Supporting Information

On-Demand Droplet Fusion: A Strategy for Stimulus-Responsive Biosensing in Solution

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Competitive model for thrombin assay

Gibbs Energy calculations (SantaLucia’s nearest neighbor model):

| Bases | $\Delta H$ (kcal/mol) | $\Delta S$ (Cal/K.mol) |
|-------|-----------------------|------------------------|
| AA/TT | -7.9                  | -22.2                  |
| AT/TA | -7.2                  | -20.4                  |
| TA/AT | -7.2                  | -21.3                  |
| CA/GT | -8.5                  | -22.7                  |
| GT/CA | -8.4                  | -22.4                  |
| CT/GA | -7.8                  | -21                    |
| GA/CT | -8.2                  | -22.2                  |
| CG/GC | -10.6                 | -27.2                  |
| GC/CG | -9.8                  | -24.4                  |
| GG/CC | -8                    | -19.9                  |
| Term. G-C | 0.1                  | -2.8                  |
| Term. A-T | 2.3                  | 4.1                  |

$$\Delta G^\circ (total) = \sum n_i \Delta G^\circ (i) + \Delta G^\circ (5'\text{term}) + \Delta G^\circ (3'\text{term})$$

$$\Delta G^\circ (37^\circ C, 1M NaCl) = \Delta H^\circ - \Delta S^\circ \times (273.15 + 37^\circ C)K$$

$$\Delta G (37^\circ C, 0.15M NaCl) = \Delta G^\circ (1M NaCl) - 0.114 \times \frac{kcal}{mol} \times 4.18 \times \frac{kJ}{kcal} \times N \times \ln(0.15)^1$$

Specific sequence overlap:

5'-TGTGGTTGTTT-3'
3'-ACACCAACCCAAA-5'

| Bases | $n_i$ | $\Delta H$ (kcal/mol) | $\Delta S$ (cal/mol.K) |
|-------|------|-----------------------|------------------------|
| TG/AC | 3    | -8.5                  | -22.7                  |
| GT/CA | 3    | -8.4                  | -22.4                  |
| GG/CC | 2    | -8                    | -19.9                  |
Calculation of Dissociation constant:

\[ \Delta G = -RT \ln K_a \]

\[ K_d = \frac{1}{K_a} \]

\[ R = 8.314 \frac{J}{\text{mol} \cdot K} \]

\[ T = 310.15 \text{ K} \]

\[ \Delta G^\circ(37 \, ^\circ\text{C}, 0.15M \text{ NaCl}) = -46.5 \frac{kJ}{\text{mol}} \]

\[ K_d = 15 \text{ nM at } 37 \, ^\circ\text{C} \]

Equations used for the model:

\[ K_{D,\text{Thr.Apt}} = \frac{[\text{Thr}]_f [\text{Apt}]_f}{[\text{Thr}.\text{Apt}]} \approx 200 \text{ nM}^2 \]

\[ K_{D,\text{Link1.Apt}} = \frac{[\text{Link1}]_f [\text{Apt}]_f}{[\text{Link1}.\text{Apt}]} \approx 15 \text{ nM} \]

\[ [\text{Apt}]_{\text{tot}} = [\text{Apt}]_f + [\text{Link1}.\text{Apt}] + [\text{Thr}.\text{Apt}] = 180 \text{ nM} \]

\[ [\text{Thr}]_{\text{tot}} = [\text{Thr}]_f + [\text{Thr}.\text{Apt}] \]

\[ [\text{Link1}]_{\text{tot}} = [\text{Link1}]_f + [\text{Link1}.\text{Apt}] = 135 \text{ nM} \]

Where:

- \( K_{D,\text{Thr.Apt}} \): Thrombin/Aptamer disassociation constant
- \( K_{D,\text{Link1.Apt}} \): 5’chol-DNA/Aptamer disassociation constant
- \([\text{Thr}]_f\): Free thrombin concentration
- \([\text{Apt}]_f\): Free aptamer concentration
- \([\text{Link1}]_f\): Free 5’chol-DNA concentration
- \([\text{Link1}.\text{Apt}]\): 5’chol-DNA/aptamer duplex concentration
- \([\text{Thr}.\text{Apt}]\): Thrombin-aptamer concentration
- \([\text{Apt}]_{\text{tot}}\): Total aptamer concentration
- \([\text{Thr}]_{\text{tot}}\): Total thrombin concentration
- \([\text{Link1}]_{\text{tot}}\): Total 5’chol-DNA concentration
Solving five simultaneous equations to obtain concentrations of \([\text{Apt}]_f, [\text{Thr}]_f, [\text{Link1}]_f, [\text{Link1. Apt}]\) and \([\text{Thr. Apt}]\) at different thrombin concentrations. These equations were solved using a MATLAB (MathWorks) code.

MATLAB code:

```matlab
% K1 and K2 are dissociation constants for TA-TH and TA-DNA respectively
k1=200;
k2=15;

% Concentrations of total Aptamer and Thrombin respectively
TA=180;
DNA=135;
TH=0;

% free aptamer (a), free thrombin (b), free L1-DNA (c), bound aptamer-L1DNA (y), bound aptamer-thrombin
% (x)
syms a b c x y
F1=k2*y-a*c;
F2=k1*x-a*b;
F3=x+y+a-TA;
F4=x+b-TH;
F5=y+c-DNA;
S = solve(F1,F2,F3,F4,F5,a,b,c,x,y);
double(S.a)
double(S.b)
double(S.c)
double(S.x)
double(S.y)
```

**Figure S1.** Dye-labelled DNA probe shows the presence of Chol-DNA on the droplet surface; A) Cy3-labelled DNA probe (red) for Link-1 and B) FAM-labeled DNA probe for Link-2. Scale bar is 2 µm
Figure S2. Histogram of colocalization fraction \( \frac{I_G}{I_G + I_R} \) is shown for one image in each group; A) DiI only (red columns) and BPEA only (green columns) droplets have colocalization fractions near 0 and 1 respectively when imaged individually and B) Mixing of droplets without DNA (black columns) and mixing of droplets with complimentary DNA (patterned columns) – values between 0.15 and 0.85 can be considered fused droplets.

Figure S3. Average Diameter of non-fused and fused droplets compared between PEG concentrations of 8 and 40 µM. The diameters were calculated using MATLAB analysis of the droplet images. The diameter of fused droplets is higher for 8 µM PEG concentration compared to 40 µM PEG concentration.

1. SantaLucia, J., Jr. A unified view of polymer, dumbbell, and oligonucleotide DNA nearest-neighbor thermodynamics. *Proceedings of the National Academy of Sciences of the United States of America* 1998, 95 (4), 1460-5.

2. Bock, L. C.; Griffin, L. C.; Latham, J. A.; Vermaas, E. H.; Toole, J. J. Selection of single-stranded DNA molecules that bind and inhibit human thrombin. *Nature* 1992, 355 (6360), 564-566.