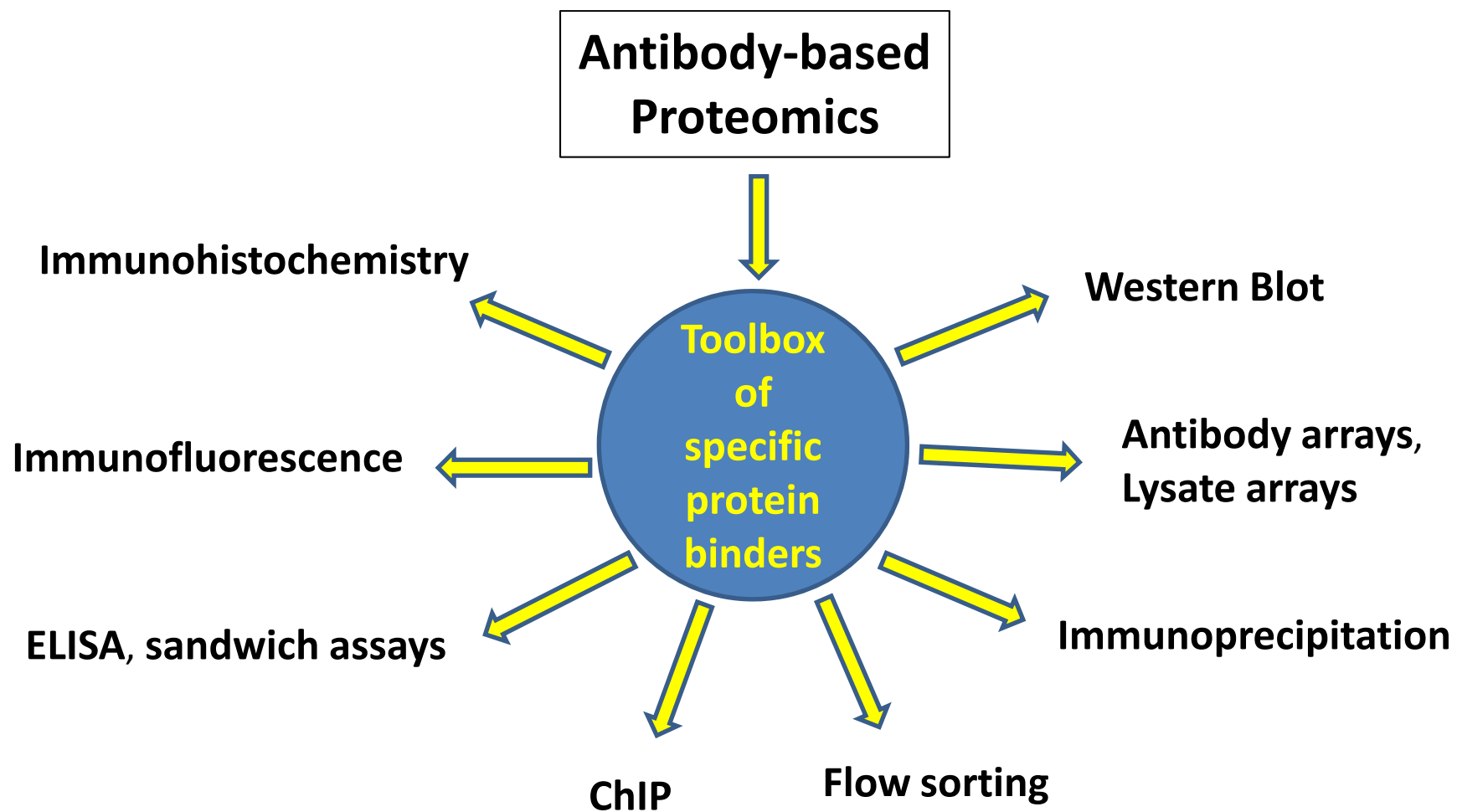


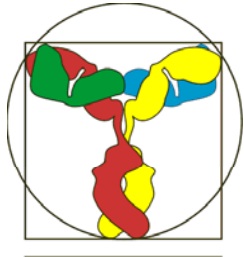
**AFFINOMICS:  
Generating European Binder  
Resources for Affinity Proteomics**

**Mike Taussig  
Babraham Bioscience Technologies  
Cambridge, UK**

Proteomic Forum, Berlin April 4th 2011

# Affinity Reagents: Universal Tools for Life Science





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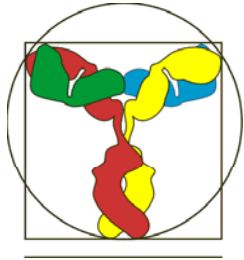
## A Proteome-wide Binder Resource: Raison d'être for Europe



Binding molecules, e.g. antibodies, are among the most essential reagents in biomedical research and an area where Europe has made breakthrough achievements (e.g. mAbs). To understand and exploit the proteome, it is essential to create a comprehensive, standardised binder collection.

**To maintain Europe's position, we should establish:**

- a European resource of *validated, quality-controlled*, binding reagents for detection of all human proteins
- centres of binder production, distribution and databases
- sets of binder-based tools to explore protein expression and function in health and disease (linking to diagnostics and personalised medicine).



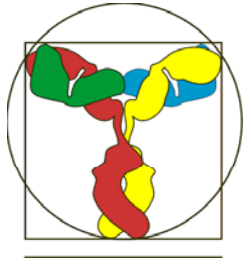
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## Scale of the Human Proteome: Inferences for binders



- 23,000 human genes but >200,000 proteins = the proteome
- Only a minor fraction of the human proteome is covered by existing binders
- At least two binders needed for each protein, and often more
- New binders (and antigens) will therefore be needed in very large ( $10^5$ ?) numbers, whether gene- or protein-centric.
- Reagents must be linked with protein detection tools capable of high sensitivity, wide dynamic range and multiplexing.

***Because of the nature and size of the proteome, the project resembles the human genome sequencing in scale and significance***



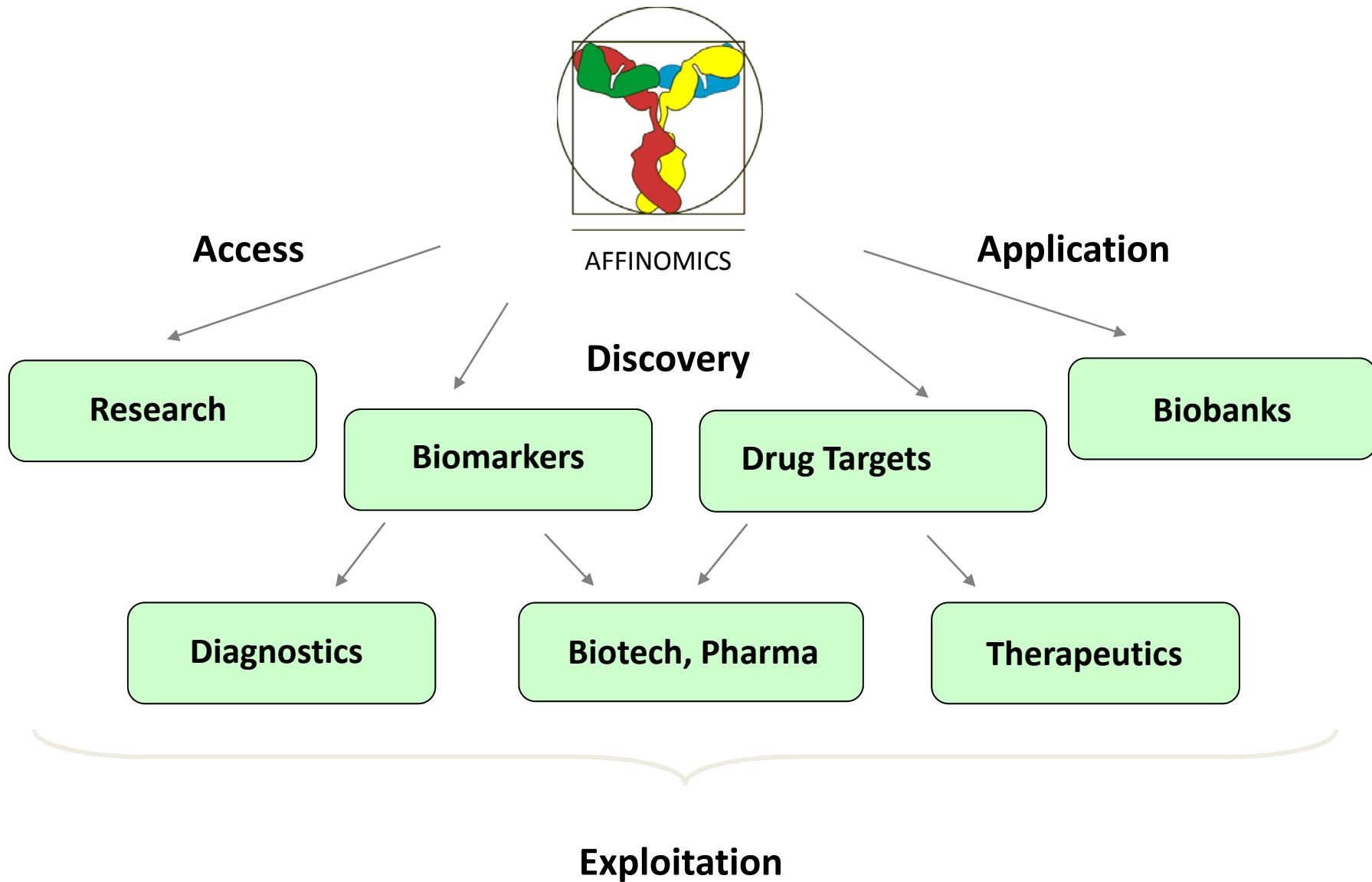
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## Quality Control: The Economic Perspective



- The global market for research antibodies is probably \$0.5 – 1.0 billion (not to mention therapeutics)
  - The quality and success rate of reagents available today from companies is highly variable
  - If about half the antibodies fail as reagents\*
    - > \$250 M is wasted annually (reagents purchased)
    - Laboratory time is wasted
    - Artefactual observations are published
  - The solution requires a combined effort by academia, industry and funders to generate reliable, validated reagents at cost
- (\*survey by Mathias Uhlén)

# Benefits of a Binders Resource

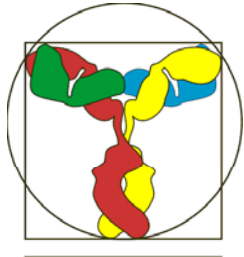


Nature Methods: 4,13-17 (2007)

## ProteomeBinders: planning a European resource of affinity reagents for analysis of the human proteome

Michael J Taussig<sup>1</sup>, Oda Stoevesandt<sup>1</sup>, Carl A K Borrebaeck<sup>2</sup>, Andrew R Bradbury<sup>3</sup>, Dolores Cahill<sup>4</sup>, Christian Cambillau<sup>5</sup>, Antoine de Daruvar<sup>6</sup>, Stefan Dübel<sup>7</sup>, Jutta Eichler<sup>8</sup>, Ronald Frank<sup>8</sup>, Toby J Gibson<sup>9</sup>, David Gloriam<sup>10</sup>, Larry Gold<sup>11</sup>, Friedrich W Herberg<sup>12</sup>, Henning Hermjakob<sup>10</sup>, Jörg D Hoheisel<sup>13</sup>, Thomas O Joos<sup>14</sup>, Olli Kallioniemi<sup>15</sup>, Manfred Koegl<sup>16</sup>, Zoltán Konthur<sup>17</sup>, Bernhard Korn<sup>13</sup>, Elisabeth Kremmer<sup>18</sup>, Sylvia Krobitsch<sup>17</sup>, Ulf Landegren<sup>19</sup>, Silvère van der Maarel<sup>20</sup>, John McCafferty<sup>21</sup>, Serge Muyldermans<sup>22</sup>, Per-Åke Nygren<sup>23</sup>, Sandrine Palcy<sup>6</sup>, Andreas Plückthun<sup>24</sup>, Bojan Polic<sup>25</sup>, Michael Przybylski<sup>26</sup>, Petri Saviranta<sup>15</sup>, Alan Sawyer<sup>27</sup>, David J Sherman<sup>28</sup>, Arne Skerra<sup>29</sup>, Markus Templin<sup>14</sup>, Marius Ueffing<sup>18</sup> & Mathias Uhlén<sup>23</sup>

ProteomeBinders is a new European consortium aiming to establish a comprehensive resource of well-characterized affinity reagents, including but not limited to antibodies, for analysis of the human proteome. Given the huge diversity of the proteome, the scale of the project is potentially immense but nevertheless feasible in the context of a pan-European or even worldwide coordination.



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## Vision and Aims for a European Binder Resource



- To provide affinity reagents against all human proteins to the research community
- To develop the required molecular resources of clones, proteins, peptides and validated paired reagents
- To establish adequate reagent QC procedures
- To develop novel tools for application of affinity reagents
- To create public database portals with binder and protein data
- To facilitate specific human proteome projects, e.g. functional annotation of the proteome; mapping of proteins in healthy and diseased tissues; plasma profiling; discovery of biomarkers



# Antibodypedia - a portal for validated antibodies

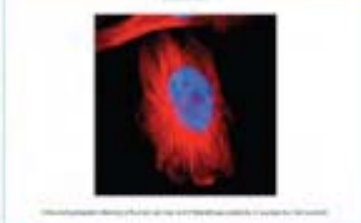


**A. EU ProteomeBinders - List of submitted binders**

ID	Antigen	Antibody	Validation	Accession
1	...	...	...	...
2	...	...	...	...
3	...	...	...	...
4	...	...	...	...
5	...	...	...	...
6	...	...	...	...
7	...	...	...	...
8	...	...	...	...
9	...	...	...	...
10	...	...	...	...

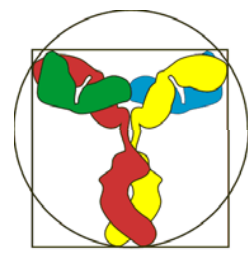
**B. EU ProteomeBinders - Details of submitted binder**

Antigen: ...  
Antibody: ...  
Validation: ...  
Accession: ...

**B. continued**



- [www.antibodypedia.org](http://www.antibodypedia.org)
- A community-based approach for sharing both antigen and antibody validation data
- Developed within the frame-work of the EU ProteomeBinders program



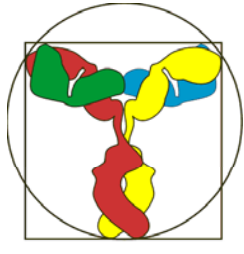
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□ Erik Björling and Mathias Uhlen  
**Antibodypedia – a portal for sharing antibody and antigen validation data**  
MCP published July 29, 2008

[Login](#) [Register](#)**EU ProteomeBinders - List of submitted binders**

Search results: 10 hits

#	Gene name	Description	Chr	Links	Binder ID	Type	Species	Antigen	Validation						S
1	ADAMTS5	ADAMTS-5 precursor (EC 3.4.24.-) (A disintegrin and metalloproteinase with thrombospondin motifs 5) (ADAM-TS 5) (ADAM-TS5) (Aggrecanase-2) (ADMP-2) (ADAM-TS 11).	21:q21.3	<a href="#">L</a> <a href="#">E</a>	<a href="#">HPA005661</a>	msAb	rabbit	PrEST	PA	IH	WB	IF	IP	OA	13
2	APP	Amyloid beta A4 protein precursor (APP) (ABPP) (Alzheimer disease amyloid protein) (Cerebral vascular amyloid peptide) (CVAP) (Protease nexin-II) (PN-II) (APPI) (PreA4) [Contains: Soluble APP-alpha (S-APP-alpha); Soluble APP-beta (S-APP-beta); C99; Beta-	21:q21.3	<a href="#">L</a> <a href="#">E</a>	<a href="#">HPA001462</a>	msAb	rabbit	PrEST	PA	IH	WB	IF	IP	OA	14
3	BACH1	Transcription regulator protein BACH1 (BTB and CNC homolog 1) (HA2303).	21:q21.3	<a href="#">L</a> <a href="#">E</a>	<a href="#">HPA003175</a>	msAb	rabbit	PrEST	PA	IH	WB	IF	IP	OA	14
4	COL18A1	Collagen alpha-1(XVIII) chain precursor [Contains: Endostatin].	21:q22.3	<a href="#">L</a> <a href="#">E</a>	<a href="#">HPA011025</a>	msAb	rabbit	PrEST	PA	IH	WB	IF	IP	OA	13
5	COL6A2	Collagen alpha-2(VI) chain precursor.	21:q22.3	<a href="#">L</a> <a href="#">E</a>	<a href="#">HPA007029</a>	msAb	rabbit	PrEST	PA	IH	WB	IF	IP	OA	15
6	CXADR	Coxsackievirus and adenovirus receptor precursor (Coxsackievirus B- adenovirus receptor) (hCAR) (CVB3-binding protein) (HCVADR).	21:q21.1	<a href="#">L</a> <a href="#">E</a>	<a href="#">HPA003342</a>	msAb	rabbit	PrEST	PA	IH	WB	IF	IP	OA	15
7	GABPA	GA-binding protein alpha chain (GABP-subunit alpha) (Transcription factor E4TF1-80) (Nuclear respiratory factor 2 subunit alpha).	21:q21.3	<a href="#">L</a> <a href="#">E</a>	<a href="#">HPA003258</a>	msAb	rabbit	PrEST	PA	IH	WB	IF	IP	OA	15
8	IFNGR2	Interferon-gamma receptor beta chain precursor (Interferon-gamma receptor accessory factor 1) (AF-1) (Interferon-gamma transducer 1).	21:q22.11	<a href="#">L</a> <a href="#">E</a>	<a href="#">HPA001535</a>	msAb	rabbit	PrEST	PA	IH	WB	IF	IP	OA	13



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## MIAPAR: Minimal Information About a Protein Affinity Reagent

Orchard et al (2010)

Nature Biotechnol, 28:650-653

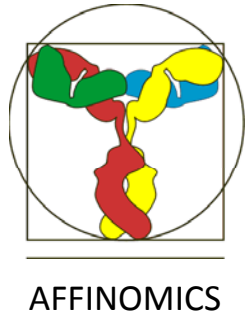


### MIAPAR – what data to describe

The Minimum Information About a Protein Affinity Reagent

Describes the information that needs to be in any article (or deposition) to enable the user to

1. To understand the strengths of the binder (target details, binding site, purity, selectivity)
2. To understand the weaknesses of a binder (selectivity)
3. To understand the correct usage of a binder (western blot, immunohistochemistry...)



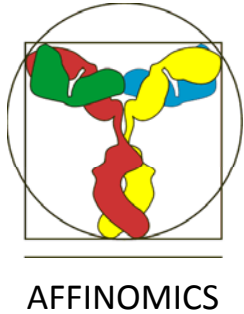
# EPIC: Epitope Selection Resource

Toby Gibson, Niall Haslam, EMBL

<http://epic.embl.de>



- A suite of software tools that aid in the experimental design of binders to human proteins
- Integrating previously existing information/ annotation and bioinformatic prediction tools
- Context dependent delivery of analysis results
- Haslam and Gibson (2010) EpiC: An Open Resource for Exploring Epitopes to Aid Antibody-based Experiments. *J. Proteome Research*, 9:3759-63.



# AFFINOMICS (AFFINity ProteOMICS)

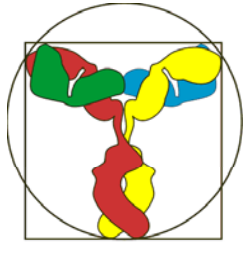


Protein Binders for Characterisation of  
Human Proteome Function:  
Generation, Validation, Application

EC Collaborative Large Scale Integrating Project

Start Date: 1<sup>st</sup> April 2010

15 partners, 5 years, 11M€



AFFINOMICS

# AFFINOMICS

## Project Essentials



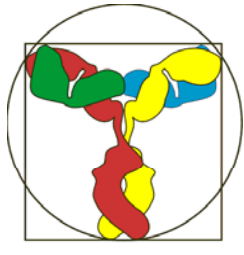
1. A binder production pipeline, including pAbs and mAbs but with particular emphasis on novel recombinant selection systems capable of proteome wide delivery.
2. Focus on 5 categories of proteins in signal transduction, cell regulation and cancer: *all protein kinases, SH2 domain-containing proteins and protein tyrosine phosphatases*, plus *proteins somatically mutated in cancers and candidate cancer biomarkers* (1000 targets)
3. High throughput analytical binder-based tools for functional analyses of signal transduction protein-protein interactions, composition of complexes, phosphorylation states, disease-specific variations and functional intracellular inhibition.

# Year 1: Target production

- Full length proteins
- Individual domains (e.g. SH2)
- PrESTs (50-100 amino acid peptides)
- Peptides (c. 20 amino acids)

Started in Year 1 at SGC Oxford, KI Stockholm, CPR Copenhagen:  
180 full length proteins or individual (SH2) domains of selected

- Kinases
- Phosphatases
- Methyltransferases
- Ubiquitin ligases
- Adaptor proteins
- Exchange factors



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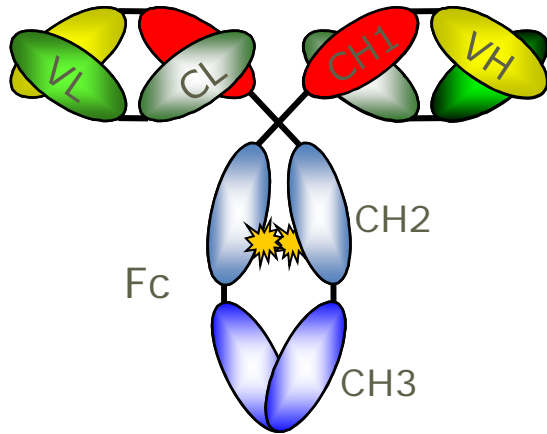
# Binder types in AFFINOMICS

## 1. Antibodies

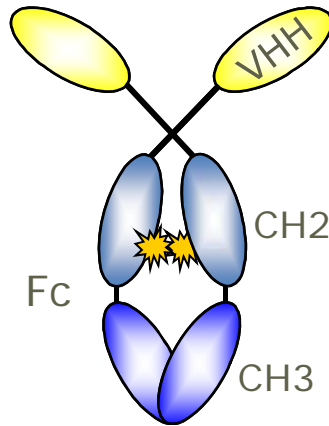


Classical

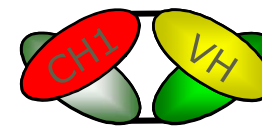
... and newer versions



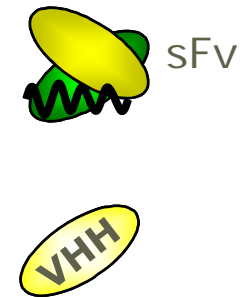
Polyclonal,  
monoclonal



Camelid

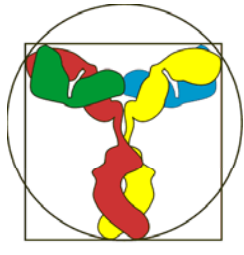


Fragments



Single chain,  
Single domain  
nanobody





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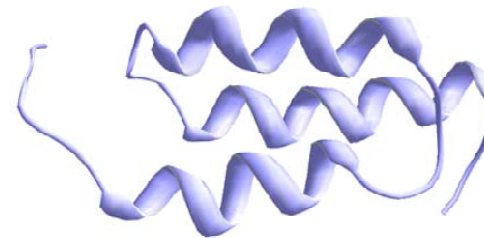
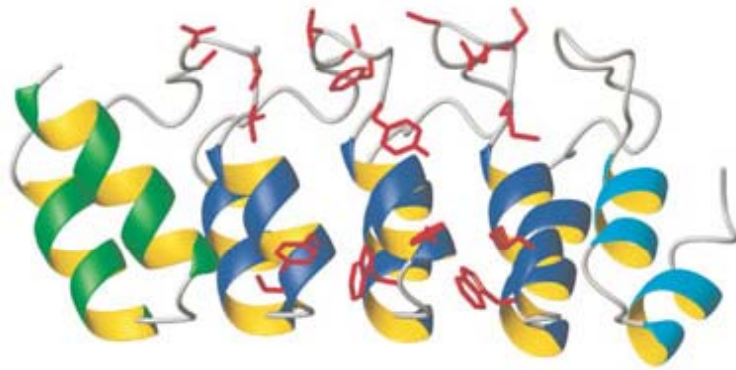
# Binder types in AFFINOMICS

## 2. Next generation alternative scaffolds



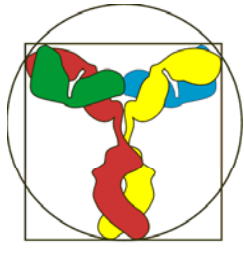
DARPinS

Affibodies



Designed Ankyrin  
Repeat Proteins

Engineered Protein A

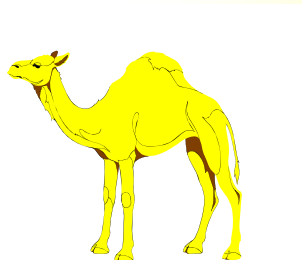


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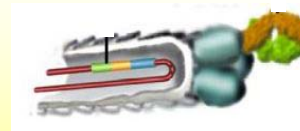
# Binder production in AFFINOMICS: Classical and Modern Methods



**Classical:  
immunisation**

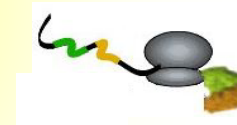


**Phage  
display**



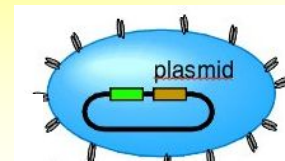
**Libraries with  
huge repertoire  
of binder genes**

**Ribosome  
display**

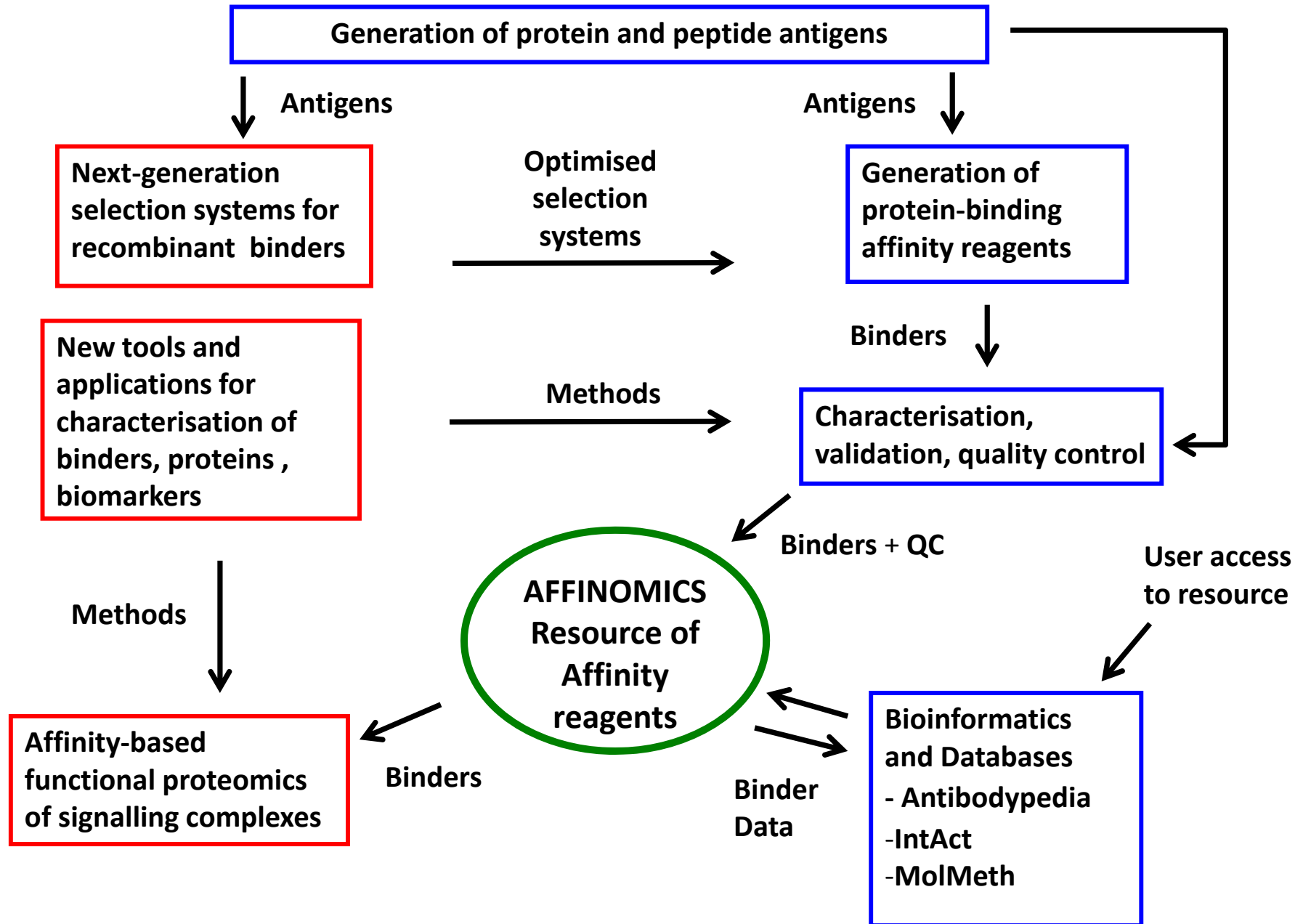


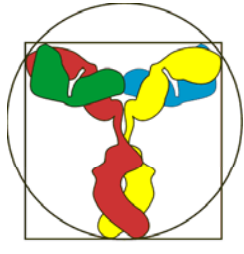
**Modern:  
Recombinant  
Molecular  
Methods**

**Cellular  
display**



# AFFINOMICS Reagent Production Pipeline





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## AFFINOMICS: Novel binder based tools for proteomic applications with high throughput potential

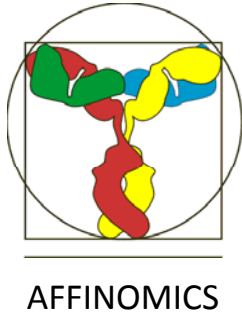


- *Binder arrays*: proteome profiling, biomarkers, diagnostics
- *Functional protein arrays*: protein-protein interactions, binder profiling, autoantibodies
- *Reverse lysate arrays*: high throughput detection of protein markers in cancer cells
- *Proximity ligation*: linkage to DNA for ultrasensitive detection of proteins and intracellular complexes *in situ*
- *Intrabodies*: intracellular expression for knockdown and dynamic localisation

## Transformative technologies needed for personalised medicine

- Sequencing genomes of individuals
- Better protein-capture agents for protein assays
- Targeted proteomic assays for all human proteins
- Microfluidic chips to quantify thousands of proteins from a droplet of blood
- Single-cell analysis for assessing quantized populations of cells rather than averaging populations

Leroy Hood, Nature Biotechnology 29, 191 (2011)



Thanks to AFFINOMICS partners ....

Ulf Landegren, Uppsala  
Mathias Uhlen, Stockholm  
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Henning Hermjakob, Hinxton  
Gianni Cesareni, Rome  
Stefan Dubel, Braunschweig  
Fritz Herberg, Kassel  
Marius Ueffing, Munich  
Susanne Muller-Knapp, Oxford  
Joerg Hoheisel, Heidelberg  
Serge Muyldermans, Brussels

