

Supporting Information

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Mixing Time Considering Only Simple Diffusion

The 3D diffusion time is given by

$$t = \frac{d^2}{6D},$$

where d is the distance traveled by diffusing solutes and D is the diffusion coefficient. With the diffusion coefficient of 1.5×10^{-5}

cm^2s^{-1} for DCIP and an average droplet size $13 \mu\text{m}$ in diameter, the calculated mixing time was 19 ms.

Speculation About the Nature of HDX in Bradykinin

The +2 charge state of bradykinin contains 17 labile hydrogen atoms plus two H^+ atoms bound to two $-\text{NH}_2$ sites (1). We speculate that the fastest exchange comes from the $-\text{COOH}$ group (2) and the other two from the two $-\text{NH}_3^+$ groups. It should be also considered that the exchange rates are influenced by neighboring residues through Coulombic repulsion between charges and by steric effects.

1. Katta V, Chait BT (1991) Conformational changes in proteins probed by hydrogen-exchange electrospray-ionization mass spectrometry. *Rapid Commun Mass Spectrom* 5(4):214–217.

2. Morgan CR, Engen JR (2009) Investigating solution-phase protein structure and dynamics by hydrogen exchange mass spectrometry. *Curr Protoc Protein Sci* Chap 17 (November):Unit 17.617.

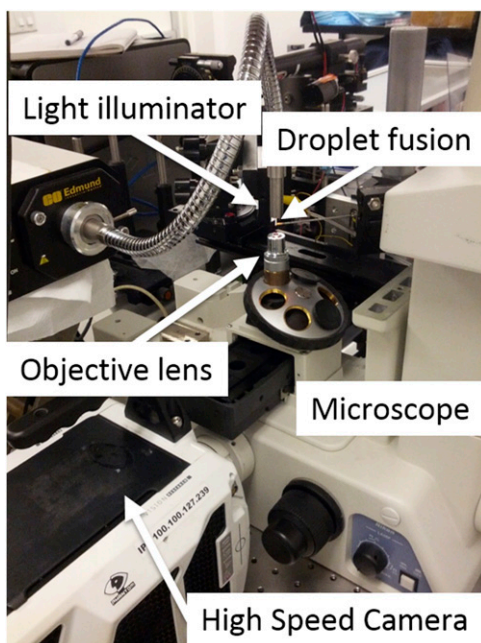


Fig. S1. Setup for high-speed camera imaging of fusion and trajectories of liquid droplets.

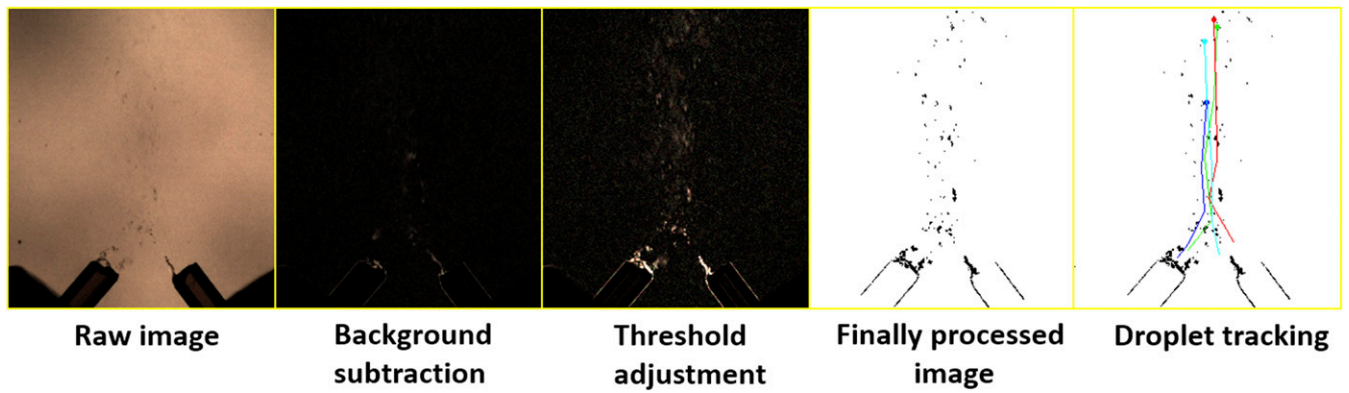


Fig. S2. Procedure of image analysis for tracking of droplet trajectories. A background image where no droplets were present was subtracted from raw images containing droplets. After adjusting the threshold level for a better visibility, the images were finally inverted and turned into black-and-white images. The droplets were tracked with imageJ software (NIH) and a manual tracking plugin.

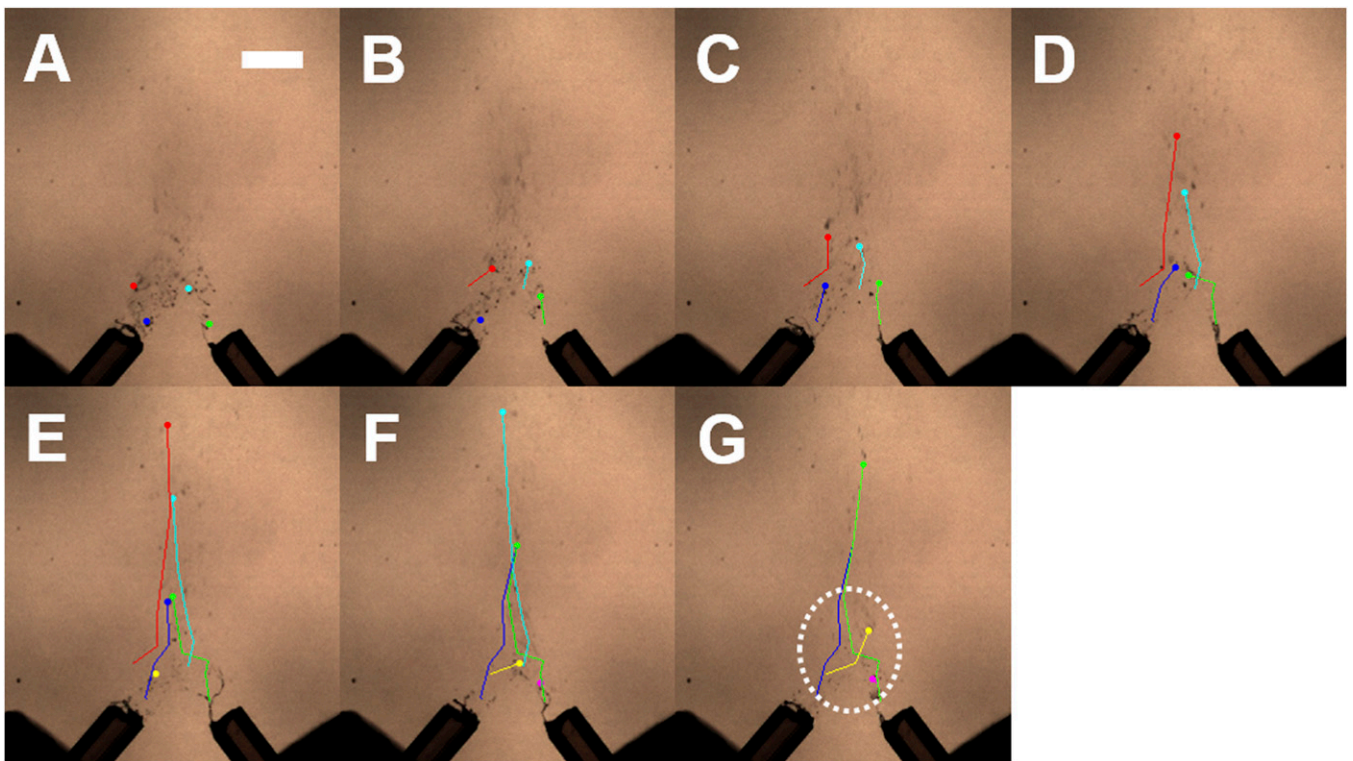


Fig. S3. (A–G) Series of time-lapse images taken from Movie S2. The time gap between each image was $8.33 \mu\text{s}$. Most of the droplet fusion occurred in the region indicated with a white circle in G. (Scale bar, $500 \mu\text{m}$.)

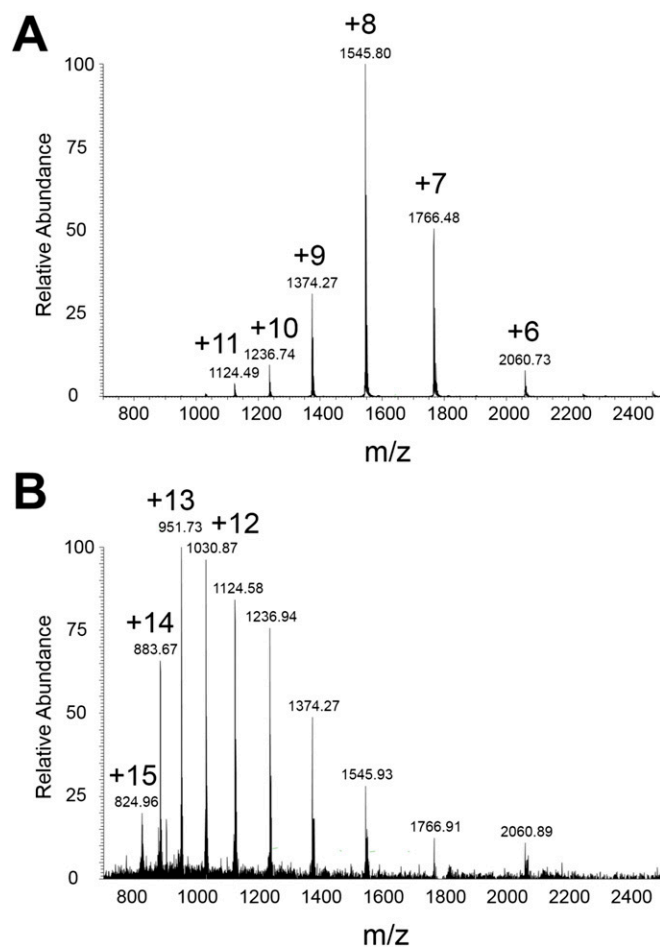


Fig. S4. Mass spectra of cytochrome c in H₂O at (A) pH 7.0 and (B) pH 3.0.

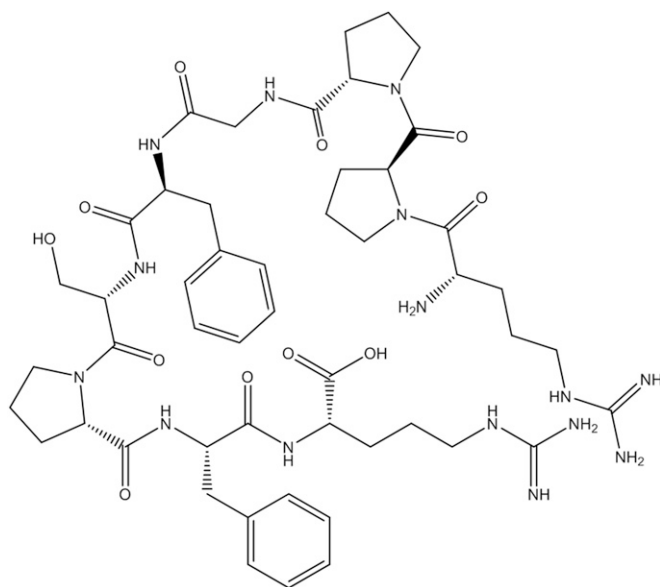


Fig. S5. Structure of the bradykinin peptide.

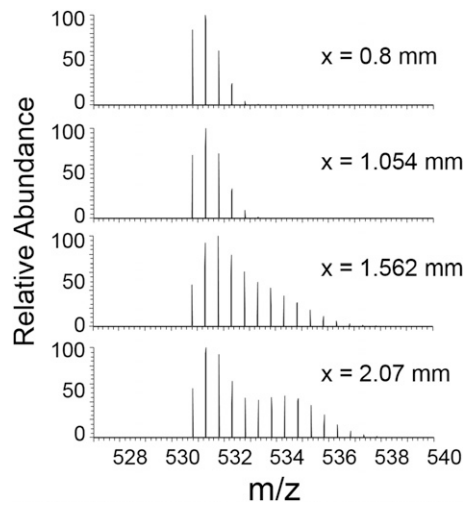
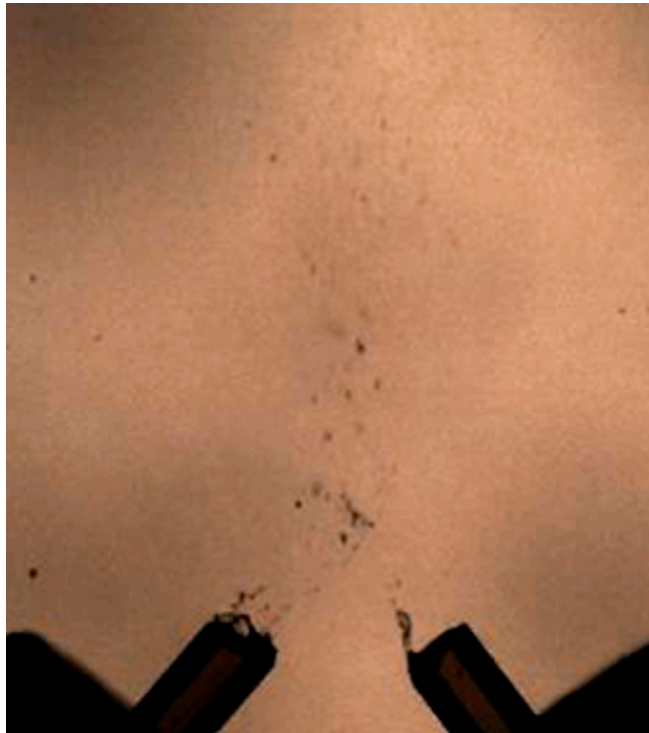


Fig. S6. Mass spectra of doubly charged bradykinin at different distances x obtained by fusing together droplets with $1\ \mu\text{M}$ of bradykinin solution with droplets containing 99.9% D_2O .

Table S1. Average diameter of fused droplets of pure water over the distance x

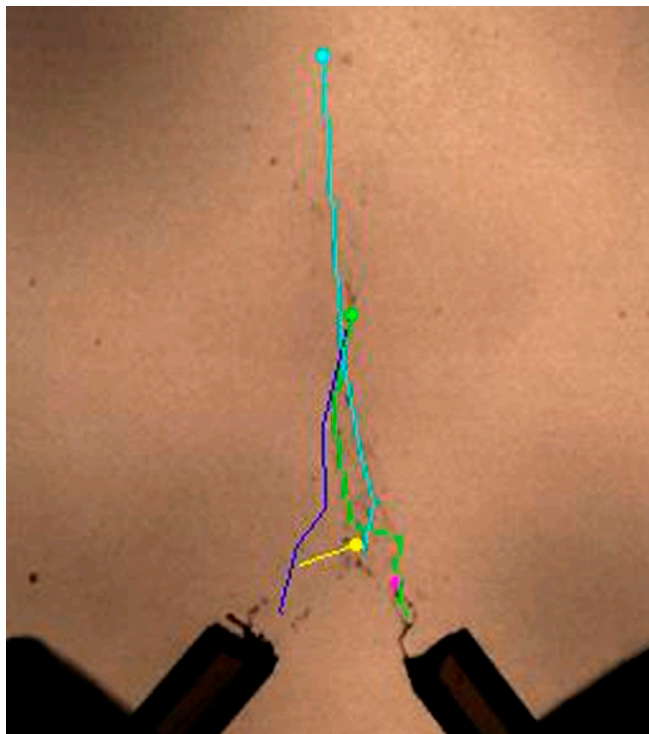
Distance x , mm	Average diameter of fused droplets, μm
0.5	12.5 ± 4.0
1.5	12.9 ± 4.9
2.5	13.0 ± 3.9
3.5	13.0 ± 3.8
4.5	12.8 ± 5.6
5.5	12.5 ± 4.7
6.5	11.8 ± 4.4
7.5	11.2 ± 2.7

Uncertainties represent the SD calculated from four measurements.



Movie S1. Movie of droplet fusion using high-speed camera recorded at 120,000 fps and played at 10 fps. The pixel size is 256×288 .

[Movie S1](#)



Movie S2. Movie of real-time tracking of droplets. Colored dots and lines indicating tracked droplets were overlaid.

[Movie S2](#)