CLAIMS

What is claimed is:

1. A microfluidic device comprising:

a first fluidic input and a second fluidic input;

a fluidic intersection channel to receive fluid from the first fluidic input and the second fluidic input, wherein the fluidic intersection channel opens into a first mixing chamber on an upper region of a first side of the first mixing chamber, wherein the first mixing chamber has a length, a width, and a depth, wherein the depth is greater than about 1.5 times a depth of the fluidic intersection channel; and

an outlet channel on an upper region of a second side of the first mixing chamber, wherein the outlet channel has a depth that is less than the depth of the first mixing chamber, and wherein an opening of the outlet channel is offset along a width of the second side of the first mixing chamber relative to the fluidic intersection.

2. A microfluidic device comprising:

a first fluidic input channel and a second fluidic input channel, wherein the first and second fluidic input channels converge to a fluidic intersection channel having width and a depth extending from a top surface to a first bottom surface;

a first mixing chamber having a depth extending from the top surface to a second bottom surface, a width extending from a first side to a second side, and a length, wherein the depth of the first mixing chamber is greater than the depth of the fluidic intersection channel and the width of the first mixing chamber is greater than the width of the fluidic intersection channel, and wherein the first mixing chamber is fluidly connected to the fluidic intersection channel at the top surface and proximate the first side; and

an outlet channel, wherein the outlet channel is fluidly connected to the first mixing chamber at the top surface and proximate the second side of the first mixing chamber.

3. A microfluidic device comprising:

a first fluidic input and a second fluidic input; and

a fluidic intersection channel to receive fluid from the first fluidic input and the second fluidic input, wherein the fluidic intersection channel opens into a first mixing chamber on an upper region of a first side of the first mixing chamber, wherein the first mixing chamber has a width, a length and a depth, wherein the depth is greater than about 1.5 times a depth of the fluidic intersection channel;

a connection channel on an upper region of a second side of the first mixing chamber, wherein the connection channel has a depth that is less than the depth of the first mixing chamber, further wherein an opening of the connection channel is offset along the width of the second side of the first mixing chamber relative to the fluidic intersection, wherein the connection channel opens into a second mixing chamber; and

an outlet channel extending from the second mixing chamber.

4. The microfluidic device of any of claims 1-2, wherein the first mixing chamber is configured as a single mixer in which the outlet channel forms a mixer output without connecting in series to an additional mixing chamber.

5. The microfluidic device of any of claims 1-4, wherein the depth of the first mixing chamber is between about 2 times and about 4 times the fluidic intersection channel depth.

6. The microfluidic device of any of claims 1-5, wherein the depth of the first mixing chamber is about 3 times the fluidic intersection channel depth.

7. The microfluidic device of any of claims 1-6 wherein the width of the first mixing chamber is between about 1.5 times and about 3 times the length of the first mixing chamber.

8. The microfluidic device of any of claims 1-7, wherein the width of the first mixing chamber is about 2 times the length of the first mixing chamber.

9. The microfluidic device of any of claims 1-8, wherein the length of the first mixing chamber is between about 2 times and about 4 times a length of the fluidic intersection channel.

10. The microfluidic device of any of claims 1-9, wherein the length of the first mixing chamber is about 3 times the length of the fluidic intersection channel.

11. The microfluidic device of any of claims 1-10, wherein the fluidic intersection channel, first mixing chamber and outlet channel are all within a first layer, and wherein the top surface of the fluidic intersection channel, first mixing chamber and outlet channel is part of a second layer.

12. The microfluidic device of any of claims 1-11, wherein the outlet channel fluidly communicates with the first mixing chamber at a first length of the mixing chamber and the fluidic intersection channel fluidly communicates with the mixing chamber at a second length of the mixing chamber.

13. The microfluidic device of any of claims 1-12, wherein the first mixing chamber has rounded corners.

14. The microfluidic device of any of claims 1-13, wherein the first mixing chamber has a corner radius of between about 65 and about 85 µm.

15. The microfluidic device of any of claims 1-14, wherein a change in fluid pressure through the first mixing chamber at a flow rate of between 0.25 and 5 ml/min is between about 6.9 kPa and about 206.8 kPa.

16. The microfluidic device of any of claims 1-15, wherein the width of the first mixing chamber is between about 150 and about 600 µm, the depth of the first mixing chamber is between about 150 and about 500 µm, and the length of the first mixing chamber is between about 500 µm and about 1000 µm.

17. The microfluidic device of claim 3, wherein the connecting channel has a width that is between about 75 µm and about 225 µm, a depth that is between about 75 µm and about 225 µm, and a length that is between about 225 and about 550 µm.

18. The microfluidic device of any of claims 1-2, further comprising:

a second mixing chamber having a depth extending from the top surface to a bottom surface of the second mixing chamber, a width extending from a first side to a second side of the second mixing chamber, and a length, wherein the depth of the second mixing chamber is greater than the depth of the outlet channel and the width of the second mixing chamber is greater than a width of the outlet channel, further wherein the second mixing chamber is fluidly connected to the outlet channel at the top surface and proximate the second side; and

a second outlet channel having a depth and a width, wherein the second outlet channel is fluidly connected to the second mixing chamber at the top surface and proximate the first side of the second mixing chamber.

19. The microfluidic device of any of claims 1-18, further comprising one or more fluid pumps to pump fluid from the fluidic intersection channel into the first mixing chamber by deflecting at least a portion of an elastic membrane within the microfluidic device.

20. The microfluidic device of any of claims 1-18, further comprising one or more fluid pumps, wherein the one or more fluid pumps are to pump fluid from the fluidic intersection channel into the first mixing chamber by deflecting at least a portion of an elastic membrane within the microfluidic device.

21. The microfluidic device of any of claims 1-2, wherein the microfluidic device comprises a plurality of fluidly connected mixing chambers including the first mixing chamber.

22. The microfluidic device of any of claims 1-21 further comprising a plurality of pressure ports to deflect an elastic layer in the microfluidic device to drive fluid between through the first mixing chamber.

23. The microfluidic device of any of claims 1-22, further comprising a flow restrictor in fluid communication with the first fluidic input, wherein the flow restrictor comprises a serpentine elongate fluidic channel.

24. A microfluidic mixing apparatus comprising:

a mixing chamber comprising a base defining a bottom surface, side walls, and an upper surface enclosing the mixing chamber;

a mixing inlet channel comprising an opening into the mixing chamber at a first side wall of the mixing chamber; and

a mixing outlet channel comprising an opening into the mixing chamber at a second side wall of the mixing chamber;

wherein a vertical dimension of the mixing chamber is larger than a vertical dimension of the mixing inlet channel and is larger than a vertical dimension of the mixing outlet channel.

25. The microfluidic apparatus of claim 24, wherein the first side wall and the second side wall are opposing side walls of the mixing chamber.

26. The microfluidic apparatus of any of claims 24-25, wherein the mixing inlet channel and the mixing outlet channel connect to the mixing chamber at offset locations along the first side wall and the second side wall.

27. The microfluidic apparatus of any of claims 24-26, wherein a height of the opening of the mixing inlet channel and a height of the opening of the mixing outlet channel are the same.

28. The microfluidic apparatus of any of claims 24-27, wherein a width of the opening of the mixing inlet channel and a width of the opening of the mixing outlet channel are the same.

29. The microfluidic apparatus of any of claims 24-28, wherein the opening of the mixing inlet channel is disposed at a height of the first side wall adjacent to the upper surface of the mixing chamber, and the opening of the mixing outlet channel is disposed at a height of the second side wall adjacent to the upper surface of the mixing chamber.

30. The microfluidic apparatus of any of claims 24-29, wherein the mixing inlet channel has a first terminus comprising a fluidic intersection and a second terminus comprising the opening into the mixing chamber.

31. The microfluidic apparatus of claim 30, wherein the fluidic intersection further comprises a first fluidic input channel and a second fluidic input channel that intersect the mixing inlet channel at the fluidic intersection.

32. The microfluidic apparatus of any of claims 24-31, wherein the first fluidic channel and the second fluidic channel intersect at the fluidic intersection at an angle smaller than about 180 degrees with respect to each other.

33. The microfluidic apparatus of any of claims 24-32, wherein the first fluidic channel and the second fluidic channel intersect at the fluidic intersection at an angle greater than about 30 degrees with respect to each other.

34. The microfluidic apparatus of any of claims 24-33, wherein the mixing chamber is a first mixing chamber, the mixing inlet channel is a first mixing inlet channel, and the mixing outlet channel is a first mixing outlet channel, and wherein the microfluidic apparatus further comprises a second microfluidic mixing apparatus comprising:

a second mixing chamber comprising a base defining a bottom surface, side walls, and an upper surface enclosing the second mixing chamber;

a second mixing inlet channel comprising an opening into the second mixing chamber at a first side wall of the second mixing chamber; and

a second mixing outlet channel comprising an opening into the second mixing chamber at a second side wall of the second mixing chamber,

wherein a vertical dimension of the second mixing chamber is larger than a vertical dimension of the second mixing inlet channel and is larger than a vertical dimension of the second mixing outlet channel.

35. The microfluidic apparatus of any of claims 24-34, further wherein:

the first side wall and the second side wall of the second mixing chamber are opposing side walls of the second mixing chamber;

the second mixing inlet channel and the second mixing outlet channel connect to the second mixing chamber at offset locations along the first side wall and the second side wall of the second mixing chamber;

a height of the opening of the second mixing inlet channel and a height of the opening of the second mixing outlet channel are the same;

a width of the opening of the second mixing inlet channel and a width of the opening of the second mixing outlet channel are the same;

the opening of the second mixing inlet channel and the opening of the second mixing outlet channel are disposed at a height of the respective first side wall and second side wall of the second mixing chamber adjacent to the upper surface of the second mixing chamber; or

any combination thereof.

36. The microfluidic apparatus of any one of claims 24-35, wherein the second mixing outlet channel comprises a first terminus at the opening into the second mixing chamber.

37. A microfluidic apparatus comprising cascading microfluidic mixers, wherein each of the cascading microfluidic mixers comprises:

a mixing chamber comprising a base defining a bottom surface, side walls, and an upper surface;

a mixing inlet channel comprising an opening into the mixing chamber at a first side wall of the mixing chamber,

a mixing outlet channel comprising an opening into the mixing chamber at a second side wall of the mixing chamber,

wherein a vertical dimension of the mixing chamber is larger than a vertical dimension of the mixing inlet channel and is larger than a vertical dimension of the mixing outlet channel; and

wherein cascading microfluidic mixers are connected to one another in a series so that the mixing inlet channel of each of the cascading microfluidic mixers after a first microfluidic mixer in the series is connected to the mixing outlet of a prior microfluidic mixer in the series.

38. A microfluidic apparatus comprising:

a first plate and a second plate;

an elastic layer disposed between the first plate and the second plate; and

a microfluidic path between the first plate and the second plate, wherein the microfluidic path comprises:

a plurality of blending chambers each comprising a fixed volume separated by a portion of the elastic layer, wherein a portion of the elastic layer is to deflect to drive fluid between blending chambers of the plurality of blending chambers;

a first microfluidic mixer, wherein the first microfluidic mixer comprises:

a first fluidic input and a second fluidic input;

a fluidic intersection to receive fluid from the first fluidic input and the second fluidic input, wherein the fluidic intersection opens into a first mixing chamber on an upper region of a first side of the first mixing chamber, wherein the first mixing chamber has a depth that is greater than 1.5 times a depth of the fluidic intersection;

a connection channel on an upper region of a second side of the first mixing chamber, wherein the connection channel has a depth that is less than the depth of the first mixing chamber, further wherein an opening of the connection channel is offset along a width of the second side of the first mixing chamber relative to the fluidic intersection, wherein the connection channel opens into a second mixing chamber on an upper region of a first side of the second mixing chamber, further

wherein the second mixing chamber has a depth that is greater than 1.5 times a depth of the connection channel; and

an output channel from the second mixing chamber on an upper region of a second side of the second mixing chamber wherein the second side of the second mixing chamber is opposite from the first side of the second mixing chamber.

39. The microfluidic apparatus of claim 38, wherein an upper surface of the fluidic intersection is substantially level with an upper surface of the first mixing chamber.

40. The microfluidic apparatus of any of claims 38-39, wherein an upper surface of the connection channel is configured to be level with an upper surface of the first mixing chamber and an upper surface of the second mixing chamber.

41. The microfluidic apparatus of any of claims 38-40, further comprising one or more fluid pumps to pump fluid from the blending chamber into the first microfluidic mixer by deflecting at least a portion of the elastic layer.

42. The microfluidic apparatus of any of claims 38-41, further comprising one or more fluid pumps between the plurality of blending chambers and the first microfluidic mixer, wherein the fluid pumps are to pump fluid from the blending chamber into the first microfluidic mixer by deflecting at least a portion of the elastic layer.

43. The microfluidic apparatus of any of claims 38-42, wherein the microfluidic apparatus comprises a plurality of microfluidic mixers including the first microfluidic mixer.

44. The microfluidic apparatus of any of claims 38-43, further comprising a plurality of pressure ports into the first plate configured to deflect the elastic layer to drive fluid between the blending chambers and through the first microfluidic mixer.

45. The microfluidic apparatus of any of claims 38-44, further comprising a flow restrictor in fluid communication with the first fluidic input, wherein the flow restrictor comprises a serpentine elongate fluidic channel.

46. The microfluidic apparatus of claim 45, further comprising a pair of blending chambers having a fixed volume, each blending chamber disposed between the first plate and the second plate, and wherein a portion of the elastic layer divides each chamber into a fluid-contacting side in the second plate and a pressure-receiving side in the first plate.

47. A method of formulating a therapeutic mRNA with a delivery vehicle, the method comprising mixing the therapeutic mRNA and the delivery vehicle in a microfluidic mixing chamber of a microfluidic device at a temperature that is between about 2 and about 20 degrees C, wherein the temperature is selected using at least a composition of the therapeutic mRNA, a composition of the delivery vehicle, or a combination thereof.

48. The method of claim 47, further comprising selecting the temperature using at least: a polynucleotide sequence of the therapeutic mRNA, a sequence of the delivery vehicle, a molecular weight of the delivery vehicle, a molecular weight of the therapeutic mRNA, a charge of the delivery vehicle, a charge of the therapeutic mRNA, a molecular weight of the delivery vehicle, a molecular weight of the therapeutic mRNA, a flow rate of the therapeutic mRNA and/or the delivery vehicle within the microfluidic mixing chamber, and a dimension of the microfluidic mixing chamber, or any combination thereof.

49. The method of claim 47, wherein mixing comprises mixing in a microfluidic device comprising the microfluidic mixing chamber.

50. The method of claim 47, further comprising separately maintaining the temperature of the mixing chamber to be between about 2 and about 20 degrees C, relative to the rest of the microfluidic device.

51. The method of claim 47, wherein mixing in the microfluidic mixing chamber comprises passing the therapeutic mRNA and the delivery vehicle through a first opening into the mixing chamber of a microfluidic device so that the therapeutic mRNA and the delivery vehicle are driven against a wall of the mixing chamber and driven out of a plane of the first opening to a depth of greater than one times the depth of the first opening.

52. The method of claim 51, wherein passing comprises driving the therapeutic mRNA and the delivery vehicle against the wall of the mixing chamber and out of a plane transverse to the first opening to the depth of greater than about 2.5 times the depth of the first opening.

53. The method of claim 51, wherein the therapeutic mRNA and the delivery vehicle are driven against the wall of the mixing chamber and out of a plane transverse to the first opening to a depth of 3 or more times the depth of the first opening.

54. The method of claim 51, wherein a top of the first opening is in line with a top of the mixing chamber.

55. A method of mixing, the method comprising:

passing a first fluid and a second fluid through a first opening into a mixing chamber in a microfluidic device, so that the first and second fluids are driven against a wall of the mixing chamber and driven out of a plane of the first opening to a depth of greater than one times the depth of the first opening to form a mixed fluid; and

passing the mixed fluid out of an outlet opening out of the mixing chamber,

wherein the mixing chamber is maintained at a temperature of between about 2 and about 20 degrees C.

56. A method of mixing, the method comprising:

passing a first fluid and a second fluid through at least one opening into a mixing chamber within a microfluidic device, so that the first and second fluids are driven against a wall of the mixing chamber and driven out of a plane of at least first opening as a mixed fluid; and

passing the mixed fluid out of an outlet opening out of the mixing chamber,

wherein the mixing chamber is maintained at a temperature of between 2 and 20 degrees C.

57. A method of mixing, the method comprising:

passing a first fluid containing oligonucleotide molecules and a second fluid containing delivery vehicle chemistry through at least one opening into a mixing chamber in a microfluidic device, so that the first and second fluids are driven against a wall of the mixing chamber and driven out of a plane of an opening; and

passing the mixed fluid out of an outlet opening out of the mixing chamber,

wherein the mixing chamber is maintained at a temperature of between about 2 and about 20 degrees C.

58. A method of mixing within a microfluidic device, the method comprising:

passing a first fluid and a second fluid through a first opening into a mixing chamber within a microfluidic device, so that the first and second fluids are driven against a wall of the mixing chamber and out of a plane transverse to the first opening to a depth of greater than about 2.5 times the depth of the first opening to form a substantially mixed fluid; and

passing the mixed fluid out of an outlet opening out of the mixing chamber, wherein the outlet opening is opposite from the first opening but is offset from the first opening,

wherein the mixing chamber is maintained at a temperature of between about 5 and about 20 degrees C.

59. The method of claim 58, wherein the passing the first fluid and the second fluid through the first opening into the mixing chamber comprises passing the first and second fluids so that the first and second fluids are driven against the wall of the mixing chamber and out of the plane transverse to the first opening to the depth of greater than about 2.5 times the depth of the first opening.

60. The method of claims 58-59, further comprising maintaining the temperature of the mixing chamber at between about 5 and about 15 degrees C.

61. The method of claims 58-60, further comprising maintaining the temperature of the mixed fluid at between about 5 and about 15 degrees C.

62. The method of claim 58-61, further comprising maintaining a temperature of the mixed fluid at about 10 degrees C.

63. The method of claims 58-62, further comprising passing the mixed fluid from the outlet opening into a second opening into a second mixing chamber, so that the fluid is driven against a wall of the second mixing chamber and driven out of a plane of the second opening to a depth of greater than one times the depth of the second opening to further mix the mixed fluid.

64. The method of claims 58-63, wherein the fluid is driven against the wall of the mixing chamber and out of the plane transverse to the first opening to a depth of about 3 or more times the depth of the first opening.

65. The method of claims 58-64, wherein a top of the first opening is in line with a top of the first mixing chamber.

66. The method of claims 58-65, wherein the outlet opening has a cross-section area that is equal to a cross-sectional area of the first opening.

67. The method of claims 58-66, wherein the mixing chamber is between a first layer and a second layer of the microfluidic device.

68. The method of claims 58-67, wherein the mixing chamber has a length that is greater than a width, and wherein the length is greater than about 2 times a width of the first opening.

69. A method of forming a composition, the method comprising:

synthesizing one or more therapeutic mRNAs in a microfluidic device, wherein the one or more therapeutic mRNAs are within a first fluid and a delivery vehicle for the one or more therapeutic mRNAs is within a second fluid;

passing the first fluid and the second fluid through a first opening into a mixing chamber in the microfluidic device, so that the first and second fluids are driven against a wall of the mixing chamber and driven out of a plane of the first opening to a depth of greater than one times the depth of the first opening to form a mixed fluid, wherein the mixing chamber is maintained at a temperature that is selected to enhance mixing of the therapeutic mRNA and delivery vehicle; and

passing the mixed fluid out of an outlet opening out of the mixing chamber.

70. The method of claim 69, wherein the mixing chamber is maintained at the temperature that is selected to enhance mixing of for the therapeutic mRNA and delivery vehicle and is between 2 and 20 degrees C.

71. The method of claim 69, further comprising selecting an enhanced mixing temperature of the mixing chamber.

72. The method of claim 71, wherein selecting the enhanced mixing temperature comprises modeling mixing within the mixing chamber *in vitro* or *in vivo*.

73. The method of claim 71, wherein selecting the enhanced mixing temperature comprises selecting a temperature between about 2 and about 20 degrees C based on the delivery vehicle and the one or more therapeutic mRNAs.

74. The method of claim 69, wherein the passing the first fluid and the second fluid through the first opening into the mixing chamber comprises passing the first and second fluids so that the first and second fluids are driven against the wall of the mixing chamber and out of a plane transverse to the first opening to the depth of greater than about 2.5 times the depth of the first opening.

75. The method of claim 69, wherein the fluid is driven against the wall of the mixing chamber and out of a plane transverse to the first opening to a depth of about 3 or more times the depth of the first opening.

76. The method of claim 69, wherein a top of the first opening is in line with a top of the mixing chamber.

77. The method of claim 69, wherein the outlet opening has a cross-section area that is equal to a cross-sectional area of the first opening.

78. The method of claim 69, wherein the mixing chamber is between a first layer and a second layer of the microfluidic device.

79. The method of claim 69, wherein the mixing chamber has a length that is greater than a width, further wherein the length is greater than 2 times the width of the first opening.

80. A method of forming a therapeutic composition, the method comprising:

passing one or more therapeutic mRNAs within a first fluid and a delivery vehicle for the one or more therapeutic mRNAs within a second fluid through a first opening into a mixing chamber in a microfluidic device, so that the first and second fluids are driven against a wall of the mixing chamber and driven out of a plane of the first opening to a depth of greater than one times the depth of the first opening to form a mixed fluid comprising the therapeutic composition;

maintaining a temperature of the mixing chamber at an enhanced mixing temperature determined to enhance mixing; and

passing the mixed fluid out of an outlet opening out of the mixing chamber.

81. The method of claim 80, wherein the enhanced mixing temperature is between about 2 and about 20 degrees C.

82. The method of claim 80, wherein maintaining comprises determining the enhanced mixing temperature for the one or more therapeutic mRNAs and/or the delivery vehicle.

83. The method of claim 82, further comprising determining the enhanced mixing temperature by modeling mixing in the mixing chamber *in vitro* or *in vivo*.

84. The method of claim 82, further comprising determining the enhanced mixing temperature by selecting a temperature between about 2 and about 20 degrees C having

greater mixing as compared to mixing at other temperatures between about 2 and about 20 degrees C.

85. The method of claim 80, wherein the passing the first fluid and the second fluid through the first opening into the mixing chamber comprises passing the first and second fluids so that the first and second fluids are driven against the wall of the mixing chamber and out of a plane transverse to the first opening to the depth of greater than about 2.5 times the depth of the first opening.

86. The method of claim 80, wherein the fluid is driven against the wall of the mixing chamber and out of a plane transverse to the first opening to a depth of about 3 or more times the depth of the first opening.

87. The method of claim 80, wherein a top of the first opening is in line with a top of the mixing chamber.

88. The method of claim 80, wherein the outlet opening has a cross-section area that is equivalent to a cross-sectional area of the first opening.

89. The method of claim 80, wherein the mixing chamber is formed between a first layer and a second layer of the microfluidic device.

90. The method of claim 80, wherein the mixing chamber has a length that is greater than a width, further wherein the length is greater than 2 times a width of the first opening.

91. A method of mixing, the method comprising:

passing a first fluid and a second fluid through a first opening into a mixing chamber in a microfluidic device, so that the first and second fluids are driven against a wall of the mixing chamber and driven out of a plane of the first opening to a depth of greater than one times the depth of the first opening to form a mixed fluid; and

passing the mixed fluid out of an outlet opening out of the mixing chamber.

92. The method of claim 91, wherein the passing the first fluid and the second fluid through the first opening into the mixing chamber comprises passing the first and second fluids so that the first and second fluids are driven against the wall of the mixing chamber and out of the plane transverse to the first opening to the depth of greater than about 2.5 times the depth of the first opening.

93. The method of claims 91-92, wherein the mixed fluid is substantially mixed by the mixing chamber and the mixing chamber is configured as a single mixer that does not connect to a second mixing chamber.

94. The method of claims 91-92, further comprising passing the mixed fluid from the outlet opening into a second opening into a second mixing chamber, so that the fluid is driven against a wall of the second mixing chamber and driven out of a plane of the second opening to a depth of greater than one times the depth of the second opening to further mix the mixed fluid.

95. The method of claims 91-92, wherein the fluid is driven against the wall of the mixing chamber and out of the plane transverse to the first opening to a depth of about 3 or more times the depth of the first opening.

96. The method of claims 91-95, wherein the outlet opening has a cross-section area that is equal to a cross-sectional area of the first opening.

97. The method of claims 91-96, wherein the mixing chamber has a length that is greater than a width, and wherein the length is greater than about 2 times the width of the first opening.

98. The method of claims 91-97, wherein the mixing chamber has rounded corners.

99. The method of claims 91-98, wherein a change in fluid pressure through the mixing chamber at a flow rate of between 0.25 and 5 ml/min is between about 6.9 kPa and about 206.8 kPa.

100. The method of claims 91-99, wherein a width of the mixing chamber is between about 150 and about 600 µm, the depth of the mixing chamber is between about 150 and about 500 µm, and the length of the mixing chamber is between about 500 µm and about 1000 µm.

101. A method of mixing within a microfluidic device, the method comprising:

passing a first fluid and a second fluid through a first opening into a mixing chamber within a microfluidic device, so that the first and second fluids are driven against a wall of the mixing chamber and out of a plane transverse to the first opening to a depth of greater than about 2.5 times the depth of the first opening to form a substantially mixed fluid; and

passing the mixed fluid out of an outlet opening out of the mixing chamber, wherein the outlet opening is opposite from the first opening but is offset from the first opening,

wherein the mixed fluid is substantially mixed by the mixing chamber and the mixing chamber is configured as a single mixer that does not connect to a second mixing chamber.

CLAIMS

1. A microfluidic device comprising:

a first fluidic input and a second fluidic input;

a fluidic intersection channel to receive fluid from the first fluidic input and the second fluidic input, wherein the fluidic intersection channel opens into a first mixing chamber on an upper region of a first side of the first mixing chamber, wherein the first mixing chamber has a length, a width, and a depth, wherein the depth is greater than about 1.5 times a depth of the fluidic intersection channel; and

an outlet channel on an upper region of a second side of the first mixing chamber, wherein the outlet channel has a depth that is less than the depth of the first mixing chamber, and wherein an opening of the outlet channel is offset along a width of the second side of the first mixing chamber relative to the fluidic intersection.

2. A microfluidic device comprising:

a first fluidic input channel and a second fluidic input channel, wherein the first and second fluidic input channels converge to a fluidic intersection channel having width and a depth extending from a top surface to a first bottom surface;

a first mixing chamber having a depth extending from the top surface to a second bottom surface, a width extending from a first side to a second side, and a length, wherein the depth of the first mixing chamber is greater than the depth of the fluidic intersection channel and the width of the first mixing chamber is greater than the width of the fluidic intersection channel, and wherein the first mixing chamber is fluidly connected to the fluidic intersection channel at the top surface and proximate the first side; and

an outlet channel, wherein the outlet channel is fluidly connected to the first mixing chamber at the top surface and proximate the second side of the first mixing chamber.

3. A microfluidic device comprising:

a first fluidic input and a second fluidic input; and

a fluidic intersection channel to receive fluid from the first fluidic input and the second fluidic input, wherein the fluidic intersection channel opens into a first mixing chamber on an upper region of a first side of the first mixing chamber, wherein the first mixing chamber has a width, a length and a depth, wherein the depth is greater than about 1.5 times a depth of the fluidic intersection channel;

a connection channel on an upper region of a second side of the first mixing chamber, wherein the connection channel has a depth that is less than the depth of the first mixing chamber, further wherein an opening of the connection channel is offset along the width of the second side of the first mixing chamber relative to the fluidic intersection, wherein the connection channel opens into a second mixing chamber; and

an outlet channel extending from the second mixing chamber.

4. The microfluidic device of any of claims 1-2, wherein the first mixing chamber is configured as a single mixer in which the outlet channel forms a mixer output without connecting in series to an additional mixing chamber.

5. The microfluidic device of claim 3, wherein the connecting channel has a width that is between about 75 μm and about 225 μm, a depth that is between about 75 μm and about 225 μm, and a length that is between about 225 and about 550 μm.

6. The microfluidic device of any of claims 1-2, further comprising:

a second mixing chamber having a depth extending from the top surface to a bottom surface of the second mixing chamber, a width extending from a first side to a second side of the second mixing chamber, and a length, wherein the depth of the second mixing chamber is greater than the depth of the outlet channel and the width of the second mixing chamber is greater than a width of the outlet channel, further wherein the second mixing chamber is fluidly connected to the outlet channel at the top surface and proximate the second side; and

a second outlet channel having a depth and a width, wherein the second outlet channel is fluidly connected to the second mixing chamber at the top surface and proximate the first side of the second mixing chamber.

7. The microfluidic device of any of claims 1-2, wherein the microfluidic device comprises a plurality of fluidly connected mixing chambers including the first mixing chamber.

8. A microfluidic mixing apparatus comprising:

a mixing chamber comprising a base defining a bottom surface, side walls, and an upper surface enclosing the mixing chamber;

a mixing inlet channel comprising an opening into the mixing chamber at a first side wall of the mixing chamber; and a mixing outlet channel comprising an opening into the mixing chamber at a second side wall of the mixing chamber;

wherein a vertical dimension of the mixing chamber is larger than a vertical dimension of the mixing inlet channel and is larger than a vertical dimension of the mixing outlet channel.

9 The microfluidic apparatus of claim 8, wherein the first side wall and the second side wall are opposing side walls of the mixing chamber.

10. The microfluidic apparatus of any of claims 8-9, wherein the mixing inlet channel and the mixing outlet channel connect to the mixing chamber at offset locations along the first side wall and the second side wall.

11. A microfluidic apparatus comprising cascading microfluidic mixers, wherein each of the cascading microfluidic mixers comprises:

a mixing chamber comprising a base defining a bottom surface, side walls, and an upper surface;

a mixing inlet channel comprising an opening into the mixing chamber at a first side wall of the mixing chamber,

a mixing outlet channel comprising an opening into the mixing chamber at a second side wall of the mixing chamber,

wherein a vertical dimension of the mixing chamber is larger than a vertical dimension of the mixing inlet channel and is larger than a vertical dimension of the mixing outlet channel; and

wherein cascading microfluidic mixers are connected to one another in a series so that the mixing inlet channel of each of the cascading microfluidic mixers after a first microfluidic mixer in the series is connected to the mixing outlet of a prior microfluidic mixer in the series.

12. A microfluidic apparatus comprising:

a first plate and a second plate;

an elastic layer disposed between the first plate and the second plate; and

a microfluidic path between the first plate and the second plate, wherein the microfluidic path comprises:

a plurality of blending chambers each comprising a fixed volume separated by a portion of the elastic layer, wherein a portion of the elastic layer is to deflect to drive fluid between blending chambers of the plurality of blending chambers;

a first microfluidic mixer, wherein the first microfluidic mixer comprises:

a first fluidic input and a second fluidic input;

a fluidic intersection to receive fluid from the first fluidic input and the second fluidic input, wherein the fluidic intersection opens into a first mixing chamber on an upper region of a first side of the first mixing chamber, wherein the first mixing chamber has a depth that is greater than 1.5 times a depth of the fluidic intersection;

a connection channel on an upper region of a second side of the first mixing chamber, wherein the connection channel has a depth that is less than the depth of the first mixing chamber, further wherein an opening of the connection channel is offset along a width of the second side of the first mixing chamber relative to the fluidic intersection, wherein the connection channel opens into a second mixing chamber on an upper region of a first side of the second mixing chamber, further wherein the second mixing chamber has a depth that is greater than 1.5 times

a depth of the connection channel; and an output channel from the second mixing chamber on an upper region of a second side of the second mixing chamber wherein the second side of the second mixing chamber is opposite from the first side of the second mixing chamber.

13. The microfluidic apparatus of claim 12, wherein an upper surface of the fluidic intersection is substantially level with an upper surface of the first mixing chamber.

14. The microfluidic apparatus of any of claims 12 - 13, wherein an upper surface of the connection channel is configured to be level with an upper surface of the first mixing chamber and an upper surface of the second mixing chamber.

15. A method of formulating a therapeutic mRNA with a delivery vehicle, the method comprising mixing the therapeutic mRNA and the delivery vehicle in a microfluidic mixing chamber of a microfluidic device at a temperature that is between about 2 and about 20 degrees C, wherein the temperature is selected using at least a composition of the therapeutic mRNA, a composition of the delivery vehicle, or a combination thereof wherein mixing in the microfluidic mixing chamber comprises passing the therapeutic mRNA and the delivery vehicle through a first opening into the mixing chamber of a microfluidic device so that the therapeutic mRNA and the delivery vehicle are driven against a wall of the mixing chamber and driven out of a plane of the first opening to a depth of greater than one times the depth of the first opening.

16. The method of claim 15, wherein passing comprises driving the therapeutic mRNA and the delivery vehicle against the wall of the mixing chamber and out of a plane transverse to the first opening to the depth of greater than about 2.5 times the depth of the first opening.

17. The method of claim 15, wherein the therapeutic mRNA and the delivery vehicle are driven against the wall of the mixing chamber and out of a plane transverse to the first opening to a depth of 3 or more times the depth of the first opening.

18. The method of claim 15, wherein a top of the first opening is in line with a top of the mixing chamber.

19. A method of mixing, the method comprising:

passing a first fluid and a second fluid through a first opening into a mixing chamber in a microfluidic device, so that the first and second fluids are driven against a wall of the mixing chamber and driven out of a plane of the first opening to a depth of greater than one times the depth of the first opening to form a mixed fluid; and

passing the mixed fluid out of an outlet opening out of the mixing chamber, wherein the mixing chamber is maintained at a temperature of between about 2 and about 20 degrees C.

20. A method of mixing, the method comprising:

passing a first fluid and a second fluid through at least one opening into a mixing chamber within a microfluidic device, so that the first and second fluids are driven against a wall of the mixing chamber and driven out of a plane of at least first opening as a mixed fluid; and

passing the mixed fluid out of an outlet opening out of the mixing chamber, wherein the mixing chamber is maintained at a temperature of between 2 and 20 degrees C.

21. A method of mixing, the method comprising:

passing a first fluid containing oligonucleotide molecules and a second fluid containing delivery vehicle chemistry through at least one opening into a mixing chamber in a microfluidic device, so that the first and second fluids are driven against a wall of the mixing chamber and driven out of a plane of an opening; and

passing the mixed fluid out of an outlet opening out of the mixing chamber, wherein the mixing chamber is maintained at a temperature of between about 2 and about 20 degrees C.

22. A method of mixing within a microfluidic device, the method comprising:

passing a first fluid and a second fluid through a first opening into a mixing chamber within a microfluidic device, so that the first and

second fluids are driven against a wall of the mixing chamber and out of a plane transverse to the first opening to a depth of greater than about 2.5 times the depth of the first opening to form a substantially mixed fluid; and

passing the mixed fluid out of an outlet opening out of the mixing chamber, wherein the outlet opening is opposite from the first opening but is offset from the first opening, wherein the mixing chamber is maintained at a temperature of between about 5 and about 20 degrees C.

23. The method of claim 22, wherein the passing the first fluid and the second fluid through the first opening into the mixing chamber comprises passing the first and second fluids so that the first and second fluids are driven against the wall of the mixing chamber and out of the plane transverse to the first opening to the depth of greater than about 2.5 times the depth of the first opening.

24. A method of forming a composition, the method comprising:

synthesizing one or more therapeutic mRNAs in a microfluidic device, wherein the one or more therapeutic mRNAs are within a first fluid and a delivery vehicle for the one or more therapeutic mRNAs is within a second fluid;

passing the first fluid and the second fluid through a first opening into a mixing chamber in the microfluidic device, so that the first and second fluids are driven against a wall of the mixing chamber and driven out of a plane of the first opening to a depth of greater than one times the depth of the first opening to form a mixed fluid, wherein the mixing chamber is maintained at a temperature that is selected to enhance mixing of the therapeutic mRNA and delivery vehicle; and

passing the mixed fluid out of an outlet opening out of the mixing chamber.

25. The method of claim 24, wherein the mixing chamber is maintained at the temperature that is selected to enhance mixing of for the therapeutic mRNA and delivery vehicle and is between 2 and 20 degrees C.

26. The method of claim 24, further comprising selecting an enhanced mixing temperature of the mixing chamber.

27. The method of claim 26, wherein selecting the enhanced mixing temperature comprises modeling mixing within the mixing chamber *in vitro* or *in vivo.*

28. The method of claim 26, wherein selecting the enhanced mixing temperature comprises selecting a temperature between about 2 and about 20 degrees C based on the delivery vehicle and the one or more therapeutic mRNAs.

29. The method of claim 24, wherein the passing the first fluid and the second fluid through the first opening into the mixing chamber comprises passing the first and second fluids so that the first and second fluids are driven against the wall of the mixing chamber and out of a plane transverse to the first opening to the depth of greater than about 2.5 times the depth of the first opening.

30. The method of claim 24, wherein the fluid is driven against the wall of the mixing chamber and out of a plane transverse to the first opening to a depth of about 3 or more times the depth of the first opening.

31. The method of claim 24, wherein a top of the first opening is in line with a top of the mixing chamber.

32. The method of claim 24, wherein the outlet opening has a cross-section area that is equal to a cross-sectional area of the first opening.

33. The method of claim 24, wherein the mixing chamber is between a first layer and a second layer of the microfluidic device.

34. The method of claim 24, wherein the mixing chamber has a length that is greater than a width, further wherein the length is greater than 2 times the width of the first opening.

35. A method of forming a therapeutic composition, the method comprising:

passing one or more therapeutic mRNAs within a first fluid and a delivery vehicle for the one or more therapeutic mRNAs within a second fluid through a first opening into a mixing chamber in a microfluidic device, so that the first and second fluids are driven against a wall of the mixing chamber and driven out of a plane of the first opening to a depth of

greater than one times the depth of the first opening to form a mixed fluid comprising the therapeutic composition;

maintaining a temperature of the mixing chamber at an enhanced mixing temperature determined to enhance mixing; and

passing the mixed fluid out of an outlet opening out of the mixing chamber.

36. The method of claim 35, wherein the enhanced mixing temperature is between about 2 and about 20 degrees C.

37. The method of claim 35, wherein maintaining comprises determining the enhanced mixing temperature for the one or more therapeutic mRNAs and/or the delivery vehicle.

38. The method of claim 37, further comprising determining the enhanced mixing temperature by modeling mixing in the mixing chamber *in vitro* or *in vivo.*

39. The method of claim 37, further comprising determining the enhanced mixing temperature by selecting a temperature between about 2 and about 20 degrees C having greater mixing as compared to mixing at other temperatures between about 2 and about 20 degrees C.

40. The method of claim 35, wherein the passing the first fluid and the second fluid through the first opening into the mixing chamber comprises passing the first and second fluids so that the first and second fluids are driven against the wall of the mixing chamber and out of a plane transverse to the first opening to the depth of greater than about 2.5 times the depth of the first opening.

41. The method of claim 35, wherein the fluid is driven against the wall of the mixing chamber and out of a plane transverse to the first opening to a depth of about 3 or more times the depth of the first opening.

42. The method of claim 35, wherein a top of the first opening is in line with a top of the mixing chamber.

43. The method of claim 35, wherein the outlet opening has a cross-section area that is equivalent to a cross-sectional area of the first opening.

44. The method of claim 35, wherein the mixing chamber is formed between a first layer and a second layer of the microfluidic device.

45. The method of claim 35, wherein the mixing chamber has a length that is greater than a width, further wherein the length is greater than 2 times a width of the first opening.

46. A method of mixing, the method comprising:

passing a first fluid and a second fluid through a first opening into a mixing chamber in a microfluidic device, so that the first and second fluids are driven against a wall of the mixing chamber and driven out of a plane of the first opening to a depth of greater than one times the depth of the first opening to form a mixed fluid; and

passing the mixed fluid out of an outlet opening out of the mixing chamber.

47. The method of claim 46, wherein the passing the first fluid and the second fluid through the first opening into the mixing chamber comprises passing the first and second fluids so that the first and second fluids are driven against the wall of the mixing chamber and out of the plane transverse to the first opening to the depth of greater than about 2.5 times the depth of the first opening.

48. A method of mixing within a microfluidic device, the method comprising:

passing a first fluid and a second fluid through a first opening into a mixing chamber within a microfluidic device, so that the first and second fluids are driven against a wall of the mixing chamber and out of a plane transverse to the first opening to a depth of greater than about 2.5 times the depth of the first opening to form a substantially mixed fluid; and

passing the mixed fluid out of an outlet opening out of the mixing chamber, herein the outlet opening is opposite from the first opening but is offset from the first opening,

wherein the mixed fluid is substantially mixed by the mixing chamber and the mixing chamber is configured as a single mixer that does not connect to a second mixing chamber.