# UNRAVELING THE CONFORMATIONAL DYNAMICS OF STAPHYLOKINASE

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## BACKGROUND

The structural proteomic approach, employing hydrogen-deuterium exchange coupled with mass spectrometry (HDX-MS), supported by bioinformatics and computational modelling can unravel a plethora of information about protein dynamics. Using this state-of-the-art technique in understanding the in-solution conformational dynamics of a potential thrombolytic drug, Staphylokinase (SAK), will play a crucial role in providing important targets for protein engineering to develop a highly efficient, and alternative thrombolytic drug to Alteplase, which remains the sole FDA-approved drug for the treatment of ischemic stroke .[1]

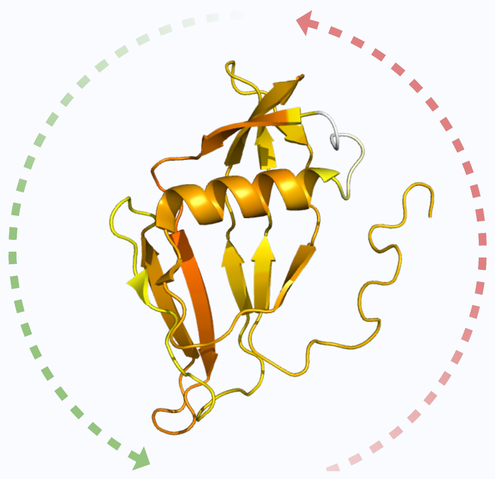
## OBJECTIVES

- To decipher the in-solution dynamics of SAK in:
  - Apo form (wild-types and mutants)
  - Holo form (wild-types and mutants with interaction partners: plasmin and plasminogen)
- To understand the impact of mutations on SAK dynamics.

## METHODOLOGY

### In-solution conformational dynamics of SAK

- **Hydrogen-deuterium exchange coupled with mass spectrometry**
  The structure of SAK in its unbound and bound forms with plasmin and plasminogen will be determined.
- This will provide baseline data to further improve the design of mutants.



### Protein engineering and biochemical characterization

- **Computational and experimental approaches**: Affilib, Ribosome display, machine learning, RF diffusion
- **Computational validation** by MD simulations
- **Fibrin plate assay** : clot dissolving activity
- **Pharmacokinetics**: to check half-life
- **Immunogenicity testing**: *in-vitro* assays

## EXPERIMENTAL DESIGN

### Peptide mapping

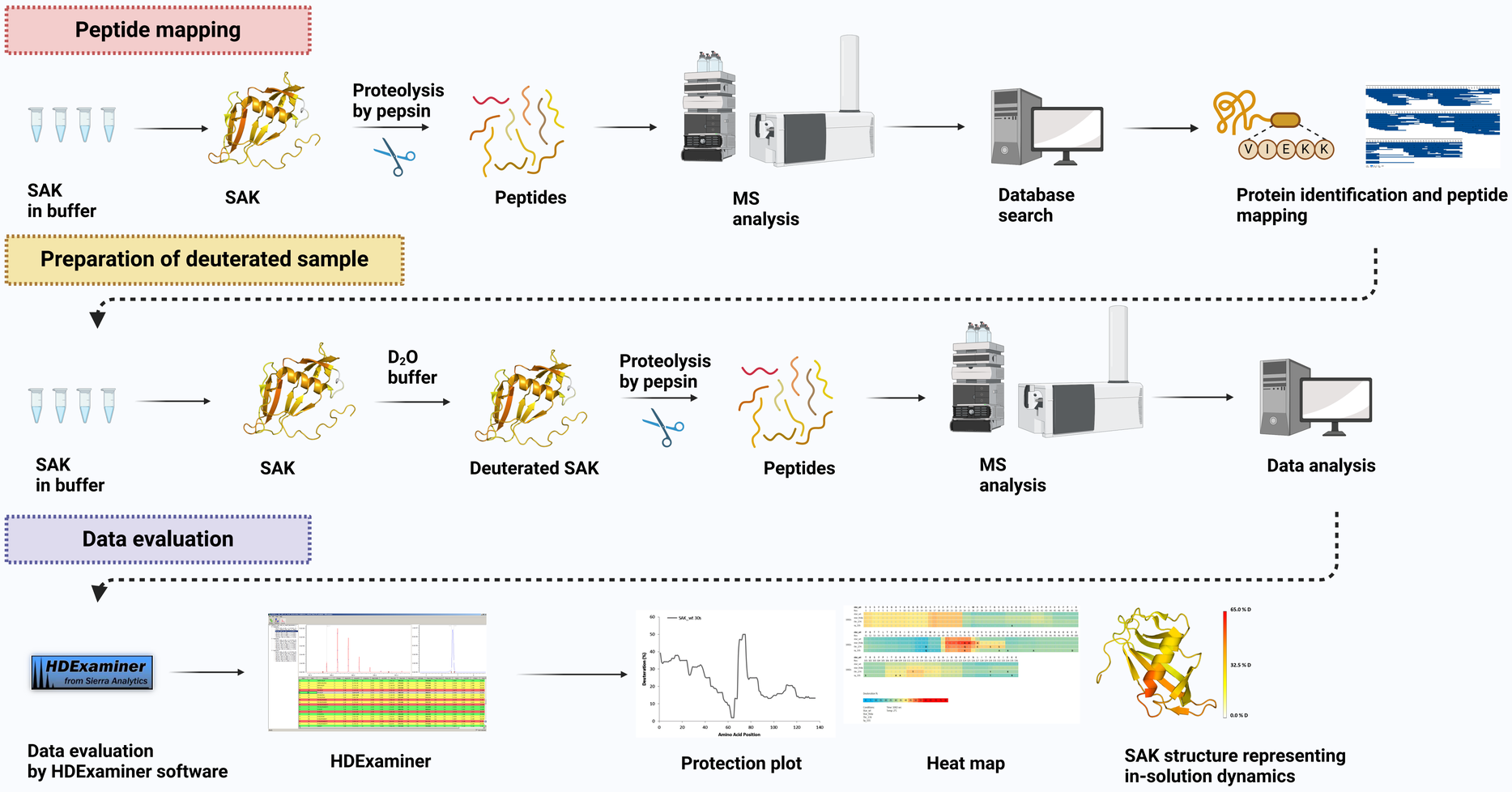
- primary sequence of the protein
- sequence coverage of the sample
- peptide list for HDX data analysis

### H/D exchange

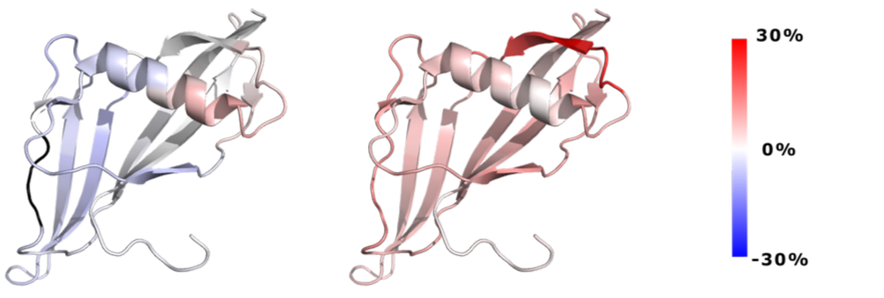
- amide $H^+$ replaced by D
- depends on the strength of H-bond
- reveals the conformational dynamics

### Data evaluation

- HDExaminer software
- calculation of single amino acid deuterium uptake in peptides
- tools to visualize evaluated data




## PRELIMINARY RESULTS




**(A) SAK SY155** **(B) SAK THR144** **Deuteration %**

***3D models of SAK mutants illustrating variations in deuterium uptake***

These 3D models of SAK show the variations in deuterium uptake **(red-maximum, blue-minimum, and black-uncovered peptides)** of SAK mutants compared to the wild-type.
**(A) SAK SY155** exhibits overall decreased solvent accessibility despite higher deuteration observed in α-helix region while **(B) SAK THR144** is more flexible with a significant increase in deuteration in the loop and exhibits higher solvent accessibility.

## OUTCOMES

- In-solution conformational dynamics of SAK.
- In-solution conformational dynamics of SAK with interacting partners.
- Physiologically compatible conformation of SAK for enhanced and efficient thrombolysis.

REFERENCES:

1. Nikitin, D. *et al.* Computer-aided engineering of staphylokinase toward enhanced affinity and selectivity for plasmin. *Comput. Struct. Biotechnol. J.* **20**, 1366–1377 (2022).

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