# Liquid Crystal as a Sensing Platform for Detection of Structurally Different $A\beta_{1-42}$ Oligomers

**Aayush**

A dissertation submitted for the partial fulfilment of
BS-MS dual degree in Science


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# Certificate of Examination

This is to certify that the dissertation titled **"Liquid Crystal as a Sensing Platform for Detection of Structurally Different $A\beta_{1-42}$ oligomers"** submitted by **Aayush** (Reg. No. MS12087) for the partial fulfilment of BS-MS dual degree programme of the Institute, has been examined by the thesis committee duly appointed by the Institute. The committee finds the work done by the candidate satisfactory and recommends that the report be accepted.

Dr. Samrat Mukhopadhyay Dr. Sabyasachi Rakshit Dr. Santanu K. Pal
(Supervisor)

Dated: April 21, 2017

# Declaration

The work presented in this dissertation has been carried out by me under the guidance of Dr. Santanu K. Pal at the Indian Institute of Science Education and Research Mohali. This work has not been submitted in part or in full for a degree, a diploma, or a fellowship to any other university or institute. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due acknowledgement of collaborative research and discussions. This thesis is a bonafide record of original work done by me and all sources listed within have been detailed in the bibliography.

Aayush

(Candidate)

Dated: April 21, 2017

In my capacity as the supervisor of the candidate's project work, I certify that the above statements by the candidate are true to the best of my knowledge.

Dr. Santanu K. Pal

(Supervisor)

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# Abbreviations

| | |
|---|---|
| LC | Liquid crystal |
| POM | Polarized optical microscope |
| DMOAP | Dimethyloctadecyl[3-(trimethoxysilyl)propyl]ammonium chloride |
| 5CB | 4-Cyano-4'-pentylbiphenyl |
| PBS | Phosphate- buffered saline |
| POPC | 1-palmitoyl-2-oleoly-sn-glycero-3-phosphocholine |
| POPG | palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1′-rac-glycerol) (sodium salt) |
| OCo | OC active oligomer |
| A11o | A11 active oligomer |
| ThT | Thioflavin T |
| AFM | Atomic force microscope |
| TEM | Transmission electron microscope |
| HFIP | Hexafluoroisopropanol |
| PBST | Phosphate- Buffered Saline with Tween 20 |

# Contents

# Chapter 1

# INTRODUCTION

## 1.1 Amyloid-β Oligomers:

Alzheimer's disease is characterized by accumulation of different neurotoxic assemblies of Aβ peptide in the form of fibrils and oligomers. It had been observed that soluble oligomers are more neurotoxic than the fibrils and correlate better with the progress of dementia over age.

There are many different pathways which can be taken up *in vivo* during oligomerization and fibril formation. Although, it's the on-pathway oligomerization which leads to formation of structured oligomers and pose maximum toxicity.[1]

There are two different kinds of oligomers based on the presence of distinct structure (generic epitope): prefibrillar oligomers and fibrillar oligomers. As the name suggests, fibrillar oligomers are conformationally similar to fibrils whereas, prefibrillar oligomers are formed before fibril formation and have different conformation as compared to the fibrillar oligomers (even though they have possible size overlaps).[2] Prefibrillar oligomers convert to fibrillar state by changing the conformation either in a concerted manner or in blocks, whereas fibrillar oligomers grow without any conformation change but just by addition of monomers to the ends to form fibrils (although there is a possibility that monomer may undergo conformation change before adding) (fig. 1).

Conformation specific antibodies had been reported which detects mutually exclusive generic epitopes of oligomers: A11 antibody detects prefibrillar oligomers whereas OC antibody detects fibrillar oligomer as well as fibrils, regardless of the sequence of peptide being used as antigen.[2] Since, these antibodies are conformation specific, therefore, detection of both fibrillar oligomer and fibrils by OC antibody implies that they share same conformation and that similarity was found to be in-register parallel β sheet structure.[3] The distinction between fibrils and fibrillar oligomer is simply based on size difference as they share the same epitope.

As mentioned before, the main culprit in amyloid pathogenesis had been shown to be soluble oligomers rather than insoluble fibrils. Out of the two classes of oligomers, it was

shown that A11 active oligomer, i.e. a prefibrillar oligomer (Aβ*56-a 56kDa dodecameric $A\beta_{1-42}$ assembly) appears before plaques form in the brain and is more tightly associated with memory declination, whereas, OC active oligomers, i.e. fibrillar oligomers are generated only after plaque formation.[3,4] Analysis of localization of these oligomers in brain parts revealed that, A11 active oligomer is dispersed and it localizes independent of plaques whereas the OC active oligomer localizes around dense-core plaques. This localization is supposed to be a key determinant in neutralizing the toxic effects of latter as compared to former.[3]

The nature of occurrence of prefibrillar oligomers show that they are not mandate intermediates during fibril formation, unlike fibrillar oligomers which are associated with fibrils spatially, temporally and in conformation. These different oligomers pose different level of toxicity and possibly have different mechanisms of pathogenesis,[5] so if an optical tool could be developed for differentiating them, that will provide a whole new platform for their easier characterizations and elucidating their pathogenesis.

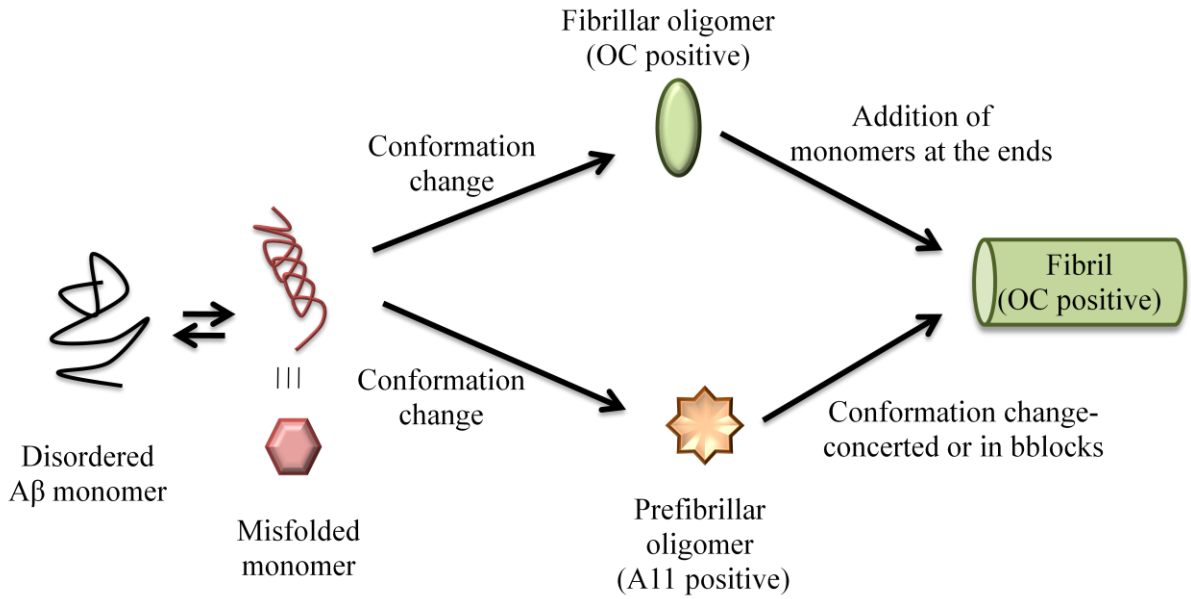



**Fig. 1-** Schematic of generation of two structurally different kinds of oligomers and the corresponding mechanisms to convert into fibrils.

## 1.2 Interaction of Aβ peptide with lipid membrane:

It had been observed that the rate of amyloid formation increases by many folds due to interaction with lipid membranes. The kind of interaction that Aβ peptide has with lipid membrane depends on many factors- (i) Aβ assembly, i.e. whether it's in monomeric or oligomeric or fibrillar form; (ii) charge on lipid membrane; (iii) pH of subphase.

Monomer had been shown to insert into the lipid membrane which in turn increases the rate of fibril formation.[6,7] Oligomers may have various interaction with the lipid membrane and thus, have toxic effects on the cell- (i) binding and disturbing lipid membrane packing; (ii) causing unregulated influx/efflux by forming annular pores and inserting in the membrane; (iii) binding with cell membrane receptors and altering their normal functions.[1] There are many reports with ambiguous results upon the kind of interaction that oligomers have with lipid membrane. Some had shown that they increase permeability of membrane by interacting peripherally[8,9] while others show that they do that by inserting into the membrane.[6,10] All these reports have consensus on the fact that oligomers do increase permeability of lipid membrane and this effect had not been observed in case of monomers or fibrils.[8]

The characterization of interaction of Aβ peptide with lipid membrane may throw light on the role of lipid in the mechanism of pathogenesis of a given peptide assembly *in vivo*.

## 1.3 Theme of the present project:

In this project, we attempted to use liquid crystal (LC) as a tool for differentiating prefibrillar and fibrillar oligomers based on their interaction with lipid monolayer. This work provides an insight into the interaction of the two kinds of oligomers with lipid membrane and the toxic effect that they may have on it. We didn't choose any particular size of oligomers for these analyses, rather a range of sizes in a mixture was analyzed for each oligomer to get a generalized result which is just based on difference in conformation.

A lipid monolayer was deposited at the LC-aqueous interface. The message of slightest of disturbance due to interaction of peptide with lipid, strong enough to perturb orientation of LC molecules at the interface is transmitted throughout the bulk and a change in optical response is observed.

Using LC as a probing tool gives one the benefit of easy readout of the events occurring at the interface over time as change in optical response is recorded. The detection limit of LC is very low (from nM up to pM), thus, even very low concentrations which are physiologically relevant could be analyzed through this.

# Chapter 2

# EXPERIMENTAL SETUP AND PROCEDURE

## 2.1 Use of LC as biosensor:

LC had long found applications in various optoelectronics devices, but quite recently it had emerged as a very important tool in the field of biosensors.[11] Interaction with various analytes at the interface under specific conditions leads to change in orientation order of LC and that message is transmitted through the bulk across the depth of few microns leading to a change in optical appearance (fig. 2). LC molecules, in the case of LC filled gold grid placed on top of DMOAP coated glass slide are aligned perpendicular to the glass slide (homeotropic alignment) due to hydrophobic interaction between long aliphatic tail of DMOAP and the hydrophobic tail of LC molecule (5CB), thus, the optical appearance is dark under cross polar (fig. 2a). When the system is immersed in phosphate-buffered saline (PBS) buffer at pH 7.4, the LC molecules at the interface with buffer become parallel to glass slide (planar alignment) which in turn, causes a change in orientation even in the bulk, changing the optical appearance from dark to bright (fig. 2b). After lipid vesicle solution/ lipid monolayer is introduced in the buffer then the lipid molecules realign themselves at the LC-aqueous interface due to hydrophobic interaction between lipid molecule's long aliphatic chain and LC, which brings back the LC molecules into homeotropic alignment marked by dark optical appearance (fig. 2c). LC have provided label-free detection technique, low detection limits, high sensitivity, easy optical readout and doesn't require any complex instruments.

LC is an anisotropic material, i.e. some of its physical properties depend on direction. In layman's terms not all directions of the material are equal towards an observer, here, observer would be electric field, magnetic field, electromagnetic wave etc. To have a frame of reference, one of the directions is called "director" of LC and behaviour of LC changes as the direction of application of field (electric, magnetic etc.) changes with respect to the director. Because of this property, LC behaves as birefringent material, i.e., property of light (speed, consequently refractive index) changes depending on its direction of propagation and polarization with respect to the director. This property of light is used to observe changes in orientation order of LC using a Polarized Optical Microscope (POM) which will be discussed in detail in section 2.3.1.

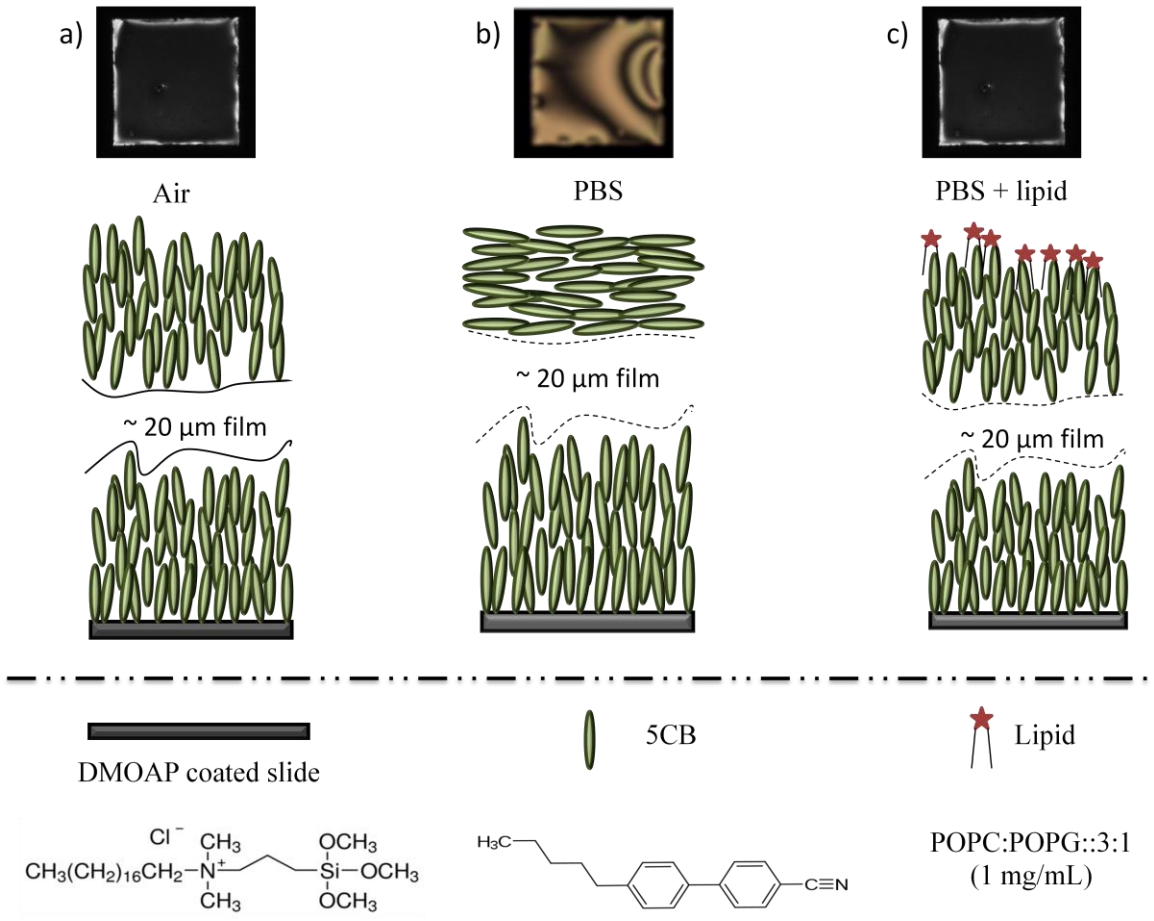



**Fig. 2**- Schematic and POM images of changes occurring at **a)** LC-air interface; **b)** LC-aqueous interface; **c)** upon deposition of lipid vesicle/ monolayer at the LC-aqueous interface.

## 2.2 Importance of monolayer and various parameters associated with it:

We prepared lipid laden LC-aqueous interface for probing peptide dynamics mimicking the process occurring *in vivo*, as there are several reports showing the enhanced rate of an otherwise very slow aggregate formation due to interaction with lipid,[6] also some reports claim it to be lipid membrane associated process.[6]

Moreover, since we are probing conformationally distinct oligomers of Aβ-42 and aggregates, interaction with lipid plays a very important role as it had been shown that different oligomers can disrupt the cell membrane to different extent.[9,14] Thus, it was though that not only to mimic the natural condition but, using lipid will also help in differentiating oligomers based on their interaction with lipid.

There are two widespread methods for forming layer of lipid at the LC - aqueous interface: vesicle fusion and Langmuir Schaefer transfer. Vesicle fusion method was tried first. The minimum concentration of lipid that will cause LC molecules to reorient themselves into homeotropic alignment from planar alignment (in aqueous medium) due to binding with lipid molecules was found. At this concentration, addition of peptide solution (even at very high concentration- around 45 μM) didn't lead to any change in orientation although a published report had shown change in optical response when monolayer was used instead of vesicle fusion (section 3.2).[12] So, further experiments were carried by using lipid monolayer laden LC-aqueous interface which was achieved by using Langmuir-Schaefer transfer technique (the details of which will be discussed in section 2.3.2).

## 2.3 Instrumentation:

### 2.3.1 Polarized Optical Microscope (POM)-

As discussed earlier, LC being anisotropic material leads to a change in polarization of light passing through it depending on its polarization and direction of propagation with respect to the director of the material (it's optical axis). Uniaxial LC (nematic phase), has two principal refractive indices, ordinary and extraordinary. Former is measured for the polarized light with electric field vibrations perpendicular to the optical axis and latter for the one having electric field vibrations parallel to the optical axis. If the polarized light entering the material is parallel to either ordinary or extraordinary axis, then, it passes without any alteration (i.e. remain linearly polarized in the same direction) otherwise it's broken into two components (parallel and perpendicular to the director) and final polarization depends on the how out of phase the two components are, giving either linear or more commonly elliptical polarized light, meaning the emerging light will have rotated polarization around the direction of propagation.

This property of material is used to probe changes in orientation order of LC by using polarized light which can be obtained by using a polarizer. The polarization of light emerging from the material will be a representative of its orientational order, so to get an idea on how much the polarization changed, another polarizer (called analyzer) is placed at the end (fig. 3).

This ensemble of polarizer and analyzer is present in POM which is used to study experiments pertaining to this field of research.(fig. 4) Most of the POMs come with the freedom to keep the analyzer either parallel or perpendicular (cross) to the polarizer giving us bright field and polarized image respectively which helps in determining the changes in orientation order occurring at different steps of experiment.

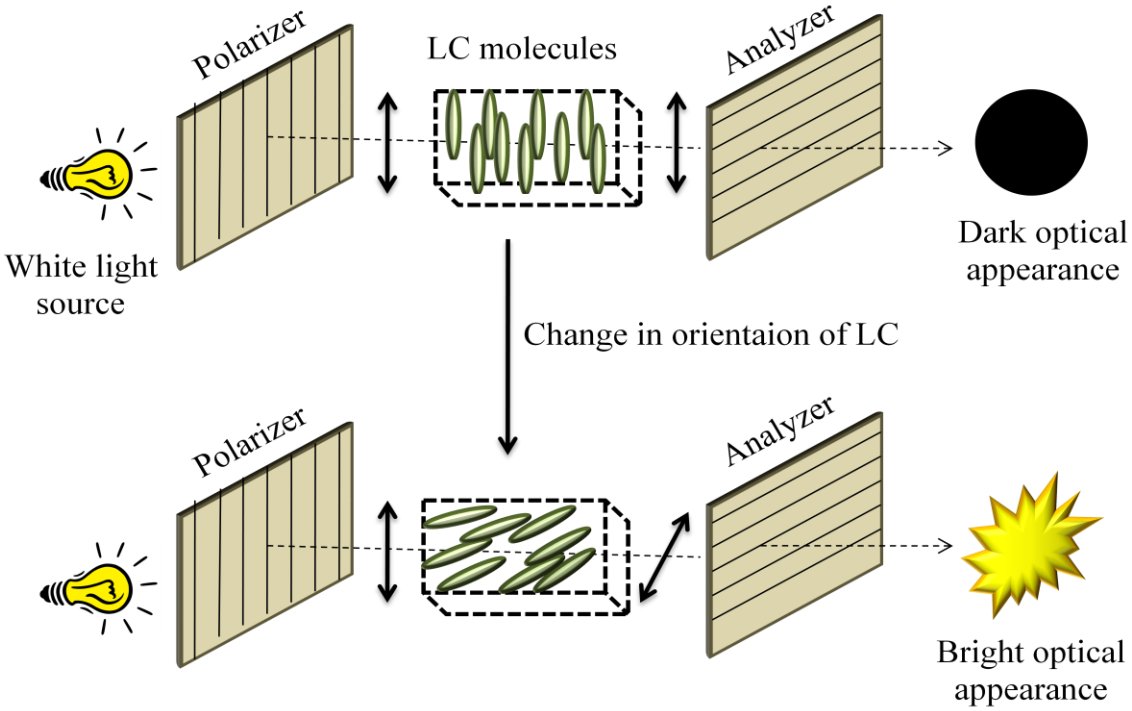



**Fig. 3-** Schematic of the change in optical response observed upon change in orientation order of LC molecules as seen under cross polarizers.

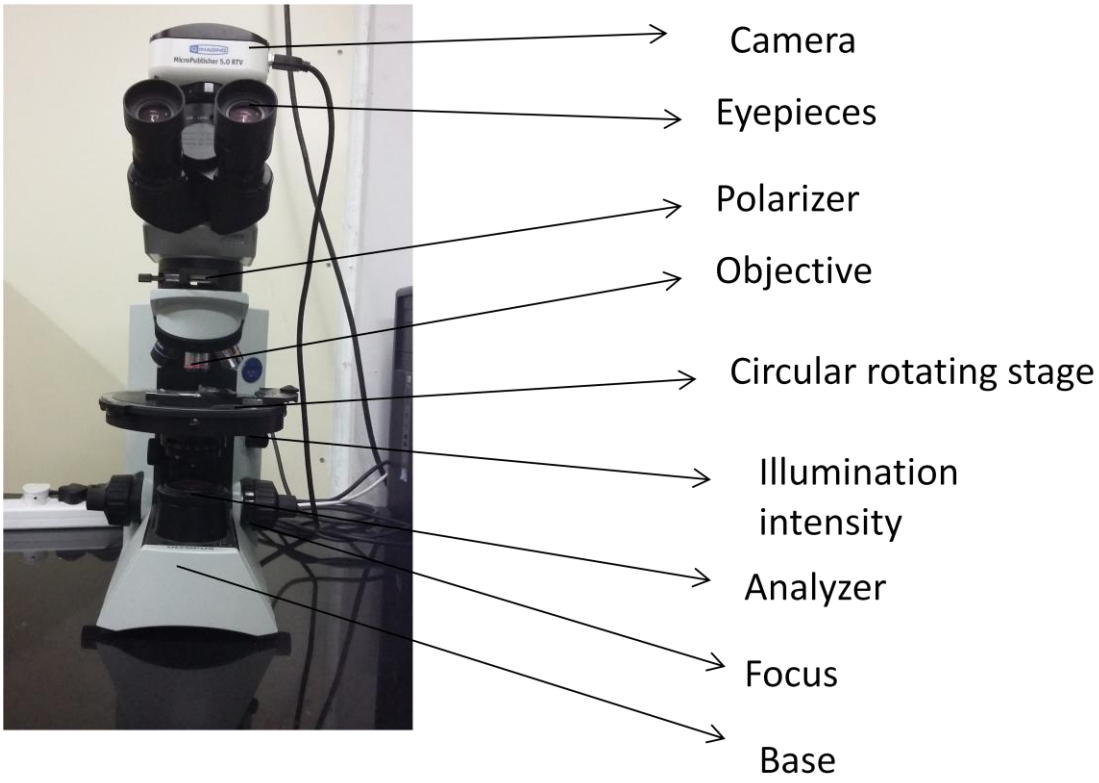



**Fig. 4-** Polarized optical microscope (POM).

### 2.3.2 Langmuir-Schaefer Transfer -

*Langmuir films*- Amphiphilic substances having both - hydrophobic part and hydrophilic part orient themselves at aqueous-air interface in a manner such that the hydrophilic part remains immersed in the water whereas the hydrophobic tail sticks out in air. Since, most of the amphiphilic substances are insoluble in aqueous medium, thus, spreading them over aqueous surface could be attained by dissolving them in appropriate organic solvent and depositing over the aqueous surface in a drop wise fashion forming an insoluble monolayer. The monolayer formed is a one molecule thick layer at the aqueous-air interface, and is also known as Langmuir films.

*Surface Pressure-Area isotherm*- Measuring the surface pressure as a function of area of aqueous surface available to each amphiphilic molecule is an indicator of the monolayer properties. To obtain good isotherms, it is recommended to do the recordings of surface pressure at constant temperature as the compression of the film is ongoing by closing barrier at a very slow rate (speed-3-5mm/min) (fig. 5a).

A typical isotherm consists of a number of phases during the process of compression which majorly depends upon- composition and temperature of subphase, physical and chemical properties of amphiphile. In general following phases are observed as compression is performed- gaseous , liquid expanded , liquid condensed , solid. Further compression of the solid phase lead to collapse of the monolayer into three- dimensional structures accompanied by rapid fall of pressure (or horizontal break in the isotherm if the monolayer is in liquid state) (fig. 5b).

*Factors affecting monolayer quality*- Some of the factors have been discussed here which may plague any experimentalist while trying to form monolayer and during deposition (more details could be found in[13])

1. speed of compression- the speed of compression should be low enough to avoid any surface local overpressure (leading to development of defects).
2. deposition pressure- molecules must have just reached the molecular packing of solid phase when the deposition is performed.
3. Temperature of subphase - To obtain a homogeneous monolayer, the presence of gaseous phase (pure fluid stage) of the monolayer in the beginning is compulsory which can't be attained if the temperature is below a certain limit. If the

temperature is too low, it will lead to nucleation and growth of condensed material and only irregular shaped structures are developed rather than a uniform monolayer.[14]

*Langmuir-Schaefer transfer*- It is a method of transferring Langmuir monolayers onto some solid substrate horizontally while the surface pressure of the layer is constant (fig. 5a). Commonly, the transfer is carried while lipid is in the solid phase (below the breakpoint). The quality of transfer depends upon- type and nature of substrate, deposition rate (comprises of both- speed and area of deposition).[13]

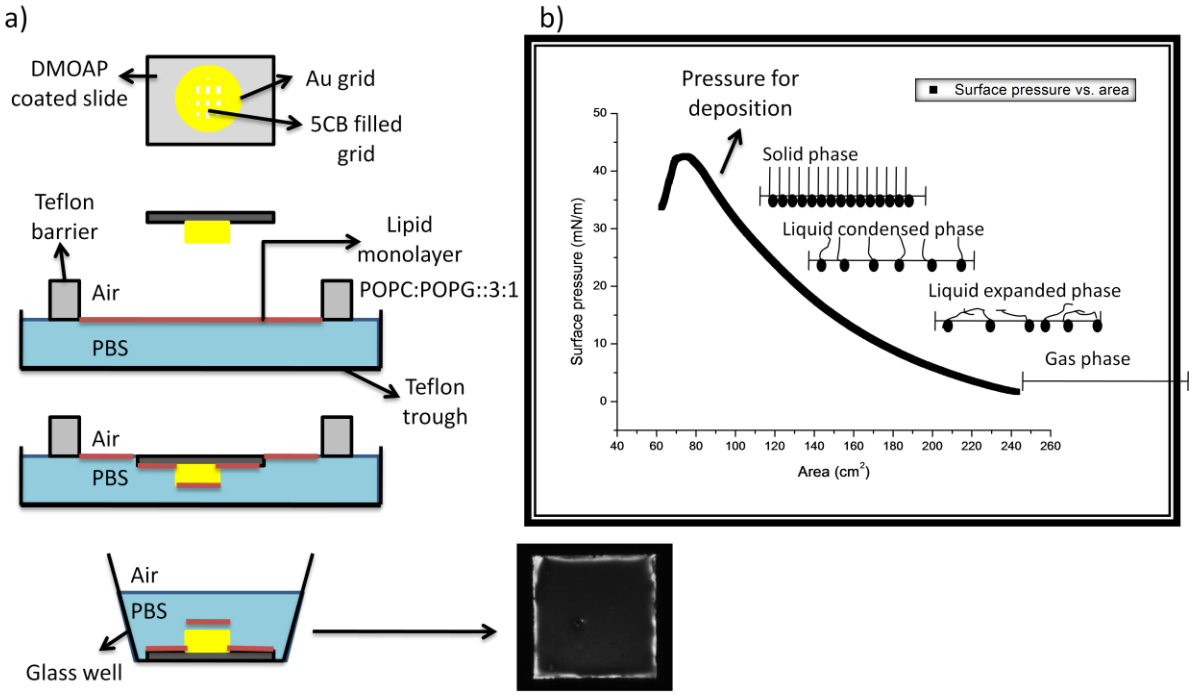



**Fig. 5- a)**Schematic of the experimental setup for transferring monolayer from air- PBS interface on to LC- PBS interface using Langmuir-Schaefer Transfer technique. **b)**Corresponding surface pressure-area isotherm obtained with a schematic of changes in the phase of lipid layer at the PBS-air interface as the surface pressure increases upon decrement of area.

## 2.4 Procedure:

The use of LC as a reporter of binding of analytes at interface lie at the core of this research. The information of any change in orientation occurring at the interface is passed on to the bulk (even up to distances ranging in μms) and thus amplified which can be detected by optical instruments (here, using POM). It had been already proven that LC gives distinct response towards different kinds of peptides[12] but, through this project, it was showed that LC have the capability to report different conformations of oligomers by giving distinct response.

For this, two structurally different oligomers- prefibrillar and fibrillar oligomers were prepared and characterized by Priyanka Madhu (from Dr. Samrat Mukhopadhyay's lab at IISER Mohali). Then, the experiments were performed at 25 °C using 5CB (4-Cyano-4'-pentylbiphenyl) in it's nematic phase. To represent biological membrane system, lipid monolayer of 1-palmitoyl-2-oleoly-sn-glycero-3-phosphocholine (POPC):1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1′-rac-glycerol) (sodium salt) (POPG) 3:1 mixture (1 mg/mL) and a subphase of phosphate–buffer saline (PBS) buffer with pH 7.4 were used.

Langmuir film was made on PBS buffer by compressing the lipid monolayer (in gas phase) slowly at the rate of 3-5mm/min and then it was deposited onto the LC films prepared on DMOAP coated glass slide at a surface pressure of 38mN/m (in solid phase) using Langmuir-Schaefer transfer (fig.5). At this point, the LC molecules attained homeotropic alignment giving dark appearance under cross polar with bright edges due to interaction with the walls of the gold grid. This was immersed in a glass well filled with 2mL of PBS and peptide solution was added to it for further characterization of its interaction with the lipid monolayer as any change occurring at the interface is amplified by LC showing a change in optical appearance.

# Chapter 3

# RESULTS AND DISCUSSION

## 3.1 Standardizing concentration of lipid to be used:

This was done by preparing lipid vesicles of the mixture POPC:POPG :: 3:1 (1 mg/mL) (section 4). Three different systems were prepared, each consisting of gold grid placed on pieces of DMOAP coated glass slides, filled with 5CB. At this stage, it was made sure that it was giving dark appearance under cross polar due to homeotropic alignment of LC molecules (fig. 6a-c). After that, the whole ensemble of slide, gold grid and LC was immersed in a glass well filled with PBS (2 mL) and change from dark to bright appearance was observed as the molecules reoriented themselves into planar arrangement (fig. 6d-f). Lipid vesicle solution (2 mg/mL) was introduced into the wells, such that final concentration reached 0.1 mg/mL, 0.5 mg/mL and 1mg/mL and changes were recorded over a period of 2 hrs (fig. 6g-i).

It was observed that the optical response for the well filled with 1mg/mL lipid vesicle solution changed from bright to completely dark but others remained bright even after 2 hrs (data not shown). Through this experiment, the lowest concentration of lipid vesicle solution to be used was found out to use in further experiments.

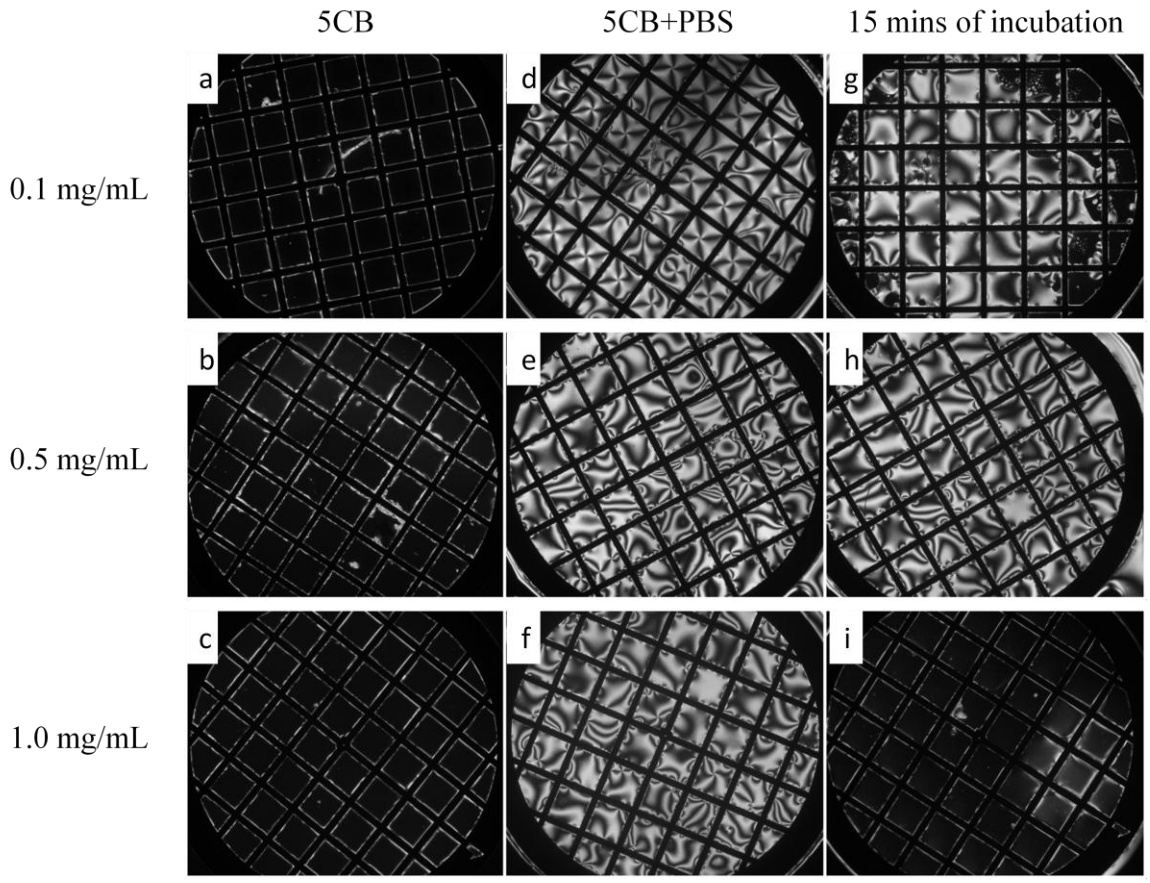



**Fig. 6- (a-c)** POM images of 5CB filled gold grids placed on DMOAP coated glass slide. **(d-f)** POM images of 5CB filled gold grids placed on DMOAP coated glass slide immersed in PBS solution. **(g-i)** POM images of response by LC towards 0.1 mg/mL(g), 0.5mg/mL(h) and 1.0 mg/mL(i) of lipid vesicles composed of POPC:POPG::3:1 (1 mg/mL).

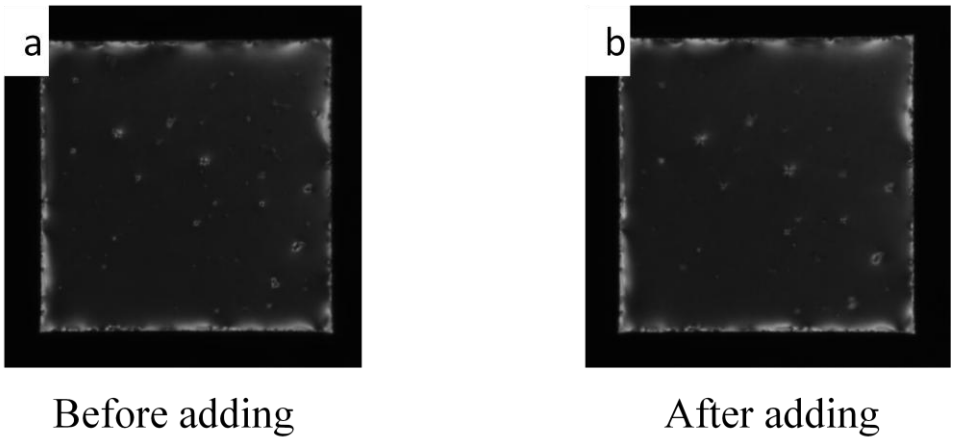



**Fig. 7- a)** POM image before adding monomer. **b)** POM image 3hrs after adding monomer.

## 3.2 Interaction with peptide:

After keeping the slide immersed in the lipid vesicle solution till it became completely homeotropic, it was taken out and immersed in fresh PBS containing well. It remained homeotropic even at this stage (fig. 7a). Monomer solution was introduced in the well and no changes occurred at all for any concentration of the peptide (up to 45 μM), even after days (fig. 7b), although there are reports showing changes occurring when peptide solution is introduced to lipid monolayer laded LC-aqueous interface.[12]

The difference lied in the method of forming layer of lipid at aqueous-LC interface. The report involved using lipid monolayer whereas here, lipid vesicle fusion was used in which there is almost no control on density of lipid being deposited over the interface. It had been shown that high density of lipid may hinder peptide from penetrating the lipid layer and reaching the LC surface to change its orientation order.[15] So, it was decided to work with lipid monolayer for which Langmuir Schaefer transfer was deemed perfect.

### 3.2.1 Surface Pressure- area isotherm for POPC:POPG :: 3:1 mixture (1 mg/mL)-

Surface Pressure vs. area isotherm for the mixture of lipids POPC and POPG present in a solution of chloroform in the ratio 3:1 making up the concentration of 1 mg/mL was obtained by spreading the solution over PBS subphase in a teflon trough and then compressing it slowly (fig. 5a). As can be seen from the isotherm, the breakpoint was 43.2 mN/m (fig. 5b) and thus, the monolayer was deposited at the surface pressure of 38 mN/m when the monolayer was in solid phase (fig. 5a).

### 3.2.2 Peptide solutions-

$A\beta_{1-42}$ in three assembly states had been analyzed- monomer, OC active oligomer and A11 active oligomer. The concentrations used were 250nM, 100nM and 50nM. For all the conformers, three concentrations were analyzed multiple times. 250 nM was kept as the highest concentration to be analyzed for mimicking biologically relevant concentrations and the lower limit of detection for this system had not been found yet.

The peptide solutions were stored at -80 °C for 24 hrs after they were prepared. The solutions were brought out of -80 °C and kept in ice for further use in the experiment. It was made sure that the solutions didn't spend more than 30-45 min in ice, as there have

been changes in conformation observed even at such low temperature when kept for long (data not shown).

*Confirmatory tests for peptides: dot blot assay* -

Two oligomers were prepared which differed in terms of both- conformation and toxicity based on the disruption that they can cause to cell membrane.[8,9] These oligomers were probed by conformation specific OC and A11 antibodies and were characterized using dot blot technique. Sequence specific antibody 6E10 (recognizes N-terminus of Aβ, residues 3-10) was used to confirm proper loading of each oligomer (fig. 8). [The preparation of oligomers and their characterizations were performed by Priyanka Madhu (from Dr. Samrat Mukhopadhyay's lab at IISER Mohali)]

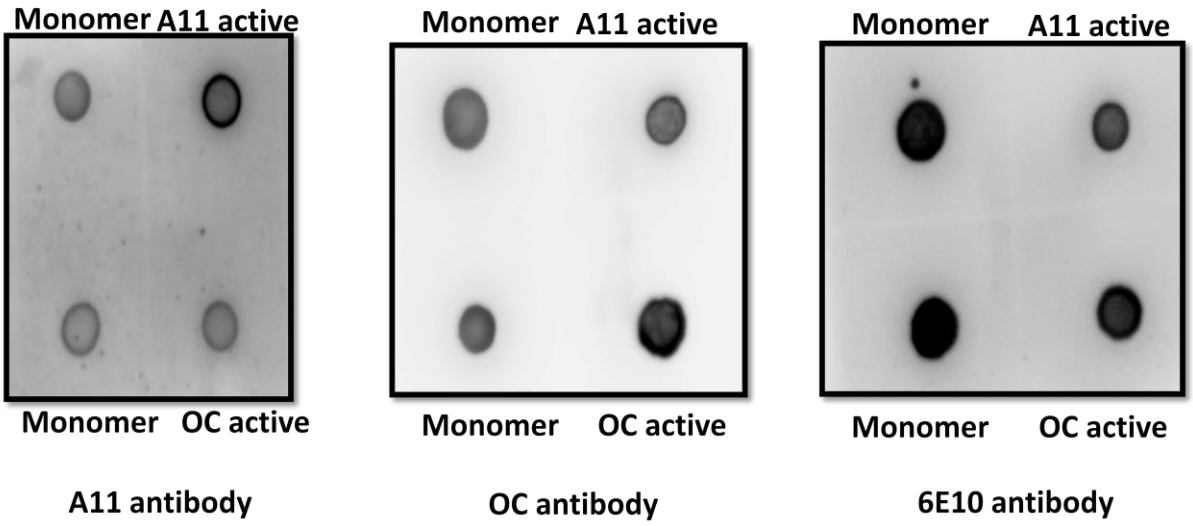



**Fig. 8**- Dot Blot assays of different oligomers using conformation-specific antibodies- A11 and OC antibody.

### 3.2.3 Interaction of different $A\beta_{1-42}$ assemblies with lipid monolayer laden LC-aqueous interface-

A. Monomer-

Three wells, each containing DMOAP coated slide with gold grid filled with 5CB having lipid monolayer at aqueous-LC interface immersed in 2 mL PBS buffer were prepared. Three different concentrations of monomer solution were prepared- 250nM, 100nM and 50nM in the three different wells by adding appropriate volume of stock monomer solution of 45 μM. Then the changes in optical response were recorded at regular intervals (fig. 9).

It was observed that after a few minutes, bright branch like structures started appearing which grew over time.

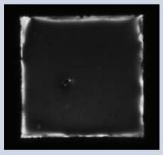
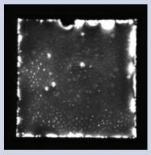
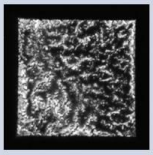
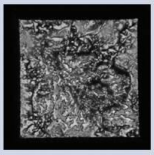
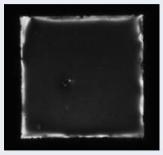
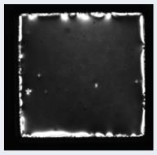
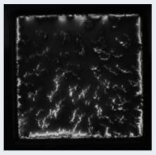
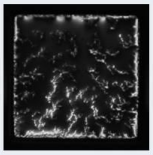
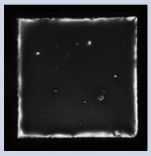
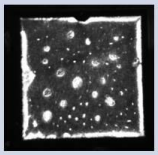
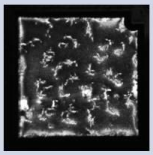
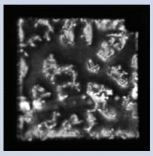
| Conc. (nM) | Before | Start | Just After start | 1h. 30min. | 3h. |
|---|---|---|---|---|---|
| 250 |  | 4 min. |  |  |  |
| 100 |  | 6 min. |  |  |  |
| 50 |  | 10 min |  |  |  |

**Fig. 9-** POM images showing change in optical response of lipid monolayer laden LC-aqueous interface towards $A\beta_{1-42}$ monomer.

B. OC active oligomer (OCo)-

Similar systems as mentioned above were prepared. A 45 μM stock solution of OCo was prepared and was used to make final concentrations of 250 nM, 100 nM and 50 nM in three different wells. The optical response was recorded at regular intervals of time (fig. 10).

This also showed appearance of bright branch like structure after a few minutes which grew over time.

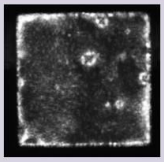
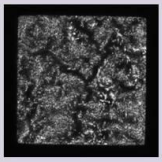
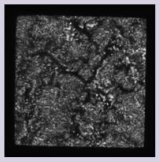
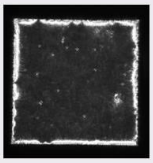
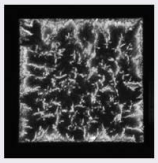
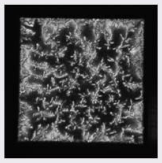
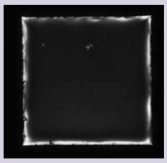
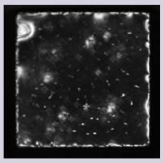
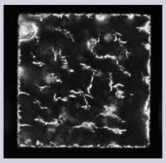
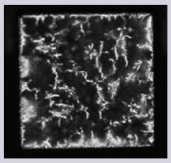
| Conc. (nM) | Before | Start | Just after start | 1h. 30min. | 4h. 30min. |
|---|---|---|---|---|---|
| 250 |  | 5 min. |  |  |  |
| 100 |  | 7 min. |  |  |  |
| 50 |  | 26 min. |  |  |  |

**Fig. 10-** POM images showing change in optical response of lipid monolayer laden LC-aqueous interface towards OCo.

C. A11 active oligomer (A11o)-

The systems prepared in case of monomers were prepared again and 45 μM stock solution of A11o was prepared. Appropriate volume of peptide stock solution was added to the wells to reach a final concentration of 250 nM, 100 nM and 50 nM and change in optical response was recorded over time (fig. 11).

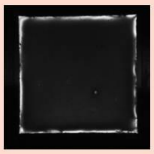
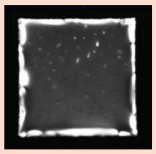
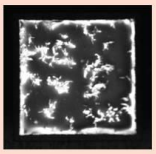
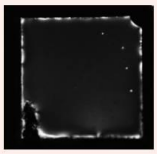
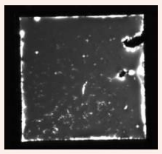
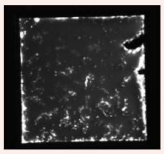
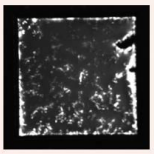
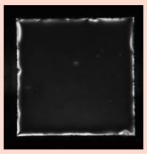
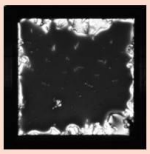
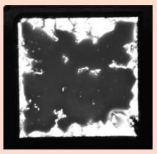
| Conc. (nM) | Before | Start | 1h. 30min. | 3h. | 7h. 30min. |
|---|---|---|---|---|---|
| 250 |  | 1h. 7min. |  | |  |
| 100 |  | 1h. 30min. |  |  |  |
| 50 |  | 2h. 32min. | |  |  |

**Fig. 11-** POM images showing change in optical response of lipid monolayer laden LC-aqueous interface towards A11o.

### 3.2.4 Thioflavin T assay

Once, the changes in optical response seemed to have reached saturation, thioflavin-T (ThT) binding assay was performed to check if there was any beta-amyloid fibril present (as the optical response of LC resembled the fibrillar structures of Aβ amyloid as observed in AFM analysis). ThT assay is used to confirm the presence of amyloid fibrils. ThT assay showed that the bright structures appearing in the case of OCo were indeed due to beta-amyloid fibrils (fig. 12). The bright branch like structures forming and growing over time can't be amyloid beta fibrils themselves (as they are too big to represent fibril which has the typical size of 6-10nm[16]), but can be optical amplification of the disturbance caused in the LC ordering due to fibril formation which formed by interaction of the oligomers with the lipid monolayer.

Of all the trials performed with A11o, none of them was positive towards ThT assay but this doesn't confirm the absence of fibrils. AFM studies will prove helpful in reaching a conclusion in this regard.

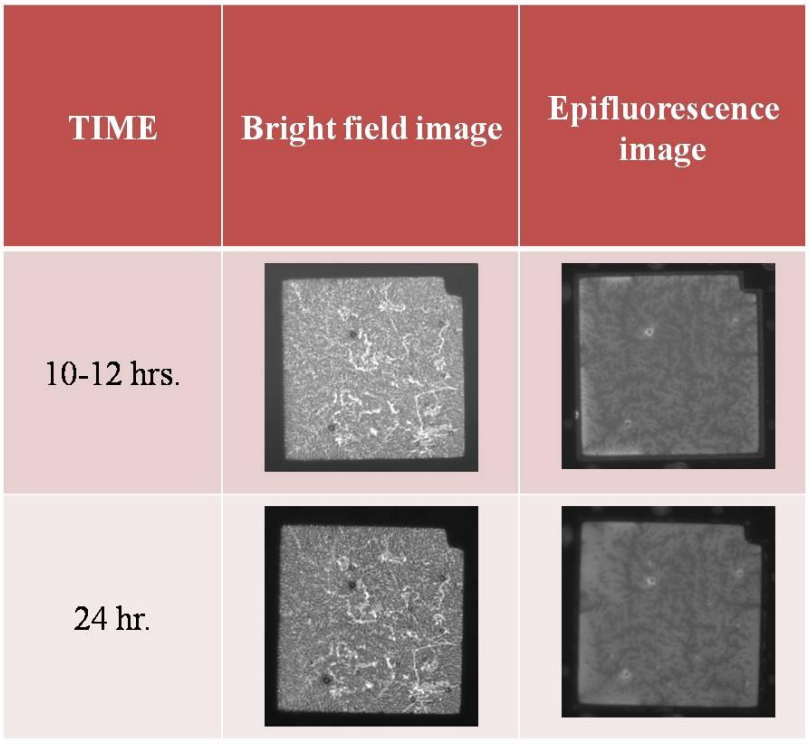



**Fig. 12-** POM and epifluorescence images recorded after incubating the system containing OC active oligomer with 5μM ThT solution. Time mentioned in the image is with respect to the start of the change in optical response observed.

# Chapter 4

# EXPERIMENTAL SECTION

*Cleaning of Glass Substrates* - Glass microscope slides were cleaned using 'piranha' solution {70:30 (%v/v) $H_2SO_4$:$H_2O_2$}, the procedure for which is described in detail elsewhere.[17] Briefly, glass slides were immersed into piranha solution and then the solution was heated up to 100 °C and kept at this temperature for an hour, after which glass slides were rinsed sequentially with Milli-Q water and ethanol and dried under a stream of nitrogen. The clean slides were stored overnight in the oven at 100 °C. All the other glassware were cleaned and dried properly prior to use.

*Preparation of DMOAP-Coated Glass Slides* - The piranha cleaned slides were dipped into 0.1% (v/v) DMOAP solution in Milli-Q water for an hour at room temperature, after which they were rinsed thoroughly with Milli-Q water to remove unreacted DMOAP from the surface. Finally, the slides were dried under nitrogen flow and stored in oven at 100 °C for 3 hours to allow cross-linking of DMOAP.

*Preparation of LC Films in TEM Grids* - The DMOAP coated slides were cut in small pieces using a glass cutter for supporting LC, then, a gold grid was placed on a piece and approximately 0.2-0.3 μL of 5CB was dispensed onto the grid to fill it completely. Excess LC was removed using a syringe to produce a planar surface.

*Optical Characterization of LC films in air and aqueous solutions* - Tapping on the birefringent character of LC, a polarizing optical microscope (Olympus CX31) with 4X objective in cross polar mode was used to characterize LC films. Images were captured using QImaging MicroPublisher 5.0 RTV camera mounted on the microscope with an exposure time of 1412.5 msec.

*Preparation of lipid vesicles* - The preparation was done according to the procedure published in detail elsewhere.[18] Briefly, chloroform solution of lipid mixture was dispensed in a round bottom flask and chloroform was left to evaporate under vacuum for at least 2h until a thin layer of the lipid formed along the inner wall of the flask. Before hydration, the lipid film in the flask was kept in nitrogen flux for 30 min. The process of hydration was carried for about an hour in PBS buffer followed by 1min of vortexing. This produced a cloudy solution indicative of large multi-lamellar vesicles. Clear solution

was obtained upon sonication of the suspension using a probe ultra-sonicator (1 x 15 min at 25 W). The vesicles were utilized within 24hr of their preparation.

*Langmuir-Schaefer transfer of POPC:POPG::3:1 Monolayers on the Aqueous-LC Interface* - According to previous studies, there are two methods of forming monolayer at the interface of thermotropic LC and aqueous interface - vesicle fusion and Langmuir-Schaefer transfer. Langmuir-Schaefer transfer was used to have control over the areal density of lipids at the interface. Preparation of Langmuir monolayer was performed on a ""Nima 602A film balance"" using filter paper Wilhelmy plate for surface pressure measurements. To prepare monolayer, the trough was filled with PBS buffer (pH=7.4) and a known volume of lipid solution in chloroform (POPC:POPG::3:1 was dissolved in chloroform at the concentration of 1mg/mL) was deposited dropwise uniformly across the surface. Chloroform was allowed to evaporate for 30 min after which compression was started at the rate of 3mm/min. Once the surface pressure reached 38mN/m, the LC filled grid supported on DMOAP-coated glass slide was inverted and passed horizontally through the monolayer towards the bottom of the trough. Finally, this ensemble was immersed in a well containing 2mL PBS buffer for further experiments and characterization. Schematic diagram of this procedure giving detailed description can be found in fig. 5.

*Preparation of Peptide Stock Solutions*

Following $A\beta_{1-42}$ peptide solutions were prepared:

1. Monomer- Peptide film of $A\beta_{1-42}$ was prepared with HFIP. 0.2 mM stock solution was prepared with 50mM NaOH and was sonicated. The resulting solution was diluted with PBS buffer at 45μM concentration and centrifuged at 22,000xg for 30 minutes.
2. A11 active oligomer- 2mM stock of $A\beta_{1-42}$ was prepared with 100mM NaOH and was sonicated. The resulting solution was diluted with PBS buffer at 45μM concentration and incubated at 25 °C for 3 days.
3. OC active oligomer- Peptide film of $A\beta_{1-42}$ was prepared with HFIP. 0.2 mM stock solution was prepared with 50mM NaOH and was sonicated. The resulting solution was diluted with PBS buffer at 45μM concentration and centrifuged at 22,000xg for 30 minutes and incubated for 3 days at 25 °C.

*Epifluorescence Imaging* - For ThT assay, ThT was added to the system (well containing glass slide supporting LC filled grid, with lipid monolayer at the LC- aqueous interface immersed in PBS buffer containing peptide solution) at the end of experiment (when saturation was approximately reached) to reach the concentration of 5μM (added from 10mM stock solution in Milli-Q water). The fluorescence images were recorded by a Zeiss (Scope. A1) fluorescence microscope. The samples were viewed using a fluorescence filter cube with a 460 nm excitation filter and a 534 nm filter emission filter. Images were obtained with an AxioCam camera.

*Peptide Characterization-Dot Blot Assay*

2 uL of sample was put on Nitrocellulose membrane. Membrane was blocked with 3% BSA in PBST (0.05% Tween-20) for 1hr at room temperature. Incubate with either OC (1:1000), A11 (1:500) or 6E10 (1:1000) in PBST buffer containing 3% BSA overnight at 4°C. HRP-conjugated secondary antibodies, goat-anti-rabbit for OC and A11 and rabbit-anti-mouse for 6E10 were applied in PBST for 1hr at room temperature. Imaged the blot with ECL.

# Chapter 5

# CONCLUSION

| | Start time | | |
|---|---|---|---|
| Concentration (nM) | Monomer | A11 active | OC active |
| 250 | 4min. | 1h. 7min. | 5min. |
| 100 | 6min. | 1h. 30min. | 7min. |
| 50 | 10min. | 2h. 32min. | 26min. |

**Table 1-** Summary of the start times at which change in optical appearance was observed for different $A\beta_{1-42}$ assemblies

This thesis provides an insight into the interaction of two different types of soluble, structured oligomers- prefibrillar (A11 active oligomer) and fibrillar oligomers (OC active oligomer) with lipid monolayer decorated at the LC-aqueous interface. The result obtained, reiterates the fact that monomers penetrate into the lipid and perturb the ordering of LC molecules.[6,12] There was a huge lag time associated with A11o in changing the orientation order of LC as opposed to OCo, in which the change was almost as fast as in the case of monomers (table 1). Although the response time matched, it can't be said that OCo also insert in to the monolayer like monomer without proper characterization as they differ from each other on many levels.

Reports on interaction of soluble oligomers with lipid membrane have led us to come up with a few hypothesis. Oligomers may either insert into the cell membrane or stay in the lipid head group area interacting peripherally depending on the lipid and subphase condition, thus, increase the permeability of membrane[6,8,9] Depending on these results, we can say that OCo is either inserting into the monolayer, thus, changing orientation order or it is getting adsorbed onto the monolayer and only changing the permeability of monolayer leading to change in ordering of LC. Since, the change in optical response observed in the case of A11o is so late as compared to OCo, it could be that it's not

interacting with the lipid monolayer initially but changes it's conformation over time and form a peptide species which interact with lipid. Another possibility is that, A11o may be interacting with the lipid monolayer initially but not so much to disturb the ordering of LC (i.e. mild peripheral interaction) and form fibrils (it could be forming fibrils without interaction too). Although fibrils are quite stable but due to shear forces they can undergo fragmentation and produce oligomers (or monomers),[1] which may not be A11o and are capable of interacting with the monolayer in a manner that it changes the orientation order of LC. Bulk studies are needed to be performed to test these hypothesis. These studies will involve using lipid vesicles and doing a temporal recording of the events occurring in the presence of different peptide assemblies.

Thioflavin T assay of OCo revealed the presence of fibrils, whereas no positive response had been obtained in the case of A11o, still, we can't be sure that fibrils are absent in this case. For confirmation of presence or absence of fibrils we will be performing AFM studies at the saturation point which will make the situation crystal clear.

As per the results obtained till now, we can say that this LC based system may prove helpful in differentiating prefibrillar and fibrillar oligomers based on the interaction that they have with lipid monolayer.

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