EMBO PPI Training Course
TGAC Norwich, 1-10-2015

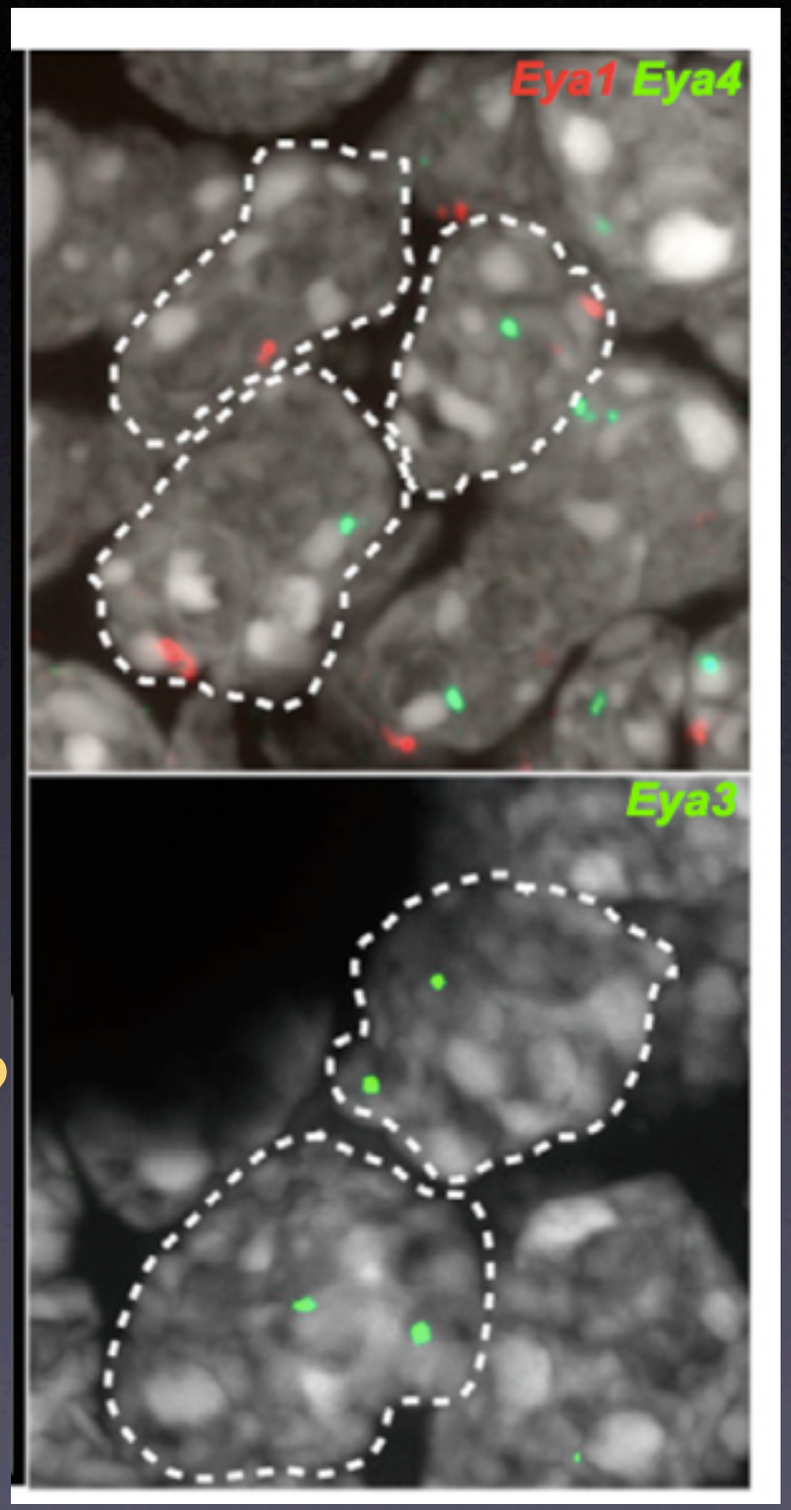
Modular Protein Architecture and the Construction of Cell Regulatory Systems

Toby J. Gibson
Structural & Computational Biology Unit
EMBL, Heidelberg

Using RNA fish, Eya4 shows random monoallelic expression (RME) in eye development

Many, many developmentally important genes show RME

Question: *Why should genes be expressed from just one of the two alleles during development?*

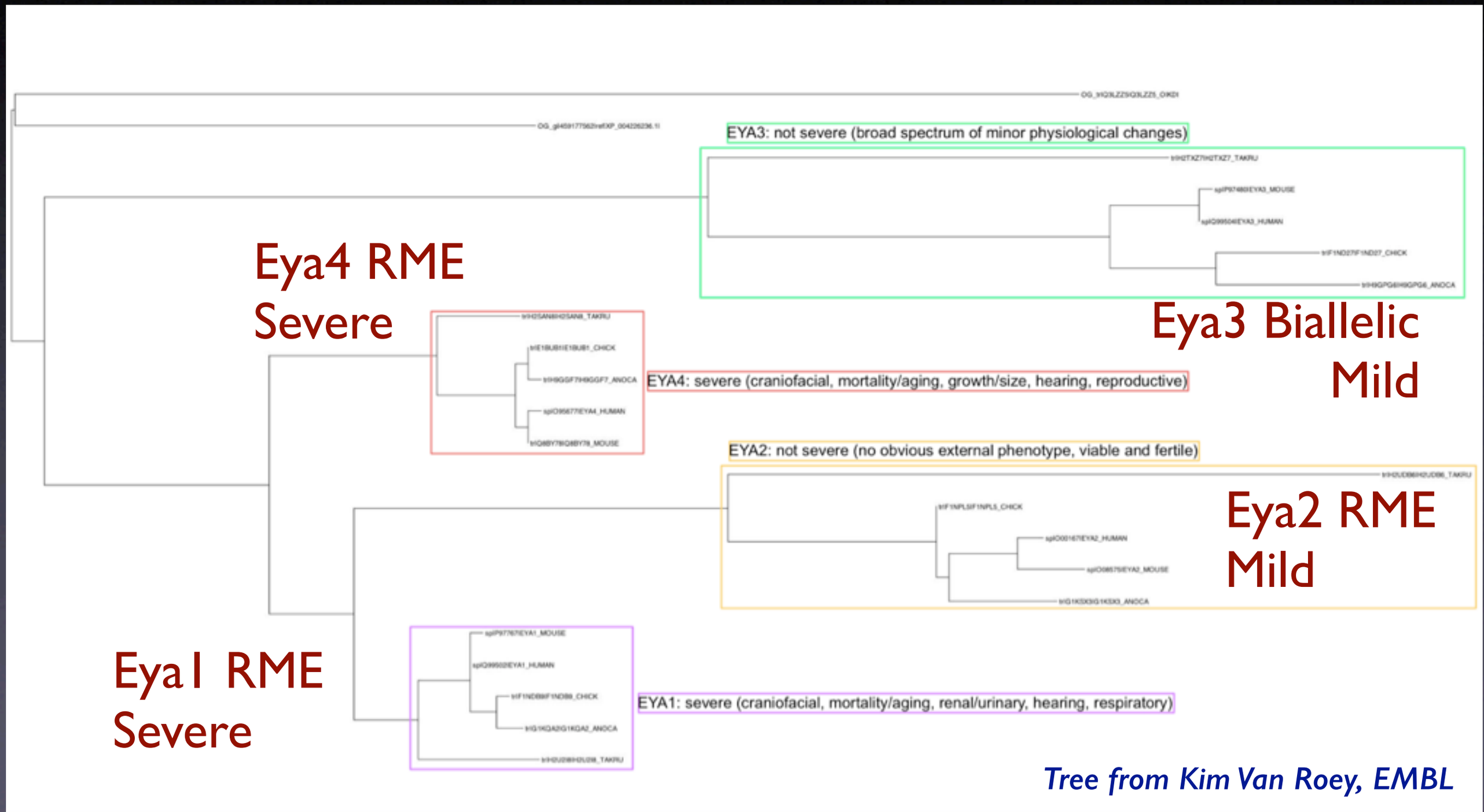


Eya1, Eya4
Monoallelic

Eya3
Biallelic

Gendrel et al. (2014) Dev. Cell 28, 366

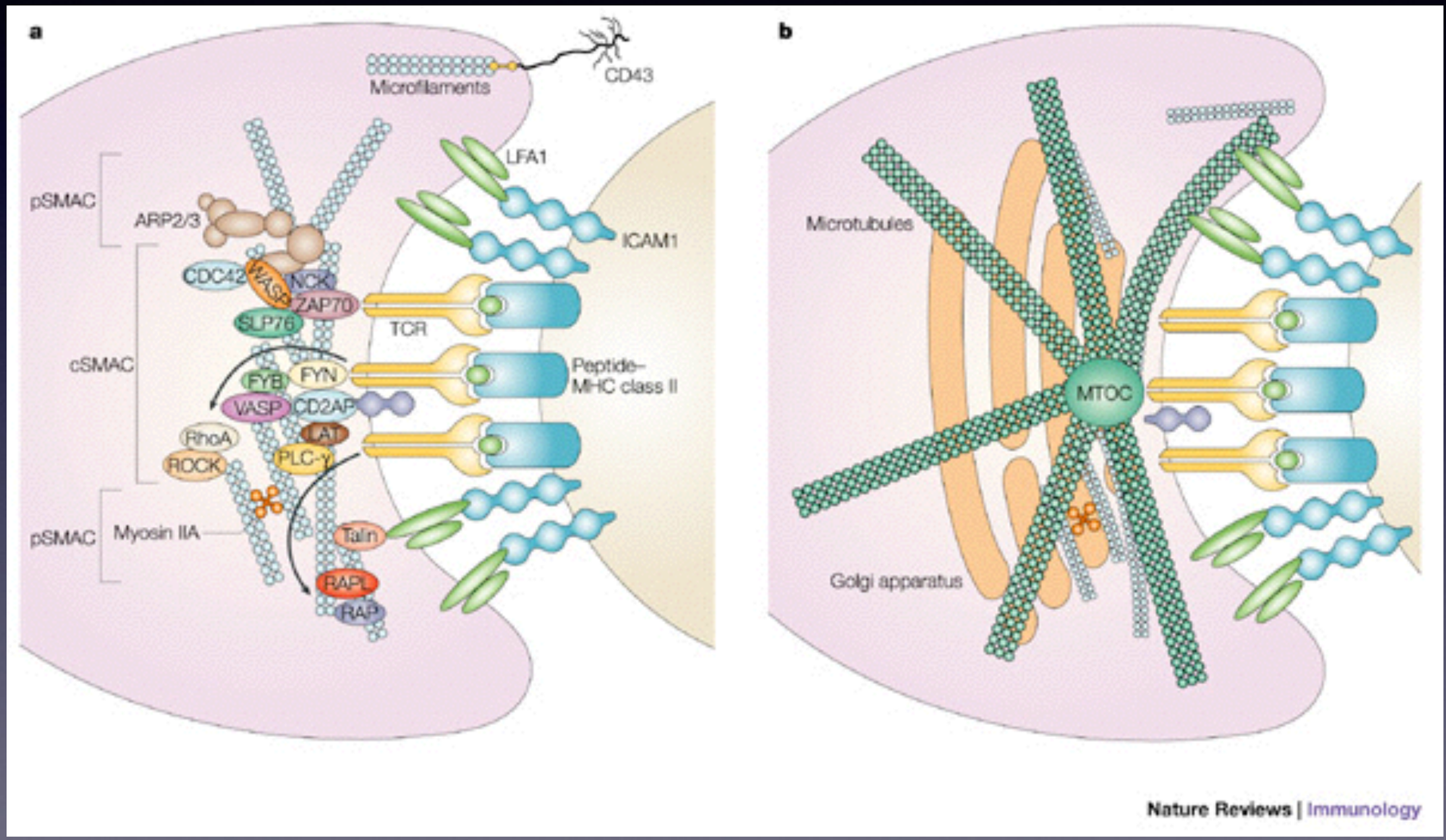
Eya paralogues are evolving at different rates. Gene knockouts have different severities. Eya1 and Eya4 heterozygotes have strong phenotypes.



Tree from Kim Van Roey, EMBL

When a signal is received by a membrane receptor, what happens next?

The Immunological Synapse - A platform for multisignal input and output in T Cell activation

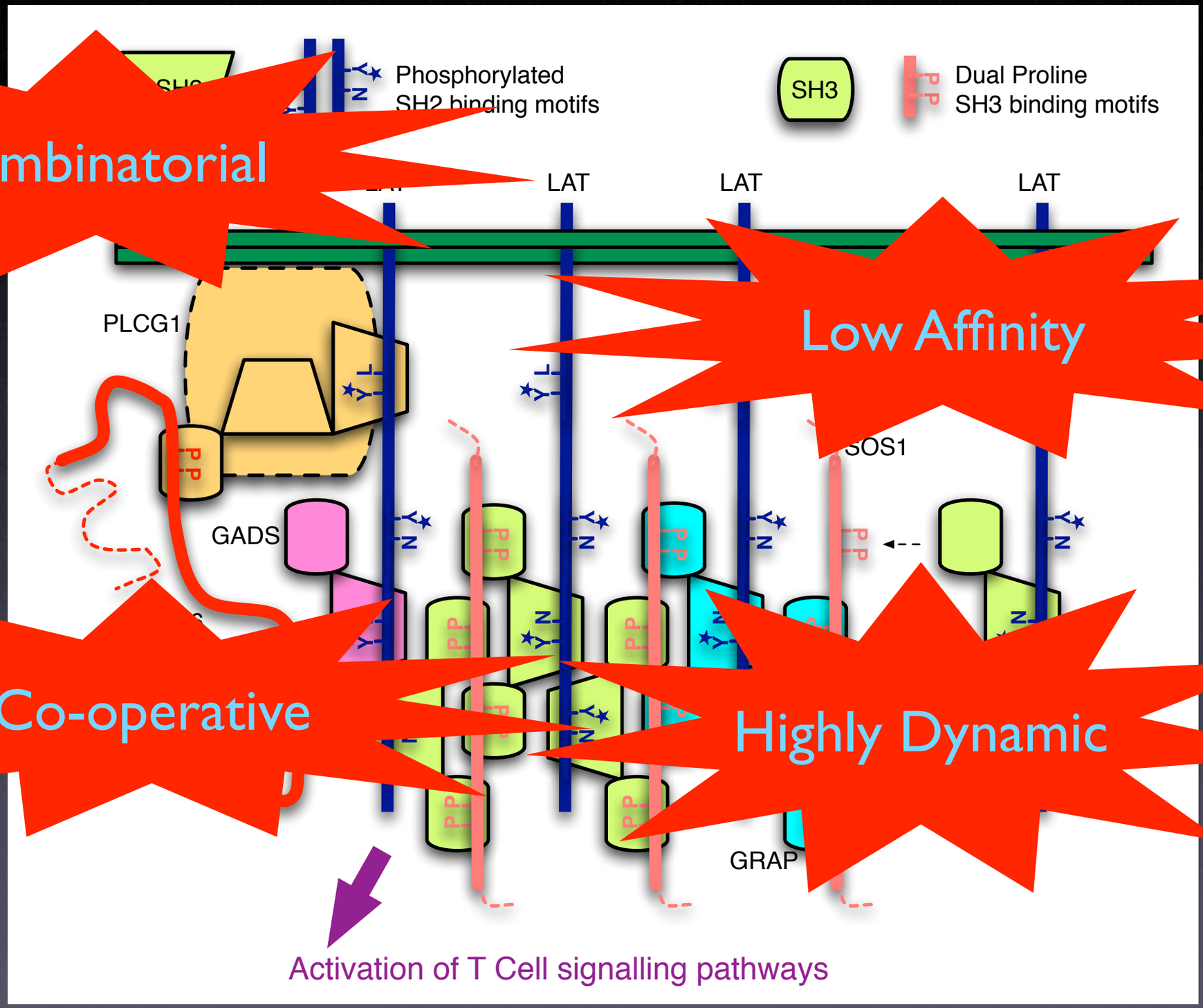


Vicente-Manzanares et al. (2004) Nat. Rev. Imm. 4, 110

Propagation of T Cell signalling

Multivalent assembly of the LAT signalling complex by short linear motifs

LAT is phosphorylated by TKs ZAP-70 and/or SYK



Combinatorial

Low Affinity

Co-operative

Highly Dynamic

After Houtman et al. (2006) NS&MB, 13, 798

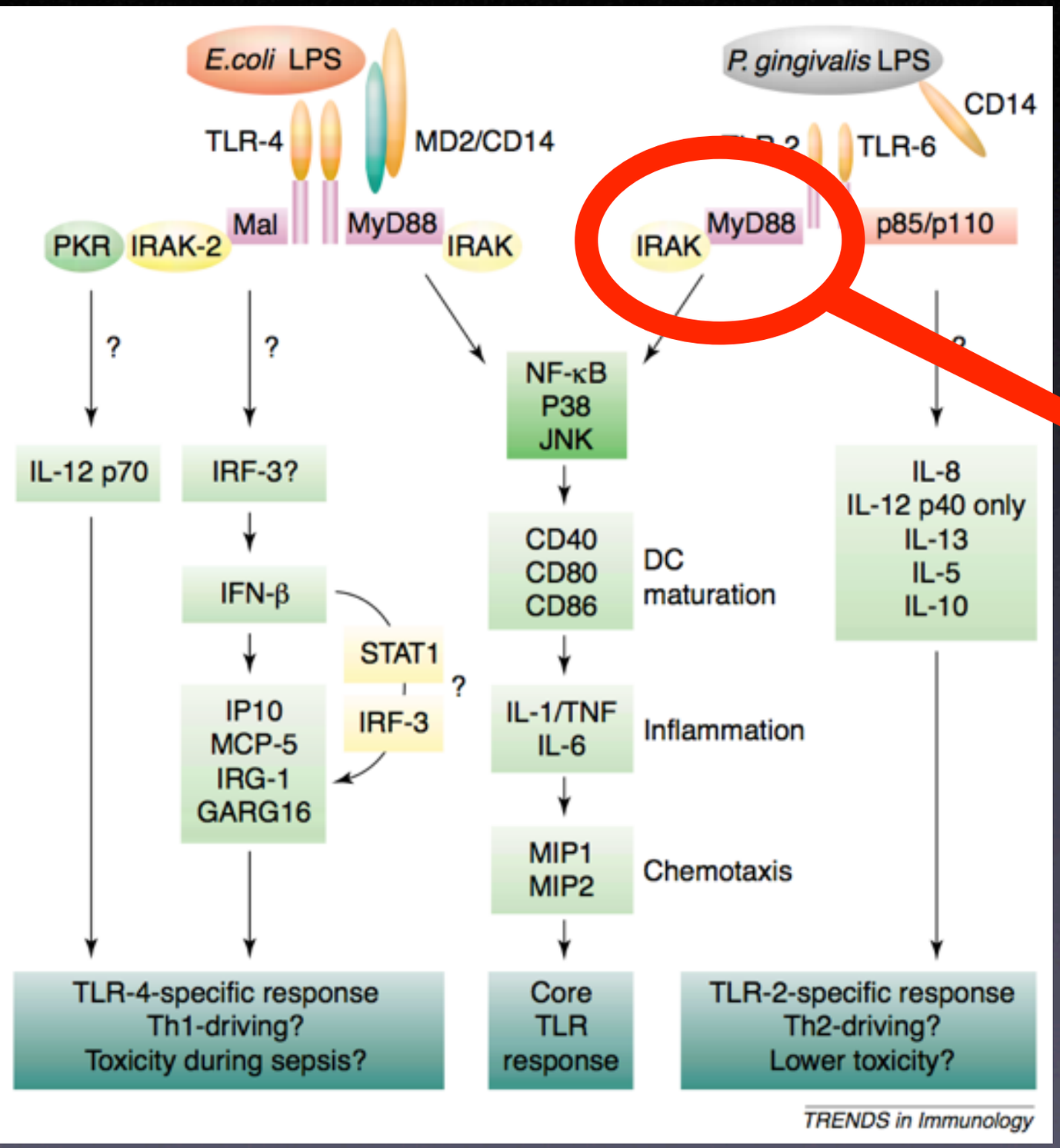
The LAT interaction fur-ball retrieved from the STRING server

Is this a good representation of the molecular details?

STRING is at http://string.embl.de/

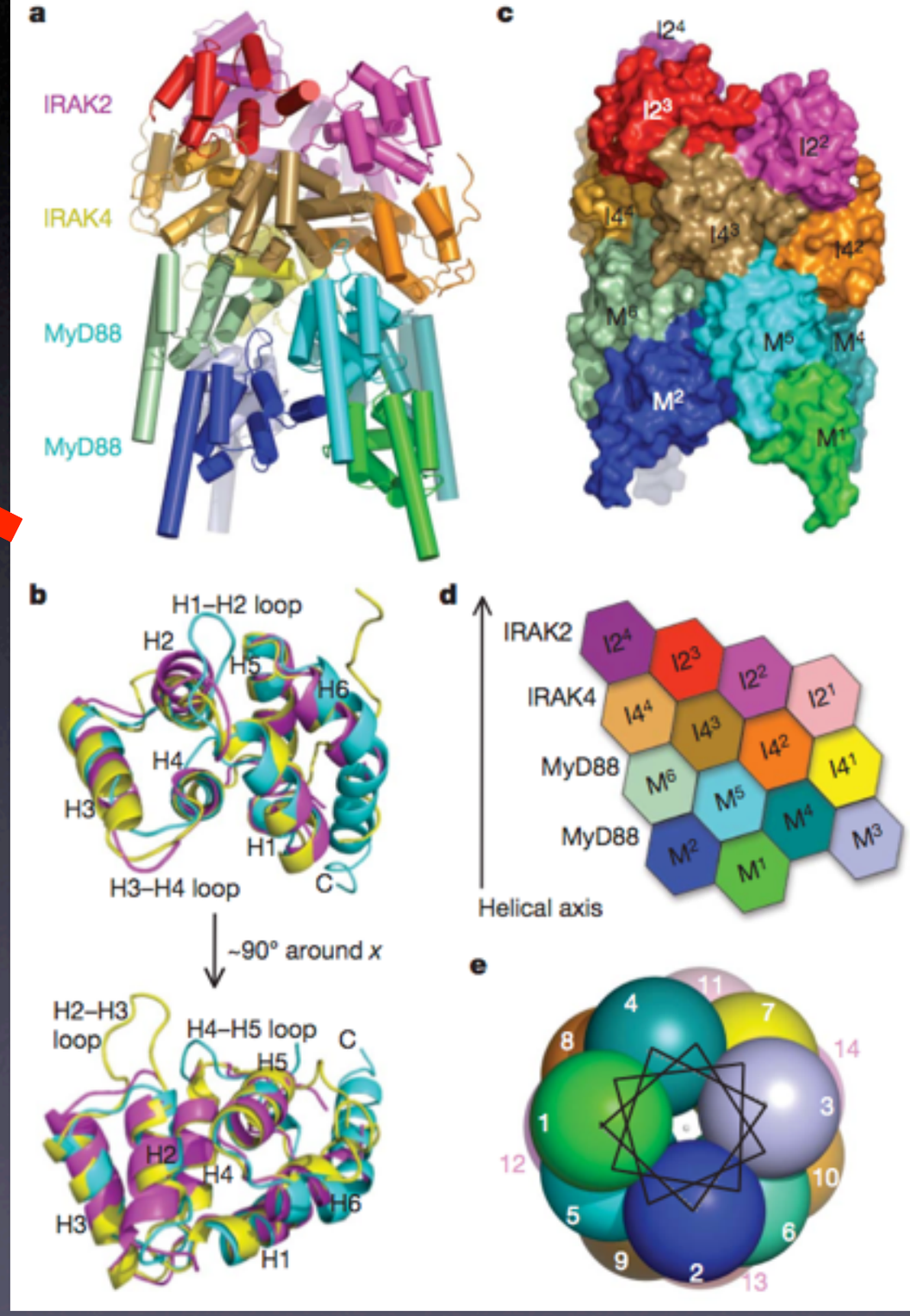
Innate Immunity

Toll-like receptor signalling



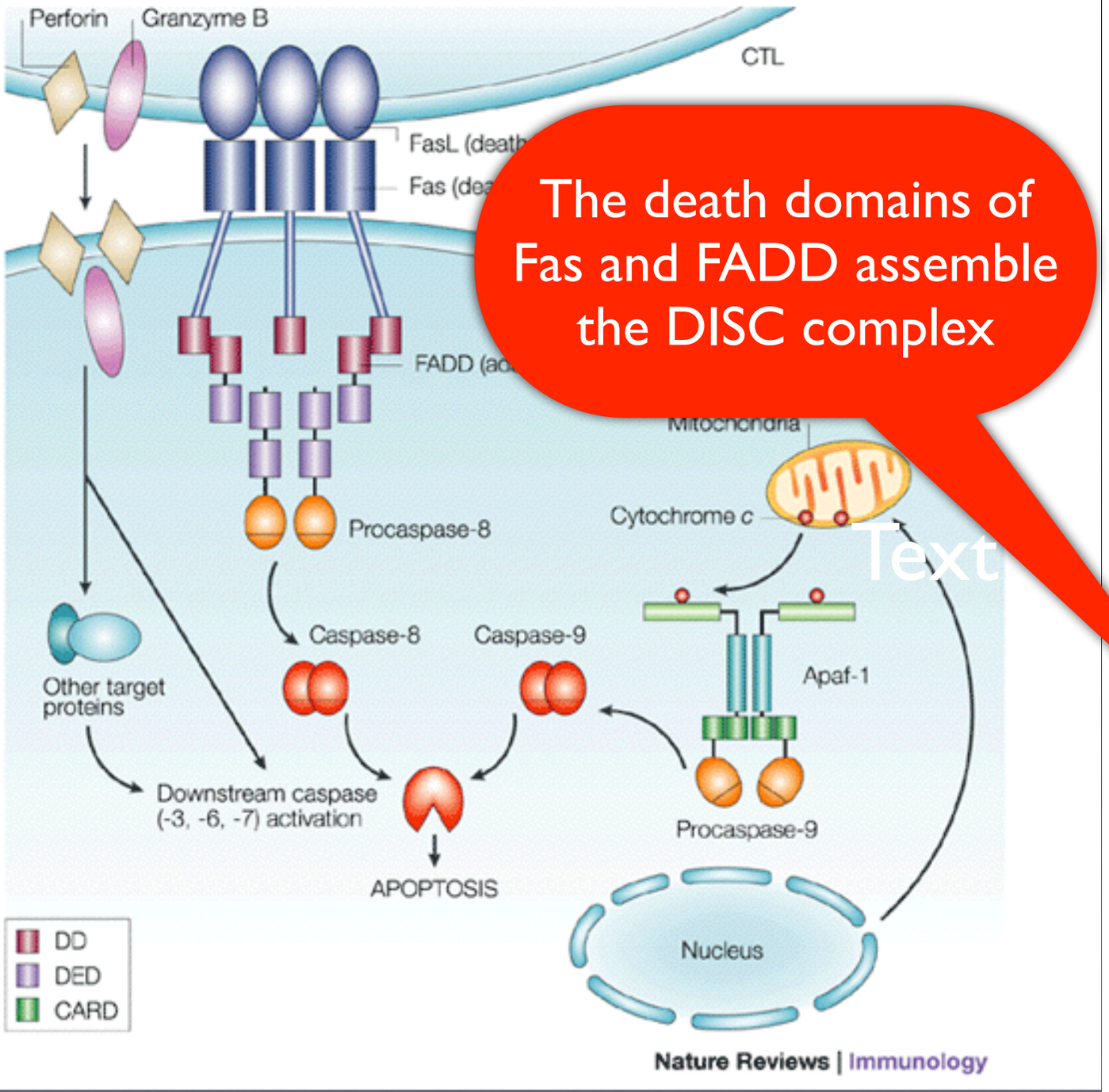
O'Neill (2002) Trends Imm. 23, 296

Assembly of the myddosome using death domains from MyD88, IRAK4, IRAK2



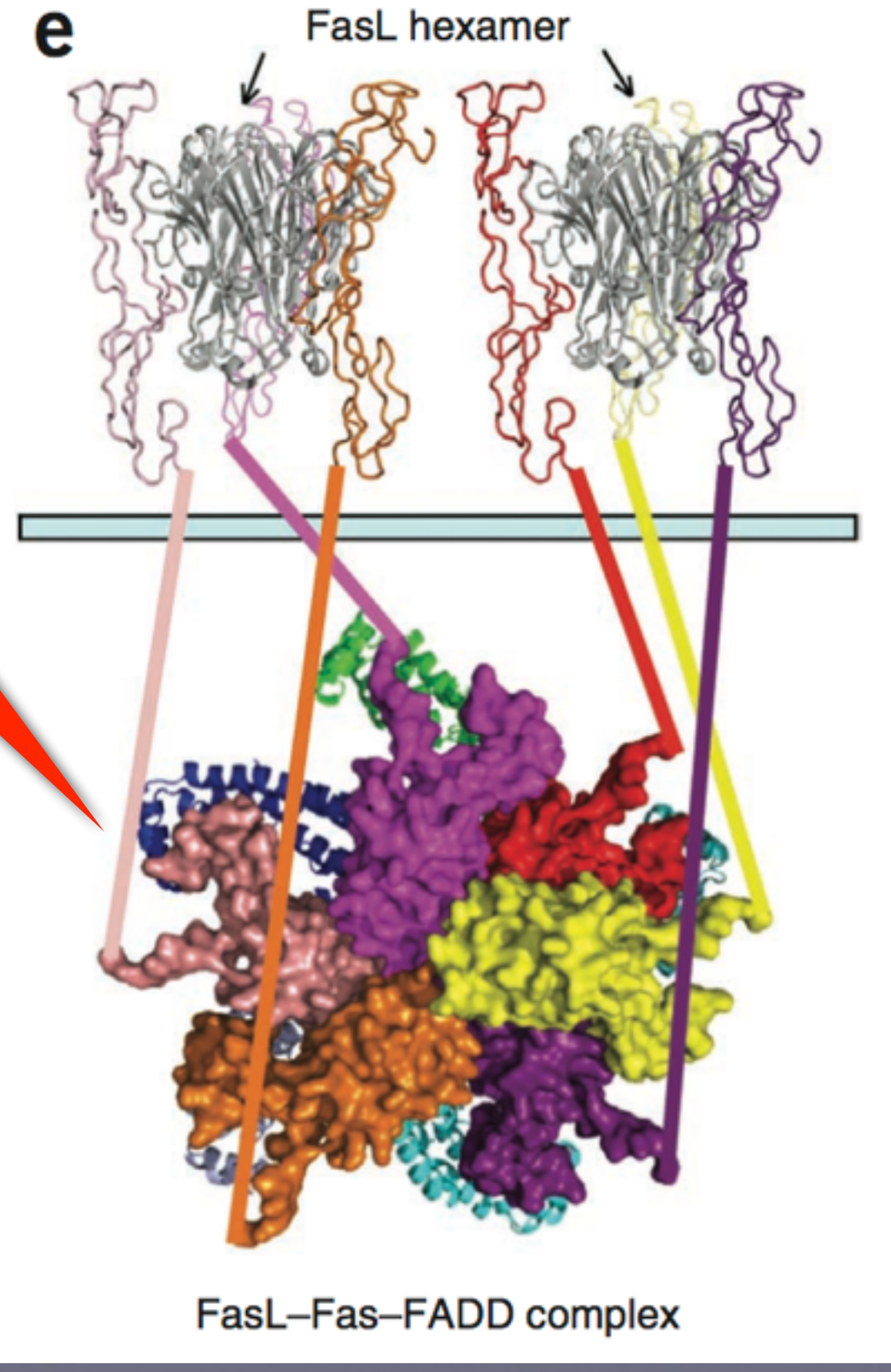
Lin et al. (2010) Nature 465, 885

Apoptotic signalling by FasL, FasR, FADD



The death domains of Fas and FADD assemble the DISC complex

*Thome and Tschopp (2001) Nat. Rev. Immunol. **1**, 50*



*Wang et al. (2010) NSMB. **17**, 1324*

When a signal is received by a membrane receptor, what happens next?

Answer

Typically, a discrete signalling platform is assembled to integrate other cell state signals so that an informed decision leads to the correct outcome

You are an engineer:

If system reliability is critical, would you design a simple system or a complex one?

Robustness of biological systems

Complexity and robustness

J. M. Carlson*† and John Doyle‡

*Department of Physics, University of California, Santa Barbara, CA 93106; and †Control and Dynamical Systems, California Institute of Technology, Pasadena, CA 91125

Carlson and Doyle (2002) PNAS, 66, 2538

...By robustness, we mean the maintenance of some desired system characteristics despite fluctuations in the behavior of its component parts or its environment....

BIOLOGICAL ROBUSTNESS

Hiroaki Kitano

Abstract | Robustness is a ubiquitously observed property of biological systems. It is considered to be a fundamental feature of complex evolvable systems. It is attained by several underlying principles that are universal to both biological organisms and sophisticated engineering systems.

Kitano (2004) Nat Rev Genet, 5, 826

CH Waddington (1905-1975)

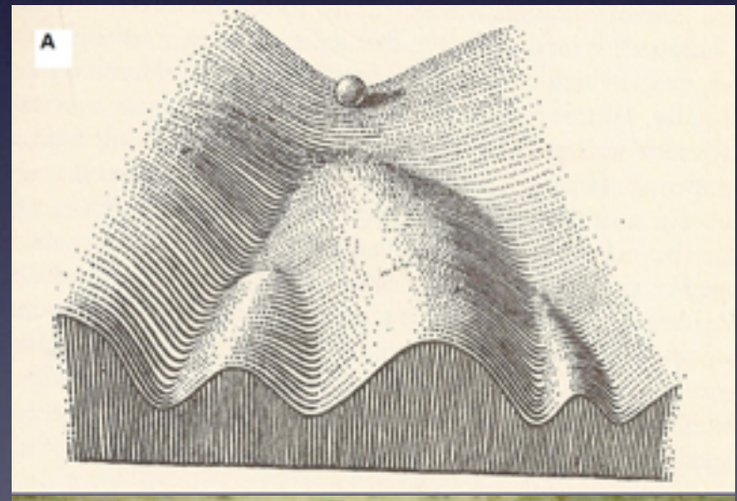
- A unifier of development and genetics

- A forefather of systems biology

- System robustness and weak phenotypes

Some of Waddington's concepts:

- Epigenetic Landscape
  - *Developmental cell fates and increasing irreversibility*
- Canalisation
  - *Robustness in developmental processes*
- COWDUNG
  - *Conventional Wisdom of the DUmiNant Group*



doi:10.1038/nature10539

A SUMOylation-defective MITF germline mutation predisposes to melanoma and renal carcinoma

Corine Bertolotto[1,2,3]*, Fabienne Lesueur[4]†*, Sandy Giuliano[1,2]*, Thomas Strub[5], Mahaut de Lichy[4], Karine Bille[1], Philippe Dessen[6], Benoit d'Hayer[4], Hamida Mohamdi[7,8,9], Audrey Remenieras[4]†, Eve Maubec[7,10], Arnaud de la Fouchardière[11], Vincent Molinié[12], Pierre Vabres[13], Stéphane Dalle[14], Nicolas Poulalhon[14], Tanguy Martin-Denavit[14], Luc Thomas[14], Pascale Andry-Benzaquen[15], Nicolas Dupin[15], Françoise Boitier[15], Annick Rossi[16], Jean-Luc Perrot[17], Bruno Labeille[17], Caroline Robert[18], Bernard Escudier[18], Olivier Caron[18], Laurence Brugières[19], Simon Saule[20], Betty Gardie[21], Sophie Gad[21], Stéphane Richard[21,22], Jérôme Couturier[23], Bin Tean Teh[24,25], Paola Ghiorzo[26], Lorenza Pastorino[26], Susana Puig[27], Celia Badenas[27], Hakan Olsson[28], Christian Ingvar[29], Etienne Rouleau[30], Rosette Lidereau[30], Philippe Bahadoran[3], Philippe Vielh[31], Eve Corda[7,9], Hélène Blanché[9], Diana Zelenika[32], Pilar Galan[33], The French Familial Melanoma Study Group‡, Valérie Chaudru[7,9,34], Gilbert M. Lenoir[4,35], Mark Lathrop[9,32], Irwin Davidson[5], Marie-Françoise Avril[15], Florence Demenais[7,8,9], Robert Ballotti[1,2,3]* & Brigitte Bressac-de Paillerets[4,7]*

both cancers, when compared with controls. Overall, Mi-E318K carriers had a higher than fivefold increased risk of developing melanoma, RCC or both cancers. Codon 318 is located in a small-ubiquitin-like modifier (SUMO) consensus site (ΨKXE) and Mi-E318K severely impaired SUMOylation of MITF. Mi-

Five-fold risk increase is an example of a genetic lesion that causes a weak phenotype

Increases in system complexity due to selection for robustness introduce a new issue: **system fragility**

A good example is the Internet which is:

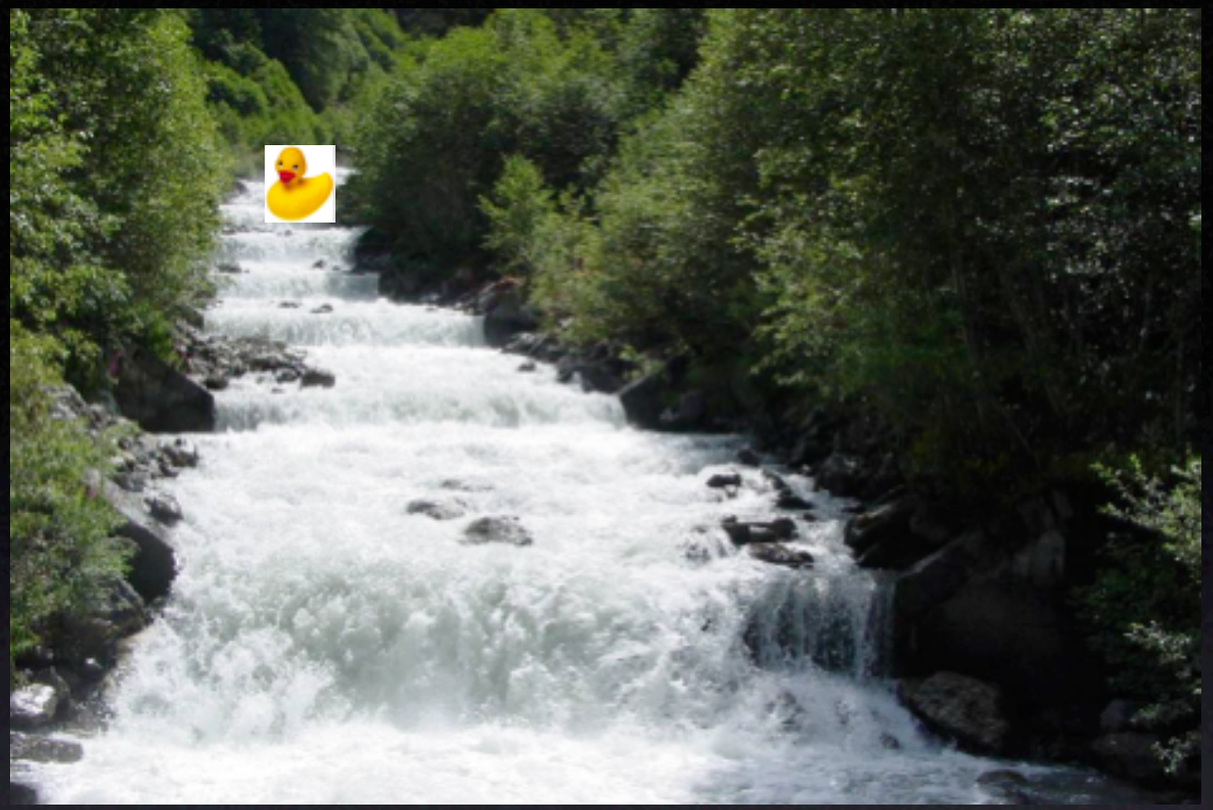
"robust yet fragile" (RYF)

that is, unaffected by random component failures but vulnerable to targeted attacks on its key components.

Cascades

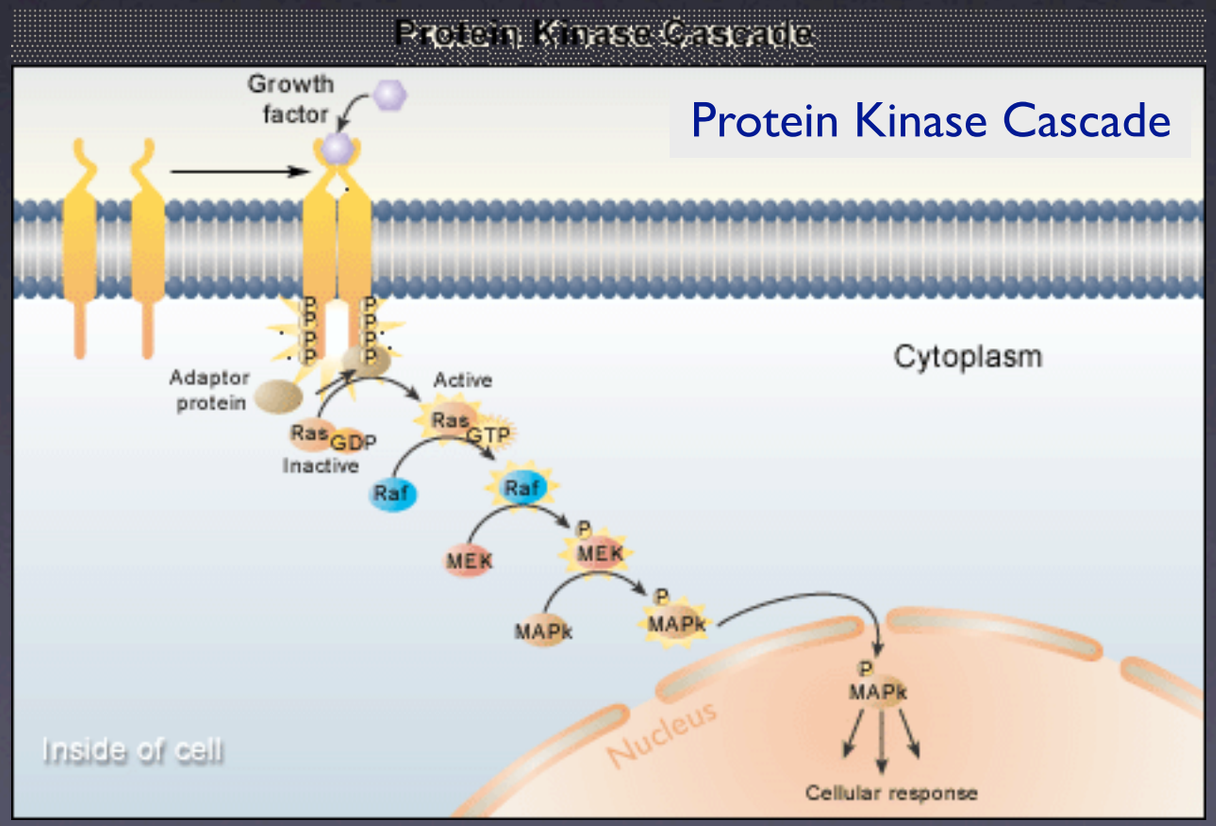
Properties

Linearity
Uneven
Accellerating
Unregulated
Uncertain end point?



Cascade in South Tyrol, source K. Amon and G. Zsoldos

Cascading mechanisms
are neither accurate
nor precise



source http://www.biology.arizona.edu/cell_BIO/

The first report of a protein kinase cascade

Cell

Result list |

Volume 25, Issue 1, July 1981, Pages 9-21

doi:10.1016/0092-8674(81)90227-0 Cite or Link Using D

Article

A mouse homolog to t[illegible]viar[illegible]rcoma virus *src* protein is a mem[illegible]of a p[illegible]ein kinase cascade

Mark Spector, Robe[illegible]B.[illegible]ins, Volker M. Vogt and Efraim Racker

Section of Bioc[illegible]str[illegible]Mole[illegible]ar and Cell Biology Wing Hall Cornell University, Ithaca, [illegible]York 14853, USA

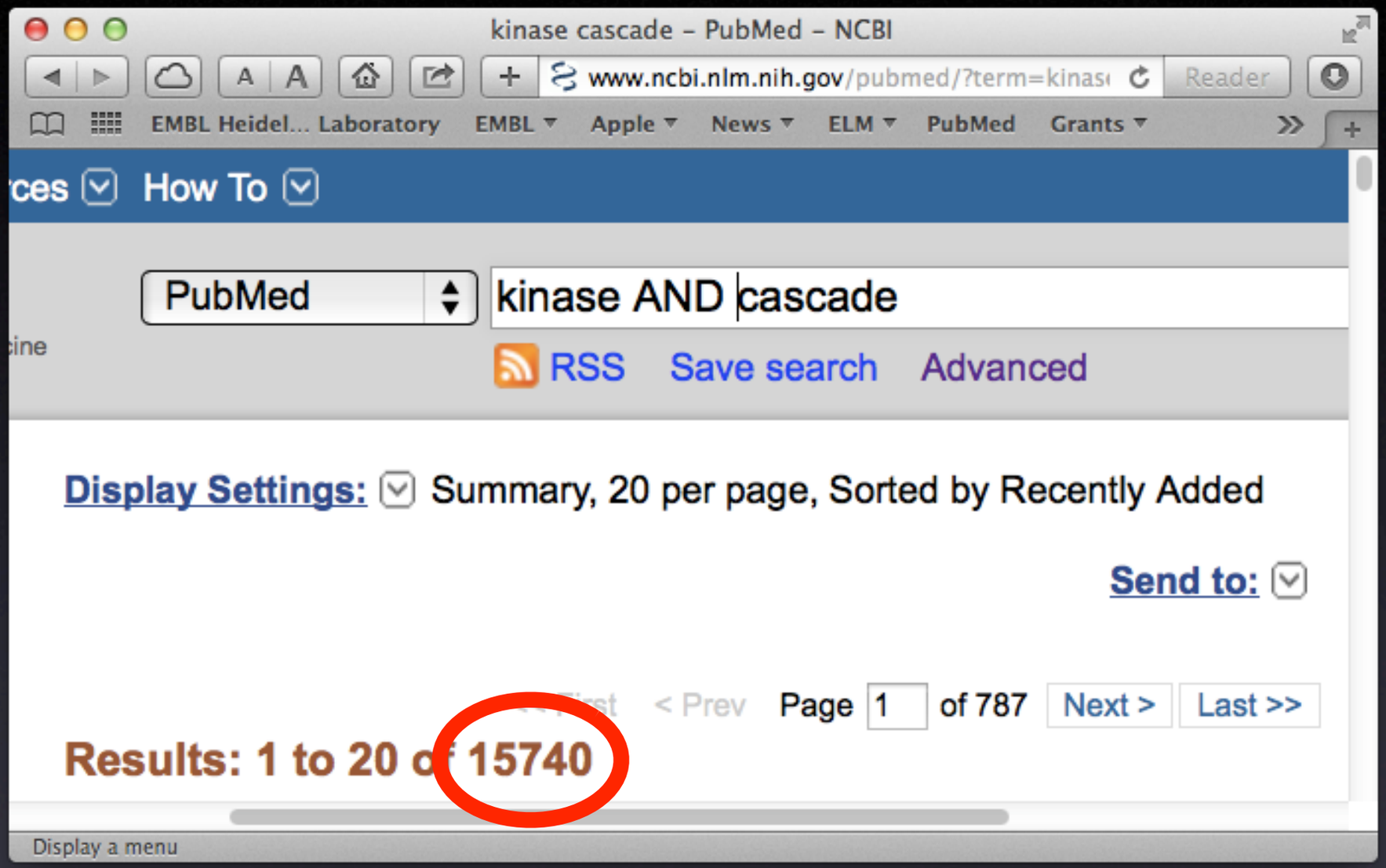
Received 2 March 19[illegible]Revised 21 April 1981. Available online 28 April 2004.

Abstract

Recent work has identified a cascade of membrane-bound protein kinases in Ehrlich ascites tumor cells. These enzymes, designated PK_L, PK_S and PK_M, are present in both Ehrlich tumor and mouse

Vast literature on kinase cascades

They must be well understood by now

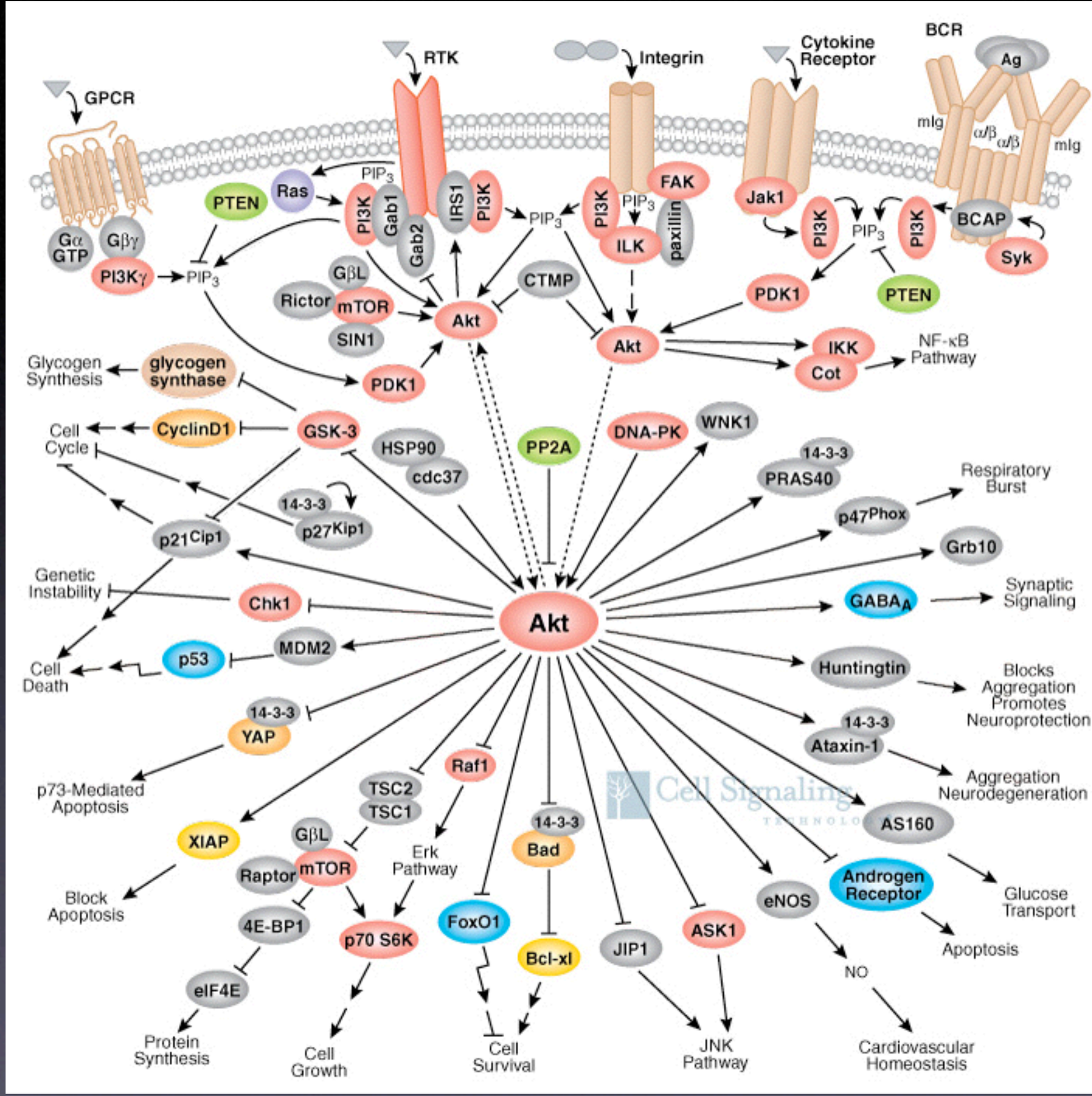


There is a Gene Ontology term too:

GO:0007243. protein kinase cascade. *A **series** of reactions, mediated by protein kinases, which occurs as a result of a single trigger reaction or compound.*

AKT / PKB Kinase Cascade

Cascade or Network?



http://www.cellsignal.com/reference/pathway/Akt_PKB.jsp

Most Tyrosine Kinases have very limited sequence specificity

- *in vivo* TK substrate detection remains difficult
- *in vivo* substrates ≠ good in *vitro* peptides
- Cannot define a simple sequence pattern at phosphosite
- Problem: how do they avoid each other's substrates?

ELSEVIER

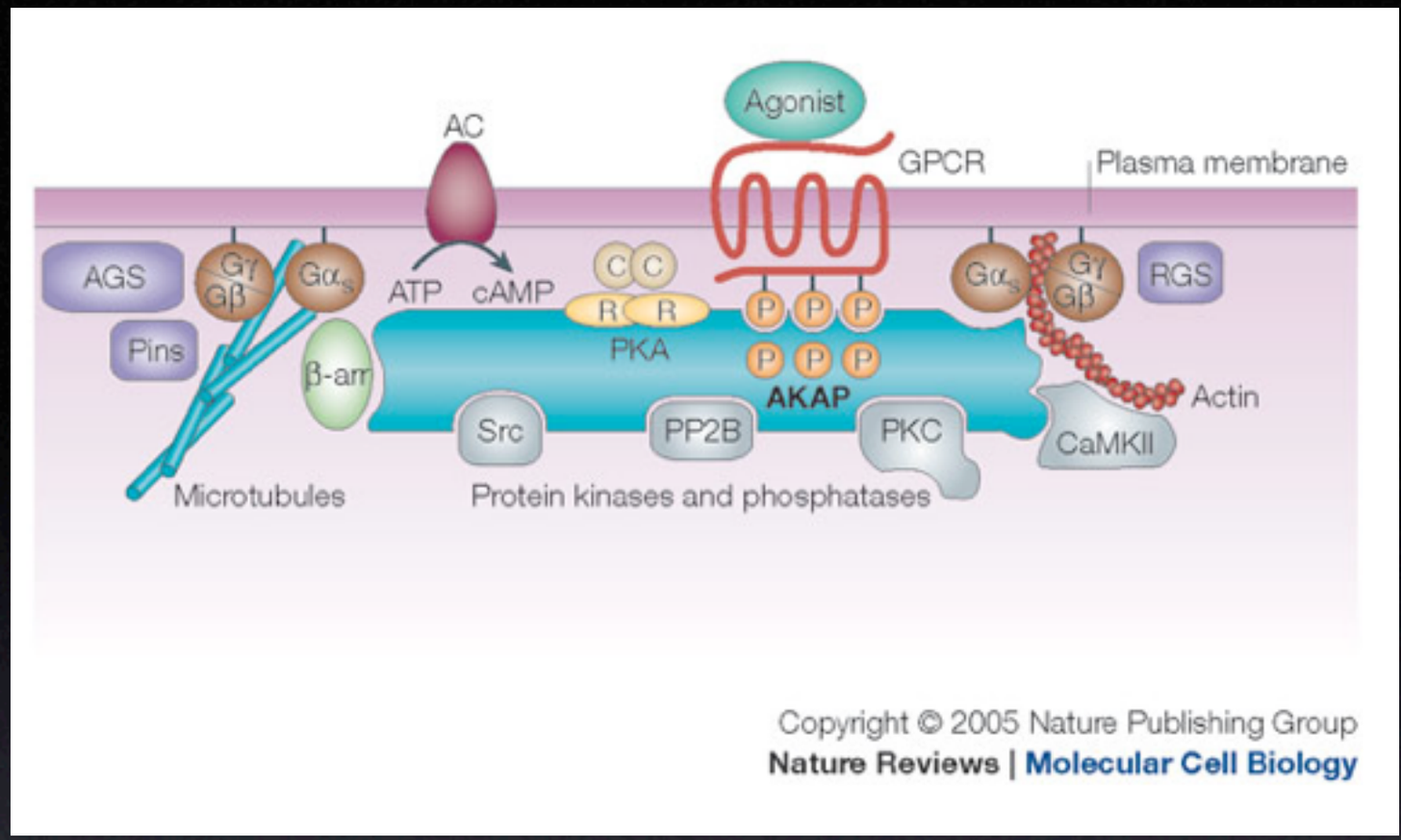
Protein tyrosine kinase–substrate interactions

Ron Bose[1,2,*], Marc A Holbert[1,*], Kerry A Pickin[1,*] and Philip A Cole[1,2]

Curr. Op. Str. Bio. (2006) 16, 668

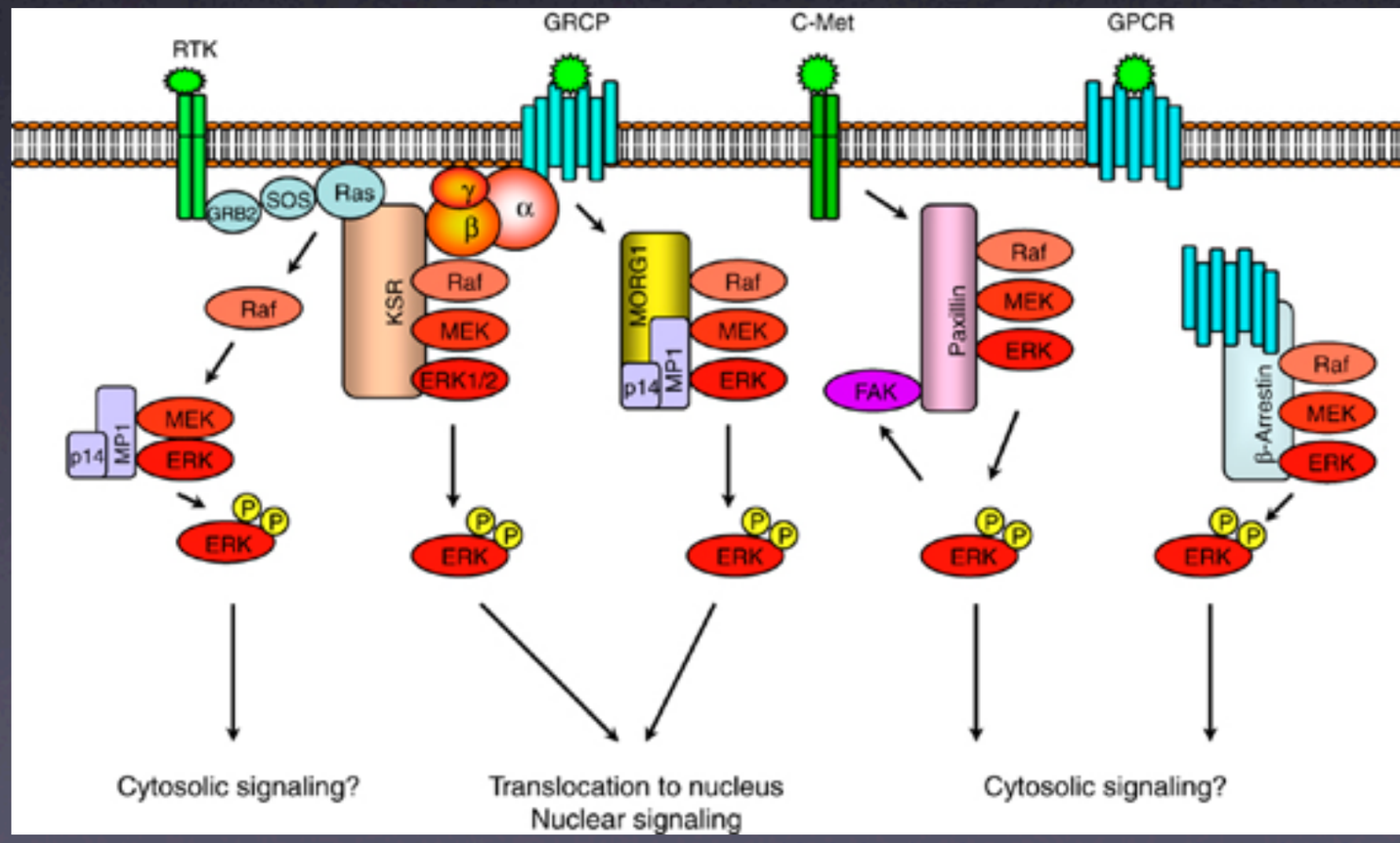
Solution to kinase substrate specificity problem: Scaffolding

PKA/Src/PKC scaffold



Malbon (2005) NRMCB, 6, 689

Map kinase scaffolds



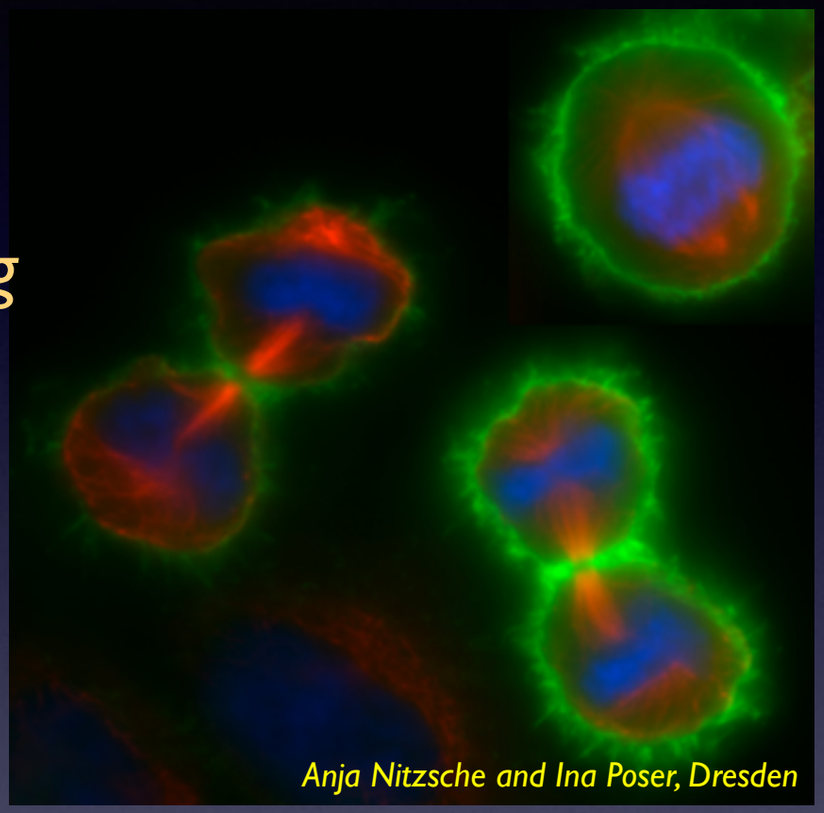
Dhanasekaran (2007) Oncogene, 26, 3185

Lots of different AKAPs scaffold the PKA kinase

Different complexes in different locations

AKAP5

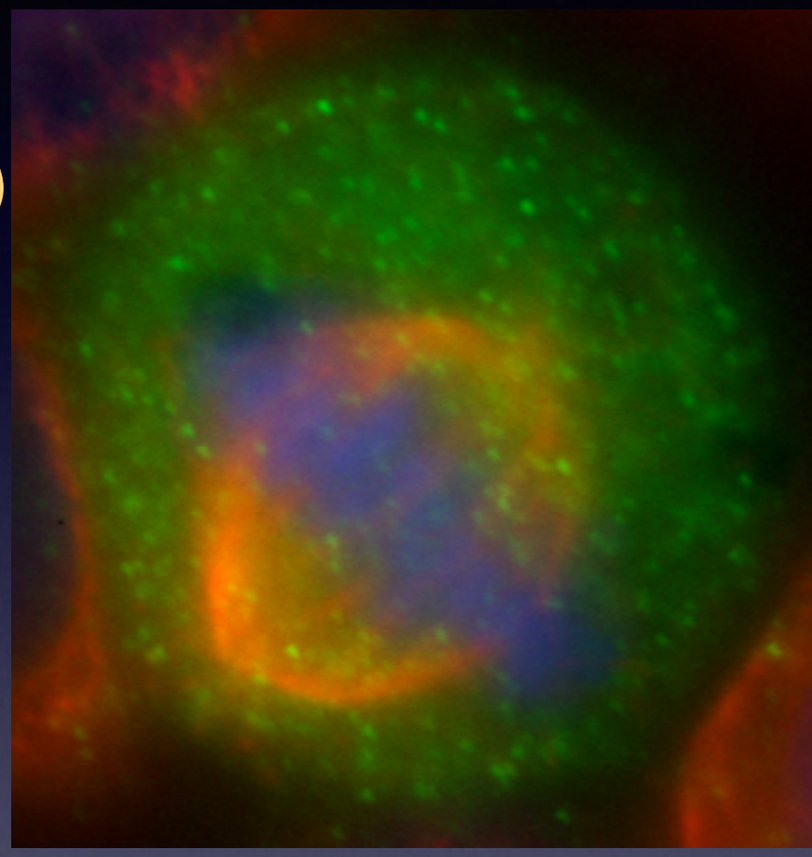
A-kinase (PKA)anchoring protein 5



Anja Nitzsche and Ina Poser, Dresden

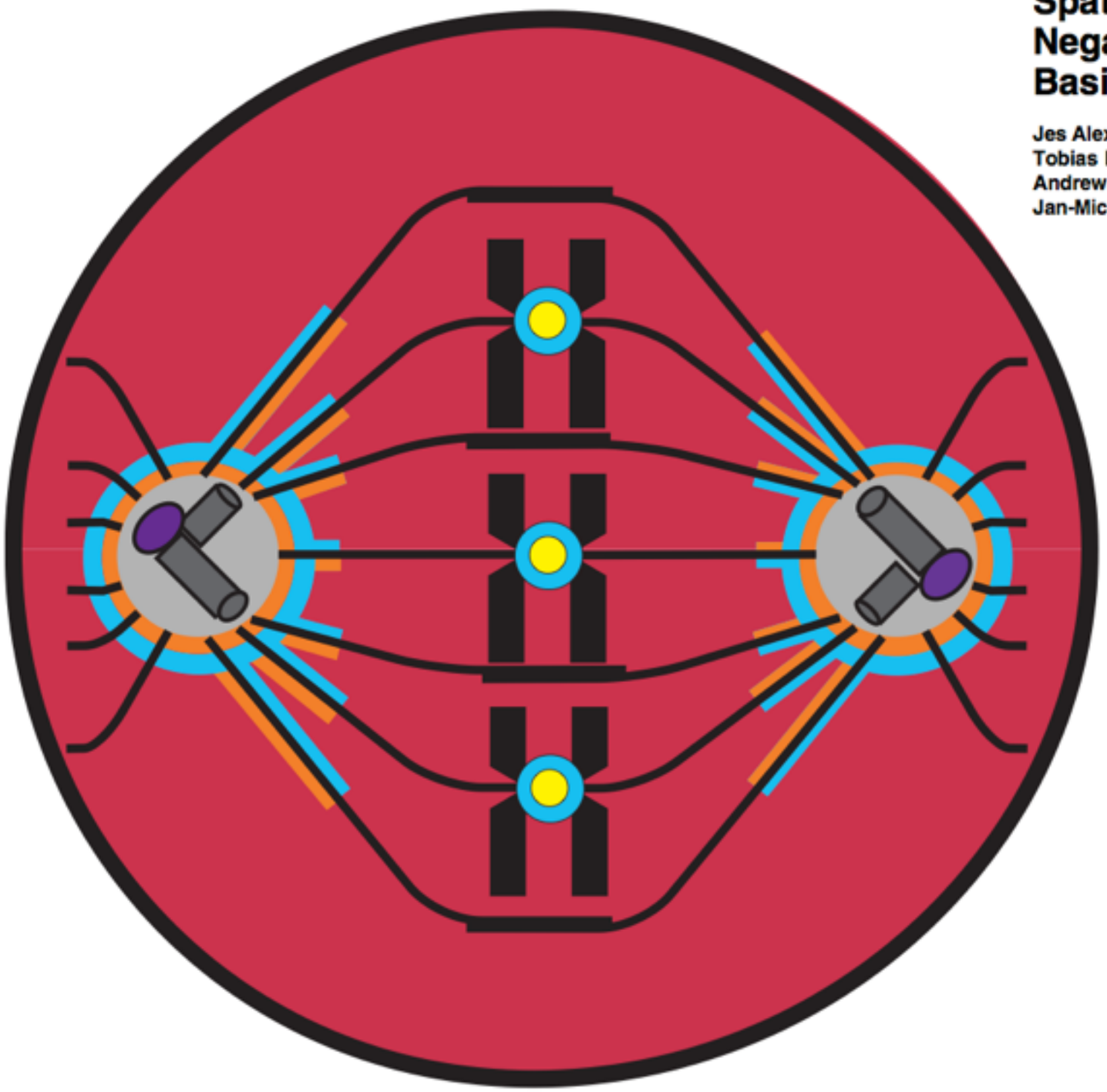
AKAP12

A-kinase (PKA) anchoring protein 12



[Tagged Cell lines made in Dresden as part of the ***Mitocheck*** and ***DiGtoP*** projects]

Spatial Exclusivity of Mitotic Kinases



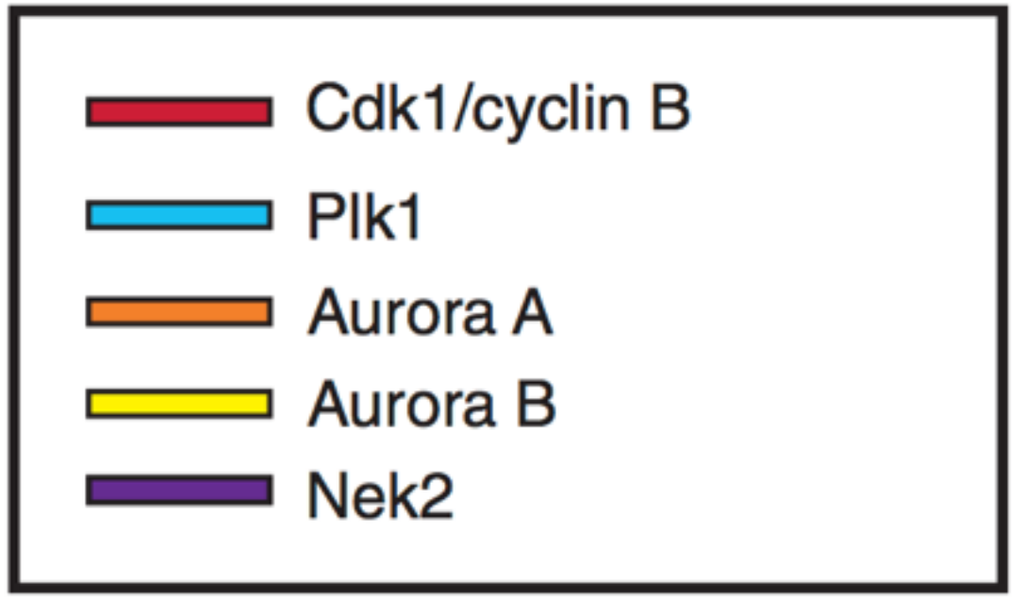
RESEARCH ARTICLE

MITOTIC KINASES

Sci. Sig., 6-2011

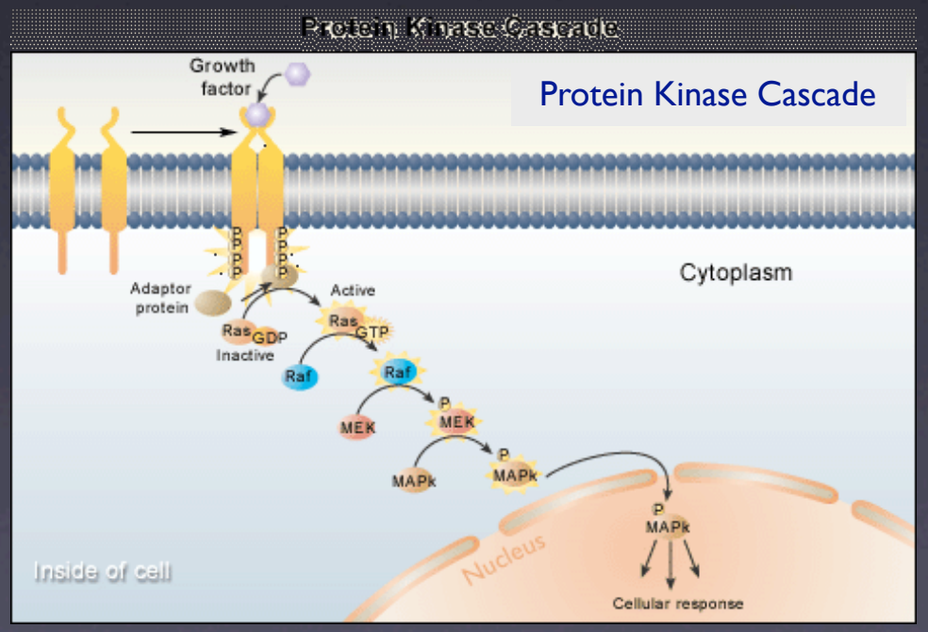
Spatial Exclusivity Combined with Positive and Negative Selection of Phosphorylation Motifs Is the Basis for Context-Dependent Mitotic Signaling

Jes Alexander,[1*] Daniel Lim,[1] Brian A. Joughin,[1] Björn Hegemann,[2†] James R. A. Hutchins,[2] Tobias Ehrenberger,[1] Frank Ivins,[3] Fabio Sessa,[4] Otto Hudecz,[2] Erich A. Nigg,[5] Andrew M. Fry,[6] Andrea Musacchio,[4] P. Todd Stukenberg,[7] Karl Mechtler,[2] Jan-Michael Peters,[2] Stephen J. Smerdon,[3] Michael B. Yaffe[1,8‡]



Kinases are networked, scaffolded and have limited or nonexistent substrate specificity

- Kinases do not find their substrates by simple free diffusion
  - Widely used Reaction-Diffusion equations are insufficient for modelling kinase signalling
- *"Kinase Cascade" is one of the worst analogies in Biology and its meme needs to become extinct*



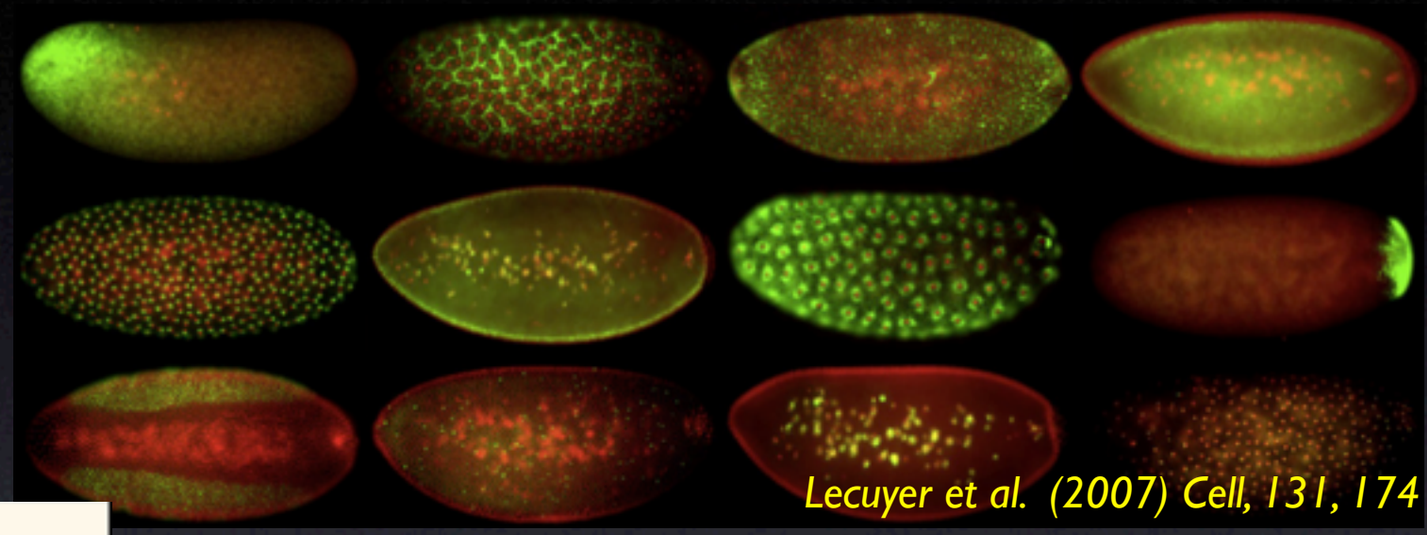
source http://www.biology.arizona.edu/cell_BIO/

Instead of measuring concentration,
[the cell] counts molecules

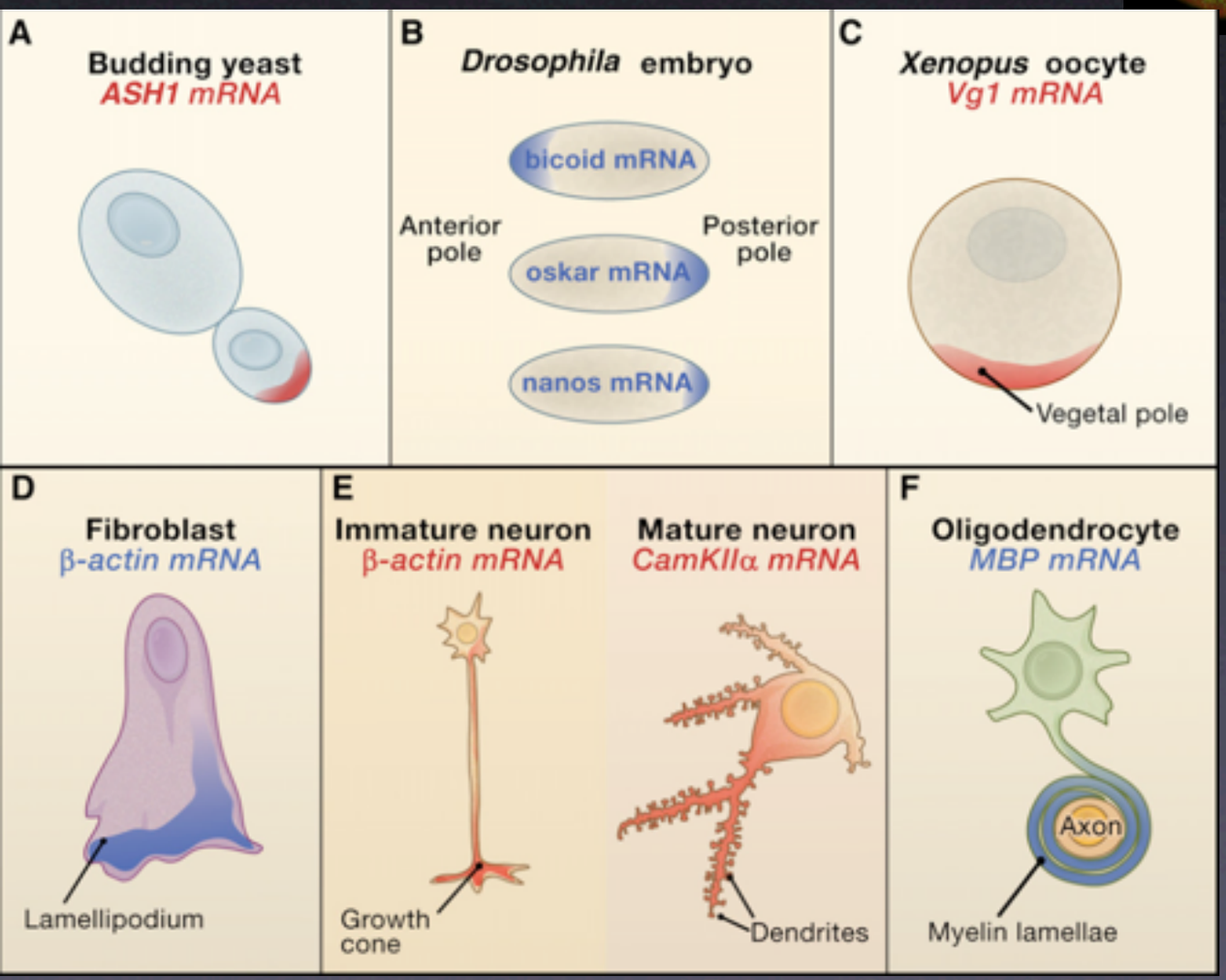
Sydney Brenner, 2007

Proteins are often made exactly where they are needed in the cell

70% of mRNAs have striking subcellular localisations in *Drosophila* embryos



Lecuyer et al. (2007) Cell, 131, 174



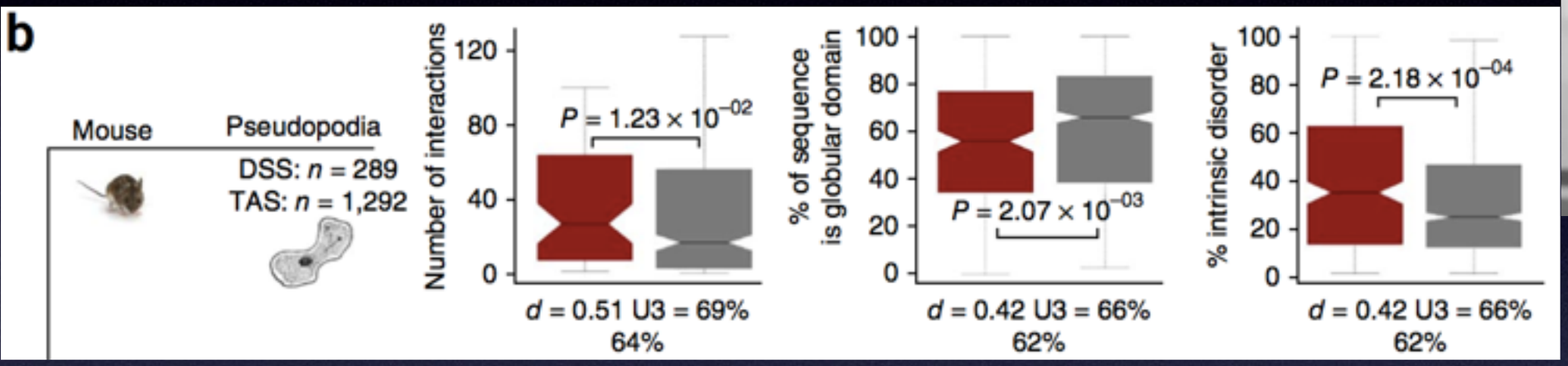
Martin and Ephrussi (2009) Cell, 136, 719

Some examples of localised mRNAs involved in spatially regulated translation

Asymmetric mRNA localization contributes to fidelity and sensitivity of spatially localized systems

Robert J Weatheritt[1,3], Toby J Gibson[2] & M Madan Babu[1]

nature structural & molecular biology

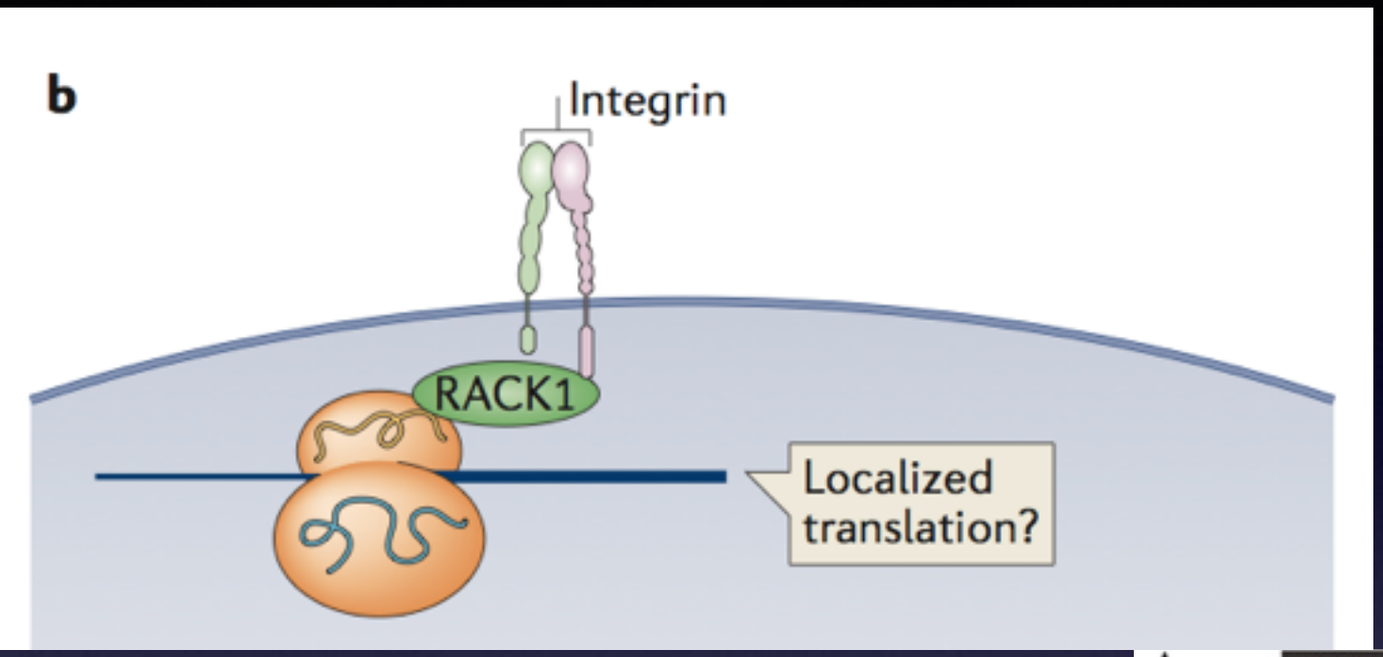


Robert Weatheritt, PhD, now in Toronto with Ben Blencowe

mRNAs in pseudopodia encode proteins enriched for intrinsic disordered regions

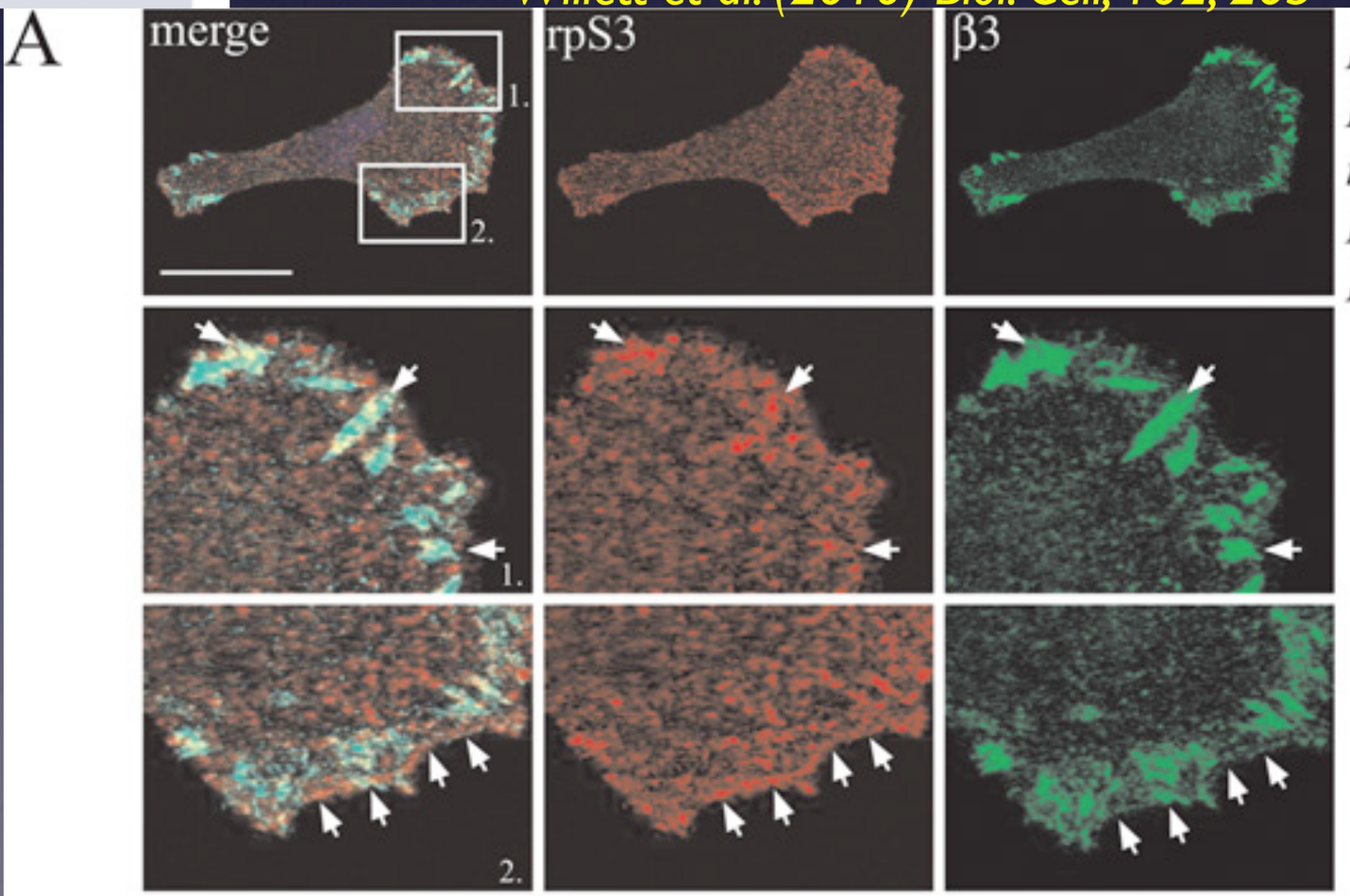
Proteins synthesised on-site often provide essential components required to activate the signalling machinery. They also tend to encode proteins that have the capacity to nucleate and form reversible, non-membranous assemblies

Ribosomal subunits colocalise with beta3 integrin at adhesion foci at the leading edge of migrating fibroblasts



Xue and Barna (2012) Nat Rev MCB, 13, 355

Willett et al. (2010) Biol. Cell, 102, 265



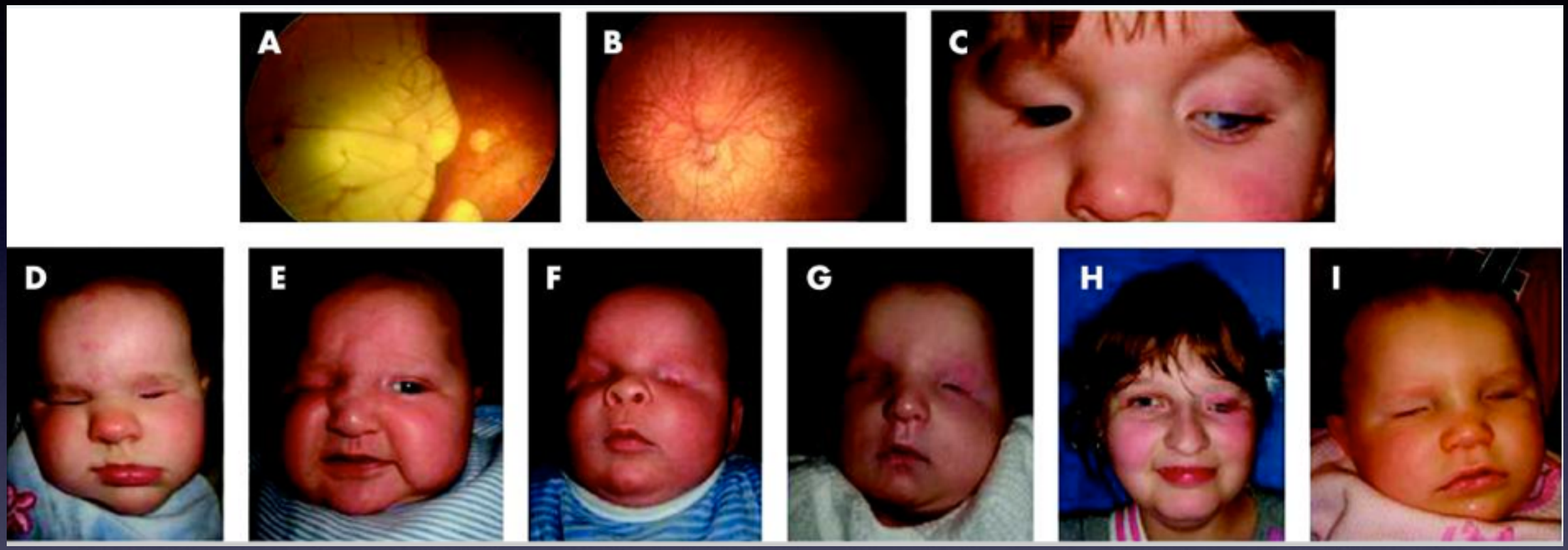
40S subunits are enriched at FACs

Spatial regulation of translation - implications

- Making proteins in the wrong place is often a bad thing
- Cells have been under continual selection pressure to develop systems for precise mRNA targeting

How many proteins can be allowed to freely diffuse in the cell?

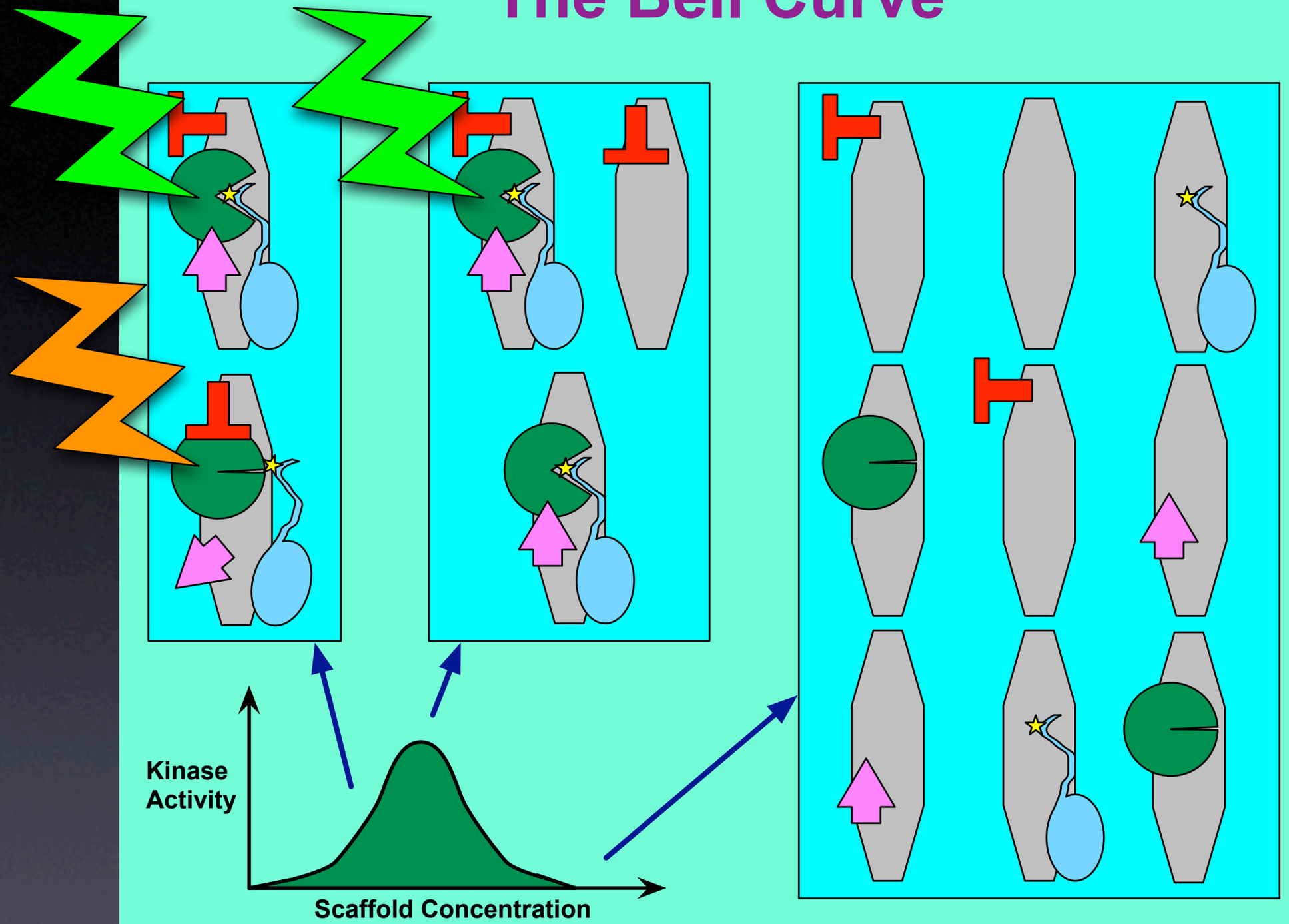
Sox2, Oct4 and Nanog are key stem cell genes



Sox2 haploinsufficiency leads to aniridia

Phenotypes can often give a misleading view of protein function. They highlight the strongest point of failure.

The Bell Curve



effect of KSR varied dramatically with the level of KSR protein expressed. In *Xenopus* oocytes, KSR functioned as a positive regulator of Ras signaling when expressed at low levels, whereas at high levels of expression, KSR blocked Ras-dependent signal transduction. Likewise, overexpression of *Drosophila* KSR blocked R7 photore-

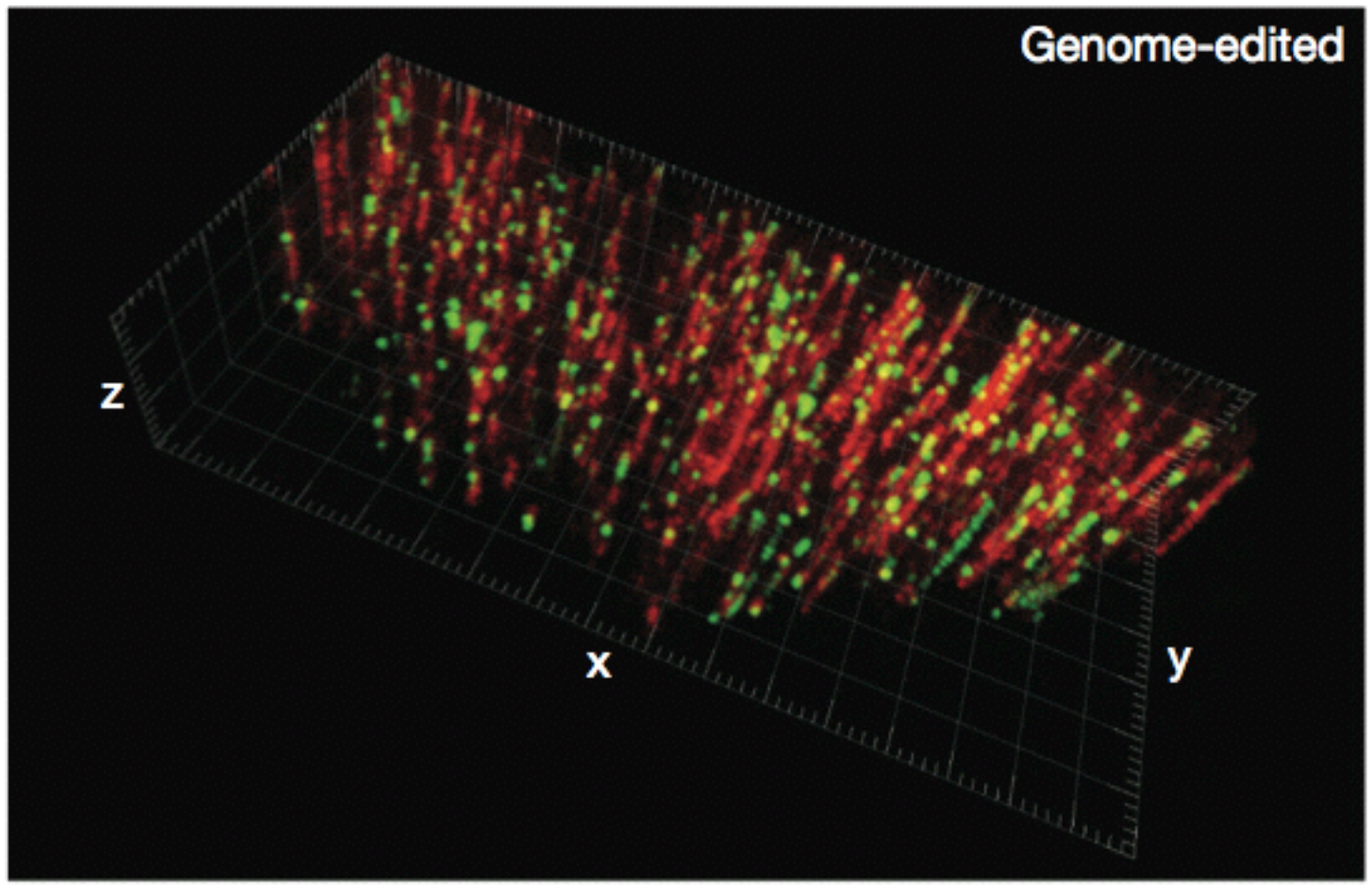
Cacace et al. (1999) MCB, 19, 229

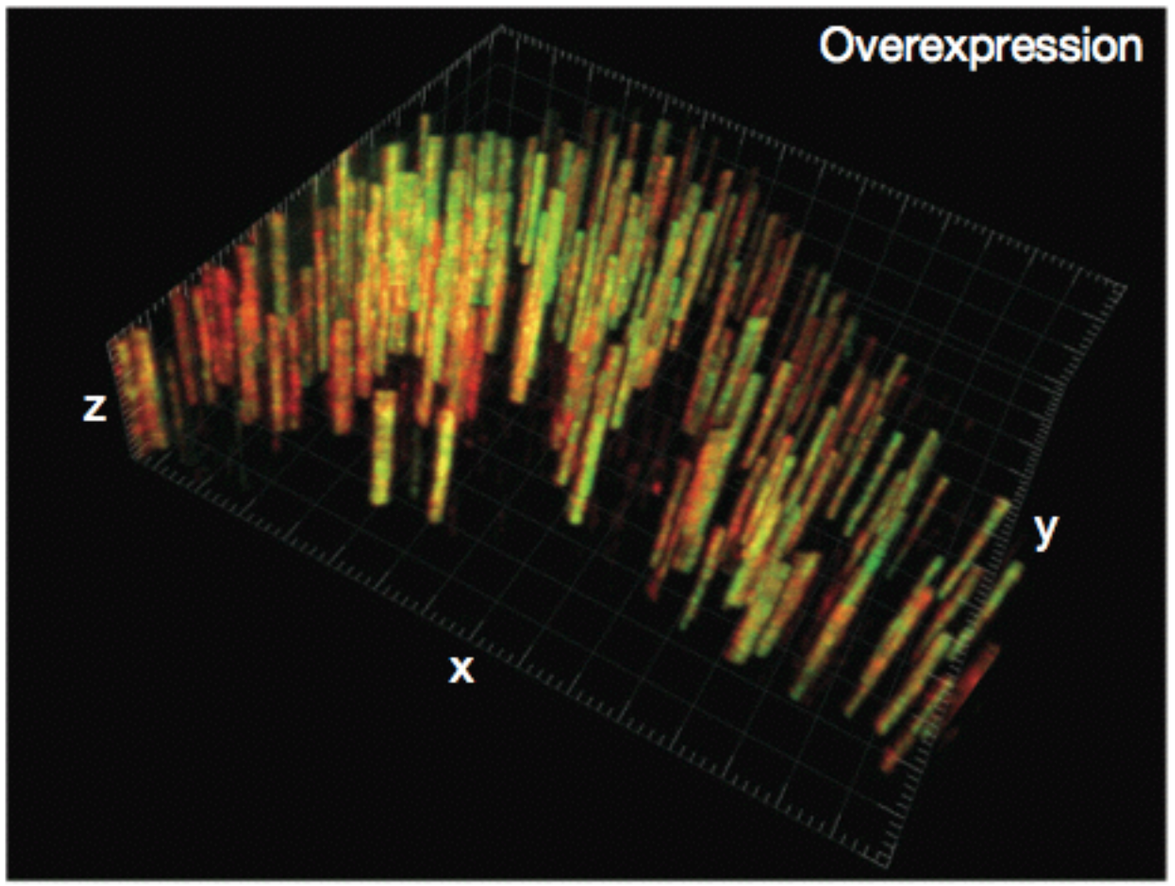
Many components of regulatory complexes exhibit balanced gene dosage

It is not just scaffolds: Foxc1 and Pax6 are, like Sox2, TFs that cannot tolerate dosage alteration in any direction during eye development

Transient overexpression experiments may give misleading results

Kymographs with red rfp-clathrin (vesicles) and bound green gfp dynamin motor proteins





DG Drubin Lab: Doyon et al. (2011) NCB 13, 331

The transience of transient overexpression

Toby J Gibson, Markus Seiler & Reiner A Veitia

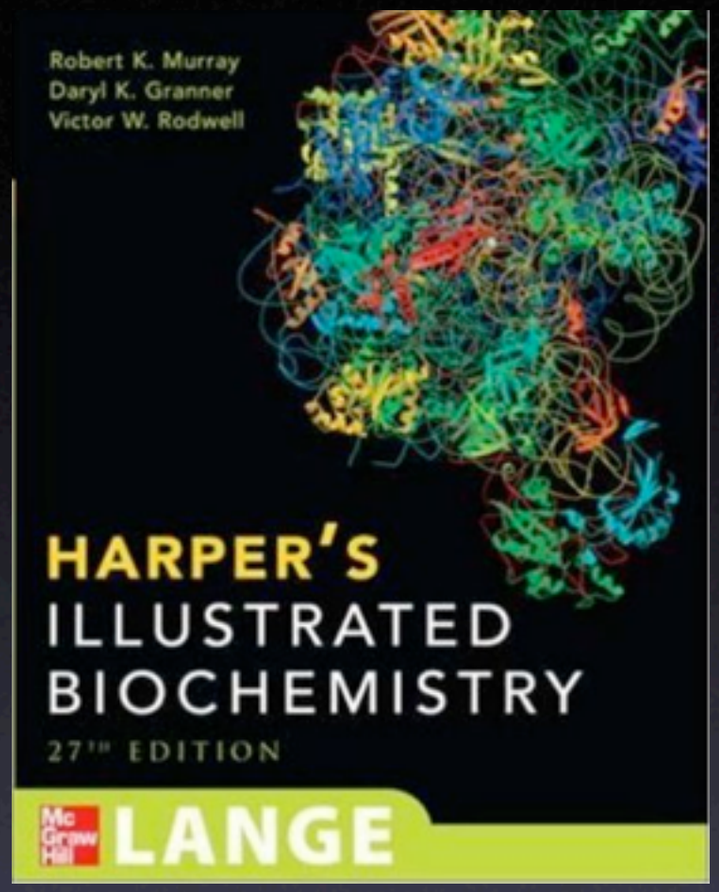
Much of what is known about mammalian cell regulation has been achieved with the aid of transiently transfected cells. However, overexpression can violate balanced gene dosage, affecting protein folding, complex assembly and downstream regulation. To avoid these problems, genome engineering technologies now enable the generation of stable cell lines expressing modified proteins at (almost) native levels.

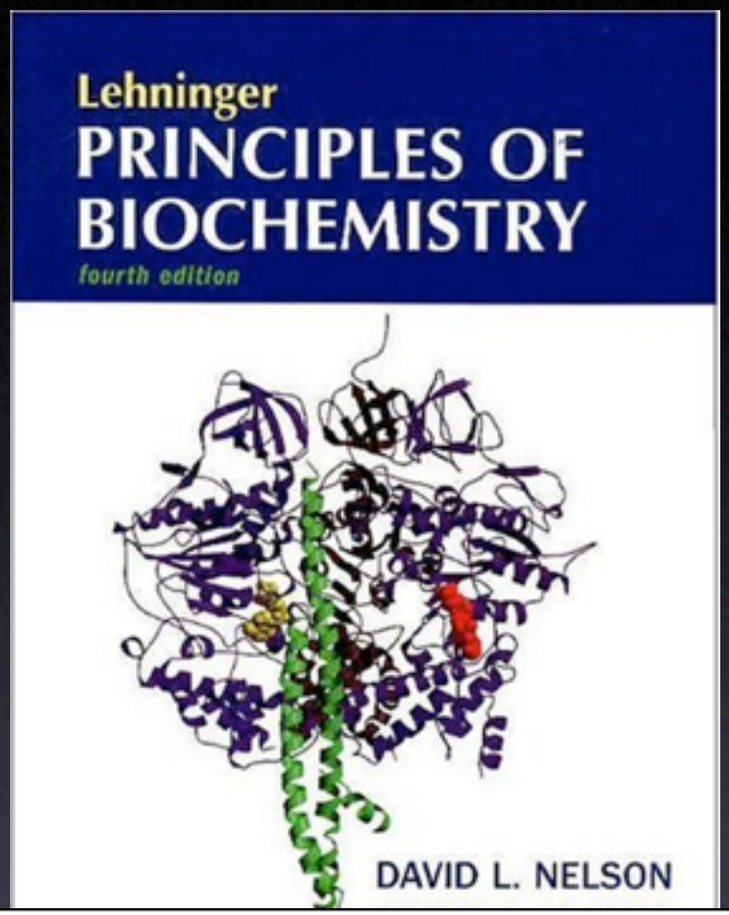
Nature Methods (2013) NCB 10, 715

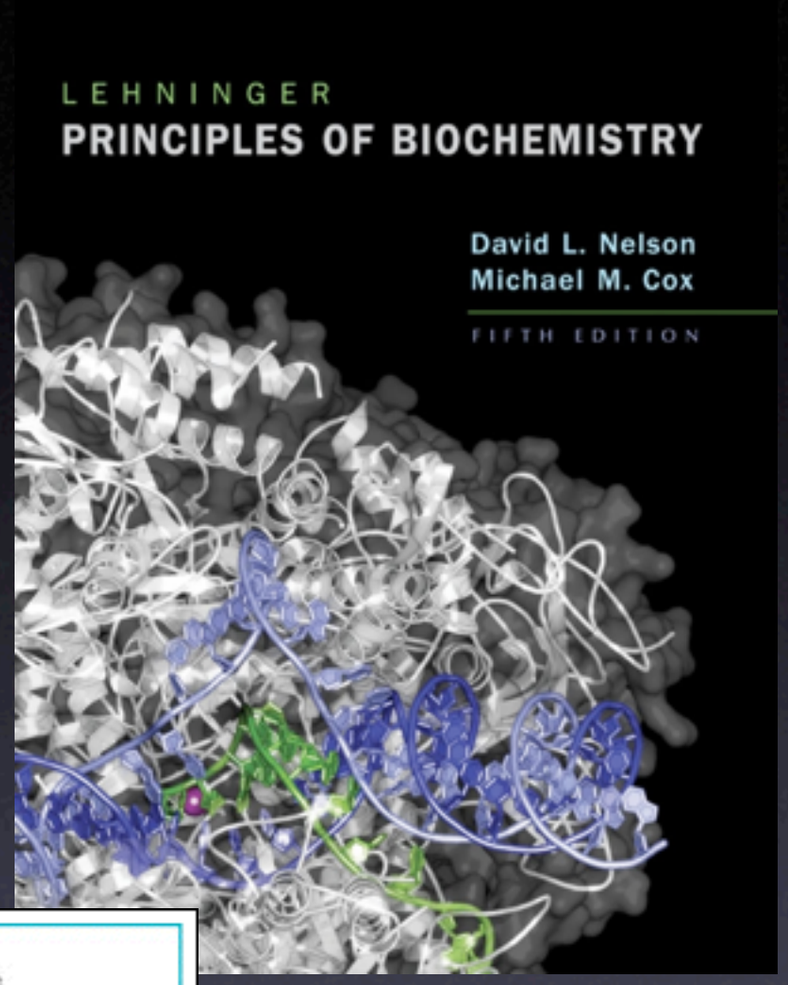
Table 2. Contrasting issues with transient overexpression experiments relative to native expression

Features of Cell Regulation / Effect on Experiment	**Over Expression**	**Native Expression**
Low molecule number (*e.g.* <1000 per cell)	X	√
Spatially arranged protein	X	√
Coupled mRNA transport / Spatial translation	Overload system	√
Mutants that are (unknowingly) unfolded	Amyloid/aggregation	?
Balanced gene dosage of regulators	X	√
Kinases and their substrates are scaffolded	X	√
Laser bleaching to study diffusion (or other motion) of a signalling protein	Meaningless	√
Protein complex by Co-IP	???	√
Proteomics	X	√
Reproducibility	??	√
Synchronised cell population	X	√
Differentiate from stem cell	X	√

Biochemistry Text Books







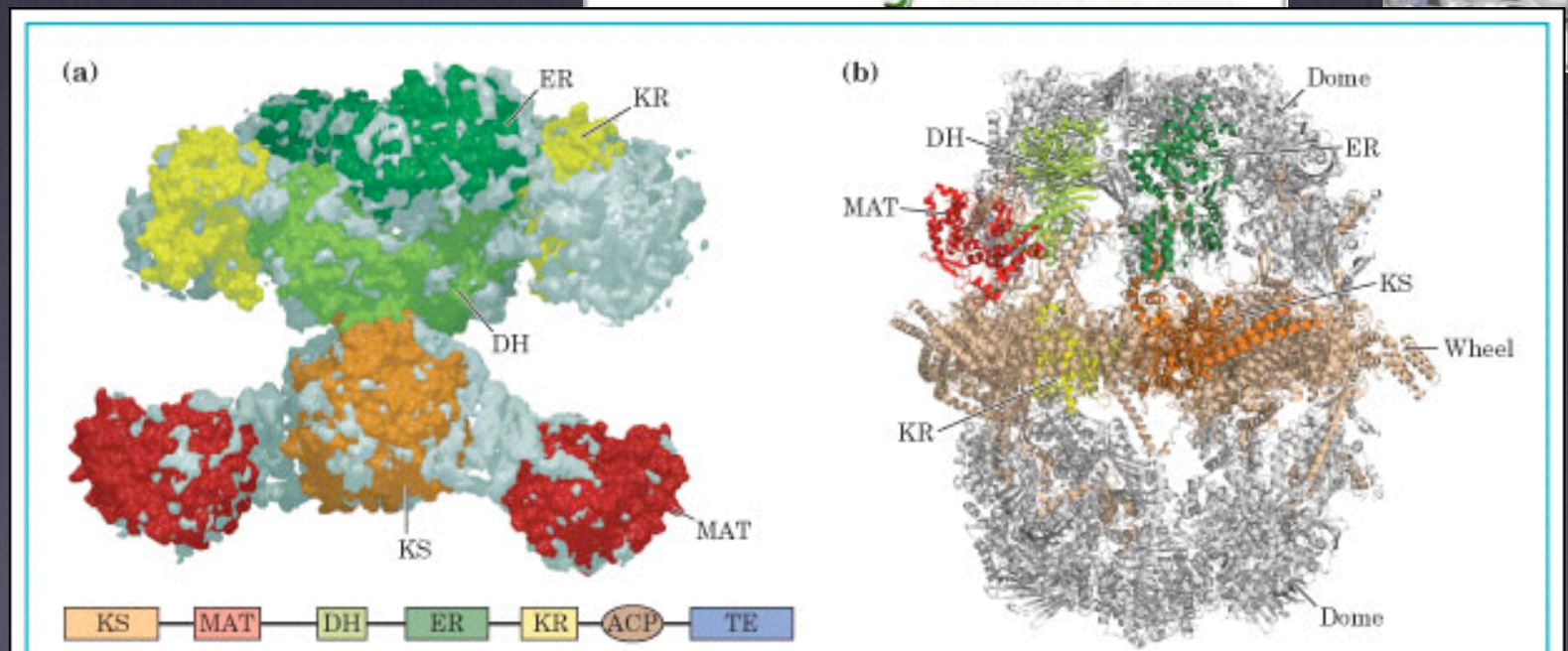


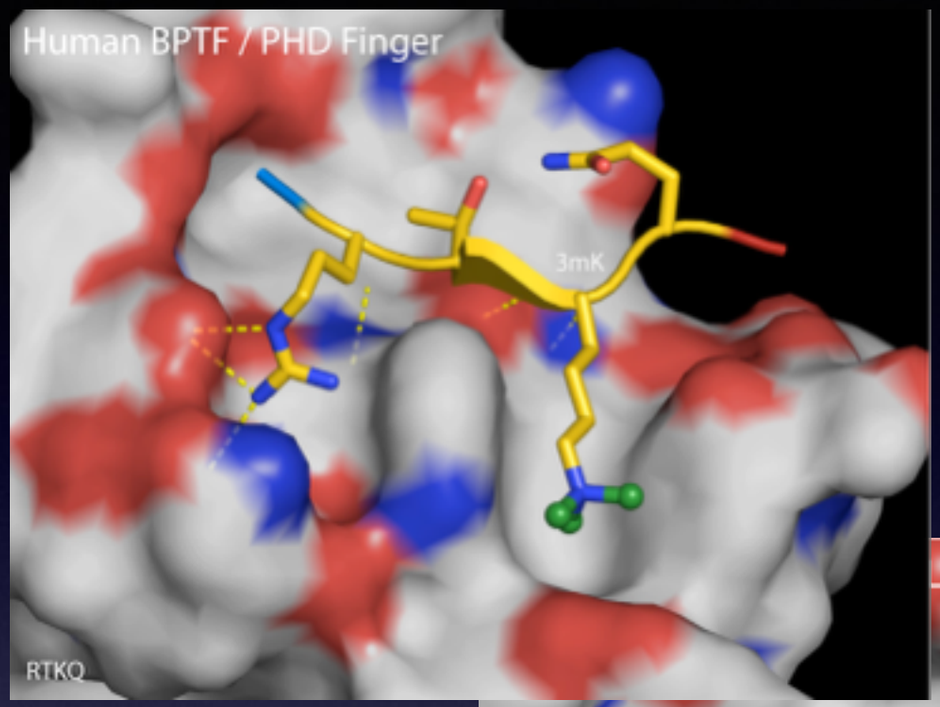
FIGURE 21-3 The structure of fatty acid synthase type I systems.

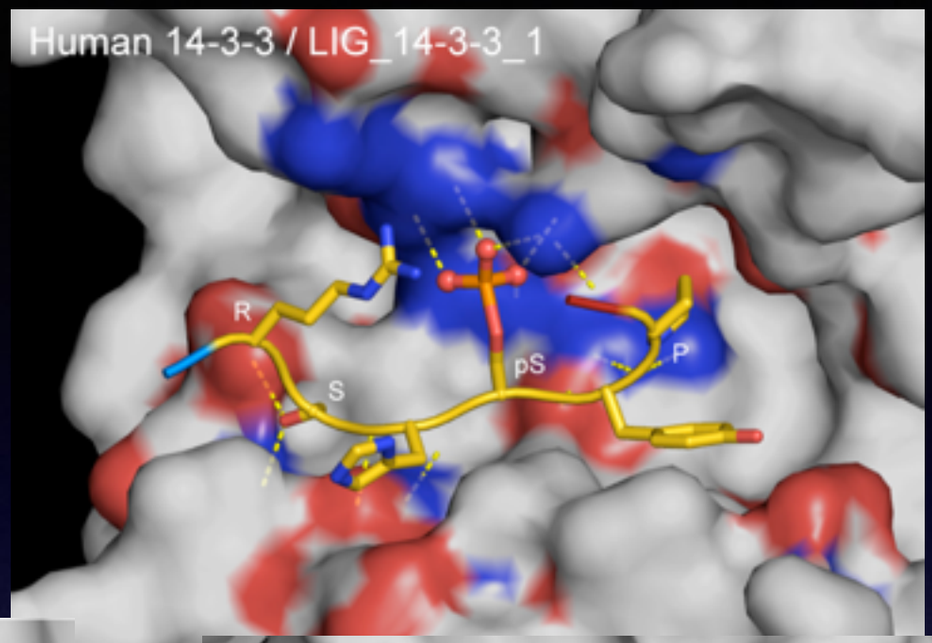
Complexes
Complexes
Complexes

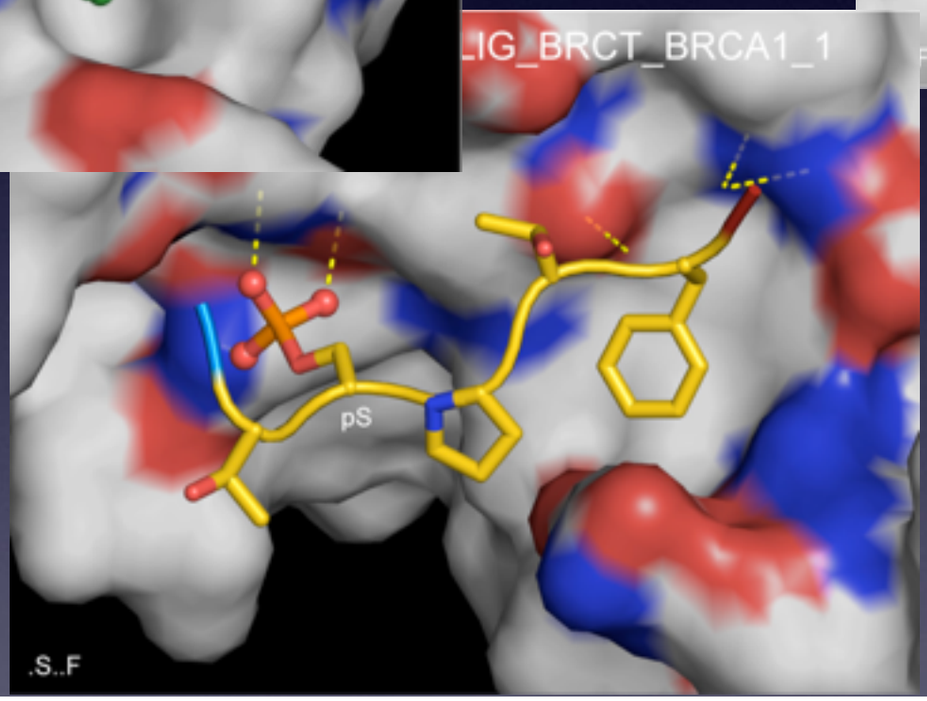
Truth and clarity are complementary

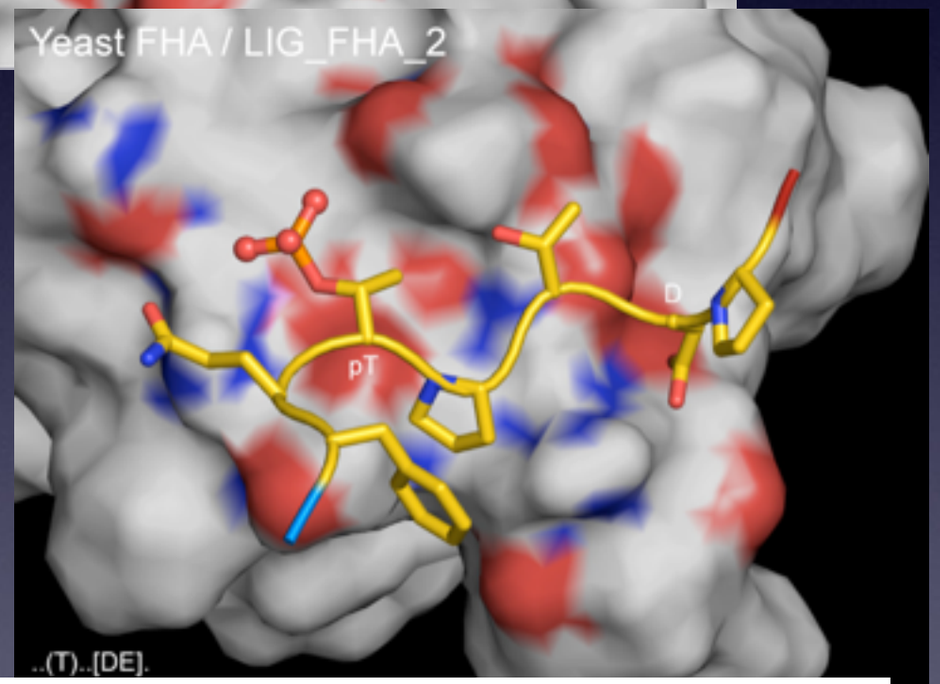
Niels Bohr

Biochemistry books are not so good on regulatory interactions









Understanding eukaryotic linear motifs and their role in cell signaling and regulation

Francesca Diella[1], Niall Haslam[1], Claudia Chica[1], Aidan Budd[1], Sushama Michael[1], Nigel P. Brown[2], Gilles Trave[3] Toby J. Gibson[1]

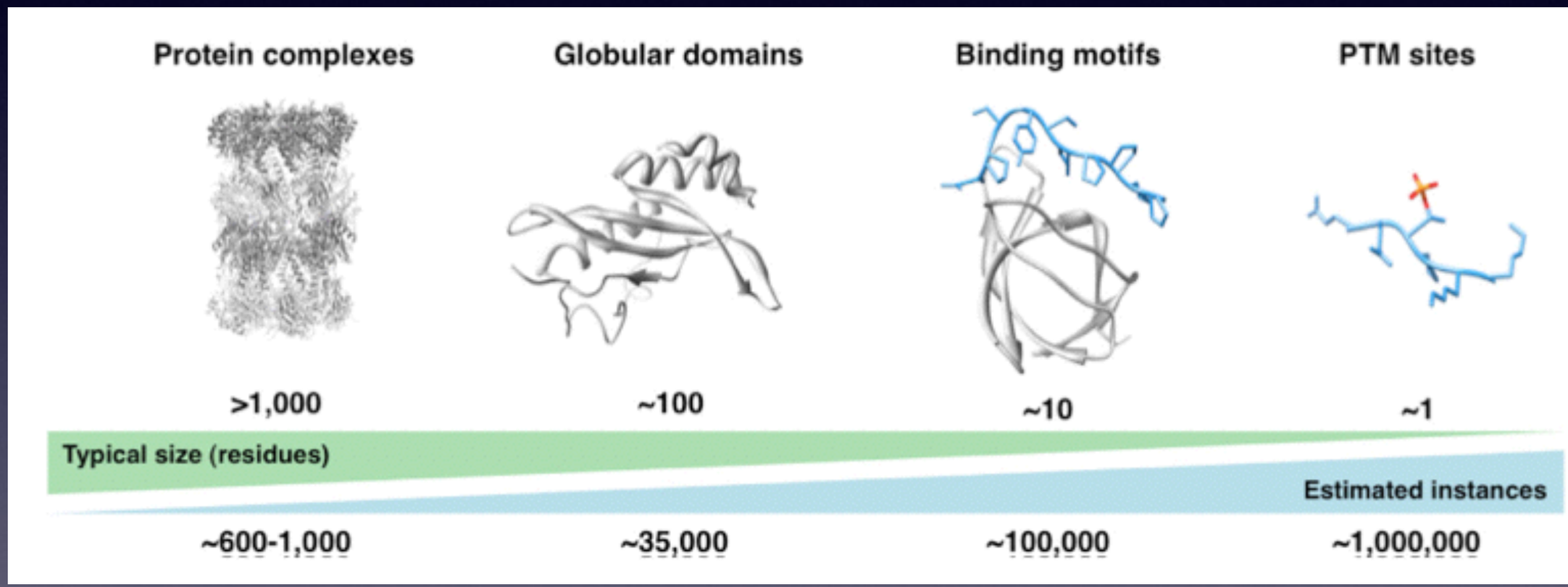
[1]Structural and Computational Biology Unit, European Molecular Biology Laboratory, 69117 Heidelberg, Germany, [2]BIOQUANT, Ruprecht-Karls-Universität Heidelberg, Im Neuenheimer Feld 267, 69120 Heidelberg, Germany, [3]ESBS, 1, Bld Sébastien Brandt, BP10413, 67412-ILLKIRCH, France 3

24 page open access review in Frontiers in Biosciences

Molecular Cell
Review
Molecular Cell 55, July 17, 2014

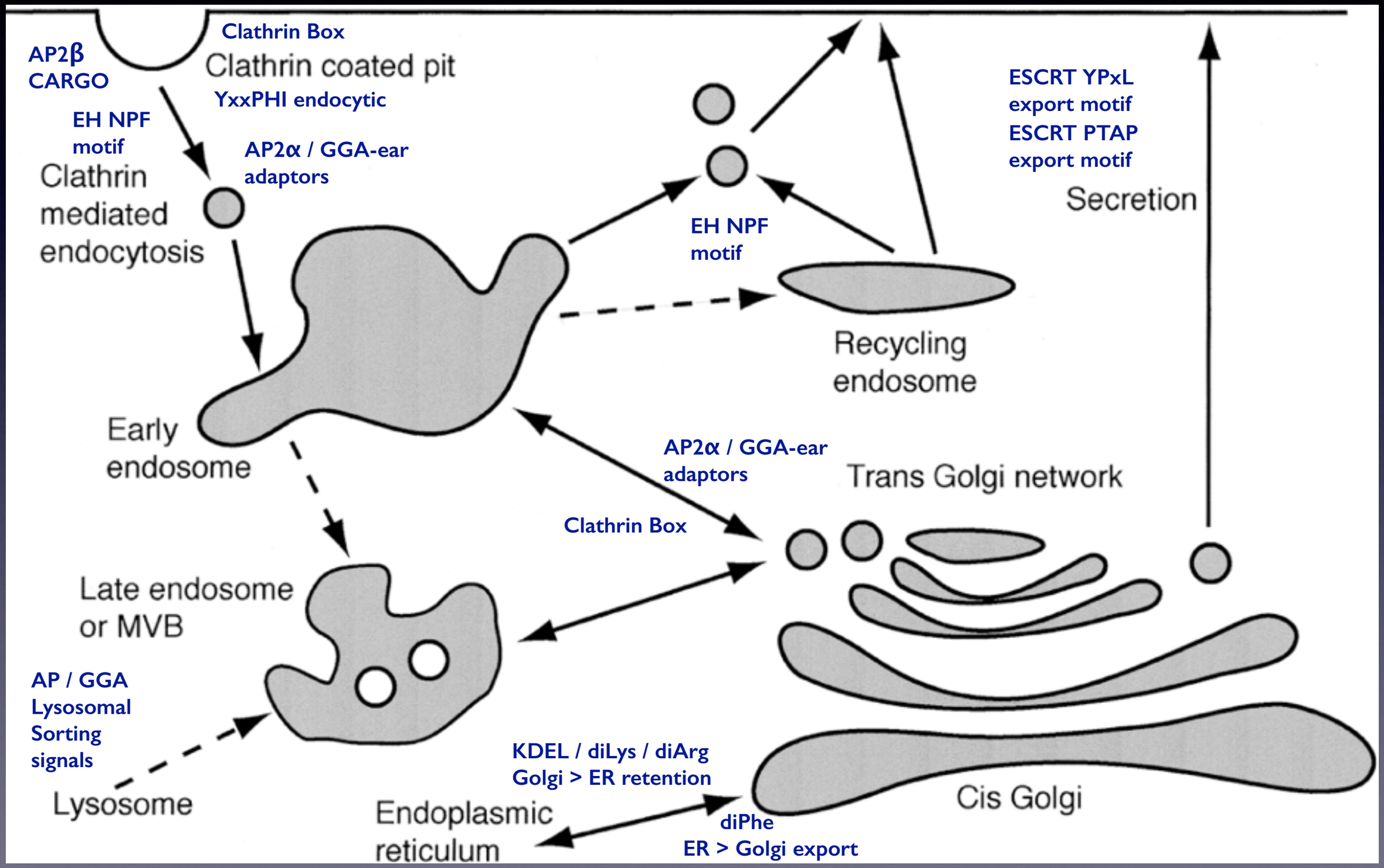
A Million Peptide Motifs for the Molecular Biologist

Peter Tompa,[1,2,*] Norman E. Davey,[3] Toby J. Gibson,[4] and M. Madan Babu[5,*]



Vesicle trafficking in the cell

The cell has to control the movement of subcellular organelles.
Complex and dynamic systems require extensive regulation.

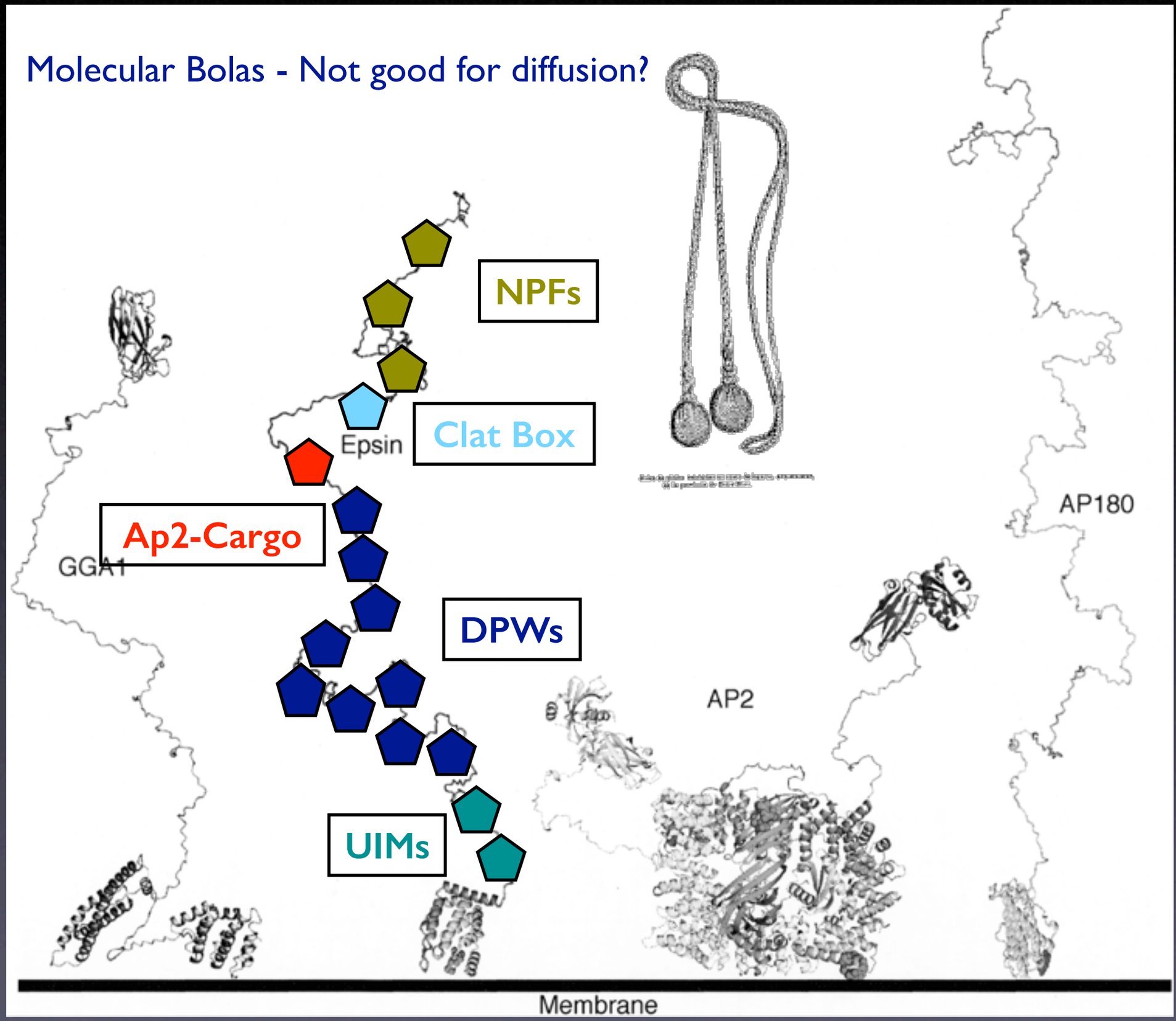


Owen DJ (2004) Biochem. Soc.Trans. 32, 1-14

Modular regulatory proteins involved in endocytosis

Most Endocytosis proteins have a mixture of **globular domains** and **natively disordered** regions. The disordered regions are proving to be rich in **Linear Motifs.**

Here the disordered regions are shown to scale with respect to the globular domains



Owen DJ (2004) Biochem. Soc. Trans. 32, 1-14.

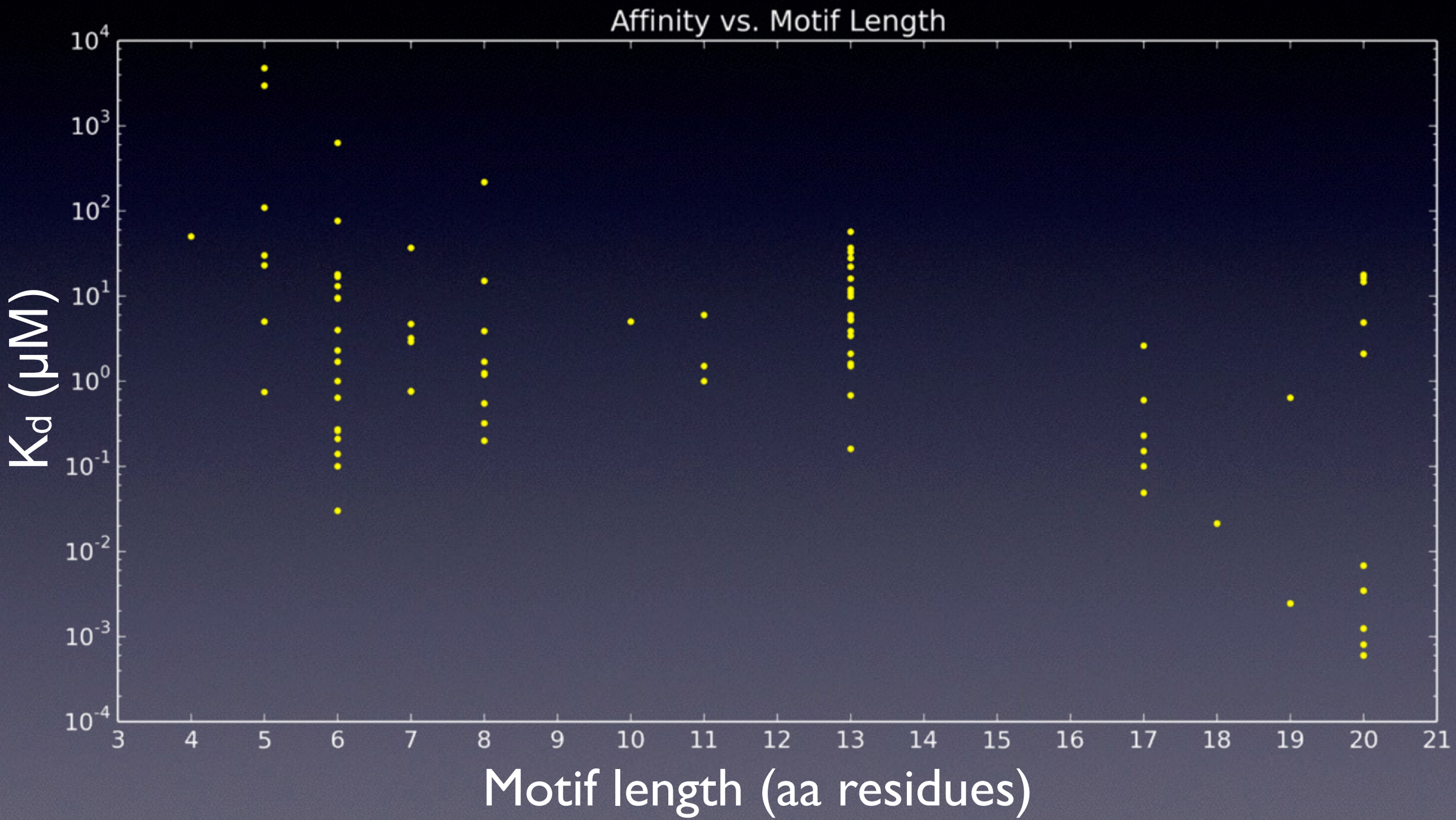
Linear Motifs - 3 is a magic number

Regular Expression	**Function of motif**
L . C . E	RB interaction motif
[RK] .{0,1}V . F	PP1 Phosphatase interaction motif
R . L .{0,1} [FLIMVP]	Cyclin binding motif
SP . [KR]	CDK phosphorylation site
L . . LL	NR Box (binds nuclear receptors)
P . L . P	MYND finger interaction motif
F . . . W . . [LIV]	MDM2-binding motif in P53
RGD	Integrin-binding motif
SKL$	Peroxisome targeting signal 1
[RK][RK] . [ST]	PKA phosphorylation site

Unfortunately matches to these LMs are not significant - providing a signal-to-noise problem for bioinformatics tools :-(

Binding Affinity vs. Motif Length

http://elm.eu.org

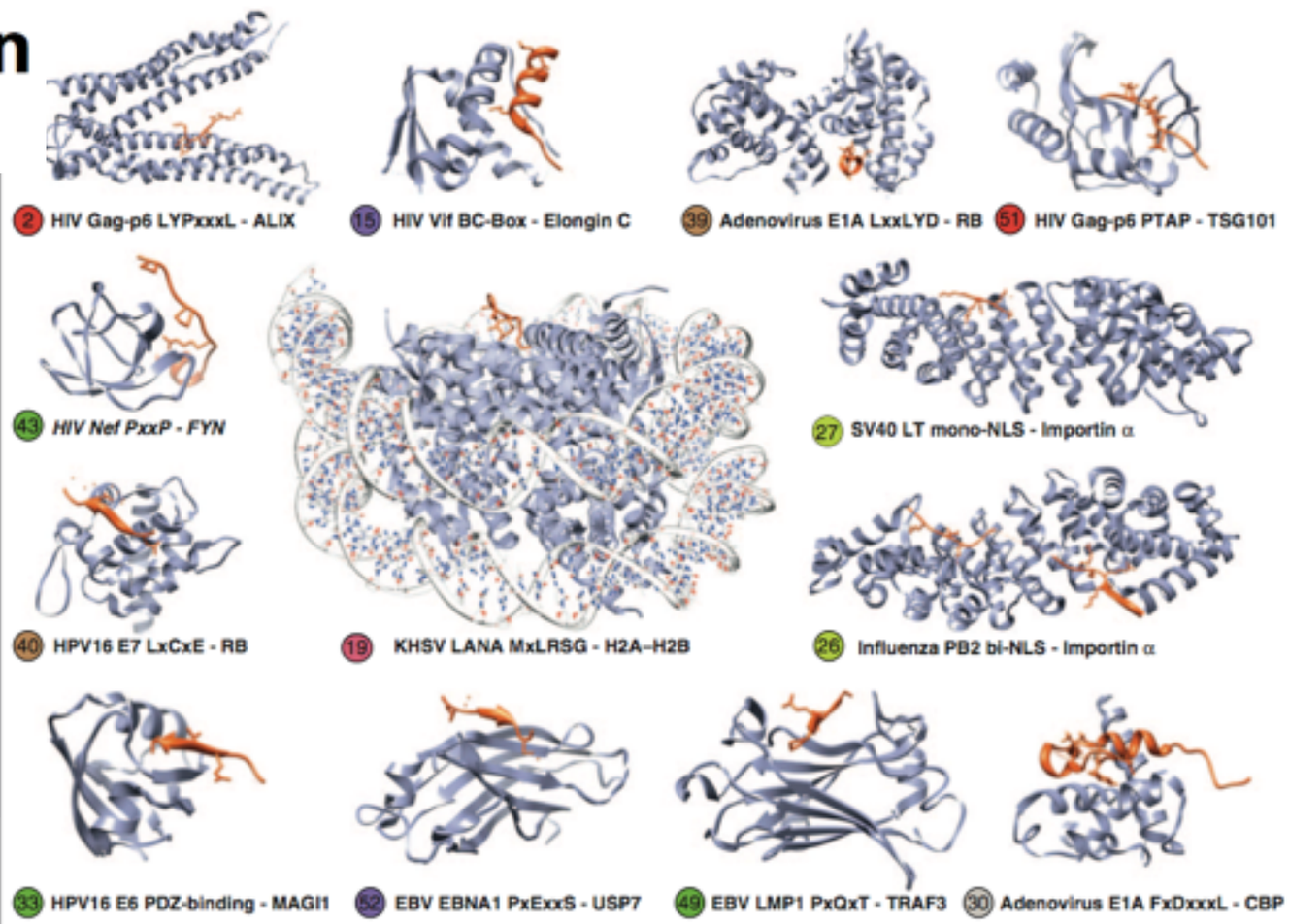


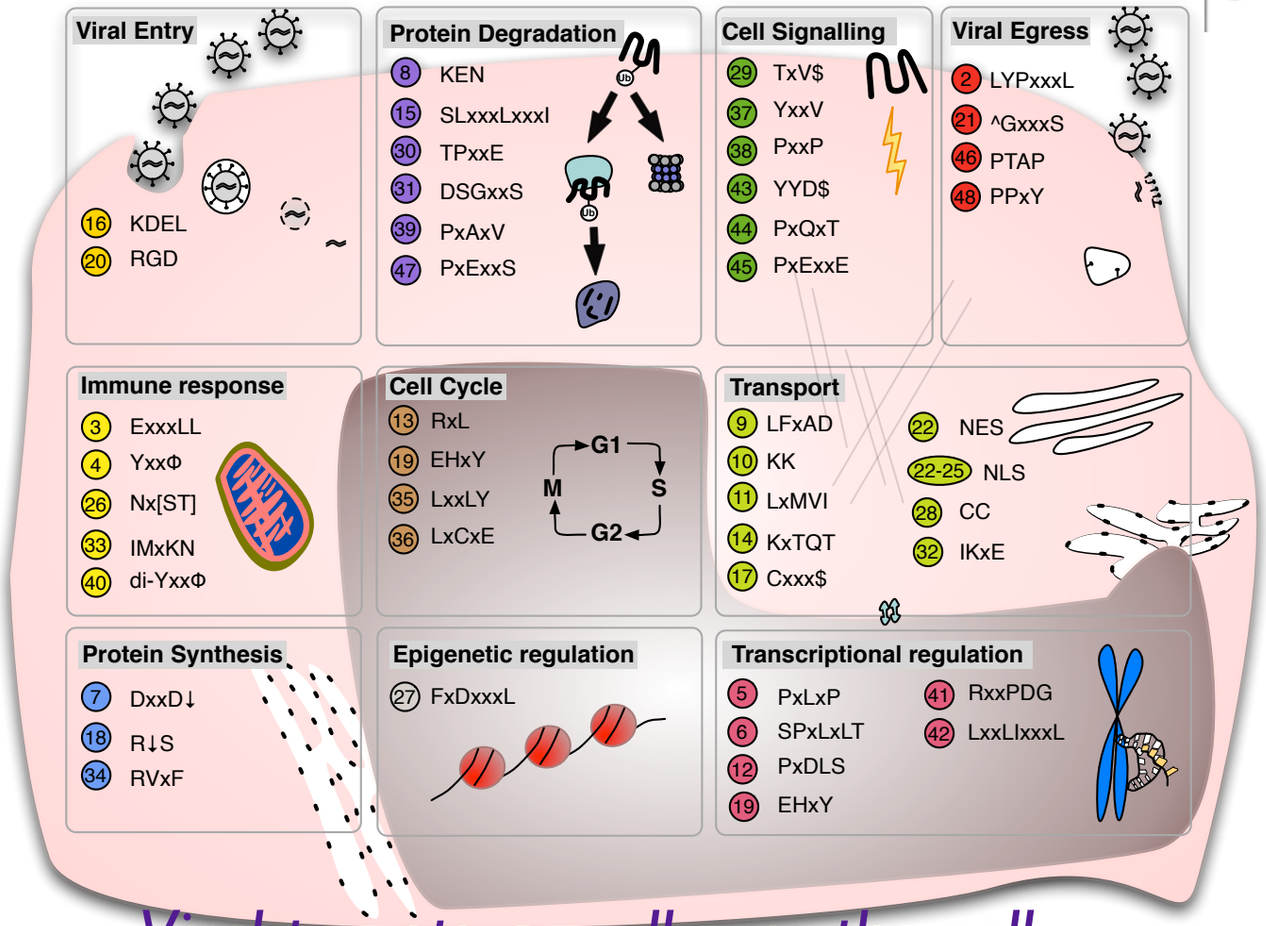
How viruses hijack cell regulation

Norman E. Davey[1], Gilles Travé[2] and Toby J. Gibson[1]

TiBS (2011) 36, 159

More than a third of the motif classes annotated in our ELM Resource (http:elm.eu.org) are already known to be used by viruses

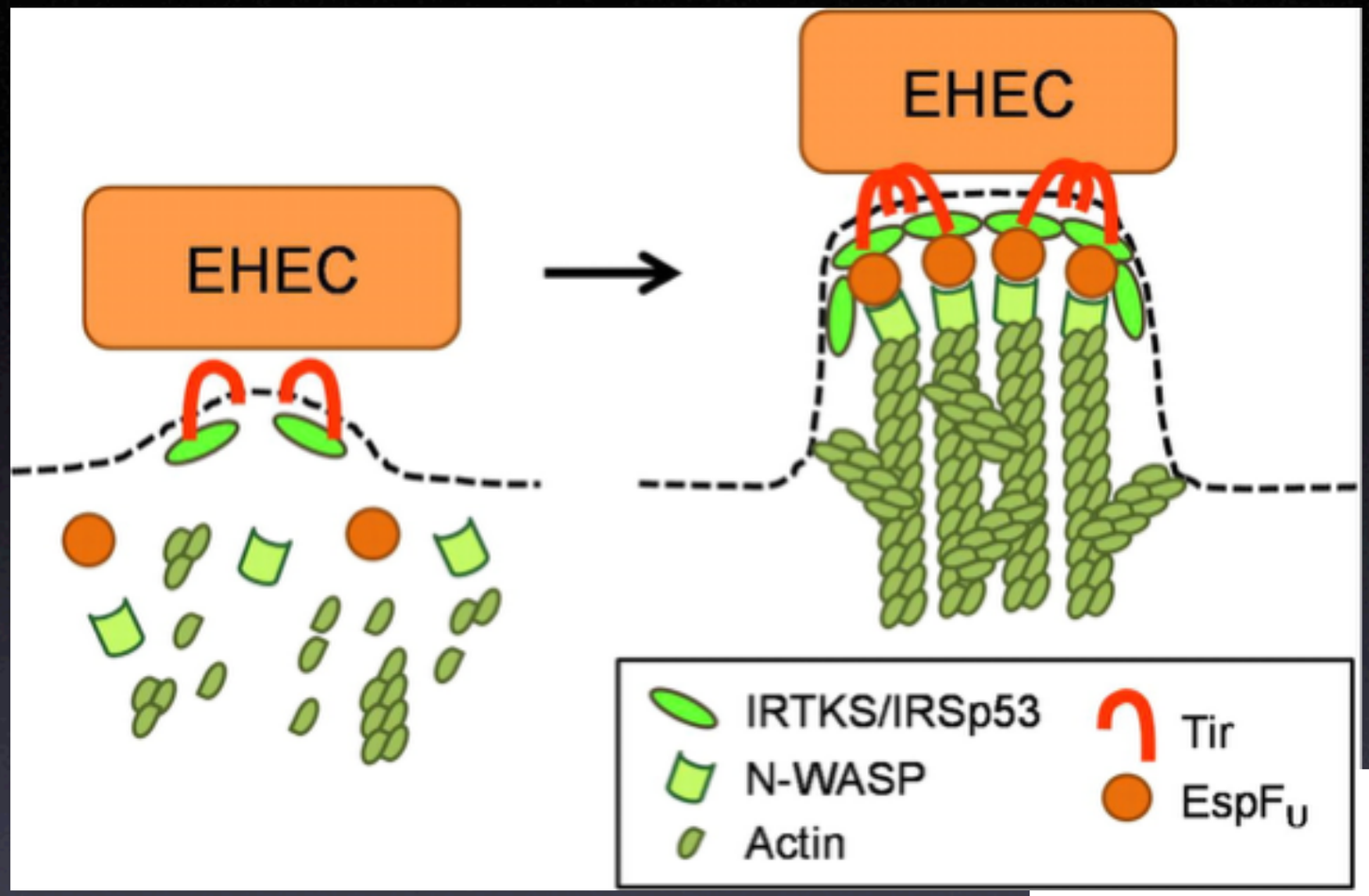




Viral targets are all over the cell

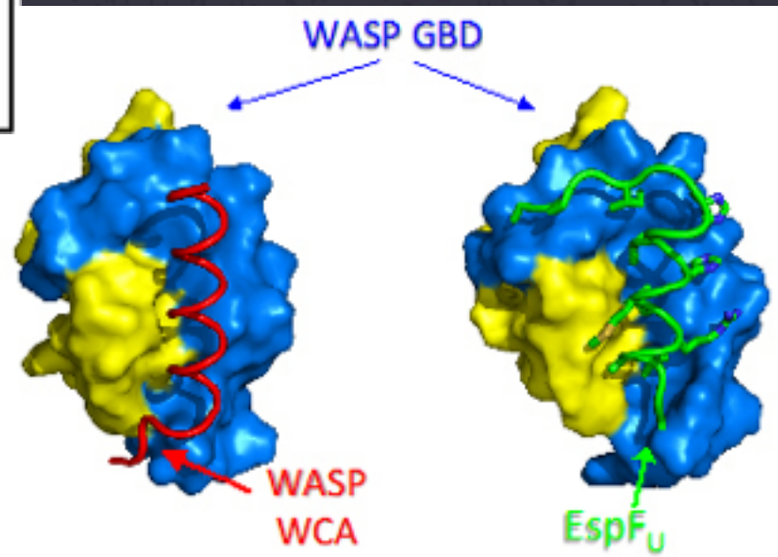
- Why is there "always" a cellular protein motif interaction for a virus to subvert?
- What does this tell us about the nature of the cell?

Pathogenic Pedestal Formation



Yi PNAS, 106, 6431 (2009)

A linear motif in *E. coli* EHEC EspFu binds N-WASP leading to Actin polymerisation



Cheng, Nature, 454, 1009 (2008)

Cell Regulation: Cooperative and Spatially arranged

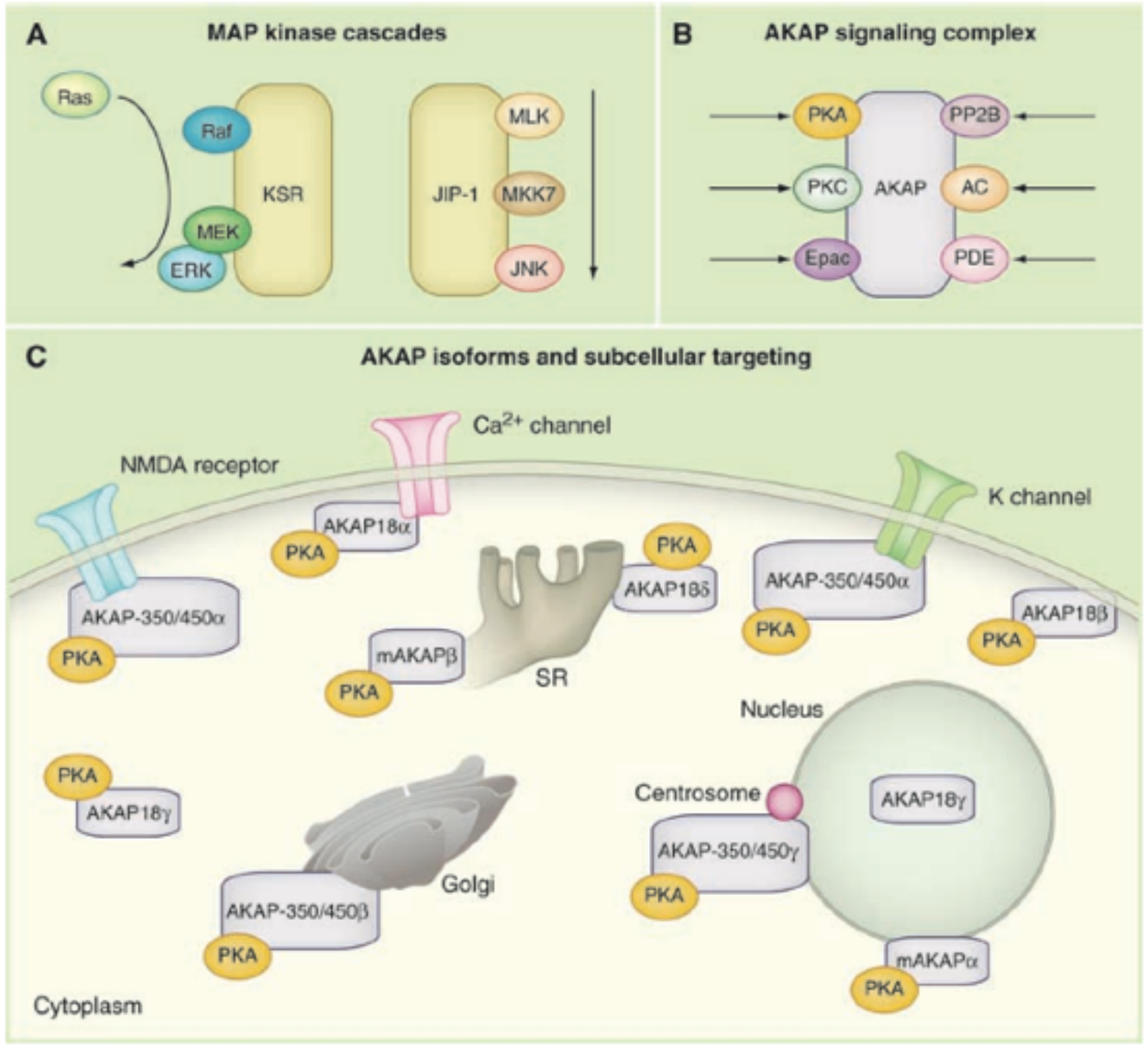
Spatial Cell Biology

REVIEW

Cell Signaling in Space and Time: Where Proteins Come Together and When They're Apart

John D. Scott[1]* and Tony Pawson[2,3]*

Science, 326, 2009



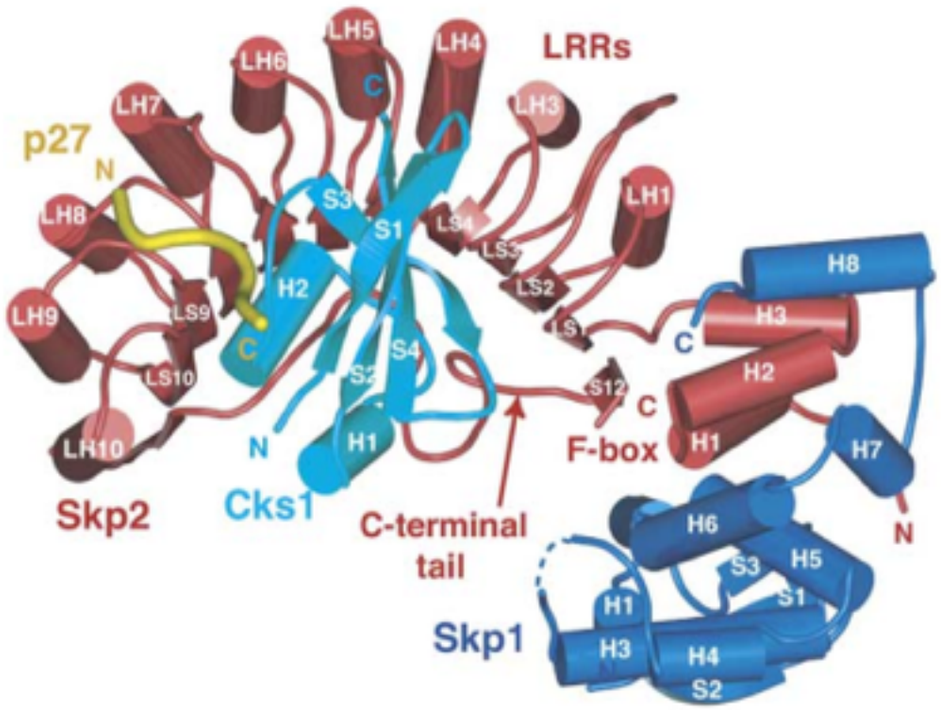
Tony Pawson, Cell (2004)

While there is still much debate about these ideas, the spatial segregation of signaling pathways is likely to be an important topic for the future.

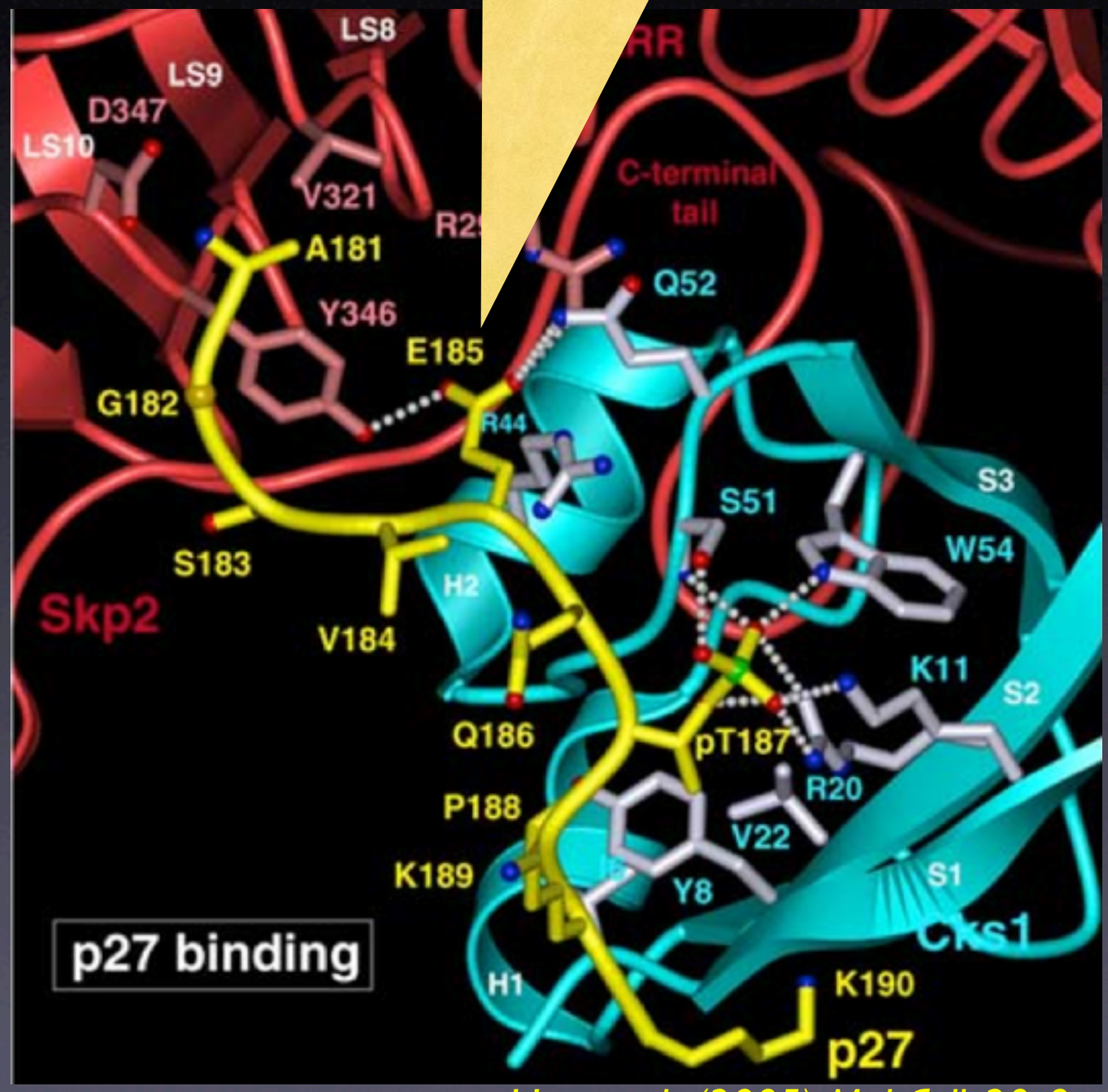
Cooperativity by preassembly: P27kip1 phosphorylated motif bound by a complex of Skp1-Skp2-Cks1

C

```
                                  181                 190
p27 human      175 SDGSPNAGSVEQTPKKPGLRRRQT
p27 pig        175 SDGSPNSASVEQTPKKPGLRRRQT
p27 mouse      175 SDGSPNAGTVEQTPKKPGLRR-QT
p27 duck       175 SEDSPSASSVEQTPKKSSPRRHQT
p27 chicken    175 SEDSPSASSVEQTPKKSSPRRHQT
p27 hamster    175 SDGSLNAGSVEQTPKKPGLRRHQT
p27 xenopus    192 TKGVHLLCPLEQTPRKK-IR
```



Glu185 is bound co-operatively by Skp2 and Cks1

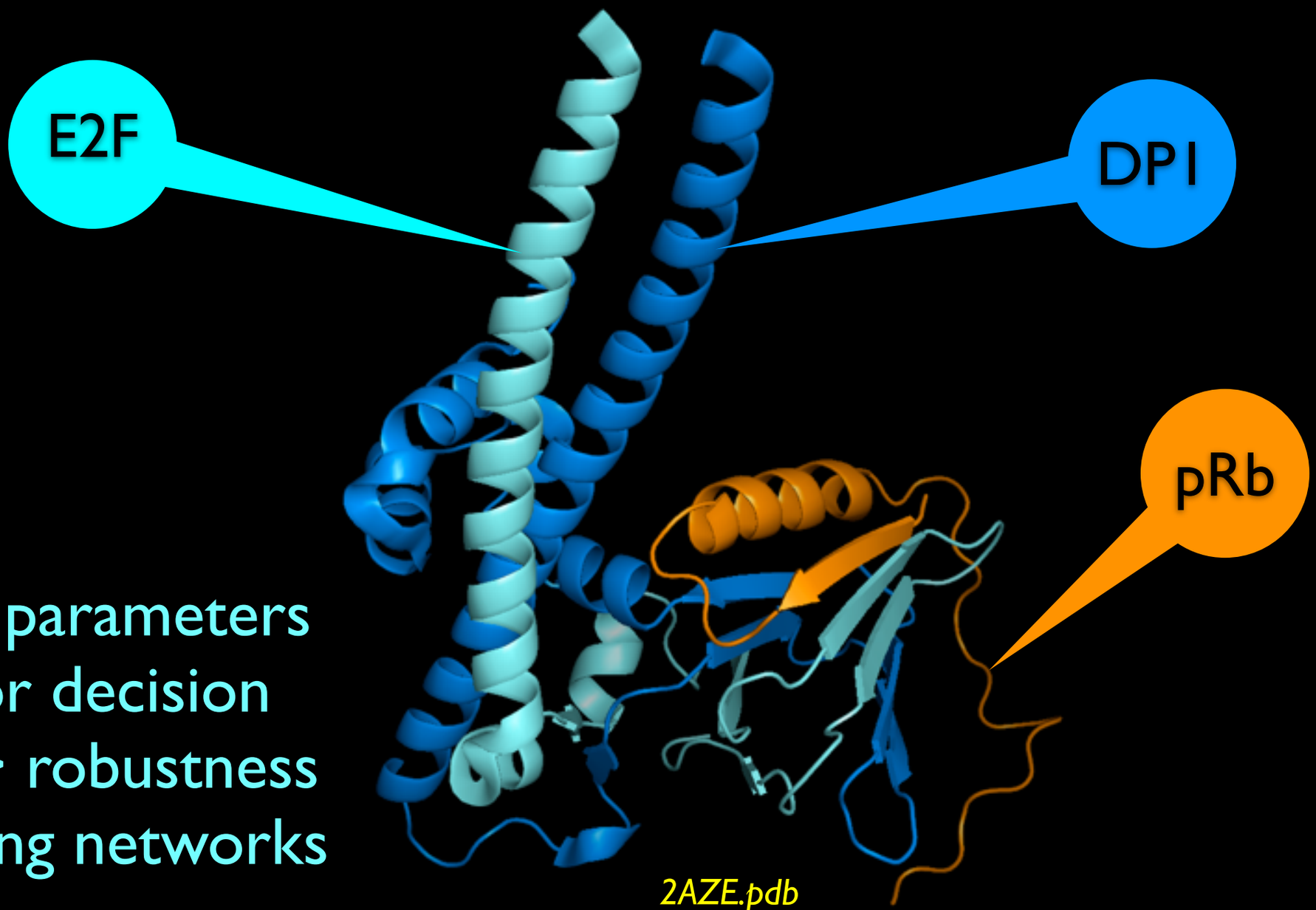


Hao et al., (2005) Mol Cell. 20, 9

Cooperativity of IDRs - Intrinsically Disordered Regions

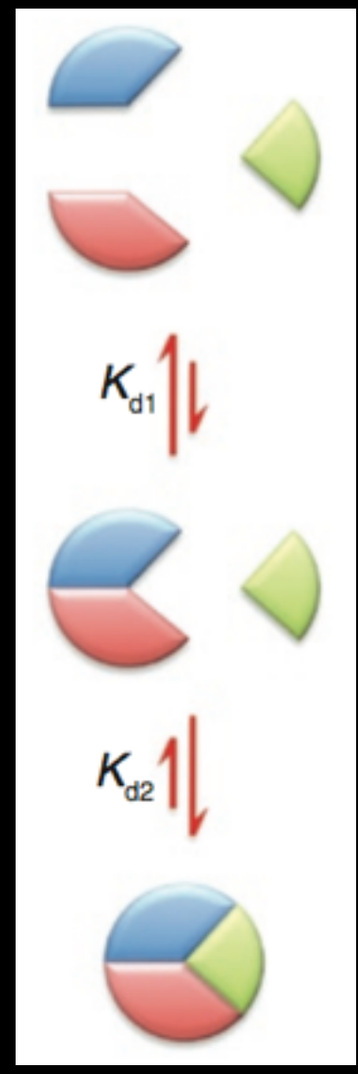
Regulation by cooperative assembly of E2F1, DP1 and Rb

Mutual induced fit assembly of a repressive heterotrimer from three natively disordered protein segments



2AZE.pdb
Rubin et al. (2005) Cell 123, 1093

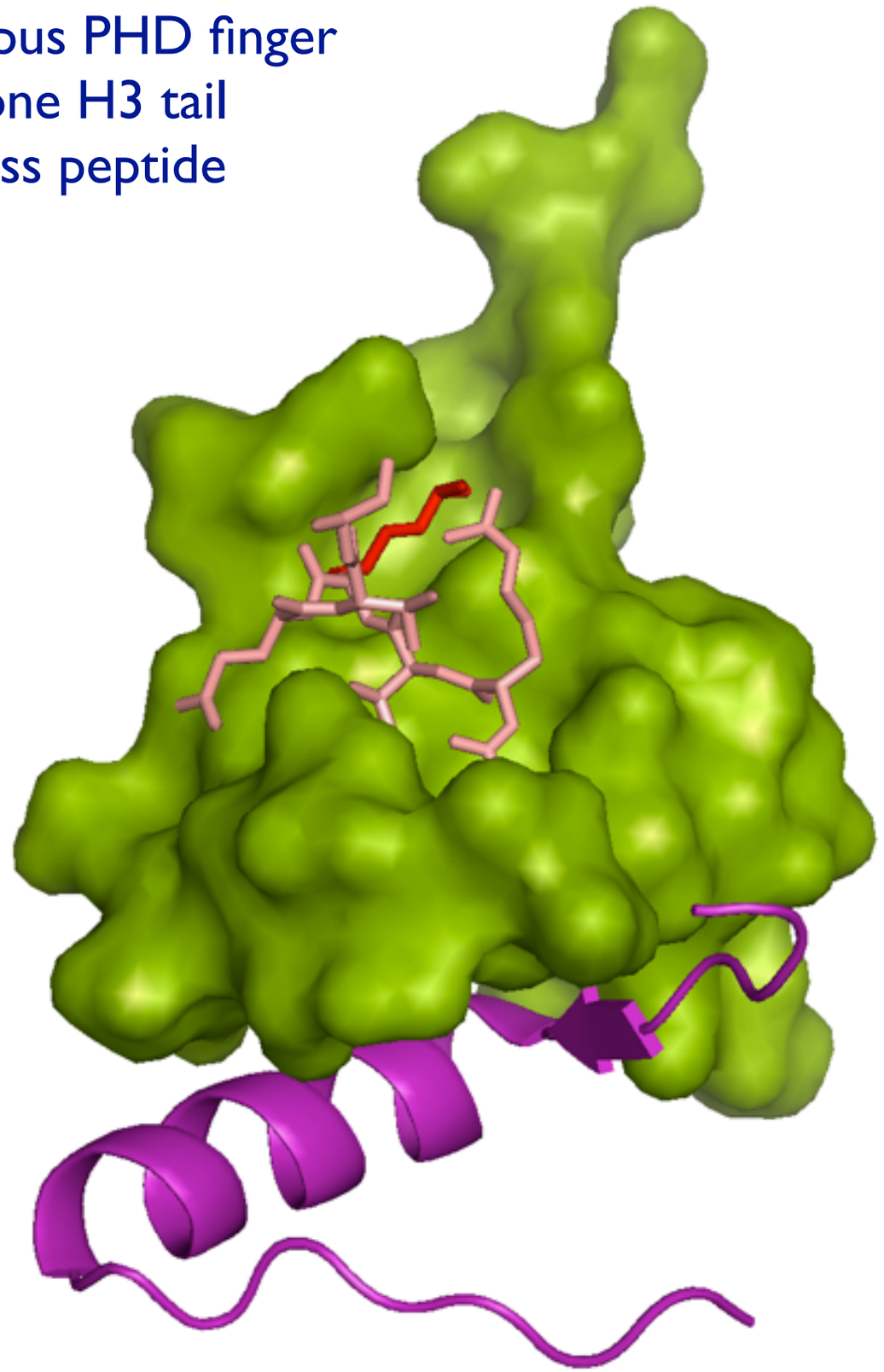
Multiple parameters used for decision making > robustness in signalling networks



Whitty (2008)
NCB, 4, 435

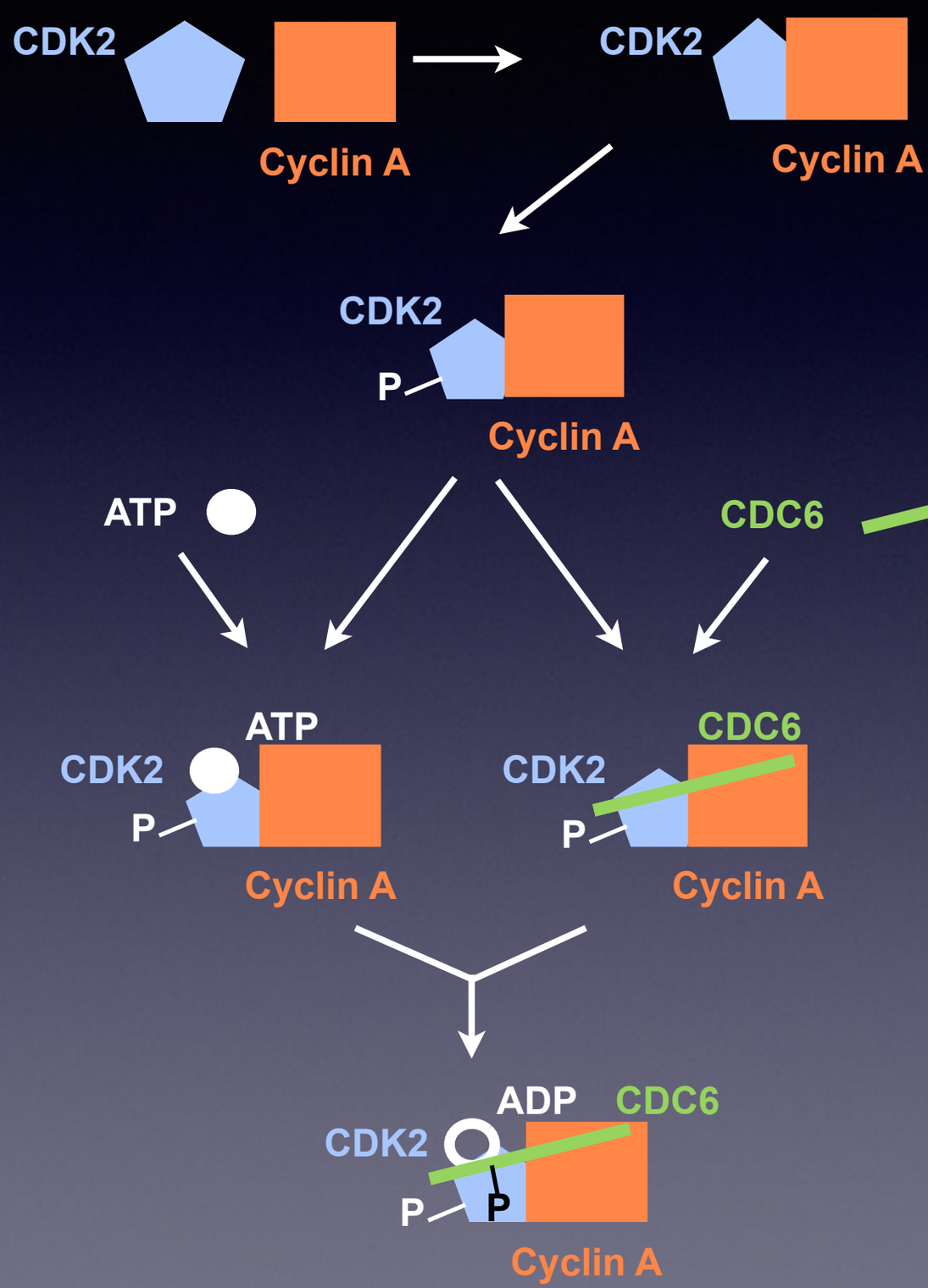
Cooperativity of SLiMs
Allostery of peptide motifs

Pygopus PHD finger
Histone H3 tail
Legless peptide



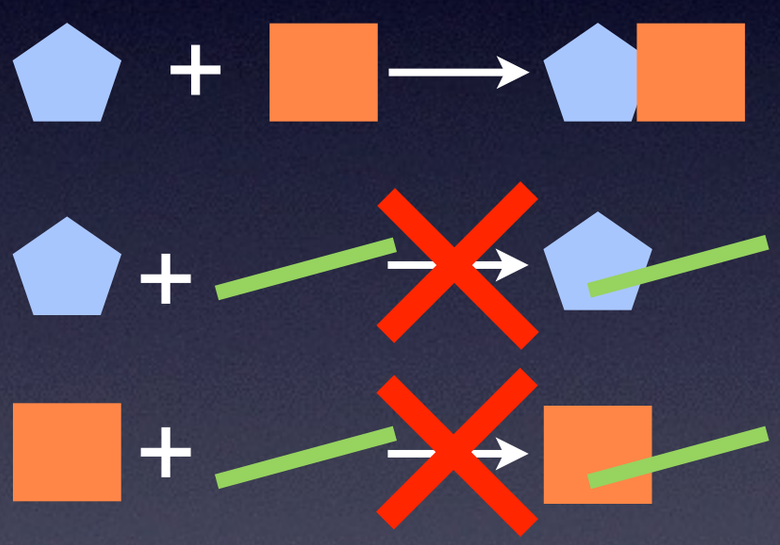
Fiedler et al. (2008) Mol Cell. 30, 507

Phosphorylation of CDC6 by Cdk2-CyclinA



How Bioinformatics interaction standards work: Capturing Phosphorylation of CDC6 by Cdk2-CyclinA

Current representation
Binary Interactions

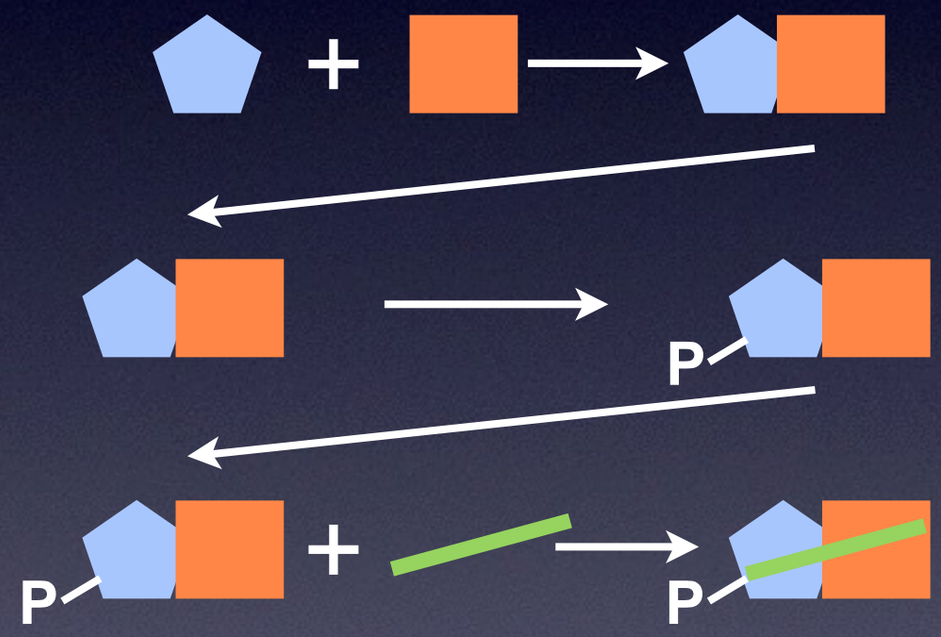


Binary

Distinct

Independent

Desired representation
Cooperative Interactions



Multivalent

Allosteric

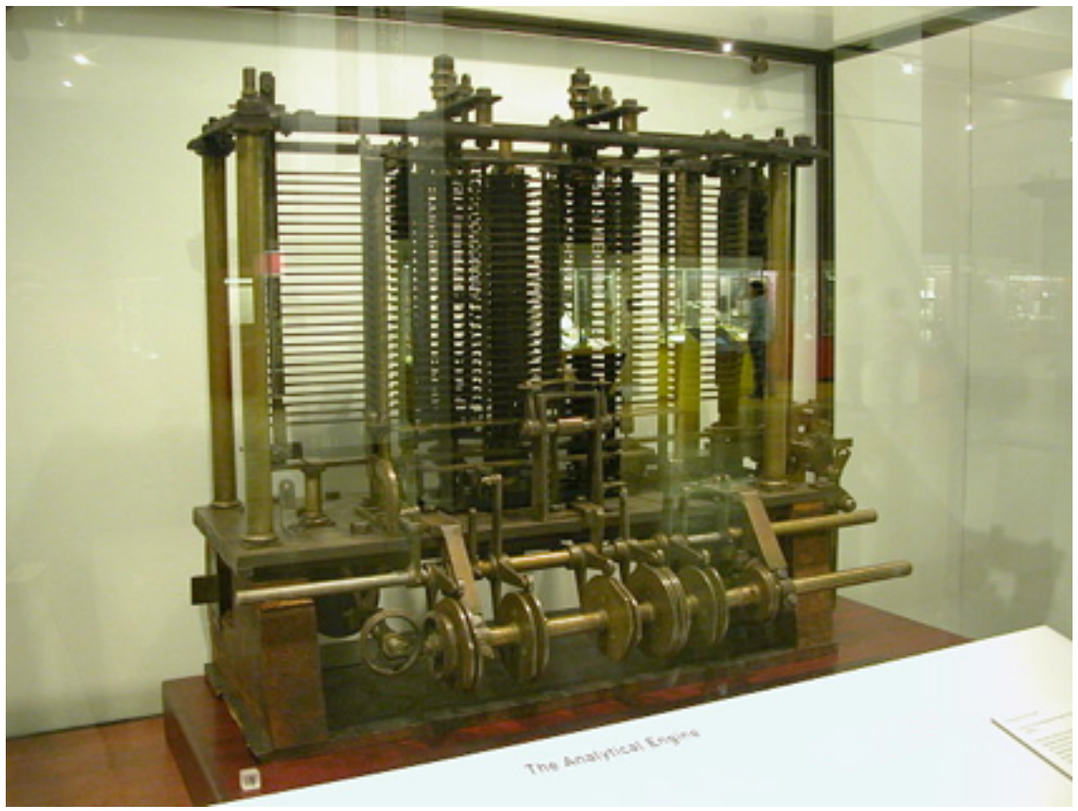
Interdependency of binding events

Allostery

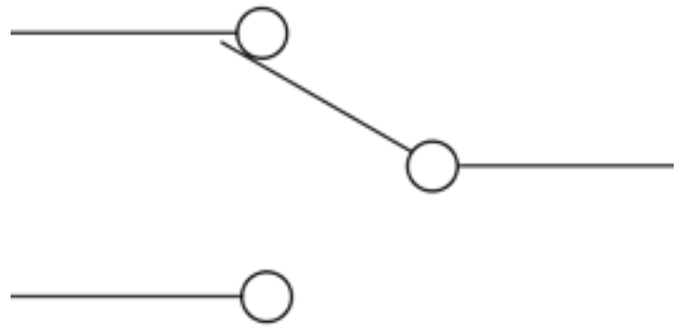
“The second secret of life”

Jacques Monod

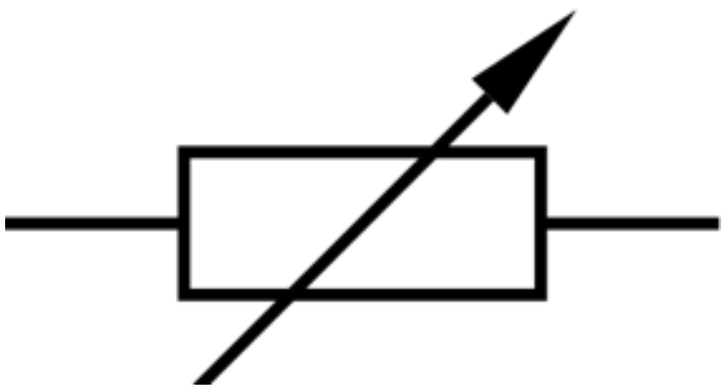
Logic processing is always done by machines with switches



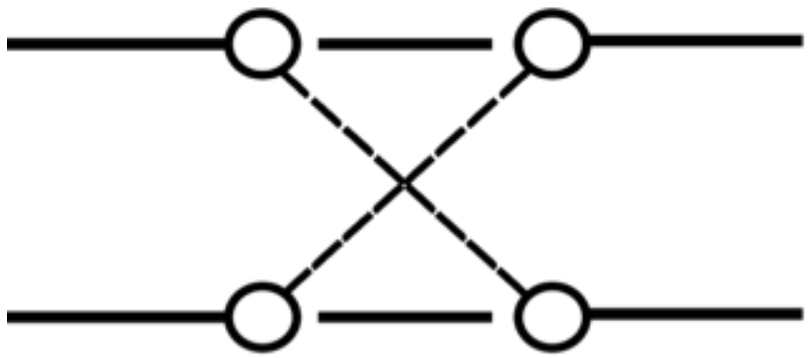
Babbage analytical engine



3-way switch



Rheostat



4-way switch

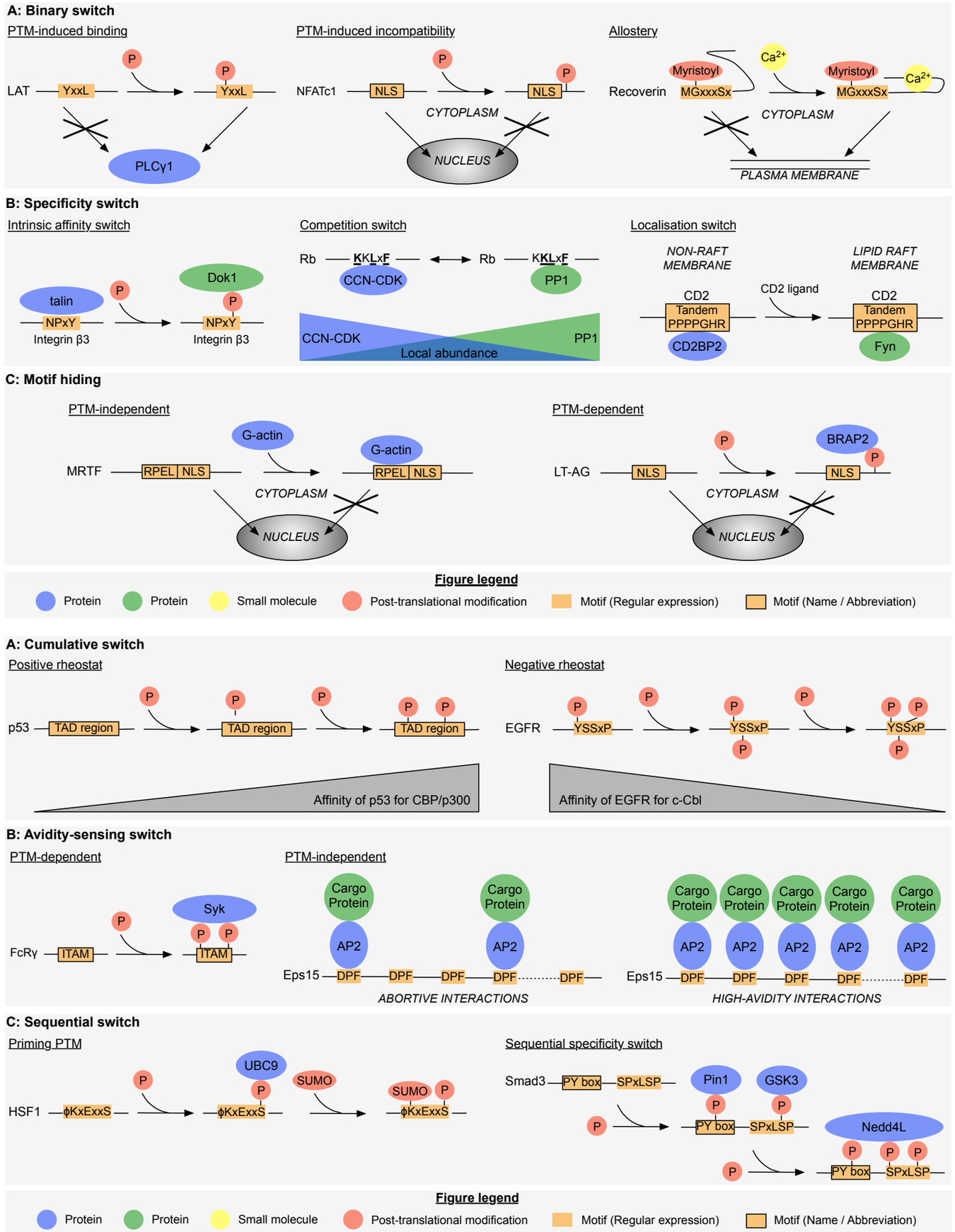
Available online at www.sciencedirect.com
SciVerse ScienceDirect
Current Opinion in Structural Biology

Switches.elm.eu.org

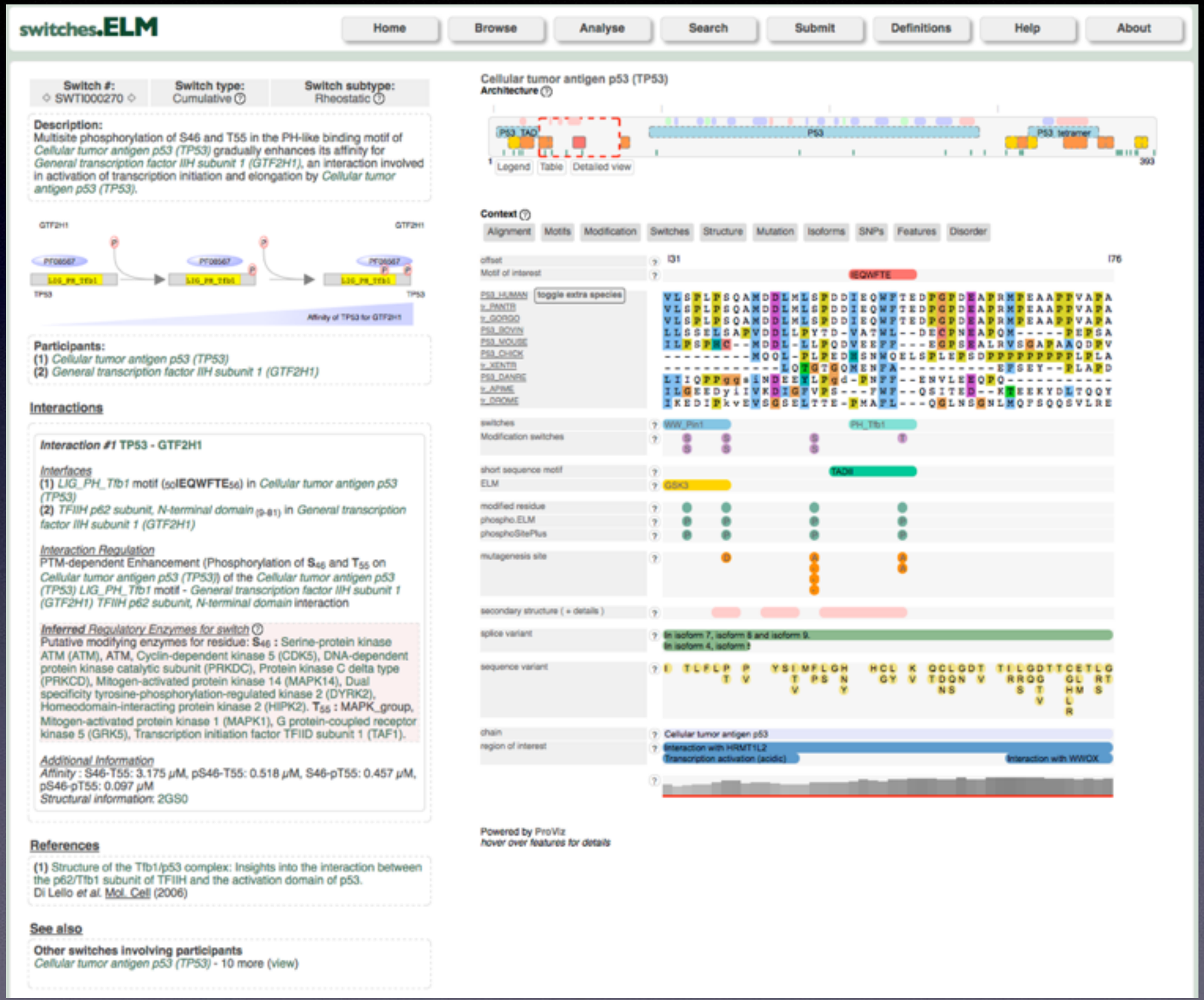
Motif switches: decision-making in cell regulation
Kim Van Roey[1], Toby J Gibson[1] and Norman E Davey[1,2]

Six classes of molecular switch involving IDP

- Binary Switch
  - Simple On-Off
- Specificity Switch
  - Multiple On states
- Motif-Hiding Switch
  - Conditional motif accessibility
- Cumulative Switch
  - Graduated rheostat-like behaviour
- Avidity sensing
  - Sharp, cooperative affinity shift
- Sequential Switch
  - Strict logical dependence of execution



switches.ELM p53 rheostatic switch example



Cell Regulatory Decisions

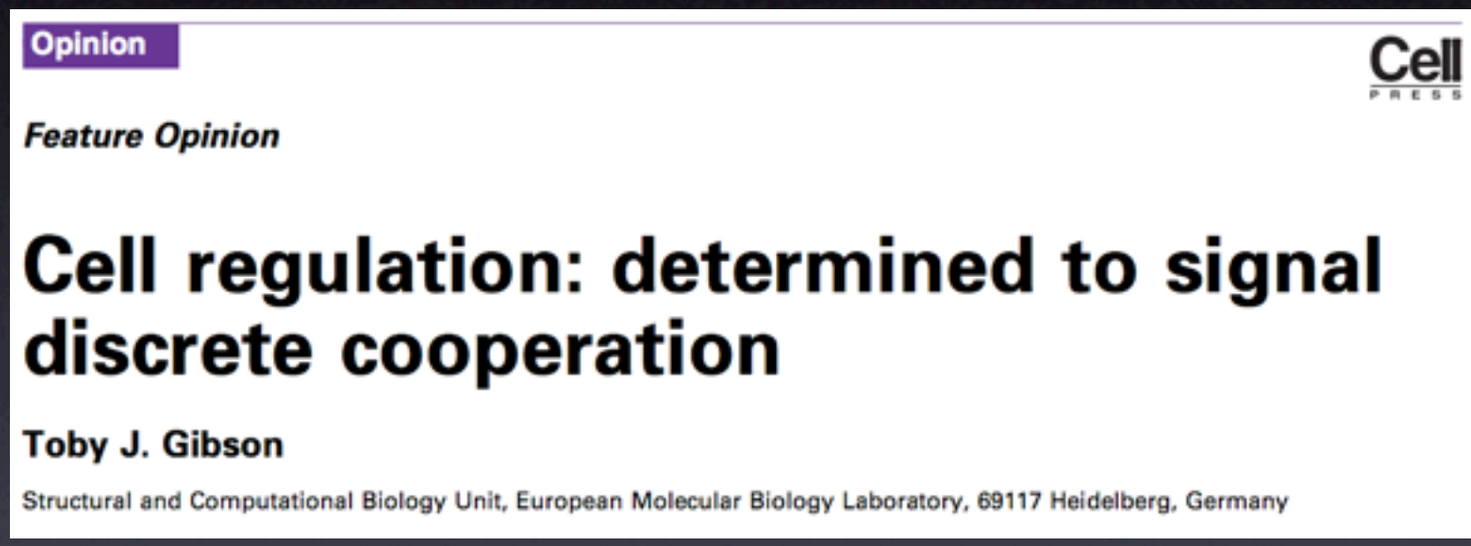
- Are made in large complexes
  - by in-complex molecular switching
    - including addition and subtraction of proteins to complexes
  - using switches assembled from low affinity interacting components
- Allostery is a major switching mechanism
- Pre-assembly is a major switching mechanism
  - and variations on pre-assembly switches include rheostats, avidity sensors, motif-hiding switches, sequential switches....

Everything should be made as simple as possible, but not simpler

Albert Einstein

Cell regulation is networked and redundant being effected by discrete, precise and cooperative molecular switches in large regulatory protein complexes

- No cellular dictator
- No master regulator
- No first among equals
- No top-down system of governance



Opinion

Cell PRESS

Feature Opinion

Cell regulation: determined to signal discrete cooperation

Toby J. Gibson

Structural and Computational Biology Unit, European Molecular Biology Laboratory, 69117 Heidelberg, Germany

TIBS 10/09

The "politics" of the Cell is Anarcho-Syndicalist

Homage to Catalonia

Some Cooperative Interactors from the past and present

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Aidan Budd
Francesca Diella (V)
Holger Dinkel
Sara Kalman
Manjeet Kumar
Vlada Milchevskaya
Grischa Tödt
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EU Grant Coordinators

SysCilia FP7 6/2010 - 15
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Martin Miller (Lyngby)
Thomas Sicheritz-Pontén (Lyngby)
Scott Cameron (Dundee)
Bernhard Kuster (Cellzome)
Lars Jensen (Bork)
Allegra Via (Rome)

Cooperative Standards
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Sandra Orchard (EBI)
Chris Workman (Copenhagen)
Olga Rigina (Copenhagen)
Fred de Masi (Copenhagen)

Clustal W/X 2.0
Mark Larkin (Dublin)
Des Higgins (Dublin)
Chenna Ramu (Berlin)
Nigel Brown (Heidelberg)
Rodrigo Lopez (EBI)
Julie Thompson (Strasbourg)

Ataxin-1 Molecular Switch
Annalisa Pastore (Mill Hill)
Cesira de Chiara (Mill Hill)

Transient overexpression
Reiner Veitia (Paris)

Million Motifs
Madan Babu (Cambridge)
Peter Tompa (Brussels)

DiGtoP BMBF/2008-2013
Wolfgang Wurst (München)
Francis Stewart (Dresden)
Matthias Mann (München)
Tony Hyman (Dresden)
Oliver Brüstle (Bonn)
.....