

Introduction

Early stage application of affinity chromatography in downstream processing is acknowledged as a desirable objective with the potential to increase yields and reduce the overall cost of goods. While protein engineering technologies are producing increasingly diverse therapeutic proteins – whole molecules, fragments, fusion proteins – advances in bioprocess development are enabling their production at industrial scale. Concomitant with these developments is an increase in the demand for sufficiently robust synthetic affinity ligands with appropriate protein binding selectivities as primary capture affinity adsorbents.

In the absence of suitable existing ligands, the development of completely new ligands has analogies with the development of novel drug compounds, but with some important distinctions:

- ▶ An affinity ligand is constrained in space by attachment to a solid support.
- ▶ An affinity ligand should not bind the target *too* tightly – we need to recover the target protein at the end of the process without destroying it.
- ▶ The immobilized ligand must be available to the protein binding site.

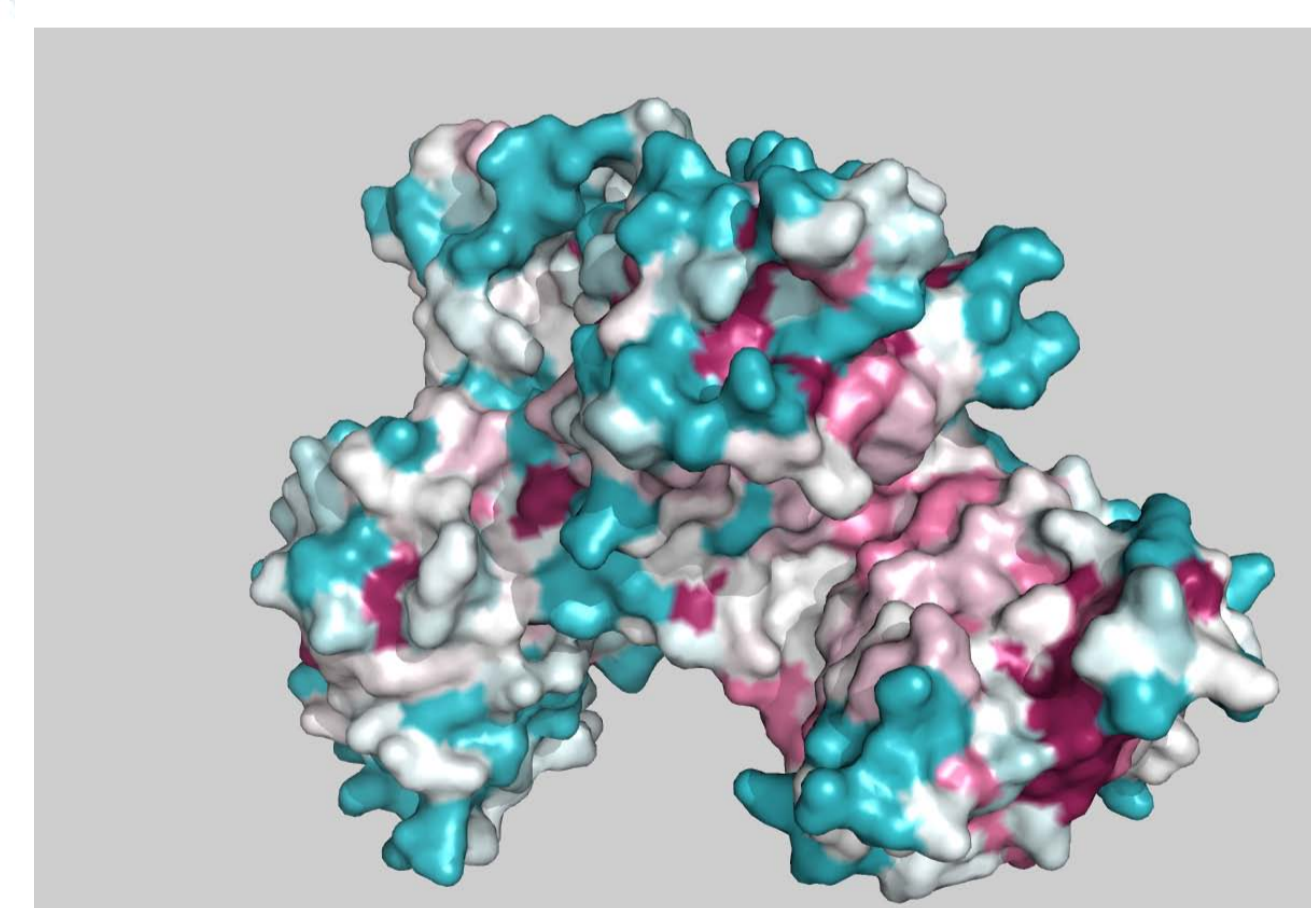
Computational techniques have become standard practice in the search for novel drug compounds and many software packages, algorithms and web services have been developed to address the drug design problem. We report here the adaptation of these techniques to various stages of the development of novel affinity chromatography ligands.

Identifying Potential Binding Sites

When dealing with novel therapeutic proteins, information on ligand binding sites may be unavailable, so a starting point for affinity adsorbent development must be sought. Where a suitable crystal structure is available, computational techniques in the form of workstation or web-based algorithms have been applied to help identify possible functional sites for targeting.

● Analysis of transferrin to guide ligand design efforts

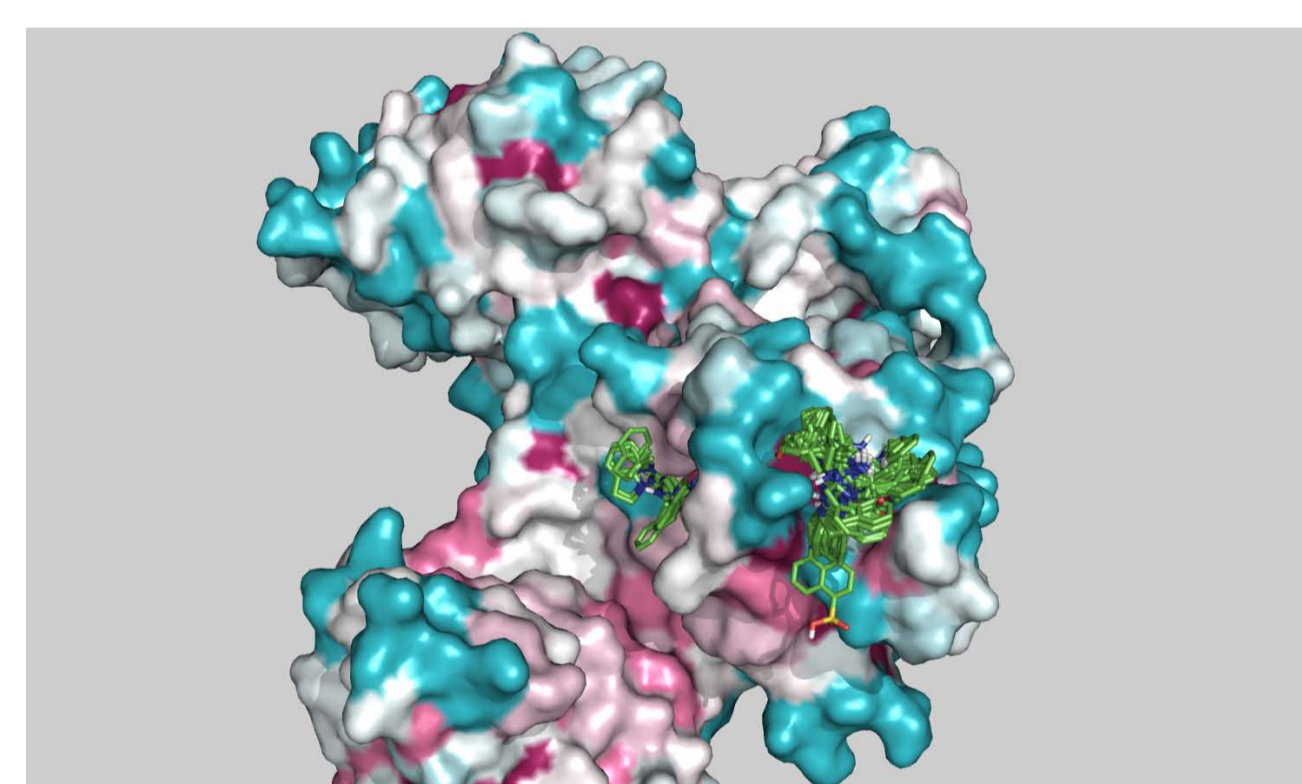
The transferrin iron carrier protein was analysed using the ConSurf server [1] which identifies **conserved** and **variable** residues – the higher the level of conservation, the greater the likelihood of functional importance.



(For more information see John Herberg's oral presentation)

● Blind docking [2]

Allowing a ligand or ligands to computationally “roam” over the entire protein surface then clustering poses – can provide useful insights into potential binding sites.

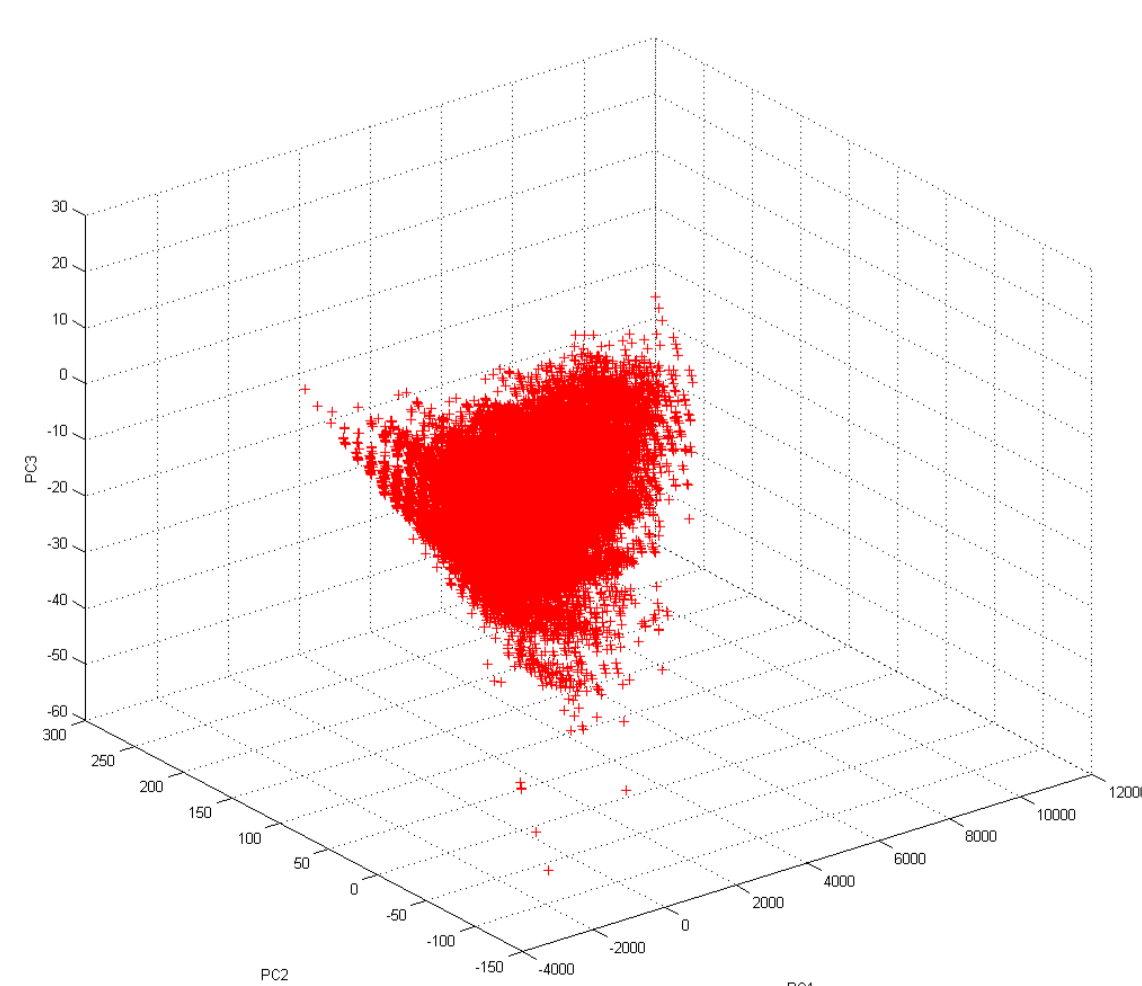


When consensus occurs between different theoretical techniques, one can be more confident about the veracity of the predictions.

Navigating “Chemical Space”

A Chemical Combinatorial Library CCL[®] has been developed containing over 100,000 triazine based compounds, shown opposite expressed in PCA space. Calculating molecular descriptors and applying dimensionality reduction techniques like Principle Component Analysis (PCA), or calculating similarity metrics has been used as a starting point for:

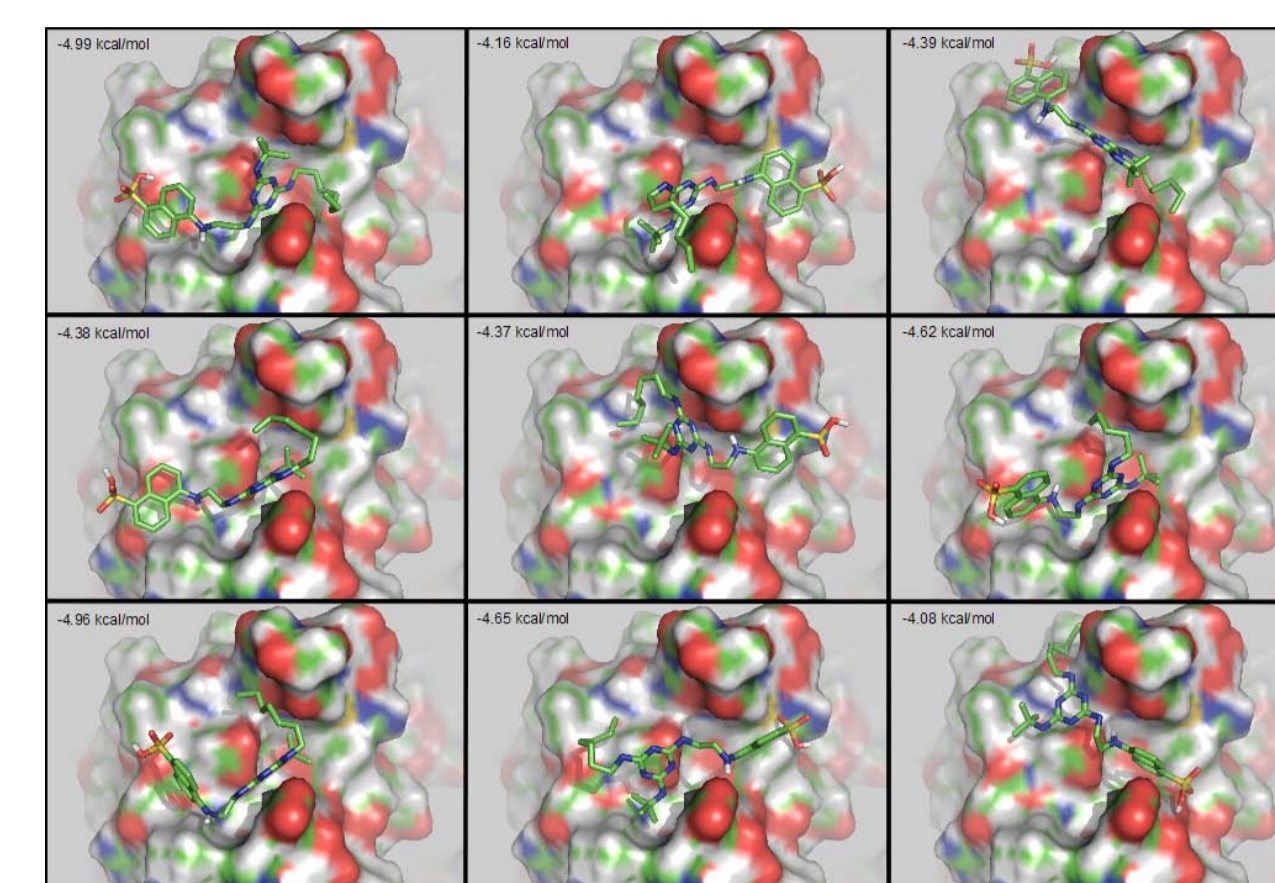
- Diverse combinatorial selection to generate exploratory screening libraries.
- Focused selection to home in on ligands showing early “activity” (favourable binding and elution properties) against a target.



Virtual Screening

Where a suitable X-Ray structure of a target protein is available, molecular docking was applied to help prioritize ligands for screening using a technique known as virtual screening (VS) due to its analogy with traditional laboratory screening.

VS algorithms place a ligand into a binding site and then “score” the resulting pose to allow screened ligands to be ranked.



Clearly the drawback of docking algorithms for the development of new chromatography ligands is that they assume the ligand is free in solution. It has therefore been necessary to develop methods to address this problem where docked poses are post-processed to remove those poses that are not accessible by an immobilized ligand.

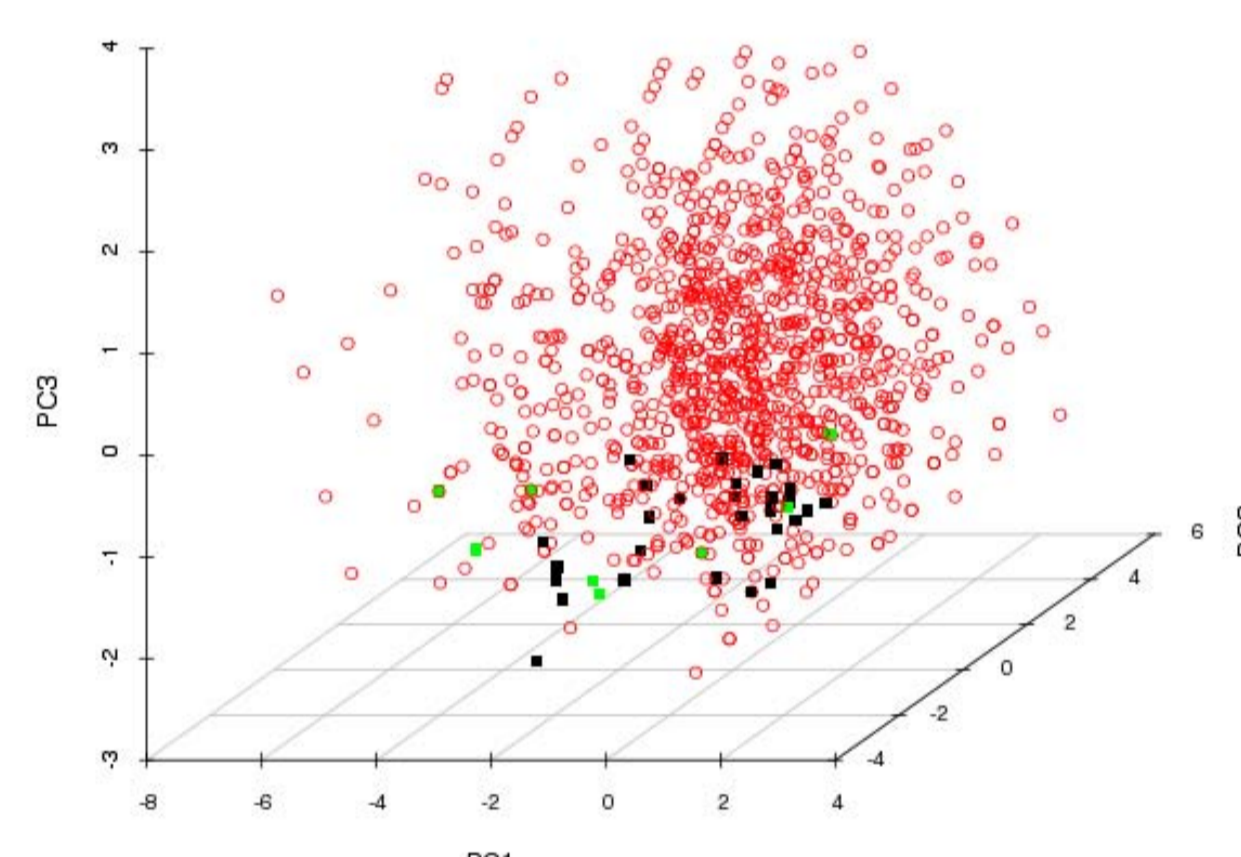
Combinatorial Library Design and Prioritization

● Combinatorial library design

In addition to the design of general diverse libraries, the development of combinatorial libraries based on early stage screening hits has been investigated. Early screening hits present a “cherry-picked” selection of candidates from which a new **combinatorial library** is developed that will further explore the surrounding chemical space.

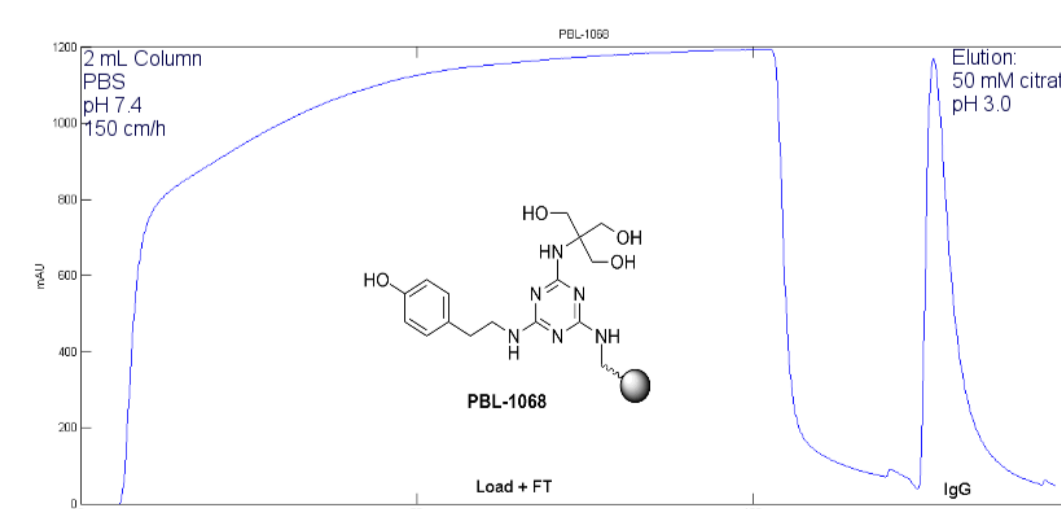
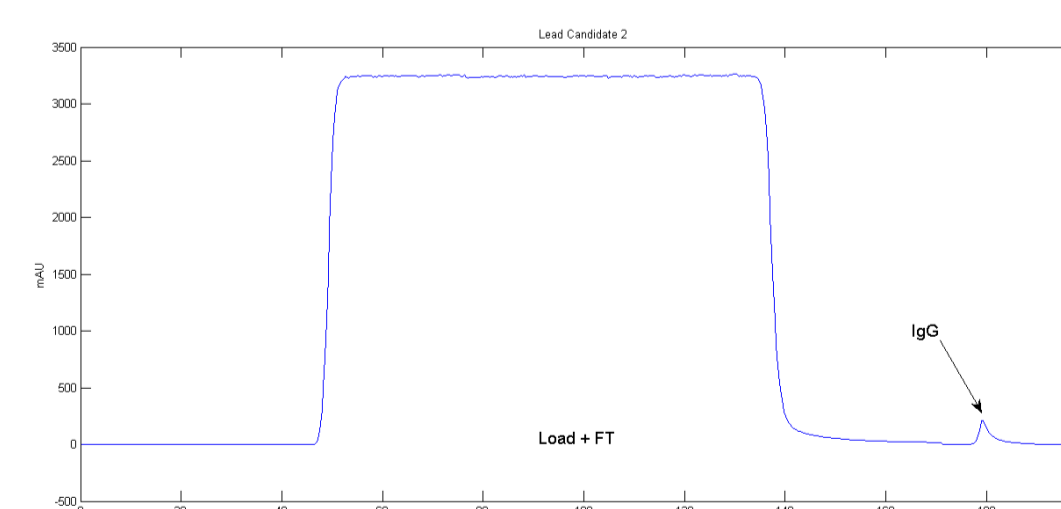
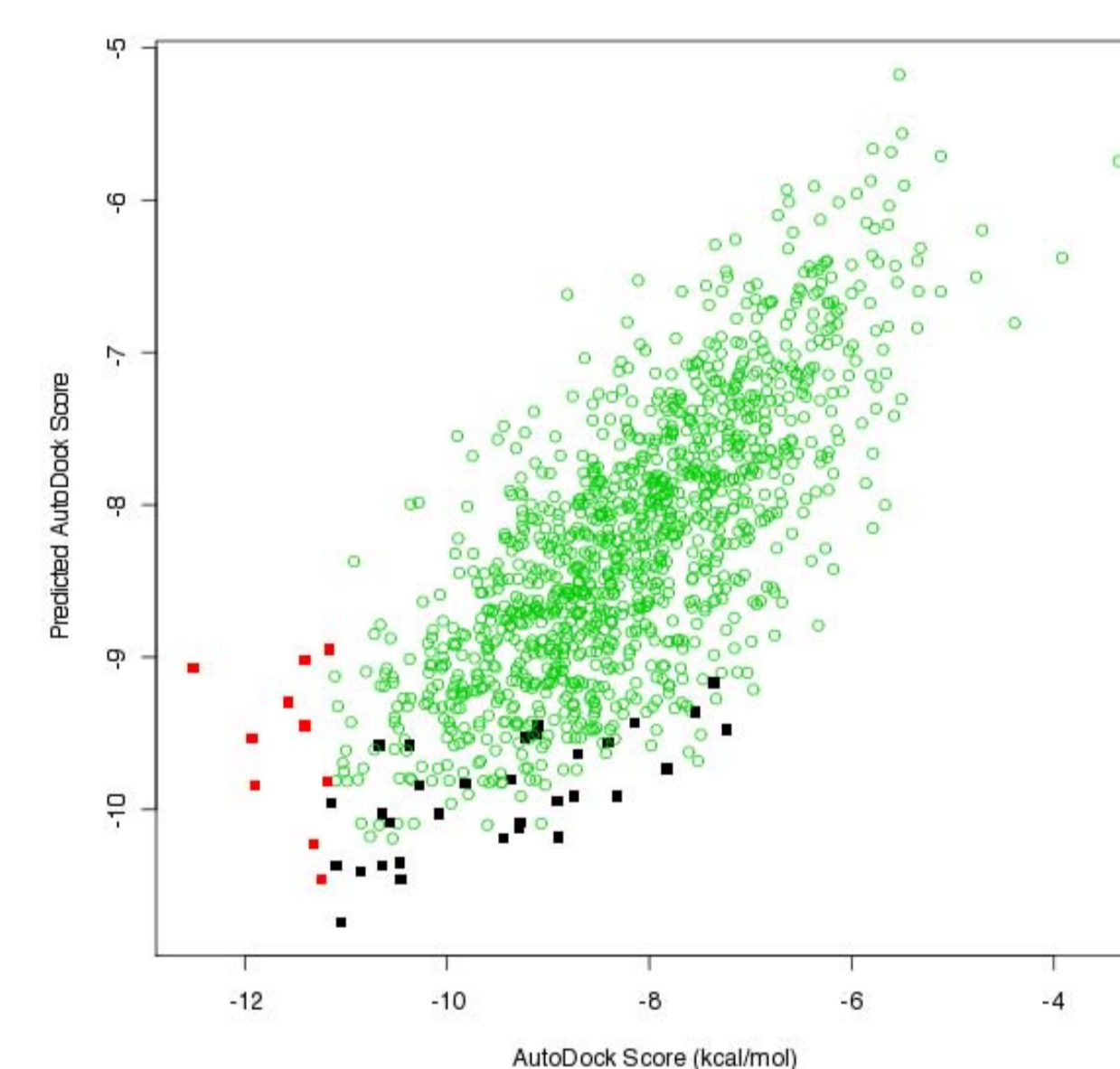
● Compound prioritization

Using the binding energy calculated by the AutoDock [3] algorithm as a surrogate for experimentally determined binding affinities, it has been demonstrated that the docking of a small, diverse library into a potential binding site (slow) can be extended to the rest of the virtual library using quantitative structure activity (QSAR) modelling (very fast) to quickly rank the remaining compounds.



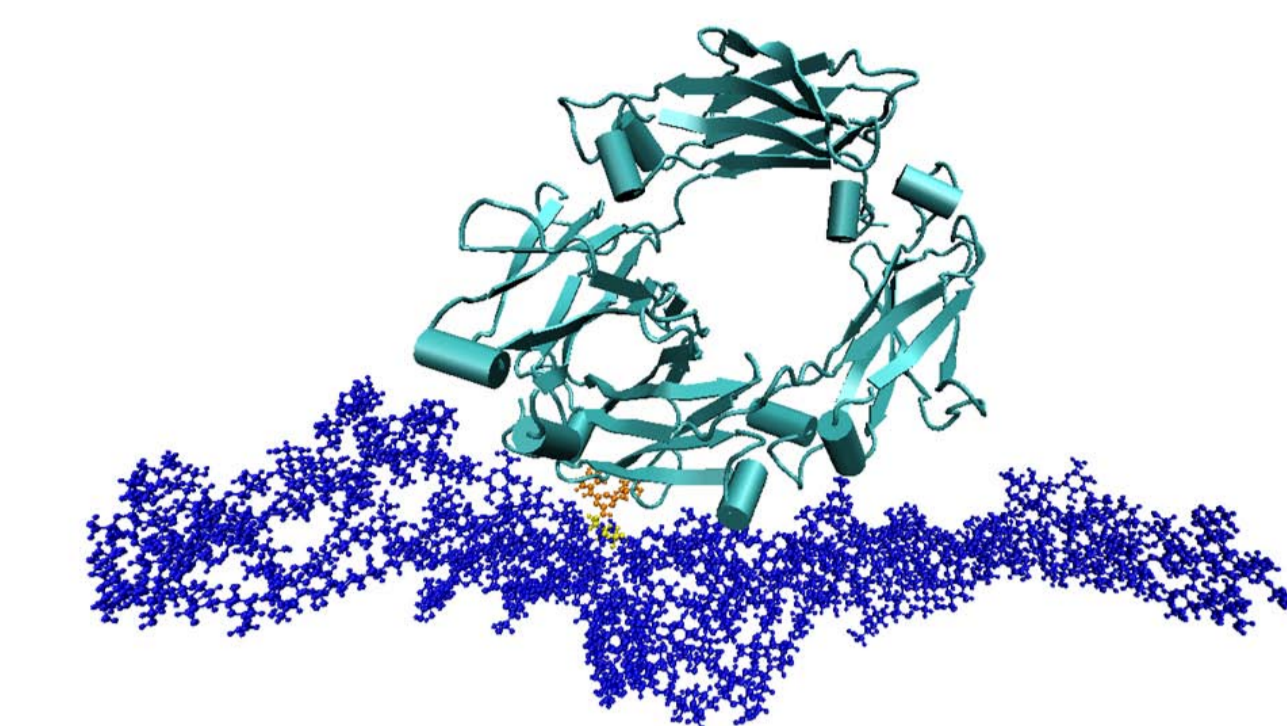
● Exploration of “hit” chemical space

Clustering techniques were applied to the two ligands identified as human IgG binders. These ligands contained two chemical groups which consistently gave rise to selective IgG binding (determined by ligand library screening) and consequently formed interesting targets for exploration of substituent group effects on ligand performance. To this end, the chemical space of each substituent was clustered and two combinatorial libraries created by taking the cluster center as a probe compound. From this work a new ligand PBL-1068 was identified. The chromatograms opposite demonstrate the capture and elution of IgG1 using an early stage lead and then the PBL-1068 ligand identified from library clustering using CHO cell culture supernatant.



Molecular Dynamics Modeling of Adsorbent/Target Interaction

A recent collaboration with Carlo Cavallotti's group in Politecnico di Milano involved molecular dynamics calculations to investigate the effects of the support, spacer arm and surface chemistry on the performance of MAbsorbent[®] A2P for affinity purification of Immunoglobulin G (IgG) [4].



This work helped to explain the observed differences in performance of various formulations of the MAbsorbent[®] A2P adsorbent in the presence of Pluronic F-68, and helped us design Pluronic resistant spacers. In addition it demonstrated the use of computational techniques to obtain detailed knowledge of the behaviour of a highly complex system and the extent to which these techniques can be applied.

Summary

In some cases *in silico* techniques translate well from their origins in drug design, while in others, more work is needed to account for the nature of affinity ligand and adsorbents. The techniques range in scale from the highly reductionist, ligand only view for rapid screening and prioritization, to the highly complex dynamic model of a particular adsorbent used to probe its behaviour under different experimental conditions.

Computational techniques have been used to inform a number of stages in the affinity ligand design process, and to gain an understanding of both the target protein and the adsorbent system. These insights will be invaluable if we are to continue to meet the demands of the biopharmaceuticals industry for newer, cheaper methods for downstream processing of novel biotherapeutics.

References

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- [3] Morris, G. M., Goodsell, D. S., Halliday, R. S., Huey, R., Hart, W. E., Belew, R. K., and Olson, A. J. *Journal of Computational Chemistry* **19**(14), 1639–1662 (1998).
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