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Macromolecular Crystallization with Microfluidic Free-Interface Diffusion

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Expert Review of Proteomics

Title:

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Summary:

Fluidigm released the Topaz 1.96 and 4.96 crystallization chips in the fall of 2004. Topaz 1.96 and 4.96 are the latest evolution of Fluidigm's microfluidics crystallization technologies that enable ultra low volume rapid screening for macromolecular crystallization. Topaz 1.96 and 4.96 are similar to each other but represent a major redesign of the Topaz system and have of substantially improved ease of automation and ease of use, improved efficiency and even further reduced amount of material needed. With the release of the new Topaz system, Fluidigm continues to set the standard in low volume crystallization screening which is having an increasing impact in the field of structural genomics, and structural biology more generally. In to the future we are likely

to see further optimization and increased utility of the Topaz crystallization system, but we are also likely to see further innovation and the emergence of competing technologies.

Running Title: Microfluidic Macromolecular Crystallization

Keywords: Topaz, microfluidics, crystallization, structural genomics, crystallography, high throughput, nanoliter, free-interface diffusion

Expert Opinion:

The new Topaz crystallization chips are one example of a parade of powerful new technologies that have emerged following the advent of structural genomics and represent the early returns on investment in structural genomics [1, 2, 3, 101]. The vision of structural genomics itself was brought on by the convergence of several key technologies and the success of the human genome project [2,3]. The genome project paved the way for large scale discovery based biology research but also revealed a challenge still lye ahead to understand the proteome. Established and emerging technologies hinted at a way forward [2]. With recombinant techniques many more proteins became available for study, not just naturally abundant proteins. The rapid increase in computer speed and the increase availability of computer resources, along with increasingly sophisticated software tools, made rapid structure determination by x-ray crystallography possible even for non-experts. With the development of cryo-crystallography techniques, MAD phasing, CCD x-ray detectors and the increased available of synchron x-rays sources it

appeared only the availability of diffraction quality crystals would limit the rate at which crystal structures could be generated.

Even prior to the NIH announcement of the PSI [101] or the start of the other international structural genomics programs [1] structural biologists were working on new enabling technologies for rapid structure determination. A significant portion of the total effort in structural genomics has focused on miniaturization, parallelization, and automation. Some of the earliest innovations in high throughput crystallography, predating structural genomics by as much as a decade, were in automation of crystallization [4, 5] and in low volume crystallization [6,7]. The Impax robot (Douglas Instruments), automated parallel, small volume microbatch crystallization [5]. Later, at the Hauptman-Woodward institute, investigators would adapt the Hydra robot to dispense microbatch experiments in much higher density formats [6]. CyberLab (now owned by Gilson) introduced the C200 to automate the most common approach to crystallization at the time, hanging drop vapor diffusion [102, 8]. A robot developed at the Lawrence Berkley national lab automated sub-microliter sitting drop vapor diffusion [9, 10]. With the release of the new Topaz crystallization chips for crystallization by free-interface diffusion we have the full complement of approaches available in miniaturized, parallel formats and automateable. This is important because each of the commonly used techniques, microbatch, vapor diffusion, and free-interface diffusion, explore protein solubility phase space in fundamentally different ways. More importantly, the Topaz crystallization chips establishes the benchmark for low volume crystallization, requiring only a tenth as much protein per experiment as the next lowest volume achievable with

other approaches. Minimizing the volume of material used for crystallization effects the whole upstream process of protein expression and purification.

Advantages of microfludics free-interface diffusion

The new Topaz crystallization chips bring many advantages in addition to the ultra low volume. The key advantages of microfluidics free-interface diffusion with the new Topaz chip are:

- Low volume: Topaz 1.96 and 4.96 chips use only 1.5 microliters, or 15nL consumed per experiment, of protein to screen 96 reagent conditions [11]. Each crystallization experiment in the chip is carried out with only 2.5nL of crystallization reagent and less than 1nL of protein stock. The remainder of the material fills the lines that deliver the material to the mixing chamber (see below for a description of the chip).
- Fast assay times: It is not entirely clear why but it is empirically true that crystals form in the crystallization chip more rapidly compared to other approaches. Crystals form in a few days after setup compared to many days or weeks with sitting drop using 1uL drops (unpublished results). It is reported that nano-volume vapor diffusion experiments also yield crystals much faster [9] though this is presumable due to the rapid evaporation. Evaporation does occur through the microfluidics chip but at a much slower rate. The increase rate of crystal formation greatly accelerates lead development time and feedback optimization.
- Free-interface diffusion: the development of an automation ready platform for miniaturized, parallel, free-interface diffusion [8] is itself a significant innovation.

Free-interface diffusion explores phase space in a fundamentally different way that other methods and can be thought of as a complement the existing portfolio of tools for high throughput crystallization screening. Moreover, free-interface diffusion yielded 2-7 fold higher hit rate [12] than sitting drop vapor diffusion.

- Elastomer construction: Topaz is made from laminated elastomer layers, which is critical to the functioning of the chip (figure 1), but also brings specific advantages for crystallization [13]. The Topaz chips can be "dead-end loaded" (figure 1) because the elastomer is gas permeable and air voids can be forced out by pressing liquid in to the chip under pressure [14]. The fact that vapor escapes from the chip also increases the phase space sampled by experiments carried out in the chip (see comparison to vapor diffusion below).
- Ease of imaging: The elastomer used to make the chip is highly translucent making it trivial to acquire undistorted images of ongoing crystallization experiments. Problems like drop finding or condensation forming on the seal that you often have with vapor diffusion are eliminated with the chip. Fiducials embedded in the chip aid the superposition of a series images acquired of a single experiment which enables image analysis by image subtraction and aids automated feature recognition. Crystals can be imaged easily with any of a number of good quality stereo microscopes common in crystallography labs and with robotic CCD microscopes.

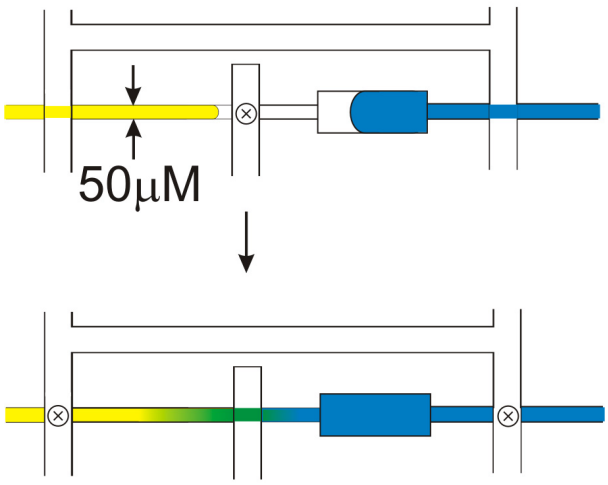

Figure 1. Loading the mixing chambers and initiation of a free-interface diffusion in the Topaz crystallization chip. The chip is made up of two layers of elastomer [13]. The top layer contains two control channels while the bottom contains the reagent channels. An interface control channel sits between the reagent chambers while interconnected lines of the containment control channel reside on either side of the reagent chambers. To start an experiment, crystallization cocktail (blue) and protein (yellow) are dispensed in to loading ports. Reagents are then pushed through the reagent lines and in to in the mixing chambers under pressure. During loading the interface control valve is closed and the containment control valves allowing reagent to flow in to the chambers. Air voids at the ends of the chamber are forced out through the gas permeable elastomer in a process called “dead-end loading” [14]. Finally, the containment valves are closed and the interface valve is opened, initiating free-interface diffusion.

Issues associated with use of microfluics crystallization

With all the advantages the new Topaz screening chips bring, there are also a number of issues:

- Cost: the costs of manufacturing the microfludics chip, and therefore the costs to the consumer, are substantially higher than for producing other consumable labware. One Topaz 1.96 chip may cost as much as 50x more than a sitting drop polystyrene plate for example. Unless substantial cost savings or a substantial profit can be realized elsewhere Topaz will have a limited share of the consumables market for crystallization. The ultra low volume does provide a significant cost saving in protein production but significant retooling of protein production facilities is required to take full advantage of this cost savings (see five year view). Retooling incurs an additional short run cost.
- Crystal retrieval: When a crystal forms in the crystallization chip it is often difficult if not impossible to retrieve the crystal from the chip. In addition, because of the scale of the experiments carried out in the chip, crystals are very small and often not suitable for further study. The fact that crystals obtained in the chip are not directly usable for diffraction experiments relegates the chip to the role of crystallization screening. Another method must be applied to follow up and further develop any initial leads discovered through screening with the chip.
- Translation to macro scale: the crystallization seems ideally suited to be used for initial screening and lead development, which would then be followed by lead optimization using another technique. Unfortunately, the results obtained with the chip do not directly translate to other methods like vapor diffusion or microbatch.

Conditions generally can be found in other systems once a protein is known to crystallize but significantly more effort is needed to develop strategies to translate leads obtained with Topaz into usable crystals. There are a number of factors that likely confound translation to other methods for scale up (see alternative methods below). Larger scale free-interface diffusion methods such as capillary counter diffusion [15] may provide a means for direct scale up, though this is yet to be demonstrated.

Evolution of the Topaz crystallization chip

The new Topaz crystallization chip has been substantially redesigned; in fact one might say completely overhauled (figures 2 and 3). The new chip designs have eliminated or dramatically reduced many of the difficulties associated with using the previous generation chip. The new Topaz system has substantially improved ease of use, it is automation ready, and it takes advantage of the knowledge gained from extensive use of the previous generation chip to improve the efficiency of crystallization screening. In addition, the new chip system is launched with new support hardware and software that also contribute to improved ease of use.

Ease of Use

Topaz 1.96 and 4.96 are now contained in their own carrier, eliminating the need to mount the chip in the carrier and hook it up to control lines. The new chips do not require the use of umbilicals to the pressure controller, which simplifies the handling and storage of the chip. The biggest improvement is the introduction of loading ports in the chip carrier. The collection of ports make up a loading manifold that is self contained within

the chip assembly. Ports are easily accessed with standard pipettes or liquid handling instruments. The previous version of the chip required considerable dexterity and good eye sight to inject reagents, one at a time, manually, in to small ports punched directly in to the elastomer (figure 2). A user also had to be careful to wet the bottom of a loading port and retract the pipette tip as reagent was dispensed to avoid back pressure pushing the reagent out of the port. It was a common experience, at least for new users, that a number of experiments would be lost due to partial filling of the mixing chambers. The new loading ports are not sensitive to air bubbles and lost experiments due to reagent loss is a rare occurrence.

Improved Efficiency

The new Topaz crystallization chips are designed to search crystallization parameter space with even greater efficiency than previous generations of the chip. The new chips use even less material and have a higher hit rate than the previous chip [12]. The mixing chambers on each chip are smaller and there is only a single mixing ratio for each of 96 reagent cocktail screened in each chip (figure 3). It was previously thought that exploring mixing ratios as a parameter would enable the exploration of a new dimension of crystallization parameter space. Empirically, it was discovered that the outcomes within a set of experiments for a given combination of reagent cocktail and protein were highly correlated [12]. This meant that the different mixing ratios were not exploring orthogonal regions of crystallization space but rather related regions of parameter space and therefore that using multiple mixing ratios, at least in the fixed set provide, was inherently inefficient. It has been observed that for a variety of proteins across a range of protein concentrations, the 1:3 protein to precipitant ration gave the highest average

success rate and the highest hit rate outright, as much as 2.5 fold higher, for 4 out of 5 proteins [12]. It is also true that experiments traverse a longer trajectory in phase space in the 1:3 ratio compared to the 1:1 or 1:3 ratios (see other methods below) which may account for the higher hit rate observed for the 1:3 ratio. The Topaz 1.96 crystallization chip does away with the multiple mixing ratios, retaining just the most successful one, thereby enabling the exploration of a greater diversity of cocktail combinations and a more efficient sampling of crystallization parameter space.

Automation

The new Topaz 1.96 and 4.96 chips are automation ready. The chips conform to the SBS footprint, simplifying storage and manipulated with off the shelf robotics. Liberation from the umbilical also greatly facilitates the use of automation to manipulate and store chips. By far the greatest improvement enabling automation though is the introduction of the loading ports. The loading ports are regularly spaced and they are amply large for most liquid handing robots to access for dispensing liquids. The manifold of loading ports is not arranged in the standard 8x12 array with 9mm offsets however, so synchronous 96-tip dispensing robots like the Hydra cannot be used to load the chip. Previous versions of Topaz were not automation ready. To use the Topaz 1.48 crystallization chip a highly automated lab would take a major loss in throughput and an increase in man hours required to setup crystallization experiments. With the new chips, a lab setup with automated liquid handling can utilize the existing robotics and likely maintain most of the preexisting capacity.

Moving to the new Topaz crystallization chips from other highly automated processes will require some retooling. To use the Topaz chip a lab must be equipped with the

instrument that operates the valves and loads the channels and chambers, the Topaz FID Crystallizer [101]. The Topaz FID Crystallizer is a replacement for the older pressure controller, which is incompatible with the new chips. Imaging systems may need reprogramming to inspect the crystallization chips as well, though existing optics should be adequate on most systems. Fluidigm sells their own imager, the Topaz AutoInspeX workstation [101], which of course is build to accommodate the chip configuration, and takes advantage of the other features of the chip that enable crystal, or at least feature recognition. The purchase a Topaz FID Crystallizer and a Topaz AutoInxpeX workstation is a substantial investment for most labs, particularly if there has already been an investment in to other automation.

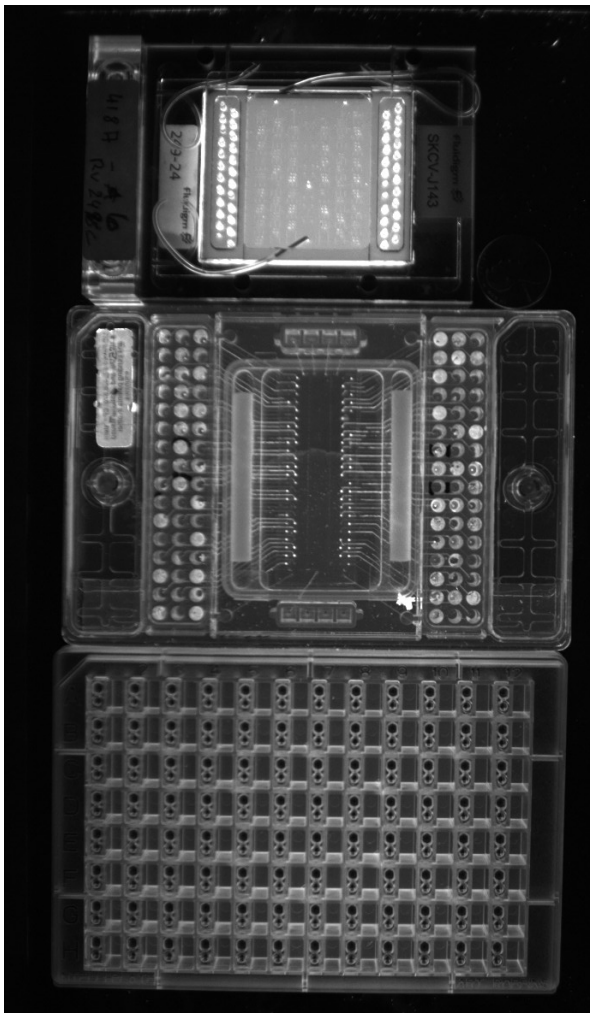

Figure 2. Footprint comparison of Topaz 1.96 crystallization chip (middle) with a previous generation of the chip, Topaz 1.48 (above) and an SBS footprint sitting drop, the IntelliPlate[103] plate (bottom).

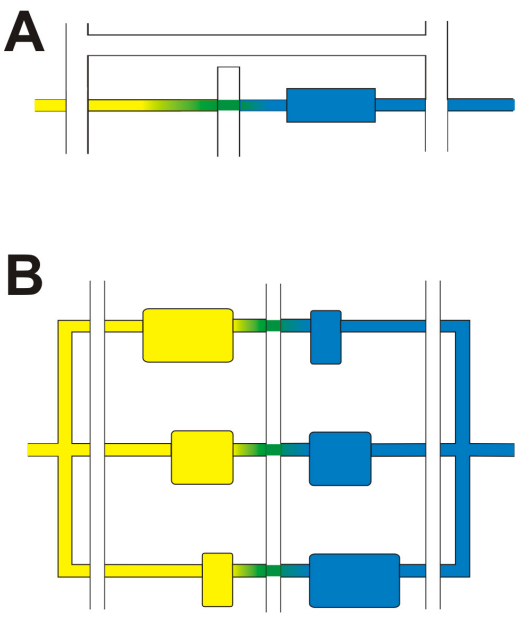

Figure 3. Comparison of the Topaz 1.96 and 1.48 chip layout. (A) a schematic of one set of mixing chambers in the Topaz 1.96 crystallization chip is shown, roughly to scale, with a schematic of a set of mixing chambers in the previous generation crystallization chip, Topaz 1.48 (B). Note the smaller mixing chambers in the Topaz 1.96 and elimination of two of three mixing ratios.

Using Topaz

Extensive description of the use of the new Topaz crystallization chips is provided in the users manual [11], available at the Fluidigm web site [104]. To use the new Topaz 1.96 the user follows a simple sequence of steps. The chip is manually hydrated then initialized for use through a prep routine which activates the control valves. Next protein and crystallization cocktails are dispensed to the chip and reagents are pushed in to the mixing chambers. Finally, one of a number of preprogrammed routines is run to initiate free-interface diffusion. Each routines opens the interface valve for some prescribed length of time, typically between an hour to just over an hour and a half, then closes the

interface valve [11]. Chips may be removed from the controller while the interface valve is open to allow for other chips to be loaded or initialized. The interface valve can also be opened and closed with separate routines, allowing for a user prescribed length of time that the interface valve is open. Accumulators in the chip, one for each control channel, maintain pressure in the chip during incubation. As described above, the chip may be loaded by robotic liquid handlers, though a liquid handling robot could quickly outstrip the capacity of the Crystallizer to execute the loading protocols.

Alternative methods

How does crystallization screening in the Topaz crystallization chip compare to other methods used for high throughput crystallization? The key advantages of using the crystallization chip have already been discussed (see above). Here some of the practical differences will be discussed as well as differences in how phase space is explored by various methods, the orthogonallity (or similarity) of methods, and the relative hit rates either, expected or observed, using the different methods.

Vapor diffusion is probably the most commonly used technique [8]. Labware and reagents are relatively inexpensive and there are numerous options for consumables available from numerous providers. There are also numerous providers of instrumentation and even fully integrated robotic systems for automating vapor diffusion crystallization. The most serious limitation of the vapor diffusion method is the volume of protein required per experiment. There has been tremendous progress driving down the lower limit [10] of volume that can used in vapor diffusion, and we may not yet know the limit that can be reached. In fact what we consider the limit to be today may be as much

determined by available liquid handling instruments and labware as it is by the inherent limits of the method. In the end of days however, the drop is dispensed in to open air and is subject to evaporation until the chamber is sealed (figure 4). The smaller the volume of the drop, the more serious the evaporation problems becomes. Typically, the drop size may range from a few hundred nano-liters to a few microliters in size. There is another potential problem inherent to the vapor diffusion method. When the protein and the reagent cocktail are combined, there is a period before equilibrium of mixing is reached, when the components of the mixture are experiencing wild gradients of solute concentrations. Micro precipitates can form and become kinetically trapped during the turbulent mixing phase and these precipitates may be irreversible, if they contain denatured protein for example. These micro-precipitates may also nucleate further disordered precipitation.

Microbatch is a third method used for high throughput crystallization [4,6]. Just as with vapor diffusion, labware and reagents are readily available and affordable for microbatch. Automation of microbatch crystallization is relatively simple compared to other methods since there are fewer transfer steps and no specialized labware is required. A number of different robotics systems are available for automated microbatch [4]. Microbatch experiments can also be setup in much higher density than vapor diffusion experiments since there is no reservoir. The microbatch method gets around the problem of evaporation that vapor diffusion suffers from by dispensing reagents under oil. Mixing under oil may lead to more rapid mixing as well, due to the surface tension of aqueous droplets under oil (George DeTitta, personal communication), getting around some of the problems associated with turbulent mixing in vapor diffusion experiments. Initially,

microbatch methods were not well suited for crystallization screening but with clever manipulation of the properties of the oil and the development of semi-permeable oil mixtures, microbatch methods have proven as successful as vapor diffusion [4].

Free-interface diffusion, vapor diffusion, and microbatch may be thought of as complementing methods. Each method explores phase space in a different way and in that sense might be thought of as different tools for crystallization screening (figure 5). It is not clear how orthogonal the various methods are to each other. There are few rigorous studies directly comparing the methods and none in the literature that would help to determine if one method is particularly well suited for one class or proteins or some subset of reagents. It is common to find testimonials to the merits of one method over the others, claiming that only with a particular method was it possible to crystallize a particular protein of high importance. Such an assertion is impossible to prove and seems highly unlikely to be true. If a protein is known to crystallize with one approach it seems likely that it can be made to crystallize using another approach. Methods can be directly compared to each other however by empirical hit rates for crystallization screening. From a small study comparing hit rates free-interface diffusion gave a 2-7 fold higher hit rate than vapor diffusion [12]. Several studies have shown the hit rate to be similar between vapor diffusion and microbatch [4]. By deduction then, free-interface diffusion is likely to yield a higher hit rate than microbatch as well, though this should be confirmed and quantified through experiments.

Each of the methods samples phase space differently, this likely explains the observed difference in hit rate between methods (figure 5). In vapor diffusion experiments, after the initial mixing of the drop, the drop and the reservoir slowly approach an equilibrium

in solute concentration through vapor diffusion. It is possible that the starting point of the vapor diffusion experiment is past the precipitation point, causing immediate heavy precipitation. And it is possible that the equilibrium point is to the left of the phase transition so that the protein never precipitates. With the batch method, direct mixing of protein stock and crystallization cocktail is expected create a supersaturated condition [8] immediately and, in essence, the starting point is the end point. Microbatch with semi-permeable oils, or "modified batch" (also called "batch diffusion"), mimics vapor diffusion experiment, traversing phase space toward the upper right and greatly extend the utility of microbatch as a screening method [16]. Modified batch differs from vapor diffusion in that there is no end point, which avoids the problem of having experiments that never precipitate. In the free-interface diffusion experiment, protein mixes slowly with the reagent cocktail and traverses the phase boundary. All of the experiments that would result in immediate heavy precipitation in vapor diffusion have some chance of resulting in crystallization in free-interface diffusion. In the Topaz crystallization chip, experiments that reach equilibrium well to the left of the phase boundary and never precipitate still have a chance to precipitate, or crystallize, because of evaporation through the elastomer. Avoiding the effects of turbulent mixing, free-interface diffusion likely provides greater reproducibility and better quality crystals as well, though this needs to be demonstrated and quantitatively compared through experiments. The fundamental differences between methods also helps to explain the difficulty in translating conditions found in the chip to other methods. It should be noted that the simple phase diagram does not capture all of the subtleties of the phenomena occurring in

the chip. For instance, the chip being semi-permeable, there is some cross talk between adjacent experiments as solvent can diffuse through the chip.

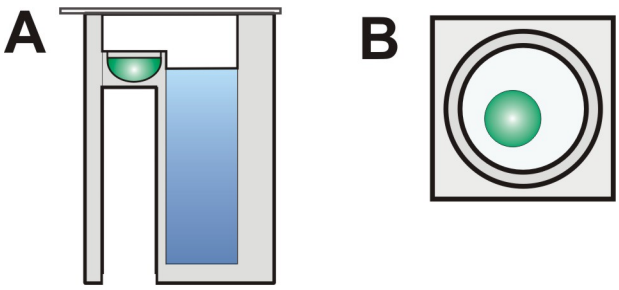

Figure 4. Two commonly used methods for high throughput crystallization screening. (A) Shows a drawing of a typical sitting drop experiment and (B) shows a drawing of a microbatch experiment. Note: if these drawings were to scale, (B) would be ~1/4 the size shown.

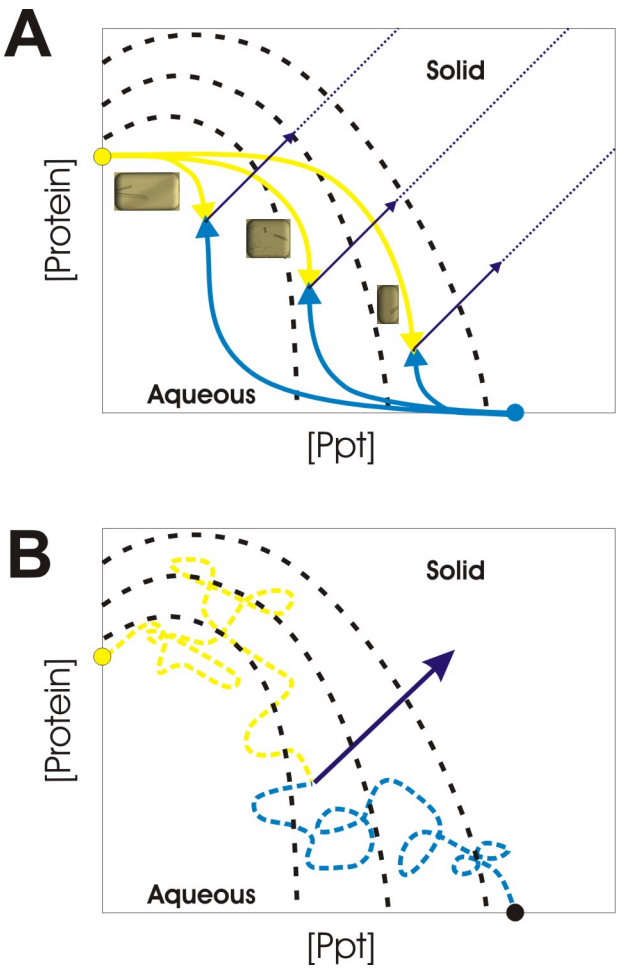

Figure 5. Exploration of phase space with microfluidics free-interface diffusion compared to sitting drop vapor diffusion. Shown above is a 2D projection of phase space, with black dotted curves defining the separation between aqueous and solid phase. There is a series of black curves because the location of the phase dividing line is unknown. The circles on the two axes show the hypothetical starting points of the experiment. The yellow and blue curves show a hypothetical experimental course mapped on to the phase diagram. (A) The three curves in the diagram represent the course for three mixing ratios using free-interface diffusion. The arrows emanating from the equilibrium point toward the upper right represent the effect of water vapor escaping through the elastomer. (B) Represent the same phase space as in A but as it would be explored using vapor

diffusion. The convoluted dotted yellow and blue curves represent the wild gradients of concentration the protein and precipitating agent go through during turbulent mixing.

Five Year View:

It is safe to say that the full impact of the Topaz crystallization chip on the field of structural biology has not been realized. The availability of the chip has had a profound impact on what scientists assume possible and on what scale things need to be pursued. The possibility to carry out crystallization screening at ultra low volumes enables new a approach to structural genomics or structural biology. Crystal screening at the nano-scale will translate to significant changes in upstream processes of protein expression and purification and drive innovation toward miniaturizing these processes and making them massively parallel. With massively parallel, miniaturized processes, and with the accelerated assay times achieved with the crystallization chip one can envision a highly sophisticated multi-path process in which every target is pursued in multiple forms (expressed constructs or homologues for example) and through multiple paths to expressed purified protein. Crystallization screening can then be the final selection that determines how a target protein should be produced. Thought of another way, the Topaz crystallization chip will be used to partition constructs of proteins in to crystallizing and non-crystallizing; crystallizing constructs would be pursued further. If you assume each construct or homologue of a particular target of interest behaves independently of every other and you assume that one in twenty soluble proteins yields a crystal, then you have only to develop a system to produce twenty constructs of a target of interest and pursue them in parallel through to crystallization screening to have a better and 98% chance of

getting at least one of the constructs crystallized. Losses along the way are anticipated and taken to mean only that a particular construct was not well suited for structure determination. If only microgram quantities of protein are required for the initial crystallization screen, only milliliter volumes of culture are required to get sufficient protein yield for crystallization, at least for robust expressing constructs. Several hundred expression cultures could be processed in parallel in a modest space. Achieving massively parallel lysis, purification, and concentration, and assessing yield and purity along the way for hundreds of targets is a significant challenge however and will require further innovation.

To fully realize the possibilities however, aside from all the requirements just outlined, a full commitment has to be made to relying on nano-scale crystallization as the core approach to crystallization screening. This requires a weighty decision because whole facilities need to be retooled, which requires a substantial short term investment and may reduce the value of existing investments. In addition to retooling for miniaturized processes, running a massively parallel, multi-path process adds a significant level of complexity to protein production. To further add to the complexity, a larger scale production process will likely need to be maintained to prep constructs that crystallize and are to be pursued further. In addition one would need very sophisticated automated scheduling tools and resources to manage a parallel process to keep the workflow balanced. The availability of the Topaz crystallization chip is profoundly important precisely because it allows us to place hard targets on process requirement for all processes upstream of crystallization. It defines the requirement for what needs to come from upstream processes. Unfortunately, any mature process like the one envisioned

above is likely some time off. Few facilities will have the resources and/or vision to tackle such an ambitious plan to retool their whole process. The cost of the Topaz crystallization chip alone may be a significant deterrent to pursuing such a plan.

For the very near term the Topaz crystallization chip is likely to be more and more recognized for the key advantages it brings, though it will not likely be seen as a fully orthogonal technology for crystallization screening. The hit rate advantage of free-interface diffusion will likely be more well establish. The Topaz crystallization chip is likely to become more and more the tool of choice to screen high value targets for which there is precious little material available, such as membrane proteins [17]. Large protein complexes may fit in to the category as well [18]. For individual researchers, particularly researchers on a modest budget, the Topaz crystallization chip is likely to be one of several tools used to obtain lead crystallization conditions.

In the short term we are likely to see further maturation of existing microfluidics crystallization technology. We may see a rigorous comparison to other methods that quantify success rates and perhaps identify subsets of proteins or protein classes for which micro-fluidics crystallization is especially well suited. As demand for the crystallization chip grows, we can hope that the economy of scale and/or cheaper manufacturing drives down the price of the chips, creating the incentive to retool other upstream processes. We can expect new research leading to better strategies for translation of initial conditions developed in the chip to conditions in macro scale method. We can also expect an evolution of methods and reagents and perhaps a further evolution of chip designs maximizing hit rates for different reagent classes used in the chip.

In the medium term we are likely to hear of research in to competing technologies. The new Topaz 1.96 and 4.96crystallization chips have set a new mark for what achievable, this alone will cause people to think about crystallization in new ways and explore other approaches to miniaturized crystallization, free-interface diffusion in particular. The cost of the Topaz crystallization chip and the new investment in structural genomics around the world will drive innovation to fill an obvious market. We are likely to see further adaptation of known methods to miniaturized forms.

Key Issues:

- The advent of Structural Genomics has spurred new investment and speculation in industries providing new technologies for high throughput protein crystallography. A significant effort has focused on miniaturization, parallelization, and automation. The Topaz crystallization chip miniaturizes free-interface diffusion crystallization in a parallel 96-well format using microfluids and sets the benchmark for low volume crystallization.
- The new Topaz screening chip has several key advantages over other high throughput crystallization approaches, most notably the extreme low volume of material needed for crystallization and the use of free-interface diffusion. Other advantages include increased assay rates and ease of imaging.
- The advantages and evolution of the chip not withstanding, there are several significant challenges. Crystals grown in the chip are not easily retrieved from the chip and conditions obtained with the chip do not directly translate to other methods. Cost may be the single most significant issue.

- The new Topaz system is a significant redesign compared to previous generations of the chip, increasing the efficiency, further reducing the volume of material needed per experiments, and greatly enhancing the ease of use.
- The new Topaz crystallization chip is automation ready. The chip can be loaded with any number of liquid handling robots and manipulated with off the shelf plate handling robots.
- Topaz crystallization chips provide a fundamentally different exploration of crystallization phase space. This leads to a higher hit rate in the chip.
- The full impact of Topaz crystallization chip has not been realized. The ultra low volume enables a completely redesigned process miniaturized at every step proceeding crystallization. However, centers are not likely to miniaturize their entire process until the crystallization chip proves to be an all-in-one technology and until the chips become more affordable.
- Success with ultra-low volume crystallization will drive further innovation. We are likely to see competing technologies that approach the benchmark set by Topaz or perhaps even exceed it.

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