Computational modeling of molecular processes in proteins

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Overview

Methods

Applications

- Transient kinetics in hydrolysis of guanosine triphosphate (GTP)
- Aspartoacylase: Role of protein dimer
- Molecular mechanisms of chromophore formation and decomposition in the green fluorescent protein (GFP)

Concluding remarks

Acknowledgements

Applications

Significance of every project can be easily justified

- **GTP hydrolysis by human Ras: relevance to cancer**
- **Aspartoacylase: the key enzyme in brains**
- **Green fluorescent protein: *in vivo* imaging**

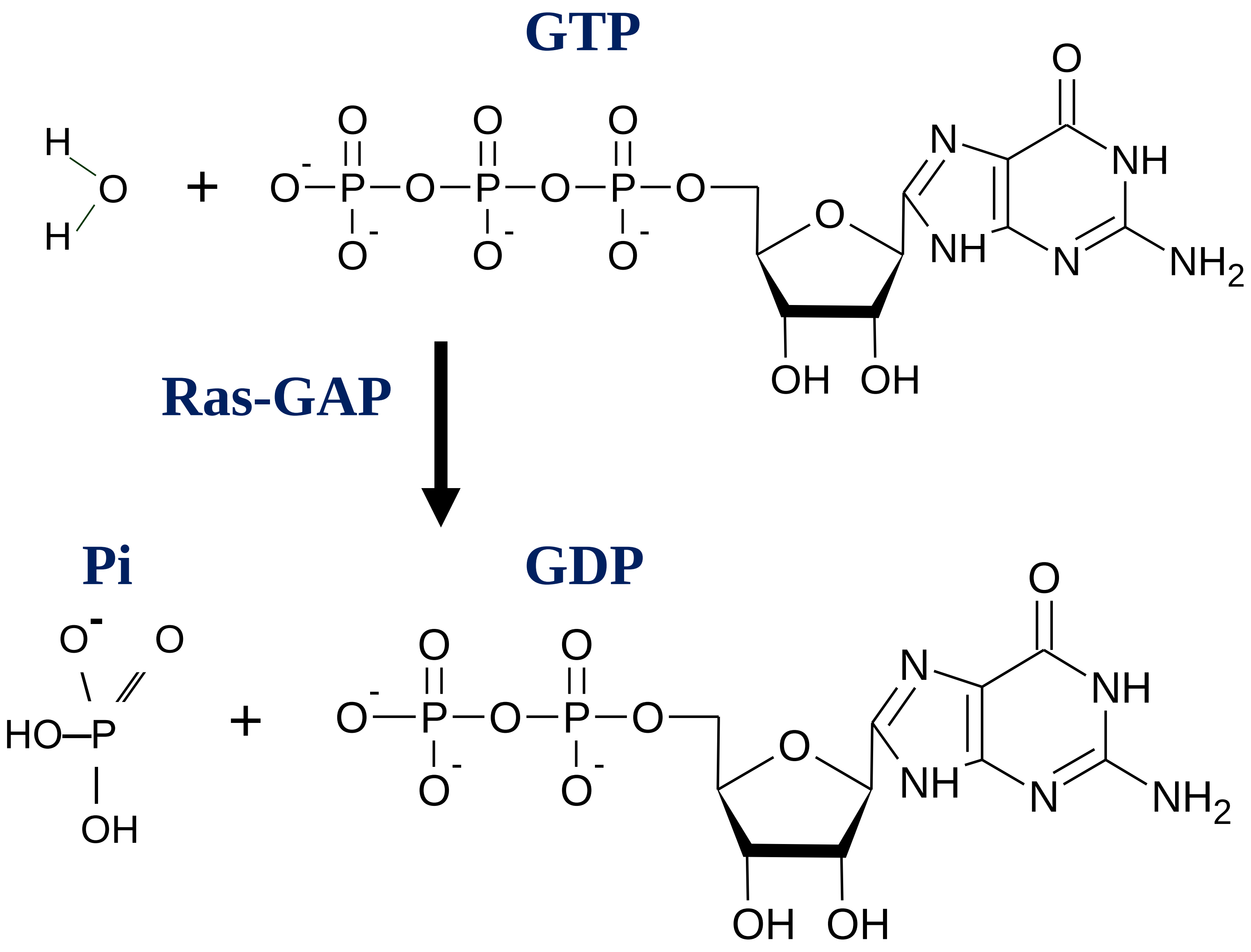
Methods

- **QM(DFT)/MM for modeling bond breaking and making**
- **QM(CASSCF and above)/MM for modeling photoexcitation**
- **QM/MM MD**
- **Classical MD including Markov State Model and Dynamical Network Analysis**

Programs: GAMESS(US)/TINKER
QChem, NWChem, CP2K, ORCA
NAMD

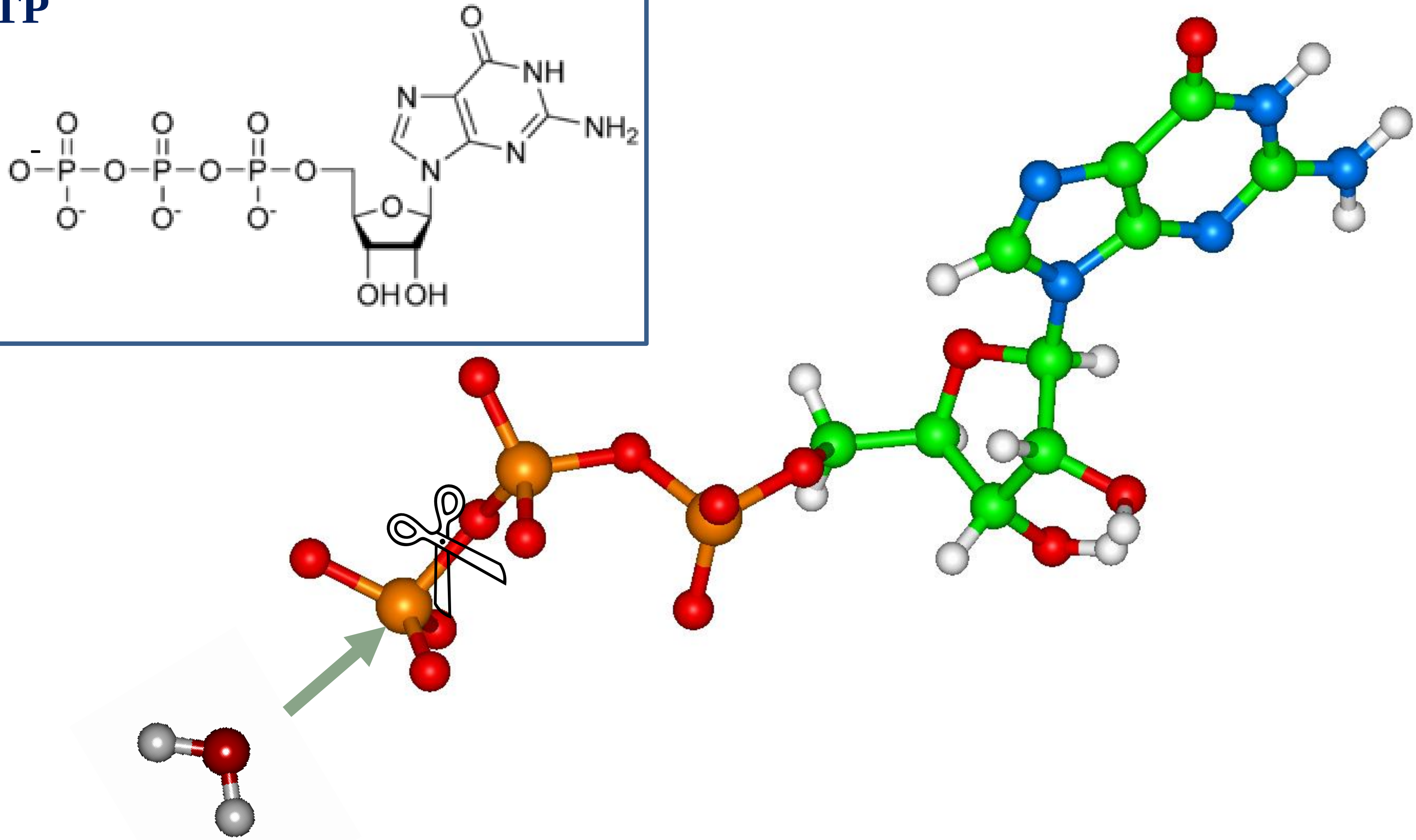
Initial source of atomic coordinates: Protein Data Bank (PDB) entries

Application: GTP hydrolysis by Ras-GAP

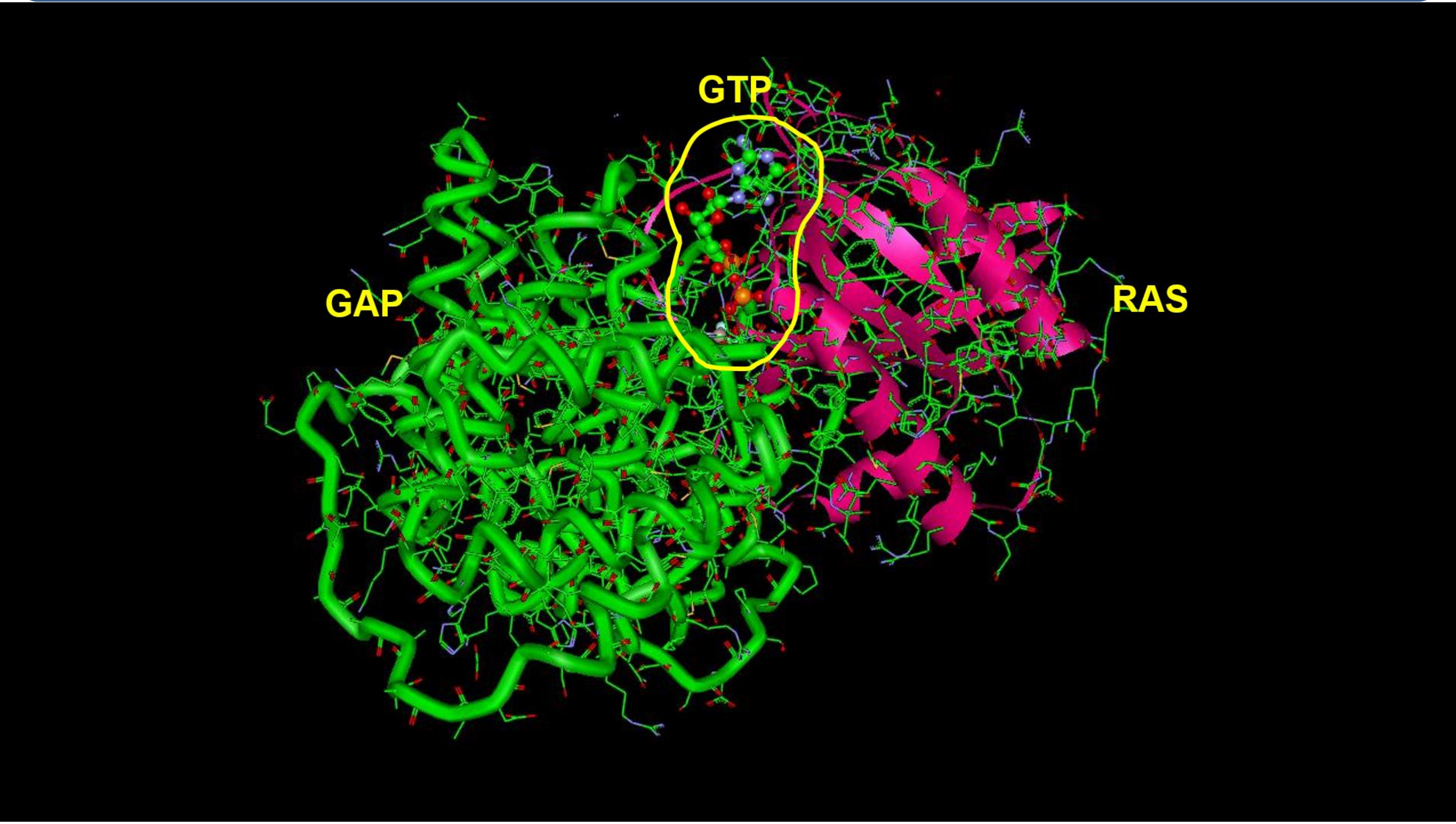


Application: GTP hydrolysis by Ras-GAP:

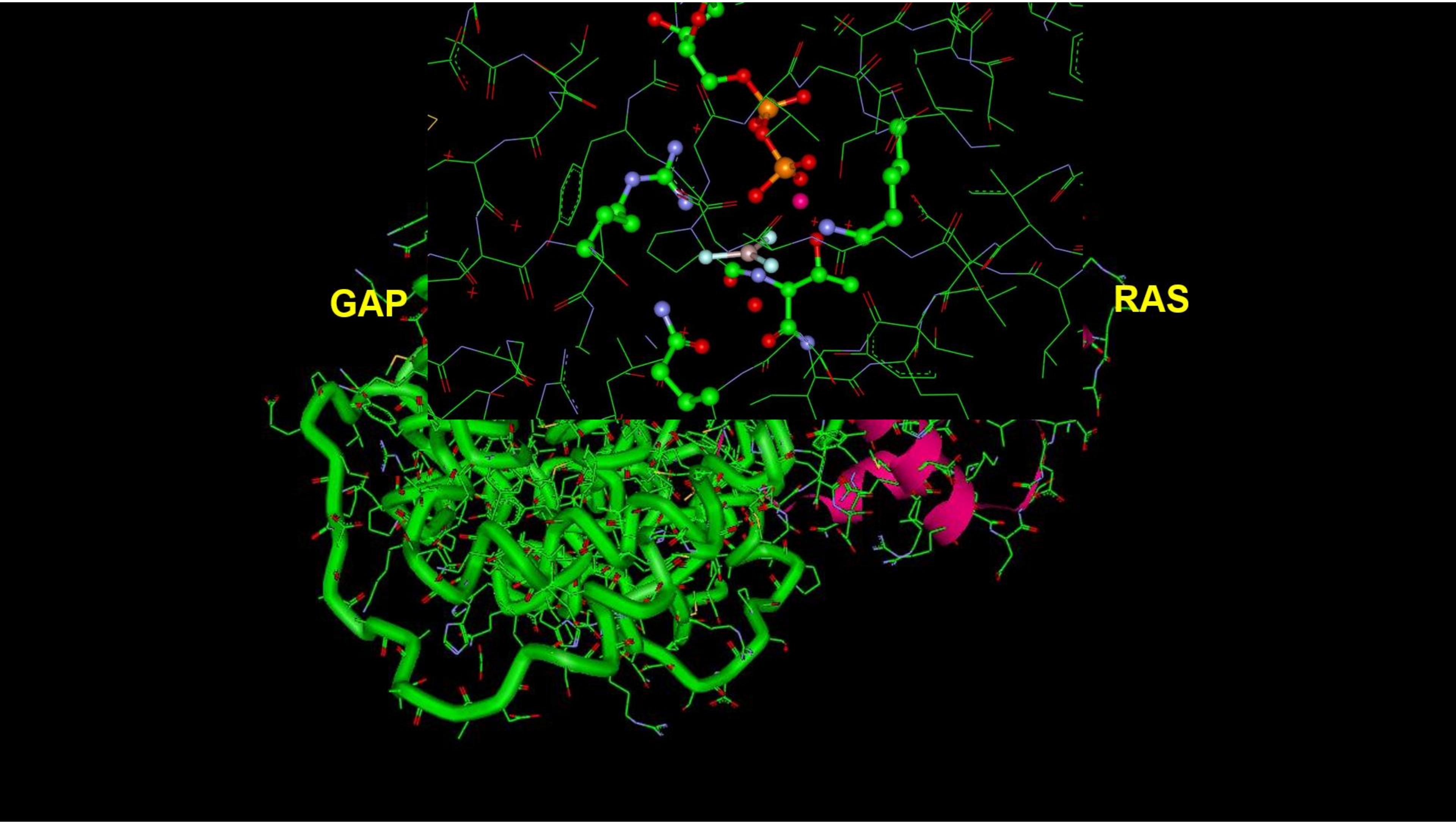
GTP



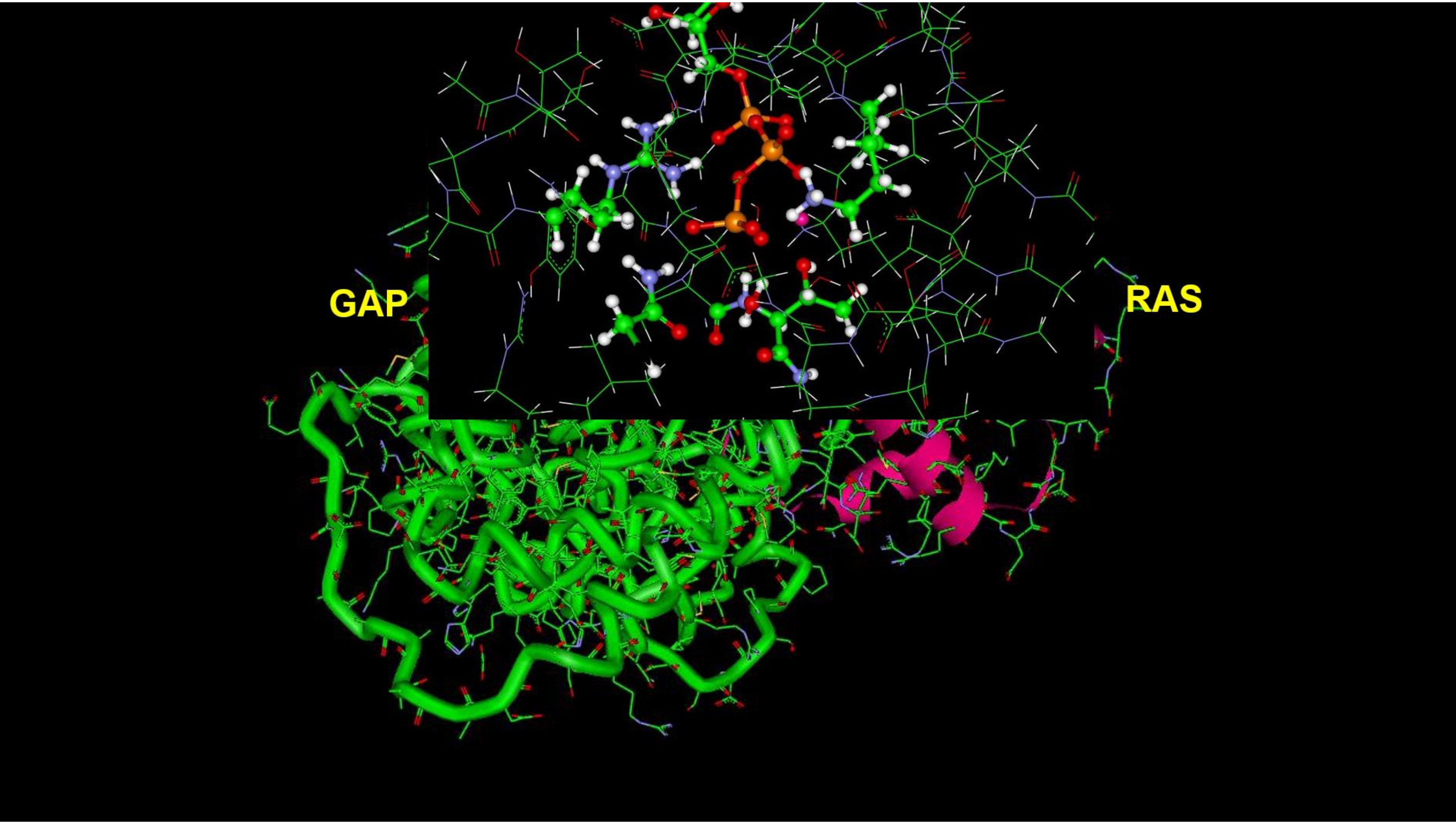
GTP hydrolysis catalyzed by Ras-GAP



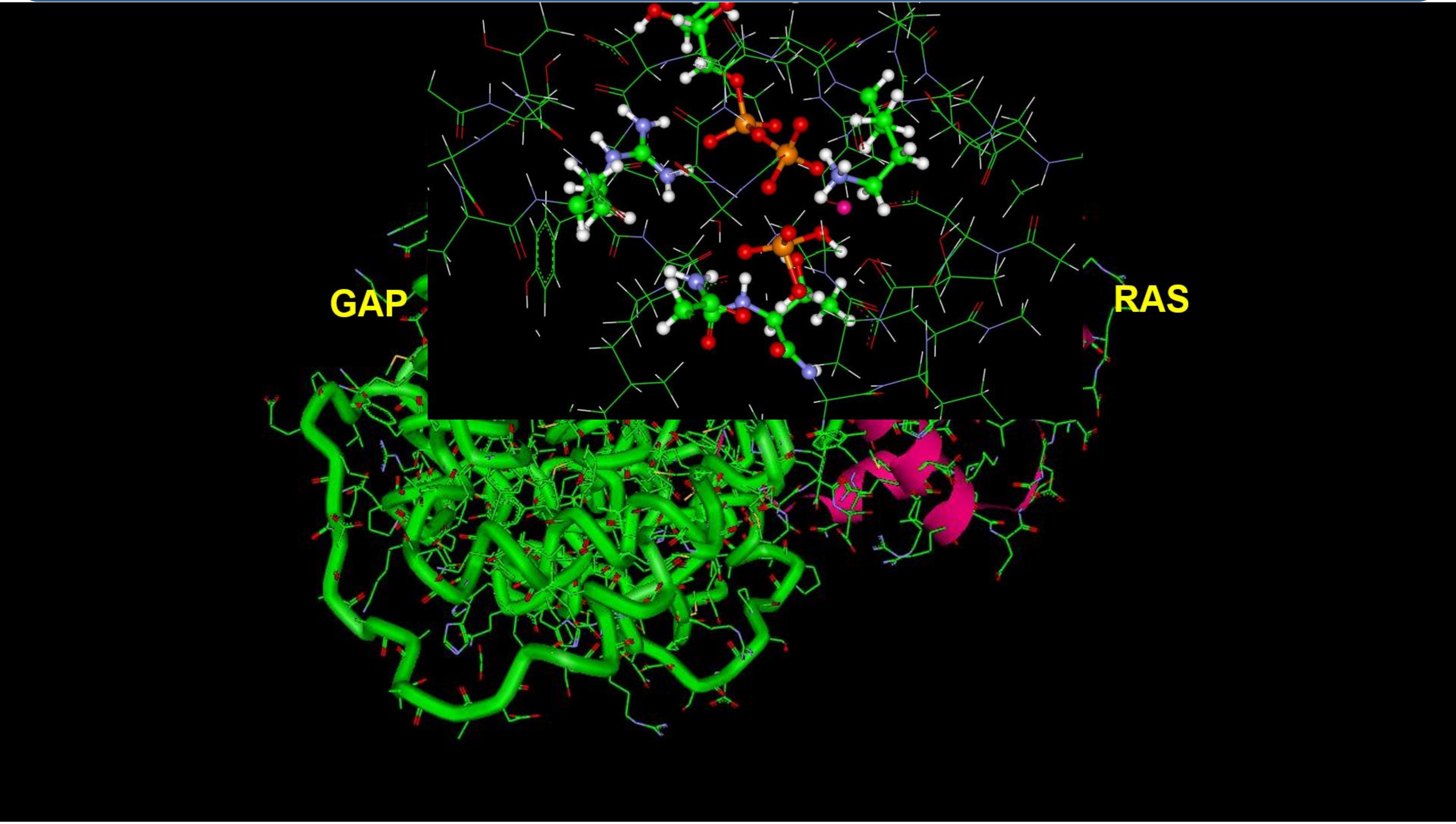
GTP hydrolysis in Ras-GAP: Initial PDB structure



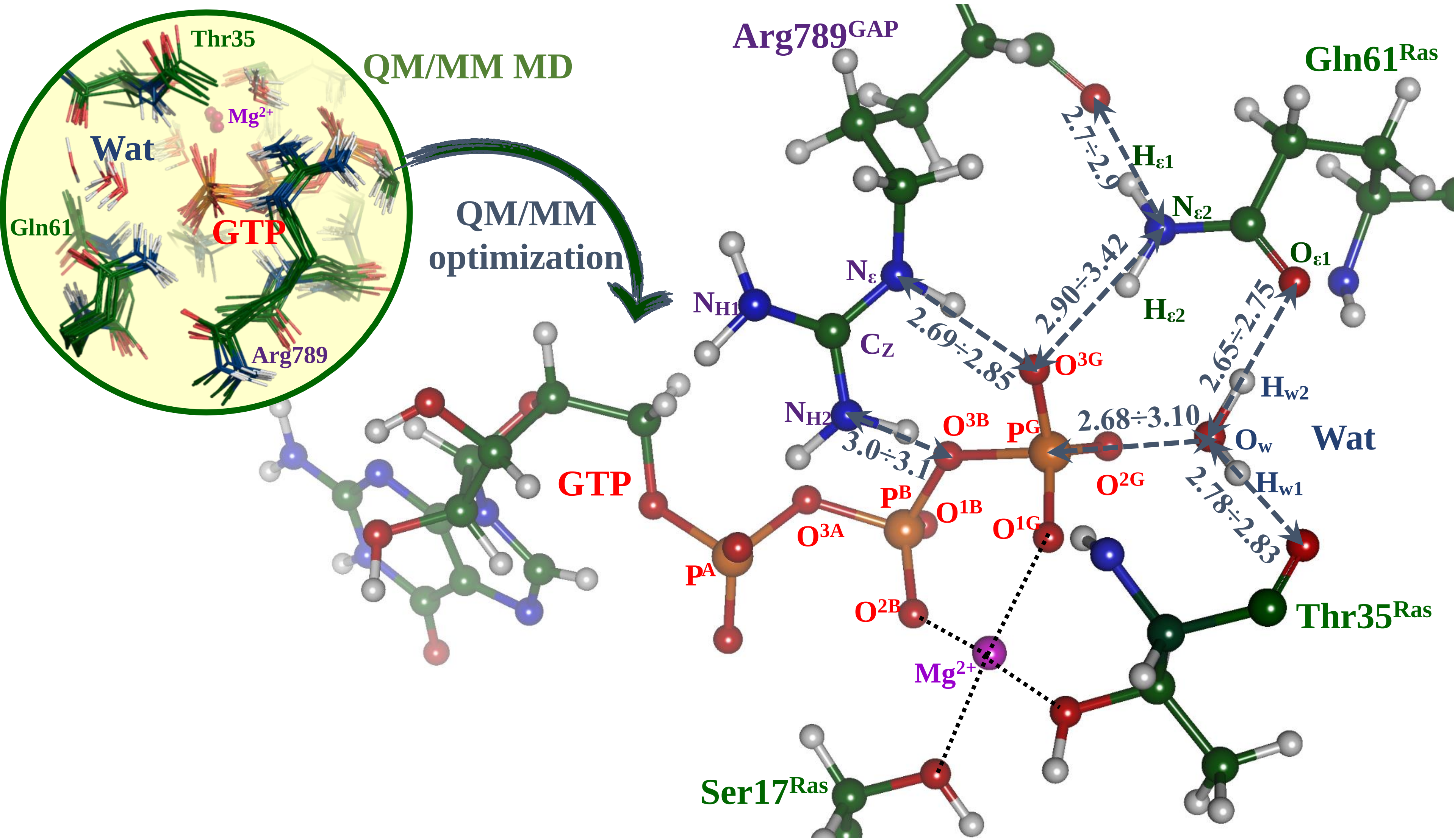
GTP hydrolysis in Ras-GAP: ES complex restored



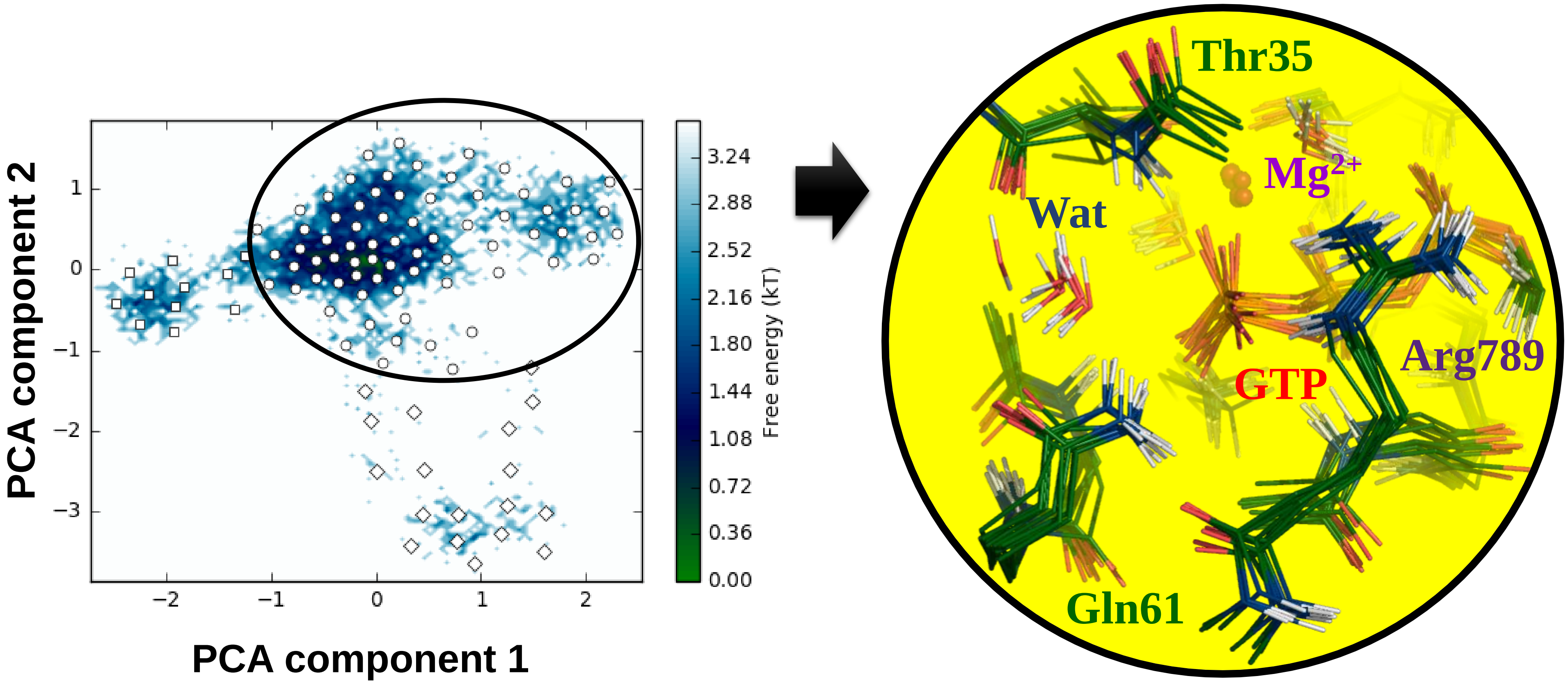
GTP hydrolysis in Ras-GAP: Reaction products



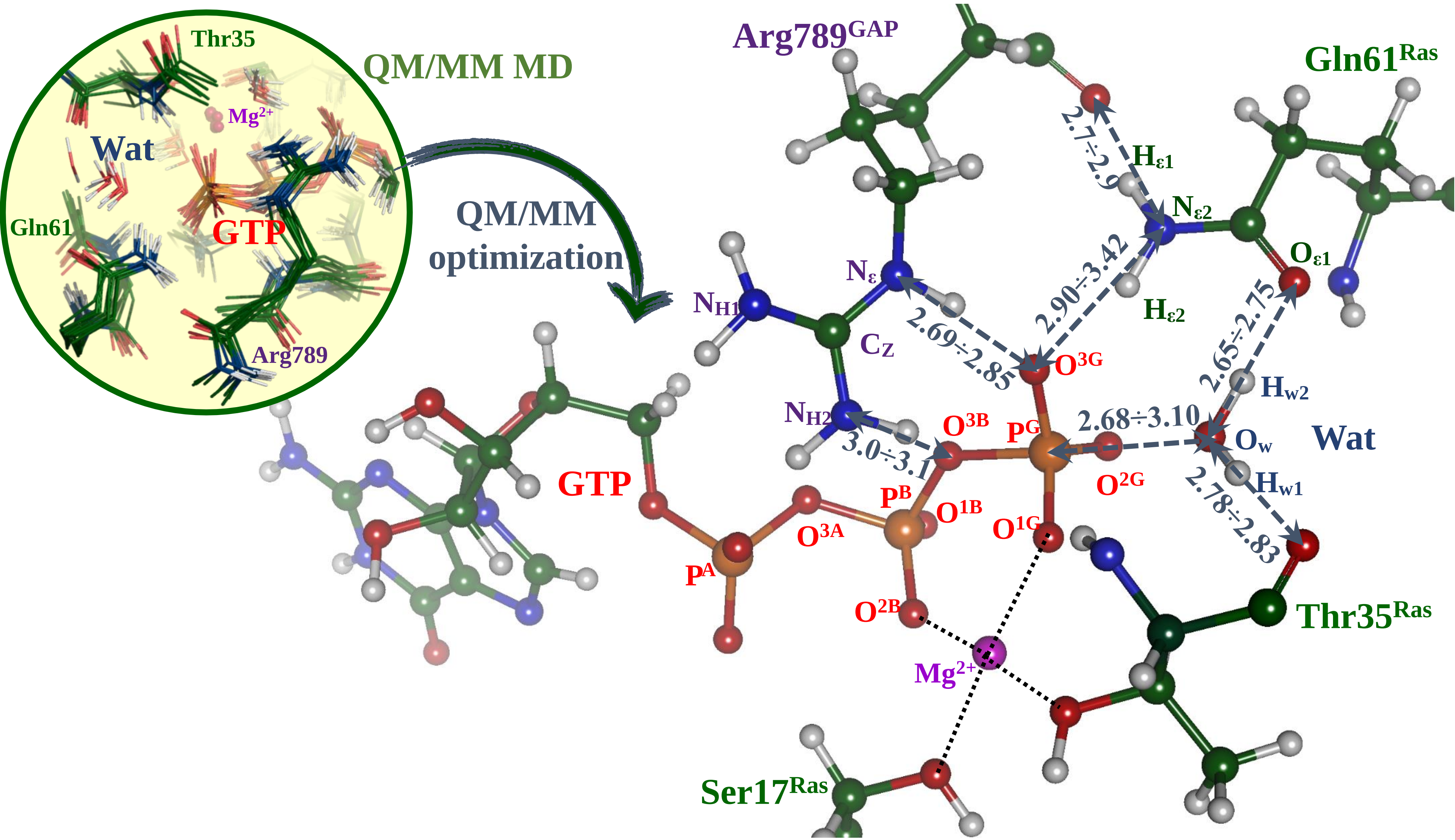
The active site: Molecular model of ES



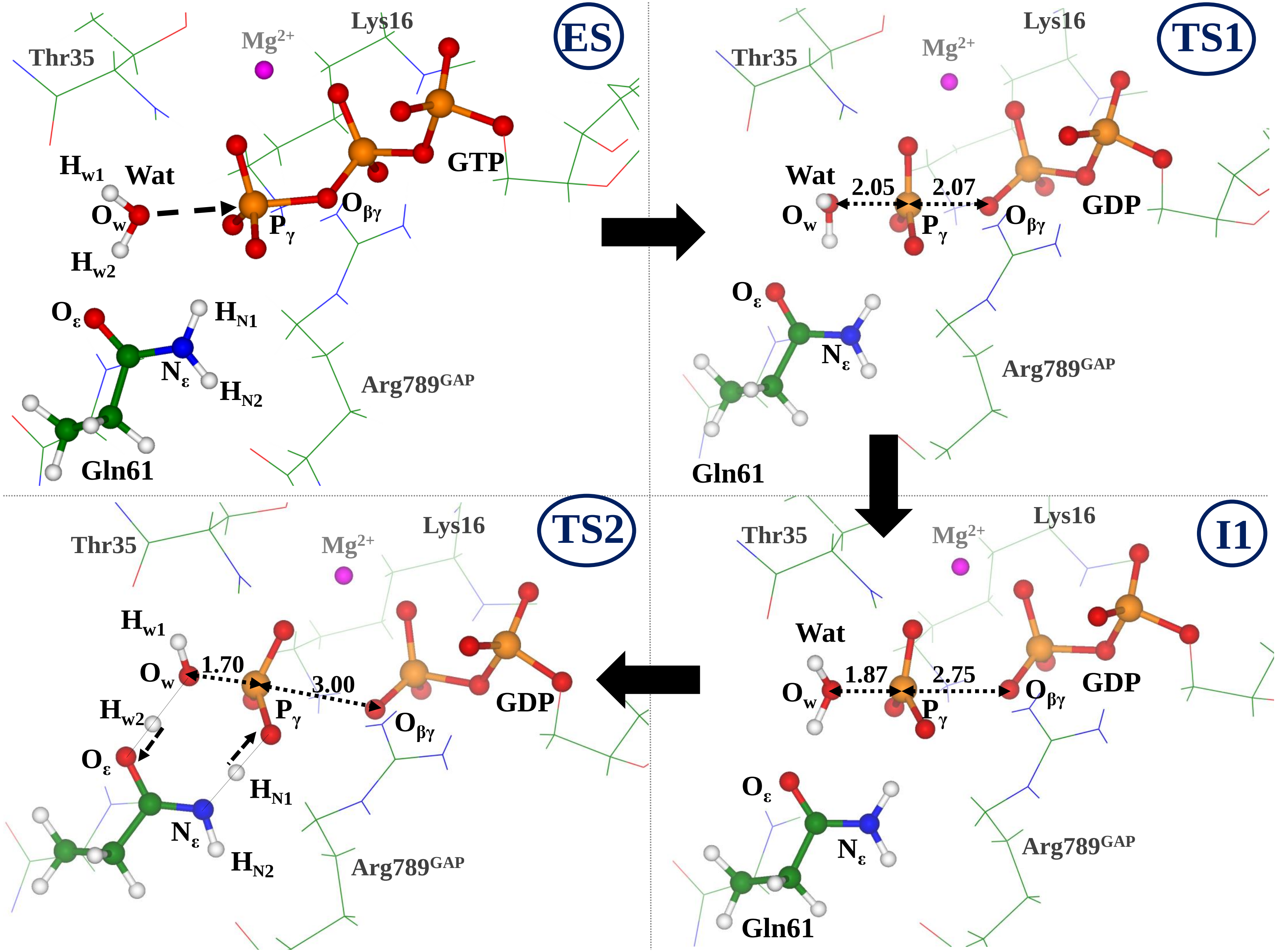
On the use of QM/MM MD



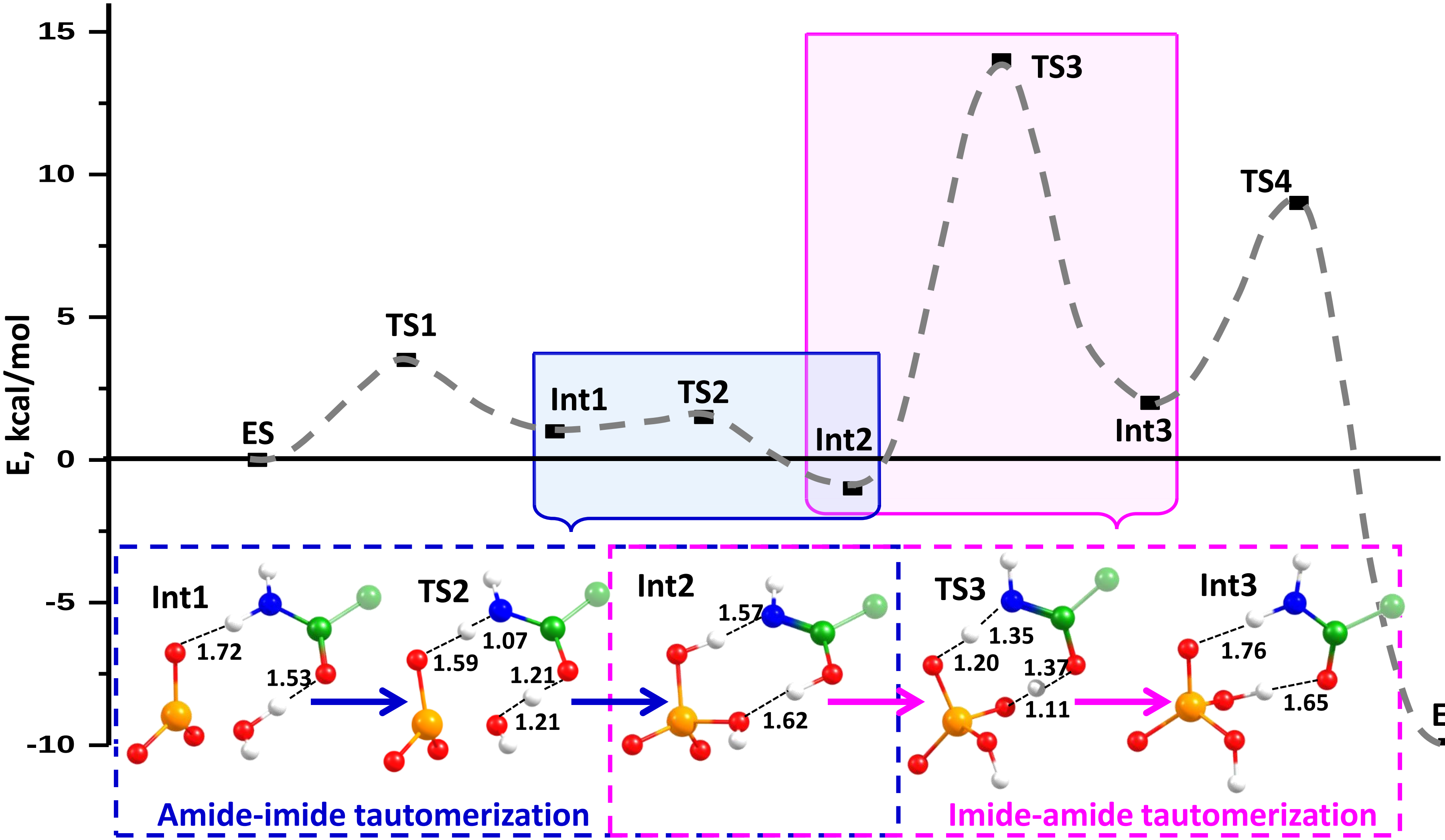
The active site: Molecular model of ES



Molecular mechanism

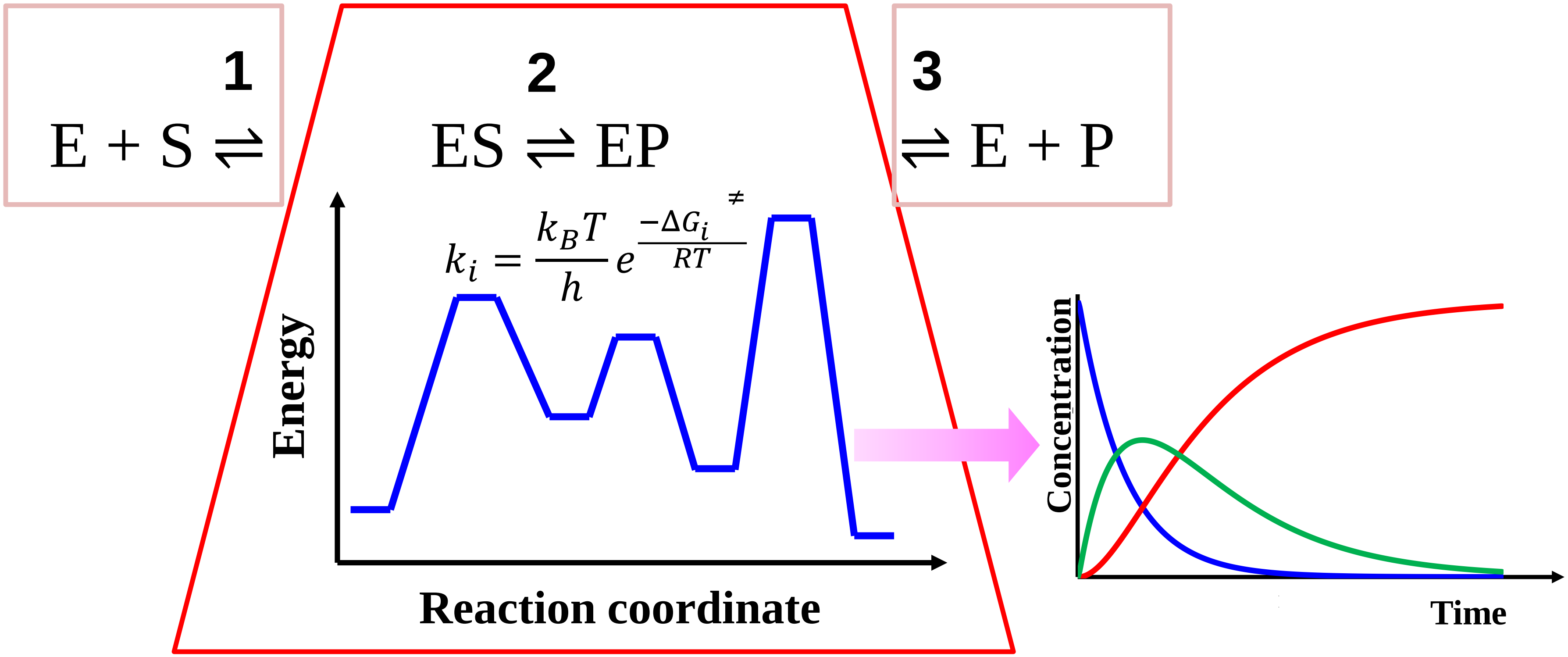


GTP hydrolysis by Ras-GAP: Gln amide-imide tautomerization

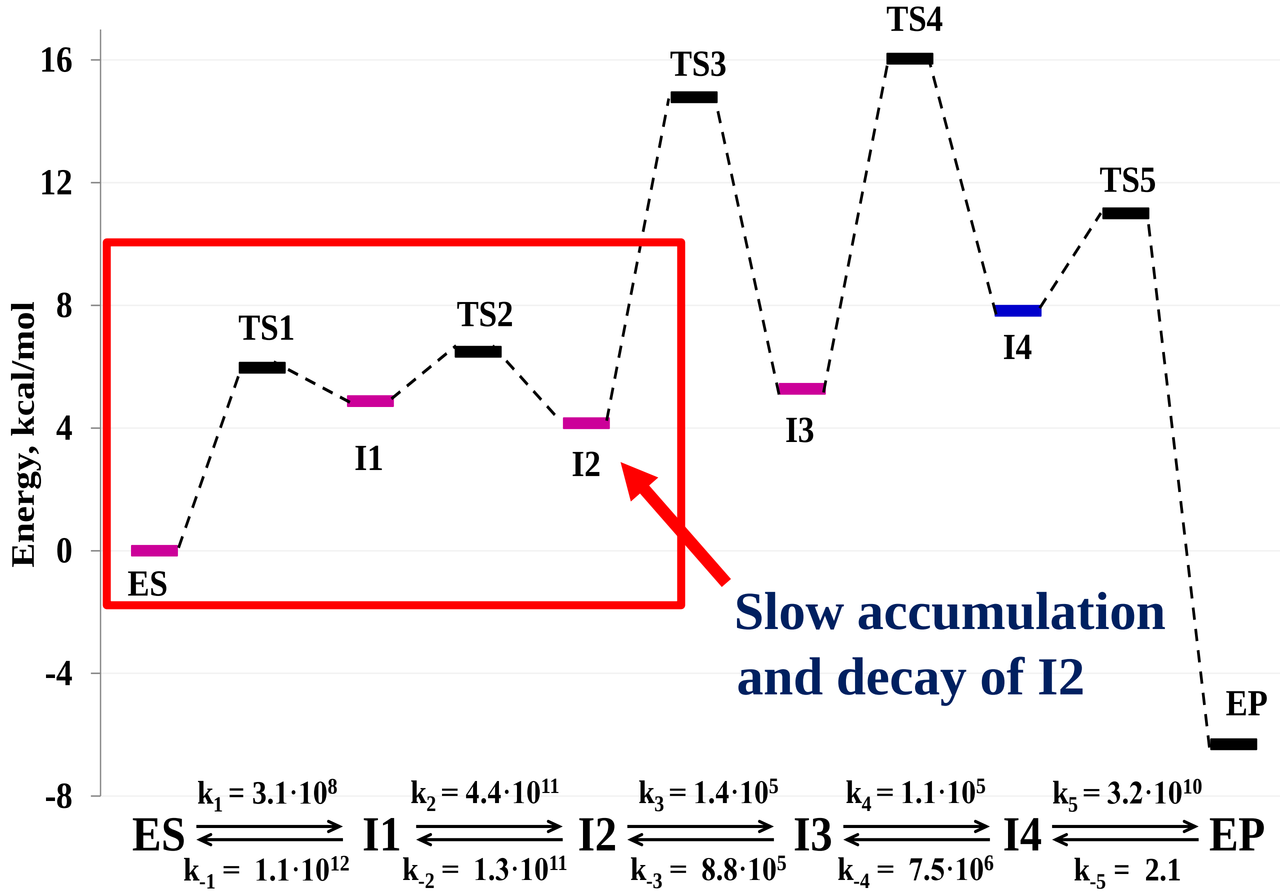


Towards direct simulation of kinetics curves

$$E + S \overset{1}{\rightleftharpoons} ES \overset{2}{\rightleftharpoons} EP \overset{3}{\rightleftharpoons} E + P$$



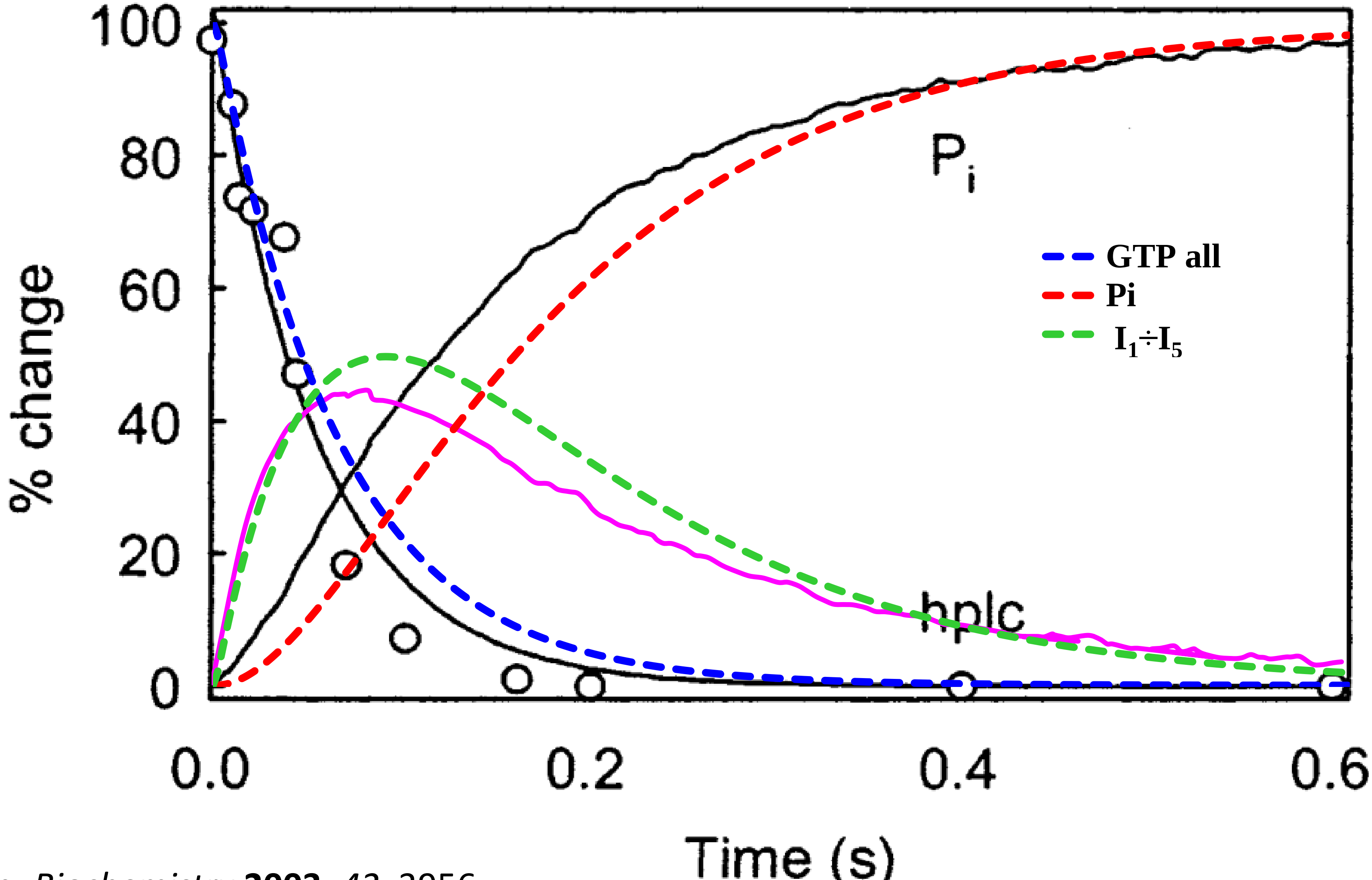
GTP hydrolysis by Ras-GAP: Modeling transient kinetics



M. Khrenova, B. Grigorenko, A. Kolomeisky, A. Nemukhin
Hydrolysis of Guanosine Triphosphate (GTP) by the Ras·GAP Protein Complex: Reaction Mechanism and Kinetic Scheme // J. Phys. Chem. B 119 (2015) 12838.

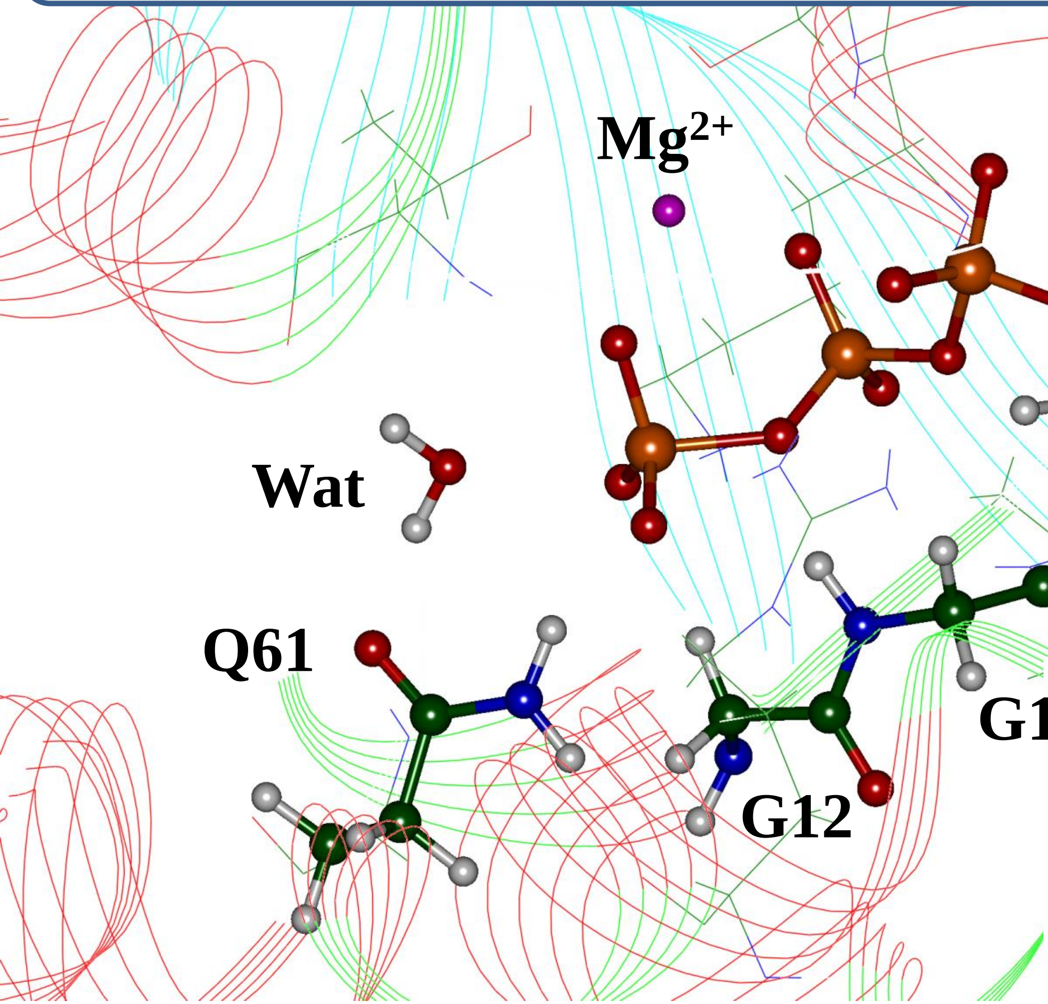
GTP hydrolysis by Ras-GAP: Direct comparison exp vs calc

Calculated $k_{eff} = 15\ s^{-1}$ (exp $19.5\ s^{-1}$)



Exp: *Biochemistry* **2003**, *42*, 3956

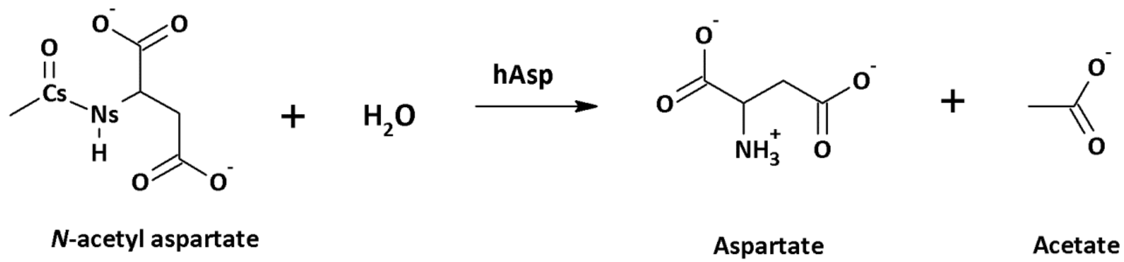
Why GTP hydrolysis catalyzed by Ras-GAP is important?

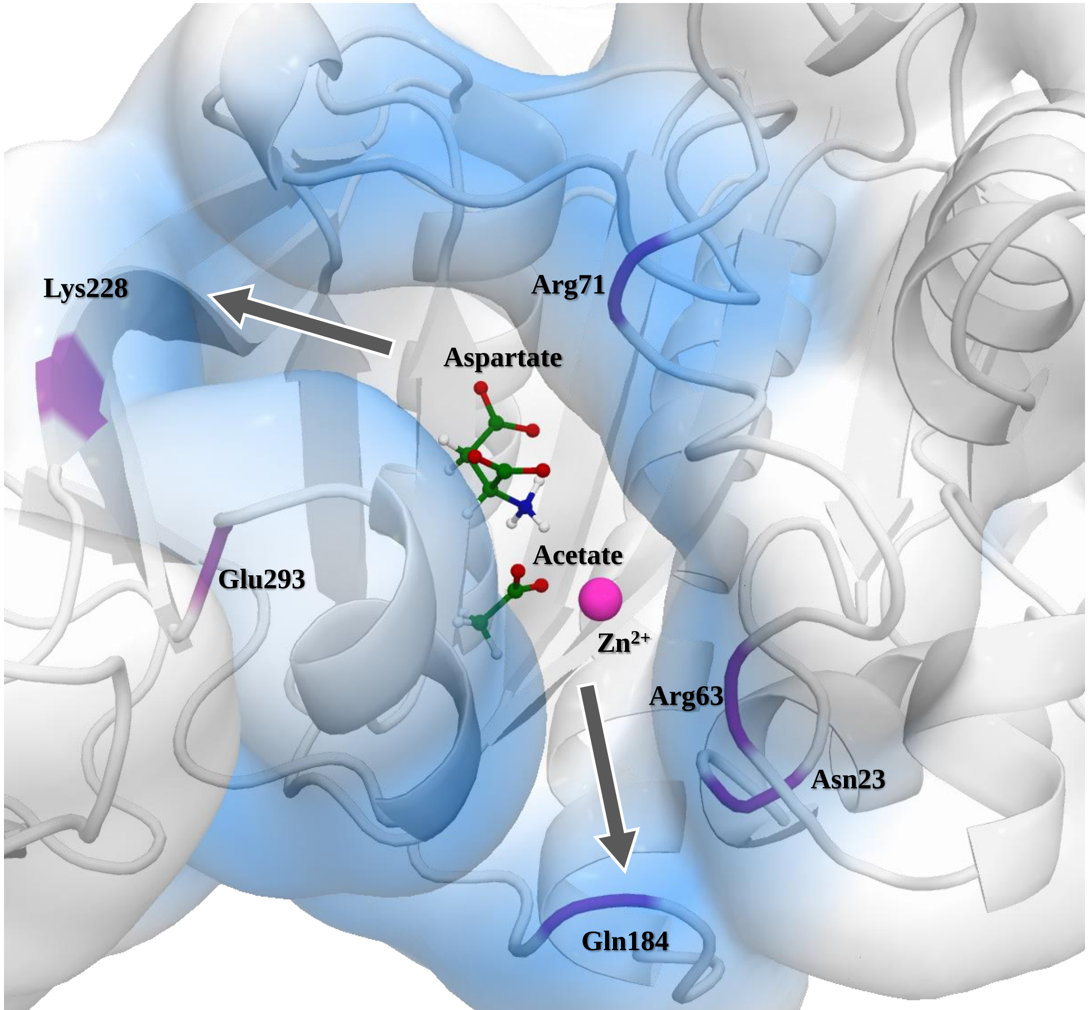


G12 (Gly12)	G13 (Gly13)	Q61 (Gln61)
G12V (239)	G13R (23)	Q61R (81)
G12S (52)	G13D (9)	Q61L (66)
G12D (34)	G13V (9)	Q61K (31)
G12C (16)	G13S (8)	Q61H (13)
G12R (12)	G13C (5)	Q61J (6)
G12A (3)	G13G (1)	Q61P (3)
		Q61R (2)
		Q61E (1)
		Q61Q (1)
		Q61R (1)

Oncogenic mutations

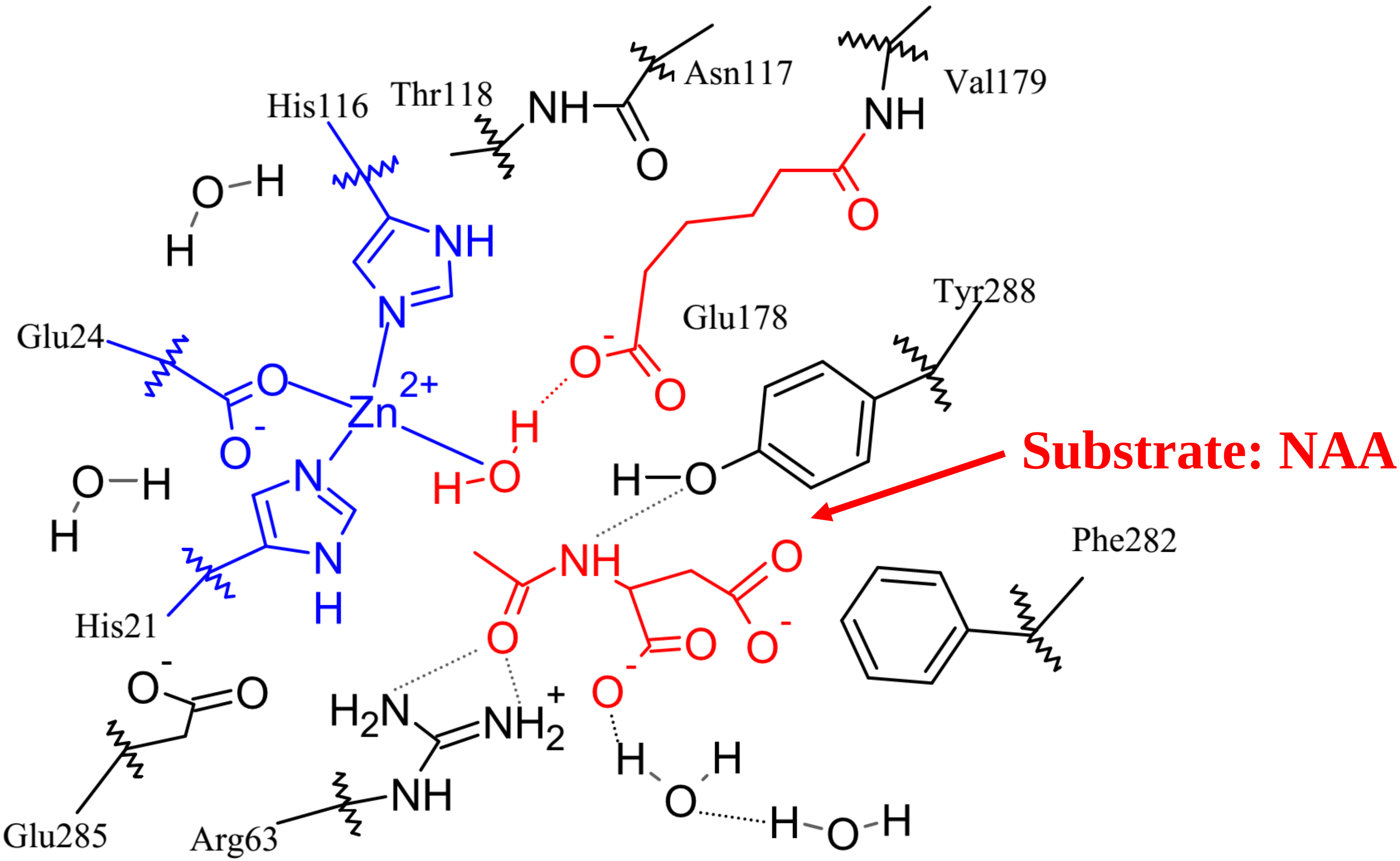
Human Aspartoacylase (hAsp): Processes in human brain



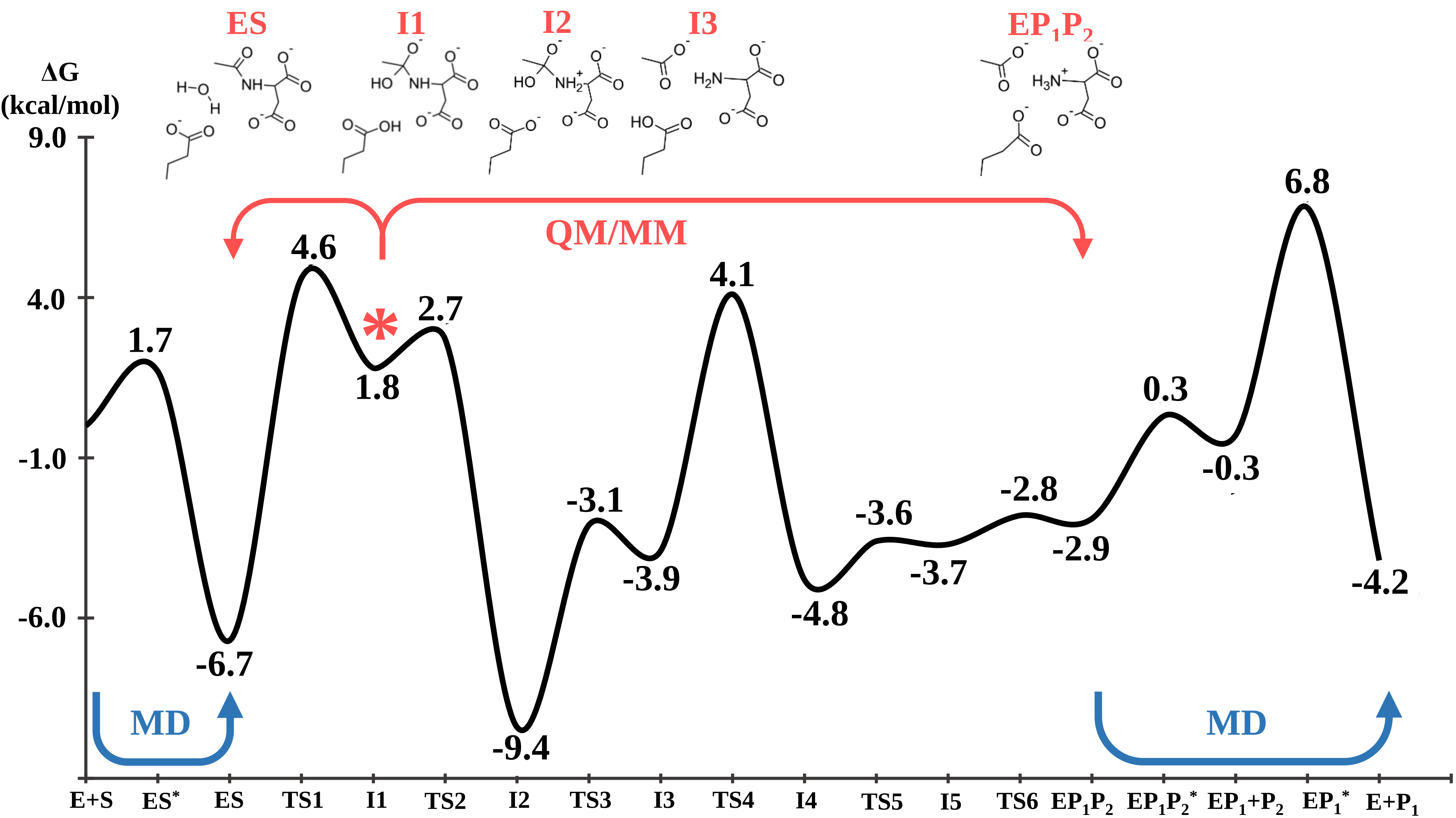


Full Catalytic Cycle of hAsp: Chemistry at the active site

Enzyme active site (QM-subsystem in simulations)

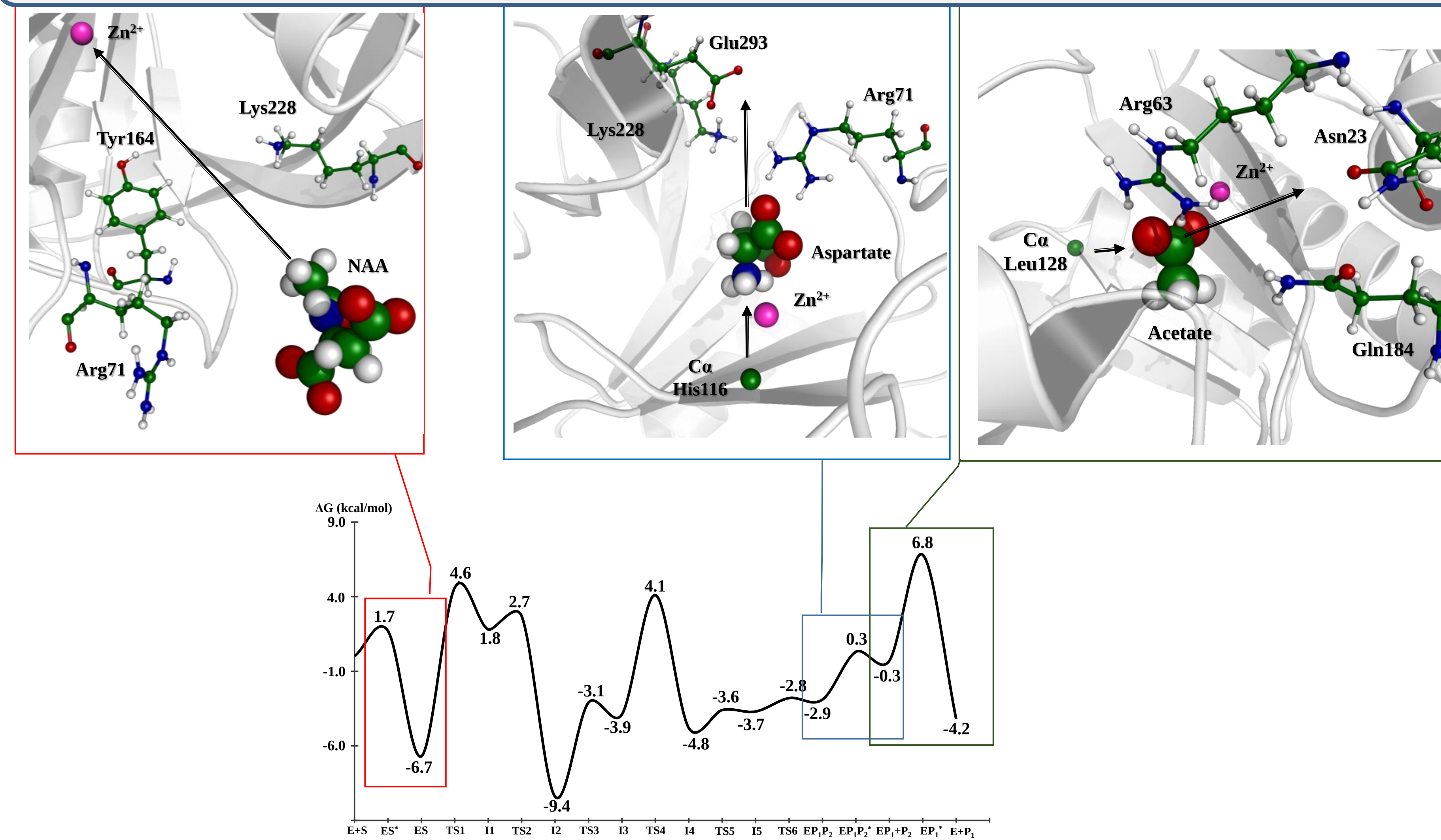


Full Catalytic Cycle of Aspartoacylase



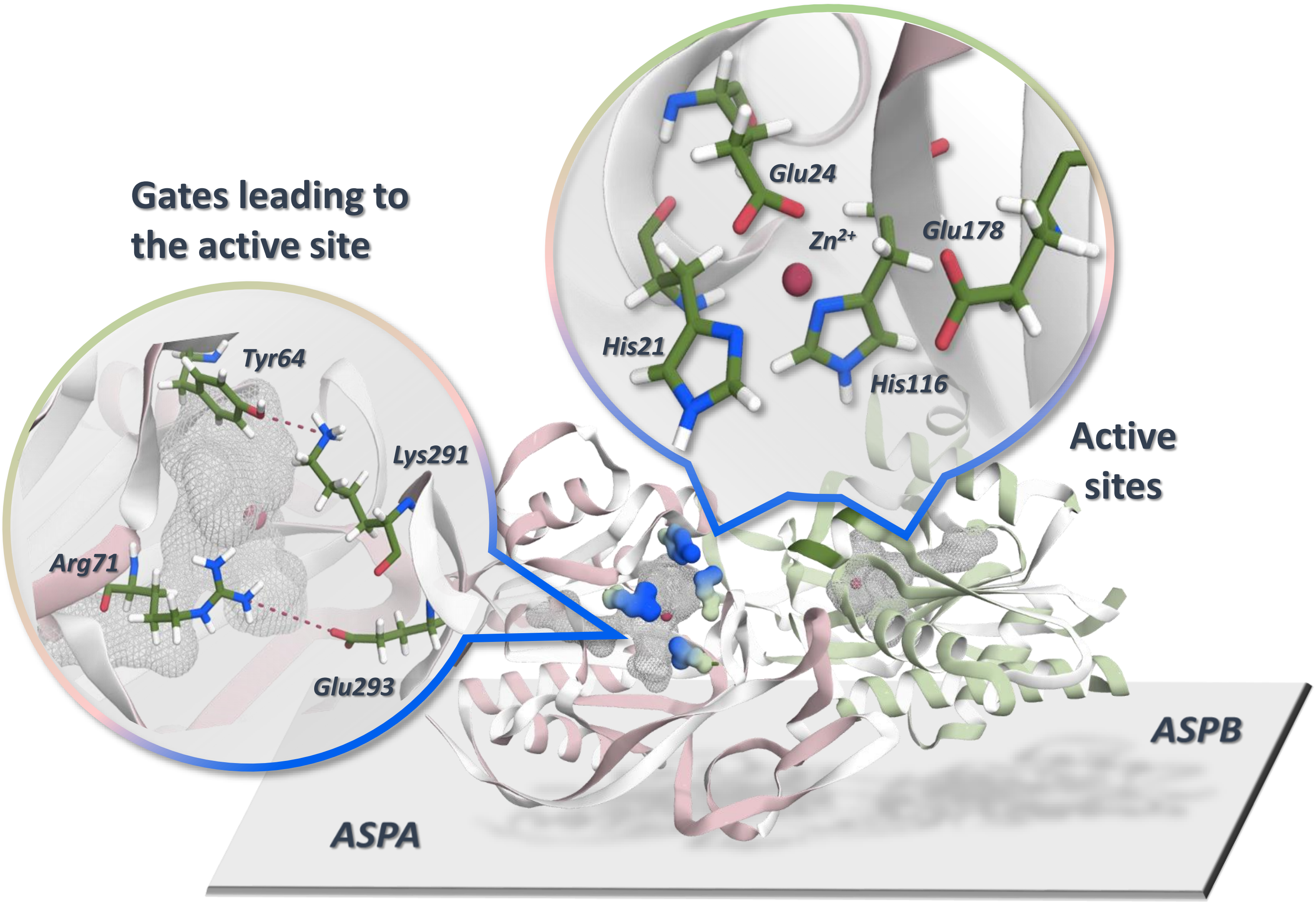
E. Kots, M. Khrenova, S. Lushchekina, S. Varfolomeev, B. Grigorenko, A. Nemukhin, Modeling the Complete Catalytic Cycle of Aspartoacylase // J. Phys. Chem. B 120 (2016) 4221.

Substrate deposition and product release

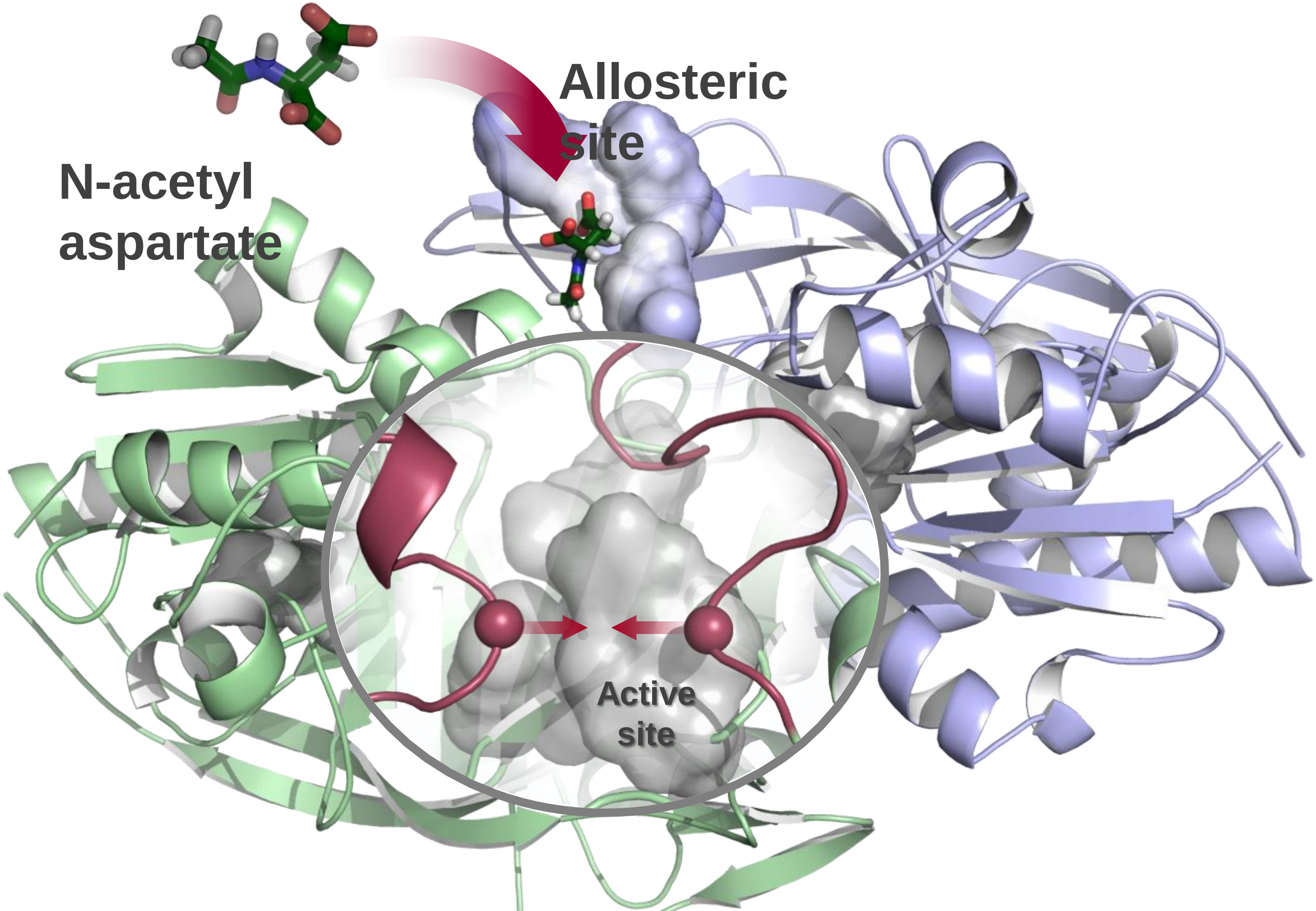


MD calculations with the replica-exchange umbrella sampling technique

hAsp appears as a dimer composed of monomers ASPA, ASPB



Substrate binding at allosteric sites controls entrance to the active site

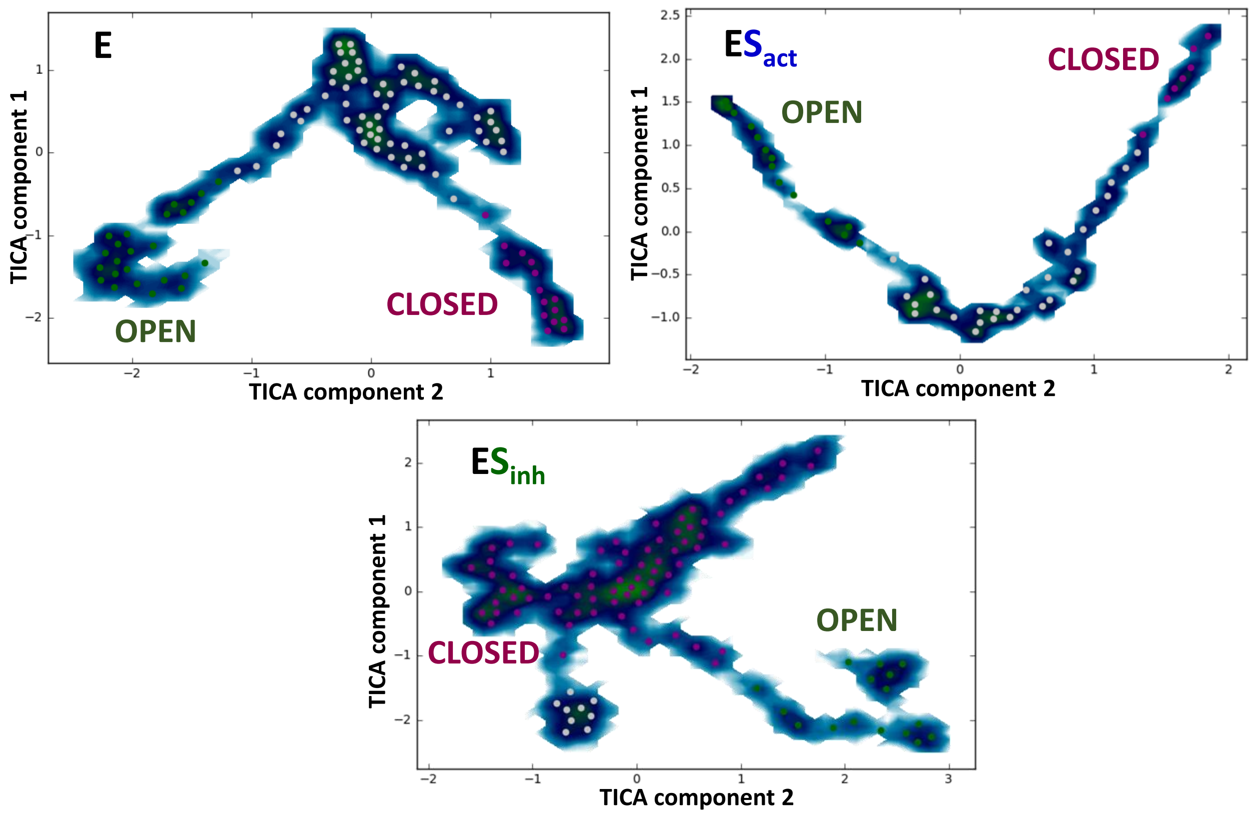


E. Kots, S. Lushchekina, S. Varfolomeev, A. Nemukhin, Role of the Protein Dimeric Interface in Allosteric Inhibition of N-Acetyl-Aspartate Hydrolysis by Human Aspartoacylase // J. Chem. Inf. Model. , 2017, 57, 1999

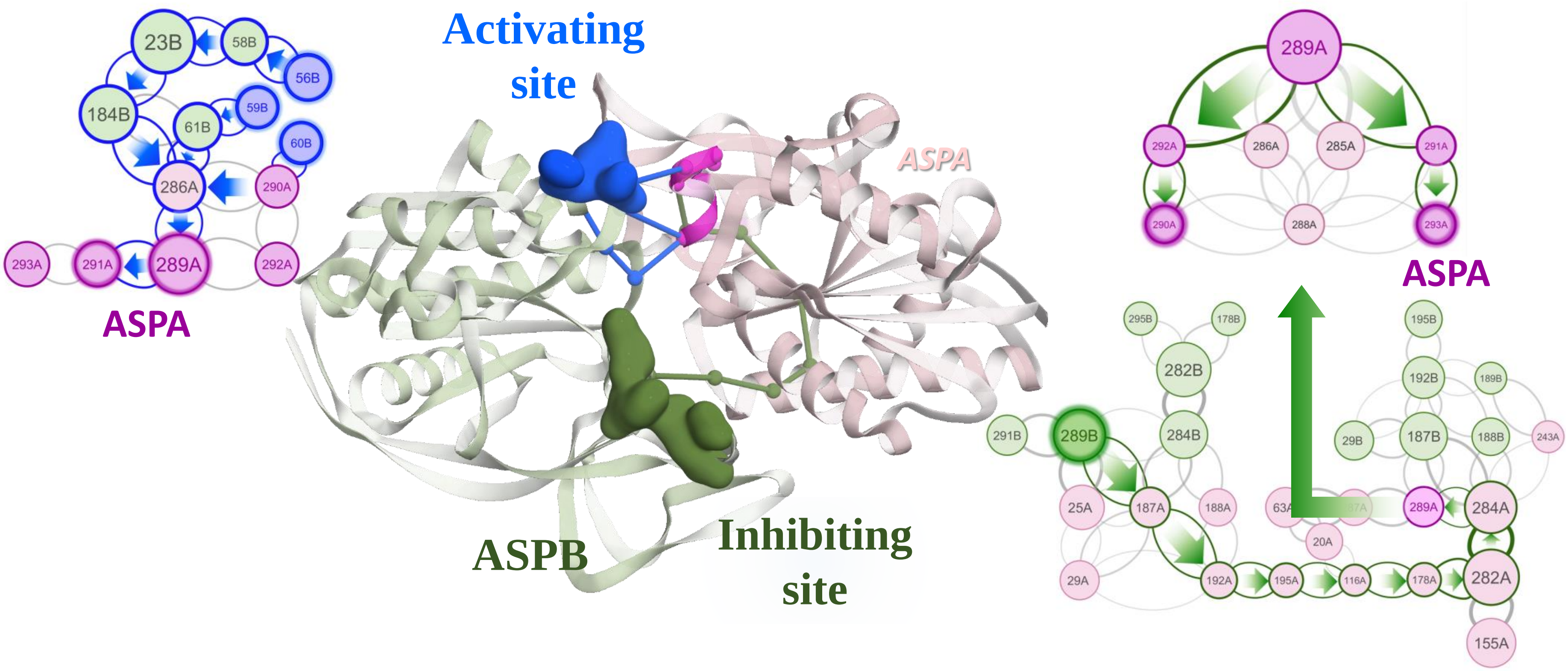
Markov State Model for transitions between conformations with open and closed gates to the active site

$$[\mathrm{CLOSED}] \underset{}{\overset{k_o}{\rightleftharpoons}} [\mathrm{OPEN}]$$

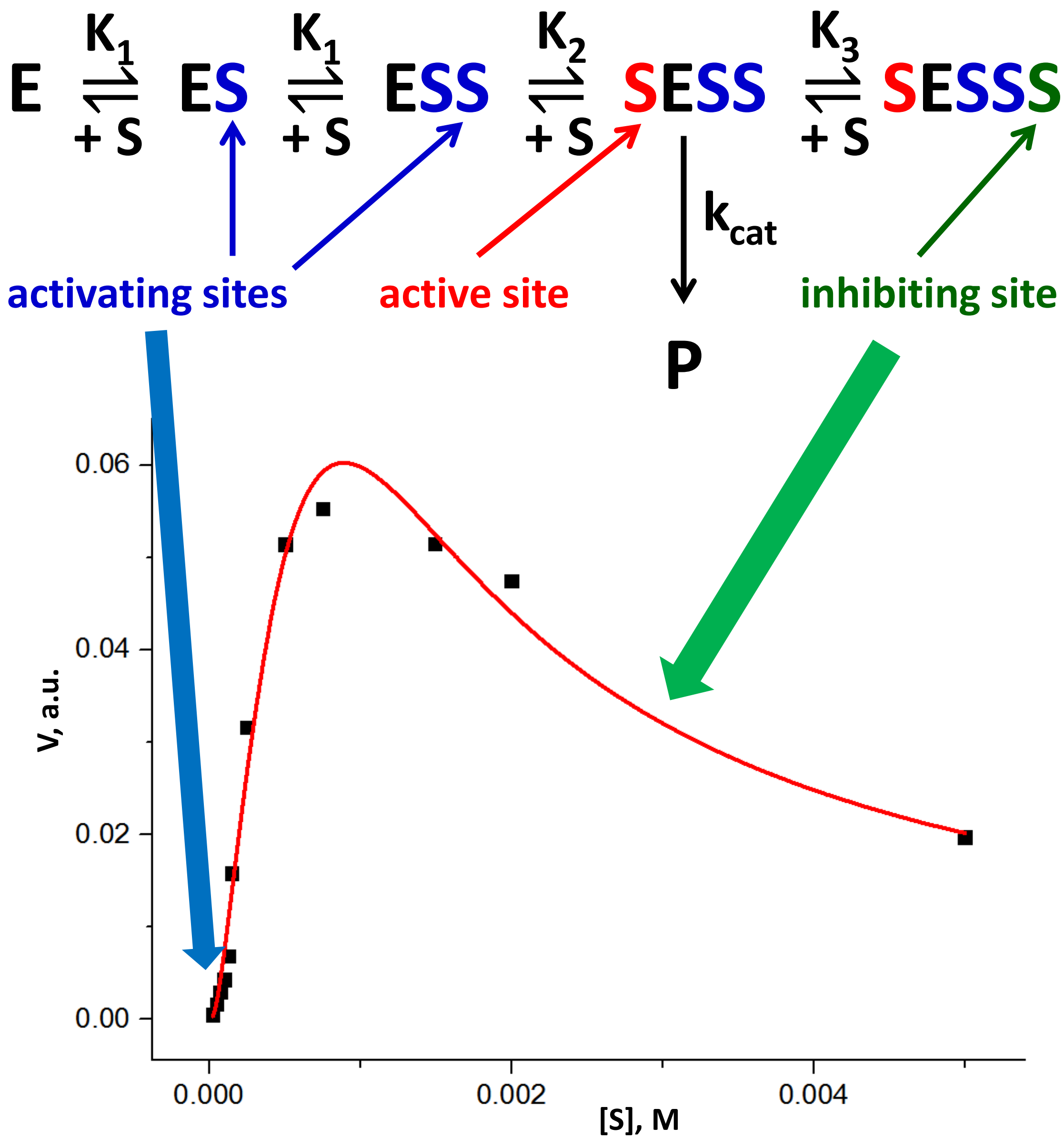
System	k_o, s^{-1}	k_c, s^{-1}	K
E	$6.7 \cdot 10^{6}$	$6.1 \cdot 10^{6}$	1.1
ES_{act}	$1.0 \cdot 10^{7}$	$5.0 \cdot 10^{5}$	20
ES_{inh}	$6.3 \cdot 10^{7}$	$1.1 \cdot 10^{9}$	0.06



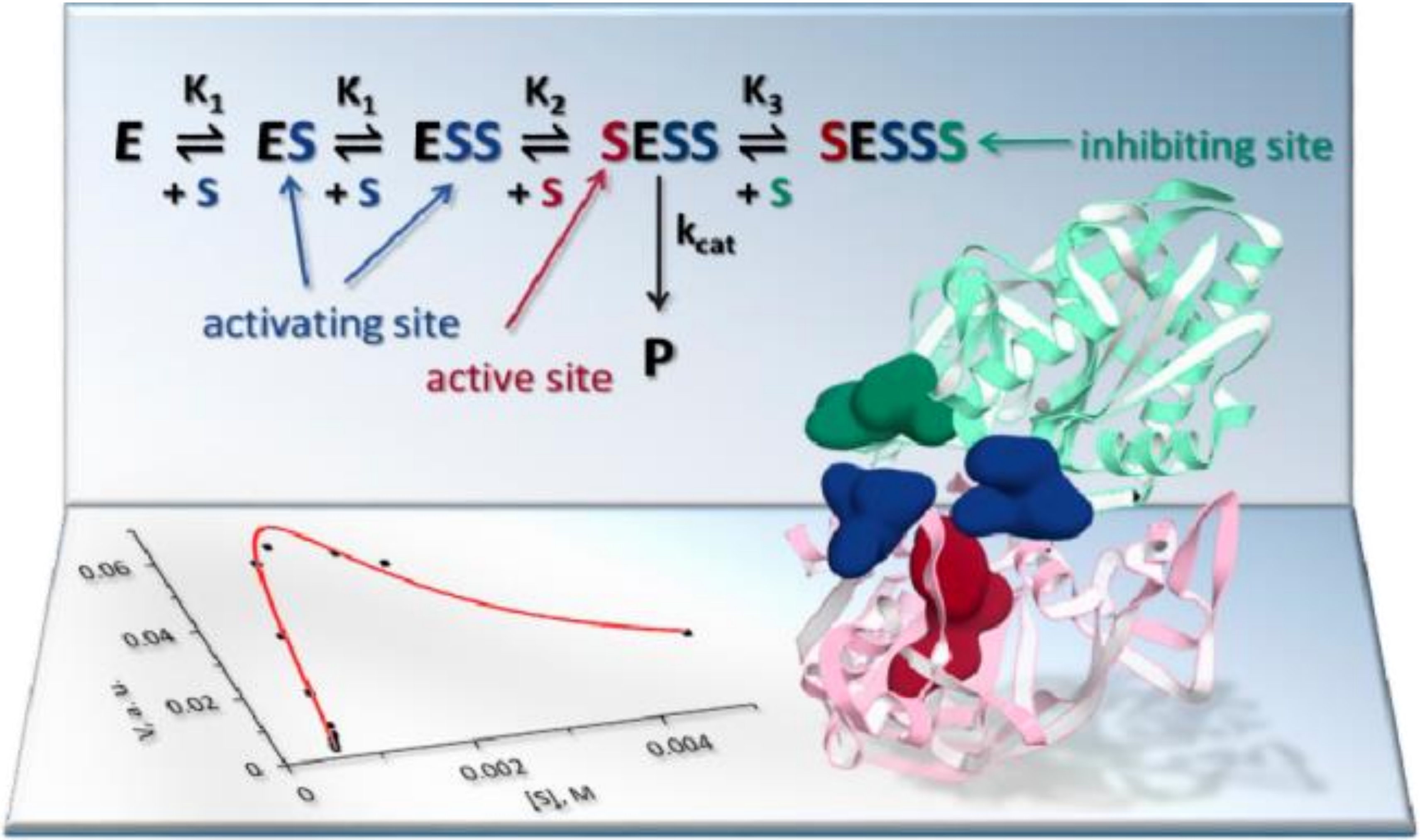
Substrate binding at allosteric sites controls entrance to the active site: Dynamic network analysis



Proposed kinetic mechanism: Quantitative description of kinetic data



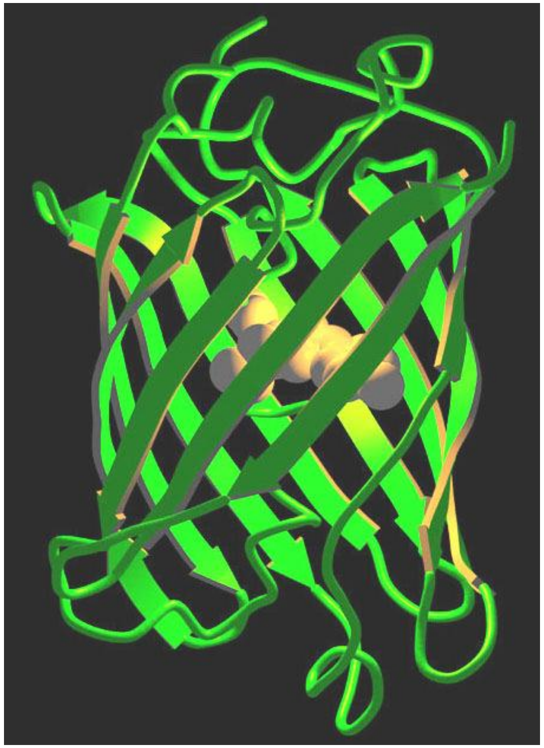
Three faces of N-acetylaspartate: activator, substrate, and inhibitor of human aspartoacylase

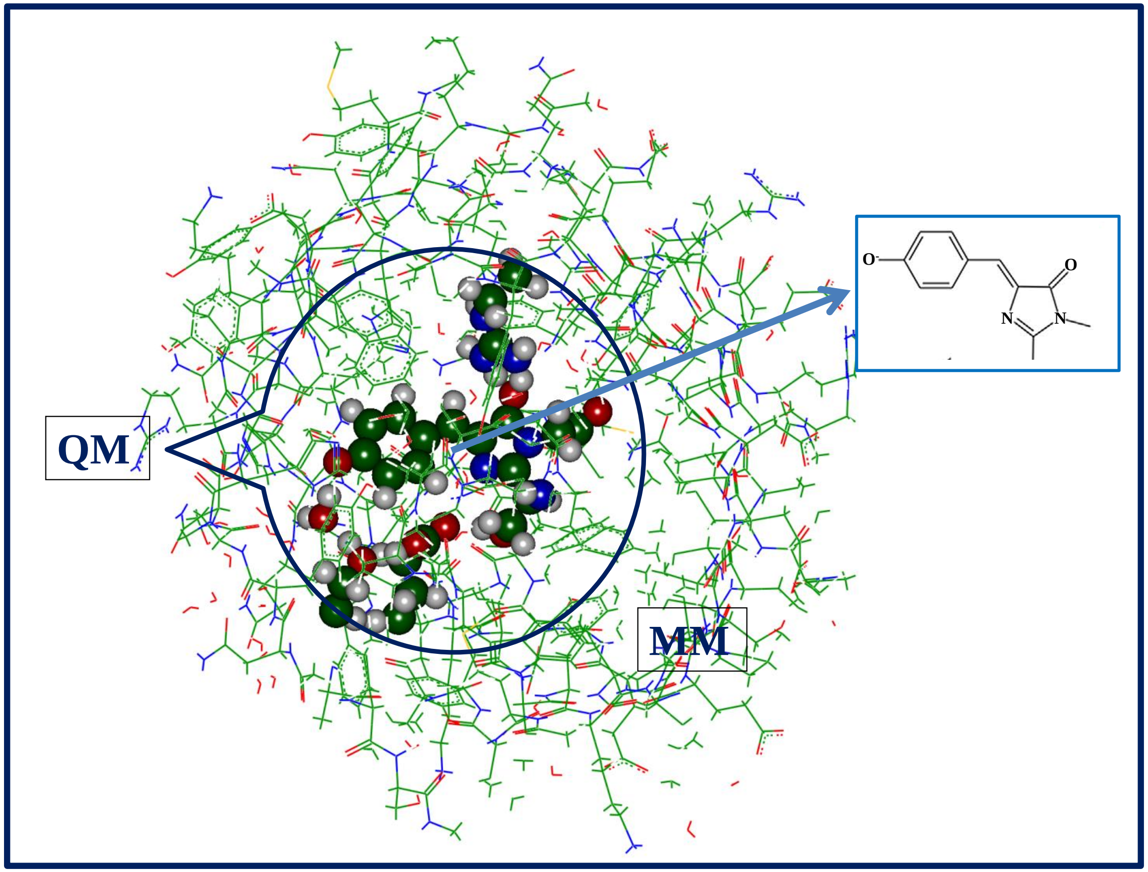


M. Khrenova, E. Kots, S. Varfolomeev, S. Lushchekina, A. Nemukhin, // J. Phys. Chem. B, 2017, 121, 9389

Chromophore formation and decomposition in the Green Fluorescent Protein (GFP)

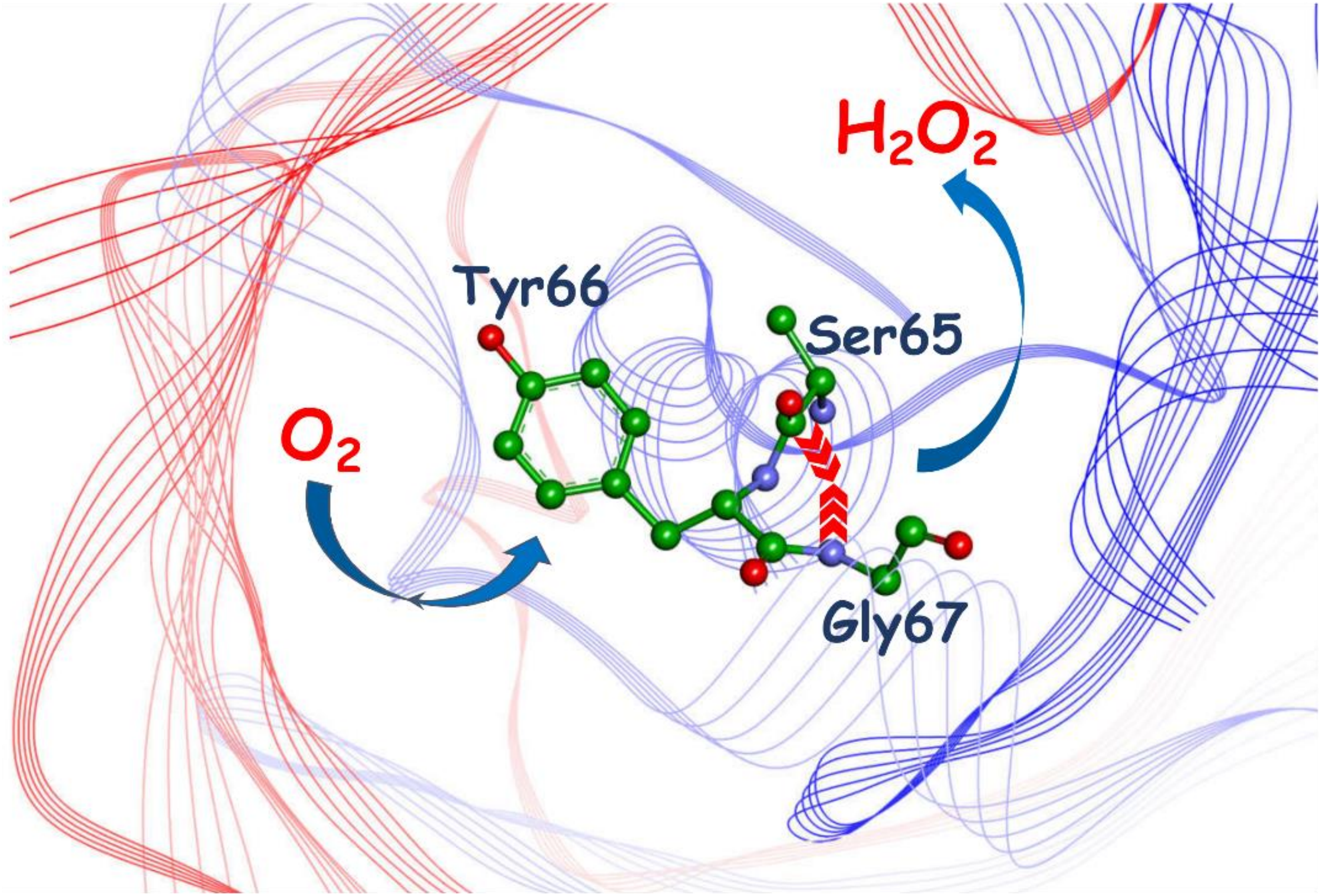
Part of the project on modeling fluorescent proteins



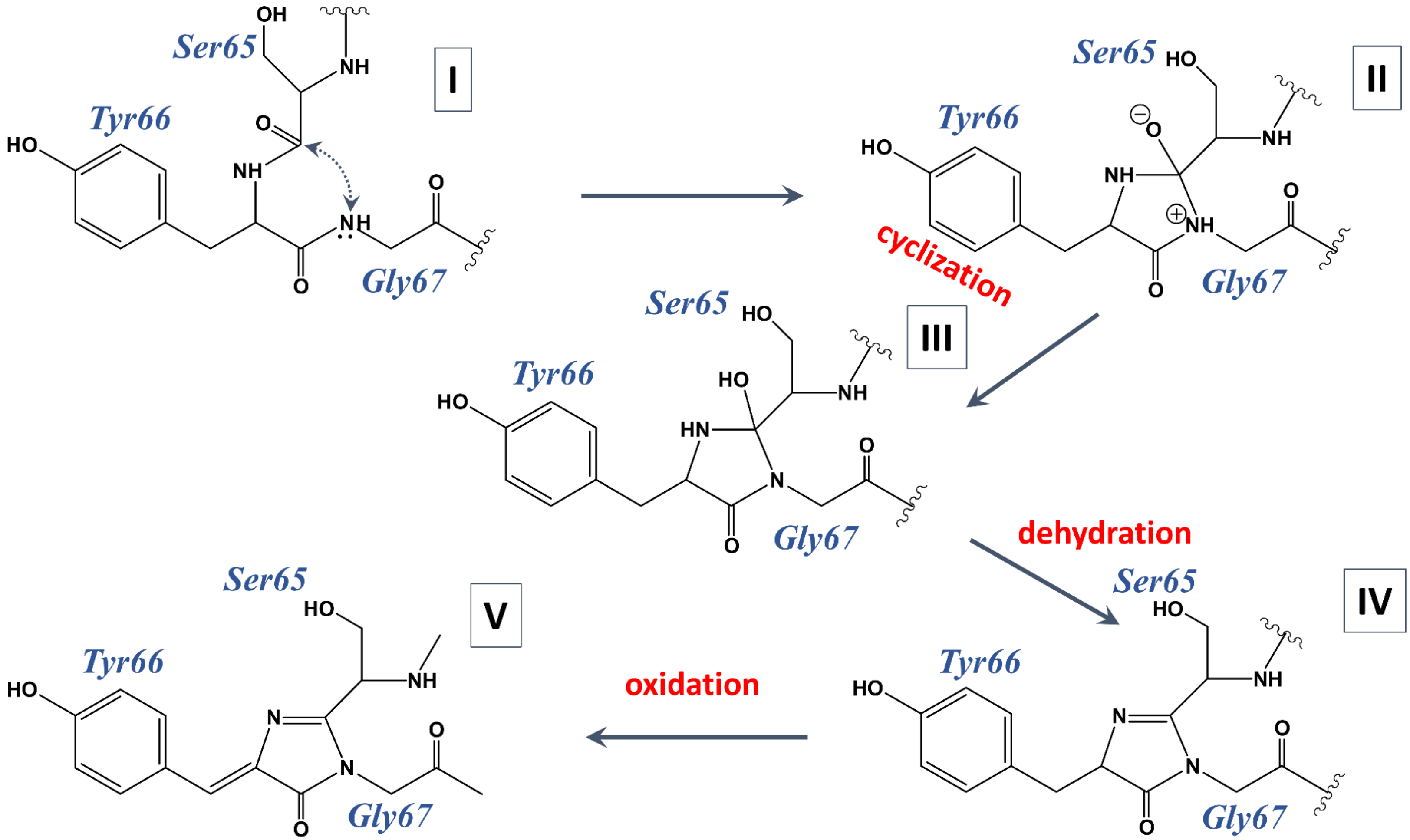


A. Acharya, A. Bogdanov, B. Grigorenko, K. Bravaya, A. Nemukhin, K. Lukyanov, A. Krylov, Photoinduced chemistry in fluorescent proteins: Curse or blessing?
// *Chem. Rev.*, 2017, *117, 758.*

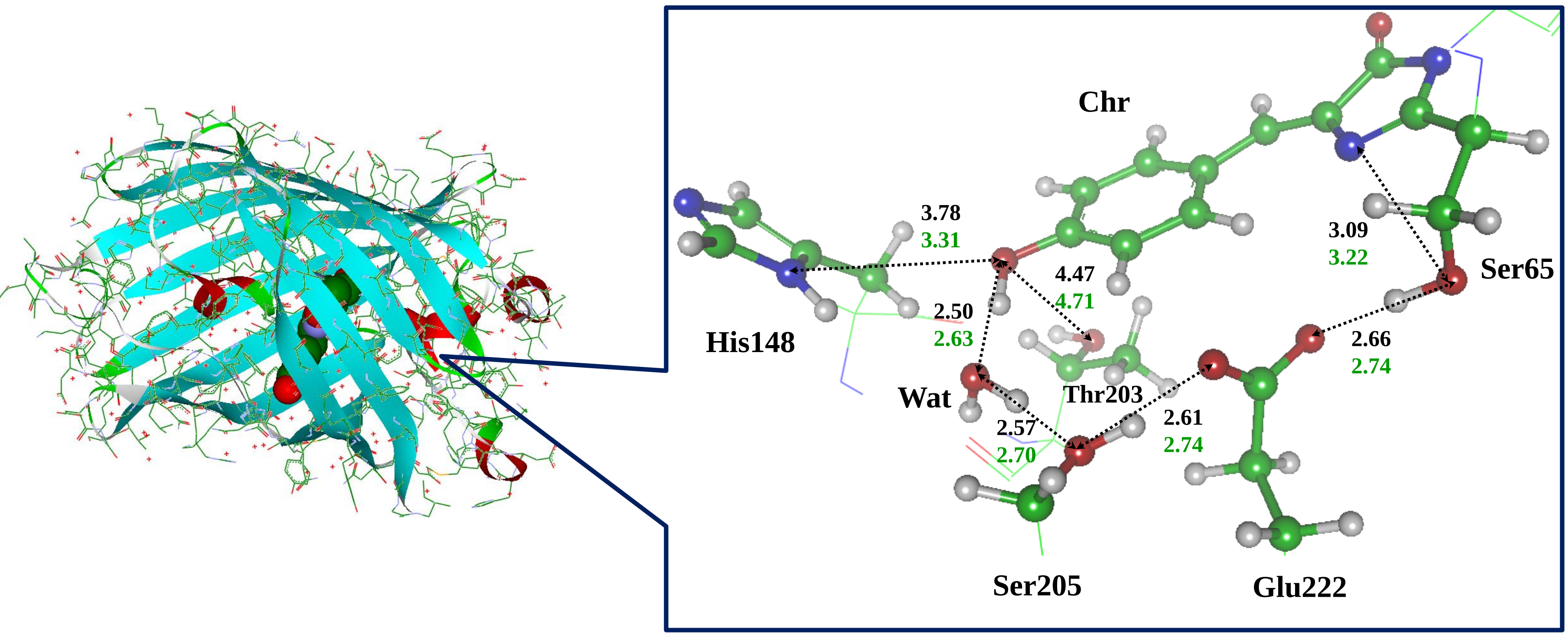
Chromophore maturation in GFP



Chromophore formation in GFP: Textbooks (R. Tsien et al.)

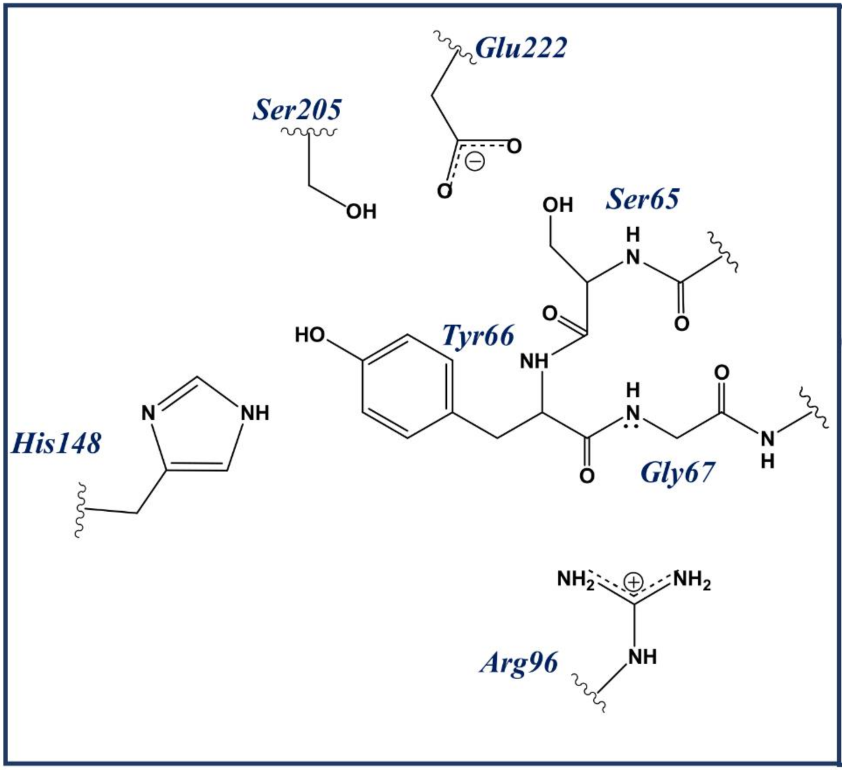


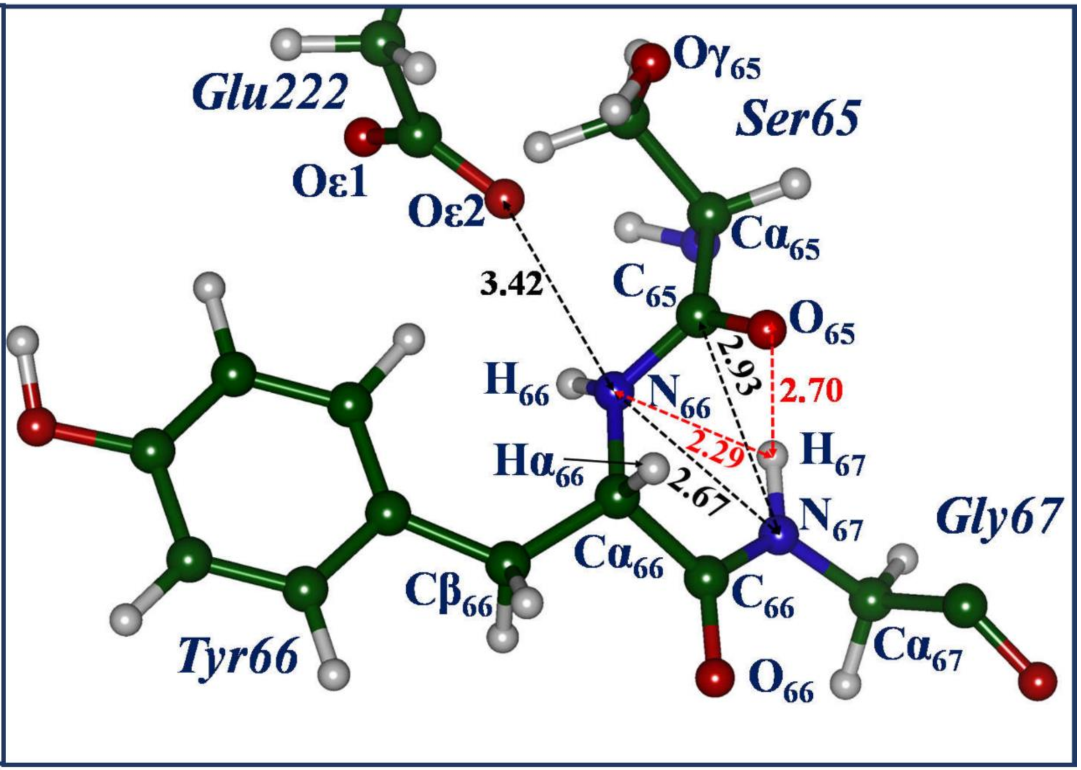
Chromophore formation in GFP: Model based on prior work



B.L. Grigorenko, A.V. Nemukhin, I.V. Polyakov, D.I. Morozov, A.I. Krylov, First-principle characterization of the energy landscape and optical spectra of the green fluorescent protein along A→I→B proton transfer route // *J. Am. Chem. Soc.*, 2013, *135*, 11541

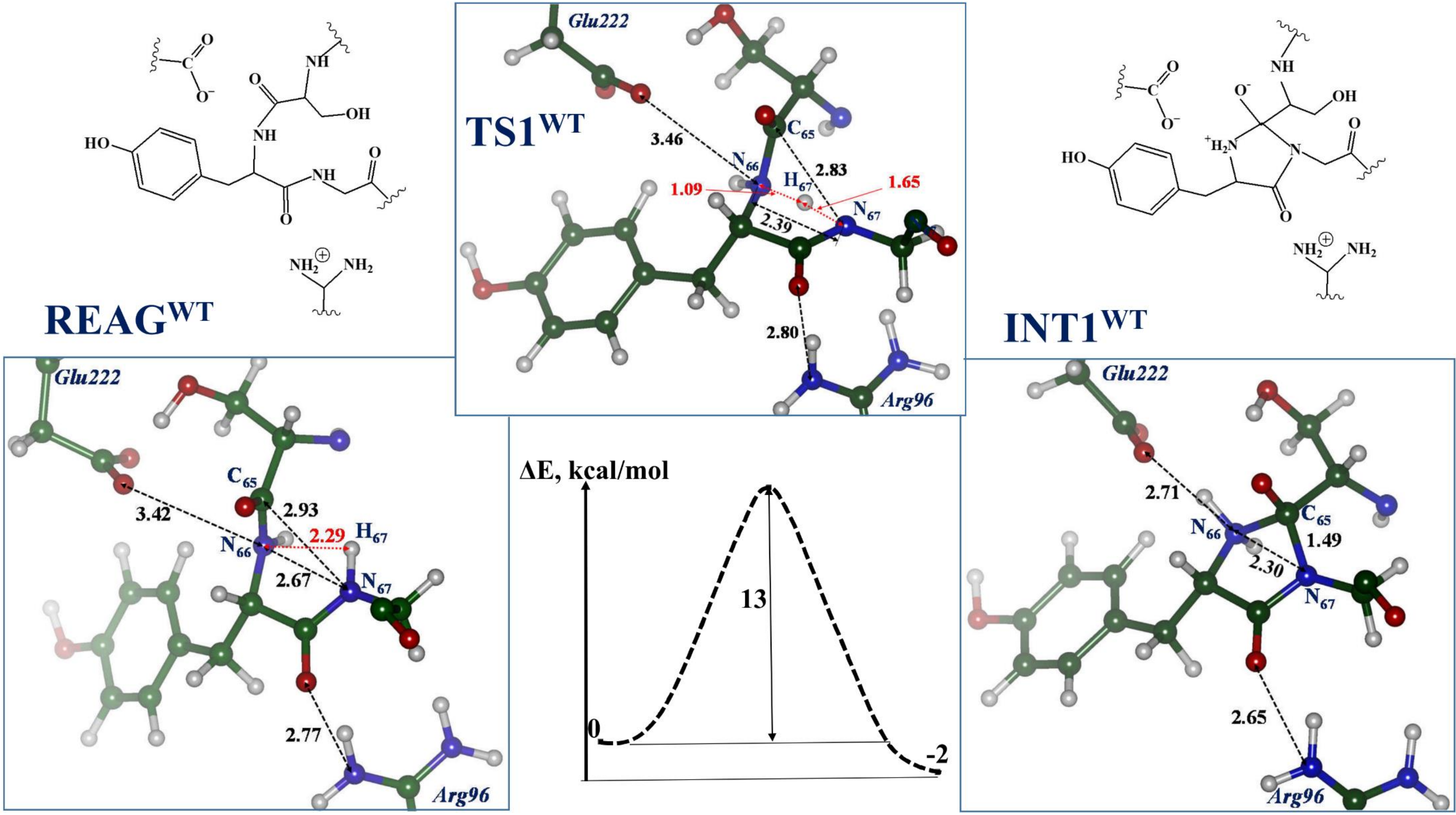
Chromophore formation in GFP: Starting REAG structure contains tripeptide Ser65-Tyr66-Gly67



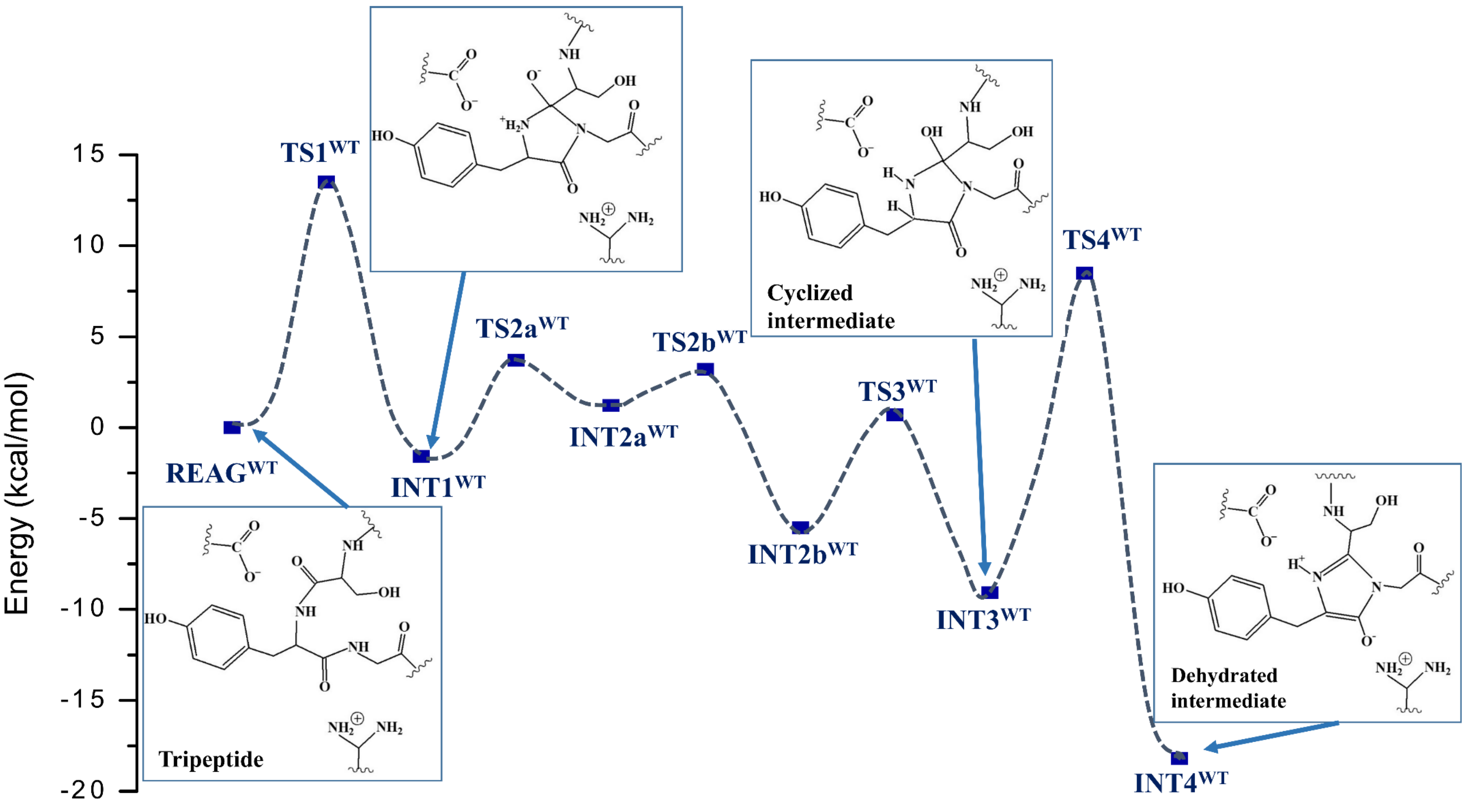


Computational protocol: QM(PBE0/6-31G*)/MM(AMBER)

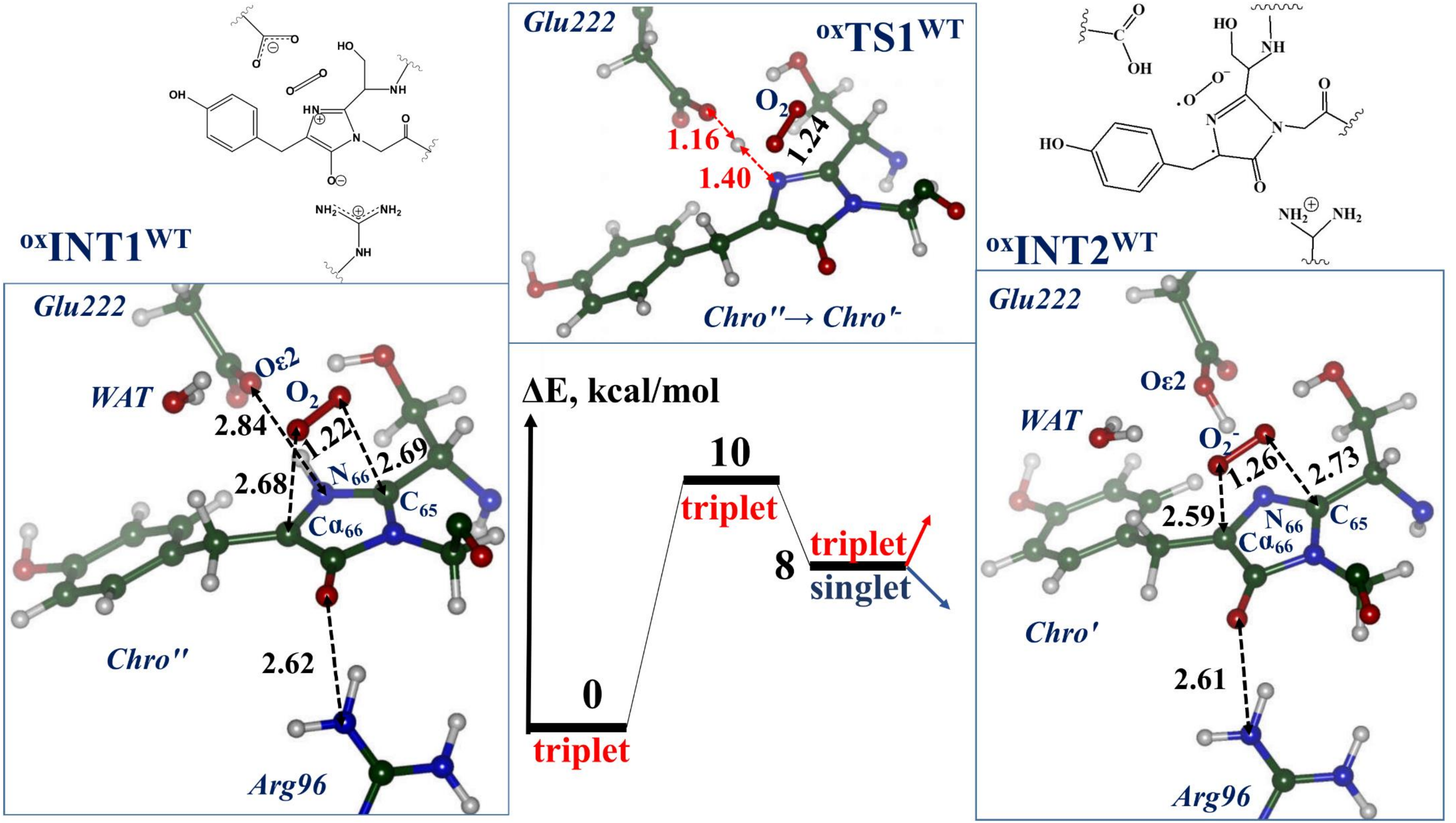
Chromophore formation in GFP: Chain of elementary steps



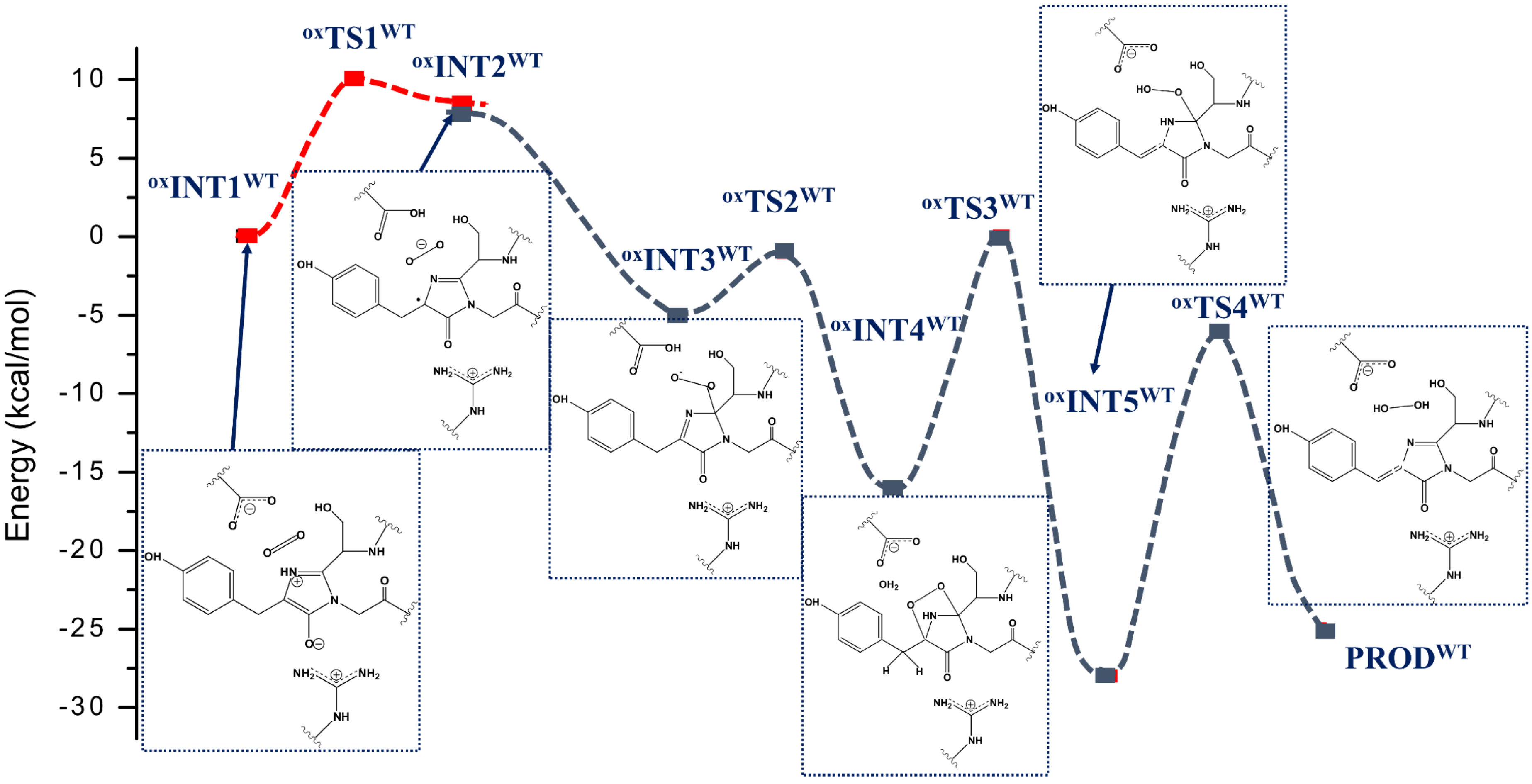
Chromophore formation in GFP: Computed QM/MM energy profile for cyclization-dehydration



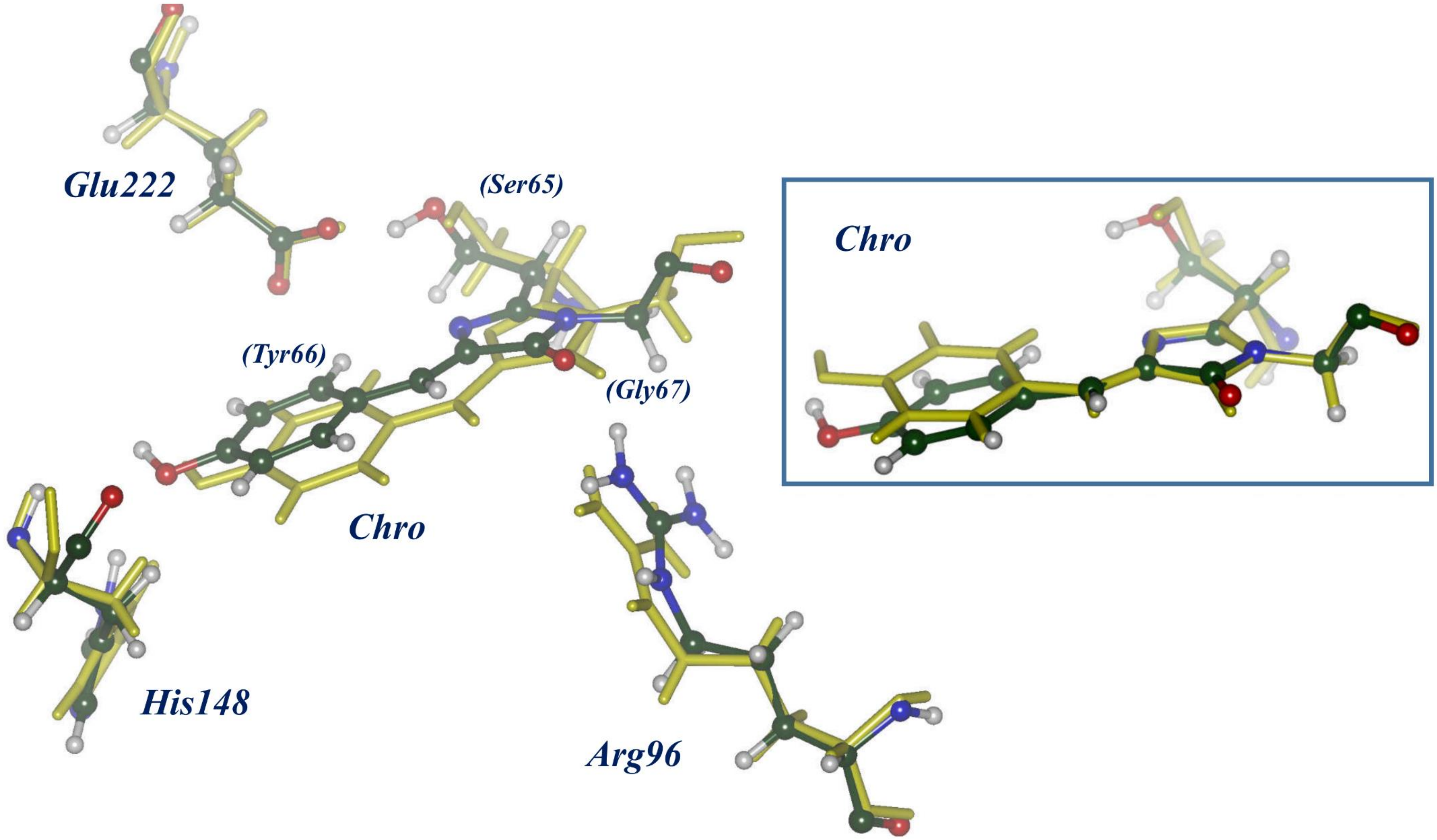
Chromophore formation in GFP: Modeling oxidation



Chromophore formation in GFP: Computed QM/MM energy profile for oxidation



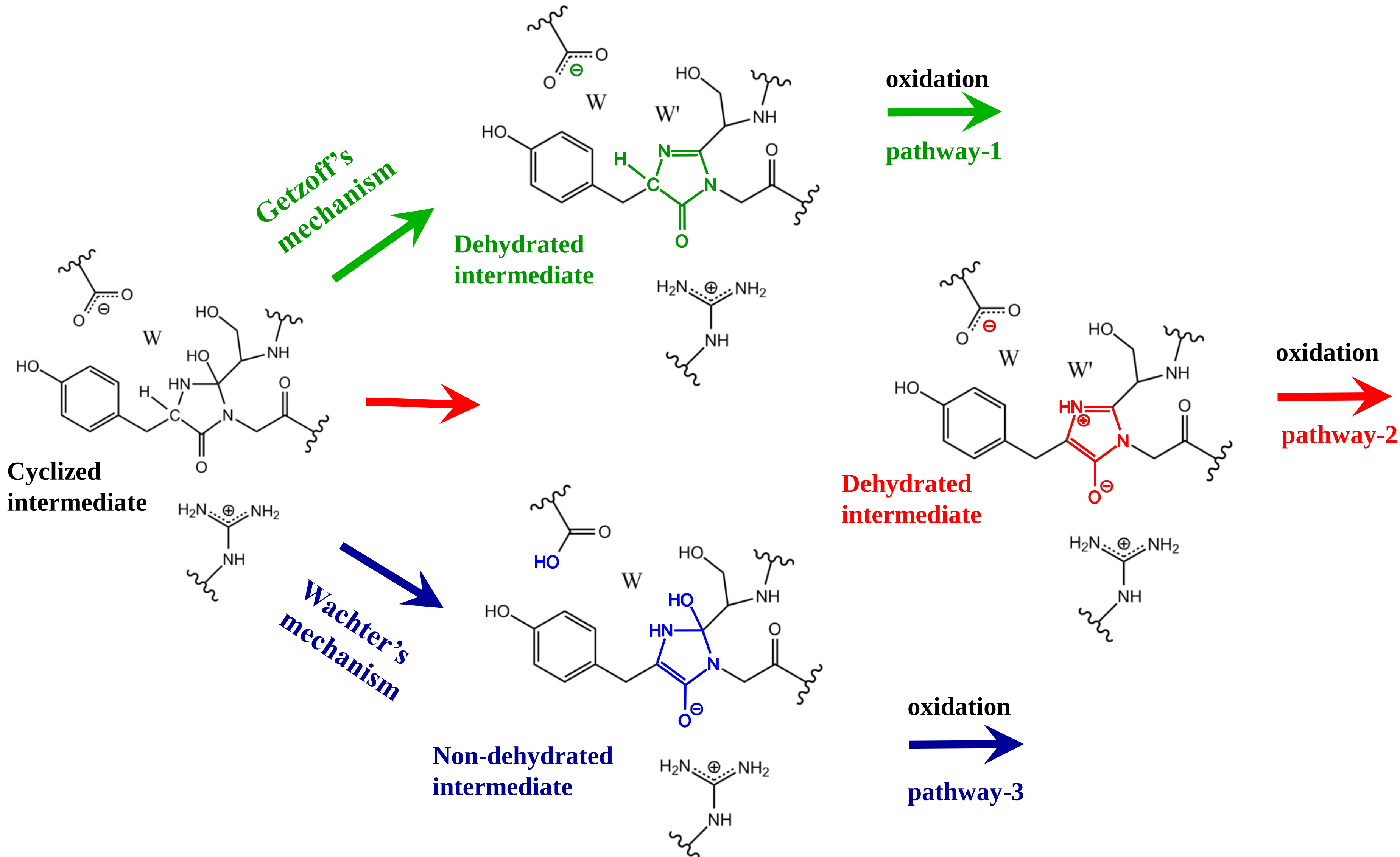
Chromophore formation in GFP: Comparison to the high-resolution crystal structure (yellow sticks)



Chromophore formation in GFP: Comparison to the results of kinetics studies

Reaction Step	Rate constant, s^{-1}	Experimental (TST) energy barrier, kcal/mol	Calculated energy barrier, kcal/mol
Cyclization-dehydration	0.0038	20.7	17
Oxidation	0.00015	22.7	21

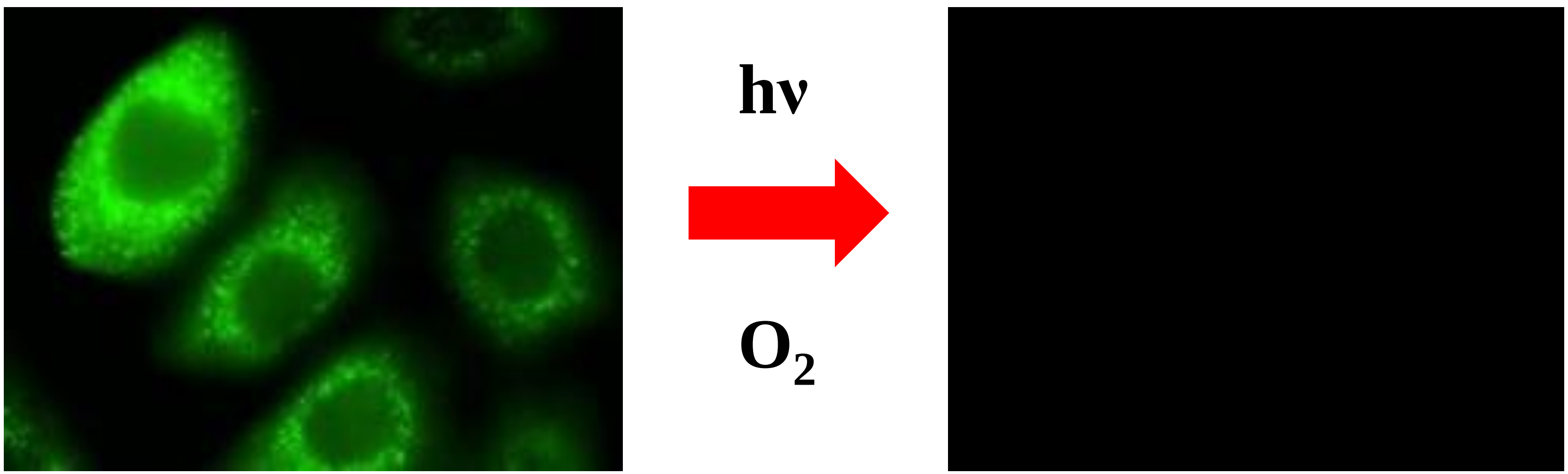
Chromophore formation in GFP: Summary of scenarios



B.L. Grigorenko, A.I. Krylov, A.V. Nemukhin, Molecular modeling clarifies the mechanism of chromophore maturation in the green fluorescent protein // *J. Am. Chem. Soc.*, 2017, *139*, 10239

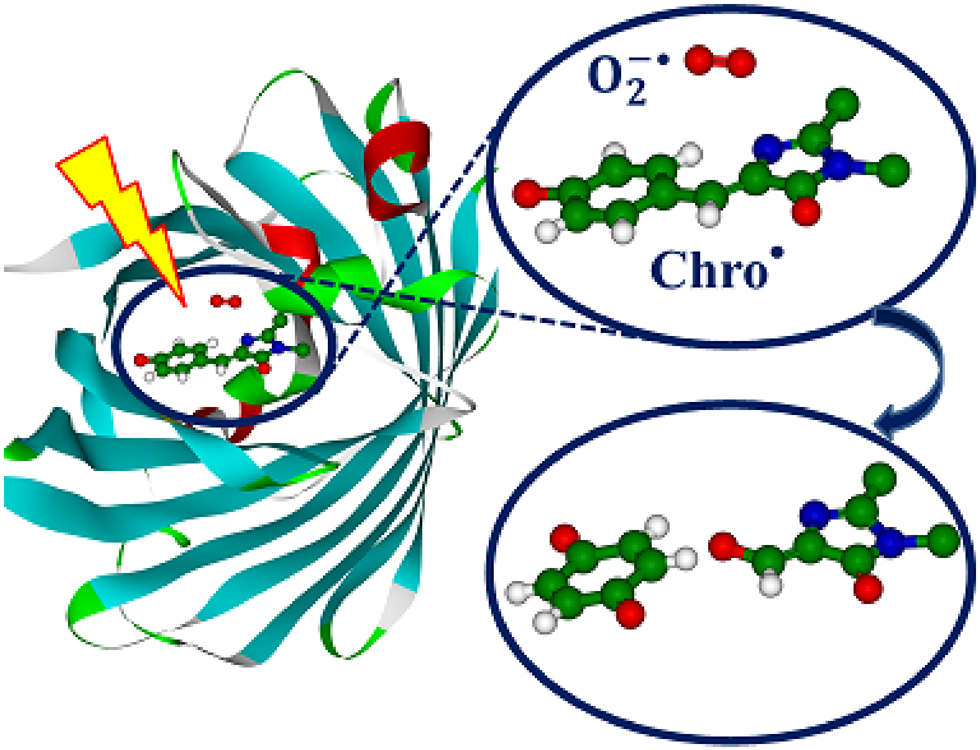
Modeling photobleaching in GFP

Light-induced reaction with oxygen leads to irreversible photobleaching in FPs



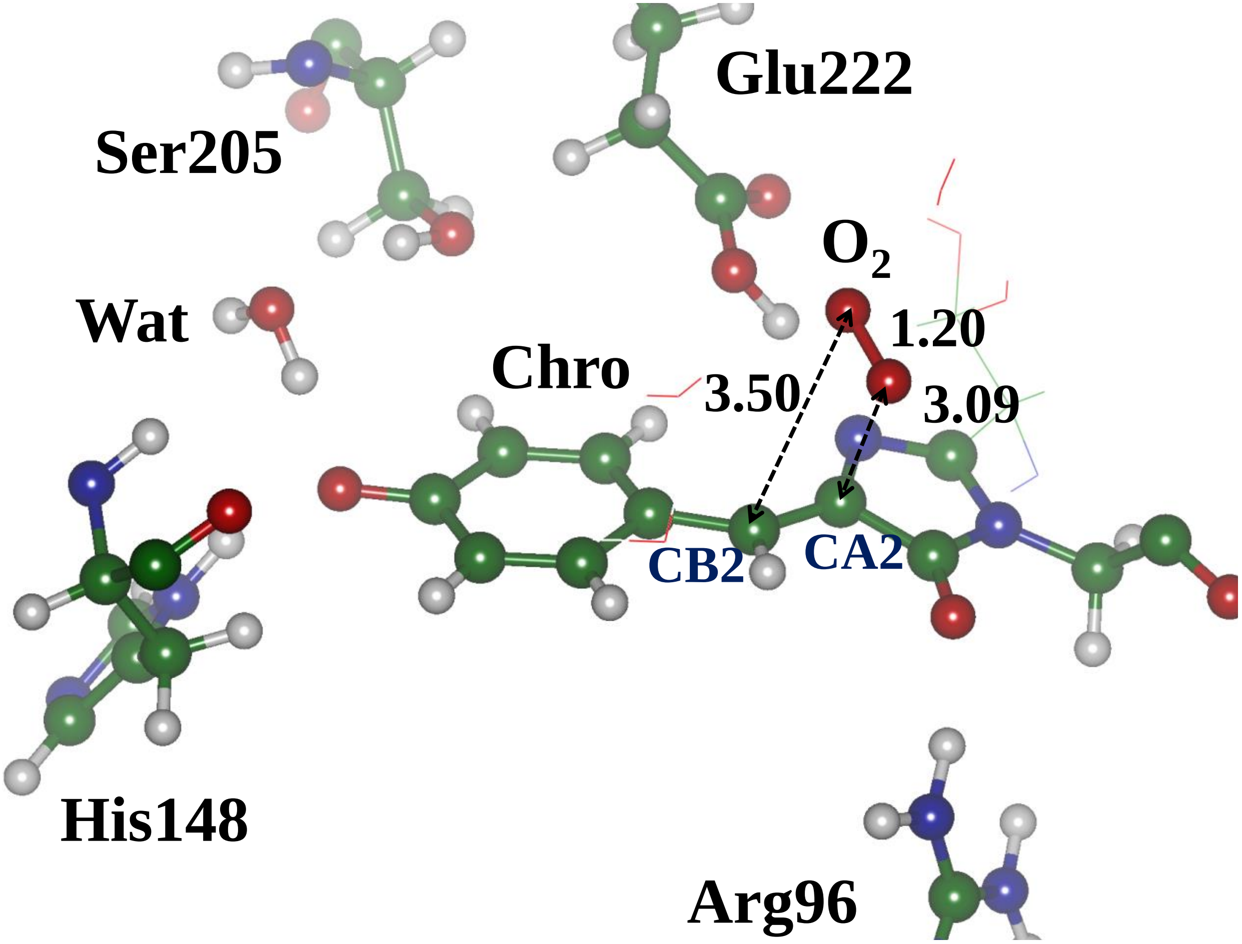
Modeling photobleaching in GFP

Light-induced reaction with oxygen leads to <u>decomposition of the chromophore</u> and irreversible photobleaching in FPs

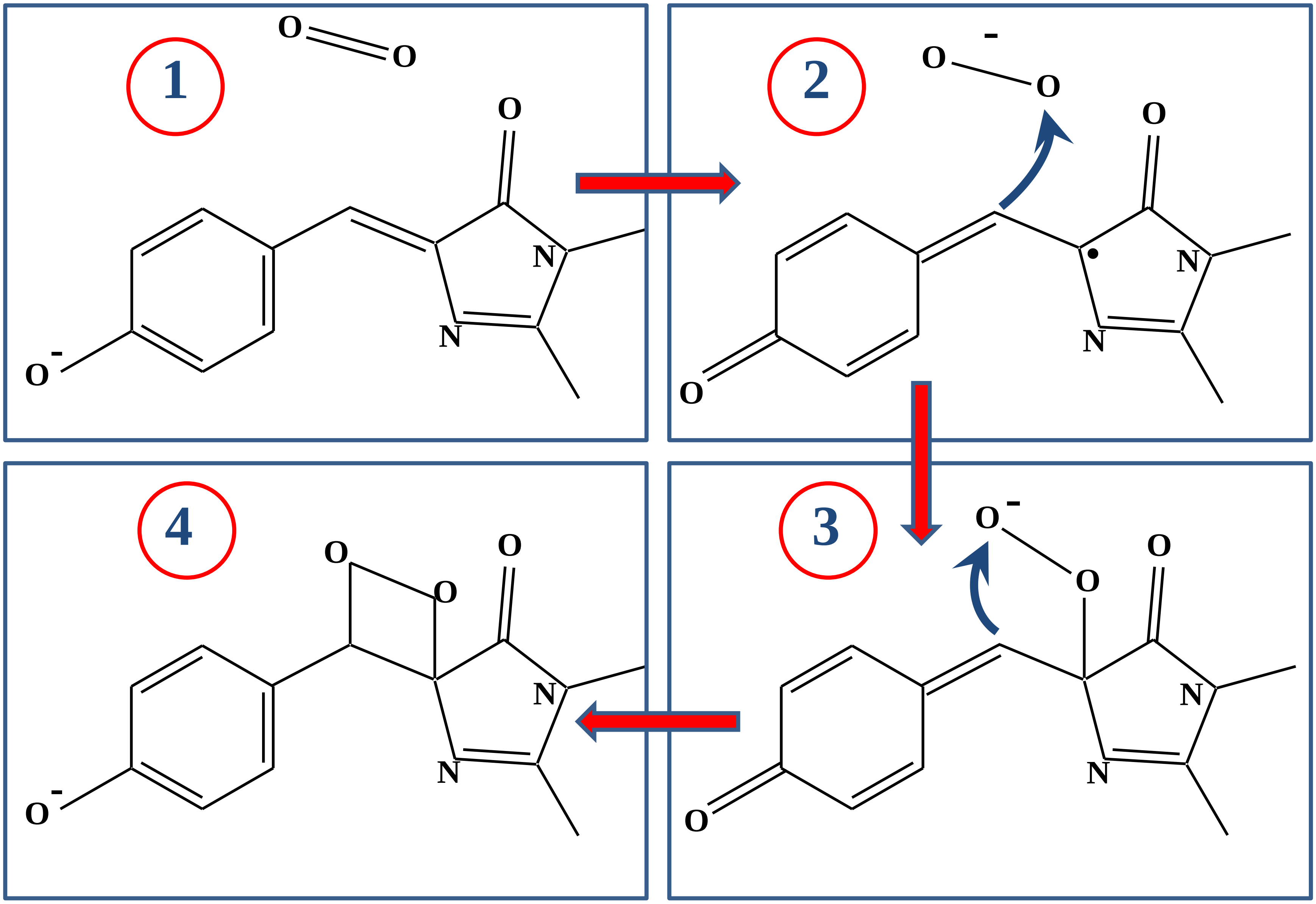


Grigorenko B., Nemukhin A., Polyakov I., Khrenova M., Krylov A.I., A light-induced reaction with oxygen leads to chromophore decomposition and irreversible photobleaching in GFP-type proteins // *J. Phys. Chem. B*, 2015, *119*, 5444-5452

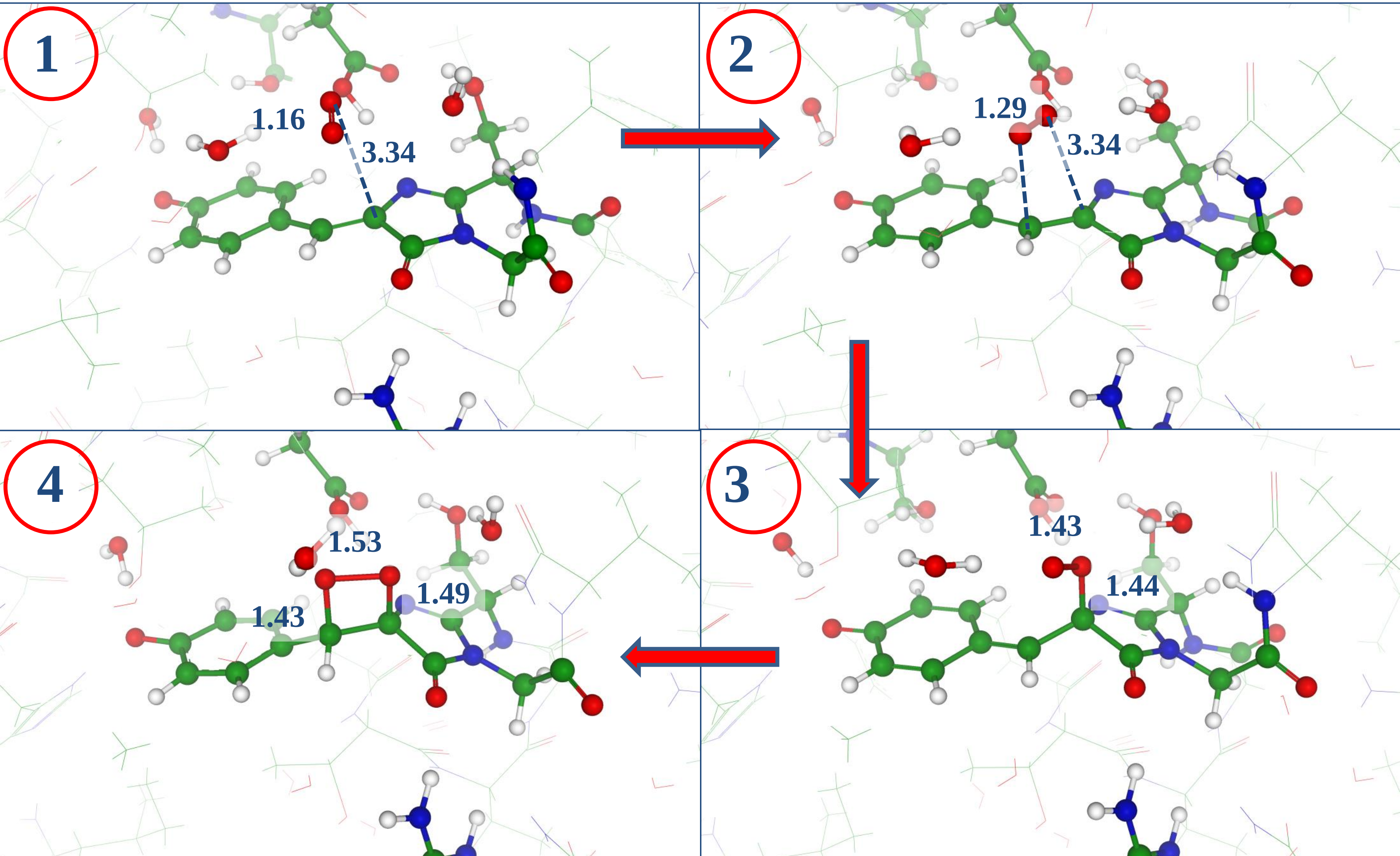
Chromophore-containing pocket in GFP plus O_2 molecule



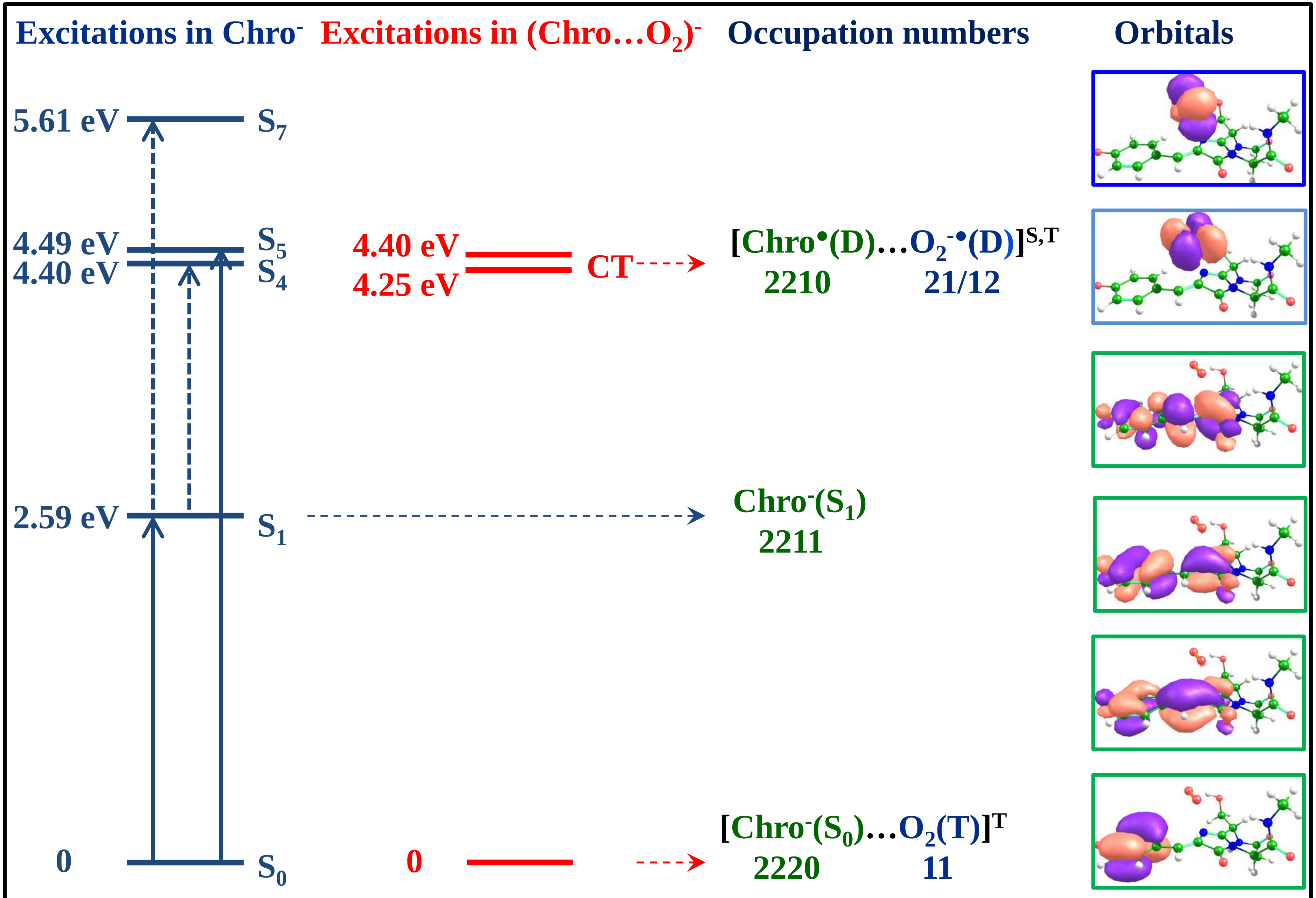
First elementary steps: Chemistry



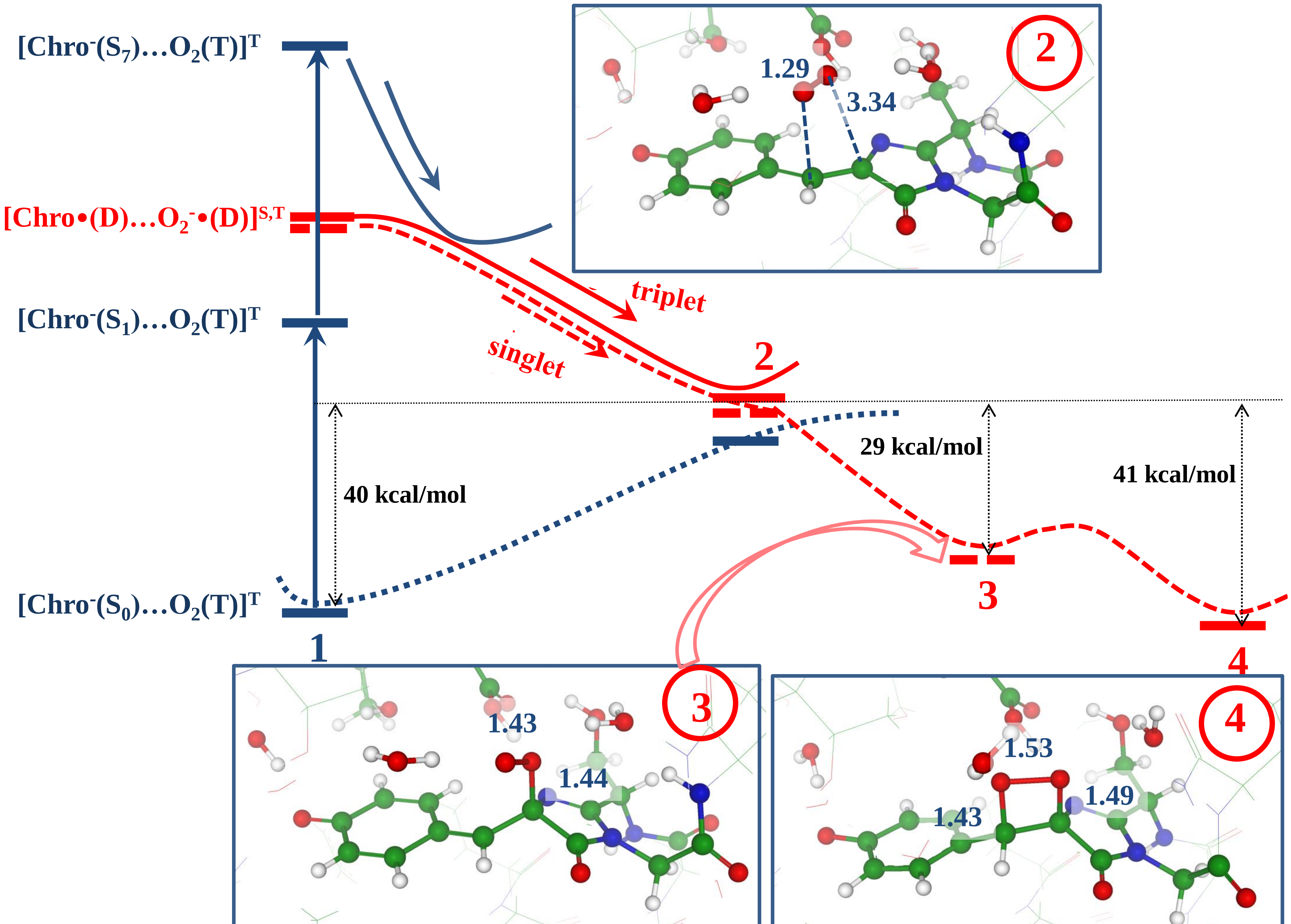
First elementary steps: Molecular models



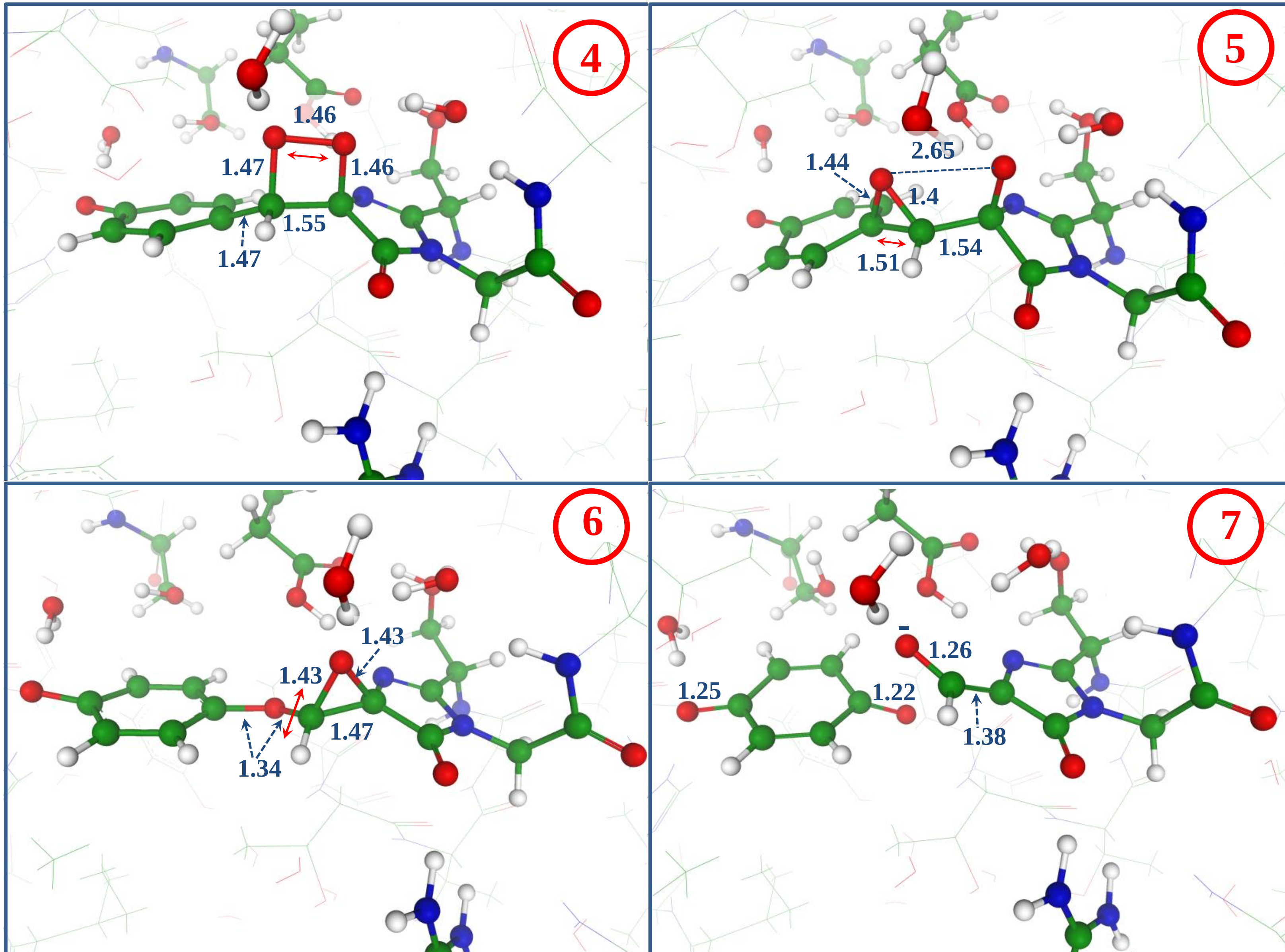
Excitation to the charge-transfer (CT) state is essential



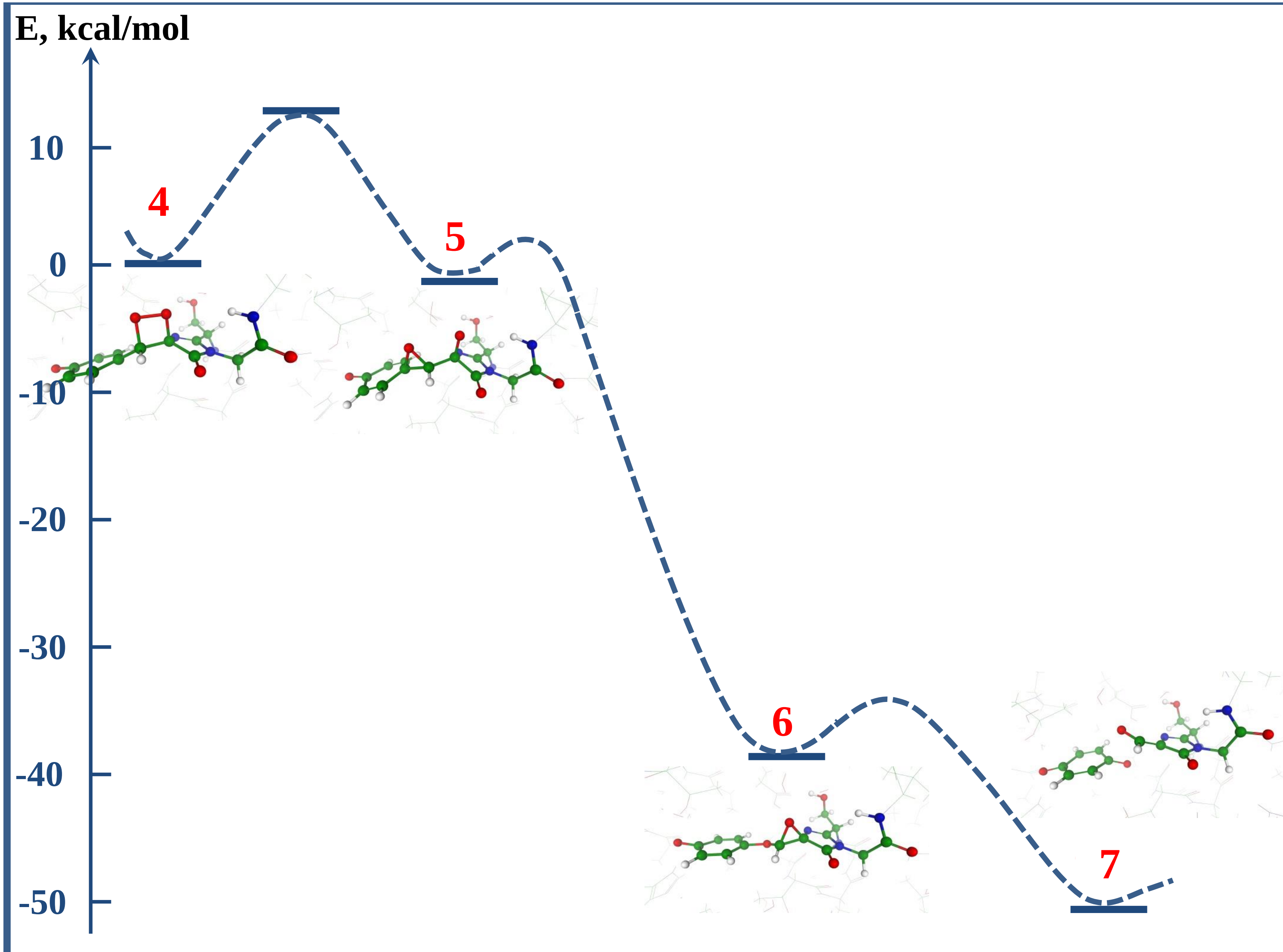
Evolution of the system after excitation



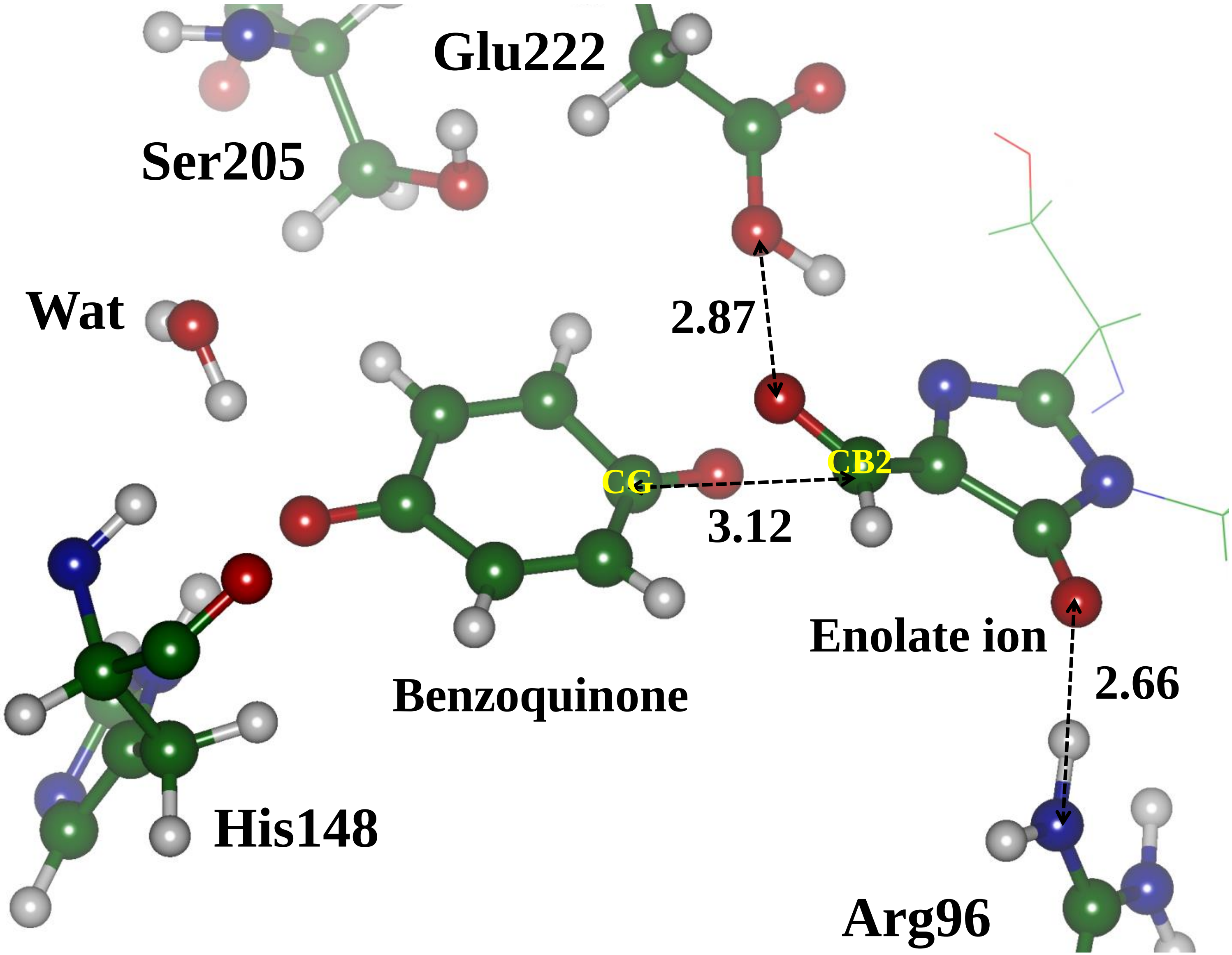
Evolution of the system after excitation



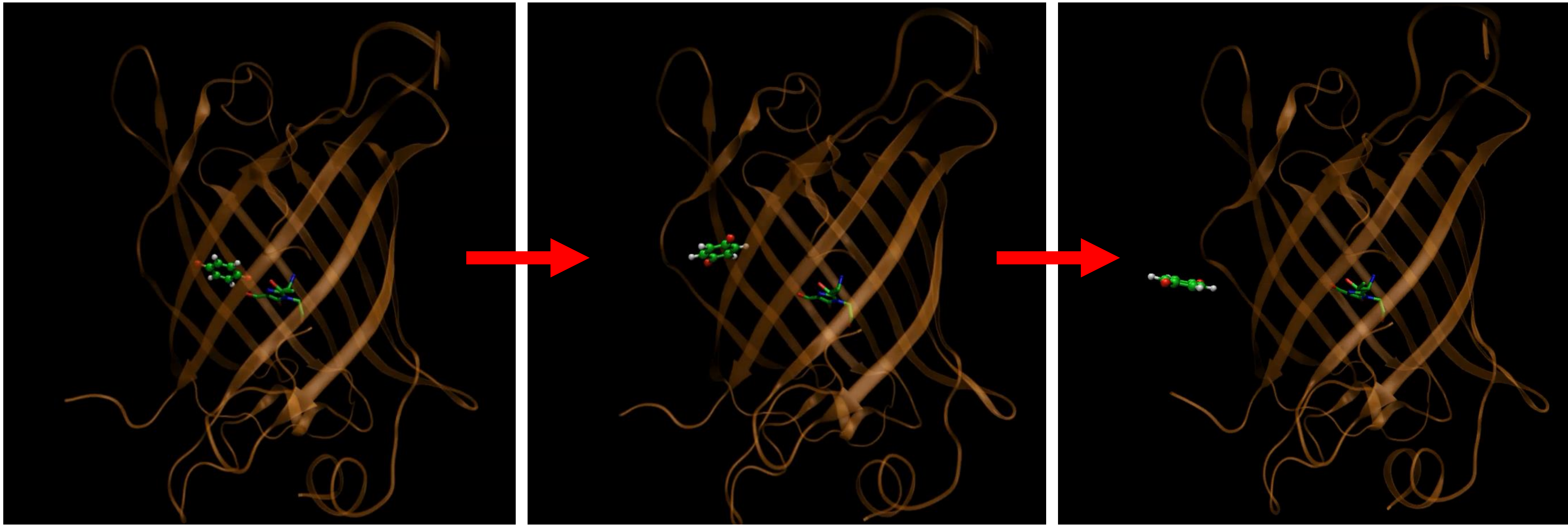
Computed energy profile



Basic qualitative result – decomposition of the chromophore



MD simulations: exit of benzoquinone from the GFP barrel



So far: Materials of the presentation at WATOC 2017, Munich

WATOC 2017

11th Triennial Congress of the World Association of Theoretical and Computational Chemists

WATOC 2017

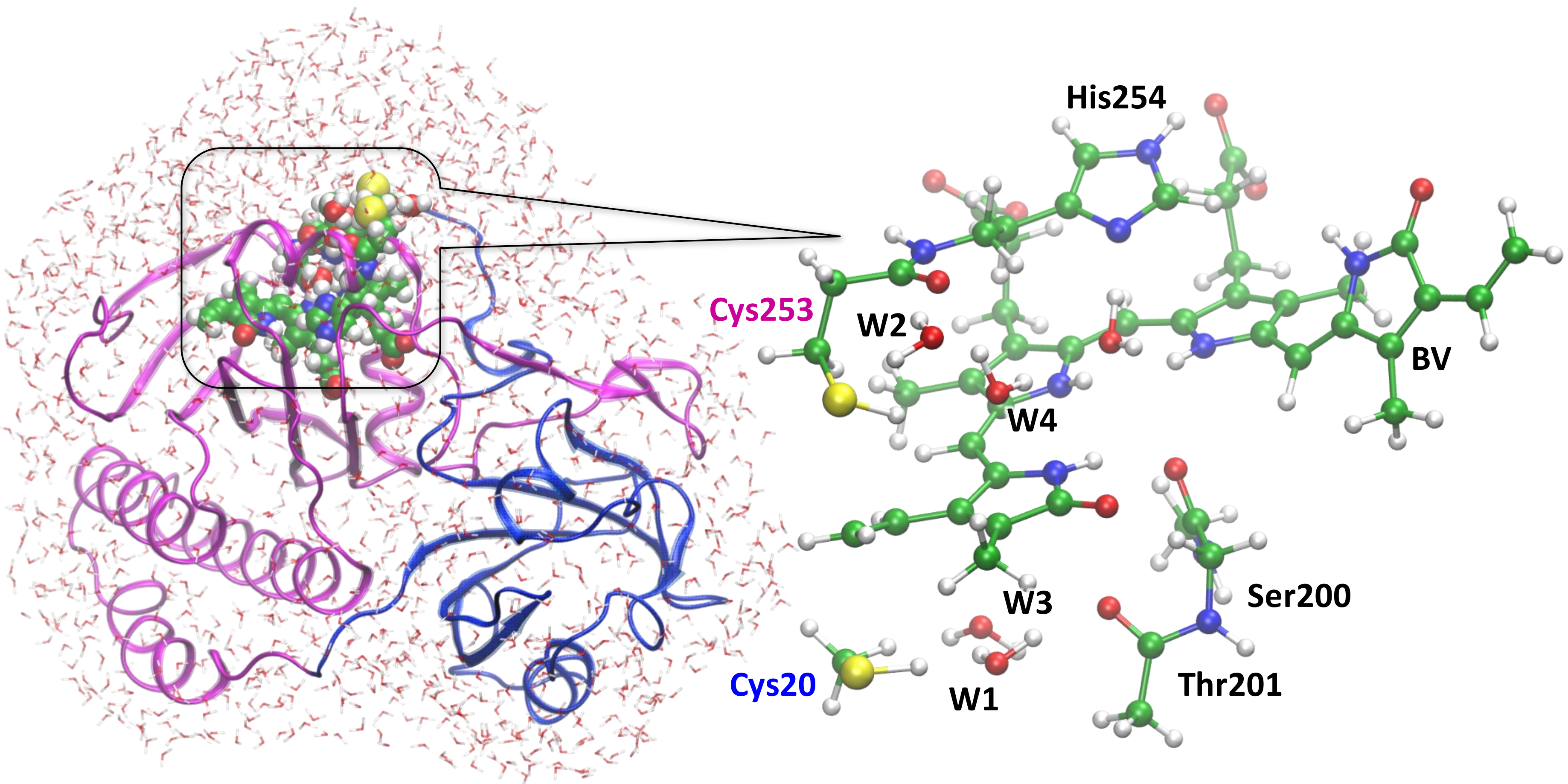
Words of Welcome

On behalf of the Local Scientific Committee, it is my pleasure to welcome you to the **11th Triennial Congress of the World Association of Theoretical and Computational Chemists** during the week of August 27 to September 1, 2017 in Munich, Germany. With about 1500 registered participants from all over the world and 12 plenary, 215 invited, 136 contributed speakers, as well as over 920 posters, WATOC2017 is the largest WATOC so far.

This shows both the increasing importance of theoretical and computational chemistry across the disciplines, and the central (and easy to reach) location of Munich in the heart of Europe. We have set up an exciting program covering a wide variety of cutting edge research topics ranging from method developments to applications pushing the limits of modern theoretical and computational chemistry, biochemistry, nanotechnology, and materials sciences.

WATOC2017 is held in the city center of Munich with both plenary and parallel sessions in the Gasteig cultural center under one roof. Besides great science, we hope that you will also find some time to explore the city of Munich and its surroundings which offer fascinating possibilities for both cultural and outdoor activities.

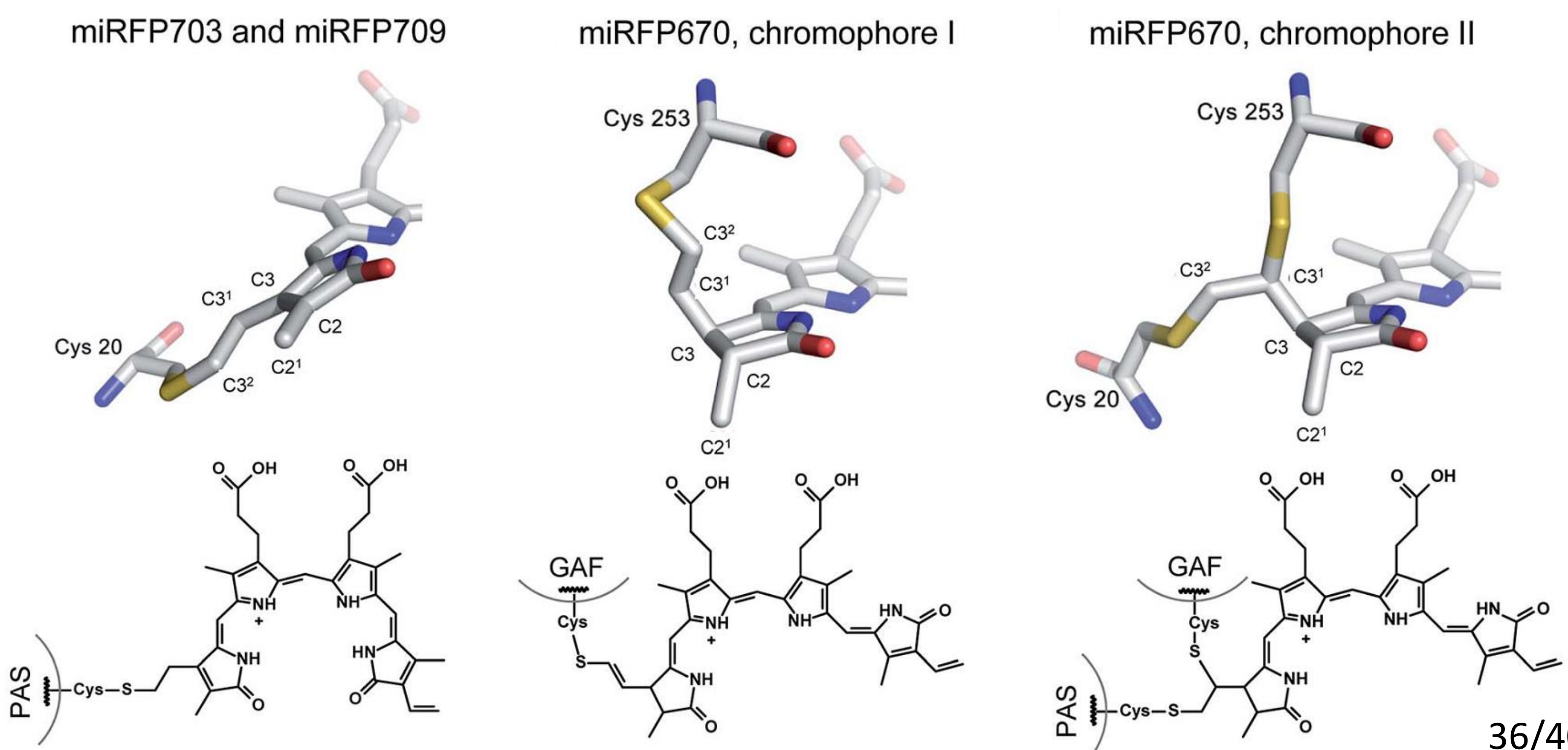
<u>Project of 2018</u> Competition Between Two Cysteines in Covalent Binding of Biliverdin to Phytochrome Domains



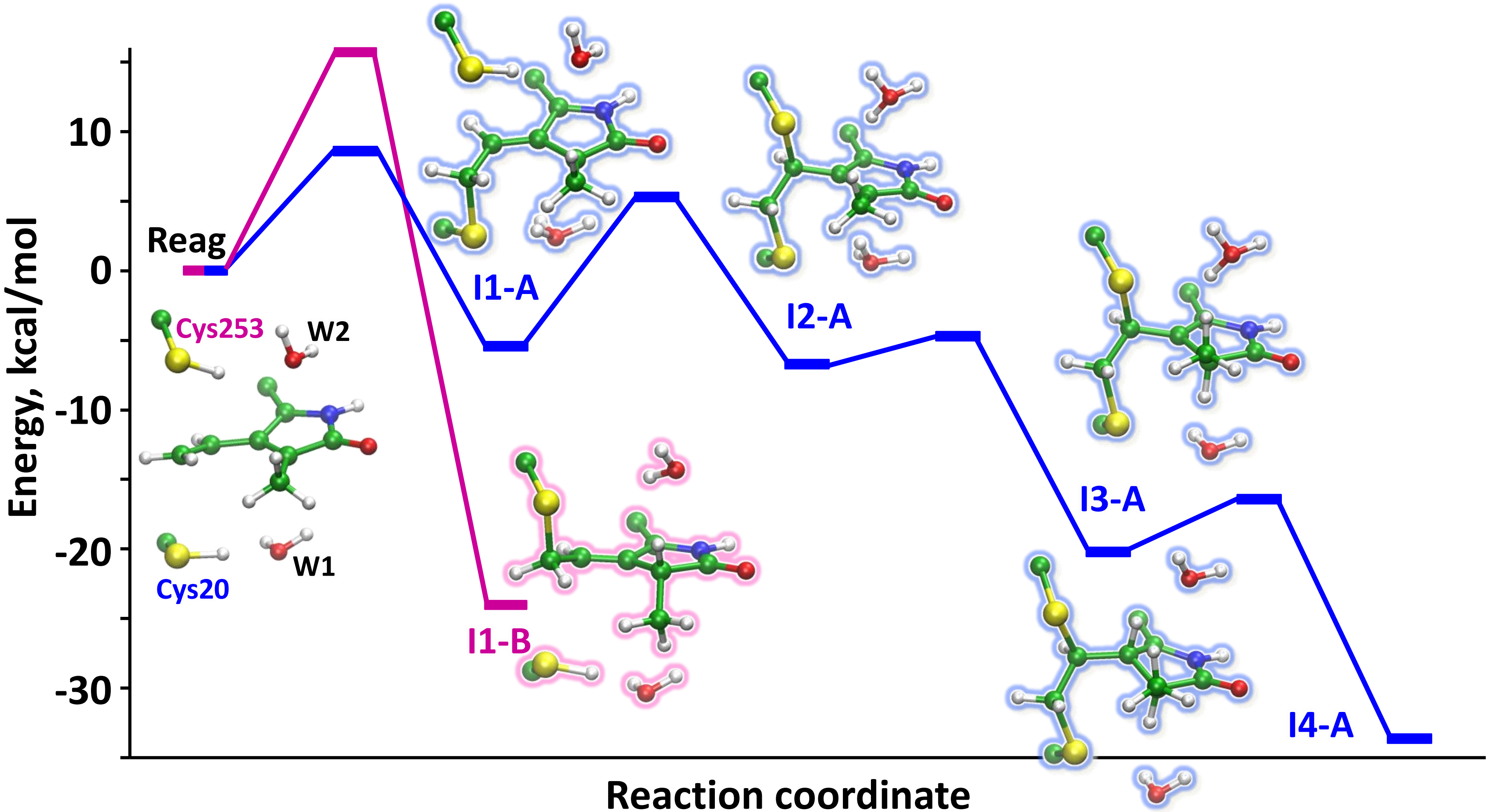
Chem. Sci., 2017, 8, 4546
DOI: 10.1039/c7sc00855d

Designing brighter near-infrared fluorescent proteins: insights from structural and biochemical studies†

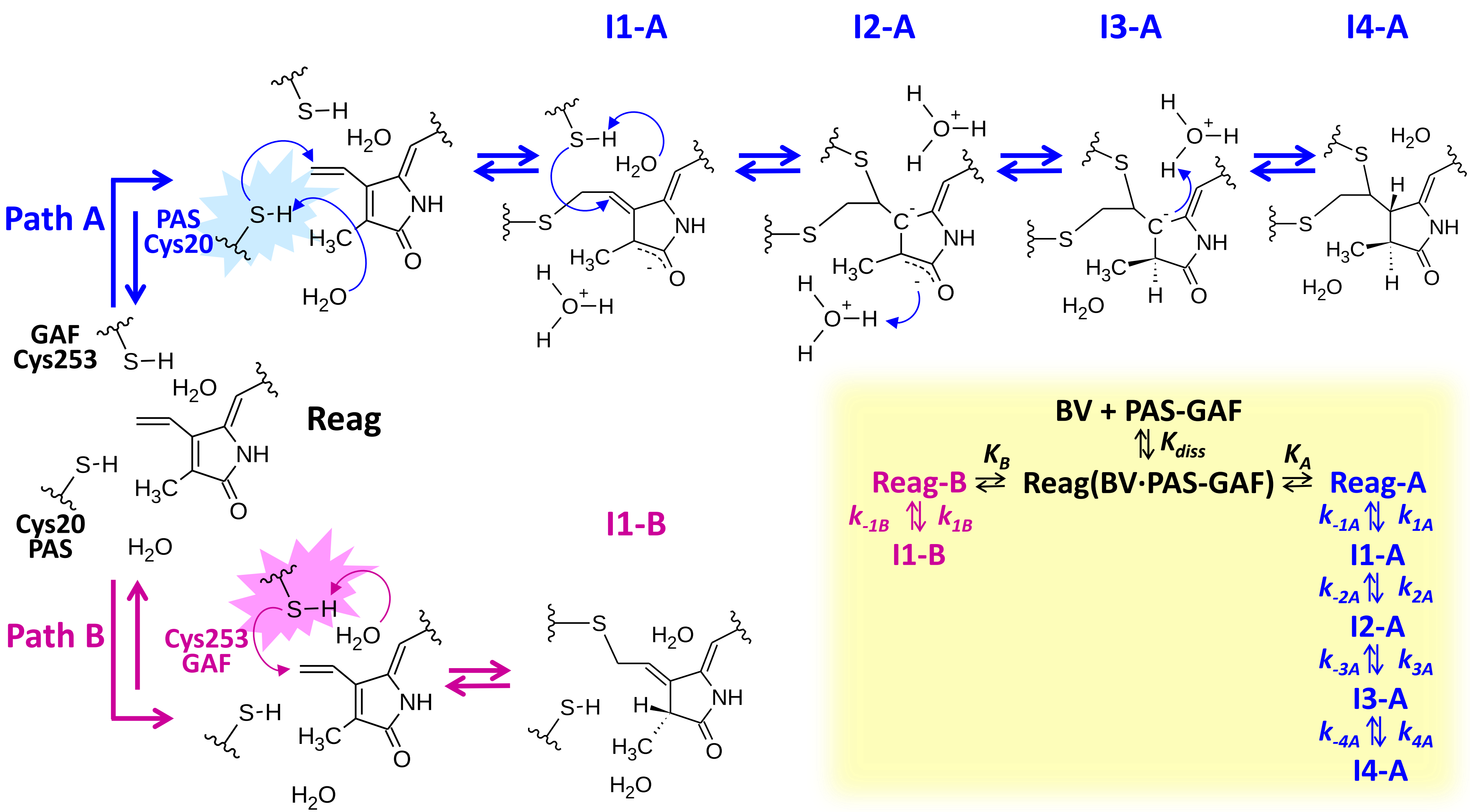
Mikhail Baloban,‡[a] Daria M. Shcherbakova,‡[a] Sergei Pletnev,‡[b] Vladimir Z. Pletnev,[c] J. Clark Lagarias[d] and Vladislav V. Verkhusha*[ae]



Competition Between Two Cysteines in Covalent Binding of Biliverdin to Phytochrome Domains



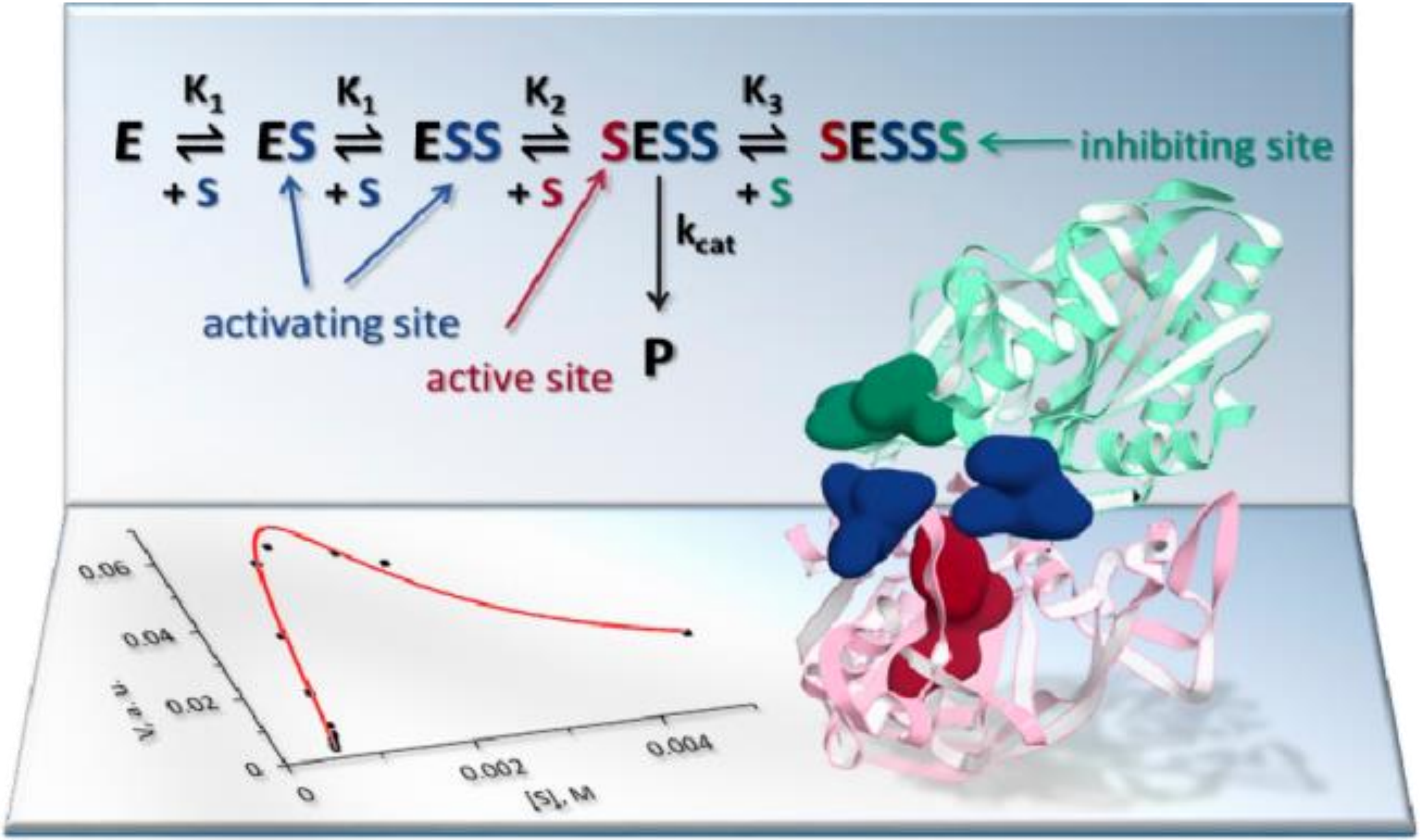
Competition Between Two Cysteines in Covalent Binding of Biliverdin to Phytochrome Domains



Khrenova M., Kulakova A., Nemukhin A. // *Organic & Biomolecular Chemistry*, 2018, *16*, 7518

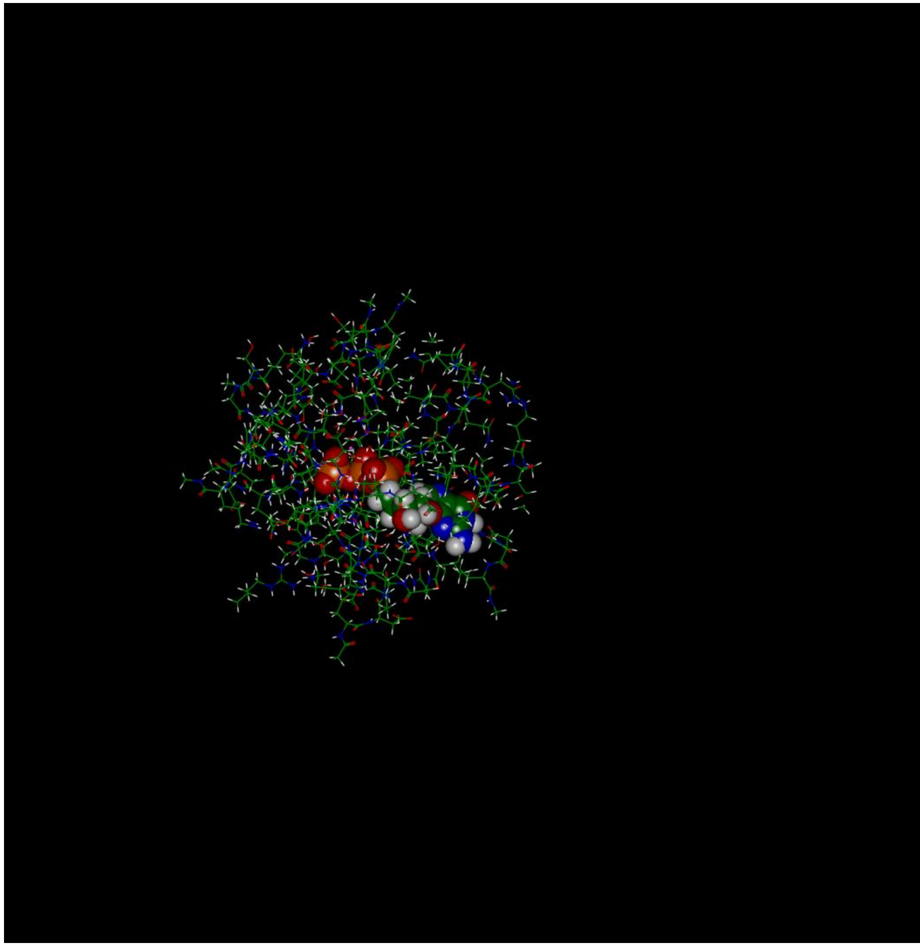
Concluding remarks

We are approaching an ambitious goal to simulate kinetic curves for reactions in proteins



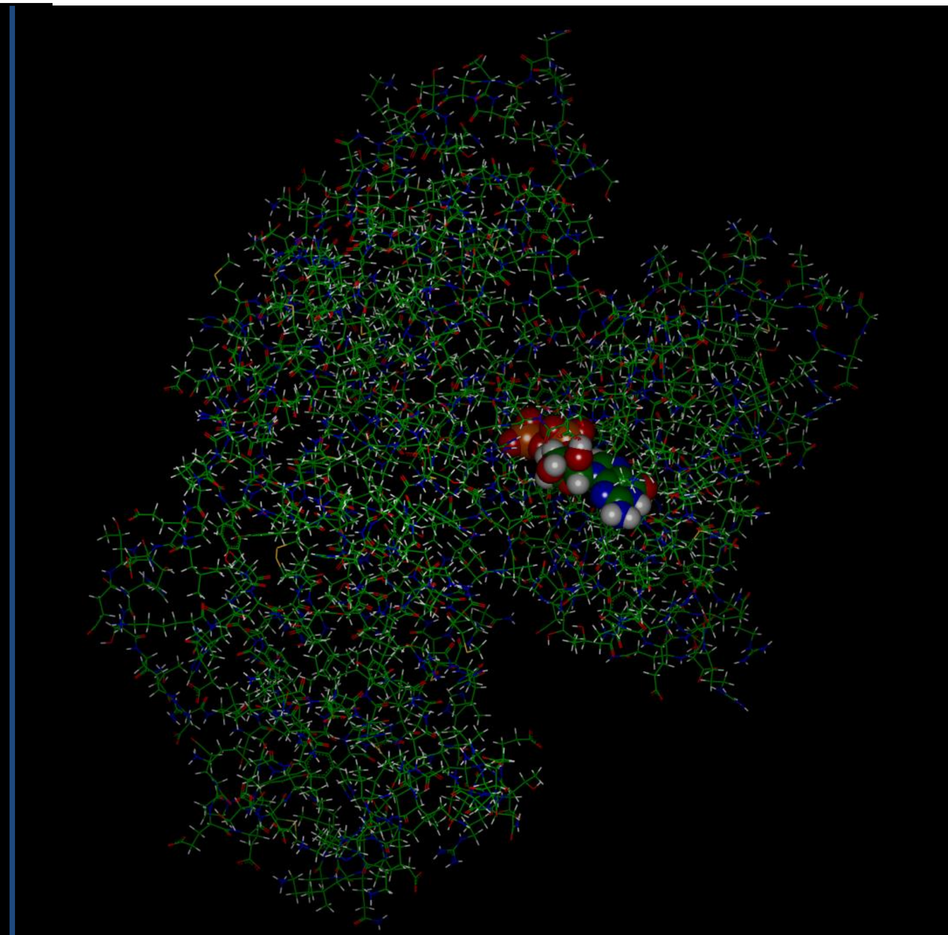
Progress in models and methods: GTP-Ras-GAP

Model of 2005



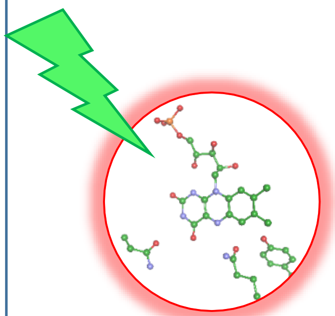
1,700 atoms in total
43 QM atoms, RHF/6-31G

Ten Years Later: Model of 2015

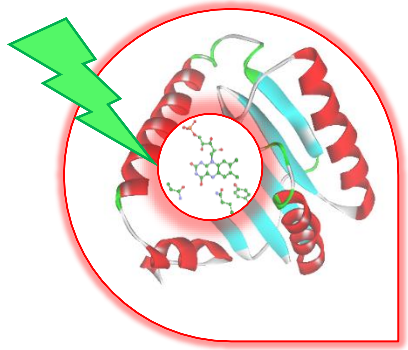


8,000 atoms in total
86 QM atoms, DFT(PBE0)/cc-pVDZ

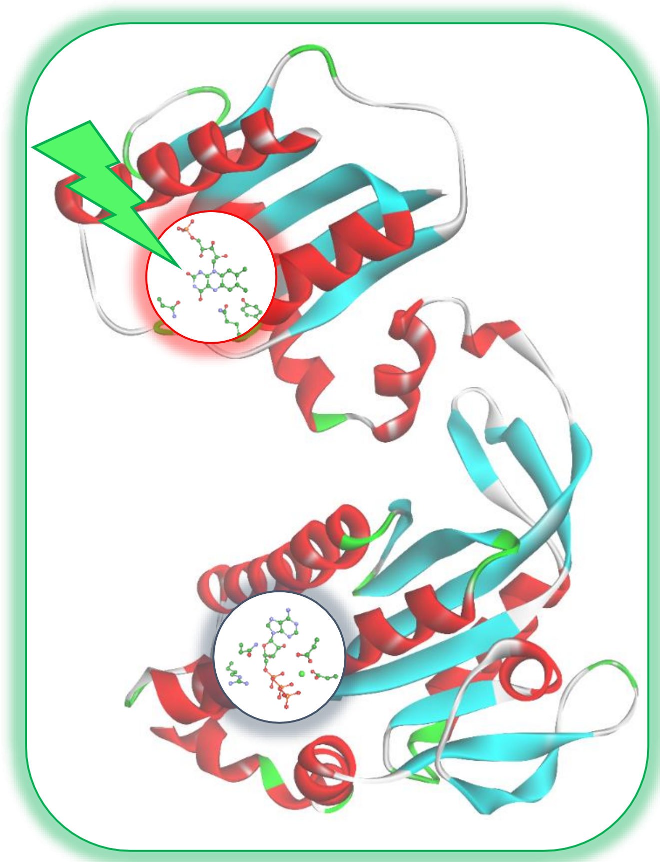
The way forward



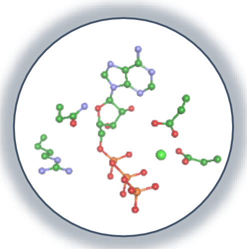
Chromophore excitation
(Quantum chemistry)



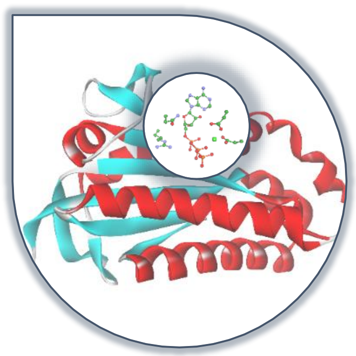
Modeling photoreceptor proteins
(QM/MM + QM/MM MD)



Photoactivated multidomain proteins
(QM/MM + MM + MD + QM/MM MD)



Chemical reactions in enzyme active sites
(Quantum chemistry)



Chemical reactions in enzymes, allosteric regulation
(QM/MM + MM + MD + QM/MM MD)

Progress (years, computer power,...)

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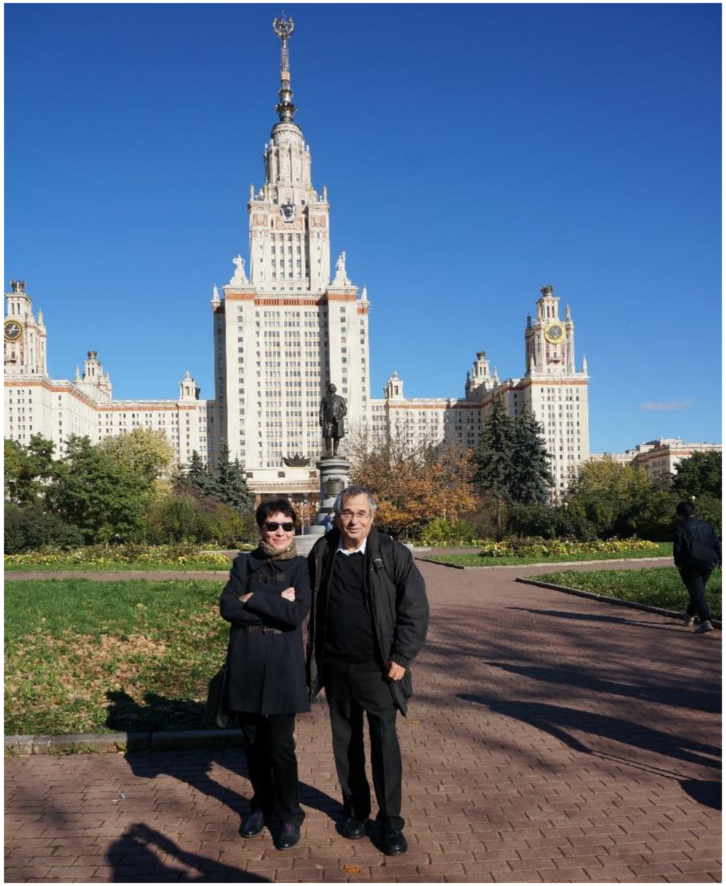

Bella Grigorenko & Arieh Warshel
Moscow 2016