

## Experiment Title

Fluorescence (HDC) and UV-Vis

①

NLS-dL5\*

Prepare 1 mM MG Probes from 5 mM DMSO stock:

600  $\mu$ l 5 mM to 24 ml ACN (except MG-I<sub>2</sub>: 100  $\mu$ l to 40  $\mu$ l ACN)

②

Prepare BSA 5 mM in DPBS (pH 7.4): 1.1 mg BSA in 4 ml DPBS.

③

Prepare FAP (NLS-dL5\*) 5 mM in DPBS (pH 7.4): stock 1.2628 mg/ml, 22.96  $\mu$ M  
(<sup>have been</sup> dialyzed in DPBS for 3 times)1372  $\mu$ l 22.96  $\mu$ M FAP + 4928  $\mu$ l DPBS to 6300  $\mu$ l  
divided to 6 tubes (900  $\mu$ l each), one tube (800  $\mu$ l)1. ~~AB~~ UV-Vis 10 mM probes in ACN9  $\mu$ l MG-Cl<sub>2</sub>, MG-Br<sub>2</sub>, MG-I<sub>2</sub> ethyl to 900  $\mu$ l ACN

Test at 3 timepoints: 0h, 2.5h, 8h.

2. UV-Vis, PL, PLE of probes in 5 mM BSA

9  $\mu$ l MG-Cl<sub>2</sub>, MG-Br<sub>2</sub>, MG-I<sub>2</sub> ethyl to 900  $\mu$ l BSA

UV-Vis time-points: 0h, 3h.

PL, PLE at: 1h.

650/680, 660/690, 680/710

3. UV-Vis, PL, PLE of probes in 5 mM NLS-dL5\*

9  $\mu$ l MG-H<sub>2</sub>, MG-HBr, MG-Cl<sub>2</sub>, MG-Br<sub>2</sub>, MG-I<sub>2</sub>, MG-I<sub>2</sub> ethyl to 900  $\mu$ l  
650/680 660/690 680/710 680/710 680/710 680/710 NLS-dL5\*

PL, PLE timepoints: PL 0min, 3min, 1h, 3h

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UV-Vis at: 3h.

— before PL/PLE (3h)

4. Titration MG-I<sub>2</sub>/NLS-dL5\*

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800  $\mu$ l 5 mM NLS-dL5\*, add 2  $\mu$ l 1 mM MG-I<sub>2</sub> each time, capture PL after 3 min -  
incubation, last one to 12.5 mM, wait for 3 min more to capture another spectra (6 min)

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Book No:

Cont'd from page

Experiment Title

Fluorescence (HOC) and UV-Vis

dLS\*

① Prepare 1 mM MgProbes from 5 mM stock in DMSO

6  $\mu$ l 5 mM stock + 24  $\mu$ l ACN  $\Rightarrow$  30  $\mu$ l 1 mM

② Prepare 5 mM BSA from powder

1.9 mg BSA to 7 ml DPBS (pH=7.4)  $\Rightarrow$  7 ml③ Prepare 5  $\mu$ M dLS\* 3 (dialyzed in DPBS) from 11.49 mM stock2.828 ml 11.49 mM stock + 3.671 ml DPBS (pH=7.4)  $\Rightarrow$  6.5 mlMg-H<sub>2</sub>, Mg-HBr, Mg-Cl<sub>2</sub>, Mg-Br<sub>2</sub>, Mg-I<sub>2</sub>, Mg-I<sub>2</sub>ethyl

Mg-BSA:

UV-Vis: 10 mm 0 h, 3 h.

PL, PLE: 1 h (1.2 h indeed, slit 1/5 nm)

Mg-dLS\*:

UV-Vis: 10 mm 3 h

PLP, PLE: 3 min, 45 min, 1 h 45 min, 3 h 10 min - 30 min

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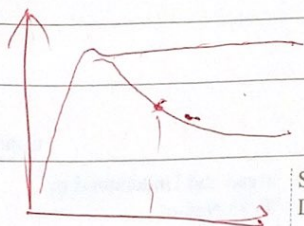
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658

Prepare MG stock in DMSO

	used conc. mM	100X stock in DMSO mM	Volume
A	2000	200	120ul = 48ul(500) + 72ul
B	<del>1000</del> 100	100	114ul = 57ul(200) + 57ul
C	500	50	108ul = 54ul(100) + 54ul
D	200	20	120ul = 48ul(50) + 72ul
E	100	10	114ul = 57ul(20) + 57ul
F	50	5	106ul = 53ul(10) + 53ul
G	20	2	115ul = 46ul(5) + 69ul
H	10	1	110ul = 55ul(2) + 55ul
I	5	0.5	104ul = 52ul(0.1) + 52ul
J	2	0.2	110ul = <del>44</del> <sup>44</sup> ul(0.5) + 66ul
K	1	0.1	102ul = 51ul(0.2) + 51ul
L	0.5	0.05	84ul = 42ul(0.1) + 42ul
M	0.2	0.02	60ul = 24ul(0.05) + 36ul
N	0	0	60ul DMSO

MGHBV, MGIV and MGIV



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Project 17/3/2022

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Book No:

Cont'd from page

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## Binding Affinity

Prepare 50 nM NLS-dL<sup>+</sup> (FAP) : 65.4  $\mu$ l 1.2628 mg/ml stock (same as 16/3/2022)  
to 30 ml DPBS (pH 7.4)

Add 6  $\mu$ l MG (A  $\rightarrow$  N) to 600  $\mu$ l 50 nM FAP

10:43 - 10:57 : MG-HBr adding, 11:37 tested (40 min) : Ex/Em = 656/696, high.

11:15 - 11:21 : MG-I<sub>2</sub> adding, 11:55 tested (35 min) : Ex/Em = 676/716

12:00 - 12:05 : MG-Cl<sub>2</sub> adding, 12:40 tested Ex/Em = 678/718, high/medium } high)  
(result : MG-Cl<sub>2</sub> and MG-I<sub>2</sub> show two sharp change from 200 to 500 nm)

Prepare MG-Br<sub>2</sub> and MG-I<sub>2</sub>ethyl (A  $\rightarrow$  M)

Prepare 50 nM NLS-dL<sup>+</sup> (FAP) : 43.6  $\mu$ l 1.2628 mg/ml stock (another stock tube)  
to 20 ml DPBS (pH = 7.4)

Add 6  $\mu$ l MG (A  $\rightarrow$  N) to 600  $\mu$ l 50 nM FAP

17:44 - 17:49 : MG-Br<sub>2</sub> adding, 16:23 tested (35 min, Ex/Em = 678/718, high)

15:57 - 16:02 MG-I<sub>2</sub>ethyl adding, 16:35 tested (33 min, Ex/Em = 678/718, high)

Cont'd on page

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ADP ABDA bleaching  
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Book No:

Cont'd from page

Experiment Title 0 min 1 min 2 min 3 min 4 min 5 min 6 min 8 min 10 min

1 DMSO

2 MG-H<sub>2</sub>

3 MG-HBr

4 MG-Cl<sub>2</sub>5 MG-Br<sub>2</sub>6 MG-I<sub>2</sub>7 MG-I<sub>2</sub>ethyl

8 MB

Probe conc. 5  $\mu$ M (1:100 from 0.5 mM stock) $200 \mu\text{l} \times 9 = 1800 \mu\text{l}$  $1800 \mu\text{l} \times 8 = 14.4 \text{ ml}$  (5  $\mu$ M dL5\* from 11.49  $\mu$ M stock dL5\* 3 DPBS)6.2663 ml 11.49  $\mu$ M stock + 8.1337 ml DPBS + 14.4 ml ABPA

ADPA

1:1000

14.4 ml DPBS alone + 14.4 ml ABPA

Add 18  $\mu$ l 0.5 mM Probe to 1.8 ml DPBS19  $\mu$ l to each well of 96-well plate dL5\* 3 - DPBS (5  $\mu$ M)660 nm irradiation from 0 - 10 min. transfer 18  $\mu$ l to another 96-well plate

Test: Microplate reader Abs.

15:30 - 15:33 adding 18  $\mu$ l probe to 1.8 ml dL5\* 3 - DPBS (5  $\mu$ M)

15:48 - 16:03 light irradiation. decrease

16:30 Abs at 380 nm very weak signal for MG Probes, MB very fast bleach (&lt;1 min)

Use cuvette (10 mm):

ADP ABDA 8  $\mu$ M in DPBS + 5  $\mu$ M MG-I<sub>2</sub> or dL5\* (5  $\mu$ M) + 5  $\mu$ M MG-I<sub>2</sub>

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ABPA 8  $\mu$ M in DPBS + 10  $\mu$ M MG-I<sub>2</sub>435  $\mu$ l + 565  $\mu$ l DPBS  
11.49  $\mu$ M

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10  $\mu$ M dL5\* + 10  $\mu$ M MG-I<sub>2</sub>870  $\mu$ l + 130  $\mu$ l DPBS

## Experiment Title

## Fluorescence of new purified NLS-dLS\*

Stock conc. 0.9983 mg/ml  $\sim$  18.15  $\mu$ M (55 kD)

Prepare 10  $\mu$ M NLS-dLS\* in DPBS (pH 7.4) 800  $\mu$ l = 44  $\mu$ l stock + 360  $\mu$ l DPBS

Prepare 10  $\mu$ M MgI<sub>2</sub> in DPBS (7.4): 400  $\mu$ l DPBS + 0.8  $\mu$ l MgI<sub>2</sub> (10 mM) (16.0  $\mu$ M)

Prepare 1 mM MgI<sub>2</sub> in ACN: 5  $\mu$ l 5 mM stock + 20  $\mu$ l ACN  $\Rightarrow$  25  $\mu$ l 1 mM

18:00

1. Mix 400  $\mu$ l 10  $\mu$ M NLS-dLS\* and 400  $\mu$ l 2h-aged 10  $\mu$ M MgI<sub>2</sub>, incubate 5 min  
Ex/Em = 680/710, slit 5/5.  $\sim$  200 a.u.

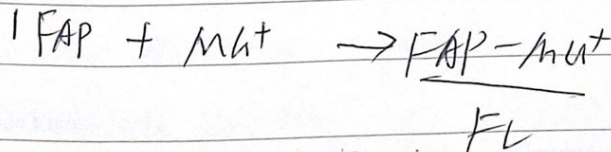
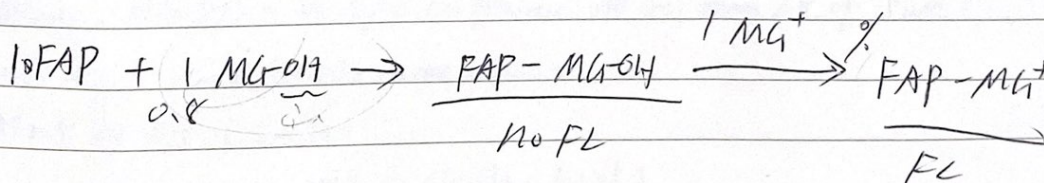
Add 4  $\mu$ l 1 mM MgI<sub>2</sub> more, incubate 5 min,  $\sim$  580 a.u.

Then test again after 15 min,  $\sim$  580 a.u.

2. 2/3 ~~FAP~~ NLS-dLS\* - MgI<sub>2</sub> (5  $\mu$ M/10  $\mu$ M), add 4  $\mu$ l MgI<sub>2</sub> more, incubate 5 min,  $\sim$  300 a.u.

3. 400  $\mu$ l 10  $\mu$ M NLS-dLS\* + 400  $\mu$ l DPBS + 4  $\mu$ l 1 mM MgI<sub>2</sub>, incubate 5 min,  $\sim$  600 a.u.

Add 2  $\mu$ l 1 mM MgI<sub>2</sub>, incubate 5 min,  $\sim$  610 a.u.



Cont'd on page

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## Experiment Title

## Absolute Fluorescence Yield. (CME G1)

Prepare 1mM MG Probes: 6ul 5mM stock in DMSO + 24ul ACN  $\Rightarrow$  30ul

Prepare 5uM NLS-dLS\*: 3.333ml 19.51 uM stock + 9.667ml DPBS (pH 7.4)  $\Rightarrow$  13ml

14:00

2ml 5uM NLS-dLS\* + 10ul 1mM MG Probes  $\Rightarrow$  1:1 binding.

14:25 H<sub>2</sub> 13.3%

14:37 H<sub>2</sub> 7.3%

14:44 HBr 12.9

14:58 Br<sub>2</sub> 7.6%

15:06 I<sub>2</sub> 8.5%

15:13 I<sub>2</sub> ester 7.9%

16:45 ~ 2.75h later, test the UV-Vis (10nm cuvette)

## Fluorescence CHOC

19.51uM NLS-dLS\* 800ul

8ul 2mM MG-I<sub>2</sub> : 1:1 binding 5min

Ex = 680nm, slit 5/2.5 or 5/5 (~900 a.u. or less than 2x of 5uM)

Then add 4ul 2mM MG-I<sub>2</sub> more, 5min

slit 5/2.5 or 5/5 (similar)

Test 10 min, 15 min : still in similar level

30 min later UV-Vis ~ 1a.u.

3h (2.5h) late UV-Vis

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and UV-Vis of 5uM FAPPNLS-dLS\* itself.  
of 5uM BSA

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Experiment Title

ADPA bleach.

Probe conc. 0.5 mM stock

ADPA conc. 100 mM stock

FAP(MS-dLS\*\*) conc. 34  $\mu$ M.DMSO, Mg-H<sub>2</sub>, Mg-HBr, Mg-Cl<sub>2</sub>, Mg-Br<sub>2</sub>, Mg-I<sub>2</sub>, Mg-I<sub>2</sub> ester. (7) MB200  $\mu$ l Time 0, 1, 2, 3, 4, 5, 6, 8, 10 (9 p in total)

$$(200 \mu\text{l} \times 9) = 1800 \mu\text{l}$$

$$(1800 \mu\text{l} \times 7) = 12600 \mu\text{l} = 12.6 \text{ ml}$$

$$V(34 \mu\text{M FAP}) = \frac{14.4 \times 5}{34} = 2.1176 \text{ ml}$$

$$V \text{ DPBS} = 12.2824 \text{ ml}$$

$$V(100 \text{ mM ADPA}) = \frac{14.4 \times 10 \times 10^{-3}}{100} = 1.44 \times 10^{-3} \text{ ml} = 1.44 \mu\text{l} \text{ (or } 14.4 \mu\text{l } 10 \text{ mM stock)}$$

Divided to 8 tubes 5  $\mu$ M FAP + 10  $\mu$ M ADPA solution:

$$(200 + 10) \mu\text{l} \times 9 = 1890 \mu\text{l}$$

$$1890 \times 7 = 13.230 \text{ ml} = \begin{cases} V(34 \mu\text{M FAP}) = \frac{13.230 \times 5}{34} = 1.9456 \text{ ml} \\ V \text{ DPBS} = 11.2844 \text{ ml} \\ V(100 \text{ mM ADPA}) = 1.323 \mu\text{l} \text{ or } V(10 \text{ mM ADPA}) = 13.23 \mu\text{l} \end{cases}$$

7 tubes of FAP-ADPA, each 200  $\times$  9 = 1800  $\mu$ l (for Mg probes)one tube of DPBS-ADPA, 1800  $\mu$ l (for MB)Add 18  $\mu$ l 0.5 mM Probe to each FAP-ADPA solution. Mix well, incubate 20 min at 37°C. incubator. or pm.② ~~transf~~ ~~200~~ <sup>190</sup>  $\mu$ l to each well for 0-10 min light irradiation.180  $\mu$ l for FL test (380 nm / 420 nm) Constant

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(669 nm / 8760 nm)

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Book No:

Cont'd from page

Experiment Title

## Binding Affinity

	used (nM)	100 x stock (uM)	Volume
A	2000		
B	1000		
C	500		
D	300		
E	200		
F	100		
G	50	100 uM	100 uM
H	30	<del>50</del> 100	$100 = 60 \mu\text{l} (50) + 40 \mu\text{l}$
I	20		$60 = 40 \mu\text{l} (30) + 20 \mu\text{l}$
J	10		$60 = 30 \mu\text{l} (20) + 30 \mu\text{l}$
K	5		$60 = 30 \mu\text{l} (10) + 30 \mu\text{l}$
L	2		$60 = 24 \mu\text{l} (5) + 36 \mu\text{l}$
M	1		$60 = 30 \mu\text{l} (2) + 30 \mu\text{l}$
N	0.5		$60 = 30 \mu\text{l} (1) + 30 \mu\text{l}$
O	0.2		$60 = 24 \mu\text{l} (0.5) + 36 \mu\text{l}$
P	0		

17:535 - 17:39 adding  
test

high and medium  
medium, Ex/Em = 654/694 nm

result kcal 60-75 kcal

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Book No:

Cont'd from page

Experiment Title

Fluorescence HOC

10<sup>10</sup> M MGS (8 ul 5 mM + 32 ul ACN → 40 ul)

10 ul to ACN 1 ml, PL, PLE, Dh

10 ul to DPBS 1 ml, PL, PLE, Dh.

Slit 5/5 nm

	ex	em (nm)
MG-H <sub>2</sub>	606	
MG-HBr	616	
MG-HI	616	
MG-Cl <sub>2</sub>	630	
MG-Br <sub>2</sub>	630	
MG-I <sub>2</sub>	628	
MG-I <sub>2</sub> ester	629	

Cont'd on page

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## Experiment Title

## UV-Vis of MGs

1 mM MG stock: 40  $\mu$ l = 8  $\mu$ l 5 mM stock + 32  $\mu$ l DMSO

(HBr,  $\text{OBr}_2$  test twice)

① Abs. of 10  $\mu$ M 10  $\mu$ l + 1 ml ACN

② Abs of 0, 2.5, 7.5, 10, 12.5, 15, 17.5, 20, 22.5  $\mu$ M  
7.5 +2.5 +2.5 +2.5 +2.5 +2.5 +2.5

0h: 15:00 test

3h: 18:<sup>20</sup>~~15~~ test

③ Abs. of 10  $\mu$ M 10  $\mu$ l + 1 ml DPBS

0h: 16:45 test

3h: 19:45 test

Cont'd on page

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Date

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## Experiment Title

## Kinetics - auto

1. MG1<sub>2</sub> <sup>Abs</sup> 628nm

- ① { Duration 21 min  
Interval 180s  
Delay Time 0s

Sample 2.5ul (ACN, -80°C) to 800ul DPBS

1.5ul (ACN, -80°C) to 1ml DPBS 0min 0.495 a.u. X

3ul (ACN, -80°C) to 1ml DPBS 0min 0.939 a.u. → 0.75

9 min later

Duration 60 min

Interval 600s

Delay Time 0s

NEW: Sample 3ul (5mM in DMSO) to 1ml DPBS 0min 0.424 X

8ul

to 1ml DPBS

1.142 a.u.

→ 0.85

9 min later

- ② { Duration 60 min (0.687 → 0.308 (40 min higher than 30 min))  
Interval 600s  
Delay Time 0s

③ Then 60 min interval ~~manually~~ (130 min, 210 min, 270 min)

Abs. 616nm

MG-H1 3.6ul (5mM in DMSO) to 1ml DPBS 0min 0.954 a.u.

① 21 min, interval 180s (0.954 → 0.702)

② 9 min later: 60 min, 600s interval (0.614 → 0.356)

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③ 1h later: 180 min, 360s interval (0.274)

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MG-HI kinetics

5 $\mu$ M MG-HI  $\oplus$ + 5 $\mu$ M NLS-dL5\* (14.39  $\mu$ M not pure)1mM MG-HI stock (7  $\mu$ l 5mM stock + <sup>16</sup>~~8~~  $\mu$ l ACN)5 $\mu$ M NLS-dL5\* (800  $\mu$ l = 278  $\mu$ l 14.39 $\mu$ M stock + 522  $\mu$ l DPBS)slit 5/5, ex = 660, em = ~~70~~ 685

Time setting

Stage	cycle (min)	Stop (min)
1	1	2
2	1	7
3	2	22
4	3	62
5	30	182

At 1.01 min, add 8  $\mu$ l 1mM MG-HI to NLS-dL5\* solution (take ~ 20s for mix)

mix before each scan: pipetting 3 times

2min to 652 a.u.

Cont'd on page

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Print Name

Date

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## Experiment Title

Abs. FL of Probes

Prepare 5 mM FAP (MS-dLS\*\*)

2D 1.2628 mg/ml stock, 22.96  $\mu$ M8 ~~73~~ ml 5 mM =  $\frac{1.634}{1.742}$  ml stock +  $\frac{5.866}{6.258}$  ml DPBSMG-H<sub>2</sub>, MG-HBr, MG-HIMG-Cl<sub>2</sub>, MG-Br<sub>2</sub>, MG-I<sub>2</sub>, MG-I<sub>2</sub> ester

Prepare 1 mM MG from 5 mM stock

25  $\mu$ l  $\times$  1 = 5  $\mu$ l stock in DMSO + 20  $\mu$ l ACN

1 mM stock

5 mM

UV-Vis and PL/PLE slit 5/5 nm

 $\frac{5 \times 1000}{1000} = 5 \text{ nm}$ MG-H<sub>2</sub> 650 680 (910) (950) MG-Cl<sub>2</sub> 680 710 (440) (450)MG-HBr 660 690 (890) (950) MG-Br<sub>2</sub> 680 710 (460) (478)MG-HI 660 685 (810) (780) MG-I<sub>2</sub> 675 710 (530) (510)~~MG-I<sub>2</sub>~~MG-I<sub>2</sub> ester 675 710 (501) (500)

FAP only 440 nm

5  $\mu$ l FAP to 1 ml FAP, 0 (10 min), 1, 3 hem  
(550 - x nm)

(ex x - 800 nm)

Cont'd on page

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Date

Print Name

Date

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2022. 5. 29

$$\frac{10 \mu\text{M} \times 980 \mu\text{l}}{9.8 \mu\text{l}} = 1000 \mu\text{M} = (1 \text{ mM})$$

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Book No:

Cont'd from page

Experiment Title

ADPA bleaching

0 min 2 min 4 min 6 min 8 min

980  $\mu\text{l}$  MG-FAP, mix with 4.9  $\mu\text{l}$  2 mM ADPA stock100 mM ADPA stock 10  $\mu\text{l}$  + 90  $\mu\text{l}$  DPBS to 100  $\mu\text{l}$  1 mM ADPA10 mM ADPA stock 20  $\mu\text{l}$  + ~~4.9~~<sup>4.80</sup>  $\mu\text{l}$  DPBS to 2 mM ADPA180  $\mu\text{l}$  for light (675 nm, 330  $\text{W}/\text{m}^2$ ) irradiation, 160  $\mu\text{l}$  for reading fluorescence intensity

ADPA: 360/420 nm

MGs: 660/700 nm

Cont'd on page

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Date

Print Name

Date

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## Experiment Title

Abs of dL5\*-3 (DPBS) - MG

Sample dL5\*-3 (DPBS) dialyzed in 12/3 in DPBS then concentrated from 35 ml to 26 ml

dL5\*-3 (DPBS)

dL5\*-4 (25/2)

conc. 0.3677 mg/ml, 11.26  $\mu$ M (23.65 KD)conc. 0.2293 mg/ml, 9.70  $\mu$ M3.35 ml (~~28.11  $\mu$ M~~) (37.72  $\mu$ M)1 ml (9.70  $\mu$ M)

Prepare 1 mM MG from 5 mM stock:  $\uparrow$  2.5  $\mu$ l 5 mM stock + 10  $\mu$ l ACN  $\Rightarrow$  12.5  $\mu$ l  
<sup>in DMSO</sup>

Prepare 6  $\mu$ M FAP (dL5\*) : 3.35 ml 11.26  $\mu$ M stock (37.72  $\mu$ M) + 2.95 DPBS  
 to 6.3 ml, divide to 850  $\mu$ l

2022. 6. 29.

1 mg in 3.64 ml

Abs. dL5\*-4 (25/2)

Abs. BSA 1.1 mg in 4 ml DPBS to 5  $\mu$ Mconc. 0.2293 mg/ml, ~~7.18  $\mu$ M~~ 8.47  $\mu$ M stock5  $\mu$ M : 472  $\mu$ l + 328  $\mu$ l DPBS

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## Experiment Title

## ADPA bleaching

(b) 0 min 2 min 4 min 6 min 8 min 10 min

1.2 ml DPBS, mixed with <sup>6</sup>50 ~~50~~ ul 2 mM ADPA stock to 10  $\mu$ M100 mM ADPA stock 10  $\mu$ l + 90  $\mu$ l DPBS  $\Rightarrow$  10 mM~~20  $\mu$ l 10 mM ADPA + 80  $\mu$ l DPBS  $\Rightarrow$  2 mM~~DMSO ~~ADPA~~, MgH<sub>2</sub>, MgHBr, Mg-HI, Mg-Cl<sub>2</sub>, Mg-Br<sub>2</sub>, Mg-I<sub>2</sub>, Mg-I<sub>2</sub> ester  
8 samples in total1) ①  $8 \times 1.2 \text{ ml} = 9.6 \text{ ml}$  10  $\mu$ M ADPA in DPBS9.6 ml DPBS + 9.6  $\mu$ l 10 mM ADPA2) 1140  $\mu$ l DPBS with ADPA + 1.14  $\mu$ l 5 mM MG to 5  $\mu$ M MG - 10  $\mu$ M ADPA  
Incubate at 37°C for 10 min3). Divided to 96-well plate, 180  $\mu$ l each for 620 nm light irradiation4). Transfer 160  $\mu$ l to another plate for microplate reader reading

FL: ADPA: 380/420 nm 500 vol

500 vol. MGs: 660/700 nm

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## Experiment Title

## Absolute Fluorescence Yield (CMC GI)

(refer to 30/3/2022)

Prepare 1mM MG Probes : 6ul 5mM stock in DMSO + 24 ul ACN  $\Rightarrow$  30ulPrepare 5mM NLS-dLS\* : 1.6ml 19.5mM stock + 4.6ml DPBS  $\Rightarrow$  6.2ml2ml 5mM NLS-dLS\* + 10ul 1mM MG Probes  $\Rightarrow$  1:1 BindingA MG-HBr  $\sim 12.9\%$ B MG-HI  $\sim 10.1\%$ C MG-Izester  $\sim 7.5\%$ 

18:28 mix

Cont'd on page

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## Experiment Title

Fluorescence (HOC)

1 mM MG (2.5  $\mu$ l) 5 mM in DMSO + 10  $\mu$ l ACNGlycerol : H<sub>2</sub>O = 7:310  $\mu$ l 1 mM MG to 1 ml Glycerol/H<sub>2</sub>O solution = 10  $\mu$ MMG-H<sub>2</sub> (460)

ex 430 20/20, ex 340 10/20, ex 340 10/10

MG-HB<sub>1</sub> (454)

ex 440 ex 430 20/20, em

MG-HI (457)

ex 430 20/20

MG-Cl<sub>2</sub> (430)MG-Br<sub>2</sub> (429)

ex = 410

MG-I<sub>2</sub> (433)

5 mM stock in DMSO

ACN as solvent

40  $\mu$ l 5 mM stock to 1 ml ACN : 200  $\mu$ M

0.5 Scan rate (nm/min) : 60, Avg Average Time 0.05, Scan 0.5 nm

MG-H<sub>2</sub> : ex 440 20/20, peak 500 nm

em 500 20/20, peak 357 nm

ex 360 10/10, peak 400 nm, 500 nm

MG-HB<sub>1</sub> : ex 440 20/20, em 500 20/20, 350 10/20, ~~10/20~~

MG-HI : ex 440 20/20, em 500 20/20, 350 10/10, 10/20, 20/20

MG-Cl<sub>2</sub> : ex 430 20/20, em 490 20/20, 360, 10/10,MG-Br<sub>2</sub> : ex 430 20/20, em 490 20/20, 360, 10/10

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MG-Br<sub>2</sub> : ex 430 20/20, em 490 10/20, 360,

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## Experiment Title

dL5<sup>++</sup>: 7.16  $\mu$ M stock (25/2)NLS-dL5<sup>++</sup>: 19.51  $\mu$ M stock (16/3)

Prepare 20 mM of MG-H<sub>2</sub>, MG-Me, MG-Me<sub>2</sub>, MG-Et<sub>2</sub> stock solution.

1.90 mg (240 $\mu$ L)	1.26 mg (157 $\mu$ L)	3.70 mg (445 $\mu$ L)	2.23 mg (250 $\mu$ L)
--------------------------	--------------------------	--------------------------	--------------------------

Prepare 4.2  $\mu$ M of dL5<sup>++</sup> by adding 2.463 ml stock to 1.737 ml DPBSPrepare 4.2  $\mu$ M of NLS-dL5<sup>++</sup> by adding 0.904 ml stock to 3.296 ml DPBS.1) UV-vis of MG and MG-FAP MG-FAP 5  $\mu$ M MG to ~~1~~ proteinMG-H<sub>2</sub>MG-H<sub>2</sub>-FAP

MG-Me

MG-Me-FAP

MG-Me<sub>2</sub>MG-Me<sub>2</sub>-FAPMG-Et<sub>2</sub>MG-Et<sub>2</sub>-FAP

5/4.2 = 4.2/3.5

2) PL / PLE of MG-FAP

MG-H<sub>2</sub> (Abs 646)

Ex 645, Em 677. Ex = 650 (FAP)

Ex = 650, Em 677 (dL5<sup>++</sup>)

MG-HMe (Abs 642)

Ex = 645, Em 670

MG-Me<sub>2</sub> (Abs 650)

Ex = 650, Em = 679

MG-Et<sub>2</sub> (Abs 649)

Ex = 650, Em = 676

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Experiment Title 2022.10.811

DHR123 ( $^{\circ}\text{O}_2$  detector) - FAP-MG-H1Prepare 5  $\mu\text{M}$  NIS-d $^{15}\text{N}$  (FAP)1.2628 mg/ml stock, 22.96  $\mu\text{M}$ 

$$190 \times \frac{1.2}{1140} = 950 \text{ nl} \times 5 \mu\text{M} / 22.96 = \frac{248}{207} \text{ nl stock} + \frac{892}{743} \text{ nl DPBS}$$

Prepare 5  $\mu\text{M}$  FAP-MG-H1Add  $\frac{1.2}{1140}$  5  $\mu\text{M}$  MG-H1 to 950 nl 5  $\mu\text{M}$  FAP solution, incubate 15 min at 37°C.Prepare 10  $\mu\text{M}$  DHR123 in { MG-H1 in DPBS ( $\frac{1.2}{1140}$  to 950 nl)By adding  $\frac{1.2}{1140}$  5 nl 10  $\mu\text{M}$  stock } FAP-MG-H1 in DPBS (950 nl)  
in DPBS ~~to~~  $\text{O}$ 

light irradiation (660 nm) = 0, 2, 4, 6, 8, 10 min

to 190 nl in ~~new~~ 96-well plate for irradiation

transfer 180 nl to another 96 well plate for microplate reader.

cann. dissolve (1M)

DHR123: (100  $\mu\text{M}$ ), 180 nl in DPBS Abs scan. weak abs > 400 nm  
10  $\mu\text{M}$ 

	FAP-MG-H1	MG-H1	DPBS only
0	↓		
2			
4	stable	linear	
6	from 2-10 min	↓	
8			

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ex = 480, 500 nm

ex = 500, 520 nm

Km = 520

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Experiment Title 2022. 10. 11DHR123 200  $\mu$ M 3.1 ml: 62  $\mu$ l 10 mM stock + 3.1 ml DPBS1  $\mu$ M Eosin Y: 1  $\mu$ l 1 mM stock + 4  $\mu$ l DMSO + 1  $\mu$ l 1M NaOH + 1 ml DHR123 + 1  $\mu$ l 1M NaOH1  $\mu$ M DBF-NHSAc2: 1  $\mu$ l 1 mM stock + ...0  $\mu$ M (DMSO): 5  $\mu$ l DMSO + ...

very strong FL turn on, saturated signal in Ex/Em = 480/520 nm

1/10 diluted: still saturated

1/100 diluted: OK.

By comparing <sup>2 min</sup> FAP-MG-H1 / DPBS only and DBF-NHSAc2 / DMSO, DBF is  
~ 600 fold higher than FAP-MG-H1

2022. 10. 14

DHR 123 MG-H1 at (620 nm).

10  $\mu$ M5  $\mu$ M

4

6 x 2 = 120

Prepare 10  $\mu$ M DHR 123: 1.4  $\mu$ l 10 mM stock to ~~4~~ 5.6 ml DPBS{ 2.6 ml 10  $\mu$ M DHR 123 + 2.6  $\mu$ l 5 mM MG-H1{ 2.6 ml 10  $\mu$ M DHR 123 + 2.6  $\mu$ l DMSO190  $\mu$ l in 96-well plate for 620 nm irradiation 0, 2, 4, 6, 8, 10 min,  
transfer 180  $\mu$ l to another plate for microplate reader.

Ex/Em = 480/520 nm, 500 vol for DHR 123

~~10  $\mu$ M~~

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Experiment Title

2022. 10. 12

0, 1, 2, 3, 4 min ( $5 \times 200 = 1 \text{ mL}$   $\emptyset$ ,  $1 \text{ mL} \times 6.2 = 6.2 \text{ mL}$ )ADPA  $200 \mu\text{M}$   $6.2 \text{ mL}$ :  $10 \text{ mM}$  stock  $124 \mu\text{l}$  to  $6.078 \text{ mL}$  DPBS

hoe-EG3-DBF

hoe-EG1-DBF

hoe-C4-DBF

Ac2DBF MHS

Eosin Y

DMSO

 $1 \mu\text{l}$   $1 \text{ mM}$  probe stock +  $4 \mu\text{l}$  DMSO +  $1 \mu\text{l}$   $1 \text{ M}$  NaOH +  $1 \text{ mL}$  ADPA in DPBS  
+  $1 \mu\text{l}$   $1 \text{ M}$  HClGreen light irradiation ( $200 \mu\text{l}$  in 96-well plate) for 0-4 min, transfer  
 $120 \mu\text{l}$  for reading (Abs.  $380 \text{ nm}$ )

FL 480/520 nm auto

FL 480/530 nm - 500 vol

Result: hoe-DBF no ADPA bleaching and very weak FL.

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Experiment Title 2022. 10. 12

10  $\mu$ M probe in ~~DMSO~~ <sup>EtOH</sup> ACN and DPBS (0.5% DMSO)

Abs.

FL.

Size distribution

10  $\mu$ M probe ~~turn~~ in ACN: turn to yellow after adding NaOH (1M),  
back to colorless after adding HCl (1M) and become insoluble  
(except DBF ~~always~~ ~~too colored~~ that also colored in HCl)

10  $\mu$ M probes in ~~ACN~~ DPBS = Abs

FL in Cytosol

AD	DBF	Abs	ex	em	slit
	DBF	506	506	516-650	5/5. <del>10/5</del> 10/5 saturated
	C4	515	515	515-650	5/5, 10/5
	EG1	515	515	515-650	5/5, 10/5
	EG3	517	517	527-650	5/5, 10/5

Then take the above solution to test the size distribution  
Since the size of EG1 and C4 are too high ( $\sim 2 \mu$ m)  
EG3 and DBF are too small ( $\sim 10$  nm)

compare

EG3 without deprotection { 2.5  $\mu$ M in 0.5% DMSO  
10  $\mu$ M in 0.5% DMSO  
10  $\mu$ M in 1% DMSO

EG1 and C4 without deprotection 10  $\mu$ M in 0.5% DMSO

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test EG3, EG1, C4 (deprotected) again

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10  $\mu$ M in 0.5% DMSO (similar result)

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Experiment Title

2022. 10. 13

DLS

Yesterday 10  $\mu$ M DBF E43 E41 C4  
 $\sim 10$  nm  $\sim 2$   $\mu$ m

today 1  $\mu$ M DBF E43 E41 C4

all &lt; 10 nm

DLS of protected version

E43/E41/C4 = 0  $\mu$ M, 0.1  $\mu$ M, 2.5  $\mu$ M, 5  $\mu$ M

all &lt; 10 nm

(yesterday)  
10  $\mu$ ME43  $\sim 30$  nmE41  $\sim 80$  nmC4  $\sim 20$  nm

all in two major intensity peaks

Abs and fluorescence in microplate reader

DBF / E43 / E41 / C4

0  $\mu$ M, 0.1  $\mu$ M, 0.5  $\mu$ M, 1  $\mu$ M, 2.5  $\mu$ M, 5  $\mu$ M, 10  $\mu$ M, 600  $\mu$ l (3  $\mu$ l DM30)

↑ ↑ ↑ ↑ ↑ ↑ ↑  
 stock 0 20  $\mu$ M 100  $\mu$ M 200  $\mu$ M 500  $\mu$ M 1 mM 2 mM

(2+8 (10  $\mu$ l) 5+5 (8  $\mu$ l) 4+6 (5  $\mu$ l) 5+5 (5  $\mu$ l) 5+5 (5  $\mu$ l) 10  $\mu$ l (5  $\mu$ l) from 4+6 0.5 mM

~~3  $\mu$ l~~ 180  $\mu$ l x3 in 96-well plate

one plate of 1  $\mu$ M add ~~0.5~~ 0.5  $\mu$ l 100 mM hairpin DNA (0.5 mM)  
 2.5  $\mu$ M

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## Experiment Title

Abs and FL in microplate reader

EG3/EG1/C4

~~200x3 = 600nt~~Stock 0 200  $\mu$ M 100  $\mu$ M 200  $\mu$ M 500  $\mu$ M 1mM 2mMto 0 0.1  $\mu$ M 0.5  $\mu$ M 1  $\mu$ M 2.5  $\mu$ M 5  $\mu$ M 10  $\mu$ M

Volume 1 1 1 1 1 1 1

Add  $\mu$ l  $\left( \begin{smallmatrix} 0.5+2 \\ 1+1 \end{smallmatrix} \right)$  (1+1)  $\left( \begin{smallmatrix} 1+1.25 \\ 0.5+2 \end{smallmatrix} \right)$   $\left( \begin{smallmatrix} 1.25+1.25 \\ 1 \end{smallmatrix} \right)$  101  $\mu$ l Probe + 1  $\mu$ l 1M NaOH + 200  $\mu$ l DPBS, transfer 180  $\mu$ l to microplate readerPrepare 0.5mM hairpin DNA: 34.6  $\mu$ l 0.1x TE buffer to solid hairpin DNA  
+  
34.6

FL: 500 vol, ex/em = 520/560 nm

First: 180  $\mu$ l probe FLThen: Add 0.9  $\mu$ l 0.5mM hairpin DNA (to 2.5  $\mu$ M), test FL againThen: Add 1.8  $\mu$ l 0.5mM hairpin DNA (to 5  $\mu$ M), test FL again

Then: after 10-min incubation, test FL again (no change)

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## Experiment Title

Prepare 0.5 mM MG-H1 from 5 mM stock with DPBS (2.5  $\mu$ l stock in DMSO solution turn from green to purple immediately + 22.5  $\mu$ l DPBS)

Incubate at R.T. dark from 12:15 -

Prepare ~~0.5 mM~~ MG-H1 from 5 mM stock with ACN (2  $\mu$ l stock + 6  $\mu$ l ACN)

Prepare 5  $\mu$ M FAP (NLS-dL5\*\*) (800  $\mu$ l x 2)

~~19.5  $\mu$ M stock 1.2628 mg/ml (22.96  $\mu$ M) 174  $\mu$ l + 626  $\mu$ l DPBS~~

One add 2 mM (1.68  $\mu$ l) to 800  $\mu$ l FAP to 4.2  $\mu$ M MG-5  $\mu$ M FAP

One add 0.5 mM (6.72  $\mu$ l) to 800  $\mu$ l FAP to 4.2  $\mu$ M MG-5  $\mu$ M FAP

Prepare 2 mM MG-H1 from 5 mM stock with ACN (2  $\mu$ l stock + 3  $\mu$ l ACN)

Prepare 4.2  $\mu$ M FAP (NLS-dL5\*\*) (1700  $\mu$ l)

1.2628 mg/ml (22.96  $\mu$ M) 311  $\mu$ l + 1389  $\mu$ l DPBS

① 800  $\mu$ l + ~~1.68  $\mu$ l~~ 2  $\mu$ l (2 mM) MG-H1 slit 5/5 mm ~~ex = 660 / 685~~ <sup>PURPLE</sup>

② 800  $\mu$ l + 8  $\mu$ l (0.5 mM) MG-H1

slit 5/5, ex = 660 nm

① incubate 5 min, scan, ~~25 min~~ 25 min later, scan again. then add 2  $\mu$ l (2 mM) MG-H1, 5 min incubation, scan.

② incubate 5 min, scan, then add 2  $\mu$ l (2 mM) MG-H1, incubate 5 min, scan.

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Experiment Title 2023.1.5

EG3/EG1/C4

stock	0	20 $\mu$ M	100 $\mu$ M	200 $\mu$ M	500 $\mu$ M	1 mM	2 mM
to	0	0.1 $\mu$ M	0.5 $\mu$ M	1 $\mu$ M	2.5 $\mu$ M	5 $\mu$ M	10 $\mu$ M
volume	1	1	1	1	1	1	1 $\mu$ l (for 200 $\mu$ l)
	3	3	3	3	3	3	3 (for 600 $\mu$ l)

+ 2  $\mu$ l 0.1 M NaOH, mix, add 600  $\mu$ l DPBS.

3 trials, each 180  $\mu$ l @ Abs 515 nm

FL 505/545 nm, 500 vol

Mix 3 trials (600  $\mu$ l), add <sup>180</sup>60  $\mu$ l 2 mM hairpin DNA to 20  $\mu$ M, vortex well.

Incubate 10 min, 3 trials, each 180  $\mu$ l Abs 515 nm

FL 505/545 nm, 500 vol

repeat, to 40  $\mu$ M.

Incubate 10 min scan: Abs 515 nm, FL 505/545 nm, 500 vol

Incubate <sup>more</sup> 20 min scan: FL 505/545, 500 vol

Incubate 2h scan: FL 505/545, 500 vol

$$\frac{6 \times 2000}{600} = 20$$

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Experiment Title 2023.1.5

EG3/EG1/C4

Prepare 1  $\mu$ M probe: 2.3  $\mu$ l 1 mM probe + 2  $\mu$ l 0.1 M NaOH + 2.3 mL DPBShairpin DNA stock to add to 200  $\mu$ l solution

	A	2 mM	100 $\mu$ M	10 $\mu$ l	
<del>40 <math>\mu</math>l</del>	B	2 mM	50 $\mu$ M	5 $\mu$ l	
	C	2 mM	20 $\mu$ M	20 $\mu$ l	
	D	0.5 <del>0.5</del> mM	10 $\mu$ M	<del>10</del> 4 $\mu$ l	} 2.8 13.3 10.5 + 50.5 = 13.3
D	E	0.5 <del>0.5</del> mM	5 $\mu$ M	20 $\mu$ l	
	F	0.1 <del>0.1</del> mM	2 $\mu$ M	4 $\mu$ l	} 1.5 24 22.5 + 3.5 = 26
	G	0.1 <del>0.1</del> mM	1 $\mu$ M	20 $\mu$ l	
	H	0.1 mM	0.5 $\mu$ M	10 $\mu$ l	} 3.5 7 2.02 $\mu$ l $\times$ 3.5 = 7
	I	0.02 <del>0.02</del> mM	0.2 $\mu$ M	20 $\mu$ l	
	J	0.02 <del>0.02</del> mM	0.1 $\mu$ M	10 $\mu$ l	$\times$ 3.5 = 3.5 $\mu$ l

incubate 10 min

K

0 mM

Abs 515 nm

FL 505/545, 500 vol

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## Experiment Title

E43 / E41

Prepare 1  $\mu$ M Probe : 2.3  $\mu$ l 1 mM Probe (prepared in 2023.1.5) + 2  $\mu$ l  $\text{D}$   
 0.1 M NaOH + 2.3 ml DPBS

hairpin DNA stock to add (to 200  $\mu$ l)

A	2 mM	100 $\mu$ M	10 $\mu$ l	
B	2 mM	50 $\mu$ M	5 $\mu$ l	
C	2 mM	20 $\mu$ M	2 $\mu$ l	
D	0.5 mM	10 $\mu$ M	4 $\mu$ l	} $6 \times 2 = 12$ (12 + 5 = 17 = 4.25 + 12.75) (2 mM)
E	0.5 mM	5 $\mu$ M	2 $\mu$ l	
F	0.1 mM	2 $\mu$ M	4 $\mu$ l	} $7 \times 2 = 14$ (14 + 3 = 17 = 3.4 + 13.6) (0.5 mM)
G	0.1 mM	1 $\mu$ M	2 $\mu$ l	
H	0.1 mM	0.5 $\mu$ M	1 $\mu$ l	
I	0.02 mM	0.2 $\mu$ M	2 $\mu$ l	} $3 \times 2 = 6$ (8 $\mu$ l = 1.6 $\mu$ l + 6.4 $\mu$ l) (0.1 mM)
J	0.02 mM	0.1 $\mu$ M	1 $\mu$ l	
K	-	0 $\mu$ M		

Abs @ 15 nm

FL : 505 / 545, 500 vol.

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Experiment Title 2023. 2. 3

EG3/EG1/C4

Prepare 1mM Probe:

2.3ul 1mM Probe (prepared 2023.1.5) + 2ul 0.1M NaOH + 2.3ml DPBS

hairpin DNA

	Stock	to	add (to 200ul) /ul	
A	2mM	100uM	10	} 17x3 = 51 (860)
B	2mM	50uM	5	
C	2mM	20uM	2	
D	0.5mM	10uM	4	} 8x3 = 24 (30 = 7.5 + 22.5)
E	0.5mM	5uM	2	
F	0.1mM	2uM	4	} 7x3 = 21 (25 = 5 + 20)
G	0.1mM	1uM	2	
H	0.1mM	0.5uM	0.1	
I	0.02mM	0.2uM	2	} 3x3 = 9 (10 = 2 + 8)
J	0.02mM	0.1uM	1	
K	-	0uM		

1st EG3/EG1/C4

FL ex/em 505/545, 500vol, Abs 515

2nd EG3/EG1/C4

⊙

same

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Experiment Title 2023.4.13 FL UV-VIS DBF Acid (PH)

Prepare 0.1M KCl : 300mL,  $0.03 \text{ mol} = 0.03 \times 74.54 = 2.2362 \text{ g}$

0.98 (HCl)

↓ 1:10 dilute (0.1M KCl)

1.98

↓ 1:10 dilute

2.92

↓ 1:10 dilute

3.83

↓ 1:10 dilute

~~6.88~~ tested 6.88 cannot stable (5)

↓ 1:10 dilute

4.6

↓ 1:10 dilute

7

PH : 1, 2, 3, 4, 5, 6, 7 (HCl)

PH : 13, 12, 11, 10, 9, 8, 7.7 (KOH)

Neutral 0.1M KCl (7)

11.62

Prepare 0.1M KOH

↓ 1:10 dilute 10mL,  $0.1 \times 10 \times 10^{-3} \times 56.11$

$= 56.11 \times 10^{-3} \text{ g}$

↓ 1:10 dilute  $= 56.11 \text{ mg}$

~9.6 6.96 81.6 mg / 150 mL

PH 13.13

↓ 1:10 cannot stable

tested = 11.09 (12.7) ~~7.7~~

↓ 1:10 (11)

11

↓ 1:10

10

↓ 1:10

9

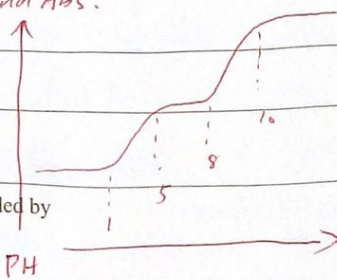
↓ 1:10

8

↓ 1:1 dilute

7.7

FL and Abs.



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2023. 5.5

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Experiment Title

## hairpin DNA

from IDT powder:  $MW = 8571.6 \text{ g/mol}$ ,  $T_m = 65.9^\circ\text{C}$ 

$$464.0 \text{ bp} = 1.875 \frac{\mu\text{mol}}{\text{mg}} = 15.64 \text{ mg}$$

to 10 mM stock: add 182.5  $\mu\text{l}$  0.1x TE bufferto 5 mM stock: add  $182.5 \times 2 = 365 \text{ } \mu\text{l}$  0.1x TE buffer

↓  
not clear, so add more 0.1x TE

home-made from:  
1x TE buffer (10 mM Tris 8.0  
+ 1 mM EDTA 8.0)

to 2 mM stock: (+ 547.5  $\mu\text{l}$ )

Save in two 1.5 ml tubes.

1:10 dilute with NF H<sub>2</sub>O

2023. 5.6

10  $\mu\text{l}$  → 1 ml

hoe-C4 APBF (5 mM stock)

Prepare 18  $\mu\text{M}$  (0.1% DMSO) in 20  $\mu\text{M}$  hairpin DNA @ in DPBS buffer  
 (1.6  $\mu\text{l}$  probe + 1  $\mu\text{l}$  0.1 M NaOH) 1) 0  $\mu\text{M}$  hairpin DNA in DPBS buffer  
 } + 8  $\mu\text{l}$  DMSO 2 min (20  $\mu\text{l}$  2 mM DNA + 980  $\mu\text{l}$  DPBS  
 2.4 then add 0  $\mu\text{M}$  DPBS } 20  $\mu\text{l}$  0.1x TE + 980  $\mu\text{l}$  DPBS

hoe-EG1-Ac2 APBF (5 mM stock) (800  $\mu\text{l}$ )

162.06  $\mu\text{l}$  Probe + 1  $\mu\text{l}$  0.1 M NaOH  
 } + 7.04  $\mu\text{l}$  DMSO (800  $\mu\text{l}$ )  
 2.4

$$\frac{10 \times 40}{[A][B]} \Rightarrow [AB]$$

hoe-Eh3-Ac2 APBF (2.85 mM stock)

2.8  
 } 350  $\mu\text{l}$  Probe + 1  $\mu\text{l}$  0.1 M NaOH  
 } 1.50  $\mu\text{l}$  DMSO (800  $\mu\text{l}$ )  
 1.2

hoechst (16.8 mM stock)

0.988  $\mu\text{l}$  Probe + 1  $\mu\text{l}$  0.1 M NaOH (1600  $\mu\text{l}$ )  
 } 7.92  $\mu\text{l}$  DMSO

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Book No:

Cont'd from page

Experiment Title

ex em

C4: 346 (0.181) 518 (0.196)

347 (0.163) 518 (0.173)

C4-DNA: 15 min

1h

350 (0.240) 513 (0.260) 349 (0.232) 513 (0.247)

emission: ex <sup>347</sup>~~350~~ ex ~~513~~ 518 slit 5/10 nm (C4)

ex 350 ex 513 slit 5/10 nm (C4-DNA, 1h)

EG1: 340 (0.150) 517 (0.180)

emission EG1: ex 340 ex 517 slit 5/10 nm

EG1-DNA: 1h

345 (0.172) 514 (0.206)

emission: ex 345 ex 514 slit 5/10 nm

EG3: 338 (0.147) 517 (0.184)

emission: ex 338 ex 517 slit 5/10 nm

EG3-DNA: 1h

348 (0.156) 513 (0.196)

emission: ex 348 ex 513 slit 5/10 nm

hoechst 33342: <sup>340</sup>~~350~~ (0.292)

emission: ex 340 slit 5/10 nm

hoechst-DNA: ex 351 (0.289)

emission: ex 351 slit 5/10 saturated slit 5/5 nm ok

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