Claims:

1. A system for rapid protein evolution, comprising:

a microfluidic device; wherein the microfluidic device comprises:

a housing comprising a base and a microfluidic structure disposed on a surface of the base, wherein the base and the microfluidic structure define a region for holding a first liquid medium and micro-objects suspended therein;

further wherein the microfluidic structure of the microfluidic device comprises a flow path for the first liquid medium and a plurality of chambers; wherein both the flow path and the plurality of chambers are fluidically interconnected with the region; and

a microfluidic system component configured to:

import a plurality of beads into the microfluidic device, each bead of the plurality comprising (i) one nucleic acid sequence of a first plurality of nucleic acid sequences, and (ii) a barcode, wherein each nucleic acid sequence of the first plurality of nucleic acid sequences encodes a sequence variant of a protein of interest;

dispose each of the plurality of beads into each of the plurality of chambers;

incubate the plurality of beads disposed in each of the plurality of chambers under conditions conducive to expression of a corresponding protein encoded by the one nucleic acid sequence of each bead of the plurality of beads, thereby producing a plurality of corresponding proteins; and

assay for a desired property in the plurality of corresponding proteins produced from the nucleic acid sequences of the plurality of beads; and

wherein the microfluidic system further comprises a computational component configured to

correlate results from the desired property assay with individual nucleic acid sequences of the first plurality of nucleic acid sequences; and

based upon the correlation, design a second plurality of nucleic acid sequences, each encoding for a further sequence variant of the protein of interest.

2. The system of claim 1, further comprising a plurality of protein aggregation beads which specifically bind to an epitope of the protein of interest or a protein tag.

3. The system of claim 1, further comprising a nucleic acid synthesis component configured to synthesize the first plurality of nucleic acid sequences.
4. The system of claim 3, wherein the system further comprises a bead preparation component configured to connect each of the first plurality of nucleic acid sequences to one of the plurality of beads.
5. The system of any one of claims 1 to 4, further comprising a barcode detection component configured to correlate the nucleic acid sequence of each bead of the plurality of beads with the location of the corresponding bead of the plurality of beads within the microfluidic device.
6. The system of claim 5, wherein the barcode detection component comprises:
an optical subsystem of the microfluidic system component, configured to detect the barcode visually; or
a nucleic acids sequencing component configured to determine the barcode by sequencing.
7. The system of claim ~~3~~1, wherein the computational component is further configured to communicate the design of the second plurality of nucleic acid sequences to the nucleic acids synthesis component.
8. The system of any one of claims 1 to 7, wherein each chamber of the plurality comprises a sequestration pen; and further wherein each sequestration pen comprises:
an enclosure; and
a single opening to the flow path, and
wherein the enclosure encloses an interior space structured to hold a micro-object suspended in a second liquid medium.
9. The system of claim 8, wherein each sequestration pen of the plurality comprises an isolation region and a connection region that fluidically connects the isolation region to the flow path, and wherein the isolation region is an unswept region in the microfluidic device.
10. The system of any one of claims 1 to 9, wherein the microfluidic device further comprises a dielectrophoretic (DEP) configuration comprising:
a first electrode;
an electrode activation substrate; and
a second electrode,

wherein the first electrode is part of a first wall of the housing and the electrode activation substrate and the second electrode are part of a second wall of the housing, and wherein the electrode activation substrate comprises a photoconductive material, semiconductor integrated circuits, or phototransistors.

11. A process for evolving a protein, the process comprising:

disposing a first library of nucleic acid sequences within a microfluidic device,

wherein the microfluidic device comprises: a housing comprising a base and a microfluidic structure disposed on a surface of the base, wherein the base and the microfluidic structure define a region for holding a first liquid medium and micro-objects suspended therein~~;~~, wherein the microfluidic structure of the microfluidic device comprises a flow path for the first liquid medium and a plurality of chambers~~;~~, wherein both the flow path and the plurality of chambers are fluidically interconnected with the region~~;~~, and further wherein each nucleic acid sequence of the first library comprises one or more variations from a nucleic acid sequence encoding a protein sequence of interest,

wherein each nucleic acid sequence of the first library is linked to a corresponding bead of a first plurality of beads, and disposing the first library of nucleic acid sequences within the microfluidic device comprises introducing individual beads of the first plurality of beads into each of the plurality of chambers;

introducing a phenotypic reporter into the microfluidic device and disposing it in proximity to each nucleic acid sequence of the first library, wherein the phenotypic reporter comprises a solution phase reagent and/or a plurality of micro-objects, and is configured to provide a phenotypic readout from the protein sequence of interest;

introducing a reagent mixture into the microfluidic device and disposing it in proximity to each nucleic acid sequence of the first library, wherein the reagent mixture is configured to express a corresponding protein sequence from each nucleic acid sequence of the first library;

expressing the corresponding protein sequence from each nucleic acid sequence of the first library;

detecting the phenotypic readout from a region proximal to one or more nucleic acid sequences of the first library;

identifying individual nucleic acid sequences from the first library having a corresponding proximal region with a desired phenotypic readout; and

determining the nucleotide sequence of the identified nucleic acid sequences of the first library.

12. The process of claim 11 further comprising:

correlating the individual nucleic acid sequences of the first library with the phenotypic readout detected in the corresponding proximal regions; and

designing a second library of nucleic acid sequences, wherein each nucleic acid sequence of the second library comprises one or more variations from the nucleic acid sequence encoding the protein sequence of interest, the one or more variations of each nucleic acid sequence of the second library selected in light of the correlation.

~~13. The process of claim 11 or 12, wherein each nucleic acid sequence of the first library is linked to a corresponding bead of a first plurality of beads; wherein disposing the first library of nucleic acid sequences within the microfluidic device comprises introducing individual beads of the first plurality of beads into each of the plurality of chambers.~~

14<u>3</u>. The process of claim 13<u>1 or 12</u>, wherein the process further comprises:

designing the nucleic acid sequences of the first library to vary from the nucleic acid sequence encoding the protein sequence of interest;

synthesizing the nucleic acid sequences of the first library; and

connecting each nucleic acid sequence of the first library to a corresponding bead of the first plurality of beads, thereby forming ~~the~~<u>a</u> first library of nucleic acid bearing beads.

15<u>4</u>. The process of claim 13~~ or 14~~, wherein each bead of the first plurality of beads further comprises a corresponding distinct barcode.

16<u>5</u>. The process of claim 15<u>4</u>, wherein the process further comprises reading the barcode of each bead of the first plurality of beads after disposing the first library of nucleic acid sequences in the microfluidic device, thereby identifying a location of each bead and its corresponding nucleic acid sequence within the microfluidic device.

17<u>6</u>. The process of any one of claims 11 to 16<u>5</u>, wherein the process further comprises:

disposing a plurality of protein aggregation beads within the microfluidic device and disposing one or more protein aggregation beads of the plurality in proximity to each nucleic acid sequence of the first library,

wherein the protein aggregation beads of the plurality specifically bind to (i) an epitope of the protein sequence of interest or (ii) an epitope tag encoded by each

nucleic acid sequence of the first library so as to be functionally comprised by the corresponding protein sequence expressed therefrom.

187. The process of claim 176, further comprising capturing the corresponding protein expressed from each nucleic acid sequence of the first library to the one or more protein aggregation beads disposed in proximity thereto.

198. The process of claim 16 or 17, wherein the process further comprises flowing a flushing medium through the microfluidic device after expressing the corresponding protein sequence, thereby displacing the reagent mixture, and wherein introducing the phenotypic reporter and disposing it in proximity to each nucleic acid of the first plurality is performed after flowing the flushing medium through the microfluidic device.

2019. The process of any one of claims 11 to 198, wherein introducing the phenotypic reporter and disposing it in proximity to each nucleic acid of the first plurality is performed after disposing the reagent mixture in proximity to each nucleic acid sequence of the first library.

210. The process of any one of claims 11 to 2019, wherein each chamber of the plurality comprises a sequestration pen; and further wherein the sequestration pen comprises:

an enclosure; and

a single opening to the flow path;

wherein the enclosure encloses an interior space structured to hold a biological micro-object suspended in a second liquid medium.

221. The process of any one of claims 11 to 210, wherein the microfluidic device further comprises a dielectrophoretic (DEP) configuration comprising:

a first electrode;

an electrode activation substrate; and

a second electrode,

wherein the first electrode is part of a first wall of the housing and the electrode activation substrate and the second electrode are part of a second wall of the housing, and wherein the electrode activation substrate comprises a photoconductive material, semiconductor integrated circuits, or phototransistors.

232. The process of any one of claims 11 to 221, wherein the protein sequence of interest comprises an enzyme or comprises a domain which binds to a cell surface marker.

243. The process of any one of claims 11 to 232, wherein the phenotypic reporter comprises:

a solution phase reagent providing a detectable signal when contacted by the protein sequence of interest; or

micro-objects including binding sites for the protein of interest, reporter cells configured to report a function of the protein of interest, or micro-objects providing enzymatic substrates for an enzymatic activity of the protein of interest.

2~~5~~4. The process of any one of claims 12 to 24~~3~~, wherein correlating the individual nucleic acid sequences of the first library with the phenotypic readout detected in the corresponding proximal regions is performed by a first computer component configured to identify a function of the corresponding protein sequence expressed from the identified nucleic acid sequences that is more desirable than the function of the protein sequence of interest, wherein the first computer component employs a machine learning algorithm to perform the correlation.

2~~6~~5. The process of claim 2~~5~~4, wherein designing the second library of nucleic acid sequences is performed by a second computer component, based on the correlation of the expressed protein function with the nucleic acid sequences of the first library of nucleic acid sequences.

2~~7~~6. The process of any one of claims 11 to 2~~6~~5, wherein the reagent mixture comprises a cell free protein expression reaction mixture.

2~~8~~7. The process of any one of claims 12 to 2~~7~~6, wherein each of the second library of nucleic acid sequences is linked to a corresponding bead to form a second library of nucleic acid bearing beads.

2~~9~~8. The process of any one of claims11 to 2~~8~~7, wherein the first library of nucleic acid sequences comprises variations within a sub-region of the nucleic acid sequence encoding the protein sequence of interest, wherein the sub-region of the nucleic acid sequence encoding the protein sequence of interest encodes for a region of the protein producing a function of interest.

3~~0~~29. The process of claim 2~~9~~8, wherein the sub-region encodes for an enzymatic active site or a binding site of the original protein sequence of interest.

~~31. A kit for rapid evolution of a protein of interest, the kit comprising:~~

~~a microfluidic device, wherein the microfluidic device comprises: a housing comprising a base and a microfluidic structure disposed on a surface of the base, wherein the base and the microfluidic structure define an interior chamber for holding a first liquid medium and micro-objects suspended therein; and~~

~~a phenotypic reporter.~~