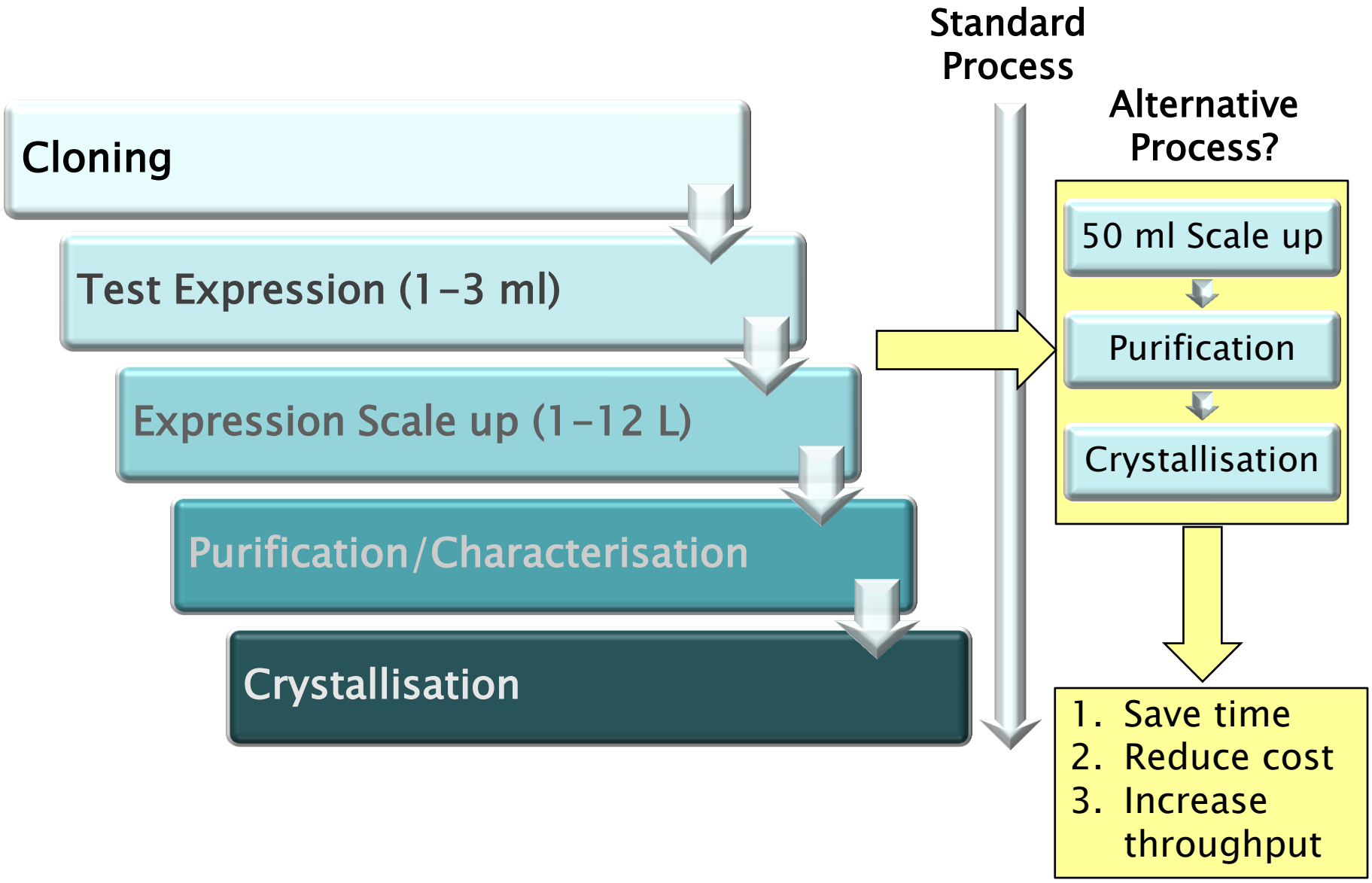
A High-Throughput Baculovirus Pipeline Enabling Structural Determination of Integral Membrane Proteins

Nicola Burgess-Brown
PI Biotechnology
SGC, Nuffield Department of Clinical Medicine
University of Oxford, United Kingdom

Miptec, 24 – 25 September 2012

SGC Toronto SGC Oxford

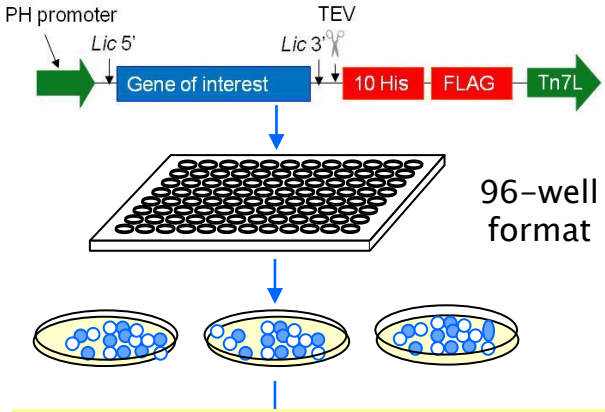
SGC Pipeline



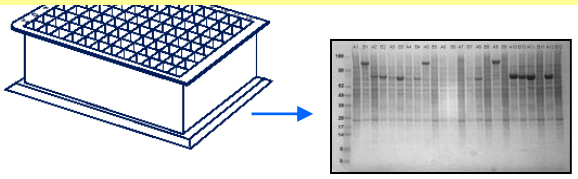
Baculovirus Expression Process

- Ligation independent cloning
- Transformation in *E. coli* DH10Bac
- Blue-white colony selection
- Bacmid DNA miniprep
- Transfection of Sf9 cells (P0 virus)
- Generation of P1 virus
- 3 ml Test purification of P1 cells
- P2 virus amplification
- Scale up to 1 L – 10 L (Sf9 or HiFive)

6-8 weeks

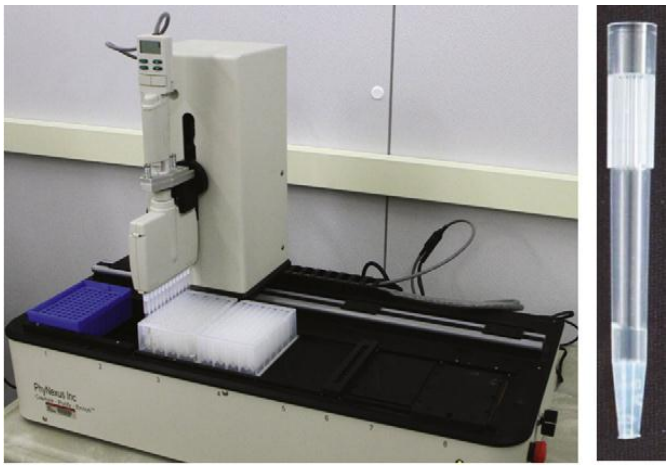


- 50-100 ml expression
- IMP pellets used for 12-detergent screening step
- Soluble protein pellets are discarded
- These could be purified for protein characterisation and/or crystallisation



METHOD DEVELOPMENT – PHYNEXUS PHYTIPS

PhyNexus Robot and Tip



Hopkins *et al* (2010) J. Struct. Biol.

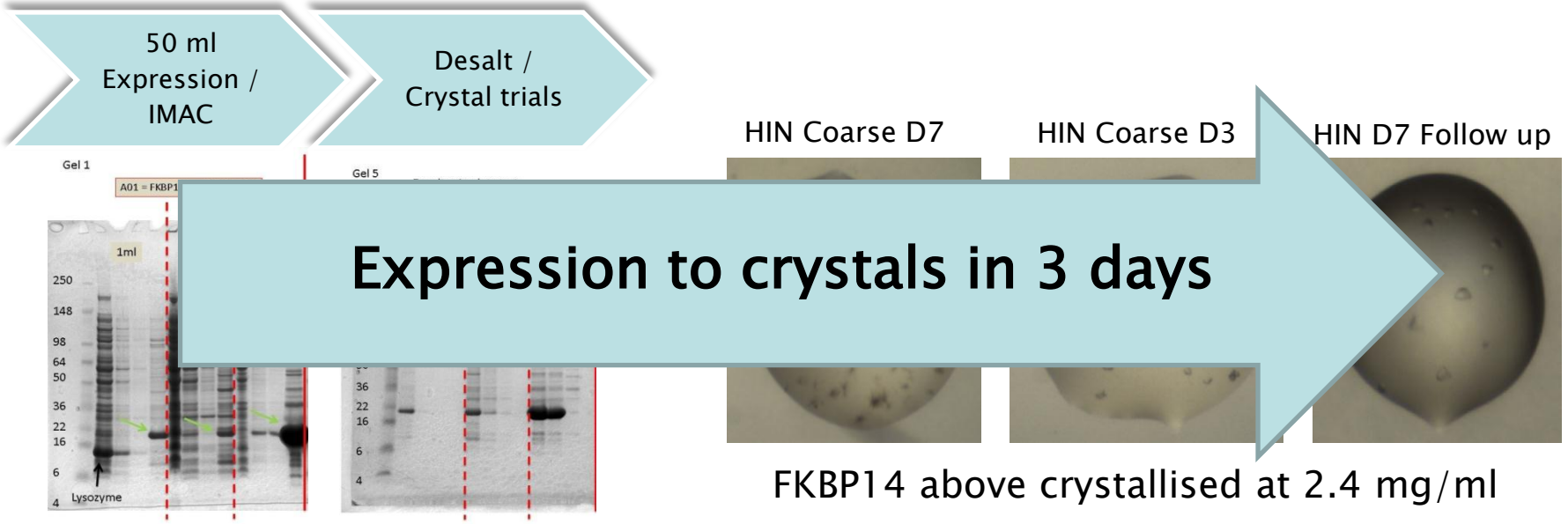
- Received on loan for 8 months
- Chromatography using automated bench top platforms (for up to 12 samples in parallel)
- Selection of column bed sizes and chemistries (affinity, ion exchange, gel filtration, normal phase and reverse phase)
- Enhanced capture and elution of target proteins (in small volumes = higher conc.)

Our aims:

- To screen multiple constructs in crystallisation trials or biophysical characterisation in parallel from small volumes of culture
- To make use of the 50-100 ml pellets resulting from baculovirus amplifications
- To automate the purification process and free up time for other work
- To increase throughput in integral membrane protein (IMP) detergent screening

TESTING PHYTIPS ON EXISTING TARGETS

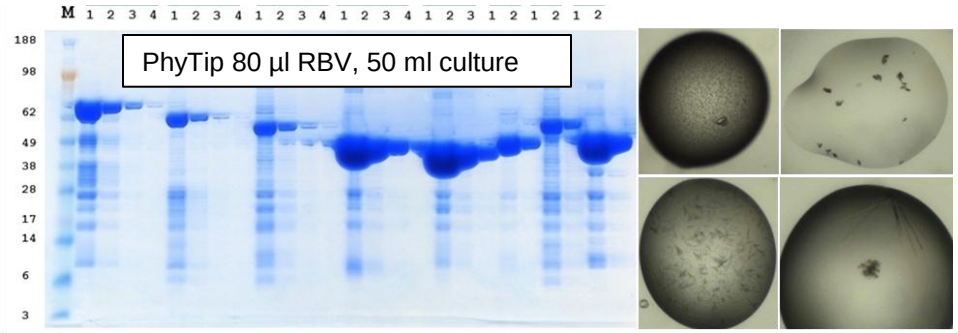
- Initial tests in *E. coli* on previously solved proteins
- Methods: Sonication (+ Benzonase), IMAC purification (eluted in 3x RBV) followed by desalting on spin columns
- 50 ml volume was the minimal quantity required for crystallisation experiments
- Low expressing proteins were not purified sufficiently
- Med/high expressing proteins gave concentrations up to 20 mg/ml



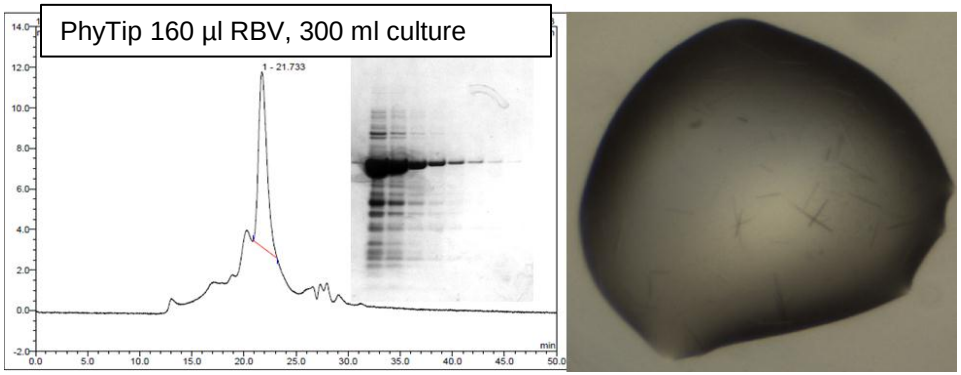
FKBP14 above crystallised at 2.4 mg/ml

George Berridge

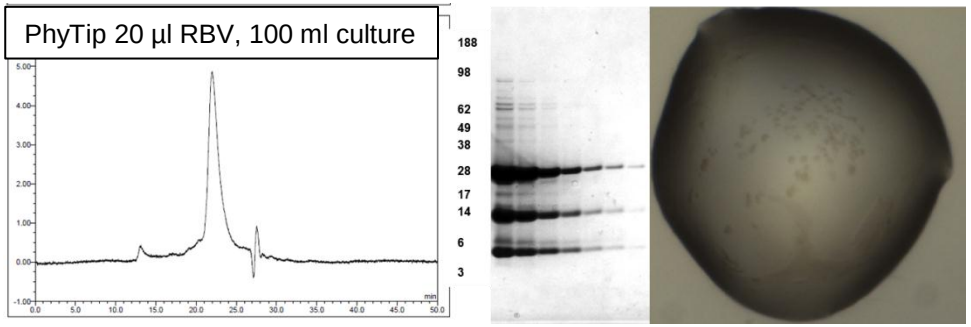
PhyTips for Novel Targets (*E. coli*)



- 8 constructs of a novel myosin protein tested in parallel
- In less than a week, 8 constructs purified from 50 ml cultures and 2 of these resulted in crystals



- 8 constructs of a novel demethylase tested in parallel
- 3 successfully purified, but only 1 construct resulted in crystal hits (conditions similar to those used for other demethylases) – diffracted to 2.6 Å at Diamond



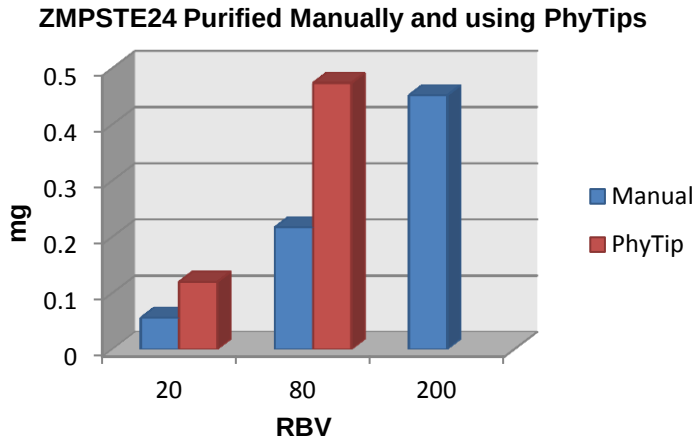
- A novel 3 protein kinase complex purified to 8.3 mg/ml gave crystal hits

George Berridge, Daniel Trindade, Catrine Johansson

PhyTips for Screening IMPs – Preliminary Data

- **Aim**: To test the use of PhyTips for automated purification of IMPs for monodispersity studies by gel filtration

- **Method**: 100–250 ml insect cell culture tested, membrane prep, 20 µl bed volume of Ni–NTA resin or Talon resin



Conclusion:

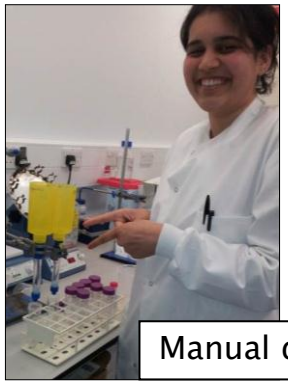
- The purity resulting from PhyTips was comparable to the manual batch method

- Total yield recovered from the PhyTips was 2-fold higher for an equivalent resin bed volume (RBV)

Conclusions so far:

- Buffer screen for 3 ABC transporters gave useful conditions and freed up researcher time

- Also suitable for larger lysate volumes (e.g. 10 ml purified in 3 h)



Manual drip columns

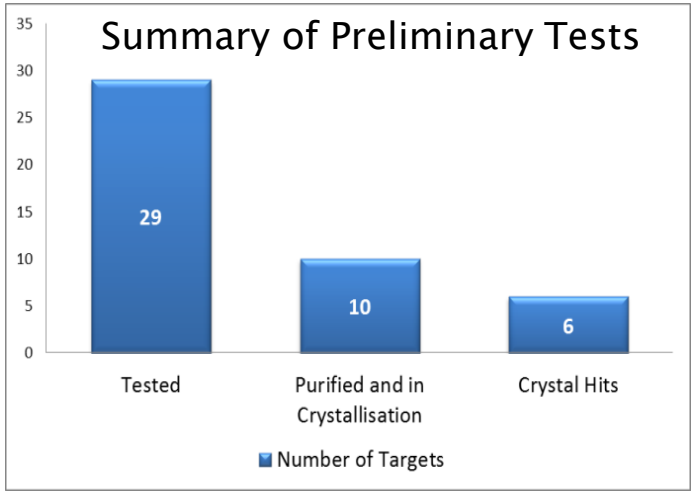
George Berridge, IMP Group Members

PhyNexus Tips – Conclusions So Far

- Potential for higher throughput expression screening to crystal trials (using robotics)
- Allows us to obtain small amounts of protein **at high concentration**, because of the small elution volume
- Suitable for med/high expressers from 50 ml bacterial expression
- Useful for screening multiple constructs of 1 target in parallel
- Potential for increasing throughput of IMP screening (buffers, detergents, ligands, etc)

Still to test/optimise:

1. Baculovirus pellets
2. On column tag cleavage
3. HTP desalting
4. Other resins/chemistries
5. Using our Hamilton robot with PhyTips



Acknowledgements

Biotechnology
Nicola Burgess-Brown
Georgina Berridge
Rod Chalk
Leela Shrestha
Solenne Goubin
Claire Strain-Damerell
Grazyna Kochan
Pravin Mahajan
Shubhashish Mukhopadhyay
Emilia Hapka
Aleksandra Szykowska

Integral Membrane Protein Group
Liz Carpenter
Ashley Pike
Andrew Quigley
Liang Dong
Yin Dong
Xiaobing Xia
Chitra Shintre
Mariana Grieben

Everyone else at Oxford SGC

Ex-SGC members
Juin-In Kim
Sanjan Das
Beatrice Li
Jing Yang
Ivan Alfano
James Bray
Binesh Shrestha
Alastair Barr

Chas Bountra (CSO Oxford)
Aled Edwards (CEO)
Cheryl Arrowsmith (CSO Toronto)
Opher Gileadi (Genome Integrity & previous Biotech PI)
Stefan Knapp (Kinases, Epigenetics)
Alex Bullock(Growth Factor Signalling)
Paul Brennan (Medicinal Chemistry)
Frank von Delft (Crystallography)
Brian Marsden (Research Informatics)
Wen Hwa Lee (Scientific Coordinator
Susanne Müller-Knapp (Epigenetics)
Wyatt Yue (Organelle Biogenesis)
Scott Thomson (Administrator)
Udo Oppermann (Metabolic Enzymes, Epigenetics)
Johan Weigelt (former CSO Stockholm)

Diamond Light Source Ltd

Funding Partners

The Canadian Institutes for Health Research, the Canada Foundation for Innovation, Genome Canada, GlaxoSmithKline, Lilly Canada, the Novartis Research Foundation, Pfizer, Takeda, the Ontario Ministry of Economic Development and Innovation, and the Wellcome Trust.

www.thesgc.org

CONTACT: nicola.burgess-brown@sgc.ox.ac.uk

ABOUT THE SGC

The SGC is an international public-private partnership (UK charity number 1097737) that aims to carry out basic science of relevance to drug discovery, placing all information, reagents and know-how into the public domain without restriction.