The Biophysicist

Intrinsically Disordered Proteins as an Instrument for Research-Integrating Teaching

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With increasing demands for inquiry-competent problem-solvers and emerging evidence that active problem-solving promotes a deeper understanding of science (1–3), universities are facing a need to rethink teaching styles to promote students as active contributors to exploring and solving scientific problems (4, 5). One way is through *integrating research in teaching*. This method involves a dynamic process of realization for both the student and the researcher and is centered around a scientific area of interest to both (6). In a course on intrinsically disordered proteins (IDPs) (7), we explored how teaching and research can be integrated in ways that not only confer deep learning (8) and research-related skills, but also create new scientific insights.

IDPs are a group of recently discovered proteins. Unlike other proteins, the function of IDPs is not tied to a three-dimensional form. Instead, they exist as dynamic ensembles of disordered structures relevant to their function. The disordered dynamics and discord of function from a specific shape challenges a >60-yr-old paradigm (9) that has shaped the established scientific knowledge on what proteins look like and how they function (10–12) (Fig 1a). IDPs are not an oddity but make up 30-40% of the human genome (13) with key roles in health and disease (14). They are subject to an emerging interest, not only in basic research (15, 16), but also from industries, having an interest in their roles in diseases and cures and in using them as novel biomaterials. The limited knowledge and potential for important

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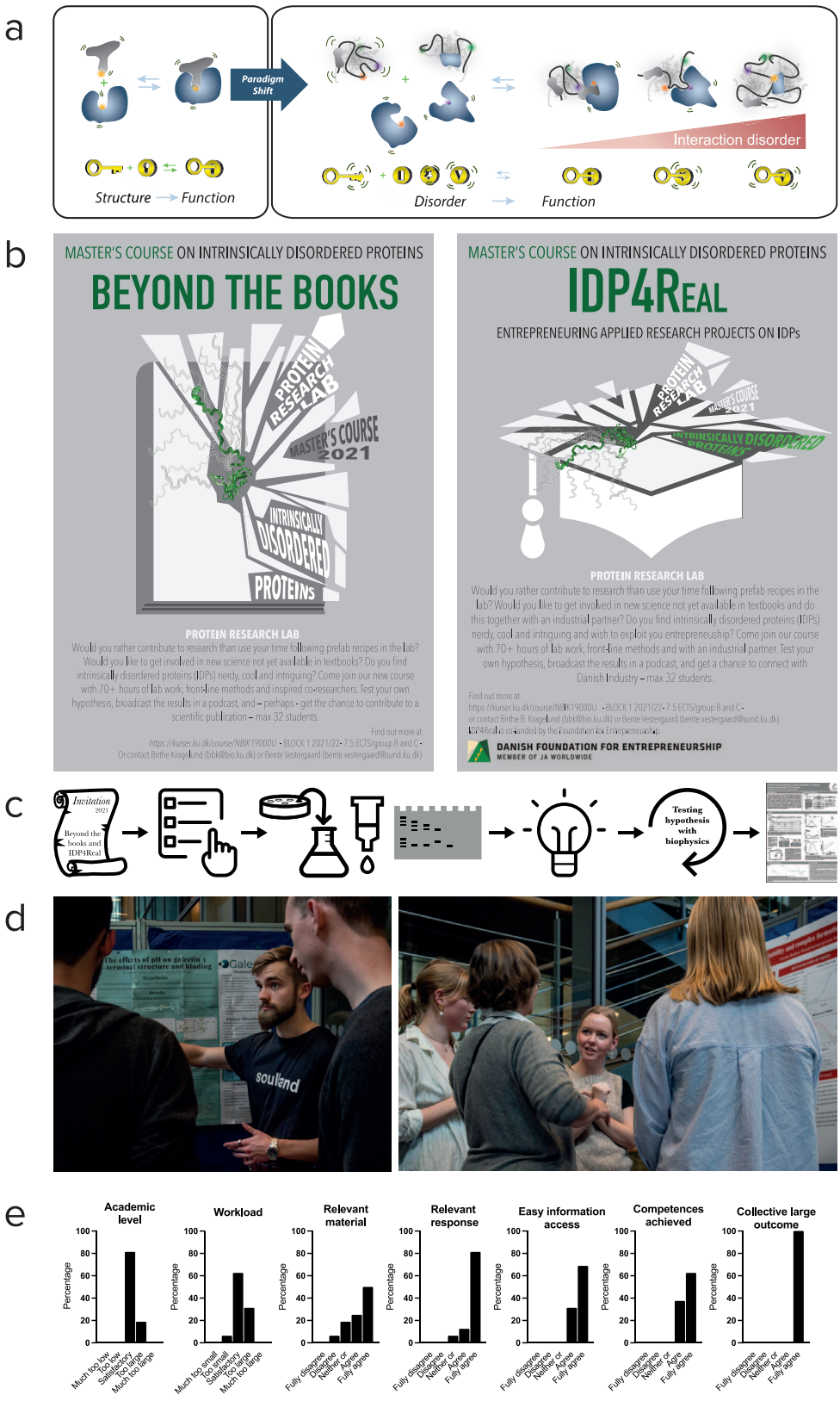

Fig 1. Research-integrating teaching platform based on IDPs. (a) IDPs can remain disordered in their complexes, which expands the interaction potential and broadens the palette of molecular communication mechanisms, challenging the structure function paradigm linking function to form. (b) Posters inviting the students to join the research-integrating course. (c) Workflow of the research-integrating teaching laboratory. An invitation from two fictive famous scientists, selecting the students to participate and setting the scene, initiates the

discoveries make IDPs a well-suited topic for integrating research in teaching. Not least because they are still underrepresented in textbooks, IDPs spur students' inquiries, and because they can be simple to produce, they are ideal for handling by students in the lab (17).

The challenge of integrating research and teaching

The coupling of research and teaching is a university landmark, but its meaning is not carved in stone. Today, research and teaching increasingly happen in separate spaces (18). Even if efforts are made to pave the way for more research in teaching, this is not a simple endeavor (6, 19). Education scholars point out that the most common ways of integrating research and teaching tend to leave little room for students' engagement (4, 6, 19). They show that much teaching linking to research maintains students as a passive audience to research or, at best, as guided participants in the research process (4, 6, 19). This system tends to ignore the research potential of the students. The reasons for a lack of integration are many and are often linked to resources. However, *making students active researchers* in teaching should be made a priority for several reasons. An example from the University of Copenhagen, Denmark, shows that it both improves student engagement and benefits research.

A platform for research-integrating teaching

The laboratory course on IDPs grew from the research center REPIN—Rethinking Protein Interactions, dedicated to IDP research, and was designed to promote students as co-researchers (6, 20). The ambition was to develop an inquiry-based course on IDPs that would offer synergies between students, researchers, and industry. Targeting students in biochemistry and molecular biomedicine, the idea was to explore IDPs in a hands-on course, which allowed for developing skills of innovation and entrepreneurship. Before the course, we mapped academic and industrial interest and established collaborations with partners on relevant IDPs. The course was two-pronged, linking students with either an industry or an academic partner. Inspired by posters (Fig 1b) and the course description (https://kurser.ku.dk/course/nbik22003u/2022-2023), 17 masters' students (fourth year at university) signed up. The students received a humorous invitation letter from Prof. Dr. MockUp and Prof. Dr. Chaos. Combined with a 1.5-h introductory lecture, this material outlined the inquiry-based framework of the course.

Students were presented with a list of 25 (precloned) IDPs, which had never or only sparsely been studied by biophysics. In groups of two to four, the students were free to select their research object from the list. Provided only with a colony of bacteria, the available literature, and a book of methods (21) students were given 80 h to (a) develop a purification protocol, (b) purify the protein, (c) post a hypothesis, and (d) experimentally test the hypothesis (Fig 1c). A teaching lab was transformed into a research lab, where the design of experiments was decided by the students. They could order columns and chemicals from the local stock for the next day and their thoughts and decisions were supported by mentors who were not giving answers but asked explorative questions. Once the students obtained a pure protein preparation, they were given access to advanced research equipment such as fluorescence, circular dichroism and nuclear magnetic resonance (NMR) spectroscopy, isothermal titration calorimetry, and small-angle x-ray scattering (SAXS), which could be booked as do other researchers. The students were trained at sites by technical mentors, hand-held in operation when needed, but they conducted the

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Fig 1. (continued) course. The students then select an unstudied IDP from the list, which they express and purify and for which they formulate a testable hypothesis. With the use of advanced biophysics to test the hypothesis, students generate and analyze data and present the results in a scientific poster. (d) Students presenting their discoveries and research in the form of a scientific poster to an interested crowd of students, PhD students, post docs, mentors, and a poster committee. (e) Course evaluation with anonymous reporting and its outcome is based on >90% responses.

analyses themselves. The costs were covered by the university, the research center, and grants dedicated to research- and inquiry-based teaching.

We created the course with the aim to be open to the unexpected and dependent on the decisions made by the students. Key to the inquiry-based framework was that students were not given protocols, premade buffers, or pre-tested—or expected—results. The idea was that students should decide, design, and produce everything themselves. The course opened with a lecture, but all other teaching was through research and took place in the lab. University professors and instructors were not acting as teachers, but as mentors. They engaged in open discussions with the students, answering questions with counterquestions to stimulate the thinking and decision-making capabilities of the students. When students experienced obstacles, they were met by encouragement and questions for furthering their thoughts. Often, teaching went overtime because of the eagerness of the students. As part of the course, students noted observations and ideas to create hypotheses interactively that they then tested, which was accomplished by advanced biophysical methods conveying authenticity. The setup led the students to do research based on their own ideas and hypotheses and teaching thus became both inquiry-based and student-led.

> I found it so motivating and exciting that no one had previously biophysically investigated the IDP we had gotten. This also made it feel more meaningful than if we would just have done the experiments with proteins that had already been well characterized. (David, Molecular Biomedicine)

From exam to authentic evaluation and dissemination formats

To ensure that the students achieved the predefined learning goals and competencies, we opted out of an exam because exam preparation can become a primary student goal, restricting learning and creativity. To increase authenticity (18), we instead developed an evaluation form comparable to a real research environment, wherein scientists present their results to peers and reviewers, and ideas, hypotheses, and interpretations are tested in scientific forums. First, the students presented and discussed the purification procedures and results with their peers and mentors. This part was evaluated by the lecturers and by peers asking questions about details of the experiments. Second, and as a first-time experience, they presented their experimental data in a poster session (Fig 1d). Students here each explained and discussed their hypothesis, experimental data, analyses, and interpretations with a poster committee, professors, and third year bachelor students. Collaborators from academia and industry engaged as well, and the best poster was celebrated. Finally, the students created a podcast targeting other students (e.g., https://www1.bio.ku.dk/formidling/biosfaeren/). A final evaluation of each student was based on active participation in the lab, engagement in discussions, and the two different formats of communication of results leading to a pass/no pass. There were several arguments for not grading students in this course. First, research on assessment formats in research-based teaching suggests that connecting research with teaching is best supported by authentic assessment formats where students get experience with realistic performance situations (18). Second, a process-oriented approach to student learning and engagement with research requires a process-oriented approach to assessment (22). Third, because the results of the students would be highly dependent on their choice of proteins, they could become internally unaligned. However, the chosen assessment form has advantages and limitations. It requires self-driven students with a focus on the scientific process and carries the risk of loss of engagement for a minor group of students. The form may also challenge the expectations by future employers, but a major advantage is that it tones down the professor-student hierarchy, contributing to transforming teaching into mentoring and discussions between scientists, a highly important goal of the approach.

> It was a real-life dive into scientific research since I've been faced with many of the frustrations of moving into an unknown/uncharacterized area of science. I had to 'think out of the box', look up papers on similar topics for guidance, and discuss the problem with my mentor, which gave me a grasp of how to go from theory to working on an uncharacterized topic. (Christoffer, Molecular Biomedicine)

Why bother?

With so many positive outcomes, why does integrating research in teaching not play a larger role in higher education? As mentioned, one important reason is budget and man-hour restraints. However, with the increased focus on establishing research centers with robust infrastructure and resources, research-integrating teaching could be explored within such environments. Our approach makes teaching attractive, also to the professors. Not only are the students given more freedom, so are professors. When students participate in scientific discussions, they pose questions that are more stimulating than those derived from predictable cook-book teaching. Often, these questions are conceptually intriguing and of interest to the professors' own research.

Promoting students to grow both scientifically and personally and supporting them through the frustrations they experience when transiting from passive to active contributors is mutually rewarding. Asking the students to reflect on the course, typical comments were: "the feeling of being independent in the lab," "the transition from teaching to mentoring," and "to examine proteins that have not been examined before." From their evaluations, the approach paid off (Fig 1e). Students took ownership and made important steps toward becoming independent researchers. As an essential outcome, the research of the students brought new knowledge (Fig 2). Although their data are preliminary, and as all good science would need to be repeated and validated, they discovered that a predicted intrinsically disordered region of ubiquilin 1 forms a molten globule-like structure (Fig 2a), one region of VAR2CSA is disordered and another region forms oligomeric structures (Fig 2b), a disordered region of the interleukin 22 receptor A1 (IL22RA1) undergoes *cis*-t*rans* isomerization (Fig 2c), a disordered, lipidated tail of KRAS forms higher order structures (Fig 2d), a disordered tail of galectin 3 interacts weakly with lactose and galactose (Fig 2e), and Rap16c forms a disordered dimer (Fig 2f). These results demonstrate the strong research capacity of the students. When motivated to be active enquirers exploring real scientific problems, students can make great achievements in a very short time. Orchestrating teaching this way, where students learn just in time (23) is fulfilling because their ways of thinking stimulate our thinking in unexpected ways.

In conclusion, the use of IDPs as an instrument for developing inquiry-based research-integrating teaching has been both amenable and successful. Because of the novelty of the topic, the associated technologies, and the results obtained, the students made links with both industrial and academic researchers and grew from exam-focused students to researchers who will be attractive to future employers and collaborators. As a natural consequence of the course style, students participated in the writing of this report, and they are co-authors with contributing data (Fig 2). We advocate that IDPs make a well-suited object for research-integrating teaching, offering opportunities for new insights for both students and researchers. The unpredictable character of IDPs invites open-ended and flexible processes that are key to both learning and research. Many other topics in biophysics (e.g., machine learning, membraneless organelles, and cryo-EM tomography) have similar attractive and open-ended opportunities and can make appealing cornerstones in future integration of research and teaching.

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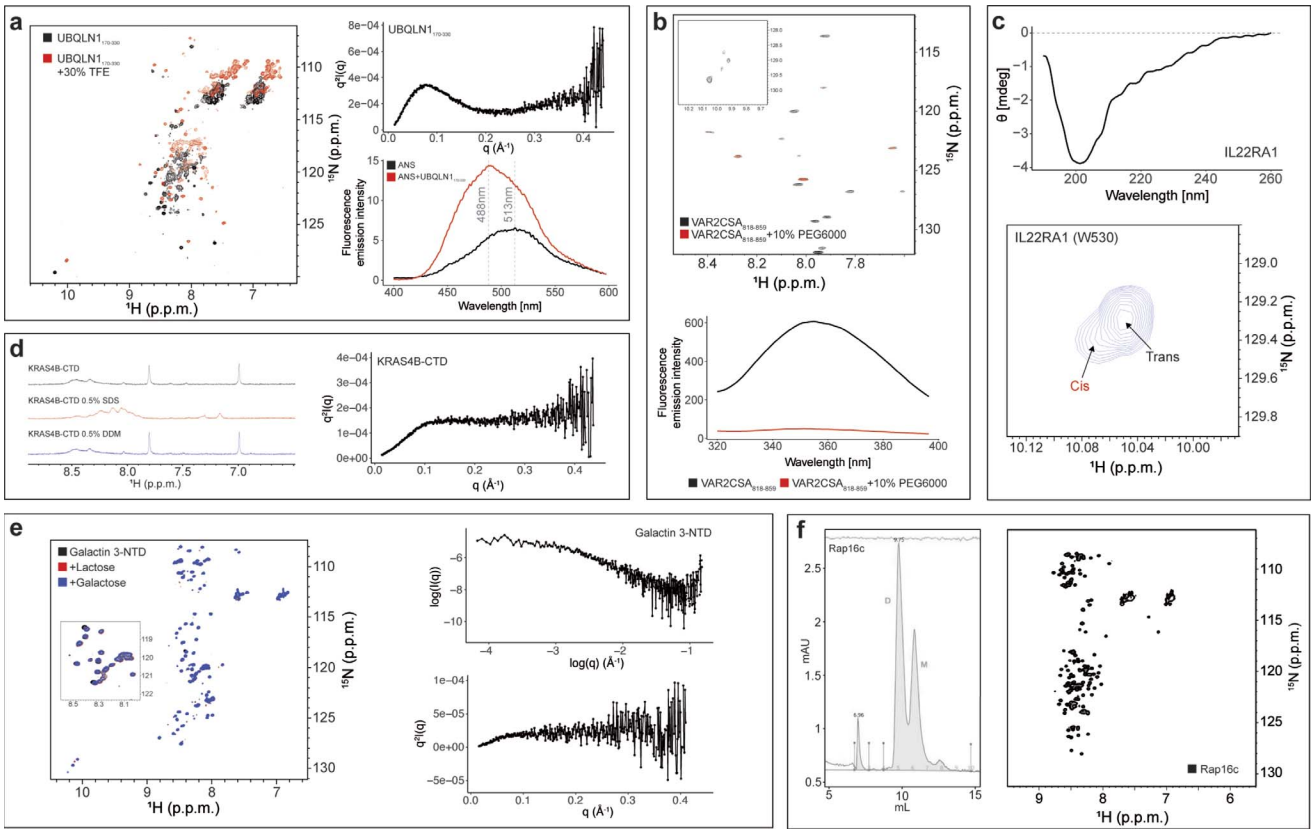

Fig 2. Research integration in teaching leads to research results. (a) The stress-inducible phosphoprotein 1 (STI1) domain of ubiquilin 1 ($UBQLN1_{170–330}$) shows a large diversity in peak intensities in the ^{15}N–heteronuclear single quantum coherence (HSQC) spectrum, which is indicative of heterogeneity in the ensemble. The addition of 2,2,2-trifluoroethanol (TFE) as well as SAXS and 1-anilino-8-naphthalene sulphonate (ANS) binding supports a molten globule character. (b) The homology block 1 (HB1) domain of VAR2CSA ($VAR2CSA_{818–859}$) shows heterogeneity and oligomerization, as seen from the very few peaks in the NMR spectrum and the diversity of peaks originating from the single tryptophan (see figure insert). Addition of crowding agents as polyethylene glycol led to disappearance of the signals and fluorescence quenching, without visible precipitation. (c) A region of the intracellular domain of interleukin 22 receptor A1 ($IL22RA1_{L447–L532}$) was confirmed to be disordered by NMR and circular dichroism, with distinct *cis-trans* isomerism. (d) A lipidated, disordered C-terminal tail of KRAS form oligomers leading to broad peaks in the ^{1}H-NMR spectrum, that can be partly resolved by sodium dodecyl sulfate addition, but with an increasing SAXS Kratky plot indicating the presence of remaining disorder. (e) The N-terminal domain of galectin 3 is disordered as evidenced by the low dispersion in the 15N-HSQC and the increasing SAXS Kratky plot. NMR data indicate that it binds lactose and galactose very weakly at low pH. (f) The NMR signals of dehydrin Rab16c shows a low dispersion in the ^{15}N-HSQC spectrum, indicative of disorder, and elutes in two populations on a Sephadex S75 column, corresponding in sizes to the presence of a monomer–dimer equilibrium. The results presented in this figure are all preliminary data and would need repetition and validation.

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