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A problem-based learning activity for enhancing inquiry skills and facilitating conceptual change in a biological chemistry course†

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ABSTRACT

When teaching STEM courses, it is important to introduce state-of-the-art techniques. Students need to learn how to conduct experiments, analyse data and choose the most effective approaches to address meaningful situations. Here we present the assessment of the implementation of a structured inquiry-based activity aimed at teaching students about protein mass and size. This activity emerges as an intervention in our educational module, designed to create a cognitive conflict that effectively drives a conceptual change. To evaluate the efficacy of this module, we collected data on students' perceived and actual knowledge through pre- and post-class surveys (n=36 and 34, respectively, mean age 26 ± 2). Additionally, we evaluated lab reports using a detailed rubric. Results indicate that the practical innovation we propose is a challenging activity that promotes the accomplishment of our learning objectives. The activity led to improvements both in confidence and in actual mastery of theoretical concepts and techniques. After completing the activity, students were able to choose the most appropriate technique to solve specific problems. Furthermore, we found that the use of a structured questionnaire in lab reports helped students to accurately analyse and process experimental data. It also allows them to demonstrate understanding of technical limitations, while integrating the knowledge and skills acquired during the module. Overall, this activity provides notions that are conceivable and profitable, thus leading to successful conceptual changes.

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INTRODUCTION

In a survey conducted by Goodey & Talgar (2016), research-active biochemistry professionals considered five skills to be the most important for preparing students in order to be successful in research positions after graduation, namely, a mastery of basic math calculations; understanding the concepts behind the methods, procedures, and assays they work with; the ability to look up information by themselves; possessing problem-solving skills; and the ability to learn new methods. A large amount of evidence shows that strategies that promote interactions and cognitively engage students with content lead to learning gains and attitude improvements in STEM courses (Singer, Nielsen, and Schweingruber 2012; Freeman et al. 2014; Cox-Paulson et al. 2012). In recent years, many studies have suggested that inquiry-based learning activities increase conceptual understanding and improve students' attitudes toward learning (Orosz et al. 2023; Gillies et al. 2014). The inquiry-based method of learning is a student-centred strategy where participants take an active part in their learning process. In this approach, which mimics the way scientists work, students collect data related to a problem, propose possible answers, and communicate results. Learning is driven by a process of enquiry with the main goal of acquiring new knowledge and skills (Szalay and Tóth 2016). Inquiry-based learning can be a powerful tool for engaging students in the learning process and promoting critical thinking skills.

Here we present the assessment of the implementation of a practical innovation in an educational module centred on protein mass and size, two key properties important for research and industrial applications. The concepts of molecular weight (MW), oligomeric state, diffusional coefficient (D), second virial coefficient (A_2), and hydrodynamic or Stokes radius (Rh) are discussed in terms of their physical meaning and the techniques for their precise measurement. This study shows that challenging students with a focused, practical, active learning activity incorporating a cognitive conflict, results in significant knowledge and skill gains in a university course covering protein characterization methods.

Theoretical background

With the intervention we propose in this work, we aim to facilitate deeper learning and more meaningful conceptual change by actively involving students in their learning process and offering opportunities for exploration, questioning, and challenging existing knowledge and beliefs. In the following paragraphs, we provide

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an overview of the theories that underlie our study to frame the research problem, its foundation, and our chosen analysis approach.

Constructivist learning theory asserts that individuals actively construct their own knowledge from their experiences and interactions with the world, connecting new ideas to what they already know (Bada and Olusegun 2015; Cakir 2008; 1999). In a nutshell, this theory posits that students should be active participants in the learning process through their own experiences, rather than passive recipients of information, and take responsibility for their own learning. These *active learning* approaches emphasize student-centred learning, with instructors acting as facilitators rather than traditional lecturers. Active learning encompasses a diverse range of methods, including problem-based learning, collaborative learning, and inquiry-based learning, which might be brought about through a multitude of engaging activities, such as hands-on experiences, group work, problem-solving exercises, and other interactive learning opportunities (Bonwell and Eison 1991). Some instructors may be resistant to adopting active learning due to concerns about increased workload, difficulty in managing large classes, and the need for additional resources and training (Kirschner, Sweller, and Clark 2006). Certainly, questions have been raised about the extent to which learners can construct knowledge independently of external influences, and the role of the teacher in facilitating the learning process. Yet, there is a growing body of literature that supports the effectiveness of active learning over traditional lecture-based instruction in terms of retention of information and development of critical thinking skills. Freeman *et al.* (2014) conducted a meta-analysis of 225 studies comparing active learning with traditional lecture-based instruction in STEM disciplines and found that it was associated with increased exam scores, reduced failure rates, and increased retention of material. Another meta-analysis by Prince (2004) found that active learning increased student performance and engagement across a variety of disciplines.

There are quite a few active-learning pedagogical methods available. Among them, inquiry-based learning emphasizes the exploration and investigation of interrogations and problems that are meaningful and relevant to students, who are encouraged to ask questions, design experiments, gather data, and draw conclusions based on their own observations and experiences. This approach encourages the development of critical thinking skills, problem-solving abilities, and scientific inquiry skills. While inquiry-based instruction has been shown to have a positive effect on students' academic achievement in STEM subjects (Kaçar et al. 2021), it does not necessarily lead to more successful learning in terms of subject-content acquisition (Reid and Amanat 2020). This method

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best suits older and more able learners who need less support because of their developed cognitive structures, greater prior knowledge and less propensity to be overwhelmed due to working-memory overload (Kirschner, Sweller, and Clark 2006; Reid and Amanat 2020), which may be the case for activities involving reduced teacher participation, leading to a decreased level of understanding and the emergence of misconceptions (note that throughout this paper we use the term misconceptions, which is also referred to as 'alternative conceptions', to refer to students' conceptions that are often inconsistent with the scientific notions generally agreed). In addition, some educators have expressed concerns about cultural or socioeconomic factors that impact students' effective engagement, and the potential lack of structure and guidance in this approach, which can produce confusion and frustration. To address these concerns, researchers have proposed various models and frameworks for implementing inquiry-based learning in the classroom, such as the 5E—*engage*, *explore*, *explain*, *elaborate*, *evaluate*—instructional model proposed by Bybee *et al*. (2006), which provides a structured framework for guiding students through the inquiry process (Bybee 2014).

It is worth noting that under a constructivist pedagogical approach, students may choose to reject new knowledge when it does not fit with existing knowledge. Prior knowledge helps to decrease cognitive load leading to good learning performance (Myhill and Brackley 2004; Mihalca et al. 2011; van Riesen et al. 2022); however, such preconceptions might be either waymarks or obstacles to learning. Misconceptions—erroneous and naïve preconceptions—are not simply wrong knowledge but might involve fully developed belief systems that hinder students' understanding (Faraj, Rossi, and Montes 2019; Posner et al. 1982; Leonard, Kalinowski, and Andrews 2014; Miller and Brewer 2010). This is why it is frequently observed that misunderstandings are resistant to change (Malińska et al. 2016; Sendur and Toprak 2013). The repair process where the misconception is corrected is generally called *conceptual change* and involves the reconstruction of pre-existing knowledge structures to resolve conflicts with new information and to create new mental models (or conceptual systems) that are more accurate and comprehensive (Hewson and Hewson 1984). Since first described, conceptual change has emerged as one of the main research areas in science education, and many studies have been carried out to improve students' learning (Cakir 2008; Liaw, Chiu, and Chou 2014). These theories posit that the development of knowledge from misconceptions to scientific understanding goes through a series of stages: dissatisfaction with currently held concepts, encountering new and plausible concepts, and accommodating new concepts (Chiu, Chou, and Liu 2002;

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Eckstein and Shemesh 1993; Posner et al. 1982). Conceptual-change teaching is not the same as finding and eradicating students' misconceptions: the constructivist understanding is that students' conceptual knowledge develops gradually, and misconceptions will disappear naturally as students gain expertise (Cakir 2008). di Sessa (2014) emphasizes the complex and multifaceted nature of conceptual change and the importance of using a variety of approaches and methods to facilitate it effectively, and provides a discussion of different theoretical perspectives on conceptual change and the methods used to facilitate it, in particular, the knowledge-in-pieces perspective. By considering the individual's prior knowledge and understanding, the nature of the new information or experiences, and the context in which learning occurs, educators can help learners develop a deeper and more meaningful understanding of the world around them. Conceptual change can be facilitated by exposure to new information or experiences—for example, through cognitive conflict or the use of bridging analogies—active engagement and participation in the learning process (including cooperative and shared learning to promote collective discussion of ideas) and metacognitive reflection on one's learning and understanding through the so-called Socratic dialogue. This approach might focus on the restructuring of existing knowledge frameworks, the refinement or expansion of existing concepts, or the replacement of prior concepts with new, scientifically accurate ones (Limón 2001; Gafoor and P.T 2013; Pacaci, Ustun, and Ozdemir 2023; Planinic et al. 2005).

Conceptual or *cognitive conflict* is an important instructional strategy for promoting conceptual change. It occurs when an individual's existing knowledge or beliefs are challenged or contradicted by new information or experiences, producing a state of mental discomfort or disequilibrium that can motivate the individual to re-examine their existing knowledge and understanding, and seek new information or perspectives that can help them to reconcile conflicting ideas (Hewson and Hewson 1984; Chi and Roscoe 2002). According to Piaget's theory of cognitive development, cognitive conflict is necessary for learning and growth as individuals attempt to resolve these inconsistencies and modify their existing mental schema. Recent research on cognitive conflict has explored various aspects of the phenomenon, including its impact on learning outcomes, the factors that influence the experience of cognitive conflict, and the role of emotions in the process. Nevertheless, the optimal level of conflict needed for learning is an important matter of debate. While too much cognitive conflict can be overwhelming and lead to frustration and disengagement, some researchers suggest that a moderate level of conflict is necessary for optimal learning outcomes (Kang et al. 2005).

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Together, these concepts emphasize the importance of active engagement, inquiry, and meaningful learning experiences in constructing new knowledge and understanding. Beliefs in personal efficacy also play a crucial role in determining the initiation of coping behaviour, the level of effort expended, and the duration of persistence in the presence of obstacles and challenges. Consistent engagement in activities that may initially appear threatening results in the acquisition of mastery experiences, further enhancing self-efficacy and consequently reducing defensive conducts (Bandura 1977).

Activity background

The protein mass and size module in our bioorganic chemistry course had had a long tradition of lecture-based teaching to introduce the use of light scattering techniques, followed by the demonstration of the determination of the MW and Rh of the model protein bovine serum albumin (BSA). This straightforward strategy easily fitted into the course: the lecture component satisfactorily allowed presenting most of the underlying theory and the laboratory demonstration allowed students to have some contact with a *real* experiment. However, we eventually noticed that this kind of approach focused mainly on lower-level cognitive skills to the exclusion of analysis and critical thinking (Momsen et al. 2010), and did not reach any further than the *comprehension* level (Bloom, Krathwohl, and Masia 1956). For example, students were able to effectively recall the definition of most key concepts such as MW or Rh, they could readily state the advantages and disadvantages of LS over SEC, list several reasons for a protein to exhibit an unexpected behaviour in one or another technique (e.g. the possibility of protein oligomerization or quaternary structure acquisition, matrix-protein interactions, and conformational changes that might lead to protein expansion), and were perfectly capable of applying the equation of a regression line to obtain the MW of a protein assessed by SEC. However, when challenged with an atypical result they were unable to produce a reasonable hypothesis to explain it or to suggest a modification to the experimental conditions that would help better understand the situation, despite having been provided with—and having shown knowledge of—all necessary factual elements.

In this regard, we aimed to count with an inquiry-based activity that allowed students to face actual dilemmas when trying to determine proteins' molecular weight, size, and oligomeric state, and to have first-hand evidence of the advantages and limitations of different techniques available. Although there is not a unique classification in the literature, four levels of inquiry-based learning can be distinguished, depending on the amount

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of information provided to the learners and the teacher's involvement in the activity. Recent studies have explored the effectiveness of different forms of inquiry-based learning in diverse contexts, highlighting the importance of tailoring the approach to specific learning goals and student needs (Zion and Mendelovici 2012; Bell, Smetana, and Binns 2005; Orosz et al. 2023). The highest level of inquiry-based learning is *open inquiry*, where the teacher's role is merely to define the subject and learning goals, and students have the freedom to select the problem and research questions they want to explore and design their own investigations. This approach entails a high level of responsibility, granting considerable autonomy and requiring advanced inquiry skills, reasoning ability, and creativity. Nevertheless, it can pose difficulties for students lacking sufficient background knowledge or research expertise, leading to varying learning outcomes. The next level in terms of students' autonomy is termed *guided inquiry*. In this approach, the teacher introduces the problem, while all ensuing phases of the process, such as crafting hypotheses and devising experiments, are carried out by the students. These activities encourage collaborative learning, allowing greater space for student creativity and the honing of skills and capabilities. Guided inquiry provides students with more structure and support in the form of prompts, scaffolds, and teacher guidance, aiding them in cultivating the necessary research proficiencies and leading to more consistent educational outcomes. Nevertheless, it might be less captivating and inventive compared to open inquiry and might not offer the same extent of student autonomy. The following level is referred to as *structured inquiry*, wherein the teacher presents the problem and experimental methods, while the students propose a solution based on their observations and measurements. This kind of activity presents restricted room for independent thought, as students are directed towards a predefined path leading to the solution. However, it proves beneficial for practising inquiry skills like observation, measurement, data interpretation, result discussion, and documentation. In the context of this article, structured inquiry involves a clearly outlined research question and a set of procedures designed to address it. This structured and supportive method eases students' involvement in the research process, leading to consistent learning outcomes. At the base level of inquiry-based learning lies *confirmatory inquiry*, where learners are aware of the experiment's outcome beforehand, and their sole objective is to replicate the results. Confirmatory inquiry is a methodical procedure that enhances comprehension of research principles and underscores the significance of empirical evidence. Through conducting experiments and scrutinizing outcomes to authenticate prevailing theories, hypotheses, or ideas, students acquire critical thinking skills and grasp the scientific method.

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The key for success in inquiry-based learning is to find the right balance of structure and freedom to meet the needs of individual students and learning objectives. We chose to adopt a structured approach to inquiry-based learning in our laboratory practical because of the module's specific learning objectives, students' existing background knowledge and research skills, and the available resources. This format offers a clearly defined and manageable research question for students to explore. Moreover, crafting an experiment is a time-consuming endeavour and comes with the possibility of errors, potentially leading to the need for procedure adjustments during the activity; given that our undergraduate students are not yet equipped with the necessary research skills to undertake open or guided inquiry in such matters, we considered that the structured form is preferable. Furthermore, this approach allowed us to incorporate a knotty scenario that would induce a cognitive conflict by challenging students' existing beliefs on the relationship between protein size and mass by experimental results that contradicted expected ones in a SEC run, which would not necessarily arise if a guided inquiry approach were implemented. This motivates them to re-examine their existing knowledge and understanding about the several features that might affect molecular weight determinations on the basis of the chosen technique, and seek new information or perspectives that can help them reconcile conflicting ideas (Chi and Roscoe 2002). Also, note that a guided approach would have probably impeded the use of SEC and LS, which are the experimental techniques we want to apply in this module.

We designed a laboratory module based on an experiment in which students make use of analytical size exclusion chromatography (SEC) and light scattering (LS)—both static (SLS) and dynamic (DLS)—to determine the MW and Rh of a set of proteins: BSA, hypoxanthine phosphoribosyltransferase from *Trypanosoma cruzi* (HPRT) and human frataxin (FXN). These proteins provide a challenging scenario: BSA presents multiple aggregation states, HPRT is a tetramer with a monomer-like behaviour (Valsecchi et al. 2016), and FXN is a negatively-charged protein that experiences significant electrostatic repulsion with the gel matrix (Faraj et al. 2013). This experiment demonstrates that the determination of MW and Rh by SEC may be misleading, highlighting the limitations of the methods under study by means of an obvious discrepancy between the results that are expected to be obtained by each technique—something that was not achieved prior to this intervention when only BSA was used, and results were *explained* by the instructor rather than *deduced* by students.

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Consequently, the activity challenges learners to confront their preconceived notions and prompts them to revise their existing knowledge structures to account for the new experiences.

Aims of the study

In this paper, we present the results of an educational study performed in 2018, 2019 and 2022 within a course focused on protein chemistry, structure, and interactions. One of our aims was to contribute meaningful and high-level data to the debate regarding the benefits of inquiry-based learning approaches. We hypothesized that incorporating challenging proteins that create a cognitive conflict as the learning method in the context of an inquiry-learning activity leads to beneficial changes in actual knowledge outcomes compared to traditional lectures. This work was guided by the following questions:

- What do students understand by *size* and *mass*, both before and after the module?
- How comfortable do students feel with different concepts and techniques, before and after the educational module?
- How do students' self-perception relate to their actual capacity to understand experimental data before and after the educational module?
- What does an inquiry-based activity with a challenging scenario contribute to a module comparing mass and size characterization techniques?
- To what extent does the pedagogical strategy lead to a conceptual change?

To test the hypothesis and address the research questions, we set the following main learning objectives (LO) for our students:

1. *Gain further understanding of the concepts of mass and size.*

   We propose a set of exercises in which students are asked to analyse MW or Rh data and apply complementary knowledge to reach conclusions such as the oligomeric state or the compactness of a protein. We seek to foster students' skills for constructing meaning from information, as well as the ability to contextualize data in order to reach a proper interpretation of results.

2. *Develop a strong conceptual and operational understanding of SEC and LS techniques.*

   We aim to promote student understanding and appreciation of light scattering and to recapitulate and deepen previous knowledge of SEC in a practical way. The practical exercise includes deciding whether some given

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data (e.g., the order of elution of three proteins in a SEC experiment) is enough evidence to support or disprove a conclusion. Students critique possible explanations, arguing for or against the options.

3. *Gain criteria to choose the most adequate technique to solve a given problem.*

   By the end of the module, students are expected to know how to design an experiment based on the complexity of the sample. The activity helps to build connections between different kinds of information and use it to make predictions in quite new situations. For this specific learning goal, students must be able to recognize relevant information and discern which method is the most appropriate to find the MW and Rh of the components of mixtures of different complexity.

4. *Learn how to carry out an experiment, analyse data, and present experimental results.*

   Students carry out an experiment and process collected data to obtain information. They need to make tables and figures to appropriately present their findings and generate further hypotheses. Besides, they are expected to spot inconsistencies in the results and explain them considering the chemical and physical principles of each technique. The attainment of these competencies is enriched by writing a laboratory report.

To assess the impact of this intervention we collected information on perceived and actual knowledge through pre- and post-class surveys, and by evaluating lab reports using a detailed rubric.

METHODOLOGY

Participants

Here we report results obtained during four offerings of a course dealing with methods on protein chemistry, structure, and interactions, which were given in 2018, 2019 and 2022 in the Department of Biological Chemistry of the Faculty of Pharmacy and Biochemistry from the University of Buenos Aires. Learners are typically last-year students from the 6-year clinical biochemistry professional degree program, who have already completed several courses in chemistry and biology, or graduates already pursuing an advanced degree. This module takes place midway through the course.

There is ample evidence in the literature that a host of classroom characteristics and practices by the instructor will influence the implementation of any curriculum and the resulting student outcomes (Jennings and

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Greenberg 2009; Roehrig and Garrow 2007; Cronin-Jones 1991). Thus, the teaching approaches used to implement the module and the instructional staff, which include a professor and two instructors per session, were the same over the four offerings of the course analysed in this study.

The pre- and post-surveys were completed by 36 and 34 students, respectively. The mean age of the participants was 26 ± 2. Our institution is an independent, government-funded research university, with non-selective admission and no tuition fees for undergraduates. Note that in Argentina neither socioeconomic status nor gender is generally regarded as relevant in terms of academic performance at this level of instruction.

Assessment of the implementation

To evaluate the efficacy of this module, we collected data employing voluntary and anonymous pre- and post-class surveys, which were administered before the introductory recitation class and after the *Data analysis session*, respectively. The questions on pre- and post-survey instruments were identical. Students were not told about the upcoming post-survey during the module, nor were questions on the pre-survey directly addressed by instructors before the post-class assessment. This work followed the ethical guidelines suggested by Taber (2014). Students were informed of the research purposes and could withdraw from the research at any stage of the research. The importance of providing thoughtful answers was stressed by explaining that responses were going to be used to understand their learning process and to improve future offerings of the course. Students were forewarned that survey results would not have any impact on their grades.

All surveys used to obtain information for this paper were identical and could be completed thoughtfully within 20 minutes. The instrument was designed to assess both perceived and actual knowledge. The first two questions (*A1* and *A2*) focused on self-efficacy, i.e., students' beliefs about their knowledge and capacity to carry out the steps necessary to produce specific performance attainments (Bandura 1977). The subsequent questions (*Q1* to *Q4*) examined actual knowledge and higher-order thinking skills. The questions were crafted to assess specific learning objectives (see *Introduction*), covering core concepts such as molecular weight and size, SEC, SLS, and DLS, along with core competencies such as conducting experiments, data analysis, and choosing the most suitable technique to solve a given problem. Each self-perception question was paired with at least one actual-

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knowledge question (see *Results*), allowing us to evaluate the extent to which perceived gains aligned with actual gains.

The assessment instrument included a variety of exercises designed to challenge students at different levels of thinking complexity. In question *A1* students were asked to report their self-efficacy on each of the three discussed techniques (SEC, SLS, and DLS) by selecting from five confidence statements (see Figure 7). Question *A2* had students rate their perceived understanding of several core concepts and parameters (MW, Rh, A_2, *D*, oligomeric state, and *Fa*) with four confidence statements (see Figure 4). For actual-knowledge question *Q1*, students were required to analyse a SEC profile (chromatogram) and match each peak to one of three proteins of known MW; in this question, students chose one of four possible options (see Figure 8). Question *Q2* provided MW data of a protein obtained by SEC and SLS, and calculated from the amino acid sequence, and asked students to establish the oligomeric state of the protein (Figure 5). In question *Q3*, students were given the Rh of a protein determined by SEC and DLS, and asked to decide how the actual degree of compaction of the protein compares with the predicted by using an empirical correlation of the Rh with the MW; in this question students chose one correct answer from three options (see Figure 6). Finally, question *Q4* presented three statements about experimental setups for determining the MW of protein samples of varying complexity; students were to indicate if each statement was true or false (see Table 4).

The face validity of the survey instrument was established through input from instructors teaching the module and senior faculty overseeing the course. In the offering prior to the implementation of our intervention, we carried out a pilot test that helped us understand how students interpreted the questions and assignments. Based on this pilot test, we made necessary amendments and modifications to the survey instrument.

Self-efficacy is a concept that refers to an individual's belief in his or her ability to perform a specific task or achieve a particular goal. It is an important factor in learning and achievement because individuals with higher levels of self-efficacy are more likely to set goals, persist in the face of challenges, and ultimately succeed in their endeavours. In the context of learning, self-efficacy can significantly affect motivation, effort, and persistence. Lin and Wu (2021) proposed that self-efficacy serves as the initial catalyst for learner engagement and motivation in the field of chemistry. If an activity results in increased self-efficacy, learners may be more inclined to engage with the material in the future, ultimately leading to greater success in their chemistry learning journey (Bandura

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1977; Lin and Wu 2021; Panadero, Jonsson, and Botella 2017). We conducted an exploration of learners' self-efficacy before and after the activity. Our aim was to assess whether students find motivation through the cognitive conflict incorporated into our practical activity, which, in turn, encourages them to engage in a genuine and challenging learning experience.

Students' self-perception and actual knowledge before and after the laboratory module were compared. To aggregate data, when appropriate, responses were classified into discrete categories to group similar answers. "Don't know", "Don't remember" and "—" were aggregated with wrong answers, and the distribution of responses was analysed. There were no cases in which all answers were left blank. In terms of invalid (poor-quality) responses, we considered respondents who repeatedly selected the same answer choice, who fell beyond the range of answers from other students, or who offered nonsensical feedback in open-ended questions; we identified only one respondent who answered unthoughtfully, and their survey was excluded from the analysis. We spotted a few inconsistent responses but preferred not to remove them given that they may have been due to lack of understanding, not to careless answering. As descriptive statistics of our data, we calculated the median to find the central tendency and the range to indicate the variability. Analysis of statistical significance was performed using the two-tailed Wilcoxon signed-rank test on the 34 respondents who answered both surveys (Lewis 2014).

We calculated odds ratios (ORs) to compare the relative likelihood of correctly answering actual knowledge questions in the high self-efficacy group compared to the low self-efficacy group. The OR is a statistical measure used to assess the strength and direction of the association between two binary outcomes. To calculate it, we divided the odds of correctly answering an actual knowledge question given high self-efficacy by the odds of correctly answering the same question given low self-efficacy (Szumilas 2010). To do this, we created a contingency table with two binary variables (*correct* vs. *incorrect* answer, and *high* vs. *low* self-efficacy), which defines four groups: (a) students with high self-efficacy who answered the question correctly, (b) students with low self-efficacy who also answered correctly, (c) students with high self-efficacy who did not answer correctly, and (d) students with low self-efficacy who also did not answer correctly. Then, we calculated the odds of students with high self-efficacy answering correctly (a/c) and the odds of students with low self-efficacy answering correctly (b/d). The OR is computed as (a/c) / (b/d). An OR greater than 1 indicates that students with high self-efficacy have higher odds of answering correctly, while an OR less than 1 suggests the opposite.

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Additionally, we asked students to provide feedback to obtain information on their appreciation of the learning experience. In this regard, we asked students to provide their opinions by means of a survey administered after the final exam.

Lab reports

We used two formats for lab reports. The *original format* required students to write a traditional report in which they freely presented results and their analysis and conclusions. The *new format* consists of a structured questionnaire included in the laboratory handout, which students complete and hand in (see *Supplementary information*). In both instances, this activity was optional. The shift from the *original* format to the *new* format occurred during the last course offering. This change was prompted by the low adherence and the weak correlation between report grades and post-class survey results. Both report formats could be evaluated using almost identical rubrics. The assessment for both formats was divided into three categories: *data processing*, which is focused on making figures and presenting data; *results analysis*, which involves identifying peaks in a chromatogram from a SEC experiment and making comparisons between SEC and LS results; and *conclusions*, where students integrate ideas to formulate a feasible explanation for experimental results. Students could earn up to 5 marks for each category, with the final grade (A being the highest and E the lowest) determined by combining marks obtained in each category, following the specifications outlined in the rubric (see *Supplementary information*).

Instructional activity schedule

This module is divided into the following four main components:

Recitation: This class introduces or extends the concepts of MW, oligomeric state and Rh, and discusses the techniques for their determination. An important issue we address is the distinction between mass and size measurements, which are sometimes unthinkingly mixed. Most students would have already studied different techniques that allow mass and size determination—such as SDS-PAGE, mass spectrometry, gel filtration chromatography, analytical ultracentrifugation, and nuclear magnetic resonance—so we devote some time to clarifying misconceptions and discussing in which conditions those measurements are done, with their consequent advantages and limitations. We present a few situations in which SEC has not led to correct information and

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discuss different aspects of the technique and of the sample that may affect results, for example, due to researchers being unaware of the fact that gel filtration matrixes exhibit charges that might distort the chromatographic run (Folta-Stogniew and Williams 1999; Allen and Ullman 1994; Uversky 1993). Besides, we introduce the physical phenomenon of light scattering and describe how it is exploited to obtain absolute determinations of MW, with a special focus on the use of SLS and DLS to obtain information about protein size and mass. It is worth noting that in this session we focus solely on explaining the techniques' functionality, without delving into exceptions, which will be covered in the *Data Analysis* session.

Laboratory practical: The wet-lab component session begins with a brief review of the experiment to be performed. A laboratory guide with a detailed explanation of the practical is made available to students in advance (see *Supplementary information*). Students learn about the equipment and all their components, alternatives for carrying out experiments, and how the sample should be prepared before being loaded and injected into the system. Students work in groups of approximately 5 during chromatographic experiments and LS data acquisition.

Data analysis session: Next, we carry out a brief tutorial on data analysis, in which students learn how to analyse data with the appropriate software. During this unit, students are encouraged to discuss their data analysis approaches and share ideas with peers and instructors for interpreting results. These discussions often reveal cognitive conflicts and initiate the process of conceptual change. To support this, we provide guidelines for interpreting the most challenging results. This guidance helps students in the learning process by preventing incomplete observations and inaccurate conclusions, which might incite the reinforcement of existing misconceptions or even the generation of new ones (Kirschner, Sweller, and Clark 2006).

Lab report: Students are required to prepare a report; each student is expected to hand in an individual piece of work, although interaction among them is common and welcome. The laboratory handout includes a step-by-step description of how to analyse and present data. Teachers are available during the following weeks for assistance on any issue students might face while writing their reports. Most frequently asked questions by students revolve around data processing using spreadsheets (such as how to plot results informatively and find specific information like maximum absorbance value, average MW, or Rh values), data interpretation (including explanations for unexpected observations or comparisons of different values in a table), or factual knowledge (such as the physical foundations of techniques or the impact of molecular mass, size, and interactions on experimental results). Finally,

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reports are graded, and students receive feedback on their work on a one-to-one basis and in a group discussion in which the instructor guides them in the assessment of their learning outcomes.

Student guidance and support

Throughout this activity, students had the chance for independent work, which allowed them to develop problem-solving skills and cultivate autonomy in their learning journey. To support their exploration, we provided them with bibliographic materials to delve into the content independently, nurturing their ability to investigate and discover new ideas.

In addition, we encouraged a culture of collaboration and idea-sharing among students and teachers. We allocated dedicated time for group discussions, facilitating the exchange of perspectives and deepening their understanding through constructive dialogue and valuable feedback. This collaborative approach promoted a conducive learning environment, promoting the sharing of experiences and knowledge for the collective benefit of all students.

Moreover, the activity spurred active student participation by creating an atmosphere of inquiry and reflection. Students were prompted to raise questions and seek answers, sparking their curiosity and facilitating a comprehensive grasp of the concepts being addressed. Furthermore, they were motivated to reflect on their learning process, enabling them to identify strengths and areas for improvement and, subsequently, adapt their study strategies to enhance their overall learning experience.

The laboratory module

In this practical, students characterize two protein samples of different complexity. In doing so, they face the fact that specimens may not be pure or simple, develop a good working knowledge of the concepts of MW, Rh, and oligomeric state, acquire quantitative skills, understand to what extent SEC, SLS, and DLS are appropriate techniques to solve a given problem and learn to interpret data.

SEC is a simple yet powerful and widespread method for protein mass and size estimation, commonly taught in most biological chemistry courses. However, actual SEC results may not always accurately describe the molecule under study, given that this technique relies on the calibration with reference molecules and assumes

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certain approximations and ideal conditions. SLS and DLS are two alternative methods based solely on the interaction of the molecule with light. SLS allows the absolute determination of the MW, and the addition of a DLS (aka, quasi-elastic light scattering, QELS) module allows the determination of *D*, and subsequent calculation of the Rh.

Practical experimental details

FXN and HPRT were purified as described elsewhere (Valsecchi et al. 2016; Faraj et al. 2016). BSA was purchased from *Sigma* (USA). Protein purity was verified by SDS-PAGE and concentration was assessed by UV absorbance. MW and Rh determinations were performed by SEC and LS in continuous flow mode. Our system consists of a set of devices arranged in tandem as depicted in Figure 1A, which allows the simultaneous determination of the MW and the value of *D*. Briefly, the sample is loaded in a 100 µl loop and injected into a SEC column (Superdex S200 or Sepharose 12). The flow rate of the buffer (20 mM Tris-HCl, 100 mM NaCl, pH 7.4) is set constant at 0.3 ml min^{-1}. The eluate passes through the UV detector (*Jasco* UV 2075 plus), which allows determining the elution volume (Ve) of each peak, and through the multi-angle LS detector (*miniDAWN Tristar, Wyatt Technology*), which measures the amount of light scattered to calculate the absolute molar mass of the sample by SLS. An optical fibre receiver is mounted in the 90° detector and coupled to the DLS module for the determination of *D* and subsequent calculation of Rh (*WyattQELS, Wyatt Technology*). Collected data are transferred to a computer where they are processed with ASTRA software (*Wyatt Technology*). Typical SEC, SLS and DLS results are shown in Figure 1B.

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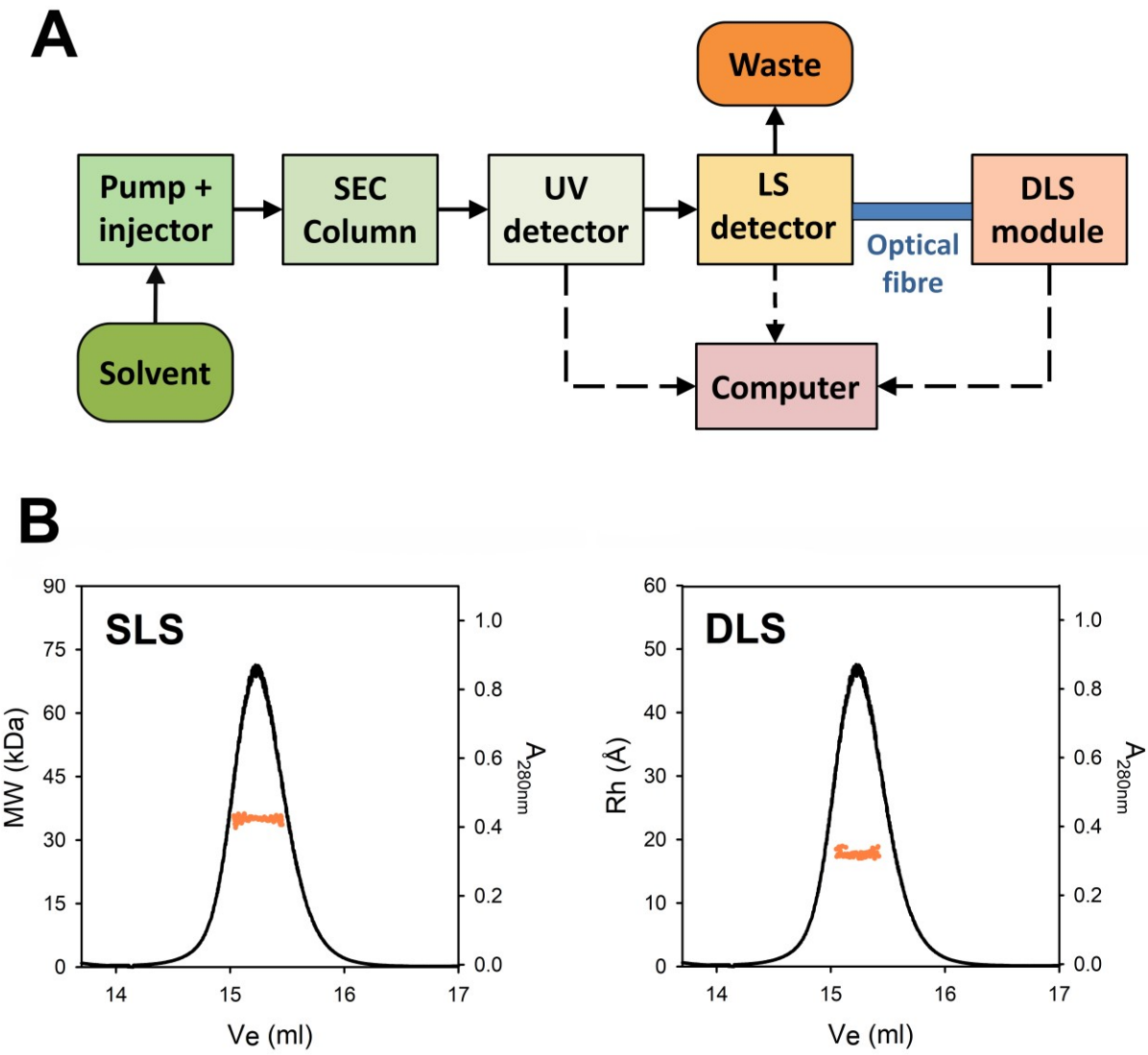


Figure 1. Protein size and mass determination. (**A**) Schematic representation of the system. The sample is loaded into a loop of known volume and injected into a SEC column. The eluate passes through the UV and LS detectors. The optical fibre (blue) transfers data (fluctuations in the intensity of the scattered light) to the DLS module. Collected data from each piece of equipment are transferred to a computer. (**B**) Typical protein chromatographic profile (—) and LS results (●). MW (left) and Rh (right) values were obtained by SLS and DLS, respectively.

Laboratory practical results and data analysis

During the wet-lab session, students perform three chromatographic experiments, as described in detail in the *Laboratory handout* (see *Supplementary information*), corresponding to the following samples:[1]

[1] The data sets used to illustrate results throughout this paper are available for teachers who do not have access to a LS equipment, but desire to apply this activity in a data-analysis exercise. In addition, teachers can take advantage of the enzymes

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A. Gel filtration standards (a mixture of 5 proteins ranging from 1,350 to 670,000 Da)

B. A mixture of BSA and FXN

C. A solution of pure HPRT

Sample *A* is a mixture of proteins of known molecular weight and is used to obtain a calibration curve for the gel filtration column. Samples *B* and *C* contain the proteins we wish to characterize. Figure 2 summarizes the algorithm for calculating the MW of a protein using SEC.

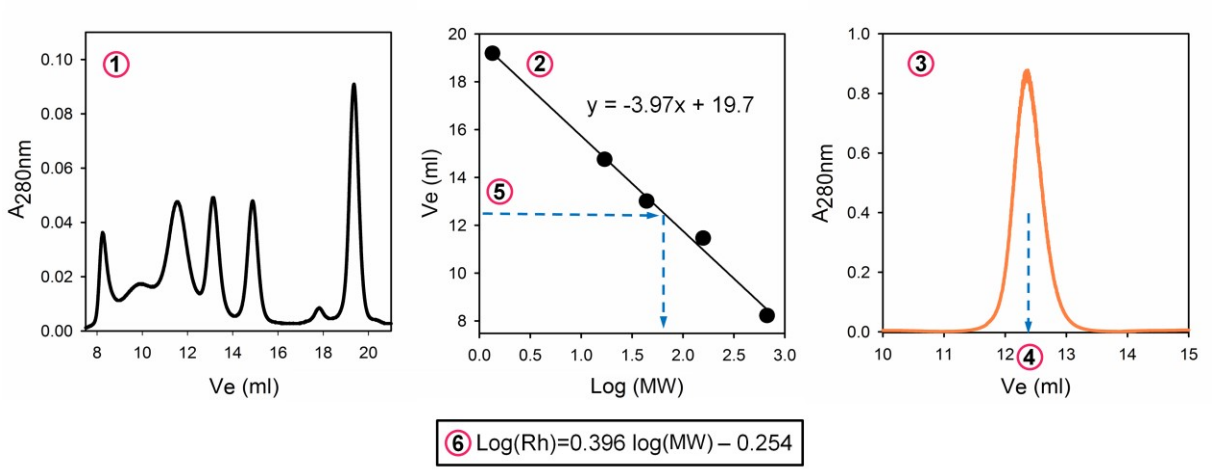


Figure 2. Calculating the MW of a protein using SEC. The SEC profile of a sample of molecular weight markers is obtained (1) and the Ve of each species is plotted as a function of log MW to calculate the line of best fit (2). The SEC profile of the problem protein is also obtained (3), and the Ve (4) is used to calculate its MW using the best-fit line for the molecular weight markers (5). Additionally, the value of Rh can be approximately calculated (6), for example, using the correlation presented by Uversky (1993).

Data obtained for samples *A* and *B* are presented in Figure 3. First, students are asked to identify peaks in SEC profiles (UV absorbance), and to calculate the MW and the Rh of the species in samples *B* and *C* using the best-fit line obtained for data from the molecular weight markers (Table 1). In the case of sample *B*, results show that (at least) three species coexist even though the sample consists of only two pure proteins. Considering that the BSA monomer has a sequence-based MW of 66.5 kDa, students readily assign peak 2 to these species. Students may suggest several interpretations to explain peak 1, which corresponds to the BSA dimer, in accordance with the calculated MW and the well-known propensity of BSA to oligomerize. Peak 3 must correspond to FXN, which

here analyzed or make use of other proteins that show expected (e.g., BSA, ovalbumin or lysozyme) or anomalous hydrodynamic behaviors (e.g., yeast enolase, Folta-Stogniew and Williams, 1999).

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has a sequence-based MW of 13.7 kDa. In turn, sample *C* presents only one peak. Given that the only protein in this sample is HPRT, with a sequence-based MW of 26.6 kDa, students conclude that HPRT is a monomer under these working conditions. To calculate Rh, we use an equation that establishes the relationship between MW (sequence-based or estimated by SEC) and Rh (Uversky 1993) as described in Figure 2; note that in order to obtain Rh in Ångstroms (Å), the MW must be input in Da (not kDa).

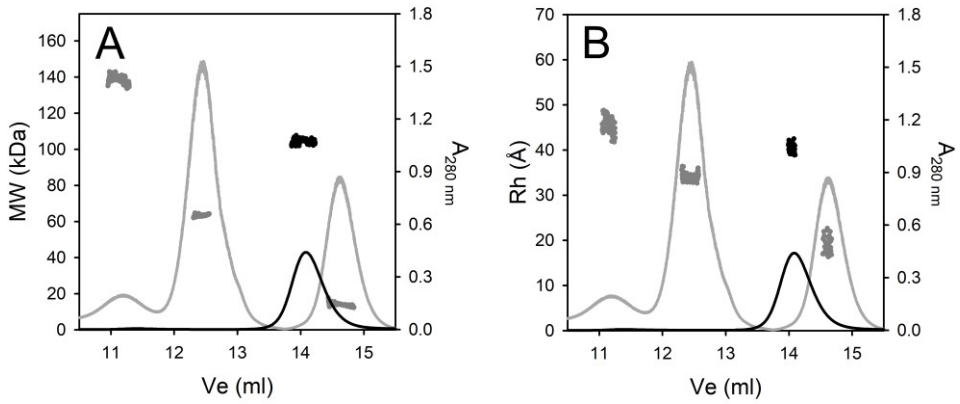


Figure 3. Typical results of the experience. SEC chromatographic profiles visualized by UV absorbance of samples B (▬) and C (▬), together with (**A**) MW data calculated by SLS for samples B (●) and C (●), and (**B**) Rh values calculated from DLS data obtained for samples B (●) and C (●).

Table 1. Analysis of SEC results.

Sample		Peak (left to right)	SEC Ve (ml)	SEC MW (kDa)	SEC Rh (Å)	SLS MW (kDa)	DLS Rh (Å)
(B)	**BSA+FXN**	1	11.1	140.6	44.2	138.7 ± 2.1	45.4 ± 1.6
(B)	**BSA+FXN**	2	12.4	67.9	33.8	63.4 ± 0.5	33.9 ± 0.8
(B)	**BSA+FXN**	3	14.6	19.2	21.2	13.9 ± 0.7	18.6 ± 1.8
(C)	**HPRT**	1	14.2	24.8	23.3	104.3 ± 1.3	40.3 ± 0.9

Sequence-based MWs for monomers are 66.5 kDa (BSA), 13.7 kDa (FXN) and 26.6 kDa (HPRT).

In the following step of the analysis, students make use of LS data to characterize the samples. Figure 3A shows MW values for each peak obtained by SLS, and Figure 3B presents Rh values calculated from DLS data. Results are summarized in Table 1. Values for peaks 1 and 2 from sample *B* coincide with SEC data. In contrast, values for peak 3 from sample *B* differ from those calculated by SEC, but the obtained MW is similar to what is expected based on the protein's sequence. This discrepancy arises from the fact that FXN presents an elution volume slightly lower than expected for a protein of its MW due to its acidic surface that produces its exclusion

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from the gel matrix due to charge repulsion (Faraj et al. 2013); this effect is rectified at higher salt concentrations (Figure S1). Alternatively, students may be provided or asked to retrieve the protein sequences (e.g., from UniProt) to identify outstanding features, such as the prevalence of Asp and Glu residues endowing strong acidic character to the protein, and its consequences on SEC, considering the residual negative charges on the column matrix. The peak in sample *C* exhibits a MW value inconsistent with HPRT being a monomer as deduced from SEC, but instead, it indicates a tetramer. Correspondingly, the Rh value obtained by DLS closely matches that of a tetramer (39.9 Å). This deviation is attributed to the positively charged nature of HPRT, which may interact favourably with the matrix; indeed, increasing NaCl concentration reverses the abnormal behaviour of HPRT, without altering its apparent MW (Valsecchi et al. 2016)—*cf.* Figure S1. In this context, students can be provided or tasked with retrieving HPRT sequence, analysing the abundance of Lys and Arg residues, calculating its pI value, and thereby deducing its basic character. These results highlight that using SEC alone could lead to an overestimation of the MW of FXN and an erroneous assignment of HPRT as a monomer. Such observations are discussed with students during the *Data analysis* session in terms of the variables that may influence the hydrodynamic behaviour of proteins, such as unusual compactness or matrix-protein interactions, and the possibility of using salt ions to attenuate the effect of their charged character.

RESULTS

To evaluate the accomplishment of our learning objectives, we administered pre- and post-class surveys. We were interested in whether students' self-efficacy responses correlated with the actual acquisition of analytical skills and content understanding. Therefore, the survey instrument included both self-perception and knowledge-based questions on different contents addressed in the module.

While actual-knowledge responses are straightforward to interpret because answers are objectively right or wrong (allowing for possible partially correct answers that permit us to evaluate aspects of a problem), self-perception responses are inherently subjective. To assess the "honesty" of students' responses to the latter, we introduced a fictitious parameter called the *Avece factor (Fa)* as an experimental control. Naturally, we expected perceived mastery of *Fa* to be null in both pre- and post-class surveys. Interestingly, while the most popular answer in pre-class surveys was "*I have never heard about this parameter*" (Figure 4), by the end of the module, a

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surprising 38 % of students reported having heard about it, with 15 % claiming to understand the information it provides, and even one student answered they could determine the value of such a parameter.

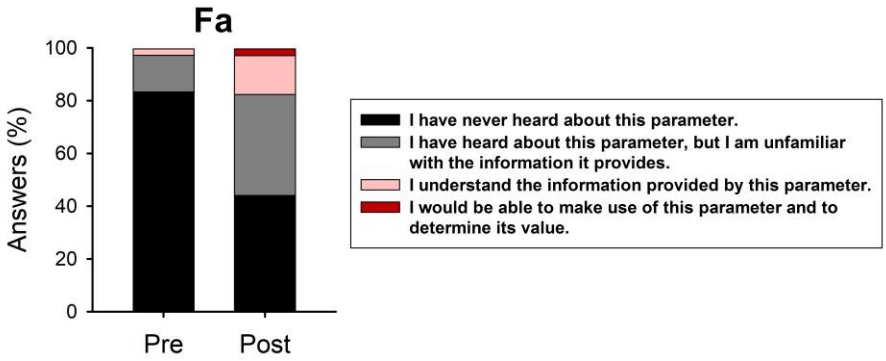


Figure 4. Genuineness of self–perception answers. Students were asked to report their perceived understanding of the fictitious parameter *Fa* (question *A2*).

This result raises a host of questions about what can and cannot be concluded from self-perception assessments, which should be analysed and considered with caution. However, we must keep in mind that these findings only highlight a trend in self-efficacy responses, which are subjective and do not reflect actual understanding. Considering improvements in self-efficacy by the end of the module, a *real* greater confidence in most topics might drag students to state they also gained in their understanding of even a non-existent factor. Given that unquestionably no one had ever heard about the *Fa* parameter, the fact that some people stated they had heard about it before the module, while many more said so afterwards, shows that students are more likely to admit ignorance at the beginning of the module than at the end—obviously, under the assumption that they were not being *cheated*. We believe the most probable explanation for this observation is that answers are biased toward what students feel they *should know* rather than what they feel they *actually know*. This apparent dishonesty might have its basis in the notion that not knowing a concept after an educational experience that presumably introduced it is embarrassing or may disappoint teachers.

LO1: Gain further understanding of the concepts of mass and size

In this module, our primary focus revolves around the topics of protein mass and size. Most students taking this course are already familiar with a general definition of both concepts, even though they have not faced the practical challenges of experimental work. During the recitation, both concepts are discussed in terms of their practical definition, the different techniques for their determination, the actual meaning of apparent values, and

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the factors that affect calculations. In this study, understanding of mass was assessed by inquiring about the notion of MW and its applicability to determine the oligomeric state of proteins exhibiting quaternary structure.

Self-efficacy on molecular size was assessed through questions on Rh, *D*, and A_2 (question *A2*). Results are shown in Table 2. Pre-class surveys reveal a higher perceived understanding of mass indicators than those of size, something expected given that it is a more complex idea. Interestingly, despite being two closely related concepts, while 3 out of 5 students reported the maximum level of confidence for MW in the pre-class survey, only 1 out of 4 said so for the oligomeric state (Figure S2). Post-class surveys show an increase in self-efficacy in all assessed categories. By the end of the module, most students reported feeling able to make use of the information provided by MW, oligomeric state, and Rh, but not of that provided by *D* and A_2 (Figure S2). These results are unsurprising since *D* and A_2 are new concepts and require a greater level of abstraction—note, however, that Rh is a theoretical calculation based on the empirical value of *D*—besides, they were not discussed in as much depth as the other three parameters throughout the module.

Table 2. Mean pre- and post-class survey scores for self-efficacy on mass and size (question *A2*).

		Median value (min-max, range)		*p*-value
	Parameter	**Pre-class survey**	**Post-class survey**	
Mass	**MW**	4 (3-4, 1)	4 (3-4, 1)	0.0034
	Oligomeric state	3 (1-4, 3)	4 (2-4, 2)	< 0.0001
Size	**Rh**	3 (1-4, 3)	4 (3-4, 1)	< 0.0001
	A_2	1.5 (1-3, 2)	3 (2-4, 2)	< 0.0001
	D	3 (1-4, 3)	3 (2-4, 2)	0.0002

Students chose from a list of confidence statements (see Figure 4). For this analysis, the lowest confidence level was assigned a value of 1 and the highest level a value of 4. Significance was determined by the two-tailed Wilcoxon signed-rank test.

In addition to evaluating students' self-perception, we also aimed to determine whether they had successfully attained this learning objective. We asked them to assess the potential supramolecular organization of a protein for which the MW has been experimentally obtained by SEC and SLS (question *Q2*). This assessment was conducted through an open-ended question asking students to describe the oligomeric state in whatever way they were able to. Even when answers were not correct, understanding of this concept was shown by giving a plausible response. Possible answers to this question included stating that the protein was a *tetramer* (if SLS data were used), a *monomer* (if SEC data were used), or any other description of the number of subunits that comprise the quaternary structure of a protein such as *two molecules* or *more than one subunit*. In the pre-class survey, 2 out of 5 students did not respond to this question, despite the majority reporting some level of understanding of the

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concept (as indicated by their selection of the two most positive options in the self-efficacy question, Figure 5). After completing the module, few students left the question unanswered and there was a significant increase in those using SLS data to respond. This change aligned with their improved self-efficacy regarding the concepts of oligomeric state (Figure 5). It is noteworthy that, in the pre-class survey, approximately half of the students chose to answer the question based on SLS data (*tetramer*), despite most of them claiming not to understand the technique (*cf.* Figure 7). This raises concerns about a potential appeal to novelty bias. In fact, our calculation of ORs for selecting the SLS-based option among students with high self-efficacy regarding oligomeric state yielded values of 0.8 in pre-class surveys and 3.4 in post-class surveys. Similarly, when calculating ORs for selecting the SLS-based option among students with high self-efficacy regarding MW, we obtained values of 0.5 in pre-class surveys and 3.1 in post-class surveys. These findings suggest that the significant use of SLS data by students in the pre-class surveys was likely casual (low self-efficacy students most probably used SEC data than high self-efficacy students), and highlight the consistency between self-perceived and actual knowledge in post-class surveys.

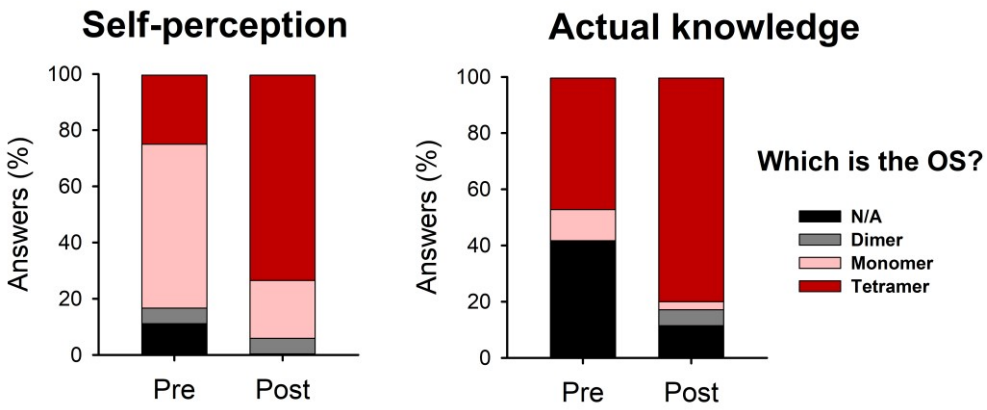


Figure 5. Perceived versus actual understanding of oligomeric state. *(Left)* Students were asked to report their perceived understanding of oligomeric state (OS); bars colours represent the same as in Figure 4, red being the highest and black the lowest confidence level. *(Right)* Students were assessed on their ability to make use of experimental data to establish the oligomeric state of a protein (question *Q2*).

To assess actual knowledge of molecular size, we asked students to decide whether a protein exhibited a typical degree of compaction based on the Rh value obtained by SEC and DLS (question *Q3*); this was a multiple-choice question in which students answered by selecting between the options "*It is more compact than a typical globular protein*", "*It is as compact as a typical globular protein*" or "*It is less compact than a typical globular protein*". In pre-class surveys, most answers were unsatisfactory (Figure 6), with one-third of students leaving the

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assignment unanswered and each of the three options being almost equally chosen, in sharp contrast with the fact that the majority of students reported a good understanding of the topic, but in agreement with most of them being unfamiliar with DLS technique (*cf.* Figure 7). After the module, about 2 out of 3 students were able to answer this challenging question correctly, in line with their increased confidence.

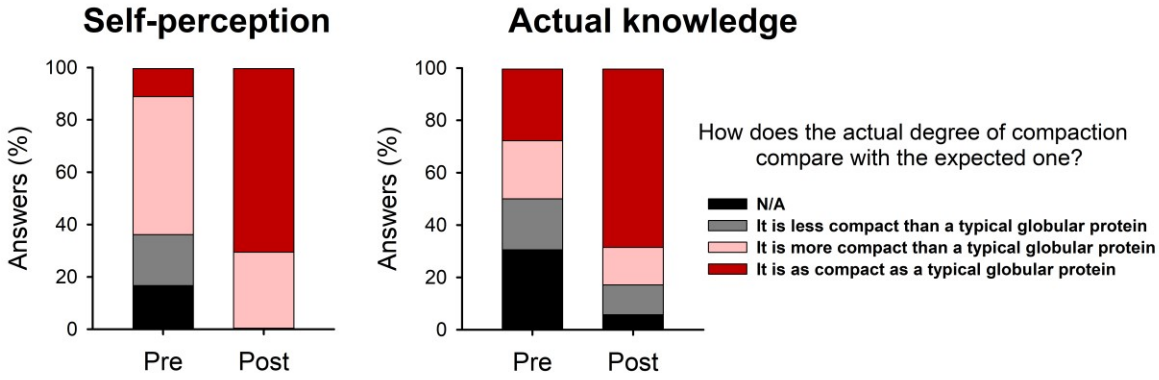


Figure 6. Perceived versus actual understanding of Rh. *(Left)* Students were asked to report their perceived understanding of Rh; bars colours represent the same as in Figure 4, red being the highest and black the lowest confidence level. *(Right)* Students were assessed on their ability to make use of the value of Rh to establish the degree of compaction of a protein (question *Q3*).

In terms of LO1, we observed increased self-efficacy and improved analytical skills following the activities, indicating the module's success. Although pre- and post-class surveys revealed that students perceived a greater understanding of the notions of mass and size than they actually demonstrated in knowledge-based questions, it is interesting to note that self-reported understanding did not correlate well with actual knowledge before the module but did so after students completed the proposed activities.

LO2: Develop a strong conceptual and operational understanding of SEC and LS techniques

Our second major goal was to effectively convey the foundations of the two most popular techniques for native mass and size determination. Here, we assessed self-perceived understanding of SEC, SLS, and DLS by asking students to rate themselves using a five-level scale (Figure 7 and Table 3).

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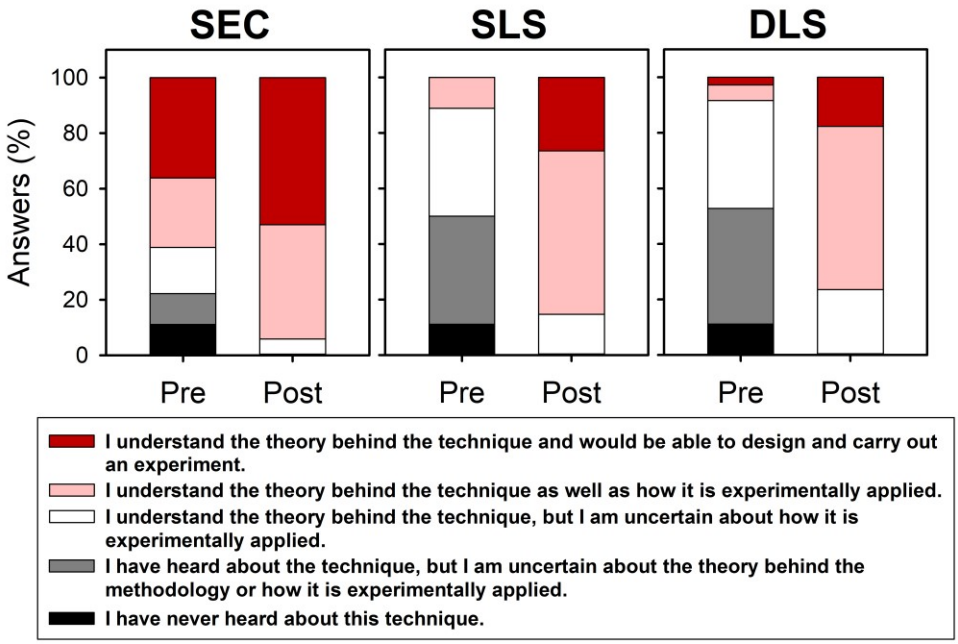


Figure 7. Self-reported understanding of the three main techniques discussed during the module. Students were asked to rate their understanding of these techniques using the given scale. Bar graphs show the percentage of students who indicated each response on pre- and post-class surveys (question *A1*).

Pre-class surveys showed a better self-perceived understanding of SEC than any of the LS-based techniques. Conversely, self-perception of the three techniques was high by the end of the module.

Table 3. Mean pre- and post-class survey scores for self-efficacy on SEC, SLS and DLS (question *A1*).

Technique	Median value (min-max, range)		*p*-value
	Pre-class survey	Post-class survey	
SEC	4 (1-5, 4)	5 (3-5, 2)	< 0.0001
SLS	3 (1-4, 3)	4 (3-5, 2)	< 0.0001
DLS	2.5 (1-5, 4)	4 (3-5, 2)	< 0.0001

Students self-reported their understanding of each of the discussed techniques by choosing from a list of confidence statements (see Figure 7). For this analysis, the lowest confidence level was assigned a value of 1 and the highest level a value of 5. Significance was determined by the two-tailed Wilcoxon signed-rank test.

To evaluate actual knowledge of SEC, we asked students to assign peaks from a chromatogram to three proteins (question *Q1*). This was a multiple-choice question with four possible answers (Figure 8): (*i*) the standard SEC peak assignment (*larger proteins elute first*); (*ii*) the inverse order (*smaller proteins elute first*); (*iii*) an assignment based on protein absorbance (*higher MW yields higher absorbance*); and (*iv*) unable to tell (*information provided is not enough to decide*).

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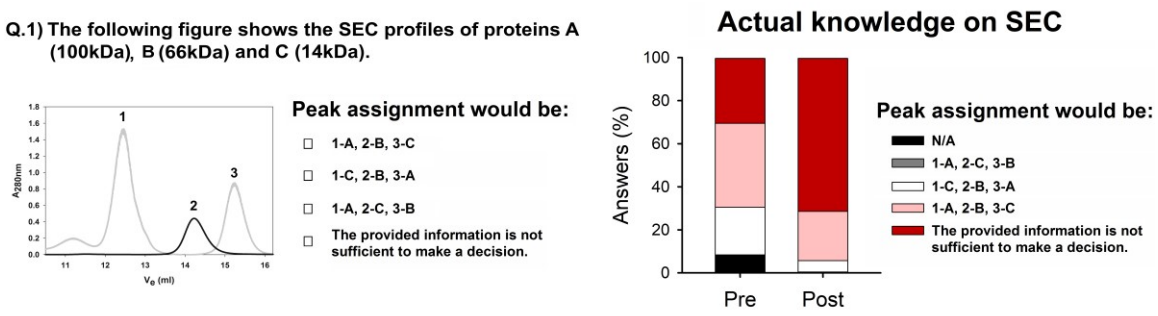


Figure 8. Interpretation of SEC results. Students were evaluated on their ability to characterize a set of proteins using information from SEC. Standard peak assignment A/B/C (□), inverse order peak assignment C/B/A (□), absorbance-based peak assignment A/C/B (■), unable to decide (■), and no answer (■) (question *Q1*).

Since students usually become familiarized with SEC in basic biological chemistry courses, we expected the "*Larger proteins elute first"* option to be the most popular before the class. However, in pre-class surveys, it was only slightly more chosen than the "*Not enough information"* option—an alternative more closely associated with expertise in SLS. Conversely, by the end of the module, while the standard option was still acceptable, we expected students to be aware of the limitations of the technique and realize that certain additional experiments are necessary to validate the information obtained by SEC. That was indeed the case, also in line with higher self-efficacy regarding SLS (Figure 7). Moreover, all the students answered the question in the post-class survey, showing increased confidence by the end of the experience. None of the students selected the absorbance-based option, neither in the pre-class survey nor in the post-class survey.

We wondered whether the "*Not enough information"* option was genuinely chosen before the module, or if some students who did not know how to approach the problem guessed it might be the expected option based solely on the wording. To assess the coherence between self-perceived and actual knowledge, we calculated the ORs for the selection of this option by students with high self-efficacy in SLS. Our findings revealed ORs of 0.92 in pre-class surveys and 2.15 in post-class surveys. These results indicate that there was no alignment between self-perceived and actual understanding before the module, suggesting that the prevalence of this choice in pre-class surveys (Figure 8) was likely coincidental. In contrast, coherence between self-perceived and actual understanding improved in post-class surveys.

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Our results show that the practical activity was effective in terms of gains in actual understanding of SEC and LS techniques. Also, by the end of the module, students' self-efficacy suggests an improvement in their self-calibration, indicating that they became more aware of their learning process and its outcome.

LO3: Gain criteria to choose the most adequate technique to solve a given problem

This objective aimed to evaluate the ability to appropriately select methods for characterizing samples based on size, concentration, and complexity. To achieve this, we designed a *true-or-false* questionnaire with three statements (question *Q4*). The first one evaluated actual knowledge about SEC; students were asked to determine whether a specific SEC experimental design could yield the MW of the proteins in a sample (Table 4, *statement A*). This statement is false since there is no one-size-fits-all solution for SEC: the type of matrix should effectively resolve all the proteins in the mixture, adequate MW markers must be available, and potential interactions between the matrix and proteins should be considered. Results show an increase in the knowledge about this topic, suggesting that students have learnt to use SEC and to discriminate whether it can provide the desired information (Table 4). These findings align with those presented in Figure 8, which demonstrates a significant improvement in students' critical judgment regarding the validity of SEC results.

Table 4. Percentage of correct answers (question *Q4*).

	Statement	Pre	Post
A	*Given a mixture of proteins of different sizes, a SEC column attached to a UV detector would always allow obtaining the MW of each of them.*	36	63
B	*A light scattering device (685 nm laser) with a single 90° detector may be adequate to obtain the MW of a monodisperse solution of a polymer with a mean radius of 15 nm.*	25	51
C	*In order to obtain the MW of a solution of a small, pure protein, a light scattering device (685 nm laser) with a single 90° detector (online with a SEC column) would be a convenient choice.*	56	83

Statements B and *C* assessed the knowledge acquired regarding LS. Both statements presented similar scenarios in which a protein sample is analysed by LS using batch (*B*) or flow (*C*) modes. While the sample composition remained the same for both scenarios, the language used to describe it differed: in *B*, the sample was described as a *monodisperse solution of a polymer with a mean radius of 15 nm* (involving technical details), whereas in *C* the sample was described as a *small, pure protein* (using more everyday language). The percentage

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of correct answers to both statements increased in post-class surveys, showing significant gains in actual knowledge. The different success with which students evaluated statements *B* (51 %) and *C* (83 %) are most probably due to the terminology used, suggesting that selecting technical language that requires abstraction, rather than relying on intuitive characteristics (*pure* or *small*), provides a further challenge.

While this task has shown to be challenging, the number of correct answers, as well as the percentage of students who attempted the exercise (pre / post: *A*: 86/94%, *B*: 72/91%, *C*: 81/94%), increased by the end of the module. This reflects not only gains in knowledge but also in students' confidence. These results reveal that the proposed activities successfully achieved LO3, helping students understand the limitations of each technique and how to choose appropriate experimental setups that will provide valid results.

LO4: Learn how to carry out an experiment, analyse data, and present experimental results.

Both analytical SEC and LS-based techniques have advantages and limitations. A proficient user should be able to select the most suitable experimental method for acquiring specific information, and to analyse data effectively. Therefore, understanding what information each setup provides, and the accuracy of measurements and calculations is crucial. The contrasting results obtained by different techniques during this practical activity make students realize the importance of assessing data acquisition methods and their relevance to specific questions. Following the wet-lab session, students receive a step-by-step data processing tutorial. Next, they are tasked with analysing the collected data, drawing conclusions from results, and submitting a laboratory report (optional but highly encouraged).

Our initial experience with traditional laboratory reports was not up to par. About 1 out of 3 of the students handed in their work but failed to produce a complete analysis, especially regarding the discussion of results, which proved to be a rather challenging task. Those observations, while not systematic, were particularly worrisome to us given that post-class surveys depicted a completely different scenario: students stated they were confident about the concepts and the use of techniques, and effectively demonstrated so in actual knowledge questions. Consequently, we changed the free-response report modality (original format) for a short-answer questionnaire (new format), in which students write down their results and analysis, and sketched graphs. While not intended to make elaborate conclusions, students were asked to examine results by making relevant

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comparisons. With such a change, we sought to alleviate the challenge and to get a positive response from students by motivating them to perform to the best of their ability. To standardise report grades across different years, a rubric was used to assess reports.

In the original format, students managed to explain MW results obtained by SLS, but failed to analyse Rh data obtained by DLS, which was usually left incomplete. We identified two possible reasons for this observation: on the one hand, the value of Rh does not have an obvious meaning to most students, and they may struggle to determine which pieces of information may be compared or not; on the other hand, students found themselves in the need of applying their previous knowledge of Rh in the context of LS, which is a completely new topic for most of them.

In contrast, preliminary data obtained using the new structured format helped students produce a more complete analysis. Being designed with short-answer questions and blank spaces where it explicitly indicates what kind of information is expected, it prevents the omission of certain calculations or comparisons, and encourages the discussion of challenging topics. Moreover, the new format requires less elaboration, improving adherence to the task. It is worth noting that all students engaged in the new report modality.

We believe that using a structured questionnaire with clear instructions helps students to *(i)* succeed in processing the experimental data, *(ii)* understand technical limitations, such as that certain challenging proteins cannot be well described by SEC, and *(iii)* integrate concepts, skills, and knowledge acquired in this module. All these statements provide evidence of the achievement of LO4.

DISCUSSION

When teaching STEM courses, it is important to introduce state-of-the-art techniques that students will likely encounter at the beginning of their professional careers. Numerous curriculum reform reports from around the world (AAAS 2011; Rieckmann 2012; UNESCO 2021; European Education Area (European Union) 2020; Tawil and Locatelli 2015; OECD 2018; Furman, Llach, and Tiramonti 2017) state that practising science entails not only grasping basic concepts but also developing and applying relevant skills. Gross (2004) suggested that our goal as educators should be to equip students with the cognitive tools necessary to investigate questions that pique their interest. Students need to learn how to conduct experiments, analyse data and select the most suitable

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approaches to efficiently address meaningful situations. Clearly, the application of course concepts to authentic science dilemmas will lead to a greater understanding of scientific practices (Russell and Weaver 2011; Hoffman et al. 2016). In this regard, various approaches for engaging students differ in their level of student involvement. Here we presented an educational study of a structured inquiry-based learning activity, in which we provide students with an initial question and an outline of the experimental protocol. Students are tasked with formulating explanations of their findings by evaluating and analysing the data they collect. In doing so, they activate their prior knowledge and connect it to new ideas, thereby increasing the number of connections to the learned concept, and facilitating future knowledge retrieval (Schwartz and Bransford 1998).

With the intervention we made to our educational module, we sought to foster four main learning objectives: (LO1) gain further understanding of the concepts of mass and size, (LO2) develop a strong conceptual and operational understanding of SEC and LS techniques, (LO3) gain criteria to choose the most adequate technique to solve a given problem, and (LO4) learn how to carry out an experiment, analyse data, and present experimental results. We measured its effectiveness employing an assessment instrument administered both before (pre-class survey) and after (post-class survey) the class, as well as through lab reports.

Even though a few interesting articles have proposed pedagogical activities on the fundamental principles of static light scattering and their application in polymer science (Thompson, Kozimer, and Stockwell 1970; Mougan et al. 1995; Matthews 1984), and even some have reported instructional success in the application of both static and dynamic techniques to biochemical systems (Santos and Castanho 1996; Lorber et al. 2012), none of them presents a systematic assessment of the pedagogical innovation.

Our results indicate that the educational module was effective at helping students achieve the learning objectives. The activity led to improvements both in confidence and in the actual mastery of theoretical concepts (LO1) and the discussed techniques (LO2). Interestingly, students also reported improved self-efficacy on the fictitious *Fa* factor in post-class surveys. One possible explanation for this apparent dishonesty is that not remembering a concept is embarrassing and will probably disappoint instructors. It has also been reported that college students often overestimate their knowledge (Dörrenbächer and Perels 2016; Dunlosky and Rawson 2012). Furthermore, the improved general confidence in the subject after completing the educational module may also bias responses toward more positive evaluations, even when discussing an inexistent factor. Additionally, the

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tendency to select positive responses more frequently is a well-documented bias in survey research (Watson 1992) and might explain why some students claimed to know the meaning of the *Fa* parameter even before the class.

However, it is worth noting that while there was no coherence between self-perception and actual knowledge before the module, by the end of the activity this was indeed the case, suggesting an improvement in learners' self-calibration, i.e., what they think they know better approximates what they actually know (Osterhage et al. 2019).

Certainly, a pedagogical benefit of pre-class surveys is that they make students aware of their difficulties in solving specific problems and help as a stimulus to focus their attention on certain contents during the class. This was also observed and thoroughly analysed by Sana *et al.* (2020), who proposed the implementation of pre-testing as an active method to enhance the learning process. However, while this holds for familiar concepts like MW and Rh, the same does not apply to lesser known or completely new ideas such as the fictitious *Fa* factor, which probably went virtually unnoticed.

The challenging scenario that students face when analysing SEC and LS results from BSA, HPRT and FXN creates a desirable cognitive conflict in terms of expected results. This conflict prompts learners to feel dissatisfied with their existing conceptions and encourages thinking outside the box to explain experimental observations using advanced knowledge in the field. By the end of the module, students were able to successfully discern when to use SEC to obtain information, as well as to consider LS-based techniques as a more versatile alternative (LO3). Furthermore, we observed that abstraction adds a further challenge to students' comprehension, even if they have already developed a reasonable understanding of these techniques. This observation aligns with the idea that the use of technical language tends to hamper nonexperts from interpreting scientific literature (Dolan 2007).

Misconceptions present a significant hurdle to students' grasp of scientific explanations. To understand scientific concepts, students must shift beyond their intuitive reasoning and adopt the scientific perspective. Traditional research often assumes that learners already possess coherent mental models akin to those of scientists. This assumption implied that dissatisfaction with their existing conceptions was necessary to motivate the acquisition of new knowledge and the development of more credible theories, forming the basis of the classical cognitive conflict approach (Chen et al. 2020). Our instructional approach incorporates cognitive conflict as a means to promote conceptual change in students' understanding of the topic. It revolves around expanding and clarifying existing concepts related to protein molecular weight and size. By engaging learners in a process that

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prompts them to confront their preconceived notions and re-evaluate their comprehension of the subject matter, we aim to facilitate a deeper understanding.

The model proposed by Posner *et al.* (1982) underscores the importance of cognitive conflict in changing students' preconceptions. While it has been influential in conceptual change research, it has been shown that this model has limitations. Solely relying on cognitive conflict may not always be enough to drive conceptual change. Other factors, including students' motivation, metacognitive strategies, and prior knowledge, also play significant roles in the learning process (2016; Pintrich, Marx, and Boyle 1993). Even when learners experience cognitive conflict, they may resist changing their preconceptions due to emotional attachment to existing beliefs or the perceived coherence of their current knowledge structures. Some argue that conceptual change is more likely when learners actively engage in the learning process, engage in self-reflection, and have opportunities to connect new information with their existing knowledge and experiences (Zhou 2010). Therefore, educators and researchers should explore diverse instructional strategies and interventions to facilitate conceptual change and help learners develop accurate, scientifically informed understandings of the subject.

Besides, it has been suggested that the process of constructing one's own explanation has a positive impact on learning (Chi et al. 1989). In connection with this, laboratory reports offer students the opportunity to present and explain their results, additionally providing effective evidence of learning success. However, students' weak performance in laboratory reports when using the original format did not reflect their quite good results in post-class surveys. Márquez Bargalló (2005) recognizes that it is frequent for students to perceive themselves as knowledgeable in certain topics while being unable to successfully communicate that knowledge. Teachers are typically able to guess what students attempt to explain, and therefore, neglect to encourage learners to develop good communication skills (while also complaining about the lack of this competence). In this regard, she points out that science teachers have the responsibility to improve students' language skills by teaching them to express phenomena in their own words. Also, it has been suggested that without sufficient teacher support and discussion, learners tend to make incomplete observations and draw incorrect conclusions, which may then lead to the reinforcement of existing misconceptions or even the generation of new ones (Kirschner, Sweller, and Clark 2006). With this in mind, we developed a structured questionnaire to reduce the discouraging burden of writing full laboratory reports, while still requiring students to produce accurate explanations about their experimental results.

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In this way, working in the so-called *zone of proximal development* (Vygotsky et al. 1978) students could successfully analyse and process experimental data, demonstrate an understanding of technical limitations, and integrate the knowledge and skills acquired in the module (LO4).

Learning goals are met and exceeded when students find the topics engaging, exciting, and worthwhile. A way to achieve this is to work with everyday life examples in the hopes that students will find them interesting and stimulating. They often show more sophisticated reasoning when working in familiar contexts, and we can build on their knowledge from these contexts as we explore new material (Ambrose et al. 2010). Some curiosities about the techniques under study and the physical phenomena behind them such as *why is the sky blue?*, *why are clouds white?,* or *why are sunsets orange or red?* conquer the interest and attention of learners and provide good opportunities to talk about science (Santos and Castanho 1996).

In this regard, we noticed that communicating that this practical experience is based on our own research is inspiring for students, who were eager to learn more about the systems we work with. By the end of the module, it is common for graduate students to be interested in applying this technique to their own work. When asked for their opinions on the practice, students produced constructive feedback. For example, they were positive about the data analysis session: *"I found it useful that data processing was reviewed after the class since many times during the main lecture one does not fully understand (or even ask oneself) how to calculate a certain parameter. In addition, it works as a reinforcement to grasp some difficult concepts".* Additional comments stated that *"Those practicals* [throughout the course] *that included questionnaires that were commented by the professors have been very useful to assimilate basic concepts. They should be implemented for all modules, time permitting.", "I suggest that some activities with questions and interpretation of results be implemented during the practicals and recitations of the other topics. In this way, one can identify if something was not well understood from the explanation, and if one can really interpret results from graphs.",* and *"I would've liked to have had more practice analysing figures from real articles."* Besides, our observations indicate that students enjoyed learning about the LS phenomenon because of its accuracy and simplicity. For instance, one student remarked: "*It was a very interesting lab and theoretical lesson. I was interested in learning how to experimentally obtain the MW and Rh by SLS and DLS, although I was aware that it could be difficult to apply the concepts without previous experience in the subject, as was my case. However, I found the module extremely useful."*

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Limitations and future research directions

The main purpose of this study was to assess the effectiveness of an active learning activity in enhancing students' comprehension of protein mass and size characterization. Through the evaluation of outcomes, we aim to gain valuable insights into the efficacy of this instructional strategy. These insights will be instrumental in shaping our future teaching practices, with the ultimate goal of facilitating conceptual change among students. However, it is essential to acknowledge the potential challenges and limitations associated with evaluating the strategy's effectiveness. Factors such as variations in individual learning styles, the duration of the intervention, and external influences should be considered when interpreting the results. Note that the assessment of the implementation of the activity was performed in four offerings of a low-enrolment course. Therefore, the size of the sample may affect the generalizability of results. Nevertheless, this initial experience lays the foundation for conducting a larger-scale study and demonstrates that using engaging examples makes the activity valuable to students.

While self-efficacy measurements are commonly used in educational research to assess students' beliefs in their own capacities to understand or perform specific tasks, it's important to recognize their inherent bias and limitations. For example, people may underestimate or overestimate their abilities in response to external or internal factors (Müller-Pinzler et al. 2019). Indeed, here we presented results that show that students' beliefs in their knowledge of an inexistent parameter notoriously increased after the module. This observation highlights the need for caution when analysing self-efficacy studies to avoid drawing far-fetched conclusions. However, this does not hinder the usefulness of this type of study. First, it is unlikely that students will answer in the same way when reporting self-efficacy regarding familiar concepts; when faced with completely unknown concepts (real or not), people lack a reference point to estimate their ability to work with them. Second, self-efficacy gains applicability when combined with actual knowledge data, for it can be used to assess correlations between self-perception and actual knowledge, as well as how self-calibration evolves as students learn more about a topic (Osterhage et al. 2019). Nonetheless, our results emphasize the importance of considering the potential impact of self-report measures on the validity of the study as they are susceptible to various forms of bias that may affect the findings' accuracy. Incorporating control groups or conducting comparative analyses can contribute to a more comprehensive evaluation of the strategy's efficacy. Furthermore, a follow-up study to assess the long-term

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retention of the acquired knowledge and the enduring impact of the approach over time would yield valuable insights.

Also, regarding the assessment of the nonsense parameter *Fa*, the increase in "*I have heard about this parameter*" responses after the module may be due to students having been exposed to the term in the pre-class survey, rather than genuinely feeling they learnt about it during the activity. However, this pre-exposure to the term does not explain why some students claimed proficiency in calculating the fabricated parameter. To gain a deeper understanding of this matter and mitigate potential confounding factors, future research could explore alternative approaches. For instance, a study might include a control group that does not receive the activity, allowing for results comparison. Another approach could involve introducing two different invented concepts instead of repeating the same one in both the pre- and post-class surveys. These methods may provide further insights into the impact of such activities on students' responses and perceptions.

The intervention presented here showed to facilitate the understanding of less challenging ideas like MW, Rh and oligomeric state. However, less straightforward concepts such as *D* and A_2 require more scaffolding to grasp fully. It's important to note that these less obvious concepts are explicitly addressed in the lecture component of the module but are not directly covered in the practical activity. On the implementation level, this highlights the need for teachers to be mindful not to assume that pedagogical approaches will effortlessly connect well-known concepts to less familiar ones. Providing additional support and guidance may be necessary for the latter.

Exploring the impact of metacognitive strategies and metaconceptual awareness on the development and retention of conceptual knowledge is yet another intriguing avenue of investigation. Metacognitive strategies refer to how individuals manage their own cognitive processes, including aspects of learning, thinking, and problem-solving. Conversely, metaconceptual awareness pertains to an individual's comprehension and awareness of the underlying concepts within a particular subject area, involving the ability to reflect on and monitor their own understanding of these concepts. Promoting metaconceptual awareness often involves the use of metacognitive strategies, such as self-questioning, self-monitoring, and self-evaluation. Awareness of one's own thought and learning processes allows individuals to better assimilate new information into their existing knowledge structures. Similarly, awareness of the fundamental concepts and ideas in a subject area enables individuals to learn and apply new knowledge more effectively. Although our research primarily focused on the development of conceptual

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knowledge, it is plausible that it also influenced the development of metaconceptual awareness; assessing this would require specific assessment. Indeed, this study could have benefitted from a more comprehensive investigation of the pre-existing concepts that students brought to the activity, and how these concepts were impacted by the activity. Further research is warranted to delve this aspect of learning in greater depth.

IMPLICATIONS

The present study explored the effects of integrating an inquiry-driven learning approach with a cognitive conflict activity, in which students must experience a conceptual change in order to address an otherwise unsolvable experimental problem. The comparison of pre- and post-class surveys revealed that the intervention resulted in a significant acquisition of scientific notions and the correction of a variety of misconceptions that are commonly fixed due to exposure to simplified explanations in introductory courses. These results also highlight how *conflicting* experimental outcomes can prompt conceptual change, helping students discerning their misconceptions from scientifically accepted concepts. For chemistry educators, this underscores the value of constructivism as a solid theoretical foundation for science pedagogy.

Regarding self-efficacy, an important insight is that it becomes a more reliable indicator of knowledge and understanding after the class. However, as observed with the fictitious *Fa* factor, self-efficacy questions have limitations in predicting students' understanding. Yet, they offer valuable information when combined with actual knowledge assessment data.

Student-centred methods, which empower learners to construct their own knowledge, demand more effort than teacher-centred approaches. Therefore, it is important to provide learners with familiar and relevant contexts (Jong 2008). Considering that students may achieve varying levels of conceptual change when confronted with challenging experiences, the positive outcomes indicate that the context was well-suited and made the lessons more relevant to them. We hope that our results will encourage teachers to align their laboratory practicals with our learning objectives, which aim to foster integrative and critical thinking. This approach is based on the desire to facilitate conceptual change, enabling students to incorporate research skills and conceptual knowledge.

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CONCLUSION

In this paper, we presented a challenging yet realistic and cost-effective laboratory exercise. It enables students to gain a comprehensive understanding of how some of the most employed techniques for protein characterization function, along with the potential challenges encountered when working with real samples. Based on results obtained from pre- and post-class surveys and laboratory reports, we can conclude that our students have acquired significant knowledge related to SEC, DLS and SLS, and have met the established learning objectives. Compared to past experiences when the practical component involved a simple demonstration using a prototypical protein, our students displayed higher levels of engagement, actual knowledge acquisition, and competency mastery. This activity, which transfers actual research into an educational experience, provides notions that are conceivable and profitable, thus leading to successful conceptual changes. We crave that others who teach and design biological chemistry laboratory courses will find this work useful and inspiring.

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Electronic supplementary information for

A problem-based learning activity for enhancing inquiry skills and facilitating conceptual change in a biological chemistry course

Wanda M. Valsecchi, José M. Delfino, Javier Santos, Santiago E. Faraj

This file contains the following materials:

- ***Supplementary figures*** with additional result analysis.
- The ***Assessment instrument*** used for pre- and post-questionnaire.
- The ***Laboratory handout*** used by students to carry out experiments and with instructions on how to produce their lab reports, and the assessment ***Rubric*** used to grade lab reports.

Data sets used to illustrate results throughout this paper, which can be used in a data-analysis exercise, can be found in the public repository https://github.com/wanvalsecchi/Data_Set_SEC_LS.git

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SUPPLEMENTARY FIGURES

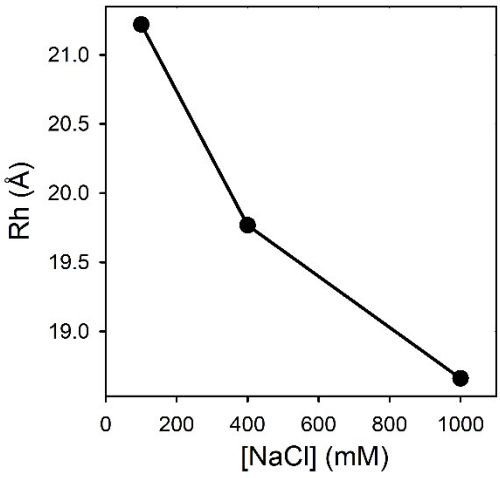


Figure S1. Apparent hydrodynamic behavior of FXN. The Rh of FXN was calculated from SEC data obtained at different NaCl concentrations. Experiments were carried out at room temperature using a Superose 12 prepacked column equilibrated with buffer 20 mM Tris·HCl, pH 7.0, 1 mM EDTA, and varying concentrations of NaCl.

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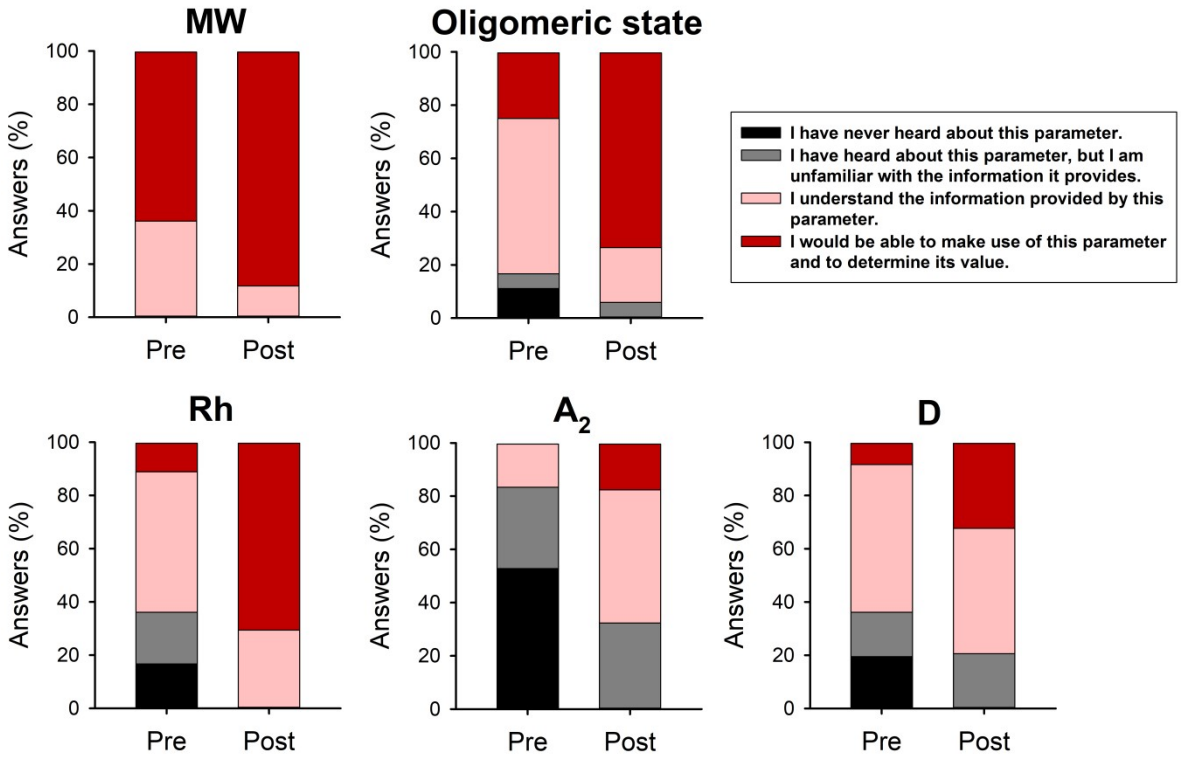


Figure S2. Self-perception. Students were asked to report on their perceived understanding of different parameters discussed throughout the module. This questionnaire was presented as a multiple-choice survey and was answered by 36 respondents in pre-test and 34 respondents post-tests.

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Assessment instrument

(Please note that the actual questionnaire was administered in Spanish)

DETERMINATION OF PROTEIN MOLECULAR WEIGHT AND SIZE

A1) Choose the option that better describes your understanding of each of the following techniques: analytical size exclusion chromatography (SEC), static light scattering (SLS) and dynamic light scattering (DLS).

	SEC	SLS	DLS
I understand the theory behind the technique and would be able to make experimental use of it.	☐	☐	☐
I understand the theory behind the technique as well as how it is experimentally applied.	☐	☐	☐
I understand the theory behind the technique, but I am uncertain about how it is experimentally applied.	☐	☐	☐
I have heard about the technique, but I am uncertain about the theory behind the methodology or how it is experimentally applied.	☐	☐	☐
I have never heard about this technique.	☐	☐	☐

A2) Choose the option (1-4) that you feel best describes your situation with respect to each of the following parameters of a protein.

Molecular weight (MW) _____

Hydrodynamic radius (Rh) _____

Second Virial Coefficient (A_2) _____

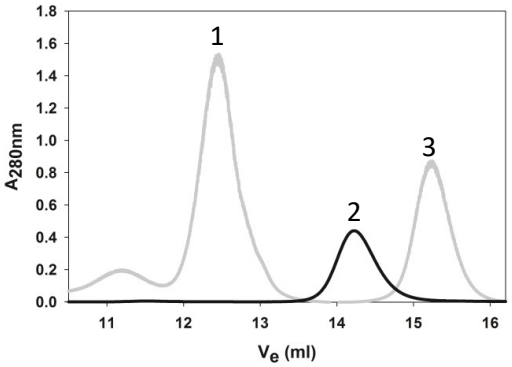
Avece factor (Fa) _____

Diffusional coefficient (D) _____

Oligomeric state _____

1. I would be able to make use of this parameter and to determine its value
2. I understand the information provided by this parameter
3. I have heard about this parameter, but I am unfamiliar with the information it provides
4. I have never heard about this parameter

Q1) The following figure shows the SEC profiles of proteins A (100kDa), B (66kDa) and C (14kDa).



Peak assignment would be:

☐ 1-A, 2-B, 3-C

☐ 1-C, 2-B, 3-A

☐ 1-A, 2-C, 3-B

☐ The provided information is not sufficient to make a decision.

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Q2) The molecular weight of a protein was determined by SEC and SLS. Results were the following:

SEC: 25.8 kDa

SLS: 105.3 kDa

The molecular weight of a monomer calculated from the amino acid sequence is 26.6 kDa.

Which is the oligomeric state of the protein?

……

Q3) The hydrodynamic radius (Rh) of a protein was determined by SEC and DLS. Results were the following:

SEC: 23.3 Å

DLS: 40.1 Å

The Rh was estimated at 39.9 Å using an empirical correlation between the Rh and the molecular weight.

How does the actual degree of compaction of the protein compare with the expected one?

☐ It is more compact than a typical globular protein ☐ It is as compact as a typical globular protein ☐ It is less compact than a typical globular protein

Q4) State whether the following statements are true or false:

_____ *Given a mixture of proteins of different sizes, a SEC column attached to a UV detector would always allow obtaining the MW of each of them.*

_____ *A light scattering device (685 nm laser) with a single 90° detector may be adequate to obtain the MW of a monodisperse solution of a polymer with a mean radius of 15 nm.*

_____ *In order to obtain the MW of a solution of a small, pure protein, a light scattering device (685 nm laser) with a single 90° detector (online with a SEC column) would be a convenient choice.*

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Determination of protein molecular weight and size

Laboratory handout

In this practical we will apply size exclusion chromatography, static light scattering (SLS) and dynamic light scattering (DLS) to obtain the molecular weight (MW) and the hydrodynamic or Stokes radius (Rh) of a set of proteins.

Laboratory guide

MW and Rh determination will be performed by size exclusion chromatography (SEC) and light scattering (LS) in a continuous flow mode. Our system consists of a set of devices arranged in tandem as outlined in Fig. 1, which allows the simultaneous determination of the MW and the value of the diffusional coefficient (D). The samples will be thoroughly centrifuged, loaded into a 100 µl injector loop and filtered through a Sepharose 12 SEC column. The flow rate of the buffer (20 mM Tris-HCl, 100 mM NaCl, pH 7.4) will be set constant at 0.3 ml min^{-1}. As shown in Figure 1, the eluate passes through the UV detector (*Jasco UV 2075 plus*), which allows determining the elution volume (Ve) of each peak, and through the multi-angle LS detector (*miniDAWN Tristar, Wyatt Technology*), which measures the amount of light scattered to calculate the absolute molar mass of the sample by SLS. An optical fiber receiver is mounted in the 90° detector and coupled to the DLS module for the determination of D and posterior calculation of Rh (*WyattQELS, Wyatt Technology*). Collected data is transferred to a computer where it is processed with ASTRA software (*Wyatt Technology*).

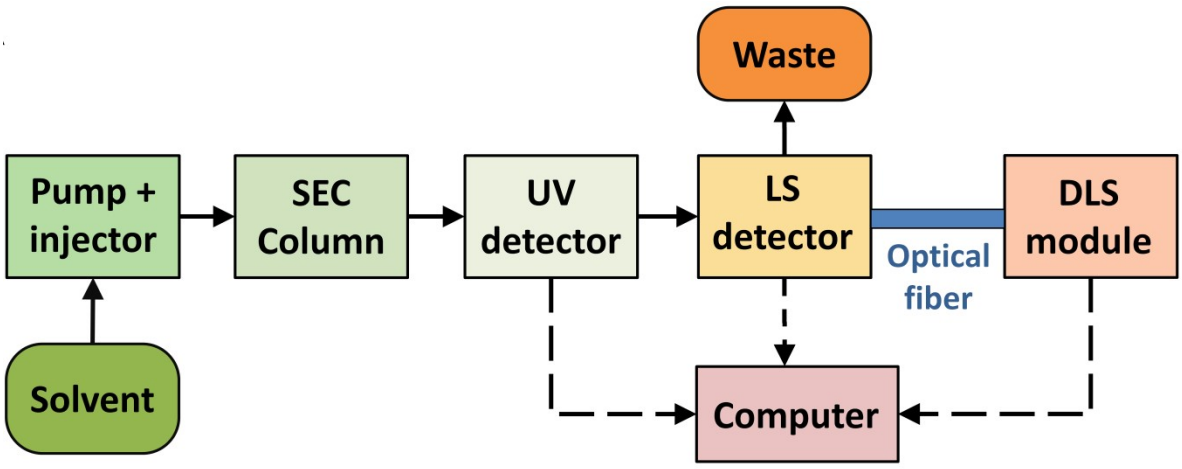


Fig. 1

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We will perform three chromatographic runs during this practical:

Sample A: Molecular mass standards that will be used to calibrate the SEC column:

- Thyroglobulin (bovine) 670 kDa
- γ-globulin (bovine) 158 kDa
- Ovalbumin (chicken) 44 kDa
- Myoglobin (horse) 17 kDa
- Vitamin B12 1.35 kDa

Sample B: A mixture of bovine serum Albumin (BSA) and human Frataxin (FXN):

- BSA 66 kDa *
- FXN 14 Da *

Sample C: A solution of Hypoxanthine phosphoribosyl transferase (HPRT) from *T. cruzi*:

- HPRT 27 kDa *

*The molecular weights shown are those of a monomer of the protein, calculated using the amino acid sequence.

Data from experiments will be exported as spreadsheet files.

Data analysis

Open the spreadsheet file. You will find three sheets, one for each run. Identify the columns within each spreadsheet:

- Column A is the independent variable **time**, expressed in minutes.
- Column B is the **absorbance at 280 nm** of the SEC column eluate collected by the UV detector.
- Column C is the **MW** obtained using data collected by the LS detector, expressed in Daltons.
- Column D is the **Rh**, calculated using the diffusional coefficient value (D) obtained by the DLS module, expressed in nanometers.

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Part 1: MW and Rh determination by SEC

First, we will calculate the MW and Rh using only elution volumes of the different species.

Use data obtained for **Sample A** (MW standards).

1. Using the buffer flow rate, calculate the Ve for each time. Plot the chromatogram using Ve as the independent variable: absorbance (in ordinate y axis) vs. Ve (in abscissa x axis).
2. Identify the peaks on the chromatogram and assign them to the different species present in the mixture of MW standards. Note that the components of the mixture elute according to their MW.
3. Make note of the Ve of the maximum absorbance signal for each of the peaks.
4. Plot Log(MW) vs. Ve for each MW standard and find the best-fitting line to the data. This will be the characteristic equation of the system in our working conditions.

Use UV absorption data (column B) obtained for **Samples B and C**.

5. Using the buffer flow rate, calculate the Ve for each time. Plot the chromatograms using Ve as the independent variable (absorbance vs. Ve).
6. Identify the peaks on the chromatograms and find the Ve that yielded the maximal absorbance signal for each of them.
7. Using the equation of the best-fitting line calculate the MW of each of the peaks.
8. In the work by Uversky (1993)*, identify the equation that correlates Rh with MW for proteins in native conformation, and use it to estimate the Rh of each species from the value of MW obtained for each peak (assume that each peak corresponds to a single species). Use MW values in Da (not kDa).

Part 2: MW and Rh determination by light scattering

Using data collected by LS and UV detectors, we can obtain the MW of polymers that elute from the size exclusion column (SLS). Additionally, the DLS module allows obtaining the value of the diffusion coefficient (D), which is used to calculate the Rh value. Since we performed an on-line experiment (flow mode), components present in the sample are fractionated in the column and reach the light scattering detector cell at different times. In this fashion, many light scattering data are obtained for each peak. We will use the MW and Rh data from **Samples B and C** (columns C and D in the spreadsheets).

9. Calculate the mean value and standard deviation of the MW and Rh for each peak.

* Uversky VN, Use of fast protein size-exclusion liquid chromatography to study the unfolding of proteins which denature through the molten globule (1993), *Biochemistry* 32(48):13288-98.

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Laboratory report *[structured questionnaire – new format only]*

Follow the instructions in the *Data analysis* section. Then answer the following questions.

The chromatograms obtained for **Samples B and C** are shown in Fig. 2. *Note that absorbance values might not exactly correspond to those you obtained.*

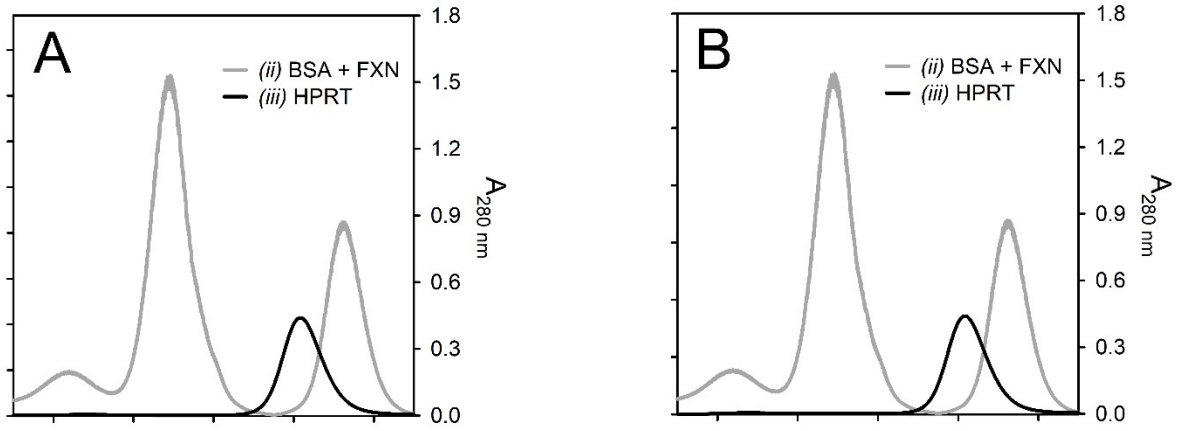


Fig. 2

1 Using the left (primary) axes, plot **MW values obtained by SLS in Fig. 2A** and **Rh values obtained by DLS in Fig. 2B.** Suggest suitable labels and units for the graph axes when appropriate.

2 Identify which peaks correspond to each protein, **BSA, HPRT and FXN**, and clearly label them on Fig. 2.

3 Complete the missing spaces in Table 1 with the MW values obtained by SEC and Rh values calculated using the equation that describes the dependence of Rh with the MW for proteins in their native conformation proposed by Uversky (1993):

$$\log(Rh) = -(0.254 \pm 0.002) + (0.369 \pm 0.001)\log(MW) \qquad \textbf{Eq.1}$$

Bear in mind that you should use MW values in Da (not kDa) when using this equation.

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Table 1. MW values obtained by SEC and Rh values calculated using an empirical correlation for each peak obtained for Samples B and C.

Sample	Peak (left to right)	MW obtained by SEC (kDa)	Calculated Rh (Å)
B	1	……	……
B	2	……	……
B	3	……	……
C	1	……	……

Table 2 shows sequence-based MW values for several possible species of the proteins under study.

Table 2. Calculated MW and Rh values obtained for several species of BSA, FXN and HPRT.

Protein		Sequence-based MW (Da)	Calculated Rh (Å)
BSA	Monomer	66 300	……
	Dimer	132 600	……
	Trimer	198 900	……
FXN	Monomer	13 700	……
	Dimer	27 400	……
HPRT	Monomer	26 600	……
	Dimer	53 200	……
	Tetramer	106 400	……

4 Complete the missing spaces in Table 2 with the Rh values calculated using **Eq. 1**.

5 Using information in Tables 1 and 2, and considering that FXN does not interact with BSA, suggest the most probable scenario for species (protein and oligomeric state) present in each peak.

Sample B, peak 1:……

Sample B, peak 2:……

Sample B, peak 3:……

Sample C, peak 1:……

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6 Complete the missing spaces in Table 3 with MW values obtained by SLS and Rh values obtained by DLS.

Table 3. MW values obtained by SEC and Rh values calculated using an empirical correlation for each peak obtained for Samples B and C.

Sample	Peak (left to right)	MW obtained by SLS (kDa)	Rh obtained by DLS (Å)
B	1	.....	.....
B	2	.....	.....
B	3	.....	.....
C	1	.....	.....

7 Using information in Tables 2 and 3, and considering that FXN does not interact with BSA, suggest the most probable species (protein and oligomeric state) present in each peak.

Sample B, peak 1:.....

Sample B, peak 2:.....

Sample B, peak 3:.....

Sample C, peak 1:.....

8 Compare results obtained by SEC, SLS and DLS (use the mean ± SD as a comparison criterion) and suggest possible reasons for any discrepancy you find. In view of all the information you have analyzed, suggest the most probable species (protein and oligomeric state) in each peak and their MW and Rh value. Explain your answer in full.

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Sections	Relevant information	Inadequate = 1	Adequate = 2	Good = 3	Very good = 4	Excellent = 5
Data processing	Figures: chromatograms Abs. vs Ve for MW markers; calibration curve; LS results; DLS results. Tabulated information: MW and Rh values calculated by sequence; SEC; and LS.	Most of the figures and tabulated information is missing. Figures or tables contain unclear information. *Orig. Format: figures have no or inadequate title or legend. Axes are not named or lack labels.	Some of the figures or tabulated information is missing or contains unclear information. *Orig. Format: figures have no or inadequate title or legend. Axes are not named or lack labels.	All figures are presented. Some of the tabulated information is missing or contains unclear information. *Orig. Format: figures have no or inadequate title or legend. Axes are not named or lack labels.	Most the required information is included. There is still room for improvement. *Orig. Format: figures have adequate titles and legends. Axes are named and correctly labeled.	All the required information is included in an effective way to convey results. *Orig. Format: figures have adequate titles and legends. Axes are named and correctly labeled.
Results analysis	Identification of peaks; Description of BSA, HPRT and FXN behaviors; Comparison of SEC with sequence-based values; Comparison of LS with sequence-based values; Comparison between SEC and LS results.	Most components are missing.	Some components are missing. Topics are weakly mentioned: e.g., no comparison is done. Some values are wrongly calculated.	Some of the components are missing. Topics are weakly mentioned: e.g., when contradiction between SEC and LS, the student ignores it or makes no effort for explaining it. Most values are correctly calculated.	Most components are analyzed, and all or most values are well calculated. Good general analysis but there is still room for improvement.	All components are very well analyzed. In some cases, values can be wrongly calculated or analyzed, but this is compensated with a very good general analysis and conclusions.
Conclusions	HPRT and FXN behave anomalously; Compaction hypothesis; Matrix interaction hypothesis.	Poorly organized. Reviewed of analysis but not proper discussion. Most key items are missing.	Conclusions are too brief. Student states MW and Rh by SEC without taking into account LS results. No hypothesis is proposed to explain apparently contradictory data. No discussion exists on Rh results.	Conclusions are brief. Student hypothesizes that FXN and HPRT behave anomalously, but does not propose any hypothesis. MW analysis is performed, but a discussion on Rh results is missing.	Conclusions are brief. Student finds discrepancies between SEC and SLS MW data and proposes some hypotheses. The Rh discussion is vague only about SEC, and does not compare it with LS.	Excellent conclusions. Student finds discrepancies between SEC and SLS MW data and proposes all or most of the expected hypotheses. The Rh discussion is complete, comparing SEC with LS in full.

*Requirements considered in the original format.

Letter grade	Criteria
A	Earned 5 in all sections.
AB	Earned at least 4 in *Data processing* AND *Results analysis*, AND 5 in *Conclusions.*
B	Earned at least 4 in all sections.
BC	Earned at least 3 in *Data processing* AND *Results analysis*, AND at least 4 in *Conclusions.*
C	Earned at least 3 in all sections.
D	Earned at least 2 in all sections.
E	Does not meet the minimum criteria for grade D

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Electronic supplementary information for

A problem-based learning activity for enhancing inquiry skills and facilitating conceptual change in a biological chemistry course

Wanda M. Valsecchi, José M. Delfino, Javier Santos, Santiago E. Faraj

This file contains the following materials:

- ***Supplementary figures*** with additional result analysis.
- The ***Assessment instrument*** used for pre- and post-questionnaire.
- The ***Laboratory handout*** used by students to carry out experiments and with instructions on how to produce their lab reports, and the assessment ***Rubric*** used to grade lab reports.

Data sets used to illustrate results throughout this paper, which can be used in a data-analysis exercise, can be found in the public repository https://github.com/wanvalsecchi/Data_Set_SEC_LS.git

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SUPPLEMENTARY FIGURES



Figure S1. Apparent hydrodynamic behavior of FXN. The Rh of FXN was calculated from SEC data obtained at different NaCl concentrations. Experiments were carried out at room temperature using a Superose 12 prepacked column equilibrated with buffer 20 mM Tris·HCl, pH 7.0, 1 mM EDTA, and varying concentrations of NaCl.

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Figure S2. Self-perception. Students were asked to report on their perceived understanding of different parameters discussed throughout the module. This questionnaire was presented as a multiple-choice survey and was answered by 36 respondents in pre-test and 34 respondents post-tests.

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Assessment instrument

(Please note that the actual questionnaire was administered in Spanish)

DETERMINATION OF PROTEIN MOLECULAR WEIGHT AND SIZE

A1) Choose the option that better describes your understanding of each of the following techniques: analytical size exclusion chromatography (SEC), static light scattering (SLS) and dynamic light scattering (DLS).

	SEC	SLS	DLS
I understand the theory behind the technique and would be able to make experimental use of it.	☐	☐	☐
I understand the theory behind the technique as well as how it is experimentally applied.	☐	☐	☐
I understand the theory behind the technique, but I am uncertain about how it is experimentally applied.	☐	☐	☐
I have heard about the technique, but I am uncertain about the theory behind the methodology or how it is experimentally applied.	☐	☐	☐
I have never heard about this technique.	☐	☐	☐

A2) Choose the option (1-4) that you feel best describes your situation with respect to each of the following parameters of a protein.

Molecular weight (MW) _____

Hydrodynamic radius (Rh) _____

Second Virial Coefficient (A_2) _____

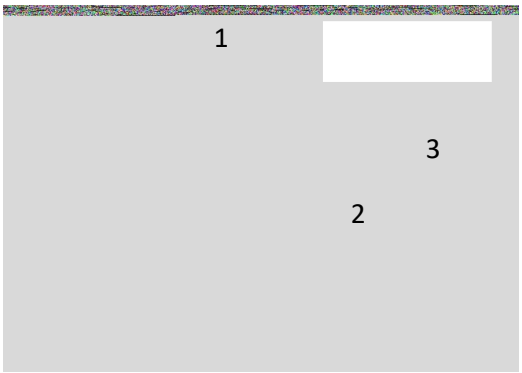
Avece factor (Fa) _____

Diffusional coefficient (D) _____

Oligomeric state _____

1. I would be able to make use of this parameter and to determine its value
2. I understand the information provided by this parameter
3. I have heard about this parameter, but I am unfamiliar with the information it provides
4. I have never heard about this parameter

Q1) The following figure shows the SEC profiles of proteins A (100kDa), B (66kDa) and C (14kDa).



Peak assignment would be:

☐ 1-A, 2-B, 3-C

☐ 1-C, 2-B, 3-A

☐ 1-A, 2-C, 3-B

☐ The provided information is not sufficient to make a decision.

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Q2) The molecular weight of a protein was determined by SEC and SLS. Results were the following:

SEC: 25.8 kDa

SLS: 105.3 kDa

The molecular weight of a monomer calculated from the amino acid sequence is 26.6 kDa.

Which is the oligomeric state of the protein?

……

Q3) The hydrodynamic radius (Rh) of a protein was determined by SEC and DLS. Results were the following:

SEC: 23.3 Å

DLS: 40.1 Å

The Rh was estimated at 39.9 Å using an empirical correlation between the Rh and the molecular weight.

How does the actual degree of compaction of the protein compare with the expected one?

☐ It is more compact than a typical globular protein ☐ It is as compact as a typical globular protein ☐ It is less compact than a typical globular protein

Q4) State whether the following statements are true or false:

_____ *Given a mixture of proteins of different sizes, a SEC column attached to a UV detector would always allow obtaining the MW of each of them.*

_____ *A light scattering device (685 nm laser) with a single 90° detector may be adequate to obtain the MW of a monodisperse solution of a polymer with a mean radius of 15 nm.*

_____ *In order to obtain the MW of a solution of a small, pure protein, a light scattering device (685 nm laser) with a single 90° detector (online with a SEC column) would be a convenient choice.*

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Determination of protein molecular weight and size

Laboratory handout

In this practical we will apply size exclusion chromatography, static light scattering (SLS) and dynamic light scattering (DLS) to obtain the molecular weight (MW) and the hydrodynamic or Stokes radius (Rh) of a set of proteins.

Laboratory guide

MW and Rh determination will be performed by size exclusion chromatography (SEC) and light scattering (LS) in a continuous flow mode. Our system consists of a set of devices arranged in tandem as outlined in Fig. 1, which allows the simultaneous determination of the MW and the value of the diffusional coefficient (D). The samples will be thoroughly centrifuged, loaded into a 100 µl injector loop and filtered through a Sepharose 12 SEC column. The flow rate of the buffer (20 mM Tris-HCl, 100 mM NaCl, pH 7.4) will be set constant at 0.3 ml min^{-1}. As shown in Figure 1, the eluate passes through the UV detector (*Jasco UV 2075 plus*), which allows determining the elution volume (Ve) of each peak, and through the multi-angle LS detector (*miniDAWN Tristar, Wyatt Technology*), which measures the amount of light scattered to calculate the absolute molar mass of the sample by SLS. An optical fiber receiver is mounted in the 90° detector and coupled to the DLS module for the determination of D and posterior calculation of Rh (*WyattQELS, Wyatt Technology*). Collected data is transferred to a computer where it is processed with ASTRA software (*Wyatt Technology*).



Fig. 1

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We will perform three chromatographic runs during this practical:

Sample A: Molecular mass standards that will be used to calibrate the SEC column:

- Thyroglobulin (bovine) 670 kDa
- γ-globulin (bovine) 158 kDa
- Ovalbumin (chicken) 44 kDa
- Myoglobin (horse) 17 kDa
- Vitamin B12 1.35 kDa

Sample B: A mixture of bovine serum Albumin (BSA) and human Frataxin (FXN):

- BSA 66 kDa *
- FXN 14 Da *

Sample C: A solution of Hypoxanthine phosphoribosyl transferase (HPRT) from *T. cruzi*:

- HPRT 27 kDa *

*The molecular weights shown are those of a monomer of the protein, calculated using the amino acid sequence.

Data from experiments will be exported as spreadsheet files.

Data analysis

Open the spreadsheet file. You will find three sheets, one for each run. Identify the columns within each spreadsheet:

- Column A is the independent variable **time**, expressed in minutes.
- Column B is the **absorbance at 280 nm** of the SEC column eluate collected by the UV detector.
- Column C is the **MW** obtained using data collected by the LS detector, expressed in Daltons.
- Column D is the **Rh**, calculated using the diffusional coefficient value (D) obtained by the DLS module, expressed in nanometers.

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Part 1: MW and Rh determination by SEC

First, we will calculate the MW and Rh using only elution volumes of the different species.

Use data obtained for **Sample A** (MW standards).

1. Using the buffer flow rate, calculate the Ve for each time. Plot the chromatogram using Ve as the independent variable: absorbance (in ordinate y axis) vs. Ve (in abscissa x axis).
2. Identify the peaks on the chromatogram and assign them to the different species present in the mixture of MW standards. Note that the components of the mixture elute according to their MW.
3. Make note of the Ve of the maximum absorbance signal for each of the peaks.
4. Plot Log(MW) vs. Ve for each MW standard and find the best-fitting line to the data. This will be the characteristic equation of the system in our working conditions.

Use UV absorption data (column B) obtained for **Samples B and C**.

5. Using the buffer flow rate, calculate the Ve for each time. Plot the chromatograms using Ve as the independent variable (absorbance vs. Ve).
6. Identify the peaks on the chromatograms and find the Ve that yielded the maximal absorbance signal for each of them.
7. Using the equation of the best-fitting line calculate the MW of each of the peaks.
8. In the work by Uversky (1993)*, identify the equation that correlates Rh with MW for proteins in native conformation, and use it to estimate the Rh of each species from the value of MW obtained for each peak (assume that each peak corresponds to a single species). Use MW values in Da (not kDa).

Part 2: MW and Rh determination by light scattering

Using data collected by LS and UV detectors, we can obtain the MW of polymers that elute from the size exclusion column (SLS). Additionally, the DLS module allows obtaining the value of the diffusion coefficient (D), which is used to calculate the Rh value. Since we performed an on-line experiment (flow mode), components present in the sample are fractionated in the column and reach the light scattering detector cell at different times. In this fashion, many light scattering data are obtained for each peak. We will use the MW and Rh data from **Samples B and C** (columns C and D in the spreadsheets).

9. Calculate the mean value and standard deviation of the MW and Rh for each peak.

* Uversky VN, Use of fast protein size-exclusion liquid chromatography to study the unfolding of proteins which denature through the molten globule (1993), *Biochemistry* 32(48):13288-98.

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Laboratory report *[structured questionnaire – new format only]*

Follow the instructions in the *Data analysis* section. Then answer the following questions.

The chromatograms obtained for **Samples B and C** are shown in Fig. 2. *Note that absorbance values might not exactly correspond to those you obtained.*

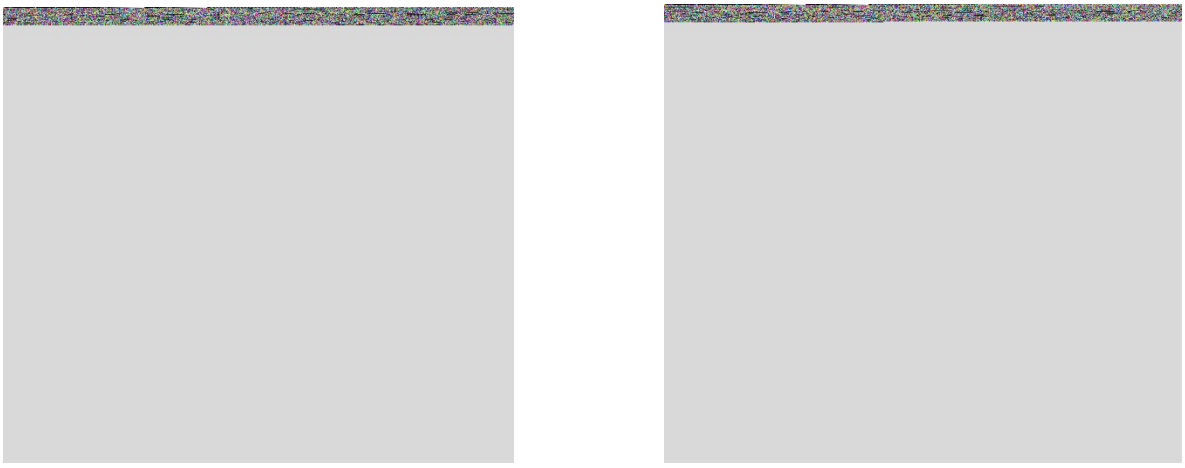


Fig. 2

1 Using the left (primary) axes, plot **MW values obtained by SLS in Fig. 2A** and **Rh values obtained by DLS in Fig. 2B.** Suggest suitable labels and units for the graph axes when appropriate.

2 Identify which peaks correspond to each protein, **BSA, HPRT and FXN**, and clearly label them on Fig. 2.

3 Complete the missing spaces in Table 1 with the MW values obtained by SEC and Rh values calculated using the equation that describes the dependence of Rh with the MW for proteins in their native conformation proposed by Uversky (1993):

$$\log(Rh) = -(0.254 \pm 0.002) + (0.369 \pm 0.001)\log(MW) \qquad \textbf{Eq.1}$$

Bear in mind that you should use MW values in Da (not kDa) when using this equation.

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Table 1. MW values obtained by SEC and Rh values calculated using an empirical correlation for each peak obtained for Samples B and C.

Sample	Peak (left to right)	MW obtained by SEC (kDa)	Calculated Rh (Å)
B	1	……	……
B	2	……	……
B	3	……	……
C	1	……	……

Table 2 shows sequence-based MW values for several possible species of the proteins under study.

Table 2. Calculated MW and Rh values obtained for several species of BSA, FXN and HPRT.

Protein		Sequence-based MW (Da)	Calculated Rh (Å)
BSA	Monomer	66 300	……
	Dimer	132 600	……
	Trimer	198 900	……
FXN	Monomer	13 700	……
	Dimer	27 400	……
HPRT	Monomer	26 600	……
	Dimer	53 200	……
	Tetramer	106 400	……

4 Complete the missing spaces in Table 2 with the Rh values calculated using **Eq. 1**.

5 Using information in Tables 1 and 2, and considering that FXN does not interact with BSA, suggest the most probable scenario for species (protein and oligomeric state) present in each peak.

Sample B, peak 1:……

Sample B, peak 2:……

Sample B, peak 3:……

Sample C, peak 1:……

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6 Complete the missing spaces in Table 3 with MW values obtained by SLS and Rh values obtained by DLS.

Table 3. MW values obtained by SEC and Rh values calculated using an empirical correlation for each peak obtained for Samples B and C.

Sample	Peak (left to right)	MW obtained by SLS (kDa)	Rh obtained by DLS (Å)
B	1	.....	.....
B	2	.....	.....
B	3	.....	.....
C	1	.....	.....

7 Using information in Tables 2 and 3, and considering that FXN does not interact with BSA, suggest the most probable species (protein and oligomeric state) present in each peak.

Sample B, peak 1:.....

Sample B, peak 2:.....

Sample B, peak 3:.....

Sample C, peak 1:.....

8 Compare results obtained by SEC, SLS and DLS (use the mean ± SD as a comparison criterion) and suggest possible reasons for any discrepancy you find. In view of all the information you have analyzed, suggest the most probable species (protein and oligomeric state) in each peak and their MW and Rh value. Explain your answer in full.

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Conclusions	HPRT and FXN behave anomalously; Compaction hypothesis; Matrix interaction hypothesis.	Poorly organized. Reviewed of analysis but not proper discussion. Most key items are missing.	Conclusions are too brief. Student states MW and Rh by SEC without taking into account LS results. No hypothesis is proposed to explain apparently contradictory data. No discussion exists on Rh results.	Conclusions are brief. Student hypothesizes that FXN and HPRT behave anomalously, but does not propose any hypothesis. MW analysis is performed, but a discussion on Rh results is missing.	Conclusions are brief. Student finds discrepancies between SEC and SLS MW data and proposes some hypotheses. The Rh discussion is vague only about SEC, and does not compare it with LS.	Excellent conclusions. Student finds discrepancies between SEC and SLS MW data and proposes all or most of the expected hypotheses. The Rh discussion is complete, comparing SEC with LS in full.

*Requirements considered in the original format.

Letter grade	Criteria
A	Earned 5 in all sections.
AB	Earned at least 4 in *Data processing* AND *Results analysis*, AND 5 in *Conclusions.*
B	Earned at least 4 in all sections.
BC	Earned at least 3 in *Data processing* AND *Results analysis*, AND at least 4 in *Conclusions.*
C	Earned at least 3 in all sections.
D	Earned at least 2 in all sections.
E	Does not meet the minimum criteria for grade D

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