

INVESTIGATING THE RECOGNITION
AND INTERACTIONS OF NON-POLAR
 α HELICES IN BIOLOGY.

A THESIS SUBMITTED TO THE UNIVERSITY OF MANCHESTER
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY
IN THE FACULTY OF LIFE SCIENCES

2016

James Baker

Contents

Abstract	6
Declaration	7
Copyright Statement	8
Acknowledgements	9
List of publications	10
1 Introduction	11
1.1 The importance of membranes and transmembrane proteins.	11
1.2 Biological membranes	12
1.3 Transmembrane helix sequence composition	12
1.4 The “Positive-Inside” rule.	13
1.5 Biogenesis of transmembrane proteins.	14
1.6 Spontaneous membrane insertion	14
2 The “negative-not-inside” rule	16
3 Tail-anchored protein discovery	17
4 Not all transmembrane helices are the same	18
5 The good & the bad helices	19
6 Conclusions	20

List of Tables

List of Figures

- 1.1 **Cartoons of helices in the membrane.** (A) A cartoon showing the general components of the membrane and TMH. Dark grey areas denote the area composed typically of polar or charged amino acid groups. These areas are often described as flanking regions, and are often in contact with the aqueous interface of the membrane. The curved black lines represent the residue chain outside of the membrane. The helix core is mostly composed of hydrophobic groups and is illustrated here in dark orange. More recently the hydrophobic group region has been associated with cell localisation and a broad range of biochemical functions [5, 9]. Note that the definition of an α -helix is not entirely clear; how far the helix rises into the water-interface region to qualify as a TMH for example [7]. (B) A cartoon depicting various problematic, yet biologically observed topologies and lengths that the α helices can adopt. From left to right: a typical and traditional TMH, an exceptionally long TMH, a TMH that lies flat in the interface region, a kinked helix that enters and exits the bilayer on the same leaflet, a TMH that is not long enough to span the entire membrane. Note that these exceptional formations present a challenge for topology predictions of the loop regions. 15

The University of Manchester

James Baker

Doctor of Philosophy

Investigating the Recognition and Interactions of Non-Polar α Helices in Biology.

May 26, 2016

Transmembrane α helix containing proteins make up around a quarter of all proteins, as well as two thirds of drug targets, and contain some of the most critical proteins required for life as we know it. Yet they are fundamentally difficult to study experimentally due to the very features that make them so biologically influential: their hydrophobic transmembrane helices. What is missing in the current literature is a complex, nuanced understanding of this helix composition. Currently it is known that the properties of transmembrane protein α helices underpin membrane protein insertion mechanisms and furthermore can be used to predict presence of function in the transmembrane helix itself. By leveraging large datasets of transmembrane proteins, this thesis is focussed on characterising features of α helices en masse, particularly regarding their topology, membrane-protein interactions, and intra-membrane protein interactions.

Herein we expand on the core understanding of the biophysicochemical properties of these helices. We find evidence of a universal, “negative-not-inside” rule that complements the famous “positive-inside rule” as well as intramembrane leucine propensity for the inner leaflet.

Declaration

No portion of the work referred to in the thesis has been submitted in support of an application for another degree or qualification of this or any other university or other institute of learning.

Copyright Statement

- i. The author of this thesis (including any appendices and/or schedules to this thesis) owns certain copyright or related rights in it (the “Copyright”) and s/he has given The University of Manchester certain rights to use such Copyright, including for administrative purposes.
- ii. Copies of this thesis, either in full or in extracts and whether in hard or electronic copy, may be made **only** in accordance with the Copyright, Designs and Patents Act 1988 (as amended) and regulations issued under it or, where appropriate, in accordance with licensing agreements which the University has from time to time. This page must form part of any such copies made.
- iii. The ownership of certain Copyright, patents, designs, trade marks and other intellectual property (the “Intellectual Property”) and any reproductions of copyright works in the thesis, for example graphs and tables (“Reproductions”), which may be described in this thesis, may not be owned by the author and may be owned by third parties. Such Intellectual Property and Reproductions cannot and must not be made available for use without the prior written permission of the owner(s) of the relevant Intellectual Property and/or Reproductions.
- iv. Further information on the conditions under which disclosure, publication and commercialisation of this thesis, the Copyright and any Intellectual Property and/or Reproductions described in it may take place is available in the University IP Policy (see <http://documents.manchester.ac.uk/DocuInfo.aspx?DocID=487>), in any relevant Thesis restriction declarations deposited in the University Library, The University Library’s regulations (see <http://www.manchester.ac.uk/library/aboutus/regulations>) and in The University’s Policy on Presentation of Theses.

Acknowledgements

So long! And thanks for all the fish. I wish my thesis title was “The ins-and-outs of greasy peptides”.

List of publications

Chapter 1

Introduction

1.1 The importance of membranes and transmembrane proteins.

Transmembrane Proteins (TMPs) underpin almost every biological process directly, or indirectly, from photosynthesis to respiration. Integral TMPs are encoded by around 30% of the genes in the human genome which reflects their biological importance [1].

More recently, the insertion and formation of the unusually orientated TMHs and of the more traditional TMHs have been shown to be underpinned by complex thermodynamic equilibria [2]. TMHs have been identified as regulators of protein quality control and trafficking mechanisms, shifting the idea away from TMHs broadly simply functioning as anchors [3]. The story is not as simple as originally thought. There is a contingency in the field of biological membranes that despite progress over the last decade, there is a lack of information regarding their structure, assembly, and the behaviour of TMHs in the lipid bilayer; the native biological environment of TMHs [2, 4].

Properties that can be analysed by bioinformatics, the sequence complexity and hydrophobicity, of the TMH have been used to predict the role of the TMH as either functional or structural, and as a discrete cluster from other SCOP annotated helices [5]. Those findings demonstrated that sequence of the TMH holds valuable information regarding biological roles, and forms the basis of our interest in the link between the polarity of a helix and functional activity beyond structural anchorage.

1.2 Biological membranes

The compartmentalisation of cellular biochemistry is arguably one of the most significant events to have occurred in the evolution, and is certainly one of the fundamental prerequisites for life [6].

1.3 Transmembrane helix sequence composition

In the 1990s and early 2000s the Transmembrane Helix (TMH) was a rather simple topological story. TMPs were thought to contain membrane spanning bundles of non-polar α -helices, with each TMH having a consistent orientation of being perpendicular to the membrane surface. Although this is broadly true, hundreds of high quality membrane structures have elucidated that TMHs can adopt a plethora of lengths and orientations within the membrane. TMHs are capable of partial spanning of the membrane, spanning using oblique angles, and even lying flat on the membrane surface [7, 8] (Figure 1.1).

Because of the experimental hinderence, the story of transmembrane proteins has been relatively slow to emerge. In the 1990s and early 2000s the story was seemingly uncomplicated. There were membrane-spanning bundles of non-polar α -helices of roughly 20 residues length, with a consistent orientation of being perpendicular to the membrane surface. Since the mid-2000s the elucidation of many more intramembrane helix structures implied a far richer variety of transmembrane helices existed than previously thought, with a range of orientations and intra-membrane biophysical variations.

The language used to describe TMHs varies somewhat across the literature, primarily due to a changing understanding of TMH general structure and relevance to function over the last 15 years or so. There is a general composition of a TMH despite specific protein and membrane constraints [10].

A study by Baeza et al. from 2013 [11] looked at TMHs in 170 integral membrane proteins from a manually maintained database of experimentally confirmed TMPs; MPTopo [12]. The group examined the distribution of residues along the TMHs. As expected, half of the natural amino acids are equally distributed along Transmembrane (TM) helices whereas aromatic, polar, and charged amino acids along with proline are

biasedly near the flanks of the TM helices [11]. Transitions between the different types of amino acid at the ends of the hydrophobic core occur in a more defined region on the cytosolic side than at the extra cytosolic face. This is probably reflecting the different lipid composition of both leaflets of biological membranes [11]. A larger study using 1192 human and 1119 yeast predicted TMHs that were not structurally validated further explored the difference in TMH and leaflet structure by exploiting the evolutionarily conserved sequence differences between the TMH in the inner and outer leaflets [10]. TMHs from vertebrates and invertebrates were found to be reasonably similar compositionally. The differences in consensus TMH structure implies that there are general differences between the membranes of the golgi and ER. The abundance of serines in the region following the luminal end of golgi TMDs probably reflects the fact that this part of many golgi enzymes forms a flexible linker that tethers the catalytic domain to the membrane [10].

1.4 The “Positive-Inside” rule.

Two publications by von Heijne coined the “Positive-Inside” rule demonstrated the practical value of positively charged residue sequence clustering in topology prediction of transmembrane helices in bacteria [13, 14]. It was clearly defined and shown that positively charged residues more commonly were found on the “inside” of the cytoplasm rather than the periplasm of *E. coli*.

More recently still large scale sequence analysis of transmembrane helices from different organelle membrane surfaces in eukaryotic proteomes, show the clustering of positive charge being cytosolic [10, 11].

Whilst the “inside” was an imprecise term used to indirectly refer to the cytoplasmic space. To understand why the cytoplasm is the key part, one must recall how the membranes are thought to be synthesised.

As the idea of positive residues inside the cytoplasm emerged, so did the idea of negative residues working in concert with TMH orientation. It was shown that removing a single lysine residue reversed the topology of a model *Escherichia coli* protein, whereas much higher numbers of negatively charged residues are needed to reverse topology [15]. One would also expect to see a skew in negatively charged

distribution if a cooperation between oppositely charged residues orientated a TMH, however there is no conclusive evidence in the literature for an opposing negatively charged skew [10, 11, 16, 17]. However, in *E. coli* negative residues do experience electrical pulling forces when travelling through the SecYEG translocon indicating that negative charges are biologically relevant [18].

1.5 Biogenesis of transmembrane proteins.

1.6 Spontaneous membrane insertion

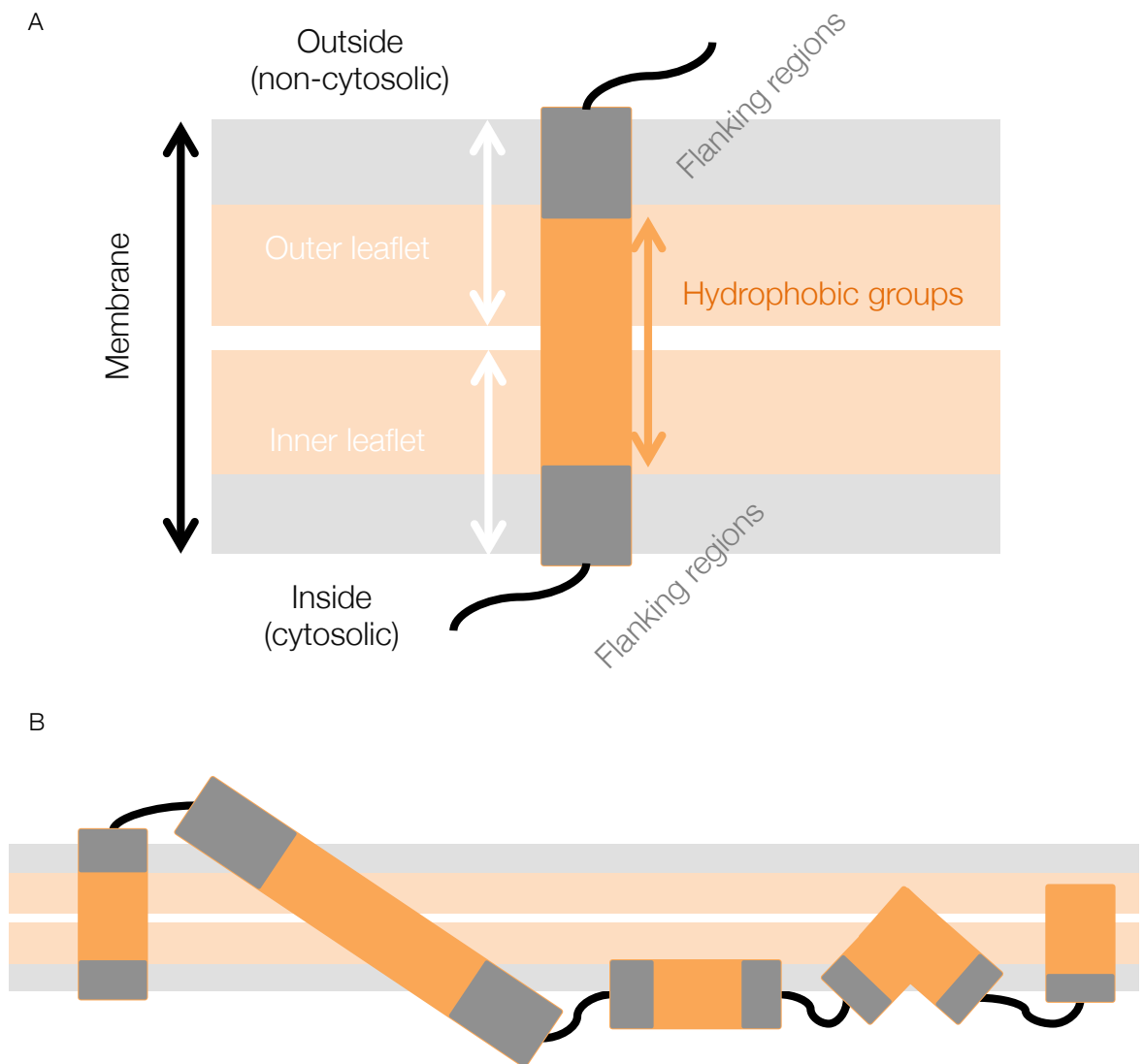


Figure 1.1: **Cartoons of helices in the membrane.** (A) A cartoon showing the general components of the membrane and TMH. Dark grey areas denote the area composed typically of polar or charged amino acid groups. These areas are often described as flanking regions, and are often in contact with the aqueous interface of the membrane. The curved black lines represent the residue chain outside of the membrane. The helix core is mostly composed of hydrophobic groups and is illustrated here in dark orange. More recently the hydrophobic group region has been associated with cell localisation and a broad range of biochemical functions [5, 9]. Note that the definition of an α -helix is not entirely clear; how far the helix rises into the water-interface region to qualify as a TMH for example [7]. (B) A cartoon depicting various problematic, yet biologically observed topologies and lengths that the alpha helices can adopt. From left to right: a typical and traditional TMH, an exceptionally long TMH, a TMH that lies flat in the interface region, a kinked helix that enters and exits the bilayer on the same leaflet, a TMH that is not long enough to span the entire membrane. Note that these exceptional formations present a challenge for topology predictions of the loop regions.

Chapter 2

The “negative-not-inside” rule

$$c_r = \frac{(a_{K,r} + a_{R,r}) - (a_{D,r} + a_{E,r})}{N}$$

$$p_{i,r} = \frac{a_{i,r}}{\max_r(a_r)}$$

$$q_{i,r} = \frac{100a_{i,r}}{a_i}$$

Chapter 3

Tail-anchored protein discovery

Chapter 4

Not all transmembrane helices are
the same

Chapter 5

The good & the bad helices

Chapter 6

Conclusions

Bibliography

1. Almén, M., Nordström, K. J., Fredriksson, R. & Schiöth, H. B. Mapping the human membrane proteome: a majority of the human membrane proteins can be classified according to function and evolutionary origin. *BMC Biology* **7**, 50. ISSN: 1741-7007 (Jan. 2009).
2. Cymer, F., Von Heijne, G. & White, S. H. Mechanisms of integral membrane protein insertion and folding. *Journal of Molecular Biology* **427**, 999–1022. ISSN: 10898638 (Sept. 2015).
3. Hessa, T. *et al.* Protein targeting and degradation are coupled for elimination of mislocalized proteins. *Nature* **475**, 394–397. ISSN: 0028-0836 (July 2011).
4. Ladokhin, A. S. Membrane Protein Folding & Lipid Interactions: Theory & Experiment. *The Journal of Membrane Biology* **248**, 369–370. ISSN: 0022-2631 (June 2015).
5. Wong, W.-C., Maurer-Stroh, S., Schneider, G. & Eisenhaber, F. Transmembrane helix: simple or complex. *Nucleic acids research* **40**, W370–5. ISSN: 1362-4962 (July 2012).
6. Koshland, D. E. Special essay. The seven pillars of life. en. *Science (New York, N.Y.)* **295**, 2215–6. ISSN: 1095-9203 (Mar. 2002).
7. Von Heijne, G. Membrane-protein topology. *Nature Reviews Molecular Cell Biology* **7**, 909–918. ISSN: 1471-0072 (Dec. 2006).
8. Elofsson, A. & von Heijne, G. Membrane protein structure: prediction versus reality. *Annu Rev Biochem* **76**, 125–140. ISSN: 0066-4154 (Jan. 2007).

9. Junne, T., Kocik, L. & Spiess, M. The hydrophobic core of the Sec61 translocon defines the hydrophobicity threshold for membrane integration. *Molecular biology of the cell* **21**, 1662–70. ISSN: 1939-4586 (May 2010).
10. Sharpe, H. J., Stevens, T. J. & Munro, S. A Comprehensive Comparison of Transmembrane Domains Reveals Organelle-Specific Properties. *Cell* **142**, 158–169. ISSN: 00928674 (July 2010).
11. Baeza-Delgado, C., Marti-Renom, M. A. & Mingarro, I. Structure-based statistical analysis of transmembrane helices. *European Biophysics Journal* **42**, 199–207. ISSN: 01757571 (Mar. 2013).
12. Jayasinghe, S., Hristova, K. & White, S. H. MPtopo: A database of membrane protein topology. *Protein Science* **10**, 455–458. ISSN: 0961-8368 (2001).
13. Von Heijne, G. Control of topology and mode of assembly of a polytopic membrane protein by positively charged residues. en. *Nature* **341**, 456–458. ISSN: 0028-0836 (Oct. 1989).
14. Elofsson, A. & von Heijne, G. Membrane protein structure: prediction versus reality. *Annu Rev Biochem* **76**, 125–140. ISSN: 0066-4154 (May 2007).
15. Nilsson, I. & von Heijne, G. Fine-tuning the topology of a polytopic membrane protein: Role of positively and negatively charged amino acids. *Cell* **62**, 1135–1141. ISSN: 00928674 (Sept. 1990).
16. Granseth, E., Von Heijne, G. & Elofsson, A. A study of the membrane-water interface region of membrane proteins. *Journal of Molecular Biology* **346**, 377–385. ISSN: 00222836 (Feb. 2005).
17. Nilsson, J., Persson, B. & von Heijne, G. Comparative analysis of amino acid distributions in integral membrane proteins from 107 genomes. *Proteins* **60**, 606–616. ISSN: 1097-0134 (2005).
18. Ismail, N., Hedman, R., Lindén, M. & von Heijne, G. Charge-driven dynamics of nascent-chain movement through the SecYEG translocon. en. *Nature Structural & Molecular Biology* **22**, 145–149. ISSN: 1545-9993 (Feb. 2015).

Appendix A

Big tables

.....