Bacterial Chemotaxis

Using mutagenesis to assist in NMR assignment of chemotaxis receptor

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Biochemistry

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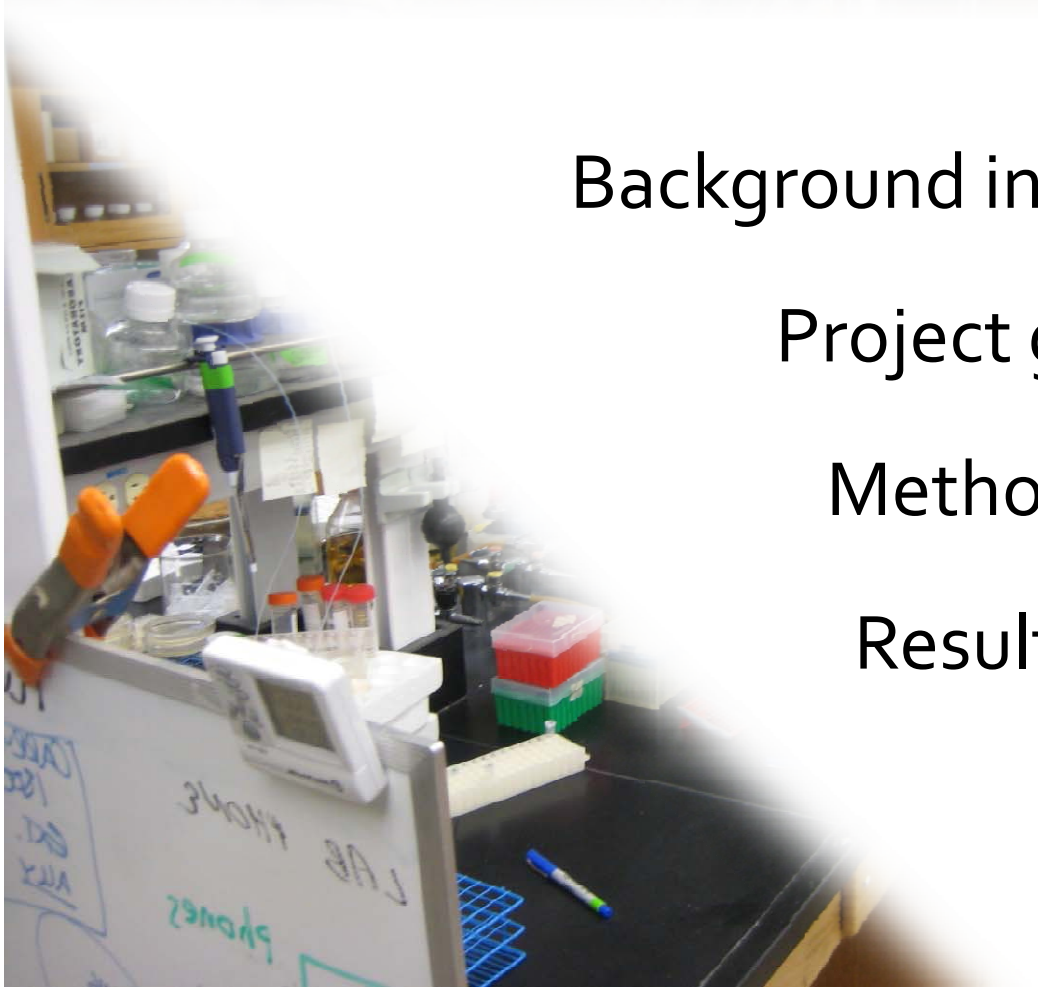
UCSB

Presentation Outline



Background information

Project goal

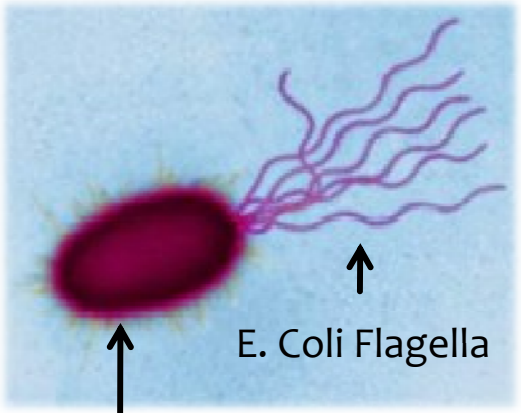
Methods

Results

Bacterial Chemotaxis

What is chemotaxis?

Movement of bacteria towards or away from chemical stimuli.



Gather knowledge

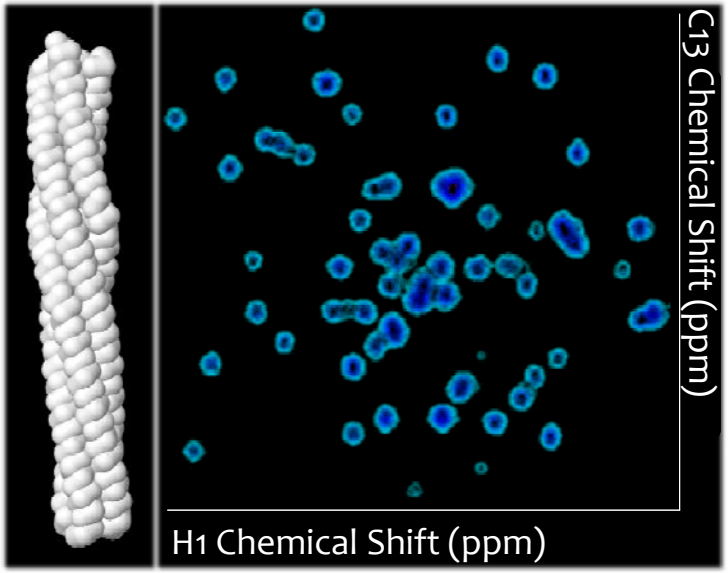
Applications

Prevent/inhibit diseases

Chemotaxis receptor;
TM14 Brian R. Crane et. al.

Project Goal

To create a single mutation in the chemotaxis protein receptor to assist in methyl side chain assignment of the receptor protein.



Sample 2D NMR spectrum

Chemotaxis receptor TM14; http://www.pdb.org

Project Goal

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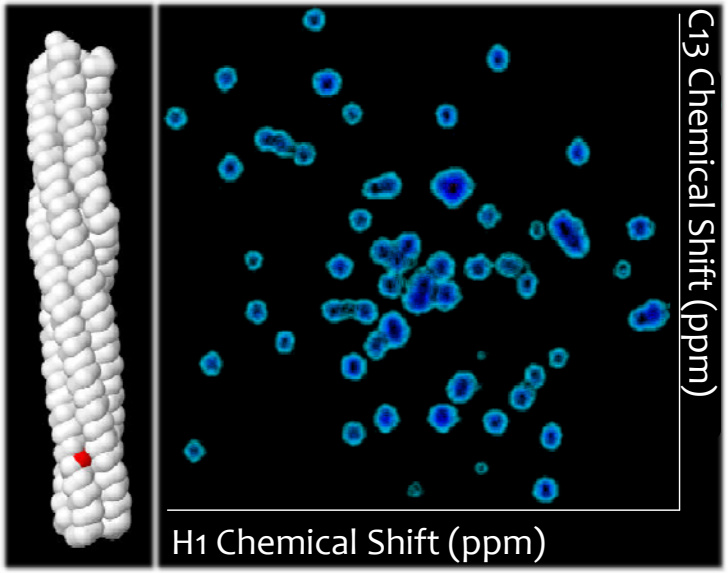
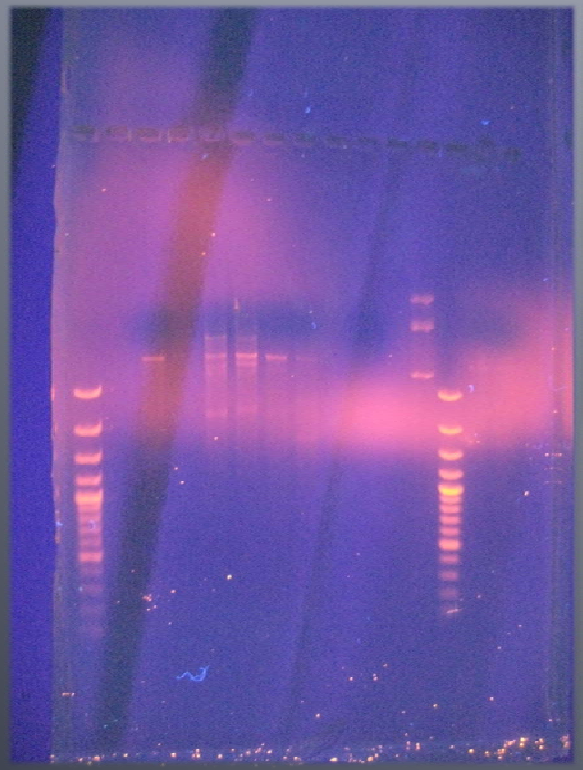
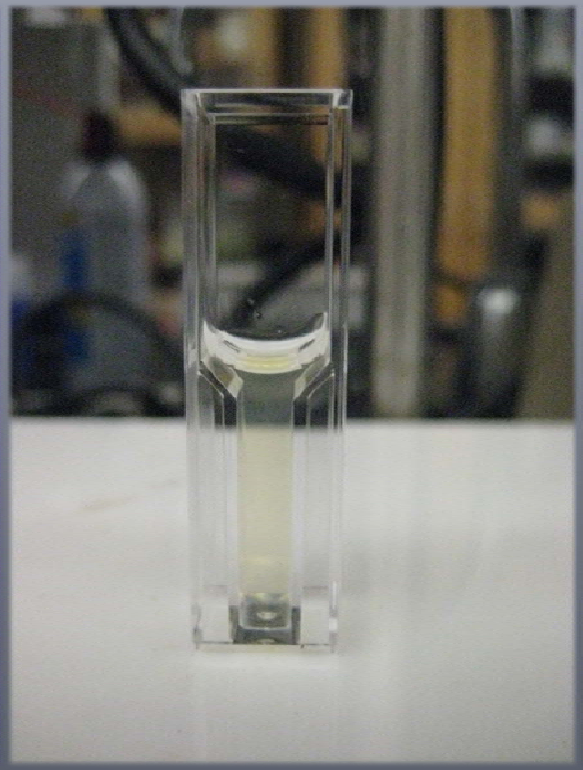


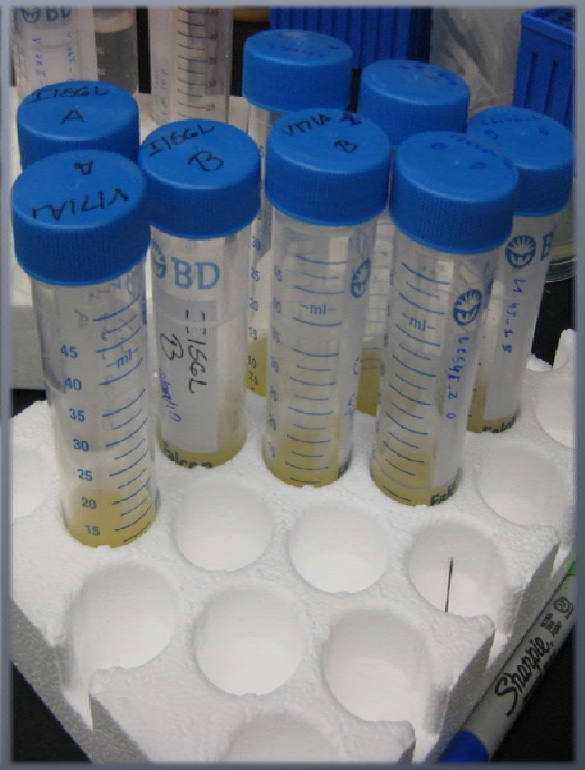
Sample 2D NMR spectrum

Chemotaxis receptor TM14; http://www.pdb.org

Research Methods





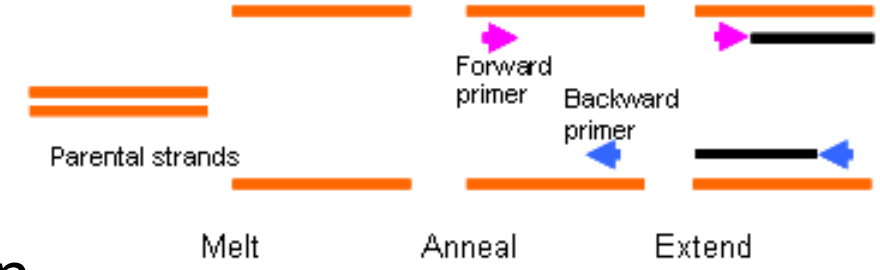


Various pictures from our lab at UCSB

Mutagenesis

Create mutant receptors using PCR

- Custom primers
- Single nucleotide mutation
- Single amino-acid mutation
- Ex: TTA to ATA (Leucine to Isoleucine)



PCR in our lab

PCR diagram: http://www.obgynacademy.com/

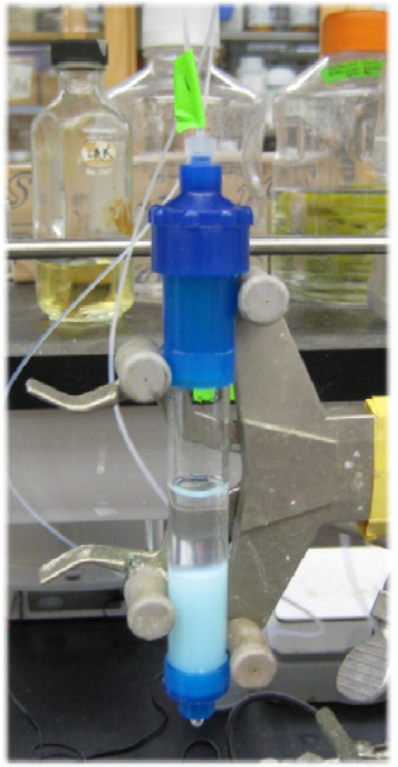
Expression and Purification

Protein expression

- Expression =
  Production of desired protein
  by the bacteria.

Protein purification

- Using nickel column to bind protein.
- Elute protein from column using
  another chemical that competes
  for nickel binding.

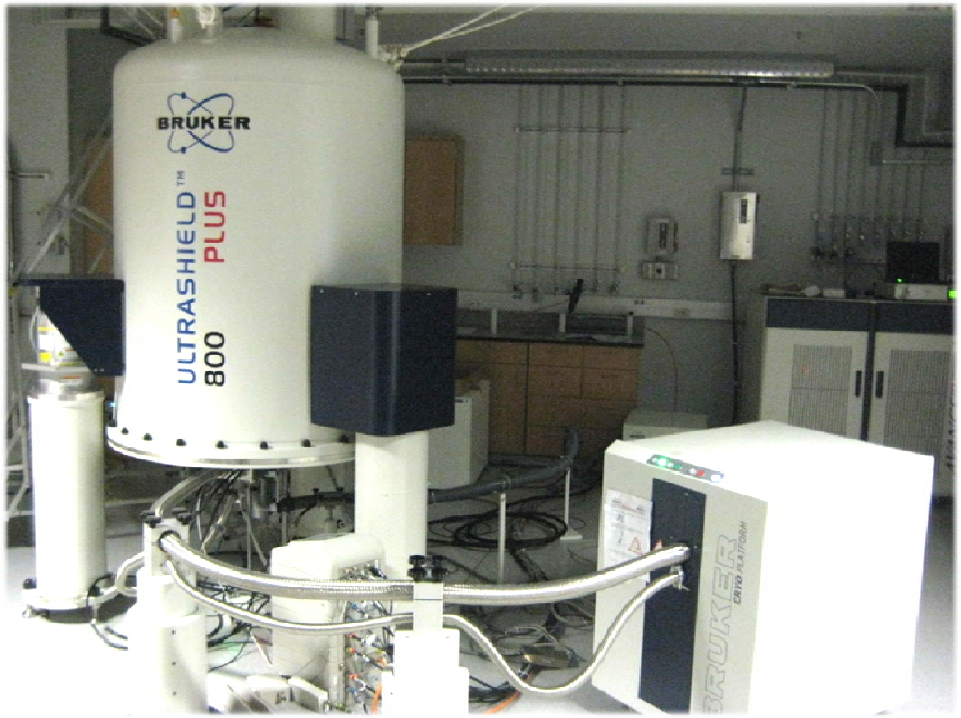


Nickel column in our lab

Nuclear Magnetic Resonance

Obtain NMR spectrum

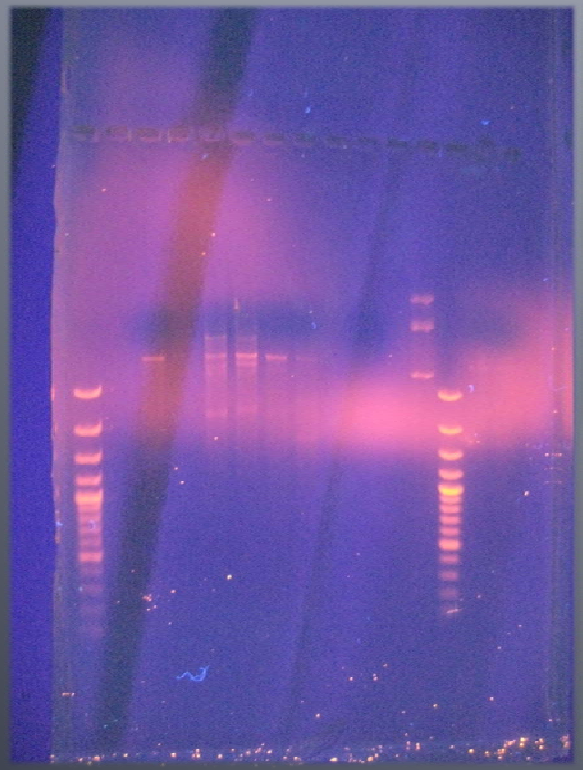
- 2D $^{1}H - ^{13}C$ NMR
- Each nucleus will give a different signal based on its environment

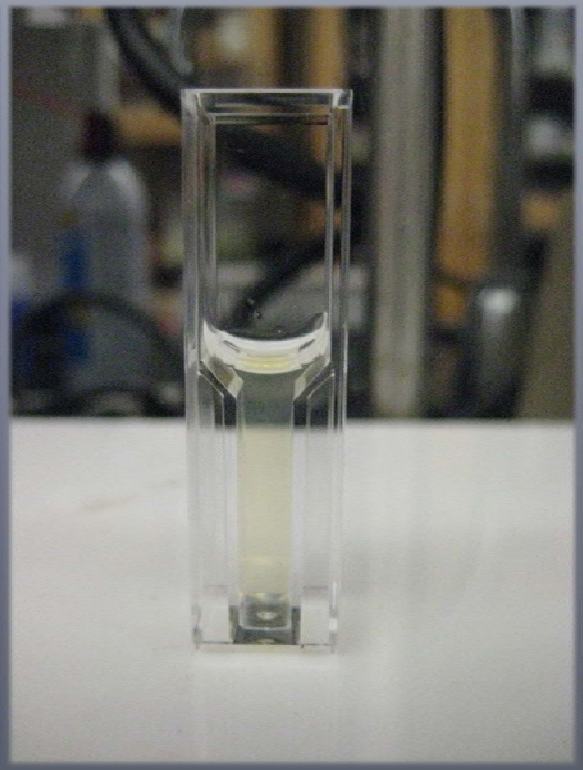


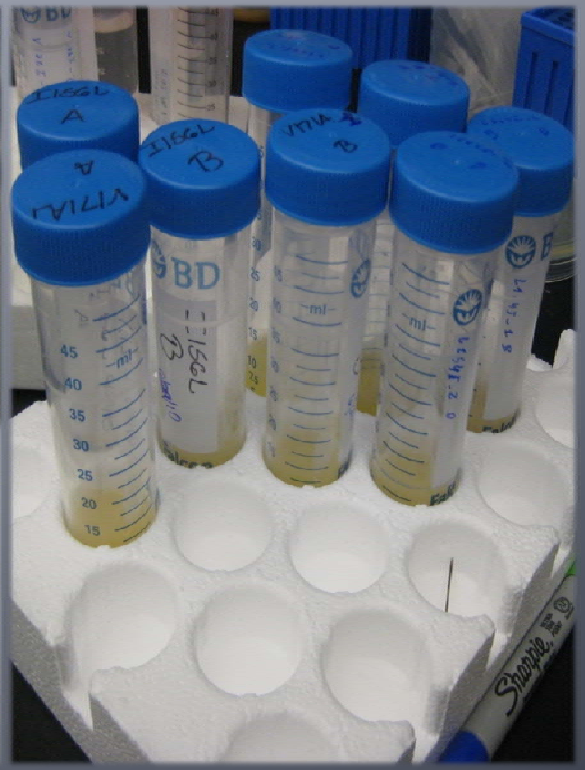
Our NMR Machine at UCSB

- Comparing spectrum from wildtype and mutant will allow us to verify correct assignment of peaks

Results







Various pictures from our lab at UCSB

Successful Mutants

Successfully created mutant receptors

- Single amino-acid mutation
- Results verified using DNA sequencing.

```
..GEAGKGFMLVANEVQNLSNETN..
..GEAGKGFMIVANEVQNLSNETN..
..******** ************..

Single amino-acid mutation
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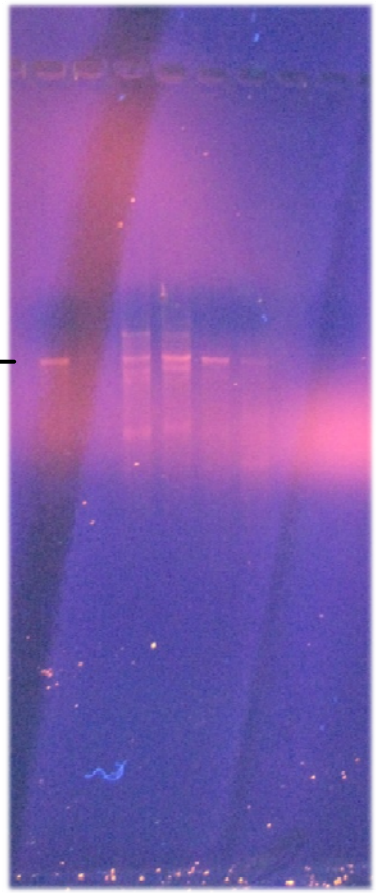
DNA Plasmid
~5kbp

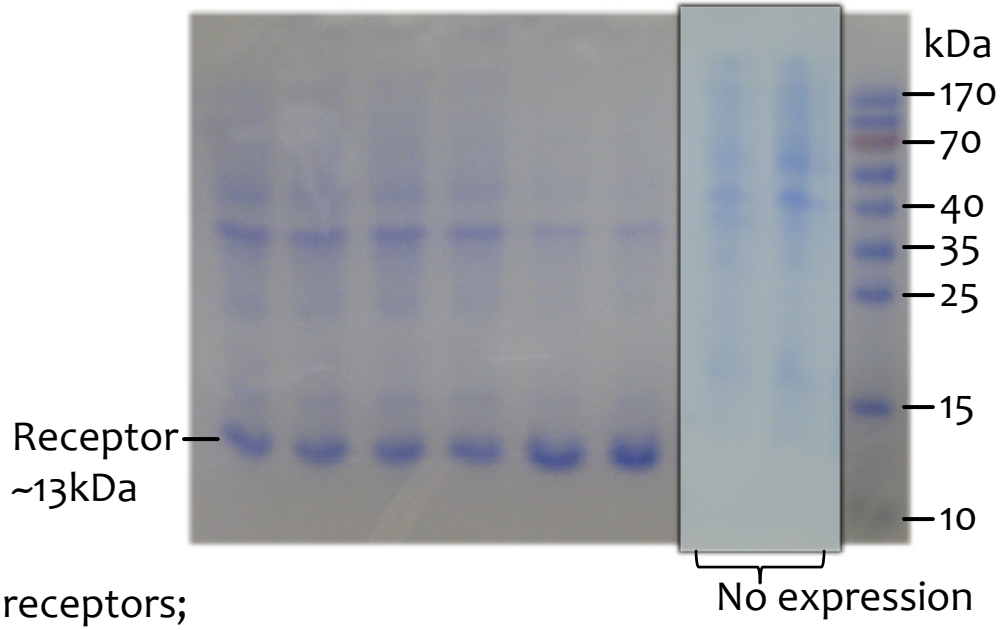


Agarose gel 1%, purified DNA plasmid. Taken at our lab.

Protein Expression

Successfully expressed mutant receptors

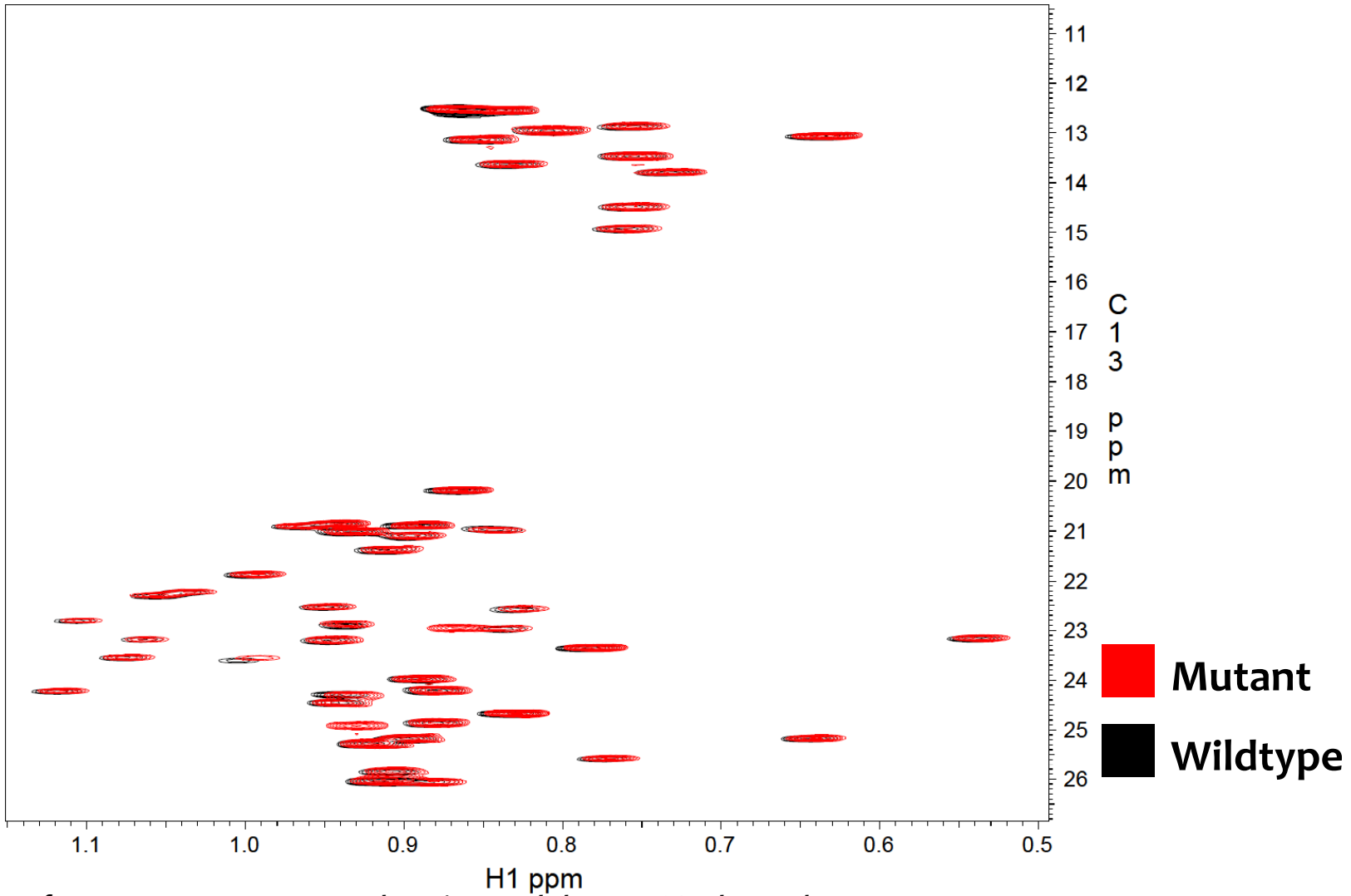
- Our receptor is clearly expressed (compared to no expression).



SDS-PAGE of expressed receptors;

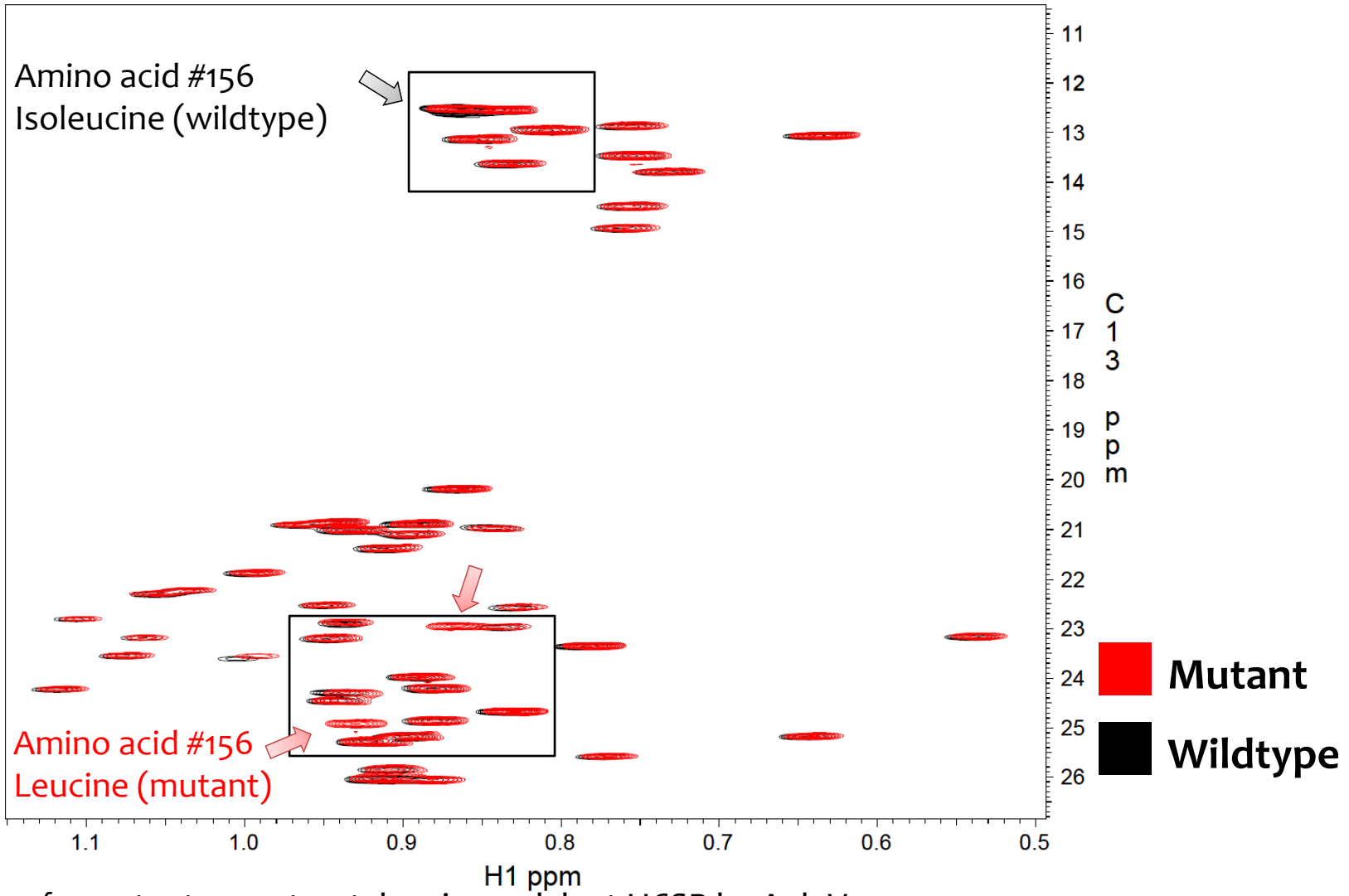
SDS-PAGE of unexpressed receptors. Taken at our lab.

NMR Results



NMR spectrum of a mutant receptor, taken in our lab at UCSB by Anh Vu.

NMR Results



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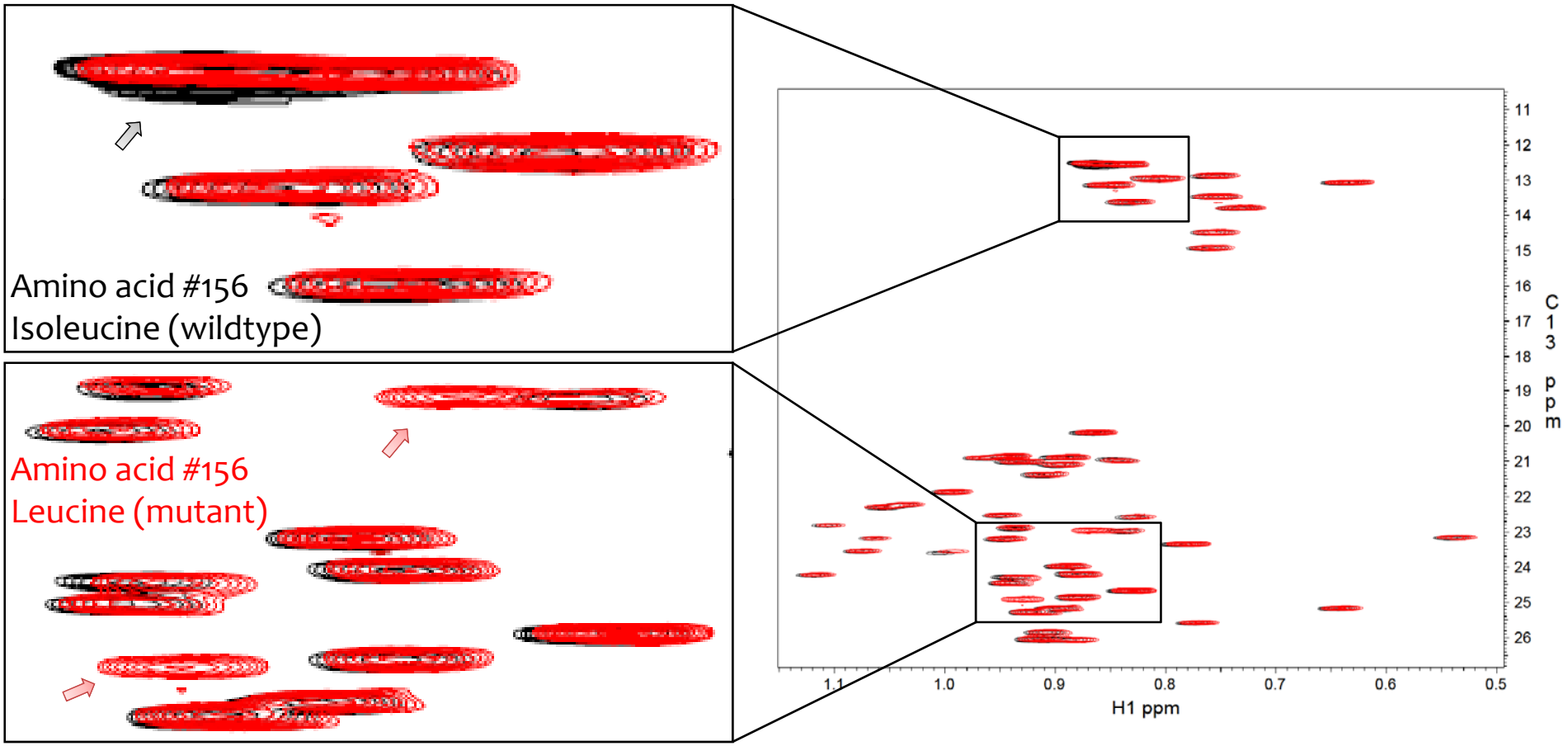


NMR spectrum of a mutant receptor, taken in our lab at UCSB by Anh Vu.

Acknowledgments

Dahlquist's Lab

Mentor: Anh Vu

Lab Partner: Melanie Shelton

(NMR spectrum is of Melanie's mutant)

Dr. Frederick Dahlquist

INSET Program

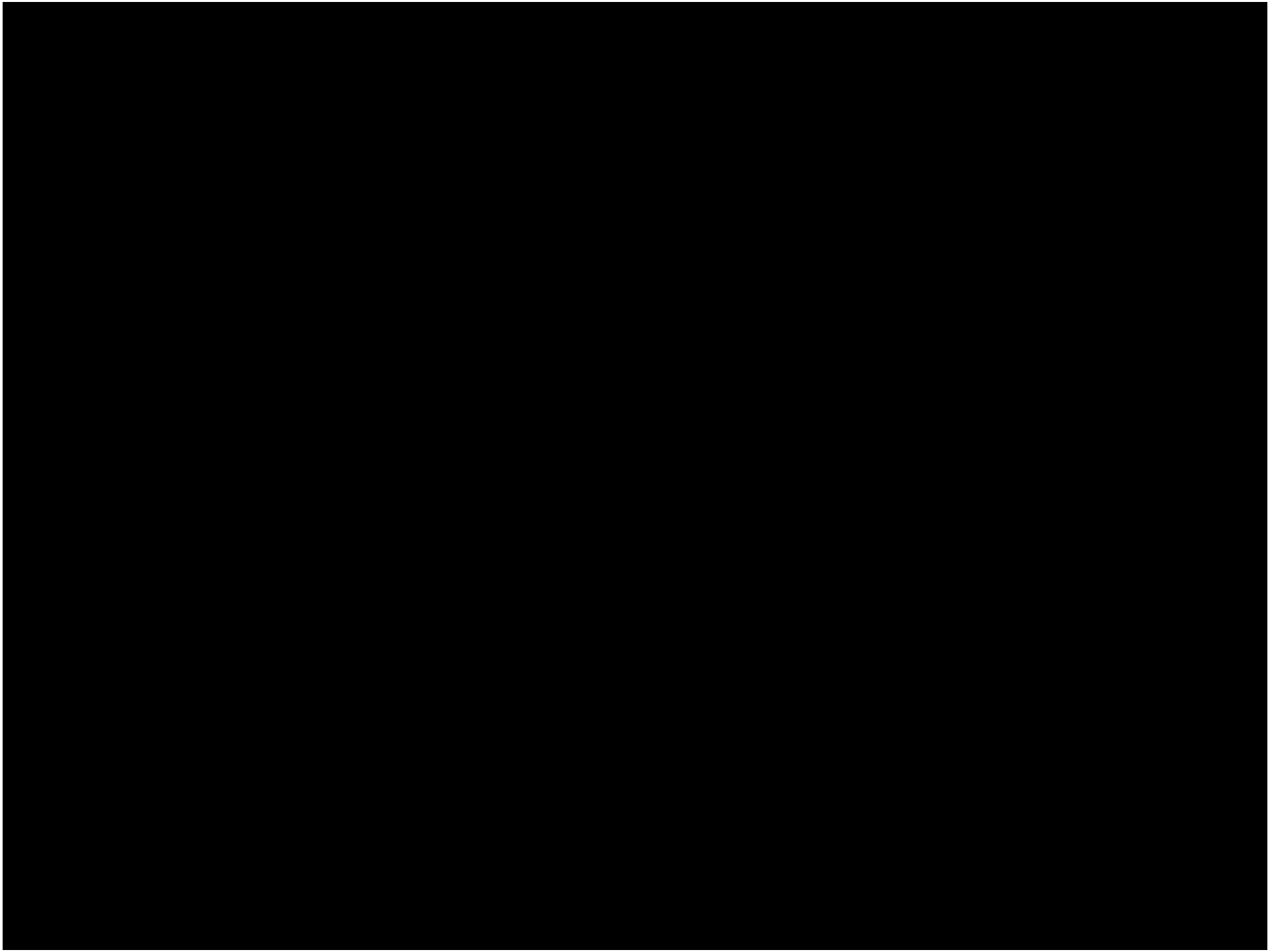
Dr. Jens-Uwe Kuhn

Dr. Nicholas Arnold

Dr. Arica Lubin

UCSB

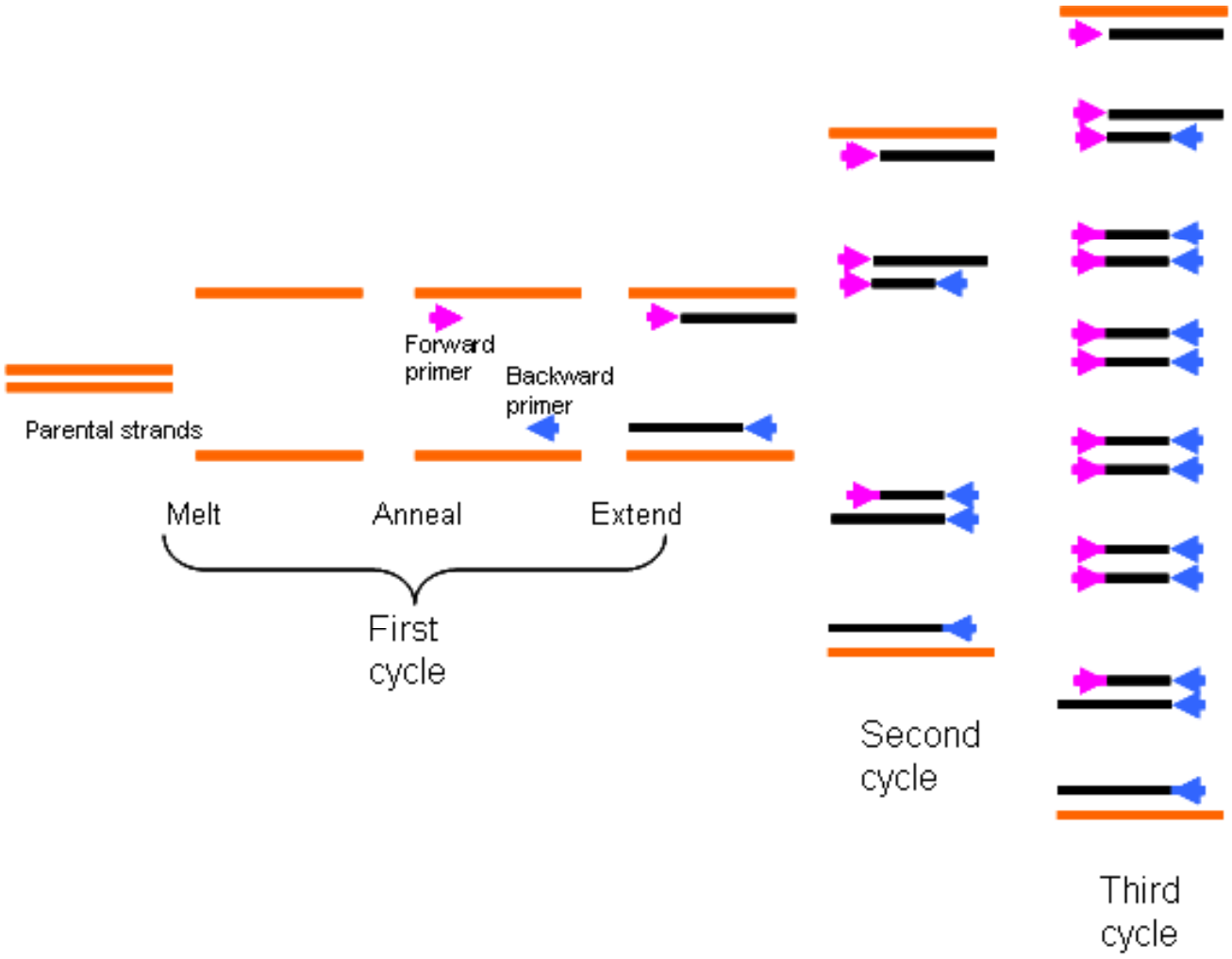
Questions?



Chemotactic Pathogenic Bacteria

Infected species	Chmotactic Bacteria	Symptoms/ Diseases
Various crops (potato, chicory)	Dickeya dadantii	Necrosis
Mammals (inc. humans)	Spirochetes	Lyme disease, relapsing fever, syphilis
Fish, humans	Vibrio	Various infections
Humans	Helicobacter pylori	Inflammation, Ulcers

PCR Outline


Forward primer
Backward primer
Parental strands
Melt
Anneal
Extend
First cycle
Second cycle
Third cycle

Mutant Sequencing Results

```
001 MGSYHHHHHHSSGLVPRGSHMKSGTNVDQIVERVKEASSQIGETLENIRSIEKLIQNIMR
001 MGSYHHHHHHSSGLVPRGSHMKSGTNVDQIVERVKEASSQIGETLENIRSIEKLIQNIMR
001 **************************************************************

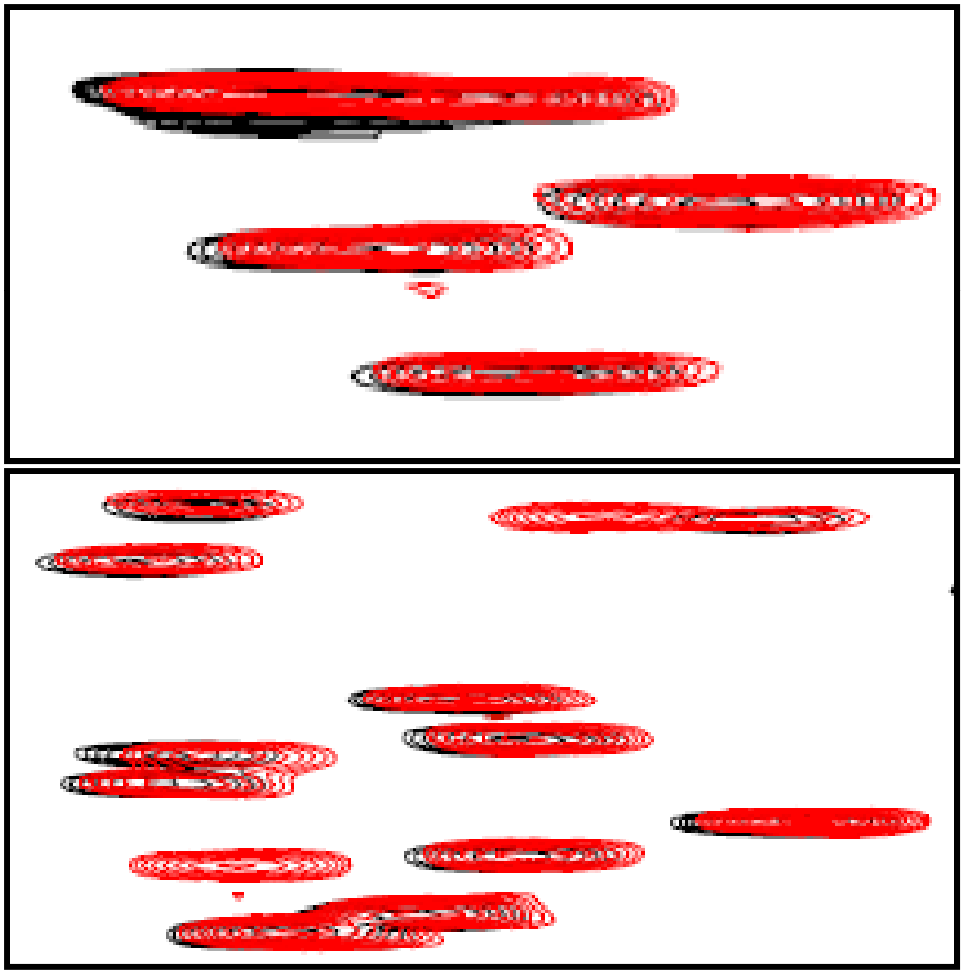
061 IARETNILALNATIEAARAGEAGKGFMLVANEVQNLSNETNEVTKQIVEKAREILESSQR
061 IARETNILALNATIEAARAGEAGKGFMIVANEVQNLSNETNEVTKQIVEKAREILESSQR
061 ************************** **************************************

121 SLENLEFMANLFETVGKT
121 SLENLEFMANLFETVGKT
121 ******************
```

Top- Mutant
Bottom- Wildtype

Single amino-acid mutation.

NMR Results



Mutant

Wildtype

NMR spectrum of a mutant receptor, taken in our lab at UCSB.