

In the format provided by the authors and unedited.

# Acoustic biosensors for ultrasound imaging of enzyme activity

Anupama Lakshmanan<sup>1,5</sup>, Zhiyang Jin<sup>2,5</sup>, Suchita P. Nety<sup>1</sup>, Daniel P. Sawyer<sup>3</sup>,  
Audrey Lee-Gosselin<sup>1</sup>, Dina Malounda<sup>1</sup>, Mararet B. Swift<sup>1</sup>, David Maresca<sup>1,4</sup> and Mikhail G. Shapiro

 <sup>1</sup> 

---

<sup>1</sup>Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA, USA. <sup>2</sup>Division of Engineering and Applied Sciences, California Institute of Technology, Pasadena, CA, USA. <sup>3</sup>Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA, USA. <sup>4</sup>Present address: Department of Imaging Physics, Delft University of Technology, Delft, Netherlands. <sup>5</sup>These authors contributed equally: Anupama Lakshmanan, Zhiyang Jin. ✉e-mail: [mikhail@caltech.edu](mailto:mikhail@caltech.edu)

## SUPPLEMENTARY INFORMATION

### Acoustic Biosensors for Ultrasound Imaging of Enzyme Activity

Anupama Lakshmanan, Zhiyang Jin, Suchita P. Nety, Daniel P. Sawyer, Audrey Lee-Gosselin, Dina Malounda, Margaret B. Swift, David Maresca and Mikhail G. Shapiro.

Correspondence to: mikhail@caltech.edu

## CONTENTS

Supplementary Table 1  
References for Supplementary Information

Plasmid Name	Description and Purpose	Transcriptional regulators	Output gene product(s)	Insertions/Tags (including linkers)	Reference Information
WT C-His Ana GvpC in pET28a	Ana <i>gvpC</i> used as wild-type control for TEV and calpain sensor	pT7, LacO	WT C-His Ana GvpC	SLE-His6 at C-terminus	Addgene ID# 85732
WT N-His Ana GvpC in pET28a	Ana <i>gvpC</i> used as wild-type control for ClpXP sensor	pT7, LacO	WT N-His-Ana-GvpC	G-His6-SG at N-terminus	Addgene ID# 153294
C-His-GvpC-TEV	Ana <i>gvpC</i> with TEV cleavage site	pT7, LacO	C-His Ana GvpC with TEV cleavage site	SLE-His6 at C-terminus, GSGSGS-ENLYFQG-SGSGSG in GvpC repeat 2	Addgene ID# 153296
C-His-GvpC-Calpain	Ana <i>gvpC</i> with calpain cleavage site	pT7, LacO	C-His Ana GvpC with calpain cleavage site	SLE-His6 at C-terminus, GSGSG-QQEVYGMMPR D-GSGSG in GvpC repeat 2	Addgene ID# 153295
N-His-GvpC-ssrA	Ana <i>gvpC</i> with ssrA degradation tag	pT7, LacO	N-His Ana GvpC with ssrA degradation tag	G-His6-SG at N-terminus, SG-AANDENYALAA at C-terminus	Addgene ID# 153297
pBEST_OR2_OR1-Pr-UTR1_ClpP-T500	<i>clpP</i> plasmid for use in the cell-free TX-TL system	OR2-OR1-Pr	ClpP		Addgene ID# 153302
pACYC-FLAG-dN6-His	<i>clpX</i> plasmid for use in the cell-free TX-TL system	pT7, LacO	Flag-ClpXdeltaNLinkedHexamer-His6	Flag tag at N-terminus His6 at C-terminus L20 linkers	Addgene ID# 22143
pET28a_T5-ARG1	Original acoustic reporter gene construct ( <i>ARG<sub>WT</sub></i> )	pT5, LacO	Ana GvpA, WT Ana GvpC, Mega GvpR-U		Addgene ID # 106476

pET28a-T5-ASG_ClpXP	Acoustic sensor gene for ClpXP ( <i>ASG<sub>ClpXP</sub></i> )	pT5, LacO	Ana GvpA, dGvpC, Mega GvpR-U	SG-AANDENYALAA at C-terminus	Addgene ID# 153299
pKD3	Frt-flanked cat cassette for recombineering		CAT		Addgene ID # 45604
pKD46	Plasmid that carries the Lambda Red recombineering system	pBAD, araBAD operon	Gam, Beta, Exo		Coli Genetic Stock Center
pE-FLP	<i>FLP</i> recombinase to remove the integration module in recombineering	pE	FLP		Addgene ID # 45978
araBAD-BCD20-ClpP-BCD17-ClpX	Expression of <i>clpX</i> and <i>clpP</i> under araBAD promoter	pBAD, araBAD operon	ClpX, ClpP		Addgene ID # 153301
pTetR-BCD2-Ana GvpC	Wild-type Ana <i>gvpC</i> under Tet promoter	pTet, TetO	WT Ana GvpC		Addgene ID # 153298

### Supplementary Table 1: List and features of genetic constructs used in this study.

pKD3<sup>1</sup> was a gift from Barry L. Wanner (Addgene plasmid # 45604 ; <http://n2t.net/addgene:45604> ; RRID:Addgene\_45604). pKD46<sup>1</sup> was obtained from the Coli Genetic Stock Center (CGSC, <https://cgsc.biology.yale.edu/Site.php?ID=64672>).

pE-FLP<sup>2</sup> was a gift from Drew Endy & Keith Shearwin (Addgene plasmid # 45978; <http://n2t.net/addgene:45978> ; RRID:Addgene\_45978).

The pBEST\_OR2-OR1-Pr-UTR1\_ClpP-T500 was a gift from Zachary Sun and Richard Murray<sup>3</sup>.

pACYC-FLAG-dN6-His<sup>4</sup> was a gift from Robert Sauer (Addgene plasmid # 22143 ; <http://n2t.net/addgene:22143> ; RRID:Addgene\_22143)

The pTARA backbone was modified to make the araBAD-BCD20-ClpP-BCD17-ClpX and pTetR-BCD2-Ana GvpC constructs. pTARA<sup>5</sup> was a gift from Kathleen Matthews (Addgene plasmid # 31491 ; <http://n2t.net/addgene:31491> ; RRID:Addgene\_31491).

### REFERENCES FOR SUPPLEMENTARY INFORMATION:

1. Datsenko, K.A. & Wanner, B.L. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proceedings of the National Academy of Sciences* **97**, 6640-6645 (2000).
2. St-Pierre, F. et al. One-step cloning and chromosomal integration of DNA. *ACS Synthetic Biology* **2**, 537-541 (2013).
3. Zachary, Z.S., Jongmin, K., Vipul, S. & Richard, M.M. Protein degradation in a TX-TL cell-free expression system using ClpXP protease. *bioRxiv* (2015).
4. Martin, A., Baker, T.A. & Sauer, R.T. Rebuilt AAA + motors reveal operating principles for ATP-fuelled machines. *Nature* **437**, 1115-1120 (2005).
5. Wycuff, D.R. & Matthews, K.S. Generation of an AraC-araBAD promoter-regulated T7 expression system. *Analytical Biochemistry* **277**, 67-73 (2000).