

protein reagents for coating the cartridges may need to be continuously available in large quantities, as well as considerably time consuming, as fresh cartridges may need to be prepared on a regular basis. For example, particular detection molecules may need to be prepared and then attached to the matrix before it can be used, which may require a number of process steps. And some of the necessary reagents, such as commercial antibodies or antigens, may be very costly to purchase in the quantities needed to prepare sufficient coated matrix. Thus, if the matrix can be re-used, much time and resources might be saved.

[005] The disclosure herein describes, *inter alia*, methods for preparing cartridges comprising a matrix that are re-usable, for example, several times, and also that can be stored in a dry state between uses for easier handling, for the detection of analytes in solutions, including in complex solutions such as cell lysates, as well as the associated re-usable cartridges. The disclosure herein also describes exemplary methods of using cartridges described herein for detection of particular analytes in a solution sample, such as major histocompatibility complex Class I (MHC-I) molecules and other proteins in biological fluids and cell lysates.

SUMMARY

[006] In one aspect, ~~T~~this disclosure includes, *inter alia*, a reusable cartridge for detecting an analyte in a solution, wherein the cartridge comprises: a) a matrix, b) detection molecules attached to the matrix, wherein the detection molecules specifically bind to the analyte, and wherein the detection molecules are optionally cross-linked to the matrix; and c) at least one cryoprotectant, wherein the cartridge can be used at least 10, 20, 50, or 100 times for detection of the analyte in a solution, and wherein, after each use the matrix is optionally stored with cryoprotectant in a dry state. In some aspectexamples, the cartridge is a tube open at both ends, a tube open at one end, a well, a plate with or without wells, or a chip capable of containing the matrix. In some aspectexamples, the matrix comprises particles (e.g. beads, grains, chips, or pellets) comprising silica or agarose, wherein the particles are optionally magnetic. In some cases, the matrix comprises protein A, protein G, and/or protein L. In some cases, the detection molecules are antibodies. In some cases, the detection molecules are cross-linked to the matrix. In some cases, more than one type of detection molecule is attached to the matrix, e.g., two different antibodies for detecting

two different antigen analytes in a solution. In some [aspectexamples](#), the detection molecules are cross-linked to the matrix with a crosslinker selected from selected from dimethylpimelimidate (DMP), a cyanate ester, an NHS ester, azlactone, carbonyl diimidazole (CDI), maleimide, iodoacetyl, pyridyl disulfide, hydrazide, or a carbodiimide. In some [aspectexamples](#), the analyte detectable by the detection molecules on the matrix is a protein or peptide. For example, in some cases, the analyte is a major histocompatibility complex I (MHC-I) molecule. In some cases, the cartridge can be used at least 10 times for detection of a protein analyte in a cell lysate or biological fluid. In some cases, the reusable cartridge is stored in the dry state. In some [aspectexamples](#), the cartridge can be stored in the dry state after preparation, and then used at least 10 times for detection of a protein analyte in a cell lysate or biological fluid after storage in the wet state after each use. In some [aspectexamples](#), the cartridge can be used at least 10 times for detection of a protein analyte in a cell lysate or biological fluid after storage in the dry state after each use. In some [aspectexample](#)s, the cartridge can be stored in the dry state after preparation, and then used at least 20 times for detection of a protein analyte in a cell lysate or biological fluid after storage in the wet state after each use. In some cases, the cartridge can be used at least 20 times for detection of a protein analyte in a cell lysate or biological fluid after storage in the dry state after each use. In some [aspectexamples](#), the cartridge can be stored in the dry state after preparation, and then used at least 50 times for detection of a protein analyte in a cell lysate or biological fluid after storage in the wet state after each use. In some cases, the cartridge can be used at least 50 times for detection of a protein analyte in a cell lysate or biological fluid after storage in the dry state after each use. In some [aspectexamples](#), the cartridge can be stored in the dry state after preparation, and then used at least 100 times for detection of a protein analyte in a cell lysate or biological fluid after storage in the wet state after each use. In some cases, the cartridge can be used at least 100 times for detection of a protein analyte in a cell lysate or biological fluid after storage in the dry state after each use. In some [aspectexamples](#), the matrix is stored at acidic pH. In some [aspectexamples](#), the matrix is stored at 2-8 °C. In some [aspectexamples](#), the matrix is stored in a wet state. In some [aspectexample](#)s, the matrix is stored at acidic pH, and optionally at 2-8 °C. In some cases, the cryoprotectant comprises one or more of

sucrose, trehalose, ethylene glycol, propylene glycol, glycerol, 2-methyl-2,4-pentanediol (MPD), or dimethyl sulfoxide (DMSO).

[007] The present disclosure also relates to methods of preparing a previously used cartridge, for instance, as described above or otherwise herein, for re-use, comprising, after analyte has been eluted from the cartridge, washing the matrix in 1-10% acetic acid, 1% formic acid, or 1% trifluoroacetic acid (TFA), washing the matrix in a buffer comprising cryoprotectant, and allowing the matrix to dry. In one aspect, the disclosure provides a method of preparing a previously used cartridge as disclosed herein for re-use, comprising, after analyte has been eluted from the cartridge, washing the matrix in 1-10% acetic acid, 1% formic acid, or 1% trifluoroacetic acid (TFA), washing the matrix in a buffer comprising cryoprotectant, and allowing the matrix to dry. In some aspectexamples, the cryoprotectant comprises one or more of sucrose, trehalose, ethylene glycol, propylene glycol, glycerol, 2-methyl-2,4-pentanediol (MPD), or dimethyl sulfoxide (DMSO). In some cases, the matrix is allowed to dry by heating the cartridge to at least 30 °C, followed by storage at room temperature for at least 1 hour. In some cases, the matrix is allowed to dry by heating the cartridge to 37 °C, followed by storage at room temperature for at least 1 hour. In some cases, the matrix is allowed to dry, the cartridge is stored in a dry state at 2-8 °C until re-use.

[008] The present disclosure further relates to methods of preparing a reusable cartridge, as described above or otherwise herein, comprising obtaining a cartridge comprising a matrix, contacting the matrix with the detection molecules, and crosslinking the detection molecules to the matrix. In one aspect, the disclosure provides a method of preparing a reusable cartridge as disclosed herein, the method comprising obtaining a cartridge comprising a matrix, contacting the matrix with the detection molecules, and crosslinking the detection molecules to the matrix.

[009] The disclosure further relates to methods of detecting an analyte in a solution, comprising contacting the matrix of a reusable cartridge described above or otherwise herein with a solution comprising the analyte, and optionally eluting the analyte from the cartridge. In one aspect, the disclosure provides a method of detecting an analyte in a solution, comprising contacting the matrix of the reusable cartridge as disclosed herein with a solution comprising the analyte, and optionally eluting the analyte from the cartridge. In some aspectexamples, the analyte is a peptide or protein. In some aspectexamples, the analyte is an MHC-I molecule. In some cases, the solution is a biological fluid or cell lysate. In some cases, the solution is filtered or treated to remove cell debris and membranous materials prior to contact with the matrix. In some cases, the cartridge has previously been used to detect the analyte at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 50, or 100 times prior to its use in the method. In some cases, following elution of analyte, the cartridge is treated by methods of preparing a previously used cartridge herein, such as those described above.

[0010] The present disclosure also encompasses kits comprising at least one reusable cartridge as described herein, such as those described above. In one aspect, the disclosure provides a kit comprising at least one reusable cartridge as disclosed herein. In some aspectexamples, a

kit comprises at least one cartridge as described herein as well as: (a) at least one buffer; (b) at least one control cartridge without detection molecule or with a control detection molecule; (c) reagents for preparing the cartridge for storage and reuse; and/or (d) instructions for use

[0011] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the claims. The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate certain ~~embodiments~~ examples or aspects, and together with the description, serve to explain the principles described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] The present US provisional application contains at least one drawing executed in color. If the present US provisional application is made available to the public in future due to the publication of a nonprovisional or international application claiming priority to it, then copies of this provisional application with color drawings will be provided by the Office upon request and payment of the necessary fee.

[0013] **Figs. 1A-E** show an overview of the use of reusable cartridges as described herein in a method for detecting MHC-I complexes. **Fig. 1A** provides an overview of the stages in MHC-I associated peptide (MAP) endogenous processing and presentation enrichment workflow, and mass spectrometry-based analysis and quantification. In canonical presentation, intracellular proteins are polyubiquitinated within the cell and translocated to the proteasome for degradation. The resulting peptide fragments are transported into the endoplasmic reticulum via TAP proteins. These are then loaded into MHC-I complexes (consisting of an MHC-I heavy chain and B2M), further trimmed by ERAP, and the stable MHC-I peptide complex is translocated to the cell surface. The display level of each unique peptide is determined by its abundance, degradation rate, and affinity for the MHC-I allele(s) in a given cell. Enrichment involves applying lysate containing MHC-I complexes to a solid support containing anti-MHC-I antibodies, washing away unbound contaminants, and then eluting the MHC-I proteins and associated peptides *via* acid treatment. Analysis involves peptide isolation and desalting, chromatographic separation, and analysis by mass spectrometry followed by