https://ntrs.nasa.gov/search.jsp?R=20170012499 2019-08-30T16:41:34+00:00Z

National Aeronautics and Space Administration

# Enzymatic Activity Detection via Electrochemistry for Enceladus

Lucy Studemeister[1,2,3], Jessica Koehne[2], Richard Quinn[2,3]
[1] Santa Clara University; [2] NASA Ames; [3]SETI Institute
lstudemeister@scu.edu

## Abstract

Electrochemical detection of biological molecules is a pertinent topic and application in many fields such as medicine, environmental spills, and life detection in space. Proteases, a class of molecules of interest in the search for life, catalyze the hydrolysis of peptides. Trypsin, a specific protease, was chosen to investigate an optimized enzyme detection system using electrochemistry. This study aims at providing the ideal functionalization of an electrode that can reliably detect a signal indicative of an enzymatic reaction from an Enceladus sample.

## Methods[1]

A macro-scale electrode system was researched and employed to study optimal enzymatic detection. The main functionalization components are: a redox-labeled sensing probe (1a), thiol spacer (1b), and a backfill molecule (1c). Method of detection is illustrated by Figure 2: Trypsin is introduced to the sample and the electrochemical signal decreases over time due to cleavage of the sensing probe via Trypsin. The sensing probe (Fig 3) was synthesized in lab.

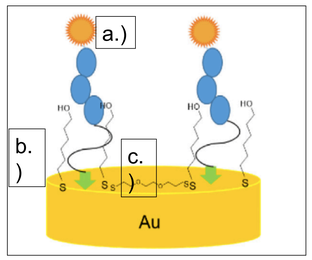



**Figure 1: Functionalization of gold electrode surface[1].** Redox labeled sensing probe (a), dithiol spacer (b), backfill mercaptohexanol molecule (c).

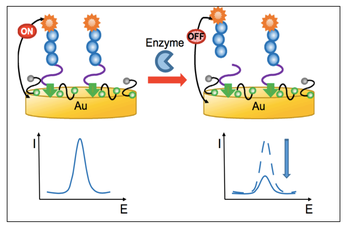



**Fig 2: Method of trypsin detection[1].** Enzyme cleaves peptide and signal decrease is observed.

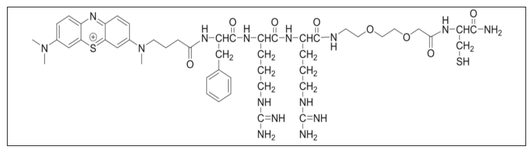


**Figure 3: Sensing probe[1].** From left to right: methylene blue, phenylalanine, arginine, arginine, polyethylene glycol, cysteine.

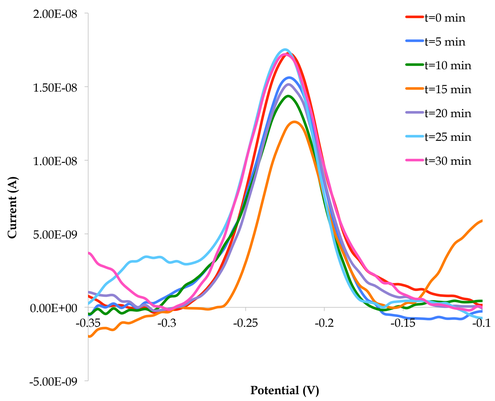



**Figure 4**: Cyclic voltammogram of fully functionalized gold electrode over time in 1X PBS.

**Table 1:** Mean peak heights (n=4), standard deviation, and change in peak height over time

| Time (min) | Mean Peak Height (Amps) | Standard Deviation | Percent Change (%) |
|---|---|---|---|
| t=0 | 1.2E-08 | 5.5E-09 | N/A |
| t=5 | 1.1E-08 | 4.6E-09 | -3 |
| t=10 | 1.1E-08 | 3.3E-09 | -5 |
| t=15 | 9.1E-09 | 3.3E-09 | -23 |
| t=20 | 1.1E-08 | 4.8E-09 | -9 |
| t=25 | 1.1E-08 | 5.7E-09 | -5 |
| t=30 | 1.0E-08 | 5.5E-09 | -15 |

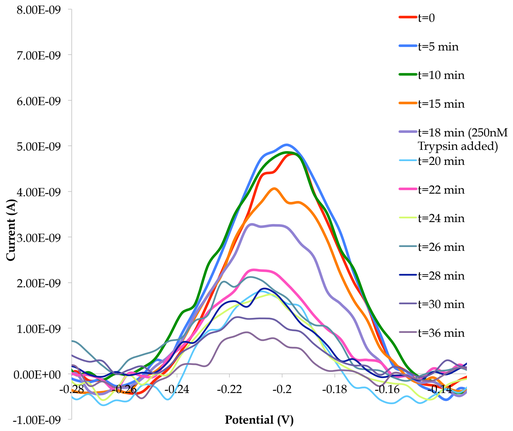
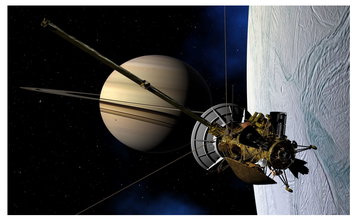



**Figure 5**: Cyclic voltammogram of fully functionalized gold electrode over time in 1X PBS, with Trypsin introduced at t=18 minutes.

**Table 2**: Percent change of peak height of fully functionalized gold electrode over time in 1X PBS. At t=18 minutes, 250nM Trypsin is introduced

| Time (min) | Percent Change (%) |
|---|---|
| 5 | 4 |
| 10 | -3 |
| 15 | -13 |
| 18 | -28 |
| 20 | -54 |
| 22 | -55 |
| 24 | -56 |
| 26 | -58 |
| 28 | -64 |
| 30 | -76 |



## Results

An optimal functionalization procedure resulted in the stable assembly of molecules on a gold surface. Figure 1 shows both the kinetic dynamics of thiols on gold over time and the stability of the sensing probe over time. Stability results found that the maximum amount of peak height decrease over 30 minutes was 30%. Table 1 shows that the overall mean decrease of peak height from several trials was 15%. In comparison, with Trypsin present, a 76% peak height decrease was measured after 30 minutes (Table 2). Results demonstrate that the proposed functionalization assembly, although fluid and dynamic, can successfully detect enzymatic activity.

## Future Work

The methods described will be applied to detect a variety of enzymes, including the 20S Proteasome, a potentially ubiquitous enzyme of life. With regards to the sensing probe, a variety of redox labels and fluorophores will be investigated, such as ferrocene, for simpler assembly and detection. Methods for covalent attachment of the sensing probe onto a carbon electrode will be researched in order to decrease the kinetic dynamics which occur with thiols on gold.

## Conclusion

In conclusion, enzymatic activity is detectable via the described electrochemical mechanism. Although the active and kinetic dynamics of thiols produce a fluid electrochemical signal, the functionalization method is still stable in comparison, and the addition of Trypsin demonstrates a major decrease in signal when present. This research presents the opportunity to be applied to a general class of enzymes for not only the field of space, but even medicine and environmental science.

## Acknowledgements

This work was supported in part by grants from the National Aeronautics and Space Administration, the SETI Institute, as well as by the immense support of Dr. Jessica Koehne and Dr. Richard Quinn.

## References

1. González-Fernández, Eva, Nicolaos Avlonitis, Alan F. Murray, Andrew R. Mount, and Mark Bradley. "Methylene Blue Not Ferrocene: Optimal Reporters for Electrochemical Detection of Protease Activity."*Biosensors and Bioelectronics* 84 (2016): 82-88. Web.