

**STUDY OF DIALYSIS-RELATED B2-
MICROGLOBULIN AMYLOID FIBRILLATION
AND ITS INHIBITION**

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by

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Kusuma School of Biological Sciences

Submitted

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*Dedicating to my dear family, my teachers, and my friends
for their everlasting love and support.*

CERTIFICATE

This is to certify that the thesis entitled “**Study of dialysis-related β 2-microglobulin amyloid fibrillation and its inhibition**” being submitted by **Mr. Devanshu Mehta** to the **Kusuma School of Biological Sciences, Indian Institute of Technology Delhi** for the award of the degree of “**Doctor of Philosophy**” is a record of the bonafide research work carried out by him, prepared under my supervision, in conformity with the rules and regulations of the ‘Indian Institute of Technology Delhi’. The research report and the results present in the thesis have not been submitted to any other University or Institute for the award of any other degree or diploma.

Date:

Place:

Dr. Tapan K. Chaudhuri

Professor

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“Hope begins in the dark, the stubborn hope that if you just show up and try to do the right thing, the dawn will come. You wait and watch and work: you don’t give up.”

– Anne Lamott

When I joined my Ph. D., I had a very different view of a Ph.D. research. I had not, at all, anticipated the extreme limits to which the physical, emotional, and mental capabilities of an individual can get appraised during this time. I believe HOPE was, has been and still is one of my closest companions and my strongest source of motivation. Being here in this moment makes me feel proud to know that I have contributed, albeit an extremely humble, yet a portion of my research to the science. Ph.D. has been an extensive, involving, and evolving journey. With time and energy that I invested through all these years, I believe that now I stand relatively wiser, more composed, and more patient than before. And this all would not have been possible if I had not gotten the trust, love, support, and guidance from some very important people around me.

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Devanshu Mehta

Abstract

In the present work, the *in vitro* process of β 2 microglobulin (β 2m) amyloid fibrillation and the molecular insights into its inhibition orchestrated by a rationally designed peptidomimetic have been investigated. β 2m protein is involved in dialysis-related amyloidosis (DRA), a protein misfolding disorder that affects renal failure patients when subjected to prolonged hemodialysis. DRA involves the accumulation of long fibrillar aggregates (called amyloids) of β 2m specifically into the musculoskeletal system of the patients. The deposition of β 2m amyloids results in the inflammation of joints and bones, followed by their degradation causing chronic pain. Treatment methods currently available to manage DRA include surgery to remove amyloid deposits, medications for symptomatic relief, improved dialysis columns for β 2m removal from the patient's serum, and kidney replacement therapy. However, these methods are plagued with the high cost and low success and only provide palliative care to the patients. With no drug-based treatment method currently available for managing DRA, our study explores the potential of a rationally designed peptidomimetic against *in vitro* process of β 2m amyloid fibrillation.

Peptidomimetics are a class of molecules that may constitute a molecular scaffold intrinsically employed in their structure to provide them a specific overall structure. Such specific conformation can help a peptidomimetic mimic the PPI interfaces of species which are typically generated during the amyloid fibrillation process and help interfere with amyloid-competent intermolecular associations (Haridas *et al.*, 2012; Pelay-Gimeno *et al.*, 2015; Srivastava *et al.*, 2017; Goyal *et al.*, 2017; Singh *et al.*, 2021). The B(LVI)₂ peptidomimetic investigated in this study constitutes a centrally located, hydrophobic bispidine moiety to which hydrophobic amino acids leucine (L), valine (V), and isoleucine (I) have been attached on both sides. The bispidine scaffold provides B(LVI)₂ an overall structure that mimics the PPI interfaces typically observed during amyloid fibrillation. With its intrinsic hydrophobic nature and a β -strand conformation, B(LVI)₂ was hypothesized to interfere with hydrophobic, amyloid-competent, β -sheet driven intermolecular associations among β 2m amyloid precursor species to inhibit the formation of long and ordered amyloid-like fibrils.

This thesis investigates and introduces a peptidomimetic-based approach that may help develop future therapeutics against DRA and amyloidogenesis in general.

The first part of this study explores the effect of B(LVI)₂ against *in vitro* developed process of β 2m amyloid fibrillation. Thioflavin-T, SDS-PAGE, and transmission electron microscopy analysis show that B(LVI)₂ significantly reduces the yield and extends the time required to generate long and straight amyloid-like β 2m fibrils in a dose-dependent manner. β 2m is observed to develop into morphologically distinct short and straight amyloid-like fibril species in the presence of B(LVI)₂, indicative of an altered route from typical β 2m amyloid fibrillation. The second part of this study delves into the molecular mechanism of B(LVI)₂-effected inhibition of β 2m amyloid fibrillation. In the absence of B(LVI)₂, circular dichroism data shows that β 2m species undergo typical β -sheet-rich amyloid-competent conformational rearrangements. However, in the presence of B(LVI)₂, β 2m species exist in a non-amyloidogenic, non- β -sheet containing conformation indicative of β 2m incompetence to undergo required conformational rearrangements for generation of control-like long and ordered amyloid-like fibrils. B(LVI)₂ is further observed using dynamic light scattering to interfere with the amyloid-competent intermolecular associations among β 2m species. Our study demonstrates that the long and straight β 2m amyloid-like fibrils are cytotoxic to human synovium cell lines, while the morphologically distinct B(LVI)₂-induced non- β -sheet containing soluble, non-fibrillar β 2m species and β -sheet rich, insoluble, fibrillar β 2m species are found to be non-cytotoxic. B(LVI)₂ peptidomimetic is non-cytotoxic at lower concentrations (250 μ M) which, as per DRA relevant serum β 2m levels, may prove promising for future study under *in vivo* setup.

Overall, this thesis reports the anti-fibrillation property of a novel rationally designed peptidomimetic 'B(LVI)₂' against dialysis-related β 2m amyloid fibrillation, which may help bring a potential shift in therapeutic approaches against DRA.

सार

वर्तमान कार्य में, $\beta 2$ माइक्रोग्लोबुलिन ($\beta 2m$) अमाइलॉइड फ़िब्रिलेशन की इन विट्रो प्रक्रिया और तर्कसंगत रूप से डिज़ाइन किए गए पेप्टिडोमिमेटिक द्वारा ऑर्केस्ट्रेटेड इसके निषेध में आणविक अंतर्दृष्टि की जांच की गई है। $\beta 2m$ प्रोटीन डायलिसिस से संबंधित अमाइलॉइडोसिस (DRA) में शामिल है, एक प्रोटीन मिसफॉल्डिंग विकार जो लंबे समय तक हेमोडायलिसिस के अधीन होने पर गुर्दे की विफलता के रोगियों को प्रभावित करता है। डीआरए में विशेष रूप से रोगियों के मस्कुलोस्केलेटल सिस्टम में $\beta 2m$ के लंबे तंतुमय समुच्चय (अमाइलॉइड कहा जाता है) का संचय शामिल है। $\beta 2m$ अमाइलॉइड के जमाव के परिणामस्वरूप जोड़ों और हड्डियों में सूजन हो जाती है, जिसके बाद उनका क्षरण होता है जिससे पुराना दर्द होता है। डीआरए को प्रबंधित करने के लिए वर्तमान में उपलब्ध उपचार विधियों में एमिलॉइड जमा को हटाने के लिए सर्जरी, रोगसूचक राहत के लिए दवाएं, रोगी के सीरम से $\beta 2m$ हटाने के लिए बेहतर डायलिसिस कॉलम और किडनी रिप्लेसमेंट थेरेपी शामिल हैं। हालांकि, ये विधियां उच्च लागत और कम सफलता से ग्रस्त हैं और केवल रोगियों को उपशामक देखभाल प्रदान करती हैं। डीआरए के प्रबंधन के लिए वर्तमान में कोई दवा-आधारित उपचार पद्धति उपलब्ध नहीं होने के कारण, हमारा अध्ययन $\beta 2m$ अमाइलॉइड फ़िब्रिलेशन की इन विट्रो प्रक्रिया के विरुद्ध तर्कसंगत रूप से डिज़ाइन किए गए पेप्टिडोमिमेटिक की क्षमता की पड़ताल करता है।

पेप्टिडोमिमेटिक्स अणुओं का एक वर्ग है जो एक विशिष्ट समग्र संरचना प्रदान करने के लिए उनकी संरचना में आंतरिक रूप से नियोजित आणविक पाइ का गठन कर सकता है। इस तरह की विशिष्ट रचना एक पेप्टिडोमिमेटिक प्रजातियों के पीपीआई इंटरफेस की नकल करने में मदद कर सकती है जो आम तौर पर एमिलॉयड फाइब्रिलेशन प्रक्रिया के दौरान उत्पन्न होती हैं और एमिलॉयड-सक्षम इंटरमॉलिक्युलर एसोसिएशन (हरिदास एट अल।, 2012; पेले-गिमेनो एट अल।, 2015; श्रीवास्तव एट) के साथ हस्तक्षेप करने में मदद करती हैं। अल।, 2017; गोयल एट अल।, 2017; सिंह एट अल।, 2021)। इस अध्ययन में जांच की गई (बीएलवीआई)₂ पेप्टिडोमिमेटिक एक केंद्रीय रूप से स्थित, हाइड्रोफोबिक बिस्पिडीन की मात्रा का गठन करती है, जिसमें हाइड्रोफोबिक अमीनो एसिड ल्यूसीन (एल), वेलिन (वी), और आइसोल्यूसीन (आई) दोनों तरफ से जुड़े होते हैं। बिस्पिडीन मचान (बीएलवीआई)₂ को एक समग्र संरचना प्रदान करता है जो आम तौर पर एमिलॉयड फाइब्रिलेशन के दौरान देखे गए पीपीआई

इंटरफेस की नकल करता है। इसकी आंतरिक हाइड्रोफोबिक प्रकृति और एक β -स्ट्रैंड संरचना के साथ, (बीएलवीआई)₂ को हाइड्रोफोबिक, एमिलॉयड-सक्षम, β -शीट संचालित इंटरमोलेक्यूलर एसोसिएशन के साथ β 2m एमिलॉयड अग्रदूत प्रजातियों के बीच हस्तक्षेप करने के लिए परिकल्पित किया गया था ताकि लंबे और आदेशित एमिलॉयड के गठन को रोका जा सके।

यह थीसिस एक पेप्टिडोमिमेटिक-आधारित दृष्टिकोण की जांच और परिचय देता है जो सामान्य रूप से डीआरए और एमाइलॉयडोजेनेसिस के खिलाफ भविष्य के चिकित्सीय विकसित करने में मदद कर सकता है।

इस अध्ययन का पहला भाग β 2m अमाइलॉइड फाइब्रिलेशन की इन विट्रो विकसित प्रक्रिया के खिलाफ (बीएलवीआई)₂ के प्रभाव की पड़ताल करता है। थियोफ्लेविन-टी, एसडीएस-पेज, और ट्रांसमिशन इलेक्ट्रॉन माइक्रोस्कोपी विश्लेषण से पता चलता है कि (बीएलवीआई)₂ उपज को काफी कम कर देता है और खुराक पर निर्भर तरीके से लंबे और सीधे एमिलॉयड-जैसे β 2m फाइब्रिल उत्पन्न करने के लिए आवश्यक समय बढ़ाता है। β 2m को (बीएलवीआई)₂ की उपस्थिति में रूपात्मक रूप से अलग छोटी और सीधी अमाइलॉइड जैसी तंतु प्रजातियों में विकसित होने के लिए मनाया जाता है, जो विशिष्ट β 2m अमाइलॉइड फ़िब्रिलेशन से परिवर्तित मार्ग का संकेत है। इस अध्ययन का दूसरा भाग (बीएलवीआई)₂ के आणविक तंत्र में β 2m एमाइलॉयड फाइब्रिलेशन के 2-प्रभावित निषेध पर चर्चा करता है। (बीएलवीआई)₂ की अनुपस्थिति में, परिपत्र द्वैतवाद डेटा से पता चलता है कि β 2m प्रजातियां विशिष्ट β -शीट-समृद्ध अमाइलॉइड-सक्षम गठनात्मक पुनर्व्यवस्था से गुजरती हैं। हालांकि, (बीएलवीआई)₂ की उपस्थिति में, β 2m प्रजातियां एक गैर-एमिलॉयडोजेनिक, गैर-बीटा-शीट में मौजूद होती हैं, जिसमें β 2m अक्षमता का संकेत होता है, जो नियंत्रण-जैसे लंबे और ऑर्डर किए गए एमाइलॉयड-जैसे फाइब्रिल की पीढ़ी के लिए आवश्यक गठनात्मक पुनर्व्यवस्था से गुजरना पड़ता है। (बीएलवीआई)₂ को आगे β 2m प्रजातियों के बीच अमाइलॉइड-सक्षम अंतर-आणविक संघों के साथ हस्तक्षेप करने के लिए गतिशील प्रकाश प्रकीर्णन का उपयोग करके देखा जाता है। हमारे अध्ययन से पता चलता है कि लंबे और सीधे β 2m अमाइलॉइड जैसे तंतु मानव सिनोवियम सेल लाइनों के लिए साइटोटॉक्सिक हैं, जबकि रूपात्मक रूप से अलग (बीएलवीआई)₂-प्रेरित गैर- β -शीट जिसमें घुलनशील, गैर-फाइब्रिलर β 2m प्रजातियां और β -शीट समृद्ध हैं। , अघुलनशील, तंतुमय β 2m प्रजातियां गैर-साइटोटॉक्सिक पाई जाती हैं। (बीएलवीआई)₂ पेप्टिडोमिमेटिक कम सांद्रता (250 माइक्रोन) पर गैर-

साइटोटॉक्सिक है, जो डीआरए प्रासंगिक सीरम β_2m स्तरों के अनुसार, विवो सेटअप के तहत भविष्य के अध्ययन के लिए आशाजनक साबित हो सकता है।

कुल मिलाकर, यह थीसिस डायलिसिस से संबंधित β_2m एमाइलॉयड फाइब्रिलेशन के खिलाफ तर्कसंगत रूप से डिजाइन किए गए पेप्टिडोमिमेटिक (बीएलवीआई)₂' की एंटी-फाइब्रिलेशन संपत्ति की रिपोर्ट करती है, जो डीआरए के खिलाफ चिकित्सीय दृष्टिकोण में संभावित बदलाव लाने में मदद कर सकती है।

Table of Contents

<i>CERTIFICATE</i>	<i>ii</i>
<i>Acknowledgements</i>	<i>iii</i>
<i>Abstract</i>	<i>vii</i>
<i>Table of Contents</i>	<i>ix</i>
<i>List of Figures</i>	<i>xii</i>
<i>List of Tables</i>	<i>xiv</i>
<i>Abbreviations and Symbols.....</i>	<i>xv</i>
<i>Chapter 1 Introduction.....</i>	<i>2</i>
1.1. Protein folding, misfolding, and aggregation	2
1.2. Amyloid	7
1.3. Amyloidosis	9
1.4. Dialysis-related β 2 microglobulin amyloidosis (DRA)	12
1.5. DRA: Pathophysiology	14
1.6. DRA: Current treatment methods	17
1.7. Thesis scope and significance	19
1.8. Thesis Objectives.....	22
<i>Chapter 2 Review of Literature</i>	<i>2</i>
2.1. β 2m amyloid fibrillation.....	2
2.2. Inhibitors of β 2m amyloid fibrillation	4
2.3. Peptidomimetics as potential inhibitors of amyloid fibrillation	8
2.4. Bispidine-based peptidomimetics and amyloid inhibition	10
<i>Chapter 3 In vitro investigation of the role of rationally designed bispidine peptidomimetic analogue B(LVI)₂ in the process of β2m amyloid fibrillation</i>	<i>30</i>

3.1. Background	30
3.2. Materials and methods	32
3.2.1. B(LVI) ₂ peptidomimetic synthesis	32
3.2.2. Recombinant his-tagged β 2m (H β 2m) preparation	33
3.2.3. Recombinant H β 2m expression	35
3.2.4. Recombinant H β 2m purification	35
3.2.5. H β 2m amyloid fibrillation condition	36
3.2.6. Thioflavin-T (ThT) based assessment of H β 2m amyloid fibrillation	37
3.2.7. Transmission electron microscopy (TEM)	38
3.2.8. Congo red (CR) dye assay	38
3.2.9. SDS-PAGE and densitometric analysis	38
3.2.10. Statistical analysis and plotting	39
3.3. Results	39
3.3.1. Subcloning, overexpression, and purification of his-tagged β 2m (H β 2m)	39
3.3.2. Characterization of purified H β 2m	41
3.3.3. Preliminary screening of compounds against H β 2m aggregation	44
3.3.3. Effect of B(LVI) ₂ on amyloid fibrillation kinetics of H β 2m	46
3.3.4. Effect of B(LVI) ₂ on the morphology and population of generated amyloid-like H β 2m fibrils	50
3.4. Conclusions	55
Chapter 4 Characterization of rationally designed B(LVI)₂ peptidomimetic effect on the conformation, size, and cytotoxicity of the species developed during the in vitro β2m amyloid fibrillation	55
4.1. Background	55
4.2. Materials and methods	57
4.2.1. Far-UV Circular Dichroism (CD) spectroscopy	57
4.2.2. 8-Anilino-1-naphthalene sulfonic acid (ANS) fluorescence assay	57
4.2.3. Transmission electron microscopy (TEM)	57
4.2.4. Dynamic light scattering (DLS) experiment	58
4.2.5. Methyl Thiazol diphenyl Tetrazolium Bromide (MTT)-based cell viability assay ..	58

4.2.6. Statistical analysis and plotting.....	58
4.3. Results.....	59
4.3.1. Effect of B(LVI) ₂ on the secondary structure of soluble species during Hβ2m amyloid fibrillation.....	59
4.3.2. Effect of B(LVI) ₂ on size and morphology of Hβ2m during amyloid fibrillation	63
4.3.3. Cytotoxicity of soluble and fibrillar Hβ2m end-products and B(LVI) ₂ peptidomimetic	65
3.4. Conclusions	68
Chapter 5 Discussion.....	73
Thesis Summary	79
Concluding remarks.....	79
Future prospects.....	80
References.....	1
Appendices.....	83
Appendix I: List of consumables and equipment.....	101
Appendix II: Media and antibiotic preparation	104
Appendix III: Preparation of gels, buffers, and dye solutions.....	105
Appendix IV: DNA and protein sequence	110
Author's Resume	111

List of Figures

FIGURE 1.1. THE FREE-ENERGY PROTEIN FOLDING LANDSCAPE.	3
FIGURE 1.2. PROTEIN QUALITY CONTROL SYSTEM (PQC)	4
FIGURE 1.3. PROTEIN AGGREGATION MODEL.....	5
FIGURE 1.4. FREE ENERGY PROTEIN FOLDING AND PROTEIN AGGREGATION LANDSCAPE	6
FIGURE 1.5. AMYLOID FIBRIL STRUCTURE.....	7
FIGURE 1.6. HIERARCHY OF AMYLOID STRUCTURE.....	8
FIGURE 1.7. HLA-UNBOUND NATIVE B2M STRUCTURE	13
FIGURE 1.8. SCHEMATIC REPRESENTATION OF DIALYSIS-RELATED B2M AMYLOIDOSIS.	14
FIGURE 1.9. SALIENT STRUCTURE OF BISPIDINE PEPTIDOMIMETIC ANALOGUE 'B(LVI) ₂ '	20
FIGURE 1.10. FIRST GROUND OF OUR STUDY HYPOTHESIS.	21
FIGURE 1.11. SECOND GROUND OF OUR STUDY HYPOTHESIS.....	21
FIGURE 2.1. SCHEMATIC ILLUSTRATING POSSIBLE STEPS FOR POTENTIAL INHIBITOR MEDIATED INTERVENTION OF AMYLOID FIBRILLATION PROCESS.....	7
FIGURE 2.2. DESIGN AND SYNTHESIS OF BISPIDINE CHEMICAL SCAFFOLD	10
FIGURE 3.1. SCHEMATIC REPRESENTATION OF THE SIGNIFICANT EVENTS DURING TYPICAL NUCLEATION-DEPENDENT POLYMERIZATION KINETICS OF AMYLOID FIBRILLATION.....	31
FIGURE 3.2. SCHEMATIC REPRESENTATION OF THE CHARACTERISTIC RED-SHIFT IN ABSORBANCE OBSERVED FOR CONGO-RED DYE IN THE PRESENCE OF AMYLOID-LIKE FIBRILLAR SPECIES.....	32
FIGURE 3.3. SUBCLONING C-TERMINAL HIS-TAGGED B2M (Hb2M).....	39
FIGURE 3.4. OVEREXPRESSION AND PURIFICATION OF Hb2M.....	40
FIGURE 3.5. AFFINITY CHROMATOGRAPHY-BASED PURIFICATION OF Hb2M.	41
FIGURE 3.6. NATIVE STRUCTURE OF B2M (IADANZA ET AL., 2018).....	42
FIGURE 3.7. FAR-UV CD SPECTRUM OF PURIFIED Hb2M.....	42
FIGURE 3.8. INTRINSIC FLUORESCENCE SPECTRUM OF PURIFIED Hb2M.	43
FIGURE 3.9. SDS-PAGE PROFILE OF Hb2M UNDER REDUCING AND NON-REDUCING CONDITIONS.	43
FIGURE 3.10. SEC-HPLC-BASED CHARACTERIZATION OF PURIFIED Hb2M.	44
FIGURE 3.11. THIOFLAVIN T FLUORESCENCE BASED PRELIMINARY SCREENING OF DIFFERENT CLASSES OF PEPTIDOMIMETICS.	45
FIGURE 3.12. EFFECT OF CURCUMIN ON THE PROCESS OF Hb2M AMYLOID FIBRILLATION..	46

FIGURE 3.13. EFFECT OF B(LVI) ₂ AND A CONTROL PEPTIDOMIMETIC ON THE KINETICS OF Hb2M AMYLOID FIBRILLATION.	47
FIGURE 3.14. EFFECT OF B(LVI) ₂ ON DIFFERENT KINETIC PARAMETERS OF Hb2M AMYLOID FIBRILLATION.....	48
FIGURE 3.15. EFFECT OF B(LVI) ₂ ON THE FINAL PHASE ThT FLUORESCENCE VALUES OF Hb2M AMYLOID FIBRILLATION KINETICS AND YIELD OF FORMED Hb2M AMYLOID-LIKE FIBRILS.	49
FIGURE 3.16. MORPHOLOGY OF Hb2M FIBRILS GENERATED UNDER CONTROL (1:0) CONDITIONS.	51
FIGURE 3.17. LENGTH AND DISTRIBUTION ANALYSIS OF Hb2M FIBRILS GENERATED UNDER CONTROL CONDITIONS.. ..	52
FIGURE 3.18. MORPHOLOGY OF Hb2M FIBRILS GENERATED UNDER 1:1 AND 1:10 B(LVI) ₂ -TREATMENT CONDITIONS.	52
FIGURE 3.19. EFFECT OF B(LVI) ₂ ON MORPHOLOGY AND POPULATION OF Hb2M FIBRILS AT 1:20 B(LVI) ₂ -TREATMENT CONDITIONS.	53
FIGURE 3.20. CONGO RED (CR) DYE ABSORBANCE-BASED TEST OF THE AMYLOID-LIKE CHARACTERISTIC OF GENERATED Hb2M FIBRILS.	54
FIGURE 3.21. CHANGE IN THE MORPHOLOGY OF B(LVI) ₂ -INDUCED SHORT, THIN, AND CURVED Hb2M FIBRILS TO SHORT, THICK, AND STRAIGHT FIBRILS UPON PROLONGED INCUBATION.	55
FIGURE 4.1. SCHEMATIC REPRESENTATION OF THE CONFORMATIONAL CHANGES IN AMYLOID PRECURSOR SPECIES DURING TYPICAL AMYLOID FIBRILLATION PROCESS.	56
FIGURE 4.2. CHANGES IN THE FAR-UV CD SPECTRA OF SOLUBLE Hb2M SPECIES UNDER CONTROL CONDITIONS DURING FIBRILLATION.	59
FIGURE 4.3. CHANGES IN THE FAR-UV CD SPECTRA OF SOLUBLE Hb2M SPECIES UNDER 1:20-B(LVI) ₂ TREATMENT CONDITION DURING FIBRILLATION.	60
FIGURE 4.4. MORPHOLOGY OF SOLUBLE END-PRODUCT Hb2M SPECIES ISOLATED FROM CONTROL AND 1:20-REACTION CONDITIONS OF AMYLOID FIBRILLATION.....	61
FIGURE 4.5. EFFECT OF B(LVI) ₂ ON THE SECONDARY STRUCTURE OF DIFFERENT Hb2M CONFORMATIONS.	62
FIGURE 4.6. EFFECT OF B(LVI) ₂ ON SIZE AND MORPHOLOGY OF Hb2M DURING AMYLOID FIBRILLATION.	64
FIGURE 4.7. CYTOTOXICITY ASSOCIATED WITH SOLUBLE Hb2M END-PRODUCTS.	66
FIGURE 4.8. CYTOTOXICITY ASSOCIATED WITH FIBRILLAR Hb2M END-PRODUCTS.....	67
FIGURE 4.9. CYTOTOXICITY ASSOCIATED WITH B(LVI) ₂ PEPTIDOMIMETIC.	68
FIGURE 5.1. SCHEMATIC REPRESENTATION OF B(LVI) ₂ -ORCHESTRATED INHIBITION OF ACID-INDUCED Hb2M AMYLOID FIBRILLATION.. ..	76

List of Tables

TABLE 1.1. AMYLOID PROTEINS AND THEIR PRECURSORS IN AMYLOIDOSIS.	10
TABLE 1.2. FACTORS REPORTED BEING RESPONSIBLE FOR INDUCTION AND PROGRESSION OF DRA.....	15
TABLE 1.3. TREATMENT OPTIONS FOR MANAGEMENT OF DRA IN RENAL FAILURE PATIENTS	18
TABLE 2.1. MOLECULES/TECHNIQUES AGAINST IN VITRO B2M AMYLOID FIBRILLATION PROCESS.....	5
TABLE 3.1. REACTION MIXTURE FOR PCR AMPLIFICATION OF PET23A B2M GENE	34
TABLE 3.2. PCR AMPLIFICATION PROTOCOL	34
TABLE 3.3. REACTION MIXTURE FOR RESTRICTION DIGESTION ASSAY	34
TABLE 3.4. KINETIC PARAMETERS OF Hb2M AMYLOID FIBRILLATION AT DIFFERENT Hb2M: B(LVI) ₂ MOLAR RATIOS.	50

Abbreviations and Symbols

α	Alpha
β	Beta
~	Approximately
μ	Micro
μg	Microgram
μL	Microlitre
μm	Micrometer
μM	Micromolar
3D	Three-dimensional
%	Percent
$^{\circ}\text{C}$	Degree Celcius
λ	Wavelength
λ_{max}	Fluorescence emission maxima
a.a	amino acid
$\text{\AA}$	Angstrom
A_{280}	Absorbance measured at 280 nm
A_{260}	Absorbance measured at 260 nm
ANS	8-anilino-1-naphthalene-sulfonic acid
APS	Ammonium persulphate
$\beta 2\text{m}$	Beta 2 microglobulin
B(LVI)_2	Bispidine peptidomimetic analogue
CD	Circular dichroism
CR	Congo red
CV	Column volumes
DLS	Dynamic light scattering
DMEM	Dulbecco's Modified Eagle's Medium
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic Acid

DRA	Dialysis related amyloidosis
DTT	1, 4-Dithiothreitol
EDTA	Ethylene diamine tetra acetic acid
EtBr	Ethidium bromide
g	Gram
h	Hours
Hβ2m	His-tagged Beta 2 microglobulin
IPTG	Isopropyl- β- D-1-thiogalactopyranoside
kb	Kilobase pair
kDa	KiloDalton
LA	Luria Bertani agar
LB	Luria Bertani broth
M	Molar
mg	Milligram
min	Minute
mM	Millimolar
mol. wt.	Molecular weight
MRE	Molar residue ellipticity
MTT	MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide)
NDP	Nucleation dependent polymerization
Ni-NTA	Nickel-nitrilotriacetic acid
nm	Nanometer
O.D. ₆₀₀	Optical density (Absorbance) measured at 600 nm
O/N	Overnight
PAGE	Polyacrylamide gel electrophoresis
PBS	Phosphate Buffered Saline
PCR	Polymerase chain reaction
PDB	Protein data bank
PMSF	Phenyl methyl sulfonyl fluoride
PPIs	Protein-protein interactions
PTM	Post-translational modifications

rpm	Revolutions per min
RT	Room temperature
SDS	Sodium dodecyl sulphate
SW-982	Human synovium cells
s	Second
TAE	Tris-acetate-EDTA
TEM	Transmission electron microscopy
TEMED	N, N, N', N'-Tetramethylethylenediamine
ThT	Thioflavin-T
Tris	Tris(hydroxymethyl)aminomethane
UV	Ultra-violet
Wt.	Wild type