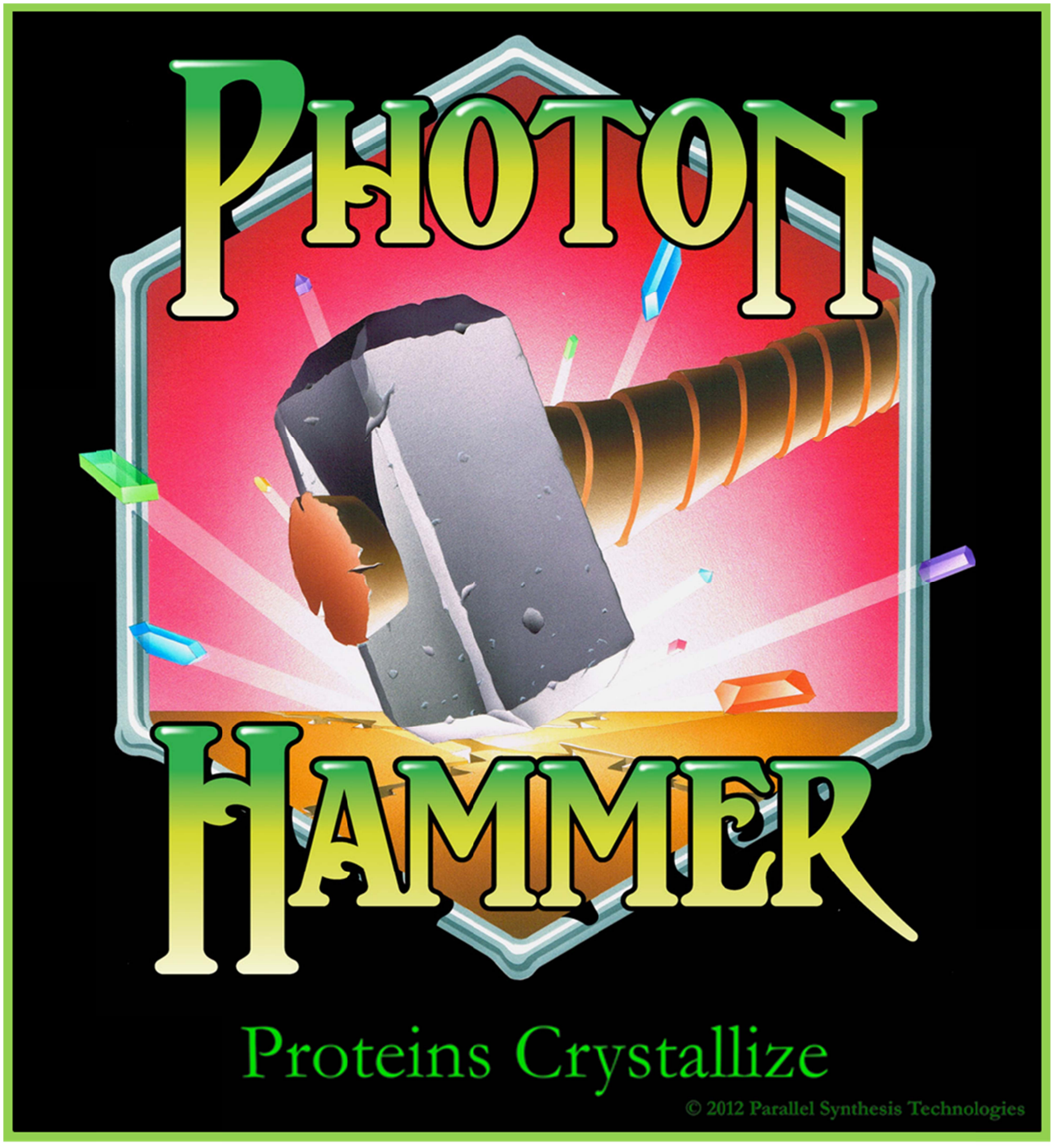
PHOTON
HAMMER
Proteins Crystallize
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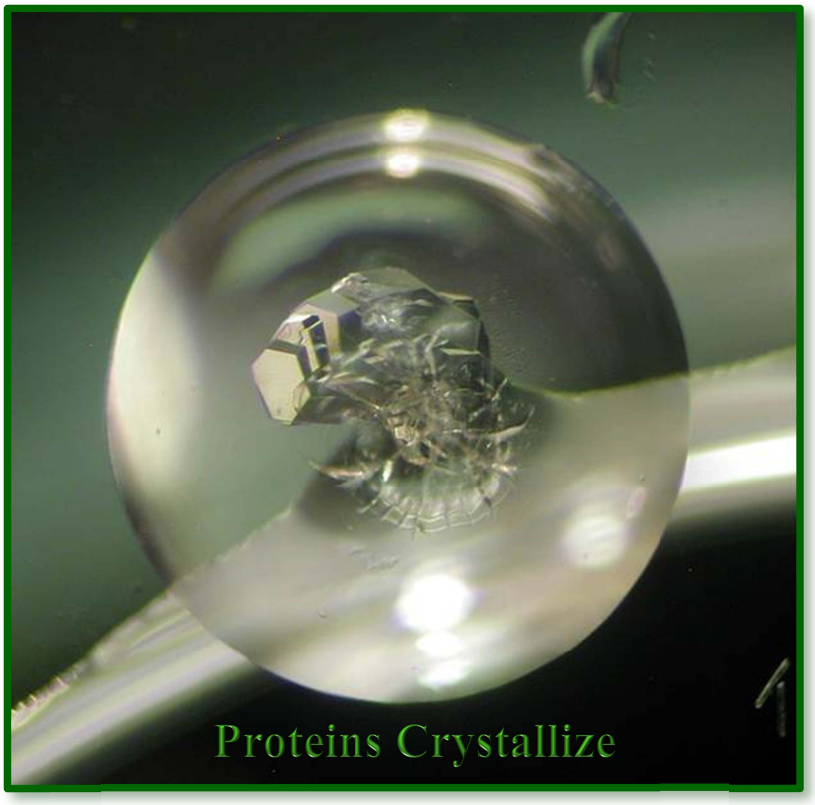
Photon Hammer



Photon Hammer™ Substrates (PHS) for Protein Crystallization

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Photon Hammer™ Substrates (PHS) for Protein Crystallization

1. Introduction and Technical Background

1.A Introduction to the Photon Hammer Substrates (PHS)

Parallel Synthesis Technologies, Inc. is very pleased to offer its unique Photon Hammer heterogeneous nucleation technology to facilitate the crystallization of proteins in a convenient self-contained cartridge. Using a new patent-pending laser processing technique called the Photon Hammer, myriad micro-cracks are fabricated within a small, spatially well-defined region of a glass substrate which, when placed in contact with a protein solution to be crystallized, can act as potent heterogeneous nucleants for the crystallization of proteins. Photon Hammer Substrates (PHS) cartridges are now available for all familiar protein crystallization experiment protocols including Vapor Diffusion, Liquid Diffusion and GPCR in LCP.

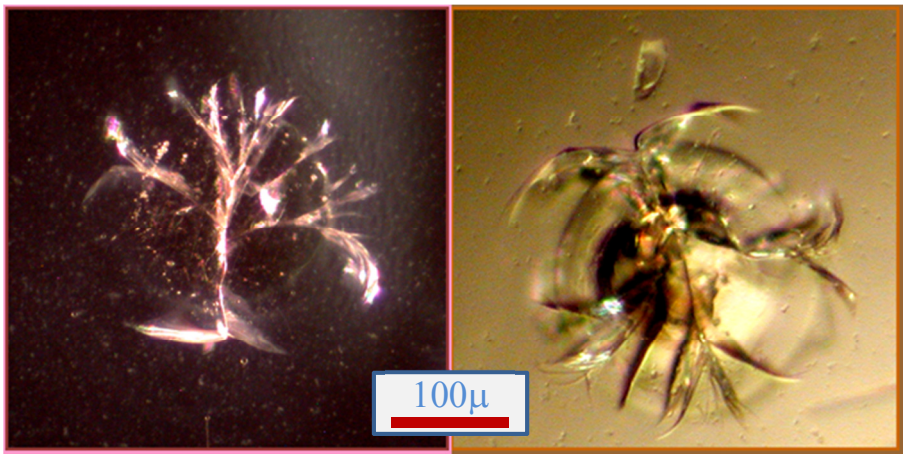

Fig. 1 Images of bare, unreacted heterogeneous nucleants prepared with using the Photon Hammer laser processing technology. The nucleation sites, each of which possesses countless microscopic cracks, are present within each well of the Photon Hammer Substrates (PHS) cartridge. Note that when the cracks emanating from the central damage zone propagate into the undamaged substrate the cracks are deflected and terminate thereby limiting the nucleant to a small, spatially well-defined area. Each variable width crack tapers from a finite width in the damage zone to *zero width* at its termini both perpendicular and parallel to the plane of the substrate. Therefore, cracks ranging in size from microns to zero width are present within each nucleant. We hypothesize that protein molecules may lose translational motion and begin ordering when absorbed into a crack of an appropriate size and shape thereby forming the first stages of an incipient crystal nucleus.

The Photon Hammer heterogeneous nucleants are fabricated from glass. A glassy material is particularly well suited to the formation of cracks. If cracks are initiated in a crystalline material it would likely propagate along the cleavage planes through the substrate resulting in mechanical failure of the material into pieces. Likewise, polymers may melt or decompose and metals are damaged in ways that do not result in fractures. As shown in **Fig. 1**, the cracks initiated from the laser pulse terminate a short distance from the damage zone. Unlike the "debris-based" heterogeneous nucleants, which are comprised of bits of broken or ground glass, horsehair, polymer spheres for seeding, seaweed and the like, the Photon Hammer technology provides a distinct, integral and well-localized heterogeneous nucleant. Since the nucleant is integral to PHS slide, it does not require any manipulation, loading into the crystallization trial or separation from your protein crystals after growth like the debris-based nucleants.

To provide contact between the nucleant and the protein solution polymer wells configured on a 384-well format are laminated onto these glass heterogeneous nucleation substrates to provide convenient 25mm x 75mm Photon Hammer Substrate (PHS) slide cartridges in which the protein crystallization experiments are performed (see below). You just select the type of crystallization protocols desired (vapor diffusion, liquid diffusion or GPCR/LCP), dispense your solutions and precipitants into the wells manually or automatically and seal. Crystallization progress is monitored from either side of the transparent Photon Hammer Substrate cartridge. With only minor changes to your current crystallization protocols you can take advantage of the Photon Hammer heterogeneous nucleation technology and, with *free* screening buffers shipped with each PHS purchase, it may be possible to reduce your screening costs.

1.B Technical Background for Heterogeneous Nucleation

It has long been known that certain solid additives, i.e., a heterogeneous nucleant (HN), can facilitate the deposition of crystals from solutions or melts. Often times, in spite of the fact that the solution to be crystallized is supersaturated with respect to solute, an initial nucleation event does not occur and therefore there is no subsequent crystal growth. An efficacious heterogeneous nucleant can assist, by decreasing the translational motion of species in solution by adsorbing them onto its surface, in overcoming the entropic barrier which allows ordering of the solution species into a 3-D crystal. Examples include the seeding of solutions with the crystals to be formed, chemists scratching glass flasks to induce crystallization, the rapid freezing of supercooled water by the addition of ice and the formation of rock candy on strings dipped into concentrated sugar solutions. More recently, “debris-based nucleants” such as ground glass, seaweed, hair, polymer spheres and other detritus, which could exert unknown deleterious effects on the crystallizing proteins, have been used in attempts to increase crystallization efficacy.

Since the structural information obtained by determining the 3-D X-ray crystal structures of crystallized proteins is invaluable in determining protein function, and there is generally a very low chance of obtaining X-ray diffraction quality single crystals in any given crystallization trial, there is an urgent and immediate need for materials to initiate and facilitate the crystallization of proteins. One way to encourage, control, facilitate, accelerate, and provide selectivity in terms of polymorph crystallized, is to use a heterogeneous nucleant.

Homogeneous and Heterogeneous Nucleation In order for any crystal to form from solution there must be an initial phase where there is only sub-nanometer sized solid assemblages (nuclei) of the soluble species (e.g., protein molecules) aggregating into a lattice in an ordered 3-D arrangement in space called a crystal. Most often, the reason that a given soluble species cannot crystalize, when the solution is already saturated or supersaturated, is that there is no efficient nucleation mechanism available to induce the initial order required to commence crystal growth. In principle, this nucleation event may occur within the solution itself (homogeneous nucleation) or on the surface of the solution container or on an adventitious particle within the solution (heterogeneous nucleation). The homogeneous nucleation depends on factors such as temperature, concentration, pH or solution composition whereas the heterogeneous nucleation depends on these parameters but also on the nature of the surface on which the initial nucleation events takes place. In order to increase the chances of obtaining useful crystals beyond homogeneous nucleation it would be necessary to use heterogeneous nucleation since the homogeneous nucleation is already a subset of every heterogeneous nucleation experiments.

Heterogeneous Nucleation Mechanisms There is no universally accepted explanation for the mechanism by which a heterogeneous nucleation catalyst performs its function of facilitating protein crystallization. It is clear, since the crystal is comprised of a regular spatial array of molecules or substituents, and these incipient crystal components are well separated in the

solution or melt, that the constituents must lose translation degrees of freedom and some amount of entropy in order to form the solid. So in some sense, the heterogeneous nucleation catalysts likely provide a surface onto which the soluble constituents may attach themselves thereby reducing their translation freedom. Once absorbed a certain amount of surface mobility is required of the protein. The heterogeneous nucleant must allow enough surface diffusion of the proteins molecules for them to "anneal" into their preferred orientation in the lattice. If the adsorption is too weak and fleeting to induce order, or so strong that the protein is adsorbed in an irreversible manner in a random orientation, then there is no crystalline nucleus onto which the protein molecules may adsorb to grow the crystal.

Since it is conceptually difficult to envision the initial nucleation events ordering the soluble constituents into a long one dimensional (1-D) string as the first step on the path to a 3-D crystal, a more likely initial structure evolving on the way to a 3-D crystal is a 2-D "raft" of protein molecules on the surface of the heterogeneous nucleant. Once the ordered raft or sheet forms it may act as a template or seed crystal to facilitate further crystal growth. Therefore, it could be expected that a structure that had spatially confined 2-D spaces (e.g., cracks, fissures, crevices, slits, clefts, holes and other forms of damage) might act as particularly effective nucleants and would be expected to have a higher degree of efficiency than a pore in a debris-based nucleant because the pores offer little restraints in the third dimension. Note that, of course, the crack-like features must also have a third dimension, however limited in size the third dimension may be, or it would not be possible for protein molecule to enter the feature. For example, a thin crack would provide more constraint and diffusional hindrance (i.e., it would be easier for the soluble species to lose their motion and come together to form the crystal) to the nucleating 2-D raft as compared to a 3-D pore. Also, since the largest dimension of the crack is directly exposed to the nutrient crystal growth solution, when a nucleant forms in a crack the incipient crystal has good diffusional access to the nutrient solution.

Indications of Heterogeneous Nucleation A heterogeneous nucleant (HN) can influence crystal growth in many ways but the fundamental effect must be to form a single type of nucleus at the earliest stages of nucleation and crystal growth. It seems clear that once a given type of nucleus forms, other crystalline polymorphs or nuclei of differing habit cannot form and are all other nuclei polymorphs but one are completely suppressed. There are many examples in the Image Gallery showing the strong influence of the Photon Hammer nucleants on crystal growth in the ways enumerated below. In all of the examples shown, which provides a summary of hundreds of heterogeneous nucleation experiments, there was never an example of two different crystal habits, types or polymorphs observed within the same crystallization solution. This is consistent with only one polymorph existing in the solution in the presence of the heterogeneous nucleant.

A heterogeneous nucleant is generally a solid state material which, when in contact with a solution containing the soluble species to be crystallized, alters the course of crystallization as compared to the course in the absence of the HN in ways such as:

1. A higher percentage of crystals form in screening trials with a HN than without a HN
2. Crystals form faster in the presence of a HN than without a HN
3. Crystals nucleate and form on the region of the substrate where the HN is physically located and not on other regions of the same substrate
4. Crystallization, or a particular crystalline polymorph, is suppressed in the presence of a heterogeneous nucleant
5. Crystal of a different habit or polymorph form in the presence of a HN but not in the absence a HN

6. Crystallization onto heterogeneous nucleants may be more reproducible than homogeneous nucleation
7. Fewer larger crystals, rather than more smaller crystals, form in the presence of a HN but not in the absence of a HN
8. Higher quality crystals form in the presence of a HN than without a HN
9. Crystals that have proven difficult or impossible to crystallize crystalize more readily in the presence of a HN

In order to see these heterogeneous nucleation phenomena displayed with protein crystals, please visit *www.photon-hammer.com/Image_Gallery*.

2. Photon Hammer Substrates (PHS)

Using the Photon Hammer heterogeneous nucleation technology for protein crystal growth is as simple as pipetting your protein solution into the wells of a small microfluidic cartridge and sealing the cartridge. The Photon Hammer heterogeneous nucleants are present within each of the wells of the various types of PHS cartridge devices such that no loading or manipulation of the nucleant is required. It is possible to perform any type of familiar protein crystallization experiment including Vapor Diffusion, Liquid Diffusion, GPCR in LCP and evaporation experiments using our convenient, ready to use 25mm x 75mm Photon Hammer Substrates (PHS) nucleation slides. For automated liquid dispensing, four of the 25mm x 75mm PHS slides are placed into an adapter than can be handled as an SBS 96 well plate. Either 14 or 28 samples may be screened on the 25mm x 75mm x 3mm Photon Hammer Substrates and the PHS stored at a very high density for screening or archiving purposes. The PHS are sufficiently inexpensive that you can enjoy the added benefits of heterogeneous nucleation, the ease of viewing crystal growth and harvesting as well as the convenience and small sample volumes of a microfluidic slide cartridge. And with *free* high quality crystallization screening buffers with each PHS purchase you never need to purchase screening buffers again to perform your sparse matrix screening protocols.

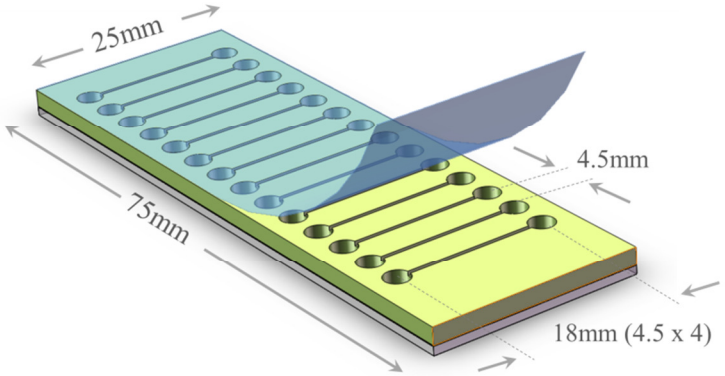

Fig. 2 A Photon Hammer Substrate (**PHS-VD**) cartridge, which is used for vapor diffusion crystallization trials, contains integral heterogeneous nucleants within each of 14 wells. The PHS-VD slide has the nucleants on a glass slide (*grey*) onto which the acrylic wells (*yellow)* have been laminated. The protein solutions are placed into one row and the vapor equilibrant solution placed into the wells of the adjacent row. The cartridge is tape sealed (*blue*) and the small channel connecting the two wells allows the water vapor to equilibrate between them.

To screen your protein crystallization conditions with the heterogeneous nucleants contained within the PHS you simply add your protein solution into a well and the precipitant or buffer solution into an adjacent well and seal the wells. The wells are interconnected by either a liquid channel (for Liquid Diffusion experiments) or a vapor channel (for Vapor Diffusion trials). Crystal growth is monitored from either side of the transparent cartridge and the crystals may be harvested by simply cutting the tape seal and removing the crystals. For the growth of GPCR materials, the membrane protein is mixed with LCP or other media (see below), dispensed into the well and the sample sealed.

The below sections describe the PHS, how the cartridges are used for protein crystal growth, the types of screening crystallization protocols that may be performed, how the cartridges are stored as well as the viewing and harvesting the protein crystals.

2.A Vapor Diffusion with Photon Hammered Substrates (PHS-VD Cartridges)

Vapor diffusion is a common method for growing protein crystals where a protein in a buffer is equilibrated, within the same sealed well or vessel, against another solution of greater osmotic strength than the protein-containing solution. Water is transported from the dilute, protein-containing solution to the more concentrated solution with a concomitant increase in the concentration of the protein which encourages crystallization. On traditional large crystallization plates the vapor diffusion trial is usually performed in either a sitting or hanging drop geometry.

Using the PHS-VD Cartridges In the **PHS-VD** cartridges (**Fig. 2**) the protein-containing solution is placed into one of 14 wells in the **PHS-VD** slide using a manual or automatic pipetting system. The PHS slide is a glass-polymer laminated stack with the heterogeneous nucleant created with the Photon Hammer on the surface of the glass substrate. The glass substrate is attached to an acrylic well array such that each of the 14 wells in a row is in registry with the heterogeneous nucleant on the underlying glass slide. Thus, the glass substrate with the exposed heterogeneous nucleant forms the bottom of each well. The solution of higher osmotic strength against which the protein solution will be equilibrated is placed into an adjacent well and is in contact with the protein solution via a small channel. This allows the water vapor pressure above the two connected wells to equilibrate but prevents the liquids in the wells from mixing as shown in **Fig. 3**.

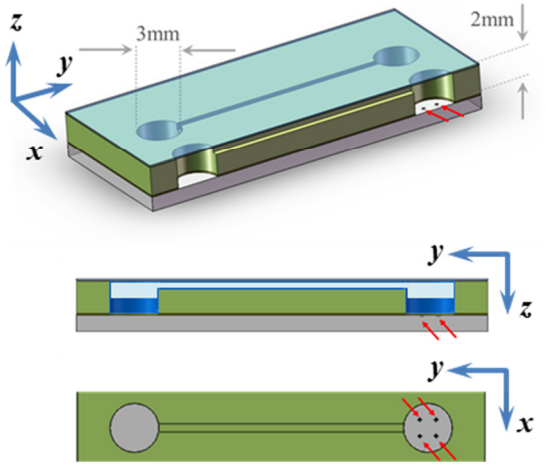

Fig. 3 In the **PHS-VD** slide, the protein solution in a well (volume = 10-15µL) containing the heterogeneous nucleants (*red arrows*) and the equilibrant in the adjacent well are connected by a 100µm x 100µm x 15mm vapor conducting channel (channel not shown to scale). After sealing with tape, the solutions equilibrate and the crystals harvested by removing or cutting the tape.

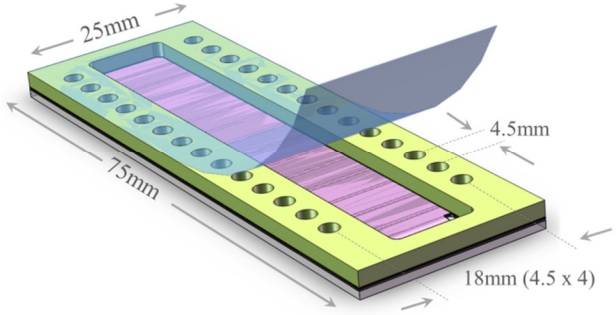

Fig. 4 A Photon Hammer Substrate (**PHS-LD**) cartridge, which is used for liquid-liquid diffusion crystallization trials, contains integral heterogeneous nucleants within each of 14 wells. The **PHS-LD** slide has the nucleants on a glass slide (*gray*) onto which the acrylic wells (*yellow)* have been laminated with PSA (*black*). The protein solutions are placed into one row and the precipitant placed into the wells of the adjacent row. The cartridge is tape sealed (*blue*) and the small channel connecting the two wells allows the two solutions to slowly intermix.

After loading is completed the wells are tape sealed and solutions allowed to equilibrate whereupon the water is transferred from the protein solution to the equilibrant thereby slowly concentrating the protein-containing solution. The crystallization trials can be easily monitored by viewing the slides from either top or bottom and the slides efficiently stored at a high density during crystallization. The crystals may be harvested and removed from the **PHS-VD** slide by simply cutting or removing the tape.

For pricing and ordering information please see the Purchase page on this website. Please remember that when you purchase either the **PHS-VD** or **PHS-LD** substrates you will receive *free crystallization screening buffers* – you'll never need to purchase screening buffers again.

2.B Liquid Diffusion with Photon Hammered Substrates (PHS-LD)

A liquid-liquid diffusion crystallization experiment is performed by placing a solution of the material to be crystallized in one container and this connecting solution to a second solution, which will induce crystallization of the desired material upon admixture of the two solutions, by a thin channel of sufficient length such that the rate of diffusional mixing is very slow. Liquid diffusion is a more common crystallization technique in crystal growth areas outside of protein crystallography - perhaps because of the lack of convenient and inexpensive crystallization devices. Since steep compositional gradients may be obtained over short distances within a liquid diffusion system, many different effective crystallization conditions may be sampled within a very small volume. For example, if the ideal concentration of a particular precipitant is not known, then a liquid-liquid diffusion experiment may be performed and, since many possible concentration ratios exist between the pure protein and pure precipitant solutions along the diffusion pathway interconnecting the wells, the best concentration may be easily located in a single experiment.

The liquid diffusion taking place within the **PHS-LD** slides (**Fig. 4**), where the precipitant solution slowly diffuses into the protein solution, is fundamentally different than the vapor equilibration and evaporation taking place in the **PHS-VD** slides. Since there is no mixing or addition of different compounds in the vapor diffusion experiment, the vapor diffusion experiment has no analogue, corollary or resemblance to the liquid-liquid diffusion screens accessible using the **PHS-LD**. Therefore, the **PHS-LD** slides offer a fundamentally different and complementary alternative to the familiar sitting and hanging drop vapor diffusion protocols. Crystals demonstrating recalcitrance in previous vapor diffusion crystallization attempts may be amenable to crystallizing under the myriad and diverse liquid diffusion conditions available.

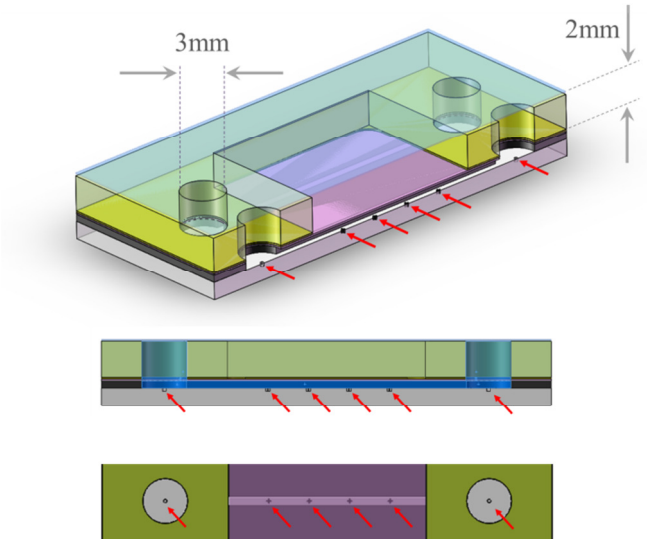

Fig. 5 Sectional views of wells in adjacent rows in the PHS-LD slide showing the two wells, which contain the protein solution and precipitant, connected by a channel (200µm x 250µm x 15mm) which allows the solutions to slowly interdiffuse (channel not shown to scale). The heterogeneous nucleants (*red arrows*) in the glass layer are present in both wells and the channels. Crystal growth may be viewed form the top or bottom of the slide and crystals harvested by simply cutting the tape.

Using the PHS-LD Cartridges Parallel is now pleased to offer a convenient, low volume and inexpensive Photon Hammer Substrate for Liquid Diffusion - the **PHS-LD** slide (**Fig. 4**). The protein solution is placed into one of 14 wells and the precipitant solution added to the adjacent well. As depicted in **Fig. 5**, the two wells are configured such that they communicate by means of a 200µm x 250µm x 15mm channel. When the slide is loaded and diffusion commences, the exact concentration and diffusion rates of the two liquids are not known so the heterogeneous nucleants are placed in both wells and along the diffusion channel

as shown in **Fig. 5**. As the crystallization trial proceeds the slides may be monitored from either the top or bottom side of the cartridge and the ripe crystals harvested by removing the tape seal.

Please remember that whether you are purchasing the **PHS-VD** for vapor diffusion experiments or screening samples with the **PHS-LD** liquid diffusion cartridges that you receive *free* crystallization buffers with each PHS order.

2.C Crystallizing GPCR in Lipidic Phases with Photon Hammered Substrates (PHS-GPCR Cartridges)

For various proteins such as GPCR, membrane proteins (MP) and other proteins normally found in a lipidic environment, the aqueous phase experiments described for the vapor phase and liquid phase diffusion experiments are inappropriate as the membrane proteins are often neither soluble nor have the ability to maintain their structural integrity or function in aqueous solutions. These proteins require a hydrophobic environment similar to that found in cell membranes. One popular and successful method to obtain crystals of the lipid-soluble proteins is to mix them with a lipidic phase material with the appropriate physical and chemical properties to allow the proteins to diffuse within the lipidic phase, form a nucleus and subsequently crystallize. For these type of experiments with oleophilic proteins we provide a Photon Hammer Substrate (**PHS-GPCR** cartridge) onto which the MP – lipidic phase may be dispensed and subsequently monitored for the development of crystal growth.

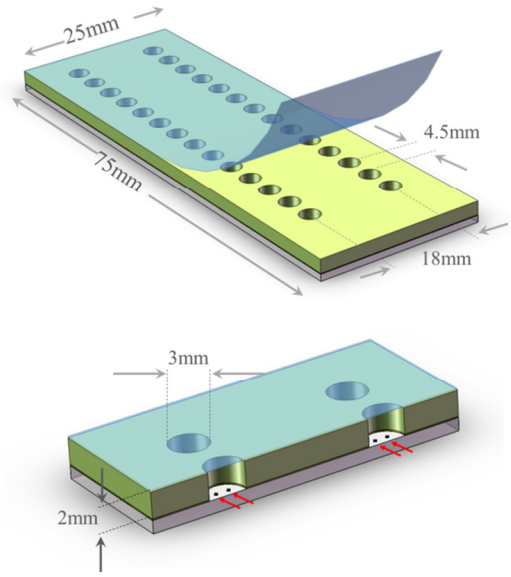

Fig. 6 A Photon Hammer Substrate (PHS-GPCR) cartridge, which is used for crystallization screening trials for GPCR and other membrane proteins, contains integral heterogeneous nucleants within each of 28 wells of the PHS-GPCR. The GPCR is mixed with some lipidic material, dispensed into the well and onto the nucleant and the wells sealed with tape. Crystal growth is monitored from the top or bottom of the PHS slide and any suitable crystal isolated by simply cutting or removing the tape seal.

The first step in the crystallization of a membrane protein or GPCR is mix the protein with the lipidic phase material that will be used for the crystallization medium. Like nearly all crystallization methods a means to slowly produce the crystal from the growth media is desirable. In this case, the hope is that a protein crystal nucleus will form in the well-dispersed protein in the lipidic phase and that the nuclei will coarsen and grow as the protein molecules in the lipid slowly diffuse to the growing crystal.

Using the PHS-GPCR Cartridges Once the oleophilic protein is dispersed into the lipidic phase chosen for the crystallization trial the protein-lipidic phase dispersion is dispensed into one of 28 wells on the **PHS-GPCR** slide and the slide tape sealed. Each of these wells, which possess integral heterogeneous Photon Hammer nucleants on the glass well bottom, has a total volume of approximately 14μL. The wells may be inspected for crystal growth from the top or bottom of the slide cartridge and the crystals removed from the well when ready to harvest by simply cutting the tape seal.

These same **PHS-GPCR** slides can be used to for GPCR diffusion screening (i.e., to determine the diffusion rate of the membrane protein in the lipidic phase). If the GPCR is not observed to diffuse within the lipidic phase according to some predetermined parameters, then there would

be no need to set up screening trials for a protein that cannot diffuse within the crystallization matrix.

2.D Use of Automated Dispensing with the Photon Hammer Substrates

These PHS-GPCR cartridges can be easily configured for use with automated liquid or GPCR dispensing protocols as depicted in **Fig. 7**. As shown in **Figs. 2, 4** and **6**, the wells on any PHS slide are related by multiples of the 4.5mm spacing of the SBS 384-well format. To facilitate handling by automated dispensing equipment Parallel provides a simple inexpensive adapter to allow four 25mm x 75mm PHS slides to be robotically handled and as SBS-formatted microtiter plate.

2.E Custom Photon Hammer Substrates

Since Parallel Synthesis Technologies designs all PHS substrates and components at our Santa Clara location, we can provide any type of custom design to fit your current crystallization protocols and allow incorporation of the Photon Hammer heterogeneous nucleants. Starting from your desired work flow and concepts we can design plates, apparatus and devices which can incorporate the PHS into your existing processes.

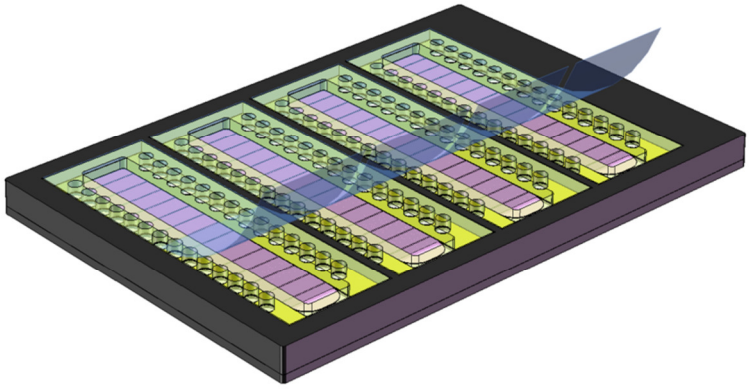

Fig. 7 Four PHS slides can be placed into an adapter that may be manipulated by automated liquid dispensers as an SBS-formatted microtiter plate.

All types of PHS cartridges are manufactured by Parallel in Santa Clara CA from the very best materials sourced from carefully qualified local vendors and suppliers. Your assurance of the quality, manufacturing consistency and reproducibility of each and every component purchased from Parallel is a direct results of our rigorous inspection and quality assurance procedures.

Please contact us for a confidential and complimentary discussion.

3. Protocols and Methods

The section describes how to simply, quickly and conveniently load all types of PHS cartridges. The sample and precipitant/buffer are pipetted into adjacent wells, which are in communication via a water vapor-conducting conduit for the **PHS-VD** slides and a liquid bridge for the **PHS-LD** slides, and the slide sealed to await crystal growth as the two solutions equilibrate.

3.A Vapor Diffusion Experiments with PHS-VD Cartridges

Using the PHS loading fixture (please see Purchase), or a suitable holder from your laboratory, the **PHS-VD** slide is used to screen protein crystallization conditions as follows:

(a) One of two types of **PHS-VD** slide is selected depending on the volume of equilibrant desired: the **PHS-VD-15** has a protein sample well of 15µL volume and an equilibrant well of 15µL or the **PHS-VD-50** has a sample well of 15µL and an equilibrant well of 50µL volume;

(b) The **PHS-VD** slide is placed into the PHS loading fixture, the chosen buffer tubes into the buffer slots and the protein tubes into the chilled aluminum block.

(c) The row of wells along one side of the slide, which contain the PH nucleants, is filled with the protein solution;
(d) There are Photon Hammer heterogeneous nucleants in the well containing the protein solution *only*. Make sure the protein solution is placed into the well with the PHS nucleant present;
(e) The wells should not be filled more than ~70% full to avoid sealing issues;
(f) The row of wells along the other side of the slide is filled with the equilibrant solutions;
(g) The backing is removed from the precut sealing tape and the tape used to seal the wells;
(h) Crystal growth may be monitored optically from either surface of the slide;
(i) To harvest the developed crystals the tape seal is simply cut or removed for retrieval

3.B Liquid Diffusion Experiments with PHS-LD Cartridges

Using the PHS loading fixture (please see Purchase), or a suitable holder from your laboratory, the **PHS-LD** slide is used to screen protein crystallization conditions as follows:

(a) The **PHS-LD** slide is placed into the PHS loading fixture, the chosen buffer tubes into the buffer slots and the protein tubes into the chilled aluminum block.
(b) The row of wells along one side of the slide is filled with the protein solution;
(c) Since both rows of wells and the intervening liquid channel all possess PH nucleants it makes no difference which row of the **PHS-LD** slide is selected for the protein and which row for the precipitant.
(d) The wells should not be filled more than ~70% full to avoid sealing issues;
(e) The row of wells along the other side of the slide is filled with the equilibrant solutions;
(f) The backing is removed from the precut sealing tape and the tape used to seal the wells;
(g) Crystal growth may be monitored optically from either surface of the slide;
(h) To harvest the developed crystals the tape seal is simply cut or removed for retrieval

3.C GPCR Diffusion Experiments with PHS-GPCR Cartridges

Using the PHS loading fixture (please see Purchase), or a suitable holder from your laboratory, the **PHS-GPCR** slide is used to screen protein crystallization conditions as follows:

(a) If desired, the GPCR or membrane protein can first be screened to see if the protein has a sufficiently high diffusion rate within the lipidic phase to allow subsequent crystal growth to occur at a reasonable rate. This is accomplished by placing a very small concentrated GPCR sample in the well and covering the protein with dispensed lipidic phase. If the protein does not diffuse into the surrounding lipidic phase at some predetermined rate, then this sample will always be incapable of crystallizing and can be eliminated from further screening trials.
(b) A suitable GPCR or membrane protein is mixed a Lipidic Cubic Phase (LCP) or some other lipidic phase.
(c) The **PHS- GPCR** slide is placed into the PHS loading fixture.
(d) All wells contain heterogeneous nucleants and all wells are filled with a mixture of the GPCR and lipidic phase.
(e) The wells should not be filled more than 80% full to avoid sealing issues;
(f) The backing is removed from the precut sealing tape and the tape used to seal the wells;
(g) Crystal growth may be monitored optically from either surface of the slide;
(h) To harvest the developed crystals the tape seal is simply cut or removed for retrieval

4. Protein Crystal Image Gallery

In the Image Gallery section, images of protein crystals are shown whose growth has been influenced in various ways by the Photon Hammer heterogeneous nucleants. The sections are arranged according to what type of heterogeneous nucleation effect was observed (see list of

effects below). When browsing the image gallery clicking an image load a larger view and mousing over the larger image zooms in and allows panning about the image.

A heterogeneous nucleant (HN) can influence crystal growth in many ways but the fundamental effect must manifest itself in creating one and only one type of nucleus at the earliest stages of nucleation and crystal growth. There are several examples given below where it is clear that once a given type of nucleus forms other crystalline polymorphs or nuclei of differing habit cannot form and are completely suppressed. In all of the examples shown in the Image Gallery, which provides a summary of hundreds of heterogeneous nucleation experiments, there was never an example of two different crystal habits, types or polymorphs observed growing within the same solution.

A heterogeneous nucleant is a solid state material which, when in contact with a solution containing the soluble species to be crystallized, alters the course of crystallization as compared to the course in the absence of the HN in ways such as:

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- Crystallization, or a particular crystalline polymorph, is suppressed in the presence of a heterogeneous nucleant
- Fewer larger crystals, rather than more smaller crystals, form in the presence of a HN but not in the absence HN
- Higher quality crystals form in the presence of a HN than without HN
- Crystals that have proven difficult or impossible to crystallize crystalize more readily in the presence of an HN

5. Crystallization of Non-proteinacious Materials

While there is a very great importance attached to the crystallization and structure determination of proteins, which is obvious because of their scientific, technical and commercial attributes, crystals of non-proteinacious materials have received far less attention. We have found that the enhanced nucleation effects associated with the Photon Hammer Substrates not only increases the efficacy of the protein crystallization process but likewise also exhibits a strong nucleating effect on a variety of other materials undergoing crystallization from solution. Although there are many technology areas, such as polymorph control of crystals of small molecule drugs or purification by crystallization, where influence on the speed, selectivity and reproducibility of a crystallization process could be of great benefit, the influence of heterogeneous nucleants on the growth of these materials is far less studied.

Since the cracks that are responsible for increased efficacy of the Photon Hammer heterogeneous nucleants taper to zero width as the terminate with the glassy substrates with the PHS cartridges, no matter how small the incipient unit cell may be there is a crack of similar dimensions since all molecular dimensions are present with the Photon Hammer nucleants.

Please see the Image Gallery for some beautiful and fascinating images of inorganic materials and small molecules nucleated on Photon Hammer Substrates.

6. Ordering the PHS and Accessories

Please visit the Purchase section of this website for the PHS slides, accessories and your *free* crystallization screening buffers.