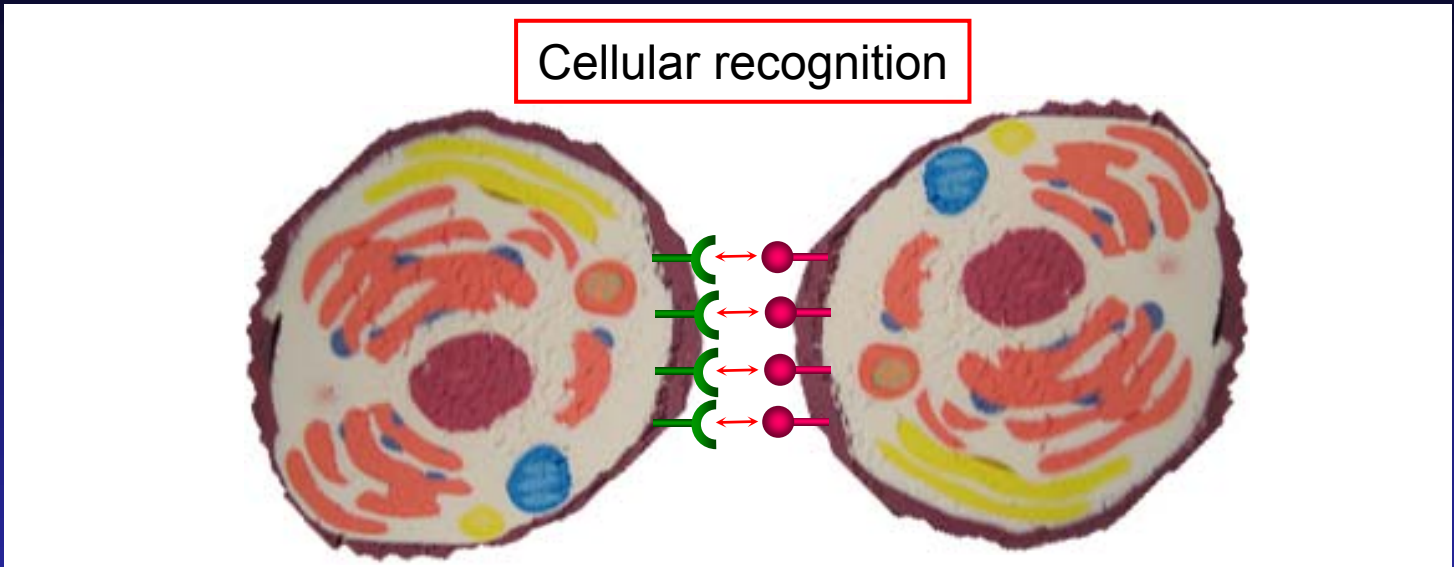
Large-scale detection of low affinity extracellular protein interactions

Gavin J. Wright

Cell Surface Signalling Laboratory
Wellcome Trust Sanger Institute
Cambridge UK

gw2@sanger.ac.uk

Extracellular protein interactions in cellular recognition



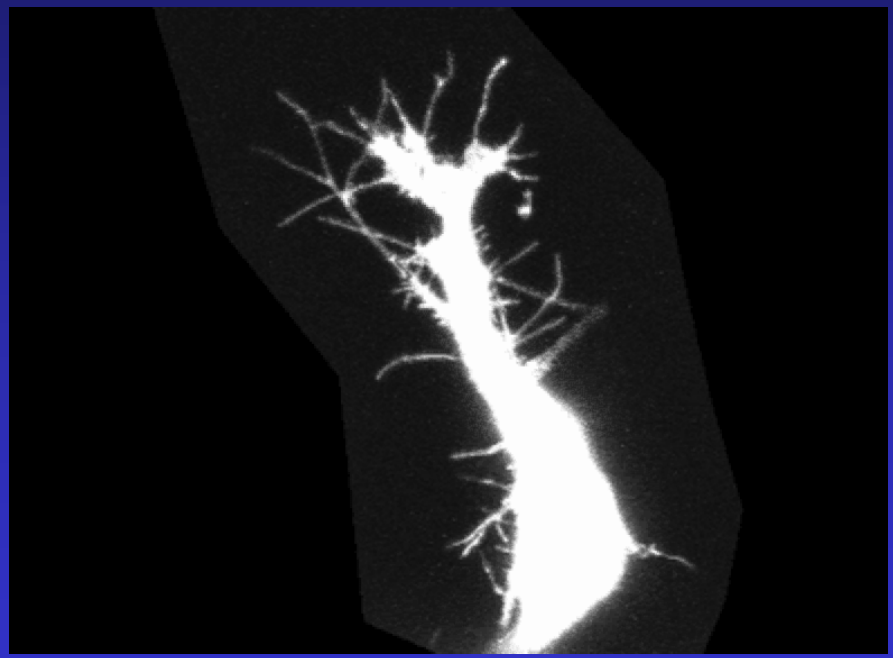
- Specific cellular recognition required in normal development and tissue homeostasis, host-pathogen interactions etc…

- Receptor interactions responsible for cellular recognition processes, including those underlying many diseases are often unknown

- Extracellular protein interactions between membrane-embedded receptor proteins are technically difficult to identify
    - Posttranslational modifications
    - Very weak interaction strengths

Transient cell surface interactions allow independent cellular motility

- Interactions between membrane-embedded receptor proteins can be very transient: (μM range, $t_{1/2}$ < 1 s)



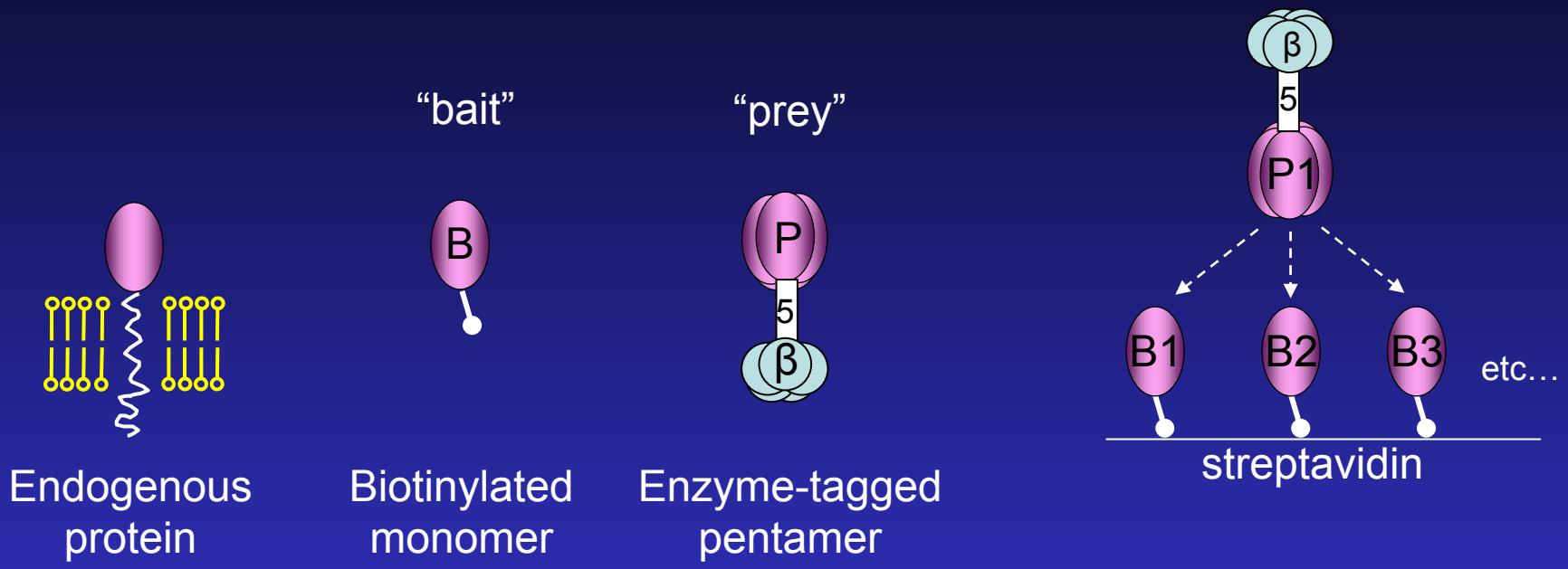
1 frame every 30 s

20 minutes “real time”

Jon Leslie, CRUK

- Aim to develop a systematic high throughput assay to detect low affinity extracellular protein interactions

Detecting transient extracellular protein interactions

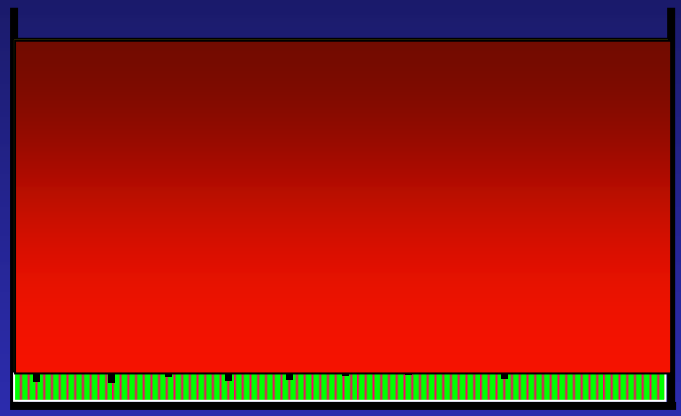


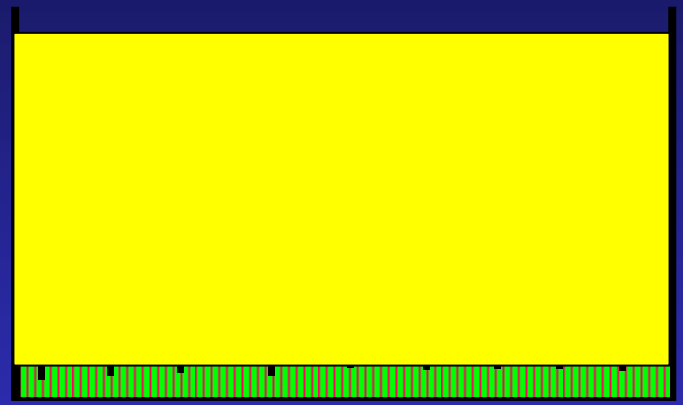
- All proteins expressed by transient transfection in mammalian cells (to add PTMs) and levels normalised

- Prey pentamerised by peptide from cartilage oligomeric matrix protein (COMP) increases the avidity of the interaction

Schematic description of AVEXIS

AVidity-based EXtracellular Interaction Screen

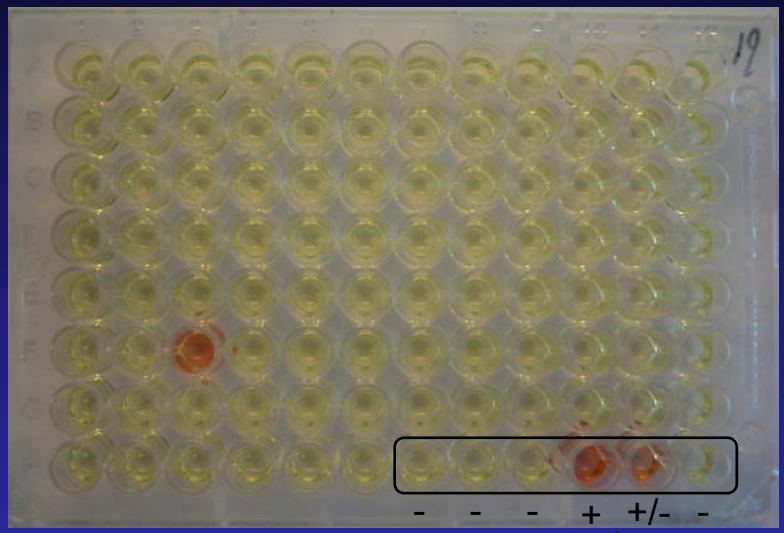




1) Streptavidin
2) Capture monomeric biotinylated bait
3) Add normalised enzyme-tagged multimeric prey
4) Wash
5) Add enzyme substrate

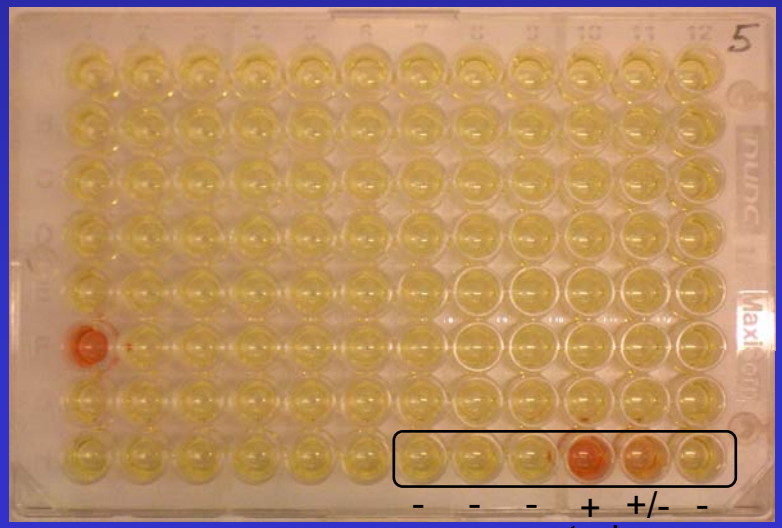
AVEXIS screening positives

Prey: Slit-like2



controls:

Prey: Islr2



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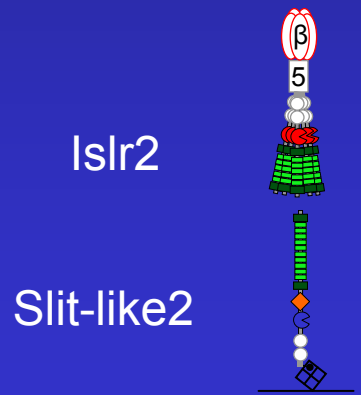


AVEXIS technical parameters

- What is the affinity threshold for detection?
  - Surface plasmon resonance ($t_{1/2} \leq 0.1$ s)
- False positive and negative rates were bench-marked against well known published positive and negative interactions from the human CD2 family (Gen. Res. 18 622)
- How frequently were interactions detected?
  - 0.6% of interactions tested are positive (1 every 166)
  - 71% are detectable in both orientations
- How much protein do you need?
  - 500fmol / well (60kDa = 30ng)
  - ~3µg for 100 tests

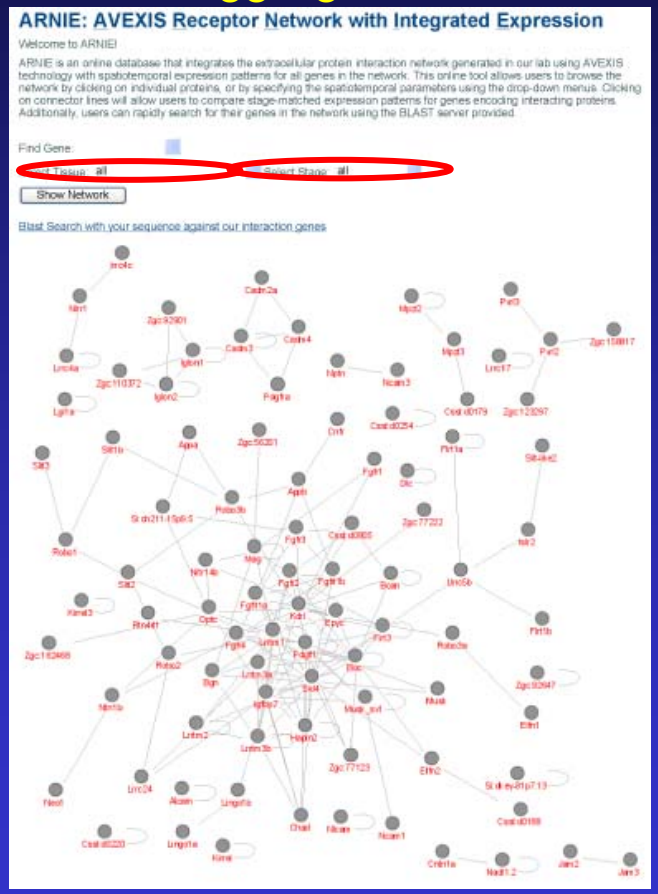
Applying AVEXIS to problems of cellular recognition

- We have initially applied this technique to understand cellular recognition processes during early vertebrate development
- Systematically screened for interactions within a library of 252 zebrafish cell surface receptor and secreted proteins (> 31,000 unique potential interactions)
- This model vertebrate enables rapid distribution and loss of function analysis

ARNIE: AVEXIS Receptor Network with Integrated Expression

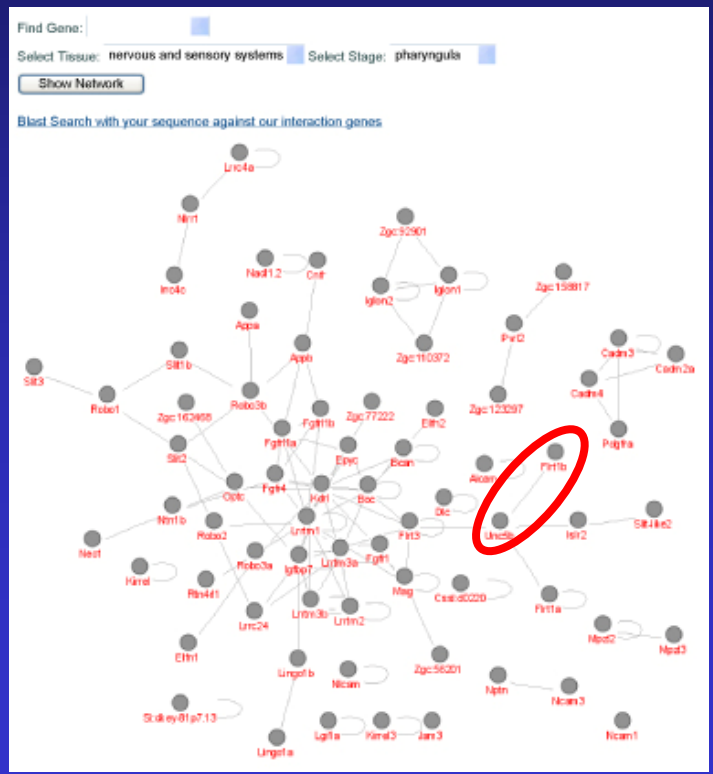
- Spatiotemporal expression patterns of genes within the protein library by wholemount in situ hybridisation

Whole “aggregate” network

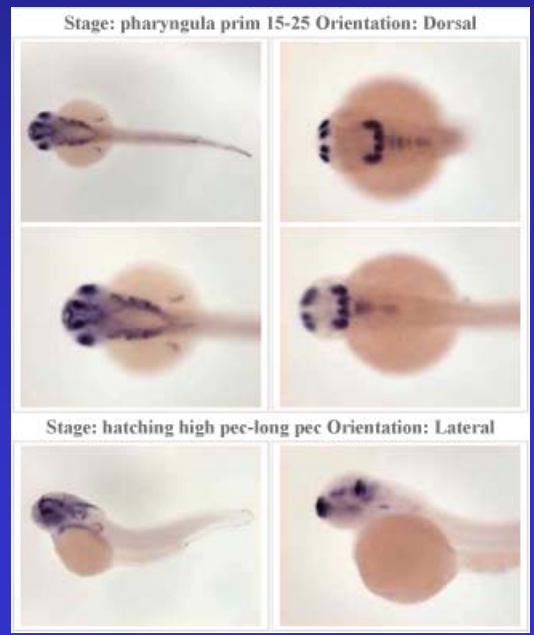


118 interactions
between 92 proteins

Tissue & stage-specific network
5 different stages



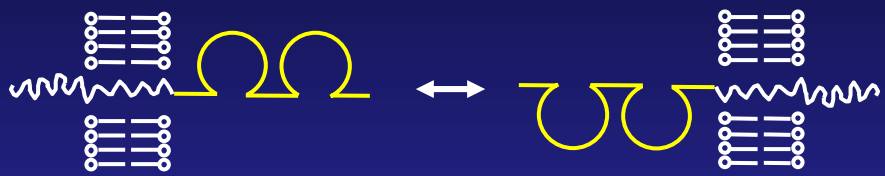
Detailed biological information for each interaction

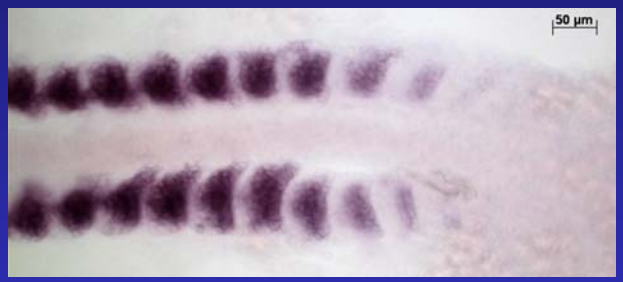


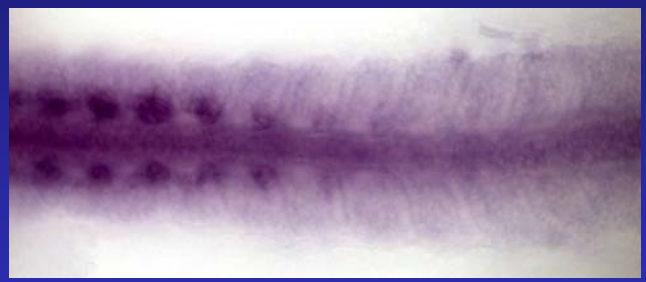
http://www.sanger.ac.uk/arnie

Understanding the function of individual interactions

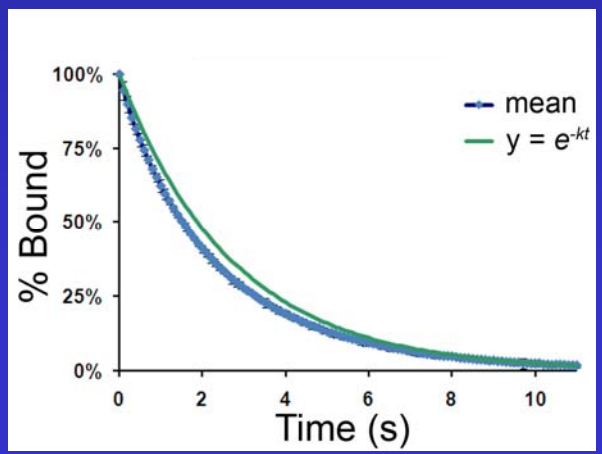
- Hypotheses regarding the function of individual interactions can be made based on their expression patterns







in situ hybridisation 14 hrs pf

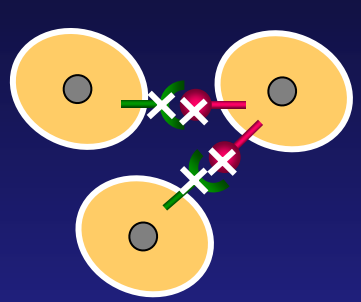


$k_d = 0.37\ s^{-1}$

$t_{1/2} = 1.9\ s$

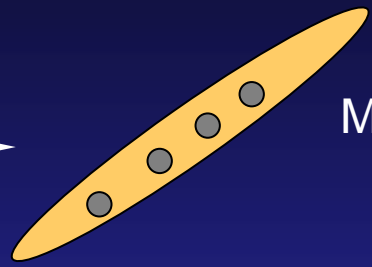
$K_D \sim 2.4\ \mu M$

A receptor-ligand pair necessary for myoblast fusion

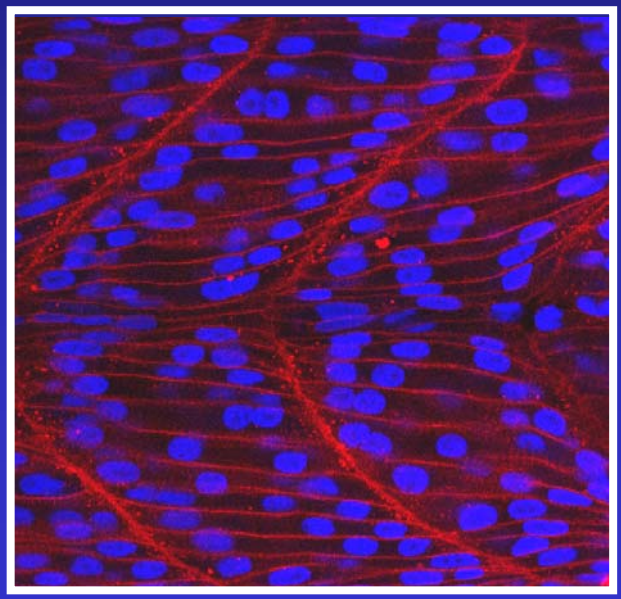


Cellular recognition

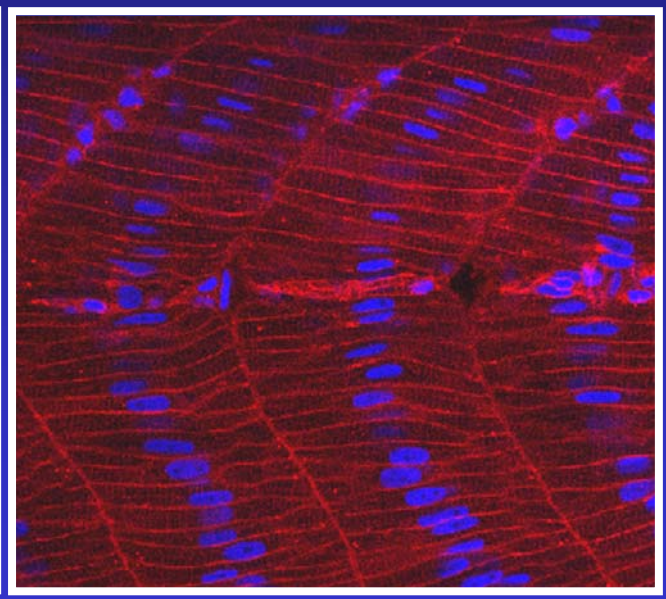
Fusion



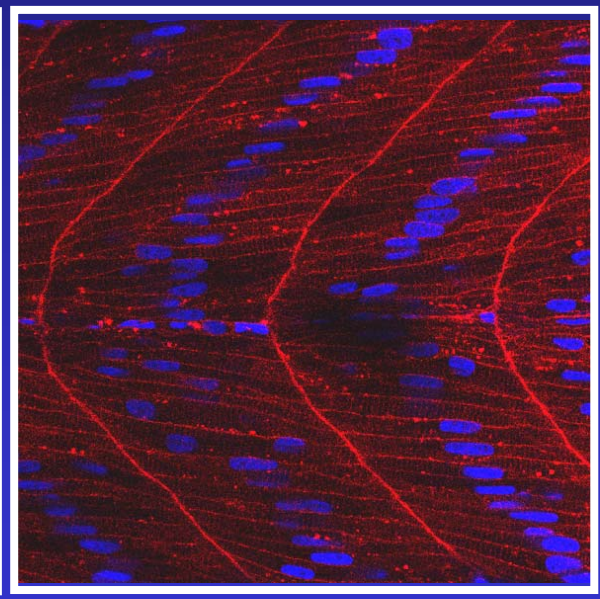
Multinuclear skeletal muscle fibre



Wild type



Ligand mutant



Receptor mutant

Summary

- Development and application of a scalable assay to detect binary, low affinity extracellular protein interactions
- Construction of a large extracellular protein interaction network of >100 interactions
- Zebrafish model enables rapid wholemount gene expression studies enabling resolution of static, aggregate networks into stage and tissue-specific networks
- Functional validation of interactions using mutant zebrafish

Acknowledgements

Cell Surface Signalling Laboratory

Zebrafish screening
- Christian Söllner
- Steve Martin
- Mark Bushell
- Gareth Powell

Malaria
- Cécile Crosnier
- Madushi Wanaguru
- Josefin Bartholdson

Other cellular recognition projects
- Jason Kerr
- Yi Sun

Collaborators

Christine et Bernard Thisse

(Charlottesville. USA)

Yod Charoensawan, Boris Adryan and Sarah Teichmann

(LMB, Cambridge)

Sanger Institute zebrafish mutation resource

(Derek Stemple)

Funding sources: Wellcome Trust, NIH, HFSP