# An Unbiased Approach on The Conformational Dynamics of In-Solution Fibrinogen and 

## Its Physiological Implications.

A Senior Honors Thesis Presented to the

Faculty of the Department of Biology and Biochemistry

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In Partial Fulfillment of the Requirements for the Degree of Bachelor of Science

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# An Unbiased Approach on The Conformational Dynamics of In-Solution Fibrinogen and 

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## EPIGRAPH

"Nothing has such power to broaden the mind as the ability to investigate systematically and truly all that comes under thy observation in life."
-Marcus Aurelius

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#### Abstract

By itself, Fibrinogen (Fng) stands out as one of the most complex hematopoietic proteins in the cardiovascular system for multiple species in nature. Upon its activation and further cascade mechanisms, Fng can polymerize into fibrin and contribute to blood clot formation and substantial growth. Fng's interactions with fibrinolytic proteins aggregate into a conglomerate of different fragments in blood bodily mechanisms. Any form of dysregulation in any of these pathways can lead to several complications not only within the cardiovascular system but throughout the entirety of the body. Understanding the crux of Fng's functions and interactions with itself along with other proteins ultimately can be traced back to its inherent dynamic structure. In this study, I aim to probe the intrinsic flexibility that is beset on Fng by way of its multi-domain composition, allowing it to withstand incredible mechanical forces as well as being highly dynamic in its physiological form. Thus, extending the key biological concept that structure and flexibility that comes with it determine functions. Through an unbiased approach by implementing protein structural studies as well as computational dynamic simulations, in-solution Fng dynamics were studied in their totality.


## TABLE OF CONTENTS

EPIGRAPH ..... III
ACKNOWLEDGMENTS ..... IV
ABSTRACT ..... VI
TABLE OF CONTENTS ..... VII
LIST OF FIGURES ..... IX
ABBREVIATIONS ..... X
CHAPTER I: PHYSIOLOGICAL SIGNIFICANCE OF FIBRINOGEN ..... 1
FIBRINOGEN ..... 1
FIBRINOGEN STRUCTURES ARE COMPOSED OF A, B AND Г CHAINS ..... 3
FIBRINOGEN POLYMERIZATION AND BLOOD PROCESSES ..... 4
FIBRINOGEN MUTATIONS ..... 5
FURTHER DETAILS OF FIBRINOGEN INTERACTIONS ..... 6
SPECIFIC AIM ..... 8
CHAPTER II: MATERIALS AND METHODS ..... 9
MULTIPLE SEQUENCE ALIGNMENT OF FIBRINOGEN A, B, AND Г CHAINS. ..... 9
SYNCHROTRON SAXS MEASUREMENTS ..... 10
AB INITO MODELLING OF FIBRINOGEN ARCHITECTURE ..... 10
CORAL MODELLING OF FIBRINOGEN STRUCTURE ..... 11
NEGATIVE STAIN ELECTRON MICROSCOPY IMAGING FOR IN-SOLUTION FIBRINOGEN. ..... 11
ANGLE MEASUREMENTS BETWEEN RIGID BODIES ..... 12
CHAPTER III: DATA-ASSISTED PROBING OF THE IN-SOLUTION FIBRINOGEN FLEXIBILITY. ..... 13
PURIFIED FIBRINOGEN MOLECULE AND STRUCTURAL METHODS. ..... 13
HDX-MS OF FIBRINOGEN AT ROOM TEMPERATURE. ..... 15
SUBSECTION ON EX-2 AND THE ABILITY TO PROBE THERMODYNAMICS. ..... 18
NEGATIVE STAIN ELECTRON MICROSCOPY IMAGING OF FIBRINOGEN. ..... 21
X-RAY SCATTERING OF FIBRINOGEN ..... 24
ARCHITECTURAL FLEXIBILITY OF FIBRINOGEN ..... 27VISUAL INSIGHTS INTO THE FIBRINOGEN MODELS THROUGH LOW-RESOLUTIONDATA COMPARISON.33
INTERSPECIES ALIGNMENT OF THE A, B AND Г FIBRINOGEN CHAINS ..... 36
CHAPTER IV: DISCUSSION ON THE INTRINSIC DYNAMICS OF IN-SOLUTIONFIBRIONGEN.41
APPENDIX ..... 50
BIBLIOGRAPHY ..... 82

## LIST OF FIGURES

FIGURE 1.1: FIBRINOGEN ARCHITECTURE ..... 1
FIGURE 3.1: FIBRINOGEN PURIFICATION \& STRUCTURES ..... 14
FIGURE 3.2: TIME DEPENDENT HDX-MS HUMAN FIBRINOGEN EXPOSURE ..... 17
FIGURE 3.3: CHAIN DEPENDENT HDX-MS EXPOSURE FOR HUMAN FIBRINOGEN ..... 19
FIGURE 3.4: NEGATIVE STAIN ELECTRON MICROSCOPY PARTICLES \& ENSEMBLES. ..... 22
FIGURE 3.5: CLASS AVERAGES FOR NEGATIVE STAIN EM FIBRINOGEN IMAGES ..... 23
FIGURE 3.6: DATA COLLECTION REDUCTION FOR FIBRINOGEN SEC-SWAXS ..... 25
FIGURE 3.7: SUPERIMPOSITION OF GASBOR FIBRINOGEN MODELS. ..... 26
FIGURE 3.8. CLUSTER ANALYSIS OF THE RIGID BODY MODELS GENERATED BY CORAL ..... 29
FIGURE 3.9 CHI DIVERGENCE GRAPH FOR CORAL RUNS ..... 31
FIGURE 3.10: ANGLE DISTRIBUTION CALCULATION FOR CORAL MODELLING RUNS OF SPECIFIC FIBRINOGEN REGIONS ..... 32
FIGURE 3.11: CROSS-CORRELATION OF NEGATIVE STAIN EM, CORAL AND GASBOR FIBRINOGEN MODELLING. ..... 35
FIGURE 3.12: FIBRINOGEN INTERSPECIES SEQUENCES PERCENT SIMILARITY \& DIVERGENCE ..... 38
FIGURE 4.1: FIBRINOGEN CRYSTAL STRUCTURES SUPERIMPOSITION AT SPECIFIC REGIONS. ..... 42
FIGURE 4.2: IDENTIFIED FIBRINOGEN MUTATIONAL SITES ..... 47

## ABBREVIATIONS

| Fng | Fibrinogen |
| :--- | ---: |
| FPA | Fibrinopeptide A |
| FPB | Fibrinopeptide B |
| SAXS-SEC | Small Angle X-Ray Scattering Size Exclusion Chromatography |
| EM | Electron Microscopy |
| TD-HDXMS | Temperature-Dependent Hydrogen Deuterium Exchange Mass Spectrometry |
| VLDLR | Very-Low-Density-Lipoprotein-Receptor |
| AFM | Atomic Force Microscopy |
| CD | Circular Dichroism |
| NMR | Nuclear Magnetic Resonance |
| LC | Liquid Chromatograph |
| ESI | Electrospray Ionization |
| MS | Mass Spectrometry |
| CID | Collision-Induced Dissociation |
| CORAL | Complexes with Random Loops |
| COM | Center of Mass |

## CHAPTER I: PHYSIOLOGICAL SIGNIFICANCE OF FIBRINOGEN

## Fibrinogen

Fibrinogen's homodimeric structure incorporates two sets of three distinct amino acid chains $(A \alpha B \beta \gamma)_{2}$ with an overall molecular weight of 340 kDa . All three $\mathrm{A} \alpha, \mathrm{B} \beta$, and $\gamma$ chains are connected via five disulfide bridges merged at six different portions in the E region portion


FIGURE 1.1: FIBRINOGEN ARCHITECTURE
(A) Domain organization of Fibrinogen. Inter-subunit and intra-disulfide bonds are shown in dotted and straight lines, respectively. Each domain is colored different. (B) Fibrinogen cartoon encompassing an accurate depiction of the overall structure of the molecule's regions. Parts of the $\mathrm{E} \& \mathrm{D}$ regions comprise the coiled-coil domain and $\beta \& \gamma$ nodules conform the globular domains. Angles that provide flexibility are shown in double-arrowed arcs.
of each individual set of chains, and dimerization occurs also at these sections (Figure 1.1). Its concentrations are around 2-5 g/L, making it one of the most abundant biomolecules in human plasma (Weisel \& Litvinov, 2017). Soluble Fng is converted into insoluble fibrin, when thrombin cleaves fibrinopeptide A (FpA, the first 16 N -terminal residues of the $\mathrm{A} \alpha$ chain) and, in a slower reaction, fibrinopeptide $\mathrm{B}(\mathrm{FpB}$, the first 14 N -terminal residues of the $\mathrm{B} \beta$ chain) (Riedel et al., 2010). Once FpA has been cleaved off, individual fibrin molecules can polymerize into a 3-dimensional elastic mesh, serving as the mechanical net that allows blood clot formations (Feller et al., 2021).

Fng plays a major role in the formation of clot growth throughout the body's circulatory system. Subsequent activation of several mechanisms leads to fibrinogen deposition to vasculature or cleavage and the formation of fibrin. These Fng events are related to the formation of thrombus and subsequent cascade reactions that dramatically differ in function from those represented by fibrinogen (Litvinov \& Weisel, 2016). Still, Fng and fibrin hold a similar relationship regarding the polymerization of what is considered the mechanical backbone of blood clots.

FpA and FpB are small fragments, and their thrombin-cleavage also diversifies Fng/Fibrin functions and endows them with differentially regulated interactions with a vast binding-partner repertoire. Fng is pivotal in both thrombosis and immunity, and thus, involved in a range of pathologies from thrombotic to autoimmune disorders such as inflammatory bowel disease, pericellular fibrosis, and atherosclerosis (Lee et al., 2016) (Ho et al., 2010). Given that Fng undergoes several processes to polymerize into fibrin, it comes as no surprise that there is a clear distinction between the hematologic and immunological functions between Fng and fibrin. (Weisel \& Litvinov, 2013). Given that Fng-both soluble or insoluble-, and its
polymerized form of fibrin are present through the circulatory system, it comes as no surprise that both molecules can interact with a conglomerate of other ligands and molecules throughout the blood system (Yakovlev \& Medved, 2017).

## Fibrinogen Structures are Composed of $\alpha, \beta$ and $\gamma$ chains

Fng's structure has been studied using electron microscopy (EM) (Veklich et al., 1993), X-ray crystallography (Kollman et al., 2009) (Yang et al., 2001) (Spraggon et al., 1997), atomic force microscopy (AFM) (Protopopova et al., 2017) (Zavyalova et al., 2011), circular dichroism (CD) (Tsurupa et al., 2002), and nuclear magnetic resonance (NMR) (Burton et al., 2007). In the earliest structural studies for Fng, EM images revealed that the molecule was comprised of three main regions that would mirror each other beginning form a central nodule. The protruding arms are in highly dense distal globular regions, which are known as E \& D regions, respectively (Figure 1.1). In homodimerization, the N -terminus portion of the amino acid chains is connected by disulfide bonds that conform to the two pairs of trihelices. The curling of these three helices around each other makes up the coiled-coil domain. (Kollman et al., 2009) (Yang et al., 2001) (Medved \& Weisel, 2009). In electron densities collected from the Fng crystals, the C-terminus of the $\alpha$-chains (residues 200-610), along with the N -termini of each chain- (A $\alpha 1-26$ (containing FpA), B $\beta 1-57$ (containing FpB), and $\gamma 1-13$ ) —were not visible (most likely averaged out), suggesting high conformational flexibility at these regions (Kollman et al., 2009) (Doolittle \& Kollman, 2006). This "large section" of the $\alpha$-chain that has not been visualized by any structural studies is known as the $\alpha \mathrm{C}$ region, which can be further subdivided into the $\alpha \mathrm{C}$ connector ( $\alpha 221-391$ ) and $\alpha \mathrm{C}$ domain ( $\alpha 392-610$ ) (Figure 1.1 B). The $\alpha \mathrm{C}$ connector is mostly populated with serine, proline, and glycine as determined by proteolysis and HDX
studies (Tsurupa et al., 2002) (Medved et al., 2009). This being the major reason for the highly dynamic nature of the $\alpha \mathrm{C}$ region. The $\alpha \mathrm{C}$ connector is a highly flexible loop, thus helping the $\alpha \mathrm{C}$ domain to move away from the main molecular structure and effectively facilitating the rapid fibrin polymerization (Doolittle \& Kollman, 2006) (Protopopova et al., 2017) (Zavyalova et al., 2011). In EM and AFM images, globular and compact $\alpha \mathrm{C}$ domain structures have been observed, at acidic $\mathrm{pH}(\mathrm{pH}<5)(V e k l i c h ~ e t ~ a l ., ~ 1993) ~(P r o t o p o p o v a ~ e t ~ a l ., ~ 2017) ~(T s u r u p a ~ e t ~ a l ., ~$ 2002). In-solution NMR studies, however, showed, that due to the limited spatial NOE constraints, two $\beta$-hairpin structures between the lone disulfide bond and the C -terminal section of the $\alpha \mathrm{C}$ domain are present (Burton et al., 2007) (Burton et al., 2006).

## Fibrinogen Polymerization and Blood Processes

During the process of coagulation, Fng heavily relies on its interactions with platelet integrin receptor $\alpha \mathrm{IIb} \beta 3$. The KQAGDV binding motif is in the C -terminus of the $\gamma$ chain and is responsible for the binding of $\alpha \mathrm{IIb} \beta 3$, thus allowing it to properly regulate adequate platelet aggregation. (Litvinov \& Weisel, 2015) (Litvinov et al., 2016). This binding motif is always accessible, facilitating a rapid Fng polymerization into fibrin. In consequence, the process of polymerization, via conformational change of the $\alpha \mathrm{IIb} \beta 3$ receptors, is allosterically regulated, which gives rise to distinct mechanisms and interactions necessary for platelet aggregation.

Furthermore, there are two other potential $\alpha \mathrm{IIb} \beta 3$ binding sites - RGD sites are available in the $\alpha$-subunit - the first is in the middle of the coiled-coil region (RGD sequence position $\alpha 95-\alpha 97$ ), and the second ( $\alpha 572-\alpha 574$ ) is towards the end of the $\alpha$ C-region. $\alpha$ IIb $\beta 3$ and other RGD-binding integrins selectively bind to one of the two RGD motifs in the $\alpha$-chain of Fng. In clotting, $\alpha$ IIb $\beta 3-\alpha-{ }^{95} \mathrm{RGD}^{97}$ interaction involves in platelet adhesion and retraction,
and, contrary to the $\gamma$-KQAGDV motif, it does not contribute to platelet aggregation. Simply, the $\alpha-{ }^{95}$ RGD ${ }^{97}$ site appears to be cryptic. However, how the accessibility of the $\alpha-{ }^{95} \mathrm{RGD}^{97}$ site is encrypted to its structure remains to be determined. Similar events were also noted for sites for fibrinolytic enzymes tPA and plasminogen (Yakovlev et al., 2000), (Schielen et al., 1989). Fng and fibrin also present several different binding sites that can also interact with a vast array of hematopoietic proteins such as integrins $\alpha v \beta 3$, and $\alpha v \beta 8$ (Suehiro et al., 2000) (Smith et al., 1990) (Chernousoy \& Carey, 2003), fibronectin (Engvall et al., 1978), VE-cadherin [CDH5] (Gorlatov \& Medved, 2002), very low-density lipoprotein receptor (VLDLR) (Yakovlev et al., 2012), heparin (Martino et al., 2013), and amyloid- $\beta$ (Ahn et al., 2010), among others. The mechanism of these interactions at molecular and atomic levels remains to be investigated.

## Fibrinogen Mutations

Multiple mutation sites across different Fng genes have been characterized leading to a spectrum of diseases. Congenital genetic Fng disorders are divided into three different groups, afibrinogenemia, dysfibrinogenemia, and hypofibrinogenemia. These disorders have been traced to induce severe bleeding episodes in patients as well as the expression of thrombotic pathologies. Given that these congenital disorders can be characterized by the partial or complete absence of Fng, bleeding episodes can happen throughout the integumentary system, genitourinary tracks, and even present themselves as intracranial hemorrhages (Simurda et al., 2021) (Casini et al., 2015) (Taira et al., 2017). However, there have also been mutational incidences in which some of the patients presenting these conditions can stay asymptomatic (or mild symptoms) for the entirety of their life (Casini et al. ,2021).

The major question is how and why mutations display different pathologic phenotypes?

My major hypothesis is that some of these mutations' sites that do not impair Fng folding and eliminate its secretion are altering the in-solution dynamics of Fng and induce different kinetics in clot formation. Also, I predict that any change in Fng topology and conformational kinetics will have a direct effect on the aforementioned Fng interactions. Furthermore, it is also conceivable that mutations can alter the overall intrinsic flexibility, thus, differentially exposing the critical binding sites for any of Fng's cascade mechanisms. This might be essential for creating cryptic sites like the previously mentioned $\alpha-{ }^{95} \mathrm{RGD}^{97}$. Alteration of such flexibility, by any means (e.g., post-translational modifications) can have severe consequences (BolligerStucki et al., 2001). Briefly, understanding the relevance of where mutations are most prevalent goes hand in hand with understanding which regions along the Fng molecule are more flexible or more rigid since these regions are involved in the proper folding, expression, functioning, and maintenance of Fng kinematics.

## Further details of Fibrinogen Interactions

Additionally, Fng is also responsible for the correct trafficking of leukocytes across the circulatory system through its interactions with molecules important central to immunity, such as ICAM-1 (IntraCellular Adhesion Molecule-1), leukocyte-specific integrin $\alpha \mathrm{M} \beta 2$, along with its sister homologs $\alpha \mathrm{X} \beta 2$ and $\alpha \mathrm{D} \beta 2$. Fng's dynamism, which most likely endows, and differentially expose these bindings sites, appears to be pivotal in Fng-dependent immunity and thrombogenesis. For instance, the unmasking of the cryptic binding site for integrin $\alpha \mathrm{M} \beta 2$ in the gamma C-domain of fibrinogen is regulated, yet molecular details of this regulatory mechanism are yet to be explored (Ryu et al., 2009). Furthermore, $\alpha \mathrm{M} \beta 2, \alpha \mathrm{X}, \beta 2$, and $\alpha \mathrm{D} \beta 2$ share a high sequence similarity of over $60 \%$, allowing them to bind different epitopes (Ugarova
\& Yakubenko, 2006) (Loike et al., 1991) (Lishko et al., 2002). What differentiates their binding behaviors at a molecular level is another immediate question in the field.

Involvement of Fng in pathologies ranging from thrombotic to autoimmune disorders, as well as its association with several integrin molecules mediated by the accessibility of the receptor binding sites suggests that Fng and its complement fibrin are constrained and regulated through their structural conformations (Ryu et al., 2018). Nonetheless, there is still a range of unknowns when it comes to the variability of Fng's structure, more specifically on its structural states in solution, polymerization, immobilization, and transition.

The mechanical and dynamic integrities of Fng molecules among different species have captured the intricacies of the protein in "snapshots" as opposed to a physiological "film" setting (Brown et al., 2000) (Yang et al., 2000). To understand the intrinsic dynamics of Fng, studies on molecular dynamics (MD) simulations (Köhler et al., 2015) (Zhmuroy et al., 2018) and Hydrogen/Deuterium Exchange Mass Spectrometry (HDXMS) (Marsh et al., 2013) were performed, however, this led to a dilemma within conflicting views from these structural biology studies, with the formation of dramatic, acutely bending Fng. Much is still unknown about the in-solution structural dynamics of Fng, the immobilized structure of Fng, the polymerized structure of fibrin, and the structural transitions between these states. Given that the previously mentioned Fng interactions with cell surface receptors appear to be mediated by conformational changes, in-solution dynamic studies that elucidate conformational transition would provide more physiologically relevant structural snapshots of Fng and a better understanding of molecular mechanisms that tune and regulate Fng interactions and functions.

## Specific Aim

Fng, one of the most abundant proteins in plasma, carries multi-faceted functions in various physiological events ranging from thrombus formation to immunity. Despite being highly studied in biological experiments Fng has very complicated structure and structural dynamics, and molecular links between Fng-in solution dynamism and functions remain to be elaborated at the molecular level.

My goal in this project is to use an integrative structural biology approach-including temperature-dependent hydrogen-deuterium exchange mass spectrometry (TD-HDXMS), small-angle X-ray scattering (SAXS), negative stain electron microscopy (EM), and interspecies sequence alignment-to quantitatively probe in-solution dynamics of human Fng. Although structural biology techniques employed here provide a low-resolution assessment of Fng structure in solution (around $20 \AA$ ), outcomes of my findings reveal major in-solution populations of Fng. Cross-correlation between these techniques, together with TD-HDXMS and sequence alignments create an unbiased, data-assisted, and integrative strategy for depicting the in-solution conformational ensembles of human Fng for the first time.

In short, assessing the true physiological dynamics of Fng will provide an outlet for understanding how its intrinsic flexibility and mechanics influence its function and performance throughout the cardiovascular system. This project serves as a template that can be adapted to the understanding of different bodily molecules that play a primary role in the physiology of organisms.

## CHAPTER II: Materials and Methods

## Multiple Sequence Alignment of Fibrinogen $\alpha, \beta$, And $\gamma$ Chains.

All Fng sequences, from lamprey (jawless hagfish) to humans, that are available from Uniprot.org were aligned using ClustalX2 (Larkin et al., 2007) (Appendix 1.1 - 1.3). The multiple sequence alignment parameters used in the sequence analysis are set to a gap opening of 10 , a gap extension of 0.2 , and a DNA transition weight of 0.5 . For the protein weight matrix, Gonnet series was used. A custom-made coloring scheme that shows amino acid similarities was used to highlight sequence alignment (Appendix 1.1 - 1.3). The secondary structure based on 3ghg and globular homology were mapped onto alignments with ESpript 3.0 at default settings. Sequence similarities were based on \% equivalence with a global score of 0.7. The helical propensity was calculated through PredictProtein webserver using default settings and incorporated into the sequence alignment.

Epitopes identified on the $\alpha, \beta$, and $\gamma$ Fng chains that have been reported to interact with other molecules are noted in sequence alignments, as follows; locations for binding epitopes for A $\alpha$ 95-98 RGDF integrin site and $\mathrm{A} \alpha$ 572-575 RGD for the matrix- integrin binding sites, $\mathrm{A} \alpha$ 148-160, $\beta 15-42$ : Heparin-binding site, $\beta 15-42$ : VE-cadherin, $\gamma 312-324 \mathrm{tPA}$, and $\gamma 400-411: \alpha \mathrm{IIb} \beta$ was acquired (Mosseson et al., 2001). Binding epitopes $\alpha 239-421$ : $\mathrm{A} \beta$-amyloid, and $\beta 366-414$ : $\mathrm{A} \beta$-amyloid locations were also sourced (Zamolodchikov et al., 2016). The fibronectin-binding site at position $\alpha 221-391$ was subsequently gathered (Makogonenko et al., 2007). Additionally, the VLDR epitope at $\beta 15-66$ was cited (Yakovlev \& Medved, 2017). The ICAM-1 site located was found to be in positions $\gamma 117-133$ (Boyd et al., 2008). The $\alpha_{M} \beta_{2}$ interaction site located in positions $\gamma 190-202$, referred to as P 1 , is subject to disagreement but was previously identified
(Ugarova et al., 1998). The $\alpha_{\mathrm{M}} \beta_{2}$ interaction site at locations $\gamma 377-395$ is referred to as P2 (Ugarova et al., 2003).

## Synchrotron SAXS Measurements

Purified Fng purchased from the Enzyme Research Laboratories were shipped on ice to the synchrotron and incubated at ambient temperature for $15-20 \mathrm{~min}$ before x -ray solution scattering measurements were performed. For the SEC-SAXS experiment, $200 \mu \mathrm{~L}$ of $10 \mathrm{mg} / \mathrm{mL}$ Fng was injected into the Superdex S200 column for each experimental run. The mobile phase was 20 mM HEPES, 150 mM NaCl , and pH 7.4 . The SAXS experiments on the prepared samples were collected using National Synchrotron Light Source-II (NSLS-II) Beamline 16-ID (LiX) at Brookhaven National Laboratory. $I(0)$ and the pair distance distribution function $P(r)$ were calculated by circular averaging of the scattering intensities $I(q)$ and scaling using the software GNOM (Svergun, 1992). Fng scattering data was processed to a $q\left(A^{-1}\right)$ of 0.09 and 1.15 . (Appendix 1.4,1.5).

## ab inito Modelling of Fibrinogen architecture

By using GASBOR - program for ab initio reconstruction of protein structure by a chainlike ensemble of dummy residue-, wide-angle scatterings were used to generate ab initio models and then superimposed using DAMAVER (Volkov \& Svergun, 2003). GASBOR algorithm differs from other SAXS modeling tools in that the protein structure is depicted as by an ensemble of dummy residues (corresponding to average residue densities) placed anywhere in continuous space with a preferred number of close distance neighbors for each atom. The centers of these residues aim to approximate the positions of the $C \alpha$ atoms in the protein structure. Parameters for $a b$ inito modeling used are the followings: the number of residues in a homodimer is 2898 and
the Fng diameter is 448 Ang (Appendix 1.6).

## CORAL Modelling of Fibrinogen structure

Complexes with Random Loops (CORAL) is a computational modeling software that allows the creation of SAXS-based rigid body modeling with flexible or "missing" hinge regions. These regions could be interdomains in a protein architecture that helps define freely moving sections (Petoukhov et al., 2012). SAXS profiles generated from SEC-SAXS experiments for Fng were subcategorized into 20 different rigid bodies. Rigid bodies for CORAL models were defined as follows; For A $\alpha$ chain: V20-L94, R104-P203, V214-S569, and N316-S569. For the $\beta$ chain, the following residues were paired as rigid bodies: C65-E136, Y142-T198, and C211-F458. As for the $\gamma$ chain residues: R1-D58, N64-K127, and C140-I381 were selected to act as rigid bodies. In crystal structures, the beta and gamma nodules always stay in the same relative configuration to each other, so we kept single-bond constraints between these two domains. Additionally, the E- and D-region sections of the coiled-coil connectors also had a rigid quaternary structure, so we included three single-bond constraints between $\alpha-\beta, \beta-\gamma$, and $\gamma$ - $\alpha$ chains for the E- and D-region sections of the coiled-coil connectors. Via simulated annealing, the most optimal positions, and orientations of high-resolution Fng molecules were obtained with the input parameters for the modeled rigid domains and the missing peptide portions of the flexible linker residues (Appendix 1.7).

## Negative Stain Electron Microscopy Imaging for in-solution Fibrinogen.

For negative stain EM grid, Fng, within 2 hours after Superdex 200 10/30 column purification of $\sim 30 \mu \mathrm{~g}$ of Fng in 20 mM Hepes pH 7.4 and 150 mM NaCl , were adsorbed to glow discharged carbon-coated copper grids, stained with uranyl formate, and inspected with an

Jeol2100 operated at 200 kV . Low-dose images were acquired with a nominal magnification of $12,000 \mathrm{X}$ or $52,000 \mathrm{X}$ using a defocus of $-1.5 \mu \mathrm{~m}$. About 3,000 particles were interactively picked, windowed into individual images using the BOXER module of EMAN (Ludtke et al., 1999) and subjected to 10 cycles of multi-reference alignment and $K$-means classification into 50,20 , or 10 classes using SPIDER (Frank et al., 1996) as described (Sen et al., 2018).

## Angle Measurements Between Rigid Bodies

Angles between rigid bodes/domains were calculated for models that were generated by CORAL under SAXS constraints using PyMOL (The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC). A PyMOL script was generated with the name of Angle_between_domains which first defined the center of mass (COM) for each individual rigid body and subsequently the angle between the COM of each rigid body was calculated along with their displacement in terms of $\AA$ (Appendix 1.9). A total of seven different set of angles were generated for the entire dimeric Fng structure, beginning with the angle between the coiled-coil E region connector and the coiled-coil D region connector (Figure 1.1). All globular nodules were characterized as subsequent rigid bodies, the $\alpha \mathrm{C}, \beta$ and $\gamma$ nodules were paired with the second part of the coiled-coil section pertaining to the D-region connector to calculate both angle rotation and body displacement (Both $\beta$ and $\gamma$ were treated as a single rigid body). Other angle parameters which were calculated included the angle between the initial sections of the coiledcoil E region connectors of both sides of the dimer interphase. The same parameters and rigid body structures/parings were used for the second part of the dimer to keep a consistent depiction of the flexible variants among the 100 SAXS generated Fng CORAL models.

# CHAPTER III: Data-Assisted Probing of the in-solution Fibrinogen Flexibility. 

## Purified Fibrinogen Molecule and Structural Methods.

The Full-length human Fng molecule containing the $\alpha, \beta$ and $\gamma$-chains spanning from Ala1-P625, Q1-Q461, and Y1-V411, was studied. This protein was sufractionated, and the major 1st peak, containing two platelet-binding 'gamma-A' gamma-chain isoforms, is free from any Fng-binding molecules such as factor XIII was used. In each of the structural methods used in the following sections, Fng was further purified to homogeneity by size-exclusion chromatography (Figure 3.1A. This data was produced in the Hudson laboratory, East Caroline University); Fng gave single, dimeric $(\mathrm{A} \alpha \mathrm{B} \beta \gamma)_{2}$ peak and in SDS-PAGE of gel filtrations fractions showed the bands corresponding only to the $\alpha, \beta$ and $\gamma$-chains (Figure 3.1B - This data was produced in the Hudson laboratory, East Caroline University).

Since the $\alpha$ C-region from residue 360 to 580 showed reasonable interspecies similarity as well as secondary structure propensity, in modelling the Fng dimer, we generated its 3D structure model (Figure 3.1C) using I-TASSER (Yang \& Zhang, 2015), which detects structure templates using fold-threading from a protein data bank and reassembles the query using replica exchange Monte Carlo simulations. Parent structures used form modelling were 1PCL, 2BAF, 1W0R, 1N7D, and 1W0S, generating a single globular domain with multiple parallel $\beta$-strands. Moreover, the model displayed a spatial correlation between Cys442 and Cys472 of only 1 nm , without imposing any disulfide bond constrains, which corresponded well to the actual presence of a disulfide bond between these residues (Zauner et al., 2012).


FIGURE 3.1: FIBRINOGEN PURIFICATION \& STRUCTURES
(A) Size exclusion chromatography of full-length fibrinogen molecule, including $\alpha, \beta$ and $\gamma$ in a dimeric state corresponding to intact fibrinogen purification and extraction. Elution volume is 48 mL . Purification was performed at $25^{\circ} \mathrm{C}$ (B) SDS-PAGE gel of the purified fibrinogen, showing $\alpha, \beta$ and $\gamma$ chains. Molecular weights for the corresponding chains are shown in S lane, labels for all three chains are shown in lane 1. (C) Fibrinogen structure containing highlighted gamma-A isoforms of $\alpha \mathrm{C}$ domain predictions from I-TASSER. $\gamma$-A isoforms are highlighted in red.

Additionally, the size of the loose $\beta$-helix structure corresponds well to the size of the $\alpha \mathrm{C}$ regions previously observed in EM and AFM studies (Protopopova et al., 2015). AlphaFold2 similarly predicts some beta-stand structures like the those from I-TASSER predictions.

## HDX-MS of Fibrinogen at Room Temperature.

Flexibility of human Fng was previously characterized by hydrogen-deuterium exchange-mass spectrometry (HDX-MS) at $0^{\circ} \mathrm{C}$ (Marsh et al., 2013). Under these conditions, $\mathrm{D}_{2} \mathrm{O}\left(75 \%\right.$ in $\left.\mathrm{H}_{2} \mathrm{O}\right)$ has a freezing point at ca. $3^{\circ} \mathrm{C}$; thus, $\mathrm{D}_{2} \mathrm{O}$ crystals could form at $0^{\circ} \mathrm{C}$, and thus complicate the HDX-MS analysis. Given that HDX-MS is responsive to temperature, we sought to examine the exchange behavior of Fng at multiple temperatures, including room temperature, to reveal the inherent flexibility and thermal motions of Fng in solution. From pepsin digests, tandem LC-MS/MS supported the assignment of 170, 101, and 119 peptides from the $\alpha, \beta$, and $\gamma$ chains of Fng, providing 70, 81, and $74 \%$ coverage, respectively.

These coverage maps are comparable to those previously reported (Marsh et al., 2013), when using pepsin and moderate concentrations of guanidinium hydrochloride as a chaotrope post-quench. Most notable was the large gap in coverage of the $\alpha$ domain, residues 250-349, that represents the $\alpha \mathrm{C}$-connector, a flexible linker between the $\alpha \mathrm{C}$ and coiled-coil domains (Figure 3.2). This $\alpha$ C-connector was likewise absent in previous HDX-MS analysis (Marsh et al., 2013). In addition, a significant portion of the uncovered regions in $\alpha$ (9-53), $\beta$ (42-86), and $\gamma$ (10-28) chains represent the compact region of the central domain which is connected through several post-translational disulfide linkages. This compacted, cysteine rich region, however, is well structured in the X-ray crystal structure and is anticipated to have minimal exchange with $\mathrm{D}_{2} \mathrm{O}$. Two additional regions, 361-374 in $\beta$ and 44-56 in $\gamma$, were also
uncovered due to the presence of the N -linked glycosylation sites, N364 in $\beta$ and N 52 in $\gamma$ chain.


FIGURE 3.2: TIME DEPENDENT HDX-MS HUMAN FIBRINOGEN EXPOSURE
Structural model of fibrinogen molecule is colored according to the figure legend at the bottom of the image. Exposure at $25^{\circ} \mathrm{C}$ was measured for time intervals for $10 \mathrm{~s}, 10 \mathrm{~min}$, and 2 h for (A), (B) and (C) respectively. Uncolored regions were not subjected to MS characterization. (This data was collected in the Offenbacher Laboratory, East Caroline University)

From this list, a set of $23(\alpha), 23(\beta)$ and $21(\gamma)$ non-overlapping peptides was selected for data reduction purposes. Initial $\mathrm{HDX}-\mathrm{MS}$ samples were incubated in ${ }^{2} \mathrm{D}_{2} \mathrm{O}$ buffer at $25^{\circ} \mathrm{C}$ and over nine time points ( 10 s to 2 h ). For each peptide, the HDX-MS data reported herein is corrected for peptide-specific back-exchange (see Methods for more details). Overall, the trends in the exchange behavior are like previous reports at $0^{\circ}$ C. (Marsh et al.,2013). The HDX data presented here are also consistent with high exchange ( $>80 \%$ ) values for the $\alpha$ C region of Fng. These data support the hypothesis that the $\alpha \mathrm{C}$ domain is highly flexible and potentially showing more "breathing" characteristics (Collet et al., 2005). Further, the $\beta$ - and $\gamma$-nodules mostly exhibit low to moderate exchange, suggesting a more structured, rigid architecture. A few notable differences in the absolute HDX values are seen in the coiled-coil helices, in which the exchange values reported herein at $25^{\circ} \mathrm{C}$ are slightly elevated at longer time scales, relative to those previously reported at $0^{\circ} \mathrm{C}$. This behavior is most notable for the coiled-coil E domain [HNE2] that is located adjacent to the central nodule.

## Subsection On Ex-2 And the Ability to Probe Thermodynamics.

To resolve differences in Fng structure and flexibility between room and reduced temperatures, we also collected HDX-MS for Fng at $10^{\circ} \mathrm{C}$. From analysis of the entire dataset, three classes of temperature dependent HDX behavior emerged (Figure 3.3). Class I is characterized by low exchanging behavior $(<40 \%)$ that exhibits no significant differences in HDX between 10 and $25^{\circ} \mathrm{C}$. Class II is characterized by high exchange behavior (>60\%) that likewise exhibits no significant differences in HDX between the two temperatures. Class III is typically characterized by moderate exchange accompanied by temperature sensitive apparent rates of exchange and/or extent of exchange (i.e., protein motions are thermally activated).

All individual chains, for Fng $(\mathrm{A} \alpha \mathrm{B} \beta \gamma)_{2}$ were subjected and characterized for their rate of HDX percentage exchange in a time and temperature-dependent manner. The molecule was subjected to runs at $10^{\circ} \mathrm{C} \& 25^{\circ} \mathrm{C}$. Class I represent the gamma chain; Class III represent the


## FIGURE 3.3: CHAIN DEPENDENT HDX-MS EXPOSURE FOR HUMAN FIBRINOGEN

All individual chains, for fibrinogen $(\mathrm{A} \alpha \mathrm{B} \beta \gamma)_{2}$ were subjected and characterized for their rate of HDX percentage exchange in a time and temperature-dependent manner. The molecule was subjected to runs at $10^{\circ} \mathrm{C} \& 25^{\circ} \mathrm{C}$. Class I represent the gamma chain; Class III represent the beta chain and Class II represent the alpha chain. Two distinct views can be seen through a $90^{\circ}$ rotation angle of the molecule. (This data is collected in the Offenbacher Laboratory, East Caroline University)
beta chain and Class II represent the alpha chain. Two distinct views can be seen through a $90^{\circ}$ rotation angle of the molecule (Figure 3.3).

The exchange classes were mapped onto the Fng structural model. From this view, class I is almost completely centered on the $\beta$ - and $\gamma$ - nodules, further supporting that these regions of protein are rigid and well structured, and their motions are not thermally activated. Class II is mostly restricted to the $\alpha$ C region of Fng. This is consistent with the highly dynamic behavior of this region. Conversely, the coiled-coil is almost predominantly characterized by class III (purple) behavior. One exception to this behavior is a short sequence ( $\alpha: 74-89$ and $\beta: 106-115$ ) that exhibits temperature-independent, high exchange consistent with class II behavior. While the X-ray model supports well-structured $\alpha$-helices for these peptides our TD-HDX data implicate that this localized region is highly dynamic and could cause "flexing" of the Fng molecule as previously described by MD simulations (Madrazo et al., 2001).

## Negative Stain Electron Microscopy Imaging of Fibrinogen.

To visualize Fng single particles on the electron microscopy, intact Fng molecule was examined by negative stain EM in 150 mM NaCl and 20 mM HEPES pH 7.4. In high resolution images, most Fng particles are elongated and asymmetrical (Figure. 3.4A).

To further categorized the overall conformations, more than 3000 particles were picked and then, images were subjected to 10 cycles of multireference alignment into 50, 20, and 10 sub-classes using SPIDER (Frank et al., 1996). Image averages revealed a heterogenous conformational ensembles that show obtuse bends along the major axis (Figure 3.4C). In 50subclass, Fng micrograph averages consist of a linear array of two distinct nodules at each end that are dimeric and both nodules are held together with a thin thread, which approximately have a diameter of 10 to $20 \AA$ (Figure 3.5). Both end nodules are comparable in size. It is conceivable that both end-nodules herein represents the $\beta \mathrm{C}$ and $\gamma \mathrm{C}$ nodules. Relative orientation of this doublet is invariantly consistent in crystal structures (Spraggon et al., 1997) (Spraggon et al., 1998) (Kollman et al., 2009) (Figure 4.1) and in our cryoEM averages (not shown). These distinct structural features, when the number of averages is enforced to 20 and 10 , disappear and become fuzzier (Figure 3.5a, b).


FIGURE 3.4: NEGATIVE STAIN ELECTRON MICROSCOPY PARTICLES \& ENSEMBLES.
(A) Individual fibrinogen particles that were selected to generate averages, intact particles in a good quality state contributed to the clarity of classes. (B) Clustered EM fibrinogen particles were not chosen because class averages would have been undeceivable. (C) Four prominent structures were observed for all the individual particles that were selected. Classification was given to each one along with highlighted areas of interest.


FIGURE 3.5: CLASS AVERAGES FOR NEGATIVE STAIN EM FIBRINOGEN IMAGES
(a) Multireference alignment of 50 class averages. (b) Multireference alignment of 20 class averages. (c) Multireference alignment of 10 class averages. All multireference alignments were generated using EMAN and the classified particles were aligned and averaged in separate iterative processes for each class average. The number of particles per panel are displayed on the bottom right of each individual window. The architectural classification for each individual panel is displayed on the top right of each window. Fibrinogen architectural classifications are defined as the following: A) Straight, B) Straight-bent, C) Central-bent, D) Trans, E) Unique, ND) Not Defined. The overall number of particles for their respective classification is displayed for the multireference alignment 50 class-average

## X-Ray Scattering of Fibrinogen

We assessed the overall in-solution shape of Fng using size-exclusion SEC-SWAXS. Scattering profiles of the fractions that corresponds to the tip of the eluting peak, corresponding to the SAXS frames from 560 to 580 were normalized and averaged using the synchrotron software (Figure 3.6A, B) (Appendix 1.5). The Kratky plot (Figure 3.6D) shows a bell-shape peak in the low-q region and does not converge to the $q$-axis, showing that Fng fractions used in our SAXS data collection was monomeric and multi-domain protein with a pronounced domain-domain flexibility. Despite very limited and short Guinier region (Figure 3.6B), the Kratky plot of Fng showed a folded multidomain protein with an intrinsic flexibility (Figure 3.6D). $D_{\text {max }}$ calculated from $X$-ray scattering is around $448 \AA$ (Figure 3.6C), which is consistent with the previously reported Fng length, $450 \AA$ (Estis et al., 1980).

To gain insight into the conformation of Fng in solution, we first generated ab initio models reconstructed by a chain-like ensemble of dummy residues without any secondary structure definition and generated 100 different SAXS models with explicit solvent and electron density maps using GASBOR (Svergun et al., 2001). Superimposed models revealed a Yshaped, predominantly asymmetrical residue distribution (Figure 3.7), and clustering models into groups containing similar volumetric distribution results in four major classes, differences of which result from rotational pivots locations on the central axis (Figure 3.8).



FIGURE 3.7: SUPERIMPOSITION OF GASBOR FIBRINOGEN MODELS
100 different GABSOR models were superimposed to one another to show the most prominent structures and orientations. Residues colored in red represent areas of prominent water hydration and end nodules represent those for all three sets of amino acid chains $(\mathrm{A} \alpha \mathrm{B} \beta \gamma)_{2}$.

In the central long axis of the superimposed GASBOR models, a prominent hydration (red beads) was observed (Figure 3.7). When examined separately, the central axis adopts a highly flexible structure and the calculated dummy atom distributions, with two enlarged and globular nodules were similarly observed in EM class averages (Figure 3.5). The central longaxis has an overall width around $20 \AA$, which is very similar to the coiled-coiled structures in crystal lattices (Stafford et al., 2011), and the diameter of the both nodules combined is about $90 \AA$, which corresponds to the diameter of the dimeric $\beta \mathrm{C}$ - and $\gamma \mathrm{C}$-nodules. We observed a globular protrusion from the central axis, which mostly likely represent the globular $\alpha \mathrm{C}$-region. Although the previous NMR studies showed a slower cooperativity in motion for the $\alpha \mathrm{C}$-region, heavy resonance overlaps with high degree of ambiguity were inconclusive in determining the structure of the $\alpha$ C-region (Burton et al., 2006). Thus, structural definition of the $\alpha \mathrm{C}$-region has not been evident. Since our secondary structure prediction and homology modelling also suggest a potential domain for residues in the range of 350-570; further structural studies are required to define the structure of the $\alpha \mathrm{C}$-region.

## Architectural Flexibility of Fibrinogen

For flexible protein scaffolds like Fng, modelling in-solution states as an ensemble of conformers rather than a single structure represents better views of the conformational dynamism. Thus, to gain further insights for the in-solution dynamics of Fng, we next used an unbiased data-assisted assembly approach-using our HDX, EM and sequence alignments-to demarcate the extent of flexibility under SAXS constraints. In generating SAXS-based rigid body models of Fng, linker regions were experimentally defined as the following loops: V1V20, L95-R103, V204-P213, 235M-S315 for the $\alpha$-chain, V1-C64, Y137-E141 and C199-T210
for the $\beta$-chain, and P59-N64 and K127-C140 for the $\gamma$-chain, resulting in 20 different monomeric rigid modules. Via simulated annealing, translation, and rotation of these rigid modules for optimal positions and orientation within Fng homodimer scaffold that conform restraints from the experimental X-ray scattering models were generated using CORAL (Petoukhov et al., 2012). The observed ensembles are asymmetrical and contain bends along the central axis (Figure 3.8). Models generated showed a well-diverged $\chi$-values (Figure 3.9) and clustered into an into major four sets of conformationally related subfamilies (Figure 3.8).


Next, we calculated angle distributions between the defined globular modules on the dimeric Fng molecules modelled to quantitatively describe the relative angle deviations along the Fng homodimer scaffold-axis. Histograms constructed via our Matlab scripts (Matlab version 2021b-Appendix 1.8) (Appendix 1.9) show the angle distributions and fits between:
i. The E-region coiled-coils at the homodimer interface,
ii. The E- and D-region coiled-coils,
iii. The D-region coiled-coil and the dimeric $\beta \mathrm{C}$ - and $\gamma \mathrm{C}$-nodules and
iv. The D-region coiled-coil and the $\alpha \mathrm{C}$-domain.

The center of Fng homodimer where five disulfide bridges connect all Fng N-terminals sample into two different conformers, one angle with a nearly straight $\left(178^{\circ} \pm 14^{\circ}\right)$ and the other at an obtuse angle $\left(132^{\circ} \pm 11^{\circ}\right)$. Flexibility introduced by the patch in the coiled-coil connector with class-II TD HDX resulted three major conformers adopting angles $\left(177^{\circ} \pm 22^{\circ}\right.$, $94^{\circ} \pm 12^{\circ}$ and $130^{\circ} \pm 14^{\circ}$ ) (Figure 3.9 A-B).

Relative orientation between the D-region coiled-coil and the dimeric $\beta \mathrm{C}$ - and $\gamma \mathrm{C}$ nodules appears to adopt a single mode with an angle of $129^{\circ} \pm 26^{\circ}$ (Figure 3.9C). Bimodal and highly broad histogram was observed for the $\alpha \mathrm{C}$-domain relative to the coiled-coil connector $\left(180^{\circ} \pm 30^{\circ}\right.$ and $\left.101^{\circ} \pm 31^{\circ}\right)$ (Figure 3.9 D). The major flexion point on the central axis of Fng is in the center of the coiled-coil connector. The $\alpha \mathrm{C}$-domain appears to move almost independently as our gaussian fit show angles in each value. Perhaps, this flexibility is the reason behind why the $\alpha \mathrm{C}$-domain EM image classes was averaged out in crystal structures (Figure 3.5, Figure 3.10) (Tsurupa et al., 2002).


## FIGURE 3.9 CHI DIVERGENCE GRAPH FOR CORAL RUNS

As part of ATSAS software on the creation of Complexes with Random Loops bases on SAXS rigid body modeling of complexes. CORAL runs compare the scattering curves to the backcalculated scattering curves of the model being generated in each cycle. For each individual run, evolution of the chi value for the best target function for each model is given and plotted in the graph above.


## FIGURE 3.10: ANGLE DISTRIBUTION CALCULATION FOR CORAL MODELLING RUNS OF SPECIFIC FIBRINOGEN REGIONS

(A) Angle distributions between the coiled-coil connector for E-region of one side of the dimer vs the other. Two distinct populations of angles were observed along the distribution of angles. (B) Angle distribution between the coiled-coil connector for E-region \& D-region, multiple populations were observed with the most prominent one pertaining to a straight configuration. (C) Angle distribution between coiled-coil connector for D-region and fixed $\gamma$ and $\beta$ nodules, only one full distribution was observed for this angle population. (D) Angle distribution for coiled-coil connector for D -region and $\alpha \mathrm{C}$ globular domain, the biggest range of angles was observed in this distribution along with a vast number of angle populations.

## Visual Insights into The Fibrinogen Models Through Low-Resolution Data Comparison

Analyses of our EM averages and SAXS-data assisted clustering of CORAL models are consistent, identifying four overall shapes of Fng:
i. Linear
ii. Centrally bent,
iii. One-arm bent or straight bent and
iv. Trans homodimers.

Although single $a b$ initio GASBOR models could not replicate each state alone, the 3Dvolumetric clustering via DAMCLUST could reproduce the general features of the four structural states. Straight configurations were clearly observed through the central regions ( E region and part of D regions) of most Fng molecules in EM class averages, in ab-initio GASBOR models as well as in CORAL models (Figure 3.11A, B, D - panels 1 to 4). A significantly smaller number of EM class averages and SAXS models demonstrated more clear bending patterns in the central regions of Fng molecules (Figure 3.11C). Several EM averages as well as SAXS generated Fng molecules demonstrated bent conformations in the regions pertaining to the coiled-coil connectors. However, some of raw unfiltered images have a faint density protruding from the central axis (Figure 3.4 A, B) The homodimer interface appears mostly linear, and the E- and D-regions appears to have a single pivoting region, which is consistent with many crystal structures (Figure 3.11) (Figure 4.1).

Higher cross correlation values were observed for those molecules which had those type of bending patterns in the previously mentioned regions (Figure 3.11A, D-panels 3 to 4). Suggesting that such bending and dynamic patterns amongst specific regions are more
accurately represented than others. Even thought there was not enough representations for trans configuration, EM class averages and SAXS models were still observed for these types of architectural conformations (Figure 3.11D - Panels 1 to 4).

Additionally, ab-initio models generated by GASBOR were cross correlated with CORAL rigid body SAXS models. Lower cross correlation values were observed for all four prominent Fng molecules as compared to those between negative stain EM imagining and CORAL models. The coiled-coil domain interfaces for both CORAL and GABSOR models matched one another rather plainly, showing a preferred structural conformation for the coiledcoil domains between central and distal regions (Figure 3.11 A, B\&D-panels 5). It is also interesting to note that, some of the more highly dynamic regions of the Fng models such as the $\alpha \mathrm{C}$ connector and the $\alpha \mathrm{C}$-domain, as well as some of the regions pertaining to the coiled-coil connectors between the E and D regions do not match as distinctly as some of the more rigid structures of the molecule. Perhaps, that could be one of the reasons as to why the crosscorrelation values between CORAL and GASBOR models were significantly lower (Figure 3.11 A, B \& D - panels 5).


## Interspecies alignment of the $\alpha, \beta$ and $\gamma$ fibrinogen chains

Fng is one of the most structurally and functionally complex blood proteins, with its $\alpha$ and $\gamma$-subunits having two different splice isoforms, $\mathrm{A} \alpha$ and $\mathrm{A} \alpha \mathrm{E}$, and $\gamma$ and $\gamma^{\prime}$, respectively, as well as numerous posttranslational modifications (Appendix 1.1, 1.3). The sequence-structure alignment between fibrinogen chains is surprisingly straightforward, the structure-based alignment for each chain arranged accordingly with the correct sequences. Given that Fng is a vertebrate specific molecule, we aligned fibrinogen molecules from human to lamprey; representative alignments are presented in (Appendix 1.1-1.3). Based on our Fng divergence calculated by Fng sequence comparison in units in relation to the constructed phylogeny, fibrinogen divergence appears highly stepwise and could be reliably split into categories: lamprey, as the only jawless fish is the most diverged, then aves (birds) and mammals in comparison to the human Fng (Figure 3.12B).

The $\alpha$-chain contains two $\operatorname{RG}(\mathrm{D} / \mathrm{E})$ sequences, the first one is located at the end of the coiled-coil connector wherein the helical propensity is temporarily reduced and is also well conserved in all mammals (Figure 3.12A). The second is a more degenerate site and not wellconserved across the Fng-alignment and falls just outside the predicted fold of the $\alpha \mathrm{C}$-domain (Appendix 1.1). In the $\beta$-chain, the binding epitope of heparin is in residues $15-42$ and is conserved across vertebrates. Epitopes of the Very-Low-Density-Lipoprotein-Receptor (VLDLR) that promotes trans-endothelial leukocyte migration and VE-Cadherin that regulate cellular interaction exclusively in vasculature are located more upstream, highly flexible-not well-resolved in crystal structures (Nawroth et al., 2002)—Arg-Lys rich region in positions 3066 (Luyendyk et al., 2019) and are invariant mammals. Both VE- cadherin and VLDLR are urochordate specific molecules. In the $\gamma$-chain, ICAM- 1 and tPA binding sites are observed to
be highly conserved (Appendix 1.3). $\alpha \mathrm{M} \beta 2$ integrin-binding site was shown to be the ${ }^{390}$ NRLTIG $^{395}$ with Arg and Leu having the greatest importance, which is invariant in all Fg alignments except lamprey (Appendix 1.3) (Podolnikova et al., 2015). It is interesting to note here that Lamprey or jawless and cartilaginous fishes do not carry vertebrate-specific $\alpha \mathrm{I}$ -integrins- $\alpha \mathrm{M} \beta 2$ belongs to $\alpha \mathrm{I}$-leukointegrin family-that has an extra inserted $\alpha \mathrm{I}$-domain in their $\alpha$-subunit.

## FIGURE 3.12: FIBRINOGEN INTERSPECIES SEQUENCES PERCENT SIMILARITY \& DIVERGENCE

The percentages for similarities and divergence for all three fibrinogen chains were assembled and analyzed for the relationship between sequences amongst several individuals from different species including mammals (humans and primates), birds, reptiles, and fish (jawless). (A) Percent similarity (identities/length of the alignment) values range from 15-97, the latter representing the highest percent similarity value amongst species. (B) Divergence (The divergence distances-the number of base substitutions per site-were computed using the maximum composite likelihood method using MegAlign, Lasergene Core Suite 10.1.2; DNASTAR) values ranging from 0.7-85.3 were observed indicating that the higher values represent a greater degree on sequence divergence.



## CHAPTER IV: DISCUSSION ON THE INTRINSIC DYNAMICS OF INSOLUTION FIBRIONGEN.

In invertebrates, the major role of Fng-like molecules is non-specific immunity (Hanington and Zhang, 2011). Fng first emerged in urochordates as triple chain moleculethus, representing a recent evolutionary event (Jiang \& Doolittle, 2003). It appears that the $\alpha$ chain amongst species has a higher divergence since Fng $\beta$ and $\gamma$ chains of lampreys-jawless fish that is the origin of the neural crest and other vertebrate traits-shares at least $50 \%$ sequence similarity to human Fng $\beta$ and $\gamma$ chains, however, similarity between their $\alpha$-chains does not exceed beyond $15 \%$. The $\alpha$-chain, in comparison to other two, contains more interactions epitopes. Many convoluted pathways, such as blot clotting, angiogenesis, and immune response are well-tuned for Fng sequences. For instance, birds that have high-pressure cardiovascular systems with high fluid shear forces in their arterial vasculature do not have complete RGD binding sites on their $\alpha$-chain, which are required for thrombocyte aggregate formation in mammals (Doolittle et al., 2012). Shear force in the blood in human and mammals are comparable, and based on the back of the envelope calculations, it will be in the fN range even in the birds. However, the high-pressure system could create a better pull-on fibrin through platelets and effectively expose the ${ }^{95} \mathrm{RGD}^{97}$ site. This is probably why the ${ }^{95} \mathrm{RGD}^{97}$ site is critical for platelet retraction (fibrin-dependent), but not aggregation (Fng-dependent).

Furthermore, the KQAGDV sequence that regulates platelet interaction in all warmblooded vertebrates is not present in lamprey, instead a single RGD sequence is found in at Ctermini of the lamprey $\gamma$-chain. Also, epitopes of leukocyte integrins, cadherins, involved in cellular interactions are not also identified on the jawless fish Fng. Given that invertebrates
have neither acquired/adaptive immunity or nor employ Fng in coagulation process, such convoluted and well-refined Fng-related events must be mutually shaped and tuned by Fng molecular interactions during recent vertebrate evolution.

Previously published Fng crystal structures showed mostly linear, limited bending patterns on the coiled-coil domain regions as well as different tilt depths for globular ends as noted in Fng diagrams (Figure 4.1B). However, motions and flexibility are essential features for protein functions and macromolecular machines, thus, just as protein structures are subject


## FIGURE 4.1: FIBRINOGEN CRYSTAL STRUCTURES SUPERIMPOSITION AT SPECIFIC REGIONS.

(A) Superimposition of fibrinogen crystal structures $1 \mathrm{deq}, 1 \mathrm{ei} 3$, $1 \mathrm{fza}, 1 \mathrm{fzb}, 1 \mathrm{fzf}, 11 \mathrm{t} 9,11 \mathrm{tj}, 1 \mathrm{wu}$, $1 \mathrm{~m} 1 \mathrm{j}, 1 \mathrm{n} 73$, 1re3, 1re4, 1rf0, 1rf1, 2h43, 2oyh, 2oyi, and 3ghg at D region coiled-coil connector. Structures are colored based on their RMDS. (B) Superimposition of previously mentioned fibrinogen crystal structures at D region coiled-coil connector.
to natural selection, evolutionary pressures in these multipronged Fng events appear to tune Fng dynamics beyond the structural states observed in crystallography to adapt to new environments and enable the emergence of new functionalities.

The rod-like density for the coiled-coil connecter region sampled into three different bending ensembles in our EM and SAXS, and show high H/D exchange, suggesting an insolution bending in the coiled-coil connector. Herein, the bending is in close vicinity to the functionally important sites; i. the N -glycosylation site, $\gamma \mathrm{N} 52$, which carries altered glycan moieties during pregnancy (Maghzal et al., 2005), in response to trauma, and in select human diseases (Brennan, 2015), ii. the cryptic $\alpha$-RGD sequence that regulates adhesion and retraction of platelets rather than aggregation. Additionally, novel Fng mutations, $\alpha$-R104C (Caracas IX) and $\alpha$-R110P, $\alpha$-S112P were identified, which most likely alter flexibility in the middle of the coiled-coil connector and lead to slower kinetics of fibrin formation and reduced Fng expression (Brunclikova et al., 2022). Therefore, this local dynamic architecture in the middle of the coiled-coil connector likely plays a regulatory role for fibrin polymerization and Fng interactions. Indeed, this may explain why birds lack this RGD sequence since high shear force would eliminate this hinging motion, constantly exposing the cryptic RGD for a rapid platelet aggregation-similar mechanism normally employed by the $\gamma$-KQAGDV sequence. This conformational "shape-shifting" motion in the coiled-coil region observed in our study would provide a range of rotational and spatial flexibility during polymerization and interchangeably expose or hide the interaction epitopes and might differently regulate Fng functions in soluble and immobilized states such as rate of fiber formation, the porosity of fiber, clot growth and recruitment of platelets.

Crystal structures of the isolated "D region" of fibrin and Fng do not show any evidence
of structural rearrangements that would expose plasminogen site $\alpha 148-160$ (Yakovlev et al., 2000). In the X -ray scattering models and negative stain EM images, the dimeric $\beta \mathrm{C}$ - and $\gamma \mathrm{C}$ nodules only adopted a single angle distribution and 2D-orientation relative to the coiled-coil connector, suggesting the cryptic $\alpha \mathrm{C}$ binding site $\alpha 148-160$ is protected in a shielded location or lacks sufficient temporal exposure for the premature cleavage by plasminogen. Given that plasminogen could readily reach to this cryptic $\alpha \mathrm{C}$ binding site in the fibrin state, the rigidity of interface formed between the dimeric $\beta \mathrm{C}$ - and $\gamma \mathrm{C}$-nodules with the coiled-coil connector also appears to be functionally important. Further evidence for this mechanism comes from afibrinogenemia-a congenital disorder whereby the blood does not clot normally due to mutations that are in the flexible regions probed in our studies, such as the interface of the $\beta \mathrm{C}$ nodule and coiled-coil connector or central region of the coiled-coil region (Figure 4.2) (Simurda et al., 2022).

Despite large experimental and clinical literature revealing the epidemiology and genetics of inherited Fng pathologies, which precisely define the clinical presentations of these diseases, better understanding of in-solution stochastic dynamisms of Fng would illustrate its functional relevance in both health and Fng diseases. There exist three types of pathologies caused by Fng mutations: dysfibrinogenemia, hypofibrinogenemia and afibrinogenemia. The observed phenotypes in clinics, such as in patients with dysfibrinogenemia are heterogeneous (Casini et al., 2021); some patients have severed bleeding episodes and thrombotic phenotypes, whilst some stay asymptomatic their whole lifespan. The severity of these Fng pathologies is likely based on the location of specific residue mutations. For example, mutation $\gamma$ G165R is located at a highly flexible interface between the $\beta \mathrm{C}$ - and $\gamma \mathrm{C}$-nodules and the coiled-coil D region connector. This mutation, although it is in a highly dynamic region of the protein, leads
to asymptomatic or mild dysfibrinogenemia (Figure 4.2). Presenting only a slight reduction in the $\alpha$-helix content in comparison the WT Fng (Bolliger-Stucki et al., 2001).
$\beta$ M118K and $\beta$ L172Q mutations are positioned in the highly rigid-Class II region of HDX Fng data (Figure 3.3)-however, these mutations can lead to an impaired polymerization of fibrin, resulting in severe and abnormal clotting patterns (Figure 4.2). In other words, the severity of mutations and therefore the expressivity of the mutative phenotypes, is correlated with the location in which these changes occur. That is, the intrinsic flexibility and tensile strength of the region in which they are situated, dictates how these changes allow for proper Fng polymerization and clot architecture. For instance, mutations in more highly dynamic regions which have a propensity to be flexible such as $\gamma \mathrm{G} 272 \mathrm{R}$ and $\beta \mathrm{Q} 169 \mathrm{R}$ express asymptomatic phenotypes (Figure 4.2). Additionally, with the recent surge of the COVID-19 virus, attention has been drawn to severely ill patients that have presented increased levels of fibrin deposition/formation (Brubaker et al., 2022). Establishing the narrative that physiological conditions such as COVID-19 can affect the outcome of clotting processes which are inherent to Fng. Interestingly, it has been found that Fng levels do not directly affect clot density formations, this is rather affected by the interactions of other blood protein molecules (von Willebrand Factor \& vitronectin) in fibrin polymerization and fibrinolysis (Miszta et al., 2014) (Brubaker et al., 2022) (Wu et al., 2004). Fng can act both as an ally and a renegade in several physiological disorders throughout the human body. The high prevalence of Fng in vasculature allows it to participate pathologies such as cardiovascular disease, certain types of cancer, neurological disorders, microbial infections and allergic reactions, obesity and diabetes, and amyloidosis (Vilar et al., 2020). Several bodily disorders have been characterized as responsible for altering the structure of Fng, specifically at interface protein regions responsible for proper
platelet aggregation and leukocyte trafficking during clotting processes and immunological pathways (D'Andrea et al., 2009). Changes in Fng structure can occur at different glycosylation sites, specifically on their availability/exposure as well as binding epitopes responsible for the appropriate aggregation between Fng and other blood macromolecules (Hugenholtz et al., 2016) (Pieters et al., 2006). Understanding how certain interactive regions of Fng become exposed or obscured based on their structural dynamics could help explain as to why some of these changes become prevalent through different diseases. Thus, understanding Fng variants in terms of in-solution studies present a narrative that could help to understand the behavior of these changes/mutations and its interactions with other blood proteins and other Fng molecules.


 Figure 4.2 was generated using PyMOL.

 FIGURE 4.2: IDENTIFIED FIBRINOGEN MUTATIONAL SITES



While the Fng-to-fibrin transition is initiated by the cleavage of FpA and FpB , which unmask polymerization sites at the homodimer interface, the initiation, growth, and maintenance of polymerization are also dependent on the blood factors, including Fng and thrombin concentration, extent of TF exposure, blood pressure, vasculature length, and diameter, the density of platelets-all tuning the coagulability. For instance, the $\gamma \mathrm{C}$-nodule mutations that would aggressively impair $k_{\text {on }}$ of this interaction and the dynamics of Fng polymerization process are clearly accumulated on a single side of the $\gamma \mathrm{C}$-nodule and presented as severe pathologies. Coagulability of Fng carrying mutation(s) or chemical modifications that partly altering this polymerization dynamics shows no-to-mild pathologies despite having a unique clot structure. Multiscale mathematical modeling approaches studying fibrin polymerization accurately describe the diffusion, interactions, and concentrations of these blood factors, however, despite extensive quantitative data on from the clinical studies, the current complete mathematical models have not been adequately described the edges of growing clot and model its interaction with a blood flow (Tosenberger et al., 2015). Where they come short is most likely in quantitatively describing the internal flexibility factor of Fng during the polymerization event.

Herein, through the usage of multifaceted psychological and computational protein dynamics studies in the form of negative stain EM, HDX-MS, an unbiased approach was established to quantitatively elucidate the conformational dynamics of in-solution Fng. This has allowed for the proper characterization of the four major conformational ensembles (i. Linear, ii. Centrally bent, iii. One/arm bent or straight bent, and iv. Trans homodimer) which Fng can attain in what can be considered as true physiological conditions.

As previously mentioned, understanding the molecular basis of Fng, while considering
its mutations and post-translational modifications, can give an explanation to altered Fng functions (e.g., clot formation). These behaviors seem to be tightly woven with its intrinsic flexibility and exposure of vital residues. Highlighting the fact that, Fng dynamisms can either be severely hampered or promoted depending on its conformational states, flexibility patterns and molecular interactions. Recalling the fact that most structural studies on Fng have sought to explain the conformational components of static Fng molecules. Understanding Fng interactions and its behaviors at a true physiological state has come about through the cross correlation of the data sets presented throughout this paper (e.g., Negative stain EM, HDX-MS and SAXS). Presenting a narrative that focuses on understanding the driving factors behind the structural components of Fng in a true physiological state.

## APPENDIX

## APPENDIX 1.1: SEQUENCE ALIGNMENT FOR $\alpha$ CHAIN

A chain sequences were aligned for several species which include mammals, aves, reptiles, and fish. Alignments were made to understand conserved sequences responsible for polymerization processes and binding sites for different key molecules. HDX data alignments were also done for corresponding residue sequences.





## APPENDIX 1.2: SEQUENCE ALIGNMENT FOR $\beta$ CHAIN

$\beta$ chain sequences were aligned for several species which include mammals, aves, reptiles, and fish. Alignments were made to understand conserved sequences responsible for polymerization processes and binding sites for different key molecules. HDX data alignments were also done for corresponding residue sequences.

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## APPENDIX 1.3: SEQUENCE ALIGNMENT FOR $\gamma$ CHAIN

$\gamma$ chain sequences were aligned for several species which include mammals, aves, reptiles, and fish. Alignments were made to understand conserved sequences responsible for polymerization processes and binding sites for different key molecules. HDX data alignments were also done for corresponding residue sequences.



APPENDIX 1.4: AVERAGED SAXS DATA FROM ELUTION FRAME 560 to 580. MOST PREVALENT FNG PROFILED STRUCTURE

| 0.00500 | 46.69702 | 5.91009 |
| :--- | :--- | :--- |
| 0.00600 | 44.52024 | 4.18072 |
| 0.00700 | 41.31513 | 4.01578 |
| 0.00800 | 37.87548 | 2.55475 |
| 0.00900 | 34.49031 | 1.45202 |
| 0.01000 | 31.42774 | 0.87184 |
| 0.01100 | 28.71604 | 0.54961 |
| 0.01200 | 26.20656 | 0.33890 |
| 0.01300 | 23.95109 | 0.23387 |
| 0.01400 | 22.02373 | 0.16852 |
| 0.01500 | 20.38445 | 0.12929 |
| 0.01600 | 18.98900 | 0.10249 |
| 0.01700 | 17.78730 | 0.08445 |
| 0.01800 | 16.76470 | 0.07180 |
| 0.01900 | 15.87442 | 0.06323 |
| 0.02000 | 15.04473 | 0.05716 |
| 0.02100 | 14.26784 | 0.05065 |
| 0.02200 | 13.52260 | 0.04634 |
| 0.02300 | 12.80894 | 0.04352 |
| 0.02400 | 12.15994 | 0.04132 |
| 0.02500 | 11.53813 | 0.03826 |
| 0.02600 | 10.93500 | 0.03548 |
| 0.02700 | 10.36470 | 0.03379 |
| 0.02800 | 9.81857 | 0.03195 |
| 0.02900 | 9.31983 | 0.02991 |
| 0.03000 | 8.86881 | 0.02906 |
| 0.03100 | 8.49050 | 0.02698 |
| 0.03200 | 8.16228 | 0.02643 |
| 0.03300 | 7.87287 | 0.02494 |
| 0.03400 | 7.60292 | 0.02466 |
| 0.03500 | 7.33542 | 0.02394 |
| 0.03600 | 7.08013 | 0.02298 |
| 0.03700 | 6.85283 | 0.02208 |
| 0.03800 | 6.63829 | 0.02156 |
| 0.03900 | 6.42378 | 0.02123 |
| 0.04000 | 6.21554 | 0.02057 |
| 0.04100 | 6.00398 | 0.01988 |
| 0.04200 | 5.79218 | 0.01947 |
| 0.04300 | 5.57901 | 0.01928 |
| 0.04400 | 5.38364 | 0.01865 |
|  |  |  |


| 0.04500 | 5.20906 | 0.01826 |
| :--- | :--- | :--- |
| 0.04600 | 5.03116 | 0.01777 |
| 0.04700 | 4.84772 | 0.01761 |
| 0.04800 | 4.67160 | 0.01692 |
| 0.04900 | 4.49471 | 0.01696 |
| 0.05000 | 4.29685 | 0.01331 |
| 0.05200 | 4.06289 | 0.01130 |
| 0.05400 | 3.81378 | 0.01101 |
| 0.05600 | 3.57169 | 0.01056 |
| 0.05800 | 3.34746 | 0.01022 |
| 0.06000 | 3.14248 | 0.00991 |
| 0.06200 | 2.95599 | 0.00966 |
| 0.06400 | 2.78647 | 0.00937 |
| 0.06600 | 2.62840 | 0.00943 |
| 0.06800 | 2.47755 | 0.00942 |
| 0.07000 | 2.32997 | 0.00934 |
| 0.07200 | 2.19107 | 0.00925 |
| 0.07400 | 2.06426 | 0.00911 |
| 0.07600 | 1.94461 | 0.00897 |
| 0.07800 | 1.82931 | 0.00888 |
| 0.08000 | 1.72397 | 0.00896 |
| 0.08200 | 1.62385 | 0.00914 |
| 0.08400 | 1.53170 | 0.00918 |
| 0.08600 | 1.44895 | 0.00913 |
| 0.08800 | 1.37602 | 0.00883 |
| 0.09000 | 1.31323 | 0.00865 |
| 0.09200 | 1.25063 | 0.00861 |
| 0.09400 | 1.18449 | 0.00846 |
| 0.09600 | 1.11771 | 0.00832 |
| 0.09800 | 1.04721 | 0.00825 |
| 0.10000 | 0.96246 | 0.00620 |
| 0.10500 | 0.86122 | 0.00523 |
| 0.11000 | 0.75941 | 0.00496 |
| 0.11500 | 0.67445 | 0.00479 |
| 0.12000 | 0.60514 | 0.00466 |
| 0.12500 | 0.53897 | 0.00452 |
| 0.13000 | 0.47382 | 0.00444 |
| 0.13500 | 0.41535 | 0.00437 |
| 0.14000 | 0.36487 | 0.00442 |
| 0.14500 | 0.32331 | 0.00459 |
| 0.15000 | 0.29034 | 0.01246 |
| 0.15500 | 0.25952 | 0.01074 |
| 0.16000 | 0.22904 | 0.00966 |
| 0.16500 | 0.20480 | 0.00912 |
| 0.17000 | 0.18681 | 0.00885 |
|  |  |  |


| 0.17500 | 0.16948 | 0.00853 |
| :--- | :--- | :--- |
| 0.18000 | 0.15368 | 0.00810 |
| 0.18500 | 0.14192 | 0.00785 |
| 0.19000 | 0.13369 | 0.00762 |
| 0.19500 | 0.12663 | 0.00742 |
| 0.20000 | 0.11919 | 0.00718 |
| 0.20500 | 0.11339 | 0.00715 |
| 0.21000 | 0.11091 | 0.00699 |
| 0.21500 | 0.10835 | 0.00686 |
| 0.22000 | 0.10466 | 0.00673 |
| 0.22500 | 0.10170 | 0.00669 |
| 0.23000 | 0.10218 | 0.00653 |
| 0.23500 | 0.10294 | 0.00664 |
| 0.24000 | 0.09963 | 0.00668 |
| 0.24500 | 0.09531 | 0.00673 |
| 0.25000 | 0.09350 | 0.00667 |
| 0.25500 | 0.09323 | 0.00656 |
| 0.26000 | 0.09032 | 0.00656 |
| 0.26500 | 0.08697 | 0.00656 |
| 0.27000 | 0.08521 | 0.00647 |
| 0.27500 | 0.08471 | 0.00640 |
| 0.28000 | 0.08482 | 0.00660 |
| 0.28500 | 0.08426 | 0.00703 |
| 0.29000 | 0.08228 | 0.00733 |
| 0.29500 | 0.07971 | 0.00758 |
| 0.30000 | 0.07809 | 0.00802 |
| 0.30500 | 0.07721 | 0.00854 |
| 0.31000 | 0.07654 | 0.00920 |
| 0.31500 | 0.07637 | 0.00991 |
| 0.32000 | 0.07658 | 0.01084 |
| 0.32500 | 0.07538 | 0.01197 |
| 0.33000 | 0.07357 | 0.01737 |
| 0.33500 | 0.07264 | 0.00382 |
| 0.34000 | 0.07345 | 0.00378 |
| 0.34500 | 0.07368 | 0.00371 |
| 0.35000 | 0.07041 | 0.00365 |
| 0.35500 | 0.06634 | 0.00364 |
| 0.36000 | 0.06461 | 0.00362 |
| 0.36500 | 0.06477 | 0.00363 |
| 0.37000 | 0.06708 | 0.00358 |
| 0.37500 | 0.06822 | 0.00353 |
| 0.38000 | 0.06750 | 0.00359 |
| 0.38500 | 0.06611 | 0.00356 |
| 0.39000 | 0.06521 | 0.00357 |
| 0.39500 | 0.06570 | 0.00352 |
|  |  |  |


| 0.40000 | 0.06690 | 0.00342 |
| :--- | :--- | :--- |
| 0.40500 | 0.06886 | 0.00325 |
| 0.41000 | 0.06997 | 0.00321 |
| 0.41500 | 0.06929 | 0.00314 |
| 0.42000 | 0.06874 | 0.00309 |
| 0.42500 | 0.06847 | 0.00303 |
| 0.43000 | 0.06807 | 0.00296 |
| 0.43500 | 0.06802 | 0.00295 |
| 0.44000 | 0.06750 | 0.00288 |
| 0.44500 | 0.06667 | 0.00288 |
| 0.45000 | 0.06664 | 0.00285 |
| 0.45500 | 0.06685 | 0.00283 |
| 0.46000 | 0.06681 | 0.00279 |
| 0.46500 | 0.06718 | 0.00275 |
| 0.47000 | 0.06750 | 0.00269 |
| 0.47500 | 0.06809 | 0.00267 |
| 0.48000 | 0.06856 | 0.00264 |
| 0.48500 | 0.06838 | 0.00261 |
| 0.49000 | 0.06789 | 0.00262 |
| 0.49500 | 0.06778 | 0.00260 |
| 0.50000 | 0.06752 | 0.00209 |

## APPENDIX 1.5: FIBRINOGEN SAXS DATA REDUCTION FOR SAXS PROFILE, Dmax GRAPH AND KRATKY PLOT

\#\#\#\# GNOM Version $5.0(r 12314)$ \#\#\#\#<br>Tue Sep 8 11:53:31 2020<br>\#\#\#\# Configuration \#\#\#\#

System Type: $\quad$ arbitrary monodisperse $(j o b=0)$
Minimum characteristic size: $\quad 0.0000$
Maximum characteristic size: 448.0000
rad56: $\quad 0.0000$
Force 0.0 at $\mathrm{r}=\mathrm{rmin}: \quad$ yes
Force 0.0 at $\mathrm{r}=\mathrm{rmax}: \quad$ yes
Initial alpha: 0.0000
Initial random seed: 6714578439260132896
Points in real space: 151

Input 1: /Volumes/Meliksah_I/SAXS/304883_oct19/processed/fibrinogen_s1_05600580s.dat

First data point used: 1
Last data point used:
Scaling coefficient:
Experimental setup:
$0.1000 \mathrm{E}+01$
point collimation

## \#\#\#\# Results \#\#\#\#

Parameter DISCRP OSCILL STABIL SYSDEV POSITV VALCEN SMOOTH
$\begin{array}{llllllll}\text { Weight } & 1.000 & 3.000 & 3.000 & 3.000 & 1.000 & 1.000 & 1.000\end{array}$

| Sigma | 0.300 | 0.600 | 0.120 | 0.120 | 0.120 | 0.120 | 0.600 |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |

$\begin{array}{llllllll}\text { Ideal } & 0.700 & 1.100 & 0.000 & 1.000 & 1.000 & 0.950 & 0.000\end{array}$
$\begin{array}{lllllllll}\text { Current } & 0.365 & 2.530 & 0.001 & 0.933 & 1.000 & 0.692 & 1.962\end{array}$
$\begin{array}{llllllll}\text { Estimate } & 0.288 & 0.003 & 1.000 & 1.000 & 1.000 & 0.010 & 0.000\end{array}$

Angular range
Reciprocal space Rg: $\quad 0.1345 \mathrm{E}+03$
Reciprocal space I(0): $\quad 0.5348 \mathrm{E}+02$
Real space range: $\quad 0.0000$ to 448.0000
Real space Rg: $\quad 0.1357 \mathrm{E}+03+-\quad 0.2688 \mathrm{E}+01$
Real space $\mathrm{I}(0): \quad 0.5349 \mathrm{E}+02+-\quad 0.1651 \mathrm{E}+01$

| Highest ALPHA (theor): | $0.8667 \mathrm{E}+07$ |
| :--- | :--- |
| Current ALPHA: | $0.4313 \mathrm{E}+00$ |
| Total Estimate: | 0.5621 (a REASONABLE solution) |

\#\#\#\# Experimental Data and Fit \#\#\#\#

## S

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| $0.000000 \mathrm{E}+00$ |  |  | $0.534895 \mathrm{E}+02$ |  |
| :--- | :--- | :--- | :--- | :--- | :--- |
| $0.100000 \mathrm{E}-02$ |  |  | $0.531593 \mathrm{E}+02$ |  |
| $0.200000 \mathrm{E}-02$ |  |  | $0.521943 \mathrm{E}+02$ |  |
| $0.300000 \mathrm{E}-02$ |  |  | $0.506389 \mathrm{E}+02$ |  |
| $0.400000 \mathrm{E}-02$ |  |  | $0.485680 \mathrm{E}+02$ |  |
| $0.500000 \mathrm{E}-02$ | $0.466474 \mathrm{E}+02$ | $0.591009 \mathrm{E}+01$ | $0.460792 \mathrm{E}+02$ | $0.460792 \mathrm{E}+02$ |
| $0.600000 \mathrm{E}-02$ | $0.444879 \mathrm{E}+02$ | $0.418072 \mathrm{E}+01$ | $0.432830 \mathrm{E}+02$ | $0.432830 \mathrm{E}+02$ |
| $0.700000 \mathrm{E}-02$ | $0.412949 \mathrm{E}+02$ | $0.401578 \mathrm{E}+01$ | $0.402979 \mathrm{E}+02$ | $0.402979 \mathrm{E}+02$ |
| $0.800000 \mathrm{E}-02$ | $0.378625 \mathrm{E}+02$ | $0.255475 \mathrm{E}+01$ | $0.372398 \mathrm{E}+02$ | $0.372398 \mathrm{E}+02$ |
| $0.900000 \mathrm{E}-02$ | $0.344859 \mathrm{E}+02$ | $0.145202 \mathrm{E}+01$ | $0.342156 \mathrm{E}+02$ | $0.342156 \mathrm{E}+02$ |
| $0.100000 \mathrm{E}-01$ | $0.314286 \mathrm{E}+02$ | $0.871840 \mathrm{E}+00$ | $0.313164 \mathrm{E}+02$ | $0.313164 \mathrm{E}+02$ |
| $0.110000 \mathrm{E}-01$ | $0.287185 \mathrm{E}+02$ | $0.549610 \mathrm{E}+00$ | $0.286134 \mathrm{E}+02$ | $0.286134 \mathrm{E}+02$ |
| $0.120000 \mathrm{E}-01$ | $0.262073 \mathrm{E}+02$ | $0.338900 \mathrm{E}+00$ | $0.261559 \mathrm{E}+02$ | $0.261559 \mathrm{E}+02$ |
| $0.130000 \mathrm{E}-01$ | $0.239489 \mathrm{E}+02$ | $0.233870 \mathrm{E}+00$ | $0.239699 \mathrm{E}+02$ | $0.239699 \mathrm{E}+02$ |
| $0.140000 \mathrm{E}-01$ | $0.220208 \mathrm{E}+02$ | $0.168520 \mathrm{E}+00$ | $0.220607 \mathrm{E}+02$ | $0.220607 \mathrm{E}+02$ |
| $0.150000 \mathrm{E}-01$ | $0.203831 \mathrm{E}+02$ | $0.129290 \mathrm{E}+00$ | $0.204158 \mathrm{E}+02$ | $0.204158 \mathrm{E}+02$ |
| $0.160000 \mathrm{E}-01$ | $0.189890 \mathrm{E}+02$ | $0.102490 \mathrm{E}+00$ | $0.190080 \mathrm{E}+02$ | $0.190080 \mathrm{E}+02$ |
| $0.170000 \mathrm{E}-01$ | $0.177876 \mathrm{E}+02$ | $0.844500 \mathrm{E}-01$ | $0.178029 \mathrm{E}+02$ | $0.178029 \mathrm{E}+02$ |
| $0.180000 \mathrm{E}-01$ | $0.167649 \mathrm{E}+02$ | $0.718000 \mathrm{E}-01$ | $0.167613 \mathrm{E}+02$ | $0.167613 \mathrm{E}+02$ |
| $0.190000 \mathrm{E}-01$ | $0.158747 \mathrm{E}+02$ | $0.632300 \mathrm{E}-01$ | $0.158444 \mathrm{E}+02$ | $0.158444 \mathrm{E}+02$ |
| $0.200000 \mathrm{E}-01$ | $0.150451 \mathrm{E}+02$ | $0.571600 \mathrm{E}-01$ | $0.150179 \mathrm{E}+02$ | $0.150179 \mathrm{E}+02$ |
| $0.210000 \mathrm{E}-01$ | $0.142679 \mathrm{E}+02$ | $0.506500 \mathrm{E}-01$ | $0.142538 \mathrm{E}+02$ | $0.142538 \mathrm{E}+02$ |
| $0.220000 \mathrm{E}-01$ | $0.135224 \mathrm{E}+02$ | $0.463400 \mathrm{E}-01$ | $0.135320 \mathrm{E}+02$ | $0.135320 \mathrm{E}+02$ |
| $0.230000 \mathrm{E}-01$ | $0.128085 \mathrm{E}+02$ | $0.435200 \mathrm{E}-01$ | $0.128403 \mathrm{E}+02$ | $0.128403 \mathrm{E}+02$ |
| $0.240000 \mathrm{E}-01$ | $0.121595 \mathrm{E}+02$ | $0.413200 \mathrm{E}-01$ | $0.121738 \mathrm{E}+02$ | $0.121738 \mathrm{E}+02$ |
| $0.250000 \mathrm{E}-01$ | $0.115379 \mathrm{E}+02$ | $0.382600 \mathrm{E}-01$ | $0.115330 \mathrm{E}+02$ | $0.115330 \mathrm{E}+02$ |
| $0.260000 \mathrm{E}-01$ | $0.109349 \mathrm{E}+02$ | $0.354800 \mathrm{E}-01$ | $0.109223 \mathrm{E}+02$ | $0.109223 \mathrm{E}+02$ |
| $0.270000 \mathrm{E}-01$ | $0.103646 \mathrm{E}+02$ | $0.337900 \mathrm{E}-01$ | $0.103478 \mathrm{E}+02$ | $0.103478 \mathrm{E}+02$ |
| $0.280000 \mathrm{E}-01$ | $0.981842 \mathrm{E}+01$ | $0.319500 \mathrm{E}-01$ | $0.981552 \mathrm{E}+01$ | $0.981552 \mathrm{E}+01$ |
| $0.290000 \mathrm{E}-01$ | $0.931959 \mathrm{E}+01$ | $0.299100 \mathrm{E}-01$ | $0.932982 \mathrm{E}+01$ | $0.932982 \mathrm{E}+01$ |
| $0.300000 \mathrm{E}-01$ | $0.886877 \mathrm{E}+01$ | $0.290600 \mathrm{E}-01$ | $0.889269 \mathrm{E}+01$ | $0.889269 \mathrm{E}+01$ |
| $0.310000 \mathrm{E}-01$ | $0.849076 \mathrm{E}+01$ | $0.269800 \mathrm{E}-01$ | $0.850345 \mathrm{E}+01$ | $0.850345 \mathrm{E}+01$ |
| $0.320000 \mathrm{E}-01$ | $0.816266 \mathrm{E}+01$ | $0.264300 \mathrm{E}-01$ | $0.815842 \mathrm{E}+01$ | $0.815842 \mathrm{E}+01$ |
| $0.330000 \mathrm{E}-01$ | $0.787334 \mathrm{E}+01$ | $0.249400 \mathrm{E}-01$ | $0.785191 \mathrm{E}+01$ | $0.785191 \mathrm{E}+01$ |
| 0 |  |  |  | 0 |


|  | $0.760339 \mathrm{E}+01$ | 0.246600E-01 | $0.757683 \mathrm{E}+01$ |  |
| :---: | :---: | :---: | :---: | :---: |
| $0.350000 \mathrm{E}-01$ | 0.7 | 0. | 0. | $0.732563 \mathrm{E}+01$ |
| 360000 | $0.708050 \mathrm{E}+01$ | 0.2 | 0.7 | $0.709108 \mathrm{E}+01$ |
| $0.370000 \mathrm{E}-01$ | $0.685333 \mathrm{E}+01$ | $0.220800 \mathrm{E}-0$ | $0.686696 \mathrm{E}+01$ | 0.68 |
| 380000E-01 | $0.663886 \mathrm{E}+01$ | 0.215600 E | 0.66 |  |
| $0.390000 \mathrm{E}-01$ | 0.6 | 0.2 | 0.6 | $0.643289 \mathrm{E}+01$ |
| $0.400000 \mathrm{E}-01$ | 0.6 | 0. | 0.6 | 0.6 |
| 410000E-01 | $0.600444 \mathrm{E}+01$ | $0.198800 \mathrm{E}-01$ | $0.600535 \mathrm{E}+01$ | $0.600535 \mathrm{E}+01$ |
| $0.420000 \mathrm{E}-01$ | $0.579277 \mathrm{E}+01$ | 0. | 0.5 | $0.579442 \mathrm{E}+01$ |
| $0.430000 \mathrm{E}-01$ | 0.5 | 0.1 | 0.5 | $0.558724 \mathrm{E}+01$ |
| 0000E-01 | $0.538444 \mathrm{E}+01$ | $0.186500 \mathrm{E}-01$ | $0.538547 \mathrm{E}+01$ | 0.5 |
| $0.450000 \mathrm{E}-01$ | 0.52 | 0.1 | 0.5 | 1 |
| , | 0.5 | 0.1 | 0.5 | 0.5 |
| $0.470000 \mathrm{E}-01$ | 0.4 | 0.1 | 0.4 | 0. |
| 480000E-01 | $0.467208 \mathrm{E}+01$ | $0.169200 \mathrm{E}-01$ | $0.465704 \mathrm{E}+01$ | $0.465704 \mathrm{E}+01$ |
| 0000 | $0.449506 \mathrm{E}+01$ | 0.169600 E | 0.449659 | $0.449659 \mathrm{E}+01$ |
| $0.500000 \mathrm{E}-01$ | 0. | 0.1 | 0.4 | 0. |
| 520000E-01 | $0.406294 \mathrm{E}+01$ | $0.113000 \mathrm{E}-01$ | $0.406110 \mathrm{E}+01$ | $0.406110 \mathrm{E}+01$ |
| 促 | $0.381375 \mathrm{E}+01$ | $0.110100 \mathrm{E}-0$ | $0.380268 \mathrm{E}+01$ | $380268 \mathrm{E}+01$ |
| 60000 | $0.357167 \mathrm{E}+01$ | $0.105600 \mathrm{E}-0$ | $0.356561 \mathrm{E}+01$ | $0.356561 \mathrm{E}+01$ |
| 0000 | $0.334760 \mathrm{E}+01$ | $0.102200 \mathrm{E}-01$ | $0.334785 \mathrm{E}+01$ | $0.334785 \mathrm{E}+01$ |
| 00000 | $0.314277 \mathrm{E}+01$ | $0.991000 \mathrm{E}-02$ | $0.314727 \mathrm{E}+01$ | $0.314727 \mathrm{E}+01$ |
| .620000E | 0.295630 E | $0.966000 \mathrm{E}-02$ | 0.296169 | 0.296 |
|  | 0.2786 |  |  | $0.278813 \mathrm{E}+01$ |
| 00000E-01 | $0.262856 \mathrm{E}+01$ | $0.943000 \mathrm{E}-02$ | $0.262505 \mathrm{E}+01$ | $0.262505 \mathrm{E}+01$ |
| 80000E-01 | $0.247767 \mathrm{E}+01$ | $0.942000 \mathrm{E}-02$ | $0.247189 \mathrm{E}+01$ | 0.247189E+01 |
| , | $0.233015 \mathrm{E}+01$ | $0.934000 \mathrm{E}-02$ | $0.232863 \mathrm{E}+01$ | $0.232863 \mathrm{E}+01$ |
| 20000E-01 | $0.219148 \mathrm{E}+01$ | $0.925000 \mathrm{E}-02$ | $0.219459 \mathrm{E}+01$ | $219459 \mathrm{E}+01$ |
| 0000 | $0.206481 \mathrm{E}+01$ | $0.911000 \mathrm{E}-02$ | $0.206802 \mathrm{E}+01$ | .206802E+01 |
| 760000E-01 | $0.194503 \mathrm{E}+01$ | $0.897000 \mathrm{E}-02$ | $0.194702 \mathrm{E}+01$ | $0.194702 \mathrm{E}+01$ |
| 00 | $0.182963 \mathrm{E}+01$ | $0.888000 \mathrm{E}-02$ | $0.183082 \mathrm{E}+01$ | 0.1830 |
| 000 | $0.172425 \mathrm{E}+01$ | $0.896000 \mathrm{E}-02$ | $0.172062 \mathrm{E}+01$ | $0.172062 \mathrm{E}+01$ |
| 20000 | $0.162416 \mathrm{E}+01$ | $0.914000 \mathrm{E}-02$ | 0.161940 E | $0.161940 \mathrm{E}+01$ |
| $0.840000 \mathrm{E}-01$ | 0.153205 | $0.918000 \mathrm{E}-02$ | $0.152977 \mathrm{E}+01$ | $0.152977 \mathrm{E}+01$ |
| 60000E-01 | $0.144931 \mathrm{E}+01$ | $0.913000 \mathrm{E}-02$ | $0.145194 \mathrm{E}+01$ | $0.145194 \mathrm{E}+01$ |
| 0000 | $0.137636 \mathrm{E}+01$ | $0.883000 \mathrm{E}-02$ | $0.138286 \mathrm{E}+01$ | 0.138286E+01 |
| , | $0.131353 \mathrm{E}+01$ | $0.865000 \mathrm{E}-02$ | $0.131712 \mathrm{E}+01$ | $0.131712 \mathrm{E}+01$ |
| 920000E-01 | $0.125079 \mathrm{E}+01$ | $0.861000 \mathrm{E}-02$ | $0.125010 \mathrm{E}+01$ | $0.125010 \mathrm{E}+01$ |
| 940000E-01 | $0.118447 \mathrm{E}+01$ | $0.846000 \mathrm{E}-02$ | $0.117937 \mathrm{E}+01$ | $0.117937 \mathrm{E}+01$ |
| $960000 \mathrm{E}-01$ | $0.111765 \mathrm{E}+01$ | $0.832000 \mathrm{E}-02$ | $0.110683 \mathrm{E}+01$ | $0.110683 \mathrm{E}+01$ |
| $980000 \mathrm{E}-01$ | $0.104729 \mathrm{E}+01$ | $0.825000 \mathrm{E}-02$ | $0.103695 \mathrm{E}+01$ | $0.103695 \mathrm{E}+01$ |
| $100000 \mathrm{E}+00$ | $0.962610 \mathrm{E}+00$ | $0.620000 \mathrm{E}-02$ | $0.974361 \mathrm{E}+00$ | $0.974361 \mathrm{E}+00$ |
| $105000 \mathrm{E}+00$ | $0.861300 \mathrm{E}+00$ | $0.523000 \mathrm{E}-02$ | $0.856678 \mathrm{E}+00$ | $0.856678 \mathrm{E}+00$ |
| $0.110000 \mathrm{E}+00$ | $0.759450 \mathrm{E}+00$ | $0.496000 \mathrm{E}-02$ | $0.762293 \mathrm{E}+00$ | $0.762293 \mathrm{E}+00$ |
| .115000E+00 | $0.674530 \mathrm{E}+00$ | $0.479000 \mathrm{E}-02$ | $0.673479 \mathrm{E}+00$ | $0.673479 \mathrm{E}+0$ |

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0.120000E+00 0.605250E+00 0.466000E-02 0.604917E+00 0.604917E+00
0.125000E+00 0.539010E+00 0.452000E-02 0.539975E+00 0.539975E+00
0.130000E+00 0.473790E+00 0.444000E-02 0.472844E+00 0.472844E+00
0.135000E+00 0.415350E+00 0.437000E-02 0.415850E+00 0.415850E+00
0.140000E+00 0.364940E+00 0.442000E-02 0.364933E+00 0.364933E+00
0.145000E+00 0.323440E+00 0.459000E-02 0.323180E+00 0.323180E+00
0.150000E+00 0.290530E+00 0.124600E-01 0.292194E+00 0.292194E+00
0.155000E+00 0.259700E+00 0.107400E-01 0.259589E+00 0.259589E+00
0.160000E+00 0.229160E+00 0.966000E-02 0.228399E+00 0.228399E+00
0.165000E+00 0.204860E+00 0.912000E-02 0.205786E+00 0.205786E+00
0.170000E+00 0.186840E+00 0.885000E-02 0.186509E+00 0.186509E+00
0.175000E+00 0.169520E+00 0.853000E-02 0.169202E+00 0.169202E+00
0.180000E+00 0.153800E+00 0.810000E-02 0.154393E+00 0.154393E+00
0.185000E+00 0.142050E+00 0.785000E-02 0.141767E+00 0.141767E+00
0.190000E+00 0.133750E+00 0.762000E-02 0.133706E+00 0.133706E+00
0.195000E+00 0.126620E+00 0.742000E-02 0.126924E+00 0.126924E+00
0.200000E+00 0.119130E+00 0.718000E-02 0.118832E+00 0.118832E+00
0.205000E+00 0.113340E+00 0.715000E-02 0.113550E+00 0.113550E+00
0.210000E+00 0.110890E+00 0.699000E-02 0.110983E+00 0.110983E+00
0.215000E+00 0.108320E+00 0.686000E-02 0.108107E+00 0.108107E+00
0.220000E+00 0.104630E+00 0.673000E-02 0.104704E+00 0.104704E+00
0.225000E+00 0.101690E+00 0.669000E-02 0.101922E+00 0.101922E+00
0.230000E+00 0.102210E+00 0.653000E-02 0.102041E+00 0.102041E+00
0.235000E+00 0.102990E+00 0.664000E-02 0.102936E+00 0.102936E+00
0.240000E+00 0.996900E-01 0.668000E-02 0.997121E-01 0.997121E-01
0.245000E+00 0.953900E-01 0.673000E-02 0.952643E-01 0.952643E-01
0.250000E+00 0.935700E-01 0.667000E-02 0.939106E-01 0.939106E-01
0.255000E+00 0.932600E-01 0.656000E-02 0.929709E-01 0.929709E-01
0.260000E+00 0.902900E-01 0.656000E-02 0.902369E-01 0.902369E-01
0.265000E+00 0.869600E-01 0.656000E-02 0.871777E-01 0.871777E-01
0.270000E+00 0.852400E-01 0.647000E-02 0.852078E-01 0.852078E-01
0.275000E+00 0.847500E-01 0.640000E-02 0.847978E-01 0.847978E-01
0.280000E+00 0.848500E-01 0.660000E-02 0.848793E-01 0.848793E-01
0.285000E+00 0.842800E-01 0.703000E-02 0.840633E-01 0.840633E-01
0.290000E+00 0.823000E-01 0.733000E-02 0.822458E-01 0.822458E-01
0.295000E+00 0.797300E-01 0.758000E-02 0.799073E-01 0.799073E-01
0.300000E+00 0.781200E-01 0.802000E-02 0.781199E-01 0.781199E-01
0.305000E+00 0.773100E-01 0.854000E-02 0.773808E-01 0.773808E-01
0.310000E+00 0.766900E-01 0.920000E-02 0.768392E-01 0.768392E-01
0.315000E+00 0.765100E-01 0.991000E-02 0.764527E-01 0.764527E-01
0.320000E+00 0.766900E-01 0.108400E-01 0.763555E-01 0.763555E-01
0.325000E+00 0.754600E-01 0.119700E-01 0.754063E-01 0.754063E-01
0.330000E+00 0.736500E-01 0.173700E-01 0.736048E-01 0.736048E-01
0.335000E+00 0.727100E-01 0.382000E-02 0.728057E-01 0.728057E-01
0.340000E+00 0.735200E-01 0.378000E-02 0.735544E-01 0.735544E-01
```

| $0.345000 \mathrm{E}+00$ | $0.737500 \mathrm{E}-01$ | $0.371000 \mathrm{E}-02$ | $0.734592 \mathrm{E}-01$ | $0.734592 \mathrm{E}-01$ |
| :--- | :--- | :--- | :--- | :--- |
| $0.350000 \mathrm{E}+00$ | $0.704700 \mathrm{E}-01$ | $0.365000 \mathrm{E}-02$ | $0.704938 \mathrm{E}-01$ | $0.704938 \mathrm{E}-01$ |
| $0.355000 \mathrm{E}+00$ | $0.664400 \mathrm{E}-01$ | $0.364000 \mathrm{E}-02$ | $0.665799 \mathrm{E}-01$ | $0.665799 \mathrm{E}-01$ |
| $0.360000 \mathrm{E}+00$ | $0.647200 \mathrm{E}-01$ | $0.362000 \mathrm{E}-02$ | $0.647033 \mathrm{E}-01$ | $0.647033 \mathrm{E}-01$ |
| $0.365000 \mathrm{E}+00$ | $0.648300 \mathrm{E}-01$ | $0.363000 \mathrm{E}-02$ | $0.651518 \mathrm{E}-01$ | $0.651518 \mathrm{E}-01$ |
| $0.370000 \mathrm{E}+00$ | $0.670700 \mathrm{E}-01$ | $0.358000 \mathrm{E}-02$ | $0.668448 \mathrm{E}-01$ | $0.668448 \mathrm{E}-01$ |
| $0.375000 \mathrm{E}+00$ | $0.681900 \mathrm{E}-01$ | $0.353000 \mathrm{E}-02$ | $0.681388 \mathrm{E}-01$ | $0.681388 \mathrm{E}-01$ |
| $0.380000 \mathrm{E}+00$ | $0.675300 \mathrm{E}-01$ | $0.359000 \mathrm{E}-02$ | $0.675368 \mathrm{E}-01$ | $0.675368 \mathrm{E}-01$ |
| $0.385000 \mathrm{E}+00$ | $0.662100 \mathrm{E}-01$ | $0.356000 \mathrm{E}-02$ | $0.661105 \mathrm{E}-01$ | $0.661105 \mathrm{E}-01$ |
| $0.390000 \mathrm{E}+00$ | $0.653100 \mathrm{E}-01$ | $0.357000 \mathrm{E}-02$ | $0.655400 \mathrm{E}-01$ | $0.655400 \mathrm{E}-01$ |
| $0.395000 \mathrm{E}+00$ | $0.657700 \mathrm{E}-01$ | $0.352000 \mathrm{E}-02$ | $0.657752 \mathrm{E}-01$ | $0.657752 \mathrm{E}-01$ |
| $0.400000 \mathrm{E}+00$ | $0.669600 \mathrm{E}-01$ | $0.342000 \mathrm{E}-02$ | $0.669926 \mathrm{E}-01$ | $0.669926 \mathrm{E}-01$ |
| $0.405000 \mathrm{E}+00$ | $0.689000 \mathrm{E}-01$ | $0.325000 \mathrm{E}-02$ | $0.689227 \mathrm{E}-01$ | $0.689227 \mathrm{E}-01$ |
| $0.410000 \mathrm{E}+00$ | $0.699800 \mathrm{E}-01$ | $0.321000 \mathrm{E}-02$ | $0.697766 \mathrm{E}-01$ | $0.697766 \mathrm{E}-01$ |
| $0.415000 \mathrm{E}+00$ | $0.692700 \mathrm{E}-01$ | $0.314000 \mathrm{E}-02$ | $0.693029 \mathrm{E}-01$ | $0.693029 \mathrm{E}-01$ |
| $0.420000 \mathrm{E}+00$ | $0.687100 \mathrm{E}-01$ | $0.309000 \mathrm{E}-02$ | $0.687656 \mathrm{E}-01$ | $0.687656 \mathrm{E}-01$ |
| $0.425000 \mathrm{E}+00$ | $0.684500 \mathrm{E}-01$ | $0.303000 \mathrm{E}-02$ | $0.683805 \mathrm{E}-01$ | $0.683805 \mathrm{E}-01$ |
| $0.430000 \mathrm{E}+00$ | $0.680100 \mathrm{E}-01$ | $0.296000 \mathrm{E}-02$ | $0.680755 \mathrm{E}-01$ | $0.680755 \mathrm{E}-01$ |
| $0.435000 \mathrm{E}+00$ | $0.679300 \mathrm{E}-01$ | $0.295000 \mathrm{E}-02$ | $0.679001 \mathrm{E}-01$ | $0.679001 \mathrm{E}-01$ |
| $0.440000 \mathrm{E}+00$ | $0.674500 \mathrm{E}-01$ | $0.288000 \mathrm{E}-02$ | $0.673913 \mathrm{E}-01$ | $0.673913 \mathrm{E}-01$ |
| $0.445000 \mathrm{E}+00$ | $0.666500 \mathrm{E}-01$ | $0.288000 \mathrm{E}-02$ | $0.667495 \mathrm{E}-01$ | $0.667495 \mathrm{E}-01$ |
| $0.450000 \mathrm{E}+00$ | $0.666300 \mathrm{E}-01$ | $0.285000 \mathrm{E}-02$ | $0.666400 \mathrm{E}-01$ | $0.666400 \mathrm{E}-01$ |
| $0.455000 \mathrm{E}+00$ | $0.668500 \mathrm{E}-01$ | $0.283000 \mathrm{E}-02$ | $0.668058 \mathrm{E}-01$ | $0.668058 \mathrm{E}-01$ |
| $0.460000 \mathrm{E}+00$ | $0.668200 \mathrm{E}-01$ | $0.279000 \mathrm{E}-02$ | $0.669035 \mathrm{E}-01$ | $0.669035 \mathrm{E}-01$ |
| $0.465000 \mathrm{E}+00$ | $0.672000 \mathrm{E}-01$ | $0.275000 \mathrm{E}-02$ | $0.671491 \mathrm{E}-01$ | $0.671491 \mathrm{E}-01$ |
| $0.470000 \mathrm{E}+00$ | $0.675100 \mathrm{E}-01$ | $0.269000 \mathrm{E}-02$ | $0.675641 \mathrm{E}-01$ | $0.675641 \mathrm{E}-01$ |
| $0.475000 \mathrm{E}+00$ | $0.680800 \mathrm{E}-01$ | $0.267000 \mathrm{E}-02$ | $0.680652 \mathrm{E}-01$ | $0.680652 \mathrm{E}-01$ |
| $0.480000 \mathrm{E}+00$ | $0.685400 \mathrm{E}-01$ | $0.264000 \mathrm{E}-02$ | $0.684806 \mathrm{E}-01$ | $0.684806 \mathrm{E}-01$ |
| $0.485000 \mathrm{E}+00$ | $0.683800 \mathrm{E}-01$ | $0.261000 \mathrm{E}-02$ | $0.683650 \mathrm{E}-01$ | $0.683650 \mathrm{E}-01$ |
| $0.490000 \mathrm{E}+00$ | $0.679400 \mathrm{E}-01$ | $0.262000 \mathrm{E}-02$ | $0.679635 \mathrm{E}-01$ | $0.679635 \mathrm{E}-01$ |
| $0.495000 \mathrm{E}+00$ | $0.678600 \mathrm{E}-01$ | $0.260000 \mathrm{E}-02$ | $0.678487 \mathrm{E}-01$ | $0.678487 \mathrm{E}-01$ |
| $0.500000 \mathrm{E}+00$ | $0.675800 \mathrm{E}-01$ | $0.209000 \mathrm{E}-02$ | $0.673959 \mathrm{E}-01$ | $0.673959 \mathrm{E}-01$ |

\#\#\#\# Real Space Data
Distance distribution function of particle

## R $\quad \mathrm{P}(\mathrm{R}) \quad$ ERROR

$0.0000 \mathrm{E}+00 \quad 0.0000 \mathrm{E}+00 \quad 0.0000 \mathrm{E}+00$
$0.2987 \mathrm{E}+01 \quad 0.2732 \mathrm{E}-02 \quad 0.4704 \mathrm{E}-04$
$0.5973 \mathrm{E}+01 \quad 0.3005 \mathrm{E}-02 \quad 0.5752 \mathrm{E}-04$
$0.8960 \mathrm{E}+01 \quad 0.4327 \mathrm{E}-02 \quad 0.4132 \mathrm{E}-04$
$0.1195 \mathrm{E}+02 \quad 0.7616 \mathrm{E}-02 \quad 0.6676 \mathrm{E}-04$
$0.1493 \mathrm{E}+02 \quad 0.1101 \mathrm{E}-01 \quad 0.5699 \mathrm{E}-04$
$0.1792 \mathrm{E}+020.1309 \mathrm{E}-01 \quad 0.7479 \mathrm{E}-04$
$0.2091 \mathrm{E}+02 \quad 0.1451 \mathrm{E}-01 \quad 0.7083 \mathrm{E}-04$
$0.2389 \mathrm{E}+02 \quad 0.1623 \mathrm{E}-01 \quad 0.8801 \mathrm{E}-04$
$0.2688 \mathrm{E}+020.1785 \mathrm{E}-01 \quad 0.8253 \mathrm{E}-04$
$0.2987 \mathrm{E}+020.1847 \mathrm{E}-01 \quad 0.1044 \mathrm{E}-03$
$0.3285 \mathrm{E}+02 \quad 0.1826 \mathrm{E}-01 \quad 0.1283 \mathrm{E}-03$
$0.3584 \mathrm{E}+02 \quad 0.1812 \mathrm{E}-01 \quad 0.1246 \mathrm{E}-03$
$0.3883 \mathrm{E}+020.1829 \mathrm{E}-01 \quad 0.1330 \mathrm{E}-03$
$0.4181 \mathrm{E}+020.1833 \mathrm{E}-01 \quad 0.1233 \mathrm{E}-03$
$0.4480 \mathrm{E}+020.1812 \mathrm{E}-01 \quad 0.1565 \mathrm{E}-03$
$0.4779 \mathrm{E}+020.1803 \mathrm{E}-01 \quad 0.1440 \mathrm{E}-03$
$0.5077 \mathrm{E}+02 \quad 0.1818 \mathrm{E}-01 \quad 0.1645 \mathrm{E}-03$
$0.5376 \mathrm{E}+02 \quad 0.1818 \mathrm{E}-01 \quad 0.1791 \mathrm{E}-03$
$0.5675 \mathrm{E}+020.1792 \mathrm{E}-01 \quad 0.1948 \mathrm{E}-03$
$0.5973 \mathrm{E}+020.1772 \mathrm{E}-01 \quad 0.1752 \mathrm{E}-03$
$0.6272 \mathrm{E}+02 \quad 0.1774 \mathrm{E}-01 \quad 0.2197 \mathrm{E}-03$
$0.6571 \mathrm{E}+02 \quad 0.1757 \mathrm{E}-01 \quad 0.2092 \mathrm{E}-03$
$0.6869 \mathrm{E}+020.1697 \mathrm{E}-01 \quad 0.2293 \mathrm{E}-03$
$0.7168 \mathrm{E}+020.1638 \mathrm{E}-01 \quad 0.2366 \mathrm{E}-03$
$0.7467 \mathrm{E}+020.1629 \mathrm{E}-01 \quad 0.2348 \mathrm{E}-03$
$0.7765 \mathrm{E}+020.1648 \mathrm{E}-01 \quad 0.2441 \mathrm{E}-03$
$0.8064 \mathrm{E}+02 \quad 0.1641 \mathrm{E}-01 \quad 0.2957 \mathrm{E}-03$
$0.8363 \mathrm{E}+02 \quad 0.1602 \mathrm{E}-01 \quad 0.3011 \mathrm{E}-03$
$0.8661 \mathrm{E}+02 \quad 0.1574 \mathrm{E}-01 \quad 0.2800 \mathrm{E}-03$
$0.8960 \mathrm{E}+02 \quad 0.1579 \mathrm{E}-01 \quad 0.2921 \mathrm{E}-03$
$0.9259 \mathrm{E}+02 \quad 0.1589 \mathrm{E}-01 \quad 0.3190 \mathrm{E}-03$
$0.9557 \mathrm{E}+02 \quad 0.1577 \mathrm{E}-01 \quad 0.3117 \mathrm{E}-03$
$0.9856 \mathrm{E}+020.1557 \mathrm{E}-010.3049 \mathrm{E}-03$
$0.1015 \mathrm{E}+03 \quad 0.1550 \mathrm{E}-01 \quad 0.3483 \mathrm{E}-03$
$0.1045 \mathrm{E}+03 \quad 0.1550 \mathrm{E}-01 \quad 0.3667 \mathrm{E}-03$
$0.1075 \mathrm{E}+03 \quad 0.1544 \mathrm{E}-01 \quad 0.3996 \mathrm{E}-03$
$0.1105 \mathrm{E}+030.1533 \mathrm{E}-01 \quad 0.3947 \mathrm{E}-03$
$0.1135 \mathrm{E}+03 \quad 0.1527 \mathrm{E}-01 \quad 0.3660 \mathrm{E}-03$
$0.1165 \mathrm{E}+03 \quad 0.1521 \mathrm{E}-01 \quad 0.3187 \mathrm{E}-03$
$0.1195 \mathrm{E}+03 \quad 0.1502 \mathrm{E}-01 \quad 0.3864 \mathrm{E}-03$
$0.1225 \mathrm{E}+030.1472 \mathrm{E}-01 \quad 0.4607 \mathrm{E}-03$
$0.1254 \mathrm{E}+03 \quad 0.1446 \mathrm{E}-01 \quad 0.4603 \mathrm{E}-03$
$0.1284 \mathrm{E}+030.1432 \mathrm{E}-01 \quad 0.4780 \mathrm{E}-03$
$0.1314 \mathrm{E}+03 \quad 0.1428 \mathrm{E}-01 \quad 0.5110 \mathrm{E}-03$
$0.1344 \mathrm{E}+03 \quad 0.1428 \mathrm{E}-01 \quad 0.5128 \mathrm{E}-03$
$0.1374 \mathrm{E}+030.1425 \mathrm{E}-01 \quad 0.5108 \mathrm{E}-03$
$0.1404 \mathrm{E}+030.1408 \mathrm{E}-01 \quad 0.4600 \mathrm{E}-03$
$0.1434 \mathrm{E}+03 \quad 0.1379 \mathrm{E}-01 \quad 0.4325 \mathrm{E}-03$
$0.1463 \mathrm{E}+03 \quad 0.1358 \mathrm{E}-01 \quad 0.5284 \mathrm{E}-03$

```
0.1493E+03 0.1361E-01 0.5914E-03
0.1523E+03 0.1379E-01 0.5041E-03
0.1553E+03 0.1382E-01 0.4974E-03
0.1583E+03 0.1362E-01 0.5477E-03
0.1613E+03 0.1339E-01 0.5072E-03
0.1643E+03 0.1331E-01 0.5631E-03
0.1673E+03 0.1333E-01 0.5880E-03
0.1702E+03 0.1323E-01 0.5660E-03
0.1732E+03 0.1290E-01 0.6387E-03
0.1762E+03 0.1247E-01 0.6602E-03
0.1792E+03 0.1214E-01 0.6136E-03
0.1822E+03 0.1204E-01 0.5976E-03
0.1852E+03 0.1207E-01 0.6141E-03
0.1882E+03 0.1193E-01 0.6605E-03
0.1911E+03 0.1150E-01 0.6464E-03
0.1941E+03 0.1102E-01 0.6899E-03
0.1971E+03 0.1086E-01 0.7144E-03
0.2001E+03 0.1110E-01 0.6669E-03
0.2031E+03 0.1132E-01 0.7010E-03
0.2061E+03 0.1113E-01 0.7392E-03
0.2091E+03 0.1063E-01 0.6783E-03
0.2121E+03 0.1024E-01 0.6715E-03
0.2150E+03 0.1022E-01 0.7066E-03
0.2180E+03 0.1037E-01 0.7226E-03
0.2210E+03 0.1036E-01 0.7475E-03
0.2240E+03 0.1002E-01 0.7977E-03
0.2270E+03 0.9467E-02 0.8057E-03
0.2300E+03 0.8943E-02 0.7410E-03
0.2330E+03 0.8636E-02 0.7592E-03
0.2359E+03 0.8528E-02 0.7721E-03
0.2389E+03 0.8427E-02 0.7438E-03
0.2419E+03 0.8178E-02 0.7453E-03
0.2449E+03 0.7849E-02 0.7411E-03
0.2479E+03 0.7627E-02 0.7832E-03
0.2509E+03 0.7548E-02 0.8809E-03
0.2539E+03 0.7472E-02 0.8885E-03
0.2569E+03 0.7313E-02 0.8479E-03
0.2598E+03 0.7160E-02 0.8037E-03
0.2628E+03 0.7111E-02 0.8184E-03
0.2658E+03 0.7126E-02 0.8791E-03
0.2688E+03 0.7137E-02 0.8721E-03
0.2718E+03 0.7167E-02 0.8074E-03
0.2748E+03 0.7228E-02 0.9100E-03
0.2778E+03 0.7194E-02 0.9067E-03
0.2807E+03 0.6968E-02 0.7949E-03
```

$$
\begin{array}{lll}
0.2837 \mathrm{E}+03 & 0.6702 \mathrm{E}-02 & 0.9071 \mathrm{E}-03 \\
0.2867 \mathrm{E}+03 & 0.6668 \mathrm{E}-02 & 0.9317 \mathrm{E}-03 \\
0.2897 \mathrm{E}+03 & 0.6908 \mathrm{E}-02 & 0.8843 \mathrm{E}-03 \\
0.2927 \mathrm{E}+03 & 0.7176 \mathrm{E}-02 & 0.8349 \mathrm{E}-03 \\
0.2957 \mathrm{E}+03 & 0.7248 \mathrm{E}-02 & 0.8392 \mathrm{E}-03 \\
0.2987 \mathrm{E}+03 & 0.7148 \mathrm{E}-02 & 0.9364 \mathrm{E}-03 \\
0.3017 \mathrm{E}+03 & 0.6998 \mathrm{E}-02 & 0.9807 \mathrm{E}-03 \\
0.3046 \mathrm{E}+03 & 0.6823 \mathrm{E}-02 & 0.1018 \mathrm{E}-02 \\
0.3076 \mathrm{E}+03 & 0.6598 \mathrm{E}-02 & 0.9971 \mathrm{E}-03 \\
0.3106 \mathrm{E}+03 & 0.6372 \mathrm{E}-02 & 0.9025 \mathrm{E}-03 \\
0.3136 \mathrm{E}+03 & 0.6215 \mathrm{E}-02 & 0.8608 \mathrm{E}-03 \\
0.3166 \mathrm{E}+03 & 0.6104 \mathrm{E}-02 & 0.8094 \mathrm{E}-03 \\
0.3196 \mathrm{E}+03 & 0.5991 \mathrm{E}-02 & 0.8749 \mathrm{E}-03 \\
0.3226 \mathrm{E}+03 & 0.5915 \mathrm{E}-02 & 0.8685 \mathrm{E}-03 \\
0.3255 \mathrm{E}+03 & 0.5936 \mathrm{E}-02 & 0.8949 \mathrm{E}-03 \\
0.3285 \mathrm{E}+03 & 0.5995 \mathrm{E}-02 & 0.9964 \mathrm{E}-03 \\
0.3315 \mathrm{E}+03 & 0.5967 \mathrm{E}-02 & 0.9382 \mathrm{E}-03 \\
0.3345 \mathrm{E}+03 & 0.5837 \mathrm{E}-02 & 0.9333 \mathrm{E}-03 \\
0.3375 \mathrm{E}+03 & 0.5718 \mathrm{E}-02 & 0.9052 \mathrm{E}-03 \\
0.3405 \mathrm{E}+03 & 0.5677 \mathrm{E}-02 & 0.7964 \mathrm{E}-03 \\
0.3435 \mathrm{E}+03 & 0.5670 \mathrm{E}-02 & 0.8154 \mathrm{E}-03 \\
0.3465 \mathrm{E}+03 & 0.5651 \mathrm{E}-02 & 0.9287 \mathrm{E}-03 \\
0.3494 \mathrm{E}+03 & 0.5644 \mathrm{E}-02 & 0.9281 \mathrm{E}-03 \\
0.3524 \mathrm{E}+03 & 0.5639 \mathrm{E}-02 & 0.8682 \mathrm{E}-03 \\
0.3554 \mathrm{E}+03 & 0.5548 \mathrm{E}-02 & 0.9197 \mathrm{E}-03 \\
0.3584 \mathrm{E}+03 & 0.5323 \mathrm{E}-02 & 0.9234 \mathrm{E}-03 \\
0.3614 \mathrm{E}+03 & 0.5050 \mathrm{E}-02 & 0.8832 \mathrm{E}-03 \\
0.3644 \mathrm{E}+03 & 0.4842 \mathrm{E}-02 & 0.9924 \mathrm{E}-03 \\
0.3674 \mathrm{E}+03 & 0.4694 \mathrm{E}-02 & 0.1100 \mathrm{E}-02 \\
0.3703 \mathrm{E}+03 & 0.4518 \mathrm{E}-02 & 0.1133 \mathrm{E}-02 \\
0.3733 \mathrm{E}+03 & 0.4273 \mathrm{E}-02 & 0.9674 \mathrm{E}-03 \\
0.3763 \mathrm{E}+03 & 0.3984 \mathrm{E}-02 & 0.7957 \mathrm{E}-03 \\
0.3793 \mathrm{E}+03 & 0.3674 \mathrm{E}-02 & 0.9458 \mathrm{E}-03 \\
0.3823 \mathrm{E}+03 & 0.3357 \mathrm{E}-02 & 0.9325 \mathrm{E}-03 \\
0.3853 \mathrm{E}+03 & 0.3071 \mathrm{E}-02 & 0.9361 \mathrm{E}-03 \\
0.3883 \mathrm{E}+03 & 0.2838 \mathrm{E}-02 & 0.9928 \mathrm{E}-03 \\
0.3913 \mathrm{E}+03 & 0.2611 \mathrm{E}-02 & 0.9686 \mathrm{E}-03 \\
0.3942 \mathrm{E}+03 & 0.2345 \mathrm{E}-02 & 0.9576 \mathrm{E}-03 \\
0.3972 \mathrm{E}+03 & 0.2107 \mathrm{E}-02 & 0.8137 \mathrm{E}-03 \\
0.4002 \mathrm{E}+03 & 0.2000 \mathrm{E}-02 & 0.8048 \mathrm{E}-03 \\
0.4032 \mathrm{E}+03 & 0.1996 \mathrm{E}-02 & 0.7825 \mathrm{E}-03 \\
0.4062 \mathrm{E}+03 & 0.1946 \mathrm{E}-02 & 0.8745 \mathrm{E}-03 \\
0.4092 \mathrm{E}+03 & 0.1795 \mathrm{E}-02 & 0.9530 \mathrm{E}-03 \\
0.4122 \mathrm{E}+03 & 0.1659 \mathrm{E}-02 & 0.8651 \mathrm{E}-03 \\
0.4151 \mathrm{E}+03 & 0.1647 \mathrm{E}-02 & 0.8683 \mathrm{E}-03
\end{array}
$$

$0.4181 \mathrm{E}+030.1692 \mathrm{E}-02 \quad 0.9233 \mathrm{E}-03$
$0.4211 \mathrm{E}+030.1651 \mathrm{E}-020.8786 \mathrm{E}-03$
$0.4241 \mathrm{E}+03 \quad 0.1511 \mathrm{E}-02 \quad 0.8576 \mathrm{E}-03$
$0.4271 \mathrm{E}+03 \quad 0.1365 \mathrm{E}-02 \quad 0.9129 \mathrm{E}-03$
$0.4301 \mathrm{E}+03 \quad 0.1234 \mathrm{E}-02 \quad 0.8679 \mathrm{E}-03$
$0.4331 \mathrm{E}+03 \quad 0.1047 \mathrm{E}-02 \quad 0.8832 \mathrm{E}-03$
$0.4361 \mathrm{E}+03 \quad 0.7814 \mathrm{E}-03 \quad 0.9348 \mathrm{E}-03$
$0.4390 \mathrm{E}+03 \quad 0.5137 \mathrm{E}-03 \quad 0.1026 \mathrm{E}-02$
$0.4420 \mathrm{E}+030.2902 \mathrm{E}-03 \quad 0.9523 \mathrm{E}-03$
$0.4450 \mathrm{E}+03 \quad 0.8449 \mathrm{E}-04 \quad 0.9446 \mathrm{E}-03$
$0.4480 \mathrm{E}+030.0000 \mathrm{E}+000.0000 \mathrm{E}+00$

## APPENDIX 1.6: AB-INITIO FOR GASBOR MODEL RUN PARAMETERS.

$===$ GASBOR 2.3i (r12592) started on ..... 16-Sep-2020 12:31:02Computation mode: User
Project identifier ..... run_93
Project description:Initialized random seed as7259000901118836360
GNOM file name

$\qquad$
: fibrinogen_s1_0560-0580sDmax448.out
Data set title
Maximum diameter of the particle ..... 448.0
Radius of gyration ..... 135.7
Number of GNOM data points ..... 156
Maximum s value [1/angstrom] ..... 0.5000
Number of Shannon channels ..... 71.30
Reduced s maximum ..... : 0.4950
Reduced number of Shannon channels ..... 70.59
Number of knots in the curve to fit ..... 141
Point symmetry of the particle ..... P1
Number of equivalent positions ..... : 1
Total number of residues ..... : 2898
Packing radius of dummy atoms ..... : 1.900
Number of dummy waters ..... 2585
Excluded volume per residue ..... : 28.73
Radius of the search volume ..... : 224.0
Histogram penalty weight ..... : $1.000 \mathrm{E}-03$
Bond length penalty weight ..... $: 1.000 \mathrm{E}-02$
Discontiguity penalty weight ..... : 1.000E-02
Peripheral penalty weight ..... : 1.000
Expected particle anisometry ..... : Unknown
Contrast of the hydration layer ..... : 3.000E-02
Histogram penalty value ..... : 36.22
Bond length penalty value ..... : 0.1910
Initial DRM \# of graphs ..... : 2830
Discontiguity value ..... 6.873
Peripheral penalty value ..... : 0.3041
Weight: 0-2 = s^2, 3-5 = s, $6=\log$ ..... : 2
*** Accounting for constant background ..... ***
Initial scale factor ..... : 2.759E-08
Constant background subtracted ..... 4.922E-02
Initial R^2 factor ..... : 0.3042
Initial R factor ..... : 0.5515
Initial penalty ..... : 0.4109
Initial fVal ..... 0.7151
R-factor fixing threshold ..... : 0.0
Fixing threshold for PenCha ..... : 0.0
Fixing threshold for PenLen ..... : 0.0
Initial annealing temperature ..... : 1.000E-03
Annealing schedule factor ..... : 0.9000
\# of independent atoms to modify ..... : 1
Max \# of iterations at each T ..... : 265000
Max \# of successes at each T ..... : 26500
Min \# of successes to continue ..... : 265
Max \# of annealing steps ..... : 100

## APPENDIX 1.7: RIGID BODY CORAL MODEL RUN PARAMATERS

$===$ CORAL05 started at $\quad 06-$ Sep-2021 12:49:49
Computation mode $\qquad$ : Expert
Project description : config
Initialized random seed as : 5543010344313606652
File name with objects info
File name with objects info
File name with objects info
File name with objects info
File name with objects info
File name with objects info
File name with objects info
File name with objects info $\qquad$ : config.con
Coordinates of the 1 -st subunit evaluated from . $\qquad$ : fibA_cA_f2.pdb 616 atoms read, center at 203.83208 .86376 .04
Coordinates of the 2-nd subunit evaluated from $\qquad$ : fibA_cA_f3.pdb
832 atoms read, center at 216.28201 .69271 .70
Coordinates of the 3-rd subunit evaluated from ......... : fibA_cA_f4b.pdb 183 atoms read, center at 192.49193 .20286 .12
Coordinates of the 4-th subunit evaluated from $\qquad$ : fibA_cA_f5.pdb 1876 atoms read, center at $192.10 \quad 100.34294 .82$
Coordinates of the 5-th subunit evaluated from . $\qquad$ : fibB_cB_f2.pdb
577 atoms read, center at 207.66216 .15361 .81
Coordinates of the 6 -th subunit evaluated from . $\qquad$ : fibB_cB_f3.pdb
464 atoms read, center at 222.79200 .39266 .39
Coordinates of the 7-th subunit evaluated from $\qquad$ : fibB_cB_f4_v2.pdb
2003 atoms read, center at 200.91182 .27234 .80
Coordinates of the 8-th subunit evaluated from ......... : fibG_cC_f1.pdb
463 atoms read, center at 204.39213 .03363 .99
Coordinates of the 9-th subunit evaluated from $\qquad$ : fibG_cC_f2.pdb
517 atoms read, center at $222.17 \quad 207.15269 .82$
Coordinates of the 10 -th subunit evaluated from .. : fibG_cC_f3_v2.pdb
1940 atoms read, center at 217.20168 .01196 .79
Coordinates of the 11 -th subunit evaluated from $\qquad$ : fibA_cD_f2.pdb
616 atoms read, center at 192.15212 .27420 .85
Coordinates of the 12-th subunit evaluated from ..
832 atoms read, center at 180.24223 .61525 .82
Coordinates of the 13-th subunit evaluated from ........ : fibA_cD_f4b.pdb
183 atoms read, center at 202.74211 .77513 .40
Coordinates of the 14 -th subunit evaluated from $\qquad$ : fibA_cD_f5.pdb
1876 atoms read, center at 205.95119 .70500 .61
Coordinates of the 15 -th subunit evaluated from $\qquad$ : fibB_cE_f2.pdb
577 atoms read, center at 188.72221 .19434 .14
Coordinates of the 16 -th subunit evaluated from $\qquad$ : fibB_cE_f3.pdb

464 atoms read, center at 173.71222 .85530 .77
Coordinates of the 17 -th subunit evaluated from $\qquad$ : fibB_cE_f4_v2.pdb
2003 atoms read, center at 196.59216 .28566 .30
Coordinates of the 18-th subunit evaluated from ........ : fibG_cF_f1.pdb
463 atoms read, center at 192.10217 .84432 .23
Coordinates of the 19-th subunit evaluated from ........ : fibG_cF_f2.pdb
517 atoms read, center at 174.20229 .37525 .12
Coordinates of the 20 -th subunit evaluated from .. $\qquad$ : fibG_cF_f3_v2.pdb 1940 atoms read, center at 181.42213 .81607 .35
Subunit 1 was grouped with 5 and 8.
Subunit 2 was grouped with 6 and 9 .
Subunit 5 was grouped with 1 and 8.
Subunit 6 was grouped with 2 and 9 .
Subunit 7 was grouped with 10 .
Subunit 8 was grouped with 1 and 5 .
Subunit 9 was grouped with 2 and 6.
Subunit 10 was grouped with 7 .
Subunit 11 was grouped with 15 and 18.
Subunit 12 was grouped with 16 and 19.
Subunit 15 was grouped with 11 and 18.
Subunit 16 was grouped with 12 and 19.
Subunit 17 was grouped with 20.
Subunit 18 was grouped with 11 and 15.
Subunit 19 was grouped with 12 and 16.
Subunit 20 was grouped with 17.
Number of backbone atoms generated 2896
Averaged formfactors of DRs used
DR formfactor multiplier 1.200

Point symmetry of the particle ......................... : P1
Number of equivalent positions ......................... : 1
Cross penalty .......................................... : 7.795E-02
Cross penalty weight ................................... : 200.0
Shift penalty .......................................... : 11.55
Shift penalty weight ................................... : 10.00
Contacts conditions file name $\qquad$
Condition \# 1: Distance 10.000
Between chain \# 3, Residues from CYS 6 to CYS 6
and chain \# 6, Residues from CYS 6 to CYS 6
Contacts conditions penalty ............................ : 0.0
Contacts penalty weight ................................ : 10.00
Total number of scattering curves ...................... : 1
*** Accounting for constant background $* * *$
1 -st construct, the first and the last residues: 1 and 2896
File name, 1-st experimental data $\qquad$ : fibrinogen_s1_0560-0580s_edited1.dat
Number of experimental points found : 151
Experimental radius of gyration ..... : 116.4
Number of points in the Guinier Plot ..... : 0
Maximum s-vector in master grid ..... : 0.5000
Number of points in partial amplitudes ..... : 101
Maximum order of harmonics ..... : 14
Total penalty ..... : 131.1
1-st curve:
NEXP reduced to ..... : 150
Theoretical points from 1 to ..... 101 used
The best Chi^2 values: 9856.7Initial fVal: 9988.
Spatial step in angstroems ..... : 5.000
Angular step in degrees ..... : 20.00
Initial annealing temperature ..... : 20.00
Annealing schedule factor ..... : 0.9500
Max \# of iterations at each T ..... : 125500
Max \# of successes at each T ..... : 12550
Min \# of successes to continue ..... 125
Max \# of annealing steps ..... : 100

## APPENDIX 1.8: ANGLES VALUES BETWEEN RIGID BODY DOMAINS CALCULATED USING PYMOL FROM CORAL MODEL RUNS.

All the displayed angles were calculated via PyMOL. Angles were calculated based on the displacement between defined rigid bodies from CORAL modelling. Classification for each angle column goes as follows: A) E and D region coiled-coils 1, B) D-region and $\alpha$-C domain 1, C) E and D region coiled-coils 2, D) D-region and $\alpha$-C domain 2, E) E-region and E-region, F) D-Coil and $\beta \gamma$ fixed domain 1, G) D-Coil and $\beta \gamma$ fixed domain 2. These angles were used in order the calculate their distribution patterns via MATLAB by Angles_between_domains script.

| Coral <br> Runs | $\mathbf{A}$ | $\mathbf{B}$ | $\mathbf{C}$ | $\mathbf{D}$ | $\mathbf{E}$ | $\mathbf{F}$ | $\mathbf{G}$ |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 1 | 103.57 | 169.25 | 148.69 | 109.05 | 177.87 | 144.64 | 124.65 |
| 2 | 162.24 | 140.52 | 97.3 | 75.74 | 178.96 | 133.12 | 109.94 |
| 3 | 170.2 | 177.76 | 144.1 | 88.97 | 178.9 | 114.97 | 157.5 |
| 4 | 177.13 | 162.6 | 159.22 | 136.47 | 143.63 | 105.39 | 117.53 |
| 5 | 85.02 | 82.24 | 137.82 | 137.37 | 158.9 | 94.12 | 96.57 |
| 6 | 156.24 | 130.6 | 126.09 | 128.79 | 162.17 | 142.76 | 105.65 |
| 7 | 162.54 | 171.26 | 107.11 | 104.12 | 157.8 | 171.34 | 141.01 |
| 8 | 102.4 | 167.37 | 175.16 | 166.77 | 175.27 | 117.8 | 155.85 |
| 9 | 118.91 | 162.67 | 142.82 | 87.64 | 172.18 | 107.7 | 144.8 |
| 10 | 177.53 | 91.63 | 151.44 | 65.8 | 178.54 | 91.98 | 141.31 |
| 11 | 172.65 | 152.76 | 168.01 | 99.59 | 169.56 | 138.68 | 150.17 |
| 12 | 140.94 | 144.67 | 125.61 | 65.45 | 179.79 | 118.57 | 142.66 |
| 13 | 126.05 | 93.32 | 170.46 | 163.02 | 167.28 | 143.7 | 114.14 |
| 14 | 155.39 | 140.99 | 121.71 | 115.08 | 118.99 | 158.63 | 99.57 |
| 15 | 112.54 | 121.73 | 178.03 | 173.29 | 163.14 | 120.36 | 101.23 |
| 16 | 128.39 | 111.22 | 160.02 | 37.48 | 170.41 | 174.68 | 102.42 |
| 17 | 139.41 | 87.13 | 163.37 | 120.44 | 145.9 | 106.99 | 113.46 |
| 18 | 110.58 | 118.69 | 121.64 | 150.25 | 136.79 | 172.05 | 120.05 |
| 19 | 118.65 | 148.63 | 170.1 | 149.48 | 169.15 | 136.49 | 138.78 |
| 20 | 172.19 | 157.32 | 95.31 | 145.72 | 178.48 | 146.01 | 121.71 |
| 21 | 80.16 | 170.97 | 165.29 | 175.54 | 129.74 | 150.76 | 127.65 |
| 22 | 124.95 | 176.59 | 140.2 | 48.04 | 168.91 | 76.94 | 140.64 |
| 23 | 137.08 | 75.09 | 164.98 | 175.03 | 169.36 | 109.57 | 163.68 |
| 24 | 170.13 | 111.04 | 140.1 | 179.82 | 126.14 | 119.66 | 175.64 |
| 25 | 161.01 | 67.5 | 141.54 | 113.68 | 176.53 | 176.71 | 59.56 |
| 26 | 87.4 | 130.77 | 106.41 | 157.36 | 174.63 | 113.88 | 77.66 |
| 27 | 150.08 | 76.72 | 173.59 | 89.54 | 159.87 | 134.16 | 151.13 |
| 28 | 155.36 | 102 | 134.04 | 63.62 | 172.99 | 115.28 | 108.58 |
| 29 | 89.3 | 159.39 | 168.17 | 161.65 | 179.83 | 173.3 | 117.52 |


| 30 | 119.31 | 104.27 | 159.98 | 55.54 | 142.28 | 158.11 | 109.39 |
| :--- | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 31 | 141.03 | 163.28 | 177.95 | 148.76 | 156.43 | 106.71 | 145.56 |
| 32 | 176.27 | 109.31 | 168.48 | 115.46 | 176.55 | 111.3 | 122.38 |
| 33 | 140.49 | 161.63 | 98.5 | 125.29 | 157.14 | 170.93 | 136.82 |
| 34 | 65.48 | 154.24 | 172.89 | 179.45 | 172.5 | 136.97 | 107.64 |
| 35 | 176.56 | 161.64 | 134.67 | 64.14 | 178.92 | 95.27 | 140.96 |
| 36 | 145.55 | 151.73 | 169.92 | 122.28 | 160.33 | 106.17 | 138.69 |
| 37 | 115.98 | 101.73 | 178.76 | 42.82 | 166.22 | 100.02 | 107.43 |
| 38 | 165.79 | 174.23 | 130.68 | 92.22 | 143.78 | 92.02 | 128.44 |
| 39 | 124.7 | 149.81 | 148.54 | 174.22 | 177.28 | 152.79 | 118.98 |
| 40 | 55.75 | 178.84 | 115.42 | 121.65 | 156.72 | 125.35 | 77.48 |
| 41 | 93.76 | 73.21 | 123.93 | 171.27 | 121.99 | 130.79 | 58.65 |
| 42 | 160.53 | 84.23 | 118.88 | 174.23 | 178.32 | 168.55 | 101.22 |
| 43 | 95.81 | 160.36 | 113.38 | 106.06 | 149.58 | 117.6 | 126.33 |
| 44 | 173.02 | 166.96 | 144.75 | 153.77 | 172.39 | 113.78 | 120.55 |
| 45 | 171.29 | 95.44 | 169.5 | 73.3 | 170.67 | 131.06 | 107.57 |
| 46 | 151.48 | 166.34 | 148.47 | 170.91 | 179.77 | 136.06 | 132.5 |
| 47 | 176.51 | 131.94 | 106.73 | 134.12 | 173.9 | 160.89 | 113.18 |
| 48 | 128.13 | 94.96 | 146.8 | 179.9 | 175.62 | 136.69 | 120.5 |
| 49 | 178.08 | 117.77 | 176.63 | 124.54 | 177.66 | 114.97 | 126.26 |
| 50 | 132.22 | 106.42 | 146.8 | 176.92 | 178.66 | 110.22 | 88.08 |
| 51 | 119.42 | 111.36 | 154.21 | 114.38 | 168.61 | 107.38 | 124.05 |
| 52 | 173.54 | 125.66 | 174.01 | 125.73 | 166.5 | 103.09 | 54.47 |
| 53 | 90.74 | 142.6 | 91.05 | 96.22 | 178.29 | 112.12 | 106.19 |
| 54 | 105.87 | 164.93 | 105 | 173.46 | 161.59 | 133.93 | 142.9 |
| 55 | 177.35 | 72.42 | 133.39 | 143.4 | 114.99 | 136.88 | 121.7 |
| 56 | 175.25 | 151.78 | 40.42 | 172.55 | 152.44 | 120.9 | 159.99 |
| 57 | 176.35 | 136.77 | 138.74 | 106.06 | 158.36 | 106.16 | 84.06 |
| 58 | 57.67 | 173.8 | 127.05 | 95.02 | 161.27 | 87.06 | 132.88 |
| 59 | 174.55 | 139.18 | 112.24 | 158.23 | 162.85 | 109.76 | 129.81 |
| 60 | 135.09 | 172.64 | 156.58 | 130.86 | 168.01 | 65.14 | 98.78 |
| 61 | 170.35 | 161.47 | 145.86 | 50.72 | 167.99 | 103.61 | 139.64 |
| 62 | 105.52 | 63.12 | 165.7 | 149.57 | 168.04 | 96.44 | 87.82 |
| 63 | 161.99 | 61.66 | 152.21 | 144.63 | 163.38 | 133.05 | 156.44 |
| 64 | 96.73 | 161.08 | 112.18 | 161.28 | 162.86 | 118.03 | 167.06 |
| 65 | 126.29 | 57.54 | 116.96 | 117.96 | 141.78 | 70.44 | 140.86 |
| 66 | 141.96 | 49.24 | 163.82 | 178.91 | 155.69 | 158.95 | 169.99 |
| 67 | 123.27 | 97.44 | 95.76 | 87.04 | 172.82 | 111.79 | 78.52 |
| 68 | 120.15 | 153.76 | 161.94 | 162.87 | 171.97 | 173.66 | 128.46 |
|  |  |  |  |  |  |  |  |
| 38 |  |  |  |  |  |  |  |
| 36 |  |  |  |  |  |  |  |


| 69 | 83.43 | 172.02 | 90.99 | 148.95 | 170.99 | 98.52 | 130.85 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 70 | 110.44 | 147.66 | 173.95 | 157.42 | 175.13 | 110.65 | 116.08 |
| 71 | 151.36 | 125.79 | 120.4 | 123.75 | 170.82 | 104.88 | 123 |
| 72 | 177.34 | 101.98 | 145.17 | 167.7 | 158.62 | 90.2 | 97.91 |
| 73 | 137.27 | 128.42 | 107.79 | 175.51 | 159.57 | 131.37 | 164.08 |
| 74 | 117.79 | 165.1 | 172.02 | 45.19 | 172.96 | 146.35 | 115.42 |
| 75 | 123.49 | 153.47 | 103.21 | 92.46 | 118.62 | 150.52 | 72.22 |
| 76 | 167.51 | 114.56 | 170.53 | 105.29 | 143.4 | 122.26 | 104.08 |
| 77 | 132.37 | 177.68 | 151.8 | 114.5 | 131.09 | 89.44 | 88.97 |
| 78 | 39.04 | 168.18 | 155.22 | 175.43 | 156.42 | 132.21 | 153.85 |
| 79 | 178.49 | 86.09 | 125.15 | 121.7 | 131.61 | 175.54 | 114.14 |
| 80 | 147.23 | 155.36 | 162.94 | 144.65 | 151.68 | 124.1 | 134.25 |
| 81 | 141.54 | 119.89 | 149.43 | 134.27 | 124.26 | 124.49 | 89.27 |
| 82 | 178.64 | 82.52 | 143.75 | 132.59 | 171.25 | 97.21 | 97.8 |
| 83 | 179.73 | 176.46 | 162.34 | 147.63 | 173.6 | 132.3 | 148.88 |
| 84 | 139.57 | 143.44 | 103.16 | 120.44 | 170.19 | 117.45 | 163.98 |
| 85 | 131.96 | 158.63 | 132.22 | 161.79 | 154.96 | 176.48 | 114.85 |
| 86 | 153.27 | 120.09 | 134.59 | 171.77 | 170.31 | 141.88 | 108.98 |
| 87 | 138.14 | 146.03 | 89.65 | 65.8 | 164.48 | 125.81 | 165.98 |
| 88 | 153.48 | 116.65 | 160.78 | 164.78 | 163.5 | 132.03 | 124.83 |
| 89 | 131.01 | 179.15 | 148.41 | 105.39 | 165.99 | 161.61 | 151.41 |
| 90 | 129.31 | 58.05 | 173.45 | 127.34 | 175.08 | 127.29 | 147.77 |
| 91 | 165.5 | 165.29 | 147.21 | 153.03 | 179.34 | 100.06 | 133.21 |
| 92 | 136.85 | 171.27 | 173.95 | 131.28 | 175.3 | 133.26 | 111.66 |
| 93 | 121.64 | 136.84 | 46 | 161.47 | 167.89 | 168.12 | 139.05 |
| 94 | 134.08 | 111.39 | 169.77 | 77.41 | 158.34 | 141.23 | 66.28 |
| 95 | 111.49 | 156.53 | 169.65 | 94.78 | 171.03 | 126.61 | 164.44 |
| 96 | 163.23 | 172.45 | 162.86 | 142.34 | 141.44 | 124.15 | 125.1 |
| 97 | 164.24 | 172.82 | 154.32 | 176.97 | 176.77 | 96.52 | 134.31 |
| 98 | 147.52 | 53.35 | 170.72 | 165.08 | 170.95 | 141.02 | 140.42 |
| 99 | 163.61 | 127.86 | 160.1 | 167.72 | 164.96 | 148.87 | 110.62 |
| 100 | 137.27 | 146.59 | 105.69 | 120.44 | 173.46 | 138.84 | 103.16 |
| 101 | 125.19 | 160.96 | 131.44 | 124.14 | 177.5 | 176.83 | 148.18 |
|  |  |  |  |  |  |  |  |

# APPENDIX 1.9: MATLAB ANGLE_BETWEEN_DOMAINS SCRIPT. HISTOGRAM DISTRIBUTION FOR CORAL 100 MODELS 

```
table=readtable('coral_m3_angles_v3.xlsx');
    %%% Isolating data %%%
% E_coil to E_coil
N_terminus_and_N_teminus=table(:,14);
%D_coil to alpha C
C_terminus_and_alpha_C_domain=table(:,5);
C_terminus_and_alpha_C_domain1=table(:,10);
%E coil to D coil
N_terminus_and_C_terminus=table(:,4);
N_terminus_and_C_terminus1=table(:,9);
%Beta alpha to pdules coiled
D_Coil_fixed_domain1= table(:,16);
D_Coil_fixed_domain2= table(:,17);
```

$\% \% \%$ converting to vector $\% \% \%$
\%E_coil to E_coil
N_terminus_and_N_teminus=N_terminus_and_N_teminus $\{:,:\} ;$
N_terminus_and_N_teminus_makeup $=360-\mathrm{N} \_$terminus_and_N_teminus;
N_terminus_and_N_teminus_total=sort([N_terminus_and_N_teminus;N_terminus_and_N_te
minus_makeup]);
\%D_coil to alpha C
C_terminus_and_alpha_C_domain=C_terminus_and_alpha_C_domain $\{:,:\} ;$
C_terminus_and_alpha_C_domain1=C_terminus_and_alpha_C_domain $1\{:,:\} ;$
D_coil_to_alpha_C=[C_terminus_and_alpha_C_domain;C_terminus_and_alpha_C_domain1]
;
D_coil_to_alpha_C_makeup= 360-D_coil_to_alpha_C;
D_coil_to_alpha_C_total=[D_coil_to_alpha_C;D_coil_to_alpha_C_makeup];
\% E coil to D coil
N_terminus_and_C_terminus=N_terminus_and_C_terminus $\{:,:\} ;$
N_terminus_and_C_terminus1=N_terminus_and_C_terminus1 $\{:,:\} ;$
E_coil_to_D_coil=[N_terminus_and_C_terminus;N_terminus_and_C_terminus1];
E_coil_to_D_coil_makeup= 360-E_coil_to_D_coil;
E_coil_to_D_coil_total=[E_coil_to_D_coil;E_coil_to_D_coil_makeup];
\%Beta alpha to pdules coiled

D_Coil_fixed_domain1= D_Coil_fixed_domain1\{:,:\};
D_Coil_fixed_domain2=D_Coil_fixed_domain2\{:,:\};
Beta_alpha_to_Pdules_coiled=[D_Coil_fixed_domain1;D_Coil_fixed_domain2];

```
figure(1)
h=histogram(N_terminus_and_N_teminus,'FaceColor','#0072BD');
alpha(.80);
h.BinWidth=3;
hold on
x1=(100:1:180);
%pd1=fitgmdist(N_terminus_and_N_teminus_total,3);
n1=makedist('Normal',178.0314160226039,sqrt(191.6944));
n2=makedist('Normal',132.2285131250540,sqrt(124.5796));
y=700*(0.727069575988753*pdf(n1,x1)+0.074810405723011*pdf(n2,x1));
plot(x1,y,'LineWidth',5)
plot(x1,700*0.727069575988753*pdf(n1,x1),'LineWidth',5)
plot(x1,700*0.074810405723011*pdf(n2,x1),'LineWidth',5)
title('E-region to E-region')
xlabel('angle')
ylabel('recurrent')
legend('Location','northwest');
mu=mean(N_terminus_and_N_teminus);
mi=std(N_terminus_and_N_teminus);
mylegend = 'Mean: ' + string(mu) + ' Std: ??' + string(mi);
legend(mylegend);
hold off
```

figure (2)
h2=histogram(D_coil_to_alpha_C,'FaceColor','\#0072BD');
alpha(.80);
h2.BinWidth=5;
hold on
n1=makedist('Normal',180,sqrt(914.6532));
n2=makedist('Normal',101.2664180546399,sqrt(942.4305));

```
x2=(35:1:180);
y2=1500*(0.541474149994141*pdf(n1,x2)+0.235405468003058*pdf(n2,x2));
```

plot(x2,y2,'LineWidth',5)
plot(x2,1500*0.541474149994141*pdf(n1,x2),'LineWidth',5)
$\operatorname{plot}\left(\mathrm{x} 2,1500 * 0.235405468003058 * \operatorname{pdf}(\mathrm{n} 2, \mathrm{x} 2),{ }^{2}\right.$ 'LineWidth',5)
title('D-region to ??C domain')
xlabel('Angle')
ylabel('Recurrence')
legend('Location','northwest');
mu=mean(D_coil_to_alpha_C);
mi=std(D_coil_to_alpha_C);
mylegend = 'Mean: ' $+\operatorname{string}(\mathrm{mu})+$ ' Std: ??' $+\operatorname{string}(\mathrm{mi})$;
legend(mylegend);
hold off
figure (3)
h3=histogram(E_coil_to_D_coil,'FaceColor','\#0072BD');
alpha(.80);
h3.BinWidth=3;
hold on
\%pd3=fitgmdist(E_coil_to_D_coil_total,5);
n1 = makedist('Normal',177.09987667524514,sqrt(1582.8));
n2=makedist('Normal',167.0512078349467,sqrt(1654.6));
\%n3=makedist('Normal',pd3.mu(3),sqrt(pd3.Sigma(3)));
n4=makedist('Normal',110.5293378651434,sqrt(1399));
\%n5=makedist('Normal',pd3.mu(5),sqrt(pd3.Sigma(5)));
\%n6=makedist('Normal',pd3.mu(6),sqrt(pd3.Sigma(6)));
\%n7=makedist('Normal',pd3.mu(7),sqrt(pd3.Sigma(7)));
\% $\mathrm{p}=\mathrm{pd} 3$.ComponentProportion;
x3=(39:1:180);
$\mathrm{y} 3=1500 *(0.300742305341801 * \operatorname{pdf}(\mathrm{n} 1, \mathrm{x} 3)+0.258639052312525 * \operatorname{pdf}(\mathrm{n} 2, \mathrm{x} 3)+0.03984187148$
4796*pdf(n4,x3));

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