Substrate Engineering Enabling Fluorescence Droplet Entrapment for IVC-FACS-Based Ultrahigh-Throughput Screening

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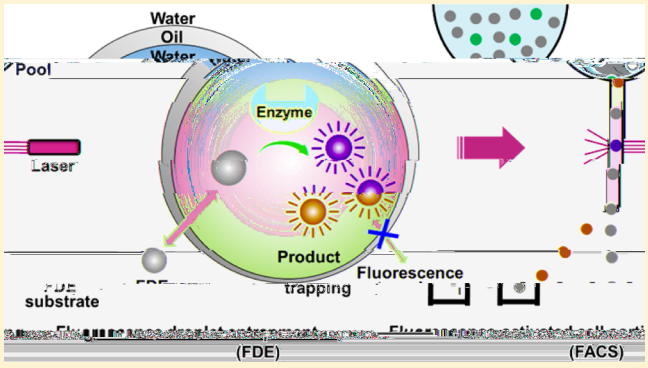
[†] K , , B , J , 800 D , 200240, C

[‡]D C , B C , 2036 , , B C 6 1Z1, C

[#] C I C B (CICB), E C , 130 , 200237, C

Supporting Information

ABSTRACT: I - fl - (I C-FAC) . H , ffi I C-FAC fl ff , . H fl (FDE) . , - fl . FDE , , , , ffi FDE 900- : I C-FAC . , FDE - , fl . I I C-FAC , , .



D fi , . [1,2] H , ffi . , - fl - (I C-FAC) - . [3,4] I C-FAC - , , fl - - -w (w/ /w) , - . fl w - (>10[7] /), . I , I C-FAC , , [3] β- , [4] , [5] β- - , [6] , [7] , [8] , [9] G- , [10] . [11] I I C-FAC fl . H , ( ) ff [12,13] ( ) . [14,15] - - B A [16] fl . [17,18] H , ff / fl , 7- -4- 3- -7- -4- - . [10,19] - fl , fi - . [20,21] H ,

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ff .
I C-FAC -
/ , **before** .
C , fl ,
.
A
. .
.[7] C
, ,
fl .
fl (FDE).
FDE
,
fl
. **after** .
,
, - , -
fl , I C-
FAC .
I , FDE ff
fi , , , . A
fi , fl
- ff
. ,
FDE ff .
FDE ff fi ,
FDE
fl
fl .
fl
, FDE I C-
FAC
, .

EXPERIMENTAL SECTION

Synthesis. fl
3- -7- -4-
(CH C, **4**), 7- -3- (HCCA, **6**),
7- -4- (HC , **13**)
I 1.
fl (**14**) fl
(**15a** **15b**)
I 2.
D (ffi)
ACD/I- (ACD/I-
2.0, 5.0.0.184, :// . . / 2/ .).

Cleavage of 1, 2, and 3 by Phosphotriesterase GkaP and Mutants. - *Ge-baci a hi* H A426 (G)
26A8, 26A8 , 26A8C
fi .[22] E
- ( )
405 4- -2550
( ). E **1**,
2, **3** (F 1)
F-7000
F (H) E /E
385/450 , 400/450 , 340/467 , .
( ) fi
1
. A 1× B (H 7.4)
fi
0.2 .

fl
ffi . ffi 4-
16000 /(·),
fl
ffi **4**, **6**, **13** (
1, **2**, **3**,)
2554 F /μ ,
3992 F /μ , 504 F /μ , (
I 3 4).
E. c i
, G -26A8
.
50 μ (B ,
D600 = 5.0) 50 μ B ( )
37 °C 10 .
100 μ 1.5
E 400 μ B ;
fi 0.2 . A
37 °C 2 , fl
(I 5).

Retention of Modified Coumarin Fluorophores in Droplets. C **4**, **6**, **13** 1× B
(H 7.4) 100 μ
/ /
.[11]
fl , 100-
.
fl
ff . F fl
, fl
(BD FAC A II) BD FAC D .
F C > 10 000 C > 10 000. fl **4**, **6**, **13**
DA I (375 - fi
450/40).

Single-Cell Enzymatic Reaction in w/o/w Double Emulsion Droplets. *E. c i* G
/ /
-
.[11] , **1**
2 fi
0.5 . **3**
(-
E. c i , I 5),
.
B , **3** 0.5 . / /
B B ( )
37 °C, 500 30 , .
F fl , 100-
fl **4**, **6**, **13** .

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Enrichment Analysis of GkaP-26A8-Expressing Cells. , C fl G (I 6). G -26A8- C - C18 , fl C G -26A8 *b* , C18 . G -26A8- C - C18 *E. c i* 10G, , - C fl . C G -26A8 (*E. c i* 10G G -26A8 C fl) G - (*E. c i* 10G C - C18) 1:10, 1:100, 1:1000 . / / ,

2 (0.5) 37 °C 30 fl (I 8). D fl 2 E 0.2 B . G -26A8- fi 96- .

Evaluation of FDE Behavior of Substrates 14 and 15 in Droplets. I FDE **14** **15**, *E. c i* B 21 (DE3) C ( ) AFE (E 28 -AFE)

°C. , 10 μ **14** **15** (10
D) 200 μ
37 °C. F fl ,
fl , **16**,
A F 430 , 375
- fi 530/30 .

RESULTS AND DISCUSSION

Determination of the Critical Hydrophobicity for Oil Permeability.

/ . I
fl , . C ,
3- 4-
7- (**4**,
6, **13**) ff (F 1).
fi
ffi (*D*), ff
.[21] / ff
fl . / .
- .[11] - -
fi .[3] A ,
-
fl [24−26]
. I C-FAC
, / 100
μ fl 100-
. A F 2,
4 - ,
(F
2A). A ff / ,
- . fl
fl
<0.33 >3.0 fl , 34%
F 2A.
fl 50% 1
(F 2D), fl
. **4** .
(**6**), ,
. 3.9%
fl 3-
(F 2B), 70% fl
1 (F 2E). C **13**,
, 1.2%
(F 2C) 90% fl
1 (F 2F).
, **4** < **6** < **13**,
. F
6 (*D* −5.92) **13** (*D* −2.41)
ffi 1 I C-FAC ,
, ,
4 (*D* 1.67) . F
, fi , *D*
>1.67 <
D < −2.41 .
-
,[7]
ffi . ,
(E) . *D*
fl (F 1).
1 (*D* 2.66), **2** (*D* −2.13),
3 (*D* −4.59) fl
fl , ,
ffi (F 1).
/
E . I
, fl
. G -26A8 .[22] A 60 ,
1 **2** fl
,
H , **3**
(I 7),
. G
D (F 1),
D > −2.13
. C
fi , “ *D* ”
−2.13 −2.41;
, *D* −2.13,
, *D*
−2.41, fi
. “ *D* ”
. “ ” “ ”
/ ,
- ff
. H ,
.

Compound 2 Is an Effective FDE Substrate for IVC-FACS of Phosphotriesterase.

/
G -26A8- *E. c i* . I
, **1** **2**
, **3**
. A
F 3, **4**
fl
1 I C-FAC . B ,
, -
3 fl
-
. **2**, , ffi
, ,
fl
. ,
2 (**6**) -
, /
, - I C-FAC

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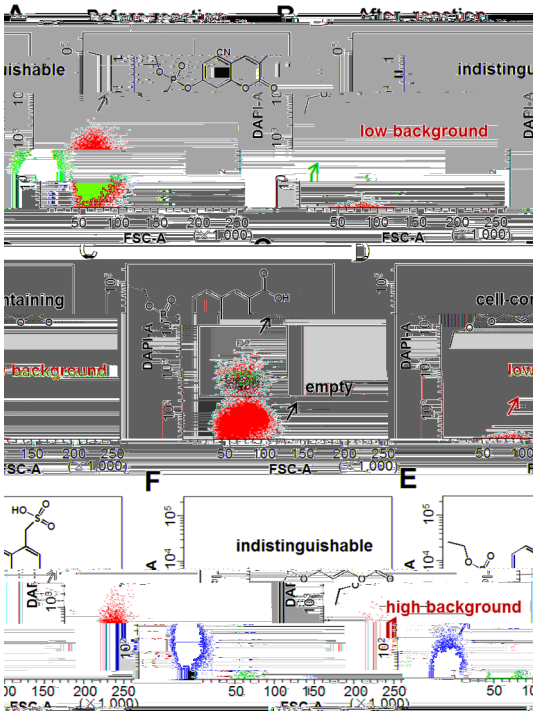

Figure 3. H 1, 2, 3 G -26A8- *E. c i* w/ /w . B , 1 2 w fl (A, C) w 3 w (E). A 60 , fl (B,D,F). H w , w - w 1 (B) . F 3, w - w fl (F). 2 fl - . I w ~0.1 . : 1 2 w w , w 3 w w . F , 20 000 w .

. I FDE ff 2 , , w , [3,7,11] w fl . A w F 4.

(E), ffi . [10,22] G . I C-FAC 1 w S - , (DE C C) w w . [10] w 2 I C-FAC , *Ge baci a hi* H A426 (G) 26A8, 26A8 , 26A8C. F , G w 2 E w

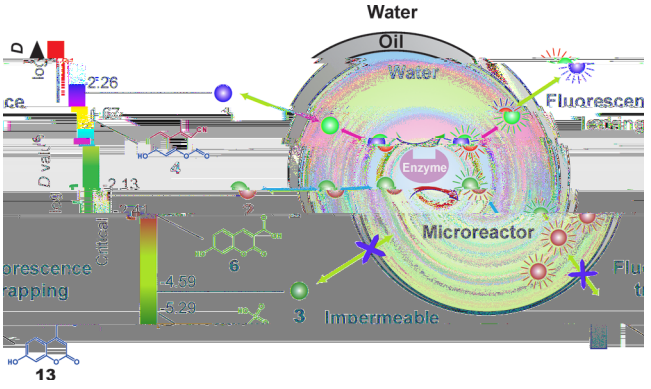

Figure 4. ff 1, 2, 3 w/ /w . w/ /w : D , . 1 2 ff w , w 3 w . C 4, 1, ff , *f e ce ce eq i g.* I 2, 6 , . 2 *f e ce ce d e e a e (FDE).*

1× B (H 7.4) 37 °C. A w F 5A, w 2 w

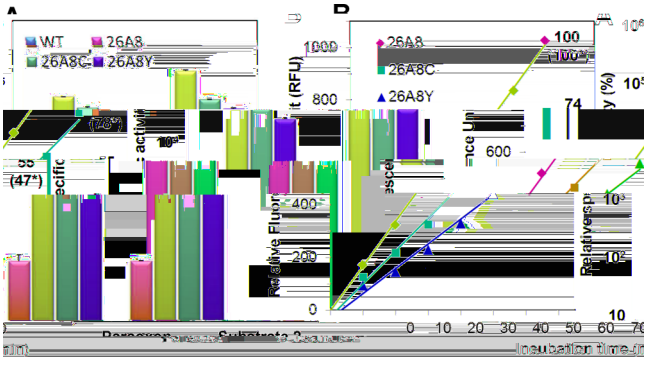

Figure 5. C 2 . (A) G w - (26A8, 26A8 , 26A8C) w 2. F , fi

, 2 . I fi FAC , G - *E. c i* w , w 2, fl w . A F 5B, w , . F ,

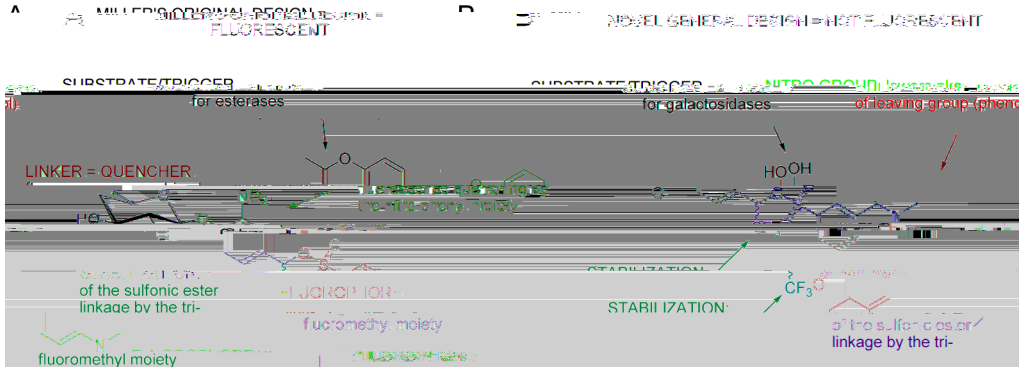

Figure 6. D ’ fl (A) FDE (B).

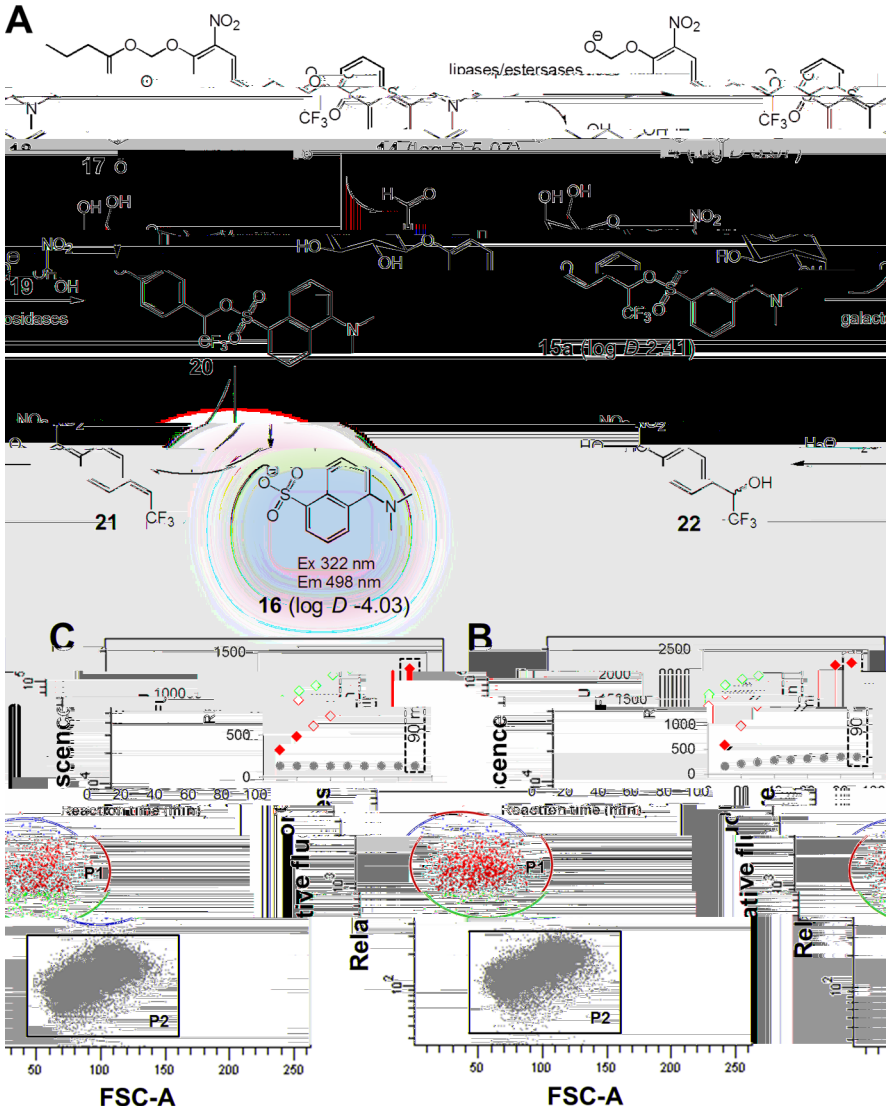

Figure 7. (A) fl . A , fl **16**. (B) F - **14** . (C) F - **15a** . I (B) (C), - fl - (1,) (2,) 90 .

, . *E. c i* G -26A8 **2** . E- ff I C-FAC /
2, E- . ,

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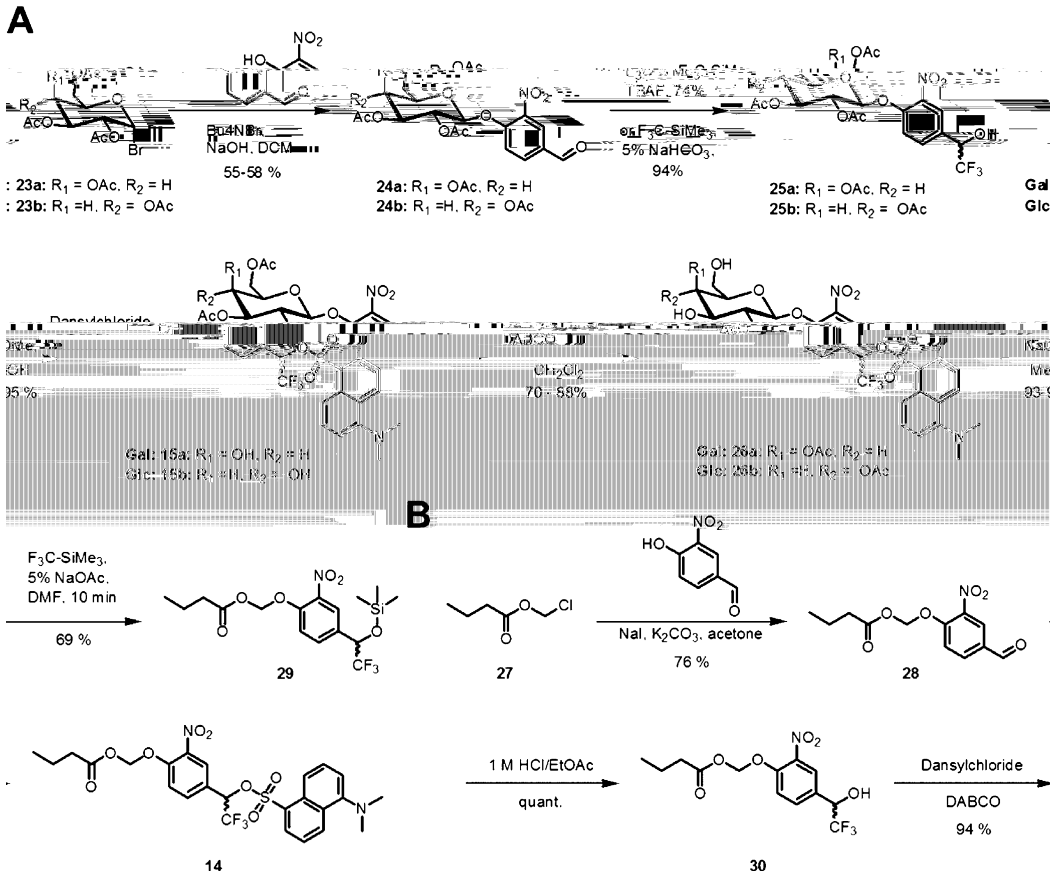
Scheme 1. Chemical Synthesis of 15a and 15b (A), and 14 (B)



fl , C , fl - (I 6). A **2** 37 °C 30 , (I 8) . 1. I 10% 1% 97% . E 0.1% 90% (900-). ffi - , 40- 330- ,[3,4,7,11] fi **2** I C-FAC E .

Design of a General Fluorophore for FDE Substrates.

A , FDE ff fl . H , FDE E , - ffi . . FDE ff fi , fl . I

H , . I fl - " " . (F 6) . " -fl " .[27] A , fl **16** 1,6- **20** (F 7A). F **16** H (K = 0.22 ± 0.4), D −4.03 (ACD/I-) / / . A fi - K . A fl ( ) ,[28] fl 200- ( ). A fl .[29] , -fl **14**, **15a**, **15b** " - " fl . C " " . I **14**, - (F 7A).[30]

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, . FDE
,
, fl .
A I C-FAC
, FDE
[32,33]
fi ,
, .

■ ASSOCIATED CONTENT

Supporting Information

I
AC D I: 10.1021/ . -
.6 01712.
A (DF)

■ AUTHOR INFORMATION

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Present Address

§(.F.) G H, B 10, A-6250 K , , A

Notes

fi .

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B C (973 , . 2012CB721000), F C (. 31470788 31100611), E C C , G C , G B C . F . .D. A J . .F. A F (F F) E F (J3293-B21). Z I & C B , J fl .

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