Prediction of Binding Free energies: Application to Class 1 MHC- Peptide binding Free Energies Predictions based on homology models using Discovery Studio and Pipeline Pilot

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Abstract

During a disease process, cells produce associated proteins (or antigens) which, after proteolysis, are transported to the cell surface as peptides. At the cell surface, the Major Histocompatibility Complex (MHC) I proteins display these peptides to immune cells known as Cytotoxic T cell lymphocytes (CTLs) which act as a defence mechanism and destroy cells with unfamiliar antigens e.g., those containing viral proteins. These T cells contain receptors or TCRs which recognize peptides when they are expressed in complexes with MHC Class I molecules. For the TCR to bind a peptide-MHC complex, two conditions must be met. Firstly, the TCR must have a structure which allows it to bind the peptide-MHC complex. Secondly, the accessory molecule CD8, must bind to the alpha-3 domain of the MHC Class I molecules. To investigate the selectiveness of TCRs, the binding of MHC Class I proteins to different peptides was studied. We have used molecular dynamics simulation methods using CHARMm to evaluate the binding free energies for several MHC-protein peptide complexes. This procedure may be automated in Pipeline Pilot to allow routine assessment and evaluation of protein-peptide complexes. The method and protocols used are described. Based on such studies, one can consider designing peptide based immunotherapy.

Protein Interactions in the Immune System
- Distinguish self from non-self
- Memorise "data" from past invaders
- Defend organisms against invaders

Protein-Protein Interactions
- Antibody –antigen complexes
- Presentation of peptides
- Large diversity and selectivity of protein-protein interaction types
- Achieve high specificity in antigen recognition
  - But still a "lock and key" mechanism

Focusing on Major Histocompatibility Proteins
- Two types of MHC proteins
  - Class I : These respond to Intracellular pathogens
    - eg., viruses
  - Class II : These respond to extracellular antigens
    - eg., Venoms

Focusing on Intracellular pathogens: Viruses
- T cells destroy the infected cells
  - Virus invades the cells
  - Fragments of these viral proteins are chopped into peptides that appear in the cell
  - Fragments displayed on surface in complex by a protein of Major Histocompatibility complex (MHC protein)
  - T Cells recognise the MHC-peptide complex, proliferate and destroy cells containing the target antigen

Molecular Dynamics Simulations is used in a number of ways
- Predicting thermodynamics properties of binding
- Predicting complex behaviour
- Protein-Protein interactions
- Predicting ligand binding in drug design
- Understanding protein mutation and deletions
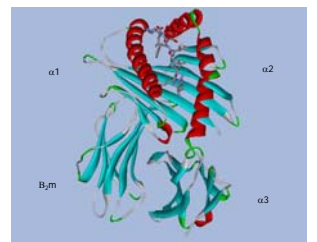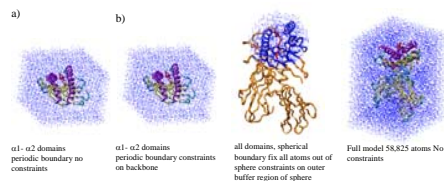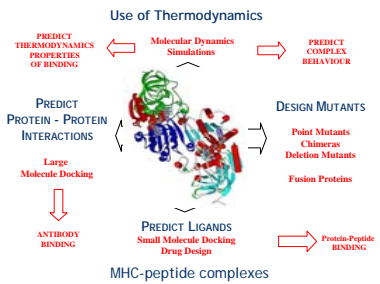(Figure 1)

Aim:
- If one considers peptide MHC binding is similar to the binding of drugs (i.e. small molecules) to other receptors, using molecular dynamics (MD) simulation can we predict MHC-peptide interactions and determine the binding free energies using homology models
- How can this procedure be automated in Pipeline Pilot using Discovery Studio and CHARMm
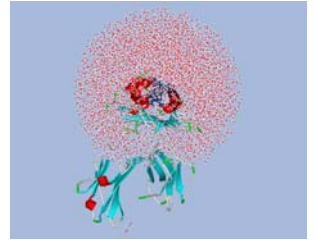
Previous work
- Rognan et al. (1992) *Proteins* 13, 70-85: 1- α2 domains periodic boundary and no constraints
- Meng et al. (1997) *Int. Immunol.* 9, 1339-1346: α1- α2 domains periodic boundary constraints on backbone
- Michielin et al. (2002) *J. Mol. Biol.* 324, 547-569: All domains spherical boundary fix all atoms out of sphere constraints on outer buffer region of sphere
- Nojima et al., *Chem Pharm Bull* (Tokyo) 2002 50(9), 1209-1214. Full model with no constraints

Methodology
- Perform a a sequence alignment of Class 1-MHC protein-peptide complex
  - 1W0V.pdb as a template (see Figure 2)
- Perform sequence alignment of Class 1 MHC protein-peptide complex
  - Use 1W0V.pdb as template sequence and pdb template (figure 2)
- For MD Simulation
  - Solvate protein-peptide complex using Explicit Spherical Boundary with Harmonic restraints (38A sphere) using the peptide as Centre of Mass
  - Create Fixed Atom Constraints for atoms outside the spherical water boundary to keep the rest of the protein restrained during the simulation cascade.
  - For the simulation cascade;
    - Steepest Descents Minimisation (500 steps, RMS gradient 0.1)
    - Adopted Basis-set Newton Raphson (ABNR) Minimisation (500 steps, RMS gradient 0.0001)
    - Heating (2000 steps, initial temperature 50K, final temperature 300K)
    - Equilibration (120 ps, 1fs time step, coordinates saved every 250 steps)
    - Production (120 ps, 1fs time step, 300 K, NVT ensemble, nonbond cutoff 14A, Switching function applied between 10 and 12A, coordinates saved every 150 steps)
    - Calculate the binding free energy of a protein-ligand complex using MM-GBSA
      - From energy minimized structures
      - From a molecular dynamics trajectory
- Perform MM-PB(GB)SA:
  - Developed by Kollman, Case and co-workers (*Acc. Chem. Res.* 2000, 33, 889-897)
  - MD simulation in explicit solvent often using periodic box with waters and counterions
  - Appropriate long-range electrostatics effects
  - Save set of representative structures
- Post-process structures
  - Remove solvent and counterions
  - Calculate free energy
    - PB(GB)SA free energy is an average free energy over a number of states
  - Entropic terms using quasiharmonic analysis or NMA
    - Can be ignored if ligands small or similar to each other
- Molecular Mechanics-Poisson Boltzmann(Generalized Born) Surface Area
(Equation 1)
  - G_{intra} → Intramolecular energy
  - G_{inter} → Intermolecular energy
  - G_{pol} → Polar contribution to solvation free energy
  - G_{np} → Nonpolar contribution to solvation free energy

Use of Thermodynamics

PREDICT THERMODYNAMICS PROPERTIES OF BINDING

PREDICT COMPLEX BEHAVIOUR

Molecular Dynamics Simulations

PREDICT PROTEIN - PROTEIN INTERACTIONS

DESIGN MUTANTS
Point Mutants
Chimeras
Deletion Mutants

Large Molecule Docking

Fusion Proteins

ANTIBODY BINDING

PREDICT LIGANDS
Small Molecule Docking
Drug Design

Protein-Peptide BINDING

MHC-peptide complexes

a) α1- α2 domains periodic boundary no constraints

b) α1- α2 domains periodic boundary constraints on backbone

all domains, spherical boundary fix all atoms out of sphere constraints on outer buffer region of sphere

Full model 58,825 atoms No constraints

α1

α2

B₂m

α3

X-ray structure of MHC-Class 1 peptide in complex with a peptide (1w0v.pdb)

X-ray structure of MHC-Class 1 peptide in complex with a peptide (1w0v.pdb) solvated with a pherical boundary and the rest of the protein held rigid

$$\Delta G_{bind} = G_{complex} - G_{ligand} - G_{protein}$$

$$G = \langle G_{intra}\rangle + \langle G_{inter}\rangle + \langle G_{pol}\rangle + \langle G_{np}\rangle - T\Delta S$$

Equation1: Method to calculate Binding Free Energy
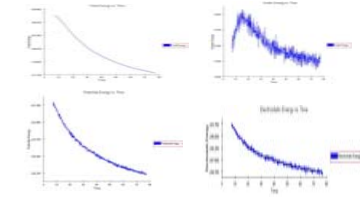
Results
- A comparison of the peptide conformation obtained from a homology model vs a low energy single frame obtained during the production phase were made. Figure X(a), X(b) and X© Shows Hydrogen bond observed in homology models as well as during the production phase of the simulations.

Backbone rmsd (0.59A)

Yellow = frame (686) from the production phase
Gray = Homology model from Modeller
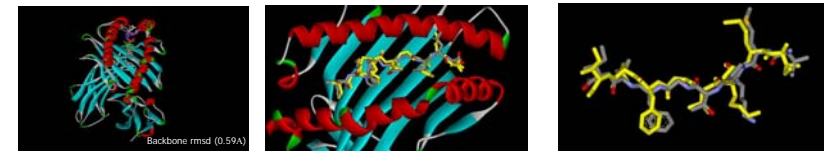
Backbone superposition of Modeler model and one frame from the CHARMm production phase

Yellow = Homology model from Modeller

Hydrogen bonds formation between the MHC-1 protein and peptide

Yellow = frame (686) from the production phase
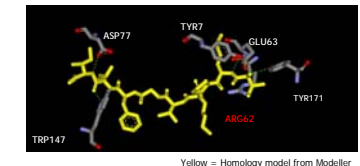
Hydrogen bonds formation between the MHC-1 protein and peptide during the production phase

Energy profiles during a ~80ps of production phase in the CHARMm simulation phase

- Customisation of Discovery Studio with Discovery Studio and Pipeline Pilot components

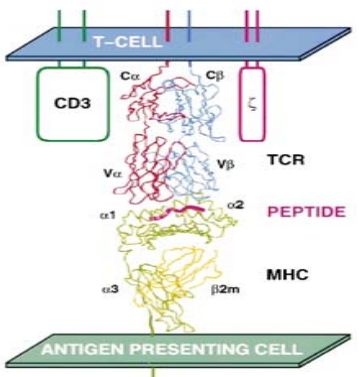Example Custom Protocol Built in Pipeline Pilot - "For homology modeling "

Example Custom Protocol Built in Pipeline Pilot - "For measuring binding energies " CHARMm GBSA PBSA example

These new Custom Protocol Built in Pipeline Pilot can then incorporated in the DS "Protocol" explorer so all users can run the new experiment through the DS client.

- Results for two MHC protein –peptide model complexes

PEPTIDE	Expt. Δ G*	Homology model Δ G	Method
FRFNGYIHR	-48.51	-34.832	Minimisation of homology model of peptide-protein complex with solvent cap around the peptide
IMPKTGFLI	-26.56	-12.439	80 ps MD with solvent cap around peptide-protein complex. Free energy measured for a single frame

Results from free energy calc. using CHARMm Scripts (CHARMm

*Logean. A., Sette. A., and Rognan. D. Bioorganic & Medicinal Chem. Lett. 11, (2001) 675-679

Conclusion:

The results demonstrate that combining homology modelling and molecular dynamics simulations of Major Histocompatibility Class I (MHC) protein-peptide complexes provides a detailed understanding of the protein-peptide interactions. Structural analyses and calculation of binding free energies afford a means to quantify the atomic interactions observed at the microscopic level. Based on such studies, one can consider designing peptide based immunotherapy.

We are in the process of implementing workflows using Pipeline Pilot to allow routine and automated assessment and evaluation of protein-peptide complexes. In addition the calculated binding free energies are being compared to conventional docking scores from vHTS techniques.