Lab on a Chip

PAPER – Supplementary Data

On-chip automation of cell-free protein synthesis: New opportunities due to a novel reaction mode

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Figure S1: Engineering drawing of the microfluidic device with the microfluidic patterns and heating zones.

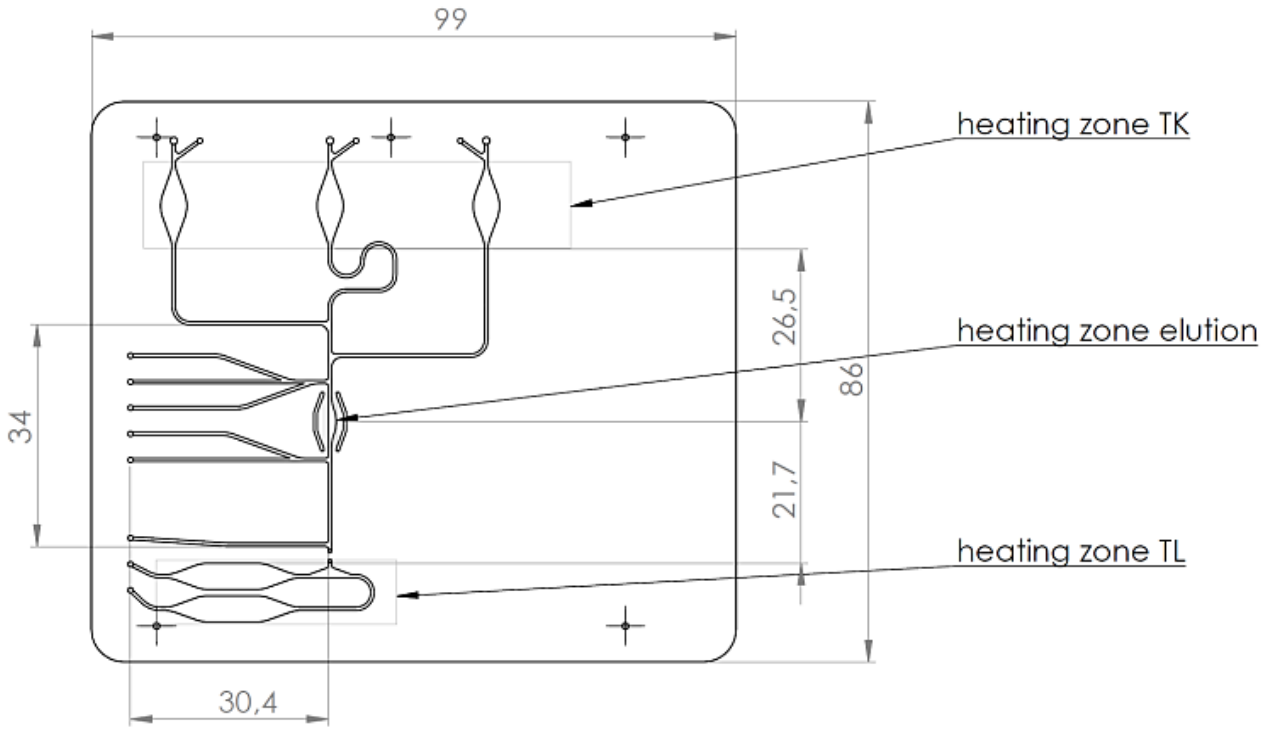


Fig S1 Engineering drawing of the microfluidic device with the microfluidic patterns and heating zones. All dimensions in mm. The microfluidic pattern was micro-milled into 1.55 mm high amorphous thermoplastic Cyclic Olefin Copolymer COC (TOPAS 6013-S04) substrate. The rectangular channels and reaction chambers are 500 µm deep. The area of the TK chambers is 29.5 mm^2, of the elution chamber is 8 mm^2 and of TL chambers is 59.4 mm^2. The air slots (1.55 mm deep → breakthrough) were drilled around the elution chamber to ensure proper heat insulation (verified by IR thermography) between the different temperature zones. The channels and air slots are 500 µm wide.

Figure S2: The apparatus for magnet movement

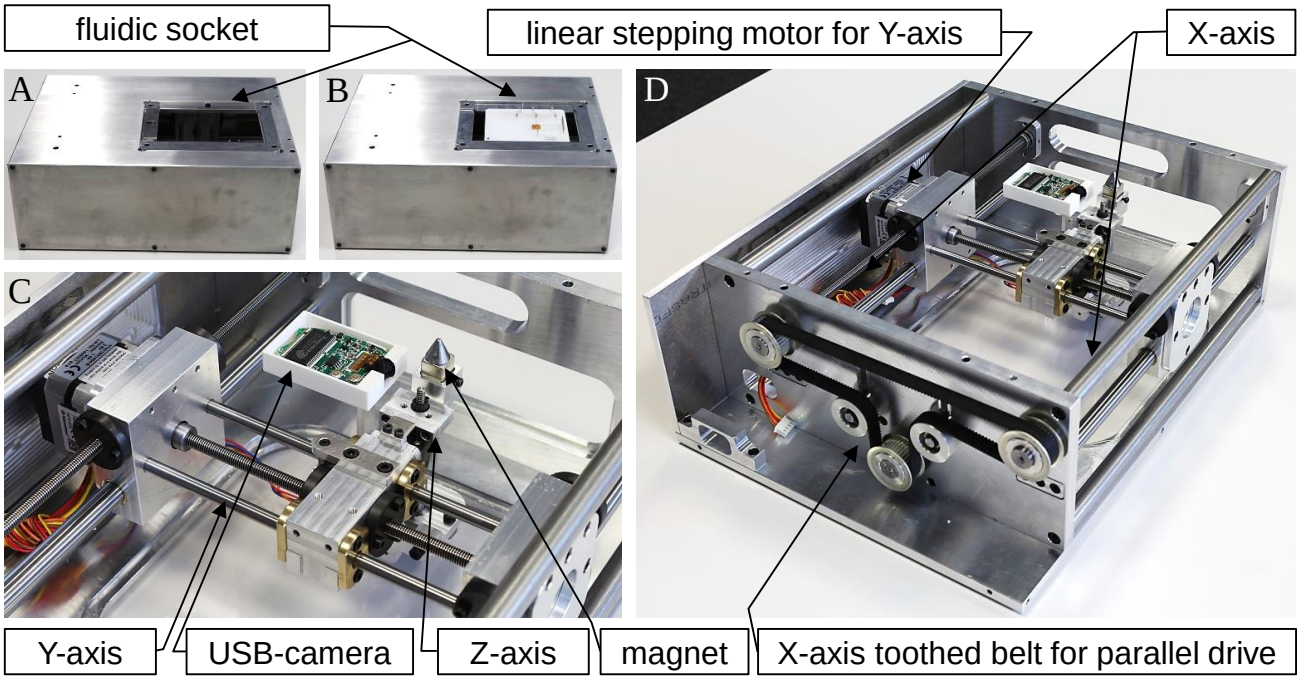


Fig S2 Photographs of the apparatus for magnet movement. (A) Device with aluminum housing. (B) Device with aluminum housing and microfluidic chip in the fluidic socket. (C) Device without aluminum housing. Detail view on the mounting system holding the permanent magnet with a cone-shaped add-on piece. The movement of the magnet is driven by three axes. An integrated USB-camera is used for observation of the magnet movement and the ongoing process in the microfluidic structure. (D) Device without aluminum housing – overview of the whole xyz-positioning system. Three stepping motors are driving trapezoidal spindles, translating rotational motion into linear movement of the axes carriages. Two parallel x-axes are powered by a toothed belt.

Functional description of the xyz-positioning system

The xyz-positioning system is able to move a mounting system holding the permanent magnet in all three dimensions (Fig **S2** D). With a maximum range of movement of 130mm x 90mm in x- and y-direction the device covers the plane area of a microwell plate (Fig **S2** B). The z-stroke is 37mm. An integrated USB-Camera with 0.3 megapixels gives the opportunity to observe the movement of the magnet and to follow the ongoing process in the fluidic structure. The camera is focused to the tip of the magnet (Fig **S2** C). Three stepping motors are driving trapezoidal spindles, translating rotational motion into linear movement of the axes carriages. Two parallel x-axes are powered by a toothed belt (Fig **S2** D). The repeat accuracy is assured through backlash compensation and an axial-radial bearing combination. Linear guide shafts with slide bearings are used in each axis to guarantee a precise and defined movement. The xyz- positioning system is handy and transportable (Fig **S2** A). Moreover the mechanical components and the electric wires are protected against water splashes through the aluminum housing. The power supply is standard 230V and the communication and control software interfaces by a USB interface.

Detailed description of chip operation

In general, fluid actuation was realized by pressure differences caused by syringe pumps. All syringes were actuated using a syringe pump array developed in-house that was controlled by a LabVIEW program.

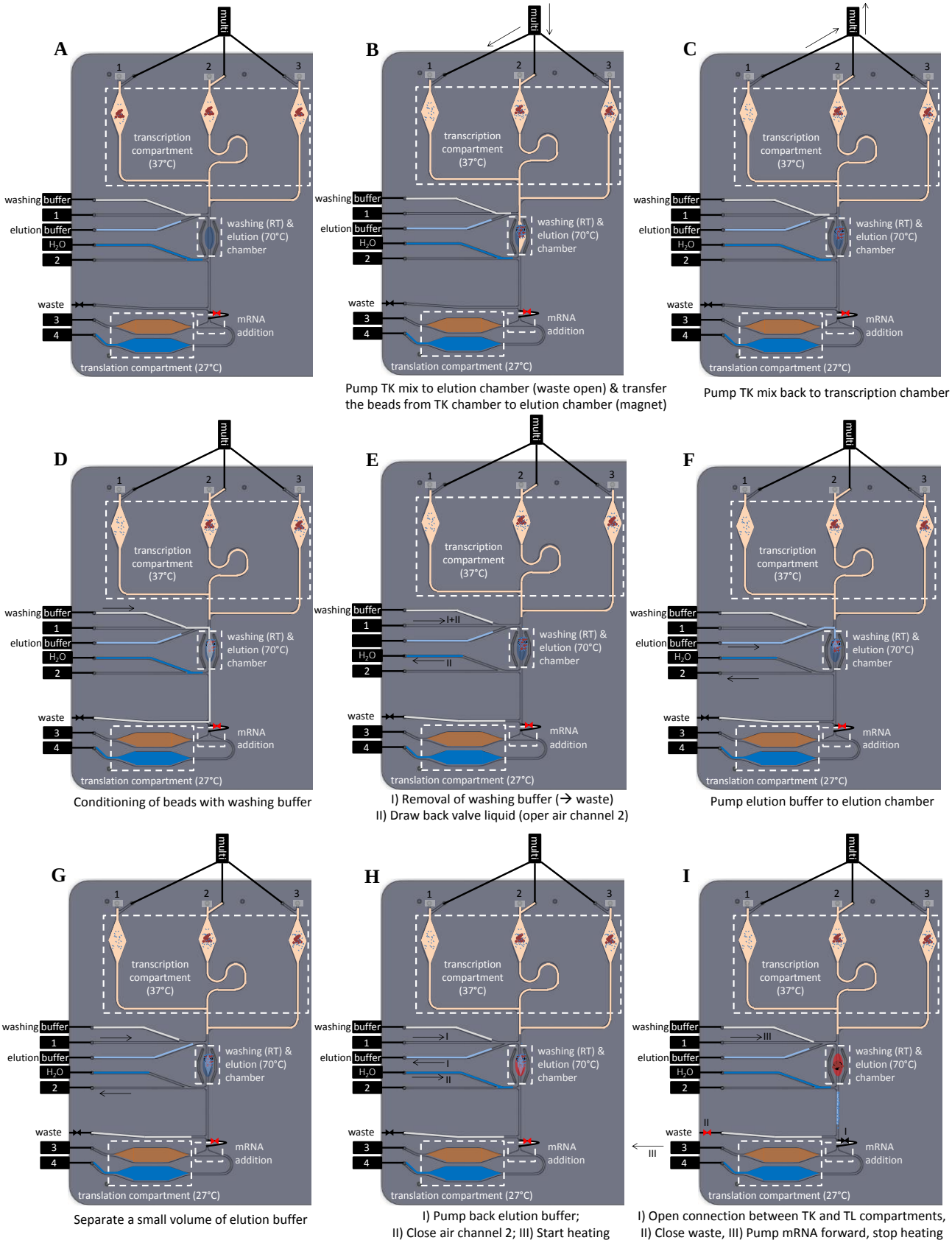
The waste exit and the connection between TK and TL compartment were mechanically closed or opened by clamping the tubings (using a simple in-house fabricated valve). In this way pressure differences could be realized in an open or closed system.

As air is compressible, liquid pumping media (tetradecane or water) were used to fill the syringe pumps. However, air was used as separation medium between the individual reaction mixtures and solutions and also to separate the pumping media.

The channels were coated by fluoro-acrylate-polymer to increase hydrophobicity and decrease the adherence of the particles to the channel wall.

The particles were transferred in a liquid medium (for instance TK mixture), the liquid was then drawn back while the particles are locally fixed by the magnet, and the particles were resuspended in the new solution (for instance washing buffer). The different solutions were separated by an air gap.

The precise chip operation including transfer of particles in liquid media and separation of reaction mixtures and buffers with air is described in Fig S3.



Pump TK mix to elution chamber (waste open) & transfer the beads from TK chamber to elution chamber (magnet)

Pump TK mix back to transcription chamber

Conditioning of beads with washing buffer

I) Removal of washing buffer (→ waste)
II) Draw back valve liquid (oper air channel 2)

Pump elution buffer to elution chamber

Separate a small volume of elution buffer

I) Pump back elution buffer;
II) Close air channel 2; III) Start heating

I) Open connection between TK and TL compartments,
II) Close waste, III) Pump mRNA forward, stop heating

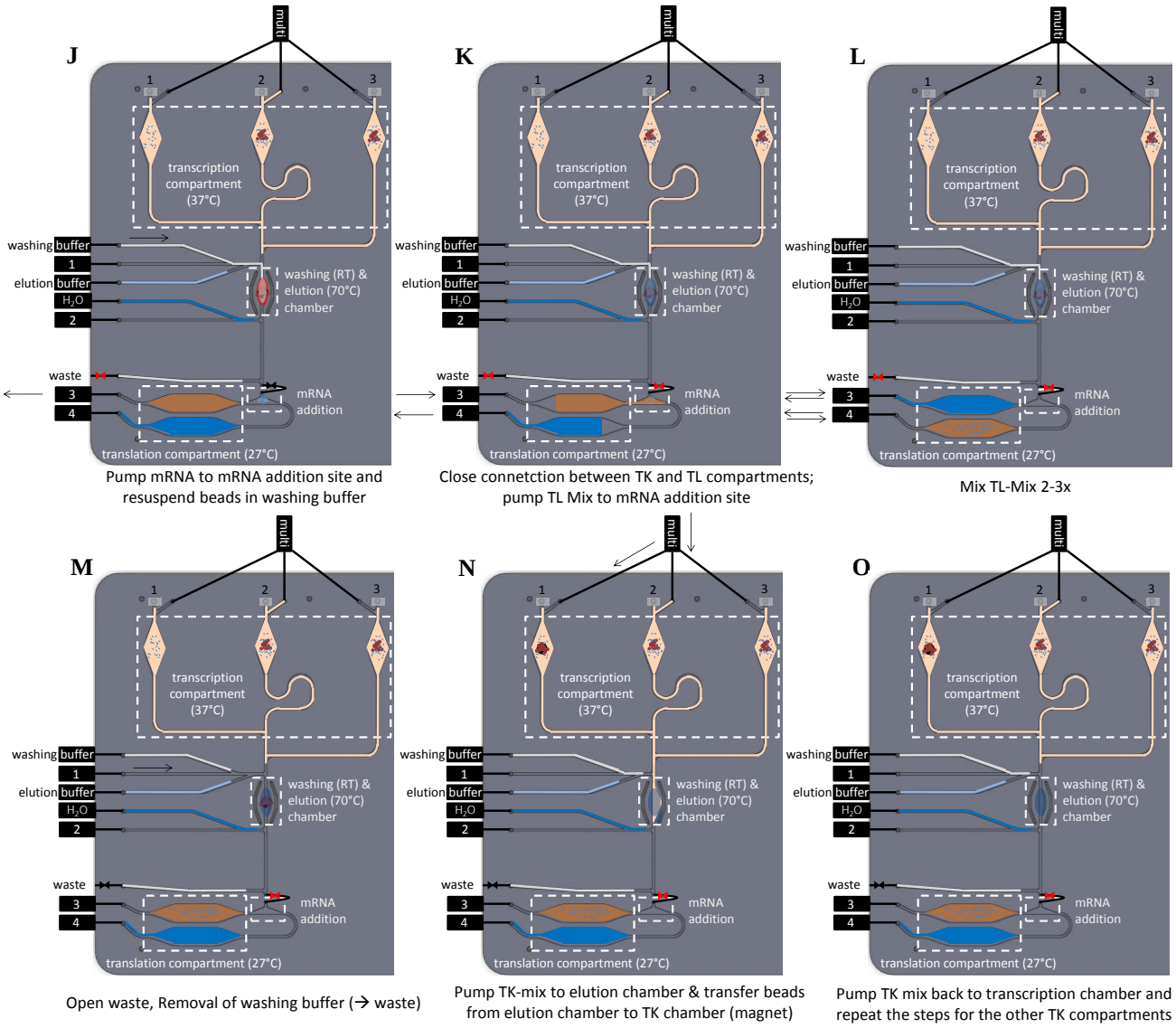


Fig S3: Illustration of the precise chip operation including transfer of particles in liquid media and separation of reaction mixtures and buffers with air:
(A) The buffers and solutions were filled in the compartments of the chip with the syringe pumps or a pipette tip, and the filling ports were closed. **(B)** In the first run, the TK mixture (beige) was pumped and the particles (functionalized magnetizable particles with immobilized mRNA) of the first TK chamber were transferred to the washing and elution chamber. **(C)** The TK mixture was pumped back to the TK compartment and **(D)** the particles were conditioned using washing buffer (withe). Washing removes residuals of the TK mixture and prepares the particles for elution. **(E)** Washing buffer was removed and **(F+G)** particles were resuspended in a small volume of elution buffer (light blue). The air channel was closed with valve liquid (water or tetradecane (mid blue)) to avoid that bypassing liquid is pressed into the channel. (**H)** mRNA was eluted by temperature (70°C) and low salt driven elution. **(I+J)** The mRNA was pumped to the TL compartment and **(K+L)** mixed with the TL mixture (brown). **(M-O)** Finally the particles were transferred back to the first TK chamber.
In the following runs, the same procedure was repeated with the particles in the second and the third TK chamber and the first chamber again and so on. During transfer of the particles of the other chambers, mRNA is again immobilized to the particles in the resting chamber.