Class core values

1. Be **respect**ful to yourself and others
2. Be **confident** and believe in yourself
3. Always do your **best**
4. Be **cooperative**
5. Be **creative**
6. Have **fun**
7. Be **patient** with yourself while you learn
8. Don't be shy to **ask "stupid" questions**

Week 2, Lecture 1

Fantastic proteins & How to measure them

Learning Objectives

1. Identify protein properties required to modify for different applications
2. Describe methods that can be used to measure given properties
3. Critically evaluate the limitations of methods for each property and propose alternative methods

Protein engineering is a growing field
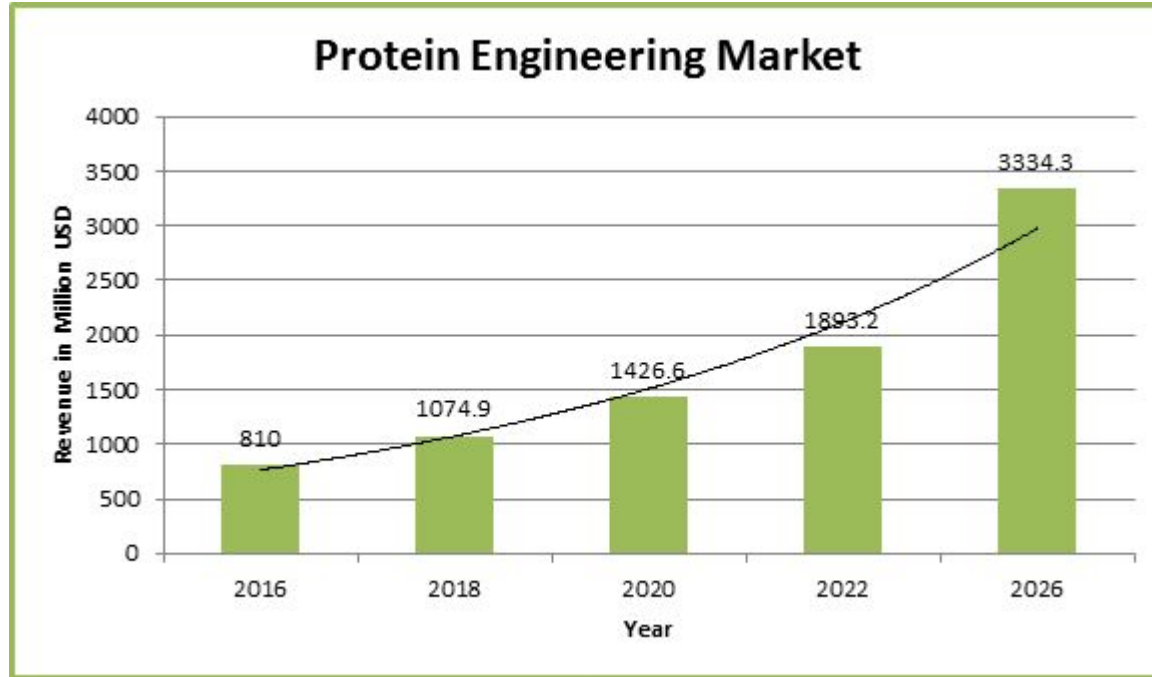
Protein Engineering Market

Protein engineering aims to improve different properties of proteins based on the application

Protein engineering aims to improve different properties of proteins based on the application

Engineering function

Protein engineering aims to improve different properties of proteins based on the application

Engineering function

- Binding to other proteins
- Enzymatic activity
- Protein-based material

Protein engineering aims to improve different properties of proteins based on the application

Engineering function

- Therapeutics: Binding to other proteins
- Enzymatic activity
- Protein-based material

Engineering stability

Protein engineering aims to improve different properties of proteins based on the application

Engineering function

- Therapeutics: Binding to other proteins
- Enzymatic activity
- Protein-based material

Engineering stability

- Thermal stability
- Protease stability
- Organic solvent tolerance

To assess our success, we need to be able to measure these properties

Engineering function

- Binding to other proteins
- Enzymatic activity
- Protein-based material

Engineering stability

- Thermal stability
- Protease stability
- Organic solvent tolerance

Measurements can be quantitative or qualitative

Measurements can be quantitative or **qualitative**

Qualitative measurements are often "binary"

Starting point

Qualitative measurements are often "binary"

Engineered protein

Starting point

Qualitative measurements are often "binary"

Engineered protein

Starting point
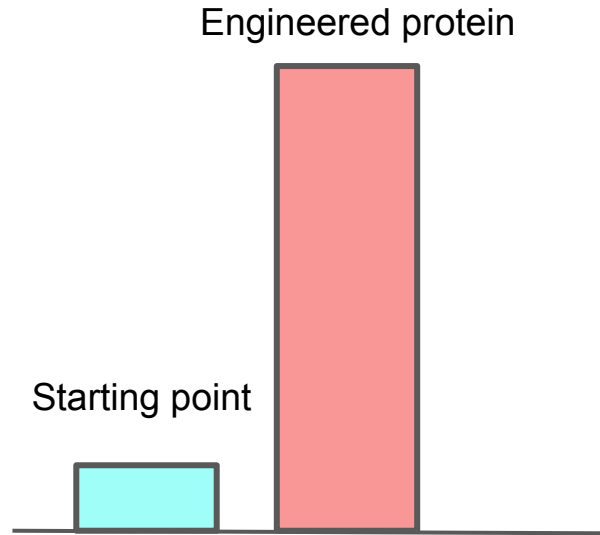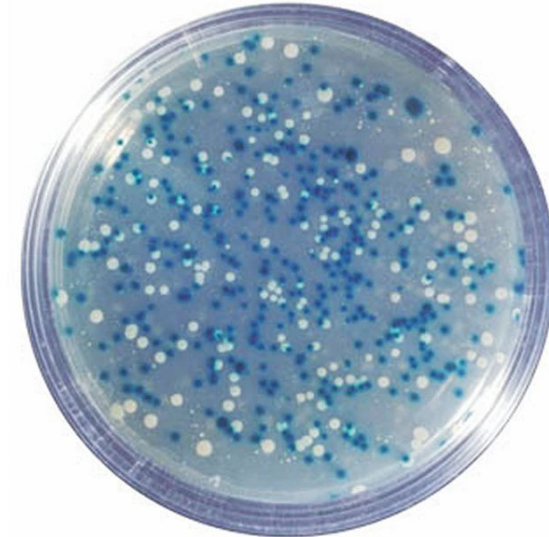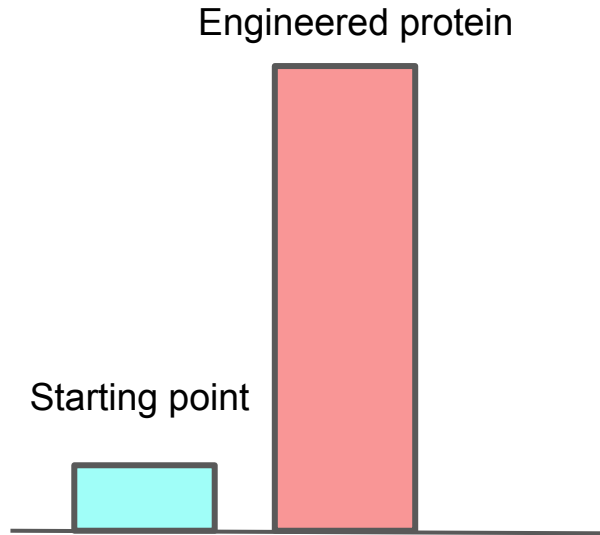
Blue-white selection

Measurements can be **quantitative** or qualitative

Quantitative measurements require monitoring a given property over a range
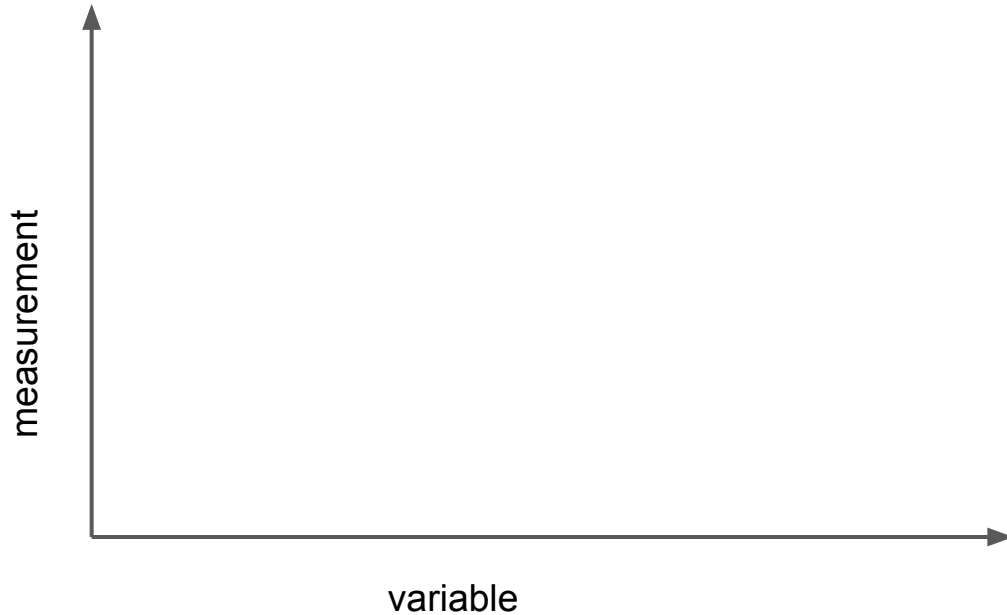
Quantitative measurements require monitoring a given property over a range
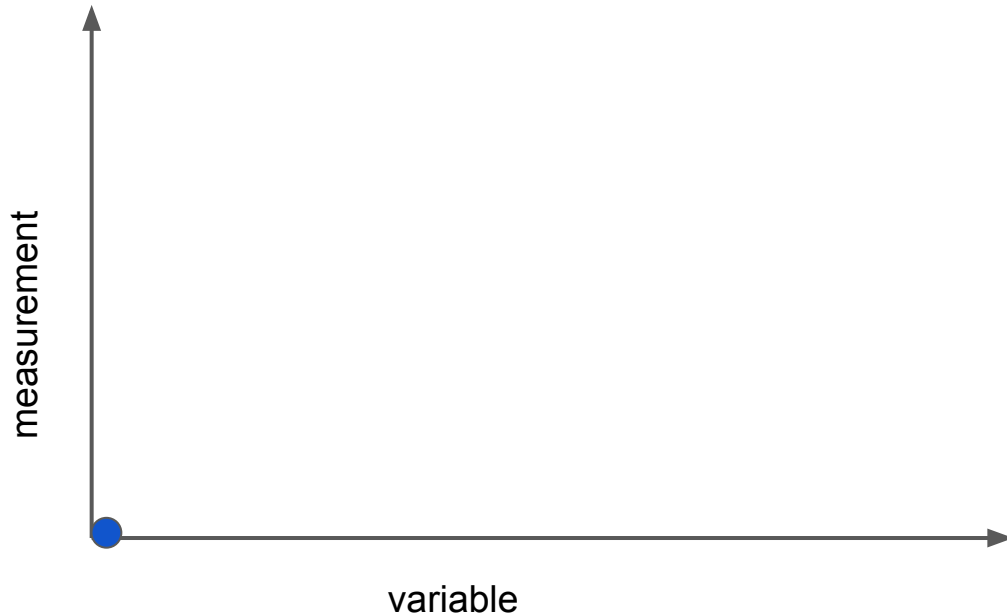
Quantitative measurements require monitoring a given property over a range
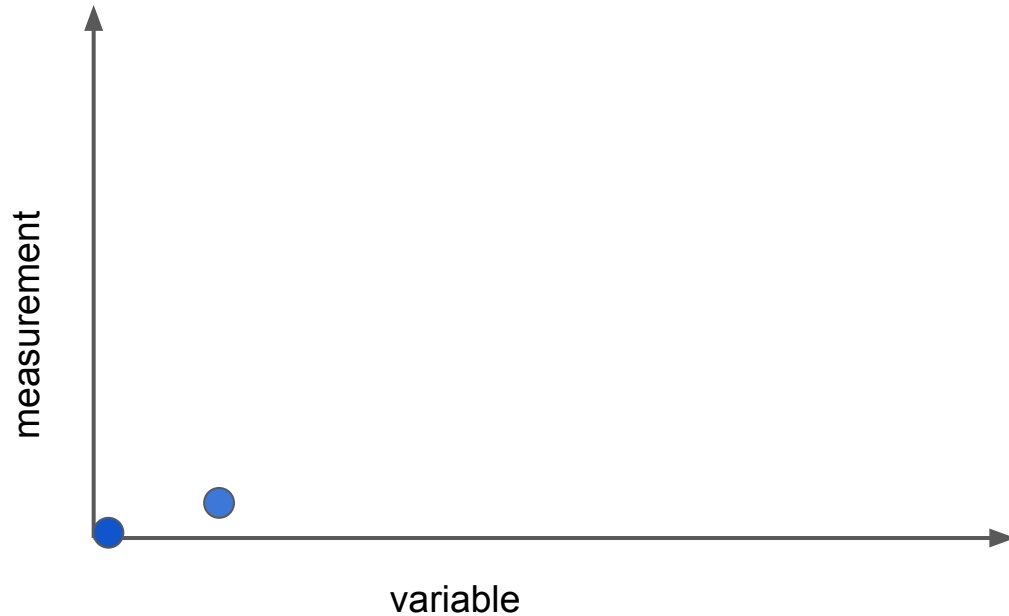
Quantitative measurements require monitoring a given property over a range
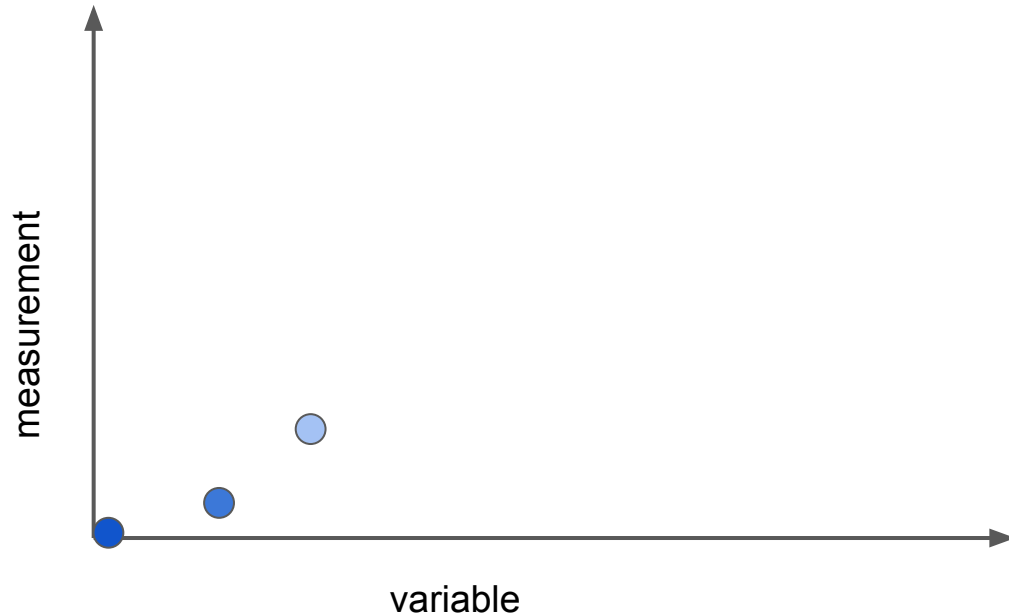
Quantitative measurements require monitoring a given property over a range

Measurements can be high, medium or low throughput

Measurements can be **high**, medium or low throughput
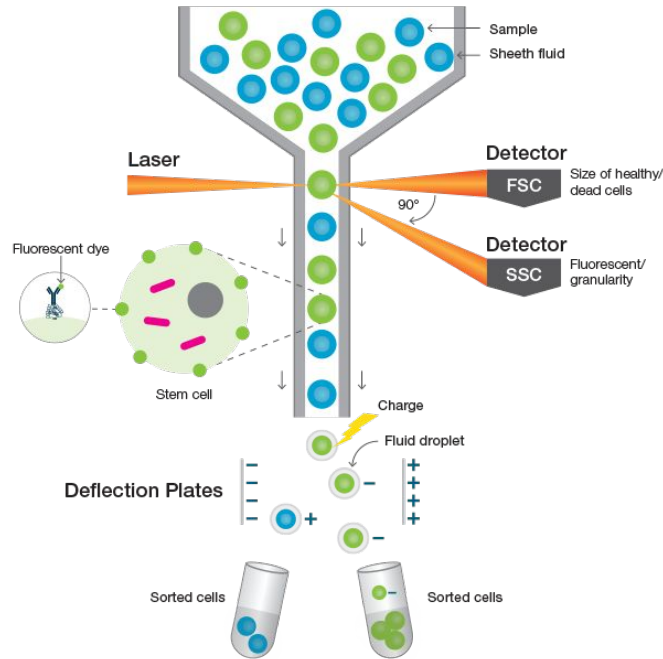
Measurements can be high, **medium** or low throughput
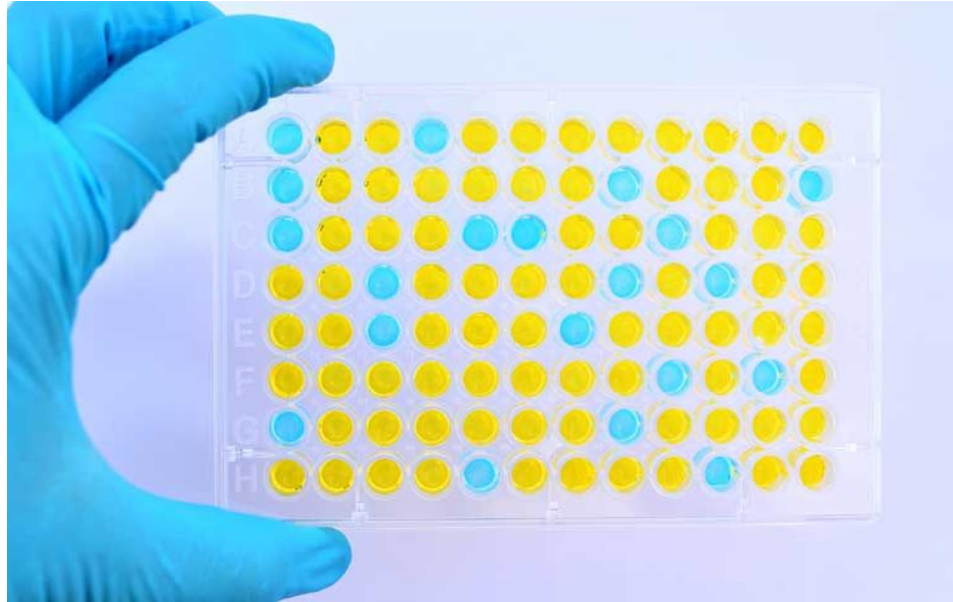
Measurements can be high, medium or **low** throughput

Measuring enzyme activity
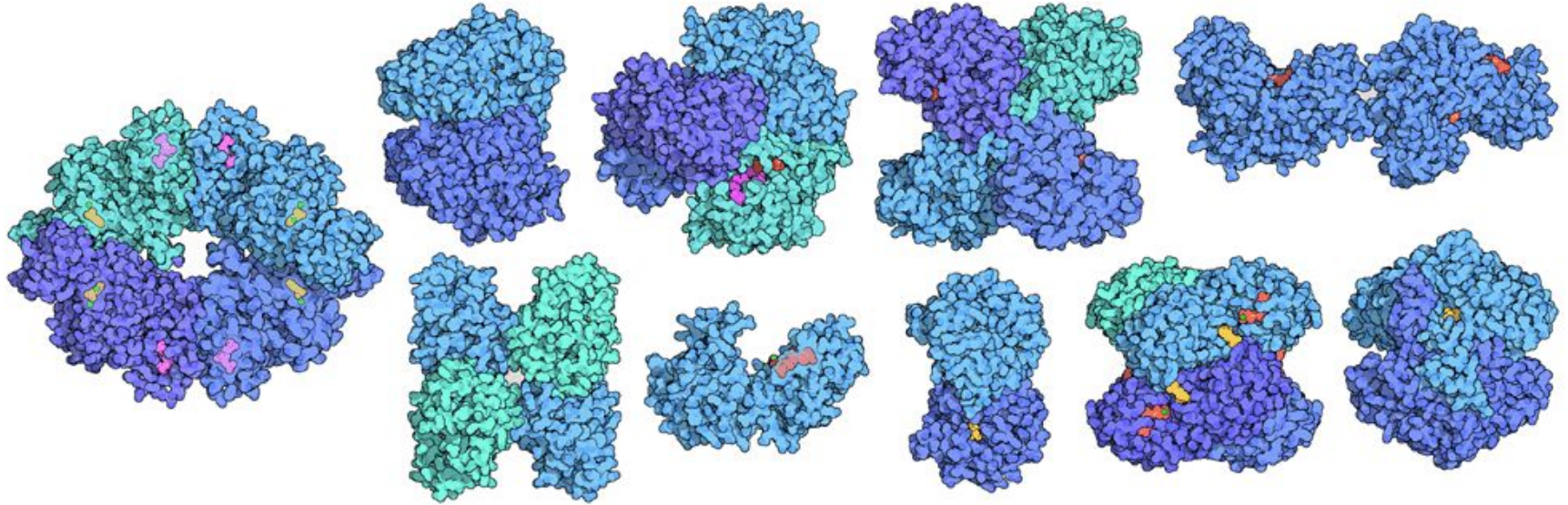
Glycolytic enzymes (from PDB 101)

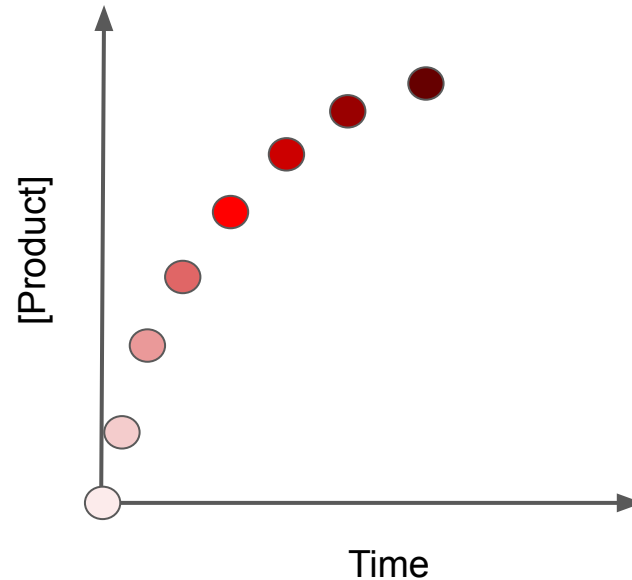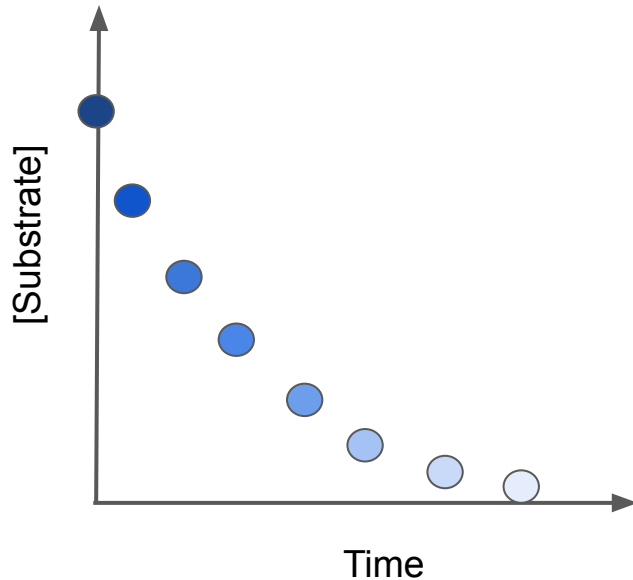# Measuring enzyme activity = measuring the substrate consumption or product generation
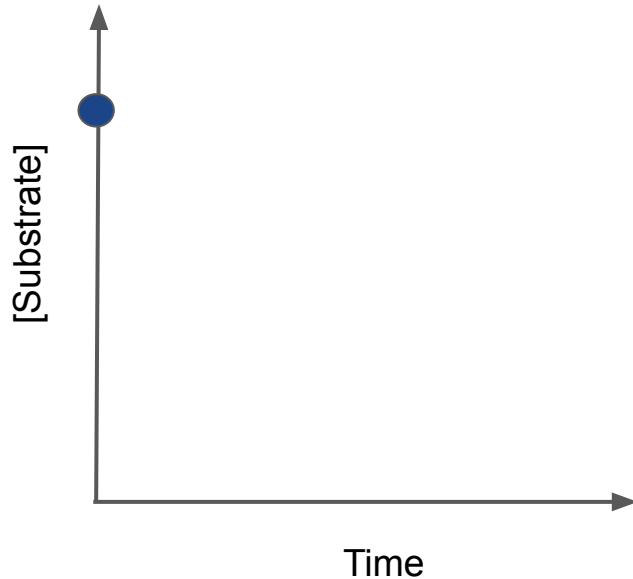
Measuring enzyme activity = measuring the substrate consumption or product generation
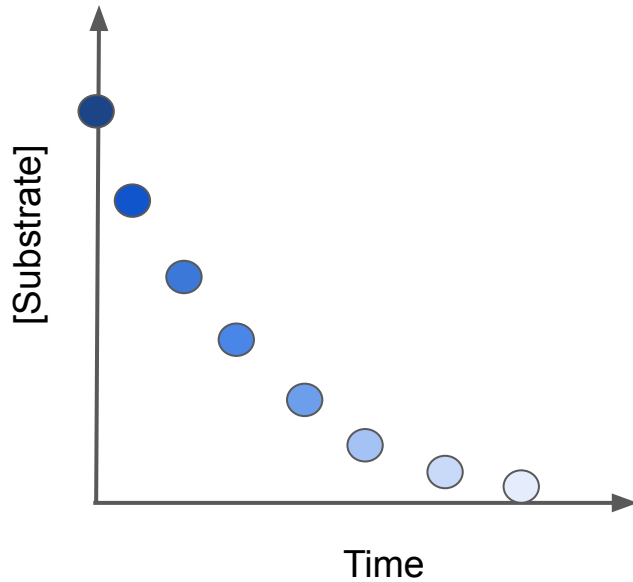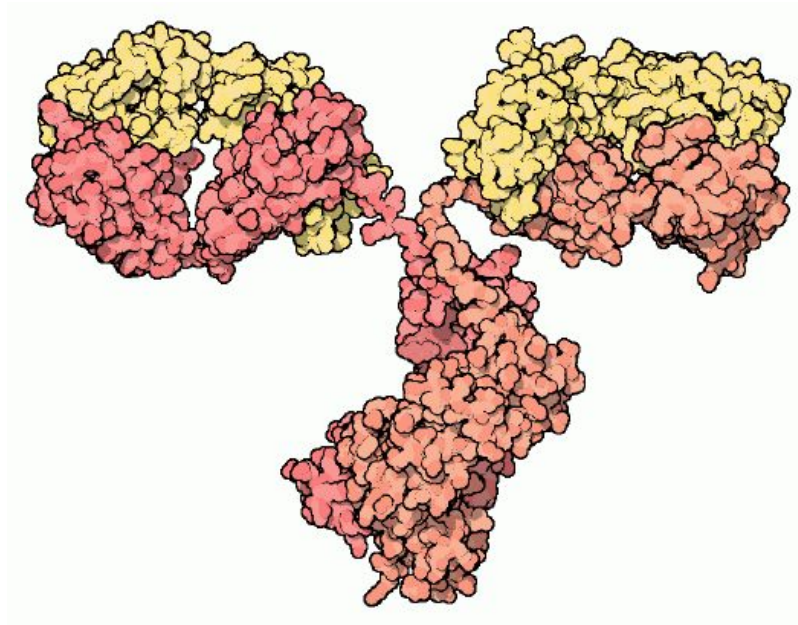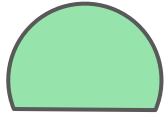
Measuring enzyme activity = measuring the substrate consumption or product generation
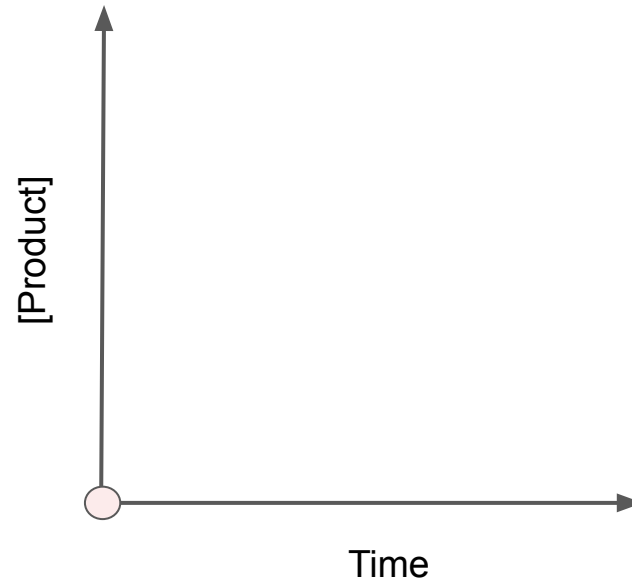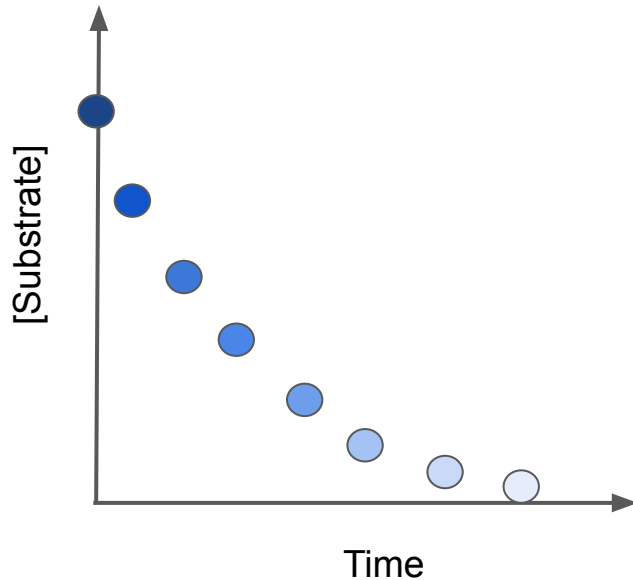
Measuring enzyme activity = measuring the substrate consumption or product generation
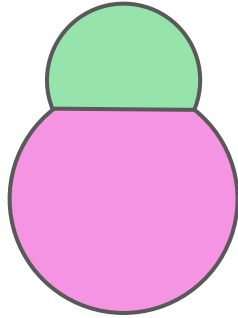
Measuring binding

Glycolytic enzymes (from PDB 101)

Binding measurements follow changes in the properties of single protein vs complex
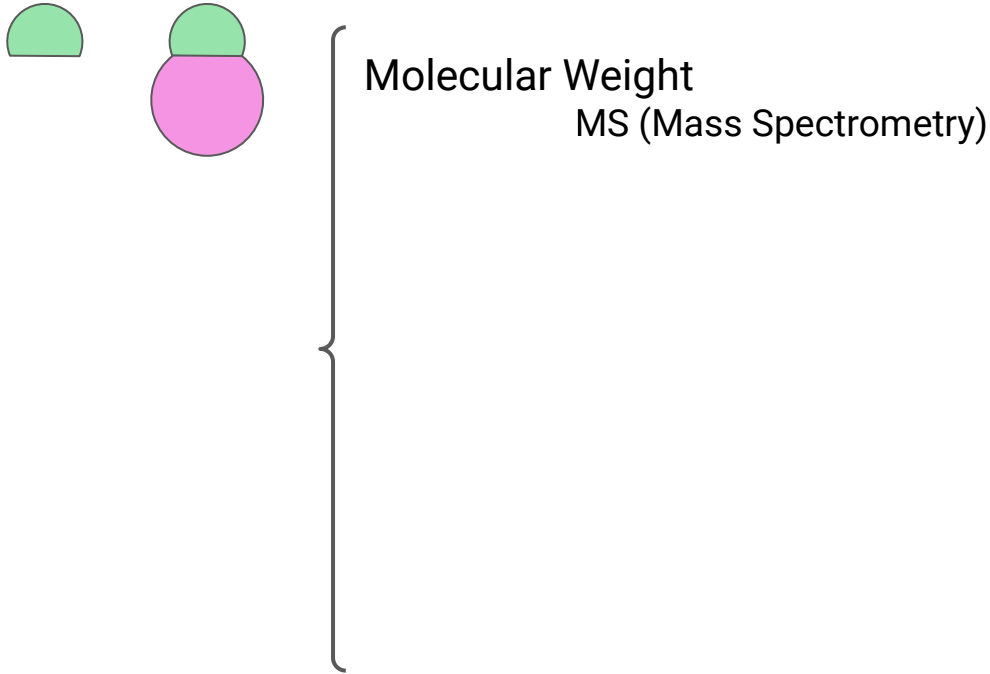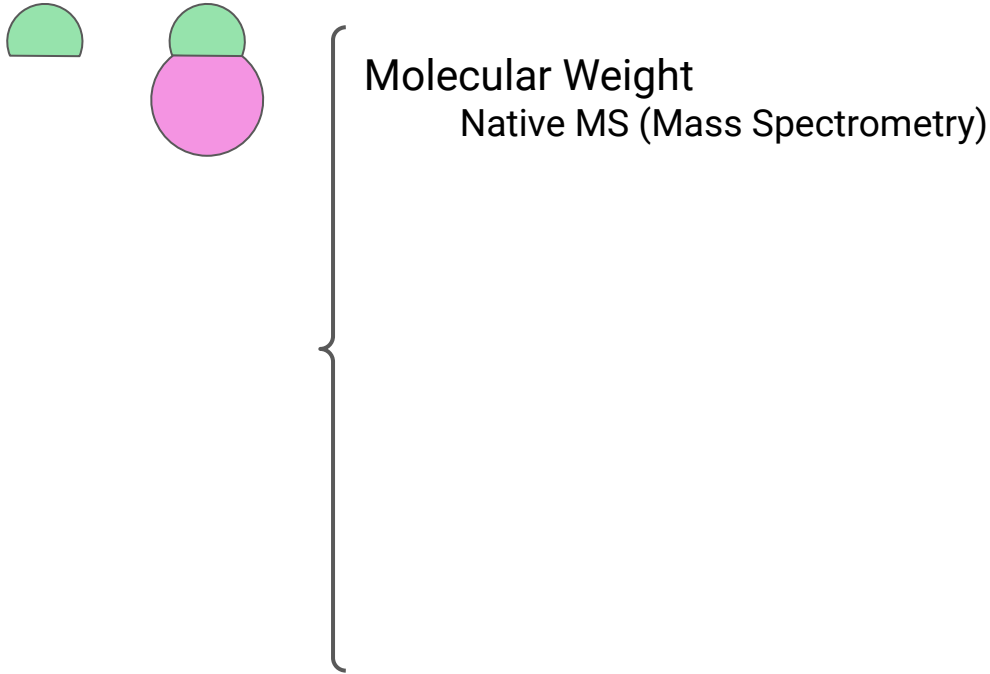

Free

Complex

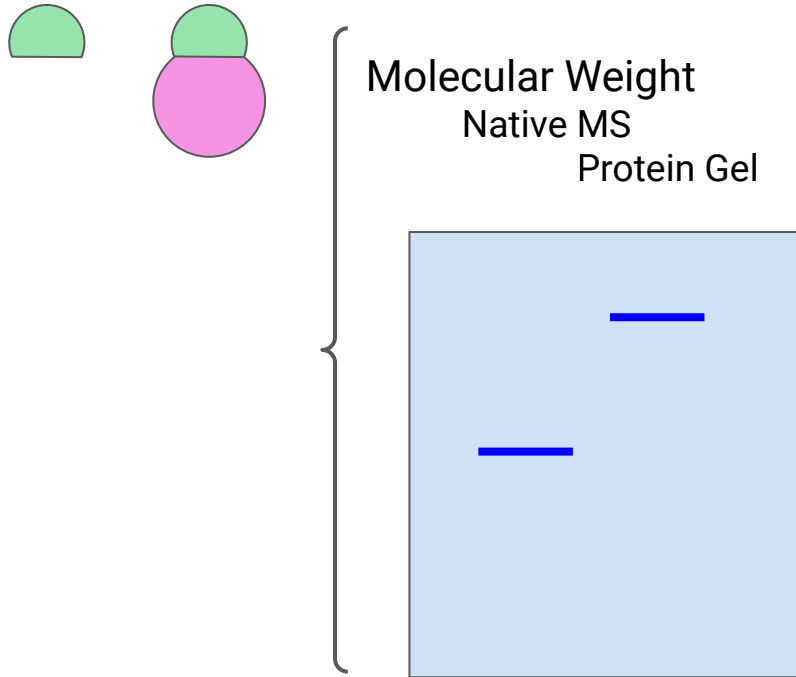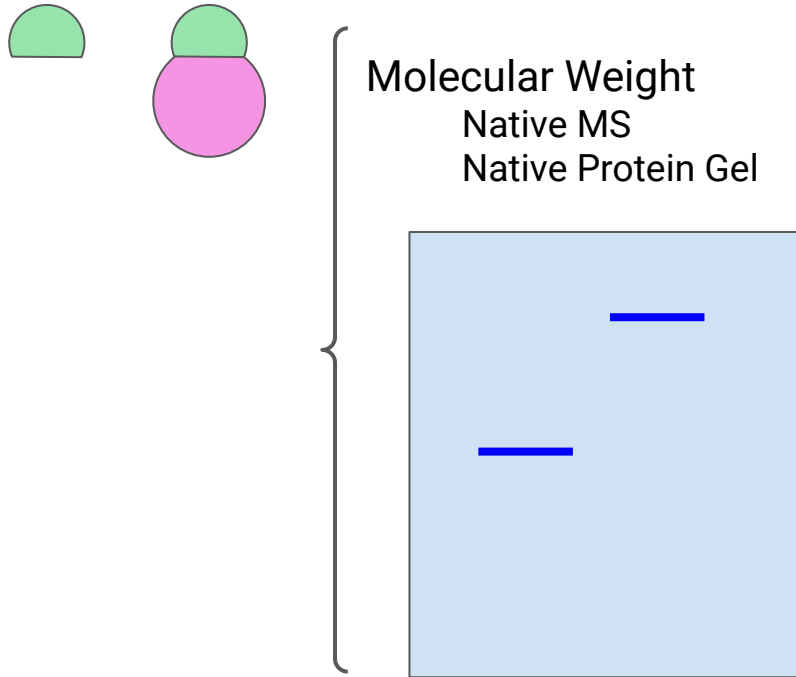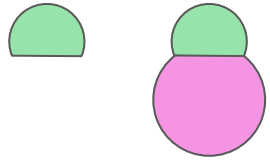# Binding measurements follow changes in the properties of single protein vs complex

Molecular Weight

MS (Mass Spectrometry)

Binding measurements follow changes in the properties of single protein vs complex

Molecular Weight
Native MS (Mass Spectrometry)

Binding measurements follow changes in the properties of single protein vs complex

Molecular Weight
  Native MS
    Protein Gel

Binding measurements follow changes in the properties of single protein vs complex

Molecular Weight
    Native MS
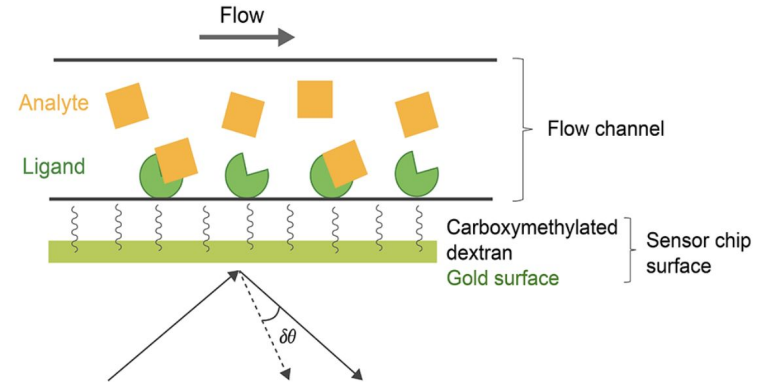   Native Protein Gel

Binding measurements follow changes in the properties of single protein vs complex
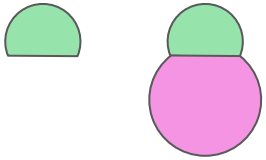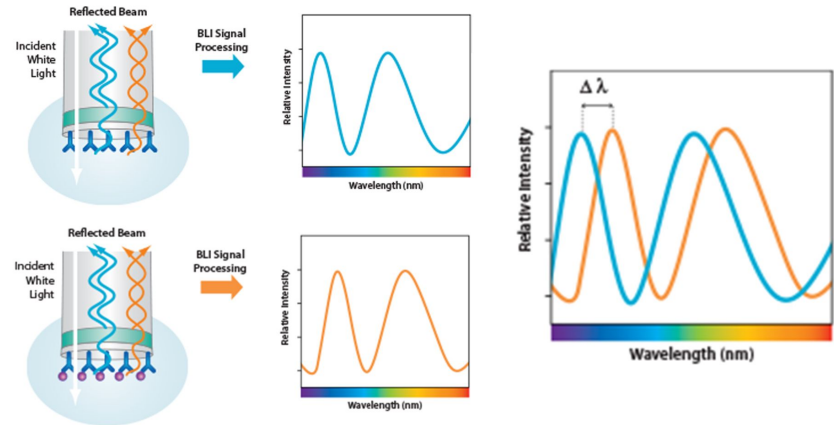
Molecular Weight
    Native MS
    Native Protein Gel

Light diffraction properties
    SPR (Surface Plasmon Resonance)

Binding measurements follow changes in the properties of single protein vs complex
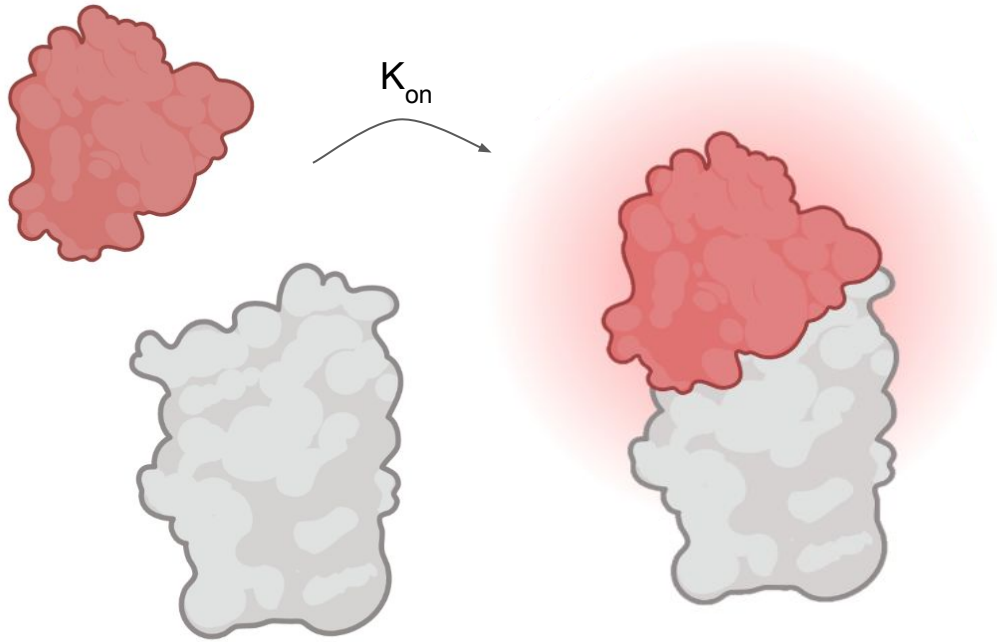

Molecular Weight
    Native MS
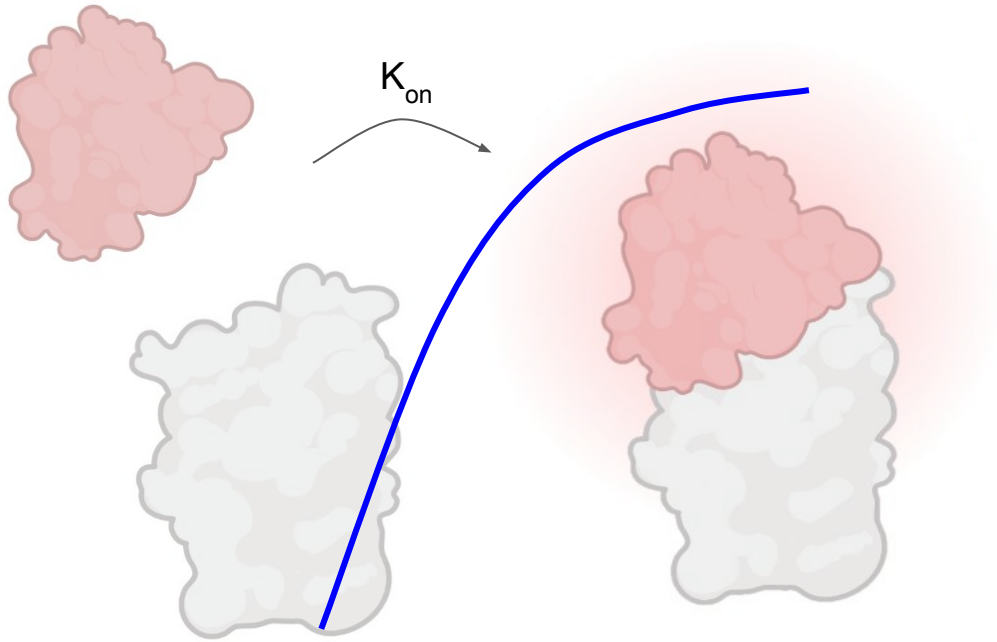   Native Protein Gel

Light diffraction properties
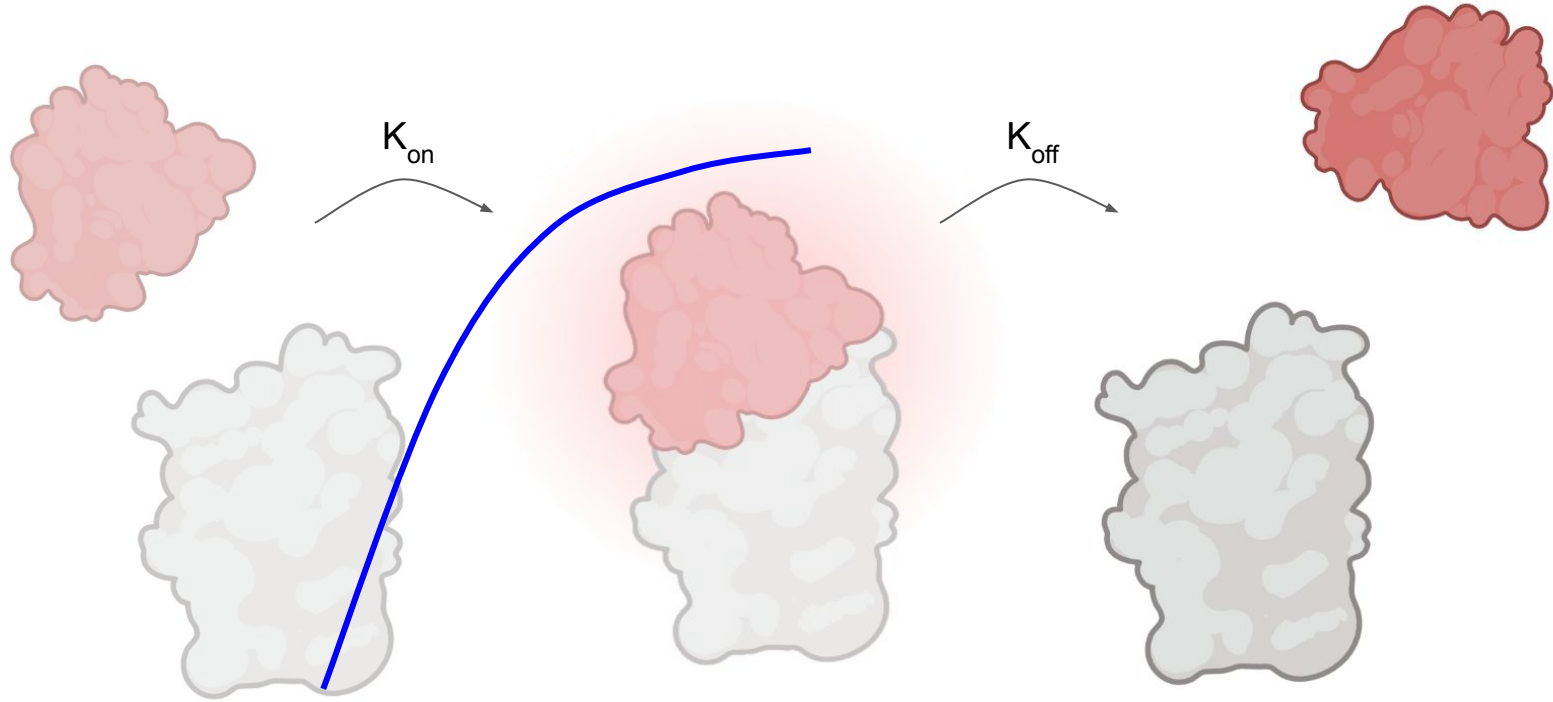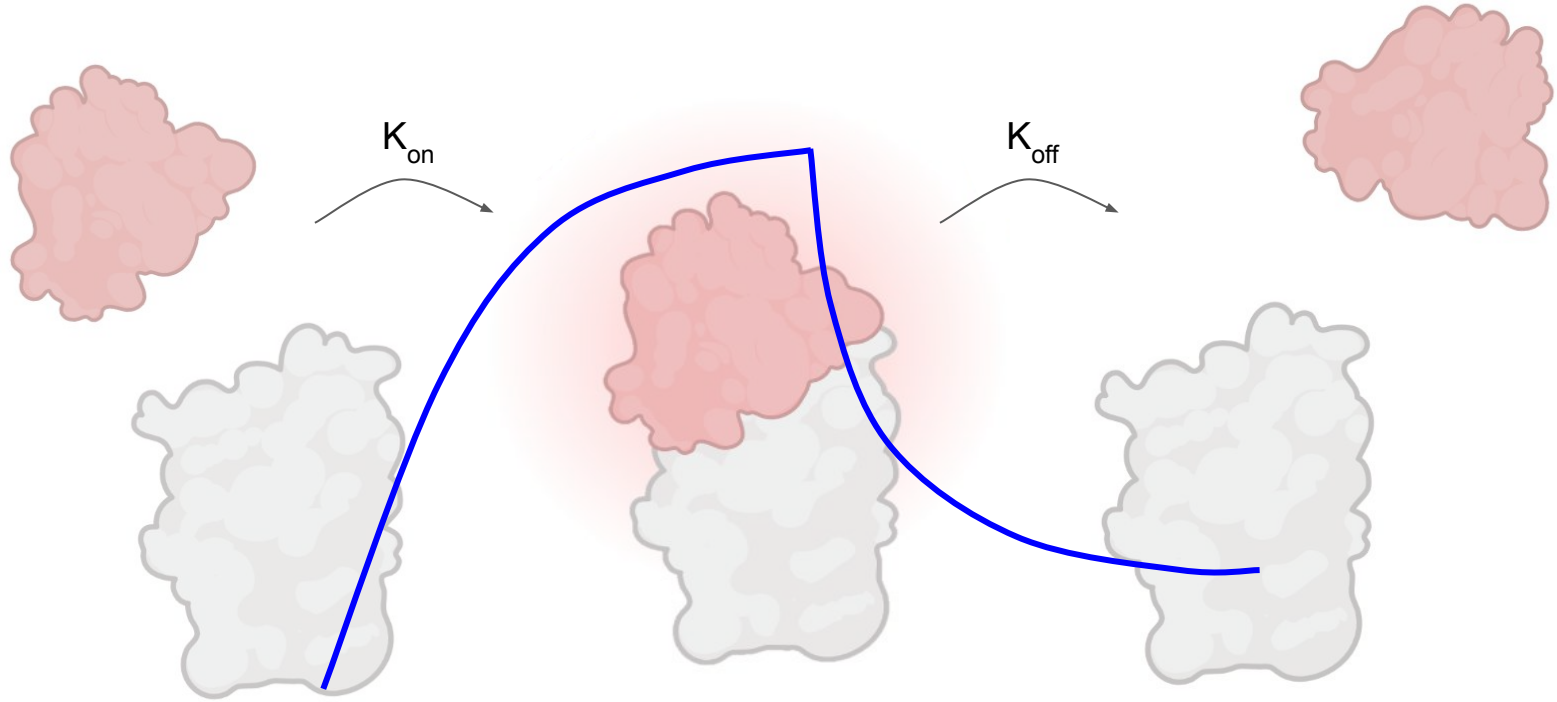   SPR
    BLI (Biolayer Interferometry)

Binding is an equilibrium process

Binding is an equilibrium process
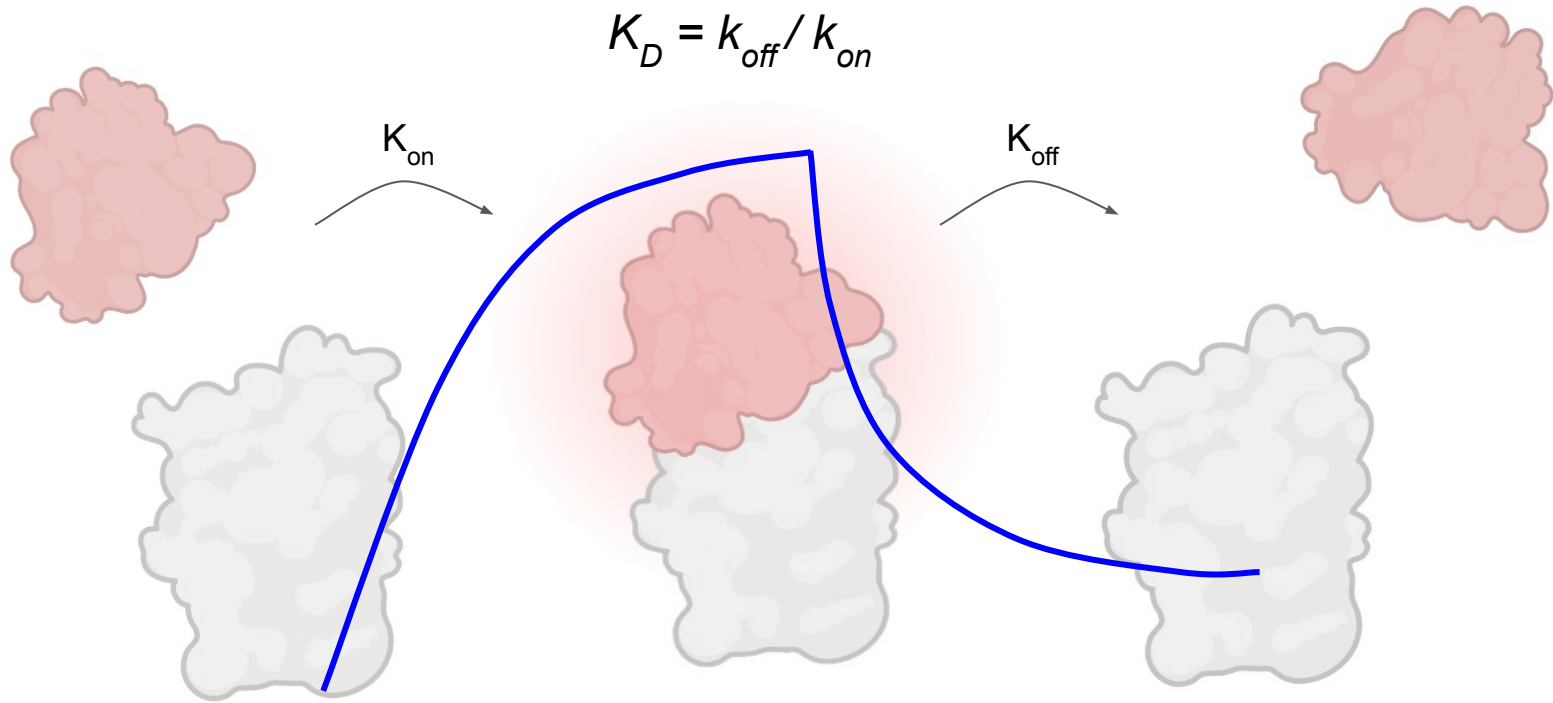
Binding is an equilibrium process

Binding is an equilibrium process

Binding is an equilibrium process

$K_D = k_{off} / k_{on}$

K_{on}

K_{off}
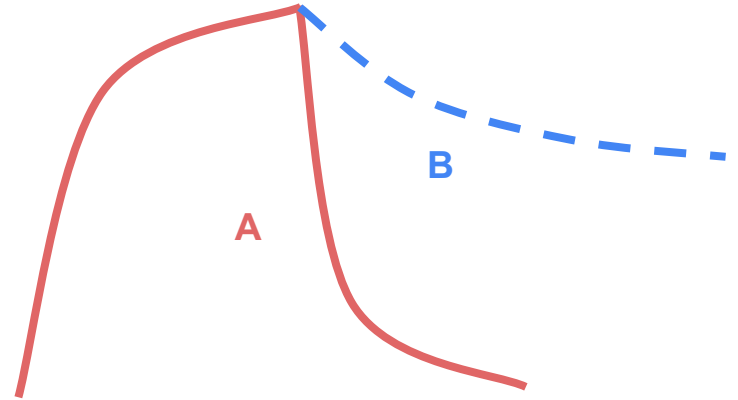
In-class activity

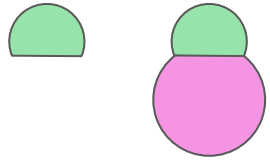Which one has lower K_D(ie higher affinity)?
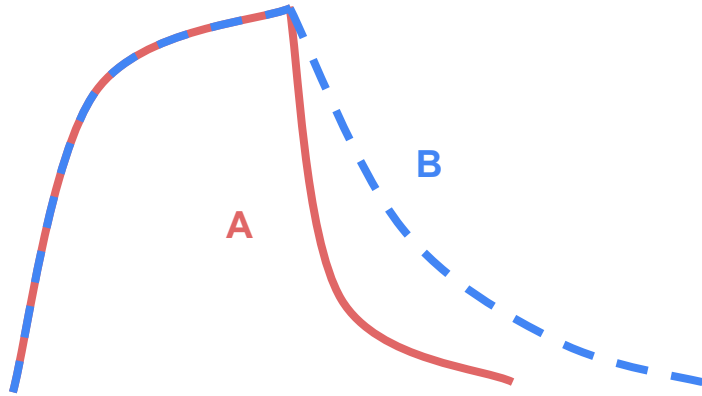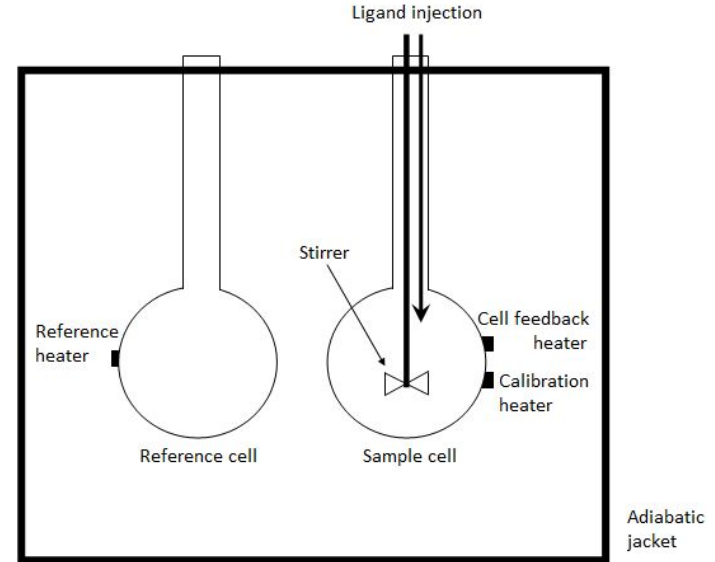
Which one is a covalent binder?

Binding measurements follow changes in the properties of single protein vs complex

Molecular Weight
Native MS
Native Protein Gel

Light diffraction properties
SPR
BLI

Changes in free energy
ITC (Isothermal Titration Calorimetry)

$$\Delta G = -RT \ln K_a = \Delta H - T\Delta S$$

$$c = n * Ka * M$$

Binding measurements follow changes in the properties of single protein vs complex

Molecular Weight
    Native MS
    Native Protein Gel
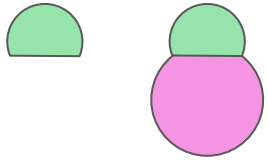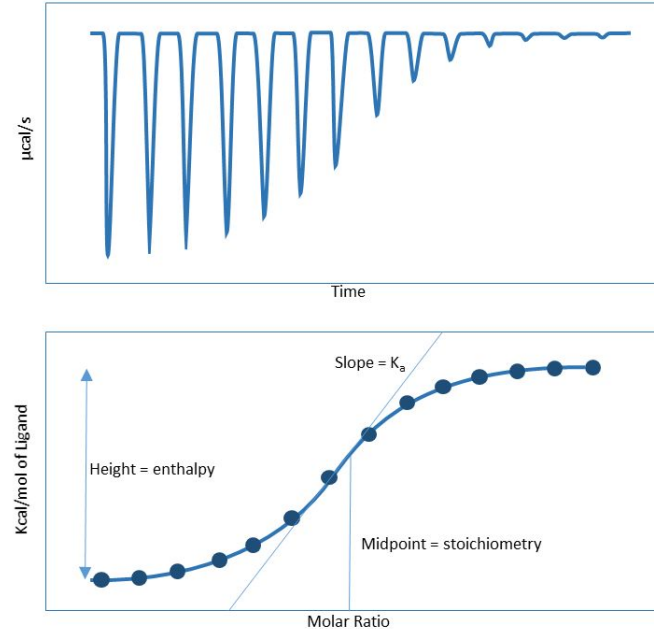
Light diffraction properties
    SPR
    BLI

Changes in free energy
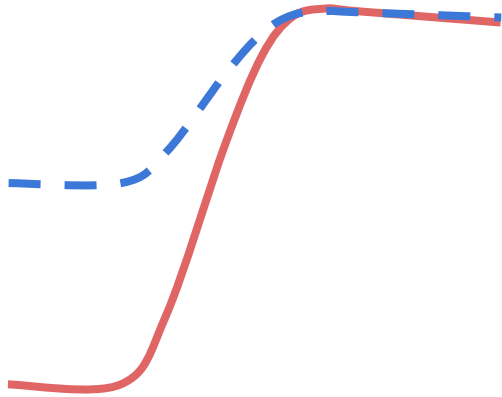   ITC (Isothermal Titration Calorimetry)

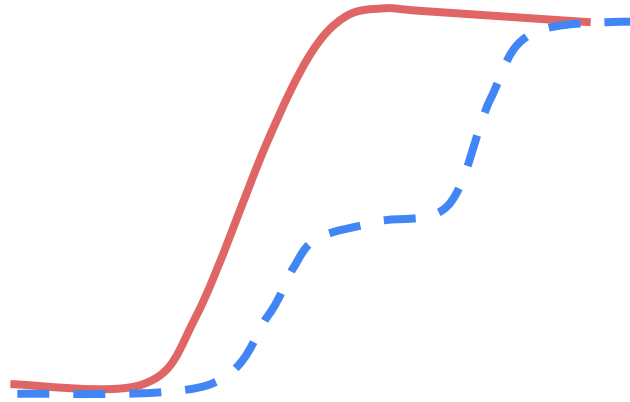# In-class activity

Enthalpy vs Entropy driven

One vs two binding sites

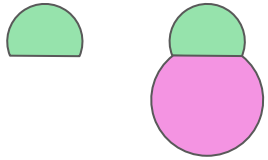# In-class activity

Enthalpy vs Entropy driven

One vs two binding sites

Binding measurements follow changes in the properties of single protein vs complex
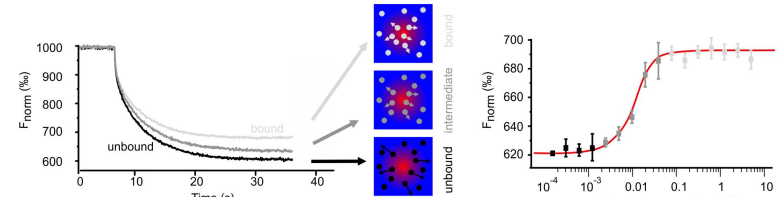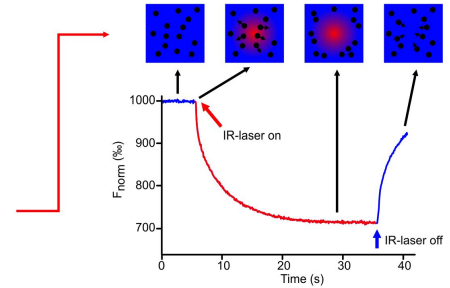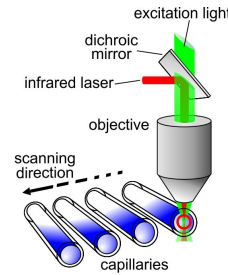

Molecular Weight
   Native MS
   Native Protein Gel

Light diffraction properties
   SPR
   BLI

Changes in free energy
   ITC

Movement
   MST (Micro Scale Thermophoresis)

Summary of binding methods

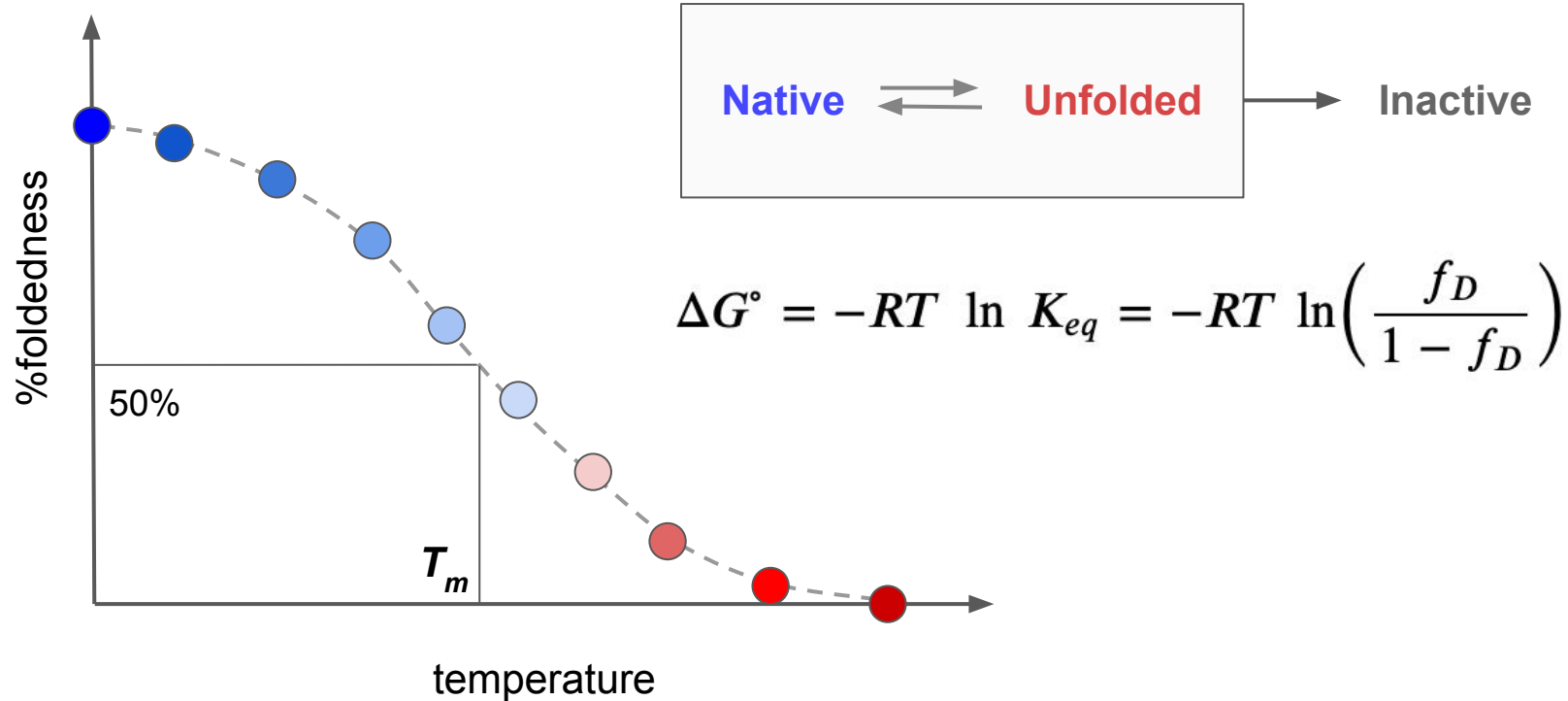|	SPR	ITC	BLI	MST
Label/Immobiliz.	N/Y	N/N	N/Y	Y/N
Throughput	Medium	Low	Medium	Medium/High
Accuracy	High	Thermodynamics	Low	High (but sens.)
Range	Wide	Limited	Wide	Wide (complex)
Sample quantity	Small	Large	Medium	Small
Cost/expertise	High	Medium	Medium	Medium
Maintenance	High	Medium	Low	Medium

High throughput binding measurements

High throughput binding measurements can be achieved by linking binding to fluorescence, or life/death

Measuring Stability

Thermal stability is often correlated to proper folding of the protein
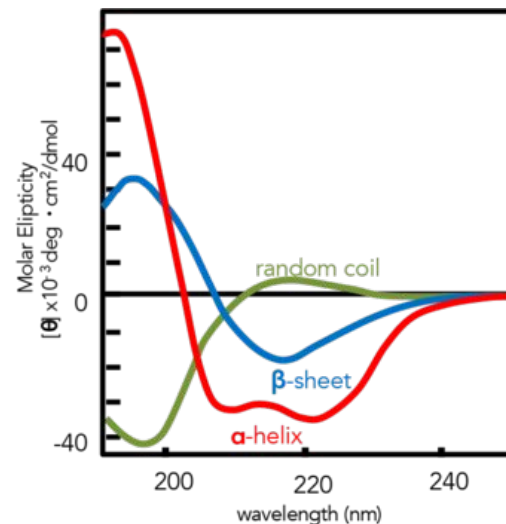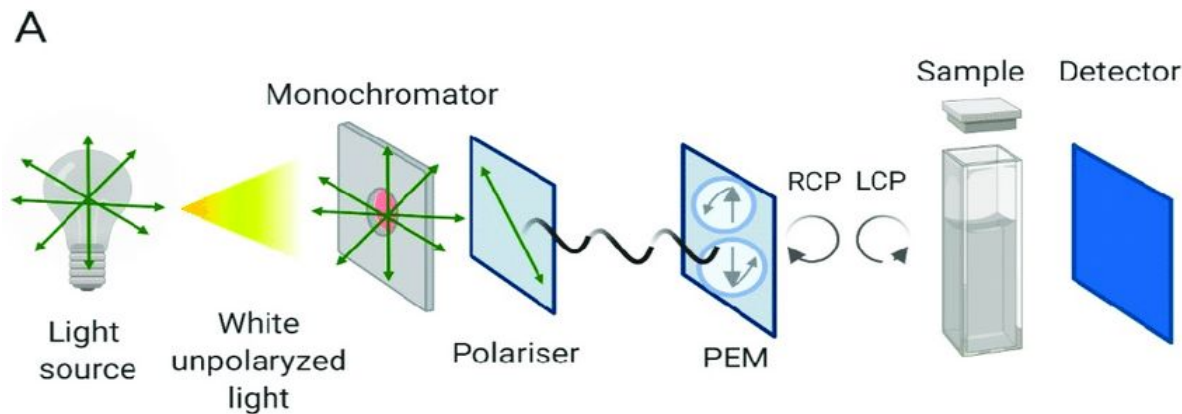

%foldedness

50%

T_m

temperature

Native ⇌ Unfolded → Inactive

$$\Delta G° = -RT \ \ln K_{eq} = -RT \ \ln\left(\frac{f_D}{1 - f_D}\right)$$

Protein fold can be assessed by measuring …
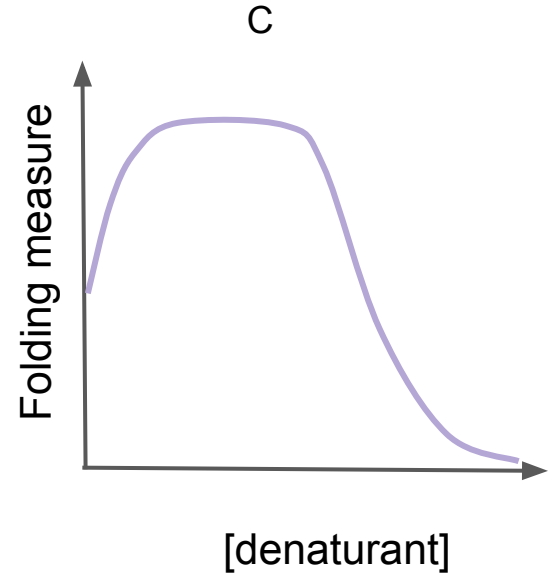
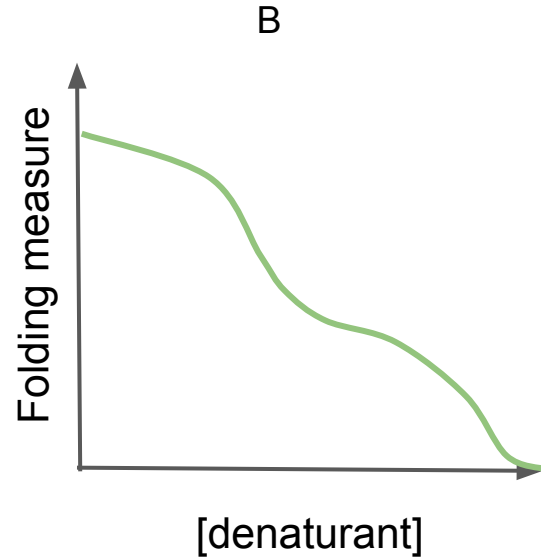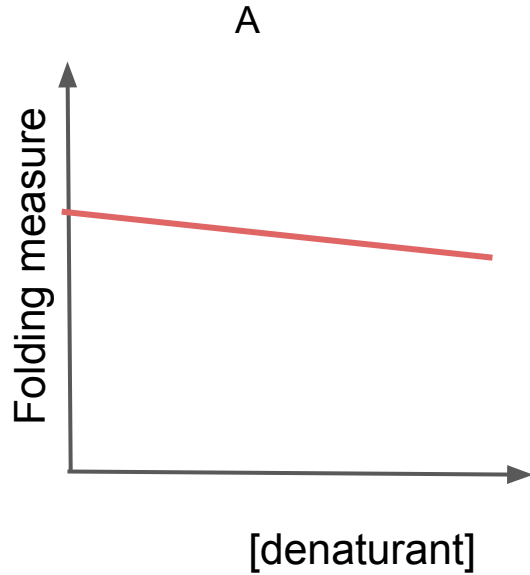# Protein fold can be assessed by measuring its activity

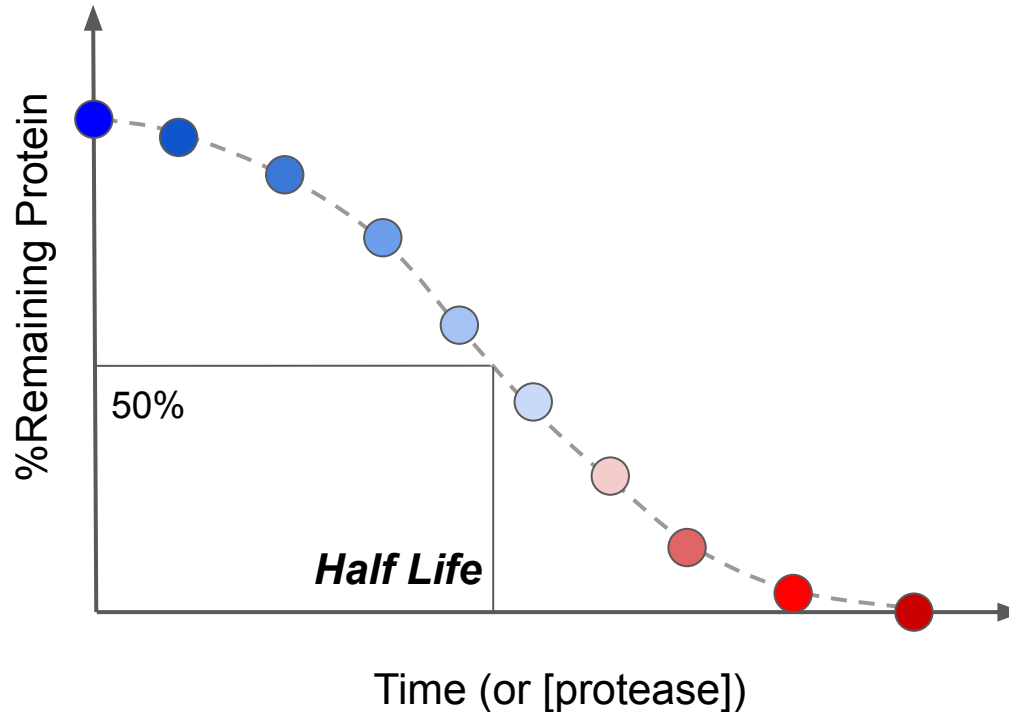# Protein fold can be assessed by measuring its activity or its secondary structure content
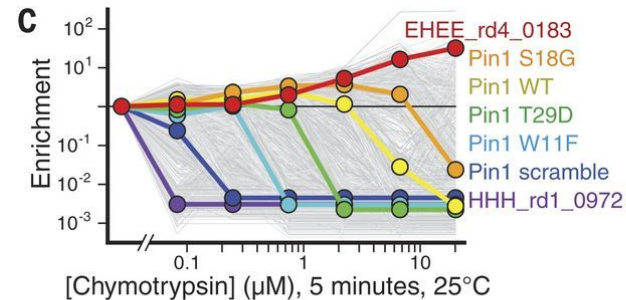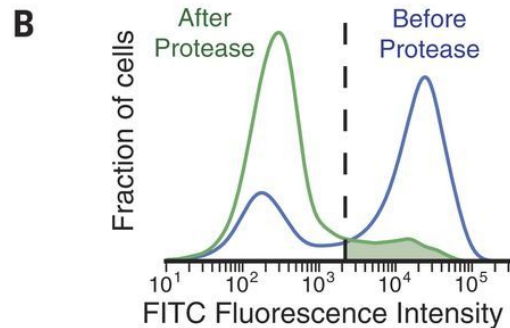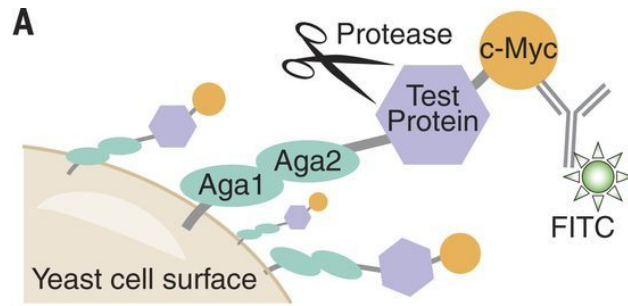

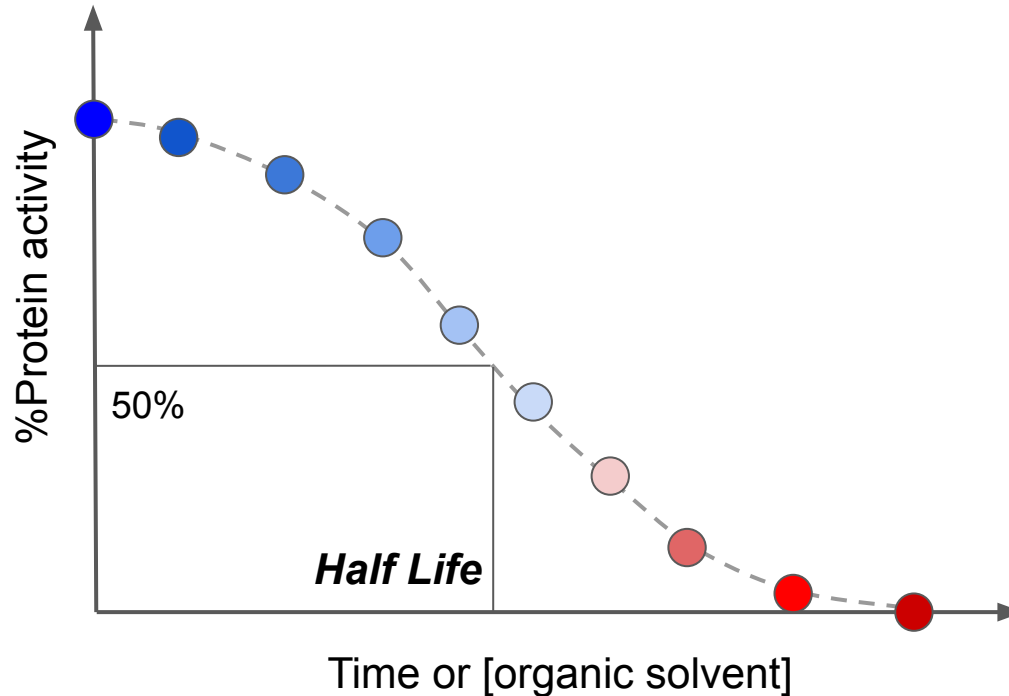(Adapted from N. Greenfield, 1969)

In-class activity

Protease stability monitors protein degradation over time

Protease stability monitors protein degradation over time and can be made high throughput

To measure stability against harsh conditions, we often measure activity

For the next lecture:

1. Pre-class assessment for the next lecture
   Needs to be done before the start of class, will be available after this class

2. Post-class assignment
   The one from W1L2 due next lecture
   This lecture assignment: Proposal write-up
   Read journal for the next lecture

3. Make sure foldx is installed!

Next lecture:

Rational design of proteins guided by structure