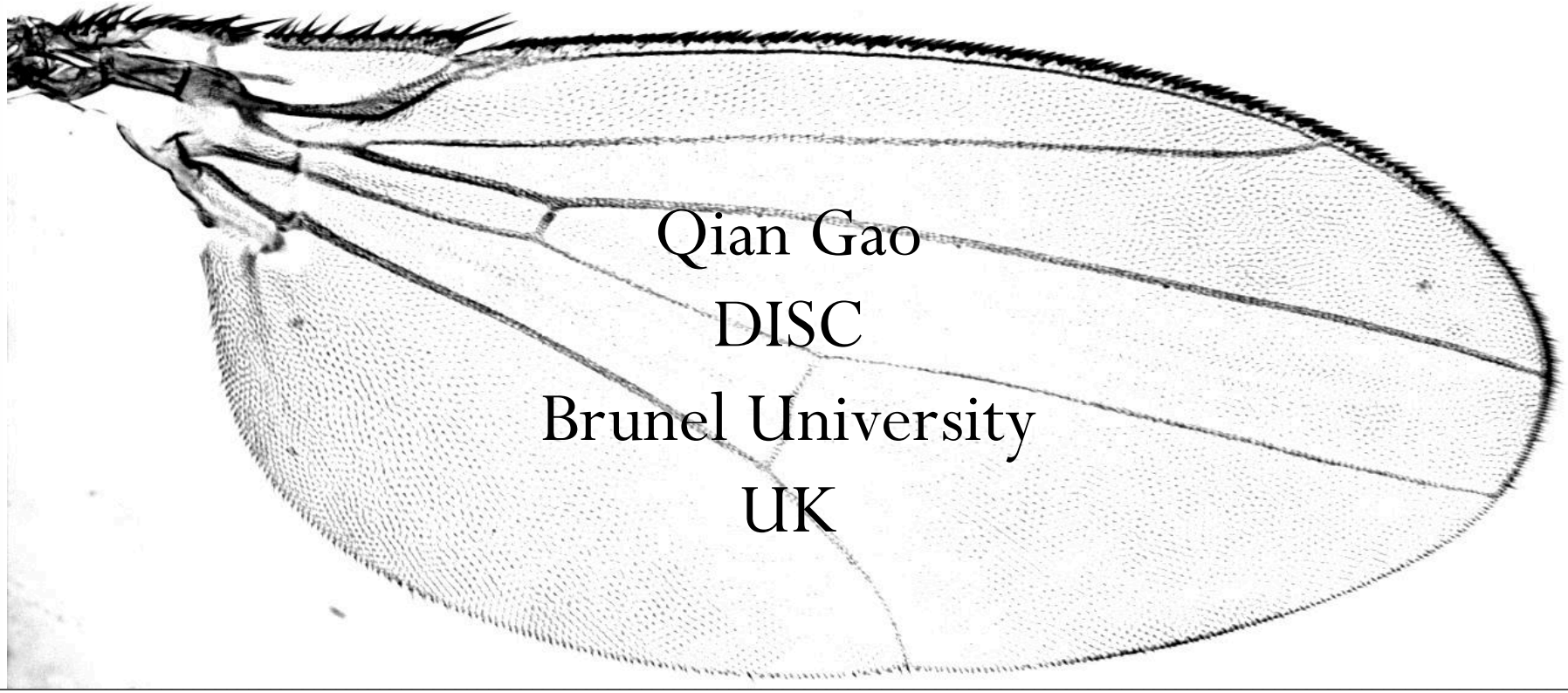
Investigating Planar Cell Polarity by Modelling



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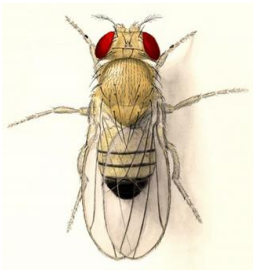
Overview



1. Planar cell polarity
2. Modelling approach
3. Some progress in modelling

Planar Cell Polarity Signalling

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Department of Biosciences
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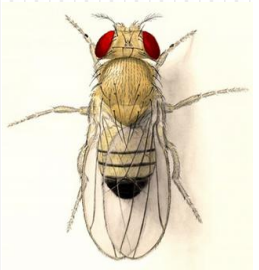
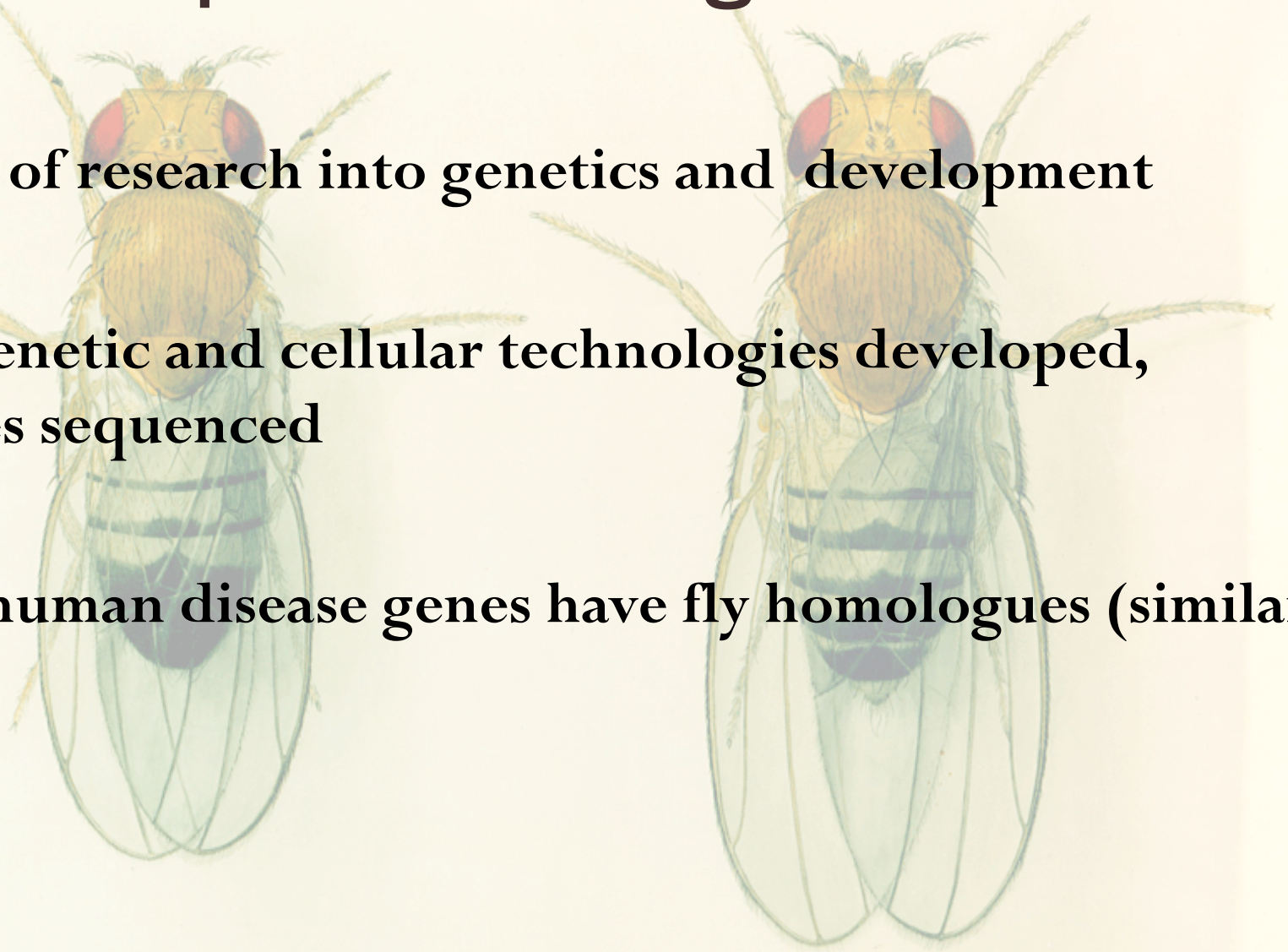


Aims and Objectives

- The role of Drosophila Melanogaster as a system model
- To outline Planar Cell Polarity (PCP)

Drosophila melanogaster



- History of research into genetics and development
- Many genetic and cellular technologies developed, genomes sequenced
- 61% of human disease genes have fly homologues (similar)

The procedures

What is great about flies:

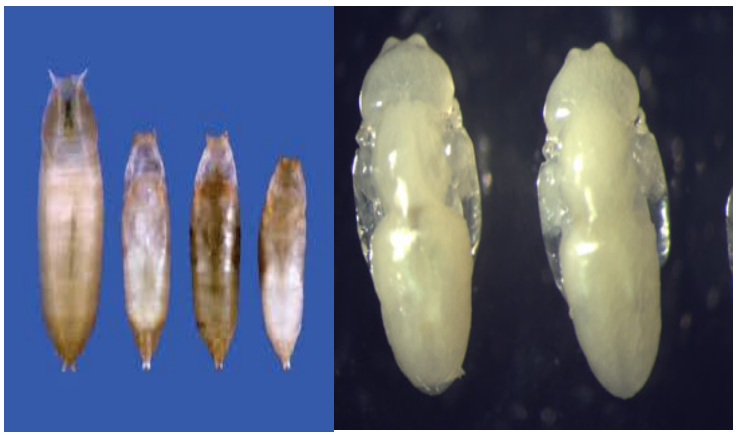
- **Short life cycle** - 10 days in a single vial
- **Cheap** - small, simple food source
- '**Small' genome** (100 megabases)
  - Around 14,000 genes
  - Only 3 major chromosomes
- Good vertebrate model
  - 75% human genes found in *Drosophila*

Disadvantages

- Cannot be frozen/stored **- require continuous culture**
- **No targeted mutagenesis** - e.g. homologous recombination
- **Able to fly**!

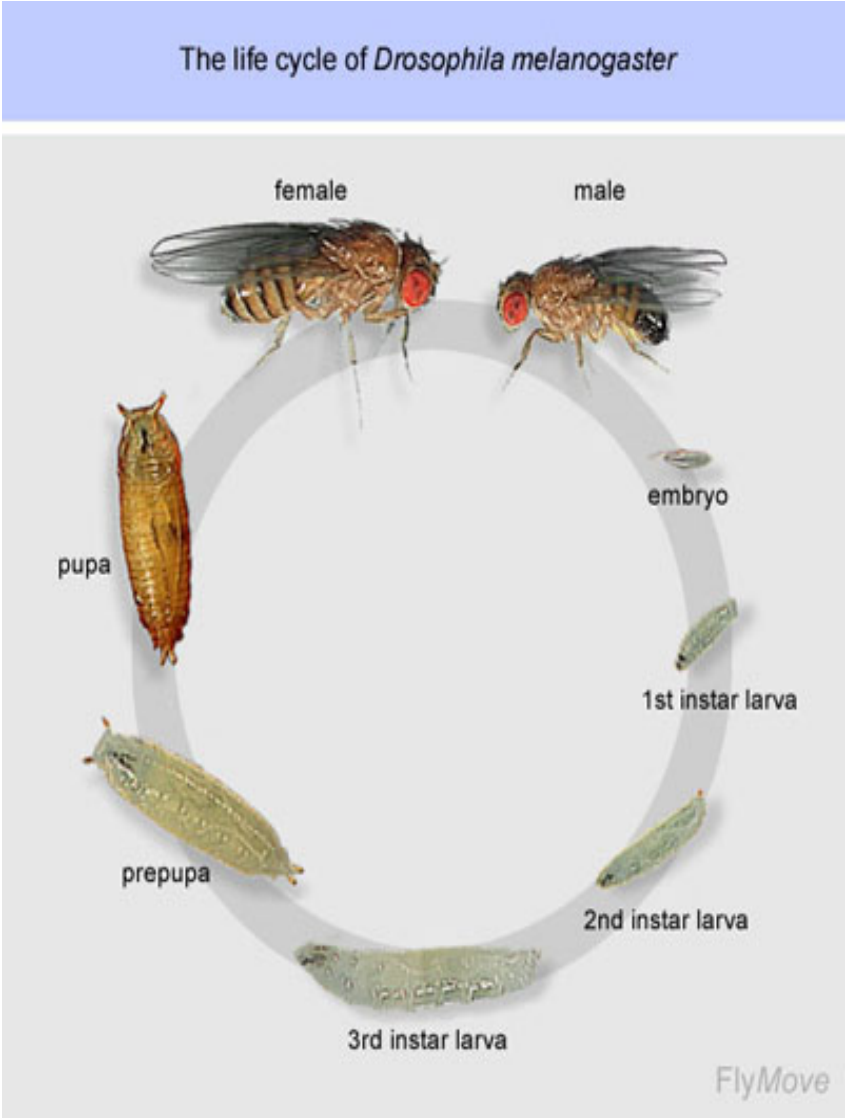
Dissecting fly wings to analyse wing hair patterns: knock-out and knock-in genes

Immuno-fluorescence imaging: imaging of a developing wing



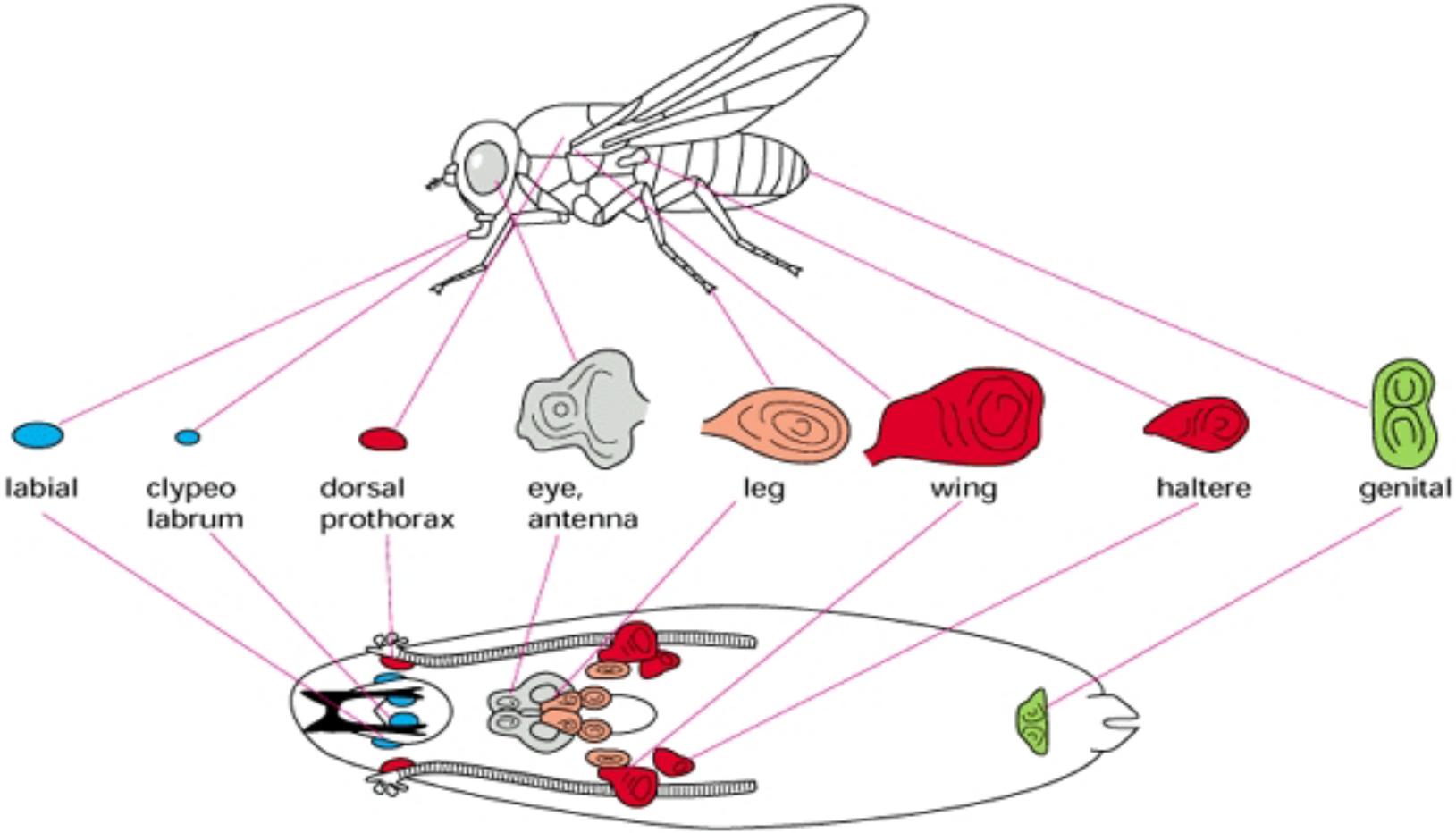
Drosophila melanogaster: life cycle

- **Embryo stage**: 24 hours
- **Larva stage**: 4 days (*Pupariation)*
- **Pupa stage**: 4 days
- **Adult stage**
- Life cycle: ~9-10 days



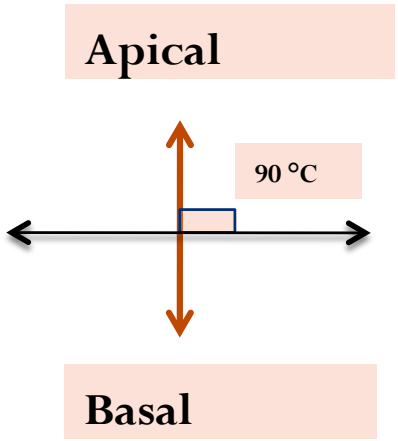
Drosophila Development

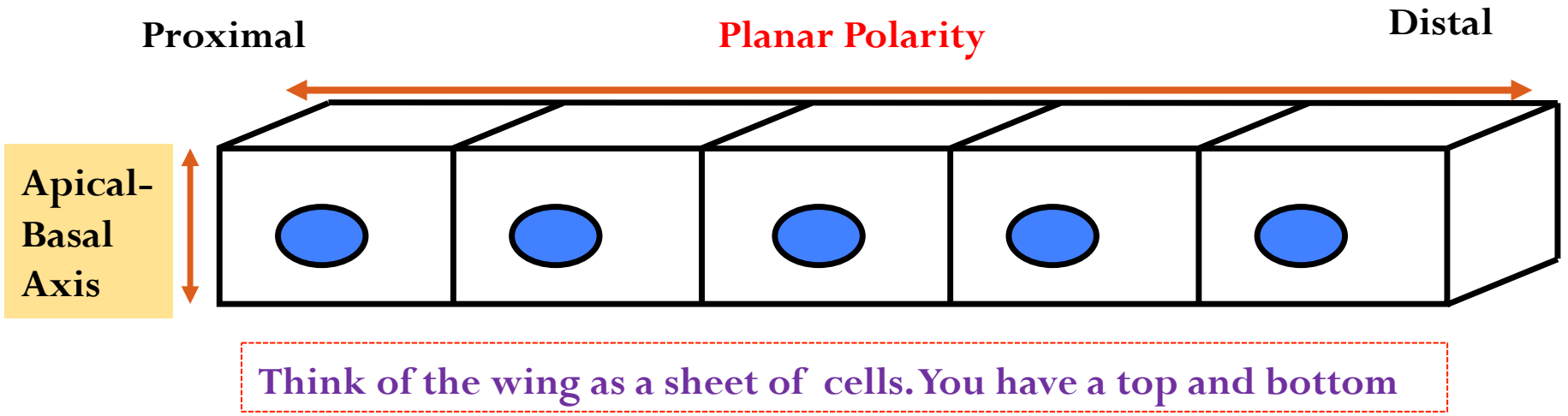
- Patterning: groups of cell aggregate and later become organs



Planar Cell Polarity

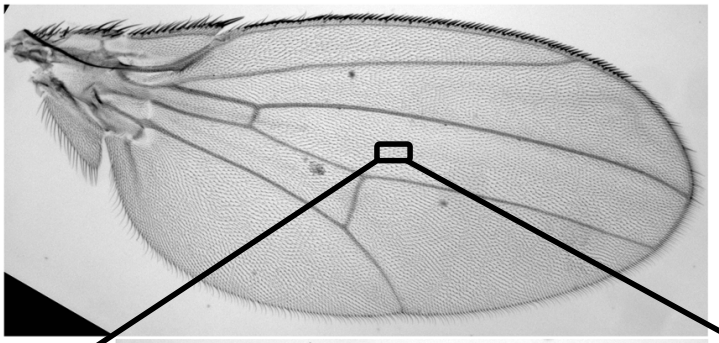
- **Mechanism through which a number of tissue types determine the polarity of their cells perpendicular to their cellular apical-basal axis.**
  - in mammals: ear (sensory hair cell), eye (Equipotent R3/R4 cells) epithelia

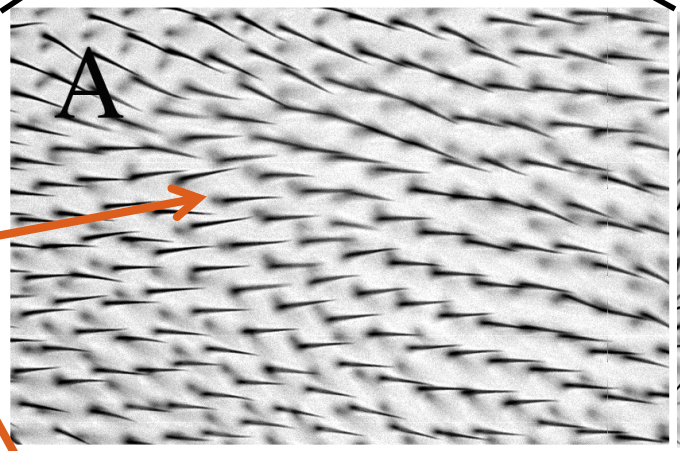


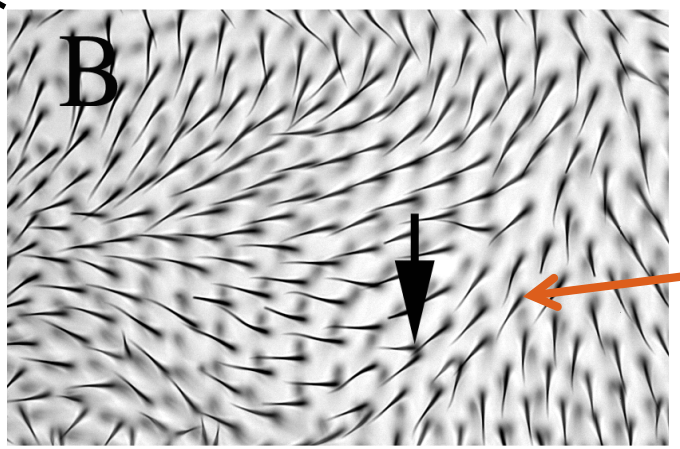


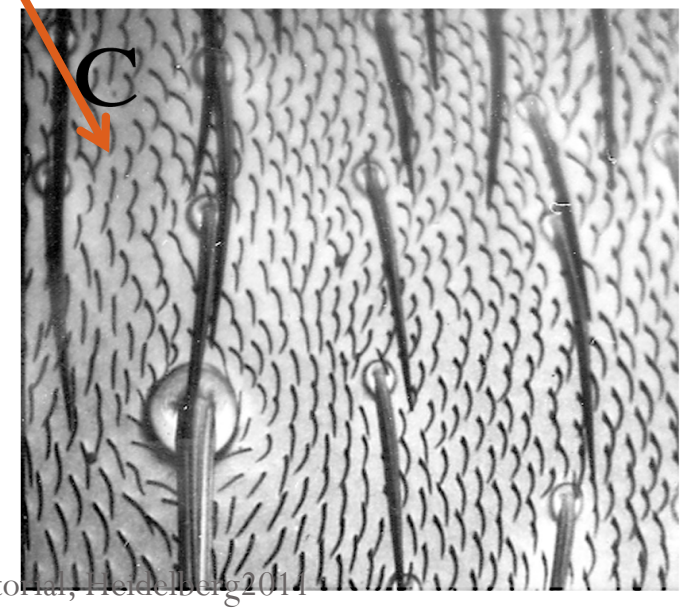
Think of the wing as a sheet of cells. You have a top and bottom

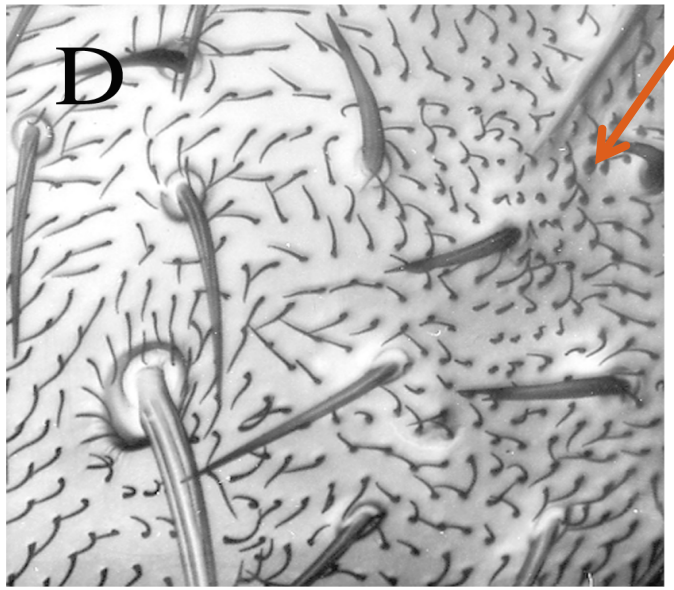
PCP: Phenotypes









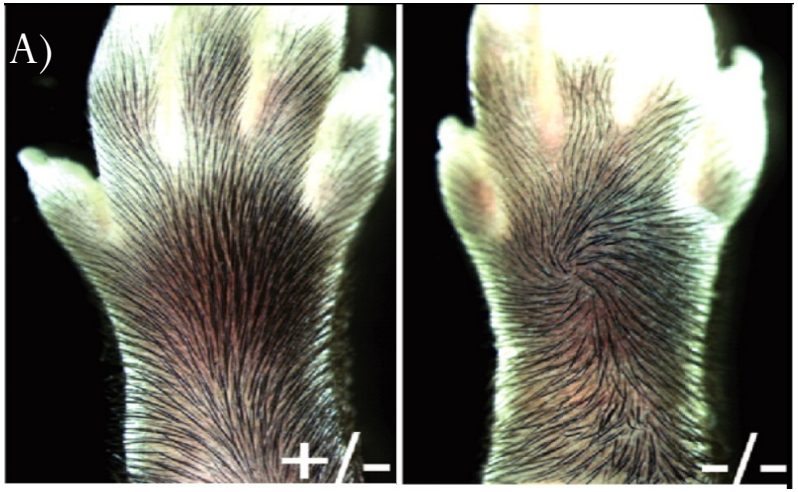


Wild type or 'normal' phenotype

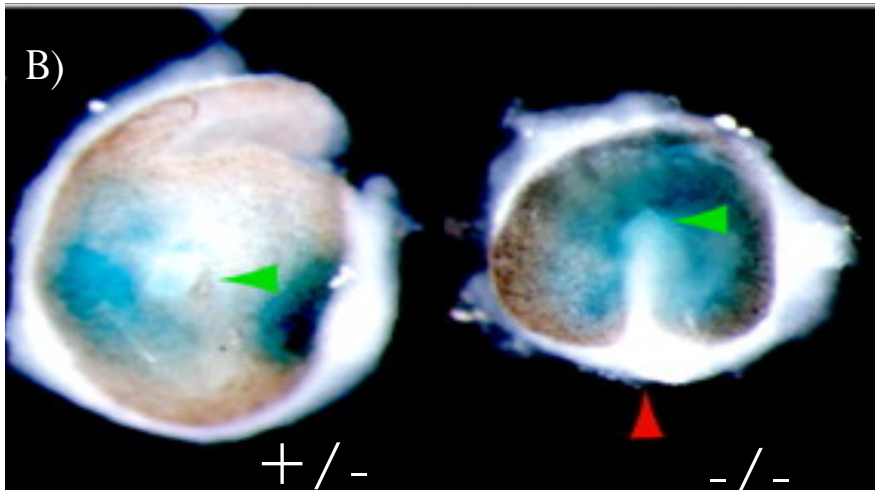
Mutant phenotype

PCP: Vertebrate Phenotypes

Fz6 Knockout: Hair Polarity

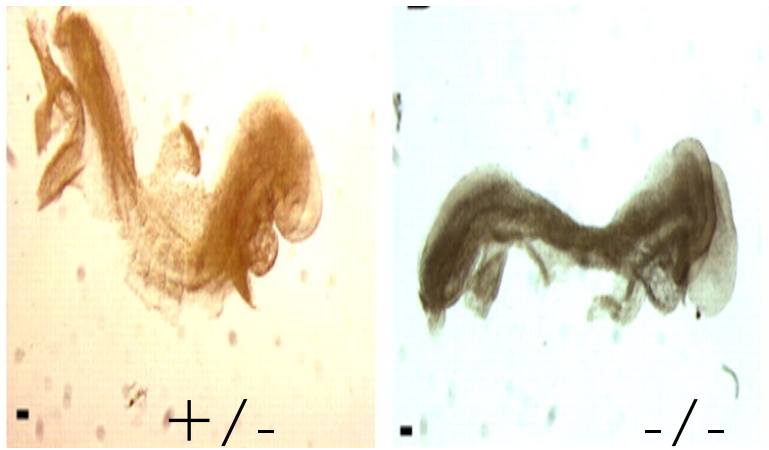


Fz5 Knockout: Coloboma

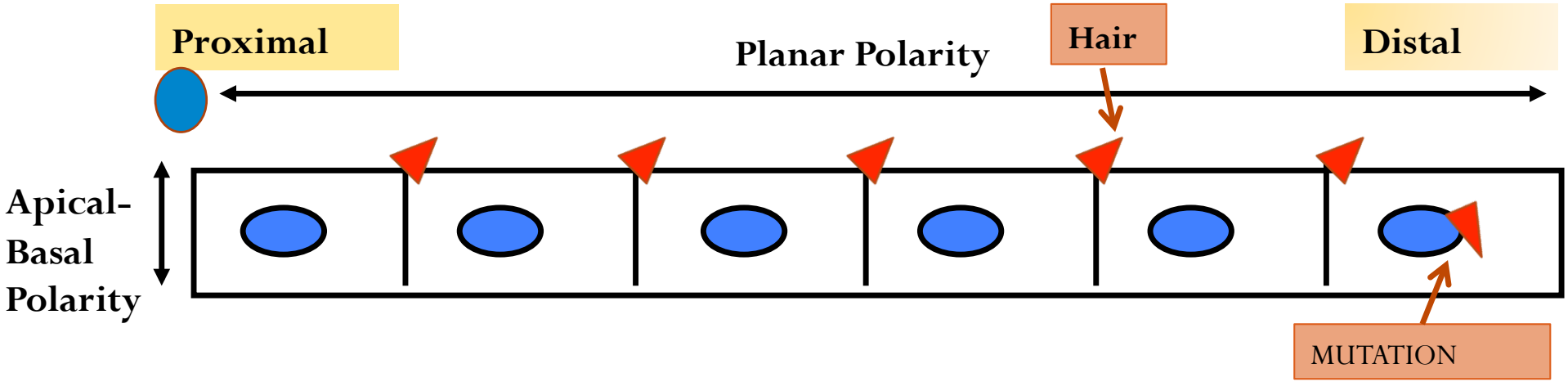


Dact1 Knockout: Urogenital Defects

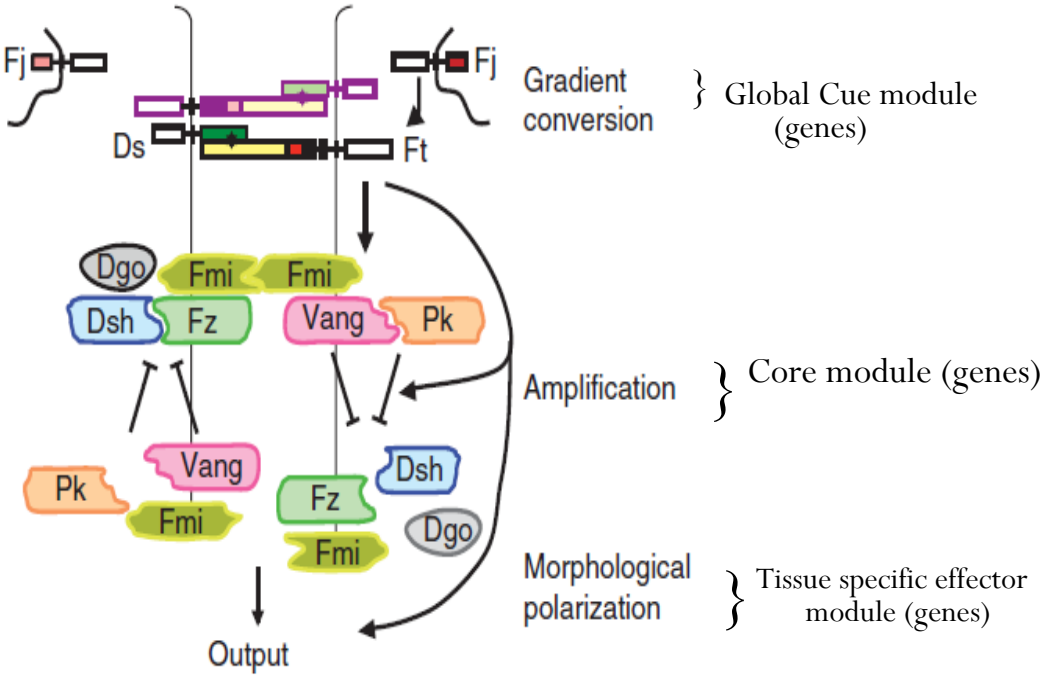
Vangl2 Knockout: Impairs Cranial Neurulation



Planar Cell Polarity: in the fly wing

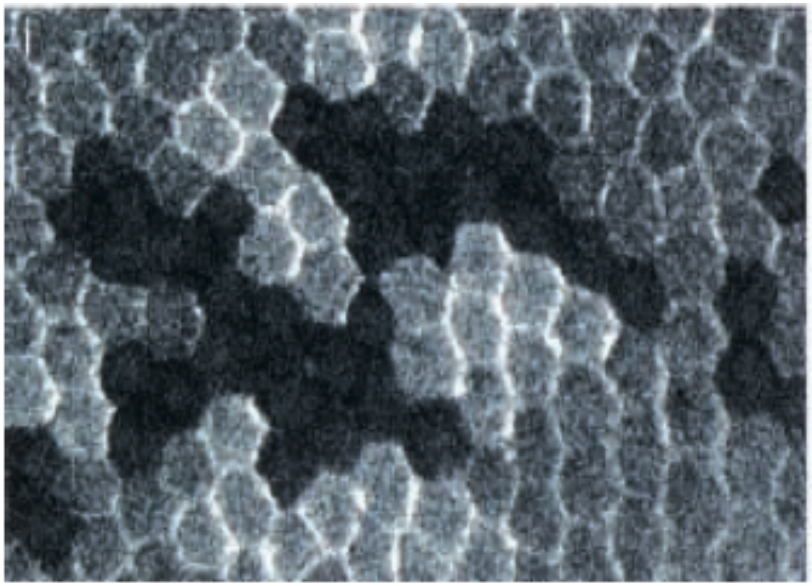


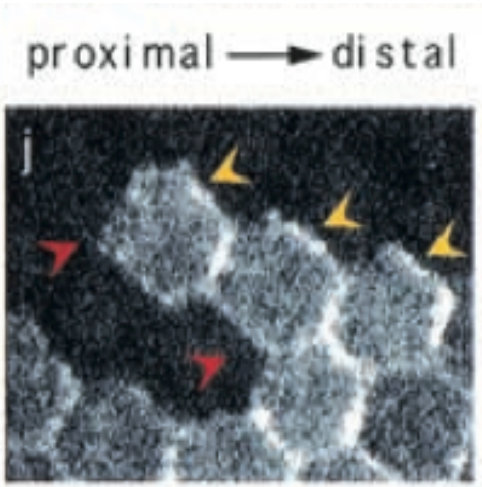
- Physical interactions/ signalling relationships

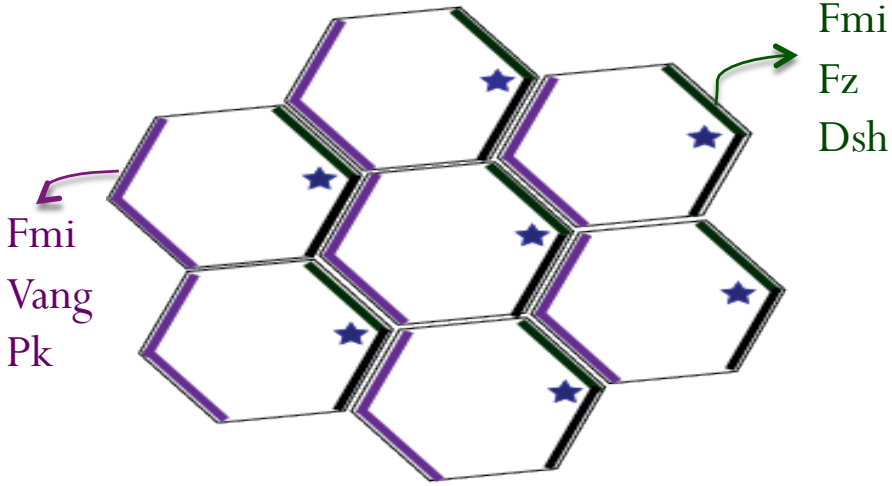


Core : Asymmetric Protein Localisation

GFP- GREEN FLUORESENCE PROTEIN

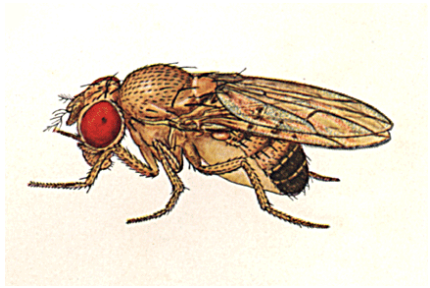


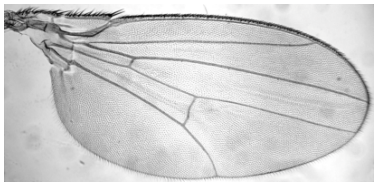


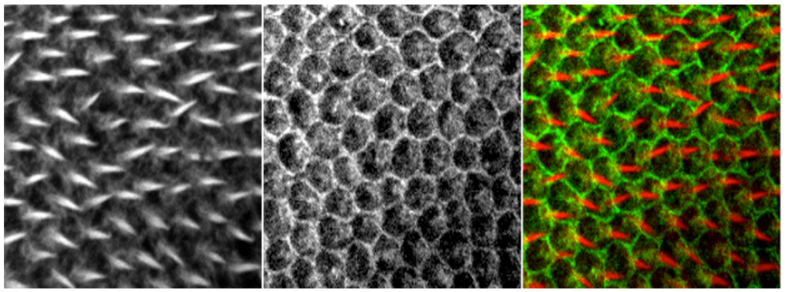


Asymmetric distribution

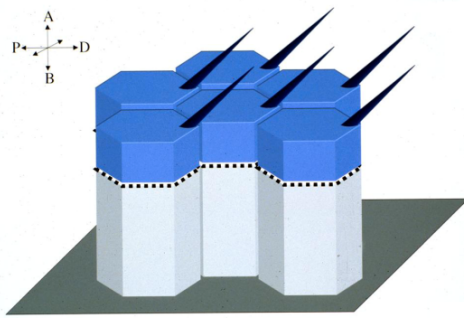
Multiscale from signalling to organs

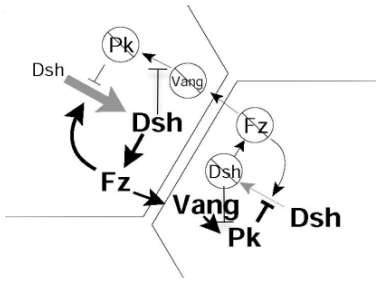




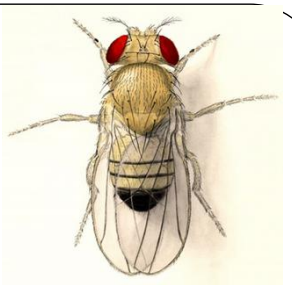


- Drosophila wing hairs point distally virtually error free.
- Hexagonally packed, planar (30,000)
- Planar cell polarity: the polarization of a field of cells within the plane of a cell sheet.
- Human pathology:
  - ✓Cochlear hair cells
  - ✓Spina bifida
  - ✓Oncogenic Wnt pathway

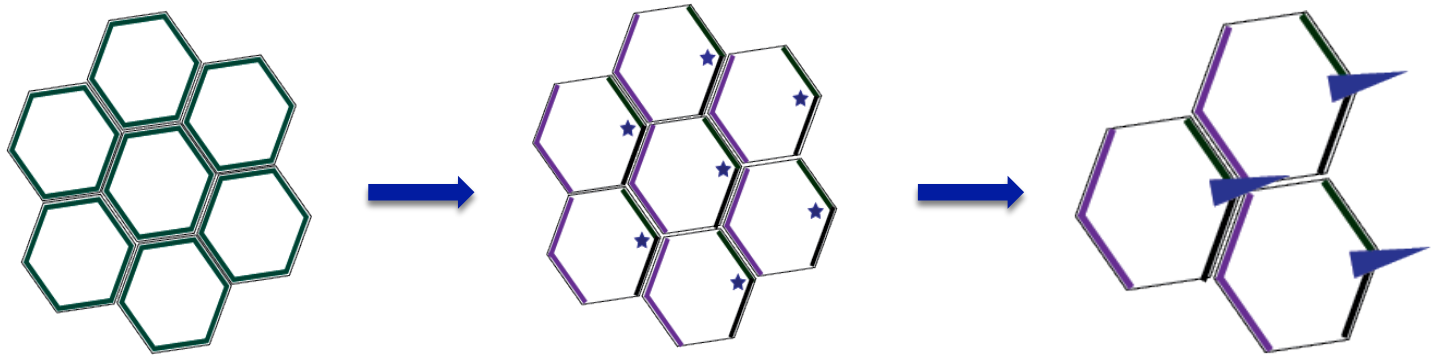




Biological Model



- **PCP proteins** involved in the intercellular signalling: Frizzled (Fz), Dishevelled (Dsh), Prickle (Pk), Flamingo (Fmi) and Van-Gogh (Vang) .
- **A core machinery** mediates a competition between the proximal and distal proteins: Frizzled (Fz), Dishevelled (Dsh), Prickle (Pk) and Van-Gogh (Vang). Flamingo (Fmi) localises at both distal and proximal edges.
- **Feedback loops**: cells tent to align cell polarity as asymmetric distribution.



Symmetric distribution

Asymmetric distribution

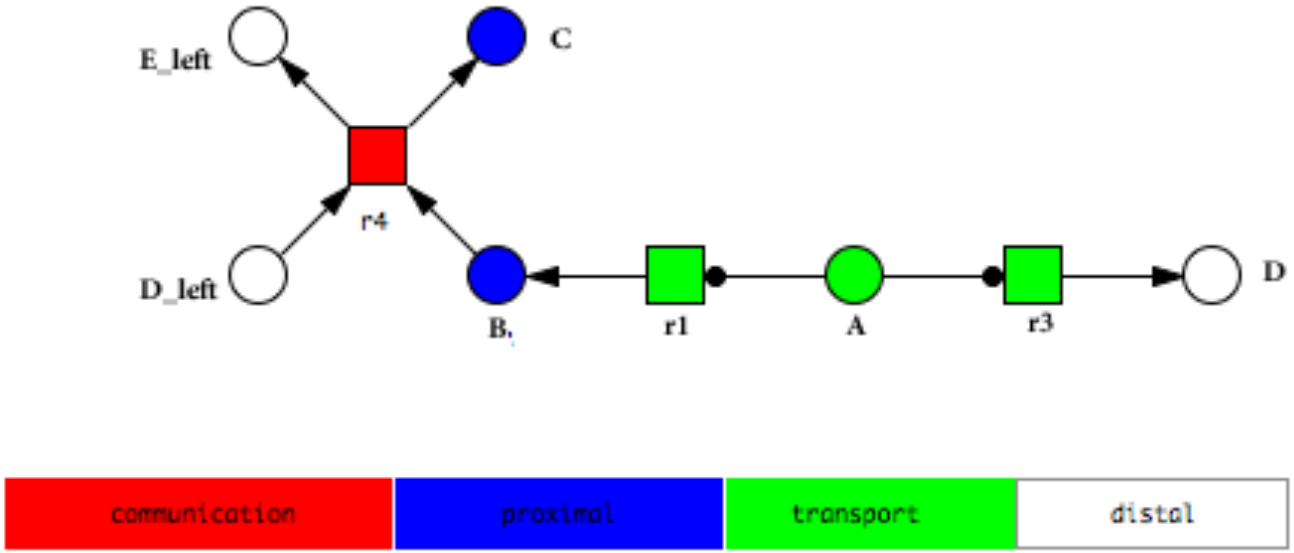
Prehair formation

Coloured Petri Nets (CPN)

- Tokens distinguished via their colours.
- Each place gets a colour set, specifying the kind of tokens which can reside on the place.
- Each transition gets a guard, specifying which coloured tokens are required for firing.
- Each arc gets an arc expression specifying the kind of tokens flowing through it

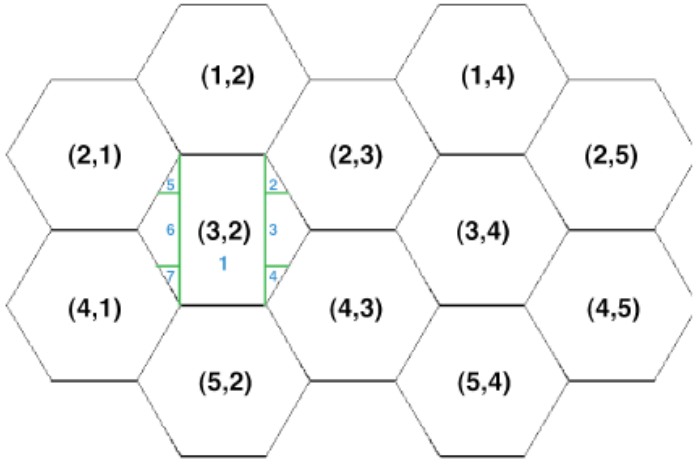
- Allows for the discrimination of species (molecules, metabolites, proteins, secondary substances, genes, etc.).
- Colours can be used to distinguish between sub-populations of a species in different locations (cytosol, nucleus and so on).

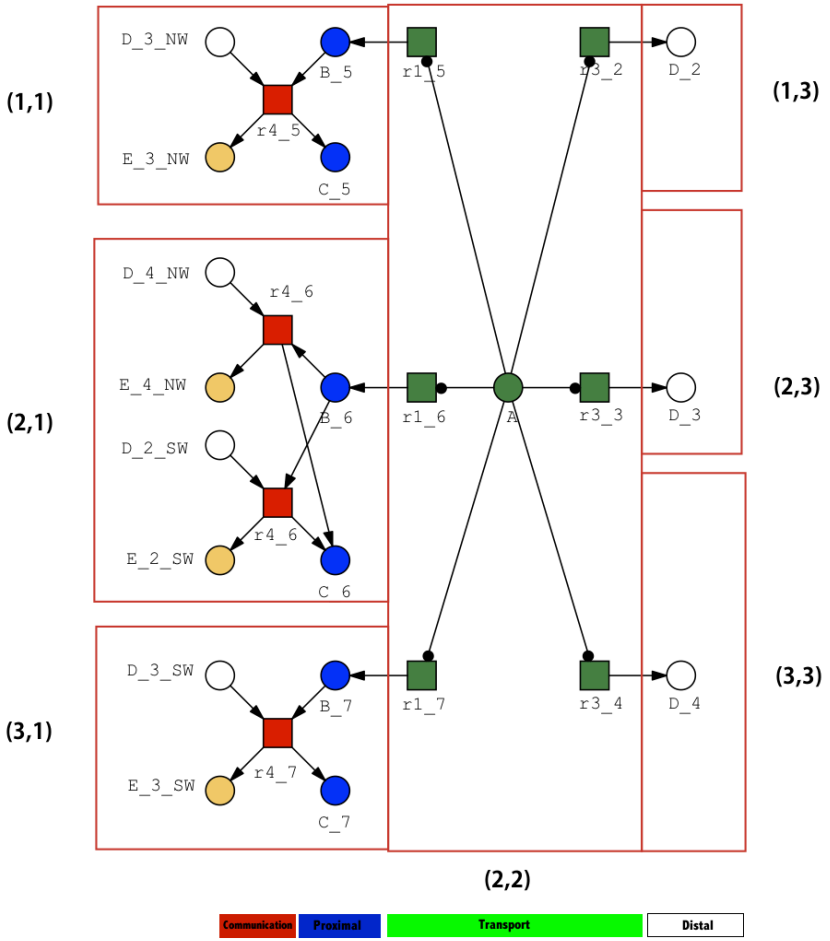
Single cell
Abstract level



- Four spatial regions as labelled *(Labelled colours are **not** CPN colour sets)*
- D_left & E_left: two molecular species (places) from the left-hand side neighbouring cell(s)

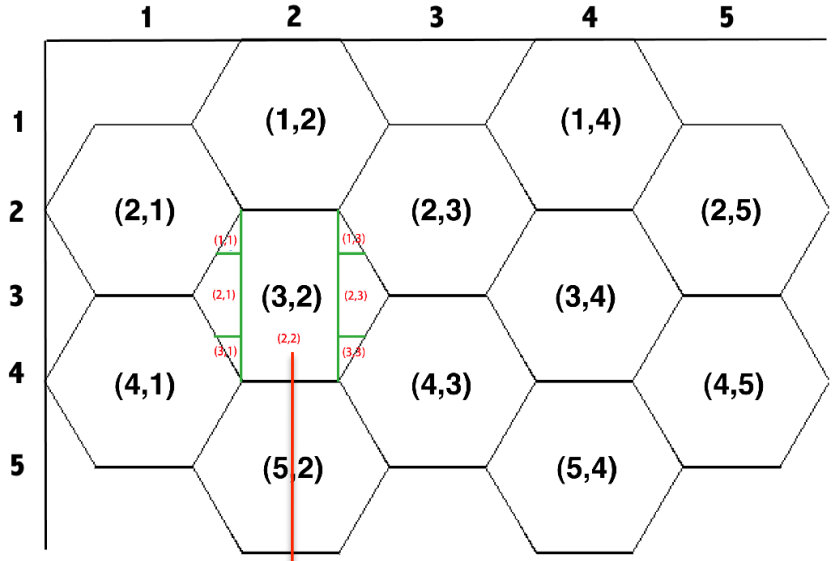
Petri net model for a single cell





Hierarchical organisation

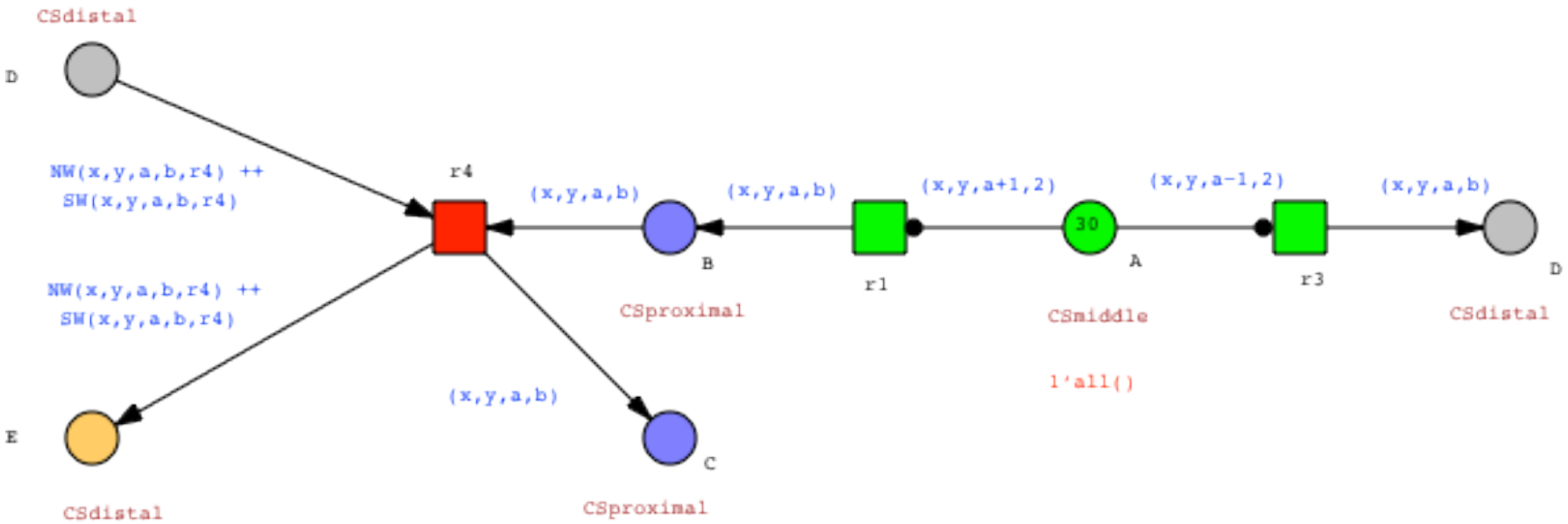
- Hierarchically coloured



(3,2)(1,1)	(3,2)(1,2)	(3,2)(1,3)
(3,2)(2,1)	(3,2)(2,2)	(3,2)(2,3)
(3,2)(3,1)	(3,2)(3,2)	(3,2)(3,3)

Colourset = {…, {((3,2)(1,1)), ((3,2)(1,2)), ((3,2)(1,3)),……((3,2)(3,3))}, …

CPN model of cells with 7 compartments in a 2-D lattice



- 4 spatial regions: communication, proximal, transport and distal.
- Seven virtual compartments ((1, 1), (2, 1),…, (3,3)).
- Each place or transition belongs to a specific compartment.
- NW and SW denote two left neighbours of the current cell.

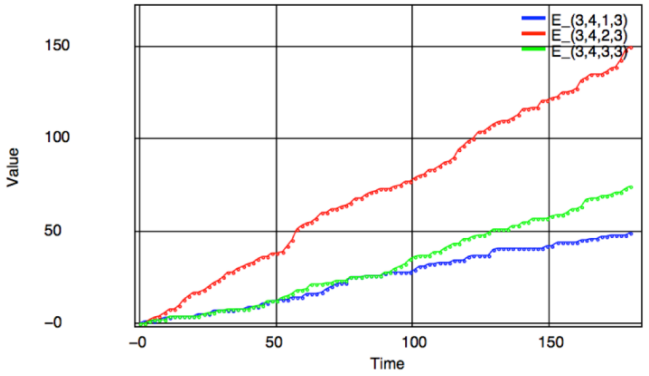
Declarations for th CPN model

Declaration

Constant M = int with 5 ;
Constant N = int with 5;
Constant C = int with 3;
Constant R = int with 3;
colourset Row = int with $1 - M$;
colourset $Column$ = int with $1 - N$;
colourset $ComR$ = int with $1 - R$;
colourset $ComC$ = int with $1 - C$;
colourset $CSr4$ = enum with $c5, c6\_1, c6\_1, c7$;
colourset $CS1$ = product with $Row \times Column$;
colourset $CS2 = CS1$ with $x\%2 = 1\&y\%2 = 0|x\%2 = 0\&y\%2 = 1$;
colourset CS = product with $Row \times Column \times ComR \times ComC$;
colourset $CS4 = CS3$ with $x\%2 = 1\&y\%2 = 0|x\%2 = 0\&y\%2 = 1$;
colourset $CSdistal = CS4$ with $b = 3$;
colourset $CSproximal = CS4$ with $b = 1$;
colourset $CSmiddle = CS4$ with $b = 2$;
Variable x : Row;
Variable y : $Column$;
Variable a : $ComR$;
Variable b : $ComC$;
Variable $r4$: $CSr4$;
Function $CSproximal\ NW(Row\ x, Column\ y, ComR\ a, ComC\ b)$;
Function $CSproximal\ SW(Row\ x, Column\ y, ComR\ a, ComC\ b)$;

Analysis Results

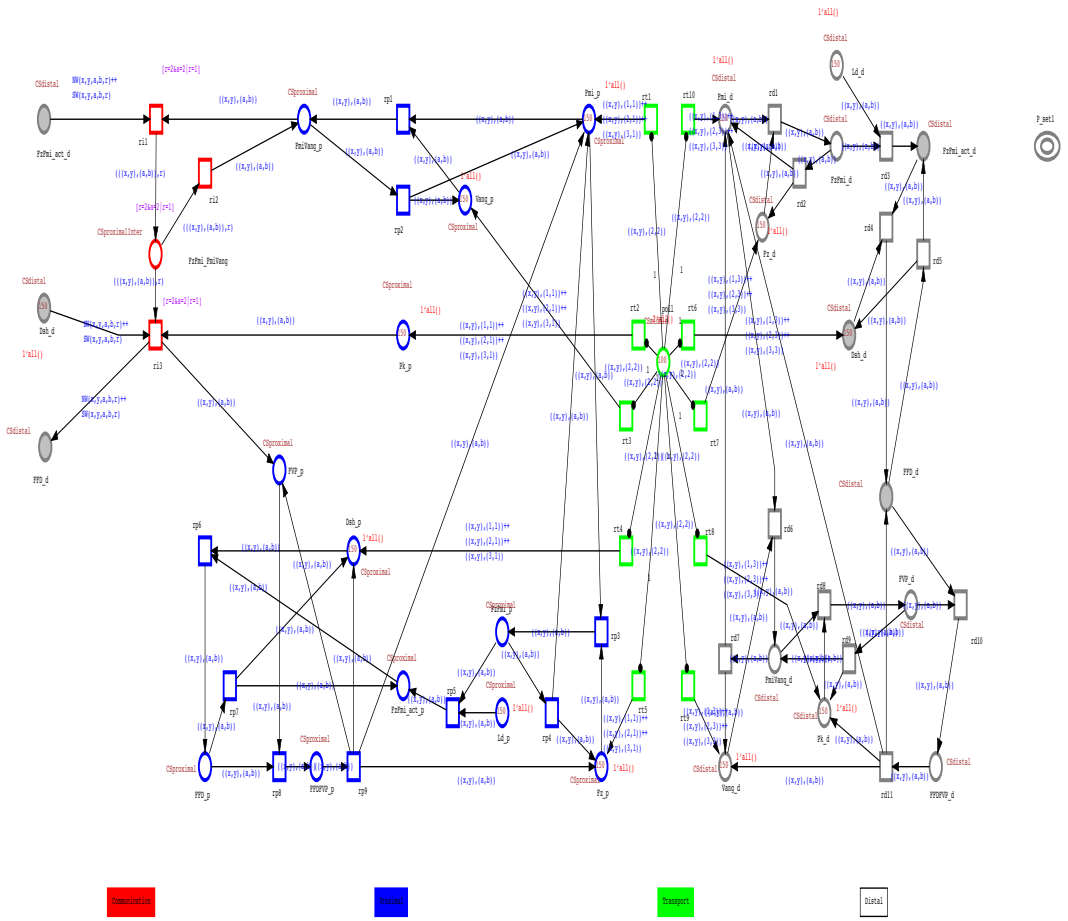
Stochastic simulation results



			0				0	
	72	(1,2)	**97**		69	(1,4)	**77**	0
(2,1)	**135**		49	(2,3)	**148**		45	(2,5) 0
	46		58		45		50	0
		(3,2)	**168**			(3,4)	**170**	
	46		48		49		39	0
(4,1)	**167**		42	(4,3)	**165**		43	(4,5) 0
	76	(5,2)	**88**		68	(5,4)	**84**	0
			0				0	

- Q. Gao, F. Liu, D. Tree & D. Gilbert. A Multi-cell Modelling Using Coloured Petri Nets Applied to Planar Cell Polarity. **BioPPN 2011**, Newcastle, UK.

CPN model of PCP signalling



Declarations for the coloured Petri net model

Type	Declaration
con	M = int with 15;
con	N = int with 15;
con	R = int with 3;
con	C = int with 3;
cs	Row = int with $1-M$;
cs	$Column$ = int with $1-N$;
cs	$CS1$ = product with $Row \times Column$;
cs	$CS\_Cell = CS1$ with $x\%2 = 1 \& y\%2 = 0 \mid x\%2 = 0 \& y\%2 = 1$;
cs	$ComR$ = int with $1-R$;
cs	$ComC$ = int with $1-C$;
cs	CS_ComP = product with $ComR \times ComC$;
cs	$CS2$ = product with $CS\_Cell \times CS\_ComP$;
cs	$CSdistal = CS2$ with $b = 3$;
cs	$CSproximal = CS2$ with $b = 1$;
cs	$CSmiddle = CS2$ with $a = 2 \& b = 2$;
cs	$CSInter$ = int with $1-2$;
cs	$CS3$ = product with $CSproximal \times CSInter$;
cs	$CSproximalInter = CS3$ with $r = 2 \& a = 2 \mid r = 1$;
var	x : Row;
var	y : $Column$;
var	a : $ComR$;
var	b : $ComC$;
var	r : $CSInter$;
fun	$CSproximal\ NW$ $(Row\ x, Column\ y, ComR\ a, ComC\ b, CSInter\ r)$ {[(!(x=1|y=1))&(r=1&a=1&b=1|r=2&a=2&b=1)] ((x-1,y-1),(a+1,b+2));}
fun	$CSproximal\ SW$ $(Row\ x, Column\ y, ComR\ a, ComC\ b, CSInter\ r)$ {[(!(x=M|y=1))&(r=2&a=2&b=1|r=1&a=3&b=1)] ((x+1,y-1),(a-1,b+2));}
fun	$bool\ MutReg(Row\ x, Column\ y)$ {x>=4&x<=8&y>=3&y<=7;}

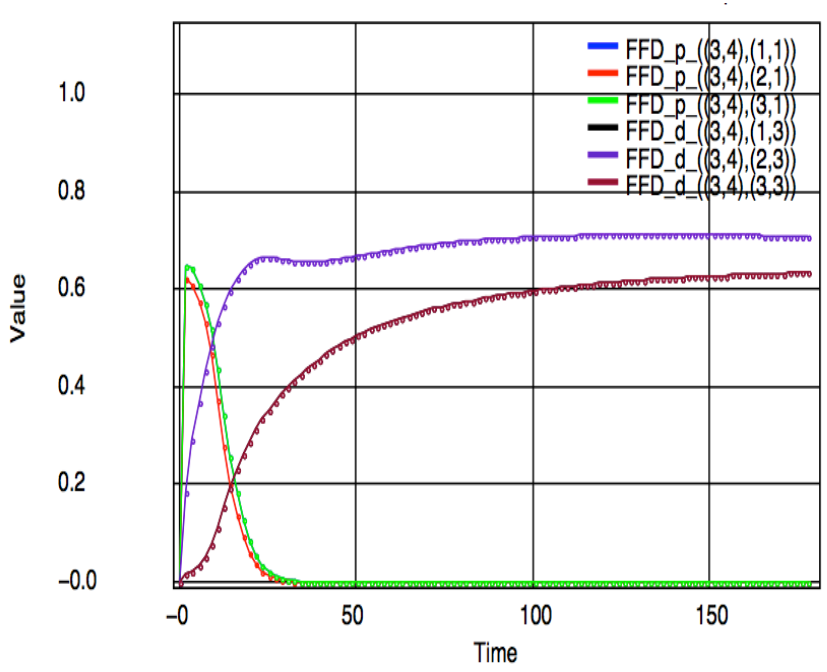
Some Statistics

Size				Time (seconds)			
Grid $(M \times N)$	Cells	Places	Transitions	Unfolding	Unfolding/Cells	Simulation	Simulation/Cells
5×5	12	924	984	0.665	0.0546	1.192	0.0993
10×10	50	3,850	4,100	2.528	0.0506	5.152	0.1030
15×15	112	8,624	9,184	6.108	0.0545	11.952	0.1067
20×20	200	15,400	16,400	11.705	0.0585	25.272	0.1264
50×50	1,250	96,250	102,500	139.594	0.1117	220.735	0.1766

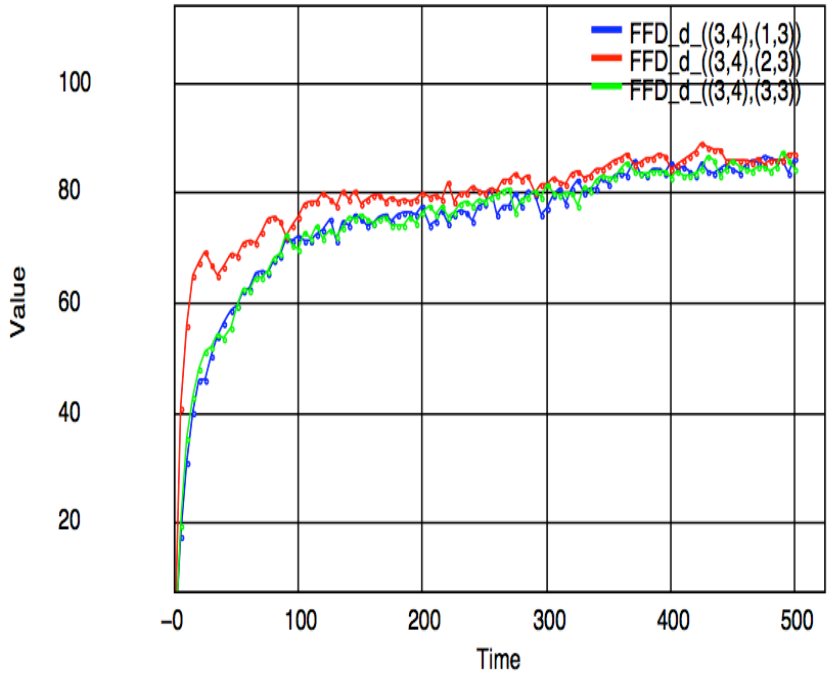
[a] done on PC Windows Vista (TM) business Intel(R) Xeon(R), CPN 2.83GHz, Memory(RAM) 4.00 GB;

Simulations

Continuous simulation

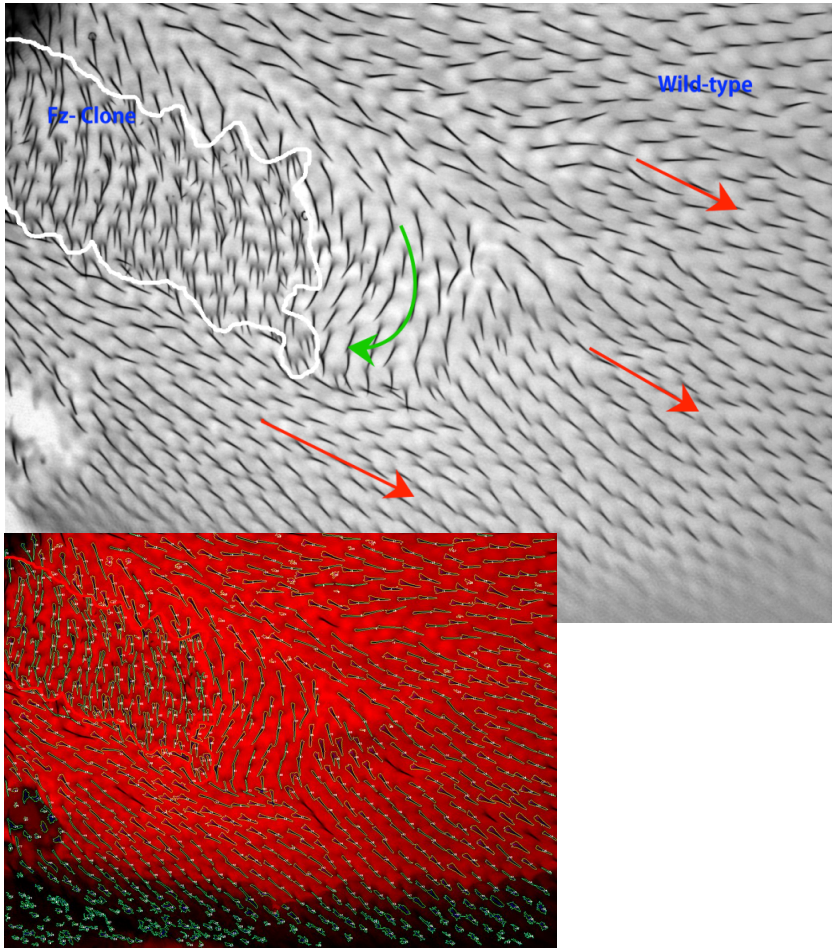


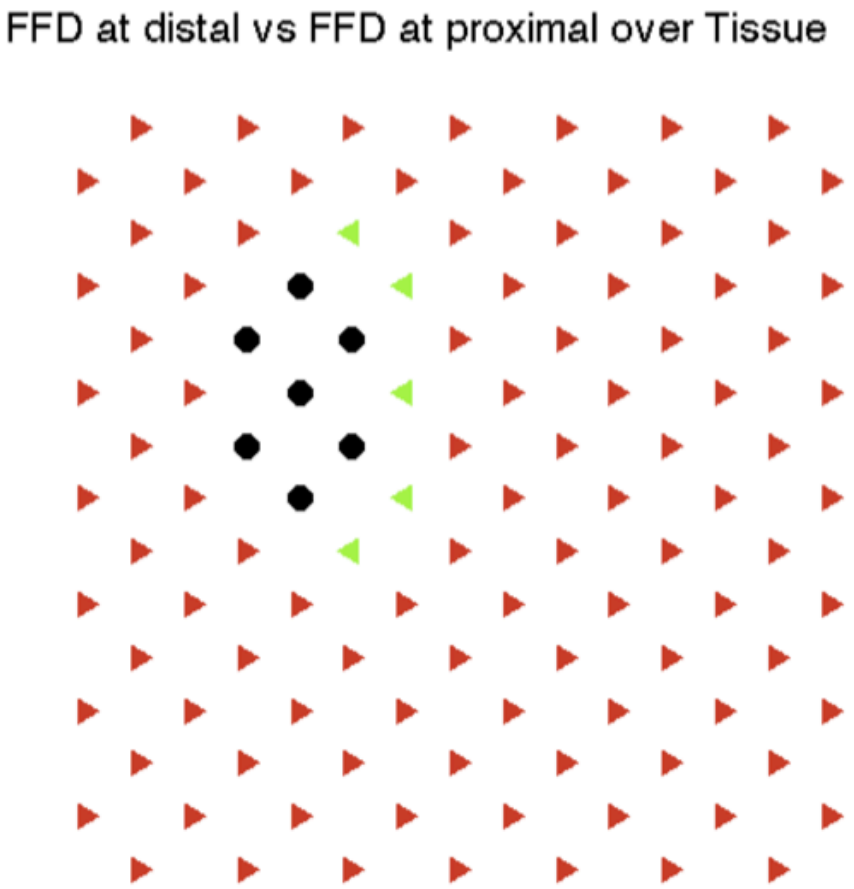
Stochastic simulation (average of 10 runs)



FFD accumulates at the distal edge of the cell rather than the proximal edge at the end of signalling.

Experimental vs In-silico





- Q. Gao, F. Liu, D. Gilbert, M. Heiner & D. Tree. A Multscale Approach to Modelling Planar Cell Polarity in Drosophila Wing Using Hierarchically Coloured Petri Nets. **CMSB 2011**, Paris, France.

Downloads

- Snoopy for standard PN & CPN:

  http://www-dssz.informatik.tu-cottbus.de/DSSZ/Software/Snoopy

- CPN models for PCP:

  http://people.brunel.ac.uk/~cspgqqg

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The End…

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