Rule of Three Approach to Hypothesis Development

From Hypothesis to Predictions; The Three Questions

Three Questions

1. What do I need to Measure?
2. What will I change?
3. How will I know if what I am measuring has changed and is consistent with the hypothesis ?

1. Your third "Something" from the previous panel tells you what you need to measure: some aspect of structure or function

There are usually several ways to measure a given aspect of structure or function- focus here on what you need to measure – in the proposal module, T4, you will focus on how you will measure it.

2. Your second "Something" from the previous panel tells you what you need to change

This will often be some structural aspect of the protein or ligand but could be some condition that changes the basis of the interaction.

3. To know if what you are measuring has changed as a result of the above perturbation you will need to have measured the unperturbed as well as the perturbed system with a large enough sample size to get statistically significant data sets for each and have conducted appropriate control experiments to ensure that alternative explanations for any observed change are not responsible for the change.

Choosing the Three Somethings in Hypothesis Development

Something does **Something** that contributes to **Something**

That relates back to your Big Picture

All Proteins have two things in common:

1) **They have structure (Ligands too have structure)**

(Primary, secondary, tertiary and in some cases quaternary)
Structure is not necessarily static and the dynamics of the structure may contribute to function

&

2) **They bind(interact with) something**

(A Ligand, could be a small molecule (substrate, regulator etc or another protein),
which leads to their biological function (if they are an enzyme, this leads to catalysis)
Binding has two fundamental parameters: specificity & Affinity in addition to stoichiometry.
Remember, proteins are large complex molecules with many internal interactions that govern structure, and hence function.

Structure of a testable hypothesis:
Something, (some aspect of protein or ligand structure) does (interacts) with Something (some aspect of protein or ligand structure, give mechanistic detail, eg hydrogen bonds etc) that contributes to Something (some aspect of structure or function: eg catalysis, affinity, specificity, structure etc)

How to Measure what you need to measure?

This again stems from the third **"Something"** in the first panel and usually involves measuring some aspect of structure or function, and there are usually several ways to measure a given aspect of structure or function briefly outlined below.

Wet Lab Techniques to show aspects of Structure:

Secondary Structure
- CD Spectroscopy
- IR Spectroscopy
- NMR Spectroscopy

Tertiary Structure
- Fluorescence Spectroscopy
- UV Spectroscopy
- NMR Spectroscopy
- Chemical Modification Approaches

Quaternary Structure
- Size Exclusion Chromatography
- Dynamic Light Scattering
- Native PAGE
- CrossLinking & SDS PAGE

Stability
- Global
  - Chemical or thermal Denaturation (with DLS, CD or Fluorescence- with tDenaturation can follow using simple kinetics or FbTSA)
- Local
  - HDEx with Mass Spectrometry

Wet Lab Techniques to show aspects of Function:

Catalytic Activity
- Initial Rate Measurements
- Pre-SteadyState Measurements
  - Either can be combined with Isotope Effect Measurements

Ligand Binding
- Specificity
  - Compare K_D for different ligands
- Affinity
  - Equilibrium Dialysis
  - Spectroscopy Techniques
  - Protection Techniques
    - (vs Chemical Modification, or FbTSA)
- Stoichiometry
  - Equilibrium Dialysis
  - Spectroscopy Techniques
- Sidechain pKa Determination
  - NMR
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Computational Approaches to Explore Aspects of Structure and Function

Molecular Dynamics:	Local Motion & Response to Ligand Binding
HawkDock:	Local Interactions in Protein-Protein Complexes
SwissDock:	Ligand Binding Sites and Affinities
H++:	Sidechain pKa Values from structure
Deep ΔΔG:	Impact of Mutations on global stability from structure
HINT:	NonCovalent Interactions involved in structure or ligand-protein complexes
MolView:	Construction of Small Molecules, Calculation of Molecular Properties