



Ферменты-совершенные макромолекулярные машины

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Московский государственный университет*



Кафедра химической энзимологии

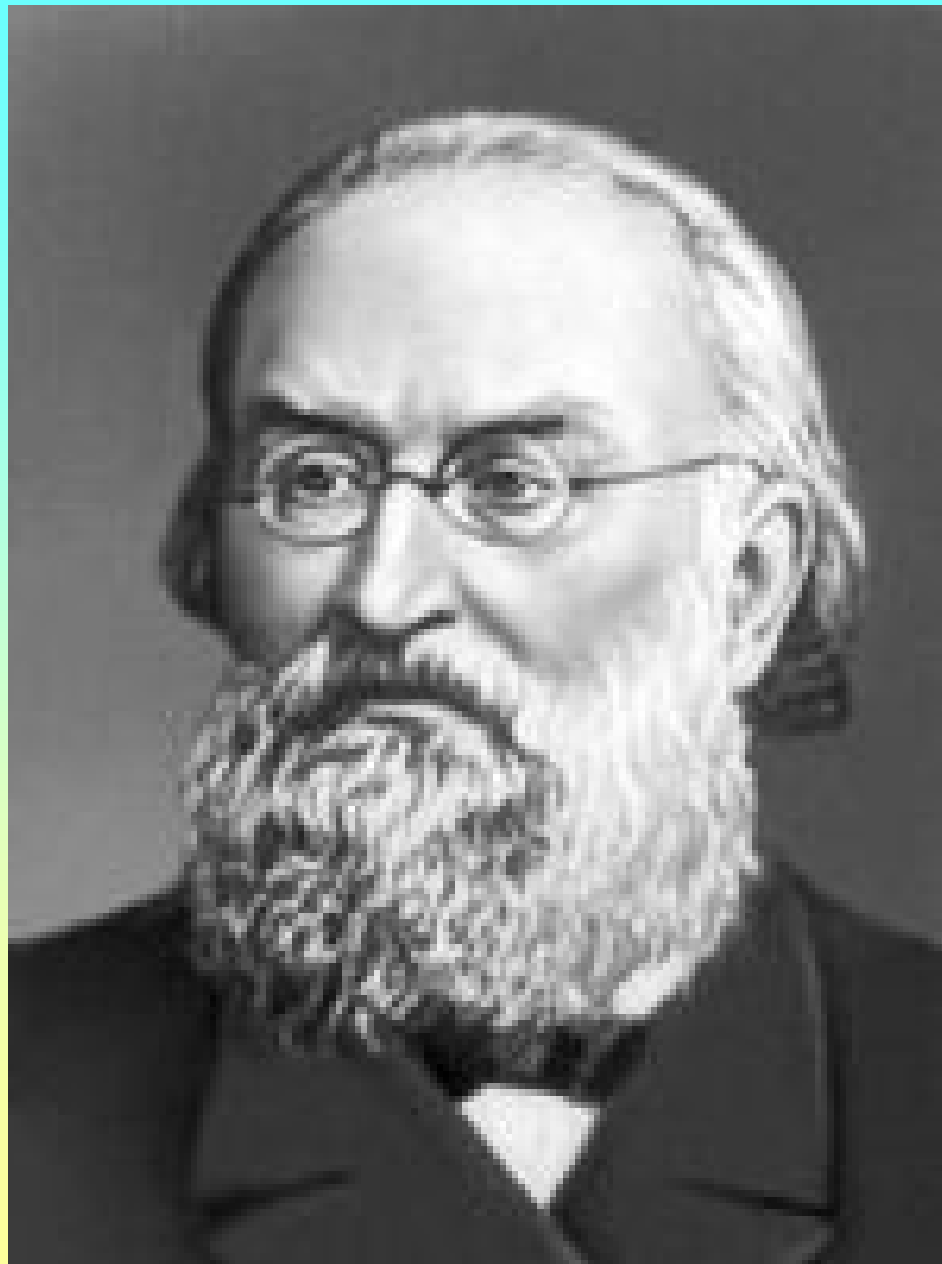


Кирхгофф Константин Сигизмундович

Родился 19 февраля 1764
года

г. Тетеров (Мекленбург-
Шверин, Германия)

1792-1802 – помощник
директора и директор
главной аптеки Санкт-
Петербурга



1811

Открытие реакции гидролиза крахмала под действием минеральных и органических кислот (серная, соляная, азотная, щавелевая)

Доказательство того, что кислота не вступает в реакцию в качестве реагента - нейтрализация кислоты мелом после окончания реакции

Первая каталитическая реакция

1814

Доклад в Академии наук «*Über Zucker bildung
beim Malzen des Gestreides und beim Bebrühen
seines Mehl mit kochendem Wasser*»

1815, опубликовано в *Journal für Chemie und
Physik* (Швейцария)

Осахаривание крахмала под действием амилазы
клейковины и солода



Biocatalysis

*«In the living and animals thousand of **catalytic processes** go on between the tissues and the fluids, and produce the amount of dissimilar chemical syntheses for whose formation from the common raw material»*

*«В живых растениях и животных в тканях и жидкостях протекают тысячи **каталитических процессов**, осуществляя большое количество различных химических синтезов из общего исходного материала»*

Berzelius, 1836



- **Modern trends**

- *Identification of ALL enzymes in ALL genomes*
- *Structural information on ALL enzyme active sites*
- *Molecular polymorphism of human enzymes*
- *Kinetic studies and intermediates identification on ALL types of catalytical sites*
- *Molecular mechanics and molecular dynamic analysis*
- *Quantum chemical analysis of elementary acts*

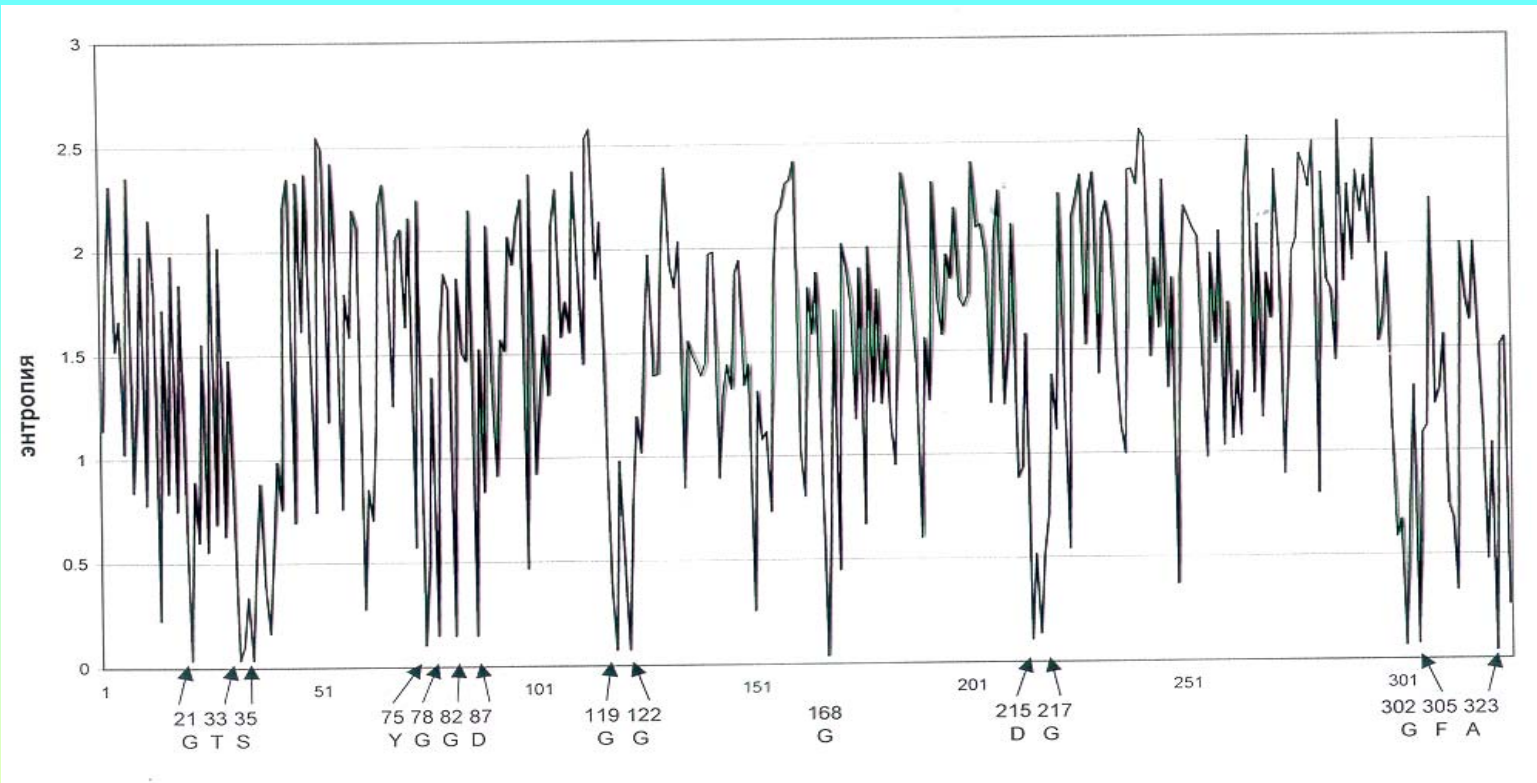
Basis: Information technologies and high efficiency of computer operations

Principles of catalytical structures formation

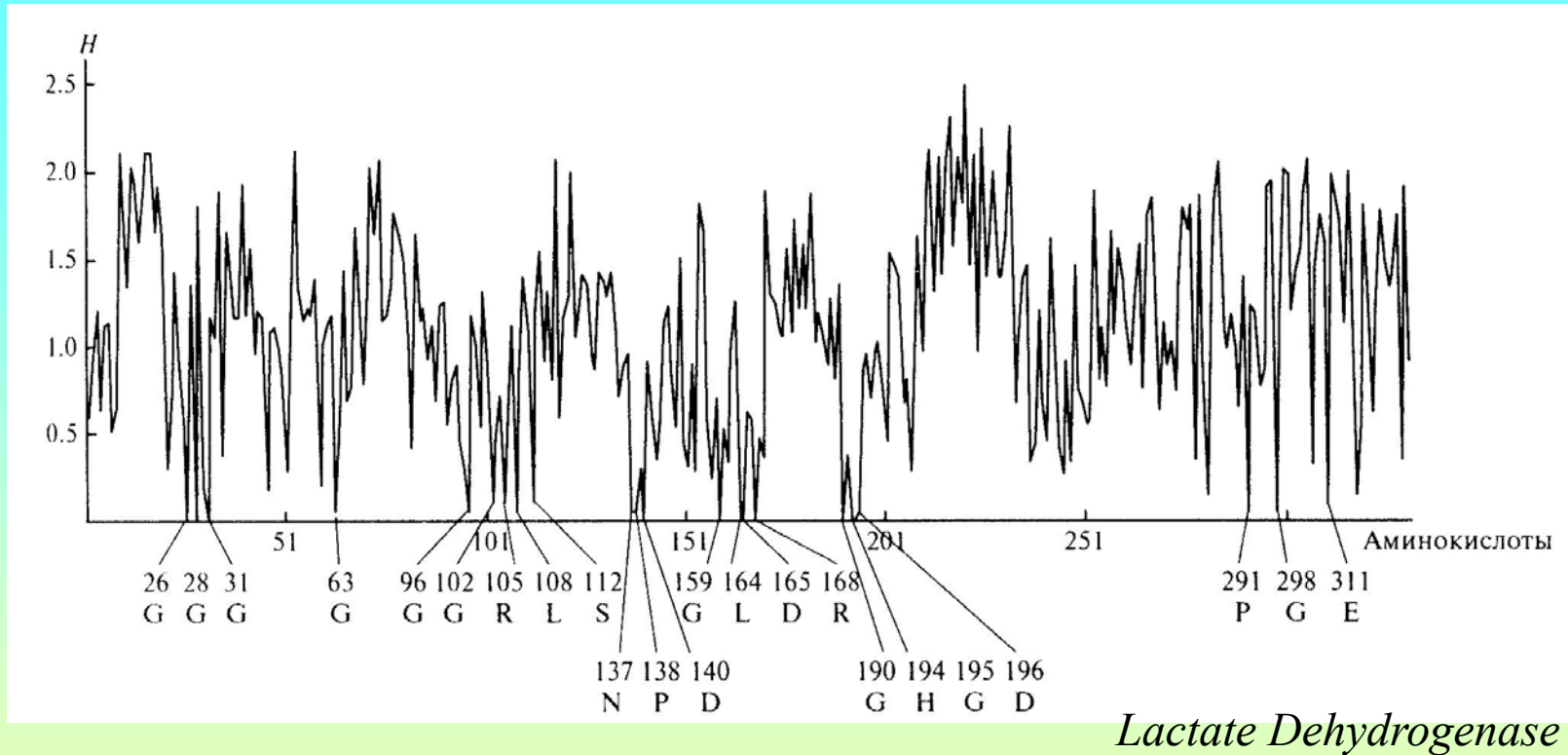
- How to find the catalytical structure forming aminoacids and variable positions in enzyme from sequence data?

- *To construct of information (Shenon's) entropy portret for enzyme family*

- S.Varfolomeyev,Mendeleev
Comm.(2004)5,185-189

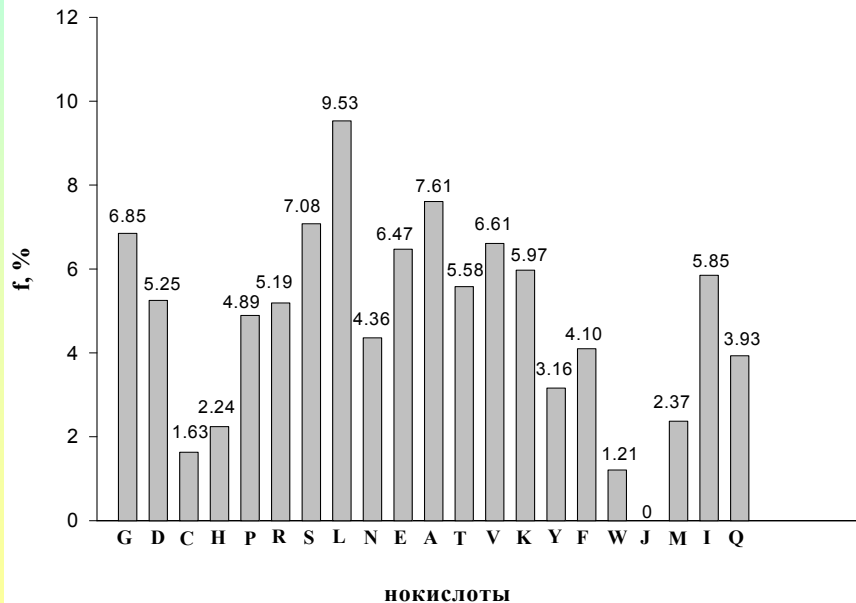
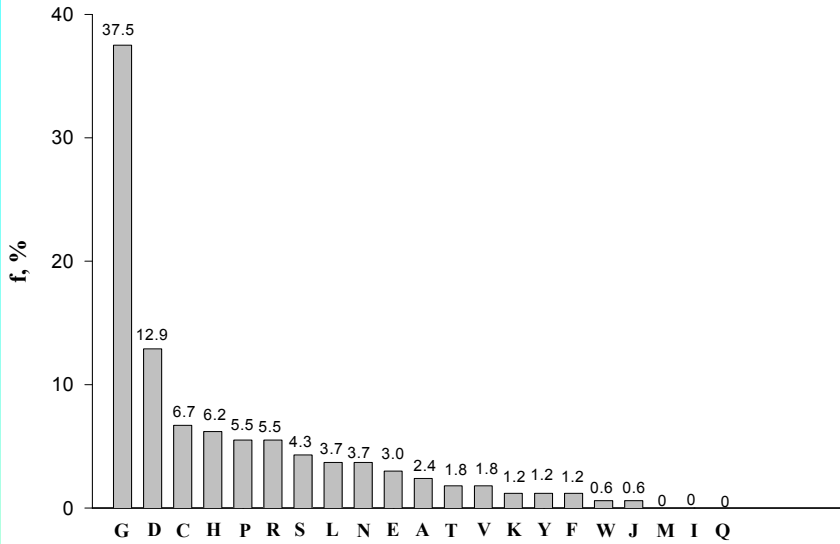


- A large fraction of aminoacids in polypeptide chain of related enzymes (90-95%) is highly variable ($H > 2$)
- There are several positions which are conservative ($H \sim 0$)

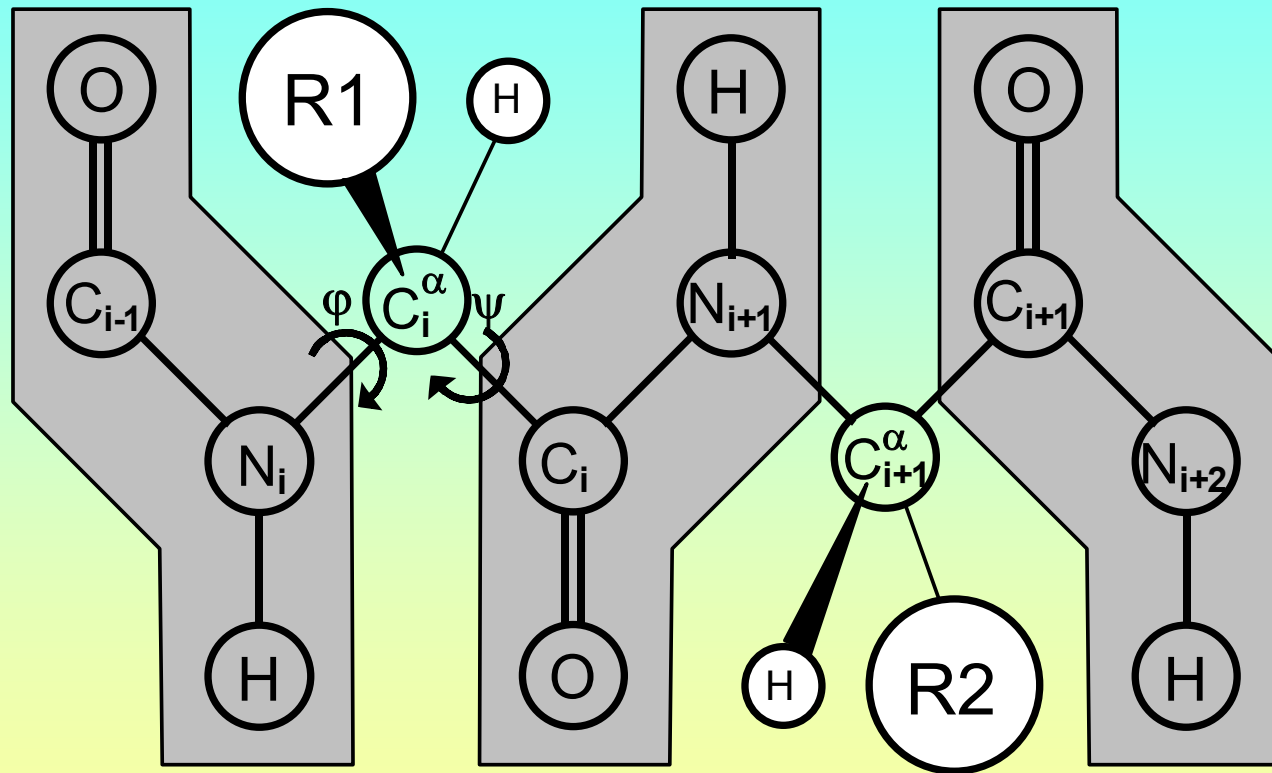


- The amino acids forming a catalytical site always manifested themselves as conservative amino acids

The method for identification of catalytically active groups
from sequence data



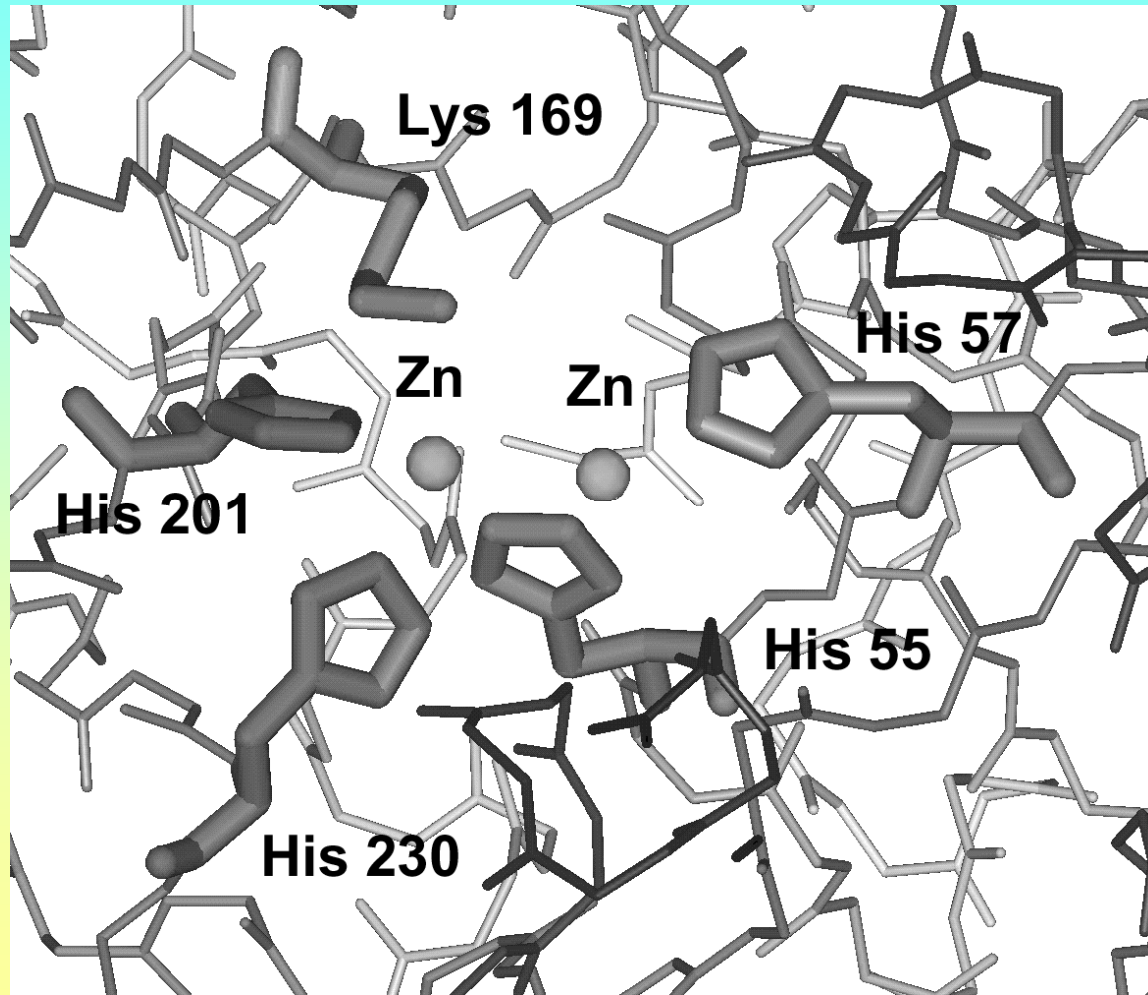
- Rating of conservative aminoacids
 - *Glycine is the most important aminoacid in the structure of enzymes*
 - *Aspartic acid*
 - *Histidine*
 - *Arginine*



- Conservative structure forming amino acids (Gly, Cys, Pro)
 - His 57 Asp 102 Ser 195 (Chymotrypsin)
 - Asp 32 His 64 Ser 221 (Subtilysin)

Put together the catalytical groups

2 Metal ions (2 Zn)\HDB Parathion hydrolase (1DPM)



From sequence to three-dimensional structure (semi-empirical approach)

- *Computer comparison of primary structures of relative enzymes in large family*
- *Identification of prototype with known 3-D structure*
- *Molecular mechanics, energy optimization of amino acids changes*



Identification of catalytical site in macromolecule

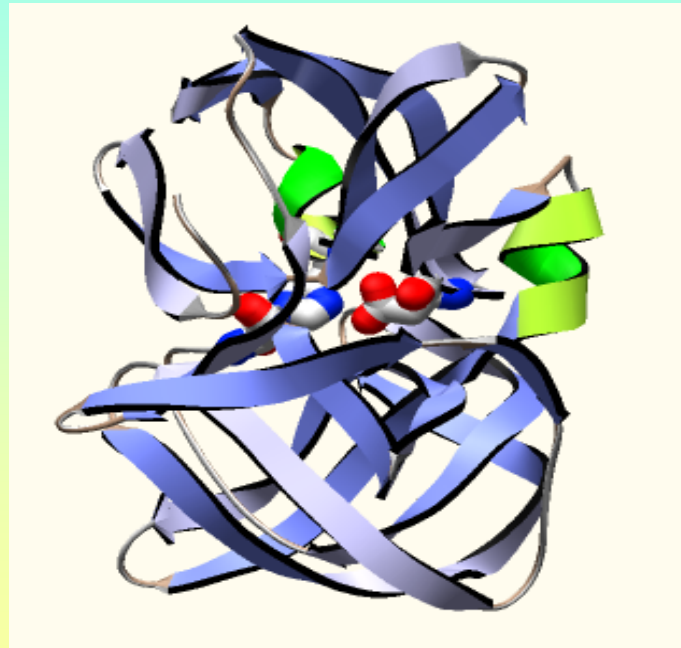
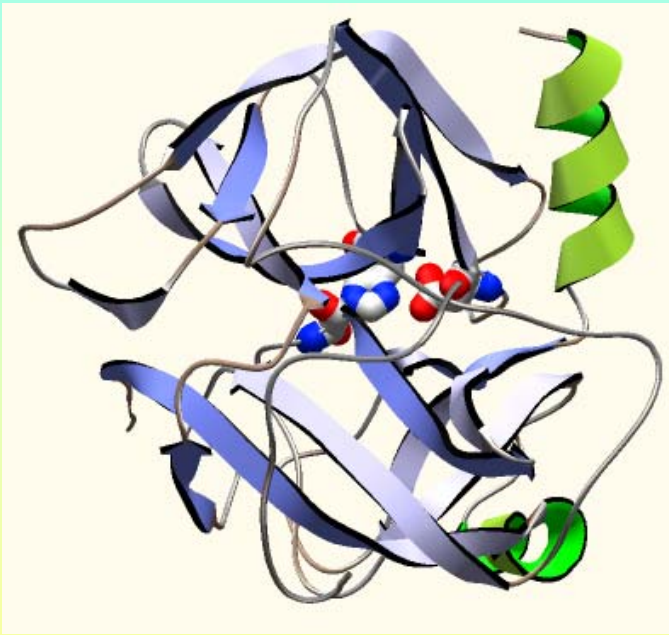
Computer comparison

3D structures, templates construction

*I. Gariev .S. Varfolomeev .Bioinformatics, 2006, Oct
15, 20(22) 2574*

Chymotrypsin and Streptogrisin

- Primary structures are absolutely different :



- Catalytical sites are the same

Search template

residues

```
H    is    His
S    is    Ser
D    is    Asp or Glu
```

atoms

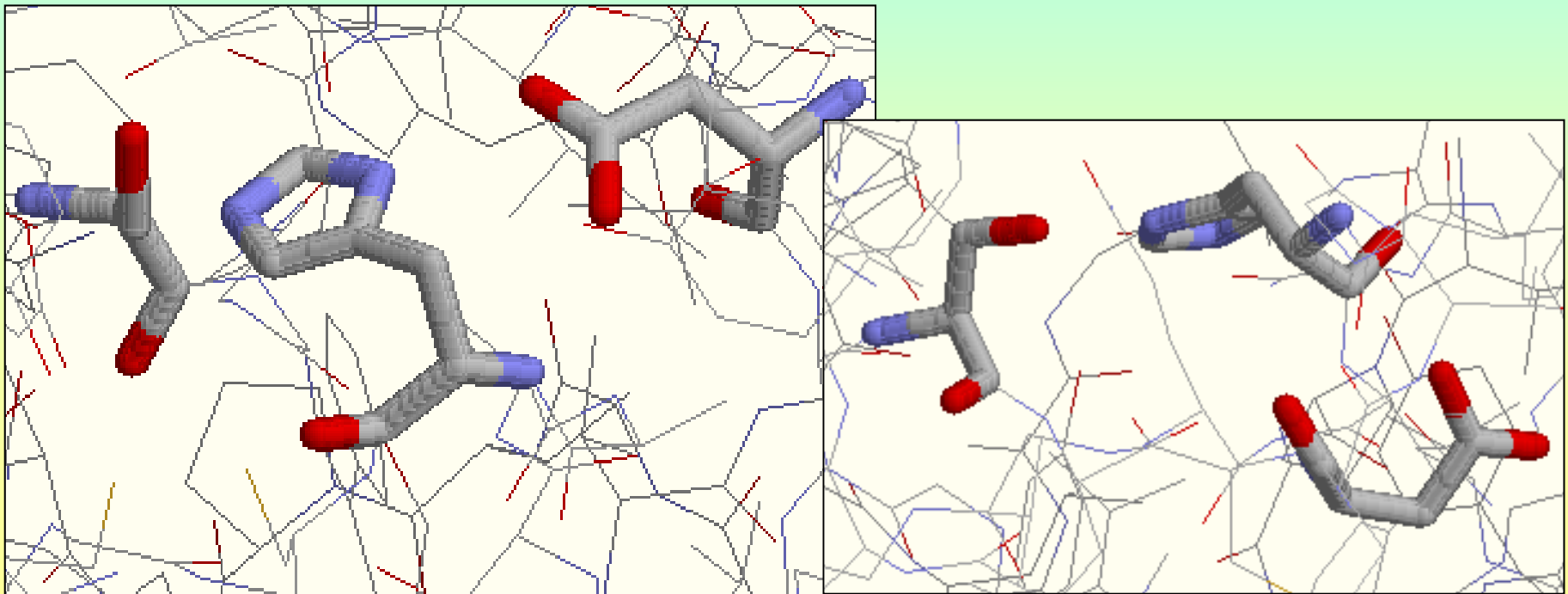
```
O1   is    D.OD1, D.OD2, D.OE1 or D.OE2
```

constraints

```
distance(H.NE2, S.CB)           between  2.7      and  4.2
distance(O1, H.ND1)             between  2.3      and  3.9
angle(H.C, H.CG, S.CA)          between  95       and 138
angle(D.CB, D.C, H.CE1)         between  34       and  78
angle(D.CB, S.CA, H.CA)         between  20       and  46
angle(H.CA, H.C, S.CA, S.C)     between  10       and 120
angle(S.CB, S.C, O1)            between  30       and  95
angle(S.CB, S.C, H.CA)         between   5       and  90
angle(D.C, H.C, D.CB)          between   5       and  37
```

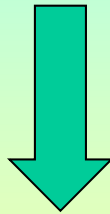
- *Template-the set of constrains on the type of amino acids,types of atoms and geometric parameters,determening the space orientation*

*Automatic method for
identification of the catalytical
sites for the enzymes of different
classes*



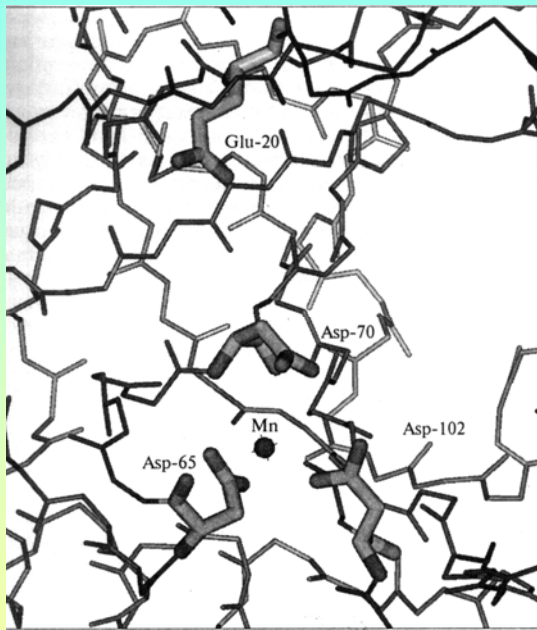
Main structural paradox of enzymatic catalysis

The great diversity of protein primary structures

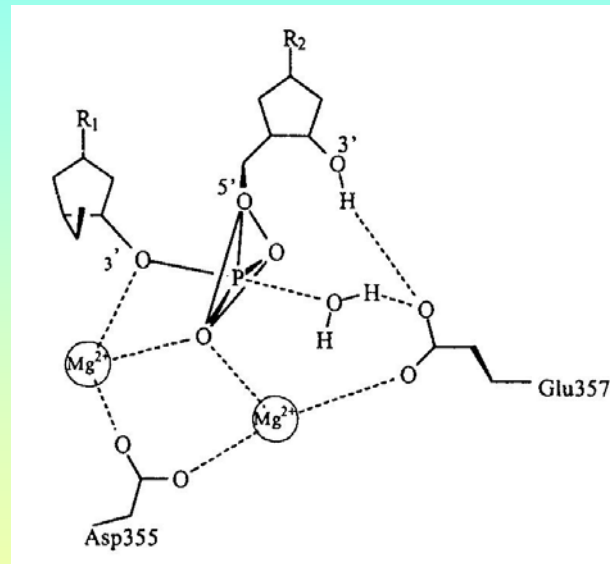


The very restricted number of catalytical site structures

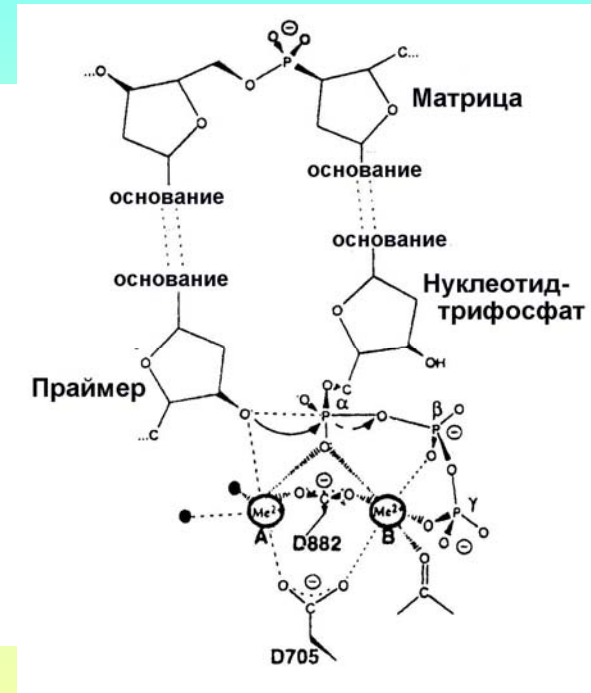
Structural Unity of catalytical sites



Pyrophosphatase



Exonuclease

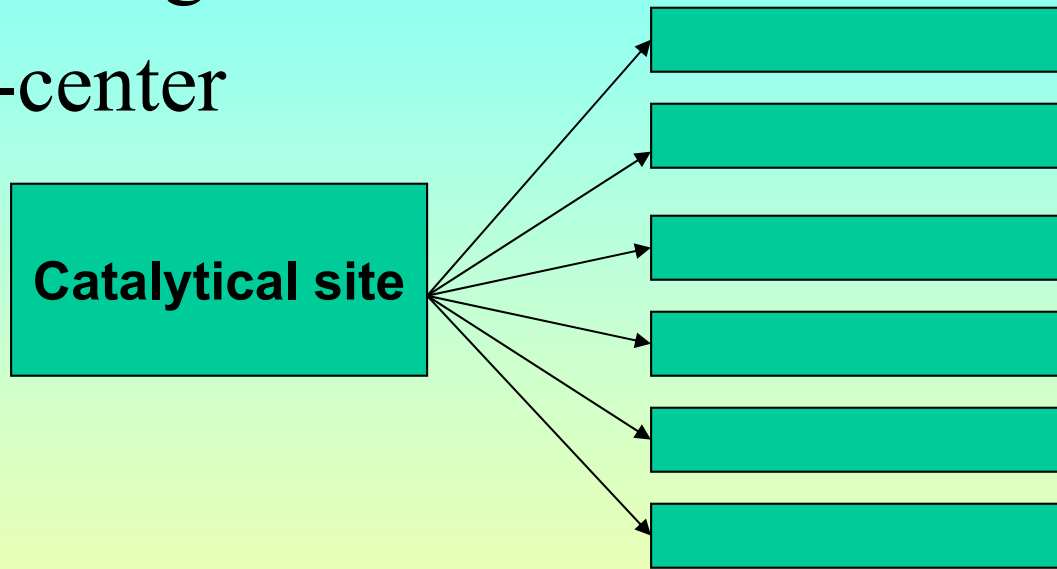


DNA-polymerase

Structural Unity of catalytical sites

activating
sub-center

selecting
sub-center

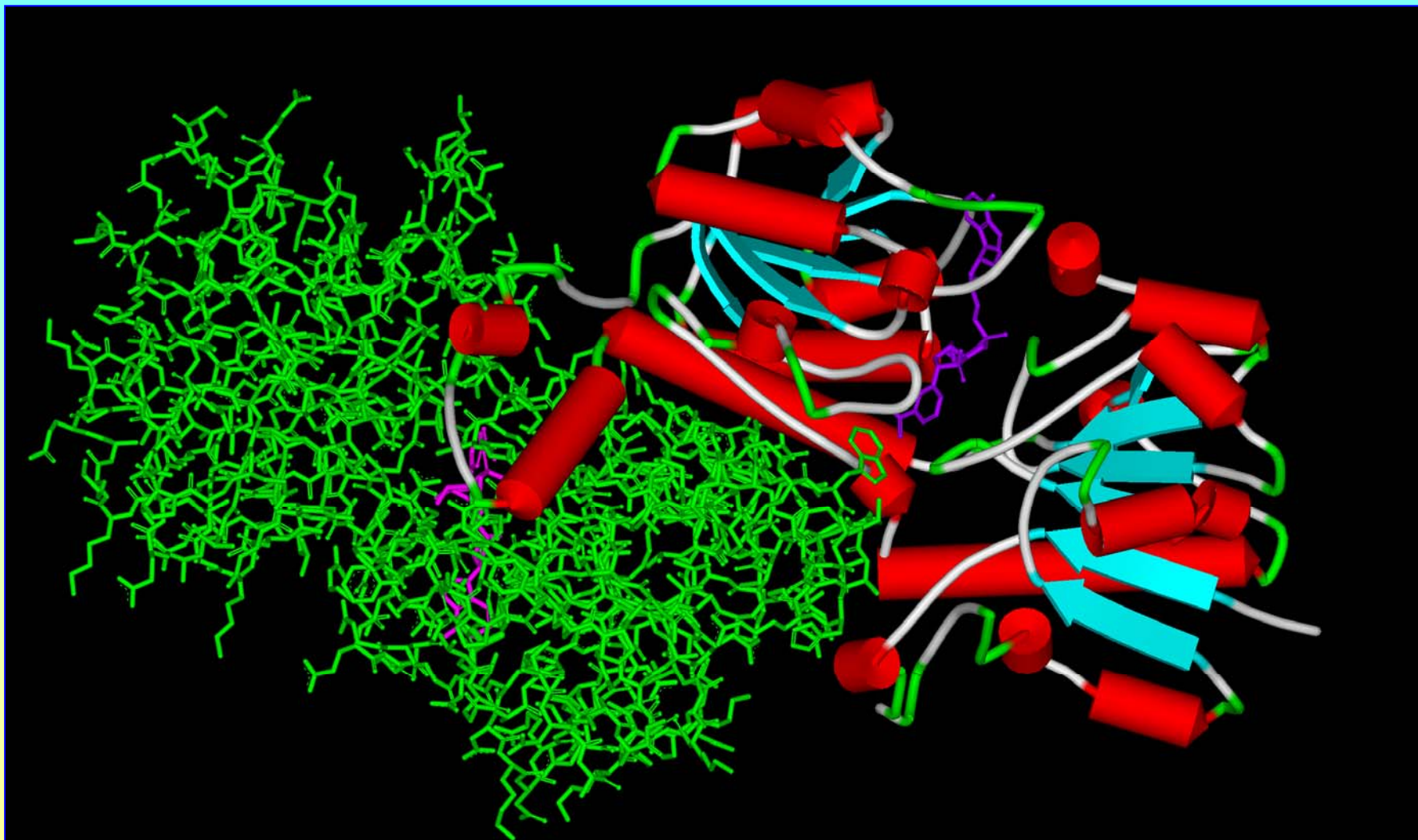


uniformed
(50-60 for all
enzymes)

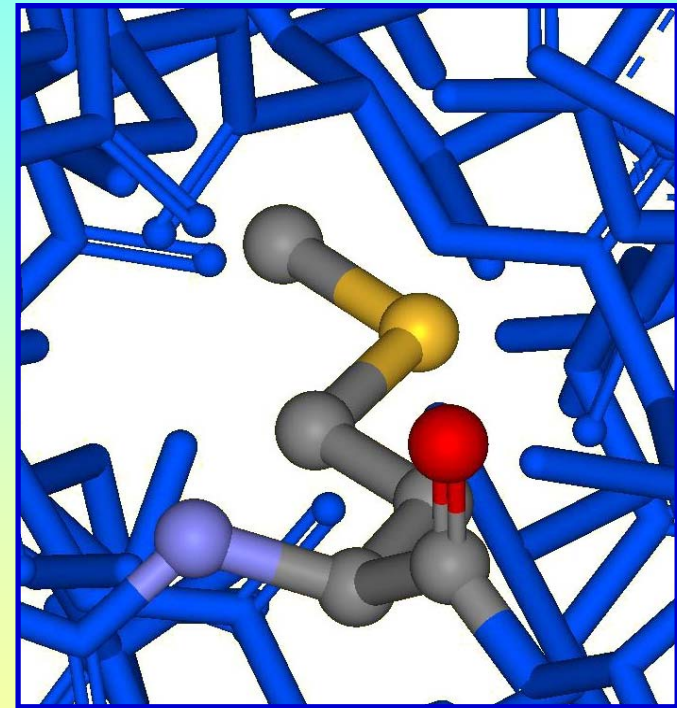
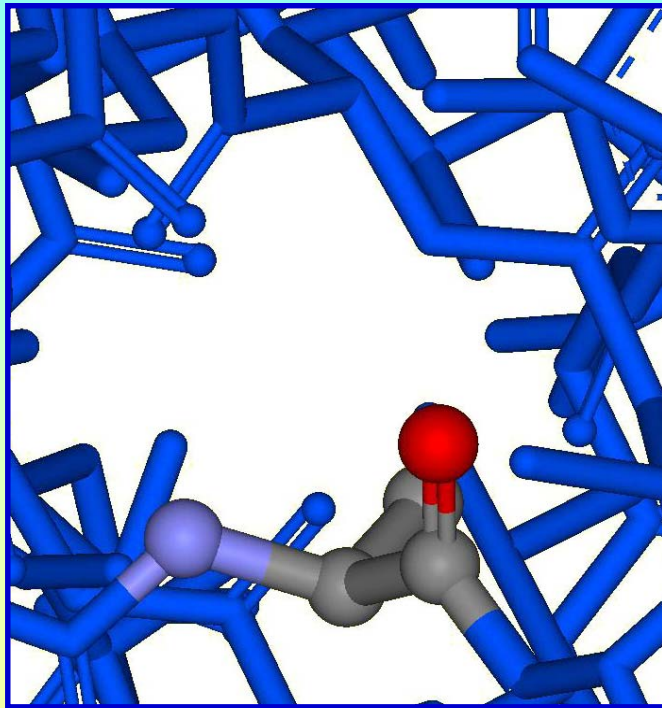
variable

- *Protein engineering, creation of new enzymes*

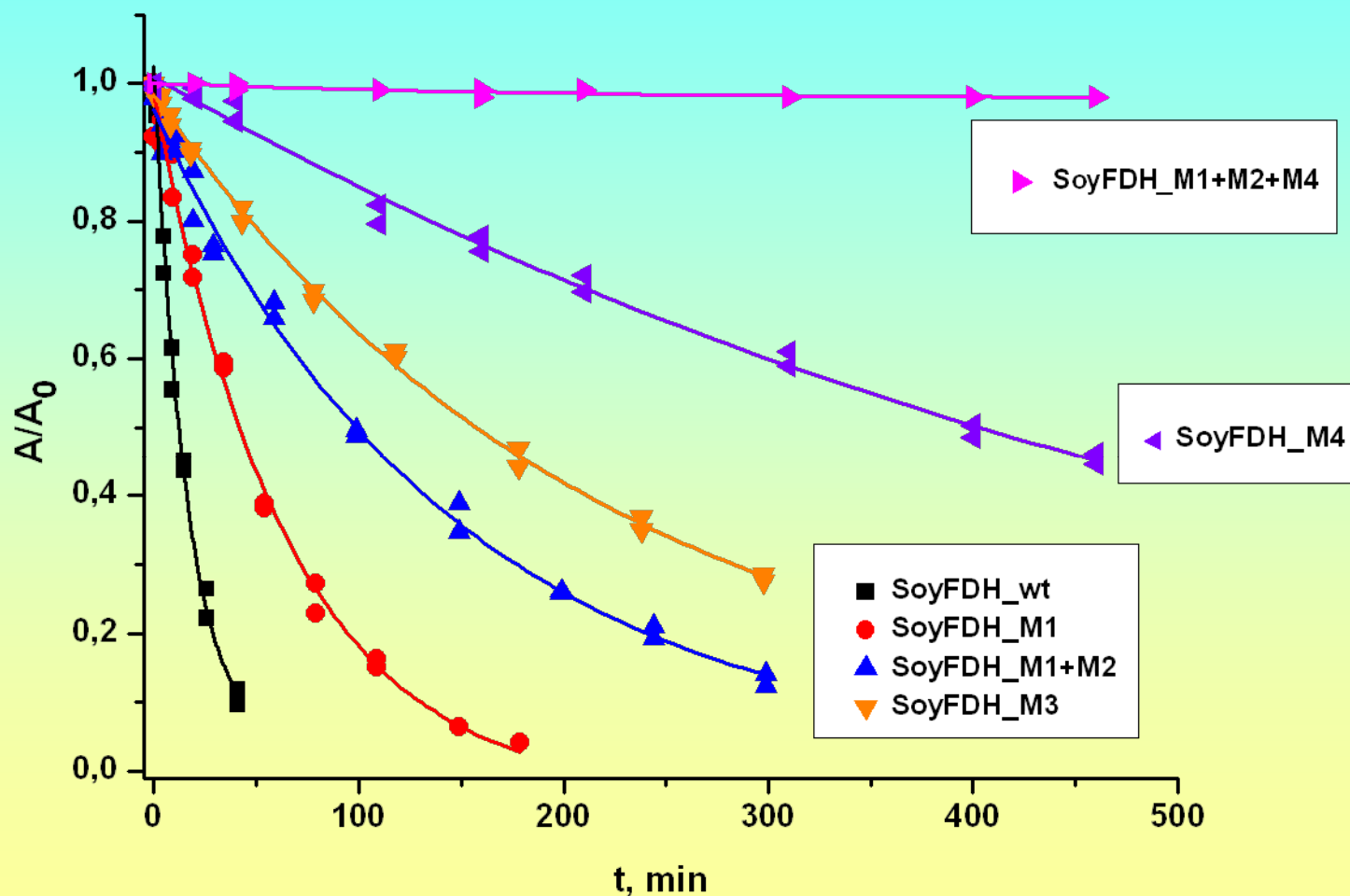
Structure of ternary complex FDH-NAD⁺-Azid (1.9Å resolution)



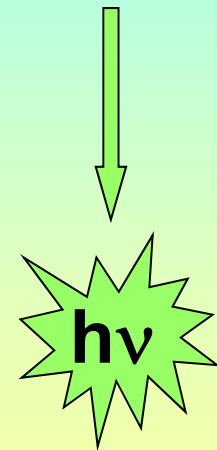
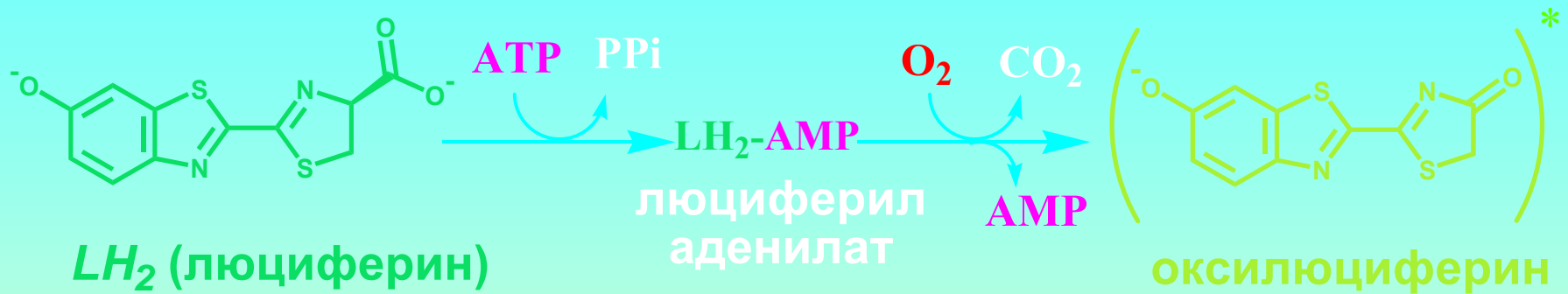
Компьютерное моделирование аминокислотной замены **Ala-Met** по заполнению полости внутри глобулы SoyFDH для стабилизации фермента



Термостабильность рекомбинантной SoyFDH и полученных мутантов

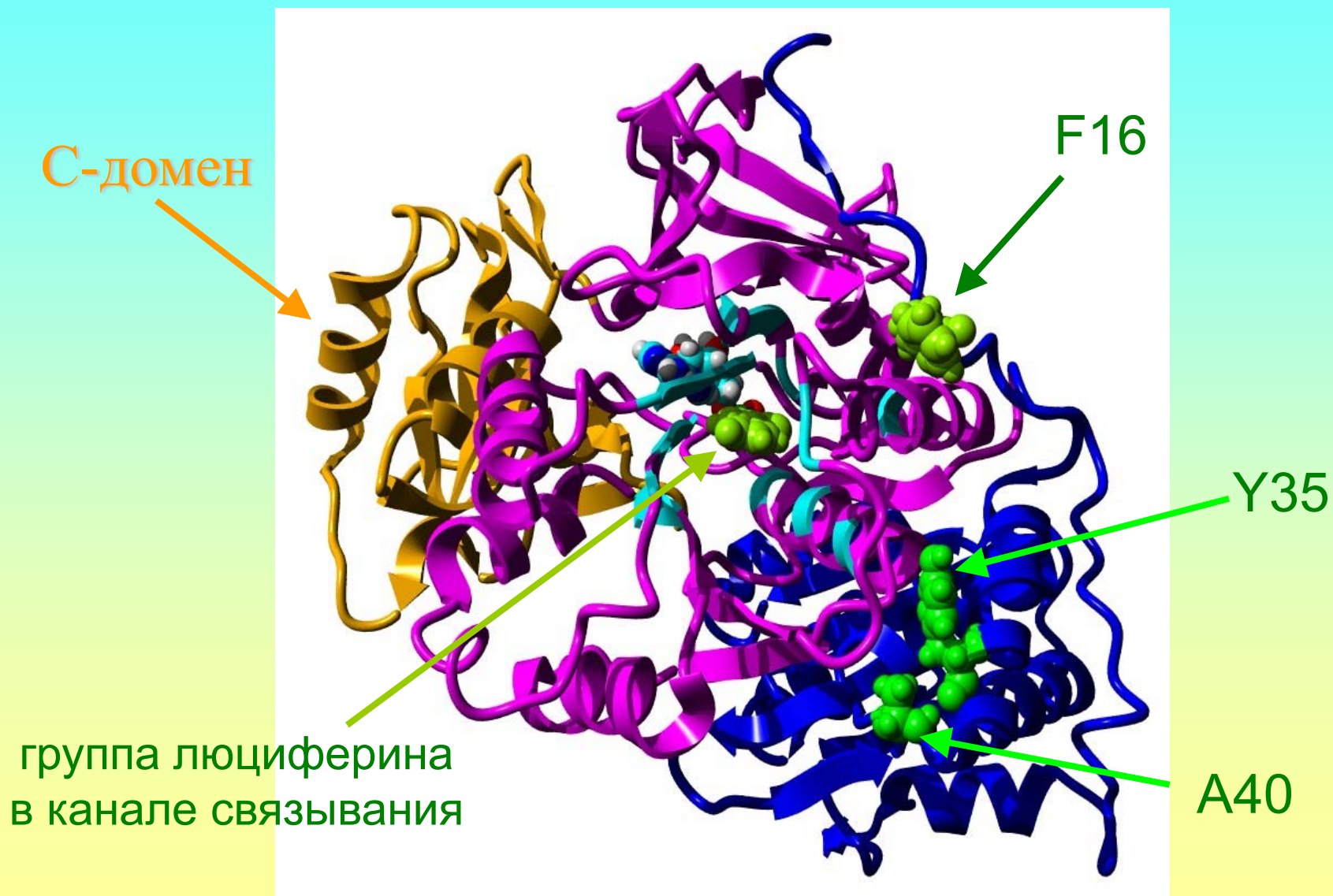


Luciferase-photon producing enzyme



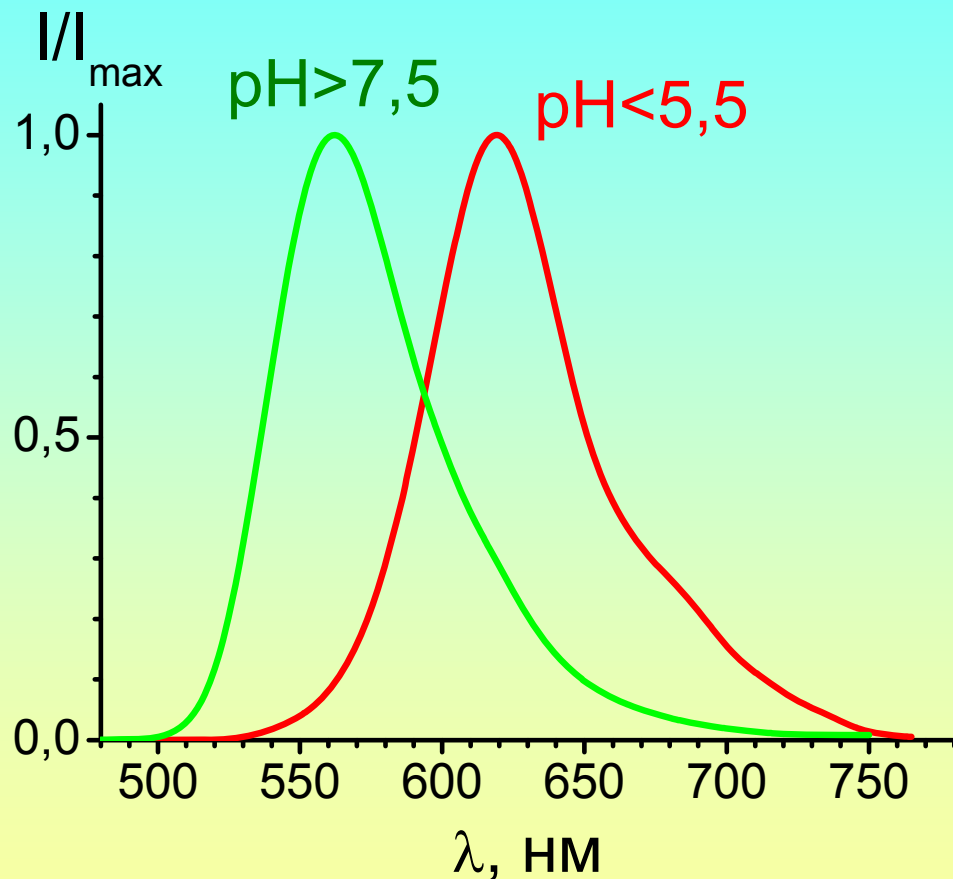
$\lambda_{max} = 540-570 \text{ nm}$

Расположение ключевых мутаций, понижающих рН-чувствительность спектра биолюминесценции

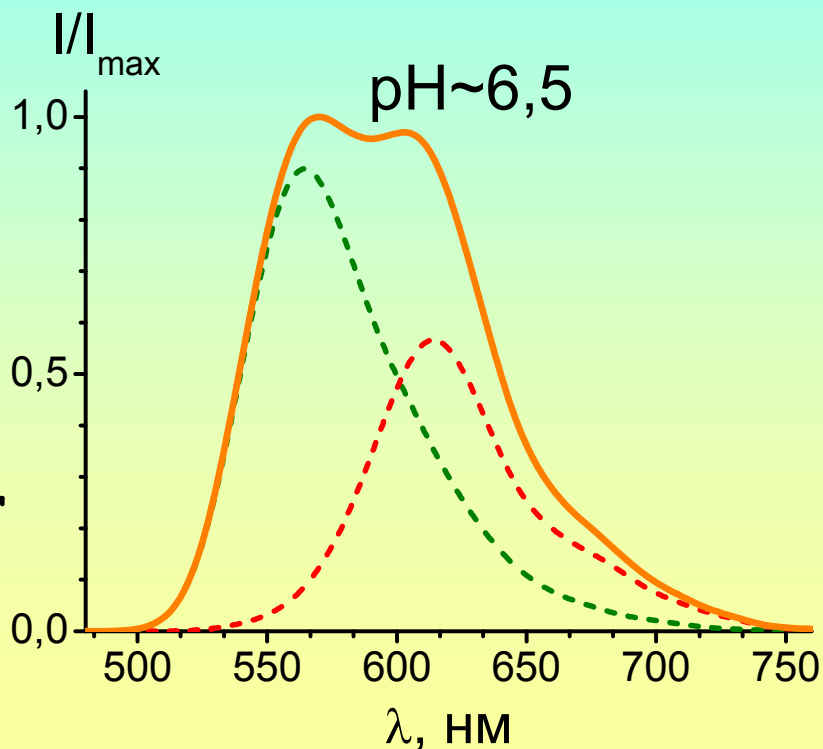


L. cruciata crystal structure: Nakatsu et al, 2006

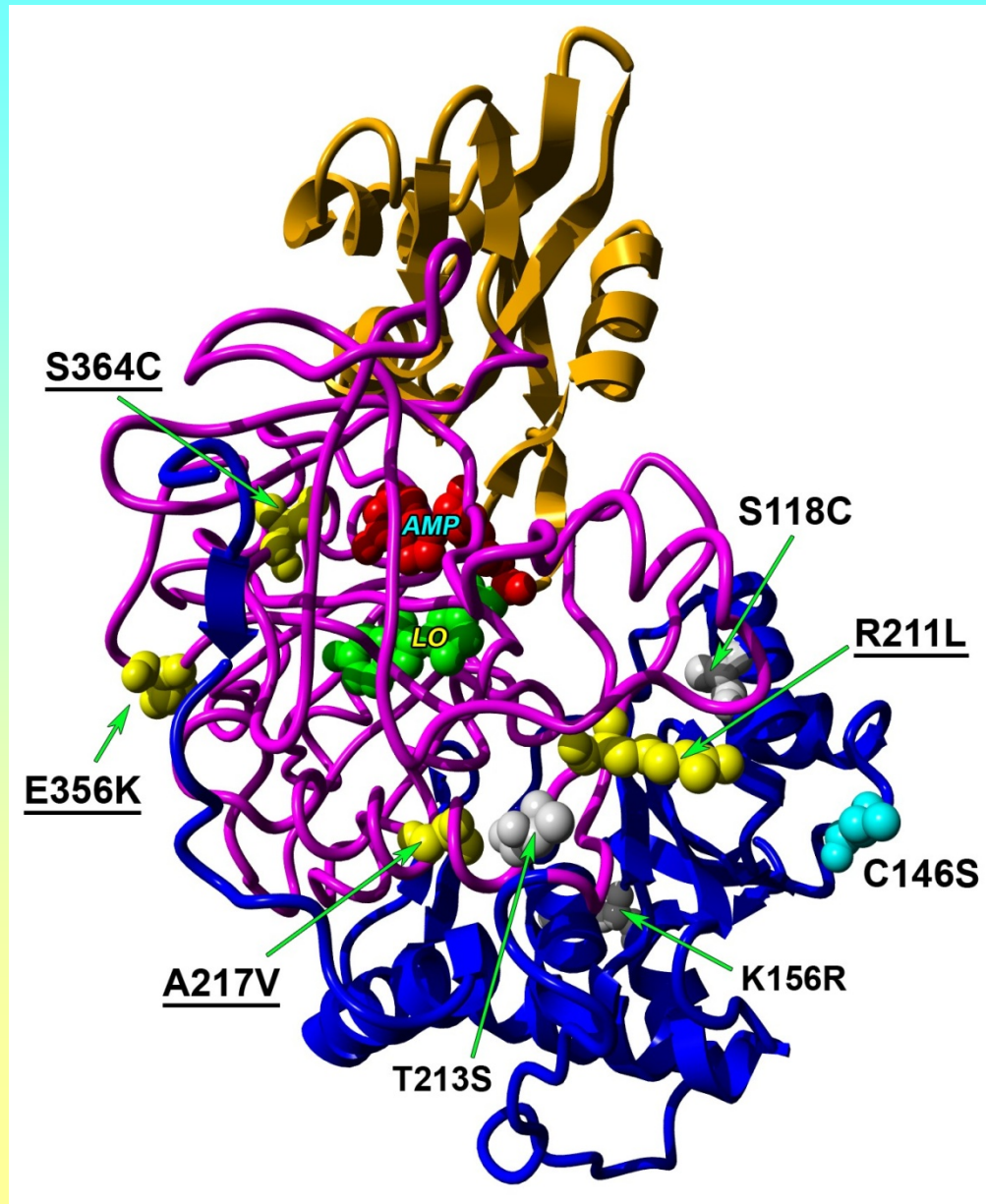
Спектры биолюминесценции люциферазы светляков *L. mingrelica* в зависимости от pH



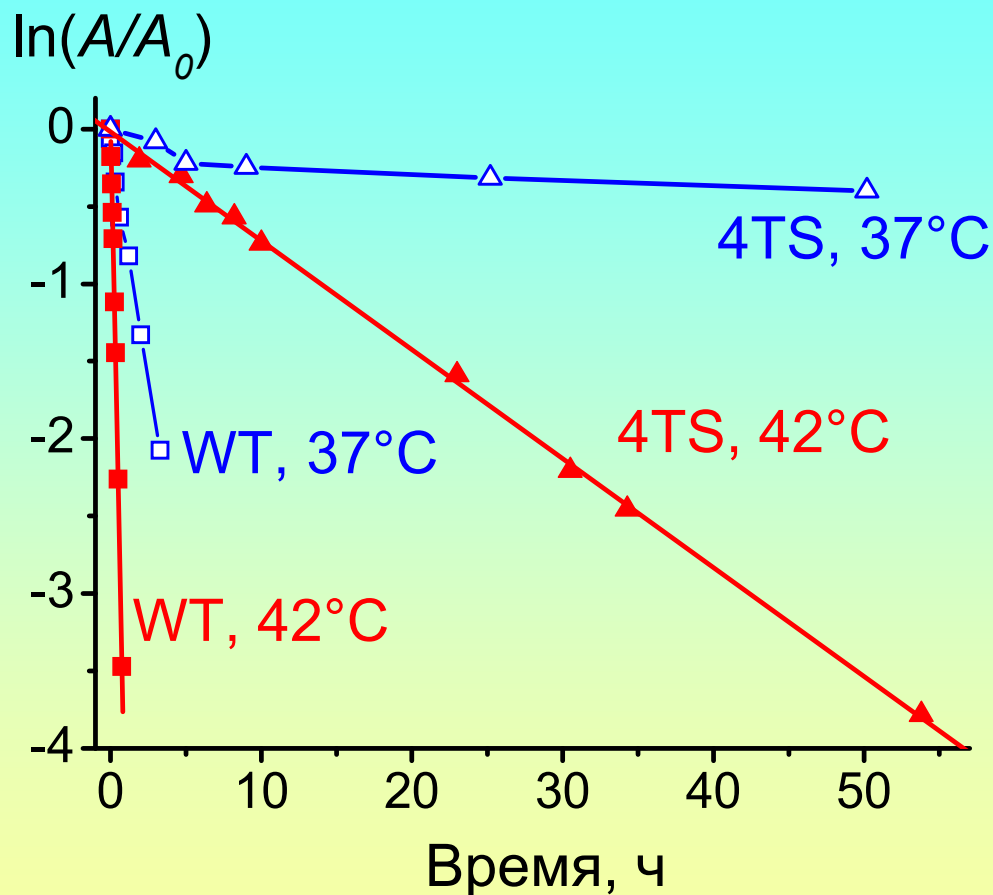
Бимодальный спектр при промежуточных условиях



Engineering of LUCIFERASE

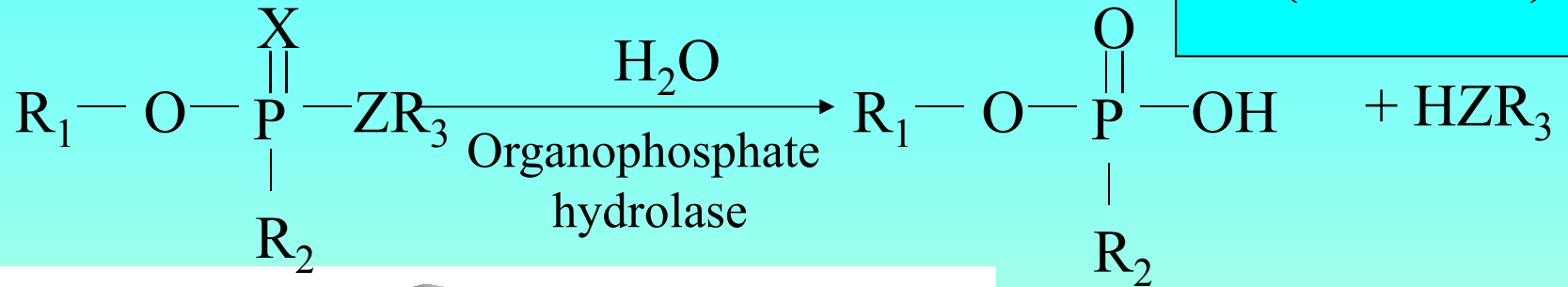


Кривые необратимой термоинактивации мутанта 4TS и люциферазы дикого типа (WT)



Условия: 50 мМ трис-ацетат, 20 мМ $MgSO_4$, 2 мМ ЭДТА, 0,2 мг/мл БСА, рН 7.8, 0,01 мг/мл люциферазы

*Reaction catalyzed by
organophosphate hydrolase (EC 3.1.8.1)*

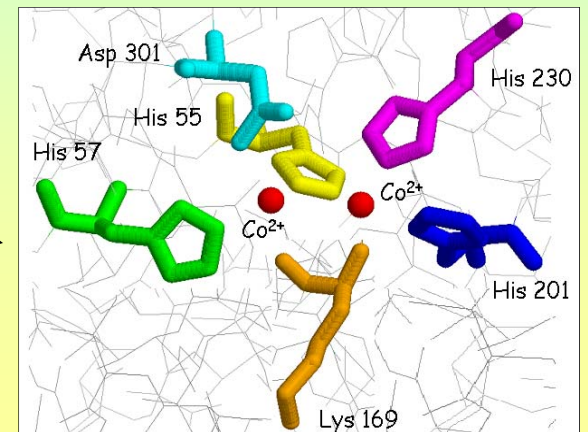
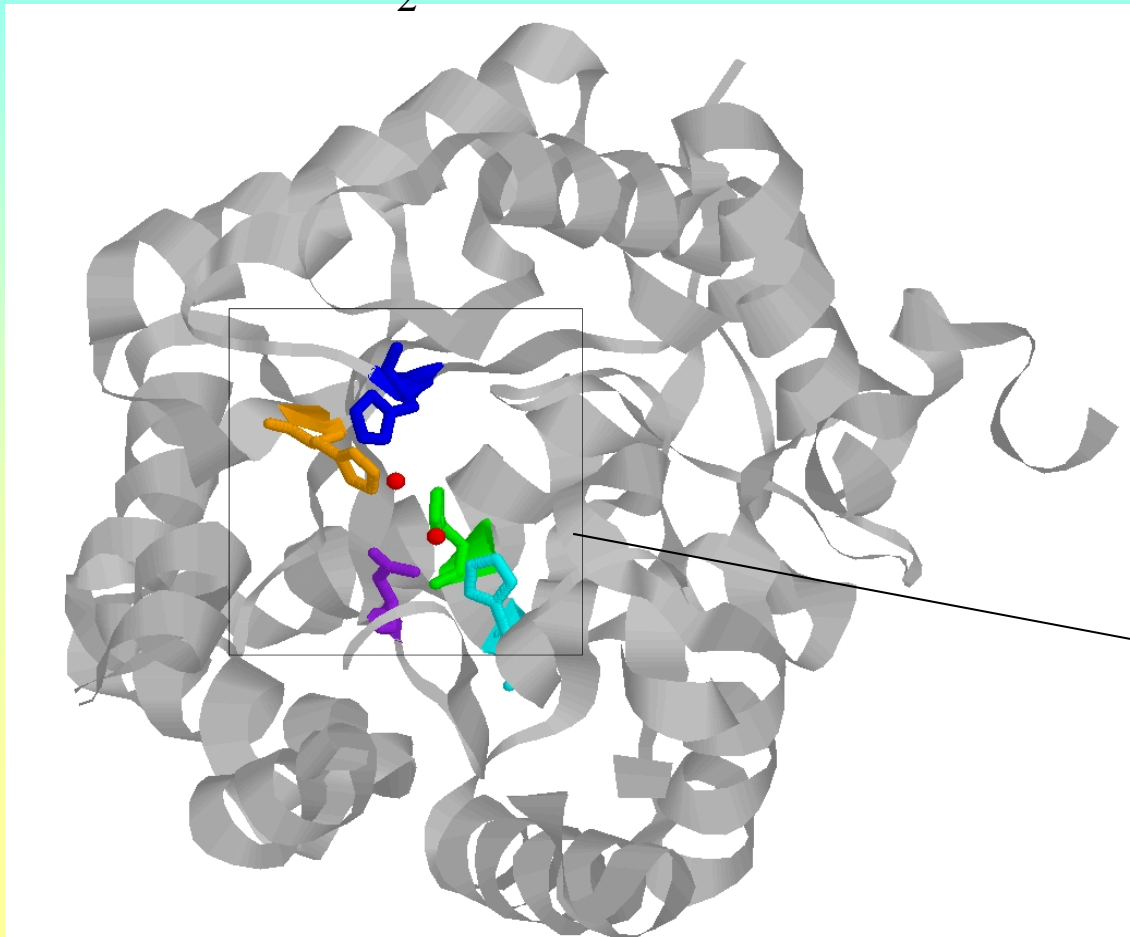


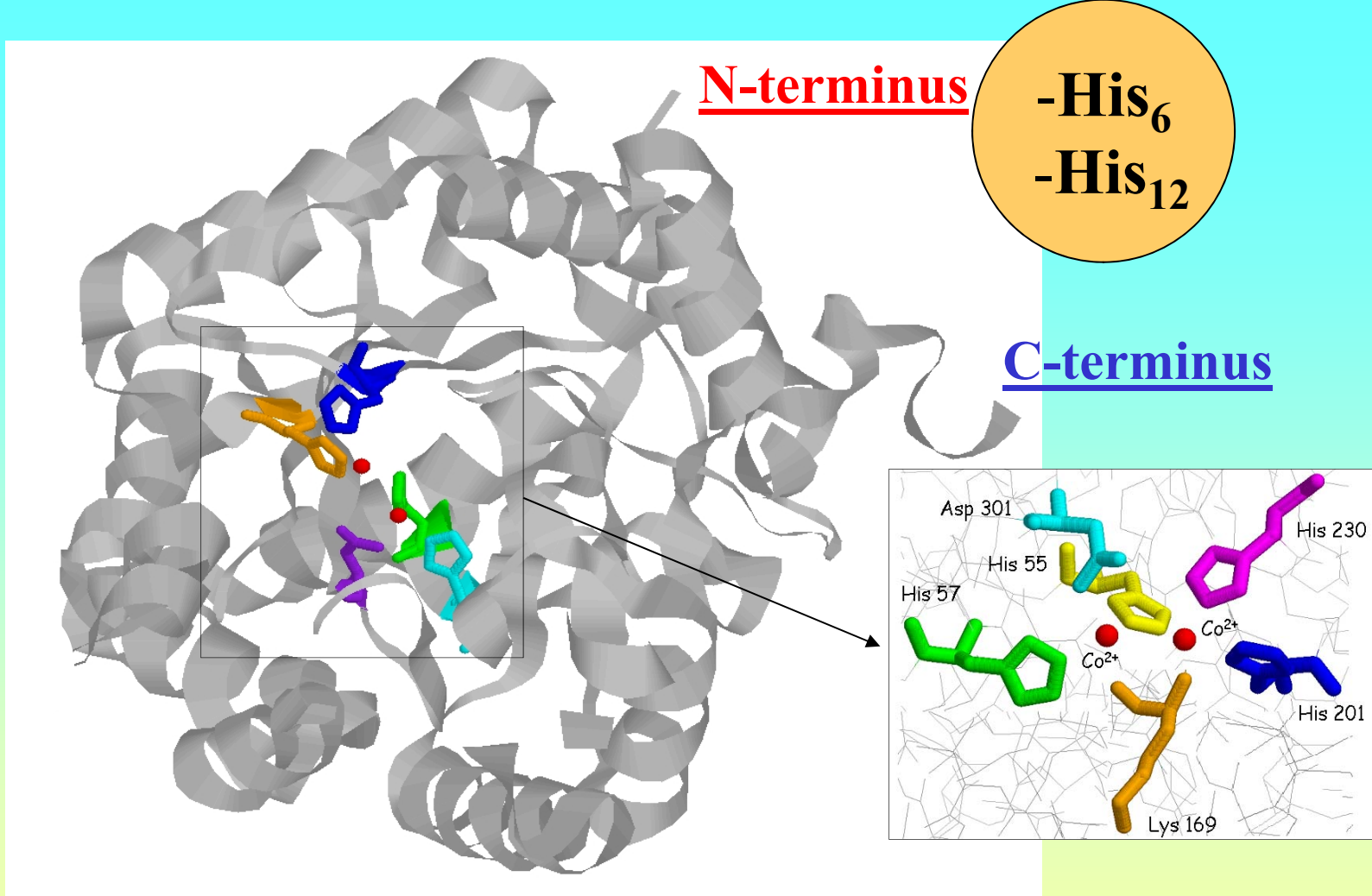
**Organophosphate
hydrolase
(EC 3.1.8.1)**

X = O, S

Z = O, S and F,

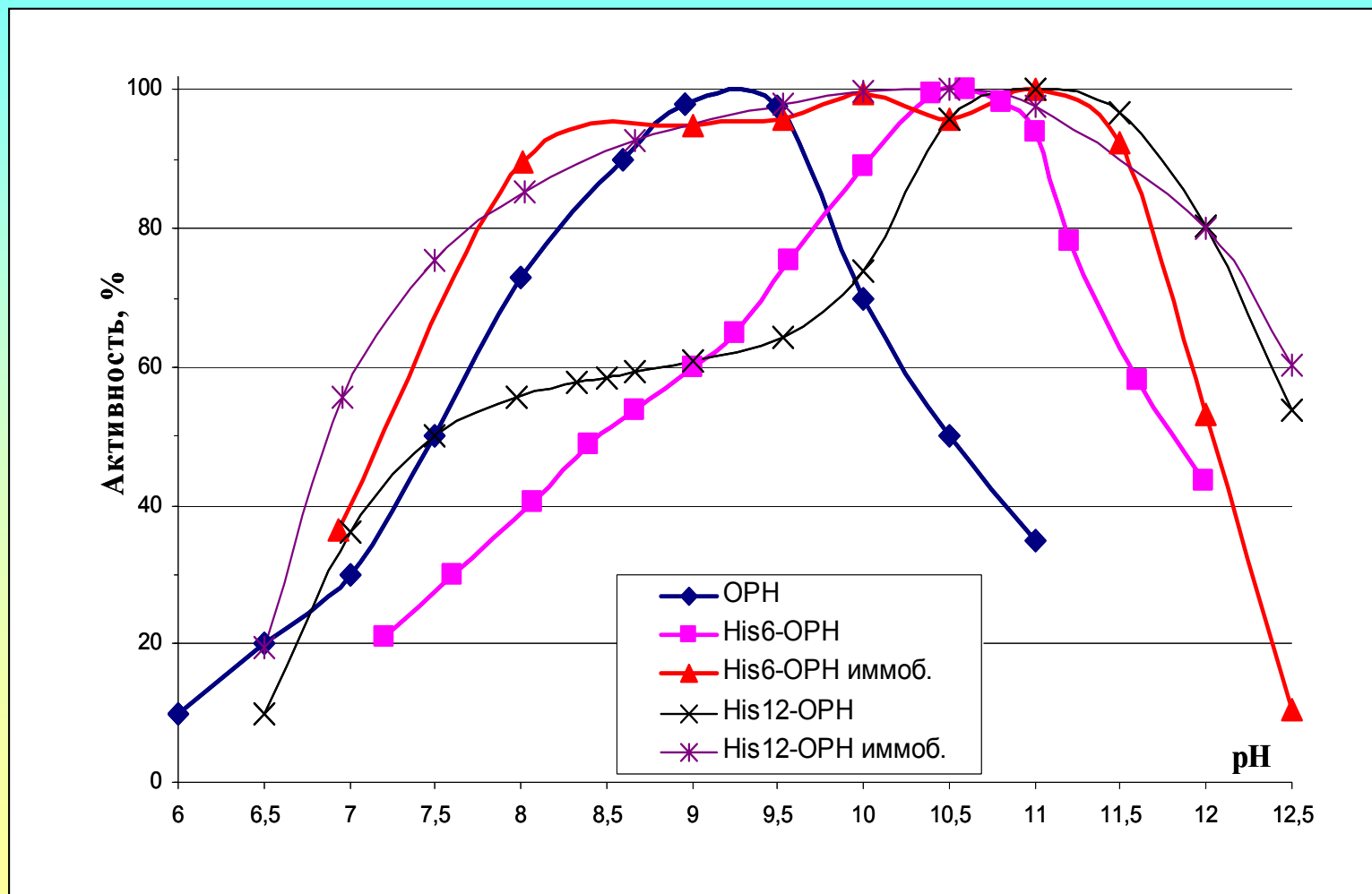
when R₃ is absent





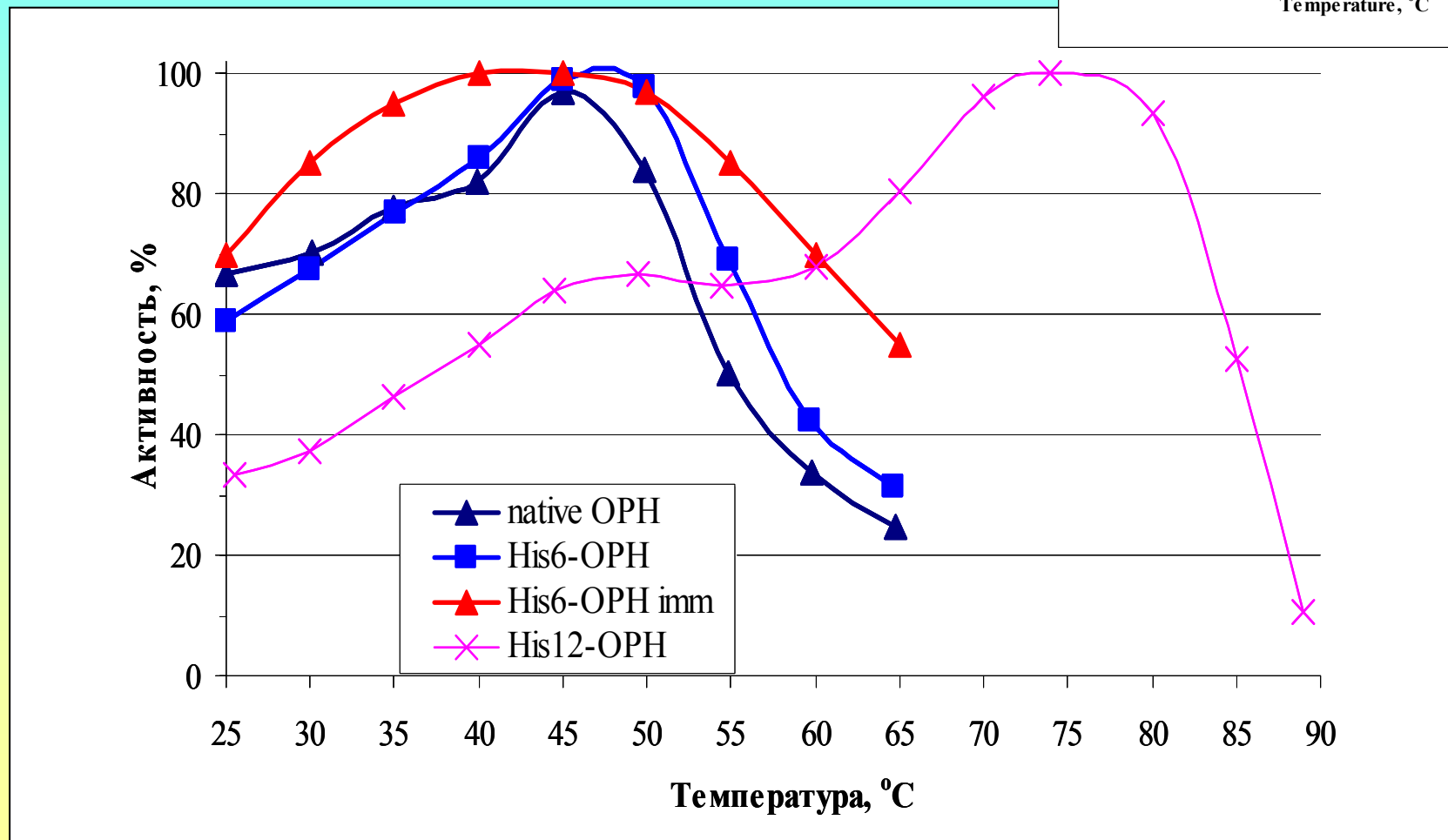
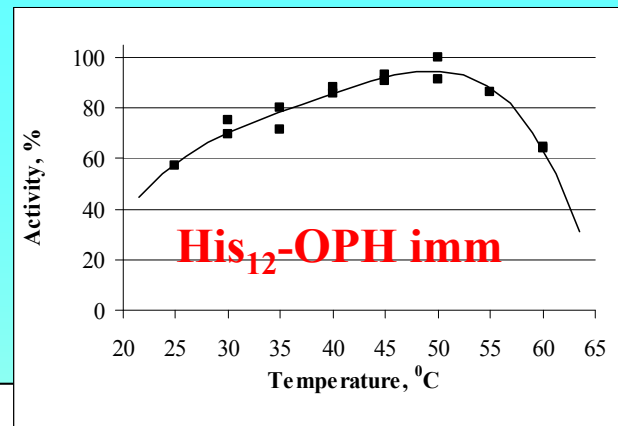
- Presence of affine tags in the protein structure
- Systems for highly efficient expression of proteins (plasmids and strains)
- Optimized conditions for high yield of enzyme synthesis in the soluble active form

pH-optimum of native and polyhistidine-containing OPH in the soluble and immobilized forms (Cu-IDA-cryoPAAG)



Conditions: 25°C, 2 mM Paraoxon, 50 mM buffers

Temperature optimum of native and polyhistidine-containing OPH in the soluble and immobilized forms (Cu-IDA-cryoPAAG)



Conditions: pH 10.5; 2 mM Paraoxon, 50 mM buffers

Molecular modelling



Elementary acts

Quantum chemistry and mechanism of catalysis



Dr. Nemukhin A.V.



Dr. B. Grigorenko

QM/MM Modeling of the G117H BuChE Catalyzed Echothiophate Hydrolysis Reaction Mechanism

Lushchekina S.V.¹, Masson P.^{2,3}, Nachon F.²,

Nemukhin A.V.⁴, Varfolomeev S.D.^{1,4}

¹N.M. Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, Moscow, Russia

²Institut de Recherche Biomédicale des Armées-CRSSA, La Tronche, France

³University of Nebraska Medical Center, Eppley Institute, Omaha, USA

⁴Department of Chemistry, M.V. Lomonosov Moscow State University, Moscow, Russia

sofya.lushchekina@yandex.ru



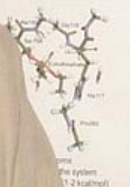
Introduction

OP compounds, forming of BuChE mutants of OP compounds will be toxic to the nervous system. The OP compounds at slow hydrolytic mechanism should be of high catalytic activity. The catalytic mechanism of the G117H BuChE catalyzed hydrolysis of echothiophate was published in the present work to explore all aspects of the reaction.



Methods

QM/MM



His117 orientation



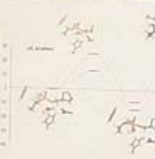
Along the trajectory His117 ring changes orientation and forms H-bond with Echothiophate substrate.

Main stationary points configuration

Water molecule attack on phosphorus atom leads to the formation of pentacoordinated intermediate, which dissociates releasing either free enzyme, or aged conjugate.



Energy



[1] Millard C.B. et al., Biochemistry 34 (1995)

[2] Nachon F. et al., Biochemical Journal 424 (2009)

[3] Rybnik E.J. et al. (2009), MChEm, A Comp

[4] Philippe J.C. et al., Journal of Computational

Ackno

All calculations were performed at Skiff MSU.

The work was supported by RFBR grant No 10-03-00000.



Supercomputers for QM/MM calculations

- SRCC MSU
 - 60 TFlop/s
 - 1250/5000
 - processors/cores
 - (Intel Xeon 3.0 HHZ)



- JSCC RAS
 - 95,04 TFlop/s
 - 990/3960 (Intel Xeon 3.0 HHZ)

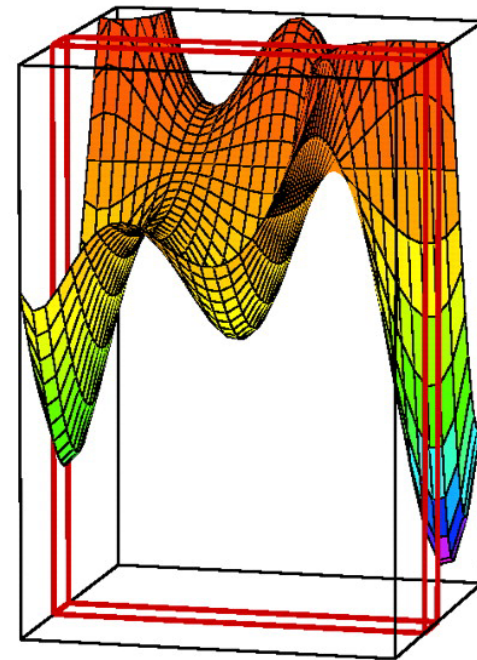


-
- **Расчёт многомерных**
- **поверхностей потенциальной**
- **энергии**

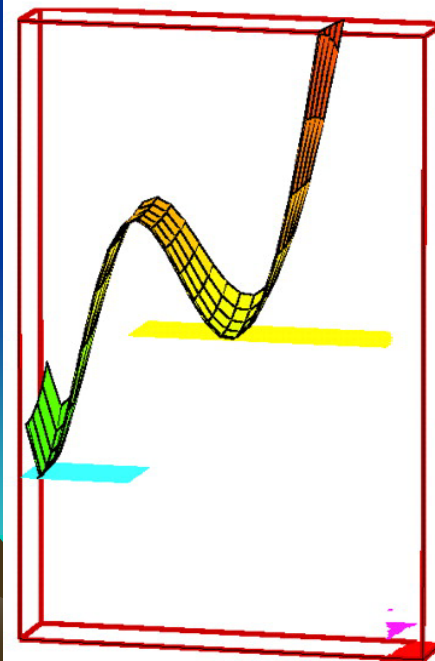
- **Исследование и идентификация**
- **минимумов и переходных**
- **состояний**

- **Построение энергетических**
- **профилей химической реакции**

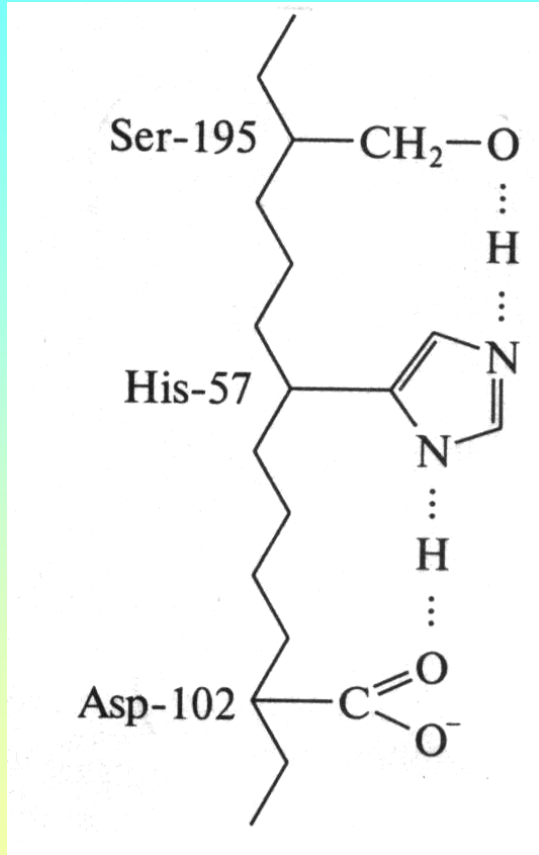
- **Идентификация молекулярного**
- **механизма реакции**



с



Charge relay system

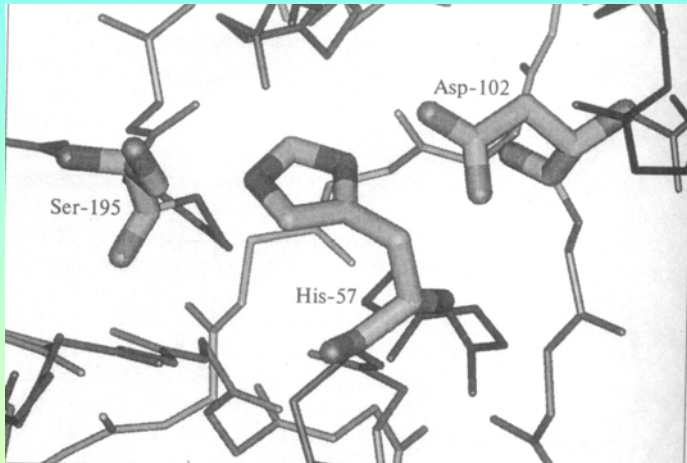


- Matthews B.W., Sigler P.B., Henderson R., Blow D. (1967)
- Blow D., Birktof T.A., Hartley BS (1969)

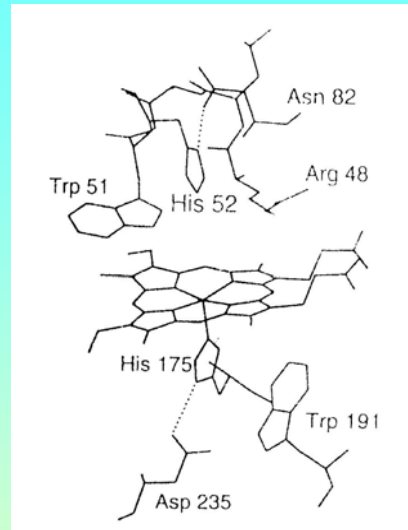
Asp⁻ His Ser ---> Asp His Ser⁻
mechanism that increases
the nucleophilicity of Ser

Structural Unity of catalytical sites

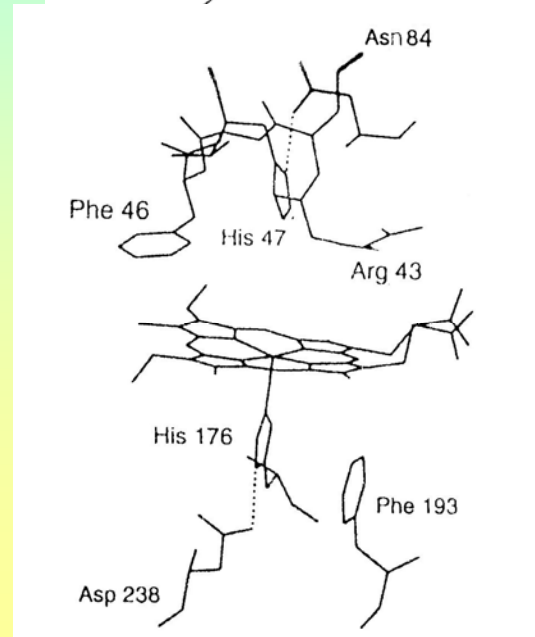
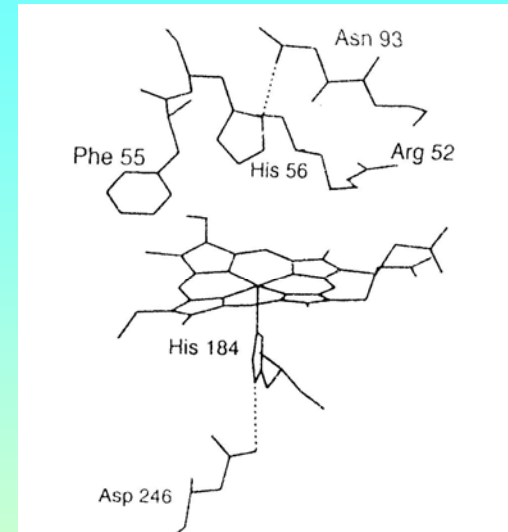
"charge relay system"



Chymotrypsin

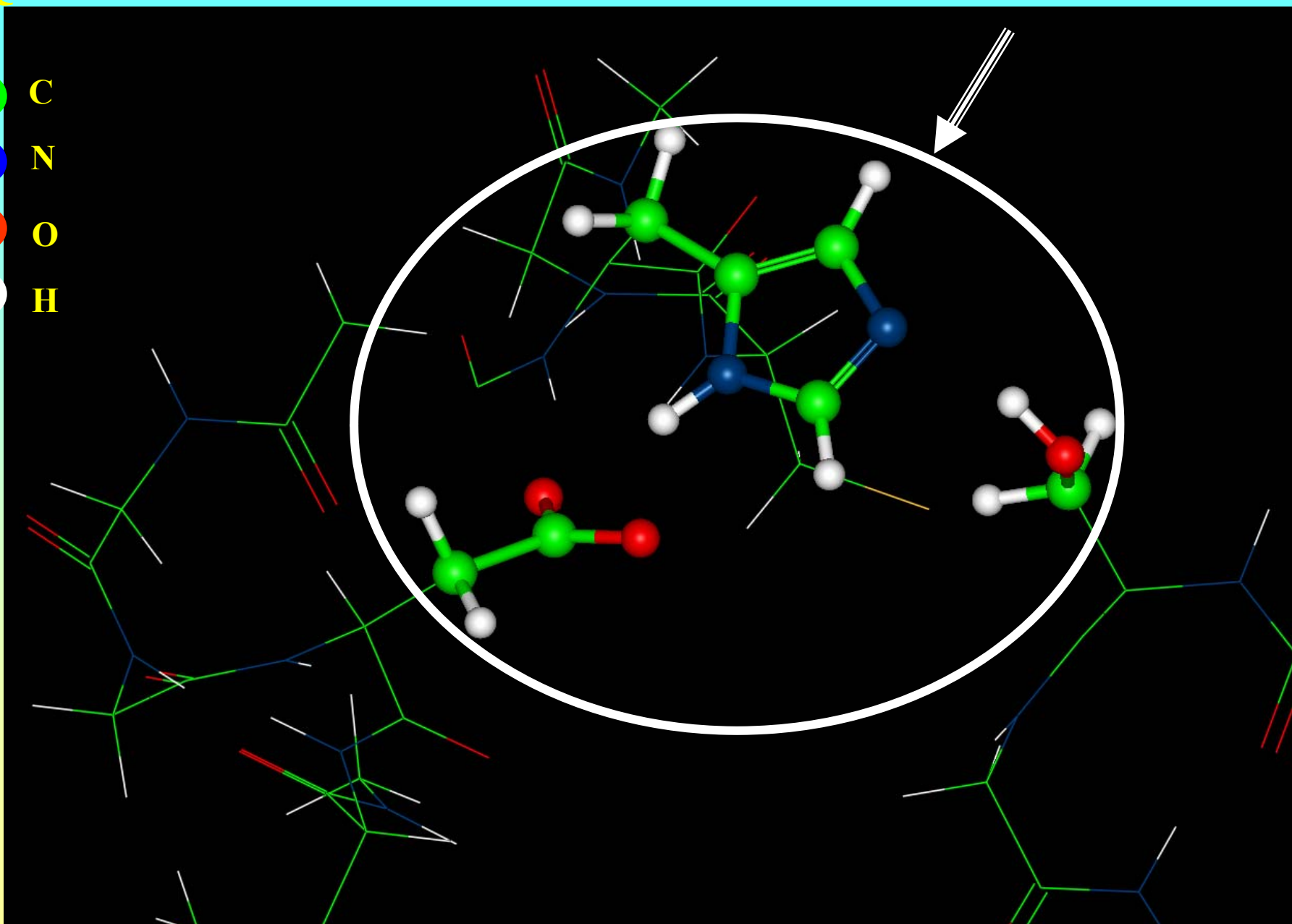


Peroxidases



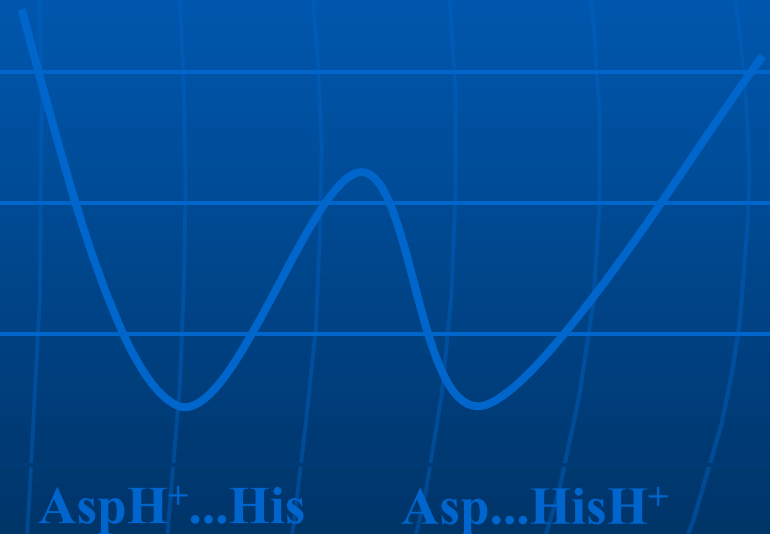
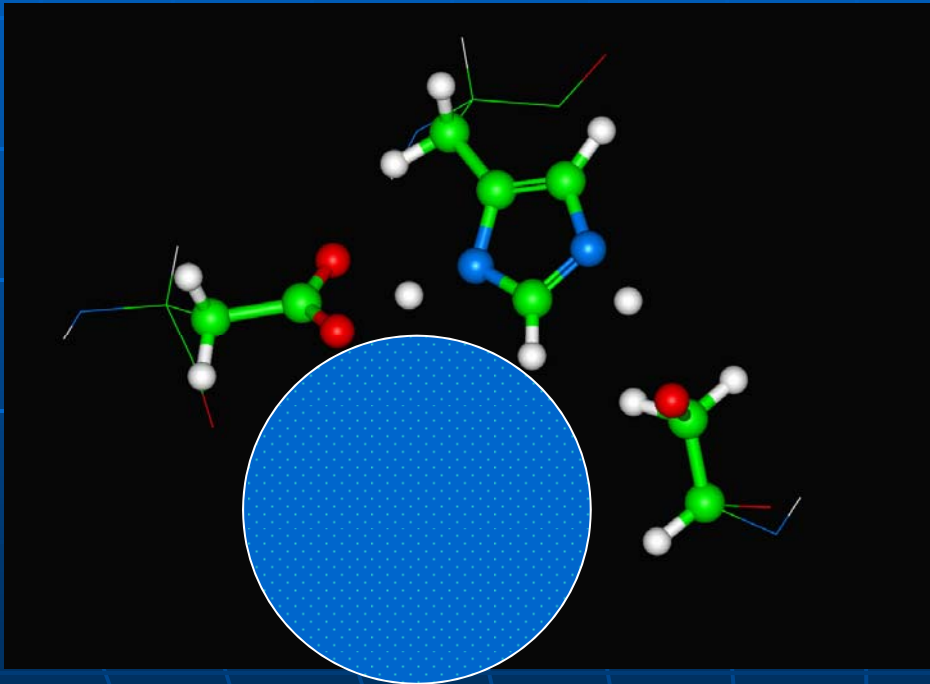
QM/MM:

- C
- N
- O
- H



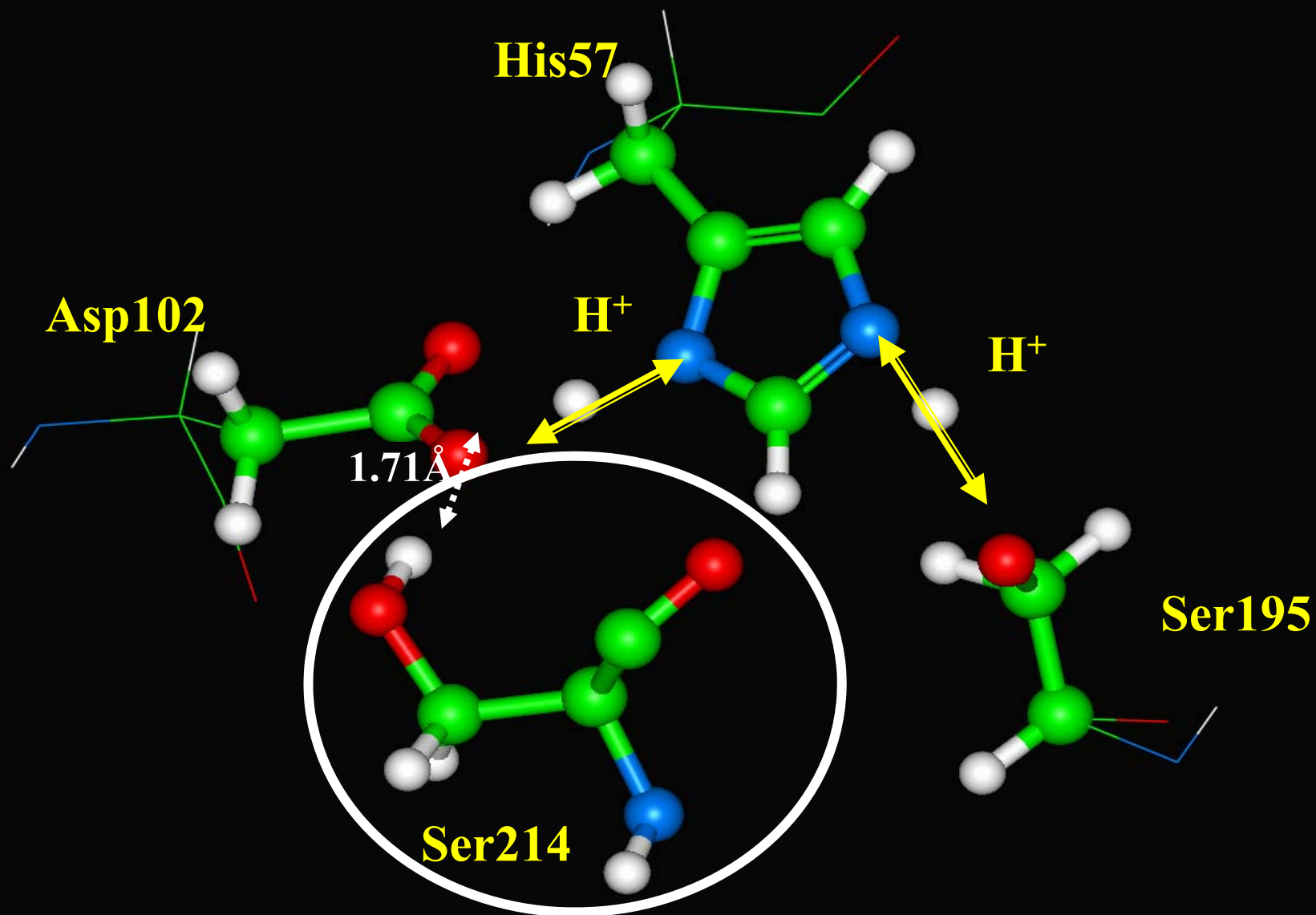
Одно-протонный или двух-протонный механизм: ключевая роль окружения

**Квантово-химические расчёты энергетического профиля:
Ser214 не включён в систему**



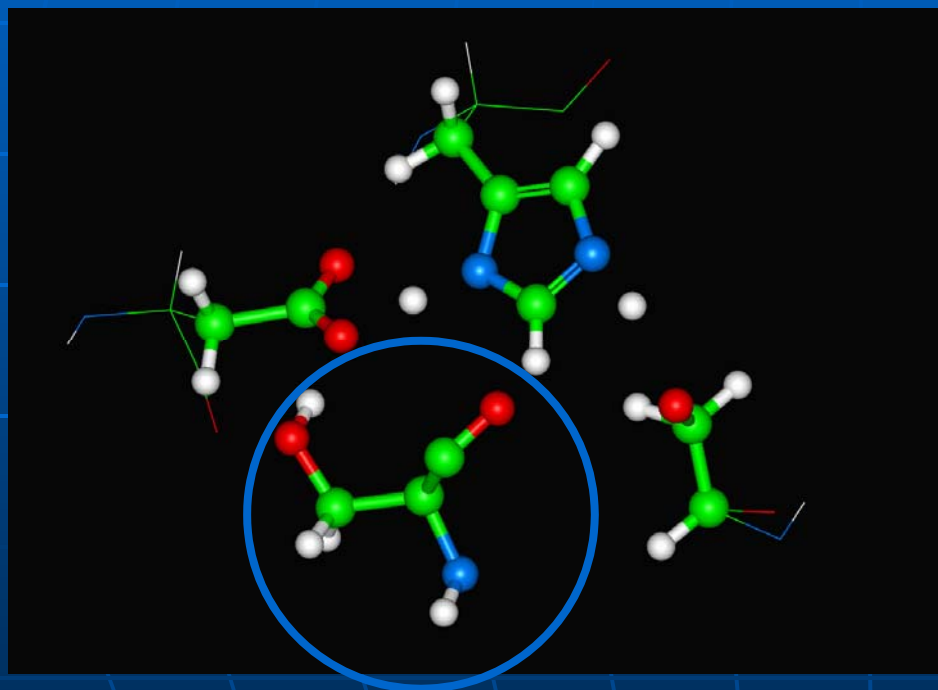
Возможен последовательный двух-протонный механизм

Одно-протонный или двух-протонный механизм: ключевая роль окружения



*Одно-протонный или двух-протонный
механизм: ключевая роль окружения*

Квантово-химические расчёты
энергетического профиля:
Ser214 включён в систему



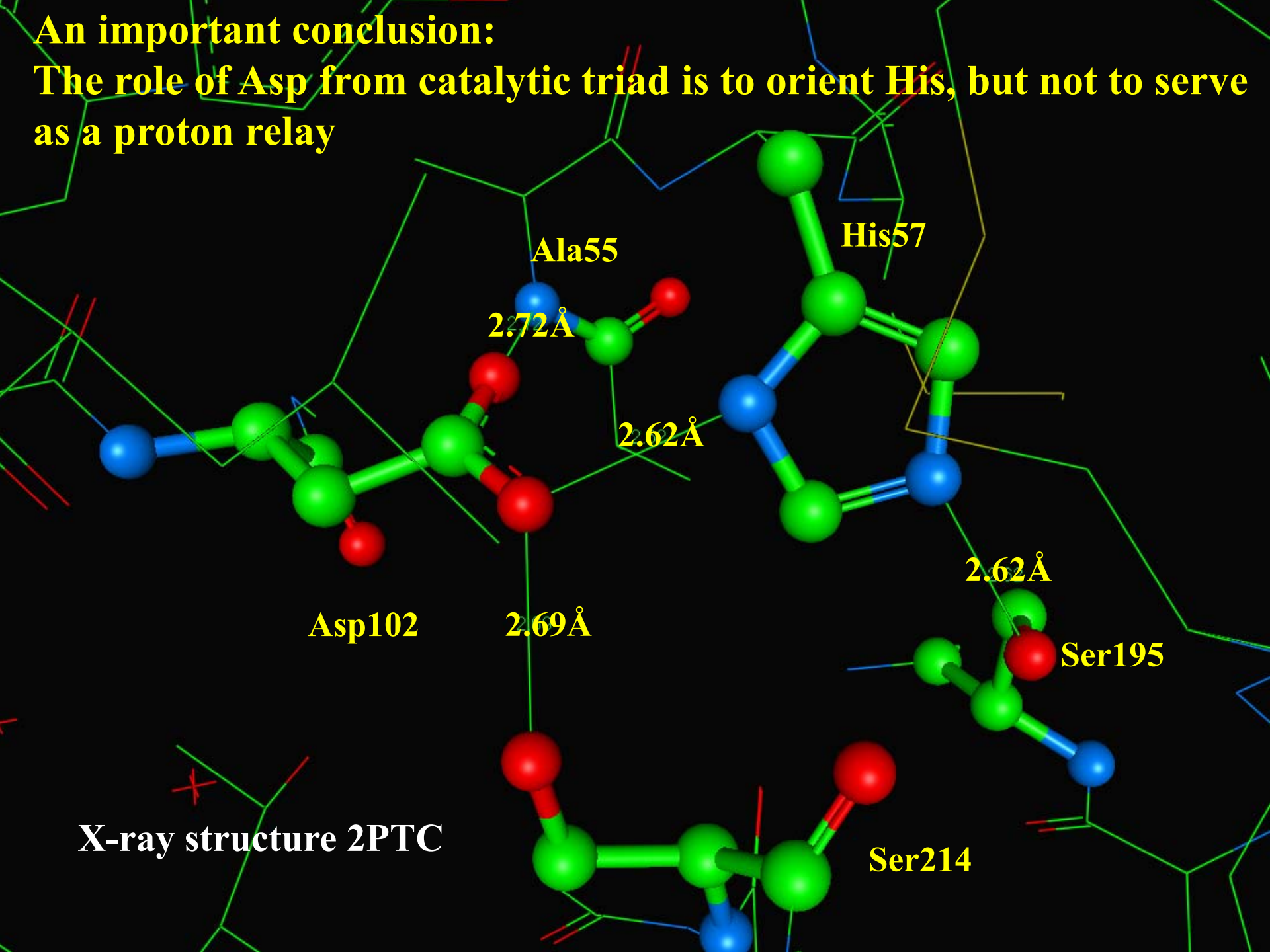
~~AspH⁺...His~~

Asp...HisH⁺

Одно-протонный механизм

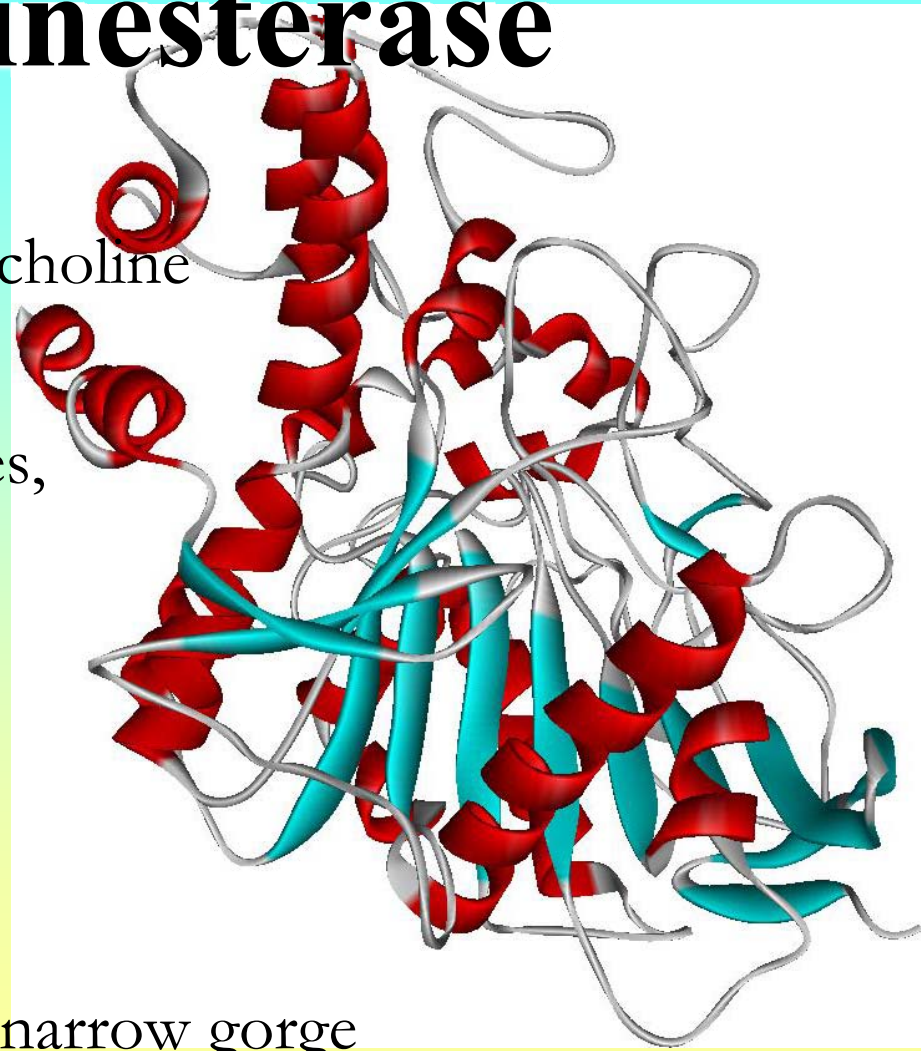
An important conclusion:

The role of Asp from catalytic triad is to orient His, but not to serve as a proton relay



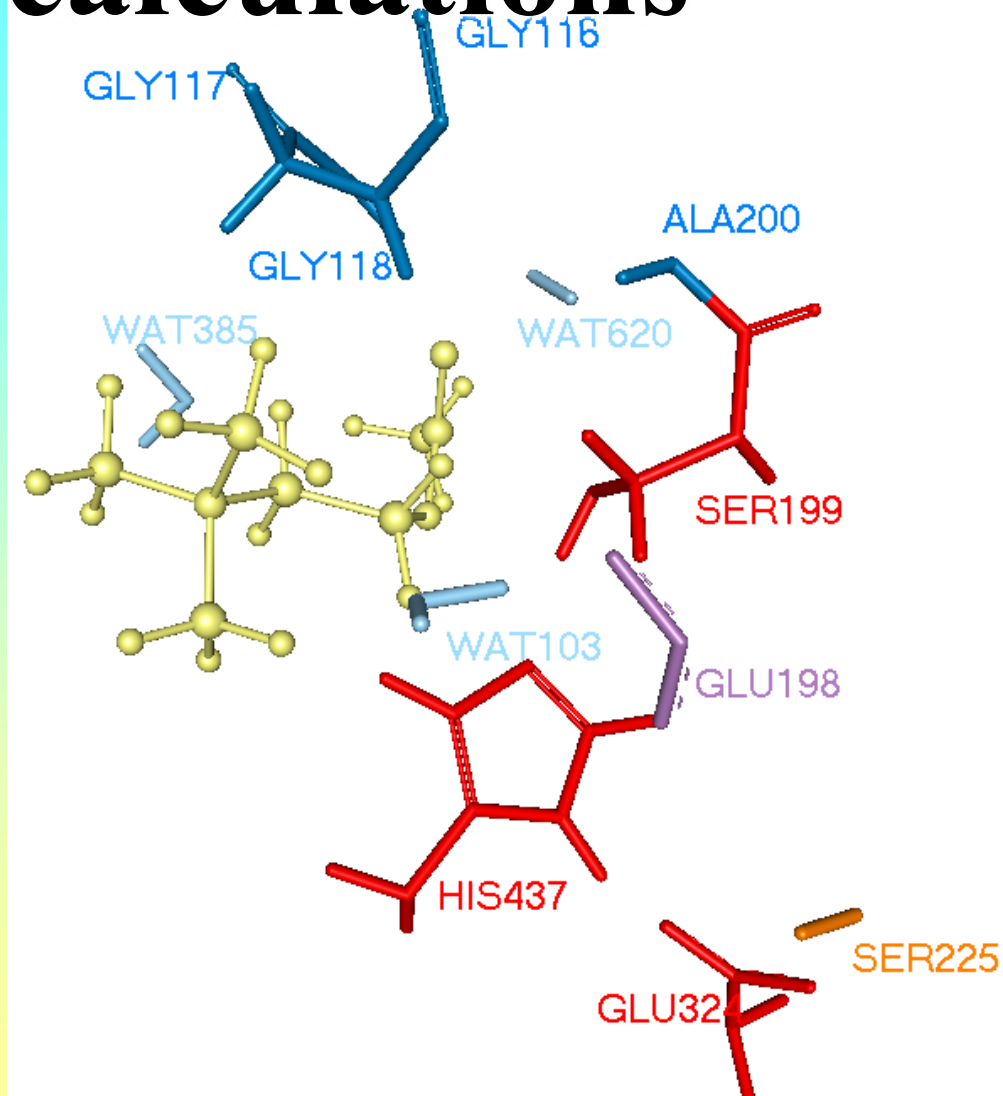
Acetylcholinesterase

- Enzyme of central nerve system, degrades the neurotransmitter acetylcholine
- Seryne hydrolase
- Structure: 537 aminoacid residues, 12 β -sheets, 14 α -helix
- Catalytically perfect enzyme:
$$k_{\text{cat}}/K_{\text{M}} \sim 2 \times 10^8 \text{ M}^{-1}\text{s}^{-1}$$
- Active site at the bottom of 20 Å narrow gorge

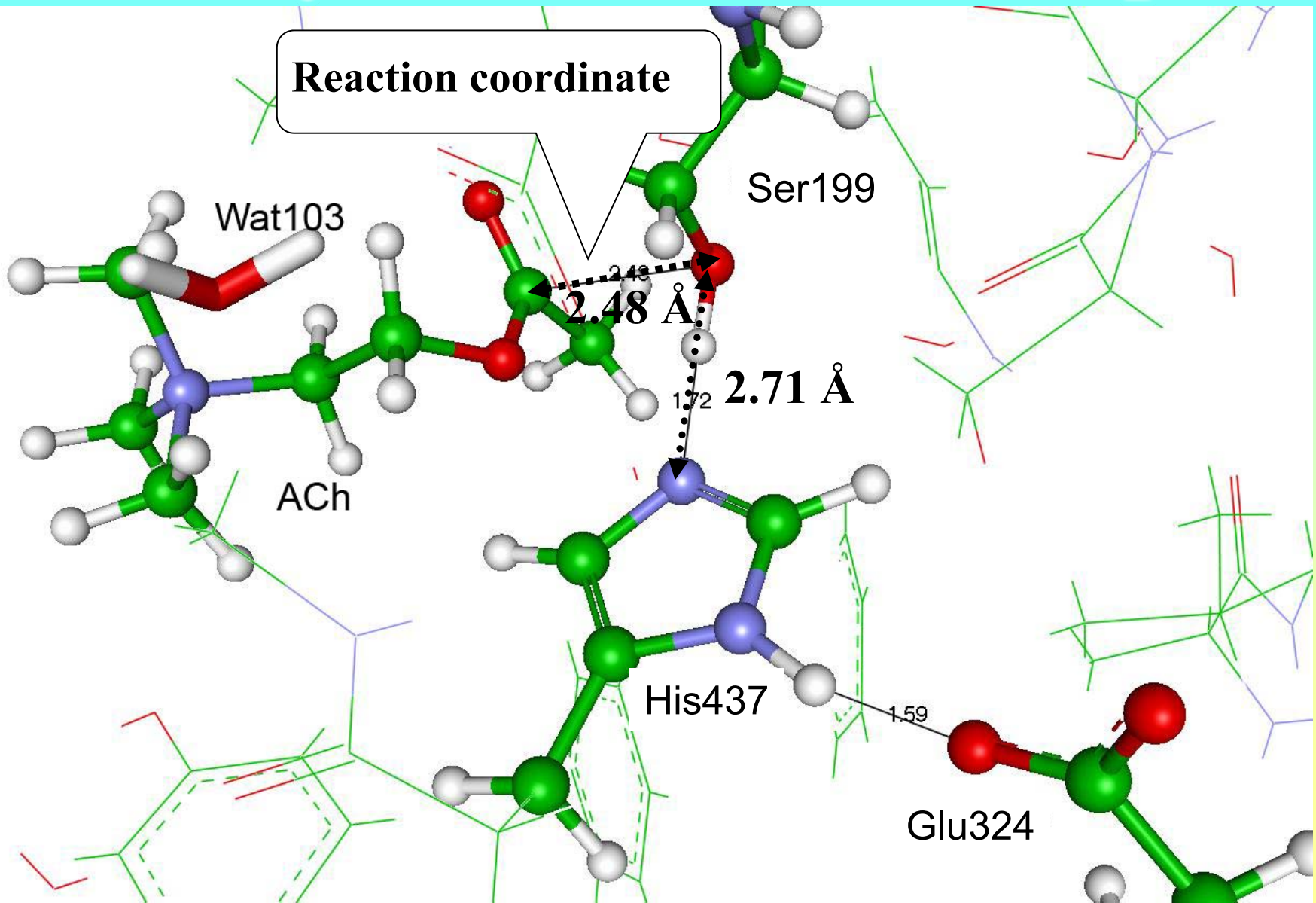


QM/MM calculations

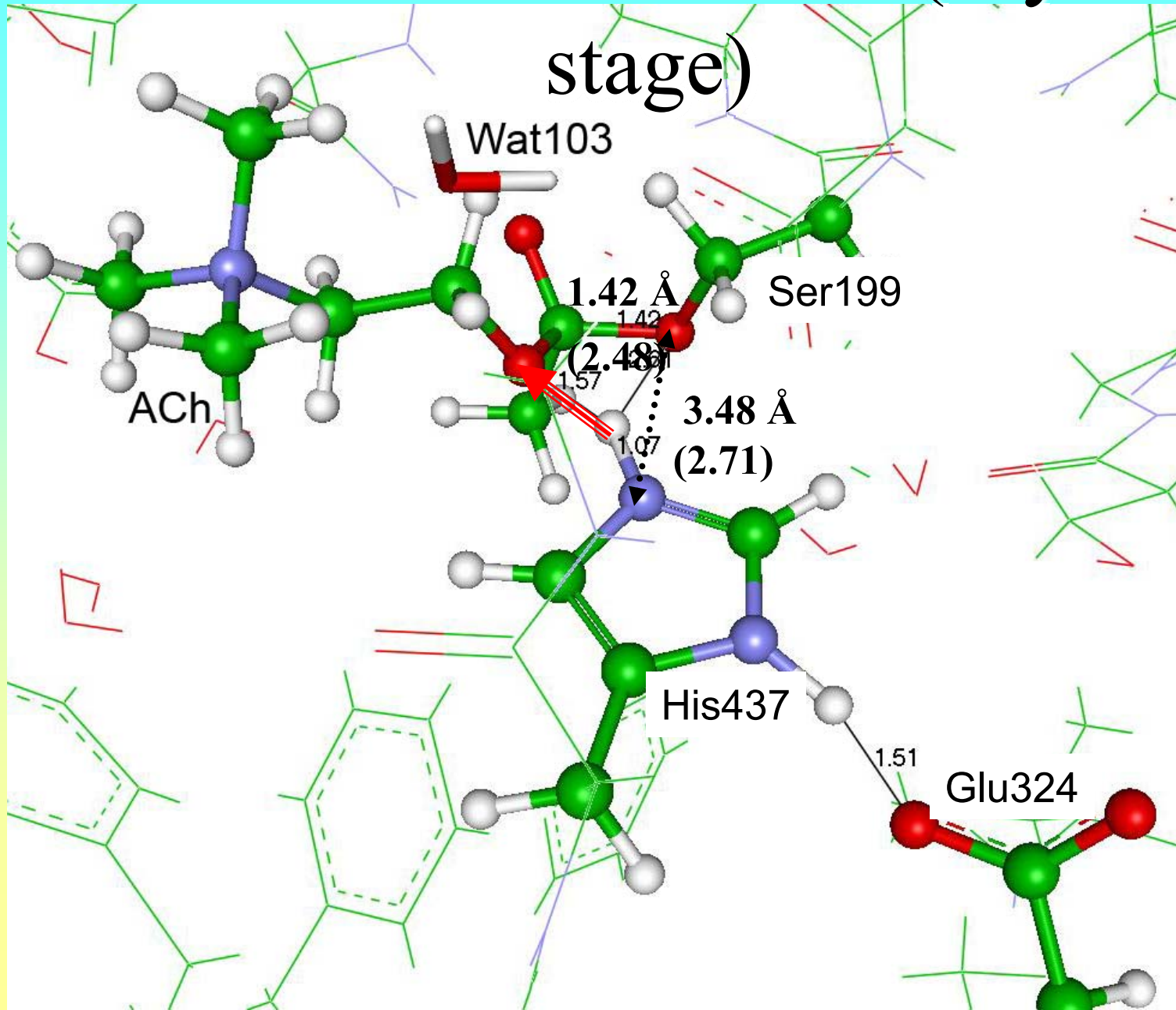
- **Quantum subsystem:**
 - Ach molecule
 - Atoms of Ser199, His437, Glu324, Gly117, Gly118, Ala200, Glu198, Ser225 residues
 - 3 water molecules
- **QM/MM run:**
 - DFT(PBE0)/6-31G*,
 - 713 basis functions
 - Amber99 force field



Enzyme-substrate complex

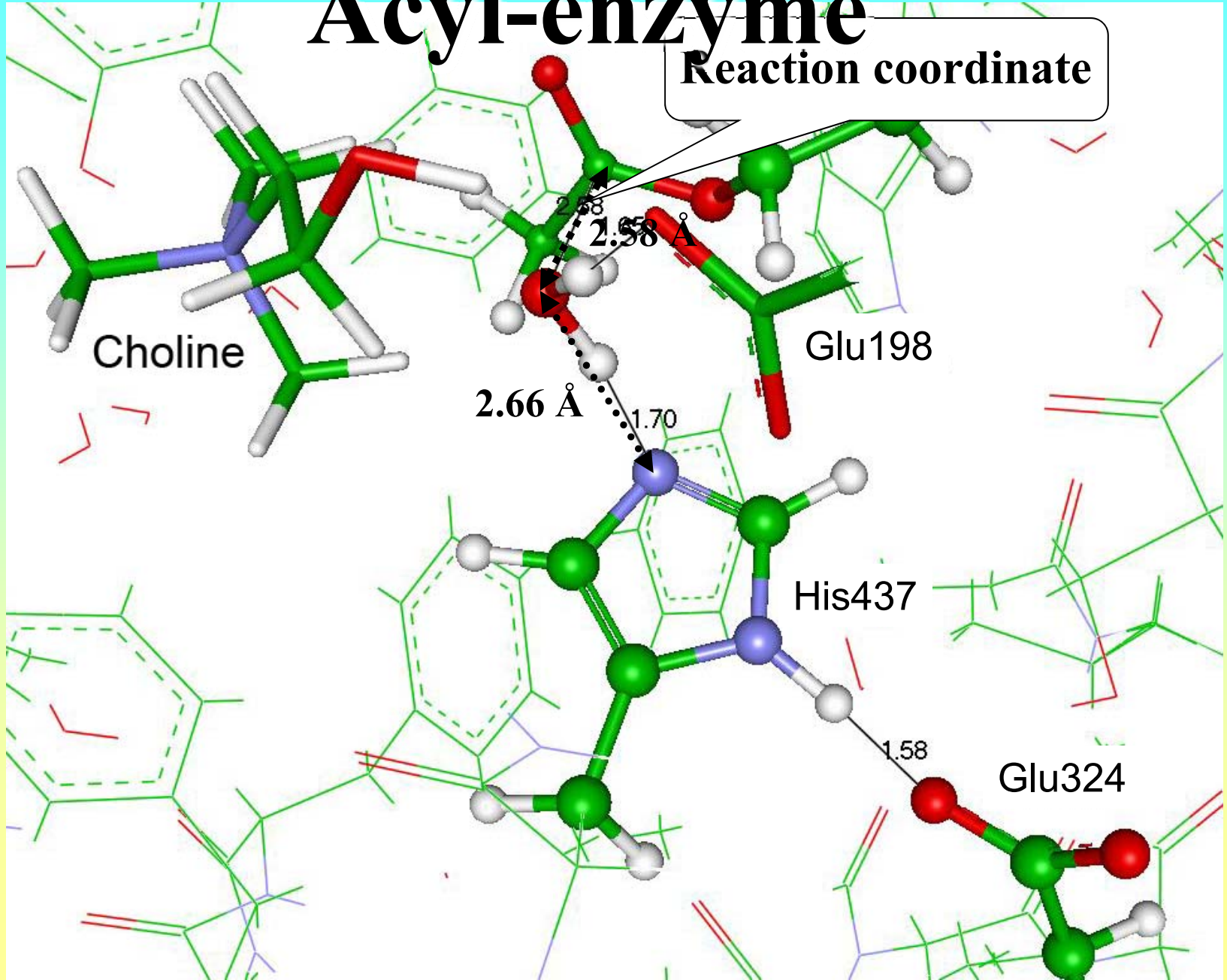


Tetrahedral intermediate (acylation stage)

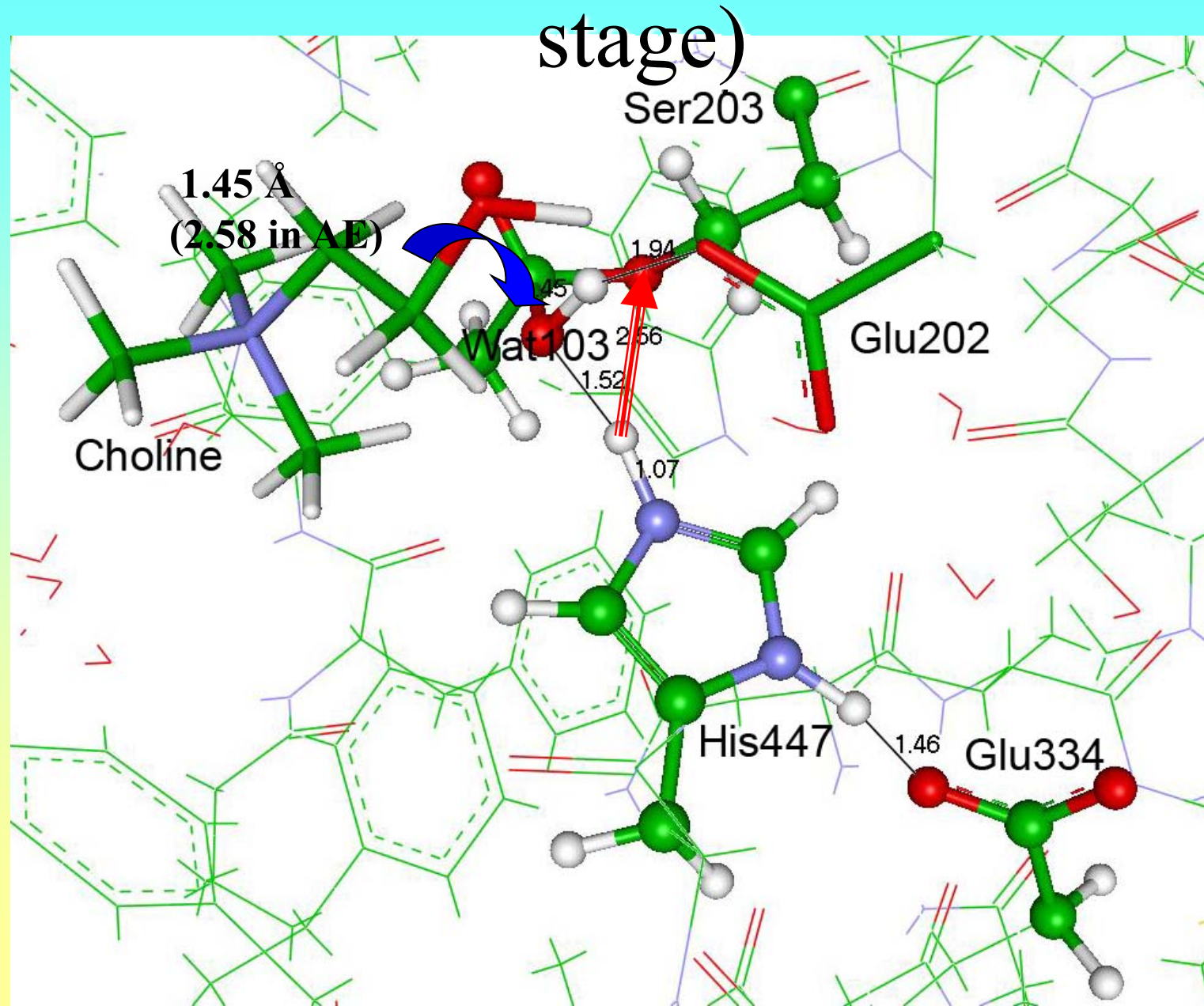


Acyl-enzyme

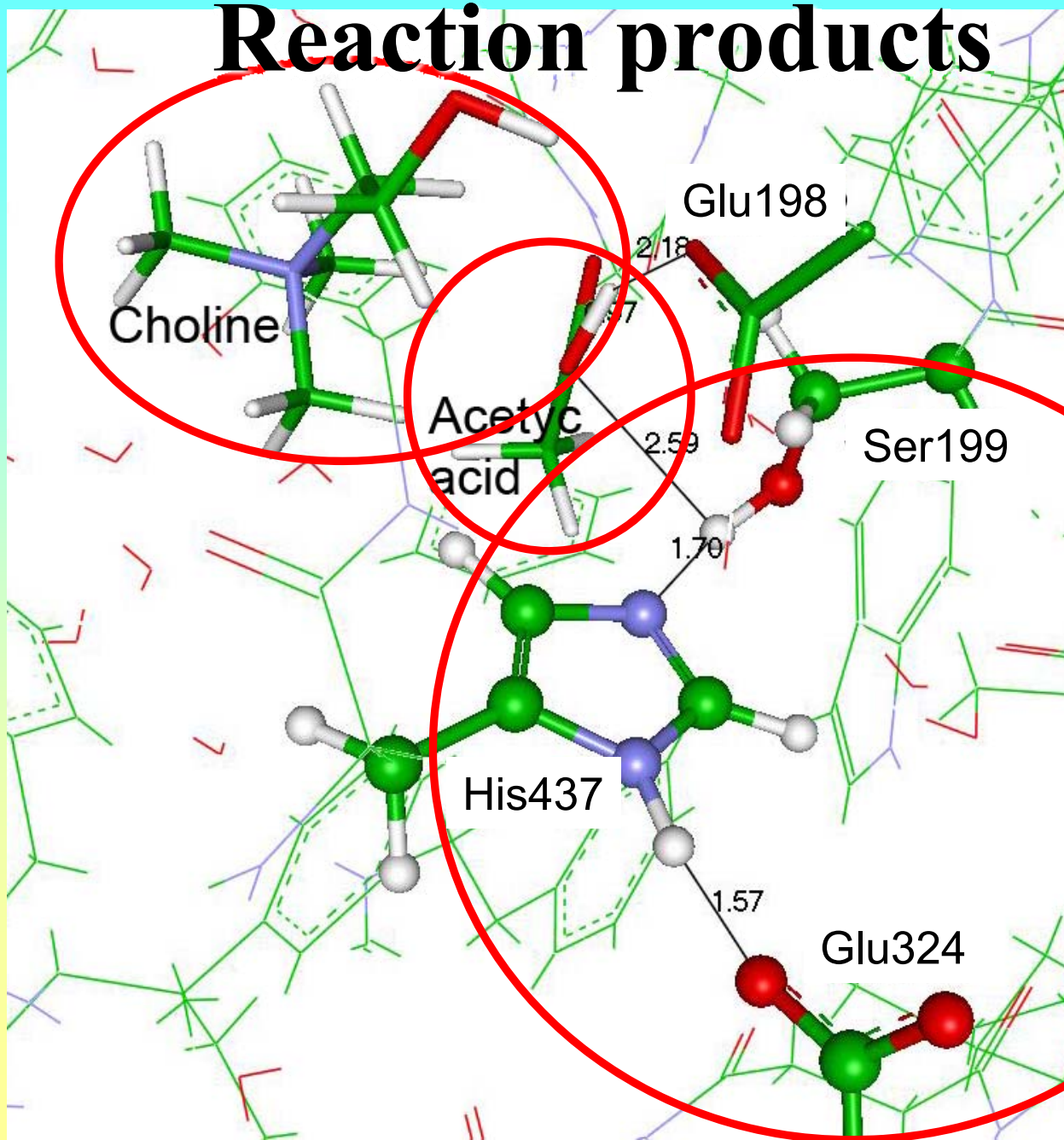
Reaction coordinate



Tetrahedral intermediate (deacylation stage)



Reaction products



Программированная молекулярная машина

МОЛЕКУЛЯРНЫЙ СТАНОК

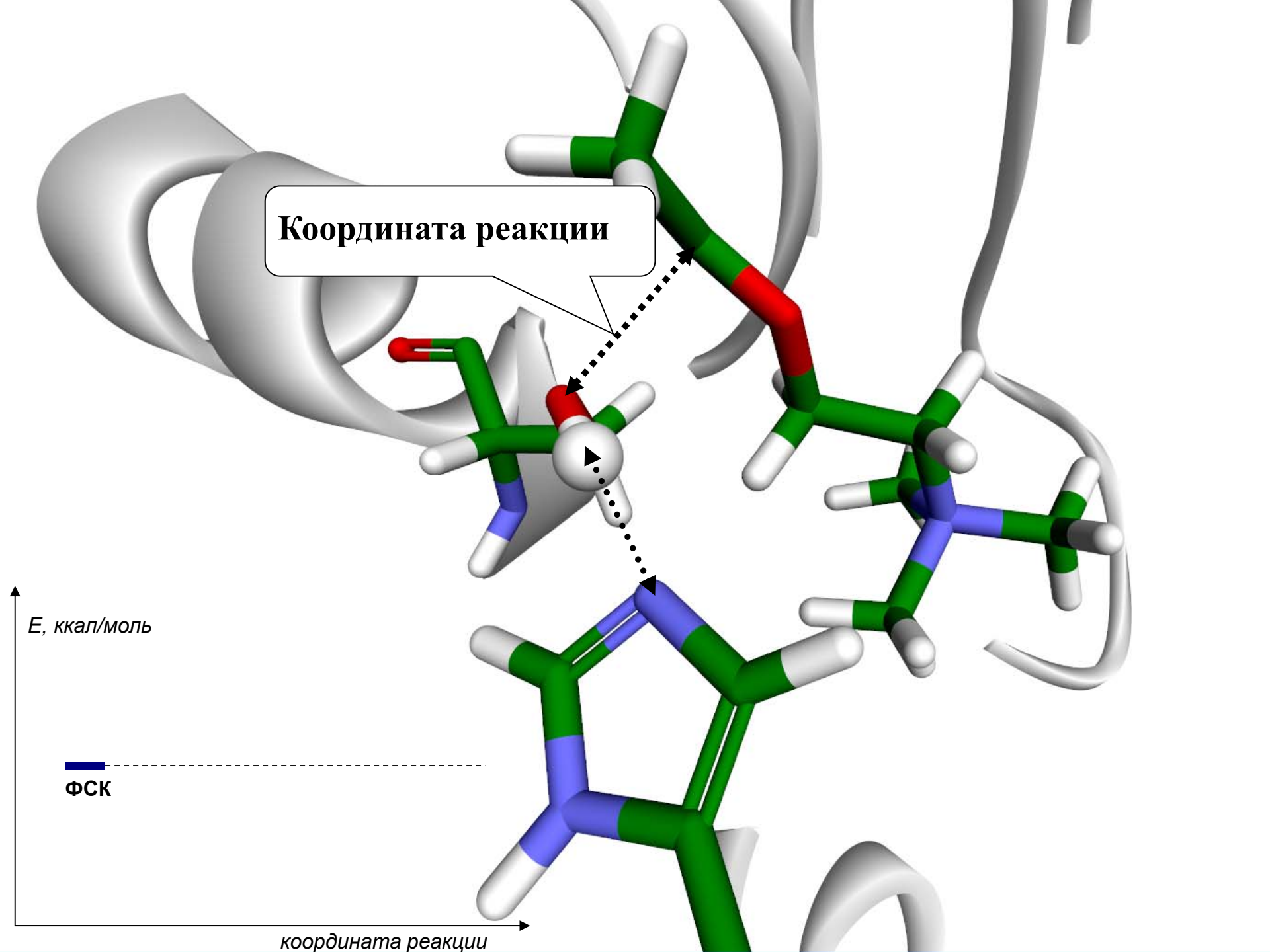
Стадия ацилирования

Координата реакции

E , ккал/моль

ФСК

координата реакции

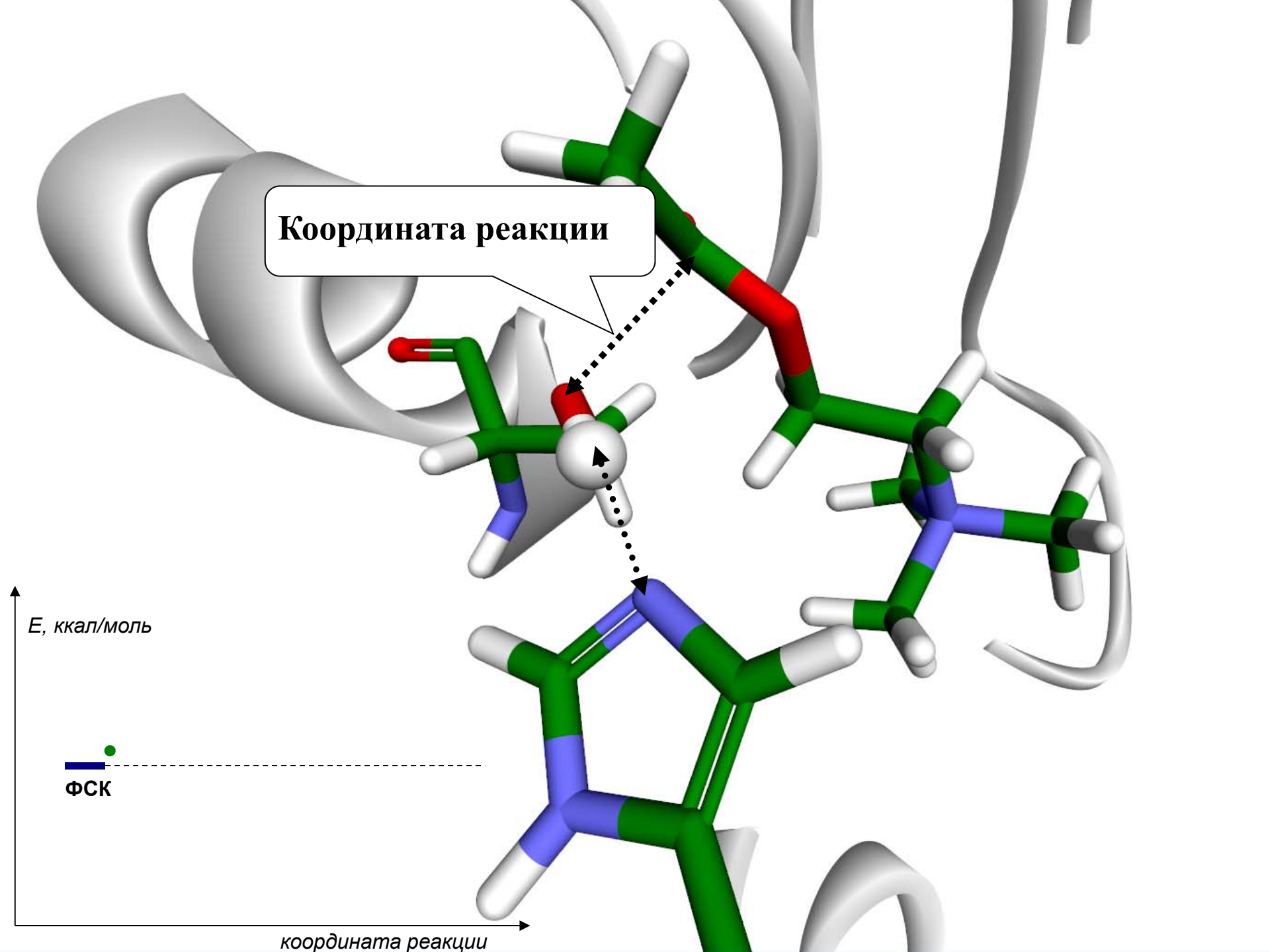


Координата реакции

E , ккал/моль

ФСК

координата реакции

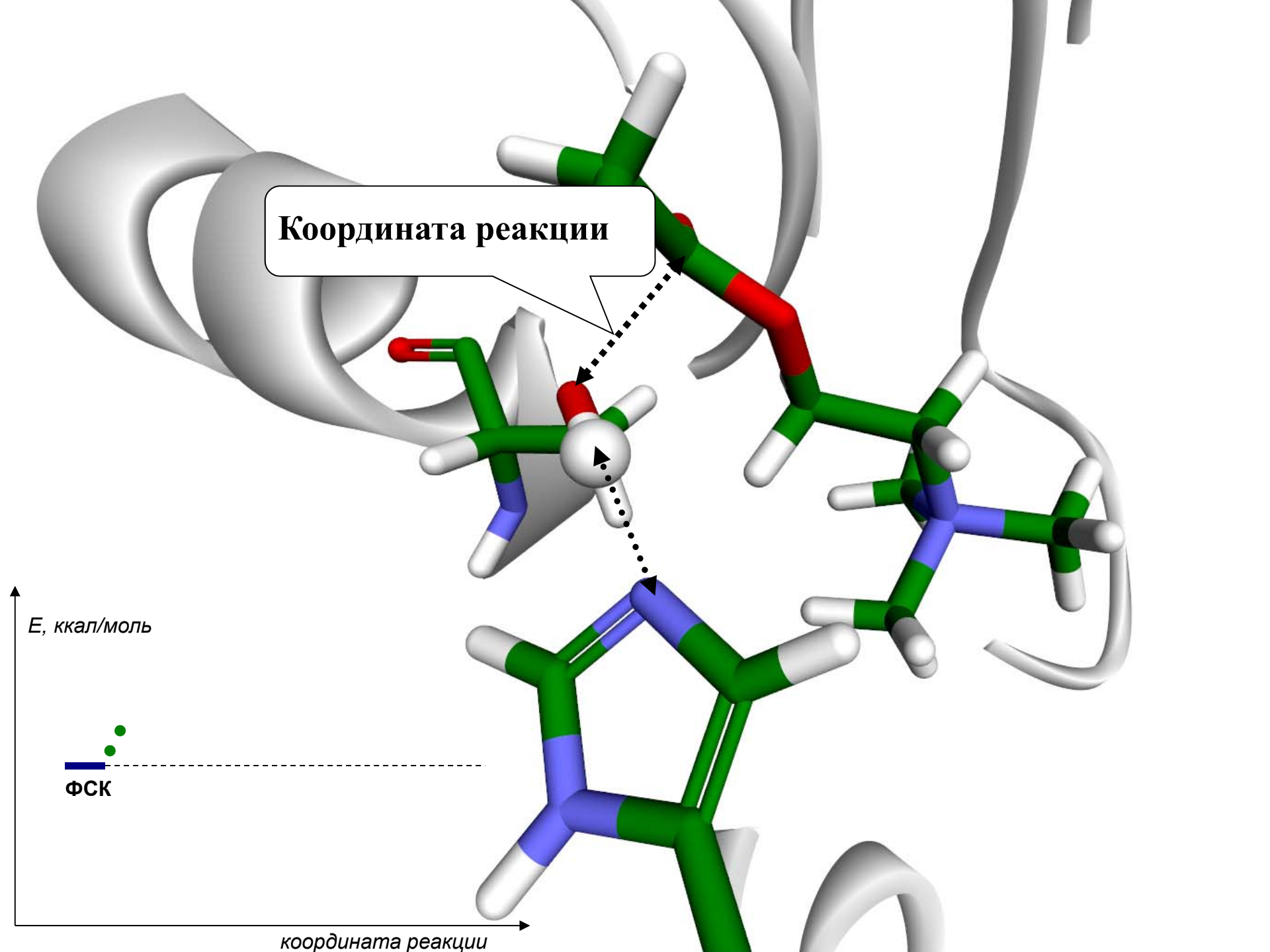


Координата реакции

E , ккал/моль

ФСК

координата реакции

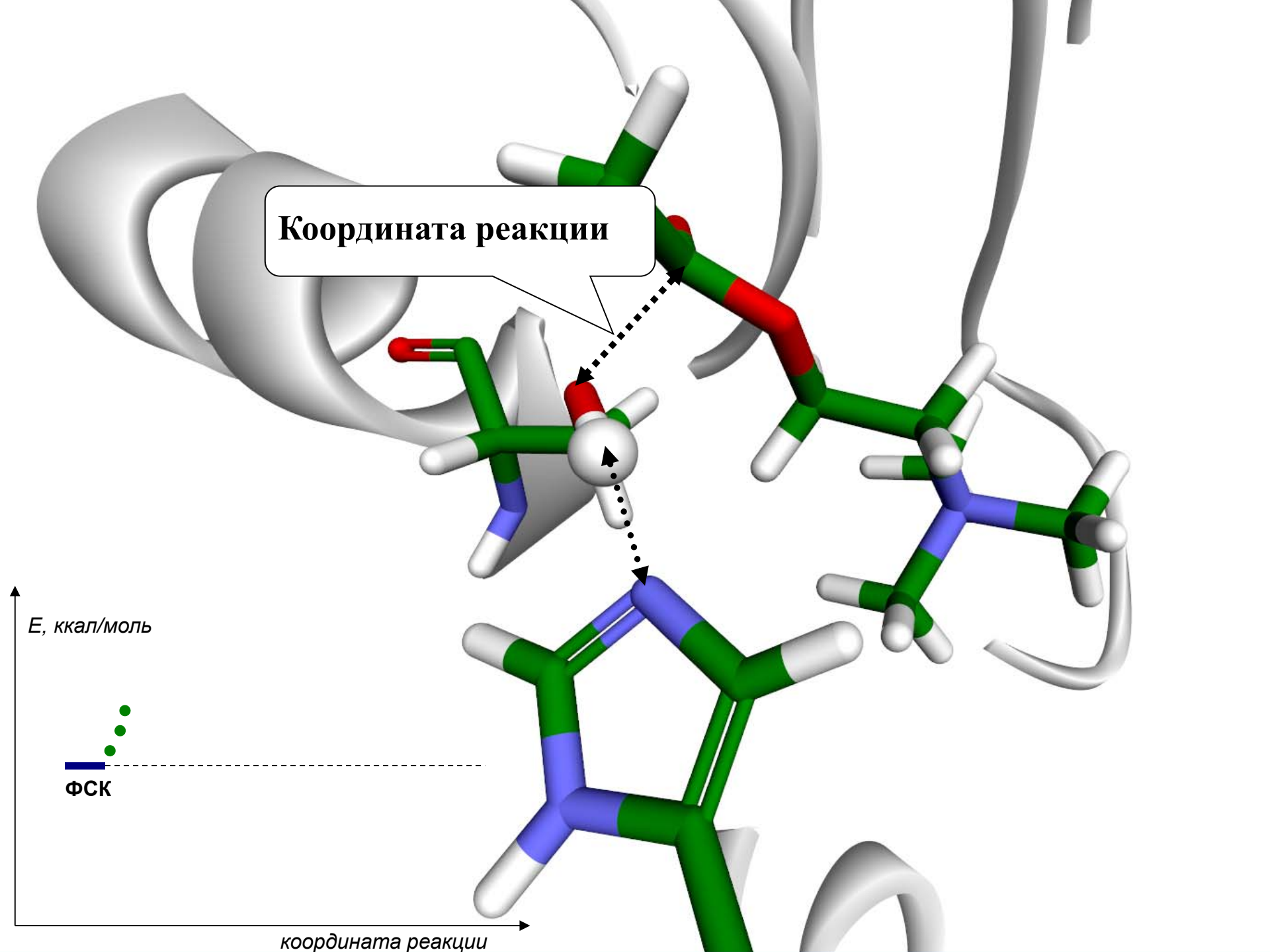


Координата реакции

E , ккал/моль

ФСК

координата реакции

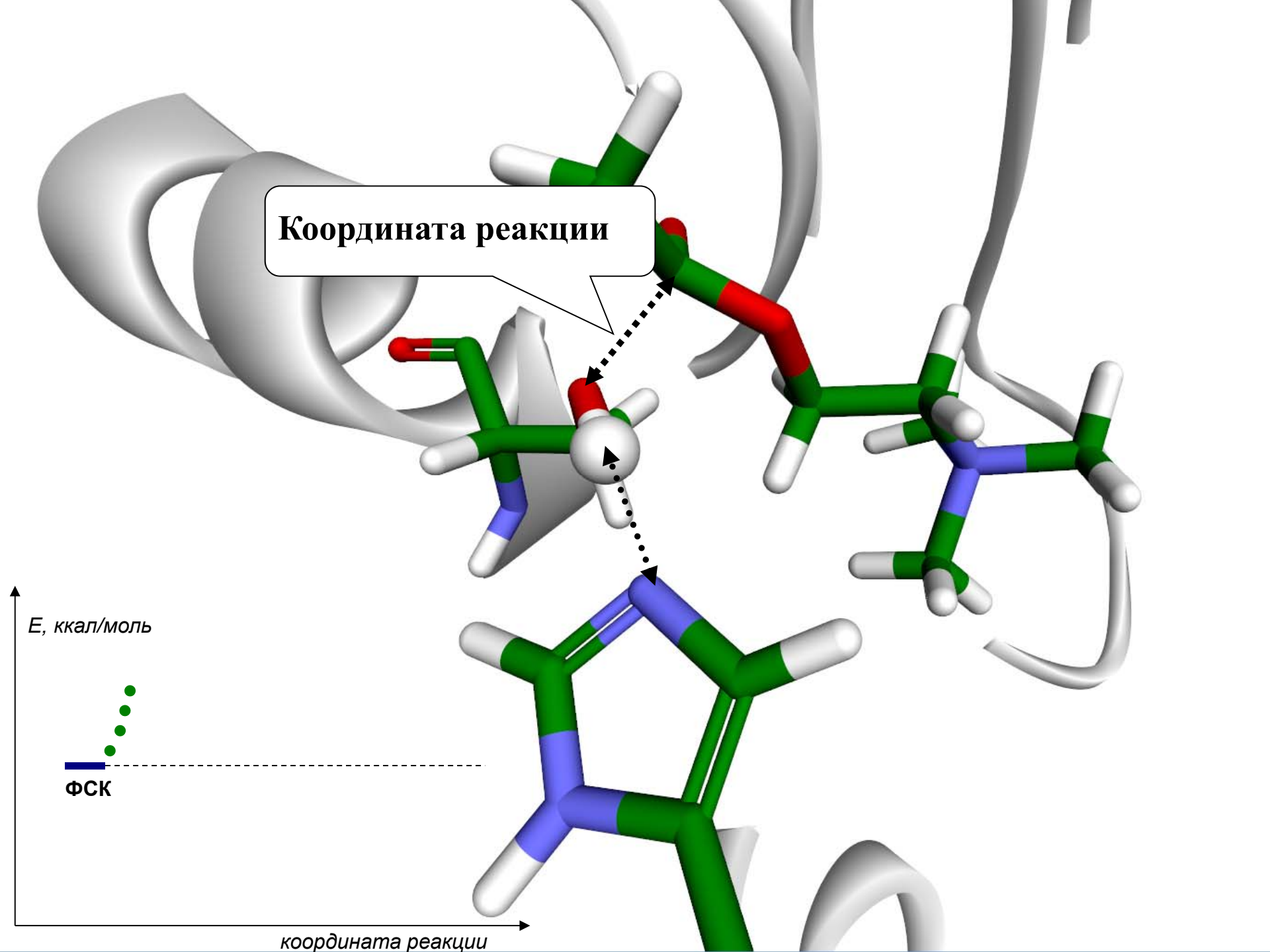


Координата реакции

E , ккал/моль

ФСК

координата реакции

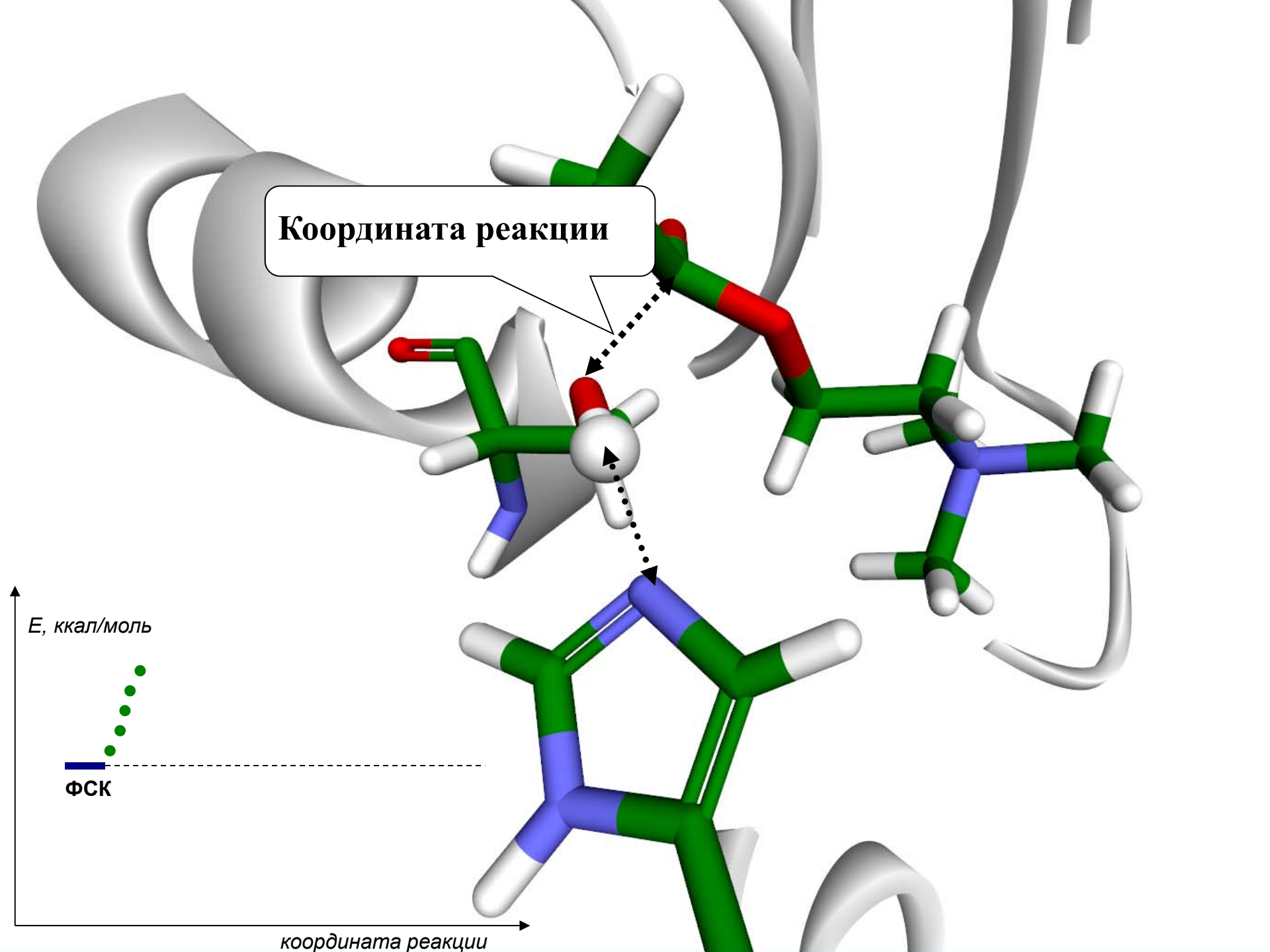


Координата реакции

E , ккал/моль

ФСК

координата реакции



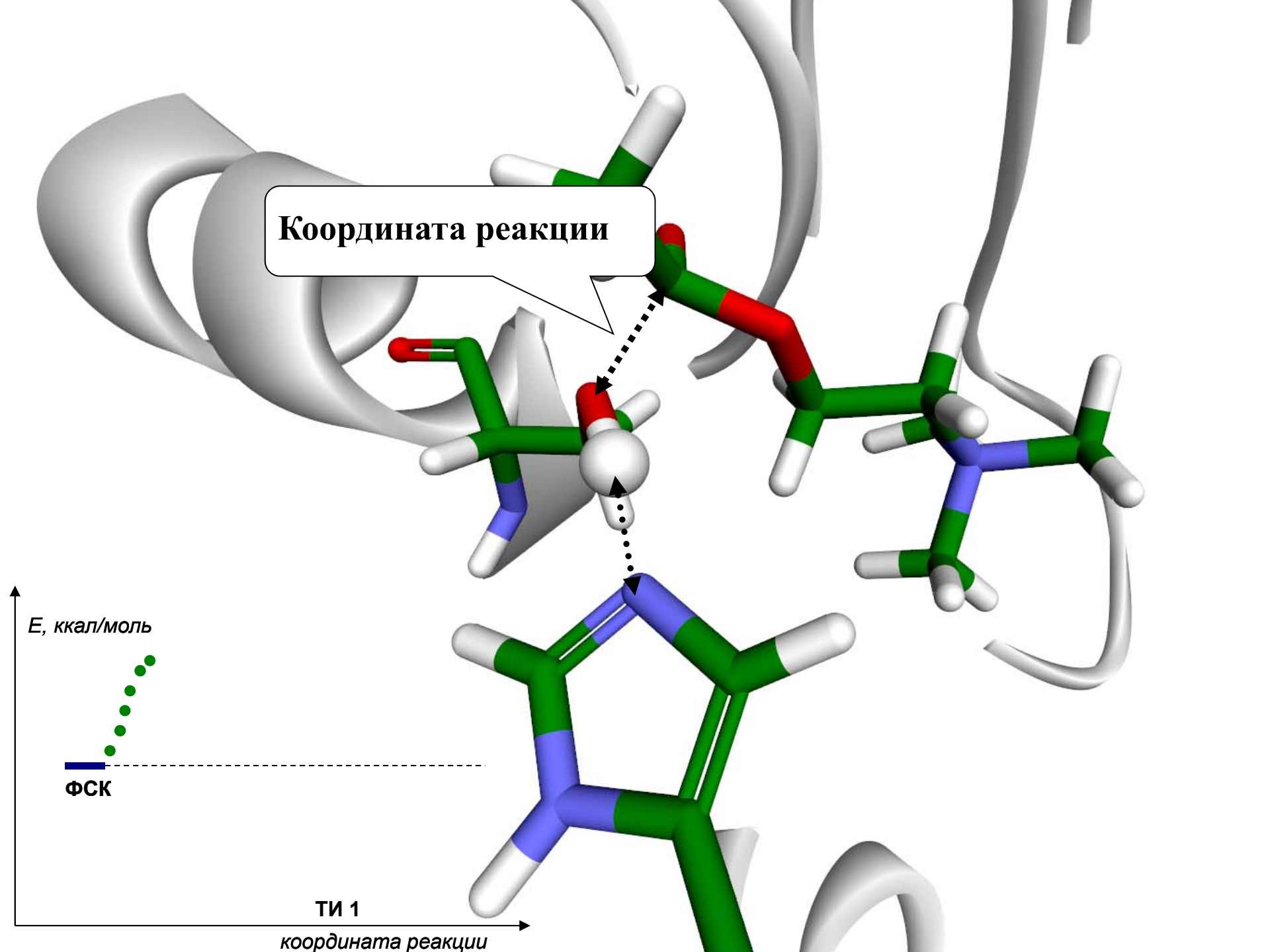
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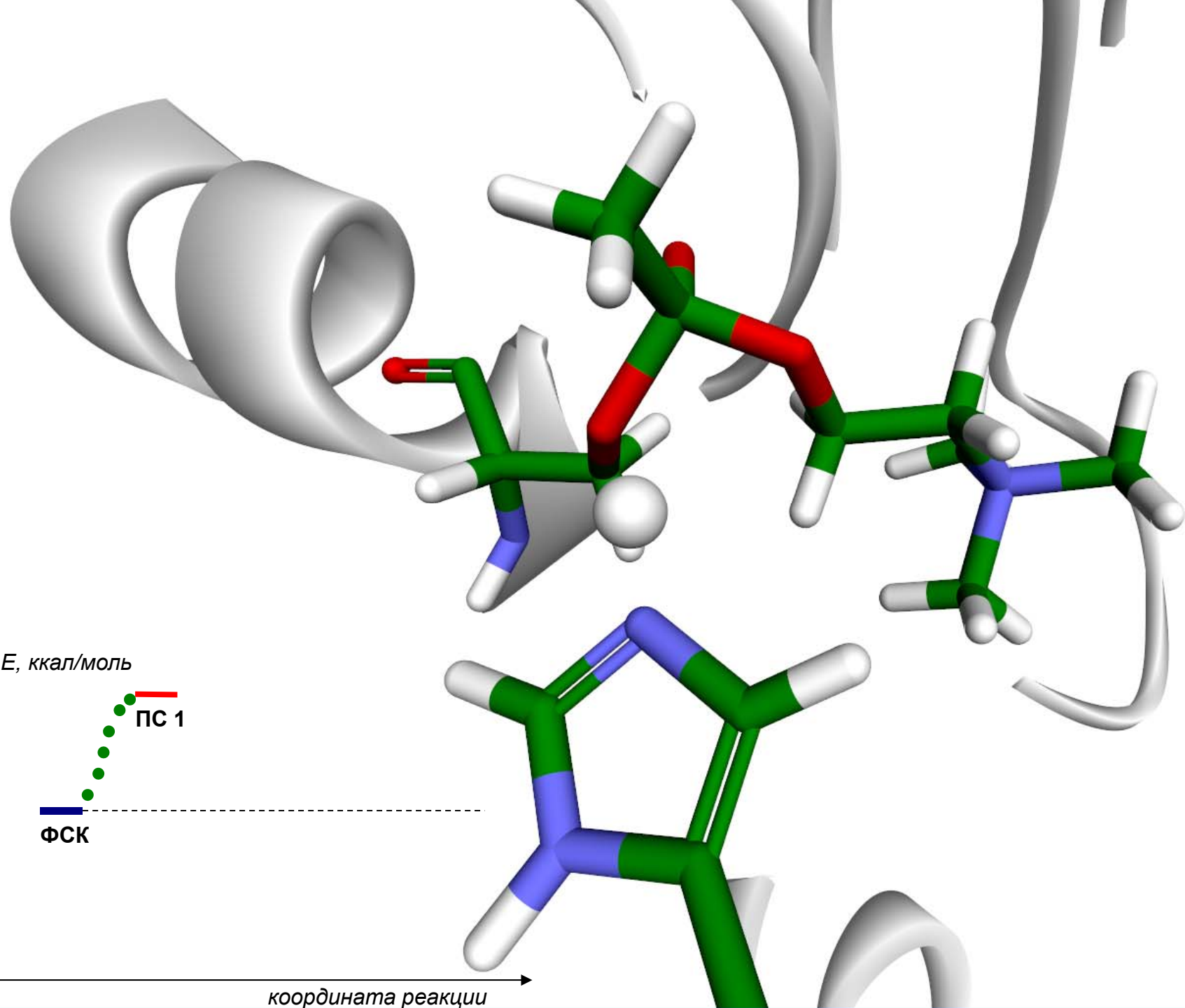
E , ккал/моль

ФСК

ТИ 1

координата реакции



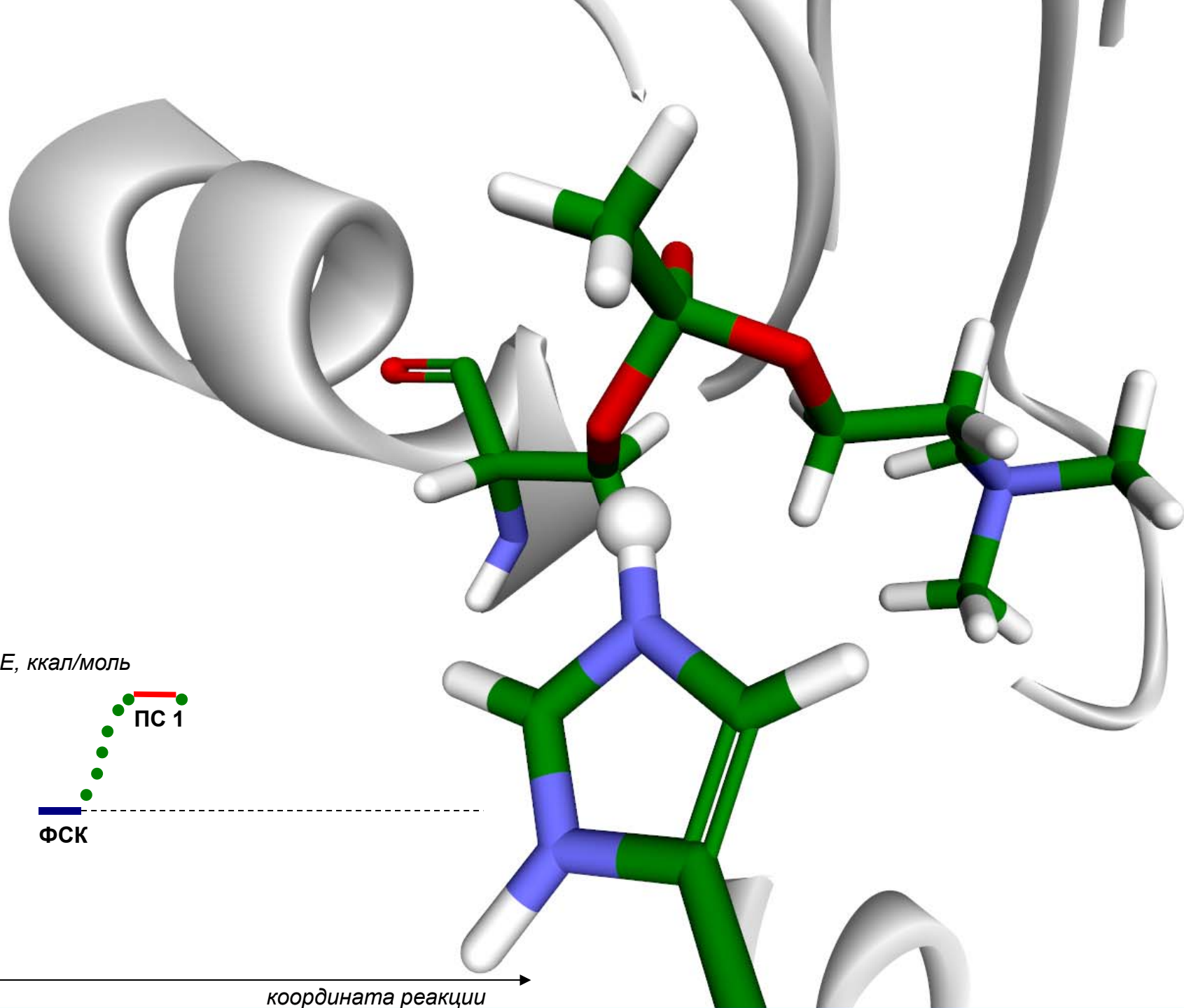


E , ккал/моль

ПС 1

ФСК

координата реакции

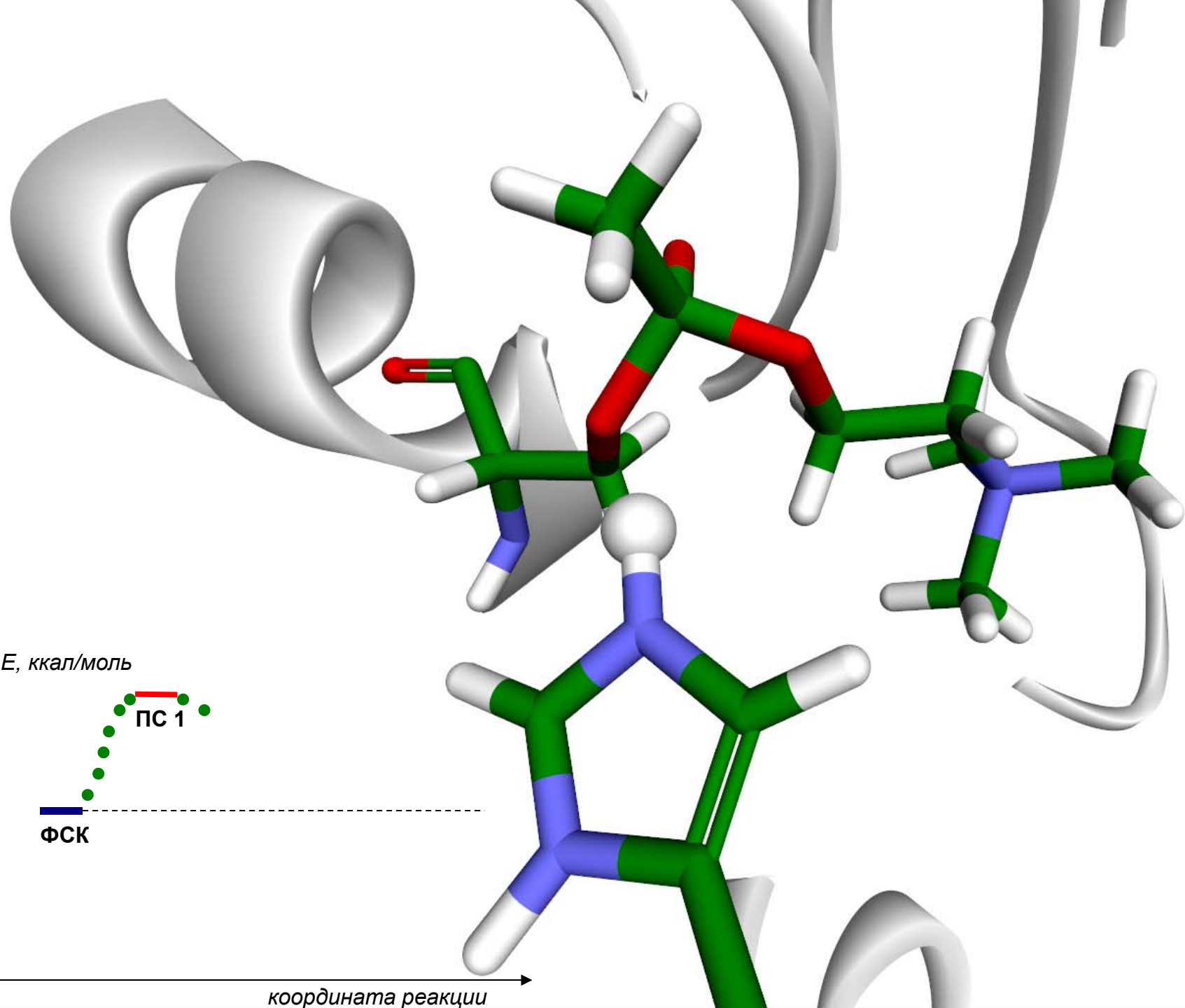


E , ккал/моль

ПС 1

ФСК

координата реакции

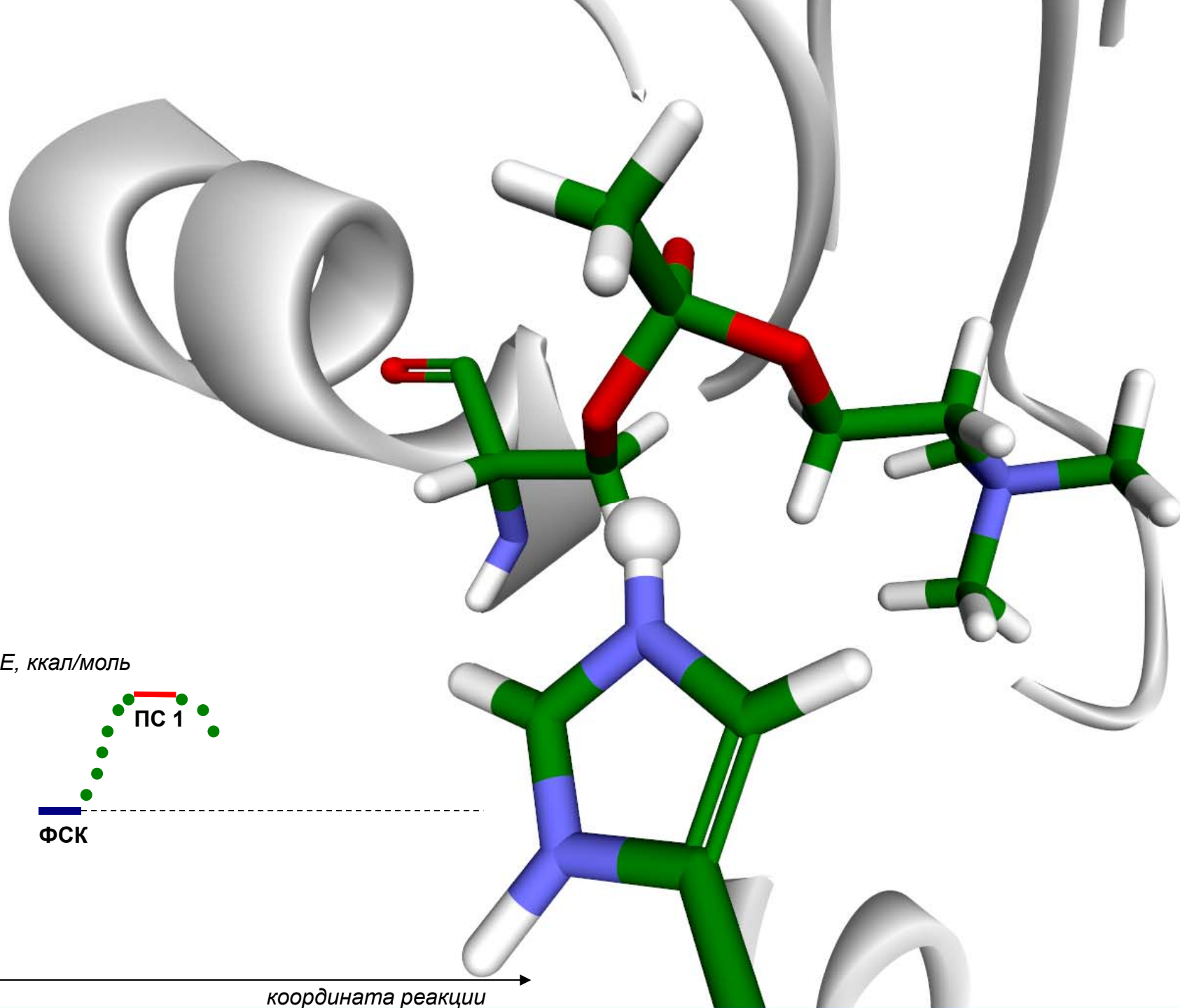


E , ккал/моль

ПС 1

ФСК

координата реакции

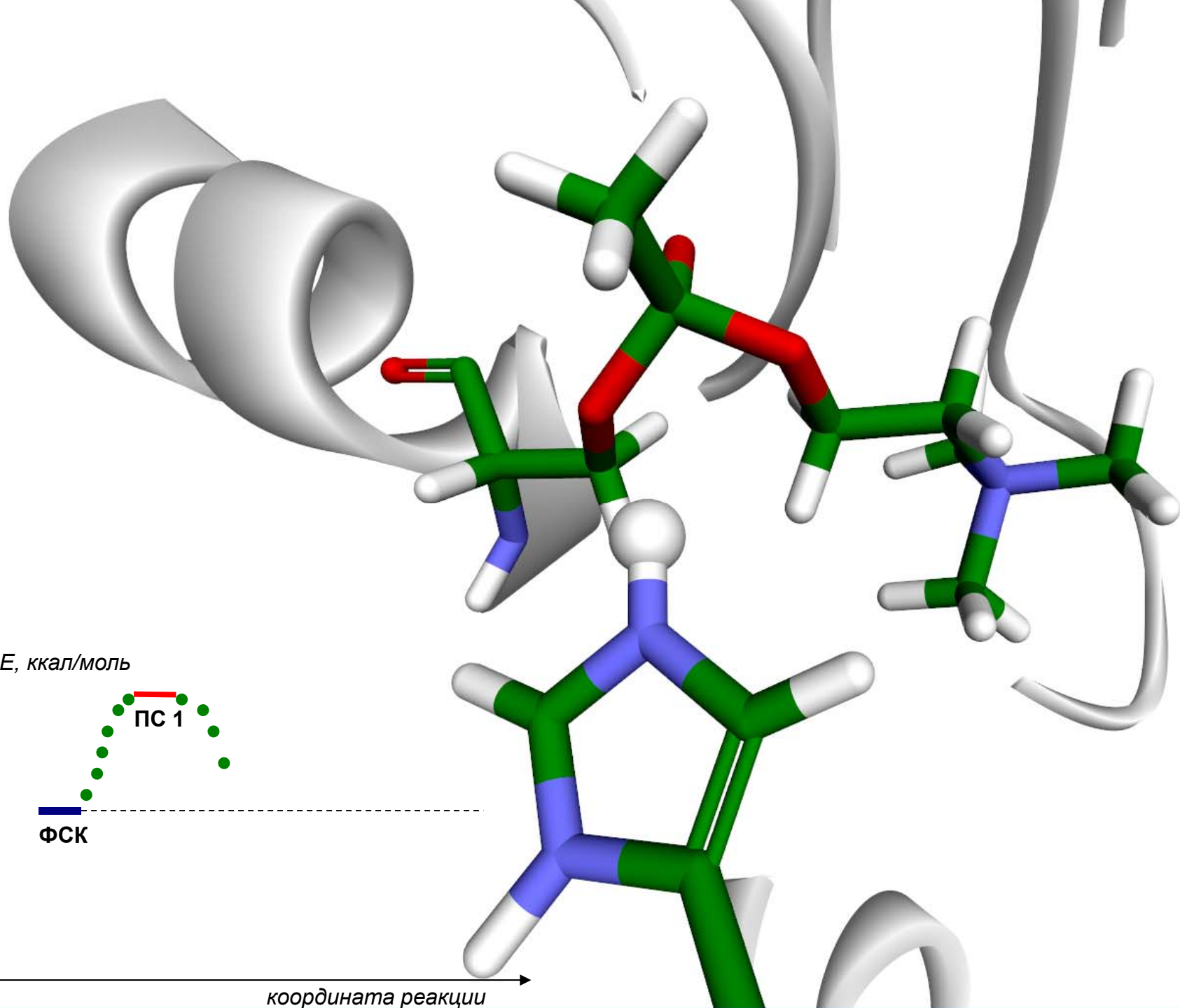


E , ккал/моль

ПС 1

ФСК

координата реакции

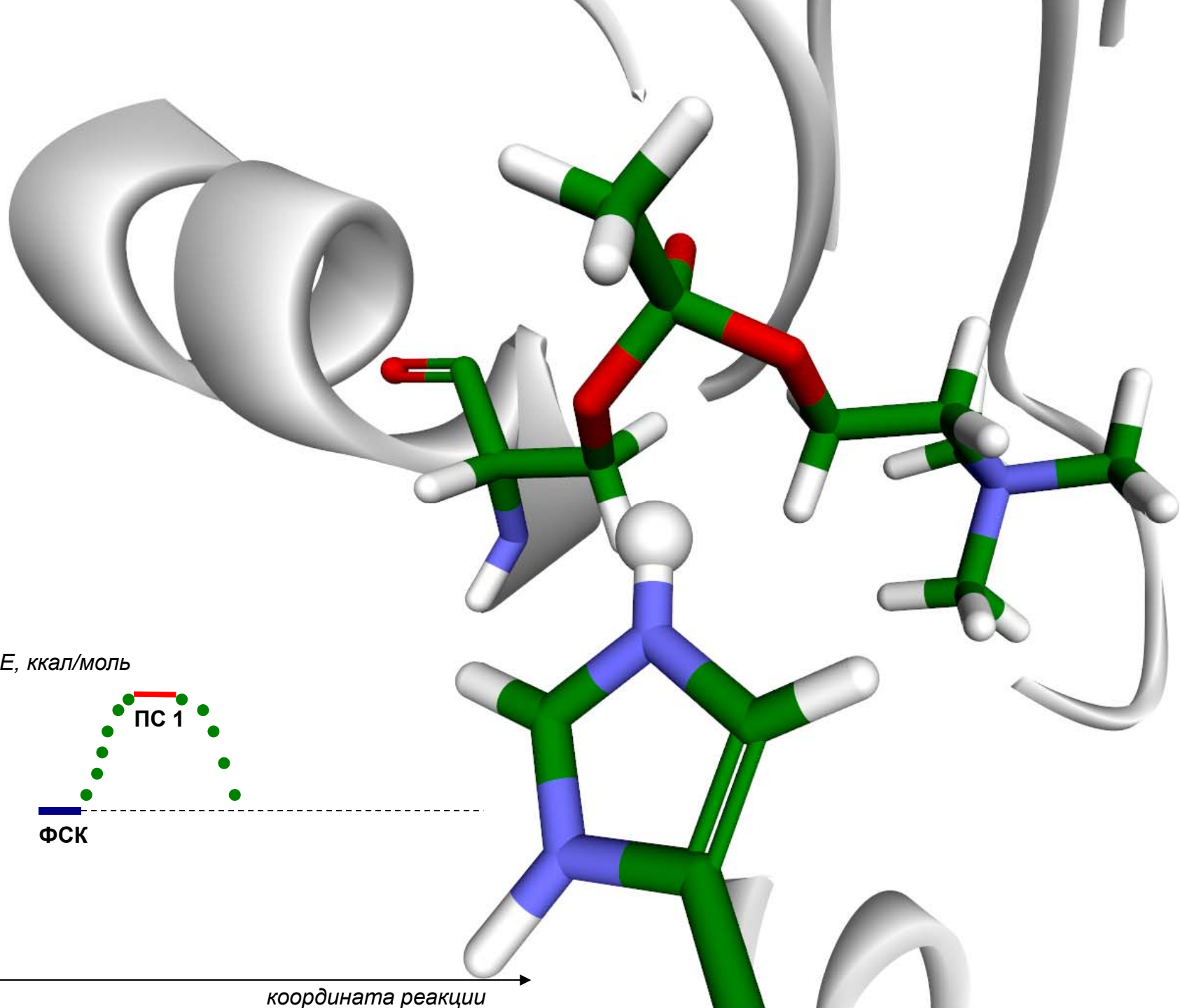


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ФСК

координата реакции

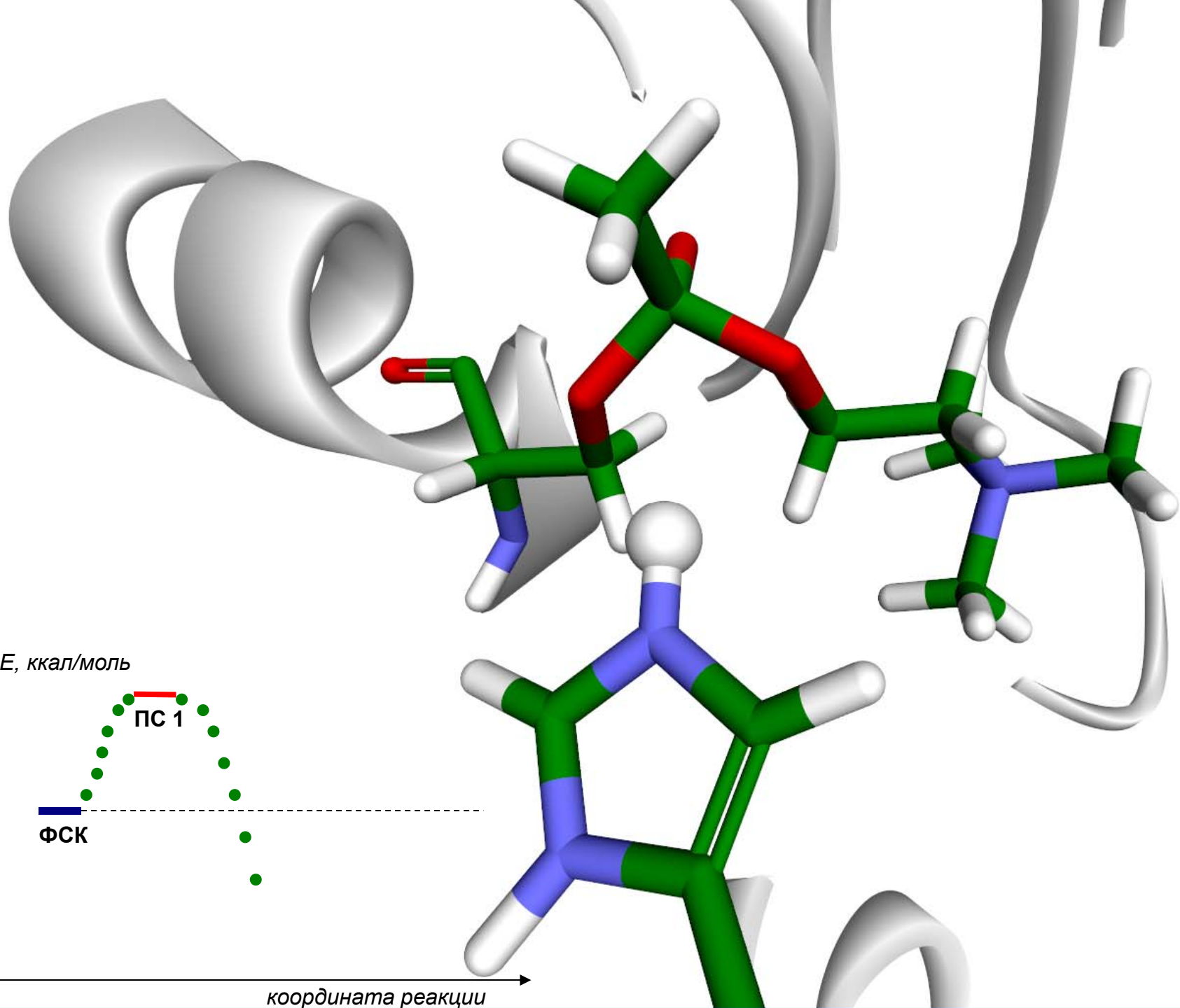


E , ккал/моль

ПС 1

ФСК

координата реакции

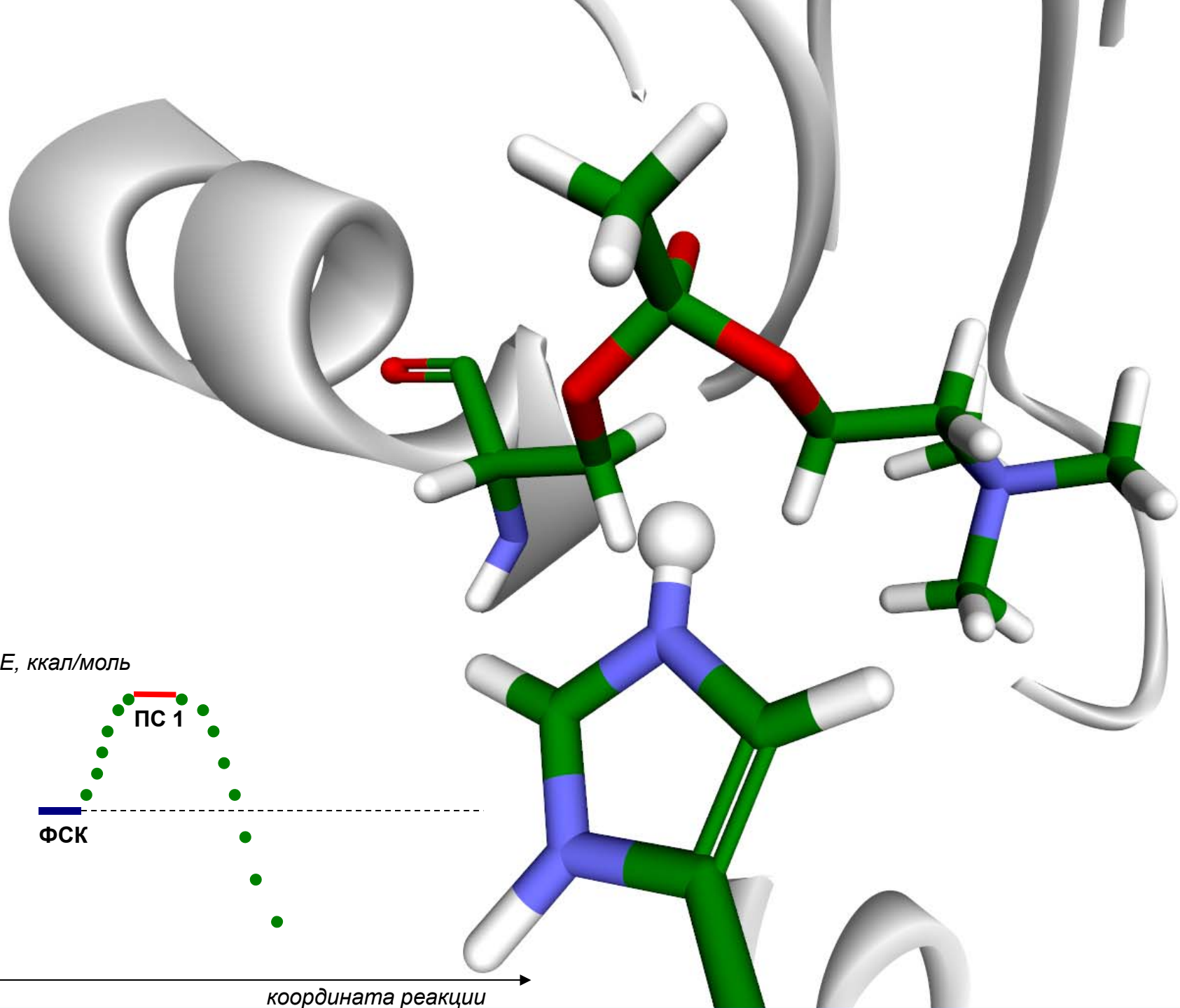


E , ккал/моль

ПС 1

ФСК

координата реакции

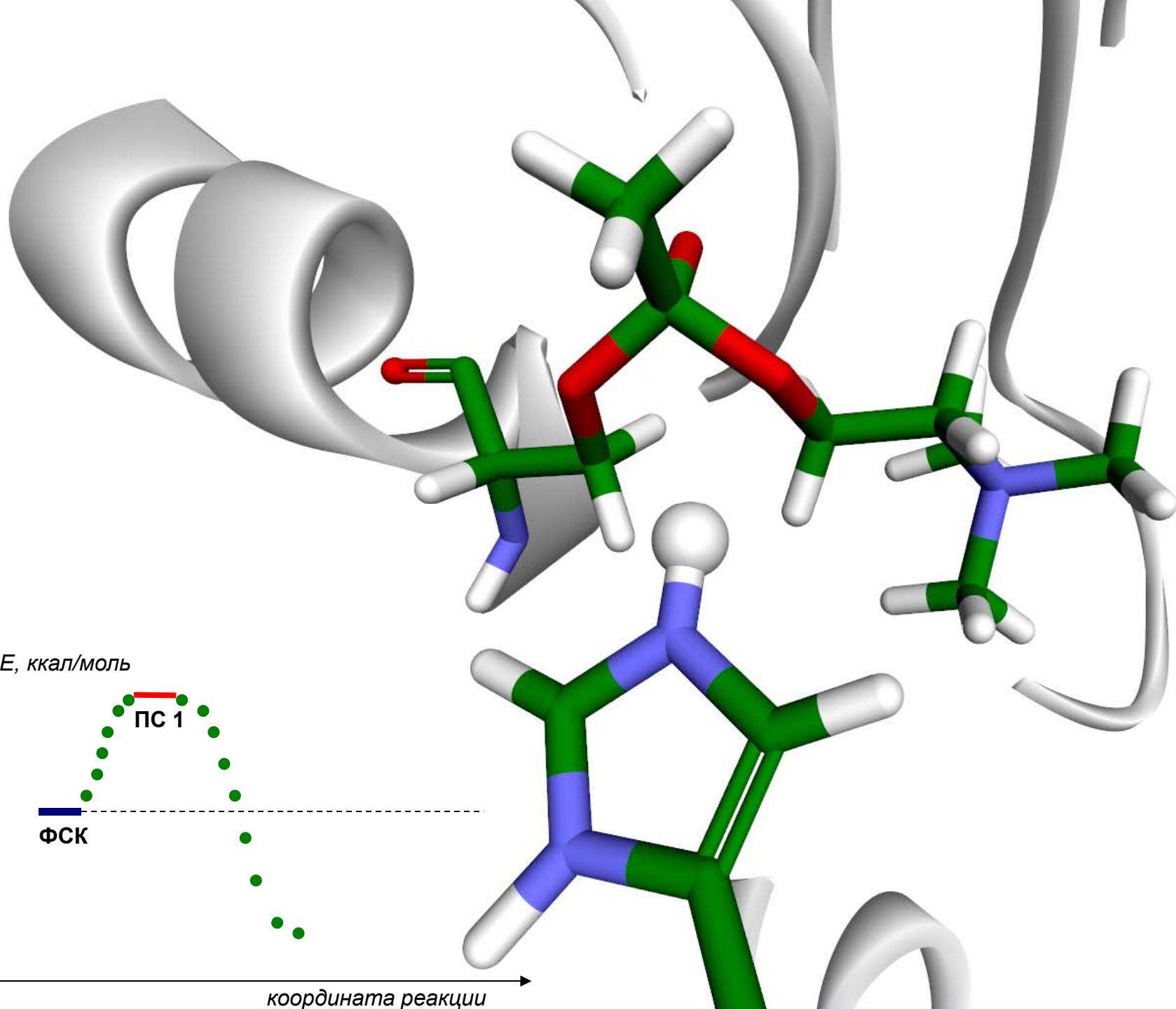


E , ккал/моль

ПС 1

ФСК

координата реакции

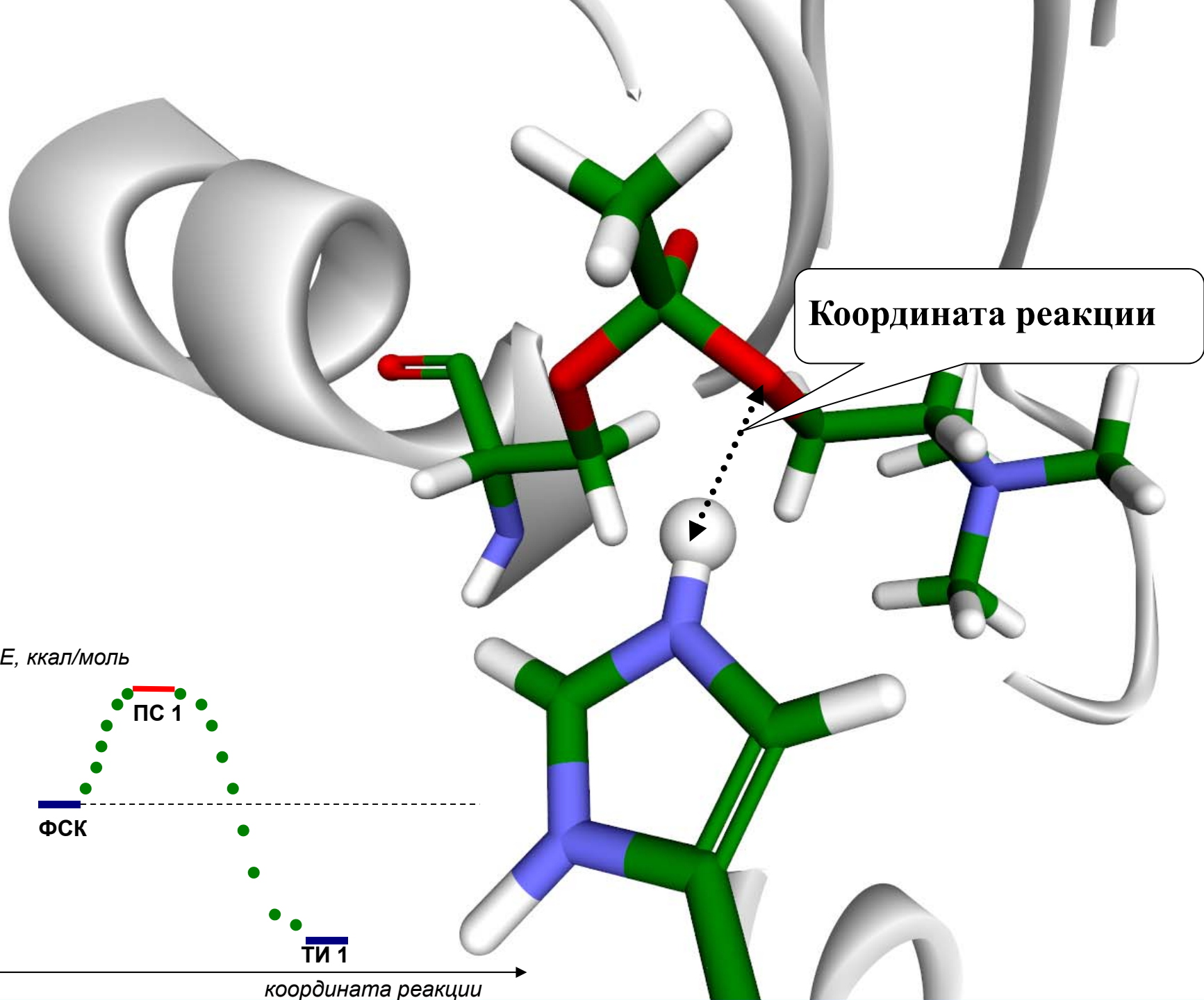


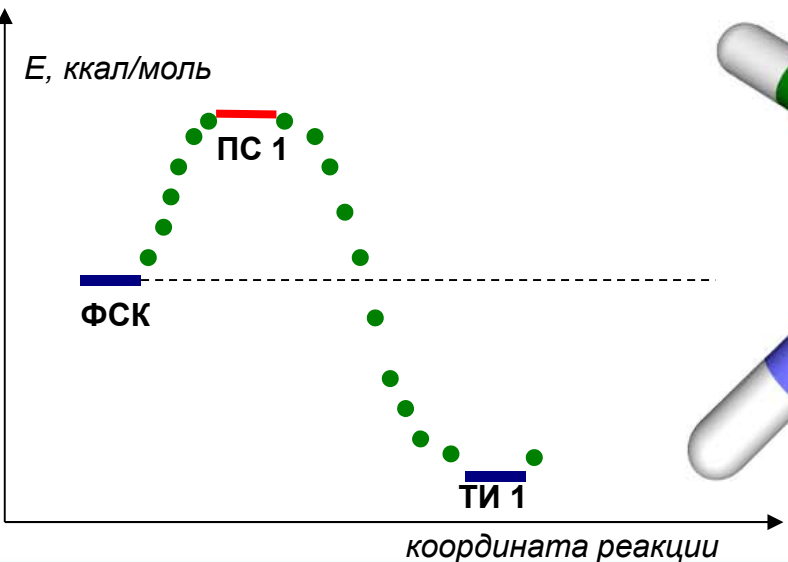
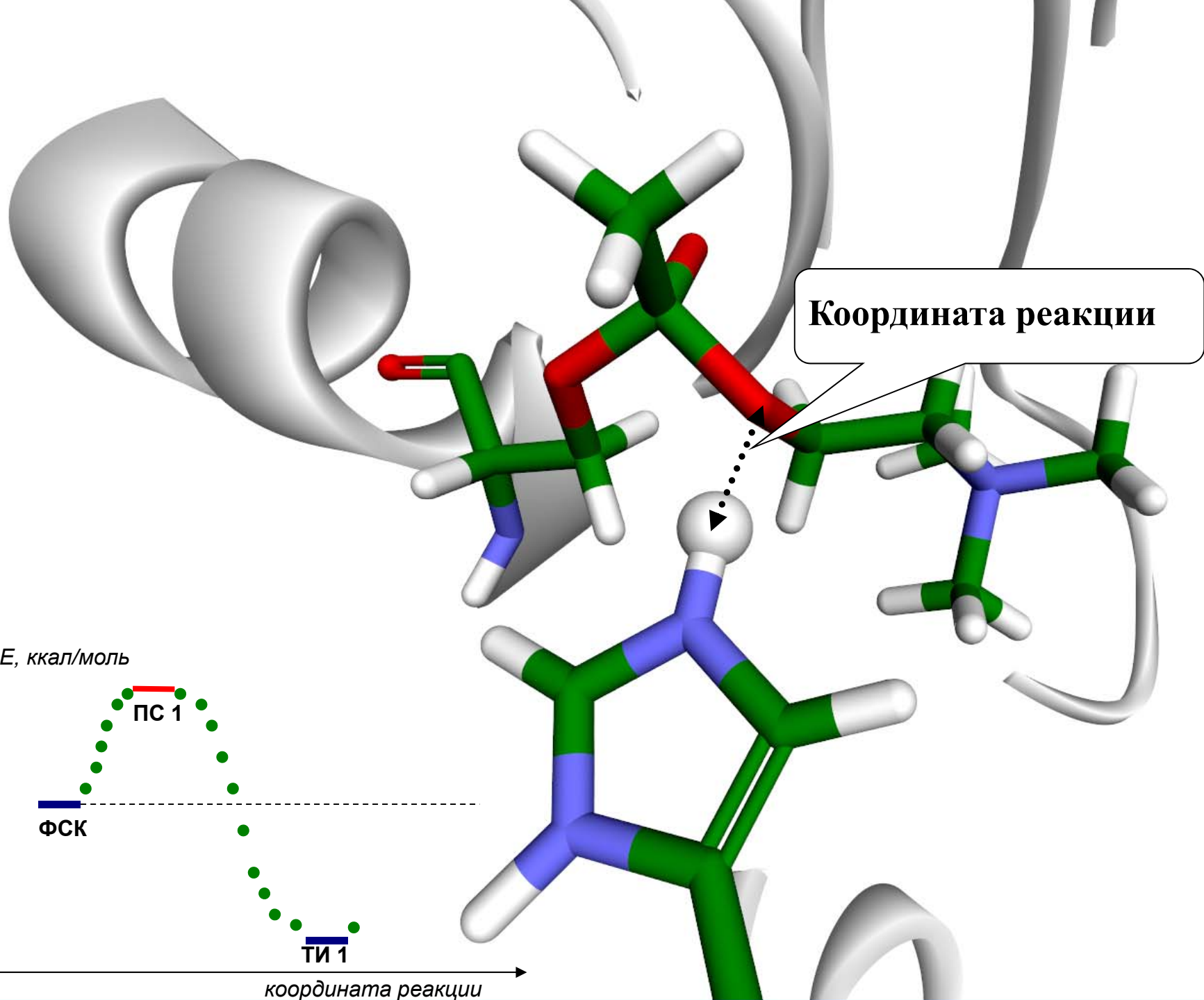
E , ккал/моль

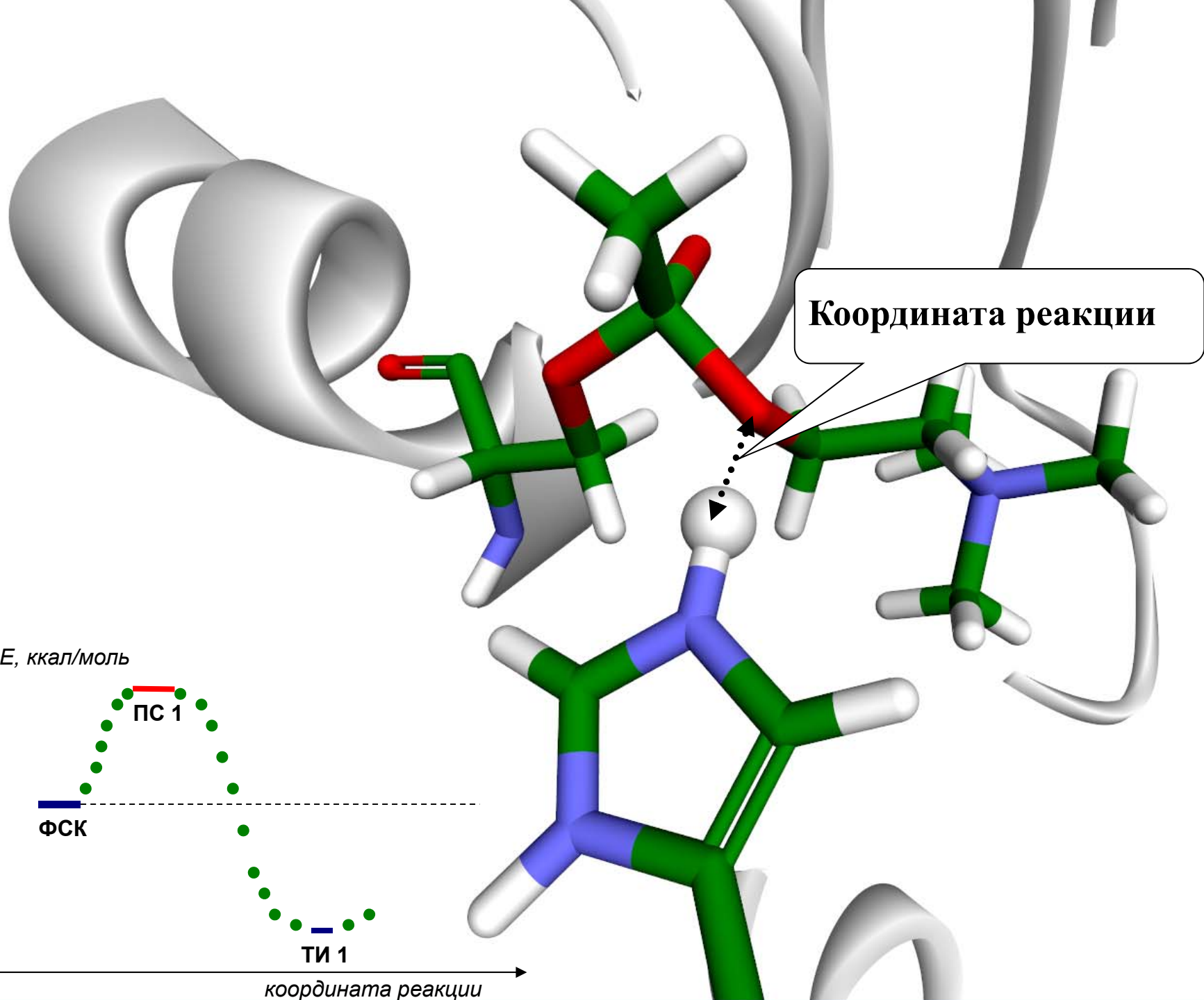
ПС 1

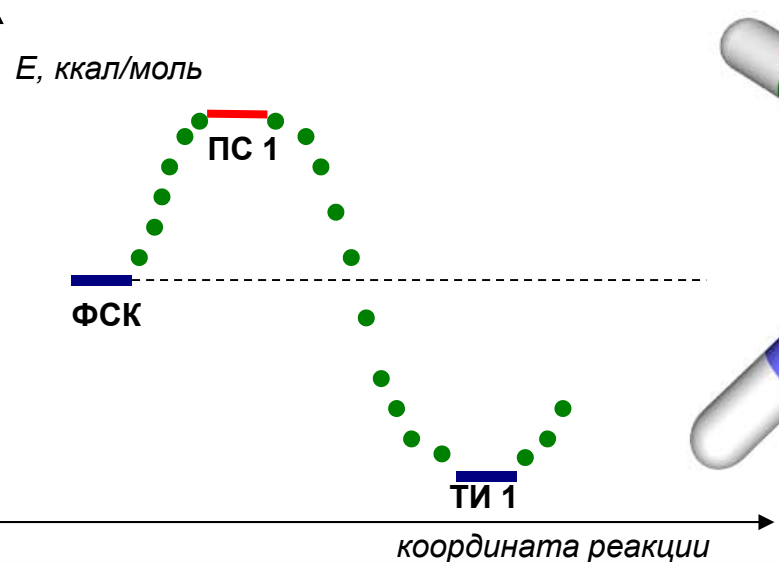
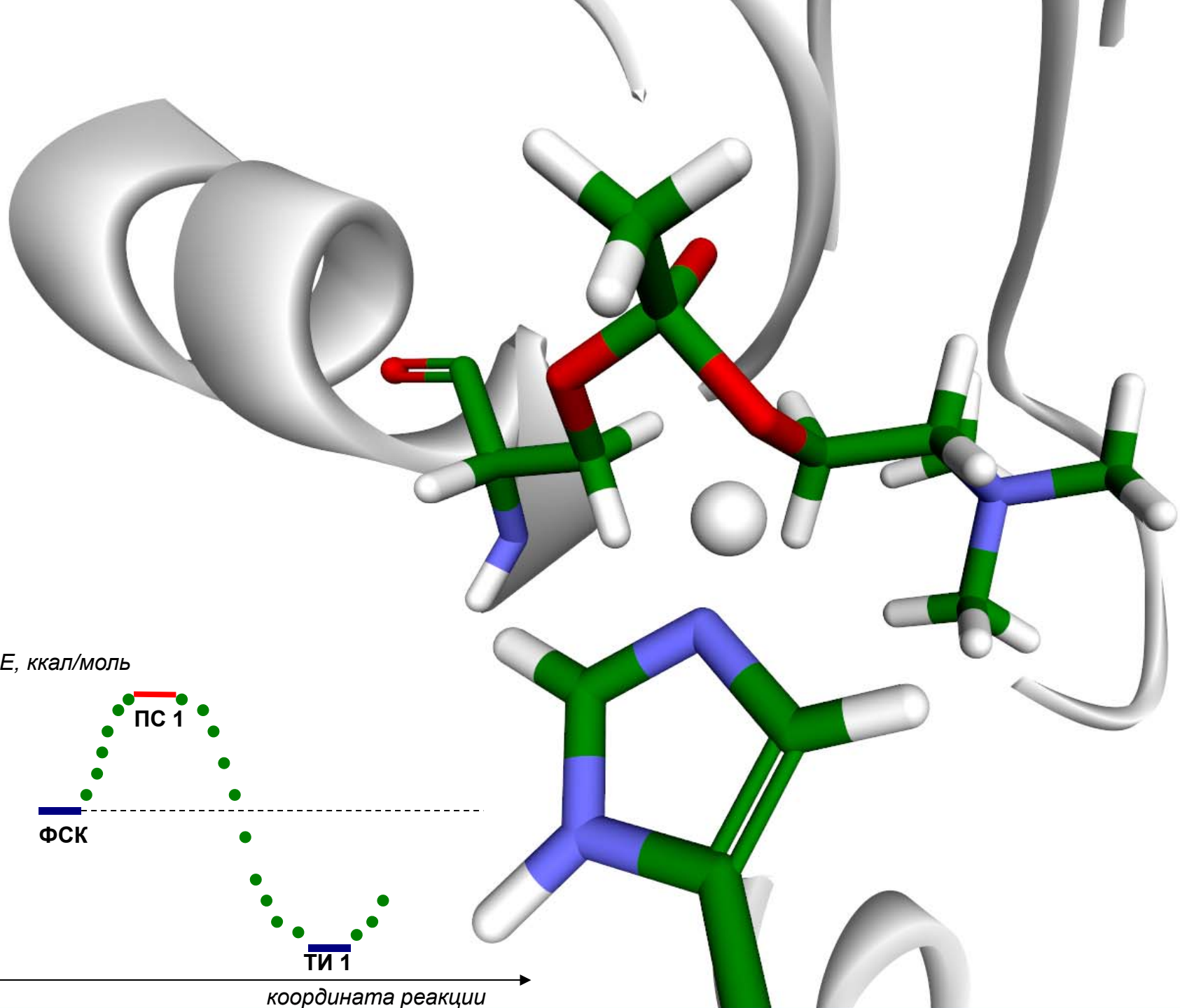
ФСК

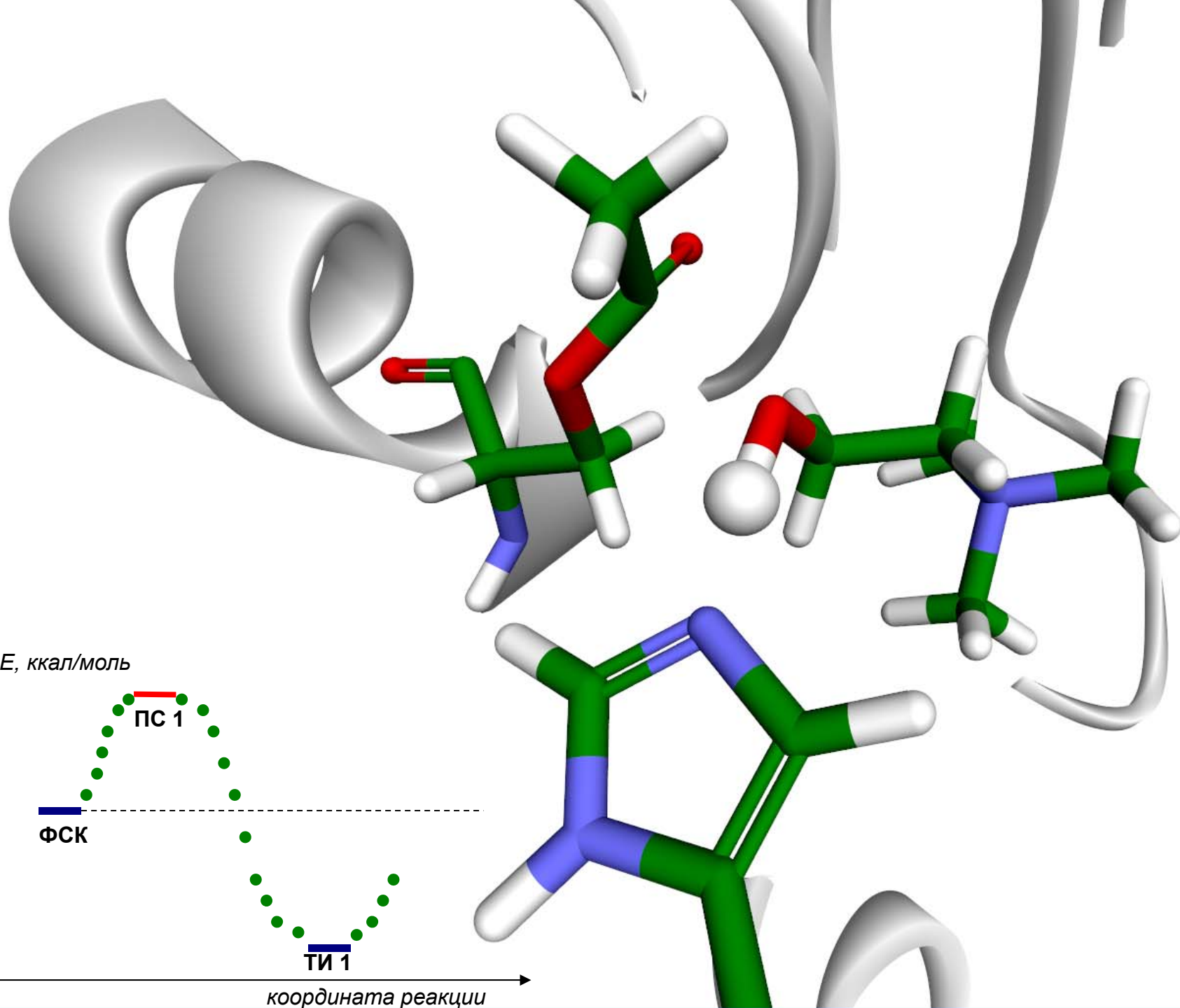
координата реакции











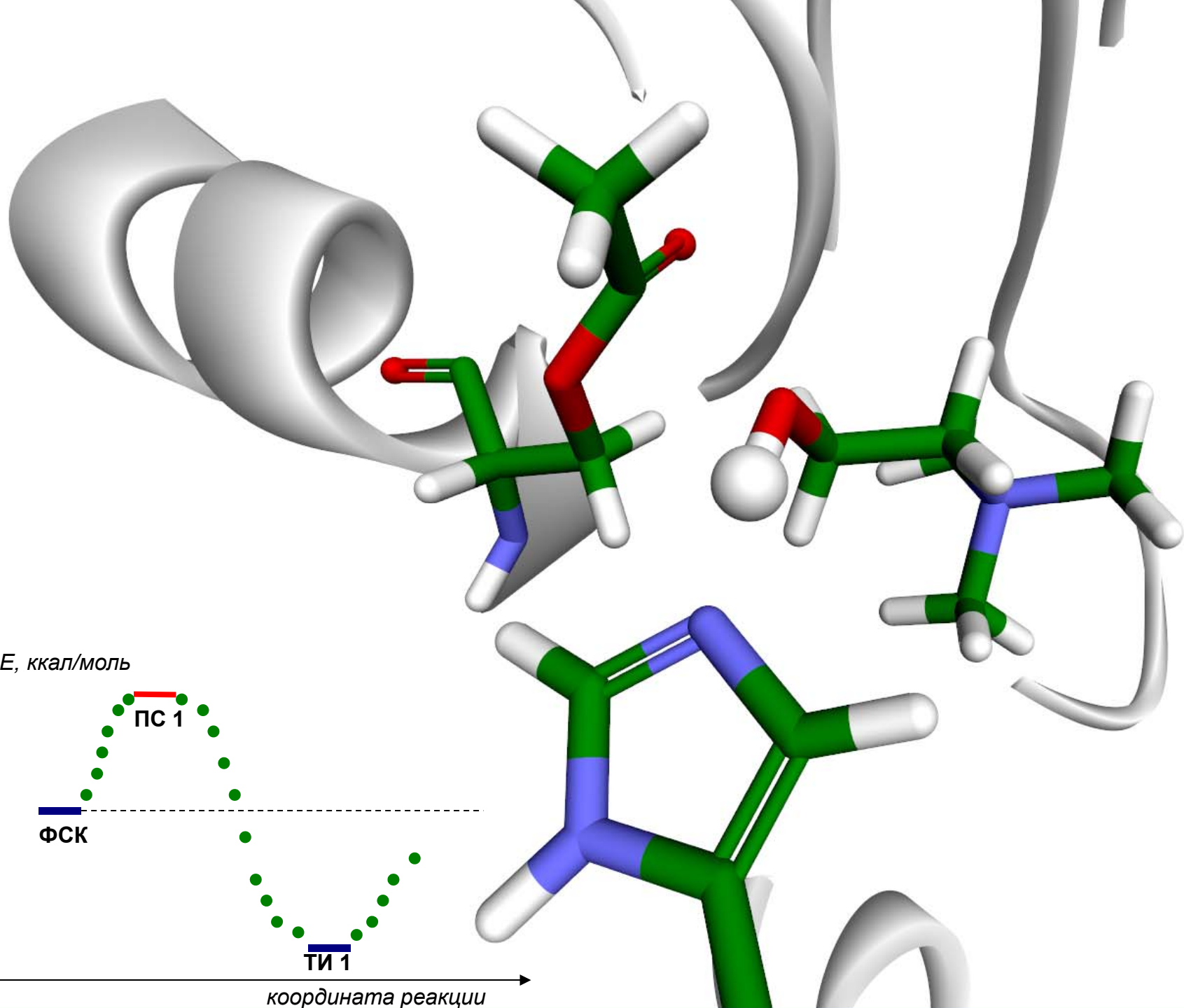
E , ккал/моль

ПС 1

ФСК

ТИ 1

координата реакции



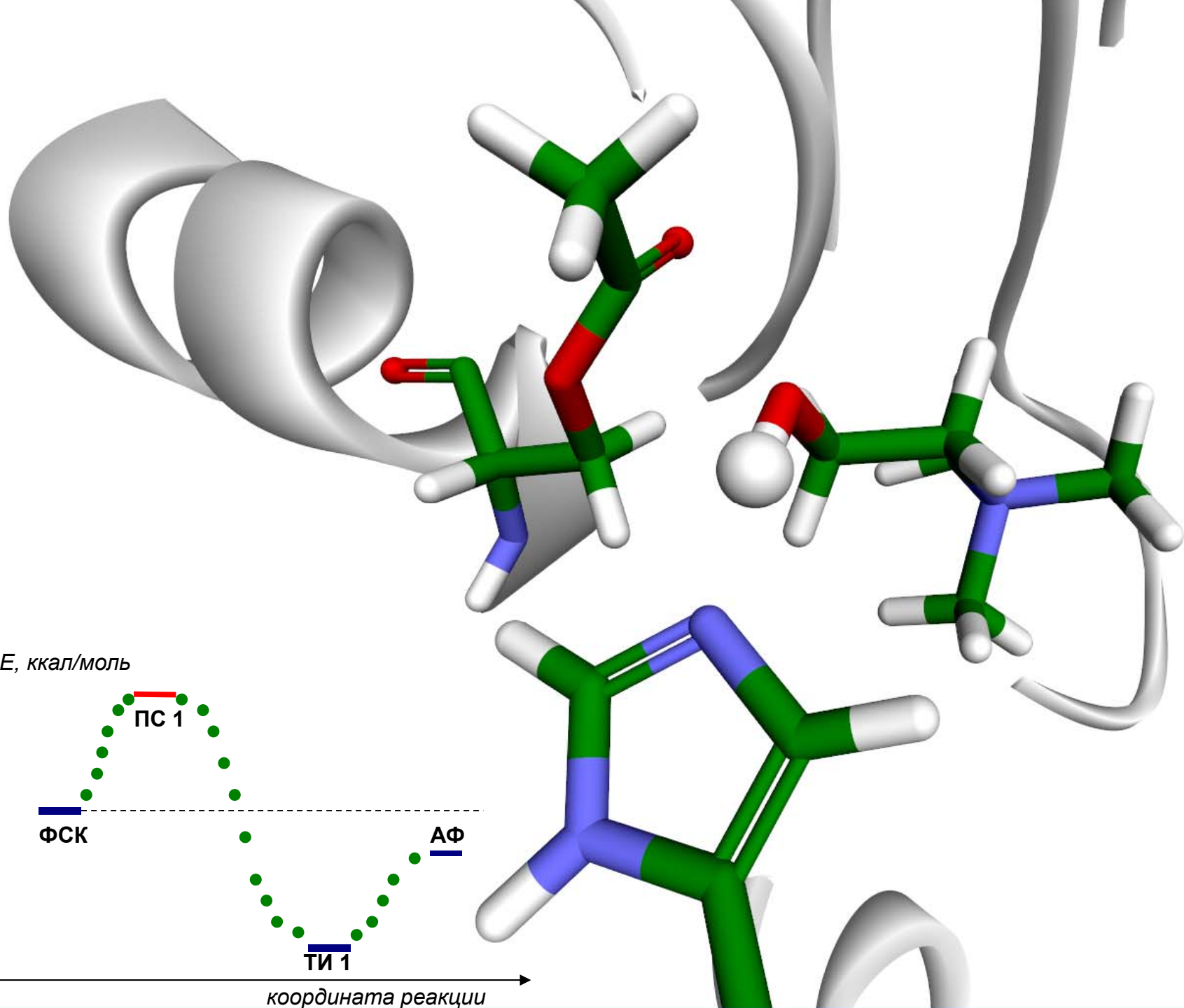
E , ккал/моль

ПС 1

ФСК

ТИ 1

координата реакции



E , ккал/моль

ПС 1

ФСК

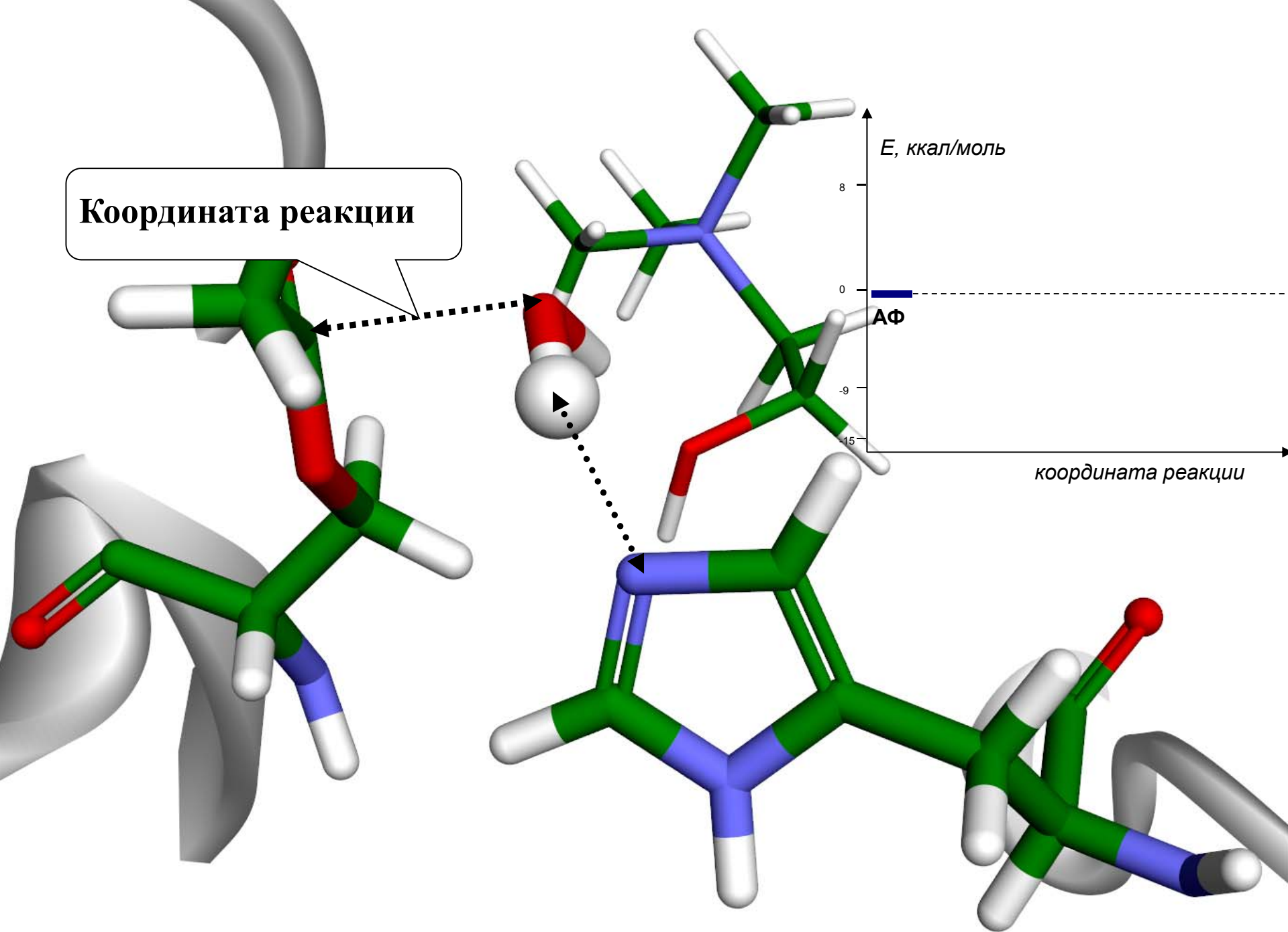
АФ

ТИ 1

координата реакции

Стадия деацилирования

Координата реакции



E , ккал/моль

8

0

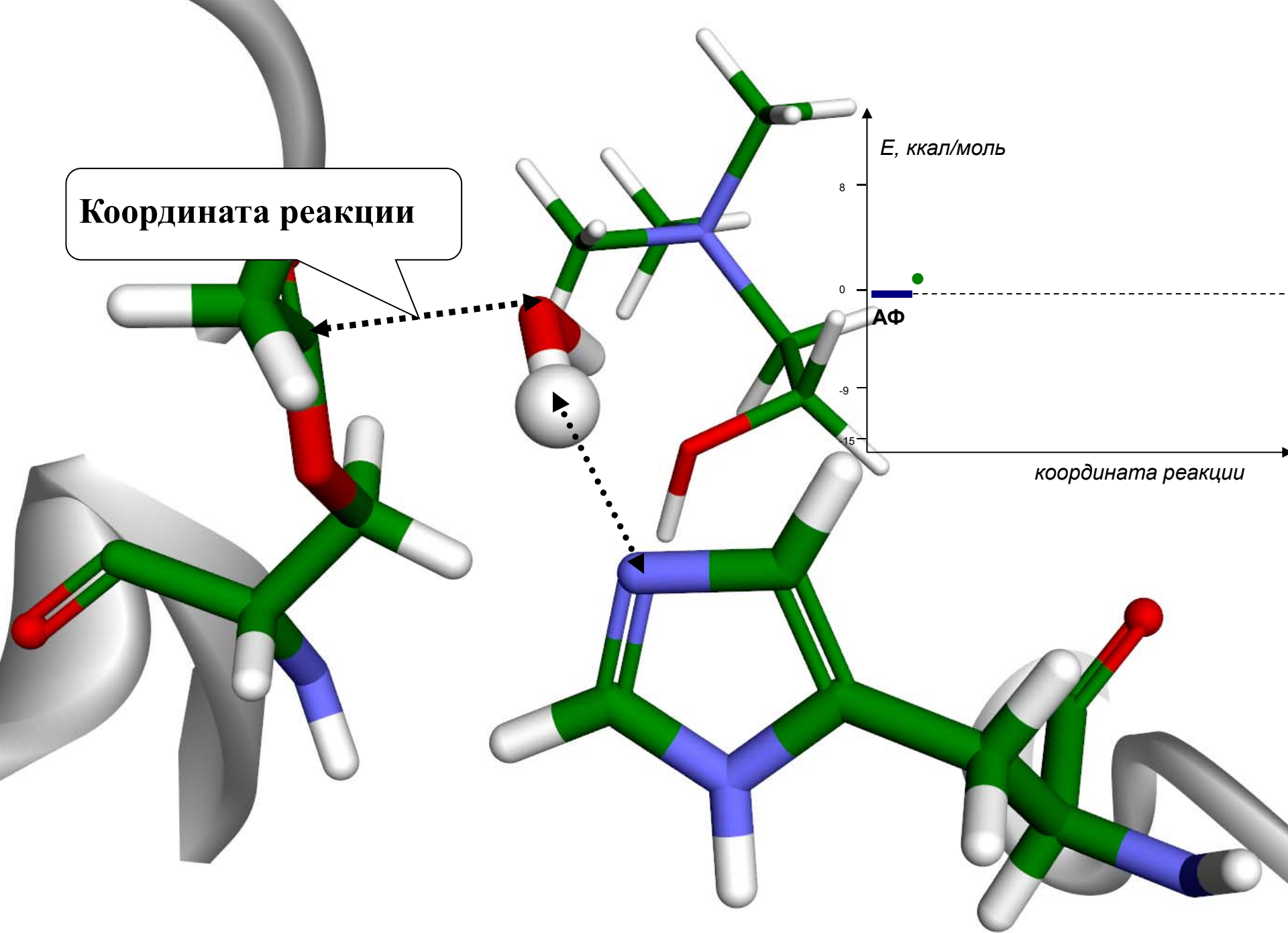
АФ

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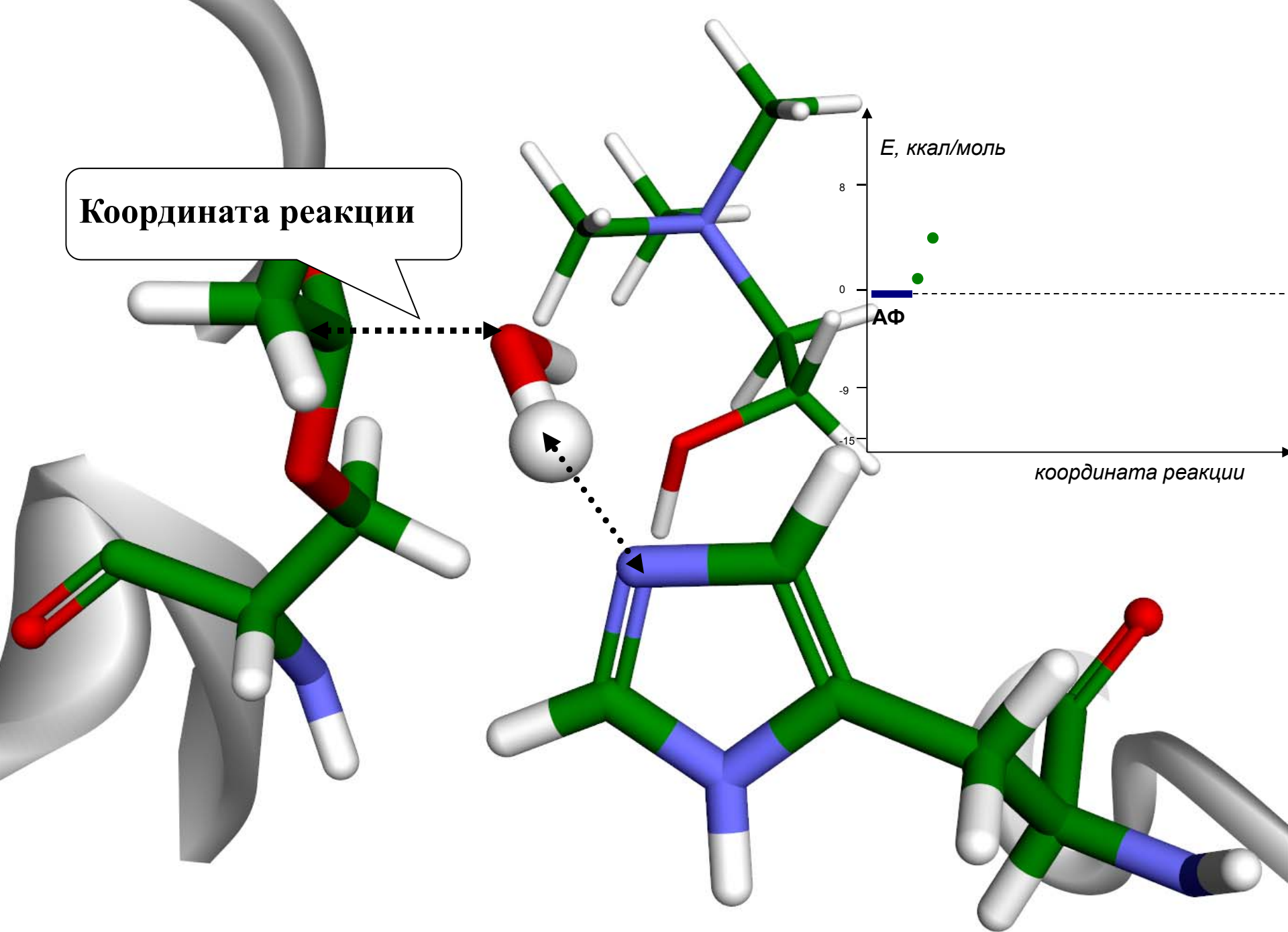
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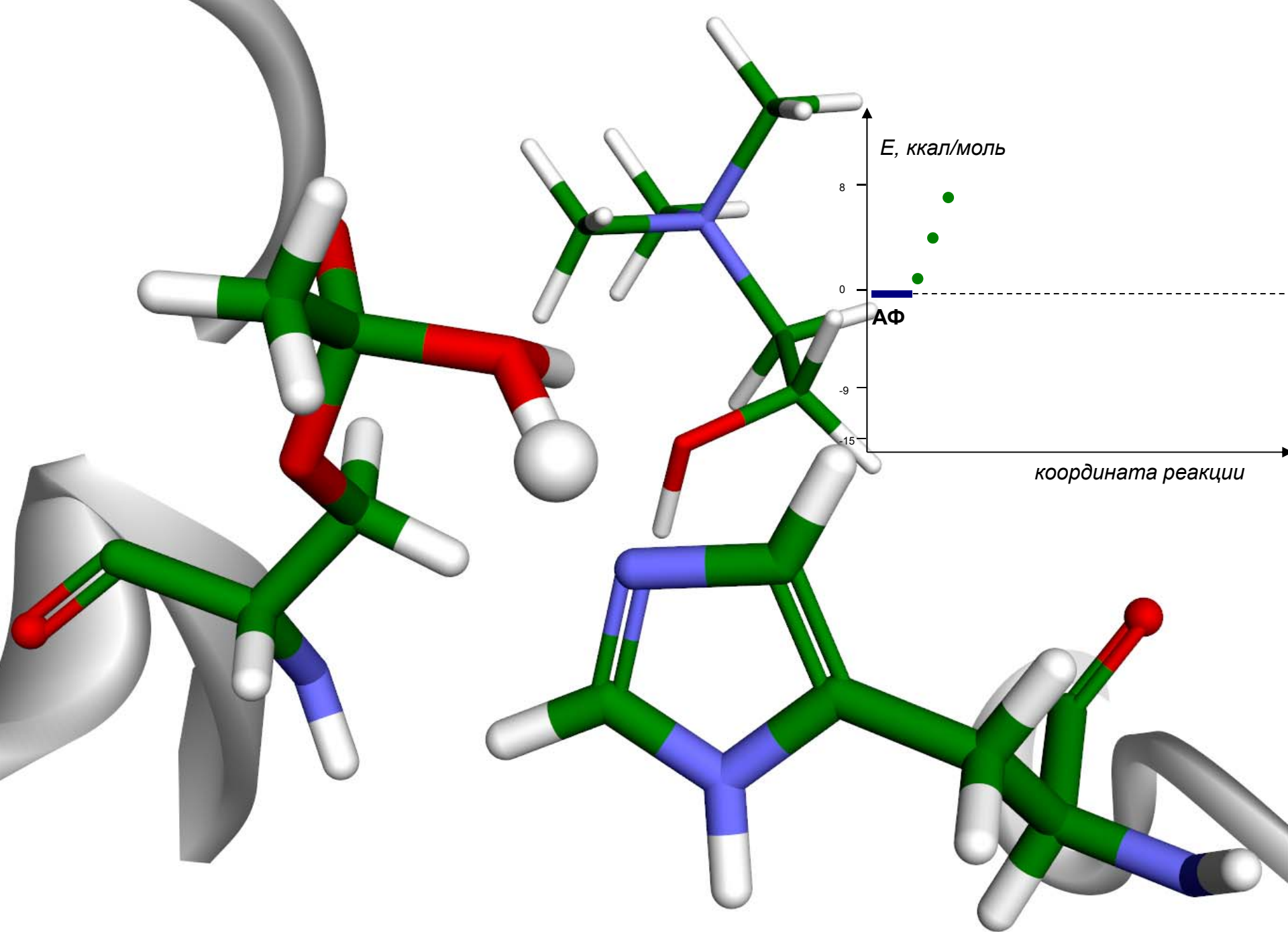
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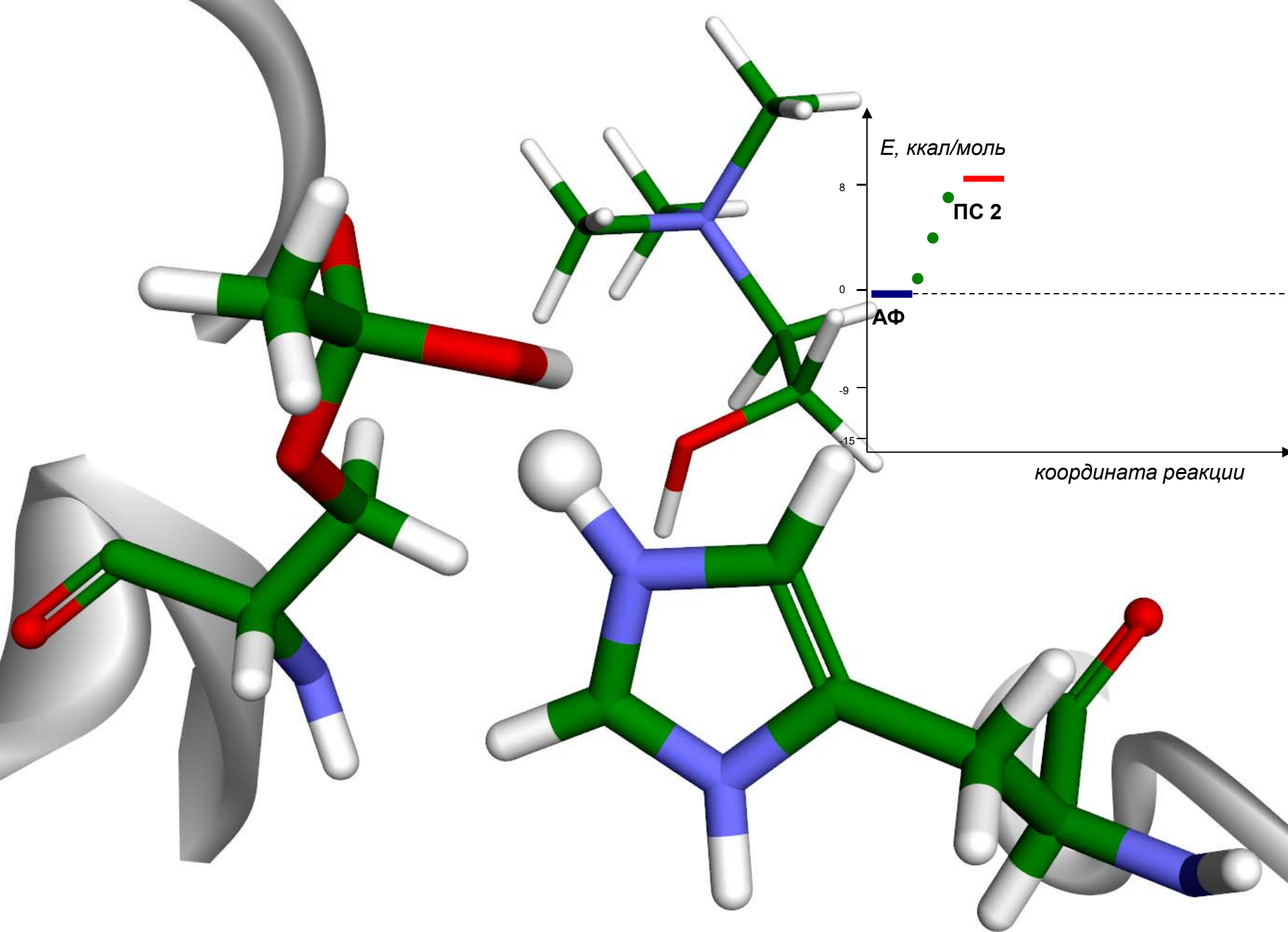
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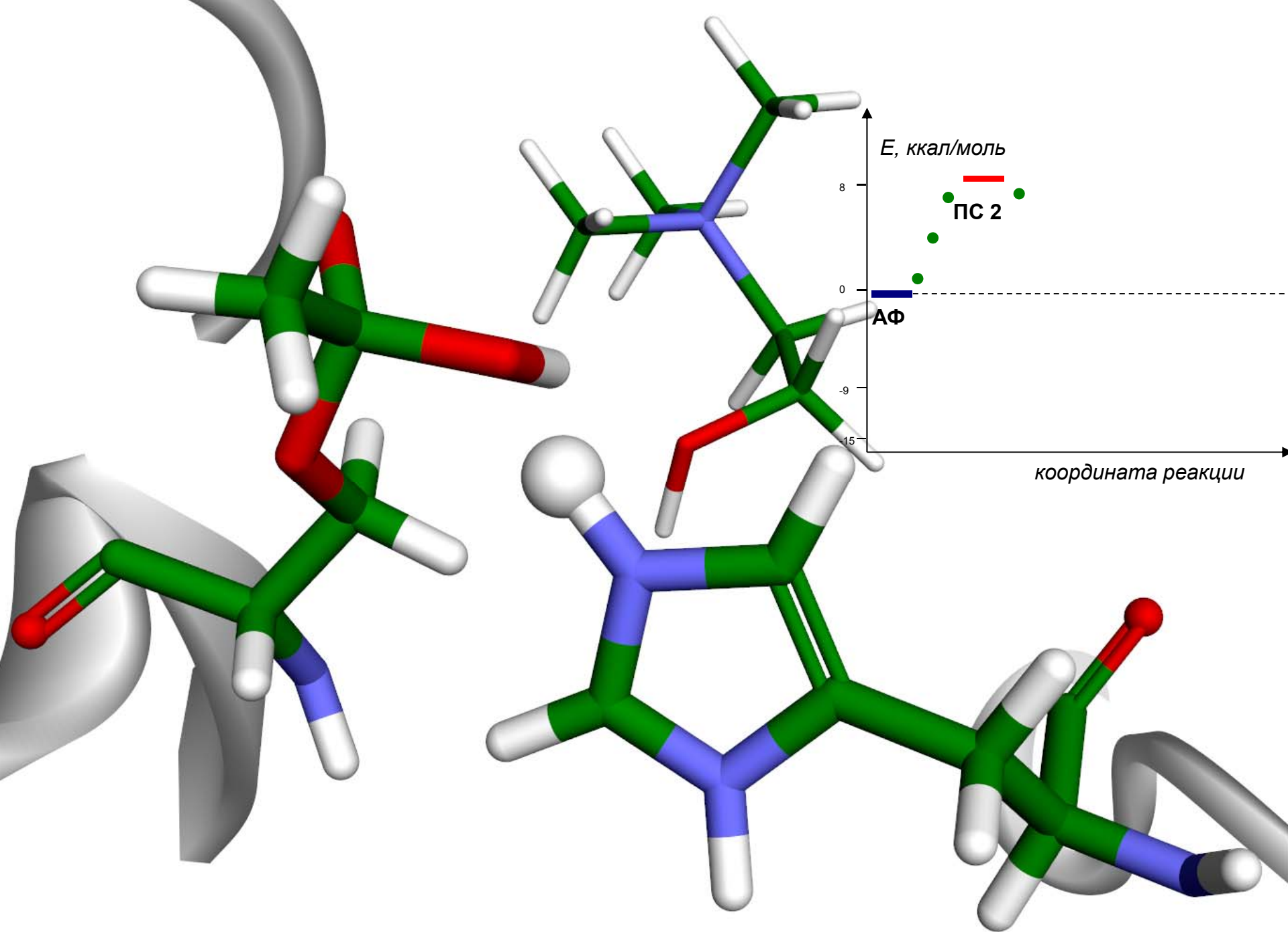


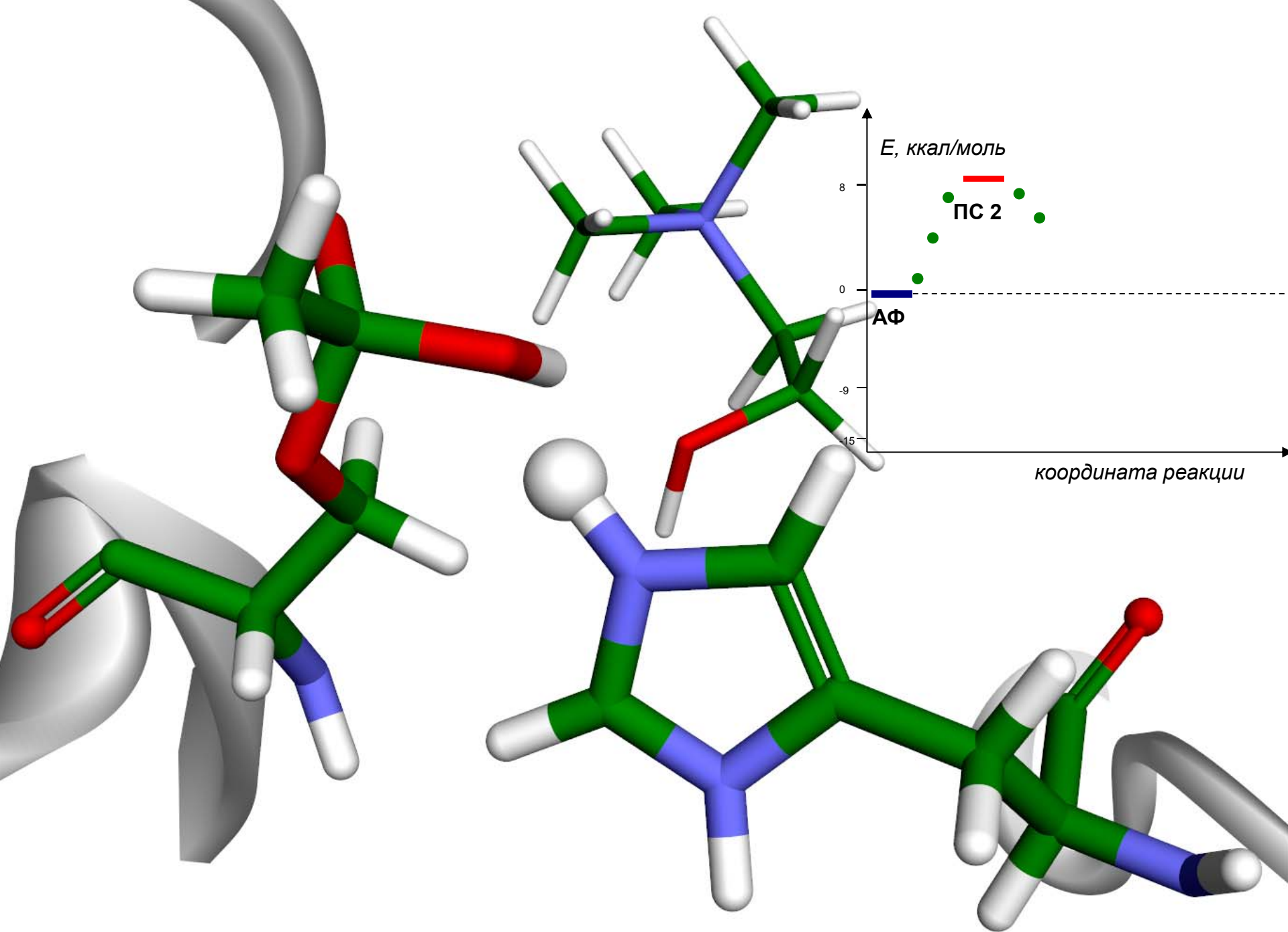
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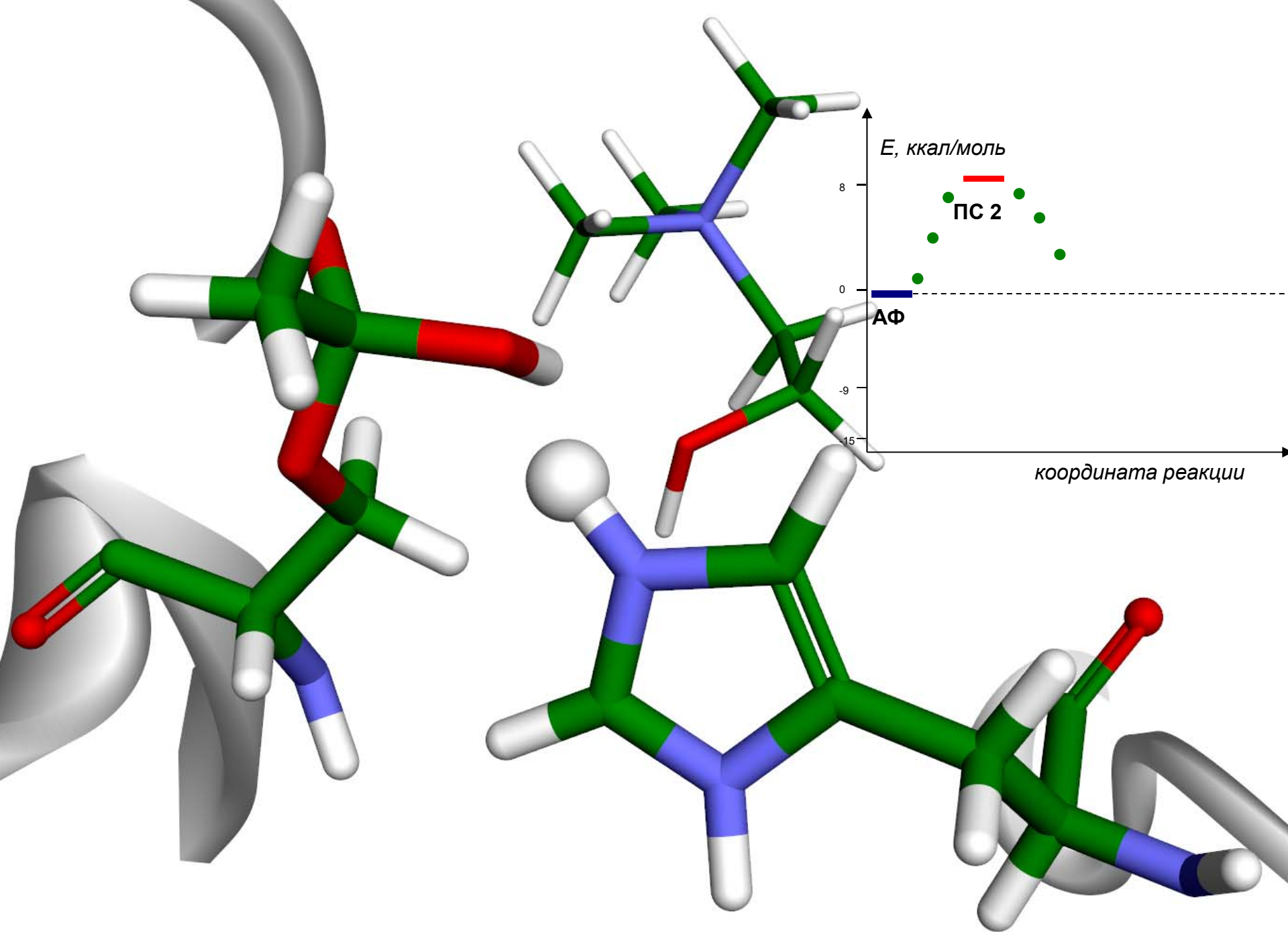


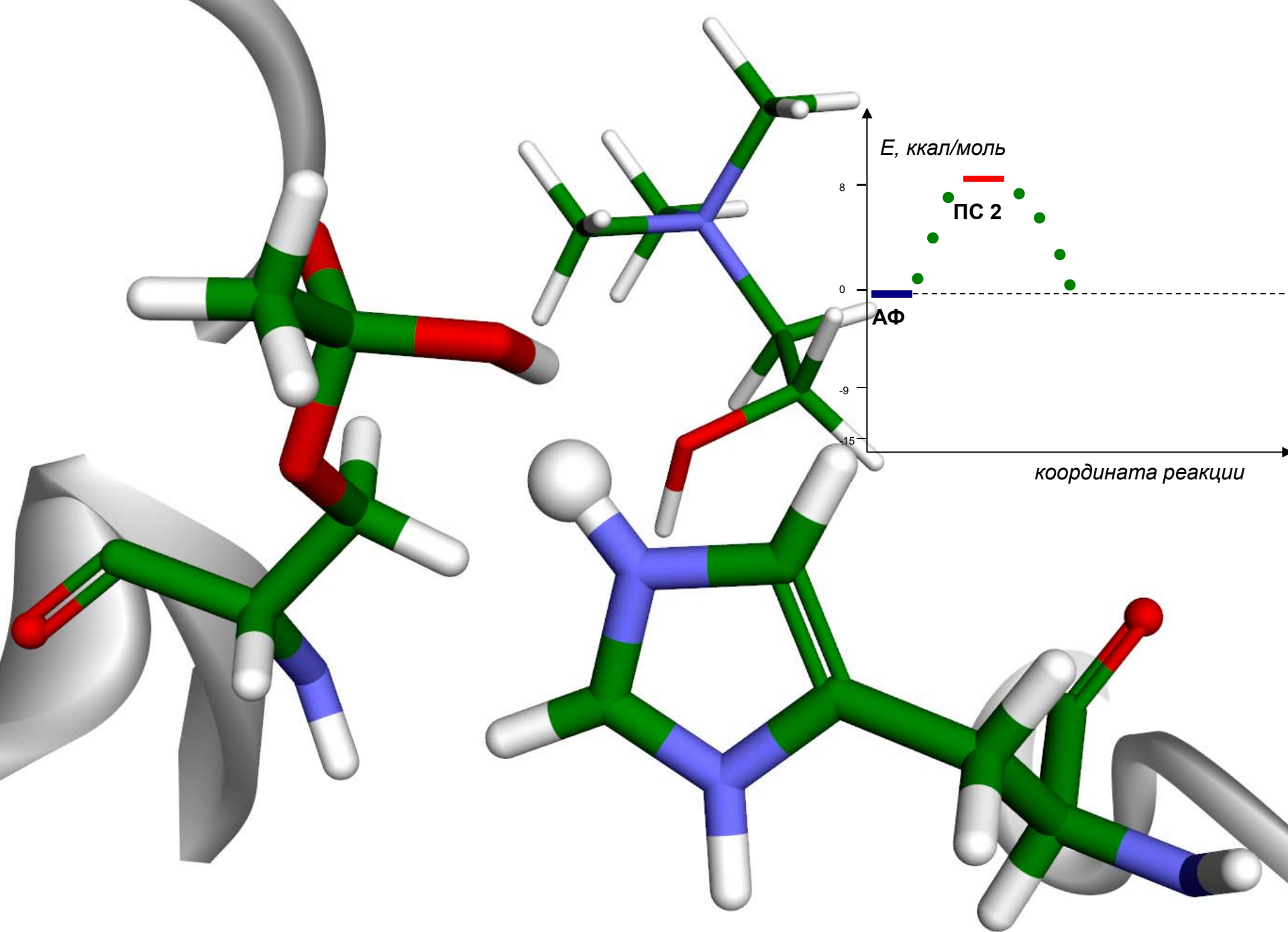










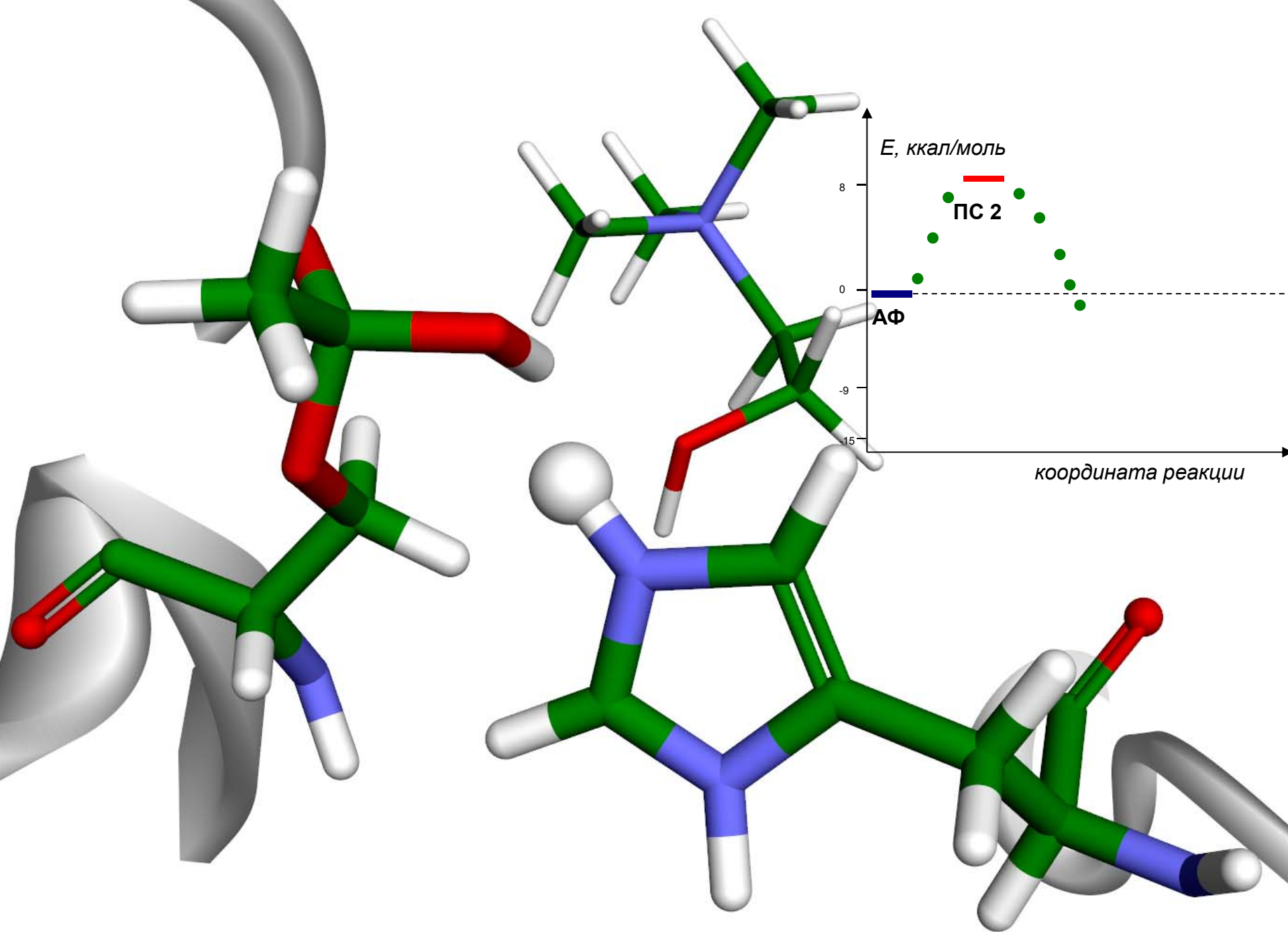


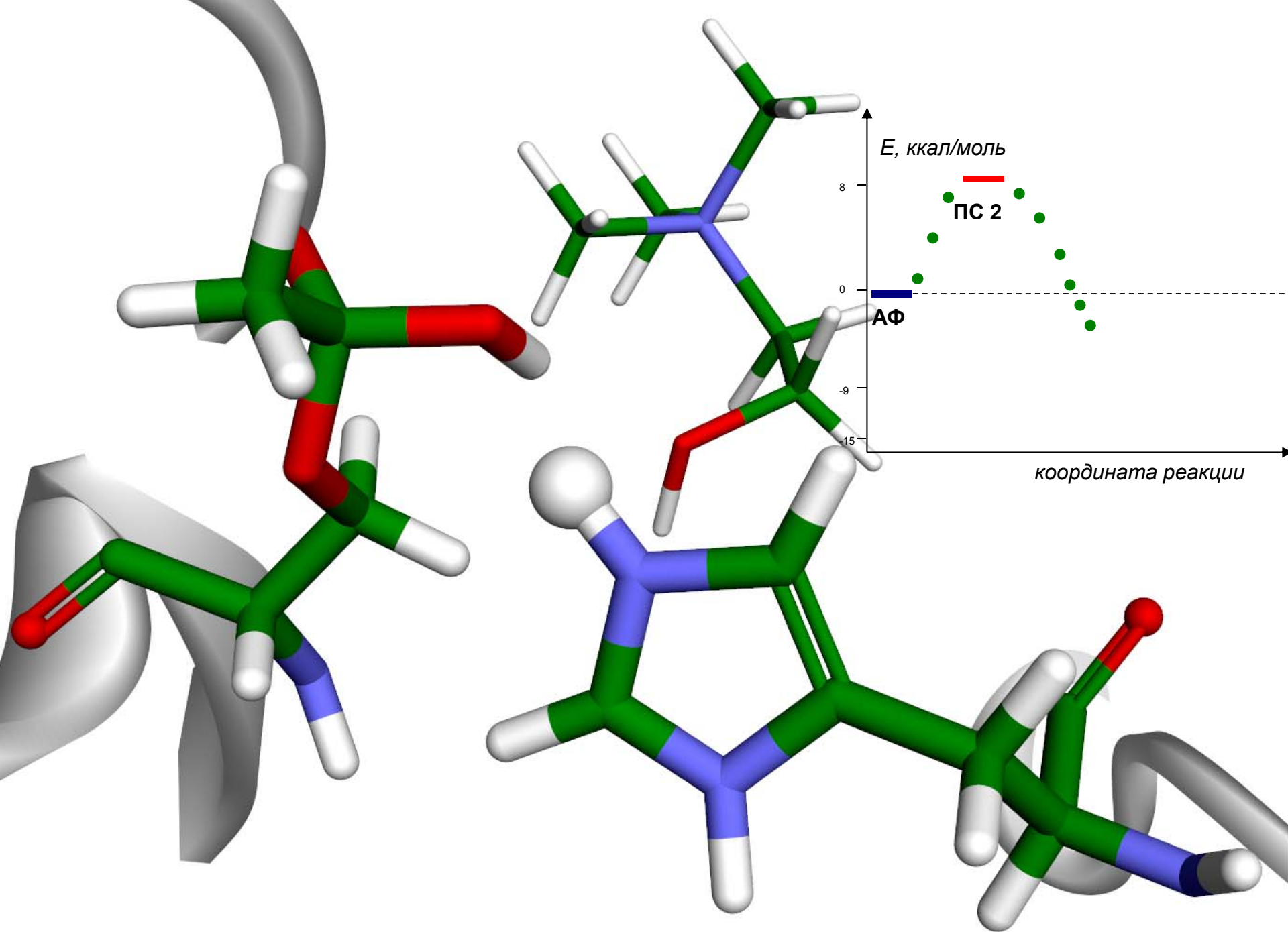
E , ккал/моль

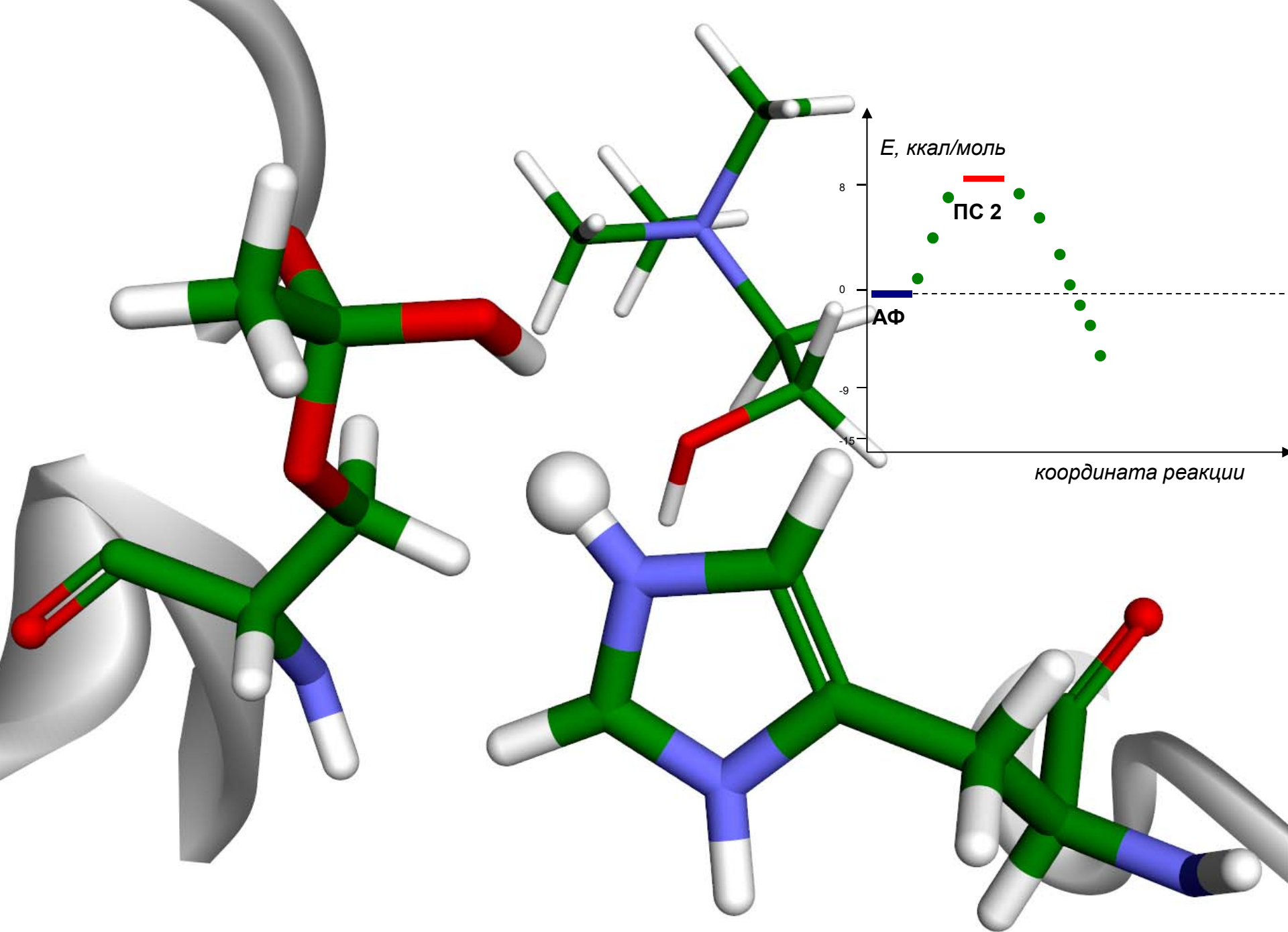
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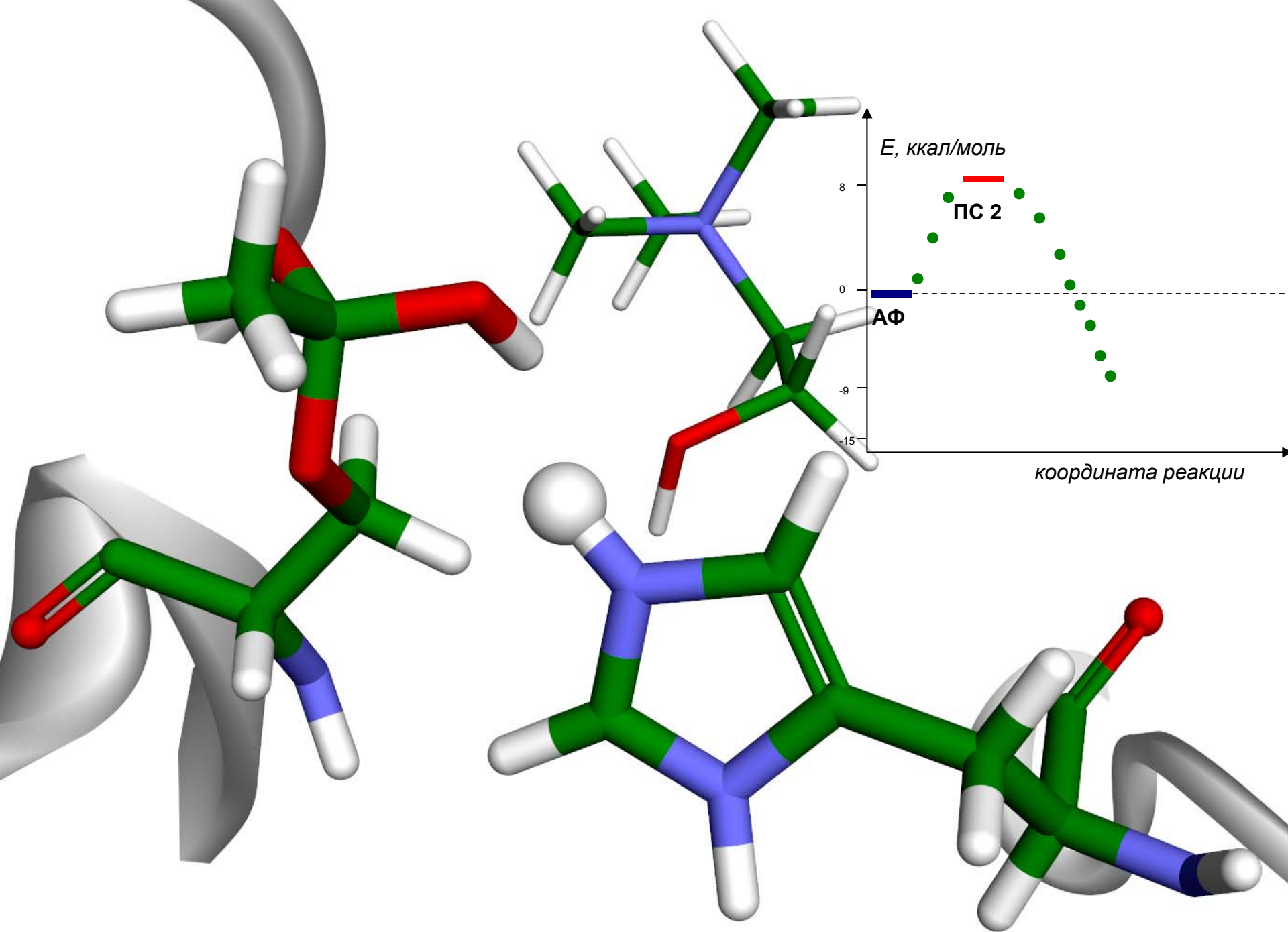
АФ

координата реакции







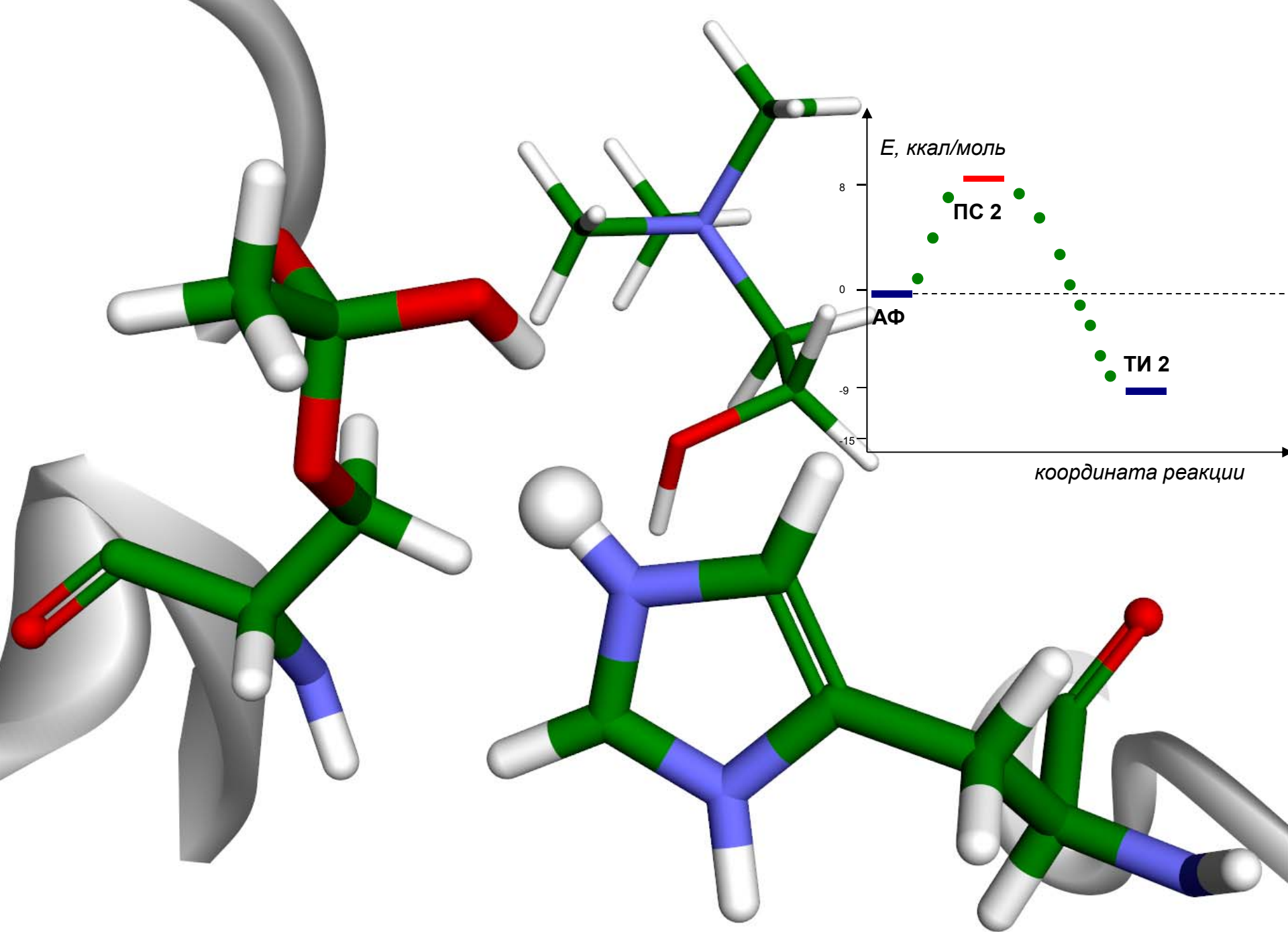


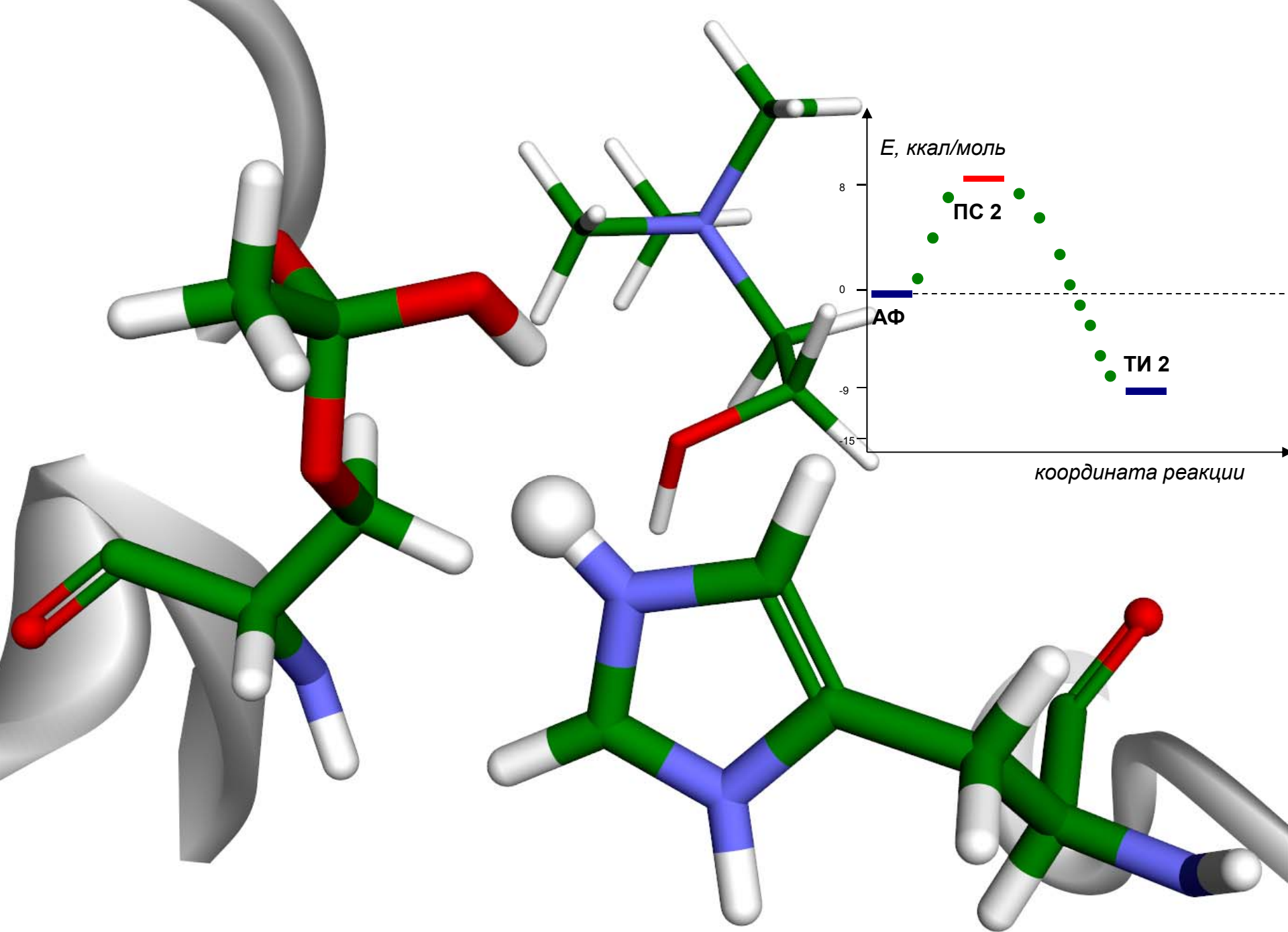
E , ккал/моль

ПС 2

АФ

координата реакции





E , ккал/моль

8

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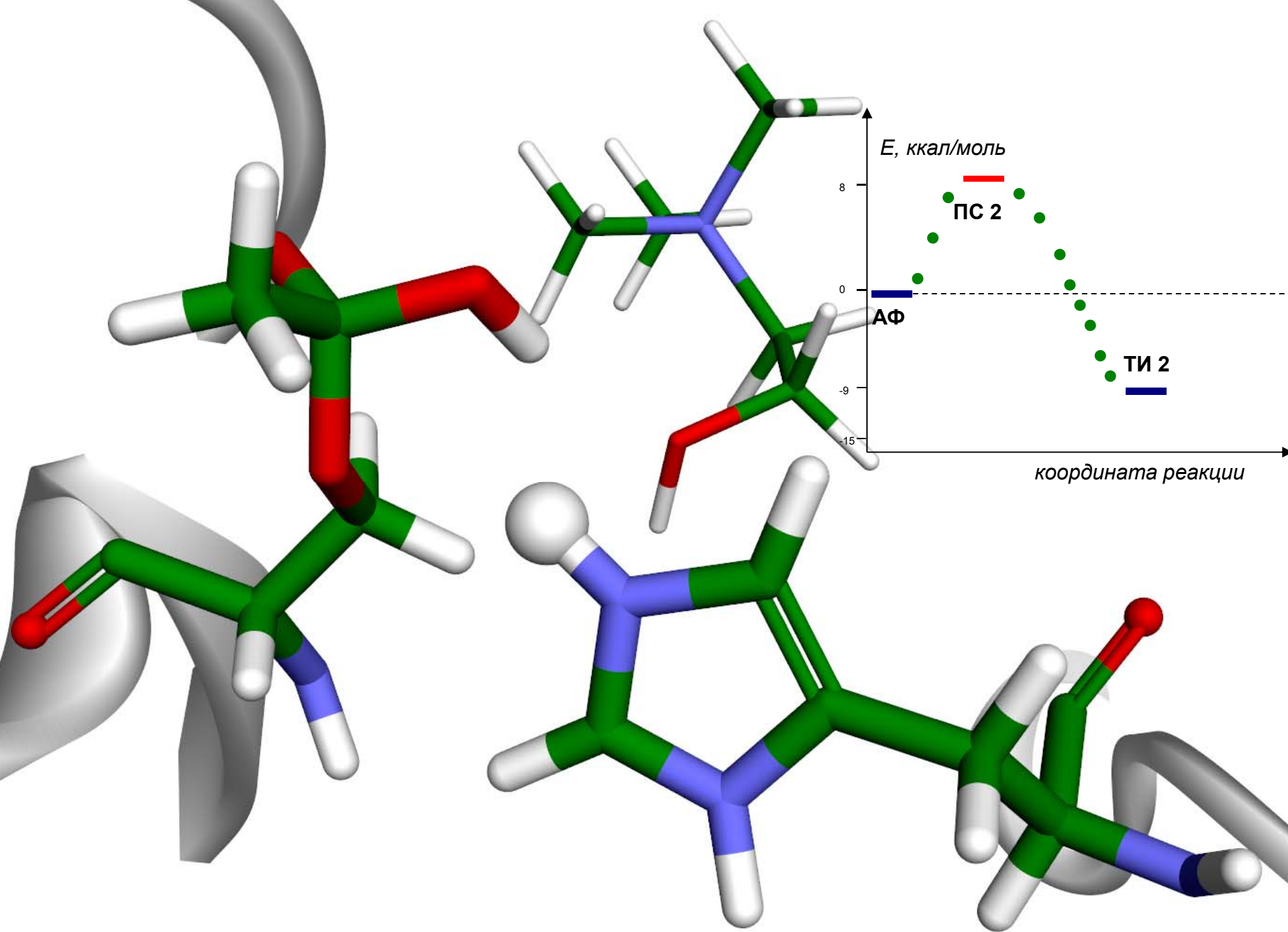
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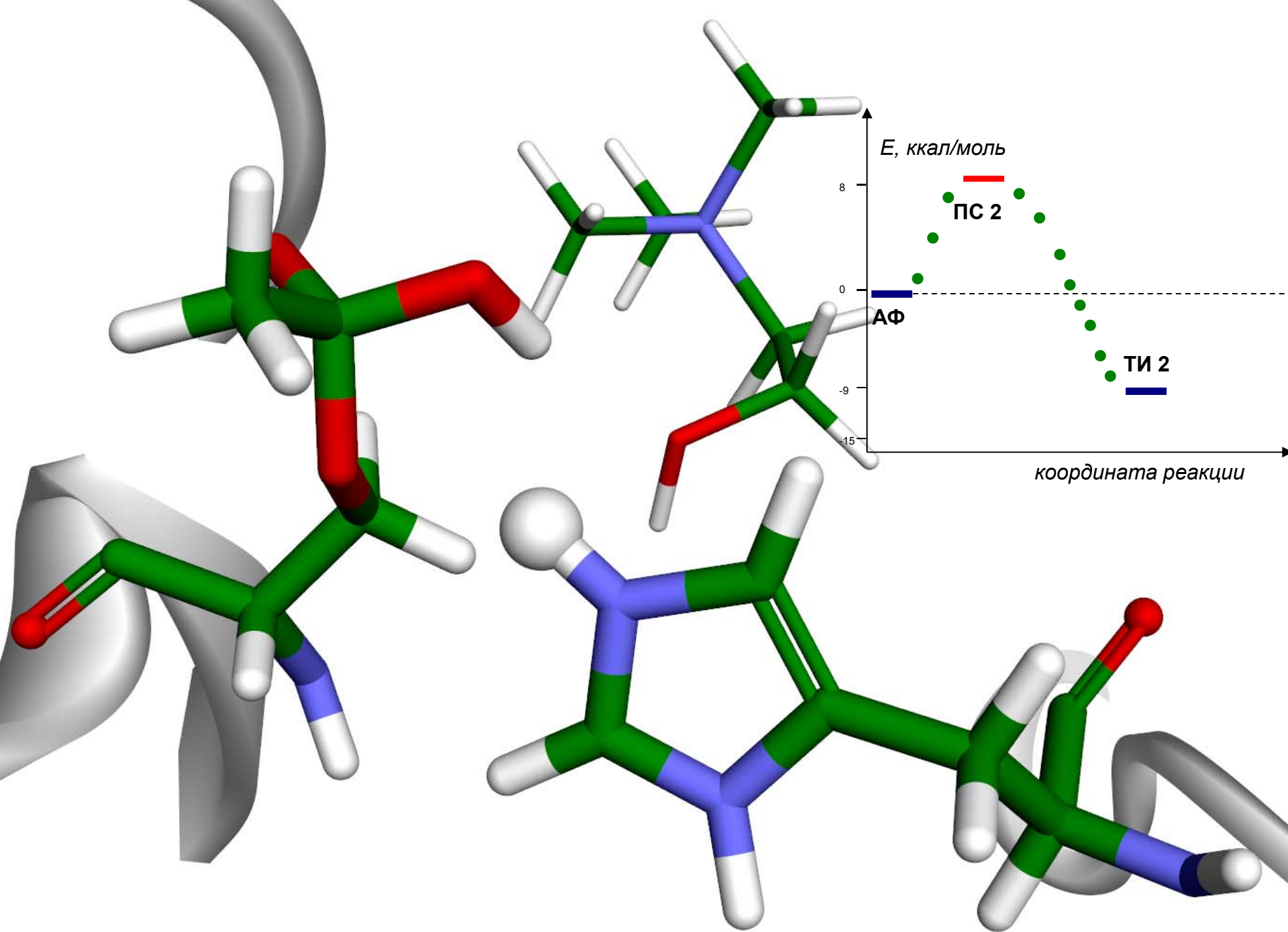
ТС 2

АФ

ТИ 2

координата реакции





E , ккал/моль

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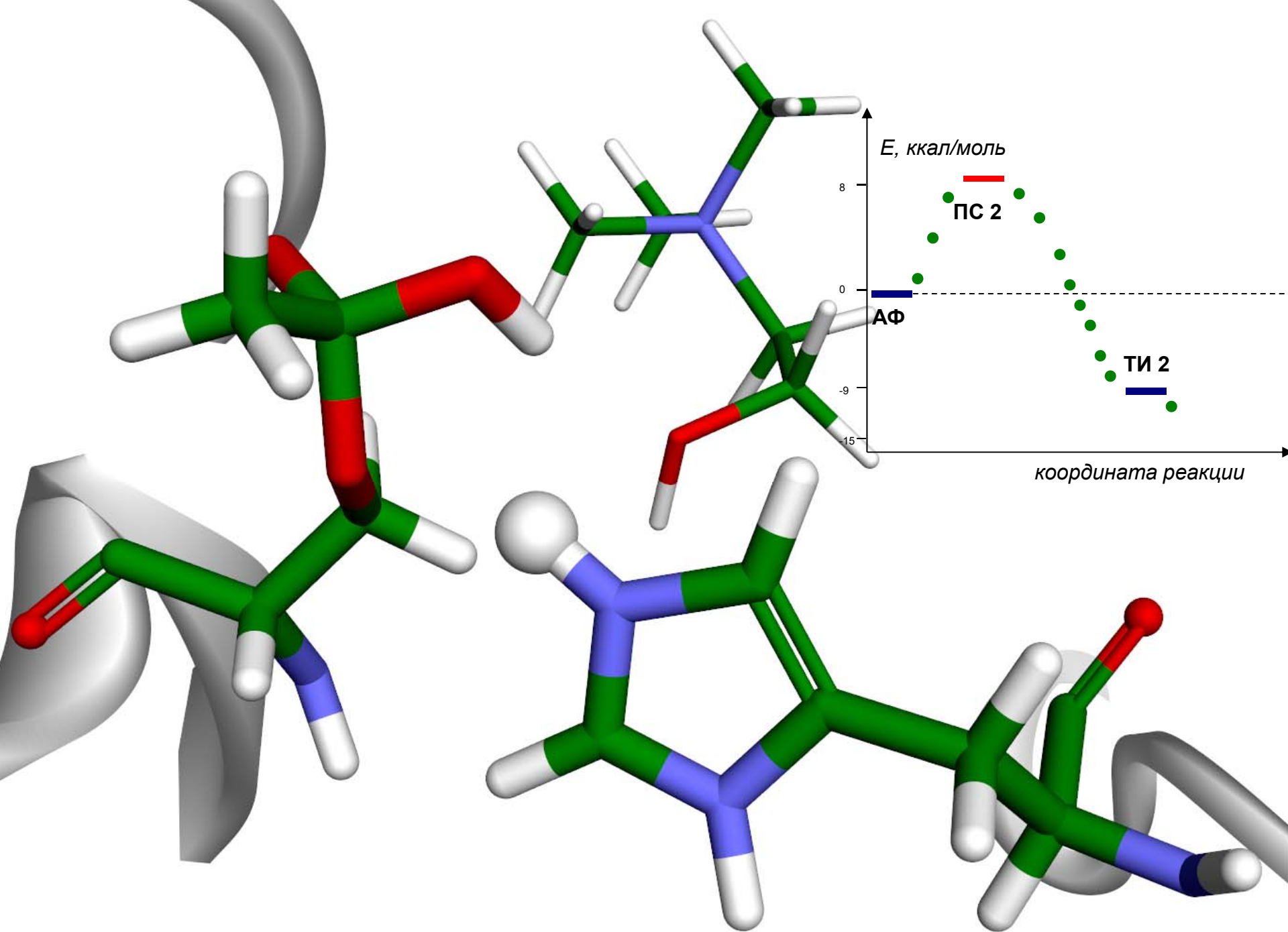
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ПС 2

АФ

ТИ 2

координата реакции



E , ккал/моль

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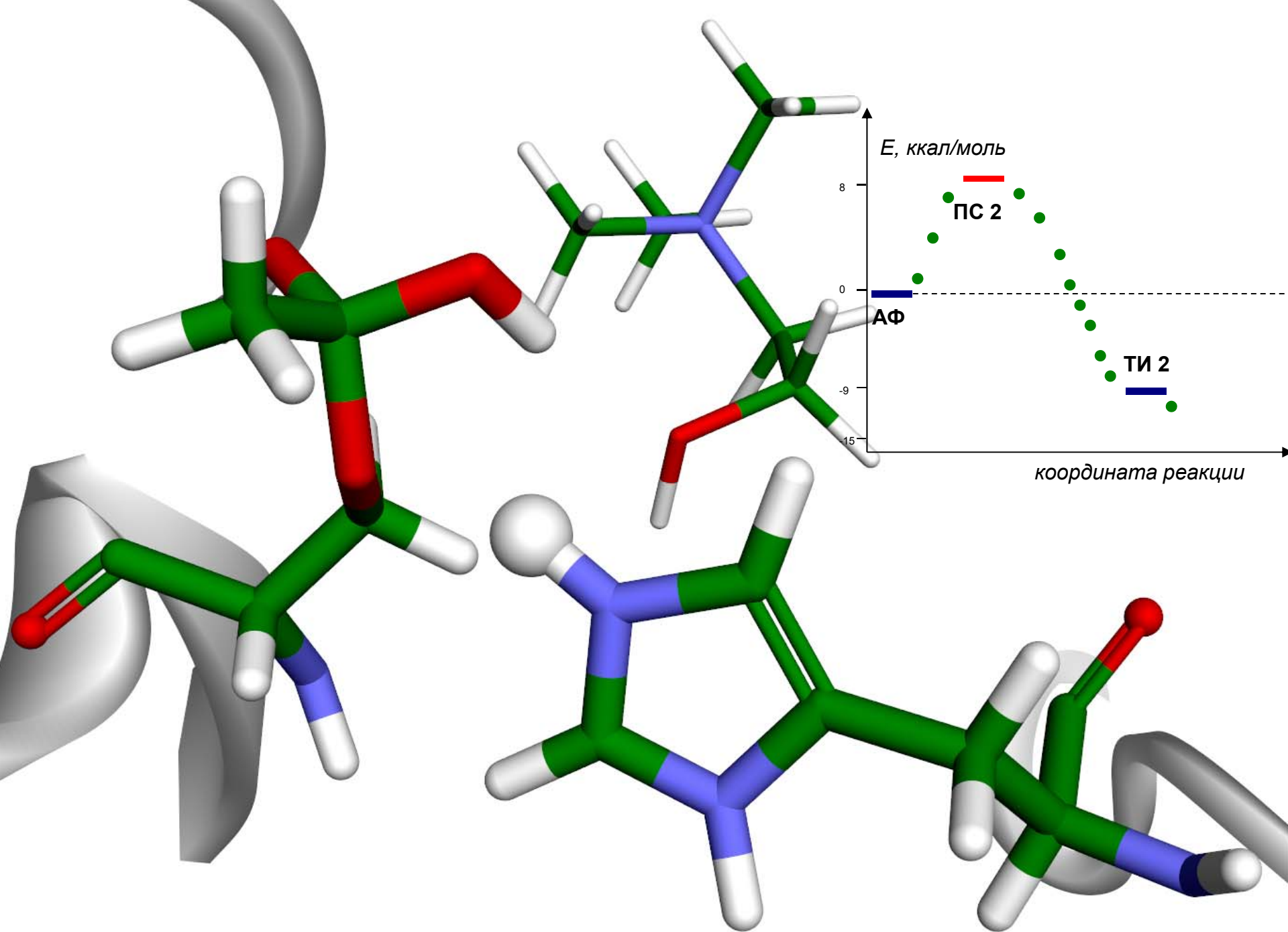
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ПС 2

АФ

ТС 2

координата реакции



E , ккал/моль

8

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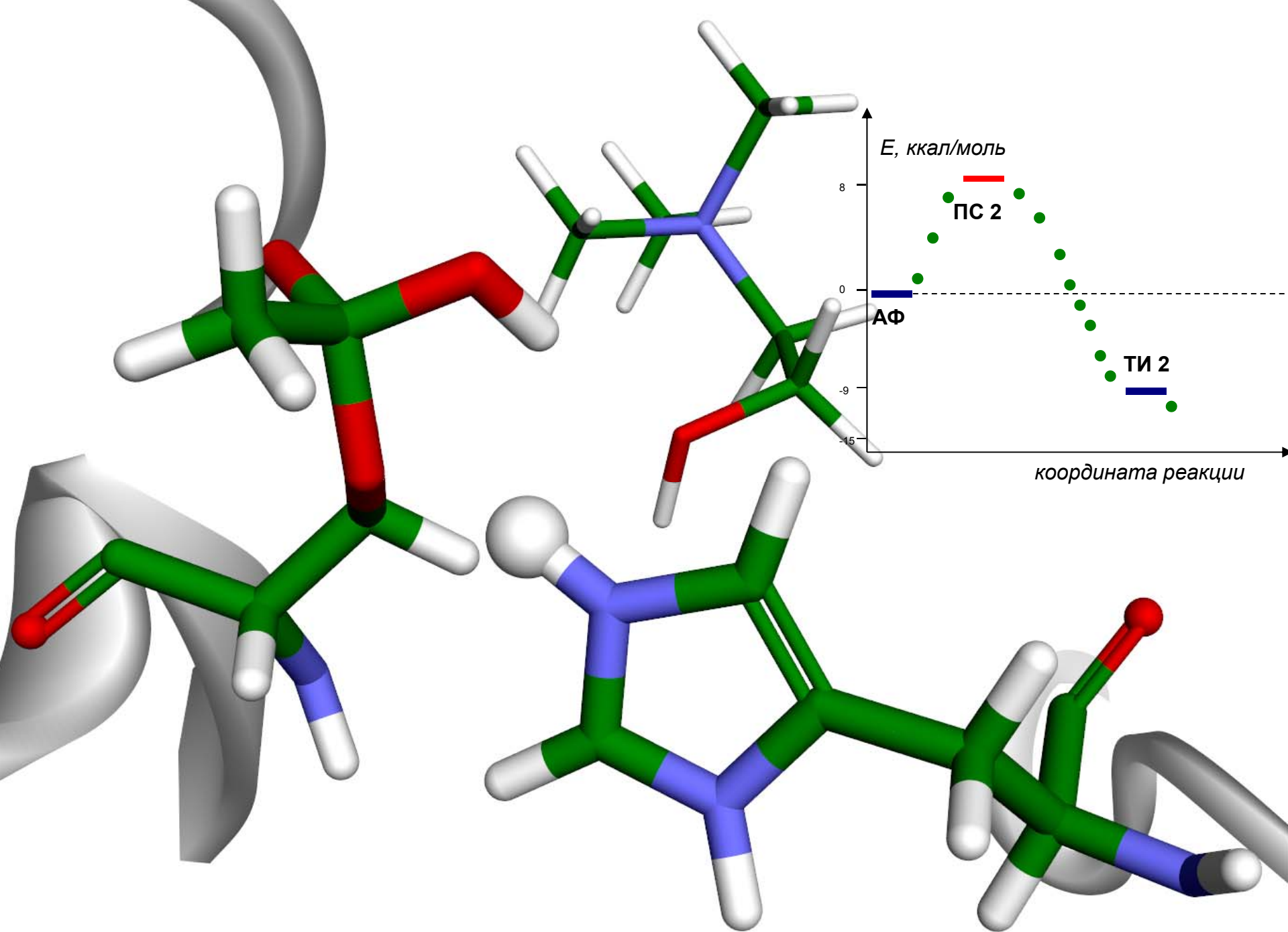
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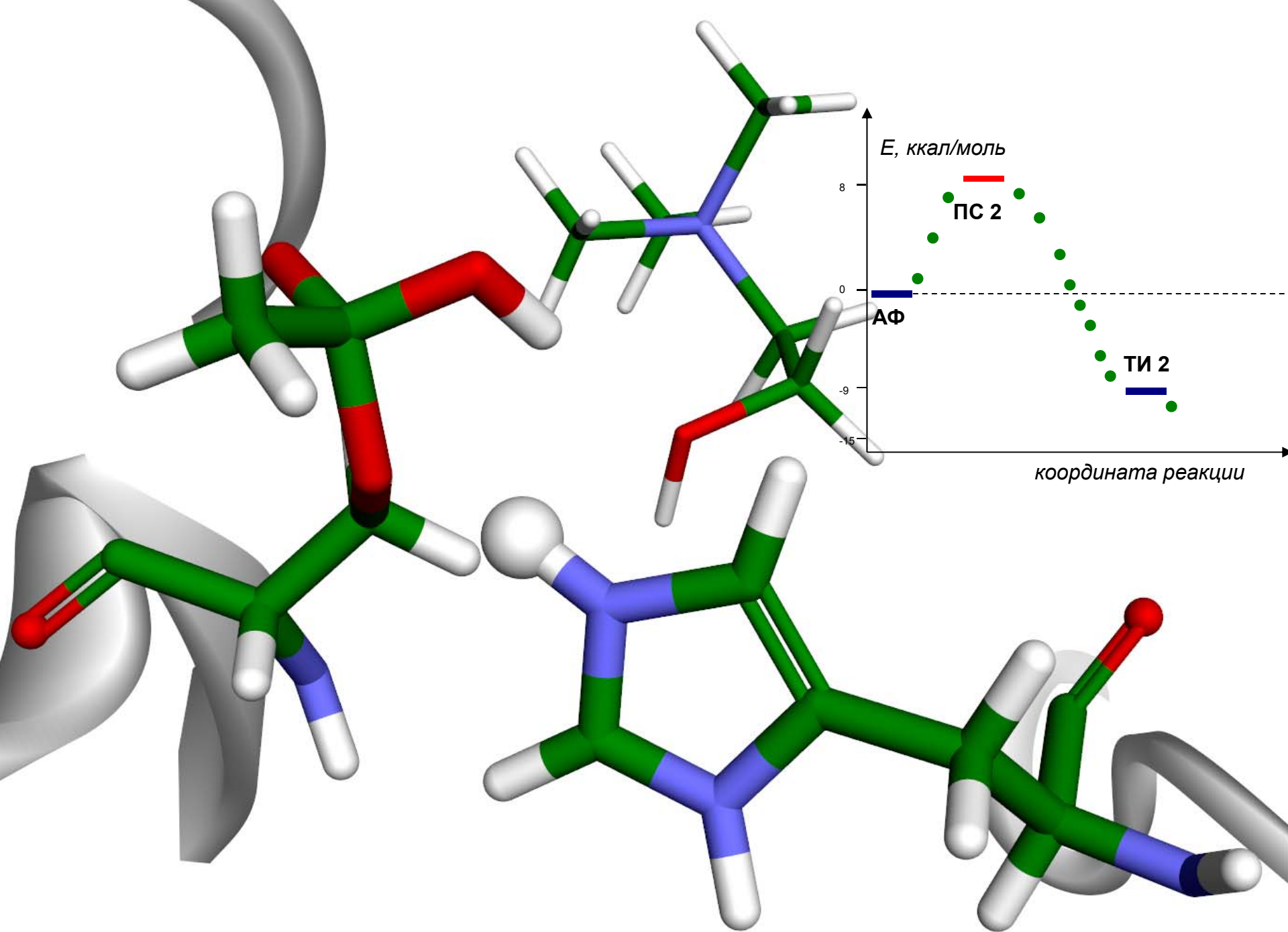
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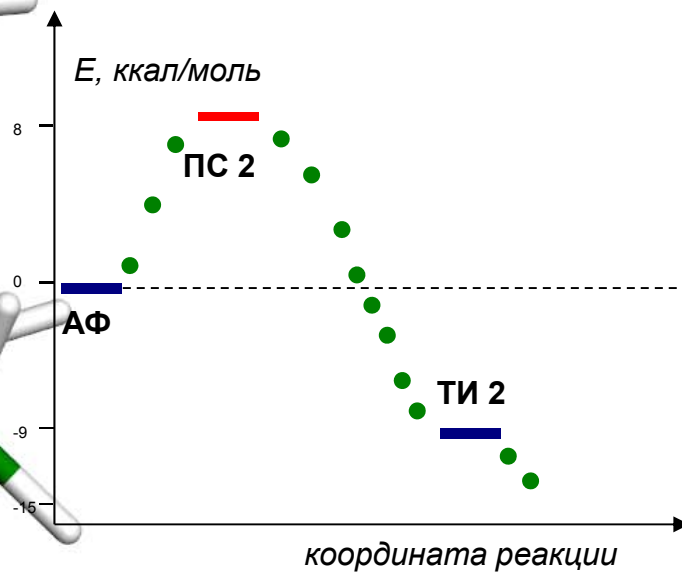
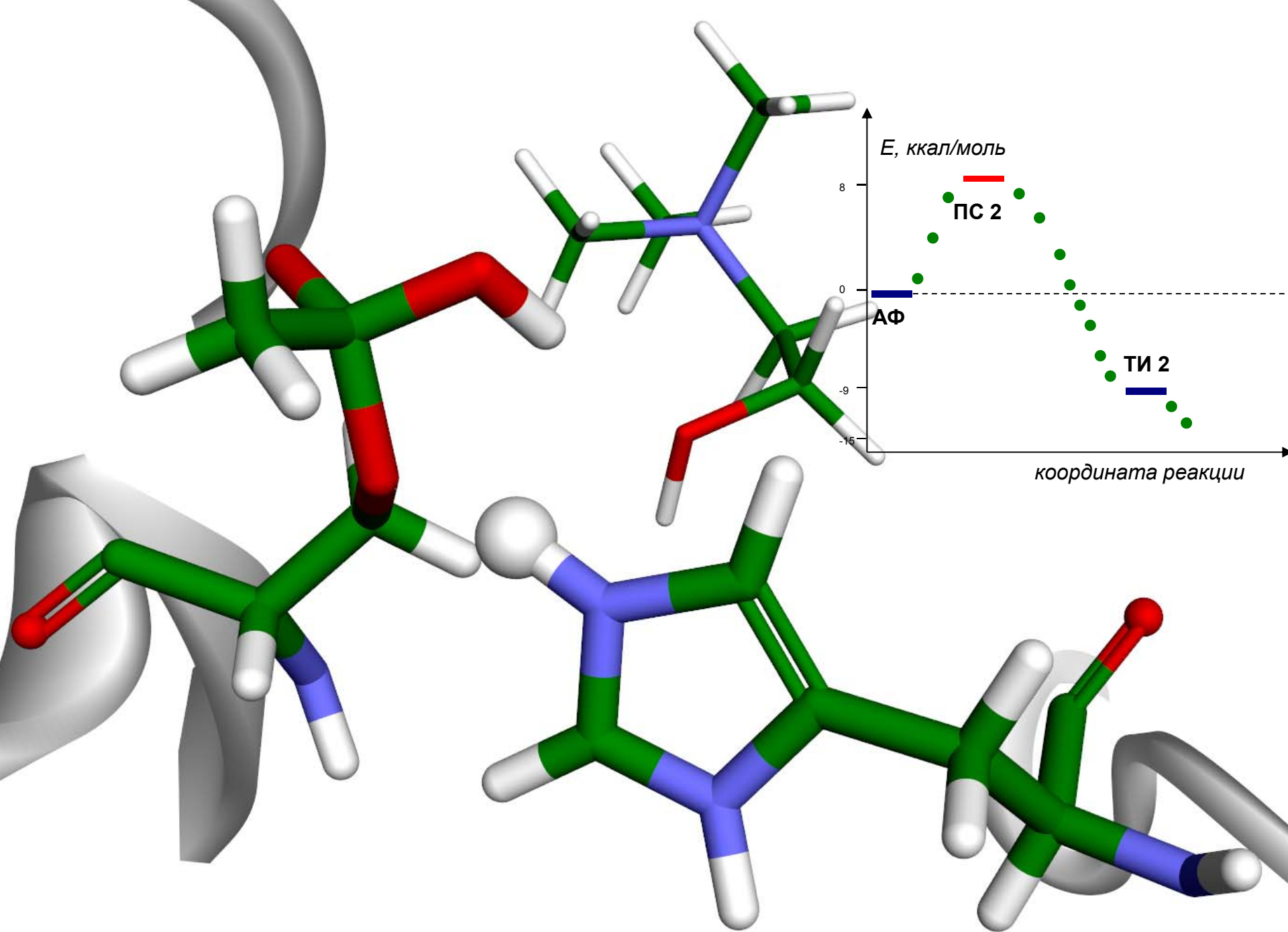
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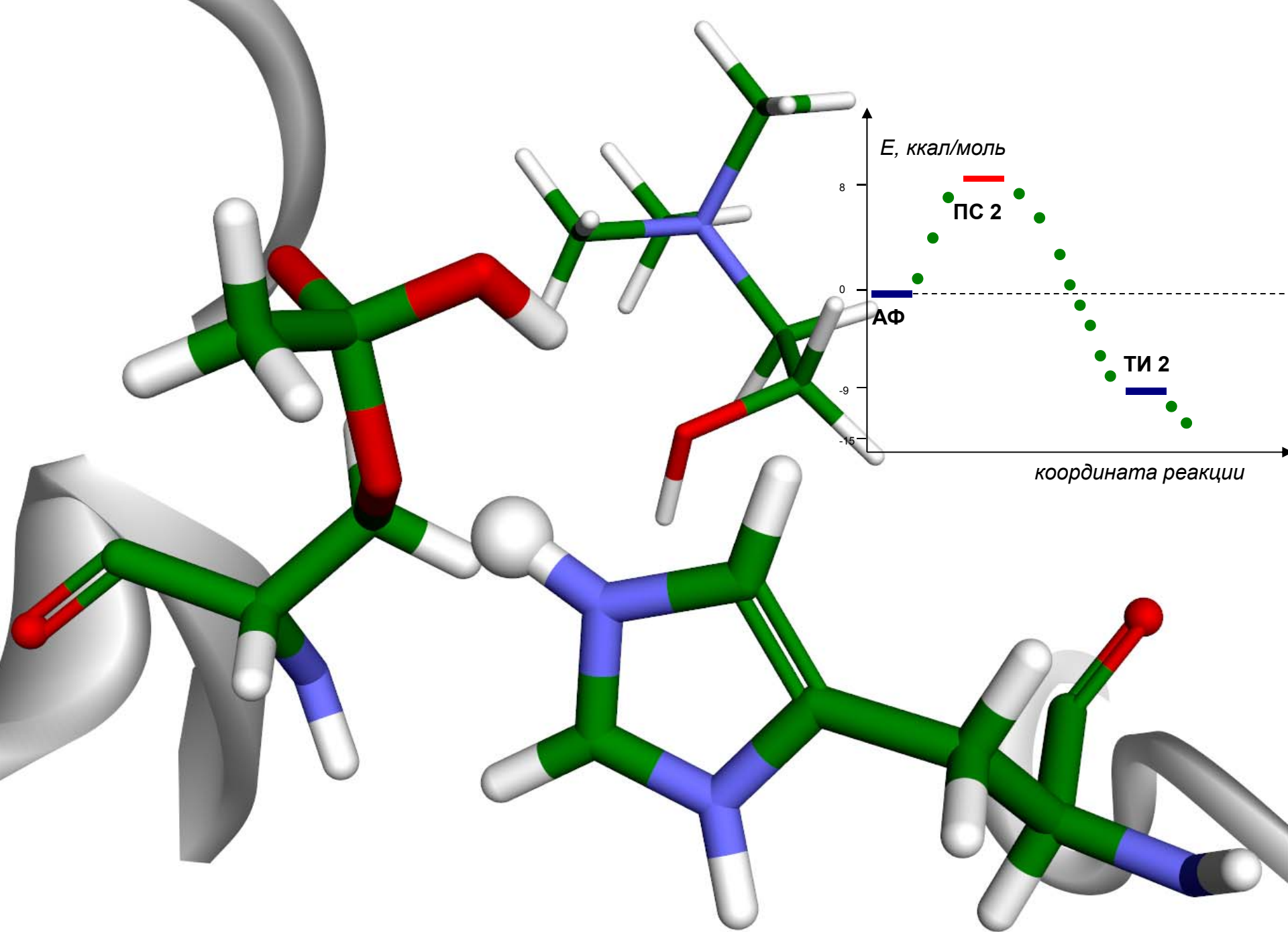
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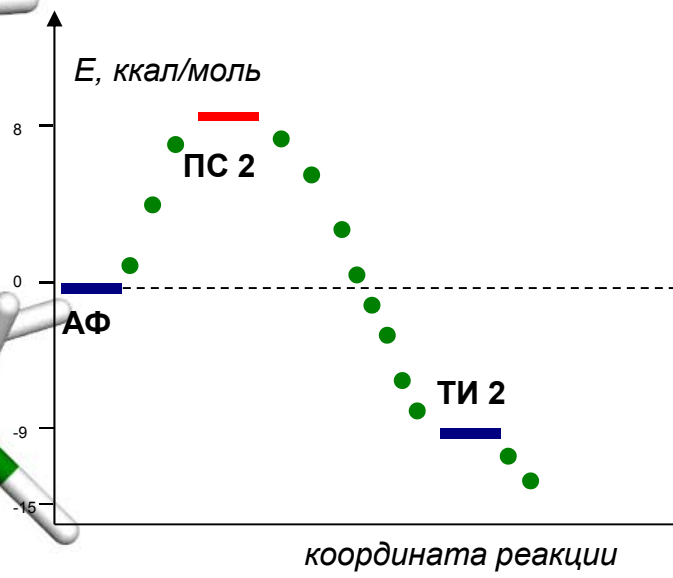
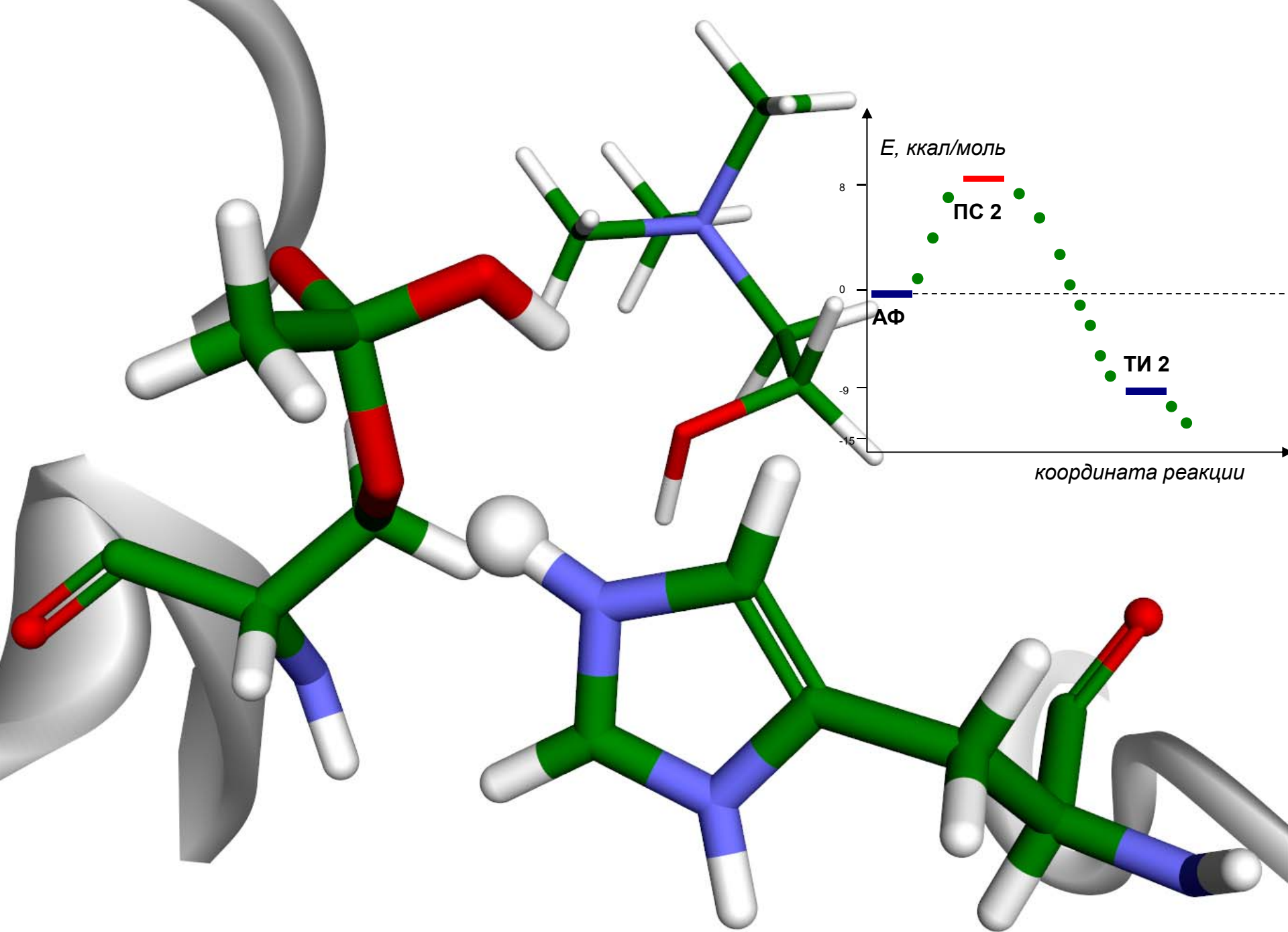
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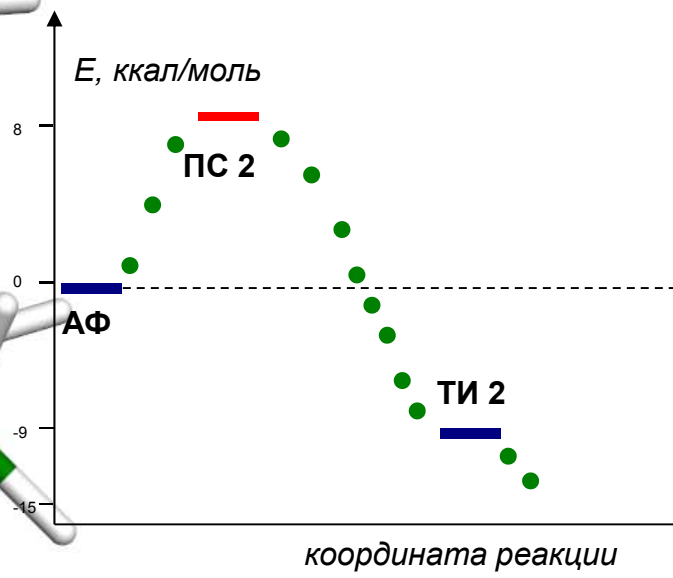
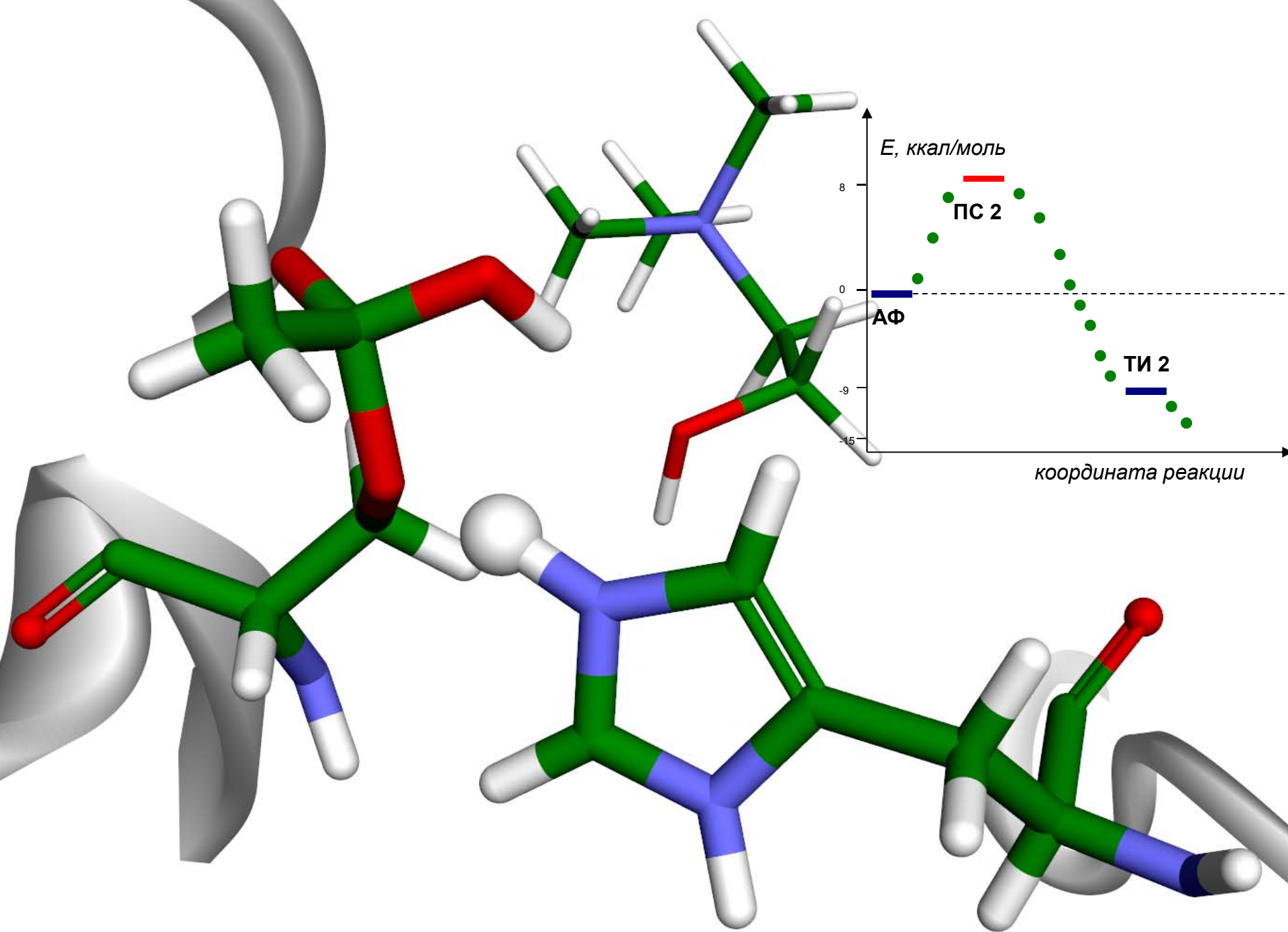


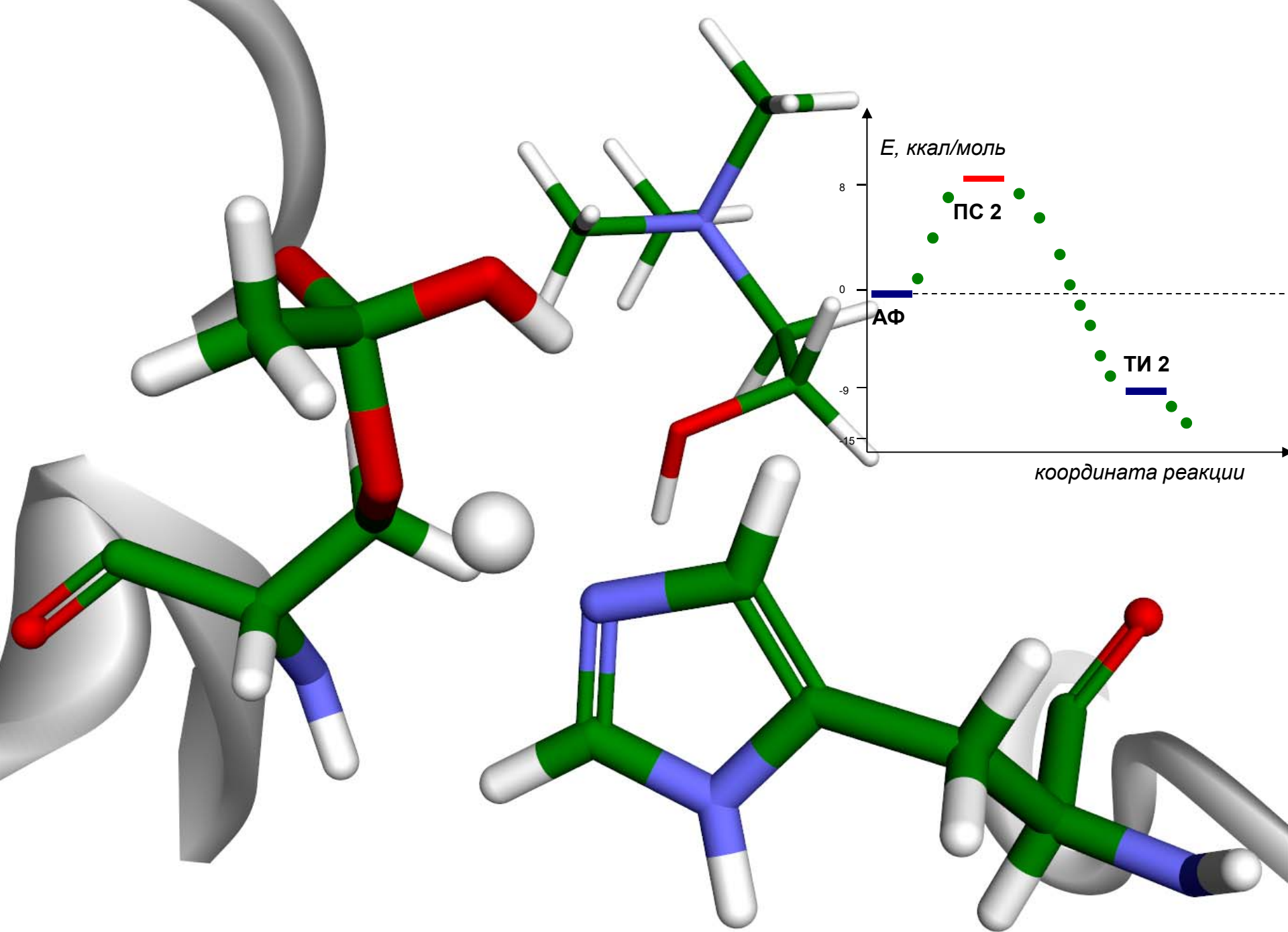


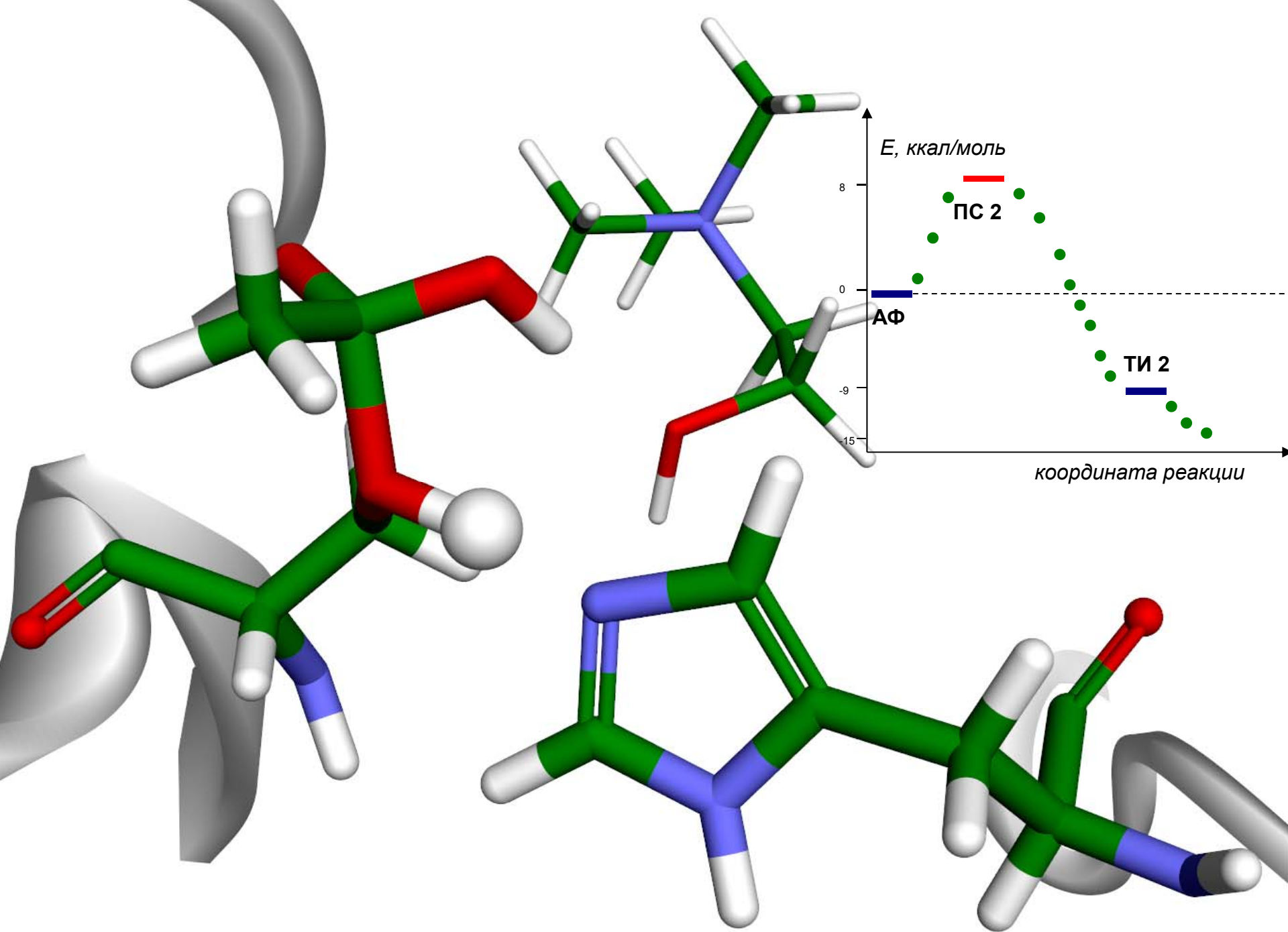


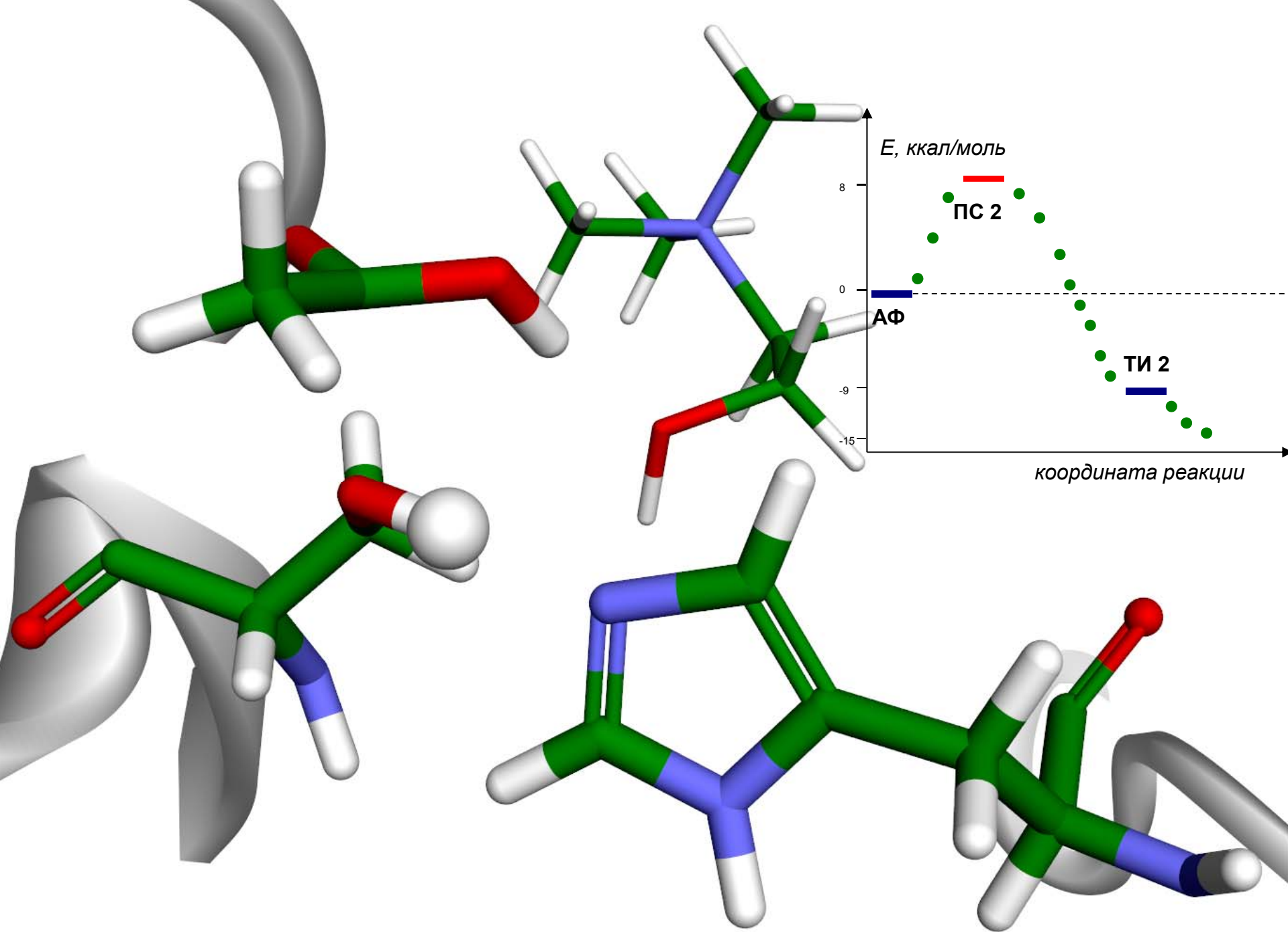


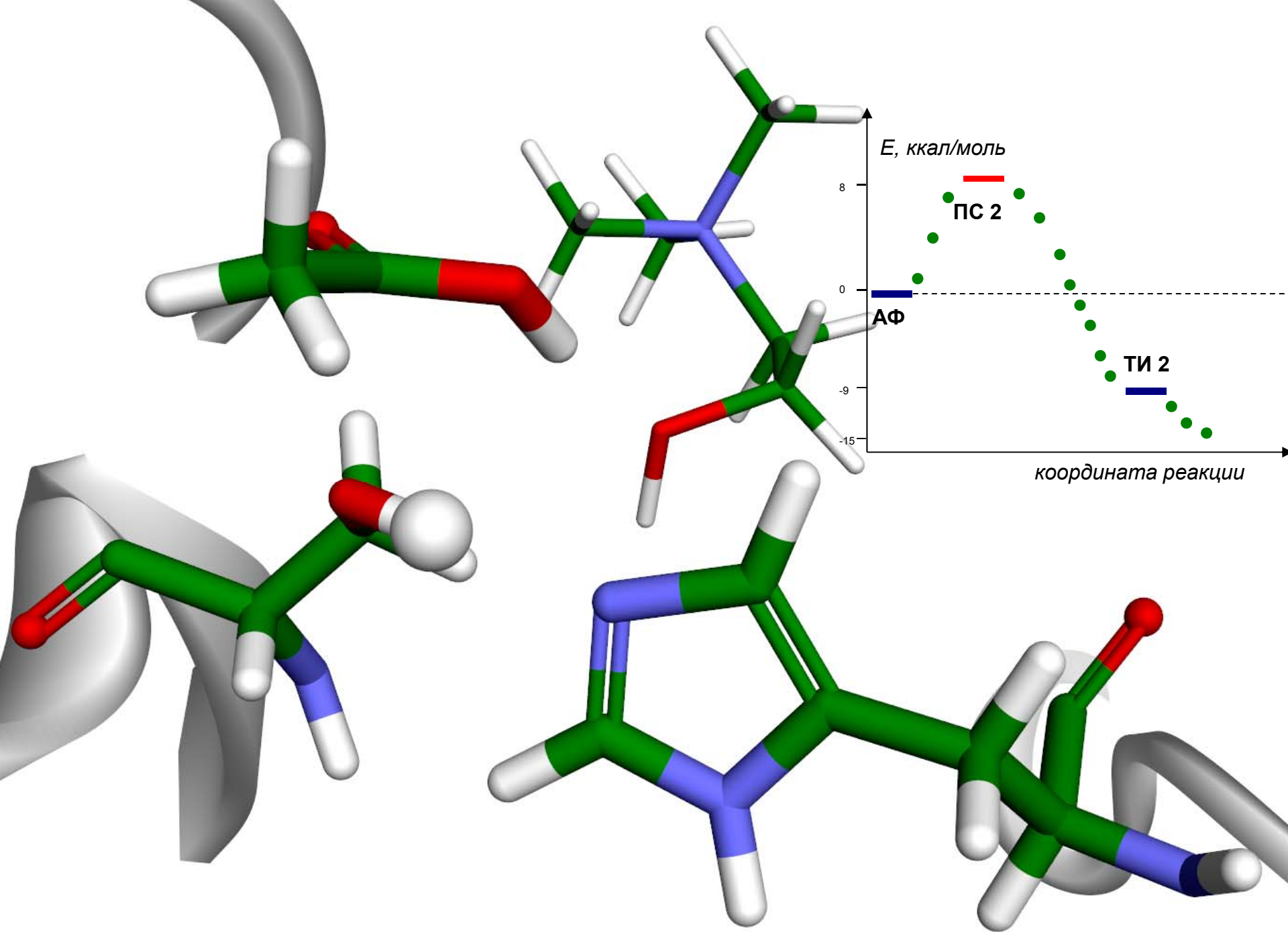


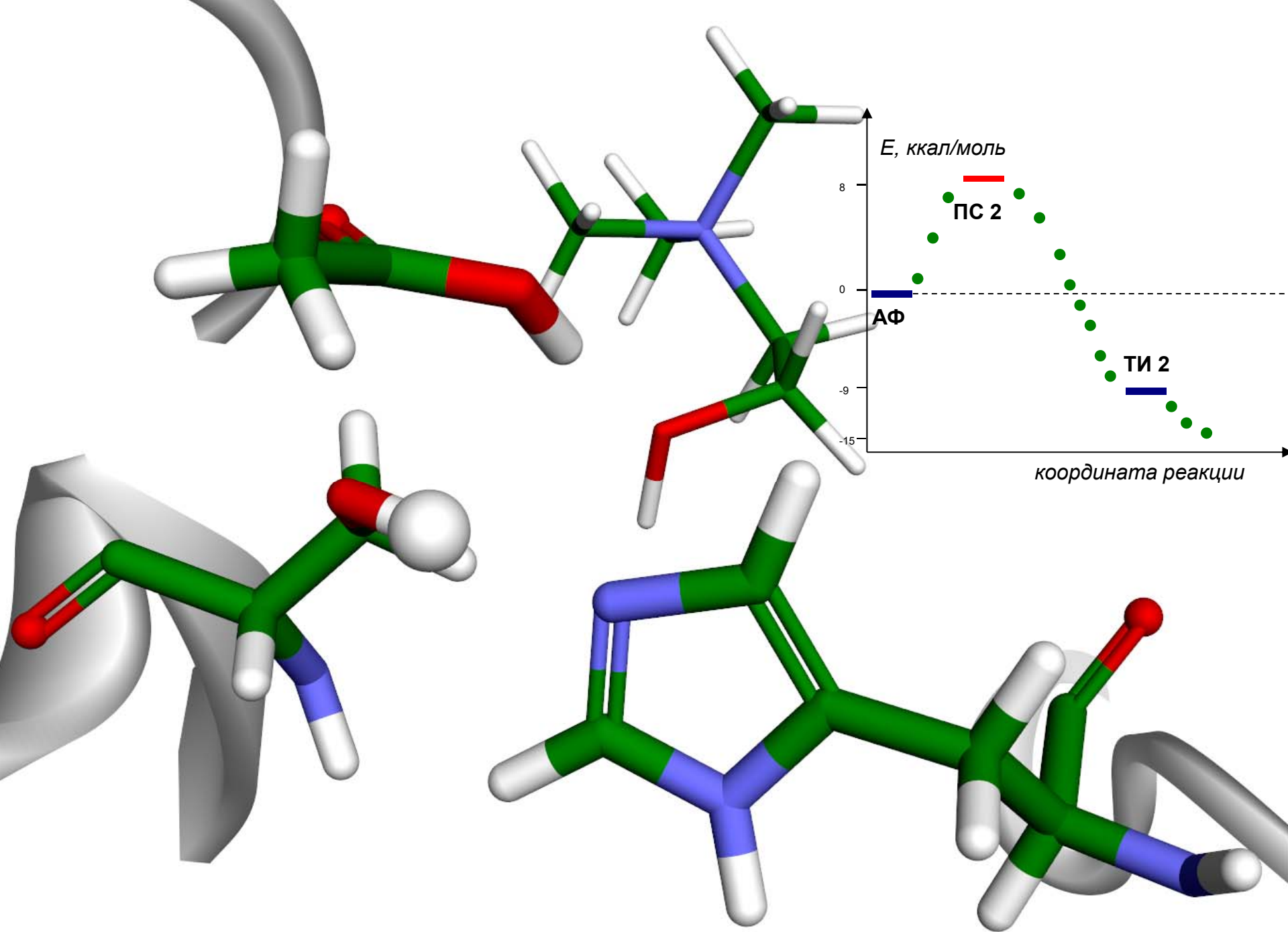


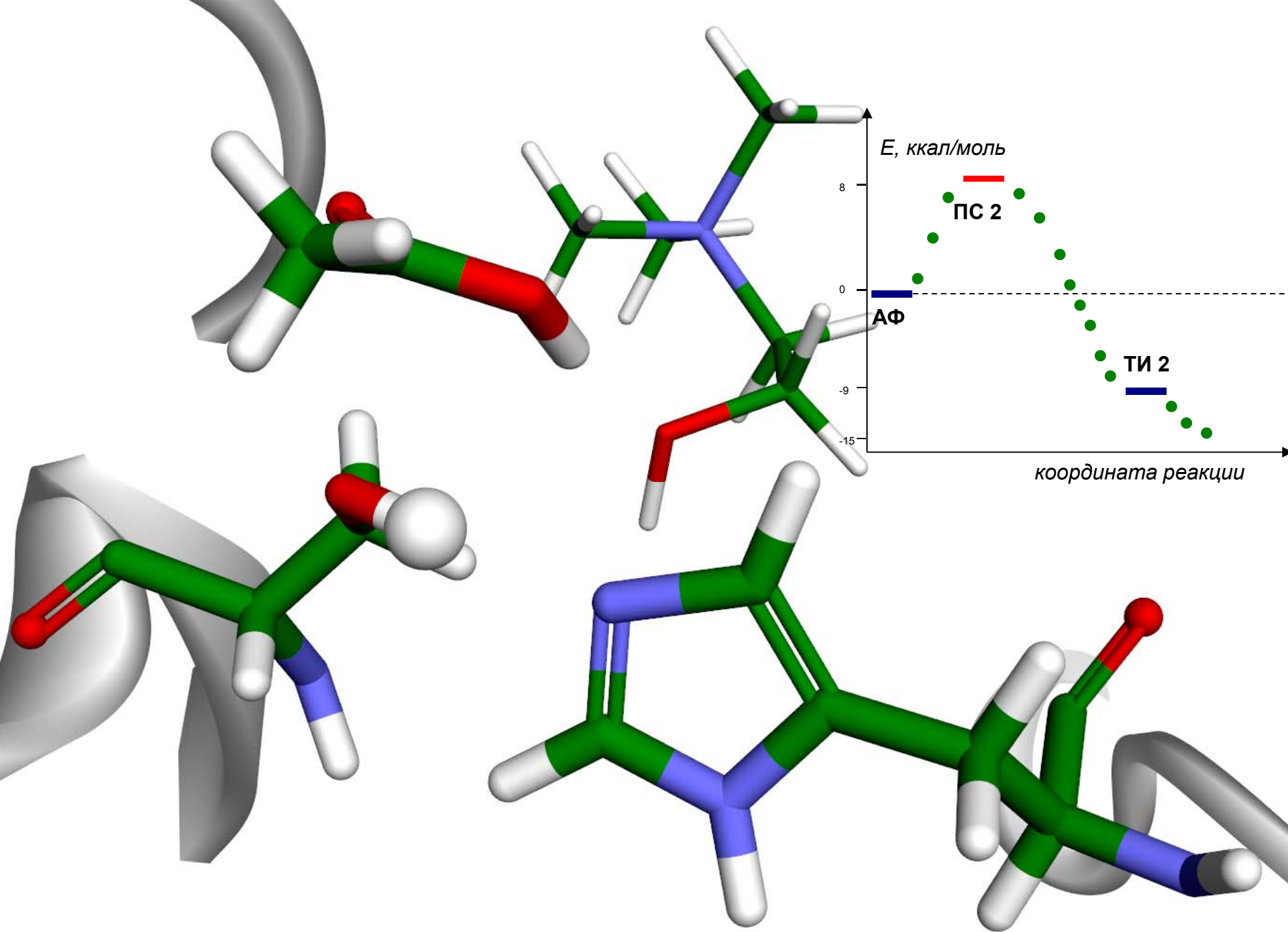


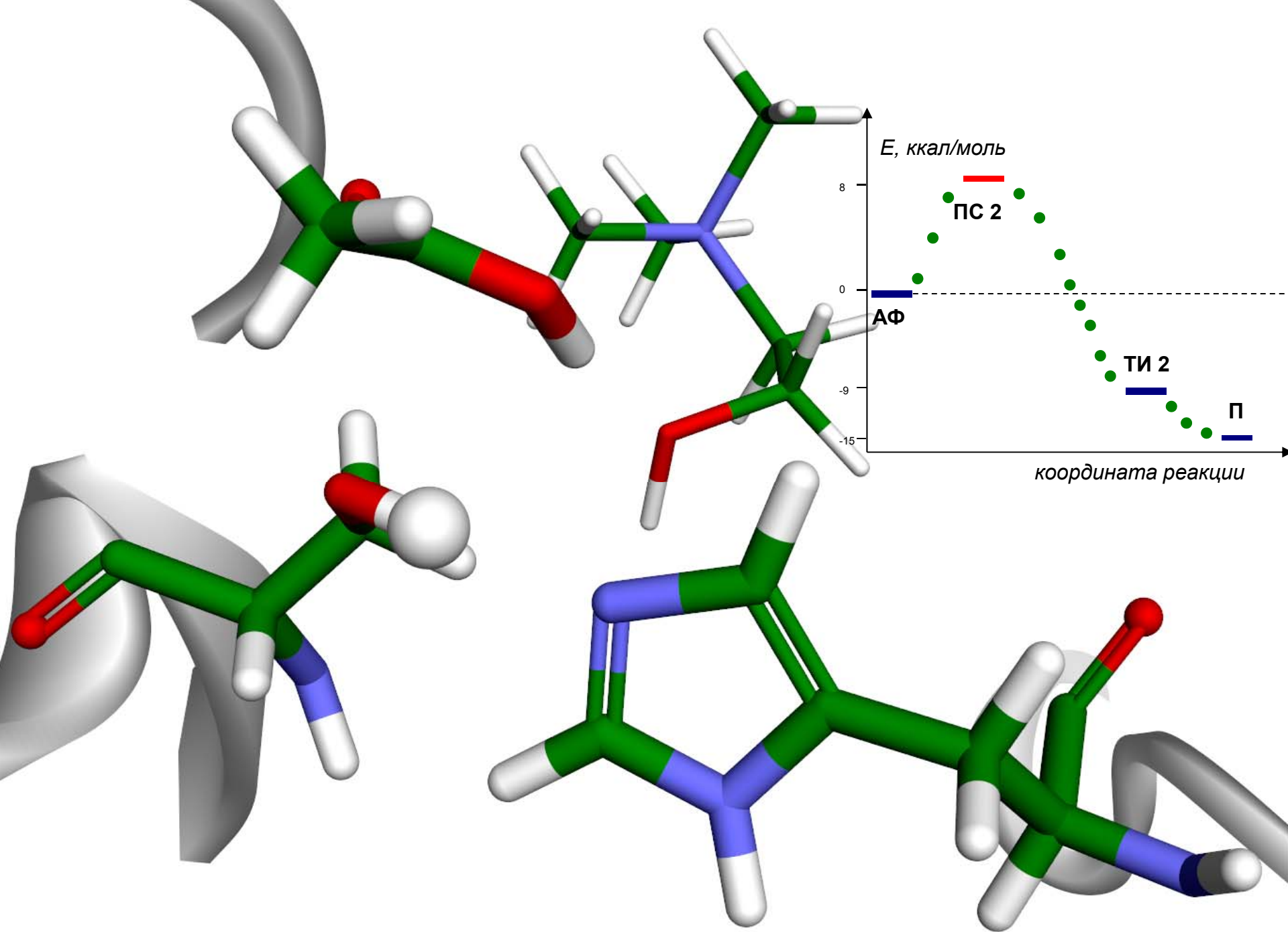












Post genomic chemistry

- One of the most interesting problem of the modern chemistry, biology and medicine _____
- *Individualization of the genomic and proteomic diversity of human biomacromolecules*

Pure Appl. Chem., Vol. 77, No. 9, pp. 1641–1654, 2005.

DOI: 10.1351/pac200577091641

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INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY

ORGANIC AND BIOMOLECULAR CHEMISTRY DIVISION*
CHEMISTRY AND HUMAN HEALTH DIVISION**

POSTGENOMIC CHEMISTRY

(IUPAC Technical Report)

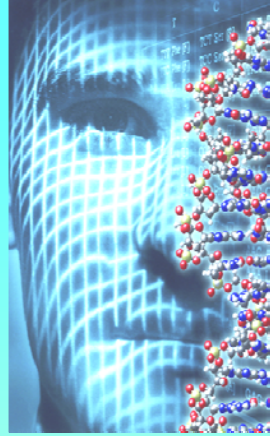
Prepared for publication by

SERGEY VARFOLOMEYEV^{1,‡}, ELENA EFREMENKO¹, IRINA BELETSKAYA¹,
IVANO BERTINI², G. MICHAEL BLACKBURN³, ALEXEY BOGDANOV⁴, RAIMOND CUNIN⁵,
JUTTA EICHLER⁶, IGOR GALAEV⁷, VADIM GLADYSHEV⁸, DAVID O'HAGAN⁹,
THOMAS HAERTLE¹⁰, JAAK JARV¹¹, ARKADIY KARYAKIN¹, ILIA KUROCHKIN¹,
MARIAN MIKOLAJCZYK¹², VLADIMIR POROIKOV¹³, IVAN SAKHAROV¹,
FRIEDRICH SPENER¹⁴, NORMAND VOYER¹⁵, AND JAMES WILD¹⁶

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Molecular polymorphism-genetic
predisposition to different diseases

Genetic predispositions

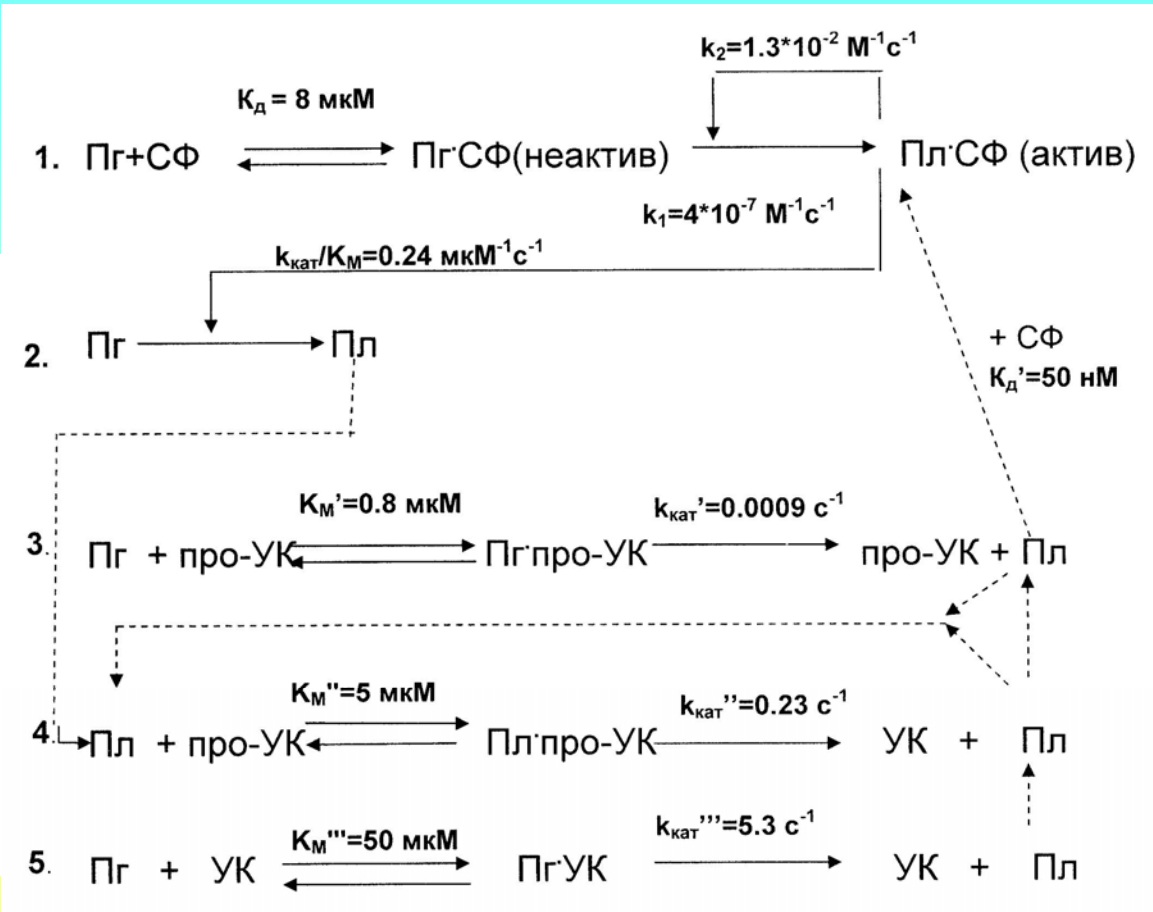
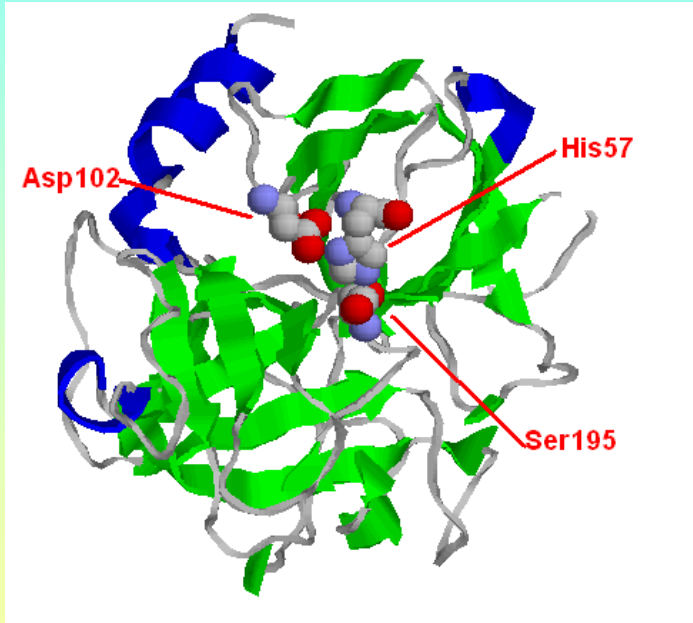


- *Cardiovascular diseases*
- *Oncology*
- *Respiratory diseases*
- *Diabetes*
- *Infections*

Project-Molecular polymorphism of human enzymes

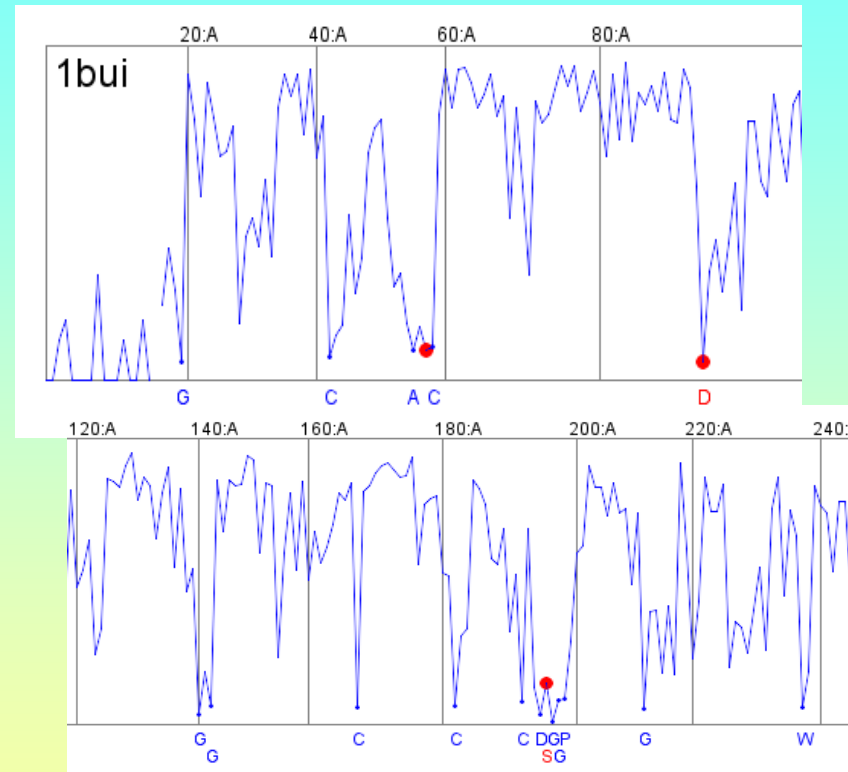
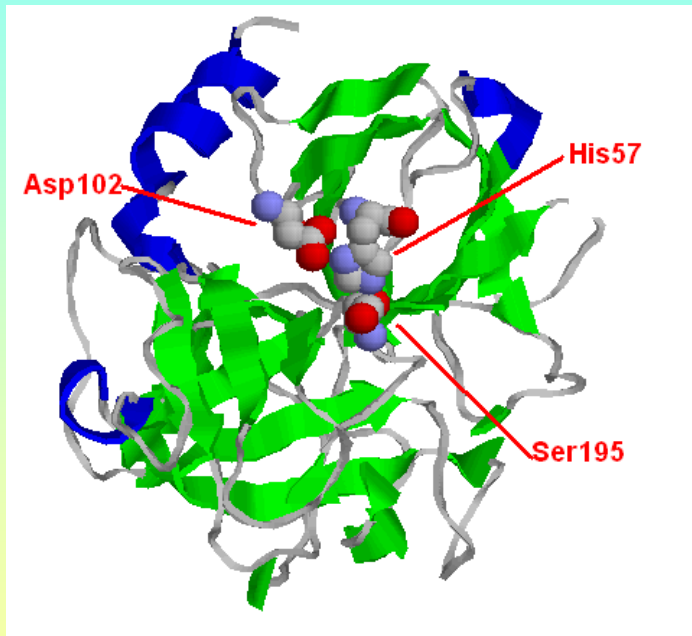
- *Enzymes-basis of transformation of all substances in organism*
- *Enzymes-the most investigated biopolymers*
- *Restricted number of genes (2800)*
- *Kinetic basement of biosystems*

Плазминоген-плазмин – ключевая система очистки сосудов от тромбов



- R.Aisina, L. Mukhametova, S. Varfolomeev, Biochimica et Biophysica Acta 1725 (2005), 370-376
- R.Aisina, L. Mukhametova, S. Varfolomeev, J. Thromb. Haemost. 2005, V3, 1385

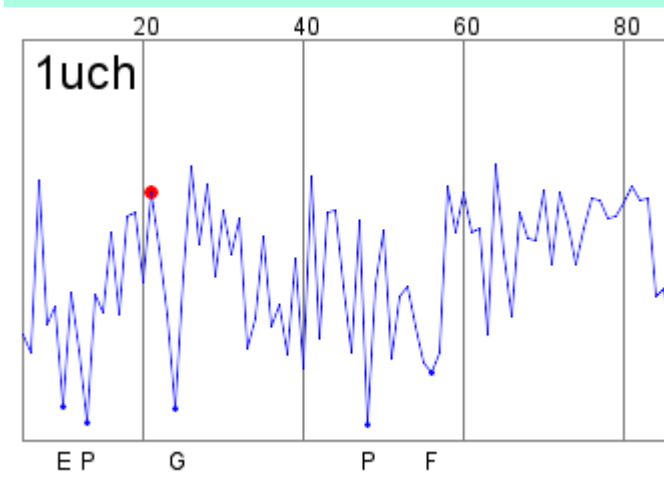
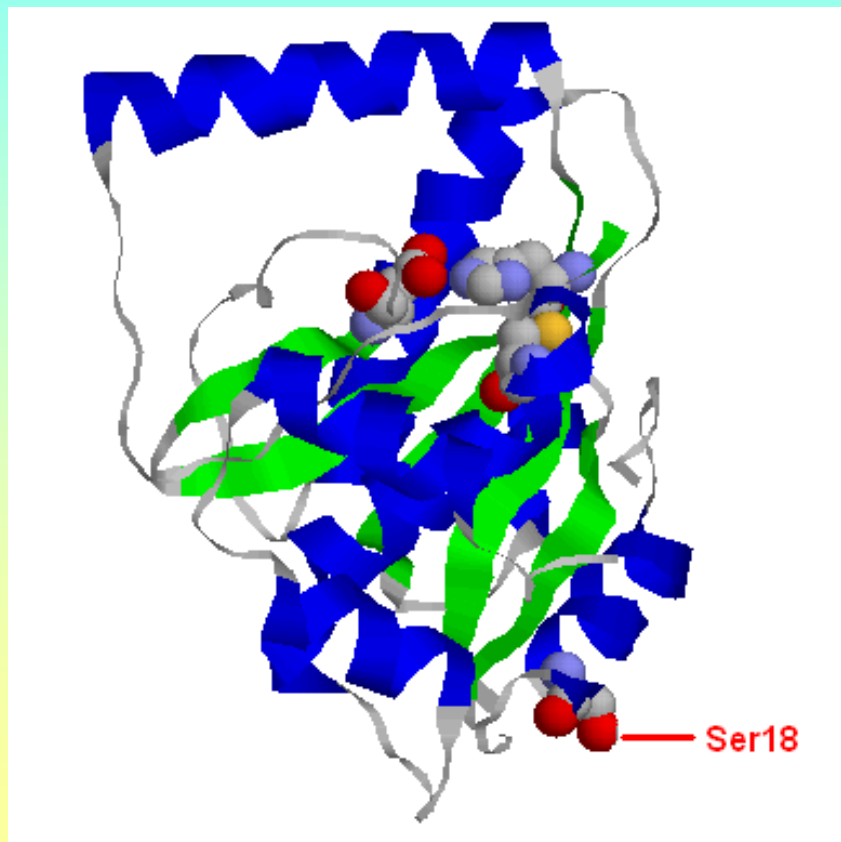
Плазмин-плазминоген



- Ключевая система очистки сосудов от тромбов.
- Единичные замены-риски развития сердечно-сосудистых заболеваний

Молекулярный полиморфизм ферментов человека

- **Карбокси-концевая убикитин-гидролаза**
Биологическая функция фермента: Тиоловая гидролаза, отщепляет убикитин, ковалентно соединенный через С-концевой остаток Gly.

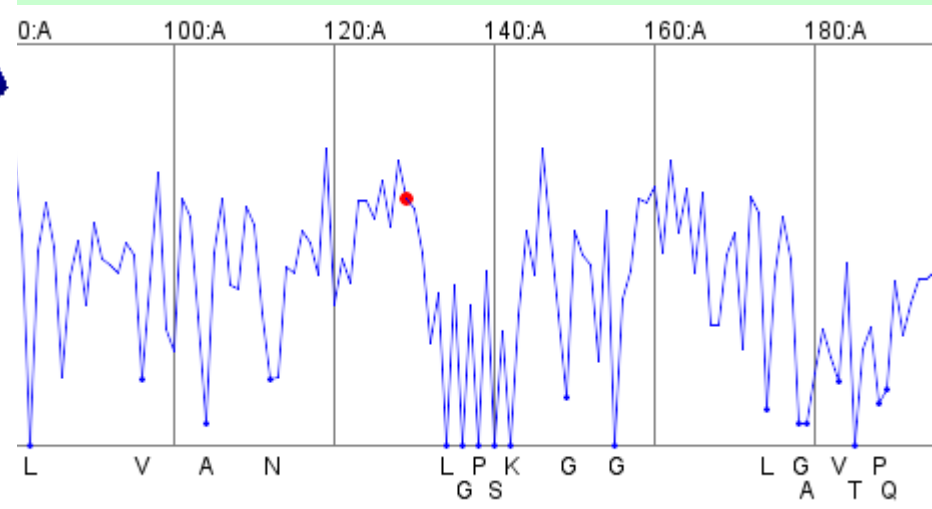
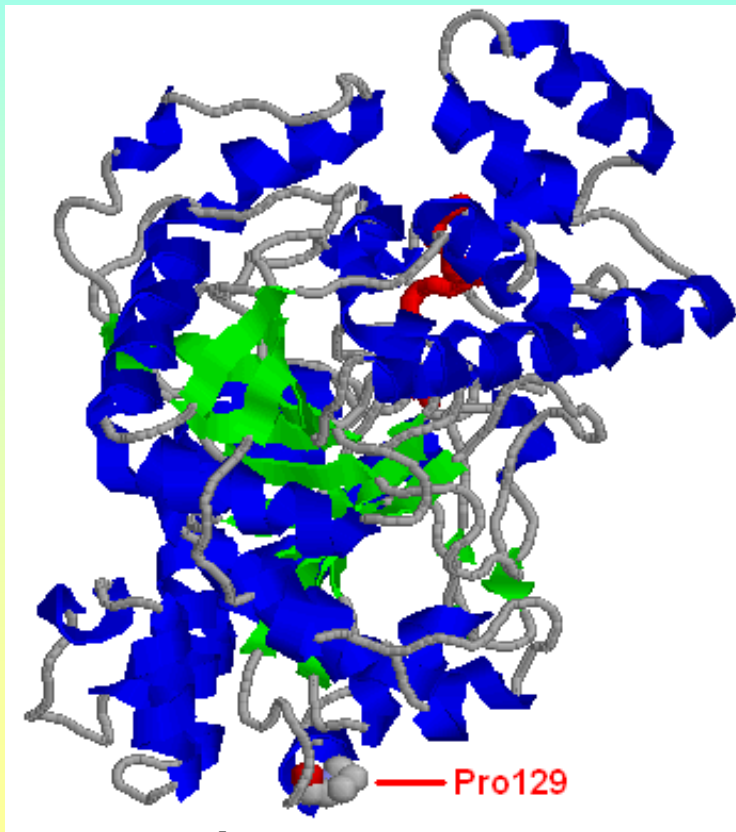


Фактор риска развития болезни Паркинсона

Молекулярный полиморфизм ферментов человека

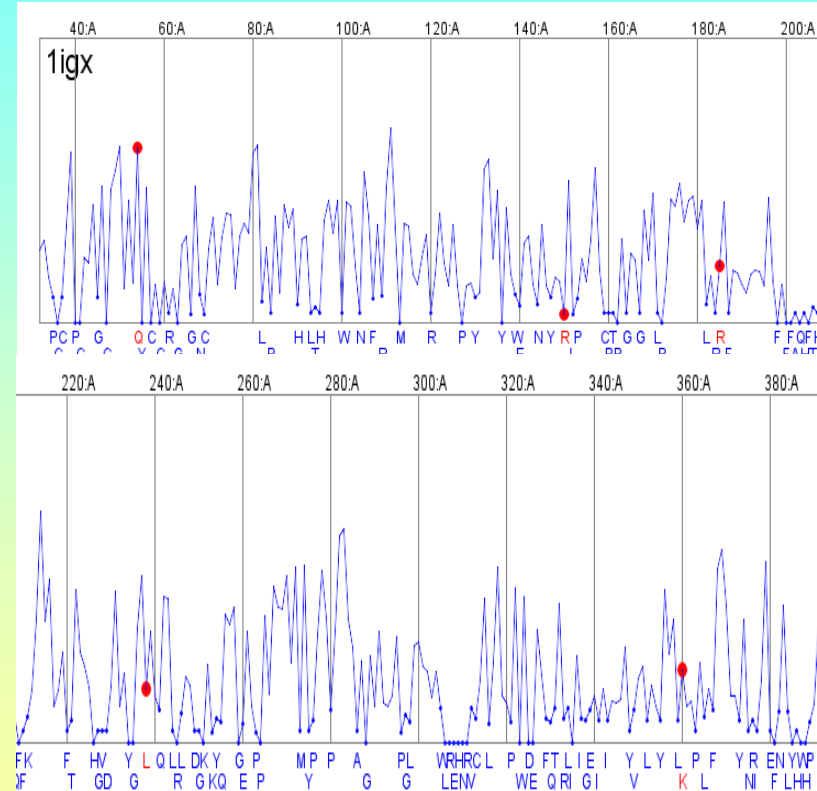
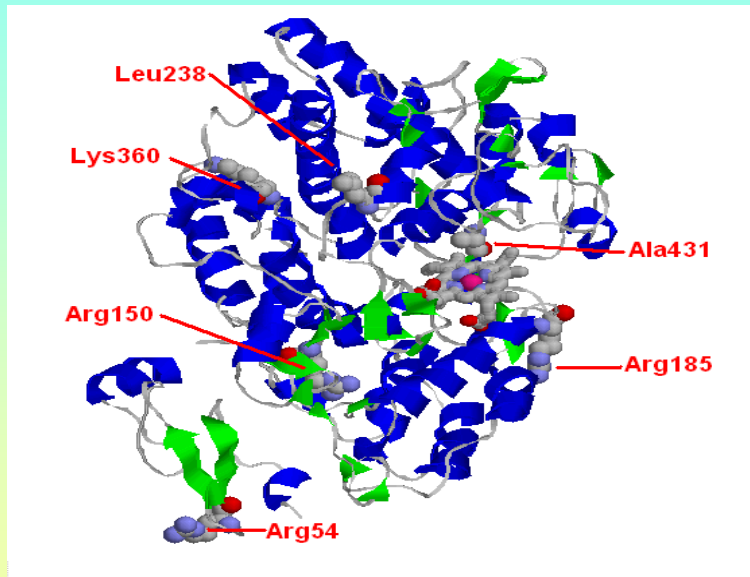
- **Гидролаза амидов жирных кислот**

Биологическая функция фермента: Инактивирование нейротрансмиттера – анандамида. Анандамид является природным лигандом каннабиоидного рецептора, другим лигандом является действующее вещество марихуаны – тетрагидроканнабиол.



Фактор риска злоупотребления алкоголем и наркотиками

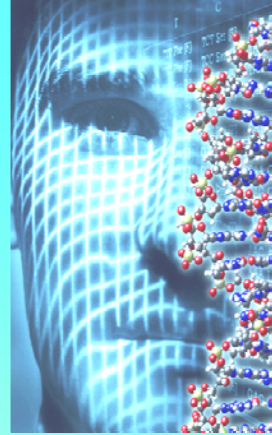
Простагландин-Н синтаза- лимитирующий фермент синтеза простагландинов



- Индивидуальная чувствительность к противовоспалительным препаратам (аспирин, анальгин, бруфен и др.)

Molecular polymorphism of human enzymes

PGH – syntase (COX 1, COX 2)

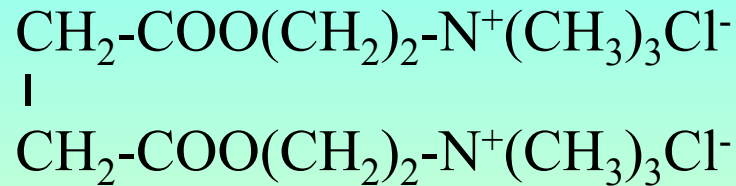


Cox 1	-842 A>G	Reduced sensitivity to aspirin
	Leu 257 Met	Increased risk of colon cancer
	Arg 8 Trp Pro 17 Leu	Reduced sensitivity to aspirin
Cox 2	-765 G>C	Reduced risk of colorectal cancer, squamous carcinoma
	-1290 A>G, -1195 G>A	Increased risk of esophageal dis.
	-8473T>C	Increased risk of lung cancer
	Val 511 Ala	Increased risk of bowels cancer
	Arg 298 His	Increased risk of diabetes - 2

Индивидуальная
чувствительность к
лекарственным препаратам

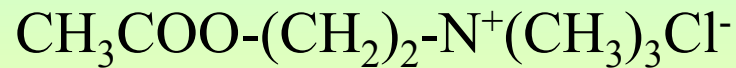
Butyrylcholinesterase

Individual sensitivity to drugs



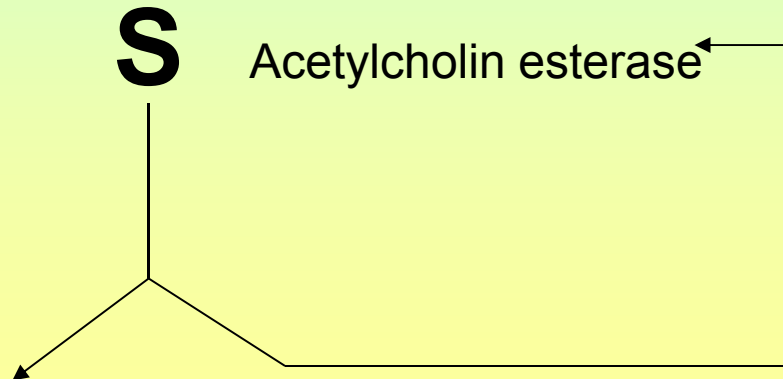
Succinyl choline-agent for
anesthesiology

Acetylcholine-neuromediator



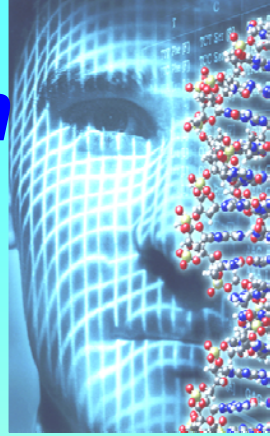
Defective gene

Enzyme inhibition
(CWA)



Hydrolysis

Molecular polymorphism of human enzymes

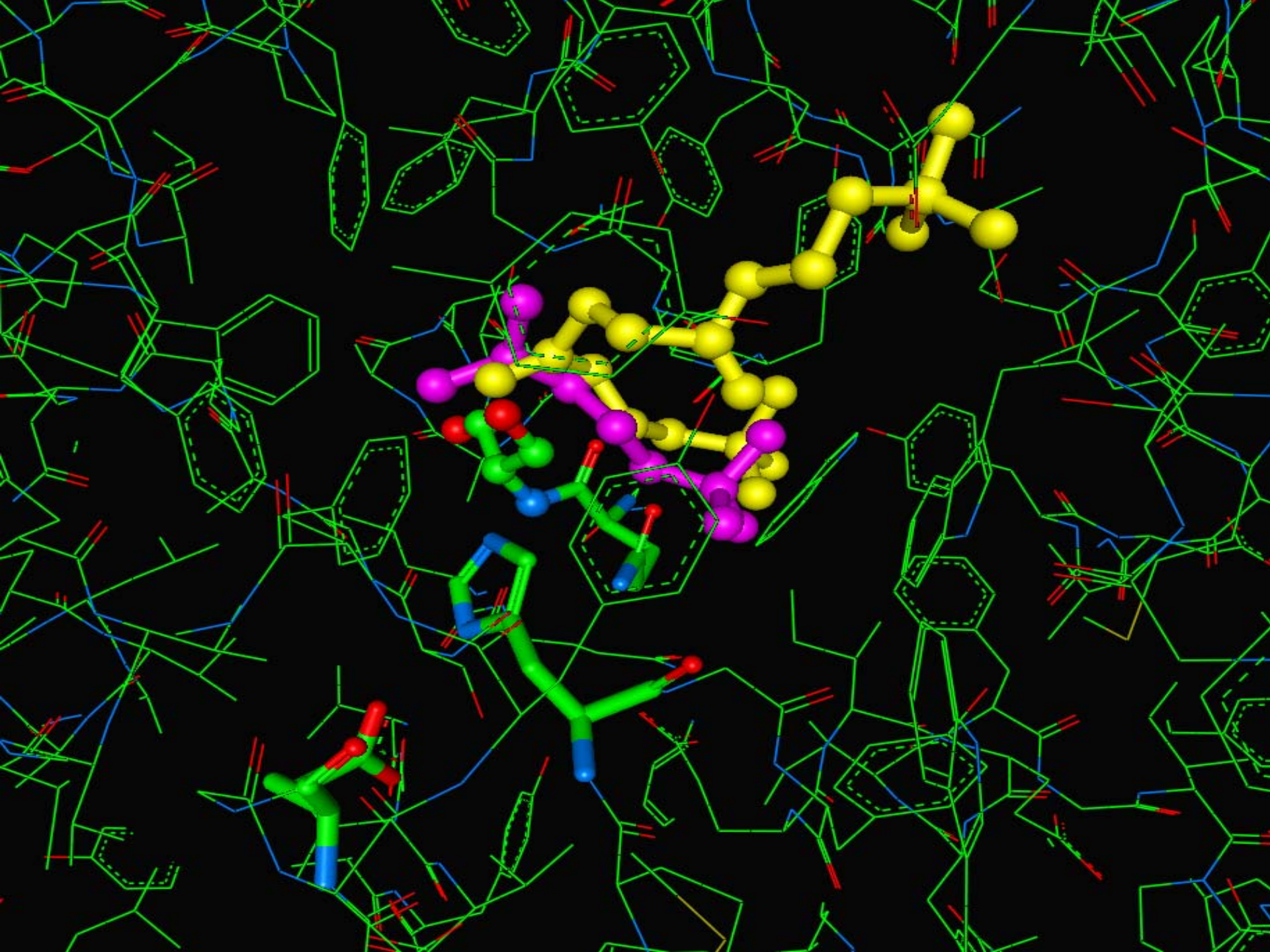


Butyrylcholin esterase

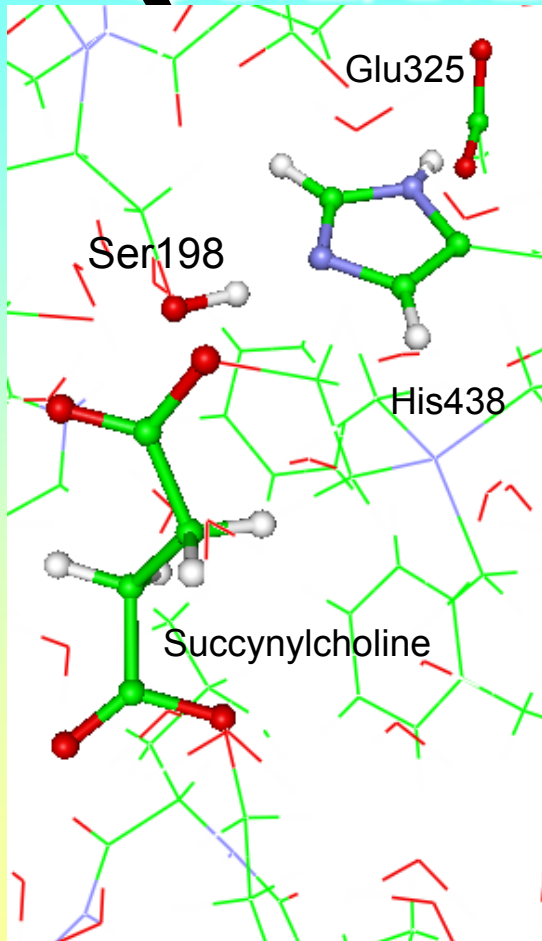
Ala 567 Thr, Asp 98 Gly, Asp 98 His, Glu 525 Val,
Gly 418 Val, Len 335 Pro, Len 358 Ile, Thr 271 Met,
Tyr 156 Cys

“Atypical” butyrylcholin esterase Asp 98 His
Asp 98 Gly

“Apnoe” and paralysis



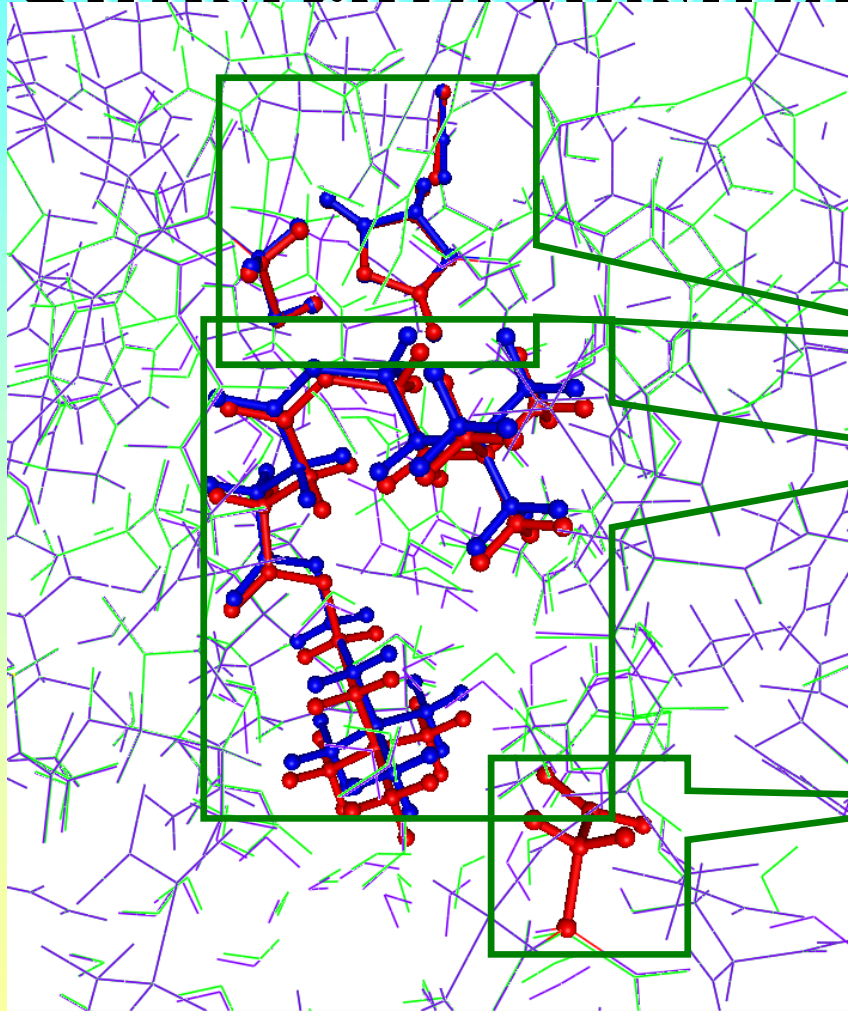
QM/MM calculations



- Quantum subsystem:
 - Restricted Hartree-Fock method
 - cc-pVDZ basis set
 - 25 atoms in QM subsystem
 - 335 basis functions
 - Amber99 force field

Substrate position difference

ES



— WT BuChE

— Asp70Gly
BuChE

Catalytic triad

Substrate

Asp70

Asp70Gly SNP

- $K_m(\text{snp}) \setminus K_m(\text{n}) = 100$
- $K_3(\text{n}) \setminus k_3(\text{snp}) = 8100$
- ---
- *Very low reactivity*
- ---
- Very toxic for human polymorphants
(one among 2000)