{-20} J}{1.381 \times 10^{-23} J \bullet K^{-1}} \left(\frac{1}{277K} - \frac{1}{300K} \right) \right)$$

$k^{decay}_{protein,F} = 6.1 \times 10^{-3} \text{min}^{-1}$

Let's calculate the time it takes an *in vitro* protein of the same concentration as example 1 to decay to 10% its original value, $N = 0.1 N_0$:

$N = 0.1 N_0 = N_0 \exp\left(-k^{decay}_{protein,F} t \right)$, which gives

$t = -\ln(0.1) / k^{decay}_{proein_F} = -\ln(0.1) / 6.1 \times 10^{-3} \text{min}^{-1} = 377 \text{min}$, which is longer, but not substantially longer for a biochemist trying to keep a protein solution stable.

Example 3: Suppose the temperature is kept at room temperature, but the protein were reduced by 100 times. Assume that the original protein concentration is

$\left(\frac{N}{V}\right)_i = 4\times10^6 \frac{proteins}{\mu m^3}$, as shown in class the average protein separation is $\overline{\ell}_i = \left[\left(\frac{N}{V}\right)_i\right]^{-1/3} = \left[4\times10^6 \frac{proteins}{\mu m^3}\right]^{-1/3} = 6.3\times10^{-3}\mu m$. The final diluted concentration is 100 times more dilute, $\left(\frac{N}{V}\right)_F = \frac{1}{100}\left(\frac{N}{V}\right)_i = 4\times10^4 \frac{proteins}{\mu m^3}$. This gives a final average protein separation of $\overline{\ell}_F = \left[\left(\frac{N}{V}\right)_i\right]^{-1/3} = \left[4\times10^4 \frac{proteins}{\mu m^3}\right]^{-1/3} = 2.9\times10^{-2}\mu m$.

$k^{decay}_{protein,i} = \frac{A_0 T}{\overline{\ell}_i^2}\exp\left(-\frac{\Delta E}{k_B T}\right)$ and $k^{decay}_{protein,F} = \frac{A_0 T}{\overline{\ell}_F^2}\exp\left(-\frac{\Delta E}{k_B T}\right)$, and taking the ratio:

$$\frac{k^{decay}_{protein,F}}{k^{decay}_{protein,i}} = \frac{\overline{\ell}_i^2}{\overline{\ell}_F^2} = \frac{\left(6.3\times10^{-3}\mu m\right)^2}{\left(2.9\times10^{-2}\mu m\right)^2} \rightarrow$$

$$k^{decay}_{protein,F} = 0.0173\min^{-1}\frac{\left(6.3\times10^{-3}\mu m\right)^2}{\left(2.9\times10^{-2}\mu m\right)^2} = 8.16\times10^{-4}\min^{-1}$$.

Let's calculate the time it takes an *in vitro* protein of the same concentration as example 1 to decay to 10% its original value, $N = 0.1N_0$:

$N = 0.1N_0 = N_0\exp\left(-k^{decay}_{protein,F}t\right)$, which gives

$t = -\ln(0.1)/k^{decay}_{proein_F} = -\ln(0.1)/8.16\times10^{-4}\min^{-1} = 2820\min$, or about 47 hr.

How many ribosomes in a cell Read the following

http://book.bionumbers.org/how-many-ribosomes-are-in-a-cell/