Neurotoxic aggregating protein, such as APP, alpha-synuclein (aSyn), Tau, extracellular matrix (ECM) and prions, share the same behavior from gene activation, to protein synthesis, to folding, misfolding, toxicity and aggregation:

- Translation is up-regulated at the iron responsive element (IRE) by Fe and down-regulated by Iron-Regulatory Protein 1 (IRP-1).
- At normal concentrations they have a benign function.
- At high concentrations they form toxic oligomers.
- Oligomers in the cytoplasm can incorporate into the nuclear envelope and degrade the DNA.
- Cells sequester these toxic oligomers into amyloid aggregates in the cytoplasm.

Posiphen affects translation at the IRE/IRP-1 interface

**Posiphen’s Mechanism of Action**

**Figure 2:** Posiphen increases IRP-1 binding to the 5' untranslated region (5'-UTR) of APP mRNA

Posiphen’s mechanism of action was studied at the molecular level in the laboratory of Jack Rogers. Iron influx increases the translation of APP and aSyn via an IRE RNA stem loop in its 5'-UTR region. IRP-1, but not IRP-2, selectively binds the APP IRE with an affinity of 35 pM. When IRP-1 is bound to the stem loop, it prevents it from binding to the ribosome and prevents translation of the mRNA. The addition of Posiphen to SH-SY5Y human neuroblastoma cells increases the affinity of IRP1 to the stem loop structure from 35 pM to 15 pM and decreases the rate of translation of the mRNA by the ribosomes.

In summary, Posiphen enhances the binding of IRP1 to the APP stem loop in the 5'-UTR of its own mRNA without changing the binding to the IRP of H-ferritin mRNA, thereby lowering mRNA synthesis and the generation of the toxic protein. The presence of Posiphen increases the affinity of IRP-1 resulting in decreased APP mRNA translation rate.

**Confirmation of Mechanism of Action in Tissue Culture, Mice and Cognitively Impaired Patients**

Posiphen was shown to lower APP/Ab, Tau/p-Tau and aSyn levels in human and mouse lines of neurodegenerative cells.

- **Wild type normal mice**
- **Transgenic Alzheimer mice**
- **Tauopathies**
- **Transgenic Parkinson mice**
- **Human mildly cognitive impaired patients**
- **Posiphen rescues long term potentiation in brain slices in a dose dependent manner**

**Figure 3:** Assessing Posiphen’s ability to rescue synaptic dysfunction in hippocampal slices

Posiphen rescues long term potentiation in brain slices in a dose dependent manner.

- **APP/PS1** + veh vs WT+veh, p<0.0001
- **APP/PS1** + 0.1 mg Posiphen vs WT+veh, p<0.0001
- **APP/PS1** + 0.5 mg Posiphen vs APP/PS1 + 25 mg, p=0.0004

**Figure 4:** Testing in contextual fear conditioning

Posiphen (10 and 25 mg/kg oral) ameliorates the contextual fear memory deficit in APP/PS1 transgenic mice (p=0.0035)

**Figure 5:** Effect on spatial memory in radial water maze

Posiphen totally recovers memory of the double transgenic mice as compared to wild type normal mice. It statistically improves the spatial memory in a dose dependent way (p=0.0034).

**Figure 6:** Staining for senile plaque in treated and untreated double transgenic mice

Posiphen inhibits plaque formation in the brains of the transgenic mice. Posiphen is a promising drug to treat neurodegenerative disorders, such as AD and PD.

**Conclusion**

Posiphen totally restores brain function, cognition and memory in transgenic Alzheimer mice treated chronically and inhibits plaque deposition in their brains. Posiphen shows target engagement in all species tested and reverses cognitive deficits in AD mice. Posiphen is a promising drug to treat neurodegenerative disorders.