


# ATM-CHK2 SIGNALLING IN MAMMALIAN AGEING

Dr. Ashley Craig

2004 RSE/Lloyds TSB Foundation for Scotland Personal  
Research Fellowship

Cell Signalling Unit  
Cancer Research Centre  
University of Edinburgh

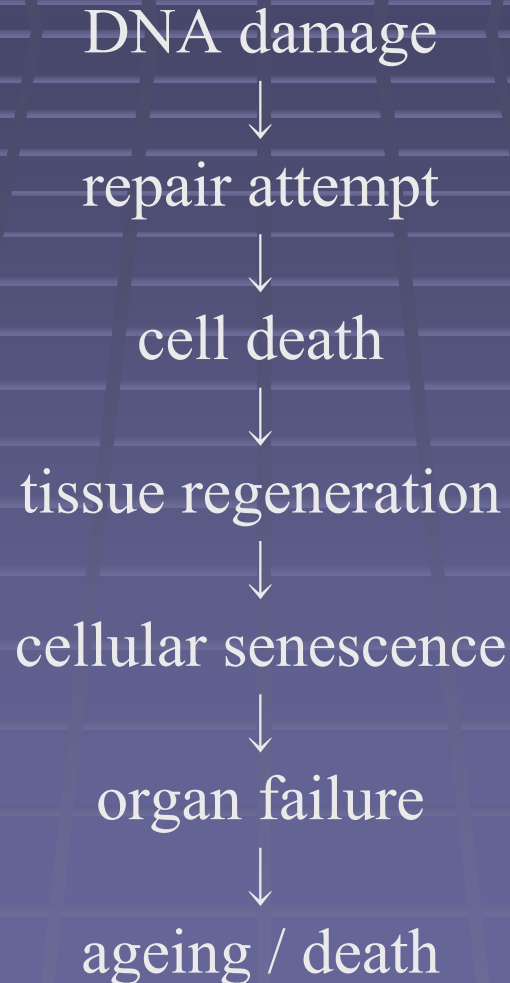
# Ageing-related health problems

- Decline in mental health
- Loss of muscle strength
- Reduced wound healing 

Elucidating the damage-induced signalling pathways causing cell death is essential to design rational drug therapies to improve wound healing in the elderly

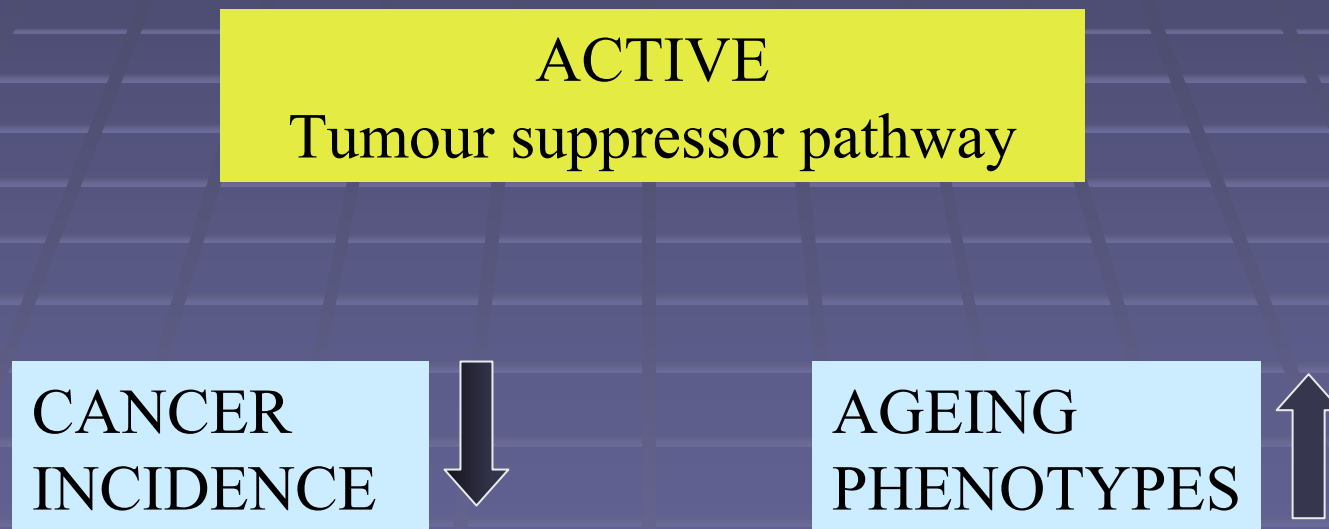
# Normal ageing pathway:

Loss of efficiency of damage repair systems causes ageing



Tumour suppressors can kill damaged cells

# Age-related damage increases activity of tumour suppressors



# The p53 tumour suppressor pathway

- "Tumour suppressors" become activated in ageing tissues due to accumulated cellular damage
- p53 is a damage-activated "master switch" that controls the expression of genes to protect from cancer initiation / progression events
- p53 activation can kill or stop proliferation of damaged cells
- Loss of cell number can accelerate ageing

# p53 has a central role in ageing

## Prolonged damage signals due to:

- genomic instability
- telomere erosion
- reduced DNA repair

'Superactive p53' mouse

inputs?



INACTIVE p53



ACTIVATED p53

p66<sup>SHC</sup>

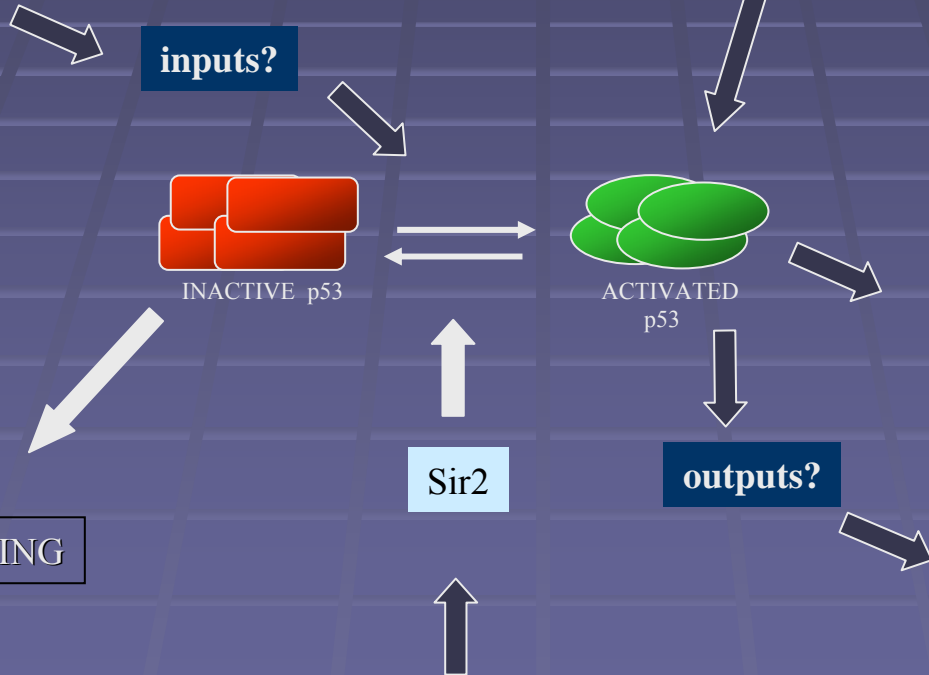
outputs?

NORMAL AGEING

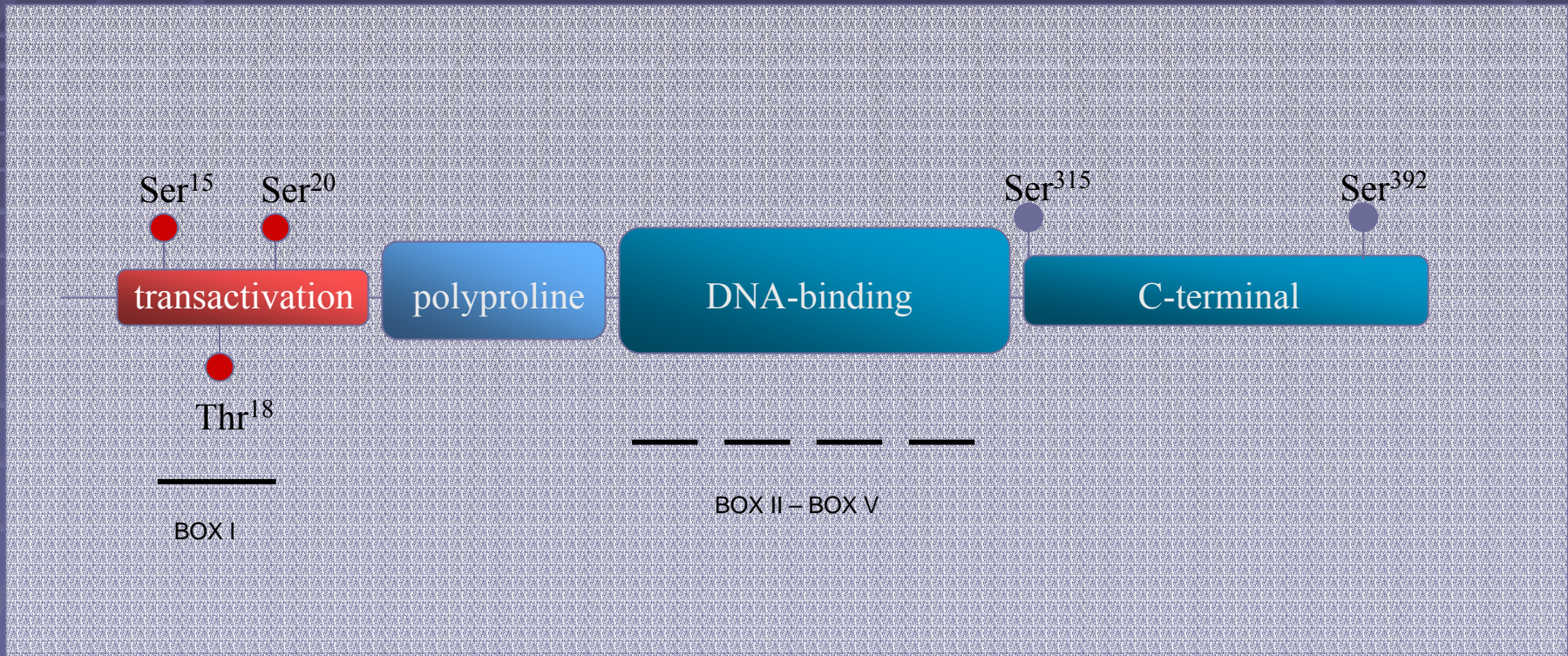
Sir2

PREMATURE AGEING

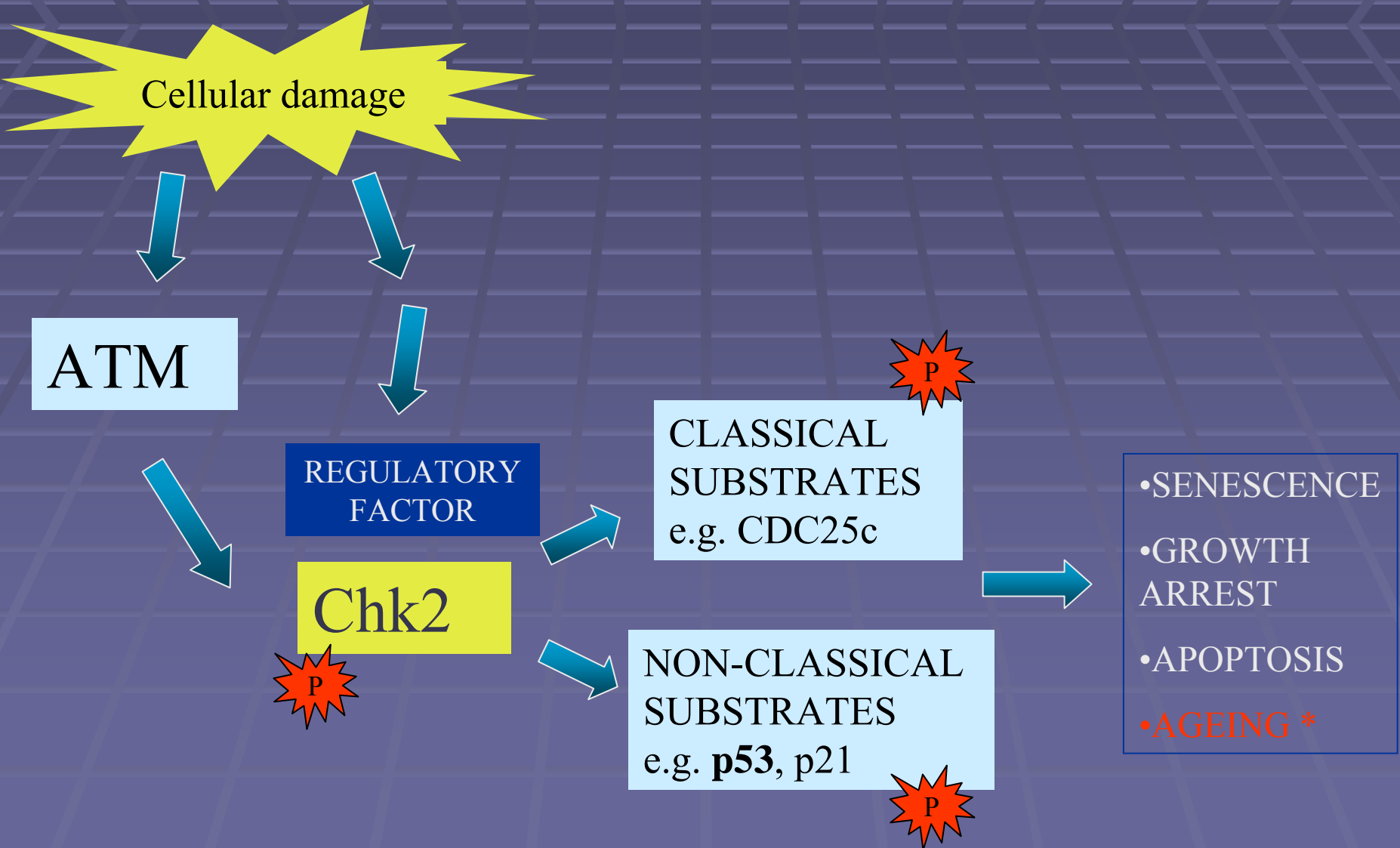
Calorie restriction



# p53 structure



# ATM - Chk2: a major p53 activation pathway



# Outline of talk:

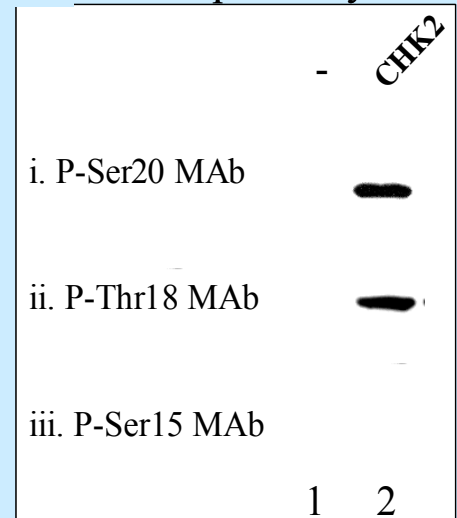
1. Chk2 is a p53 kinase
2. ATM signalling in ES cells
3. The role of Chk2 in mammalian ageing

# I. Chk2 is a p53 kinase

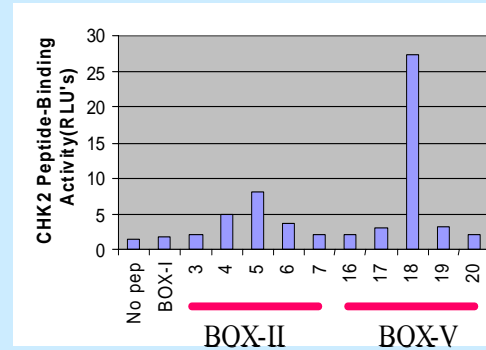
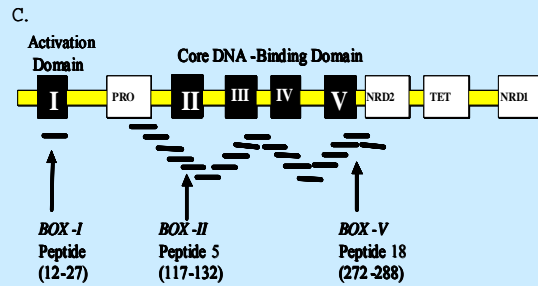
## Chk2 targets:

Chk1-tide		L	V	R	Y	P	S	F	V	I	T	A	K
Chk2-tide		L	E	R	Q	D	S	I	F	Y	P	K	K
Cdc25A	123	L	K	R	S	H	S	D	S	L	D	H	D
	178	T	Q	R	Q	N	S	A	P	A	R	M	L
	292	T	K	R	R	K	S	M	S	G	A	S	P
Cdc25C	216	S	G	L	Y	R	S	P	S	M	P	E	N
BRCA-1	988	L	F	P	I	K	S	F	V	K	T	K	C
E2F-1	364	L	S	R	M	G	S	L	R	A	P	V	D
PML	117	L	Q	R	R	L	S	V	Y	R	Q	I	V
p53	18	P	L	S	Q	E	T	F	S	D	L	W	K
	20	S	Q	E	T	F	S	D	L	W	K	L	L
Chk2	383	T	S	L	M	R	T	L	C	G	T	P	T
	387	R	T	L	C	G	T	P	T	Y	L	A	P
MDM2	????												
Plk-3	????												

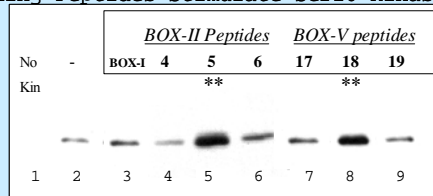
## CHK2 Specificity



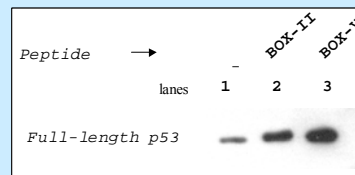
# Chk2 is activated by p53 peptides



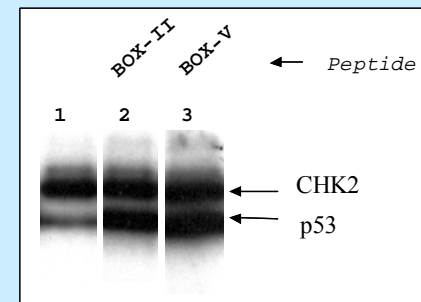
## D. Docking Peptides Stimulate Ser20 Kinase Activity



## E. Docking Peptides Stimulate Thr<sup>18</sup> Kinase Activity



## F. Effects of CHK2-Docking Peptides on 32-P Kinase activity



# Chk2 docking peptides form a continuous surface on the p53 core

## BOX II

<sup>127</sup>SPALNKMFCQLAKTC<sup>141</sup>

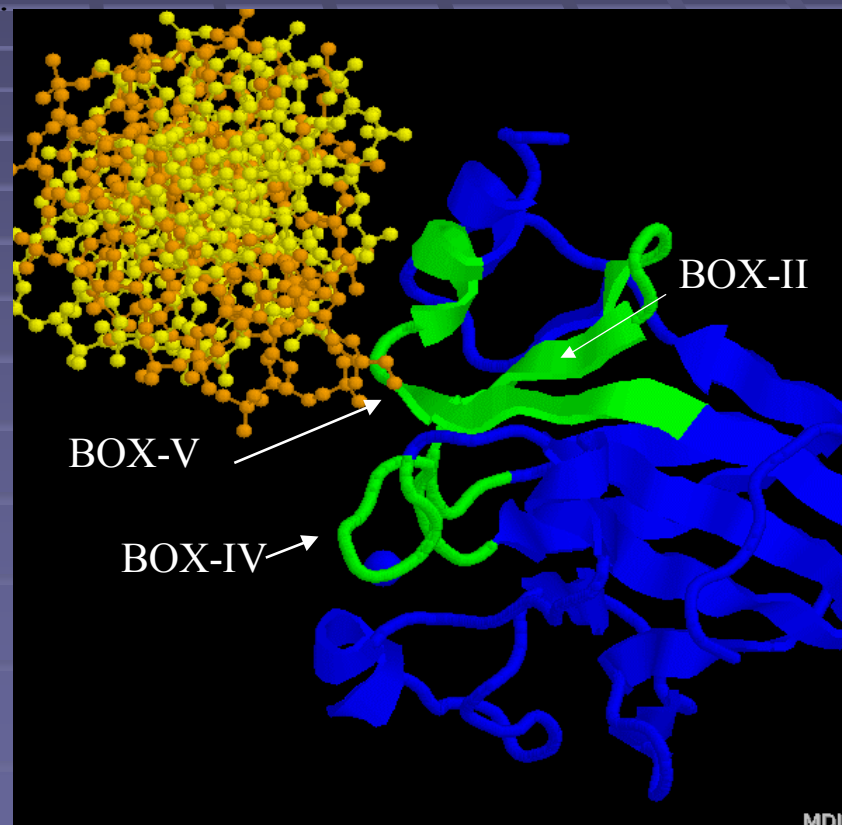
## BOX IV

<sup>237</sup>MCNSSCMGGMNRRPI<sup>251</sup>

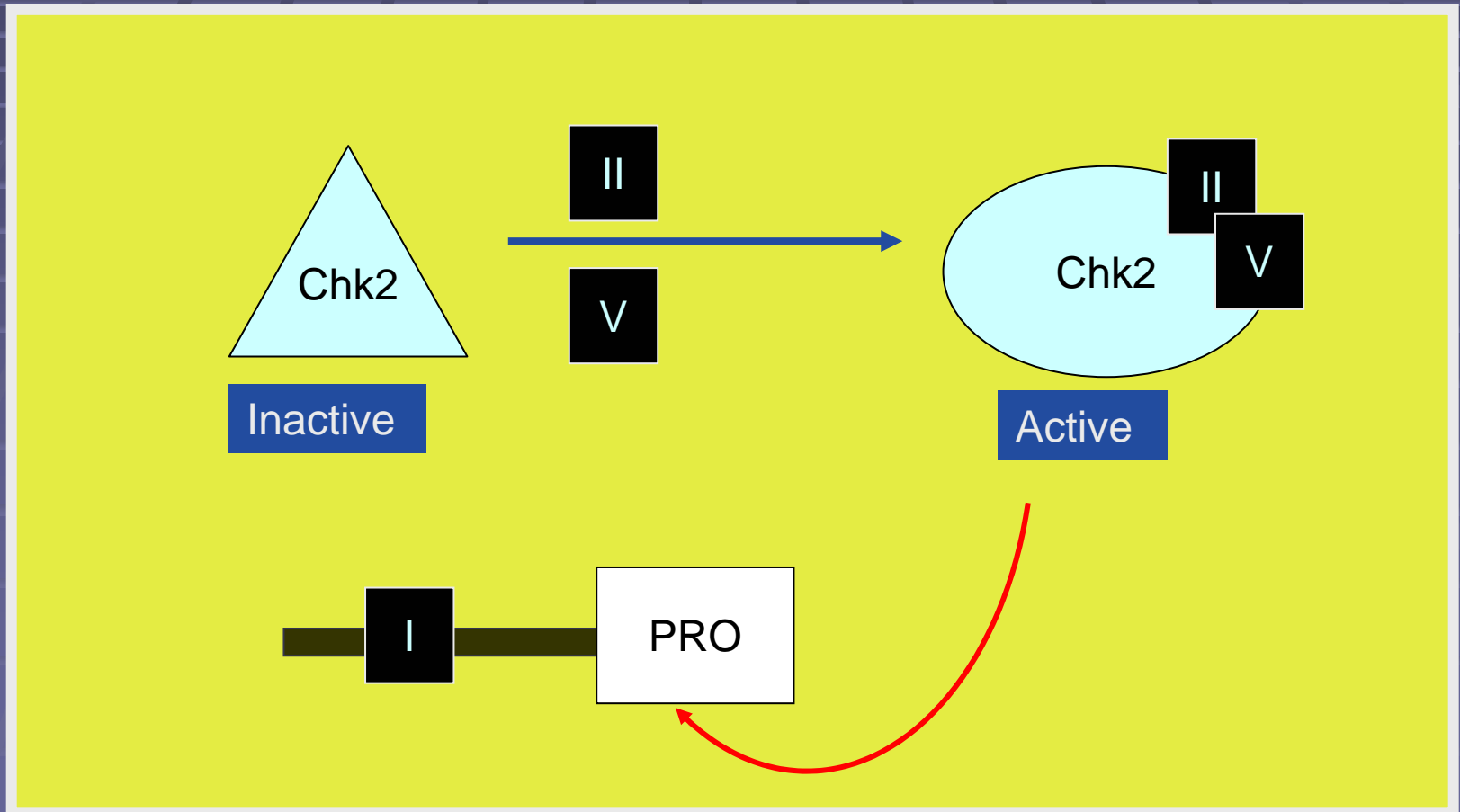
## BOX V

<sup>267</sup>RNSFEVRVCACPGRD<sup>281</sup>

B.



# Allosteric model for Chk2 regulation by p53



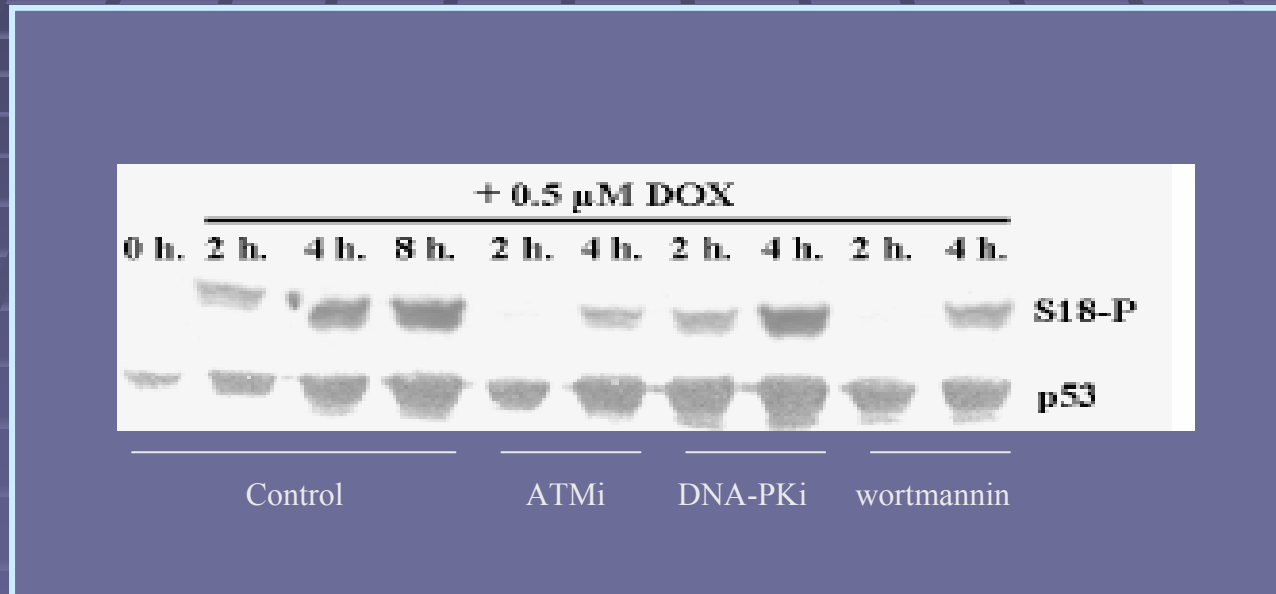
# Summary I

- Chk2 is a p53 kinase
- The phosphorylation sites on p53 do not conform to the Chk2 consensus sequence
- Chk2 binds to the core DNA-binding domain of p53
- This results in the allosteric activation of Chk2 towards a non-classical substrate

## II. ATM signalling to p53 in normal cells

- KU-55933 - specific ATM inhibitor developed by KuDOS Pharmaceuticals
- We have used the ATM inhibitor in a pilot study to delineate ATM signalling in embryonic stem cells

# The ATM pathway is active in ES cells

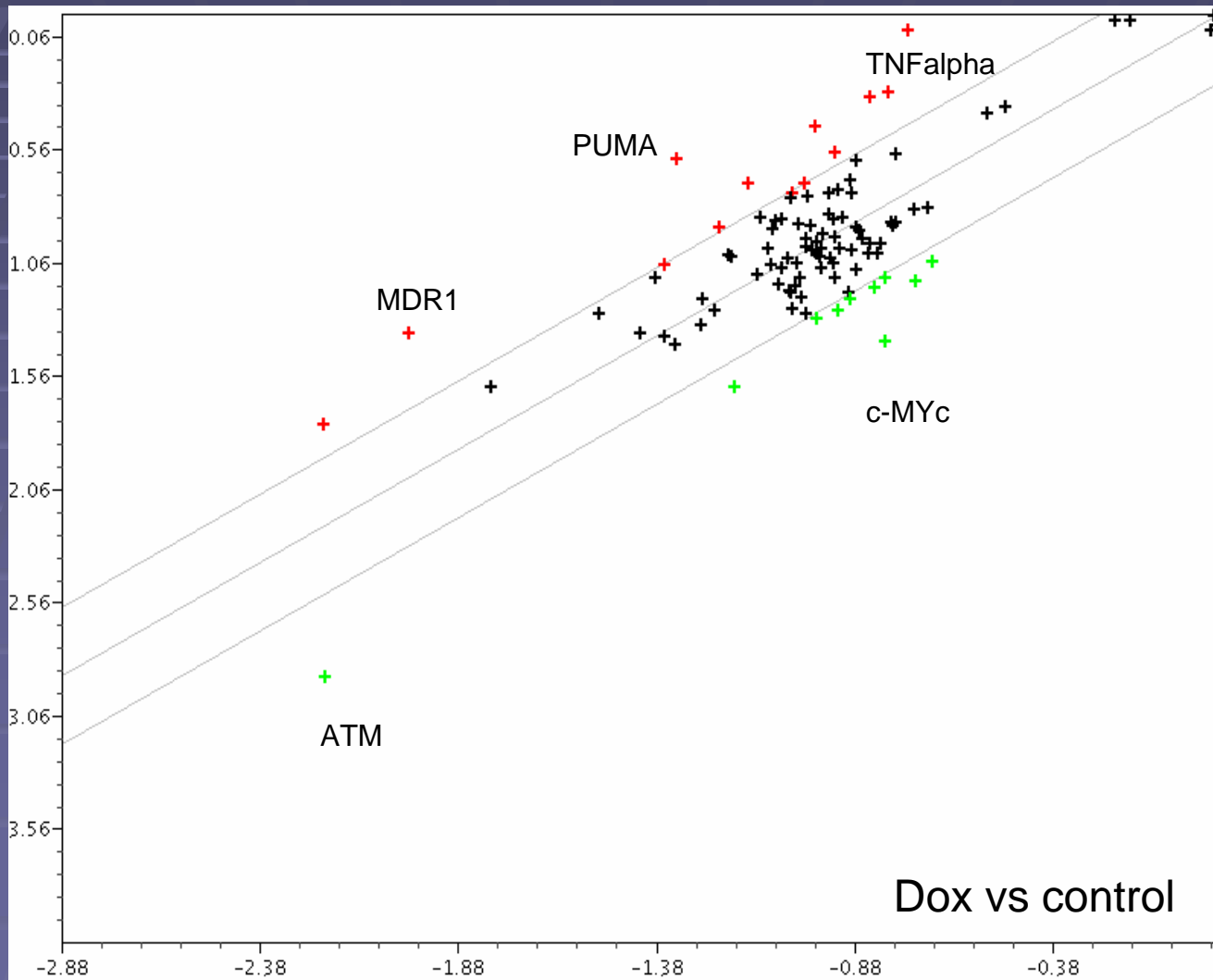


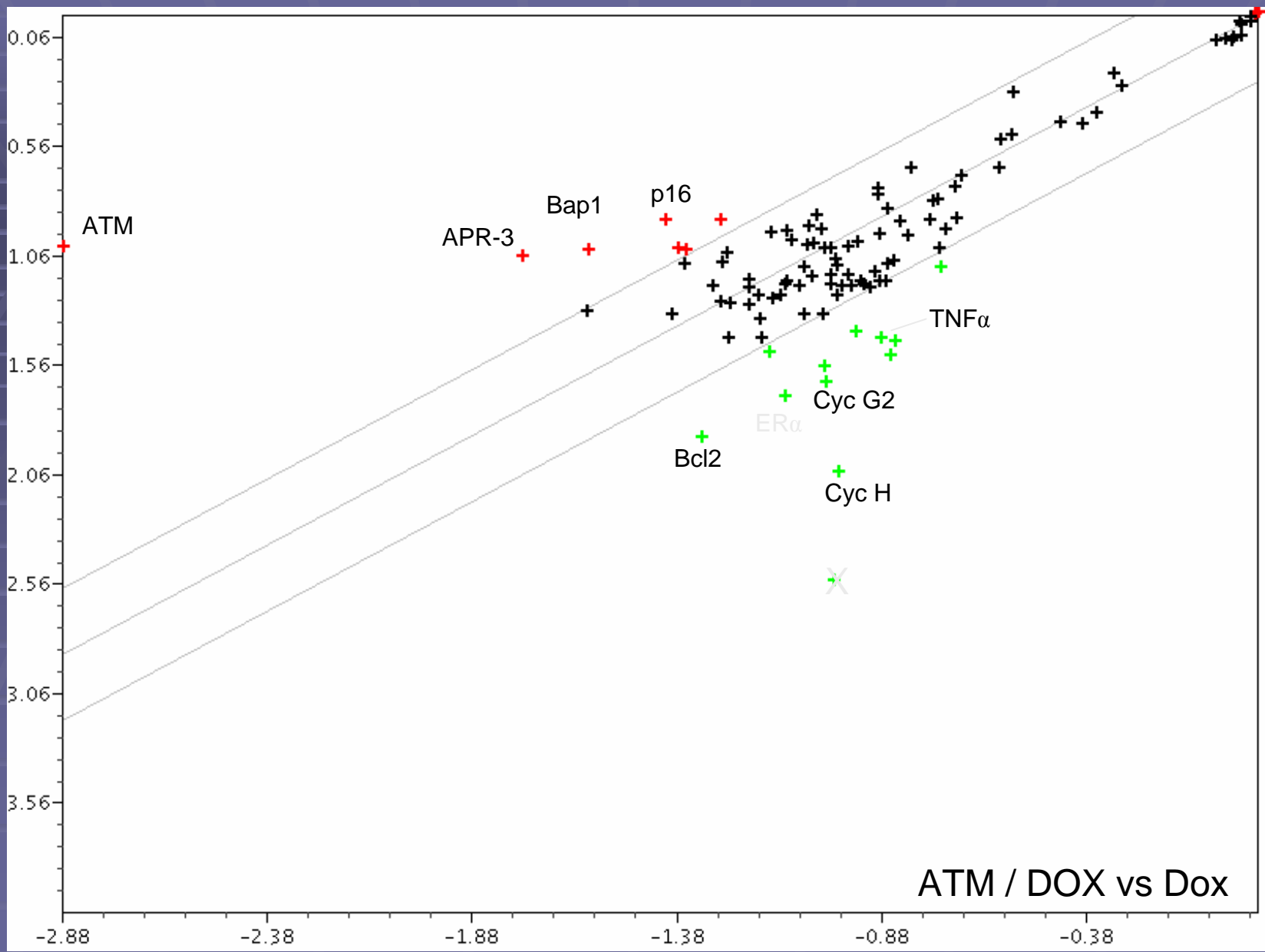
Damage-dependent p53 ser18 phosphorylation is blocked by ATM inhibition



What is the effect on gene expression?

# Superarray analysis



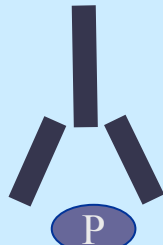


# Summary II

- The ATM signalling pathway is active in ES cells
- Damage-dependent p53 phosphorylation is blocked by ATM inhibition
- Preliminary data suggests that ATM activity feeds back to control ATM gene expression.

# III. Is Chk2 activity affected by ageing?

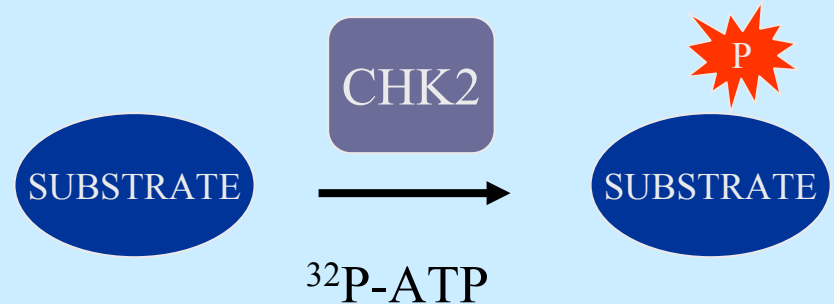
INDIRECT measurement



CHK2...<sup>69</sup>LSSLETVSTQELCSIPEDQ<sup>87</sup>....

Using phospho-Chk2 antibody

DIRECT measurement

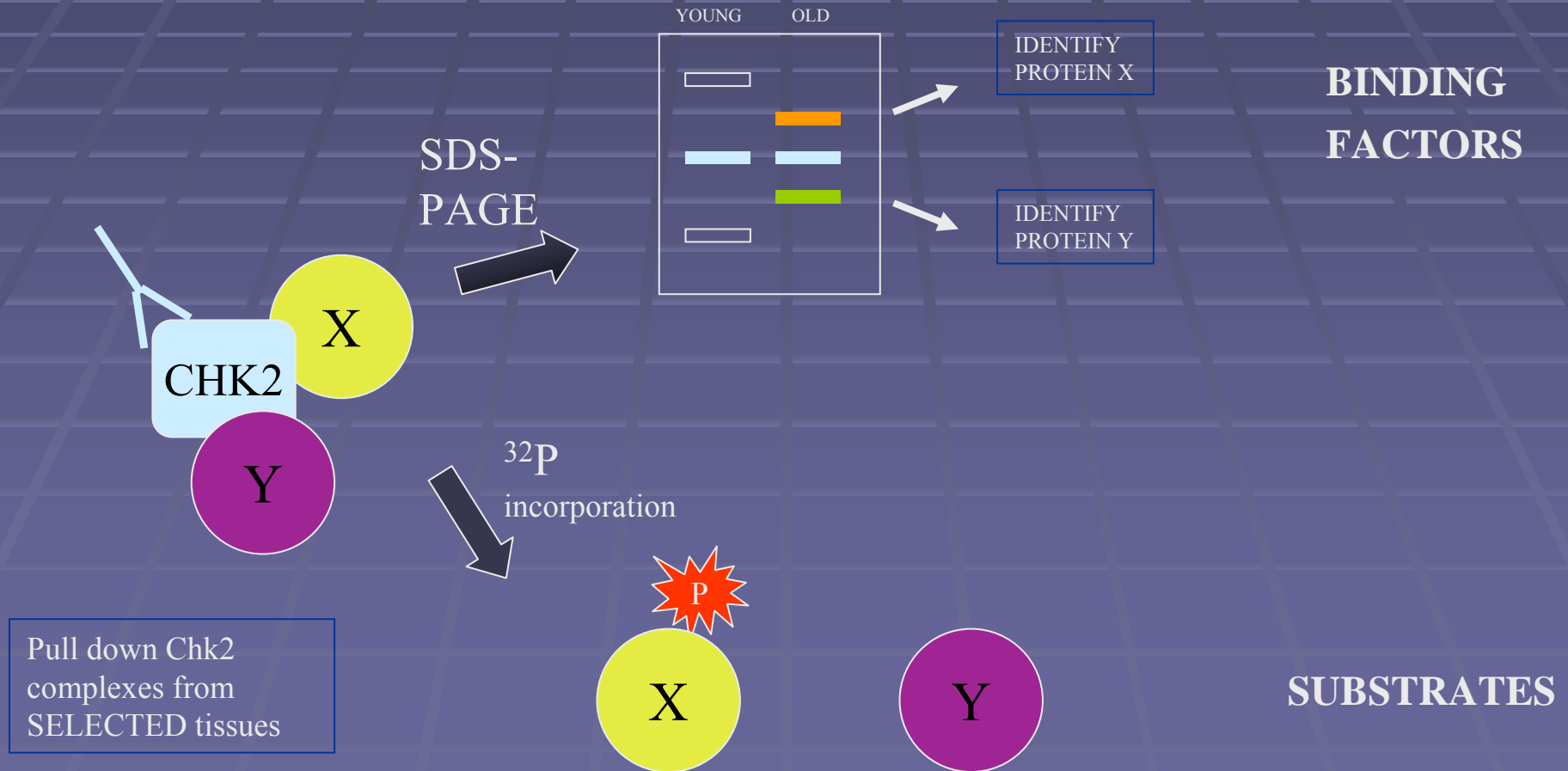


Kinase assay



Screen mouse tissues for age-specific increase in Chk2 activity

# Identification of age-specific Chk2 signalling components



# Functional analysis of age-specific Chk2 pathway components

Age-specific Chk2 pathway components:

- regulatory factors
- substrates

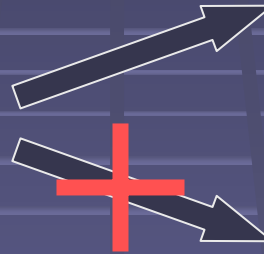
How do the novel regulators affect Chk2 activity in human cells?

These are potential drug targets for inhibiting Chk2 – p53 signalling pathways in ageing

# Action of anti-Chk2 drug

**ACTIVATED CHK2**

**INJURED TISSUE**

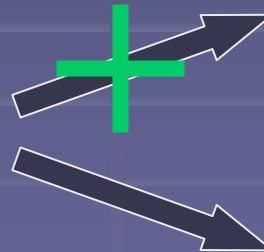


**CELL DEATH**

**TISSUE REPAIR**

**NOVEL DRUG**

**INJURED TISSUE**



**CELL DEATH**

**TISSUE REPAIR**

**Drugs that BLOCK CHK2 ACTIVITY in elderly patients could IMPROVE WOUND HEALING following surgery.**

# Summary III

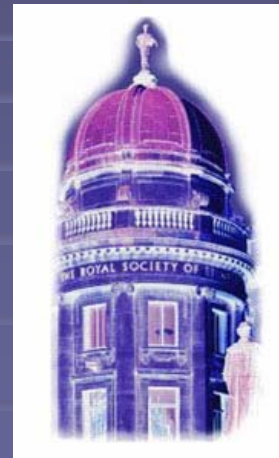
- Age-related changes in Chk2 activity will be investigated using a mouse model system
- Age-specific binding proteins and substrates will be identified
- These are potential drug targets for rational therapies to inhibit Chk2 activity in the elderly
- Short-term use of these drugs could improve post-operative wound healing

# Aknowledgements

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