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David Baker's Lecture Part 2:
Design of New Protein Functions

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Protein design, epitope-focused vaccine design, protein engineering

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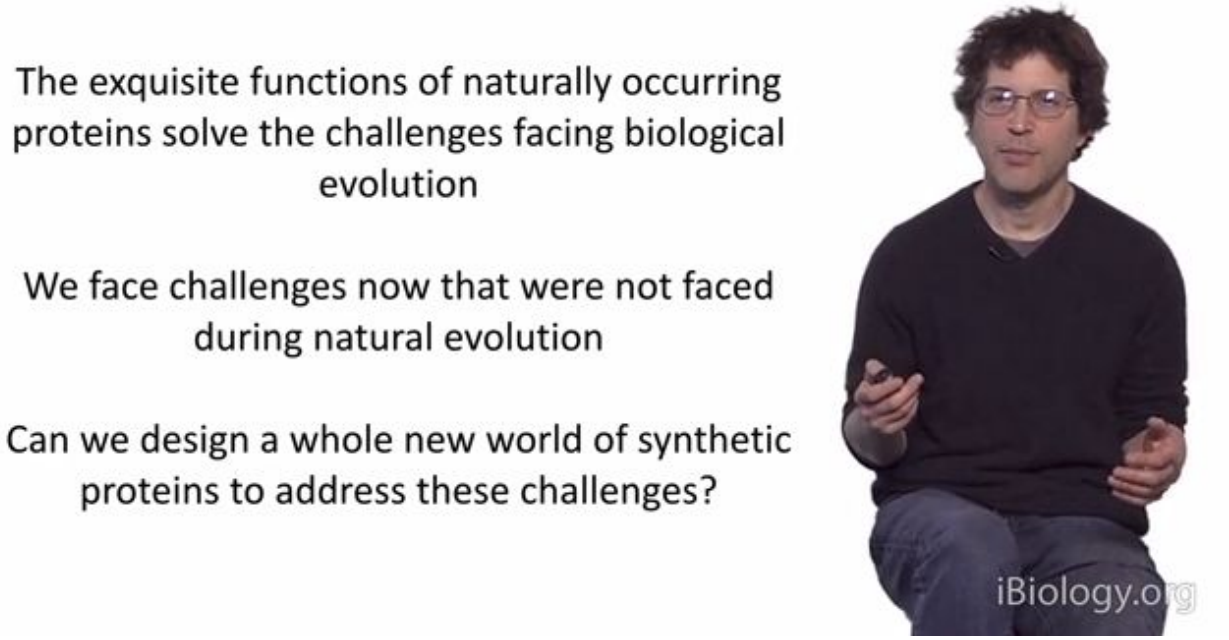
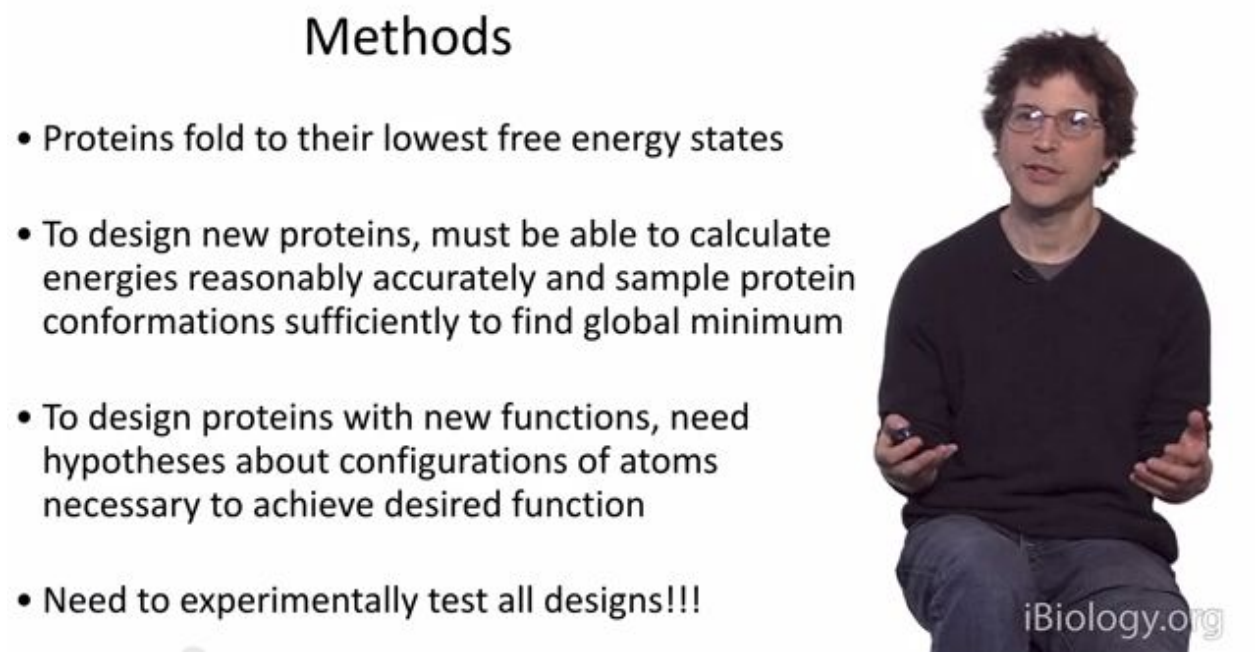
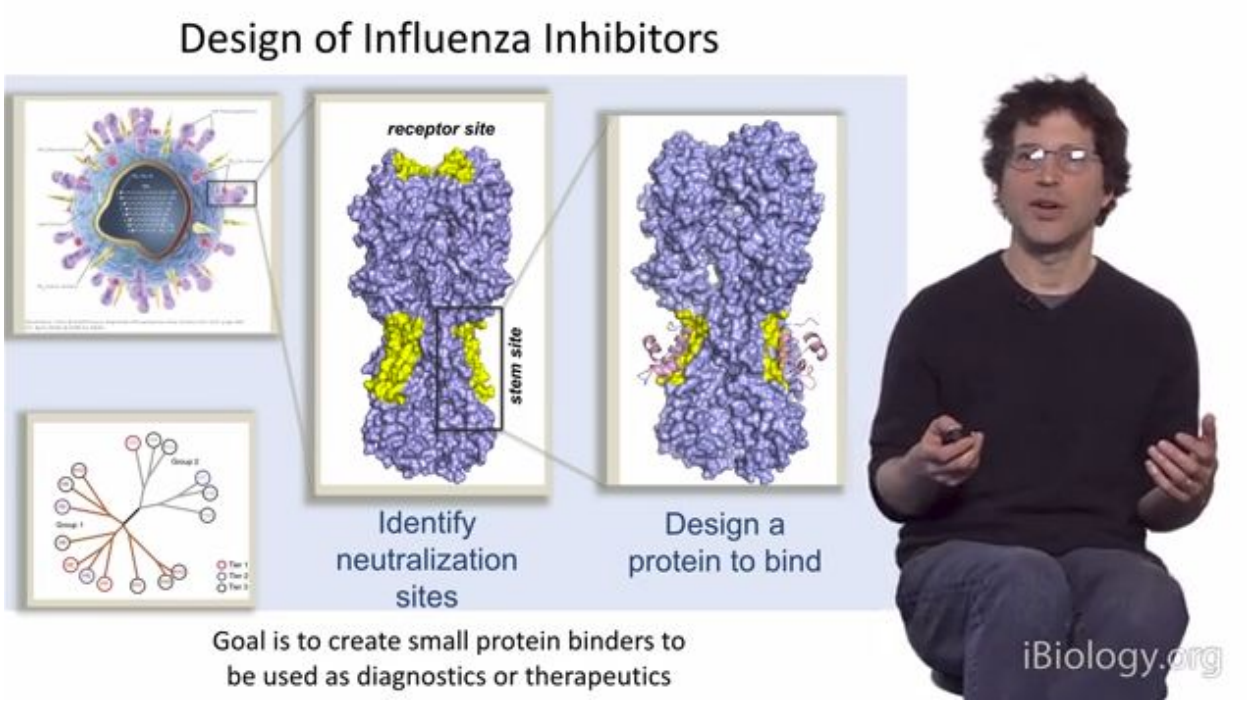


0:50—
Evolved proteins developed specific functions over time as determined by the forces of natural selection. To create proteins that tackle modern challenges we need a faster approach than evolution and one that selects for our desired functions.

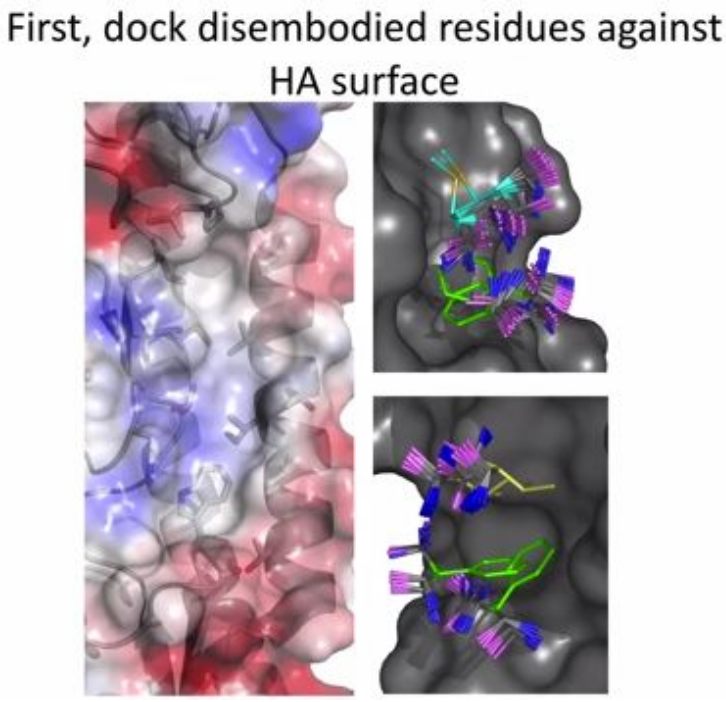


2:35—
Computational enzyme design can tackle this challenge by calculating the energies of various protein folding states.
Proteins are designed computationally and validated by experimental methods and structure determination.



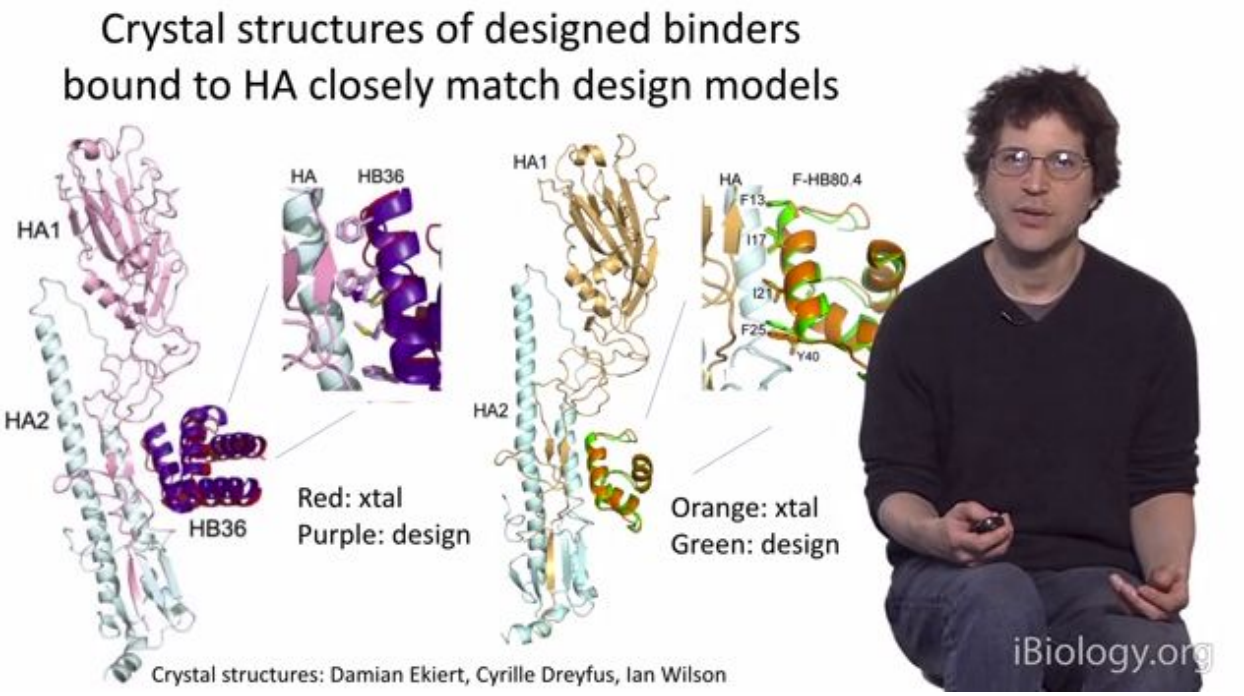
4:46—

Influenza epitope design:
2 parts of influenza hemagglutinin protein don't change: receptor site and stem site. We can design proteins that bind these regions to act as epitopes for antibody production.



6:06—

Influenza epitope design:
Binders to the HA site are designed by placing amino acids into a cavity on hemagglutinin and creating a backbone that holds these amino acids in place. Charged residues can help strengthen interactions.

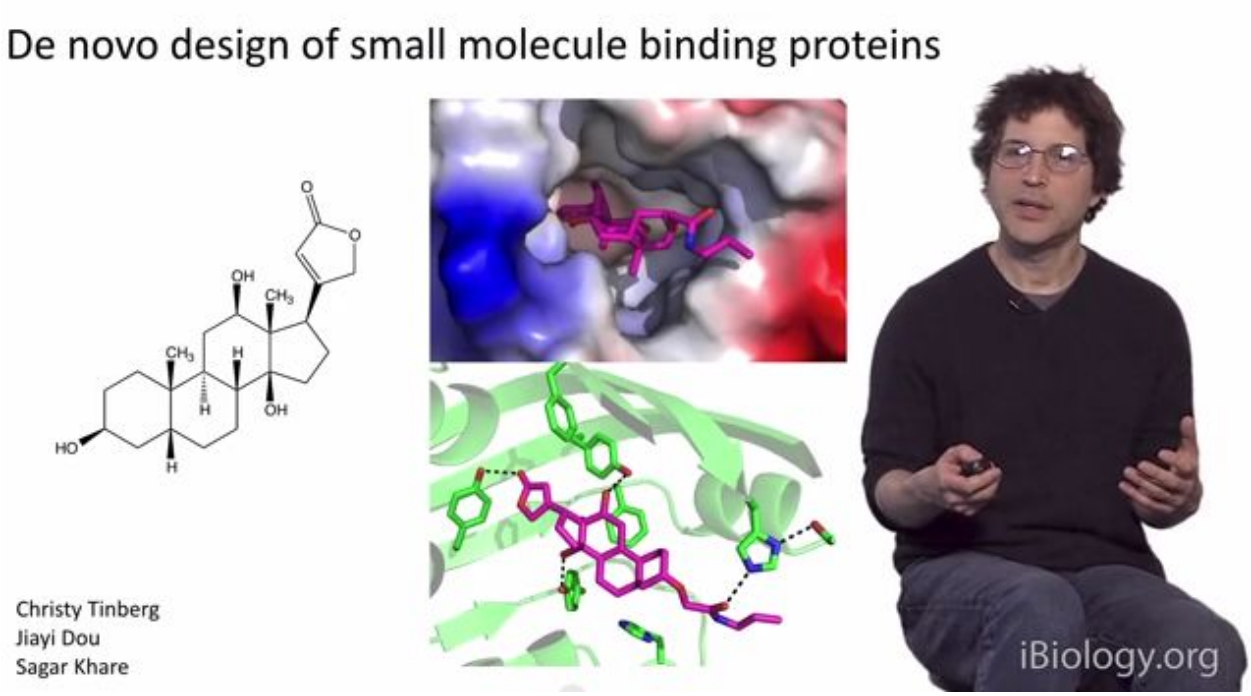


10:02—

Only a fraction of designs work when tested. This is because our calculations are not good enough to predict accurate folding.

When designs do seem to work we can validate the structure by crystallography. Here, crystal structures of a two of many HA designs seem to accurately match design models.

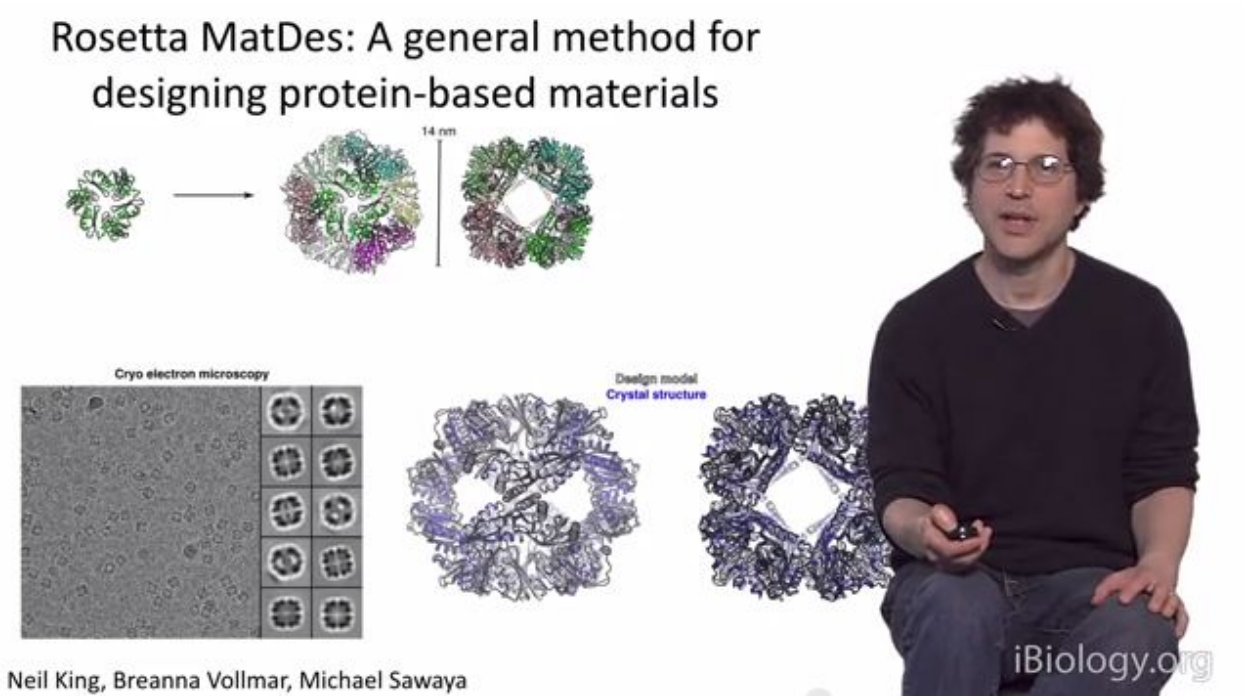
Function is validated in mouse models.



12:05—

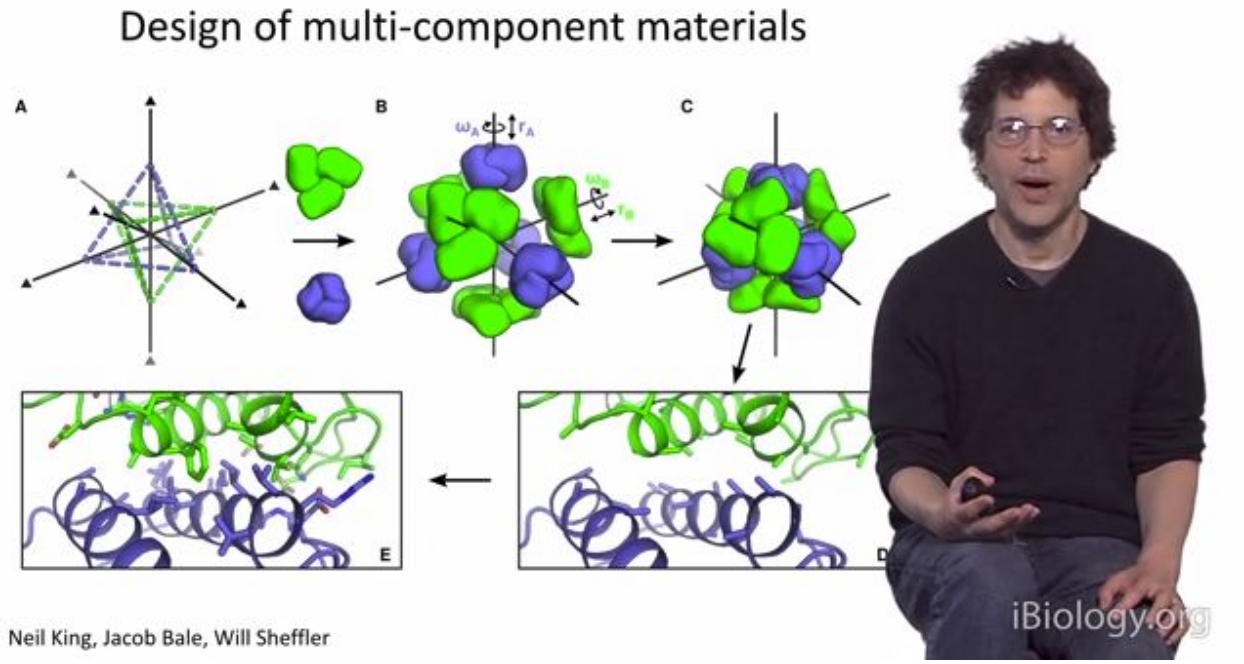
We can design proteins that bind small molecules in a similar way. These proteins act as biosensors that sense the presence and concentration of a small molecule. They can also sequester small molecules/drugs and thereby act as a “therapeutic sponge”.

Here, a small molecule DIG is bound by a protein (green).



15:18—

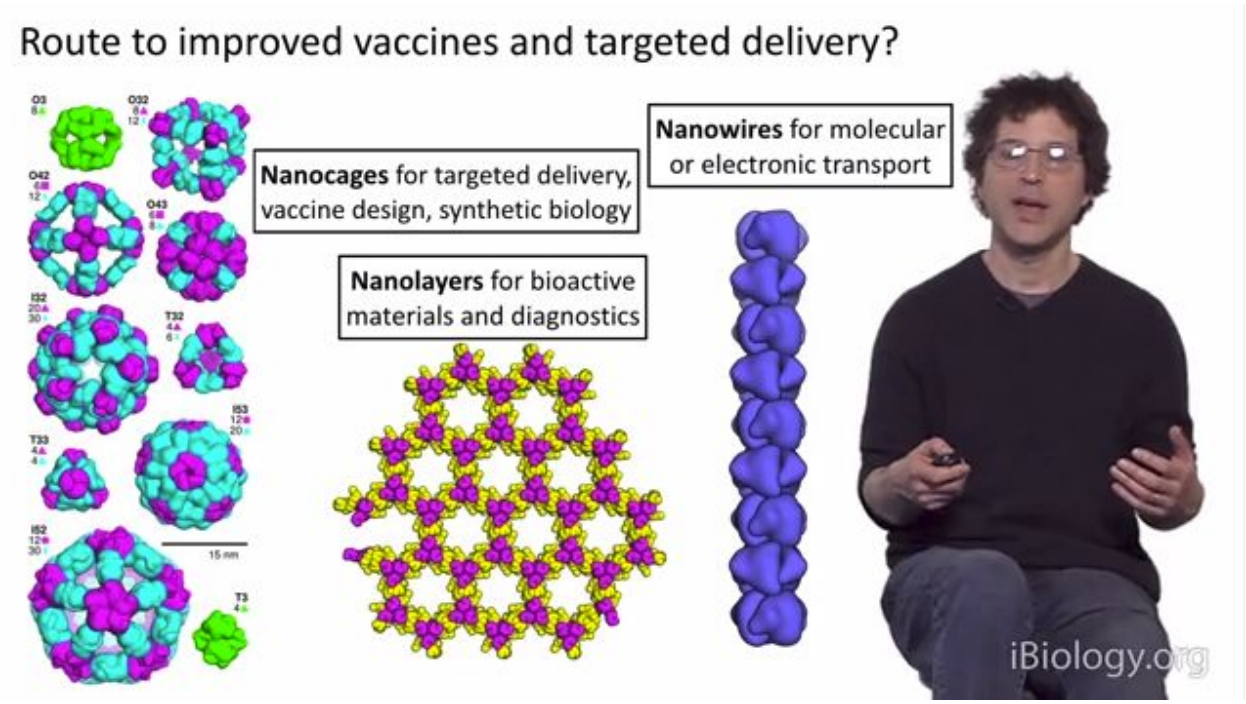
Self-assembling protein complexes can be designed in a number of shapes for nanomaterial productions.



19:15—

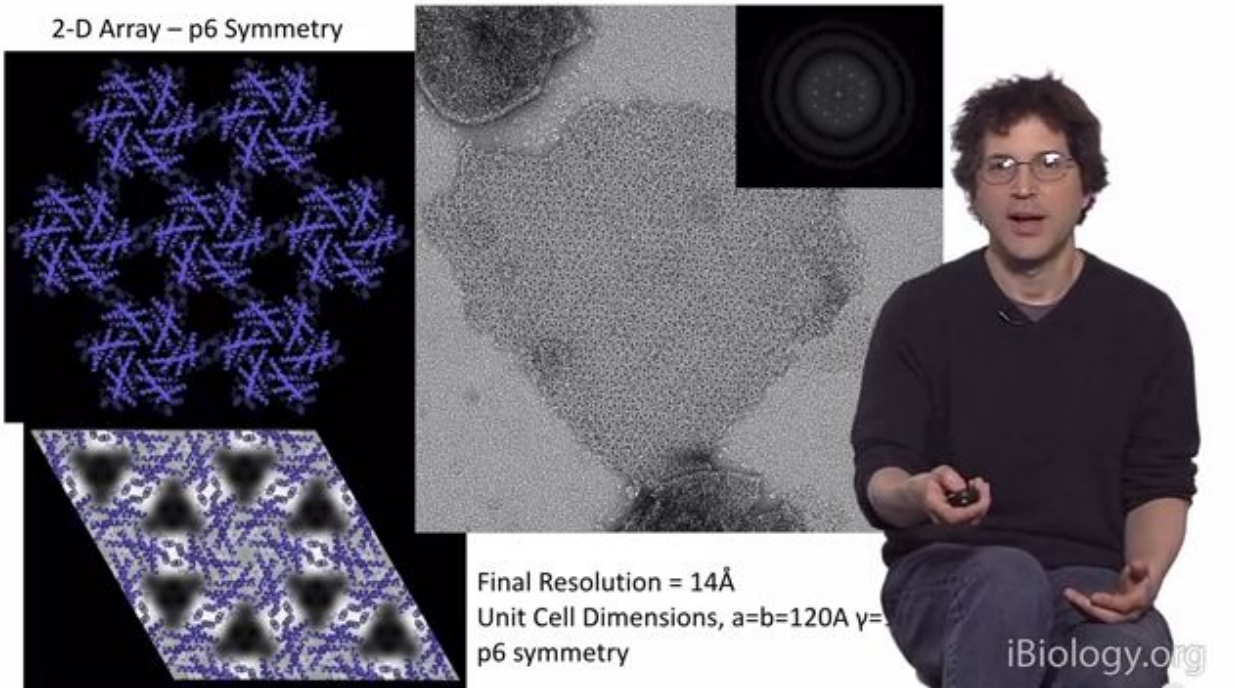
We can design symmetrical, three dimensional structures like a protein icosahedron.

First, protein shapes are designed to fit together. Next, sequences are designed to preferentially fold proteins into our desired shapes.



23:26—

We can design protein nanocages and nanotubes for many purposes, including the delivery of drugs, genetic material, and electron transport.



25:07—
Self-assembling proteins can be used to
design even larger structures. Here,
protein design was used to create a two dimensional
array that can be seen as a
protein wafer in the electron micrograph

3. Review Questions:

1. How does the Baker lab validate computationally designed proteins?
2. How are vaccines traditionally developed for viruses such as influenza? How does protein
3. design overcome roadblocks posed by traditional vaccine development?
4. What is the purpose of designing a small-molecule binder? What are challenges associated
5. with designing this kind of enzyme?
6. What are some applications for self-assembling protein nanomaterials?

4. Answers to Review Questions

1. Structure determination of a designed protein will tell us if the protein sequence folds correctly into the design structure. Experimental methods (i.e. in vivo testing of epitope-focused vaccine design) allow us to analyze the function of the enzyme and ensure that it fits the initial function of the design.
2. Vaccines are traditionally made for influenza by exposing humans to an attenuated or dead version of the virus to stimulate antibody production. However, the most exposed regions of the viral coat proteins are highly dynamic. Immunity does not outlast speedy viral evolution in these regions, and live virus exposure is often unsafe for infants, the elderly, and the immune-compromised. Computational enzyme design allows us to create epitopes matching the less-exposed regions of the virus that are resistant to evolutionary change. These can safely stimulate antibody production that should create long-lasting immunity.
3. By designing a small molecule binder we can create a biosensor for the presence of the small molecule. A high affinity binder can be used as a “therapeutic sponge”, i.e. a protein that can soak up excess drug molecules in vivo. The main challenge with designing a small molecule binder is to create high affinity bonds with a molecule that may not form many naturally strong interactions (i.e. many hydrogen bonds).
4. Self-assembling nanostructures can be used to create artificial viruses or cages that can be used for the delivery of drugs or genetic material to tissues.

5. Discussion Questions

1. Why can we not simply express a part of the HA protein to use as an epitope for antibody production?
2. Baker discusses a number of protein design projects: ligand-binding proteins, self-assembling proteins, repeat proteins, and other nanomaterials. How might these proteins be useful in other areas of scientific research, i.e. medicine or environmental science?
3. How can you increase the complexity of engineered nanomaterials? Why would you want to do this?

6. Answers to Discussion Questions

1. These proteins are complex and oftentimes regions that are close together in the protein structure are very far apart in the sequence and in the polypeptide chain. It is impossible to cut out a structure region of amino acids that are not connected.
2. One example might be to create a ligand-binding protein that can modulate the concentration of a drug in vivo. Another ligand-binding protein could function as a fluorescent biosensor for the presence of a toxic compound in drinking water supply, i.e. in lake water or groundwater. Self-assembling proteins might be used to build elaborate structures for the delivery of chemical and genetic therapeutics.
3. One way might be to increase the number of protein designs that assemble together, allowing for the creation of more complex shapes. More complex shapes would allow for the building of various scaffolds to bind to in vivo proteins and deliver small molecules/DNA. Another way to increase engineering complexity might be to design in binding sites for chemical interactions. This could allow for tethering of a small molecule or metal along the nanomaterial surface to orient drug delivery or catalytic surfaces.

7. Explain or Teach These Concepts to a Friend

1. Explain the differences between traditional vaccine development and computational epitope-focused vaccine design
2. Explain why only a limited set of protein designs are experimentally functional

8. Questions for Discussion Paper

Discussion Paper:

Correia, B. E. Proof of principle for epitope-focused vaccine design. 2014. Nature. 507: 201–206.

1. How does epitope-focused vaccine design work? What are the advantages of this method compared to traditional vaccine design methods?

2. Briefly describe the computational method of protein design discussed in this paper (Fold From Loops). How many FFL designs were ultimately chosen for filtering and human-guided optimization?

3. The authors then did an immunological evaluation of specific FFL designs they optimized. What evidence did the authors have to support and/or go against the clinical relevance of their designed FFL scaffolds?

4. What evidence did the authors have from their antibody characterization that their designed scaffolds can “re-elicit” neutralizing antibodies?

5. Do you think there is enough evidence supporting the efficacy of FFL scaffolds for use as a vaccine against RSV? If no, which additional experiments are required? Comment on the feasibility of this approach for developing vaccines against other viruses, i.e. HIV or Ebola.

9. Answers to Questions for Discussion Paper

1. Epitope-focused vaccine design involves designing an immunogen for a specific binding area of a virus—an epitope. The binding of the immunogen would lead to the induction of protective neutralizing antibodies. Many viruses have resisted vaccines designed by traditional methods even though such viruses have identified vulnerable epitopes. This is due to the variability amongst different strains of the same virus and thus difficulty in identifying a specific immunogen to target the epitope. The advantage of epitope-focused vaccine design would be the ability to design an ideal immunogen protein that would overcome this setback.

2. Once the epitope-antibody complex has been identified for the virus of interest, the Fold Form Loops (FFL) method selects the functional motif of the epitope and matches it up with its target topology. Next, backbone conformations are designed for the epitope that match the target topology. Low-energy amino-acids are then selected for the backbone conformation of interest. Finally, a series of filtering and optimization are carried out to identify the best designs that will be selected for use. (Also, refer to Figure 1)

3. The authors tested the binding of human sera from six RSV-positive patients and found that three sera reacted to one of the designed epitopes, FFL_001. This suggests that FFL_001 is clinically relevant for RSV. (Also, refer to Figure 3)

4. The authors analyzed the monoclonal antibodies generated by an immunized macaque with the FFL_001 epitope and found that two monoclonal antibodies (17-HD9 and 31-HG7) generated have high affinity for the scaffolding protein of FFL_001. They also saw that the two antibodies target the same helix-turn-helix compared to mota and pali (two control RSV antibodies). (also, refer to Figure 4)

5. This is an open-ended question up to the students. Possible experiments the students propose would be to take this vaccine to the clinical stage for testing in humans. Likewise, further experiments could be done in animals as well. The epitope-focused approach to designing vaccines would be especially suitable for diseases such as HIV, as HIV is highly antigenically variable. Ideal proteins would thus allow researchers to design a protein that will bind specifically to the epitope of a virus, ensuring that an immune response is elicited.