2nd Canadian Peptide and Protein Community Virtual Symposium

CPPC 2024

Sciforum no. # 109225

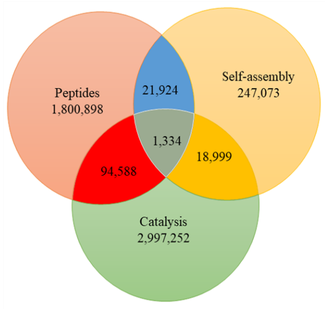
Artificial Hydrolase based on Self-assembled Peptide and Their Potential Applications

Kalpana Kumari[a]* and Vibin Ramakrishnan[a,b]

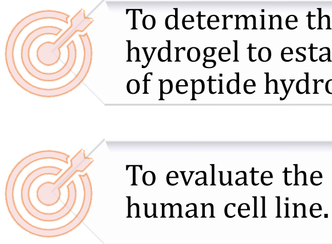
[a]Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati, Assam-781039, India

[b]Mehta Family School of Data Science and Artificial Intelligence, Indian Institute of Technology Guwahati, Guwahati, Assam-781039, India

MOTIVATION AND INTRODUCTION



SciFinder, as of 5th March 2018



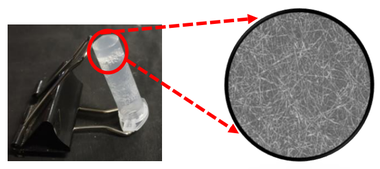
Steps involved in a typical heterogeneous catalytic reaction

Why peptide catalyst?

1. Self-assembly
2. Low immunogenic
3. Cost-effectiveness
4. Inherent biological origin
5. Biocompatible and Biodegradable

Characteristics of Hydrogels

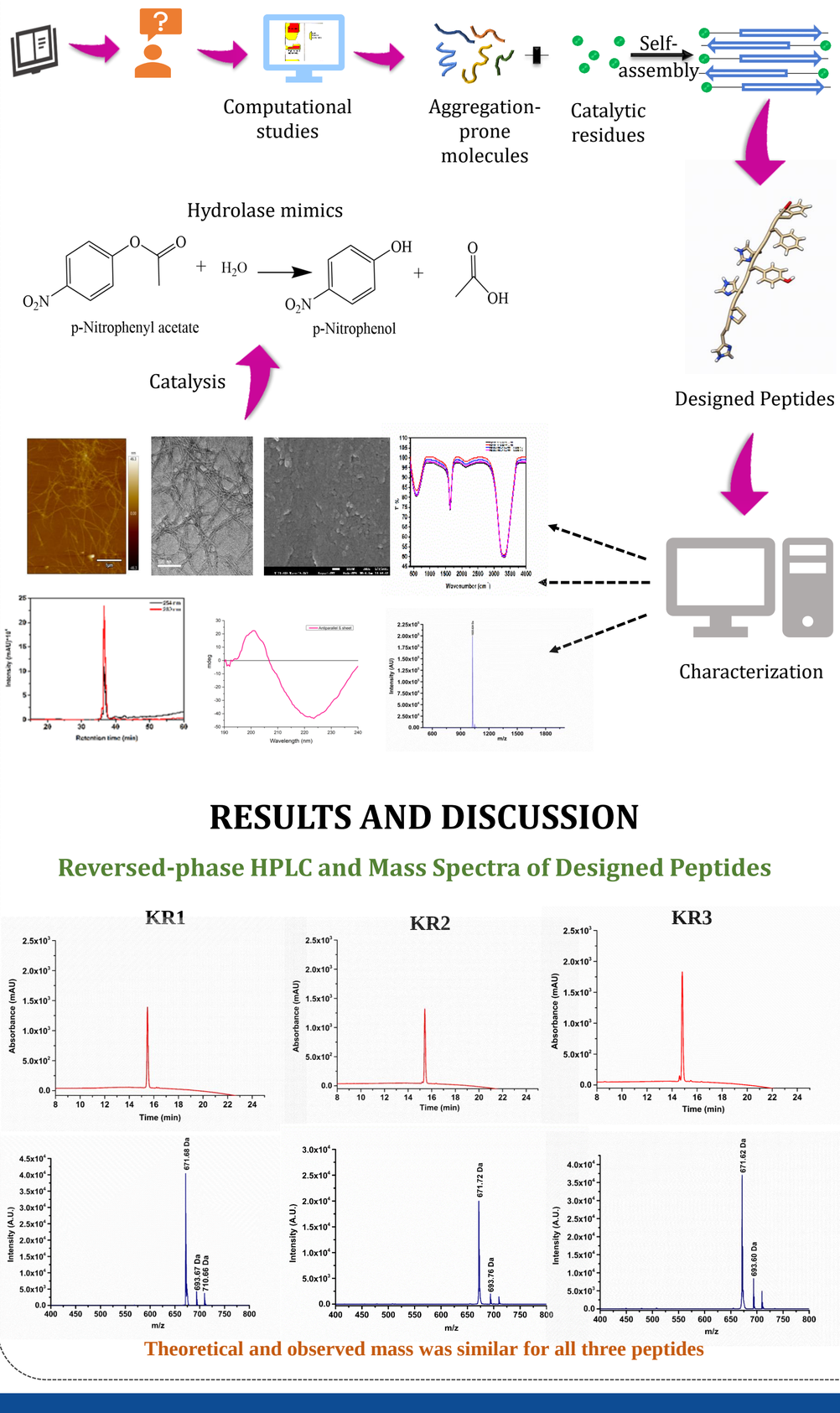
1. High-water content
2. Microporous structure
3. Tunable mechanical stability



OBJECTIVES

- Design, synthesis and characterization of short self-assembled tripeptide based on amino acids present in the active site of Hydrolase.
- To evaluate the efficiency of catalytic activity by performing p-Nitrophenyl acetate hydrolysis followed by determination of conversion rate.
- Verify the stability of peptide nano-assembly at different pH and temperatures.
- To verify substrate specificity of Hydrolase mimics by using a series of substrates that differ in the length of their side chain.
- To determine the rheological aspects of designed peptide hydrogel to establish the co-relation between the strength of peptide hydrogel and its catalytic activity.
- To evaluate the biocompatibility of peptide with the normal human cell line.

METHODOLOGY

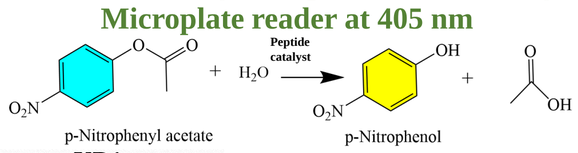


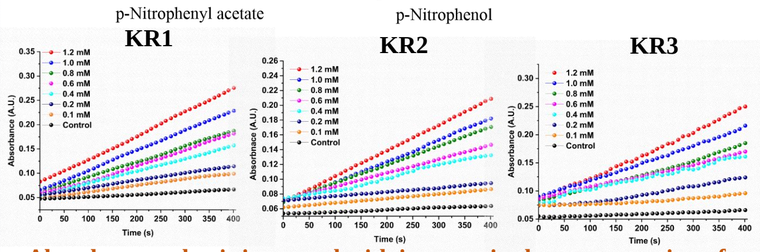
RESULTS AND DISCUSSION

Reversed-phase HPLC and Mass Spectra of Designed Peptides

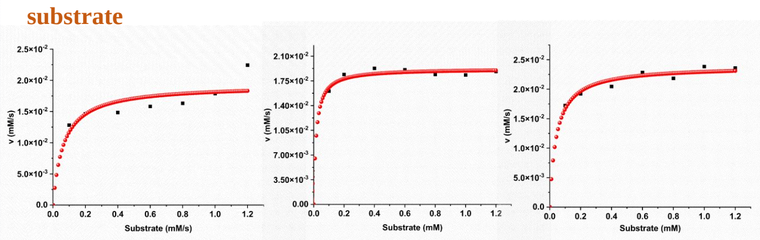
Theoretical and observed mass was similar for all three peptides

Esterase Activity of Peptide Monitored by Microplate reader at 405 nm





Absorbance value is increased with increase in the concentration of substrate

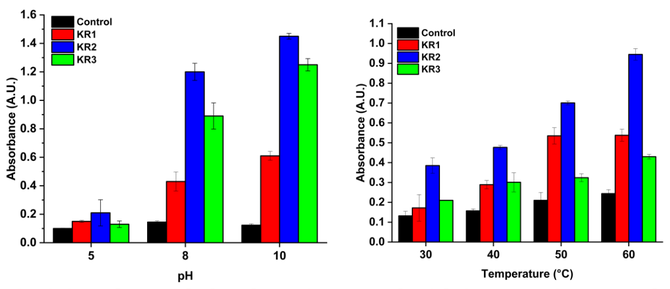


Kinetic Parameters of Biomolecular Artificial Mimics of Hydrolase

Kinetic parameters	KR1	KR2	KR3
V_{max} (mMs^{-1})	0.004±0.00	0.02±0.00	0.05±0.00
K_M (mM)	0.43±0.17	0.03±0.02	0.020±0.02
K_{cat} (s^{-1})	0.04±0.00	0.23±0.00	0.54±0.00
Catalytic efficiency ($mM^{-1}s^{-1}$)	0.10±0.00	7.57±0.07	26.94±0.17

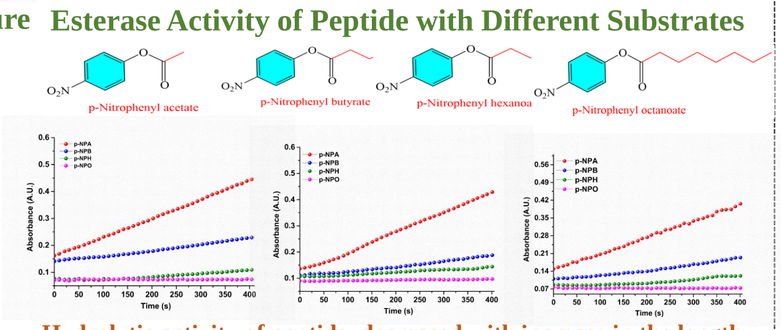
Catalytic efficiency: KR3 > KR2 > KR1

Esterase Activity of Peptide at Varying pH and Temperature



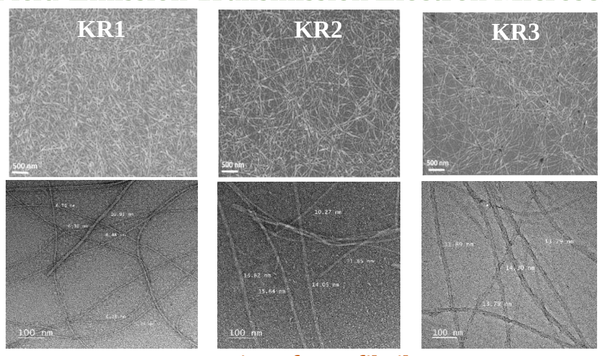
Activity of peptide has been increased with increase in pH and temperature

Esterase Activity of Peptide with Different Substrates



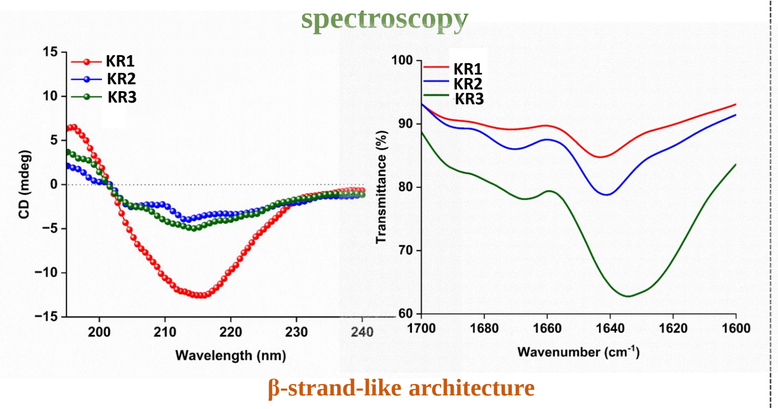
Hydrolytic activity of peptide decreased with increase in the length of side chain of substrate

Morphological Characterization of Catalytic Peptides by Field-Emission Scanning Electron Microscopy and Field-Emission Transmission Electron Microscopy



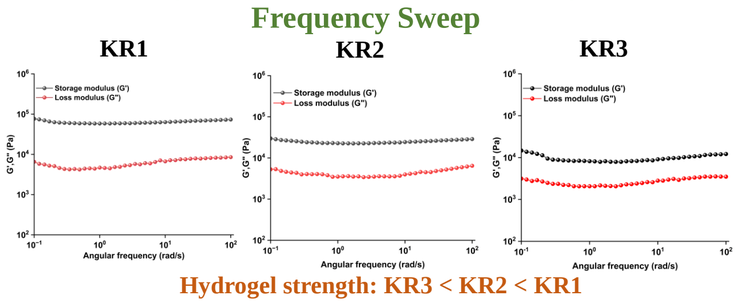
Formation of nanofibrils

Secondary Structure Characterization of Peptide by Circular dichroism and Fourier Transform Infrared spectroscopy



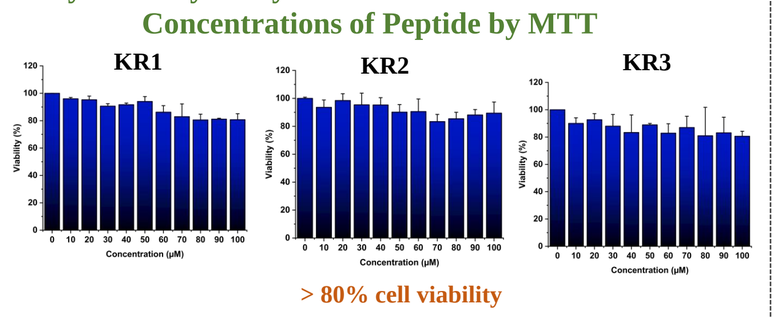
β-strand-like architecture

Rheological Studies of Peptide Hydrogels by Frequency Sweep



Hydrogel strength: KR3 < KR2 < KR1

Cytotoxicity Assay with HEK cell line at Different Concentrations of Peptide by MTT



> 80% cell viability

CONCLUSION

Rationally designed peptide mimics of hydrolase was successfully synthesized. Primary characterization- HPLC, Mass spectrometry has been performed. Studies of secondary characterization confirmed the formation of beta-strand like architecture of peptide hydrogel.

Peptide KR3 has shown the best catalytic performance, whereas peptide KR1 has shown the least catalytic activity. However, the strength of peptide hydrogel P1 is highest, whereas least for P3. Our findings established an inverse relationship between catalytic activity and the strength of peptide hydrogel.

All designed peptides have shown pH and thermal stability that implies distinct advantages over the natural hydrolase. Peptide catalysts have also shown good substrate specificity and promoted hydration activity.

These peptides have shown >80% cell viability with normal human cell lines, which offers a great advantage of using them in various diseased conditions in the future. Such peptide catalysts can be a promising alternative to natural hydrolase for various applications.

ACKNOWLEDGEMENTS

PMRF
Prime Minister's Research Fellowship

Department of Biosciences and Bioengineering
Indian Institute of Technology Guwahati

REFERENCES

1. Zozulia, O., Dolan, M. A., and Korendovych, I. V. (2018) Catalytic peptide assemblies, *Chem. Soc. Rev.*, 47, 3621–3639.
2. Fleming, S., and Ulijn, R. V. (2014) Design of nanostructures based on aromatic peptide amphiphiles, *Chem. Soc. Rev.*, 43, 8150-8177.
3. Y. S. Moroz et al.(2015) New Tricks for Old Proteins: Single Mutations in a Nonenzymatic Protein Give Rise to Various Enzymatic Activities, *J. Am. Chem. Soc.*, 137, 47, 14905–14911.
4. D. Röthlisberger et al.(2008) Kemp elimination catalysts by computational enzyme design, *Nature*, 453, 7192, 190–195.
5. Rufo et al. (2014) Short peptides self-assemble to produce catalytic amyloids, *Nat. Chem.*, 6, 4, 303–309.