DE-NOVO DETERMINATION OF PROTEIN TERTIARY STRUCTURE IN A HHPNX 3D -FACE CENTERED CUBIC LATTICE WITH MEAN FIELD MULTI-AGENT REINFORCEMENT LEARNING

by

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I declare that this dissertation is my own work and that the work of others is acknowledged and indicated by explicit references.

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May 2020

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Abstract

The protein folding problem is the search for a function that maps a proteins primary structure, composed of a string of discrete amino acid residues to their respective native conformation in 3D space denoted as the protein's tertiary structure. Recent breakthroughs in the field have utilize techniques such as multiple sequence alignment coupled with residual convolutional neural networks to derive candidate posteriors over the distribution of inter-residue distances from which multiple energetically favourable tertiary structures can be generated; these results are typically annealed to produce a structure of lowest conformational energy. In this work I propose an alternative deep reinforcement learning system that does not rely on the pre-existing data of other sequences and instead approximates the pair-wise local interactions of the residues on a 3D Face-Centered Cubic Lattice model. I show that mean-field approximations effectively model the the free energy landscape of the system with respect to the expectation of the distribution of rewards in the local neighbourhood for each agent. I propose that the architecture of this system effectively incorporates inductive bias into the problem formulation and thus provides a richer training signal. Additional techniques are also employed in combination to reduce the sample complexity of the search space.

Acknowledgements

Write any personal words of thanks here. Typically, this space is used to thank your supervisor for their guidance, as well as anyone else who has supported the completion of this dissertation, for example by discussing results and their interpretation or reviewing write ups. It is also usual to acknowledge any financial support received in relation to this work.

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Glossary

A_f	The source message, being a sequence of f source symbols $a_1 a_2 \ldots a_f$
a_i	The i^{th} symbol in the source message, where $a_i \in S_m$
B_g	The decoded message, being a sequence of g source symbols $b_1 b_2 \ldots b_g$
b_i	The i^{th} symbol in the decoded message, where $b_i \in S_m$
C_h	The transmitted (compressed) message, being a sequence of h Tunstall codewords $c_1 c_2 \ldots c_h$
c_i	The i^{th} codeword in the transmitted message, where $c_i \in T_n$
D_h	The received (compressed) message, which for a complete Tunstall code is a sequence of h Tunstall codewords $d_1 d_2 \ldots d_h$
d_i	The i^{th} codeword in the received message, where $d_i \in T_n$ for a complete Tunstall code

Abbreviations

MDP	Markov Decision Process
DQN	Deep-Q-Learning
PSP	Protein Structure Prediction
PDB	Protein Data Bank
CASP	Critical Assessment of Structure Prediction
KL	Kullback Liebler
ΔH	Change in enthalpy
ΔS	Change in entropy
T	Temperature

Chapter 1

Introduction

1.1 Problem Background

Proteins are biological molecules that carry out specific functions within the body, they are assembled out of chemical bonds between smaller sub units called amino acid residues to form a poly-peptide chain. Proteins carry out their functions by conforming into specific shapes and fitting into substrates, this is referred to as the "lock and key" model of proteins. A protein's three-dimensional structure has been proven [reference] to been entirely determined from the ordering of a discrete set of 22 residues in a sequence of arbitrary length. The ability to infer a protein's precise[1] three dimensional structure directly from the sequence of residues would unlock the potential for designer drugs that carry out specific actions within the body; it would also open a path for the treatment of illnesses that arise from malformed proteins such as huntington's disease [reference]. This has however been an open problem, as the search space of possible conformations for a given protein is vast and subject to numerous local minima. Traditional methods of protein structure determination such as X-Ray Crystallography [reference] are extremely costly[2] and time consuming, sometimes taking up to *four years* to determine the structure of a protein. This has given rise to multiple computational approaches [reference] as means to drive down cost and increase the productivity of researchers. Naturally, many approaches using deep learning have been proposed to solve the problem, recently a breakthrough by DeepMind [reference] propelled them to success at the bi-annual Critical Assessment of Structure Prediction (CASP) competition. Though their results were state of

[1] Within 1Å

[2] In the order of millions [reference]

the art, their methods still relied on building a predictive model of the properties of available proteins in the Protein Data Bank (PDB)[reference]; as opposed to inferring the tertiary structure from the primary sequence alone.

1.2 Project Aims and Objectives

Over the course of this dissertation I aim to explore and contrast modern deep learning approaches for approximating the native conformations of proteins on a discrete lattice structure. I will then go on to propose a novel algorithm based on mean-field approximations that addresses some of the drawbacks and biases inherent in the approaches I have reviewed. The following is a list of the project's aims overall:

1. Provide a succinct introduction to the molecular mechanics that govern the conformations of proteins
2. Introduce and compare different approaches to modelling the problem computationally
3. Provide an extended analysis of lattice models for proteins and their ability to encode correct conformations
4. Introduce reinforcement learning and progress to modern Deep Q-Learning
5. Building on Deep Q-Learning, introduce improvements to the algorithm since it's inception
6. Compare and contrast deep learning approaches to the lattice model with an emphasis on reinforcement learning methods
7. Introduce multi-agent learning within the framework of stochastic games
8. Introduce mean-field approximations in particular reference to mean field games
9. Formulate the conformation of residues on a lattice as a cooperative game of incomplete information
10. Provide a novel approach to de-novo structure determination using mean-field multi-agent learning
11. Benchmark my approach against the results of other groups on the same proteins to evaluate the effectiveness of the novel algorithm

1.3 Success Criteria

In order to evaluate the utility of both my findings and subsequent algorithm, I have defined the following high-level requirements that must be satisfied to mark this project as a success.

1. Provide comprehensive overview of the underlying problem of protein folding
2. Describe markov decision processes (MDPs) and its ties to reinforcement learning
3. Highlight drawbacks to the default DQN algorithms and notable improvements to address those
4. Demonstrate multi-agent learning as a generalisation of single MDPs into markov games
5. Successfully benchmark my multi-agent approach against similar lattice-based approaches to protein folding

1.4 Structure of Report

1. **Introduction**

   In this section I provided an overview the the protein folding problem and introduced the components that I will be synthesising into novel approach.

2. **Literature Review**

   In this section I will provide an overview as-well as an introduction to select topics and that comprise the necessary background knowledge. This section ends with a comparative analysis of related work on the problem.

3. **System Requirements and Specification**

   In this section I will analyse the drawbacks of methods utilized in related work, and from this evaluation derive the requirements of a systems required to address these limitations.

4. **System Design**

   This section seeks to unify the selected systems and concepts into an integrated learning algorithm that coherently reflects the underlying problem's structure.

5. **Testing & Validation**

   In order to verify the efficacy of the learning agents, a set of lattice structure of known proteins will be calculated and evaluated against the results for the same set of proteins in related work.

6. **Discussions**

   Limitations of my implementation are discussed here and possible solutions and research directions are proposed.

7. **Conclusion**

   Here I will present my concluding thoughts and results of my final analyses of the architecture.

Chapter 2

Literature Review

2.1 Proteins

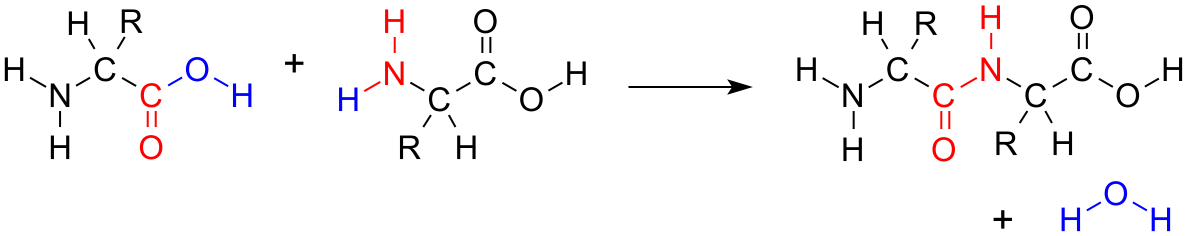
2.1.1 Amino Acids & Poly-Peptides

Proteins are the mechanism by which executive function takes place in the cell. This is includes the implementation of activities such as *"metabolism, growth, architecture and regulation of cell and organism"* -(Lesk 2019). Proteins themselves are composed of discrete molecular units termed amino acids, the precise way in which they amino acids arrange themselves is dependent of the genetic sequence of the parent organism itself. There exist 20 unique amino acids (examples given below) that can be combined that can be sequenced into strings of arbitrary length of any order following chemical laws. These molecules come together to form peptide

Table 2.1

Examples of proteins and their codes	
Glycine	G
Alanine	A
Serine	S
Cysteine	C
Threorine	T
Proline	P
Valine	V
Leucine	K

Figure 2.1: Peptide Bond

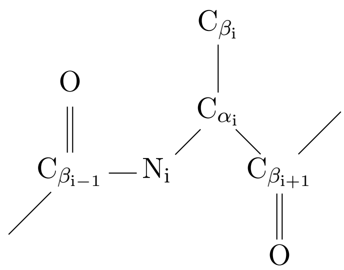


†Image Source: `https://en.wikipedia.org/wiki/Peptide_bond`

bonds, this occurs when the carboxyl group ($^{-}COOH$) bonds to the *amino* group ($^{+}NH_3$) of another amino acid producing water as a by-product of the reaction (Lesk 2019). Despite all amino acids possessing an amino and carboxyl group, every amino acid has a unique side chain, an additional molecular group attached to the main chain. The unique chemical properties of these side chains are responsible for the interactions in that molecule's *neighbourhood*; a term that is expanded on in the next few sections.

2.1.2 Protein Structures

Figure 2.2: Structure of a peptide unit

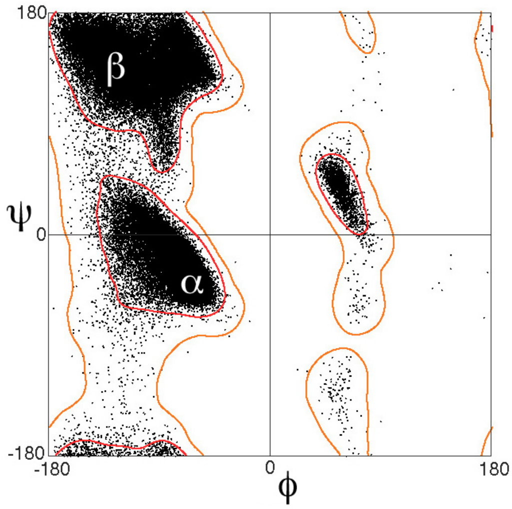


The carbon atom that joins the side chain to the main chain is denoted as the α carbon atom and the carbon atoms in immediate contact with the α carbons are known as β carbons[1]. The bond angles between the $C_{\alpha_i} - C_{\beta_i+1}$ and $C_{\alpha_i} - N_i$ groups are commonly denoted as ψ and ϕ respectively. Stereochemically feasible angles can be described by a Ramachandran plot as in figure 2.3.

Poly-peptide conformations can be described by three structures:

[1]In figure 2.2 subscript i denotes membership to a unique unit

Figure 2.3: Ramachandran plot



†Source: `https://proteopedia.org/wiki/index.php/Ramachandran_Plots`

1. **Primary Structure**

   This is the encoding of a protein as a 1-D sequence of its constituent amino acids. I.e:

   $$DTYGYWEPYT$$

2. **Secondary Structure**

   This encoding represents local, repeating structures with respect to the entire conformations. These structures satisfy chemical restraints common to most proteins[2]. In particular, the formation of structures known as α[3] helices and β[4] sheets largely facilitate the energetic and conformational constraints imposed by the interactions amongst the side-chains orthogonal to the backbone. These structures form the dense regions in the Ramachandran plot.

3. **Tertiary Structure**

   A protein's tertiary structure are its 3D atomic coordinates in space. The protein's torsion backbone (main-chain) traces out a curve through space parameterised by all pairs (ϕ, ψ) for each residue pair. Hydrogen bonds, which arise when neighbouring residues are in close proximity although not directly connected by the backbone, hold together the various

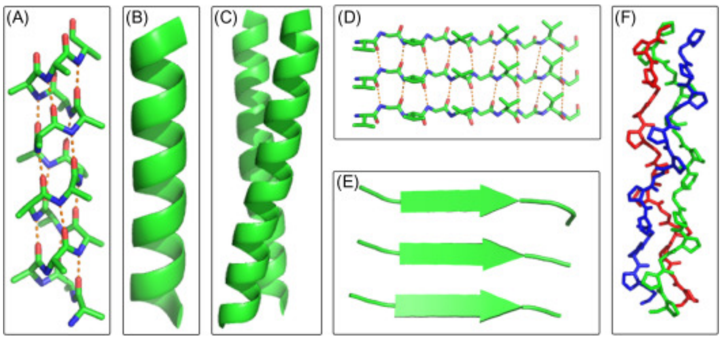
[2]The hydrogen bonding potential of main-chain $N-H$ and $C=O$ groups (Lesk 2019)

[3]B in fig 2.4

[4]E in fig 2.4

secondary structures in specific conformations such that the global energy of the system is minimized (Yang, Ji & Liu 2013).

Figure 2.4: Secondary and tertiary structures



†Source: (Boyle 2018)

The primary goal of protein structure prediction is the *ab initio* determination of a protein's tertiary structure from its primary structure (Yang et al. 2013) which can be described as a mapping:

$$\mathbf{X}^n := \text{Set of all sequences of length } n \tag{2.1}$$

$$\mathbf{F} : \mathbf{X}^n \rightarrow ((\phi, \psi)_1 \ldots (\phi, \psi)_n) \tag{2.2}$$

(Anfinsen 1972) et al showed that the protein's tertiary structure is entirely encoded by its primary structure; the implications of this are explored next.

2.1.3 The Protein's Energy Landscape

Due in part to the continuous spectrum of stereochemically plausible values of (ϕ, ψ), the possible number of conformations for a given sequence are extraordinarily large. Given that each possible conformation has an associated free energy, Levinthal's paradox states that if all conformations were equally energetically favourable, then protein would essentially have to undergo a random walk along the *Gibbs free energy* surface until it has found its native conformation. The free energy surface in this respect refers to the manifold that is formed by taking the *distribution* over conformations $((\phi, \psi)_1 \ldots (\phi, \psi)_n)$ at every time-step and ΔG at every point to form a surface in $2n + 1$ dimensional space. Given that common proteins have in the order of 100-1000+

constituent residues, the combinatorial size of the state space would prohibit any efforts to find a specific conformation by random walk with vanishing probability.
(Yang et al. 2013) address both the paradox and subsequent critiscisms; part of their work can be summarised by the following lemmas:

Lemma 1. *Not all conformations are equally energetically favourable.*

Proof. Proteins fold spontaneously in the order of *nanoseconds* into their native conformation in a solvent at constant temperature, thus they cannot be traversing the whole state space. □

Lemma 2. *There must therefore be a driving reaction that narrows down the search space.*

Proof. Folding appears to undergo two consecutive stages, tier 1 occurs of a timescale of *nanoseconds* and tier 2 appears to occur over a scale of *picoseconds*, a reduction in 3 orders of magnitude. Thus the peptide must undergo a slower interaction that constrains the state space followed by a final, faster "relaxation" into the native state. □

Lemma 3. *By undergoing hydrophobic collapse, the remaining residues have restricted degrees of freedom, thus a reduced subspace consisting of only energetically feasible conformations is explored, within this subspace there exists one unique solution whose energy is minimized.*

Proof. The relative timescales of the tier 1 and tier 2 interactions indicate a smooth slope in conformational space that greatly constrains the possible conformations, followed by a second tier of faster interactions that enables the system to quickly overcome the local maxima and minima which gives way to the global optimum at the bottom of the subset. □

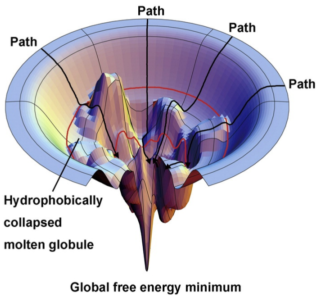
Lemma 4. *If there exists only one unique conformation whose Gibbs free energy is minimal amongst all possible conformations, the manifold must be "funnel shaped".*

Proof. The nature of the hydrophobic collapse "drops" the full state space into a smaller subspace, the unique global minimum lies within this subspace. If there existed more than one unique solution, then a given sequence could form into multiple possible native conformations, this would violate the marginal stability property of the native state so there cannot exist more than one unique solution. □

$$\Delta G = \Delta H - T\Delta S$$

By the second law of thermodynamics, the entropy (amount of disorder) in a closed system can never decrease over time and can remain constant only if the system is in equilibrium, thus a state of maximum entropy (Jaffe 2018). The work of (Yue & Dill 1993, Yang et al. 2013) show that the overall increase in entropy of the peptide-solvent system is primarily motivated by a phenomenon known as the *hydrophobic collapse* whereby all the water produced as by-product of the peptide bond [5] "squeezes" the hydrophobic residues into globular core. Following **Lemma 4**, the process appears to be guided by a heuristic search in this subspace, enabling it to swiftly reach its native state.

Figure 2.5: Gibbs free energy funnel



†Source: (Yang et al. 2013)

Critically, the notion of ΔG is with respect to the distribution over conformations, indicating that at any point the protein exists as an ensemble of possible conformations, and the native state consists of a collection of conformations whose joint distribution minimises the value of ΔG. I reformulate this property as maximum likelihood objective:

Theorem 1.

1. *Let $T \in \{\mathbf{X}\}^n$ denote the native state as a tuple of n torsion angles (follows from eq. 2.1).*

2. *Let $P(\mathbf{F}(\mathbf{X}^n; \theta))$ denote the distribution over all functional mappings from a primary sequence to a tuple of n torsion angles with parameters θ (follows from eq. 2.2).*

The search for a function that maps the protein's primary structure to its tertiary structure can be formulated as the maximization of the posterior probability that function $\mathbf{F}$ with parameters θ

[5] See fig 2.1

maps sequence $\mathbf{X}^n$ *to state* T.

$$P(T|\mathbf{F}(\mathbf{X}^n;\theta)) = \frac{P(\mathbf{F}(\mathbf{X}^n;\theta)|T) \cdot P(T)}{P(\mathbf{F}(\mathbf{X}^n;\theta))} \tag{2.3}$$

This formulation is further explored in the **System Requirements and Specification** section, however an interesting detail to note is that this is equivalent to sampling the maximum likelihood estimate from the posterior of a *Gaussian process* [6].

2.1.4 Bioinformatics

There exists experimental techniques to determine the protein's native (or *crystalline*) structure[7], however these approaches are out of the scope of this project and are not given a detailed analysis. Otherwise, these techniques can often require years of trial and error and can be very costly (Alberts 2002).

In recent years, advancements in both hardware and processing power have enabled computational methods to play increasingly significant role in the PSP problem. I will focus briefly what's known as homology modelling as this is relevant to understanding related supervised learning methods, and then I will then provide extended review of another class of models used for *ab initio* structure prediction, lattice models.

2.1.4.1 Homology Modelling

Homology modelling, otherwise known as template modelling focuses on trying to predict the tertiary structure of an unknown protein from the structures of closely related (homologous) proteins. This involves measuring the Hamming distance of closely related proteins and trying to deduce secondary structure elements from sub-strings within the primary sequence that show high overlap with sub-strings in related sequences.

[6] See appendix A.1

[7] Such as X-Ray Crystallography (Chayen 2005)

2.1.4.2 Lattice Models

2.1.4.3 HP Model

2.1.4.4 hHPNX Model

2.2 Reinforcement Learning

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2.2.4.3 Exploitation

2.2.4.4 Rainbow DQN

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2.3.1 Stochastic Games

2.3.1.1 Game Theory

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