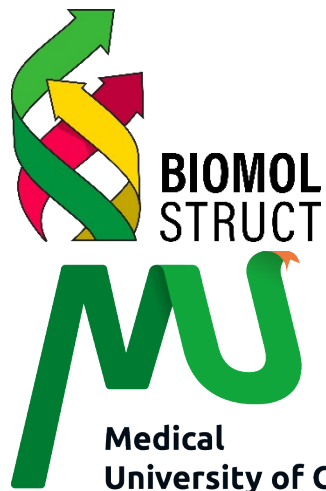


Deciphering the intricate role of intrinsically disordered regions in transcription factor regulation

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Medical University Graz, Austria



Gottfried Schatz
Research Center

February 16th 2023



BioTechMed[®]
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Disclosures

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Scientific Director Core Facility Functional Omics

Co-founder Cleara Biotech

Consultant for Matterhorn Biosciences and Bayer/Steigerwald

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Thanks to Prof. Gilbert Reibnegger and Amtsrat Gerhard Ledinski



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Emil Spreitzer

Sinem Usluer

Qishun Zhou

Technicians/students

Greta Bramerdorfer

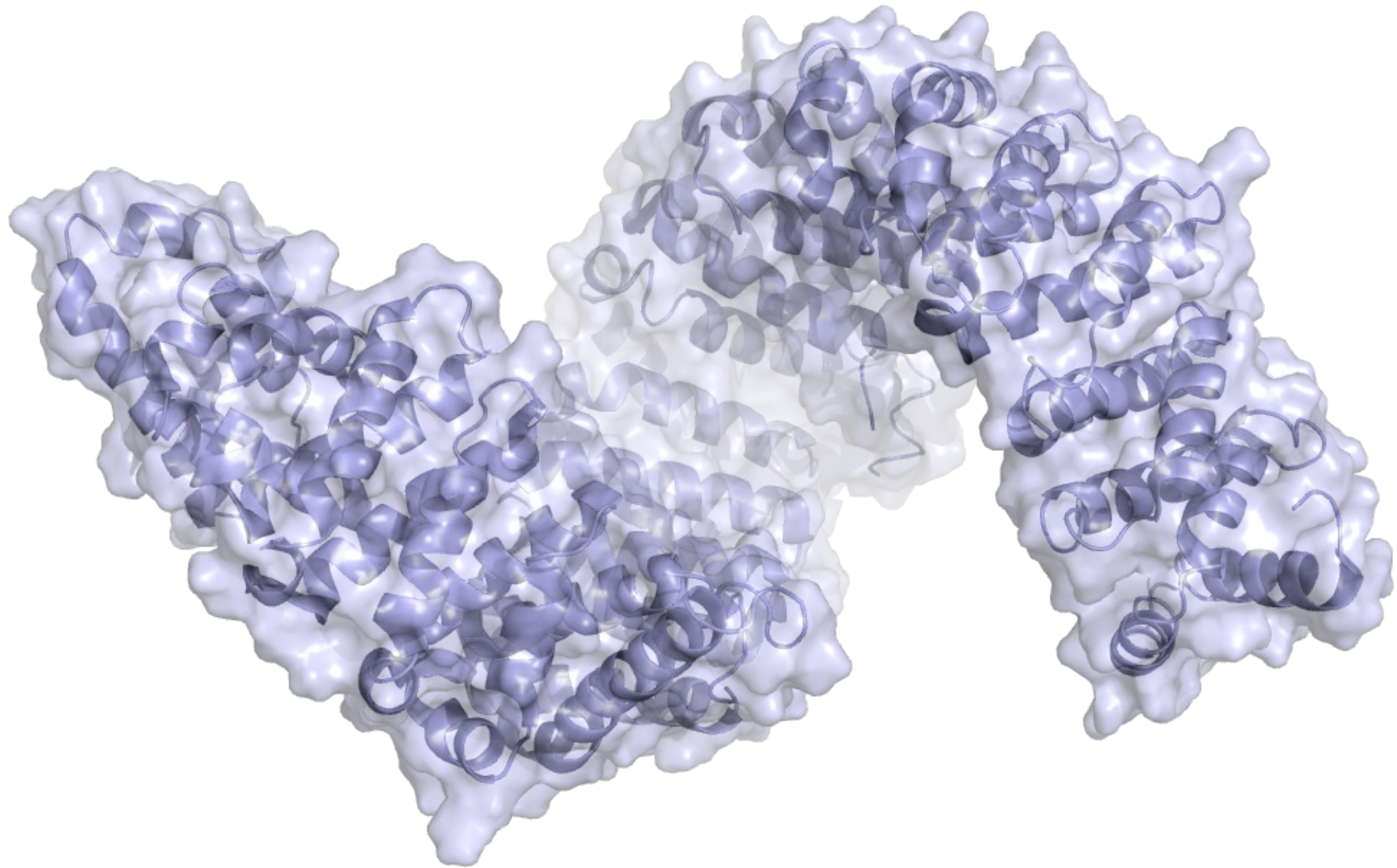
Vivian Eber

Melanie Grissmann

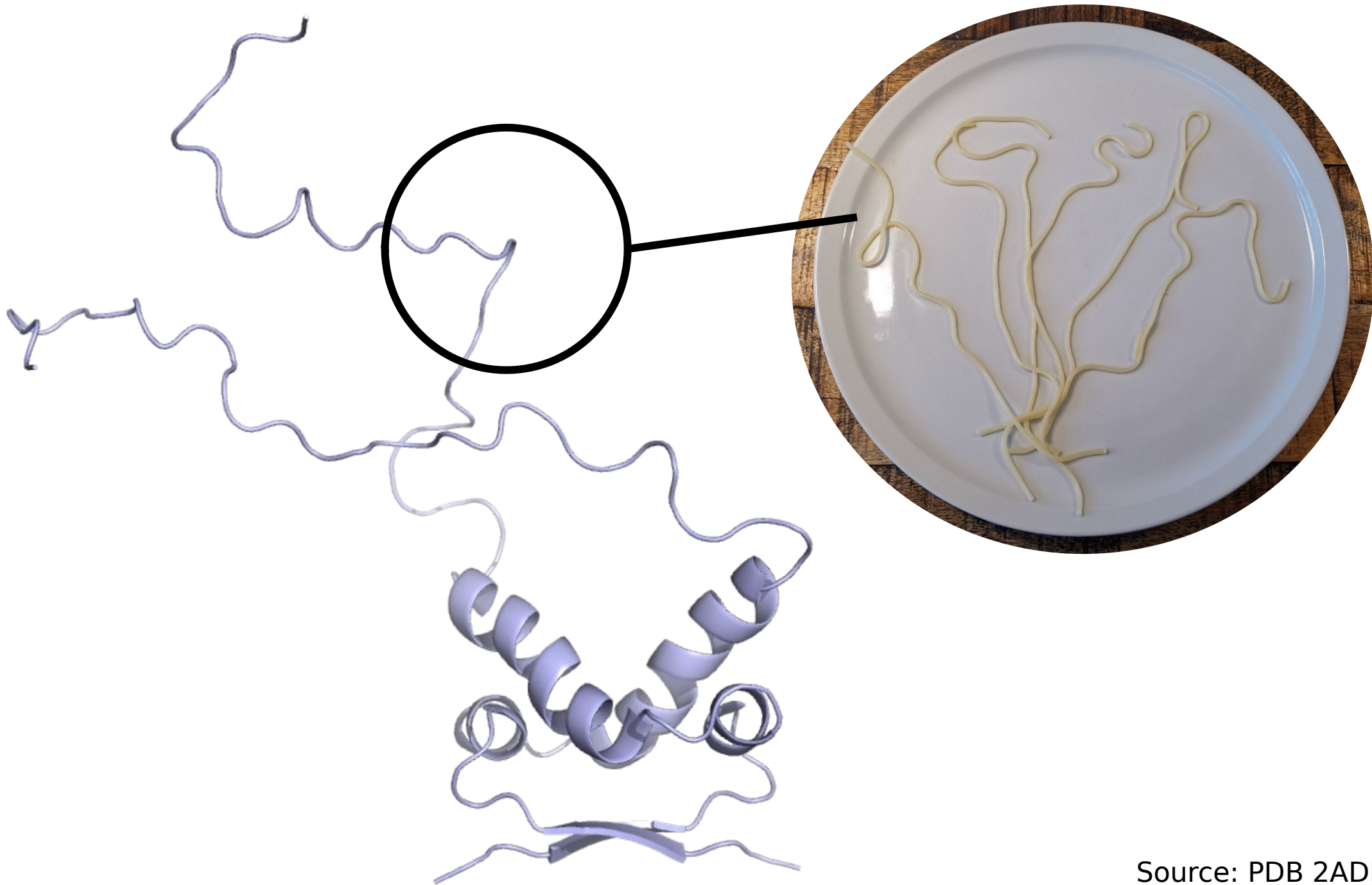
Hansjörg Habisch



#madllab on Instagram and Twitter



Source: PDB 2Z5J



Source: PDB 2ADL

Hallmarks of Ageing

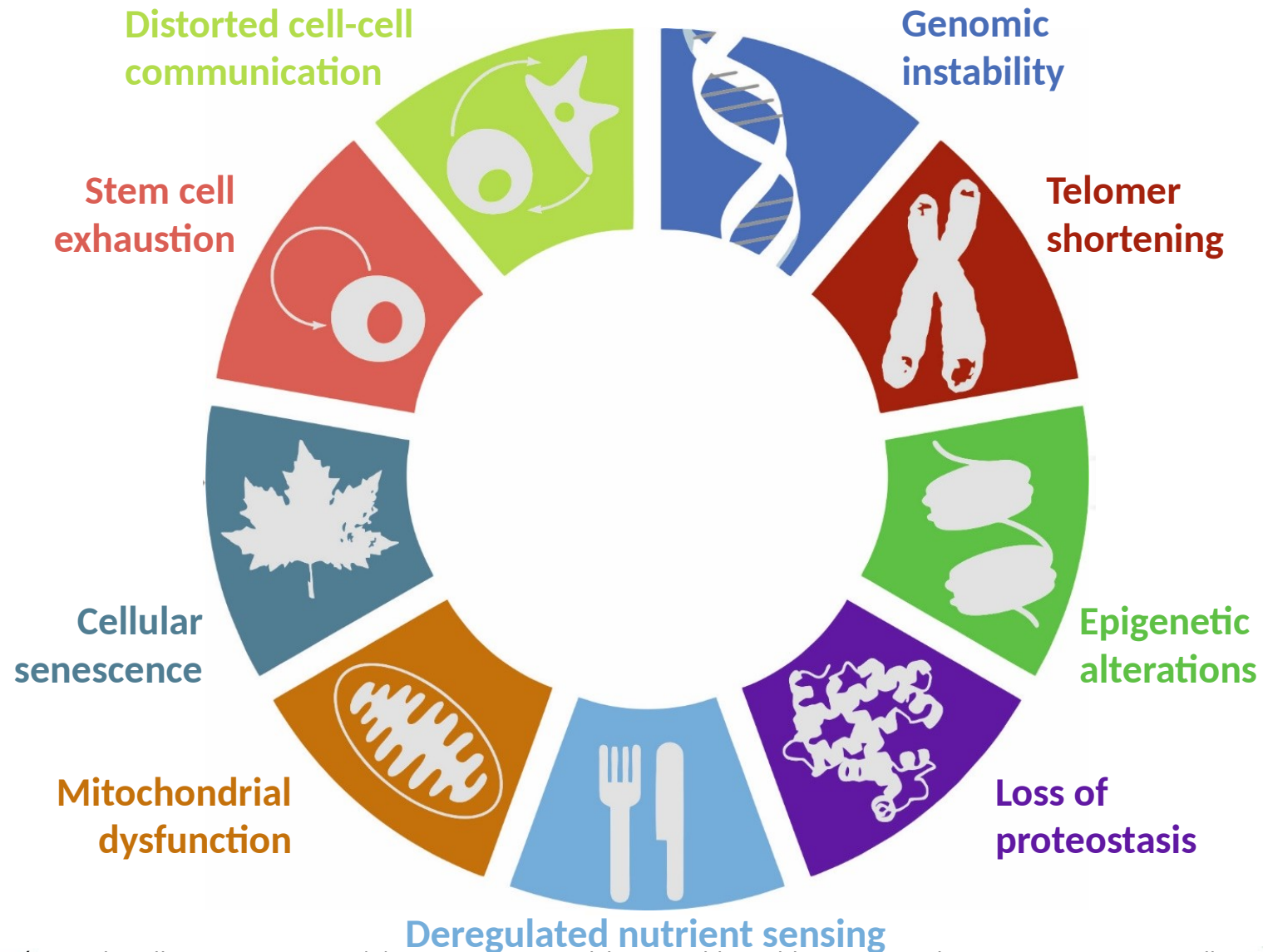


Problems with garbage disposal
(loss of proteostasis)

Problems with cellular switches
(epigenetic alterations)

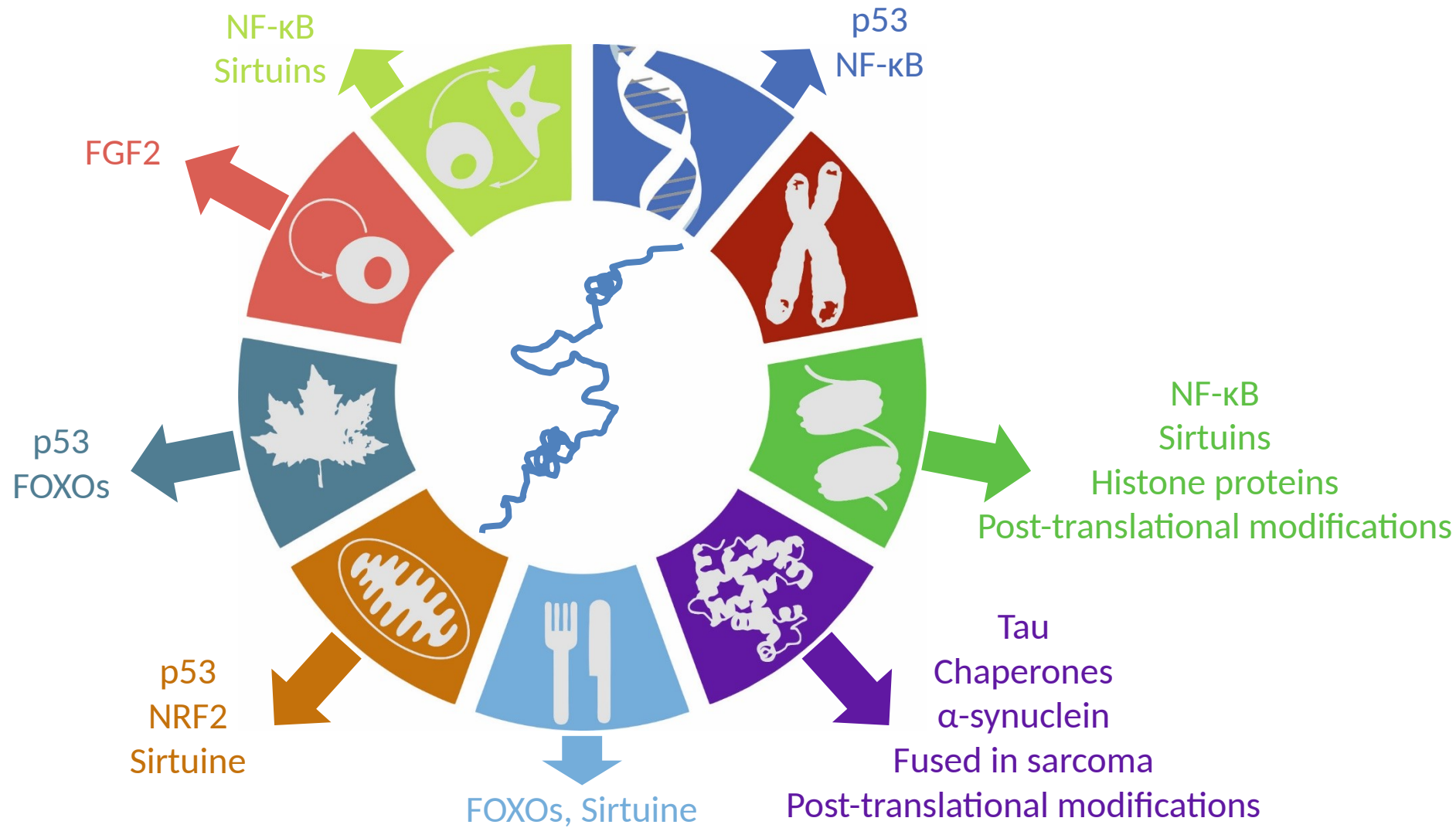
Problems with DNA repair
(genomic instability)

Hallmarks of Ageing



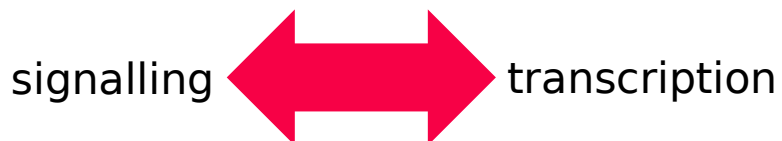
López-Otín *et al.* Cell. 2013 Jun 6;153(6):1194-217; Campisi J, Kapahi P, Lithgow GJ, Melov S, Newman JC, Verdin E. From discoveries in ageing research to therapeutics for healthy ageing. Nature. 2019 Jul;571(7764):183-192. doi: 10.1038/s41586-019-1365-2. Epub 2019 Jul 10. PMID: 31202552

Disordered proteins are key players in ageing



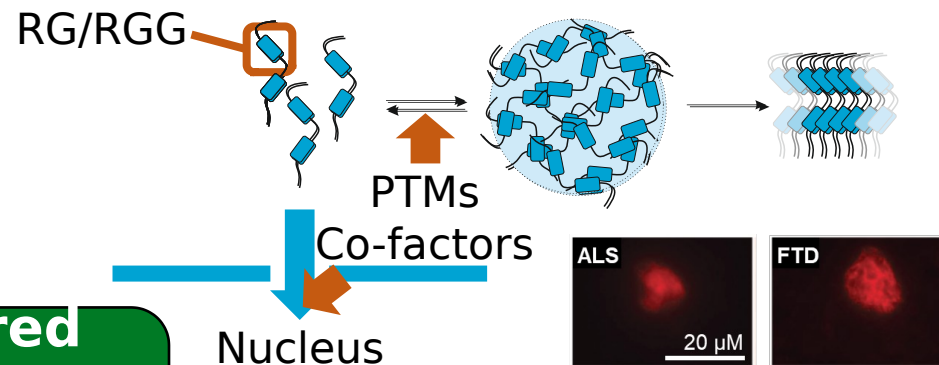
Our vision - deciphering and targeting key molecular mechanisms of ageing

Wnt signalling and intersecting pathways



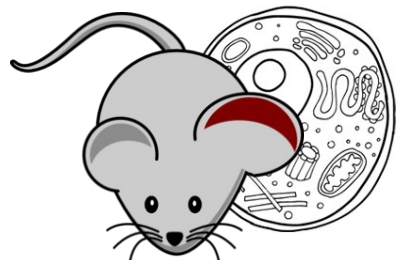
CLEARA FOXO/p53/Wnt pathways
Phase separation
Misregulation in human diseases

Regulation of RNA-binding proteins



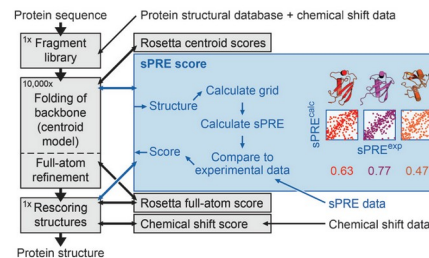
Disordered proteins in health and disease

Metabolomics

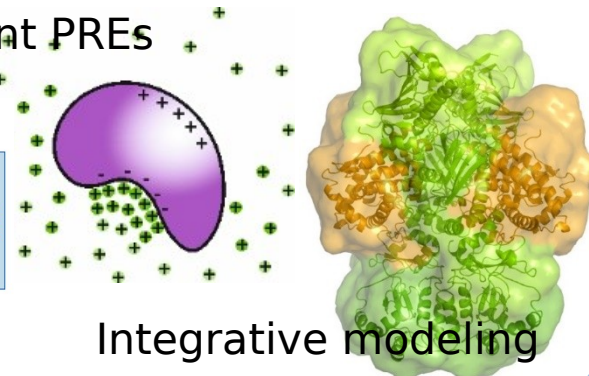


Method development

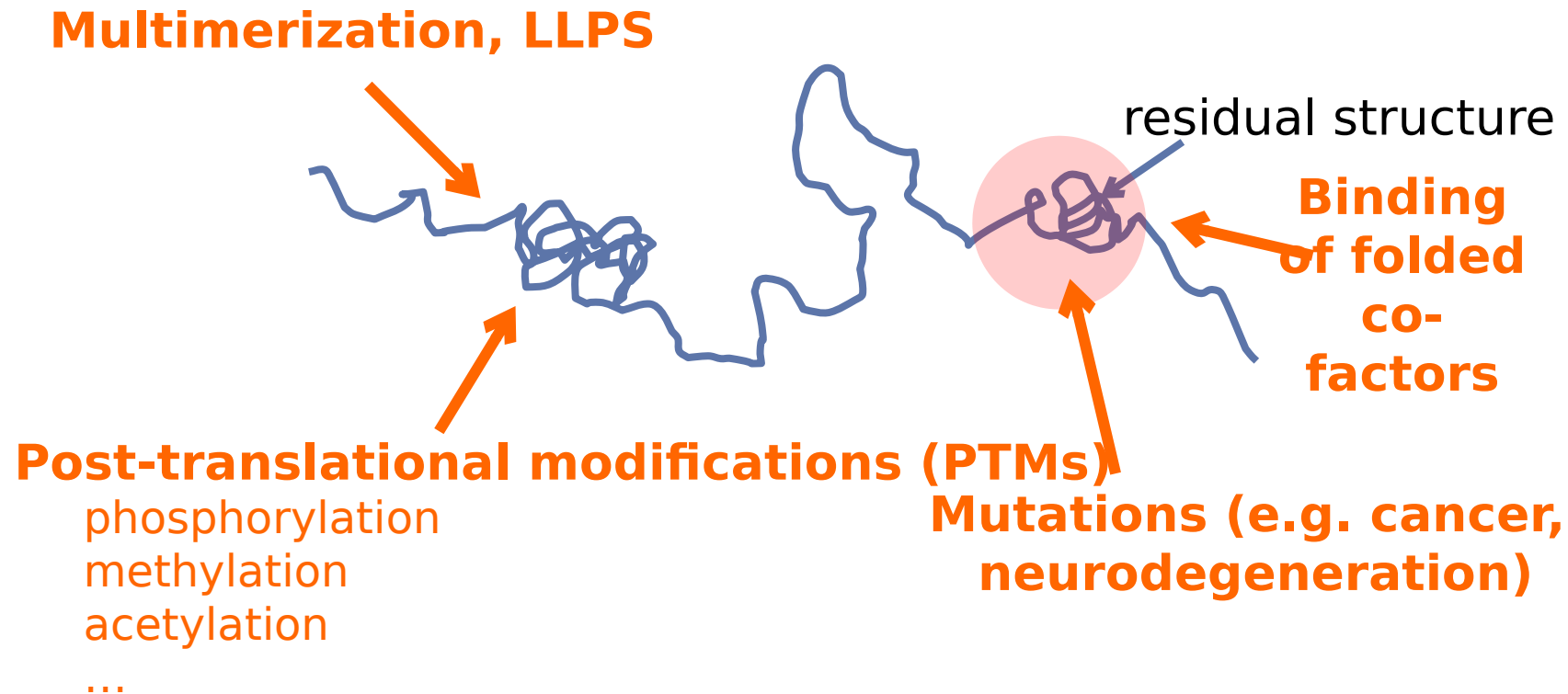
Rosetta



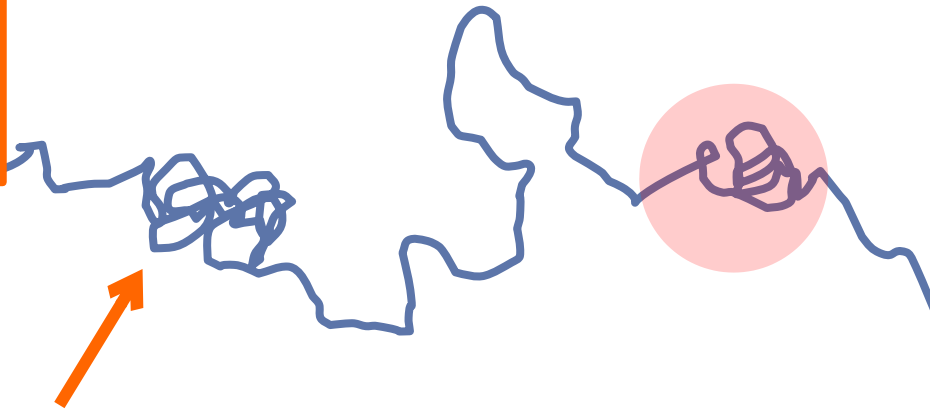
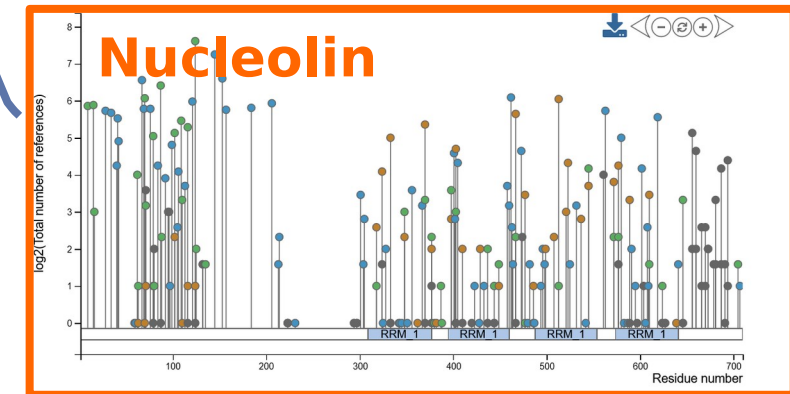
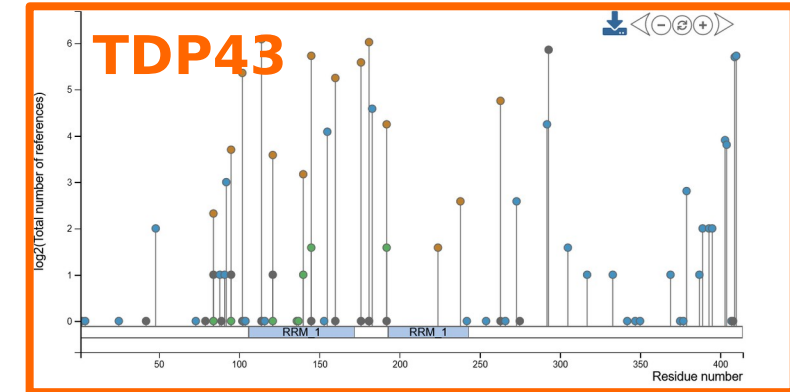
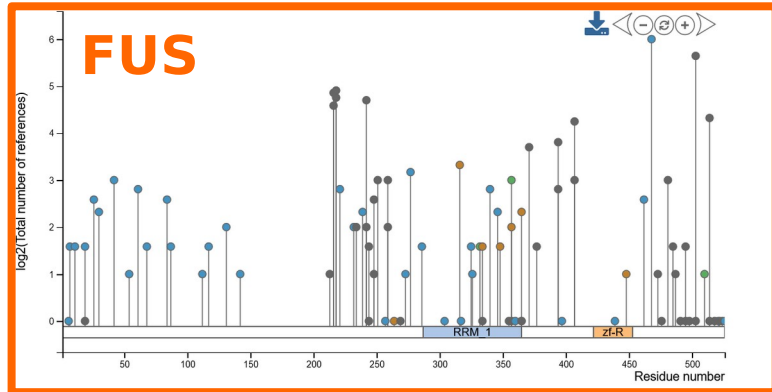
Solvent PREs



Intrinsically disordered proteins/regions have unique properties

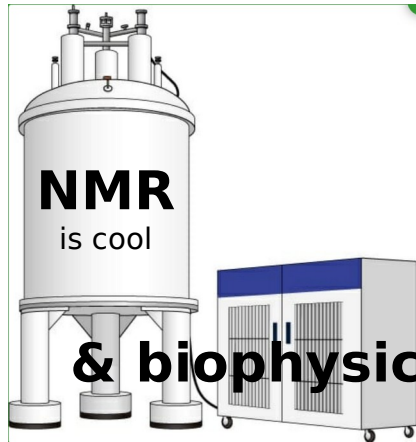


Intrinsically disordered proteins/regions are highly modified



post-translational modifications (PTMs)

- phosphorylation
- methylation
- acetylation
- ...



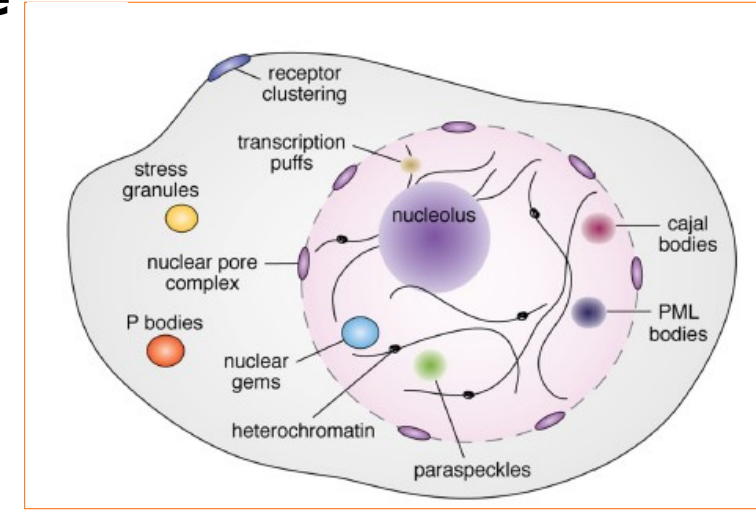
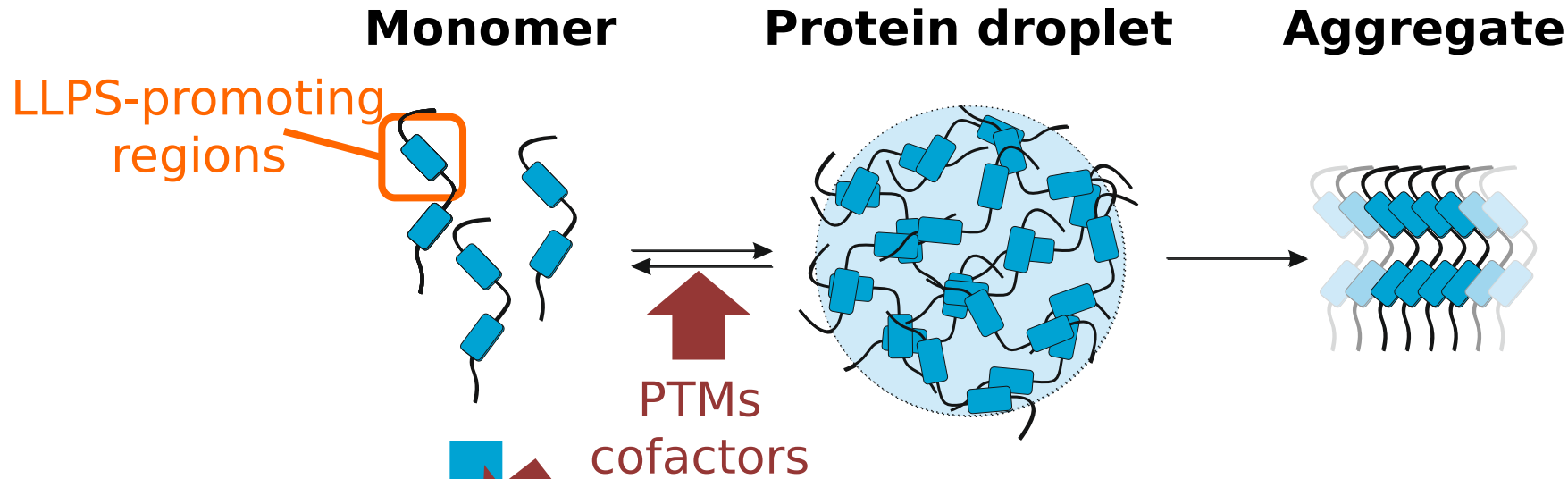
& biophysical assays

Legend: PTM sites

- Phosphorylation
- Acetylation
- Ubiquitylation
- Other

<https://www.phosphosite.org>

Disordered regions play key roles in import & LLPS



Gomes et al., JBC (2019)

Zhang et al., Progr Neurobiol (2023)

Lenard et al., Cells (2022)

Zhou et al., Protein Sci (2021)

Zhang et al., Cell Rep Meth (2021)

Hutten et al., Cell Rep (2020)

Bourgeois et al., PNAS (2020)

Hofweber et al., Cell (2018)

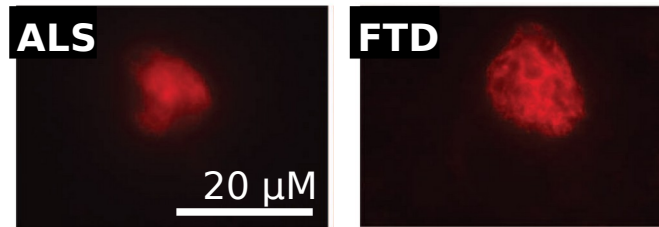
Suárez-Calvet et al., Acta Neuropathologica (2016)

Dormann et al., EMBO J (2012)

Aneta Lenard
Sinem Usluer
Qishun Zhou

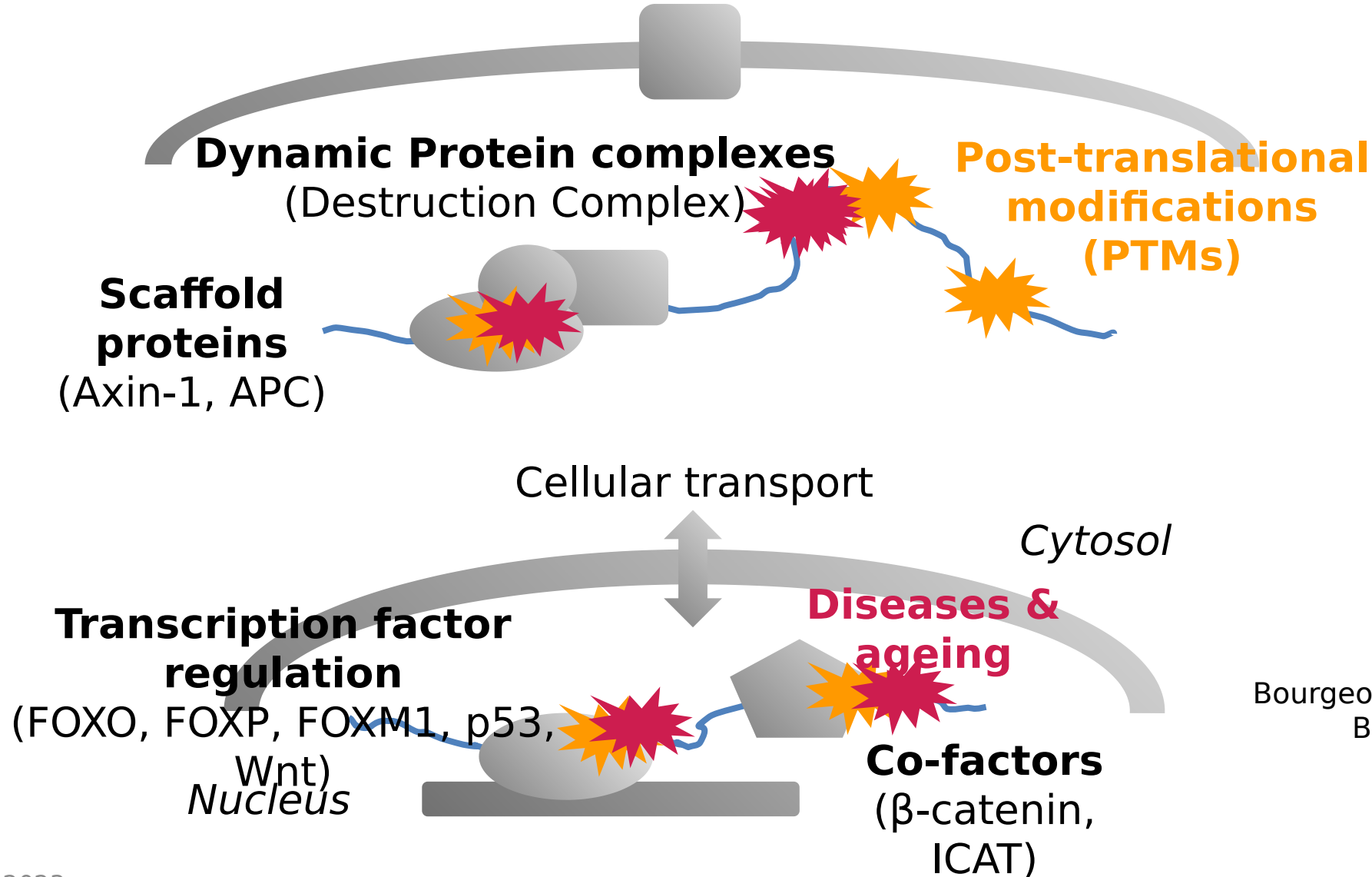
Nucleus

Fused-in-sarcoma (FUS)



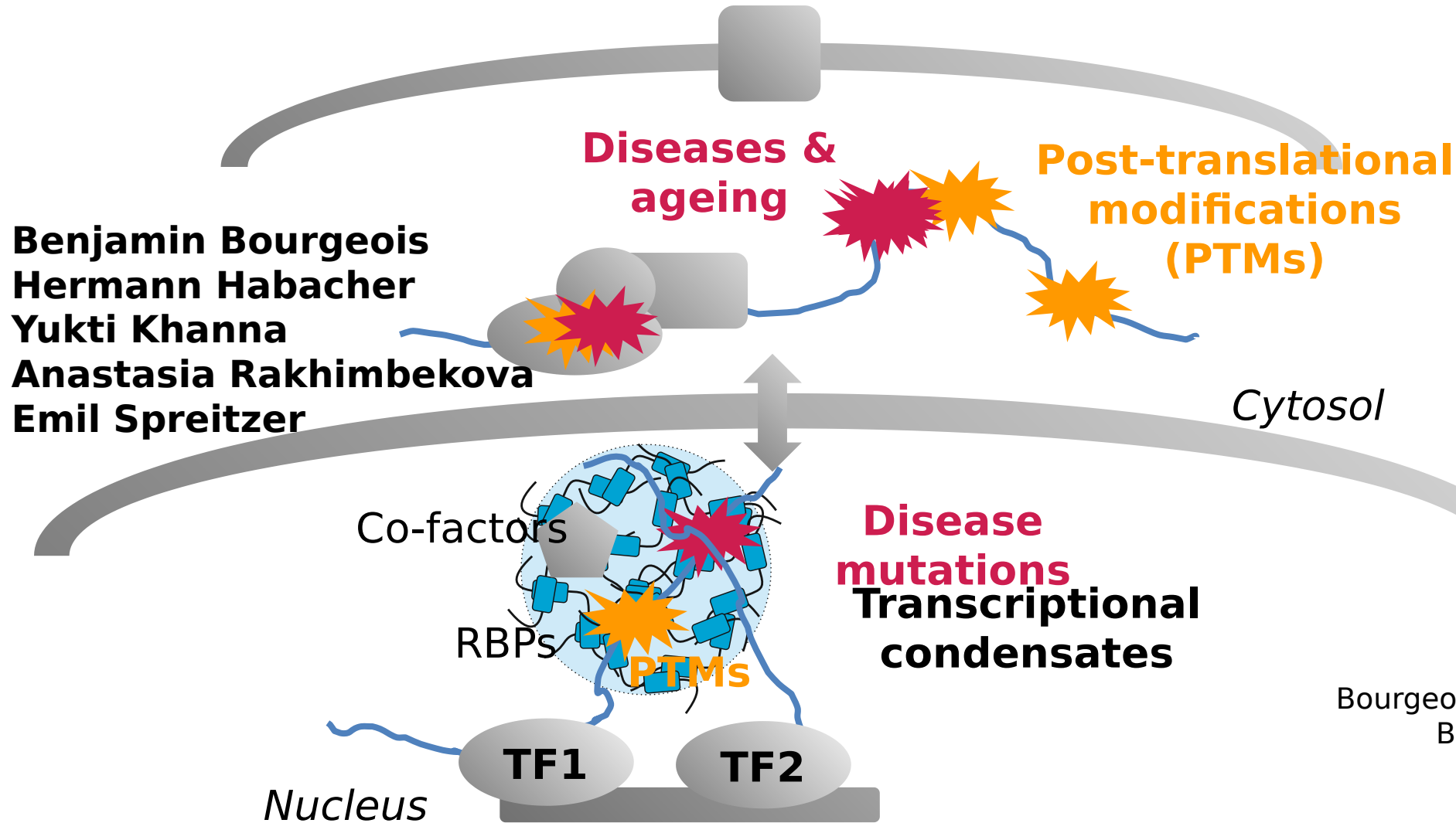
Inclusions in ALS & FTD patients

Disordered regions play key roles in signaling



Alderson et al., JMRO (2022)
Spreitzer et al., CRISB (2022)
Usluer et al., IJMS (2021)
Bourgeois et al., Cell Rep (2021)
Richter et al., FEBS J (2021)
Göbl et al., Redox Biol (2020)
Merle et al., JMB (2019)
Hartmüller et al., JBNMR (2019)
Bourgeois and Madl, FEBS Lett 592 (2018)
Bomblies et al., Plos One 12 (2017)
Anvarian et al., NSMB 23 (2016)
Baar et al., Cell 169 (2017)
Putker et al., Mol Cell 49 (2013)

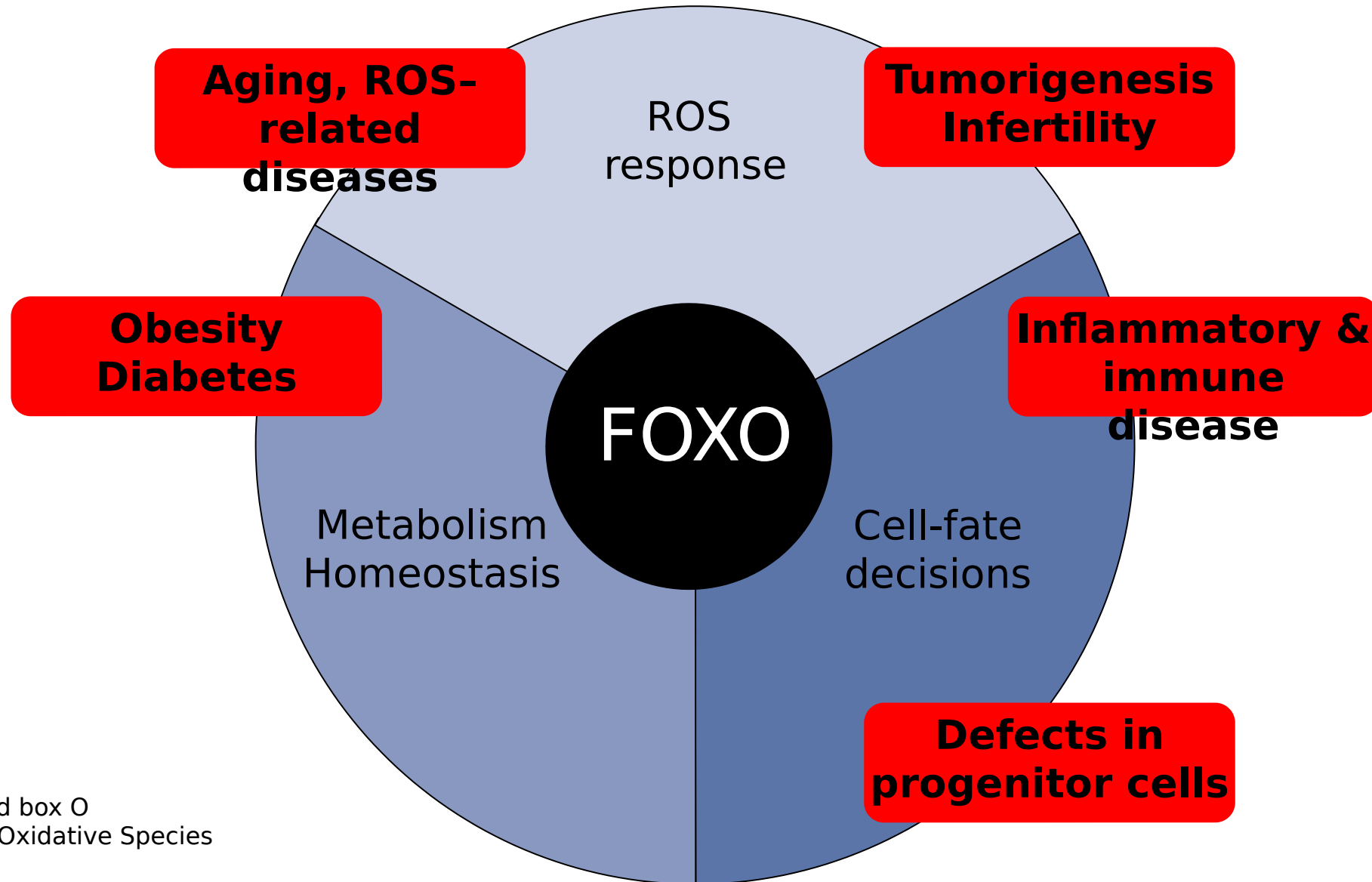
Disordered regions play key roles in signaling



Benjamin Bourgeois
Hermann Habacher
Yukti Khanna
Anastasia Rakhimbekova
Emil Spreitzer

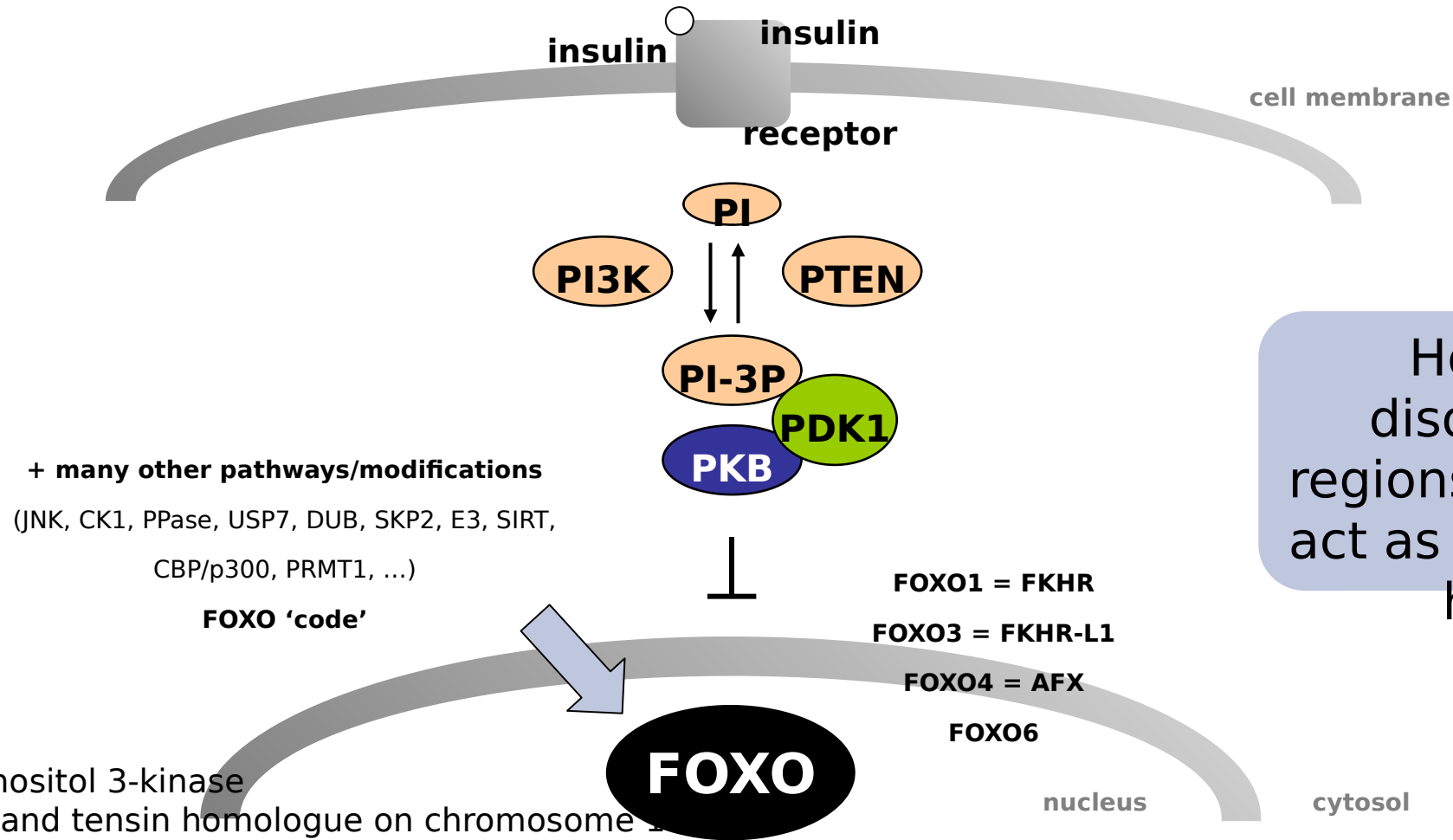
Alderson et al., JMRO (2022)
Spreitzer et al., CRISB (2022)
Usluer et al., IJMS (2021)
Bourgeois et al., Cell Rep (2021)
Richter et al., FEBS J (2021)
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Bomblies et al., Plos One 12 (2017)
Anvarian et al., NSMB 23 (2016)
Baar et al., Cell 169 (2017)
Putker et al., Mol Cell 49 (2013)

FOXOs are master regulators

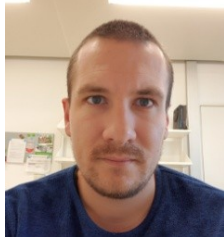


FOXO: Forkhead box O
ROS: Reactive Oxidative Species

The PI3K/PKB/FOXO module



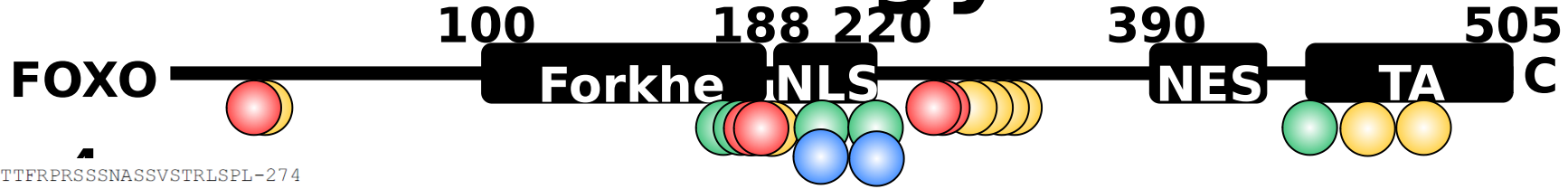
How do disordered regions of FOXOs act as regulatory hub?



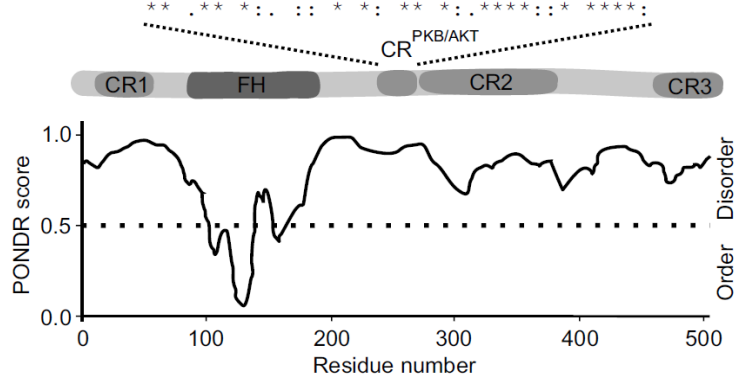
Benjamin Bourgeois

PI3K: phosphatidylinositol 3-kinase
PTEN: phosphatase and tensin homologue on chromosome 10
PKB: protein kinase B
PDK1: phosphoinositide-dependent kinase-1

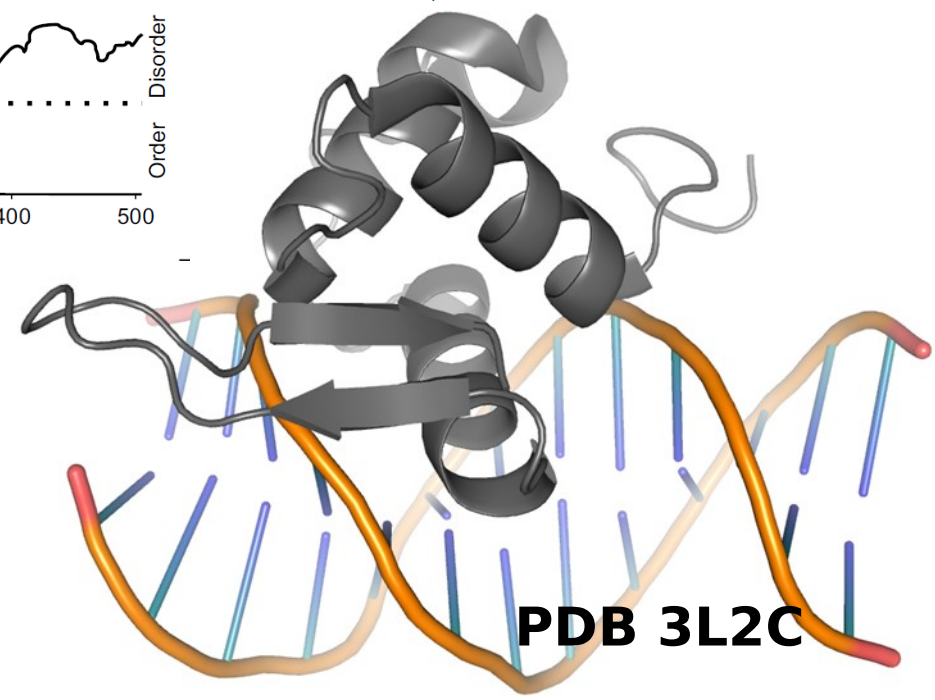
FOXOs are challenging for structural biology



FOXO4 237-KWSGSPCSRNRREEDMWTTFRPRSSSNASSVSTRLSPL-274
 FOXO1 294-KWPASPGSHSNDFFDNWSTFRPRTSSNASTISGRLSPI-331
 FOXO3 290-KWPGSPTSRSDDELDAWTFDRSRTNSNASTVSGRLSPI-327



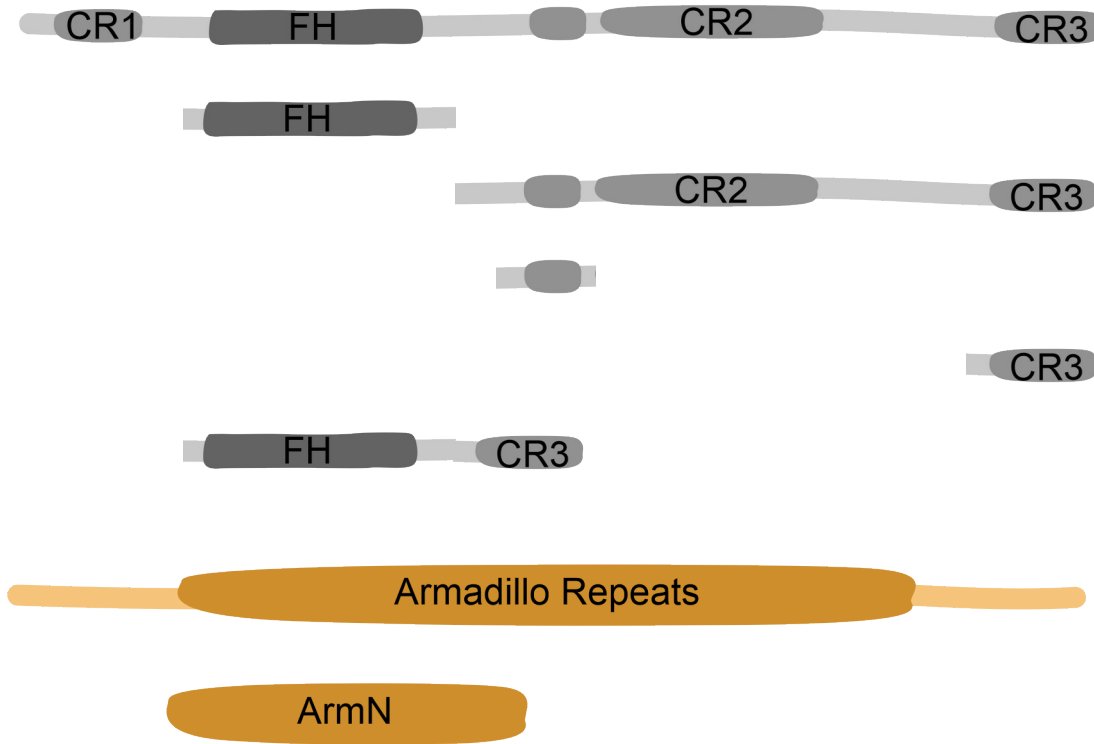
DNA
 binding



PTM 'code'

- Phosphorylation
- Acetylation
- Ubiquitination
- Methylation

β -catenin binds FOXO4 disordered region



FOXO4

FOXO4^{FH}

FOXO4^C

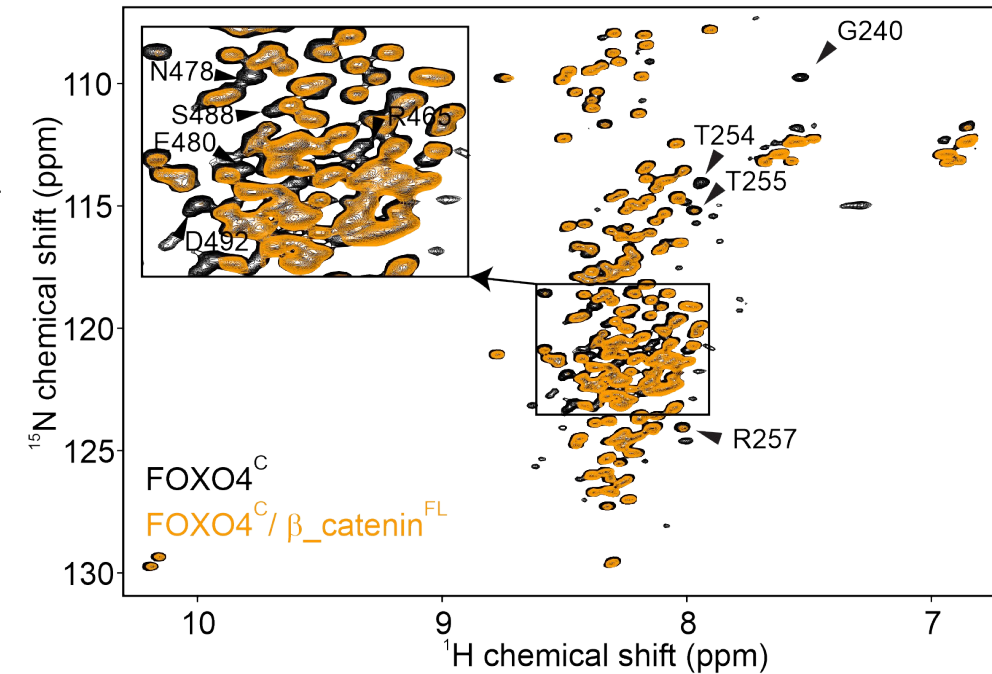
FOXO4^{CR-PKB/AKT}

FOXO4^{CR3}

FOXO4^{FH-CR3}

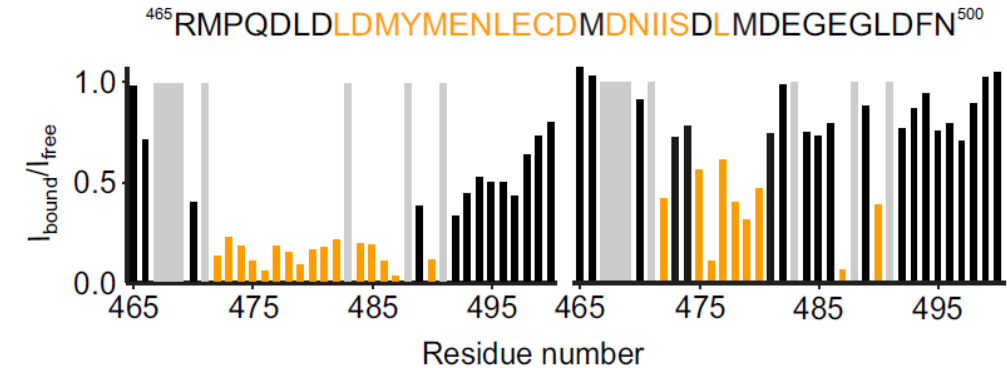
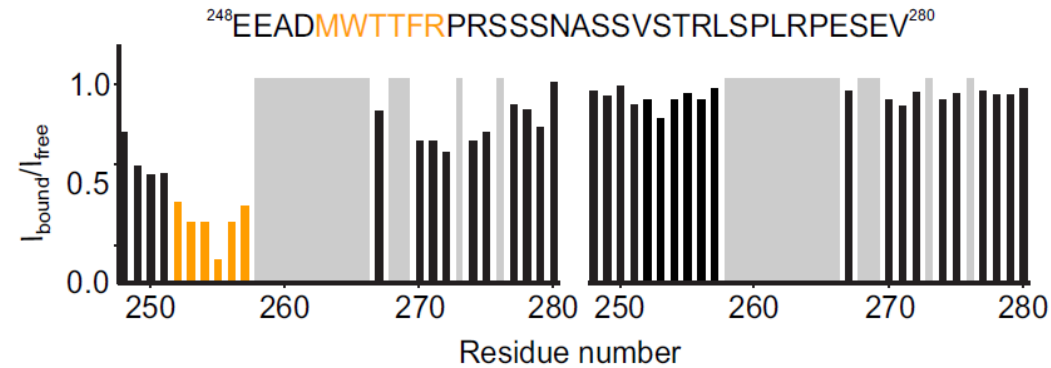
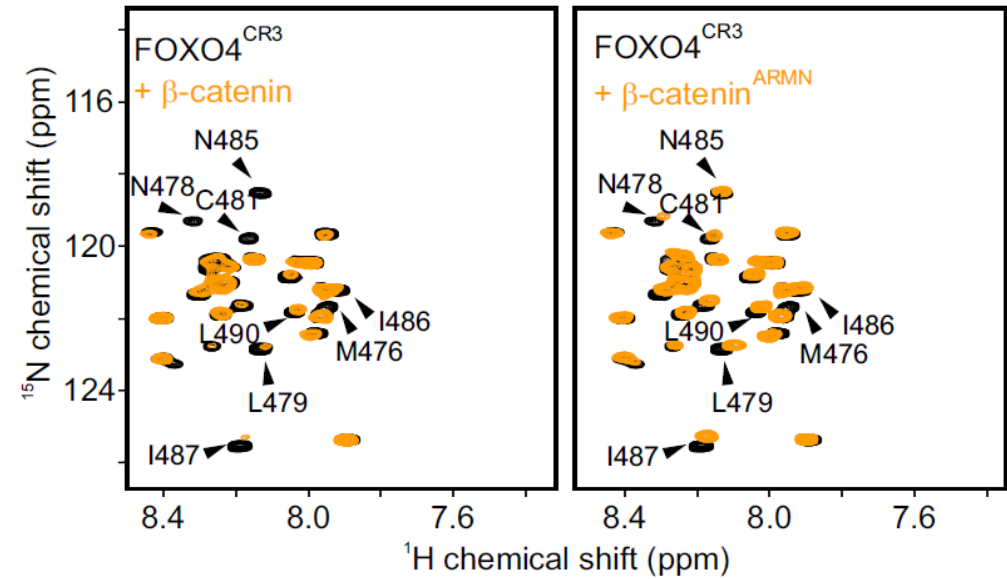
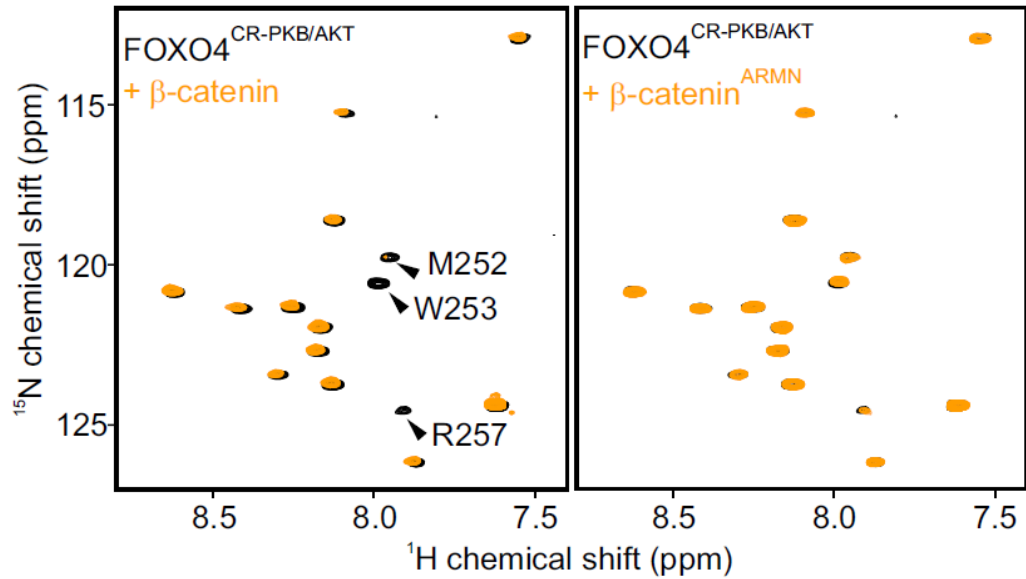
β -catenin

β -catenin^{ARMN}

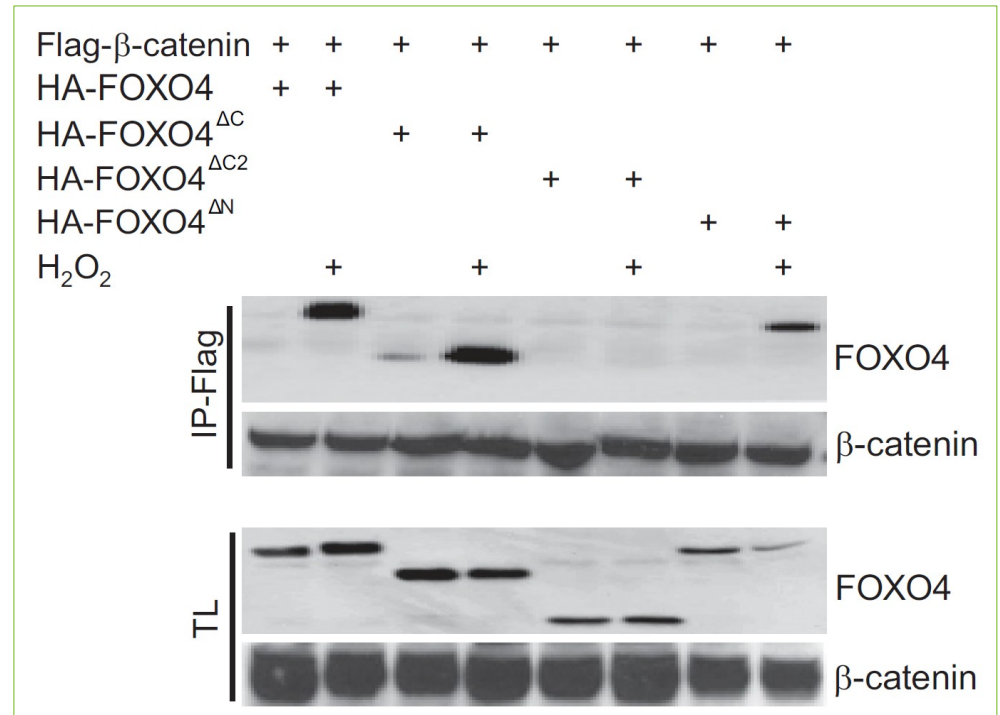
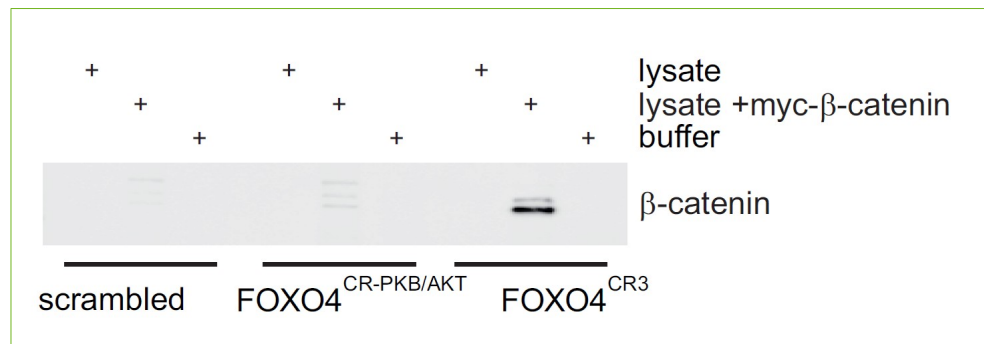
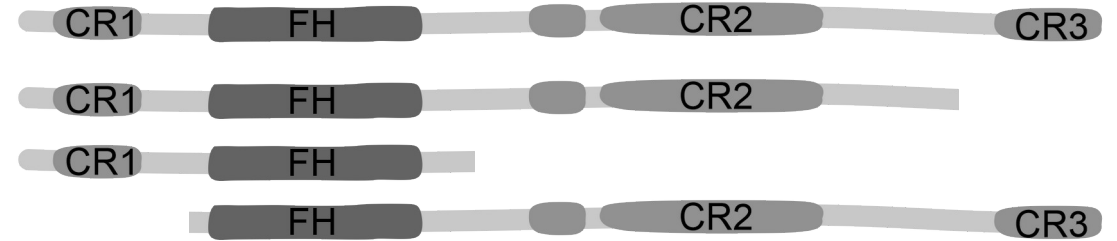
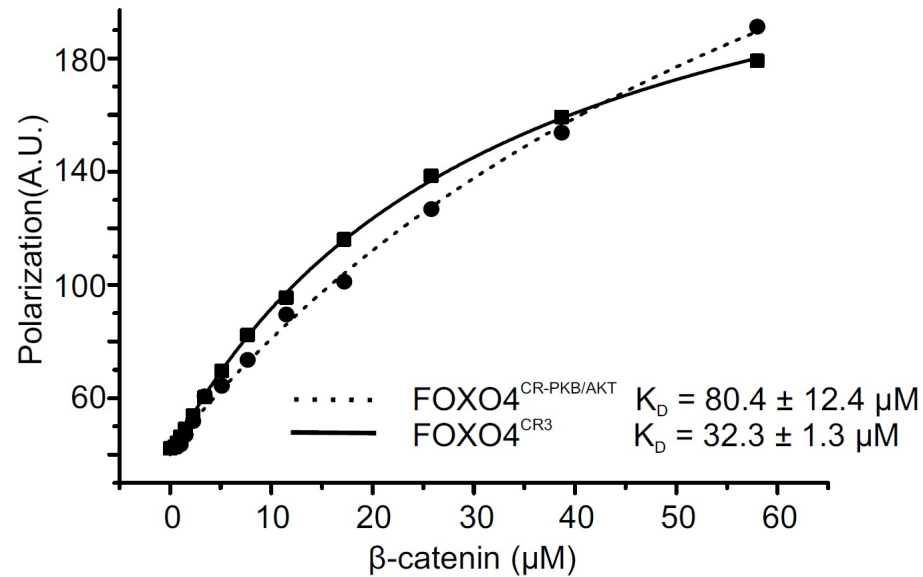


Bourgeois B, Gui T, Hoogeboom D, Hocking HG, Richter G, Spreitzer E, Viertler M, Richter K, Madl T, Burgering BMT. Multiple regulatory intrinsically disordered motifs control FOXO4 transcription factor binding and function. Cell Rep. 2021 Jul 27;36(4):109446. doi: 10.1016/j.celrep.2021.109446. PMID: 34320339.

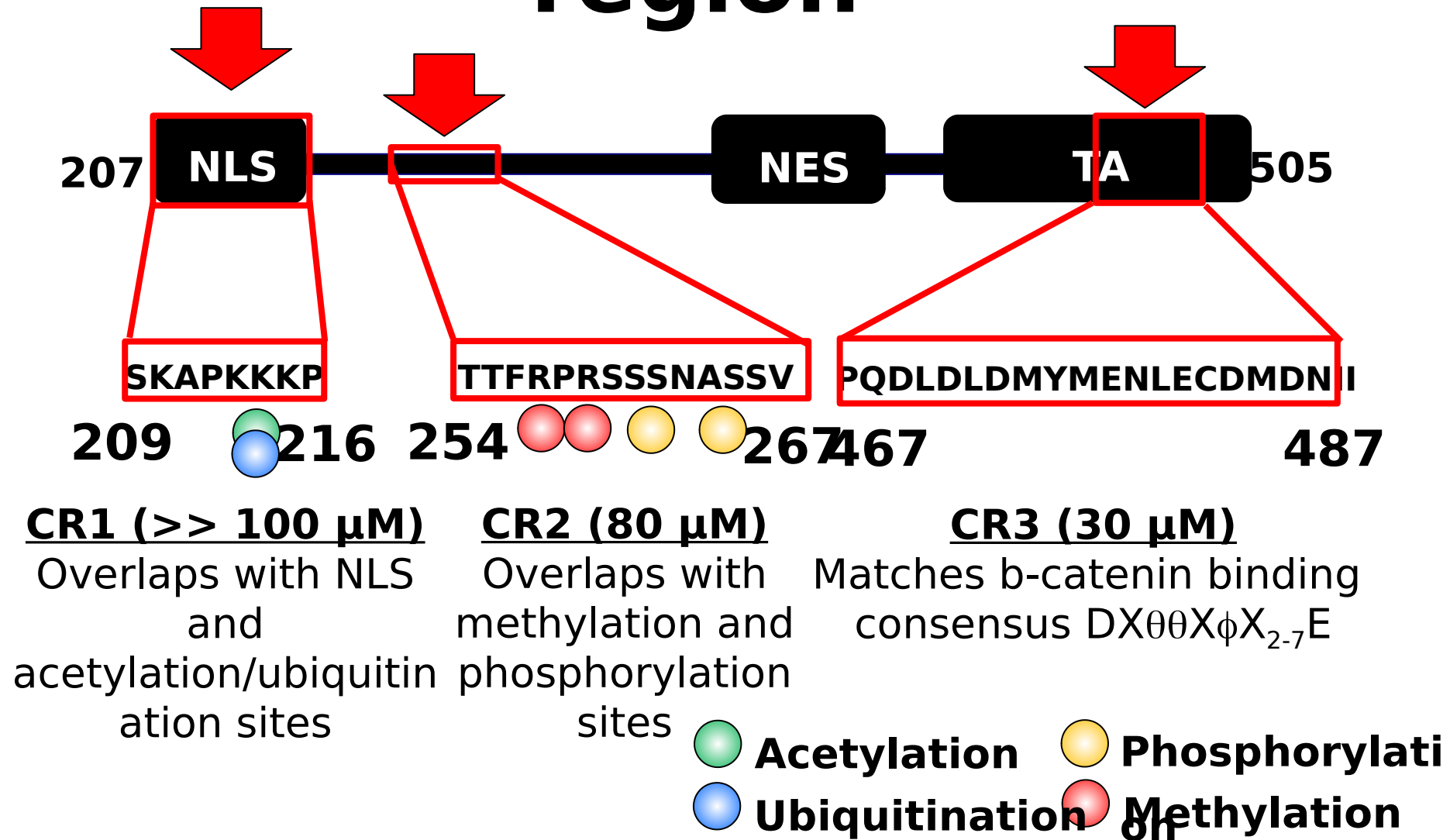
β -catenin binds FOXO4 disordered region



β -catenin binds FOXO4 disordered region

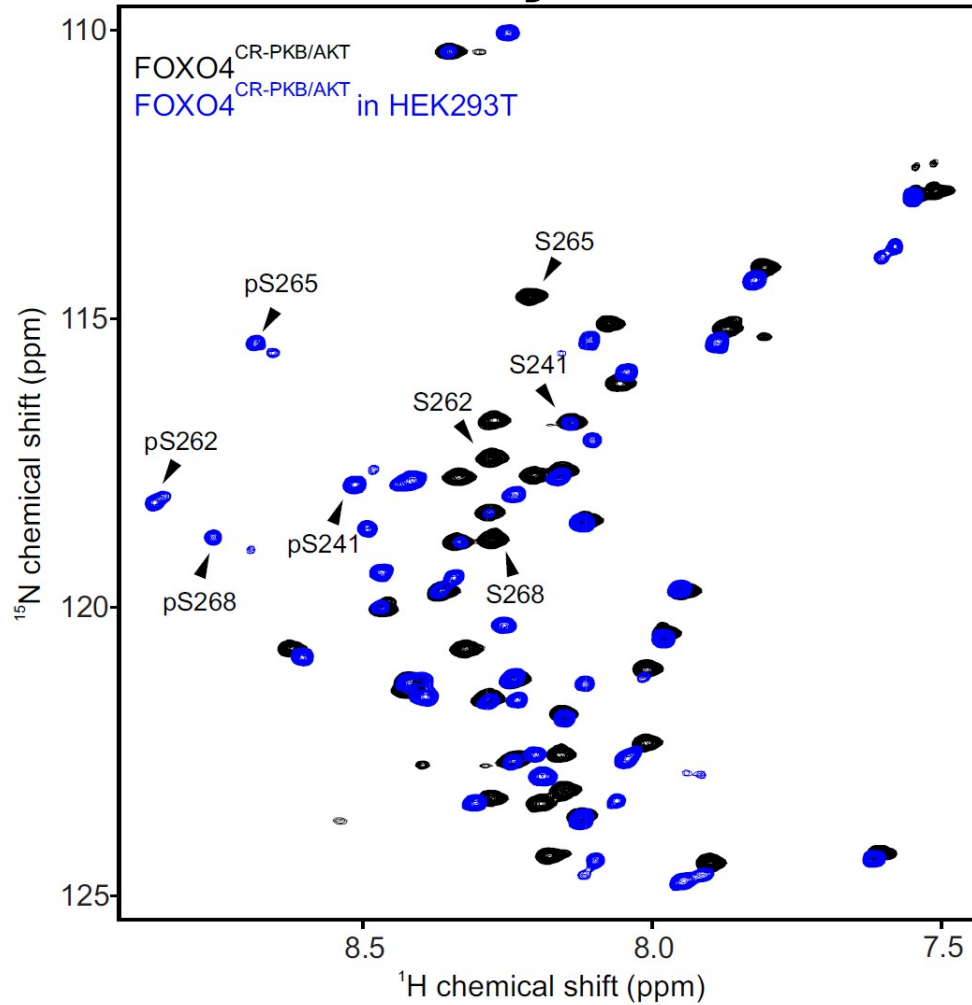


β -catenin binds FOXO4 disordered region

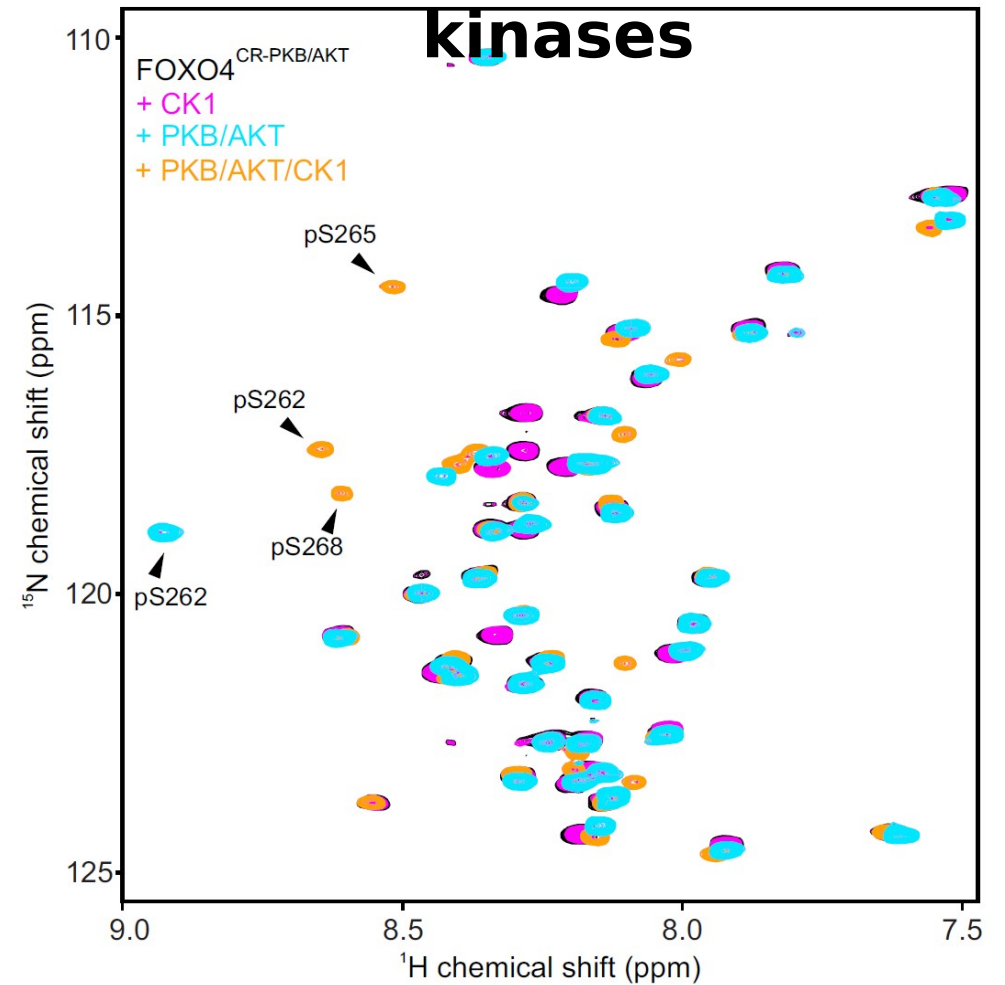


FOXO4 is phosphorylated at the CR^{PKB/AKT}

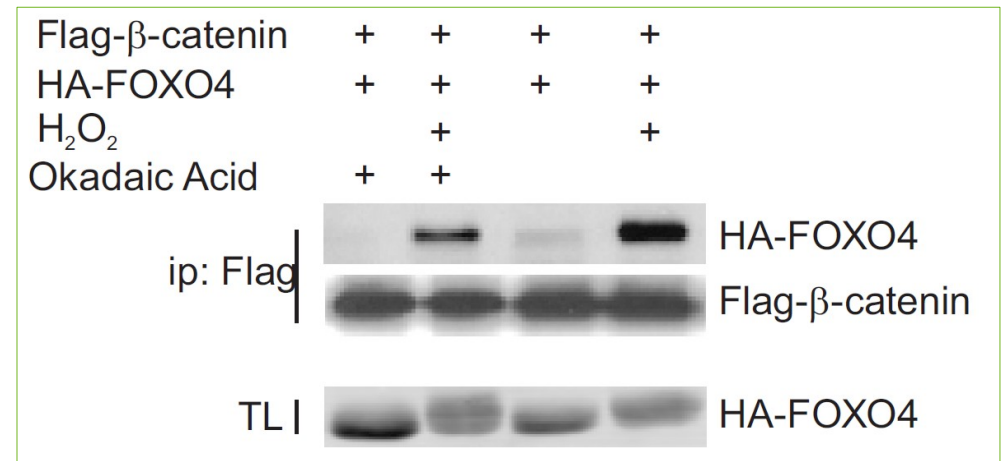
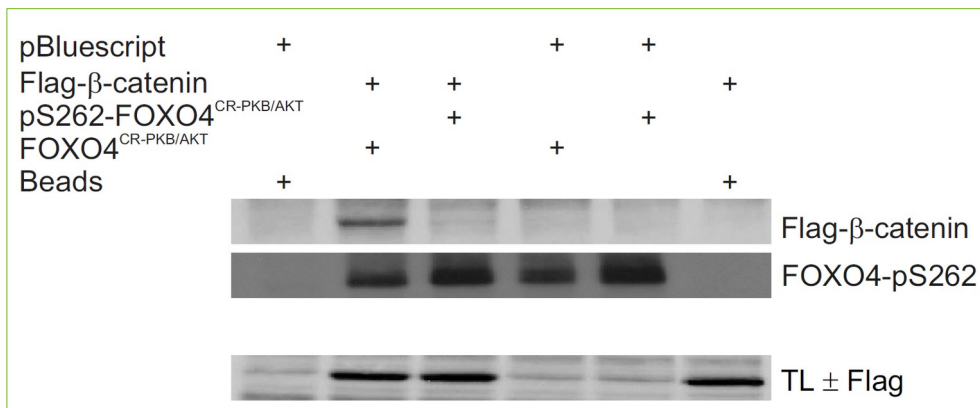
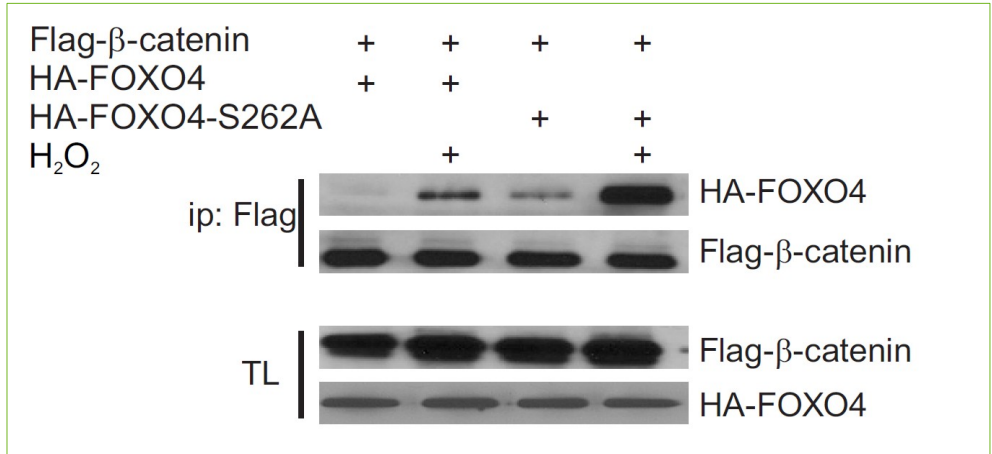
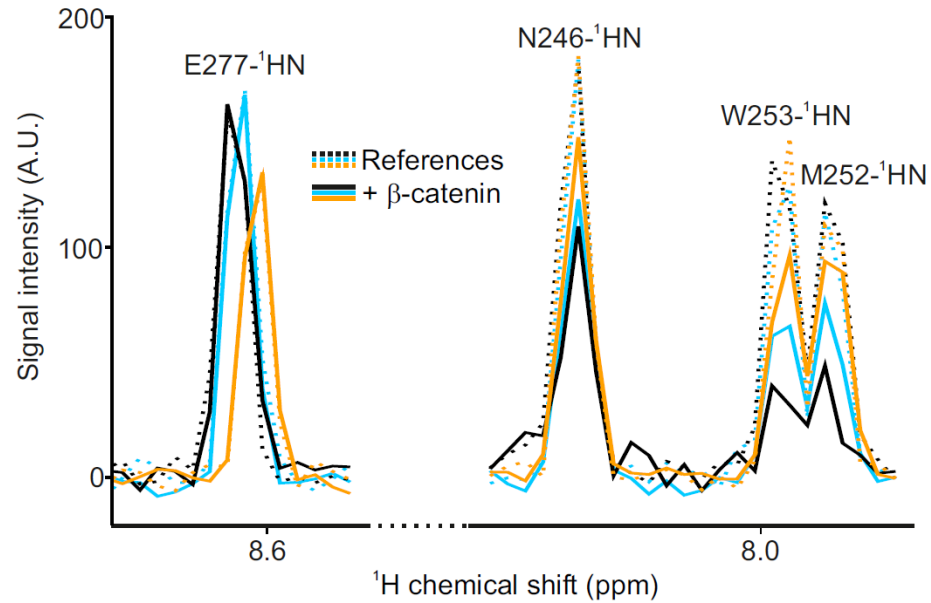
Cell lysate



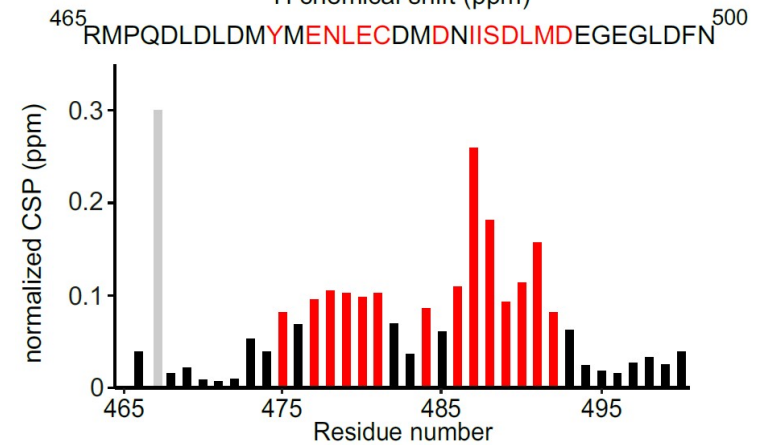
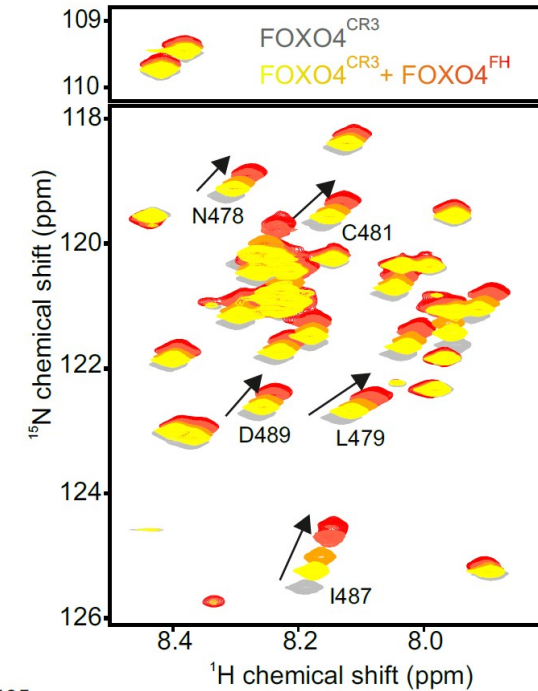
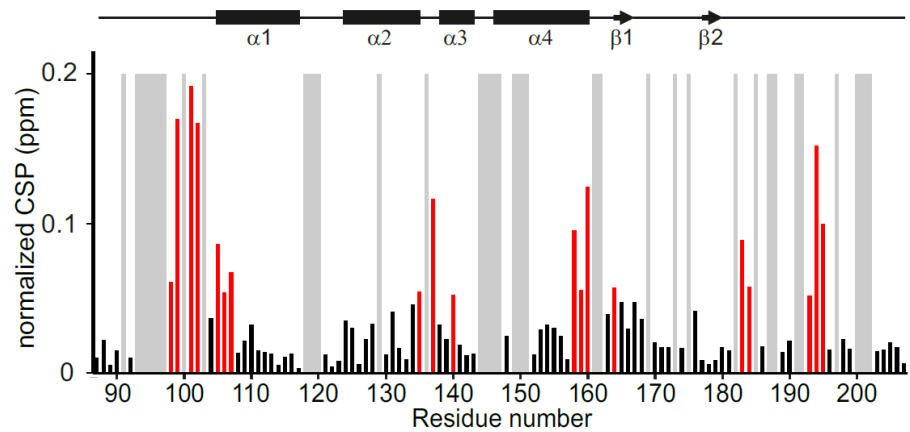
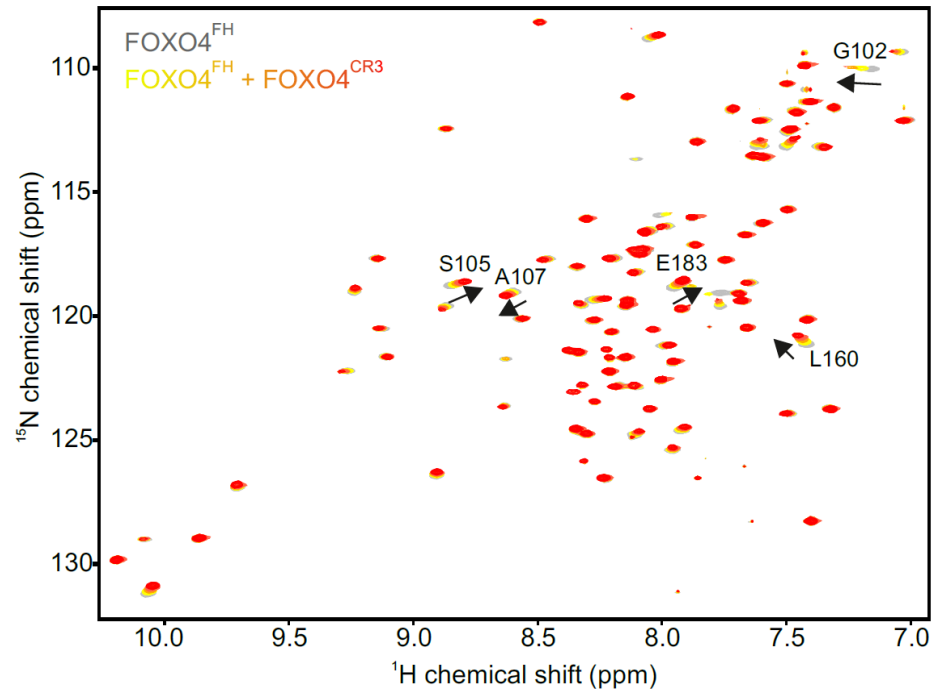
Recombinant



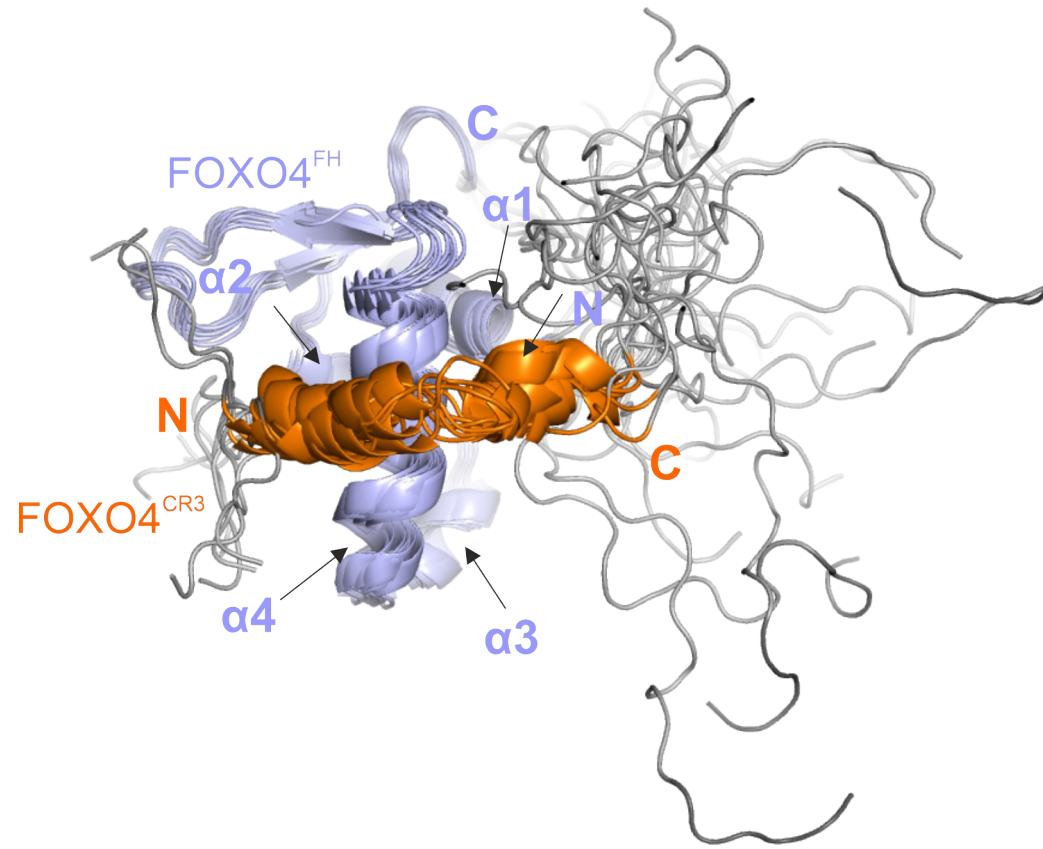
Phosphorylation blocks β -catenin binding



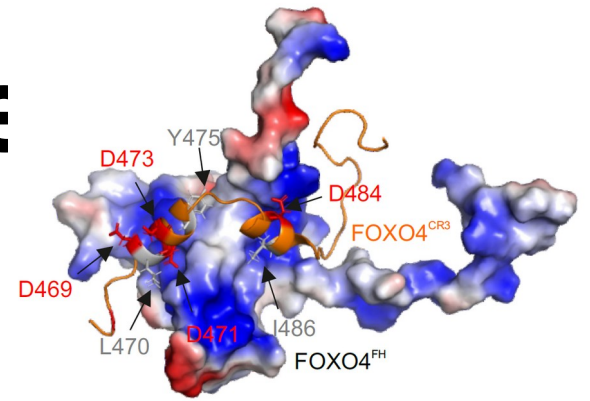
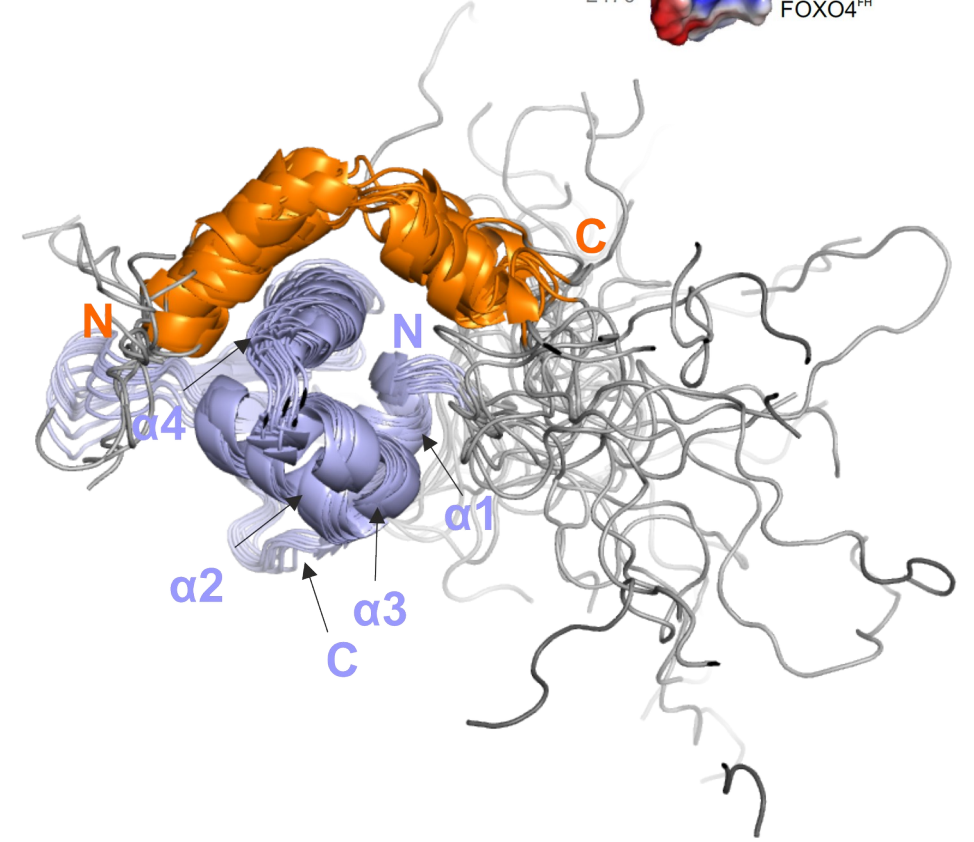
FOXO4 is auto-inhibited



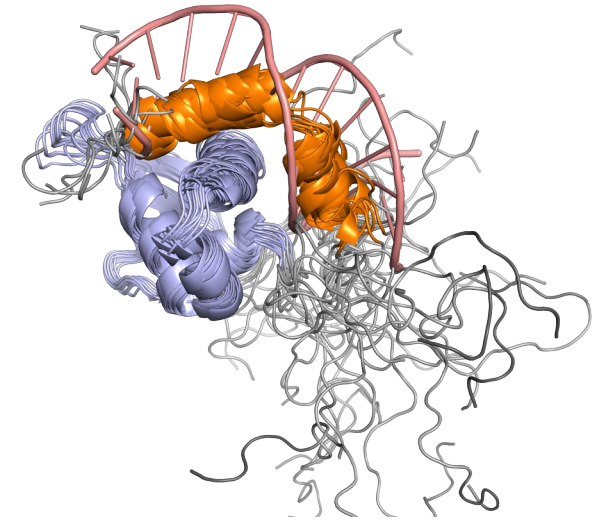
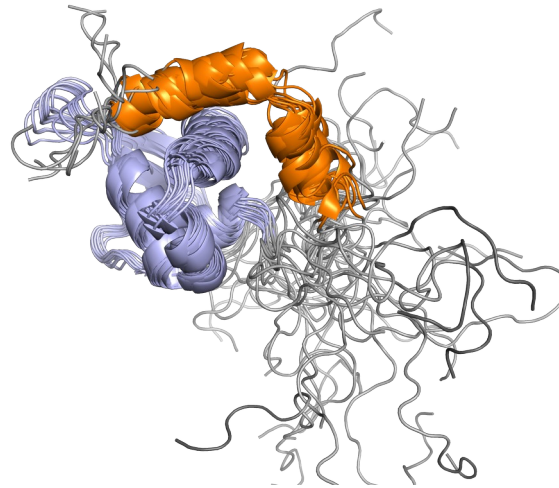
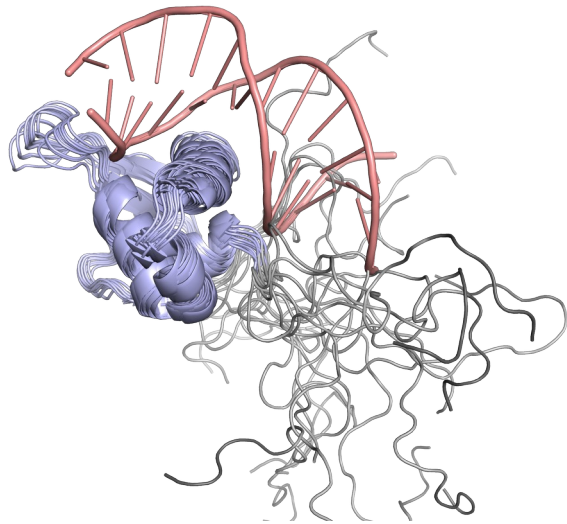
FOXO4 is auto-inhibited



90°



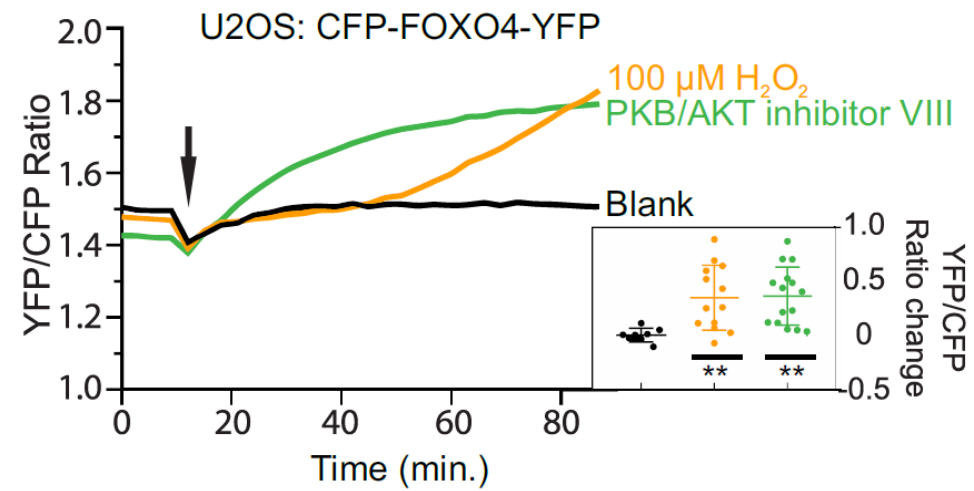
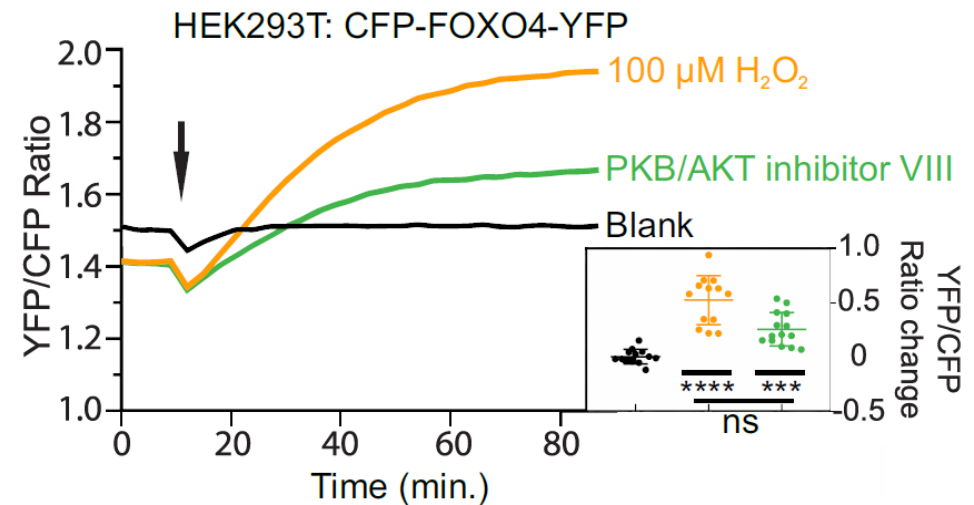
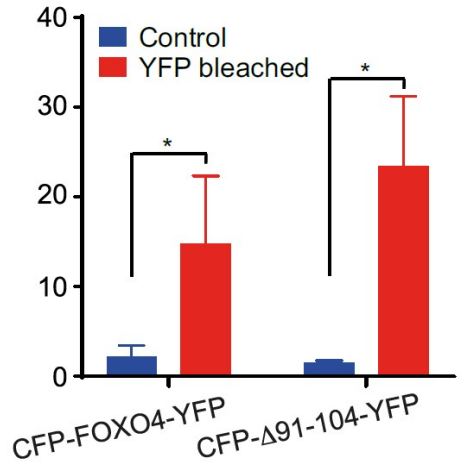
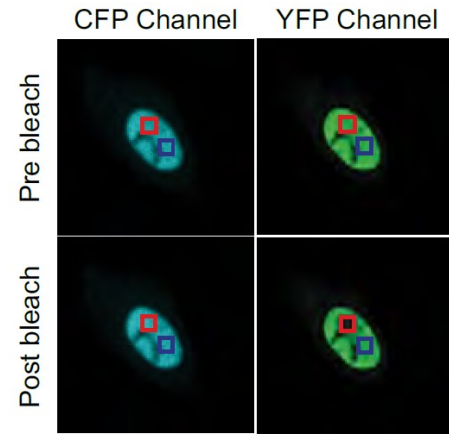
FOXO4 is auto-inhibited



FOXO4 ^{FH}	DNA	200 ± 17 nM
FOXO4 ^{FH-CR3}	DNA	2090 ± 382 nM
FOXO4 ^{FH-CR3} + β -catenin	DNA	277 ± 8 nM

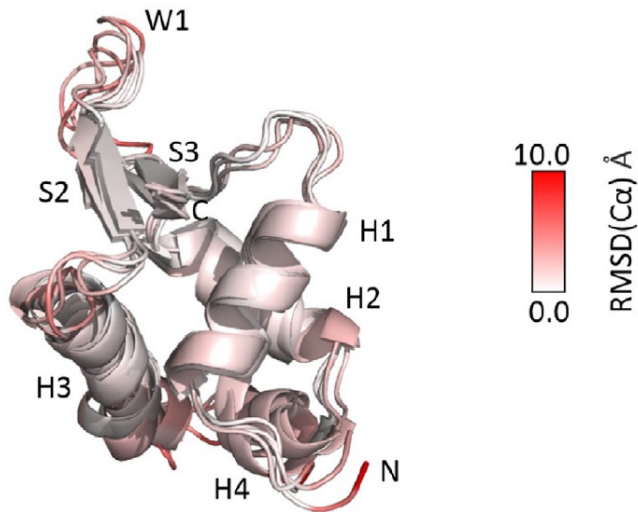
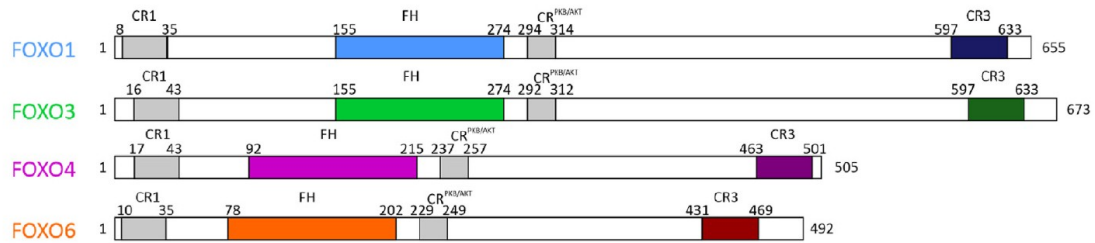
PDB 3L2C

In cell FRET confirms FOXO4 conformational change

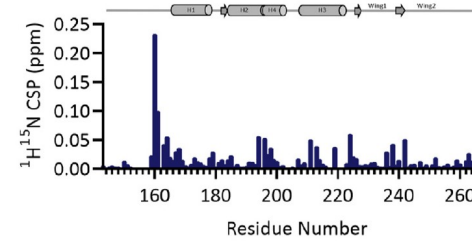
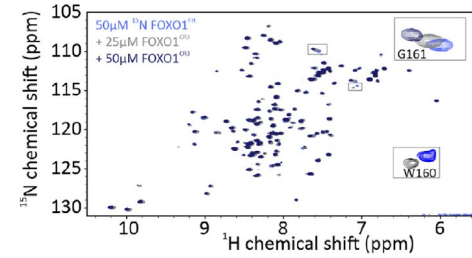


Tianshu Gui and Boudewijn Burgering

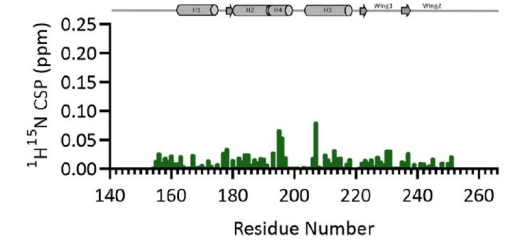
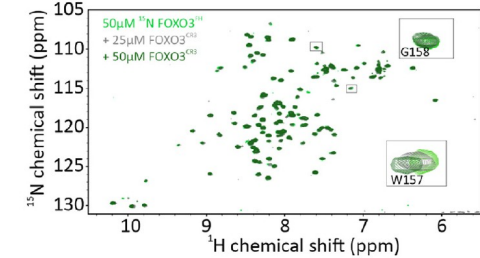
Auto-inhibition is conserved among FOXOs



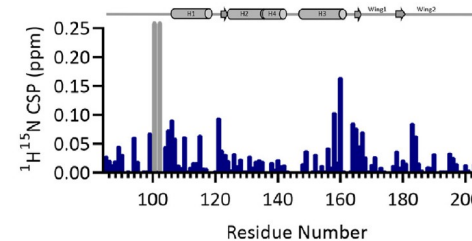
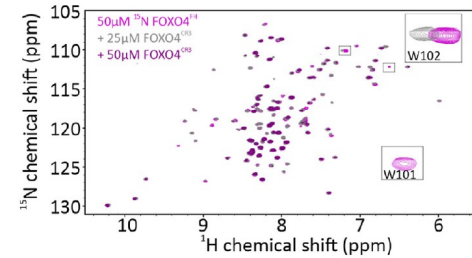
B



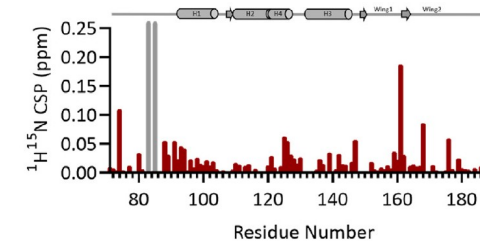
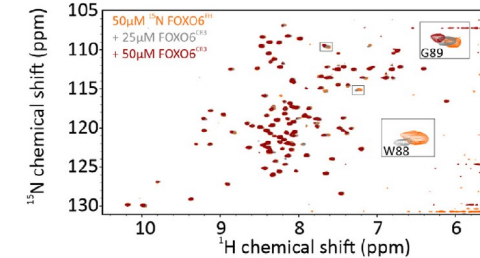
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D

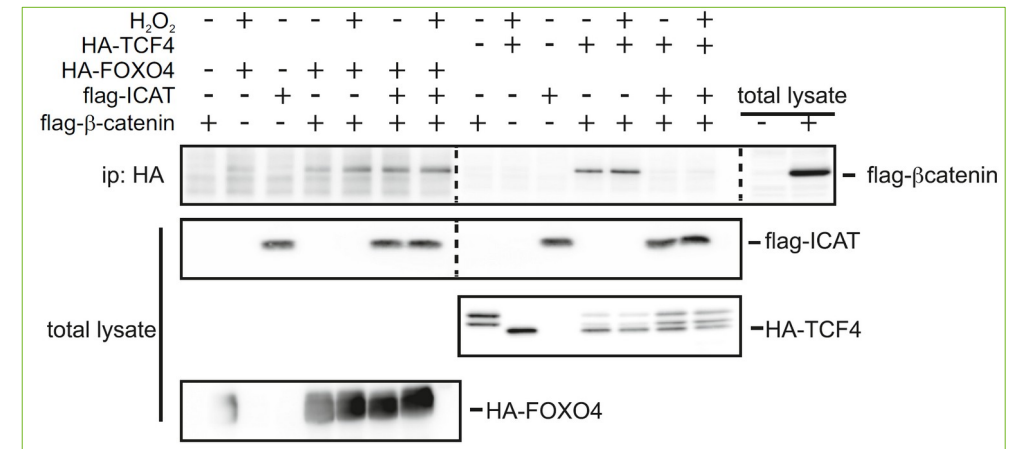
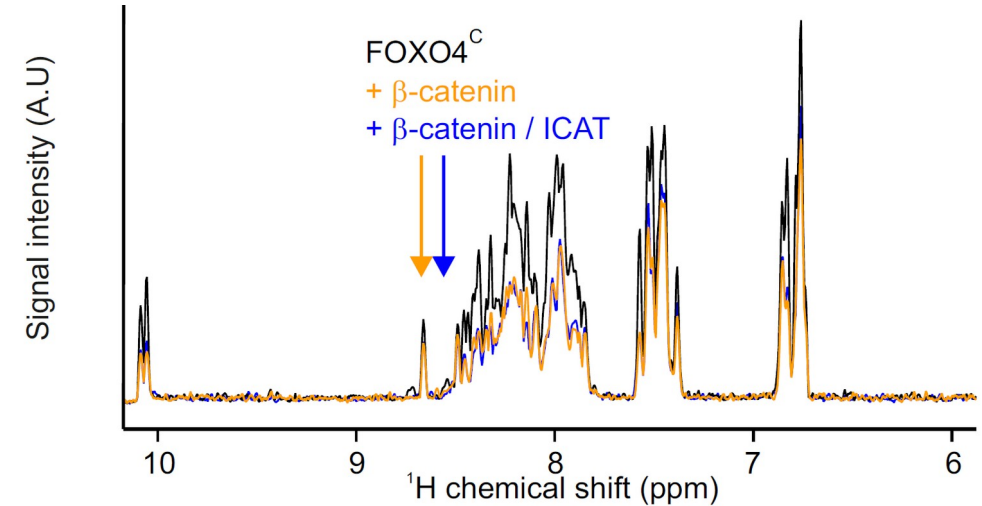
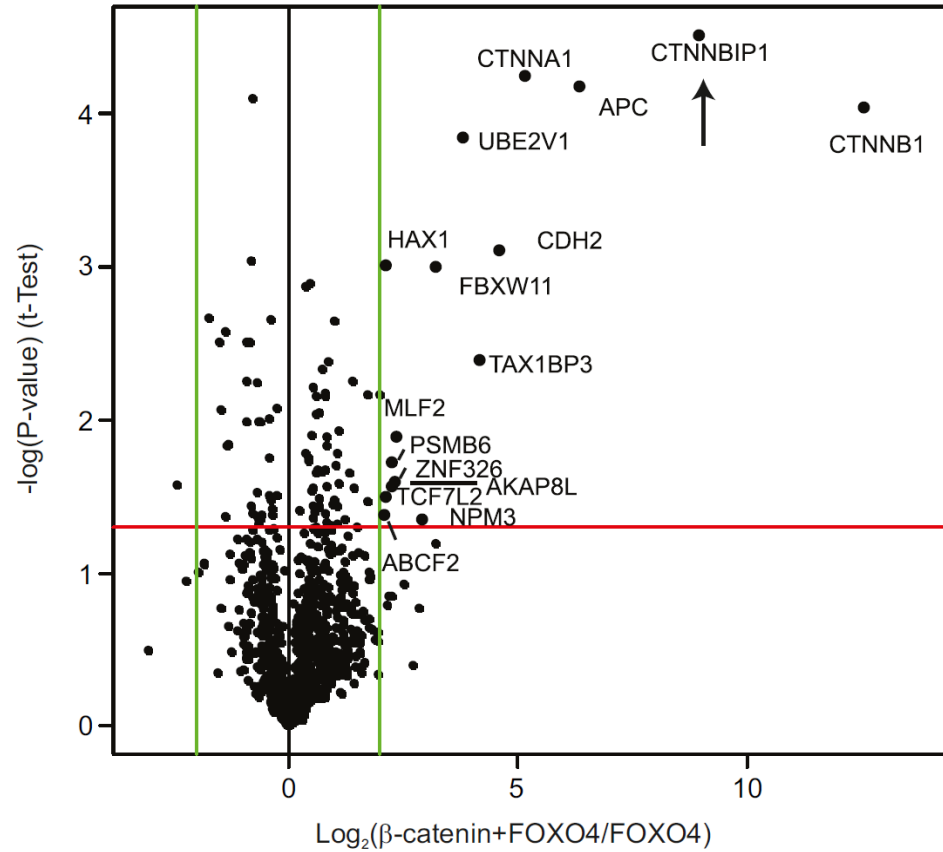


E



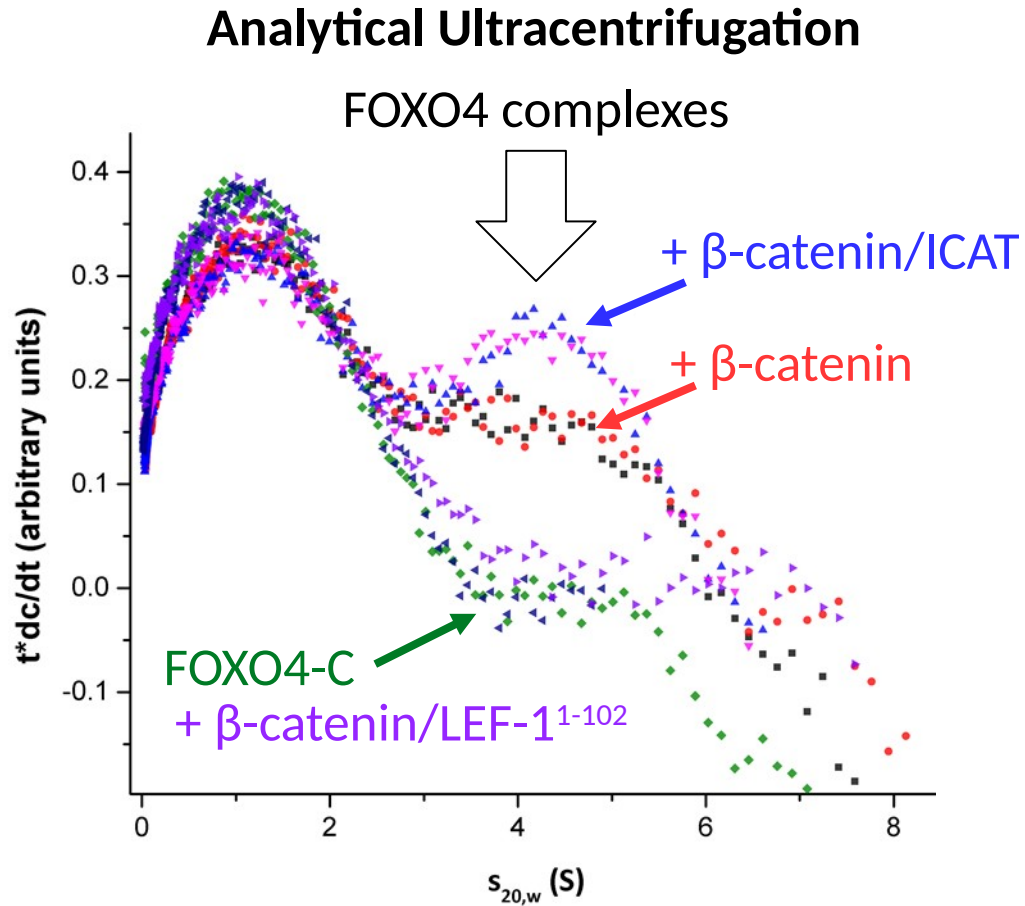
Spreitzer E, Alderson TR, Bourgeois B, Eggenreich L, Habacher H, Brahmersdorfer G, Pritisanac I, Sánchez-Murcia PA, Madl T. FOXO transcription factors differ in their dynamics and intra/intermolecular interactions. *Curr Res Struct Biol*. 2022 Apr 27;4:118-133. doi: 10.1016/j.crstbi.2022.04.001. PMID: 35573459

ICAT regulates FOXO4/TCF/LEF binding to β -catenin



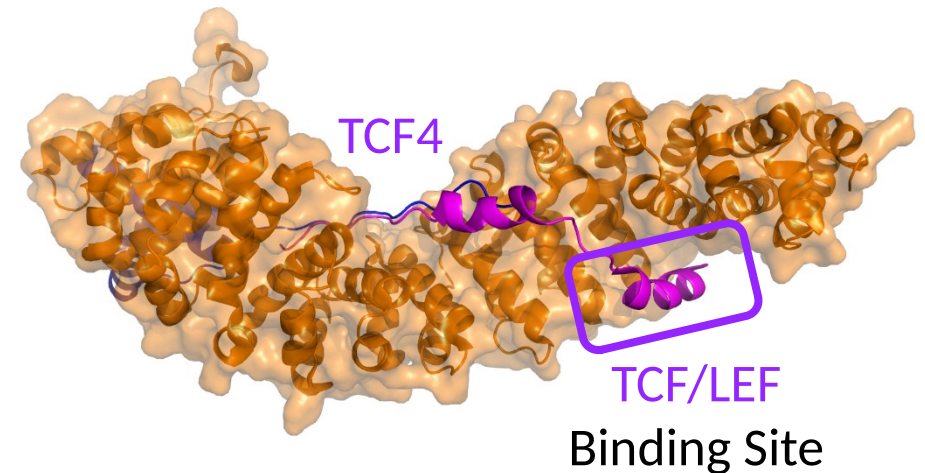
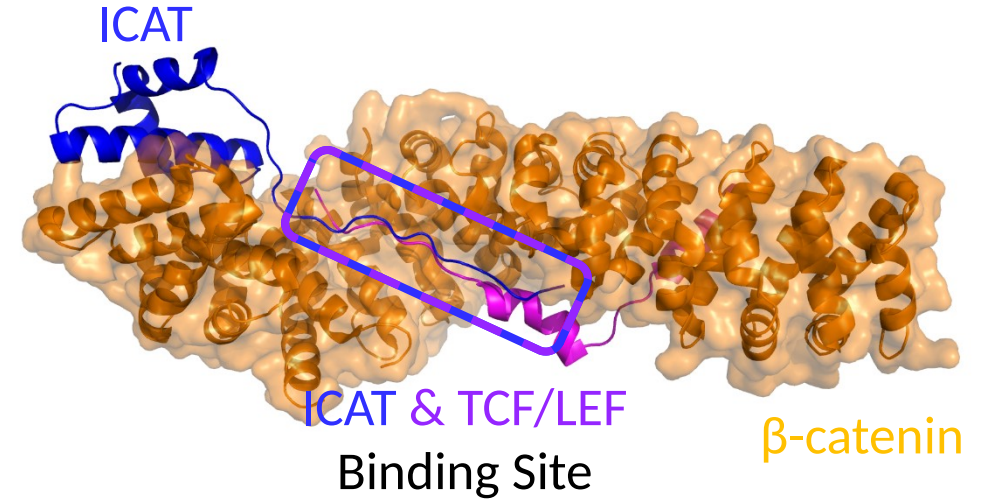
Tianshu Gui and Boudewijn Burgering
Klaus Richter

ICAT regulates FOXO4/TCF/LEF binding to β -catenin



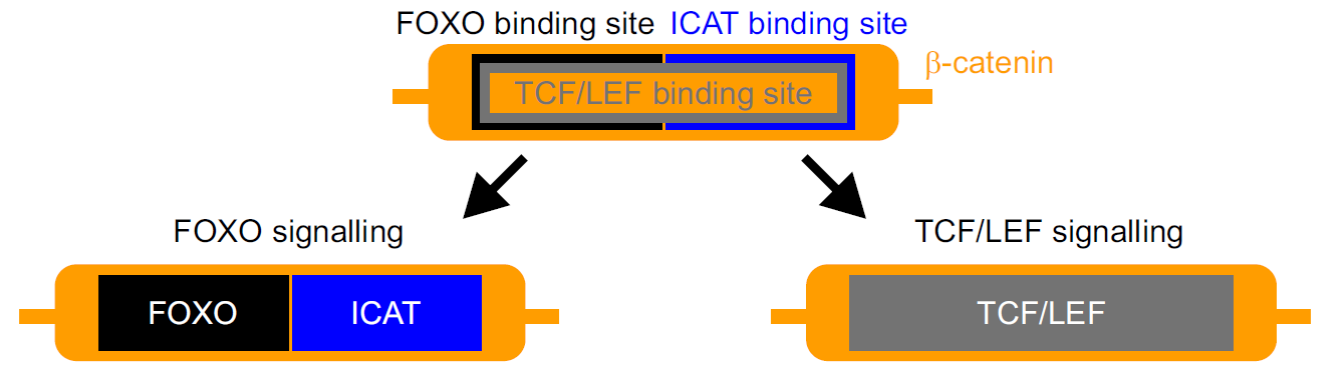
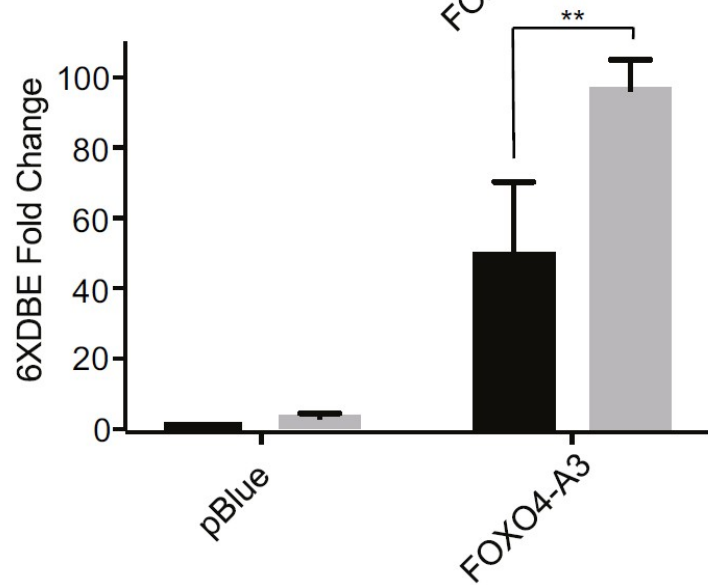
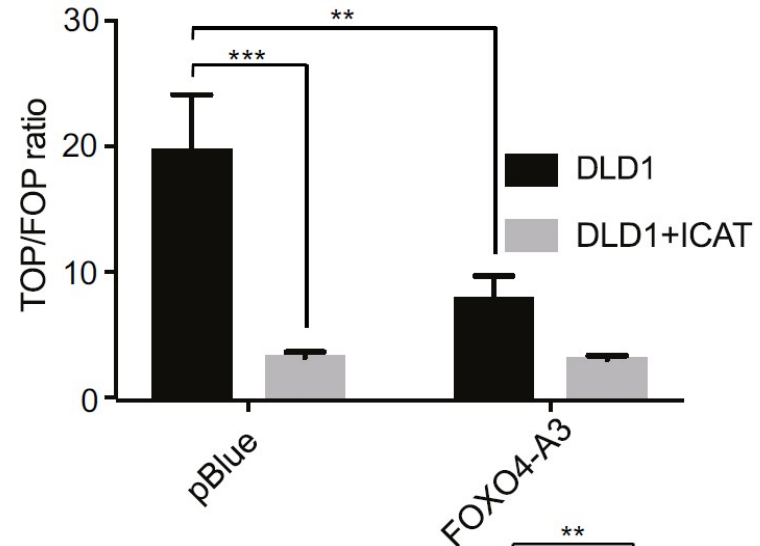
Molecular mass

(collaboration Klaus Richter, TUM)

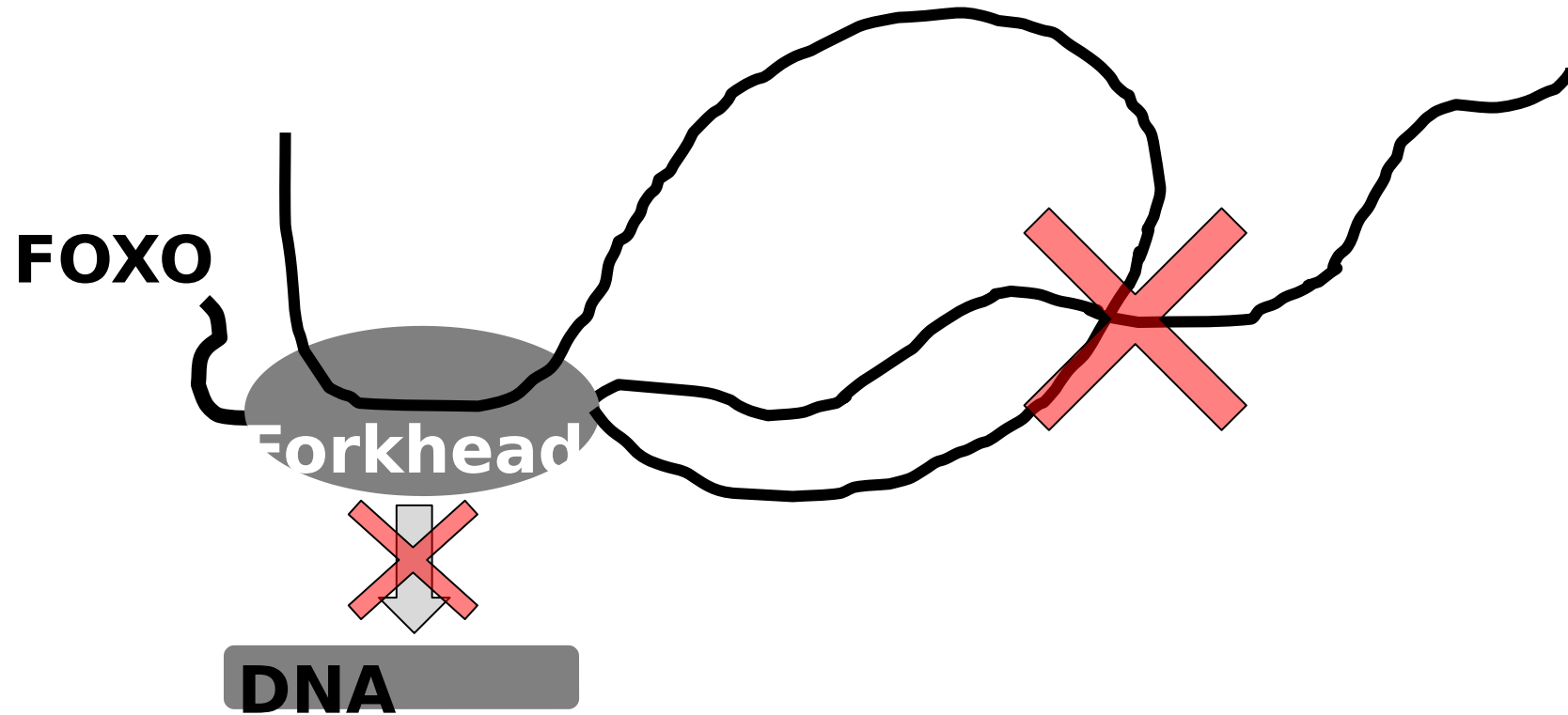


PDB 1LUJ, 1G3J

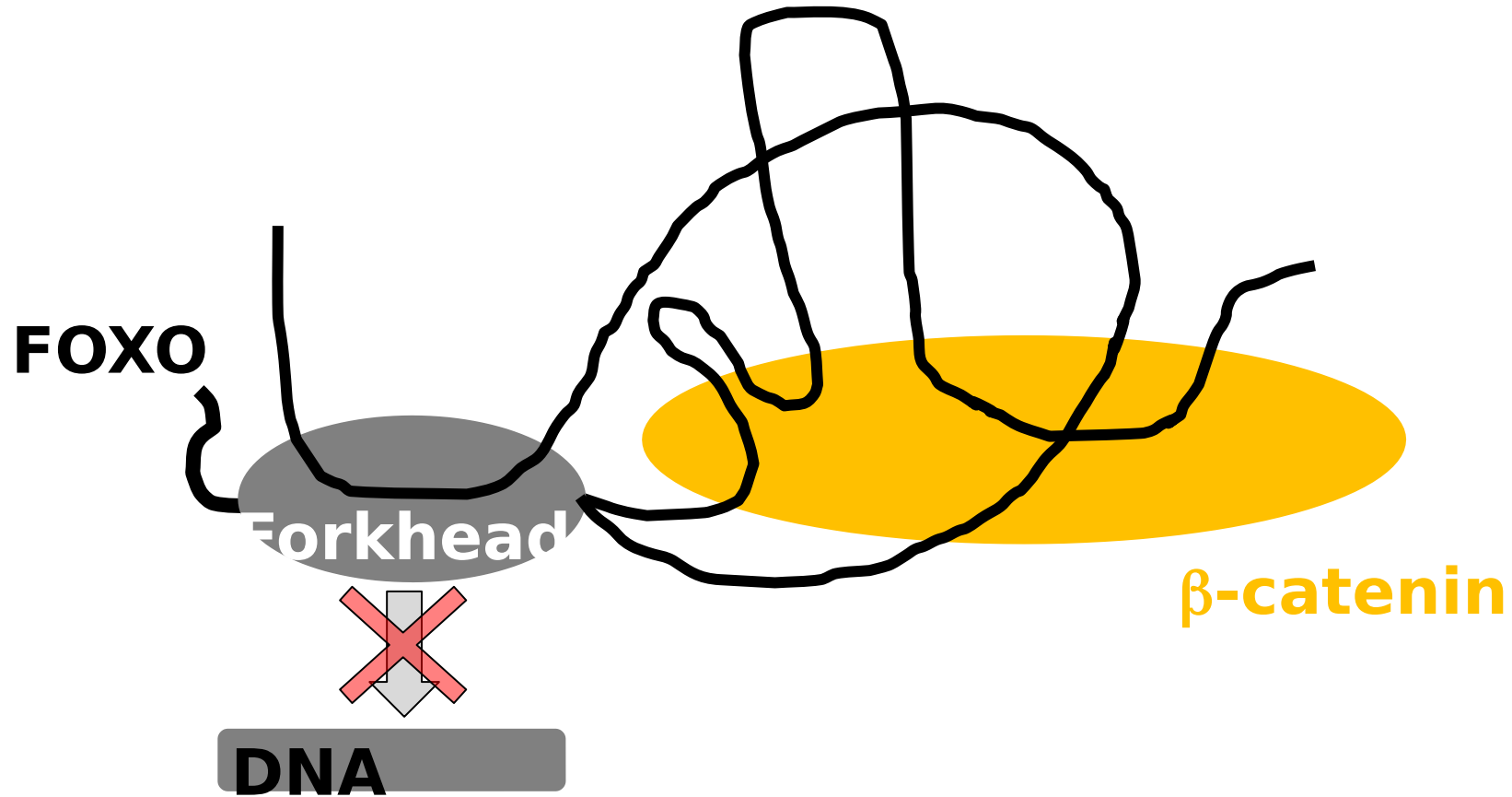
ICAT regulates FOXO4/TCF/LEF signaling



β -catenin competes with FOXO auto-inhibition

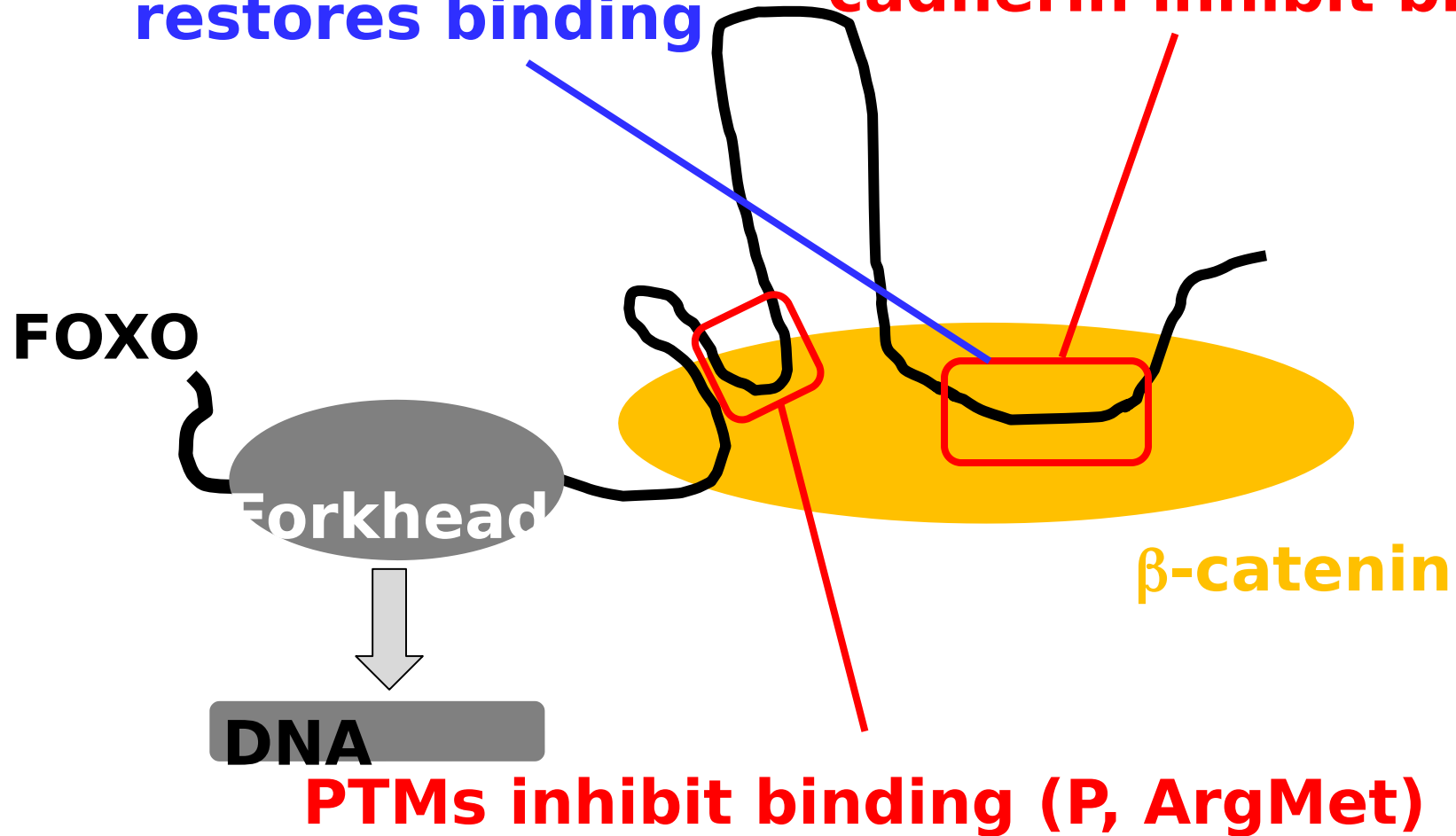


β -catenin competes with FOXO auto-inhibition

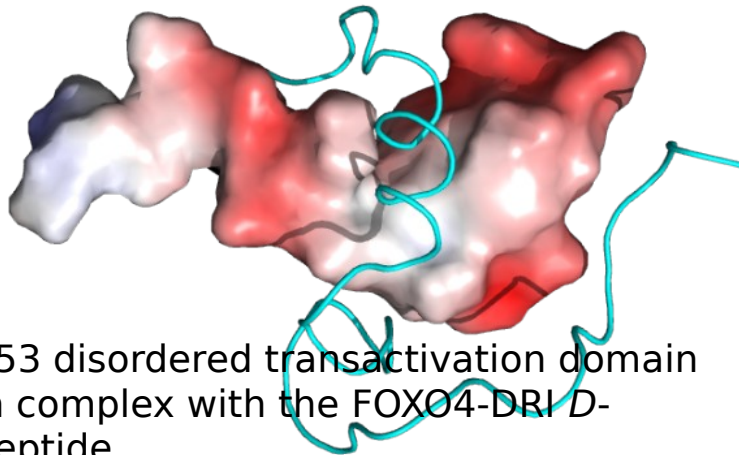
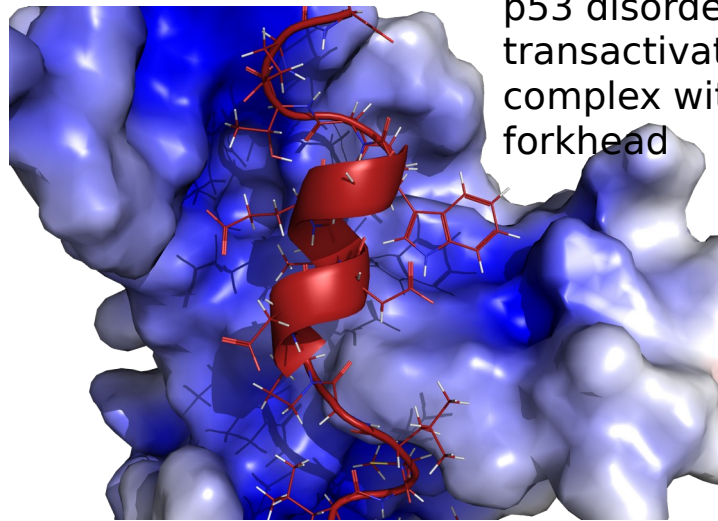


β -catenin - FOXO interaction network

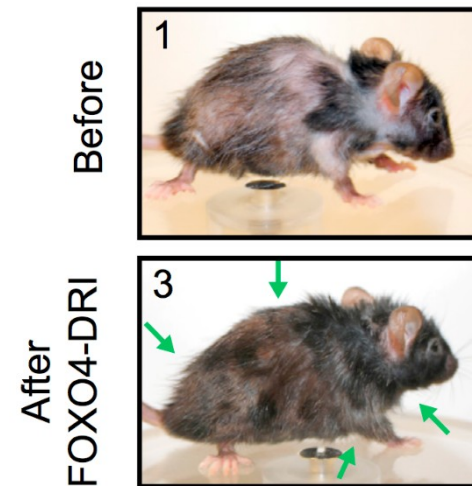
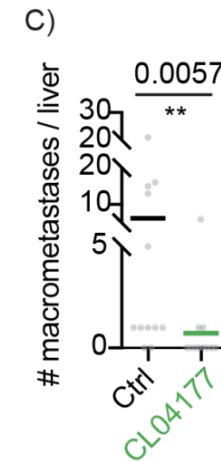
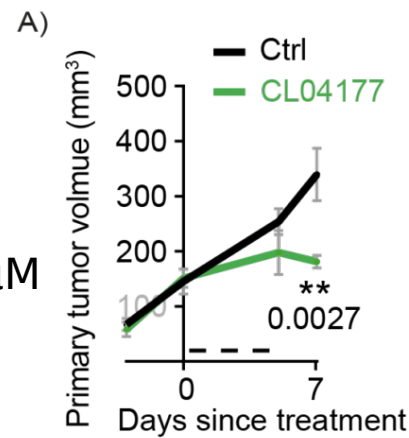
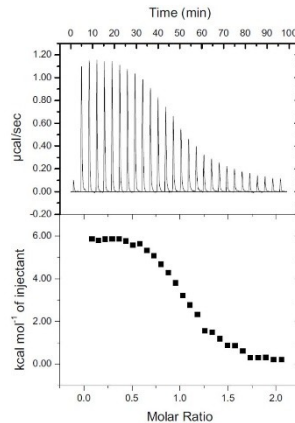
ICAT blocks TCF/LEF and restores binding **TCF/LEF, Axin-1, APC, cadherin inhibit binding**



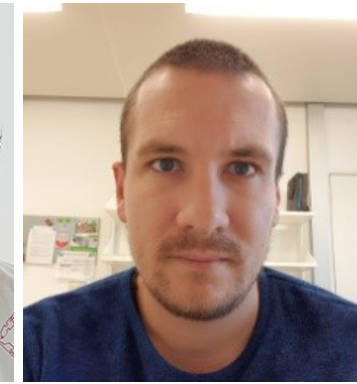
Targeting disordered regions of transcription factors



$K_D: 1.8 \pm 0.2 \mu\text{M}$



Emil Spreitzer



Benjamin Bourgeois

Collaboration
Peter de Keizer

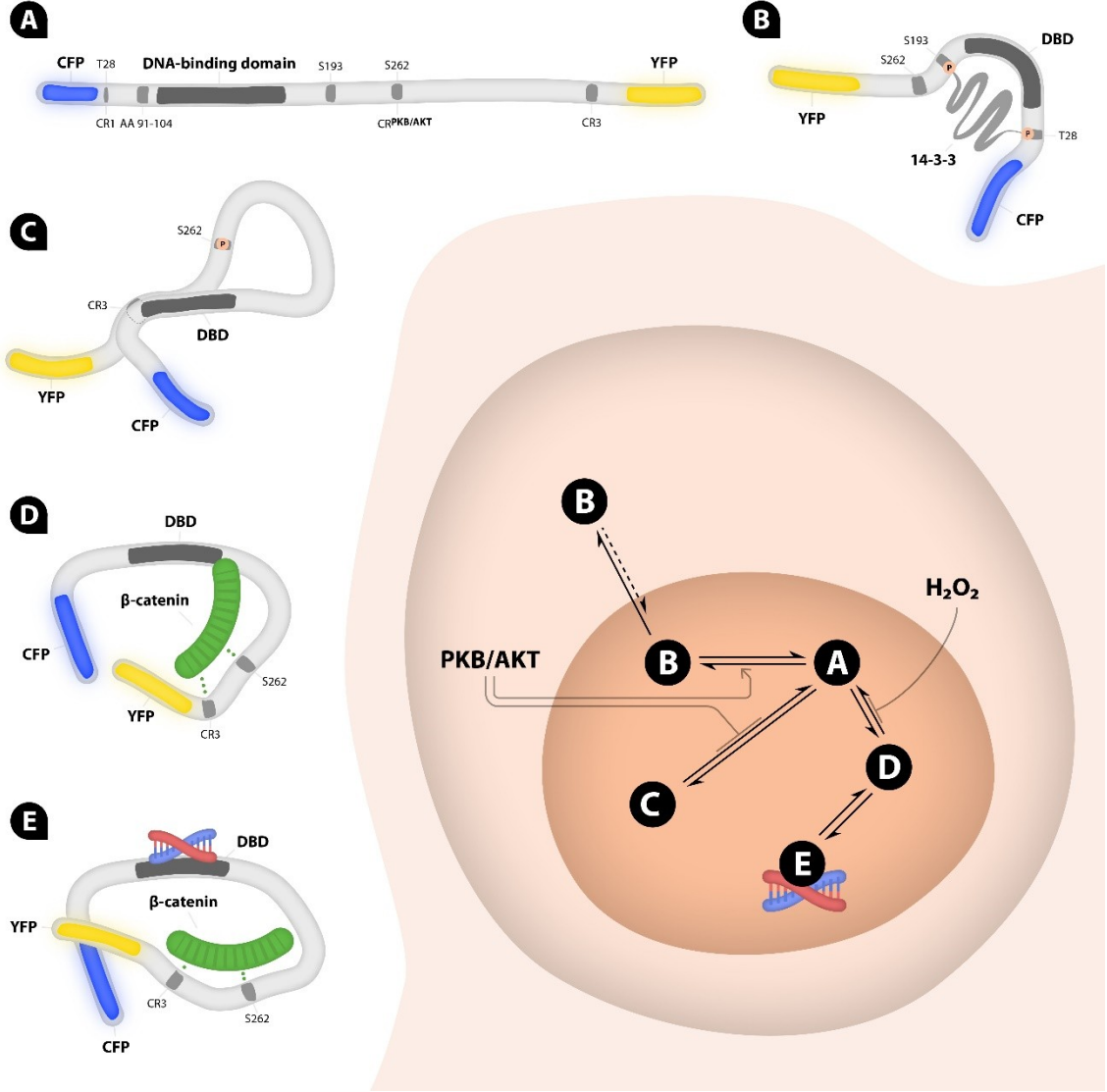


UMC Utrecht



WO 2021/165538 Improved anti-senescence compounds and uses thereof
DeKeizer, Campisi, Hoeijmakers and Madl labs - Cell 169 (2017) 137

Here we are and there we go



The interaction network between FOXO4 and β -catenin was deciphered

FOXO4 auto-inhibition interferes with DNA binding and is counter-acted by β -catenin

FOXO4 exists in multiple conformations regulated by phosphorylation and co-factors

Challenging disordered p53 transcription factor can be targeted with peptides

Targeting p53 eliminates senescent cells *in vitro* and *in vivo*

Discoveries and new concepts might enable targeting of other promising disordered proteins in ageing

Thank you!

Madl lab, Med Uni Graz

Benjamin Bourgeois

Hermann Habacher
Hansjörg Habisch

Yukti Khanna

Aneta Lenard

Anastasia Rakhimbekova

Emil Spreitzer

Sinem Usluer

Fangrong Zhang

Qishun Zhou

Pedro Sanchez Murcia lab

De Keizer lab, Utrecht

Peter de Keizer

Marjolein Baar

Burgering lab, Utrecht

Boudewijn Burgering

Maria J Rodriguez Colman

