Computational Study of the Attack of ·OH radicals on Aromatic Aminoacids

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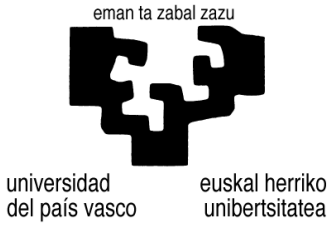


Contents

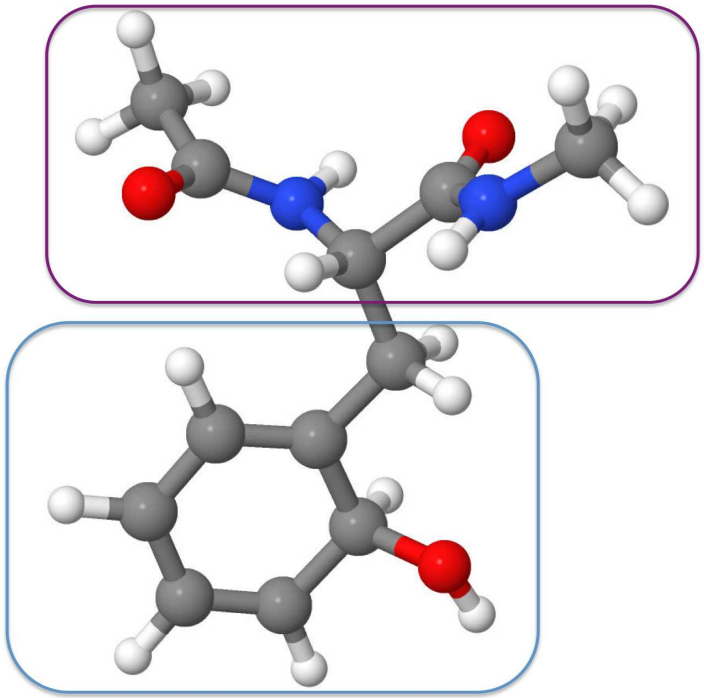
1. Introduction

ROS and biomacromolecules

- ROS: abundant among radical species (RNS, RCS, RSS)
  - $\cdot OH$, $\cdot OOH$, $\cdot O_2^-$, $^1O_2$.
- Biomacromolecular targets:
  - Proteins, cell membrane phospholipids, DNA, RNA...
- Attack may cause...
  - Alteration of the function (function loss) of cell macromolecules
  - If not controlled, cell death
- Deseases
  - Neurodegenerative: Alzheimer, Parkinson etc...
  - Among others, diabetes mellitus, etc...

·OH Attack to Proteins

- Radical attack to the protein back-bone
  - Leads to breaking of back-bone
- Radical attack to the amino acid side chains
  - Leads to many different possibilities



Global Research Project

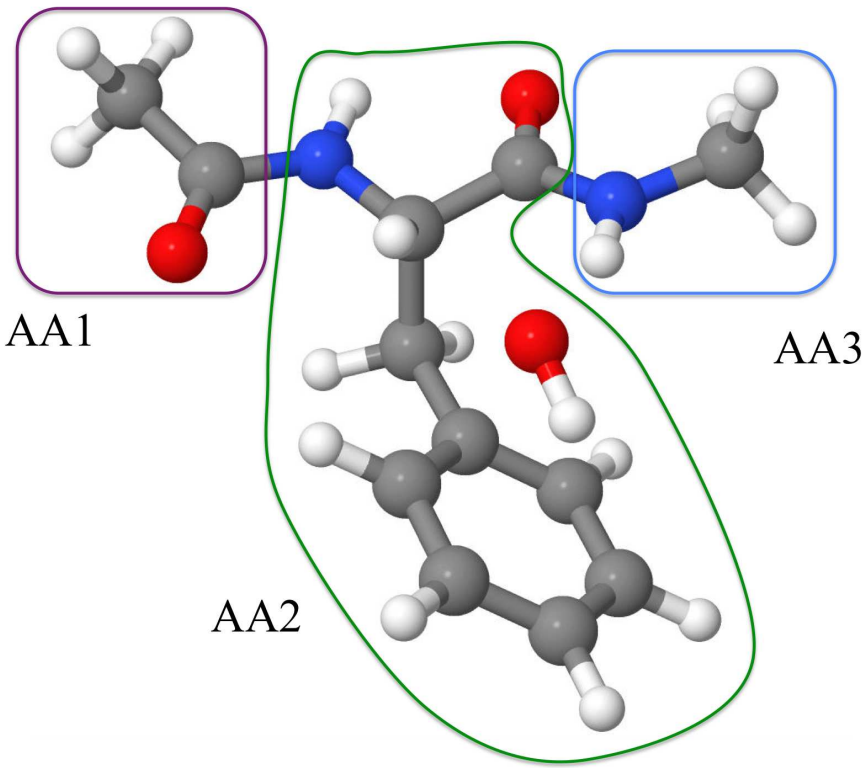
- Study reactivity with small models (DFT)
  - Attack to different possitions of the back-bone:
    - Influence of the side chain important?
    - Influence of the protein folding? (α-helix, β-sheet...)
    - Influence of the protein environment? (different dielectrics)
  - Attack to different possitions of the side chain
    - Influence of the protein folding?
    - Influence of the protein environment?
- Future QM/MM simulations on proteins

2. Model and Methods

Model

- Tripeptide model:
  - Two peptide bonds (α-helix, β-sheet...)
  - Side chain only at central amino acid.
  - Lateral amino acids truncated at C_α
- Disadvantages:
  - α-helix not properly described
- Advantages:
  - One side chain (R)

- This presentation
  - R: Phe, Tyr, Trp
  - β-sheet



Methods

- Geometry Optimizations & Frequency Calculations:
  - MPWB1K / 6-31+G(d,p)
- Singlepoints at $\varepsilon = 4, 80$ (IEFPCM):
  - MPWB1K / 6-311++G(2df,p)
  - $H_{\epsilon}^{298} = E_{\epsilon} + H_{cont}^{gas}$
- Kinetics: $\Delta H_{\epsilon}^{TS} = H_{\epsilon}^{TS} - H_{\epsilon}^{React}$
- Thermodynamics: $\Delta H_{\epsilon}^{Prod} = H_{\epsilon}^{Prod} - H_{\epsilon}^{React}$
- Gaussian09

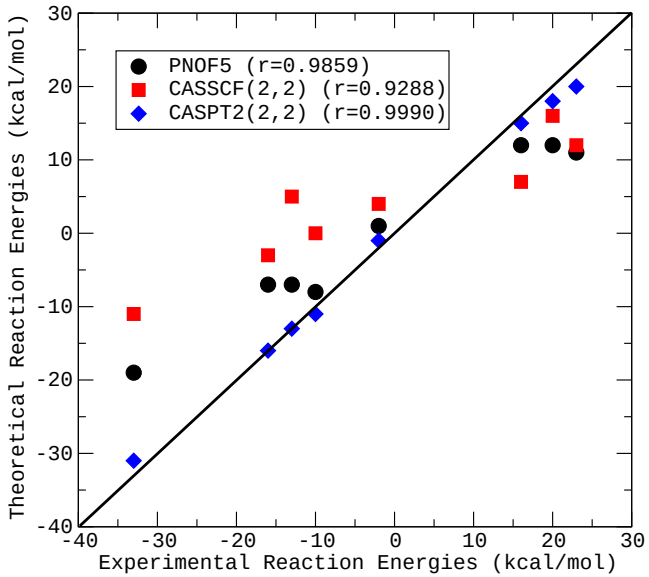
Method Validation

Reaction	Computed	Experimental	Difference
Bond dissociation			
$CH_4 \rightarrow CH_3^{\cdot} + {}^{\cdot}H$	112.4	113.0	−0.6
$NH_3 \rightarrow NH_2^{\cdot} + {}^{\cdot}H$	114.2	115.9	−1.7
$H_2O \rightarrow {}^{\cdot}OH + {}^{\cdot}H$	122.3	126.0	−3.7
$C_2H_6 \rightarrow C_2H_5^{\cdot} + {}^{\cdot}H$	107.9	109.4	−1.5
$H_2O_2 \rightarrow HOO^{\cdot} + {}^{\cdot}H$	89.1	92.7	−3.6
$C_2H_6 \rightarrow 2\,CH_3^{\cdot}$	98.7	96.6	2.1
$H_2O_2 \rightarrow 2\,{}^{\cdot}OH$	49.4	55.0	−5.6
MAD			−2.1
H abstraction			
$CH_4 + {}^{\cdot}OH \rightarrow CH_3^{\cdot} + H_2O$	−9.9	−13.0	3.1
$NH_3 + {}^{\cdot}OH \rightarrow NH_2^{\cdot} + H_2O$	−8.2	−10.1	1.9
$C_2H_6 + {}^{\cdot}OH \rightarrow C_2H_5^{\cdot} + H_2O$	−14.4	−16.6	2.2
$H_2O_2 + {}^{\cdot}OH \rightarrow HOO^{\cdot} + H_2O$	−33.3	−33.3	0.0
MAD			1.8

Do not forget: DFT good results due to cancellation of errors!

PNOF5 and Radicals

- Energetics of radical mediated reactions
  J. Chem. Theor. Comput. 8, 2646 (2012)

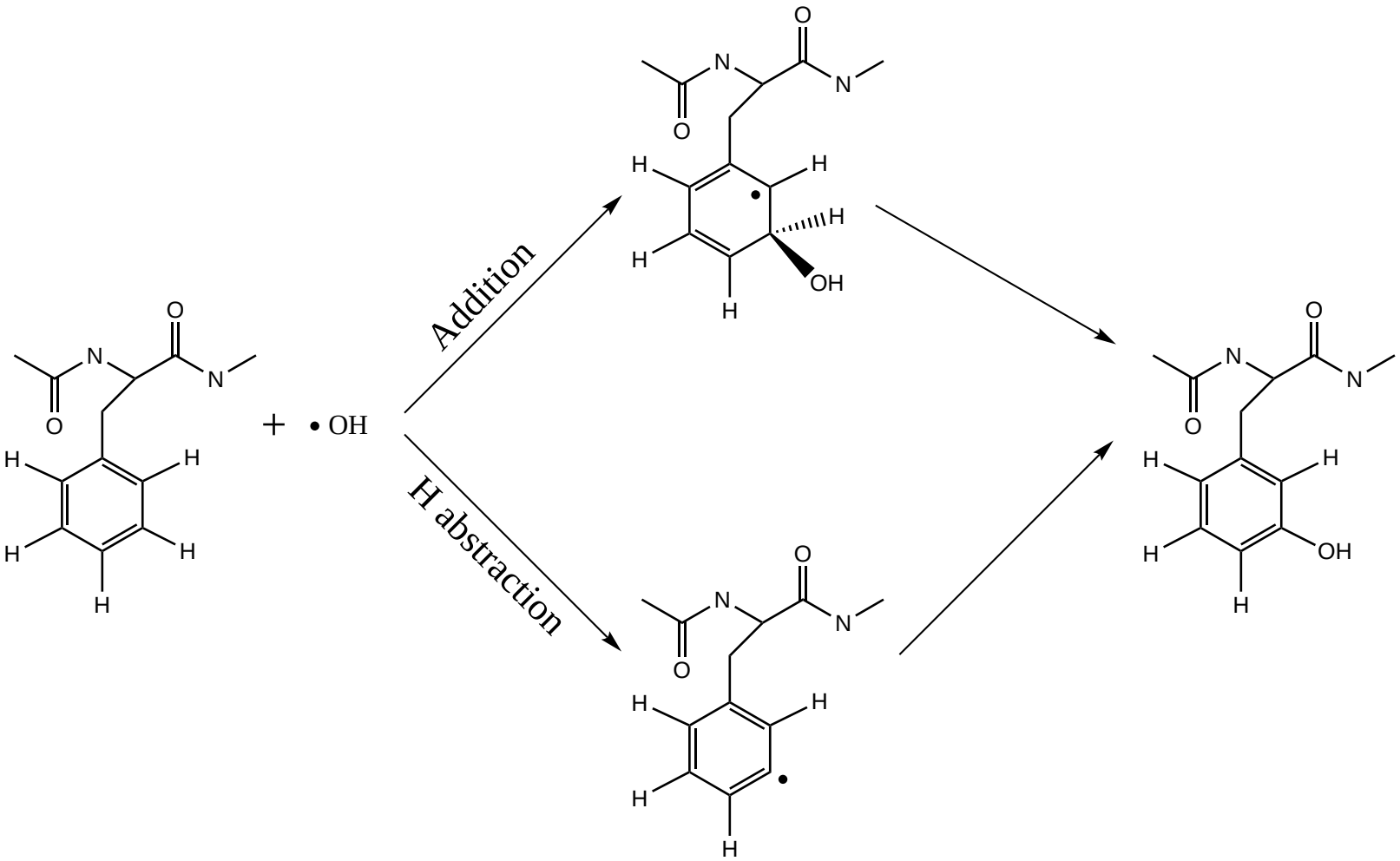


- Sign of reaction energetics improved from CASSCF to PNOF5

3. Results

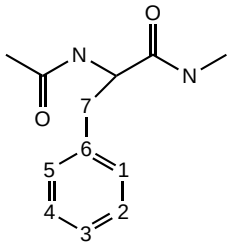
Mujika et. al., Chem. Eur. J 19, 6862 (2013)

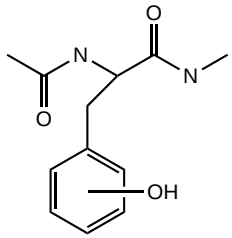
Reactivity of ·*OH* on Aromatic Rings

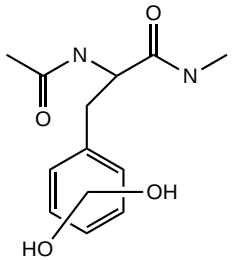


Atom Labeling and Experimental Products

Phenylalanine

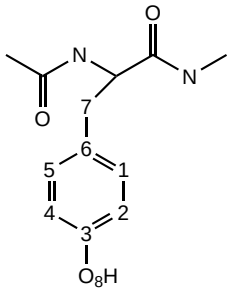


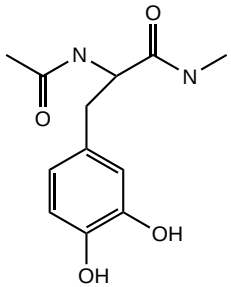


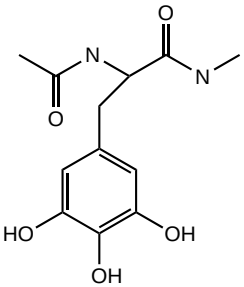


2-,3-, or 4-hydroxyphenylalanine

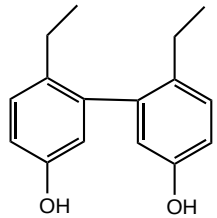
Tyrosine





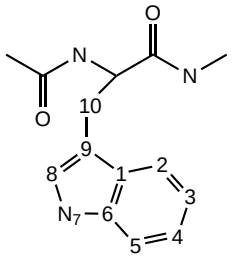


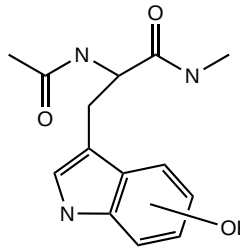
DOPA

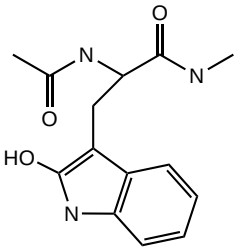


Dityrosine

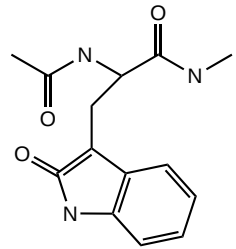
Tryptophan





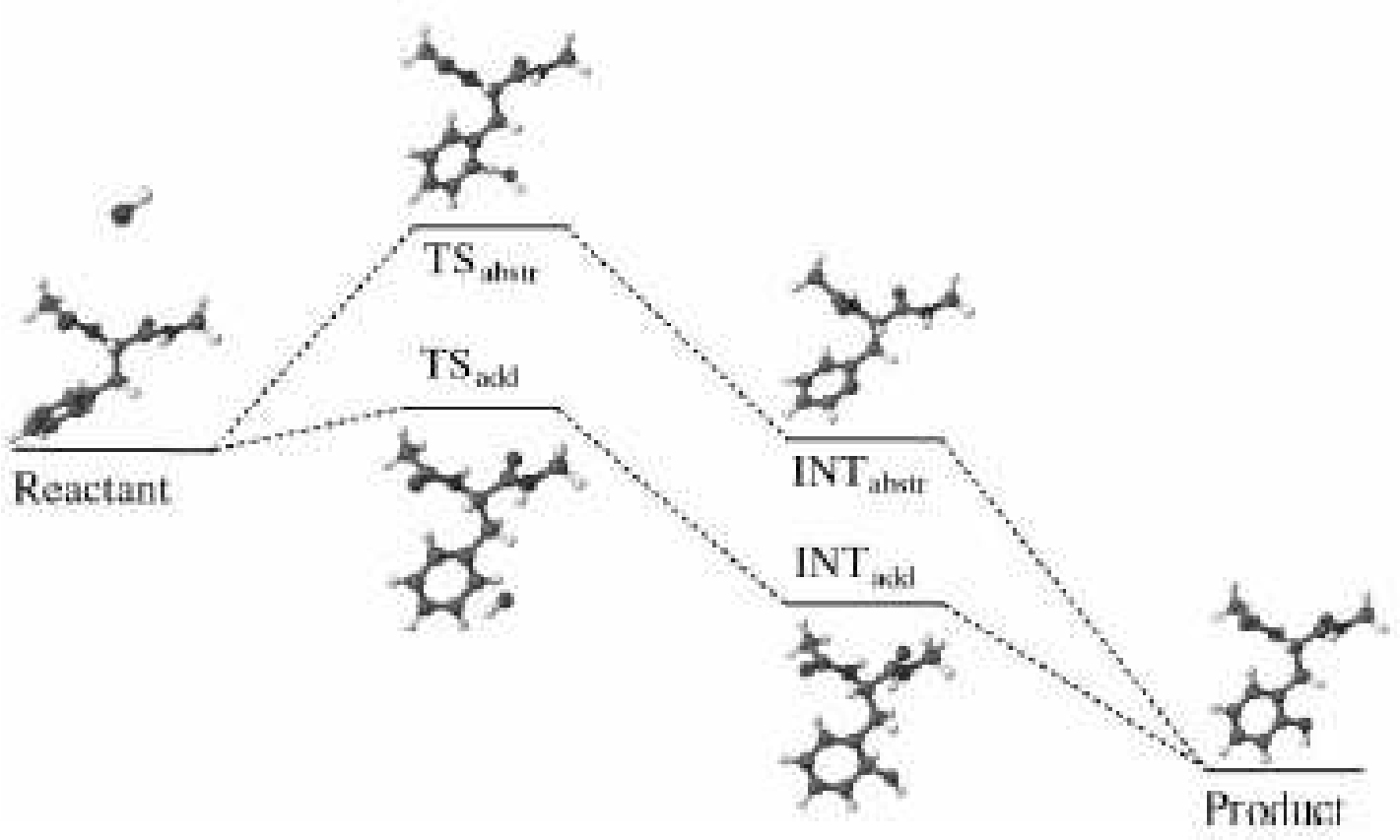


hydroxytryptophan

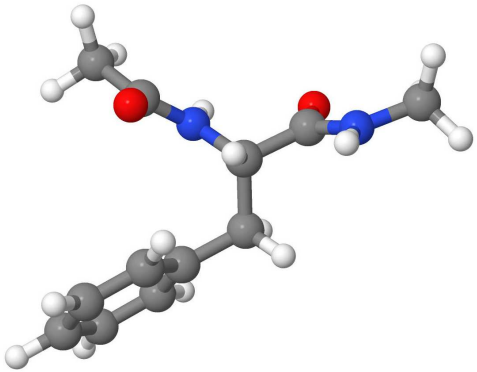


Oxindolylalanine

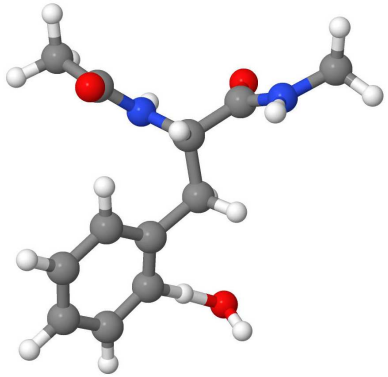
Phenylalanine



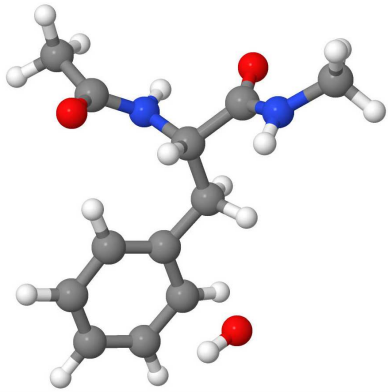
Phenylalanine



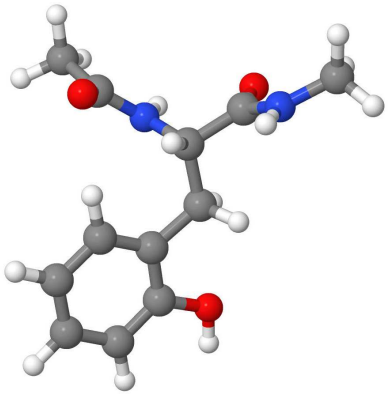
Reactant



TS^{C1ab}_{Phe}



TS^{C1add}_{Phe}



$Prod^{C1}_{Phe}$

Phenylalanine (1^{st} $\cdot OH$)

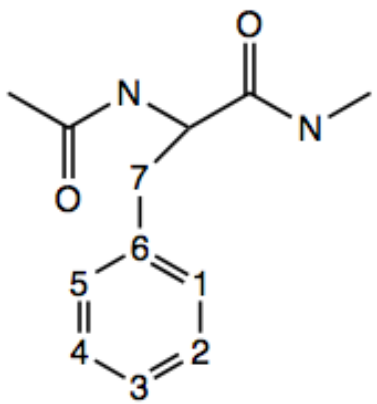
	$\cdot OH$ Addition					H Abstraction			
	ΔH_4^{TS}	ΔH_4^{Int}	ΔH_{aq}^{TS}	ΔH_{aq}^{Int}		ΔH_4^{TS}	ΔH_4^{Int}	ΔH_{aq}^{TS}	ΔH_{aq}^{Int}
C1	-0.4	-18.0	1.1	-17.2	C1	4.1	-4.6	4.9	-4.9
C2	1.0	-16.3	1.7	-16.0	C2	3.7	-4.7	4.1	-5.5
C3	0.4	-18.0	1.0	-17.0	C3	4.1	-4.2	4.7	-4.9
C4	1.0	-16.5	1.7	-15.5	C4	5.3	-5.4	6.7	-6.2
C5	-4.5	-17.7	-1.3	-15.1	C5	2.2	-4.9	5.4	-5.4
C6	-0.5	-18.6	0.6	-14.9	C7	0.1	-29.7	3.2	-29.1

- ϵ=4, 80 similar conclusions.
- Addition kinetically and thermodynamically favored
- Exception: H abstraction from C_β

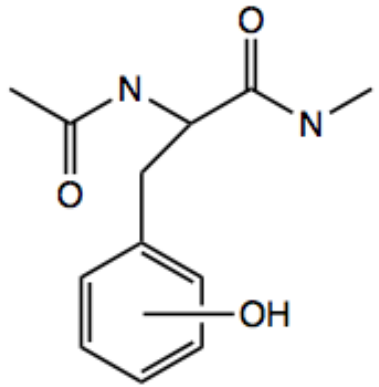
Phenylalanine (2^{nd} $\cdot OH$)

	$\triangle H_4^{298}$
$Prod_{Phe}^{C1}$	-117.1
$Prod_{Phe}^{C2}$	-116.9
$Prod_{Phe}^{C3}$	-116.5
$Prod_{Phe}^{C4}$	-116.4
$Prod_{Phe}^{C5}$	-115.9
$Prod_{Phe}^{C7}$	-111.0
$Prod_{Phe}^{C7\alpha}$	-102.5

Labeling



Exper. Products



2-,3-, or 4-hydroxyphenylalanine

- Addition + Abstraction
- Steric effects

Tyrosine (1^{st} $\cdot OH$)

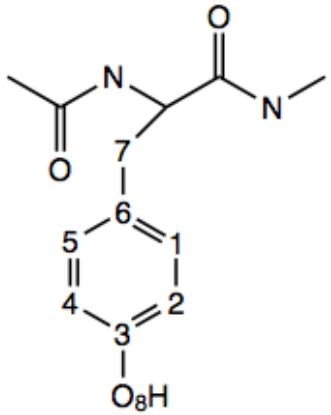
$\cdot OH$ Addition			H Abstraction		
	ΔH_4^{TS}	ΔH_4^{Int}		ΔH_4^{TS}	ΔH_4^{Int}
C1	0.9	-16.7	C1	5.2	-3.7
C2	0.6	-18.1	C2	6.2	-2.2
C3	2.5	-18.9	C4	5.8	-2.6
C4	1.1	-18.2	C5	3.0	-3.8
C5	-3.4	-17.0	C7	0.9	-29.6
C6	-1.8	-19.5	O8	2.4	-29.3

- Addition kinetically and thermodynamically favored
- Exception: H abstraction from C_β and O8

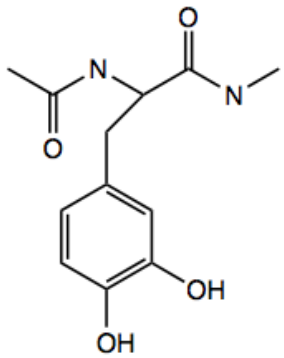
Tyrosine (2^{nd} $\cdot OH$)

	$\triangle H_4^{298}$
$Prod_{Tyr}^{C1}$	-116.5
$Prod_{Tyr}^{C2}$	-114.9
$Prod_{Tyr}^{C4}$	-114.1
$Prod_{Tyr}^{C5}$	-116.4
$Prod_{Tyr}^{C7}$	-111.0
$Prod_{Tyr}^{C7\alpha}$	-101.3
$Prod_{Tyr-Tyr}^{OO}$	-37.3
$Prod_{Tyr-Tyr}^{CC}$	-107.7

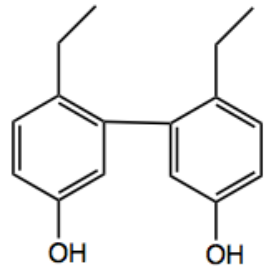
Labeling



Exper. Products



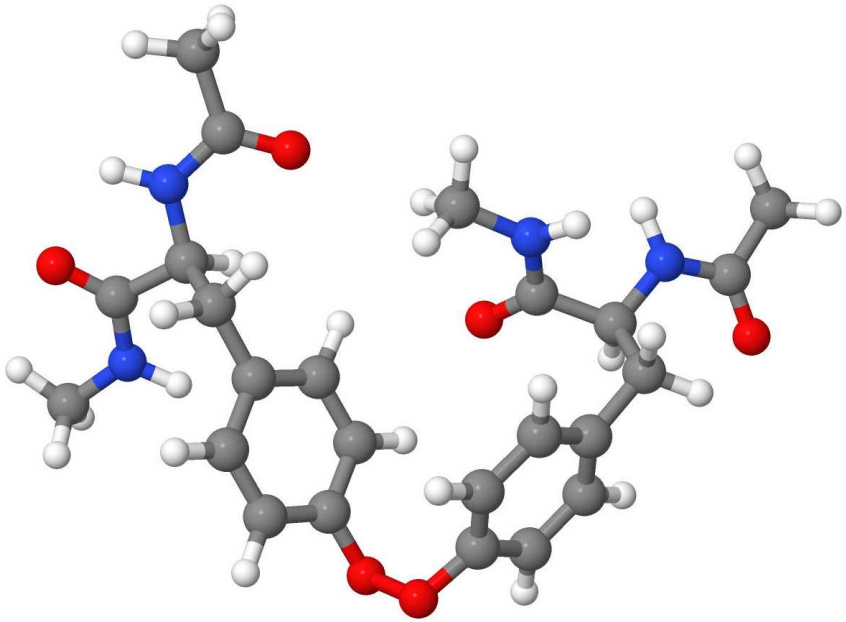
DOPA

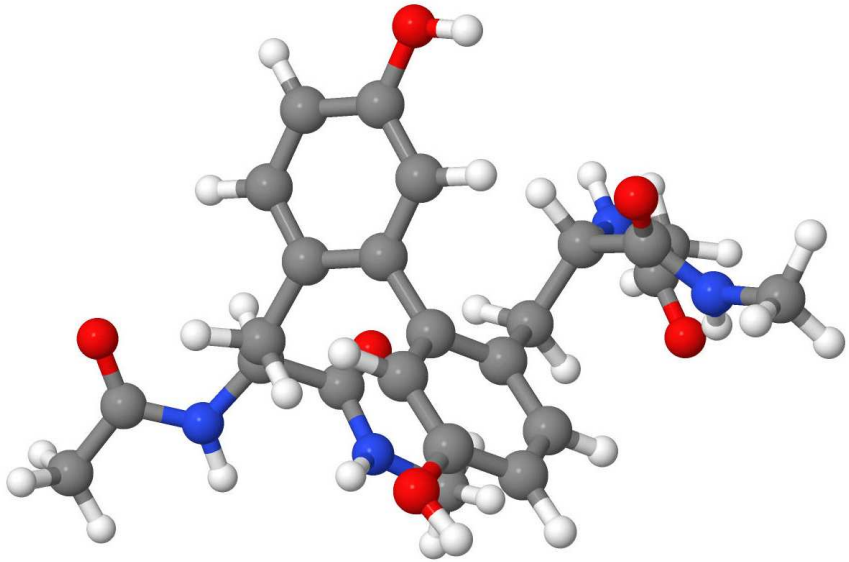


Dityrosine

- DOPA: Addition + Abstraction
- Dityrosine: 2 H abstractions
- Thermodynamic effects

Tyrosine (2^{nd} $\cdot OH$)





Tryptophan (1^{st} $\cdot OH$)

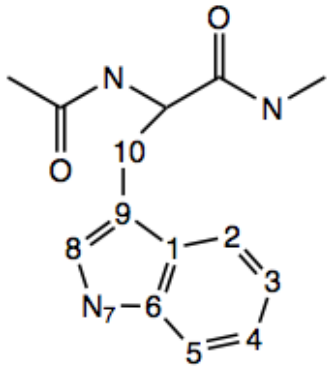
$\cdot OH$ Addition			H Abstraction		
	ΔH_4^{TS}	ΔH_4^{Int}		ΔH_4^{TS}	ΔH_4^{Int}
C2	0.1	-24.4	C2	1.7	-4.4
C3	0.7	-15.3	C3	4.7	-3.1
C4	-0.4	-17.7	C4	4.9	-3.3
C5	-0.8	-19.8	C5	5.8	-2.1
C8	-5.7	-28.6	N7	2.2	-24.5
			C8	-	3.3
			C10	-1.6	-28.7

- Addition kinetically and thermodynamically favored
- Exception: H abstraction from C_β and N7

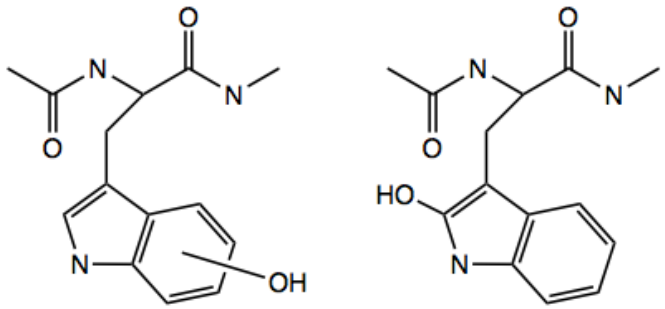
Tryptophan (2^{nd} $\cdot OH$)

	$\triangle H_4^{298}$
$Prod_{Trp}^{C2}$	-116.2
$Prod_{Trp}^{C3}$	-113.9
$Prod_{Trp}^{C4}$	-114.6
$Prod_{Trp}^{C5}$	-114.6
$Prod_{Trp}^{N7}$	-74.7
$Prod_{Trp}^{C8}$	-123.5
$Prod_{Trp}^{C10}$	-111.6
$Prod_{Trp}^{10\alpha}$	-104.6

Labeling



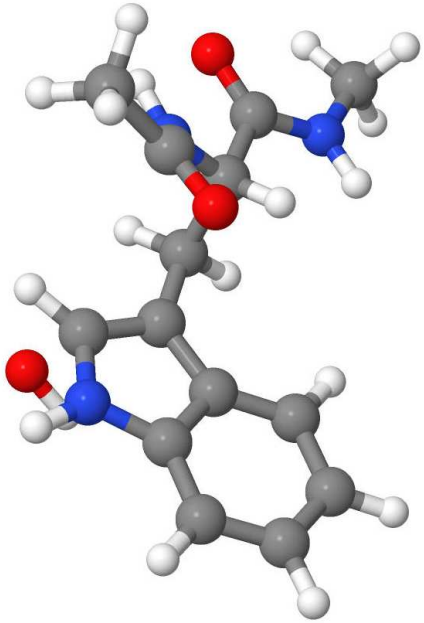
Exper. Products



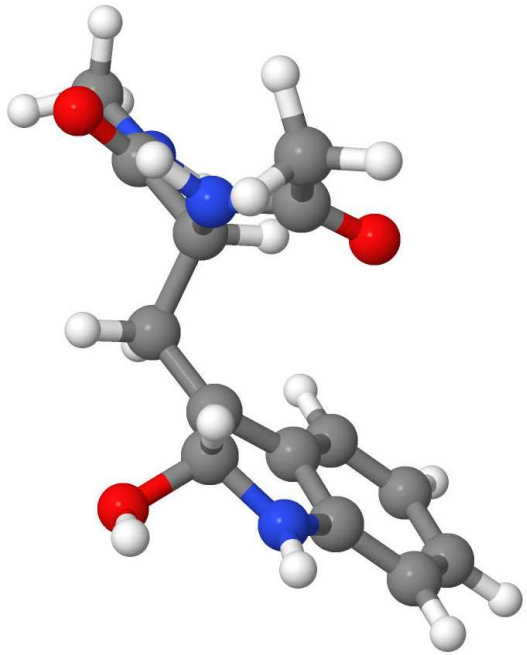
hydroxytryptophan

- Addition + Abstraction
- Thermodynamic effects

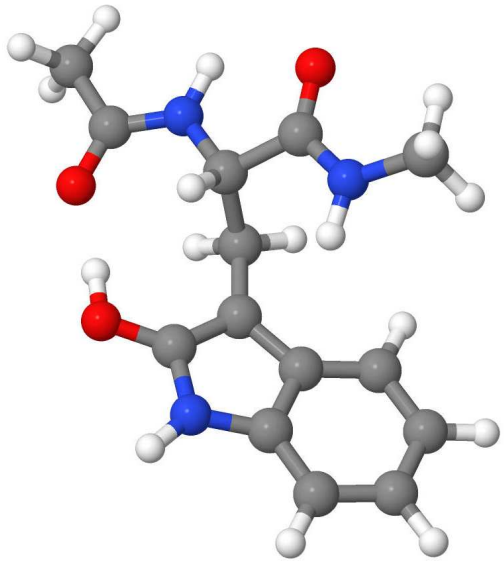
Tryptophan



TS_{Trp}^{C8add}



Int_{Trp}^{C8add}



$Prod_{Trp}^{C8}$

4. Conclusions

Conclusions

- All aromatic amino acids target.
- Thermodynamics governs over the kinetics
- Other factors, radical concentration and steric effects, important
- Two-step mechanism:
  - the first ·OH radical addition
  - the second ·OH radical abstracts a hydrogen.
- Results in agreement with experiments

Acknowledgments

Theoretical Chemistry Group

- The SGI/IZO–SGIker (UPV/EHU)
  - Arina supercomputer
- Funding
  - Basque and Spanish Governments
- Organizing committe
- Audience!