Title: NANOVOLUME MICROCAPILLARY CRYSTALLIZATION SYSTEM

Abstract: A nanovolume microcapillary crystallization system allows nanoliter-volume screening of crystallization conditions in a crystal card that allows crystals to either be removed for traditional cryoprotection or in situ X-ray diffraction studies on protein crystals that grow within. The system integrates formulation of crystallization cocktails with preparation of the crystallization experiments. The system allows the researcher to select either gradient screening in crystallization experiments for efficient exploration of crystallization phase space or a combination of sparse matrix with gradient screening to execute one comprehensive hybrid crystallization trial.
NANOVOLUME MICROCAPILLARY CRYSTALLIZATION SYSTEM

CROSS-REFERENCE TO A RELATED APPLICATION

This application claims the benefit of U.S. Provisional Patent Application No. 61/061536, filed June 13, 2008, which is incorporated herein by reference.

STATEMENT OF GOVERNMENT LICENSE RIGHTS

This subject matter was made, at least in part, with Government support as provided for by the terms of NIGMS U54 GM074961, awarded by the National Institute of General Medical Sciences. The Government has certain rights in the subject matter.

BACKGROUND

The field of structural biology is generating technologies that increase throughput and efficiency each year. Such advances have inspired progression from gene to three-dimensional structure in three days. In an effort to improve efficiency, it is desirable to minimize the volume of protein required such that sufficient material for crystallization screening and optimization can be obtained from cell-free synthesis. With the “three day” structure goal in mind, it is desirable to develop several technologies to increase efficiency in the gene to structure pipeline.

SUMMARY

This summary is provided to introduce a selection of concepts in a simplified form that are further described below in the Detailed Description. This summary is not intended to identify key features of the claimed subject matter, nor is it intended to be used as an aid in determining the scope of the claimed subject matter.

One aspect of the discussed subject matter includes a system for protein crystallization, which comprises a pumping system and pieces of software configured to execute on the protein crystallization system to control the pumping system. The system further includes one or more crystal cards coupled to the pumping system, each configured to house a mixer and a microfluidic capillary that is coupled to the mixer to facilitate storage and inspection of protein crystallization.

Another aspect of the subject matter includes a method for gradient screening, which comprises regulating aqueous streams by independently controlling each aqueous stream with a pumping system exercised by pieces of software. The method further comprises mapping out crystallization phase space of a protein to illustrate transition
from precipitation, to microcrystals, to single crystals in a protein crystallization experiment.

A further aspect of the subject matter includes a method for hybrid screening, which comprises pre-forming precipitant plugs and pre-forming plug spacers, each separating two precipitant plugs from each other. The method further comprises forming gradients by merging precipitant plugs, plug spacers, and a protein stream. The method further includes mapping out crystallization phase space of a protein to illustrate transition from precipitation, to microcrystals, to single crystals in a protein crystallization experiment.

A further aspect of the subject matter includes a method which comprises receiving a crystal card with capillaries, coating capillaries with a reagent to reduce the surface energy, and removing the reagent.

In another aspect, the subject matter includes a crystal card, which comprises a substrate configured to house a mixer circuit and an inspection circuit. The crystal card further includes a layer bonded to the substrate and configured to peel from the substrate.

DESCRIPTION OF THE DRAWINGS

The foregoing aspects and many of the attendant advantages of this invention will become more readily appreciated as the same become better understood by reference to the following detailed description, when taken in conjunction with the accompanying drawings, wherein:

FIGURE 1 is a block diagram illustrating an exemplary nanovolume microcapillary crystallization system;

FIGURE 2 is a block diagram illustrating an exemplary pumping system of the nanovolume microcapillary crystallization system;

FIGURE 3A is a pictorial diagram illustrating an exemplary user interface for configuring the pumping system;

FIGURE 3B is a pictorial diagram illustrating an exemplary user interface for priming fluids to a crystal card of the system;

FIGURE 3C is a pictorial diagram illustrating an exemplary user interface for specifying the production of nanoplug in the crystal card wherein the nanoplug are of equal size and equal content according to one embodiment of the subject matter;

FIGURE 3D is a pictorial diagram illustrating an exemplary user interface for specifying the production of nanoplug in the crystal card wherein the nanoplug have
varying concentrations of protein and precipitant according to one embodiment of the subject matter;

FIGURE 3E is a pictorial diagram illustrating an exemplary user interface for specifying the production of nanoplugfs in the crystal card wherein the nanoplugfs have varying size and concentration for multiple precipitants according to another embodiment of the subject matter;

FIGURE 4A is a pictorial diagram illustrating a top isometric view of one embodiment of a crystal card; FIGURE 4B is a pictorial diagram illustrating a bottom isometric view of one embodiment of a crystal card; FIGURE 4C is a pictorial diagram illustrating a top view of one embodiment of a crystal card; FIGURE 4D is a pictorial diagram illustrating a side view of one embodiment of a crystal card; FIGURE 4E is a pictorial diagram illustrating a bottom view of one embodiment of a crystal card;

FIGURE 5A is a pictorial diagram illustrating a top isometric view of another embodiment of a crystal card; FIGURE 5B is a pictorial diagram illustrating a bottom isometric view of another embodiment of a crystal card; FIGURE 5C is a pictorial diagram illustrating a top view of another embodiment of a crystal card; FIGURE 5D is a pictorial diagram illustrating a side view of another embodiment of a crystal card; FIGURE 5E is a pictorial diagram illustrating a bottom view of another embodiment of a crystal card;

FIGURE 6A is a pictorial diagram illustrating a top isometric view of a third embodiment of a crystal card; FIGURE 6B is a pictorial diagram illustrating a bottom isometric view of a third embodiment of a crystal card; FIGURE 6C is a pictorial diagram illustrating a top view of a third embodiment of a crystal card; FIGURE 6D is a pictorial diagram illustrating a side view of a third embodiment of a crystal card; FIGURE 6E is a pictorial diagram illustrating a bottom view of a third embodiment of a crystal card;

FIGURE 7A is a pictorial diagram illustrating a top isometric view of a fourth embodiment of a crystal card; FIGURE 7B is a pictorial diagram illustrating a bottom isometric view of a fourth embodiment of a crystal card; FIGURE 7C is a pictorial diagram illustrating a top view of a fourth embodiment of a crystal card; FIGURE 7D is a pictorial diagram illustrating a side view of a fourth embodiment of a crystal card; FIGURE 7E is a pictorial diagram illustrating a bottom view of a fourth embodiment of a crystal card;
FIGURE 8A is a pictorial diagram illustrating a top isometric view of a fifth embodiment of a crystal card; FIGURE 8B is a pictorial diagram illustrating a bottom isometric view of a fifth embodiment of a crystal card; FIGURE 8C is a pictorial diagram illustrating a top view of an embodiment of a crystal card; FIGURE 8D is a pictorial diagram illustrating a side view of a fifth embodiment of a crystal card; FIGURE 8E is a pictorial diagram illustrating a bottom view of a fifth embodiment of a crystal card;

FIGURE 9A is a pictorial diagram illustrating a top isometric view of a sixth embodiment of a crystal card; FIGURE 9B is a pictorial diagram illustrating a bottom isometric view of a sixth embodiment of a crystal card; FIGURE 9C is a pictorial diagram illustrating an exploded isometric view of a sixth embodiment of a crystal card; FIGURE 9D is a pictorial diagram illustrating a top view of a sixth embodiment of a crystal card; FIGURE 9E is a pictorial diagram illustrating a side view of a sixth embodiment of a crystal card; FIGURE 9F is a pictorial diagram illustrating a bottom view of a sixth embodiment of a crystal card;

FIGURE 10 is a pictorial diagram illustrating one embodiment of a three-plus-one mixer of one embodiment of a crystal card;

FIGURE 11 is a pictorial diagram illustrating another embodiment of a three-plus-one mixer of one embodiment of a crystal card;

FIGURE 12 is a pictorial diagram illustrating a third embodiment of a three-plus-one mixer of one embodiment of a crystal card;

FIGURE 13 is a pictorial diagram illustrating a fourth embodiment of a three-plus-one mixer of one embodiment of a crystal card;

FIGURE 14 is a pictorial diagram illustrating a cross section through one embodiment of a crystal card; and

FIGURES 15A-15V are process diagrams illustrating an exemplary method for crystallizing molecules using a nanovolume microcapillary crystallization system:

DETAILED DESCRIPTION

Various embodiments of the subject matter describe a nanovolume microcapillary crystallization system which comprises a pump, software configured to control the pump, and a crystal card that houses a mixer circuit and an inspection circuit. The crystal cards are suitably manufactured using materials that include one or more properties selected from a group consisting of X-ray transmission, optical clarity, moldability, chemical resistance and surface energy. The crystal cards house macromolecular crystals in
various phases enabling either extraction of crystals from the crystal card or in situ X-ray diffraction. The crystals are promoted inside the crystal cards by formation of nanoplugs by the nanovolume microcapillary crystallization system. Nanoplugs are formed by combining streams of aqueous solutions with an immiscible and biologically inert carrier fluid, such as fluorocarbon solution. Streams of aqueous solutions, such as those composed of a target molecule, buffer, and precipitant solutions, are combined at the mixer circuit to form nanoplug crystallization experiments. The nanoplugs are incubated and monitored for crystallization. Nanoplug crystallization experiments can be suitably used to shed light on scientific questions regarding protein crystal nucleation and growth and to generate crystals for novel structure solution.

The nanovolume microcapillary crystallization system facilitates two screening styles: gradient mode and hybrid mode. As used herein, the term gradient mode includes any suitable screening method that provides various crystallization phases of molecules. The gradient mode allows a crystallographer to finely scan a crystal card to reveal crystallization phase space of a particular molecule. Because each stream of aqueous solution used in the nanovolume microcapillary crystallization system can be independently controlled using the pump via the software, concentration gradients of desired granularity over a series of nanoplugs are suitably formed by changing the flow rates of the individual streams. As a precipitant stream decreases in flow rate, the nanovolume microcapillary crystallization system increases a flow rate of a buffer stream such that the sum of the flow rates remains constant. Using the gradient mode, crystallization phase space of a particular molecule, such as a protein, can be mapped out to show a transition from precipitation, to microcrystals, to single crystals.

As an enhancement to gradient mode, hybrid mode combines gradients with sparse matrix screening on one crystal card. Sparse matrix screening of molecule crystals in nanoplugs can be achieved by generating a pre-formed cartridge of different crystallizing agents. As used herein, the term hybrid mode includes hybrid screening, including any suitable screening method that includes pre-formed cartridges. The hybrid mode extends the concept of sparse matrix screening by pre-forming precipitant nanoplugs, separated by a nanoplug spacer (gas bubble), and forming a concentration gradient as they are merged with a molecule stream. Similar to the gradient mode, the hybrid mode generates gradients by coordinating flow rate change between the pre-formed precipitant nanoplugs and the buffer stream. By performing sparse matrix
and gradient screening together on one crystal card, the hybrid mode is able to sample a
large area of the crystallization phase space, generating 20-40 experiments from each
pre-formed precipitant nanoplug.

As used hereinabove and hereinbelow, the term "nanoplug" refers to a
nanoliter-volume sized drop, such as a 10-20 nL aqueous drop, that fills a microfluidic
channel of the system. Each nanoplug comprises a distinct microcrystallization
experiment. As used hereinabove and hereinbelow, the term "mixer circuit" means the
inclusion of a circuit having three aqueous channels and one carrier fluid channel that
come together at a point upstream of the inspection circuit of a crystal card. Additional
configurations are possible, such as mixers having four or five aqueous channels and one
carrier fluid channel. The aqueous channels come together and suitably intersect the
carrier fluid channel at a 90 degree angle. As used herein, the term "macro-micro
interface" means the inclusion of a coupling between the syringes and a crystal card. In
some embodiments, the syringes are connected to the mixer circuit or the inspection
circuit via tubing, such as Teflon® (PTFE) tubing. In other embodiments, the tubing is
connected to the mixer circuit or the inspection circuit using connectors configured to
fluidically connect the inlets and outlets of the circuits with the tubing. As used
hereinabove and hereinbelow, the term "inspection circuit" refers to a capillary or channel
where fluids come together and form aqueous nanopugs that flow inside the capillary.
The inspection circuit can also be used to inspect the nanopugs for crystal formation.
Further, the inspection circuit can also be used to store crystals formed by the methods of
the subject matter, and is therefore also referred to herein as a storage capillary. As used
herein, the term "main channel" refers to the area of the inspection circuit that locates
downstream of the mixer where the aqueous solutions and carrier fluid combine to form
aqueous nanopugs. As used hereinabove and hereinbelow, the term "molecule" includes
small molecules, such as organic compounds and/or chemicals, and macromolecules.
The term "biological molecule" refers to a molecule that is derived from, modeled on, or
corresponds to a molecule from a biological source. The term also includes molecules
synthesized or produced in vitro, such as by cell-free synthesis, and/or in vivo, such as
recombinant proteins, mutant proteins, and artificial proteins, natural and artificial nucleic
acid molecules, and other biological molecules that do not occur in nature. As used
herein, the term "macromolecule" includes biopolymers such as nucleic acids, proteins,
carbohydrates and lipids. For simplicity, the terms "protein" and "protein solution" are used herein to encompass other types of molecules in addition to proteins.

A nanovolume microcapillary crystallization system 100 useful for molecule crystallization is shown in FIGURE 1. A prepared sample 102 comprising a molecule for which the crystal structure is desired, for example a prepared protein sample, is provided in an aqueous solution 104. Additional aqueous solutions are also provided; for example, a buffer solution and a precipitant solution. The buffer solution may comprise the buffer used to prepare the biological sample 102. A carrier fluid is also provided. The carrier fluid is immiscible with the aqueous solutions. Suitable examples of carrier fluids include fluorinated oils; for example, FC-40 (3M Corp., St. Paul, Minnesota). The aqueous solutions and carrier fluid 104 are provided to one or more syringes 106 which are connected to one or more pumps 108. The pump 108 is controlled by software executed on a nanoplug-forming computer 110. The software executed on the nanoplug-forming computer 110 regulates the flow of the aqueous solutions and carrier fluid 104 in a crystal card 112. The flow of the aqueous solutions and carrier fluid in the crystal card 112 are observed through a magnifying device such as a microscope 114.

A pumping system 200 useful for regulating the flow of various fluids through a crystal card is shown in FIGURE 2. Pump 1202 controls syringe 1204 and syringe 2208. Syringe 1204 is loaded with an aqueous solution such as buffer 206. Syringe 2 is filled with an aqueous solution such as a precipitant reagent 210. Pump 2212 controls syringe 3214 and syringe 4218. Syringe 3214 is filled with an immiscible fluid such as a carrier fluid 216. Syringe 4218 is filled with an aqueous solution containing a molecule, such as a protein of interest 220. Suitable pumps include Harvard Twin 33 syringe pumps (Harvard Apparatus, Holliston, MA). In some embodiments, the syringe pumps have been modified by the manufacturer to provide better accuracy. Suitable syringes include Hamilton syringes, such as an 1800 series Hamilton Gas Tight syringe. Suitable syringe volumes range from 10 ul to 100 ul. The pumping system 200 is controlled by software executed on a nanoplug-forming computer 110.

Suitable software is provided for controlling the pumping system 108. FIGURES 3A-3E illustrate representative user interfaces of the software of the system showing various modes that control the pumping system 200. FIGURE 3A shows a representative user interface 300 of a configuration mode of the software. FIGURE 3B
shows a representative user interface 302 of a prime mode of the software. FIGURE 3C
shows a representative user interface 304 of a constant mode of the software.
FIGURE 3D shows a representative user interface 306 of a gradient mode of the
software. FIGURE 3E shows a representative user interface 308 of a hybrid mode of the
software of the system.

Referring now to the crystal cards of the subject matter disclosed herein, a
representative example of one embodiment of a crystal card is shown in
FIGURES 4A-4E. The crystal card 400 is configured to be about the same size as a
standard microscope slide, being about 76.20 mm long and about 25.40 mm wide (or
about 3 inches long by about 1 inch wide). The crystal card 400 is about 1.0 to 1.5 mm
thick. The crystal card is manufactured of transparent polycarbonate by injection
molding (Siloam Biosciences, Inc.).

Referring now to the embodiment shown in FIGURES 4A and 4B, the crystal
card 400 has an upper surface 402 and a lower surface 414 that is parallel to the upper
surface 402. The crystal card 400 further comprises a substrate configured to house a
mixer circuit 404 and a storage and inspection circuit 406. The mixer circuit 404 is
comprised of four microfluidic channels 421, 422, 424, and 426. See FIGURE 4C.
Channels 421, 422, and 424 come together and intersect channel 426 at a 90 degree angle.
Each channel comprises an inlet 410. See FIGURE 4E. The inspection circuit 406
comprises a long microfluidic capillary channel that locates just downstream of the
mixer 404 and ends at an outlet 412. The length of the microfluidic capillary 406 is about
67 cm. The microfluidic capillary channel 406 is also referred to as an inspection circuit,
in which crystals produced in the card may be stored in the channel 406 until subjected to
in situ X-ray diffraction analysis or extracted for cryocooling. The microfluidic
channels 421, 422, 424, 426 and the capillary channel 406 are substantially square in
cross-section and have an inner diameter of about 200 micrometers (µm) x 200 µm.
However, other configurations of the channels are possible.

Referring now to FIGURE 4D, the crystal card 400 further comprises a layer 420
that is thermally bonded to the substrate and configured to peel from the substrate. The
peelable layer 420 is thermally bonded to the substrate surface 414. In other
embodiments, the peelable layer 420 may be chemically bonded to the substrate. The
peelable layer 420 is about 0.10 to 0.14 mm thick. The peelable layer 420 is suitably
configured such that removal of the peelable layer 420 exposes the interior space of the
inspection circuit channel 406. The crystal card 400 further comprises a macro-micro interface that connects the syringes to the crystal card. In one embodiment, the macro-micro interface includes sections of rigid plastic tubing 430 (for example, tubing made of PEEK™ polymer) that are connected at one end to the inlets 410 and outlet 412, and are connected at the other end to slip fit connectors 432 made of flexible silicone tubing. The slip fit connectors 432 are configured to accept Teflon® tubing (PTFE) (not shown). The other end of the tubing is connected to a syringe of the system. The Teflon® tubing has an inner diameter of 360 um and an outer diameter of 760 um (ID/OD 360/760), whereas the connector 432 has an inner diameter of 760 um, thereby forming a gas and liquid tight seal when the Teflon® tubing is inserted into the connector 432.

In operation, the channel 421 is connected to tubing that is filled with an aqueous solution, such as a buffer that is used in the protein solution of interest. Channel 422 is connected to tubing that is filled with a precipitant solution. As used herein, it is understood that the term precipitant is interchangeable with the term crystallant. Channel 424 is connected to tubing that is filled with a solution containing a target molecule of interest. In one embodiment, the target biological molecule is a protein. Channel 426 is connected to tubing that is filled with a carrier fluid. Suitable examples of carrier fluids include fluorinated oils or fluorocarbons, such as FC-40, although others are possible. The carrier fluid is immiscible with the aqueous fluids and preferentially wets the walls of the inspection circuit microchannel, thereby separating segments of the combined aqueous solution into nanoplugs that span the width of the channel. In one embodiment, the aqueous nanoplugs are about 10-20 nL in volume.

Referring now to FIGURES 5A-5E, a representative example of another embodiment of a crystal card of the subject matter is shown. Similar elements between different figures have similar reference numbers, wherein the first digit increases by one and corresponds to the figure number. For the sake of brevity, elements that are similar between the different Figures will not be described further. In the embodiment shown in FIGURES 5A-5E, the inlet 510 is located in a shallow cylindrical depression 508 located in a top surface 502 of the crystal card 500. The cylindrical depression 508 is configured for attaching a connector (not shown) that connects tubing to the inlets 510 and outlet 512. The dimensions of the crystal card 500 are shown in FIGURE 5E. The crystal card 500 is 76.2 mm long and 25.4 mm wide. The inlets 510 are spaced 4.5 mm
apart. The parallel channels of the inspection circuit 506 are 2.0 mm apart. However, as
will be appreciated by a person skilled in the art, other suitable configurations are
possible.

Referring now to FIGURES 6A-6E, a representative example of a third
embodiment of a crystal card is shown. For the sake of brevity, similar elements that are
described in previous figures are not described here. In the embodiment shown in
FIGURES 6A-6E, the inlets 610 and outlet 612 are positioned below a cylindrical
projection 608 that is connected to and extends outwardly from the surface 602. The
projection 608 is configured for attaching a connector (not shown) that connects tubing to
the inlets 610 and outlet 612. Digressing, the crystal cards illustrated in the embodiments
shown in FIGURES 4-6 are manufactured from transparent polycarbonate plastic by
injection molding (Siloam Biosciences, Inc.).

Returning now to FIGURES 7-9, representative embodiments of a second type of
crystal card will be described. FIGURES 7A-7E illustrate a representative example of
another embodiment of a crystal card of the subject matter. For the sake of brevity,
similar elements that are described in previous figures are not described here. In the
embodiment shown in FIGURES 7A-7E, the top surface 702 of the crystal card 700
further comprises two rows of ports 708. The ports are configured to receive a plastic
connector (not shown) that is suitable for connecting tubing to the inlets 710 and
outlets 712 located beneath the port 708. The surface 702 comprises 28 ports 708.
However, different numbers of ports are possible depending on the design of the crystal
card 700. The port 708 extends about 2.5 mm above the surface 702 of the crystal
card 700. A hole is suitably drilled in the bottom center portion of the port 708 such that
it aligns with and is in fluidic connection with the inlets 710 and outlets 712. The center
of the ports are spaced about 4.5 mm apart. The hole drilled in the bottom of the port 708
is about 0.2 mm (200 um) in diameter. It will be understood that not every port is
connected to the circuit channels such that only desired ports to connect tubing to the
inlets 710 and the outlets 712 need be drilled. In other embodiments, a laser is used to
drill holes through the peelable layer 720 before it is bonded to the bottom surface 714.
The laser-drilled holes are configured to be in fluidic connection with the inlets 710 and
the outlets 712. Tubing is connected to the laser-drilled holes using a specially designed
crystal card holder (not shown).
Referring still to FIGURES 7A-7E, the crystal card further comprises two separate asymmetrical microfluidic channel circuits 706A, 706B. In 706A, the inspection circuit is about 270 mm long. In 706B, the inspection circuit is about 306 mm long. In both circuits 706A and 706, the outlet 712 is located on the opposite side of the circuit from the inlets 710 and the mixer circuits 704A, 704B. The embodiment shown in FIGURES 7A-7E comprises two separate configurations of the mixer circuit 704A, 704B. As shown in more detail in FIGURE 10, the mixer circuit 704A comprises a short neck region approximately 0.20 mm long between the aqueous channels and the carrier fluid channel. As shown in more detail in FIGURE 11, the mixer circuit 704B lacks a neck region between the aqueous channels and the carrier fluid channel. The mixer circuit 704A was found to be suitable for aqueous nanoplug formation in a crystal card.

Referring now to FIGURES 8A-8E, a representative example of another embodiment of a crystal card of the subject matter is shown. For the sake of brevity, elements that are similar to previously described elements are not further described here. The crystal card 800 comprises two separate symmetrical microfluidic channel circuits 806. In this embodiment, the outlet 812 is located on the same side of the circuit 806 as the mixer 804 and the inlets 810.

Referring now to FIGURES 9A-9E, a representative example of another embodiment of a crystal card of the subject matter is shown. For the sake of brevity, elements that are similar to previously described elements are not further described here. A crystal card 900 comprises a single microfluidic circuit comprising one mixer circuit 904 and a long inspection circuit 906. The inspection circuit 906 is about 665 mm long. FIGURE 9C illustrates an exploded view of the crystal card 900. Piece 930 comprising ports 908 is bonded to piece 940 comprising the microfluidic circuit channels. The peelable layer 920 is thermally bonded to the bottom surface 914 of piece 940. However, in other embodiments, the peelable layer 920 may be chemically bonded to the substrate surface 914. The peelable layer 920 is suitably configured such that removal of the peelable layer 920 exposes the interior space of the inspection circuit channel 906. Digressing, the crystal cards illustrated in the embodiments shown in FIGURES 7-9 are manufactured from transparent cyclic olefin copolymer (COC) or comparable plastic (ThinXXS Microtechnology AG, Germany).

Returning now to FIGURES 10-13, representative examples of mixer circuits will now be described. FIGURE 10 shows a representative example of one embodiment of a
mixture circuit that corresponds to the mixer 704A shown in FIGURE 7. The mixer circuit 1000 comprises three aqueous channels 1021, 1022 and 1024. The aqueous channels are separated from the carrier fluid channel 1026 by a neck region 1007. The channels are oriented such that the three channels 1021, 1022, 1024 containing aqueous solutions come together and intersect the channel 1026 containing the carrier fluid at a 90 degree angle. The mixer 1000 further comprises a portion of an inspection circuit 1006.

Referring still to FIGURE 10, the dimensions of the mixer 1000 will now be described. The neck region 1007 is about 0.2 mm long. Channel 1021 is about 0.2 mm in diameter. Channels 1022, 1024 are about 0.141 mm in diameter. Channels 1006, 1026 are about 0.2 mm in diameter. However, other suitable dimensions for a mixer circuit are possible.

FIGURE 11 shows a representative example of another embodiment of a mixer circuit that corresponds to the mixer circuit 704B shown in FIGURE 7. The mixer circuit 1100 comprises three aqueous channels 1121, 1122 and 1124. The aqueous solution channels connect directly to the carrier fluid channel 1106 in the absence of a neck region. The mixer circuit feeds into the inspection circuit 1126. The channels are oriented such that the three channels containing aqueous solutions come together and intersect the channel containing the carrier fluid at a 90 degree angle. The diameter of channel 1121 is about 0.2 mm. The diameter of channels 1122, 1124 is about 0.141 mm. The diameter of the junction region between the aqueous channels and the carrier fluid channel 1126 is about 0.285 mm. However, other suitable dimensions for a mixer are possible.

Referring now to FIGURE 12, another view of the mixer circuit 704A described in FIGURE 7 is shown. The mixer circuit 1200 comprises three aqueous channels 1221, 1222 and 1224 that are connected by a short neck region to the carrier fluid channel 1226. The channels are oriented such that the three channels containing aqueous solutions come together and intersect the channel containing the carrier fluid at a 90 degree angle. Each channel has an inlet 1210. Downstream of the mixer circuit 1204, the solutions feed into a portion of an inspection circuit 1206. Referring still to FIGURE 12, the dimensions of the mixer circuit 1200 will now be described. The inlets 1210 are located about 4.4 mm from the channels 1206, 1226. The aqueous channels 1221, 1222, 1224 make a right angle turn about 2.9 mm from the inlet. The right angle turn has an inner radius RO.300 and an outer radius RO.500. The portion of channels 1221, 1222, 1224 that are disposed in a plane parallel to channel 1206 are about
1.300 mm from channel 1206. The aqueous channels 1221, 1222, 1224 make a 45 degree turn before connecting with each other upstream of the neck region. The inner diameter of channel 1206 is about 0.200 mm (200 um). The parallel portions of channel 1206 are about 1.2 mm apart. However, other suitable dimensions are possible.

FIGURE 13 shows a representative example of another embodiment of a mixer that corresponds to the mixer circuits 804 and 904 shown in FIGURES 8 and 9. The mixer circuit 1300 comprises aqueous channels 1321, 1322, and 1324. The aqueous channels are separated from the carrier fluid channel 1306 and the inspection circuit 1326 by a short neck region. The diameter of the neck region is about 0.200 mm. However, other suitable dimensions are possible. The channels are oriented such that the three channels containing aqueous solutions come together and intersect the channel containing the carrier fluid at a 90 degree angle. Downstream of the mixer circuit, the solutions flow into the inspection circuit 1326.

FIGURE 14 shows a representative example of a cross-section through a crystal card similar to the embodiment illustrated in FIGURE 9. The crystal card 1400 is comprised of three layers 1420, 1430 and 1440. Layer 1430 comprises the ports as shown in FIGURES 7-9. Layer 1430 is about 0.4 mm thick. Layer 1440 comprises the microfluidic channel circuit and is about 1.5 mm thick at the edge. Layer 1420 comprises the peelable layer attached to the bottom surface of the crystal card 1400, and is about 0.14 mm thick.

FIGURES 15A-15V illustrate a method 5000 for crystallizing molecules using a nanovolume microcapillary crystallization system. From a start block, the method 5000 proceeds to a set of method steps 5002, defined between a continuation terminal ("Terminal A") and an exit terminal ("Terminal B"). The set of method steps 5002 describes the preparation of a crystal card and the connection of the crystal card to a pump.

From Terminal A (FIGURE 15B), the method 5000 proceeds to a set of method steps 5008 where the crystal card is manufactured from a suitable material, such as polydimethylsiloxane (PDMS) or plastic by injection molding. The method then returns to a point of invocation. The method 5000 next proceeds to a set of method steps 5010 defined by a continuation terminal ("Terminal A2"). The set of method steps 5010 treats the microcapillary surface of the crystal card to reduce the surface energy.
From Terminal A2 (FIGURE 15C), the method 5000 proceeds to decision block 5014 where a test is performed to determine whether the crystal card is manufactured from plastic. If the answer to the test is NO, the method proceeds to another continuation terminal ("Terminal A4"). If the answer to the test at decision block 5014 is YES, the method proceeds to another decision block 5016 where another test is performed to determine whether the plastic is polycarbonate. If the answer to the test at decision block 5016 is NO, the method 5000 proceeds to another continuation terminal ("Terminal A5"). If the answer to the test at decision block 5016 is YES, the method 5000 proceeds to another continuation terminal ("Terminal A6").

From Terminal A4 (FIGURE 15D), the method 5000 proceeds to block 5018 where the method treats the crystal card as manufactured from PDMS. The method proceeds to block 5020 where the microcapillary surface is treated with a perfluorinated silane solution for 2 hours at room temperature. The method then proceeds to block 5022 where the perfluorinated silane solution is removed by vacuum. At block 5024, the microcapillary surface of the crystal card is dried using a gas such as air under pressure at 5-10 psi for 1 hour. The method then returns to the point from which the steps of Terminal A2 were invoked, and proceeds to another continuation terminal ("Terminal A3"). See block 5012.

From Terminal A5 (FIGURE 15E), the method 5000 proceeds to block 5026 where the method treats the crystal card as made of a plastic comprising cyclic olefin copolymer (COC) or comparable plastic. At block 5028, the microcapillary surface is treated with a reagent to reduce the surface energy (hydrophobicity) of the plastic for 2 hours at room temperature. Suitable reagents for reducing the surface energy include fluorinated copolymer solutions, but other reagents are possible. Suitable fluorinated copolymer solutions include a two percent fluorinated copolymer solution in a fluoro solvent, such as Cytonix PFC 502AFA (Cytonix Corp., Beltsville, MD). Cytonix PFC 502AFA is manufactured to adhere to polycarbonate and reduce the surface energy to 6-10 dyne/cm. To apply the fluorinated copolymer solution, the crystal card is filled from the outlet with the Cytonix PFC 502AFA solution. At block 5030, the fluorinated copolymer solution is removed by vacuum. At block 5032, the microcapillary surface is dried using a gas such as air under pressure of 5-10 psi for 1 hour. The method 5000 then proceeds to block 5034 where the crystal card is heated to 60°C for 1 hour. The method
then returns to the point of invocation of the steps of Terminal A2. See block 5012 at Terminal A3.

From Terminal A6 (FIGURE 15F), the method 5000 proceeds to block 5036 where the crystal card is pre-chilled on ice. At block 5038, the microcapillary surface is treated with a fluorinated copolymer solution such as Cytonix PFC 502AFA for 2 hours on ice. The polycarbonate crystal card inlets may be prone to cracking if incubated with the 502AFA solution at higher temperatures. The method then proceeds to continuation terminal A5 where it skips to block 5030 and performs the steps in blocks 5030, 5032, and 5034. The method then returns to a point at which the steps of Terminal A2 were invoked. See Terminal A3 at block 5012. The set of method steps at block 5012 couples the crystal card to the pump.

From Terminal A3 (FIGURE 15G), the method 5000 proceeds to block 5040 where syringe 1 is filled with a buffer or aqueous solution. At block 5042, syringe 2 is filled with a precipitant solution. At block 5044, syringe 3 is filled with a carrier fluid. A representative example of a suitable carrier fluid includes a fluorinated carbon solution. Suitable examples of a fluorocarbon fluid include FC-40. FC-40 has a high surface tension with the detergents used in solubilizing membrane proteins. The surface tension enables nanoplug formation and crystallization. In a representative embodiment, the carrier fluid is a fluorinated oil which is immiscible with aqueous fluids. The carrier fluid surrounds and separates the aqueous nanoplug as they are formed, moving them forward through the crystal card during the method. At block 5046, syringe 4 is filled with a protein solution containing the protein of interest in a suitable buffer. At block 5048, suitable tubing such as Teflon® tubing is attached to the needle of each syringe. At block 5050, syringes 1 and 2 are attached to pump 1, and syringes 3 and 4 are attached to pump 2. At block 5052, the tubing is connected to the crystal card via a macro-micro interface. Suitable connections for the macro-micro interface are described above. The method then proceeds to exit Terminal B.

From Terminal B, the method 5000 proceeds to a set of method steps 5004, defined between a continuation terminal ("Terminal C") and an exit terminal ("Terminal D"). The set of method steps 5004 receives instructions to regulate fluid flow through the crystal card to obtain crystals. From Terminal C (FIGURE 15H), the method 5000 proceeds to a set of method steps 5054, defined by a continuation terminal ("Terminal C1"). The set of method steps 5054 configures the pump.
From Terminal C1 (FIGURE 151), the method 5000 proceeds to block 5060 where the method receives instructions on the type of syringe pump model to be controlled by the system. Suitable pumps include Harvard Apparatus Twin Syringe Pump Model 33 (Harvard Apparatus, Holliston, MA), which has been modified by the manufacturer to provide better accuracy. As illustrated by FIGURE 2, each syringe pump controls two syringes. At block 5062, the method receives instructions on the serial communication port of a computer used to control the pump system. The communication ports are configured such that each syringe pump receives instructions at the same time, thereby preventing time delays and allowing the solutions to flow through the crystal card simultaneously. The method proceeds to block 5064 where the method receives instructions on the volume of each syringe connected to the pumps. At block 5066, the method determines the diameter of each syringe connected to the pumps. The method then proceeds to return to a point at which the steps of the Terminal C1 were invoked.

From block 5054, the method 5000 proceeds to a set of method steps 5056 defined by a continuation terminal ("Terminal C2"). The set of method steps primes fluids to the mixer circuit of the crystal card. From Terminal C2 (FIGURE 15J), the method 5000 proceeds to block 5068 where the method receives instructions on which syringe will be used to dispense fluids into the mixer of the crystal card. At block 5070, the method receives instructions on the flow rate from each syringe. At block 5072, the method receives instructions on the volume of fluid to be dispensed by the syringe. At block 5074, the method dispenses or aspirates fluid from a fluidic channel upstream of the mixer circuit. The method then continues to another continuation terminal ("Terminal CA").

From Terminal C4 (FIGURE 15K), the method 5000 proceeds to decision block 5076 where a test is performed to determine whether the syringe is dispensing an aqueous fluid. If the answer to the test at decision block 5076 is NO, the method proceeds to another continuation terminal ("Terminal C5). If the answer to the test at decision block 5076 is YES, the method proceeds to block 5078 where the method receives instructions to stop the aqueous fluid at the mixer circuit and before the fluid enters the inspection circuit. The method then continues to Terminal C2 and repeats the above identified process steps for the next syringe. From Terminal C5 (FIGURE 15K), the method 5000 proceeds to block 5080 where the method receives instructions to stop the carrier fluid downstream of the mixer circuit and slightly inside the inspection circuit.
The method then proceeds to return to a point from which the steps of Terminal C2 were invoked.

Digressing, an illustrative process for priming aqueous solutions and the carrier fluid to the mixer of the crystal card will now be described in detail. First, the empty crystal card mixer circuit is positioned on the microscope stage for observation during priming. The method receives instructions to dispense a solution, for example buffer, from syringe 1 to the mixer. The buffer is dispensed into the fluid channel connected to syringe 1 until the user observes that the solution has reached the region of the mixer just upstream of the junction between the fluidic channels. The method then receives instructions to stop dispensing the solution. Solution may be removed from the channel by instructing the method to aspirate the reagent. It is suitable to refrain aqueous solutions from entering the inspection circuit of the crystal card. The method is repeated for each of the three fluid channels connected to syringes dispensing aqueous solutions; for example, syringe 4 (protein solution) and syringe 2 (precipitant solution). The carrier fluid is then dispensed into the fourth fluid channel connected to syringe 3. The carrier fluid is dispensed into the fourth fluid channel until the fluid travels through the mixer junction and just slightly enters the inspection circuit (fifth channel) of the crystal card. The method then receives instructions to stop dispensing the carrier fluid.

Returning to block 5056, the method 5000 proceeds to a set of method steps 5058 defined by a continuation terminal ("Terminal C3"). The set of method steps receives instructions to produce aqueous nanoplugs in the inspection circuit of the crystal card. From Terminal C3 (FIGURE 15L), the method 5000 receives instructions on which nanoplug formation protocol will be performed at block 5082. The method then proceeds to decision block 5084 where a test is performed to determine whether the instruction received was to perform the constant mode. If the answer to the test at block 5084 is NO, the method proceeds to another continuation terminal ("Terminal C6"). If the answer to the test at decision block 5084 is YES, the method proceeds to block 5086 where the method receives instructions on the flow rate for each syringe. The method then proceeds to block 5088 where the method receives instructions on the total volume of fluid to pass through the mixer circuit. At block 5090, the method produces aqueous nanoplugs inside the inspection circuit of the crystal card wherein each nanoplug is suitably of equal size and has the similar concentration of protein and precipitant. The method then proceeds to return to a point of invocation. From block 5058, the method proceeds to exit terminal D.
From Terminal C6 (FIGURE 15M), the method 5000 proceeds to decision block 5092, where a test is performed to determine whether the method was instructed to perform gradient mode. If the answer to the test in block 5092 is NO, the method proceeds to another continuation terminal ("Terminal C7"). If the answer to the test in decision block 5092 is YES, the method proceeds to block 5094 where the method receives instructions on the maximum flow rate for the syringes with variable flow. In one embodiment, the variable flow syringes contain the buffer and precipitant. In another embodiment, syringes 1 and 2 are the variable flow syringes. However, the method can designate any syringe to be a variable flow syringe. In one embodiment, the combined flow rate of the variable flow syringes equals the maximum flow rate. For example, in one embodiment, the method provides instructions for the flow rate of syringe 1 to equal 2 µl/min, whereas the method provides instructions for the flow rate of syringe 2 to equal 0 (zero) µl/min. In this embodiment, the maximum flow rate equals 2 ul/min (2 + 0 µl/min). The method then proceeds to block 5096 where the method receives instructions on the constant flow rate for the syringe controlling the carrier fluid. In one embodiment, syringe 3 controls the carrier fluid. In one embodiment, the carrier fluid flow rate equals the total flow rate of the aqueous solutions (buffer, precipitant, and protein solutions). In another embodiment, the flow rate for the carrier fluid may be selected to be slower or faster than the total flow rate of the aqueous fluids. Slower carrier fluid rates generate larger aqueous nanoplug with smaller segments comprising carrier fluid between nanoplug. Faster carrier fluid rates generate smaller aqueous nanoplug with larger carrier fluid segments between the nanoplug. The method then proceeds to block 5098 where the method receives instructions on the constant flow rate for the syringe controlling the protein solution. In one embodiment, syringe 4 controls the carrier fluid. In one embodiment, the protein flow rate equals the sum of the flow rate of the other aqueous solutions (buffer and precipitant). Changing the flow rate of the protein solution changes the ratio of protein-to-crystallization conditions in each nanoplug. The method then proceeds to block 6000 where the method receives instructions on the total aqueous volume to be dispensed during a single iteration or cycle of the method. The method then proceeds to another continuation terminal ("Terminal C8").

From Terminal C8 (FIGURE 15N), the method 5000 proceeds to block 6002 where the method receives instructions on the volume of each aqueous nanoplug that will
be dispensed into the inspection circuit. At block 6004, the method receives instructions on the total number of iterations or cycles to be performed (i.e., the number of times the gradient screening steps are repeated). In one embodiment, if the method receives instructions to run zero iterations, the pumps will stop when the total aqueous volume selected at block 6000 is dispensed. In another embodiment, if the method receives instructions to run one or more iterations, the pumps will stop when the process steps described above have been repeated the desired number of times. At block 6006, the method reciprocally varies the flow rate of the buffer and precipitant solutions such that the sum of the buffer and precipitant solution flow rates equals the maximum flow rate selected at block 5094. For example, in one embodiment, at block 5094 the method provides instructions for the flow rate of syringe 1 to equal 2 µl/min and provides instructions for the flow rate of syringe 2 to equal 0 µl/min, such that the maximum flow rate equals 2 µl/min. When the method starts, the flow rate from syringe 1 will begin at 2 µl/min and ramp down to 0 µl/min, while the flow rate from syringe 2 will simultaneously ramp up from 0 µl/min to 2 µl/min. At block 6008, the method produces a series of aqueous nanopluggs inside the inspection circuit wherein each drop is of equal size but varies in the concentrations of protein and precipitant in each drop. At block 6010, the method terminates after the desired number of iterations or cycles has been performed. The method then returns to block 5058 where the method proceeds to exit terminal D.

From Terminal C7 (FIGURE 150), the method 5000 proceeds to decision block 6012 where a test is performed to determine whether the method was instructed to perform hybrid mode. If the answer to the test at block 6012 is NO, the method proceeds to another continuation terminal ("Terminal C9"). If the answer to the test at block 6012 is YES, the method proceeds to another decision block 6014 where a test is performed to determine whether a precipitant cartridge has been prepared. If the answer to the test at decision block 6014 is NO, the method proceeds to another continuation terminal ("Terminal C10"). If the answer to the test at block 6014 is YES, the method proceeds to another continuation terminal ("Terminal CH").

From Terminal C9 (FIGURE 15P), the method 5000 proceeds to decision block 6016 where a test is performed to determine whether the method was instructed to perform the pulsatile mode. If the answer to the test at decision block 6016 is NO, the method returns to Terminal C3 where the above identified steps are repeated. If the
answer to the test at decision block 6016 is YES, the method proceeds to block 6018
where the method receives instructions on performing the pulsatile mode. The method
then returns to block 5058. From block 5058, the method exits to Terminal D.

From Terminal C10 (FIGURE 15Q), the method 5000 proceeds to block 6020
where a syringe is connected to tubing, such as Teflon® tubing, containing carrier fluid.
The method then proceeds to block 6022 where the syringe is connected to a syringe
pump. At block 6024, the method receives instructions to enter a defined volume, for
example, about 40 nL, and aspirates an air bubble of about 40 nL into the tubing. At
block 6026, the method aspirates a defined volume, for example, about 120 nL, of a
precipitant solution into the tubing. At block 6028, the method repeats the above two
steps until a suitable number of precipitants are loaded into the tubing. For example, a
suitable number of precipitants can range from 1-24 or more. At block 6030, the method
aspirates carrier fluid, about 1 µL, into the open tip of the tubing. At block 6032, the
tubing is connected to the precipitant inlet of the crystal card. The method then proceeds
to continuation Terminal C1I.

At Terminal C1II (FIGURE 15R), the method 5000 proceeds to block 6034 where
the method receives instructions on the starting flow rate of the buffer solution
(syringe 1). At block 6036, the method receives instructions on the change in the flow
rate (step size) of the buffer solution. The step size is the change in the rate of flow that
will be applied at each ramp up or down of the method. At block 6038, the method
receives instructions on the starting flow rate of the precipitant cartridge (syringe 2). At
block 6040, the method calculates the change in the flow rate (step size) of the precipitant
solution. In one embodiment, the step size for the buffer equals the step size for the
precipitant. At block 6042, the method sums the buffer and precipitant flow rates to
determine the total flow rate. At block 6044, the method receives instructions on the
starting flow rate for the carrier fluid (syringe 3). At block 6046, the method receives
instructions on the change in the flow rate (step size) of the carrier fluid. The method
then proceeds to another continuation terminal ("Terminal C12").

From Terminal C12 (FIGURE 15S), the method 5000 proceeds to block 6048
where the method receives instructions on the constant flow rate of the protein solution
(syringe 4). The method then proceeds to block 6050 where the method receives
instructions on the number of ramp up steps (rate of flow changes) for each precipitant.
At block 6052, the method sets the number of ramp down steps to equal the number of
ramp up steps for each iteration or cycle of the method. At block 6054, the method receives instructions on the number of iterations or cycles to be performed. In one embodiment, one iteration or cycle corresponds to a single precipitant loaded in the precipitant cartridge. At block 6056, the method receives instructions on the duration of each ramp step. For example, in one embodiment, the duration of each ramp step is 1.5 seconds. At block 6058, the method reciprocally varies the buffer and precipitant flow rates such that the sum equals the starting rates. The method then proceeds to another continuation terminal (“Terminal C13”).

From Terminal C13 (FIGURE 15T), the method 5000 proceeds to block 6060 where the method varies the flow rate of the carrier fluid. The method then proceeds to block 6062 where the method produces a series of nanoplug inside the inspection circuit wherein each drop has equal amounts of protein and varying amounts of precipitant and buffer. In one embodiment, the method provides a varied amount of precipitant with a constant amount of protein for each cycle. Table 1 illustrates one embodiment of the method described above for the hybrid mode. The method then proceeds to Terminal D.

<table>
<thead>
<tr>
<th>One cycle up</th>
<th>Step</th>
<th>Buffer (ul/min)</th>
<th>Precipitant (ul/min)</th>
<th>Carrier Fluid (ul/min)</th>
<th>Protein (ul/min)</th>
<th>Sum Aqueous</th>
<th>Duration (sec)</th>
<th>Total time (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.2</td>
<td>0.6</td>
<td>2.2</td>
<td>0.6</td>
<td>1.4</td>
<td>1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ramp up</td>
<td>1</td>
<td>0.3</td>
<td>0.5</td>
<td>2.0</td>
<td>0.6</td>
<td>1.4</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.4</td>
<td>0.4</td>
<td>1.8</td>
<td>0.6</td>
<td>1.4</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.5</td>
<td>0.3</td>
<td>1.6</td>
<td>0.6</td>
<td>1.4</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.6</td>
<td>0.2</td>
<td>1.4</td>
<td>0.6</td>
<td>1.4</td>
<td>1.5</td>
<td>6.0</td>
</tr>
<tr>
<td>Ramp down</td>
<td>1</td>
<td>0.5</td>
<td>0.3</td>
<td>1.6</td>
<td>0.6</td>
<td>1.4</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.4</td>
<td>0.4</td>
<td>1.8</td>
<td>0.6</td>
<td>1.4</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.3</td>
<td>0.5</td>
<td>2.0</td>
<td>0.6</td>
<td>1.4</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.2</td>
<td>0.6</td>
<td>2.2</td>
<td>0.6</td>
<td>1.4</td>
<td>1.5</td>
<td>6.0</td>
</tr>
<tr>
<td>Step size</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>stable</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Values specified by the method in hybrid mode.
From Terminal D at block 5004, the method 5000 proceeds to a set of method steps 5006, defined between a continuation terminal ("Terminal E") and an exit terminal ("Terminal F"). The set of method steps 5006 performs diffraction experiments on the crystals obtained from the crystal card. From Terminal E (FIGURE 15U), the method 5000 proceeds to decision block 6064 where a test is performed to determine whether crystals were extracted from the inspection circuit of the crystal card prior to diffraction. If the answer to the test at block 6064 is NO, the method proceeds to another continuation terminal ("Terminal E1"). If the answer to the test at block 6064 is YES, the method proceeds to block 6066 where a peelable layer is removed from the bottom surface of the crystal card. In one embodiment, the peelable layer is bonded to the plastic part of the crystal card that contains the microfluidic channels. The bond is designed to be strong enough to prevent fluid from leaking out of the microfluidic circuit but weak enough to be manually peeled off. In one embodiment, the bond is a thermal bond. In another embodiment, the bond is a chemical bond. Removal of the peelable layer exposes the interior of the microfluidic channels of the crystal card, allowing access to the aqueous nanoplugs. In another embodiment, the aqueous nanoplugs that contain crystals are retained in the microfluidic channels of the crystal card after the peelable layer is removed. At block 6068, the crystal formed in the inspection circuit is extracted from the crystal card using a cryoloop. In one embodiment, the cryoloop is a nylon cryoloop. At block 6070, the crystal is cryocooled, and diffraction data is obtained. The method then proceeds to exit Terminal F where the method terminates execution.

From Terminal E1 (FIGURE 15V), the method 5000 proceeds to block 6072 where the crystal card containing crystals is mounted onto the goniometer of an X-ray source. At block 6074, the method obtains diffraction data from crystals located in situ inside the inspection circuit. The method then proceeds to block 5006 and exit Terminal F. The method then terminates execution.

The above described crystal extraction steps can be used in combination with the gradient screening of various embodiments of the subject matter to generate crystals of methionine-R-sulfoxide reductase. Crystals were removed from the crystal card using a cryoloop and then cryocooled for diffraction experiments. As an example, a 1.7 Å data set was collected at SBC-CAT beamline 19BM located at the Advanced Photon Source at Argonne National Laboratories and the structure was subsequently solved and refined.
The final coordinates and structure factors were deposited to the Protein Data Bank (accession code 3CXK).

The crystal card of various embodiments of the subject matter is also suitable for in situ diffraction. In situ diffraction allows the crystallographer to assess the quality of a crystal before being altered by the cryoprotection process. For robust crystals, it can allow complete diffraction data to be collected. The crystal card is sufficiently X-ray transparent to be mounted onto the goniometer of an X-ray source for diffraction data collection at room temperature. For example, a simple test was conducted to analyze the absorption of the X-Rays by the crystal card. The beam current in the ion chamber normalized to the APS ring current (1/I₀) was measured with and without the crystal card inserted at a wavelength of 0.979261Å (12.66099 keV). 1/I₀ without the crystal card measured 1.91671 E-6 and I/I₀ with the crystal card measured 1.5511 E-6. This constitutes a 19% X-ray absorbance by the crystal card. Further, the crystal card can be translated along its X and Y axis to collect data from multiple crystals to be combined for a complete data set. To demonstrate this technique, a crystal card containing Lysozyme crystals was mounted on the goniometer head at NE-CAT beamline 24ID-C located at the Advanced Photon Source at Argonne National Laboratories. Data were collected at room temperature from three crystals in the crystal card. Crystallographic data are provided in Appendix A.

Regarding structure determination, data sets were collected at the Advanced Photon Source: beamline 19BM at IOOK for methionine-R-sulfoxide reductase and beamline 24-IDC at room temperature for lysozyme. Data were integrated and scaled with HKL2000. For the lysozyme structure, intensities were integrated separately for each of the three data sets using the mosflm package. The structures of lysozome and methionine-R-sulfoxide reductase were solved by molecular replacement using Molrep and PDB entries HEE and 3CEZ as the search models, respectively. Structures were refined with Refmac5 and model building was performed with Coot.

While illustrative embodiments have been illustrated and described, it will be appreciated that various changes can be made therein without departing from the spirit and scope of the invention.
APPENDIX A

Crystallographic Data:

<table>
<thead>
<tr>
<th>Data Collection</th>
<th>Lysozyme</th>
<th>methionine-R-sulfoxide reductase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unit Cell (Å)</td>
<td>(a=79.18, \ c=38.38)</td>
<td>(a=42.00, \ b=45.17)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(c=45.40, \ \alpha=88.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(\beta=83.7, \ \nu=69.1)</td>
</tr>
<tr>
<td>Space group</td>
<td>(P4_2_2_1) (No. 96)</td>
<td>(P1) (No. 1)</td>
</tr>
<tr>
<td>Resolution (Å)</td>
<td>50 – 1.90</td>
<td>50 – 1.70</td>
</tr>
<tr>
<td>Wavelength (Å)</td>
<td>0.97950</td>
<td>0.97932</td>
</tr>
<tr>
<td>Total Reflections</td>
<td>54,338</td>
<td>118,181</td>
</tr>
<tr>
<td>Unique Reflections</td>
<td>10,151</td>
<td>32,539</td>
</tr>
<tr>
<td>(I/\sigma(I))</td>
<td>11.4 (2.9)</td>
<td>23.1 (2.2)</td>
</tr>
<tr>
<td>(R_{merge}) (%)</td>
<td>13.7 (58.4)</td>
<td>6.8 (42.3)</td>
</tr>
<tr>
<td>Completeness (%)</td>
<td>98.8 (98.5)</td>
<td>95.3 (87.4)</td>
</tr>
<tr>
<td>Redundancy</td>
<td>5.4 (5.0)</td>
<td>3.6 (3.3)</td>
</tr>
<tr>
<td>Wilson B factor (Å²)</td>
<td>24.1</td>
<td>22.1</td>
</tr>
</tbody>
</table>

Refinement

| Resolution (Å) | 50 – 1.90 | 50 – 1.70 |
| Reflections (working/test) | 9,412 / 480 | 30,828 / 1,650 |
| \(R_{work}\) / \(R_{free}\) (%) | 19.6 / 23.0 | 16.6 / 19.9 |
| Number of atoms (protein / water) | 1001 / 45 | 2082 / 180 |
| r.m.s. deviation bond length (Å) | 0.016 | 0.015 |
| r.m.s. deviation bond angle (degrees) | 1.607 | 1.408 |
| Average B factor (Å²) (All atoms) | 28.8 | 28.3 |
| Average B factor (Å²) (Protein) | 28.5 | 27.5 |
| Average B factor (Å²) (Water) | 35.9 | 37.0 |
| Coordinate error (Å) Based on \(R_{free}\) | 0.149 | 0.095 |

Ramachandran Analysis (%)

| Most Favored (chain A/B) | 89.4 | 91.7 / 90.8 |
| Additionally Allowed (chain A/B) | 10.6 | 7.3 / 8.3 |

* Parenthesis indicates values for the 2.00 Å to 1.90 Å resolution shell for lysozyme and 1.76 Å to 1.70 Å shell for methionine-R-sulf oxide reductase.
CLAIMS

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. A protein crystallization system, comprising:
   a pumping system;
   pieces of software configured to execute on the protein crystallization system to control the pumping system; and
   one or more crystal cards coupled to the pumping system, each configured to house a mixer and a microfluidic capillary that is coupled to the mixer to facilitate storage and inspection of protein crystallization.

2. The protein crystallization system of Claim 1, wherein the pumping system includes a syringe pumping system or a pressure pumping system, wherein the syringe pumping system includes four-channel syringe pumps to regulate aqueous solutions being conveyed into the one or more crystal cards through second, third, and fourth microfluidic channels, and fluorous solutions being conveyed into a fifth microfluidic channel.

3. The protein crystallization system of Claim 1, wherein the pieces of software facilitate control of each pump of the four-channel syringe pump and control of each channel of second, third, fourth, and fifth microfluidic channels to generate granular gradients of flow of aqueous solutions.

4. The protein crystallization system of Claim 1, wherein the one or more crystal cards are formed from materials having properties that are selected from a group consisting of X-ray transmissive, optical clarity, modable, chemical resistive, suitable surface energy, and a combination of two or more of the foregoing recited properties.

5. The protein crystallization system of Claim 1, wherein the mixer includes a junction of second, third, fourth, and fifth microfluidic channels where aqueous plugs are formed, the second, third, fourth, and fifth microfluidic channels being formed from microfluidic channels that are approximately 200 by 200 micrometers.
6. The protein crystallization system of Claim 5, wherein the junction defines a hydrophobic surface that supports formation of aqueous plugs, which are approximately in a range of 10 nanoliters to 20 nanoliters, and wherein the microfluidic capillary transports the aqueous plugs away from the junction.

7. The protein crystallization system of Claim 2, wherein the one or more crystal cards are formed from plastic configured for fine gradient screening and are alternatively formed from PDMS/Teflon® configured for hybrid screening and membrane proteins.

8. The protein crystallization system of Claim 1, further comprising syringes with needles coupled to the pumping system, and yet further comprising tubings having distal ends and proximal ends configured to act as macro-micro interface between the needles of the syringes and the one or more crystal cards, each tubing having an inner diameter of about 360 micrometers and an outer diameter of about 760 micrometers, the distal end of a tubing configured to slide onto a needle and the proximal end of the tubing configured to coupled to the one or more crystal cards.

9. A method for gradient screening, comprising:
regulating aqueous streams by independently controlling each aqueous stream with a pumping system exercised by pieces of software; and
mapping out crystallization phase space of a protein to illustrate transition from precipitation, to microcrystals, to single crystals in a protein crystallization experiment.

10. The method of Claim 9, wherein the act of regulating includes forming concentration gradients over a series of aqueous plugs by changing flow rates of each aqueous stream.

11. The method of Claim 10, wherein the act of regulating includes regulating aqueous streams selected from proteins, crystallization agents, fluorocarbons, precipitants, ligands, protein partners, DNA complexes, buffers, and cryoprotectants.

12. The method of Claim 11, wherein the act of regulating includes increasing a flow rate of an aqueous stream of a buffer when a flow rate of an aqueous stream of a precipitant decreases so that a sum of flow rates remains constant.
13. A method for hybrid screening, comprising:
   pre-forming precipitant plugs;
   pre-forming plug spacers, each separating two precipitant plugs from each other;
   forming gradients by merging precipitant plugs, plug spacers, and a protein stream;
   mapping out crystallization phase space of a protein to illustrate transition from precipitation, to microcrystals, to single crystals in a protein crystallization experiment.

14. The method of Claim 13, wherein pre-forming plug spacers includes pre-forming using gas bubbles.

15. The method of Claim 13, wherein forming gradients includes coordinating flow rate change between a stream formed from the precipitant plugs, plug spacers, and a buffer stream.

16. The method of Claim 13, wherein each precipitant plug is about 100 nanoliters.

17. A method comprising:
   receiving a crystal card with capillaries;
   coating capillaries with a reagent to reduce a surface energy; and
   removing the reagent.

18. The method of Claim 17, further comprising incubating the crystal card on ice for a predetermined number of hours.

19. The method of Claim 17, wherein the capillaries include inside surfaces and the act of coating capillaries includes coating the inside surfaces of the capillaries to reduce surface energy to about six to ten dynes per centimeter.

20. The method of Claim 17, wherein the fluorinated copolymer solutions include two percent fluorinated copolymer solutions in fluorosolvent.

21. The method of Claim 17, wherein removing the fluorosolvent includes vacuuming the crystal card.
22. The method of Claim 17, further comprising an act of forcing clean, dry air through the crystal card, which is executed at five psi for about one hour.

23. The method of Claim 17, further comprising an act of baking the crystal card, which is executed at about sixty degree Celsius for about one hour.

24. The method of Claim 17, further comprising:
peeling a thin layer bonded to a substrate of a crystal card;
extracting crystals by a cryoloop from microfluidic circuitry housed on the substrate;
cryocooling the crystals; and
performing diffraction experiments on the crystals to obtain diffraction data.

25. The method of Claim 17, further comprising:
mounting a crystal card with microfluidic circuitry to a goniometer;
radiating the crystal card with X ray; and
collecting diffraction data.

26. The method of Claim 25, further comprising translating the crystal card along x and y axes to collect the diffraction data from multiple crystals stored by the microfluidic circuitry.

27. A crystal card, comprising:
a substrate configured to house a mixer circuit and an inspection circuit; and
a layer bonded to the substrate and configured to peel from the substrate.

28. The crystal card of Claim 27, wherein the layer is either thermally bonded to the substrate or chemically bonded to the substrate.

29. The crystal card of Claim 27, wherein the substrate and the layer are formed from a group consisting of an amorphous polymer, Cyclic Olefin Copolymer, a thermalplastic polymer, and Polycarbonate.

30. The crystal card of Claim 27, wherein the substrate includes a thickness of about one millimeter and the layer includes a thickness in a range of about 100 to 150 micrometers.
31. The crystal card of Claim 27, wherein the mixer circuit includes first, second, third, and fourth summand channels, each summand channel including a distal end and a proximal end, the distal end of each summand channel defining an opening configured to fluidly receive solutions, the proximal end of each summand channel defining an opening configured to fluidly communicate aqueous plugs or plug spacers, each summand channel having a first part being coupled to the distal end and a second part of the first, second, and third summand channels being coupled to the proximal end, the first part of each summand channel being spaced apart and oriented in parallel with another summand channel, the second parts of the first and third summand channels angled so that their proximal ends intersect, the second parts of the second and fourth summand channels continuing in parallel until the proximal end of the second summand channel intersects with the proximal ends of the first and third summand channels to form a vertex, a third part of the fourth summand channel continuing from the second part of the fourth summand channel at a ninety degree angle where its proximal end intersects with the vertex at another ninety degree angle.

32. The crystal card of Claim 31, wherein the first part of each summand channel is spaced apart from the first part of another summand channel by about 4.50 millimeters.

33. The crystal card of Claim 31, wherein the substrate includes a first side, a second side, a third side, and a fourth side, the second side of the substrate being spaced apart from the distal end of the third summand channel by about 3.70 millimeters, a length of the first and third side being approximately 25.40 millimeters, a length of the second and fourth side being about 76.20 millimeters, the first side of the substrate being spaced apart from the distal ends of the summand channels by about 6.00 millimeters.

34. The crystal card of Claim 33, wherein the second side of the substrate is spaced apart from the distal end of the third summand channel by about 3.70 millimeters, the first side of the substrate being spaced apart from the distal ends of the summand channels by about 6.00 millimeters, the third side of the substrate being spaced apart from the inspection circuit by approximately 6.00 millimeters.
35. The crystal card of Claim 31, wherein the inspection circuit includes a summation channel, a serpentine body, and a tail channel which terminates in an opening configured to fluidly communicate, the summation channel being coupled to the vertex and continued in a direction that is collinear with the proximal end of the fourth summand channel until the summand channel reaches an axis that is collinear with the first part of the third summand channel at which the summation channel makes a ninety degree turn to join with the serpentine body of the inspection circuit.

36. The crystal card of Claim 35, wherein the serpentine body of the inspection circuit is formed from a compound curve having multiple convex turnings coupled to each other by a serpentine channel to facilitate fluid communication, one convex turning being spaced apart from a subsequent convex turning by about 53.31 millimeters, each convex turning having a length of about 2.00 millimeters.

37. The crystal card of Claim 36, wherein a last convex turning of the serpentine body is coupled to the tail channel.

38. The crystal card of Claim 37, wherein a length of the summation channel, the serpentine body, and the tail channel is collectively about 67 centimeters, wherein cross-sectional dimensions of the summation channel, the serpentine body, and the tail channel are about 200 by 200 micrometers.

39. The crystal card of Claim 27, wherein the substrate is configured to house two mixer circuits and two inspection circuits.

40. The crystal card of Claim 39, wherein the substrate houses multiple annular ports that project upwardly, some of which multiple annular ports are adapted to fluidly receive solutions or fluidly communicate aqueous plugs or plug spacers.
Fig. 3A.
START A METHOD FOR MACROMOLECULE CRYSTALLIZATION USING A NANODROP CRYSTALLIZATION SYSTEM

A CRYSTAL CARD IS PREPARED AND CONNECTED TO A PUMP (FIG. 15B-15I)

THE METHOD RECEIVES INSTRUCTIONS TO REGULATE FLUID FLOW THROUGH THE CRYSTAL CARD TO OBTAIN CRYSTALS (FIG. 15H-15T)

DIFFRACTION EXPERIMENTS ARE PERFORMED ON THE CRYSTALS (FIG. 15U-15V)

FINISH

Fig.15A.
THE CRYSTAL CARD IS MANUFACTURED FROM PDMS OR PLASTIC BY INJECTION MOLDING

RETURN

THE MICROCAPILLARY SURFACE OF THE CRYSTAL CARD IS TREATED TO REDUCE SURFACE ENERGY

RETURN

THE CRYSTAL CARD IS CONNECTED TO THE PUMP

RETURN

Fig.15B.
IS THE CRYSTAL CARD MANUFACTURED OF PLASTIC?

IS THE PLASTIC POLYCARBONATE?

Fig. 15C.
THE METHOD TREATS THE CRYSTAL CARD AS MANUFACTURED FROM PDMS

THE MICROCAPILLARY SURFACE IS TREATED WITH A PERFLUORINATED SILANE SOLUTION FOR 2 HOURS AT ROOM TEMPERATURE

THE PERFLUORINATED SILANE SOLUTION IS REMOVED BY VACUUM

THE MICROCAPILLARY SURFACE IS DRIED USING A GAS UNDER PRESSURE (5-10 PSI FOR 1 HOUR)

RETURN

Fig. 15D.
THE METHOD TREATS THE CRYSTAL CARD AS MADE OF A PLASTIC COMPRISING CYCLIC OLEFIN COPOLYMER (COC) OR COMPARABLE PLASTIC

THE MICROCAPILLARY SURFACE IS TREATED WITH A FLUORINATED COPOLYMER SOLUTION FOR 2 HOURS AT ROOM TEMPERATURE

THE FLUORINATED COPOLYMER SOLUTION IS REMOVED BY VACUUM

THE MICROCAPILLARY SURFACE IS DRIED USING A GAS UNDER PRESSURE (5-10 PSI FOR 1 HOUR)

THE CRYSTAL CARD IS HEATED TO 60°C FOR 1 HOUR

RETURN

Fig.15E.
THE CRYSTAL CARD IS PRE-CHILLED ON ICE

THE MICROCAPILLARY SURFACE IS TREATED WITH A FLUORINATED COPOLYMER SOLUTION FOR 2 HOURS ON ICE
SYRINGE 1 IS FILLED WITH A BUFFER OR AQUEOUS SOLUTION

SYRINGE 2 IS FILLED WITH A PRECIPITANT SOLUTION

SYRINGE 3 IS FILLED WITH A CARRIER FLUID

SYRINGE 4 IS FILLED WITH A PROTEIN SOLUTION

TUBING, SUCH AS TEFLO® TUBING, IS ATTACHED TO THE NEEDLE OF EACH SYRINGE

SYRINGES 1 AND 2 ARE ATTACHED TO PUMP 1, AND SYRINGES 3 AND 4 ARE ATTACHED TO PUMP 2

THE TUBING IS CONNECTED TO THE CRYSTAL CARD VIA A MACRO-MICRO INTERFACE

Fig. 15G.
THE METHOD RECEIVES INSTRUCTIONS TO CONFIGURE THE PUMPS

RETURN

THE METHOD RECEIVES INSTRUCTIONS TO PRIME FLUIDS TO THE MIXER OF THE CRYSTAL CARD

RETURN

THE METHOD RECEIVES INSTRUCTIONS TO PRODUCE AQUEOUS NANOPLUGS IN THE CAPILLARY OF THE CRYSTAL CARD

RETURN

Fig. 15H.
THE METHOD RECEIVES INSTRUCTIONS ON THE PUMP MODEL TO BE CONTROLLED BY THE SYSTEM

THE METHOD RECEIVES INSTRUCTIONS ON THE SERIAL COMMUNICATION PORT USED TO CONTROL THE PUMP

THE METHOD RECEIVES INSTRUCTIONS ON THE VOLUME OF EACH SYRINGE CONNECTED TO THE PUMPS

THE METHOD DETERMINES THE DIAMETER OF EACH SYRINGE CONNECTED TO THE PUMPS

RETURN

Fig.15I.
THE METHOD RECEIVES INSTRUCTIONS ON WHICH SYRINGE WILL BE USED TO DISPENSE FLUIDS INTO THE MIXER OF THE CRYSTAL CARD

THE METHOD RECEIVES INSTRUCTIONS ON THE FLOW RATE FROM THE SYRINGE

THE METHOD RECEIVES INSTRUCTIONS ON THE VOLUME OF FLUID TO BE DISPENSED BY THE SYRINGE

THE METHOD DISPENSES OR ASPIRATES FLUID FROM A FLUIDIC CHANNEL UPSTREAM OF THE MIXER

Fig.15J.
IS THE SYRINGE DISPENSING AN AQUEOUS FLUID?

NO

THE METHOD RECEIVES INSTRUCTIONS TO STOP THE AQUEOUS FLUID AT THE MIXER AND BEFORE THE FLUID ENTERS THE CAPILLARY

YES

THE METHOD RECEIVES INSTRUCTIONS TO STOP THE CARRIER FLUID DOWNSTREAM OF THE MIXER AND SLIGHTLY INSIDE THE CAPILLARY

RETURN

Fig. 15K.
C3

THE METHOD RECEIVES INSTRUCTIONS ON WHICH NANODROP FORMATION PROTOCOL WILL BE PERFORMED

5082

5084

WAS THE INSTRUCTION TO PERFORM CONSTANT MODE?

NO

C6

YES

5086

THE METHOD RECEIVES INSTRUCTIONS ON THE FLOW RATE FOR EACH SYRINGE

5088

THE METHOD RECEIVES INSTRUCTIONS ON THE TOTAL VOLUME OF FLUID TO PASS THROUGH THE MIXER

THE METHOD PRODUCES AQUEOUS NANODROPS INSIDE THE CAPILLARY OF EQUAL SIZE HAVING THE SAME CONCENTRATION OF PROTEIN AND PRECIPITANT

5090

RETURN

Fig. 15L.
WAS THE INSTRUCTION TO PERFORM GRADIENT MODE?

**NO**

C7

**YES**

THE METHOD RECEIVES INSTRUCTIONS ON THE MAXIMUM FLOW RATE FOR THE SYRINGES WITH VARIABLE FLOW (BUFFER AND PRECIPITANT)

5094

THE METHOD RECEIVES INSTRUCTIONS ON THE CONSTANT FLOW RATE FOR THE SYRINGE CONTROLLING THE CARRIER FLUID

5096

THE METHOD RECEIVES INSTRUCTIONS ON THE CONSTANT FLOW RATE FOR THE SYRINGE CONTROLLING THE PROTEIN SOLUTION

5098

THE METHOD RECEIVES INSTRUCTIONS ON THE TOTAL AQUEOUS VOLUME TO BE DISPENSED DURING A SINGLE ITERATION

6000

C8

Fig. 15M.
THE METHOD RECEIVES INSTRUCTIONS ON THE NANODROP VOLUME

THE METHOD RECEIVES INSTRUCTIONS ON THE TOTAL NUMBER OF ITERATIONS TO BE PERFORMED

THE METHOD RECIPROCALLY VARIES THE FLOW RATE OF THE BUFFER AND PRECIPITANT SOLUTIONS SUCH THAT THE SUM EQUALS THE MAXIMUM FLOW RATE

THE METHOD PRODUCES A SERIES OF AQUEOUS NANODROPS INSIDE THE CAPILLARY OF EQUAL SIZE AND VARYING CONCENTRATIONS OF PROTEIN AND PRECIPITANT

THE METHOD TERMINATES AFTER THE DESIRED NUMBER OF INTERATIONS

Fig. 15N.
WAS THE INSTRUCTION TO PERFORM HYBRID MODE?

IS A PRECIPITANT CARTRIDGE PREPARED?

Fig.150
WAS THE INSTRUCTION TO PERFORM PULSATILE MODE?

THE METHOL RECEIVES INSTRUCTIONS ON PERFORMING PULSATILE MODE

RETURN

Fig. 15P.
A SYRINGE IS CONNECTED TO TUBING, SUCH AS TEFLOW® TUBING, CONTAINING CARRIER FLUID

THE SYRINGE IS CONNECTED TO A PUMP

THE METHOD RECEIVES INSTRUCTIONS TO ENTER A DEFINED VOLUME (E.G., ~40 nL) AND ASPIRATES AN AIR BUBBLE (~40 nL) INTO THE TUBING

THE METHOD ASPIRATES A DEFINED VOLUME (E.G., ~120 nL) OF A PRECIPITANT SOLUTION INTO THE TUBING

THE METHOD REPEATS THE ABOVE TWO STEPS UNTIL A SUITABLE NUMBER OF PRECIPITANTS, SUCH AS UP TO 24, ARE LOADED INTO THE TUBING

THE METHOD ASPIRATES CARRIER FLUID (~1.0 μL) INTO THE OPEN TIP OF THE TUBING

THE TUBING IS CONNECTED TO THE PRECIPITANT INLET OF THE CRYSTAL CARD

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Fig. 15Q.
THE METHOD RECEIVES INSTRUCTIONS ON THE STARTING FLOW RATE OF THE BUFFER SOLUTION (SYRINGE 1)

THE METHOD RECEIVES INSTRUCTIONS ON THE CHANGE IN THE FLOW RATE (STEP SIZE) OF THE BUFFER SOLUTION

THE METHOD RECEIVES INSTRUCTIONS ON THE STARTING FLOW RATE OF THE PRECIPITANT CARTRIDGE (SYRINGE 2)

THE METHOD CALCULATES THE CHANGE IN THE FLOW RATE (STEP SIZE) OF THE PRECIPITANT SOLUTION

THE METHOD SUMS THE BUFFER AND PRECIPITANT FLOW RATES TO DETERMINE THE TOTAL FLOW RATE

THE METHOD RECEIVES INSTRUCTIONS ON THE STARTING FLOW RATE FOR THE CARRIER FLUID (SYRINGE 3)

THE METHOD RECEIVES INSTRUCTIONS ON THE CHANGE IN THE FLOW RATE (STEP SIZE) OF THE CARRIER FLUID

Fig. 15R.
THE METHOD RECEIVES INSTRUCTIONS ON THE CONSTANT FLOW RATE OF THE PROTEIN SOLUTION (SYRINGE 4)

THE METHOD RECEIVES INSTRUCTIONS ON THE NUMBER OF RAMP UP STEPS (RATE OF FLOW CHANGES) FOR EACH PRECIPITANT

THE METHOD SETS THE NUMBER OF RAMP DOWN STEPS TO EQUAL THE NUMBER OF RAMP UP STEPS FOR EACH ITERATION

THE METHOD RECEIVES INSTRUCTIONS ON THE NUMBER OF ITERATIONS TO BE PERFORMED

THE METHOD RECEIVES INSTRUCTIONS ON THE DURATION OF EACH RAMP STEP

THE METHOD RECIPROCALLY VARIES THE BUFFER AND PRECIPITANT FLOW RATES SUCH THAT THE SUM EQUALS THE STARTING RATES

Fig. 15S.
THE METHOD VARIES THE FLOW RATE OF THE CARRIER FLUID

THE METHOD PRODUCES A SERIES OF NANO DROPS INSIDE THE CAPILLARY HAVING EQUAL AMOUNTS OF PROTEIN AND VARYING AMOUNTS OF PRECIPITANT AND BUFFER

D

Fig. 15T.
WERE CRYSTALS EXTRACTED PRIOR TO DIFFRACTION?

A PEELABLE LAYER IS REMOVED FROM THE CRYSTAL CARD

THE CRYSTAL IS EXTRACTED FROM THE CRYSTAL CARD USING A CRYOLOOP

THE CRYSTAL IS CRYOCOOLED AND DIFFRACTION DATA IS OBTAINED

Fig. 15U.
THE CRYSTAL CARD CONTAINING “DIFFRACTION-READY” CRYSTALS IS MOUNTED ONTO THE GONIOMETER OF AN X-RAY SOURCE

DIFFRACTION DATA IS OBTAINED FROM THE CRYSTAL IN SITU