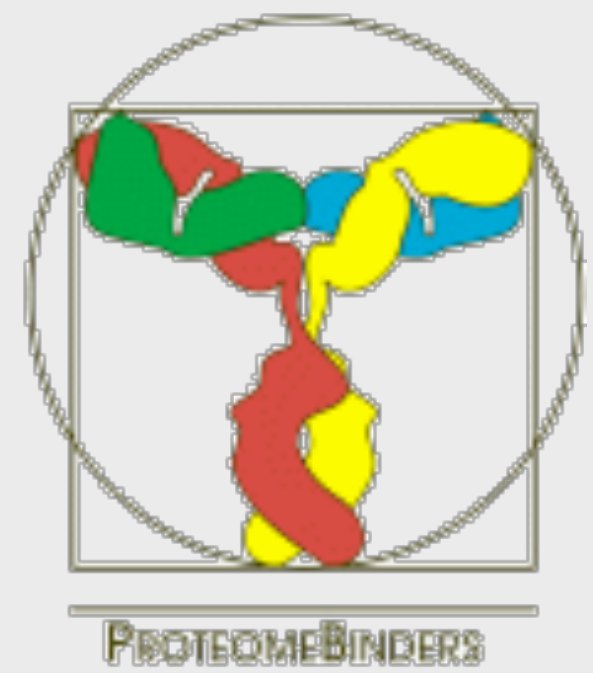


# DAS Workshop 2009

Niall Haslam EMBL Heidelberg



# ProteomeBinders



- Co-ordinate infrastructure for the generation of binding molecules for detection of the human proteome
- Create platform for the systematic development and quality control for these essential reagents
- Consistently characterised binders (QC), required to detect all the relevant human proteins in tissues and fluids in health and disease
- Protein State, modifications, splice variants etc.

# Moving from Bench to Bioinformatics

**Table 2.** Protein epitope selection choices

Questions at the bench	Answered through epitope targeting for	Applications and technologies
Does the binding reagent need to recognise the native protein?	Full length expressed protein	Protein–protein interaction studies (coprecipitation), protein expression (capture arrays)
Will a binder targeted to a globular domain be sufficient?	Complex epitopes from folded globular domains	Binders for families of proteins sharing domains
Are binders to the unfolded (denatured) globular domains also needed?	Nonnative linear epitopes	Monitoring of unfolded protein; Western blotting, IHC
Would a linear epitope, from natively unstructured polypeptide regions, be desirable?	Linear, natively unstructured peptide epitope	Targeting of functional linear motifs
Are binders against posttranslationally modified variants (e.g. phosphoproteins) required?	Epitopes with and without PTMs	Tracking of signaling events influencing PTMs
Can the binder distinguish splice variants?	Epitope in modules alternatively spliced in or out	Detection of splice variants
Should the binder interfere (agonistically or antagonistically) with a function of the protein?	Functional site as epitope	Stimulatory/inhibitory binders for membrane receptors; competitive inhibition of protein–protein interactions; intrabodies

Proteomics.  
2007 Jul 19;  
Affinity reagent  
resources for  
human  
proteome  
detection:  
Initiatives and  
perspectives.

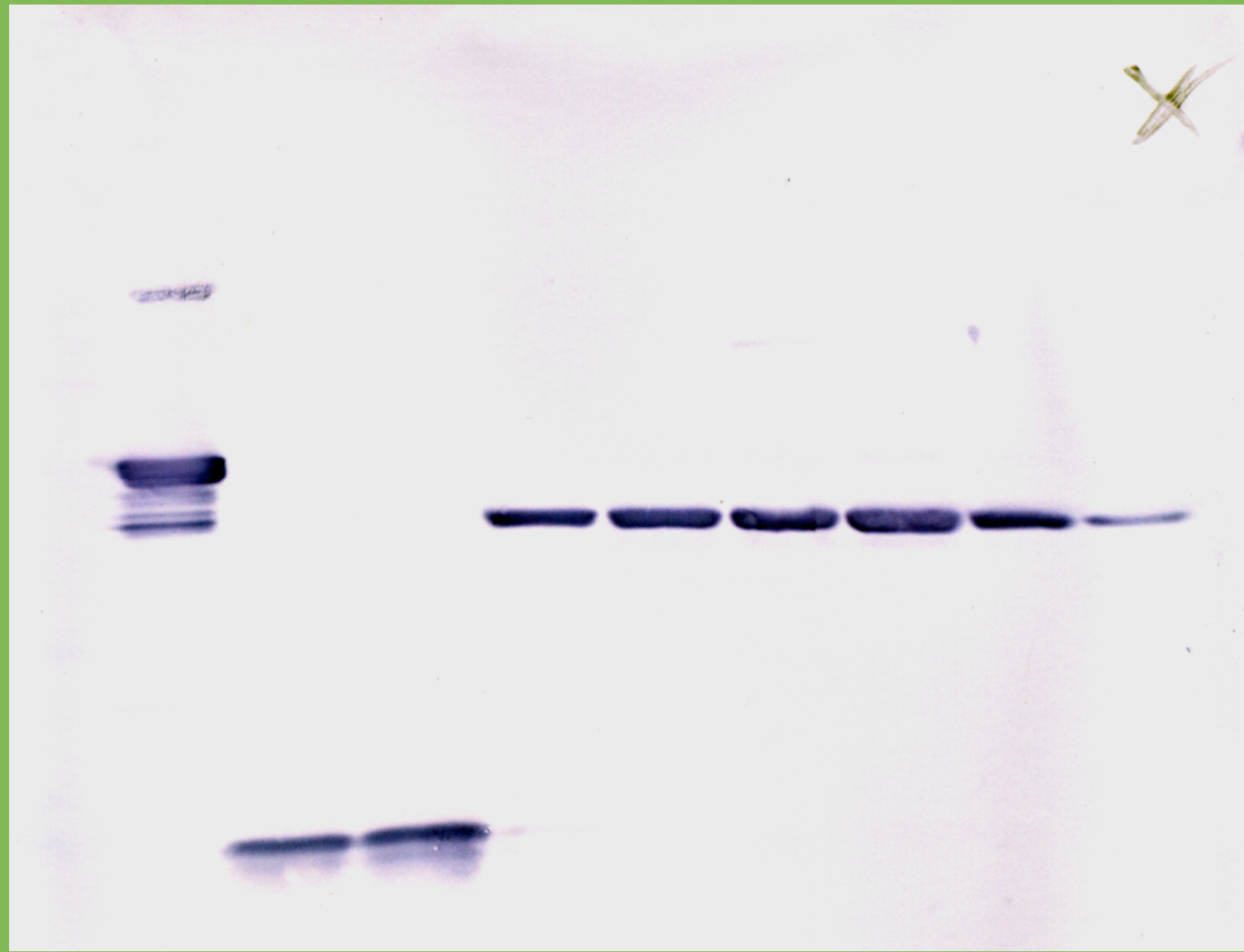
Taussig. M.





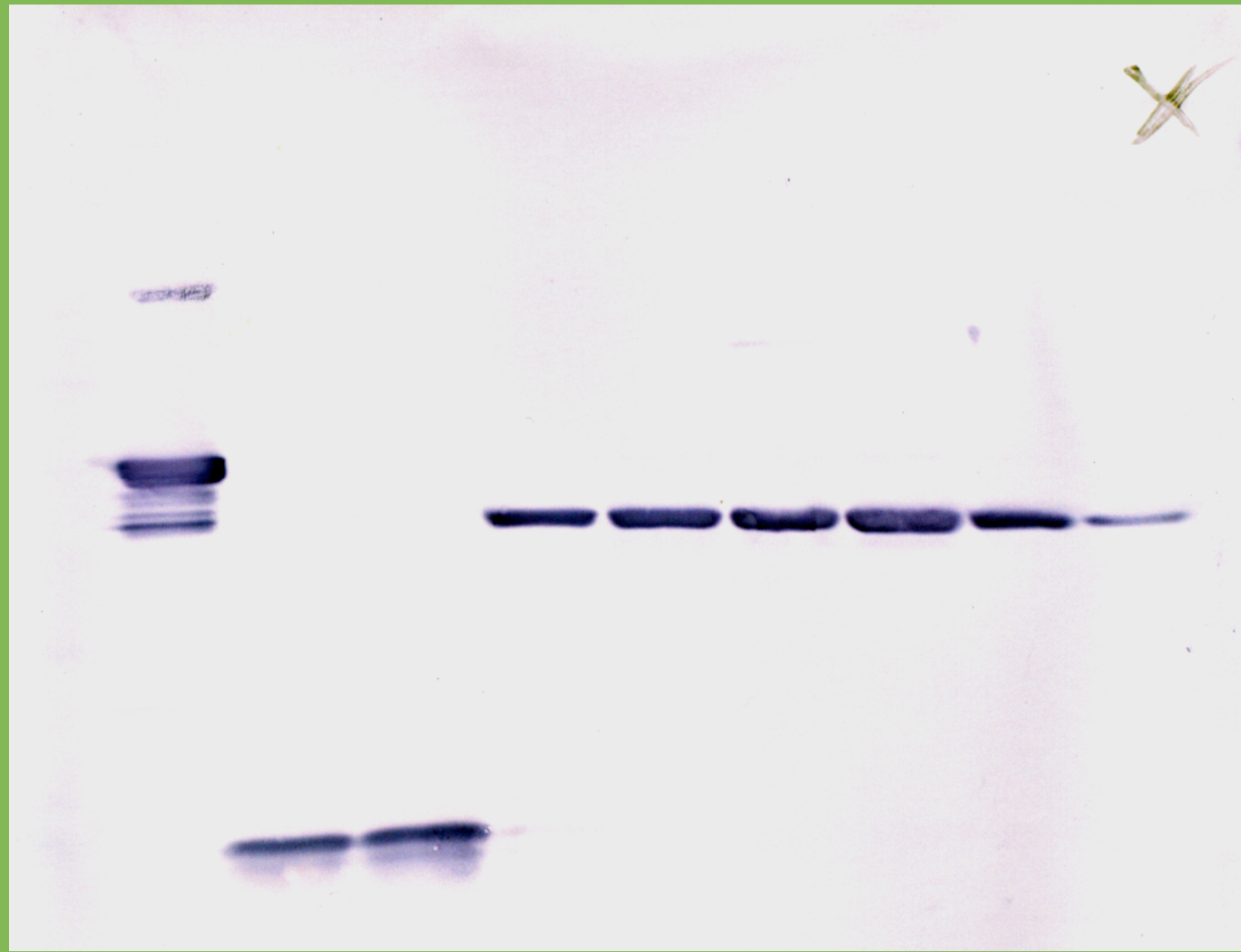
# Mapping requirement to experiment

## Western Blot

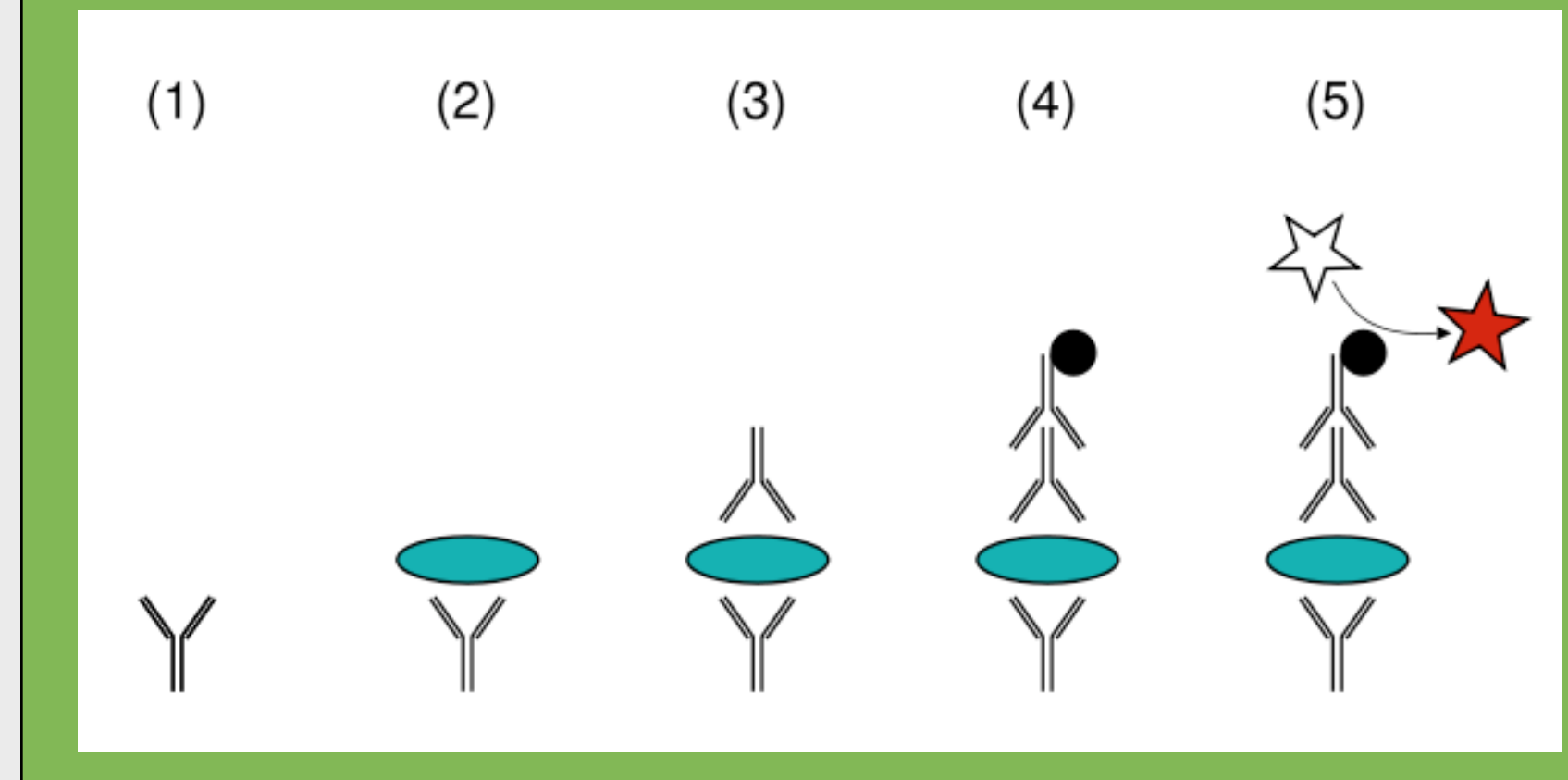


# Mapping requirement to experiment

## Western Blot

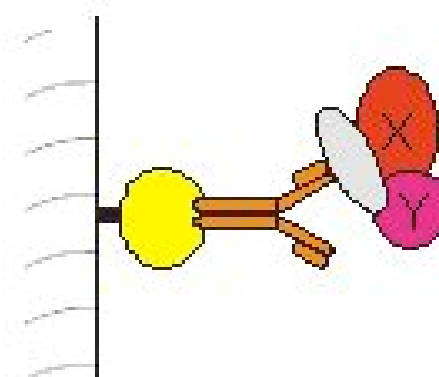


## "Sandwich" ELISA Experiment



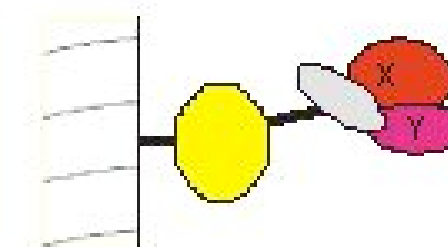
## Pull-down

### Co-immunoprecipitation



● Protein- A

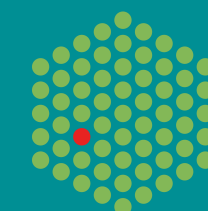
### Fusion protein pull-down



● Fusions-moiety

○ Antigen, known viral protein

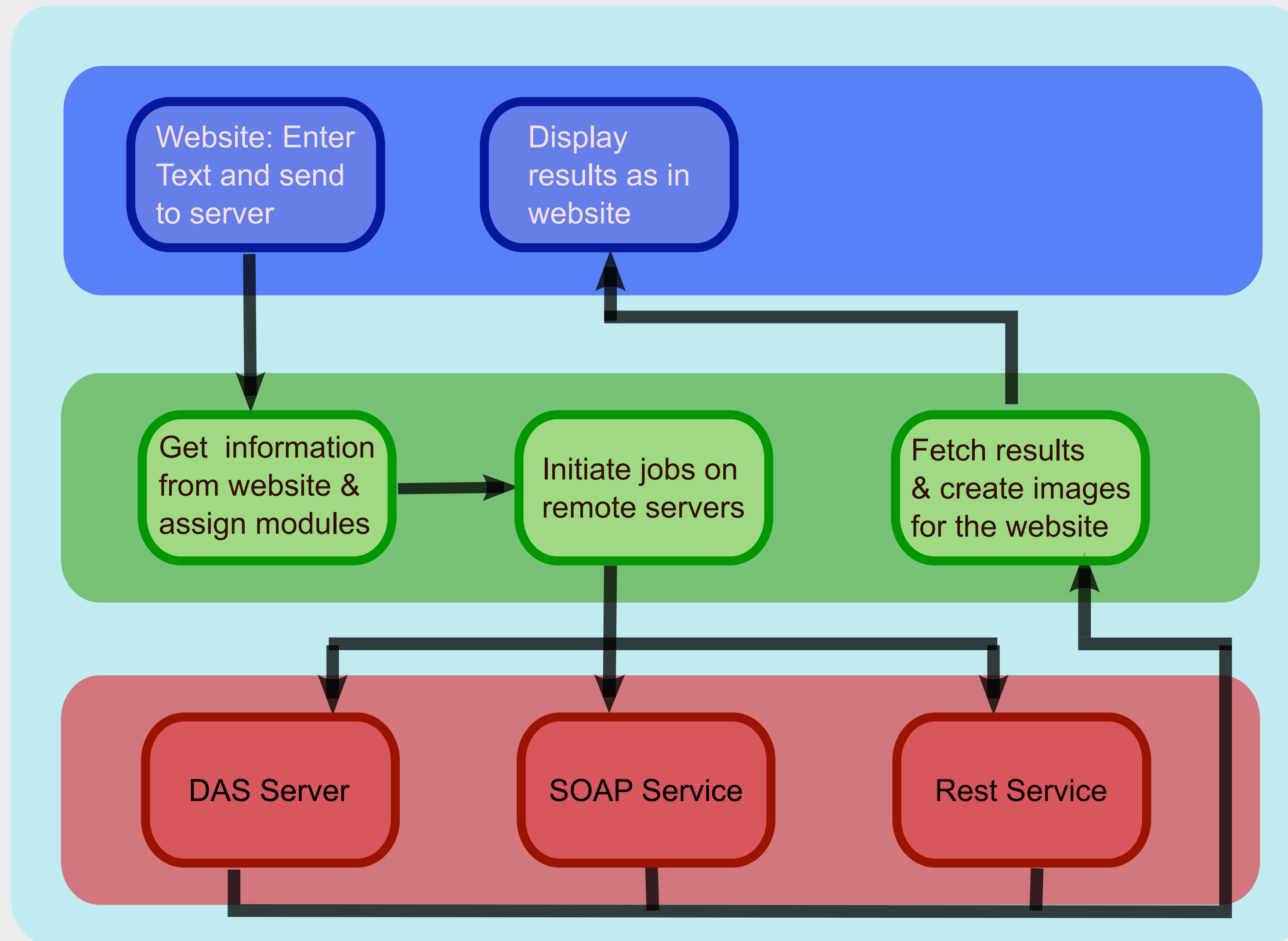
● Unknown ligands



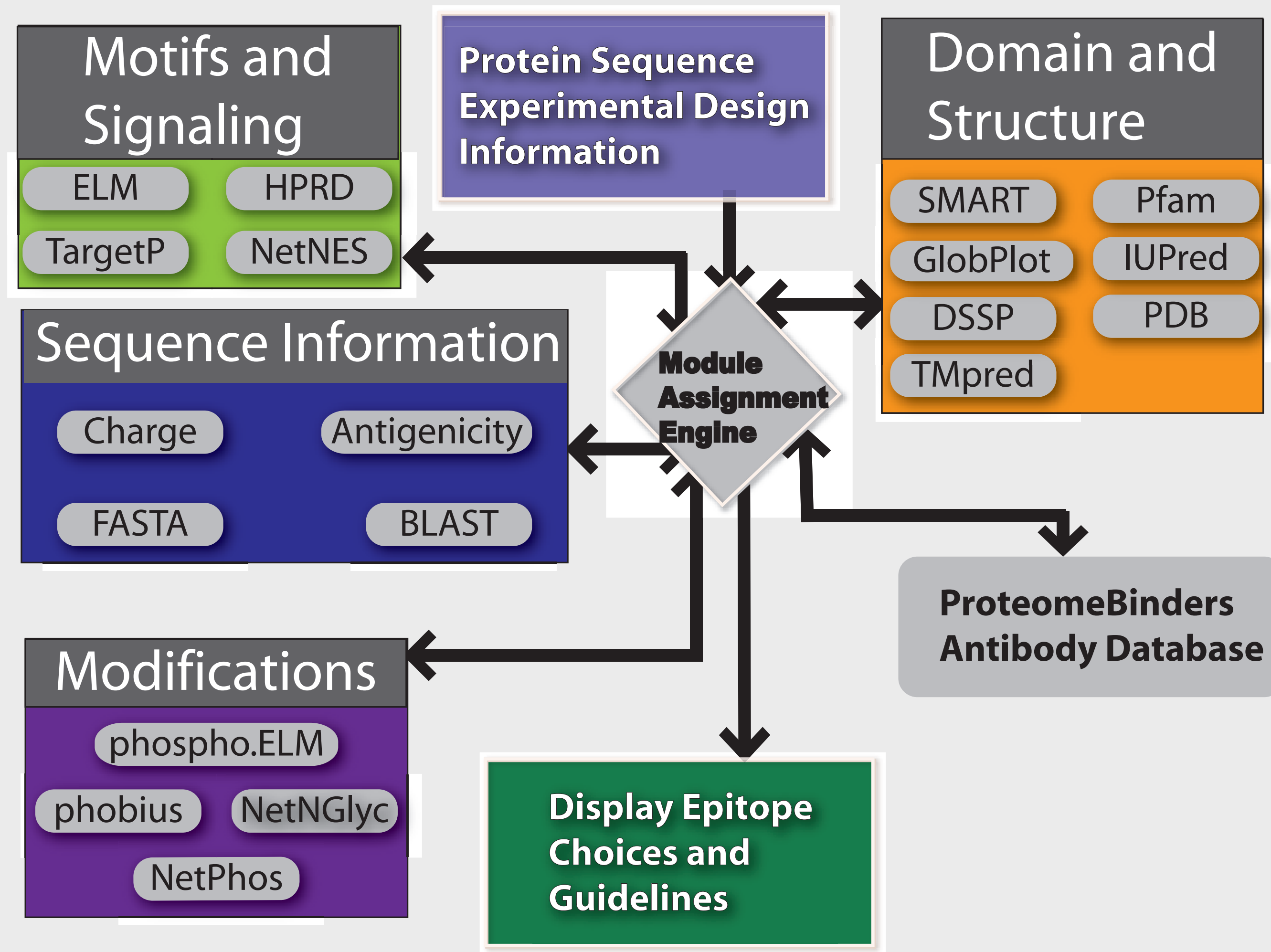
# Why distributed collection of information

- Impossible or impractical to maintain databases in-house
- Difficult to deploy a range of clients to each server
- Stability of APIs in web available sources
- Speed - server is relatively small and underpowered
- DAS and webservices make it possible for small server to achieve complex analysis in acceptable time

# Overview of web and server interaction



# Module Assignment Engine





# Collecting the information

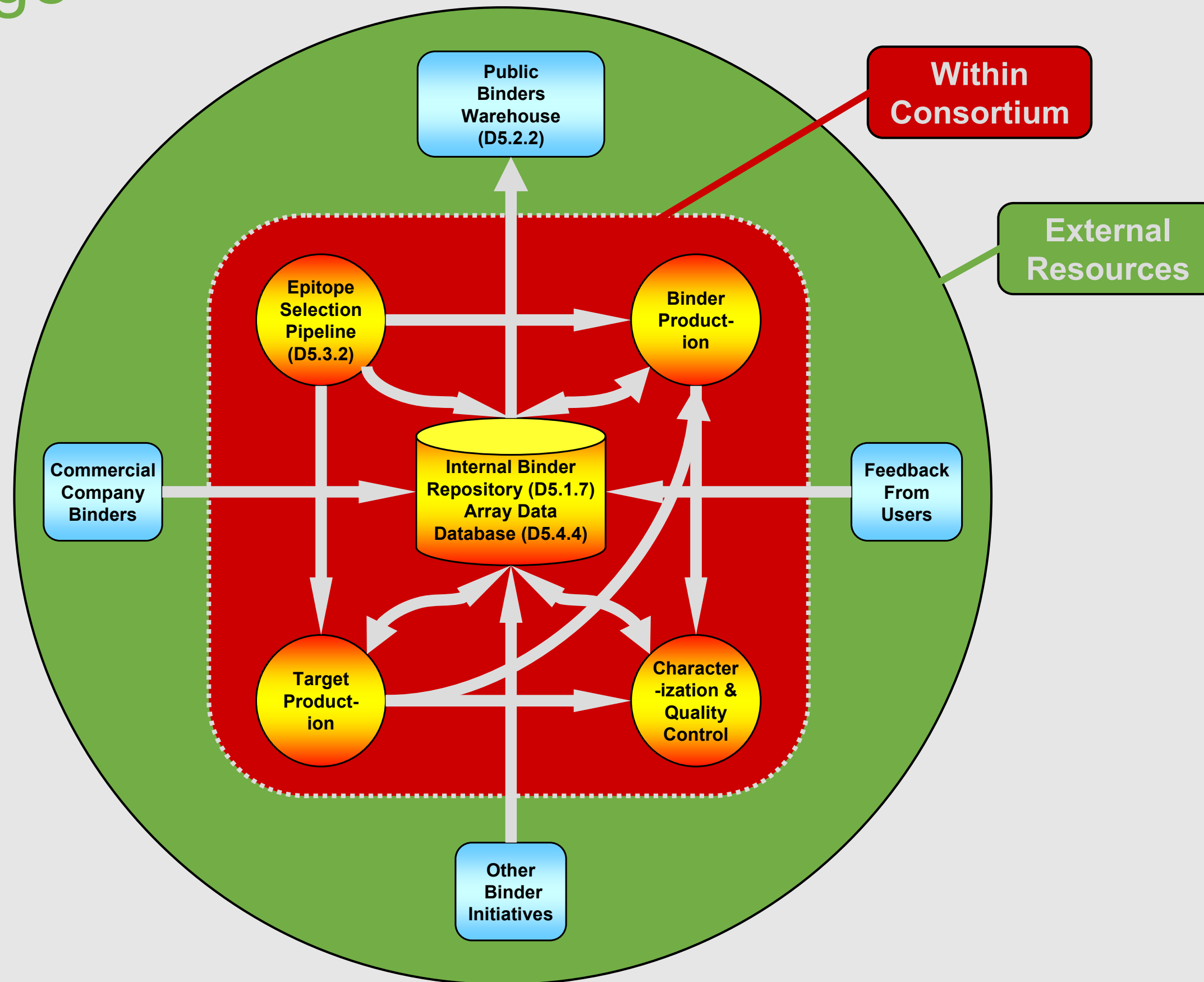
- Ajax interface to collect the results
- RPC Calls to a tomcat server
- Job creation/management by the server
- Parsing of results
- Send result objects back to GUI to be drawn there
- Responsive and interactive GUI

# Webservices integration to DAS objects

- Return results of webservices run analysis into DAS Objects
- These can be displayed in the Ajax GUI easily too
- Object in Ajax coded behaves like DAS Object
- Has properties like co-ordinates, description, name, id, version, notes
- Specifically - the objects sent/recieved are List<DasObject> or List<String> or String - whatever

# Protein Standards Initiative Ontology: PSI - Protein Affinity Reagents (PAR)

Exchange  
/Share  
Events



- David Gloriam
- Louisa and Sandra
- Sandrine Palcy
- EBI and Uni of Bordeaux



# DAS Sources Used





# User Interface

**About the Protein** About the Experiment Explore Epitopes Help

Please enter the protein information here: Hide

Please enter the UniProt ID and click Fetch:  
e.g. P04637 Fetch ?

Alternatively enter the raw sequence by click on the button below:  
Click to enter Protein Sequence

Questions about subcellular localisation: Hide

Are you interested in signal peptide information?  
 Are you interested in localisation information?  
 Are you interested in nuclear localisation information?

Questions about the state of the protein: Hide

Please select the state of the protein in the experiment: Native ?

Next

# User Interface

**About the Protein** About the Experiment Explore Epitopes Help

Please enter the protein information here: Hide

Please enter the UniProt ID and click Fetch:

P04637 done ?

Alternatively enter the raw sequence by click on the button below:

Sequence Retrieved

Questions about subcellular localisation: Hide

- Are you interested in signal peptide information?
- Are you interested in localisation information?
- Are you interested in nuclear localisation information?

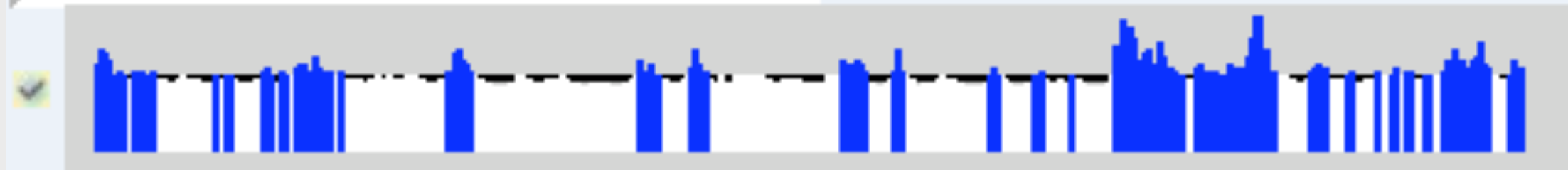
Questions about the state of the protein: Hide

Please select the state of the protein in the experiment: Native ▼ ?

Next

# Results Page

## Surface and Flexibility Predictions



## Antigenicity Predictions



## Hydrophilicity Predictions



## Hydrophobicity Predictions



# Highlighting graphs

Configuration Module for the Graphs.

Enter selection to highlight	Enter selection to Blast
<input type="text" value="56"/>	<input type="text" value="56"/>
and	and
<input type="text" value="207"/>	<input type="text" value="200"/>
<input type="button" value="Highlight"/>	<input type="button" value="b"/>
<input type="button" value="Remove"/>	
<input type="text" value="b"/>	

>> < + -

Legend

- Undefined 
- Polypeptide Domain 
- Motif 
- Alternative Sequence Site 
- Secondary Structure Element 
- Low Complexity or Disorder 



# Highlighting graphs

Configuration Module for the Graphs.

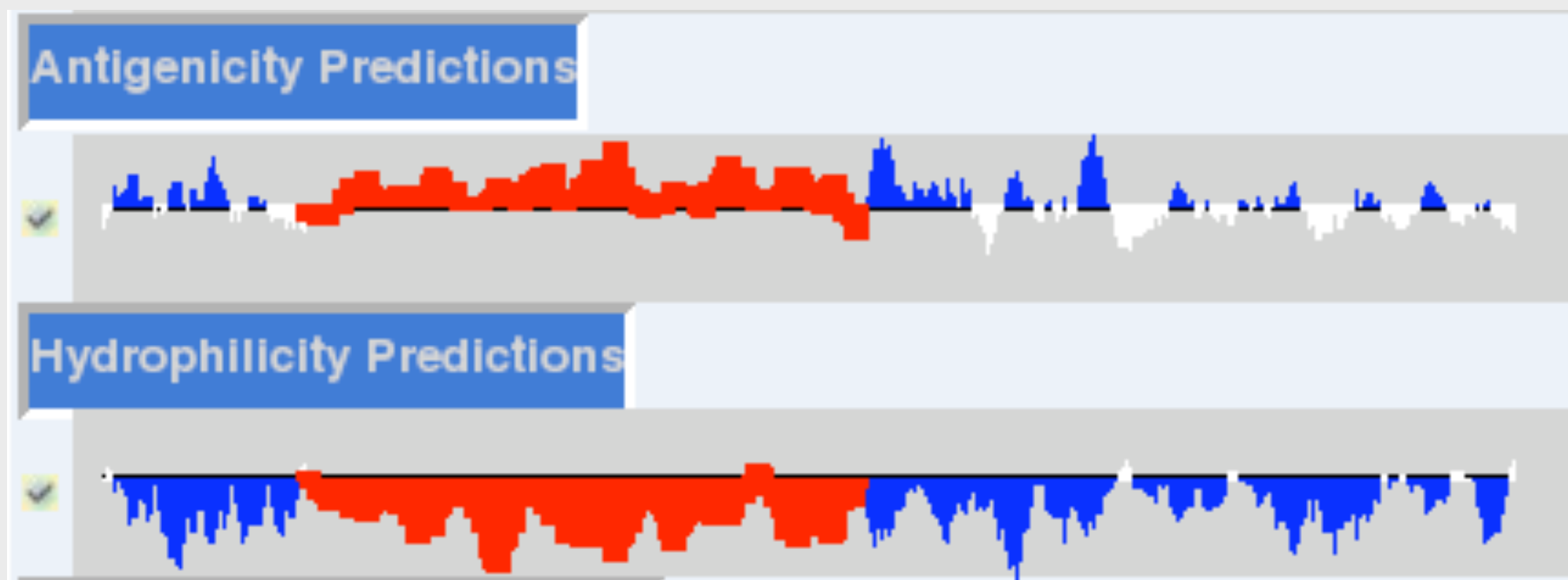
Enter selection to highlight: 56  
and: 207  
Highlight  
Remove  
b

Enter selection to Blast: 56  
and: 200  
b

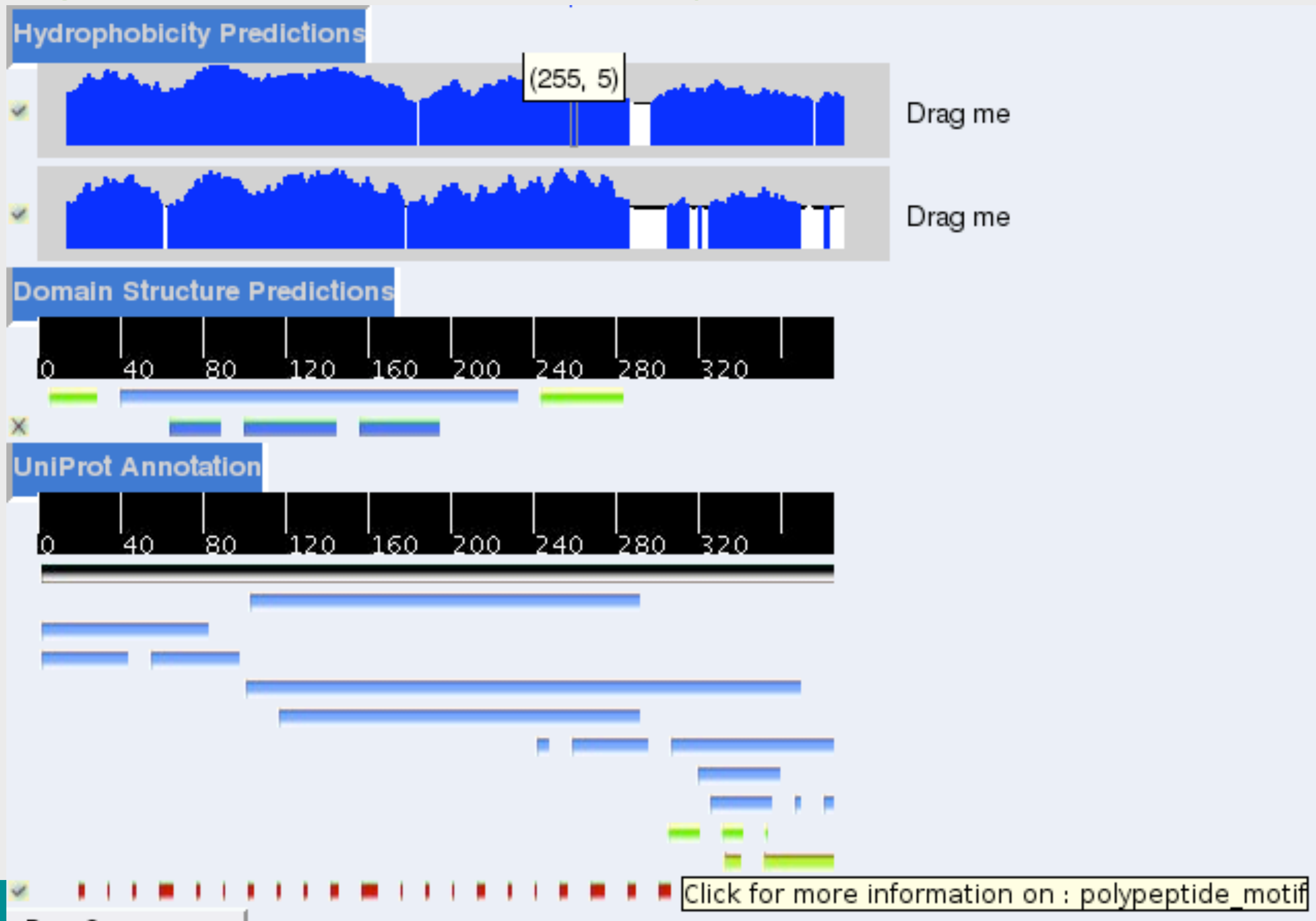
>> < + -

Legend

- Undefined
- Polypeptide Domain
- Motif
- Alternative Sequence Site
- Secondary Structure Element
- Low Complexity or Disorder



# Diagrams on the block objects below



# Diagrams on the block objects below

The image shows a bioinformatics interface with several panels and a detailed description of a block object.

**Hydrophobicity Predictions**

- Two hydrophobicity plots are shown, each with a checkmark icon on the left. The top plot has a tooltip showing "(25)".

**Domain Structure Predictions**

- A domain structure diagram showing a protein sequence from 0 to 240. A blue bar highlights a domain from approximately position 305 to 321.

**UniProt Annotation**

- A UniProt annotation diagram showing the same protein sequence with various features highlighted in blue.

**Block Object Description**

Id: (25)  
Description: polypeptide\_motif  
Note: Bipartite nuclear localization signal  
Score: 0.0  
No link available  
[Original DAS Server Response](#)  
Start: 305 End: 321  
Version: a4339fbbe40c69e28b37c4eb4c4fad74  
Method: UniProt  
Type ID: SO:0001067  
Type Category: inferred by curator (ECO:0000001)  
Method OLS Def: A sequence motif is a short (up to 20 amino acids) region of biological  
Such motifs, although they are too short to constitute functional domains, share sequence  
and are conserved in different proteins. They display a common function (protein-binding  
location etc.).  
Feature Sequence: KRALPNNTSSSP QPKK  
Version Status:

Buttons: x B

# Diagram on the pop ups.

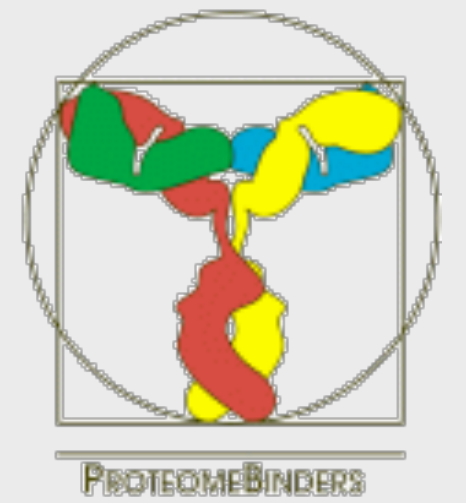
- Mention that this controls the blast interface
- Version check from md5checksum
- Ontology lookup for Experimental Code and definitions of features



# Conclusions

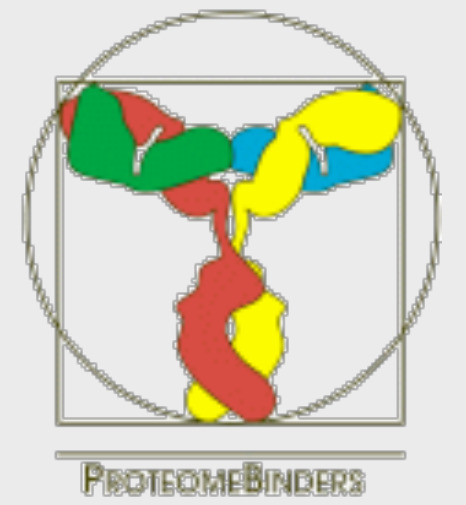
- DAS Sources speed up the delivery of information
- Alignment of objects in GUI much easier
- Ability to check provenance of information available in DAS
- Webservices require one client per app - DAS One client - many apps
- Wealth of information on there
- Would be nice to have updates on newly added DAS Sources
- facebook app as well.

# Acknowledgements



- ProteomeBinders - EU Framework 6 Programme
- EMBL Heidelberg - Structural and Computational Biology Unit
- Toby Gibson's Team
- Henning Hermajakob (EBI), David Gloriam(EBI), Erik Bjorling(KTH), Sandrine Palcy(Uni Bordeaux), David Sherman (Uni Bordeaux), Julie, Antoine - (Uni Bordeaux)
- SPICE, Dasobert, GoogleWebToolkit, biojava, tomcat

# Acknowledgements



## Comments or Questions