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# Improving teaching in the laboratory by open questions: A case study

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## Introduction

In the field of biochemistry and molecular biology the laboratory is the place where the science is converted from the ideas to reality. Lab exercises for students are therefore a crucial part for their development. In many cases the students enter the laboratory with high interest, where they believe to find a new world to satisfy their own curiosity and let them develop own creativity, critical thinking and team work. The laboratory should give the easiest way for the student's deep learning. There is no way for the student to fulfill the task without being active. Anyway teachers can often observe the situation: "hands on – mind off" (Rienecker, Jørgensen, Dolin, & Ingerslev, 2015). The reason causing this (kind of) situations might be a cognitive overload for the student. Modifying the observation of Johnston and Wham, (Johnstone & A.J.B., 1982), there might be different factors and combinations thereof: a) unfamiliar environment and actions: the student enters with the laboratory an unknown room with specialized equipment and behaving rules, like safety issues. especially in facultative courses a new social environment could also occur on top. b) untrained manual skills to perform the experiments. at the same time the student needs to self-reflect on his own actions. c) transfer of theoretical knowledge and resulting imaginations of the experiment with the reality and d) keeping track of the expectations for the experiment and the scientific question the experiment should solve. The result is a micro-organizing student with the aim to fulfill now context-less step by step a given recipe – the protocol for the experiment – and leave the student on the uni-structural low level of understanding (see Structure of the

Observed Learning Outcome taxonomy (SOLO) Biggs and Tang (2007)). One way to reduce the student's cognitive overload is the preparation for the tasks before going into the laboratory (Tamir, 1989). Therefore in many cases the teachers prepare students beforehand with handing out the protocol and having colloquia to secure, that the steps of the protocol have been understood and the expectations for the outcome of the experiment are clear. Nevertheless, dependent on the type of colloquia, this might not support the student's overall development in learning how to think scientifically, improve the general ability to ask the right scientific questions, keep the curiosity and with this the motivation to do science or the general self-awareness. To facilitate this, Tamir proposed 1989 the concept of open questions. The idea of this concept is quite simple. The open questions demand from the student a self-elaborated and comprehensive answer. These answers can be demanded on the level of the scientific problem (what scientific question needs to be answered), on the level of "ways and means" (how can we answer the scientific question), and on the level of the answer (what is the answer?) and different combinations thereof (table 3.1). I was interested if it would be possible to improve my own teaching by open questions. Therefore I applied open questions for a preparation session for the laboratory during a summer course. To get a better feeling if the open questions indeed would change the overall learning outcome, I compared it to closed questions approach for the laboratory preparation during the same summer course.

Table 3.1: (according to Tamir (1989)): Level of openness in teaching in the laboratory

| Level | Problem | Ways and means | Answers |
|---|---|---|---|
| 0 | Given | Given | Given |
| 1 | Given | Given | Open |
| 2 | Given | Open | Open |
| 3 | Open | Open | Open |

## Background, materials and methods

The comparison between the open question approach and the closed question approach was carried out during a two weeks summer course at the department of Plant and Environmental Science (PLEN). The summer course had the topic "protein biochemistry" and was on the master's level. . Six

Danish students from different study programs -nanotechnology, biotechnology, biochemistry and biology – participated in the course. This reflects a quite diverse background in knowledge and experience for the students regarding their experiences with the laboratory work. During the course the students worked on different scientific topics, where they needed to elaborate different biological questions. The topics were integrated in ongoing research projects at PLEN and had their focus on methods for the identification of protein interaction partners and complexes. The students needed to learn to perform the different methods in the laboratory. In the end of the course the students were examined separately based on their performance during a presentation in a wrap up session, and a report of the different experiments.

To perform my study of the comparison between the open question approach and the closed approach, I gave a lab preparation session, where the students were supposed to develop their own protocol (open question approach) and another session, where they had to discuss about a given protocol (closed question approach). The sessions took place at different days for different experiments. To point out, these experiments have no connections for their knowledge background and the master students have their basics in their laboratory experience, it was not possible to take advantage from the first experiment for the second one. Observations during the lessons of the two different approaches were done by me and by two visitors (either my university pedagogical supervisors or colleagues). For the evaluation of the two approaches I took the observations from the lessons, but also the students' performance in the lab, their performance during the presentation of the wrap up session and their method knowledge by two questions in a written exam like form, although they were informed it is not an exam. The written reports were not taken into consideration, because they were only partially evaluated by me. In the lab I focused on the following points: a) how do the students act in the unfamiliar environment with new equipment and colleagues from different study programs? b) How are they performing manual skills and self-reflection on their own performances, c) How do they cope with the transfer of theoretical knowledge and resulting imaginations of the experiment with the reality. The evaluation for the wrap up session was done by judgement for the completeness and clearness of the students' explanations according to their slides, whereas the answers of the written knowledge questions were simply quoted as right or wrong. Finally the students were asked to fill out a questionnaire, what they preferred as

preparation for the lab and their impression which approach gave them a stronger learning outcome.

## The structure of closed question approach: Epitope based affinity purification

Intended Learning Outcomes:

- How to perform the method
- What are the functions of the single steps of the method and which are critical
- What results can we expect from this method and how can the results be interpreted

Lesson design for the closed approach. Remark: The students needed to read through the protocol before the session and note questions

Table 3.2: the course design for the closed approach.

| | | | |
|---|---|---|---|
| Devolution | Questions for the protocol | Group work students | 1 min |
| Action | Writing the questions for the protocol on the board | Students | 4 min |
| Institutionalization | Answering the questions | Students - support teacher | 5 min |
| Devolution | Task for the students to explain the single steps of the protocol | Teacher | 1 min |
| Action | Going through the single steps of the protocol, discussion which steps are made why | Group work | 15 min |
| Institutionalization And summary | Evaluations and clarifications for the protocol | Students together with teacher | 10 min |
| Devolution | Handing out the task to find the chemicals and consumables needed for the experiment and the safety rules belonging to them | Teacher | 1 min |
| Action | Finding the chemicals and safety instructions | Group work | 8 min |
| Break! | | | 15 min |
| Lab | | | |
| Action | Prepare the buffers, familiarize with the equipment and follow the protocol | Group work 3x2 students/ teacher supervision | 15min 3x5 min (5min for each group) |
| Institutionalization | Sum up of the morning | | 10 min |

In this case the idea was to activate the students for deep learning by the discussion together about a known protocol. The “problem” as well as the “ways and means” and the “answers” are predefined and can be checked up in material from the lecture given beforehand. Therefore the approach is fully closed.

## The structure of the open question approach: identification of proteins by mass spectrometry based proteomics

Intended Learning outcomes:

- How to perform the method
- What are the functions of the single steps of the method and which are critical
- What results can we expect from this method and how can the results be interpreted

Lesson design for the open question approach.

Table 3.3: the lesson design for the open question approach.

| | | | |
|---|---|---|---|
| Devolution (introduction) | Handing out the task to design an own protocol to identify proteins by mass spectrometry | teacher | 1 min |
| Action (introduction) | Based on the lecture given before (Tuesday Wednesday) Goal: To develop an own protocol for the identification of proteins | Group work in 2x3 people groups/exercise | 15 min |
| Action | Puzzle pieces with true steps of to get it so they can combine them to their own protocol and supporting questions | Group work | 10 min |
| Institutionalization And summary | Discussion of the now written maybe different protocols with the whole group/ comparison (why are which steps needed, what do we expect to get with this protocol, what might be the biases) | Students with teacher | 10 min |
| Action | Make a list of all chemicals and equipment what is needed | Group work | 5 min |
| Break! | Printing out of the self-made protocols | Teacher | 15 min |
| Lab | | | |
| Action | Getting the chemicals, and equipment, telling me how to proceed and where to proceed | Group work 3x2 students/ expert participant teacher | 15min 3x5 min (5min for each group) |
| | Following the self-made protocol in groups 3x2 | Group work 3x2 | 1,5 hours |
| Institutionalization | Sum up of the morning | | 10 min |

The open question approach was based on the idea that the students should be activated by the task to create their own protocol for the identification of proteins by mass spectrometry. Therefore the problem was given. Anyway the “ways and means” and also the “answers” were kept as “open” in the beginning. It was designed like a riddle, were they could ask for more and more information. In the beginning they had only the lecture material to decide what kind of method they would like to take. They could get then according to the chosen method a puzzle with headings of the single steps in the protocol (figure 3.1a). The number of puzzle pieces and headings

were random but outnumbering the necessity of any protocol and the students were informed about that. As a further step they could get written supporting hints (figure 3.1b), but of course were also allowed to discuss with me. As last step they would get again as puzzle detailed descriptions of protocol steps, which they could align with their previous protocol steps or reconstruct their protocol according to the new information. With this the student decides how open or closed he/she would like to work. The aim was in the end to get a protocol they could follow and being prepared to perform the steps of it.

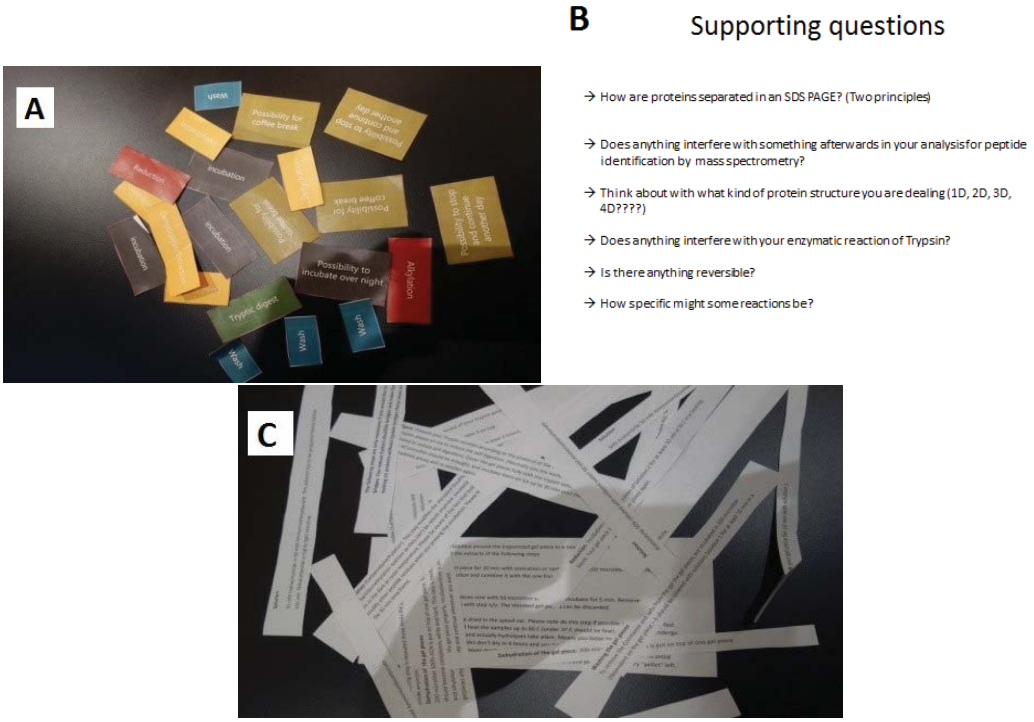



Fig. 3.1: Supporting material A: Puzzle pieces for the different protocol steps B: Supporting questions C: Puzzle pieces with detailed explanation of the protocol

## Observation for the closed question approach

The preparation part for the laboratory went overall as designed. The students participated actively to write questions to the board and discussing with each other possible answers. They also discussed to find the crucial steps in the protocol or the meaning. Nevertheless there were some important points for me. For example the time schedule for the lesson was not

kept in full length, because the students had fewer questions than expected, or they decided "I think, it is enough we should know about, let's move on" (student's quote). This indicated in my opinion, their aim was to fulfill the tasks given by the teacher without larger motivation or self-interest in it. Moreover the students preferred in many cases the action of the teacher. So I was rather asked to explain several times the points than they would think a little longer. "I think we will manage", was a final student's quote of the preparation session.

During the work in the laboratory the students took low responsibility for their own work and showed a low self-reliance. A high degree of teacher's supervision was necessary to proceeding in the protocol, like for example reminding them in which step they are now, or what they are doing. The interaction with their colleagues and the teacher can be seen by quotes from students: "what was meant again with…", "how have you done this step?", "what do we need to take first now?" They were therefore fully focused on the handling of the experiment and the teacher sometimes needed to provide the connection between the preparation session and the laboratory work. During the summary session at the end of the, they were able to recall the important functions of the protocol and the functions of the steps, but had problems with the interpretation of the results. We could conclude therefore: the intended learning outcomes were reached to a high extent but not fully satisfactory. Actually this impression was confirmed during the presentation of the wrap up session, where they were able explain very clearly, what they have done, but couldn't comment on the bottlenecks and restrictions of the method and had problems to answer critical questions about their results. The students showed the same tendency in the written exam questions, despite the fact, that during the summary and the wrap up session the points had been repeated. The question about the importance and crucial steps in the protocol was answered by 4 out of 6 students, with at least one right answer. The question for the preliminary results from the experiment could only be at least partial answered by 2 out of 6 people.

### Observations for the open question approach

As for the closed question approach the preparation part for the laboratory by open questions, went in general like designed. Important points to mention are the following: The time frame for the full open question was too much. On the opposite side, the given time for the puzzle and the supporting questions was not enough. The latter took nearly twice as long. The

students did not keep the discussion of small groups about the supporting material, but independently extended to discussions between the different groups. Moreover dialogue based support with the teacher was necessary and the students wanted to know actually the solution the teacher would choose. There was a true challenge for the students to fulfill the task ("I will never forget this", one student said lying exhausted with his upper body on the table arms widely expanded). Nevertheless they worked hard on it and none of them lost the focus. Moreover they felt proud after having managed to get the protocol. A final quote of the session was: "Yeah, let's go to the lab now". For the work in the lab they took responsibility and started for example to self-organize between the groups. So they divided the task to make the buffers to be more efficient and loose less material. They seemed to foresee the steps, actions and even safety rules could be remembered, when asked for self-reflection by the teacher. Moreover they recognized quite often, when they had made a mistake. The questions they still had were more confirmation of their own thoughts instead of asking what they should think and I had even the time to coach the nanotechnology student, who was not on the same experience level in the lab than the others. In the wrap up session they gave a very detailed and comprehensive explanation of the method (- actually too detailed for the time frame they had-). The results were presented clearly and very self-confident. The written answers to the questions for the important steps in the experiment six out of six students came up with at least one of the right answers. The same is true for the questions about the experiment. Of course there were differences in the completeness of these answers, but all of them seemed to have kept something and the intended learning outcomes have been fulfilled.

**Preferences by the students for one of the approaches**

Actually the students preferred the open questions approach and especially the puzzle. All six students believed to learn more with the open question approach. Moreover they liked to develop their own protocol step by step. But they also believed that without supporting information it would not have been possible for them to develop anything. When they were asked for the support dialogue between the teacher and them, only one of the students wanted to have short and direct answers, the others felt well with getting additional open questions to let them think themselves further.

## Evaluation of the open question approach in comparison to the classical approach

The two different approaches might have been influenced by day-dependent performance differences of the students and me. Additional some influence on the student might have been caused by me due to my excitement about the open question approach and how it would work. Nevertheless there are clear tendencies.

The two approaches started with the same basic intended learning outcomes: a) How to perform the method, b) What are the functions of the single steps of the method and which are critical c) What results can we expect from this method and how can the results be interpreted. Both approaches resulted in a similar learning outcome after the day in the laboratory regarding the basic knowledge about the protocol during the summary session. It differed on the level of interpretation of the results, where the open question approach resulted in a better outcome. On all further levels (the performance in the lab, the exams and the preference of the students) the open question approach outperformed the closed question approach. There might be various reasons, why the open question approach has caused this. The first one might be the different activation types of the students. The closed question approach caused an activity, without an authentic need for the student to be active. They could have followed the protocol without any of the activities and might have managed to perform the protocol, although the understanding would have been on a low understanding level (the unistructural level: identify, name, define mark, from the SOLO taxonomy, Biggs and Tang (2007)). Therefore it was greater effort for the teacher to get them on the level of the extended abstract understanding ("discuss, evaluate, create") and maybe it was sometimes even not possible, as we have seen by some of the students' quotes. The open question approach immediately leaded to a real authentic activity. The students would not have been able to continue in the laboratory without it. Therefore they were far more focused. And moreover it stimulated their ambition to manage the task. The possibility with the different levels of open questions seemed to provide them an environment, safe enough to proceeding, despite the challenge they felt to fulfill the task. In the end of the open question approach, they could give themselves maybe the best positive feedback they could get, a self-made protocol, which they would indeed apply in the lab afterwards. This in turn leaded to more self-confidence and a higher motivation to manage the tasks in the laboratory. The general cognitive overload during the

lab-work seemed to be strongly lowered by the challenge during the preparation before. The possibility to take own time for the performance during the puzzle seemed to support that, too. Despite all positive observations, I have to mention, that scientific curiosity was an expectation not fulfilled during this session. They were rather busy with the challenge to get any protocol. Moreover they didn't want to try out different things in the laboratory. But I hope that the given puzzle provided somehow the way of general scientific thinking we are using every day as an example (combination of the different pieces, try out different connections and come to a preliminary conclusion). Therefore the open question approach seemed to provide an easy way for learning.

Finally we also might consider something else: the differences of teaching knowledge, skills and competences (Christiansen et al., 2015). The closed question approach acted mainly on the levels of gaining knowledge and skills. But being able to take responsibility in the laboratory, as we have seen in the open question approach, for the own experiment belongs to the field of competences. And the students started to take already to take responsibility after the preparation by the open question approach. This might indicate that open question approaches deliver far easier the way to competences than closed question approaches do.

Unfortunately and of course there are also pitfalls with the described open question approach. They need attention to improve the approach. In the given case, the open question approach can switch easily to a closed one. The reason is simple. The students know the fact that a perfect answer for the protocol exists and was performed millions of times by other researchers. The students can therefore redefine - and partially they did - the open question approach to a closed question approach by demand of the perfect solution from the teacher. This tendency might become more pronounced, when in the dialogue between the students and the teacher, the teacher does not find the right balance between answering their questions and keeping the open question approach on a higher order learning level with examining and challenging questions (Rosenkvist & Hansen-Skoevmose, 2002). To summarize this part: Overall the open question for this case study delivered the way to many good quality parts for teaching (motivation of the students, reducing cognitive overload, feedback, extended abstract understanding etc.), and learning. Nevertheless there might be the pitfall for the development to a closed question approach during the session.

## Future perspectives and conclusions

This first attempt has shown the need to change the supporting material, the supporting questions and the time schedule. I had done this successfully in a second course after the herein described one (see Appendix A). This helped to keep the approach open from my side and gave the students more safety to get a good but flexible protocol setup. It might be possible in future to extend this approach to even larger classes (up to 25). In this case it might be necessary to include more peer review steps among the students instead of discussions with me. Moreover an expert protocol as comparison in the final institutionalization phase might be helpful. For classes larger than 25 people it would be necessary to program the puzzle as a computer game, so they can do it as an online group work and get feedback there. Of course there are also improvements possible for the described closed question approach. But the improvement possibilities for the open question approach might be still even more diverse. With this I would like to conclude that the described open questions had a positive impact on the laboratory work afterwards and the puzzle pieces facilitated the students' connections of the different steps in the protocol. But the puzzle pieces and the supporting material facilitate not only the understanding of the connections of the different steps in the protocol it also frames the topic the students are dealing with. This created on the one hand a safe environment for the students, where they couldn't fail to prepare their own protocol, but gives also the possibilities to the teacher to easily transfer it to complex and expensive laboratory experiments. (For example in this case the published expert protocol was the most expensive one, whereas all other possible solutions were cheaper). Of course there is a drawback for the teacher. The preparation for this approach is rather long. But in case of giving the course more than once, the time is refunded. Even when giving the course only once, the fun for the teacher to see to see the different discussion points of the student to approach a protocol and the different protocol possibilities might give enough reimbursement to go for it.

## References

Biggs, J. & Tang, C. (2007). *Teaching for quality learning at university* (3rd ed.). Open University Press/McGraw Hill.

Johnstone, A. & A.J.B., W. (1982). The demands of practical work. *Education in Chemistry*, *19*(3), 71–73.

Rienecker, L., Jørgensen, P. S., Dolin, J., & Ingerslev, G. H. (Eds.). (2015). *University Teaching and Learning* (1st ed.). Samfundslitteratur.

Rosenkvist, G. & Hansen-Skoevmose, P. (2002). *Coaching som udviklingsværktøj*. Psykologisk Forlag I.R. Stelter (ed.), coaching- læring og udvikling.

Tamir, P. (1989). Training teachers to teach effectively in the laboratory. *Science Education*, *73*, 56–69.

# A

**Supporting questions**

What are the challenges of each method (top down/in solution/in gel)?

What are the advantages of each method?

Maybe the following questions help you to decide

How many proteins are in your sample?

Do you remember the different spectra from the ion trap? What could be the challenges?

What kind of challenges does an enzyme have?

What might be biasing when you use an enzyme?

What are the substances inside a gel separation you might deal with?

What are the substances inside extracted proteins?

How do your proteins look like structure wise in a gel (might differ on the loading buffer)?

How do your proteins look like inside a protein extraction (might be dependent on the extraction)?

How can you minimize volumes of a sample? What might be the danger?

**Supporting information**

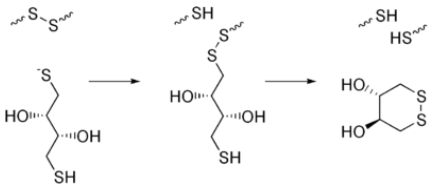
DMSO: Dimethylsulfoxide, organic solvent can be hardly evaporated

Acetonitrile: organic solvent; dehydrous

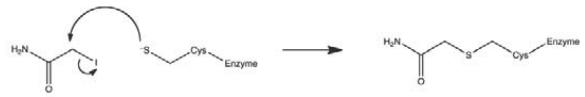
Trypsin: Serin Protease; cuts C-terminal at Arginine (R) and Lysine (K) sides in a protein except, when it is bound to a C-terminal Proline; Is pH sensitive

LysC: Endoproteinase; cuts C-terminal after Lysine (K) amino acids in a peptide. Although it has optimal pH range, it is a more robust enzyme than Trypsin regarding buffer conditions (8M Urea buffers possible). Moreover it cuts after Lysine despite Prolines.

Dithiotreitol DTT:




Iodoacetamide



Examples from the literature:

## Top Down Proteomics: Facts and Perspectives

**Adam D. Catherman**, **Owen S. Skinner**, and **Neil L. Kelleher***
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### Abstract

The rise of the "Top Down" method in the field of mass spectrometry-based proteomics has ushered in a new age of promise and challenge for the characterization and identification of proteins. Injecting intact proteins into the mass spectrometer allows for better characterization of post-translational modifications and avoids several of the serious "inference" problems associated with peptide-based proteomics. However, successful implementation of a Top Down approach to endogenous or other biologically relevant samples often requires the use of one or more forms of separation prior to mass spectrometric analysis, which have only begun to mature for whole protein MS. Recent advances in instrumentation have been used in conjunction with new ion fragmentation using photons and electrons that allow for better (and often complete) protein characterization on cases simply not tractable even just a few years ago. Finally, the use of native electrospray mass spectrometry has shown great promise for the identification and characterization of whole protein complexes in the 100 kDa to 1 MDa regime, with prospects for complete compositional analysis for endogenous protein assemblies a viable goal over the coming few years.

# Quantitative Assessment of In-solution Digestion Efficiency Identifies Optimal Protocols for Unbiased Protein Analysis*

Ileana R. León‡, Veit Schwämmle‡, Ole N. Jensen‡§, and Richard R. Sprenger‡§

**The majority of mass spectrometry-based protein quantification studies uses peptide-centric analytical methods and thus strongly relies on efficient and unbiased protein digestion protocols for sample preparation. We present a novel objective approach to assess protein digestion efficiency using a combination of qualitative and quantitative liquid chromatography-tandem MS methods and statistical data analysis. In contrast to previous studies we employed both standard qualitative as well as data-independent quantitative workflows to systematically assess trypsin digestion efficiency and bias using mitochondrial protein fractions. We evaluated nine trypsin-based digestion protocols, based on standard in-solution or on spin filter-aided digestion, including new optimized protocols. We investigated various reagents for protein solubilization and denaturation (dodecyl sulfate, deoxycholate, urea), several trypsin digestion conditions (buffer, RapiGest, deoxycholate, urea), and two methods for removal of detergents before analysis of peptides (acid precipitation or phase separation with ethyl acetate). Our data-independent quantitative liquid chromatography-tandem MS workflow quantified over 3700 distinct peptides with 96% completeness between all protocols and replicates, with an average 40% protein sequence coverage and an average of 11 peptides identified per protein. Systematic quantitative and statistical analysis of physicochemical parameters demonstrated that deoxycholate-assisted in-solution digestion combined with phase transfer allows for efficient, unbiased generation and recovery of peptides from all protein classes, including membrane proteins. This deoxycholate-assisted protocol was also optimal for spin filter-aided digestions as compared with existing methods.** *Molecular & Cellular Proteomics 12: 10.1074/mcp.M112.025585, 2992–3005, 2013.*

analyzing medium to high complexity protein samples in large-scale proteomics relies on protein digestion by using the endoprotease trypsin. Analysis and sequencing of tryptic peptides by liquid chromatography-tandem MS (LC-MS/MS)[1] then enables identification and determination of protein expression levels based on the peptide ion abundance level or the (fragment) ion intensities of identified peptides. This peptide-centric approach thus strongly relies on efficient, unbiased and reproducible protein digestion protocols. Efficiency is required to maximize the number of detectable peptides per protein (coverage) to distinguish unique proteins within protein families with similar sequences and/or sequence variants, and to detect post-translational modifications. Unbiased generation of peptides is required for the resulting data set to most accurately reflect the relative (stoichiometry) and absolute protein abundance in a sample. A particular protocol should be unbiased with respect to abundance, molecular weight, hydrophobicity and protein class. Membrane proteins for example are often suspected to be underrepresented. For MS-based proteomics approaches several critical steps can be distinguished: (a) disruption and solubilization of cells and protein complexes, (b) protein denaturation and enzymatic proteolysis, (c) MS-compatible peptide recovery, which normally entails removal of reagent leftovers and desalting before MS analysis, (d) adequate peptide separation (achieved by liquid chromatography), and (e) MS peptide analysis and sequencing (MS/MS), including the chosen data acquisition strategy.

Comparative evaluations of digestion protocols generally consist of qualitative studies using standard tandem mass spectrometry. These approaches may reveal efficiency (*i.e.* more identifications), but are unable to reveal digestion pro-

PROTOCOL

# In-gel digestion for mass spectrometric characterization of proteins and proteomes

Andrej Shevchenko[1,3], Henrik Tomas[1], Jan Havliš[1], Jesper V Olsen[2] & Matthias Mann[2,3]

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Published online 25 January 2007; doi:10.1038/nprot.2006.468

**In-gel digestion of proteins isolated by gel electrophoresis is a cornerstone of mass spectrometry (MS)-driven proteomics. The 10-year-old recipe by Shevchenko *et al.* has been optimized to increase the speed and sensitivity of analysis. The protocol is for the in-gel digestion of both silver and Coomassie-stained protein spots or bands and can be followed by MALDI-MS or LC-MS/MS analysis to identify proteins at sensitivities better than a few femtomoles of protein starting material.**