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# Novel insights in chlorine dioxide based disinfection mechanism- Investigation of the reaction with amino acids

## Supplementary information

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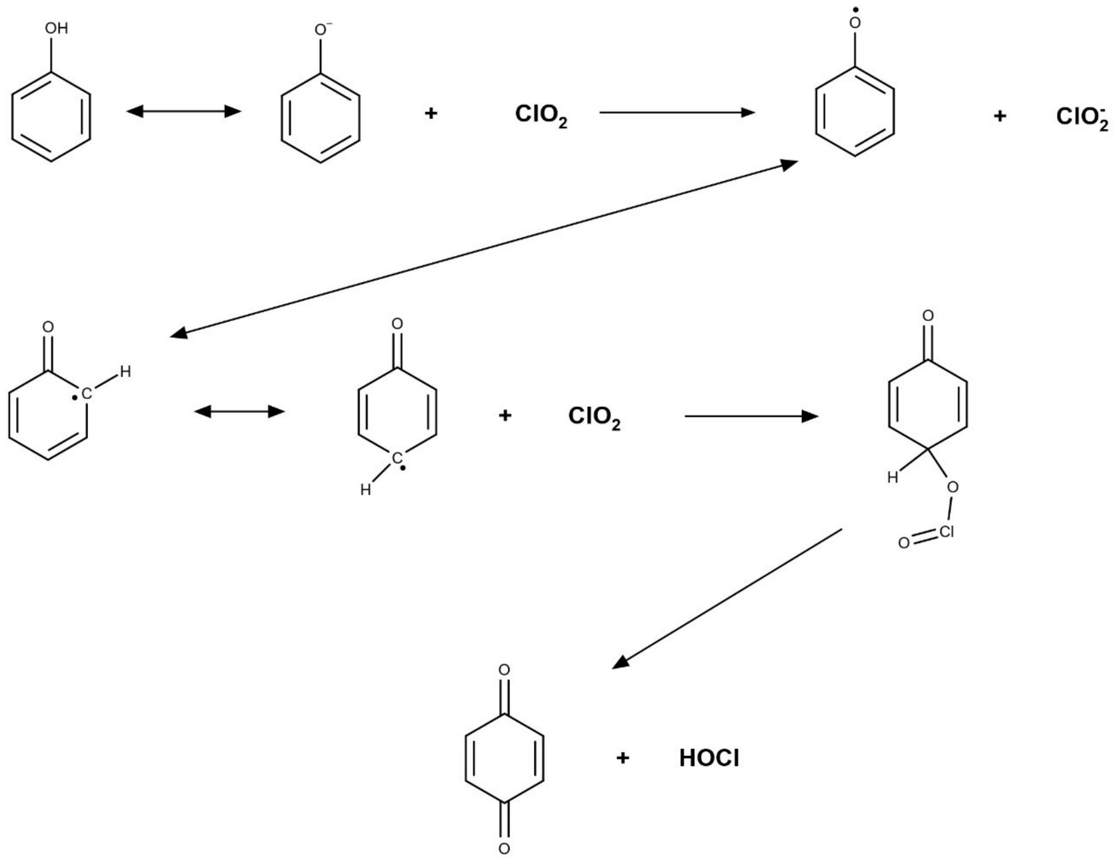

**Figure S1: Mechanistic pathway of intrinsic formation of FAC during the reaction of phenol with $ClO_2$ according to Wajon et al. 1982.[1] In the first step, phenol reacts with one molecule of $ClO_2$ under the formation of $ClO_2^-$ and a phenoxy-radical. Afterward, the radical stabilizes in the para position. The phenoxy-radical reacts with another molecule of $ClO_2$ and forms an OClO-adduct in para-position. Eventually, the adduct disproportionates to FAC and benzoquinone.**

**Table S1: Chemicals used in this research project.**

| Name | Purity [%] | Purpose of use | Manufacturer |
|---|---|---|---|
| Acetic acid | > 99.7 | Eluent (LC) | Alfa Aesar (Haverhill, Massachusetts, USA) |
| Acetonitrile | > 99.9 | Eluent (LC) | Honeywell \| Riedel-de Haen (Charlotte, North Carolina, USA) |
| Ammonium-molybdate(VI)-tetrahydrate | > 99 | Post column catalyzer | Acros Organics (Fair Lawn, New Jersey, USA) |
| Disodium phosphate | > 99 | pH buffer | Merck (Darmstadt, Germany) |
| Glycine | > 99 | HOCl Scavenger | Alfa Aesar (Haverhill, Massachusetts, USA) |
| Indol | > 99 | Competitor, model compound | Sigma-Aldrich (St. Louis, Missouri, USA) |
| Monosodium phosphate | 98 | pH buffer | Acros Organics (Fair Lawn, New Jersey, USA) |
| N-Acetyl-L-Tryptophan | ≥ 99 | Compound under study | Sigma-Aldrich (St. Louis, Missouri, USA) |
| N-Acetyl-L-Tyrosine | > 99 | Compound under study | TCI (Tokyo, Japan) |
| Nitrogen | 99.999 | $ClO_2$ production | Air Liquide (Paris, France) |
| Ortho-phosphoric acid | 85 | pH buffer | VWR (Radnor, Pennsylvania, USA) |
| Phenol | > 99 % | Competitor | Sigma-Aldrich (St. Louis, Missouri, USA) |
| Potassium iodide | > 99 | Post column reagent | Acros Organics (Fair Lawn, New Jersey, USA) |
| Sodium acetate | > 99 | Eluent (LC) | Sigma-Aldrich (St. Louis, Missouri, USA) |
| Sodium carbonate | 99.5 | Eluent (IC) | Acros Organics (Fair Lawn, New Jersey, USA) |
| Sodium chlorate | > 99 | Calibration standard | Acros Organics (Fair Lawn, New Jersey, USA) |
| Sodium chloride | > 99.5 | Calibration standard | Honeywell \| Fluka (Charlotte, Narth Carolina, USA) |
| Sodium chlorite | 80 | Calibration standard | Honeywell \| Fluka (Charlotte, Narth Carolina, USA) |
| Sodium hypochlorite | 11 – 15 % FAC | Oxidant | Alfa Aesar (Haverhill, Massachusetts, USA) |
| Sodium persulfate | > 99 | $ClO_2$ production | Carl Roth (Karlsruhe, Germany) |
| Sodium phosphate | 96 | pH buffer | Sigma-Aldrich (St. Louis, Missouri, USA) |
| Sulfamethoxazole | > 98 | Competitor | Sigma-Aldrich (St. Louis, Missouri, USA) |
| Sulfuric acid | 95 | Chemical suppressor (IC) / Post column reaction | VWR (Radnor, Pennsylvania, USA) |

**Table S2: Instruments used in this research project.**

| Name | Component | Description | Manufacturer |
|---|---|---|---|
| Ion-Chromatography | Autosampler | Dionex AS-AP | Thermo scientific (Waltham, Massachusetts, USA) |
| | Column | Asupp7 – 250mm/4.0 µm | Metrohm (Herisau, Swiss) |
| | Column department | Dionex ICS-6000 DC | Thermo scientific (Waltham, Massachusetts, USA) |
| | Conductivity detector | | Thermo scientific (Waltham, Massachusetts, USA) |
| | Pump 1 (Eluent) | Dionex ICS-6000 SP | Thermo scientific (Waltham, Massachusetts, USA) |
| | Pump 2 (Suppressor) | Dionex AXP | Thermo scientific (Waltham, Massachusetts, USA) |
| | Pump 3 (Post column reaction) | Peristaltic pump | Ismatec (Wertheim, Germany) |
| | Software | Chromeleon Console | Thermo scientific (Waltham, Massachusetts, USA) |
| | Suppressor | Dionex ACRS 500 | Thermo scientific (Waltham, Massachusetts, USA) |
| | UV detector | Dionex UltiMate 3000 Diode Array Detection | Thermo scientific (Waltham, Massachusetts, USA) |
| Liquid Chromatography | Autosampler | Dionex AS-AP | Thermo scientific (Waltham, Massachusetts, USA) |
| | Column | Acclaim Trinity P1 3µm – 2.1 µm × 150 mm | Thermo scientific (Waltham, Massachusetts, USA) |
| | Column department | Dionex ICS-6000 DC | Thermo scientific (Waltham, Massachusetts, USA) |
| | Pump 1 (Eluent) | Dionex ICS-6000 SP | Thermo scientific (Waltham, Massachusetts, USA) |
| | Software | Chromeleon Console | Thermo scientific (Waltham, Massachusetts, USA) |
| | UV detector | Dionex UltiMate 3000 Diode Array Detection | Thermo scientific (Waltham, Massachusetts, USA) |
| Photometer | Photometer | Specord 200 Plus | AnalytikJena (Jena, Germany) |
| pH-meter | pH-meter | Terminal 740 | WTW Series inoLab |

| | | | |
|---|---|---|---|
| | | | (Weilheim, Germany) |
| Balance | Balance | SM2285Di-ION-C | VWR (Radnor, Pennsylvania, USA) |
| Reaction tubes | 15 mL CellStar® tubes | Polypropylene | Greiner bio-one (Frickenhausen, Germany) |
| HPLC Vials | 1.5 mL Short thread vial | Amber glass | VWR (Radnor, Pennsylvania, USA) |
| HPLC Vials | 1.5 mL Short thread vial | Polypropylene | VWR (Radnor, Pennsylvania, USA) |

**Table S3: Liquid chromatography methods used in this research project. Eluent A = 20 mM sodium acetate buffer pH 5; Eluent B = ACN.**

| No. Method | Total time [min] | Gradient program | | | | Compound (Ret. Time [Min]) |
|---|---|---|---|---|---|---|
| | | Time | Flow rate | %A | %B | |
| 1 | 31 | 0.000 | 0.3 | 80 | 20 | Phenol (4.0), indol (7.9), SMX (10.5), NAL-Trp (22.6) |
| | | 6.000 | 0.3 | 80 | 20 | |
| | | 10.000 | 0.4 | 40 | 60 | |
| | | 22.000 | 0.4 | 40 | 60 | |
| | | 26.000 | 0.3 | 80 | 20 | |
| | | | | | | |
| 2 | 22 | 0.0 | 0.3 | 80 | 20 | Phenol (4.0), NAL-Tyr (14.6) |
| | | 6.0 | 0.3 | 80 | 20 | |
| | | 10.0 | 0.4 | 40 | 60 | |
| | | 14.0 | 0.4 | 40 | 60 | |
| | | 18.0 | 0.3 | 80 | 20 | |
| | | | | | | |

**Table S4: Ion chromatography methods used in this research project. Eluent A =pure $H_2O$; Eluent B = 4 mM $Na_2CO_3$. The retention times for Cl-Gly and $ClO_2^-$ are given for conductivity and UV detector (CD/UV).**

| No. Method | Total time [min] | Gradient program | | | | Compound (Ret. Time [Min]) |
|---|---|---|---|---|---|---|
| | | Time | Flow rate | %A | %B | |
| 1 | 40 | 0.000 | 0.75 | 70 | 30 | Cl-Gly (11.3/12.0), $ClO_2^-$ (12.3/12.9), $Cl^-$ (15.5), $ClO_3^-$ (25.3) |
| | | 16.000 | 0.75 | 70 | 30 | |
| | | 16.001 | 0.75 | 0 | 100 | |
| | | 32.000 | 0.75 | 0 | 100 | |
| | | 32.001 | 0.75 | 70 | 30 | |

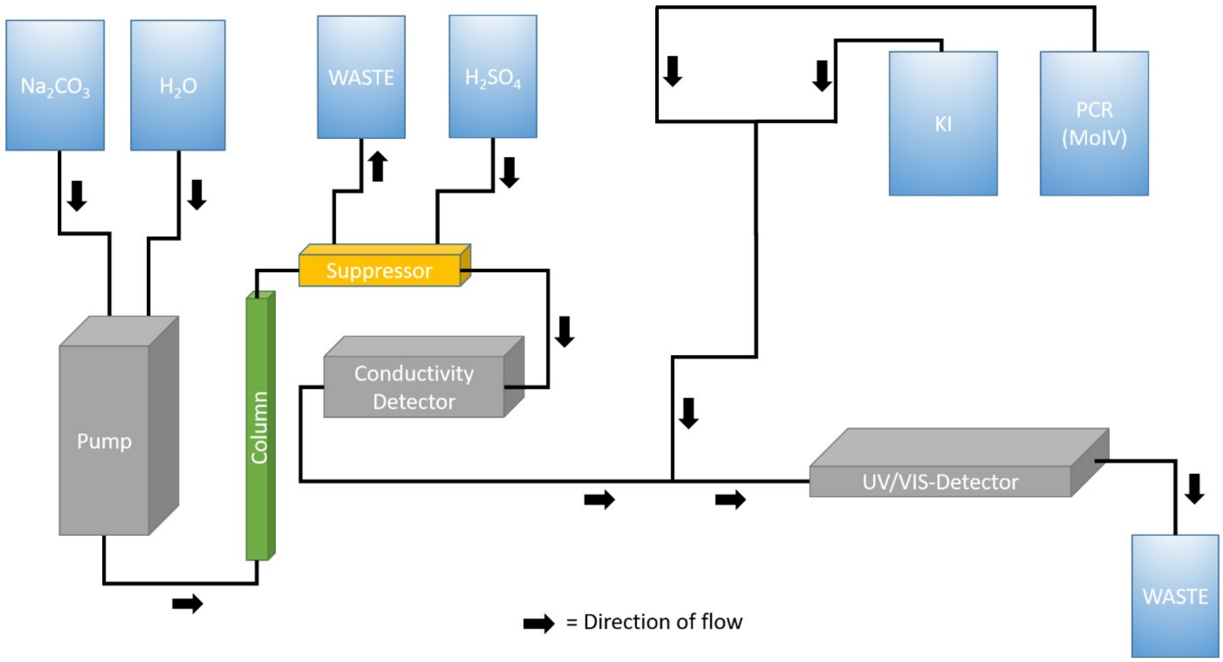

**Figure S2: Set up of IC-CD-PCR-UV. A gradient pump is transporting the eluent A: $H_2O$ and eluent B: 4 mM $Na_2CO_3$ towards the column. After the column, a chemical suppression is installed using 20 mM $H_2SO_4$ (1.0 mL/min). Then the analysts are detected by a conductivity detector. To increase the sensitivity for Cl-Gly and $ClO_2^-$ a post column reaction is installed, whereby a 0.27 M potassium iodide (KI) and a catalyzing solution (containing 0.027 mM ammonium molybdate(VI) tetrahydrate and 0.1 M $H_2SO_4$) are added to the system (0.1 mL/min each solution). During this reaction Iodide is oxidized to triiodide, which has a high absorption at $\lambda$ = 352 nm ($\varepsilon_{352}$ = 26,000 $M^{-1}$ $cm^{-1}$).**[2]

## Text S1: Calculation of necessary scavenger concentrations

In most experiments, scavengers are needed to scavenge the intrinsic formed FAC. For this purpose glycine is used ($k$ (glycine + HOCl) = $1.5 \times 10^{5}$ $M^{-1}$ $s^{-1}$)[3]. To calculate the necessary concentration of glycine ([Scavenger]), Formula S1 is used.

$$f(Scavenger + HOCl) = \frac{k\ (Scavenger + HOCl) \times [Scavenger]}{\sum(k\ (Compound + HOCl) \times [Compound])}$$

(Formula S1)

The compound under study ([Compound]) was always 0.0001 M, the reaction rate constants ($k$(compound + HOCl) are taken from Pattison et al. 2002[4] ($k$ (NAL-tyrosine + HOCl) = $4.7 \times 10^{1}$ $M^{-1}$ $s^{-1}$ & $k$ (NAL-tryptophan + HOCl) = $7.8 \times 10^{3}$ $M^{-1}$ $s^{-1}$ both at pH 7.2 – 7.4). According to Formula S1 it was calculated how much glycine was needed to scavenge a fraction (f(Scavenger + HOCl)) of 99.9 % HOCl (f = 0.999).

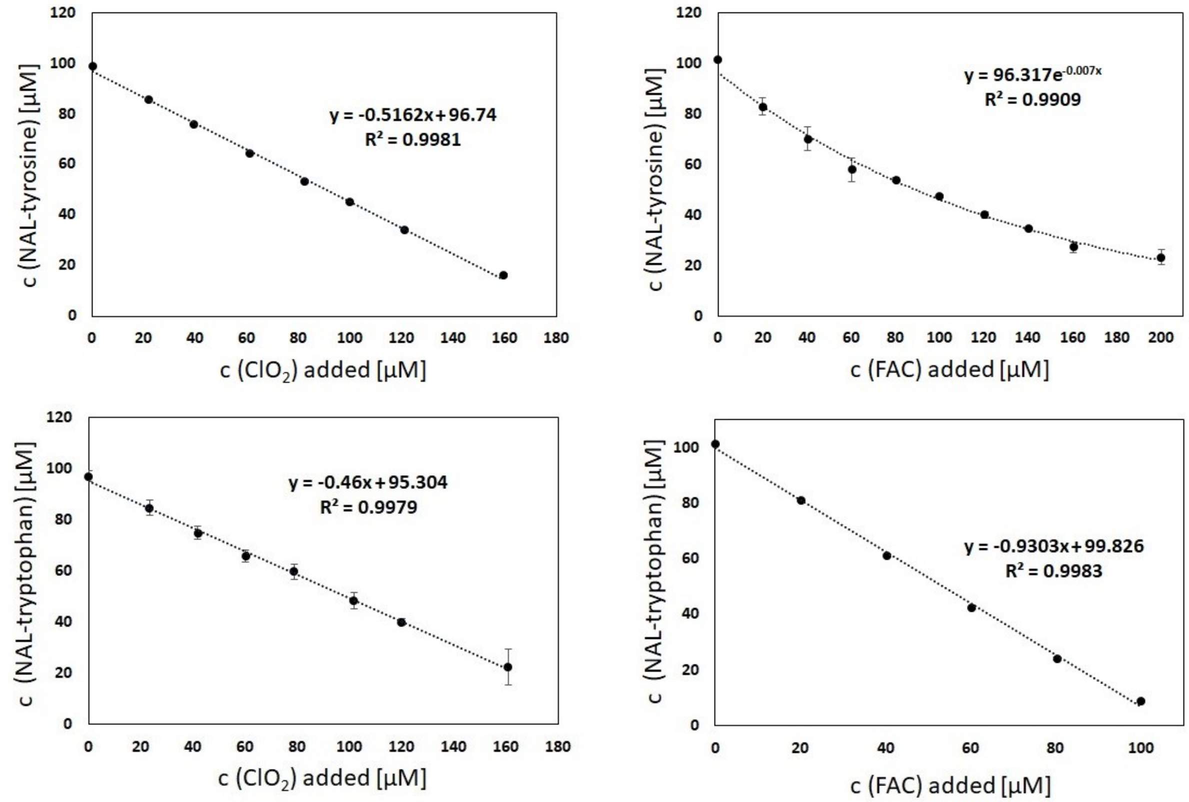

**Figure S3: Stoichiometry results. Stoichiometry has been determined for both amino acids with oxidants at pH 7. Reaction conditions: 0.1 mM of the corresponding amino acids, 5 mM of phosphate buffer, and, in the case of determining the stoichiometry for $ClO_2$, 10 mM glycine was added to scavenge intrinsic formed FAC, which would bias the final results. All experiments have been carried out in triplicates, and the error bars are representing the standard deviation of the results.**

**Table S5: Measured impurities of $ClO_2$ stock solution. Percentage is based on the initial concentration of $ClO_2$, which was measured to be 17.225 mM.**

| | $Cl^-$ | $ClO_2^-$ | $ClO_3^-$ | *FAC* | *Total* |
|---|---|---|---|---|---|
| *Impurity [%]* | 0.153 ± 0.002 | 0.118 ± 0.003 | 2.166 ± 0.038 | – | 2.438 |

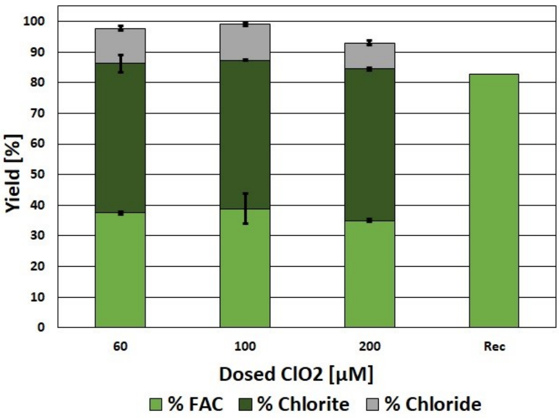

**Figure S4: Chlorine balance of $ClO_2$ during the reaction with indol at pH 7. The reaction solution contained 10 mM glycine, 5 mM phosphate buffer, and 0.1 mM indol. The experiment has been carried out in triplicates, and the error bars are representing the standard deviation of the results.**

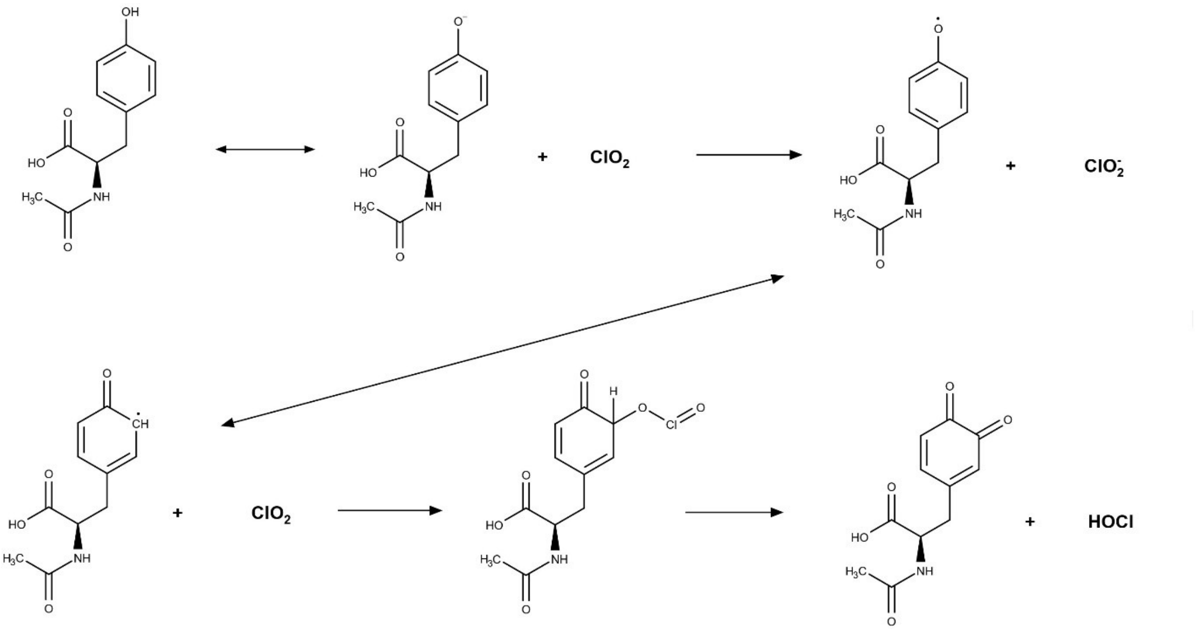

**Figure S5: Mechanistic pathway of intrinsic formation of FAC during the reaction of phenol with $ClO_2$ adapted from Napolitano et al. 2005.[5] In the first step, NAL-tyrosine reacts with one molecule of $ClO_2$ under the formation of $ClO_2^-$ and a phenoxy-radical. Afterwards the redistribution of the radical location to a more stable position takes place. The phenoxy-radical reacts with another molecule of $ClO_2$ and forms an OClO-adduct in ortho-position. Eventually, the adduct breaks down to a keto group and HOCl.**

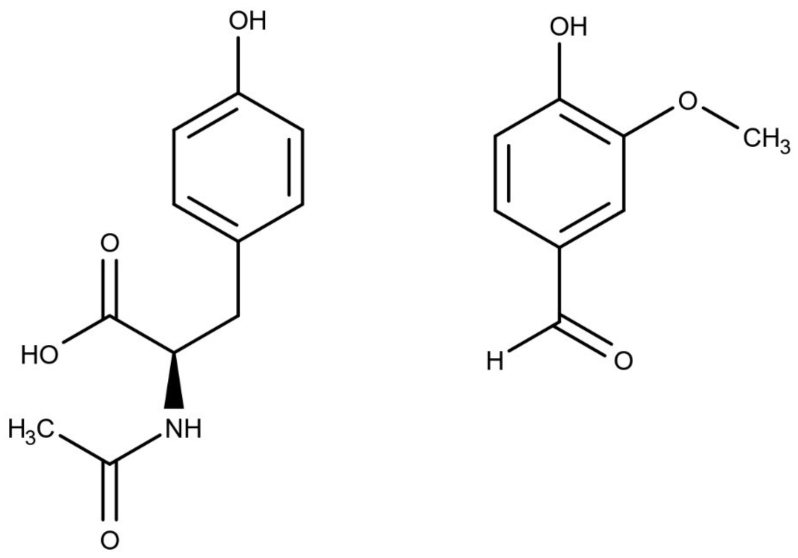

**Figure S6: Chemical structure of NAL-tyrosine (left) and vanillin (right).**

## Literature

(1) Wajon, J. E.; Rosenblatt, D. H.; Burrows, E. P. Oxidation of Phenol and Hydroquinone by Chlorine Dioxide. *Environ. Sci. Technol.* **1982**, *16* (7), 396–402.

(2) Abdighahroudi, M. S.; Schmidt, T. C.; Lutze, H. V. Determination of Free Chlorine Based on Ion Chromatography—Application of Glycine as a Selective Scavenger. *Anal. Bioanal. Chem.* **2020**, *412* (28), 7713–7722.

(3) Deborde, M.; von Gunten, U. Reactions of Chlorine with Inorganic and Organic Compounds during Water Treatment-Kinetics and Mechanisms: A Critical Review. *Water Res.* **2008**, *42* (1–2), 13–51.

(4) Pattison, D. I.; Davies, M. J. Absolute Rate Constants for the Reaction of Hypochlorous Acid with Protein Side Chains and Peptide Bonds. *Chem. Res. Toxicol.* **2001**, *14* (10), 1453–1464.

(5) Napolitano, M. J.; Green, B. J.; Nicoson, J. S.; Margerum, D. W. Chlorine Dioxide Oxidations of Tyrosine, N-Acetyltyrosine, and Dopa. *Chem. Res. Toxicol.* **2005**, *18* (3), 501–508.