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Antioxidant Potential of Anthocyanidins: A *Healthy* Computational Activity for High School and Undergraduate Students

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ABSTRACT: Molecules and Computer: Chemistry Calculations in Class (MC^4) is a computational laboratory intended for final-year high school or undergraduate students. The topic is the antioxidant potential of anthocyanidins, which is chemically related to their radical scavenging action via the mechanism of hydrogen atom transfer (HAT). This laboratory combines (bio)chemical and nutraceutical concepts with organic chemical reactions involving radical species. It allows students to apply important physicochemical (thermodynamic) concepts, such as Gibbs free energy of reaction and solvation. Finally, the procedure can easily be tailored to the resources at hand as well as the knowledge of the students. In fact, when computing facilities are not available, the whole set of molecular structures and energy data are provided as well as a simple datasheet required for their analysis. Alternatively, the whole protocol and useful scripts are provided so that students can generate their own results by experiencing the approach to computational chemistry.

KEYWORDS: *Anthocyanidins, Antioxidants, Computational Chemistry, Gibbs Free Energy of Reaction, Molecular Structure, Radicals*

■ INTRODUCTION

Nowadays, it is not unusual to find ads for healthy foods extolling their antioxidant content. Dietary supplements based on antioxidant substances are often prescribed to help prevent different diseases, ranging from a common cold to more severe pathologies.[1,2] It is also quite common to advertise the potential benefits of antioxidants for cosmetics.[3] Thus, this topic is important and of general interest. From a chemical perspective, educational emphasis should be placed on the definition of antioxidants and on the elementary reactions in which they are involved.[4] The chemistry of natural antioxidant compounds provides an excellent opportunity for classroom discussions and experiments. This is due to the fundamental concepts involved, the interdisciplinary nature of the topic, and the encouragement of critical thinking about real-world issues.

Living beings possess an endogenous antioxidant system formed by enzymes and molecular compounds (Figure 1). In the former group, we find, for example, the selenoproteins of the glutathione peroxidase (GPx) family,[5] the superoxide dismutase (SOD),[6] and the catalases (CAT);[7] in the latter, there are glutathione, alpha-lipoic acid, coenzyme Q, melatonin, and uric acid.[8] Chemically, the antioxidant action consists of reducing hydroperoxides and/or scavenging harmful radicals, such as the so-called ROS (reactive oxygen species) and RNS (reactive nitrogen species), which can damage biomolecules and nucleic acids. The antioxidant aims to keep at bay *oxidative stress*, i.e., a dangerous condition characterized by an imbalance between antioxidant and pro-oxidant species in the cell, in favor of the latter.[9] In particular, this is a true paradox of aerobic life, as Davies expertly described.[10] All higher eukaryotic aerobic organisms consume oxygen, but the oxidized products of their metabolism are harmful molecules, such as the superoxide anion, $O_2^{\bullet-}$, hydrogen peroxide, H_2O_2, and the extremely reactive hydroxyl radical $HO^{\bullet}$.

The endogenous antioxidant system is not completely effective in preventing oxidative damage, but many antioxidants are found in foods and are especially prevalent in vegetables and fruits. Among them, we find carotenoids, vitamins C and E, polyphenols, and trace elements, such as selenium and zinc, which are known as exogenous antioxidants (Figure 1). Polyphenols (Figure 2) act as scavengers and exert their actions through different mechanisms. One of the most efficient routes is hydrogen atom transfer (HAT), which is shown in eq 1 for a generic R2O$^{\bullet}$ free radical:

$$\mathrm{R1H} + \mathrm{R2O}^{\bullet} \rightarrow \mathrm{R1}^{\bullet} + \mathrm{R2OH} \qquad (1)$$

This reaction can be written for any H atom of the scavenger R1H, and all HATs are characterized by a thermodynamic

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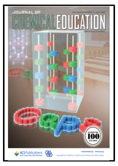

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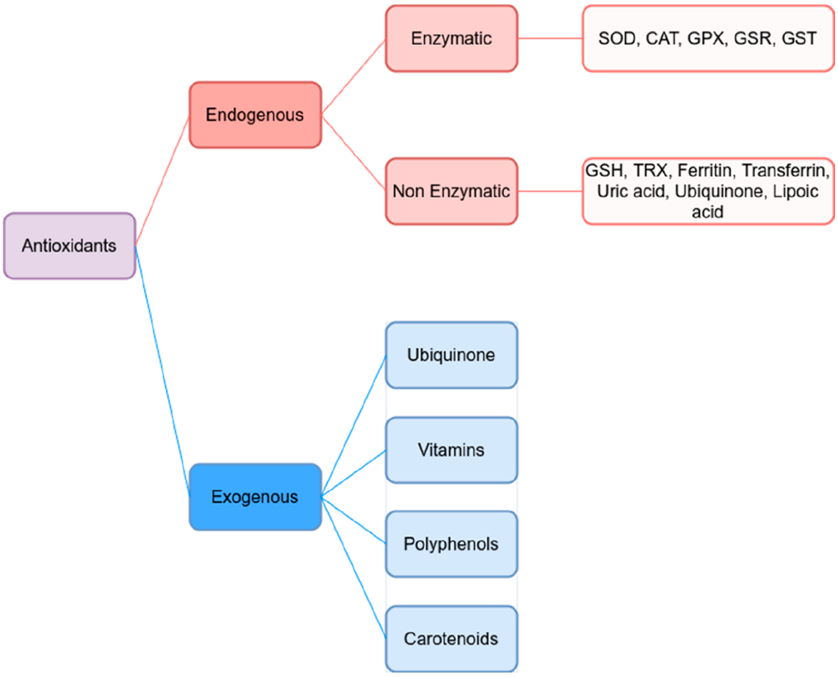


Figure 1. Classification of antioxidants. SOD = superoxide dismutase, CAT = catalase, GSR = glutathione reductase, GST = glutathione S-transferase, GSH = glutathione, TRX = thioredoxin.

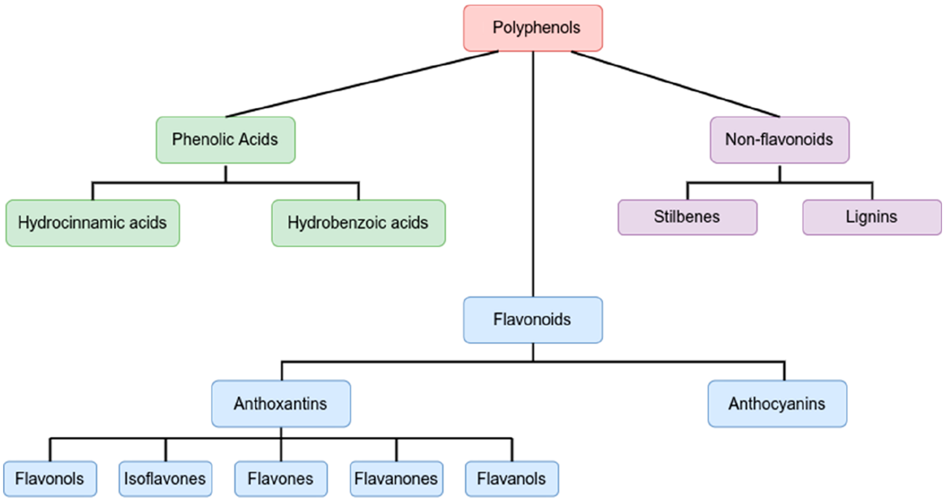


Figure 2. Classification of polyphenols. Reproduced from ref 11. Copyright 2020 MDPI.

driving force, ΔG_r(HAT), which also depends on the radical substrate and the reaction environment. In this laboratory, we propose to use five different ROSs, including examples of saturated and unsaturated molecules and oxy and hydroxy radicals, which comprise the most relevant kinds of free radicals in biology. In addition, besides the gas phase, which represents the common condition for quantum mechanical (QM) calculations, we recommend considering two solvents, benzene and water, which mimic an apolar and a polar medium, respectively.

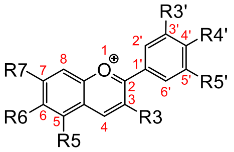
Anthocyanins, which are glycosylated derivatives of anthocyanidins and are most commonly found in plants, are widely used to introduce students to chemical concepts such as pH, chromatography, and visible spectroscopy.[12] Information on the role of anthocyanins and anthocyanidins as natural food colorants and their nutraceutical properties can be found in reviews; for example, such as those by Khoo and co-workers.[13]

The manuscript is organized into four general topics: (i) the teaching unit is first described explaining the fundamental steps of the project; (ii) the computational laboratory activity is described in detail for peonidin with all the necessary steps to reproduce it in a high school or undergraduate class; (iii) our direct experience with six high school classes is shortly reported; and (iv) conclusions are drawn, and the feedback received by the students and their teachers is included. Raw data are provided in the Supporting Information so that the laboratory activity can be conducted without the need for computational facilities.

■ RESULTS AND DISCUSSION

The teaching unit includes (i) an introductory lesson on the concept of oxidative stress and antioxidants, focusing on the class of anthocyanidins; (ii) the illustration of the objectives for

Table 1. Selected Anthocyanidins and Their Substitutions

Anthocyanidin	Basic Structure (R3,R4'=OH)	R3'	R5'	R5	R6	R7
Aurantinidin		H	H	OH	OH	OH
Capensidin		OCH_3	OCH_3	OCH_3	H	OH
Cyanidin		OH	H	OH	H	OH
Delphinidin		OH	OH	OH	H	OH
Europinidin		OCH_3	OH	OCH_3	H	OH
Hirsutidin		OCH_3	OCH_3	OH	H	OCH_3
Malvidin		OCH_3	OCH_3	OH	H	OH
Pelargonidin		H	H	OH	H	OH
Peonidin		OCH_3	H	OH	H	OH
Petunidin		OH	OCH_3	OH	H	OH
Pulchellidin		OH	OH	OCH_3	H	OH
Rosinidin		OCH_3	H	OH	H	OCH_3

the proposed activity; (iii) the presentation of specific content to fill the lack of knowledge and increase the technical competence of the class, i.e., thermochemistry and a basic description of energy and molecular structure calculations; (iv) a practical session in the computing room with training on the software use and an illustration of a case example; (v) the collection of results and the final discussion in the classroom.

Oxidative Stress and Antioxidants

A general description of the subject aims to inspire and involve students in the scientific activity proposed in this teaching unit, which covers a wide range of topics related to antioxidants. These topics include chemistry, biology, health sciences, and the nutritional importance of certain substances, which are often promoted in the media without proper scientific evidence. To start the project, some open-ended questions can be posed to the students, such as "Can you name an antioxidant substance?" or "Where can we find antioxidants in our daily lives?" Many of the students may not be able to define what an antioxidant is, but they might correctly identify some compounds and their sources. The teacher proceeds with the introductory lecture, defining the key concepts of oxidative stress, endogenous and exogenous defense systems and classification, polyphenols, and, particularly, anthocyanidins. Exhaustive literature is available on these topics, such as that by Sies[14a] and Mattioli et al.[14b] and papers cited therein. The teacher may use mind maps such as those sketched in Figures 1 and 2. Students can also prepare their own maps collecting the essential information from the lecture.

Learning Objectives of the Proposed Laboratory

The main goal of the proposed activity is to understand the action of a radical scavenger through the mechanism of hydrogen atom transfer: that is, to disclose the elementary chemical process providing the antioxidant character of an important class of polyphenols. This goal is achieved through a computational activity that requires the fulfillment of the following tasks: (1) computer-aided design of molecular structures and their radicals formed by selectively removing hydrogen atoms and their optimization; (2) calculation of the energies (electronic energy and Gibbs free energy) of the separated reactants and products of elementary HAT reactions in the gas phase, benzene, and water; (3) calculation of the Gibbs free energy of the reaction in gas phase, benzene, and water for all possible HATs. The learning objectives envisioned for the proposed laboratory are (i) understanding of organic radicals and their reactions, particularly HAT; (ii) learning fundamental concepts of thermodynamics, particularly Gibbs free energy and Gibbs free energy of reaction; (iii) computed-aided generation of three-dimensional structures of organic molecules; (iv) data management and elaboration; (v) approach to computational chemistry and supercomputing. Although (i−iv) can be proposed both to high school and undergraduate students, (v) will be used as a blackbox by the high school students to compute the energy of each reactant and product. On the contrary, depending on the degree of knowledge of quantum mechanics, fundamentals of quantum computational chemistry can be provided to undergraduate students. This topic is too wide and diverse and thus is beyond the purpose of this work; the teacher can find appropriate material in the didactic book by Cramer.[15]

Chemical and Physicochemical Background

Depending on the level of knowledge of the students, the teacher may prepare additional lectures to provide the students with the necessary background for successful participation in this laboratory. They must be familiar with the concepts of free energy and free energy of reaction to complete and fully understand the experience. Importance must be given to the relationship between the sign of the free energy of reaction and the relative stability of the radicals in the process, i.e., a positive ΔG_r(HAT) means that the more stable radical is on the reactant side and vice versa. Finally, a brief introduction to general concepts of the computational thermodynamics involving the workflow needed to obtain free energies may be useful to conclude the introductory part in the classroom.[16] In particular, the difference between electronic and free energy must be stressed, highlighting the role of the frequency calculation step in the jobs.

Practical Session in the Computing Room

The only requirements for the practical sessions in the IT room are the presence of a suitable number of PCs, at least one for each group, equipped with Avogadro and any software capable of managing spreadsheets to collect the data. In case high performance computing (HPC) facilities are available, a program capable of accessing the remote HPC nodes via a secure connection is also needed.

A total of 12 compounds (Table 1) were selected for evaluation, which is suitable for a class of 24 students. The molecular size was chosen to allow the calculations to be completed in 2 h, the allotted time for the Italian laboratory (see MC4 at the High School). The investigated anthocyanidins present a similar core structure, and three substituent

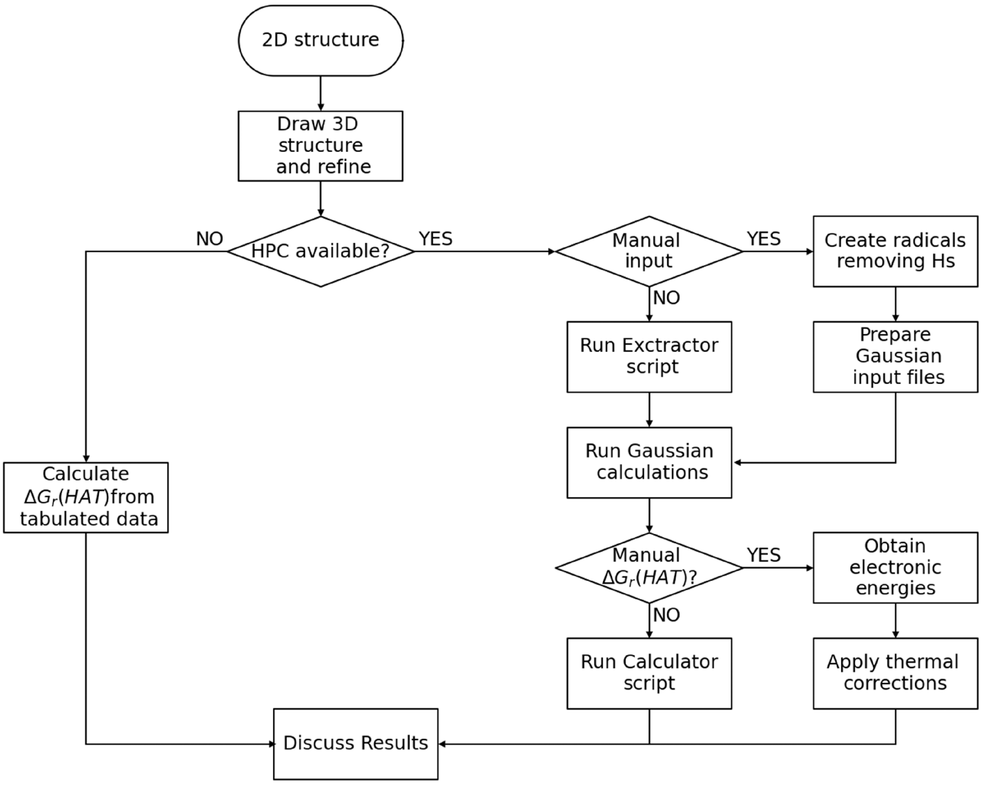


Figure 3. Flowchart of the computing laboratory session.

moieties are found, namely, −H, −OH, and−OCH_3. Five archetypal free radicals were chosen to determine the scavenging power, namely, $HO^•$, $HOO^•$, $CH_3O^•$, $CH_3OO^•$, and $CH_2{=}CHOO^•$.

A single homework compound can be assigned to a two-student group. Only the 2D structure should be given to each group, and the students can draw the 3D structure in a molecular editor. We recommend Avogadro[17] as it is free as well as cross-platform and contains an integrated molecular mechanics optimizer that can be used to refine the structure drawn by the students. The group then proceeds to create the structures of the different radicals obtained after HAT by removing H atoms from the starting molecule in the molecular editor. Students may do this manually in the classroom for the first compound to help them recognize which HAT reactions will lead to identical radicals, such as those containing methyl groups, thus gaining familiarity with the concept of chemical equivalence. For the compound chosen from those of Table 1, a script is provided that automatically deletes the H atoms one by one from the starting structure, leading to all possible HAT radicals (see Supporting Information). Thus, the students can spot chemically equivalent hydrogen atoms using numerical results instead of chemical insight. Gaussian 16 was employed to carry out the density functional theory (DFT) computations, a very popular commercial software that is typically available on computing infrastructures.[18] For those students who will actually calculate the energetics of the HAT reactions, the structure of the Gaussian input can be briefly described, recalling the concepts presented in the classroom on computational chemistry. Preparation is automated with a script (see Supporting Information) to avoid unnecessary delays caused by typos in manually prepared inputs as well as submission scripts for the calculation on HPC systems, if available. The computational protocol described here is currently applied in research papers focusing on the *in silico* assessment of scavenging activity of organic molecules, including natural substances[4,19] and drugs.[20] This protocol was validated against experimental data and evidence. As an example, the teacher can illustrate the results reported for Trolox (data computed at the same level of theory are reported by Bortoli et al.[20a]), which is an analogue of vitamin E and represents the standard in lab assays to measure the antioxidant strength, which is given in TEAC (Trolox equivalent antioxidant capacity). Geometry optimizations and frequency calculations are carried out to obtain thermal corrections on the starting molecule and the resulting radicals after HAT in the gas phase at the M06-2X/6-31G(d,p) level of theory.[21] Subsequently, a larger basis set is used to refine gas phase energies with single point calculations at the M06-2X/6-311+G(d,p) level of theory. Single points are also carried out in two different solvents, water and benzene, to highlight the effect of environmental polarity on HAT reactions. The SMD model[22] is used to include implicit solvation effects. In the classroom, for each molecule and its radicals, the absolute electronic energy is collected from the outputs of the calculations with the larger basis set in the gas phase ($E^{\mathrm{lb}}_{\mathrm{gas}}$), benzene ($E^{\mathrm{lb}}_{\mathrm{bzn}}$), and water ($E^{\mathrm{lb}}_{\mathrm{wat}}$). It is then transformed into Gibbs free energy ($G^{\mathrm{lb}}_{\mathrm{gas}}$, $G^{\mathrm{lb}}_{\mathrm{bzn}}$, and $G^{\mathrm{lb}}_{\mathrm{wat}}$ for the three solvents, respectively) adding the appropriate thermal correction obtained from the frequency calculation in the gas phase ($G^{\mathrm{corr}}_{\mathrm{gas}}$) as shown in eq 2:

$$G^{\mathrm{lb}}_{\mathrm{X}} = E^{\mathrm{lb}}_{\mathrm{X}} + G^{\mathrm{corr}}_{\mathrm{gas}} \tag{2}$$

where X refers to either the gas phase, benzene, or water.

The absolute Gibbs free energy for the chosen free radicals can be obtained analogously, and the final ΔG_{r}(HAT) can be

Table 2. Key Components to Obtain the Final Gibbs Free Energy in Solution Calculated during the Activity and Examples of Their Different Explanations for High School and Undergraduate Level Students

Key Concept	High School	Undergraduate
Total energy	(Negative) molecular energy obtained by quantum mechanical calculation (similar to a measurable quantity).	Numerical solution of the Schrödinger equation. In particular, in the DFT approximation, a numerical solution of the analogous Kohn−Sham equations.[23]
Basis set effect	Improvement in the electronic energy value as a result of a more accurate description.	Improvement in the energy value is due to a better description of the electrons in the molecule because of a larger basis set.[24]
Thermodynamic correction	Correction provided by the frequency calculation, which leads to *G* by inclusion of enthalpic and entropic corrections.	The procedure that uses gas statistical mechanics to calculate the different contributions to enthalpy and entropy and combines them to obtain *G*.[16] A frequency calculation is required for the vibrational component.
Solvation	It is defined intuitively as the difference between the electronic energy in the solution and in the gas phase. Solvent is represented as a continuum dielectric medium.	The effect of the presence of an external medium (modeled as a polarizable dielectric continuum) exerts on the quantum mechanical system charge distribution.[25]

calculated in each of the three environments from the difference in the free energy of the products and the reactants (eq 3):

$$\Delta G_r(\mathrm{HAT}) = [G_X^{\mathrm{lb}}(\mathrm{R1^\bullet}) + G_X^{\mathrm{lb}}(\mathrm{R2OH}) - G_X^{\mathrm{lb}}(\mathrm{R1H}) - G_X^{\mathrm{lb}}(\mathrm{R2O^\bullet})] \qquad (3)$$

in which, after eq 1, R1 is the anthocyanidin and $\mathrm{R2O^\bullet}$ is the free radical.

Again, for the homework compound, a script is used to automatically compute the ΔG_r(HAT) and extract the necessary data directly from the output files. In Figure 3, a flowchart summarizes the steps required to reproduce the calculations with and without the support of HPC facilities and automated scripting.

This stage ends with a homework assignment to be carried out in groups, i.e., one or more anthocyanidins from Table 1. All the computing activity can be replaced by the analysis of the raw data provided in the Supporting Information using a spreadsheet (see Collection of Results and Discussion), in the case where no computational facilities are available or the teacher does not intend to work with the students on the whole computational approach.

A difficult concept for students working through this process is the relationship between the electronic and Gibbs free energy. In quantum computational chemistry, the equations of statistical mechanics of gases are employed to obtain, from the electronic energy of a single molecule, ensemble properties, such as the Gibbs free energy. Students in high school should focus on the difference between electronic and Gibbs free energy, which justifies the need for a thermodynamic correction provided by the computer. Undergraduate students can learn more about the topic, and the teacher can find an exhaustive explanation in physical chemistry textbooks or in the computational chemistry book by Cramer.[15] Table 2 summarizes the different stages that must be illustrated to high school and undergraduate students to adequately explain the different steps necessary to obtain the Gibbs free energy in solution (a collective property) starting from the electronic energy of a single molecule.

Collection of Results and Discussion

As a final assignment, done partly in the classroom and partly as homework, each group is required to organize the data in a spreadsheet and to infer some chemically relevant properties based on the concepts given in the introductory lesson. After the numerical results have been collected, it is important to review them along with the students' comments in a final session. During this session, the teacher should analyze the numerical results to ensure that the procedure was completed successfully. Furthermore, the students' conclusions should be discussed to confirm that they have fully understood the concepts outlined in the introductory lesson. In particular, some discussion points relative to HAT reactions that should be addressed are the following: the different reactivities of free radicals, the relative reactivities of different sites in the same molecule, the relative activity of analogous moieties in different molecules, and the effect of solvation.

As an example, we report here the results for peonidin (Figure 4) and their analysis, which can be viewed as a case-compound study of laboratory activity. The chemistry concepts given in the introduction should be substantiated by the numerical results obtained from the calculations.

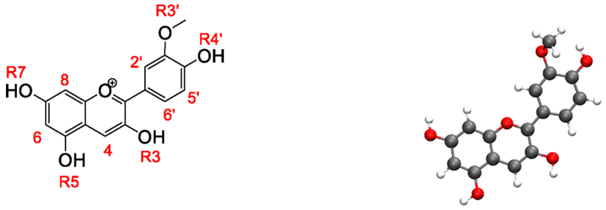


Figure 4. 2D (left) and 3D (right) structures of peonidin. In the 2D representation, the sites suitable for HAT are labeled in red after the scheme in Table 1.

The results of ΔG_r(HAT) in the gas phase show systematically lower reaction energies when scavenging oxy radicals ($\mathrm{HO^\bullet}$ and $\mathrm{CH_3O^\bullet}$). In fact, HATs involving $\mathrm{HO^\bullet}$ are found to be thermodynamically favorable or neutral for all sites, whereas the scavenging of $\mathrm{CH_3O^\bullet}$ is found to have negative free reaction energies only when HAT occurs from oxygen sites. On the other hand, the peroxy radicals show consistently positive reaction energies that in some cases reach values greater than 30 kcal mol^{-1}. Among these, $\mathrm{HOO^\bullet}$ and $\mathrm{CH_2{=}CHOO^\bullet}$ have a similarly lower ΔG_r(HAT) compared to $\mathrm{CH_3OO^\bullet}$, which is the ROS that displays the least favorable free reaction energies. A bar graph displaying all of the computed ΔG_r(HAT) is shown in Figure 5.

HATs from the various sites on the ring substituents of peonidin are computed to be largely more favorable than those from the sites in the rings. The different possible resonance structures for each formed peonidin radical confirm that the radical with the highest number of resonance structures is the most stable. In the case of peonidin, that is seen to be HAT from OH at R3. Thus, reactions involving the substituent at R3 display the lowest reaction free energy. On the contrary, the transfer from carbon at 8 is computed to have the highest reaction energies. Analysis of the resonance structures confirms that the radical generated via HAT from that site displays the

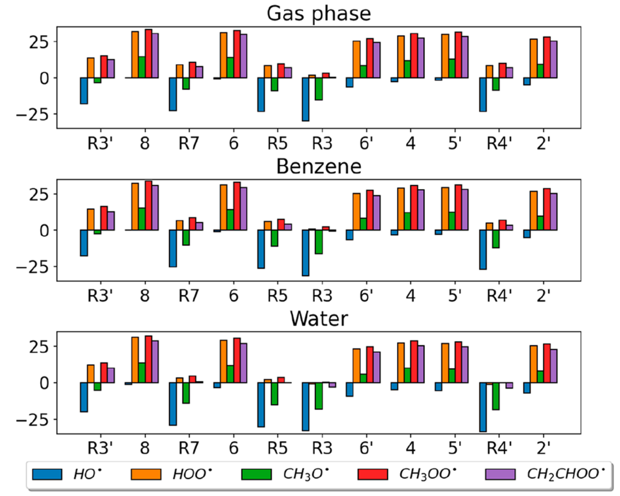


Figure 5. ΔG_r(HAT) in kcal mol^{-1} for peonidin in the gas phase, water, and benzene. Level of theory (SMD)-M06-2X/6-311+G-(d,p)//M06-2X/6-31G(d,p).

lowest number of possible resonance forms (see the Supporting Information for a complete depiction of the resonance structures of radicals formed at sites R3 and 8).

The effect of the environment polarity can also be analyzed by comparing the reaction free energies for the same site in the three different reaction media: gas phase, benzene, and water. Results show a consistent decrease in the reaction free energy as the reaction is modeled in a more polar solvent. Thus, the gas phase and water display the highest and lowest values, while benzene gives intermediate results in accordance with their relative dielectric constants. This can be explained by the fact that the stabilization of the electric field created by the solvent is stronger in the products than in the reactants, since after HAT, a radical cation is produced. Interestingly, in the case of the R3 and R4′ sites, this increased stability of the formed radical cation in water effectively results in the reactions with the five of the free radicals being thermodynamically favorable. This result provides students with the evidence that changing the reaction medium can modify the thermodynamics of a reaction, transforming an unfavorable reaction into a favorable one.

■ MC^4 AT THE HIGH SCHOOL

Before involving the students in this computational laboratory, we contacted the Italian supercomputing center CINECA to gain access to their infrastructure, which we typically use for research purposes. We obtained a significant computational budget, and four classes were invited to visit the center after the activity to see the machines on which they had run their calculations and to attend a series of introductory lectures on the technical details of HPC not covered in the classroom activities.

The computing laboratory activity described here has been tested in six classes (16−17-year-old students) from six different Italian high schools (licei and technical institutes). The educational project (Molecules and Computer: Chemistry Calculations in Class, MC^4) also provided access to national supercomputing facilities (CINECA) thanks to the complementary project mentioned above and was well received, with positive feedback from the students involved. The preparative elements given by the first part of the experience allowed students not only to successfully complete the assigned tasks in the classroom but also to repeat them on their own at home. The assessment was done by the teachers who evaluated the work in three key areas: (i) participation in the classroom and IT laboratory activity; (ii) the data correctness of the obtained data and the ability to recognize nonphysical results; and (iii) the ability to organize, analyze, and present each group's work.

Participation was very favorable, as all the teachers observed higher than usual interest, especially during the hands-on session in the IT laboratory. Moreover, all the groups managed to complete the assigned molecules, and most of the raw data obtained were found to be correct. Most of the students managed to identify the values that were not sound, and in the majority of cases, the underlying cause was seen to be a starting structure with missing atoms. Finally, the energies were usually collected in a spreadsheet, or less frequently in a text document, and presented as a scatter or line plot of site vs ΔG_r(HAT) for the three different environments.

No formal grades were given to the students since this was a pilot project, but all teachers agreed that the quality of the work of all groups was above the pass level, which is 6/10. This was based on: (i) the level of participation, (ii) the correctness of the results, which we provided to the teachers, and (iii) the quality of the final data presentation. Discussing with teachers, we concluded that the evaluation of MC^4 laboratory for the high school students can be organized by quantitatively evaluating the three key areas described above: 0−2 (i), 0−4 (ii), and 0−4 (iii), respectively. For undergraduates, it is reasonable to grade only areas ii (0−5) and iii (0−5). In order to facilitate the interpretation of the grades here proposed, which are based on the Italian grading system ranging from 1 to 10, we show in Table 3 how numbers might be assigned when evaluating.

Two of the six classes were given an anonymous survey with a single question asking about the most interesting aspects of this project. For the remaining, a similar procedure was carried out orally in a discussion session after the visit to the CINECA HPC facility. The teachers collected the written and oral responses and communicated them in an aggregated manner, highlighting the aspects of the activity that received the highest level of praise from the students. Survey, data collection, and analysis were carried out in compliance with ethical guidelines.

The aspect that most interested the students was the translation of everyday words such as "antioxidants" and "free radicals" to quantitative concepts that could be measured directly. The difficulties were easily overcome by providing direct support in the computer laboratory or via email at home. Interestingly, most errors were ascribed to incorrect preparation of the initial scavenger structure. In fact, the activity of designing a 3D molecule with an editor is *per se* a didactic task. Forgetting a hydrogen atom in a formula is a mistake for the student, but the same error when building a molecular structure prevents its optimization and results in an evident distortion, which can alert the student and challenge their knowledge of chemical bonding in organic chemistry.

Another point that increased student interest was the possibility of using Avogadro to construct and see molecules in space and to understand the structural features that give rise to differences in reactivity. This was seen as a novelty compared with the canonical 2D skeletal representation.

Teachers who gave a positive evaluation also praised the possibility of practical application, with minimal integration, concepts studied during curricular courses whose experimental

Table 3. Proposed Grading Scheme for High School and Undergraduate Students

	High School	Undergraduate
Grade	Level of Participation (0–4)	
0 Low	The student does not participate actively in the group work, the student shows no interest in the activity	Not applicable
1 Medium	The student shows interest in the activity but does not participate actively in the group work	Not applicable
2 High	The student shows interest in the activity and participates actively in the group work	Not applicable
	Correctness of the results (0–4)	
0 Not sufficient	More than 50% of the results are wrong	More than 40% of the results are wrong
1 Sufficient	Up to 50% of the results are wrong	Up to 40% of the results are wrong
2 Good	Up to 30% of the results are wrong	Up to 20% of the results are wrong
3 Very good	Up to 20% of the results are wrong	Up to 10% of the results are wrong
4 Excellent	All results are correct	
	Quality of final data presentation (0–4)	
0 Not sufficient	The datasheet is not properly filled, graphs are not clear or incorrect, the results are not interpreted correctly.	
1 Sufficient	The datasheet is properly filled, the clarity of the graphs is poor, the results are not always interpreted correctly.	The datasheet is properly filled, the clarity of the graphs is good, the results are not always interpreted correctly.
2 Good	The datasheet is properly filled, the clarity of the graphs is good, the results are not always interpreted correctly.	The datasheet is properly filled, the clarity of the graphs is very good, the results are always interpreted correctly.
3 Very good	The datasheet is properly filled, the clarity of the graphs is very good, the results are not always interpreted correctly.	The datasheet is properly filled, the clarity of the graphs is excellent, the results are always interpreted correctly.
4 Excellent	The datasheet is properly filled, the clarity of the graphs is excellent, the results are always interpreted correctly.	The datasheet is properly filled, the clarity of the graphs is excellent, the results are always interpreted correctly with a clear understanding of the effect on the HAT reactivity of the structural features and environment properties.

measurement would require instrumentation often not accessible to high school-level institutes.[26]

Some of the evaluations are available in a recent Italian conference proceedings on chemistry educational projects.[26] In general, the success of this laboratory is reported with enthusiasm and praise ("...The experience was incredible..."). It is appreciable that in MC^4 scientific skills are combined with technology because both are cornerstones for quality and cutting-edge professional development. Young students are fast learners in computer science, and it has been recognized that the MC^4 laboratory has shown that "...there are other ways to do chemistry..." especially when well equipped chemistry laboratories are not present in the school.

This activity in the class was praised by the Direzione Generale per gli ordinamenti scolastici e la valutazione del sistema nazionale di istruzione of MIUR (Ministero dell'Istruzione e del Merito), who invited us for a talk, together with two teachers and an ex-student at Job&Orienta,[27] which is an important national annual event on school, education, and future perspectives for young generations. Importantly, we also wanted to stress the subject of computational chemistry as it is relatively unknown outside academia. It was new to most of the participants who had never heard of this field of research and its possibilities compared to traditional wet laboratory practice. This project also managed to increase awareness about prospective students of the possibility that a chemistry curriculum can evolve into a computational chemistry research career. Moreover, the data calculated by the students we worked with was employed as part of the data set for the development of a machine-learning-based classification method on HAT reactions.[28]

■ CONCLUSIONS

The computing laboratory activity presented here may be modified by the teacher, depending on the level of the students and the availability of computational facilities. Thus, it can be proposed to both high school and undergraduate students. It is intrinsically multidisciplinary and encompasses concepts from different fields, such as biology, health sciences, physical chemistry, and organic chemistry. It unravels chemical details, allowing an insightful interpretation of the radical scavenging activity of anthocyanidins and providing a scientific explanation for the popular term "antioxidant". When computational facilities are available, the calculations can also be planned for other classes of compounds, taking inspiration from the scientific literature.[4,19e,20a,29] This can be easily implemented, and no changes to the scripts are required. The teacher can also plan variations to the proposed structures; for example, by introducing the effect of pH, different protonation states of the anthocyanidins can be considered.[30]

Lastly, MC^4 can be used to introduce students to computational chemistry, a discipline with great potential in research and education. The use of the computer for molecular representation, reactivity investigation, and experiment simulation is valuable and should not be considered a surrogate of the wet laboratory but rather a complementary tool to engage students, facilitate the comprehension of physical chemical concepts often too abstract, organize activities for online and/or blended courses, and approach the use of computers to the understanding and solution of chemical problems.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.2c01070.

Computed energies needed to repeat the activity if no HPC facilities are available and a brief guide to the python scripts (PDF)

Creator.py script (python script) to create inputs for radicals. Calculator.py (python script) script to calculate ΔG_r(HAT) (ZIP)

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Notes

The authors declare no competing financial interest.

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