**Supplementary Information**

**Table S1: Reaction conditions for the TdT catalysed formation of vsDNA libraries**

| | **TVS1** | **TVS2** | **TVS3** | **LVS1** |
|---|---|---|---|---|
| Initiator Sequence (F Primer 10µM) | 80µl (2 µM) | 80 µl (2 µM) | 80 µl (2 µM) | 80 µl (2 µM) |
| 5x SB1 | 1x | 1x | 1x | 1x |
| 1mM dTTP | 20 µl (50µM) | 30 µl (75µM) | 0 | 20 µl (50 µM) |
| 1mM dCTP | 10 µl (25 µM) | 0 | 30 µl (75µM) | 20 µl (50 µM) |
| 1mM dATP | 10 µl (25 µM) | 0 | 30 µl (75 µM) | 5 µl (12.5 µM) |
| 1mM dGTP | 20 µl (50 µM) | 30 µl (75 µM) | 0 | 20 µl (50 µM) |
| TdT 400 U/µl | 1 µl | 1 µl | 1 µl | 1 µl |
| Final Volume | 400 µl | 400 µl | 400 µl | 400 µl |
| Time of Incubation | 30, 60 and 120 minutes | 30, 60 and 120 minutes | 30, 60 and 120 minutes | 30, 60 and 120 minutes |

**Table S2: List of candidate thrombin binding Oligonucleotide and polynucleotide sequences with G-Quadruplex mapper scores. The initiator sequence is identified in bold.**

| Name | Sequence | Size (nt) | G-Score |
|---|---|---|---|
| **TH_01** | **ATCAGTTCGAGCAGATGAGC**AGGGGCCAGGATAGCGGCCGGATAATCGGTCCGG | **54** | **33** |
| **TH_02** | **ATCAGTTCGAGCAGATGAGC**AGGGCAGGGAGTGATCGGAGGCAGGAGCCGGAACAGGGATAGGGGGGAG | **69** | **30, 36** |
| **TH_03** | **ATCAGTTCGAGCAGATGAGC**AGTGGAGAGTAGGGCTTGGGGAAGGCGGATAGATCCAGAGATTCCGGTTG | **70** | **35** |
| **TH_04** | **ATCAGTTCGAGCAGATGAGC**AGAGGGGAAGCGGGGCGTCGGTTGG | **45** | **33** |
| **TH_05** | **ATCAGTTCGAGCAGATGAGC**ATGTGGGGAAGGAGGGGCCAGGCCCGAG | **48** | **36** |
| **TH_06** | **ATCAGTTCGAGCAGATGAGC**TGGAGGGTGGGGTACCGACGCG | **42** | **34** |
| **TH_07** | **ATCAGTTCGAGCAGATGAGC**AAGGAGGAAGGGGTAGGTCGATAATGGGAGCCCGGTAGGG | **60** | **34** |
| **TH_08** | **ATCAGTTCGAGCAGATGAGC**AGTACAGAGGGGTGTCGCGGGGGGCAGAGCG | **51** | **30** |
| **TH_09** | **ATCAGTTCGAGCAGATGAGC**AGTAGGGAGAGGTTGGCAGGGGGGCAGGTTGAACGGGAGATGAATAGTAGCGAGGGGCATTGGAGGAGAGTT | **92** | **21, 17** |
| **TH_10** | **ATCAGTTCGAGCAGATGAGC**AGGGCAGGGTGGGTCACGGAGGCAGGAGTATGACGGAGTCCCTGAGGGGGGGGGCGTGGGGCATCAGGCTGCAGCCAGCCTGGGGCCCGAAATGGAACGATGATAG | **126** | **35, 89** |
| **SC01** | GTGATGCAGTAGTCGCGAGGCTGGGTATGTAGTGTAGAGTGAAGCGCGAGAGAGAGCGAGCGAGTGAGAGAGCGTGATTGTGAGAGGAGAGAG | **126** | **N/A** |
| TBA | GGTTGGTGTGGTTGG | **22** | |
| HD22 | AGTCCGTGGTAGGGCAGGTTGGGGTGACT | **29** | |

**Table S3: List of candidate lactoferrin binding oligonucleotides and polynucleotide sequences with G-Quadruplex mapper scores. The initiator sequence is identified in bold.**

| Name | Sequence | Size (nt) | G-Score |
|---|---|---|---|
| **LF_01** | **ATCAGTTCGAGCAGATGAGC**GGGGAGGGGATGGAGGGGATAGGGAGGGGATACTAGTTGGCACAGGTGCACATGGGCCGGAGCGGGAGATTG | **92** | **102, 32** |
| **LF_02** | **ATCAGTTCGAGCAGATGAGC**AAGGGGGGTCGGAGGTGGGCGCGGTACCGGGAAGGGCGGATGGCGTGGATGGGCGAGAGGGAGAAGGGAGGCTTAGTCGAGGCTTTTGATGACAGAGGCGAGGAAGGGAGTCTTGAAATTGGAGTGGGGCG | **151** | **65, 35, 31, 27** |
| **LF_03** | **ATCAGTTCGAGCAGATGAGC**TGACAGGTGGGAGTAAGATATATGGAAGATAGTATATGCGGCGCGAAGTCGAAGGGATGGT | **81** | **25** |
| **LF_04** | **ATCAGTTCGAGCAGATGAGC**AGGTAGCGGAGGGAGTCTAGACAAGGGGGGGGAGAGTGCGCTCCCGATGCAGCGTCGGGGGGCATCGGGAGGGCCGGAGGGCCGAATAGACGTTCGGAGGGGGGGAGAGGATAG | **134** | **29,68, 35** |
| **LF_05** | **ATCAGTTCGAGCAGATGAGC**AGATAGTGGTGCGGGCCGTAGGGGTACGCCCGGGAGTGGGAACCGGATAGAAGG | **74** | **68** |
| **LF_06** | **ATCAGTTCGAGCAGATGAGC**ACGTATGAGGGGTGCTGCAG | **40** | **N/A** |
| **LF_07** | **ATCAGTTCGAGCAGATGAGC**CGGGAGTCTATGCGTGGATGGGCGGAGAGCGGGATCGTGGGGGAGGGGGGGGAGG | **75** | **96** |
| **LF_08** | **ATCAGTTCGAGCAGATGAGC**AAGAGGGGGGGGGGGGGGAAAACCGCGGGAAGGGGCCAAGAATGACTCGGGCGGGGTGGGGGGGGGGAGGCGGCAAGGGCCGTAATTATCCGCAGGGTGG | **120** | **96, 72, 23** |
| **LF_09** | **ATCAGTTCGAGCAGATGAGC**AGGGAGGGGGTTAGGGGCACAGGCAGGTAGTGGAGGGGGGAGAAGGTAGGGAGTGGAACGGGCGCCGGGATGGCG | **95** | **58, 68** |
| **LF_10** | **ATCAGTTCGAGCAGATGAGC**AGGTAGGGAGGGGGCGTCGTCGCGGAC | **47** | **35** |
| **SC02** | ATCAGTTCGACTGGGGGATATGAGAAGTGAGAGACGTCGGACCGTGAGGAATCGGGGGAAGGAGCGAGGATGAGGGAGTGGCAGGGGTACGGGAGTGGTGCCGGAGGGTTGGCGATTGGGCGTGAGGGGTGTAAGACACCGTTGGAGGCGTAACCTGAAGT | **151** | **N/A** |

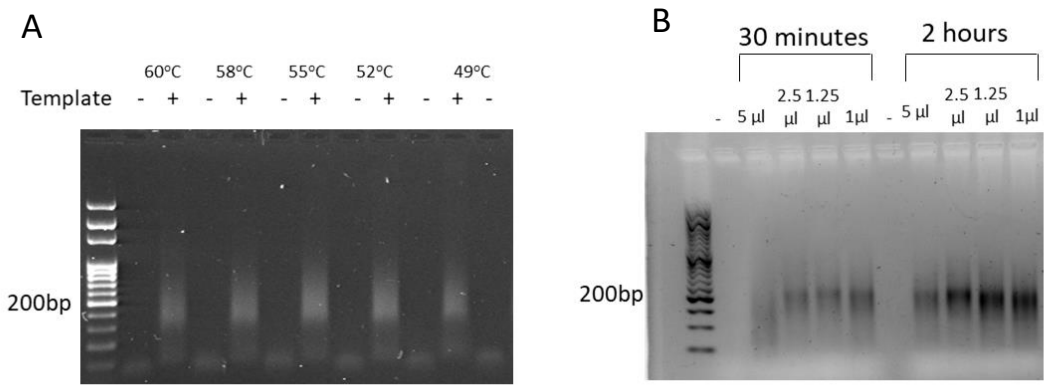
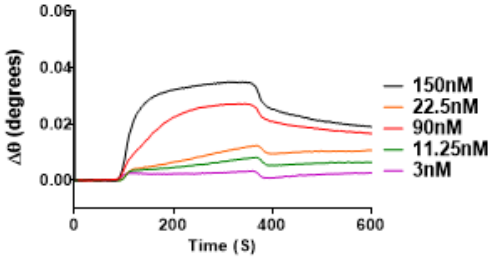
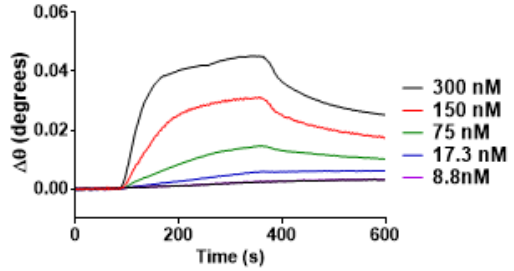



**Figure S1: 2% Agarose Gels showing the RAVE amplification of vsDNA libraries; (A) Effect of annealing temperature on the formation of PCR products using 1µl of extracted vsDNA library and (B) the effect of the volume of extracted vsDNA library (1-5 µl) on amplification efficiency.**




**D**

1:1 Binding model

| | $k_a$ (1/(M*s)) | $k_d$ (1/s) | $K_D$ (nM) |
|---|---|---|---|
| TH01 | 6.3 e4 | 1.0e-3 | 16±1.6 |
| TH05 | 5.4e4 | 1.8e-3 | 32±2.3 |
| TH07 | 1.2e4 | 1.0e-3 | 79± 0.65 |

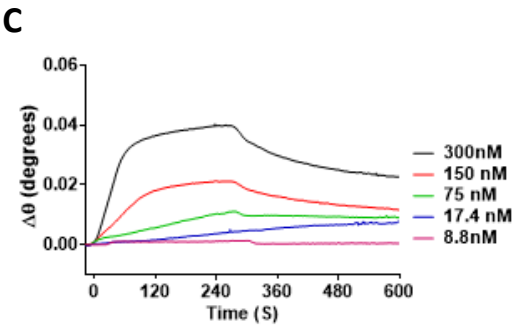
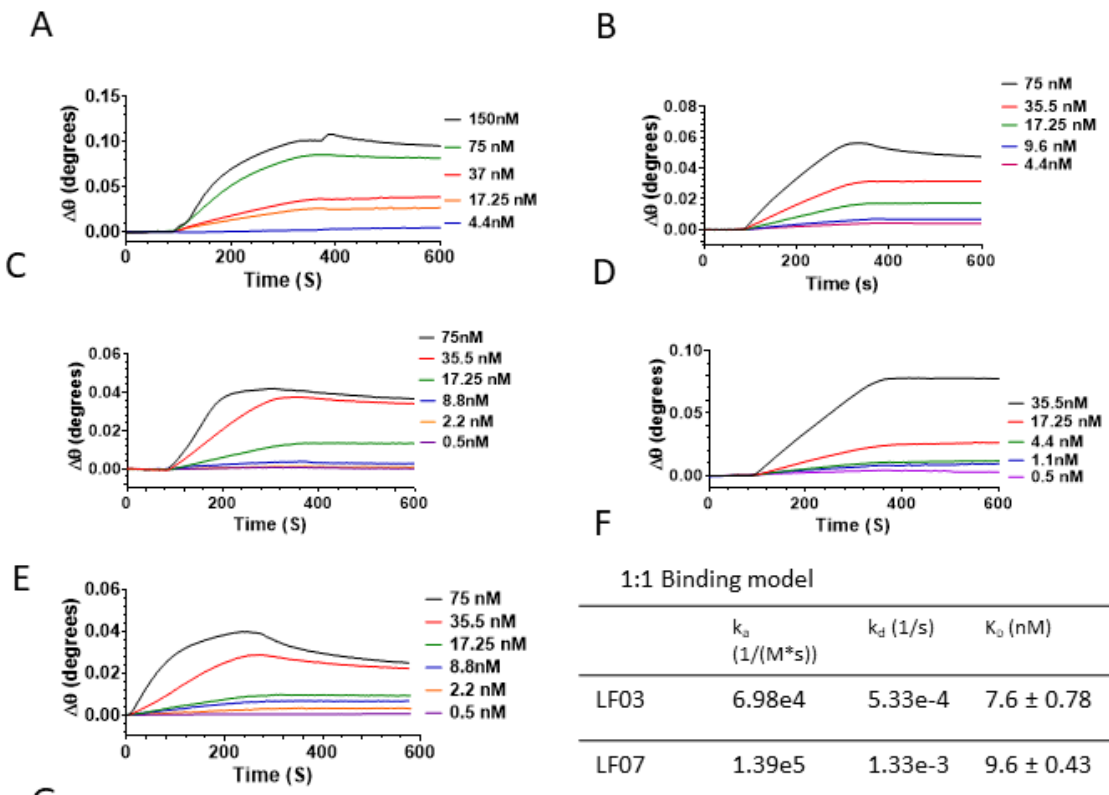



**Figure S2 SPR Analysis of thrombin binding polynucleotide sequences for (A) TH01, (B) TH05; (C) TH07 and (D) Kinetic parameters of thrombin binding sequences. All SPR experiments were performed at room temperature and in duplicate.**




1:1 Binding model

| | $k_a$ (1/(M*s)) | $k_d$ (1/s) | $K_D$ (nM) |
|---|---|---|---|
| LF03 | 6.98e4 | 5.33e-4 | 7.6 ± 0.78 |
| LF07 | 1.39e5 | 1.33e-3 | 9.6 ± 0.43 |

G

2:1 Binding model

| | $k_{a1}$ (1/(M*s)) | $k_{d1}$ (1/s) | $K_{D1}$ (nM) | $k_{a2}$(1/(M*s)) | $k_{d2}$ (1/s) | $K_{D2}$ (nM) |
|---|---|---|---|---|---|---|
| LF01 | 4.6e4 | 9.2e-4 | 19 ± 1.2 | 1.1e5 | 6.3e-4 | 2.74± 0.6 |
| LF02 | 8.8e4 | 4.7e-4 | 5.4 ± 0.7 | 7.8e4 | 2.2e-4 | 2.92± 0.4 |
| LF04 | 3.5e4 | 3.3e-6 | 0.090±0.07 | 5.6e4 | 3.3e-6 | 0.05±0.5 |

**Figure S3 SPR Analysis of human lactoferrin binding polynucleotide sequences for (A) LF01, (B) LF02; (C) LF03, (D) LF04, (E) LF07 and (F-G) Kinetic parameters of thrombin binding sequences. Run buffer: SB1, flow rate: 20 µl min$^{-1}$. All SPR experiments were performed at room temperature and in duplicate.**

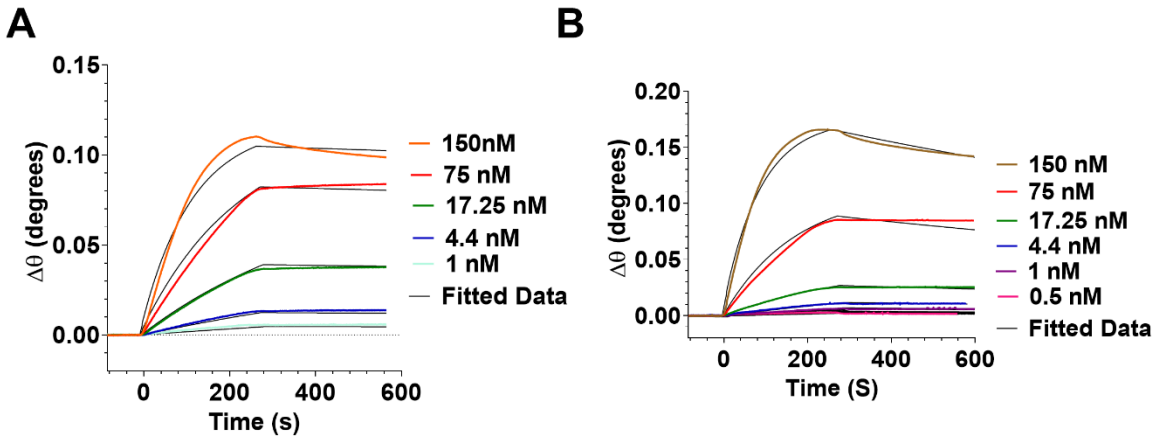
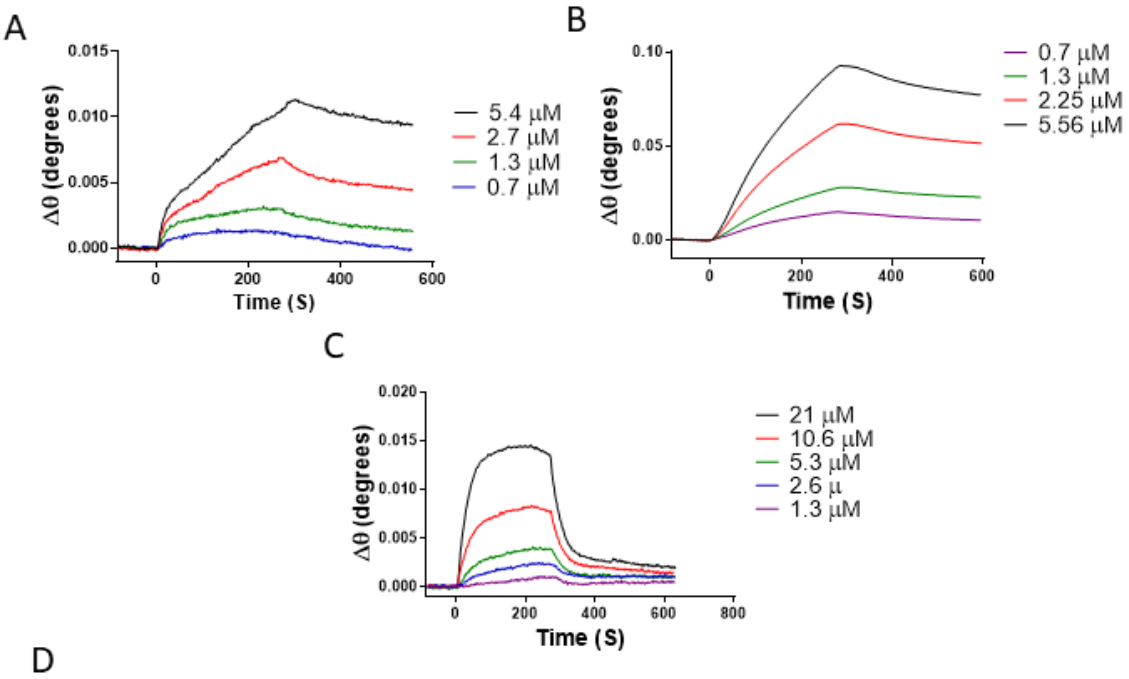



**Figure S4** ***SPR Analysis of lactoferrin binding DNA sequence for (A) LF03T and (B) LF04T.***

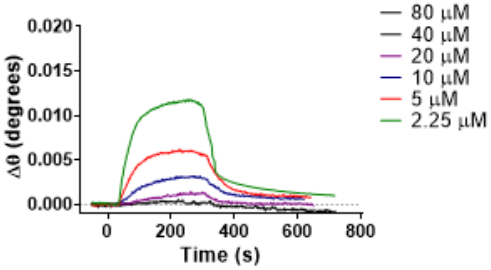



D

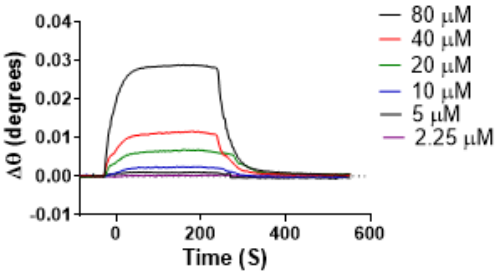
| | | $k_a$ (1/(M*s)) | $k_d$ (1/s) | $K_D$ (μM) |
|---|---|---|---|---|
| Hemoglobin | TH10T | 6.6e2 | 1.5e-3 | 2.32±0.89 |
| | SC01 | 5.7e2 | 1.2e-3 | 2.17±0.43 |
| Fibrinogen | TH10T | 3.1e2 | 1.2e-3 | 3.8e± 0.23 |
| | SC01 | 3.4e2 | 1.1e-3 | 3.1e± 0.14 |
| HSA | TH10T | 5.7e2 | 5.8 e-3 | 10.3± 0.89 |
| | SC01 | 5.9e2 | 5.4e-3 | 9.3± 0.17 |

**Figure S5 SPR Analysis of TH10T sequences for (A) Haemoglobin, (B) Fibrinogen, (C) HSA and (D) Kinetic parameters of thrombin binding. Run buffer: SB1, flow rate: 20 μl min$^{-1}$. All SPR experiments were performed at room temperature and in duplicate.**

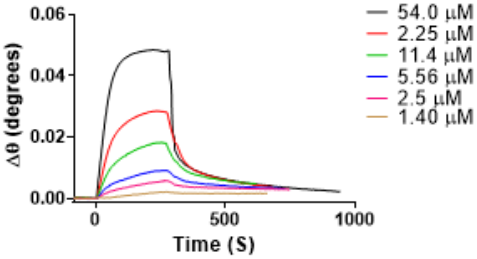
A




B




C




D

1:1 Binding model

| | | $k_a$ (1/(M*s)) | $k_d$ (1/s) | $K_D$ (μM) |
|---|---|---|---|---|
| HSA | LF02T | 1.9e3 | 6.5e-3 | 3.5± 0.45 |
| | SC02 | 1.3e3 | 9.8e-3 | 7.7± 0.23 |
| | LF03T | 2.8e2 | 2.4e-2 | 86± 0.56 |
| | SC02 | 2.7e2 | 2.1e-2 | 77± 0.83 |
| | LF04T | 3.4e2 | 6.9e-3 | 20± 0.48 |
| | SC02 | 3.8e2 | 8.91e-3 | 23± 0.93 |

**Figure S6 SPR Analysis of truncated HSA binding to (A) LF02T, (B) LF03T, (C) LF04T and (D) Kinetic parameters of HSA binding. All SPR experiments were performed at room temperature and in duplicate.**