Alzheimer's Research 2023

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Introduction

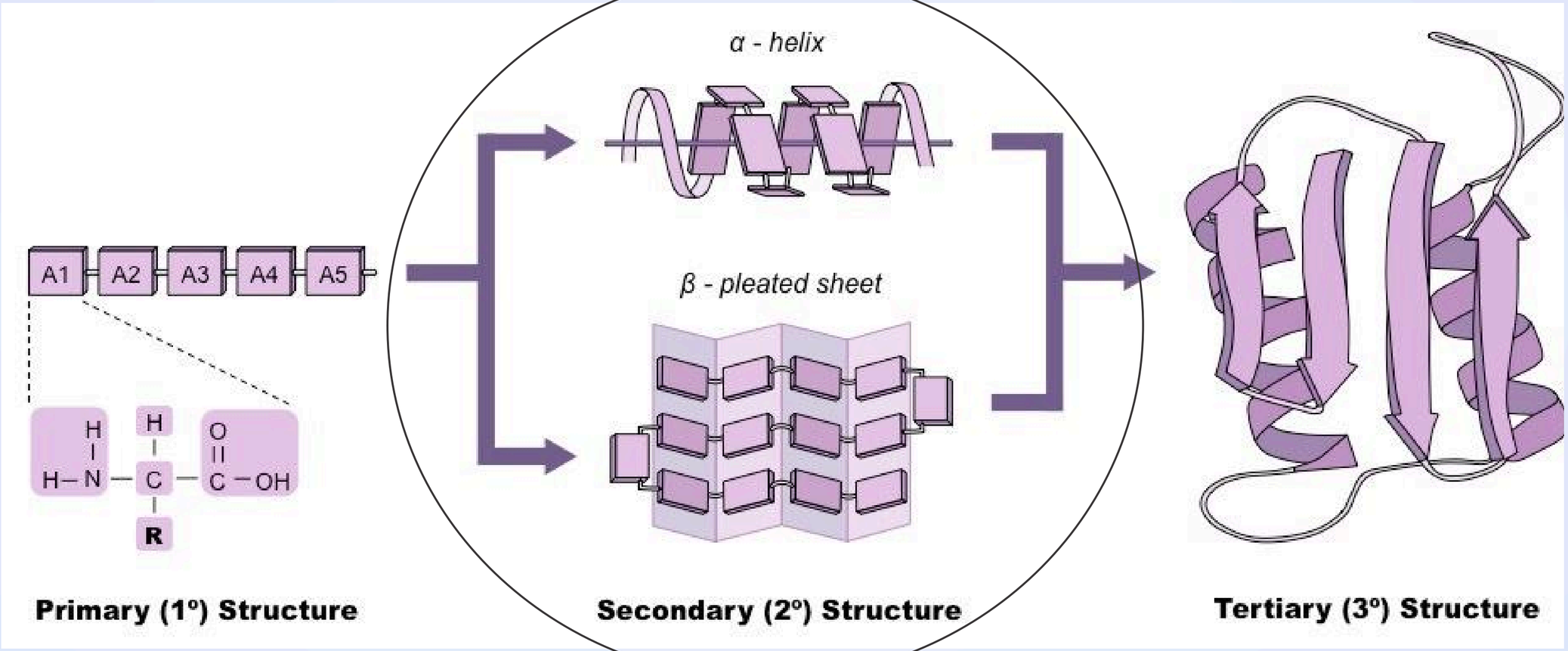
As of 2020, 5.8 million Americans were living with Alzheimer's Disease[1]

This number is expected to nearly triple to 14 million by 2060[1]

[1]Center for Disease Control and Prevention. (2020, October 26). What is alzheimer's disease? Centers for Disease Control and Prevention. Retrieved July 20, 2022, from https://www.cdc.gov/aging/aginginfo/alzheimers.htm

Protein Summary…

DNA → RNA → **PROTEINS**



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What is Alzheimer's Disease?

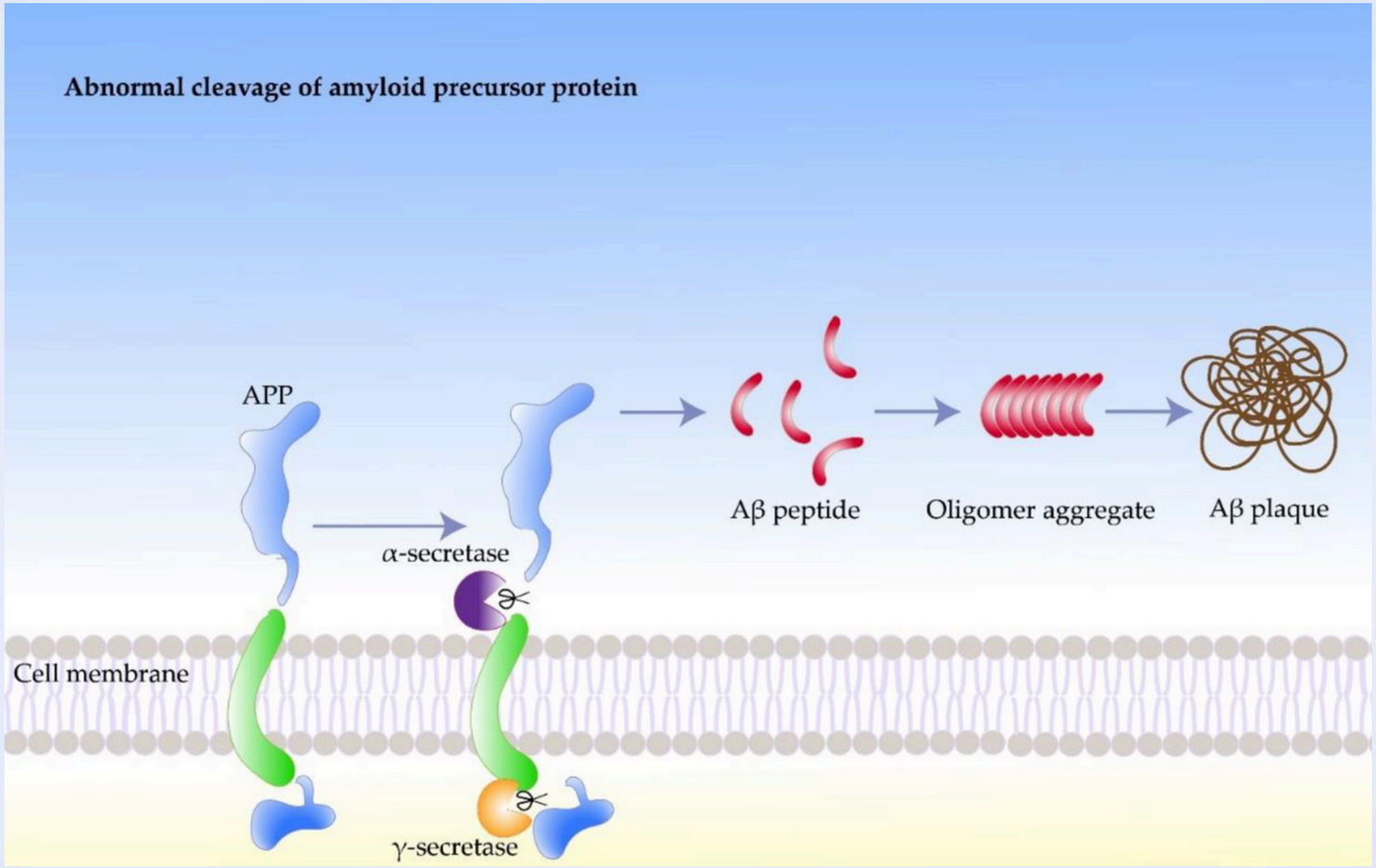
Amyloid Cascade Hypothesis[1]

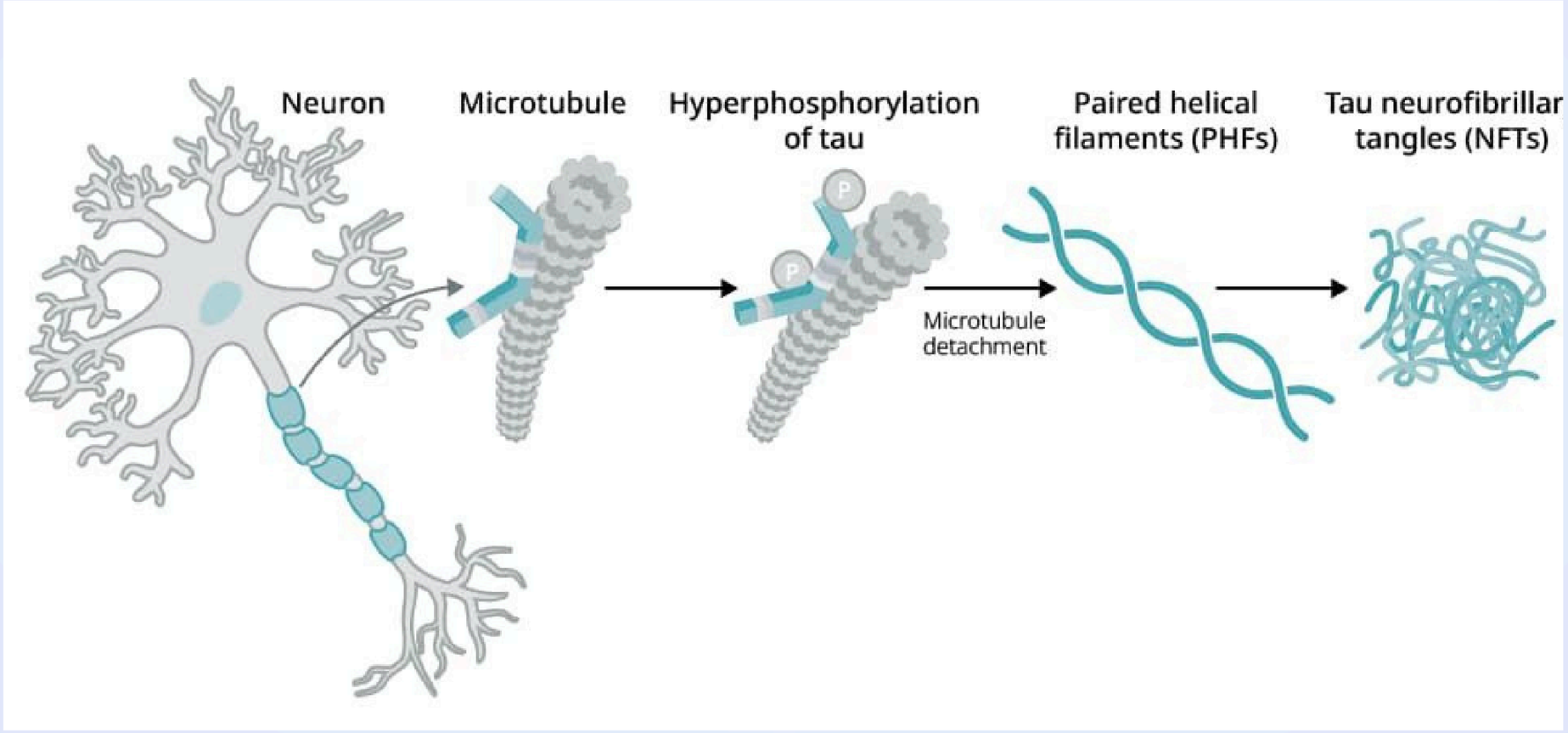
Aβ 42 → High Order Oligomers → Amyloid Plaques

TAU Hyperphosphorylation

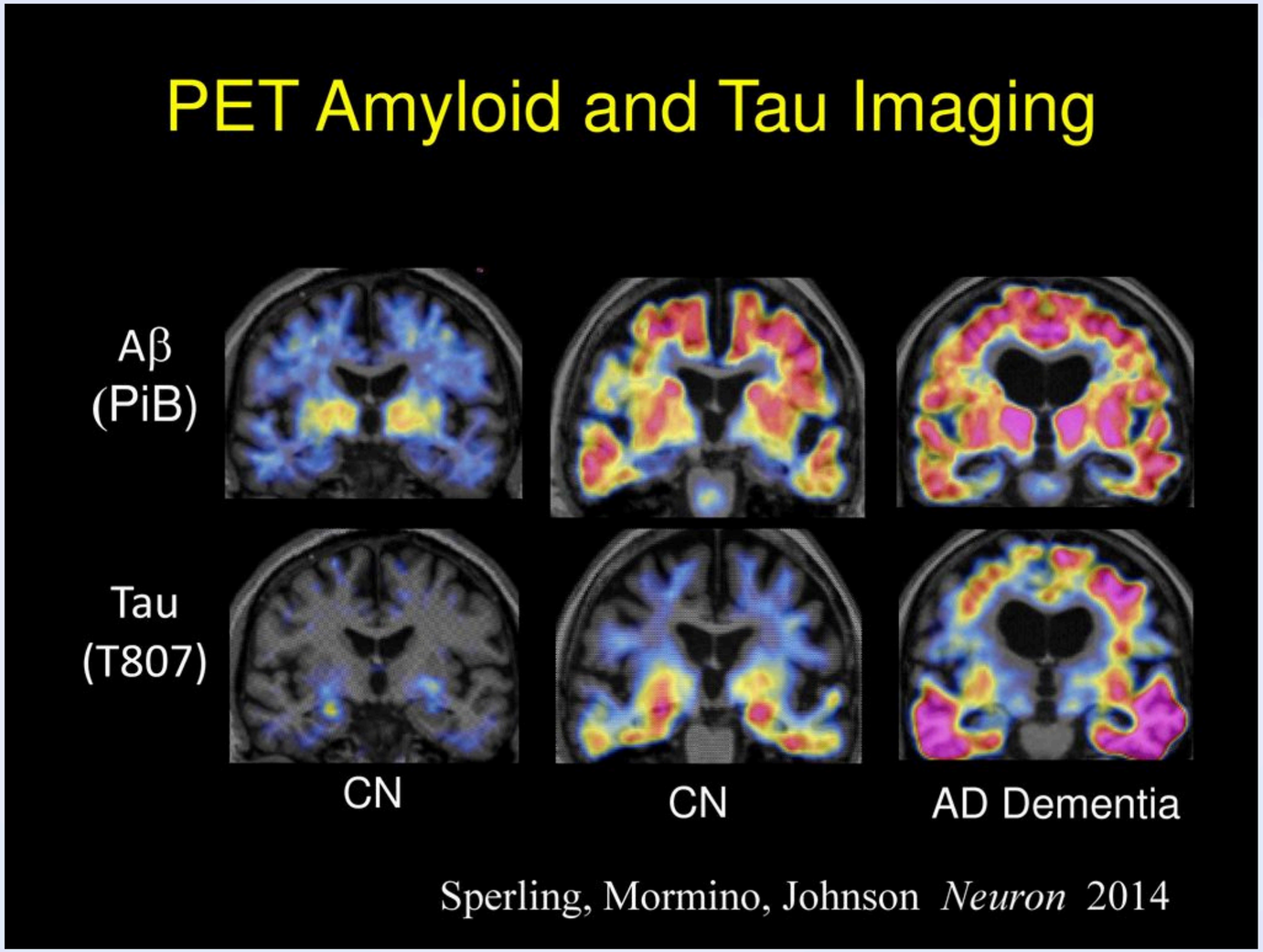
TAU → Hyperphosphorylation → NFTs

[1]Ricciarelli, R., & Fedele, E. (2017). The amyloid cascade hypothesis in alzheimer's disease: It's time to change our mind. Current neuropharmacology. Retrieved July 20, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5652035/





Tau protein aggregation assay relevance to alzheimer's disease and Tauopathies Research. Neuroscience from Technology Networks. (n.d.). https://www.technologynetworks.com/neuroscience/application-notes/tau-protein-aggregation-assay-relevance-to-alzheimers-disease-and-tauopathies-research-326535



Sperling, R., Mormino, E., & Johnson, K. (2014, November 5). The evolution of preclinical alzheimer's disease: Implications for prevention trials. Neuron. Retrieved July 20, 2022, from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4285623/

Hypothesis:

FDA approved kinase inhibitors can inhibit both the aggregation of Aβ 42 and the hyperphosphorylation of Tau.

Methodology/Results

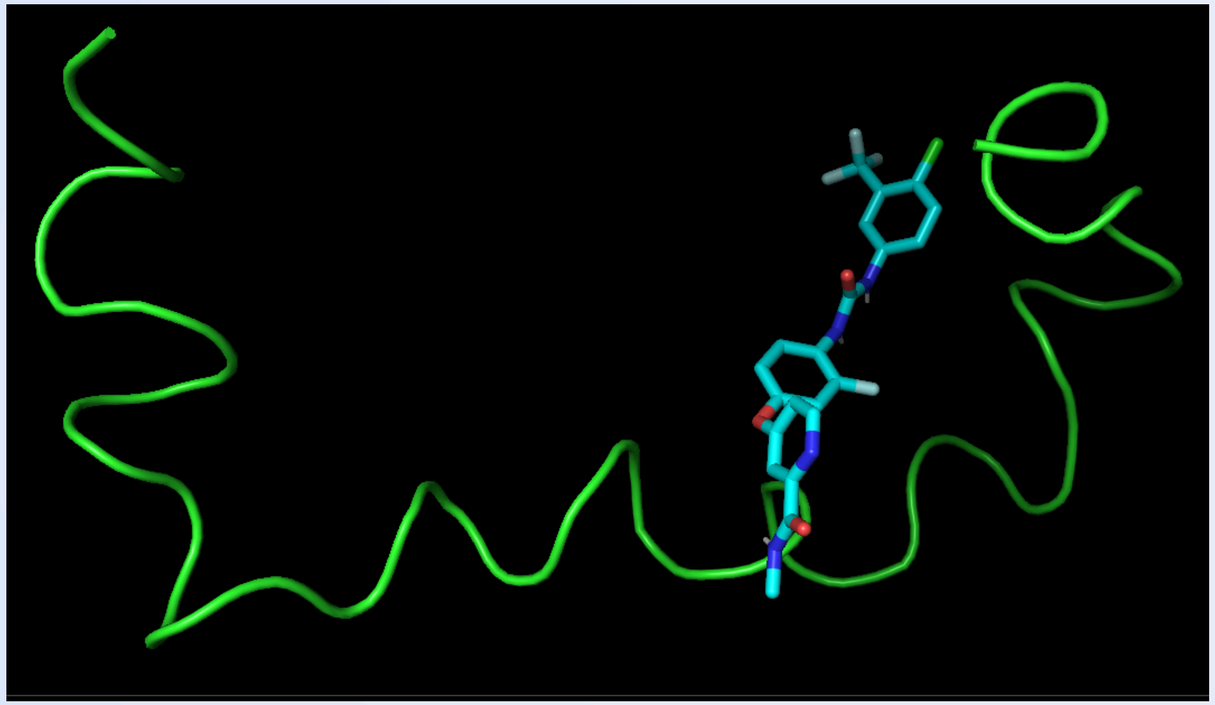

ThT Assay
PICUP and SDS-PAGE
Kinase Inhibitors and Alzheimer's Disease
Computer Modeling
Yeast
Labeling Experiments
miro

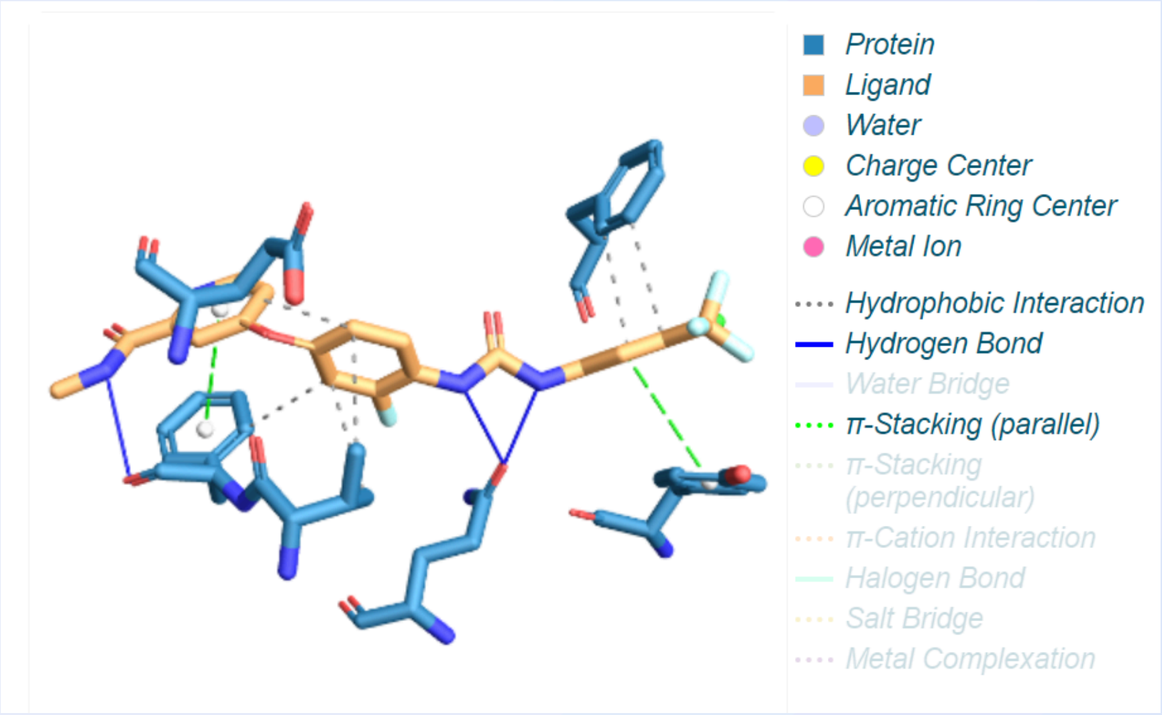
Autodock Vina

- Models Protein-ligand binding patterns
- Preliminary search for kinase inhibitors

Ligand	Average (kcal/mol)
tepotinib	-7.78375
ponatinib	-7.59325
belumosudil	-7.5445
capmatinib	-7.528
nilotinib	-7.516
umbralisib	-7.4755
imatinib	-7.469
avapritinib	-7.44675
tucatinib	-7.3985
regorafenib	-7.37825
pazopanib	-7.366

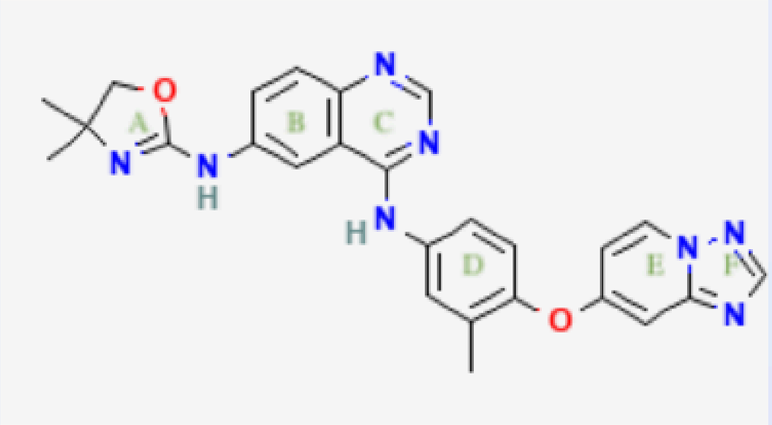
Autodock Vina





Autodock Vina

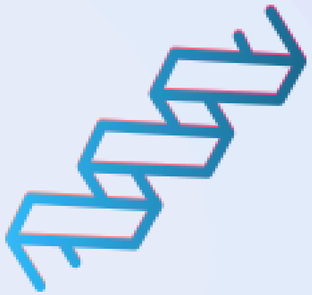
Ring A Methyl	Ring A	Ring B	Ring C	Ring D Methyl	Ring D	Ring E	Ring F
	Val18	TYR10		GLU22	ARG5	PHE19	
					PHE19		
TYR2	ASP7	TYR10	PHE4	GLU11	TYR10	PHE4	ARG5
					PHE4		ARG5
LEU17		VAL18	ARG5		ARG5	PHE19	
VAL18					PHE19	GLU22	
					GLU22		
TYR10	ASP7	TYR10	PHE4	GLU 11	PHE4	PHE4	ARG5
					TYR10	ARG5	

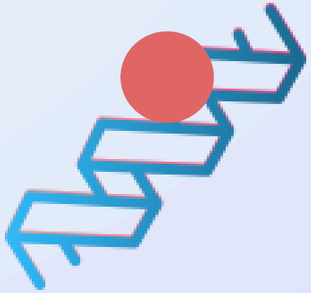


Blue	H bonds
Green	Pi stacking
Grey	Hydrophobic interations
Light Orange	Pi Cation Interactions
Green also	Pi Stacking (perpendicular)

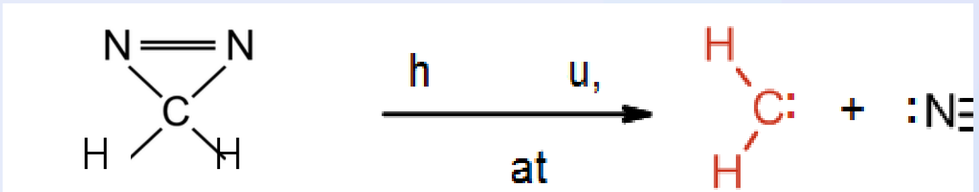
MS Labeling Experiments

1. A way to “paint” proteins
2. Also called footprinting
3. Visualize binding interactions and protein shape





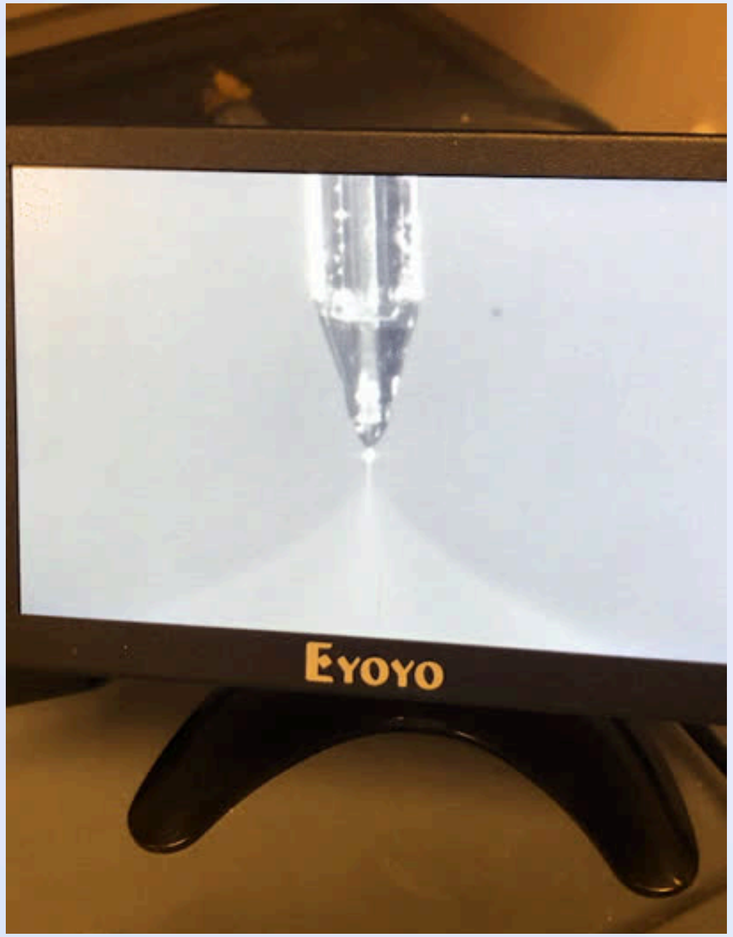
- Labeling performed in the gas phase through nanospray device

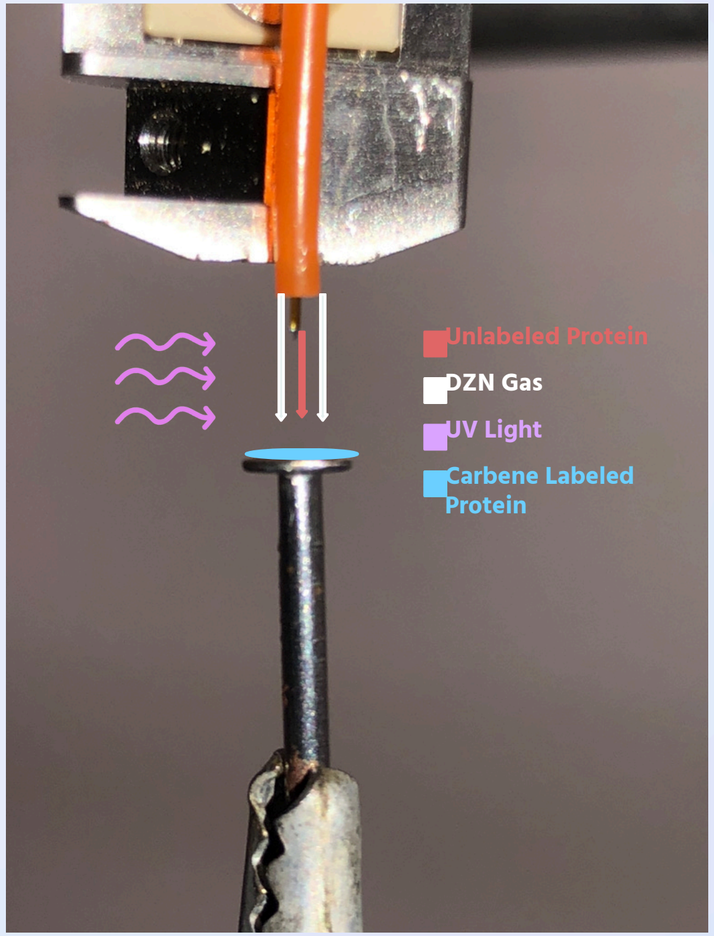


- Carbene paints protein

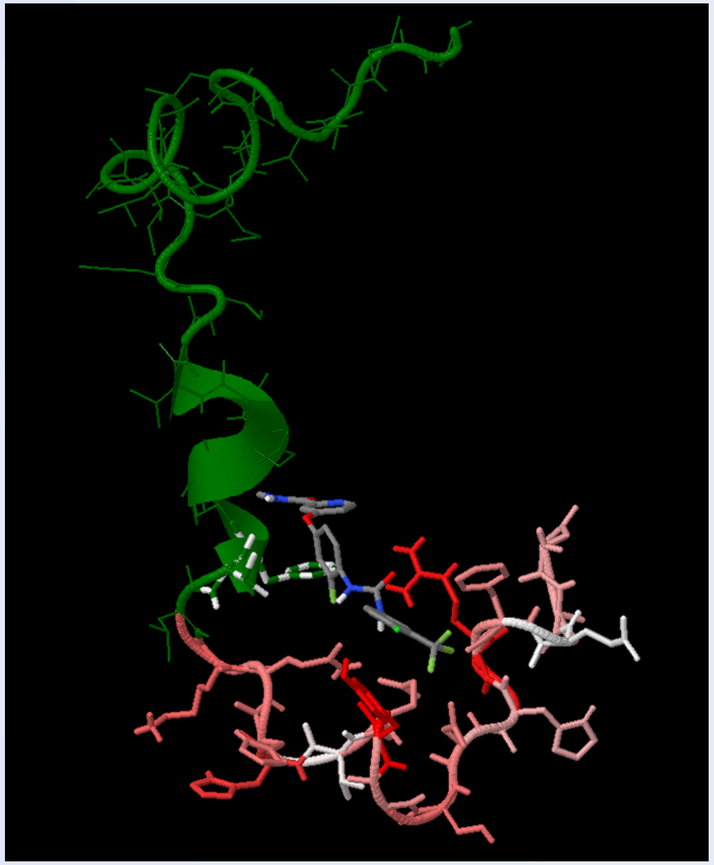
ker, M. (n.d.). Organic Chemistry II. 10.7. Additions involving cyclic intermediates | Organic Chemistry II. https://courses.lumenlearning.com/suny-potsdam-organicchemistry2/chapter/10-7-additions-involving-cyclic-intermediates/

Labeling Experiments

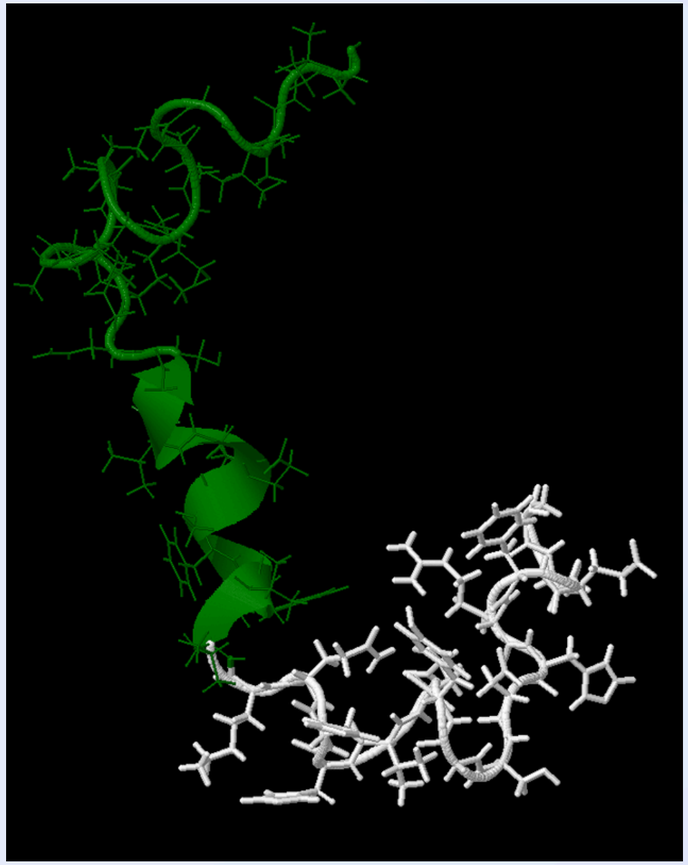




Labeled Results

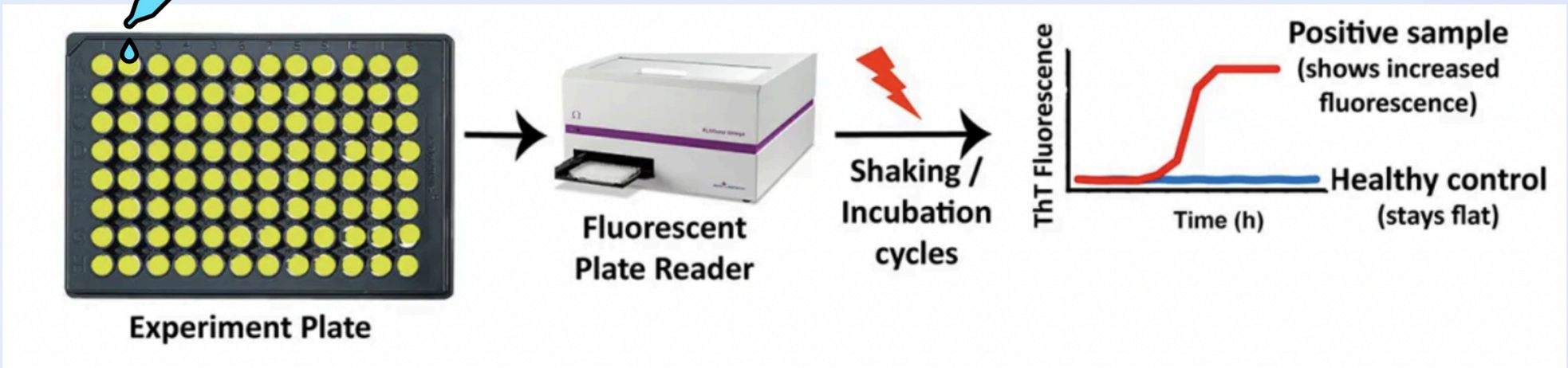


With Regorafenib



Without Regorafenib

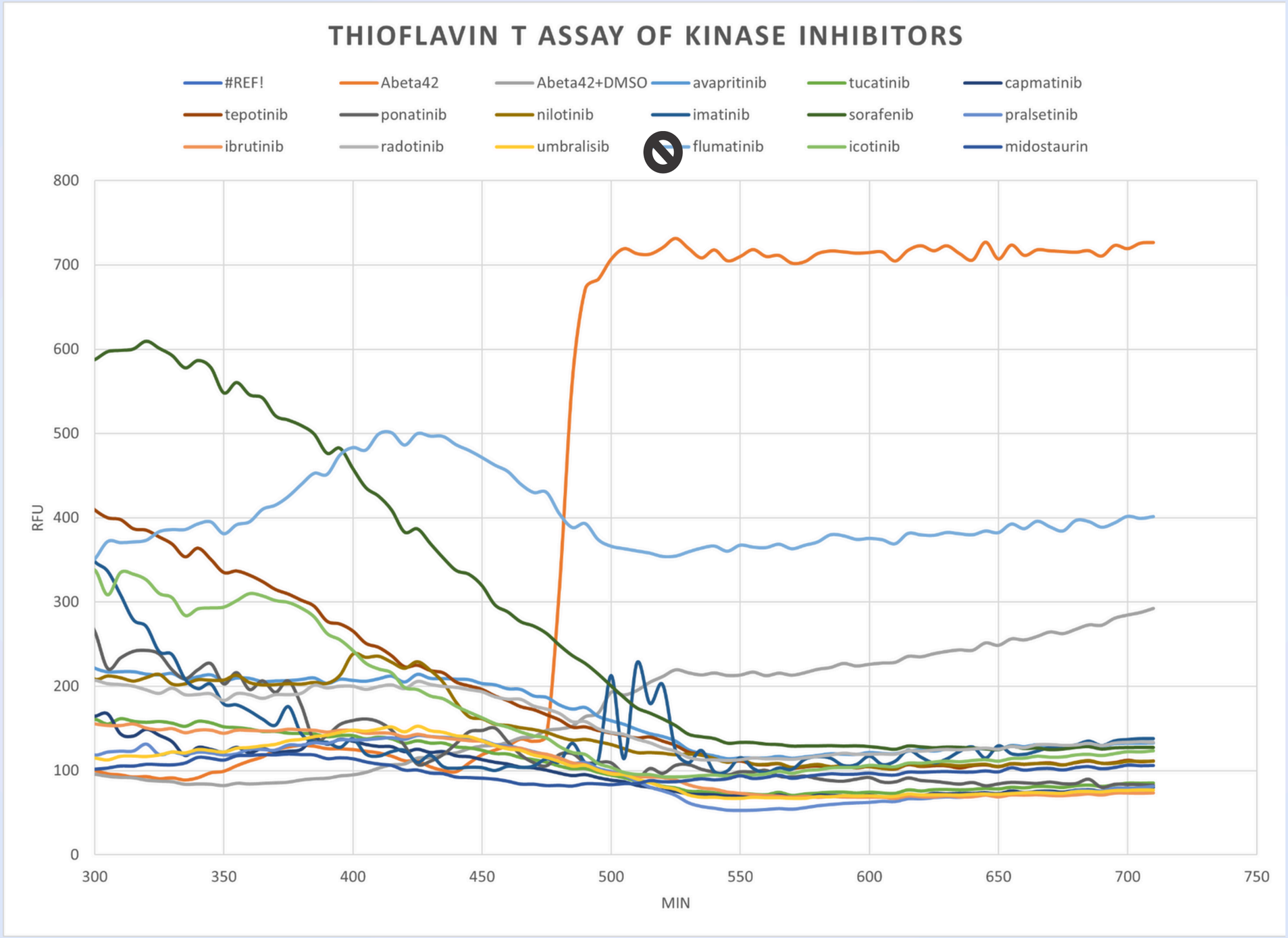
THT Explanation



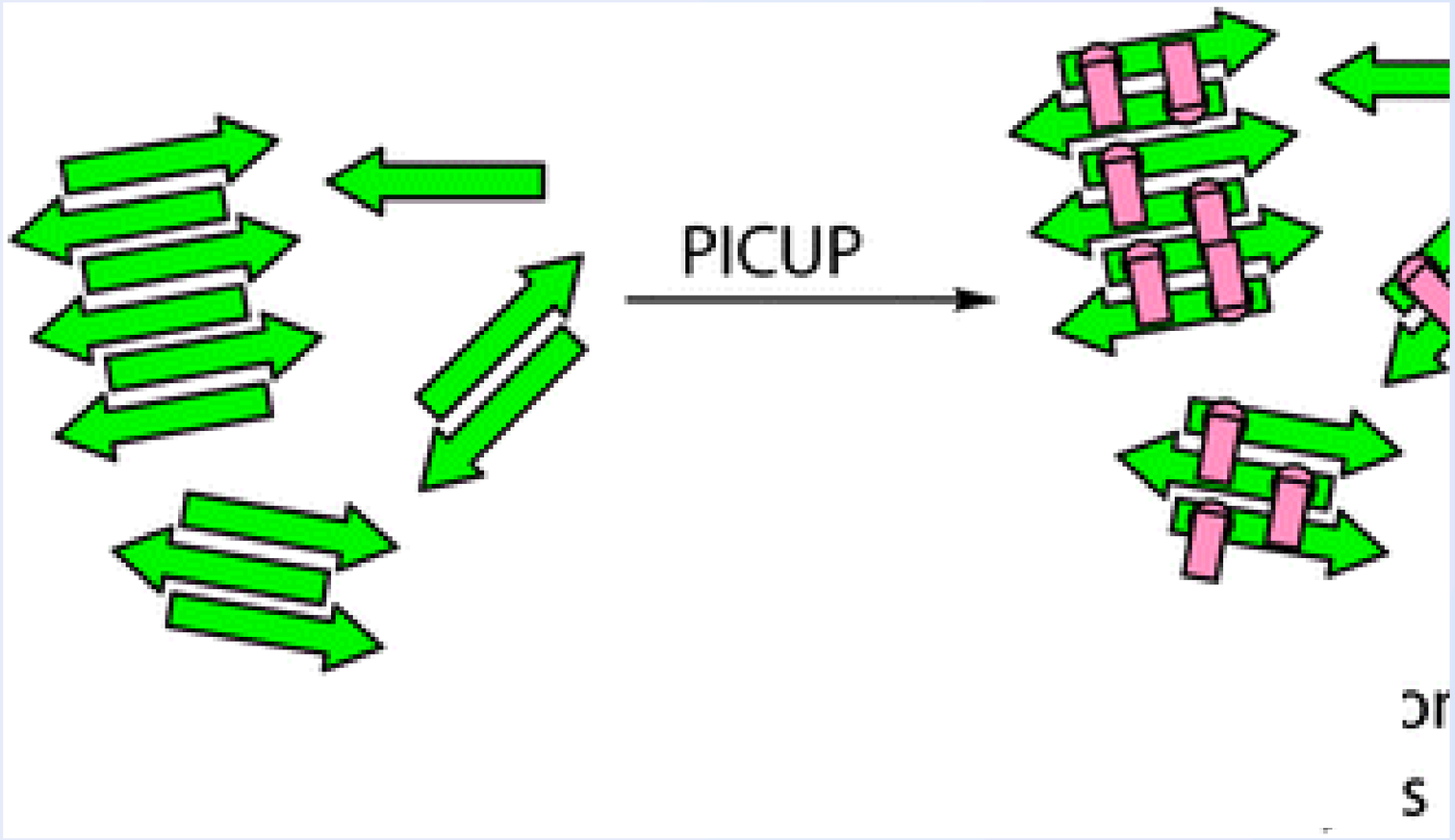
Eye care icons created by pojok d - Flaticon

Bottle icons created by Freepik - Flaticon

Protein test could lead to earlier and better diagnosis of Parkinson's (2021, December 13th, Retrieved July 6th, 2023, NUFFIELD DEPARTMENT OF CLINICAL NEUROSCIENCES Medical Sciences Division, from https://www.ndcn.ox.ac.uk/news/protein-test-could-lead-to-earlier-and-better-diagnosis-of-parkinsons


THIOFLAVIN T ASSAY OF KINASE INHIBITORS
#REF!
Abeta42
Abeta42+DMSO
avapritinib
tucatinib
capmatinib
tepotinib
ponatinib
nilotinib
imatinib
sorafenib
pralsetinib
ibrutinib
radotinib
umbralisib
flumatinib
icotinib
midostaurin
RFU
MIN
0
100
200
300
400
500
600
700
800
300
350
400
450
500
550
600
650
700
750

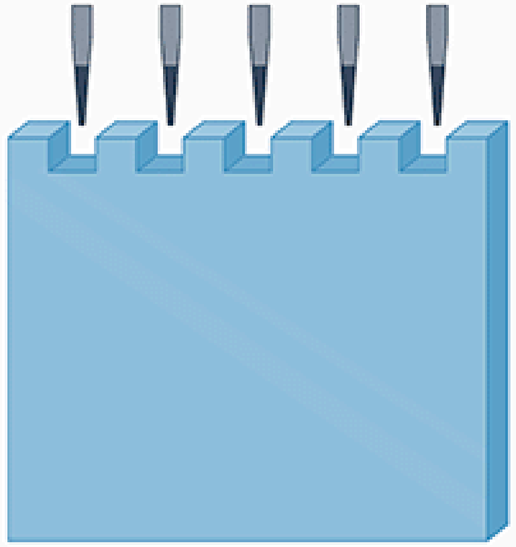
PICUP Procedure



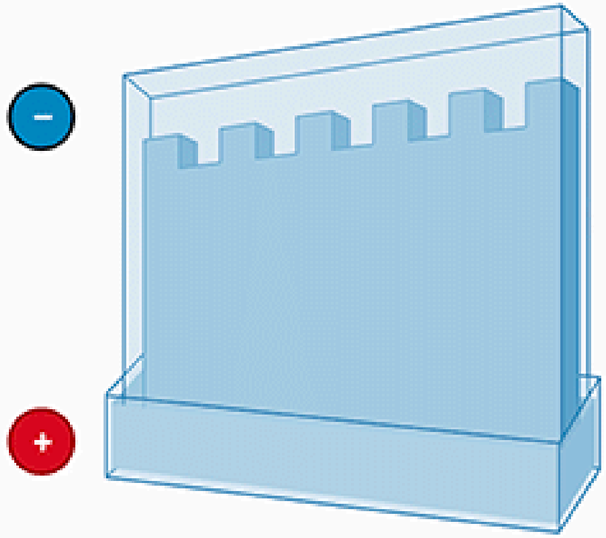
George W. Prestonab & Andrew J. Wilson (2013, February 13th) Photo-induced covalent cross-linking for the analysis of biomolecular interactions. Retrieved July 3rd, 2023, from https://pubs.rsc.org/en/content/articlehtml/2013/cs/c3cs35459h

SDS-PAGE Gels

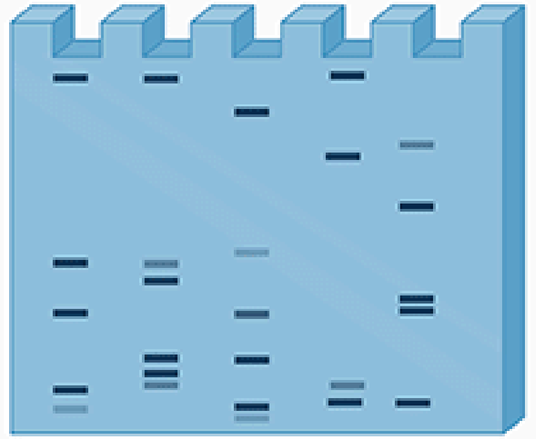
1 Load protein sample



2 Apply current

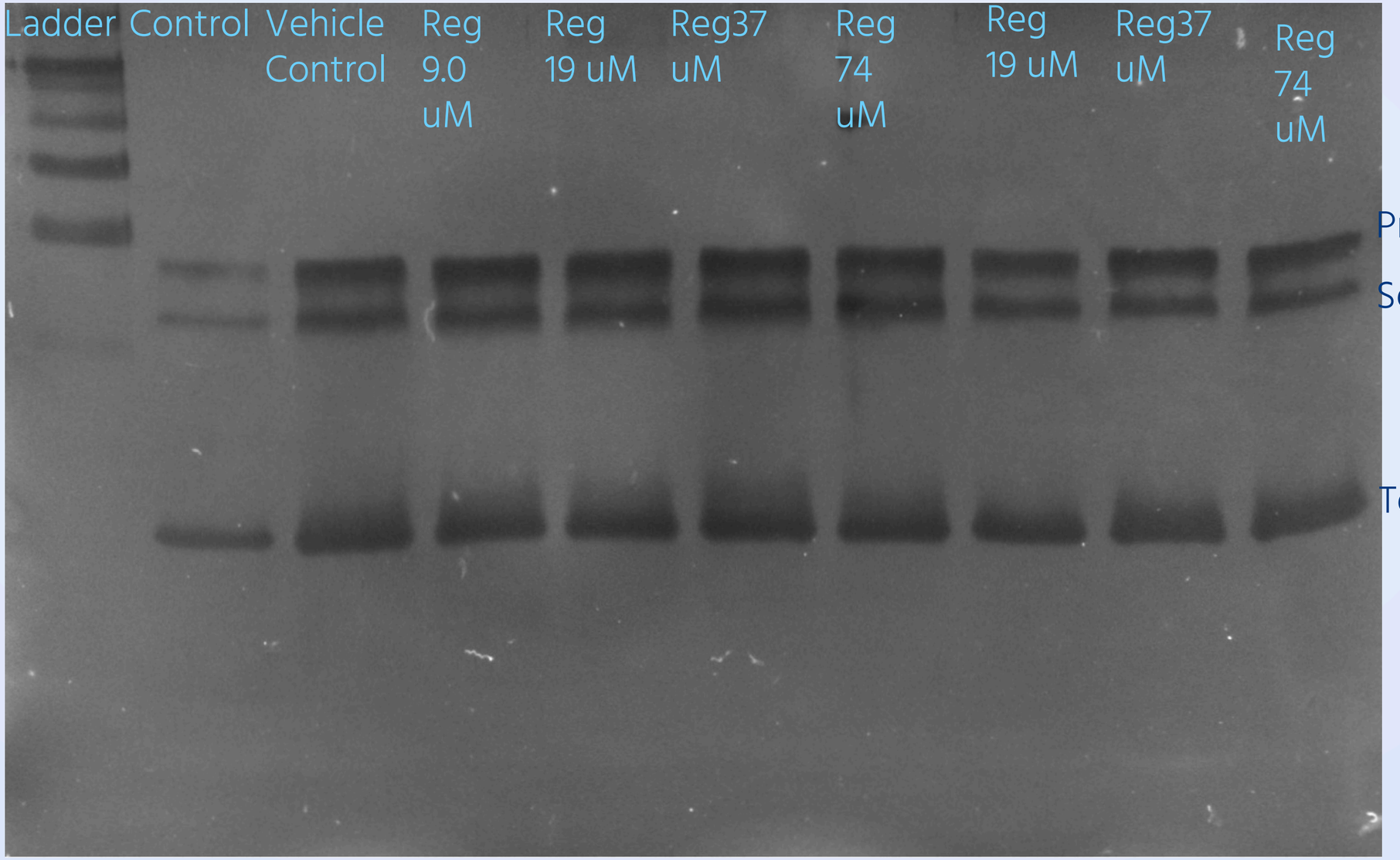


3 Analyze gel



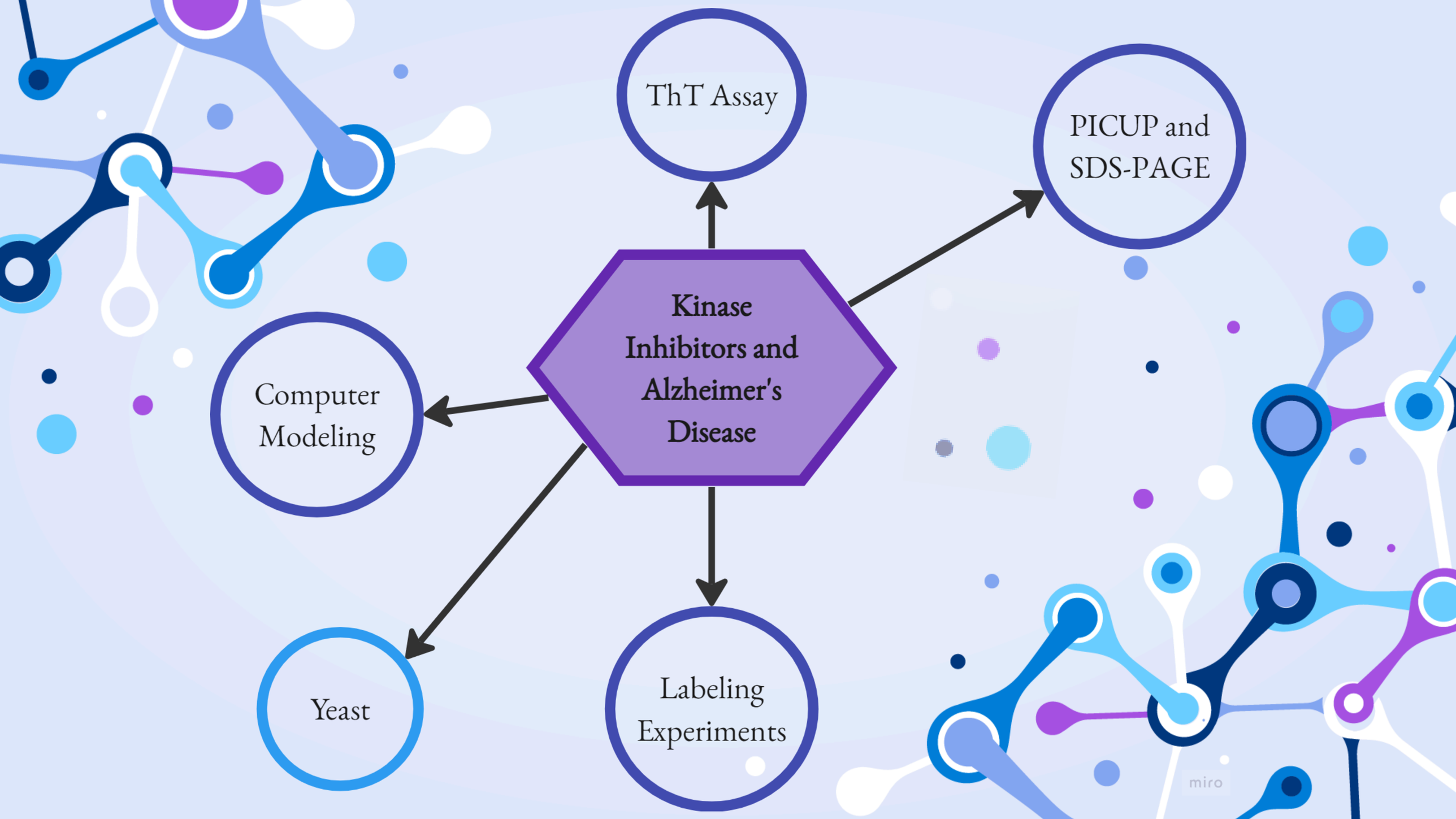
Rockland Resource Library SDS-PAGE Technique, from https://www.rockland.com/resources/sds-page-technique/

PICUP Results



Adaptations to PICUP

Bad Milli-Q Water	Staining Solutions were remade
Low rate of Aggregation in controls	Varying incubation times
Expired Regorafenib	New Regorafenib
Low Regorafenib Concentration	Increased Regorafenib Concentration
Low Protein Concentration	Increased Protein Concentration
High Protein Concentration	?
Old Peptide	New Peptide


ThT Assay
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Kinase Inhibitors and Alzheimer's Disease
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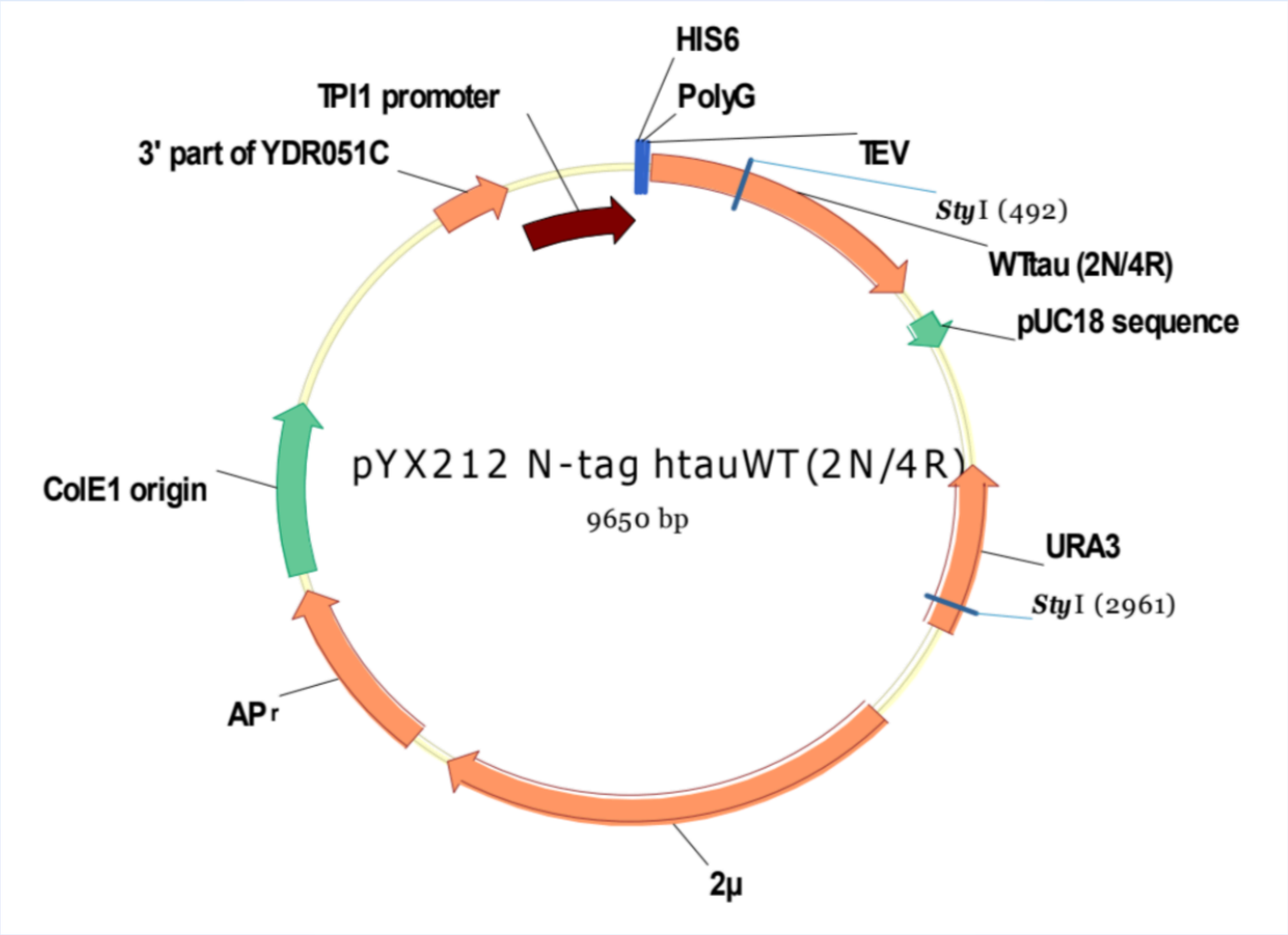
miro

Engineered Yeast Model



Graphic courtesy of Winderickx lab

Kinase Cascades

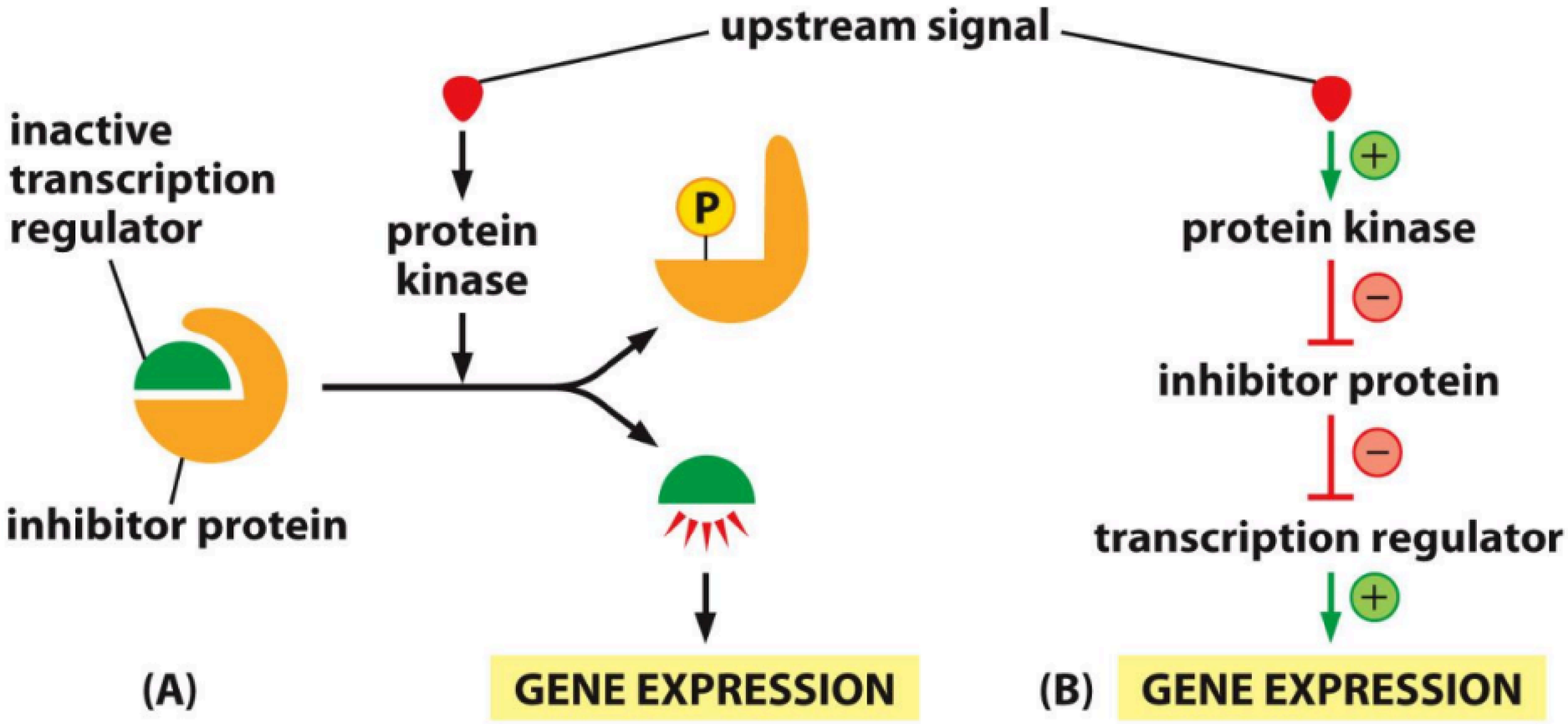


Figure 15-9 Molecular Biology of the Cell 6e (© Garland Science 2015)

Prospective Human Kinase Orthologs

Yeast has 173 kinases with a total of 282 unique phosphorylation sites[1]

HOG-1→ potential ortholog to MAPK-p38

- Protein BLAST e value = 1e-129
- 4 phosphorylation sites[2]

STE-20 → potential ortholog to PAK-1

- Protein BLAST e value = 2e-147
- 62 phosphorylation sites[3]

[1]Brinkworth, R. I., Munn, A. L., & Kobe, B. (2006). Protein kinases associated with the yeast phosphoproteome. *BMC Bioinformatics*, *7*(1). https://doi.org/10.1186/1471-2105-7-47

[2]Tyers Lab. (2023). *Saccharomyces Cerevisiae HOG1 Result Summary*. BioGRID Search for Protein Interactions, Chemical Interactions, and Genetic Interactions.https://thebiogrid.org/31384/summary/saccharomyces-cerevisiae/hog1.html

[3]Tyers Lab. (2023). *Saccharomyces Cerevisiae STE20 Result Summary*. BioGRID Search for Protein Interactions, Chemical Interactions, and Genetic Interactions.https://thebiogrid.org/36419/summary/saccharomyces-cerevisiae/ste20.html

Human Tau Kinase Orthologs

PHO-85 → ortholog to Gsk-3b[1]

- No phosphorylation sites[2]
- Deletion results in serine hyperphosphorylation at other kinases[1]

MDS-1 → ortholog to Cdk-5[1]

- 4 phosphorylation sites[3]

```
  1 MNIQSNNSPN LSNNIVSKQV YYAHPPPTID PNDPVQISFP TTEVVGHGSF
 51 GVVFATVIQE TNEKVAIKKV LQDKRFKNRE LEIMKMLSHI NIIDLKYFFY
101 ERDSQDEIYL NLILEYMPQS LYQRLRHFVH QRTPMSRLEI KYYMFQLFKS
151 LNYLHHFANV CHRDIKPQNL LVDPETWSLK LCDFGSAKQL KPTEPNVSYI
201 CSRYYRAPEL IFGATNYTNQ IDIWSSGCVM AELLLGQPMF PGESGIDQLV
251 EIIKILGTPS KQEICSMNPN YMEHKFPQIK PIPLSRVFKK EDDQTVEFLA
301 DVLKYDPLER FNALQCLCSP YFDELKLDDG KINQITTDLK LLEFDENVEL
351 GHLSPDELSS VKKKLYPKSK
```

[1]Vandebroek, T., Vanhelmont, T., Terwel, D., Borghgraef, P., Lemaire, K., Snauwaert, J., Wera, S., Van Leuven, F., & Winderickx, J. (2005). Identification and isolation of a hyperphosphorylated, conformationally changed intermediate of human protein tau expressed in yeast. *Biochemistry*, *44*(34), 11466–11475. https://doi.org/10.1021/bi0506775

[2]Tyers Lab. (2023). *Saccharomyces Cerevisiae PHO85 Result Summary*. BioGRID Search for Protein Interactions, Chemical Interactions, and Genetic Interactions. https://thebiogrid.org/36147/summary/saccharomyces-cerevisiae/pho85.html

[3]Tyers Lab. (2023). *Saccharomyces Cerevisiae Rim11 Result Summary*. BioGRID Search for Protein Interactions, Chemical Interactions, and Genetic Interactions. https://thebiogrid.org/35316/summary/saccharomyces-cerevisiae/rim11.html

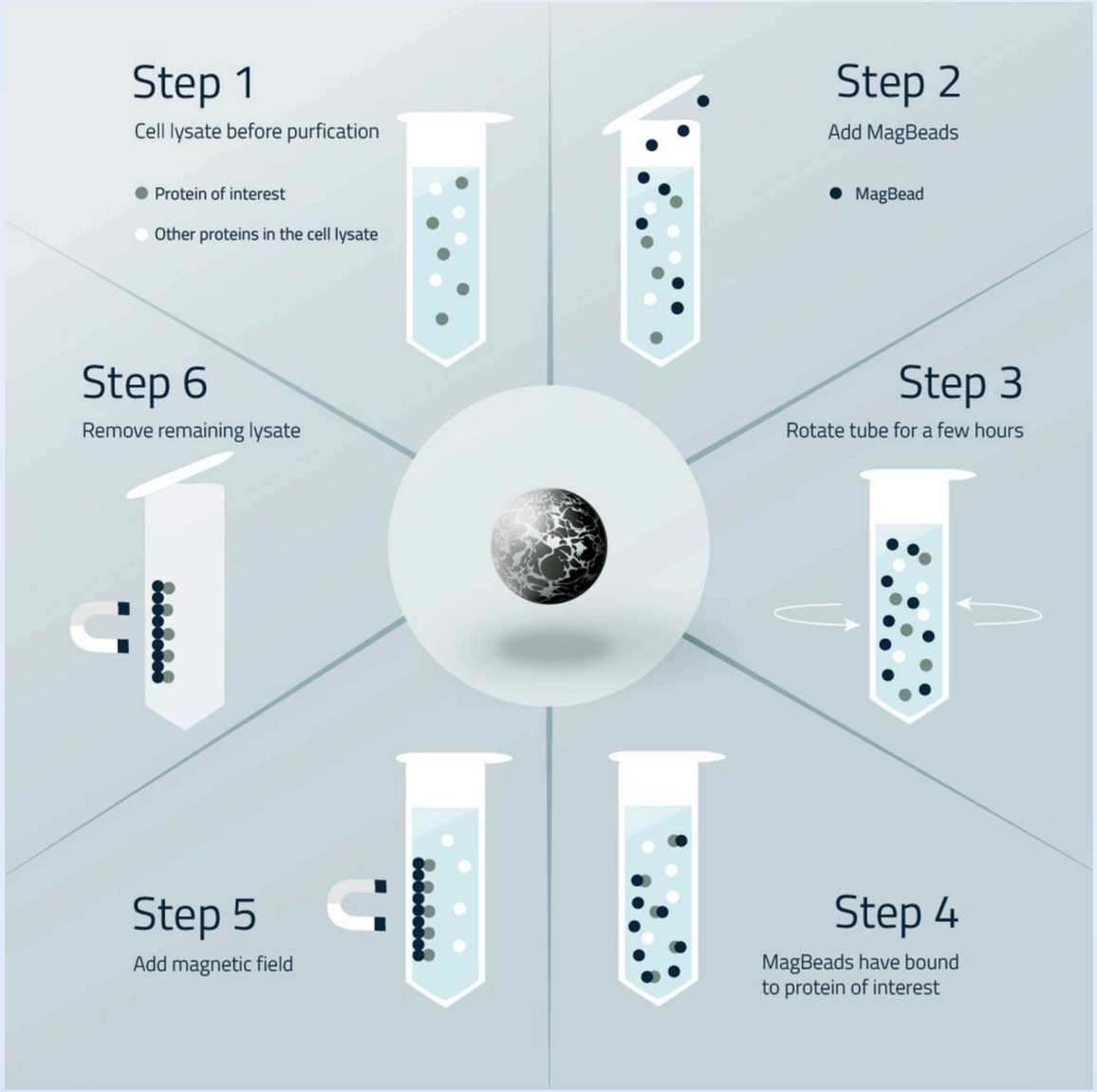
Does Regorafenib affect kinase activity responsible for Tau pathology by preventing phosphorylation?

Tau phosphorylation

1. Determine if engineered yeast can grow in the presence of Regorafenib
2. Purify Tau from yeast grown in drug and control conditions[1]
3. Develop method for phosphopeptide enrichment
4. Conduct relative quantification of phosphorylated Tau

[1]Winderickx, April 2020

Phosphopeptide Enrichment



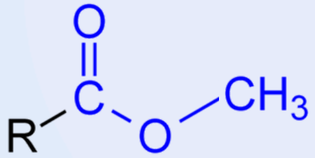
Cube Biotech https://cube-biotech.com/media/ce/22/c6/1656666760/Magbead%20Wo

Method Development

Trypsin Digest

←

Convert acidic tails to methyl esters



←

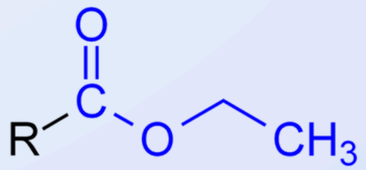
Phosphopeptide enrichment

↙

Measure on mass spec

↖

Convert acidic tails to ethyl esters



←

Phosphopeptide enrichment

←

Trypsin Digest

Method Development Results

Conducting ethyl esterification after phosphopeptide enrichment improved yield by approximately **20%**

Discussion

Relevance

- Developing new Methods
  - And Refining others
- Repurposing Medications
  - Saves Time and Money
- Address both Camp’s Problems
  - Kinase Inhibitors address Tau
  - Drug that stop aggregation

The Next Step...

- Further analysis of high scoring kinase inhibitors
  - Circular Dichroism
  - PICUP/SDS-Page
- Continue work with TAU isolation and characterization
- Assess the role of methionine oxidation on kinase activation

Acknowledgments

1. Robert Van Dyk and Family
2. Shannon Summer Research Institute
3. Biology Department
    a. Dr. Potter
4. Brandon Bate
5. Joris Winderickx