

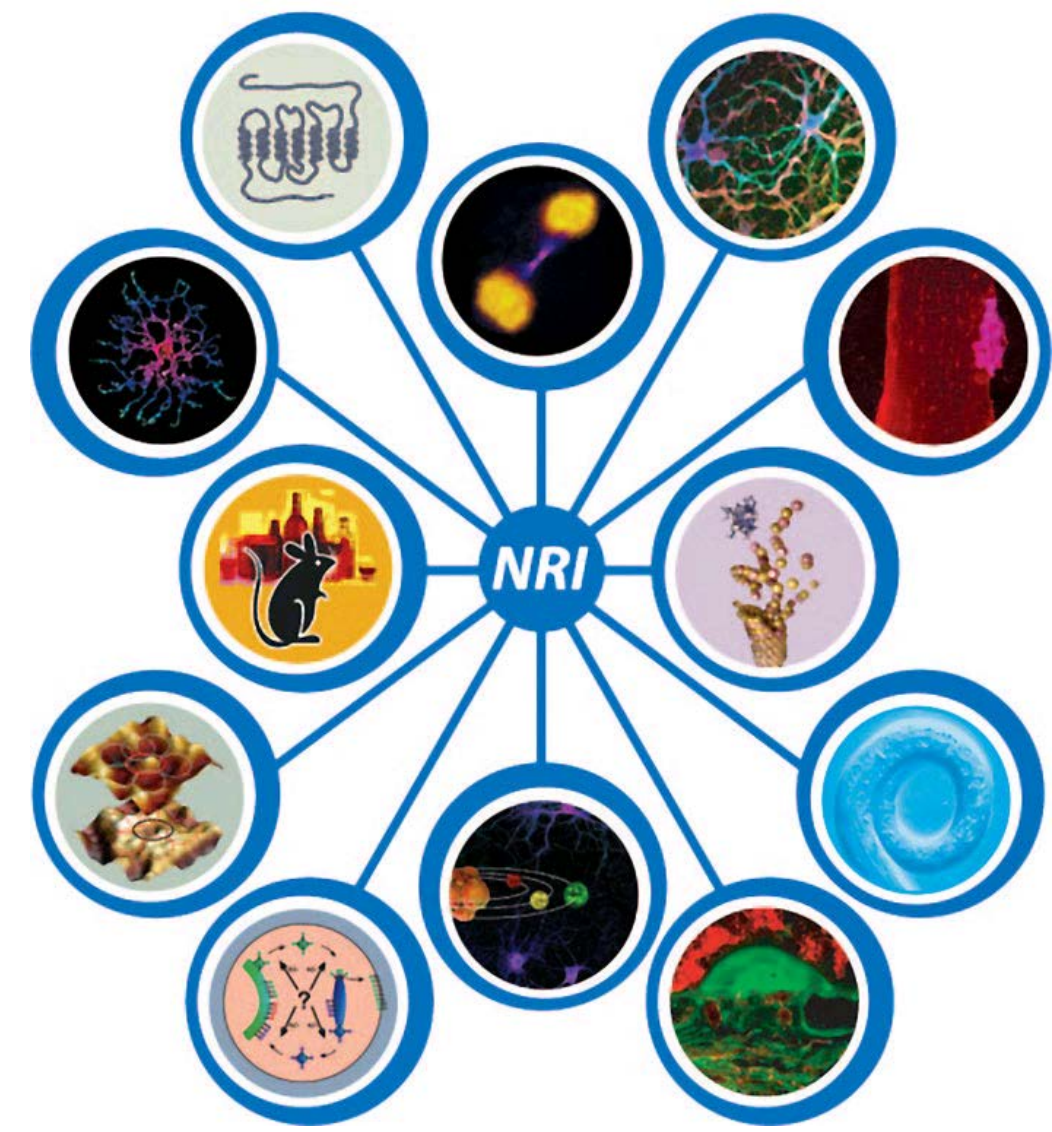
# Effects of Combinatorial Tau Pseudophosphorylation upon its Aggregation Behavior in vitro.

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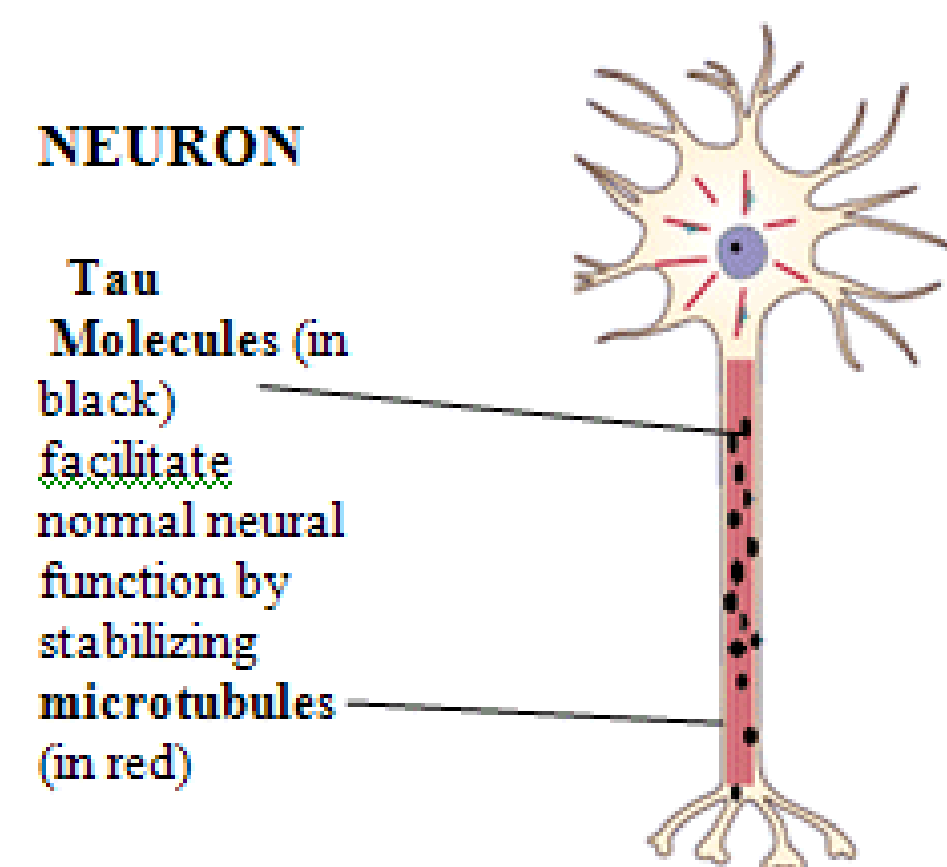
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## Normal Tau Structure and Function

- ❖ Tau is specifically expressed in neural cells and crucial for the proper development and maintenance of the nervous system.
- ❖ Mechanistically, tau binds directly to microtubules and regulates microtubule dynamics, which are crucial for numerous cellular functions, including axonal transport.



## Regulation of Tau Function: Alternative RNA Splicing and Phosphorylation

- Alternative splicing results in six tau isoforms, all of which possess either 3 or 4 imperfect repeats in the C-terminus.  
4RL N — 1 2 3 4 — C  
3RS N — 1 3 4 — C
- Tau is highly regulated by phosphorylation (~30 sites).  
N — \* \* \* \* \* — C

## Tau and Neurodegenerative Disease

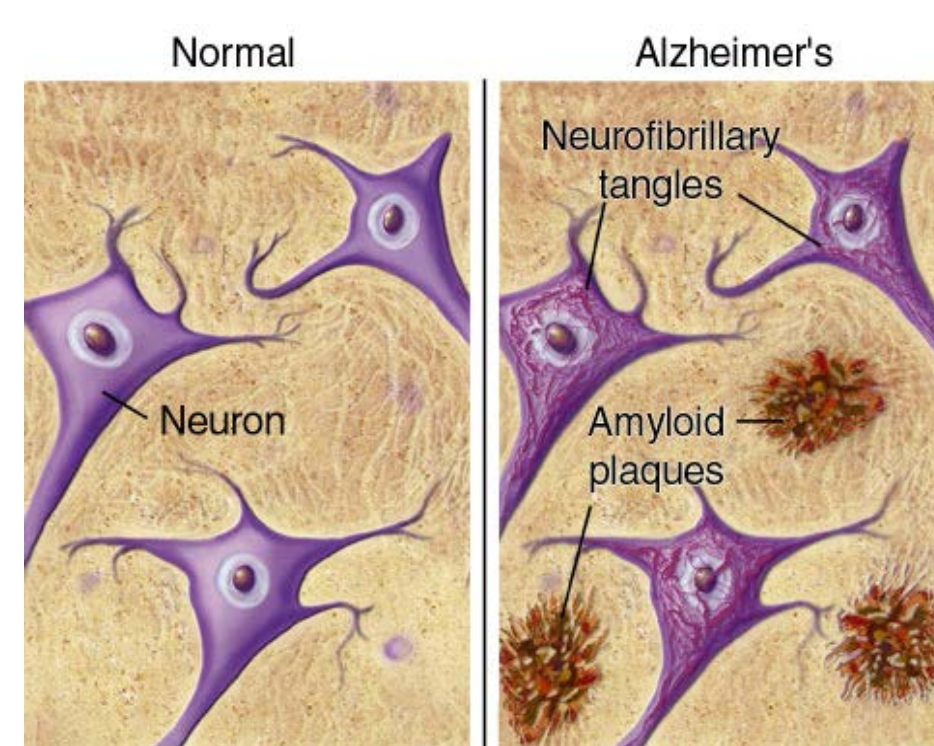
- Pathology:** Tau can form insoluble intracellular aggregates known as neurofibrillary tangles (NFTs), which are a pathological hallmark of many neurodegenerative diseases, including Alzheimers and FTDP-17.
- Genetics:** Mutations in the tau gene have been linked to a group of dominantly inherited neurodegenerative diseases known as FTDP-17.
- Biochemistry:** Abnormal Tau Phosphorylation  
❖ Tau is hyper-phosphorylated in NFTs; thus, abnormal tau phosphorylation has long been proposed as a key step in the Alzheimers pathway (Stoothoff and Johnson, 2005).

## Alzheimer's Disease (AD)

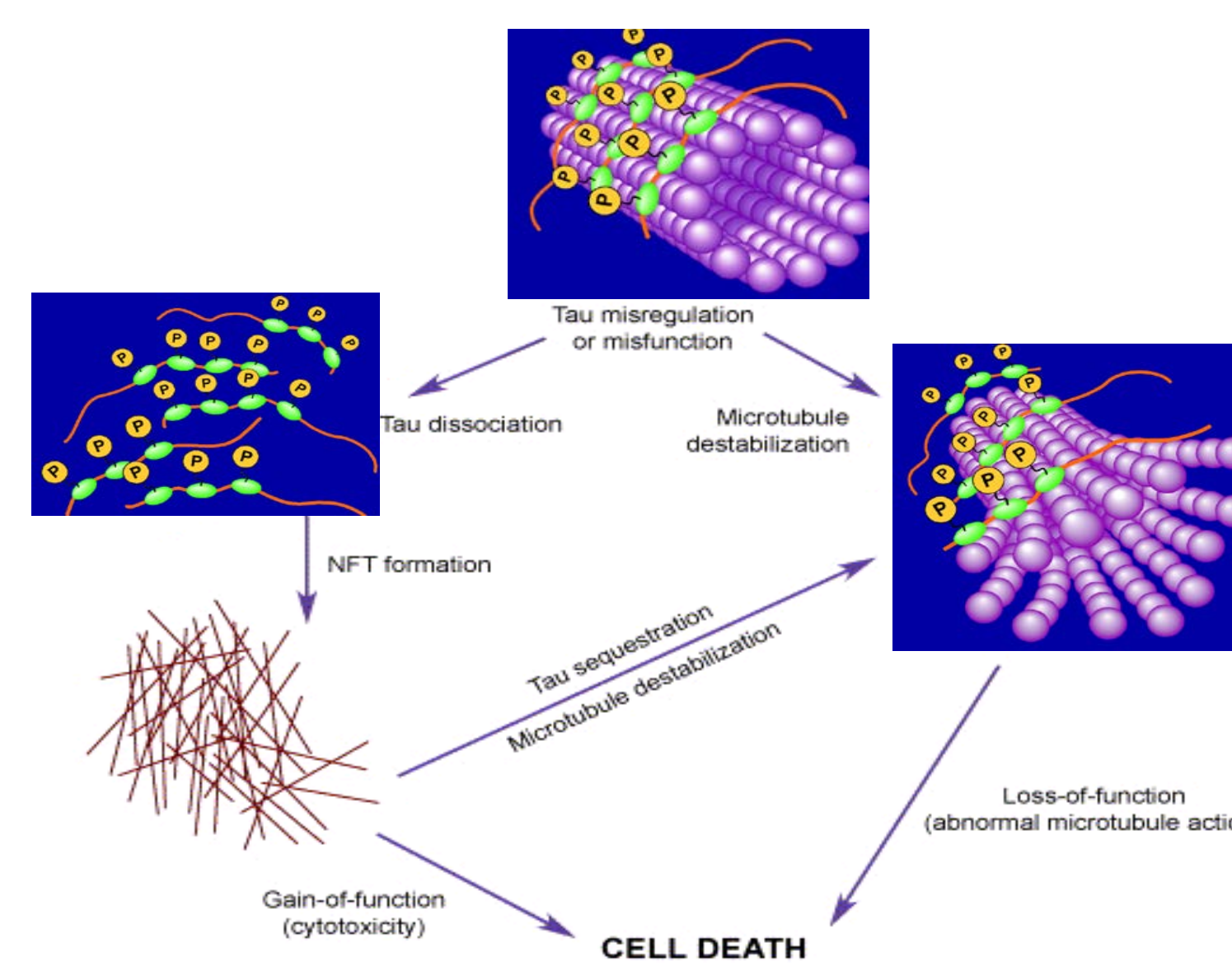
- ❖ In the USA, 4.5 Million people have Alzheimer's disease.
- ❖ It is estimated that by 2050, 13.2 million Americans will have AD if current population trends continue and no preventive treatments or cures become available.
- ❖ Alzheimer's disease is now the seventh leading cause of death in the US and the fifth leading cause of death for those over 65.

## AD Pathology

- Extensive neuronal cell death
- Two abnormal pathological features:  
1) Extracellular amyloid plaques  
2) Neurofibrillary Tangles (accumulated abnormal tau aggregates)

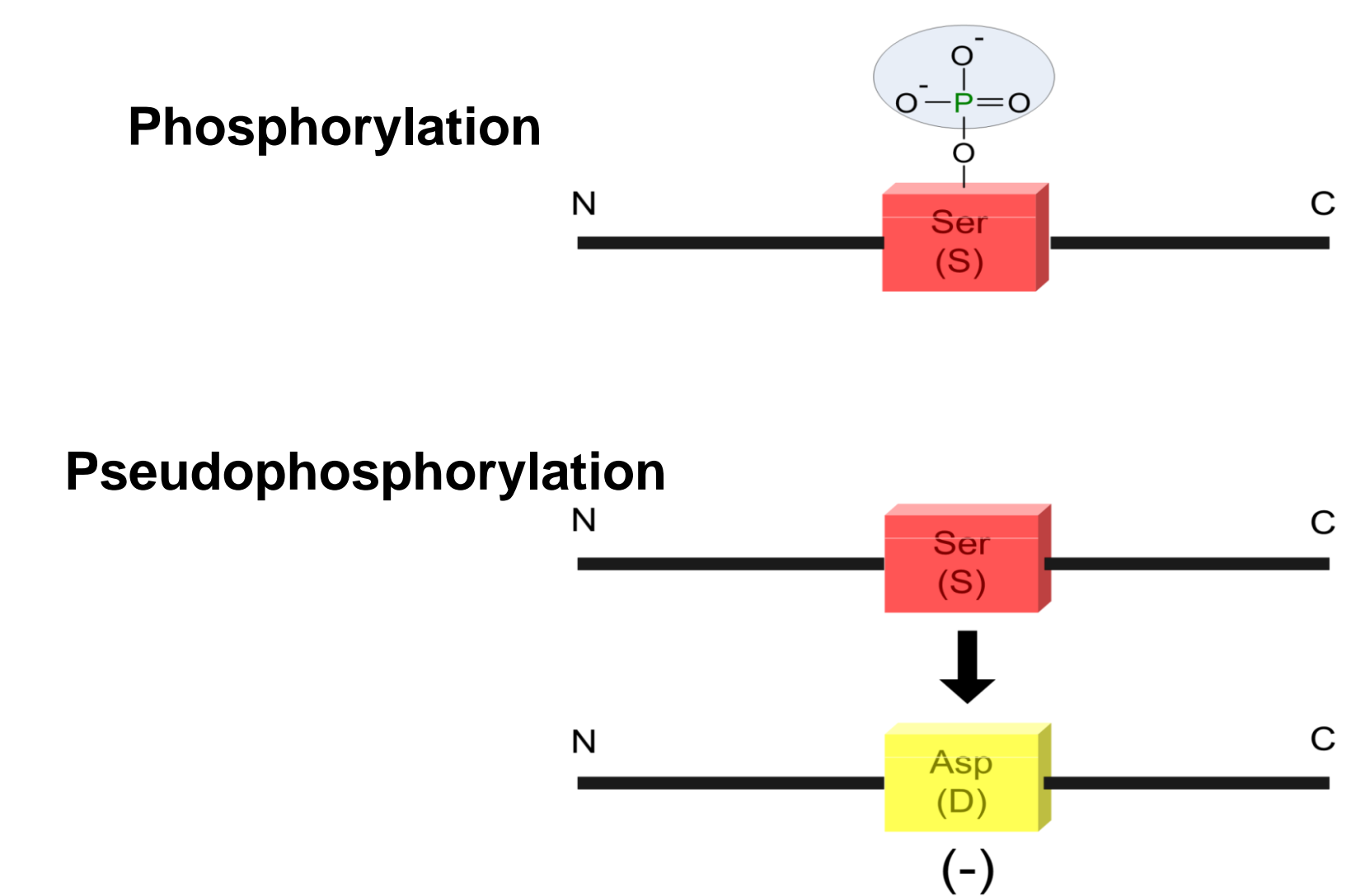


## How might Tau Dysfunction Cause Neurodegeneration?

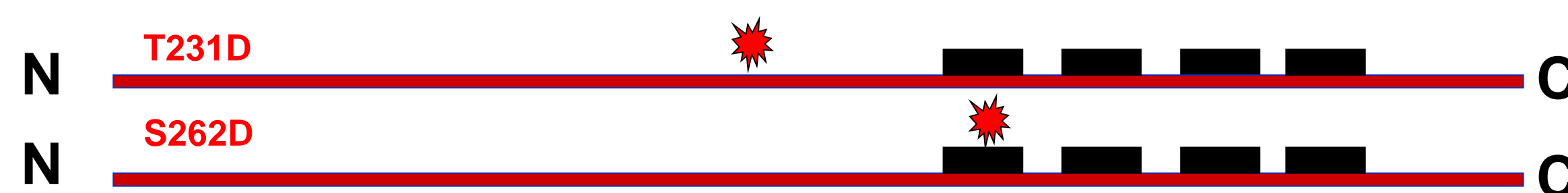


## Experimental Design - "Pseudo-phosphorylation"

"Pseudo-phosphorylation" is a widely used strategy to study phosphorylation at residues of interest. Briefly, aspartic or glutamic acid residues are substituted for particular phosphorylated amino acids, thereby mimicking their chemistry.



## Phosphorylation Sites of Interest threonine 231 and serine 262



## Research Goals

- Study the kinetics of different inducers.
- Assess the effects of individual versus combinatorial tau phosphorylation events upon its aggregation behavior.
  - How does phosphorylation affect tau aggregation?
  - Are the effects of combinatorial phosphorylation simply the sum of the individual events, or rather do they induce a distinct effect?

## DynoPro PlateReader: DLS with an Autosampler

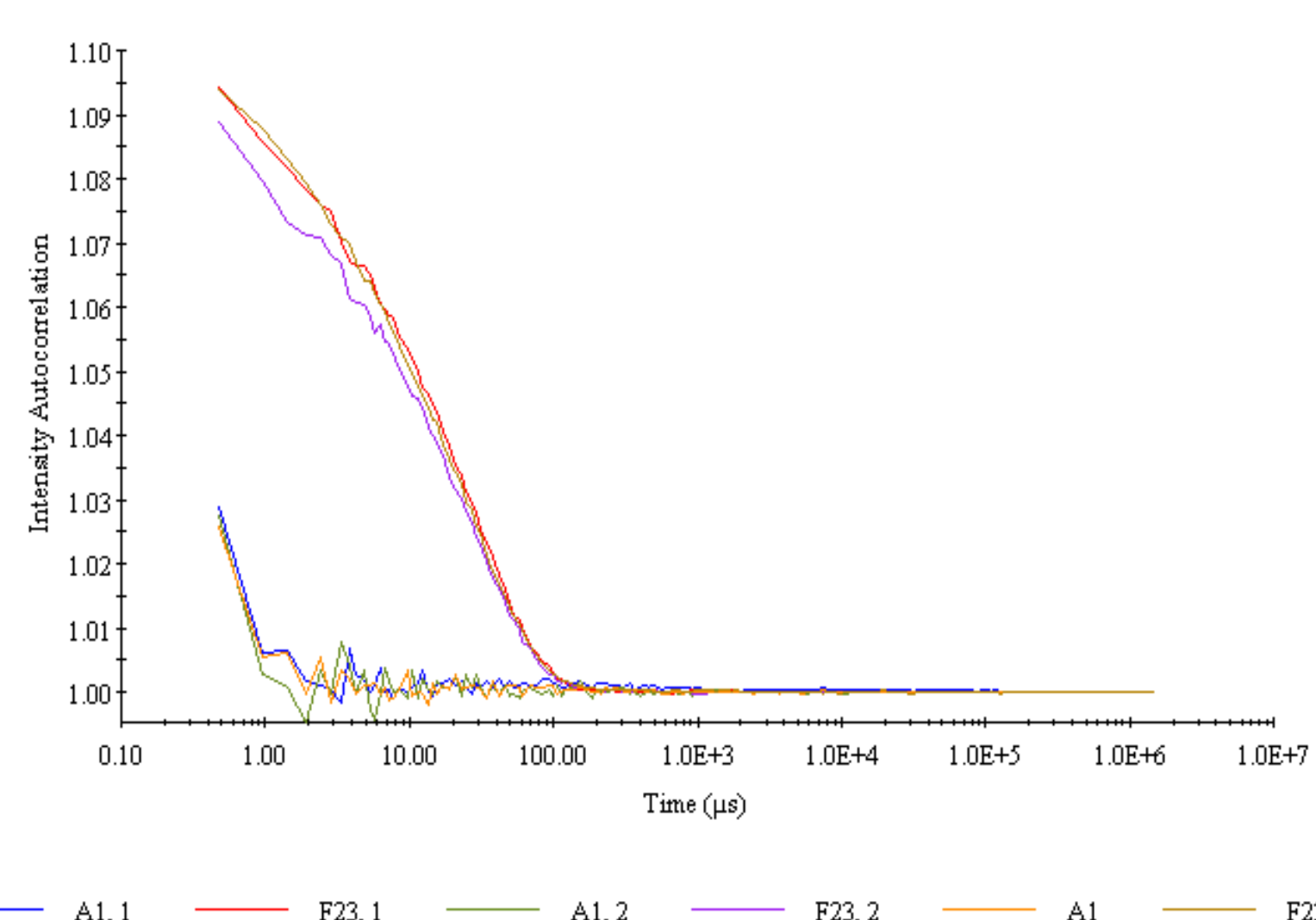


Each solution was placed in four wells  
Each well was measured 20 times during the course of 40 hours  
Each measurement contains 15 2-second acquisitions

The measurements were carried out automatically with Event Schedule in Dynamics Software.

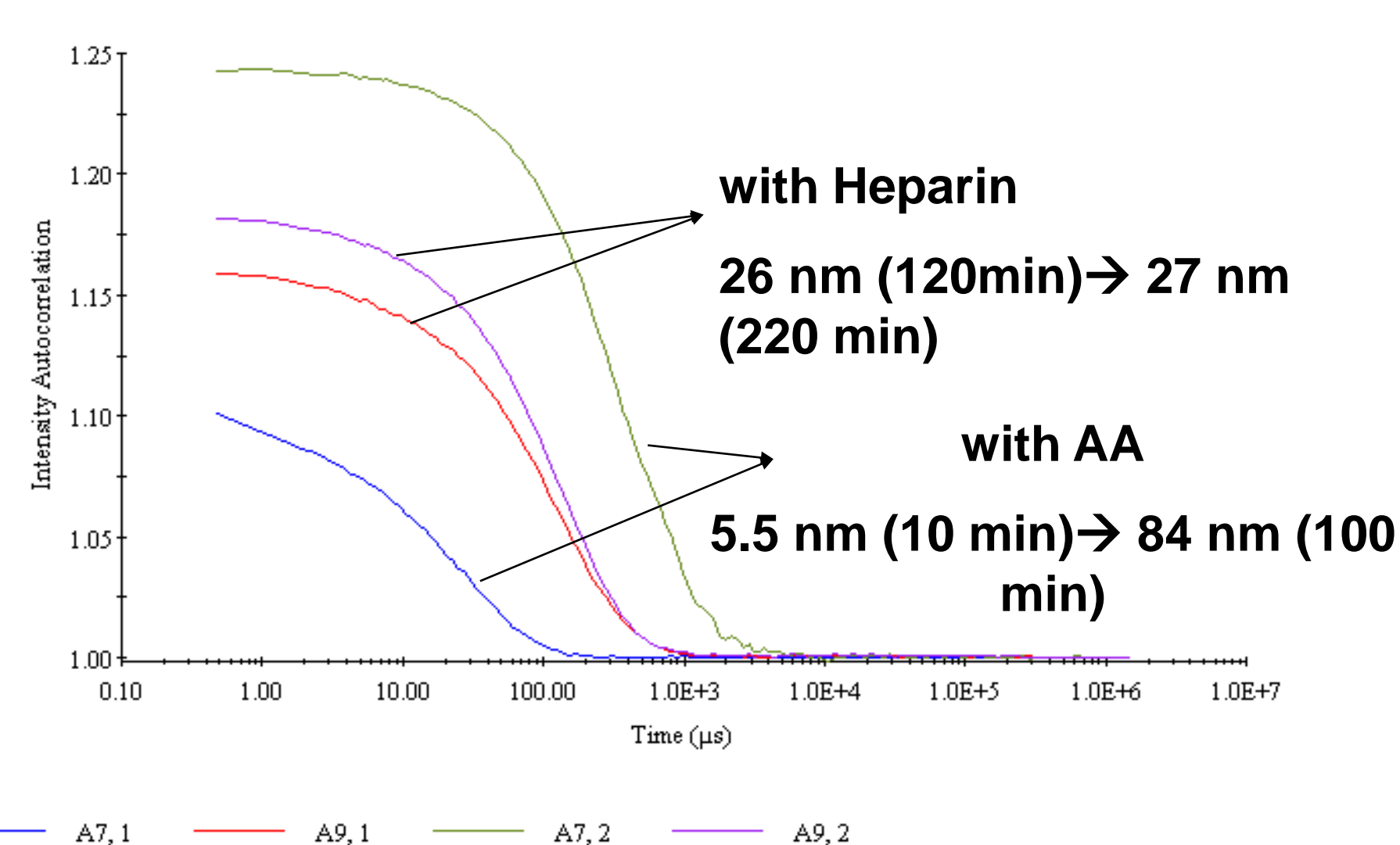
The count rate was optimized at approximately 1 million Hz using the auto-attenuator built in the DynoPro PlateReader.

## One Tau Mutant without Aggregation-Inducer 10, 100, and 2000-minute snap shots



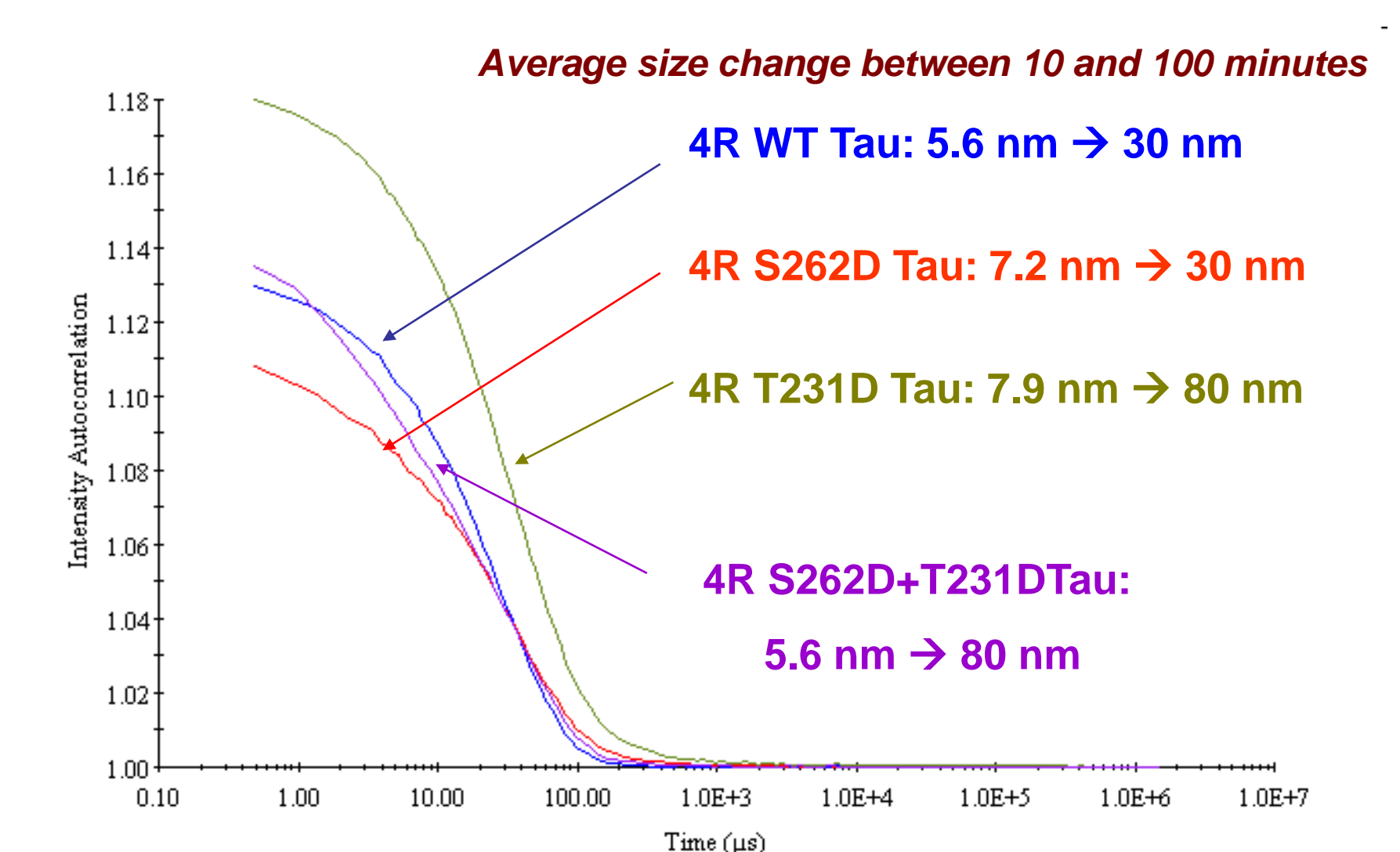
No aggregate was formed without inducer during the course of 2000 minutes.

## Heparin vs. Arachidonic Acid as the Inducer



AA induces the aggregation much faster than heparin.

## Effect of Mutation Sites with AA



The initial rate (0-10 min) of aggregation with phosphorylation at T231D was faster than at S262D and S262D+T231D.