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# Protein Splicing: an Ancient Efficient Self-Catalytic Process

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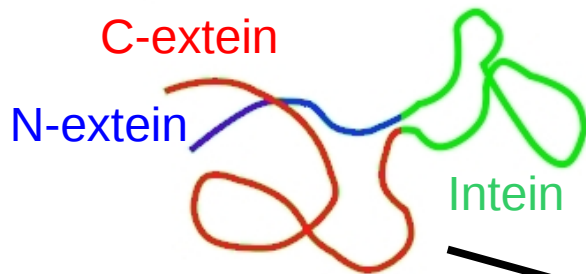
DNA

Transcription



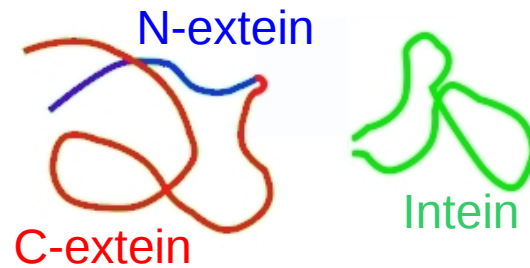
RNA

Translation



Protein  
(inactive)

Protein Splicing

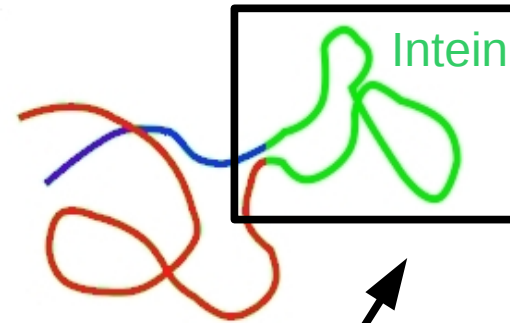


(active)

# Protein Splicing

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- In all three domains of life: archaea, bacteria and eukarya
- In unicellular organisms
- Probably an ancient evolutionary origin



- Catalyzed by amino acids located in the intein (and the first one of C-extein).
- Not coenzyme or external source of energy required.

# Intein classification

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- More than 200 inteins are known ( size of 134-650 amino acids)
- Four type of inteins:

a) Maxi-inteins:



a) Mini-inteins:



c) Ala-inteins: Ala1 instead of Cys1 or Ser1

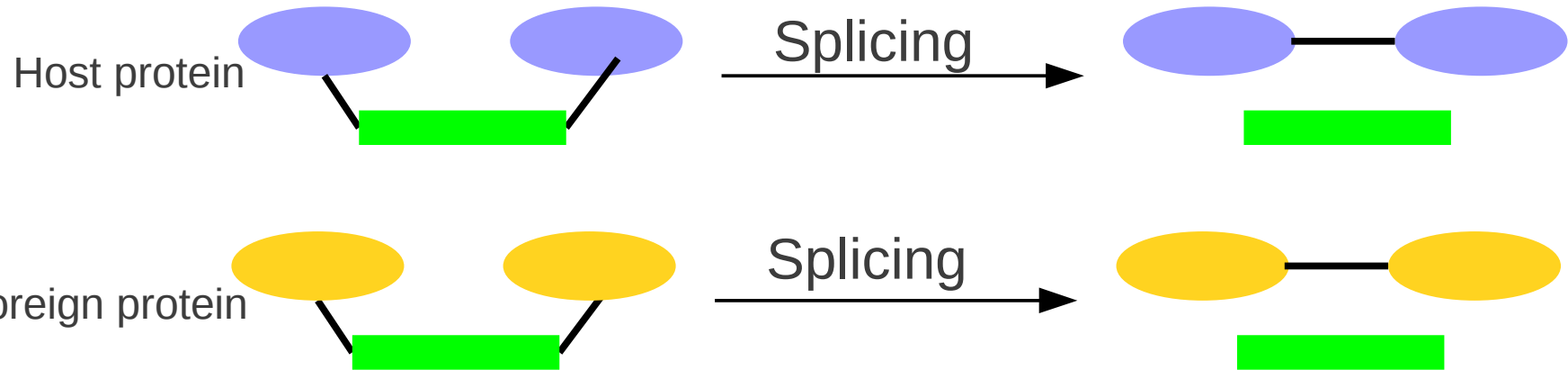
d) Trans-splicing:



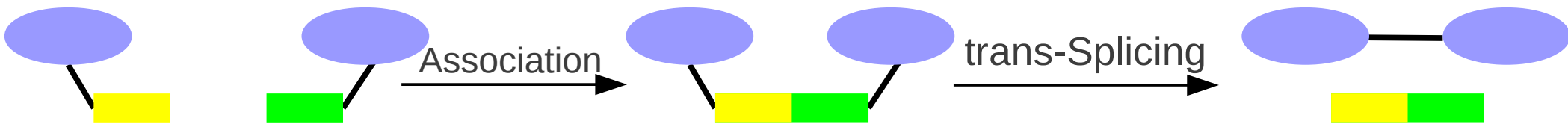
# Applications in protein engineering strategies:

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## a) Protein transferability



## b) Timing control



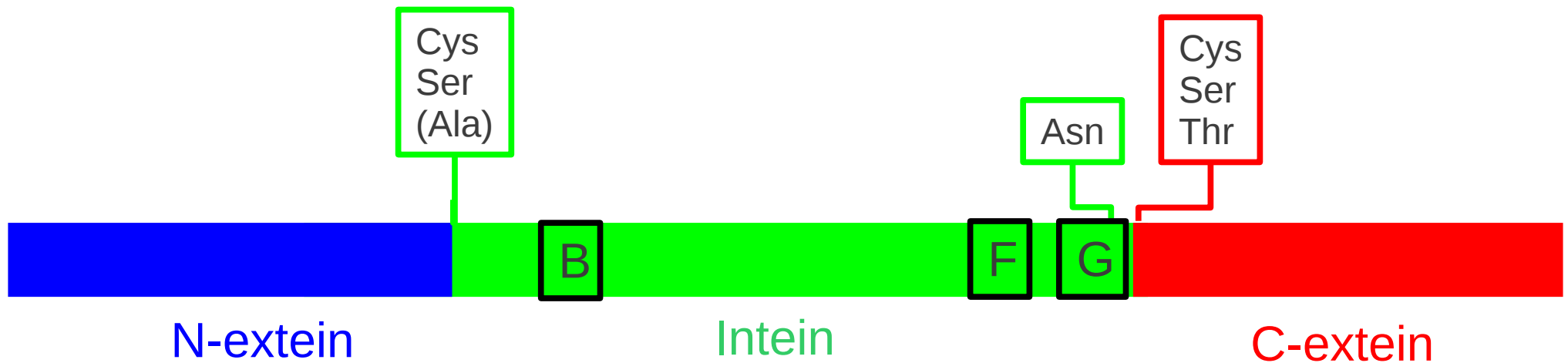
- Protein purifications
- Protein ligation
- Protein labeling
- ...

# Inteins show relatively low sequential similarity

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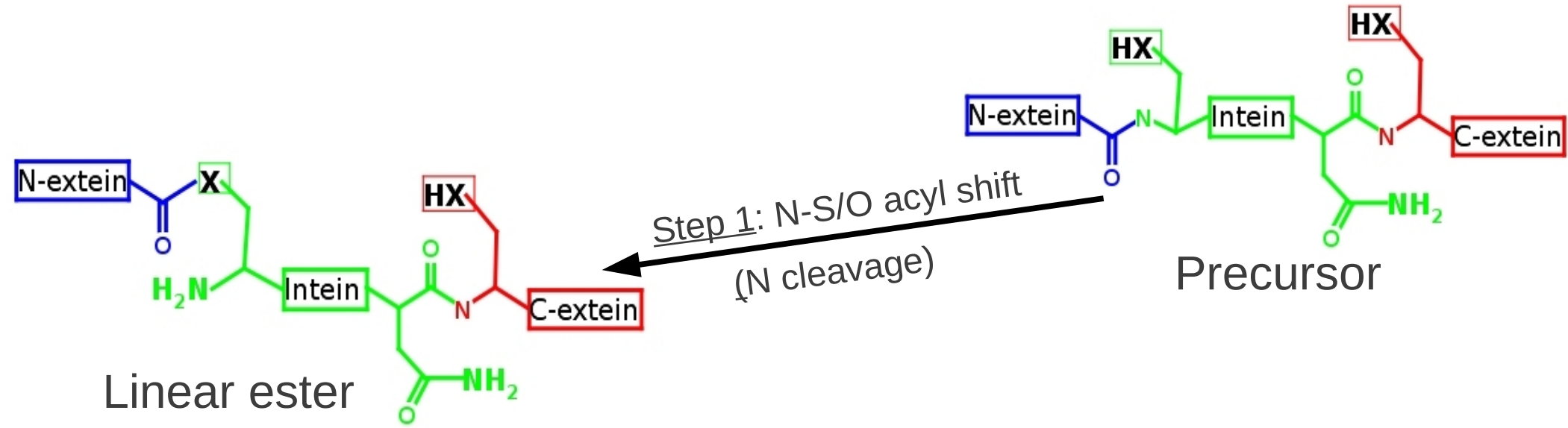
Several conserved amino acids

- Directly involved in the mechanism
- Catalytic role at a certain step

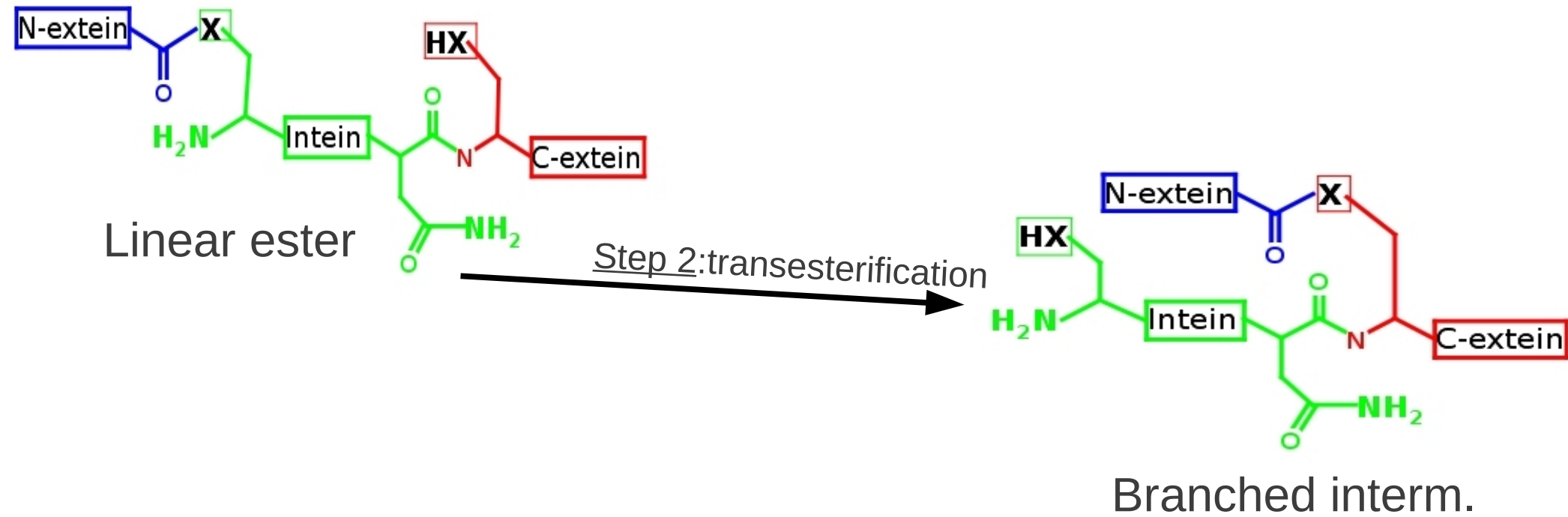


Residues located in blocks B, F and G catalyze N- and C-terminal junction cleavage

# Mechanism: independent but coordinated 4 steps

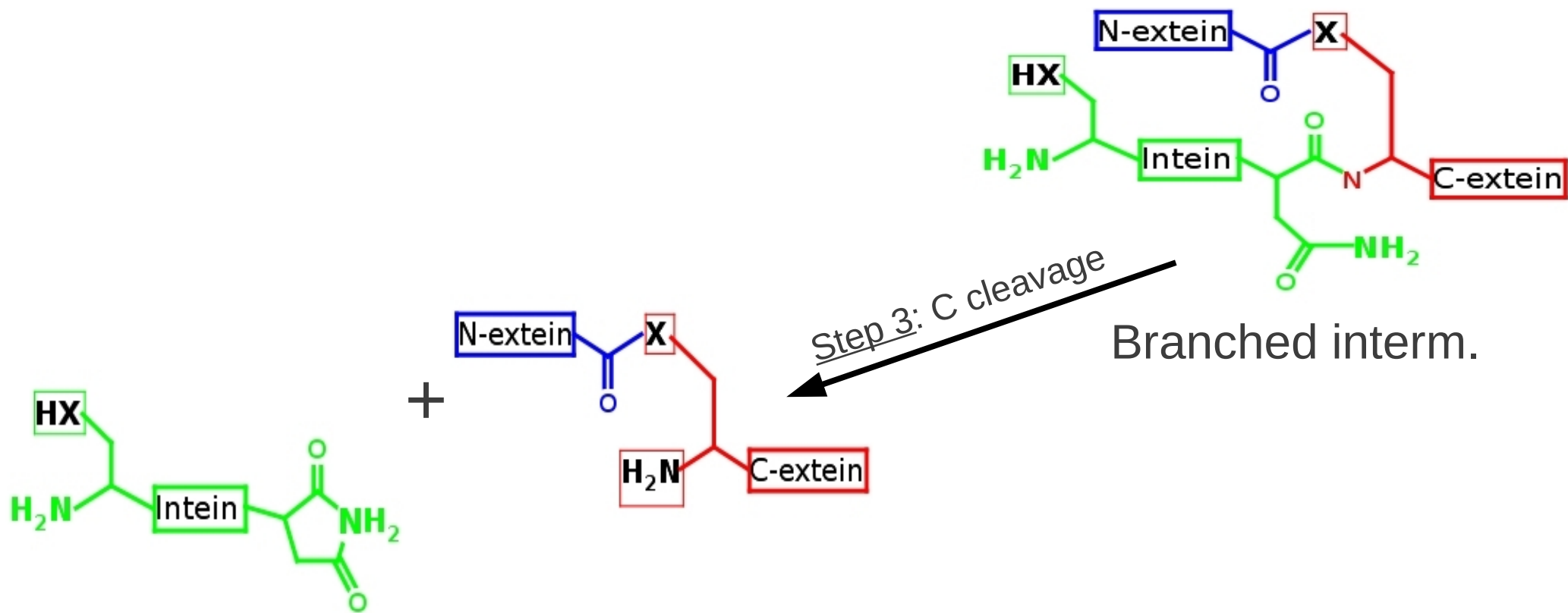


# Mechanism: independent but coordinated 4 steps

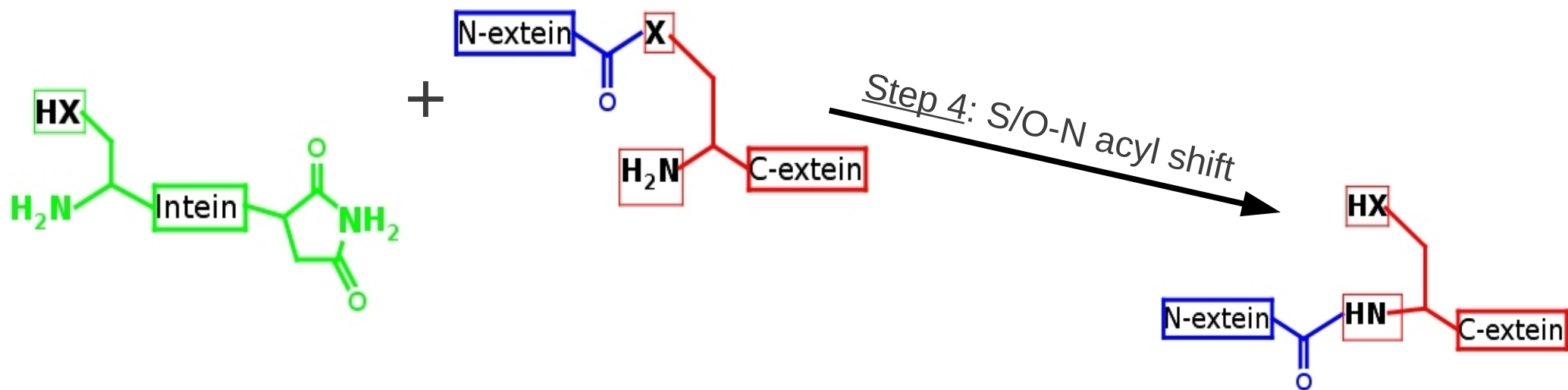




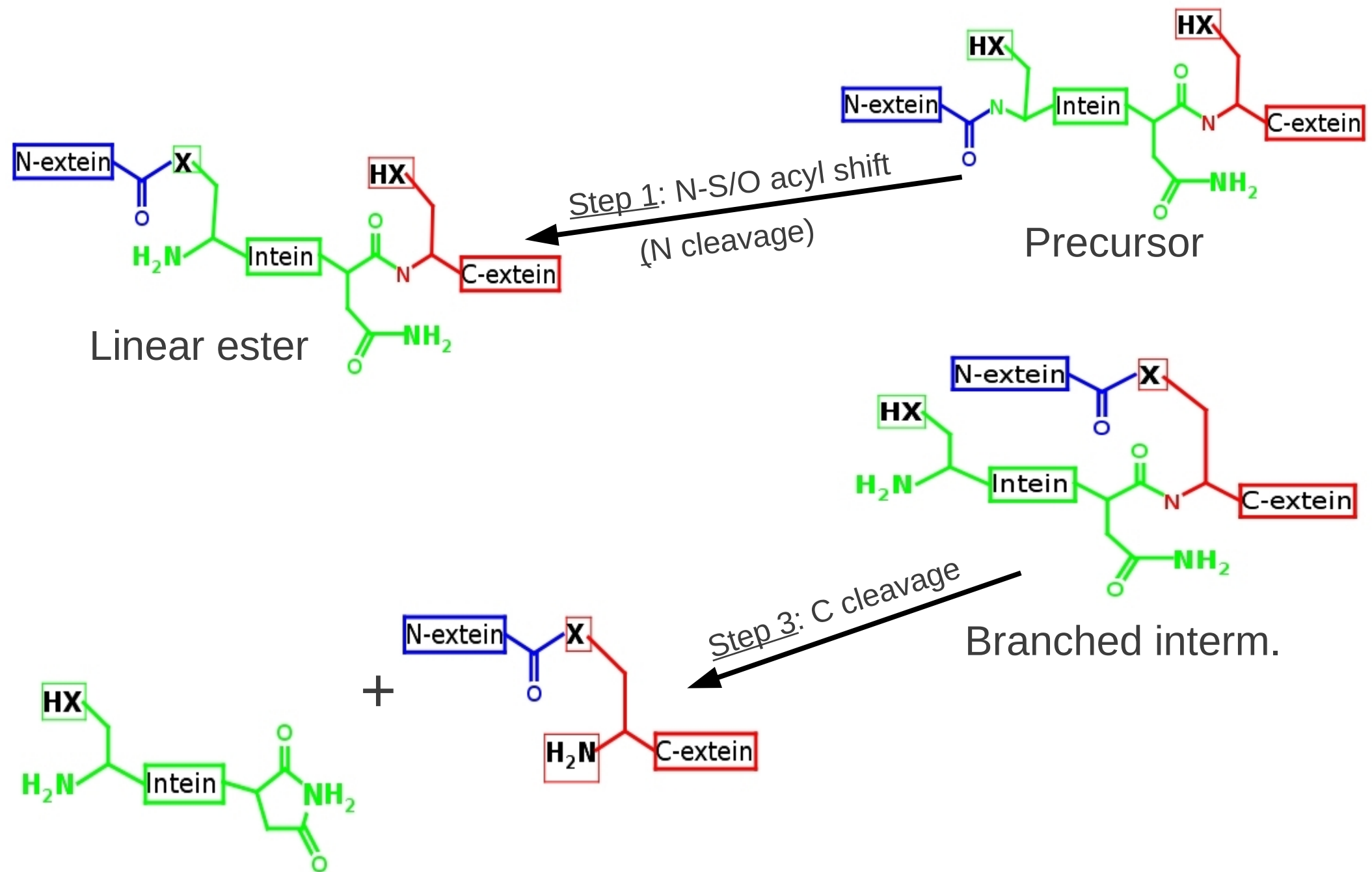
# Mechanism: independent but coordinated 4 steps



# Mechanism: independent but coordinated 4 steps



# Our research focuses on:



## Kinetic data

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N-terminal cleavage	$1 \times 10^{-4} \text{ s}^{-1}$
C-terminal cleavage	$2.8 \times 10^{-4} \text{ s}^{-1}$

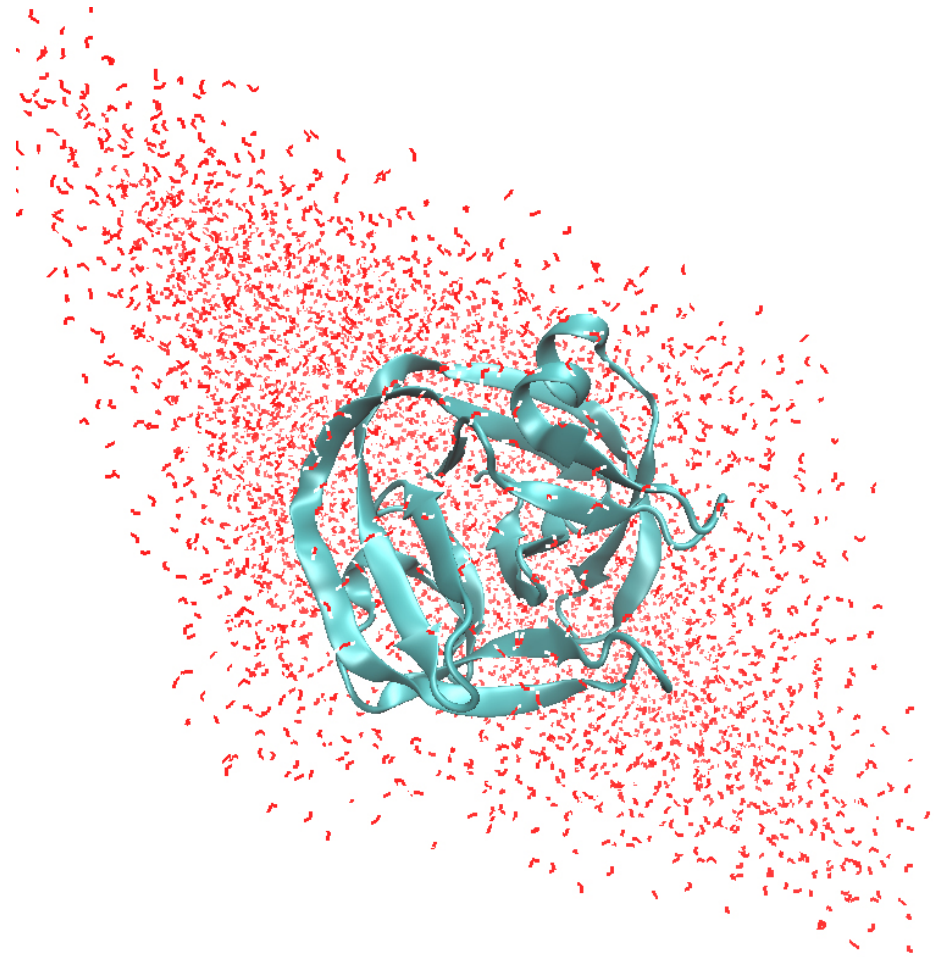
Applying Eyring equation:

N-terminal cleavage	25.6 kcal/mol
C-terminal cleavage	24.9 kcal/mol

## 1. Molecular Dynamics Simulations:

- Equilibrate the system
- Investigate histidines' protonation state

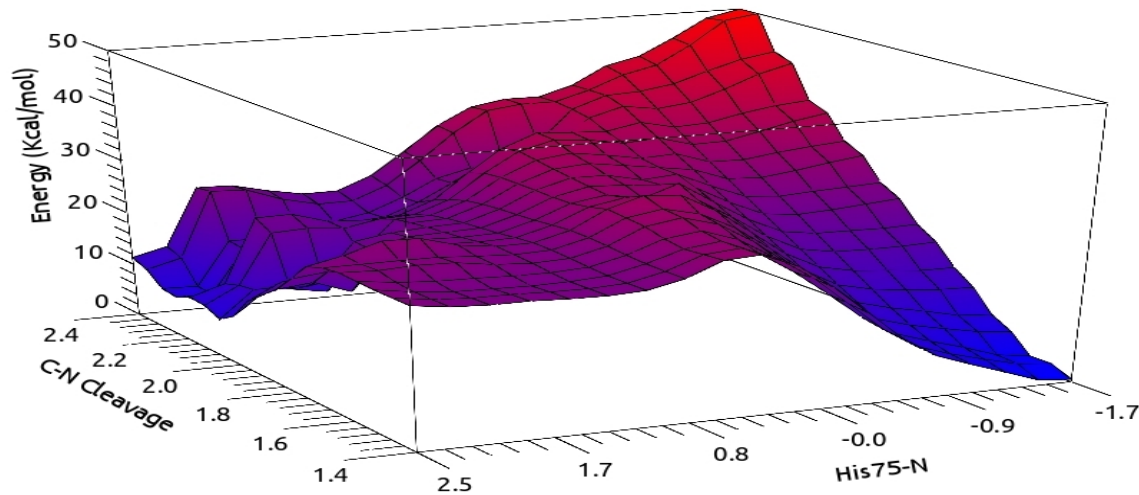
- Gromacs 4.5.3
- Charmm27 force field
- Two AAA segments added as C-extein and N-exteins
- Periodic Boundary Conditions
- NVT ensemble
- Equilibration: 2ns
- Production of 30 ns



Initial structure: recA mini-intein from *Mycobacterium tuberculosis* (2IN0 pdb code)

## 2. Potential Energy Surface (PES) characterization:

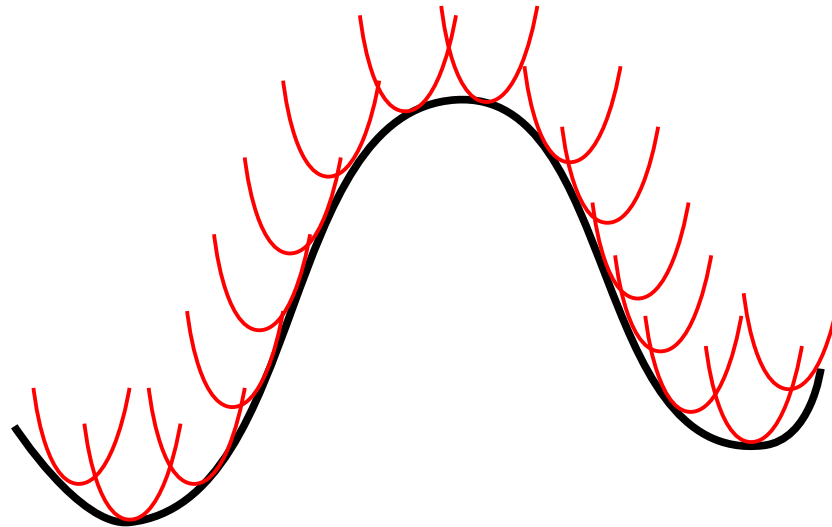
- QM/MM scheme
- Locate stationary points along a reaction pathway
- Explore alternative reaction coordinates
- Methodology testing
- High dependence on the initial conformation



- Charmm program
- QM/MM scheme
- QM part: SCCDFTB
- MM part: Charmm27
- Link atoms at the QM boundaries

## 3. Potential of Mean Force (PMF):

- QM/MM scheme
- Free energy surface characterization
- Time consuming calculations
- A better sampling
- Reaction coordinates based on PES

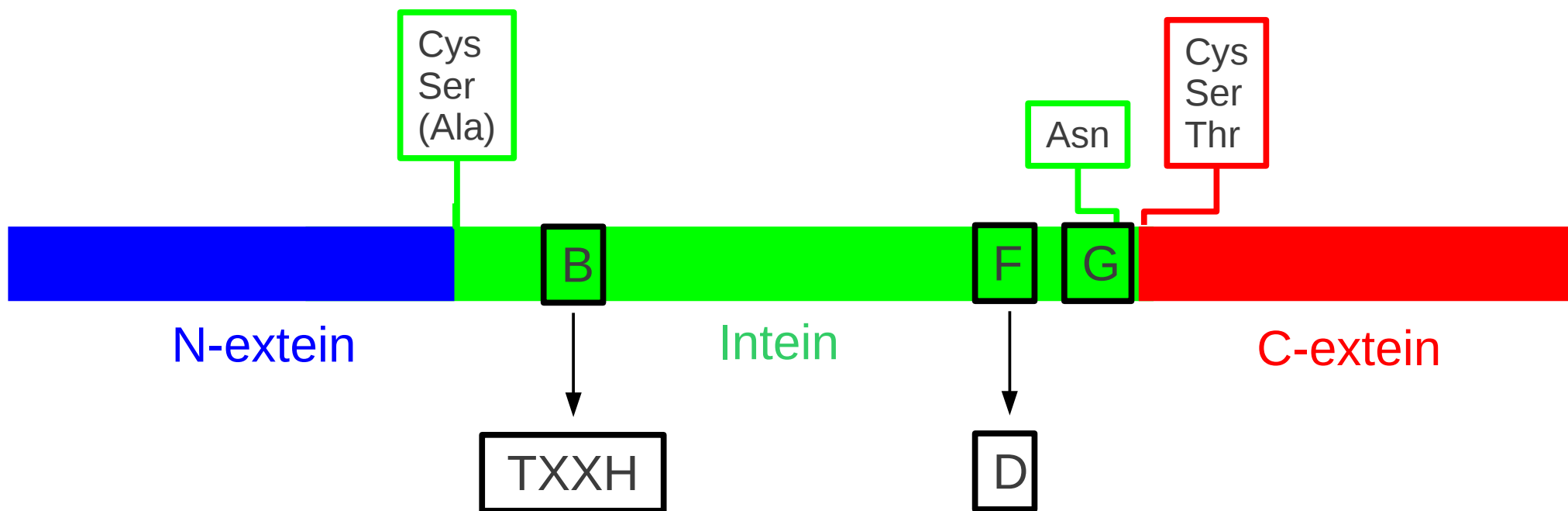
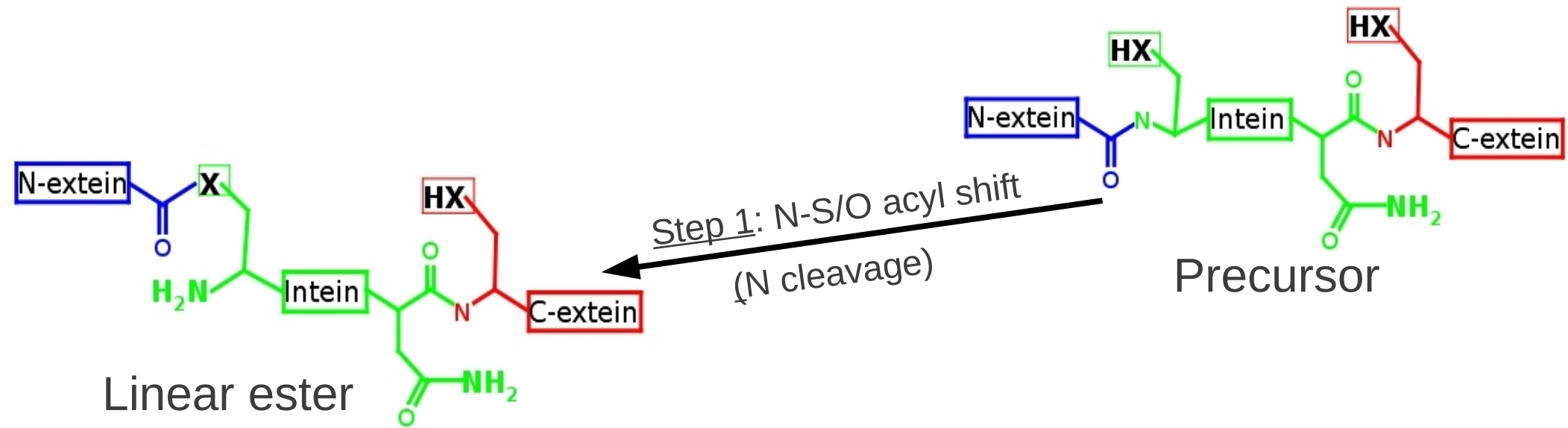


- Charmm
- SCCDFTB/Charmm27
- Link atoms at the QM boundaries
- Each window: 10 ps equilibration + 30 ps production
- Free energy profile computed with WHAM

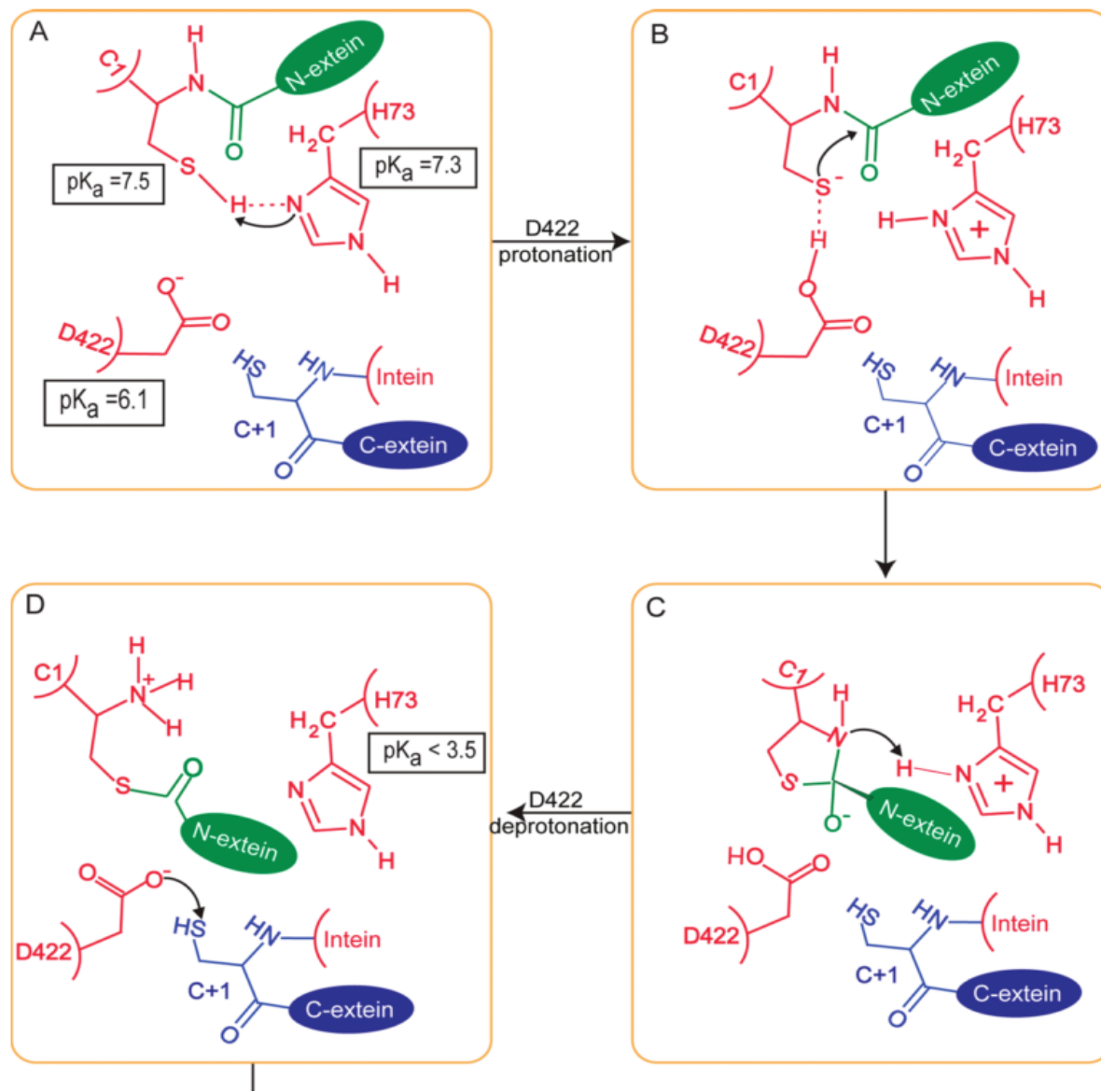
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Step1: N-S/O acyl shift

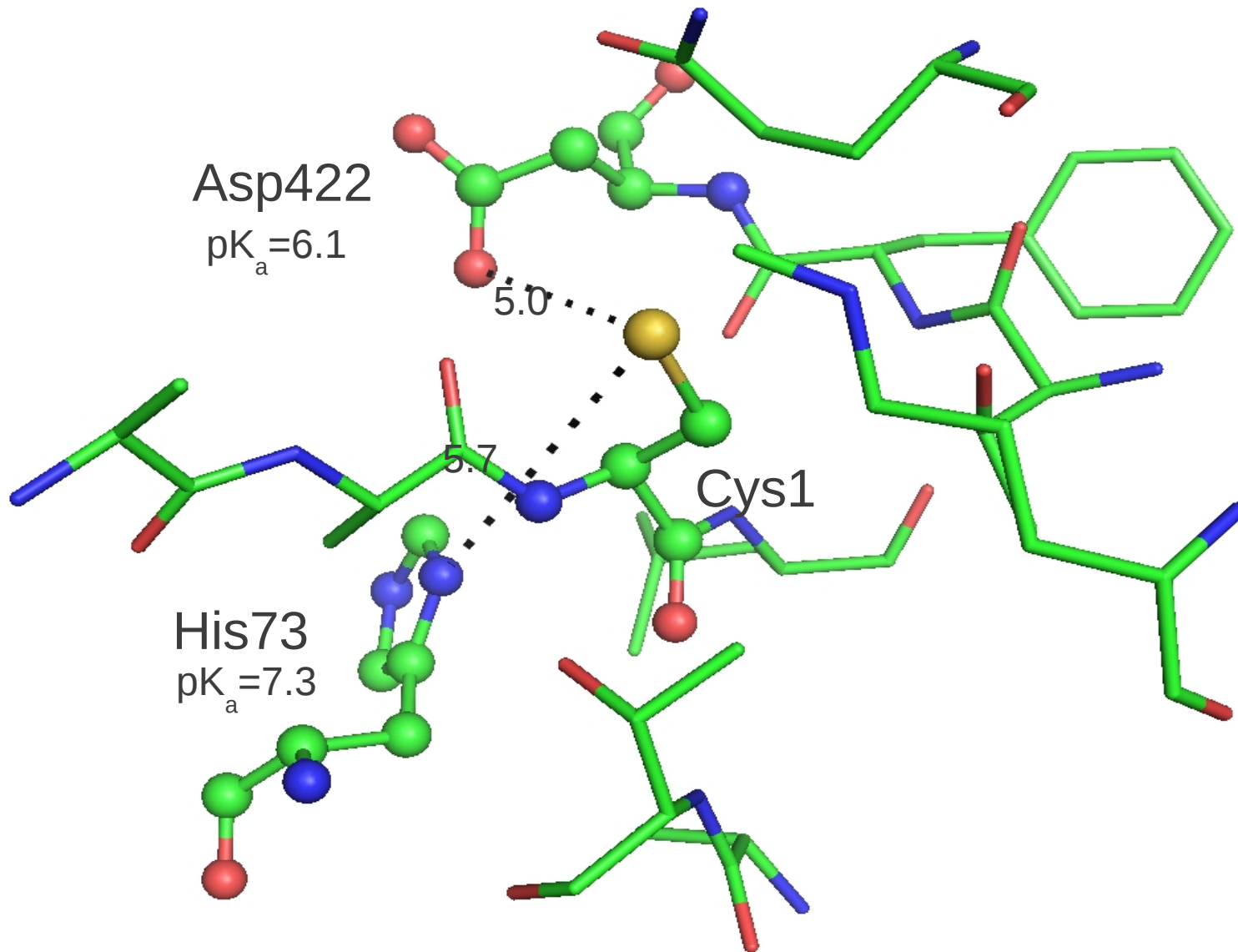




Du et al. *JACS* (2011), 133, 10275



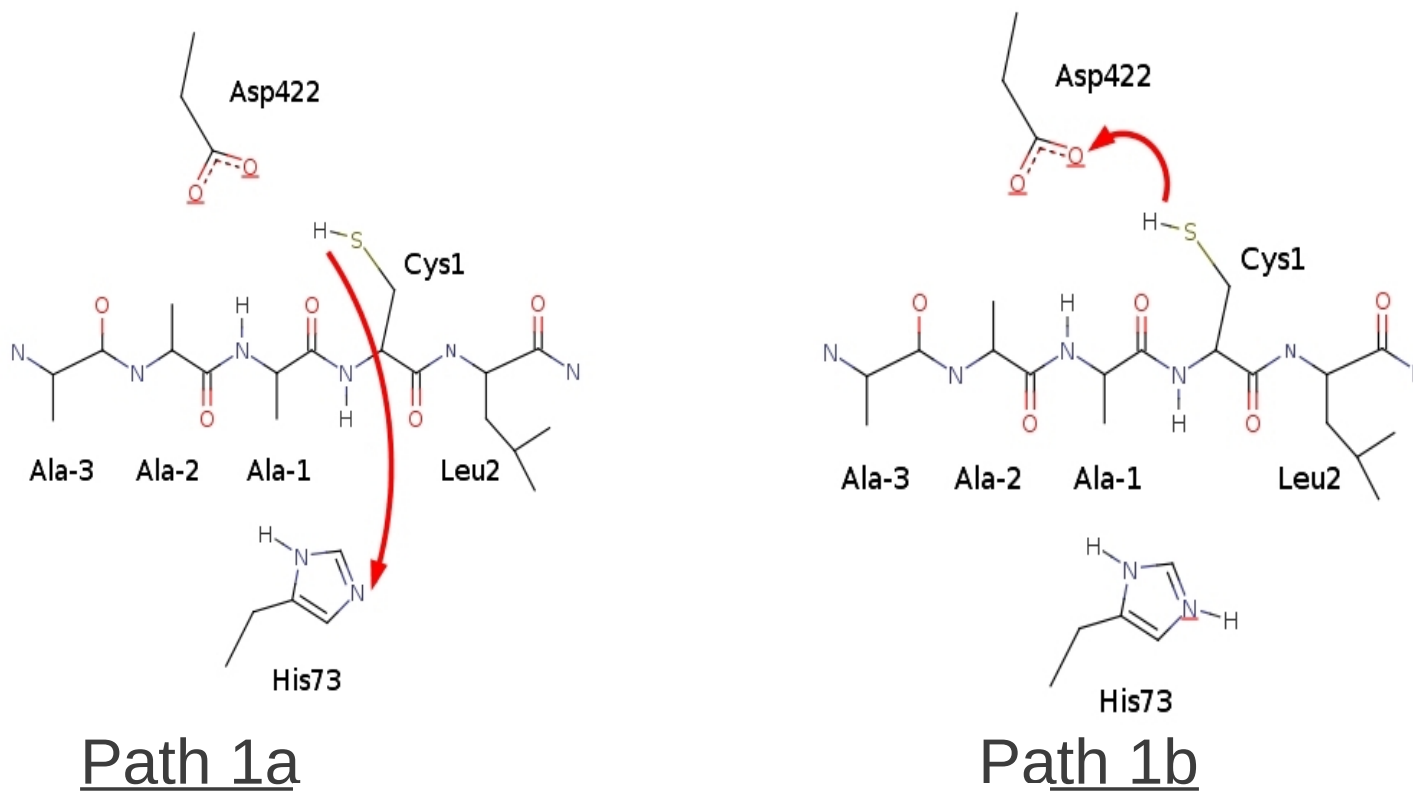
# 2IN0 X-ray crystal structure



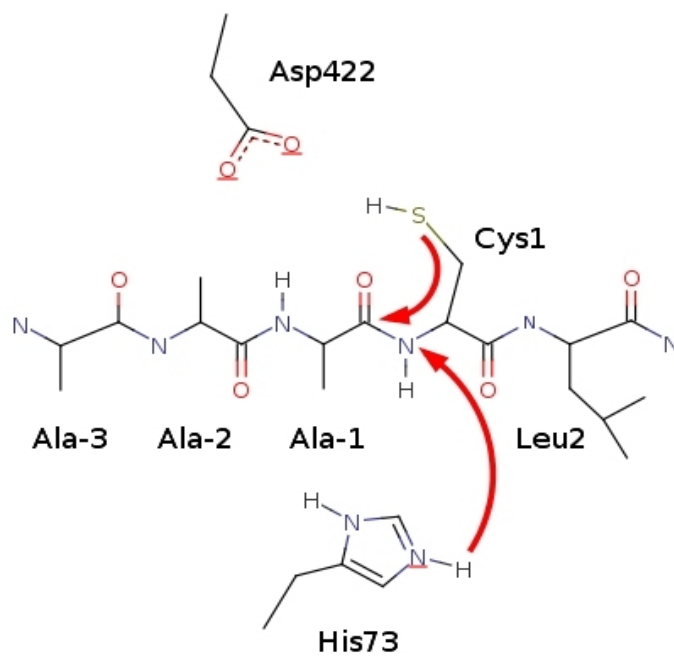
Mechanism 1: activation of Cys1 side chain by His73

Mechanism 2: activation of Cys1 side chain by Asp422

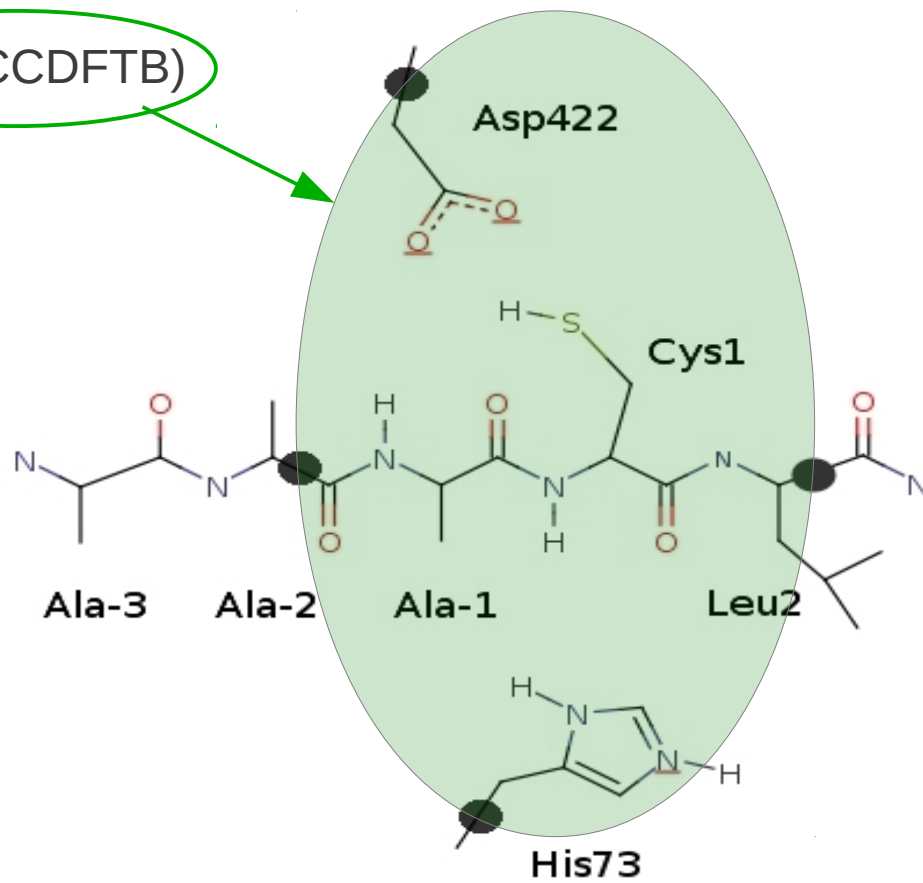
## Step 1



## Step 2

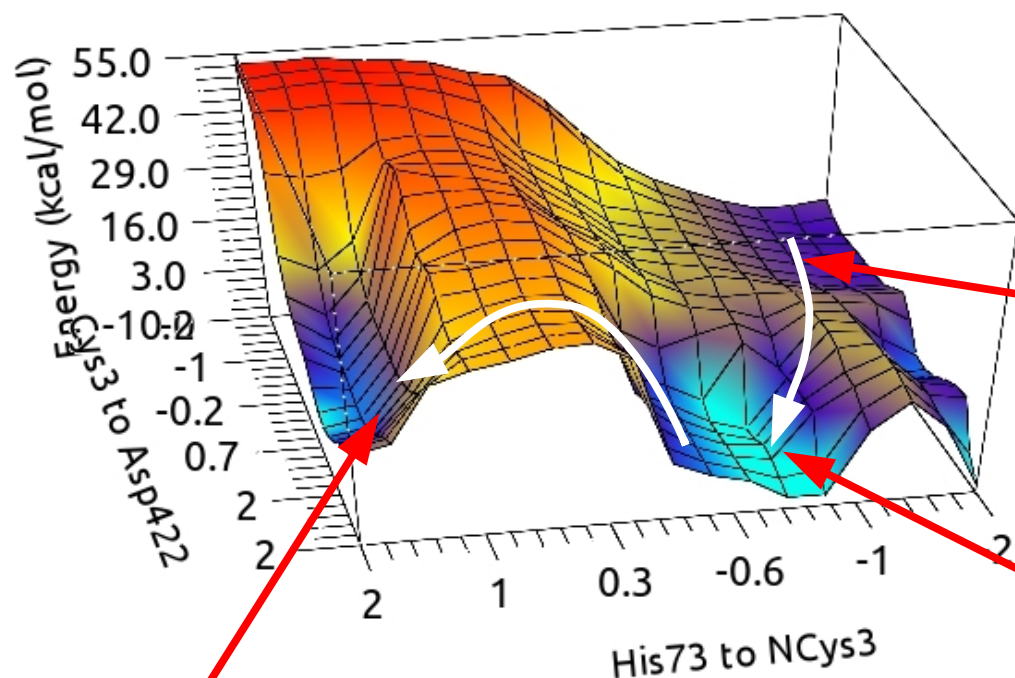


QM(SCCDFTB)

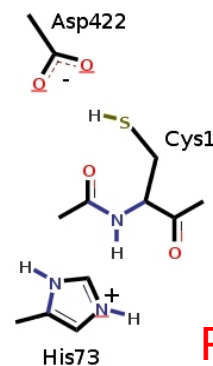


MM(CHARMM27)

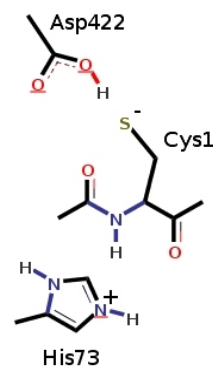
# PES characterization to determine suitable reaction coordinates



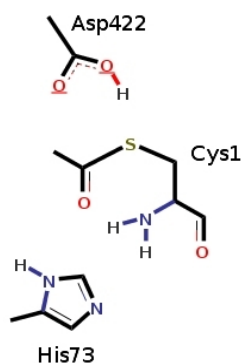
r.c.1: Cys1 activation by Asp422  
r.c.2: N protonation by His73



Reactant



Activated react.

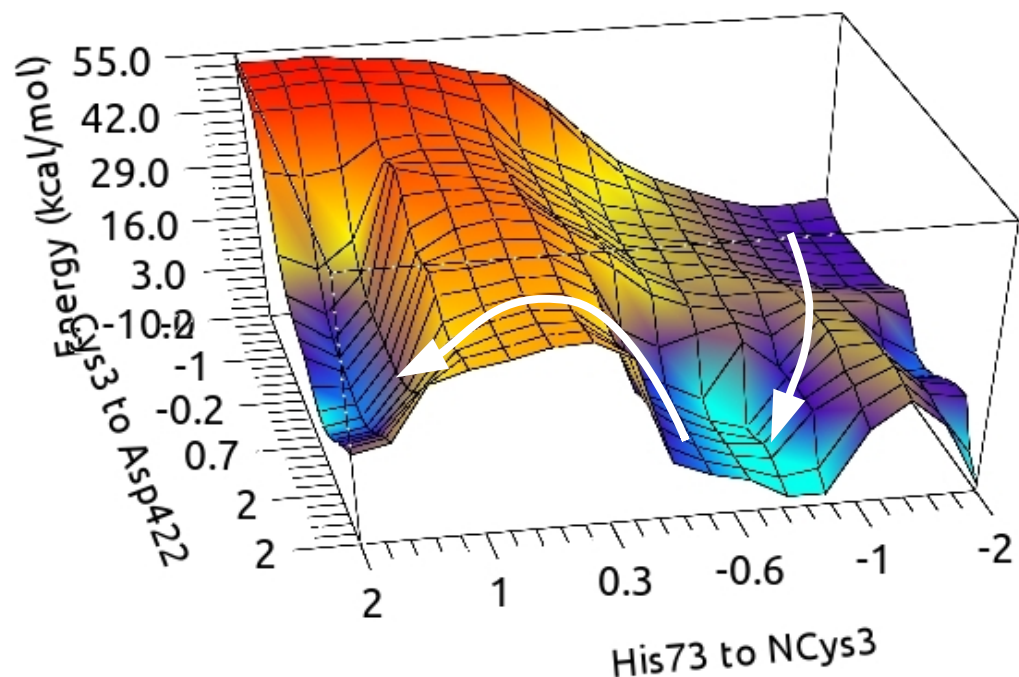


Product

- Stepwise mechanism
- 1st step: Cys1 side chain activation
- 2nd step: protonation of N by His75

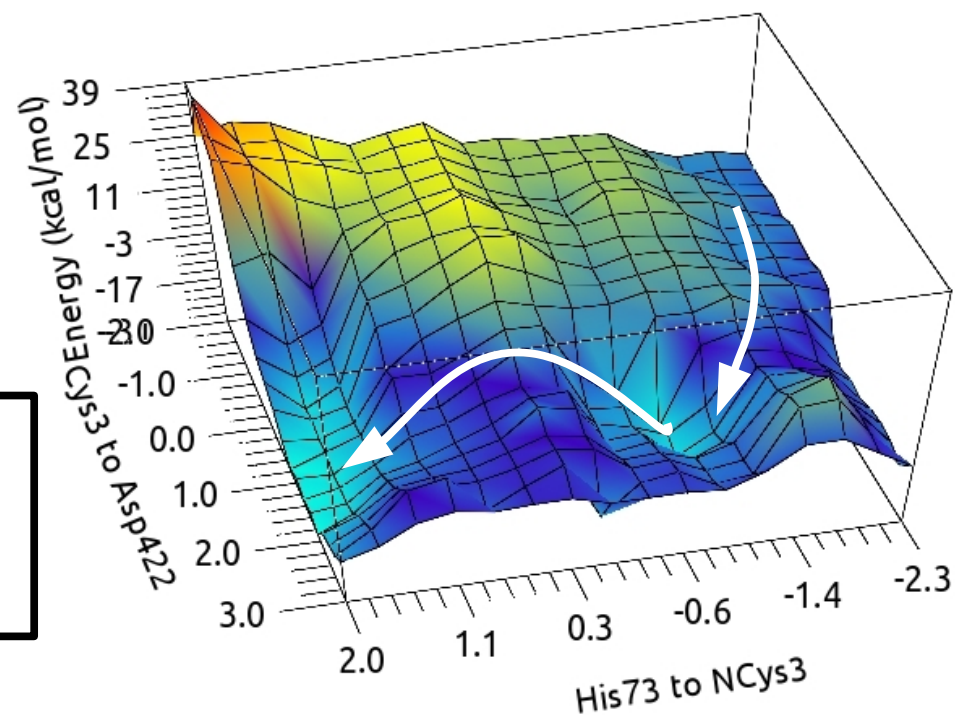
# Comparison with higher level of theory

r.c.1: Cys1 activation by Asp422  
r.c.2: N protonation by His73

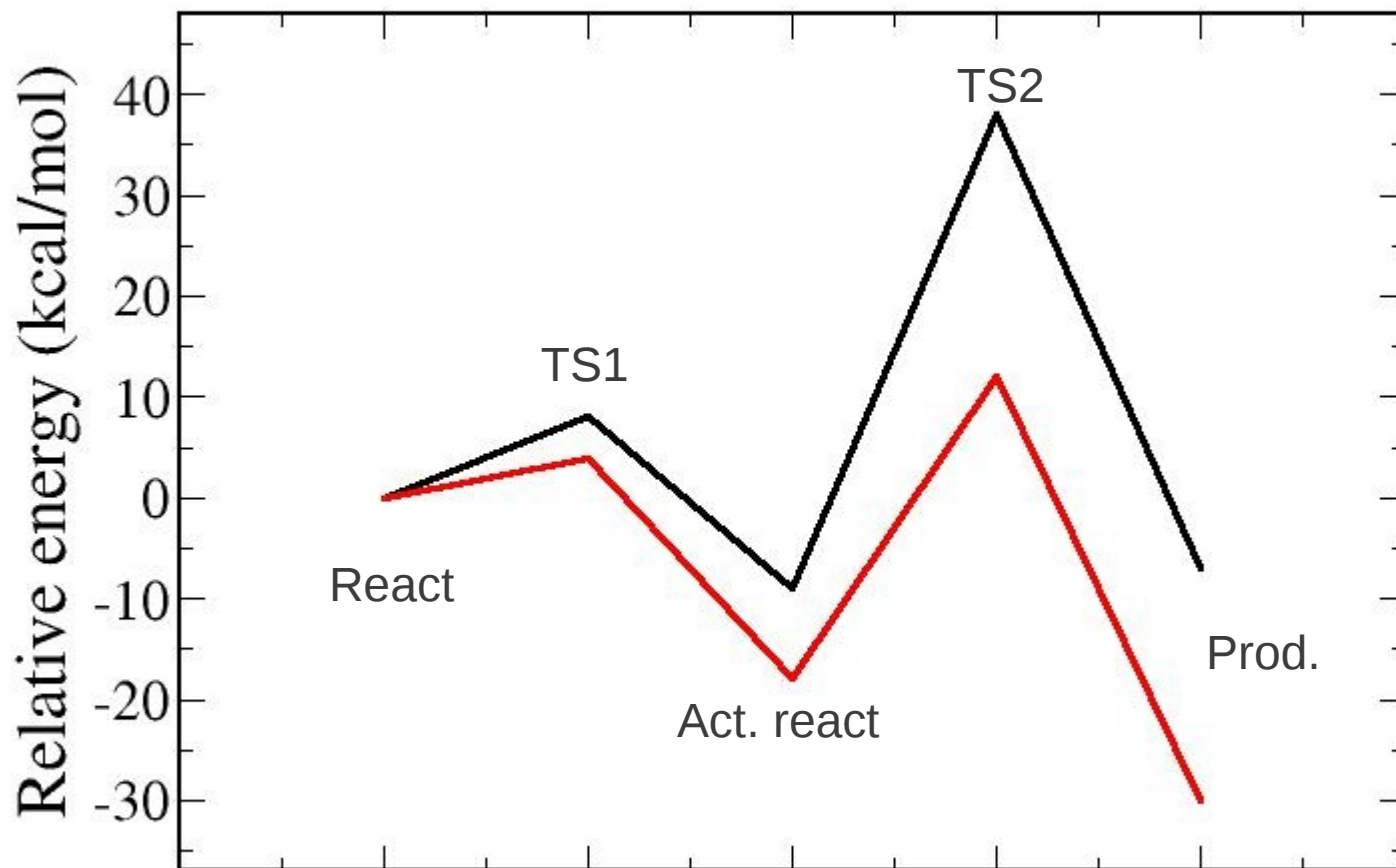


- QM contribution recalculated: single-point calculations at B3LYP/6-31+G(d)/CHARMM27
- Gaussian03

- Stationary points at similar positions
- Lower relative energy values, specially for the second step

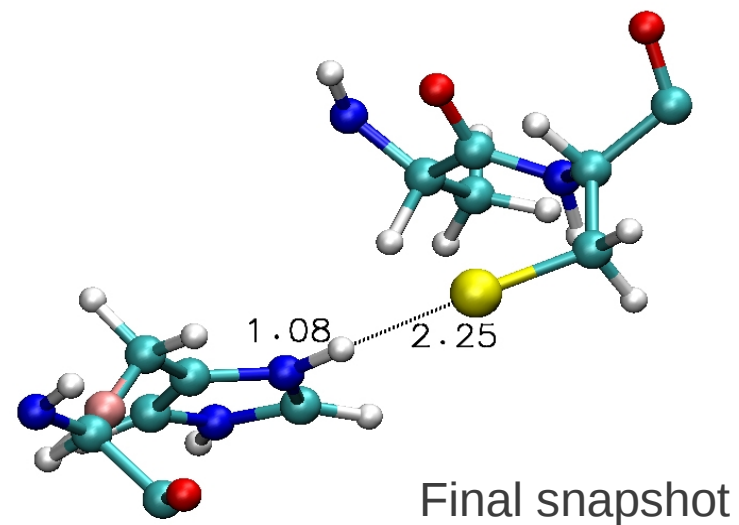
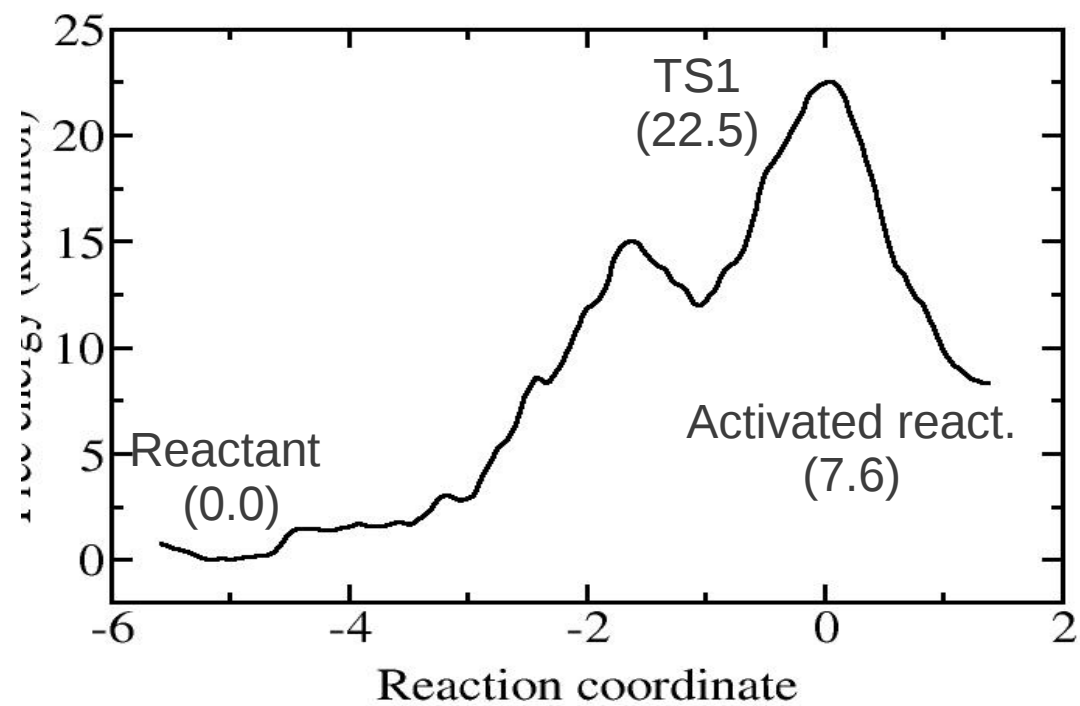
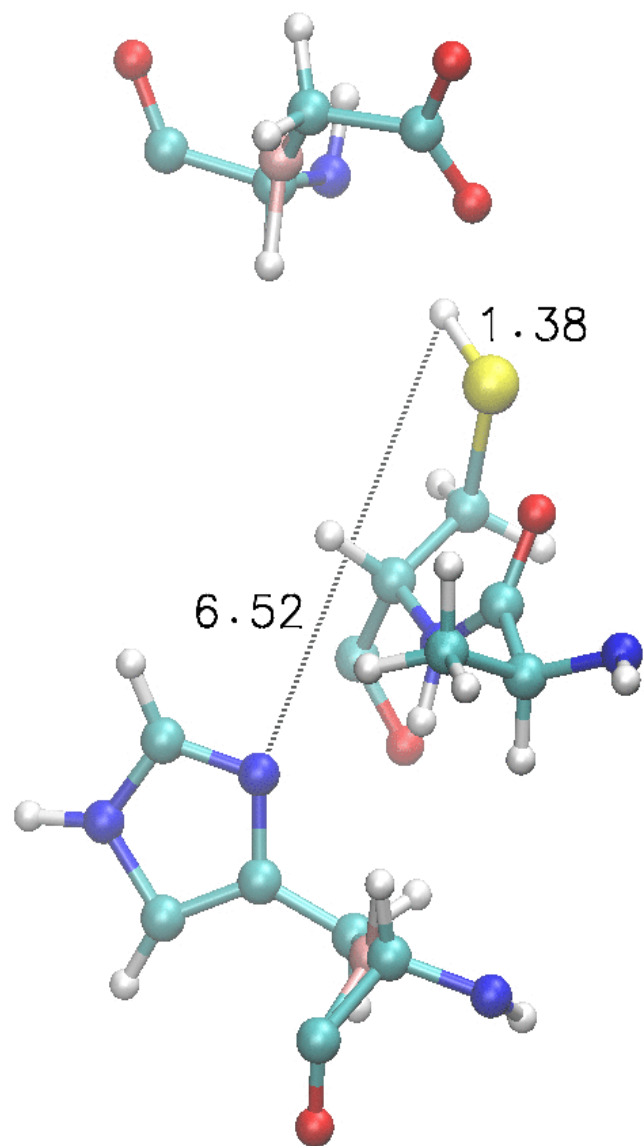


— SCCDFTB//CHARMM27  
— B3LYP/6-31+G(d)//CHARMM27

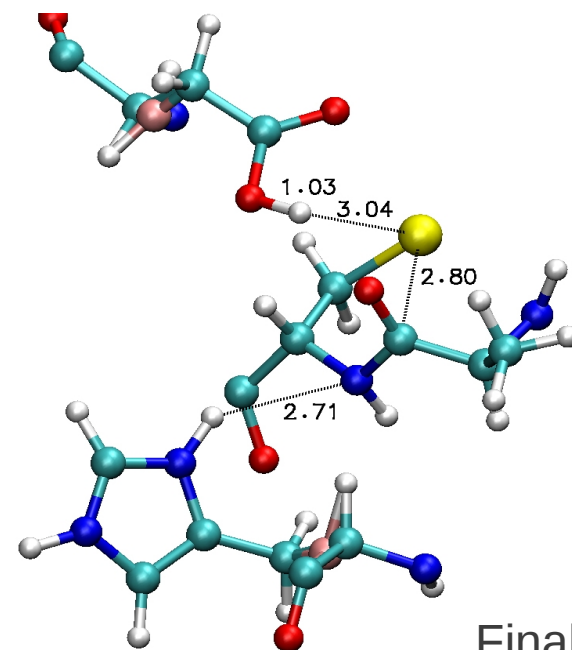
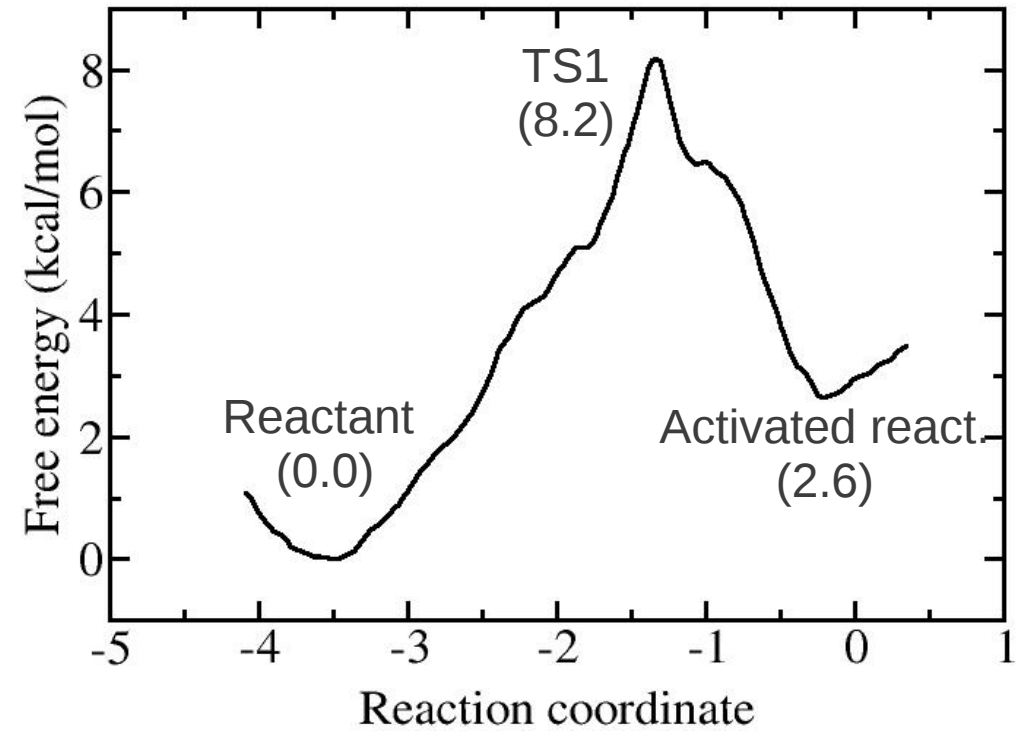
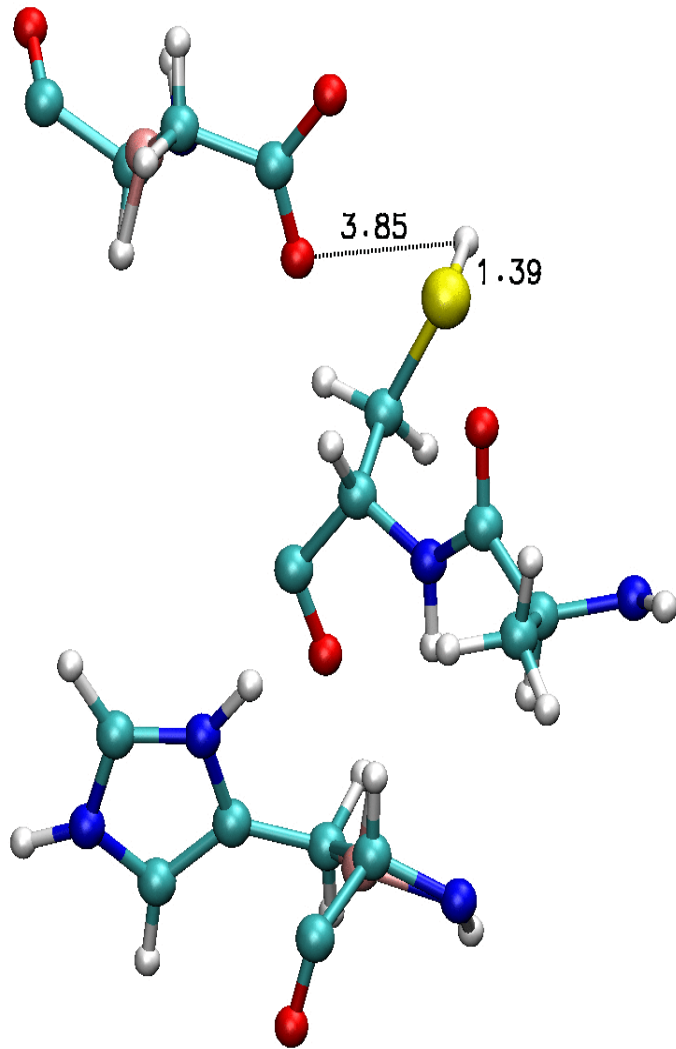




# PMF for Path 1a: Cys1 side chain activation by His73

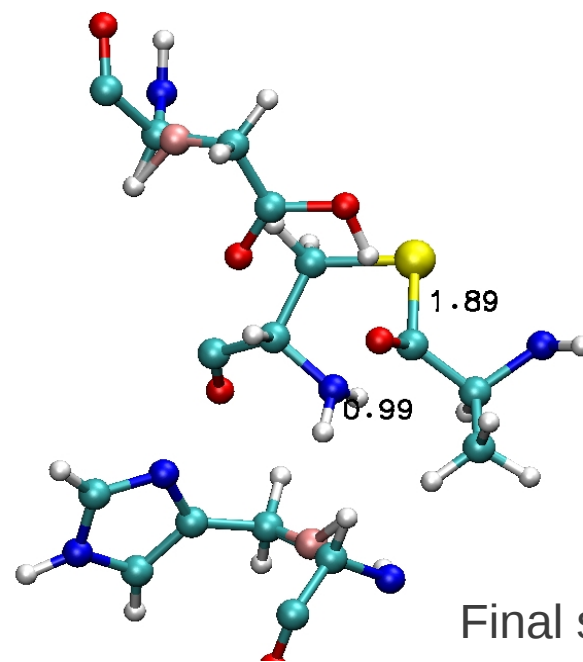
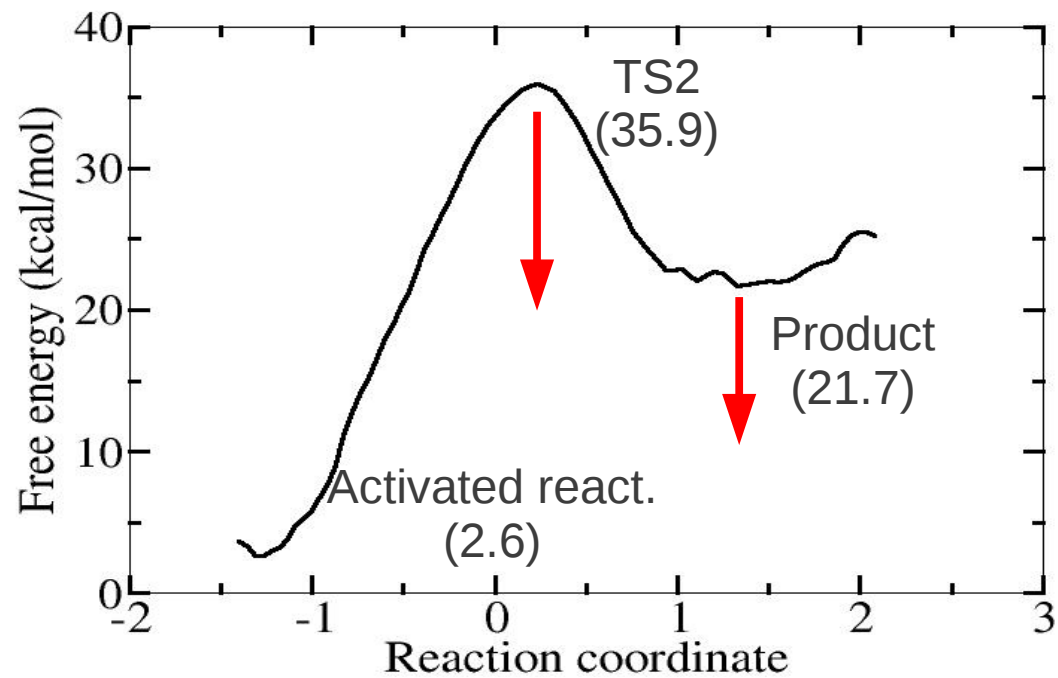
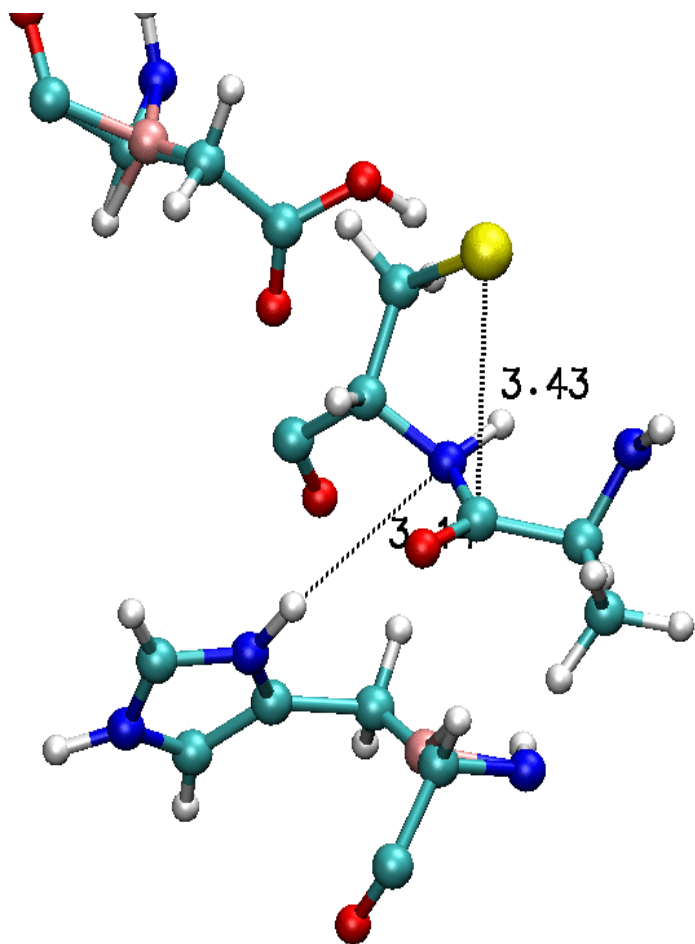


# PMF for Path 1b: Cys1 side chain activation by Asp422



Final snapshot

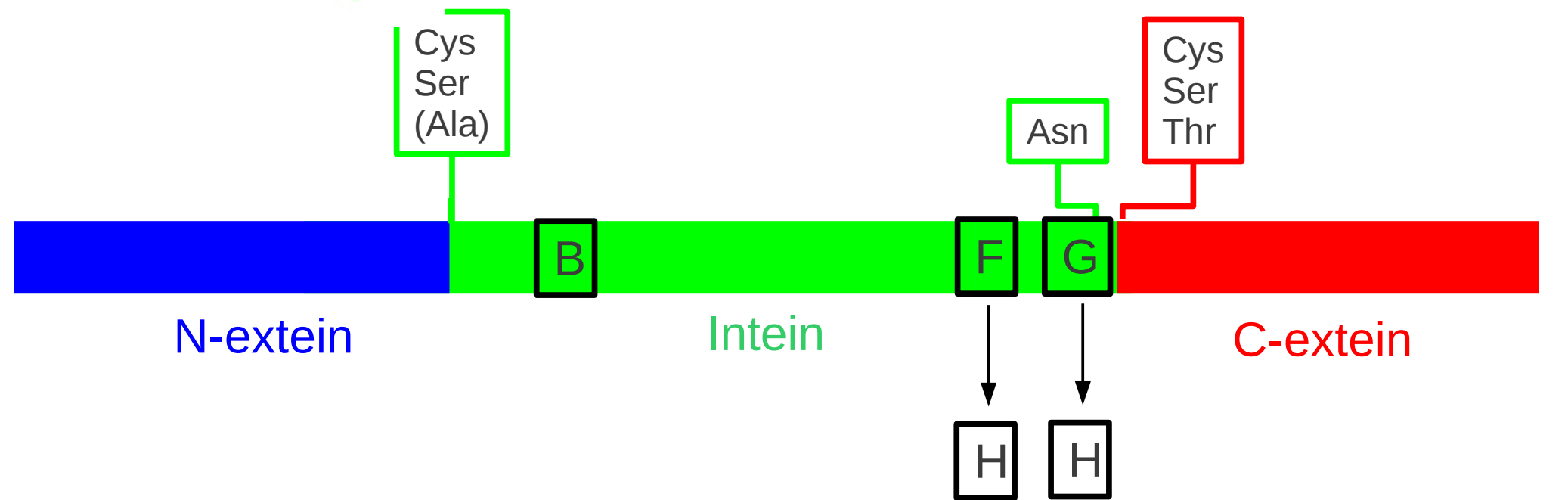
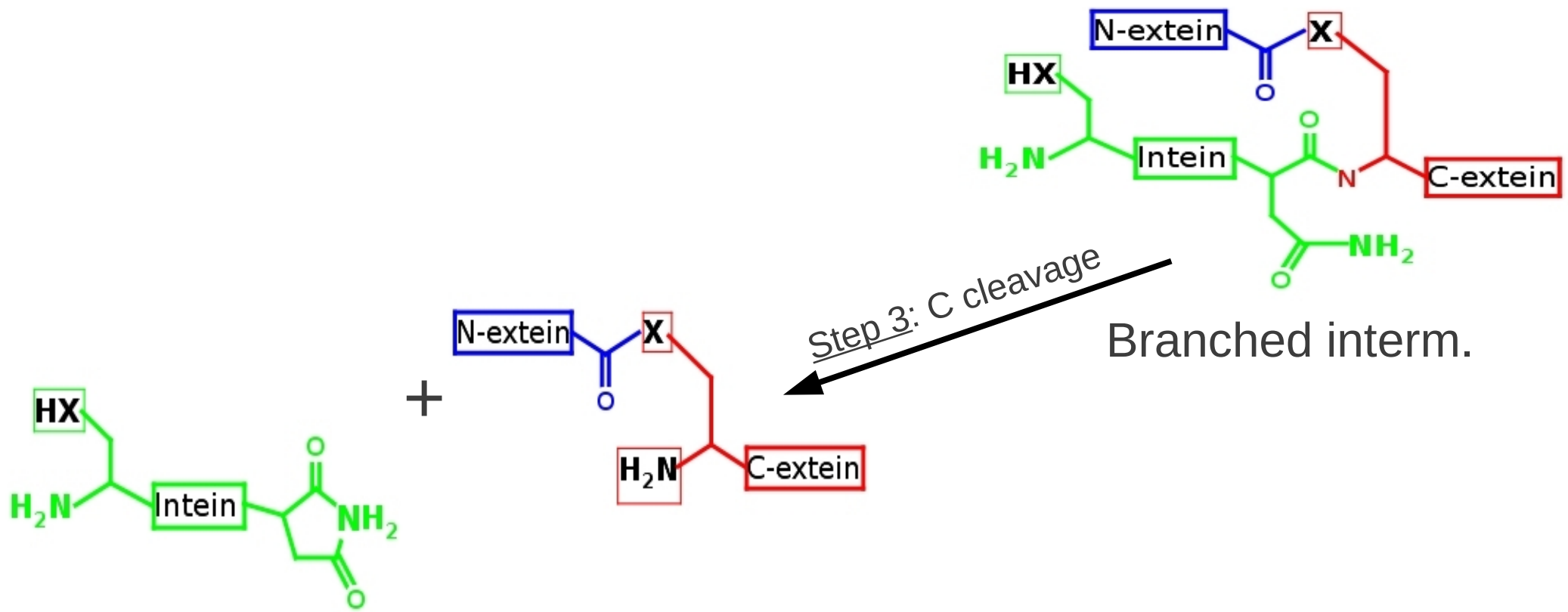
# PMF for Second step



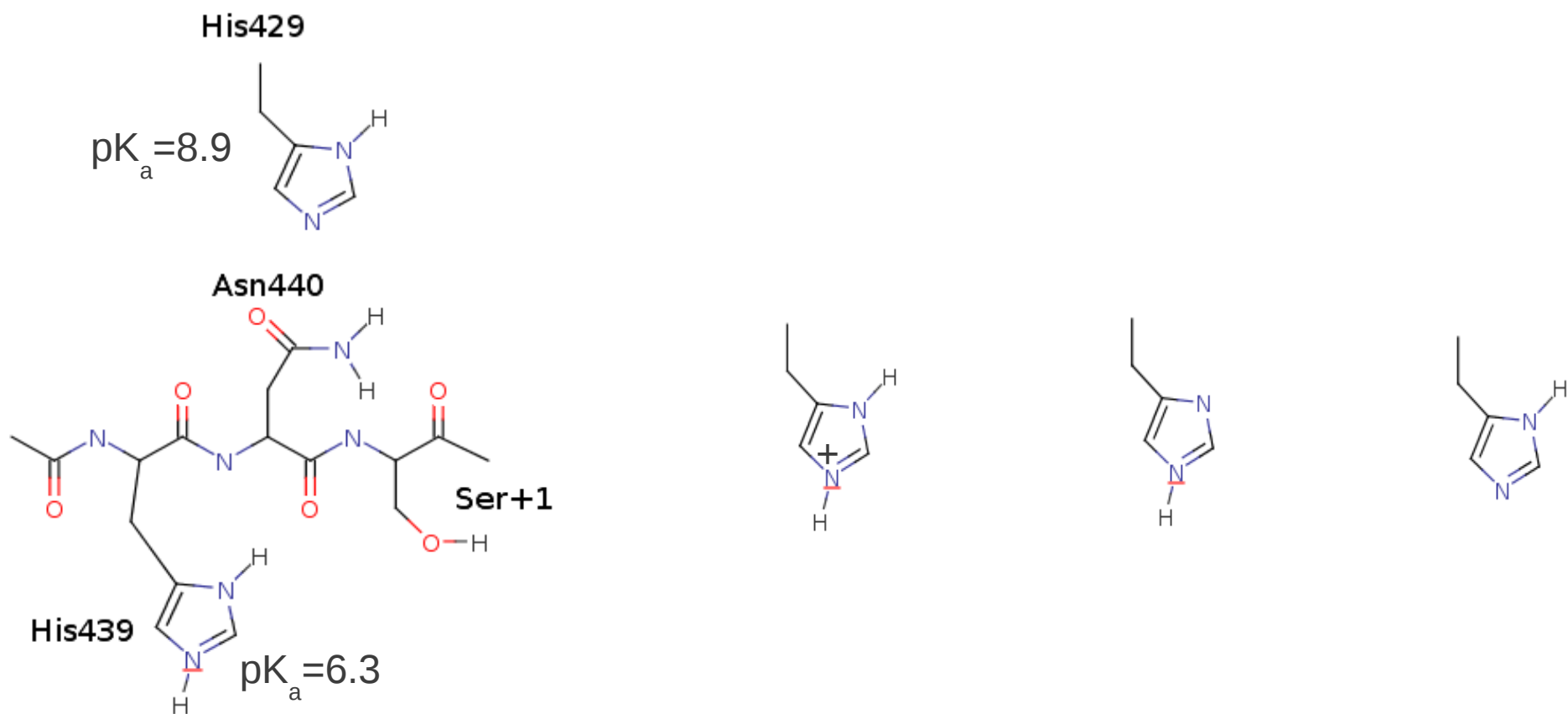
Final snapshot

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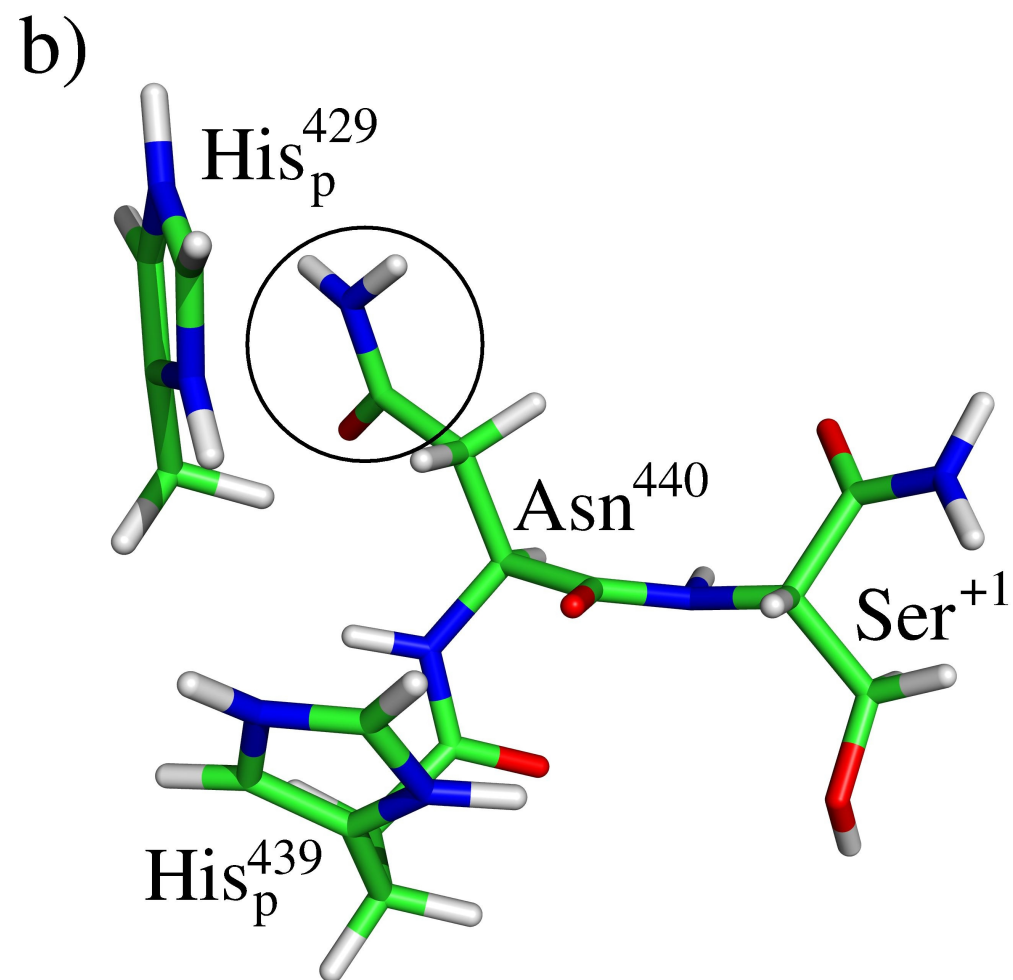
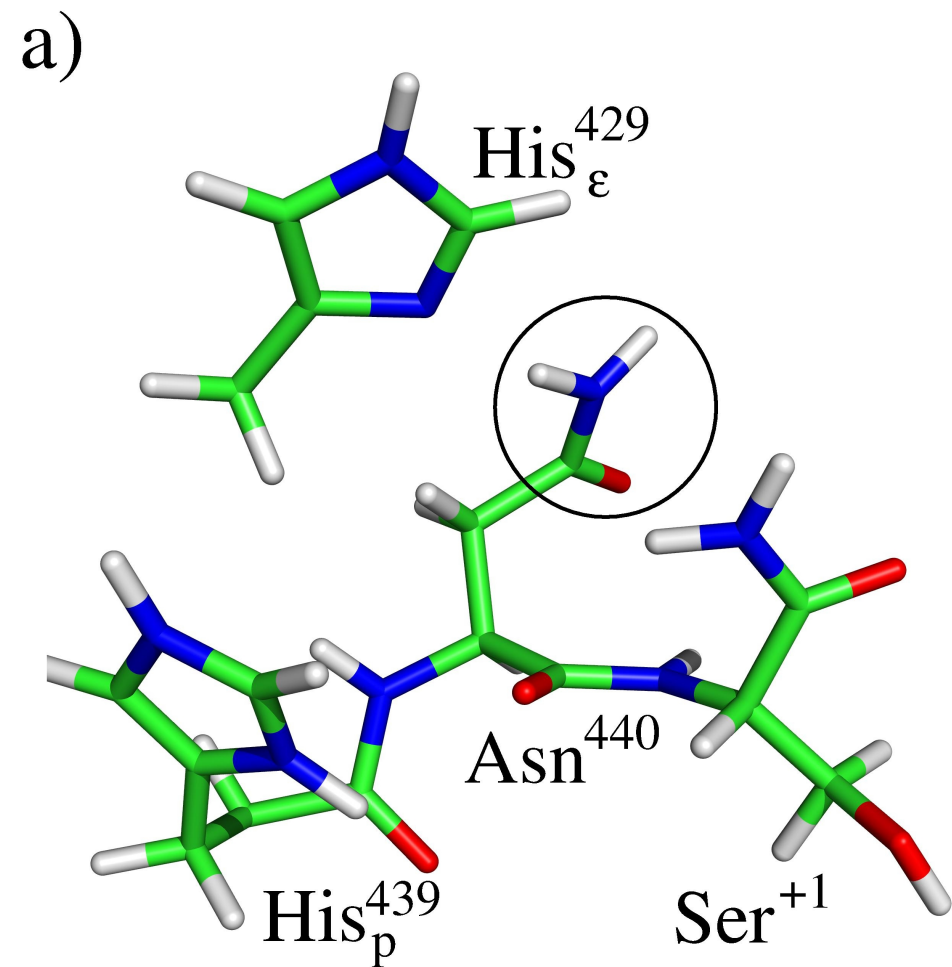
Step3: C-terminal cleavage



# But, which is the protonation state of the two histidines?

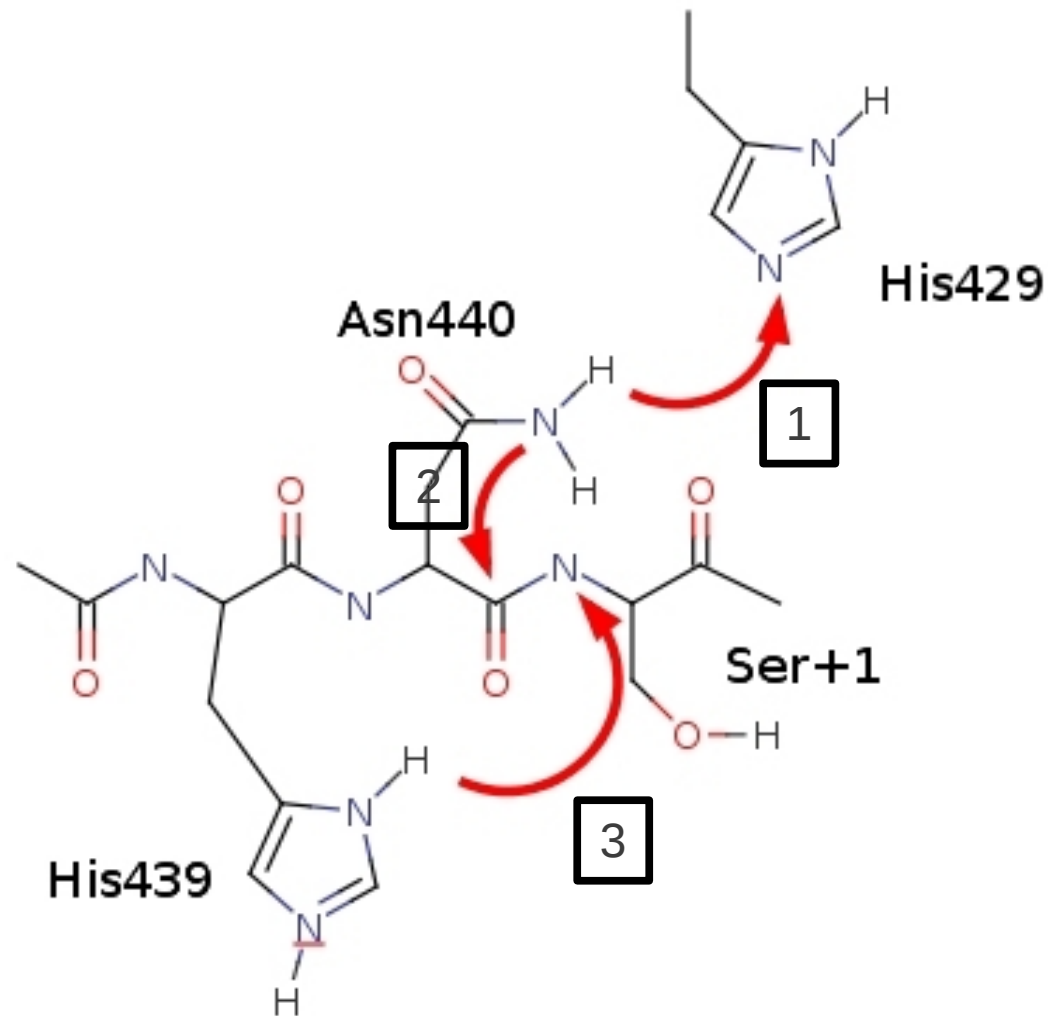


Nine molecular dynamics simulations considering the three protonation states for His429 and His439



- In eight out of nine of the MD simulations conformation b)
- Conformation a) only in one MD
- Conformation a) more suitable for the reaction

# Reaction mechanism for C-extein/intein cleavage



- The protonation of peptide bond N by His439 does not lead to the final product
- Therefore, three steps:
  - 1 Activation of Asn440 side chain
  - 2 Attack of Asn440 side chain at the peptide bond C
  - 3 Protonation of peptide bond N



QM(SCCDFTB)

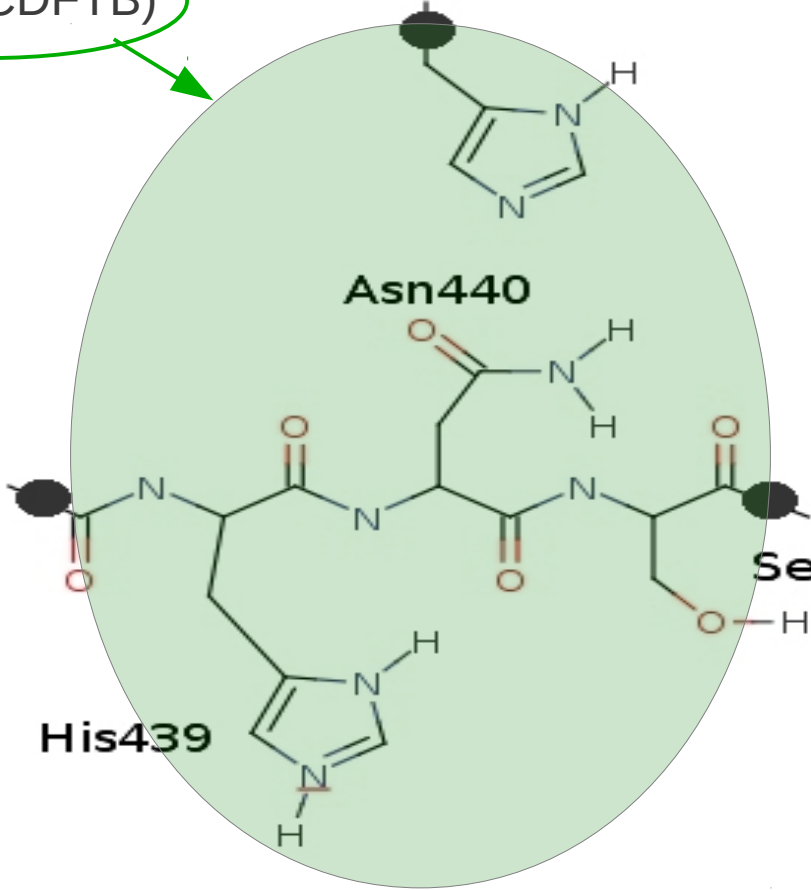
His429

Asn440

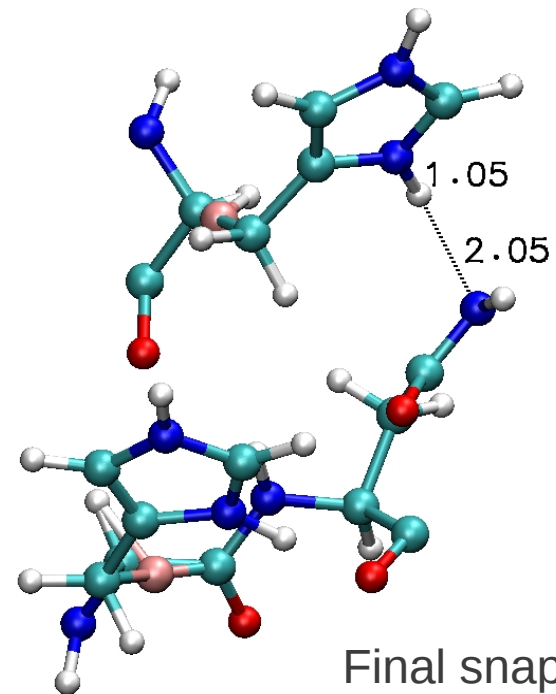
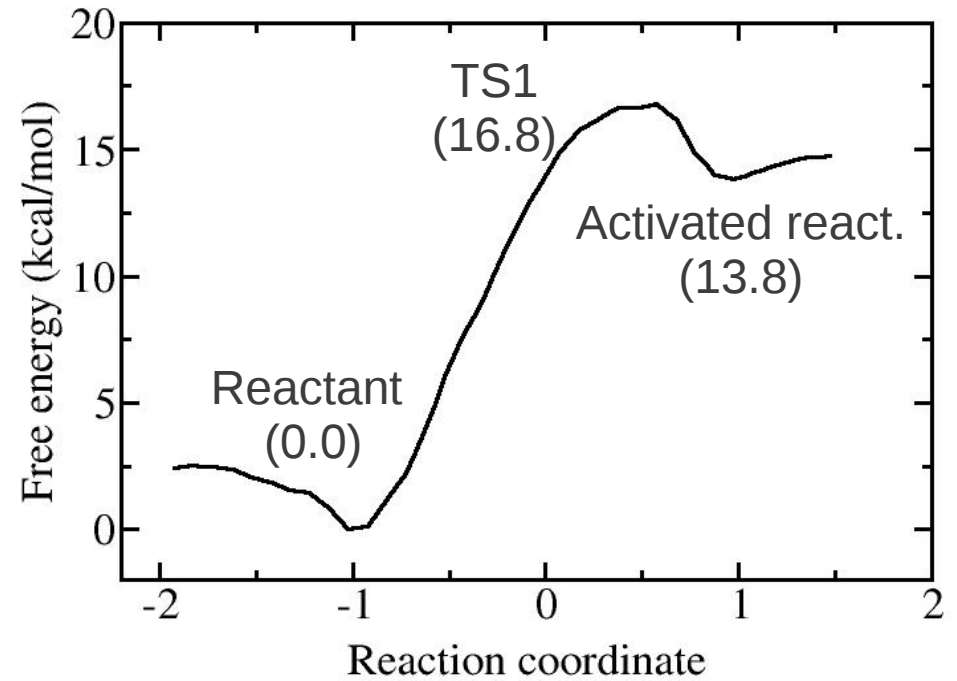
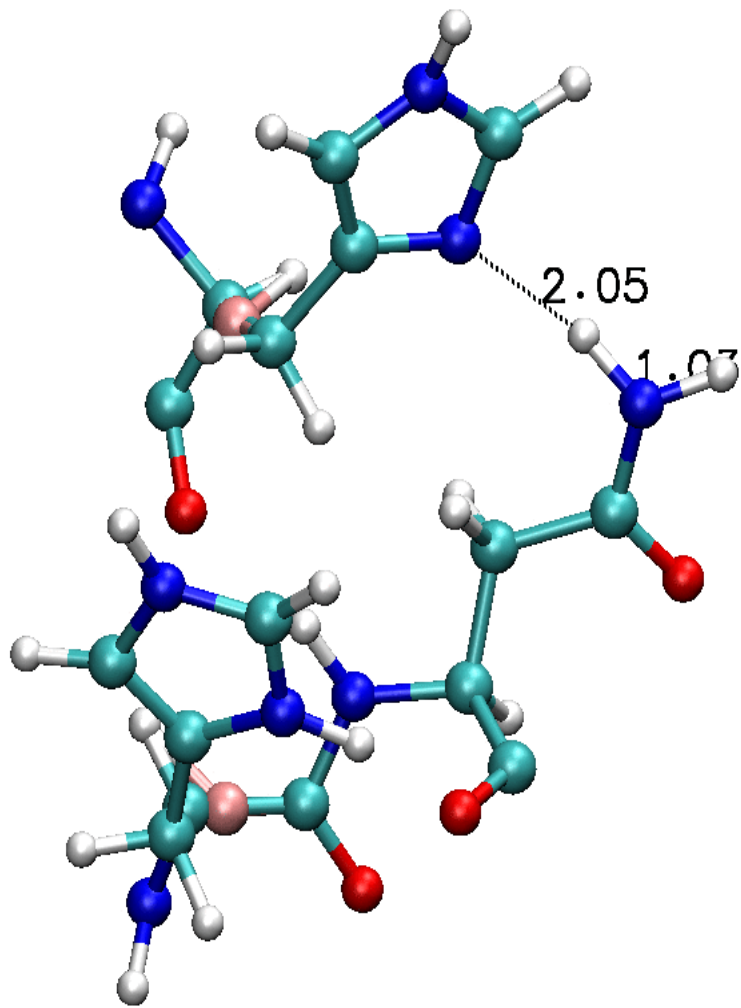
Ser+1

His439

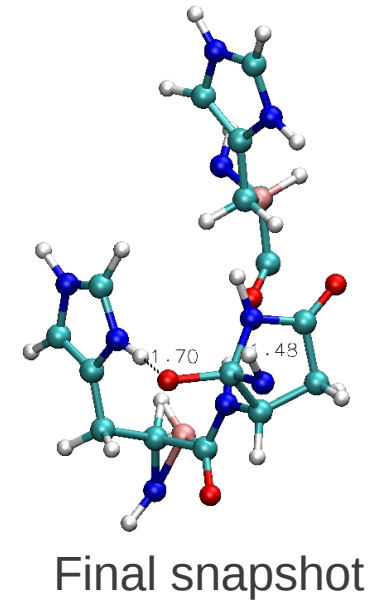
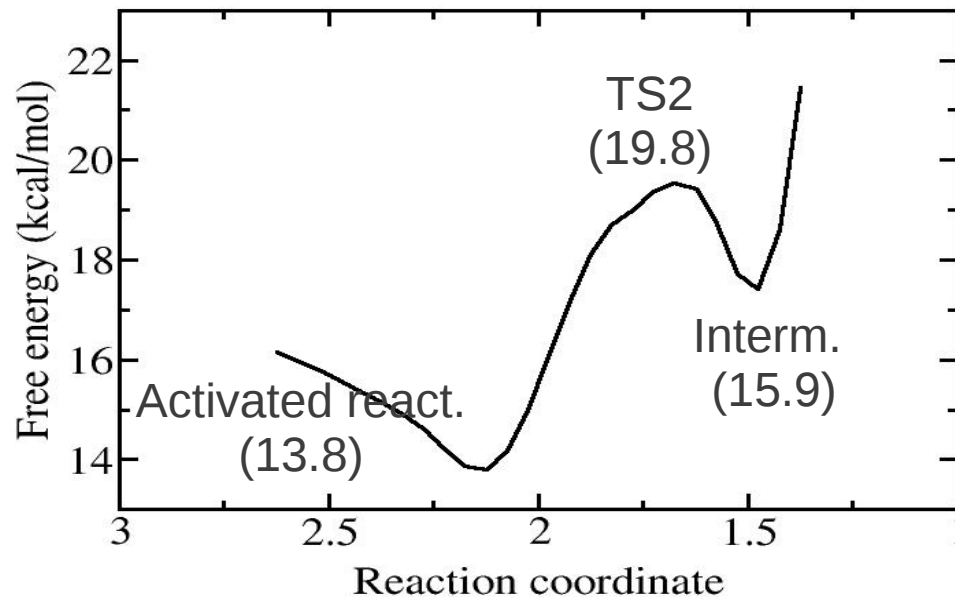
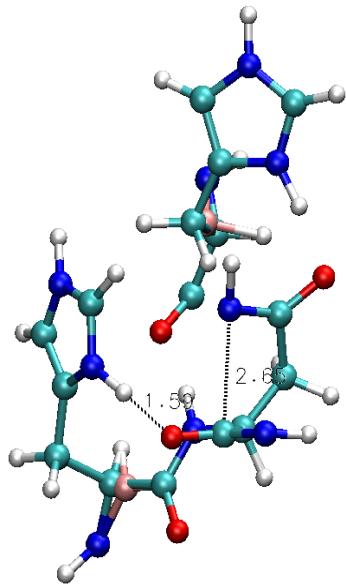
MM(CHARMM27)



# PMF for Step1: Asn440 side chain activation by His429



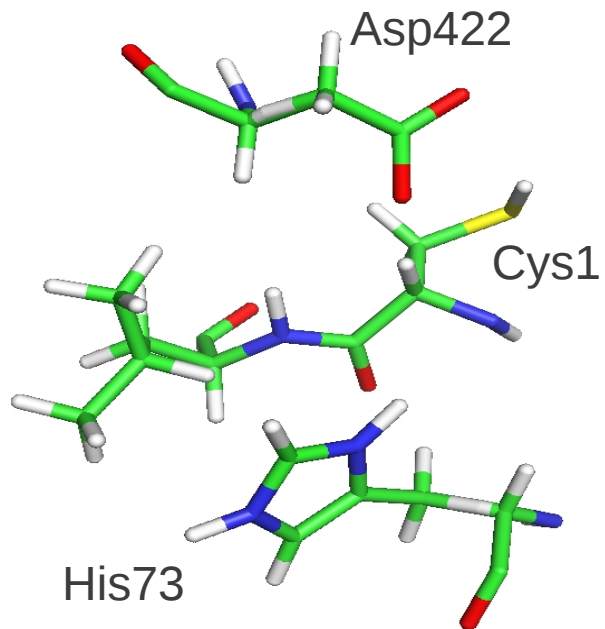
# PMF for Step2: attack of Asn440 side chain at peptide bond C



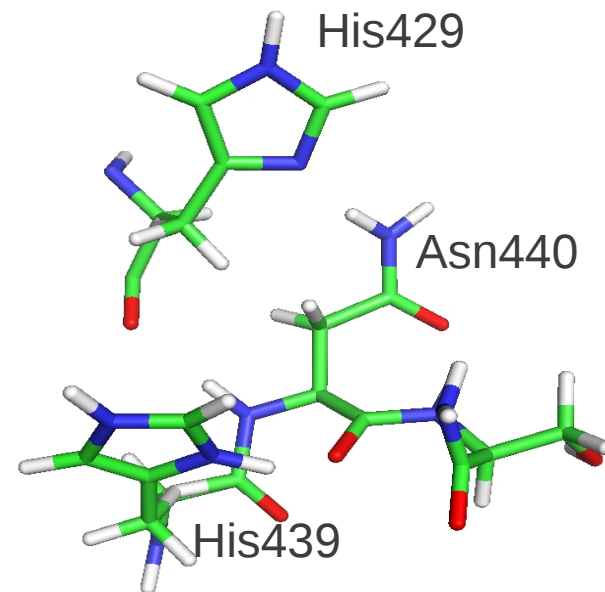
Last step: peptide bond cleavage

- Not possible to characterize the free energy profile for the protonation of N by His439.
- We hypothesize that another residue act as an acid. The protonated Asp422?

### Step1: N-terminal cleavage



### Step3: C-terminal cleavage



	N-cleavage	C-cleavage
Nucleophile	Cys1	Asn440
Base residue	Asp422	His429
Acid residue	His73	Asp422??
Main role of His	Protonate N	Stabilize oxyanion

# Conclusions

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- Similar acid-base mechanism for the N- and C-terminal cleavage.
- N-terminal cleavage: Asp422 seems a better base group than His73.
- Asp422 stabilizes the negative charge formed at peptide bond O.
- C-terminal cleavage: His429 activates the Asn440 side chain.
- Different roles for the two histidines:
  - His73 protonates the peptide bond N atom.
  - His339 stabilizes the oxyanion's negative charge.
- The SCCDFTB/CHARMM27 scheme provides reliable structures, although may overestimate the energies..

# Acknowledgments

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All of you for your attention!!