Communicating Research to the General Public

At the March 5, 2010 UW-Madison Chemistry Department Colloquium, Prof. Bassam Z. Shakhashiri, the director of the Wisconsin Initiative for Science Literacy (WISL), encouraged all UW-Madison chemistry Ph.D. candidates to include a chapter in their Ph.D. thesis communicating their research to non-specialists. The goal is to explain the candidate's scholarly research and its significance to a wider audience that includes family members, friends, civic groups, newspaper reporters, program officers at appropriate funding agencies, state legislators, and members of the U.S. Congress.

Over 50 Ph.D. degree recipients have successfully completed their theses and included such a chapter.

WISL encourages the inclusion of such chapters in all Ph.D. theses everywhere through the cooperation of Ph.D. candidates and their mentors. WISL is now offering additional awards of $250 for UW-Madison chemistry Ph.D. candidates.

Wisconsin Initiative for Science Literacy

The dual mission of the Wisconsin Initiative for Science Literacy is to promote literacy in science, mathematics and technology among the general public and to attract future generations to careers in research, teaching and public service.

UW-Madison Department of Chemistry
1101 University Avenue
Madison, WI 53706-1396
Contact: Prof. Bassam Z. Shakhashiri
bassam@chem.wisc.edu
www.scifun.org

Phase Transitions in Molecular Solids: Understanding Polymorphic Transformation and Crystal Nucleation, and Engineering Amorphous Drugs for Global Health

By

Yue Gui

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This dissertation is approved by the following members of the Final Oral Committee:

Lian Yu, Professor, Pharmacy and Chemistry (Dissertation Advisor)
Glen Kwon, Professor, Pharmacy
Sandro Mecozzi, Professor, Pharmacy
Mark D. Ediger, Professor, Chemistry

Chapter 7

Communicating Research to Non-Expert Audiences as a Part of the Wisconsin Initiative for Science Literacy Program

Yue Gui

One day last summer I was chatting with my mom over the phone and she told me some life wisdom: chocolate tastes better when kept in the refrigerator. This opinion was based on her experience with the dark chocolates she had bought a few weeks prior. She put some of them in the refrigerator and left the others on the dining table for convenience. Gradually she found those on the dining table did not taste as good as before, some turned white in color and tasted like wax, while those in the fridge became yummier than the newly-bought ones. Immediately I realized it was related to what I was studying in graduate school — phase transitions in solids — and I was excited to have the excellent chance to explain my research to my mom. That was an impressive conversation and one of the best moments in my research journey. Now I am glad to have such a platform to explain and discuss some aspects of my work to more people, thanks to the Wisconsin Initiative for Science Literacy at UW-Madison.

In this chapter, readers will develop a better understanding of solids and their microscopic structures. After reading, you will hopefully be able to answer the following questions:

(1) Why do chocolates change color and taste when stored in the summer?

(2) People usually say “diamonds are forever.” Is that true from a scientific standpoint?

(3) Why is cotton candy sweeter than traditional sugar cube?

(4) Why do we care about solid state forms in medicines?

A. What is a solid?

Solids are everywhere: our bones and teeth, eyeglasses, snowflakes, diamonds, chocolates, etc. A common feature for solids is that they do not flow to occupy the container like water, nor do they expand to fill the entire space like air. Instead, a solid can usually keep its own shape even when a little force is applied. For this reason, most medicines are designed as solids, such as tablets or capsules, for the ease of handling during storage, transportation and prescription.

How do we define a solid? For example, how can we tell whether a well-cut shining transparent solid is a real diamond or not (Figure 1)? The answer lies in their *chemical composition* and

microscopic organizations (structures). Diamonds are composed of carbon atoms. These atoms pack in a certain way to form a unit that is repeated numerous times to form a diamond. In comparison, diamond simulants are usually not composed of carbon and the atomic arrangement is also different from that in real diamonds. So by identifying the chemical compositions we can easily identify a real diamond.

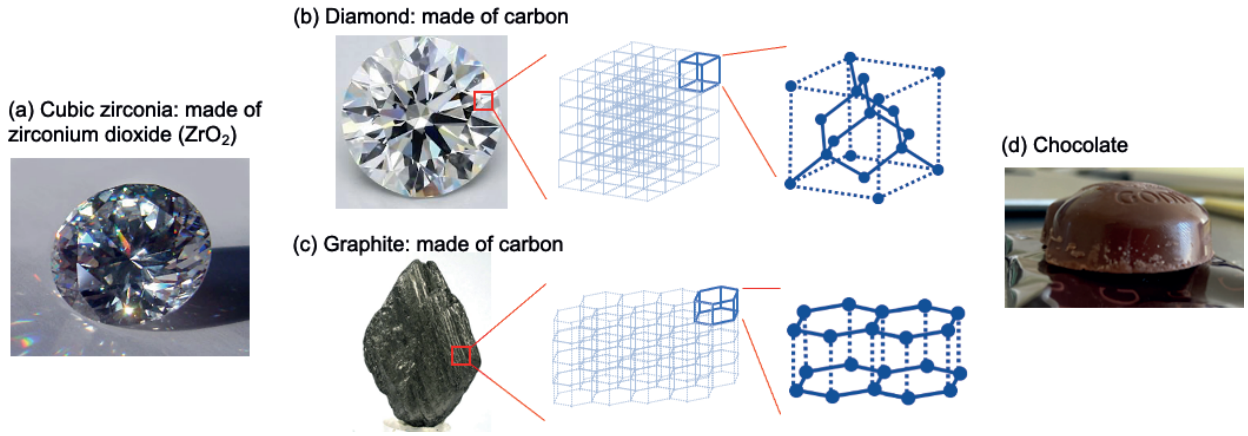

Figure 1. Various crystalline solids. (a) Cubic zirconia is an important diamond simulant. It looks almost the same as a diamond but is composed of zirconium dioxide. This is different from a real diamond, which is composed of carbon. (b, c) Diamonds and graphite are polymorphs: both are made of carbon atoms, but they have different crystal structures. (d) My chocolate is also a crystal and is polymorphic. After a long-term storage during summer it became white and did not taste as good due to a polymorph change.

Microscopic structure is another aspect we use to define a solid. Diamond and graphite are both composed of carbon atoms but are clearly very different solids – people wear diamond rings, not graphite rings. Structurally, the distinction is in their repeating units (Figure 1). Diamond has cubic units, while graphite has hexagonal units. These units define what is a diamond and what is a graphite. So even when a piece of graphite is carefully cut into the shape of a diamond, it is still a graphite. The phenomenon that the same composition (carbon) forms solids with different structures (diamond or graphite) is called "*polymorphism,*" and these solids are called "*polymorphs.*"

Solids that have repeating units are *crystals*. Solids in which there are no repeating units are "*amorphous solids*" or "*glasses*." From the name, it is easy to tell that window glasses and eyeglasses fall into this category (Figure 2). Additionally, plastics and rubbers are also in this family, despite their different appearances. This is another example where microscopic structure defines a solid: window glasses and rubbers share similar internal structures, thus they belong to the same class of solids. In amorphous solids, the molecules do not have a regular order like those in crystals; instead, they are rather disorganized, leading to distinct properties.

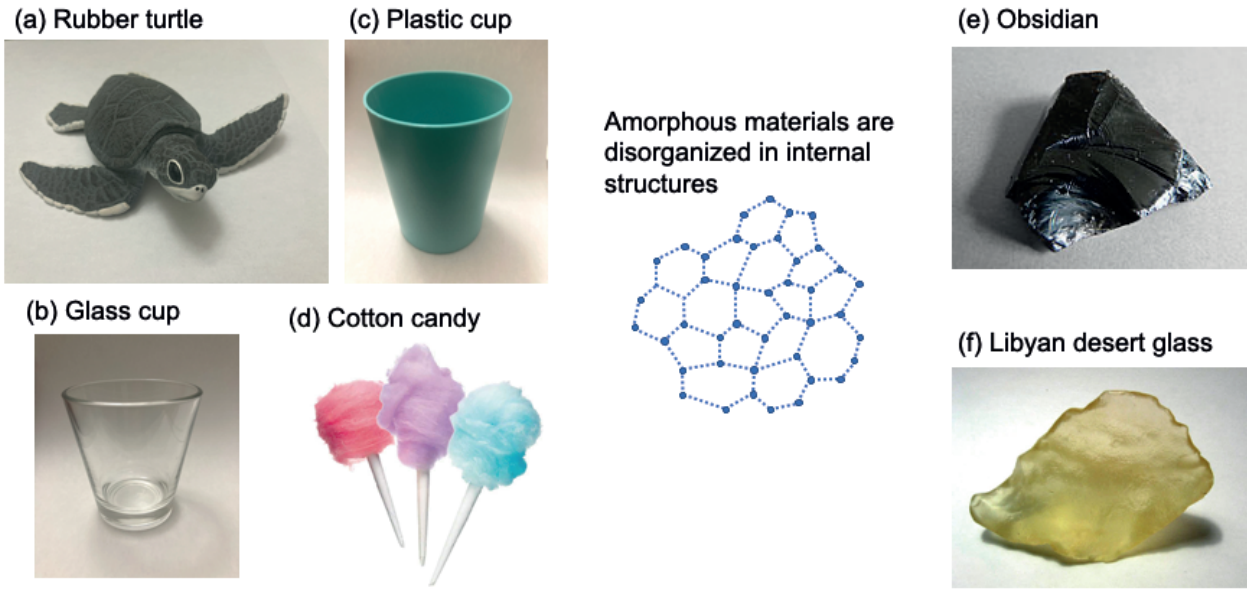

Figure 2. Amorphous solids in my home (a-d) and in nature (e, f). Rubbers (a), glasses (b), plastics (c) and cotton candies (d) are commonly seen man-made amorphous solids. Obsidian (e) and Libyan desert glass (f) are natural amorphous solids and are usually used as decorations. Unlike crystalline solids, amorphous solids do not have organized structures.

Scientists usually prepare amorphous solids by melting a crystalline solid, then quickly cooling the melt. One example in nature is the formation of obsidian from lava, which is molten rock. When a volcano erupts, it extrudes lava at a high temperature. The lava then cools rapidly on the land or in water, resulting in the natural glass obsidian. Another example is when lightning strikes

sand in the desert: crystalline sands become molten immediately, but quickly cool to form natural glasses. The process of fast cooling is the key to making glasses. If the melt (liquid) cools too slowly, it can crystallize into crystalline solids. With fast cooling, molecules do not have enough time to align well and are trapped in a similar arrangement to the liquid state. In this way, we can prepare glasses by avoiding crystallization. So amorphous solid are also referred to as "*supercooled liquids*" to indicate their disorganized liquid-like structures.

An analog to *crystalline solids* and *amorphous solids* is a scene in the bus station. Imagine a large group of people are waiting at a bus station and the weather is super nice. When the bus comes, everyone steps on the bus and finds a seat, calm and relaxed, until the bus is full. This status mimics a *crystalline solid* where all molecules (people) are well organized and relaxed. However, if this scene is on a cold winter night, people may be so eager to get into the bus that they do not have enough time to find a suitable seat, so some of them may stand in the aisle. This status mimics an *amorphous solid* where molecules (people) are not well organized and may not be relaxed. Clearly we can see *amorphous solids* are less stable than *crystalline solids*. In chemistry the stability is represented by *free energy*: the higher the free energy of the system is, the less stable the system.

B. How do solids' structures determine their properties?

People usually say, "diamonds are forever." But this is not true, scientifically. From the previous section, we already know that diamonds and graphite are polymorphs, meaning they have the same composition (carbon), but different crystal structures. Their different structures result in different properties, such as color, hardness, density, and even stability. It may surprise you to learn that diamonds are actually less stable than graphite at normal temperature and pressure. That is to say, a diamond will eventually transform into graphite given a long enough time, maybe millions of years or longer. So next time when one wants to find something that can last for a long time, graphite could even be a better choice than a diamond!

Chocolates, as mentioned in the beginning of this chapter, also have polymorphs. Composed of cocoa powder, sugar, fat and other ingredients, chocolates are crystalline solids with a unit repeating over and over again. Given different units, chocolates will present a different taste,

texture, color and melting point. Some old chocolates turn white on the surface and taste like wax, especially after melting in summertime, a result of polymorph changes after recrystallizing from the melt. So in order to make delicious chocolates, we need to control the crystallization process carefully to obtain the right polymorph, which should be solid during storage, but melt quickly in the mouth; it should look glossy and feel silky. A wrong polymorph could have an improper melting point that is too high to melt in your mouth (making it taste like wax) or too low to be stored at room temperature ("liquid" chocolate).

In pharmaceutics, polymorphism is also an essential consideration. Polymorphs of a certain drug usually have different solubilities, thus will be absorbed differently in our body. Generally speaking, a more soluble polymorph is easier to manufacture and will have a larger portion of a dose that enters blood circulation for the desired therapeutic effect. An improper polymorph can turn an effective formulation ineffective or even not manufacturable. An extraordinary example is the antiviral compound ritonavir (Figure 3). First marketed in 1996 as Form I (the only crystal form known at the time) for the treatment of Acquired Immunodeficiency Syndrome (AIDS), a new polymorph of ritonavir, Form II, appeared in some batches, and quickly dominated all manufacturing areas. Form II has solubility only one fourth of that of Form I, which forced the manufacturer Abbott to develop a new formulation based on soft-gelatin capsules. This nightmare cost Abbott nearly one billion dollars.

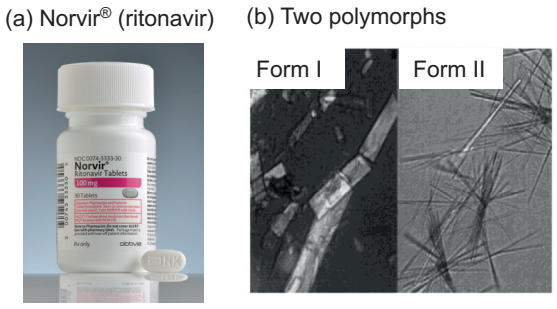

Figure 3. The antiviral drug ritonavir is polymorphic. Form I and Form II have different structures, different appearance and different solubility. The sudden appearance of the poorly soluble Form II was a big trouble to its maker.

But the ritonavir case was not good-for-nothing. It immediately convinced everybody of the importance of polymorphism in drug development and kickstarted research in this field. Soon after, researchers realized an interesting fact: almost all drugs are polymorphic. This phenomenon is a double-edged sword in pharmaceutical sciences. On the one hand, the selection of a proper polymorph could help us develop more effective drugs. On the other hand, nobody wants a second "ritonavir" case with an unexpected appearance of a new polymorph. In order to take full advantage of polymorphism and at the same time avoid risks, at present the best way is to screen for as many polymorphs as possible before marketing. Such a screening is also required by the FDA before the organization will grant patent approval. In addition, the FDA also announced that each polymorph can be patented individually, which is another big motivation for pharmaceutical companies to screen for polymorphs – they can obtain a longer protection of pharmaceutical patents in law by patenting various polymorphs of the same drug molecule. Nevertheless, such regulations greatly promote the development of drug formulations and result in better medicines. One part of my research focuses on the discovery and prediction of new polymorphs of old drugs, as will be discussed later.

In addition to polymorphs, preparing amorphous drugs is another way to enhance solubilities of drugs. As mentioned earlier, amorphous solids do not have repeating units in the structure because they are cooled too fast to allow the molecules to align well. So amorphous solids have higher free energy, meaning they are less stable, than crystalline solids. As a result, they are more soluble and dissolve faster. We can easily understand this difference by recalling the bus station analogy. When the bus arrives at the destination, passengers will get out of the bus, which mimics the dissolution process. In the messy, not organized bus (amorphous status), all passengers will try their best to get off the bus quickly, so the dissolution rate is fast. In comparison, those who are relaxed in the well-organized bus (crystalline status) will step out with no rush, suggesting a slow dissolution. At the same time, some passengers may even prefer to stay on the bus for another bus tour, so fewer passengers get out, mimicking a situation where fewer molecules dissolve, which is scientifically called low solubility.

An example of the fast dissolution of amorphous solids is the experience of licking cotton candy and sugar cube: cotton candy tastes sweet immediately, even if just a bit touches the tongue, while sugar cube may take a second to become sweet. This is because cotton candy is amorphous while

sugar cube is crystalline. The faster dissolution of amorphous solids makes cotton candy sweeter than sugar cube in the first seconds.

A key issue in developing amorphous solids, however, is that amorphous solids will eventually crystallize to become more stable crystals, which will kill all the advantages of the amorphous solid. In pharmaceutics, uncontrolled crystallization during drug manufacture and storage would be a nightmare, just like the unexpected polymorph transition as mentioned earlier. So understanding and controlling the crystallization process of amorphous drugs is an important topic in drug development.

My research focuses on the transformations among these structures, including transitions among polymorphs, transitions from amorphous to crystalline, as well as inhibition of crystallization for amorphous drugs. In the next sections, I will introduce more details about these three topics: (1) new polymorphs of an old drug and their transformations; (2) the mechanism of crystallization and (3) development of more stable and more soluble amorphous drugs.

C. Topic 1: A story on polymorph hunting

Nifedipine is an old drug for the treatment of high blood pressure. It is one of the essential medicines suggested by the World Health Organization (WHO) for global health. First patented in 1967, nifedipine has up to now over thirty cousins marketed including amlodipine (brand name Norvasc), which was the 5th most prescribed medicine in the US in 2017. Despite the long history of research and its clinical importance, nifedipine remains a mystery in polymorph studies. In over 50 years, researchers had only been able to verify the existence and structures of two polymorphs: Form α (pronounced “alpha”) and Form β (pronounced “beta”), though there was some indication that there could be more polymorphs of nifedipine. These undiscovered forms were referred to as “ghost” crystals by some researchers because people did not know what they were, when they would appear, even whether they were real polymorphs of nifedipine or not.

My work started here: identifying these mysterious polymorphs. I was so fortunate to find the first “ghost” that had rarely been reported after 1977 in my first sets of experiments. The experimental conditions were simple: melt nifedipine crystals and hold the melt at a certain temperature (212 °F)

for two minutes, then the new polymorph appears! But why, historically, was it missed for such a long time? Well, maybe because this crystal is too “shy” and it tries very hard to hide itself. My studies show it can only be obtained in a narrow temperature range and it grows slower than other polymorphs, so its crystals are small and hard to notice. Besides, it looks pretty similar to Form α from the external appearance despite its different internal structures, making it easily confused with Form α crystals. Anyway, the good news is we found it and gave it a name: Form γ’ (pronounced “gamma prime”, the symbol “prime” is to distinguish it from its low-temperature form, see below).

When I tried to “see” the internal structure of Form γ’ using a “super microscope,” a second new polymorph, γ (pronounced “gamma”), showed up. The scientific name of this “super microscope” is X-ray diffractometer, which uses a kind of special light, X-ray, to detect the accurate position of atoms and molecules in a crystal. Then through mathematical modeling, we can directly “see” what the internal structure of the crystal looks like. The workflow is similar to its medical application to generate images of our bones and joints. Typically it is easier to “see” a crystal structure at a very low temperature because the atoms are “frozen” and cannot move much during the measurement. So we tried for Form γ’ first at -320 °F, a temperature that is low enough to freeze nitrogen gas into liquid. According to our experience, such a temperature is usually friendly to most crystals and does not hurt (break) crystals. But surprisingly, in contrast to our prior experience, the Form γ’ crystals always broke into many small pieces once we exposed them to the low temperature.

This puzzle led us to think maybe the crystal changed its internal structure so much during cooling that its macroscopic shape could not handle the change, causing it to crack. With this hypothesis, we selected a smaller crystal for the measurement, hoping its internal structure change was not severe enough to cause breakage. It turned out to be an effective solution and allowed us to get the second new structure, Form γ, the low-temperature counterpart of Form γ’. Comparing the two structures, they are similar but different: Each single molecule in the two structures looks similar, but molecules in the low-temperature Form γ are much more crowded than in Form γ’, meaning the former has a smaller volume. This difference in structure explains why the first crystals always cracked during cooling: it shrank too much to keep the original shape and had to develop a fracture

in the crystal. When we used smaller crystals for such a cooling process, the volume change was not significant enough to cause this fracture.

Months later we successfully discovered the third new polymorph, β' (pronounced "beta prime") and "saw" its internal structures. We proved it was a high-temperature counterpart of Form β. Again, the two structures are similar in terms of single molecules, but different in crystal volume. The low-temperature Form β has a smaller volume than the high-temperature Form β', again, similar to the γ/γ' case. The difference between the two pairs, though, is that the volume change for β/β' is smaller than that for γ/γ', so the Form β crystal did not show much cracking during our tests. By this point in my research, we had already found, identified and knew the structures of all the so-called "ghost" crystals.

But my polymorph hunting journey still continued. A remaining mystery was that most crystals of nifedipine's cousins consist of molecules with both "arms" up (see Figure 4), while all discovered nifedipine crystals only contained molecules with one "arm" up and another down. By conducting computational calculations, we found that a single molecule actually prefers to have both "arms" up, rather than one up/one down, as in the currently known crystals. This led me to think of an innovative method for hunting: why not use nifedipine's cousin as a template, then grow nifedipine crystals on the template? It was a surprise when I saw the success of this crazy idea – we discovered a totally new structure of nifedipine with both "arms" up! To follow the previous naming, we called it Form δ (pronounced "delta").

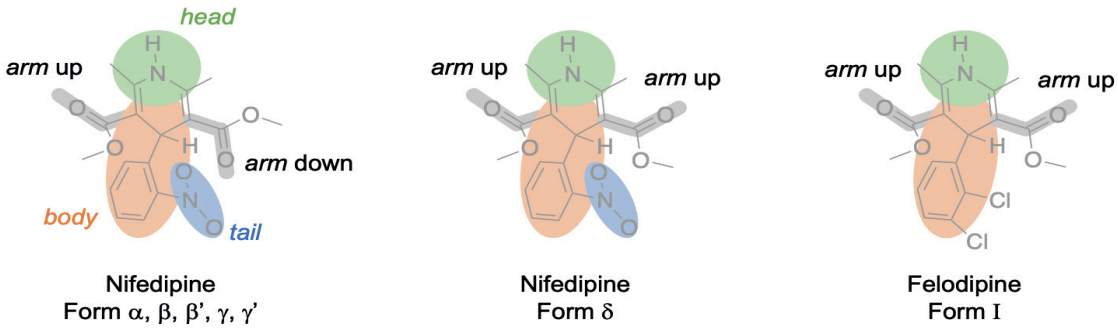

Figure 4. Nifedipine Form δ is different from all other polymorphs in that it has both "arms" up. The discovery of nifedipine Form δ employed its cousin felodipine as the template, which also has both "arms" up.

In this polymorph hunting, we successfully discovered and "saw" structures of 4 new polymorphs, bringing the total number of nifedipine polymorphs to six. Have we found them all? Unfortunately nobody can give a definite answer at present. Currently we are making efforts to predict crystal structures using a computer. That is, a computer first proposes a huge number of possible "repeating units" that constitute a crystal (Figure 1), then evaluates how stable each proposed structure is by estimating its free energy. The lower the free energy, the more stable the structure. The computer will delete all unstable structures and only leave the relatively stable ones. Finally, the computer ranks all remaining structures according to their free energy and compares experimentally known structures with the predicted ones. Ideally all known structures should be successfully predicted, and most stable polymorphs should be discovered in experiments. If not, we need to review the computer prediction process and/or screen for more polymorphs in experiments. We, as well as other scientists all over the world, believe such practices will push the polymorphism studies forward in an effective and inexpensive way, and will help pharmaceutical companies find the best polymorph for clinical use.

D. Topic 1 (continued): Why do some polymorphs transform during heating but the others do not?

In the last section we went through the journey to discover and learn the structures of 6 polymorphs of nifedipine, which involved two interesting observations: Form β transformed to Form β' during heating and the reverse transition happened during cooling; Form γ transformed to Form γ' during heating and the reverse transition happened during cooling. In contrast, there was no transition for Form α or δ. This begs the question: what causes the difference? In other words, why do some polymorphs of nifedipine undergo a transformation during temperature changes, while the others do not?

If you find it hard to picture a reversible transition, try to think of the Transformer Bumblebee (Figure 5a). It has excellent reversibility and can easily convert between Bumblebee and the yellow Beetle. The two states have exactly the same composition, but have different structures, properties, power (energy state), and shapes. This is the same as a reversible transition between polymorphs: each polymorph can easily convert into the other and present different structures and properties.

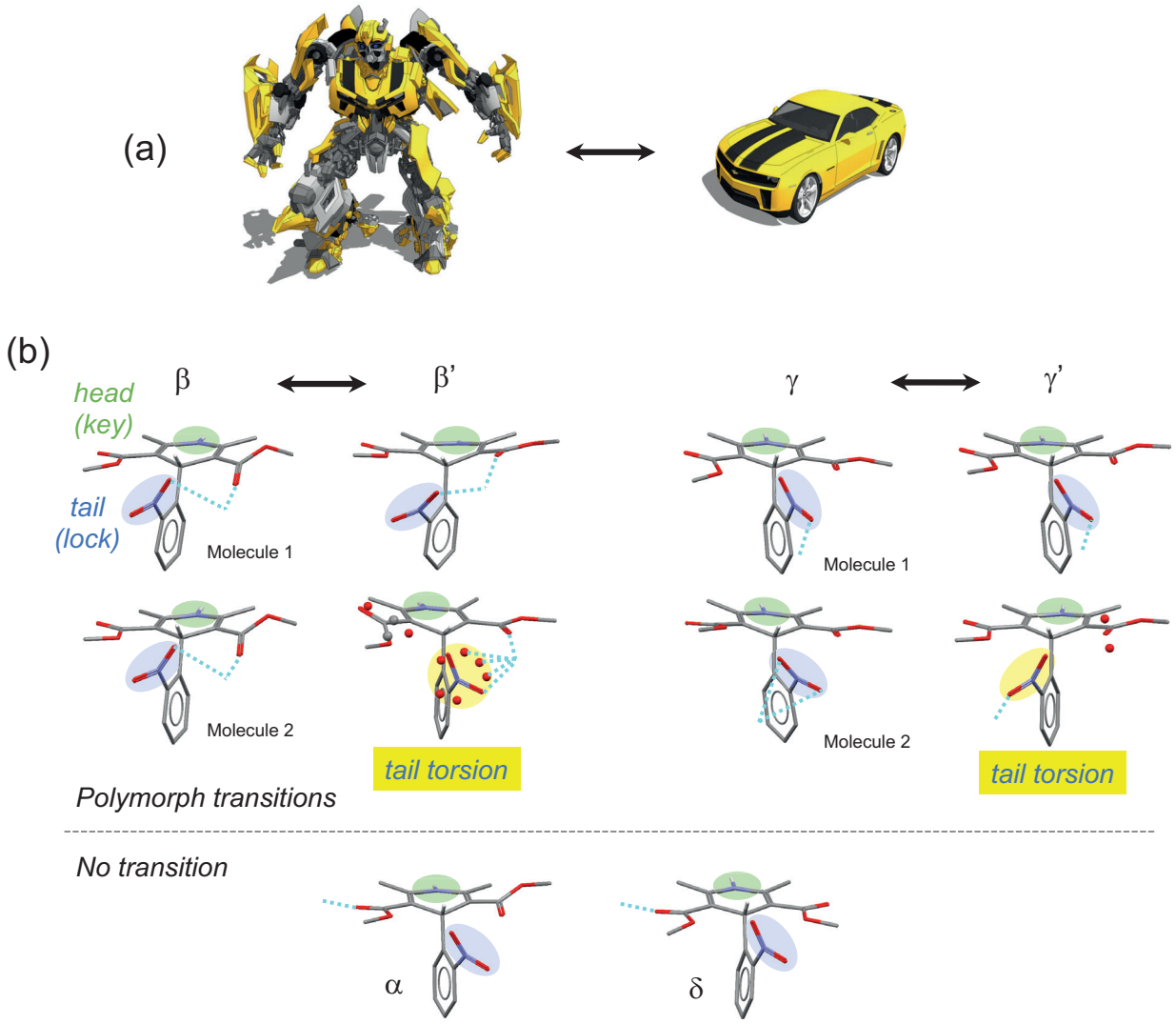

Figure 5. (a) Bumblebee can reversibly transform into the yellow Beetle, a mimic of reversible transitions in crystals. (b) Reversible transitions among nifedipine polymorphs. Some show reversible transformation (β and β', γ and γ') during heating and cooling, but the others do not (α and δ). Connected solid lines constitute a nifedipine molecule. Green areas are the "head" of the molecule acting as a key; blue and yellow areas are the tail of the molecule acting as a lock. Cyan dashed lines indicate communications between two adjacent molecules and the open end for each dashed line is the neighbor molecule's "head." In polymorphs that show transformations, the *lock* of one molecule and the *key* of its neighbor communicate well, so a *lock* rotation helps pull the two closer to make a more stable structure. In comparison, in polymorphs that do not show a transformation, the *lock* of one molecule can not communicate with the *key* of its neighbor (no dashed line around the blue area), so the molecule does not have the motivation to change the structure.

So why can Bumblebee reversibly transform, but our own yellow Beetle cannot? If we know the answer clearly, we may also be able to make our own Bumblebee. Similarly, for crystal polymorphs, we want to understand why some polymorphs undergo reversible transition, while others do not, so we are able to design more crystals that have such a phenomenon in the future.

From a scientific standpoint, it is an interesting and important research question because reversible phase transition plays a big role in materials sciences. In material sciences, crystals absorb and release heat during transitions, which can be used as an energy pocket to store and transport energy. Some crystals have a notable shape change during transitions, such as bending, twisting, and even jumping and rolling due to sudden expansion or shrinking. We can use these materials to make indicators for temperature change, or make switches that will turn on or off at different temperatures. Another application is using pressure-responsive materials, which are soft when there is no pressure, and become hard when they are compressed. We can employ these materials to design soft robotics that are soft enough when relaxed and are hard enough to lift up heavy items when compressed.

Then back to our question: what causes the difference in reversibility in polymorphs? Bumblebee can transform because it has a specific *structure* that allows it to transform, which our Beetles do not have. If we designed our Beetles the same way as Bumblebee, they would also be Transformers. Similarly, for crystals, the reversible transforming polymorphs and non-transforming polymorphs must have different *structures* that lead to their different behaviors. So next we are going to have a close look at nifedipine structures.

As shown in Figure 5b, each nifedipine molecule contains an amino group as the “head” and a nitro group as the “tail”; the “head” consists of a nitrogen atom and a hydrogen atom, acting as a *key*; while the “tail” consists of a nitrogen atom and two oxygen atoms, acting as a *lock*. A *lock* and a *key* in the same molecule can never reach each other, but a *lock* has the opportunity to reach the *key* in its neighboring molecule. In polymorphs that undergo structural change (β and β’, γ and γ’), such a connection happens, and the *key* in one molecule pairs with the *lock* in its neighbor. As the temperature changes, the *lock* rotates and the *key* opens the *lock*, making the whole structure more relaxed and more stable. In comparison, in polymorphs that do not undergo a structural

change, this connection does not happen. So the *key* fails to open the *lock*. In this case, as the temperature changes, the *lock* does not pair with the *key*, so no structural change occurs.

Up to this point, the *key-lock* model works fine in all nifedipine polymorphs: when the *lock* reaches the *key*, the *lock* prefers to rotate to better adapt with the *key*; otherwise the *lock* does not rotate. But what if the *lock* is trapped at a certain position and is not able to move? Nitroaniline is such an example (Figure 6). Similar to nifedipine, nitroaniline also has a "head" (*key*) and a "tail" (*lock*), and the *lock* in one molecule can reach the *key* in its neighbor. However, in nitroaniline, the *lock* is fixed to make itself flat and more stable. That is to say, its *lock* is not able to rotate even though it reaches the *key*. So there is no reversible transition in nitroaniline. In comparison, the nifedipine molecule is not flat. Its large "body" pushes the "tail" (*lock*) to twist out of the plane. In this case, the *lock* is not trapped in a flat mode and is able to freely rotate. The rotation of the *lock* leads to reversible transitions in some nifedipine polymorphs.

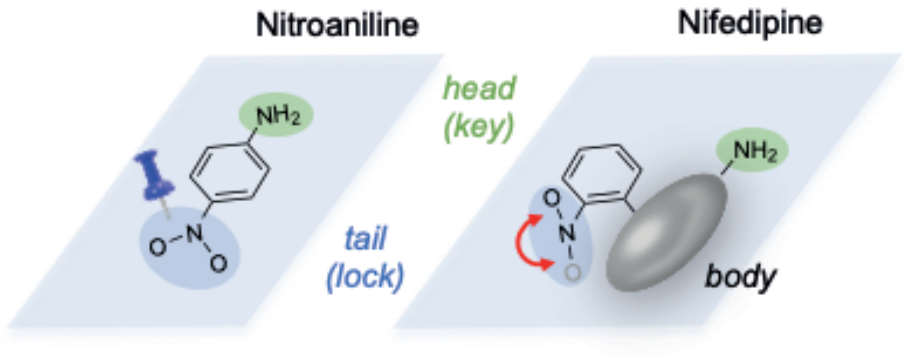

Figure 6. Comparison of nitroaniline and nifedipine. The *lock* in nitroaniline is locked to make the molecule flat, so nitroaniline does not undergo reversible transition as the temperature changes. Nifedipine is not a flat molecule and its *lock* can freely rotate, making it possible to transform.

Now we can answer our question of why some polymorphs undergo reversible transitions. That is because in these structures, the *lock* reaches the *key* and rotates to open the *key*, making the whole structure more relaxed and more stable. If the *lock* cannot reach the *key* or cannot freely rotate, then no reversible transition will occur. More generally, for any given crystal (not just nifedipine),

we expect it to undergo reversible polymorph transition if (1) its nitro "tail" (*lock*) reaches its neighbor's "head" (*key*) and (2) the nitro "tail" (*lock*) is not trapped and can freely rotate. In other words, the molecule must be willing and able to change its structure. In the future, we are going to apply our understanding of reversible transitions to design more powerful "Bumblebees."

E. Topic 2: From amorphous to crystalline: which polymorph do we get?

We already learned about the structural difference and relationship between amorphous materials and crystals: amorphous materials have disordered internal structures and will eventually crystallize into well-organized crystals. We also know that crystal structure can vary to form polymorphs. Then a natural question to ask is: which polymorph does an amorphous material crystallize into?

We care about the polymorph of crystallization products because polymorphs have different structures and properties, as discussed earlier. If we use an improper polymorph of pharmaceutics, explosives, and dyes and pigments, we may have big trouble (see Section B for examples). In addition, polymorph selection is also closely related to most lives on the Earth. For example, calcium carbonate is the primary constituent of the shells of corals and most marine organisms, and even our inner ears which control our balance. Do you know that crystals in these organisms also have polymorphs? Calcium carbonate exists in the form "calcite" in most shells, and the form "aragonite" in corals and inner ears. Organisms are so smart that they can always crystallize the desired polymorph. One goal of my research is to develop a fundamental understanding of polymorph selection during crystallization in order to prepare better medicines and pigments.

How do we know which polymorph crystallization will produce? We need to understand the crystallization process. Crystallization is similar to a potato harvest, to some extent (see Figure 7 for a comparison). To harvest daughter potatoes from a fresh parent potato, first the parent potato needs to sprout and form the so-called "potato eyes." Then each of these eyes gradually grows, eventually becoming a new, mature potato. During the process, the formation of eyes is the first step. No eyes, no new potatoes. The parent potato may take a long time to form the first eye, then a shorter time for the second one, and gradually it forms a similar number of eyes every day.

Though it sounds like we can predict the total number of eyes at a certain date, the formation of each single eye is always random. That is to say, we are not able to predict exactly when and where the next eye will form.

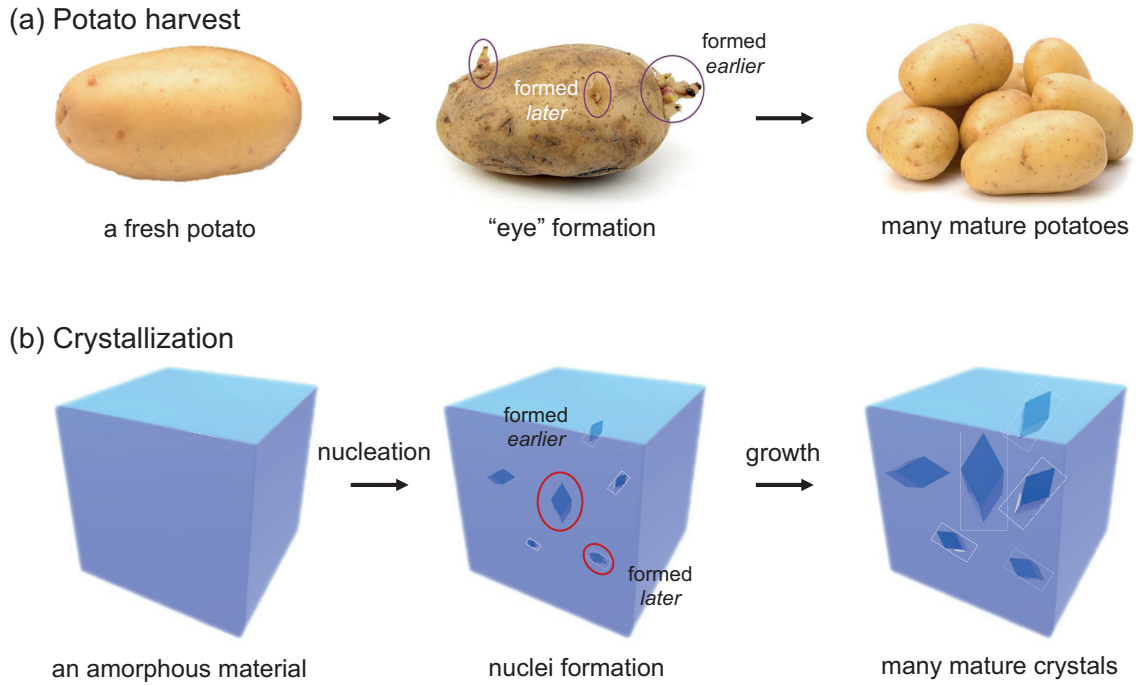

Figure 7. Potato harvest (a) and crystallization (b) have similar procedures. The first step is the formation of growing points, either potato eyes or crystal nuclei. The occurrence of each growing point is random, so they are different locations and sizes, as indicated in the circles. Larger potato eyes or nuclei formed earlier. The second step is the growth from the growing points, until forming mature potatoes/crystals.

Similarly, crystallization is also a two-step process, crystal *nucleation* and crystal *growth*. Crystal *nucleation* is the formation of tiny nuclei from the system, which is parallel to the formation of potato eyes: it must be the first step in crystallization; it usually takes a while to form the first nucleus, then a shorter time for the second one, and gradually it forms a similar number of nuclei every day; the formation of each nucleus is random and unpredictable. Then in the second step, which is called crystal *growth*, each of the nuclei gradually grows, eventually forming a mature

crystal, similar to the growth of potatoes. Again, if there is no *nucleation*, there is no *growth* and no mature crystal.

Crystal nucleation is important – it defines the final crystallization product. In most cases, a nucleus with a certain internal structure grows into a mature crystal that has the same structure, meaning they are the same polymorph. So in order to predict which polymorph we will get from crystallization, it is an essential task to understand which polymorph nucleates.

Unfortunately, despite its importance, researchers studied crystal nucleation much less than crystal growth. In past decades, scientists have only measured crystal nucleation rates for a limited number of materials. In contrast, we have abundant datasets for crystal growth rates in various systems. Why the difference? Because crystal nucleation is a random and unpredictable process compared to crystal growth, and nuclei are tiny and hard to catch – the diameter of a nucleus is about one-thousandth of the width of our hair! So compared to crystal growth, it is much harder and more inconvenient to study crystal nucleation.

My research aims to understand crystallization and its polymorph outcome by studying crystal nucleation. The two questions that we would like to answer are: (1) How fast do polymorphs nucleate? (2) If polymorphs nucleate at different rates, which one will win? Our long-term goal for the second question is to predict the fast-nucleating polymorph without doing any experiments, instead only comparing the structures and properties of polymorphs. This would immensely raise the efficiency in drug manufacturing.

After measuring how fast polymorphs nucleate, we found the nucleation rates presented an interesting inverted U-shape, as shown in Figure 8. This is because at very low temperatures, all molecules are “frozen” and can hardly move to form nuclei. At very high temperatures, the molecules are very “hot” and move too fast to maintain an organized structure, so nuclei also cannot form. When it is neither too cold nor too hot, nuclei form at the fastest rate.

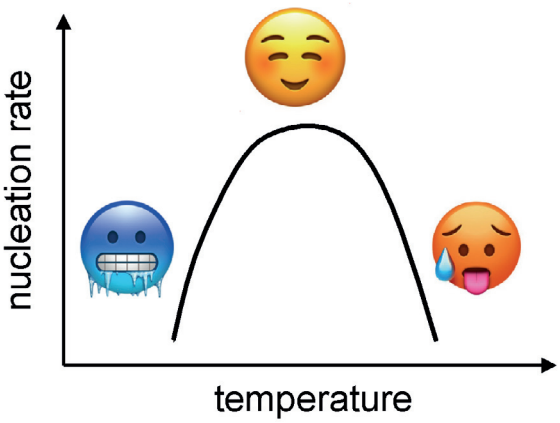

Figure 8. Nucleation rates have an inverted U-shaped relationship with temperature and reach a maximum at a moderate temperature.

Another interesting discovery is that only a few polymorphs nucleate and all other polymorphs barely nucleate. Why are they so different? By comparing these fast-nucleating polymorphs, we found they are more similar to the amorphous state than other polymorphs in terms of energy state and structure. This is consistent with our intuition that transformations between similar structures are easier than between totally different structures. Nucleation process starts from the amorphous state, so it prefers to nucleate crystals that have similar structures.

With such understanding, we will be better able to predict crystallization product. Even though at this stage we still rely on experiments to know exactly how fast polymorphs nucleate, this work does provide us some abilities to predict which polymorph nucleates fast.

F. Topic 3: For global health: make better amorphous medicines

Most medicines are available as tablets or capsules. A tablet or a capsule must first dissolve in our gastric or intestinal tract, then be absorbed into blood circulation before performing its therapeutic effect. However, more and more drug candidates in development are suffering from poor solubility. When only a limited amount of the drug dissolves in the gastrointestinal tract, its therapeutic effect is reduced. One of the most effective solutions is to make amorphous drugs to replace their

crystalline counterparts. An ideal amorphous formulation for global health applications should meet the following requirements:

(1) *High drug loading*: The majority of a tablet/capsule is the active ingredient that has therapeutic effects (as opposed to inactive additives), so patients only need to take a limited amount of medicine. High drug loading will benefit patients practically and economically.

(2) *Stability against crystallization under tropical conditions*: An amorphous formulation is stable enough for shelf storage and transportation with no concern of crystallization, especially in tropical areas (high temperature, high humidity). This will guarantee the effectiveness of the medicine globally.

(3) *Simple and inexpensive manufacturing process*: Low-tech and low-cost manufacturing will allow developing countries to produce medicines by themselves so that the drug price will be reduced and more people will have access to these medicines.

(4) *Fast dissolution*: Fast dissolution in the stomach and intestinal tract facilitates drug absorption into blood circulation to perform the desired therapeutic effect.

Currently, amorphous formulations in the market usually contain 70% - 80% additives in order to achieve enough stability and a fast dissolution rate at the expense of drug loading (only 20% - 30% drug in a tablet/capsule) and manufacturing cost. Supported by the Bill & Melinda Gates Foundation, our group has been aiming to develop high drug loading amorphous drugs with enhanced stability and dissolution at low cost, in order to help more people all over the world.

In the previous studies, our lab has already found that the most vulnerable part in amorphous drugs is their surfaces. Surface crystallization is usually much faster than interior crystallization. So if we can find a way to protect their surfaces, we may be able to protect the entire amorphous drug particles and maintain high drug loading. How do we protect surfaces conveniently?

Surface coating is a straightforward method. The famous caramel apples in Wisconsin are an excellent example of surface coating. The delicious chocolate dipped ice cream cone is another

great application. For drug particles, we can apply the same strategy to coat a thin protection layer on their surfaces without affecting the interior (Figure 9).

Figure 9. Surface coating in caramel apples, chocolate dipped cone, and amorphous drugs.

We took advantage of the acid-base chemistry in the surface coating because most drugs are either acids or bases. Acid-base reactions are carried out everyday in our kitchen: baking soda (a base) and vinegar (an acid) work together to form a salt and at the same time release a lot of carbon dioxide to leaven a cake. So for an acidic amorphous drug, a base is expected to automatically deposit to the drug particle surfaces to form a protection layer; while for a basic drug, an acid is expected to do the same.

The acid-base reaction also guarantees an extremely thin coating film with a thickness of only several nanometers, about 1/100,000th the thickness of a piece of copy paper, allowing us to prepare amorphous drugs at over 99% drug loading. That means, almost everything in a tablet has an active therapeutic effect. If a patient is taking 5 tablets per day now (assume a 20% drug loading as a typical level for the marketed amorphous drugs), he/she only needs to take 1 tablet using our new formulation.

Amorphous drugs with extremely high drug loading are also more stable. Under dry conditions (high temperature, low humidity), the coated amorphous drugs do not crystallize within at least half a year, while the uncoated ones crystallize fast. However, under tropical conditions (high

temperature, high humidity), the coated amorphous drugs also present a small amount of crystallization. We attributed the difference to the water effect: water penetrates to the interior of amorphous drugs and causes crystallization. The coating layer only works well on the surface, the interior drug is protected very little.

In order to further improve the stability of amorphous drugs under tropical conditions, we then applied the acid-base chemistry to the entire drug, not only on the surface. That is, we mixed the basic (or acidic) drug and the acidic (or basic) protection material together and let them react to form an amorphous salt. In this way, the amorphous salt of the drug is prepared at 75% drug loading and is stable with no crystallization under tropical conditions for at least half a year, overperforming other formulations. At the same time, it has outstanding dissolution performance in simulated gastric fluid and simulated intestinal fluid, indicating a better absorption in our body.

The important takeaway from this is that we developed two cutting-edge strategies, the surface coating strategy and the mixing strategy, to prepare better amorphous drugs. The new products have at least three times higher drug loading compared with current ones, up to 75% (mixing) or 99%+ (surface coating). At such high drug loading, our amorphous drugs present more remarkable stability against crystallization under tropical conditions, allowing us to apply these processes in tropical areas. In addition, our products have better dissolution, suggesting a better absorption in our body.

For future studies, we will continue to optimize the two strategies. Each of them has its own advantages: surface coating provides extremely high drug loading; mixing provides extremely great stability against crystallization. The common features are that both can be manufactured conveniently at low cost, and both can improve drug dissolution in the gastrointestinal tract. So in the future, the next optimization will be carried out by combining both strategies in order to find the best balance between drug loading and stability.

G. Summary

If I have to summarize my thesis work in one word, it would be "*transition*."

In Sections A and B, we learned about two classes of solids: *amorphous solids* and *crystalline solids*. The internal structures of amorphous solids are not well-organized, while crystals have highly ordered structures. Some materials have more than one crystalline structure, forming *polymorphs*.

In Section C, I talked about my adventures in polymorph hunting for an old drug, nifedipine, which turned out to be an excellent opportunity to discover a new mechanism of reversible *transitions* among polymorphs, as discussed in Section D. As explained in depth above, we expect a crystal to undergo reversible polymorph transition if (1) its *lock* reaches its neighbor's *key* and (2) the *lock* is not trapped and can freely rotate. Taking advantage of this mechanism, we will be able to design more powerful materials.

In Section E, we studied crystal nucleation, which is a *transition* from an amorphous to a crystalline state. We pay special attention to crystal nucleation in polymorphic systems and ask two questions: (1) How fast do polymorphs nucleate? (2) Can we predict which polymorph nucleates the fastest? We found that in all studied systems, the fast-nucleating polymorph(s) is more similar to the amorphous state in terms of energy state and structure than other polymorphs. This work allows us to better predict the crystallization product so we can more efficiently make better drugs.

In Section F, I presented the cutting-edge strategies to inhibit crystallization of amorphous drugs in order to improve global health. There were three *transitions* here: the crystallization process is a *transition*, inhibition of crystallization is another *transition*; we applied our fundamental understanding of crystallization science to solve real manufacturing problems, representing a third *transition* from knowledge to action. In our research, we developed both a surface coating technique and an amorphous drug-polymer salt approach to prepare amorphous medicines with high drug loading, outstanding stability and improved dissolution. These methods will help us make better and cheaper medicines.

In addition to the *transitions* mentioned above, graduation from a Ph.D. program is an important *transition* to me. For example I am going to be Dr. Yue Gui. (laugh) Seriously, I am going to start my career outside the campus, which will be a new experience to me. I have been a full-time student for such a long time. Now it is the time to change. Transition means opportunities and responsibilities. Best wishes!

Picture sources:

Figure 1d and Figure 2a-c are the photographs taken by Yue Gui.

Figure 1a cubic zirconia: https://en.wikipedia.org/wiki/Diamond_simulant

Figure 1b diamond: https://www.bluenile.com

Figure 1c graphite: https://en.wikipedia.org/wiki/Graphite

Figure 2e obsidian: https://en.wikipedia.org/wiki/Obsidian

Figure 2f Libyan desert glass: https://en.wikipedia.org/wiki/Libyan_desert_glass

Figure 3a: https://www.e-abbvie.com/ProductDetailView.aspx?RowId=360

Figure 3b: Bauer, J.; Spanton, S.; Henry, R.; Quick, J.; Dziki, W.; Porter, W.; Morris, J. Ritonavir: an extraordinary example of conformational polymorphism. *Pharm. Res.* **2001,** *18*, 859−866.

Figure 5a Bumblebee: https://3dwarehouse.sketchup.com/model/9948f9e461f81e433ca9fc7a34f66819/Transformers-Bumblebee-v20

Figure 7a center potato “eyes”: https://www.quirkyscience.com/chlorpropham-eye-growth-inhibitor/

Figure 9 caramel apple: https://tastesbetterfromscratch.com/caramel-apples/

Figure 9 dipped cone: https://www.dairyqueen.com