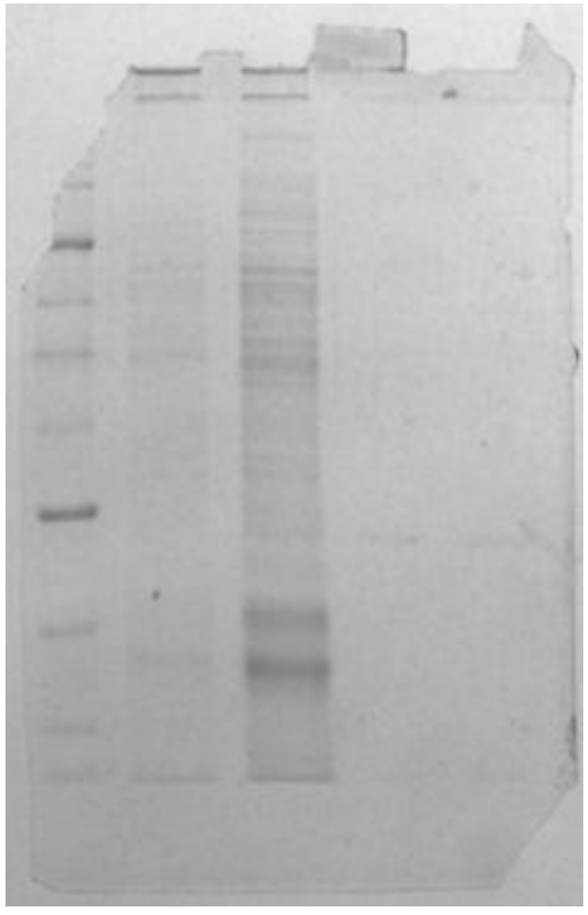
E-Futures

The Search For Biosynthetic Proteins That Assemble Photosynthetic Membranes In Cyanobacteria



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The presentation

What is the project about?

Why are we interested in the project?

What am I doing in this project?

Experimental procedure

Protein tagging

SDS-PAGE

Mass spectrometry

Results so far...

The project

GUN4 **is a protein involved in the biosynthesis of chlorophyll**

GUN4 **has been detected in** ***Synechocystis*****, but never quantified in whole cells.**

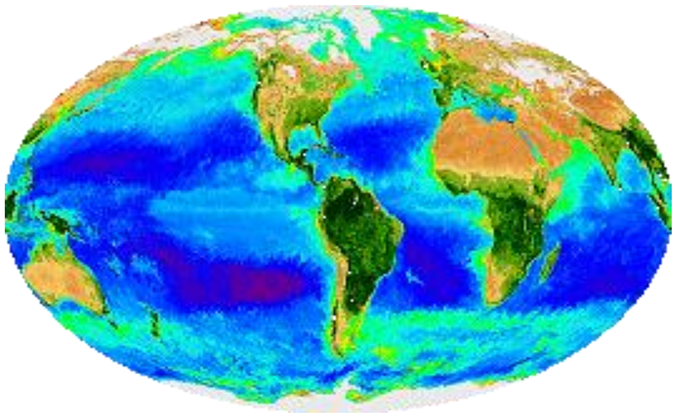
GUN4 **may have more reaction partners inside the cell which are as yet unknown**

Synechocystis **is genetically engineered to produce "tagged"** ***GUN4*** **which can be selectively pulled from the cells**

When extracted the ***GUN4*** **will stay bound to, and hence also extract, its interaction partners**

By analysing the extracted proteins it is possible to determine the interaction partners of ***Gun4*** **and their relative interaction strengths**

Scientific interest



Photosynthesis is the dominant form of solar energy harvesting on the planet.

At least 50% of global photosynthesis takes place in the oceans, as a result of cyanobacterial growth.

Photosynthetic bacteria such as cyanobacteria are therefore a major source of carbon dioxide sequestration.

With a greater understanding of the processes involved in photosynthesis it may be possible to engineer more productive energy crops and algae for biofuels.

Effects of *GUN4*

The genes responsible for *GUN4* production have been inactivated in plant cells and the chlorophyll synthesis has been suppressed

***GUN4* gene knocked out**

***GUN4* gene intact**

***GUN4* is clearly important for plant growth**

Experiment procedure

Synechocystis GUN4 is engineered with a peptide tag

Cells with tagged *GUN4* are selectively grown

Once the cells are grown they are disrupted to release proteins

Tagged proteins are retrieved from the protein mixture

Tagged protein is analysed using gel electrophoresis

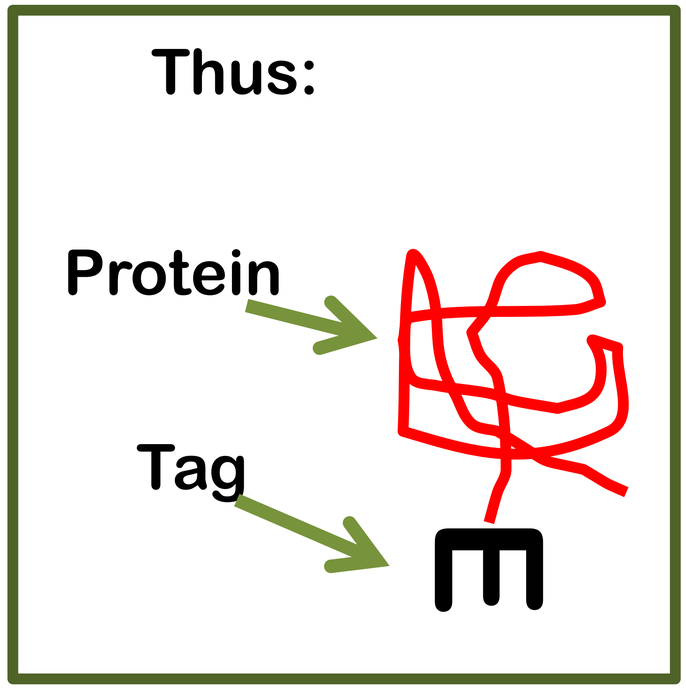
Proteins of interest are identified and digested

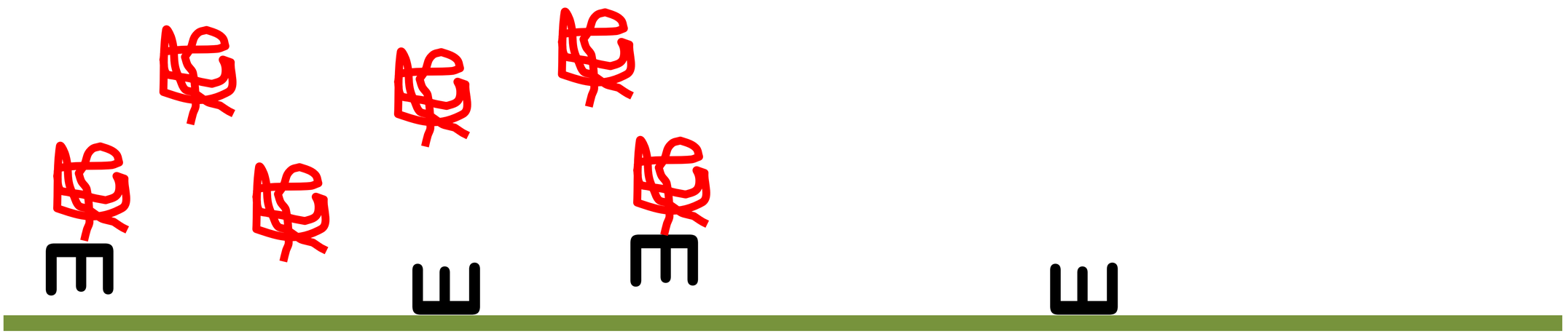
Mass spectrometry analysis

Protein tagging

The gene is modified to encode a tag usually at the N- or C-terminus of the protein of interest

Antibodies which recognise the tag can be used to pull tagged proteins out of protein mixtures.





SDS-PAGE

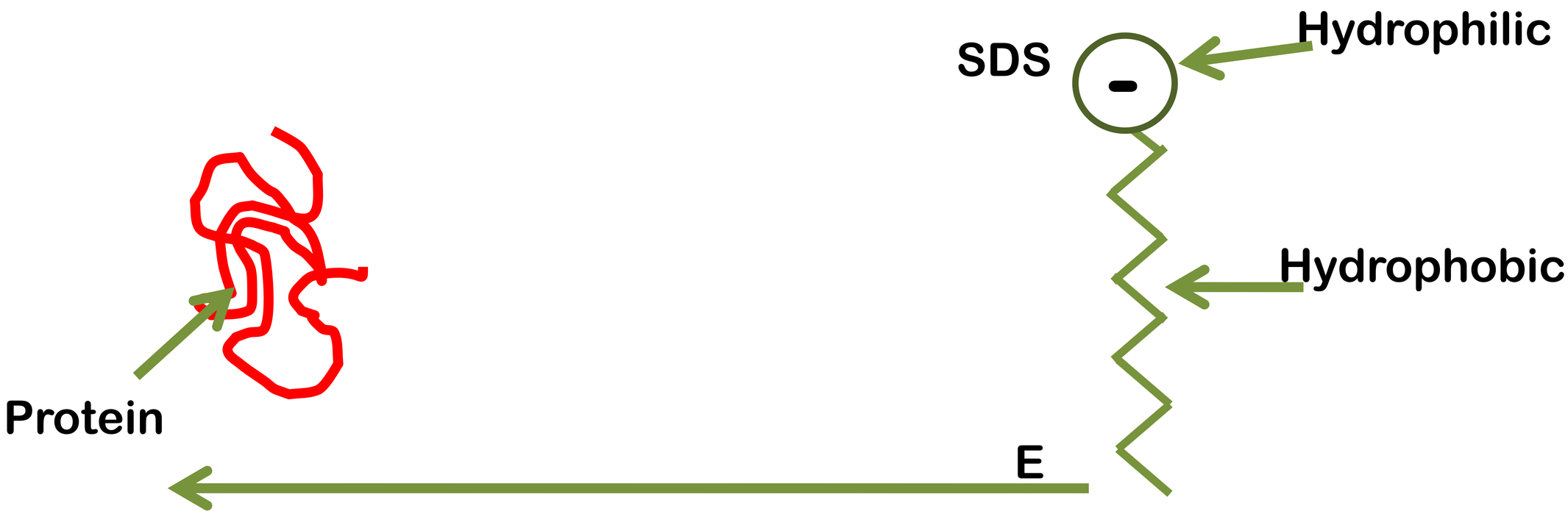
Sodium Dodecyl Sulphate PolyAcrylamide Gel Electrophoresis

Proteins are mixed with SDS

The SDS unfolds the protein

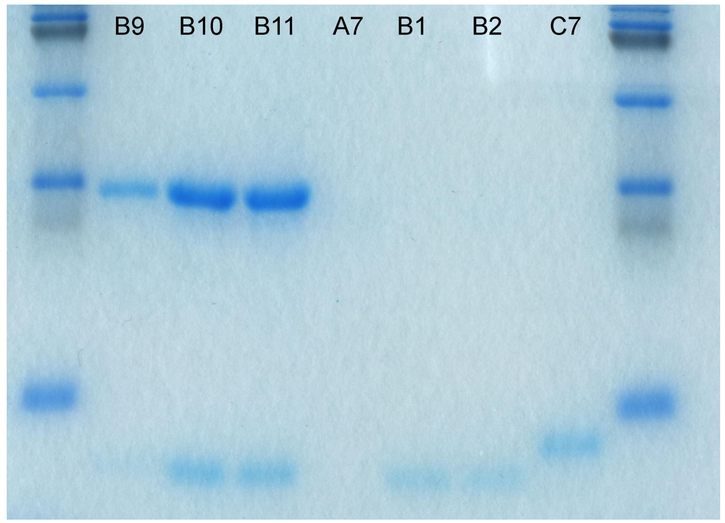
An electric field is applied across the gel, and the SDS-coated proteins enter the gel

Proteins move through the gel at a speed dependent on their mass

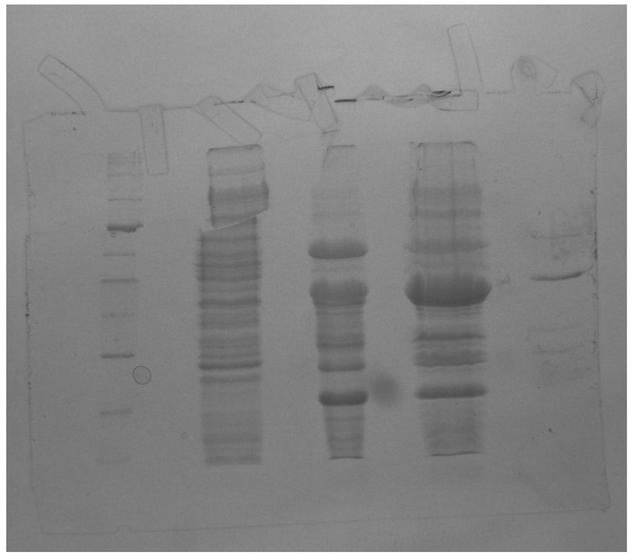


SDS-PAGE

A nice photogenic gel



My first gel

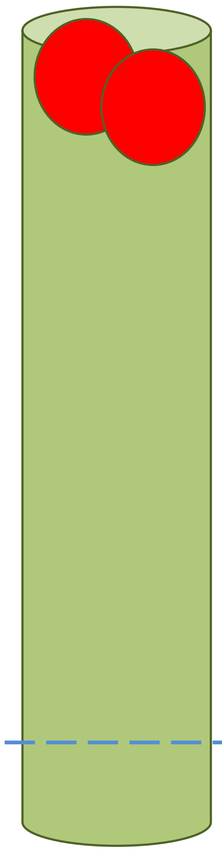


Proteins of interest can be identified and the corresponding sections of gel can be cut out.

The proteins are digested in the gel and the peptides extracted for analysis with the mass spectrometer.

Mass Spectrometry

The mass spectrometer is fed by a liquid chromatography column.



The column is washed with an increasing concentration of organic solvent

The peptides are washed out at different times depending on the strength of their binding to the column

This allows the mass spectrometer to analyse the sample one peptide at a time

Mass spectrometer

Detector

Protein identification

{MATRIX SCIENCE} Mascot Search Results

Protein View

Match to: **gi|114549**; Score: **215**
ATP synthase beta chain, mitochondrial precursor
Found in search of E:\JING\MALDI-TOF\MALDI-peaklist\c0611.txt

Nominal mass (M_r): **56525**; Calculated pI value: **5.26**
NCBI BLAST search of gi|114549 against nr
Unformatted sequence string for pasting into other applications

Taxonomy: Homo sapiens
Links to retrieve other entries containing this sequence from NCBI Entrez:
gi|106207 from Homo sapiens
gi|179281 from Homo sapiens
gi|14603307 from Homo sapiens
gi|16741373 from Homo sapiens

Variable modifications: Oxidation (M)
Cleavage by Trypsin: cuts C-term side of KR unless next residue is P
Number of mass values searched: **60**
Number of mass values matched: **28**
Sequence Coverage: **58%**

Matched peptides shown in **Bold Red**

```
    1 MLGFVGRVAA APASGALRRL TPSASLPPAQ LLLRAAPTAV HPVRDYAAQT
   51 SPSPKAGAAT GRIVAVIGAV VDVQFDEGLP PILNALEVQG RETRLVLEVA
  101 QKLGESTVRT IAMDGTEGLV RGQKVLDSGA PIKIPVGPET LGRIMNVIGE
  151 PIDERGPIKT KQFAPIHAEA PEFMEMSVEQ EILVTGIKVV DLLAPYAKGG
  201 KIGLFGGAGV GKTVLIMELI NNVAKAHGGY SVFAGVGERT REGNDLYHEM
  251 IESGVINLKD ATSKVALVYG QMNEPPGARA RVALTGLTVA EYFRDQEGQD
  301 VLLFIDNIFR FTQAGSEVSA LLGRIPSAVG YQPTLATDMG TMQERITTTK
  351 KGSITSVQAI YVPADDLTDP APATTFAHLD ATTVLSRAIA ELGIYPAVDP
  401 LDSTSRIMDP NIVGSEHYDV ARGVQKILQD YKSLQDIIAI LGMDELSEED
  451 KLTVSRARKI QRFLSQPFQV AEVFTGHMGK LVPLKETIKG FQQILAGEYD
  501 HLPEQAFYMV GPIEEAVAKA DKLAEEHSS
```

http://edoc.hu-berlin.de/dissertationen/xie-jing-2003-12-15/HTML/xie_html_2eb20d86.jpg

Peptides are matched to peptide sequences in a database

The protein can be identified without observing its entire sequence

Higher certainty is gained with greater protein sequence coverage

False identifications are predicted by checking against a dummy database

Results so far…

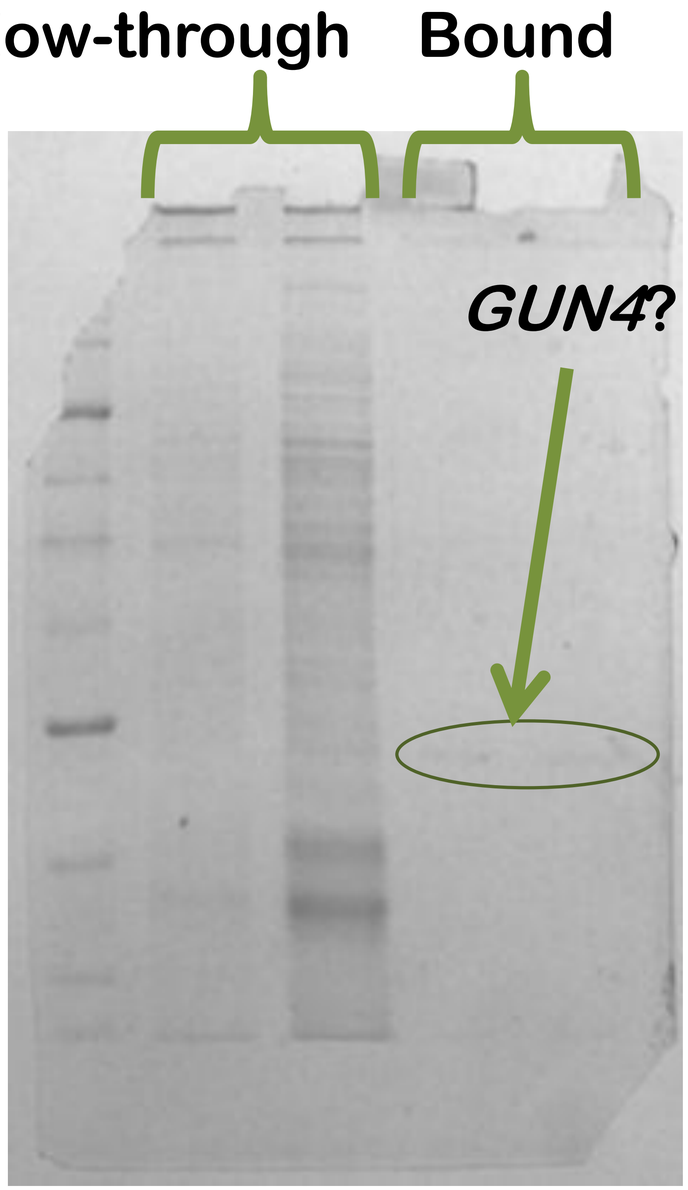
One full mass spectrometry analysis of a *Synechocystis* sample has been undertaken

Upon mass spectrometry we found the protein to be….

Trypsin!!!!

No *GUN4* found

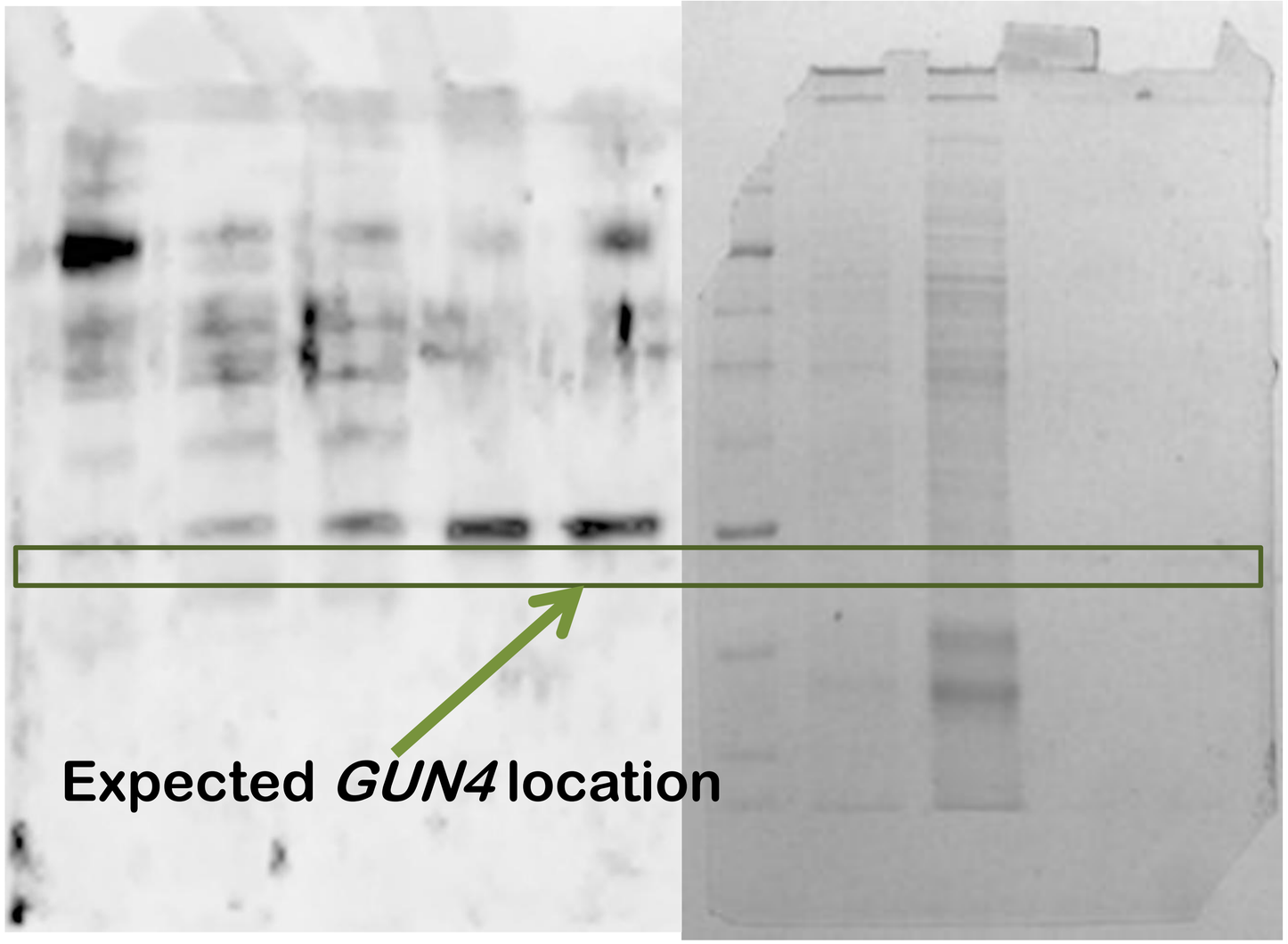
We expected to see *GUN4* so where is it?



Results so far...

A western blot was run to see if any *GUN4* was present at all

Western blot | **Gel**



Western blot showed many different proteins but no *GUN4*

This means that there may not have been enough sample to observe the *GUN4*

Also the western blot antibodies may not be working