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Laboratory Experiment

Egg with PEG—A Lysozyme Based Experiment to Teach Protein Modification and Gel Electrophoresis to Chemistry Undergraduates

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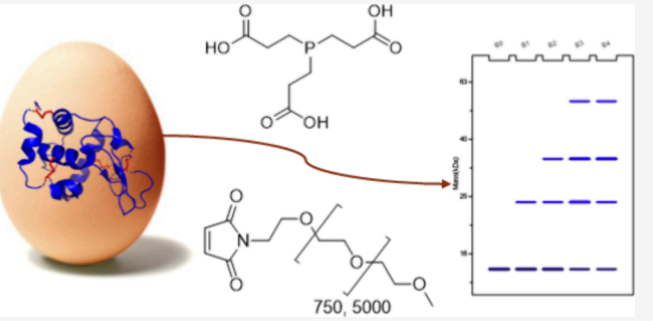
ABSTRACT: Part of the journey to excellence in any discipline is learning how to apply theory and understanding to a wide range of applications. In this article, we present a cross-disciplinary laboratory experiment designed to expose second year undergraduate chemists to the principles of protein modification and gel electrophoresis. This 6 h experiment, tested across two years, enlightens chemistry students to the working practices of biochemists/chemical biologists through the PEGylation of cysteine residues within lysozyme. PEG-maleimide in two different sizes is used to modify lysozyme following incremental reduction by tris(2-carboxyethyl) phosphine. Variation in reducing agent concentration, PEG chain length, and presence of denaturant in reaction buffer provided a range of interesting results and highlights several learning outcomes. The experiment design broadens and reinforces chemistry student's skillsets by discovering SDS-PAGE gel preparation and analysis, while relating other areas of theoretical and practical chemistry to a bioconjugation based study.



KEYWORDS: *Chemical Biology, Biochemistry, Interdisciplinary, Hands-On Learning, Laboratory Instruction, Second-Year undergraduate, Polymer Chemistry, Proteins*

INTRODUCTION

The University of Kent's School of Chemistry and Forensic Science, recruits a cohort of roughly 35 Chemistry students per year. A recent course-wide curriculum review required a total reassessment of our laboratory provision. Historically, our laboratory classes operated as traditional siloed blocks of; analytical, organic, inorganic, and physical chemistry, with most experiments fully guided in their design. One weakness in our historical course design was that the laboratory courses, in areas, struggled to nurture a student's ability to apply theory across subdisciplines at higher levels; not a unique observation.[1] Drawing on inspiration from others,[2] we have focused our laboratory course design on a multidisciplinary integrated and spiral approach.[3,4] Our new first year course (shared with forensic science students) assumes little practical knowledge and aims to reduce cognitive load by separating lecture content from the lab classes.[5] The redesign of our experimental content and course literature is now based upon an understanding that the first-year laboratory is primarily a place to focus on practical development.[6,7] Laboratories in the second year can then focus on the application of advanced combinations of skills and theory.

We wanted to give opportunities for our stage 2 chemistry students to be exposed to ways of thinking that were traditionally reserved for other practices. This report describes one of the methods we used to approach this goal; through the integration of a complex biochemistry/bioconjugation experiment, where much of the work is signposted to fundamental chemistry concepts to help drive engagement. This is now the first full day biochemistry experiment featured on our chemistry course. While there have been some examples of protein-based laboratory experiments presented in this journal,[8−11] and along a similar theme of PEGylation,[12] none were of suitable length, content, or difficulty for the constraints of our course structure and timetabling systems.

PEGylation is an important process for the development of small molecule[13] and protein based therapeutics.[14] It has been shown that the addition of a PEG group can increase solubility, reduce degradation, and increase the half-life of the molecule, all important factors in drug development. The hydrophilic nature of the PEG improves the water solubility of the PEG modified molecule, and the large PEG chains can block enzymes responsible for degradation. Common methodologies for protein PEGylation utilize modification of amino acid side chain functionality, such as primary amines of lysine and thiols

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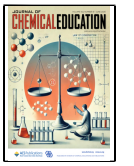

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of cysteine residues. A common post-translation modification observed with cysteine residues is the oxidative formation of disulfide bonds, a key process in oxidative protein folding and therefore understanding protein production within the cell. Due to the oxidation of cysteines to disulfides, the PEGylation of proteins utilizing cysteine thiol alkylation can require reduction of the protein prior to modification with the PEG group.

This experiment presents a method for PEGylating cysteine residues of lysozyme (hen egg-white lysozyme) with PEG-maleimides of two chain lengths. The experiment highlights the effect of denaturant (urea) on the efficiency of the PEGylation process, the role of protein size on SDS PAGE electrophoretic mobility, and the utilization of NMR to estimate the number-average molecular weight (M_n) of polymers. The experiment is designed for a 6-h second year undergraduate course (2 × 3 h. sessions) and highlights how biochemical processes are underpinned by a variety of subdivisions in chemistry. Whereas we have showcased this experiment across two years in a mixed topic chemistry laboratory module, it could be incorporated easily into a second-year undergraduate biochemistry course, or polymer chemistry course given the high loading of polymer, and macromolecular content. Table 1 lists the key techniques and theories explored during this experiment.

Table 1. Segments of This Experiment and Which Theoretical Concepts Are Addressed and Introduced by Carrying out Each Segment

Segment of Experiment	Theoretical Concepts Explored
Introduction	Protein structures, folding, activation and functionality.
Urea/Non-Urea Buffers	Denaturants and chaotropic agents. Buffering and buffer solutions.
Protein solution preparation	Beer−Lambert Law for concentration determination. UV−vis spectroscopy.
Preparation of SDS-PAGE gel	Free radical polymerization. Network copolymers, hydrogels and cross-linking.
Reduction of proteins	Main group reducing agents. Reduction of disulfides and protein unfolding. S_N2.
Addition of PEG-Mal to thiols	Bioconjugation and organic chemistry. Reactivity of cysteine residues to maleimides. 1,4-Conjugate addition.
NMR of PEG-Maleimide	Proton NMR structural assignment and end group analysis of polymers.
Running SDS-PAGE Gels	Electrophoresis. Size-exclusion principles. Mass standards and ladders.
Analysis of Gels	Structure/reactivity relationships of the protein. Staining and reading gels.

Lysozyme is a very common and relatively small enzymatic protein, with a molecular mass of 14.3 kDa. Lysozyme are found in high concentration in humans' secretions such as mucous and saliva,[15] alongside many other organisms; vertebrates, invertebrates, plants, and bacteria.[16] In this experiment we highlight to the students that the source of the lysozyme is from the egg whites of chicken (*G. g. domesticus*) eggs, where the protein makes up roughly 3.5% of the protein in the egg white. One key function of lysozyme is as a polysaccharide degrading antibacterial agent, facilitated via enzymatic cleavage of the $\beta(1-4)$-glycosidic bonds in the peptidoglycans of the bacterial cell wall.[17] Due to its innate antibacterial property, it has been thoroughly investigated as an alternative antibiotic.[15] Structurally, the chicken egg white lysozyme is composed of a single chain of 129 amino acids that adopts a tertiary structure composed of a three-stranded antiparallel β-sheet and a core of five α-helices. The structure is stabilized by 4 disulfide bridges and has no reduced cysteine residues.[18]

Lysozyme's four disulfide bonds in the native structure connect resides 6 with 127, 30 with 115, 64 with 80, and 76 with 94[19] (Figure 1A). Each of these bridges can be reduced

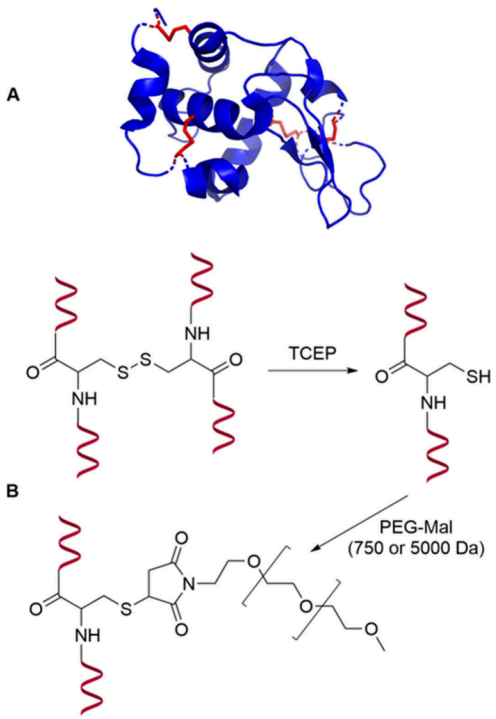

Figure 1. (A) Structure of egg-white-type lysozyme with disulfide bridges marked in red. Image created using PyMOL in conjunction with the PDB Hen Egg-White Lysozyme structure, 1DPX. (B) Scheme showing the reduction of a disulfide bridge in a protein with TCEP, and subsequent addition of a PEG-Maleimide functionality to the free thiol.

through an S_N2 mechanism by agents such as (tris(2-carboxyethyl) phosphine) (TCEP) to two free thiols[20] — one for each cysteine residue forming the disulfide bridge (Figure 1B). Through carefully controlling the equivalence of the reducing agent to protein, the students produce samples of protein with incrementally more thiol. If it is sterically possible, the generated thiols are then free to react with a suitable substrate, such as the maleimide end group of PEG-maleimide (PEG-mal 5000 or 750). The maleimide reaction with cysteine involves the 1,4-conjugate addition of the thiol to the maleimide. The mass modifications of the protein samples are relatively large and can be tracked by using SDS-PAGE electrophoresis. While these modifications to lysozyme serve no specific end-purpose in this task, the process is efficient at teaching many fundamental concepts in protein modification and general chemistry/biochemistry.

SAFETY

Students should always wear the minimum recommended PPE for this task: lab glasses, lab coat, and disposable gloves at appropriate times. The SDS-PAGE gel analysis should be supervised and carefully organized to reduce the risk of an electric shock (185 V). The experiment requires a heating

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block set to 90 °C, which presents a thermal burn risk. While the preprepared buffers, ammonium persulfate, PEG-Mal, SafeBlue stain, and protein are considered bench-safe, many of the chemicals involved are not. The neat TEMED, 2-mercaptoethanol containing loading dye, and samples containing this loading dye should be handled in the fume hood with gloves for toxicity and stench reasons. It is pertinent to mention that other somewhat safer chemicals are available to replace the 2-mercaptoethanol, such as cysteamine and 2-mercaptoethanesulfonate, but they were not compared during this body of work. The acrylamide/bis(acrylamide) solution in water is low risk for inhalation but is high risk for contact (carcinogen, skin sensitizer). Gloves should always be worn when handling this material or solutions of this. Cast gels, even when rinsed and stained, should be handled with gloves. Some safety data sheets for lysozyme list H334 as a hazard, although this is inconsistent. If listed, consider the hazards of dust inhalation and utilize fume hoods accordingly. An example COSHH[a] risk assessment can be found in the SI (Notes for Students). No other unexpected or unusually high safety hazards were encountered during this work.

EXPERIMENT OVERVIEW

Methodology

Full methodology, chemical details, and equipment information can be found in the Supporting Information files Notes for Students and Notes for Instructors.

Each student began by preparing rough 30 μM solutions of hen egg white lysozyme in one of two buffers via a 4 decimal place balance and small volumetric flasks. The buffers used for the reaction were 50 mM pH 8 tris (2-Amino-2-(hydroxymethyl)-1,3-propanediol), either without urea (reaction series "A") or with 6 M urea (reaction series "B"). In each pair, both reaction series A and B were prepared—one series per student. The solutions were accurately quantified with UV—visible spectroscopy, using the appropriate extinction coefficient ($\varepsilon^{LZ}_{280\ nm}$ = 0.036 μM^{-1} cm^{-1}), and then adjusted accordingly to the target concentration of 20 μM via accurate dilution. A set quantity (1.000 mL) of this protein solution was dispensed into each of four Eppendorf tubes. Each pair then prepared a shared solution of a reducing agent (tris(2-carboxyethyl) phosphine, TCEP, 4 mM) from a stock solution (50 mM). This was then dispensed into the solutions of protein at various equivalencies (1, 2, 3 and 4 eq.) and left to react for one hour at room temperature. A 20 mM solution of PEGylating agent (methoxypolyethylene glycol maleimide (PEG-Mal 750) or (PEG-Mal 5000), Sigma-Aldrich—claimed M_n by NMR)), was then prepared in 50 mM pH 8 tris buffer without urea, and 50 μL of this solution (an excess of 50 eq. against protein) was added to each the four reactions. The reactions were left to react for another hour at room temperature. At the end of the hour, 10 μL of each sample was transferred into its own clean Eppendorf tube, and 5 μL of the loading dye was added. This solution was then heated to 90 °C for 5 min, before being cooled and briefly spun down in a centrifuge to aggregate the droplets and condensate in the lid of the tube. During the reactions, the SDS-PAGE gel was prepared in disposable Invitrogen Mini 1 mm cassettes (Fisher, #11549156) to be run in a Invitrogen XCell SureLock Mini-Cell (Fisher, #10093492). The SDS-PAGE gels comprised a stacking portion laid on top of the resolving portion, and gels were cast with 12 crenulations. The stacking portion was roughly 20% of the total gel volume; each of the two portions took roughly 20 min to set at room temperature. The samples were loaded (10 μL), by the students: 5 crenulations per student, plus a shared ladder (3 μL) (PageRuler Plus #26619). Gels were run for between 1 and 1.5 h at 185 V (until the 15 kDa ladder marker approached the base of the gel). The gels were removed from the disposable cassettes, stained with a single step protein stain (SimplyBlue SafeStain), and photographed on a gel visualizer (although a smartphone camera was perfectly sufficient). Proton NMR spectra (Bruker Avance II, $CDCl_3$, 400 MHz) of both PEG-Mal agents were prepared by technicians and provided to the students electronically to save the cost of each student preparing their own sample.

Timings and Logistics

This experiment is designed for a 6 h class, split into two 3 h sessions on either side of a 1 h lunch break, and can be expanded or compressed slightly as per the needs of an institution. The session has not been tested as two fully separate halves. While the gels can be stored, consideration would need to be made by practitioners to the protein stability in the presence of the reducing and PEGylating agents. We ask the students to work in pairs for the task, where one student focuses on the "with urea" series and the other on the "without urea" series. The SDS-PAGE gels are shared in pairs which allows loading of each person's reaction series, plus a nonmodified protein sample and a ladder. Each pair is asked to use either the 750 or 5000 Da PEG-maleimide. Across all pairs in the group, there are therefore results for the protein in each buffer system, with each PEGylating agent, at four different equivalencies of agent.

Our students experience this experiment just after the halfway point of the year, and in our experience, students should be able to carry out many of the standard tasks independently at this stage. The most challenging skills to teach are associated with the preparation of the gel. The required techniques that were new to the students (pipetting into cassettes, removing bubbles, constructing the gel tank, and loading samples) are notoriously fiddly and require competent demonstration skills when teaching. Due to the hour-long wait times for both the reduction and PEGylation, these times can be more efficiently used for gel preparation, demonstration, and discussion of the process. The hour long wait time for the electrophoresis can be filled effectively by the students preparing tables of quantities, expected protein molecular masses, and predicted gel stains, based on the method followed. It is also a good time to discuss the NMR of the PEG-Mal in terms of structure assignment and usage of end group analysis to determine the approximate total molecular weight possible (via number average molecular mass determination). PEG-Mal can be moderately expensive to purchase for schools with small teaching budgets. At the time of writing, the 750 Da was £536 per gram, and the 5000 Da was £141 per gram from Sigma-Aldrich. The concentrations and quantities used have been selected to reduce the overall costs per student to reasonable amounts (~£2).

EXPERIMENTAL RESULTS

The students were asked to label their reactions A0 through A5 or B0 through B5 depending on whether they used buffer without or buffer with urea, respectively. Sample 0 was control lysozyme with no reducing agent or PEG-mal added, and samples 1 through 4 of each series relate to reaction with

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incrementally increasing equivalencies of TCEP to lysozyme (1, 2, 3, and 4 eq.). The quality of the gels varied hugely across a cohort, depending on the dexterity of the students and their individual propensities to tear a gel. However, in most cases, the gels provided similar results. Table 2 shows the expected results of the experiment, and Figure 2 shows the annotated gel results from using PEG-Mal 750.

Table 2. Theoretical Total Mass of Modified Lysozyme after Reaction with Varying Equivalencies of TCEP and Subsequently Either 750 or 5000 Da PEGylating Agent

Sample # (PEG-Mal used)[a]	Equivalents of TCEP to protein	Total possible protein mass (max. possible mass gain[b]) (kDa)
0 (750)	0	**14.3** (+0)
1 (750)	1	**15.8** (+1.5)
2 (750)	2	**17.3** (+3)
3 (750)	3	**18.8** (+4.5)
4 (750)	4	**20.3** (+6)
0 (5000)	0	**14.3** (+0)
1 (5000)	1	**23.3** (+10)
2 (5000)	2	**33.3** (+20)
3 (5000)	3	**43.3** (+30)
4 (5000)	4	**53.3** (+40)

[a]Sample numbers relate to either reaction series "A" or "B" as the absence or presence or urea does not affect the absolute value of theoretical mass gain. [b]Obtained from the addition of the PEG-Mal, assuming each equivalent of TCEP liberates two thiols, which each react with a PEG-Mal of the chosen molecular mass. kDa.

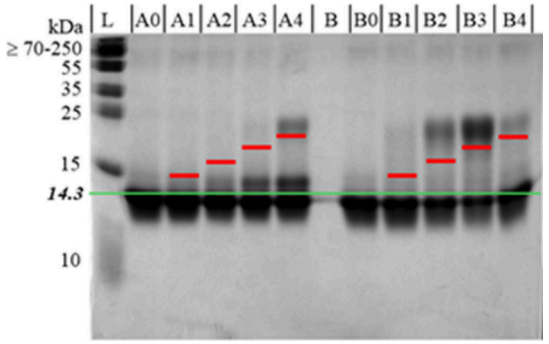

Figure 2. Example of stained SDS-PAGE gels of the protein samples without urea in buffer (A#) and with urea in the buffer (B#) after modification with TCEP and then PEG-Mal (750 Da) against the ladder (L). The green line represents the native protein mass, and red lines represent estimated total mass for each sample based on the amount of reducing agent used in the reaction and assuming that the mass of PEG is 750 Da.

The PEG-Mal 750 results (Figure 2) show PEGylation of all four disulfides/all eight thiols in the protein to varying degrees depending on condition. For the samples with urea present (B no. 1), the gels show a small portion of protein being fully PEGylated to ~ 20.3 kDa across all reduced samples, with little banding for intermediate levels of reduction and PEGylation. The without urea "A#" results in Figure 2 show much more banding at the 15.8 kDa mass position, and much less conversion to the maximum possible mass across all samples in the series. There is not much intermediate banding in either the "A" or "B" systems. Results appear to show that despite the number of equivalents of reducing agent added, PEGylation occurs on only one or four [all] of the disulfides. Presence of the urea facilitates more PEGylation of the 4 disulfides, whereas a lack of urea hinders the PEGylation to only 1 disulfide.

The results with PEG-Mal 5000 (Figure 3) were found to be better distributed. Across all samples, with and without urea,

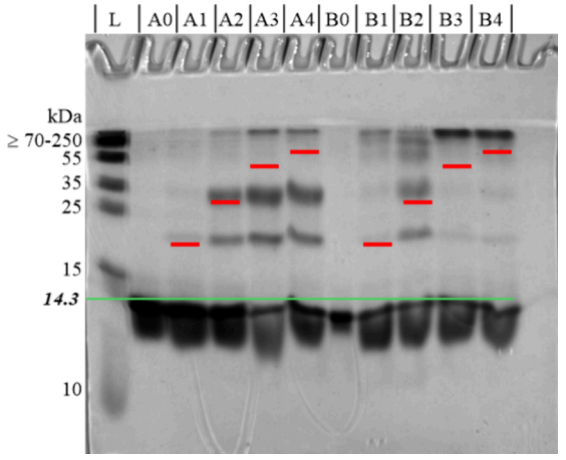

Figure 3. Stained SDS-PAGE gel of the protein samples without urea in buffer (A#) and with urea in the buffer (B#) after modification with TCEP and then PEG-Mal (5000 Da) against the ladder (L). The green line represents the native protein mass, and red lines represent estimated total mass for each sample based on the amount of reducing agent used in the reaction and assuming that the mass of PEG is 5 kDa.

there is more clear banding for all 4 increments of reducing agent added to the protein. Distinctly, in the samples without urea (A#), there is much more intermediate banding at the loci relating to the 1 and 2 eq. of reducing agent (23.3 and 33.3 kDa) regions. There is banding for the full PEGylated protein at ~53 kDa, but only for samples with 3 and 4 eq of reducing agent added. The samples with urea present show banding at all expected increments, but a much increased visibility of banding at the ~53 kDa region, alongside a loss of banding for intermediate levels of PEGylating, especially in samples 3 and 4.

The disulfide bridges in the native structure of lysozyme are formed in a specific order. The first bridge to form is between Cys 64 and Cys 80, followed by the bridges between Cys 76 and Cys 94, Cys 30 and Cys 115, and finally Cys 6 and Cys 127. The two first bridges that form are in the relatively restricted and inflexible core helices of the structure. These two bridges are in a more kinetically trapped state than the outer two, which gives rise to both a slow and a fast folding/unfolding pathway in the protein.[21] The folding of lysozyme is seen to follow a two-stage mechanism, in line with these topographic features.[22] Reports also claim that urea as a chaotropic agent is not as efficient at unfolding lysozyme as some other denaturing agents such as guanidine hydrochloride; however, it does have the advantage of not impacting the ionic strength of the system. Urea can influence small conformation changes at neutral pH, by disrupting hydrogen bonds in the protein. These changes potentially increase activity around the enzymatic sites, but do not cause unfolding.[23] Studies also found that at neutral pH and with 7.4 M urea and a supporting thiol initiator, only roughly 50% of the protein is unfolded from the native state via scrambling of the disulfides.[24] These theories reflect the gel results from the past two years; the use of urea in this experiment does assist with the overall

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performance of PEGylation of the lysozyme, as some disulfides are more difficult to access than others. With the smaller PEG-Mal 750, all increments of PEGylation are possible with or without urea. In contrast, the PEG-Mal 5000 shows much reduced reaction to the third and fourth disulfides, unless urea is present. This is likely due to the inaccessibility of the larger PEG chain to the more kinetically frustrated disulfides. With urea present, the barrier for unfolding is reduced, allowing full reduction and PEGylation. The hindrance of the large PEG 5000 species may complicate the reaction even with urea, leading to the more intermediate banding observed.

The gel results suggest a slightly higher mass modification than would be expected. For example, instead of one disulfide allowing the addition of ∼ 1.5 kDa to the protein with the PEG-mal 750, the student may identify that it added ∼2 kDa. Proton NMR end group analysis of the vinylic protons of the maleimide against the repeat unit of the polyethylene glycol is simple to carry out on this system by students. PEG-Mal 750 was found to be n = 20, Mw = 991 g·mol^{-1}. PEG-Mal 5000 was found to be n = 147, Mw = 6579 g·mol^{-1} (SI: Notes for Instructors). This helps to explain the slightly higher than estimated banding seen for each reduction increment in the gels. Reports have also shown that the relative mobility of PEGylated human serum albumin in SDS-PAGE is lower than that of a protein with a similar molecular mass. This may also be a contributing factor in the overestimation of protein size.[25]

PEDAGOGICAL JUSTIFICATIONS AND OUTCOMES

Student scores presented in this section are calculated from components of lab reports of the students who completed the work in academic years 2022 and 2023. The student assignment scores obtained for this study were collected in an aggregated manner, such that no individual student can be identified. No direct interaction with students was required, and informed consent was deemed unnecessary in accordance with the institutional Student Data Privacy Notice for the use of anonymized educational data for research purposes. All data were anonymized by removing personally identifiable information (e.g., names and student IDs) and aggregating the results to protect student privacy. Images of gels presented in this work are the Author's work, rather than the students'.

Solution Preparation and UV−Vis Analysis

We unexpectedly found this experiment challenged students in the multitude of interconversions required between the milli- and micro scales for both volume and concentrations. Additionally, physical manipulation of very small quantities (∼10 μL) with autopipettes was observed to be challenging for many. It is uncommon for our students to be exposed to using UV−vis for validation of concentration of a reagent before reaction; thus, this first step served as a useful warmup at to the experiment and as contextualized revision of the Beer−Lambert Law. Students needed only a little help with this. The lab report scores relating to equivalency and interconversion of units reflected the challenges in the theory. When asked to present a table of quantities used in each reaction, along with expected thiols formed, and overall protein mass postmodification, the class scored 67.2% on average. Low scoring students miscalculated the moles of lysozyme present in each aliquot of reaction mix and subsequently were unable to calculate equivalence and expected mass. We recommend that students prepare this table during the class going forward to improve these outcomes.

Organic Chemistry

The foundation of this experiment is in organic chemistry, and as such, there is a good opportunity to discuss and assess students on all the key reactions involved which cover radicals, reductions, and nucleophilic addition. While these mechanisms are relatively elementary for a second-year organic course, the application of these fundamentals across subdisciplines and in application of a new set of skills helps reinforce the mindset of the importance of applying knowledge across topics as an integrated learning experience.[26] When asked to show the mechanism for the reaction of a cysteine with the maleimide, the average score was only 59.5%, with some students wrongly suggesting the maleimide was the nucleophile.

Gels and Polymer Chemistry

SDS-PAGE gels can be bought preprepared or prepared by the user. We found that the value to the students of casting the gels themselves is high. At their heart the gel is simply a poly(acrylamide)-*net*-(bis(acrylamide)) hydrogel, but the theoretical basis for understating this links to second year lecture content well. Allowing students to cast the gels themselves exposes them to the phenomena of room temperature free radical polymerization in aqueous systems. The process allows opportunities for discussing the role of TEMED as a radical accelerator, the role and mechanisms of the initiator (ammonium persulfate), the mechanisms of the polymerization, and the concepts of network polymers and gels (SI: Notes for Instructors). The latter examples are concepts which are traditionally difficult to build into undergraduate lab curricula time scales, although interesting examples are becoming more widely available[27−29] since the emphasis placed on these topics by the ACS.[30] Gel design, the ratio of acrylamide to bis(acrylamide), the final network, and porosity of the gel and separation efficiency are all concepts that could be built into the assessment of the lab or live discussion. Over the past two years, 100% of students were able to produce a functioning gel during the session. Similarly, when questioned about the role of the varying components in the polymerization, 87.9% of students correctly identified the purpose of reagents. We did not directly assess the polymerization mechanisms in this experiment.

Gel Electrophoretic Analysis

Chemistry students are rarely exposed to gel electrophoresis outside lectures; therefore, this was an invaluable experience. A lot of reinforcement of a good pipet technique was required throughout, and the teamwork needed to produce and process good gels was built on communication practices. We would warn other providers that students with visual impairments or dexterity related concerns may need additional support in loading the gels, as this is an especially fiddly task which has little room for error. Backup gels prepared in advance or during the session are always a useful tool. Students are asked to present a picture of the gel they have produced with labels and ladder markers for the lab report. The class scored an average of 75.9% on this component, yet with some students failing to include the correct ladder in the labeling of gel. The scores were much lower (53.4%) when asked to describe the gel results with respect to the expected values. Some students struggled to fully understand the banding, especially where it was not neatly presented, which made an explanation difficult. Most students made attempts to explain the effect of urea to a sufficient level. The purpose of the loading dye was well understood (79.3% were able to identify and explain the

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purpose of its composition). Where time permits, for example, during the electrophoresis segment of the practical, it may be pertinent to build in a workshop-style group discussion with example gels to help bridge any gaps in understanding the conventions and intricacies of analyzing gels.

NMR End-Group Analysis

Prior to this experiment, our students will have worked through a polystyrene NMR end-group analysis experiment based on a recent literature example.[31] This experiment spirals back to those skills in NMR analysis of polymers to help evidence the results of the gel. The discrepancy in stated PEG length and NMR derived values highlights to the students that reagent quality/purity validation could be an extremely important step to take before work commences. We did not directly assess this component.

Economic Considerations

The high cost of PEG-Maleimide is always pointed out to the students so they can gain a further appreciation of the cost of certain reagents. In addition to the high cost per gram, students are asked to discuss why the high molecular mass of these PEGylating agents is a further economic concern. The high molecular mass agents give a good opportunity to reference how 1 g of the 5k PEG-maleimide is only 0.2 mmol of reactive group, and hence why they are only working with (still expensive) micromolar quantities. These concepts are often overlooked by students unfamiliar with research and experimental design.

Overall Conclusions

Figure 4 shows the distribution of marks for this experiment's lab report compared to the 9 total reports in the module. The

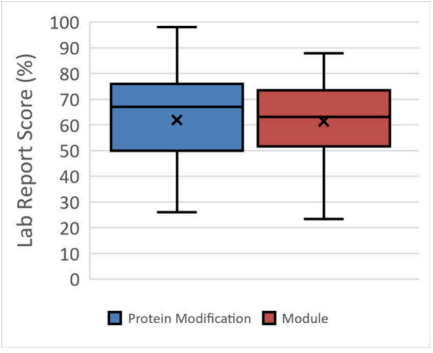

Figure 4. Plots representing the lab report scores (%) for this PEGylation experiment (blue) against the module average of 9 submitted lab reports (red).

distribution aligns well with the wider module and is centered around a similarly appropriate median score (68% vs 64% module overall). We believe that the topics covered and difficulty of assessment are pitched appropriately for the students at this point in their studies. Other practitioners should be considerate of the potentially high cognitive loading placed on students when multiple significant concepts are assessed together, such as in this example. Consideration of the removal of certain aspects of the assessment may be useful in directing the learning across a narrower set of topics. Overall, we feel this experiment gives students a strong introduction to bioconjugation, is designed with good pedagogical strategy, and gives extra value to a multidisciplinary second year course.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.4c01031.

Notes for Students, including lab manual, assessment questions, COSHH risk assessment and integrated/annotated NMR files (PDF, DOCX)

Notes for Instructors, including discussions and teaching guidance, mechanisms, and chemical/equipment lists (PDF, DOCX)

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Author Contributions

[§]A.W. and D.B. contributed equally to this work. Conceived the theoretical framework: DB. Conceived the experimental design: D.B., A.W. Tested, taught, assessed, and developed the experiment: A.W., D.B., B.J. Photographed gels, and analyzed NMR: B.J. Wrote the paper: A.W., D.B. Prepared Supporting Information: A.W., D.B., B.J.

Notes

The authors declare no competing financial interest.

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■ ADDITIONAL NOTE

[a]Control of Substances Hazardous to Health Act 2002 (UK)

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