

Title: Antibodies and their interactions

PI: Dr. Andrew C.R. Martin, UCL

Co-I: Prof. Steve Perkins, UCL and DSTL & UCB-Celltech collaborators

Aims and Objectives

This project will evaluate modelling methods for antibody structure and, by analysis of surface patches, will attempt to develop methods for predicting immunogenicity and aggregation of these proteins. This will be a collaborative project working with external groups from DSTL and UCB-Celltech as well as with Prof. Steve Perkins within the ISMB.

Biological background

Antibodies act as the 'adapter plugs' of the immune system – they interface the virtually infinite variability of antigens with the constant effector functions of the immune system. This huge range of variability, encoded within the 6 'complementarity determining regions' (CDRs, also known as hypervariable loops) enables both high affinity and specificity. These features have meant that antibodies have enormous potential in the lab, as biosensors in vitro and both as diagnostic and therapeutic agents in vivo.

Because of the difficulties of producing monoclonal human antibodies¹, mouse (or other non-human) antibodies are created, but these are generally immunogenic when used in vivo in man. Thus methods such as grafting the Fv region of a (generally) mouse antibody onto human constant domains to create chimeric antibodies, or grafting the CDRs from an antibody of interest onto a human framework ('humanization') have been used. After CDR-grafting, it is generally necessary to make further changes of human to mouse residues in order to regain binding. While many protocols are available for selecting such residues, the details of how specificity is attained are still poorly understood. Chimeric and humanized antibodies are frequently still immunogenic and aggregation can also be a problem when needing to produce antibodies in relatively high concentrations for storage and formulation for administering to patients.

This project will address some of these issues to improve our understanding of humanization, immunogenicity and aggregation.

Work which has led up to this project

I developed the first automated method for antibody modelling (Martin et al., 1989) and this was commercialized in the 1990s by Oxford Molecular as their AbM package. Part of the project will involve some novel refinements to this method building on work done by a rotation project student.

We have begun development of a method for identifying features of an antibody sequence which appear particularly human-like or mouse-like. This technique uses an evolutionary algorithm to build characteristic sequence patterns. Sequence patterns that are never seen in humans are likely to be responsible (at least in part) for immunogenicity, in particular forming T-cell epitopes. This work will be extended to 3D patterns.

Another PhD student has been starting to look at protein surfaces from the perspective of identifying patches which are characteristic of protein interfaces. It is expected that such surface features (at least to some extent) may also be characteristic of both B-cell epitopes and sites that lead to aggregation. This work will be adapted for these purposes.

Plan of Work

In the first part of this project, work performed by a rotation project student will be extended to provide an automated antibody modelling method based on previously developed methods. This will be applied to a panel of antibodies which have been humanized by DSTL. They have created 3 versions of a light chain and 3 of a heavy chain and found that only one combination binds successfully. The modelling will be used to try to understand these results and the student will be able to carry out mutations to evaluate predictions experimentally. [9 months]

1 Recent developments include using phage display and transgenic mice to create fully 'human' antibodies.

In the second part of the project, the student will extend previous work on identifying sequence features using evolutionary algorithms to look at 3D surface patches. This will allow us to identify features of the antibody surface which may be responsible for immunogenicity. This part of the project will be a collaboration with Matt Page and Andy Popplewell at UCB-Celltech who will provide data on antibody sequences investigated by them. The student will have the opportunity to work with UCB-Celltech scientists to perform mutagenesis experiments and test predictions in their in vitro assays. [12 months]

In the third part of the project, current work on looking at surface patches to predict protein interfaces will be modified for the purposes of B-cell epitope and aggregation prediction. A dataset of known B-cell epitopes is available from Blythe & Flower (2005) and will be used for this purpose. The work will also feed into the previous part of the project for predicting immunogenicity in collaboration with UCB-Celltech. Predictions on aggregation will rely on identifying unusual surface features. For example, we will calculate hydrophobicity of patches across protein surface regions known not to be involved in complexes and perform a statistical comparison with regions known to be involved in complex. We expect regions that might be involved in aggregation to have intermediate properties (since effective complexes only occur at high concentrations). This work will be tested through mutagenesis in collaboration with the Perkins group where the student will use AUC to detect complex formation. [12 months]

Interdisciplinary Aspects

This is primarily a Bioinformatics project, but will be a collaboration with experimental groups at DSTL, UCB-Celltech and the Perkins lab within the ISMB. The project will involve time spent working in each of these labs to assess and evaluate results of the predictions.

References

Blythe MJ, Flower DR (2005) Benchmarking B cell epitope prediction: underperformance of existing methods. *Protein Sci.* 14:246-248

Martin, A. C. R., Cheetham, J. C. and Rees, A. R. (1989). Modelling antibody hypervariable loops: A combined algorithm. *Proc. Natl. Acad. Sci.* 86:9269-9272.