Lipolytic enzymes in the gastric fluids of *Cancer pagurus* are capable of hydrolyzing bioplastics

Lukas Miksch, Reinhard Saborowski, Lars Gutow

Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany

Plastic in the environment

Plastic products are indispensable for daily life

Excessive use and poorly controlled discharge

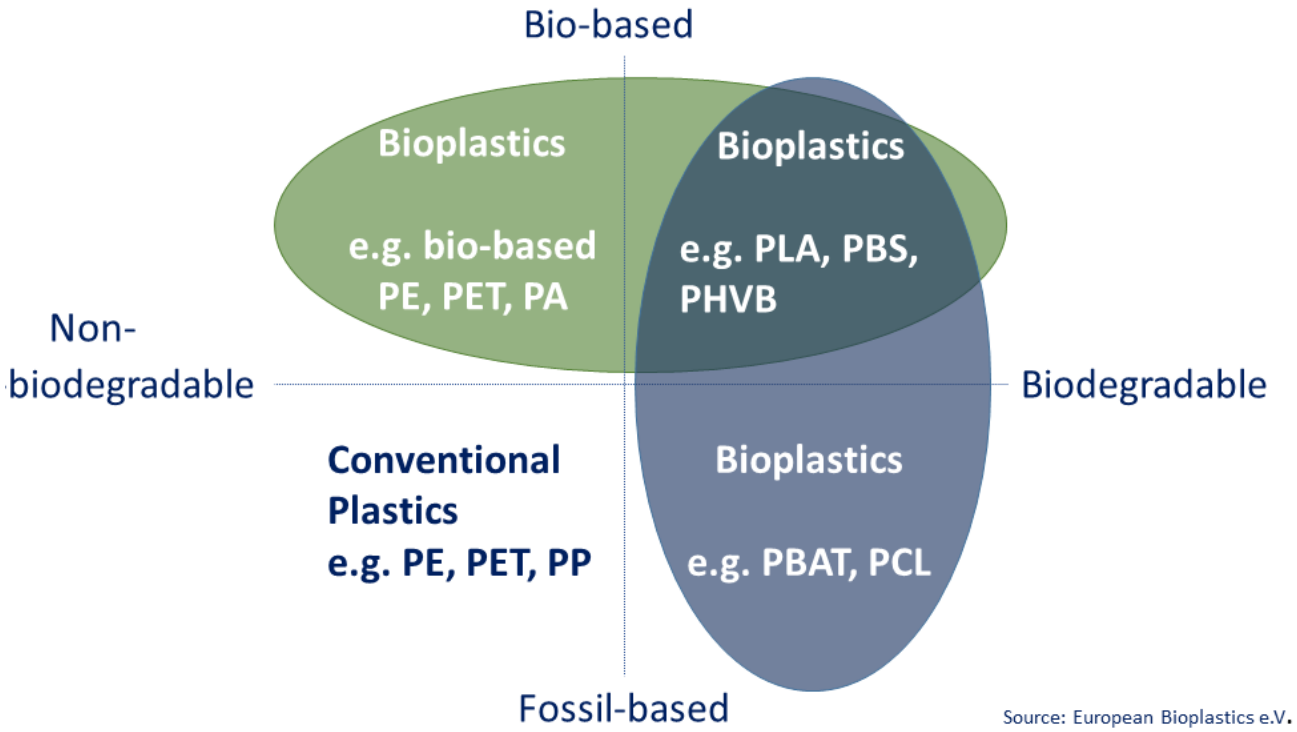
Massive contamination of ecosystems

Sources, pathways and impacts of plastic in the ocean [a]

Bioplastics

Either bio-based, biodegradable or both

Bioplastic labels from different organisations [c,d,e]



Material coordinate system of bioplastics [b]

Promising alternative to conventional plastics

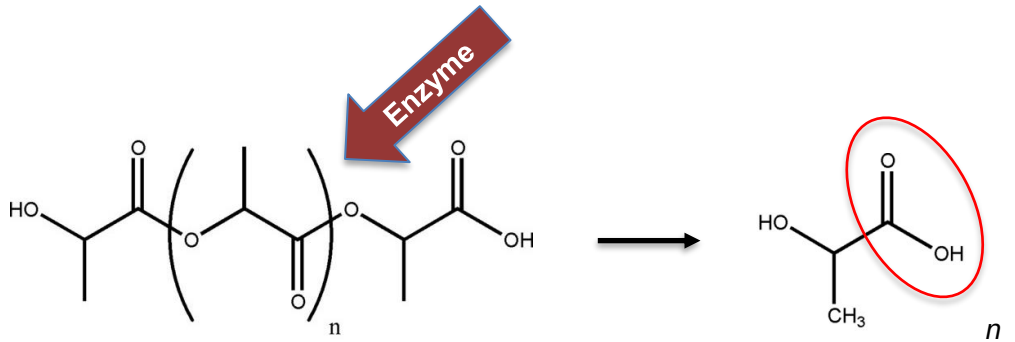
Enzymatic Degradation of Bioplastics

Degradation by hydrolytic enzymes

Hydrolytic cleavage of ester bonds

→ Release of carboxylic end groups

(acidification)



PLA **Lactic acid**

pH Stat Titration

Maintaining a constant pH by adding NaOH

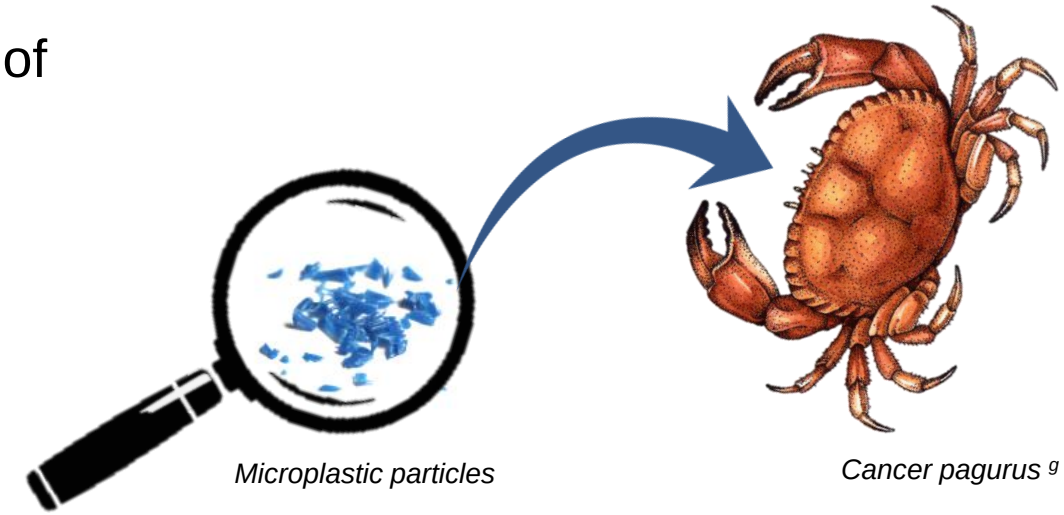
Hydrolysis can be measured by the added volume of NaOH

Titrator (TitroLine® 7000) with minichiller [f]

Cancer pagurus

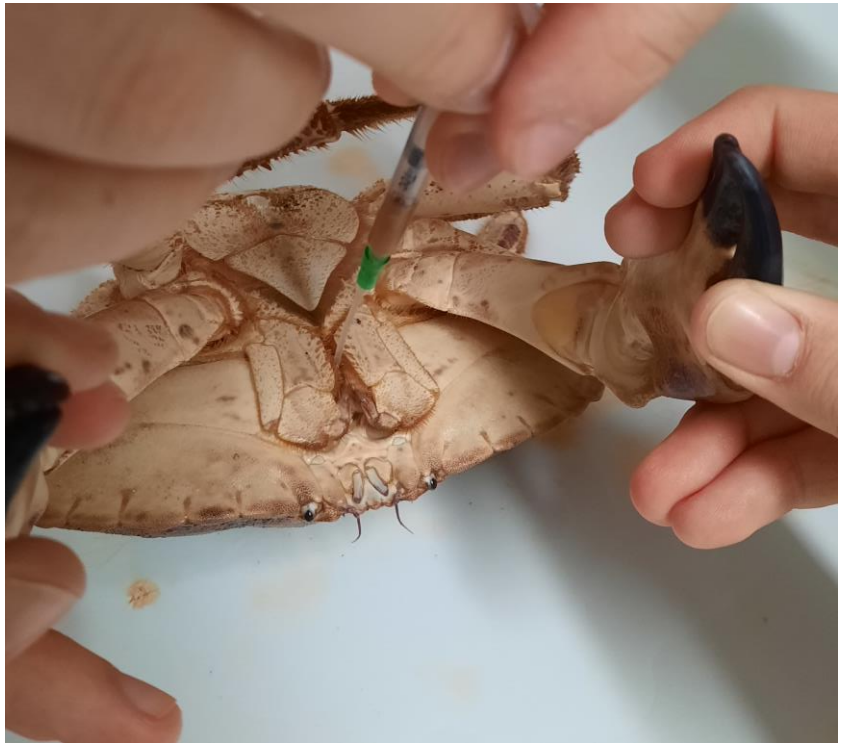
Highly active enzyme mixture in digestive fluids of decapod crustaceans

→Hydrolysis of bioplastics after ingestion?



Microplastic particles

Cancer pagurus [g]



Extraction of gastric fluids with a PTFE-tube and syringe

Extraction of fluids from gastric chamber

→ *In-vitro* degradability of bioplastics with gastric fluids

pH Stat Titration

Hydrolysis of (bio)-plastics

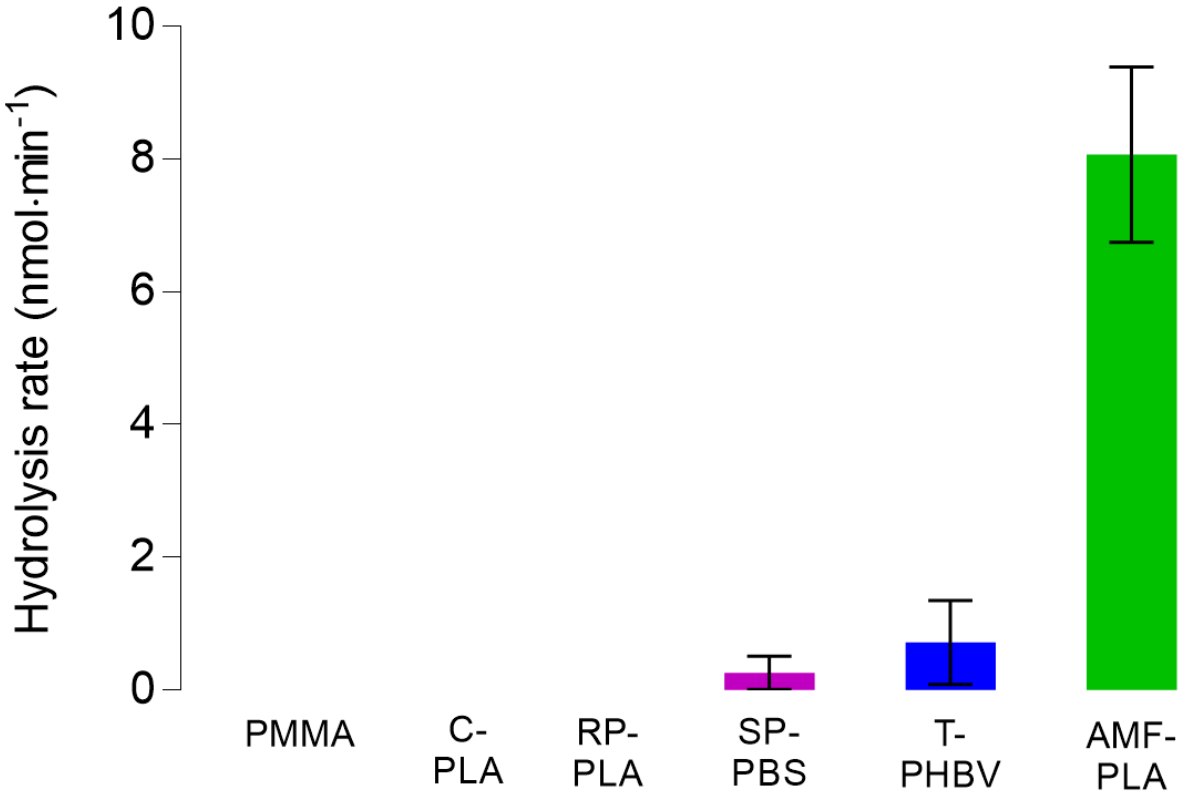
30 mg plastic particles
(<200 µm) in 10 mL seawater

100µL gastric fluid

Seawater conditions

15 °C, pH 8.2

3.2% salinity



Hydrolysis rates of different plastics with gastric fluids of C. pagurus

AMF-PLA

Bioplastic blend of
- Polylactic acid (PLA)
- Polybutylene adipate-co-terephtalate (PBAT)

Biodegradable mulch film [h]

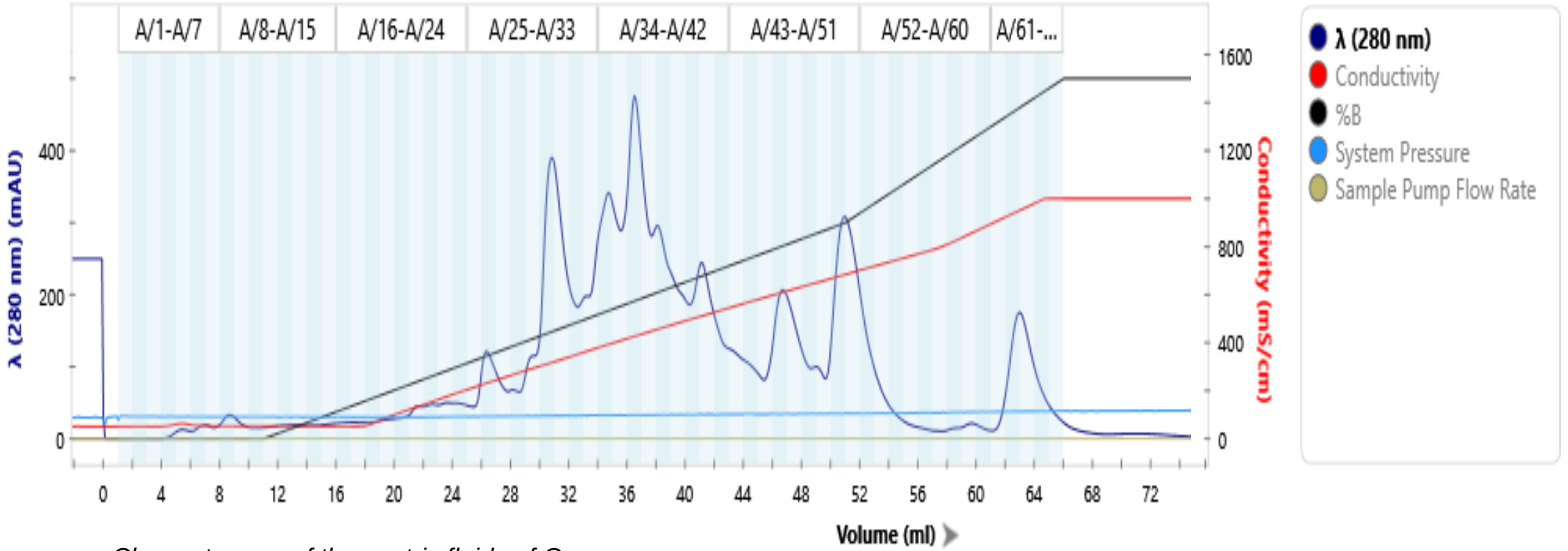
PBAT is hydrolyzed by Lipase (Herrera et al. 2002)

→ **Lipolytic enzymes in gastric fluids of *C. pagurus*?**

Anion Exchange Chromatography

Separation of the proteins in the gastric fluids by charge

→ 65 fractions of 1 mL each



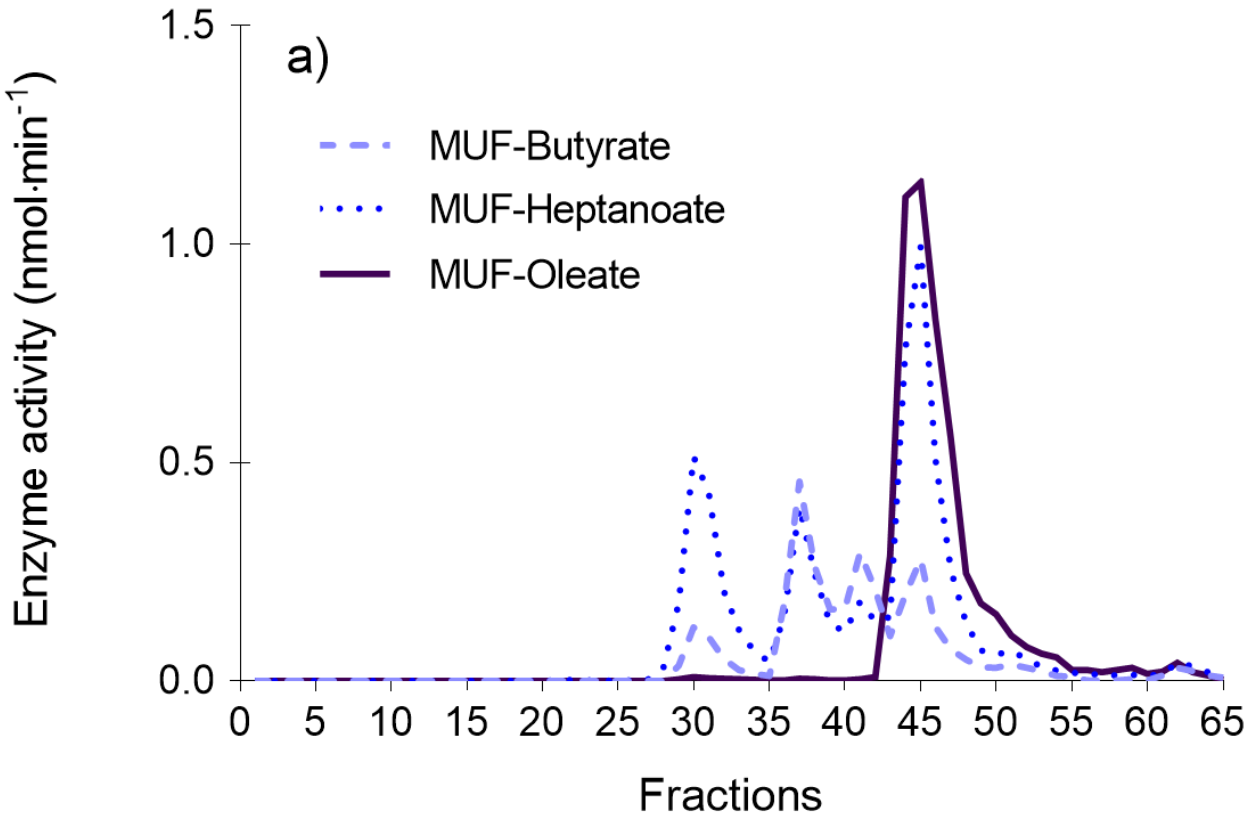
Chromatogram of the gastric fluids of C. pagurus

Enzyme Activity in the Fractions

Lipolytic activity in the gastric fluid fractions?

Methylumbelliferyl (MUF)-derivatives for esterase/lipase activity

Highest activities around fractions 30, 37, 41 and 45



Hydrolytic Potential of Gastric Fluid Fractions

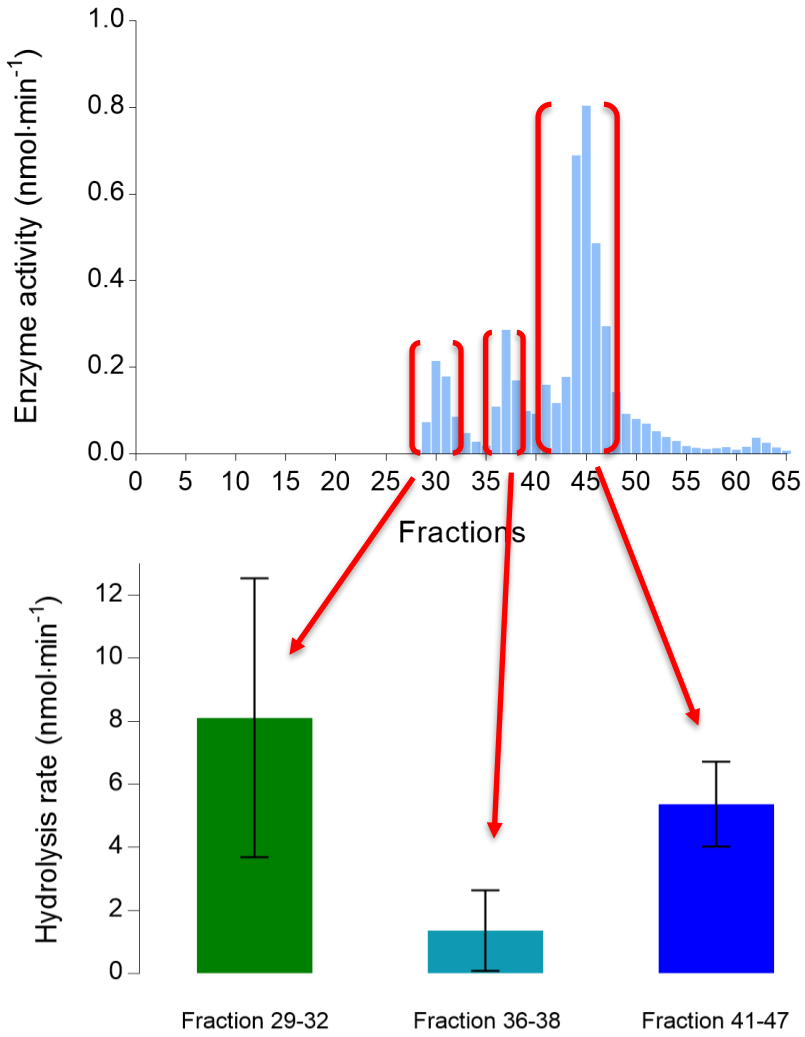
Fractions with high lipolytic activity were pooled

→ Fraction **29-32**, Fraction **36-38**, Fraction **41-47**

Concentration via ultrafiltration

Determining hydrolysis rates with pH Stat

→ All three pooled fraction hydrolyze AMF-PLA



Gel Electrophoresis (SDS-PAGE)

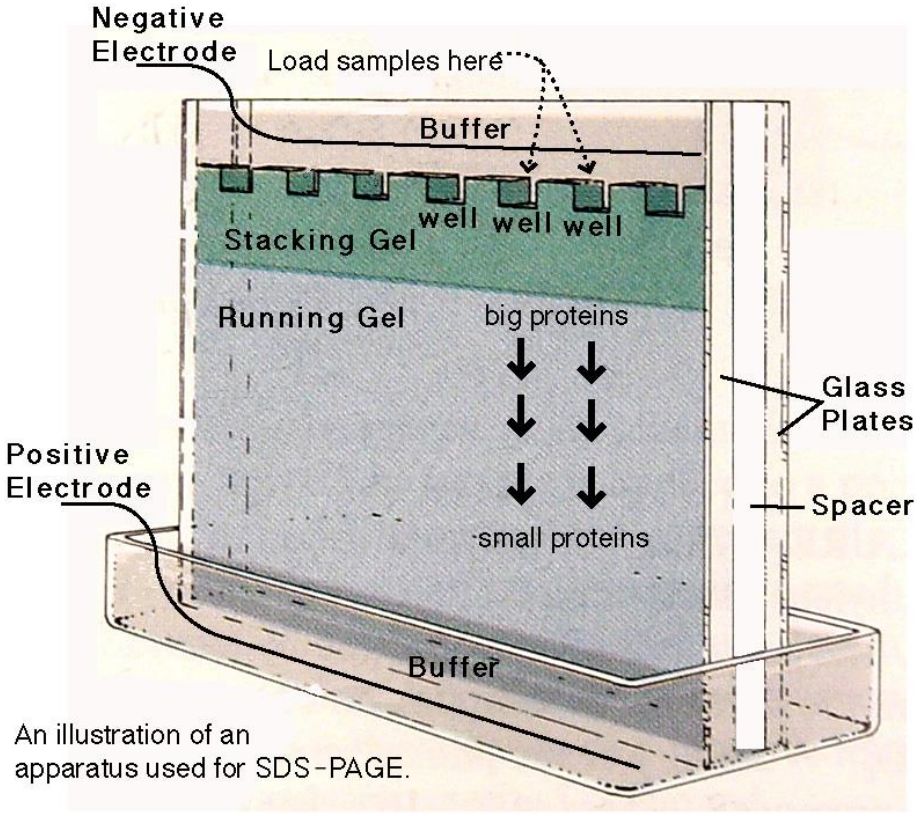
Protein separation by molecular mass

Fraction 25 – Fraction 55

After separation:

→ Soaking in fluorogenic substrate solution

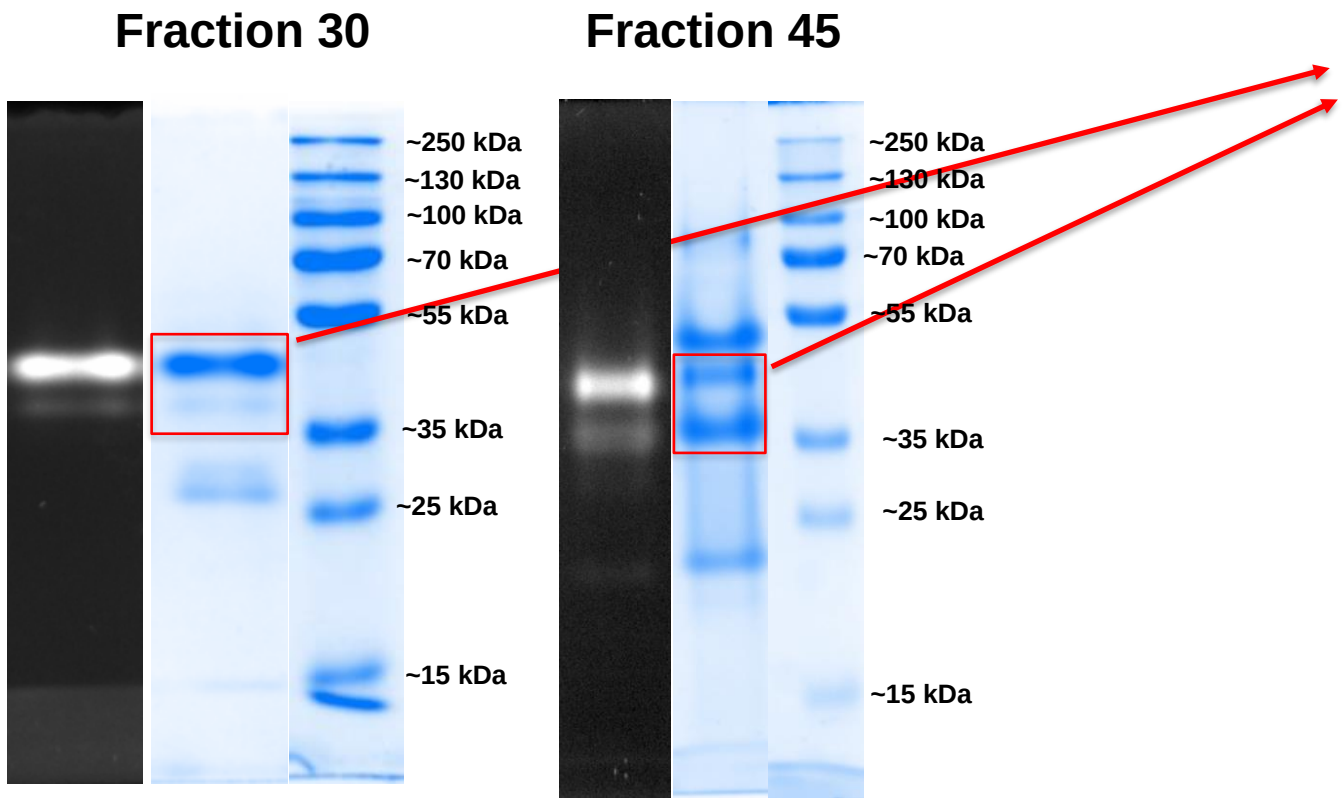
→ Protein staining with Coomassie brilliant blue



SDS-Page setup [i]

Gel Electrophoresis (SDS-PAGE)

Lipolytic activity around 45 kDA



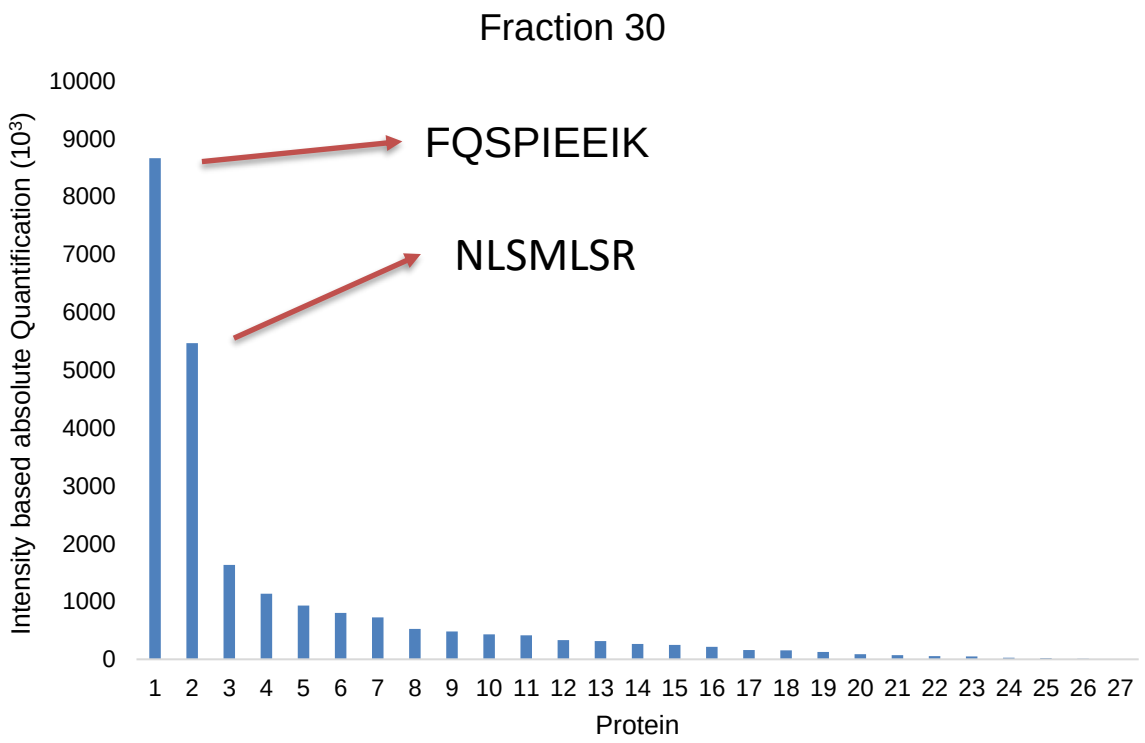
Proteome Center Tuebingen

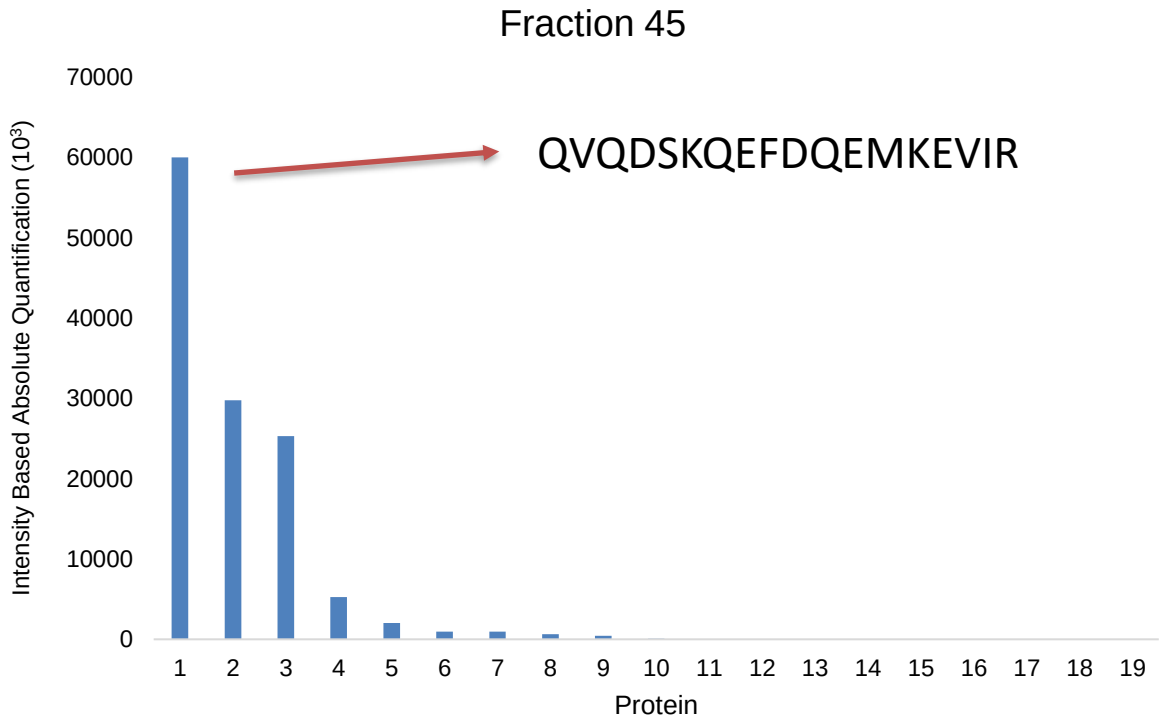
Tryptic digestion of protein bands

High resolution mass spectrometry

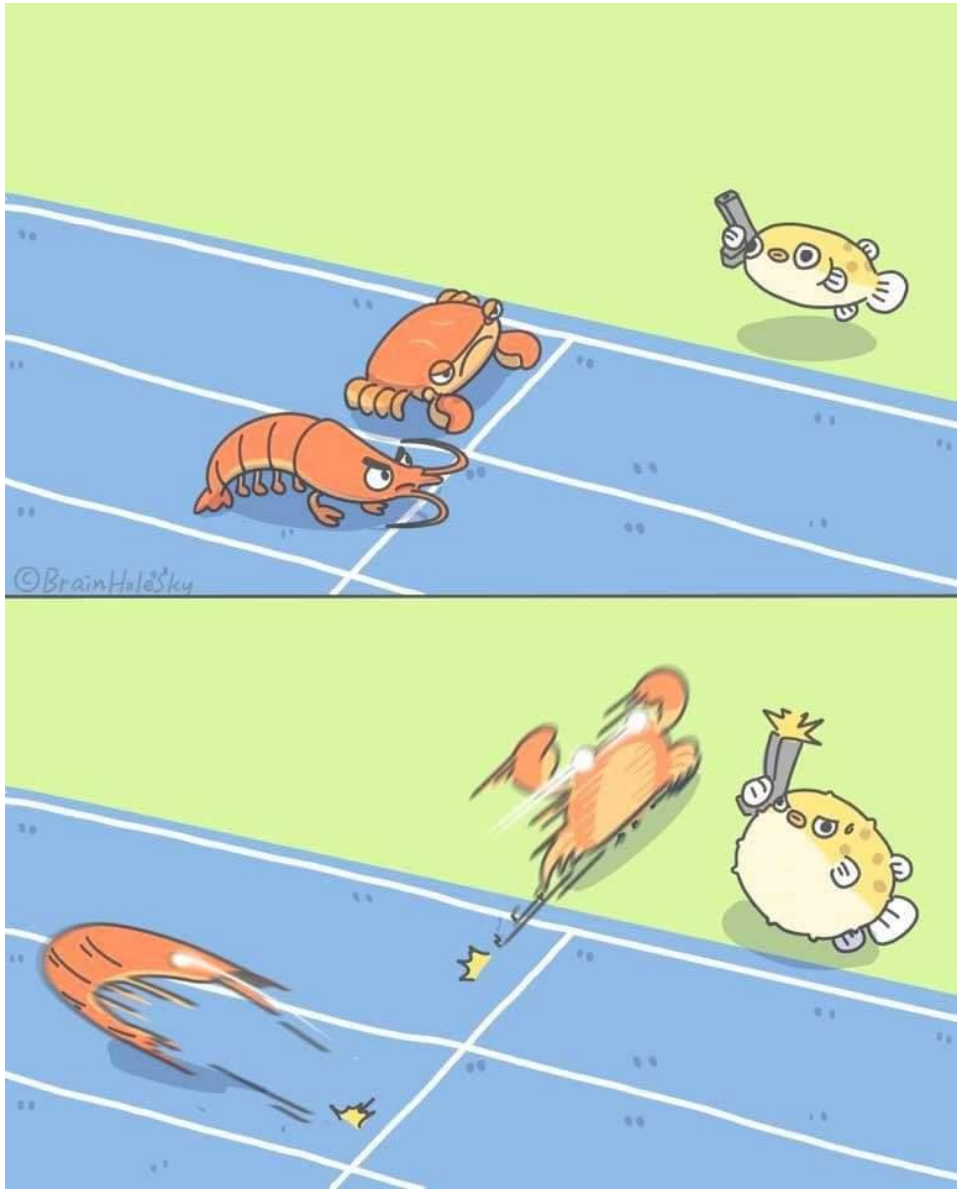
→ Sequences of peptide bonds

Quantitative Proteomics





→ Sequencing of *C. pagurus* midgut gland



Thank you for your attention!

Sources

S.B. Borrelle, J. Ringma, K.L. Law, C.C. Monnahan, L. Lebreton, A. McGivern, E. Murphy, J. Jambeck, G.H. Leonard, M.A: Hilleary, M. Eriksen, H.P. Possingham, H. De Frond, L.R. Gerber, B. Polidoro, A. Tahir, M. Bernard, N. Mallos, M. Barnes, C.M. Rochman. Predicted growth in plastic waste exceeds efforts to mitigate plastic pollution. Science. 369 (2020)1515-1518. https://doi.org/10.1126/science.aba3656.

Jambeck, J. R., Geyer, R., Wilcox, C., Siegler, T. R., Perryman, M., Andrady, A., ... & Law, K. L. (2015). Plastic waste inputs from land into the ocean. Science, 347(6223), 768-771. https://doi.org/10.1126/science.1260352

L. Lebreton, B. Slat, F. Ferrari, B. Sainte-Rose, J. Aitken, R. Marthouse, S. Hajbane, S. Cunsolo, A. Schwarz, A. Levivier, K. Noble, P. Debeljak, H. Maral, R. Schoeneich-Argent, R. Brambini, J. Reisser. Evidence that the great pacific garbage patch is rapidly accumulating plastic. Scientific Reports 8 (2018) 4666. https://doi.org/10.1038/s41598-018-22939-w.

J.A. Ivar do Sul, M.F. Costa. The present and future of microplastic pollution in the marine environment." Environmental pollution 185 (2014) 352-364. https://doi.org/10.1016/j.envpol.2013.10.036.

S. Kühn, E.L. Bravo Rebolledo, J.A. Van Franeker. Deleterious effects of litter on marine life. In: M. Bergmann, L. Gutow, M. Klages. Marine Anthropogenic Litter (2015) 75-116. https://doi.org/10.1007/978-3-319-16510-3.

A.U.B. Queiroz, F.P. Collares-Queiroz. Innovation and industrial trends in bioplastics. Polymer Reviews 49 (2009) 65-78. https://doi.org/10.1080/15583720902834759.

B. Singh, N. Sharma. Mechanistic implications of plastic degradation. Polymer Degradation and Stability 93 (2008): 561-584. https://doi.org/10.1016/j.polymdegradstab.2007.11.008.

Saborowski, R., Sahling, G., Del Toro, M. N., Walter, I., & Garcıa-Carreno, F. L. (2004). Stability and effects of organic solvents on endopeptidases from the gastric fluid of the marine crab Cancer pagurus. *Journal of Molecular Catalysis B: Enzymatic*, *30*(3-4), 109-118.

Hehemann, J. H., Redecke, L., Murugaiyan, J., von Bergen, M., Betzel, C., & Saborowski, R. (2008). Autoproteolytic stability of a trypsin from the marine crab Cancer pagurus. *Biochemical and biophysical research communications*, *370*(4), 566-571.

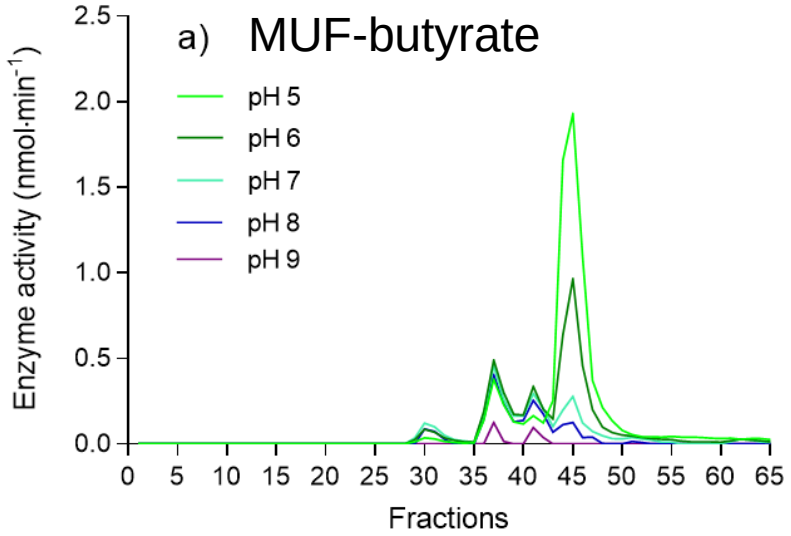
Herrera, R., Franco, L., Rodríguez-Galán, A., & Puiggalí, J. (2002). Characterization and degradation behavior of poly (butylene adipate-co-terephthalate) s. Journal of Polymer Science Part A: Polymer Chemistry, 40(23), 4141-4157. https://doi.org/10.1002/pola.10501

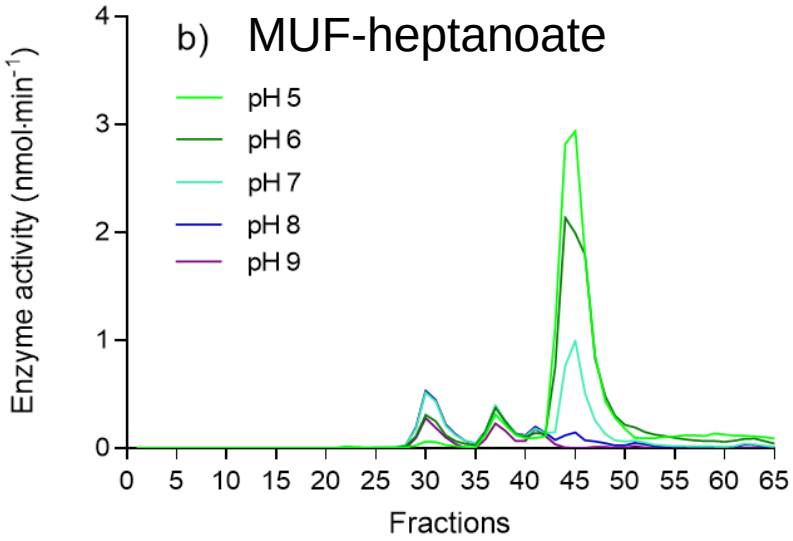
L. Miksch, L. Gutow, R. Saborowski. pH-Stat titration: a rapid assay for enzymatic degradability of bio-based polymers. Polymers 13 (2021) 860. https://doi.org/10.3390/polym13060860.

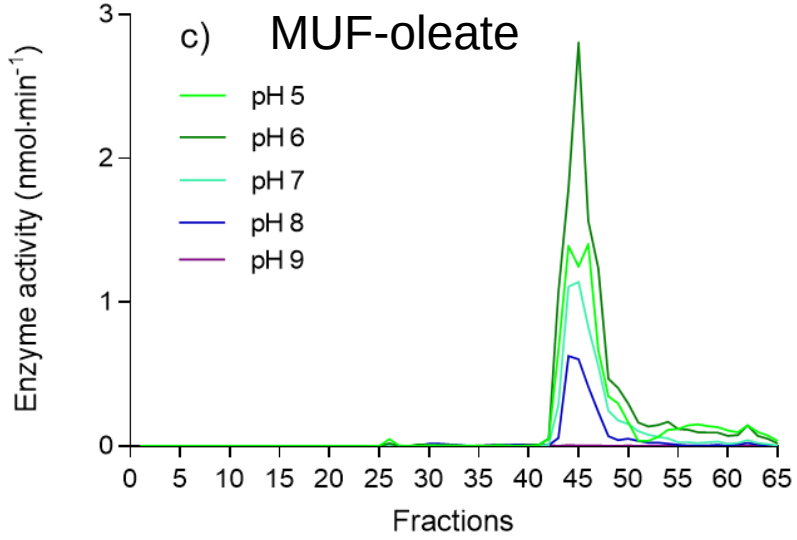
Image Sources

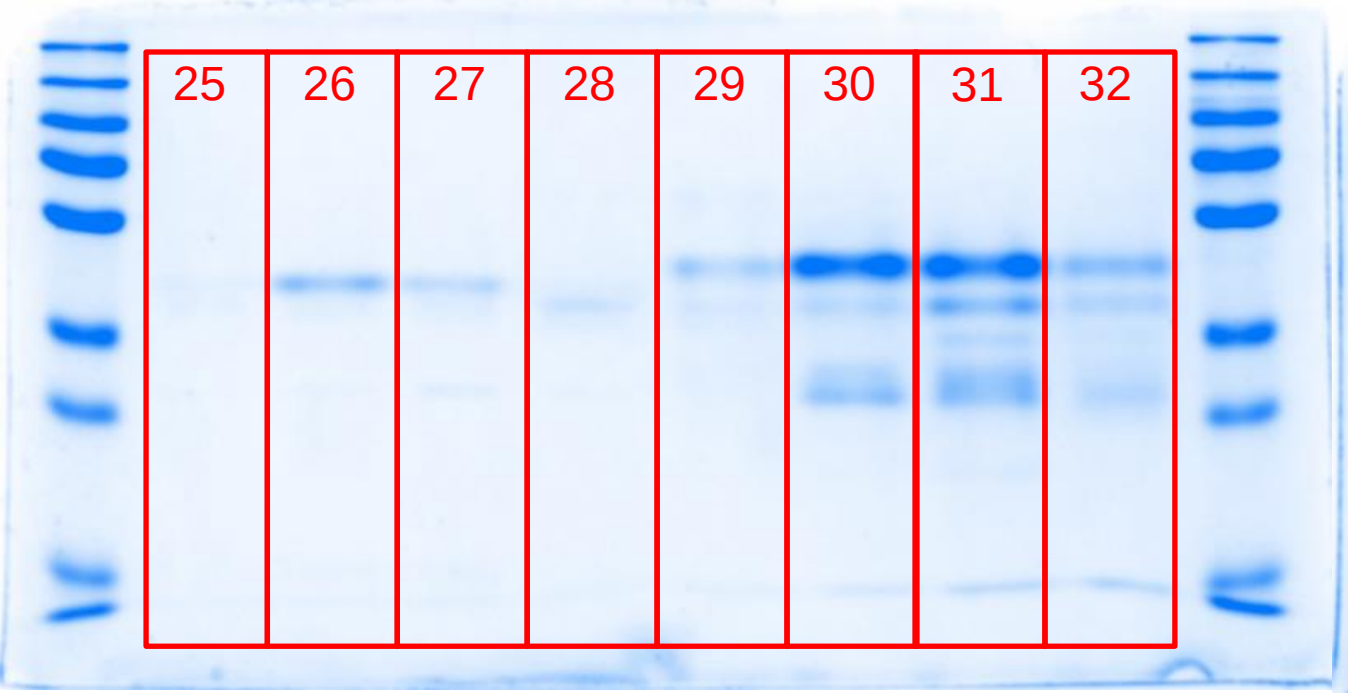
a. https://marinedebris.noaa.gov/images/plastics-ocean-infographic
b. https://www.spexsampleprep.com/6775freezermill-for-cryogenic-grinding
c. https://www.european-bioplastics.org/bioplastics/standards/labels/
d. https://apnews.com/article/lifestyle-composting-9f421243df6343a89407f423d37087cb
e. https://bio-fed.com/certifications/
f. www.european-bioplastics.org
g. https://lizzieharper.co.uk/2013/04/pen-and-ink-techniques-colour/stippled-edible-crab-illustration-showing-pen-and-ink-techniques-with-colour-by-lizzie-harper/
h. https://www.indiamart.com/proddetail/biodegradable-mulch-film-16343454991.html
i. https://experiment.com/u/2WbQ0Q

pH Profile of Esterase Activity





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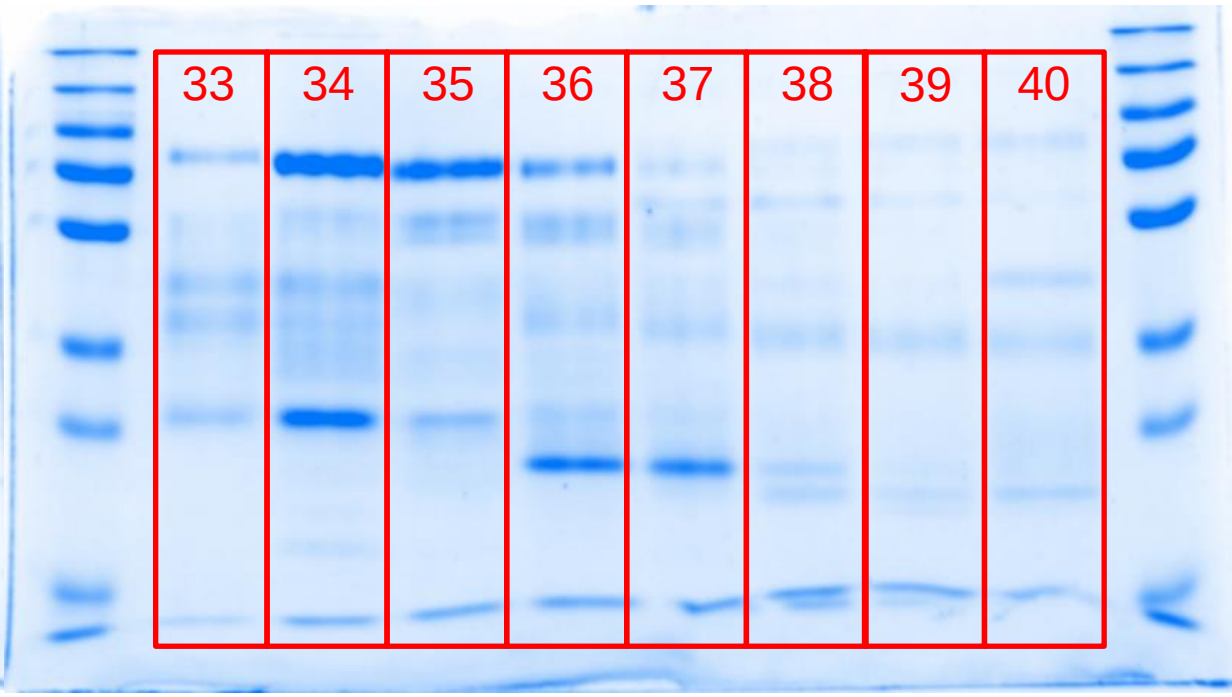
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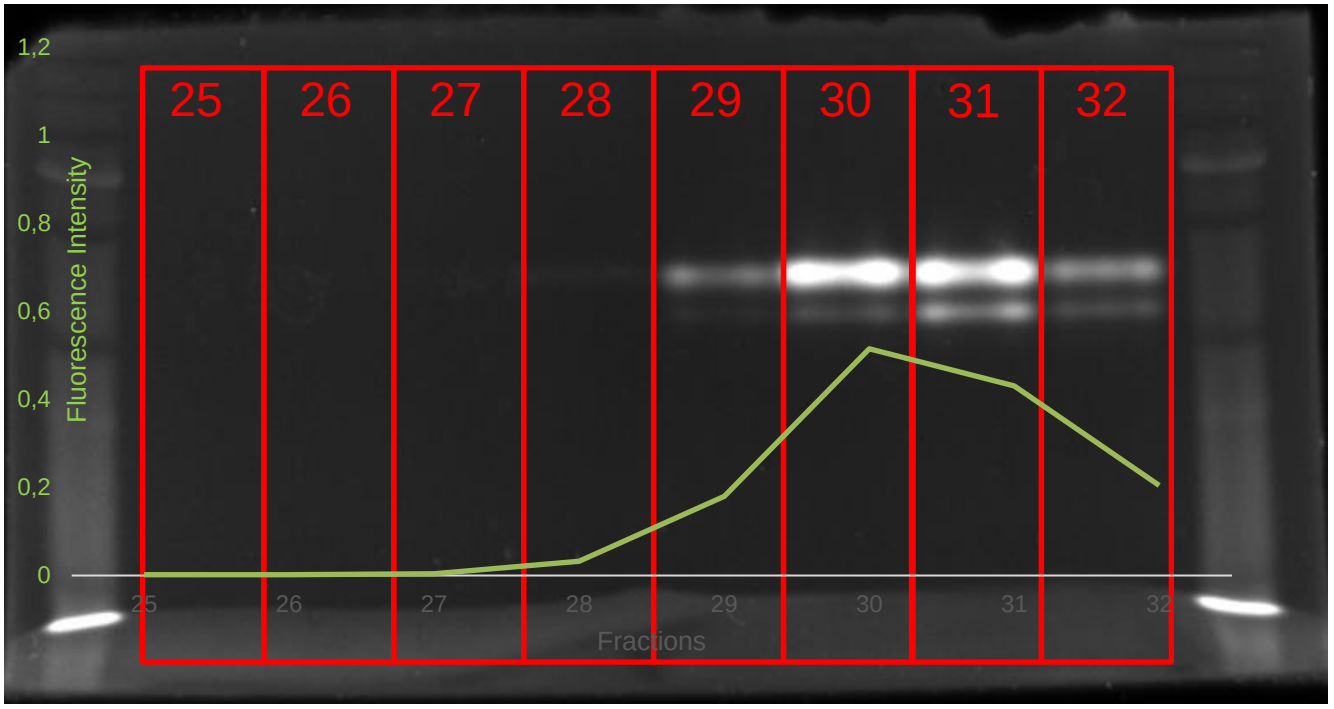
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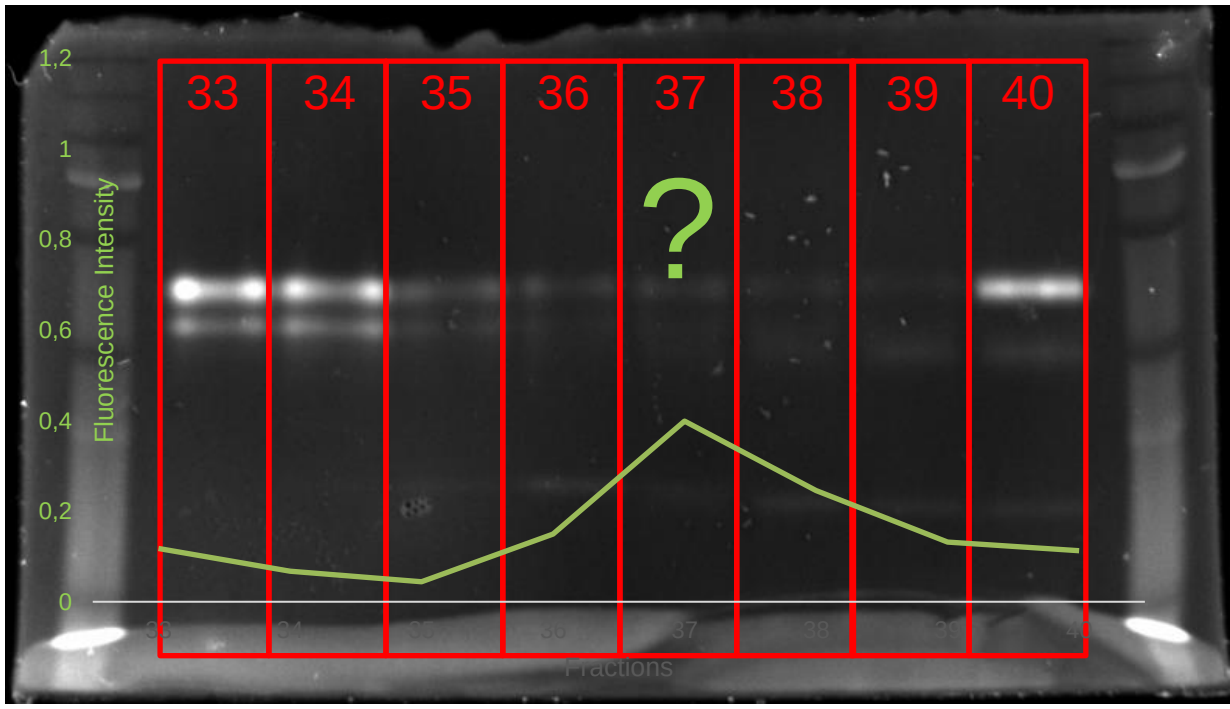
Fluorescence Intensity
Fractions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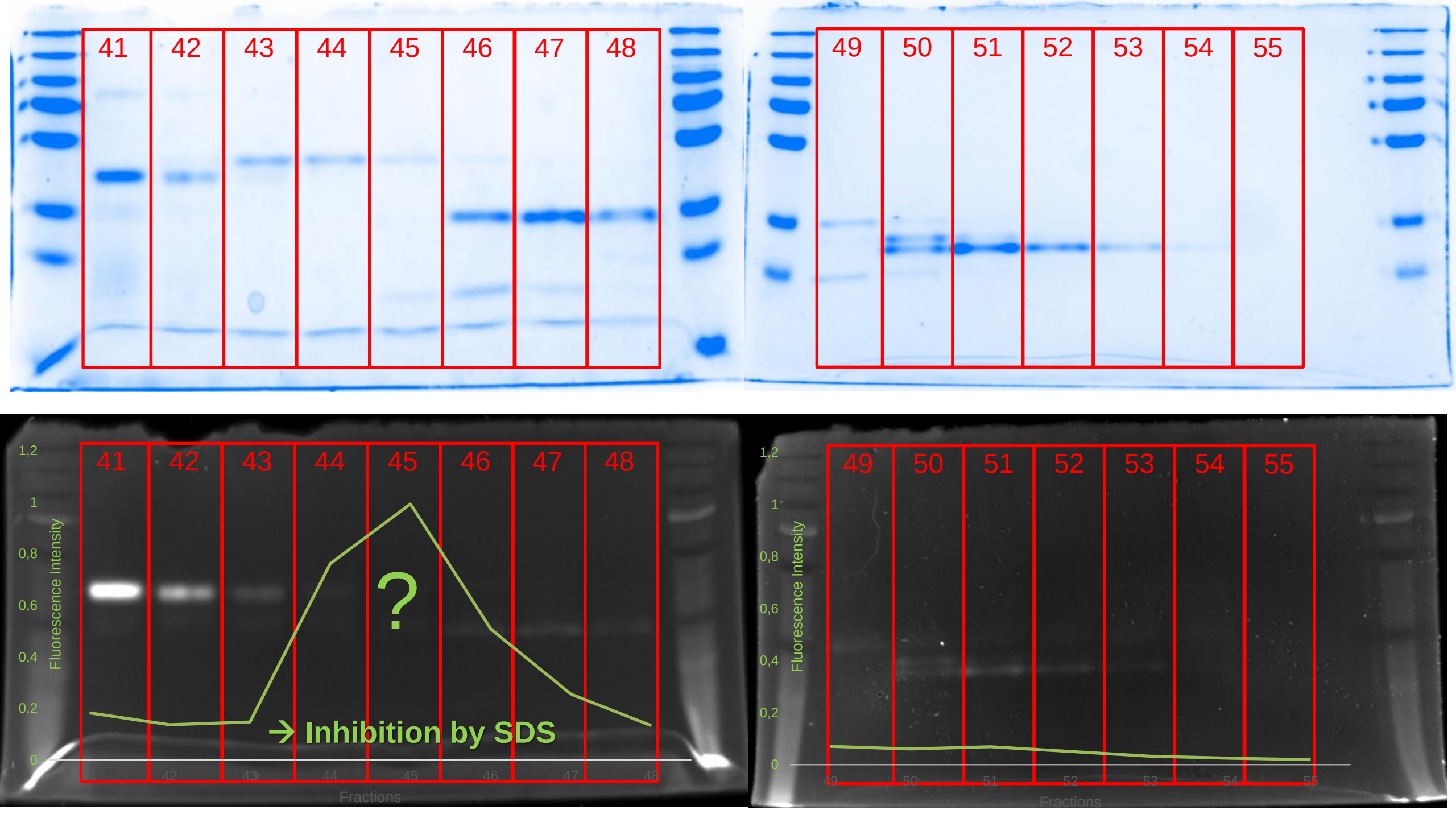
Fluorescence Intensity
Fractions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Fluorescence Intensity
?
→ Inhibition by SDS
Fractions

Effect of SDS on lipolytic activity