# Bacterial Chemotaxis

# Using mutagenesis to assist in NMR assignment of chemotaxis receptor

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## Introduction and Background

**What is Chemotaxis?**

Chemotaxis is a process that many bacteria use to direct their movement towards or away from chemical stimuli. Some bacteria also use chemotaxis to localize in their host's body and thus cause disease. Understanding how the chemotaxis system works will not only provide us with basic knowledge about this complex system, but will also allow us to implement that knowledge to inhibit chemotaxis in pathogenic bacteria and thus maybe prevent disease.

**How Does Chemotaxis Work?**

Chemotactic bacteria use their flagella (tail like projections; Fig 1) to propel themselves in their environment. There are two types of movement these bacteria exhibit: (1) straight forward movement, and (2) random tumbling (to re-orient themselves in a new direction). To produce the relatively smooth forward movement, the flagella rotates counter-clockwise (CCW). To randomly change direction by tumbling around, the flagella rotates clock-wise (CW) (Fig 2). The rotation of the flagella is powered by a molecular motor, which is controlled by the phosphorylation state of the protein CheA. CheA's phosphorylation state is controlled by the conformation change that occurs in the chemotaxis receptor when certain chemicals bind to it.

Bacteria use their chemotaxis receptors to sense the concentration of certain chemicals in their environment. When an increase in concentration of a desired chemical is sensed, the receptors will inhibit the autophosphorylation of CheA, and that will favor the counter-clockwise rotation of the flagella and thus favor smooth forward movement (in the direction of the desired chemical concentration gradient). When an increase in harmful chemical concentration or decrease in desired chemical concentration is sensed by the receptor, it will increase the rate of CheA autophosphorylation and thus cause an increase in clock-wise flagellar rotation, which will make the bacteria tumble in the solution and re-orient in a different direction

E. Coli bacteria

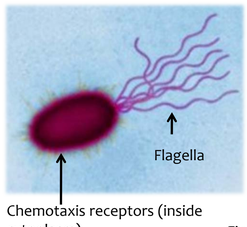
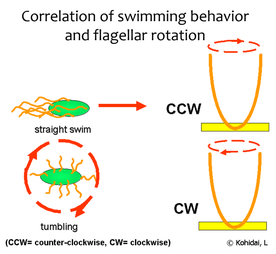



Fig 1

Correlation of swimming behavior and flagellar rotation




wikipedia.org Fig 2

## Methods

### Mutagenesis

**Overview**

To create mutant receptors we used PCR (polymerase chain reaction). PCR is a method of DNA replication, but it may also be used to introduce mutations into the DNA sequence. PCR uses man made "primers" which are small sections of DNA that serve as origins of replication. By having primers that are complementary to the DNA we want to mutate and replicate, except for a single mismatched nucleotide, we can replicate the new DNA with this single mutation in each new copy.

**Procedure**

We obtained a wildtype plasmid that contained the gene for our receptor. We used PCR to both replicate and mutate the new plasmids. Our mutation was a single nucleotide mutation, which lead to a single amino-acid mutation. For example, to mutate Isoleucine to Leucine, we only had to change one nucleotide, Adenine to Thymine (A to T):

ATA to TTA (Isoleucine to Leucine )

**Results**

To verify the success of our PCR mutagenesis we first tested the success of the PCR replication process and verified that enough copies of the DNA plasmid were produced. To test the amount of DNA that was replicated, we ran the PCR samples on a 1% agarose gel electrophoresis (Fig 4). The labeled band is the DNA plasmids. The band is clearly visible and intense, which verifies the presence of a sufficient amount of DNA plasmids.

PCR in our lab

Agarose gel 1%, purified DNA plasmid

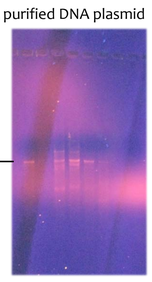



Fig 4

### Bacteria Transformation

**Overview & Procedure**

Bacteria transformation is the uptake of foreign DNA by a bacterial cell. After we have created the mutated DNA plasmid, we inserted it into bacteria. We used E. Coli competent cells (modified E. coli cells that can uptake DNA).

After we have transformed our bacteria with the mutant plasmids, we grew the bacteria on selective LB/Kan plates. As our plasmid also contained a kanamycin resistance gene, the bacterial colonies that grew on these plates had uptaken the plasmid. We selected several colonies (Fig 5) and grew them in larger liquid LB/Kan media.

To test whether the plasmid these bacteria now had contained the correct mutation, we purified the plasmid from the different samples using miniprep and sent the plasmid for DNA sequencing.

**Results**

Results from the DNA sequencing verified that the mutation was successful. The sequence bellow is a part of the amino acid sequence of our receptor protein. As the comparison shows, there is only a single mutation, and it is the desired one.

```
..GEAGKGFMLVANEVQNLSNETN..     (Mutant)
..GEAGKGFMIVANEVQNLSNETN..     (Wildtype)
..******** ************..

Single amino-acid mutation
```

E. coli colony on an LB/Kan plate

Fig 5

### Protein Expression

**Overview**

Protein expression is the production of a desired protein by bacteria. We used E. coli bacteria and the pET-28a plasmid. To induce the transcription (and thus the expression) of the mutant chemotaxis receptor protein, we added IPTG and incubated for 3 hours. After the incubation period, we prepared the samples for SDS gel electrophoresis (SDS-PAGE). We used SDS-PAGE (Fig 6) to test whether the bacteria produce enough of our protein.

**Results**

Based on the intensity of the bands on the SDS-PAGE gel (Fig 7), we could conclude that the bacteria produced a sufficient amount of mutant receptor proteins. Samples with no expression are shown as control. Each band corresponds to a different mutant receptor sample.

Running SDS-PAGE in our lab

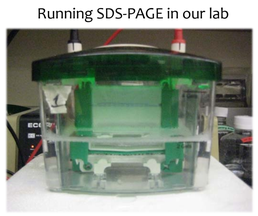
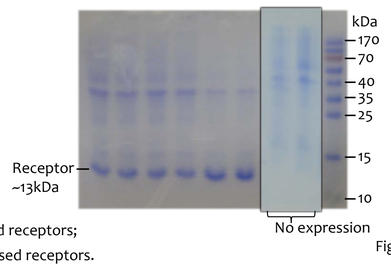
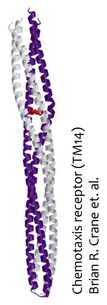


Fig 6




SDS-PAGE of expressed receptors;
SDS-PAGE of unexpressed receptors.

Fig 7



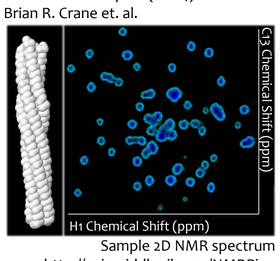

## Project Goal

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

NMR spectroscopy can be used to study the structure and interactions of various proteins (including the chemotaxis receptor protein). Before the NMR spectrum (Fig 3) can be used for such analysis, each peak on the spectrum has to be assigned to a specific amino acid on the protein. Some of these assignments can be done using standard known values, others have to be tested and assigned using other methods.

Our goal was to assist in some of the chemotaxis receptor assignments by creating chemotaxis protein receptors with a single amino acid mutation. By running an NMR scan on the mutant receptors and comparing the spectrum to the wildtype receptor we could verify the correct assignments.

Chemotaxis receptor (TM14)
Brian R. Crane et. al.




Sample 2D NMR spectrum
http://spin.niddk.nih.gov/NMRPipe
Fig 3

## Methods (continued)

### Protein Purification

**Overview**

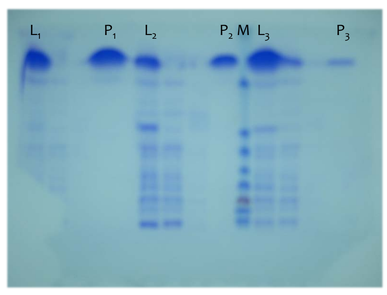
When the chemotaxis receptor protein is produced by the bacteria, its is found in a mixture of all the proteins and molecules that are inside the bacterial cytoplasm. To be able to examine the receptor alone, we need to first break the bacterial cell wall, and then purify the receptor from all the other proteins.

**Procedure**

To lyse the bacterial cell we used a cell French press at 2000 PSIG (Fig 8). We then loaded the cell lysate into a nickel column (Fig 9). Our chemotaxis receptor was labeled with a histidine tag, which gives it a strong affinity towards the nickel column. We washed the nickel column to discard any other unbound proteins and then eluted our receptor using imidazole, which is a molecule that has a strong affinity towards the nickel column, and thus it elutes our receptor. To test the purification levels, we collected samples from each stage of the purification and ran them on an SDS-PAGE gel.

**Results**

Purification of the chemotaxis receptor protein was successful. The gel bellow (Fig 10) shows 3 different mutant receptors before and after purification: L = crude cell lysate (before purification), P = purified protein (after nickel column), M = size marker.




SDS-PAGE of receptor protein before and after purification Fig 10

Cell French Press

Fig 8

Nickel Column

Fig 9

### Nuclear Magnetic Resonance (NMR)

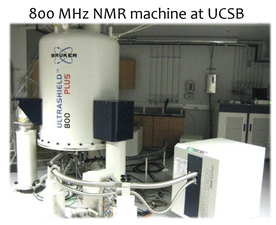
**Overview**

An NMR spectrum allows us to get information about the structure, conformation, and interactions of the chemotaxis receptor. NMR works by radiating a sample with radio frequency pulses. This energy is first absorbed by the nuclei in the sample, and is then released to the environment and recorded by the NMR machine. Each atom's nucleus will give a different signal based on the type of atom and the environment it is in.

**Procedure**

We labeled the amino acids Leucine, Isoleucine, and Valine of the chemotaxis receptor with Carbon-13 since Carbon-12 does not give an NMR signal. After purifying the receptor from all the other proteins, we ran a 2-dimension $^{1}H$ - $^{13}C$ NMR experiment (in essence it is a combination of the values of the spectra of $^{13}C$ on the Y-axis and $^{1}H$ on the X-axis; Fig 11), using a 600MHz NMR. After we ran the NMR experiment on our mutant receptor, we compared it to the wildtype NMR spectrum.

800 MHz NMR machine at UCSB




## Results

**NMR Results and Verification of Correct Peak to Amino Acid Assignment**

We suspected that the peak labeled on Fig 11 (top left, grey arrow) correlates to amino acid #156 on the chemotaxis receptor. We mutated amino acid #156 from Isoleucine to Leucine, and took an NMR spectrum. On Fig 11 (top left, grey arrow) there are 3 black peaks (somewhat hard to see since they partially overlap, yet it is clear by their intensities that there are 3 peaks). After mutating amino acid #156 and comparing the mutant spectrum to the wildtype, we can see that the mutant spectrum is missing a peak in the same area on Fig 11 (top left, grey arrow). This verified that amino acid #156 (Isoleucine) on our chemotaxis receptor correlates to that particular peak (Fig 11 , top left, grey arrow).

Two other interesting peaks on this spectrum are the new peaks (Fig 11, bottom left, red arrows) that appeared due to the new amino acid we introduced (Leucine) instead of the original amino acid (Isoleucine) in location #156 on our receptor. The new amino acid we introduced had two $^{13}C$ labels on it and thus gave 2 peaks. Notice that these peaks are not present in the wildtype spectrum.




NMR spectrum of a mutant receptor on top of the wildtype spectrum.

Fig 11