



Biotechnology, Biosensors, and Beer: The Measurement of Proteases Relevant to Brewing

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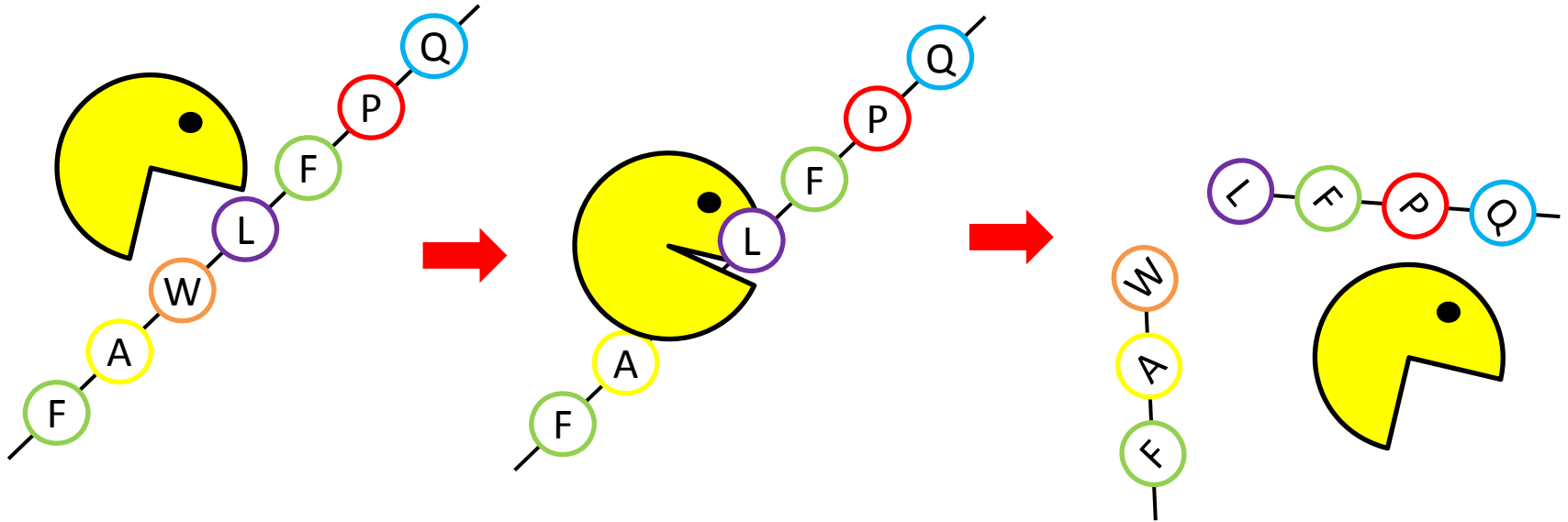
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Talk Outline

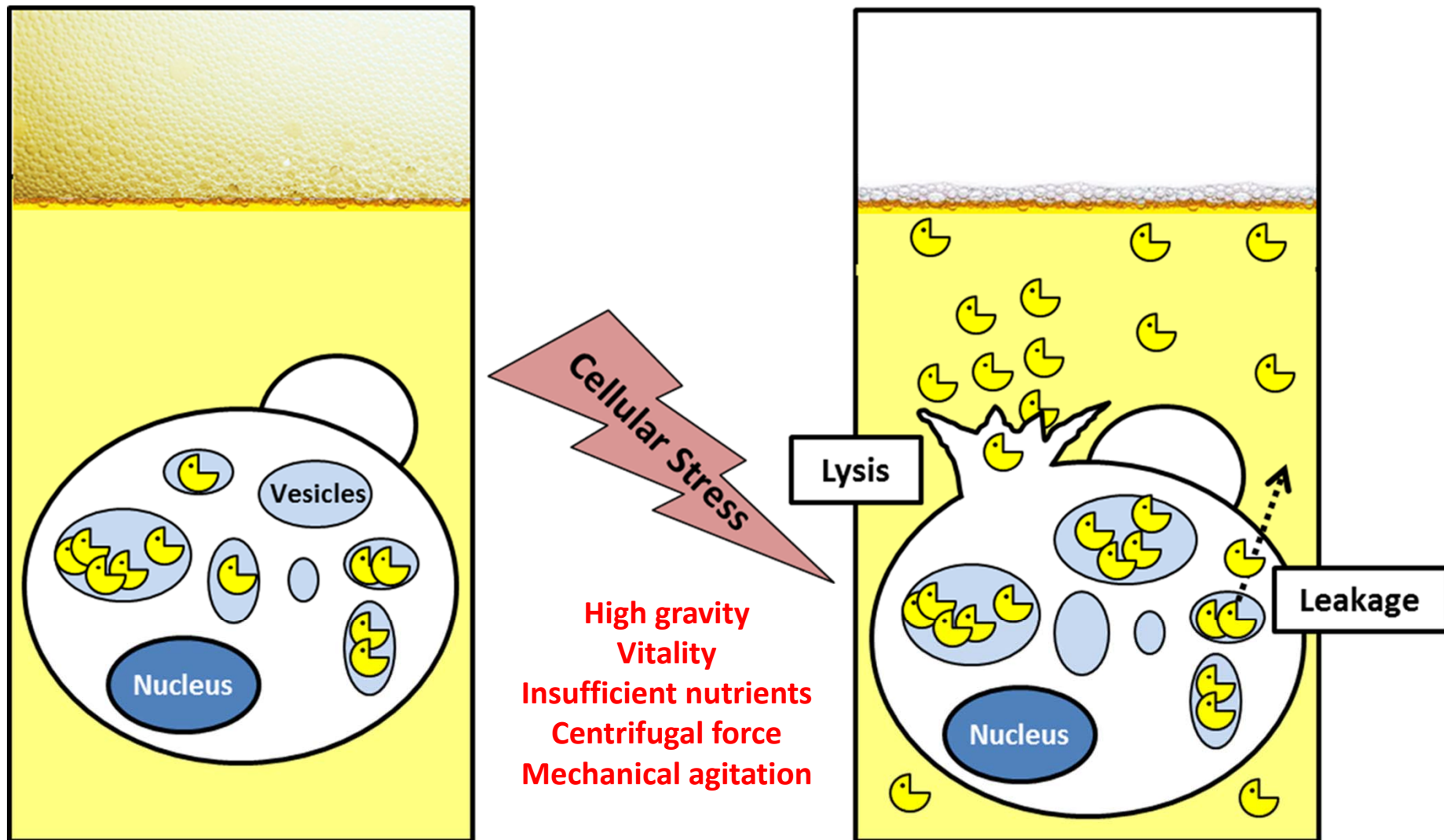
- **Background on Proteases and their implications in beer**
- **Methods for the measurement of protease activity**
- **Application of our novel biosensors to Proteinase A (PrA) in beer**
- **Application of our biosensor to proteases used in production of gluten-free beer**

What are proteases?

- Enzymes which cleave peptide bonds in target proteins.



Proteinase A: a yeast protease which is foam negative.

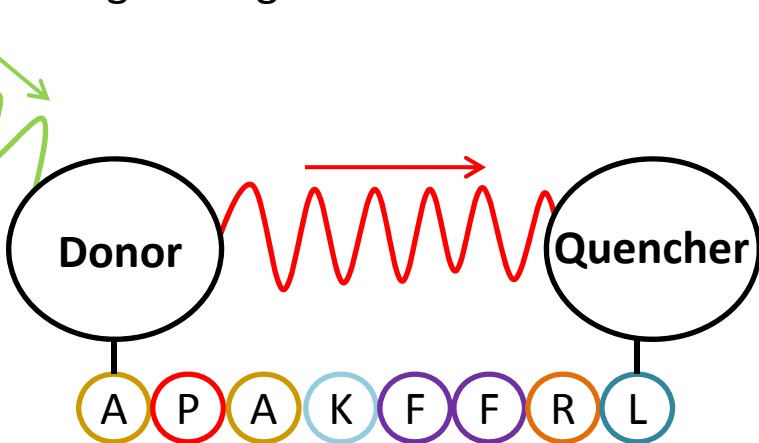


 - ProteinaseA (PrA)

Previous methods of PrA measurement in beer

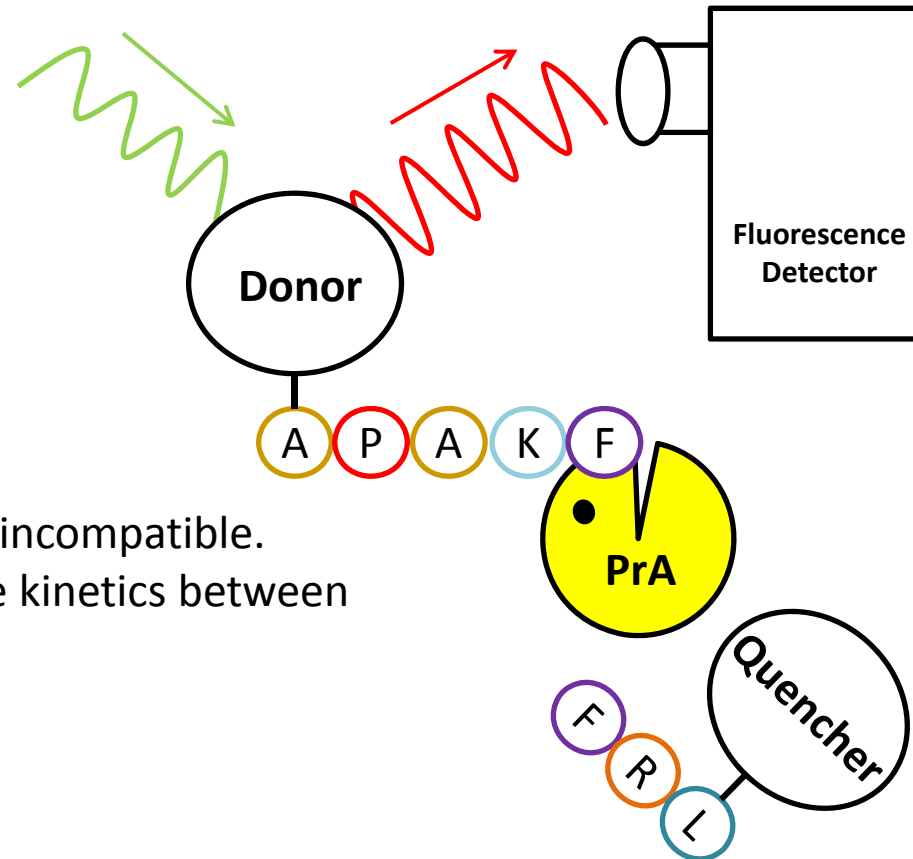
Casein, Azocasein, Resorufin-Casein

- Proteolytic digest of a purified protein.
- Non-selective cleavage
- Typically colorimetric
- Low sensitivity
- High background



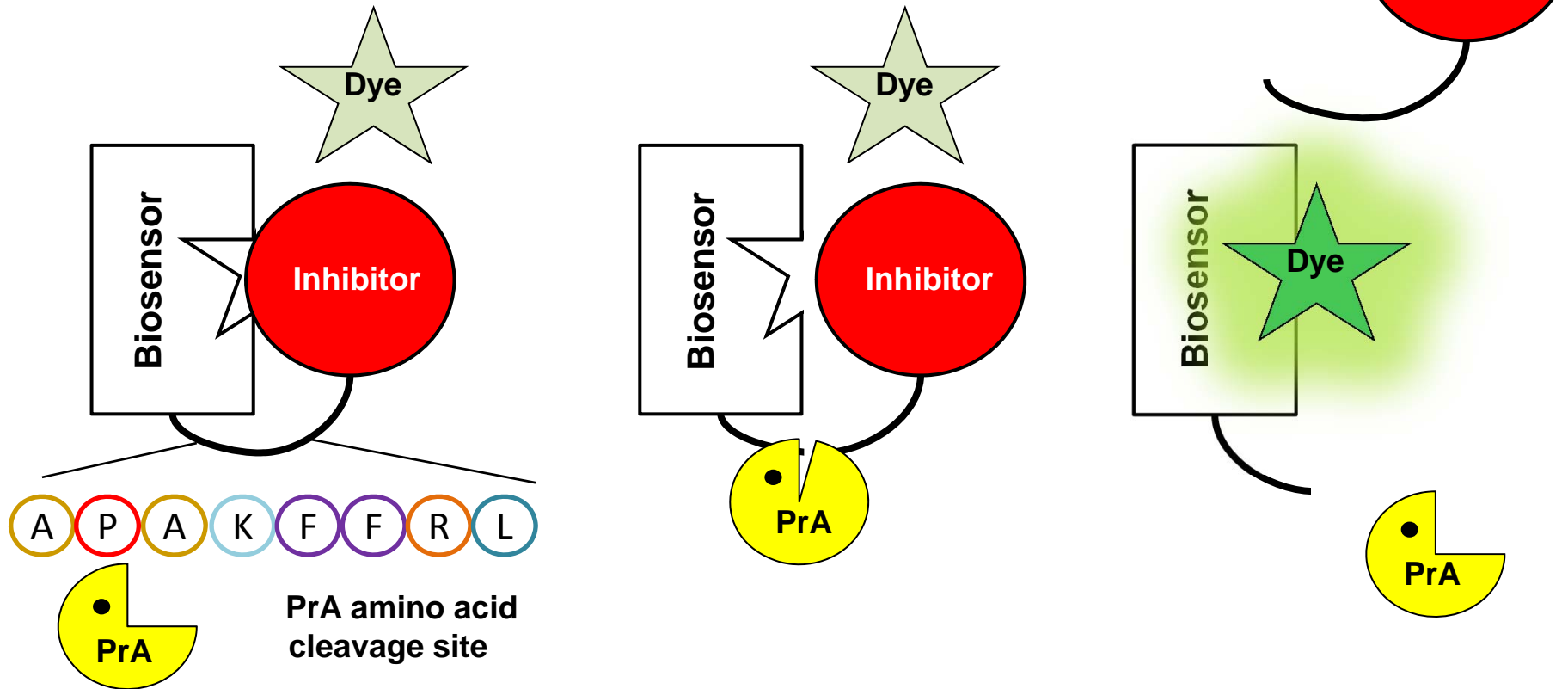
Fluorescent FRET substrates

- Proteolytic digest of a peptide
- Selectivity depends on sequence
- Fluorimetric
- High sensitivity
- Moderate background



- FRET substrates can be very expensive.
- Certain sequences are hydrophobic and thus incompatible.
- Proteases can demonstrate different cleavage kinetics between peptide substrates and protein substrates.

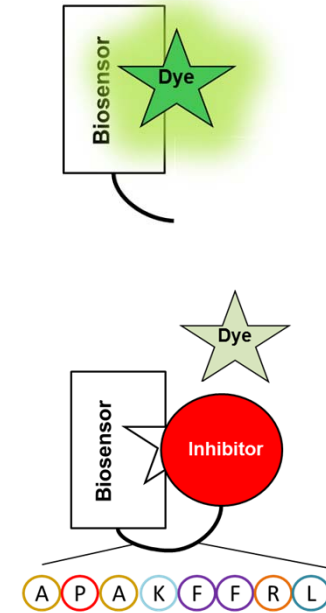
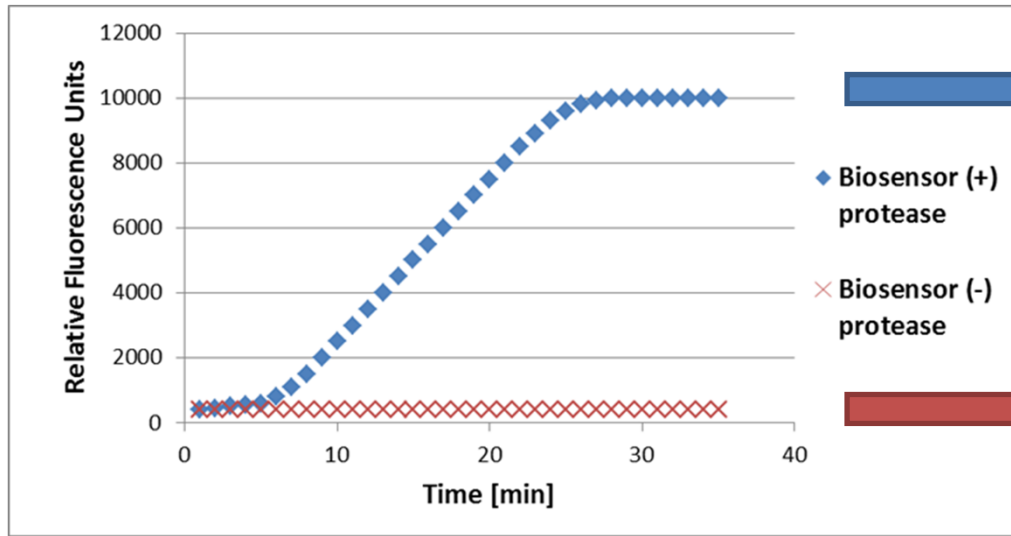
Our selective protease biosensor model system



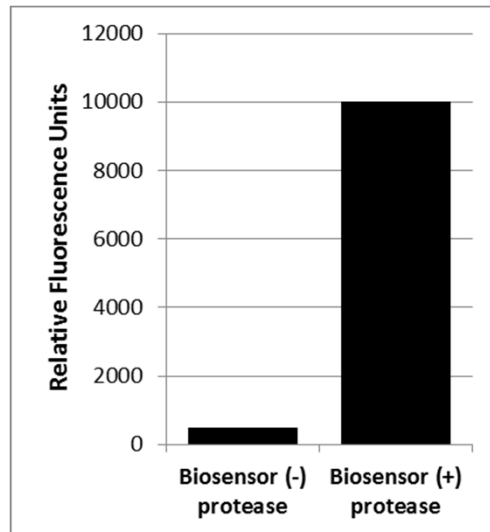
The linker sequence is modular; therefore we can build selective biosensors for any protease if an amino acid cleavage sequence is known.

Our selective protease biosensor model system

Kinetic Data

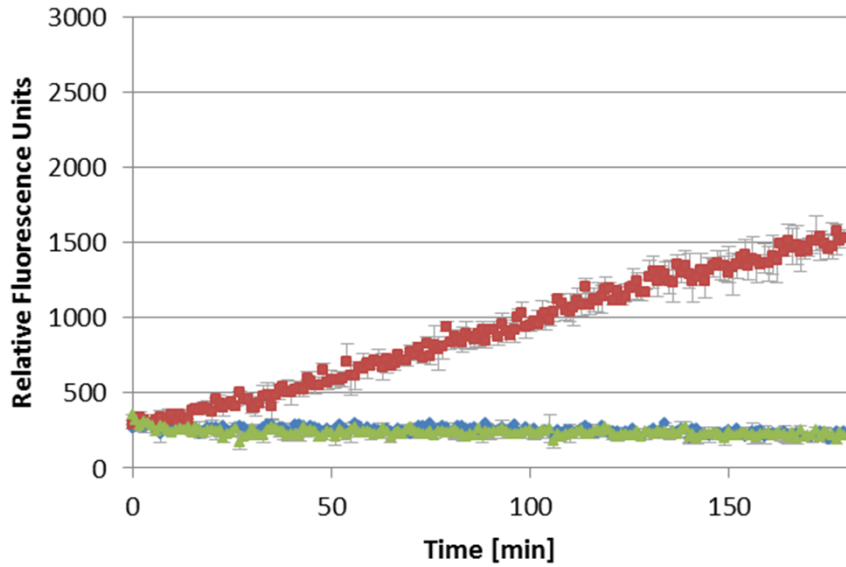


End point Data

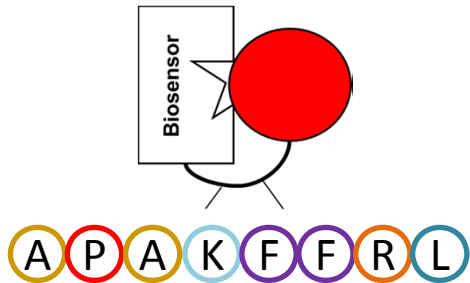


Protease measurement in beer brewed with a 5 gallon pilot system

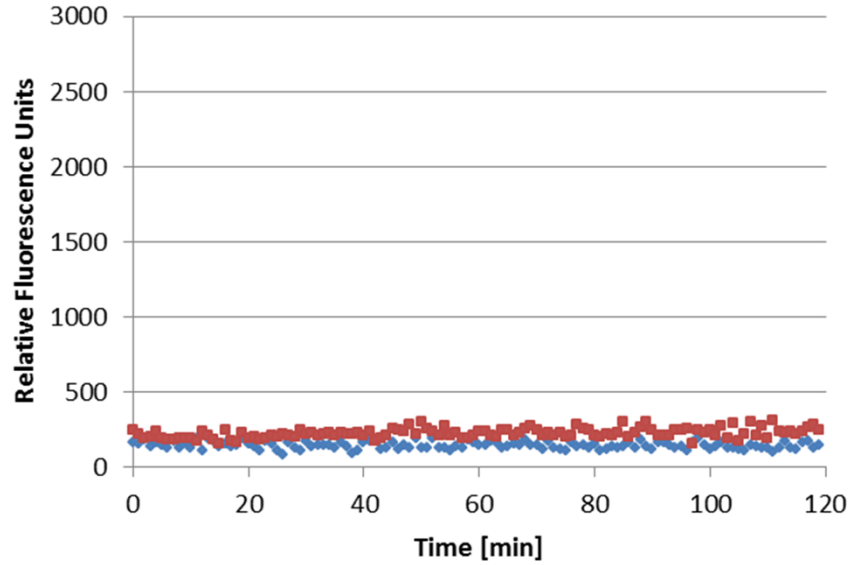
PrA biosensor



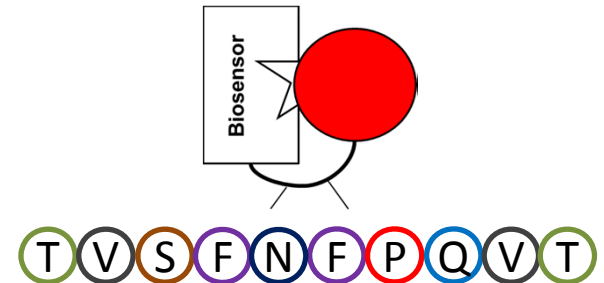
- ◆ - PrA biosensor only
- - PrA biosensor + IPA
- ▲ - PrA biosensor + porter



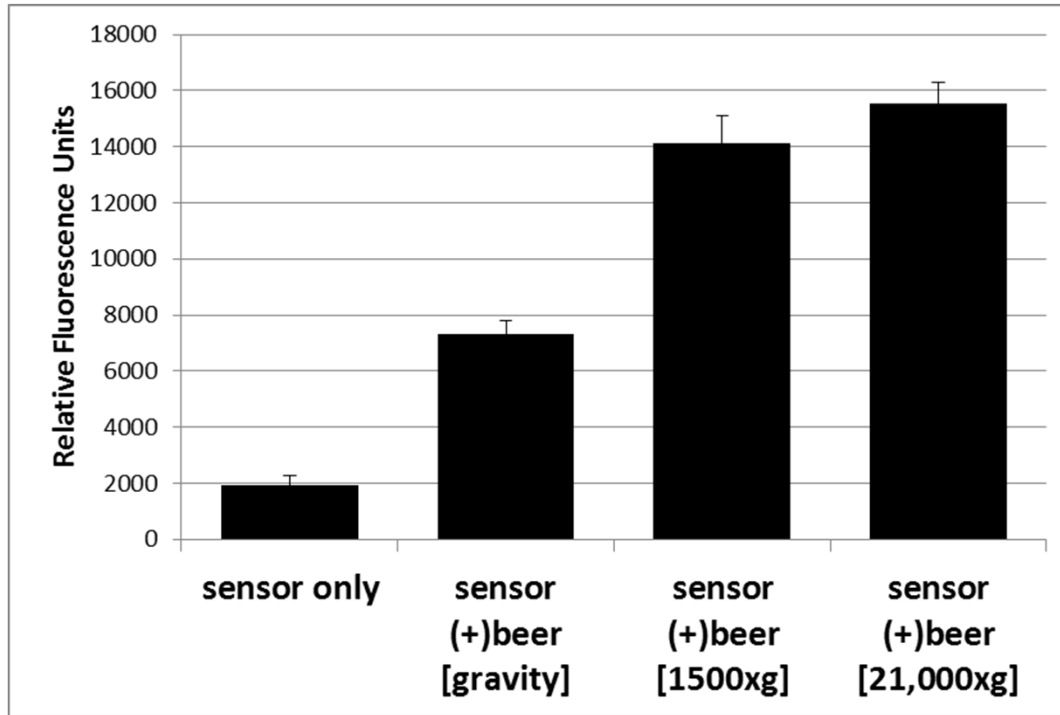
HIV protease biosensor



- ◆ - HIV protease biosensor only
- - HIV protease biosensor + IPA

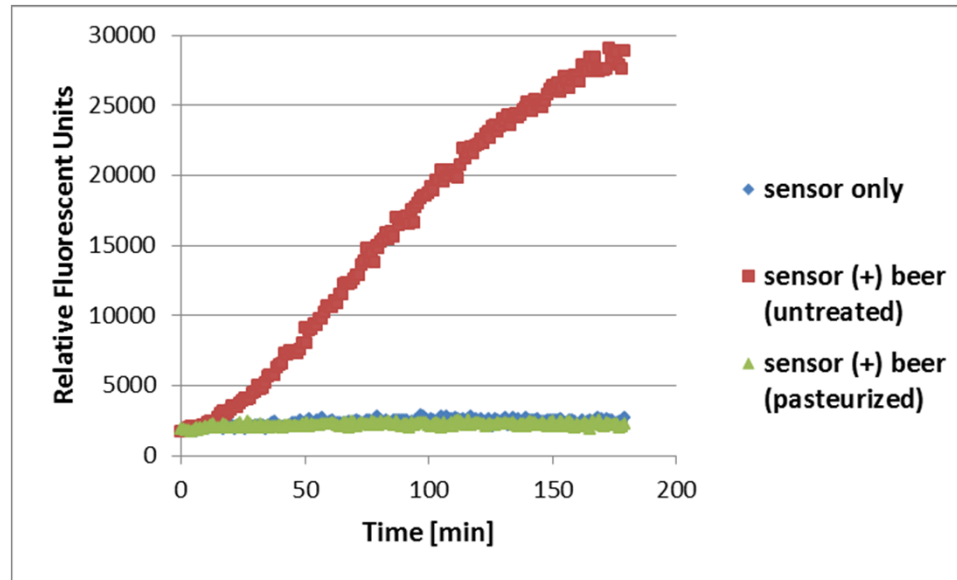


Centrifugal force increases protease activity in beer.



Endpoint fluorescent reading 2 hours after addition of beer.

Pasteurization eliminates protease activity in beer.



For pasteurization: beer incubated 2 minutes at 72°C before assay.

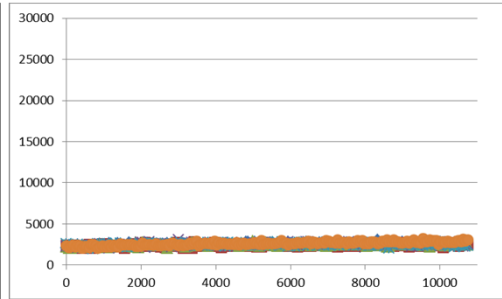
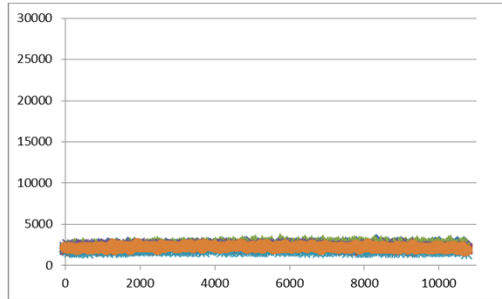
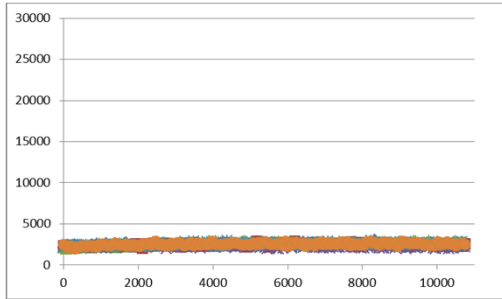
Is the release of protease into beer dependent upon yeast strain?

20°C

25°C

30°C

Week 1

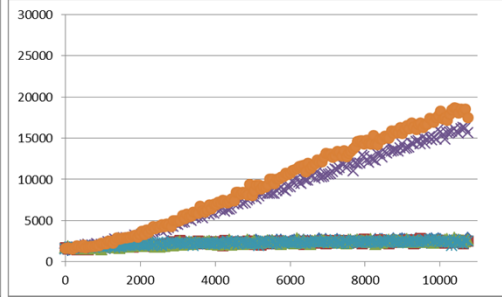
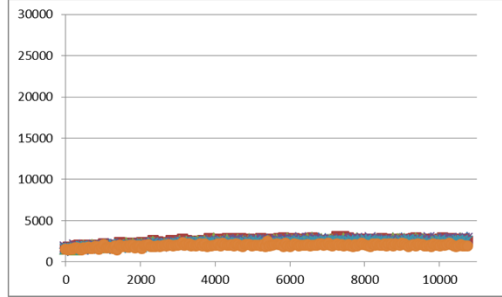
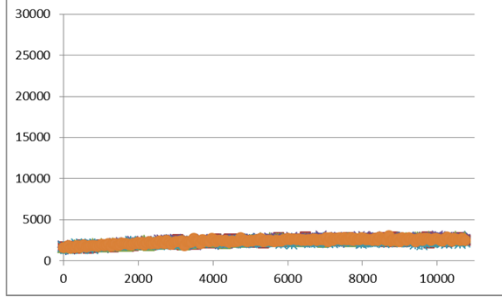


- ◆ - Sensor only
- - Wyeast American Ale I (1056)
- ▲ - Wyeast American Ale II (1272)
- ✖ - White labs Super High Gravity (WLP009)
- ✖ - White labs English Ale (WLP002)
- - Wyeast Weihenstephan Ale (3068)

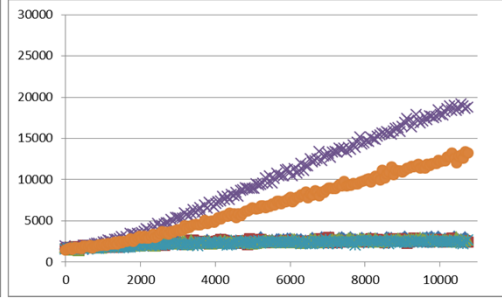
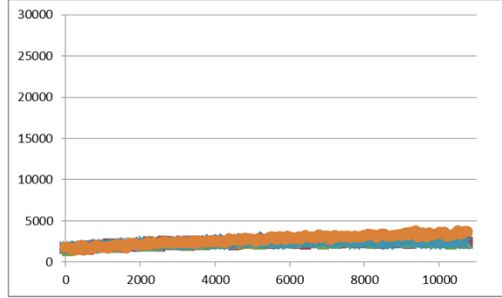
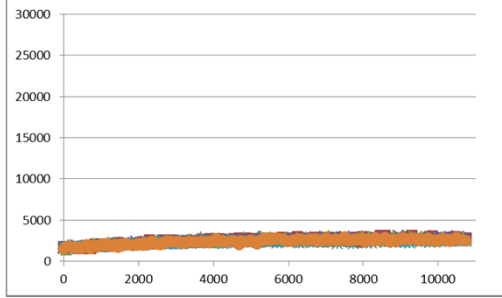
Fermentations:

10° Plato
DME only
Unhopped

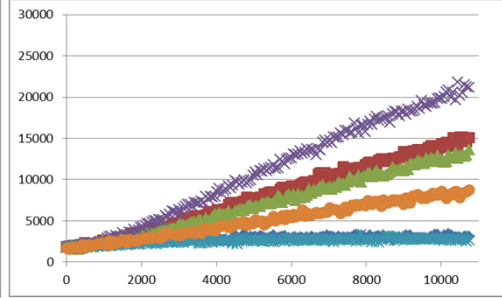
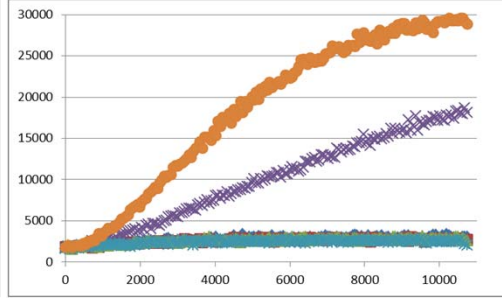
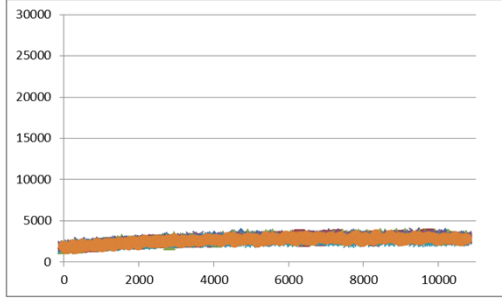
Week 2



Week 3



Week 4

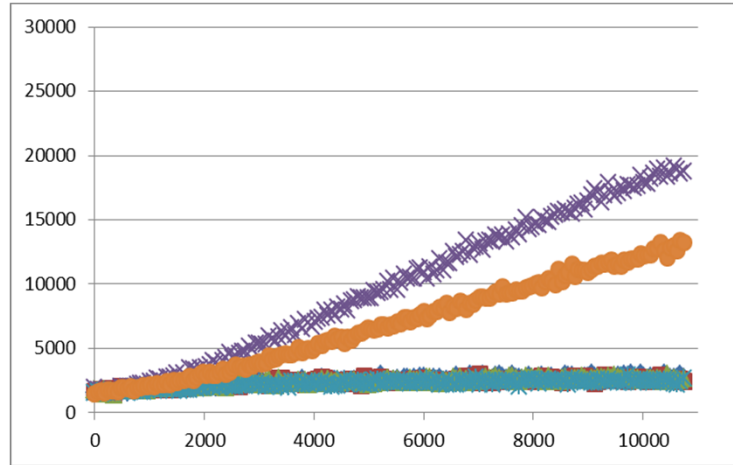
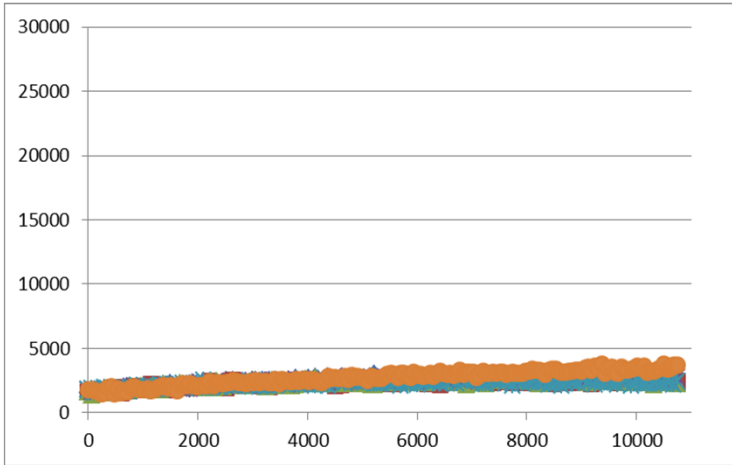


Data representative of 2-3 replicates

25°C

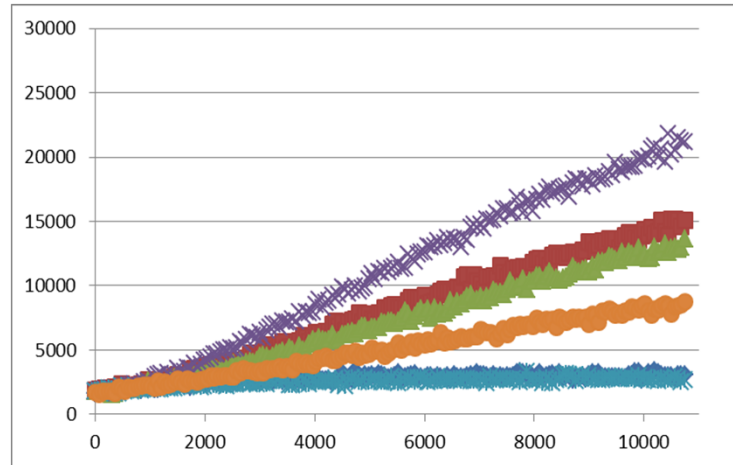
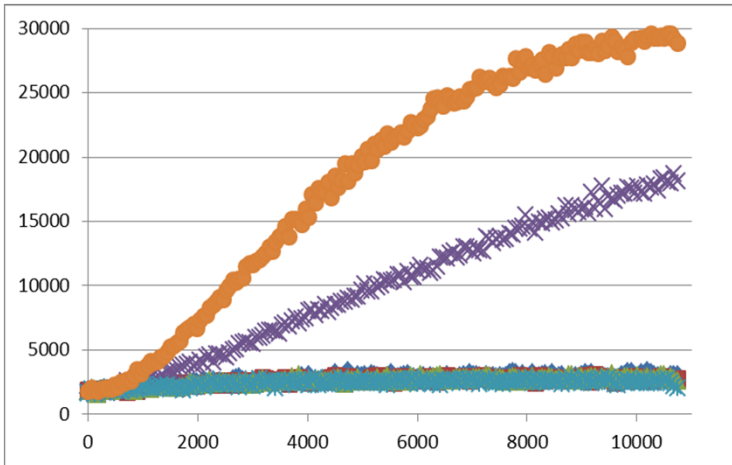
30°C

Week 3



- ◆ - Sensor only
- - Wyeast American Ale I (1056)
- ▲ - Wyeast American Ale II (1272)
- ✕ - White labs Super High Gravity (WLP009)
- ✕ - White labs English Ale (WLP002)
- - Wyeast Weihenstephan Ale (3068)

Week 4



English ale and Weihenstephan ale strains demonstrate the most protease activity.

Super High Gravity strain has no protease activity in this experiment.

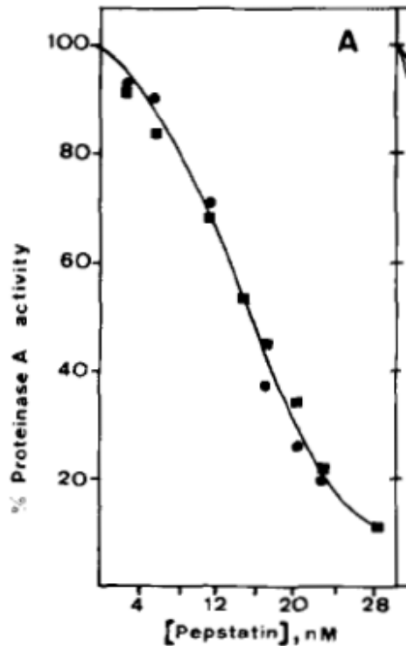
Is the PrA FRET sequence APAKFFRL selective for PrA only?

PrA = Aspartyl protease

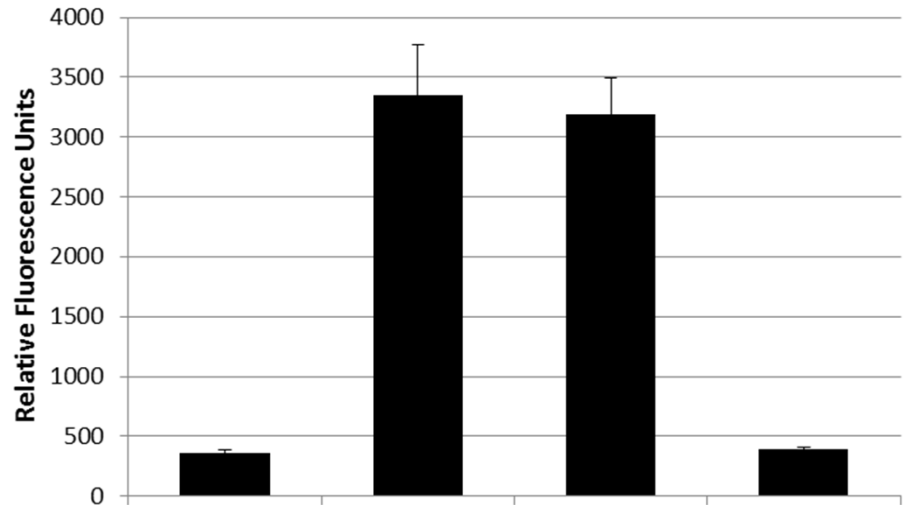
Pepstatin A = general aspartyl protease inhibitor

Chymostatin = general serine protease inhibitor

Purified protease (0.3uM)



Meussdoerffer *et al.* (1980) *JBC*

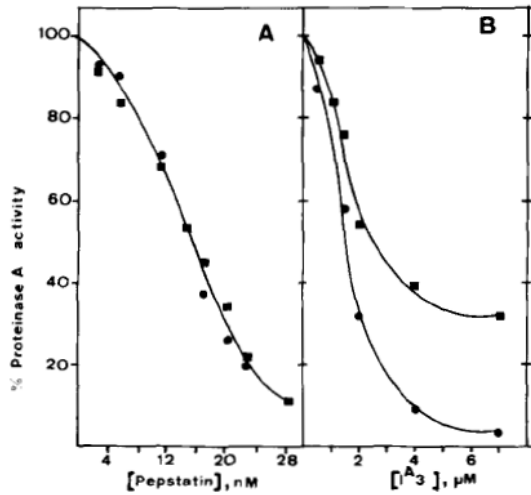


PrA biosensor -	+	+	+	+
Beer w/ Protease activity -	-	+	+	+
Pepstatin A (1uM) -	-	-	+	-
Chymostatin (1uM) -	-	-	-	+

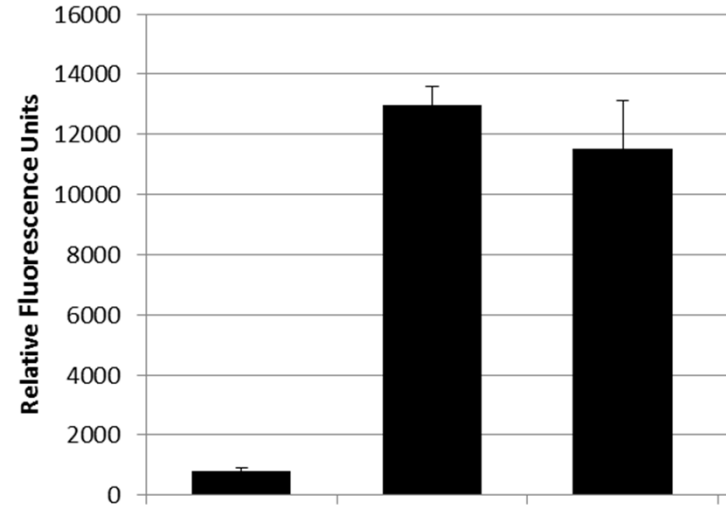
These results suggest PrA is not the active protease in the beer sample and that APAKFFRL is cleaved by another protease.

Will a more selective PrA inhibitor block cleavage of the APAKFFRL sequence?

Purified protease (2uM)



Meussdoerffer *et al.* (1980) *JBC*



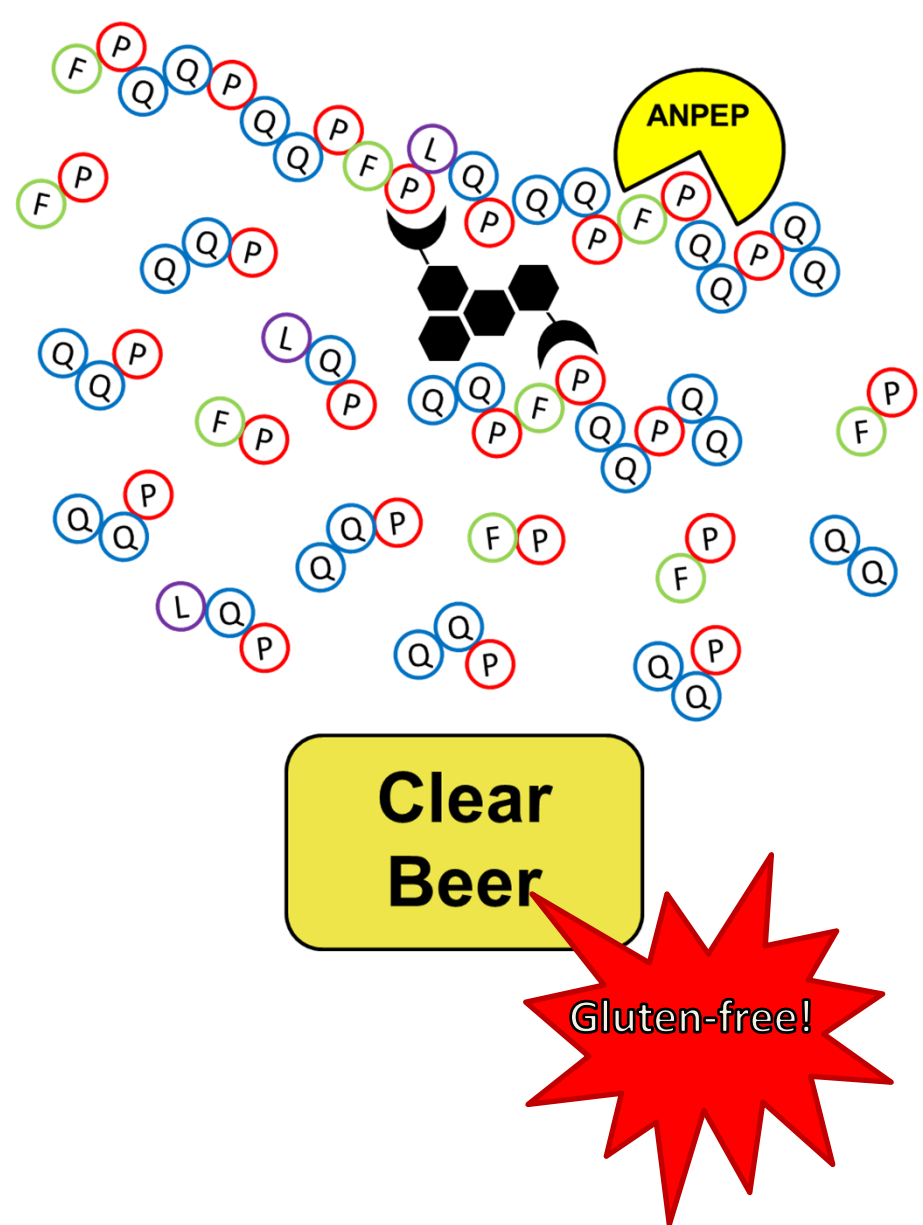
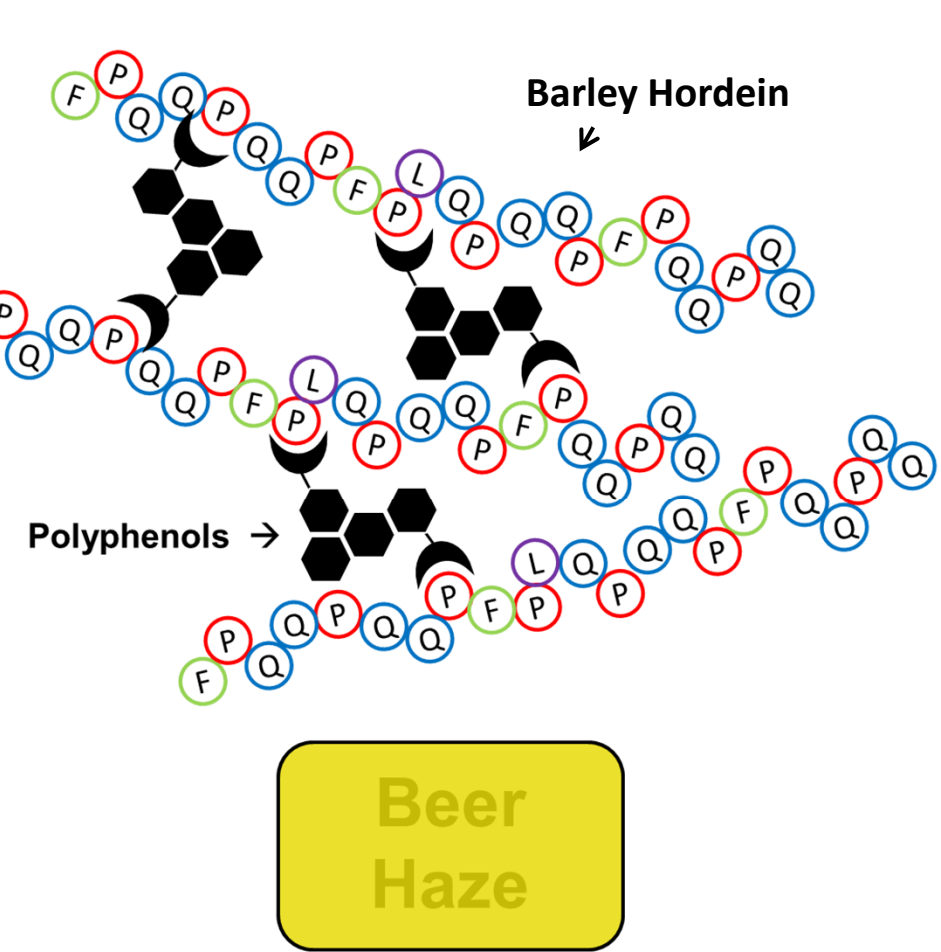
PrA biosensor -	+	+	+
Beer w/ Protease activity -	-	+	+
Recombinant IA3 (5uM) -	-	-	+

Recombinant IA3 does not inhibit cleavage of the APAKFFRL sequence in beer, suggesting another protease is responsible.

Summary Part I

- We have successfully built fluorescent biosensors for the measurement of protease activity in beer.
- The published PrA cleavage sequence, APAKFFRL, is less selective than previously suggested.
- This warrants caution when describing protease activity in beer as other proteases may be relevant.
- We are currently investigating the identity of additional proteases in beer.
- Regardless, different yeast strains exhibit varying protease activity in beer.
- Long term fermentation or conditioning on yeast may benefit from selection of yeast strains which exhibit no protease activity.

The use of *Aspergillus niger* prolyl endoprotease (ANPEP) during fermentation.



ANPEP biosensor design

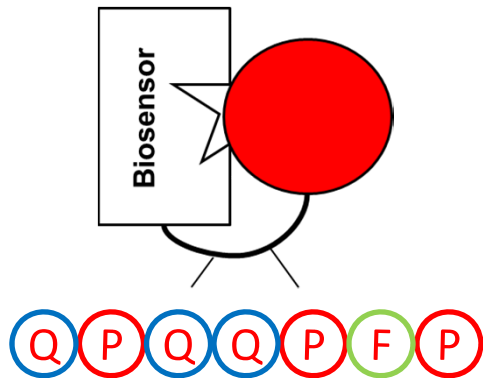
B- and C- Hordein account for over 90% of barley hordein.

- Shewrey *et al.* (1999) *Seed Proteins*

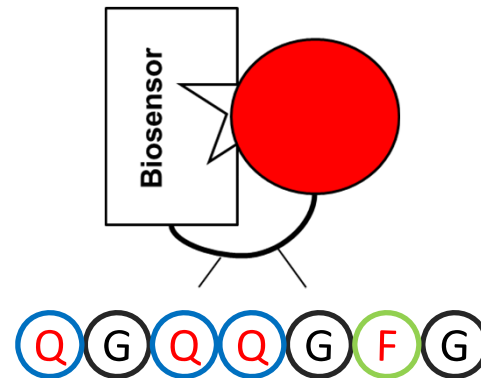
3 Peptides from gliadin, C-hordein, and secalin are responsible for 90% of all immunogenic responses in celiac patients. The responsible C-hordein peptide is: QPFPQPQQPFPQ

-Dromey *et al.* (2010) *Science Trans. Med.*

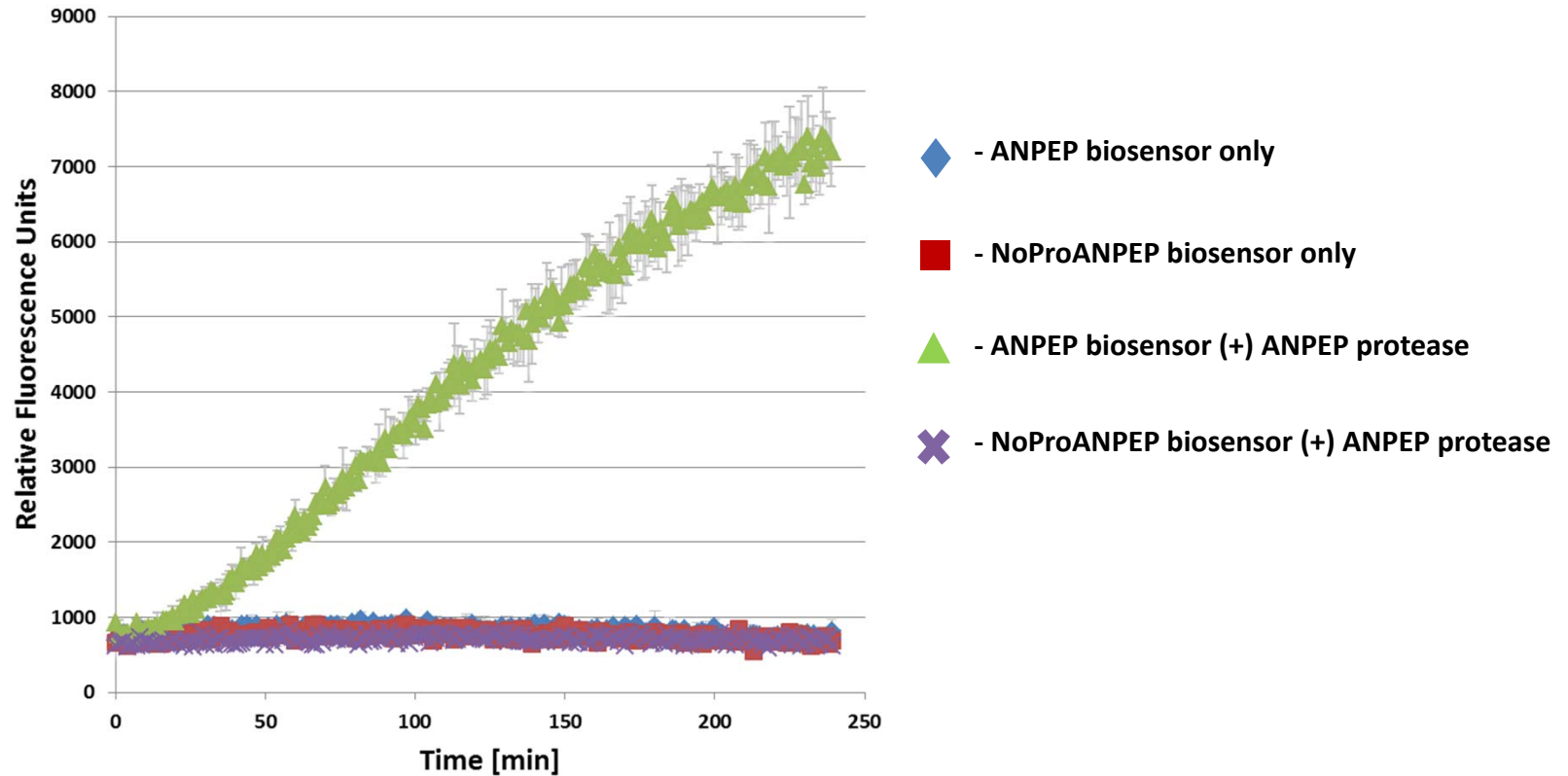
ANPEP biosensor



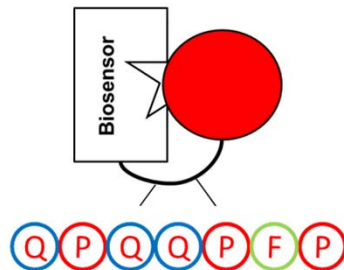
NoProANPEP biosensor



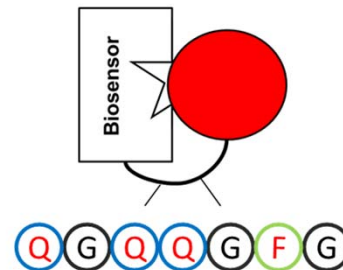
ANPEP biosensor is efficiently cleaved by ANPEP. The mutation of Prolines prevents cleavage.



ANPEP biosensor



NoProANPEP biosensor

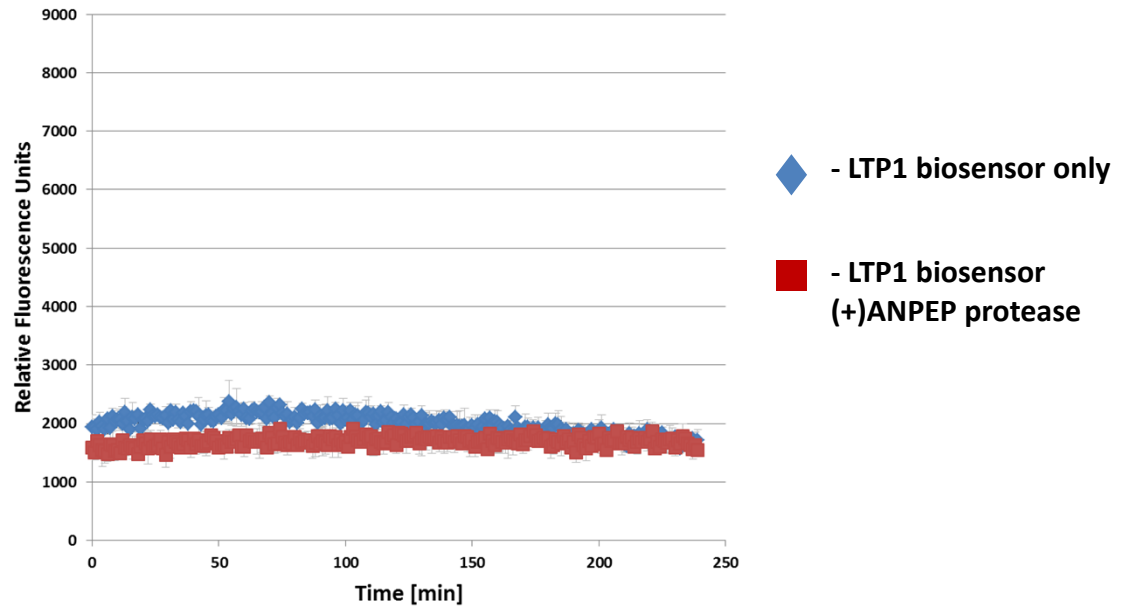
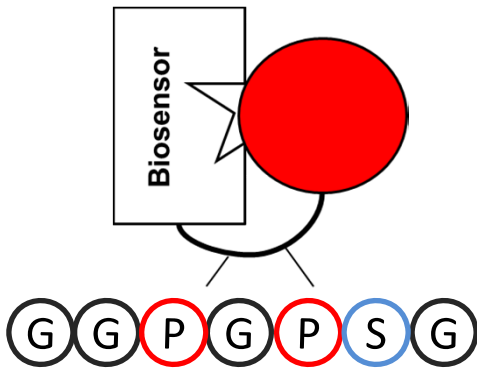


Might ANPEP exhibit non-specific cleavage of other beer proteins?

Protein sequence of barley LTP1

```
10      20      30      40      50      60
MARAQVLLMA AALVLMLTAA PRAAVALNCG QVDSKMKPCL TYVQGGPGPS GECCNGVRDL
70      80      90      100     110
HNQAQSSGDR QTVCNCLKGI ARGIHNLNLN NAASIPSKCN VNVPTISPD IDCSRIY
```

LTP1 biosensor



GGPGPSG is not cleaved by ANPEP.

Summary Part II

- ☛ We have successfully built a fluorescent ANPEP biosensor.
- ☛ The biosensor could find use in quality control of ANPEP protease activity or optimization of engineered protease variants in the future.
- ☛ We have begun experiments which examine off-target effects of ANPEP.
- ☛ The LTP1 sequence GGGPSG is not a substrate.

This is surprising as the dipeptide, Z-GlyPro-pNA has been successfully used as an ANPEP substrate in the past.

- Edens *et al.* (2005) *J. Ag. & Food Chem.*

Advantages of our protease biosensors

- Detection of nM concentrations of protease using only 4ul of beer in a 100ul reaction.
- Different protease biosensors are generated by changing the linker sequence.
- Linker length is variable with little impact on efficacy.
- Combines protein-based physiology with FRET specificity.

Acknowledgements

P.I. – Peter Berget, PhD



Interested in collaborating, contracting, or funding?

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