Usage Report for Fiscal Year 2016

Project Title:
Structural basis of CD22-sialic acid interaction by structural biology and computational chemistry

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CD22 (Sig-2) is an attractive target for autoimmune disease and non-Hodgkin's lymphoma (among 10 most common cancers) due to its pivotal role in B cells activation. Siglecs recognize only sialic-acids. The specificity for sialic-acid binding preference differs between human CD22 and mouse CD22. Although human CD22 binds to both NeuAc and NeuGc type sialic acids, mouse CD22 is known to selectively bind NeuGc. Although the 3D structure of the analogue of CD22 (Sig-1) has been resolved, the 3D structure of CD22 itself has not been determined. The difference in binding specificity between human and mouse CD22 has long been known, the molecular basis of change in sugar recognition capabilities of CD22 is not well understood. This project connects well with the other ongoing research projects in our laboratory. The other similar projects are mainly about exploring structure and function of sugar binding proteins using classical MD, NMR and X-ray. We also studied other systems like Sig-1 and Protein *O*-Mannosyl Kinase (POMK), a causative gene product of dystroglycanopathy, to explore structure and function of protein-glycan complexes.

Since the 3D structure of CD22 is now known, we used homology modeling and classical MD simulations to model CD22 structures. Thereafter we applied extensive MD simulations (using GPUs code of AMBER14) on POMK, Sig-1 and CD22/sialic-acid complexes to understand their glycan recognition capabilities. In addition to these molecular mechanics (MM) calculations, some quantum (QM) calculations for geometry optimization of the glycans were also performed using *Gaussian0 9.*

We successfully modeled the mouse Sig-1, CD22 structures and calculated their binding free-energy for NeuAc and NeuGc. The Thermodynamic Integration (TI) calculations can reproduce the sialic-acid preference data for Sig-1 and CD22.

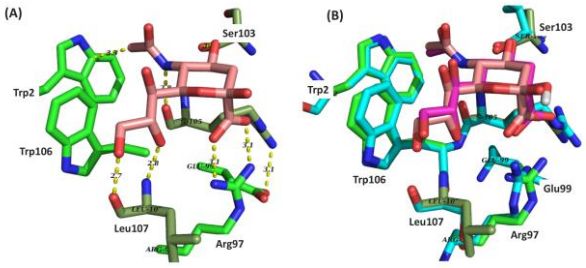

Figure 3. *Binding mode of sialic-acids in Siglec. (A) NeuAc bound to mSig-1 crystal structure. (B)Superimposition of mSig-1*

We also modeled several 3D structures of mouse Sig-1 and its mutants. The conformation of binding site residues in human and mouse Sig-1 is very similar (Fig-1). Then we predicted change in binding energy for NeuAc/NeuGc due to mutation. This helped us to understand the crucial residues involved in glycan binding. For PONK, we used computations to predict the binding mode and dynamics glycan in POMK catalytic site. This data has been published in *Genes to Cells* recently.

We successfully explored protein-glycans interactions in Sn, CD22 and POMK systems. This helps us to understand structure and function of various protein-glycan complexes correctly being studied in our laboratory. This knowledge is crucial to design new inhibitors for CD22.

In this financial year, we will perform virtual screening of drug-like compounds against CD22 receptor. Hit compounds will be further optimized (using free energy calculation approach) to enhance their binding capability. In addition to this, we will also explore protein-glycan interactions in several other protein-glycan complexes currently under investigation in Structural Glycobiology Team.

Fiscal Year 2016 List of Publications Resulting from the Use of the supercomputer

[Publication]

Masamichi Nagae, Sushil K. Mishra, Neyazaki Makiko, Rika Oi, Akemi Ikeda, Naohiro Matsugaki, Satoko Akashi, Hiroshi Manya, Mamoru Mizuno, Hirokazu Yagi, Koichi Kato, Toshiya Senda, Tamao Endo, Terukazu Nogi, Yoshiki Yamaguchi (2017). 3D structural analysis of Protein O-Mannosyl Kinase POMK, a causative gene product of dystroglycanopathy. *Genes to Cells* (***In press***) DOI:10.1111/gtc.12480