Classical Transport in Disordered Systems

Antonios Papaioannou

Graduate Center, City University of New York

How does access to this work benefit you? Let us know!

Follow this and additional works at: http://academicworks.cuny.edu/gc_etds

Part of the Biological and Chemical Physics Commons, Condensed Matter Physics Commons, and the Statistical, Nonlinear, and Soft Matter Physics Commons

Recommended Citation

http://academicworks.cuny.edu/gc_etds/1266

This Dissertation is brought to you by CUNY Academic Works. It has been accepted for inclusion in All Graduate Works by Year: Dissertations, Theses, and Capstone Projects by an authorized administrator of CUNY Academic Works. For more information, please contact deposit@gc.cuny.edu.
Classical transport in disordered systems

by

Antonios Papaioannou

A dissertation submitted to the Graduate Faculty in Physics in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York-The Graduate Center.

2016
This manuscript has been read and accepted for the Graduate Faculty in Physics in satisfaction of the dissertation requirements for the degree of Doctor of Philosophy.

(required signature)

Date Chair of Examining Committee

(required signature)

Date Executive Officer

Dmitry S. Novikov

Els Fieremans

Vadim Oganesyan

Ruth E. Stark

Supervisory Committee

THE CITY UNIVERSITY OF NEW YORK
Abstract

Classical transport in disordered systems

by

Antonios Papaioannou

Advisor: Gregory S. Boutis

This thesis reports on the manifestation of structural disorder on molecular transport and it consists of two parts.

Part I discusses the relations between classical transport and the underlying structural complexity of the system. Both types of molecular diffusion, namely Gaussian and non-Gaussian are presented and the relevant time regimes are discussed. In addition the concept of structural universality is introduced and connected with the diffusion metrics.

One of the most robust techniques for measuring molecular mean square displacements is magnetic resonance. This method requires encoding and subsequently reading out after an experimentally controlled time, a phase $\phi$ to the spins using magnetic field gradients. The main limitation for probing short diffusion lengths $L(t) \sim 1\mu m$ with magnetic resonance is the requirement to encode and decode the phase $\phi$ in very short time intervals. Therefore, to probe such displacements a special probe was developed equipped with a gradient coil capable of delivering magnetic field gradients of approximately 90 $G/cmA$. The design of
the probe is reported.

Part I also includes a discussion of experiments of transport in two qualitatively different disordered phantoms and reports on a direct observation of universality in one-dimension. The results reveal the universal power law scaling of the diffusion coefficient at the long-time regime and illustrate the essence of structural universality by experimentally determining the structure correlation function of the phantoms. In addition, the scaling of the diffusive permeability of the phantoms with respect to the pore size is investigated. Additional work presented includes a detailed study of adsorption of methane gas in Vycor disordered glass.

The techniques described in Part I of this thesis are widely used for measuring structural parameters of porous media, such as the surface-to-volume ratio or diffusive permeability. Part II of this thesis discusses the biophysical application of diffusion in disordered systems in the field of bioengineering. Elastin-based bioengineered scaffolds, which are mainly used for tissue and bone regeneration, must be able to deliver nutrients to the native tissue. It is therefore essential to quantitatively assess their structural parameters such as their surface-to-volume ratio and diffusive permeability. Part II focuses on a detailed study of structure and dynamics of elastin, the principle protein component found in tissues and one of the main components for scaffold engineering, using NMR $^{13}$C-MAS techniques. Lastly, the second half of Part II, discusses preliminary experiments of diffusion in elastin-based films.
Acknowledgements

The work I have done the past five years would not be possible without the advice, collaboration and support of the people I am gladly mentioning here.

First and foremost I want to thank my research advisor, Professor Gregory S. Boutis, who has provided me with guidance and support from day one until the last day of my studies. Greg taught me the experimental tricks of making a good measurement and data interpretation. He has also provided me with great encouragement in the lab when times were tough and the experiment wasn’t working.

Second, I would like to thank my collaborators. Dmitry S. Novikov has provided me with great understanding of the theory of transport in disordered media through many discussions. Els Fieremans always had great ideas regarding phantom design and experimental possibilities. Special thanks to Vadim Oganesyan for his guidance regarding new, exciting future projects and the next steps in my scientific career.

Last but not least, I would like to thank the people at Schlumberger Doll Research, Ravinath Kausik, Yi-Qiao Song, and Martin Hurlimann, for their special guidance during the summer of 2013. My summer in Cambridge was a blast!

Third, I would like to thank my lab mates and colleagues. I would not have survived
grad school without them.

My professors from Greece, Dimitris Emfietzoglou and Loukas Astrakas, who urged me to move to the US. Steven W. Morgan, a great friend and scientist, with whom I shared an office for nearly five years, helped me understand the basic physics of NMR (from the FID to spin-diffusion). Basant Dhital and Farhana Gul-E-Noor are great friends with whom I shared endless discussions regarding my research, during conferences and lunch breaks. Also, I would like to thank my theory-friends from the Graduate Center of CUNY, Yonatan Ben-Benjamin, George Poppe, Steven Vayl, Xing Su, Ryan Abrahams and Arthur Parzygnat. Special thanks to George and Ben-Ben with whom I explored the fascinating field of complex network theory during endless weekends working “quietly” at the GC while being on one of the busiest streets in New York City, across the street from the Empire State Building. I will never forget how “$1 pizza makes you smarter!”; unfortunately that place closed down. My first roommates, Agis Mesolongitis and Fay Rantou with whom I shared one cool year living in Brooklyn getting free brunch at Tom’s diner almost every weekend. I will never forget the owner, Mr. Gas. My Greek friends Vasilios Deligiannakis and Marios Gewrgiou with whom I shared great adventures exploring the Northeast. Stelios Tamouridis, who, during my last year in grad school, urged me to resume my love of exploring the outdoors through mountain biking. Last, but not least, my childhood friends back in Greece who supported me in their own way since we were kids.
For my parents, Nikos and Polyxeni,

my brother, Stefanos,

and life partner and friend Sofia,

who made me happy all those years and always believed in me.
"It is not the mountain we conquer but ourselves."

- Edmund Hillary
Contents

List of Figures xiii

List of Tables xv

I Diffusion in disordered systems 1

1 Introduction 2

2 Gaussian diffusion 5
   2.1 Current conservation and Fick's law 5
   2.2 Gaussian diffusion propagator 6

3 Non-Gaussian diffusion and universality classes 8
   3.1 Modified diffusion equation and the double average 9
   3.2 Disordered averaged diffusion propagator and the self energy part 11
   3.3 Double average: An illustrative example 12
   3.4 The short-time regime 13
   3.5 The long-time regime 14
## CONTENTS

3.5.1 Structure correlation function and disorder universality classes  
3.5.2 Diffusion and the dynamical exponent  
3.6 Diffusive permeability

4 Diffusion NMR and experimental setup

4.1 The cumulant expansion for Gaussian diffusion  
4.2 Gradient Echo: An illustrative example  
4.3 Gradient coil design

5 Observation of universality with diffusion

5.1 Phantom construction and experimental methodology  
5.1.1 Short-range disordered phantom  
5.1.2 Hyperuniform disordered phantom  
5.1.3 Diffusion NMR methodology  
5.2 Experimental results  
5.2.1 Structure correlation function and the dynamical exponent  
5.2.2 Universal power law scaling of the diffusion coefficient  
5.2.3 Diffusive permeability of a single barrier

6 Gas storage in disordered systems

6.1 High-Pressure Experimental Setup  
6.2 Experimental results  
6.2.1 Relaxation of free and restricted methane gas  
6.2.2 Monolayer and multilayer gas adsorption in disordered systems
## CONTENTS

<table>
<thead>
<tr>
<th>II</th>
<th>Elastin-based biomaterials</th>
<th>81</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>Introduction</td>
<td>82</td>
</tr>
<tr>
<td>8</td>
<td>$^{13}$C MAS studies of elastin upon purification</td>
<td>87</td>
</tr>
<tr>
<td>8.1</td>
<td>Sample Preparation</td>
<td>88</td>
</tr>
<tr>
<td>8.2</td>
<td>Results and Discussion</td>
<td>92</td>
</tr>
<tr>
<td>8.2.1</td>
<td>Dynamical and structural characteristics of elastin</td>
<td>94</td>
</tr>
<tr>
<td>8.2.2</td>
<td>$^{13}$C-$^1$H Rotational correlation times $\tau_c$</td>
<td>102</td>
</tr>
<tr>
<td>8.3</td>
<td>Conclusions</td>
<td>105</td>
</tr>
<tr>
<td>9</td>
<td>Diffusion in elastin-based biomaterials</td>
<td>114</td>
</tr>
<tr>
<td>10</td>
<td>Conclusion</td>
<td>119</td>
</tr>
<tr>
<td>Bibliography</td>
<td>121</td>
<td></td>
</tr>
</tbody>
</table>
List of Figures

3.1 A two dimensional example of the double average ................................. 13
3.2 One dimensional disorder classes and structure correlation function .......... 16
3.3 Instantaneous diffusion coefficient for each disorder class ........................ 18
4.1 Gradient echo pulse sequence: An illustrative example ............................ 24
4.2 Maxwell pair ...................................................................................... 26
4.3 Gradient set ....................................................................................... 27
4.4 Photograph of the constructed gradient coil ............................................ 28
4.5 Biot Savart’s solution for the gradient set .............................................. 29
5.1 Hyperuniform phantom cartoon representation ..................................... 32
5.2 NMR pulse sequences for diffusion measurements .................................. 34
5.3 Structural characterization of the phantoms ............................................ 44
5.4 Short-time regime diffusion measurements in the phantoms ..................... 45
5.5 Long-time regime diffusion measurements in the phantoms ..................... 46
5.6 Statistics for the power law least squares fit ($\chi^2$, $\vartheta$) ....................... 47
5.7 Statistics for the power law least squares fit (polynomial order, SG window) 48
LIST OF FIGURES

5.8 Diffusive permeability with respect to pore size ........................................ 49

6.1 Gas NMR experimental setup ................................................................. 53

6.2 $T_1 - T_2$ correlation pulse sequence ..................................................... 54

6.3 $T_1$ and $T_2$ relaxation times of bulk methane with respect to pressure ...... 56

6.4 $T_1$ relaxation times of bulk methane gas at various densities ................. 59

6.5 $T_1 - T_2$ correlation map for methane gas in Vycor glass ...................... 62

6.6 $T_2$ of methane gas in Vycor glass ......................................................... 64

6.7 $T_2 - T_2$ correlation maps of methane in Vycor glass ............................. 66

6.8 Methane gas stored in Vycor glass plotted as a function of pressure .......... 68

6.9 Effective Hydrogen index of gas stored in Vycor glass ............................ 69

6.10 Modified Langmuir model for the excess gas in Vycor glass ................. 71

6.11 Modified BET model for the excess gas in Vycor glass ......................... 75

6.12 Separation of the total methane gas into free and adsorbed .................. 77

8.1 Mass spectrometric spectra of hydrated elastin ....................................... 93

8.2 Direct polarization $^{13}C$ NMR spectrum of hydrated elastin ................. 108

8.3 Direct polarization $^{13}C$ NMR spectra of hydrated and lyophilized elastin .. 110

8.4 Cross polarization $^{13}C$ NMR spectra of lyophilized elastin ................. 111

8.5 Direct polarization$^{13}C$ NMR spectrum of hydrated elastin at $75^\circ C$ .... 112

9.1 Optical microscopy images of elastin based biomaterials ......................... 116

9.2 Diffusion measurements in elastin based biomaterials- short-time limit .... 117

9.3 Diffusion measurements in elastin based biomaterials- long-time limit .... 118
List of Tables

3.1 Tabulated power law exponents of the structure correlation function $\Gamma(k)k^p \sim k^\rho$ and instantaneous diffusion coefficient $D_{\text{inst}} \sim t^{-\vartheta}$ for each one dimensional disorder class. .............................................. 18

5.1 Tabulated results of the phantom characteristics: Short-range and hyperuniform phantoms ............................................................... 40

5.2 Tabulated results of the phantom characteristics: Short-range disordered phantoms ................................................................. 43

8.1 Amino acid analysis of three purified bovine nuchal ligament elastin samples .......................................................... 107

8.2 Tabulated results of bovine ligament elastin chemical shifts ......................... 109

8.3 Tabulated results of $T_{1\rho}$ and $\tau_c$ ............................................................ 113
Part I

Diffusion in disordered systems
Chapter 1

Introduction

An important question in physics is how to relate dynamical processes, such as diffusion, to the underlying geometric structure of disordered systems. The answer to the above question has a variety of applications in many interdisciplinary fields besides physics, such as biomedicine and bioengineering, oil industry, or complex networks to name a few.

Disorder is related to fundamental problems in classical systems such as the packing fraction of disordered jammed matter \([1]\) or dynamic critical phenomena \([2]\), and in quantum systems such as many body localization \([3]\) and thermalization \([4]\). Disorder was also shown to be related to creating two dimensional freeform waveguides \([5, 6]\). Scattering of X-rays or neutrons was the main technique for characterizing disorder and structure \([7, 8]\), however, dynamical processes, such as diffusion, may also reveal the salient features of the underlying geometry \([9, 10, 11, 12]\).

In the field of bioengineering, the performance of scaffolds intent for use as dermal substitutes is controlled by its underlying geometric structure. Diffusion therefore may contribute in determining structural parameters such as the surface-to-volume ratio \((S/V)\) and diffu-
sive permeability ($\kappa$). In the field of biomedicine, characterizing the underlying structural complexity of tissues may contribute to early detection of cerebral ischemia \cite{13}. In addition, diffusion may become a valuable tool in the energy industry for optimization of oil production from rock formations \cite{14}. Lastly, complex networks (Twitter or Facebook) possess an intrinsic type of disorder which affects the diffusion of information. Therefore, relating the underlying structure with the process of diffusion may contribute to the optimal transport of information on specific types of complex networks.

As of today, relating the dynamic process of diffusion with the underlying structural complexity of the system is still under exploration. In Part I of the thesis I will present both the theoretical aspects of diffusion in disordered media and experiments of diffusion in systems with controlled disorder, and show in praxis that diffusion may be used as a tool for characterizing the underlying microstructure of the medium. Chapter 2 focuses on unrestricted (Gaussian) diffusion and gives an exact derivation of the Gaussian diffusion propagator by solving the diffusion equation. Chapter 3 describes the basic concepts of diffusion in disordered systems. Specifically, the presence of disorder makes the diffusion coefficient time dependent defining two relevant time regimes. The short-time regime, where the diffusing molecules experience only the local restrictions, leading to a characteristic universal $-t^{1/2}$ dependence of the diffusion coefficient. At that limit, the surface-to-volume ratio of the medium may be measured. The long-time regime, where the diffusing molecules experience the geometry in an increasing fashion. At the long time limit, the long-range structural correlations leave a characteristic footprint in the diffusion coefficient as $t^{-\vartheta}$, where $\vartheta$ is the dynamical exponent and depends on the structural universality class of the system.
Chapter 4 discusses nuclear magnetic resonance techniques for measuring diffusion. Such measurements include spatially modulating the spins in a sample and observing the attenuation of the encoded grating over time. One of the main difficulties in measuring short diffusion times in magnetic resonance is applying short and strong magnetic field gradient pulses. For this reason a homemade gradient coil was designed. The design and characteristics of the constructed probe is discussed in the last part of Chapter 4. Chapter 5 of this thesis presents experiments of diffusion in two qualitatively different disordered phantoms, undergoing hyperuniform and short-range disorder, and reports a direct observation of universality in one dimension by monitoring the scaling of the diffusion coefficient at long times. Specifically, the structure correlation function, $\Gamma(k)$, of the two phantoms is computed and the structural exponent is determined. In addition, to investigate the transport dynamics in the controlled phantoms the diffusion coefficient was measured for a wide range of diffusion times. The presented results reveal the power law scaling of the diffusion coefficient at long times and the essence of universality. Lastly, Chapter 6 of this thesis is focused on the adsorption of gases in disordered media. Specifically, a detailed study of methane gas in Vycor is undertaken and the density and number of adsorbed layers are determined based on proposed modified Langmuir and Brunauer-Emmett-Teller (BET) models.
Chapter 2

Gaussian diffusion

Diffusion is a process arising from the random motion of the molecules associated with their internal thermal energy. A natural description of this process is through the probability density function (PDF) of position \( \vec{r} \) over time \( t \) and is associated with the diffusion equation which only contains deterministic coefficients such as the diffusion coefficient. In this chapter I will give a brief derivation of the diffusion equation based on the current conservation and Fick’s law. The free diffusion Green’s function (free diffusion propagator) will also be computed and connected with the NMR measurement.

2.1 Current conservation and Fick’s law

The conservation of the number of molecules requires,

\[
\frac{\partial}{\partial t} \Psi(\vec{r}, t) = -\nabla_{\vec{r}} j(\vec{r}, t) .
\]  

(2.1)
Physically the above equation means that the number of molecules in and out of a specific volume is changing based on the current flow. In addition, the particle current is proportional and against to the density gradient described by Fick’s law,

\[ j(\vec{r}, t) \sim -D_0 \nabla_{\vec{r}} \Psi(\vec{r}, t). \]  

(2.2)

Combining equations 2.1 and 2.2 we obtain the diffusion equation for a homogeneous fluid,

\[ \frac{\partial}{\partial t} \Psi(\vec{r}, t) = D_0 \nabla_{\vec{r}}^2 \Psi(\vec{r}, t). \]  

(2.3)

Eq. 2.3 is commonly referred to as the diffusion equation and is based on a fully deterministic macroscopic approach. The stochastic nature of the diffusion equation arises from the fact that one uses the PDF, \( \Psi(\vec{r}, t) \), to compute averages such as the average square displacement \( \langle (\vec{r}(t) - \vec{r}(0))^2 \rangle \). In the presence of disorder though, the diffusion equation needs to be modified as the diffusion coefficient is spatially dependent. The general approach for describing diffusion in disordered media will be discussed in Chapter 3.

### 2.2 Gaussian diffusion propagator

Finding the Green’s function of eq. 2.3 requires setting up,

\[ (\frac{\partial}{\partial t} - D_0 \nabla_{\vec{r}}^2)G_0(\vec{r}, t) = \delta(t) \delta(\vec{r} - \vec{r}_0). \]  

(2.4)

In the above equation \( G_0(\vec{r}, t) \) is the Green’s function, or most commonly reported in the NMR community as the diffusion propagator, and \( \delta_r \) is a point source released at time \( t \) as
required by causality. Intuitively, the Green’s function is a response function when creating a lump of particles at time \( t = 0 \) and position it at \( \vec{r}_0 \). Taking the a double Fourier transform of the above eq. 2.4, we obtain the free diffusion propagator in \( \omega-q \) space \([15, 16]\),

\[
G_0(\omega, \vec{q}) = \frac{1}{-i\omega + D_0q^2}.
\]  

(2.5)

The above Green’s function for free diffusion in the \( \omega-q \) representation has the form of a Lorentzian function. The width of this Lorentzian could be controlled by the spatial wave-vector \( q \) which experimentally is generated via the magnetic field gradients (see Chapter 4). In order to obtain the most commonly reported Gaussian shape of the free diffusion propagator we take the inverse Fourier transform of the Lorentzian in eq. 2.5 yielding,

\[
G_0(t, \vec{r}) = \theta(t)e^{-q^2t}.
\]  

(2.6)

In the above equation \( \theta(t) \) is the step function.

Note that for free diffusion the initial positions of the molecules are irrelevant. In other words, the PDF \( \Psi(\vec{r}, t) \) and therefore \( G_0(t, \vec{r}) \) are translation invariant meaning that \( \vec{r}_0 \) is arbitrary \((\vec{r}_0 = 0 \) in the above case). In the case of disordered media the translation invariance is broken and the problem needs special treatment when computing the disordered Green’s function.
Chapter 3

Non-Gaussian diffusion and universality classes

Arguably, the most interesting systems to study with diffusion methods are the ones that hinder diffusion such as, biological samples, soft tissues, porous rocks or complex networks. All these systems fall under the same category, of heterogeneous systems, as they experience some type of intrinsic disorder in their geometric structure. Classical transport in disordered systems is a hard problem, in general, due to its inherent complexity associated with structure present in the system. For this reason, various works addressing theoretical techniques on the subject exist as of today, discussing anomalous diffusion, where the mean square displacement of the diffusing molecules does not scale linearly with time, according to definition 3.4 but rather as $t^{2/d_w}$ with $d_w > 2$ [17, 18]. The latter makes the diffusion coefficient diverge or become zero at long times and is observed in finely tuned systems such as fractal geometries [19] or percolation clusters [20]. However, biological samples and porous rocks appear to have some randomness in their structure lacking this fine tuning. In this thesis only Gaussian
diffusion is considered, in which the central limit theorem holds in its original form and the diffusion coefficient is finite in both short- and long-times according to eq. 3.4. In this chapter I will present a brief introduction of the theory behind diffusion in disordered media and relations between the dynamics and the underlying structural complexity of the medium.

3.1 Modified diffusion equation and the double average

The presence of disorder breaks translation invariance and makes the solution of the diffusion equation a challenging problem to solve, in general. The difficulty arises from the fact that diffusion coefficient has spatial dependence $D(\vec{r})$. Finding the solution requires knowing the exact shape of $D(\vec{r})$, which depends on the potentially complex and/or unknown microstructure. The latter is never the case in disordered media.

In disordered media, Fick's law is modified as,

$$j(\vec{r}, t) \sim -D(\vec{r}) \nabla_r \Psi(\vec{r}, t).$$  \hfill (3.1)

and the diffusion equation becomes [16],

$$\frac{\partial}{\partial t} \Psi(\vec{r}, t) = \nabla_r D(\vec{r}) \nabla_r \Psi(\vec{r}, t).$$  \hfill (3.2)

Setting up the fundamental solution similar to eq. 2.4 we obtain,

$$\left[ \frac{\partial}{\partial t} - D_0 \nabla^2_r - \nabla_r D(\vec{r}) \nabla_r \right] Q(\vec{r}; \vec{r}_0, t) = \delta(t) \delta(\vec{r} - \vec{r}_0).$$  \hfill (3.3)

Note that the above $Q(\vec{r}; \vec{r}_0, t)$ is not translation invariant.
The definition of the diffusion coefficient in one dimension is,

\[ D(t) = \frac{\langle (x_t - x_0)^2 \rangle}{2t}. \tag{3.4} \]

Eq. 3.4 requires taking the average of the mean square displacement \( \delta x^2(t) \equiv \langle (x_t - x_0)^2 \rangle \).

The translation invariance of the diffusion propagator \( G(\vec{r}; \vec{r}_0, t) \) makes this averaging procedure a subtle point. Specifically, it requires two averaging mechanisms [21],

- **Averaging over all Brownian paths:**

\[ \langle (x_t - x_0)^2 \rangle \equiv \int dx G(x; x_0, t)(x_t - x_0)^2, \tag{3.5} \]

from a given point \( x_0 \) in the disordered system. This averaging mechanism effectively homogenizes the system into segments of length \( L(t) \) with local diffusivity \( D_j \). The resulting coarse grained system can be considered as a series of segments with slightly different local diffusivity \( D_j \) (similar to resistors in series). Note that the resulting coarse grained diffusivity \( D_j \) is smoother than the initial sharp varying \( D(x) \) (\( D(\vec{r}) \) in three dimensions).

- **Averaging over all disorder realizations:**

\[ \langle \langle (x_t - x_0)^2 \rangle \rangle \equiv \frac{1}{X} \int dx_0 \int dx G(x; x_0, t)(x_t - x_0)^2, \tag{3.6} \]

which correspond to averaging over initial positions \( x_0 \). In the above equation \( X \) corresponds to the length of the system.

The above coarse graining procedure filters out the local and sharp variations of \( D(\vec{r}) \) and results in a system with a smoothly varying \( D_j \) over the diffusion length \( L(t) \). As the diffusion time is increased, \( L(t) \) increases and therefore at sufficiently long times the system
can be effectively described by a universal diffusivity $D_\infty$.

### 3.2 Disordered averaged diffusion propagator and the self energy part

As described in the previous section any quantity is averaged over the two mechanisms, namely over all Brownian paths and over all disorder realizations. Averaging the disordered Green’s function $G(\vec{r}; \vec{r}_0, t)$, of the fundamental solution of eq. 3.3, over all disorder realizations restores the translation invariance. In the $(\omega, \vec{q})$ representation we find the disordered averaged Green’s function [16],

$$G(\omega, \vec{q}) = \frac{1}{-i\omega + D_0q^2 - \Sigma(\omega, \vec{q})}. \quad (3.7)$$

The above equation deviates from the Gaussian diffusion Green’s function (eq. 2.5) as it contains the self energy part $\Sigma(\omega, \vec{q})$, which describes any effects arising from the interactions of the molecules with the environment. The NMR signal arising from the diffusing molecules in the sample is then given by,

$$S(t, \vec{q}) = \int d\vec{r} e^{-i\vec{q} \cdot \vec{r}} G(t, \vec{r}), \quad (3.8)$$

which is similar to definition 4.4 presented in Chapter 4.
3.3 Double average: An illustrative example

Let’s illustrate the double average mechanism described by eqs. 3.5 and 3.6 by considering a system of cylinders of two different diameters imbedded in a fluid of diffusivity $D_0 = 1 \mu m^2/ms$ shown in Fig. 3.1 [21]. Note that the cylinders are made of a material with diffusivity $D_{mat}$ and that $D_{mat} << D_0$. Fig. 3.1 illustrates the disordered sample from the diffusing molecules point of view. For $t = 0 ms$ the diffusing molecules experience the local and sharp variations of geometry in the disordered sample. Increasing the diffusion time, and therefore the diffusion length (eq. 3.4 in two dimensions) $L(t) \sim \sqrt{4Dt}$, the disordered sample is coarse grained over $L(t)$ due to the double average. Specifically, for $t \simeq 50 ms$ the cylinders with the smaller diameter look practically invisible from the diffusing molecules point of view. Eventually, for $t \simeq 2 s$ the disordered sample looks homogeneous and may be described by a universal diffusivity $D_\infty$.

As illustrated in the example of Fig. 3.1, the diffusing molecules experience the disorder in an increasing fashion defining two qualitatively different time regimes for the time dependence of the diffusion coefficient. These two regimes will be discussed in the next two sections.
3.4 The short-time regime

The short-time universal scaling of the diffusion coefficient arises from the net amount of restrictions in the disordered sample. For diffusion times smaller than the typical time across a pore of diameter \( \bar{a} \), \( \tau_D = \bar{a}^2 / 2D_0 \), only a fraction of the diffusing molecules experience the restrictions leading to the universal short-time scaling of the diffusion coefficient [10],

\[
    D(t) \simeq D_0 \left(1 - \frac{4\sqrt{D_0} S}{3d\sqrt{\pi} \tau^{1/2}} t^{1/2}\right), \quad t << \tau_D.
\]  

(3.9)
In the above equation $d$ is the dimensionality and $S/V$ denotes the surface to volume ratio of the pore. The initial decay of the diffusion coefficient, has been extensively used for characterizing porous media [22], biological model systems [23] or real tissues [24], to determine the surface-to-volume ratio the medium. As mentioned earlier, the characteristic and universal $-t^{1/2}$ dependence arises from the net amount of restrictions regardless from their positions. Therefore, the short-time scaling of the diffusion coefficient provides no information regarding the intrinsic type of disorder of the medium.

Note that if $\bar{a} \simeq 1 \mu m$, as it is the case for the typical transverse size of axons, $\tau_D < 1 ms$. Probing such short diffusion times with commercially available NMR hardware is experimentally challenging and often requires homemade magnetic field gradient coils (discussed in section 4.3) or more sophisticated pulsed gradient methods, such as oscillating gradient techniques [25, 26] (not discussed in this thesis).

3.5 The long-time regime

Increasing the diffusion length, $L(t)$, the diffusing molecules probe the structural complexity of the sample in an increasing fashion. As discussed in section 3.1, solving the full problem of diffusion in a disordered sample requires having information about the structure $D(\vec{r})$. However, the double average described above yields an effective medium which, at the long time limit looks considerably simpler and may be described by a universal diffusion coefficient such as, $D(t)|_{t \to \infty} \equiv D_\infty$. All the relevant information about the microstructure and hidden in the universal limit $D_\infty$ and the long-time scaling of the diffusion coefficient.
3.5.1 Structure correlation function and disorder universality classes

Disorder may be categorized in a handful of classes based on the low-$k$ behavior of the structure correlation function in Fourier space [11],

$$\Gamma(k) \equiv \mathcal{F}(\langle n(x_0 + x)n(x_0) \rangle) = \frac{|n(k)|^2}{L} \biggr|_{k \to 0} \sim k^p, \quad (3.10)$$

In eq. 3.10, $n(k)$ is the Fourier transform of the density of the restrictions $n(x)$, $L$ is the length of the system and $p$ is the structural exponent which is determined by the low $k$ scaling of $\Gamma(k)$. Intuitively, the correlator in real space $\langle n(x_0 + x)n(x_0) \rangle$ gives the probability of finding a barrier at $x_0$ if a barrier exists at $x_0$. Figure 3.2 (original figure appears in ref. [11]) shows a series of one dimensional disorder classes made of successive permeable barriers of permeability $\kappa$ (vertical lines), and the computed structure correlation function $\Gamma(k)$. Below, I provide a description of each disorder class.

- **Periodic arrangement**

  A perfectly periodic placement of the barriers, indicated in red in Fig. 3.2, results in the Bragg peaks in the correlator $\Gamma(k)$ and a structural exponent of $p = \infty$.

- **Short-range disorder**

  Short-range (Poissonian) disorder is realized by placing the barriers in completely random and uncorrelated positions. The latter results in a plateau in $\Gamma(k)$ as $k \to 0$. In this class, the variance of the number of restrictions within a sphere grows in proportion to the sphere volume consistent with the central limit theorem. Assuming a structure factor of $v$ for the
Figure 3.2: a) One dimensional disorder classes and their structural exponents $p$. b) Structure correlation function $\Gamma(k)$ reveals the qualitative differences between the disorder classes based on the scaling at low-$k$. Reproduced from ref. [11].

barriers placed at random positions $x_{m,n}$, the structure correlation function is,

$$\Gamma(k) = \frac{1}{L} |v_k|^2 \sum_{m,n}^N e^{ik(x_m-x_n)}. \quad (3.11)$$

In the case of short-range disorder $x_m$ and $x_n$ are random variables and therefore only the diagonal terms survive resulting in,

$$\Gamma(k) = \frac{N}{L} |v_k|^2. \quad (3.12)$$
The ratio $N/L$ is equivalent to $1/\bar{a}$ and therefore $\Gamma(k)\bar{a} = |v_k|^2$ which is the resulting plateau. One realization of short-range disorder is shown in Fig. 3.2 in blue corresponding to $p = 0$.

- **Hyperuniform disorder**

A key characteristic of this disorder class is that the variance of the number of restrictions within a sphere grows slower than the sphere volume; typically as the sphere surface $[27]$. This type of disorder is a signature of the jammed state of sphere packings [1] and a key condition for creating two dimensional freeform waveguides [5, 6]. It is characterized by a power law dependence of $\Gamma(k) \sim k^2$ ($p = 2$) as $k \rightarrow 0$.

- **Strong disorder**

Strong disorder is realized by drawing the positions of the barriers from a distribution with a fat tail, $P(x) \sim x^{-(1+\mu)}$ (Levy distribution), resulting in diverging $\Gamma(k)$. This disorder class is characterized by large gaps in the placement of the barriers.

In Chapter 5 I will present experimental results of transport in two phantoms exhibiting short-range and hyperuniform disorder.

### 3.5.2 Diffusion and the dynamical exponent

For diffusion times longer than the residence time in a pore, $\tau_r \equiv \bar{a}/2\kappa$, the long-range structural correlations survive the double average, leaving a characteristic footprint in the time dependence of the instantaneous diffusion coefficient [11],

$$D_{\text{inst}}(t) \equiv \frac{\partial}{\partial t} \frac{\langle \delta x^2 \rangle}{2} \sim D_\infty + ct^{-\alpha}, \quad t > \tau_r.$$  \hspace{1cm} (3.13)
CHAPTER 3. NON-GAUSSIAN DIFFUSION AND UNIVERSALITY CLASSES

<table>
<thead>
<tr>
<th>Disorder Class</th>
<th>Structural exponent, $p$</th>
<th>Dynamical exponent, $\vartheta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Periodic</td>
<td>$\infty$</td>
<td>$\infty$</td>
</tr>
<tr>
<td>Short-range</td>
<td>0</td>
<td>$1/2$</td>
</tr>
<tr>
<td>Hyperuniform</td>
<td>2</td>
<td>$3/2$</td>
</tr>
<tr>
<td>Strong</td>
<td>$-(2 - \mu)$</td>
<td>$(\mu - 1)/2$</td>
</tr>
</tbody>
</table>

Table 3.1: Tabulated power law exponents of the structure correlation function $\Gamma(k)_{k \to 0} \sim k^p$ and instantaneous diffusion coefficient $D_{\text{inst}} \sim t^{-\vartheta}$ for each one dimensional disorder class.

Numerical results of the scaling of the instantaneous diffusion coefficient $D_{\text{inst}}$ are shown in Fig. 3.3 (original figure appears in ref. [11]) for each one-dimensional disorder class presented in Fig. 3.2. In the above equation, $\vartheta$ is the dynamical exponent and is connected with the structure via [11],

$$\vartheta = \frac{p + d}{2}. \quad (3.14)$$

The tabulated dynamical and structural exponents for each disorder class are highlighted in Fig. 3.3 and are shown in Table 3.1.

Figure 3.3: Instantaneous diffusion coefficient distinguishes between each disorder class. Original figure appears in ref. [11] and has been reproduced here with permission.
CHAPTER 3. NON-GAUSSIAN DIFFUSION AND UNIVERSALITY CLASSES

Since the derivation of eq. 3.13 and 3.14 is rather complicated I will not discuss all the details behind the proof. Instead, I will present a simple reasoning of the scaling of the diffusion coefficient at long times in the case of short-range disorder which contains the salient physics. For the full derivations the reader is referred to [11, 12].

The model lies on the averaging mechanism presented before, in which the sample is effectively coarse grained in smaller segments over the diffusion length \( L(t) \). In this effective picture, each segment has different diffusivity \( D_j \) which acts as a resistor \( R_j \sim 1/D_j \). Note that the diffusivity of each segment is different due to the different number of barriers in \( L(t) \). Averaging over the disorder realizations will yield the universal diffusion coefficient which is result of the collective effects of the disorder,

\[
\frac{1}{D_\infty} = \left< \frac{1}{D_j} \right>.
\]  

(3.15)

In the above equation the diffusivity of each segment may be expressed as,

\[
D_j = \left< D \right> + \delta D_j,
\]  

(3.16)

with \( \delta D_j \) resulting from the fluctuations in the barrier density. It is directly seen from eq. 3.16 that the long-range structural fluctuations in the barrier density will have an effect upon the instantaneous diffusion coefficient. Expressing \( D_{\text{inst}} \) in terms of \( D_\infty \) and the variance \( (\delta D_j)^2 \) yields,

\[
D_{\text{inst}} \simeq D_\infty + \frac{(\delta D_j)^2}{D_\infty}.
\]  

(3.17)

Relating the instantaneous diffusion coefficient with the structure emerges from the fact that
the variance \((\delta D_j)^2\) is proportional to \((\delta n)^2\). For the case of short-range disorder (Poissonian) \((\delta n)^2 \sim 1/L(t) \sim 1/\sqrt{t}\) and therefore, at the long time limit \(\vartheta = 1/2\),

\[
D_{\text{inst}}(t) \sim t^{-1/2}
\]  

(3.18)

For the different disorder classes the density fluctuations \((\delta n)^2\) will scale differently resulting in different power law exponents \(\vartheta\) shown in Table 3.1.

### 3.6 Diffusive permeability

The diffusive permeability of a single barrier \(\kappa \ [\mu m/ms]\), is connected with the dimensionless parameter,

\[
\zeta = \frac{SD_0}{2V\kappa d},
\]

(3.19)

which quantifies the ability of the barriers to hinder diffusion. In the above equation \(d\) is the dimensionality and \(D_0\) is the unrestricted diffusion coefficient at the corresponding temperature and pressure. The universal limit of the diffusion coefficient \(D_\infty\), is then directly connected to \(\zeta\) as [12],

\[
D_\infty \approx \frac{D_0}{1 + \zeta}.
\]

(3.20)

Therefore, determining the surface-to-volume ratio and \(D_\infty\) allows for a direct measurement of the diffusive permeability of the medium.
Chapter 4

Diffusion NMR and experimental setup

Nuclear magnetic resonance is a great tool for measuring molecular average square displacements. Diffusion is measured through the application of magnetic field gradients $g(t)_n = \partial B_z(t)/\partial n$ which imparts an evolution under the gradient Hamiltonian $\hat{H}_g = \gamma n g_a \hat{I}_z$. In the magnetic field gradient, $n$ may take any value in the cartesian coordinates and is the axis in which the magnetic field varies linearly. Lets assume that the applied magnetic field gradient is applied along the $z$ axis; in this case the spins accumulate a phase given by [15],

$$\phi = \gamma \int_0^t dt' g(t')z(t'). \quad (4.1)$$

Alternatively, the above phase may be expressed via the spatial wave vector $k(t) = \gamma \int_0^t dt' g(t')$ and the particle velocity, $v$, rather than position (integration by parts),

$$\phi = \gamma \int_0^t dt' k(t')v(t'). \quad (4.2)$$
The transverse magnetization may be expressed as a complex number $e^{-i\phi}$ and since all the spins in the sample accumulate a phase based on their position along $z$ one has to take the average of this exponential term which describes the measured signal,

$$\langle e^{-i\phi} \rangle = \langle e^{-i\gamma \int_0^t dt' k(t') v(t')} \rangle.$$  \hspace{1cm} (4.3)

### 4.1 The cumulant expansion for Gaussian diffusion

The above average in eq. 4.3 requires using the cumulant expansion [28]. The point of computing the cumulants is because they don’t depend on the raw moments. One perfect example is the variance $\sigma^2 \equiv \langle w^2 \rangle - \langle w \rangle^2$ which describes the real width of the distribution $P(w)$. On the other hand the raw second moment $\langle w \rangle^2$ which depends on $\langle w \rangle$ will not yield the real width of the distribution.

Starting from a single random variable, $w$ with probability density distribution $P(w)$ eq. 4.3 becomes,

$$\langle e^{-ikw} \rangle = \int_0^t dt' P(w) e^{-ikw},$$  \hspace{1cm} (4.4)

or equivalently with the cumulants,

$$\ln \langle e^{-ikw} \rangle = \sum_{n=0}^{\infty} \frac{(ik)^n}{n} \langle w^n \rangle_c.$$  \hspace{1cm} (4.5)

For Gaussian diffusion, where the probability density of position is given in eq. 2.6, the
above series terminates at the second term,

\[ \ln(e^{-ikw}) = ikw_0 - \frac{1}{2}k^2\sigma^2. \]  

(4.6)

Expanding the exponential of eq. 4.3 and taking the natural logarithm yields the NMR signal expressed in terms of cumulants of the molecular velocity, \( u_n \), [28],

\[
\ln(s) = \sum_{n=0}^{\infty} \frac{i^n}{n} \int u_n(t_1...t_n)k(t_1)...k(t_n)dt_1...dt_n.
\]

(4.7)

For Gaussian diffusion the Markov approximation holds, \( u_2(t_1, t_2) = 2D\delta(t_2 - t_1) \), which physically describes the lack of memory in the molecular motion, i.e. each step is independent from the previous one. That leads to the familiar expression for the signal attenuation due to the gradients [15, 29],

\[
\ln(s) = -D_0 \int_0^t dt' k^2(t') \equiv -bD_0.
\]

(4.8)

### 4.2 Gradient Echo: An illustrative example

To illustrate in praxis the above eq. 4.8 one of the simplest sequences for measuring the molecular diffusion coefficient is presented. The pulse sequence is composed of a spin echo and a gradient pulse as shown in Fig. 4.1.

During the first time interval \( \tau \) the spatial wavevector is given by \( k = \gamma \int_0^t dt' g_{\text{max}} = \gamma g_{\text{max}}\tau \) where \( g_{\text{max}} \) is the maximum applied gradient. After time \( \tau \) the maximum wavevector \( k_{\text{max}} = \gamma g_{\text{max}}\tau \) is reversed to \( k_{\text{min}} = -\gamma g_{\text{max}}\tau \) due to the \( \pi \) pulse. Finally during the last
CHAPTER 4. DIFFUSION NMR AND EXPERIMENTAL SETUP

Figure 4.1: Gradient echo pulse sequence for measuring the molecular diffusion coefficient. The sequence makes use of the formation of an echo at time $2\tau$.

time interval $\tau$ the spatial wavevector is given by $k = k_{\text{min}} + \gamma \int_0^\tau dt' g(t') = -\gamma g_{\text{max}} \tau + \gamma g_{\text{max}} t$.

Using eq. 4.8 for each of the time intervals and adding them gives the final $b$ value as,

$$\ln(s) = -D_0 \left( \frac{2}{3} \gamma^2 g_{\text{max}}^2 \tau^3 \right).$$

Varying the intensity of the applied magnetic field gradient $g_{\text{max}}$ allows for measuring the diffusion coefficient $D_0$. Probing the above exponential term in eq. 4.9 for very short time intervals $\tau$ requires using short and strong gradient pulses. The latter presents one of the main limitations in MRI. In NMR, homemade gradient coils, capable for delivering strong magnetic field gradient pulses, are used for measuring the spin diffusion constant of a solid [30, 31]. The development of such a gradient coil will be discussed in the next section.
CHAPTER 4. DIFFUSION NMR AND EXPERIMENTAL SETUP

4.3 Gradient coil design

The requirement to measure small mean square displacements of the order of $\sim 1 \mu m$ with NMR presents a challenge as it requires fast switching and strong in magnitude, gradient pulses. Such pulses are not usually available on commercial NMR probes, therefore a home-made probe was built, equipped with a gradient coil capable for delivering strong gradient pulses. In this chapter I will discuss briefly the theoretical background for designing such coils and present the characteristics of the constructed gradient coil.

Consider two closed loops with current running in opposite directions as shown in Fig. 4.2. Solving Biot Savart’s law for the magnetic field along the z axis yields,

$$B(z) = \frac{I \mu_0 \alpha^2}{2} \left[ \frac{1}{(\alpha^2 + (z - \frac{d}{2})^2)^{3/2}} - \frac{1}{(\alpha^2 + (z + \frac{d}{2})^2)^{3/2}} \right].$$  \hspace{1cm} (4.10)

In the above equation, $I$ denotes the current, $\mu_0$ the permeability of free space and $\alpha$ is the radius of the coil. Taking the higher order derivatives of eq. 4.10 is a tedious task, not presented here, but the dependence upon $d$ is apparent. Choosing $d = \sqrt{3}\alpha$ makes $\partial_z B_z$ constant through third order [32]. Adding two Maxwell pairs placed at $z_1$ and $z_2$ on the z-axis eliminates the third, fifth and higher order derivatives by choosing $z_1 = 0.44\alpha$, $z_2 = 1.19\alpha$ and $I_2/I_1 = 7.47$ [33, 34].

Figure 4.3 shows the schematic design of the gradient set with all the distances and tolerance. In this setup, the two Maxwell pairs had an average radius of $\alpha = 5.66 \ mm$, $z_1 = 2.50 \ mm$ and $z_2 = 6.72 \ mm$, and the number of loops was 45 and 6. Figure 4.4 shows a photograph of the coil along with the blocks that hold it in place. The blocks are made of
Figure 4.2: Simple maxwell pair with current flowing in opposite directions.

acrylic.
Figure 4.3: Schematic of the gradient set. The distances and coil loops were chosen such that the criteria for a homogeneous magnetic field gradient, described in the text, were met.
The uniformity of the constructed gradient set within a sphere of radius $r$ is important for studying molecular diffusion as higher order derivatives will artificially alter the measured diffusion coefficient. To study the uniformity, Biot-Savart’s law was solved numerically [33] and the results for a single Maxwell pair and two Maxwell pairs are shown in Fig. 4.5. For a single Maxwell pair (Fig. 4.5b) of $\alpha = 5.66\; mm$ placed at $z_1 = 2.50\; mm$, the generated magnetic field gradient deviates by approximately 15% from the ideal gradient with a sphere of $r = 0.28\alpha$. On the other hand, for two Maxwell pairs (Fig. 4.5a), the same deviation is approximately 0.3%. Note that the latter calculation is an approximation as the actual distances will deviate from the values above. However, it shows that the constructed gradient set is capable of delivering reliable and strong magnetic field gradients.
Figure 4.5: Percent deviation of the produced magnetic field gradient from the ideal linear gradient for one (a) and two (b) Maxwell pairs. The uniformity is shown to increase dramatically when using two pairs.

The gradient set was calibrated using H$_2$O and a spin-echo sequence resulting a gradient constant of approximately $90 \frac{G}{cmA}$. The inductance of the coil was approximately 48 $\mu$H and the resistance was 1.0 $\Omega$. 
Chapter 5

Observation of universality with diffusion

In this chapter I will present experiments of transport in one-dimensional phantoms exhibiting two types of disorder as discussed in section 3.5.1. Diffusion NMR techniques were used to monitor the diffusion coefficient of H$_2$O diffusing through two qualitatively different phantoms, undergoing hyperuniform and short-range disorder. A direct experimental observation of universality of the diffusion coefficient in one dimension is reported which emerges from long-range structural correlations at the mesoscopic scale. The barrier density autocorrelation function in Fourier space of the underlying geometric structure of the two systems at low $k$ is experimentally investigated in order to reveal the class of disorder. In addition, the time dependence of the diffusion coefficient of H$_2$O at times beyond the pore residence time is monitored. Remarkably, the experimental results reveal the distinctly different and universal power law tails of the diffusion coefficient for the two disorder classes as discussed in Chapter 3.
5.1 Phantom construction and experimental methodology

For the construction of the phantoms two sets of commercial porous polycarbonate films (barriers) were purchased from Sterlitech Corporation (Kent, WA). The pore diameters in the two sets of films were 15 \( \text{nm} \) (green-Fig. 5.3d) and 45 \( \text{nm} \) (blue/red-Fig. 5.3d). The films had a thickness of approximately 6 \( \mu \text{m} \), and pore density of approximately 8 pores/\( \mu \text{m}^2 \) as revealed via atomic force microscopy shown in Fig. 5.3c.

5.1.1 Short-range disordered phantom

The polycarbonate films were cut by hand in rectangular pieces of 0.95 \( \text{mm} \) in width and 7 \( \text{mm} \) in length with a scalpel and stacked in a layered geometry along the \( z \) axis as shown in Fig. 5.3d. The films were then placed in a rectangular NMR glass tube purchased from F&D Glass (Millville, NJ) (1 \( \text{mm} \) in height, 1 \( \text{mm} \) in width, 9 \( \text{mm} \) in length), filled with water and sonicated for 3 minutes. The phantoms were inspected under a microscope to avoid bubbles which may distort the RF and gradient fields. Two phantoms were constructed: one using the films with 15 \( \text{nm} \) pore diameter (green) and one using the films with 45 \( \text{nm} \) pore diameter (blue). Each phantom exhibiting short-range disorder had approximately 87 films stacked along \( z \) resulting in a predicted \( \bar{a} \approx 5.5 \mu \text{m} \). A representative optical microscopy image of the phantom created with the 45 \( \text{nm} \) pore diameter films, exhibiting short-range disorder (blue) is shown in Fig. 5.3a.
5.1.2 Hyperuniform disordered phantom

The same set of 45 nm pore diameter polycarbonate permeable films was used to create the phantom exhibiting hyperuniform disorder. In addition to the films, a customized set of spacers was used to achieve a semi-periodic geometry. The spacers were designed in the lab using Google Sketchup and built by Micron Solutions LLC (Salt Lake City, UT); the spacers had a length of 7mm, 0.95 mm width, and a thickness of 45 µm. The thickness of the films was chosen so that the experimental errors in the placement of the films act as random drifts resulting in hyperuniformity (see main text) [27]. The spacers were made of copper and designed with an open end to minimize eddy currents. The resulting phantom was made by placing the films and spacers in an alternating fashion as shown in the cartoon of Fig. 5.1b. An optical microscopy image of the phantom is shown in 5.3b.

![Figure 5.1: a) Cartoon representation of the sample with hyperuniform disorder. The red sheets represent the permeable films and the yellow parts act as a separation to achieve the hyperuniformity. b) Cartoon representation of the copper plates that were inserted in the sample in order to create the desired geometry. The thickness of the plates was ~ (43 ± 4) µm. The parts were constructed with an open loop to avoid Eddy current. The copper plates were custom designed and manufactured by Micron Solutions (Salt Lake City, UT).](image-url)
5.1.3 Diffusion NMR methodology

All experiments were performed on a 4.2 Tesla Tecmag Apollo system using a homemade probe equipped with high magnetic field gradients (section 4.3). The temperature was set to 25°C and was regulated to within 0.5°C; at this temperature the theoretical diffusion coefficient is $D_0 = 2.30 \mu m^2/ms$ [35]. Sample heating due to the application of high magnetic field gradients was tested with methanol [36] and found to be less than 2%.

Fig. 5.2 highlights the pulse sequences used in this work. The applied magnetic field gradients ranged from approximately 5 G/cm to 1,000 G/cm and were calibrated using the theoretical diffusion coefficient at 25°C. The time $\Delta$ was varied for each experiment from approximately 50 µs to 4.5 s. It should be noted that the diffusion time reported includes the time interval $\Delta$, gradient pulse widths ($\delta$), and delays ($\tau$). The gradient pulse width was $\delta = 120 \mu s$ for diffusion times of 1 to 1.3 ms and $\delta = 220 \mu s$ for diffusion times ranging from 1.4 ms to 4.5 s. The $\pi/2$ pulse had a duration of 5 µs and the $\pi$ a duration of 10 µs. The delay $\tau$ was 50 µs for the pulse sequence in Fig. 5.2a and 500 µs for the pulse sequence in Fig. 5.2b. The crushing gradient $\delta_c$ had a duration of 150 µs and is used to spatially encode any single quantum coherence left after the $\pi/2$ pulse. The time interval $T$ between the $\pi/2$ pulses in Fig. 5.2a was 2ms and is used to push the echo further in time to mitigate ring down effects.

There are four basic causes that artificially lower the measured diffusion coefficient: Eddy currents, magnetic field inhomogeneities, surface relaxation, and higher order terms in the cumulant expansion [28]. Sinusoidal pulses were used to mitigate ring-down effects which may result in slight errors in the k-space trajectory [29]. In addition, the $\pi$ pulses refocus
evolution under the background field gradients at time $4\tau$. The surface relaxation was also found to be minimal due to the relatively long spin-lattice relaxation times of H$_2$O imbedded in the polycarbonate films ($T_1 \simeq 1.8$ s). Lastly, higher order cumulants were suppressed by keeping the product $bD_0 < 1$ so that $\ln S = -bD_0$ for all diffusion times probed in this work.

Figure 5.2: NMR pulse sequences used in this work. Shaped pulses were used to mitigate ringdown effects which introduce imperfections in the gradient pulses and result in errors in the k-space trajectories [29]. In addition, the $\pi$ pulses refocus evolution under the background gradients colinear with the Zeeman field at time $4\tau$. a) NMR pulse sequence used for measuring diffusion for times between 1 to 50 ms [37]. The use of bipolar magnetic field gradient pulses compensate for Eddy currents. The phases of the RF pulses for this pulse sequence are given in [37]. b) NMR pulse sequence used for measuring diffusion for times between 50 ms to 4.5 s [38, 39]. The use of assymmetric magnetic field gradient pulses cancels the cross term between the applied magnetic field gradient $g$ and the background gradient, $g_b$, after decoding. The phases of the RF pulses for this pulse sequence are given in [38].

The pulse sequence used for diffusion times between 1 to 50 ms is shown in Fig. 5.2a
The use of bipolar gradients compensate for the Eddy currents induced by the fast changing current. It should be noted here that the background gradient $g_0$ was estimated based on the measured line width, $f = 350Hz$, and sample size of $L \approx 1mm$, and found to be $g_0 \approx 0.8G/cm << g_m$, where $g_m$ is the maximum applied gradient. Therefore the background gradients are not expected to introduce any artificial fluctuations in the measured diffusion coefficient at short times.

The shaped gradient pulses alternate the form of $b$ given in ref. [37]. For the pulse sequence shown in Fig. 5.2a the signal attenuation was computed as in the example give in section 4.2 and is given by,

$$S(g_m) = -D_0 \left( 16g_m^2\gamma^2\delta^2 + 22g_m^2\gamma^2\delta^3 + 16g_m^2\gamma^2\delta^2\Delta \right) \frac{1}{\pi^2},$$

(5.1)

In eq. 5.1, $g_m$ is the maximum gradient strength and $\gamma$ is the $^1H$ gyromagnetic ratio. Note that the term proportional to $\Delta$ will dominate for $\Delta > 8 ms$. The phase cycling used for the short-time measurements is provided in [37].

The pulse sequence used for diffusion times between 50 ms to 4.5 s is shown in Fig. 5.2b [38, 39]. In the long time limit, $g_0$ is comparable to $g_m$ and therefore the background gradients may alter the $k$-space trajectory. Assymetric pulses ($g_{m1} > g_{m2}$) were used to mitigate the term proportional to $g_0g_m$ in the cumulant expansion. It should be noted that long diffusion times correspond to long diffusion lengths and therefore the diffusing molecules may experience different background gradients $g_0$ ($g_0 \neq g_0'$) during the encoding and decoding periods. The signal attenuation for the pulse sequence shown in 5.2b is given by,
\[ S(g_m) = -D_0 \left( (g_0^2 + g_0'^2) \left( \frac{2\tau^3\gamma^2}{3} + 2\tau^2\gamma^2\delta + 2\tau\gamma^2\delta^2 + \frac{2\gamma^2\delta^3}{3} \right) + \left[ -2g_{m2}\gamma\delta - 2g_{m2}\gamma\delta\eta \right] \frac{\Delta}{\pi^2} + \left[ 3\gamma^2\delta^3 + 8\gamma^2\delta^3\eta + 16\tau\gamma^2\eta^2 + 11\gamma^2\delta^3\eta^2 \right] \frac{g_{2m}}{\pi^2} \right), \]  

(5.2)

with,

\[ \eta = -\frac{\delta^2(-4 + \pi^2)}{\pi^2(4\tau^2 + 8\tau\delta + \frac{4\delta^2}{\pi^2} + 3\delta^2)}. \]  

(5.3)

In the above equation \( g_0 \) and \( g_0' \) are the values of the background gradients during the encoding and decoding time intervals and were estimated to be approximately 0.8-1 G/cm based on the linewidth. The ratio \( \eta = g_{m2}/g_{m1} \) is defined by eq. 5.3; choosing an appropriate \( \eta \) suppresses the \( g_0g_m \) term in the signal attenuation. Note that the term proportional to \( \Delta \) dominates for \( \Delta > 20\text{ms} \). The phase cycling for long-time measurements is provided in [38].

## 5.2 Experimental results

### 5.2.1 Structure correlation function and the dynamical exponent

Two phantoms exhibiting short-range disorder (SR) were constructed by stacking flat permeable barriers in uncorrelated positions in a glass tube a described in section 5.1. The correlator, \( \Gamma(z) \) for this class is characterized by a fast decrease beyond the correlation length. In Fourier space, the latter corresponds to a finite plateau in \( \Gamma(k)|_{k \rightarrow 0} \sim k^0 \) with a structural exponent \( p = 0 \) (Poissonian disorder). The proof regarding this plateau at low \( k \) values is given in Chapter 3 (section 3.5.1). A representative optical microscopy image...
of the phantom is shown in Fig. 5.3a revealing an average spacing between the barriers of \( \bar{a} \simeq 6.0 \mu m \). A digitized cut-out of part of the phantom is is shown in Fig. 5.3d in green and blue, corresponding to two different realizations of short-range disorder.

Hyperuniform disorder (HYP) is characterized by reduced long-range structural fluctuations, and is defined by performing random drifts in the placement of the barriers of an otherwise periodic arrangement (see Chapter 3 section 3.5.1). In our phantom, experimental errors in the placement of the barriers act as random drifts resulting in hyperuniformity. Fig. 5.3b shows a phantom exhibiting hyperuniform disorder revealing an average film spacing of \( \bar{a} \simeq 45.0 \mu m \). The equivalent digitized cut-out of part of the phantom is shown in Fig. 5.3d in red; note that for Fig. 5.3d, \( \bar{a} \) was rescaled for comparison to the two short-range disorder phantoms. Note that the three phantoms (A-B-C) appearing in Fig. 5.3d appear similarly disordered but can we distinguish their qualitative differences using a bulk diffusion measurement?

To address the above question, first we experimentally determine \( \Gamma(k) \) (eq. 3.10) of the three phantoms using the optical microscopy images shown in Fig. 5.3a-b. The latter correlator is shown in Fig. 5.3e. A plateau in \( \Gamma(k) \sim k^0 \) is observed as \( k \to 0 \) for the phantoms in green and blue, corresponding to a structural exponent of \( p = 0 \) verifying the expected short-range disorder. On the other hand, a power law scaling of \( \Gamma(k) \sim k^2 \) was observed for the phantom in red corresponding to a structural exponent of \( p = 2 \) implying hyperuniformity. Note that the fluctuations at high \( k \) values of \( \Gamma(k) \) result from the local barrier structure profile in signal processing. The two experimentally determined structural exponents, \( p \), set the expected dynamical exponents via eq. 3.14 based on the theory (see
Table 3.1). For short-range disorder in one dimension $p = 0$, and according to eq. 3.14, $\vartheta|_{SR} = 1/2$. On the other hand for hyperuniform disorder, $p = 2$, and $\vartheta|_{HYP} = 3/2$.

### 5.2.2 Universal power law scaling of the diffusion coefficient

To investigate the transport dynamics of $\text{H}_2\text{O}$ through the phantoms the cumulative diffusion coefficient, $D$, was measured which is related to the instantaneous diffusion coefficient via,

$$
D(t) \equiv \frac{\langle \delta z^2 \rangle}{2t} = \frac{1}{t} \int_0^t dt' D_{\text{inst}}(t').
$$

Both the cumulative and instantaneous diffusion coefficients were measured for a wide range of diffusion times ranging from $1.0 \text{ ms}$ to $4.5 \text{ s}$ corresponding to mean square displacements ranging from $2.0 \mu\text{m}$ to $144.0 \mu\text{m}$. This wide dynamic range enabled us to probe both the short- and long-time transport dynamics in the three phantoms and directly observe their universality class via the dynamical exponent, $\vartheta$, in eq. 3.13.

Fig. 5.4a highlights the universal $-t^{1/2}$ dependence of the cumulative diffusion coefficient for $t \ll \tau_D$, as described in Chapter 3 (section 3.4) ($\tau_{DSR} \simeq 5.5 \text{ ms}$, $\tau_{DHYP} \simeq 380.0 \text{ ms}$). For the two phantoms exhibiting short-range disorder (green-blue) the average spacing of the barriers was determined from the surface-to-volume ratio ($S/V \equiv 2/\bar{a}$), and found to deviate by $\sim 9\%$ from the value expected from the images acquired via optical microscopy. Similarly, the average spacing of the barriers exhibiting hyperuniform disorder deviated by approximately $20\%$ from the predicted value. The deviation of the measured $\bar{a}$ with respect to the value revealed by optical microscopy may be due to the water molecules trapped between the barriers and the spacers.
Fig. 5.4b shows the initial decay of $D_{\text{inst}}$ computed using the ill-posed definition,

$$D_{\text{inst}} = \partial [t D(t)]$$ \hspace{1cm} (5.5)

to the experimental data; the inset highlights the deviation between the computed value from the $t << \tau_D$ limit using the measured $S/V$, and $D_0$ obtained from least squares fit of the cumulative diffusion coefficient Fig. 5.4a (dashed lines). Reasonable agreement was found between the two definitions of the diffusion coefficient ($D$, $D_{\text{inst}}$) in the short-time limit.

As mentioned earlier, the universal power law scaling of $-t^{1/2}$ emerges from the net amount of restrictions, regardless of their positions. Therefore, the qualitative differences between the two disorder classes resulting from the long-range structural correlations are not apparent in the short-time limit.

Fig. 5.5a highlights the time dependence of the cumulative diffusion coefficient of unrestricted H$_2$O (cyan circles) and H$_2$O diffusing through the three constructed phantoms (green-blue-red). We observe no time dependence in the cumulative diffusion coefficient, $D$, of unrestricted H$_2$O whereas a characteristic time dependence of

$$D(t) \sim D_\infty + t^{-\tilde{\vartheta}},$$ \hspace{1cm} (5.6)

was observed for H$_2$O diffusing through the phantoms as a result of disorder. Note that the definition 5.4 may mask the true dynamical exponent given by eq. 3.13; if $\vartheta > 1$ as it is the case for hyperuniform disorder, a power law exponent $\tilde{\vartheta} = 1$ will become apparent in the
cumulative diffusion coefficient and one has to compute $D_{\text{inst}}$ using definition 5.5. On the other hand if $\vartheta < 1$, as it is the case for short-range disorder, then $\tilde{\vartheta} = \vartheta$.

<table>
<thead>
<tr>
<th>Phantom</th>
<th>$\bar{a}$ (µm)</th>
<th>Pore diam. (nm)</th>
<th>Disorder Class</th>
<th>$p$</th>
<th>$\tilde{\vartheta}$</th>
<th>$\vartheta$</th>
<th>$\tilde{\vartheta}_{th}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (green)</td>
<td>6</td>
<td>15</td>
<td>Short-range</td>
<td>0</td>
<td>0.59 ± 0.09</td>
<td>0.49 ± 0.16</td>
<td>1/2</td>
</tr>
<tr>
<td>B (blue)</td>
<td>6</td>
<td>45</td>
<td>Short-range</td>
<td>0</td>
<td>0.56 ± 0.11</td>
<td>0.44 ± 0.15</td>
<td>1/2</td>
</tr>
<tr>
<td>C (red)</td>
<td>45</td>
<td>45</td>
<td>Hyperuniform</td>
<td>2</td>
<td>0.99 ± 0.14</td>
<td>1.48 ± 0.12</td>
<td>3/2</td>
</tr>
</tbody>
</table>

Table 5.1: Tabulated results of the phantom characteristics, disorder classes and measured universal exponents. The expected power law exponents based on the theory are also shown as $\tilde{\vartheta}_{th}$. For phantoms A,B,C the reader is referred to Fig. 5.3.

For short-range disorder (green,blue), and for diffusion times greater than the residence time between the films, $\tau_r \equiv \bar{a}/2\kappa$ ($\tau_{r\text{SR}}|_{15\text{nm}} \simeq 37.0\text{ ms}$, $\tau_{r\text{SR}}|_{45\text{nm}} \simeq 23.0\text{ ms}$), we observe a dynamical exponent of $\tilde{\vartheta} = 0.59 \pm 0.09|_{15\text{nm}}$ and $\tilde{\vartheta} = 0.56 \pm 0.11|_{45\text{nm}}$ in agreement with the theory [12, 11]. Fig. 5.5b shows the computed $D_{\text{inst}}$ using the definition 5.5. As expected, $D_{\text{inst}}$ reaches its universal limit $D_{\infty}$ according to eq. 3.13 with $\vartheta = 0.57 \pm 0.12|_{15\text{nm}}$ and $\vartheta = 0.53 \pm 0.15|_{45\text{nm}}$, consistent with the scaling of the cumulative diffusion coefficient ($\tilde{\vartheta} \simeq \vartheta$), and the theory. Tabulated results of the observed dynamical exponents, $\vartheta$ and $\tilde{\vartheta}$, structural exponents, $p$, disorder classes and expected power law exponents based on the theory are shown in Table 5.1.

For $t > \tau_{r\text{HYP}} \simeq 162.0\text{ ms}$ a dynamical exponent of $\tilde{\vartheta} = 0.99 \pm 0.14$ was observed for H$_2$O diffusing through the phantom exhibiting hyperuniform disorder as described by definition 5.4. Using definition 5.5, $D_{\text{inst}}$ reveals the true dynamical exponent $\vartheta = 1.47 \pm 0.18$, is in excellent agreement with the theory.

Our experiments reveal similarities between the two phantoms exhibiting short-range disorder (Fig. 5.3d, A-green, B-blue) via the structural exponent $p$, and dynamical exponent
verifying the essence of universality. The coefficients \( c \) and \( D_\infty \), for the two phantoms, are different due to local variations in the spacing, as well as the variations in the diffusive permeability \( \kappa \), while the dynamical exponent remains the same due to long-range structural correlations. On the other hand, qualitative differences were revealed between the phantoms exhibiting short-range (A-green, B-blue), and hyperuniform disorder (C-red) based on the dynamical exponent \( \tilde{\eta} \), verifying that diffusion could distinguish the disorder class of the medium.

Statistics of the power law least squares fit

Cumulative diffusion coefficient \((D)\)

A least squares power law fitting procedure with three degrees of freedom was used to estimate the dynamical exponent \( \tilde{\eta} \), the universal diffusion coefficient at long times \( D_\infty \), and the coefficient \( c \). The results are shown as solid lines in Fig. 5.5b and the \( \chi^2/DOF \) for all three fitting procedures was approximately 0.8. The resulting \( \tilde{\eta} \) from the least squares fitting procedure is shown in Table 5.1.

Instantaneous diffusion coefficient \((D_{\text{inst}})\)

The computation of \( D_{\text{inst}} \) reported in Fig. 5.5c requires taking the derivative of the measured cumulative diffusion coefficient as defined in 5.5. This operation amplifies the experimental noise and therefore a Savitzky-Golay (SG) filter with a dynamical window that increases with respect to time was implemented [41, 42]. Figure 5.7 shows the statistics of SG window size and polynomial order for the \( \chi^2/DOF \) and \( \tilde{\eta} \pm \epsilon_{\tilde{\eta}} \) for the phantom exhibiting hyperuniform disorder. The statistics for different SG window size (as percentage) are shown in Fig. 5.6a.
A two degrees of freedom least squares fit was used for \( \vartheta \) and \( c \) by using the \( D_\infty \) value from the cumulative diffusion coefficient fit. The resulting \( \vartheta \) was observed to converge to the expected theoretical values as the SG window is increased. The computed \( \chi^2/DOF \) is shown to decrease with respect to the SG window and plateaus to a reasonable value \((\simeq 3)\) for short-range disorder shown in blue and hyperuniform disorder \((\simeq 2)\) shown in red. On the other hand, for the other phantom exhibiting short-range disorder (green) the \( \chi^2/DOF \) had a higher value \((\simeq 7)\) due to the increased fluctuations in the experimental data. The minimum SG window size and polynomial order were chosen such that the \( \chi^2/DOF \) and the error in \( \vartheta \) is minimum. We chose an SG filter with a polynomial order of 2, and a filtering window of \( \pm 60\% \) of the central time point on the fitting polynomial. The results of the least squares fit procedure for \( \vartheta \) with respect to the initial conditions are shown in Fig. 5.6b-c-d and show a reasonable Gaussian shape with a tail for the phantoms A-B (green-blue) and a bimodal distribution for C (red).

### 5.2.3 Diffusive permeability of a single barrier

As discussed in chapter 3.6 the universal limit of the diffusion coefficient, \( D_\infty \), is related to the diffusive permeability of the barriers \((\kappa, \frac{\mu m}{ms})\) via eq. 3.20. To determine the scaling of the diffusive permeability with respect to pore size the barriers were stacked in a layered geometry at random positions so that the phantoms exhibit short-range disorder. Four sets of barriers were used to construct the four samples, each set with different pore diameter \( f \) \((15 \text{ nm}, 45 \text{ nm}, 65 \text{ nm}, \text{reference})\). The reference set was made of the same starting material (polycarbonate) as the other sets but no pores were formed \((f \simeq 0)\); the diffusive
permeability of this barrier therefore would correspond to the material and not the pores. The diffusion coefficient of H₂O was measured along z using diffusion NMR techniques described above. Fig. 5.8a highlights the scaling of the cumulative diffusion coefficient of H₂O in the four phantoms as well as unrestricted H₂O. As expected, no time-dependance was observed for unrestricted H₂O. On the other hand, for short-range disorder the cumulative diffusion coefficient was observed to scale as a power law with power law exponent of approximately 1/2. Performing a least squares fit procedure \( D_\infty \), coefficient \( c \) and the dynamical exponent \( \vartheta \) may be determined. The tabulated results of the three component fit along with the diffusive permeability, pore diameter, disorder class and \( \bar{a} \) are shown in table 5.2. Fig. 5.8b highlights the scaling of the diffusive permeability with respect to pore diameter. Unfortunately, no model exists for the prediction of the scaling of the diffusive permeability with respect to pore diameter. To predict this scaling one needs to modify Purcell’s model [43] for finite permeable cylinders. Since this is an ongoing project I will not include any discussion in this thesis.

<table>
<thead>
<tr>
<th>( \bar{a} ) (( \mu m ))</th>
<th>( f ) (nm)</th>
<th>Dis. Cla.</th>
<th>( \kappa ) (( \mu m/\text{ms} ))</th>
<th>( \vartheta )</th>
<th>( D_\infty ) (( \mu m^2/\text{ms} ))</th>
<th>( c )</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>reference</td>
<td>Short-range</td>
<td>0.07 ± 0.01</td>
<td>0.43 ± 0.15</td>
<td>0.33 ± 0.04</td>
<td>2.98 ± 0.86</td>
</tr>
<tr>
<td>6</td>
<td>15</td>
<td>Short-range</td>
<td>0.10 ± 0.02</td>
<td>0.59 ± 0.09</td>
<td>0.42 ± 0.04</td>
<td>5.72 ± 1.62</td>
</tr>
<tr>
<td>6</td>
<td>45</td>
<td>Short-range</td>
<td>0.13 ± 0.02</td>
<td>0.56 ± 0.11</td>
<td>0.58 ± 0.03</td>
<td>3.79 ± 1.18</td>
</tr>
<tr>
<td>6</td>
<td>65</td>
<td>Short-range</td>
<td>0.20 ± 0.02</td>
<td>0.53 ± 0.08</td>
<td>0.73 ± 0.03</td>
<td>3.19 ± 0.82</td>
</tr>
</tbody>
</table>

Table 5.2: Tabulated results of the phantom characteristics, least squares fit parameters and diffusive permeability.
Figure 5.3: Structural characterization of the constructed phantoms exhibiting short-range and hyperuniform disorder as revealed by the low $k$ behavior of the autocorrelation function $\Gamma(k) \sim k^p$. a) Representative optical microscopy image of the phantom exhibiting short-range disorder. b) Optical microscopy image of the phantom exhibiting hyperuniform disorder. As discussed in the text (see Methods) rectangular copper plates (shown in Fig. S-5.1 were inserted to achieve the hyperuniformity. c) Atomic Force Microscopy image of a single polycarbonate film used to construct the disorder classes. d) Digitized 1d cut-outs of the phantoms constructed in this work shown in a) and b): two different realizations of short-range disorder (A-B) and hyperuniform disorder realization (C). Diffusion was measured along the $z$ axis using Nuclear Magnetic Resonance techniques. e) Autocorrelation function in Fourier space, $\Gamma(k)$, reveals the qualitative differences between the disorder classes as $k \to 0$. A plateau is observed for the phantoms exhibiting short-range disorder (green-blue); on the other hand $k^2$ scaling is observed for hyperuniform disorder (red).
CHAPTER 5. OBSERVATION OF UNIVERSALITY WITH DIFFUSION

Figure 5.4: Universal short-time behavior of the diffusion coefficient as proposed in [10, 40] reveals the mean distance between the permeable films $\bar{a}$ and the unrestricted diffusion coefficient $D_0$. However, the qualitative differences between the disorder classes are not revealed. a) Universal short-time scaling behavior of the cumulative diffusion coefficient of H$_2$O diffusing through the samples exhibiting short-range (green-blue) and hyperuniform (red) disorder show the characteristic $t^{-1/2}$ power law footprint for $t << \tau_D \equiv \bar{a}^2/2D_0$. b) Universal short-time scaling behavior of the instantaneous diffusion coefficient shows the same characteristic $t^{-1/2}$ power law footprint. Inset: Instantaneous diffusion coefficient as calculated with the parameters obtained from least squares fitting procedure of the cumulative diffusion coefficient (a). Reasonable agreement was found between the two definitions of eq. 5.4 and 5.5.
Figure 5.5: The dynamical exponent of the diffusion instantaneous coefficient reveals the qualitative differences between the disorder classes. a) Relative deviation of the cumulative diffusion coefficient, $D$, of H$_2$O from its tortuosity limit $D_\infty$ distinguishes between short-range and hyperuniform disorder. For short-range disorder (green, blue) the observed power law exponents were $\tilde{\vartheta} = 0.59 \pm 0.09|_{15nm}$ and $\tilde{\vartheta} = 0.56 \pm 0.11|_{45nm}$ in agreement with the theory [11, 12]. On the other hand, for hyperuniform disorder (red), the power law exponent $\vartheta = 3/2$ is masked by the $\tilde{\vartheta} = 0.99 \pm 0.14$ in the definition of eq. 5.4. b) For $t > \tau_r$, $D_{inst}$ reveals the qualitative differences between the two disorder classes. Experimental $D_{inst}$ of H$_2$O diffusing through the phantoms exhibiting short-range disorder (green, blue) reveals power law exponents $\vartheta = 0.49 \pm 0.18|_{15nm}$ and $\vartheta = 0.44 \pm 0.15|_{45nm}$ consistent with the cumulative diffusion coefficient and the theory. Experimental $D_{inst}$ of H$_2$O diffusing through the phantom undergoing hyperuniform disorder (red) reveals a power law of $\vartheta = 1.48 \pm 0.12$ consistent with the theory.
Figure 5.6: Statistics of the Savitzky-Golay smoothing procedure and least squares power law fit with two degrees of freedom. a) Resulting power law least squares fit and error, $\vartheta \pm \epsilon_\vartheta$, with respect to Savitzky-Golay filtering window of the three disorder classes. As discussed in the text increasing the filtering window reduces the noise, and thus the error, revealing the true exponent $\vartheta$. Reduced $\chi^2$ with respect to Savitzky-Golay filtering window of the three disorder classes. The chosen dynamic window for the three data sets was $\pm 0.6$ (60%) of the central time point of the window. b-c-d) Statistics for the power law fit and reduced $\chi^2$ of the filtered $D_{\text{inst}}$ for the three disorder classes for multiple initial conditions. The resulting least squares fit parameters, $\vartheta$ and $c$ (not shown) were robust for short-range disorder (blue) and hyperuniform disorder (red) as shown from the reduced $\chi^2$ distribution. The resulting least squares fit parameters for short-range (green) don’t show large deviation with respect to the initial conditions but the reduced $\chi^2$ is high due to the increased scattering in the data.
Figure 5.7: Statistics of the Savitzky-Golay smoothing procedure (percentage) with respect to the polynomial order for the phantom exhibiting hyperuniform disorder (C-red). a) $\chi^2/DOF$ diverges as the polynomial order is increased. b) Least squares power law fit with two degrees of freedom reveals that the fitted value for $\vartheta$ converges to the expected power law for low polynomial order and converges for high polynomial order.
Figure 5.8: a) Cumulative diffusion coefficient of H₂O diffusing through the four short-range disordered phantoms with respect to the diffusion time. The measured power law exponents are consistent with the theory for short-range disorder in one dimension. b) Diffusive permeability with respect to pore diameter $f$. 
Chapter 6

Gas Storage in Vycor disordered glass

This Chapter deviates from the field of diffusion in disordered media and highlights results published in ref. [44]. It discusses gas adsorption in Vycor glass, and describes two models of adsorption which may be used for determining the density of the adsorbed layer and the number of layers in adsorption processes; namely a modified Langmuir model for monolayer adsorption and a modified BET model for multilayer adsorption.

The storage properties of methane gas in Vycor porous glass (5.7 nm) are characterized in a wide pressure range from 0.7 MPa-89.7 MPa using Nuclear Magnetic Resonance (NMR). We demonstrate the capability of high field NMR relaxometry for the determination of the methane gas storage capacity and the measurement of the Hydrogen Index, to a high degree of accuracy. This helps determine the excess gas in the pore space which can be identified to exhibit Langmuir properties in the low pressure regime of 0.7 MPa to 39.6 Mpa. The Langmuir model enables us to determine the equilibrium density of the monolayer of adsorbed gas to be 8.5% lower than that of liquid methane. We also identify the signatures of multilayer adsorption at the high pressure regime from 39.6 Mpa to 89.7 Mpa and use
the Brunauer-Emmet-Teller (BET) theory to determine the number of adsorbed layers of methane gas. We show how these measurements help us differentiate the gas stored in the Vycor pore space into free and adsorbed fractions for the entire pressure range paving way for similar applications such as studying natural gas storage in gas shale rock or hydrogen storage in carbon nanotubes.

NMR has become an invaluable tool, especially in oil and gas exploration applications where it is routinely applied for downhole logging and laboratory rock core analysis. NMR has also found broader applications in energy research including investigations of methane gas in shale rocks [45, 46, 47] and hydrogen storage in carbon nanotubes and other nanoporous materials [48, 49].

The storage of gas molecules in porous media has seen renewed interest especially due to recent advances in natural gas production from gas shale reservoirs. Gas shales are organic-rich mudstones characterized by the presence of nanometer size organic kerogen pores, hosting natural gas. The gas molecules in the nanopores coexist in two phases: free gas that has bulk-like properties and adsorbed gas that undergoes strong interactions with the pore walls [47]. These two phases are in fast exchange during the time scales of the NMR experiment [50]. In addition, the nature and dynamics of these two phases depend on the temperature and pressure of the system, making adsorption studies of great importance for understanding natural gas storage and extraction. Though NMR of fluids in porous media is a well-established field covering studies such as those of diffusion of supercritical fluids in nanoporous media using pulsed field gradient techniques [51, 52, 53] and methane gas adsorption on graphite [54], the storage properties of natural gas in nano pores at pressures
close to the highest pressures encountered in gas shale reservoirs (~ 90 MPa) have not been studied to our knowledge.

In this work we describe a detailed study of methane gas storage in nanoporous Vycor glass. We confirm spin-rotation to be the dominant contributor to the relaxation Hamiltonian of bulk methane gas, up to ultra-high pressures of 70 MPa. We demonstrate the capability of high field NMR to determine the gas capacity, Hydrogen-Index (HI) (sec. 6.2.1), adsorbed gas density, and enable the separation of free and adsorbed gas quantities in Vycor nanoporous glass at a wide range of pressures (0.7 MPa-89.6 MPa). We show how this analysis is enabled by the unique spin-spin relaxation times of the methane gas in the pore space. The quantification of methane gas stored in the nanopores together with the pore volume allow for a direct measurement of the effective Hydrogen-Index. We determine the excess gas in the pore space due to adsorption by using either monolayer or multilayer adsorption models for the appropriate low pressure and high pressure regimes. This enables the determination of the equilibrium density of the adsorbed layer and the number of adsorbed layers permitting the separation of the total gas into free and adsorbed fractions.

6.1 High-Pressure Experimental Setup

All experiments were performed on a Bruker 400MHz system equipped with high-pressure apparatus as shown in the schematic in Fig. 6.1. The main pressure generation was carried out through the syringe pump and the hand crank pressure generator, which were connected to the high pressure NMR sample tube. The hand crank pressure generator had a volume of 18 ml and was purchased from High Pressure Equipment Company (HIP). The syringe
Figure 6.1: Schematic representation of the experimental setup used in this work. The apparatus is composed of two pressure generators, the hand crank pump and the syringe pump. The upper pressure limit for this setup is 206.8 MPa. The pressure is regulated digitally to within 0.35 MPa.

A hand pump (Daedalus Innovations LLC) with a capacity of 6.7 ml and pressure rating of 413.7 MPa was used for secondary compression. The NMR sample tube (Daedalus Innovations) made of zirconium, had outer and inner diameters of 5.0 mm and 3.0 mm respectively, and was 92 mm long. Methane gas of 99.9% purity was purchased from Air-Gas (Radnor Township-PA) in a cylinder pressurized to 13.8 MPa. A three way valve was used to direct the methane gas to either the sample tube or the vacuum pump. All the connections were well evacuated before the experiments to purge out all oxygen in the system and avoid any additional relaxation effects. The high pressure components were connected with pressure tubing and valves rated to 413.7 MPa.

Vycor nanoporous glass manufactured by Corning Inc (New York, USA), was purchased
in the form of cylindrical rods of diameter 2.96 mm. They were cut into samples of 11.50 mm to fit in the center of the NMR coil (16.68 mm). The pore size of the Vycor rods was 5.7 nm and the porosity 28% of the sample volume, as determined by BJH adsorption studies.

The transverse relaxation times ($T_2$) were measured using the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence [55, 56]. The two dimensional $T_1$-$T_2$ correlation experiments were performed by using the inversion recovery pulse sequence in the indirect dimension and the CPMG pulse sequence in the direct dimension, as shown in Fig. 6.2. The time interval $\tau$ for encoding in the indirect dimension was varied from 10ms to 60s (the time at which the magnetization is fully relaxed). The number of $\pi$ pulses was chosen to be 32,000 to cover the entire $T_2$ spectrum and the echo time $T$ was 200$\mu$s. Diffusion effects were confirmed to be negligible at such short echo times by observing the attenuation of the first five echoes at various echo times [57]. The last delay (repetition time) between scans was varied for each pressure from 20s to 60s. The temperature was kept constant at 22$^\circ$C throughout all the experiments with an accuracy of $\pm$1$^\circ$C.

The RF probe and the receiver were calibrated to measure the number of $^1H$ nuclei in
the sample by carrying out $T_2$ measurements of $5\mu L$ to $40\mu L$ of $H_2O$ samples at $22^\circ C$ and atmospheric pressure. We determined the accuracy of the calibration to be within 4% by comparing the NMR signals of bulk methane gas at various pressures with its density values from National Institute of Standards and Technology (NIST) [58].

6.2 Experimental results

6.2.1 Relaxation of free and restricted methane gas

The transverse ($T_2$) and longitudinal ($T_1$) relaxation times of bulk methane gas were measured for a wide range of pressures from 3.5 MPa to 68.9 MPa at $22^\circ C$. The critical pressure and temperature of methane are 4.6 MPa and -82.75$^\circ C$ respectively, and therefore most experiments were conducted in the super critical state. The measured $T_1$ relaxation times are consistent with the literature data at the low pressure range measured by Watson et al. [59] as shown in Fig. 6.3. The relaxation times of bulk methane gas are dominated by the spin rotation mechanism at low to moderate pressures while at high pressures, due to the increased density, the mean free path decreases and inter molecular dipolar interactions could start playing a role [60, 44].

In the motional narrowing limit the relationship between the relaxation rates is given by,

$$\frac{1}{T_1} = \frac{1}{T_2} = \frac{2(C^2_{\parallel} + 2C^2_{\perp})I_1\tau_F}{3h^2} kT.$$  \hspace{1cm} (6.1)

In the above expression $\tau_F$ is the rotational correlation time, $k$ is the Boltzmann’s constant, $I_1$ is the moment of inertia of the spherical molecule, $T$ is the temperature and $C^2_{\parallel}$,
Figure 6.3: Longitudinal ($T_1$) and transverse ($T_2$) relaxation times of bulk methane gas at various pressures at 22°C. To convert the magnetization decays into relaxation distributions, a fast inverse Laplace transform algorithm was used [61] and each experimental point corresponds to the log mean of the entire 1D distribution. The reduced value of $T_2$ in comparison to $T_1$ is because of the interactions with the sample tube walls. The literature values of $T_{1\text{literature}}$ (magenta) are taken from Ref. [59].
are the principal components of the spin rotation tensor \(C_\perp\). The correlation time, \(\tau_F\), is given by,

\[
\tau_F = \frac{3I_1D}{4a^2kT},
\]

(6.2)

where \(D\) denotes the diffusion coefficient of the molecule which depends on the viscosity as \(D = kT/6\pi\eta a\), with \(a\) being the radius of the molecule and \(\eta\) the viscosity.

Bulk methane gas is in the motional narrowing limit at the Larmor frequency of the experiments \((f = 400 MHz)\) and therefore \(T_1\) should be equal to \(T_2\). However, as shown in Fig. 6.3 the \(T_2\) values are consistently shorter than the \(T_1\) values at all measured pressures. This is because the spin-spin relaxation times \((T_2)\) are sensitive to the slow motions of methane molecules caused by their interaction with the NMR sample tube walls. This may be understood by inspecting the frequency dependence of the relaxation rates \(J(\omega)\),

\[
T_1^{-1}(\omega) = \left(\frac{\mu_0}{4\pi}\right)^2 \frac{3\gamma^4h^2I(I + 1)}{2r^6} \left[\frac{1}{4}J^{(1)}(\omega) + J^{(2)}(2\omega)\right],
\]

(6.3)

and,

\[
T_2^{-1}(\omega) = \left(\frac{\mu_0}{4\pi}\right)^2 \frac{3\gamma^4h^2I(I + 1)}{2r^6} \left[\frac{1}{4}J^{(0)}(0) + \frac{1}{2}J^{(1)}(\omega) + \frac{1}{4}J^{(2)}(2\omega)\right],
\]

(6.4)

where \(\mu_0\) is the vacuum permeability, \(I\) is the spin number, \(\gamma\) is the gyromagnetic ratio and \(r\) is the internuclear vector between the \(^1H\) nuclei. The spectral densities \(J^{(n)}(\omega)\) can be obtained by the Fourier transform of the autocorrelation function \(G(t) \equiv \langle B(t)B(t + \tau)\rangle\)
which describes the time dependent fluctuations of the local magnetic field $B(t)$. The $T_2$ relaxation times are dominated by the $J^{(0)}(\omega = 0)$ term and are therefore very sensitive to the low frequency or slow motions while the $T_1$ relaxation times are sensitive to the much higher Larmor frequency ($\omega_0 \& 2\omega_0$).

Assuming Gaussian diffusion in 1D, the mean displacement of the methane gas molecules may be calculated as, $\langle \Delta x(t) \rangle = (2Dt)^{1/2}$, where $D$ is the bulk diffusion coefficient of methane and $t$ is the diffusion time period. At a pressure of 20.7 MPa the spins have a self-diffusion coefficient of $7.7 \times 10^{-8} m^2/s$ [64] and a spin lattice relaxation time of 5.2s [65], resulting in displacements of 0.9 mm during their $T_1$ lifetimes. As the high pressure tube has an inner diameter of 3.0 mm, a fraction of the spins interact with the tube walls. This makes the magnetization decay of the $T_2$ measurement (CPMG sequence) multi-exponential. The $T_2$ relaxation times shown in Fig. 6.3 are the log mean values of the $T_2$ distributions. The spin lattice relaxation times ($T_1$), being only sensitive to motions that occur around the Larmor frequency ($f = 400 MHz$), are not affected and the corresponding magnetization decays are mono exponential.

The total Hamiltonian for the spin ensemble in the rotating frame may be written as, $H_{total} = H_{ij}^{DD} + H_{SR}$ where $H_{SR}$ describes the spin rotation interaction and $H_{ij}^{DD}$ is the dipolar Hamiltonian respectively. At the pressure of 68.9 MPa the density of bulk methane is still 26% lower than that of liquid methane. At low pressures where the total Hamiltonian is completely dominated by spin rotation interaction an empirical relation between the spin lattice relaxation times and the methane gas density was obtained [67].
Figure 6.4: Longitudinal ($T_1$) relaxation times of bulk methane gas at various densities at 22°C. The 1D data were processed using the ILT algorithm [66] and each experimental point (blue circles) corresponds to the log mean of the entire 1D distribution. The solid red line corresponds to the empirical prediction of $T_1$ relaxation times for spin rotation interaction taken from [67]. As discussed in the text, the empirical prediction fits the data implying that spin rotation interactions dominate the relaxation Hamiltonian at even such high densities for bulk methane.
\[ T_{1SR} = c \cdot \rho \cdot T^{-3/2}, \]  

where \( \rho \) is the density in g/cm\(^3\), \( T \) is the temperature in K and the constant \( c = 1.57 \cdot 10^5 [cm^3K^{3/2}sg^{-1}] \). This empirical relation was obtained by Sho-Wei et al. [67] using experimental data of longitudinal relaxation times of methane gas for pressures up to approximately 20 MPa.

In Fig. 6.4 we compare our measured \( T_1 \) relaxation times with the computed values using the empirical relation given by eq. 6.5 for the entire pressure range from 3.4 MPa to 68.9 MPa. Excellent agreement (\( \chi^2/dof \simeq 1 \) [68]) was found between the empirical prediction of eq. 6.5 right from lower pressures up to 68.9 Mpa (0.307 g/cm\(^3\)) demonstrating that \( H_{total} \) is dominated by the spin rotation interaction throughout this pressure range.

It should be noted here that no phase transition effects were observed when traversing the critical pressure (\( P_c = 4.6 \) MPa) at \( T = 22^\circ C \) as indicated by the \( T_1 \) and \( T_2 \) relaxation times. Previous studies across the critical point (traversing both the critical pressure and temperature) showed changes in the relaxation times due to critical density fluctuations [69, 70] and distribution of correlation times [71], but such effects were not seen as we are far from the critical points in our experiments.

**Relaxation of methane gas in Vycor glass**

Vycor porous glass is composed of of 96% \( SiO_2 \) and 4% \( Br_2O_3 \) and the samples used in this study had a porosity of 28% and an average pore size of 5.7 nm. This results in a high surface to volume ratio of 1.25\( nm^{-1} \) that leads to enhanced surface interaction of the
confined methane molecules with the pore walls. The NMR relaxation of spin 1/2 nuclei in porous media generally occurs through two dominant pathways. The first is the slow motions at the surfaces resulting in enhanced relaxation caused by mechanisms such as Reorientation Mediated by Translational Diffusion (RMTD) [72]. This effect is due to the geometrical characteristics of the confining system. The second is the dipolar relaxation due to the presence of paramagnetic impurities in the porous medium. As Vycor does not host significant quantities of paramagnetic impurities [73], the methane gas relaxation in this case is expected to be dominated by the slow motions at the pore walls [44].

2D $T_1 - T_2$ relaxation

The results of the $T_1 - T_2$ correlation experiments of methane in Vycor at 27.6 MPa are shown in Fig. 6.5. The relaxation times were measured using an inversion recovery pulse sequence followed by the CPMG sequence as shown in Fig. 6.2 [15] and the results were analyzed using an Inverse Laplace Transform (ILT) algorithm [61]. Three well distinguishable peaks were observed in the direct dimension ($T_2$). On the other hand, the indirect dimension shows a single $T_1$ peak at a value of about 4.0s. This is because the $T_1$ relaxation mechanism is only sensitive to motions at the Larmor frequency ($f = 400 MHz$) and is therefore not affected by the slow motions of methane gas in the different environments.

The different peaks in the $T_2$ dimension are a result of the methane molecules in different environments experiencing different relaxation pathways. In the following sections we identify the origin of the different components of relaxation in Vycor glass and use this information to quantitatively assess the adsorption processes at the high and low pressure
Figure 6.5: $T_1 - T_2$ correlation map for methane gas in Vycor glass at a pressure of 27.6 MPa and temperature of 22°C is shown. While only one peak can be resolved in the $T_1$ dimension, the $T_2$ dimension allows for separation of different components in the form of three different peaks. The right most peak correspond to the annulus gas while the center and the left most correspond to the methane gas in the Vycor pore space. The intensity bar on the right is in [a.u].
CHAPTER 6. GAS STORAGE IN DISORDERED SYSTEMS

regimes.

Spin-spin relaxation

The pressure dependence of the $T_2$ relaxation times of methane gas in Vycor is shown in Fig. 6.6. The peak with the longest relaxation time, labeled $\gamma$ in Fig. 6.6, corresponds to the free methane gas in the annulus of the NMR tube (between the Vycor cylinder and the sample holder) and above the Vycor glass cylinder. This free methane gas relaxes mainly via spin rotation interaction resulting in relaxation times comparable to those of the bulk gas. The small difference in the relaxation times of these methane molecules from that of the bulk (2.4s instead of 2.9s at 27.6 MPa) is due to surface relaxation occurring on the Vycor rod surface and the NMR tube walls. The identity of this phase was further confirmed by carrying out experiments with a teflon tape covering the annulus space, resulting in drastic reduction of this long relaxing peak.

The $\beta$ component of Fig. 6.6 corresponds to the methane gas in the pore space of Vycor. The significant reduction in the spin-spin relaxation time of the $\beta$ peak with respect to the annulus gas ($\gamma$ component) is because of the enhanced surface interaction of the methane molecules with the pore walls. The strong pressure dependent increase in the intensity of this peak compared to bulk methane gas is due to adsorption of methane molecules on the pore walls. Adsorbed gas has much higher density compared to bulk gas resulting in higher spin density in the pores. As the time scale of exchange between the free and adsorbed gas is much faster than the NMR relaxation time scale [50], these two components cannot be separated and result in a single $T_2$ distribution for the free and adsorbed phases. The
Figure 6.6: Transverse relaxation times ($T_2$) of methane gas in Vycor are shown for various pressures at a constant temperature of 22°C. The pressure dependence of the signal intensity and the $T_2$ values for the different components can be identified. The $\alpha$ component is zoomed in the inset to highlight the background signal of Vycor.

$\alpha$ component corresponds to the small number of methane gas molecules trapped in pore spaces of smaller dimensions. Vycor porous glass has a pore size distribution that peaks at 5.7 nm and also hosts a few smaller pores due to geometrical disorder. These molecules undergo even higher surface interactions resulting in relaxation times on the order of 1ms. The smaller tortuous, dead-end channels can also reduce molecular mobility [74]. A small background signal with the same relaxation time as the $\alpha$ component was observed before gas injection which may correspond to trapped water molecules that could not be evacuated by the vacuum procedure and is shown as an inset in Fig. 6.6.
CHAPTER 6. GAS STORAGE IN DISORDERED SYSTEMS

2D \(T_2 - T_2\) relaxation

To further understand the dynamics of the different \(T_2\) distributions [44], diffusive coupling of the methane gas between the different environments was probed through the two dimensional \(T_2 - T_2\) correlation measurements [75, 76, 77]. This experiment consists of the measurement of spin-spin relaxation time in the indirect dimension ("evolution time") using a CPMG sequence, followed by storage of the magnetization in the z-axis ("storage time"), during which the spins relax by \(T_1\) relaxation. The latter is followed by measurement of the spin-spin relaxation time during acquisition using another CPMG sequence. The number of CPMG echoes in the indirect dimension was varied to acquire a 2D \(T_2 - T_2\) plot for different storage times. As the spin lattice relaxation times (\(T_1 \sim 2\)s) at 400MHz are very long compared to the \(T_2\), the methane gas molecules could diffuse from one environment to another during the storage intervals resulting in non diagonal peaks in the 2D \(T_2 - T_2\) map.

The exchange between two coupled environments may be quantified via the variation of the cross peak intensities with respect to the storage time, only in the slow exchange limit, i.e. the exchange is slow during the evolution times [75]. This condition is not satisfied in our experiments with significant exchange occurring between the coupled environments during the CPMG sequence (evolution time), and therefore accurate exchange times could not be extracted. However, the experimental data shown in Fig. 6.7 suggest that all three environments are coupled at the time scales of 100ms, as cross peaks appear in the \(T_2 - T_2\) correlation plots. This indicates that the different gas species are coupled and that the \(\alpha\) and \(\beta\) peaks together correspond to the gas molecules in the Vycor pore space and therefore should be integrated to obtain the total stored gas.
Figure 6.7: Two dimensional map of $T_2 - T_2$ correlation experiment of methane in Vycor glass acquired with a storage time of 100ms. The diffusive coupling between different geometrical compartment results in non diagonal cross peaks. The first two peaks with the shorter relaxation times in the diagonal axis correspond to the gas stored in the Vycor pore space while the right most peak correspond to the gas in the annulus space.
Methane gas Hydrogen-Index in porous media

The gas stored in Vycor glass pore space is the sum of the $\alpha$ and $\beta$ $T_2$ distributions shown in Fig. 6.6 [44]. In Fig. 6.8 the methane gas stored in the Vycor pore space as a function of pressure is plotted. Note that the background signal as shown in the inset of Fig. 6.6 was subtracted to obtain the signal from the gas stored in the Vycor pore space. The stored gas was quantified using the calibration of the system to the signal from the $^1H$ nuclei in pure water. The results of the calibration of the system based on various volumes of pure water are shown as an inset of Fig. 6.8. It should be noted that by using a Vycor sample 2.96 in diameter leads to a low filling factor. However, the results shown in Fig. 6.8 indicate that the low filling factor presents no challenge.

The Hydrogen Index (HI) of a fluid is an important parameter for oil and gas exploration as it is used for the interpretation of various downhole logging measurements, including NMR, to determine the quantity of fluids such as hydrocarbons and water. The HI is defined as the density of $^1H$ in the sample, relative to that of water [47]. In the case of gas filled rock samples the hydrogen index of the gas phase is given by [78],

$$HI_g = \frac{\rho_g n_{Hg}}{\rho_w \cdot MW_g},$$

(6.6)

where $\rho_w = 0.11$ moles $\cdot$ cm$^{-3}$ and $\rho_g$ are the molar and mass densities of the water and gas, $MW_g$ is the molecular weight of methane gas and $n_{Hg}$ is the number of hydrogens in a single methane molecule ($n_{Hg} = 4$).

While the HI and its pressure dependence for bulk oil, water and natural gas are well known [78], the values for natural gas in shale nanopores are difficult to determine. This is
Figure 6.8: Methane gas stored in Vycor plotted as a function of pressure. The stored methane gas is shown to increase steeply at lower pressures and slowly saturates as the fluid density approaches that of liquid methane. The calibration of the signal intensity to the number of protons was carried out by using various volumes of H₂O and is shown in the inset.
CHAPTER 6. GAS STORAGE IN DISORDERED SYSTEMS

Figure 6.9: Effective Hydrogen index (HI) computed based on the measurement of the gas stored in the Vycor pore space. The free methane gas hydrogen index at various pressures is also shown as a comparison.

because the gas in the shale pores exists as free and adsorbed phases whose densities also have a complex pressure dependence.

For petrophysical and log interpretation applications an effective HI is defined which is a weighted average of the free and adsorbed gas hydrogen indices in the porous medium. The hydrogen index of bulk methane gas occupying a pore volume equivalent to that of the Vycor sample (length of 11.50 mm, volume of 0.022 ml) is compared with the measured HI of methane gas in Vycor in Fig. 6.9. The effective Hydrogen Index of methane in the porous media is consistently higher than the bulk methane gas under the same conditions (pressure-volume-temperature) due to the increased density of the adsorbed gas. The measurement of the effective HI on real rock sample cores at formation temperatures and pressures using this methodology potentially enables direct log interpretation for obtaining fluid quantities.
6.2.2 Monolayer and multilayer gas adsorption in disordered systems

The gas inside the Vycor pore space exists in two phases, namely as adsorbed gas on the pore walls and as free gas in the center of the pore [44]. As the free and adsorbed gas are in fast exchange in the NMR relaxation time scales (1 ms to 50 ms), the measured $T_2$ relaxation times of the gas in the pores are a weighted average of the two phases. The pressure dependence of the free gas can be approximated by that of bulk methane while that of the adsorbed gas is more complex.

The quantity $N_{\text{excess}}$ can be defined as the excess number of gas molecules in the pore space accommodated due to adsorption, compared to the number of bulk gas molecules that would have occupied the same pore volume in the absence of adsorption. This quantity is given as [79, 46],

$$N_{\text{excess}} = N_G - \rho_{\text{free}} V_{\text{pore}},$$

where $N_G$ is the total gas in the porous medium, $\rho_{\text{free}}$ is the density of the bulk methane gas and $V_{\text{pore}}$ is the volume of the pore space as specified by the manufacturer. The total gas in the pore space corresponds to the sum of the $\alpha$ and $\beta$ contributions of the $T_2$ distributions (Fig. 6.6). The second term $\rho_{\text{free}} V_{\text{pore}}$ corresponds to the gas in the pore space assuming no adsorption.

The excess gas in Vycor pore space may also be written as [44],

$$N_{\text{excess}} = N_{\text{ads}} - \rho_{\text{free}} V_{\text{ads}} = N_{\text{ads}} \left(1 - \frac{\rho_{\text{free}}}{\rho_{\text{ads}}} \right),$$

(6.8)
Figure 6.10: The quantity $N_{\text{excess}}$ as a function of pressure determined experimentally is fit to the modified Langmuir isotherm (eq. 6.10) at the low pressure regime. This least squares fit with three degrees of freedom helps determine the adsorbed density layer, $\rho_{\text{ads}}$, Langmuir pressure, $P_L$, and the maximum number of adsorbed molecules, $N_L$. The inset figure shows $N_{\text{excess}}$ for the entire pressure regime, which can be described by multilayer adsorption models as described in the subsequent section.
where \( N_{\text{ads}} \) is the number of adsorbed gas molecules, \( \rho_{\text{free}} \) and \( \rho_{\text{ads}} \) are the densities of the free and adsorbed gas phases and \( V_{\text{ads}} \) is the volume of the pore space occupied by the adsorbed gas. The pressure dependence of \( N_{\text{ads}} \) helps identify the adsorption process due to its unique characteristics for monolayer [80] or multilayer adsorption [81] corresponding to the low or high pressure regimes. In the following sections we identify the pressure regimes corresponding to the Langmuir monolayer adsorption and BET multilayer adsorption and determine the density and volume occupied by the adsorbed layer, \( \rho_{\text{ads}} \) and \( V_{\text{ads}} \), as well as the number of adsorbed layers formed at higher pressures.

**Monolayer adsorption**

In the low pressure regime (0.7 Mpa < \( P < 39.7 \) MPa) the adsorption process is established by a monolayer of adsorbed molecules which can be described by the Langmuir isotherm given as [80, 44],

\[
N_{\text{ads}} = N_L \frac{P}{P + P_L},
\]  

where \( P \) is the applied pressure, \( N_L \) the maximum number of adsorbed molecules at infinite pressure and \( P_L \) the pressure at which half of the adsorption sites are occupied.

The resulting excess number of gas molecules can then be described by the modified Langmuir isotherm, obtained by combining eq. 6.8 and eq. 6.9 as [79, 44],

\[
N_{\text{excess}} = N_L \frac{P}{P + P_L} \left(1 - \frac{\rho_{\text{free}}(P,T)}{\rho_{\text{ads}}} \right).
\]  

The \( N_{\text{excess}} \) computed using eq. 6.7 is shown in Fig. 6.10 up to \( P = 39.6 \) MPa where the
Langmuir monolayer model still holds. The three unknown parameters $N_L$, $P_L$ and $\rho_{ads}$ were obtained by performing least squares fit of the data to eq. 6.10 with $\chi^2/dof = 2.4$ [68, 42]. The values obtained by the fit for the Langmuir pressure, Langmuir volume and the equilibrium adsorbed gas density were, $P_L = 10.80 \pm 0.43$ [MPa], $N_L = (2.16 \pm 0.06) \cdot 10^{-4}$ [mol · g$^{-1}$] and $\rho_{ads} = (2.43 \pm 0.02) \cdot 10^{-2}$ [mol · ml$^{-1}$] and agree well with the values reported earlier for adsorption studies in activated carbon [79]. The density of the adsorbed layer ($\rho_{ads}$) was determined to be 8.5% lower than the density of liquid methane.

It can be seen in Fig. 6.10 that the quantity $N_{excess}$ increases at low pressures to reach a maximum at approximately 13.8 MPa and then decreases at higher pressures. This is because of the increase in the number of adsorbed molecules at lower pressures ($P < 13.8$ MPa), due to the presence of a large number of vacant surface sites. At pressures greater than 13.8 MPa the number of adsorbed molecules slowly plateaus out, resulting in a slow decrease in $N_{excess}$. At higher pressures and for monolayer adsorption, as the density of the free gas in the center of the pore approaches that of the adsorbed gas, $N_{excess}$ should asymptotically approach zero. However, at pressure values of $P > 39.6$ MPa we observe that the quantity $N_{excess}$ starts to slowly increase again as shown in the inset of Fig. 6.10. This constitutes a deviation from the Langmuir isotherm behavior resulting from the formation of multiple adsorbed layers on the pore surface. In the following section we analyze the entire data set up to 89.7 MPa using a generalized isotherm, accounting for the excess number of molecules in the pore space due to multilayer adsorption.
Multilayer adsorption

In this section we consider the Brunauer-Emmett-Teller (BET) model which describes multilayer adsorption to describe the adsorption of methane in Vycor porous glass up to pressures of 89.7 MPa. The number of adsorbed molecules for multilayer adsorption process is given by [81, 44],

\[
N_{\text{ads}} = N_L \frac{c x}{(1 - x)} \left( 1 - (N + 1)x^N + N x^{N+1} \right).
\] (6.11)

In the above equation, \( x = P/P_0 \), where \( P_0 \) is the saturation pressure, \( N \) is the number of adsorbed layers, \( N_L \) is the number of molecules in a monolayer and \( c \propto e^{(E_1-E_N)/RT} \) is a unitless constant related to the heat energies of the first and \( N \) layers (\( E_1, E_N \)). Substituting eq. 6.11 into eq. 6.8 we obtain the modified BET isotherm given as,

\[
N_{\text{excess}} = \frac{N_L c P}{P_0 - P} \left[ 1 - (N + 1)\left(\frac{P}{P_0}\right)^N + N\left(\frac{P}{P_0}\right)^{N+1} \right] \frac{1 + (c - 1)\left(\frac{P}{P_0}\right) - c\left(\frac{P}{P_0}\right)^{N+1}}{1 + (c - 1)\left(\frac{P}{P_0}\right) - c\left(\frac{P}{P_0}\right)^{N+1}} \cdot \left( 1 - \frac{\rho_f}{\rho_{\text{ads}}} \right).
\] (6.12)

It should be noted that eq. 6.12 reduces to a form of the Langmuir equation in the limit of \( P \ll P_0 \) and \( N = 1 \), given as,

\[
N_{\text{excess}} = N_L \frac{c P}{P_0 + c P} \cdot \left( 1 - \frac{\rho_f}{\rho_{\text{ads}}} \right),
\] (6.13)

which is equivalent to the Langmuir isotherm (eq. 6.10) for \( P_L \equiv P_0/c \).

Performing nonlinear least squares fit of eq. 6.12 for the excess gas in the pore space to the
Figure 6.11: The quantity $N_{\text{excess}}$ is fit for the entire pressure range using the modified multilayer BET adsorption model (eq. 6.12). This least squares fit with four degrees of freedom enables the determination of the adsorbed layers $N$, the constant $c$, the saturation pressure $P_0$ and the number of adsorbed molecules in a single layer $N_L$. The value for $\rho_{\text{ads}}$ obtained from the monolayer Langmuir adsorption fit in the low pressure regime (fig 10) was used as an input in the fit.

entire data set (shown in Fig. 6.11) up to pressures of 89.7 MPa we obtain, $P_0 = 168.0 \pm 14.1$ [MPa], $N_L = (1.4 \pm 0.3) \cdot 10^{-4}$[mol · g$^{-1}$], $c = (21.6 \pm 4.6)$, $N = (9.7 \pm 3.1)$. The fit had $\chi^2/dof = 0.7$ [42, 68]. The value for $\rho_{\text{ads}}$ obtained for the monolayer Langmuir adsorption in the low pressure regime (see sec. 6.2.2) was used as a constant in eq. 6.13 during least squares minimization in order to reduce the complexity of the fit.

The constant $c$ obtained by the fit results in a $(E_1 - E_N) = 7.5kJ/mol$ energy gap between the adsorbed layers at $T = 22\degree C$, where $E_1$ is the activation energy of adsorption. This value agrees with the activation energy values reported earlier for methane adsorption on activated carbon [82]. The saturation pressure $P_0$ and constant $c$ obtained by fitting the
experimental data help determine the Langmuir pressure \( P_L = P_0/c \). The fit value for the Langmuir pressure is in good agreement with that obtained by the monolayer Langmuir model for the low pressure regime (sec. 6.2.2-Monolayer adsorption). The number of layers of methane with average molecule diameter of 3.9 that can be formed into the Vycor pore with an average diameter of 5.7 nm is approximately 7.4. Note that the fit overestimates the number of adsorbed layers \( N = (9.7 \pm 3.1) \) formed in the Vycor pore but the obtained theoretical value is within the experimental uncertainty.

**Separation of free and adsorbed gas**

To separate the gas in the Vycor nanopores into free and adsorbed fractions for the entire pressure range from 0.7 MPa-89.7 MPa we make use of the density of the adsorbed layer obtained from the Langmuir isotherm fit. At low pressures \( P < 39.6 \) MPa the \( N_{\text{excess}} \) can be alternatively written in terms of the density of the adsorbed layer as,

\[
N_{\text{excess}} \mid P=39.6\text{MPa} = \rho_{\text{ads}} V_{\text{ads}} - \rho_{\text{bulk}} \mid P=39.6\text{MPa} V_{\text{ads}}. \tag{6.14}
\]

From the above equation the volume of a monolayer of adsorbed gas \( V_{\text{ads}} \) can be determined using the \( \rho_{\text{ads}} \) obtained from the Langmuir fit and the known values of \( \rho_{\text{bulk}} \). Using the adsorbed gas volume together with the pore volume of Vycor, the gas stored in the Vycor nanopores can be separated into free and adsorbed gas on the pore walls, for pressures up to 39.6 MPa. For pressures higher than 39.6 MPa, \( V_{\text{ads}} \) is pressure dependent as multiple
Figure 6.12: Separation of the total gas into free and adsorbed. The blue squares correspond to $^1H$ inside Vycor. Based on the $V_{\text{ads}}$ calculated from eq. 6.14, the total number of adsorbed $^1H$ was calculated (black diamonds). The red circles correspond to the total number of free $^1H$ in the pores. The bulk methane gas in Vycor (assuming no adsorption) is also shown in magenta triangles.
layers start to form and is therefore determined as,

\[ V_{\text{ads}}(P) \bigg|_{P=39.6\text{MPa}}^{P=89.7\text{MPa}} = \frac{N_{\text{excess}}(P) \bigg|_{P=39.6\text{MPa}}^{P=89.7\text{MPa}}}{\rho_{\text{ads}} - \rho_{\text{bulk}}}. \]  

(6.15)

The results of the free, adsorbed and total methane gas in Vycor porous glass are shown in Fig 6.12. Up to a pressure of 39.6 MPa the “free gas” molecules in the pore interiors (red circles in Fig 6.12) show a bulk-like pressure dependence while the adsorbed gas fraction increases faster at lower pressures but slowly saturates at higher pressures. This indicates that at low pressures \((P < 12 \text{ MPa})\) more gas is stored in the pore space as adsorbed gas. As discussed in Sec. 6.2.2, multiple adsorbed layers start to form for pressures \(P > 39.6 \text{ MPa}\) and therefore a rapid drop is observed in the “free gas” fraction. On the other hand the adsorbed gas in the pore space shows a rapid increase up to \(P = 89.7 \text{ MPa}\), where it is approximately 91% of the total methane gas stored in the pore space. The amount of bulk methane gas that could be accommodated in the pore volume, in the absence of adsorption is also shown in magenta. This way the total gas stored in the Vycor nanopores can be separated into free and adsorbed fractions in the entire pressure range from 0.7 Mpa to 89.7 MPa using high field NMR relaxometry.

In the present work we demonstrate the application of high field NMR for characterization of the storage properties of methane gas in nanometer sized pore spaces. We carry out laboratory experiments at a wide pressure range (0.7 MPa to 89.7 MPa), that covers most conditions encountered in naturally existing shale gas and tight gas reservoirs. We demonstrate that the relaxation of the bulk methane gas, which forms the dominant fraction of natural gas, is dominated by the spin rotation Hamiltonian up to the highest studied
pressures of 89.7 MPa. The sensitivity of the $T_2$ relaxation distributions to the slow motions enable the separation of the gas molecules in the pore space and thereby aid gas storage and HI measurements as a function of pressure.

The quantity of methane gas in Vycor pore space was used to determine the excess gas due to adsorption on the pore walls. Two pressure regimes that correspond to monolayer and multilayer adsorption were identified. In the low pressure regime from $0.7 < P < 39.6$ MPa the excess gas was fit to the Langmuir isotherm and the Langmuir pressure, volume and equilibrium density of the adsorbed layer were determined. The adsorbed gas density was discovered to be 8.5% lower than that of liquid methane. In the high pressure regime from 39.6 Mpa to 89.7 MPa the pressure dependence of the excess gas shows evidence of multilayer adsorption and was modeled using BET theory to determine the number of adsorbed layers formed. Using the adsorbed gas density, the gas in the Vycor pore space was separated into free and adsorbed fractions for the entire pressure range. The adsorbed gas fraction was shown to increase sharply for pressures above 39.6 MPa due the formation of multiple adsorbed layers, while the quantity of free gas molecules in the pore space decreased correspondingly.

Vycor porous glass with 5.7 nm pore size used in these studies can be considered as a model system to understand storage properties of natural gas in shale gas and tight gas rock formations. While the pore size of the Vycor porous glass is comparable to the small pores in the shale rock samples, important differences include the low porosities, permeability and also the presence of paramagnetic impurities in the naturally occurring shale rocks. The presence of internal gradients due to paramagnetic impurities in the shale nanopores are averaged out.
CHAPTER 6. GAS STORAGE IN DISORDERED SYSTEMS

by the fast diffusing gas molecules, but can affect the annulus gas outside the rock resulting in their overlap in the relaxation distributions. The low porosity and permeability of shale rocks also make the NMR experiments time consuming in order to achieve acceptable signal to noise ratio and complete gas saturation. In spite of these challenges, the sensitivity and precision of the high field NMR relaxometry experiments make it a viable methodology for studying the gas storage and transport characteristics of natural gas in such micro and mesoporous media.
Part II

Elastin-based biomaterials
Chapter 7

Introduction

Elastin, the principle protein component of the elastic fiber found in vertebrate tissues, is a biopolymer that exhibits remarkable mechanical characteristics. Numerous examples of imbalance or degradation of elastin are associated to pathological disorders, such as aortic stenosis and Williams syndrome, in which the elastin gene is mutated [83] or cutis laxa, in which connective tissues have shown a disruption of elastin [84]. The interplay between elastin degradation and disease [85], in addition to efforts to design elastin-based biomaterials [86, 87], has for a long time been the stimulus of studies to understand the structure and source of elasticity of this biopolymer. For example, many efforts to design artificial skin rely on elastin as a mechanical scaffold [88]. Obtaining detailed structural information of this protein has posed a challenge, as elastin does not crystallize and is largely insoluble making X-ray and solution state NMR methods inapplicable. Tropoelastin, the soluble precursor of elastin, is a 60-70kDa monomer which is composed of hydrophobic domains rich in alanine, glycine, proline and valine, and cross-linking domains comprised of desmosine or isodesmosine [89, 90]. Together with fibrillin-microfibrils, elastin comprises the elastic
fiber and contributes to the elastomeric characteristics and longevity of many tissues in vertebrates.

The elasticity of elastin is currently believed to be driven by an entropic force that is mediated by conformational and/or dynamical changes [91]. Early models described elastin as a two phase system, composed of globular molecules that carry hydrophobic groups packed in the interior and hydrophilic groups on the surface [92]. Andersen et al. suggested the existence of both hydrophobic and hydrophilic groups on the surface of the macromolecule, adding an additional view to the interpretation of the entropic force that drives elasticity [93]. In particular, the underlying physics behind the entropic force is related to oscillations of the protein backbone but also the interactions between the hydrophobic domains and solvent molecules at the surface. The latter has been studied by Torchia and coworkers where experiments were carried out on purified $^{13}$C labeled elastin using nuclear magnetic resonance. Their measured relaxation times, line widths and correlation times showed that under physiological conditions all valine residues undergo rapid motion; on the other hand alanine and lysine residues exhibited both slow and fast motions [94]. Investigations by Partridge et. al. revealed that certain treatments of elastin may result in a product soluble in water that consists of two components with rather different physical characteristics [95]. Partridge also suggested that elastin may be composed of molecules in tetrahedral packing [96]. On the other hand, in earlier work, Ramachandran suggested a triple helical model for elastin similar to that of collagen [97].

More recently, studies have aimed to further understand the source of elasticity in elastin by considering short repeating peptides comprising tropoelastin. For example the repeating
motif \((VPGVG)_{n}\) has been given much attention; in human elastin this pentapeptide repeats nine times \[98\]. Urry and coworkers studied this polypeptide extensively and proposed a simple model, termed the librational entropy mechanism, for the elasticity of elastin \[99\]. According to this model elastin is composed of consecutive type II \(\beta\)-turns that make up an ‘idealized \(\beta\)-spiral’ and assuming fixed ends, the conformation of the macromolecule allows for librational motion. Upon mechanical deformation the libration of the peptide results in a change in entropy giving rise to an entropic force that mediates the elastic character.

Tamburro et al. suggested an alternate model, referred to as the sliding \(\beta\)-turn, consisting of mobile conformations \[100\]. In this model the conformation of the polypeptide changes under deformation and results in reduction of entropy which gives rise to elastimicrofibrillarity. An intriguing characteristic of the pentapeptide \((VPGVG)_{n}\) is that it undergoes coacervation (also termed the inverse temperature transition) over the temperature range of \(20^\circ\text{C}\) and \(40^\circ\text{C}\), similar to elastin \[101, 102, 91, 103\]. In practice, the inverse temperature transition may be experimentally controlled by the length or arrangement of the repeating motif \[104, 105\] and salt concentration \[106, 107, 108\]. The biomechanically tunable characteristics of elastin-like peptides of VPGVG motifs has led to many intriguing applications, including drug delivery\[109, 110\].

A well known characteristic of elastin is that its mechanical characteristics appear to be strongly related to the degree of hydration \[111\] and the polarity of the solvent \[112, 113\]. Dehydrated elastin exhibits brittle characteristics and many experimental efforts have focused on giving insight into the source of this behavior. The notion that the polymer backbone, not the solvent, must bear the entropic force arising in mechanically strained elastin was argued
in a classic work by Hoeve and Flory [114]. Molecular dynamics simulations have been used to study the entropic contribution of localized water [115] or exposed hydrophobic groups by modeling short pentapeptides undergoing extension [116]. Experiments on hydrated bovine elastin under mechanical deformation have recently provided a direct measurement of dynamical changes of localized water and the protein backbone and have confirmed that the protein backbone reduces in entropy upon extension giving rise to a restoring entropic force [117].

Recently, biomaterial engineering has gained great interest in the development of scaffolds with properties similar to native tissue used for tissue repair [118, 119]. These scaffolds must satisfy the following four criteria for optimal interaction with the native tissue. These are the so called "4F" requirements as first noted in [120]. Form: the biomaterial should interact with the native tissue and act as guide in order to fill potential defects. Function: the scaffold should be able to support everyday’s functional needs of the native tissue. Formation: is essentially the ability of the biomaterial to allow for diffusion of nutrients to the native tissue (mass transport). Mass transport in the scaffold is controlled by parameters such as permeability (Section 3.6), porosity (volume of the void) and surface-to-volume ratio. Fixation: is the final requirement and refers to the ability of the scaffold to fulfill all the above criteria in one package for ease of use. A wide variety of materials are used for synthesizing these scaffolds which may be categorized into two groups: biologically derived materials including collagen based and elastin based scaffolds [121, 122, 123] and biocompatible synthetic polymers which include thermoplastic polymers [124, 125]. In general, biologically derived scaffolds are favorable due to their properties of biological recognition.
At present, *in vitro* and *in vivo* studies have showed that scaffolds with high porosity and permeability (measured optically) enhance dermal proliferation and infiltration and increase angiogenesis and matrix reproduction [122]. On the other hand previous works reported no significant deference in bone growth between scaffolds with 500\(\mu m\) and 1600\(\mu m\) pores [118, 126]. Thus, the quantitative characterization of the pore size, permeability and surface-to-volume ratio of elastin-based scaffolds is important to effectively engineer dermal substitutes for enhancing tissue regeneration.

This Part of the thesis explores the potential biological applications of the work discussed earlier regarding diffusion in disordered systems. Chapter 8 discusses a detailed study of the effects of purification of elastin from fat, collagen and muscle using \(^{13}\)C MAS spectroscopy and it is based on ref. [127]. Chapter 9 highlights preliminary data on the diffusive permeability of artificial elastin based films intended for use as dermal substitutes.
Chapter 8

$^{13}\text{C}$ MAS studies of elastin upon purification

This chapter highlights results published in ref. [127].

A distinguishing feature of elastin is its resistance to high temperature and pH that usually denature many proteins. Various purification schemes take advantage of these characteristics by heating the tissue in order to purify it from collagen, fat and smooth muscle. A detailed comparison of the purity of the products resulting from five purification schemes has been recently documented by Daamen et al. using amino acid analysis, sulphydryl quantification, and transmission electron microscopy [128]. Their results showed that the purification methods may also result in a product that includes traces of the microfibrillar component of the elastic fiber. The present work reports on the structural and dynamical modifications of elastin upon purification using high field $^{13}\text{C}$ solid state NMR methodology. Both hydrated and lyophilized elastin samples were studied using techniques that allow for the determination of the structural features in addition to dynamical characteristics measured over the
CHAPTER 8. \textsuperscript{13}C MAS STUDIES OF ELASTIN UPON PURIFICATION

time scale of microseconds to milliseconds. Remarkably, the overall structural features revealed upon purification do not seem to vary significantly between samples, however, the purification methods we studied appear to slightly affect the dynamics and the heterogeneity of structures revealed on the NMR time scale. The process of lyophilization quenches the proteins’ dynamics and the different purification schemes appear to add an additional contribution to this change. In particular, proline rich moieties appear more rigid after purifying the tissue whereas alanine moieties are more mobile. Lastly, based on our measured correlation times, the dynamics of the glycine and proline rich motifs across the samples were observed to vary slightly across the samples studied.

8.1 Sample Preparation

Both purified and unpurified bovine nuchal ligament elastin samples were purchased from Elastin Products Company, LLC (Owensville, MO). Elastin Products Company, LLC purified the tissue using the following three methods which are summarized in reference \cite{129}.

\textbf{Sample 1: Autoclaving} \cite{130}: The tissue was washed and autoclaved in 20 volumes of distilled water for 45 minutes at atmospheric pressure. This procedure is repeated sequentially until the sample contains no further protein trace.

\textbf{Sample 2: Hot alkali} \cite{131}: Minced tissue was suspended in 0.1 N NaOH and mixed. The product is placed in boiling water for 45 minutes and stirred. The sample was stored at room temperature and washed with cold 0.1N NaOH followed by centrifugation.

\textbf{Sample 3: Starcher method} \cite{132}: 0.05 M Na\textsubscript{2}HPO\textsubscript{4} at 7.6 pH, 1\% NaCl, 0.1\%EDTA was used as a buffer to extract the tissue. The purification begins with suspending the
tissue in the buffer for 72h. The product is washed twice with distilled water and lyophilized. 200mg of the lyophilized product was suspended in 30ml of water and autoclaved for 45min at 25psi. The next step includes centrifugation and washing the tissue. The product is suspended in 30ml of 0.1M Tris buffer at a pH of 8.2, that contains 0.02 M $CaCl_2$ and incubated with 4mg of trypsin at 37°C for 18h. The sample is centrifuged and the residue is washed and suspended in 10ml of 97% of formic acid. Cyanogen bromide (200mg) is added and the suspension shaken under a hood at room temperature for 5h. The sample is then centrifuged and the residue is washed twice with water and resuspended in 30ml of 0.05 M Tris buffer at a pH of 8.0 which contains 6 M urea and $\beta$-mercaptoethanol (0.5% $v/v$). The suspension is stirred overnight at room temperature, centrifuged, washed successively with the three washes each of ethanol and acetone, and dried in vacuum over $P_2O_5$.

The unpurified bovine nuchal ligament elastin sample was cleared of muscle and fat and dried by Elastin Products Company, LLC. All samples arrived having an average mesh size of 90$\mu$m (Samples 1 to 3) and 465$\mu$m (Unpurified) and were lyophilized for 72 hours. Experiments on hydrated samples were prepared as follows: the samples were suspended in distilled water for 48 hours and then centrifuged for 3 minutes to remove bulk water for packing into the NMR rotor. The water to protein concentration of all samples was determined to be approximately 40% by volume. Standard amino acid analysis was performed by J. Myron Crawford’s group at the W.M. Keck Biotechnology Resource Laboratory at Yale University using a Hitachi L8900 analyzer. Amino acid analysis on unpurified elastin was performed by New England Peptide (Cambridge, MA) using the same instrumentation.
Quantification of desmosine concentration

Elastin hydrolysis

2.1-2.2 mg of each sample was placed in 300µL of 6N HCl and 1µL of 0.5% w/w phenol solution in sealed glass tubes and flushed with argon gas. The samples were incubated at 110 °C for 96 hours. After incubation the solvent was frozen under liquid nitrogen and then lyophilized for 6-8 hours. The sample was then re-suspended in 50µL of solution of 95.5% 0.14M sodium acetate, 0.5% triethylamine, 5% acetonitrile (v/v/v) at a pH of 7.5.

Quantification with labeled desmosine

Resuspended samples were mixed in 1:1 (v/v) ratios with standard d4-desmosine (Toronto Research Chemicals, Toronto, Canada) at a final concentration of 0.250 mg/mL. The mass spectrometric peak intensity ratio in MS2 mode of desmosine to d4-desmosine was used to quantify the relative amount of desmosine in each sample.

The solution of standard and matrix was made in the ratio of 1:9 (v/v) and stirred. The standard d4-desmosine (Toronto Research Chemicals, Toronto, Ontario, Canada) was at a final concentration of 0.250 mg/mL. In addition, 10 µL of this solution were mixed with 1 µL of resuspended solution. The ratio of natural abundance desmosine to d4-desmosine was used to quantify the amount of desmosine in the sample.

MALDI-MS quantitative analysis

MALDI-MS2 experiments were performed using a Thermo LTQ XL ion trap mass spectrometer (Thermo Scientific, Waltham, MA, USA) equipped with a vacuum MALDI source.
α-Cyano-4-hydroxycinnamic acid (CHCA) was purchased from Sigma-Aldrich (St. Louis, Missouri, USA) and used without additional purification. Solid CHCA was then mixed with a solution of 0.1% trifluoroacetic acid, 70% acetonitrile and 29.9% HPLC grade water until saturated, and used as a matrix solution. 1µL of the sample was added to 10 µL of solution containing standard desmosine and CHCA matrix solution. The solution of CHCA matrix and standard was prepared in the ratio 1:9 (1µL standard in 9µL of CHCA matrix). Finally, the solution was stirred and 1µL of the solution was spotted on the MALDI plate and allowed to dry prior to mass spectrometric analysis.

MALDI-MS² analysis was conducted using a laser energy of 2.6 µJ, for 150 scans with the AGC set to 10,000 ions. Precursor ions of unlabeled desmosine (m/z 526.3) and d₄ labeled desmosine standard (m/z 530.3) were selected in a single isolation window (m/z 529 ± 6), and fragmented with a normalized collision energy = 35, activation Q = 0.25, activation time = 30 ms. The most intense product ions, at 397 m/z for unlabeled desmosine and 401 m/z for d₄-desmosine, were used for relative quantification.

NMR experimental parameters

All the experiments were performed on a Bruker 750MHz system externally referenced to adamantane (TMS scale) [127]. The temperature in all experiments was set to regulated to within ±0.5°C. The three pulse sequences used in the experiments are shown in Figure S1 in the supporting material. In all experiments proton TPPM decoupling was implemented at 100kHz [133]. The ¹³C π/2 pulse was 5µs and the rotor spinning frequency was 10 kHz ± 5 Hz. The sample temperature at 10kHz magic angle spinning was measured with
lead nitrate and offset to account for additional heating effects. In the cross polarization experiments the contact time was set to 3ms and the recycle delay was set to 3s. For the $T_1\rho$ experiments the two spin locking fields used were 50kHz and 25kHz and the spin lock time interval was incremented from 50µs to 10ms. In the $T_1\rho$ experiments a $\pi$ pulse was used to eliminate ring down effects. The sample was packed in a 3.2mm rotor and sealed using compression style caps that prevented water loss. The extent of water loss throughout all the studies on the hydrated samples was measured to be less than 1%. The 37°C spectra were acquired by accumulating 10k scans and the 75°C temperature spectra were acquired by accumulating 28k scans. Gaussian multiplication with a broadening factor of 30Hz was used and the analysis was performed using MATLAB and matNMR [134]. The dwell time in all the experiments was 8.8µs and total number of points acquired was 17k.

8.2 Results and Discussion

Amino acid and MALDI mass spectrometry studies of elastin

Amino acid analysis was performed on all the samples in order to analyze their purity [127]. For each sample 0.8 $\frac{ml}{mg}$ of HCl was used for hydrolysis in vacuum at 110°C for 48h. After drying off the acid, each aliquot was dissolved and serially diluted to load onto the amino acid analyzer. Table 8.1 highlights the results of the amino acid analysis. The results show that elastin is largely composed of glycine, proline, alanine and valine; more than 84% of the residues in a single molecule belong to these four amino acids. Additionally, in less concentration, these measurements reveal that elastin also contains isoleucine, leucine,
phenylalanine, and glutamine. Additional traces of amino acids appearing in unpurified bovine ligament elastin, such as histidine, may be evidence of either residual collagen or smooth muscle. Note that the presence of these amino acids also skews the percentage contribution of other tabulated values. Our findings for purified samples are in agreement with the theoretical values computed from the cDNA of tropoelastin [135] and with work reported elsewhere [129].

Figure 8.1: Mass spectrometric spectra of hydrated samples treated with various purification methods: A) autoclaving (sample 1), B) hot alkali (sample 2), C) Starcher (sample 3), and D) unpurified. The relative amount of desmosine in the samples was measured by monitoring peak intensity of MS$^2$ fragmentation of unlabeled desmosine (m/z 397) to $d_4$-desmosine (m/z 401).

To compare these three purification methods for tissue samples, MS/MS experiments were performed to quantify the amount of desmosine in the samples treated with and without the purification steps. The internal standard, $d_4$-desmosine, was added prior to the MS/MS
experiments where the precursor ions for desmosine and $d_4$-desmosine were fragmented into their product ions (m/z 397 and 401, respectively), shown in Figure 8.1. In addition, Table 8.1 includes the relative concentration of desmosine in each sample studied in this work. The numbers reported are shown as the ratio of desmosine to $d_4$-desmosine, normalized to the unpurified sample. Note that this metric is thus sensitive to traces of residual fat and collagen. The results of Table 8.1 indicate that the relative desmosine concentration in the samples are very similar, with similar overlap in error bars; sample 2 (hot alkali protocol) appears to have slightly higher desmosine concentration with respect to all the other samples. The small differences between sample 2 and all the other samples may be attributed to traces of fat and collagen, or differences in the extent of cross-linking across the samples. As the starting tissue was the same in all three isolation schemes, the amount of cross-linking is presumably similar. Thus, these results would indicate that sample 2 may contain slightly less residual fat and collagen compared to the other isolation schemes studied. However, this protocol is known to cause significant peptide-bond cleavage[129], which we discuss later in the manuscript.

8.2.1 Dynamical and structural characteristics of elastin

Magic angle spinning $^{13}$C-NMR was used to investigate the structural modifications of elastin following purification [127]. Two standard pulse sequences were used in the experiments; a direct polarization experiment which allows for the observation of both highly mobile and restricted spins and a cross polarization experiment which allows for the transfer of magnetization from protons to carbon spins via the carbon-proton dipole-dipole interaction.
A signal resulting from cross-polarization implies a non-zero dipolar interaction between a $^{13}C$ nucleus and $^1H$ nuclei. Fast isotropic motion, however, results in suppression of the heteronuclear dipolar interaction, whereas slow or anisotropic motion would allow for magnetization to transfer between $^{13}C$ and $^1H$ nuclear spin baths [136].

Figure 8.2 highlights the direct polarization (DP) and cross polarization (CP) $^{13}C$ NMR spectra of hydrated bovine nuchal ligament elastin purified by the autoclaving method at 37°C. When completely hydrated, elastin is known to exhibit high mobility resulting in a low $1H$-$13C$ cross polarization signal, as noted elsewhere [137, 138]. A detailed discussion of the chemical shift and secondary structure assignments is provided in the supporting material, and summarized in Table 8.2.

**Structural and dynamical differences across purified elastins**

Subtle differences were observed in the $^{13}C$ NMR spectra acquired at 37°C of hydrated elastin purified by the three methods. Figure 8.3.a shows the direct polarization spectra of the three purified samples and the unpurified bovine nuchal ligament. The resolution of the direct polarization spectra is approximately 1-3ppm and within our experimental uncertainty the spectra appear remarkably similar in terms of the chemical shifts and relative signal intensities. However, the line width of the backbone carbonyl peak at approximately 172.0ppm seems to vary slightly across the different purification schemes and in comparison with the unpurified sample. Specifically, the backbone carbonyl of the unpurified sample appears more narrow compared with that of sample 1 indicating structural differences between the two. The signals observed in the aliphatic region of all hydrated samples (Figure 8.3.a)
are well resolved with no observed differences in terms of the chemical shifts, relative signal intensities and line widths. It should be noted that an enhanced signal intensity at approximately 70ppm was observed in the unpurified sample and was assigned to threonine-$C_\beta$.

Upon lyophilization of the samples, the dynamics of the ensemble seem to change dramatically in comparison with the hydrated samples. Dynamical changes between lyophilized and hydrated elastin were reported previously by Kumashiro and coworkers where they showed that the temperature and hydration levels seem to affect the underlying interactions and dynamics [137]. Additionally, previous studies on a small elastin peptide showed a decrease in the relaxation times, $T_{1\rho}$, with increasing the hydration level of the protein [146]. The hydration dependence is also directly reflected in the $^{13}C$ NMR spectra acquired by cross polarizing the carbon spins with the proton bath (Figure 8.4). One of the key characteristics of these NMR spectra is the broad lines of approximately 7ppm line width, which do not allow for an accurate determination of secondary structure. A second key observation is that these spectra appear remarkably similar in terms of chemical shifts which suggests that the structure is not affected upon purification.

In the spectra of the unpurified sample shown in Figure 8.4, an enhanced signal intensity is observed at approximately 53.0ppm in comparison with the purified samples. Although the spectra in Figure 8.4 are quite broad, this peak was assigned to alanine-$C_\alpha$ and according to the literature the observed chemical shift points to $\alpha$-helical structure [139, 140]. In previous NMR studies of elastin, the chemical shift of the alanine-$C_\alpha$ was observed at approximately 52.0ppm to 53.0ppm but different structures have been reported. Kumashiro et al. reported that the alanine-$C_\alpha$ signal at 53.0ppm was more $\alpha$-helical which in agreement with
our observation [147]. On the other hand, Witterbort et al. reported that the alanine-$C_\alpha$ chemical shift points to random coil/β-sheet like structure [143]. The signal enhancement of the alanine motifs in the unpurified sample suggests that the $C_\alpha$ spins experience a more rigid environment compared with that in the purified elastin and thus cross polarization is more efficient. Additionally, across the purified samples shown in Figure 8.4, the signal intensity of the alanine-$C_\alpha$ seems to vary; in sample 2 (hot alkali) the peak is no longer observed. Therefore, a portion of the alanine-$C_\alpha$ carbons seem to become more mobile upon purification with the alkaline extraction technique. This behavior may be due to peptide-bond cleavage which occurs with the hot alkali method, as pointed out by R. Mecham [129].

A more quantitative interpretation of the signal enhancement of the alanine residues in Figure 8.4 may be given by the amino acid analysis data and by normalizing the spectra to account for differences in mass (data not shown). Table 8.1 shows a higher concentration of alanine residues in purified elastin of approximately 3.8% compared to unpurified elastin. As mentioned earlier the presence of histidine, glutamine and arginine might skew the calculated concentration of alanine in the unpurified sample. While the values tabulated in table 8.1 would suggest that the signal intensity of alanine be lower in the unpurified sample the integral of the peak at 53ppm (corresponding to alanine-$C_\alpha$) of Figure 8.4D.2 is markedly larger than that of all purified elastin spectra by approximately 18%. The differences in the signal intensity of the alanine motifs between the purified and unpurified spectra may be due to dynamical changes but also due to residual amino acids from smooth muscle or collagen. For example, the glutamine-$C_\alpha$ signal arising from β-strand like motifs have a chemical shift of $(54.95 \pm 1.59)$ppm [139] (TMS) which might contribute to the observed
signal in the unpurified sample. However, this signal enhancement was not evident in the spectra obtained at 37° or 75° from elastin in the hydrated state (see below). Therefore, a more likely explanation for the differences in the spectra shown in Figure 8.4 is that the alanine motifs in the unpurified sample are structurally more rigid than that of any of the purified samples, which results in a higher cross polarization enhancement.

Subtle differences in the spectra were observed in the methyl region between 16.0ppm and 22.0ppm. Specifically, the peak at 16.1ppm that was assigned to the isoleucine-Cγ, seems to be more resolvable in the unpurified sample in comparison with the purified elastin samples (Figure 8.4-D). It should be noted here that previous studies on lyophilized elastin reported this peak as alanine-Cβ [147] or valine-Cγ [148], whereas other studies on hydrated elastin appear to be in agreement with our assignment [143]. The resolution enhancement indicates that upon purification isoleucine motifs would seem to experience a more mobile environment. Slightly more in the downfield region, the peaks at approximately 18.0ppm and 24.0ppm were assigned to alanine/valine and leucine residues respectively. The peak at 29.5ppm arises from the proline-Cβ and the small shoulder at roughly 32.0ppm was assigned to the valine-Cβ. The glycine-Cα signal was observed at 42.3ppm in all the samples with an approximate 3ppm line width. The peaks at 47.6ppm and 59.3ppm were assigned to proline-Cδ and valine-Cα which is in agreement with previous NMR studies on elastin [148]. Additionally, the small shoulder at approximately 55ppm may be a signature of the leucine-Cα carbons. According to references [139, 140] the Cα of leucine has chemical shift in the region between 55ppm to 59ppm (TMS) but our spectral resolution does not allow for a reliable structural assignment.
Figure 8.3.b shows the direct polarization spectra of the lyophilized samples studied in this work. The spectra look remarkably similar in terms of chemical shifts, however some subtle differences should be noted in the signal intensities. The peak at approximately 30.0ppm was assigned to proline-$C\beta$; however the valine-$C\beta$ signal has almost identical chemical shift as noted earlier. A larger signal at 30.0ppm was observed before purifying the tissue (Figure 8.3.b-D4). However, upon purification the relative signal intensity is reduced and the peak is broader (Figure 8.3.b-A through Figure 8.3.b-C). This observation indicates that upon purification some of the proline-$C\beta$ or valine-$C\beta$ seem to exhibit increased structural heterogeneity. Additionally, the peak at 22.7ppm was assigned to alanine-$C\beta$, which is well resolved in the purified sample and seems to be suppressed by overlapping peaks upon purification (Figure 8.3.b-D4).

High temperature $^{13}$C-NMR spectra of hydrated elastin

Previous $^{13}$C MAS NMR studies of elastin performed by Wittebort et al. showed that there is an enhancement in the spectral resolution by performing experiments at 75°C [143]. We have adopted the same approach to investigate structural variations in the purified samples. Figure 8.5 shows the $^{13}$C-NMR spectrum of hydrated bovine ligament elastin purified by the autoclaving method (Sample 1) at 75°C. One of the key observations is the enhancement of the resolution with 0.3ppm line width, in both the aliphatic region and the backbone carbonyl in comparison with the 37°C spectrum (Figure 8.2). This enhancement allows for a more accurate chemical shift and structural assignment. Narrower lines may point to higher structural homogeneity and increased mobility at high temperature. Another important
finding is that within our experimental uncertainty, both 37°C and 75°C spectra appear remarkably similar in terms of the observed chemical shifts. Experimentally, we found that the spectra of the purified and unpurified samples appeared identical in terms of chemical shifts and relative signal intensities; for that reason only one spectrum is shown in Figure 8.5.

Comparing the 37°C (Fig. 8.2) and 75°C data (Fig. 8.5) we observed subtle differences in the relative signal intensities of the valine-\(C_\gamma\)/alanine-\(C_\beta\) signals (at approximately 18.8ppm), leucine-\(C_\delta\) at 23.5ppm and isoleucine-\(C_\gamma\) signals (at 16.7ppm). These differences may be attributed to overlapping peaks in the 37°C spectra.

The backbone carbonyl has characteristic splitting at 171.8ppm and 174.0ppm. The peak at 171.8ppm was assigned to glycine residues in \(\beta\)-strand like structure, which is in agreement with previous studies [144]; however, glycine residues in random coil motifs appear very close to the observed chemical shift [139]. The peak at 174.0ppm points to glycine in \(\alpha\)-helical secondary structure, proline in random coil and/or \(\beta\)-strand, alanine in \(\beta\)-strand and valine in random coil and/or \(\beta\)-strand conformations. The small shoulder at approximately 174.7ppm points to either glycine in \(\alpha\)-helical structure, alanine in random coil and/or \(\beta\)-strand, valine in random coil and/or \(\beta\)-strand and proline in random coil and/or \(\beta\)-strand. Finally, the peak at 177.9ppm could be evidence of alanine or proline residues in \(\beta\)-strand like structures. In addition, glycine and valine residues may also contribute to this peak.

In the aliphatic region we observe more \(\beta\)-strand and random coil like structures, similar to the 37°C spectra. Specifically, starting with the upfield region we observe the isoleucine \(C_\delta\) and \(C_\gamma\) at 11.4ppm and 16.0ppm respectively. The peaks at 17.8ppm, 18.5ppm and
19.6ppm may be alanine and valine motifs in $\beta$-strand or random coil structures, which is in agreement with the observations at 37°C. According to Pometun et al. the alanine-$C_\beta$ at approximately 19.0ppm points to a random coil structure [143]. Additionally, the peaks at 22.1ppm and 23.5ppm were assigned to alanine-$C_\beta$ or leucine-$C_\delta$ respectively. The signal at 22.1ppm would imply an alanine $\beta$-strand structure. Proline-$C_\gamma$ and valine-$C_\beta$ are observed at 25.3ppm, 29.9ppm and 30.9ppm; these chemical shifts point to either $\beta$-strand, random coil or $\alpha$-helical secondary structures.

Isoleucine-$C_\beta$ was assigned to the peak at approximately 37.2ppm and this chemical shift could point to either $\beta$-strand, random coil or $\alpha$-helical structures. The peak at 40.9ppm was assigned to isoleucine-$C_\beta$ which implies a $\beta$-strand secondary structure [139]. It should be noted that at 37°C isoleucine exhibited $\beta$-strand and/or random coil conformations. Previous studies showed that isoleucine rich motifs might be in a more random coil secondary structure [143]. Additionally, leucine-$C_\beta$ contributes to the signal at 40.9ppm in either $\beta$-strand, random coil or $\alpha$-helical structure. The peak at 43.7ppm was assigned to glycine-$C_\alpha$, however as in the 37°C spectra, no assignment can be made regarding structure. The signals at 48.7ppm and 50.5ppm correspond to the alanine-$C_\alpha$ in random coil and/or $\beta$-strand structures; proline-$C_\delta$ also contributes to the signal at 50.5ppm. The peak 53.6ppm was assigned to the alanine-$C_\alpha$ and indicates an $\alpha$-helical conformation in agreement with our observation at 37°C. In addition, leucine-$C_\alpha$ and phenylalanine-$C_\alpha$ contribute to the signal at 53.6ppm in random coil and $\beta$-strand structures.

The $C_\alpha$ signals of phenylalanine and isoleucine, two of the low abundance amino acids of elastin, appear at 57.6ppm and 61.5ppm respectively and indicate either $\alpha$-helical, random
coil and β-strand structures respectively. The peak at 60.4ppm was assigned to valine-$C_\alpha$; this chemical shift points to β-strand and random coil secondary structure. In addition, proline-$C_\alpha$ in random coil or β-strand conformation appears to have a chemical shift of 60.4ppm; isoleucine in random coil structure appears to contribute to this signal. Lastly, the small peak at 70.7ppm was assigned to threonine.

8.2.2 $^{13}C-^1H$ Rotational correlation times $\tau_c$

A measurement of the dynamics of the system can be obtained by considering the average rotational correlation times of the effective carbon-proton internuclear vectors, obtained by NMR relaxation methods. Previous studies for describing the molecular motions of elastin and short elastin peptides included measurements of a single longitudinal relaxation time for each spectroscopically resolvable moiety [149, 94], following Solomon’s equations [150]. Their findings indicate that molecular motions of the backbone carbonyl carbons of elastin are in the ns time scale. However, Urry et al. showed that it is ambiguous to determine the correlation times from a single relaxation time and that this ambiguity arises from the degeneracy of $\tau_c$ with respect to $T_1$ due to the shape of the spectral density function (eq. S6) [151]. Large proteins, such as elastin are known to exhibit a distribution of dynamics on different time scales. The collective dynamical behavior of elastin and small elastin peptides have been studied by acoustic and dielectric methods that probed frequencies from 0.1 to 100kHz and 0.1 to 100MHz, respectively [152, 153, 91]. However, these motions are different than what is studied by $^{13}C$ NMR, which is sensitive to local $^{13}C-^1H$ nuclei internuclear fluctuations. The $^{13}C T_{1_\rho}$ relaxation time is sensitive to motions spanning the
dynamic range of $\mu s$ to $ms$ \cite{154}. Without knowledge of the spectral density functions $J(\omega)$ it is not possible to know how many correlation times govern the relaxation behavior of a given $^{13}$C-$^1$H fluctuation. we have therefore restricted the discussion below under the assumptions of an average single correlation time derived from two $T_{1\rho}$ measurements.

Table 8.3 summarizes the rotating frame relaxation times $T_{1\rho}$ at two different fields and the correlation times of the spectroscopically resolvable moieties of the hydrated elastin samples at $37^\circ C$.

All the measured correlation times appear to be in the $\mu s$ time scale. The spectral density function $J_n(\omega)$ contains the term $(\omega \tau_c)^2$ in the denominator which for the rotor frequency is of the order of $\sim 0.05 << 1$ (computed by substituting the average correlation time $<\tau_c> = 3.63\mu s$ from Table 3 and the rotor frequency $\omega = 2\pi \times 10^4$ rad). Therefore the interference of the molecular dynamics and the sample spinning has negligible effects on $R_2$. Referring to Table 8.3, small differences in the correlation times were observed across the samples. Specifically, the peak at approximately 23.5ppm of sample 2 that was assigned to leucine-$C_\delta$ appears to experience a more mobile environment in comparison with the other samples. Additionally, the correlation times of the proline-$C_\beta$ at 31.0ppm seem to vary slightly across the samples from 2.7$\mu s$ of sample 2 to 5.3$\mu s$ of sample 1, indicating less mobility. The glycine-$C_\alpha$ however seems to be more rigid in sample 2, with an average correlation time of 8.6$\mu s$, compared to sample 3 which has a correlation time of 3.1$\mu s$. Based on our correlation time measurements, the backbone carbonyl of the unpurified sample appears slightly more mobile in comparison with sample 1 and sample 2.

The correlation times of the alanine-$C_\beta$ (22.0ppm) of sample 2 seem to be shorter than
any other of the samples by a factor of two. These differences may be due to peptide bond cleavage which may result from the hot alkali treatment, discussed earlier. The correlation times of the valine-\(C_\alpha\) which was observed at approximately 60.4ppm were different between the unpurified sample and sample 1; the valine-\(C_\alpha\) appears more rigid in sample 1 as the correlation time is more than eight times larger in comparison with the unpurified sample. It should be noted here that the correlation times of the valine-\(C_\alpha\) of sample 2 and 3 could not be determined because of relatively large error bars in the \(T_{1\rho}\) measurements. However, the estimated values are of the order of \(\mu s\).

We note that the correlation times in hydrated bovine nuchal ligament elastin determined by a static (i.e. no sample spinning) measurement performed by us [138], and others[149, 94], appear smaller than the values reported in this study. In our previous work [138] the values reported were in units of s/cycle and need to be divided by \(2\pi\) for comparison with the units here (s/rad). In unstrained nuchal ligament elastin the average aliphatic correlation time measured was \(8.02 \times 10^{-8}\)s and for the carbonyl, measurements on nuchal ligament elastin in unstrained conditions the average correlation time was \(1.29 \times 10^{-7}\)s [117]. In a study by Torchia and coworkers the \(^{13}\)C NMR spin-lattice relaxation times of unstretched calf ligamentum nuchae in 0.15M NaCl were also measured without sample spinning. Approximately 80% of the backbone carbonyl carbons were shown to exhibit a correlation time of approximately \(4 \times 10^{-8}\)s [149]. Fleming, Sullivan, and Torchia also reported \(^{13}\)C-\(^1\)H correlation times of purified labeled chick aorta, again without sample spinning. Their correlation times ranged from 6 to \(15 \times 10^{-8}\) s for all the valine labeled residues and 5 to \(20 \times 10^{-8}\) s for 75 % of the alanine and 60% of the lysine labeled resides [94]. A likely cause for the differences
in the correlation times measured in this work, and that of the previous studies, may have arisen from the fact that we measured the relaxation times in the rotating frame. The relaxation time in the rotating frame has a different dependence on the spectral density than the spin lattice relaxation time and is therefore sensitive to different timescales of motion.

8.3 Conclusions

The present work highlights the structural and dynamical modifications resulting from three methods to isolate elastin from fat, collagen and muscle studied by high field $^{13}\text{C}$ NMR spectroscopy and relaxometry. Our results show that elastin maintains its structure upon purification, as the NMR chemical shifts are identical within our experimental uncertainty. However, small differences were observed in the dynamics and the relative signal intensities. The NMR spectra of the hydrated nuchal ligament elastin indicate high mobility of the backbone carbonyl at $37^\circ\text{C}$. By increasing the temperature to $75^\circ\text{C}$, the line width of the backbone carbonyl appears narrower allowing for some structural assignment. In the aliphatic region a comparison between the direct polarization and cross polarization experiments showed that the alanine motifs are comparatively more rigid than other residues. Additionally, glycine residues appear highly mobile resulting in narrower spectral lines in the direct polarization experiment and smaller signal intensity in the cross polarization experiment. The main feature of the cross polarization experiments on all lyophilized nuchal ligament elastins (purified & unpurified) was that the chemical shifts appear identical within our experimental uncertainty. The studies of the lyophilized samples revealed that upon purification the dynamical characteristics of the alanine motifs seem to be enhanced. Addi-
tionally, the direct polarization spectra of unpurified lyophilized elastin showed a signal at 30ppm which was assigned to proline-$C_\beta$ and/or valine-$C_\beta$ and is reduced upon purification indicating a possible structural heterogeneity in the proline or valine rich motifs or decrease in molecular motion. Across the samples, our results indicate that elastin largely maintains its structure upon purification, as well as the extent of cross linking, however the dynamics seem to be affected. Based on the line widths of the NMR spectra, the backbone appears structurally different and the backbone mobility also appears different based on the measured correlation times. The glycine rich motifs of elastin purified by alkaline extraction seem to be more rigid in comparison with elastin purified by the Starcher method [132, 129]. Lastly, our measurements reveal that the hot alkali purification protocol results in a product with relatively less fat and collagen in comparison with the other purification schemes studied. However, this method for isolating elastin from other tissues constituents has been reported to cause significant peptide-bond cleavage and appears to alter the dynamics of alanine and glycine residues.
### Table 8.1: Amino acid analysis of three purified bovine nuchal ligament elastin samples (samples 1-3, described in the text), unpurified elastin and their relative desmosine concentration.

The amino acid measurements are reported as “residues per 1000 residues (res/1000)” for ease of comparison with other published results [129]. The theoretical values, shown in column 6, correspond to the amino acids computed from cDNA of bovine tropoelastin [135]. The relative desmosine concentration represents the ratio of desmosine to $d_4$-desmosine, normalized by the unpurified sample (starting product). Note that the concentration of lysine in the theoretical value (column 6) is higher than that in samples 1-3 and the unpurified sample, as cross-links consist of lysine.

<table>
<thead>
<tr>
<th></th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Unpurified</th>
<th>Theory*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asx</td>
<td>5.6</td>
<td>4.6</td>
<td>4.1</td>
<td>4.2</td>
<td>4</td>
</tr>
<tr>
<td>Thr</td>
<td>6.9</td>
<td>5.5</td>
<td>6.8</td>
<td>1.0</td>
<td>11</td>
</tr>
<tr>
<td>Ser</td>
<td>7.1</td>
<td>6.4</td>
<td>7.3</td>
<td>0.0</td>
<td>10</td>
</tr>
<tr>
<td>Glx</td>
<td>14.5</td>
<td>14.9</td>
<td>14.0</td>
<td>19.7</td>
<td>14</td>
</tr>
<tr>
<td>Pro</td>
<td>147.5</td>
<td>149.2</td>
<td>153.2</td>
<td>121.0</td>
<td>119</td>
</tr>
<tr>
<td>Gly</td>
<td>335.9</td>
<td>332.6</td>
<td>329.7</td>
<td>325.3</td>
<td>319</td>
</tr>
<tr>
<td>Ala</td>
<td>238.3</td>
<td>237.6</td>
<td>238.4</td>
<td>229.1</td>
<td>211</td>
</tr>
<tr>
<td>Val</td>
<td>121.8</td>
<td>125.3</td>
<td>123.7</td>
<td>125.5</td>
<td>126</td>
</tr>
<tr>
<td>Cys</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Met</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>-</td>
</tr>
<tr>
<td>Ile</td>
<td>21.9</td>
<td>22.2</td>
<td>22.2</td>
<td>21.1</td>
<td>25</td>
</tr>
<tr>
<td>Leu</td>
<td>53.7</td>
<td>54.6</td>
<td>53.9</td>
<td>62.7</td>
<td>60</td>
</tr>
<tr>
<td>Tyr</td>
<td>6.9</td>
<td>7.3</td>
<td>7.2</td>
<td>17.4</td>
<td>10</td>
</tr>
<tr>
<td>Phe</td>
<td>33.2</td>
<td>34.3</td>
<td>34.6</td>
<td>38.4</td>
<td>29</td>
</tr>
<tr>
<td>His</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>10.4</td>
<td>0</td>
</tr>
<tr>
<td>Lys</td>
<td>2.4</td>
<td>1.9</td>
<td>1.9</td>
<td>10.9</td>
<td>53</td>
</tr>
<tr>
<td>Arg</td>
<td>4.3</td>
<td>3.6</td>
<td>3.0</td>
<td>13.3</td>
<td>7</td>
</tr>
<tr>
<td>Relative desmosine concentration</td>
<td>1.00±0.10</td>
<td>1.09±0.12</td>
<td>0.86±0.10</td>
<td>1.00±0.14</td>
<td>-</td>
</tr>
</tbody>
</table>
CHAPTER 8. $^{13}$C MAS STUDIES OF ELASTIN UPON PURIFICATION

Figure 8.2: a,b) Direct polarization (DP) $^{13}$C NMR spectrum of hydrated bovine nuchal ligament elastin purified by the autoclaving method (Sample 1) at 37°C. The aliphatic and carbonyl regions are presented with the same scale as the spectra acquired at 75°C (Figure 8.5) for ease of comparison. c,d) Cross Polarization (CP) $^{13}$C NMR spectrum of hydrated bovine nuchal ligament elastin purified by the autoclaving method (Sample 1) at 37°C. When completely hydrated, elastin exhibits high mobility resulting in a low $^1H -^{13}C$ cross polarization signal, as noted elsewhere [137, 138].
<table>
<thead>
<tr>
<th>13C&lt;sub&gt;α&lt;/sub&gt;</th>
<th>Observed Chemical Shift [ppm]</th>
<th>Assignment</th>
<th>Random Coil</th>
<th>α-helix</th>
<th>β-strand</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>13C&lt;sub&gt;α&lt;/sub&gt;</td>
<td>(53.3 ± 1.3)</td>
<td>Ala</td>
<td>51.14 ± 1.05</td>
<td>53.13 ± 1.05</td>
<td>49.83 ± 1.48</td>
<td>[139]&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(48.65 ± 1.5)</td>
<td></td>
<td>50.97 ± 1.94</td>
<td>53.16 ± 0.94</td>
<td>49.16 ± 1.28</td>
<td>[140]&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(57.5 ± 0.7)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Phe</td>
<td>56.28 ± 2.02</td>
<td>59.11 ± 1.90</td>
<td>54.95 ± 1.59</td>
<td>[139]&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(43.4 ± 3.2)</td>
<td>Gly</td>
<td>43.81 ± 1.05</td>
<td>45.21 ± 1.10</td>
<td>43.52 ± 1.17</td>
<td>[139]&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>43.64 ± 1.17</td>
<td>45.32 ± 0.90</td>
<td>43.38 ± 1.20</td>
<td>[140]&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>42.7</td>
<td>45.68 ± 0.90</td>
<td>-</td>
<td>[141]&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>43.1</td>
<td>43.12</td>
<td>43.2 ± 4.3</td>
<td>[142]&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(61.4 ± 0.6)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>His</td>
<td>59.33 ± 1.90</td>
<td>62.87 ± 1.74</td>
<td>58.35 ± 1.57</td>
<td>[139]&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>58.94 ± 2.08</td>
<td>62.98 ± 1.66</td>
<td>58.30 ± 1.51</td>
<td>[140]&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(53.3 ± 1.4)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Leu</td>
<td>55.22 ± 1.70</td>
<td>55.82 ± 1.23</td>
<td>52.38 ± 1.31</td>
<td>[139]&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>53.15 ± 1.79</td>
<td>55.84 ± 0.98</td>
<td>52.24 ± 1.19</td>
<td>[140]&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(60.4 ± 3.5)</td>
<td>Pro</td>
<td>61.77 ± 1.26</td>
<td>63.79 ± 1.08</td>
<td>60.94 ± 1.03</td>
<td>[139]&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>61.83 ± 1.26</td>
<td>63.82 ± 1.01</td>
<td>61.09 ± 1.22</td>
<td>[140]&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(60.4 ± 3.5)</td>
<td>Val</td>
<td>60.36 ± 2.16</td>
<td>64.46 ± 1.55</td>
<td>59.13 ± 1.68</td>
<td>[139]&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>60.10 ± 2.25</td>
<td>64.26 ± 1.39</td>
<td>59.02 ± 1.59</td>
<td>[140]&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>13C&lt;sub&gt;β&lt;/sub&gt;</th>
<th>Observed Chemical Shift [ppm]</th>
<th>Assignment</th>
<th>Random Coil</th>
<th>α-helix</th>
<th>β-strand</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>13C&lt;sub&gt;β&lt;/sub&gt;</td>
<td>(17.7 – 22.0 ± 0.7)</td>
<td>Ala</td>
<td>17.36 ± 1.26</td>
<td>16.56 ± 0.88</td>
<td>19.44 ± 2.05</td>
<td>[139]&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>17.33 ± 1.27</td>
<td>16.57 ± 1.08</td>
<td>20.02 ± 1.77</td>
<td>[140]&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>17.86</td>
<td>-</td>
<td>-</td>
<td>[143]&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(37.3 ± 2.1)</td>
<td>He</td>
<td>36.95 ± 1.69</td>
<td>35.90 ± 1.15</td>
<td>38.16 ± 1.98</td>
<td>[139]&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>36.56 ± 1.06</td>
<td>35.89 ± 1.08</td>
<td>38.39 ± 1.85</td>
<td>[140]&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>37.25</td>
<td>-</td>
<td>-</td>
<td>[143]&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(31.0 ± 1.8)</td>
<td>Pro</td>
<td>31.94 ± 0.95</td>
<td>31.46 ± 0.95</td>
<td>32.27 ± 1.20</td>
<td>[139]&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30.17 ± 0.96</td>
<td>29.38 ± 0.81</td>
<td>30.75 ± 0.93</td>
<td>[140]&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(31.0 ± 1.8)</td>
<td>Val</td>
<td>31.01 ± 1.37</td>
<td>29.79 ± 0.72</td>
<td>32.21 ± 1.61</td>
<td>[139]&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>30.98 ± 1.76</td>
<td>29.71 ± 1.74</td>
<td>32.11 ± 1.79</td>
<td>[140]&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CO</td>
<td>(174.0 – 177.9 ± 2.1)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Ala</td>
<td>175.97 ± 1.57</td>
<td>177.70 ± 1.32</td>
<td>174.39 ± 1.51</td>
<td>[139]&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>175.69 ± 1.45</td>
<td>177.88 ± 1.39</td>
<td>173.69 ± 1.61</td>
<td>[140]&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(171.8 – 174.7 ± 2.8)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Gly</td>
<td>172.19 ± 1.42</td>
<td>173.81 ± 1.23</td>
<td>170.85 ± 1.28</td>
<td>[139]&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>172.60 ± 1.80</td>
<td>174.61 ± 1.50</td>
<td>171.31 ± 2.59</td>
<td>[140]&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(174.0 – 177.9 ± 2.8)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Pro</td>
<td>175.19 ± 1.34</td>
<td>176.64 ± 1.45</td>
<td>174.48 ± 1.40</td>
<td>[139]&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>175.21 ± 1.72</td>
<td>176.64 ± 1.53</td>
<td>174.71 ± 1.50</td>
<td>[140]&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>(174.0 – 174.7 ± 1.4)&lt;sup&gt;*&lt;/sup&gt;</td>
<td>Val</td>
<td>173.96 ± 1.47</td>
<td>175.95 ± 1.38</td>
<td>173.10 ± 1.39</td>
<td>[139]&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>174.06 ± 1.63</td>
<td>176.05 ± 1.49</td>
<td>172.96 ± 1.36</td>
<td>[140]&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Table 8.2: Tabulated results of bovine ligament elastin chemical shifts observed in this work at 37°C. Additional peaks that were resolvable at 75°C are also shown and indicated with *<sup>*</sup>. As discussed in the text, the chemical shifts are identical across the samples (all three purification schemes, as well as the unpurified starting tissue) within our experimental uncertainty so one table is reported. A † indicates that the value shown was recomputed for the TMS scale [145]. The error bar reported in our measured chemical shift (column 2) corresponds to the half-width at half maximum of the corresponding lines.
Figure 8.3: a) Direct polarization (DP) $^{13}$C NMR spectra of hydrated (a) and lyophilized (b) bovine nuchal ligament elastin samples at 37°C. For each set of figures (a, or b) the spectra shown are as follows: A) sample 1 purified by the autoclaving method [130], B) sample 3 purified by the Starcher method [132], C) sample 2 purified by the alkaline extraction method [131] and D) unpurified elastin. As discussed in the text, the spectra appear remarkably similar in terms of the chemical shifts, however a signal enhancement of the valine or proline-$C_\beta$ at approximately 30.0ppm was observed in D-2. Note that differences shown in Table 8.1 in regards to amino acid concentration may not be reflected in a simple difference in signal intensity in the NMR spectra due differences in mass of the sample packed into the rotor or potential structural heterogeneity across the samples. The reader should note the different scales used in the spectra, as they were all scaled such that the standard deviation of the noise was set to unity.
Figure 8.4: Cross polarization (CP) $^{13}C$ NMR spectra of lyophilized bovine nuchal ligament elastin samples at 37°C. A) sample 1 purified by the autoclaving method [130], B) sample 3 purified by the Starcher method [132], C) sample 2 purified by the alkaline extraction method [131] and D) unpurified elastin. As discussed in the text, a signal enhancement which corresponds to alanine $C_\alpha$ at approximately 53ppm, of the unpurified sample (D) was observed.
Figure 8.5: Normalized $^{13}$C NMR direct polarization spectrum of hydrated bovine nuchal ligament elastin (sample 1) at $75^\circ C$. As discussed in the text, all the spectra from purified elastin were identical in terms of the measured chemical shifts and relative signal intensities, thus only one spectrum is shown. a) The backbone carbonyl is shown and a characteristic splitting is observed (structural assignments provided and discussed in the text). b) The aliphatic region is shown; our spectral resolution is approximately 0.3ppm and allows for a more accurate chemical shift and secondary structure assignment compared with data acquired at $37^\circ C$. 
### Table 8.3: Tabulated results of $T_1$ relaxation times at two different fields and correlation times of the spectroscopically resolved moieties of hydrated elastin samples at 37°C.

<table>
<thead>
<tr>
<th>ppm</th>
<th>$T_{1\rho}$ [ms] (50kHz)</th>
<th>$T_{1\rho}$ [ms] (25kHz)</th>
<th>$\tau_c$ [µs]</th>
<th>ppm</th>
<th>$T_{1\rho}$ [ms] (50kHz)</th>
<th>$T_{1\rho}$ [ms] (25kHz)</th>
<th>$\tau_c$ [µs]</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.8</td>
<td>5.90±0.18</td>
<td>3.83±0.10</td>
<td>3.27±0.05</td>
<td>18.8</td>
<td>9.60±0.54</td>
<td>6.50±0.23</td>
<td>3.02±0.25</td>
</tr>
<tr>
<td>19.5</td>
<td>5.62±0.12</td>
<td>4.05±0.08</td>
<td>2.65±0.02</td>
<td>19.5</td>
<td>8.88±0.35</td>
<td>6.08±0.15</td>
<td>2.95±0.18</td>
</tr>
<tr>
<td>22.0</td>
<td>3.11±0.13</td>
<td>1.95±0.06</td>
<td>3.49±0.13</td>
<td>22.0</td>
<td>3.23±0.31</td>
<td>2.76±0.11</td>
<td>1.62±0.73</td>
</tr>
<tr>
<td>23.4</td>
<td>2.51±0.08</td>
<td>1.58±0.04</td>
<td>3.46±0.08</td>
<td>23.4</td>
<td>2.91±0.15</td>
<td>2.61±0.09</td>
<td>1.30±0.24</td>
</tr>
<tr>
<td>25.2</td>
<td>1.05±0.02</td>
<td>0.71±0.01</td>
<td>3.03±0.06</td>
<td>25.2</td>
<td>2.20±0.09</td>
<td>1.69±0.05</td>
<td>2.27±0.13</td>
</tr>
<tr>
<td>30.9</td>
<td>1.12±0.02</td>
<td>0.53±0.01</td>
<td>5.35±0.01</td>
<td>30.9</td>
<td>1.74±0.05</td>
<td>1.24±0.02</td>
<td>2.71±0.15</td>
</tr>
<tr>
<td>43.3</td>
<td>0.60±0.04</td>
<td>0.27±0.01</td>
<td>5.71±0.44</td>
<td>43.3</td>
<td>1.01±0.03</td>
<td>0.32±0.02</td>
<td>8.65±0.63</td>
</tr>
<tr>
<td>60.3</td>
<td>0.76±0.06</td>
<td>0.23±0.03</td>
<td>9.11±1.05</td>
<td>60.3</td>
<td>1.92±0.02</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>172.1</td>
<td>1.14±0.02</td>
<td>0.70±0.02</td>
<td>3.62±0.14</td>
<td>172.1</td>
<td>1.94±0.14</td>
<td>0.96±0.04</td>
<td>5.03±0.42</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ppm</th>
<th>$T_{1\rho}$ [ms] (50kHz)</th>
<th>$T_{1\rho}$ [ms] (25kHz)</th>
<th>$\tau_c$ [µs]</th>
<th>ppm</th>
<th>$T_{1\rho}$ [ms] (50kHz)</th>
<th>$T_{1\rho}$ [ms] (25kHz)</th>
<th>$\tau_c$ [µs]</th>
<th>ppm</th>
<th>$T_{1\rho}$ [ms] (50kHz)</th>
<th>$T_{1\rho}$ [ms] (25kHz)</th>
<th>$\tau_c$ [µs]</th>
<th>ppm</th>
<th>$T_{1\rho}$ [ms] (50kHz)</th>
<th>$T_{1\rho}$ [ms] (25kHz)</th>
<th>$\tau_c$ [µs]</th>
</tr>
</thead>
<tbody>
<tr>
<td>18.8</td>
<td>6.12±0.22</td>
<td>3.92±0.10</td>
<td>3.36±0.13</td>
<td>18.1</td>
<td>4.44±0.14</td>
<td>3.23±0.08</td>
<td>2.59±0.08</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19.5</td>
<td>6.46±0.20</td>
<td>4.34±0.09</td>
<td>3.07±0.13</td>
<td>18.8</td>
<td>5.14±0.09</td>
<td>3.01±0.05</td>
<td>3.91±0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22.0</td>
<td>2.21±0.11</td>
<td>1.52±0.05</td>
<td>2.92±0.20</td>
<td>21.3</td>
<td>3.30±0.19</td>
<td>2.07±0.05</td>
<td>3.48±0.41</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23.4</td>
<td>2.24±0.11</td>
<td>1.21±0.03</td>
<td>4.44±0.32</td>
<td>22.7</td>
<td>3.16±0.16</td>
<td>1.71±0.05</td>
<td>4.43±0.28</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25.2</td>
<td>0.86±0.03</td>
<td>0.63±0.01</td>
<td>2.55±0.23</td>
<td>24.5</td>
<td>1.54±0.06</td>
<td>1.18±0.02</td>
<td>2.28±0.26</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30.9</td>
<td>0.97±0.02</td>
<td>0.55±0.01</td>
<td>4.12±0.03</td>
<td>30.5</td>
<td>1.36±0.05</td>
<td>0.76±0.02</td>
<td>4.21±0.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>43.3</td>
<td>0.51±0.03</td>
<td>0.34±0.01</td>
<td>3.11±0.35</td>
<td>42.9</td>
<td>1.05±0.07</td>
<td>0.58±0.02</td>
<td>4.29±0.42</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60.3</td>
<td>0.59±0.13</td>
<td>0.32±0.05</td>
<td>-</td>
<td>61.7</td>
<td>1.53±0.13</td>
<td>1.27±0.07</td>
<td>1.80±0.37</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>172.1</td>
<td>1.24±0.10</td>
<td>1.13±0.06</td>
<td>-</td>
<td>172.1</td>
<td>2.80±0.11</td>
<td>2.17±0.13</td>
<td>2.22±0.24</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Chapter 9

Diffusion in elastin-based biomaterials

In this chapter, I will discuss preliminary data of diffusion in elastin based biomaterials. In general, the techniques described in the first part of this thesis may be applied to systems that exhibit any type of intrinsic disorder in their geometry. A useful application of the findings is the characterization of biomaterials intended for use as dermal substitutes. Important parameters that describe structure and control nutrient diffusion, such as the surface-to-volume ratio and diffusive permeability, may be obtained with a bulk diffusion measurement.

Elastin based hydrogels have been studied for use as dermal substitutes as they mimic the extracellular matrix and are responsible for the diffusion of nutrients to the native tissue; an important requirement for tissue regeneration of burn victims. The process of \( \alpha \)-elastin hydrogel fabrication involves two steps: coacervation and crosslinking [155]. During the process of coacervation the \( \alpha \)-elastin molecules coacervate in a solution whereas during the crosslinking process, bond and form a mechanically stable material. After the formation of the hydrogel, several techniques have been used to control the porosity of the material, including gas foaming technology [156] or crosslinking under high pressure CO\(_2\) [155]. Controlling the
porosity or other structural parameters of the biomaterial is of critical importance for the optimal design and biophysical application. It was also shown that elastin based biomaterials produced under atmospheric pressure may exhibit anisotropy in their structure with respect to orientation [155].

The biomaterial used in this study was provided by Anthony Weiss (University of Sydney) in dry state. The principle component of the biofilms used in this work is tropoelastin produced from a large-scale Escherichia coli fermentation process [157]. Tropoelastin self-assembles and forms a stable synthetic elastin structure [158, 159, 160]. For the hydration process the produced biofilm was placed in PBS (phosphate buffer saline) of 1x concentration for 14 hours. The films were cut in rectangles of 1 \( mm \) in width and 7 \( mm \) length and placed in an NMR glass tube in a layered geometry. The films had a height of approximately 300 \( \mu m \).

Figure 9.1 highlights optical microscopy images\(^1\) of the hydrated films in three different orientations. A porous structure was observed with pore diameters ranging from 5 \( \mu m \) to 15 \( \mu m \). In addition, lower pore density was observed on the \( xz \) plane in comparison to the remaining planes, which may hinder diffusion.

\(^1\)Credit to Philip Durlik for performing the histology analysis and taking the images
Figure 9.1: Optical microscopy images of the elastin-based films used in this work: a) along the \(xy\) plane. b) along the \(yz\) plane and c) along the \(xz\) plane. The images reveal a porous matrix with pore diameter ranging from \(5 \, \mu m\) to \(15 \, \mu m\).

Figure 9.2 highlights preliminary data of the initial decay of diffusion of PBS in the films shown in Fig. 9.1a. The corresponding direction in which diffusion was measured is the \(z\) axis. A typical decay of \(-\sqrt{t}\) was observed for diffusion times \(t < 3 \, ms\), with the least squares fit to eq. 3.9 (Chapter 3) yielding an average pore diameter of \(\bar{a} = 16 \pm 4 \, \mu m\). The \(\bar{a}\) obtained from the fit seems to agree with the average pore diameters obtained from the optical microscopy images.
Increasing the diffusion time, would yield the characteristic $t^{-0}$ time-dependence of the diffusion coefficient at long times discussed in Chapter 3; most commonly $t^{-1/2}$ power law dependence corresponding to short-range disorder. Surprisingly, no time dependence was observed in any of the orientations, highlighted in Fig. 9.3 (red-green-cyan). For all three orientations the diffusion coefficient reaches the equilibrium value $D_\infty \approx 1.40 \, \mu m^2/ ms$ almost instantaneously indicating high permeability of the biofilms. It should also be noted that $D_\infty$ along the $y$ axis (cyan) is slightly lower compared to the other two orientations agreeing with the low pore density revealed from the optical microscopy in Fig. 9.1c of the $xz$ plane. Fig. 9.3 also highlights measurements of diffusion of unrestricted $H_2O$ and unrestricted PBS which show no time-dependence. The time dependence of the diffusion coefficient of $H_2O$
through the phantom described in Chapter 5 is also shown for comparison.

Figure 9.3: Time dependence of the diffusion coefficient in elastin based biomaterials with respect to orientation (red-green-cyan). No time dependence was observed in either orientation, indicating the high permeability of the biomaterial. On the other hand, the diffusion coefficient of water diffusing through a controlled phantom with pores of 15 nm in diameter, has a characteristic time dependence described in chapter 5. The diffusion coefficient of unrestricted H$_2$O (magenta) and unrestricted PBS (light green) showed no time dependence.
Chapter 10

Conclusion

In conclusion, this thesis reports on the relations between the dynamic process of diffusion and the underlying structural complexity of media. The theoretical models of diffusion in disordered media predict a universal scaling of $-t^{1/2}$ of the diffusion coefficient for times smaller than the typical time across a pore. At times beyond the residence time within the pore the scaling of the diffusion coefficient switches to distinguishable power law tails $D(t) \sim t^{-\vartheta}$, where $\vartheta$ is the dynamical exponent and depends on the low $k$ behavior of the structure correlation function. Measurements of diffusion in phantoms exhibiting controlled disorder at both short- and long-time regimes reveal the universal scaling of the diffusion coefficient and provide structural information for their disorder class, surface-to-volume ratio and diffusive permeability. The proposed methodology of diffusion in disordered media may be used to determine the diffusive permeability of elastin-based films intended for use as dermal substitutes. Preliminary data, showing the potential application of the findings are also highlighted in this thesis. In addition a detailed study of the structure and dynamics of elastin purified by different protocols is reported.
CHAPTER 10. CONCLUSION

More work needs to be done experimentally in the field of phantom construction, perhaps using 3D printing techniques or electrospinning. The latter techniques will allow for a quantitative control of the pore density, pore size and barrier spacing. In terms of applications, the proposed technique may be routinely used in petroleum industry for determining the diffusive permeability of rocks. In addition, various complex networks experience disorder which affects the diffusion of information. Studying how the structural universality class affects the diffusion of information may reveal interesting outcomes on disease spreading. Lastly, in the field of bioengineering the quantification of structural parameters of scaffolds used as dermal substitutes may be done in a more quantitative manner allowing for design optimization.

Part of this thesis reports on the adsorption of gases in Vycor disordered glass. The proposed models allow for determining the density and number of adsorbed layers using high field NMR methodology. Future work may include application of the proposed model to real rock samples, such as gas shales; a resource of natural gas. In addition, more work needs to be done numerically for studying diffusion and adsorption of gases in disordered systems.
Bibliography


[119] M. Lutolf and J. Hubbell, “Synthetic biomaterials as instructive extracellular microen-
vironments for morphogenesis in tissue engineering,” *Nature Biotechnology*, vol. 23, 


massive elastic assemblies of self-organized human protein monomers,” *Biomaterials*, 

Weiss, “Tailoring the porosity and pore size of electrospun synthetic human elastin 
scaffolds for dermal tissue engineering,” *Biomaterials*, vol. 32, no. 28, pp. 6729–6736, 
2011.

for potential application of heart valve tissue engineering,” *J Tissue Sci Eng S*, vol. 11, 

composite scaffold for functional tissue engineering of cartilage,” *Nature Materials*, 


[144] K. K. Kumashiro, M. S. Kim, S. E. Kaczmarek, L. B. Sandberg, and C. D. Boyd, “(13)C cross-polarization/magic angle spinning NMR studies of alpha-elastin prepara-


