In-silico identification of **Tyrosine nitration sites** in protein peptide sequences

Sourajyoti Datta

Supervisor: **Muhammad Nabeel Asim**

Thesis motivation

Summer Semester 2022

German Research Center for Artificial Intelligence

Post-translational modification in Proteins

- **Proteins** are biomolecules/macromolecules containing long chains of amino acid residues
  - **Perform critical functions within organisms**, such as catalyzing metabolic reactions, DNA replication, responding to stimuli, providing structure to cells and organisms, and transporting molecules.

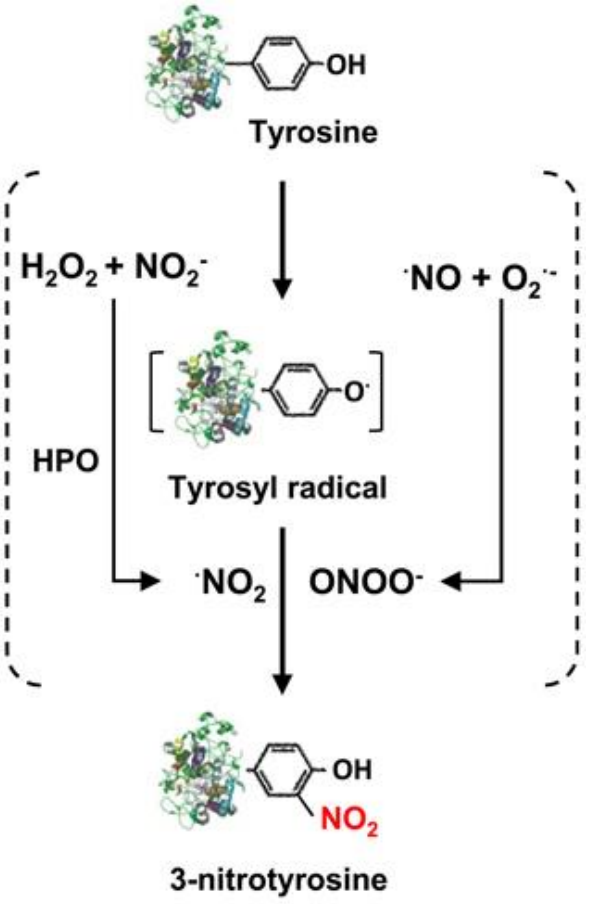
- **Protein biosynthesis** is a core biological process occurring inside cells
  - **Balances the loss of cellular proteins** in cells by producing new proteins
  - Occurs in two stages:
    - **Transcription:** A section of DNA encoding a protein (gene), is converted into a messenger RNA (mRNA).
    - **Translation:** The nucleotide sequence of mRNA is read by ribosomes to determine the sequence of amino acids in the resulting Protein

- **Post-translational modification (PTM)** is the modification of proteins after protein biosynthesis.
  - **Influences normal cell biology and pathogenesis**, and **increase the functional diversity of the proteome**
  - ***Modifications***: covalent addition, proteolytic cleavage of regulatory subunits, or degradation of entire proteins.
  - **~460** different types of PTMs have been identified

- ***Identifying and understanding PTMs is critical for studying cell biology, and disease treatment or prevention.***

Tyrosine nitration

- **Nitrotyrosine** is the product of **Tyrosine nitration**
  - It is a **covalent Post-translational modification** (PTM) in proteins
  - An **irreversible nitrative modification** of the tyrosine residue of a protein and **permanently alters the structure** of the protein
  - Result of the **substitution of a hydrogen by a nitro group** ($–NO_2$) at the ortho position of the phenolic ring of tyrosine
    - Two nitrating agents account for the nitration, i.e., **peroxynitrite** ($ONOO^-$), and **hemoperoxidases** (HPO) in the presence of hydrogen peroxide (H_2O_2) and nitrite (NO_2^-)
  - **Tyrosine nitration is selective, and not a random process**
    - **Depending on the accessibility of the tyrosine residues** to the nitrating agents (e.g., tyrosine residues exposed on the surface of proteins can become target)
    - Mostly, the nitrated tyrosine residues are in the **vicinity of a site which is generating nitrating agents**



Cause and effect of Tyrosine nitration

- **Causes:**
  - Determined by several factors, such as reactive **species**, reaction kinetics, presence of **antioxidants** and **radical scavengers**, and **compartmentalization**
  - **Two pathways for nitration:**
    - When **Nitric Oxide (NO^-) is generated in high concentration**, in the **presence of Superoxide anion** (**O_2^-**) it will lead to the rapid formation of **peroxynitrite ($ONOO^-$)**, which causes nitration
    - **Peroxynitrite-independent nitration** depends on the presence of **transition metals such as iron and copper**, through either peroxidase-dependent or Fenton-dependent pathways in the presence of **hydrogen peroxide (H_2O_2)** and **nitrite (NO_2^-)**

- **Effects:**
  - **Substantial changes** in the **biological function** of **proteins**
  - The **structural alteration** leads to **loss or augmentation of protein function**
    - Can render a protein inactive
  - Inhibits protein phosphorylation that **impedes the normal transduction pathways in cell signaling**
  - **Functional loss** of proteins in **inflammatory diseases**
  - **High correlation** with **pathogenesis of diseases**
  - **Marker** of NO-dependent, reactive nitrogen species (RNS) induced **nitrative stress**

Benefits of identifying Tyrosine nitration sites

- Causal nitration pathway inducing agents identified in various disease conditions**:**
  - **Nitric Oxide** (**NO**) is produced at a high rate in **inflammatory, stimuli-induced** conditions
  - **Nitrite ion** (**NO_2^-**) is greatly increased in **systemic inflammatory disorders** (sepsis, gastroenteritis, & hemolytic diseases)
  - Abnormal elevation of **copper ion** (**Cu^{2+}**) and free heme in the **pathogenesis** of **type 2 diabetes** mellitus, **neurological** disorders, and severe **hemolytic** diseases

- Tyrosine nitration is identified in large number of **pathological conditions**
  - **Neurodegenerative diseases** - Parkinson's and Alzheimer's, degeneration of dopamine neurons, cerebral ischemia and edema
  - **Cardiovascular diseases**
  - **Autoimmune diseases** - Rheumatoid Arthritis, Systemic Lupus Erythematosus
  - **Carcinogenesis** – Breast, Esophageal and Gastric cancer; Colorectal, Squamous cell, Adeno- and Cholangial carcinoma

- Therefore, **effective interception of protein nitration** can represent novel and critical points of **therapeutic intervention in diseases** associated with protein nitration.
  - Identifying onset and progression of the associated disease
  - Surrogate markers for the design of clinical interventions (therapeutic strategies and drugs)

Motivation for computational methods

- **PTMs** of proteins experimentally **detected** and **recorded** by a variety of techniques
  - **Experiments**: Immunohistochemical analysis, Chromatography, Mass spectrometry with prior immunoprecipitation, Eastern and Western blotting
  - **Datastores**: PhosphoSitePlus, ProteomeScout, Human Protein Reference Database, PROSITE, Protein Information Resource (PIR), dbPTM, Uniprot, O-GlcNAc Database

- **Huge amounts of data** available for knowledge discovery
  - Resulted in increasing use of Machine Learning and Deep Learning approaches

- **Wet lab experimental approaches:**
  - Technically challenging with Theoretical limitations
  - Labor intensive
  - Requires skilled laboratory experience
  - Time consuming
  - Expensive
  - Biases in proteome wide identification
  - ***Generates ground truth data***

- **Computational approaches:**
  - Easier development
  - Comparatively, much less labor intensive
  - Requires coding skills
  - Reusability saves time
  - Economical
  - Ability to balance biases in imbalanced scenarios
  - ***Requires wet-lab experiments to generate data***

Thank you for your time!

Questions?

References

1. Radi R. (2013). Protein tyrosine nitration: biochemical mechanisms and structural basis of functional effects. *Accounts of chemical research*, *46*(2), 550–559. https://doi.org/10.1021/ar300234c