MANCHESTER 1824
The University of Manchester

BioProNET

Network in Bioprocessing, a BBSRC NIBB co-sponsored by EPSRC

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# Proof of Concept Award:

A suite of web tools to predict protein solubility for the biopharmaceutical and biotechnology sectors.

Jim Warwicker and Robin Curtis

# BioProNET background

BioProNET is a network that focuses on the use of cells and their components (that is, bioprocessing) to produce biologics, which we define as products that are composed of proteins (such as antibodies), peptides, RNA, DNA or vaccines.

Such biologics could be used as therapeutics, for example as biopharmaceuticals or as non-therapeutics, for example in diagnostics, industrial enzymes, for drug screening, and for crystallization and structural studies.

BioProNET's objectives and goals are to:

- Provide leadership and vision to the UK academic and industrial community in the field of bioprocessing of biologics to usher in new collaborative models that accelerate innovation and deliver change.
- Facilitate the generation of collaborative and cross-disciplinary grant proposals and the subsequent award of major research funding from UK and international sources that ultimately generates outputs of direct benefit to the sector.
- Create an internationally recognised biologics community that is able to harness discoveries in the basic sciences for application to industrial bioprocesses and the supply chain that partners with complementary networks.
- Provide a vehicle for the delivery of proof of concept studies that lead to more competitive, collaborative, cross disciplinary and integrative funding proposals.
- Create an environment that promotes the emergence of new technologies, including synthetic biology, genomics and systems biology to allow for more rapid, flexible, predictable and cost-efficient production of biologics.
- Inspire and develop the next generation of scientists across the breath of disciplines encompassed by the network.
- Provide a mechanism for fostering community interactions and international collaboration allowing the rapid response to research challenges, policy changes, and large research calls.
- Open a route for academics to apply to industrially relevant challenges and consider the societal, environmental, economic and political ramifications of their work.

# BioProNET meeting(s)

## 2nd Annual Scientific Meeting – registration now open

## October 22nd–23rd 2015, Manchester Midland Hotel

Our second annual scientific meeting will be held at the Midland Hotel, Manchester on October 22nd and 23rd (Following on from the BRIC dissemination meeting which will be held at the same venue on October 21st and 22nd). We have an exciting programme planned including posters, workshops and presentations (see below). To register for the event please use the link below:

**https://www.surveymonkey.com/r/BioProNET_Manchester_meeting**

Day 1

**13.00-15.00 Biologics: present and future perspectives**

Susan Rosser – Universtiy of Edinburgh Synthetic Biology Centre
Thomas Scheibel – Bayreuth University, Germany
Mark Uden – GlaxoSmithKline: *Intellectual property trends in modern bioprocessing*
Andy Porter – University of Aberdeen

15.00-15.45 Coffee and networking

**15.45-17.45 Cellular production systems: coping with future demands**

Andreas Schiermeyer – Fraunhofer Institute, Germany: *Plant-based expression systems for the production of recombinant proteins*
Matthew DeLisa – Cornell University, USA
Martin Jordan – Merck Serono, Switzerland: *Rational media design in micro-scale fed batch cultures*

# Protein solubility and aggregation

**Box 3. Outstanding questions**

- Can proteins be designed to completely eliminate aggregates without sacrificing folding or function, or is aggregation unavoidable for some proteins for practical concentrations and time scales?
- Can a priori prediction of how mutations alter aggregation rates of natively folded proteins be done quantitatively or even semi-quantitatively, without a need to fit or statistically optimize against large quantitative databases?
- Will future design approaches acknowledge the importance of the solution environment and shift away from being predominantly focused on physiological conditions that are of little relevance to aggregation and stability of proteins in biopharmaceuticals and biotechnology products?
- Will it be possible to predict and design which types of aggregates form, so as to avoid aggregated forms that are more immunogenic?

CJ Roberts(2014) Trends Biotechnology 32:372

# Protein solubility prediction

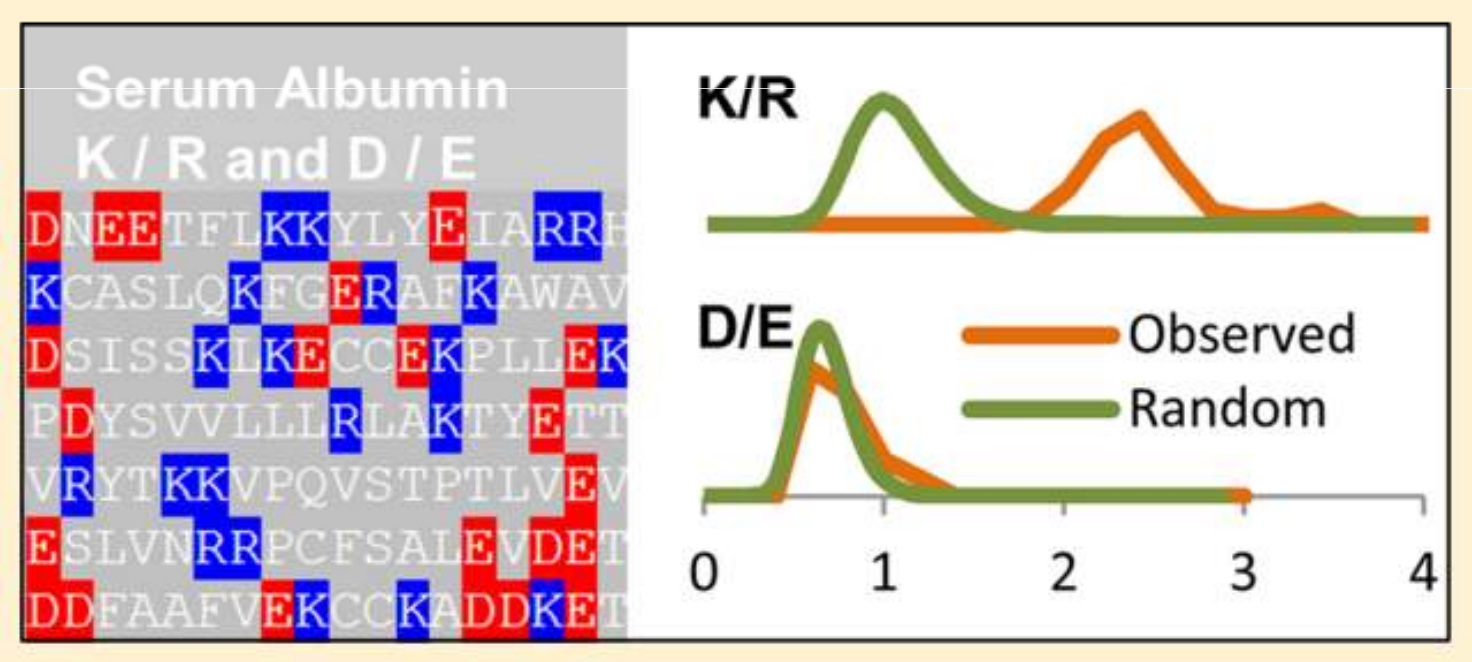
| Tool | Input | Source of Parameterization | Brief Description | Description of Validation | Applied to Improve Developability of Biotherapeutics? |
|---|---|---|---|---|---|
| PAGE[64] | Sequence | Peptides found in diseases causing amyloidogenic proteins. | Aggregation propensity calculated based on aromaticity, β-strand propensity, and charge. | Experimental data on a number of diseases causing amyloidogenic proteins was used to validate. | Yes |
| TANGO[67] | Sequence | Short aggregating and nonaggregating peptides. | Statistical mechanics-based method. Takes into account physicochemical principles behind β-sheet formation. | Experimental data on a set of 179 peptides was used to validate this method. | Yes |
| Zyggregator[70–72]/ AggreSolve | Sequence | Short peptides | Relative propensities for folding and aggregation in a given sequence region. | A number of known amyloidogenic peptides were used to validate. | Yes |
| AGGRESCAN[63] | Sequence | Aβ peptide mutants | Intracellular aggregation propensity of mutants of Aβ42 peptide (mutants were generated by single point mutation in the residues 17–21). | Experimental data on 24 fibrillar deposition-linked polypeptides was used to validate this method. | Unknown |
| SAP[11] | Structure | Accessible surface area and residue wise hydrophobicity scale were used. Note that this method does not use data on amyloidogenic peptides and proteins. | SAP finds the effective dynamically exposed hydrophobicity of a certain patch on the protein surface. High SAP values are indicative of aggregation-prone regions. | mAbs, that were redesigned based the SAP prediction showed reduced aggregation propensity in experiments. | Yes |

NJ Agrawal et al (2011) J Pharm Sci 100:5081

# Calculate: sequence properties of proteins

e.g. Compare sequences of high concentration serum albumins and paralogous proteins that occur at low concentration.

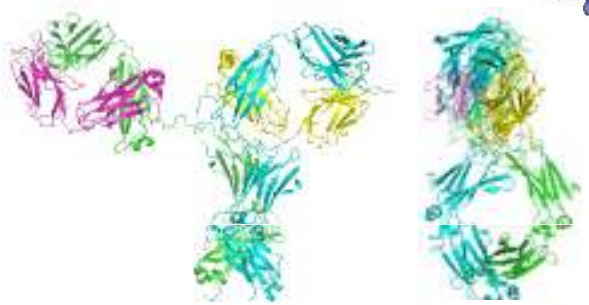
Include the distribution of a property that would be expected at random, given the proteome-wide amino acid distributions.

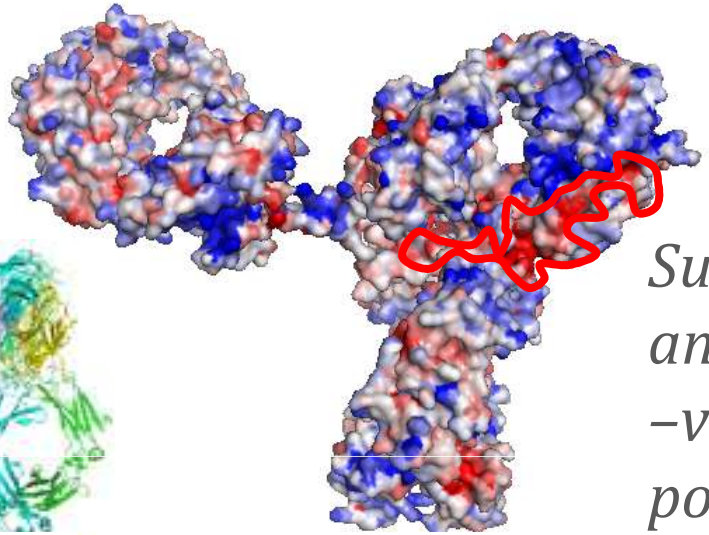



Warwicker et al (2014) Mol Pharm 11:294

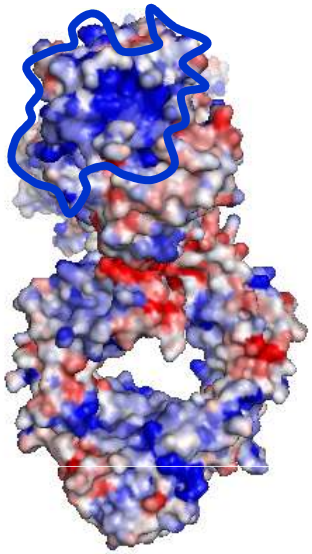
# Calculate: surface properties of poteins

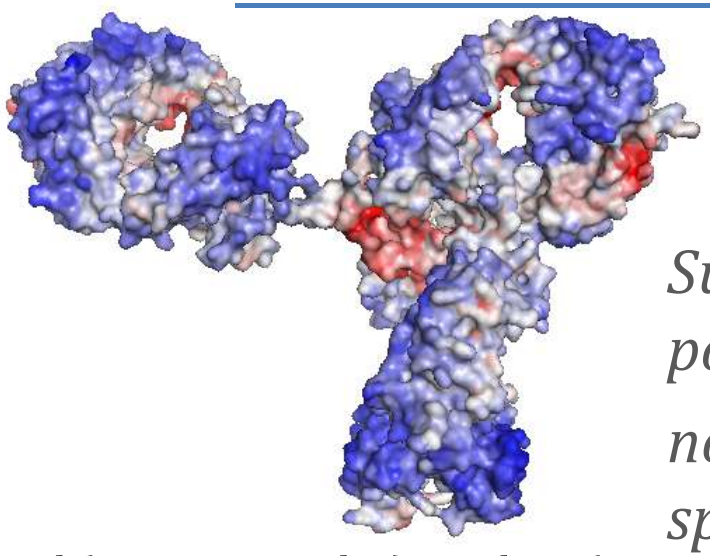
red (most negative) - white (intermediate) - blue (most positive)





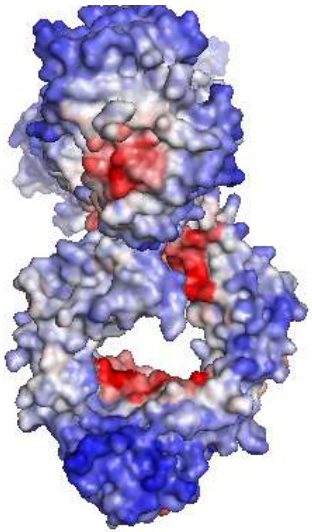
*Surface charge and patches of –ve and +ve potential*





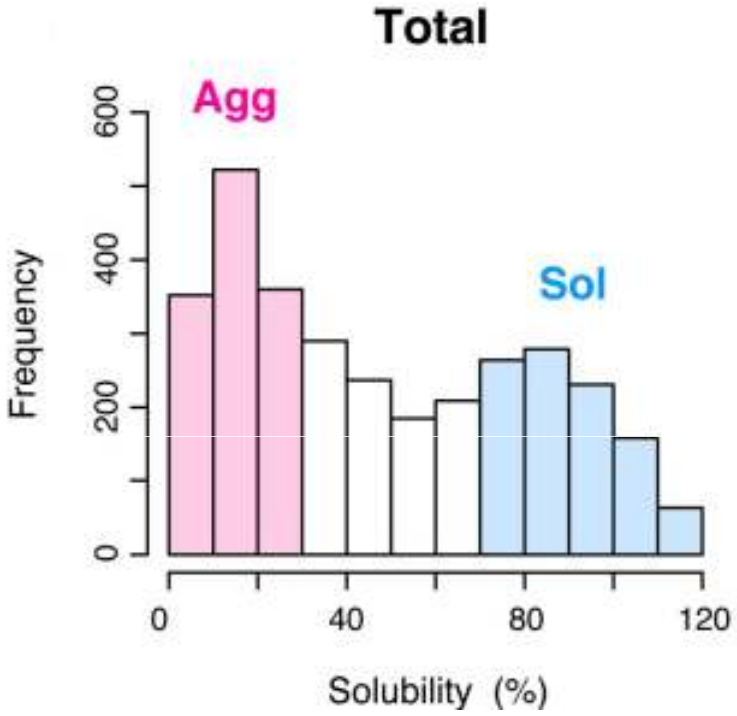
*Surface polarity*

*non-polar hot spots? (red)*



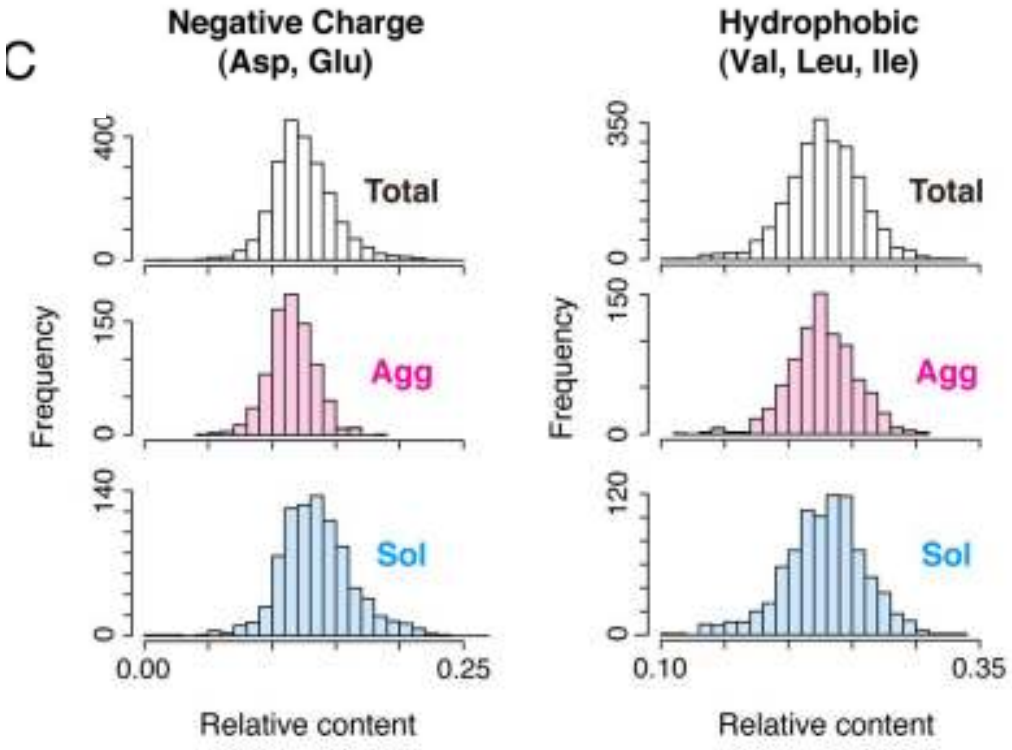
red (most non-polar) - white (intermediate) - blue (most polar)

# Data: high throughput solubility



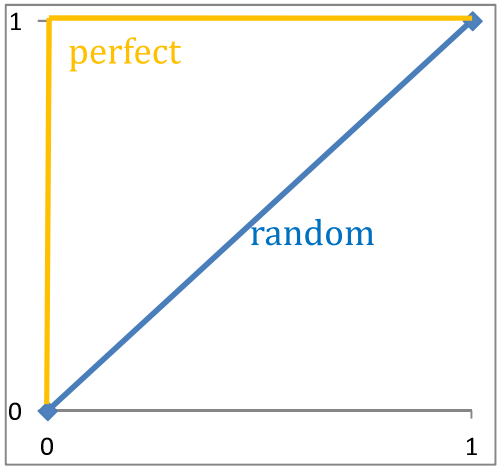

*E. Coli* protein solubility measured in a cell-free expression system shows a bimodal distribution.

Some distinction of bimodal subsets by negative charge, but little separation by hydrophobic amino acid composition.



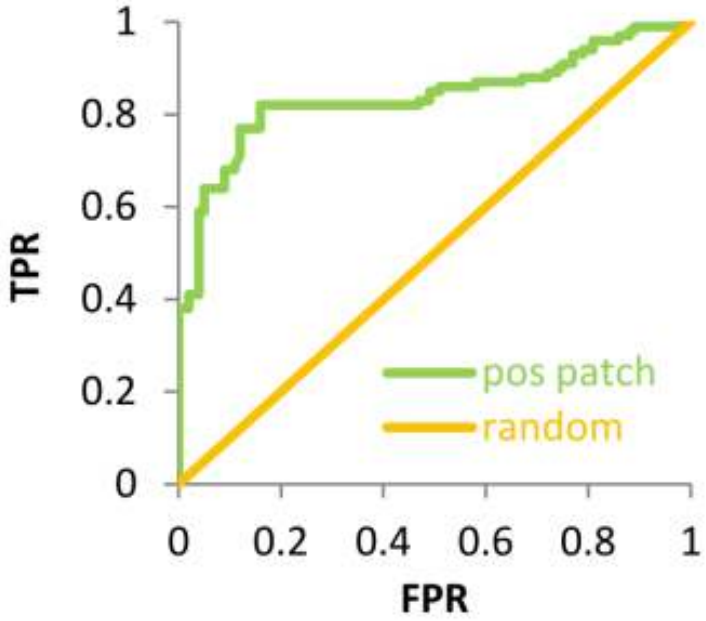

Niwa et al (2009) PNAS 106:4201

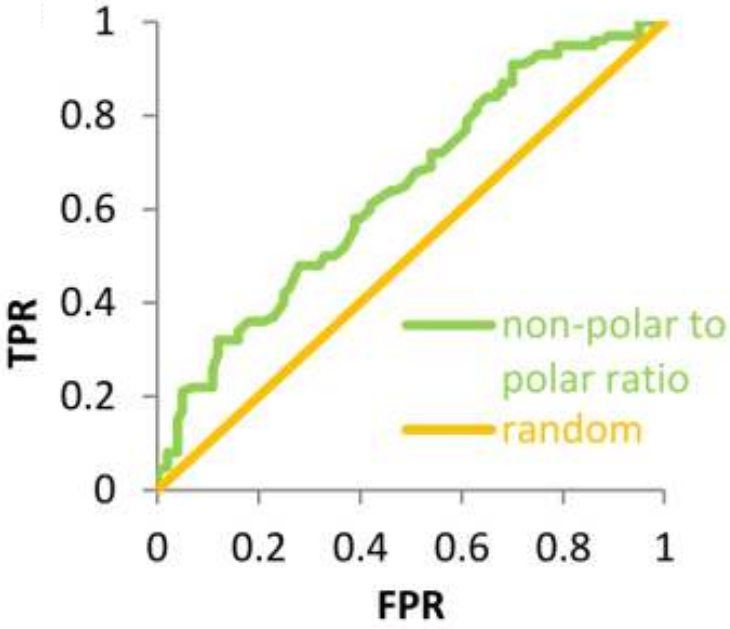
# Best separation is by 3D patches




True positive rate (TPR) vs False positive rate (FPR) ROC plots for separation of insoluble/soluble *E. coli* protein subsets, according to patches:

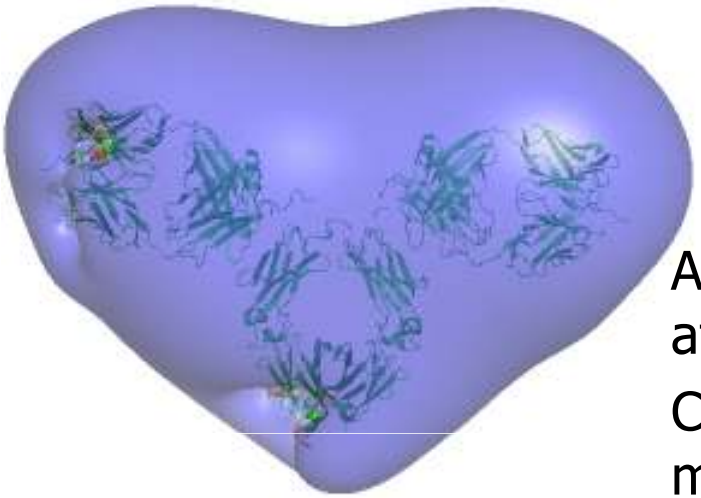
Positive patch (left) and Relative non-polarity (right)



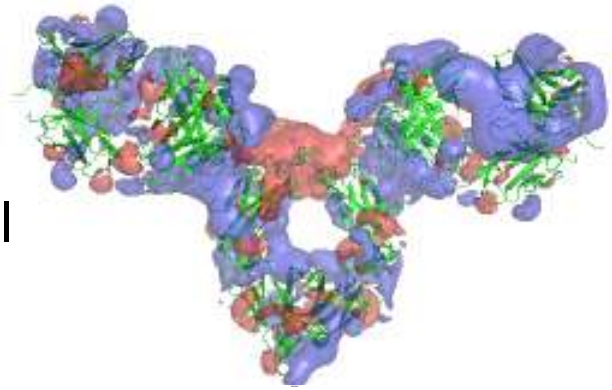

Chan et al (2013) Sci Rep 3:3333

# Future: include excipient binding



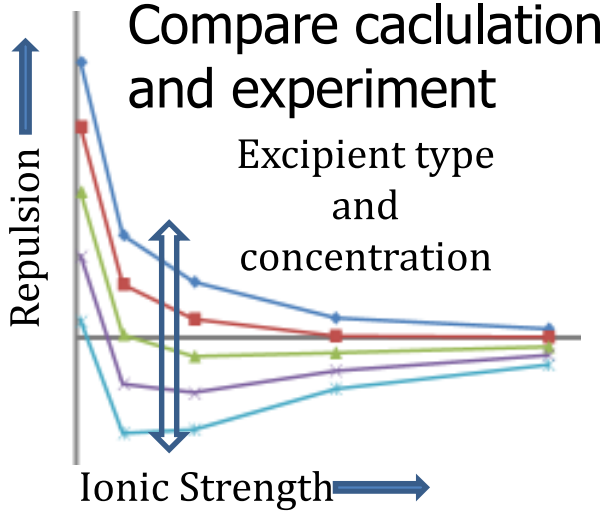
*Current Methods*

*(non-specific binding)*



Addition of 150 mM NaCl affects charge field:

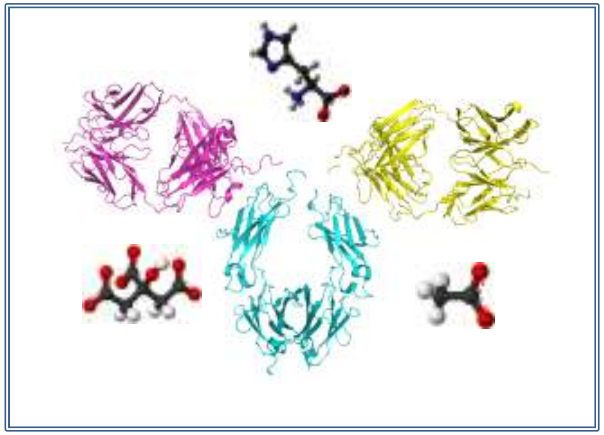
Correlate surfaces with measurement




Compare caclulation and experiment

*Proposed Methods*

*(specific binding)*



Computational screens for excipient binding to biologic e.g. Monte Carlo

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# Thank you


## BIOPHARMACEUTICALS WORK (Manchester)

| | |
|---|---|
| Spyros Charonis | PhD: Protein solubility calculation |
| Max Hebditch | PhD: Antibody structure |
| Alejandro Carballo | PhD, PDRA: Protein Engineering and solubility calculation |
| Rose Keeling | PhD (MedImmune): Antibody solubility/aggregation |
| Luke Holloway | PhD (MedImmune): Probing partial unfolded states |

Collaborating Groups (Manchester):

| | |
|---|---|
| Robin Curtis | Biophysical characterisation |
| Alan Dickson | Expression technologies |
| Jeremy Derrick | Expression, structural analysis |
| Sasha Golovanov | Structure (NMR) |
| Alain Pluen | Protein interactions |
| Jian Lu | Surface interactions |

BBSRC bioscience for the future

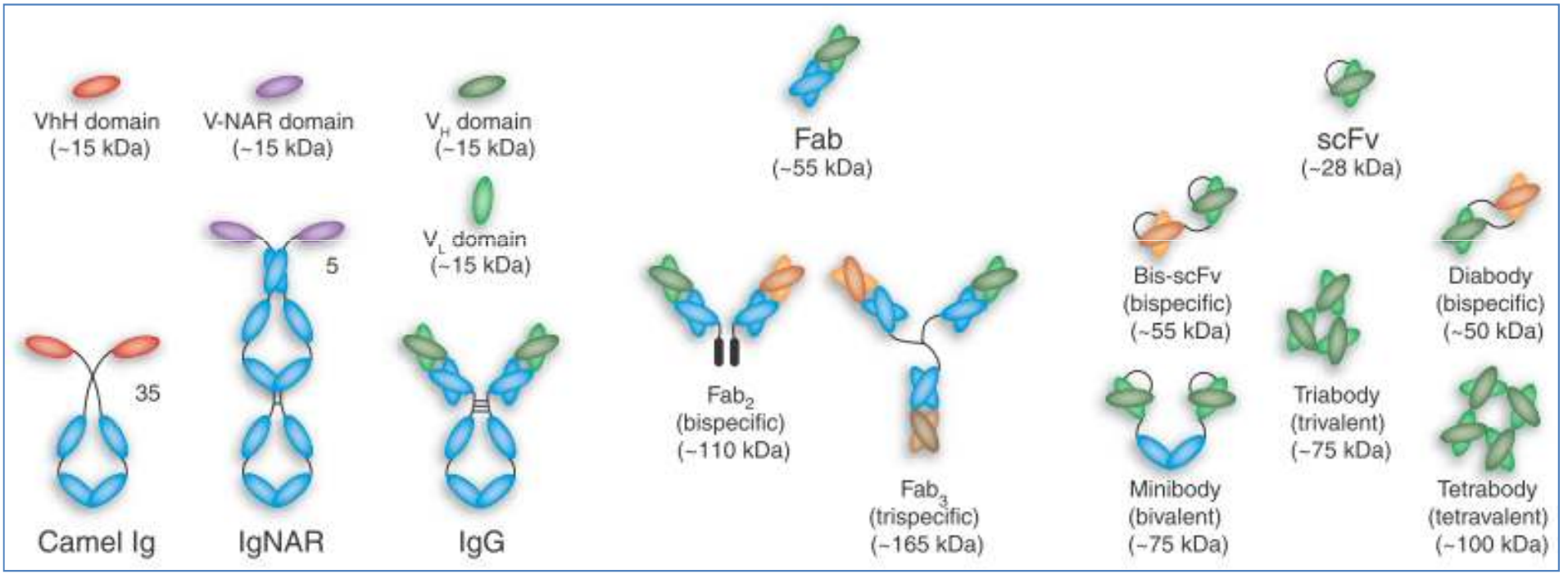
BioProNET

EPSRC

MedImmune A member of the AstraZeneca Group

FUJIFILM Diosynth biotechnologies

# A variety of protein formats




Nature Biotech 2005 23:1126