



# Improving Contraception and Fertility Treatments Through Identification of Proteins Proximal to the human FSH Receptor

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# Follicle Stimulating Hormone



- FSH is made in the Pituitary
- Plays a crucial role in human reproduction
  - Folliculogenesis
  - Spermatogenesis
- Improper FSH signaling can cause infertility

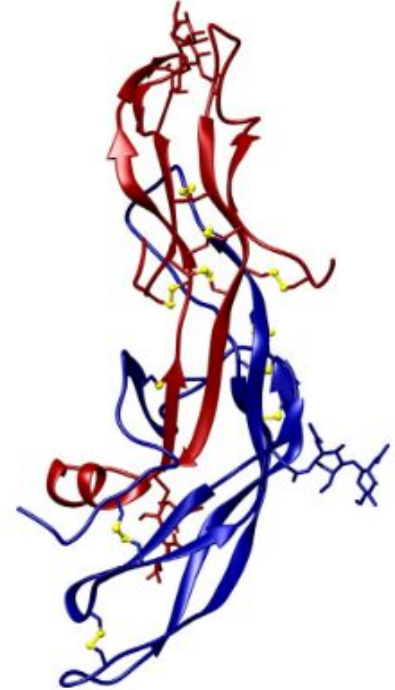


Figure 1: FSH Ribbon Structure<sup>1</sup>



# FSH Receptor



- FSH cannot act without a receptor
- G-Protein Coupled Receptor (GPCR)
- Seven transmembrane domains and extracellular binding pocket
- Binds FSH and triggers signaling cascade in the cell

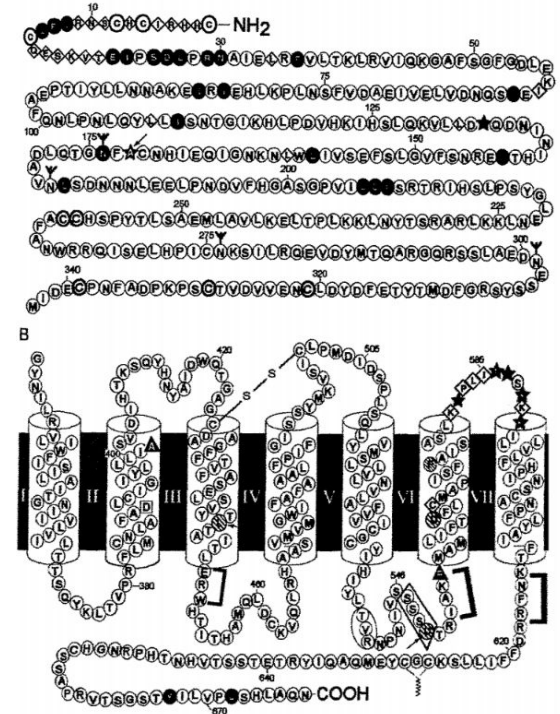


Figure 2: Amino Acid Structure of hFSHR<sup>2</sup>



# The Receptor Is Not Alone



- Thousands of proteins work together

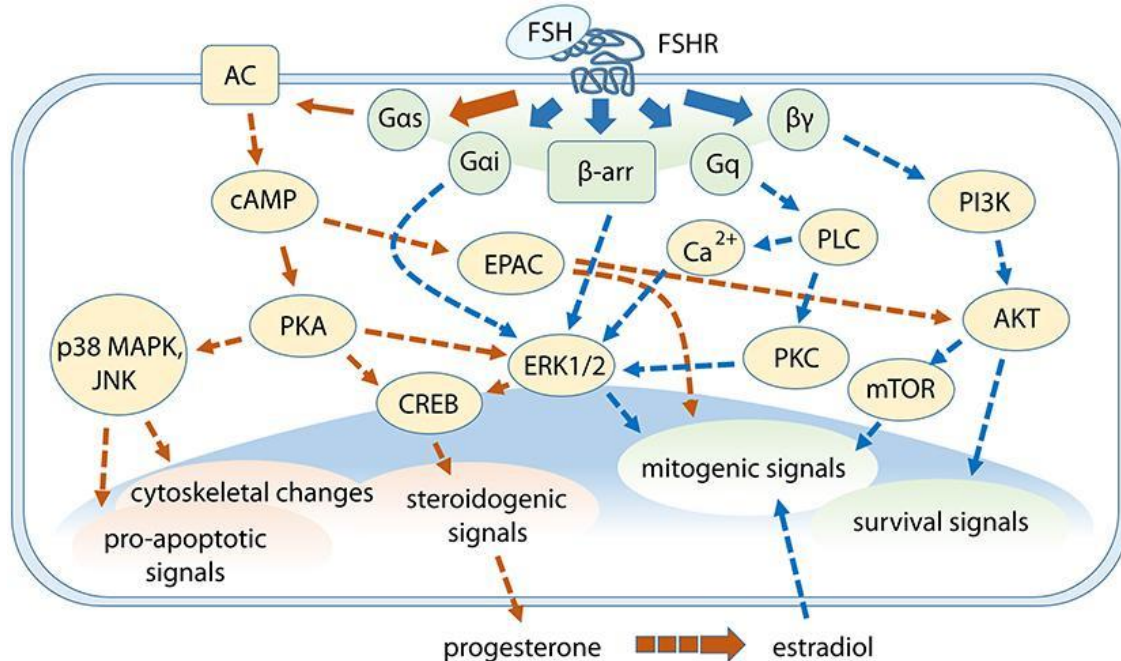


Figure 3: FSHR Intracellular Signaling Pathway<sup>3</sup>



# Finding These Proteins



- One method is immunoprecipitation
  - Tag receptor, attach antibody, and examine proteins
  - Captures a snapshot of receptor action
- Not a full picture
  - Have to target specific proteins
  - Some interactions are too weak or too short
  - Cannot differentiate between presence and lack of FSH
- Need something more encompassing and dynamic





# APEX



- Focus shifted from bound proteins to nearby proteins
- More proteins obtained this way
- Presence and Lack of ligand can be tested for

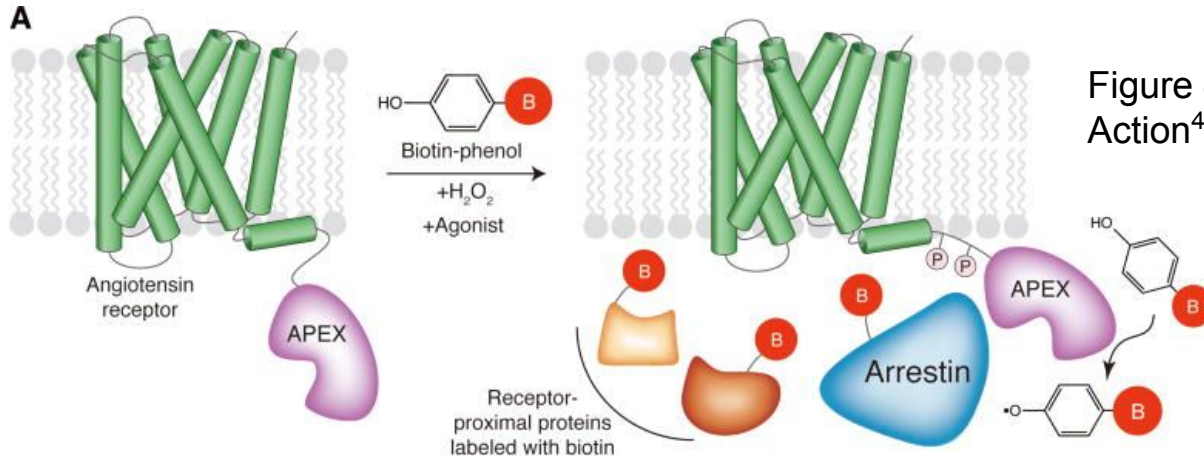


Figure 4: Illustration of APEX Technique in Action<sup>4</sup>



# Cell Preparation



- Recombinant DNA for FSH Receptor expressing the APEX tag was created
- Stably Transfected into Human Embryonic Kidney cells (HEK293)
- Resulted in a cell line that expresses the APEX tagged FSH Receptor

Thank you very much Alex Temple!





# Hypothesis

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The APEX technique can be used to identify the interacting proteins of the FSH Receptor.





# Methods

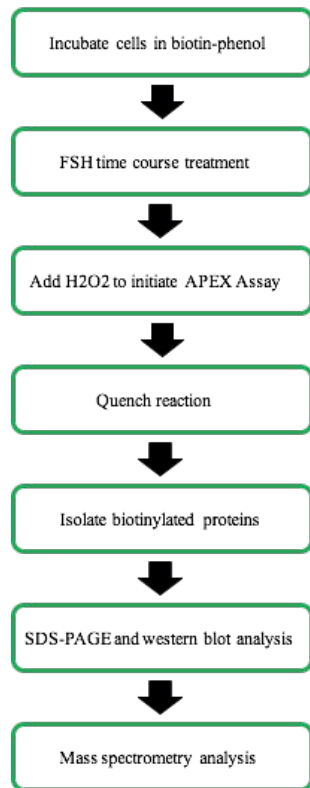


Figure 5: APEX Methods Flow Chart<sup>5</sup>

Proximity-labeling experiment

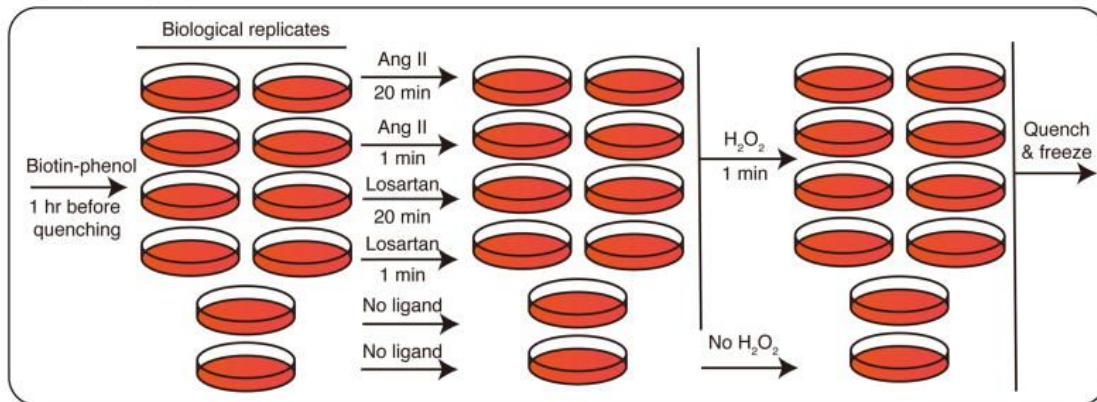


Figure 6: APEX Biotin Labeling Experiment<sup>4</sup>





# Results

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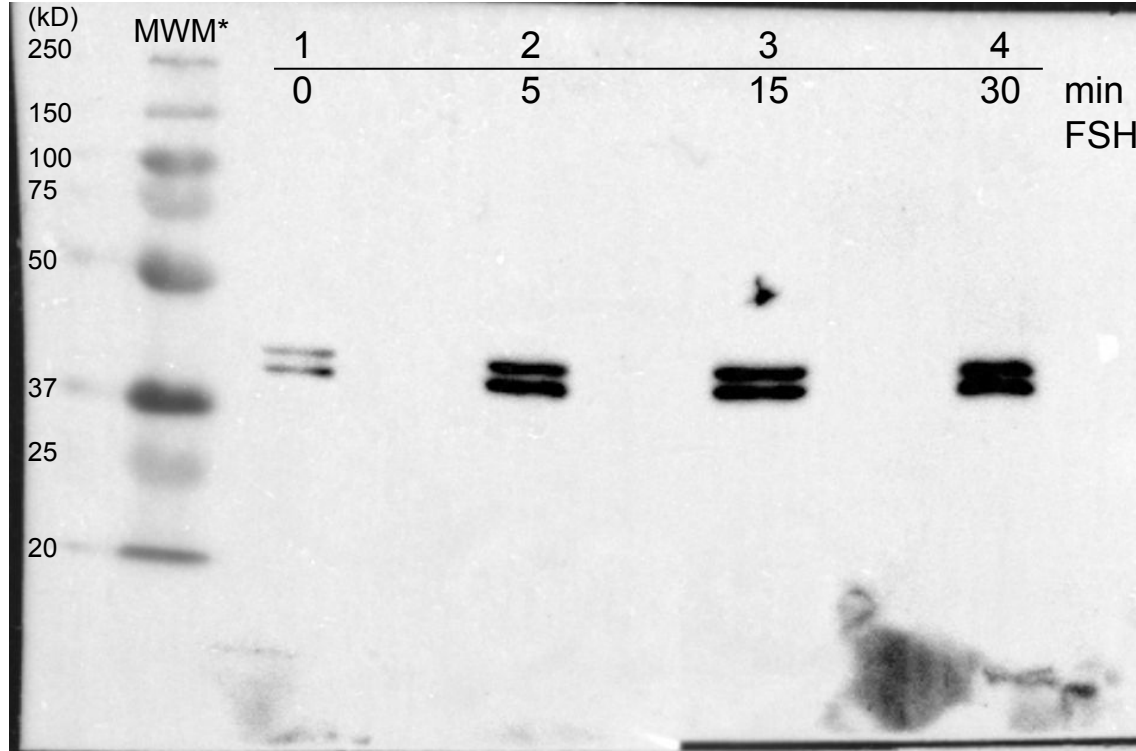


- The mutated receptor functions as normal
- The APEX reaction can successfully biotinylate proteins





# Functioning FSH Receptor



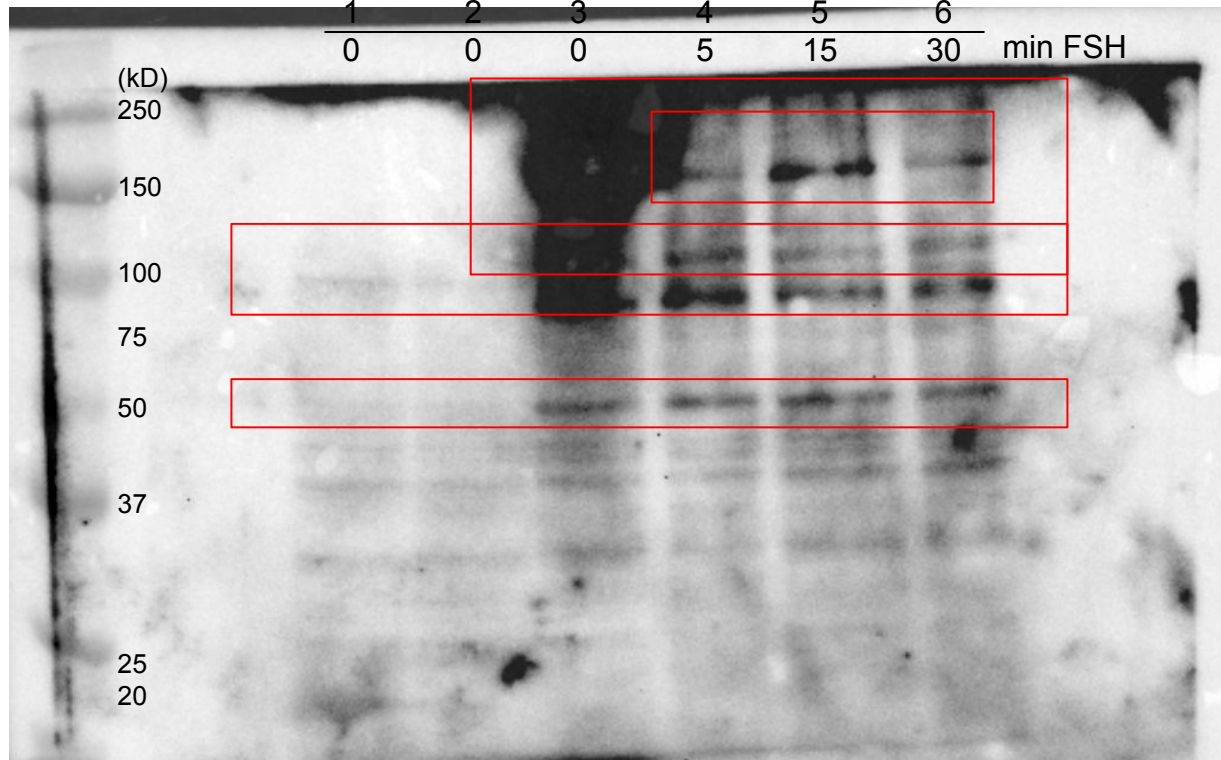
**Figure 7: p44 MAPK Presence as an Indicator of FSHR Function**  
The dark bands for samples 2-4 represent the protein p44 MAPK, a known protein involved in FSHR function. Thus, there is clear evidence that the FSHR-APEX complex is functioning as expected.

\*MWM stands for Molecular Weight Marker





# APEX Biotin Labeling



## Figure 8: APEX Biotin Labeling Yields Many Proteins Captured

The first two wells contain control, and from left to right, time with FSH present increases. There are more bands present in the experimental wells, as well as greater intensity of some bands in the experimental relative to the controls.



# Discussion



- I strove to, and succeeded, in performing the APEX technique with the FSH Receptor
- Targeting these proteins presents a new way to affect hormone signaling
  - Shown to have the same effectiveness with reduced side effects compared to traditional treatments
  - Huge potential to affect infertility treatments as well as contraceptives





# What Proteins Can Do For You



- In Vitro Fertilization (IVF) is currently very expensive due to the cost of manufactured FSH
  - Could replace FSH with more cost effective and specific activators
- Treatment for FSH Receptors with non-functional binding pocket
  - Subvert need for FSH and directly activate the Receptor





# What Comes Next?

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- Purification and Identification is the next step
- Comparison to known proteins
- Eventually, finding ways to target the proteins found





# Acknowledgements

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# References



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# Thank you!

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I hope you enjoyed it! Have a great day!



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