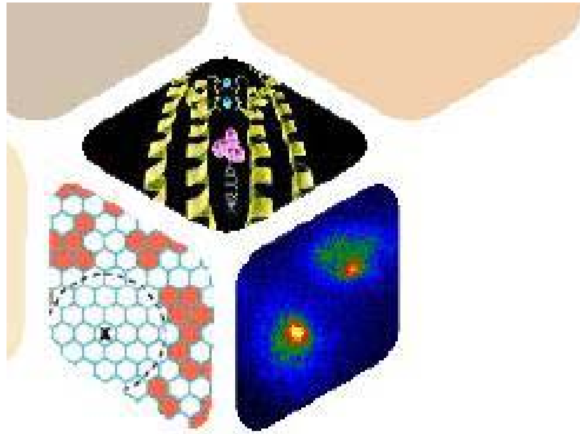

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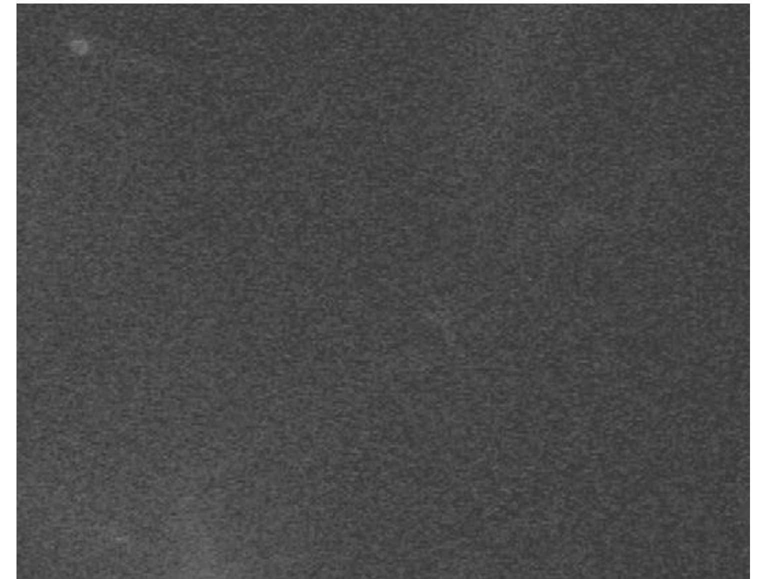


Nose diving into systems biology?

-or

From *E.coli* chemotaxis to sensory networks?

Judy Armitage

www.bioch.ox.ac.uk/sysbio

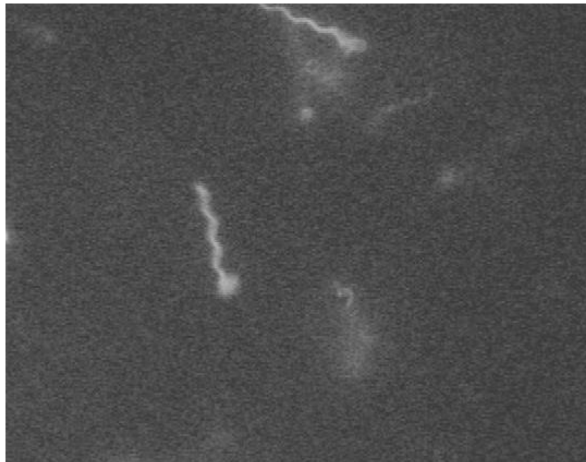
Lecture

- **First part**: *E.coli* chemotaxis, relationship to bacterial sensory networks, what we know, how we know this and development of models
- **Second part:** Attempts to extend models, the limitations and complexities of non-enteric pathways

Bacterial chemotaxis

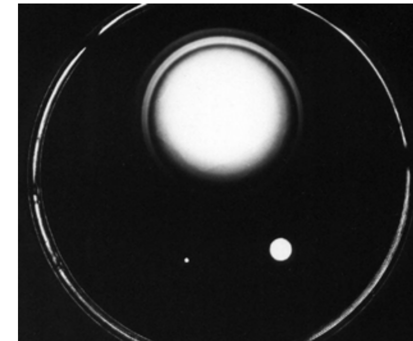
- Aim to get from:

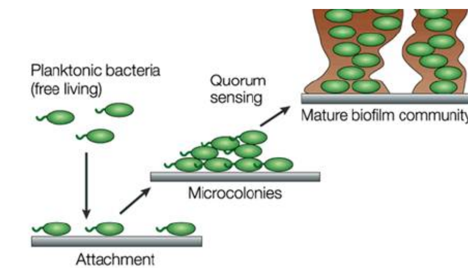
single cell behaviour

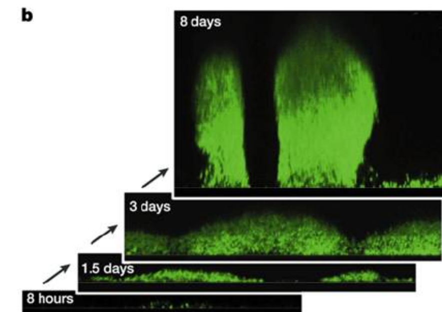


to→

Populations and population dynamics

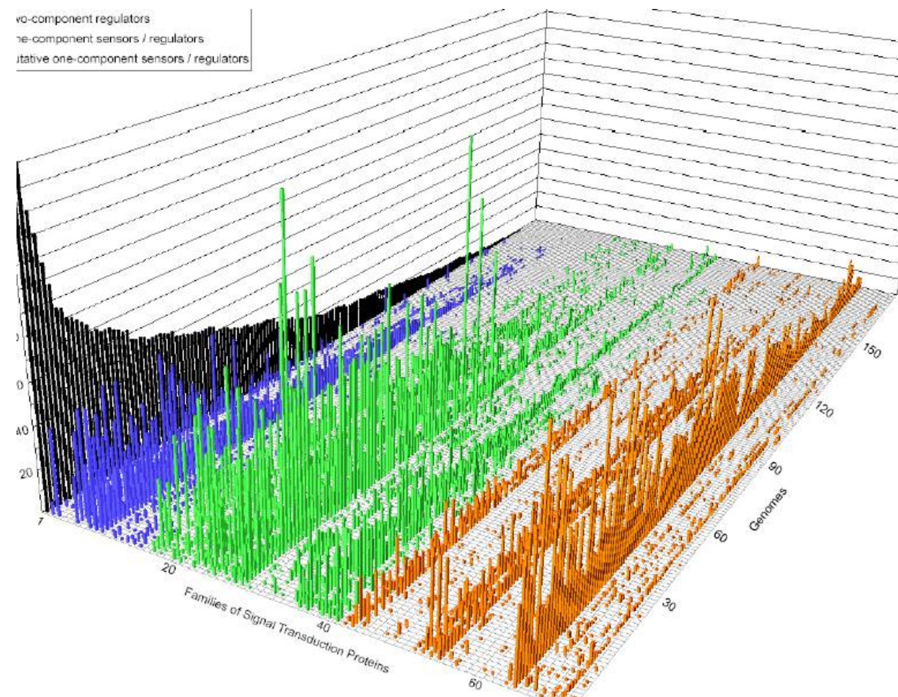




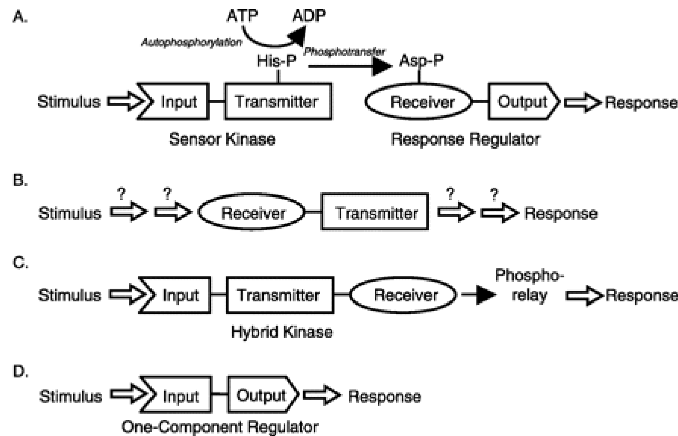


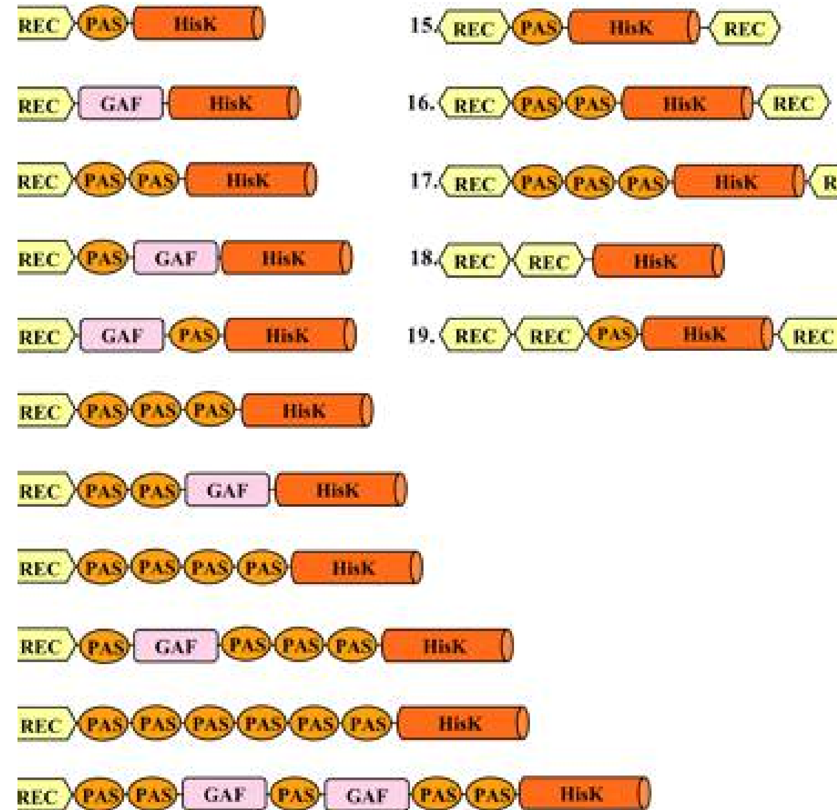
Why should we be interested?

- Closed system with obvious phenotype
- Model for other 2 component (phosphorelay) systems
- Model of sensitivity and gain (~2 molecules over ~5 orders of magnitude)--Robust
- Target for novel antimicrobials-both chemotaxis itself and 2 component pathways



Phosphorelay pathways





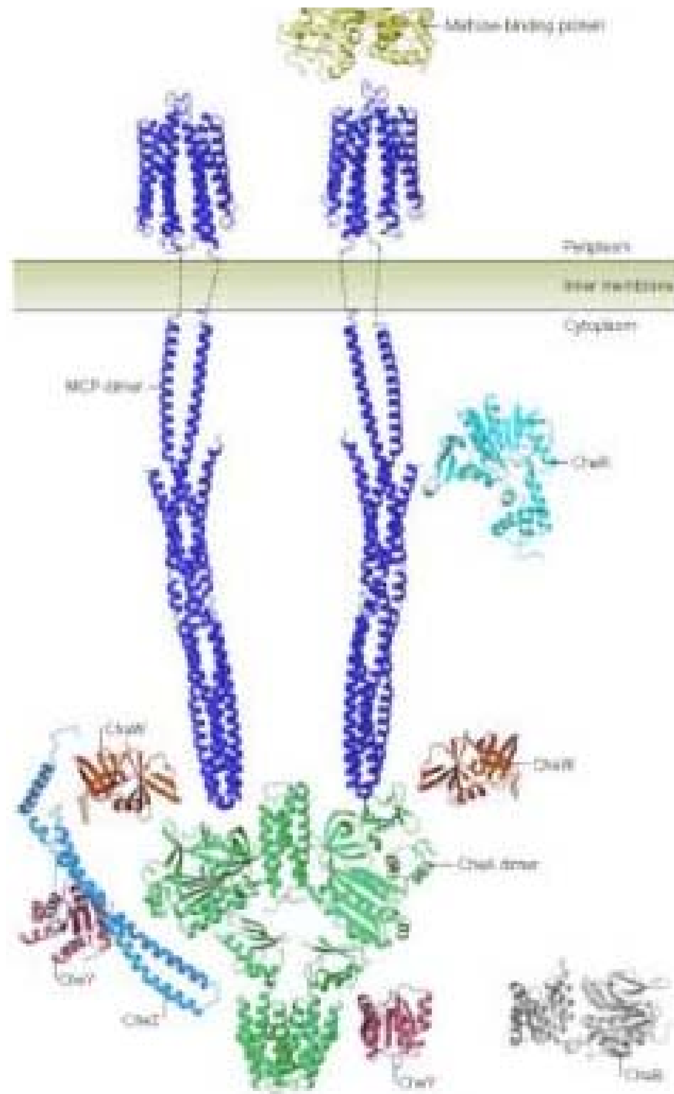
- Of ~200 genomes, most HPKs in one species 131
- Total of 4,600 RR sequences (max per species 145)
- Often together in operons
- 14 major output domains
- 66% putative DNA binding
- 14% single receiver domains

Flavours of output

E.coli chemotaxis

- Transmembrane sensing
- Phosphotransfer
- 100ms signaling to motor
- Signal termination
- Receptor adaptation – methylation/demethylation (1-5s)

The players



•Transmembrane receptor (MCP) homodimer

•Coiled-coil cytoplasmic domain with conserved glutamates

•C-terminal binding site for methyl transferase, CheR

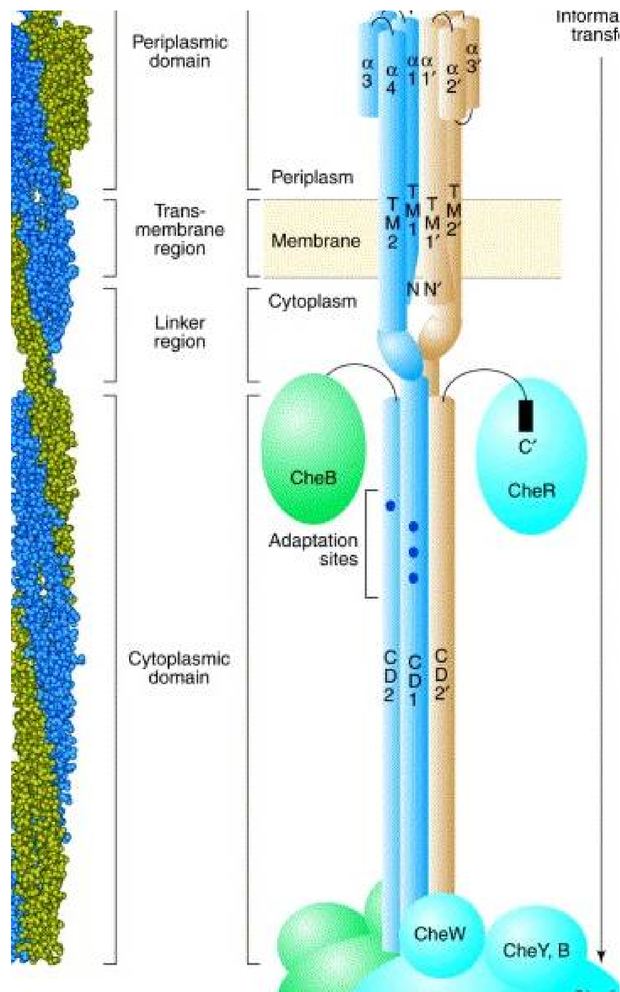
•Sensory output domain binding HPK, CheA

•CheA binds CheY (motor binding protein) and CheB (methyl esterase)

•CheZ (phosphatase) associated with CheA

•Only 7 proteins!-so how hard can it be!

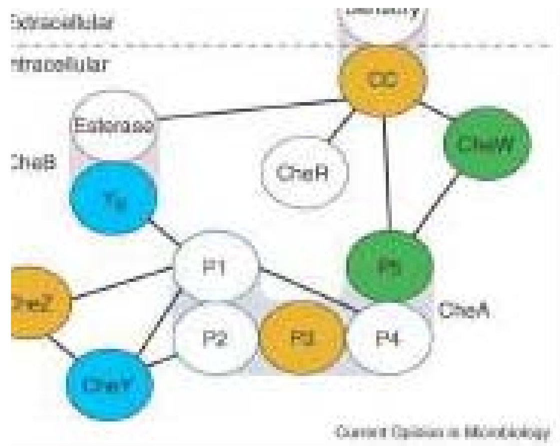
Critical steps



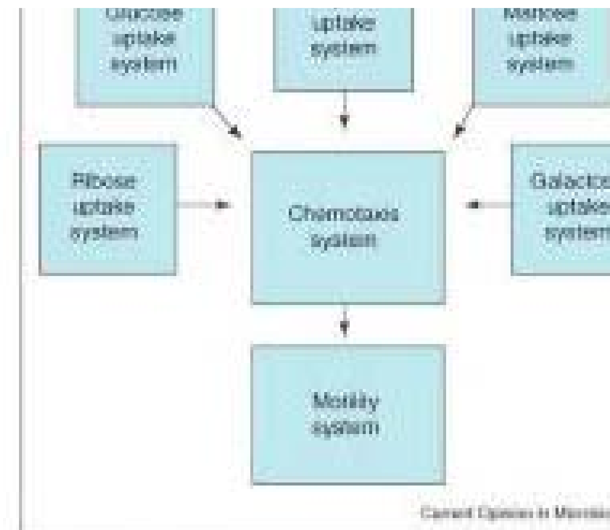
- Transmembrane signalling and HAMP domain regulates activity of CheA
- Adaptation by methylation of 4 conserved glutamates
- CheA phosphorylates CheY and CheB
- CheY-P diffuses to motor to cause switching
- Signal terminated by CheZ
- CheB-P has 100x higher esterase activity that CheB and demethylates receptors to reset

E.coli chemotaxis

Basic network



Additional input



- Know
  - Copy number of each protein (thousands)
  - Structure of each protein
  - Kinetics of phosphotransfer and methyltransfer (*in vitro* and for some *in vivo)*
  - Localisation of each protein

Stock

Some numbers

rotein concentrations (cell volumes & copy numbers)

ell volume (fl)	Notes (reference)
4	Sourjik & Berg (2002b)
32 ± 0.30	At 0.1 Osm; calculated from average amount of protein per cell (0.41 ± 0.03 pg), protein/dry weigh ratio (0.68 ± 0.07), and volume of cytoplasmic water per mg dry weight (2.19 ± 0.11 fl) (Cayley e al., 2000)
50	RP437 strain (Scharf et al., 1998)
42	AW405 strain (Scharf et al., 1998)

otein	Molecules per cell	Concentration (monomer) (μM)*	Notes (reference)
eceptors (total) r + Tar g	15000 ± 1700 14000 ± 1700 440 ± 70	18 ± 2 17 ± 2 0.52 ± 0.08	RP437 strain in rich medium; total includes an estimate for Tap + Aer (Li & Hazelbauer, 2004)
eA (total) A_L A_S	6700 ± 1100 4500 ± 940 2200 ± 520	7.9 ± 1.3 5.3 ± 1.1 2.6 ± 0.6	See above
eW	6700 ± 890	7.9 ± 1.0	See above
eY	8200 ± 310	9.7 ± 0.4	See above
eZ	3200 ± 90	3.8 ± 0.1	See above
eB	240 ± 10	0.28 ± 0.01	See above
eR	140 ± 10	0.16 ± 0.01	See above

Concentrations are based on a cell volume of 1.4 fl. See Li & Hazelbauer (2004) for da
W1 in both rich and minimal media.

heB/CheY phosphorylation and dephosphorylation

action	Rate constant	Notes (reference)
p + Y -> AA + Yp	~10^8 M^{-1} s^{-1}	With 10 mM Mg^{2+}; k_{cat}/K_M (Stewart et al., 2000)
p + B -> AA + Bp	1.5 × 10^7 M^{-1} s^{-1}	See above (Stewart, personal communication)
-> Yp	6 M^{-1} s^{-1}	With 20 mM Mg^{2+}; k_{cat}/K_M for acetyl phosphate (Silversmith et al., 2001)
-> Yp	8 M^{-1} s^{-1}	With 10 mM Mg^{2+}; k_{cat}/K_M for acetyl phosphate (Schuster et al., 2001)
-> Yp	3 M^{-1} s^{-1}	With 20 mM Mg^{2+}; k_{cat}/K_M of *Salmonella* CheY for acetyl phosphate (Da Re et al., 1999)
-> Yp	5 M^{-1} s^{-1}	With 10 mM Mg^{2+}; k_{cat}/K_M for acetyl phosphate (Mayover et al., 1999)
-> Y	0.045 s^{-1}	With 10 mM Mg^{2+} (Stewart & VanBruggen, 2004)
-> Y	0.051 s^{-1}	With 10 mM Mg^{2+} (Smith et al., 2003)
-> Y	0.085 ± 0.001 s^{-1}	From FRET between CheY and FliM *in vivo* in a *cheZ* strain (Sourjik & Berg, 2002b)
+ ZZ -> Y + ZZ	2.2 ± 0.1 s^{-1}	In the wt strain (see above) (Sourjik & Berg, 2002b)
-> Y	0.036 ± 0.006 s^{-1}	With 20 mM Mg^{2+} (Silversmith et al., 2001)
-> Y	0.044 s^{-1}	With 20 mM Mg^{2+} (Schuster et al., 2001)
-> Y	0.053 s^{-1}	With 10 mM Mg^{2+} (Schuster et al., 2000)
-> Y	0.034 s^{-1}	With 10 mM Mg^{2+} (Mayover et al., 1999)
-> Y	0.030 ± 0.002 s^{-1}	With 20 mM Mg^{2+}; *Salmonella* CheY (Da Re et al., 1999)
-> Y	0.049 s^{-1}	With 10 mM Mg^{2+} (Appleby & Bourret, 1998)

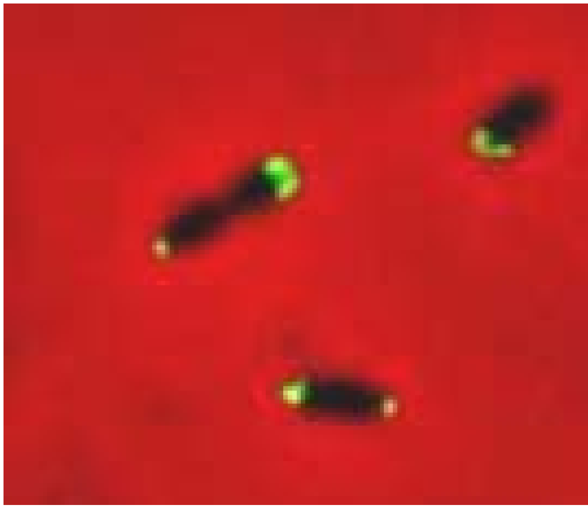
Denis Bray

Experimental Methods

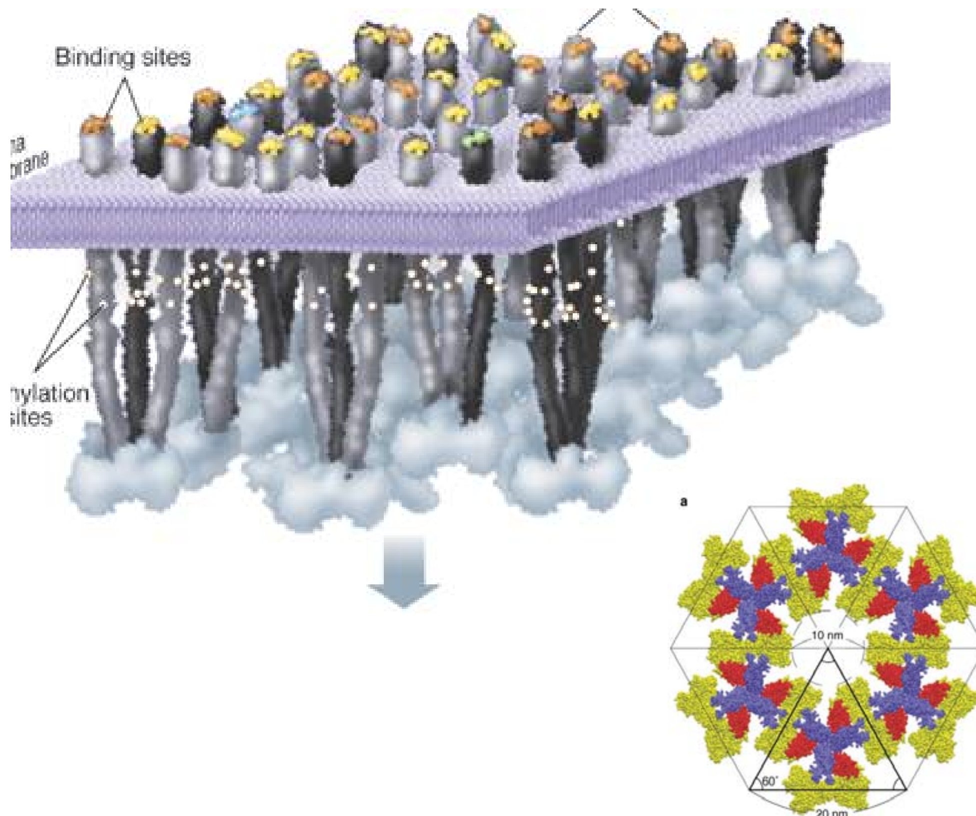
- Molecular genetics-deletion/site directed mutagenesis/cross linking/tryptophan, alanine, cysteine scanning
- Phenotype-sensitivity, response kinetics
- Quantitative protein measurement
- Structures, with and without substrate, active/ inactive conformation
- Microscopy

Model breakthrough

- Put all the *in vitro* and iin *vivo data* together and can model signal transduction, but get nowhere near understanding the amplification or sensitivity of the system
- Is amplification at receptor or at flagellar motor?
- ***In vivo* imaging provided the breakthrough**



E.coli



•Chemoreceptors cluster at the poles with CheW, CheA, CheR, CheB, CheY, CheZ

•In mixed trimers of dimers

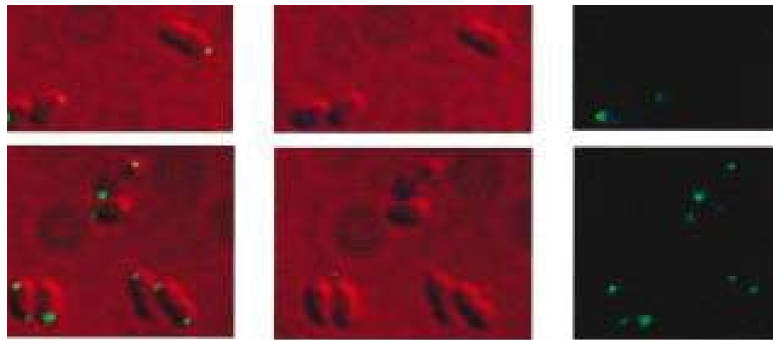
•CheR localised to C-terminal domain causing localised increase in concentration

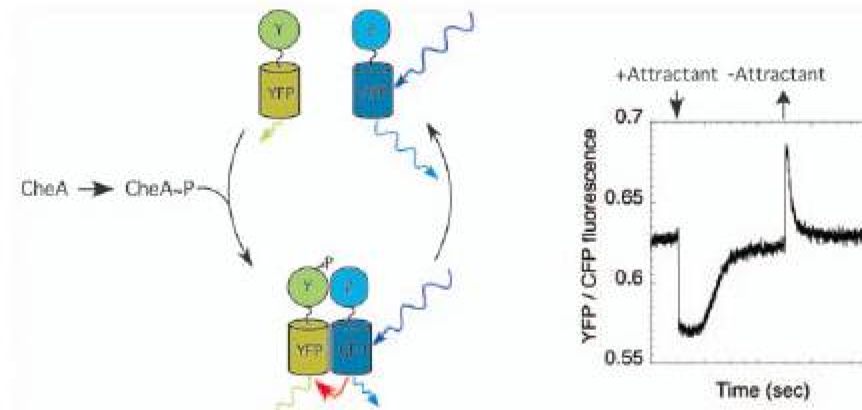
•Clustering depends on CheA and CheW not receptors

•Clustering and adaptation essential for sensitivity

•All amplification comes from the receptor cluster not target motor

In vivo kinetics





Populations of cells, but can now do on a single cell

Sourjik

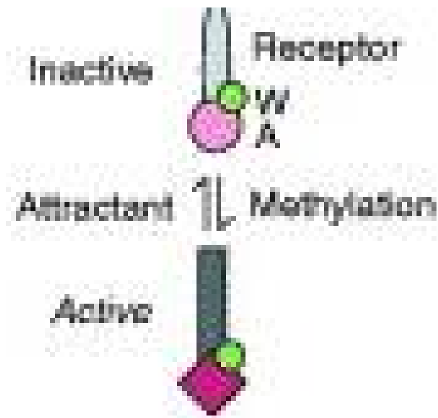
Amplification comes from CheY copy number, CheA kinetics and localised CheZ phosphatase

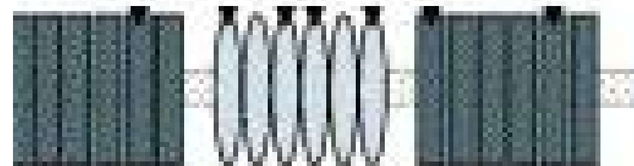
Gain comes from changes in chemoreceptor trimer of dimer clusters in response to adaptation as chemoeffector concentrations change

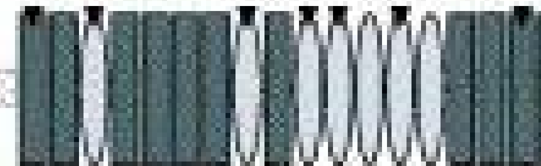
requires CheR localisation

Everything is happening within an organised receptor cluster-not at the motor

Models based on stimulus response and recruitment of neighbouring receptors through adaptation







Stochastic Model Development

Mutants

(Some of them newly isolated because of comparisons with BCT program. The program identified flaws in original genetic assignments)

Denis Bray

Deletion	Gutted	Overexpression	Mixed
T^{-}	Gutted (g)	T^{++}	$T^{-}B^{++}$
R^{-}	Y^{+} (g)	R^{++}	$T^{-}Y^{++}$
B^{-}	Y^{++} (g)	B^{++}	$T^{++}Z^{-}$
W^{-}	Y^{+++} (g)	W^{++}	$B^{-}W^{++}$
A^{-}	Y^{++++} (g)	A^{++}	$B^{-}Z^{++}$
Y^{-}	Z^{+} (g)	Y^{++}	$B^{++}Z^{-}$
Z^{-}	$Y^{+}Z^{+}$ (g)	Z^{++}	$W^{++}Z^{-}$
$T^{-}Z^{-}$	$Y^{++}Z^{+}$ (g)	$T^{++}W^{++}$	$A^{++}Z^{-}$
$R^{-}B^{-}$	$Y^{+++}Z^{+}$ (g)	$B^{++}Y^{++}$	$T^{-}B^{++}Y^{++}$
$B^{-}Z^{-}$	$Y^{++++}Z^{+}$ (g)	$W^{++}A^{++}$	$T^{-}W^{++}Z^{-}$
$W^{-}A^{-}$	$T^{+}Y^{+}$ (g)	$Y^{++}Z^{++}$	$T^{++}W^{-}Z^{-}$
$W^{-}Z^{-}$	$A^{+}Y^{+}$ (g)		$R^{-}B^{-}W^{++}$
$A^{-}Z^{-}$	$T^{+}A^{+}Y^{+}$ (g)		$R^{-}B^{-}Y^{++}$
$Y^{-}Z^{-}$	$W^{+}A^{+}Y^{+}$ (g)		
$T^{-}W^{-}Z^{-}$	$T^{+}W^{+}A^{+}Y^{+}$ (g)		
$B^{-}W^{-}A^{-}$	$T^{+}Y^{+}Z^{+}$ (g)		
$B^{-}Y^{-}Z^{-}$	$A^{+}Y^{+}Z^{+}$ (g)		
$W^{-}A^{-}Z^{-}$	$T^{+}A^{+}Y^{+}Z^{+}$ (g)		
	$W^{+}A^{+}Y^{+}Z^{+}$ (g)		
	$T^{+}W^{+}A^{+}Y^{+}Z^{+}$ (g)		
	$T^{++}Y^{++}$ (g)		
	$W^{+}Y^{+}Z^{+}$ (g)		

Reproduces stimulus response in single cells

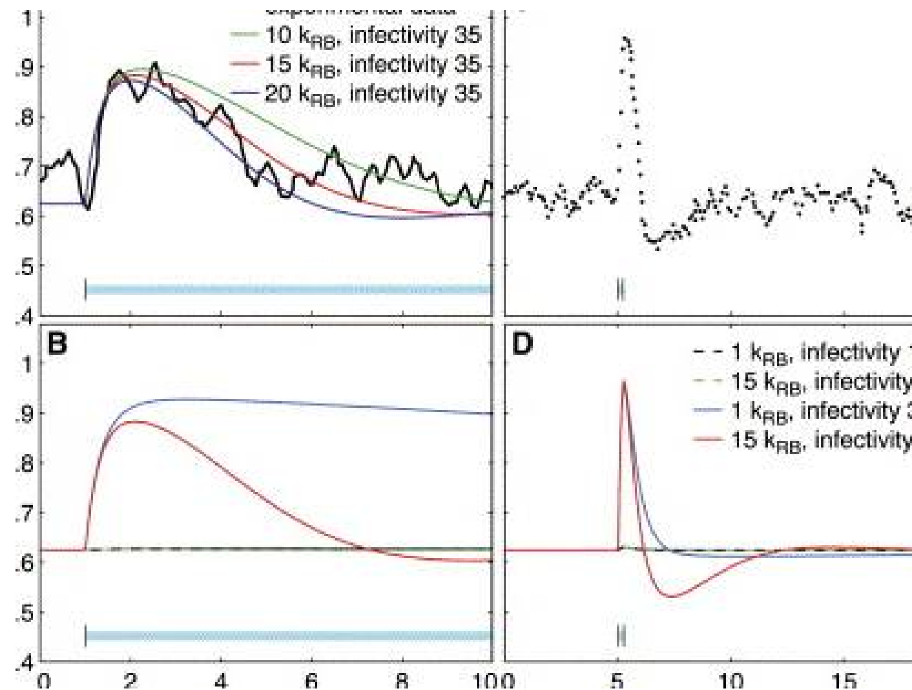
Response to Steps and Pulses of Aspartate

(A) Step increase of 15 nM aspartate. Simulations were run with different coordinate rates of methylation and demethylation (kR,B) and fixed infectivity.

(B) Same as (A) but with different levels of infectivity (see [D] for legend).

(C) Experimental impulse response

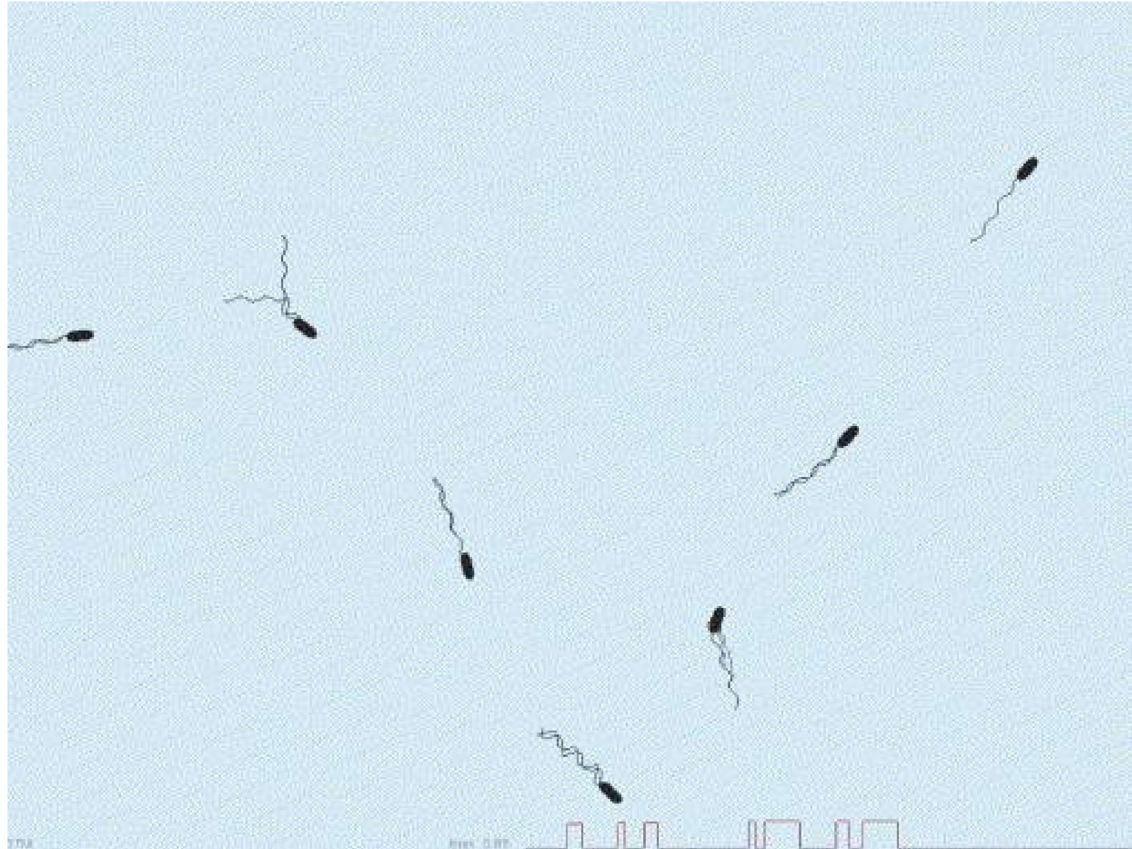
(D) Results of BCT simulations of the response to a 0.25 s pulse of 0.8 M aspartate; cells with different coordinate rates of methylation and demethylation (kR,B) and different levels of infectivity (gain).



Modeling

Simulated Swimming Bacteria

1s screen shot of a simulated *E. coli* in 0.1 μM aspartate

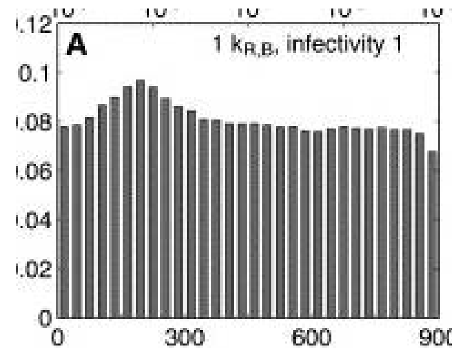


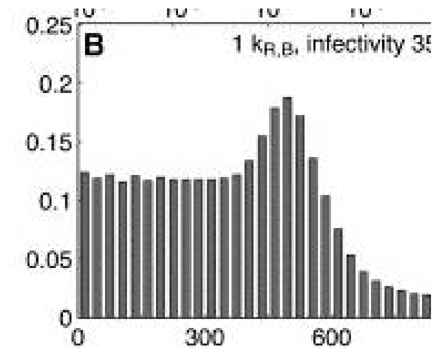
Extends to populations

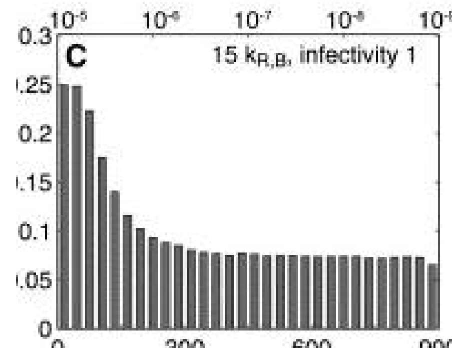
Distribution of *E. pluribus* in a Radial Gradient

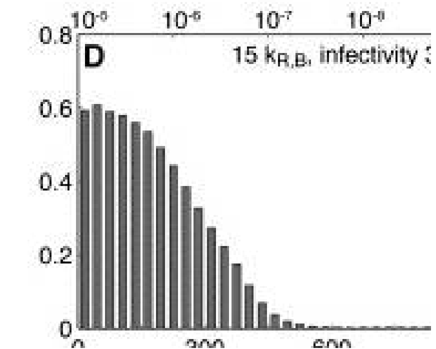
1000 bacteria in a circle of radius 900 μm. Exp gradient of aspartate, from 10−5 M to 10−9 M. After 100 s, the positions of bacteria were recorded at 2 s intervals for next 1000 s and pooled.

(A) Default with infectivity of 1.
(B) Default with infectivity of 35.
(C) Adaptation-enhanced strain, activities of CheR and CheB 15-fold greater and infectivity of 1.
(D) Same as (C) but with infectivity of 35.

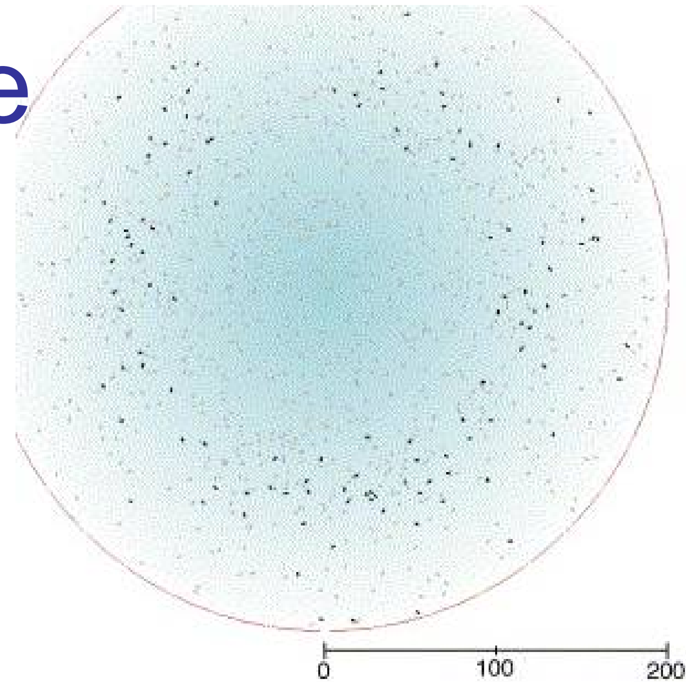






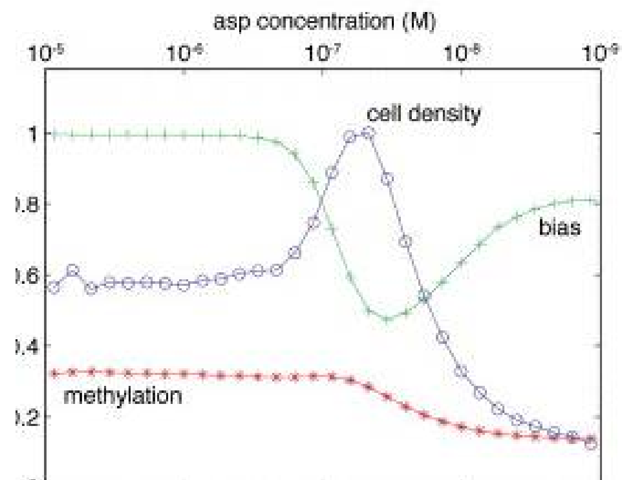


Population response

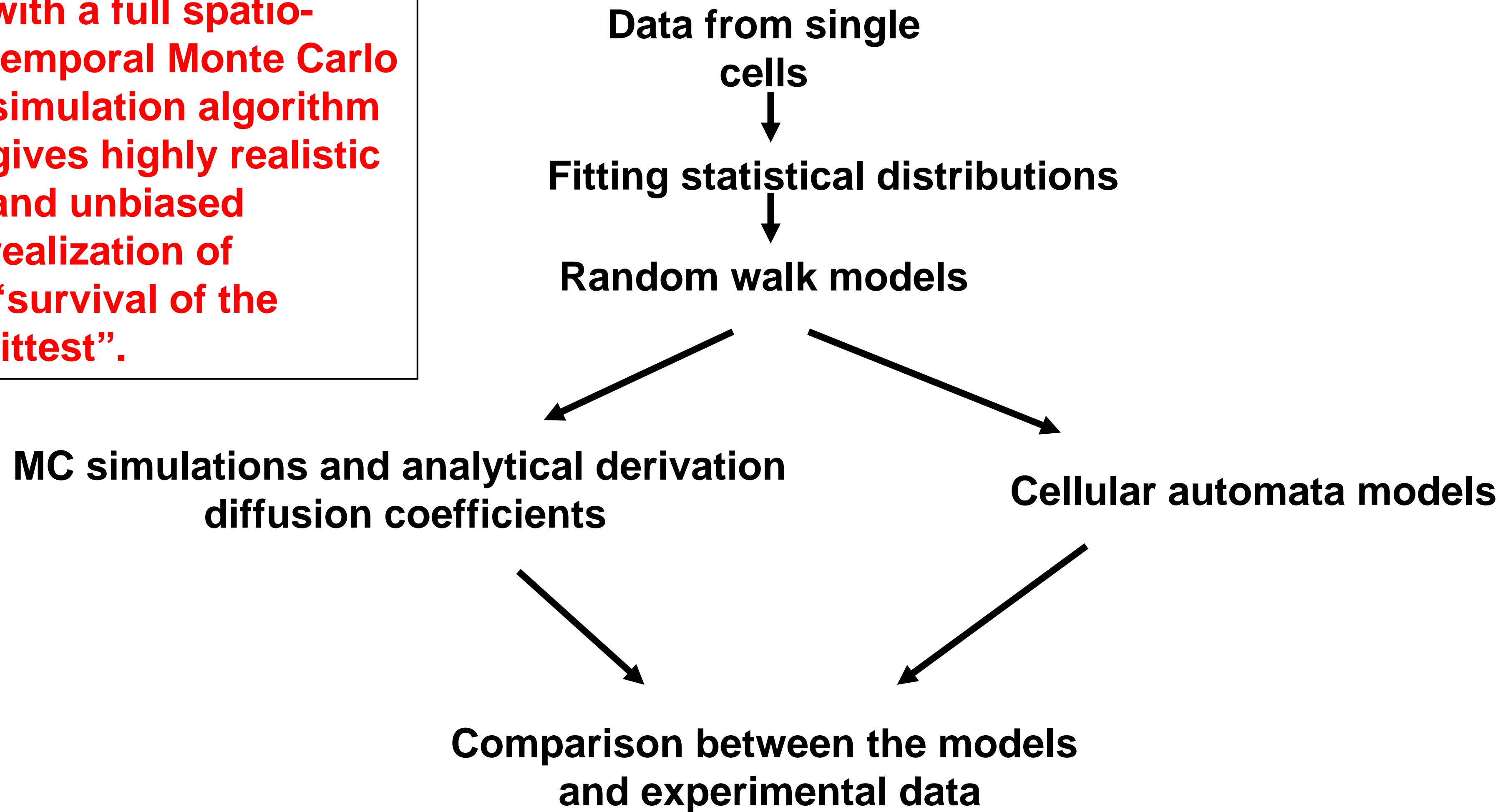


(A) A screen shot of 1000 *E. pluribus* cells distributed in a radial gradient of aspartate. Cells undergoing a tumble are shown in black; cells in a run are in gray.

(B) The same simulation depicted in (A) averaged over 20 min. Normalized cell density is represented in blue, rotational bias is in green, and methylation level is in red.



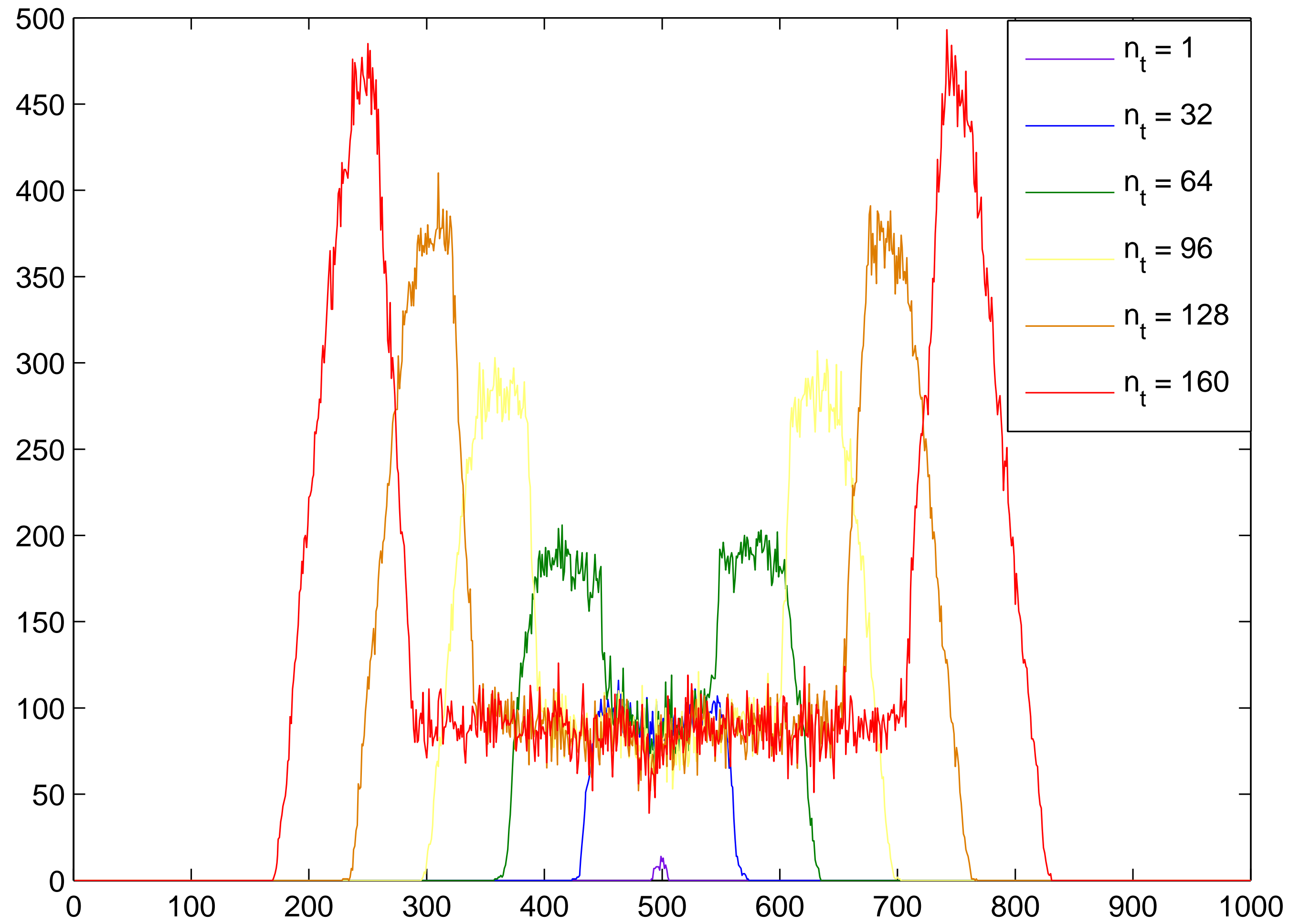
Different approaches



Cellular automaton models

- The two-dimensional model consists of an array of L x L automaton elements.
- At each time step every element is characterised by concentration of nutrient and presence or absence of bacterium.
- The joint evolution of these two characteristics is determined by local rules for bacterial motion based on temporal data.
- As characteristic lengths of molecules are very small compared with the typical size of bacteria, we can consider the nutrient concentration as a continuous field, while treating bacteria as individual discrete entities.

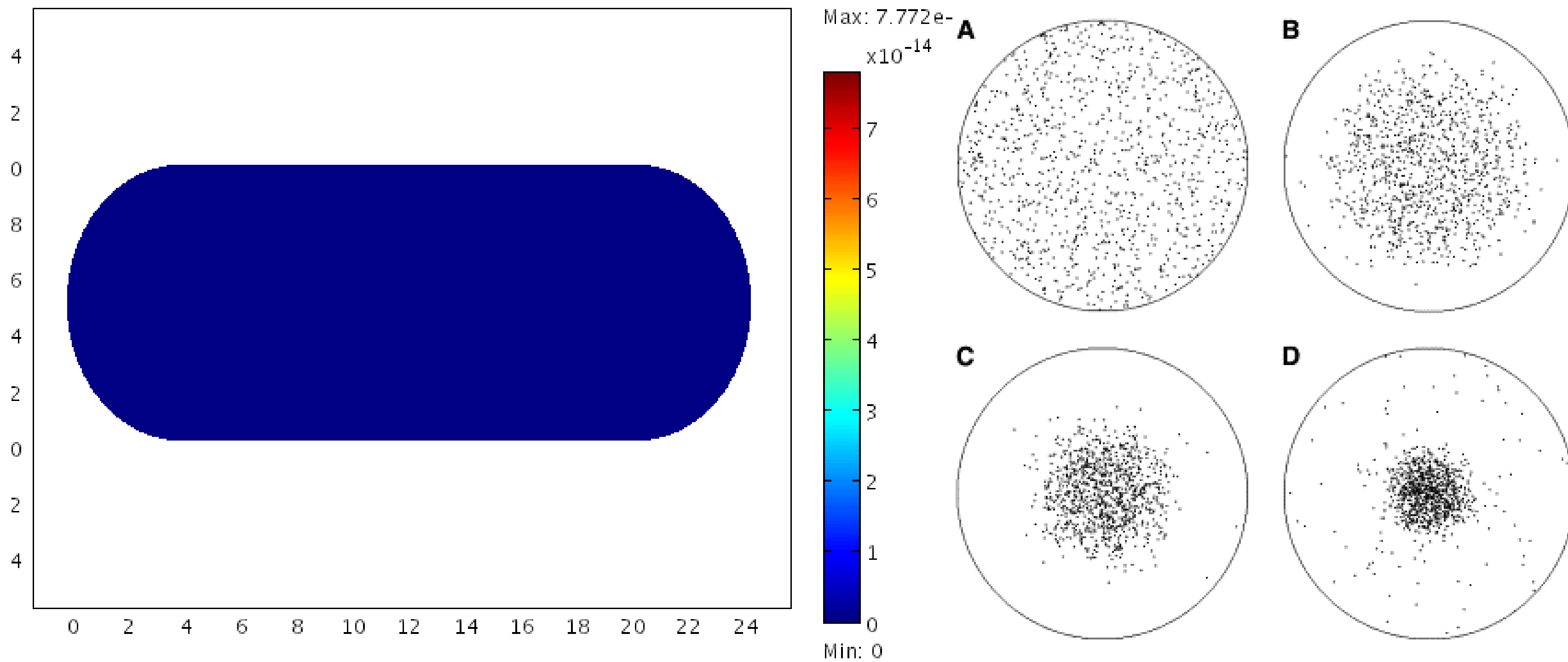
Spreading population



Comparison between CA models and experiment

Nutrient concentration range	Exponential	Weibull	Levy
Low	poor	poor	good
Medium	poor	good	best
High	good	good	poor

Reaction diffusion models



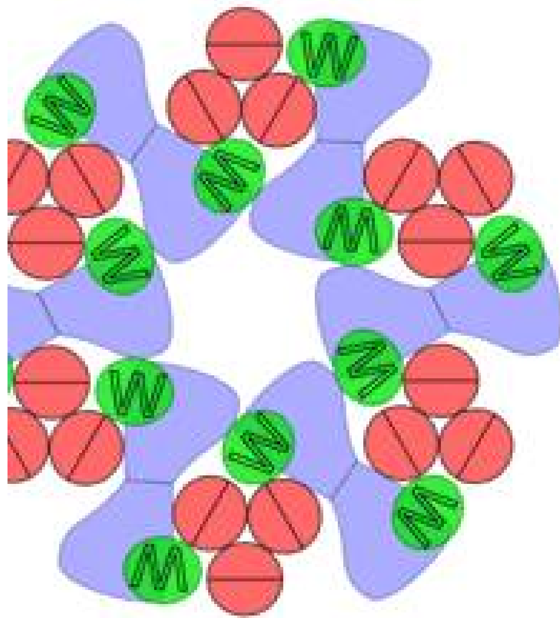
Can these *E.coli* models be extended?

- *E.coli* has 4 receptors, *Magnetospirillum magnetotacticum* has 65 (average about 20)
- Only 10% putative MCPs have CheR binding domain
- Crystals of *Thermotoga maritima* MCPs packed as dimers not trimers of dimers
- Most species encode multiple putative chemotaxis pathways-how do these control one motor?
- Do they all encode chemotaxis pathways?
- Expression of different pathways often regulated by different environmental signals
- *E.coli* signalling may not be 2 state

Thermotoga chemoreceptors

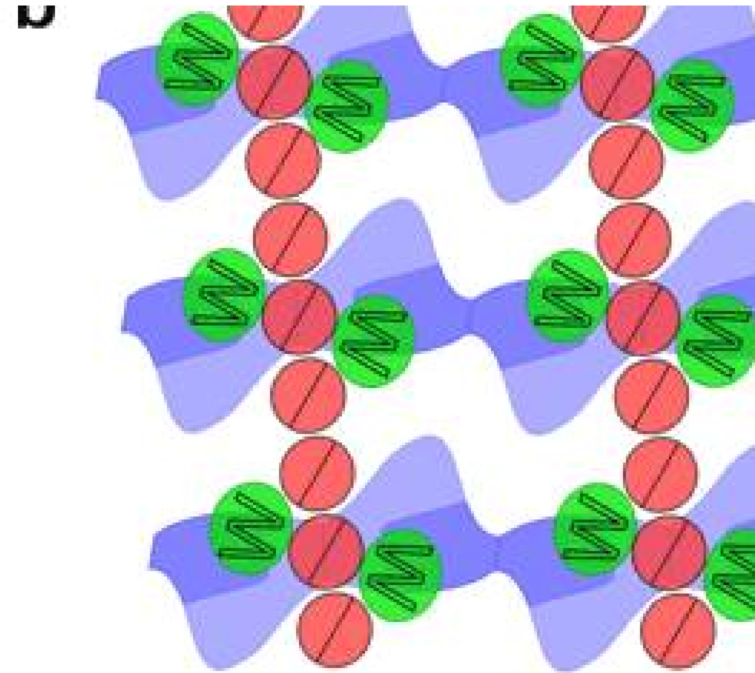
- CheR and robust adaptation is essential for all models
- Chemoreceptors and sensory proteins must all localise for signal amplification
- Localisation allows both “infectivity” between adapted receptors and high local concentration of enzymes (in solution *T.maritima* CheR to MCP has Kd>0.6mM, *E.coli* 2μM)-can’t measure MCP:CheR interaction *in vitro*, but kcat same as *E.coli*

Debate over cluster arrangement



Trimers of dimers

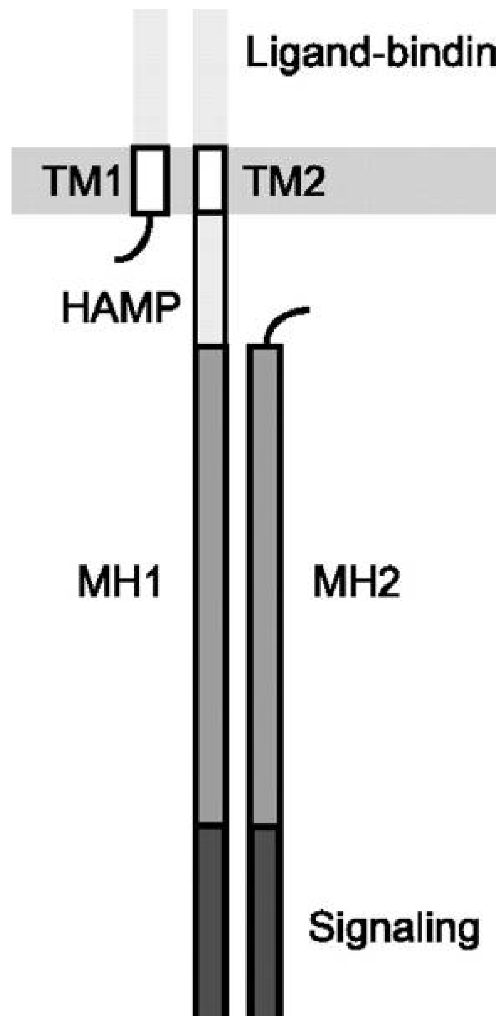
E.coli

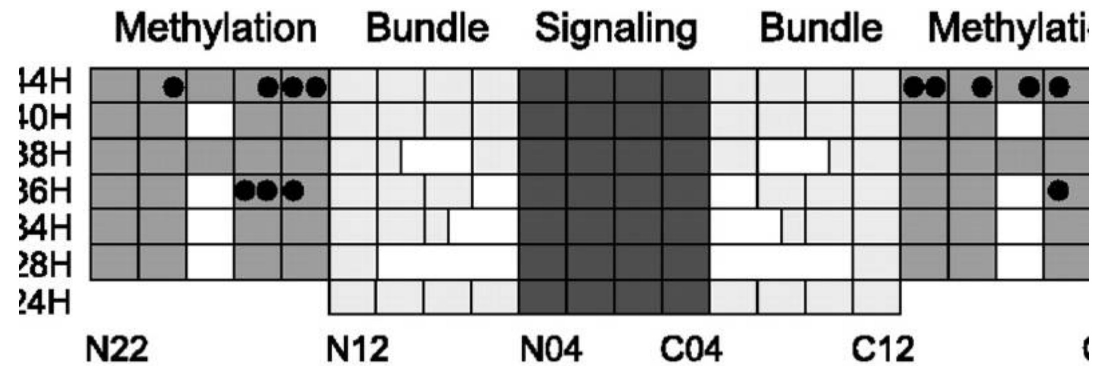


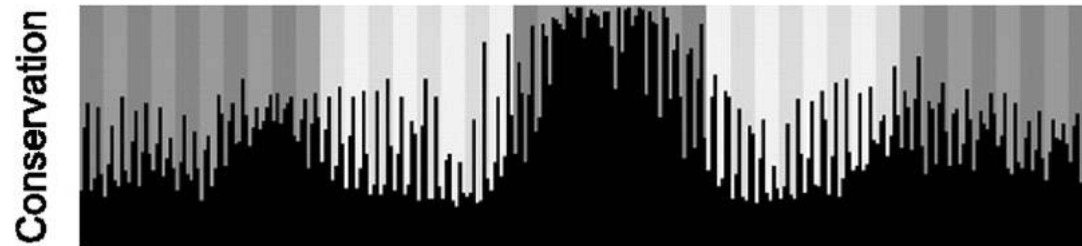
Rows of dimers

T.maritima

Chemoreceptors







Multiple sequence alignments

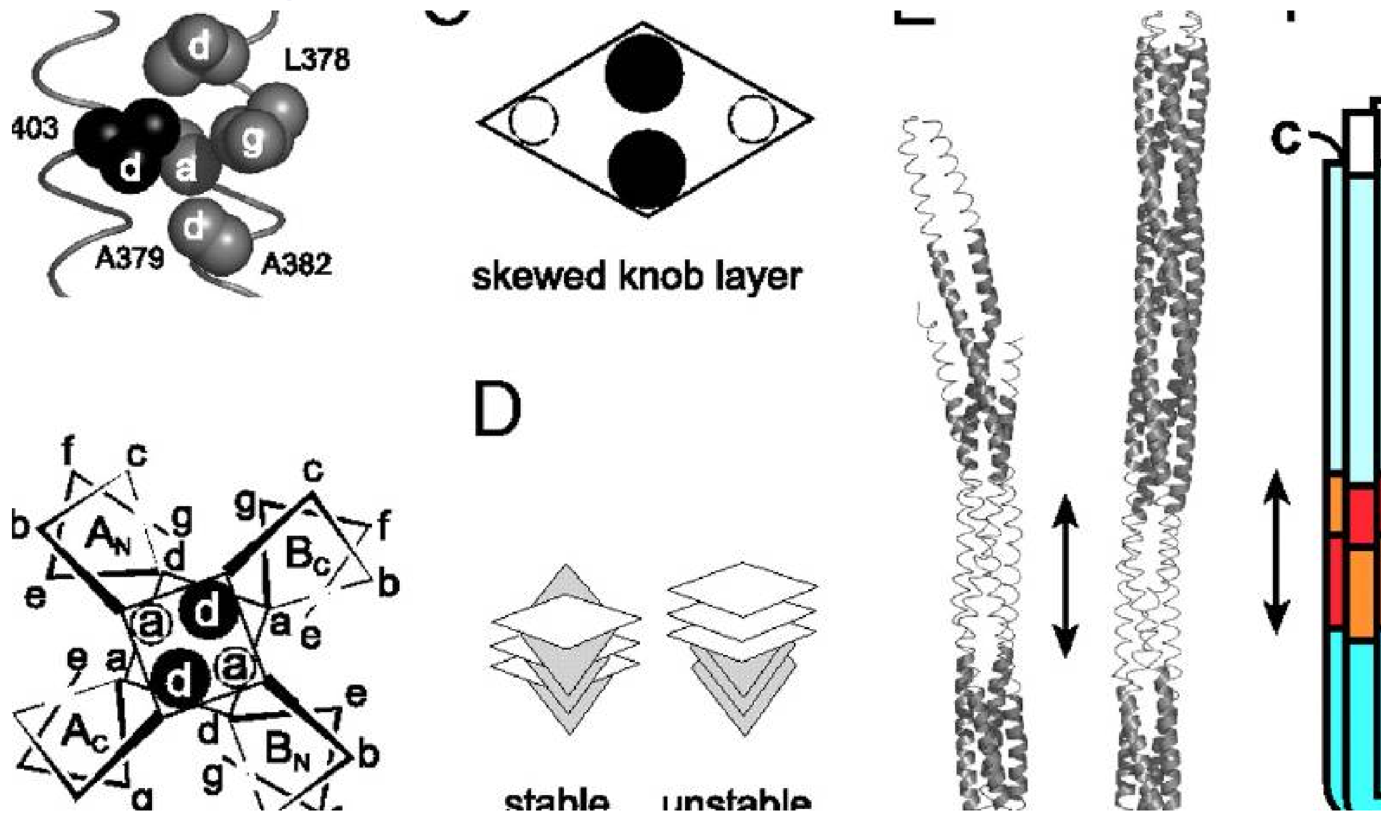
Zhulin

There are families

Alexander, Roger P. and Zhulin, Igor B. (2007) Proc. Natl. Acad. Sci. USA 104, 2885-2890

Only subset of 36H have CheR binding pentapeptide

Flexible subdomain allowing propagation of adaptation state



Alexander, Roger P. and Zhulin, Igor B. (2007) Proc. Natl. Acad. Sci. USA 104, 2885-2890

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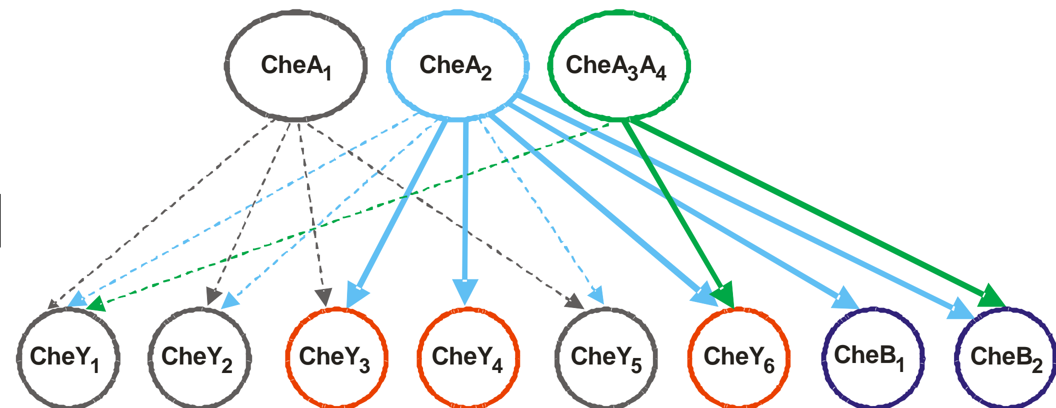
How will these MCP families cooperate in signalling?

- *E.coli* only has 5 MCPs-all 36H
- *M. magnetotacticum* has- 1 44H, 2 40H, 48 38H, 1 34H, 6 cytoplasmic
- *T.maritima* has- 6 44H, 1 cytoplasmic
- *Campylobacter jejuni* has- 1 40H, 5 28H, 1 24H, 2 cytoplasmic
- All 5 sequenced *Listeria* species have 1 44H and 1 24H

- How do these pack and signal? Are there separate clusters? Are they expressed under different conditions? Do different MCPs use different chemosensory pathways?
- Is adaptation the same? Is the sensitivity and gain similar?

Lessons from *Rhodobacter sphaeroides*

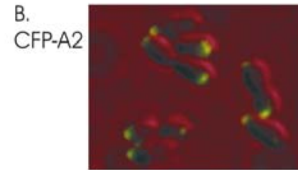
- Has operons encoding 3 complete chemosensory pathways
- *In vitro* phosphotransfer and Y2H show complex crosstalk between pathways
- 10 MCPs-1 36H, 6 38H, 3 cytoplasmic



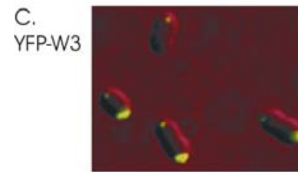
In vivo localisation

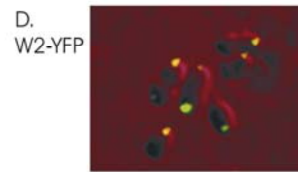
- All genes replaced in frame in-operon with fusion to *cfp* or *yfp*

Localisation of all Che proteins

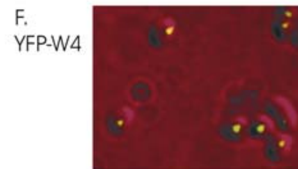


CheA2 polar

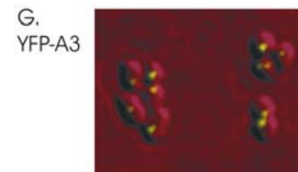


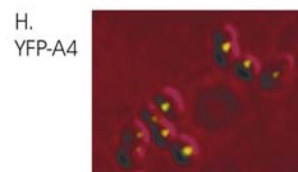


CheW2 and CheW3 polar

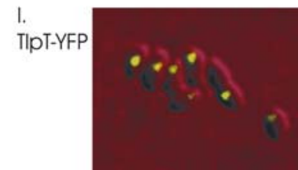


CheW4 cytoplasmic cluster





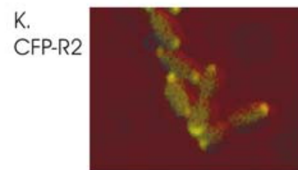
CheA3 and CheA4 cytoplasmic cluster

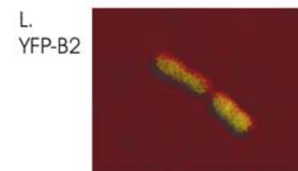


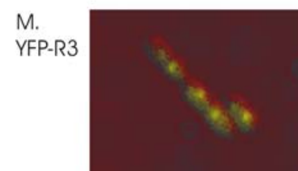
TlpT cytoplasmic cluster

Che Op2 encoded - polar

Che Op3 encoded - cytoplasmic





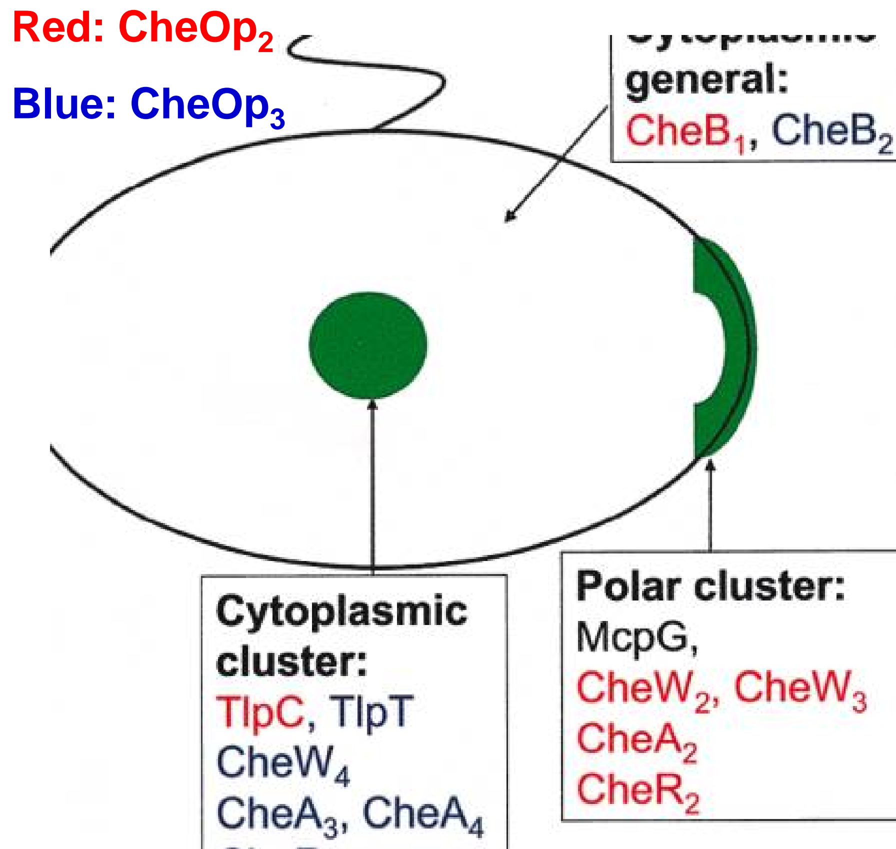


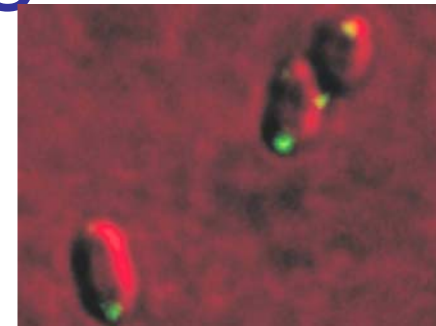
CheR2, mainly polar

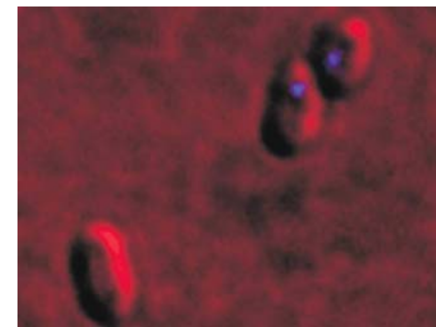
CheR3 mainly cytoplasmic cluster

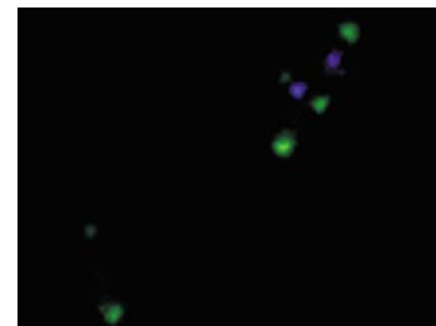
CheBs delocalised

Differentially targeted chemotaxis pathways – preventing crosstalk?









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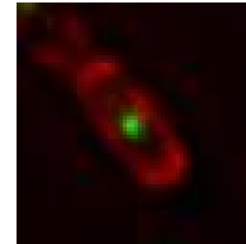
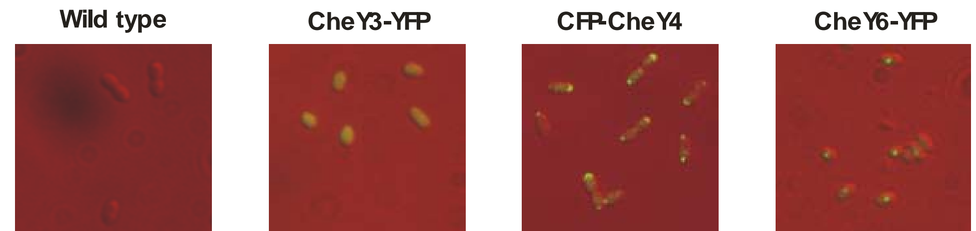
Localisation of the 2 clusters

- Clusters form independently of each other
- **When proteins for either cluster delocalise, they do not localise to other cluster (but do if overexpressed)**
- **This could happen for the different families of transmembrane receptors**



McpH-GFP, TlpT-CFP, ΔCheA2

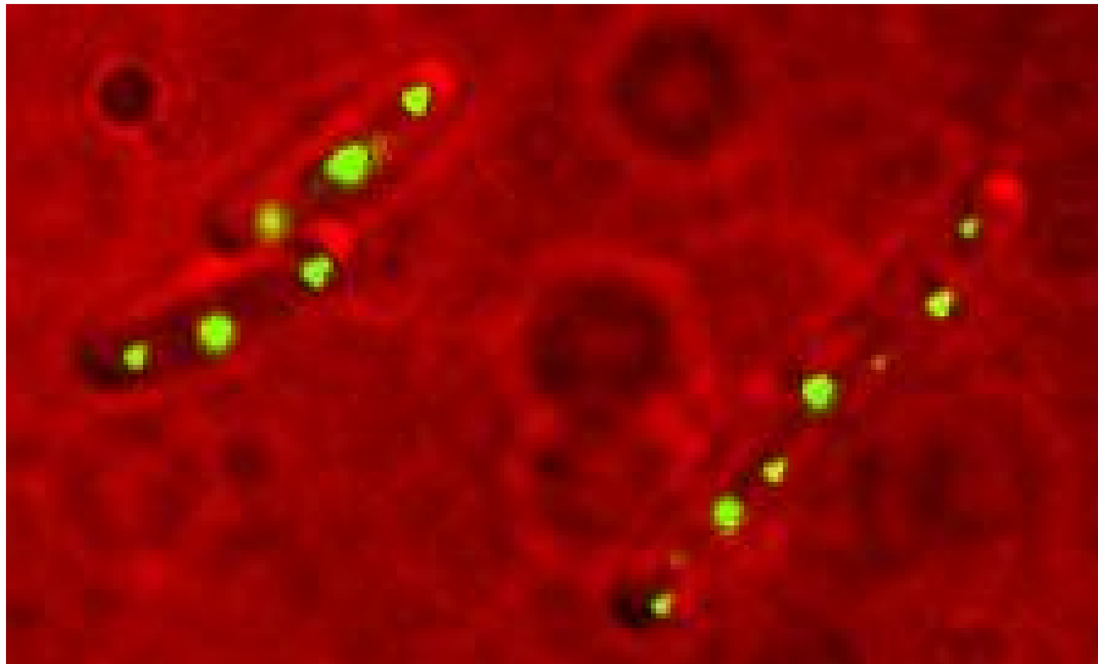


CheYs also localise to specific clusters

•Multiple "homologous" pathways can be spatially organised

•Deletion phenotype may be indirect result of disruption of complex

TlpT-YFP in filamentous cells



As with data from *E.coli* (Homma and Sourjik) chemotaxis clusters localise at defined intervals

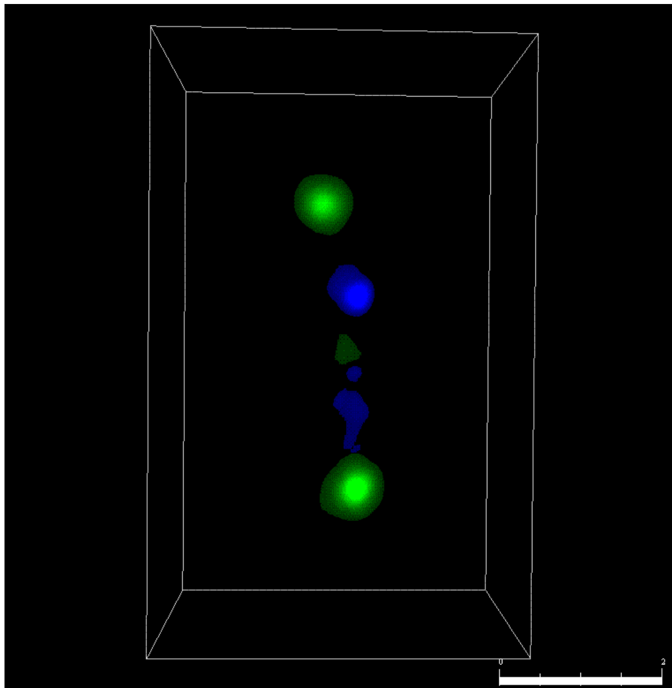
(cf- with division complexes)

Cephalexin treated

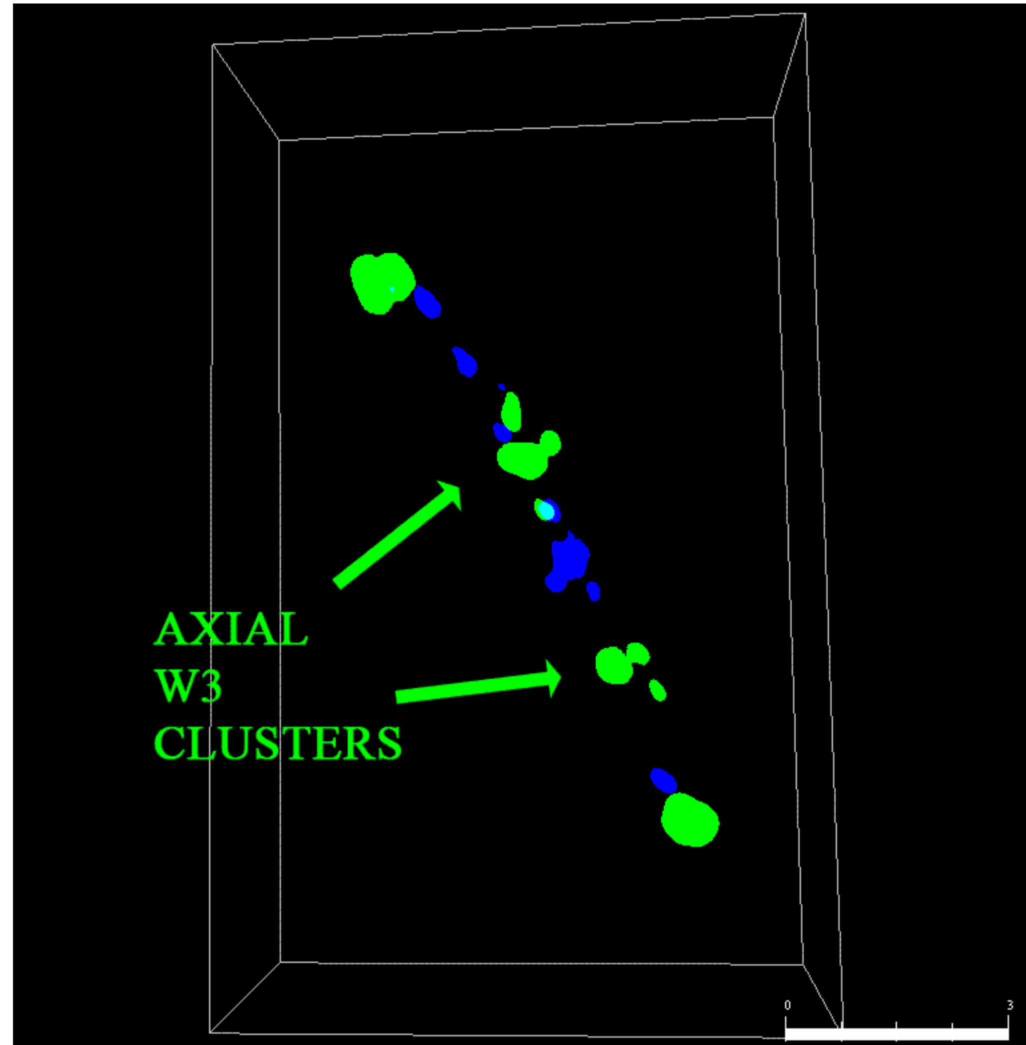
Separate signalling pathways

- Most bacterial species have not one closed chemosensory pathway, but at least 3, and as many as 8
- Each pathway may use a family of different receptors (up to 30 of each different type)
- Packing pattern of each family is probably different-in isolated membrane patches?
- Different associated kinases and different adaptation and termination kinetics
- Level of expression are environmentally regulated
- All pathway regulating switching of (usually) one type of flagellar motor
- How do the different pathways produce a balanced response?

Spatial organisation



- Cytoplasmic pathway sensing metabolic state
- Polar cluster sensing outside world
- Level of expression of each in each cell not related



Other protein complexes localise

- Divisome
- Differentiation complex PleC/PleD in *Caulobacter*

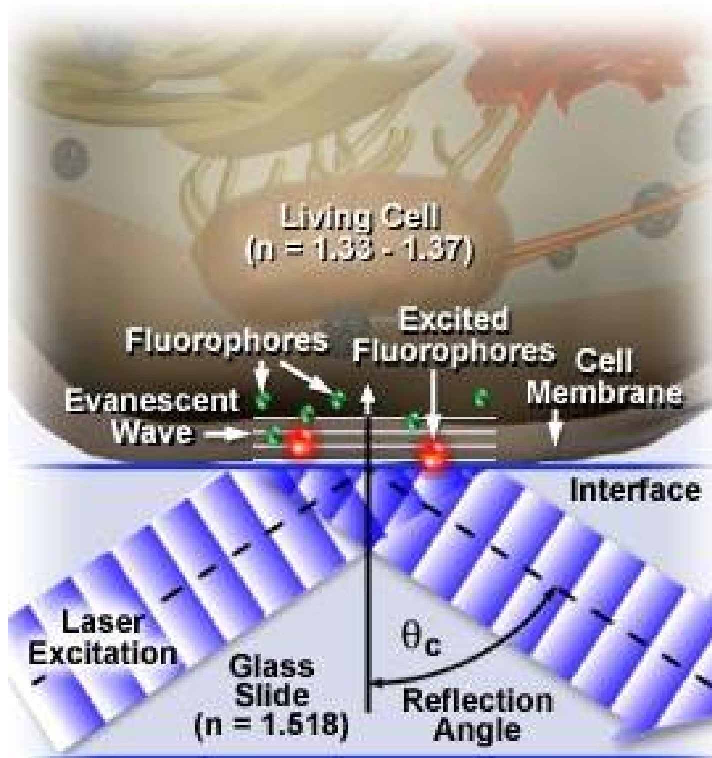
Urs Jenal

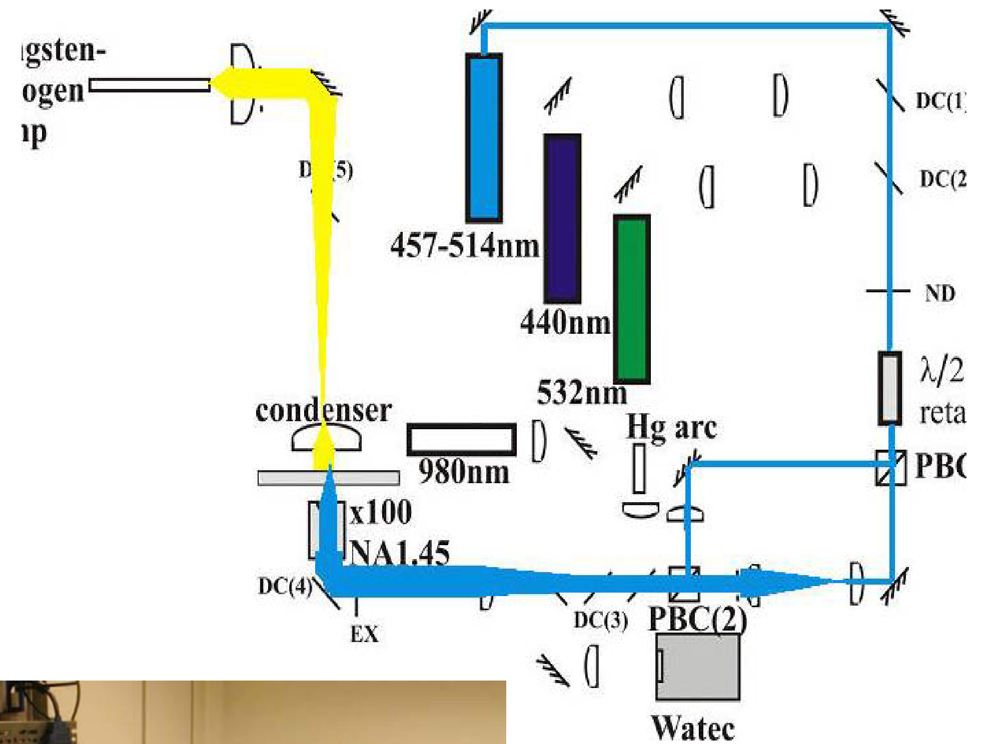
Extending the *E.coli* models to identify whether sensory pathways are linear or parallel

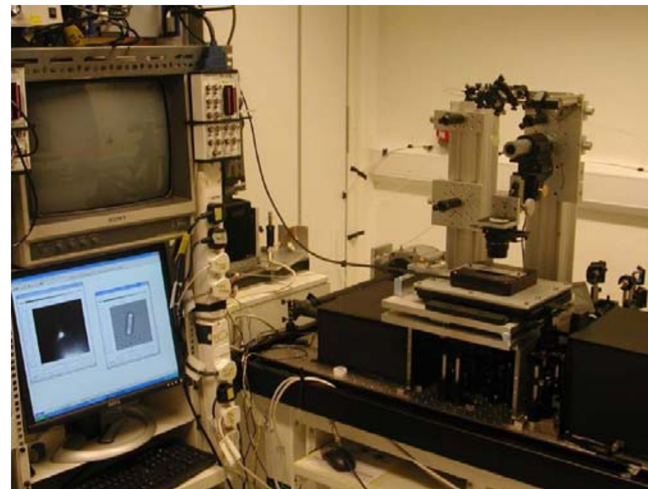
Why are the pathways differentially regulated and what does change in relative copy number mean to sensitivity?

-needs single cell analysis

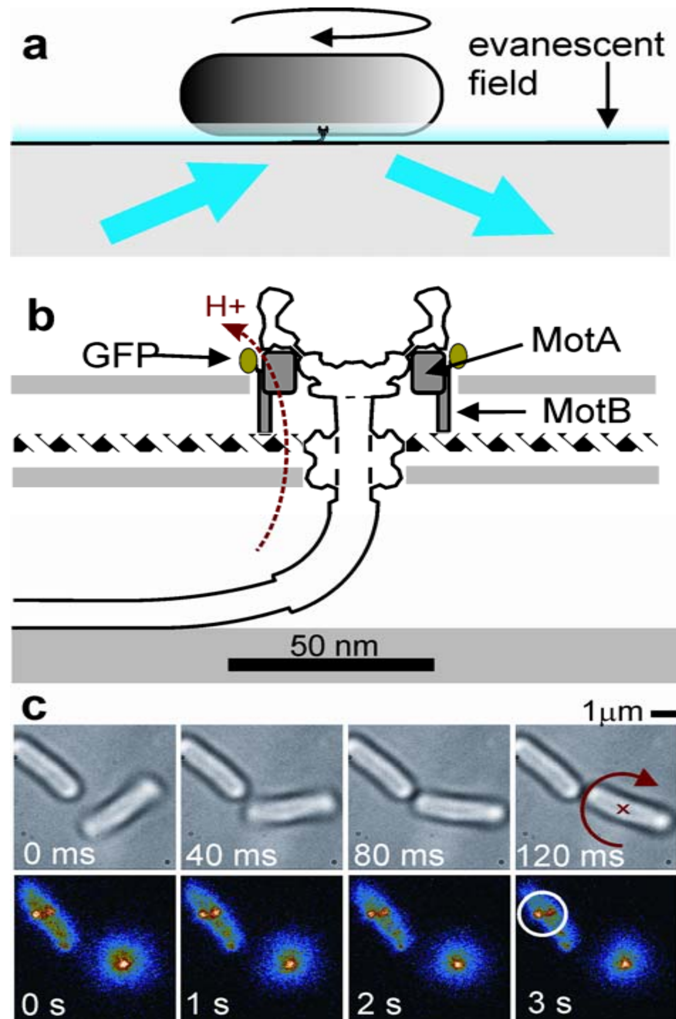
Single-cell FRET TIRF Microscopy

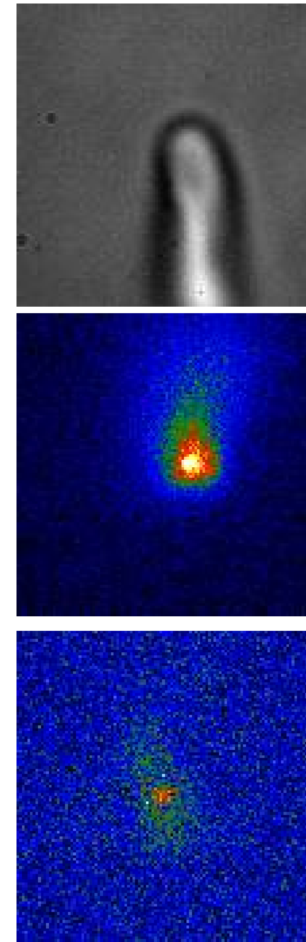






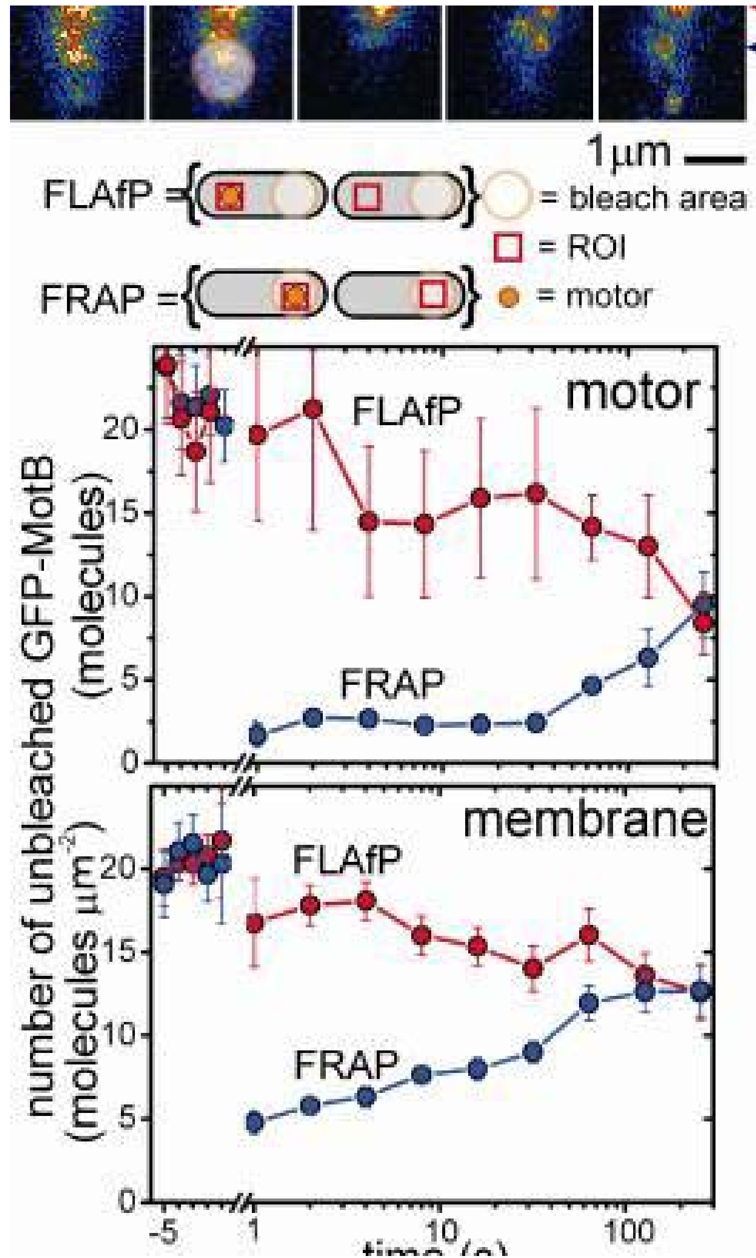
GFP-MotB: genomic replacement

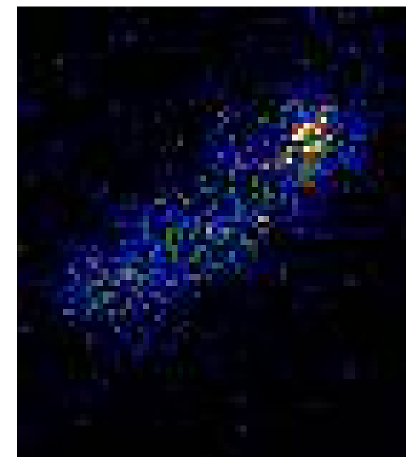




FRAP

Calculation of diffusion and turnover rate





Examining dynamics of complexes, single proteins and interaction kinetics

What will interact when
(a) overexpressed or
(b) in Y2H or B2H
or (c) pull downs
may not interact *in vivo*
and *vice versa*

Your interactome may be misleading if only validated *in vitro*

Need to know what does interact rather than what can interact

Local concentration may also not be what you measure in whole cell extracts

Biology is dynamic and organised-not a random soup of proteins

Care is needed when extrapolating from one very good model to different bacterial species

Challenge-need medium throughput *in vivo* imaging

Which range of interactions do you need to image to be reasonably confident?

To do some systems well-need “slow” throughput as well as high through put

The workers

George Wadhams
Steve Porter
Steve Thompson
Marcus Gould
Mark Roberts
Alice Ind
Nicolas Delalez
Mostyn Brown
David Wilkinson
Yo-Cheng Chang
Elaine Byles

Angela Martin
Jenny Chandler
Rachel Webster

Richard Berry
Mark Leake
Stuart Reid

Philip Maini
Marcus Tindall
Anna Chenova
Antonis Papachristodoulou
Denis Bray