

Enantioselective Ketone Reduction by Catalytic Amyloids

A thesis submitted in partial fulfilment of the
requirements for the award of the degree of

MASTER OF SCIENCES

By

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Under

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to the

DEPARTMENT OF CHEMICAL SCIENCES



INDIAN INSTITUTE OF SCIENCE EDUCATION AND RESEARCH KOLKATA

May 2025

Declaration

I hereby declare that the MS thesis "**Enantioselective Ketone Reduction by Catalytic Amyloids**" is original work done by me as a part of 2 Years Master of Science Program (MP) at IISER Kolkata. I declare that this report is the result of investigations carried out by me working as a project student under the guidance of **Dr. Dibyendu Das**, Associate Professor, IISER-Kolkata.

Date: 9/05/2025

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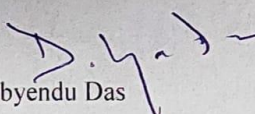
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Certificate

This is to certify that the thesis entitled “**Enantioselective Ketone Reduction by Catalytic Amyloids**” being submitted to the Indian Institute of Science Education and Research Kolkata in partial fulfilment of the requirements for the award of the 2 Years Master of Science Program (MP), embodies the research work done by **Ms. Khyati Sarma** under my supervision at IISER Kolkata. The work presented here is original and has not yet been submitted so far, in part or full, for any degree or diploma of any other institute/university.

Date: 9/05/2025



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Abstract

Through million years of evolutionary journey, extant enzymes are conferred with highly conserved three-dimensional folds that are capable of showing remarkably specific chemical transformations with great efficiency. Further, contemporary enzymes can effectively bind and activate co-enzymes/co-factors to carry out various catalytic reaction stereo-specifically. However, there remains a critical gap in understanding how early enzymatic folds, despite lacking the precisely folded binding pockets like modern enzymes, could catalyze stereoselective chemical transformations that are crucial for the emergence of homochirality during the chemical origins of life. In this context, short peptide-based self-assemblies have often been argued as early protein folds due to their capabilities to self-assemble into extended periodic nanostructures and demonstrate diverse catalytic roles.

In my thesis work, we envisage to develop highly cationic short peptide-based assemblies that can effectively recruit small negatively charged co-factors as hydride transfer agents to facilitate early oxidoreductive processes, thereby fueling the emergence of early protometabolism. We have developed catalytic peptide nanostructures that can capitalize its surface-exposed binding sites punctuated with kinetically locked cationic residues (quaternized amines). Moreover, the chiral surfaces of the nanostructures provide various interactions with hydrophobic substrates that can also effectively bind the co-enzyme, sodium borohydride leveraging the enantioselective reduction of prochiral ketones. This work underscores the enantioselective catalytic power of short peptides foreshadowing their possible roles in the emergence of homochiral chemical inventory in prebiotic earth.

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SI. No	Scheme
1	General Scheme for the synthesis of 1-(naphthalen-2-yl)ethan-1-ol

List of Abbreviations

NADH- Nicotinamide Adenine Dinucleotide (Hydrogen)

NADPH- Nicotinamide adenine dinucleotide phosphate

NaBH₄- Sodium borohydride

DMF- Dimethylformamide

DIC- N,N'-Diisopropylcarbodiimide

DIPEA- N,N-Diisopropylethylamine

TFA- Trifluoroacetic acid

DCM- Dichloromethane

ACN- Acetonitrile

HCl- Hydrochloric acid

1. Introduction

1.1. Enantioselective Reaction

Enantioselective reaction are chemical reactions which favor the formation of a specific enantiomer over the other having opposite configurations at all the chiral centers. Biological building blocks such as amino acids are present exclusively as one enantiomer. However, such enantioselective reactions are difficult to achieve as both the enantiomers may be formed at equal percentage leading to the formation of a racemic mixture. Nevertheless, enantioselective reaction highly relies on the presence of a chiral influence that directs the formation of one enantiomer preferentially over the other from the racemic mixture by selectively lowering the energy of one enantiomer's transition state over the other. This phenomenon is called asymmetric induction that can arise by introducing chiral substrate, reagent, catalyst, or from the surrounding environment.

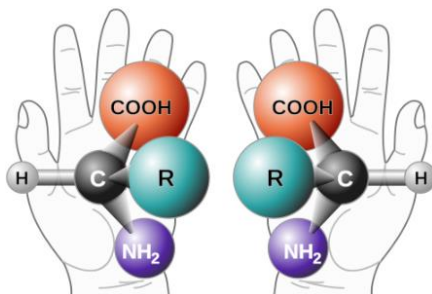


Figure 1: Two enantiomers of a generic alpha amino acid¹

1.1.1. Enantioselective Reduction Reaction

In enantioselective reduction reactions a prochiral molecule is converted into a chiral molecule favoring one enantiomer over the other forming an enantiopure or enantiomeric excess mixture. Biocatalytic reductions are commonly used in various asymmetric synthesis of wide range of alcohols with high chemical yield and optical purity. Enantiomeric reduction of prochiral ketone generating enantioenriched secondary alcohol is one of the most important reactions as these enantiopure alcohols also act as an important precursor for various natural products². One such class of enzyme is the ketoreductases (KREDs) that can efficiently reduce carbonyl groups into corresponding alcohol. These reactions are of high interests, as they can serve as chiral intermediates that are used for the synthesis of various compounds and have applications in industries like pharmaceutical and agrochemical³. These reduction reactions are usually carried out by using various hydride transferring reagents such as lithium aluminum hydride (LAH) or sodium borohydride (NaBH_4) in the presence of some chiral catalysts.

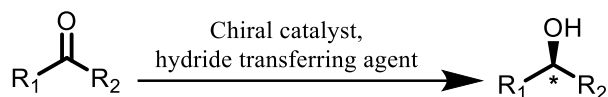


Figure 2: Asymmetric reduction of ketone

1.2. Cofactor mediated enantioselective reduction reaction

Enantioselective reduction reactions which are mediated by different cofactors, such as NADH or NADPH that exploit their catalytic abilities to reduce various prochiral substrates, resulting in the selective formation of one enantiomer over the other. In biocatalytic reactions these cofactors are harnessed by enzymes to achieve exceptional enantioselectivity. Especially, the oxidoreductase class of enzyme that catalyzes various oxidation and reduction reaction recruit NADH for asymmetric reduction of prochiral ketones. In these reduction reaction NADH act as a reductant getting oxidized to NAD^+ and the substrate gets converted into the desired product⁴. Similarly, it has been explored from several years that sodium borohydride (NaBH_4), when paired with a chiral modifier (chiral reagent or catalyst), facilitates the enantioselective reduction reaction of prochiral ketones, yielding chiral secondary alcohols with high enantiomeric excess.

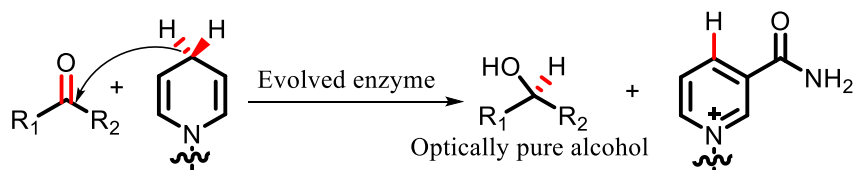


Figure 3: NADH mediated reduction of ketone

1.3. Role of asymmetric catalysts in enantioselective reduction reaction

Present day enzymes in modern biochemistry have been benefited greatly from evolutionary selection and adaptation, which have given them a given them a highly conserved and a precise three-dimensional structure that arise from the packing efficiency of long polypeptide chains. However, in the active site of these enzymes only few amino acid residues are present that play a very cooperative and crucial role depending upon their position in the active site. This ultimately leads to determining the selectivity and specificity of these extant enzymes towards a particular

molecular substrate⁵⁻⁸. These enzymes also have the capacity of working as exceptional asymmetric catalysts that can discriminate between two enantiomers by leveraging a combination of various interactions such as hydrophobic interaction, steric constraints, hydrogen bonding and charge distribution within their binding pockets⁹⁻¹². In this regard, the concept of chiral symmetry breaking introduced by these enzymes has always been thermodynamically unfavourable. However, in achieving enantioselective catalysis through such concept of symmetry breaking has become highly essential in diversifying the domain of different biological systems¹³⁻¹⁶. These evolved enzymes can recruit low molecular weight, negatively charged cofactors like NADPH or NADH that act as hydride transferring agents and energy carriers in various metabolic processes like oxidative phosphorylation, glycolysis, insulin secretion etc¹⁷⁻¹⁸. But such functions of these cofactors highly depend on the three-dimensional binding pocket of the evolved enzyme that enables hydride transfer reactions which otherwise could not proceed efficiently with cofactors alone. These negatively charged cofactors are stabilized and activated with arginine moieties present in the active site of the enzymes that facilitate Coulombic interactions and mediate different biologically relevant redox reaction (reduction/oxidation)¹⁹. Despite this understanding, there has always been a critical gap in knowing the mystery of how these primitive enzymes and simple cofactors could initiate such oxidoreductive processes such efficiently that contributed to the advent of protometabolism, emergence of compartmentalization and eventually led to the evolution of structurally complex biocatalysts²⁰⁻³³.

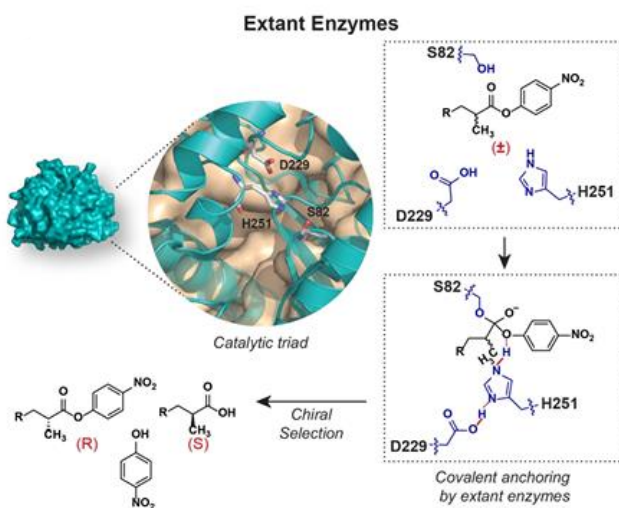


Figure 4: Covalent anchoring and enantioselective hydrolysis by *P. aeruginosalipase*¹⁶

1.4. Motivation and Objective

Oligomeric peptide fragments, despite of having a limited sequence length of amino acids, were seen to be capable of forming antiparallel beta sheet containing nanostructures. These structures were highly stable even under harsh environmental conditions and were considered as the plausible mimics of primitive protein folds. However, using such short peptide assemblies to carry out enantioselective reaction was quite challenging as they lacked the assistance of proximally located residues found in the active site of extant enzymes and performed chemical transformations only through single catalytic residues.

Hence, in this project our main motivation and objective was to exploit heptameric peptide-based nanostructures that can non-covalently anchor substrates through the exposed amino acid residues in a stereoselective manner. This would bring in asymmetric induction leading to the generation of a particular enantiomer over the other for different prochiral substrates. We also asked whether such short peptide assemblies can show some kind of interaction via its binding site exposing charged amino acid residue to carry out hydride transfer with negatively charged elementary cofactor. Eventually, this is an interaction mediated chemical transformation where chiral peptide assemblies interact with the prochiral substrate and cofactor leading to the generation of an enantioselective kinetically controlled reduced product.

2. Experimental Section

2.1. Chemicals used

2-Acetonaphthone (Sigma Aldrich), Acetophenone (SRL), 3,5-dimethoxy-4hydroxyacetophenone (Sigma Aldrich), Sodium borohydride (NaBH_4) (SRL), Hexane (HPLC graded-SRL), Isopropanol (HPLC graded- SRL), Fmoc amino acids (Sigma Aldrich), DMF (SRL), DIC (SRL), Oxyma (Nova-Biochem), DIPEA (SRL), piperidine (Hyma), TFA (SRL), DCM (SRL), Diethyl ether (Hyma), Fmoc-Rink Amide MBHA Resin (Merck), Nile Red Dye. Milli-Q water was used throughout the experiment. All chemicals were used without further purification.

2.2. Synthetic procedure of peptides

The CEM Liberty Blue peptide synthesizer was used for synthesizing all BLVFFAL-NH₂ (BET)-L, Ac-RLVFFAH (Ac-RH), Ac-ELVFFAL (Ac-EL), Im-RLVFFAL (Im-RL) and (PBS)LVFFAL (PBS-LL). Fmoc-Rink Amide MBHA Resin (loading 0.52 mmol/g) was swollen using dimethylformamide (DMF) for 30 minutes and subsequently 20% piperidine in DMF was used for Fmoc deprotection. For each Fmoc– amino acid, DIC was used as an activator and the oxyma solution in DMF for coupling step. Thereafter, acetylation of the synthesized peptides was done at free N-terminus (when required) by using acetic anhydride in DMF. Finally, resin bound peptides was washed with DMF and dichloromethane (DCM) respectively and allowed to air dry. From resin, peptides were cleaved using trifluoroacetic acid (TFA)/triethylsilane (5:0.1 V/V) at room temperature for 2 hours. The solution of peptide-TFA mixture was filtered and using high vacuum, TFA was removed. The peptides were precipitated by dropwise addition to ice-cold diethyl ether. The precipitated product was centrifuged at 8000 rpm for 15 min at 4 °C in Eppendorf centrifuge 5804 R resulting in the white pellet. Further, pellet was washed 3 times with cold diethyl ether. The crude peptides were purified using an Atlantis T3 C18 preparative reverse phase column and a preparative Waters HPLC system, a linear gradient of water with 0.1% TFA and acetonitrile with 0.1% TFA was used. Bruker Mass Spec Q-tof systems confirmed the molecular weight.

BLVFFAL-NH₂ ((BET)-L) (C₄₈H₇₁N₁₁O₈) (m/z) calculated for [M+H⁺]: 808.5177; found: 808.5172

Ac-RLVFFAH-NH₂ (C₄₆H₆₇N₁₃O₈) (m/z) calculated for [M+H⁺]: 930.5308; found: 930.5320.

Ac-ELVFFAL-NH₂ (C₄₅H₆₆N₈O₁₀) (m/z) calculated for [M+Na⁺]: 902.4799; found: 902.4752.

Im-RLVFFAL-NH₂ (C₄₉H₇₄N₁₃O₈) (m/z) calculated for [M+H⁺]: 972.5778; found: 972.5908.

(PBS)LVFFAL-NH₂ (GLU-16) (C₅₁H₇₂N₈O₁₁S) (m/z) calculated for [M+H⁺]: 1004.5041; found: 1004.5075.

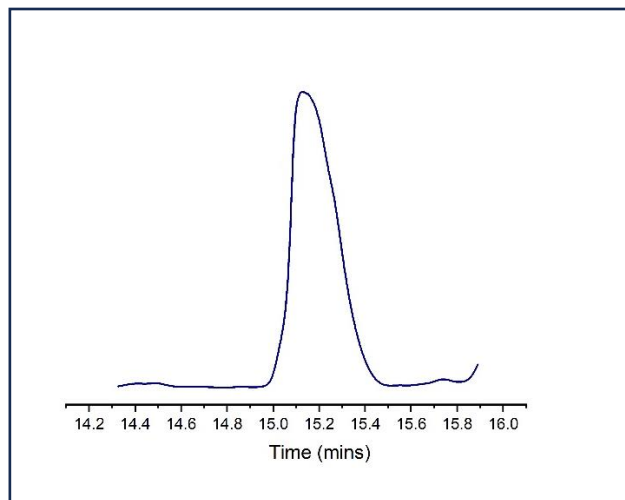


Figure 5: HPLC chromatogram of (BET)-L

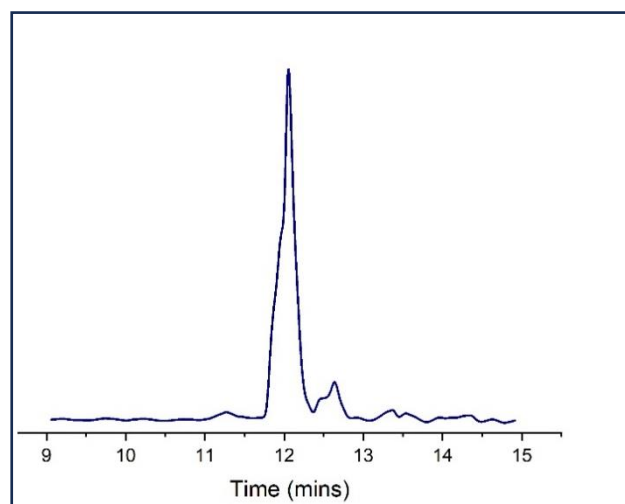


Figure 6: HPLC chromatogram of Ac-RH

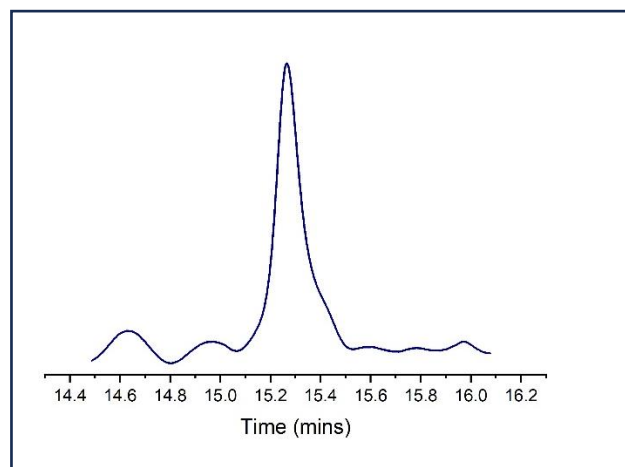


Figure 7: HPLC chromatogram of Ac-EL

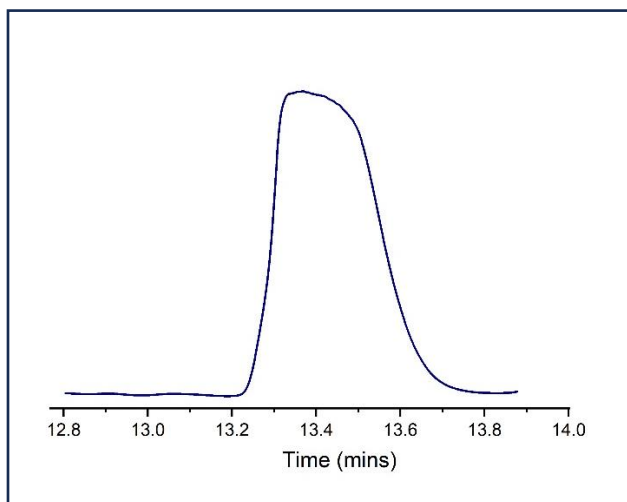


Figure 8: HPLC chromatogram of Im-RL

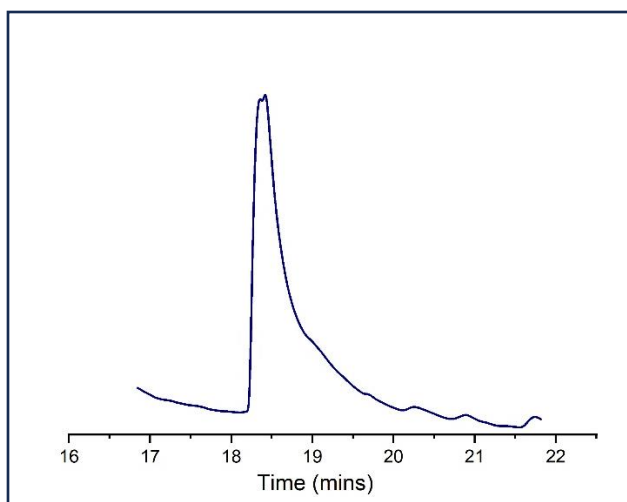
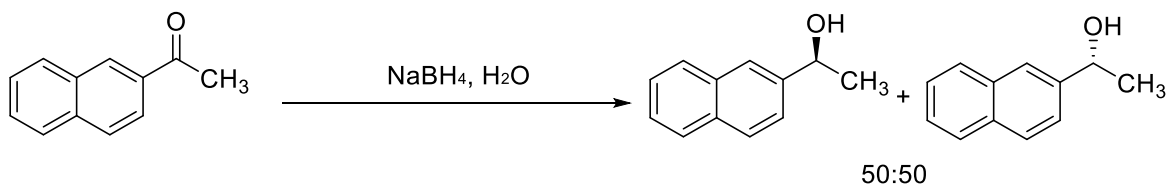


Figure 9: HPLC chromatogram of PBS-LL

2.3.Synthesis of 1-(naphthalen-2-yl)ethan-1-ol



Scheme 1: General Scheme for the synthesis of 1-(naphthalen-2-yl)ethan-1-ol

The reduced product 1-(naphthalen-2-yl)ethan-1-ol was synthesized by reacting 2-Acetonaphthone with NaBH₄ in MilliQ water. The reaction was kept for 30mins and then quenched using 1N HCl. The quenched mixture was injected in Atlantis T3 C18 preparative reverse phase column and a linear gradient of water with 0.1% TFA and acetonitrile with 0.1% TFA was used. Different retention times were obtained for both the reactant (2-Acetonaphthone) and product (1-(naphthalen-2-yl)ethan-1-ol with different UV peak pattern.

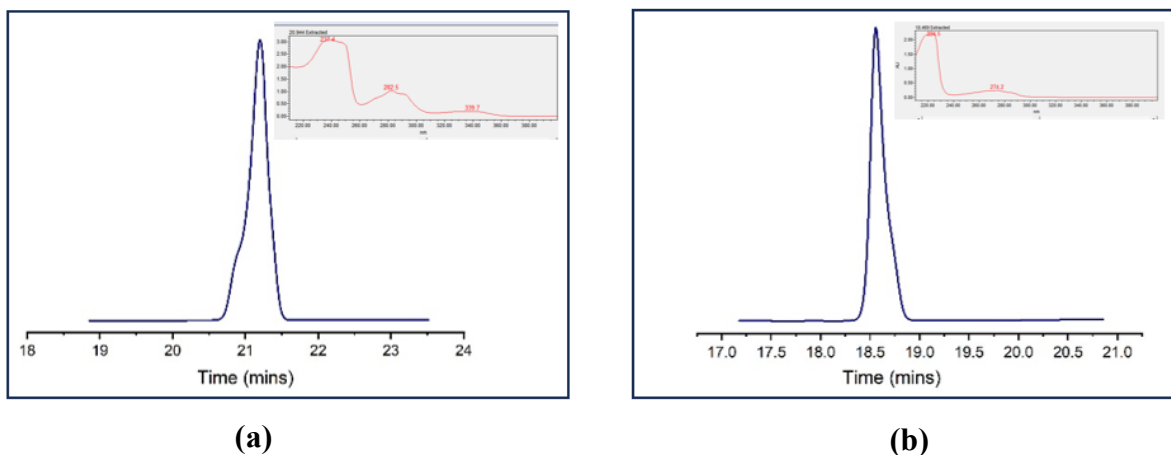


Figure 10: HPLC peak of 2-Acetonaphthone(a) and 1-(naphthalen-2-yl)ethan-1-ol(b)

2.4. Experimental Techniques

2.4.1. High Performance Liquid Chromatography (HPLC)

The enantiomeric excess for the different peptide assemblies for various substrates was determined by using Chiral High-performance liquid chromatography (HPLC) performed in Waters HPLC system. A Chiralpak OD-RH (Daicel) normal phase 4.6 × 150 mm column with particle size 5μm was used where the substrates were eluted isocratically with 85% Hexane/Isopropanol at the flow rate of 1 mL/min. All the chromatograms were extracted at 274nm.

2.4.2. Electrospray Ionization Mass Spectrometry (ESI-MS)

Mass spectra of the synthesized compounds were recorded on Waters Mass Spec Q-tof systems.

2.4.3. Peptide assembly

The air-dried peptide (BET)-L was dissolved in 40% acetonitrile-water (ACN/H₂O) containing 0.1 % trifluoroacetic acid (TFA). Then pH was adjusted by dropwise addition of NaOH and HCl. The homogeneous solution of peptide was kept in the refrigerator for a month before using³⁴⁻³⁵

2.4.4. Transmission electron microscopy Imaging (TEM)

The peptide assembly of (BET)-L (5mM) was diluted to 150 μ M using MilliQ water that was drop-casted onto a TEM grid for 1-2 min and was allowed to adsorb on the grid for 1 min. The excess solution was wicked off using a filter paper. Subsequently, 2 μ L of 1% (w/v) uranyl acetate was added, followed by a 1-minute incubation. Samples were then kept in a desiccator under vacuum for one day. TEM micrographs were recorded with a JEOL JEM-2100F with a tungsten filament at an accelerating voltage of 200 kV. The peptide (BET)-L self-assembled to form homogeneous nanostructures.

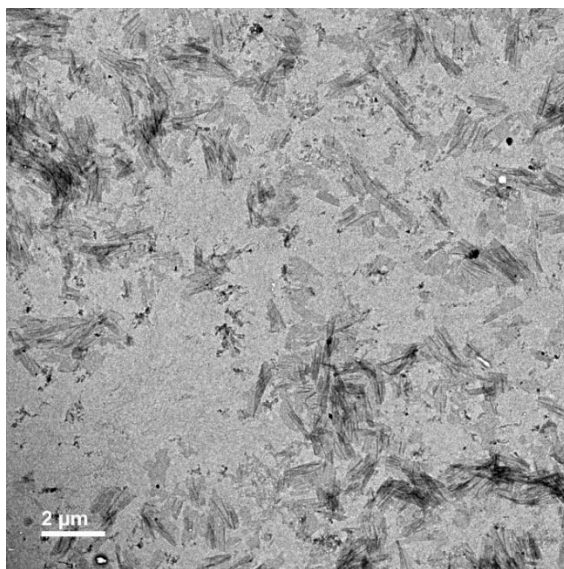


Figure 11: TEM image of (BET)-L

2.4.5. Confocal Microscopy

Peptide (BET)-L having concentration of 1mM was incubated with Nile Red (10 μ M) for 20min to probe the binding affinities of the peptide assembly. The solution was then cast on a petri plate

and enclosed with a cover glass. The confocal image was taken at a frame of 1024X1024, with 12-bit depth with a 100X, Plan Super Apochromat objective, N.A.1.4, excited by 488 nm laser line, emission bandwidth of 493-560 nm in Olympus Laser Scanning Confocal System Model FV3000 (part of the Atomic Force Microscope with Rheological Measurement and Confocal Imaging Unit Facility, supported by Swarnajayanti, SB/SJF/2020-21/08).

CLSM of fluorophore bound peptide networks were done to further enrich the peptide characterization. Apart from showing nanotubular morphology, CLSM micrographs further confirmed the strong affinity of the nanostructures for hydrophobic guest molecules such as Nile Red.

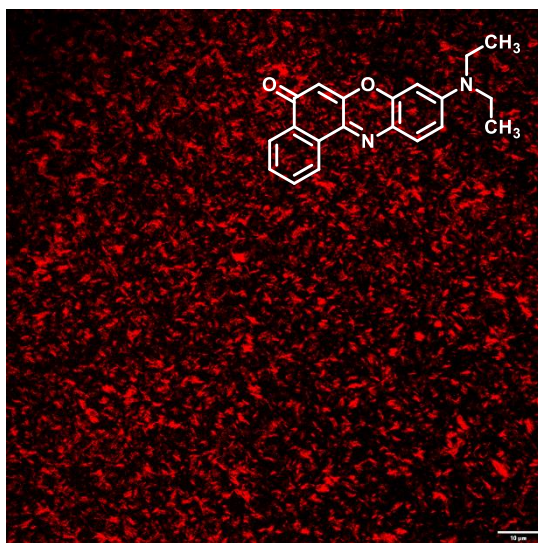


Figure 12: CLSM micrograph of Nile Red bound (BET)-L

2.4.6. Atomic Force Microscopy (AFM)

The peptide assemblies were diluted (250 µM) by using MilliQ water and adsorbed on a silicon wafer for 1 min. The excess solution was then wicked off by using filter paper and then sample was dried under vacuum for one day. Standard tapping mode probes were used for imaging the samples under ambient conditions using Oxford MFD-3D Infinity AFM.

AFM of the peptide assembly further confirmed the nanostructures formed by (BET)-L.

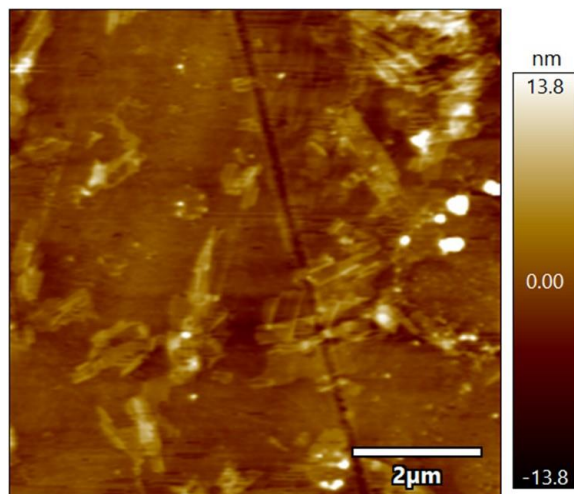


Figure 13: AFM image of (BET)-L

2.4.7. Circular Dichroism

CD spectra were recorded in the JASCO J-810 circular dichroism spectrometer. Aliquots of the peptide sample (BET)-L were taken into a quartz cuvette of 1 mm path length. The spectrum was recorded by scanning the wavelengths from 400 nm to 190 nm at a scanning rate of 100 nm/min for three successive times.

CD showed the characteristic β -sheet signature of the peptide assembly. The peptide strands were arranged in an anti-parallel out-of-register β -sheet stack fashion having a cationic residue at the N-terminal and a hydrophobic leucine residue at C-terminal. These residues were positioned outside the H-bonded β -sheet array in order to access the well-defined surfaces of the nanostructures.

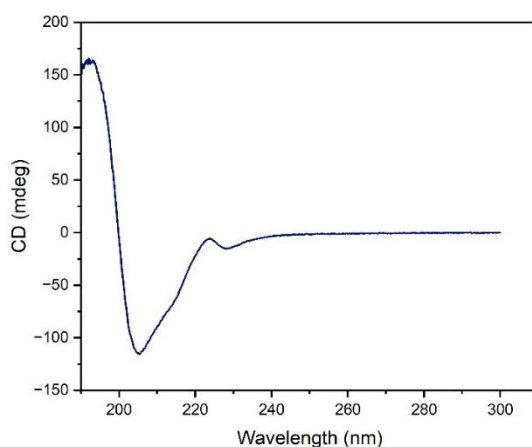


Figure 14: CD graph of (BET)-L

2.4.8. Fluorescence study

Fluorescence spectra were recorded in Fluoromax3 spectrofluorometer from Horiba Jobin Yvon. 2-Acetonaphthone (250 μ M) was added to peptide assembly (BET)-L (1mM) and placed in a cell of 10 mm path length for time dependent fluorescence measurement where the substrate and peptide assembly were incubated for 10mins in MilliQ and then NaBH₄ (5mM) was added. The sample was then excited at 300 nm.

The hydrophobic substrate 2-Acetonaphthone showed a very strong fluorescence at 434nm. However, when peptide assembly was added the fluorescence emission intensity was reduced indicating some kind of interaction of the hydrophobic substrate on the amyloid surface.

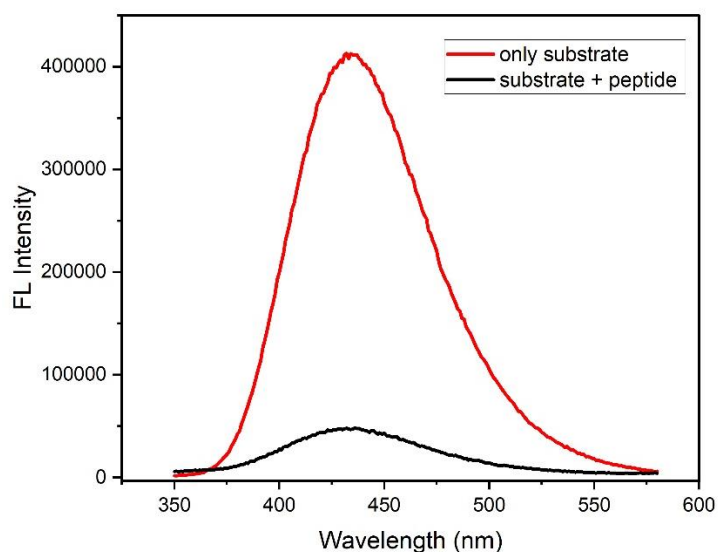


Figure 15: Fluorescence spectra of 2-Acetonaphthone in the presence and absence of (BET)-L

Along with BET peptide other charged peptide assemblies (both positive and negative) such as Ac-RLVFFAH (Ac-RH), Ac-ELVFFAL (Ac-EL), Im-RLVFFAL (Im-RL) and PBS-LLVFFAL (PBS-LL) were also synthesized to carry out the enantioselective studies of various hydrophobic substrates. A comparison was attempted between these peptides based on their interaction with the amyloid surface and proximally present substrate.

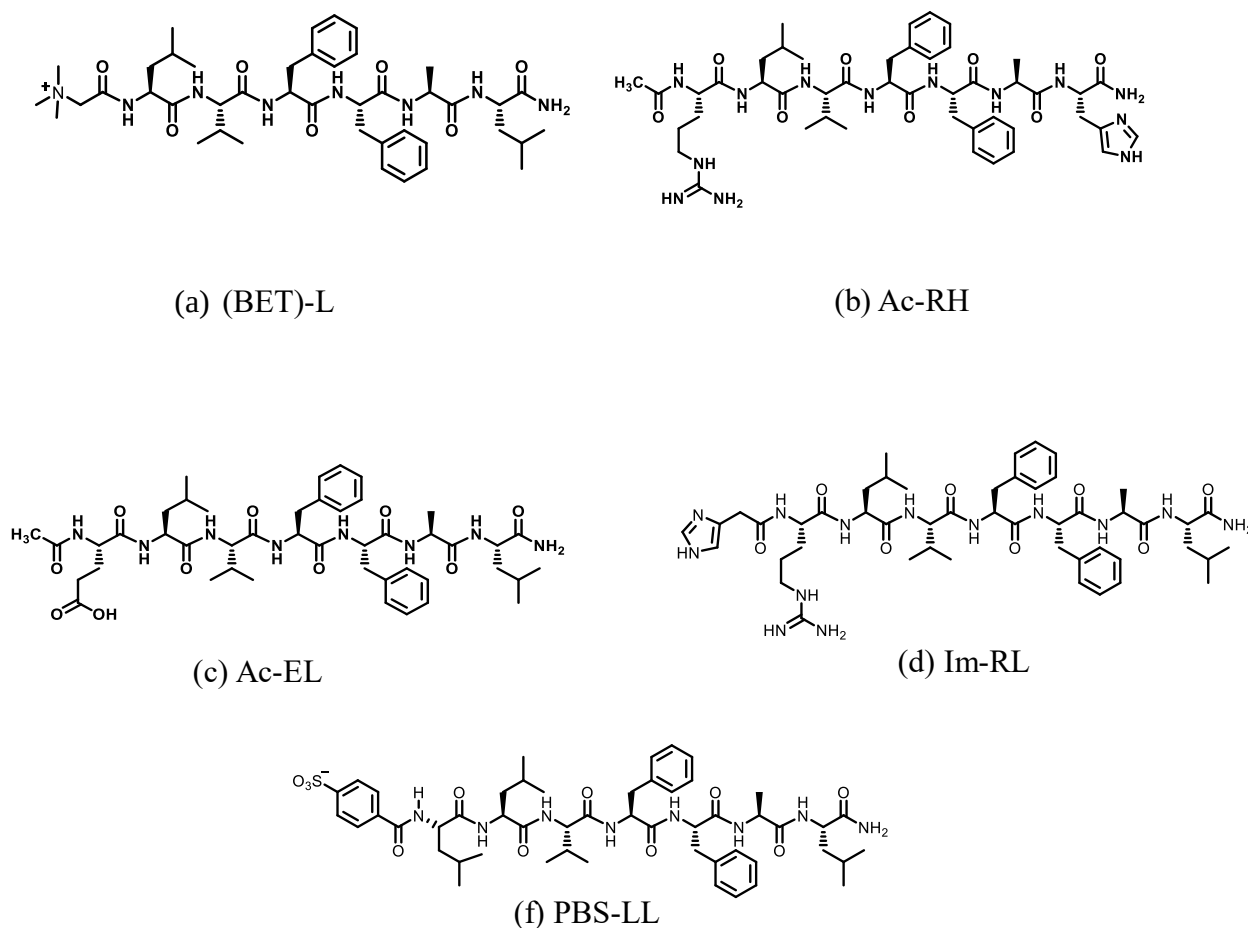


Figure 16: Different peptide assemblies used for enantioselective studies

3. Results and Discussion

3.1. Probing the enantioselective reaction at room temperature

3.1.1. Enantioselective studies of substrate 2-Acetonaphthone in the absence of peptide (BET)-L

In order to prove our hypothesis of introducing asymmetric induction in a system containing hydrophobic substrates in the presence of peptide assembly, we first did a control experiment where NaBH_4 reduced the prochiral substrate to the subsequent alcohol in the absence of peptide.

From this data it was observed that in the absence of peptide assembly there was no enantioselectivity, indicating the role of peptide assembly in generating the asymmetric induction.

Reaction Condition: 5mM 2-Acetonaphthone + 40mM NaBH₄ in water. The chromatogram was extracted at 274nm.

Reaction Procedure: The substrate was added in MilliQ water. To that NaBH₄ was added and the reaction mixture was kept for 30mins before quenching with HCl. The quenched reaction mixture with pH around 6-7 was then injected in the OD-H chiral column to obtain the separated enantiomeric peaks at 90% Hexane/Isopropanol solvent system.

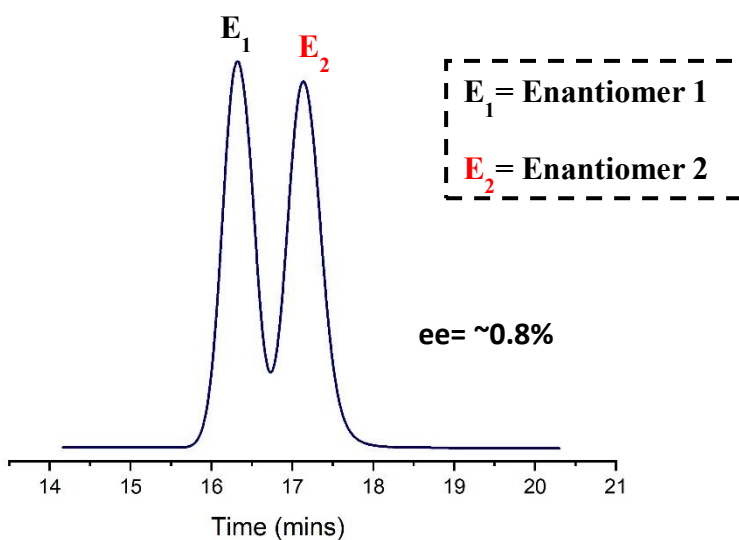


Figure 17: HPLC plot of 2-Acetonaphthone in the absence of peptide assembly at RT

3.1.2. Enantioselective studies of substrate 2-Acetonaphthone in the presence of peptide (BET)-L

The hydrophobic substrate 2-Acetonaphthone was then reacted with the peptide assembly (BET)-L to check the generation of asymmetric induction upon getting reduced by NaBH₄. The data obtained indicated the generation of enantioselectivity of about 17%.

Reaction Condition: 5mM 2-Acetonaphthone + 1mM (BET)-L + 40mM NaBH₄ in water. The chromatogram was extracted at 274nm.

Reaction Procedure: The peptide assembly and the substrate were incubated for 10mins in MilliQ water. After that NaBH₄ was added and the reaction mixture was kept for 30mins before quenching

with HCl. The quenched reaction mixture with pH around 6-7 was then injected in the OD-H chiral column to obtain the separated enantiomeric peaks at 85% Hexane/Isopropanol solvent system.

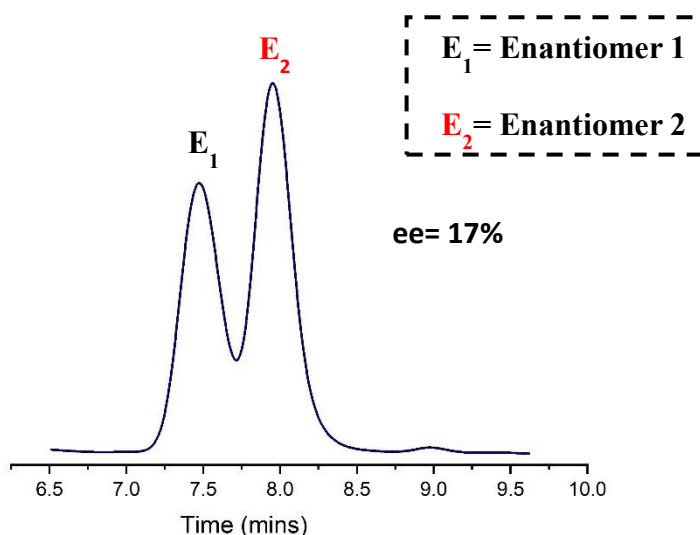


Figure 18: HPLC plot of 2-Acetonaphthone in the presence of peptide assembly at RT

Similarly, the prochiral substrate 2-Acetonaphthone was also tried against other charged peptides having different charge distribution on the surface, where peptide Ac-RLVFFAH (Ac-RH) had a positive charge exposed on its surface due to the presence of arginine moiety whereas the peptide Ac-ELVFFAL had a negative charge due to the glutamic residue. But both the assemblies gave a lower enantiomeric excess of 5% and 3%, Ac-RH giving a little more due to the presence of some electrostatic interaction with the negatively charged cofactor NaBH_4 .

System	Enantiomeric excess (%)
(BET)-L	17
Ac-RH	5
Ac-EL	3
Buffer	~0.8

Table1: Enantiomeric excess obtained against different peptide

3.1.3. Time-dependent study of 2-Acetophenone with (BET)-L

With the aim of checking the binding affinity of 2-Acetonaphthone (5mM) and NaBH₄ (40mM) on the peptide surface, a time dependent study was carried out where NaBH₄ was added at different time points.

Time (mins)	Enantiomeric Excess (%)
~0-2	16
5	14
10	13
15	13
20	13
25	13
30	14

Table 2: Enantiomeric excess obtained at different time stamps on 2-Acetophenone with (BET)-L

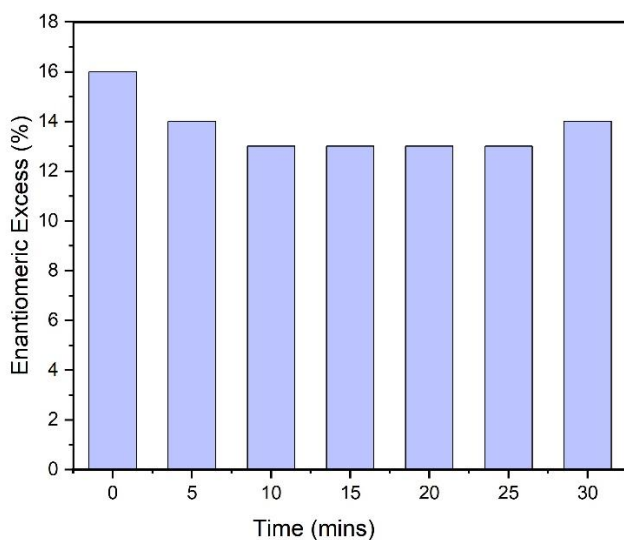


Figure 19: Bar graph showing different enantiomeric excess at different time stamps

From the time dependent data obtained, we could infer that the interaction of the prochiral hydrophobic substrate with the peptide surface was the major driving force in bringing in the enantiomeric excess, as a very high enantiomeric excess % was obtained at the initial addition of the cofactor.

3.1.4. Variations in the concentration of NaBH₄ and 2-Acetonaphthone

The concentrations of NaBH₄ and 2-Acetonaphthone were systematically varied to identify the optimal conditions that would maximize enantiomeric excess. Firstly, we varied the concentration of NaBH₄, keeping the concentration of 2-Acetonaphthone (5mM) constant.

Concentration of NaBH ₄ (mM)	Enantiomeric Excess (%)
40	17
20	16
10	16
5	18
1	14

Table 3: Variation in the concentration of NaBH₄ at RT

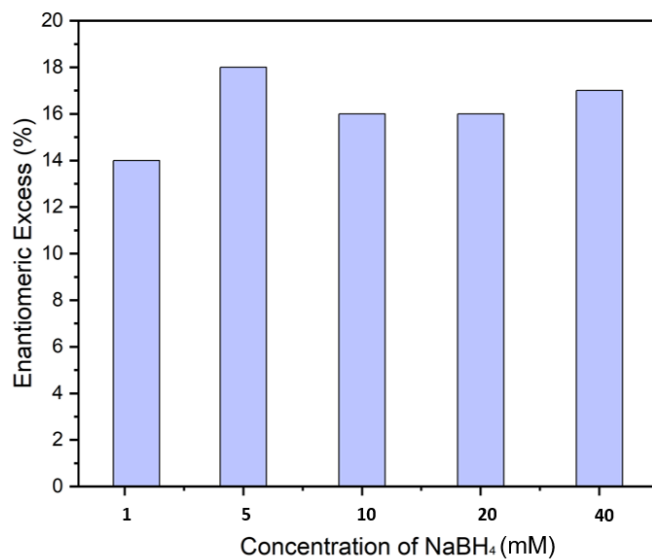


Figure 20: Bar graph showing different concentrations of NaBH₄ at RT

From this dataset, the enantiomeric excess was the highest for 5mM concentration of NaBH₄ with 18%.

Now keeping the concentration of NaBH₄ as 5mM, the substrate concentration was varied.

Concentration of 2-Acetonaphthone	Enantiomeric Excess (%)
5mM	18
1mM	19
500uM	20
250uM	22
100uM	22

Table 4: Variation in the concentration of 2-Acetonaphthone

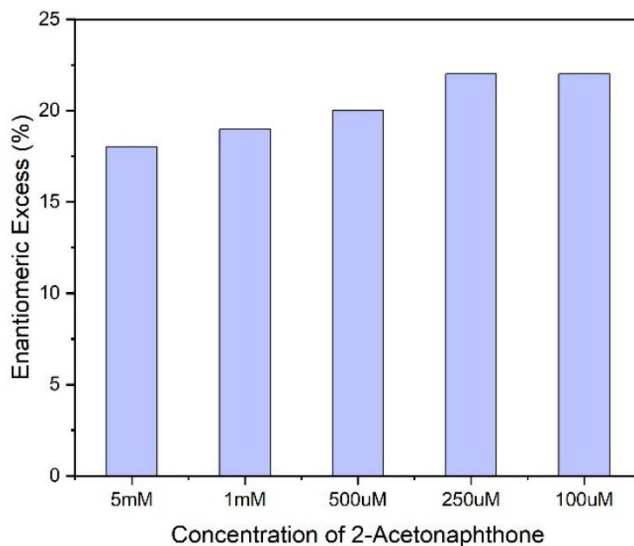


Figure 21: Bar graph showing different concentrations of 2-Acetonaphthone

The highest enantiomeric excess was observed at a substrate concentration of 250 μ M with 22%. Therefore, the concentrations of substrate (250 μ M), (BET)-L peptide (1 mM), and cofactor NaBH₄ (5 mM) were established as the optimized conditions for the reaction.

These data further supported the interaction-driven asymmetric induction of the hydrophobic prochiral substrate.

3.2. Probing the enantioselective reaction at lower temperature

Upon achieving 22% enantioselectivity under the conditions described in **section 3.1.4**, the reaction was subsequently carried out at 4 °C with the aim of reducing the reaction kinetics, thereby potentially enhancing the enantiomeric excess.

It was seen that on using the similar conditions where (BET)-L peptide concentration was 1mM, 2-Acetonaphthone 250 μ M and NaBH₄ 5mM, the enantiomeric excess increased up to 30.5%. Therefore, to further enhance enantiomeric excess, the concentration of NaBH₄ was reduced while maintaining constant concentrations of the substrate and peptide.

3.2.1. Variation in the concentration of NaBH₄

Due to the rapid kinetics of the reaction, the concentration of NaBH₄ was further reduced to slow down the reaction rate and promote improved enantiomeric excess by facilitating more efficient binding of the cofactor to the peptide surface.

Concentration of NaBH ₄	Enantiomeric Excess (%)
5mM	30.5
1mM	30.8
500μM	29.1
250μM	27.9

Table 5: Variation in the concentration of NaBH₄ at 4°C

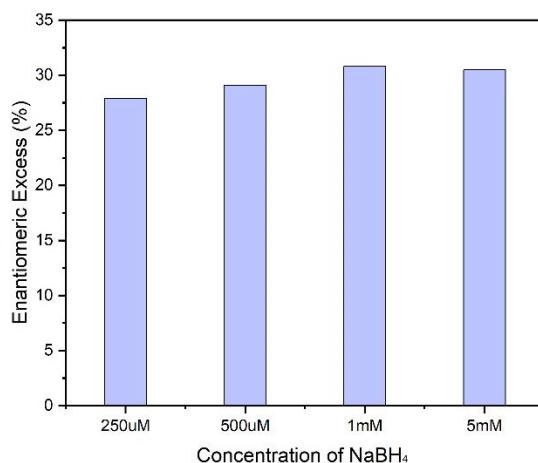


Figure 22: Bar graph showing different concentrations of NaBH₄ at 4°C

However, no significant change in enantioselectivity was observed at 1mM NaBH₄, while further reductions to 500μM and 250μM resulted in decreased enantioselectivity. Therefore, using 5mM and 1 mM NaBH₄ concentrations, a time-dependent study was conducted to evaluate the variation in enantiomeric excess over time.

3.2.2. Time-dependent study for 5mM and 1mM concentration of NaBH₄

A time-dependent study was conducted at both 5 mM and 1 mM concentrations of NaBH₄, using three different time points, while maintaining the substrate and peptide concentrations constant at 25μM and 1mM, respectively.

Time (mins)	Enantiomeric excess (%)
0	24.9
10	27.3
30	30.5

Table 6: Time-dependent study for 5mM concentration of NaBH₄ at 4°C

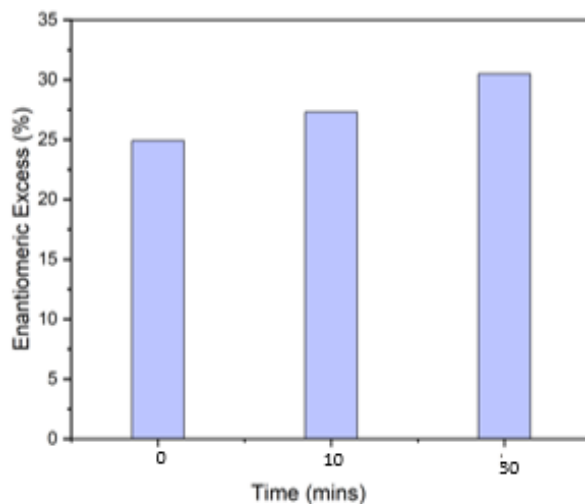


Figure 23: Bar diagram showing the time-dependent study for 5mM concentration of NaBH₄ at 4°C

Time (mins)	Enantiomeric excess (%)
0	24.5

10	26.8
30	30.8

Table 7: Time-dependent study for 1mM concentration of NaBH₄ at 4°C

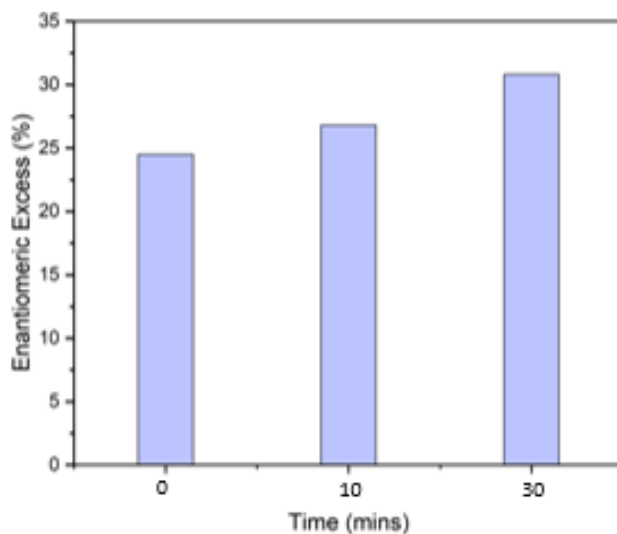


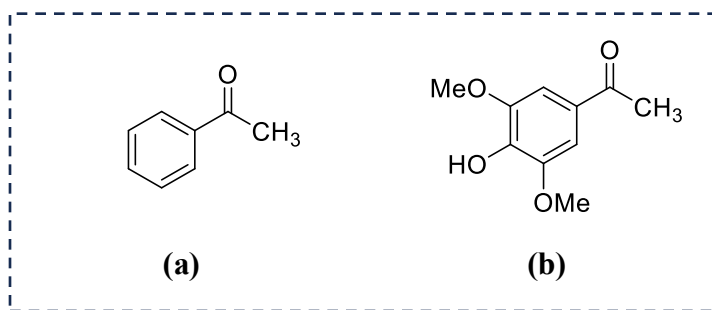
Figure 24: Bar diagram showing the time-dependent study for 1mM concentration of NaBH₄ at 4°C

From both time-dependent studies, it was observed that enantiomeric excess increased with extended reaction time at both NaBH₄ concentrations. This suggests that longer reaction times may facilitate more effective binding of NaBH₄ to the peptide surface, enabling enhanced enantioselectivity through the reduction of the prochiral substrate positioned at a proximal distance on the peptide surface.

3.2.3. Reactions performed with different substrates at 4°C

Substrate Scope

Apart from 2-Acetonaphthone, two other substrates Acetophenone (**a**) and 3,5-dimethoxy-4hydroxyacetophenone (**b**) were used to investigate the influence of hydrophobic interactions on the peptide surface.



Reaction Condition: 250 μ M (a)/(b) + 1mM (BET)-L + 5mM NaBH₄ in water. The chromatograms were extracted at 274nm.

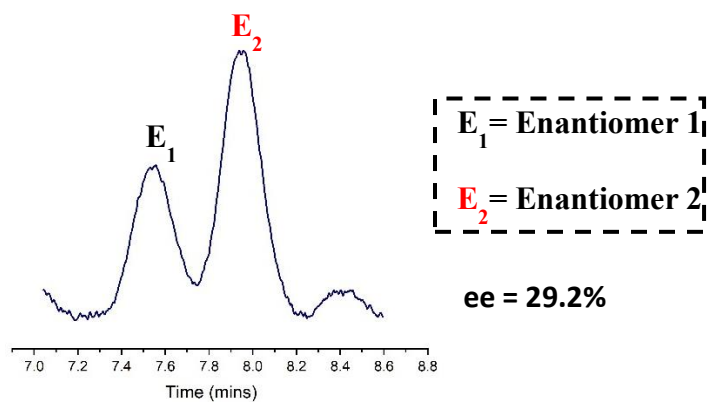


Figure 25: HPLC plot of Acetophenone in the presence of peptide assembly at 4°C

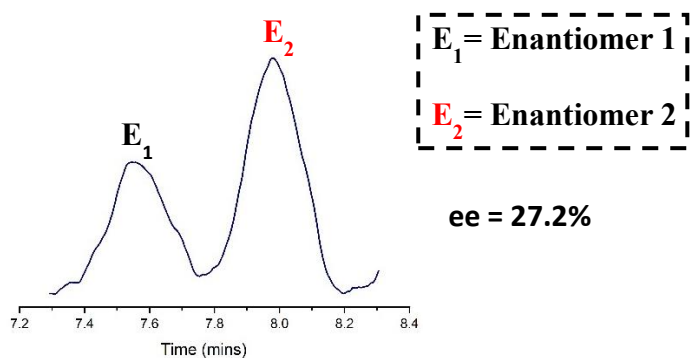


Figure 26: HPLC of 3,5-dimethoxy-4-hydroxyacetophenone in the presence of peptide assembly at 4°C

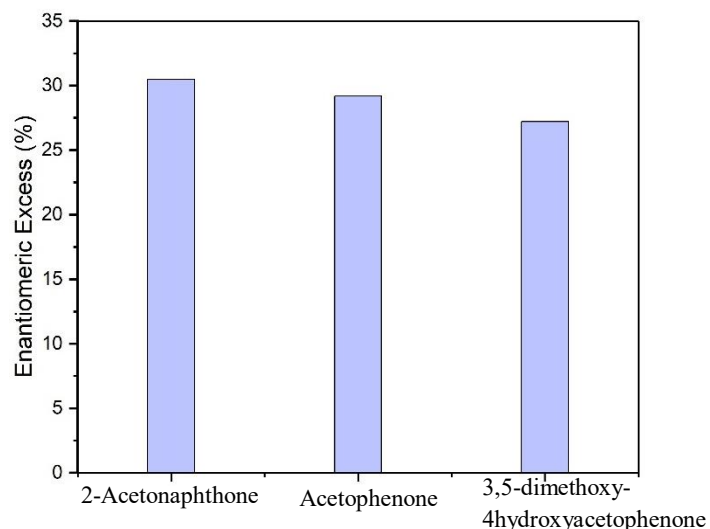


Figure 27: Bar graph showing comparison of enantiomeric excess for different substrates

Variation of the substrate scope revealed that hydrophobic interactions between the substrates and the peptide surface played a key role in driving asymmetric induction.

3.2.4. Reactions performed with different peptides at 4°C

Under the optimized reaction conditions at 4 °C with 2-Acetonaphthone as the substrate, additional reactions were performed using various peptide assemblies containing surface-exposed charged residues to assess their influence on enantioselectivity.

Reaction Condition: 250μM 2-Acetonaphthone + 1mM (b) Ac-RH/(c) Ac-EL/(d) Im-RL/(e) PBS-LL (Structures are shown under section 2.4) + 5mM NaBH₄ in water. The chromatograms were extracted at 274nm.

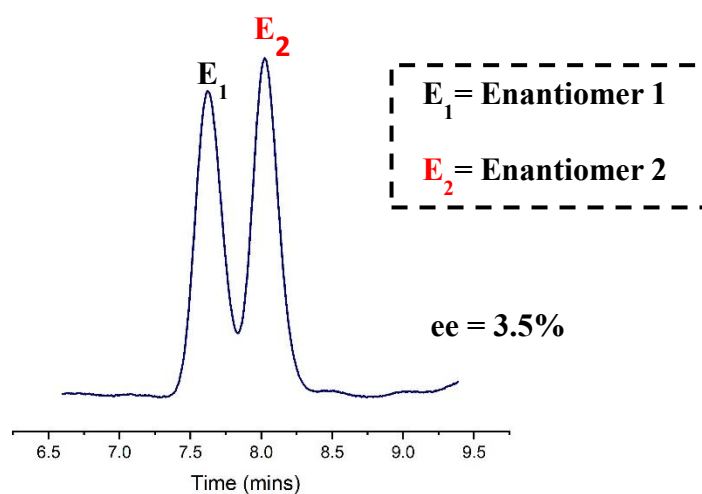


Figure 28: HPLC plot of 2-Acetonaphthone in the presence of Ac-RH

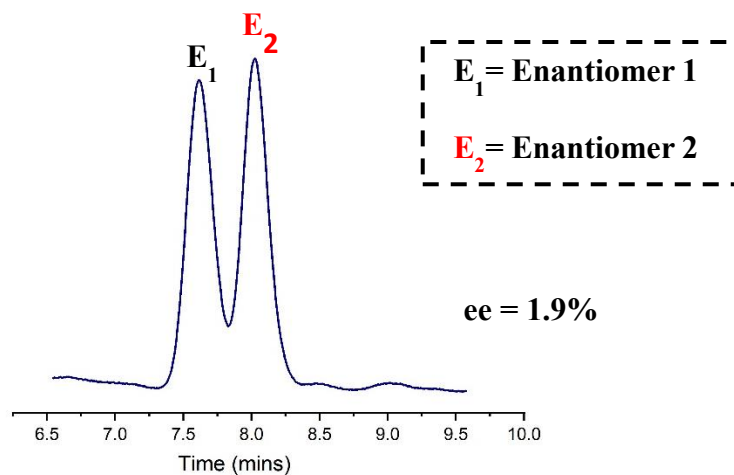


Figure 29: HPLC plot of 2-Acetonaphthone in the presence of Ac-EL

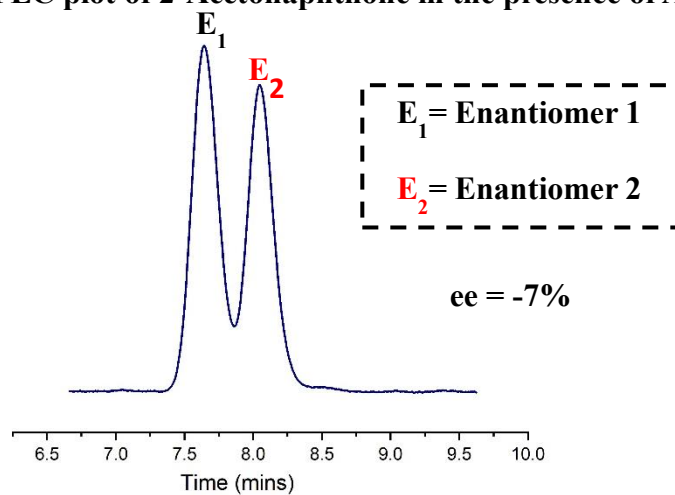


Figure 30: HPLC plot of 2-Acetonaphthone in the presence of Im-RL

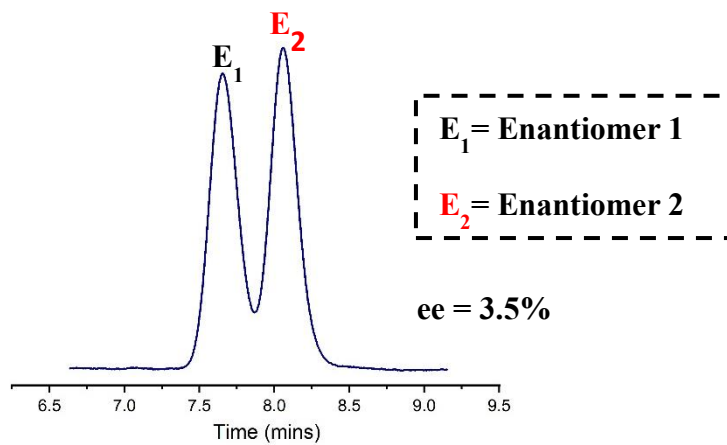


Figure 31: HPLC plot of 2-Acetonaphthone in the presence of PBS-LL

System	Enantiomeric Excess (%)
(BET)-L	30.5
Ac-RH	3.5
Ac-EL	1.9
Im-RL	7
PBS-LL	3

Table 8: Enantiomeric excess obtained against different peptides.

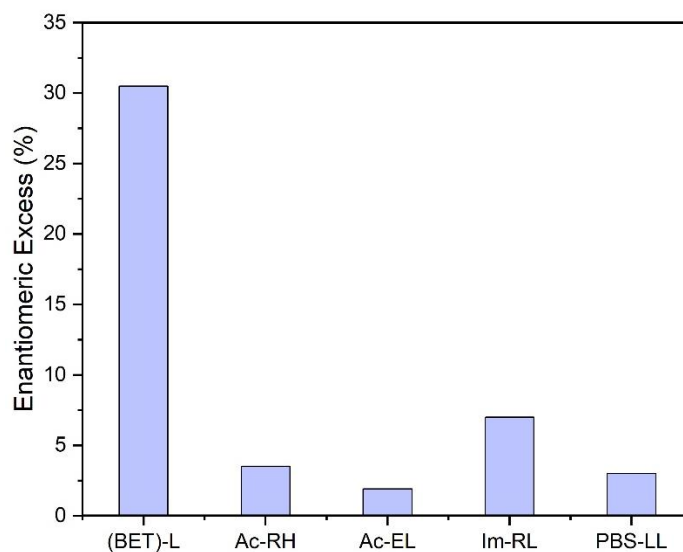


Figure 32: Bar graph showing the enantiomeric excess obtained for 2-Acetonaphthone against different peptides

This variation in peptide assemblies revealed that the nature of the surface charge plays a critical role in asymmetric induction. Specifically, peptides with negatively charged residues (Ac-EL and PBS-LL) exhibited lower enantioselectivity, with values of 1.9% and 3.0%, respectively

4. Conclusion

In conclusion, we successfully leveraged the catalytic potential of short, charged peptide-based amyloid assemblies capable of mediating enantioselective covalent catalysis through the strategic placement of functional residues. These assemblies also demonstrated the ability to recruit negatively charged cofactors, thereby facilitating catalysis at the amyloid surface. The hydrophobic nature of the prochiral substrates and the surface-exposed charge on the amyloids played a pivotal role in modulating the binding enthalpies of both the substrate and cofactor.

While the observed enantiomeric excess was lower than that achieved by fully evolved enzymes, the significance of this work lies in the use of minimalistic peptide oligomers. This study highlights the promising potential of short peptide-based amyloids to recruit cofactors and function analogously to enantioselective ketone reductases, offering insights into the possible evolutionary origins of chiral binding pockets in modern enzymes.

5. References

1. Enantioselective synthesis. *Wikipedia, The Free Encyclopedia*. Published April 6, 2025. https://en.wikipedia.org/wiki/Enantioselective_synthesis (accessed May 8, 2025).
2. Itsuno, S. *Enantioselective Reduction of Ketones*. In *Organic Reactions*; 2004.
3. Petrovičová, T.; Gyuranová, D.; Plž, M.; Myrtollari, K.; Smonou, I.; Rebroš, M. Application of Robust Ketoreductase from *Hansenula polymorpha* for the Reduction of Carbonyl Compounds. *Mol. Catal.* **2021**, 502, 111364.
4. Wang, X.; Saba, T.; Yiu, H. H. P.; Howe, R. F.; Anderson, J. A.; Shi, J. Cofactor NAD(P)H Regeneration Inspired by Heterogeneous Pathways. *Chem* **2017**, 2 (5), 621–654.
5. *Enzyme Catalysis in Organic Synthesis: A Comprehensive Handbook* (Eds.: K. Drauz, H. Waldmann), VCH, Weinheim, **2002**, pp. 1–3.
6. Jensen, R.A. Enzyme Recruitment in Evolution of New Function *Annu. Rev. Microbiol.* **1976**, 30, 409–425.
7. Pal, S.; Goswami, S.; Das, D. Cross β amyloid assemblies as complex catalytic machinery. *Chem. Commun.* **2021**, 57, 7597–7609.
8. Chatterjee, A.; Reja, A.; Pal, S.; Das, D. Systems chemistry of peptide-assemblies for biochemical transformations. *Chem. Soc. Rev.* **2022**, 51, 3047–3070.
9. Reetz, M. T. Controlling the enantioselectivity of enzymes by directed evolution: Practical and theoretical ramifications. *Proc. Natl. Acad. Sci. USA* **2004**, 101, 5716–5722.

10. Land, H.; Ruggieri, F.; Szekrenyi, A.; Fessner, W.-D.; Berglund, P. Engineering the Active Site of an (S)-Selective Amine Transaminase for Acceptance of Doubly Bulky Primary Amines. *Adv. Synth. Catal.* **2020**, 362, 812.
11. Ottosson, J.; Rotticci-Mulder, J.C.; Rotticci, D.; Hult, K. Rational design of enantioselective enzymes requires considerations of entropy. *Protein Sci.* **2001**, 10, 1769–1774.
12. Shin, J.-S.; Kim, B.G. Exploring the active site of amine:pyruvate aminotransferase on the basis of the substrate structure-reactivity relationship: how the enzyme controls substrate specificity and stereoselectivity. *J. Org. Chem.* **2002**, 67, 2848–2853.
13. Reetz, M.T.; Garcia-Borras, M. The Unexplored Importance of Fleeting Chiral Intermediates in Enzyme-Catalyzed Reactions. *J. Am. Chem. Soc.* **2021**, 143, 14939–14950.
14. Leech, A.P.; James, R.; Coggins, J.R.; Kleanthous, C. Re-evaluating the role of His-143 in the mechanism of type I dehydroquinase from *Escherichia coli* using two-dimensional ¹H,¹³C NMR. *J. Biol. Chem.* **1995**, 270, 25827–25836.
15. Sarkhel, B.; Chatterjee, A.; Das, D. Covalent Catalysis by Cross β Amyloid Nanotubes. *J. Am. Chem. Soc.* **2020**, 142, 4098–4103.
16. Singh, A.; Goswami, S.; Singh, P.; Das, D. Exploitation of Catalytic Dyads by Short Peptide-Based Nanotubes for Enantioselective Covalent Catalysis. *Angew. Chem., Int. Ed.* **2023**, 62, e202315716.
17. Pellerin, L.; Magistretti, P. J. Let There Be (NADH) Light. *Science*. **2004**, 305, 50–52.
18. Eto, K. et al. Role of NADH Shuttle System in Glucose-Induced Activation of Mitochondrial Metabolism and Insulin Secretion. *Science*. **1999**, 283, 981–985.
19. Riordan, J. F. Arginyl residues and anion binding sites in proteins. *Mol. Cell. Biochem.* **1979**, 26, 71–92.
20. Ottel , J.; Hussain, A. S.; Mayer, C.; Otto, S. Chance emergence of catalytic activity and promiscuity in a self-replicator. *Nat. Catal.* **2020**, 3, 547–553.
21. Neri, S.; Martin, S. G.; Pezzato, C.; Prins, L.J. Photoswitchable Catalysis by a Nanozyme Mediated by a Light-Sensitive Cofactor. *J. Am. Chem. Soc.* **2017**, 139, 1794–1797.
22. Liu, Q et al. Cofactor-free oxidase-mimetic nanomaterials from self-assembled histidine-rich peptides. *Nat. Mater.* **2021**, 20, 395–402.
23. Chen, K.; Arnold, F. H. Engineering new catalytic activities in enzymes. *Nat. Catal.* **2020**, 3, 203–213.

24. Robinson, W.; Daines, E.; vanDuppen, P.; deJong, T.; Huck, W.T.S. Environmental conditions drive self-organization of reaction path ways in a prebiotic reaction network. *Nat. Chem.* **2013**, *14*, 623–631.
25. Pott, M. et al. Noncanonical Heme Ligands Steer Carbene Transfer Reactivity in an Artificial Metalloenzyme. *Angew. Chem. Int. Ed.* **2021**, *60*, 15063–15068.
26. Das, D.; Roy, S.; Das, P.K. Efficient and Simple NaBH₄ Reduction of Esters at Cationic Micellar Surface. *Org. Lett.* **2004**, *6*, 4133–4136.
27. Dasgupta, A.; Mitra, R.N.; Roy, S.; Das, P.K. Asymmetric Resolution in Ester Reduction by NaBH₄ at the Interface of Aqueous Aggregates of Amino Acid, Peptide, and Chiral-Counterion-Based Cationic Surfactants. *Chem. Asian J.* **2006**, *1*, 780–788.
28. Hosseinzadeh, P. et al. Comprehensive computational design of ordered peptide macrocycles. *Science*. **2017**, 6359, 1461–1466.
29. Bian, T. et al. Electrostatic co-assembly of nanoparticles with oppositely charged small molecules into static and dynamic superstructures. *Nat. Chem.* **2021**, *13*, 940–949.
30. Lyu, Y.; Scrimin, P. Mimicking Enzymes: The quest for powerful catalysts from simple molecules to nanozymes. *ACS Catal.* **2021**, *11*, 11501–11509.
31. Liu, Q., Wang, H., Shi, X., Wang, Z. G. & Ding, B. Self-Assembled DNA/Peptide-Based Nanoparticle Exhibiting Synergistic Enzymatic Activity. *ACS Nano*. **2017**, *11*, 7251–7258.
32. Arad, E. et al. Native Glucagon Amyloids Catalyze Key Metabolic Reactions. *ACS Nano*. **2022**, *16*, 12889–12899.
33. Greenwald, J; Riek, R. On the Possible Amyloid Origin of Protein Folds. *J. Mol. Biol.* **2012**, *421*, 417–426.
34. Chatterjee, A., Goswami, S., Kumar, R.; Laha, J.; Das, D. Emergence of a short peptide-based reductase via activation of the model hydride rich cofactor. *Nat Commun.* **2024**, *15*, 4515.
35. Ghosh, C.; Menon, S.; Bal, S.; Goswami, S.; Mondal, J.; Das, D. Emergence of Catalytic Triad by Short Peptide Based Nanofibrillar Assemblies. *Nano Lett.* **2023**, *23*, 12, 5828–5835.
36. Roy, S.; Das, D.; Dasgupta, A.; Mitra, R. N.; Das, P. K. Amino Acid Based Cationic Surfactants in Aqueous Solution: Physicochemical Study and Application of Supramolecular Chirality in Ketone Reduction. *Langmuir* **2005**, *21* (22), 9744–9753.