

Effects of Tau Phosphorylation upon Microtubule Binding and Regulation of Dynamics; Implications for Alzheimer's and Related Dementias.

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Major: Physiology and Neuroscience

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Why study tau and Alzheimer's?

- **There is a direct genetic link between Alzheimer's and tau.**
- **In the USA, 4.5 Million people have Alzheimer's disease.**
(From NIH, Alzheimer's Disease: Unraveling the Mystery, 2005.)
- It is estimated that by 2050, 13.2 million Americans will have AD if current population trends continue and no preventive treatments become available.

Who funds this study?

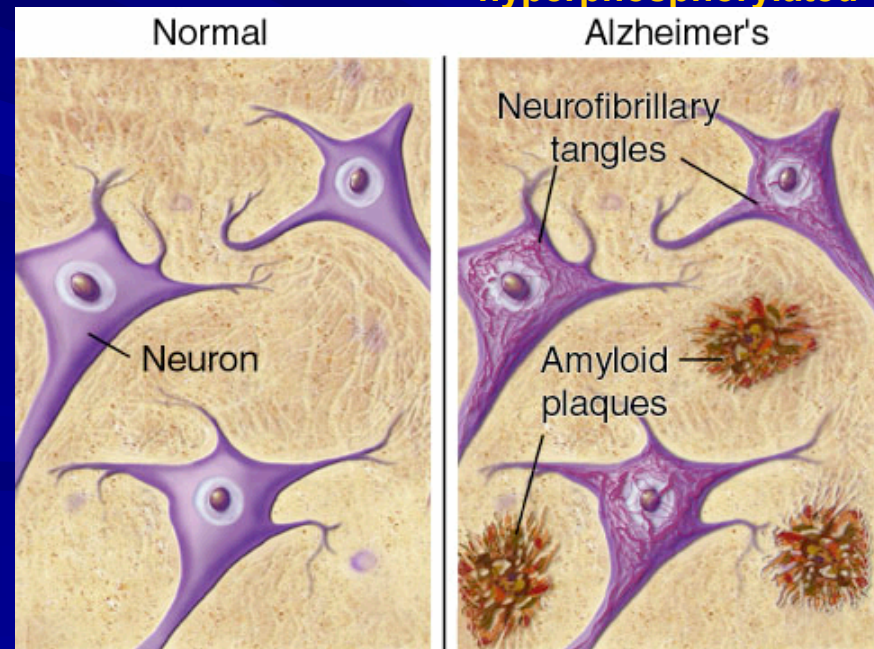
- National Institute of Health
- NSF-Information Technologies Research Project

Alzheimer's Disease Pathology

- Extensive neuronal cell death
- Two abnormal pathological features:
 - 1) Extracellular amyloid plaques - composed of $A\beta$
 - 2) **Intracellular Neurofibrillary Tangles (NFT)**

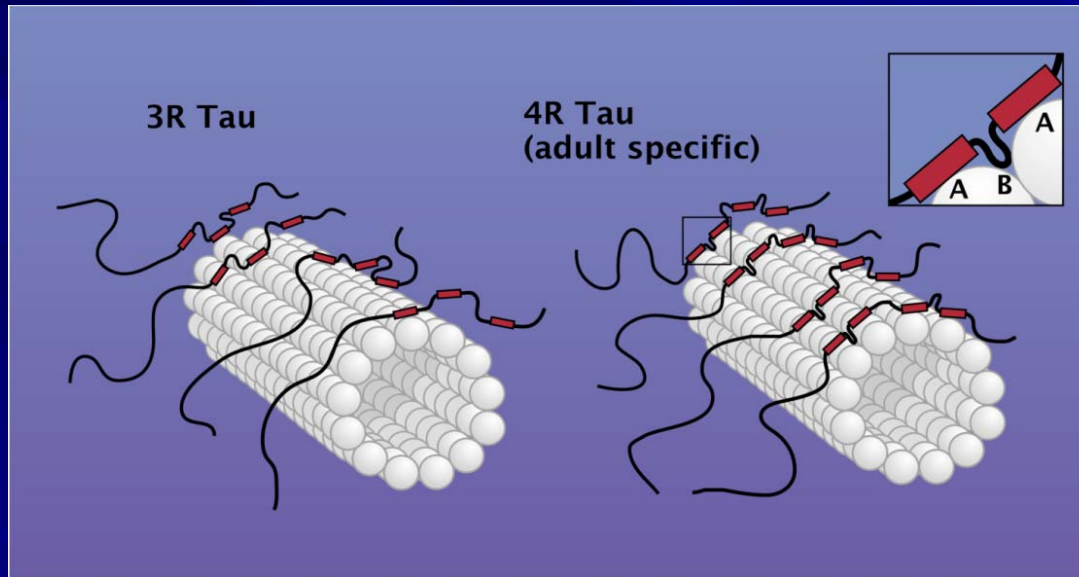
(accumulated abnormal tau fibers)

hyperphosphorylated



Tau is a Microtubule Associated Protein

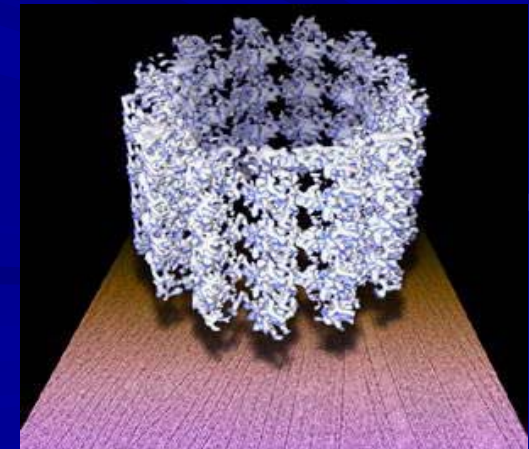
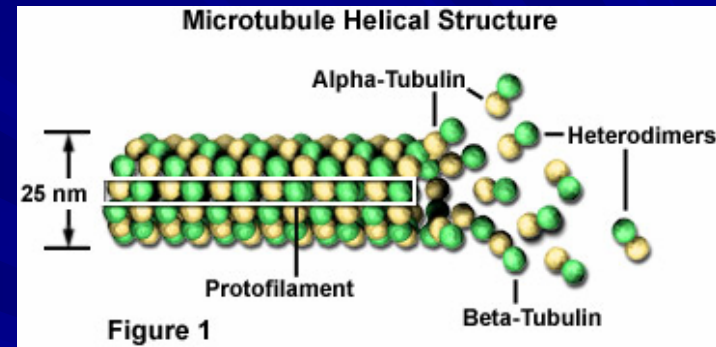
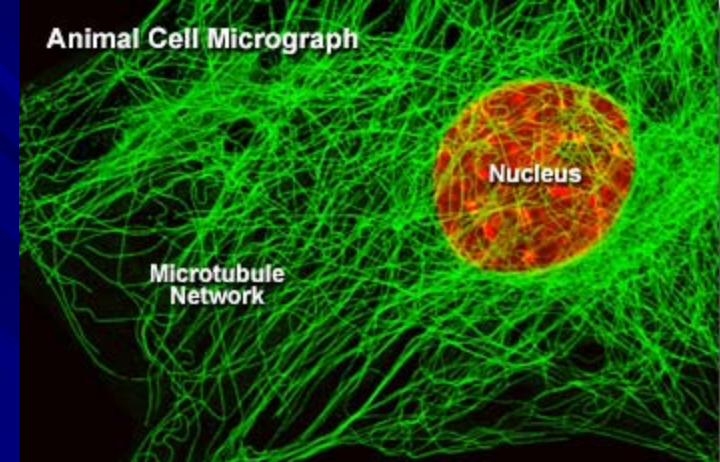
- Expression is limited to neural cells.
- Crucial protein for the proper development and maintenance of the nervous system.
- Influence axonal transport



- Tau does all of this by directly binding and regulating microtubule dynamics.

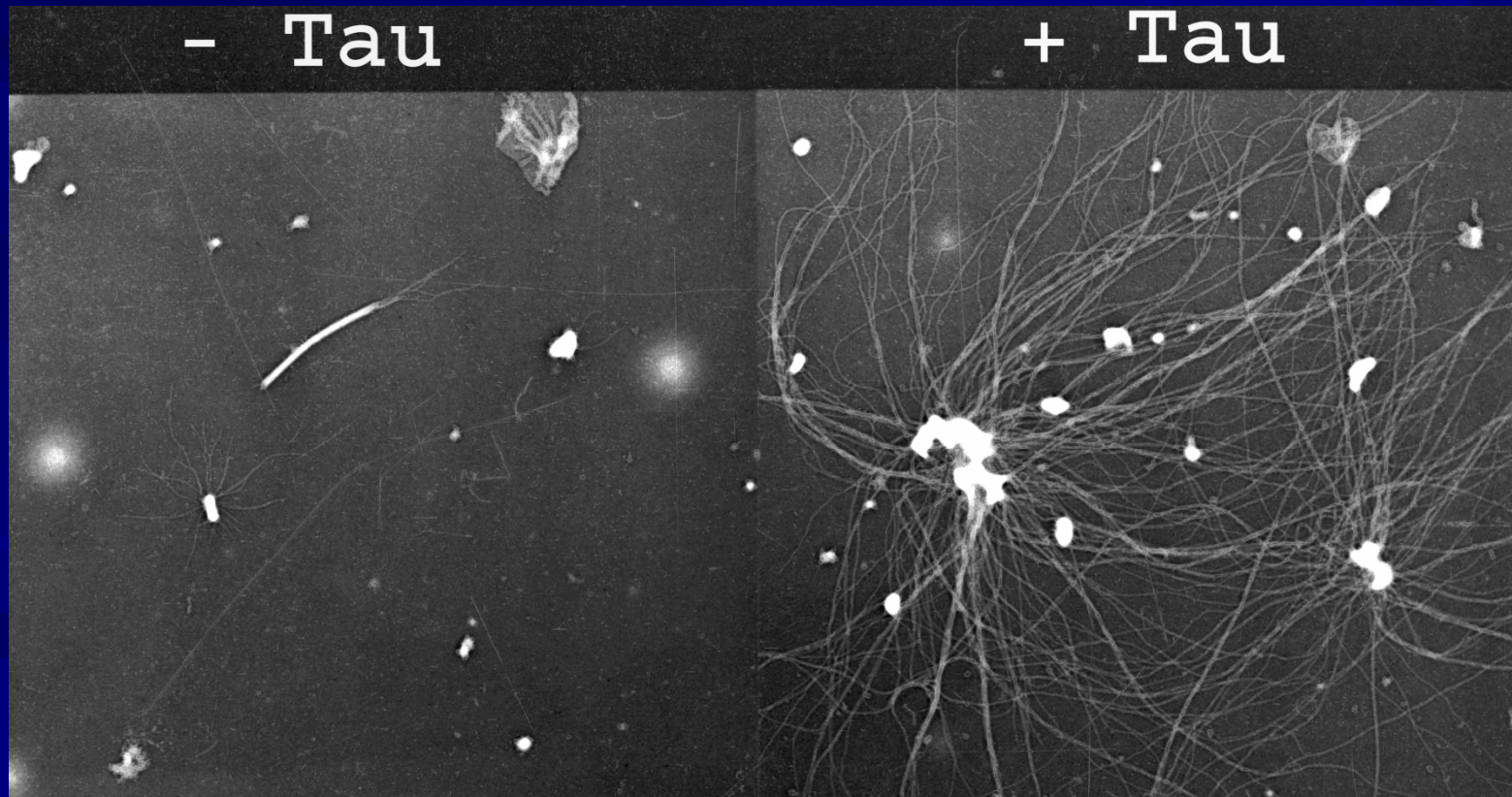
Microtubules are Dynamic Polymers

- ✓ Axonal transport
- ✓ Assembled from α and β tubulin
- ✓ Display dynamic instability
- ✓ Properly regulated microtubule dynamics are essential for cell function and viability!



Tau Function: it is well established that tau **binds directly** to microtubules, promotes **microtubule assembly** and regulates **microtubule dynamics**, which are essential for cell viability.

An example of tau action in vitro:
tau promotes microtubule assembly when mixed with tubulin.



Unpublished results of Tim Mitchison and Marc Kirschner

Our research project:

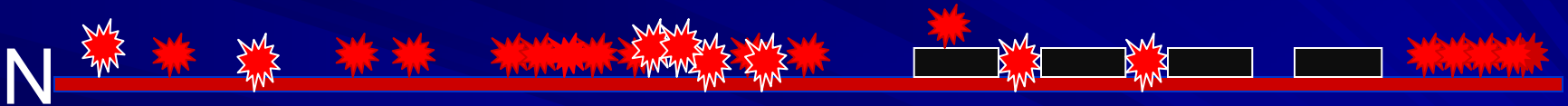
- To study single and multiple tau pseudophosphorylations in terms of binding, assembly, and its affect on microtubule dynamics in vitro.
- Historically, research on this subject has targeted single phosphorylation events.
- This project aims to study both single and combinatorial pseudophosphorylation events in order to more naturally mimic events that occur in vivo.

4R2N (Tau Protein amino acid sequence)

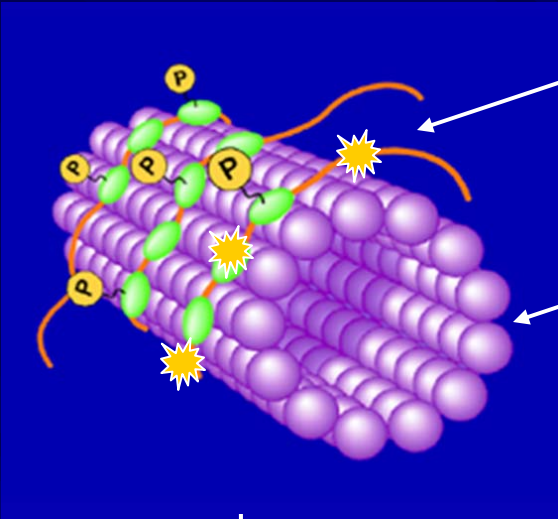


Tau Phosphorylation

4R tau has 85 amino acids that have the potential to be phosphorylated (~20% of total a.a.)

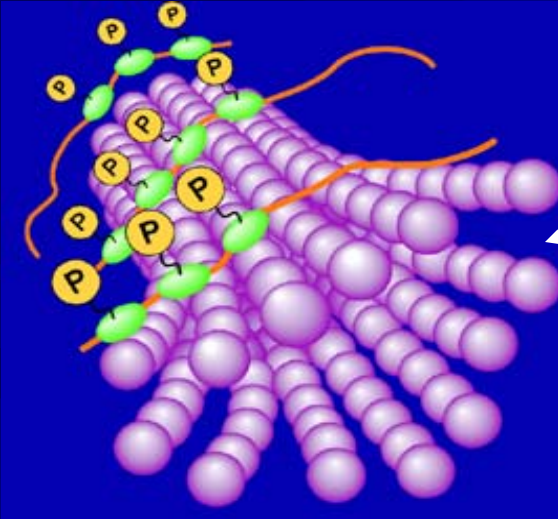


Tyr18, Thr39, Ser46, Thr50, Thr69, Thr153, Thr175, Thr181, Ser198, Ser199, Ser202, Thr205, Ser208, Ser210, Thr212, Ser214, Thr217, **Thr231**, Ser235, Ser237, Ser241, **Ser262**, Ser285, Ser305, Ser324, Ser352, Ser356, **Ser396**, Ser400, Thr403, **Ser404**, Ser409, Ser412, Ser413, Ser416 and Ser422...



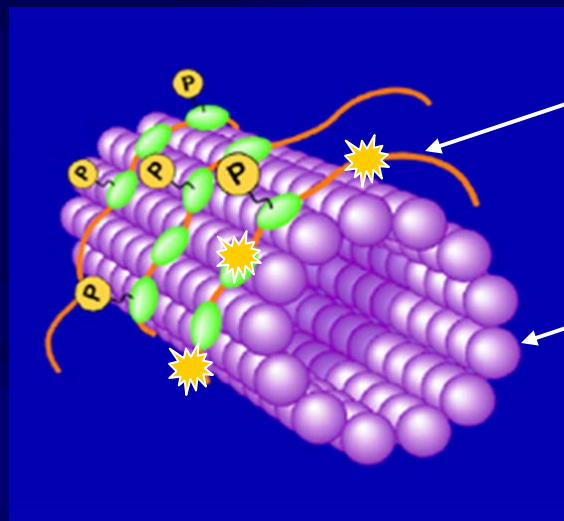
Tau protein(3R2NT231+ S262+ 396)

Microtubule (composed of tubulin)



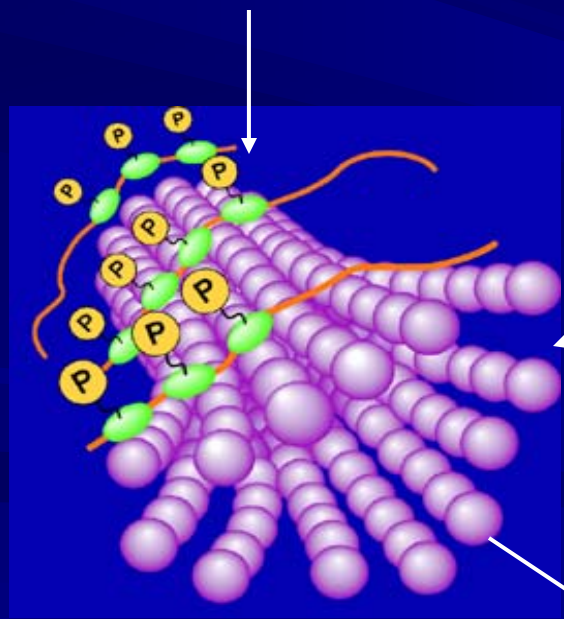
Microtubule destabilization

Hypothesis:



Tau protein(3R2N T231+ S262+ 396)

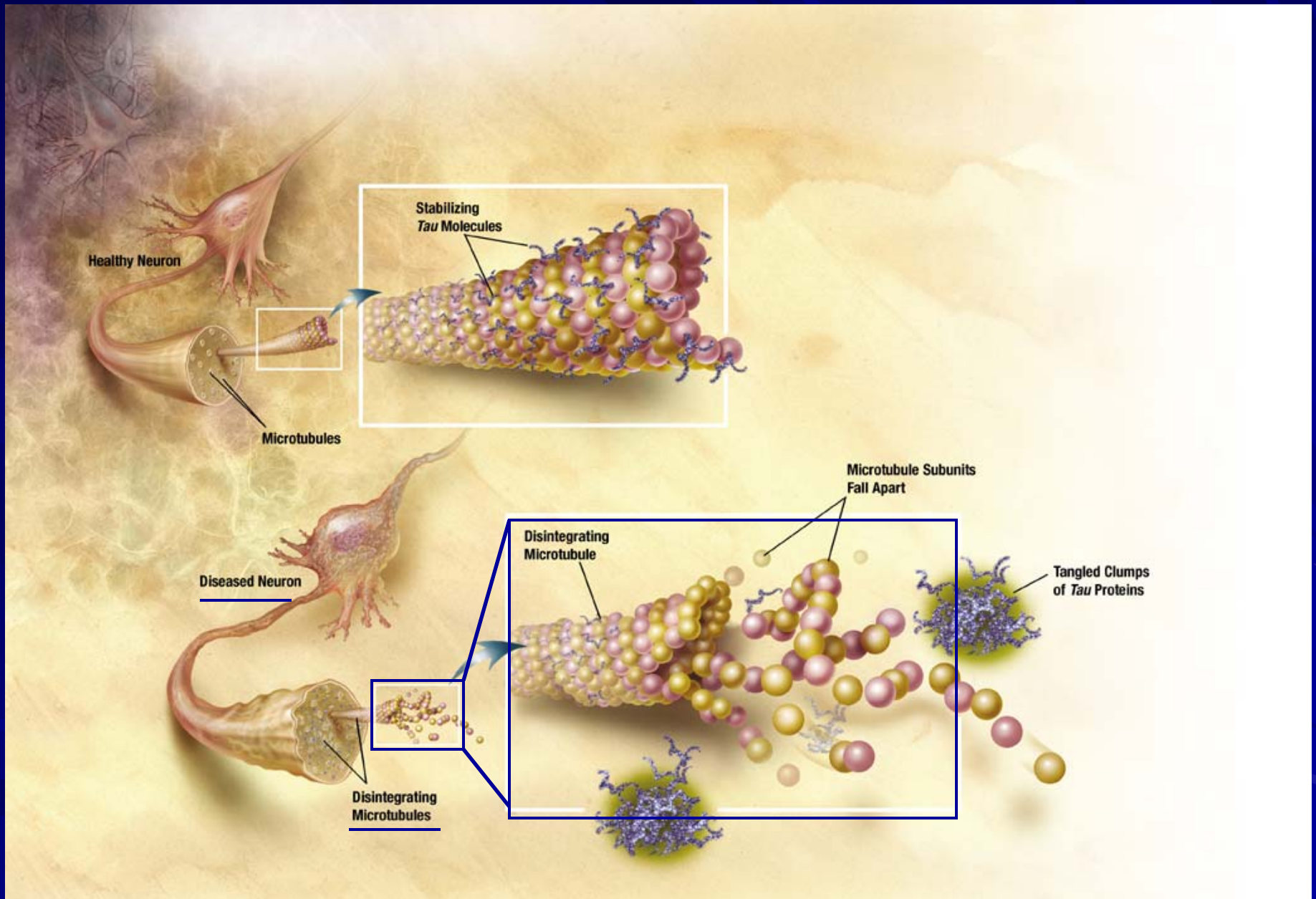
Microtubule (composed of tubulin)



Microtubule destabilization

Loss of function

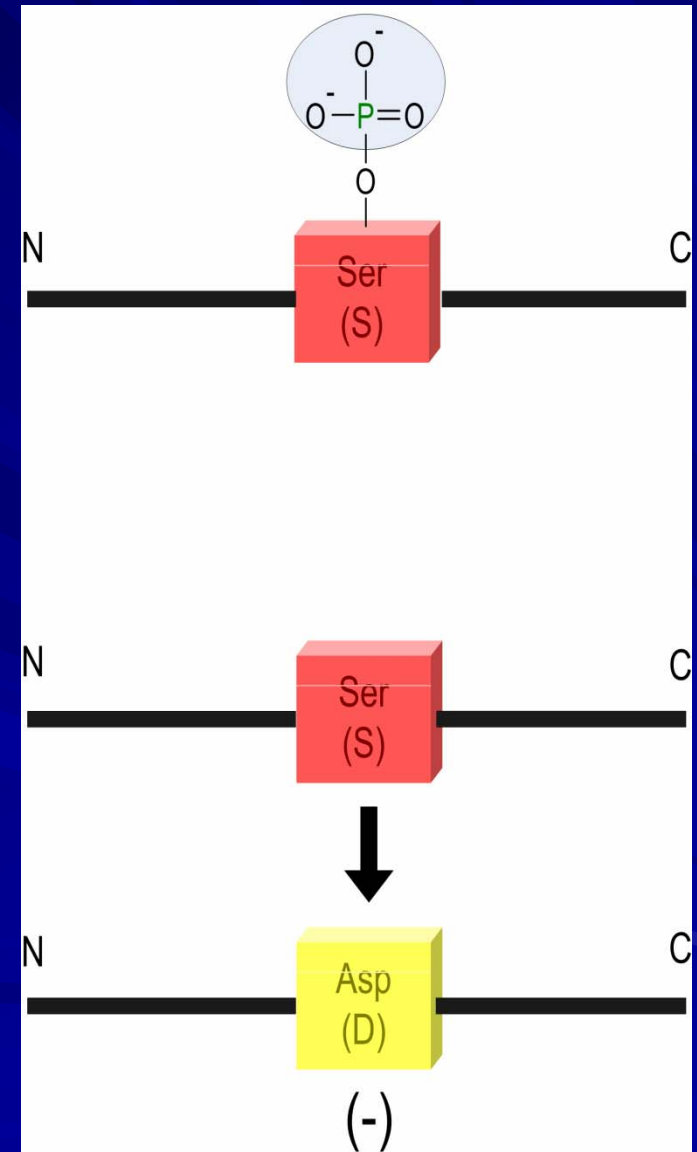
Neuronal Cell Death



(From NIH, Alzheimer's Disease: Unraveling the Mystery, 2005.)

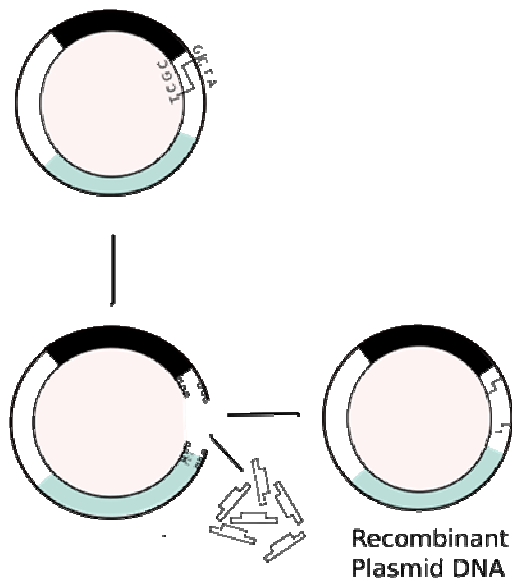
Methods:

- Site-directed mutagenesis
- PCR
- Transformation
- Plating
- Inoculation
- Induction
- Protein purification
 - **Sonication**
 - **Boiling**
 - Pre/post boil gel
 - **PC Column**
 - gel
 - **HPLC**
 - gel
 - **Determine Protein Concentrations**
- **Binding and Assembly Experiments**
- **Microtubule dynamics in vitro**
- **Aggregation**

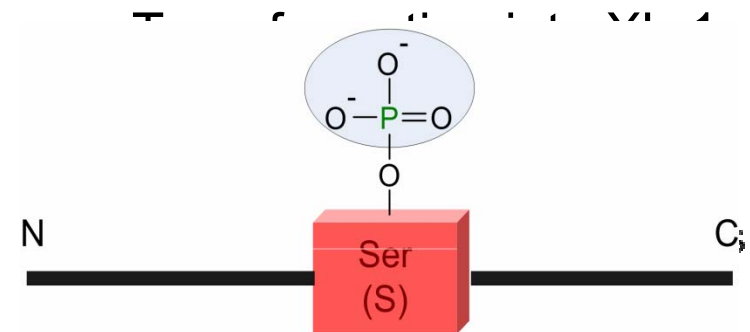


Methods: How to make pseudophosphorylated tau protein

Site-directed mutagenesis



Phosphorylation

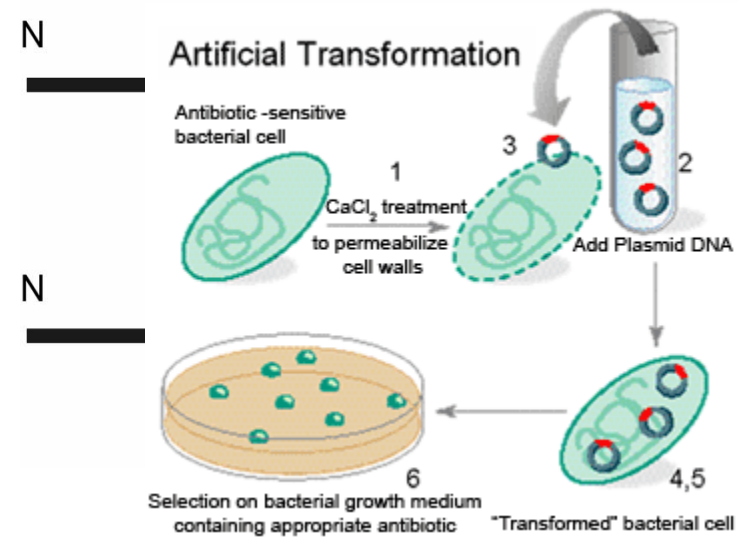


PCR

Pseudophosphorylation

It has been suggested that introducing a single (-) charge to tau protein mimics the phosphorylation event and leads to conformational change.

(Eidenmüller *et al.*, 2000; Fath *et al.*, 2002)



Constructs	XL-1	BL-21	Protein Purified	Binding Exp.	In Vitro MT Dynamics	Aggregation
4R2N T231D	X	X	X	X	/	
4R2N S262D	X	X	X	X	/	
4R2N S396D	X	X	X	X	/	
4R2N S404D	X	X	X	X	/	
3R2N T231D	X	X	X	X	/	
3R2N S262D	X	X	X	X	/	
3R2N S396D	X	X	X	X	/	
3R2N S404D	X	X	X	X	/	
4R2N T231+S262D	X	X	X			
3R2N T231+S262D	X	X	X			
4R2N S262+396D	X	X	X			
4R2N S262+404D	X	X	X			
4R2N S396+404D	X	X	X			
3R2N S262+396D	X	X	/			
3R2N S262+404D	X	X	/			
3R2N S396+404D	X	X	/			
4R2N S262+396+404D	X	X				
4R2N T231+S262+396D	X	X				
4R2N T231+S262+S404D	X	X				
4R2N T231+S396+404D	X	X				
3R2N S262+396+404D	X	X				
3R2N T231+S262+396D	X	X				
3R2N T231+S262+S404D	X	X				
3R2N T231+S396+404D	X	X				
4R2N T231+S262+396+404D						
3R2N T231+S262+396+404D						

Constructs	XL-1	BL-21	Protein Purified	Binding Exp.	In Vitro MT Dynamics	Aggregation
4R2N T231D	X	X	X	X	/	
4R2N S262D	X	X	X	X	/	
4R2N S396D	X	X	X	X	/	
4R2N S404D	X	X	X	X	/	
3R2N T231D	X	X	X	X	/	
3R2N S262D	X	X	X	X	/	
3R2N S396D	X	X	X	X	/	
3R2N S404D	X	X	X	X	/	
4R2N T231+S262D	X	X	X			
3R2N T231+S262D	X	X	X			
4R2N S262+396D	X	X	X			
4R2N S262+404D	X	X	X			
4R2N S396+404D	X	X	X			
3R2N S262+396D	X	X	/			
3R2N S262+404D	X	X	/			
3R2N S396+404D	X	X	/			
4R2N S262+396+404D	X	X				
4R2N T231+S262+396D	X	X				
4R2N T231+S262+S404D	X	X				
4R2N T231+S396+404D	X	X				
3R2N S262+396+404D	X	X				
3R2N T231+S262+396D	X	X				
3R2N T231+S262+S404D	X	X				
3R2N T231+S396+404D	X	X				
4R2N T231+S262+396+404D	X					
3R2N T231+S262+396+404D	X					

Methods:

Jellyfish

File Edit Tools Actions Help

PROJECT

- Project
 - 235 33R
 - 3R2N Y18D
 - Alignment Example
 - Eric's
 - Erkan's Sequences
 - 06 06 07 Triple's
 - 3R2N S262+396+404D
 - 3R2N S262+396D
 - 3R2N T231+262+396D
 - 3R2N T231+262+404D
 - 3R2N T231+396+404
 - 4R2N S262+396+404D
 - 4R2N T231+S262+396D**
 - 4R2N T231+S262+404D
 - 4R2NT231+S396+404D
 - 3R2NS262+396D
 - Double's
 - Jacks Sequences
 - Sequence Examples
 - TAU SEQUENCES

Manipulate Restriction Enzymes Alignment Oligos PCR Notes Genbank Statistics Web Tools

4R2N T231+S262+396D

4R2N wild ty	ATGGCTGAGCCCCGCCAGGAGTTTCAAGTGATGGAAGATCACGCTGGGACGTACGGGTGGGGGACAGGAAAAGATCAGGGGGGCTACACCATGCACCAAGACCAAGAGGGTGACACGGACGCTGGC												
Consensus	-----												
	131	141	151	161	171	181	191	201	211	221	231	241	251
New DNA Sequ	-----												
4R2N wild ty	AAGAATCTCCCTGCAGACCCCCACTGAGGACGGATCTGAGGAACCGGGCTCTGAAACCTCTGATGCTAAGAGCACTCCAACAGCGGAAGATGTGACAGCACCCCTTAGTGGATGAGGGAGCTCCCG												
Consensus	-----												
	261	271	281	291	301	311	321	331	341	351	361	371	381
New DNA Sequ	-----												
4R2N wild ty	GCAGGCTGCCCGCAGCCCCACAGCGAGATCCAGAAGGAACACAGCTGAAGAAGCAGGCATTGGAGACACCCCGCCTGGAAGACGAAGCTGCTGCTCACGTGACCCAAGCTCGCATGGTCCAG												
Consensus	-----												
	391	401	411	421	431	441	451	461	471	481	491	501	511
New DNA Sequ	-----												
4R2N wild ty	AGCAAAGACGGGACTGGAAGCGATGACAAAAAAGCCAAGGGGGCTGATGGTAAAACGAAGATCGCCACACCGCGGGAGCAGCCCTCCAGGCCAGAAGGGCCAGGCCAACGCCACCGAGGATTTCCA												
Consensus	-----												
	521	531	541	551	561	571	581	591	601	611	621	631	641
New DNA Sequ	-----												
4R2N wild ty	AAACCCCGCCCGCTCCAAGACACCACCCAGCTCTGGTGAACCTCCAAAAATCAGGGGATCGCAGCGGCTACAGCAGCCCGGGCTCCCCAGGCCTCCCGGCAGCCCGCTCCCGGCACCCCGCTCCCTTC												
Consensus	actcccgccgagccgctcccgccaccccgctcccttc												
	651	661	671	681	691	701	711	721	731	741	751	761	771
New DNA Sequ	-----												
4R2N wild ty	CCCACCCACCCGGGAGCCCAAGAAGGTGGCAGTGGTCCGTGACCCACCCAAGTCGCGCTCTTCGCCAAGAGCCCGCTGCAGACAGCCCGCTGCCATGCCAGACCTGAAGAATGTCAAGTCCAA												
Consensus	cccacccacccgggagcccaagaaggtggcagtggtccggt ccacccaagtcgccgctcttcgccaagagccgctgcagacagcccccgtgccatgccagacctgaagaatgtcaagttccaa												
	781	791	801	811	821	831	841	851	861	871	881	891	901
New DNA Sequ	-----												
4R2N wild ty	GGCGATCTGAGAACCTGAAGCACCAGCCGGGAGCCGGGAAGGTGCAGATAATTAATAAGAAGCTGGATCTTAGCAACGCTCCAGTCCAAAGTGTGGCTCAAAGGATAAATCAAACAGCTCCCGGGA												
Consensus	ggc actgagaacctgaagcaccagccgggagccgggaaggtgcagataattaataagaagctggatcttagcaacgctccagttccaaagtgtggctcaaaggataaatacaaacagctcccgggga												
	911	921	931	941	951	961	971	981	991	1001	1011	1021	1031
New DNA Sequ	-----												
4R2N wild ty	GCAGTGTGCAAAATAGTCTACAAAACAGTTGACCTGAGCAAGGTGACCTCCAAGTGTGGCTCATTAGGCAACATCCATCATAAACCCAGGAGGTGGCCAGGTGGAAGTAAAATCTGAGAAGCTTGACT												
Consensus	gcagtggtgcaaaatagtctacaaaacagttgacctgagcaaggtgacctccaagtggtgctcattaggcaacatccatcataaacccaggaggtggccaggtggaagtaaaatctgagaagcttgact												
	1041	1051	1061	1071	1081	1091	1101	1111	1121	1131	1141	1151	1161
New DNA Sequ	-----												
4R2N wild ty	GGACAGAGTCCAGTTCGAAGATTGGGTCCCTGGACAATATCACCCAGCTCCCTGGCGGAGGAAATAAAAAGATTGAAAACCCACAAGTGCACCTTCGCCGAGAAGCCCAAAGCCAAAGACAGACCACGG												
Consensus	ggacagagtccagttcgaagattgggtccctggacaatatacaccagctccctggcgaggaaataaaaagattgaaaacccacaagtgaccttcgccgagaagcccaaagccaaagacagaccacgg												
	1171	1181	1191	1201	1211	1221	1231	1241	1251	1261	1271	1281	1291
New DNA Sequ	-----												
4R2N wild ty	GAGATCGGTGACAAAGGATCCAGTGGTCTGGGGACAGCTCCACCGGCATCTCAGCAATGTCTCCTCCACCGCAGCATCGACATGGTAGACTCGCCCCAGCTCGCCACGCTAGCTGACGAGGTG												
Consensus	gagatcgggtgacaaaggatccagtggtctggggacagctccacggcatctcagcaatgtctcctccacggcagcatcgacatggtagactcgccccagctcgccacgctagctgacgaggtg												
	1301	1311	1321	1331	1341	1351	1361	1371	1381	1391	1401	1411	1421
New DNA Sequ	-----												
4R2N wild ty	CTCCCTGGCCAAGCAGGTTTGTGATCAGGCCCTTGGGGCGTCAATAATTGTGGAGGAGAGAATGAGAGAGTGTGAAAAAAGAAATAATGACCCGGGCCCGCCCTCTGCCCCACGCTG												
Consensus	cctccctggccaagcagggtttgtgatacagggccttggggcggtcaataattgtggagaggagagaatgagagagtgtaaaaaaagaaataatgacccgggcccgccctctgccccacgctg												

JELLYFISH
An Infotrieve Product

Constructs	XL-1	BL-21	Protein Purified	Binding Exp.	In Vitro MT Dynamics	Aggregation
4R2N T231D	X	X	X	X	/	
4R2N S262D	X	X	X	X	/	
4R2N S396D	X	X	X	X	/	
4R2N S404D	X	X	X	X	/	
3R2N T231D	X	X	X	X	/	
3R2N S262D	X	X	X	X	/	
3R2N S396D	X	X	X	X	/	
3R2N S404D	X	X	X	X	/	
4R2N T231+S262D	X	X	X			
3R2N T231+S262D	X	X	X			
4R2N S262+396D	X	X	X			
4R2N S262+404D	X	X	X			
4R2N S396+404D	X	X	X			
3R2N S262+396D	X	X	/			
3R2N S262+404D	X	X	/			
3R2N S396+404D	X	X	/			
4R2N S262+396+404D	X	X				
4R2N T231+S262+396D	X	X				
4R2N T231+S262+S404D	X	X				
4R2N T231+S396+404D	X	X				
3R2N S262+396+404D	X	X				
3R2N T231+S262+396D	X	X				
3R2N T231+S262+S404D	X	X				
3R2N T231+S396+404D	X	X				
4R2N T231+S262+396+404D	X					
3R2N T231+S262+396+404D	X					

Constructs	XL-1	BL-21	Protein Purified	Binding Exp.	In Vitro MT Dynamics	Aggregation
4R2NT231D	X	X	X	X	/	
4R2NS262D	X	X	X	X	/	
4R2NS396D	X	X	X	X	/	
4R2NS404D	X	X	X	X	/	
3R2NT231D	X	X	X	X	/	
3R2NS262D	X	X	X	X	/	
3R2NS396D	X	X	X	X	/	
3R2NS404D	X	X	X	X	/	
4R2NT231+S262D	X	X	X			
3R2NT231+S262D	X	X	X			
4R2NS262+396D	X	X	X			
4R2NS262+404D	X	X	X			
4R2NS396+404D	X	X	X			
3R2NS262+396D	X	X	/			
3R2NS262+404D	X	X	/			
3R2NS396+404D	X	X	/			
4R2NS262+396+404D	X	X				
4R2NT231+S262+396D	X	X				
4R2NT231+S262+S404D	X	X				
4R2NT231+S396+404D	X	X				
3R2NS262+396+404D	X	X				
3R2NT231+S262+396D	X	X				
3R2NT231+S262+S404D	X	X				
3R2NT231+S396+404D	X	X				
4R2NT231+S262+396+404D	X	X				
3R2NT231+S262+396+404D	X	X				

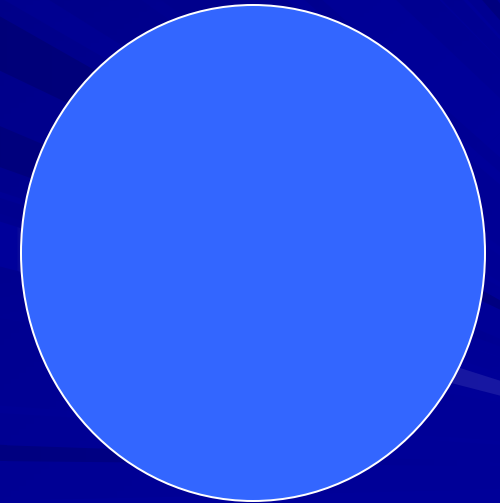
Recombinant tau protein purification:



Inoculation and autoinduction



Small bacterial colony

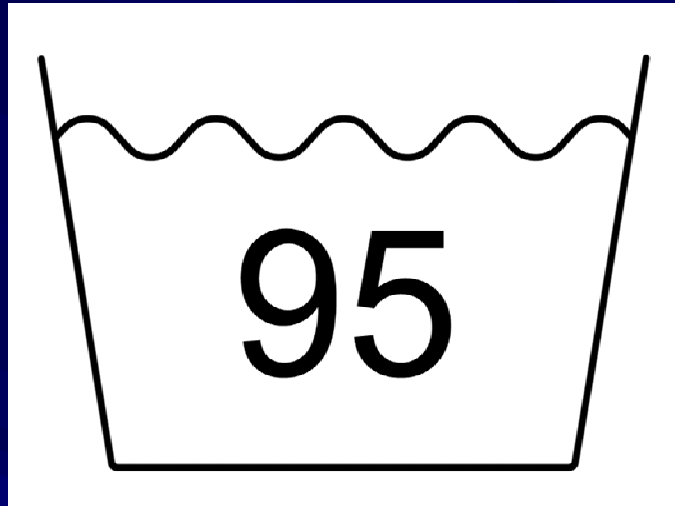


Large pellet of autoinduced
bacteria

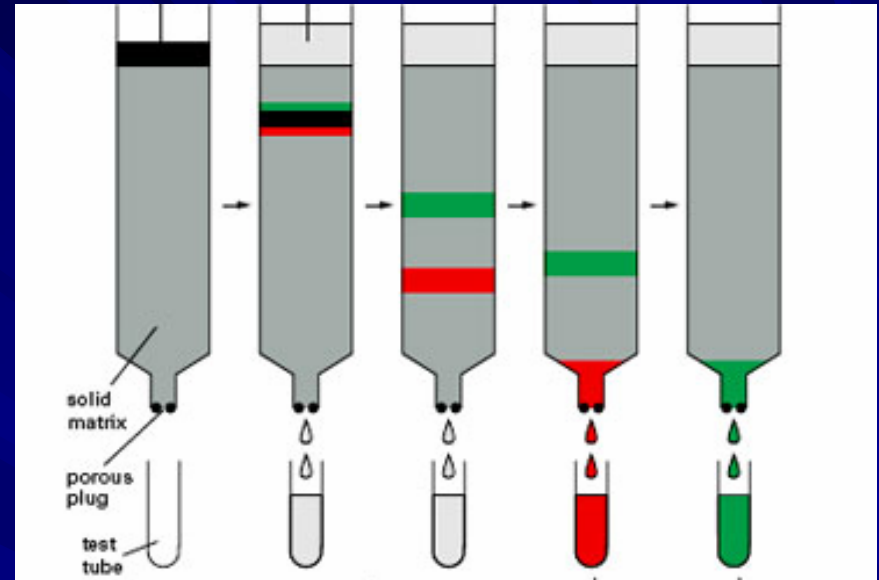


Sonication breaks down the bacterial cell walls so that the protein of interest (tau) can be obtained and purified.

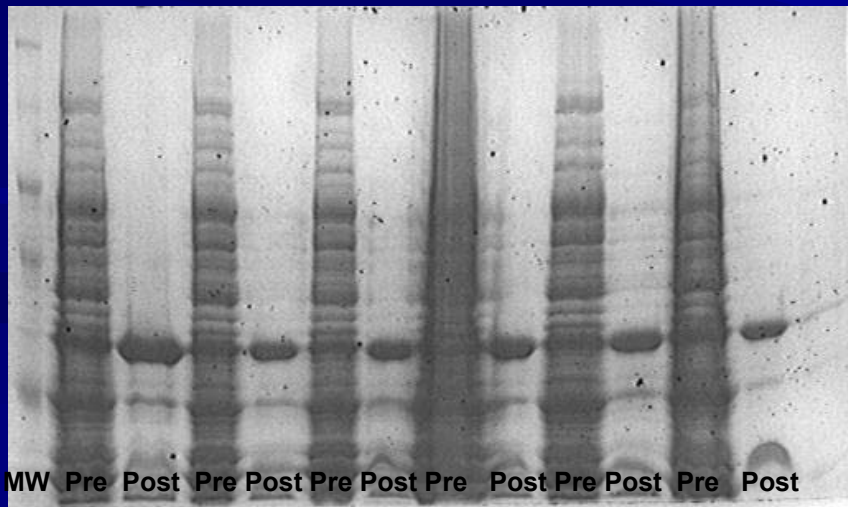
Heat at 95 °C



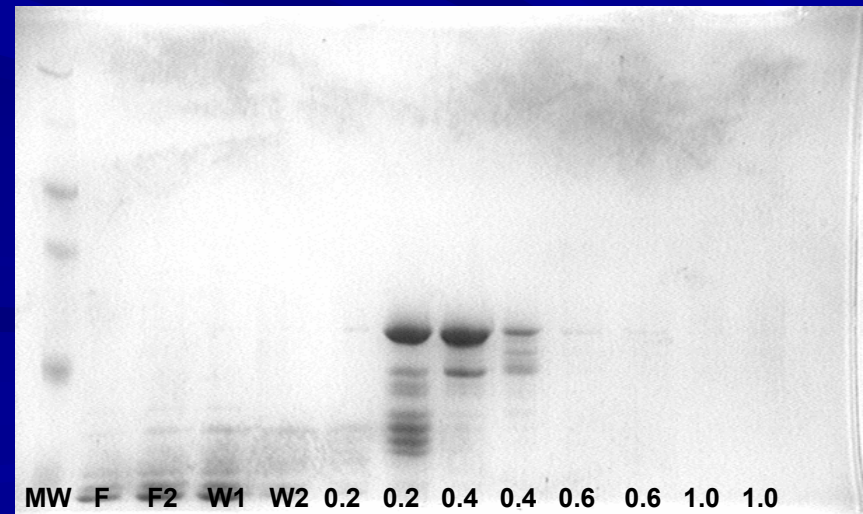
Column Chromatography



Pre/post boil SDS-PAGE gel



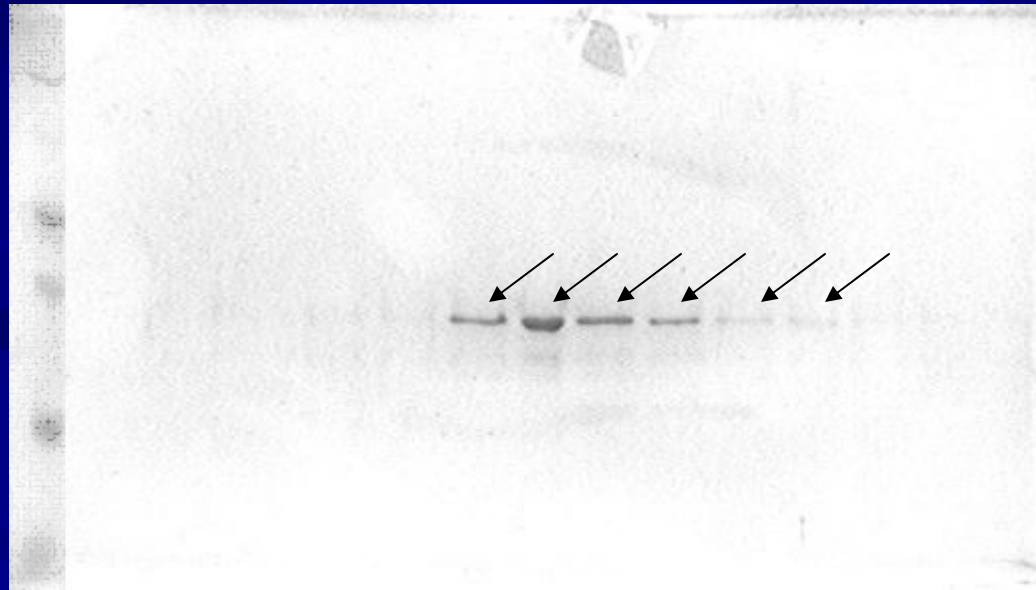
Phospho-Cellulose Column gel



High Pressure Liquid Chromatography



HPLC Gel



Constructs	XL-1	BL-21	Protein Purified	Binding Exp.	In Vitro MT Dynamics	Aggregation
4R2N T231D	X	X	X	X	/	
4R2N S262D	X	X	X	X	/	
4R2N S396D	X	X	X	X	/	
4R2N S404D	X	X	X	X	/	
3R2N T231D	X	X	X	X	/	
3R2N S262D	X	X	X	X	/	
3R2N S396D	X	X	X	X	/	
3R2N S404D	X	X	X	X	/	
4R2N T231+S262D	X	X	X			
3R2N T231+S262D	X	X	X			
4R2N S262+396D	X	X	X			
4R2N S262+404D	X	X	X			
4R2N S396+404D	X	X	X			
3R2N S262+396D	X	X	/			
3R2N S262+404D	X	X	/			
3R2N S396+404D	X	X	/			
4R2N S262+396+404D	X	X				
4R2N T231+S262+396D	X	X				
4R2N T231+S262+S404D	X	X				
4R2N T231+S396+404D	X	X				
3R2N S262+396+404D	X	X				
3R2N T231+S262+396D	X	X				
3R2N T231+S262+S404D	X	X				
3R2N T231+S396+404D	X	X				
4R2N T231+S262+396+404D	X	X				
3R2N T231+S262+396+404D	X	X				

Constructs	XL-1	BL-21	Protein Purified	Binding Exp.	In Vitro MT Dynamics	Aggregation
4R2N T231D	X	X	X	X	/	
4R2N S262D	X	X	X	X	/	
4R2N S396D	X	X	X	X	/	
4R2N S404D	X	X	X	X	/	
3R2N T231D	X	X	X	X	/	
3R2N S262D	X	X	X	X	/	
3R2N S396D	X	X	X	X	/	
3R2N S404D	X	X	X	X	/	
4R2N T231+S262D	X	X	X			
3R2N T231+S262D	X	X	X			
4R2N S262+396D	X	X	X			
4R2N S262+404D	X	X	X			
4R2N S396+404D	X	X	X			
3R2N S262+396D	X	X	X			
3R2N S262+404D	X	X	X			
3R2N S396+404D	X	X	X			
4R2N S262+396+404D	X	X	/			
4R2N T231+S262+396D	X	X	/			
4R2N T231+S262+S404D	X	X	/			
4R2N T231+S396+404D	X	X	/			
3R2N S262+396+404D	X	X	/			
3R2N T231+S262+396D	X	X	/			
3R2N T231+S262+S404D	X	X	/			
3R2N T231+S396+404D	X	X	/			
4R2N T231+S262+396+404D	X	X	/			
3R2N T231+S262+396+404D	X	X	/			

In the future...

MT Binding Assay

Co-Incubate Tau and Tubulin
(15 μ M Tubulin; varying amounts of Tau)

37°C, 2h

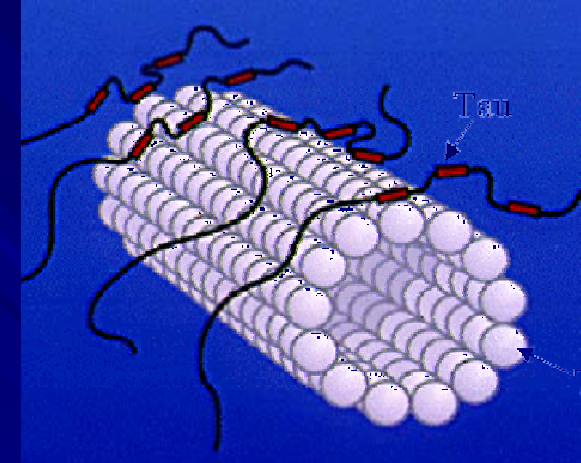
Microtubules with bound tau + Free Tubulin + Free Tau

Spin over a 50% sucrose cushion

Pellet
Microtubules and
Bound Tau

Supernatant
Free Tubulin
And Free Tau

Quantitate Tubulin and Tau in each fraction by
SDS/PAGE (for Tubulin) and
Immunoblotting (for Tau)



DIC (Differential Interference Contrast) vs. Darkfield Microscopy

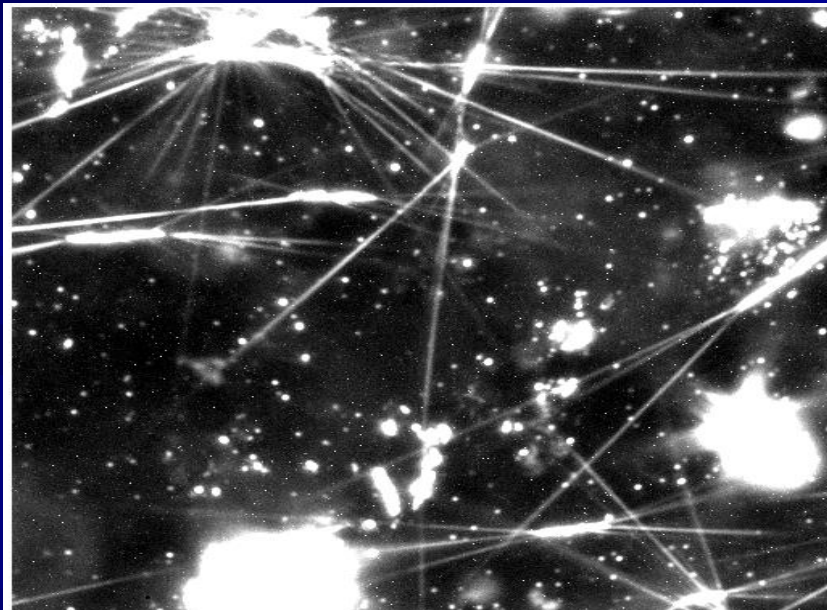
Original DIC image



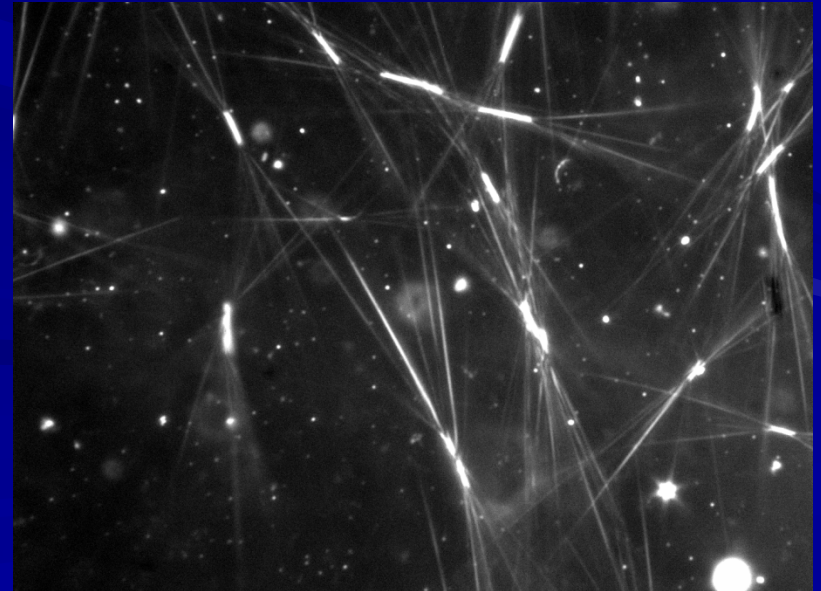
Processed DIC image



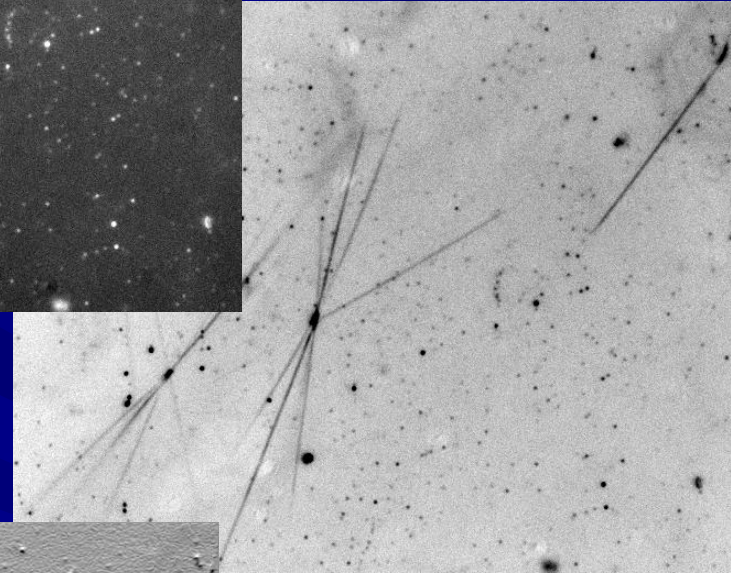
Unprocessed Darkfield Image



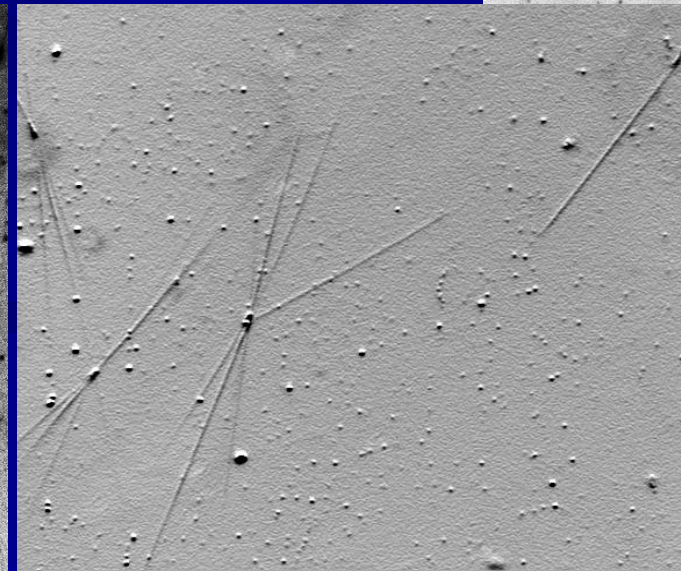
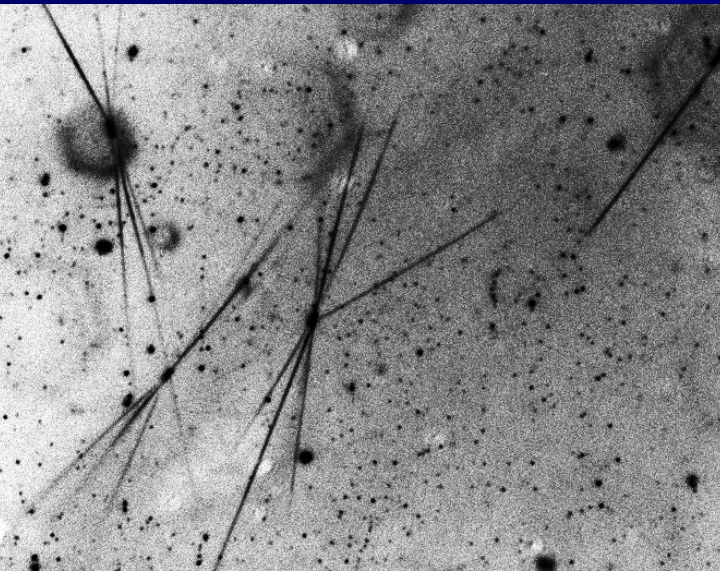
Unprocessed Darkfield Image



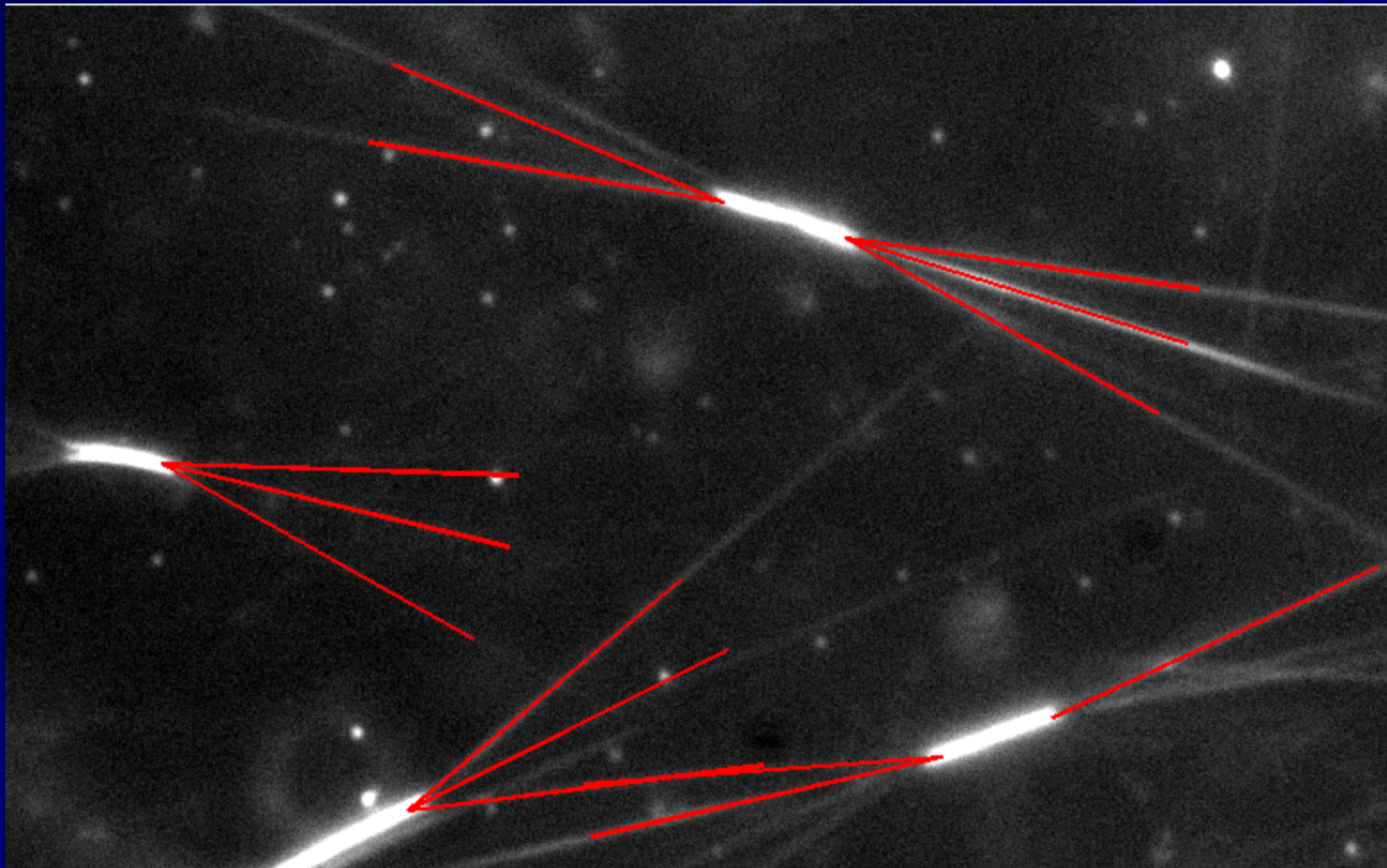
Darkfield Microscopy for In Vitro MT Dynamics



(100X magnification, oil immersion)

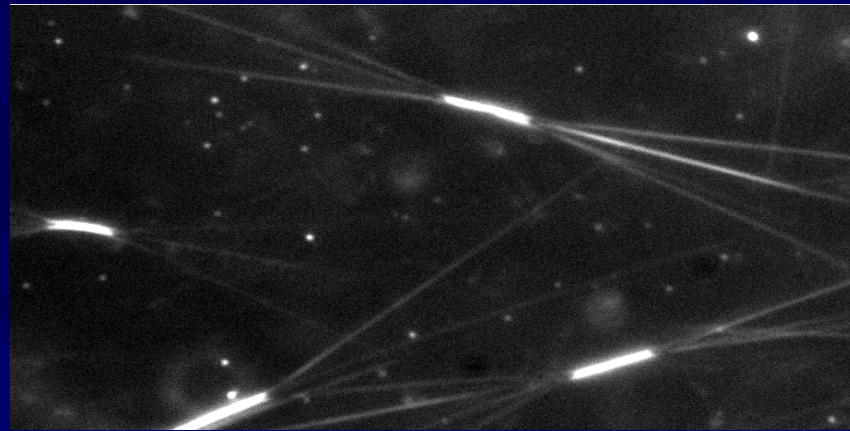


Automated detection and tracking of microtubules

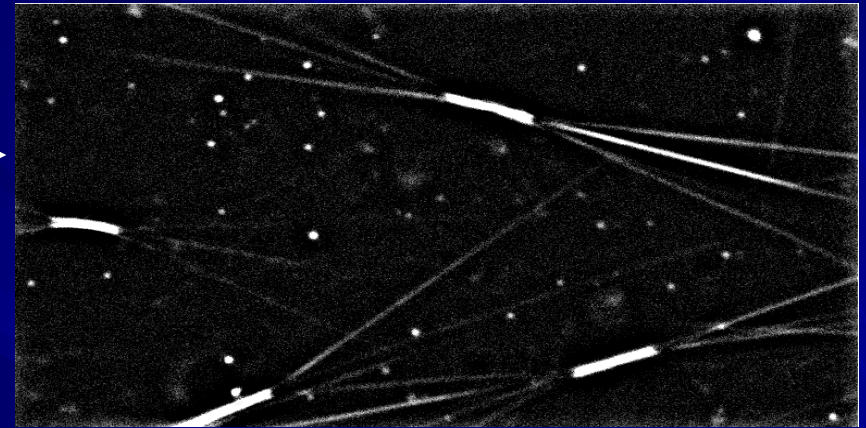


Novel automated detection and tracking methods to assess microtubule dynamics.

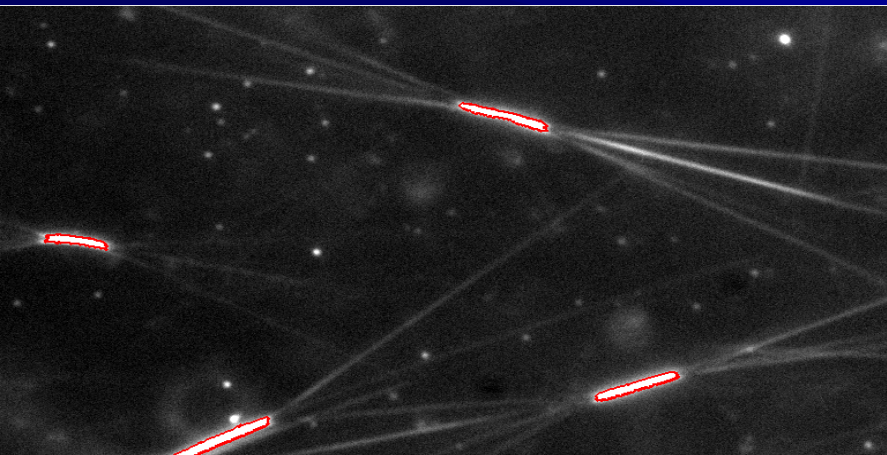
ITR project, www.bioimage.ucsb.edu



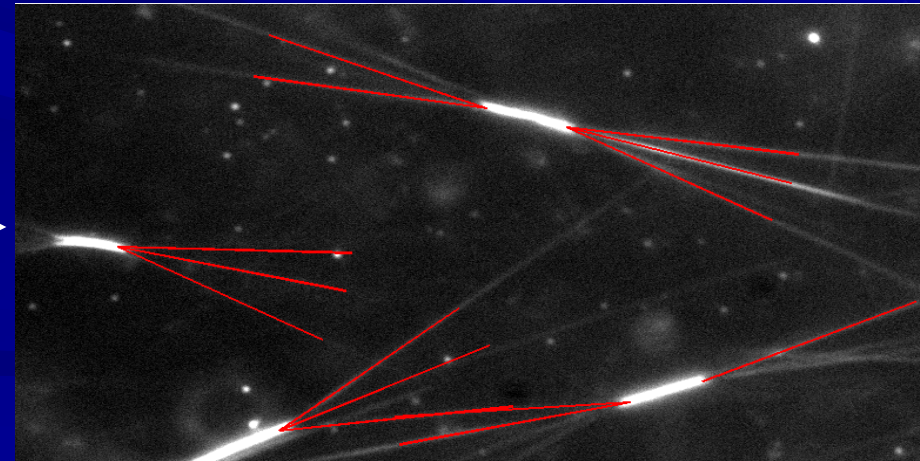
Original Darkfield Image



Processed: Background Subtraction



Detecting Axonemes



Detecting MTs for Automated Tracking