

Unfolding the Truth: Alpha-Synuclein as a Model System to Explore Gas-Phase Activation Mechanisms and Biomolecular Dynamics

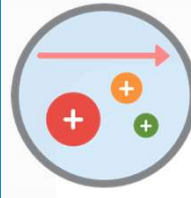
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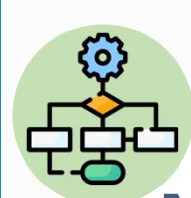
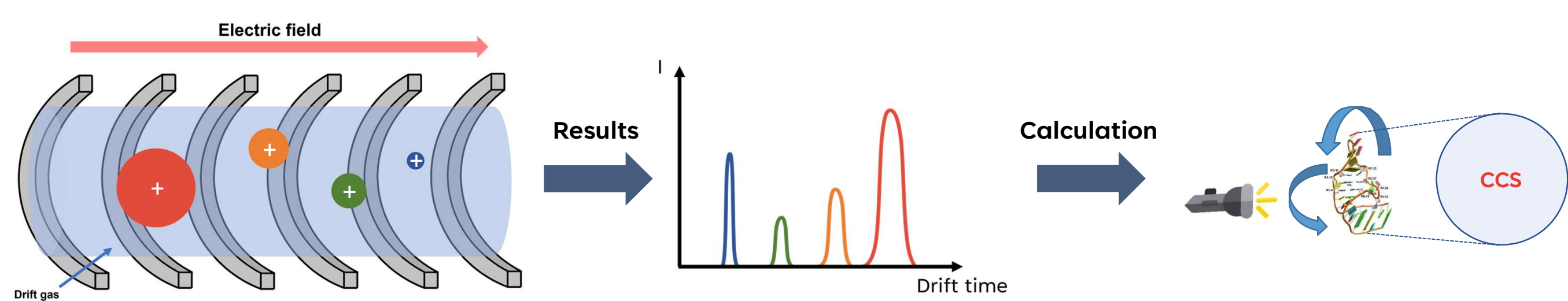
Introduction

Recent advances in **ion mobility-mass spectrometry** have enabled the rapid characterization of **biomolecular structures** in the **gas phase**, offering new perspectives beyond **traditional techniques** that **require extensive sample preparation**. **Collision-induced unfolding (CIU)** provides **energetic fingerprints** of **protein** and **protein-ligand complexes**, revealing their **stability** and **conformational dynamics** with high sensitivity. However, the **mechanisms** behind these methods are still **poorly understood**.



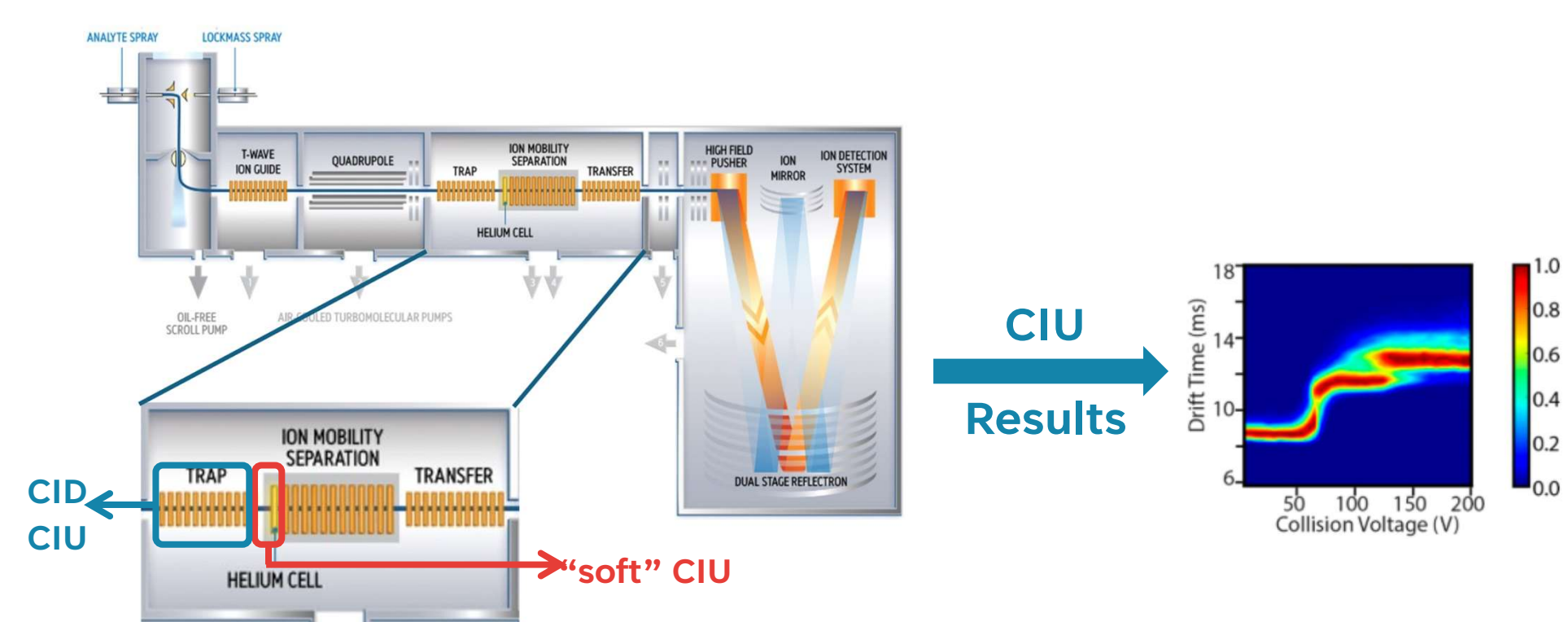
Ion mobility

- ◆ Separates **ions** based on their **charge** and **collision cross section (CCS)**.
- ◆ A **drift time** is measured and can then be used to obtain the **CCS**.



Methods and workflow Collision-induced unfolding

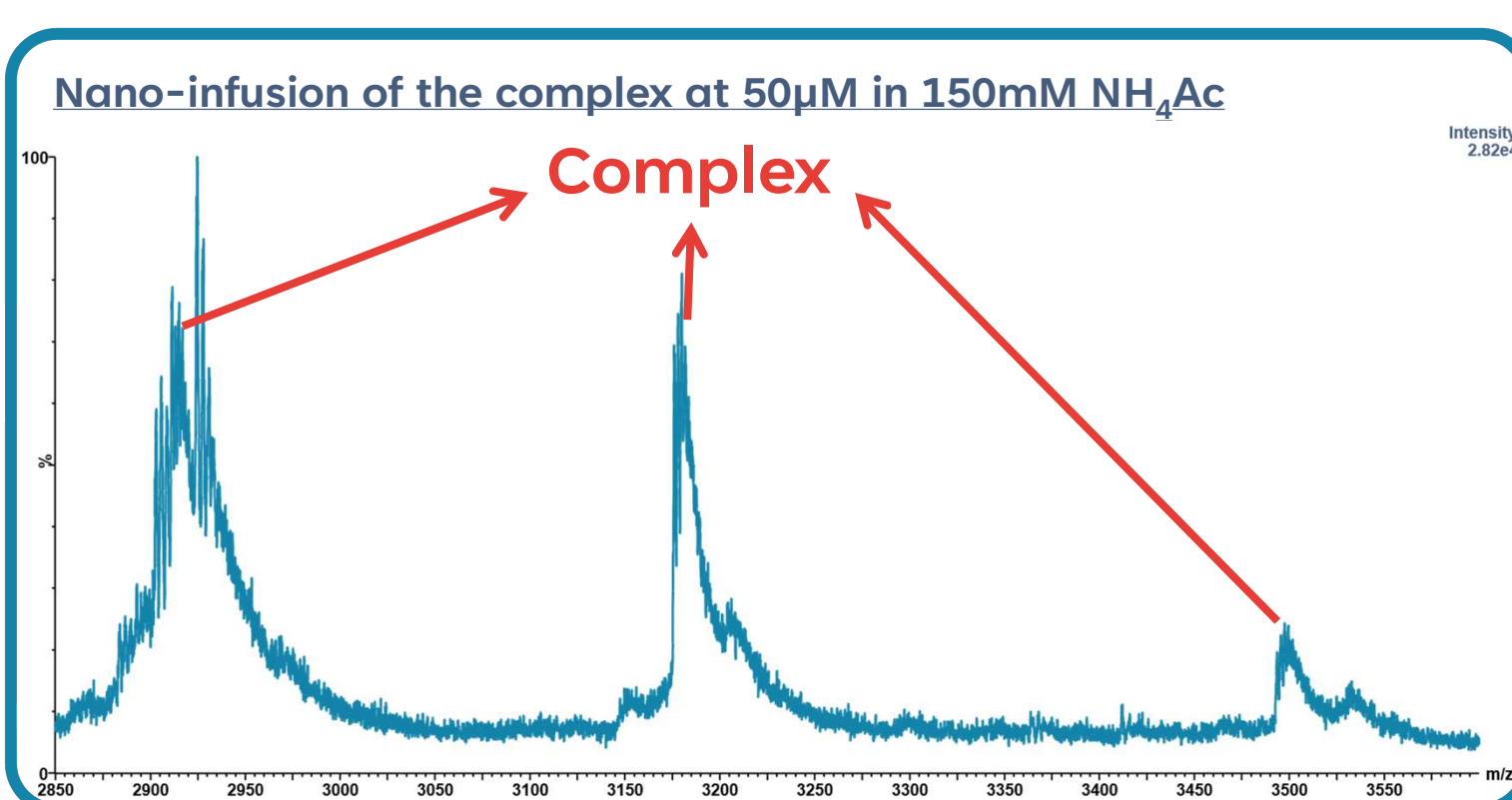
- ◆ Energy-resolved **collision** experiments monitored by **IMS**
- ◆ The aim is to induce a **structural change, not fragmentation**
- ◆ Multiple **parameters** will be modified to assess their **impact**:
 - Type of gas (N₂, He, Ar,...)
 - Gas pressure
 - Trapping time
 - Acceleration voltages



Results

An **optimization** of the **sample preparation** and the **MS parameters** was conducted.

The MS parameters were optimized in **positive ion mode** and using both **ESI** infusion and **nano-ESI**. The spectrum below displays an **optimized nano-ESI** acquisition of the **complex solution** in **positive ion mode**.



Sample preparation:

- ⌚ No incubation time needed
- 🧪 Buffer: NH₄Ac (MS compatible)
- 🧴 Desalting required



Conclusion & perspectives

- ✓ **Sample preparation was optimized for complex formation**
 - ➔ No incubation time seems to be required, unlike **solution experiments**³
 - ➔ MS compatible **buffer** was found, and a **desalting procedure** was developed
- ✓ **Protein-aptamer complex was successfully observed in MS**
 - ➔ The **complex** was observed in **positive ion mode**
 - ➔ Both **ESI** and **nanoESI** were tested, with **nanoESI** displaying **better resolution** for the **complex**
 - ➔ **Better results** were obtained on a **high mass-modified Synapt G2-S** (Waters)

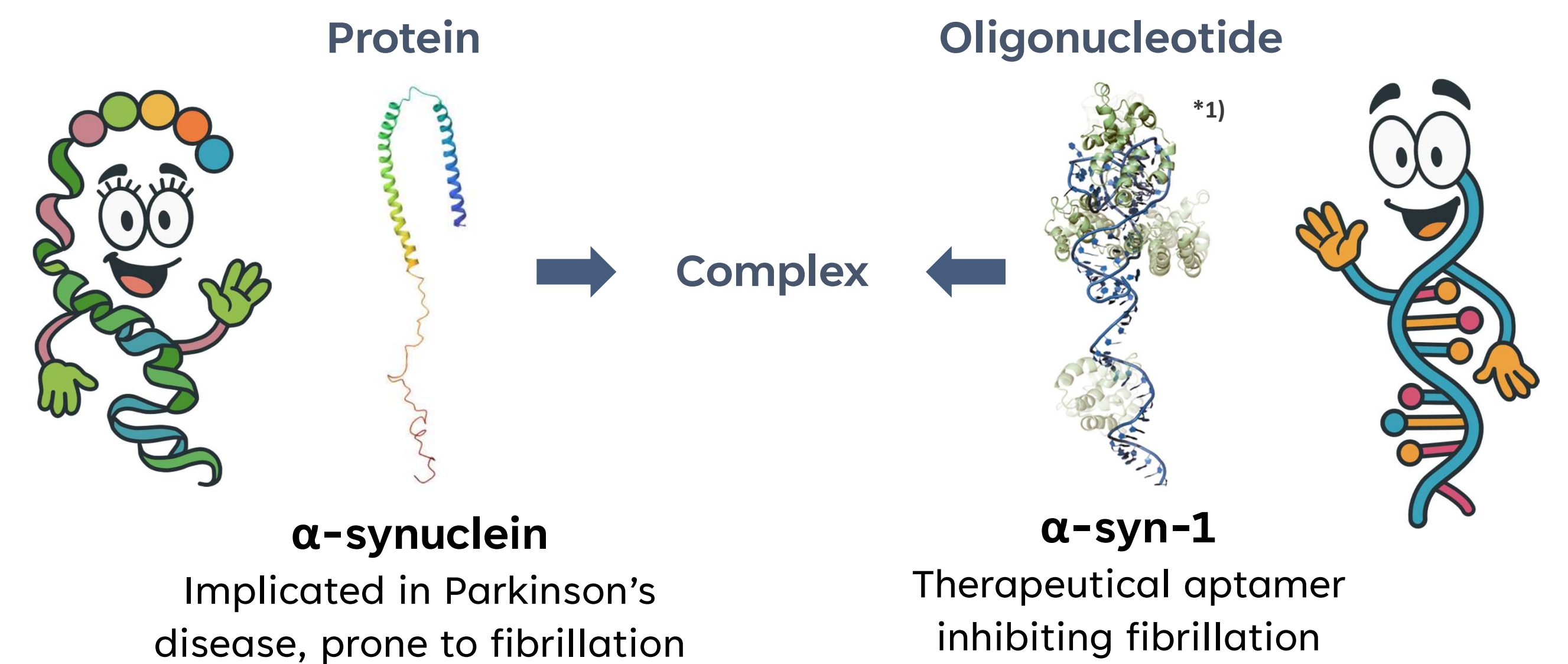


Objectives

Better understand **activation mechanisms** in the gas phase

Get insights on the **binding of the aptamer to the protein**

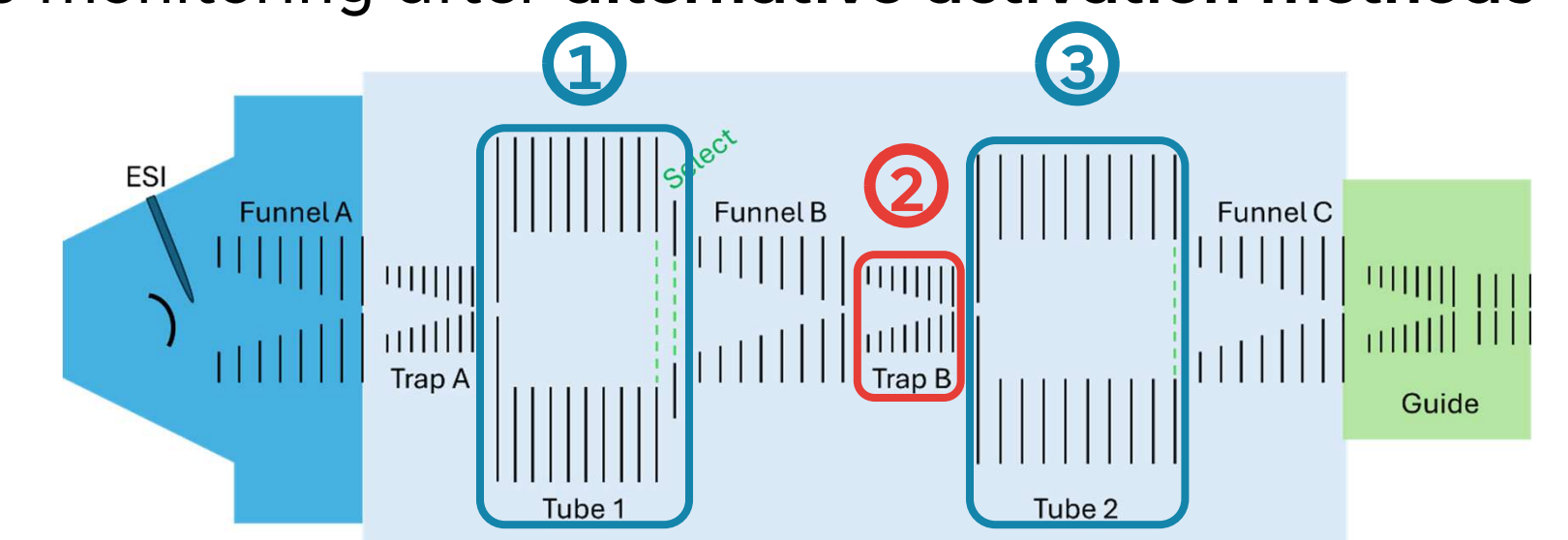
Models



*3D structure of a different aptamer, for illustration purposes

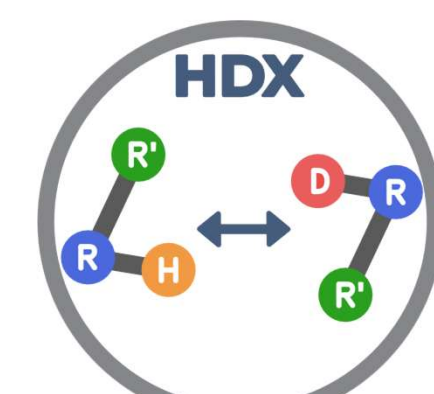
Alternative activation methods

- ◆ Utilization of a **tandem IMS** equipment for IMS monitoring after **alternative activation methods**
 - 1 Selection of ions by their mobility
 - 2 Activation: IR irradiation or heated gas
 - 3 IMS analysis of the activated ions

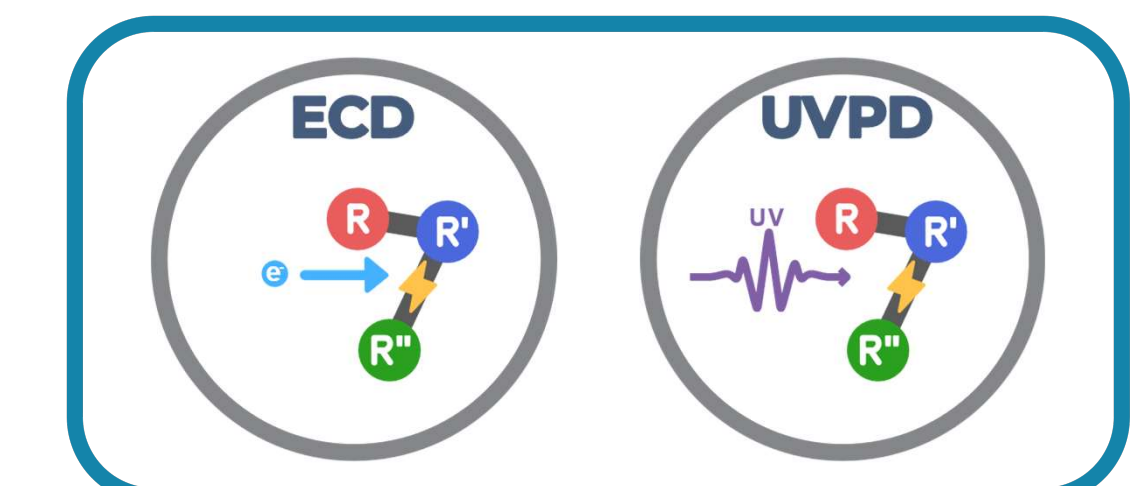


Further investigations

To obtain more information on the **binding mechanisms** and **unfolding pathways** of the models, other methods will be investigated



Gas-phase Hydrogen-deuterium exchange



Non-ergodic fragmentation methods



Potential applications

Fundamental study of the dynamics

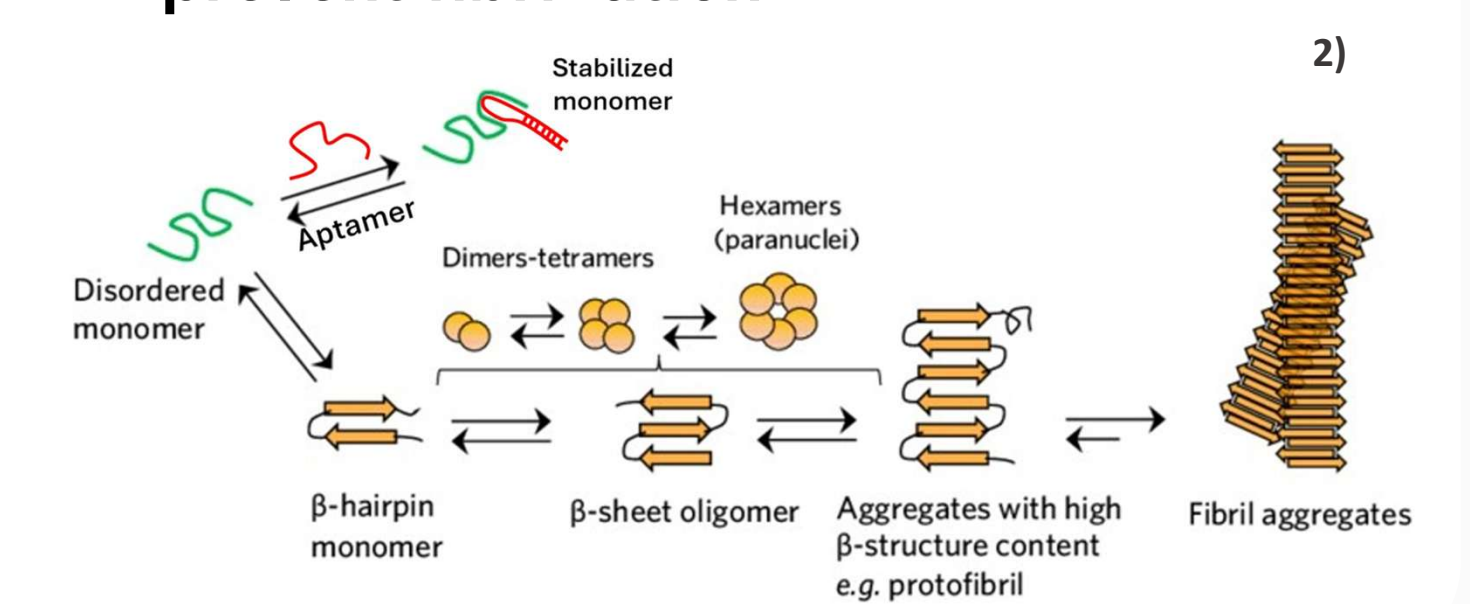
- Better understanding of **“unfolding” mechanisms**
- Study on **specific models** (IDP, ONs, complexes)

Novel analytical approaches

- Fast and sensitive method to obtain **structural information**
- Determination of the **“energetic fingerprint”** of molecules, for efficient discrimination

Biomedical and therapeutical

- Insights on the **alpha-synuclein structure** and potential **fibrillation pathways** in the **gas phase**
- Characterization of the **complex properties** in the gas phase, which will help **design more efficient ligands to prevent fibrillation**



References

- 1) Joseph, D. F. et al. *PLOS ONE* 14, e0211756 (2019)
- 2) Rahman, M. (2018), <https://www.researchgate.net/publication/329360634> [accessed 26 Jun 2025]
- 3) McConnell, E. M. et al. *Molecular Therapy Nucleic Acids* 35, (2024)

Contacts



Contact and partners information



What's next?

- Investigate **negative ion mode** in MS for the **complex**
- Conduct **IMS experiments** in **both ion modes** for the **complex** and the **monomers**
- Investigate **ESI** on the **Synapt G2-S**
- Start on the **activation experiments** with **CIU**