Supplementary Information

Microvortex-induced turbulent mixing for reconstitution of high-density lipoprotein-mimicking nanoparticles with aggregation-prone phosphatidylcholine

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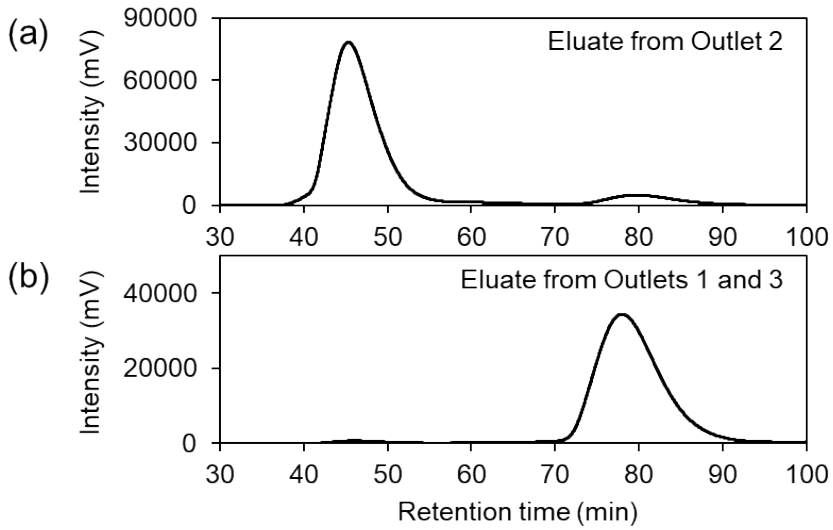

Fig. S1. Size-exclusion chromatograms of the eluates from (a) Outlet 2 and (b) Outlets 1 and 3, by the in-chip separation. The nanoparticles containing 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) were prepared at a total flow ratio (TFR) of 5.5 mL/min at 55°C. This in-chip separation facilitated the separation of the lipid nanoparticles and free protein in (a) and (b), respectively. Regarding the in-chip separation experiments, a PTFE tube with inner diameter/length of 0.25 mm/35 cm for Outlet 2 and another with inner diameter/length of 0.25 mm/45 cm for the merged side outlets were used, and the mixed solutions from Outlet 2 and the merged side outlets were collected separately.

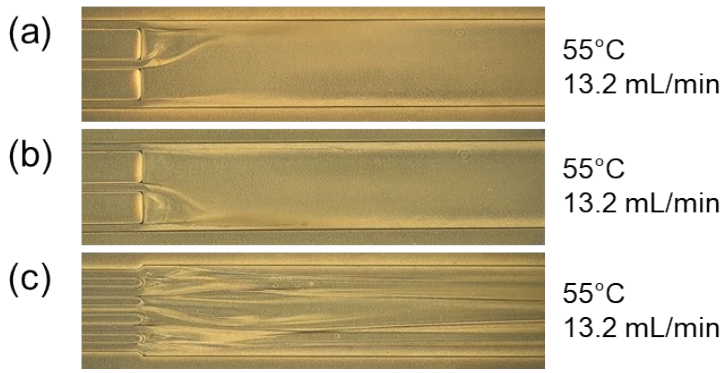

Fig. S2. Photographs of the upstream region of the mixing channel at the same total flow rate (TFR) (13.2 mL/min) and temperature (55°C). In (a) and (b), which are under the same condition, the asymmetrization occurred along both sides. In (c), the inlet and outlet were set opposite each other (water was injected from the original pre-outlets 1, 2, 4, and 5, and ethanol was injected from the original pre-outlet 3). The mixing boundary remained visible. These results indicate that this asymmetrization does not depend on the slight asymmetry in the chip structure; it depends on the inlet structure for generating the microvortices, where the three inlet channels were arranged apart.

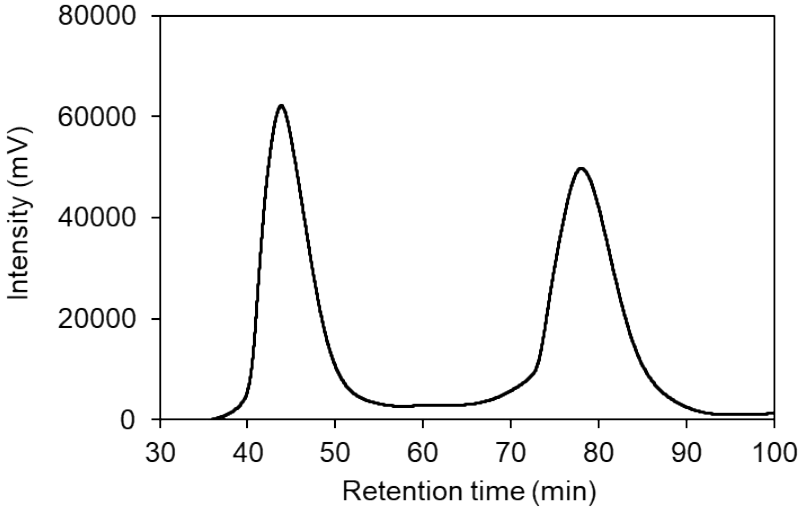

Fig. S3. Size-exclusion chromatogram of the nanoparticles containing 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) prepared under the condition where the inlet and outlet were set opposite each other (the aqueous protein solution was injected from the original pre-outlets 1, 2, 4, and 5, and the ethanolic DSPC solution was injected from the original pre-outlet 3), at a total flow rate (TFR) of 13.2 mL/min at 55°C. The protein-to-lipid flow-rate ratio was 10. The 60 min peak, assigned to high-density lipoprotein mimicking nanoparticles prepared with 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC–μHDL), was not detected. This result also corroborates the necessity of adopting asymmetrized microvortices-induced turbulence (AMT).

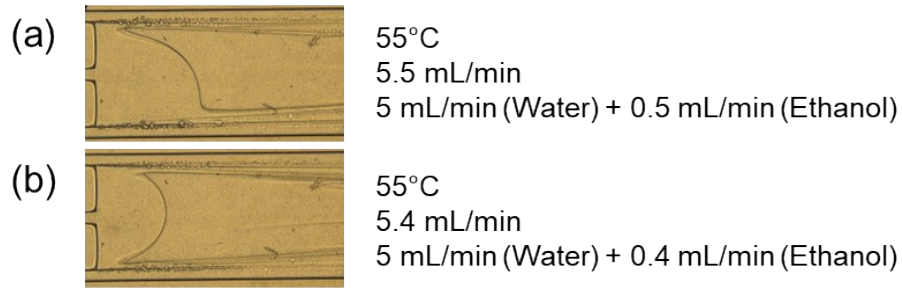

Fig. S4. Photographs of the upstream region of the mixing channel at total flow rates (TFRs) of (a) 5.5 and (b) 5.4 mL/min at 55°C. The microvortices, which was asymmetrized in (a), became symmetrized by slightly decreasing the flow rate of ethanol from 0.5 to 0.4 mL/min in (b). These results indicate that the disruption of the symmetric traveling of ethanol accounts for the aymmetrization of the microvortices.

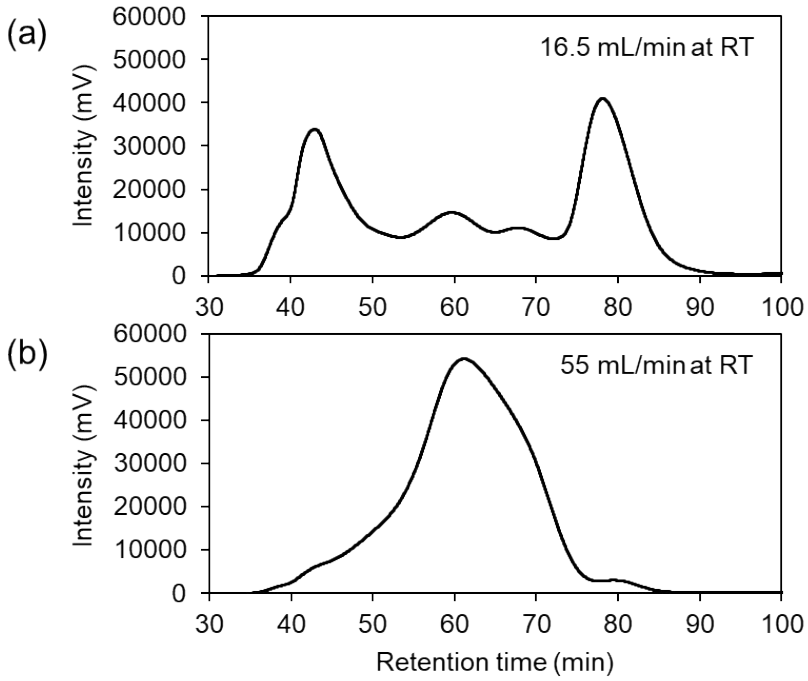

Fig. S5. Size-exclusion chromatograms of high-density lipoprotein-mimicking nanoparticle prepared with 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC–μHDL) by our asymmetrized microvortices-induced turbulence (AMT) method at total flow rates of (a) 16.5 and (b) 55 mL/min at room temperature. In (b), a high pressure-resistent chip (Zeon corporation) with the same structures of inlet channels and mixing channel as that shown in Fig. 1 was used.

Table S1. Characterization of the products in the mixture containing 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) prepared by our asymmetrized microvortices-induced turbulence (AMT) method at various total flow rates (TFRs)

TFR (mL/min)	Mean hydrodynamic diameter (nm)	Unused protein (%)[a]
5.5	33.7	69
7.7	28.2	59
11	15.4	31
13.2 (N=3)	14.4 ± 0.2	17 ± 4

[a]Values for the free protein isolated from the mixture by size exclusion chromatography

Table S2. AMT mixing at much higher total flow rate (TFR) using high pressure-resistant chips (Zeon corporation)

DSPC : cholesterol : apoA-I (mixing molar ratio) [a]	Mean hydrodynamic diameter (nm)	Yield (% protein)[b]
80 : 0 : 1	10.6	97
80 : 8 : 1	9.9	93
80 : 16 : 1	9.8	94
80 : 0 : 0	agglomerate	–
80 : 20 : 0	130.8	–
80 : 80 : 0	30.4	–

[a]Ethanol solution containing 5 mg/mL DSPC and 0, 0.24, 0.49, 0.61, or 2.4 mg/mL cholesterol was injected at flow rate of 5 mL/min. PBS solution containing 0 or 0.2 mg/mL N-terminal 43 amino acid-deleted apoA-I and 0 or 2 M urea, respectively, was injected at flow rate of 50 mL/min.
[b]Values for the 49–74.5 min fraction in our size exclusion chromatography