

H/D exchange combined with top-down ECD for protein complex structural characterization: Development and early results.

M. Duchateau, Y. Mechulam, E. Schmitt, L. Guillon, P.D. Coureux,
J. Chamot-Rooke and G. Van der Rest

Ecole Polytechnique - France

Outline

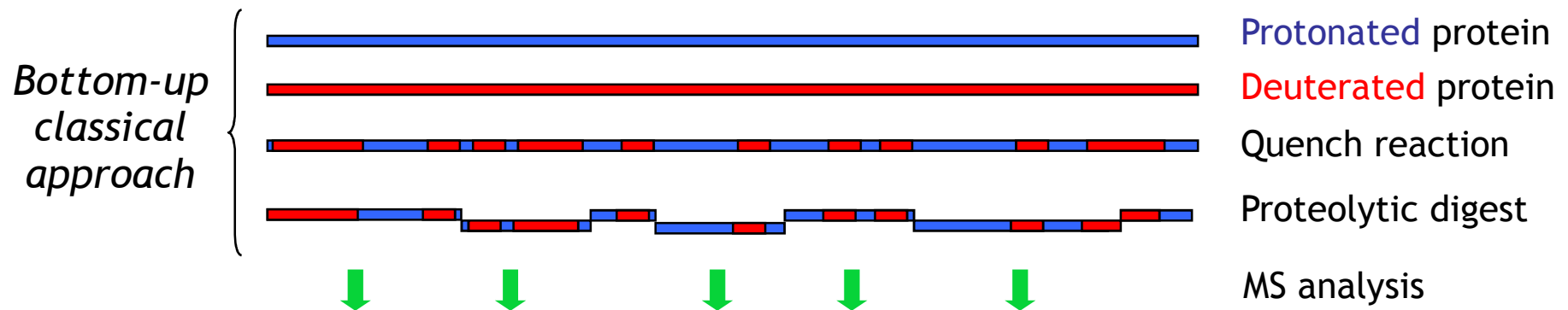
- **HDX and Mass Spectrometry:**
describe the different approaches that can be used to obtain structural information on proteins
- **Overview of a new methodology: top-down ECD HDX**
optimized set-up for real life proteins (small amounts, highly salt buffer, degrades rapidly even at low temperature)
- **Application to aIF2 (archaeal Translation Initiation Factor)**
first results for the analysis of the alpha subunit

Outline

- **HDX and Mass Spectrometry:**
describe the different approaches that can be used to obtain structural information on proteins
- **Overview of a new methodology: top-down ECD HDX**
optimized set-up for real life proteins (small amounts, highly salt buffer, degrades rapidly even at low temperature)
- **Application to aIF2 (archaeal Translation Initiation Factor)**
first results for the analysis of the alpha subunit

HDX and Mass Spectrometry

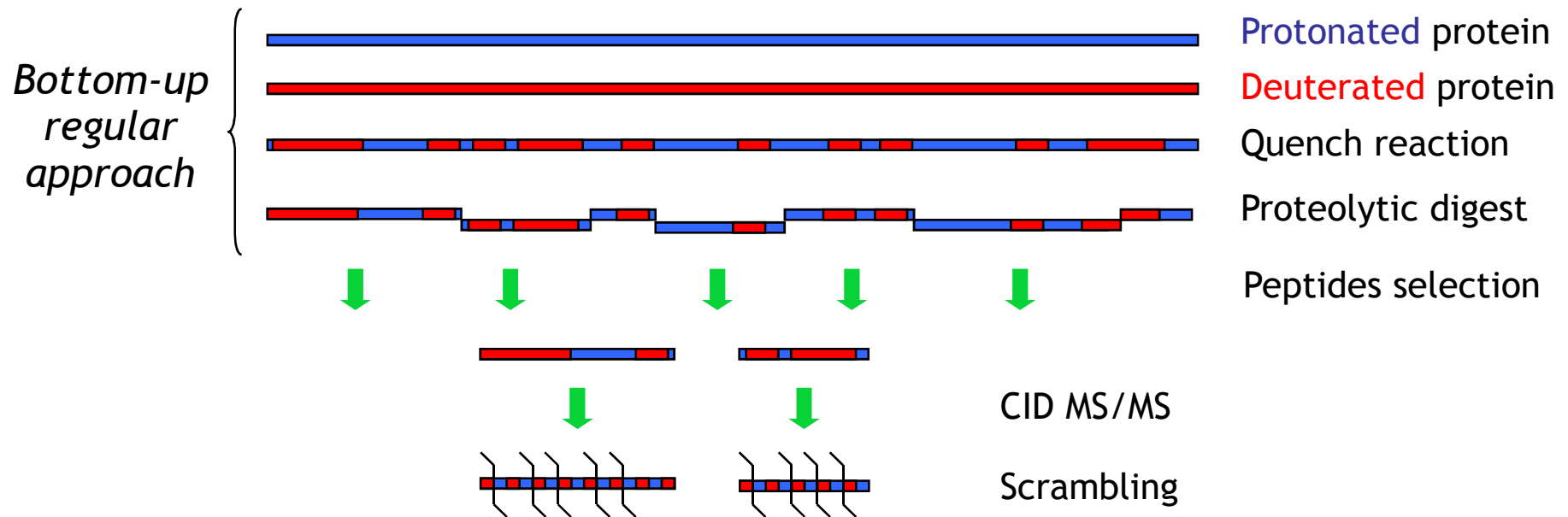
- HDX MS: a powerful experimental tool for probing both structural and dynamic features of proteins



- HDX kinetics is monitored through its mass shift as a function of time
- Exchange rate correlated both to the solvent accessibility and the local structure in the protein
- Drawback of this approach: resolution limited by the size of peptides after digestion

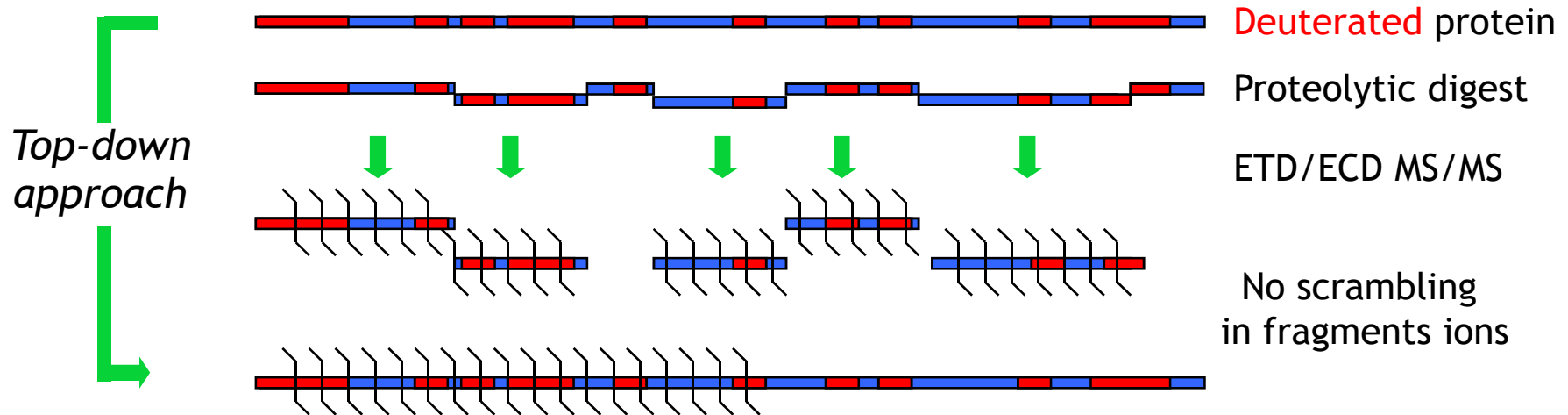
HDX and Mass Spectrometry

- Although the literature is not completely consistent - the use of CID MS/MS to locate deuterium position has not emerged as an efficient technique: deuterated peptides undergo HD scrambling before backbone fragmentation



- Electron based activation methods (ETD or ECD) seems to minimize scrambling - Their use in HDX methodology is very promising

HDX and ETD/ECD



- **Electron Transfer Dissociation (ETD)**
 - Mainly for peptide dissociation (Jorgensen)
 - Very recently for a 15 kDa model protein (Kaltashov)
- **Electron Capture Dissociation (ECD)**
 - For a small model protein (Koneremann)

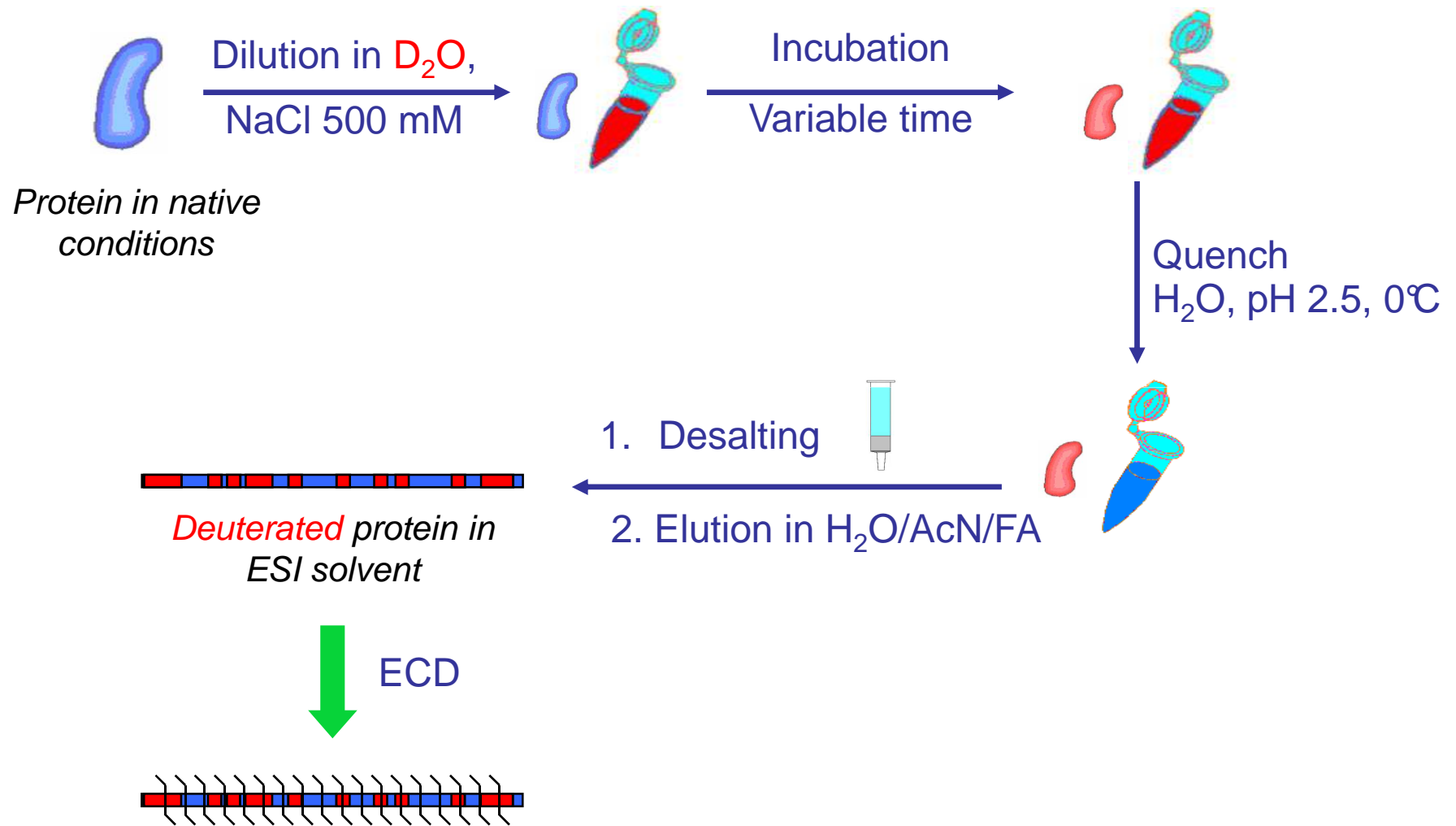
ETD and ECD are outstanding tools in combination with HDX for structural analysis of proteins

Set-up a new protocol including additional steps allowing the analysis of real biological samples

Outline

- **HDX and Mass Spectrometry:**
describe the different approaches that can be used to obtain structural information on proteins
- **Overview of a new methodology: top-down ECD HDX**
optimized set-up for real life proteins (small amounts, highly salt buffer, degrades rapidly even at low temperature)
- **Application to aIF2 (archaeal Translation Initiation Factor)**
first results for the analysis of the alpha subunit

Workflow overview



Workflow optimization

- Desalting performed at low temperature and pH to minimize back exchange
 - For electrospray: Sep-Pak C₈
 - For nanoelectrospray: ZipTip C₁₈ } Depending on the amount of protein available
- For nanoelectrospray : use of the TriVersa NanoMate system (Advion)
 - Chip-based automated infusion for a fast and reproducible spray formation
 - Cooled storage of deuteriated proteins before their analysis



- ECD parameters optimized to have enough scans to observe fragments and minimize back exchange
 - 15 scans, 50 ms irradiation (0.7 eV)



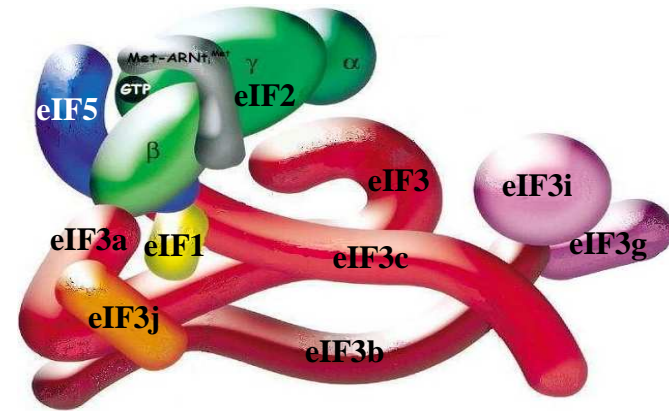
Analysis can be done in less than 3 minutes!!!

Outline

- **HDX and Mass Spectrometry:**
describe the different approaches that can be used to obtain structural information on proteins
- **Overview of a new methodology: top-down ECD HDX**
optimized set-up for real life proteins (small amounts, highly salt buffer, degrades rapidly even at low temperature)
- **Application to aIF2 (archaeal Translation Initiation Factor)**
first results for the analysis of the alpha subunit

aIF2

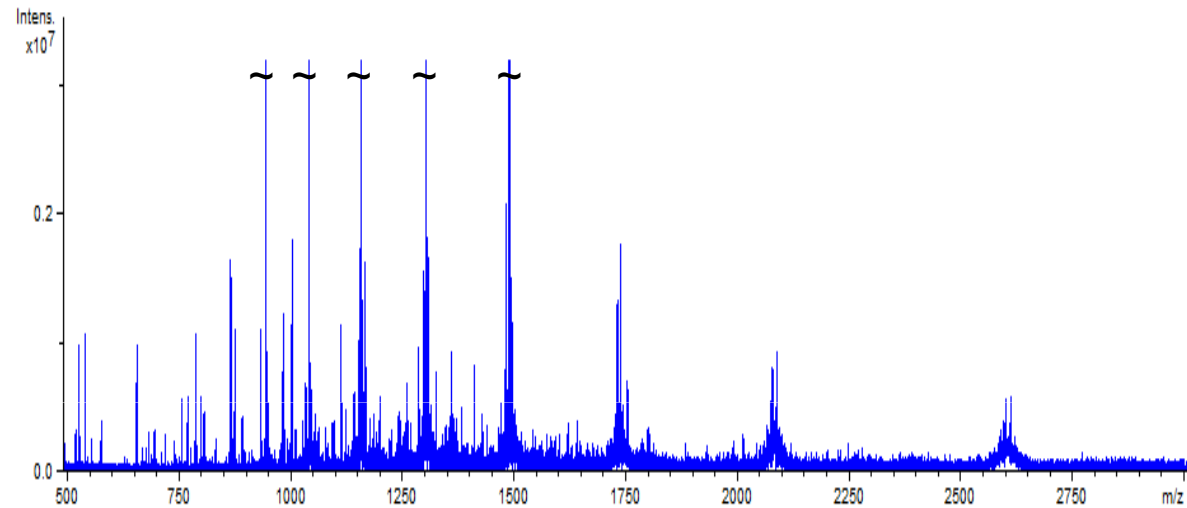
- Proposed structure of the eukaryotic multifactor complex responsible for the initiation of translation



- In archeas not all subunits are present
 - analogous complex of eIF2 with a similar role (to bring the initiator methionyl-tRNA in contact with the mRNA bound to the small subunit of the ribosome and ensure codon selection)
- 3 subunits interact together
 - γ central protein which interact both with α and β subunits
 - whereas no contact established between α and β
- Need for better knowledge of aIF2 structural organization
- Our objective: mapping the interaction surface between the different subunits of aIF2

ECD of aIF2 α 3

- ECD of aIF2 α 3 (10 kDa)



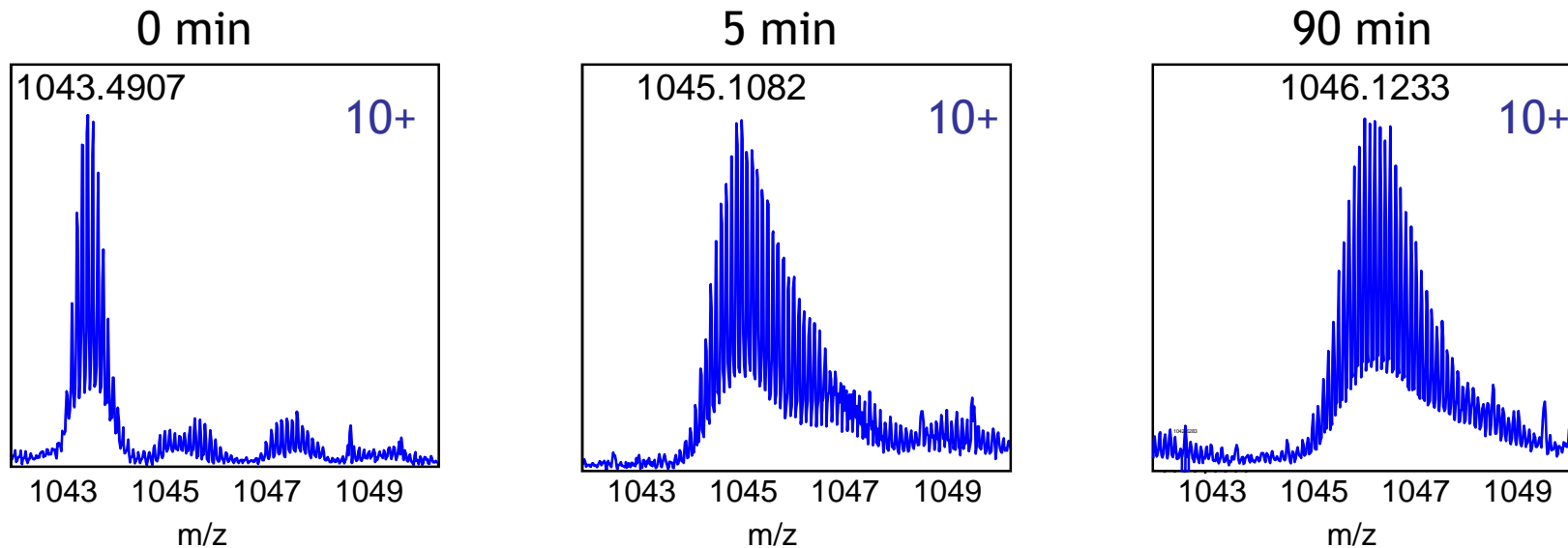
E R K V K M S G L I T V R T N E P L G V E K I K E V I S K A L
E N I E Q D Y E S L L N I K I Y T I G A P R Y R V D V V G T N
P K E A S E A L N Q I I S N L I K I G K E E N V D I S V V K K

ions zions

- 70 % sequence coverage

HDX of aIF2 α 3

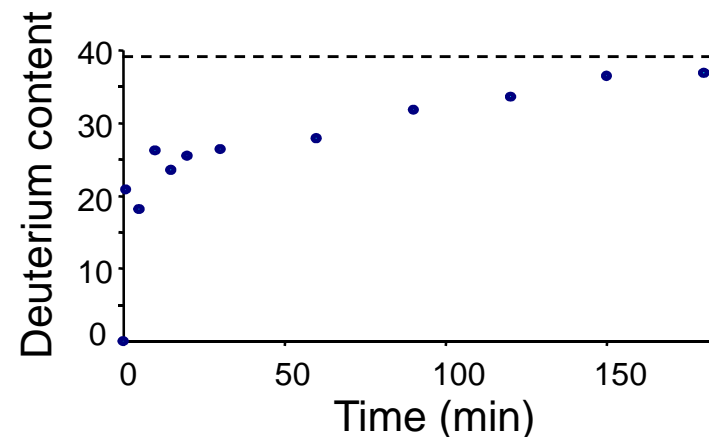
- Checked incorporation of deuterium for the entire protein



- Maximum deuteration
 - 40% in ESI
 - 66% in nanoESI using the NanoMate



Shows that the NanoMate minimizes back exchange

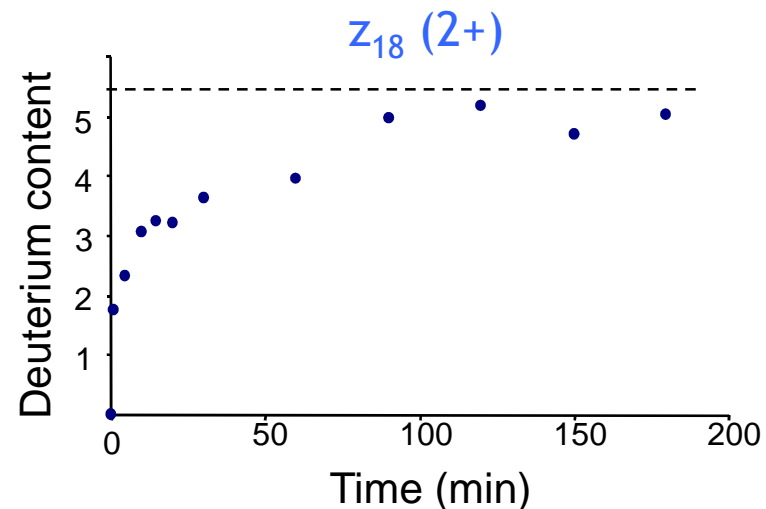
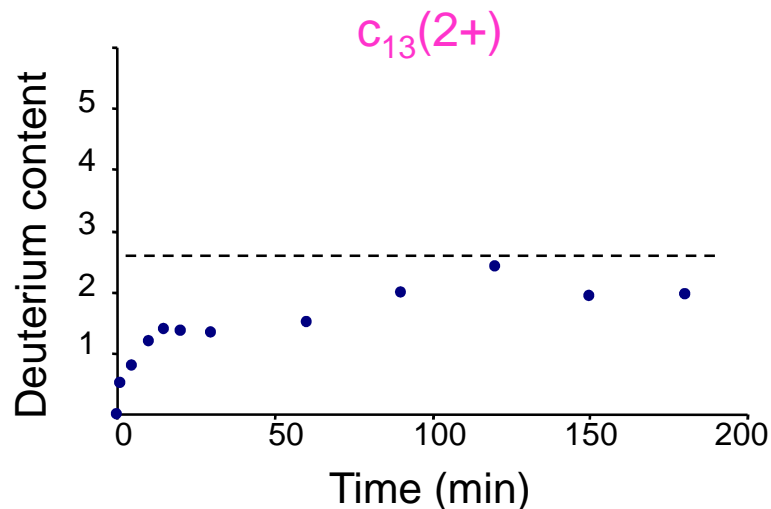


HDX top-down ECD of aIF2 α 3

- In optimized conditions for HDX ECD (15 scans) of aIF2 α 3, the sequence coverage with c/z ions is 20%

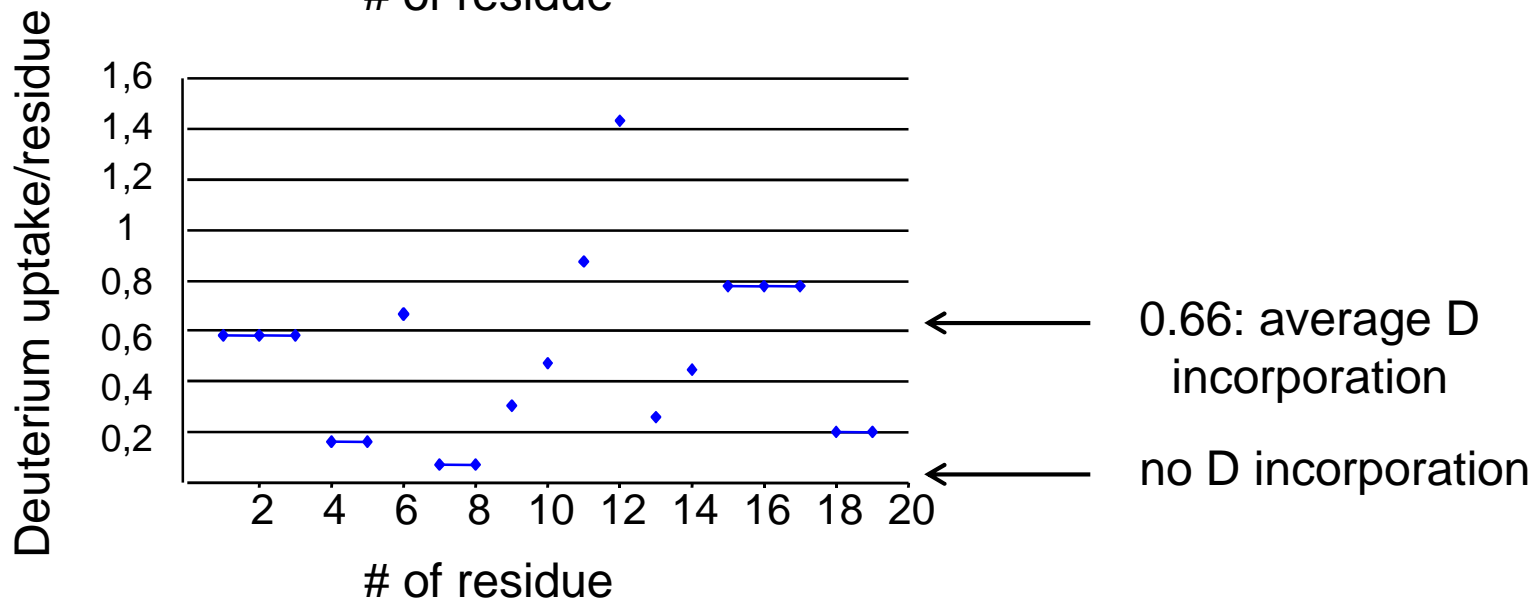
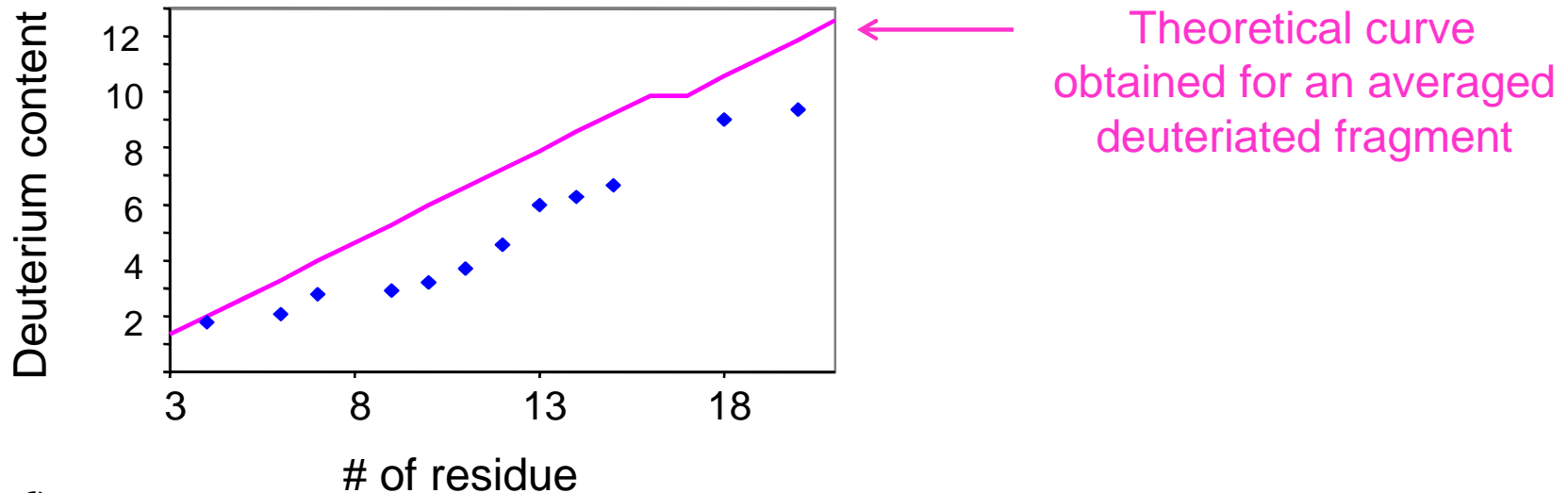


- Single residue resolution for both N and C-terminii
- Different HDX kinetics for c-type and z-type ions

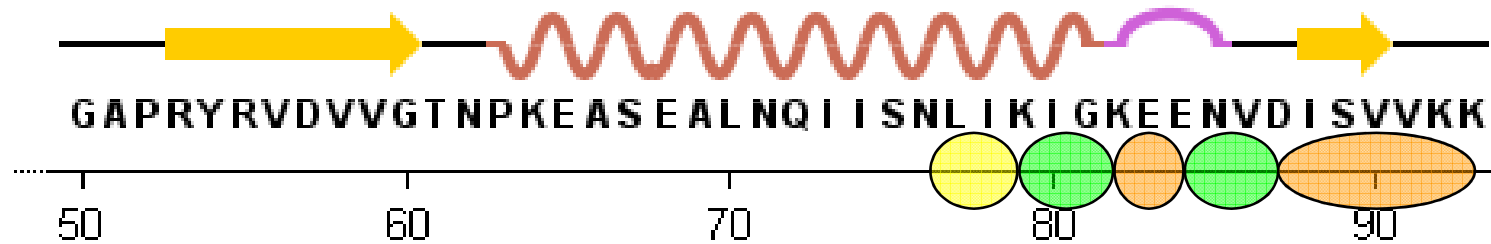


HDX kinetics for the N-terminus

- Analysis of deuterium content for c-type fragments



Structural analysis of aiF2 α 3



0 → 0.2 0.2 → 0.4 0.4 → 0.6 0.6 → 0.8 0.8 → >1

Slow exchange

Fast exchange

Conclusions

- ECD provides an alternative to CID to avoid proton/deuteron migration before backbone fragmentation
- Single residue resolution can be achieved by top-down ECD HDX, which is not feasible by bottom-up approaches
- Top-down workflow still needs optimizations, in particular to increase sequence coverage after HDX
- Next stage of this work in aIF2: other subunits alone and within the complex

Acknowledgments

- **Lab. des Mécanismes Réactionnels (Ecole Polytechnique)**

Julia Chamot-Rooke

Guillaume Van der Rest

Christian Malosse

Charlotte Boisseau

Edith Nicol

- **Lab. de Biochimie (Ecole Polytechnique)**

Yves Méchulam

Emmanuelle Schmitt

Laurent Guillon

Pierre-Damien Coureux

Christine Lazennec-Schurdevin

- **€€€: "MASTIC" Project (ANR Physique et Chimie du Vivant 2006)**