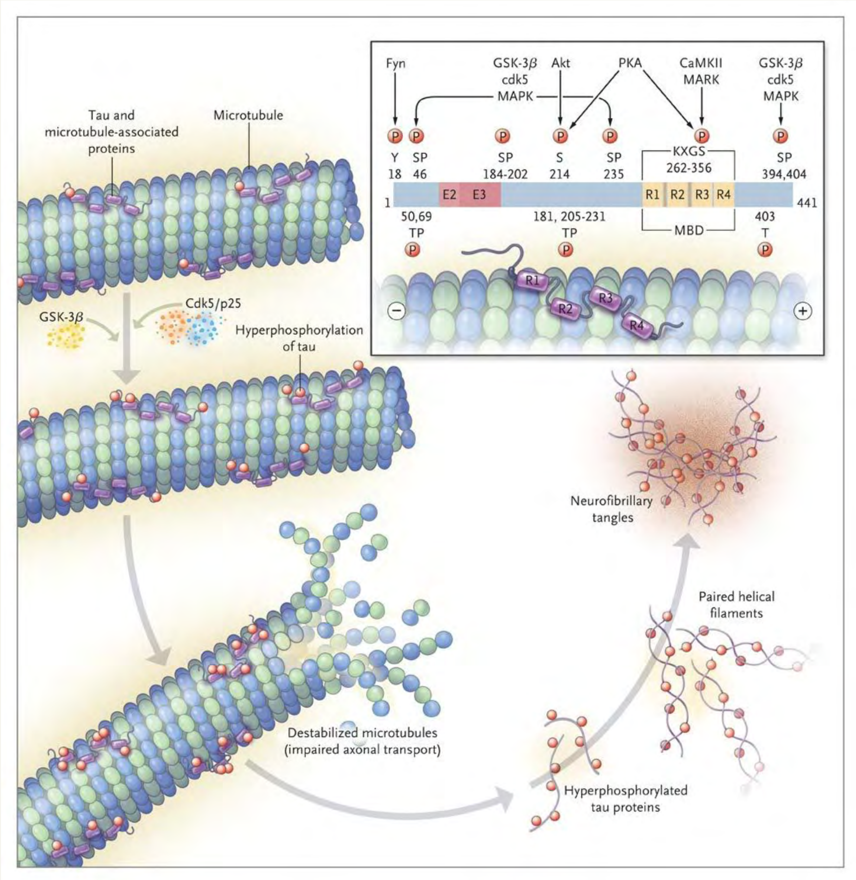
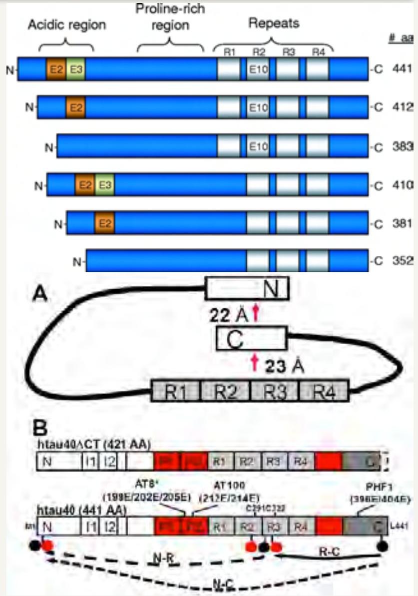
Synthesis of post-translationally modified functional domains of human MAP Tau

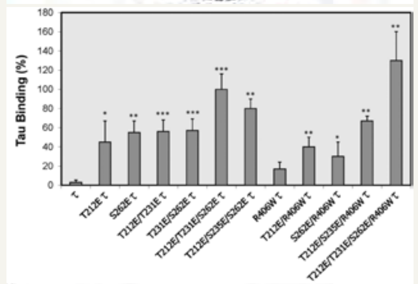


PRS Group Seminar – Lukas Wanka – July 1, 2011

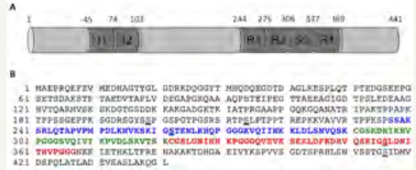
Contents

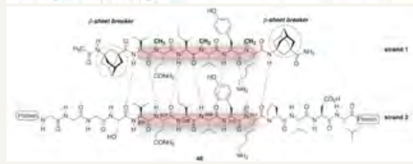


Acidic region
Proline-rich region
Repeats
aa
441
412
383
410
381
352
A
22 Å
23 Å
R1
R2
R3
R4
B
htau40ΔCT (421 AA)
htau40 (441 AA)
AT8*
AT100
PHF1
N-R
R-C
N-C



Tau Binding (%)

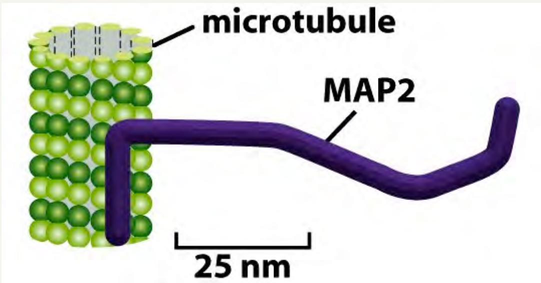


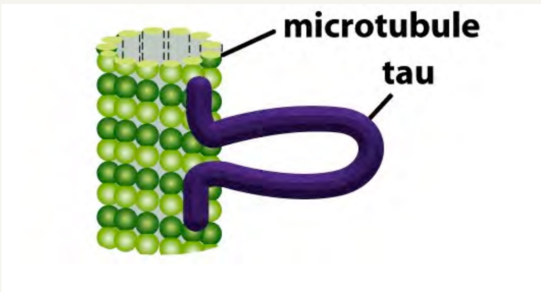


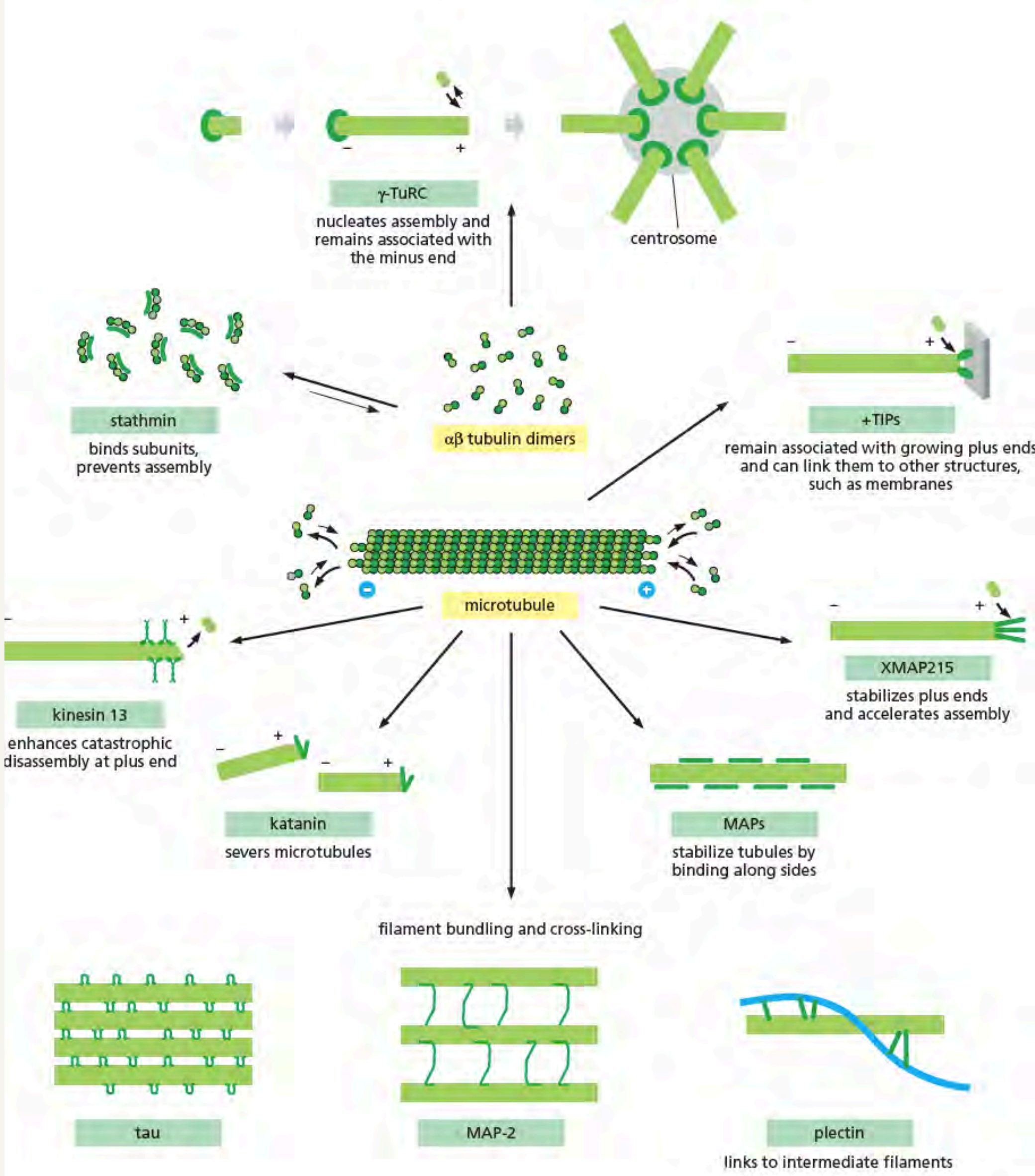
1. Human Tau protein – warrant of apprehension

• Microtubules are a highly dynamic part of the cytoskeleton
• Over 100 microtubue associated proteins, many of which probably not yet recognized
• Tau: Axon
• MAP2: Cell body and dendrites

Figure 16-40 Molecular Biology of the Cell 5/e (© Garland Science 2008)



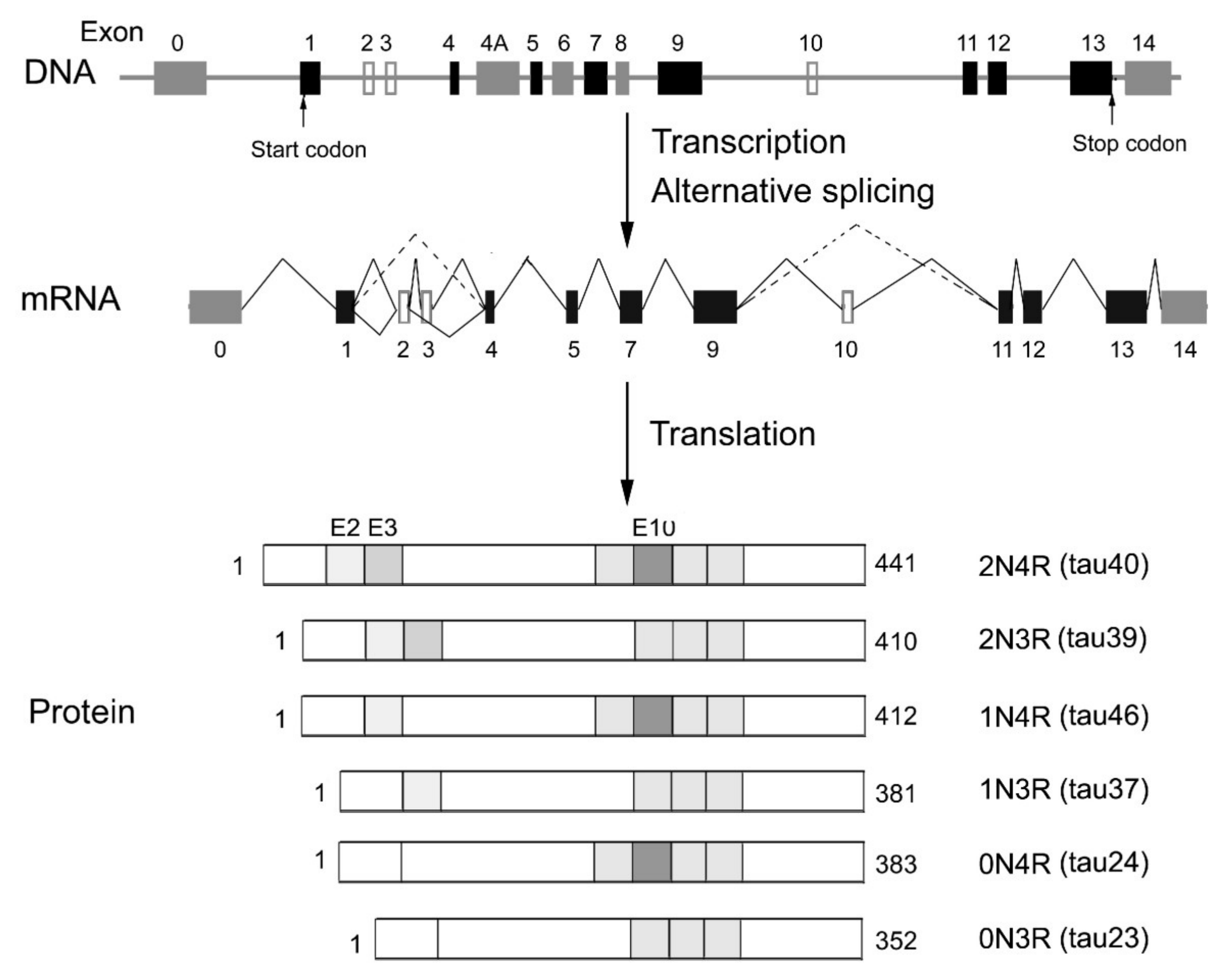




Molecular Biology of the Cell / Bruce Alberts et al., 5th ed.

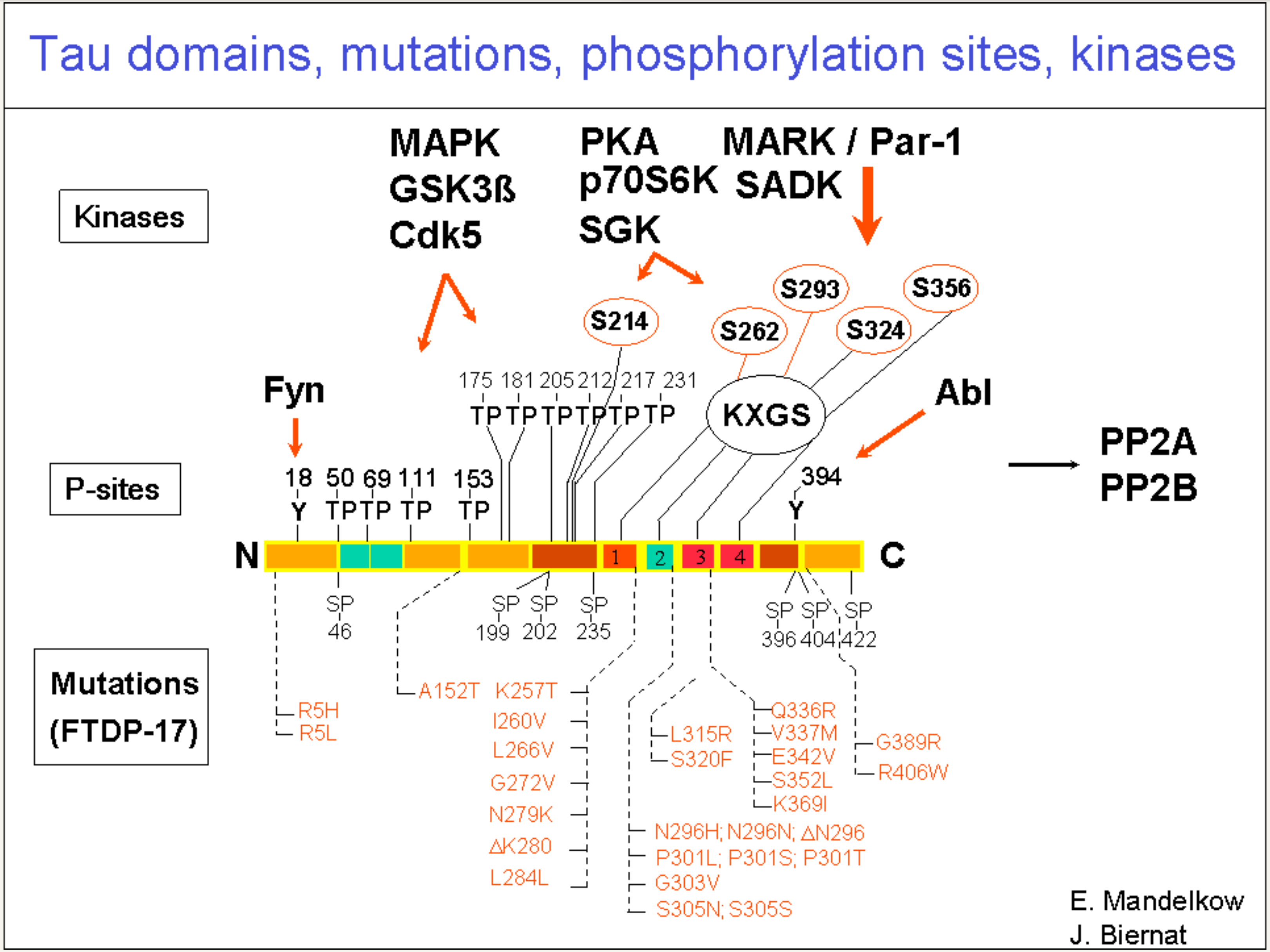
1. Human Tau protein – warrant of apprehension

Synthesis of τ domains



- Six tau isoforms expressed in adult human brain
- Single gene (chromosome 17q21)
- Alternative mRNA splicing
- presence or absence of N-terminal inserts (29 Xaa)
- One of four microtubule-binding repeats (R2) may be missing
- 3R Tau: Fetal humans
- Adult humans: [3R] = [4R]
- 4R Tau: stabilizes better
- Rodents: Only 4R tau

Phosphorylation negatively regulates tau-microtubule-interaction



In PHF, Tau is abnormally hyperphosphorylated

Recombinant proteins

- Isolation of the encoding DNA fragment from the right organism
- Insertion into an appropriate Vector
- Transfection of the vector into host organism
- Cultivation of the host organism (cloning)
- Isolation of the recombinant protein

Good: Fast, large proteins available, mature mutagenesis biochemistry

Bad: Truncation, small scale (research lab), No post-translational modifications in routine host organisms such as *e. coli*

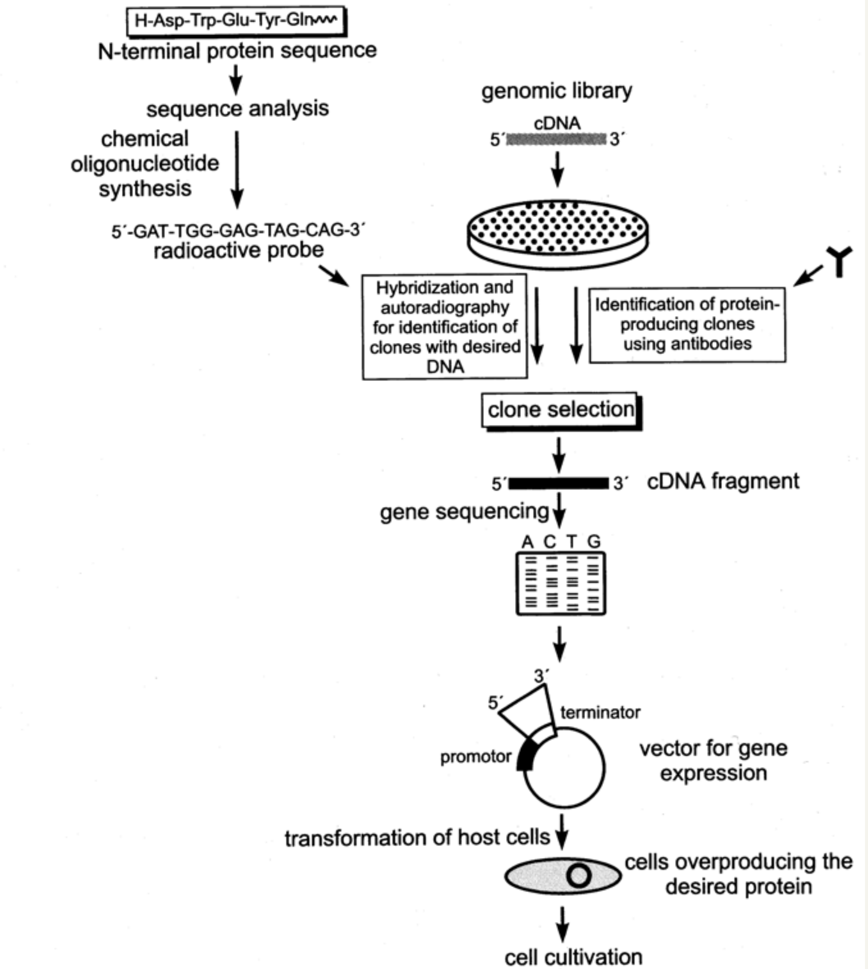
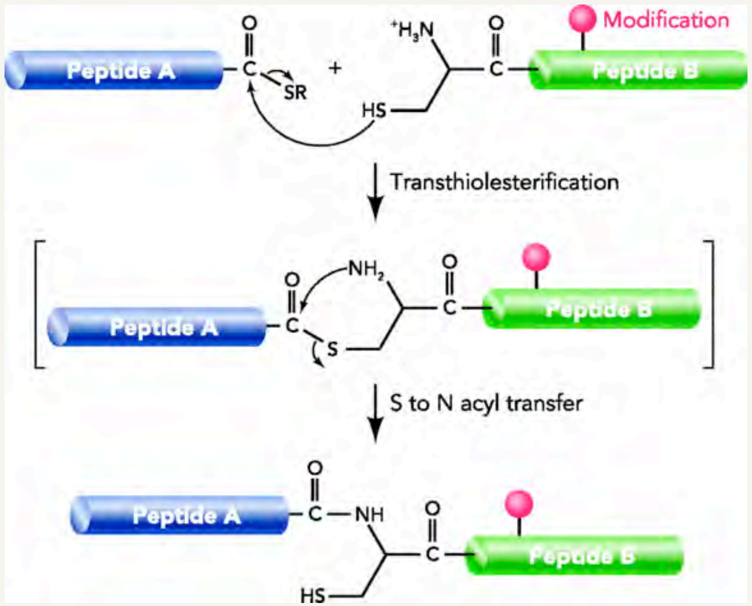


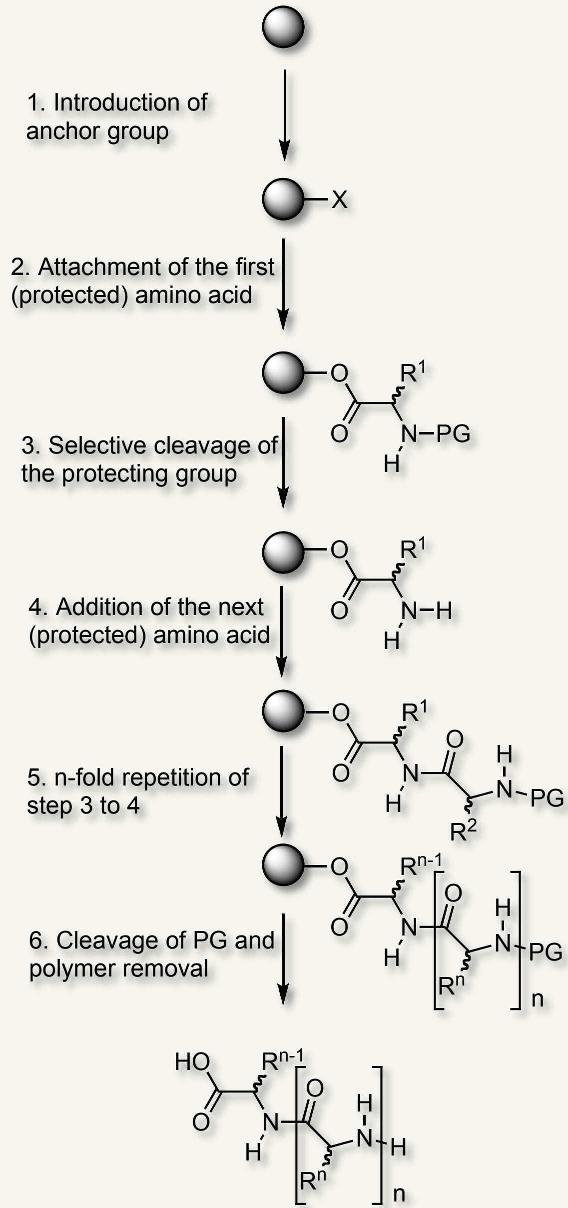
Fig. 4.41 Procedure to obtain to a recombinant protein from the gene.

Blohm, D.; Bollschweiler, C.; Hillen, H. *Angew. Chem. Int. Edit.* ***1988,*** *27,* 207-225.

Chemical peptide synthesis: SPPS stronghold

- Routine job for 30–50-mer peptides
- Unnatural Xaas easily to be incorporated
- Longer proteins: Native Chemical Ligation (NCL)



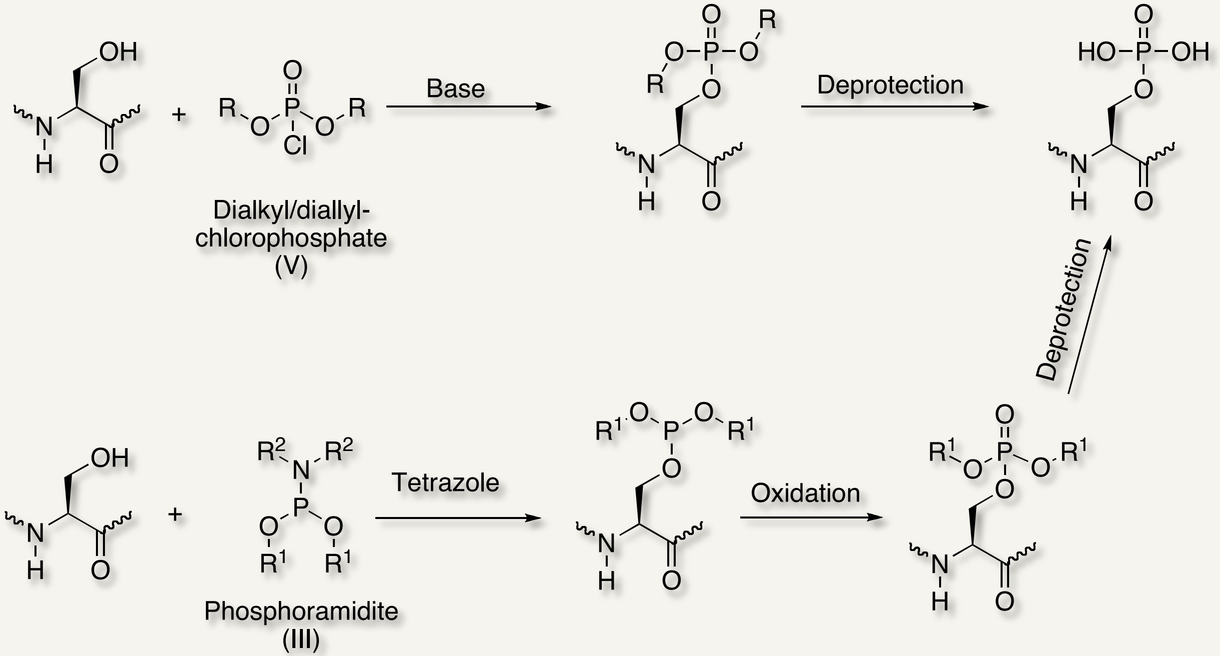


Good: Large scale possible, post-translational modifications
Bad: Laborious for longer sequences, HPLC workup

(1) Merrifield, R. B. *J. Am. Chem. Soc.* ***1963,*** *85,* 2149-2154. (2) Svobodova, J.; Cabrele, C. *ChemBioChem* ***2006,*** *7,* 1164-1168. (3) Dawson, P. E.; Muir, T. W.; Clark-Lewis, I.; Kent, S. B. *Science* ***1994, 266, 776-779.***

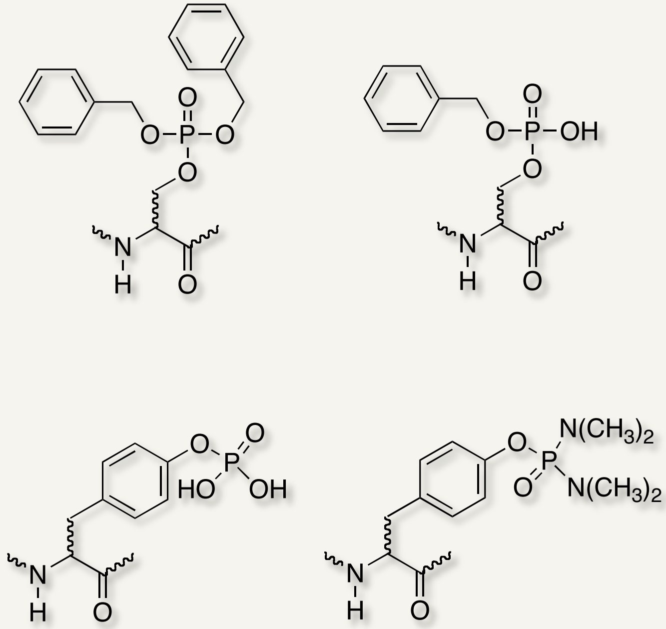
2. Access to model compounds

Post-Assembly phosphorylation:



• Needs selectively cleavable PGs
• Oxidation troublesome with Met and Cys sidechains

Building Blocks for SPPS



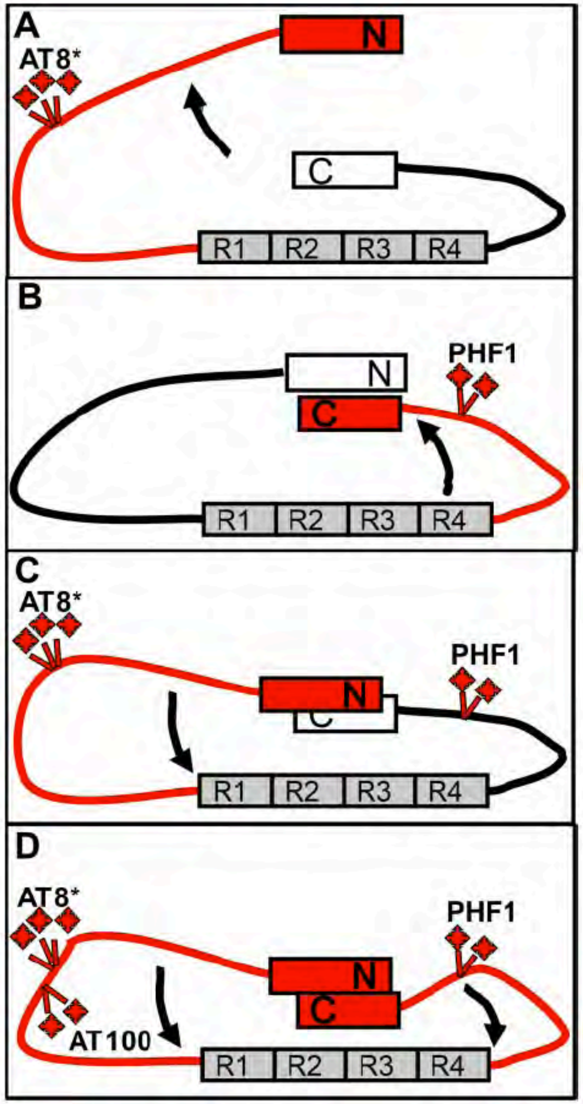
• Risk of β-elimination
• Not compatible with PyBOP
• Not compatible with carbodiimides

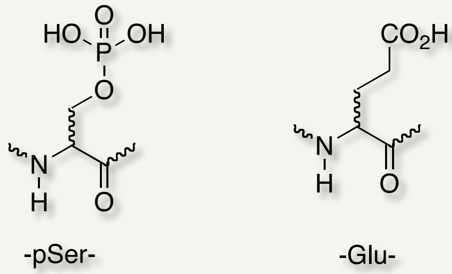
Of course, enzymatic phosphorylation is an alternative

(1) Toth, G. K.; Kele, Z.; Varadi, G. *Curr. Org. Chem.* **2007,** *11,* 409-426. (2) Attard, T.; O'Brien-Simpson, N.; Reynolds, E. *Int. J. Pept. Res. Ther.* **2007,** *13,* 447-468. (3) Vorherr, T.; Bannwarth, W. *Bioorg. Med. Chem. Lett.* **1995,** *5,* 2661-2664.

3. Insights gained using pseudophosphorylation

Pseudophosphorylation: Replaces pSer by Glu



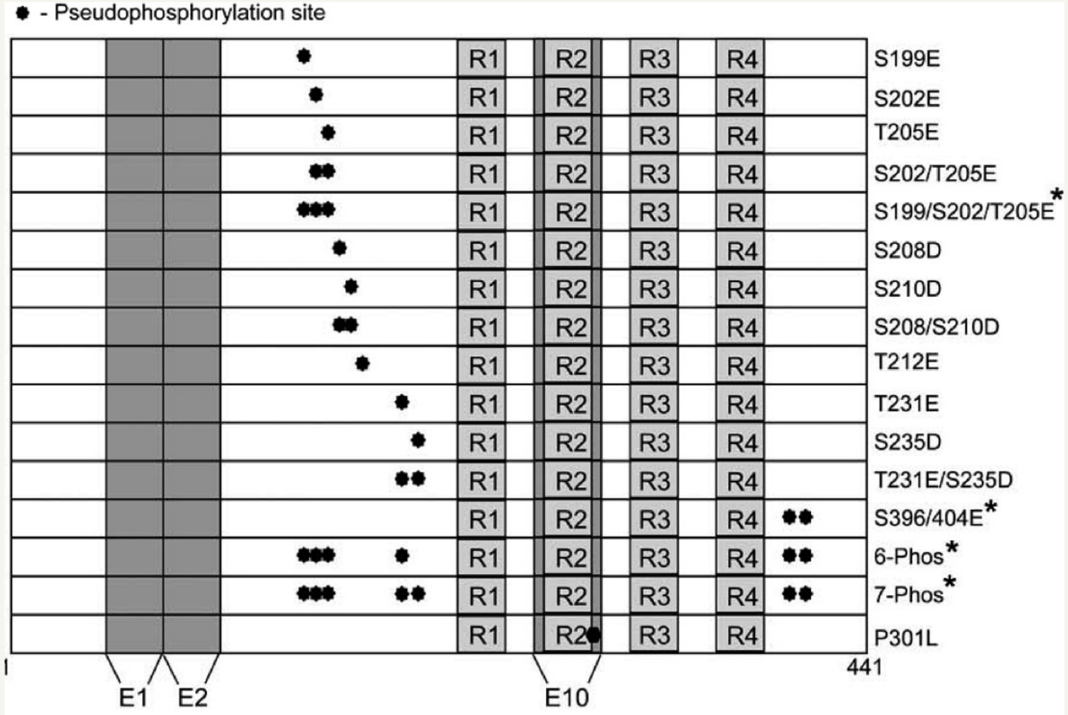


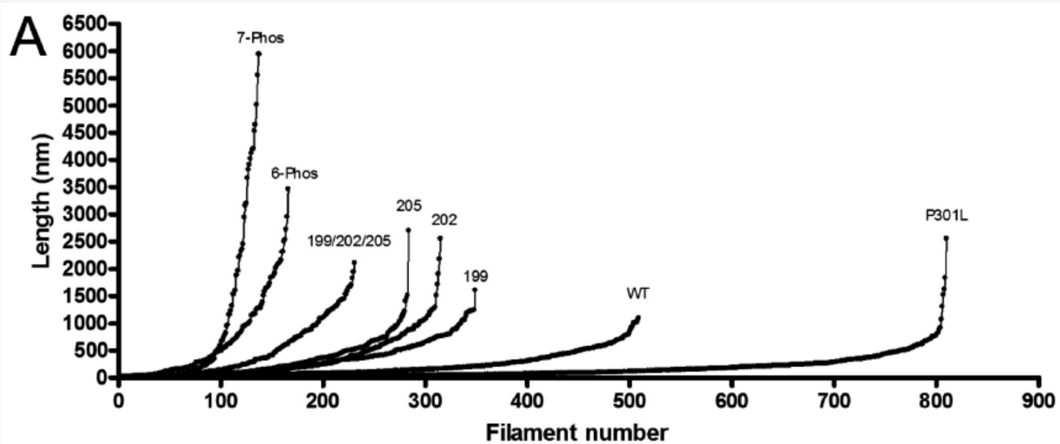
- Alters Tau conformation as monitored by FRET distance measurements
  - AT8: S199E, S202E, T205E: loosens paperclip
  - PHF1: S396E, S404E: C-terminus moves away from MTBD
  - AT8 + PHF1 pseudophosphorylation: Compacts paperclip; N-terminus approaches the MTBD
  - AT8, PHF1 and AT100 epitopes pseudophosphorylated: even more compact paperclip

Compaction forms pathological characteristics typical for AD

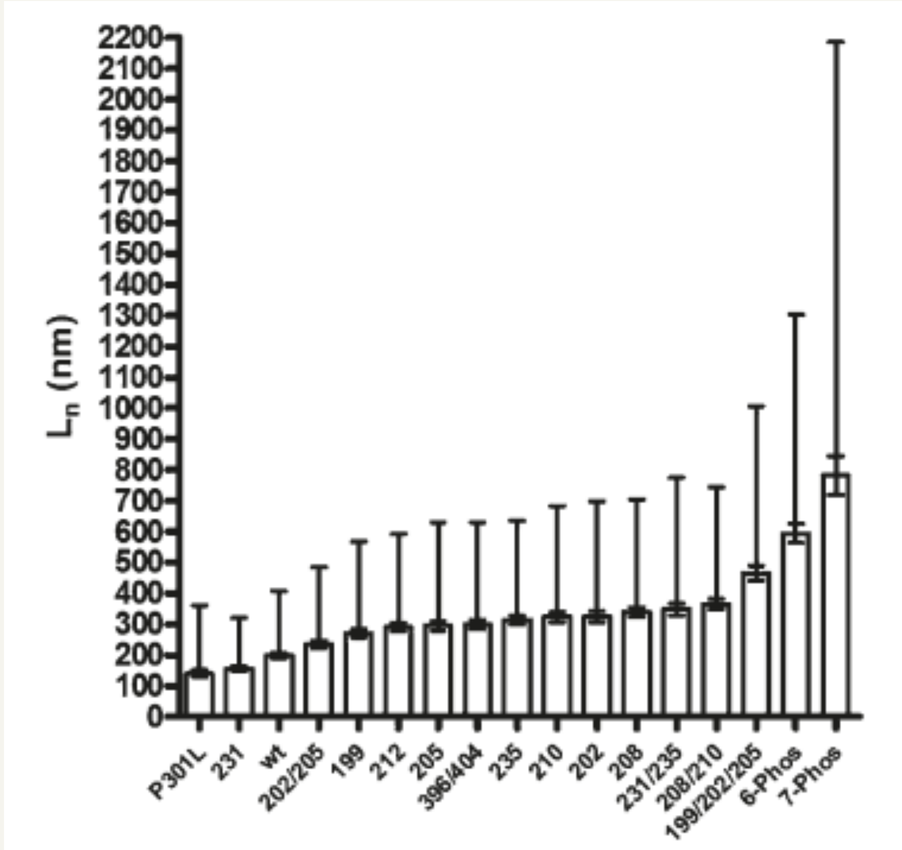
Jeganathan, S.; Hascher, A.; Chinnathambi, S.; Biernat, J.; Mandelkow, E.-M.; Mandelkow, E. *J. Biol. Chem.* **2008,** *283,* 32066-32076.

3. Insights gained using pseudophosphorylation





Pseudophosphorylated mutants form longer tangles (TEM)



Pseudophosphorylation alters Tau aggregation behavior

Sun, Q.; Gamblin, T. C. *Biochemistry* **2009,** *48,* 6002-6011.

3. Insights gained using pseudophosphorylation

Pseudophosphorylation of tau at Thr^{212}, Thr^{231}, and Ser^{262} combined causes neurodegeneration

Pseudophosphorylated Tau binds more strongly to normal Tau (enhanced "sequestration")

TUNEL staining: detects apoptosis
- Nicks in DNA are equipped with a labeled molecule by terminal deoxynucleotidyl transferase

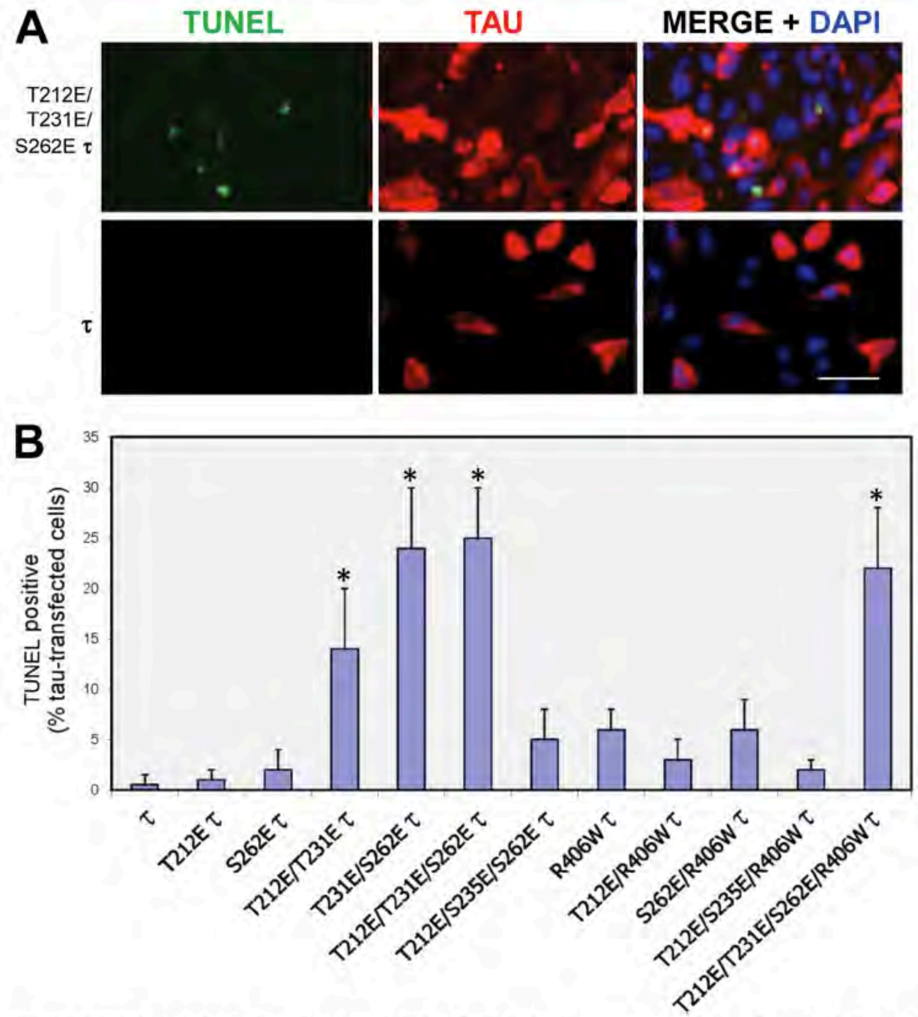


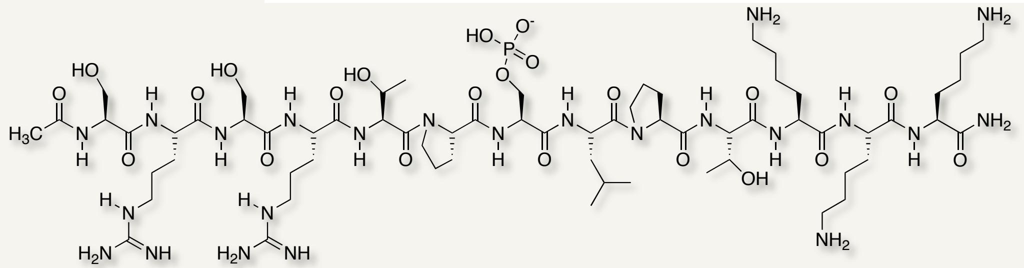
FIGURE 6. **TUNEL staining in Tau-transfected CHO cells.** *A*, CHO cells were transiently transfected with Tau and R406W Tau, and these Tau proteins pseudophosphorylated at various sites. After 48 h, the cells were double labeled with 134d (Tau) and TUNEL. Cells were counterstained with DAPI to visualize the nuclei. *B*, quantitation of cells double stained for Tau and TUNEL. The Tau construct used in each case is indicated. Approximately 300 cells were counted per Tau construct. T212E/T231E Tau, T231E/S262E Tau, T212E/T231E/S262E Tau, and T212E/T231E/S262E/R406W Tau induced apoptosis in the transfected cells. *Bar*, 50 μm. *Error bars*, SD. *, $p < 0.05$.

Alonso, A. D.; Di Clerico, J.; Li, B.; Corbo, C. P.; Alaniz, M. E.; Grundke-Iqbal, I.; Iqbal, K. *J. Biol. Chem.* **2010,** *285,* 30851-30860.

4. Chemical syntheses to date

Sequence of the longest human Tau isoform

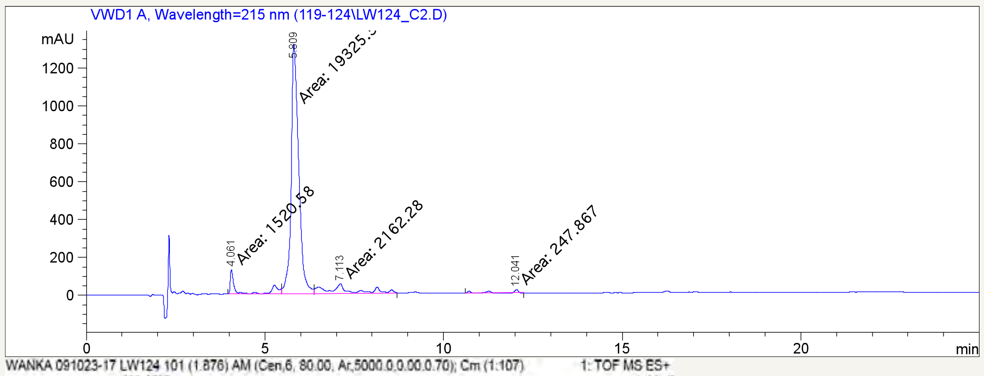
```
  1 MAEPRQEFEV MEDHAGTYGL GDRKDQGGYT MHQDQEGDTD AGLKESPLQT PTEDGSEEPG
 61 SETSDAKSTP TAEDVTAPLV DEGAPGKQAA AQPHTEIPEG TTAEEAGIGD TPSLEDEAAG
121 HVTQARMVSK SKDGTGSDDK KAKGADGKTK IATPRGAAPP GQKGQANATR IPAKTPPAPK
181 TPPSSGEPPK SGDRSGYSSP GSPGTPGSRS RTPSLPTPPT REPKKVAVVR TPPKSPSSAK
241 SRLQTAPVPM PDLKNVKSKI GSTENLKHQP GGGKVQIINK KLDLSNVQSK CGSKDNIKHV
301 PGGGSVQIVY KPVDLSKVTS KCGSLGNIHH KPGGGQVEVK SEKLDFKDRV QSKIGSLDNI
361 THVPGGGNKK IETHKLTFRE NAKAKTDHGA EIVYKSPVVS GDTSPRHLSN VSSTGSIDMV
421 DSPQLATLAD EVSASLAKQG L
```

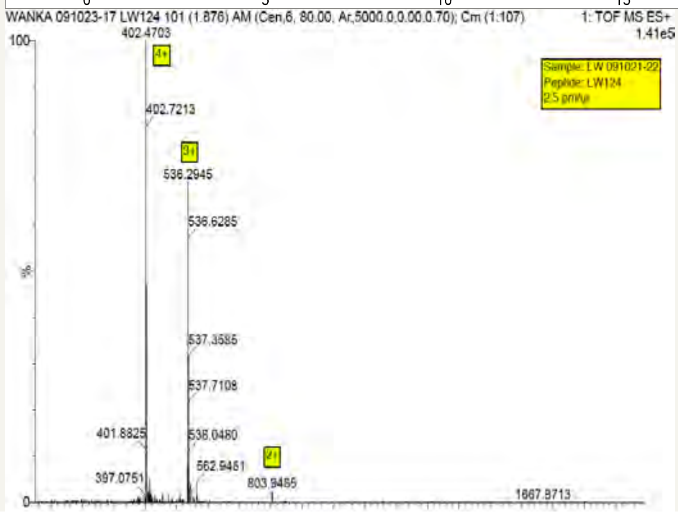


Neutral peptide:
Chemical Formula: $C_{65}H_{120}N_{23}O_{22}P$
Exact Mass: 1605.87159
Molecular Weight: 1606.76296

Pentakis-TFA salt:
Chemical Formula: $C_{75}H_{125}F_{15}N_{23}O_{32}P$
Exact Mass: 2175.83591
Molecular Weight: 2176.87971

pSer-Monoanion
Chemical Formula: $C_{65}H_{119}N_{23}O_{22}P^-$
Exact Mass: 1604.86431
Molecular Weight: 1605.75557





14 human Tau-derived 13/14-mer (phospho) peptides, here: Ac-SRSRTPpSLPTKKK-NH_2

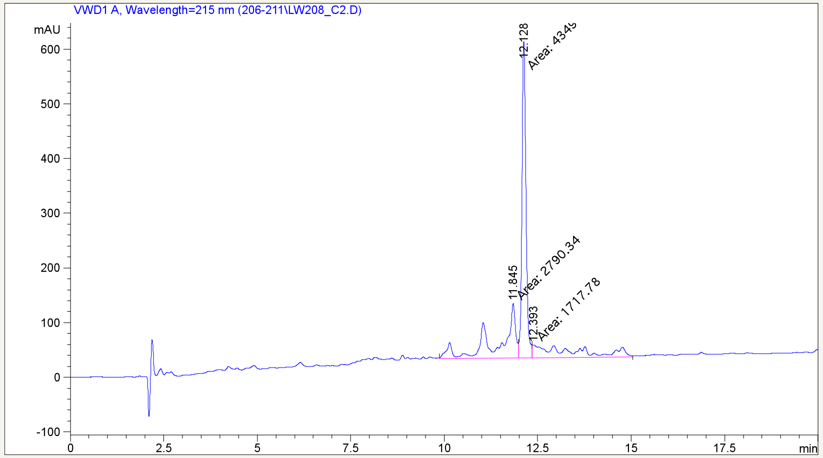
0.015 mmole scale, 2 x 8 eq. DC (2 x 30 min)
3 x 3 min Fmoc-cleavage

4. Chemical syntheses to date

Synthesis of τ domains

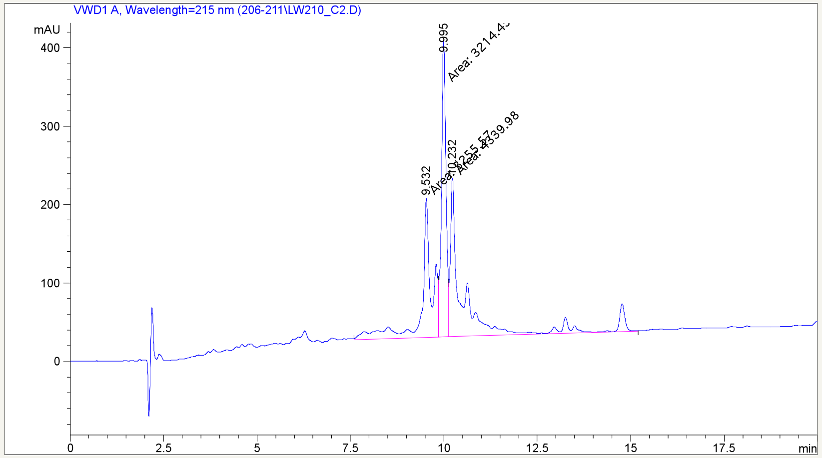
R1: Ac-Q^{244}–V^{275}-NH_2:

Ac-QTAPVPMPDLKNVKSKIGSTENLKHQPGGGK-NH_2



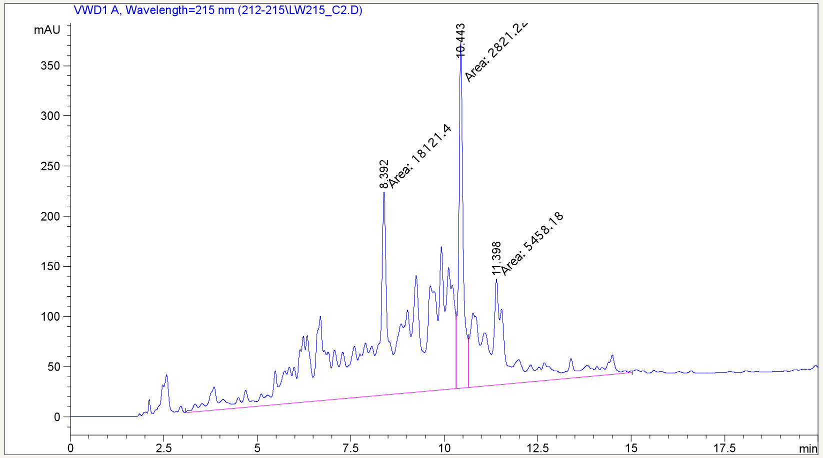
R2: Ac-V^{275}–S^{305}-NH_2:

Ac-VQIINKKLDLSNVQSKCGSKDNIKHVPGGGS-NH_2



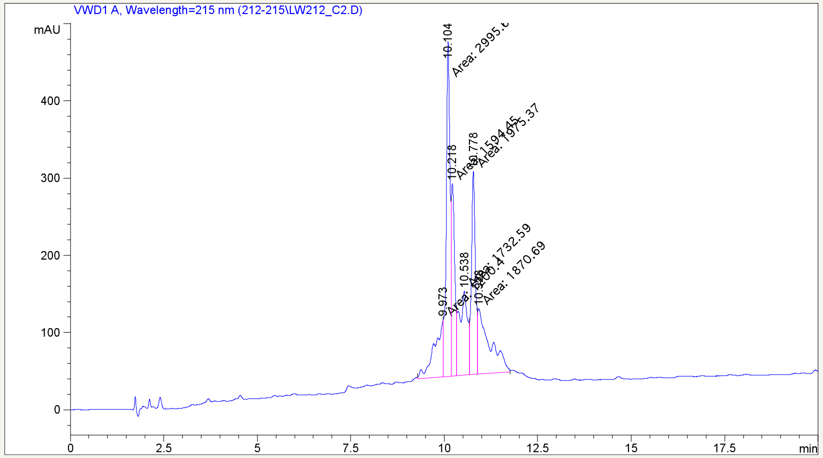
R3: Ac-V^{306}–Q^{336}-NH_2:

Ac-VQIVYKPVDLSKVTSKCGSLGNIHHKPGGGQ-NH_2



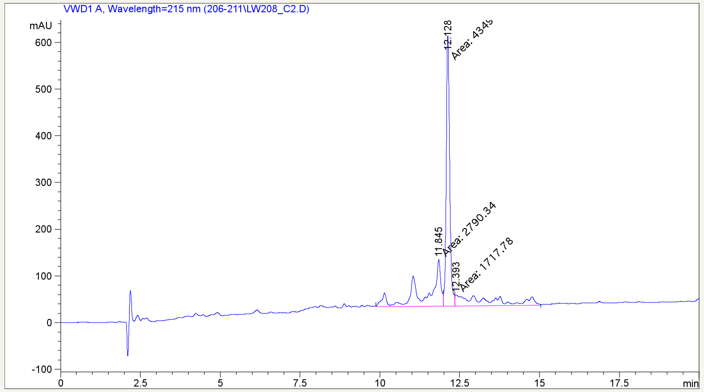
R4: Ac-V^{337}–N^{368}-NH_2:

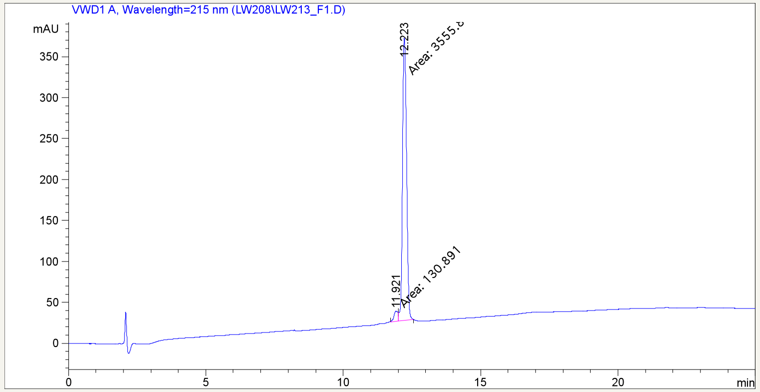
Ac-VQIVYKPVDLSKVTSKCGSLGNIHHKPGGGQ-NH_2

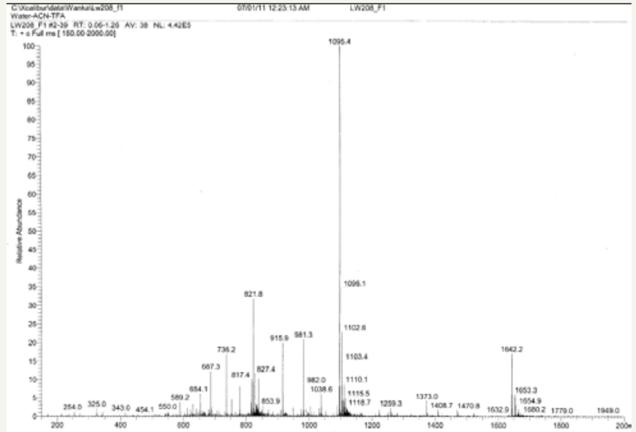


4. Chemical syntheses to date

R1: Ac-QTAPVPMPDLKNVKSKIGSTENLKHQPGGG-NH_2

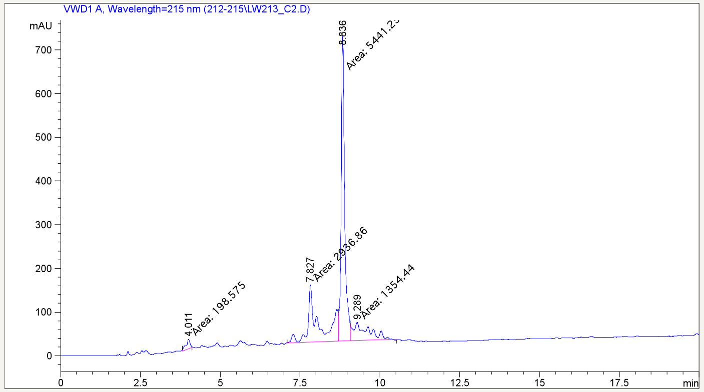


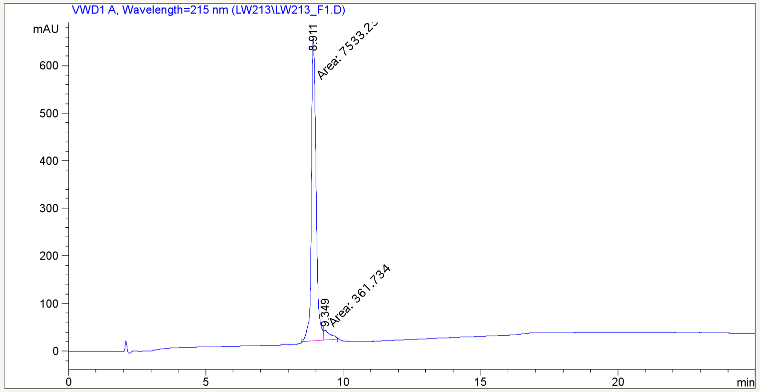


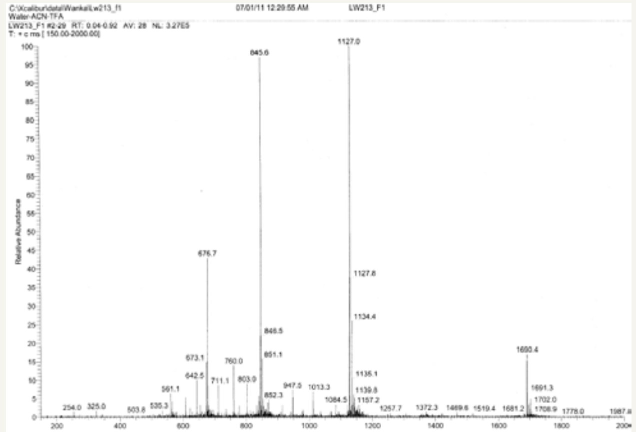


• No difficulty; elimination of water in the ESI source

Phospho-R1: Ac-QTAPVPMPDLKNVKSKIGpSTENLKHQPGGG-NH_2







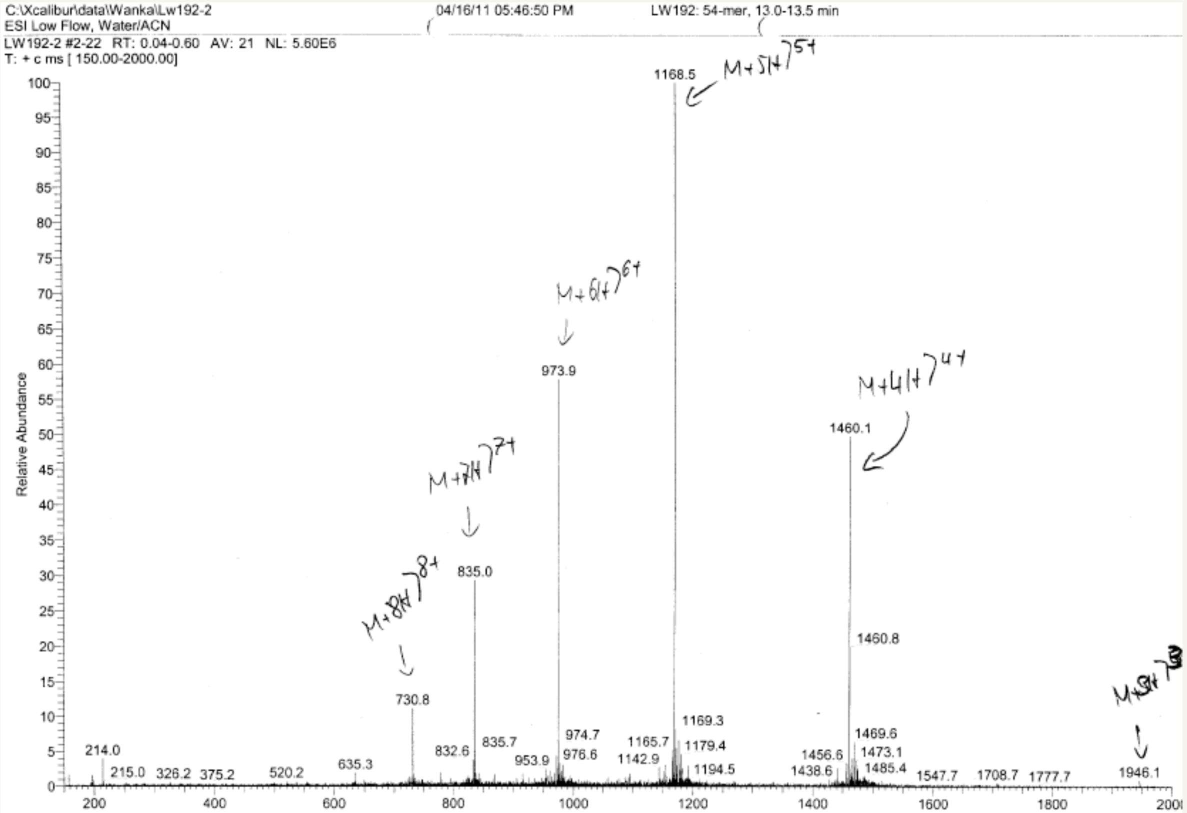
• Used Mutter's Pseudoproline building blocks; no difficulty; elimination of water in ESI source

White, P.; Keyte, J. W.; Bailey, K.; Bloomberg, G. *J. Pept. Sci.* ***2004, 10, 18-26.***

5. Current status: Optimization & Refinement of SPPS

World record for human tau: Blue fragment, 54-mer (Ac-S^{237}–K^{290}-NH_2): 8.0 mg, >96% purity

```
  1 MAEPRQEFEV MEDHAGTYGL GDRKDQGGYT MHQDQEGDTD AGLKESPLQT PTEDGSEEPG
 61 SETSDAKSTP TAEDVTAPLV DEGAPGKQAA AQPHTEIPEG TTAEEAGIGD TPSLEDEAAG
121 HVTQARMVSK SKDGTGSDDK KAKGADGKTK IATPRGAAPP GQKGQANATR IPAKTPPAPK
181 TPPSSGEPPK SGDRSGYSSP GSPGTPGSRS RTPSLPTPPT REPKKVAVVR TPPKSPSSAK
241 SRLQTAPVPM PDLKNVKSKI GSTENLKHQP GGGKVQIINK KLDLSNVQSK CGSKDNIKHV
301 PGGGSVQIVY KPVDLSKVTS KCGSLGNIHH KPGGGQVEVK SEKLDFKDRV QSKIGSLDNI
361 THVPGGGNKK IETHKLTFRE NAKAKTDHGA EIVYKSPVVS GDTSPRHLSN VSSTGSIDMV
421 DSPQLATLAD EVSASLAKQG L
```

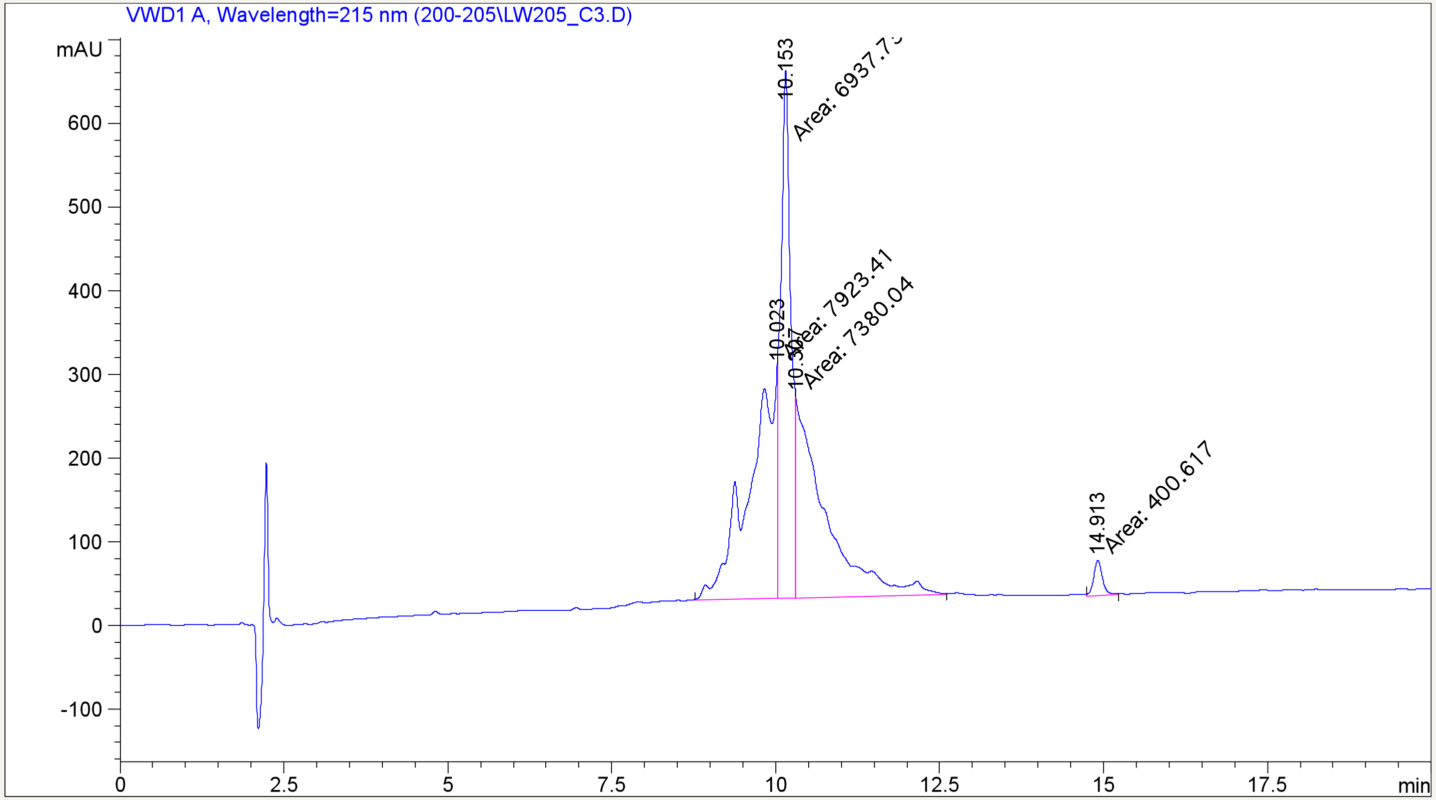


Electrospray series (positive mode) :

charge	m/z
+1	5838.7987
+2	2919.9033
+3	1946.9381
+4	1460.4556
+5	1168.5660
+6	973.9730
+7	834.9780
+8	730.7317
+9	649.6513
+10	584.7869

5. Current status: Optimization & Refinement of SPPS

New World record?: 78-mer Ac-Q^{244}–K^{321}-NH_2: Blue + green fragments

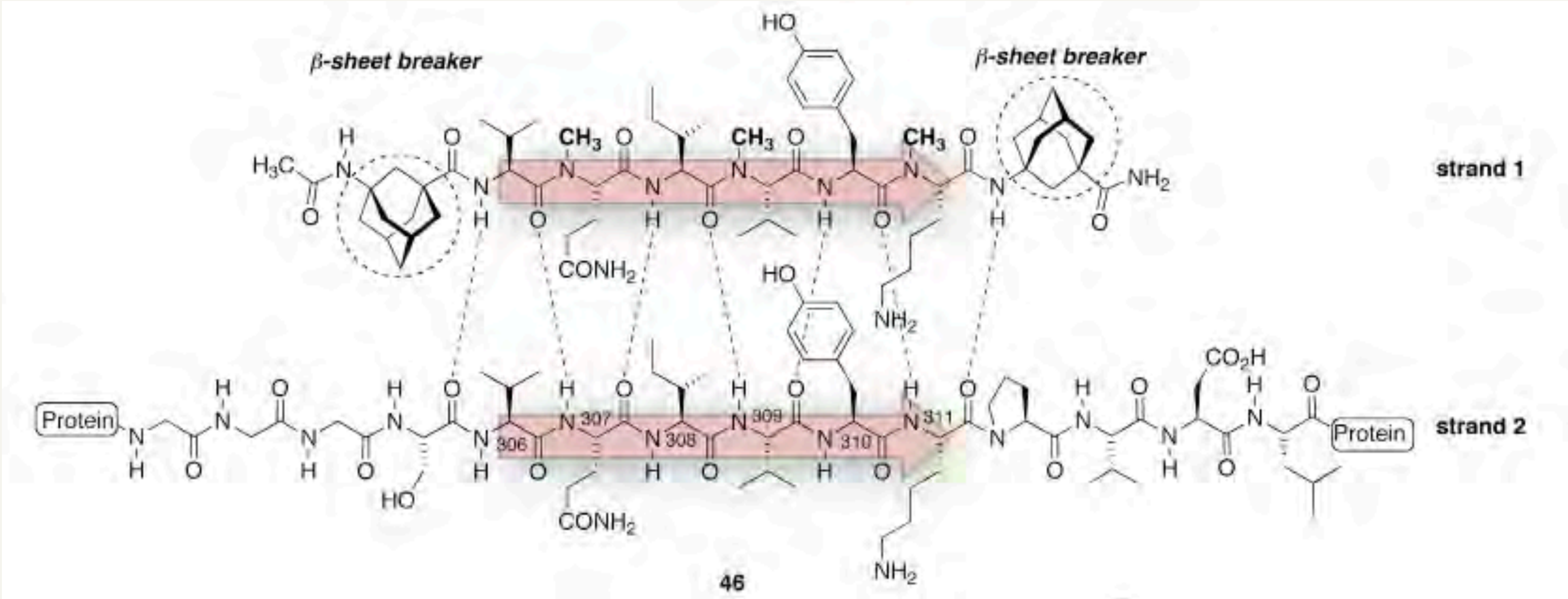


Next:

- SPPS optimization (Pseudoprolines, GGG-Trimers, GG-Dmb building blocks, acylation times...
- Synthesis of phosphoryated and unphosphorylated Repeats as well as
- Full-length (4R) Tau MTBD (>120 residues) and 3R-Tau MTBD

6. What is that for?

- Examination of STRUCTURE influence (CD, ...)
- Examination of Aggregation behavior
- Development of Assays to study ^{A}Gly-incorporating peptidic β-sheet breakers

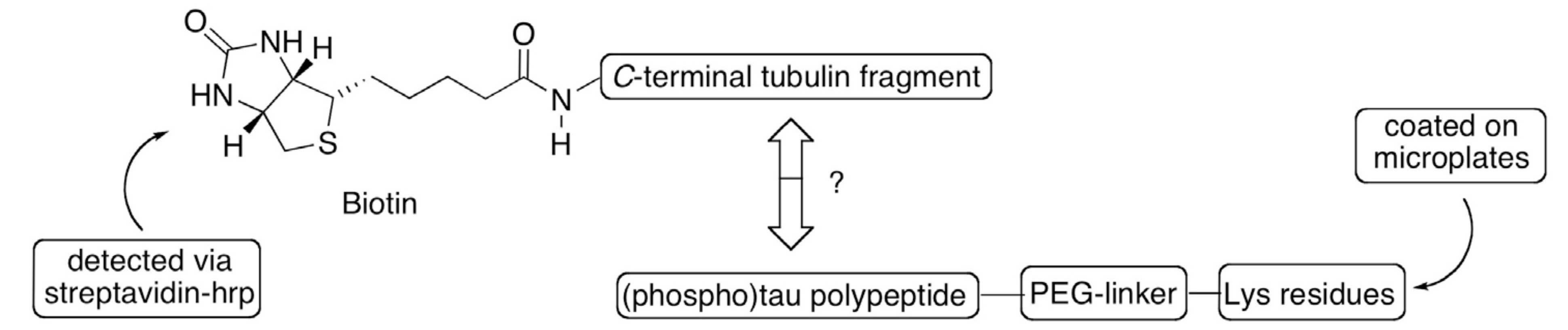


Sites of interest: Subsequences of tau prone to the formation of β-structure, including the sequence at the beginning of the third microtubule-binding repeat (^{306}Val-Gln-Ile-Val-Tyr-Lys^{311}, "PHF6”, pictured), ^{275}Val-Gln-Ile-Ile-Val-Lys^{280} ("PHF6*") at the beginning of the second microtubule-binding repeat, ^{226}Val-Ala-Val-Val-Arg^{230}, and ^{392}Ile-Val-Lys-Lys^{395}.

(1) Goux, W. J.; Kopplin, L.; Nguyen, A. D.; Leak, K.; Rutkofsky, M.; Shanmuganandam, V. D.; Sharma, D.; Inouye, H.; Kirschner, D. A. *J. Biol. Chem.* **2004,** *279,* 26868-26875. (2) Sevcik, J.; Skrabana, R.; Dvorsky, R.; Csokova, N.; Iqbal, K.; Novak, M. *FEBS Lett.* **2007,** *581,* 5872-5878. (3) von Bergen, M.; Friedhoff, P.; Biernat, J.; Heberle, J.; Mandelkow, E. M.; Mandelkow, E. *P. Natl. Acad. Sci. USA* **2000,** *97,* 5129-5134.

6. What is that for?

***In-Vitro* modelling** of the Tau–microtubule interaction



- Influence of phosphorylation on tau – tubulin interactions
- Influence of the β-sheet breakers on this interaction: would be detrimental for a drug
- SPPS of biotinylated C-terminal tubulin-fragment in-house: reatively simple.

Hoffmann, R.; Dawson, N. F.; Wade, J. D.; Otvos, L., Jr. *J. Pept. Res.* **1997,** *50,* 132-142.

Thank you very much for the attention!

Acknowledgement

- Prof. Schreiner
- PRS Group
- PS3 Synthesizer
- Edgar Reitz – fixing everything
- DFG-Referees for blaming me of a lack of intellectual capacy