

## MODEL AND QUESTIONS Due March 6, 2008

See instructions on page 2 and 3

Project

3

## MODELING INSULIN-A PEPTIDE HORMONE

Using PDB coordinate data and toobers to model a protein

```
HEADER HORMONE 08-OCT-96 2HIU
TITLE NMR STRUCTURE OF HUMAN INSULIN IN 20% ACETIC ACID, ZINC-
TITLE 2 FREE, 10 STRUCTURES
COMPND MOL_ID: 1;
COMPND 2 MOLECULE: INSULIN;
COMPND 3 CHAIN: A;
COMPND 4 MOL_ID: 2;
COMPND 5 MOLECULE: INSULIN;
COMPND 6 CHAIN: B
SOURCE MOL_ID: 1;
SOURCE 2 ORGANISM_SCIENTIFIC: HOMO SAPIENS;
SOURCE 3 ORGANISM_COMMON: HUMAN;
SOURCE 4 MOL_ID: 2;
SOURCE 5 ORGANISM_SCIENTIFIC: HOMO SAPIENS;
SOURCE 6 ORGANISM_COMMON: HUMAN
KEYWDS INSULIN, HORMONE, GLUCOSE METABOLISM
EXPDTA NMR, 10 STRUCTURES
AUTHOR Q.X.HUA,S.N.GOZANI,R.E.CHANCE,J.A.HOFFMANN,B.H.FRANK,
AUTHOR 2 M.A.WEISS
REVDAT 1 01-APR-97 2HIU 0
SPRSDE 01-APR-97 2HIU 1HIU
JRNL AUTH Q.X.HUA,S.N.GOZANI,R.E.CHANCE,J.A.HOFFMANN,
JRNL AUTH 2 B.H.FRANK,M.A.WEISS
JRNL TITL STRUCTURE OF A PROTEIN IN A KINETIC TRAP.
JRNL REF NAT.STRUCT.BIOL. V. 2 129 1995
JRNL REFN ASTM NSBIEW US ISSN 1072-8368
REMARK 1
REMARK 1 REFERENCE 1
REMARK 1 AUTH Q.X.HUA,S.E.SHOELSON,M.KOCHOYAN,M.A.WEISS
REMARK 1 TITL RECEPTOR BINDING REDEFINED BY A STRUCTURAL SWITCH
REMARK 1 TITL 2 IN A MUTANT HUMAN INSULIN
REMARK 1 REF NATURE V. 354 238 1991
```

Human insulin (PDB ID: 2HIU)<sup>1</sup>

Right: Structure data and information from the PDB website  
Below: Wireframe model of human insulin.

Images produced by T.Hata using PyMol Molecular Graphics System<sup>2</sup>

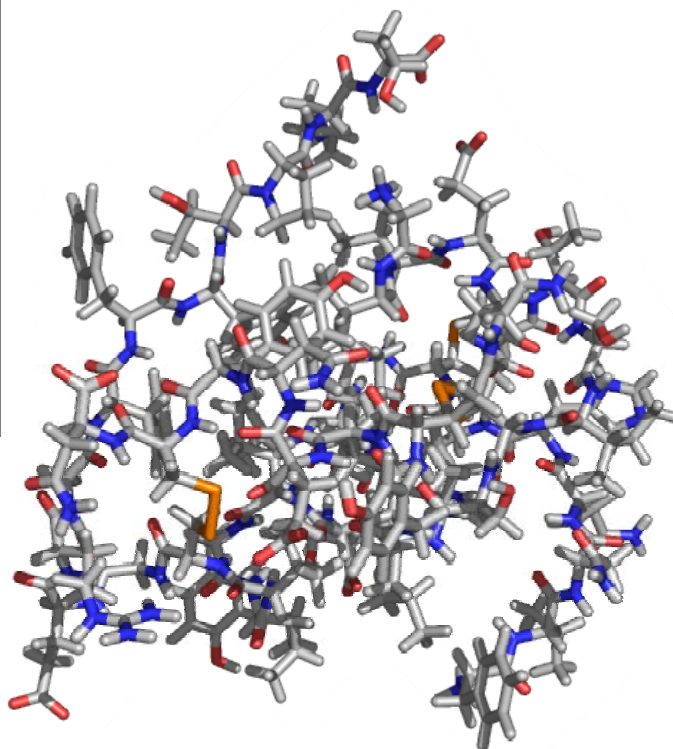
Chain B (polymer 2)

Description: INSULIN  
Chain Type: polypeptide(L)  
UniProt reference: P01300 [show this sequence below]  
Length: 30 residues  
SCOP domain assignment [hide] [reference]: Insulin: 30 residues  
DSSP secondary structure [hide] [reference]: 48% helical (1 helices; 12 residues); 3% beta sheet (1 strands; 1 residues)  
More annotations: Select

SCOP: [Diagram showing a blue bar representing the protein structure]

DSSP: [Diagram showing a red wavy line representing the secondary structure]

PDB: FVNHQLCGSHLVEALYLIVGERGFFYPCT  
PDB: 1 10 20 30



# The Pingry School Biology Honors Projects

[http://www.pingrybiology.com/honors\\_projects.htm](http://www.pingrybiology.com/honors_projects.htm)

# Introduction

## **Building an insulin model**

In Project 2 we discussed how the pro-insulin protein is processed through the endoplasmic reticulum, sorted through the golgi, and secreted from pancreatic cells as a two-chain protein-hormone. This protein is released in your body in response to the food you consume so that sugar levels in your blood can be regulated. Insulin initiates a cascade of events that allow cells to activate transmembrane channels that allow glucose into the cell to be metabolized (think cellular respiration).

You will now combine your ability to look up protein sequence data with using visualization websites to fold a toober into a scaled model of the insulin hormone. You will work with another student to complete this project. You may need to consult instructions on Project 2 on how to utilize tools on the PDB website to guide you through this Project.

Follow directions carefully. On March 6, 2008 (Thursday), your group will submit the following:

1. A single insulin model for your group.
2. A single insulin "protein map" prepared by your group.
3. Individual written (typed) responses to the questions at the end of this Project. This is NOT group work. It should be completed individually.

**The project should be submitted by 3:00PM to the Biology office in room 110. Late projects will NOT be accepted and both students of the group will be failed for the project. It is BOTH of your responsibilities to submit the project.**

**Follow these directions when preparing your individual written answers:**

- The answers you submit should have the following information on the top of the first page: your name, teacher's name, class period, and the name of your partner. On each additional page (if needed), put your name and your teacher's name on the top right corner (if using MS Word, use the "header" function).
- All numbered questions on this project should be typed on a separate sheet to submit along with the model.
- Type out the question first, then answer in complete sentences whenever appropriate. Answers should be double-spaced.
- Each student is responsible for his/her responses.
- The written portion is to be completed individually without aid from your partner.
- These responses must be submitted on hard-copy along with the model on March 6.

# Module 2.4

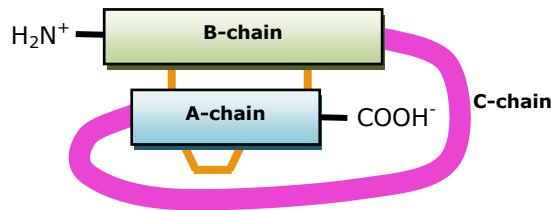
## Building an insulin model

Recall the diagram below. It shows how proinsulin, created by the ribosome, is “processed” through the endoplasmic reticulum and golgi into mature insulin. Mature insulin is a two-chain protein held together by disulfide bonds.

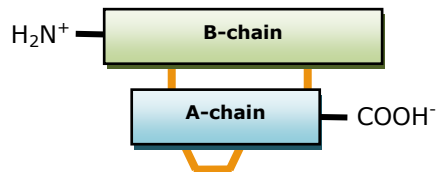
### Proinsulin as produced by the ribosome



↓ **Processing in the ER:** cleaving of signal peptide, folding, disulfide bridge formation.



↓ **Processing in the Golgi:** Transport vesicle transports to Golgi. Within Golgi and the transport vesicle moving towards the cell membrane, enzymes cleave off C-chain to create mature, active insulin.



↓ **Exocytosis out of the cell:** Transport vesicle from Golgi fuses with cell membrane and releases insulin out of the cell.

**Figure 1: Insulin production and secretion.**  
A simplified diagram of insulin “modification” before secretion out of a pancreatic cell.

## Individual work:

### Review the Insulin module on the Molecule of the Month feature on the PDB website (module for Feb 2001).

Look up the PDB file 2HIU.

1. Mature insulin is made up of two chains. How many residues is the A chain? The B chain?
2. Using the PDB "header" file, determine the amino acid sequence of both A and B chains of insulin. Write this sequence out using the 3-letter amino acid abbreviation.
3. Which regions of both chains take on an  $\alpha$ -helices secondary structure? Answer in the following format (fill in the spaces): Chain \_\_, residues \_\_ to \_\_, etc.

Open up the PDB file 2HIU using FirstGlance in Jmol at

<http://molvis.sdsc.edu/fgj/index.htm>. Adjust the view to show the backbone in "Vines..." view and remove the sidechains. Recall that each "kink" or "bend" in the main chain is the alpha-carbon of the amino acid (the carbon that the sidechain attaches to). By clicking on these bends, you should be able to identify the residue ID and its position on the chain.

4. What do the yellow lines connecting the two main chains represent?
5. With your image showing insulin in "vines" view without sidechains, screen-capture the FirstGlance window, crop out the insulin model, and insert the picture into your answer sheet. Using text boxes and arrows, identify the A and B chains. (You can crop images using Photoshop, Microsoft Image Editor, or any other software).
6. In the 1970's, diabetic patients requiring insulin supplements had to use either porcine or bovine insulin (pig or cow) usually collected from slaughtered animals. According to the Molecule of the Month module on insulin, there are small structural differences between these two animal insulins and human insulin. Considering that we have always discussed the significance of structure to function (how altering structure can alter function), why do you think these slight variations of insulin was still effective in humans? Name and describe two (2) disadvantages to using porcine or bovine insulin.
7. In 1982, Genentech (the first "biotechnology" company) got FDA approval to market the very first "recombinant DNA-based" product to market. Scientists at Genentech cloned the human insulin gene from human cells and developed a way to express the

protein in microorganisms that could be grown in large quantities for pharmaceutical production. Name and describe two (2) advantages to cloning human genes to produce recombinant DNA products for pharmaceutical use.

8. The scientists at Genetech had a number of obstacles before successfully expressing human insulin in a transgenic organism. What modifications had to be made to the human insulin gene before it could be cloned and expressed in another organism? Why? Research and identify the current methods used to create recombinant human insulin. What microorganism is used? What are the advantages of using this organism? Starting with an isolated copy of the human insulin gene, briefly describe (a bulleted flowchart is acceptable for this question) how recombinant human insulin is produced.

**Group work:**

Follow the next directions to create a physical model of insulin using the mini-toobers provided in class.

You should have received the following supplies in a bag:

- Two toobers precut to the scale of 2 cm per amino acid
- Two sets of blue and red caps to place on the amino and carboxyl ends of the peptide chains (figure out which colors belong on which ends of your chains)
- Three linkers to connect the two chains

Cut two 8½ x 11 blank sheets of paper (computer or printer paper will work) length-wise into three strips:

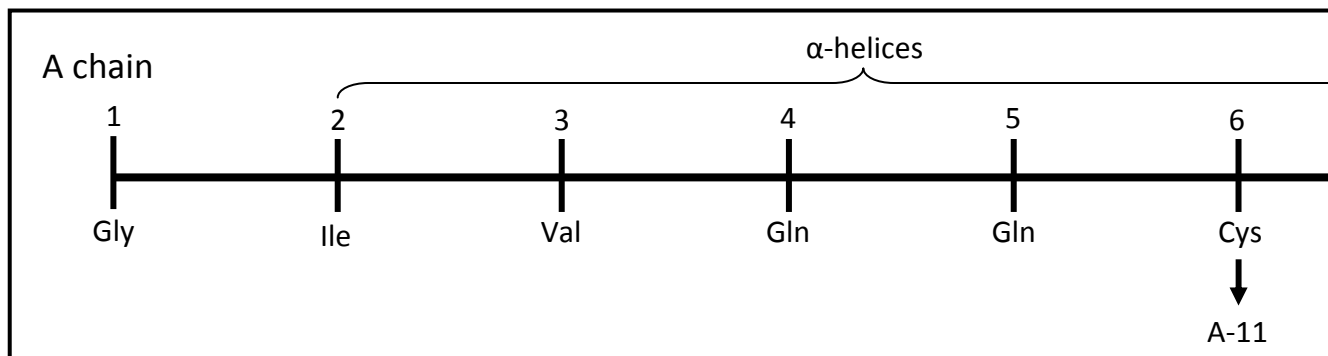


Connect two of these strips together to make one piece and three strips to make a second.



You will draw a scaled “map” of the two insulin chains onto these strips of paper. Using a ruler, draw a straight line using a scale of 2cm per amino acid. Draw this line near the center of the paper since you will be making additional marking on the top and bottom of this line. Make marks at every 2cm and label the two strips as chain A and B.

At each 2cm mark, both number and identify the appropriate amino acid in the chain. Above the line, label any secondary structure characteristics appropriate for the region. Below, mark significant tertiary interactions an amino acid may be making (mark the pairing amino acid by the chain ID and residue number).



You will use this "map" to fold the toobers into an accurate, scaled model of insulin.

Begin by carefully transferring the diagrammed scale onto the surface of the toober using a finepoint Sharpie marker or another permanent pen. You were provided with an excess length of each toober; carefully trim off segments that are unnecessary. For the regions with a secondary structure, simply coil or bend the backbone accurately to represent the structure and scale of the protein. In the other regions, kink the tuber every 2cm to represent the alpha-carbon of each amino acid. Using FirstGlance in Jmol, fold the tuber and use the appropriate linkers and caps to represent the protein's characteristics.

Through the previous Honors Projects, you have gained familiarity with resources that will further enable your understanding of insulin structure and function. It is up to you and your partner to decide what resources to use to guide your toober folding.

**Model grading criteria:**

- Accurate, scaled amino acid map created.
- Alpha-carbons should be visible as kinks on backbone where appropriate.
- Alpha-helices should be visible and coiled with uniform diameter and in correct "handedness".
- Disulfide bridges should be correctly positioned.
- The spatial positioning of the free "ends" should be accurate relative to each other and to the secondary structure motifs.

# Notes and Bibliography

1. PDB ID: 1HIU  
Hua, Q.X., S.N. Gozani, R.E. Chance, J.A. Hoffmann, B.H. Frank, and M.A. Weiss. Structure of a protein in a kinetic trap. *Nat.Struct.Biol.* **2** 129-138 (1995)
2. DeLano, Warren L., "The PyMOL Molecular Graphics System." DeLano Scientific LLC, San Carols, CA, USA. <http://www.pymol.org>
3. Research Collaboratory for Structural Bioinformatics. Berman H.M. , J. Westbrook, Z. Feng, G. Gilliland, T.N. Bhat, H. Weissig, I.N. Shindyalov, and P.E. Bourne. The Protein Data Bank. *Nucleic Acids Research*, **28** 235-242 (2000).
4. Jmol. <<http://www.jmol.org>>
5. Martz, E. FirstGlance in Jmol (2005, 2006). <<http://firstglance.jmol.org>>

The Pingry School Biology Honors Projects were developed and written by Tommie S. Hata during the 2003-2004 school year and edited each year by the biology teachers. The Projects were rewritten in 2006-2007 to reflect current findings in biology and to better reflect topics that we believe is important for our students. The Honors Projects will not be possible without the help from and dedication of the other Pingry biology teachers who continue to offer ideas and suggestions. A special thank you to Deirdre O'Mara for all the input and editing. Thank you also to Dr. Tim Herman, Dr. Mike Patrick, and others at the Center for Biomolecular Modeling at MSOE and the many other scientists that continue to provide us with the technical and intellectual support to make the Projects possible.