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Experimental Methods in Systems Biology

Part of the Coursera Certificate in Systems Biology

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Icahn School of Medicine at Mount Sinai

Outline

- Introductions
- Course Scope
  - What we will cover in depth
  - What we will cover briefly (only in Week 1 intro)
  - What we will not cover
- The “Systems Biology Loop”
- Distinctive Features of Systems Biology Experiments
- Important Features of Any Experiment

Introductions

- About me—Marc Birtwistle
- B.S. and Ph.D in Chemical Engineering
- Postdoctoral Work in Cell and Molecular Biology
- Became Assistant Professor at the Icahn School of Medicine at Mount Sinai Sept. 2012
- Our lab focuses on integrating experiments and computation in the context of cancer systems pharmacology of glioblastoma multiforme, a deadly brain tumor
- We are particularly interested in cellular heterogeneity and how that impacts disease progression and treatment response

Introductions

- I assume you have a basic knowledge of cell and molecular biology, but I review relevant concepts to the extent possible.
- I also assume you have a knowledge of basic statistics.
- Although systems biology can be mathematics and computer science heavy, this course does not go into much depth in that regard.

Introductions

- As you can guess from my training and lab focus, I am familiar with many types of experiments and how they are used in computational modeling.
- However it’s impossible for anyone to be an expert in a wide array of techniques, therefore I draw on the help of many people in creating this course.
- It’s also not possible to cover every technique in reasonable depth.
- I’ve chosen a few key techniques to focus on that will give you reasonable coverage of important items, and briefly mention many others.

Scope—In Depth Coverage

- mRNA sequencing (Week 2)
  - Uses "next-generation" massively parallel DNA sequencing to quantify gene expression in an "omic" (genome-scale) fashion
  - Mortazavi et al., 2008, Nat Meth; Wang, Gerstein, Snyder, 2009, Nat Rev Genetics
- Mass spectrometry-based proteomics (Week 3)
  - Uses state-of-the-art mass spectrometry to quantify the levels of proteins (or protein states) in an "omic" fashion
  - Schwanhausser et al., 2011, Nature

Scope—In Depth Coverage

- The previous two techniques are omic-scale, and typically applied to cell populations rather than single cells
  - However single cell mRNA sequencing is becoming more feasible—e.g. Patel et al, Science, 2014 (Regev lab), Treutlein et al., Nature, 2014;
- Things need not be omic-scale to be systems biology.
- Many systems biology experiments
  - concern single-cell behavior and dynamics/time courses, which are difficult to probe on a omic-scale
  - are focused on small subsystems, e.g. a kinase signaling cascade

Scope—In Depth Coverage

- Flow and Mass Cytometry (Week 5)
  - Uses labeled antibodies and other specific labeled reagents to stain single cells in suspension, then measures labeling intensities in these single cells relatively rapidly (often easily 100s of cells/second)
  - Allows observation of a handful of analytes in many single cells of a population at fixed time points
  - Bendall et al., Science, 2011
- Live-cell Imaging (Week 6)
  - Uses (predominantly) fluorescence microscopy to observe processes in living cells in real time.
  - Allows observation of one or a few analytes in a handful of cells with dense temporal sampling
  - Regot et al., Cell, 2014

Scope—Brief Coverage

- Nucleic acid measurements
  - Quantitative polymerase chain reaction (qPCR)
  - Microarrays
  - Whole genome sequencing
  - Exome sequencing
  - Bisulfite (methylation) sequencing
  - ChIP sequencing (epigenetics)
  - Fluorescence in situ hybridization (FISH)

Scope—Brief Coverage

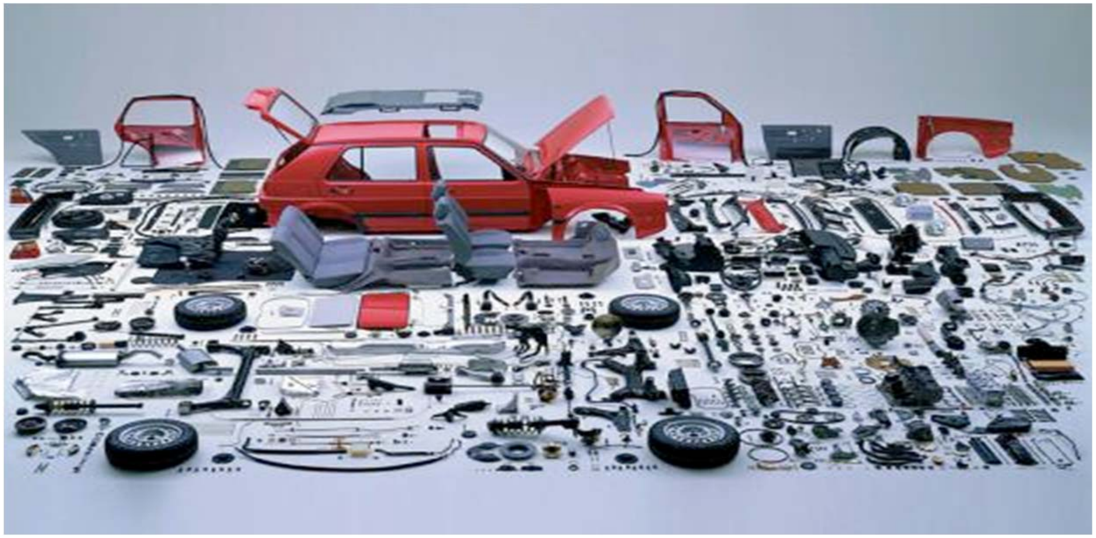
- Protein and Protein State Measurements
  - Western blot/microwestern
  - Reverse-phase Protein Array
  - Immunofluorescence

Scope—No Coverage

- Microfluidics
  - All kinds of clever devices built by microfabrication techniques that allow precise perturbation and/or capture of cells for unique measurements
  - It's relevant and interesting but we don't have time to do it justice
- Metabolomics
  - Mainly mass spec-based approaches to measure metabolites present in cells on an omic-scale
  - Again, it's quite relevant in many areas of systems biology but we just don't have time to give it proper coverage
  - We do cover mass spectrometry so its not a large stretch to consider it also applies to metabolites
- High-throughput Techniques
  - Many of the techniques we will describe have been optimized to be done in a high-throughput fashion
  - We describe the principals of the technique rather than its scaling
- Formal Design of Experiments
  - There are established statistical fields devoted to design of experiments
  - There is much ongoing work in systems biology to use quantitative computational models as part of this
  - We rather focus on the experimental techniques themselves

The Systems Biology Loop

What is Systems Biology?

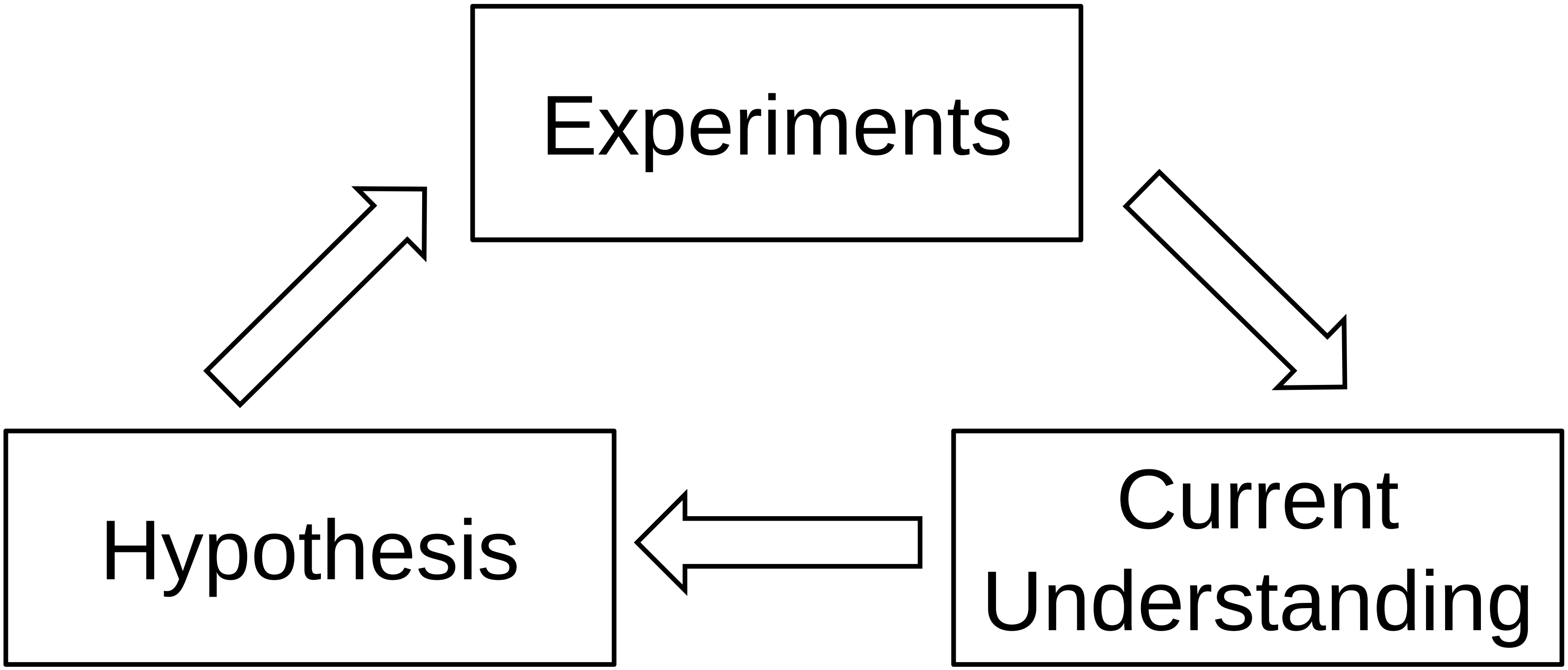


We know all the parts, so we should be able to understand how it works, right?

Biologists typically try to understand one part.

Systems biologists try to understand how the interactions between many parts give rise to function. Some say it is physiology re-invented (they're probably correct, although there are some differences).

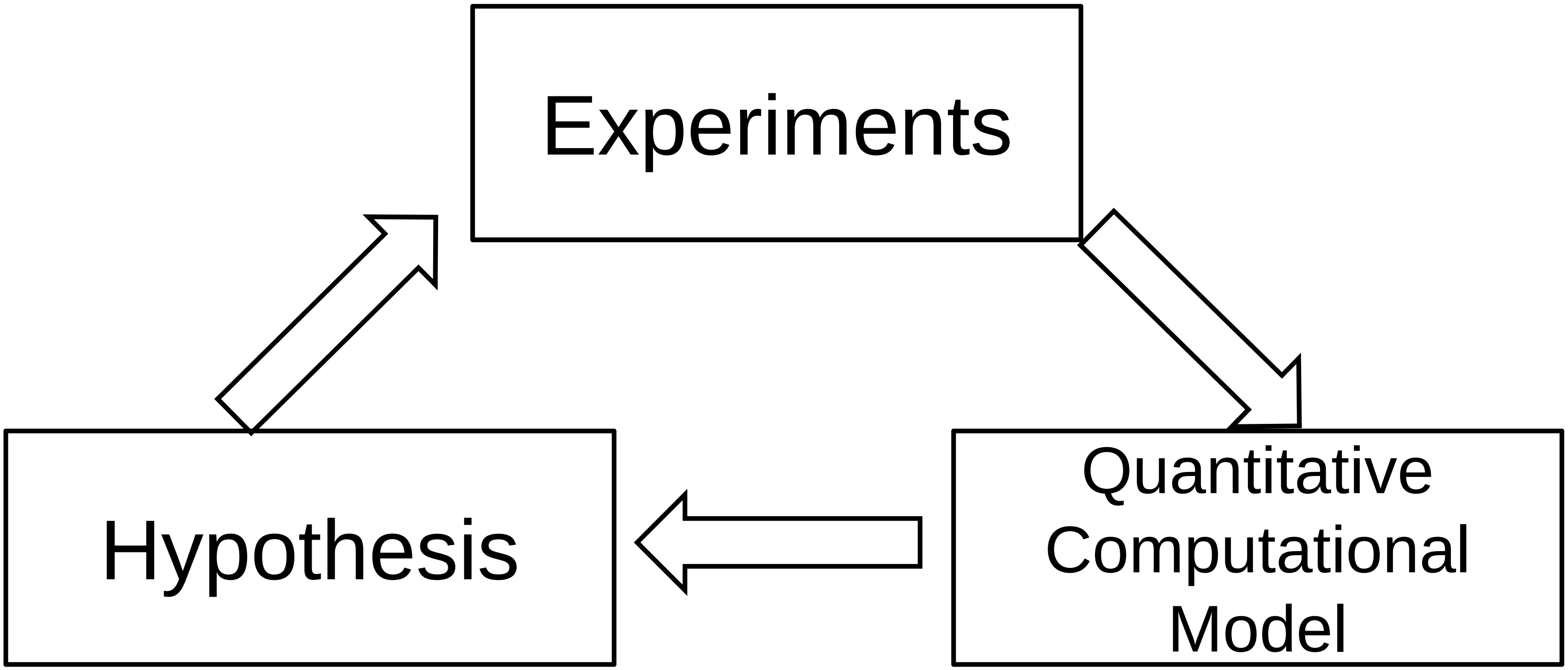
Common Scientific Workflow



Common Scientific Workflow

- In practice, one can enter this loop anywhere.
- The loop need not be completed by one group.
  - In fact, many great discoveries have been made when the loop arrows are completed by different groups sometimes long separated in time.
    - E.g. Watson and Crick with the DNA double helix structure
    - Recent discovery of the Higgs Boson in particle physics

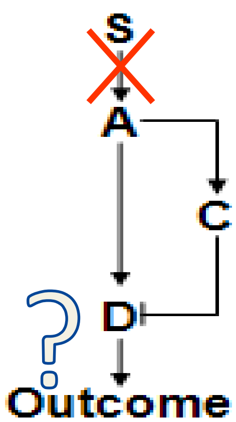
How is Systems Biology Different?



What Difference Does That Make?

- As opposed to many other scientific disciplines, “Current Understanding” in biology is often semantic or cartoon-based.
- Even though much can and has been learned in this way, it can be imprecise, even in simple cases.
- Moreover, biology can be remarkably complex.
  - This makes semantic or cartoon-based understanding even more imprecise, and sometimes even impossible.
- Many engineered systems are also remarkably complex, but the difference in biology is that we didn’t build it, so we have limited knowledge of what is in there!

Issues with Cartoon-Based Understanding



- **Need to know:**
  - **Magnitude of Effects on D**
    - A strong; C weak → D up
    - A weak; C strong → D down
  - **Dynamics of Interactions with D**
    - A slow; C fast → D down then up
    - A fast; C slow → D up then down
  - **Localization with D**
    - A local; C distant → D up
    - A distant; C local → D down

Quantitative Computational Models Allow Us To Keep Track of These Kinds of Properties in Complex Systems

Distinctive Features of Systems Biology Experiments

- Because systems biology experiments usually support development or refinement of a quantitative computational model, they tend to have the following properties
  - Quantitative
    - Many biological experiments tend not to be quantitative, either because the technique inherently disallows it, or because a great deal of extra effort is required to be quantitative.
    - We will give strong focus to how one makes experiments quantitative.
  - Dynamic
    - Often, critical information from a systems biology experiment is how the system responds to a perturbation over time, which supports development of a computational model and can support inference of causality.
    - Many important phenomena in biology are time-dependent, e.g. circadian clocks, cell cycle, developmental processes, drug responses, action potentials, etc.
- These two features transcend most systems biology experiments

Distinctive Features of Systems Biology Experiments

- Large-scale vs. small-scale systems biology
  - Although omic-level measurements are often associated with systems biology, one needn't have omic-level measurements to do systems biology
  - In fact, sometimes much is learned by "simple models" and experiments
    - E.g. Shen-Orr et al., Nat Genetics, 2002; Chang and Ferrell, Nature, 2013; Lim, Nat. Rev. Mol Cell Biol, 2010
  - Hence why we spend half the course on more "low-throughput" methods
- Spanning across systems and scales
  - Whether small or large scale, usually systems biology experiments aim to answer questions that go beyond traditional boundaries of biological disciplines, e.g.
    - Instead of looking at single proteins in a signaling pathways, understanding how coordinate regulation of many proteins within a pathway give rise to observed biological behavior and context-dependent phenotype
    - How does tissue level function (such as shape) arise from molecular level phenomena in individual cells?
  - This focus on molecular mechanisms is one way in which systems biology seems to be different from traditional physiology (the jury is still out…).

Important Features of Any Experiment

- Experiments are expensive and time consuming
- A very important part of any experiment is having a clear and precise question (hypothesis) that the experiment is intended to address.
  – If I answer this question, will the answer be significant?
  – Will the answer lead to new knowledge that drives the field forward or accomplishes an important business goal?
- There are many possible questions, very few will be worthwhile to investigate!

Important Features of Any Experiment

- Given a specific question, one can then come up with answers to the following three key properties of the experiment:
  1. What biological system?
     - E.g. Do I look at human cell lines, a mouse, yeast, etc.?
  2. What perturbation/treatment conditions?
     - E.g. What compounds should I apply to the system to elicit a relevant response?
  3. What measurements?
     - E.g. What transcripts do I need to look at, and/or do I need to look at protein levels instead?
- Often (but not always), if you can't design an experiment that only has a handful of conditions and measurements, results may be difficult to interpret
  - Usually the question is too complex or not significant
  - Exceptions are sometimes screening-based studies, but those also typically have a specific question of interest

Important Features of Any Experiment

- Positive and Negative Controls
  - You never know when your experiment will fail, but at some point it **will** happen.
  - You will want to know why it failed when it does.
  - Therefore always include positive and negative controls, no matter how simple the experiment.
- Replicates
  - Surprisingly, many studies in biology do not perform a sufficient number of replicates.
  - Especially with quantitative data, one needs AT LEAST three replicates, to estimate the mean and standard deviation of the measurement, and do proper statistical inference or hypothesis testing
  - If the experiment is too expensive to do replicates, but still worthwhile to do, then careful consideration is needed about caveats.
    - E.g. How we will determine significance of the result?

Next Time—Biological Systems