JOURNAL OF
APPLIED
CRYSTALLOGRAPHY

Volume 51 (2018)

Supporting information for article:

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Supplemental Information

Crystal Growth, a Research-Driven Laboratory Course.

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Table S1. Chemicals Used in the Crystallization Project

	Chemical Name	Chemical Formula	Solubility g/100mL (20°C, water)	*Prices (USD)
1	Ammonium Iron(II) Sulfate Hexahydrate	$(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$	26.9	$150/kg
2	Ammonium Magnesium Sulfate Hexahydrate. Mix equal amounts of: ammonium sulfate & magnesium sulfate	$(NH_4)_2Mg(SO_4)_2 \cdot 6H_2O$		$130/kg
3	Aspirin	$2\text{-}(CH_3CO_2)C_6H_4CO_2H$	0.3	$45/100g
4	Calcium Chloride hexahydrate	$CaCl_2.6H_2O$	90.8	$25/kg
5	Calcium Copper Acetate Hexahydrate. Mixture of calcium oxide and copper acetate	$CaCu(CH_3COO)_2 \cdot 6H_2O$	11.3	$27/kg $104/kg
6	Chromium Potassium Sulfate Dodecahydrate	$CrK(SO_4)_2 \cdot 12H_2O$	24.0	$92/kg
7	Copper (II) Sulfate Pentahydrate	$CuSO_4.5H_2O$	39.0	$125/kg
8	Copper Acetate Monohydrate	$Cu(CH_3COO)_2 \cdot H_2O$	7.2	$104/kg
9	Sucrose (table sugar)	$C_{12}H_{22}O_{11}$	21.2	$84/kg
10	Fructose	$C_6H_{12}O_6$	375.0	$72/kg
11	Glucose	$C_6H_{12}O_6$	90.9	$50/kg
12	Nickel (II) Sulfate hexahydrate	$NiSO_4 \cdot 6H_2O$	65.0	$137/kg
13	Oxalic acid	$C_2O_4H_2$	14.3	$62/kg
14	Potassium Aluminium Sulfate (Alum)	$KAl(SO_4)_2 \cdot 12H_2O$	11.8	$42/kg
15	Potassium Ferricyanide	$K_3Fe(CN)_6$	46.4	$150/kg
16	Potassium phosphate monobasic	KH_2PO_4	22.6	$92/kg
17	Potassium Sodium Tartrate (Rochelle salt)	$KNaC_4H_4O_6 \cdot 4H_2O$	63.0	$70/kg
18	Sodium Chloride	$NaCl$	35.9	$42/kg
19	Sodium Nitrate	$NaNO_3$	55.0	$109/kg
20	Urea	NH_2CONH_2	107.9	$90/kg
21	Lysozyme (use only as micro-crystal).	Protein sample	Solution of 5 mg/ml	$64/10g

*Average Prices as listed on Sigma-Aldrich in US dollar on Nov 9, 2015

Table S2. Laboratory Schedule and Assignments

Week	Activity	Assignment
1	Exp 1: Lewis Structure and Molecular Geometry	
2	Exp 2: Spectra and Absorption Introduction to Crystal Project	Quiz 1
3	Exp 3: Chemical Rxn: Precipitation	Quiz 2, Lab report 1
4	Exp 3: Chemical Rxn: Thermochemistry	Crystal Project Outline
5	Exp 3: Chemical Rxn: Kinetics	Quiz 3
6	Exp 3: Chemical Rxn: Le Chatelier	Quiz 4
7	Exp 4: Buffers & Acid Base Titration: Monoprotic acids	Lab report 2
	Mid-term break	
8	Exp 4: Buffers & Acid Base Titration: Polyprotic acids & Buffers	Crystal Project Proposal
9	Crystal Project: Macro-crystallization	Quiz 5
10	Crystal Project: Micro-crystallization	Quiz 6, Lab report 3
11	Crystal Project: Crystal screening	Quiz 7, Lab notebook
12	Crystal Project: Crystal analysis	
13	Crystal Project: Crystal analysis	Crystal Project Poster & Presentation/movie
14	Crystal Growth Symposium	Final scientific report, lab notebook.

Table S3. Consumables required for the micro-crystal project

Items	Description	Cat #	Size	*Prices (USD)
VDXm Plate with sealant	24 well plate with sealant for hanging drop vapor diffusion crystallization	HR3-306	50 plate / case	$239.00
Crystal Screen	50 unique crystallization reagents	HR2-110	50 reagents / 10 ml each	$324.00
Crystal Screen 2	50 unique crystallization reagents	HR2-112	50 reagents / 10 ml each	$324.00
Cover slips	22 mm circular microscope cover slide.	Any vendor	1000 slides/box	$50.00

*Average Prices as listed on Hampton Research website in US dollar on Nov 9, 2015

SUPPLEMENTARY NOTE 1

Course Structure and Budget

At New York University, the Crystal Growth laboratory is set up at a maximum of sixteen students per section. The diverse nature of the lab requires a small number of students per instructor as extra attention and support to the students is required. Even though the crystal project does not require the instructor to have a crystallography background, his or her involvement with the students is critical to ensure a safe laboratory environment. The Crystal Growth laboratory can be managed by any chemistry instructor, however, for detailed and more in-depth chemical and physical analysis of the single crystals using single crystal or powder X-ray diffraction (XRD), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDS), or any advanced analytical techniques, collaboration with local research laboratories with access to such instrument would need to be arranged. Our collaboration with different instrumentation specialists made it possible for our students to analyze their samples and gain a very valuable and remarkable experience for how research is conducted in postgraduate institutions.

With all the advancement that can be incorporated into the crystal project, the running cost for the laboratory is typically small and will vary depending on its design. For a small budget introductory level course, a simple lab can be designed based on the macro-crystal growth lab with the requirements to purchase chemicals, pH meter, and glassware. A class of sixteen students will cost less than one thousand US dollars per semester (Table S1, chemicals recommendation and pricing). For a complete experience including the macro- and micro-crystal growth, the lab set up will require the additional purchase of a stereomicroscope (Nikon SMZ18 or equivalent) and consumables that are specific for the microcrystal component (Table S3). The consumable for the microcrystal lab will add another thousand US dollars to the course budget as the same chemicals purchased for the macro-crystal lab can be used in the micro-crystal lab.

SUPPLEMENTARY NOTE 2

Crystallization Protocols

Students are initially given an overview of crystals, their structures, uses and methods for characterization, as well as the two main categories of crystals, macro- and micro-crystals (see Crystal Growth Lecture; Supporting Information). Several examples of easy-to-produce crystals are shown along with the most common methods for preparing them (Table S1), e.g. solvent evaporation (slow) vs. supersaturation (fast) and the pros and cons associated with each. Student groups propose two macro- and three micro-crystal project ideas; they are encouraged to use existing methods in literature to have as back-up should their initial novel proposal not succeed in the given time frame. It has been found that using the same chemicals for the macro- and micro-crystal components, or different chemicals but the same external factors (such as electrical field, solvent, temperature etc.), reduces the student workload for carrying out the project. Students are encouraged to think of projects that have meaning to them or that they are interested in. For instance, biology majors tend towards bio-related projects (e.g. kidney stones), physics majors lean towards physical concepts (e.g. magnetism) and engineers likewise (e.g. influence of electric fields). The variety in project ideas is immense and the blending of concepts between students of different disciplines is staggering.

In the first half of the semester and prior to the crystal project, students have all been carrying out the same experiments as everyone else in the traditional freshman lab; it is strongly advised that this portion is maintained as the diversity, and therefore potential dangers, is vastly increased during the coming project time period and lab skills and safety awareness are very important. Just before the crystal project kicks off, it is helpful to go over the proper etiquette of working in a lab. Instructors need to be especially vigilant in the early days of the project, constantly reminding the students of the value of cleaning up immediately as well as being aware of what is going on in their vicinity.

Macro-Crystallizations

The macro crystallization technique requires traditional glassware including beakers, petri dishes, and Erlenmeyer flasks. Evaporation of saturated or supersaturated solutions are used in the crystallization of a large single macro-crystal (Figure 1). For the saturated solutions:

(a) Prepare 100 ml saturated chemical solution by adding the appropriate mass of the chemical based on its solubility point in water at room temperature.
(b) Place the chemical solution in various shaped/sized containers as well as in containers made from different materials to facilitate a variety of nucleation conditions (e.g. petri-dish vs. test tube).
(c) Incubate the solution at room temperature and partially cover or uncover the container to vary the evaporation rate and as a result the crystal growth rate.
(d) Monitor the solution for crystal growth and if small size crystals are acquired, use them as seeds to grow a large single crystal (Figure 1A). The seed crystal can be suspended in solution using a thread, where the crystal will grow on the thread in all directions, or placed on the bottom of the container, where it will not grow in the direction of the container.

Supersaturated solutions:

(a) Increase the tempreture of 100 ml water to 60-80 °C using a hotplate. Avoid high boiling point temperatures as it can produce toxic chemical fumes.
(b) Disolve the required mass of the chemical to make a saturated solution based on its solubility point in water.
(c) Make a supersaturated solution by gradually adding small amounts of the chemical until the solution comes to saturation.
(d) Move the solution into a container that can be tightly sealed and allow it slowly cool down to room temperature by keeping it on the hotplate or placing it in a styrofoam box. A slow cooling rate is important to avoid the fast formation of small sized crystals.
(e) In the case of formation of small crystals, follow step d from the above section to grow a large single crystal.

A large size crystal up to 10 cm can be acquired for some of the chemicals (Table 1 and Figure 1b-d). The macro-crystal lab requires less equipment and consumables than the micro-crystal lab but it requires larger amounts of chemicals, as students have to make 100 mL of saturated or supersaturated chemical solutions with solubility ranging from 30 – 100 g/100 mL in water.

Micro-Crystallizations

A vapor diffusion method is employed in the crystallization of proteins that will crystallize only in a specific crystallization solution. Here, the hanging-drop vapor diffusion method is used for the crystallization of micro-crystals of chemical compounds or protein sample. The micro-crystallization technique includes 24-well crystallization plates, 96 different crystallization solutions, and micropipettes for utilizing the hanging drop vapor diffusion method (Figure 2a), as follows:

1) Acquire a 24-well VDX crystallization plate that is pre-greased (Hampton Research; Cat. HR3-306).
2) Add 400 μl of crystallization solution in the wells. Up to 96 different conditions can be screened in four 24-well plates utilizing the Crystal Screen and Crystal Screen 2 from Hampton Research (Cat. HR2-110 and HR@-112, respectively).
3) Prepare 1 ml 50% saturated chemical solution or 10 mg/ml lysozyme or any protein sample. Saturated chemical solution is not recommended due to the small size droplet that will evaporate very fast in the presence of crystallization solution in the well and a slow crystallization rate is desirable to achieve large single crystals.
4) Place a droplet of the chemical/protein solution on a 22 mm cover slip. Up to three different drops can be included on the same cover slip to maximize the number of samples screened in one plate. If the chemicals screened are colorless or exhibit the same color, different color markers can be used to label the cover slip next to the droplets.
5) Suspend the cover slip above the reservoir of the 24-well plate. The pre-greased well will seal the microenvironment between the drop and reservoir for equilibration with the crystallization solution.
6) Repeat steps 4 and 5 until all wells are sealed.
7) Once the plate is completed, store it in a safe place under the desired conditions and make sure not to shake or drop the plate to avoid mixing of the drops with the crystallization solution. Screen the plate periodically using a stereomicroscope for crystals that range in size from 50 μm to 5 mm; micro-crystals could appear in one to three days. Take pictures of the crystals soon after they appear, as some might not survive for long time due to continuous evaporation that can dry or damage the crystals.
8) A camera software or the microscope itself can be used to measure the size and dimension of the crystals.

In-gel Crystallizations

The single diffusion technique is used to grow crystals in agar gel, in which two different chemicals are required to grow the crystals of interest. One chemical is included in the agar gel and the other is in solution on the top of the chemical-agar gel as follow:

(a) Agar media is prepared by dissolving agar in boiling water. Once agar is completely dissolved, it should be removed from the hotplate and left to cool down. The first chemical solution is added to the agar before it solidifies; it is recommended not to add the chemical solution when the agar is very hot to avoid producing any fumes. You need to take into consideration the dilution factor after adding the chemical solution to the agar media to achieve the desired chemical concentration and to allow the gel to solidify.

(b) The chemical-agar solution is placed into a glass test-tube and filled halfway. The agar media is left to solidify overnight.

(c) The second chemical solution is placed on the top of the solidified chemical-agar gel, covered with Parafilm, and left to crystalize vertically. Crystallization conditions, e.g. pH, can be carried out at any stage of the experiment by modifying either the initial agar-chemical solution or the second chemical solution.

(d) Small micro-crystals can be observed and analyzed using a stereomicroscope.

SUPPLEMENTARY NOTE 3

Students Evaluations

The response of students has been universally positive, in most cases enthusiastic. Many have expressed the desire for more time to pursue additional variations on the crystallization conditions and additional studies on characterizing the crystals. In addition to the learning experience, the fact that they are all involved essentially in the same fundamental scientific activity means that the poster session creates a sense of competition not unlike that encountered in a true scientific setting. The open-ended nature of the laboratory creates a true atmosphere of discovery, and due to safety and manpower considerations we have often had to turn students away from spending even more afternoon and evening time in the laboratory.

As one student noted, an interesting phenomenon happens when students are allowed to run their own experiments early in their learning career. Traditional chemistry labs are structured such that students are exposed to a plethora of equipment and experimentation methods in a short time. Students learn at a steady pace, albeit often with superficial understanding and engagement. But, when they are challenged to come up with their own experiment, something special happens. A switch is flipped and at least a select few become entirely invested in their work. Now, the learning curve steepens. Every mistake is a learning experience, because it is directly related to their results. The goal changes from completion to discovery, and as a whole, the project unlocks the possibility for self-motivation among the student body.

Another group of students wrote that this project challenged them to design and perform their own research experiment that would be subjected to questioning by the faculty and student body. Although their project was biology-oriented, the crystal project is suitable for any scientific discipline and can be modified depending on the students' field of interest. Though they were given little time to draft and execute their research, the few deadlines given pushed them to be proactive and set their own dates to check-in on their progress and receive feedback from the lab instructors. Moving forward, their ability to complete what was once thought to be an impossible task gave them confidence to tackle further challenges that they may face in their college career and beyond.

Another group of students wrote that: "what really amazed us with the crystal project for the Foundations Lab was that we were given the freedom to choose what to research. This way not only we learned how to make our own crystals, but it felt like we were exploring fields that were not explored before and that we might have created crystals with properties that no one had created before."

Anonymous student comments on the Chemistry Laboratory course, specifically related to the crystal project for Spring 2015:

- The crystal project, unlike other experiments, gave us free reign in designing and conducting investigations. This was the most useful in improving our investigation and general lab skills. It also forced us to analyse data using our own knowledge.
- Crystal project was amazing! Definitely developed my understanding and creativity – overall good fun.
- The instructors were really helpful. The bi-weekly quizzes added unnecessary stress. The crystal project was probably the best because we had to do it with little guidance.
- I found the crystal project incredibly helpful for consolidating and applying my chemistry knowledge whilst exploring a biology topic in the experiment of our own design (Kidney stones).
- Crystal project is too short.
- Crystal growth was the best part.
- The beginning labs, apart from the crystal project. It felt too repetitive in having to do the same thing in FoS lecturer (sic) and the lab. I request it gets changed to more creative experiments.
- Quite a few of the labs felt quite repetitive, and therefore many students lost interest and learning was hampered. More project style labs such as the crystal project would be desirable.
- Things that could be improved is more guidance in regards to the crystal project and background. More time for the project would've been helpful, it was quite rushed.
- Loved the crystal project – definitely learned the most in that project.
- Could you make them more interesting. (sic) The crystals were the most fun to do because we had more control over what we were doing.

SUPPLEMENTARY NOTE 4

Instructor Reflection

As we reflect on the past 7 years of the crystal project, there are several aspects of the course that have become permanent fixtures which, in retrospect, would have eased the introduction of the project itself, outside of the challenges outlined in the main body of the paper. These are:

1) Ensuring key concepts were covered in the class. Until recently, the instructor for the labs was not the same as that of the class and it wasn't clear from the outset the levels of detail covered during lecture. Ensuring curriculum alignment would have eased initial stress and potentially supported lecture too.

2) Pre-crystal project experiments: Designing lab experiments prior to the crystal project that supported the experimental procedures to be used and introduce students to some of the theoretical concepts are essential for a smooth transition into the crystal project. These include short experiments on precipitation and solubility, chemical kinetics, acid-base titration and buffers, etc. In fact, for anyone seeking to implement the crystal project and with resource limitations, the selection of pre-crystal project experiments could facilitate the establishment of the principles and equipment within which the students could have freedom to explore thereafter.

3) We could never have foreseen the creativity of the student project ideas and are constantly challenged with requests for equipment, chemicals and analysis that are not currently available in the lab. While we appreciate that not everyone has sufficient resources to allow students total freedom, we ourselves limit them with chemicals that are safe (first priority) and equipment that are readily available. Having lists of these items for students to work with could better refine the scopes of the projects. However, we have found that it is generally only a couple of groups that go beyond the 'typical' pH, magnetic field, solvent effects etc.

4) Similar frustrations amongst students come up year after year, particularly regarding projects not working. Adequately preparing students to the reality of research, that in fact lots of it 'fails' is key to ensuring they do not get too disappointed. To alleviate this, grading students based on creativity, lab work and scientific decision-making during their project over the actual results may obviate this. In addition, students testing 2 to 3 chemicals in their initial crystallization trails will ensure the yield of at least one successful crystallization trial.