

June 26 - 30, 2023
Smolenice Castle,
Slovakia

BenBedPhar Training School 2023

NRF2 in non-communicable diseases: From bench to bedside



General introduction to NRF2

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Autonomous University of Madrid, Spain

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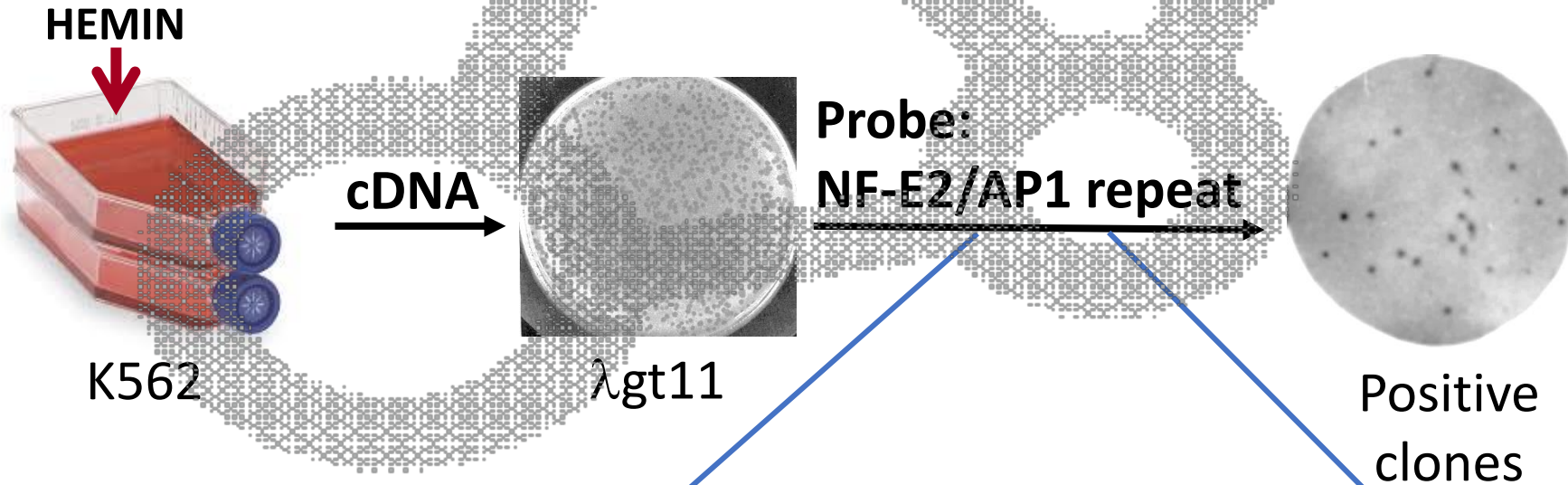
Contents

- Discovery of NRF2. Molecular evolution of NRFs.
- The antioxidant response element (ARE).
- The NRF2 transcriptional signature.
- Regulation of NRF2 at the level of protein stability.
- NRF2 in degenerative diseases.

Cloning and characterization of NRF2

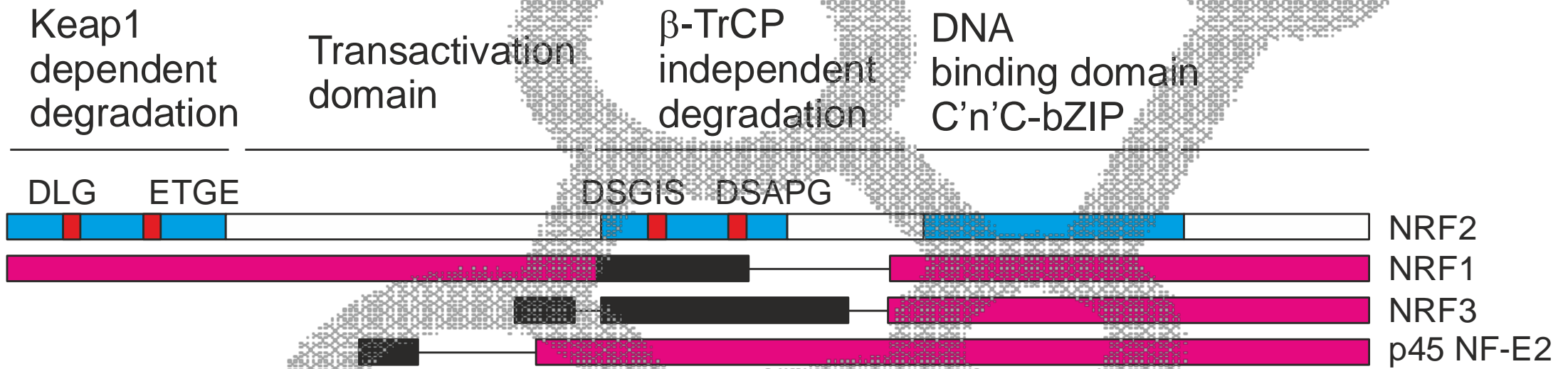
Hiper sensitive site 2 → beta-globin cluster → NF-E2/AP1 site → ?

p45NF-E2 → erythroid and megakaryocytic maturation and differentiation

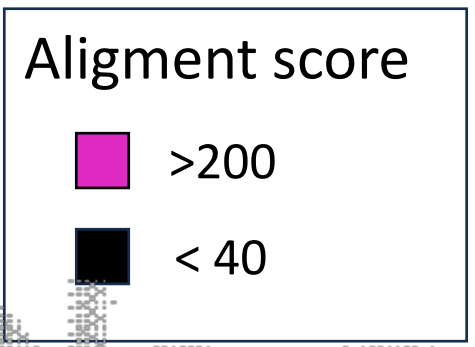


5'-GCACAGCAATGCTGAGTCATGATGAGTCA TGCTG-3'

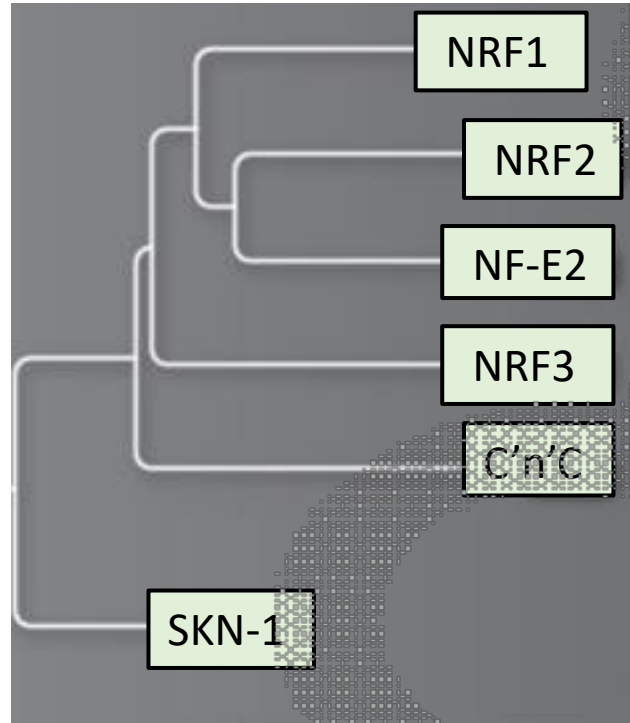
The C'n'C collar family of transcription factors



Different functions as determined in knockout mice



NRFs in Metazoa



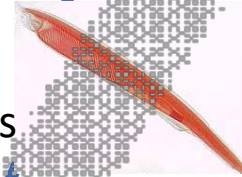
Gnathostomata

NRF1, 2, 3, NF-E2



Cephalochordata

several variations of NRFs



Deuterostomia

Variations of NRF2-like



Lophotrochozoa

NRF2-like



Cnidaria

C'n'C



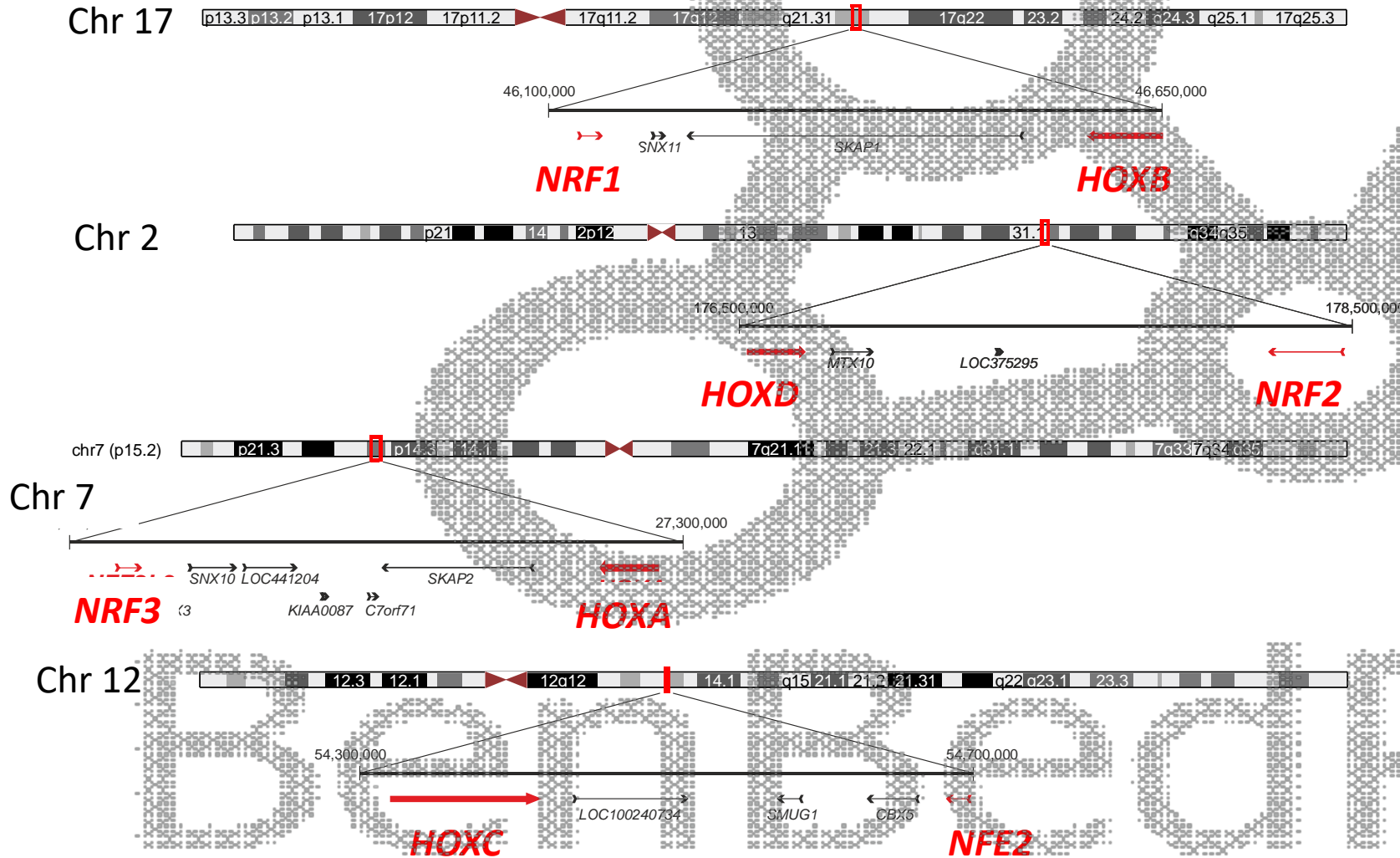
Porifera

bZip



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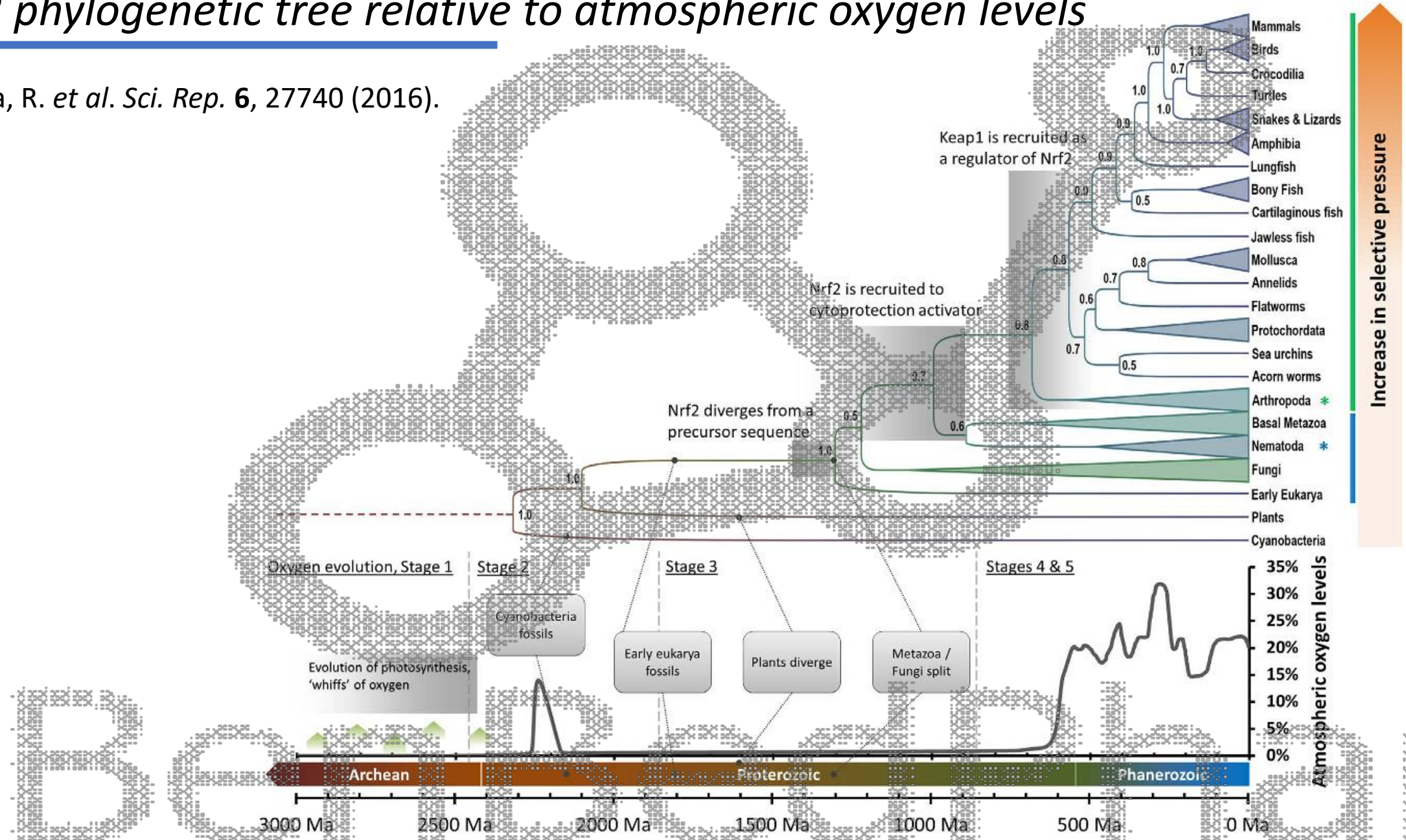
Genome duplications in the proximity to HOX genes



Jiménez et al, unpublished results

Nrf2 phylogenetic tree relative to atmospheric oxygen levels

Gacesa, R. et al. *Sci. Rep.* 6, 27740 (2016).



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Variability of the NF-E2/AP1 sites

TRE type MARE
(T-MARE) 13 bp



CRE type MARE
(C-MARE) 14 bp



NF-E2

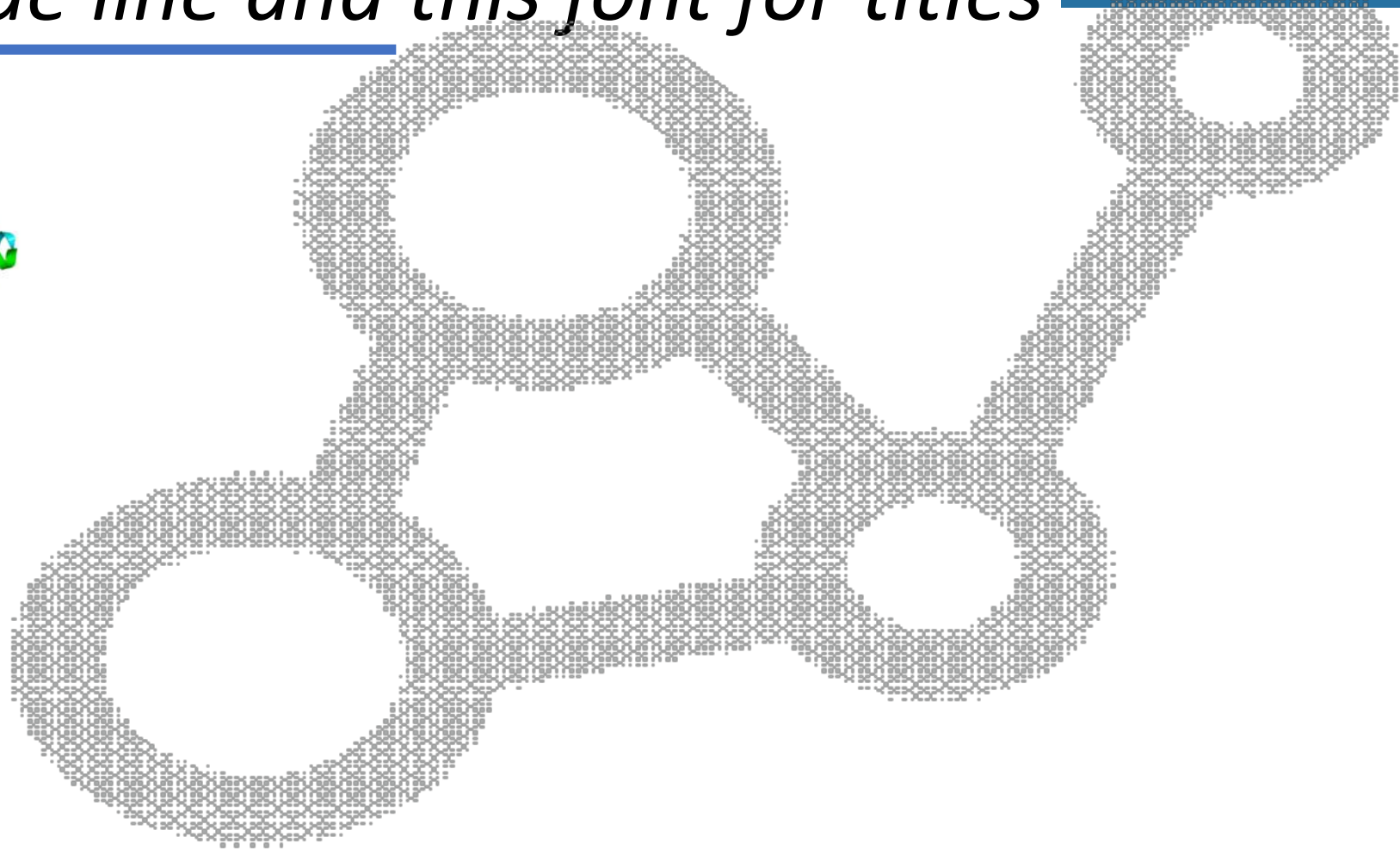
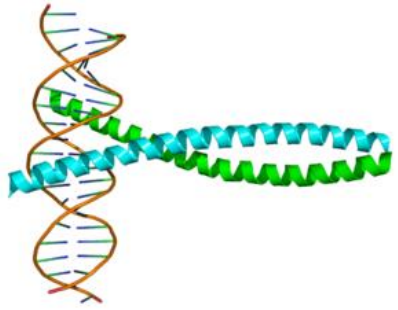


Anti-oxidant responsive
element (ARE)



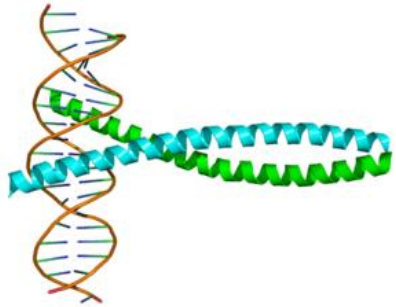
tBHQ, BHA, etc responsive
EpRE sites found in *GST*, *NQO1*

Use this blue line and this font for titles



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Small MAFs:

- MAFF
- MAFG
- MAFK

BACH:

- BACH1
- BACH2

Modified from Hirotsu et al, (2012) Nucleic Acids Res. 40:10228-39.

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NRF2 regulates 1% of the genome?

Function

Detoxification: phase I
Functional groups (-OH)

Detoxification: phase II
Conjugation

Detoxification: phase III
Extracellular transport

symbol	name
ADH7	alcohol dehydrogenase class 4 mu/sigma chain
AKR1B1	aldo-keto reductase family 1, member B1 (and 1B8 and 1B10)
AKR1C1	aldo-keto reductase family 1, member C1 (and 1C2 and 1C3)
ALDH1A1	aldehyde dehydrogenase 1 family, member A1
ALDH3A1	aldehyde dehydrogenase 3 family, member A1 (and 3A2)
ALDH7A1	aldehyde dehydrogenase 7 family, member A1
CBR1	carbonyl reductase 1 (and 3)
CYP1B1	cytochrome P450, family 1, subfamily B, polypeptide 1
CYP2B9	cytochrome P450, family 2, subfamily B, polypeptide 9 (and 10)
EPHX1	epoxide hydrolase 1, microsomal
PTGR1	prostaglandin reductase 1 (also called LTB4DH)
NQO1	NAD(P)H:quinone oxidoreductase 1
GSTA1	glutathione S-transferase class Alpha 1 (and A2, A3 and A4)
GSTM1	glutathione S-transferase class Mu 1 (and M2, M3, M4, M6 and M7)
GSTP1	glutathione S-transferase class Pi 1 (and P2)
MGST1	microsomal glutathione S-transferase 1 (and 2)
SULT1A1	sulfotransferase family, cytosolic, 1A, member 1 (2)
UGT1A1	UDP glucuronosyltransferase 1 family, polypeptide A1 (and 1A5)
UGT2B7	UDP glucuronosyltransferase 2 family, polypeptide B7 (and 2B34)
ABCB6	ATP-binding cassette, subfamily B (MDR/TAP), member 6
ABCC1	ATP-binding cassette, subfamily C (CFTR/MRP), member 1
ABCC2	ATP-binding cassette, subfamily C (CFTR/MRP), member 2
ABCC3	ATP-binding cassette, subfamily C (CFTR/MRP), member 3
ABCC4	ATP-binding cassette, subfamily C (CFTR/MRP), member 4
ABCC5	ATP-binding cassette, subfamily C (CFTR/MRP), member 5

NRF2 regulates 1% of the genome?

Function	symbol	name
Antioxidant: GSH system	<i>GCLC</i>	glutamate-cysteine ligase, catalytic subunit
	<i>GCLM</i>	glutamate-cysteine ligase, modifier subunit
	<i>GGT1</i>	gamma-glutamyltransferase 1
	<i>GLRX</i>	glutaredoxin 1
	<i>GLS</i>	glutaminase
	<i>GPX2</i>	glutathione peroxidase 2
	<i>GPX4</i>	glutathione peroxidase 4
	<i>GSR1</i>	glutathione reductase
	<i>SLC6A9</i>	glycine transporter
	<i>SLC7A11</i>	cystine/glutamate transporter
Antioxidant: PRDX/TXN system	<i>PRDX1</i>	peroxiredoxin 1
	<i>PRDX6</i>	peroxiredoxin 6
	<i>SRXN1</i>	sulfiredoxin-1
	<i>TXN1</i>	thioredoxin
	<i>TXNRD1</i>	thioredoxin reductase 1

NRF2 regulates 1% of the genome?

Function

Heme & Iron metabolism

Gene expression:
Transcription factors

Proteasome/autophagy

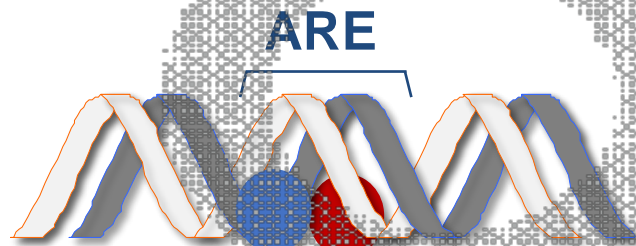
symbol	name
BLVRA	biliverdin reductase A
BLVRB	biliverdin reductase B [flavin reductase (NADPH)]
FECH	ferrochelatase
FTH1	ferritin, heavy polypeptide 1
FTHL12	ferritin, heavy polypeptide-like 12
FTHL17	ferritin, heavy polypeptide-like 17
FTL	ferritin, light polypeptide
HMOX1	heme oxygenase (decycling) 1
AHR	arylhydrocarbon receptor (AHR)
CEBPB	CCAAT/enhancer-binding protein (C/EBP), β
MAFG	MafG protein
NFE2L2	nuclear factor-erythroid 2-like 2
PPARG	peroxisome proliferator-activated receptor gamma (PPAR γ)
PPARGC1B	peroxisome proliferator-activated receptor gamma coactivator 1-beta
RXR α	retinoid X receptor alpha (RXR α , or NR2B1)
KEAP1	Kelch-like ECH-associated protein 1

NRF2 regulates 1% of the genome?

Function	symbol	name
Heme & Iron metabolism	BLVRA	biliverdin reductase A
	BLVRB	biliverdin reductase B [flavin reductase (NADPH)]
	FECH	ferrochelatase
	FTMT	ferritin, heavy polypeptide 1
Gene expression: Trasncription factors	FTHL12	ferritin, heavy polypeptide-like 12
	FTHL17	ferritin, heavy polypeptide-like 17
	FTLI	ferritin, light polypeptide
	HMOX1	heme oxygenase (decycling) 1
	AHR	arylhydrocarbon receptor (AhR)
	CEBPB	CCAAT/enhancer-binding protein (C/EBP) β
	MAFK	MaFG protein
	NFE2L2	nuclear factor-erythroid 2-like 2
	PPARG	peroxisome proliferator-activated receptor gamma (PPAR γ)
	PPARGC1B	peroxisome proliferator-activated receptor gamma coactivator 1-beta
RXR α	retinoid X receptor alpha (RXR α , or NR2B1)	
Proteasome/autophagy	KEAP1	Kelch-like ECH-associated protein 1

NRF2 is a master regulator of cellular homeostasis

> 250 ARE-genes



NRF2

MAF

F
G
K

Redox balance

HMOX1, NQO1, TrxR, cGS,
GCLc, GCLm, Gpx, GR ...

Purine metabolism

PPAT, MTHFD2

Pentoses metabolism

G6PDH, PGD, IDH1, ME

Lipid metabolism

ACC1, FAS, SCD1, ACL...

Inflammation

MARCO, CD36, IL17D

Proteostasis

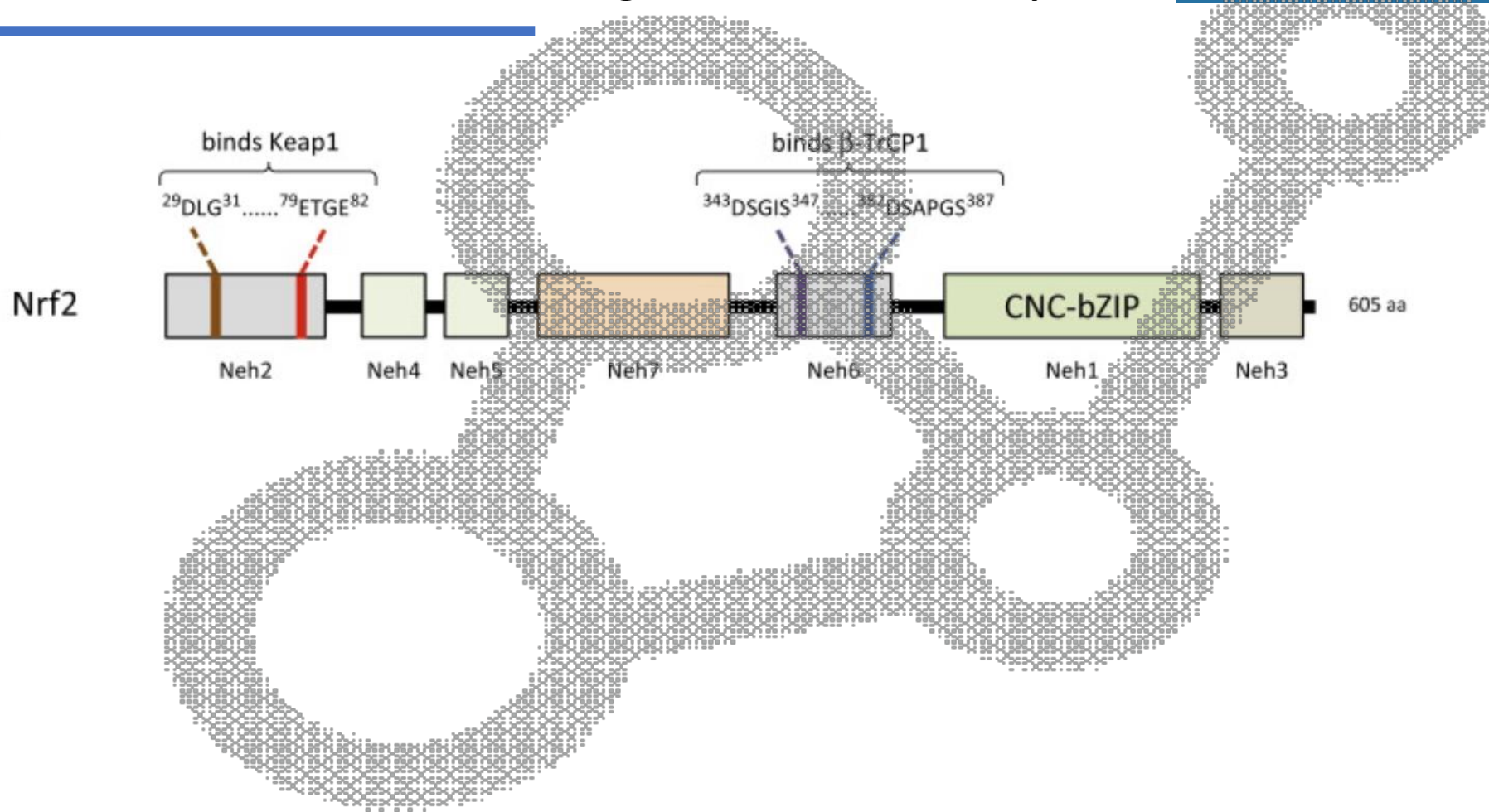
Gpx8, PSMB7, SQSTM,
CALCOCO2, ULK1.....

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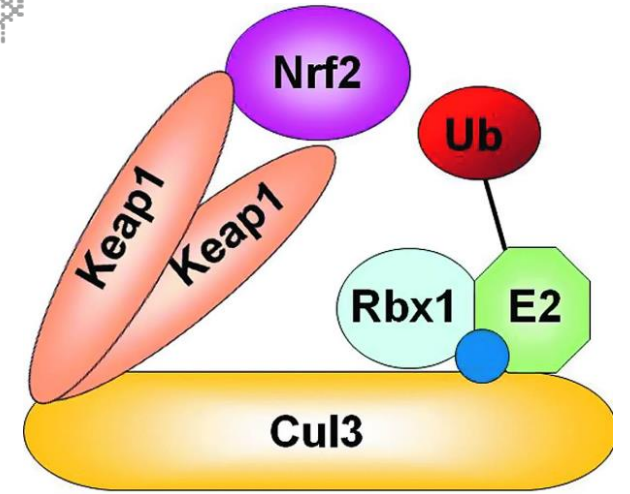
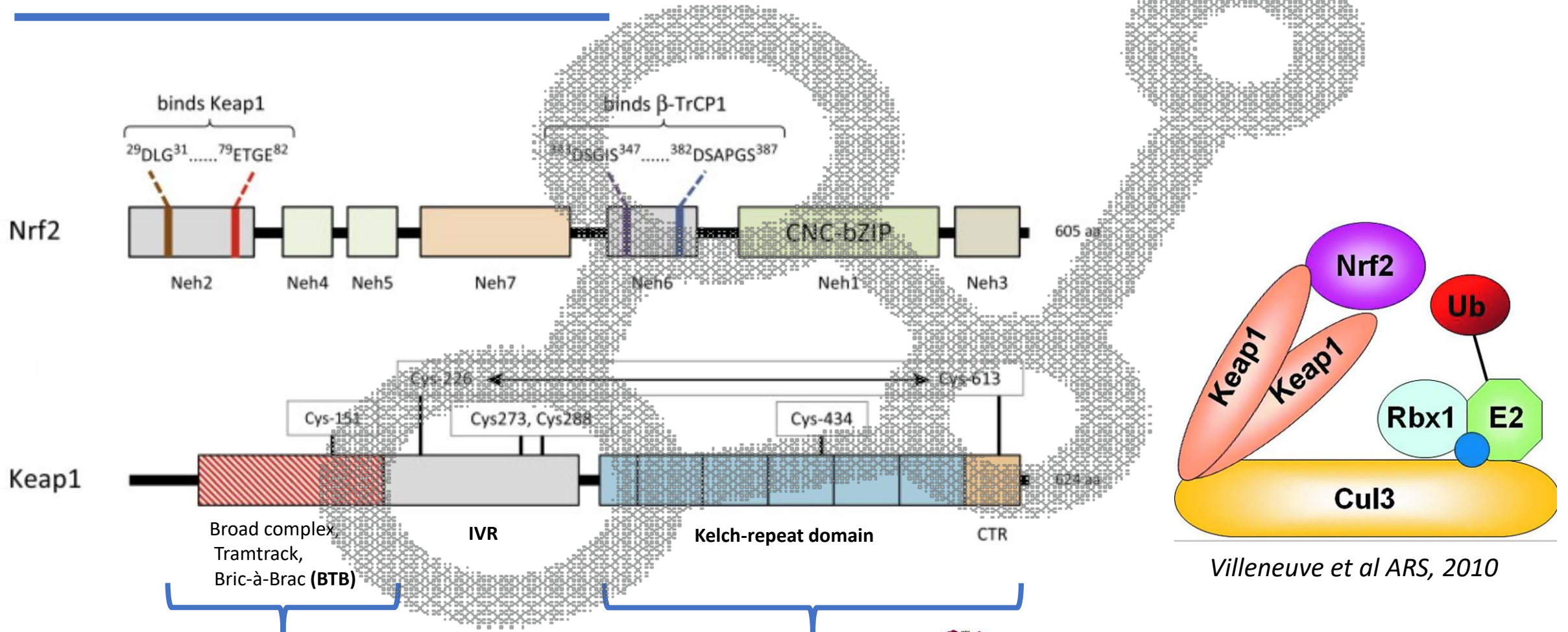
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Structure domains in NRF2 that regulate its stability



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Structure domains in NRF2 that regulate its stability



Villeneuve et al ARS, 2010

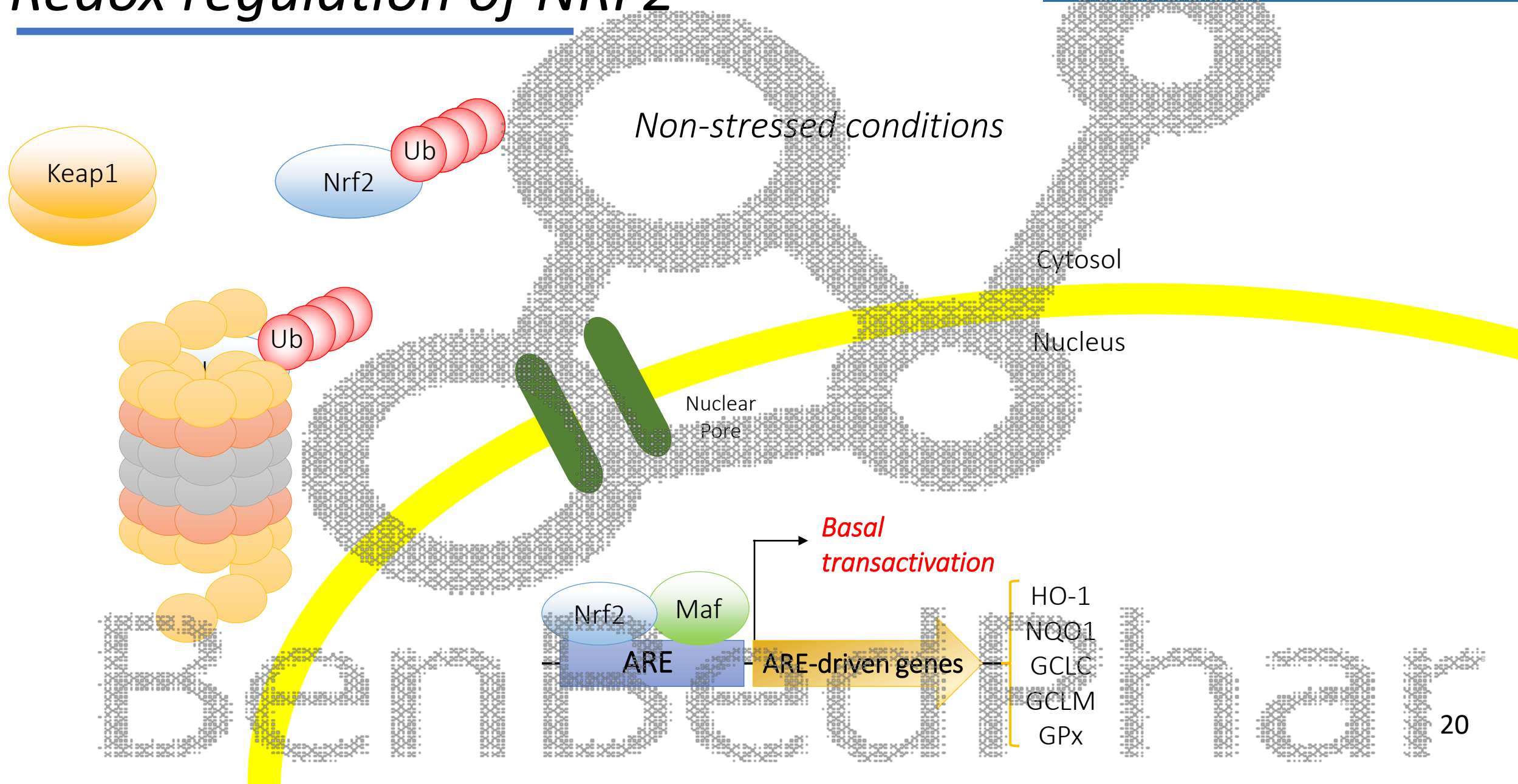
Binding to Cullin 3

KEAP1 is a redox sensor

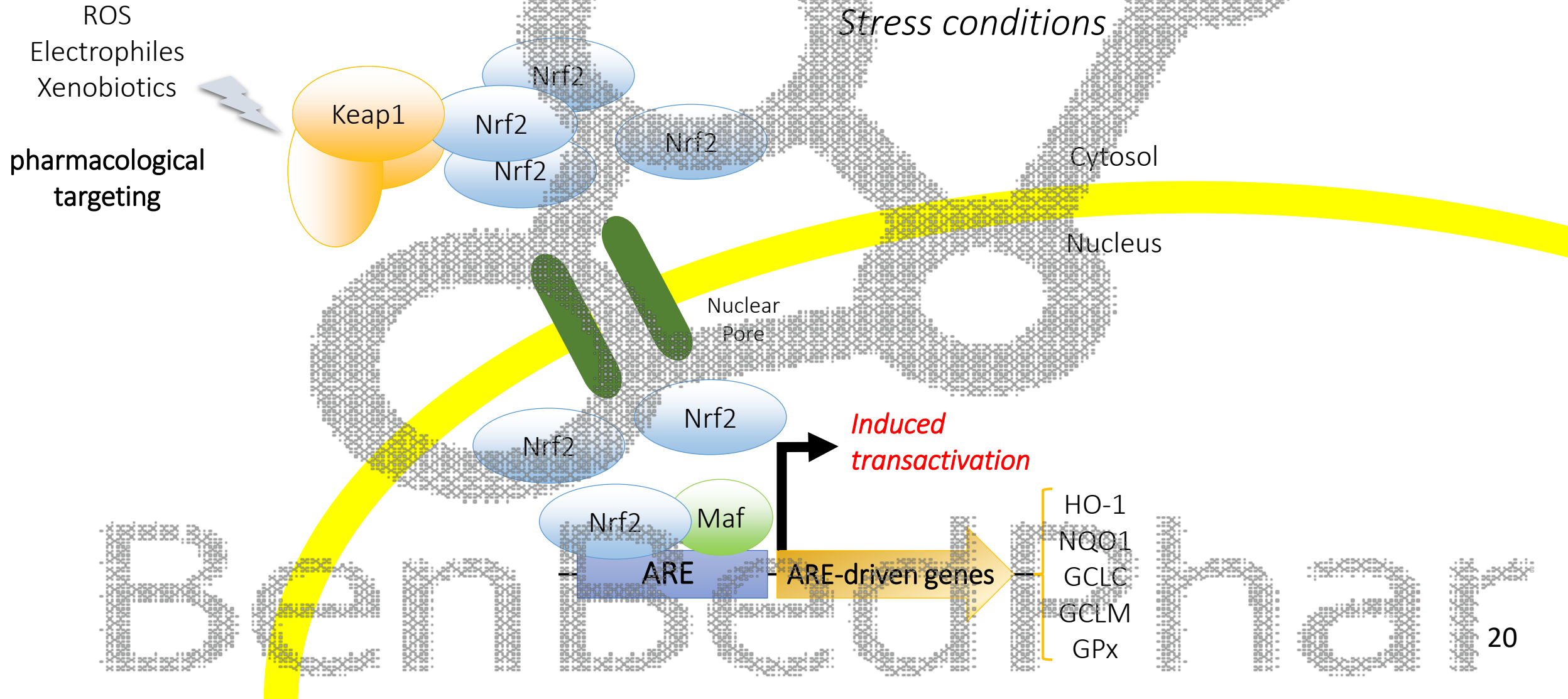
β -propeller



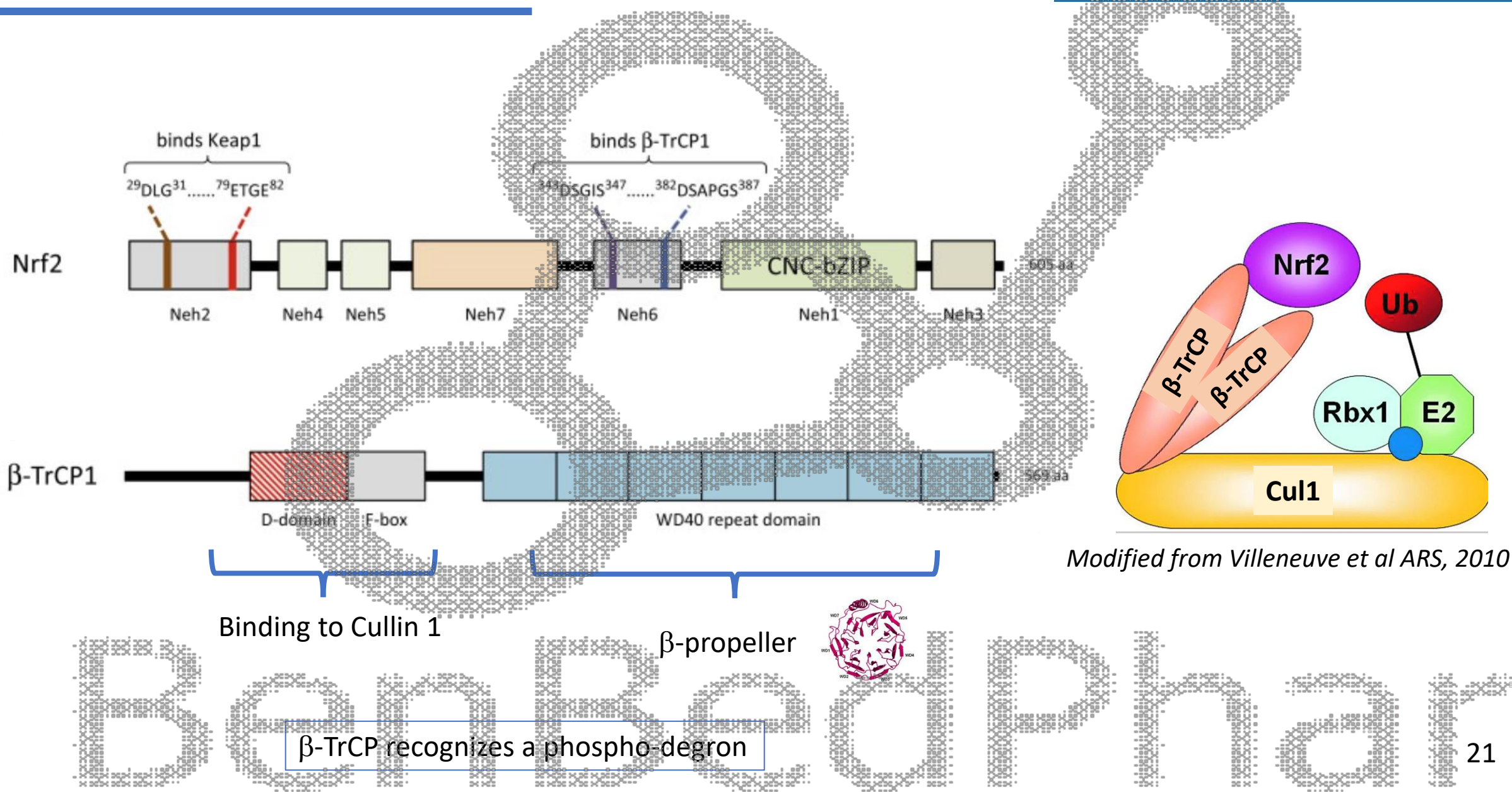
Redox regulation of NRF2



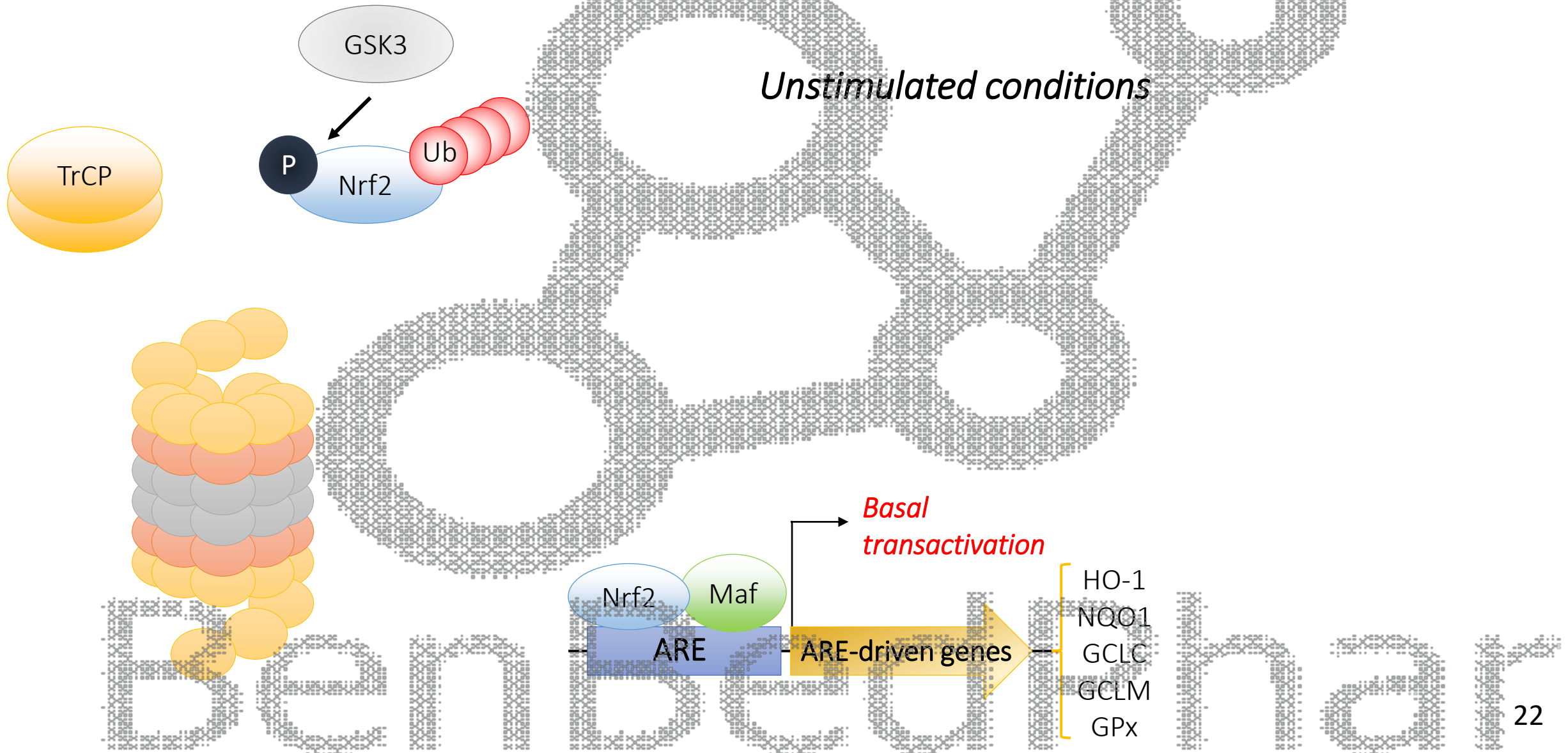
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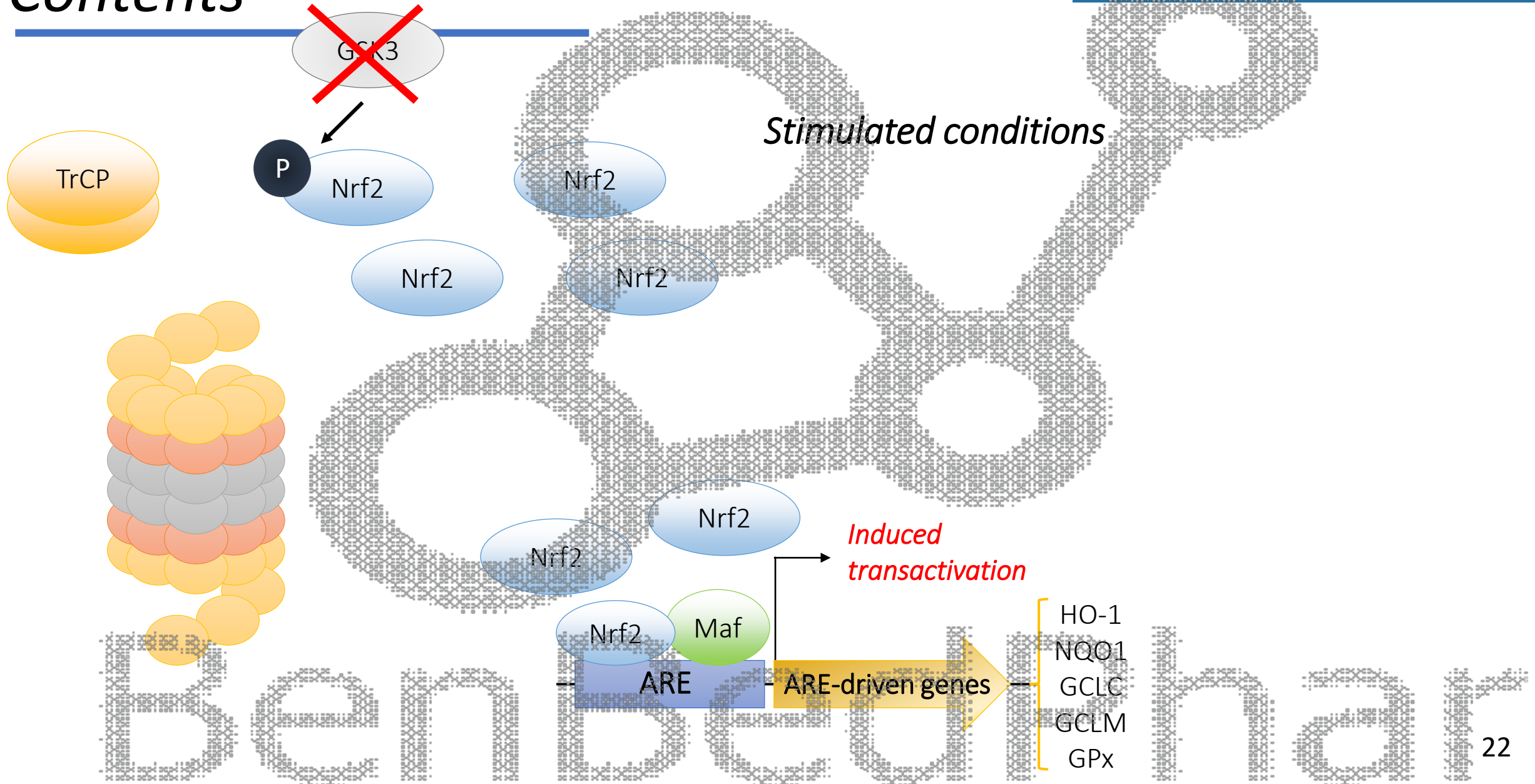
Structure domains of NRF2 that regulate its stability

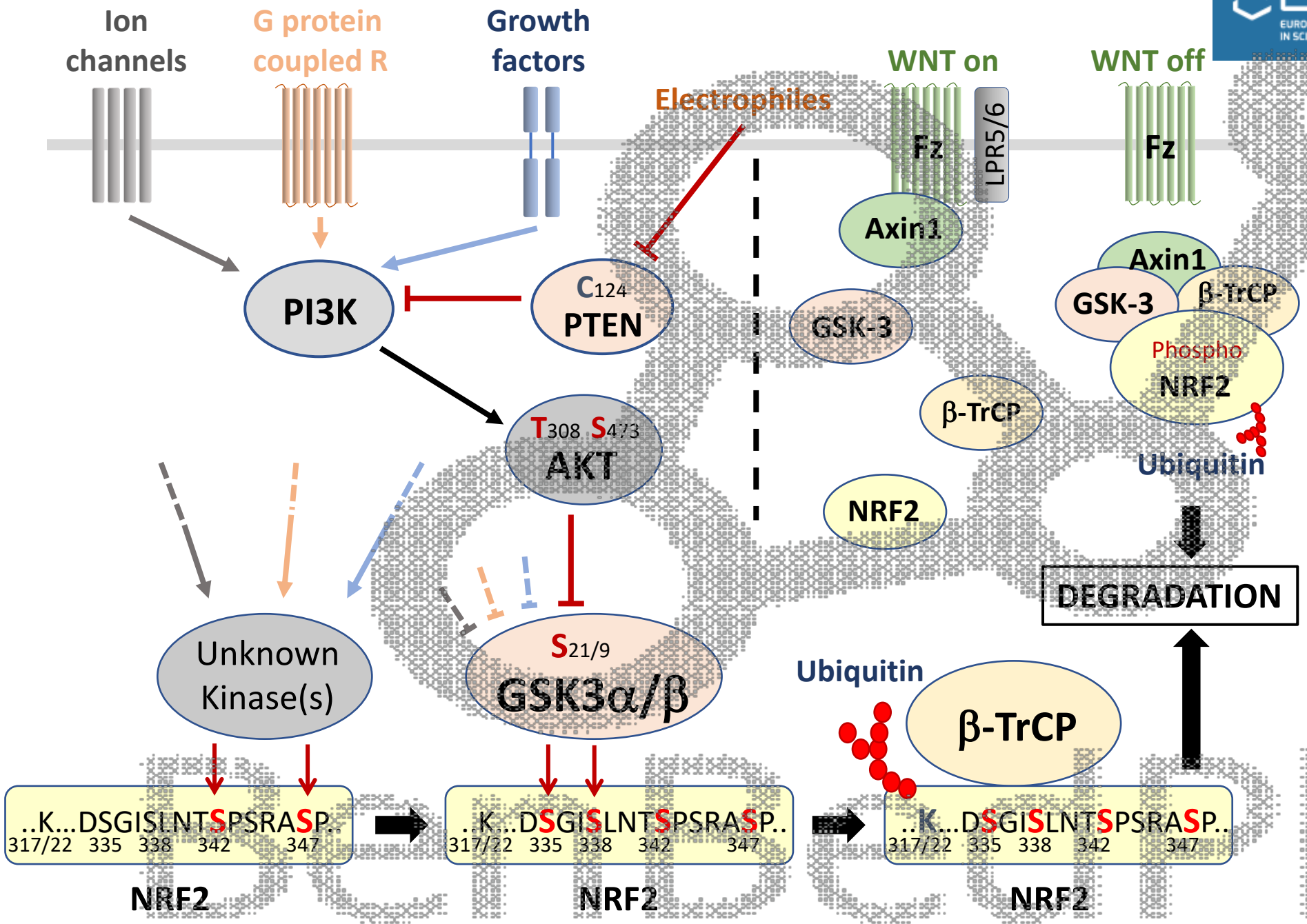


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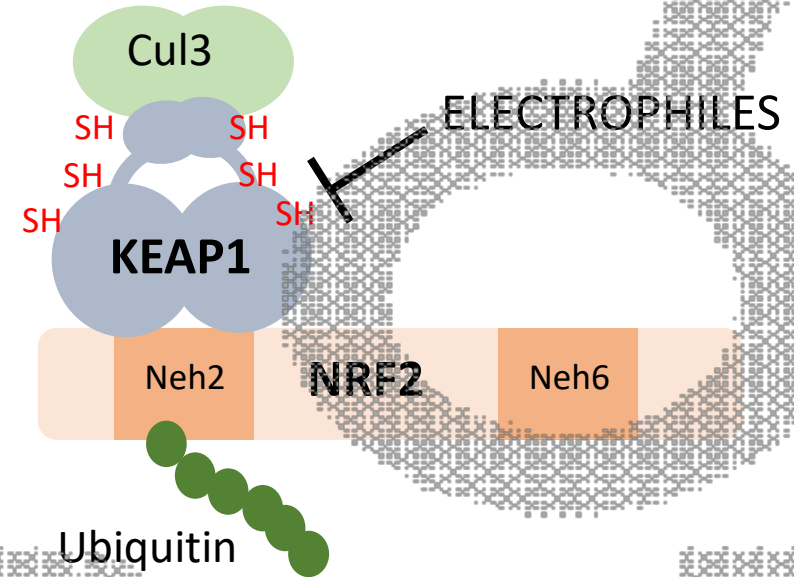
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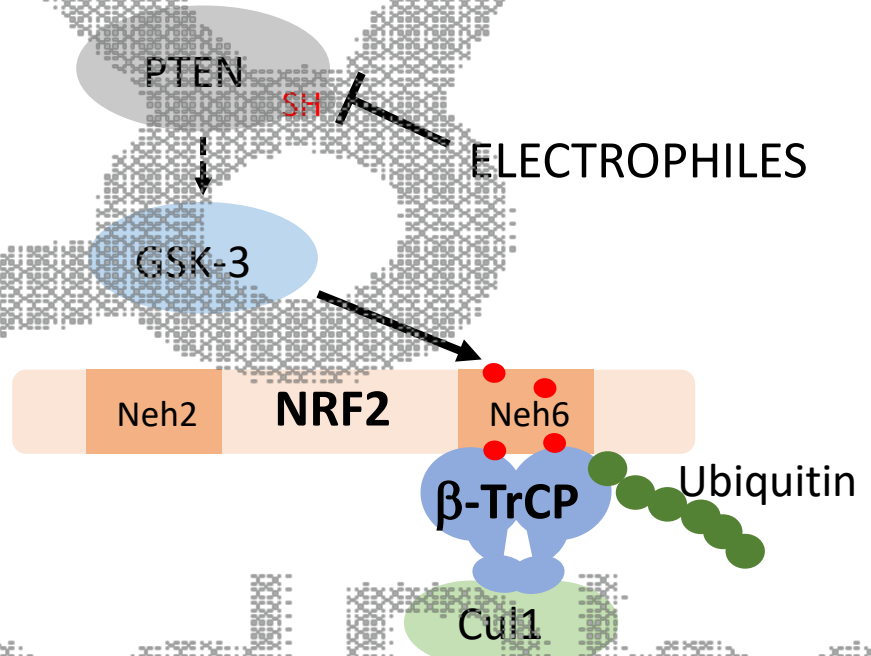


Some electrophiles may have a combined effect depending on potency and concentration

KEAP1 as electrophile sensor



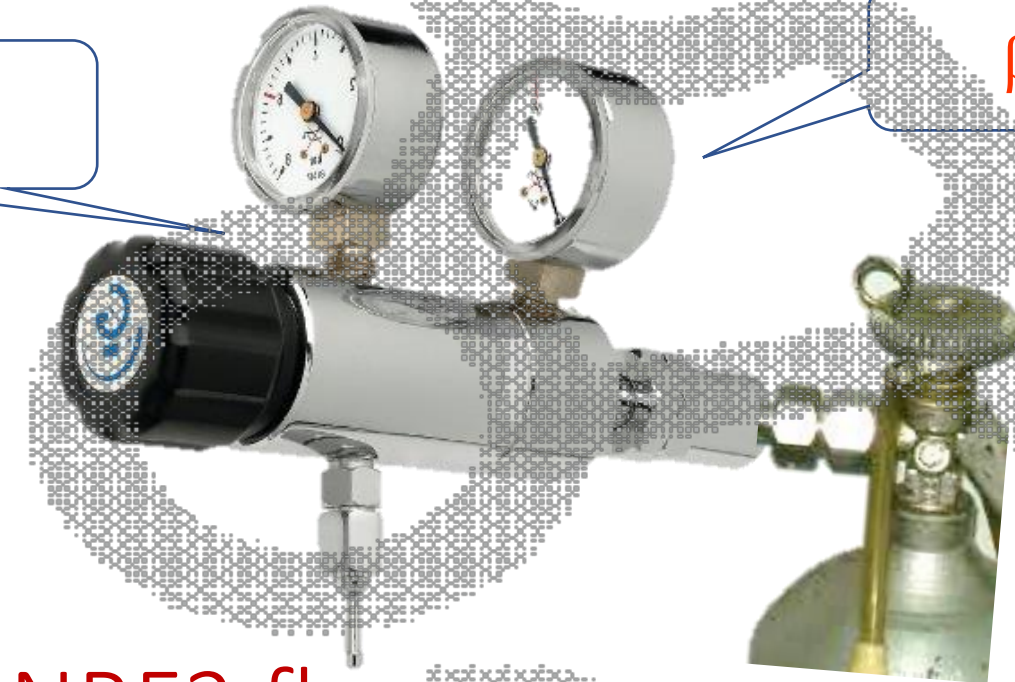
PTEN as electrophile sensor



Double flux controller

KEAP1

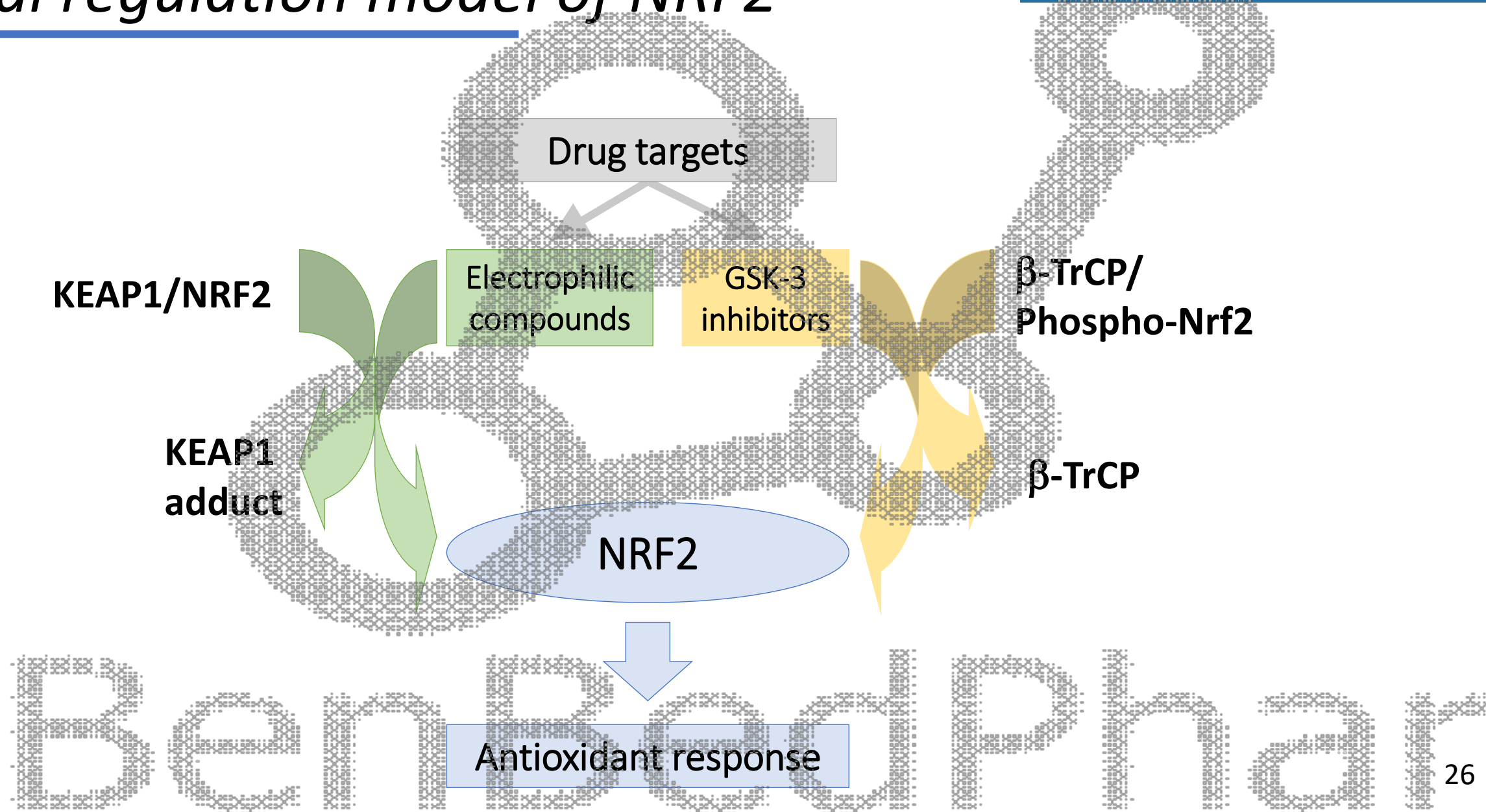
β -TrCP



NRF2 flux

Adapted to demand

Dual regulation model of NRF2

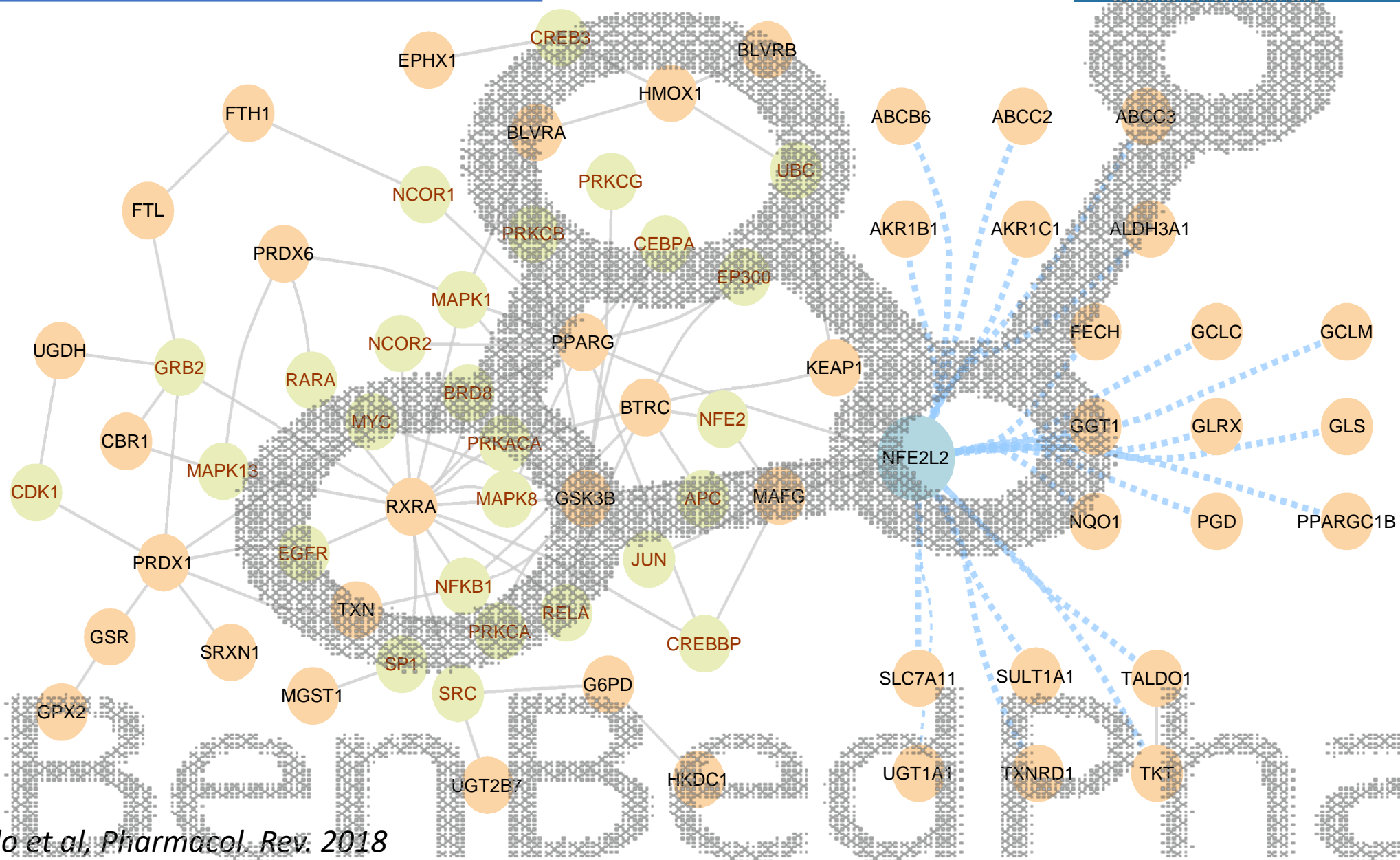


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- **NRF2 in degenerative diseases.**
- Nrf2 as a master regulator of autophagy.

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The NRF2 interactome



Lessons from the Nrf2-knockout mice

Brain:

- Stroke
- Parkinson's disease
- Alzheimer's disease
- Brain leukoencephalopathy with astrogliosis
- Spinal cord pathologies
- Hearing loss

Inflammation/immunity:

- Sepsis
- Autoimmune encephalomyelitis
- Rheumatoid arthritis
- Lupus-like autoimmune nephritis

Heart and circulation:

- Atherosclerosis (-)
- Ischemia-induced neovascularization
- Cardiomyocyte damage
- Hemolytic anemia

Carcinogenesis (-/+):

- Gastric neoplasia
- Colorectal cancer
- Mammary carcinoma
- Bladder carcinogenesis
- Lung adenocarcinomas
- Skin cancer

Metabolism:

- Lipid homeostasis
- Glucose homeostasis
- High-fat-diet induced obesity

Gastrointestinal:

- Liver injury
- Steatohepatitis
- Xenobiotic hepatotoxicity
- Acute intestinal mucosal injury
- Colitis

Airways:

- Acute lung injury
- COPD/emphysema
- Allergy and Asthma
- Pulmonary fibrosis

Kidney and bladder:

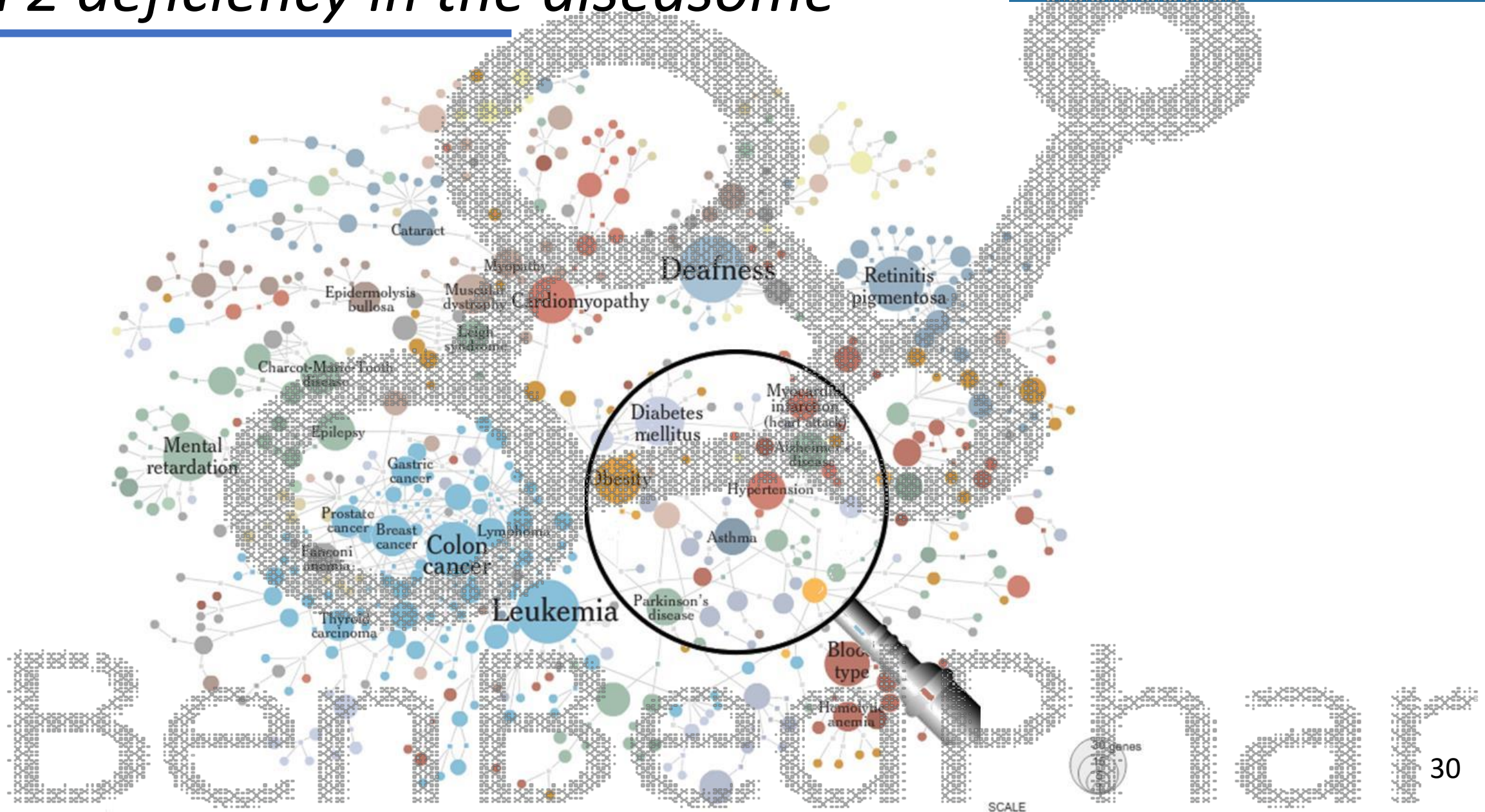
- Cisplatin nephrotoxicity
- Ischemic nephrotoxicity
- Diabetic nephropathy

Skin:

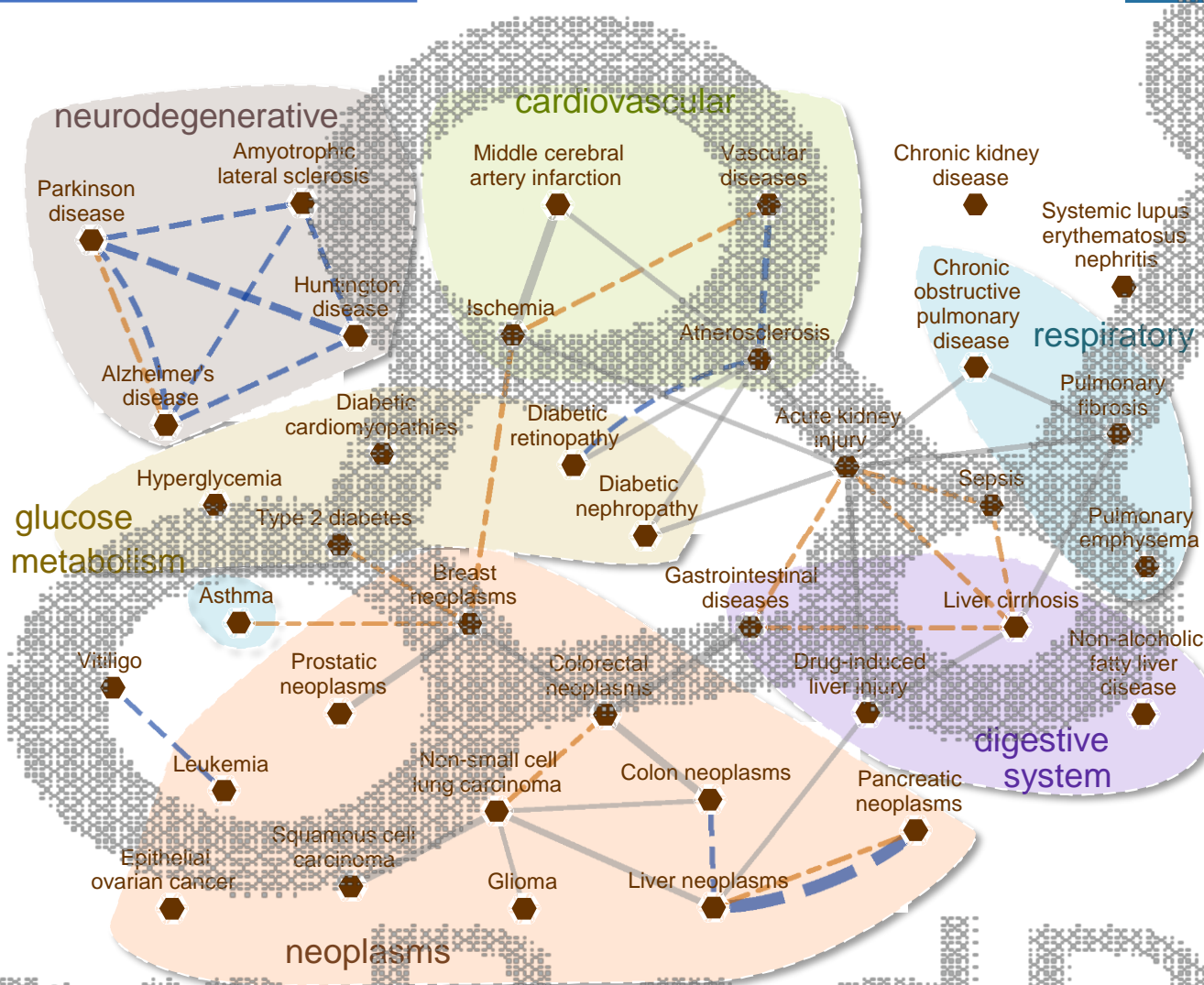
- Skin healing
- UV-induced skin aging
- Uveitis
- Retinal ischemia/reperfusion



NRF2 deficiency in the diseasome



The human NRF2 diseasome

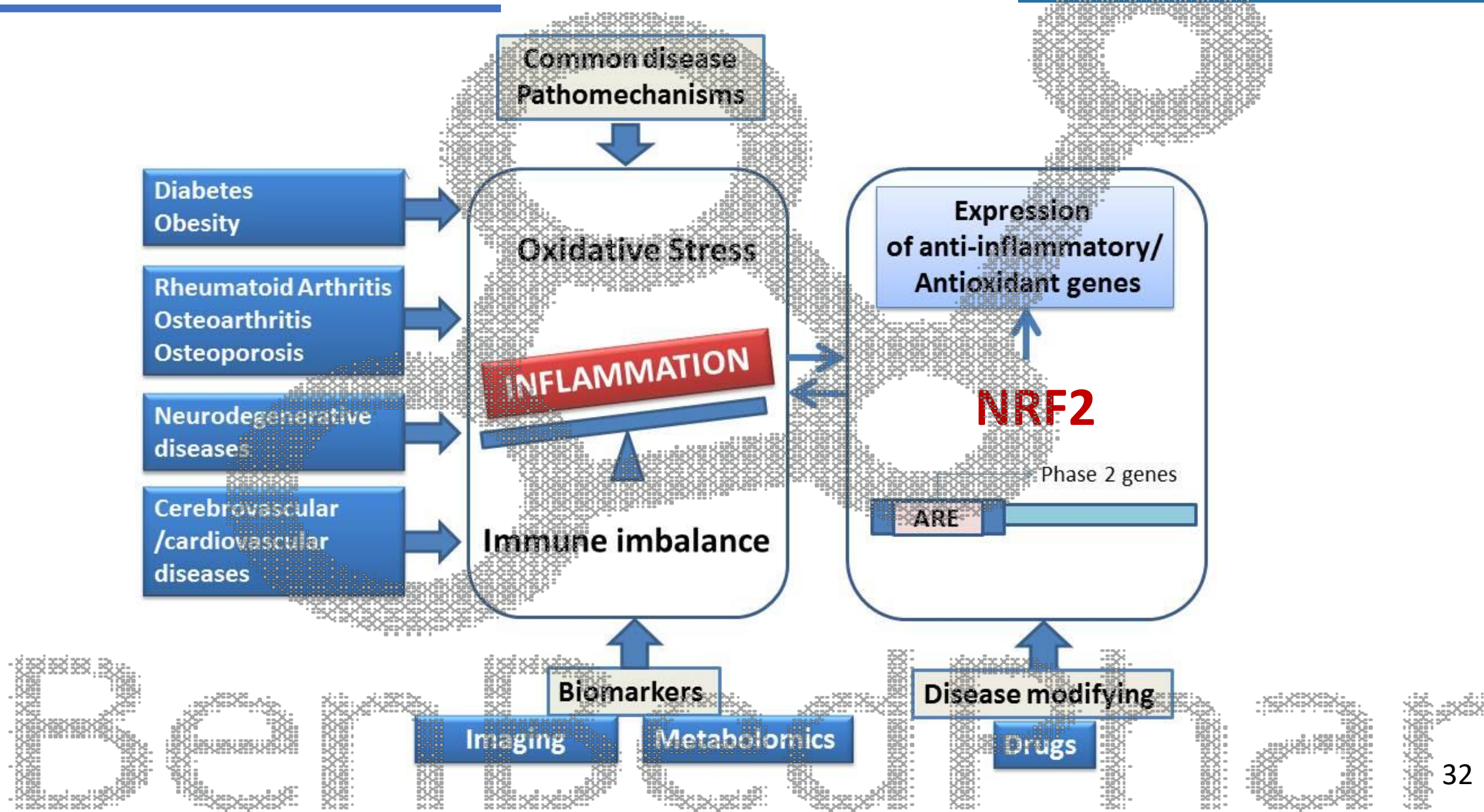


NRF2 related diseases in the diseasome

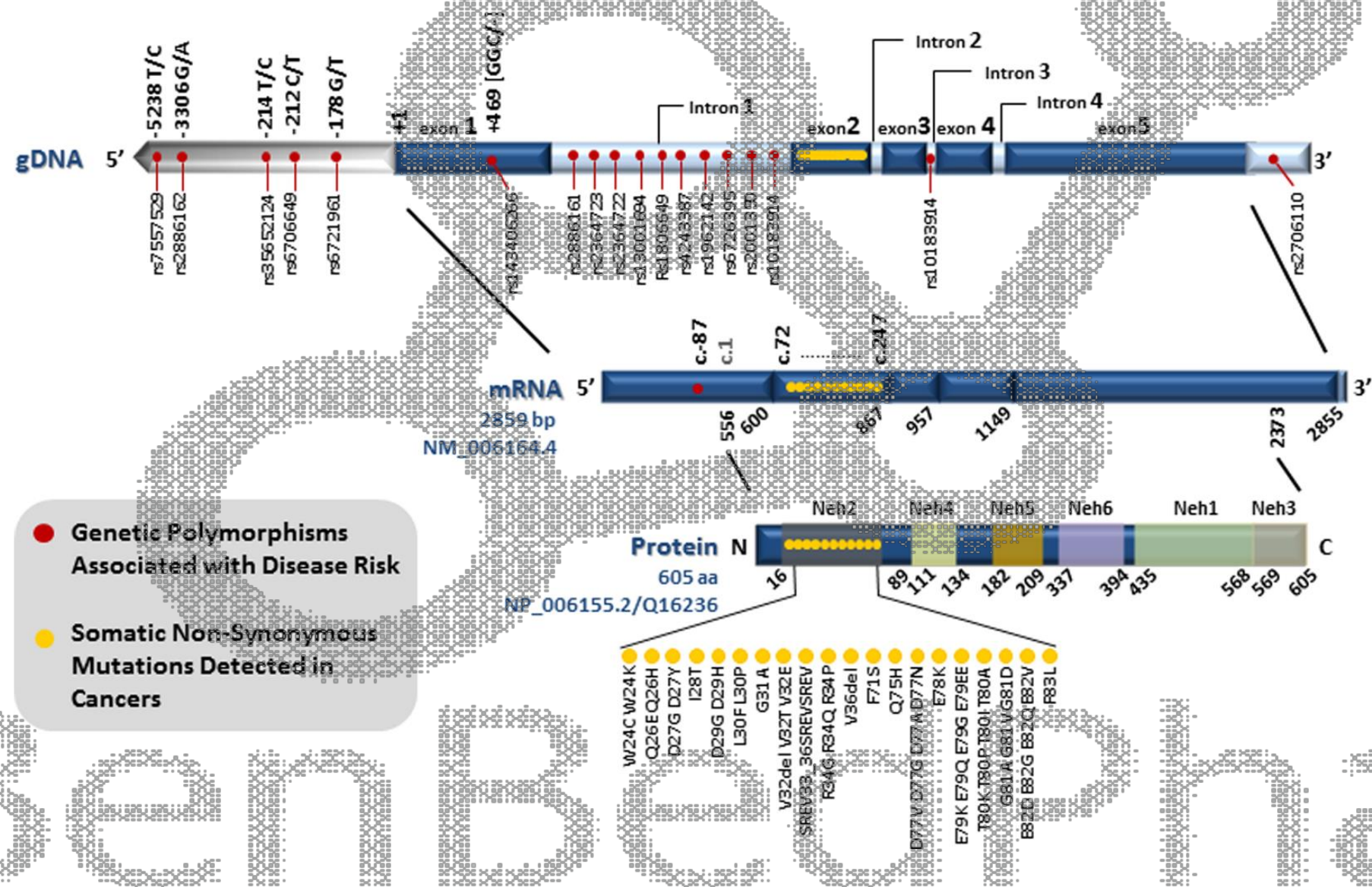
Disease-disease relationships

- shared genes
- - - symptom similarity
- - - comorbidity

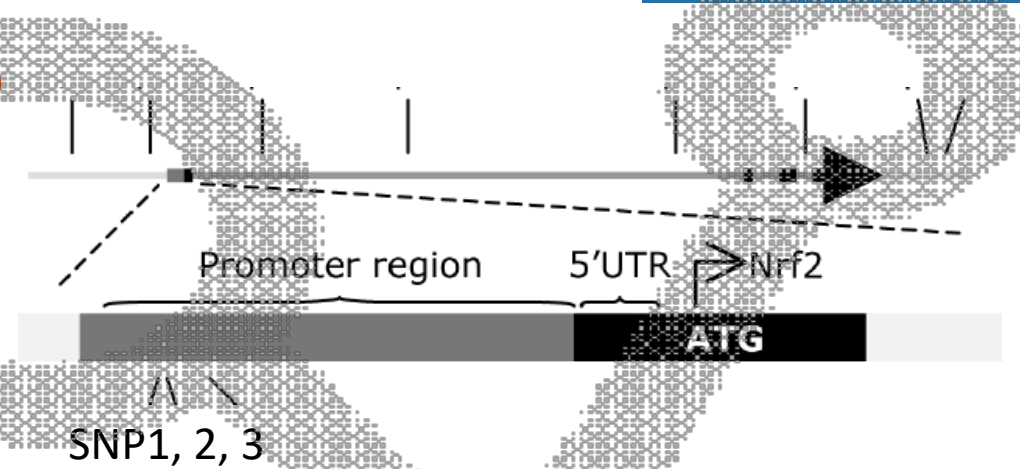
NRF2-deficiency as a common pathomechanism



Somatic and Inherited mutations in NFE2L2 (NRF2)



Gene associations with PD



von Otter *et al.* *BMC Medical Genetics* 2014, **15**:131
<http://www.biomedcentral.com/1471-2350/15/131>

BMC
Medical Genetics

RESEARCH ARTICLE

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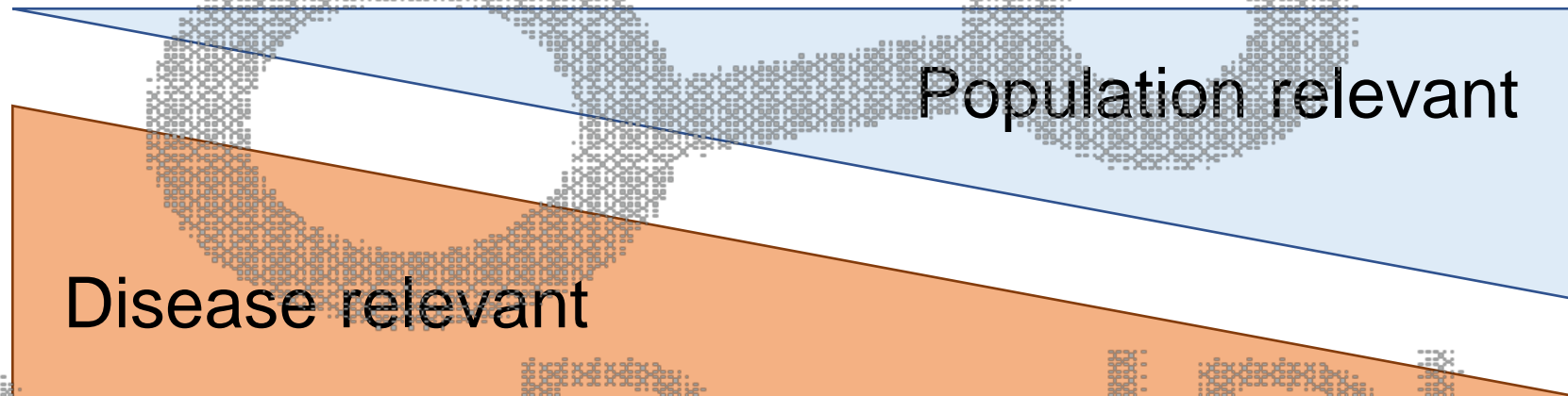
Genetic associations of Nrf2-encoding *NFE2L2* variants with Parkinson's disease – a multicenter study

Malin von Otter¹, Petra Bergström^{1*}, Aldo Quattrone^{2,3}, Elvira Valeria De Marco⁴, Grazia Annesi⁵, Peter Sderkvist⁶,
Stephanie Bezzina Wettinger⁷, Marek Drozdziak⁸, Monika Bialecka⁸, Hans Nissbrandt⁹, Christine Klein¹⁰,
Michael Nilsson^{11,12}, Ola Hammarsten¹³, Staffan Nilsson¹⁴, and Henrik Zetterberg^{1,15}

NFE2L2 and the genetics of Parkinson's disease

- Some genes have a strong impact in disease development but little effect at the population level
- Some genes have little effect on disease but strong impact at the population level

PARKs.....*GBA1*.....*NRF2*



1. NRF2 belongs to a family of bZip transcription factors that participate in multiple aspects of development, proliferation and differentiation.
2. NRF2 is a master regulator of cell homeostasis. Protection against:
 - Oxidative stress.
 - Inflammatory stress.
 - Metabolic stress.
 - etc.
3. NRF2 deficiency is a common theme in degenerative diseases.
NRF2 up-regulation is common to many tumors.
4. Activators of NRF2 may provide a therapeutic strategy for diseases with low-grade chronic inflammation.
5. Intensive search is done to find NRF2 inhibitors for some cancer types