Support information

Nanomolar fluorescent detection of c-di-GMP using a modular aptamer strategy

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Experimental section

Preparation of RNA sensors

The sequences of SP_1-6 Vc2 including a 5' T7 promoter sequence were cloned into pCU57 vector (Genscripts). The double stranded DNA templates for SP_1-6 Vc2 were created by PCR amplification. PCR products were then purified by agarose gel and used as templates for *in vitro* T7 transcription reaction. The crude RNAs were purified by denaturing PAGE.

General preparation: c-di-GMP and DFHBI concentrations were determined via UV absorbance measurements (c-di-GMP at 253 nm and DFHBI at 405 nm) and 28,600 $M^{-1}cm^{-1}$ (c-di-GMP), 11,864 $M^{-1}cm^{-1}$ (DFHBI) were used as extinction coefficients to calculate concentrations.[1, 2] RNA in buffer solution was heated to 80 ^{0}C for 5 min and cooled down to room temperature in 15 min. Then $MgCl_2$ and c-di-GMP were added and the sample was kept for 12 h at room temperature.

Fluorescence measurement: DFHBI was added to the sample and fluorescence was monitored by excitation at 496 nm and emission at 501 nm.

Table S1. RNA Sequences that were used in this study.

Name of RNA	**Sequence**
SP_1 Vc2	5'-GAC GCG ACU GAA UGA AAU GGU GAA GGA CGG GUC CAG AC GCA CAG GGC AAA CCA UUC GAA AGA GUG GGA CGC AAA GCC UCC GGC CUA AAC UUC GGU AGG UAG CGG GGU UAC C GAU CUU GUU GAG UAG AGU GUG AGC UCC GUA ACU AGU CGC GUC-3'
SP_2 Vc2	5'-GAC GCG ACU GAA UGA AAU GGU GAA GGA CGG GUC CAG GC C GCA CAG GGC AAA CCA UUC GAA AGA GUG GGA CGC AAA GCC UCC GGC

	CUA AAC UUC GGU AGG UAG CGG GGU UAC CGA GC CUU GUU GAG UAG AGU GUG AGC UCC GUA ACU AGU CGC GUC -3'
SP_3 Vc2	5'-GAC GCG ACU GAA UGA AAU GGU GAA GGA CGG GUC CAG C GCA CAG GGC AAA CCA UUC GAA AGA GUG GGA CGC AAA GCC UCC GGC CUA AAC UUC GGU AGG UAG CGG GGU UAC C G CUU GUU GAG UAG AGU GUG AGC UCC GUA ACU AGU CGC GUC -3'
SP_4 Vc2	5'-GAC GCG ACU GAA UGA AAU GGU GAA GGA CGG GUC CAG CUC GCA CAG GGC AAA CCA UUC GAA AGA GUG GGA CGC AAA GCC UCC GGC CUA AAC UUC GGU AGG UAG CGG GGU UAC C GAG CUU GUU GAG UAG AGU GUG AGC UCC GUA ACU AGU CGC GUC -3'
SP_5 Vc2	5'-GAC GCG ACU GAA UGA AAU GGU GAA GGA CGG GUC CAG UCG C GCA CAG GGC AAA CCA UUC GAA AGA GUG GGA CGC AAA GCC UCC GGC CUA AAC UUC GGU AGG UAG CGG GGU UAC CG UGG CUU GUU GAG UAG AGU GUG AGC UCC GUA ACU AGU CGC GUC -3'
SP_6 Vc2	5'-GAC GCG ACU GAA UGA AAU GGU GAA GGA CGG GUC CAG CU C GCA CAG GGC AAA CCA UUC GAA AGA GUG GGA CGC AAA GCC UCC GGC CUA AAC UUC GGU AGG UAG CGG GGU UAC CG GG CUU GUU GAG UAG AGU GUG AGC UCC GUA ACU AGU CGC GUC -3'

Sequences in black (not underlined) are found in the Spinach module (see Fig. 1, main text) and underlined sequences are in stem loops 1 and 3. Sequences in pink make up the c-di-GMP recognition module and green color represents stem loop 2, which is the transducer module.

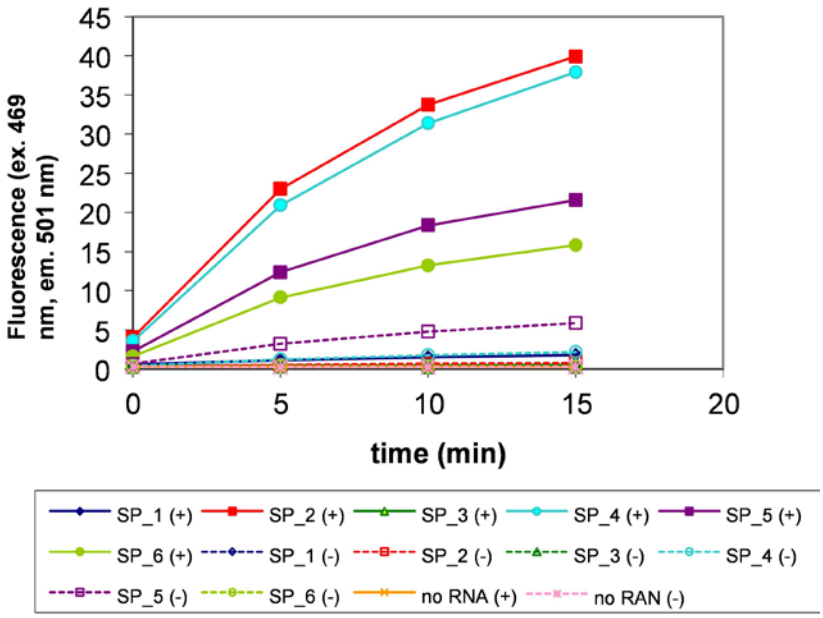

Fig. S1 Fluorescence response of various spinach-Vc2 fusion RNAs. *Conditions*: [c-di-GMP] = 10 µM, [RNA] = 1 µM, [DFHBI] =10 µM (0.05 % DMSO), Buffer: 10 mM Na-cacodylate (pH 6.8), 10 mM KCl, 6 mM $MgCl_2$. *Procedure:* RNA was heated up to 80 °C and kept at 80 °C for

5 min without $MgCl_2$. Then $MgCl_2$ and c-di-GMP were added and cooled to room temperature (24°C) over ~10 min. The mixture was then left to stand at 24 °C for 12 h. DFHBI was then added and fluorescence was monitored (Ex. 469 nm, Em. 501 nm) at 24 °C.

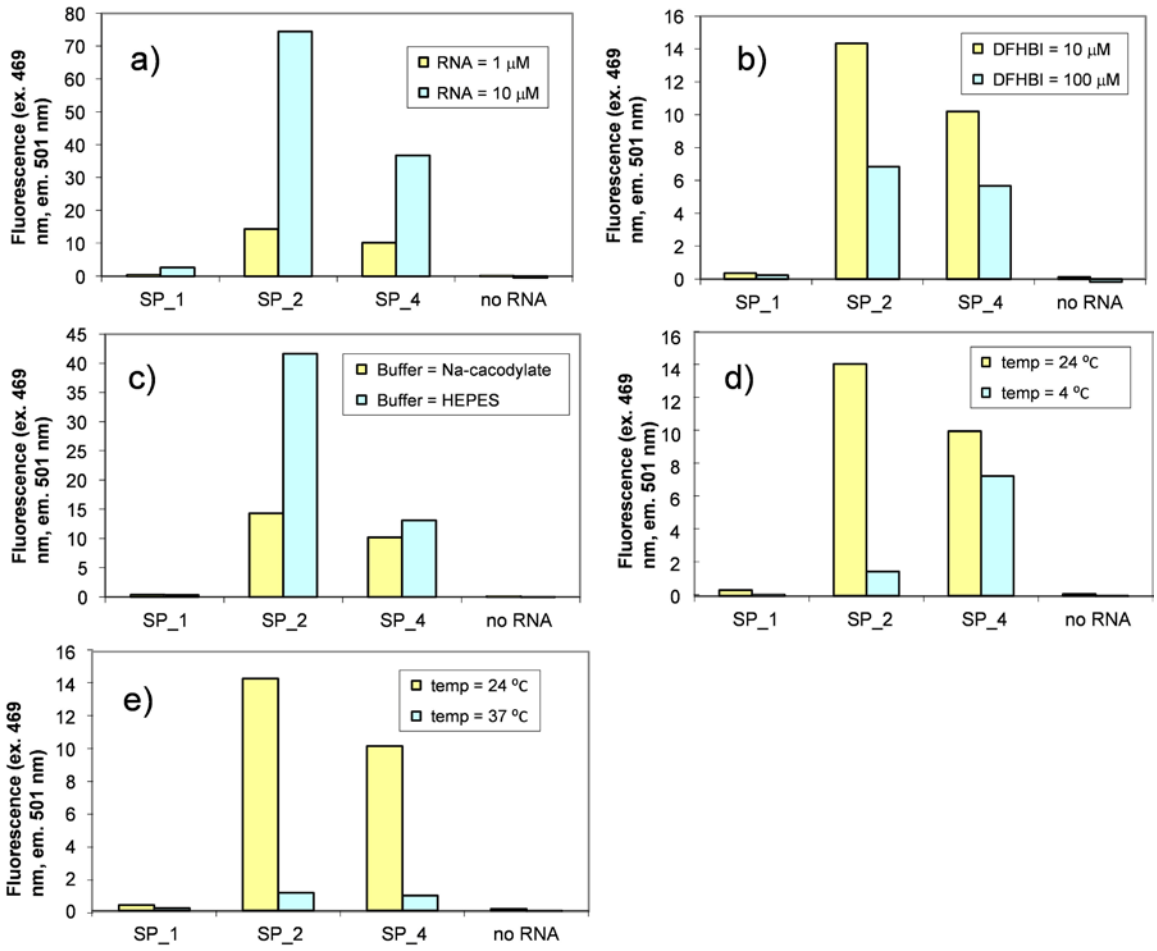

Fig. S2 Optimization assays to determine the best sensor and assay conditions. a) Different RNA concentrations (1 μM or 10 μM); b) Different DFHBI concentrations (10 μM or 100 μM); c) Testing different buffers; d) Investigating different temperatures for c-di-GMP incubation; e) Investigating different temperatures for DFHBI incubation. *General conditions*: [c-di-GMP] = 10 μM. Buffer: 10 mM Na-cacodylate (pH 6.8), 10 mM KCl, 6 mM $MgCl_2$ or 100 mM HEPES, 100 mM KCl, 100 mM NaCl. 6 mM $MgCl_2$ (pH 6.8). *Incubation process:* RNA was heated up at 80 °C for 5 min without $MgCl_2$. Then $MgCl_2$ and c-di-GMP were added. The mixture was then incubated at 24 °C for 12 h. After DFHBI was added, fluorescence was monitored (Ex. 469 nm, Em. 501 nm) at 24 °C immediately.

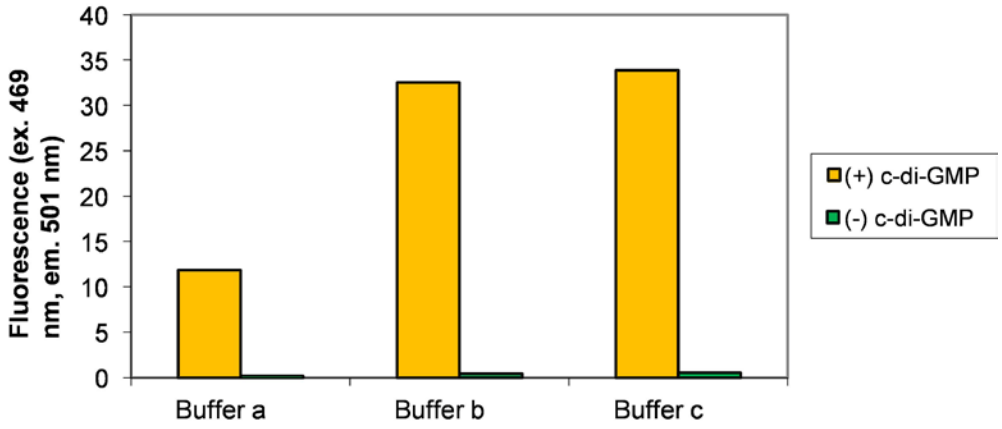

Fig. S3 Determination of best monovalent cation concentrations for assay.
Buffer a: 10 mM Na-cacodylate (pH 6.8) 10 mM KCl, 6 mM $MgCl_2$
Buffer b: 100 mM HEPES (pH 6.8), 100 mM NaCl, 100 mM KCl, 6 mM $MgCl_2$
Buffer c: 10 mM Na-cacodylate (pH 6.8), 90 mM NaCl, 100 mM KCl, 6 mM $MgCl_2$
General conditions: [c-di-GMP] = 10 µM, [RNA; SP_2 Vc2] = 1 µM, [DFHBI] = 10 µM (0.05 % DMSO),
Incubation process: Same as in Figures S1 and S2.

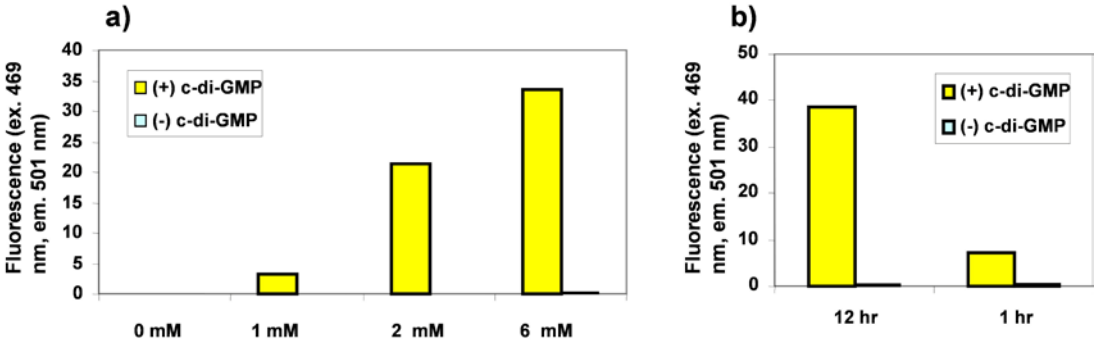

Fig. S4 a) Determination of the ideal Mg^{2+} concentration for the assay: 0-6 mM $MgCl_2$ concentration, b) Incubation time with c-di-GMP. *Conditions*: [c-di-GMP] 10 µM, [RNA; SP_2 Vc2] 1 µM, [DFHBI] 10 µM (0.05 % DMSO), Buffer: 100 mM HEPES (pH 6.8) containing 100 mM KCl, 100 mM NaCl, 6 mM $MgCl_2$. *Incubation process:* Same as Fig S1.

b) Determination of best incubation time for c-di-GMP.

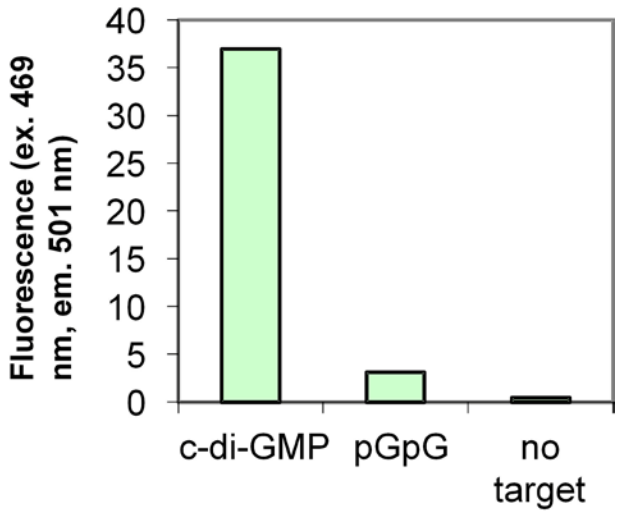

Fig. S5 Selectivity between pGpG and c-di-GMP. *Conditions*: [c-di-GMP or pGpG] = 10 µM, [RNA; SP_2 Vc2] = 10 µM, [DFHBI] = 10 µM (0.05 % DMSO). Buffer: 100 mM HEPES (pH 6.8) , 100 mM KCl, 100 mM NaCl, 6 mM $MgCl_2$. *Incubation process:* Same as Fig S1.

1. M. Gentner, M. G. Allan, F. Zaehringer, T. Schirmer and S. Grzesiek, *J. Am. Chem. Soc*, 2012, **134**, 1019-1029.
2. J. S. Paige, K. Y. Wu and S. R. Jaffrey, *Science*, 2011, **333**, 642-646.