„MODELING CATALYTIC MECHANISM OF NITRILE HYDRATASE BY SEMI-EMPIRICAL QUANTUM MECHANICAL CALCULATION” - *REVIEW OF CURRENT LITERATURE**

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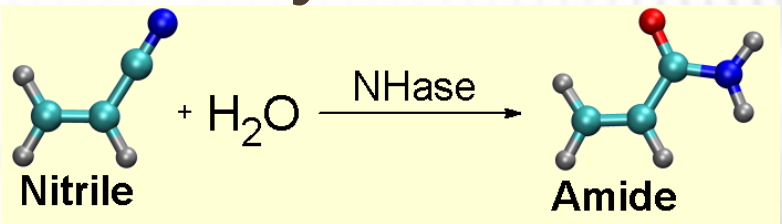
OUTLINE

- introduction
- methodology
- results and discussion
- summary

INTRODUCTION

- mechanism of nitrile hydratase



- TRITON graphical software

freely supported by the National Center of Biomolecular Research

http://ncbr.chemi.muni.cz/triton/

consisting of three modules of

MOPAC, MODELLER and DRIVER

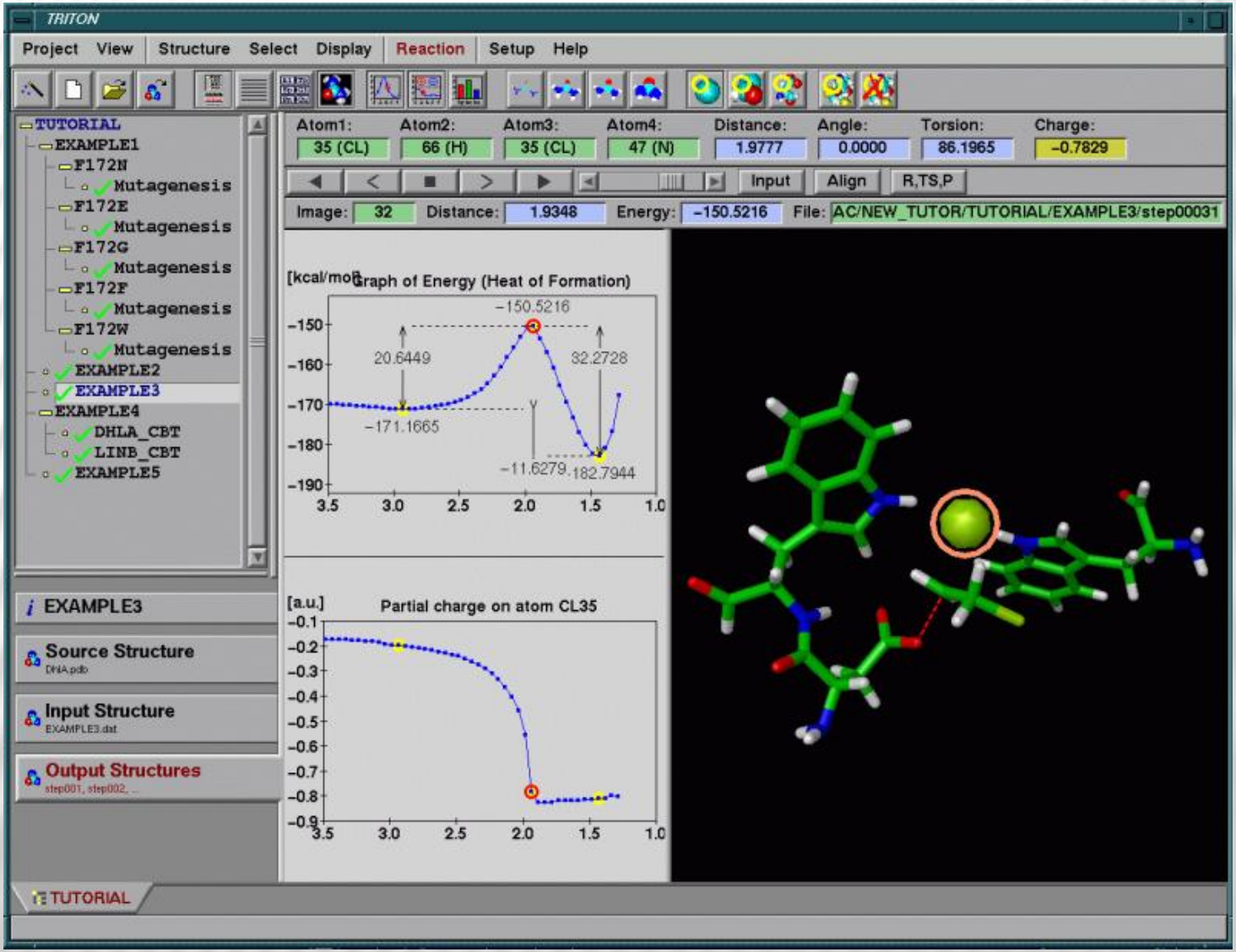
INTRODUCTION

TRITON SOFTWARE:

- modeling enzymatic reactions
- analyzing interactions between the active site residues and the substrate
- constructing protein mutants *in silico*

INTRODUCTION

TRITON SOFTWARE



METHODOLOGY

- Co-type NHase JCM 3095 (from PDB - code 1IRE)
- tartiary structure of acrylonitryle (Discovery Studio 2.0)
- docking acrylonitryle (AN) into 1IRE (AutoDock 3.0.5)
- grid with size of 80Å x 80Å x 80Å
- distance of the grid node - 0.375Å.
- searching algorithm – LGA
- Enzymatic reaction modeling - TRITON

METHODOLOGY

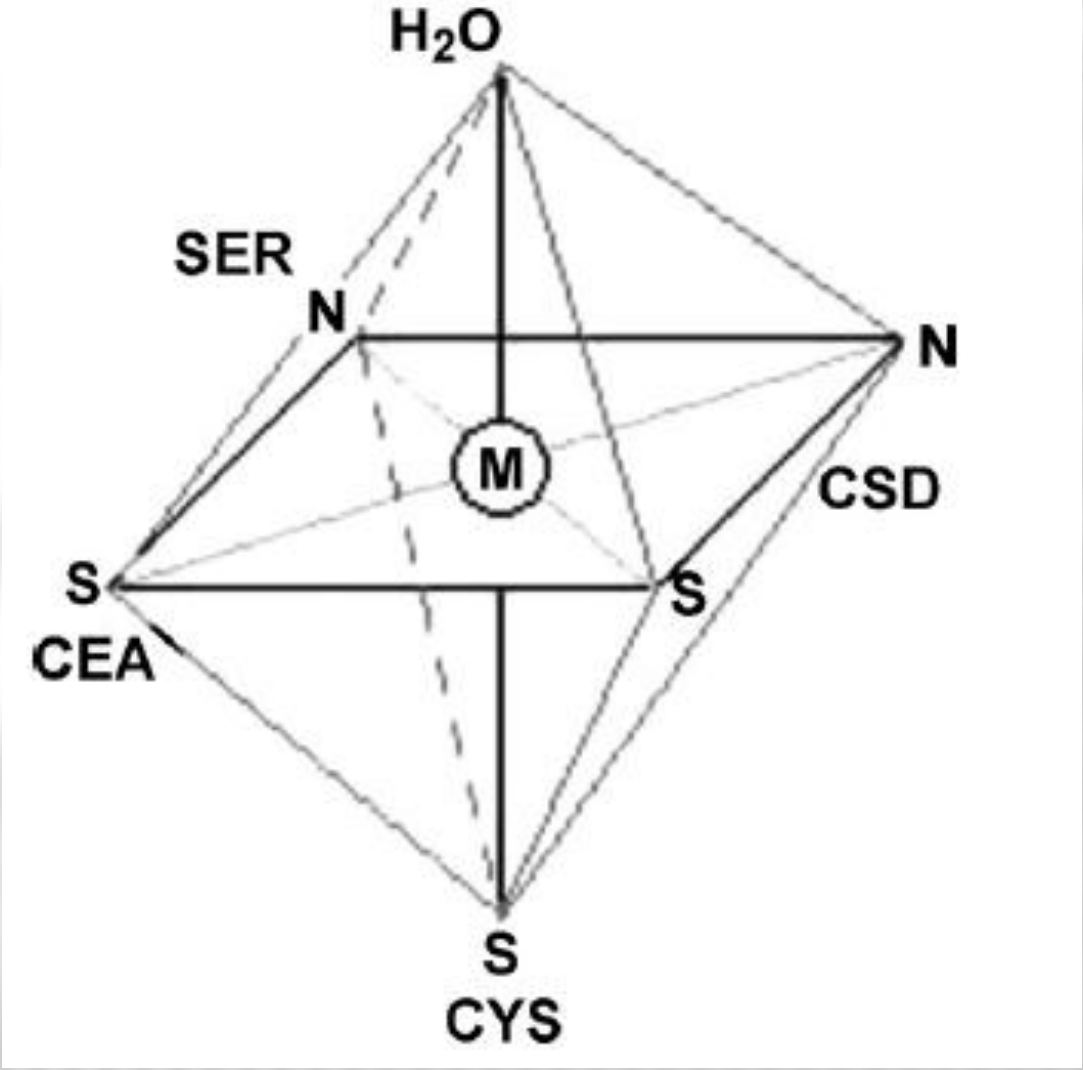
Detailed simulation process was carried out as follows:

1) load model of the enzyme–substrate complex
2) specify the catalysis substrate
3) specify functional groups composing the catalysis „cavity”
4) search the reaction pathway of atoms taking part in catalysis
5) specify the main chain atoms
6) determine the QM calculating parameters in MOPAC

RESULTS AND DISCUSSION

1) *Active site structure of NHase and docking of acrylonitrile*

Schematic octahedron structure of the active site of NHases. The ligands to the cobalt atom (M) include a water oxygen atom, two main chain amide nitrogen atoms (N) (α-SER112 and α -CEA113) and three sulfur atoms (S) of the α -CYS108, α -CSD111, and α -CEA113, where CSD is the post-translationally modified cysteinesulfunic acid and CEA is the post-translationally modified cysteine sulfenic acid.



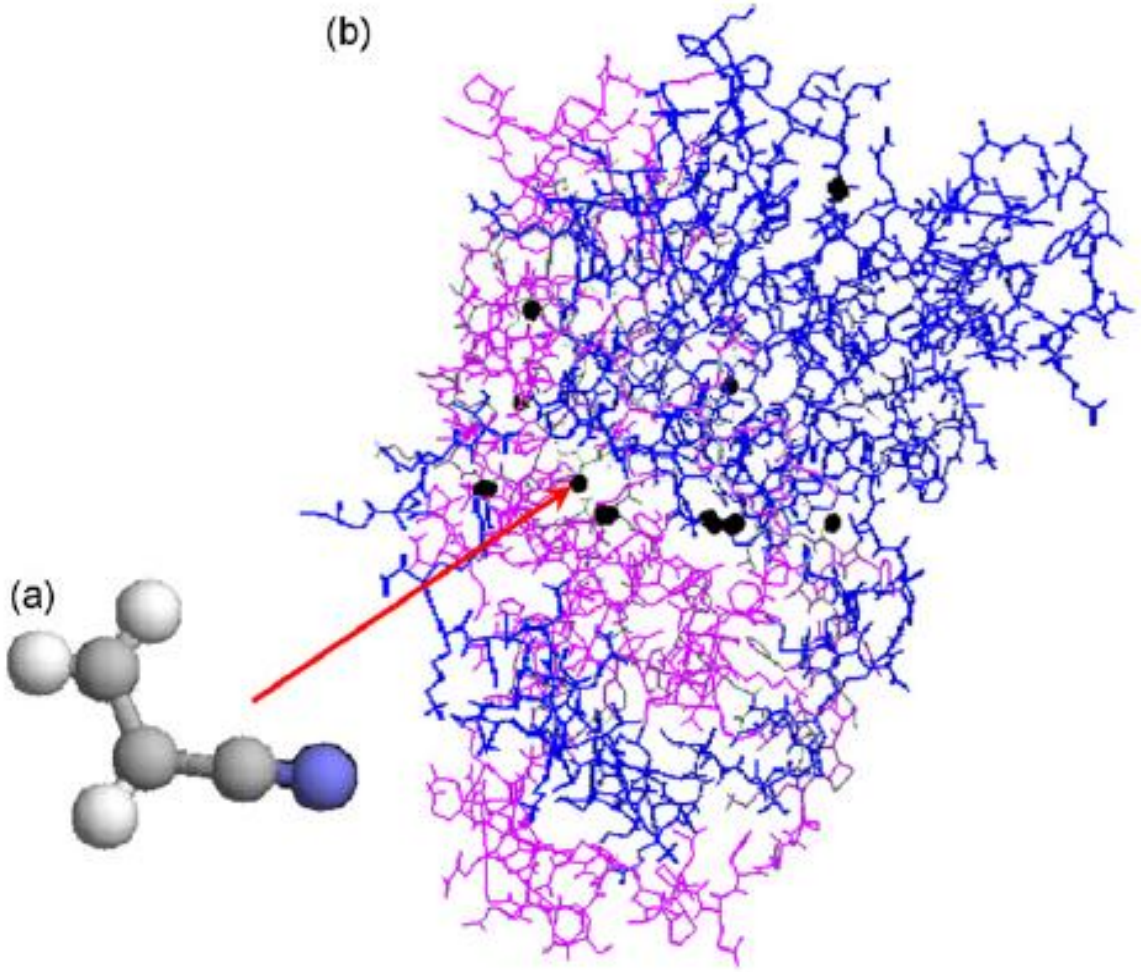
RESULTS AND DISCUSSION

1) Active site structure of NHase and docking of acrylonitrile

Autodock of the substrate acylonitrile into the active center of nitrile hydratase 1IRE:

(a) the molecule of acrylonitrile;

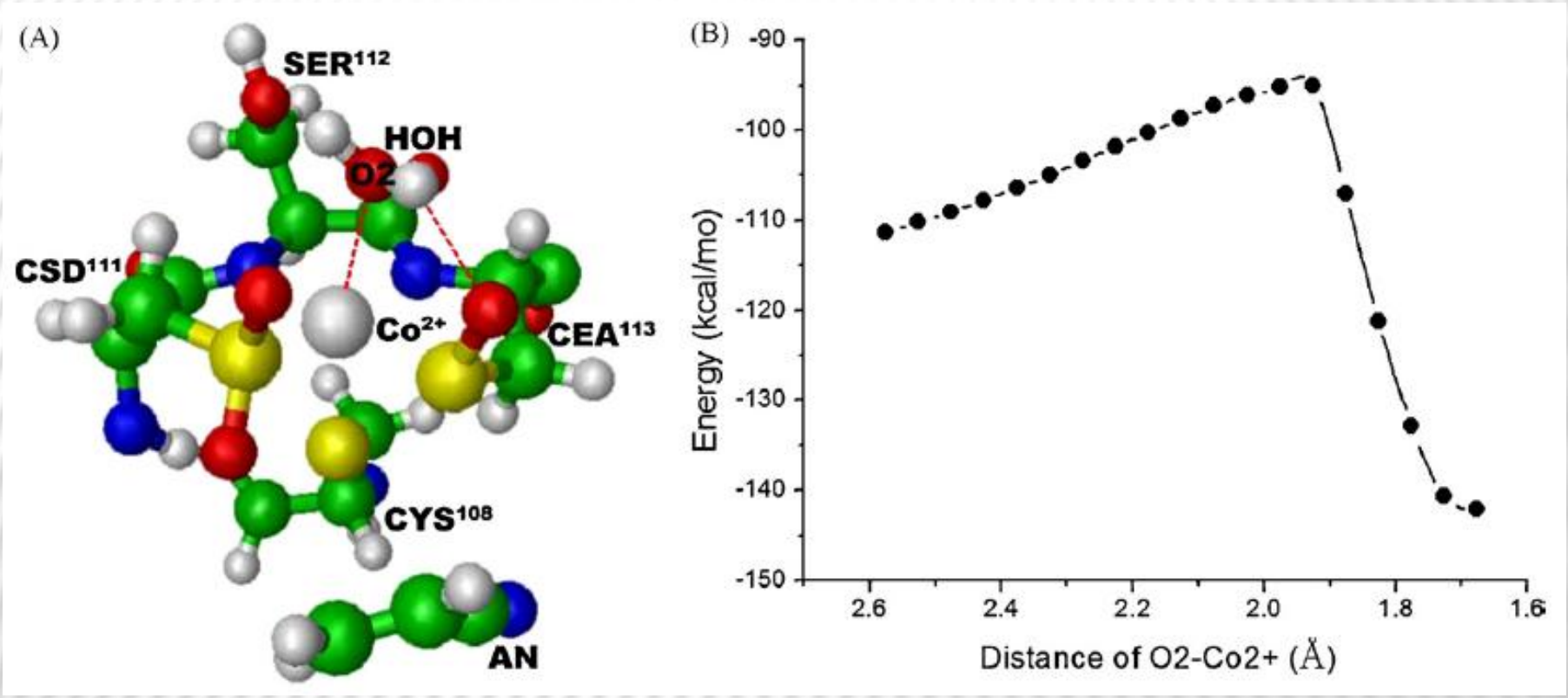
(b) the 3D structure of 1IRE



lowest binding energy of –3.86 kcal/mol

RESULTS AND DISCUSSION

2) Step 1 simulation of NHase catalysis designated as active site activation



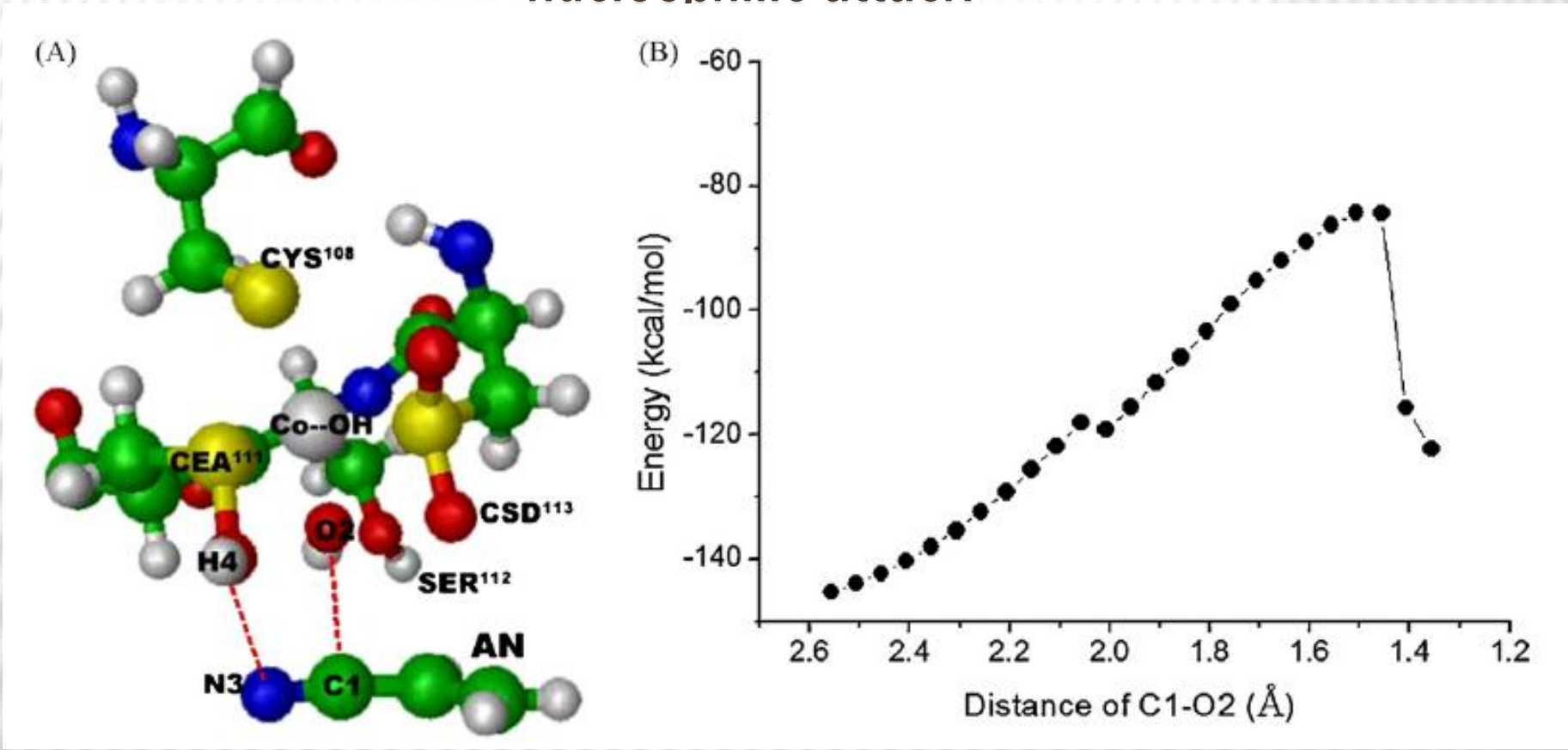
Pathway sketch map and energy changes in step 1 simulation of NHase catalysis designated as active site activation.

(A) Schematic active center structure of the O2– Co2+ reaction pathway. Major atoms of O2 (H_2O)–Co^{2+} and O (CEA)–H (H_2O)

(B) Energy (heat of formation) changes of the O2–Co^{2+} reaction pathway

RESULTS AND DISCUSSION

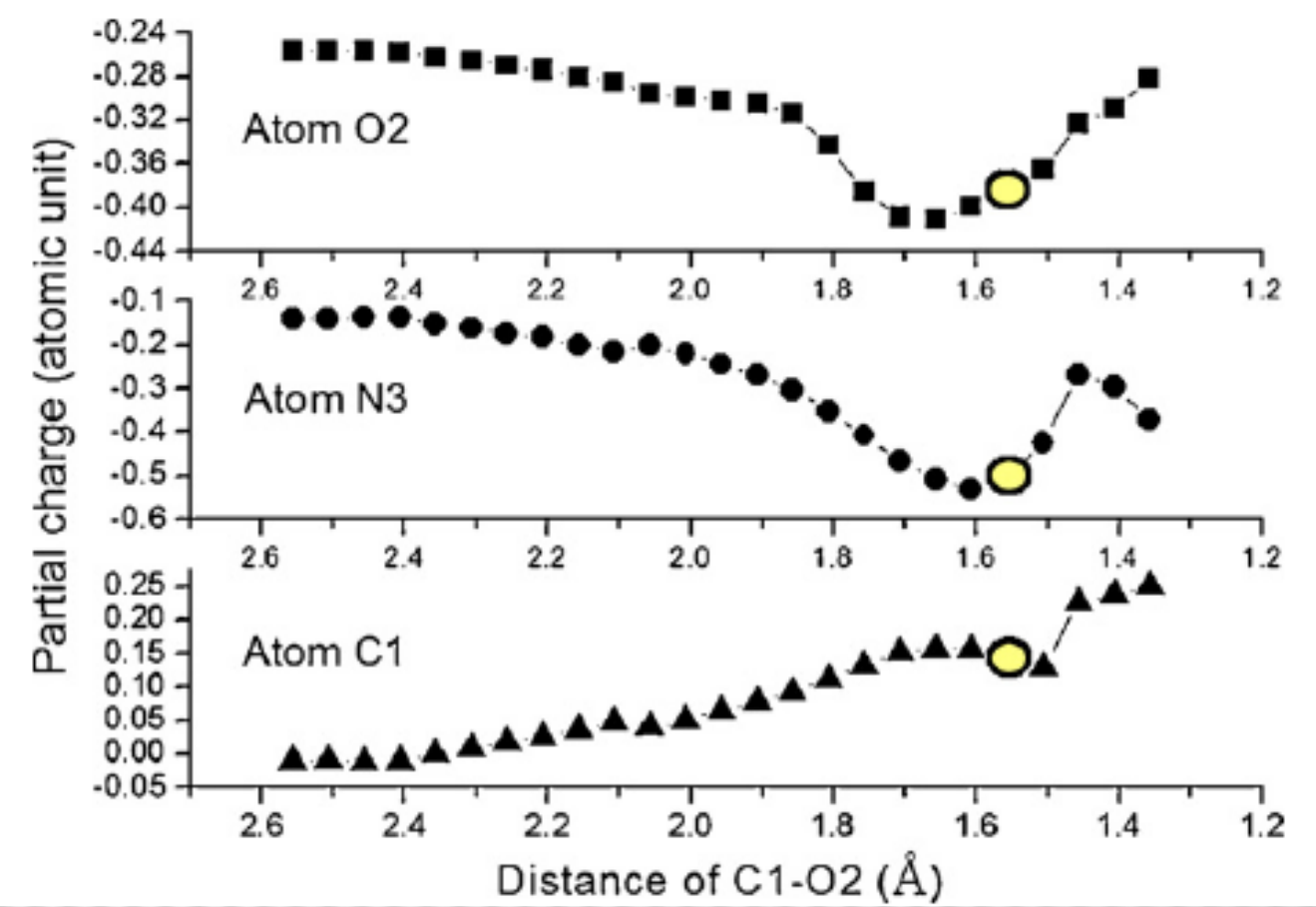
3) Step 2 simulation of NHase catalysis designated as nucleophilic attack



Pathway sketch map and energy changes in step 2 simulation of NHase catalysis designated as nucleophilic attack performed by Co^{2+}-bound –OH in C1–O2:N3–H4 pathway.

RESULTS AND DISCUSSION

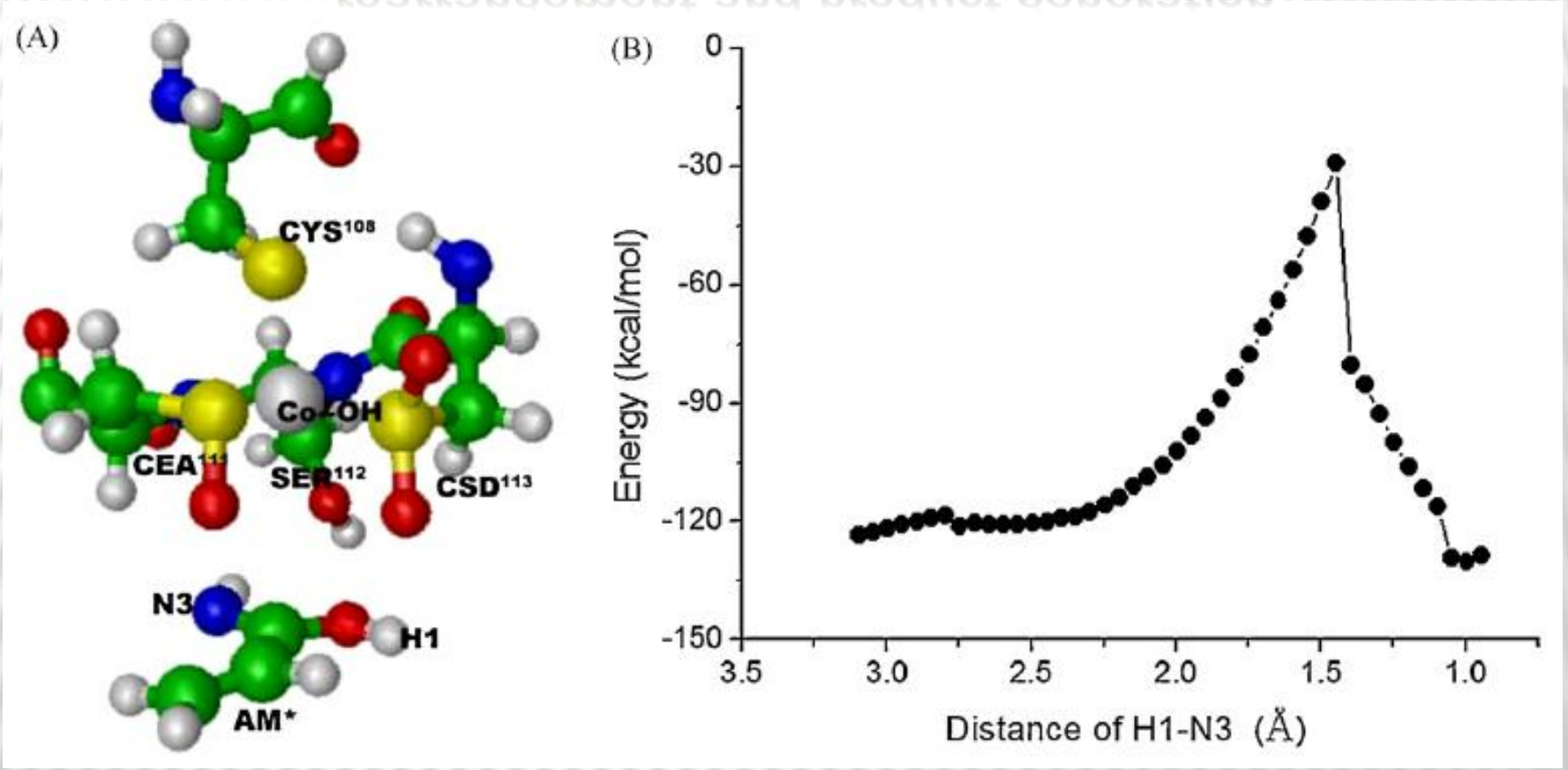
3) Step 2 simulation of NHase catalysis designated as nucleophilic attack



Changes in partial charges of atom O2, N3 and C1 in the C1–O2:N3–H4 reaction pathway of nucleophilic attack performed by Co2+-bound –OH group

RESULTS AND DISCUSSION

4) Step 3 simulation of NHase catalysis designated as proton rearrangement and product generation



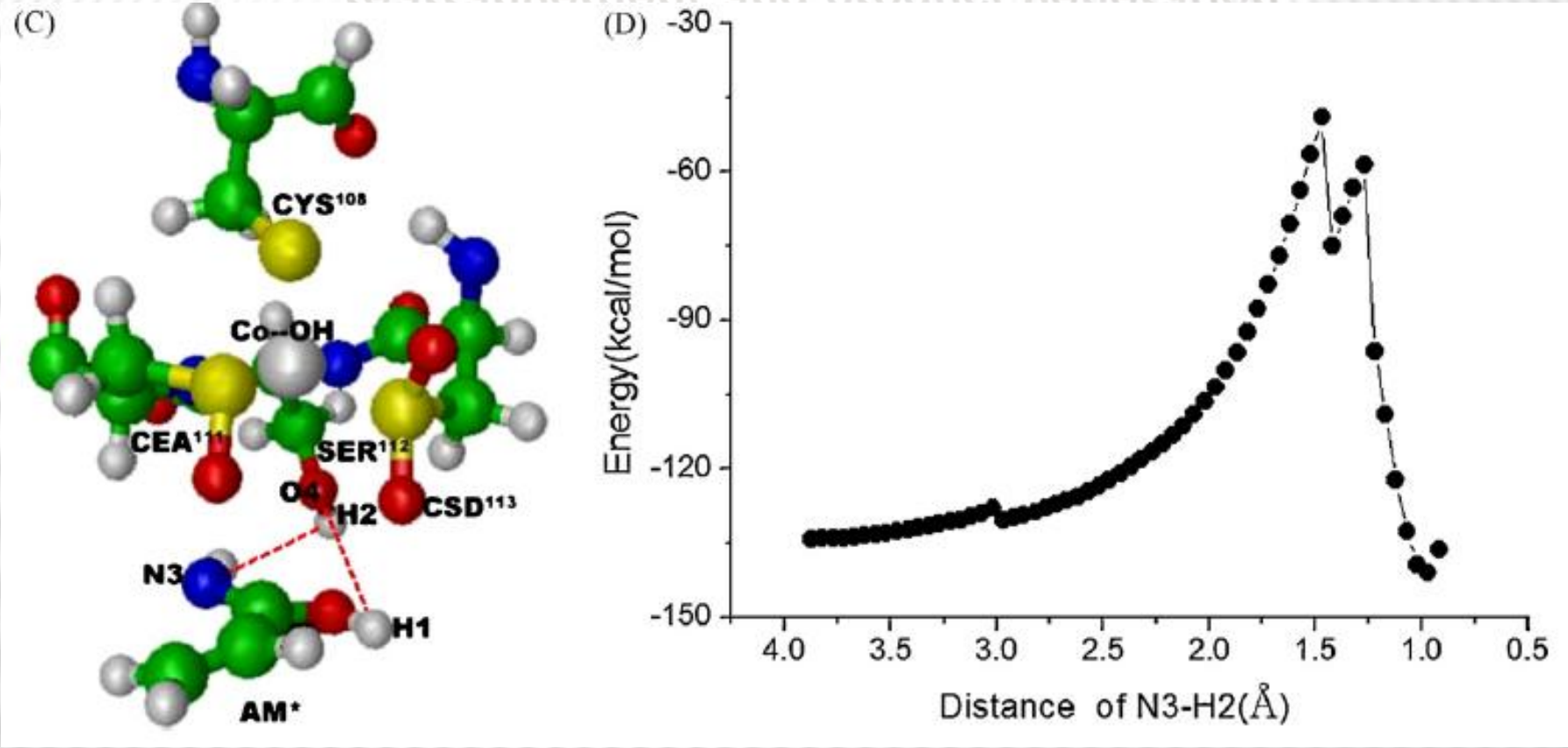
Pathway sketch map and energy changes in proton transfer/product generation simulation of NHase catalysis

(A) Schematic structure of the H1–N3 reaction pathway

(B) energy changes of the H1–N3 reaction pathway

RESULTS AND DISCUSSION

4) Step 3 simulation of NHase catalysis designated as proton rearrangement and product generation

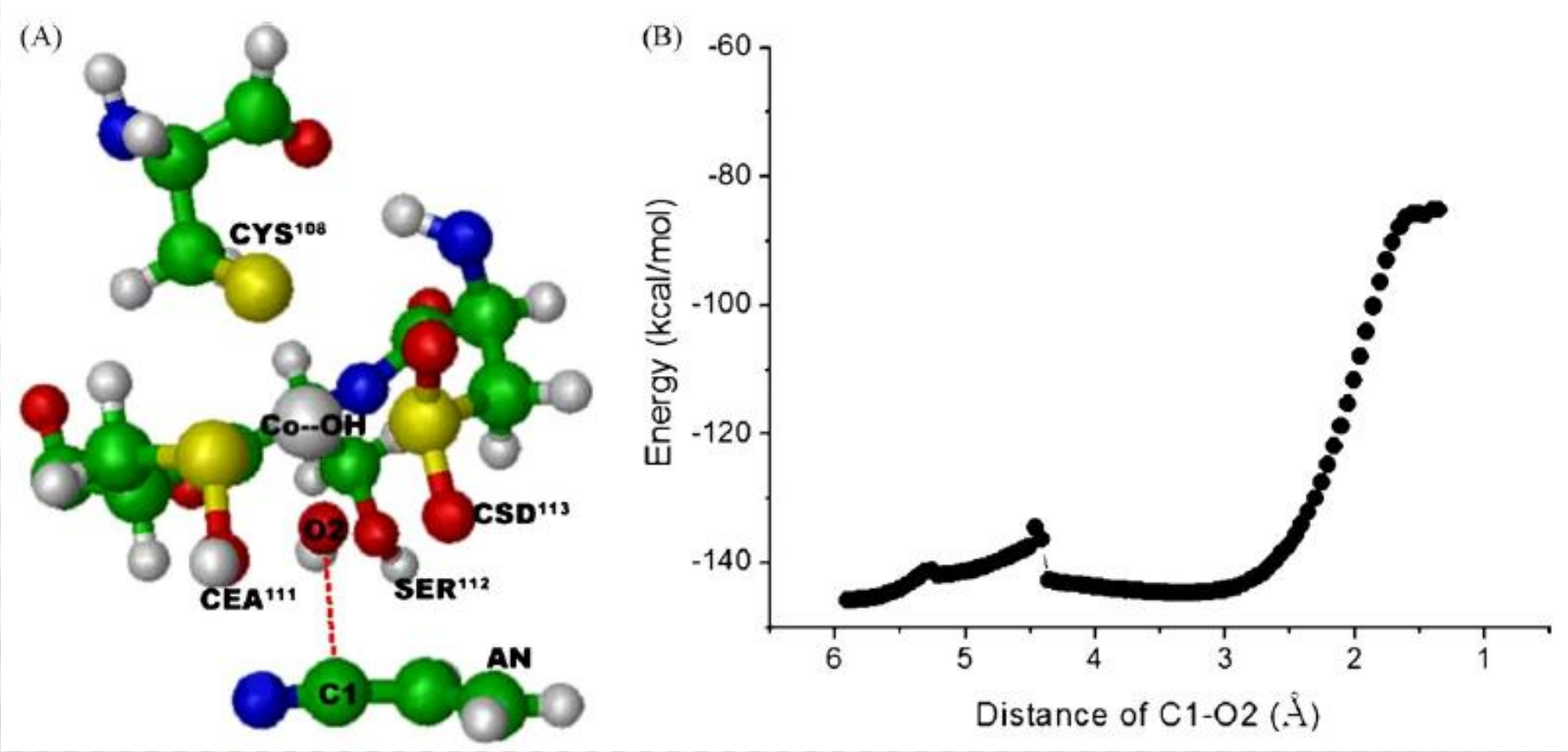


(C) schematic structure of the H2–N3:H1–O4 reaction pathway;
(D) energy changes of the H2–N3:H1–O4 reaction

Dashed lines indicate the major reaction pathways, and the metal Co^{2+} coordinated to a new water molecule. AM*, the precursor of acrylamide (AM).

RESULTS AND DISCUSSION

5) MOPAC calculation test for diverse reaction pathways approaching



Pathway sketch map and energy changes in nucleophilic attack simulation assuming that the reaction was performed by merely O2 and C1 atoms.

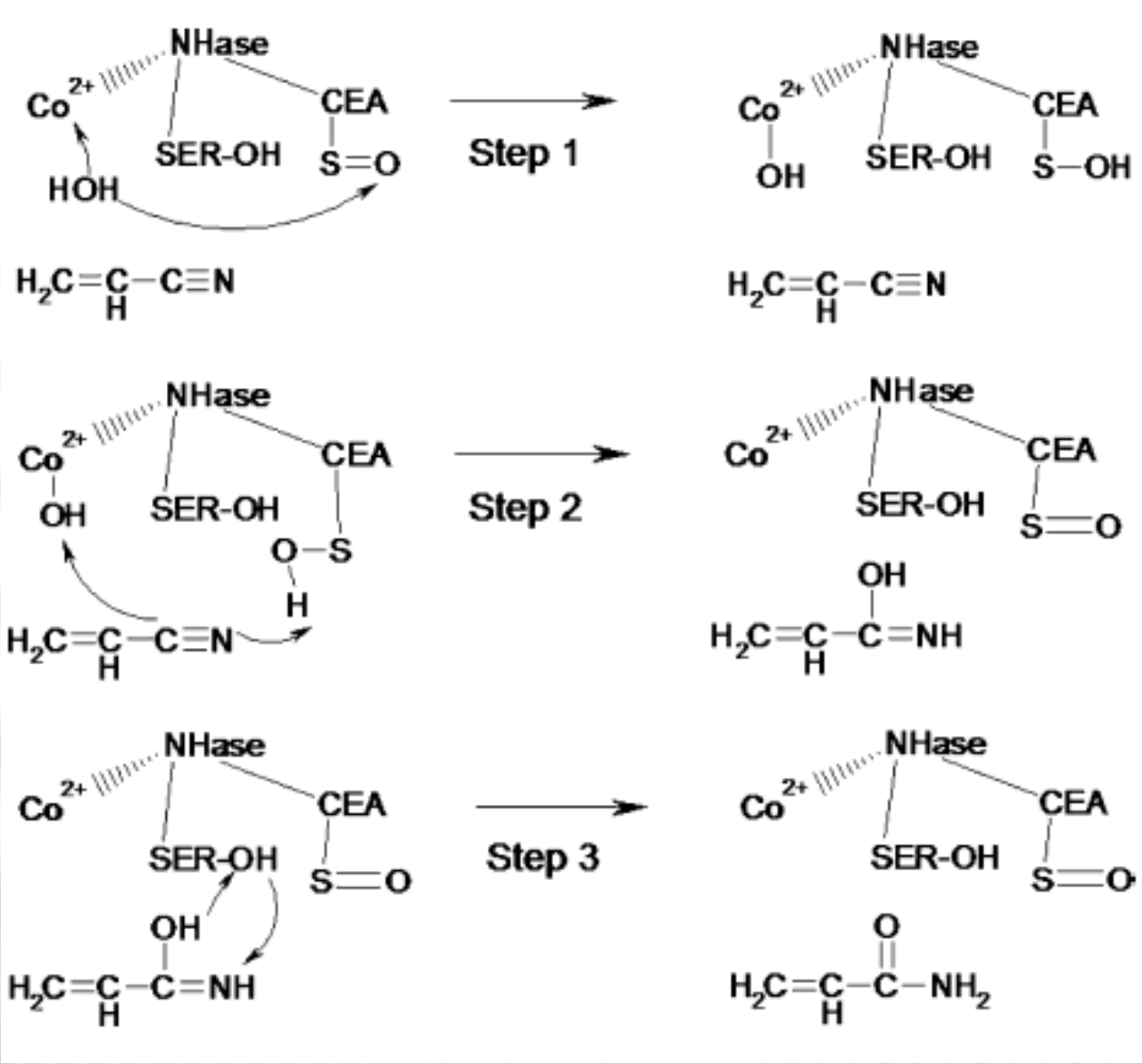
SUMMARY

Three steps

1) Active center activation

2) nucleophilic attack

3) proton rearrangement and product generation



SUMMARY

α-CEA113

- plays an important role for activating the active center

α -SER112

- firstly helping the initial release of the product from the active center
- secondly, it participates in the process of proton-rearrangement ensuring a fast and efficient transformation of the intermediate into the final product

SUMMARY

α-SER122 and α -TYR68

- located at appropriate positions prone to generate hydrogen bond interaction with the AM molecule, which would ‘‘pull’’ the AM product fast out from the active center, accordingly affect the Nhase activity

α-SER122

- is important for the activity presence of NHase

END

Thank You for Your attention