Supporting Information

**Reporter-recruiting bifunctional aptasensor for bioluminescent analytical assays**

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S1. Experimental procedures

Materials and reagents

Monitoring the course of selection by DiStRO assay
The diversity of the libraries during the selection was assessed by the DiStRO (Diversity Standard of Random Oligonucleotides) assay. Probes of 0.5 µM dsDNA, 1x SYBR Green I, 10 mM sodium cacodylate (pH 8.0), 100 mM NaCl, 20 µL in total, were taken in triplicate. The probes were run on the Rotor-Gene Q machine (Qiagen), using the following protocol: hybridization (95°C to 35°C, 0.5°C/min), hold at 35°C for 1 h, denaturation (35°C to 95°C, 0.5°C/min, recorded). Triplicates of raw melting curves were averaged, and the fluorescence of the control solution (w/o DNA) was subtracted. Resulting curves were plotted as fluorescence intensity (I) or its derivative (dI/dT) vs. temperature.

Electrophoretic mobility shift assay (EMSA)
The 5’-[32P]-labeled 2’-F-RNA probes (≥5 pmol) in 100 µL of DPBSM were incubated at 90°C for 5 min and then cooled down for 10 min at 25°C. Tween 20, BSA and polyA were added to the reaction mixture for final concentrations of 0.05 %, 0.01 % and 0.1 mg/mL, respectively. To start binding, 5 µL of the RNA solution were mixed with 5 µL of protein solution in DPBSM. After 40 min of incubation at 25°C, aliquots were withdrawn and analyzed by EMSA. Each binding assay was performed in triplicate.

An aliquot of the reaction mixture (7.5 µL) was mixed with 1.5 µL of the loading buffer (0.05 % of xylene cyanol FF and bromophenol blue dyes in 50 % aq. glycerol) and loaded onto the running non-denaturing 6 % polyacrylamide gel (48:1 acrylamide/bisacrylamide, 0.5× Tris-borate buffer). After separation, the gel was dried and exposed to the Bio-Rad phosphorimager screen overnight. The screen was scanned by the Molecular Imager FX.

To examine the affinities of RNA libraries in the presence of blocking oligodeoxyribonucleotides complementary to primer-binding sites, approximately 10-fold molar excess of the blocking oligonucleotide (50 pmol) was added to the 2’-F-RNA library before annealing. NA-protein complexes were then formed and analyzed as described above.

Solid-phase assays for binding of 2’-F-RNA aptamers with obelins
Before use, each aptamer was refolded by heating for 5 min at 90°C and cooling for 15 min at 25°C in 0.15 M NaCl, 50 mM K-Na phosphate buffer, pH 7.0 (PBS), 1 mM MgCl2. Then, Tween 20, BSA and polyA were added for final concentrations of 0.05 %, 0.01 % and 0.1 mg/mL, respectively.

The well surface of the white low volume stripwell microplate (Corning, USA) was activated with streptavidin (10 µg/mL in PBS, pH 7.5, 50 µL in each well, overnight at 4 °C) then washed ( thrice with PBS, pH 7.0, containing 0.1 % Tween 20, 1 mM MgCl2). Biotinylated 2’-F-RNA aptamer (30 nM) in the binding buffer (PBS pH 7.0, 1 mM MgCl2, 0.05 % Tween 20, 0.01 % BSA, 0.1 mg/mL polyA) or just binding buffer (control wells) was placed into the wells and incubated for 1 h at room temperature. After washing, 50 µL aliquots of His-
Obe or wt-Obe (360-0.16 nM in binding buffer) were incubated for 40 min at room temperature, and washed. Bioluminescence of surface-bound obelin was initiated by injection of 0.1 M CaCl$_2$ in 0.1 M Tris-HCl, pH 8.8 (50 μL) and measured with Mithras LB 940 plate luminometer (Berthold, Germany). The signal was integrated for 5 s. Signals from the control wells were subtracted from those obtained from the respective aptamer-containing wells.

**Solid-phase assays for optimizing of obelin concentration for assays with bi-functional aptamers**

An optimization of obelin concentration study was carried out using bi-functional 2'-F-RNA aptamer O79t1-L-H9t11. Before measurement, biotinylated derivative of O79t1-L-H9t11 aptamer was folded in DPBSM by heating at 90°C for 5 min and cooling down for 10 min at 25°C. Then Tween 20, BSA and E.coli tRNA were added as nonspecific competitors for final concentrations of 0.05 %, 0.01 %, and 0.1 %, respectively. The solution of biotinylated bi-functional 2'-F-RNA aptamer (25 nM, 50 μL) was added into streptavidin-activated wells (10 μg/mL in PBS, pH 7.5, 50 μL in each well, overnight at 4 °C) in binding buffer (DPBSM, 0.05 % Tween 20, 0.01 % BSA, 0.01 % E. coli tRNA) and incubated with shaking for 30 min at room temperature. Then, the wells were washed, and 50 μL aliquots of the mixed solution of human hemoglobin (final concentration from 100 to 1.6 nM) and His-Obe (final concentration of 300 or 100 nM) in binding buffer was added into the wells and incubated for 40 min at room temperature. Control wells contained aliquots of 300 or 100 nM His-Obe in binding buffer. After washing the wells, bioluminescence of bound obelin was initiated and measured as described above.

**S2. Selection of obelin-binding 2'-F-RNA aptamers**

The Ca$^{2+}$-regulated photoprotein obelin elongated from the N-terminus by the hexahistidine fragment (His-Obe) was used as a SELEX target, according to commonly used selection technique (see ref. 3 for a review). Previously 4, we described the preparation of His-Obe and demonstrated that it retains the main physicochemical characteristics of the wild-type obelin. According to numerous selection protocols for His-tagged proteins immobilized on Ni$^{2+}$ resins (e.g., refs. 5,6), at each SELEX round the positive selection on the protein-loaded resin is preceded by the negative selection step on the 'empty' one to discard the resin-binding aptamers. To minimize the non-specific binding with the resin and the protein target, we added the E.coli tRNA as a competitor from the first to the sixth round. Since purine-rich RNA sequences are affine to the immobilized nickel ions 7 we supposed that the presence of polyA would prevent a loss of such sequences due to resin binding. We used polyA as a competitor from 6$^{th}$ to 12$^{th}$ round. Moreover, after six rounds of selection the resin was replaced by Ni-sepharose magnetic beads to further minimize the possibility of selecting matrix-binding sequences. The selection pressure was progressively increased by decreasing the amount of protein and the time of incubation and adding more washes (Table S1).
| Round | 2’-F-RNA library, nmol | Binding buffer | Washing buffer | Negative selection | Positive selection |
|-------|------------------------|----------------|---------------|--------------------|--------------------|
| 1     | 1                      | DPBSE, 0.05 % Tween 20, 0.01 % BSA, 0.1 mg/ml *E.coli* tRNA | DPBSE | Ni sepharose (5 µl), 60 min | 130 pmol His-Obe on the Ni sepharose (5 µl) 60 min Washings: 3x150 µl |
| 2     | 1                      | DPBSE, 0.05 % Tween 20, 0.01 % BSA, 0.1 mg/ml *E.coli* tRNA | DPBSE, 0.05 % Tween 20 | Ni sepharose (5 µl), 60 min | 130 pmol His-Obe on the Ni sepharose (5 µl) 60 min Washings: 4x150 µl |
| 3     | 1                      | DPBSE, 0.05 % Tween 20, 0.01 % BSA, 0.1 mg/ml *E.coli* tRNA | DPBSE, 0.05 % Tween 20 | Ni sepharose (5 µl), 60 min | 130 pmol His-Obe on the Ni sepharose (5 µl) 45 min Washings: 5x150 µl |
| 4     | 0.8                    | DPBSE, 0.05 % Tween 20, 0.01 % BSA, 0.1 mg/ml *E.coli* tRNA | DPBSE, 0.05 % Tween 20 | Ni sepharose (5 µl), 60 min | 70 pmol His-Obe on the Ni sepharose (5 µl) 45 min, mixing Washings: 5x150 µl |
| 5     | 1                      | DPBSE, 0.05 % Tween 20, 0.01 % BSA, 0.1 mg/ml *E.coli* tRNA | DPBSE, 0.05 % Tween 20 | Ni sepharose (5 µl), 60 min | 70 pmol His-Obe on the Ni sepharose (5 µl) 30 min Washings: 5x150 µl |
| 6     | 1                      | DPBSE, 0.05 % Tween 20, 0.01 % BSA, 0.1 mg/ml *E.coli* tRNA | DPBSE, 0.05 % Tween 20 | Ni sepharose (5 µl), 60 min | 70 pmol His-Obe on the Ni sepharose (5 µl) 30 min Washings: 7x150 µl |
| 7     | 1                      | DPBSE, 0.05 % Tween 20, 0.01 % BSA, 0.1 mg/ml polyA | DPBSE, 0.05 % Tween 20 | Ni sepharose MB (10 µl), 60 min | 130 pmol His-Obe on the Ni sepharose MB (10 µl), 30 min Washings: 7x150 µl |
| 8     | 1                      | DPBSE, 0.05 % Tween 20, 0.01 % BSA, 0.1 mg/ml polyA | DPBSE, 0.05 % Tween 20 | Ni sepharose MB (10 µl), 60 min | 70 pmol His-Obe on the Ni sepharose MB (10 µl), 30 min Washings: 8x150 µl |
| 9     | 1                      | DPBSE, 0.05 % Tween 20, 0.01 % BSA, 0.1 mg/ml polyA | DPBSE, 0.05 % Tween 20 | Ni sepharose MB (10 µl), 60 min | 35 pmol His-Obe on the Ni sepharose MB (10 µl), 30 min Washings: 9x150 µl |
| 10    | 0.4                    | DPBSE, 0.05 % Tween 20, 0.01 % BSA, 0.1 mg/ml polyA | DPBSE, 0.05 % Tween 20 | Ni sepharose MB (10 µl), 60 min | 35 pmol His-Obe on the Ni sepharose MB (10 µl), 30 min Washings: 10x150 µl |
| 11    | 0.4                    | DPBSE, 0.05 % Tween 20, 0.01 % BSA, 0.1 mg/ml polyA | DPBSE, 0.05 % Tween 20, 0.5 M NaCl | Ni sepharose MB (5 µl), 60 min | 35 pmol His-Obe on the Ni sepharose MB (5 µl), 30 min Washings: 10x200 µl |
| 12    | 0.4                    | DPBSE, 0.05 % Tween 20, 0.01 % BSA, 0.1 mg/ml polyA | DPBSE, 0.05 % Tween 20, 0.5 M urea | Ni sepharose MB (5 µl), 60 min | 35 pmol His-Obe on the Ni- sepharose MB (5 µl), 30 min Washings: 10x200 µl |

The evolution after each round was estimated by analyzing RT-PCR products of negative and positive selection steps. To monitor the enrichment, we also assessed the diversities of the initial and enriched libraries by analyzing remelting profiles of dsDNA pools (DISTRO assay)\(^1\). The progressive increase in remelting temperature with the number of the round pointed to the loss of diversity, which corresponds to the enrichment of the library (Fig. S1).
To test if constant primer-binding sites of the enriched 2'-F-RNA library RNA10 participate in the target binding, we blocked them by complementary oligodeoxyribonucleotides. According to results of the EMSA, blocking of the 3'-constant region did not influence the binding of RNA10 to His-Obe while blocking of the 5'-constant region or both regions significantly decreased the amount of the RNA-protein complex (Fig. S2). Therefore, we concluded that the aptamers that are most abundant in the 2'-F-RNA library after the 10th round do not need the 3'-constant region for target binding.

Finally, we performed two additional SELEX rounds to remove possible weak binders by much more stringent washings after a positive selection step. Aside from larger volumes, the washing buffer was supplied with 0.5 M NaCl at round 11, or 0.5 M urea at the round 12, analogous to ref.6.
The enriched pools after the 6th, 10th, and 12th rounds were re-amplified with barcode-bearing primers and sequenced with Illumina NGS. The dynamics of abundances for most represented sequences in the enriched pools are shown in Fig. S3 and Table S2.

Table S2. The most represented 2'-F-RNA aptamer sequences in course of selection.

| Aptamer | Nucleotide sequence of the N_{40} region, 5'->3' |
|---------|-----------------------------------------------|
| O79     | ugugaagucgcauuuaauugcuggcgccgguuacuugcuc     |
| O3      | ugugaagucgcaucauagcuuggcgccuuacuugcuc*       |
| O1      | ugugaagucgcauuuaauugcuggcgccgguuuacuugcuc*   |
| O6      | gacgugccgggaaagaccgcacgcucuacccccauuucagcuu |
| O4      | cuagggcgugccgguccuauuacuucgccgccccucccuu    |
| O5      | guuguaacgcgguuugccauuuccgccgguuuacgguucc    |
| O11     | cagggugcuacacgauuauuacucacccccgccccggcc    |
| O80     | gacgugccgggaaagaccgcacgcucuacccccauuucagcuu |
| O15     | caggggugccuacauccgaauuaauucugcaccgccccggcc |
| O35     | uaggguacgcggacgcauuggaccgucggcagccggccc    |
| O257    | acagccccuugccuaacgcggccacggauacuucacgcuu    |
| O115    | guuggaauuaauacgcgucguuauacuccgacucccgcggg   |
| O13     | cagggugcuacacgauuuucguauucacgucgccgguugug  |
| O2      | ugggcuccaauuacgcagcauuuauuauugcgaacca       |
| O7      | aggggucgcuacccauuauuauuuauauggcgaacca       |
| O10     | agagaauugggcuucccagccuauuccauuuuuga         |
| O8      | ugggcuccaagggcuacccauuuuauuuuauauggcgaacca  |
| O9      | caggguaccuauuuuucguauuauuauuacggcagggc     |
| O12     | cagggccccacauaacuacacauuuuauuauauggcgaacca  |
| O14     | cagggugucaaucuacccauuuuauuauuauacuucgccccgcc |

* Nucleotides different from O79 are boldfaced and underlined

Figure S3. The dynamics of abundances for most represented sequences in the course of selection, calculated from mass sequencing data.
Almost half of the RNA10 library (44%) was represented by the aptamers O79, O3, and O1, which differ only by several point mutations in the random N40 region and possess similar secondary structures (Fig. S4).

Since O1 showed a progressive loss of abundance from round 6 to rounds 10 and 12, we expelled it from further studies. Aptamers O4 and O6 with entirely different sequences of the N40 region demonstrated an increase in abundance from 6th to the 10th round and represented a significant fraction in the RNA10. As mentioned above, blocking the 3'-constant region of RNA10 library did not influence its target binding properties. Therefore, we considered a possibility to delete this fragment from the aptamers that are most represented in the RNA10 (namely, O3, O4, O6, and O79). A comparison of predicted secondary structures for full-length aptamers and their truncated versions lacking the 3'-constant fragment showed that 3'-truncation does not significantly disturb the secondary structure, which is in a good agreement with the blocking experiment.

After last two SELEX rounds, O79 and O3 significantly lost their abundances, and the total fraction of O79+O3+O1 lowered from 44% to 13.3%, while O4 was dominant in the enriched pool RNA12. Moreover, O35, which represented a minor fraction in RNA6 and RNA10, gained 17.5% after the 12th round. We also took into account O5, which represented 8.8% of the enriched pool after ten rounds, and retained 7% after two final SELEX rounds with sharply increased stringency. All candidate aptamers were chemically synthesized and assessed for their binding affinity for the target protein.

S3. Examination of target binding for candidate aptamers

Candidate aptamers were tested for their binding affinity with His-Obe by the electrophoretic mobility shift assay. However, only O3t and O79t gave well-defined bands of aptamer-protein complexes (Fig. S4a and b), while for other candidates (O4t, O6t, O5, O35) the complexes were absent or registered as faint smeared bands (see, e.g., Fig. S4c). Thus, we proposed that these complexes are unstable during the native gel electrophoresis and used an alternative method to study the complex formation.

Figure S4. Binding of [32P]-labeled aptamers O3t (a), O79t (b) and O4t (c) to His-Obe examined by EMSA.

The DRaCALA technique is based on different mobilities of free nucleic acid, and NA-protein complexes in the probe spotted on the dry nitrocellulose. This method enables identifying the complexes that are unstable in the course of the gel electrophoresis. It is applicable for quantitative measurements of NA-
protein interaction and allows assessing binding affinity. To the best of our knowledge, DRaCALA has not been previously used for characterizing aptamer-protein complexes. Actually, with the DRaCALA technology, we registered in all cases aptamer-obelin complexes, clearly identified as the spots of smaller diameter inside the larger spots corresponding to the free aptamers (Fig. S5).

Fig. S5. Autoradiographs after DRaCALA assays of aptamer-protein complex formation for His-tagged and wild-type obelins.

Quantitative analysis of the obtained data allowed for calculation of equilibrium dissociation constants ($K_D$) (Table 1 in the main text). Since for the aptamers O4t, O5t, O6t and O35 the target binding is registered by DRaCALA but can hardly be seen by the EMSA, we suspected that it relies on electrostatic interactions between negatively charged aptamers and positively charged His$_6$-tag. We thus examined the binding of aptamers with the tag-free obelin wt-Obe by the DRaCALA technique (Fig. S7). The results showed that O3t, O79t and its untruncated version O79 bind to both obelins, and the values of $K_D$ depended to some extent on the type of the protein. In the case of wt-Obe, $K_D$ values increased 2-fold in $K_D$ for O79t and O79, and 5-fold for O3t. In contrast, aptamers O4t, O5, O6t, and O35 bound only to the His-tagged protein, and showed no complexes with the wtObe (Fig. S5). Since we aimed to develop aptamers that interact specifically with obelin itself and do not strictly need the His-tag for target binding, we considered O79t and O3t as most promising candidates for obelin-recruiting modules.

S4. Further truncation of O79t aptamer

Since O79t was intended to be part of a single oligonucleotide chain of a bi-modular aptamer, we found it quite reasonable to truncate it as much as possible to minimize the overall length of the construct. As mentioned above, the 3'-constant region of the aptamer is not obligatory for target binding, which was also proven by nearly the same binding affinities of O79t and the full-length parent aptamer O79 (Table S2). We
examined the possibility of further truncating O79t. For this purpose, two aptamer variants were tested: O79t1 (44 nt) and O79t2 (19 nt) (Fig. S6). In the aptamer O79t1, the longest stem was shortened to 6 bp (shown by the red line at the secondary structure, Fig. S6a), and two terminal AU pairs were replaced by GC pairs to stabilize the structure. The aptamer O79t2 represents the result of a complete deletion of the longest stem, together with an adjacent 6-bp hairpin (the site of truncation is shown by the blue line, Fig. S6a). The affinities of both aptamers were estimated in DRaCALA experiments with the wtObe. It was shown that O79t1 binds the target protein (Fig. S6b) with the KD value ~3-fold higher as compared to O79 (1.9±0.5 µM vs. 0.72±0.10 µM). A slight decrease in $K_D$ points to some destabilization of the aptamer’s structure after truncation. Meanwhile, the shortest O79t2 showed no binding affinity (Fig. S6b). We, therefore, concluded that the central part of the structure (6-bp stem and neighboring non-paired nucleotides) is obligatory for the target binding. As a result, we found the 44-nt O79t1 to be suitable as an obelin-recruiting module for bispecific constructs.

**Fig. S6.** Truncation studies of the aptamer O79t. (a) Predicted secondary structures for O79t, O79t1, O79t2. Sites of truncation are shown by red (O79t1) and blue (O79t2) lines. (b) Binding of the aptamers O79t1 and O79t2 to the wtObe: examples of DRaCALA spots and binding curve for O79t1.
A series of model experiments were carried out to study the binding of the candidate aptamers to obelin under conditions of the solid-phase bioluminescent microassay (Fig S7).

Biotinylated derivatives of the aptamers were immobilized on a streptavidin-activated surface, and bound obelin was detected by its bioluminescence, triggered by Ca\(^{2+}\). Each point in the Fig. S7 was obtained as signal differences between the sample and the corresponding control (a well without aptamer, but with the same amount of obelin introduced). Both target variants, His-Obe and wt-Obe, were examined in the concentration range of 0.49 – 120 nM. All aptamers (except O6) bound to His-Obe. Wild-type obelin was bound only by two of them: O79t in the concentration range of 13 –120 nM and O5 in the concentration range of 0.5–120 nM.

**Figure S7.** Solid-phase bioluminescent assay of aptamer–obelin binding. At the top: the assay scheme. Binding curves with His-Obe are shown in black, with wt-Obe – in red. Stavi – streptavidin; Bio - biotinyl, r.l.u. – relative light units.

Therefore, O79t binding ability is observed under conditions of both homogeneous (EMSA and DRaCALA technologies) and heterogeneous assays. Surprisingly, O5 which does not bound to wt-Obe during DRaCALA experiments showed a pronounced binding affinity in the heterogeneous bioluminescent assay.

We hypothesize that such a discrepancy originates from different formats of homogeneous (EMSA, DRaCALA) and heterogeneous plate assays. Similar differences between homogeneous and plate-based assays have been previously reported for protein A-specific aptamers\(^8\). Nevertheless, the obtained results also prove that the photoprotein retains its bioluminescent properties when bound with aptamers.
S6. Optimization of obelin concentration for solid-phase bioluminescent assays with bi-functional 2'-F-RNA aptamers

To reveal the optimal concentration of free obelin for the aptasensing systems based on bi-functional aptamers, we tested 100 nM and 300 nM His-Obe in the assay with bi-functional 2'-F-RNA aptamer O79t1-L-H9t11. Biotinylated aptamer was immobilized in the microplate wells, then the mixture of human hemoglobin (final concentration from 100 to 1.6 nM) and His-Obe (constant concentration 100 nM or 300 nM) was added, followed by the measurement of Ca^{2+}-triggered bioluminescence. The obtained results (Fig. S8) evidence that both obelin concentrations provide sufficiently high, concentration-dependent bioluminescent signal, but 100 nM obelin gives lower background signal and smaller deviation. Therefore, this concentration was chosen for the subsequent bioluminescent assays with bi-functional aptamers.

Figure S8. The solid-phase bioluminescent assay for human hemoglobin with bi-specific aptamer Apt79t1-L-H9t11 at simultaneous addition of obelin (300 nM (-□-) or 100 nM (-○-)) and hemoglobin (1.56-100 nM). Red symbols show the signals from hemoglobin-free (control) samples. Each point is the average ± standard deviation, n=3.
REFERENCES

1. T. Schütze, P. F. Arndt, M. Menger, A. Wochner, M. Vingron, V. A. Erdmann, H. Lehrach, C. Kaps and J. Glökler, *Nucleic Acids Res.*, 2009, **38**, e23.

2. G. P. Donaldson, K. G. Roelofs, Y. Luo, H. O. Sintim and V. T. Lee, *Nucleic Acids Res.*, 2012, **40**, e48.

3. T. K. Sharma, J. G. Bruno and A. Dhiman, *Biotechnol. Adv.*, 2017, **35**, 275–301.

4. V. V. Krasitskaya, A. S. Davydova, M. A. Vorobjeva and L. A. Frank, *Russ. J. Bioorganic Chem.*, 2018, **44**, 296–301.

5. M. B. Murphy, S. T. Fuller, P. M. Richardson and S. a Doyle, *Nucleic Acids Res.*, 2003, **31**, e110.

6. P. Bouvet, *Methods Mol Biol.*, 2009, **543**, 139–150.

7. B. Nastasijevic, N. A. Becker, S. E. Wurster and L. James Maher, *Biochem. Biophys. Res. Commun.*, 2008, **366**, 420–425.

8. R. Stoltenburg, P. Krafčiková, V. Víglaský and B. Strehlitz, *Sci. Rep.*, 2016, **6**, 33812.