Targeting Abeta: A Fragment Based Drug Design Approach

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Intensive Training Course (S&T) 2012
September 09-14, 2012, Ecole de Physique des Houches, Les Houches, France

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Overview

Introduction

- Rational Drug Design: targeting ordered proteins
- Intrinsically Disordered Proteins : Drug Design Approaches
- Intrinsic Disorder in Neurodegenerative Diseases
- Targeting Abeta – How??

Methodology

- Computational Mapping
- Fragment Based Drug Design

Results

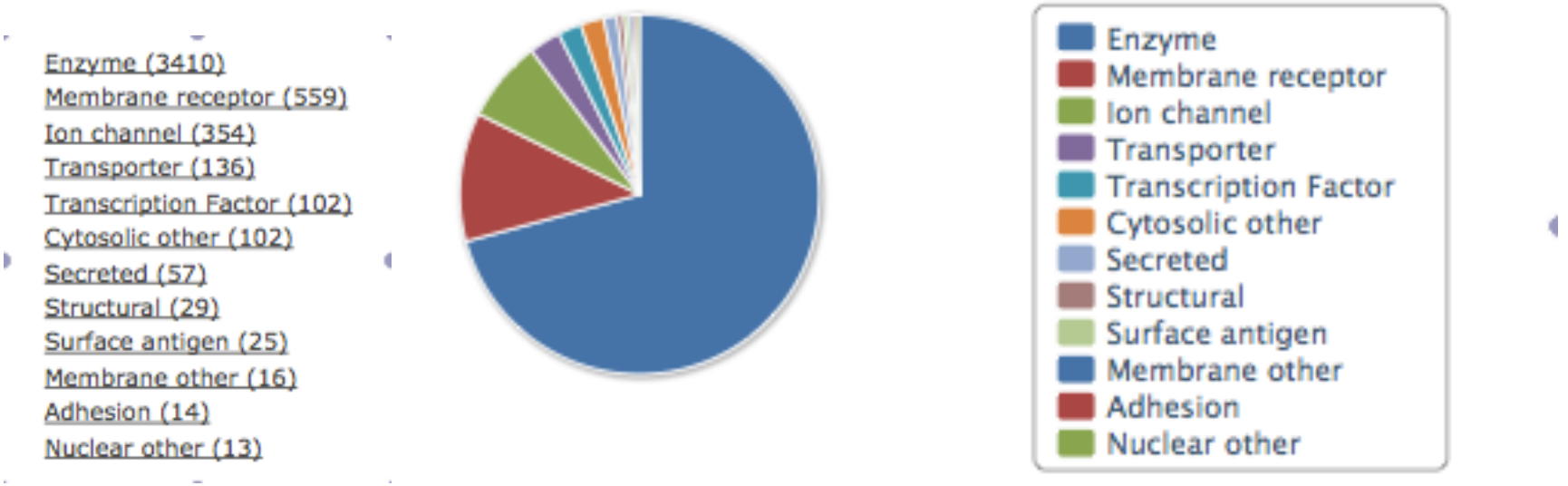
- Hotspots → Binding Pockets; Fragments

Conclusions and Future Directions

IDPbyNMR - High resolution tools to understand the functional role of protein intrinsic disorder – Project n. 264257

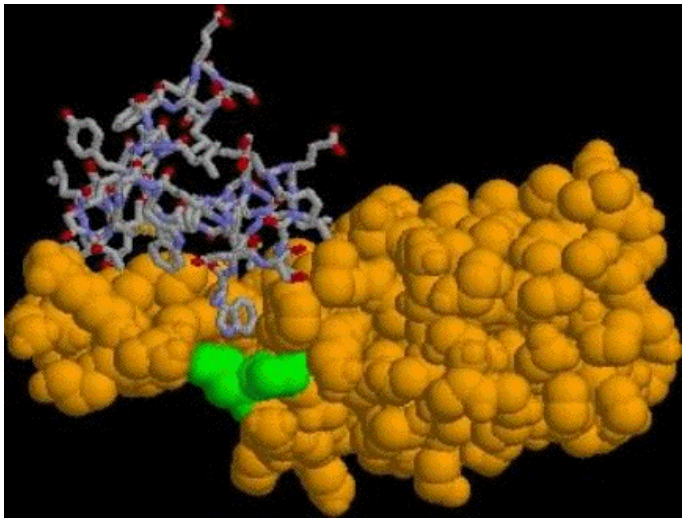
Drugs targeting ordered proteins

Inhibitors → competition with endogenous ligands or substrates at structurally defined binding sites.

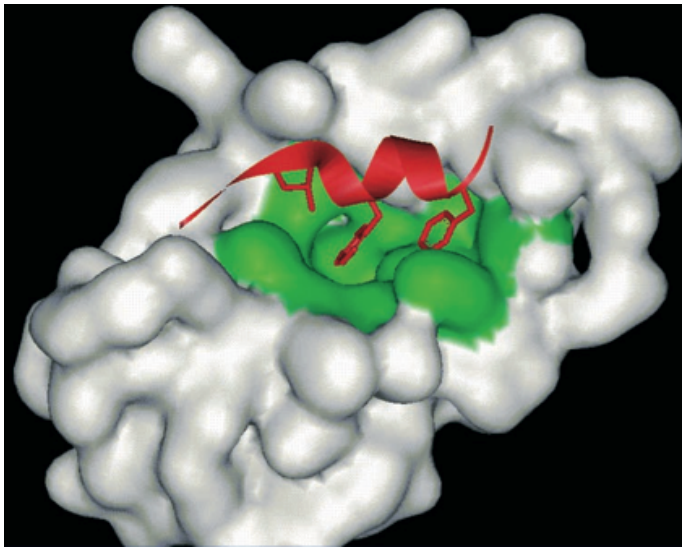


Source: ChEMBL Targets : 9,003 (2012) (https://www.ebi.ac.uk/

protein-protein interactions



Ubiquitin ligase skp1-skp2 complex



P53-mdm2

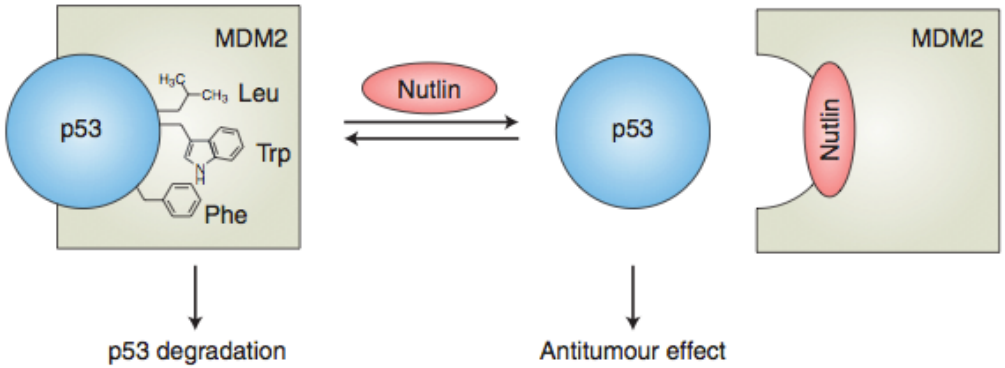
Regions – 1500-3000 Å^2

Complex binding surfaces – discontinuous epitopes or multiple continuous epitopes

Devoid of groves or pockets

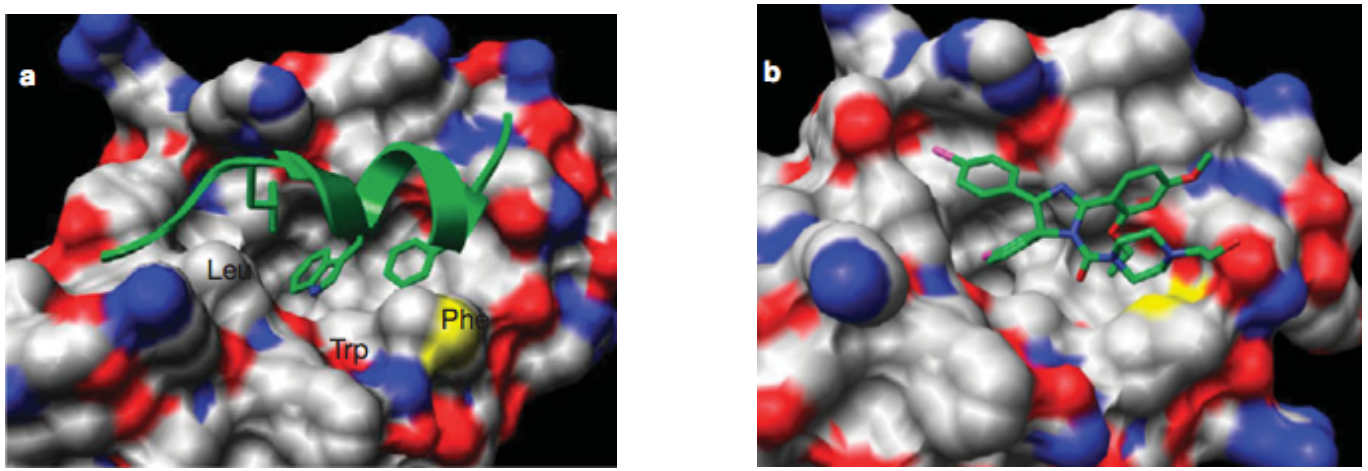
Energy of PPI not evenly distributed over a large contact area but in smaller regions

P53-MDM2



A conceptual diagram of a protein–protein interaction (PPI) inhibitor targeting a PPI (p53–MDM2) hot spot

Expert Reviews in Molecular Medicine © 2008 Cambridge University Press

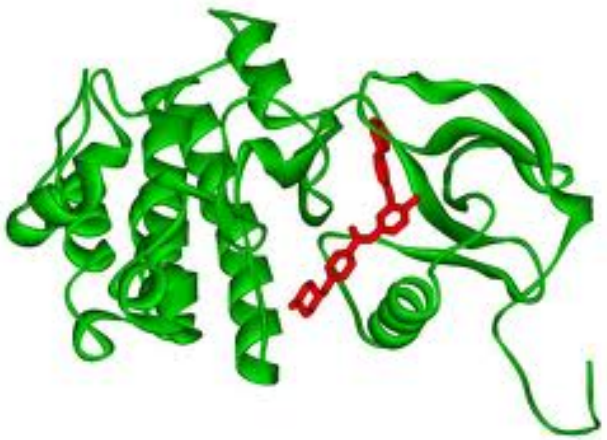


Structural analysis of the interactions of MDM2 with p53 and with a nutlin inhibitor

Expert Reviews in Molecular Medicine (2008) Cambridge University Press

Rational Drug Design

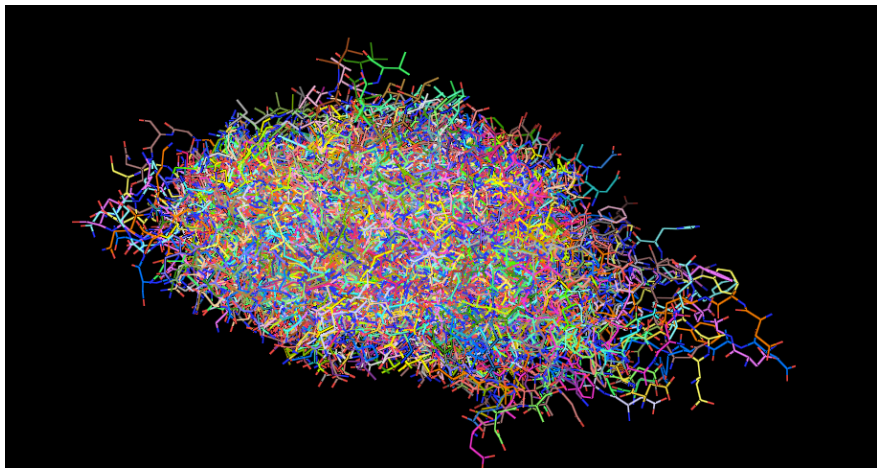
Prior knowledge of a target protein structure represents the most important prerequisite for the development of a new drug.



Intrinsically Disordered Proteins

Inherent lack of structure.

Highly dynamic conformation ensembles resembling **'protein clouds'**



Abeta40 ensemble generated by metadynamics (Alessandro Laio, SISSA)

Approaches for the development of drugs affecting the functions of IDPs

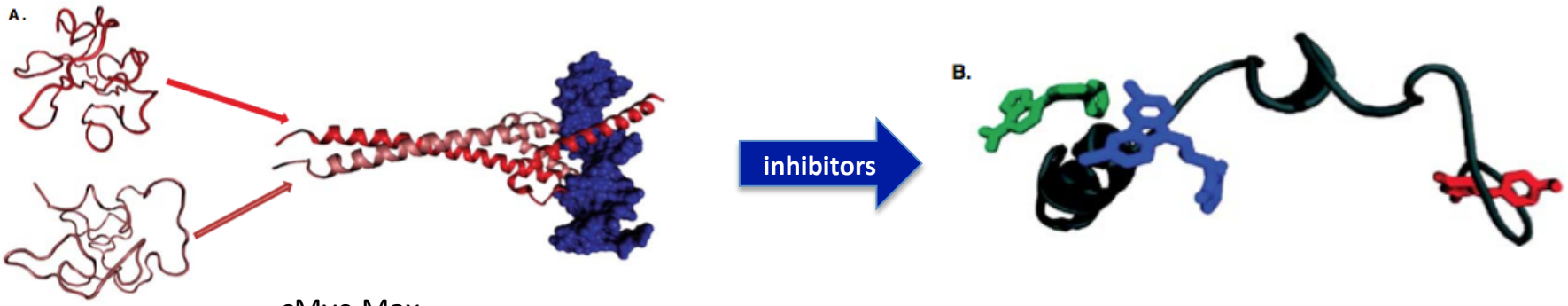
Ordered part targeted (prior knowledge of domain's 3D structure)

Block interaction sites of ordered protein (3D structural information on disordered partner)

Coupled folding and binding – mimicked by small molecules

Direct Target of IDP (!)

p53-Mdm2 interaction (nutlins)
cMyc-Max dimerization

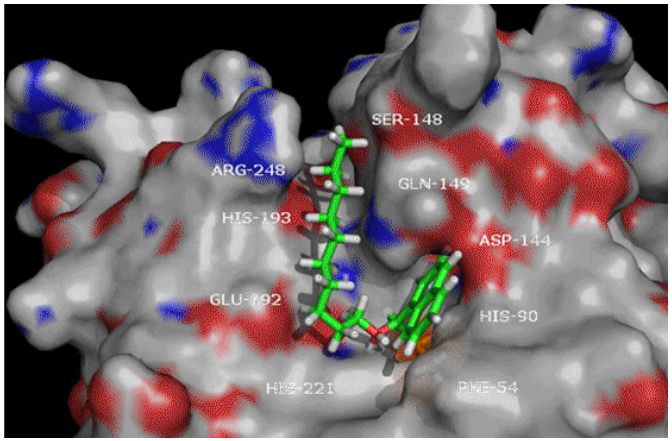


cMyc-Max

Docking small molecules to a protein is a fundamental step in Structure Based Drug Design

(i) Docking of potential ligands from a compound database (potential ligands ?)

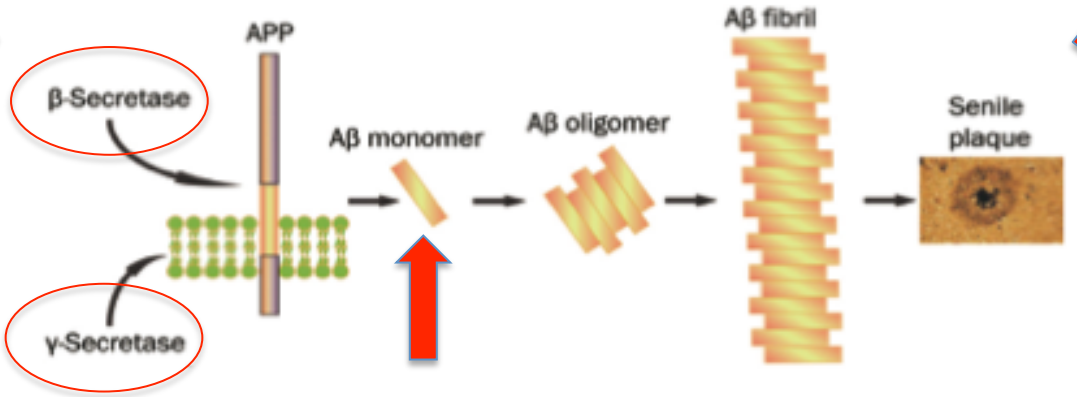
(ii) Mapping the protein for the binding site of molecular probes – small molecules and functional groups – and using the favourable positions for the construction of larger ligands. (binding **hotspots** !)



Docking of a small molecule inhibitor into human PAP
(McGeary et al. (2009) Bioorg Med Chem . Lett. 19.163-166)

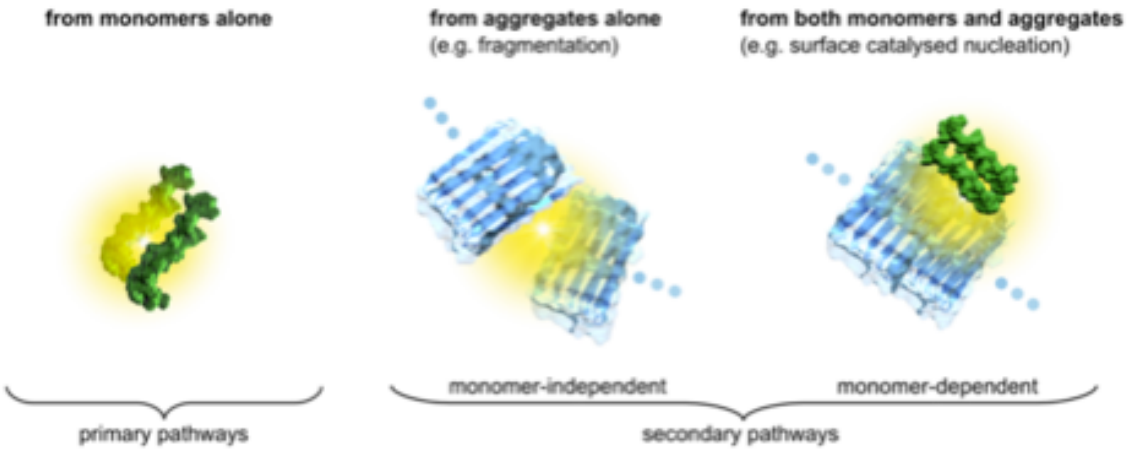
The Amyloid precursor protein (APP) is a precursor of Abeta which is cleaved by beta and gamma secretase to produce Abeta40 and Abeta42 of which Abeta42 monomers aggregate to form oligomers and toxic fibrils.

Targeting

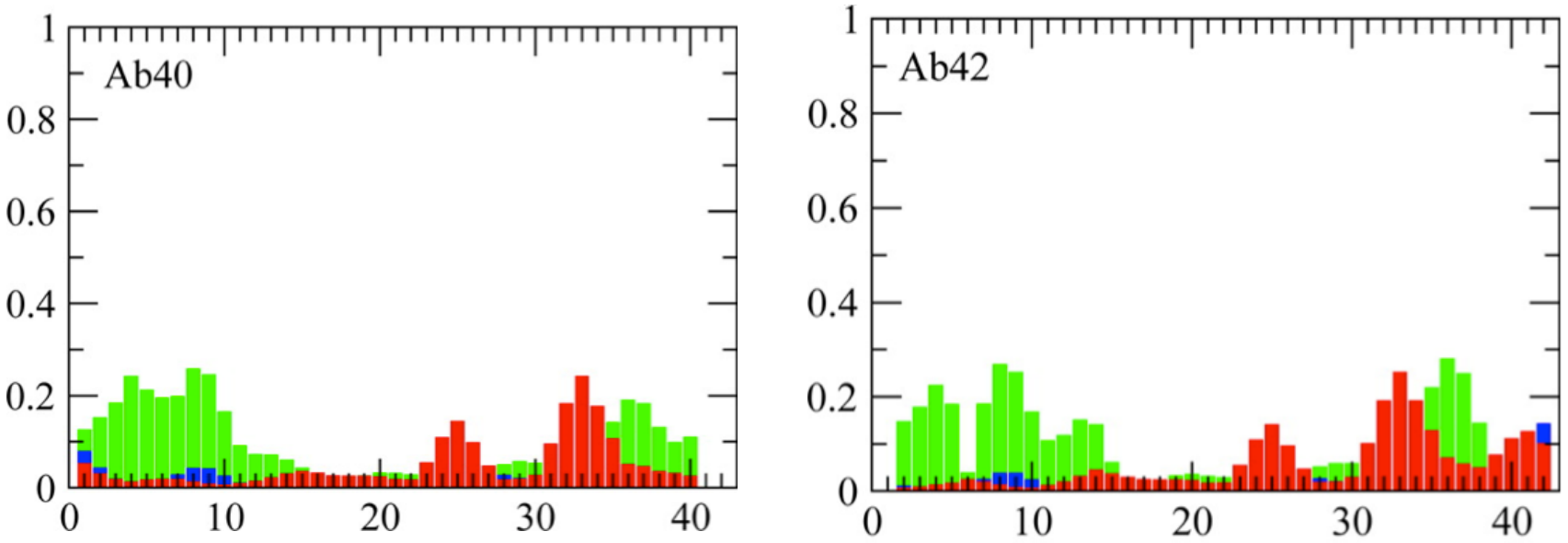


Abeta

General classes of mechanisms that create new aggregates



Secondary Structure Populations - the δ2D method



Chemical shifts have been measured(41) for the Aβ(1–40) (bmr17796) and Aβ(1–42) (bmr17794) peptides. Secondary structure populations are colored red for β-sheets, blue for α-helices, and green for PPIIs. The random coil population is not shown explicitly but can be inferred from the condition that the sum of the four types of secondary structure populations should be equal to one

Comparison of the secondary structure populations for the two main variants of the Aβ peptide obtained by the δ2D method.

Camilloni *et al*. (2012), Biochemistry

Methodology

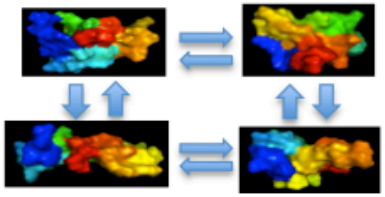
Ensemble of Abeta42 structures

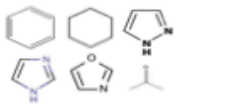
Preparation of Receptor (FRED)

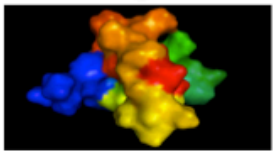
Docking (AM1-BCC charges)

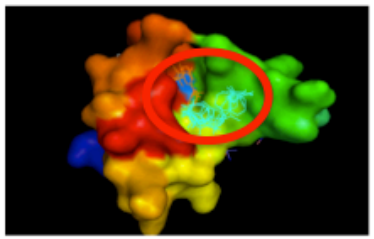
Ranking (best 300 poses of each fragment)

Hotspot analysis and Ligand Efficiency Calculation

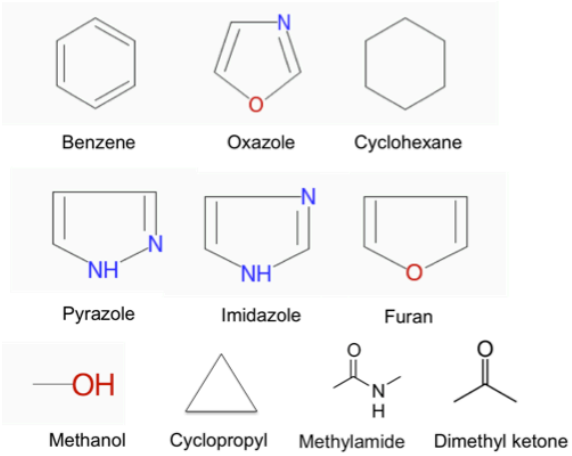








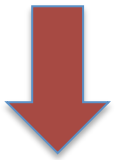
Computational Mapping - FTMap



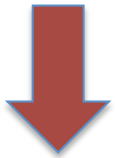
Docking - FRED

Abeta42 ensemble

Replica Exchange Molecular Dynamics (REMD) Simulations of the full length form of the Abeta42 peptides (GROMACS v3.3.3)
Duration of Simulation – 100 ns
Integration time step – 2 fs
Temperature Range (48) – 276.1-376.9 K
Force field – AMBER99SB and the TI3P water model (Yanching Chu *et al.*)

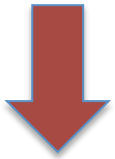


Cluster Analysis (20,000) -> GROMACS, cutoff 2A
(from 60-100 ns of the 309.4K trajectory)
Any clusters larger than 0.05% of the total population in included in cluster analysis → 45 clusters (66.8% of selected structures



FTMap screening → 10 most promising structures

Binding hotspot is defined as a small surface area where more than one fragment ligand binds to and any binding sites consisting of multiple hotspots are subsequently revealed



Ligand Efficiency

$$LE: LE_{p\text{-}f} = E_{p\text{-}f} / N_f$$

where N_f is the number of heavy atoms or non-hydrogen atoms in the fragment probe

Potential Energies here are based on the MMFF94s forcefield

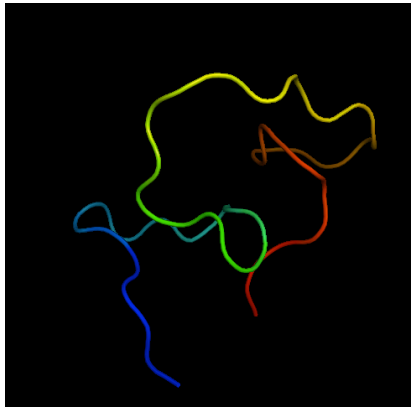
Goodness of **hotspot**

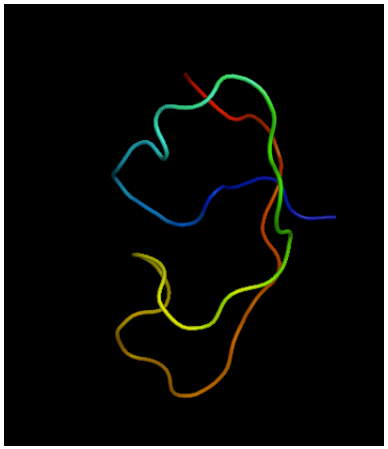
$$LE_{avg} = \sum E_{p\text{-}f} / N_p$$

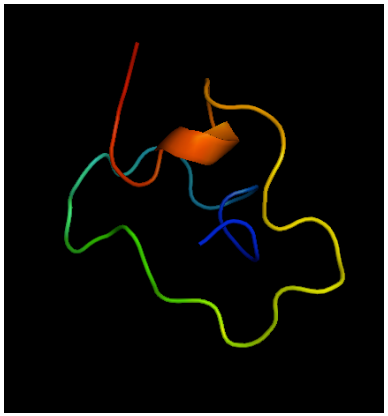
where N_p is the total number of fragment probes concerned

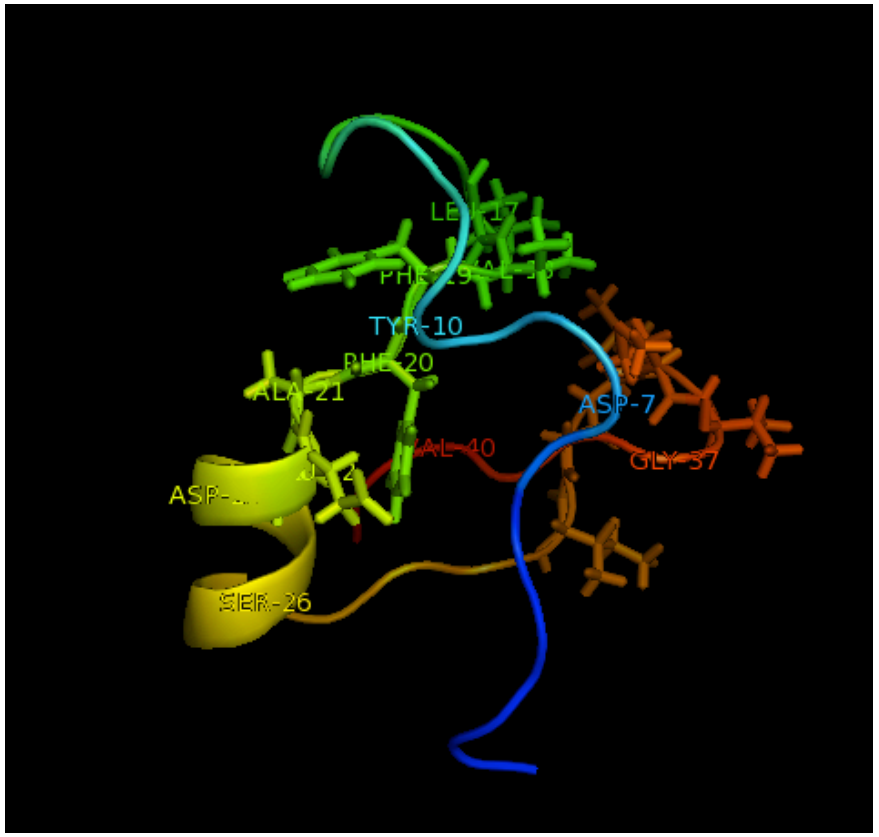
A lower ligand binding efficiency value is indicative of a better binding hotspot in energy term

Abeta42- Dynamical ensemble of conformations

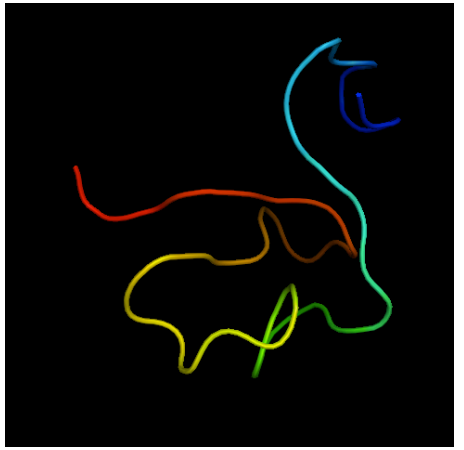


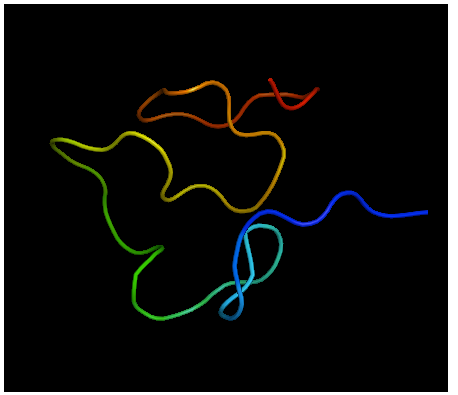


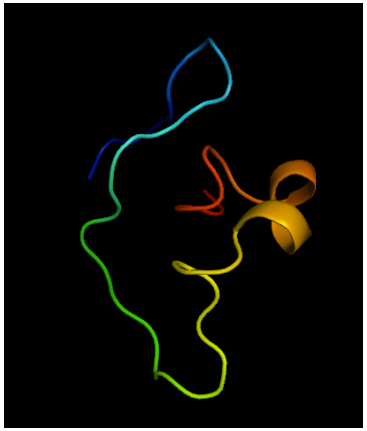




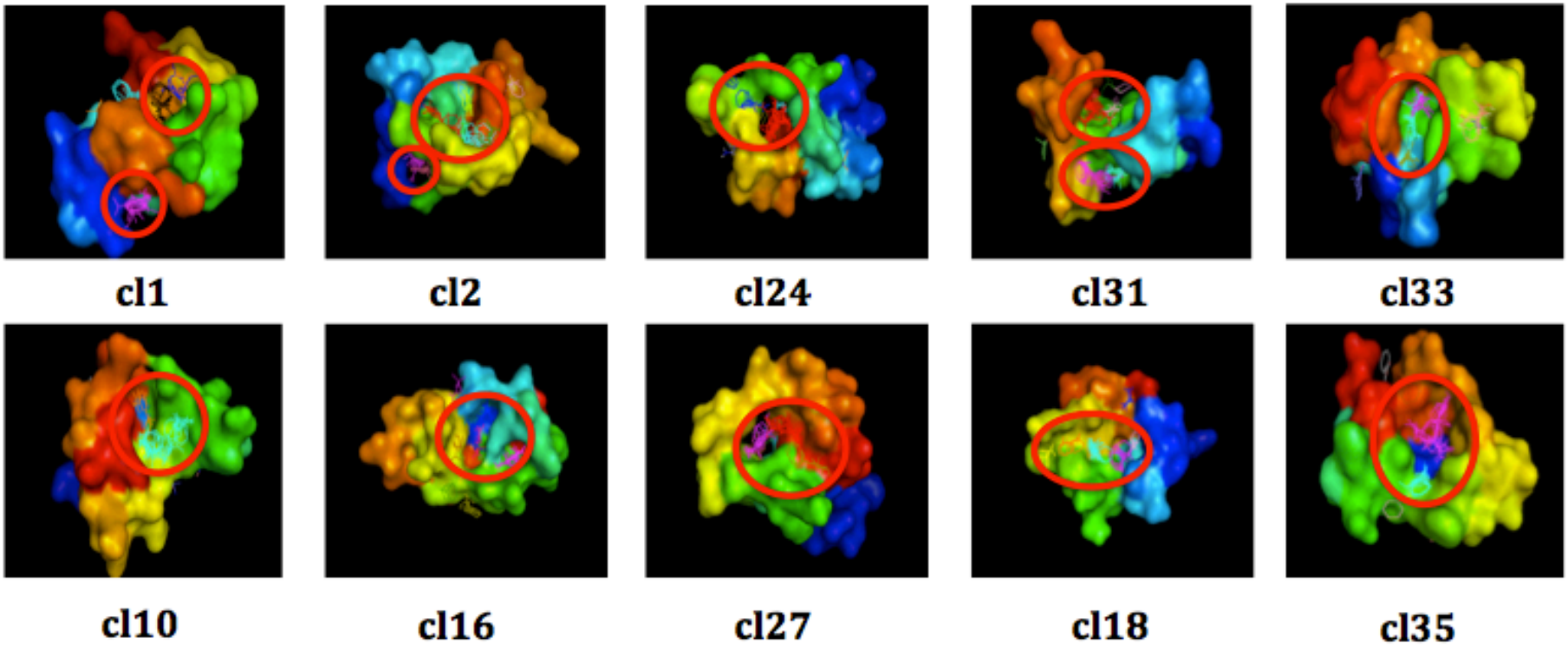
DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA







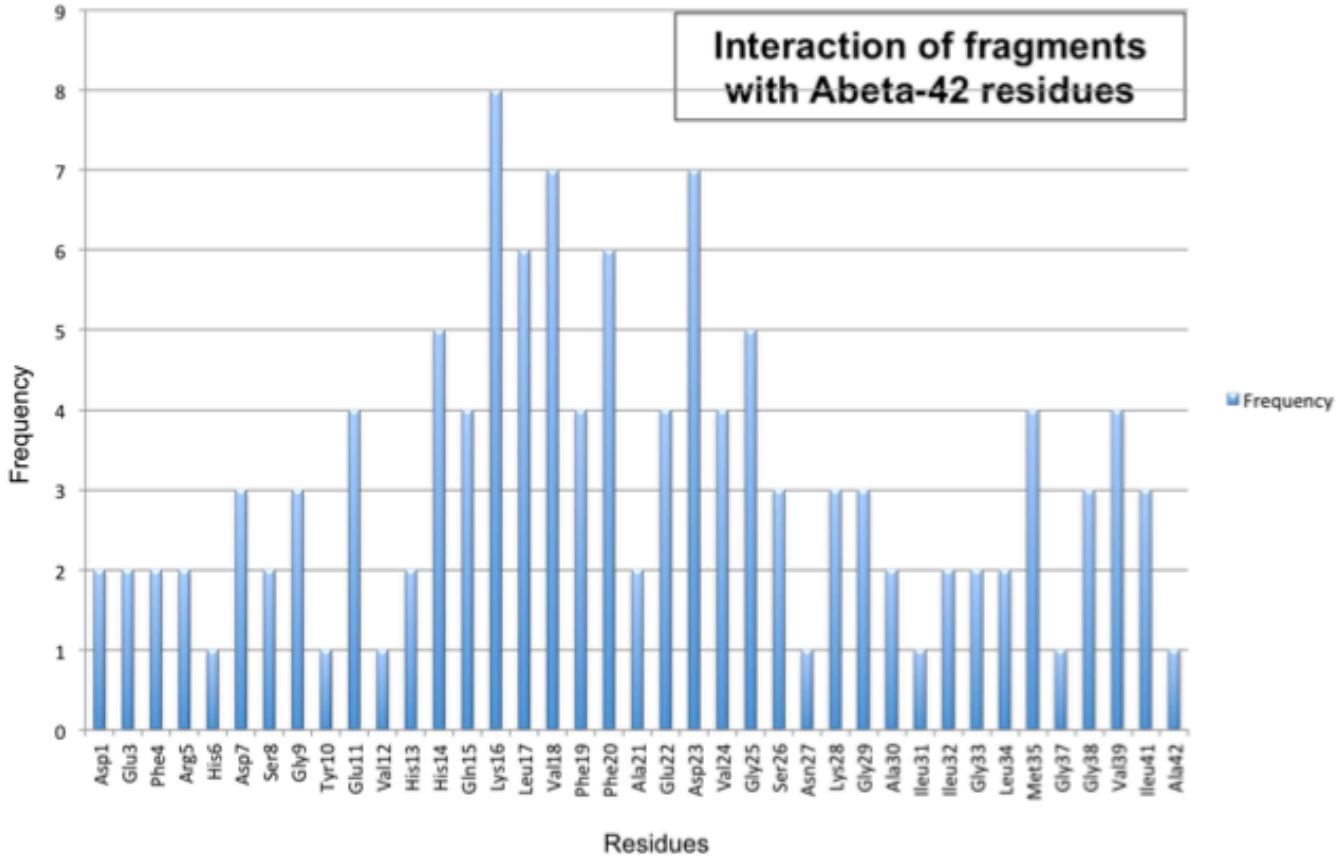
Potential binding hotspots identified by fragment based mapping of the ensemble of Abeta42 structures



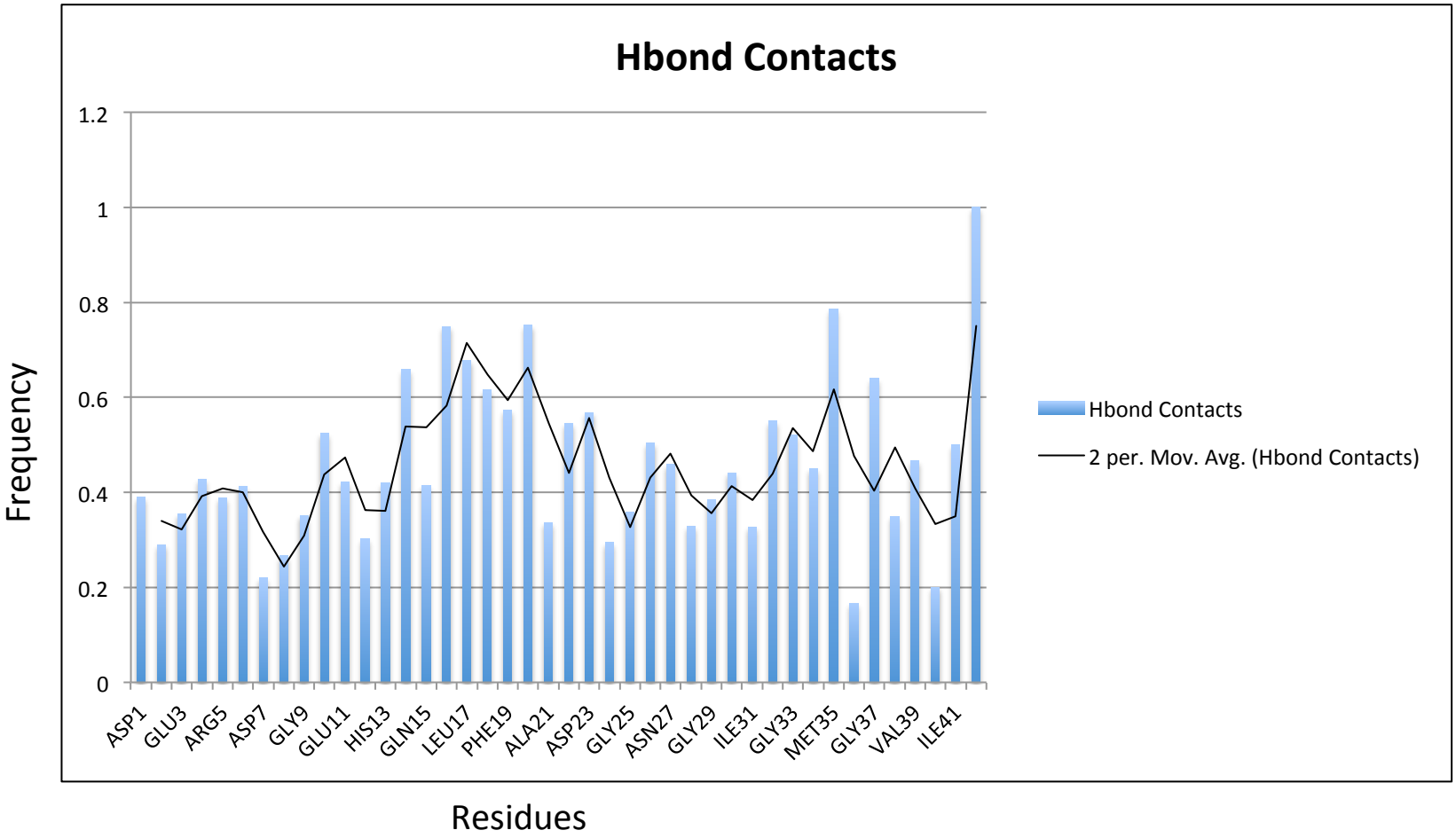
DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA

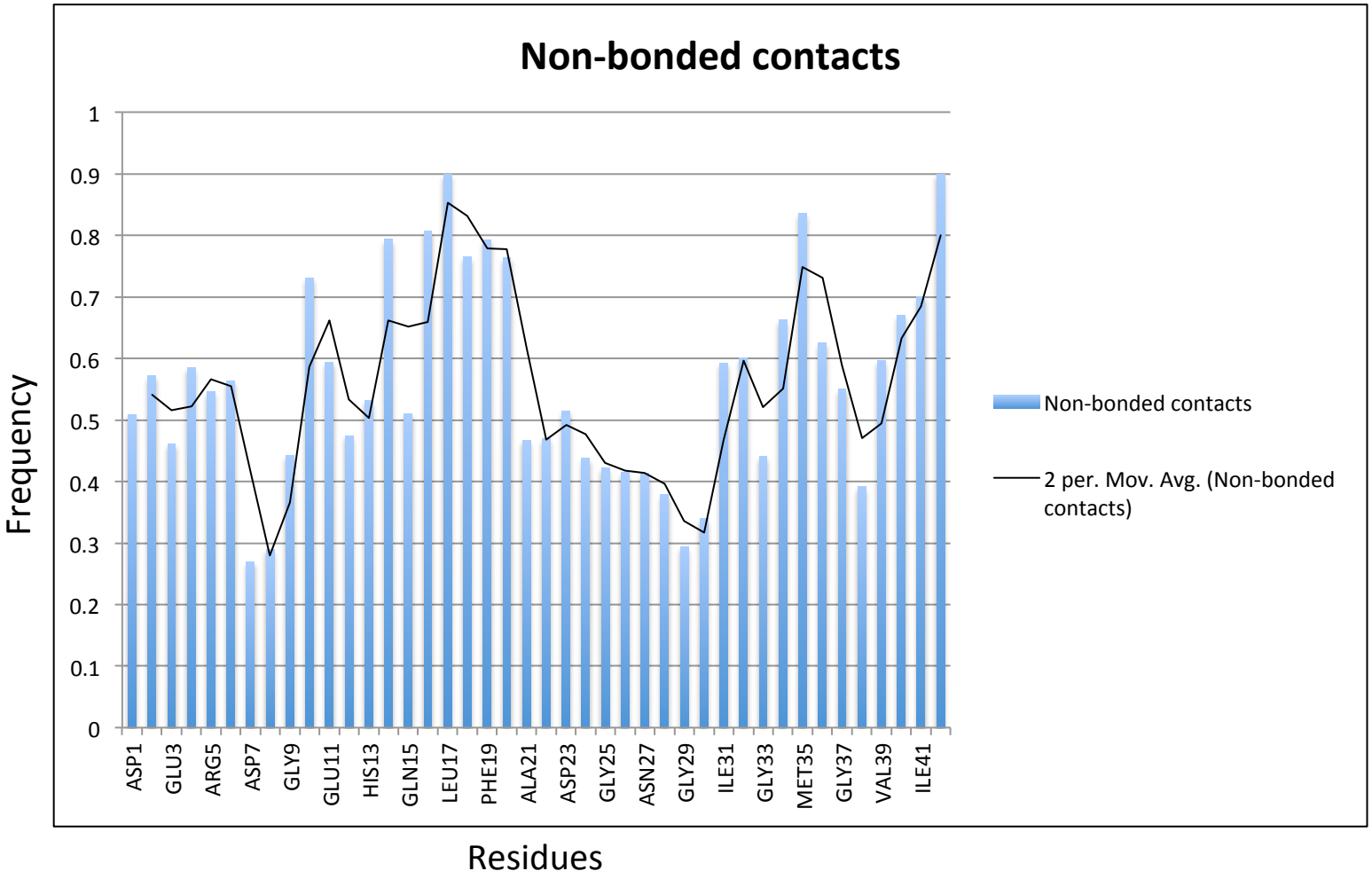
- Abeta fibril formation is controlled by specific amino acids within the Abeta peptide.

- The N-terminus, hydrophobic core, hinge or turn regions and C terminus are all crucial for Abeta41-42's ability to aggregate.



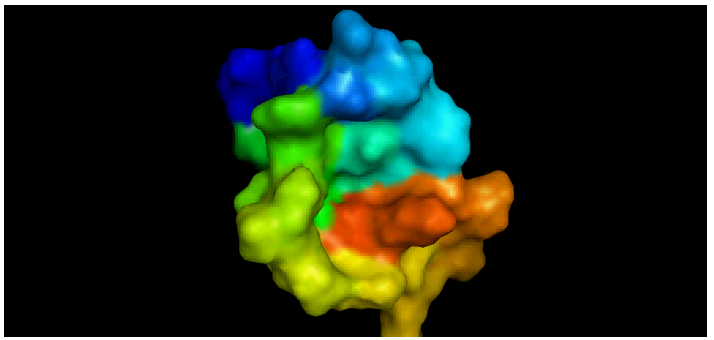
Key interactions: His14, Lys16, Leu17, Val18, Phe20, Asp23, Gly25 (>50% structures)

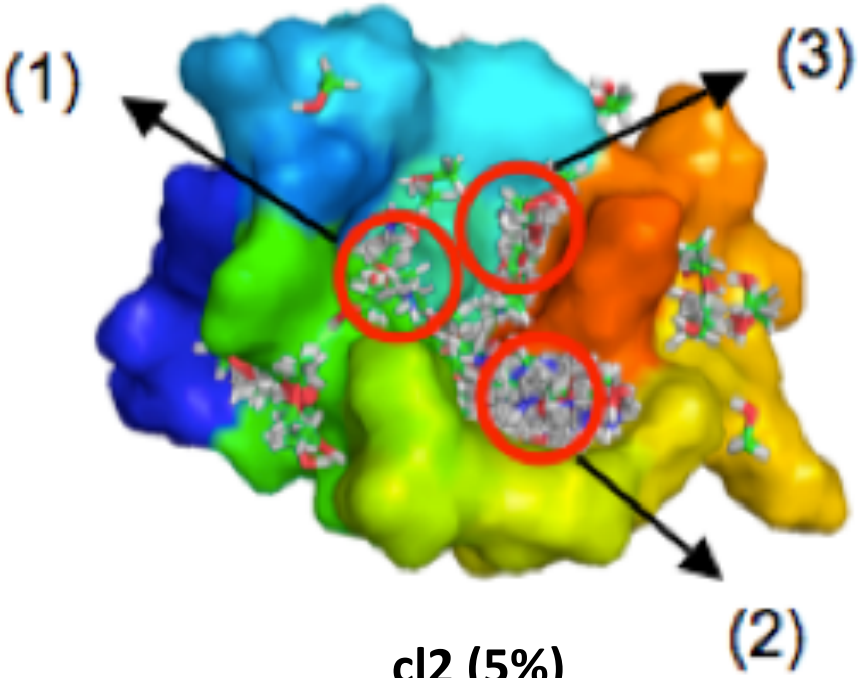

Hbond Contacts
1.2
1
0.8
0.6
0.4
0.2
0
Frequency
ASP1
GLU3
ARG5
ASP7
GLY9
GLU11
HIS13
GLN15
LEU17
PHE19
ALA21
ASP23
GLY25
ASN27
GLY29
ILE31
GLY33
MET35
GLY37
VAL39
ILE41
Residues
Hbond Contacts
2 per. Mov. Avg. (Hbond Contacts)


Non-bonded contacts
Frequency
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
ASP1
GLU3
ARG5
ASP7
GLY9
GLU11
HIS13
GLN15
LEU17
PHE19
ALA21
ASP23
GLY25
ASN27
GLY29
ILE31
GLY33
MET35
GLY37
VAL39
ILE41
Residues
Non-bonded contacts
2 per. Mov. Avg. (Non-bonded contacts)

1. Benzene
2. Cyclohexane
3. Pyrazole
4. Furan
5. Imidazole
6. Oxazole
7. Dimethylketone
8. Methylamide
9. Cyclopropyl
10. Methanol

Hotspot Analysis



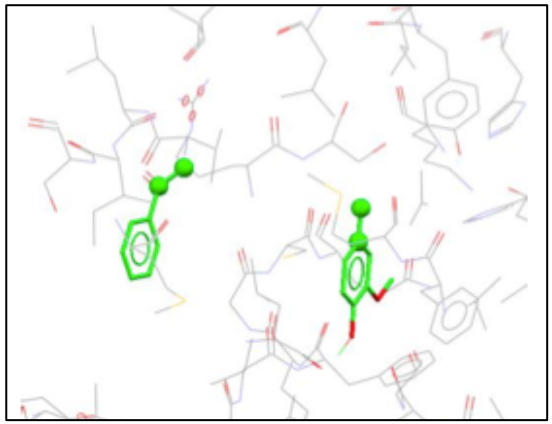


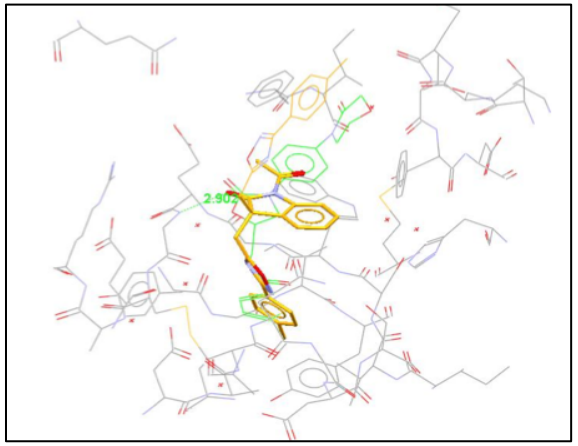
cl2 (5%)

Binding Pocket
(1) His6, His13, Leu17, Phe19 (LE: -1.04)
(2) His13, Leu17, Val36 (LE: -1.20)
(3) Tyr10, His13, Met35 (LE: -0.91)

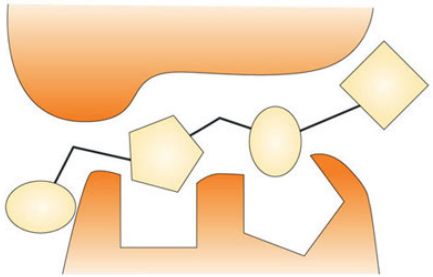
DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA

Fragment Linking and Screening





Adapted from http://www.ccdc.cam.ac.uk



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Low quality HTS Hit

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Fragment Linking

Library Screening –
Structures containing privileged substructures

Conclusions and Future Directions

1. Application of this strategy to identify binding hotspots in case of Abeta42 peptide → candidate binding pockets for virtual screening of drug like molecules that are likely to bind better at these sites.

Aim: To identify drug-like small molecules that can bind to and stabilize the native monomeric form of Abeta peptide.

1. Build a specialized library of molecules that bind to IDPs using a Fragment Based Drug Design Approach.
2. NMR experiments to validate binding and inhibitory effects of fragments and small drug like molecules to Abeta42.

Acknowledgements

Prof Michele Vendruscolo
Yanching Chu
Biao Fu
Vendruscolo and Dobson Group

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IDPbyNMR Fellows
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Thank You