Rapid Identification of Bacterial Biofilms and Biofilm Wound Models Using a Multichannel Nanosensor

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Supporting Information

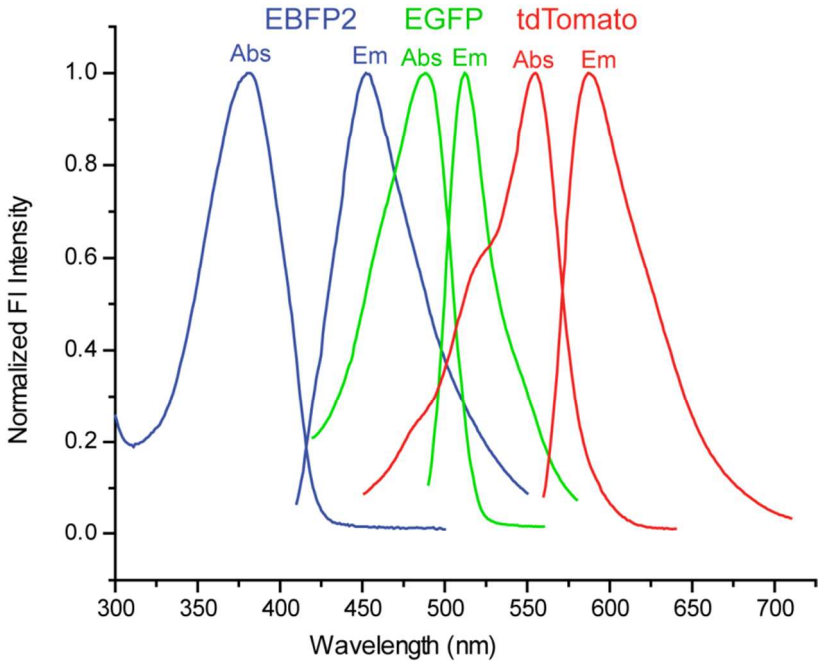

Fig. S1 . The absorption and emission spectra of the three fluorescent proteins.

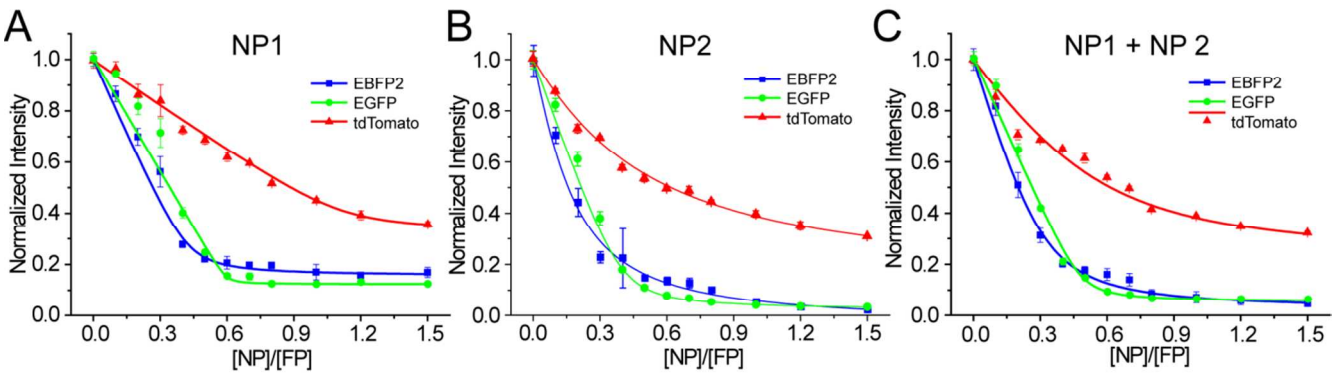

Fig. S2. Fluorescence titration of an equal-molar mixture of the three FPs by *(A)* NP1; *(B)* NP2; *(C)* the equal-molar mixture of NP1 and NP2, and the [NP] is the total concentration of NP1 and NP2. Each value is an average of three data points, and the error bars are standard deviations.

Table S1**.** The binding parameters derived from the fitting of the fluorescence titration data.

AuNP	Protein	Binding constant (K_s), M^{-1}	Association stoichiometry (n)	R^2
NP1	EBFP2	$(4.18\pm3.15)\times10^8$	2.34±0.22	0.9822
	EGFP	$(8.03\pm2.09)\times10^9$	1.68±0.08	0.9837
	tdTomato	$(5.52\pm5.24)\times10^8$	0.89±0.03	0.9830
NP2	EBFP2	$(1.54\pm1.10)\times10^7$	6.86±3.34	0.9727
	EGFP	$(2.86\pm0.50)\times10^8$	2.52±0.08	0.9938
	tdTomato	$(6.44\pm6.66)\times10^6$	4.20±2.82	0.9948
NP1+NP2	EBFP2	$(7.77\pm3.73)\times10^7$	3.18±0.49	0.9856
	EGFP	$(7.86\pm2.43)\times10^8$	2.08±0.06	0.9897
	tdTomato	$(3.31\pm2.62)\times10^7$	1.88±0.41	0.9766

Table S2. The jackknifed classification accuracy based on the parts of the triple-channel fluorescence (R: tdTomato, G: EGFP, and B: EBFP2).

Channel	Accuracy	Channel	Accuracy
R	61%	R+B	78%
G	90%	R+G	90%
B	66%	G+**B**	93%
R+G+**B**	100%		

Table S3. Identification of 24 unknown biofilm samples.

#	I/I_0			Identification	Verification
	Blue	Green	Red		
1	4.151	1.734	1.119	A.azu	A.azu
2	12.435	4.817	1.370	B.mega	B.mega
3	1.485	1.088	1.134	CD-3	CD-3
4	5.091	1.924	1.318	DH5α	DH5α
5	18.641	6.802	1.523	B.liche	B.liche
6	20.647	8.973	1.507	P.aeru	P.aeru
7	17.847	8.422	1.487	P.aeru	P.aeru
8	1.576	1.120	1.165	CD-3	CD-3
9	14.836	5.475	1.466	B.mega	B.mega
10	4.605	1.878	1.279	A.azu	A.azu
11	5.406	1.978	1.338	DH5α	DH5α
12	18.279	6.946	1.589	B.liche	B.liche
13	4.381	1.895	1.250	A.azu	A.azu
14	1.880	1.194	1.142	CD-3	CD-3
15	6.066	2.262	1.324	DH5α	DH5α
16	20.273	7.530	1.568	B.liche	B.liche
17	19.794	9.111	1.370	P.aeru	P.aeru
18	16.566	6.466	1.465	B.liche	B.mega
19	7.353	2.720	1.431	DH5α	DH5α
20	17.404	7.041	1.663	B.liche	B.liche
21	14.372	5.405	1.422	B.mega	B.mega
22	4.690	1.699	1.187	A.azu	A.azu
23	2.055	1.129	1.171	CD-3	CD-3
24	25.131	11.287	1.560	P.aeru	P.aeru

Table S4. Identification of 12 unknown co-culture samples.

#	I/I_0			Identification	Verification
	Blue	Green	Red		
1	1.77	3.54	1.74	Cell	B.liche
2	1.47	1.47	1.51	E.coli	E.coli
3	1.69	3.63	1.71	Cell	Cell
4	1.68	3.72	1.72	Cell	Cell
5	1.53	1.44	1.51	E.coli	E.coli
6	1.63	2.99	1.62	B.liche	B.liche
7	1.75	3.74	1.76	Cell	Cell
8	1.45	1.20	1.47	E.coli	E.coli
9	1.74	3.20	1.69	B.liche	B.liche
10	1.72	3.35	1.70	B.liche	B.liche
11	1.75	3.92	1.69	Cell	Cell
12	1.62	1.87	1.57	B.liche	E.coli

Table S5. The fluorescence response ratio of I/I_0 in the presence of biofilms. The sensing agent is made of 200 nM each of the three fluorescent proteins, 80 nM NP1 and 80 nM NP2 in 5 mM sodium phosphate buffer (pH=7.4).

Biofilms	Blue (EBFP2)	Green (EGFP)	Red (tdTomato)
A.azu	4.328	1.857	1.131
A.azu	4.459	1.816	1.220
A.azu	3.825	1.757	1.161
A.azu	3.962	1.751	1.245
A.azu	4.612	1.957	1.291
A.azu	4.716	1.923	1.280
B.liche	18.612	6.805	1.658
B.liche	18.579	7.218	1.402
B.liche	16.082	6.942	1.536
B.liche	18.607	6.907	1.502
B.liche	17.820	7.468	1.573
B.liche	17.372	6.517	1.662
B.mega	14.634	5.435	1.437
B.mega	15.153	5.650	1.395
B.mega	13.814	5.234	1.377
B.mega	13.918	5.168	1.442
B.mega	12.798	4.658	1.455
B.mega	16.077	5.789	1.474
DH5α	5.590	1.950	1.342
DH5α	5.579	2.068	1.292
DH5α	5.372	1.995	1.313
DH5α	5.792	2.170	1.338
DH5α	5.273	2.002	1.331
DH5α	6.634	2.321	1.348
CD-3	1.464	1.167	1.171
CD-3	0.820	1.052	1.110
CD-3	0.989	1.054	1.115
CD-3	1.568	1.125	1.159
CD-3	1.426	1.141	1.197
CD-3	1.541	1.157	1.201
P.aeru	16.557	8.234	1.497
P.aeru	19.344	9.458	1.426
P.aeru	19.126	9.204	1.584
P.aeru	17.290	8.389	1.486
P.aeru	18.109	8.699	1.577
P.aeru	21.109	9.979	1.600
Media control	1.306	1.058	1.069
Media control	1.536	1.018	1.054
Media control	1.169	1.028	1.080
Media control	1.699	1.033	1.063
Media control	1.426	1.067	1.069

Table S6. The fluorescence response ratio of I/I_0 in the presence of co-cultures. The sensing agent is made of 200 nM each of the three fluorescent proteins, 80 nM NP1 and 80 nM NP2 in 5 mM sodium phosphate buffer (pH=7.4).

Co-cultures	Blue (EBFP2)	Green (EGFP)	Red (tdTomato)
B.licheniformis	1.67	2.85	1.64
B.licheniformis	1.63	2.69	1.62
B.licheniformis	1.63	3.24	1.62
B.licheniformis	1.63	2.75	1.61
B.licheniformis	1.67	3.12	1.61
B.licheniformis	1.68	3.25	1.63
E.coli	1.39	1.27	1.36
E.coli	1.32	1.21	1.32
E.coli	1.35	1.21	1.38
E.coli	1.34	1.22	1.35
E.coli	1.34	1.24	1.37
E.coli	1.32	1.27	1.38
3T3 cell	1.64	4.19	1.63
3T3 cell	1.65	4.17	1.63
3T3 cell	1.68	3.87	1.63
3T3 cell	1.65	3.94	1.65
3T3 cell	1.64	3.83	1.63
3T3 cell	1.70	4.01	1.63

Nanoparticles synthesis

2nm diameter gold nanoparticles were synthetized by the Brust-Schiffrin two-phase methodology [1] using pentanethiol as the stabilizer; these clusters were purified with successive extractions with ethanol and acetone. A Murray place exchange reaction[2] was carried out in dry DCM to functionalize the nanoparticles with each ligand (Fig.1, ligands synthetized according to the reported procedure).[3,4] The monolayer-protected nanoparticles were redispersed in water and the excesses of ligand/pentanethiol were removed by dialysis using a 10,000 MWCO snake-skin membrane. The final concentration was measured by UV spectroscopy on a Molecular Devices SpectraMax M2 at 506nm according to the reported methodology.[5] NPs were characterized on a Malvern Nano Zetasizer to obtain hydrodynamic diameter (Fig. S3) and zeta potential for surface charge (Fig. S4).

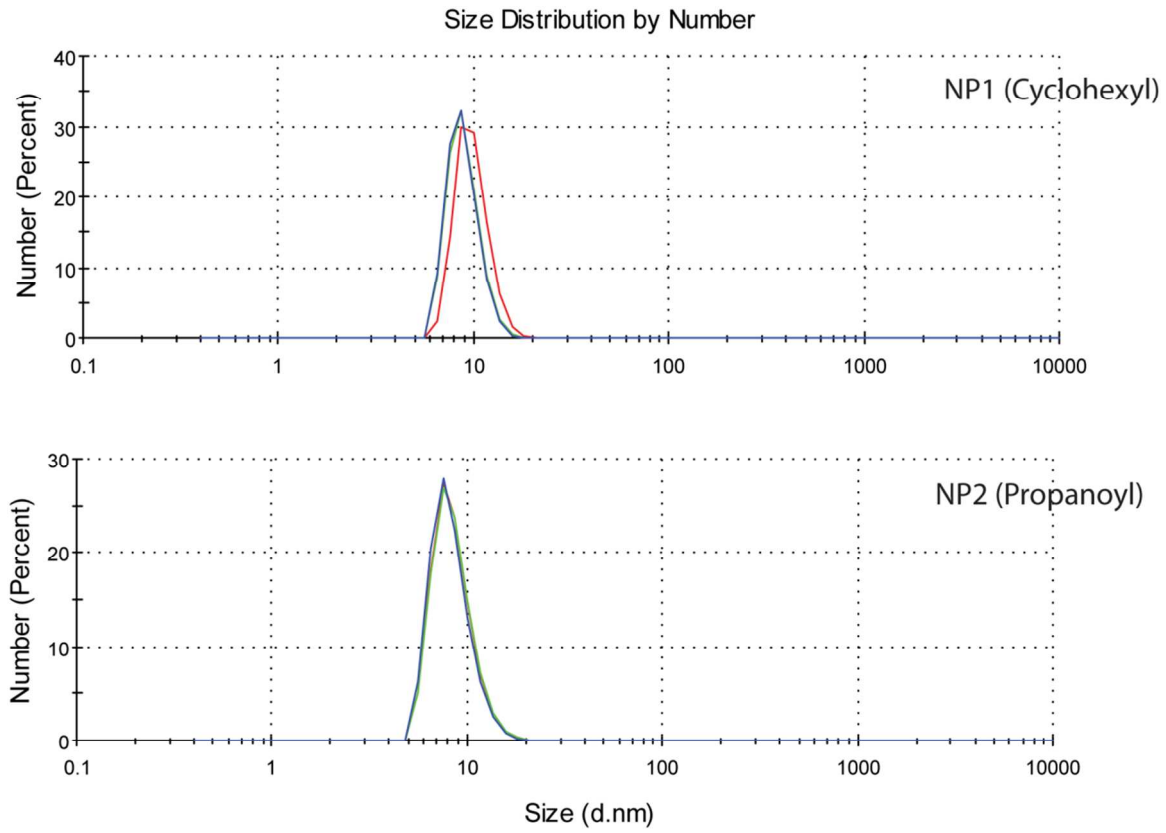

Fig. S3. DLS size distribution of NP1 and NP2.

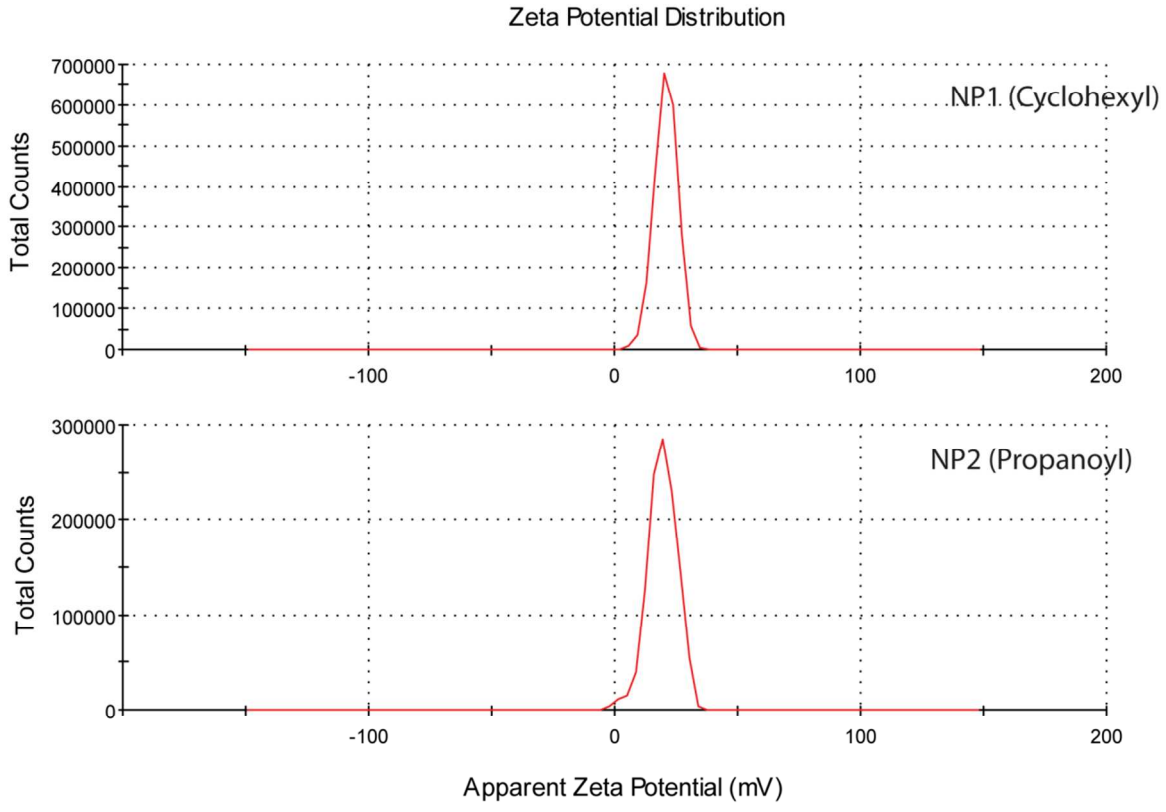

Fig. S4. Zeta potential distribution of NP1 and NP2.

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