1	IVT cell-free biosensors for tetracyclines and macrolides detection based on
2	allosteric transcription factors (aTFs)
3	
4	Huaixiu Bi <sup>a,b,#</sup> , Chen Zhao <sup>b, #</sup> , Yongkang Zhang <sup>b</sup> , Xi Zhang <sup>b</sup> , Bin Xue <sup>b</sup> , Chenyu Li <sup>b</sup> ,
5	Shang Wang <sup>b</sup> , Xiaobo Yang <sup>b</sup> , Chao Li <sup>b</sup> , Zhigang Qiu <sup>b</sup> , Jingfeng Wang <sup>b,*</sup> , Zhiqiang
6	Shen <sup>a,b,*</sup>
7	
8	<sup>a</sup> College of Food Science and Technology, Shanghai Ocean University, Shanghai
9	201306, China
10	
11	<sup>b</sup> Department of hygienic toxicology and environmental hygiene, Tianjin Institute of
12	Environmental and Operational Medicine, Tianjin 300050, China
13	
14	* Corresponding authors.
15	E-mail addresses: jingfengwang@hotmail.com; tianjinszq922@sohu.com
16	
17	<sup>#</sup> These authors contributed equally to this work.
18	

- 19 **Table S1. DNA Sequences**
- 20 Table S2. Preparations of 6.5% nondenaturing polyacrylamide gel
- 21 Table S3. Chemical components and concentration of NASBA
- 22 Table S4. Consistency and stability of the aTFs-based biosensors
- 23 Figure S1. Effect of pH on IVT cell-free biosensor
- 24 Figure S2. Arbitrary fluorescence values were converted to micromolar
- 25 equivalent fluorescein
- 26

Label	Sequence	Purpose		
		DNA template		
F	gcggataacaatttcacacaggaaacagc	amplification forward		
		primer		
		DNA template		
R	caaaaaacccctcaagacccg	amplification reverse		
		primer		
		DNA template		
Distin E	Biotin-	amplification forward		
Bloun-F	gcggataacaatttcacacaggaaacagc	primer for BIAcore		
		/EMSA		
		DNA template		
Biotin P	Riotin casasaccectesagacceg	amplification reverse		
Blottii-K	Biomi-caaaaaccecicaagaeeeg	primer for		
		EMSA/BIAcore		
Sense primers	ttaagattatgctgagtgatatccccccacatac	For NASBA		
Sense primers	acatggcaaga	TOTIMODA		
Antisense primers	cccacatactctgatgatcc	For NASBA		
	gcggataacaatttcacacaggaaacagctat			
	gaccatgattacgccaagcttgcatgcctgca			
	ggtcgactctagataatacgactcactatagga			
T7-tetO-3WJdB-	ggtccctatcagtgatagagacccacatactct	For tetracyclines detection		
Т	gatgatecgagacggtegggtecagatatteg			
	tatctgtcgagtagagtgtgggctcggatcatt			
	catggcaagagacggtcgggtccagatattcg			
	tatctgtcgagtagagtgtgggctcttgccatgt			

\_\_\_\_\_

	gtatgtgggtagcataaccccttggggcctcta	
	aacgggtcttgaggggttttttg	
	gcggataacaatttcacacaggaaacagctat	
	gaccatgattacgccaagcttgcatgcctgca	
	ggtcgactctagataatacgactcactatagga	
	gggaatataaccgacgtgactgttacatttagg	
T7 mphO	tggcccacatactctgatgatccgagacggtc	
	gggtccagatattcgtatctgtcgagtagagtg	For macrolides detection
3 W JUD-1	tgggctcggatcattcatggcaagagacggtc	
	gggtccagatattcgtatctgtcgagtagagtg	
	tgggctcttgccatgtgtatgtgggtagcataa	
	ccccttggggcctctaaacgggtcttgagggg	
	tttttg	



## Table S2. Preparation of electrophoresis gel of EMSA

Reagent	Stock Concentration	Volume
TBE Buffer (10×)	45mM	1mL
30% Acr-Bis	0.5µM	2.2 mL
Glycerol	80% (v/w)	80µL
Ammonium persulfate	10% (v/w)	90µL
TEMED	100 mM	10µL
ddH <sub>2</sub> O		6.62mL

.

## Table S3. Chemical components and concentration of NASBA

	Reagent IVT reaction solution sense and antisense primers RNase inhibit NTPs		Stock Concentration - 10 μM 40 (U/μl) 100 mM		$\mathbf{V}$	<b>Volume</b> 20 μL 0.4 μL	
]					2		
					0		
					0.5 μL Each 0.4 μL		
	d NTPs Mix		10mM each		2 µL		
		65	°C, 5 min, af	er 37 °C, 5 m	nin		
	AMV	V	10 0	J/μL		1 μL	
	RNase	H	5 U/µL		0	.1 μL	
	T7 RN	AP	200	U/µL	0.	25 μL	
7							
8							
-							
9							
9							
9 0 1							
9 0 1 2	Table S4.	Consistency a	and stability	of the aTFs-l	based biosens	ors. (n=3)	
9 0 1 2 Somplo	Table S4. G	Consistency a	and stability	of the aTFs-) Batcha 3	based biosens	ors. (n=3)	RSI
9 0 1 2 Sample	Table S4. ( Spiked (µM)	Consistency a Batche 1	and stability Batche 2	of the aTFs-l Batche 3	based biosens Batche 4	ors. (n=3) Batche 5	<b>RSI</b> (%)
9 0 1 2 Sample .nhydrot	Table S4. 0 Spiked (μM) 1	Consistency a Batche 1 0.93±0.05	and stability Batche 2 1.04±0.08	of the aTFs-1 Batche 3 0.90±0.03	based biosens Batche 4 0.95±0. 09	ors. (n=3) Batche 5 0.95±0.09	<b>RSI</b> (%) 5.60
9 0 1 2 Sample anhydrot tracyclin	Table S4.   Spiked   (μM)   1   5	Consistency a Batche 1 0.93±0.05 4. 61±0.31	<b>Batche 2</b> 1.04±0.08 4.51±0.28	of the aTFs-) Batche 3 0.90±0.03 4.16±0.39	based biosens Batche 4 0.95±0. 09 4.37±0.17	ors. (n=3) Batche 5 0.95±0.09 4.39±0.22	<b>RSI</b> (%) 5.60 3.84
9 0 1 2 Sample Inhydrot tracyclin	<b>Table S4.</b> <b>Spiked</b> (μΜ) 1 5 10	Consistency a Batche 1 0.93±0.05 4. 61±0.31 8.10±0.81	and stability Batche 2 1.04±0.08 4.51±0.28 8.80±0.92	of the aTFs-I Batche 3 0.90±0.03 4.16±0.39 9.17±0.91	based biosens Batche 4 0.95±0.09 4.37±0.17 8.71±0.71	ors. (n=3) Batche 5 0.95±0.09 4.39±0.22 8.85±0.93	<b>RSI</b> (%) 5.60 3.84 4.46

4.47±0.17 4.37±0.51

 $8.89 \pm 0.49$ 

 $4.15 \pm 0.15$ 

 $8.85 \pm 0.36$ 

 $4.32 \pm 0.47$ 

 $8.76 \pm 0.20$ 

6.58

6.58

43 **\*RSD (%)** means relative standard deviation between 5 batches .

 $8.58 \pm 0.49$ 

4.93±0.17

10.07±0.20

ycin in

milk

5

10





46 **Figure S1. Effect of pH on IVT cell-free biosensor.** MEF of biosensor at 120 min

47 under different pH of IVT without aTFs. Error bars are means and SDs from three

48 independent repeats.





49



<sup>51</sup> equivalent fluorescein (MEF). Fluorescence of different concentrations of FITC was

- 52 measured at excitation and emission wavelengths of 472 and 507 nm by the plate
- 53 reader. Error bars are SDs from nine independent repeats.