Dialysis ~114 μM CAII in PBS

1:2 dilution of 228 μM CAII stock w/ PBS Buffer in Maxi size D-tube dialyzer against 1L of PBS buffer w/ stirring @ 4°C (coldroom) O/N.

→ Need to check purity from supplier!

2011 Apr 27 9.36 am

Filtered dialysis buffer

Diluted 1 mL dialyzed protein w/ 1 mL filtered dialysis buffer to make ~ 50 μM protein stock

Diluted (1:4) 25 μL of protein soln into 75 μL filtered dialysis buffer →

$A_{280} = 0.71549$ AU

∴ ~50 μM protein soln is actually 57.2 μM by spec

$$2.1\ \text{mL} \left| \frac{40\ \mu\text{M}}{} \right| \frac{\text{mM}}{\text{M}} \left| \frac{\text{L}}{10^3\ \text{mL}} \right| \frac{\text{M}}{10^6\ \mu\text{M}} \left| \frac{}{52.7\ \mu\text{M}} \right| \frac{10^6\ \mu\text{M}}{\text{M}} \left| \frac{\text{mL}}{} \right.$$

Added 1.467 μL of 57.2 μM protein soln to 0.633 μL buffer to make ~ 2.1 mL of ~ 40 μM protein soln.

Actual A = 0.48059 for 1:4 dilution of ~40 μM stock

$A = \varepsilon b c$

$\frac{0.48059}{50,070\ M^{-1}cm^{-1}(1cm)} \times \frac{4}{1} \times 10^3 \times 10^3 = 38.4\ \mu M$ protein

Friday @ 2PM (meeting w/ Brian)

ITC

[CBS] 1782 μM

[CaCl] 40 μM

$\varepsilon_{corr} = 50,070\ M^{-1}cm^{-1}$

Made 1:2 dilution of O/N dialysis protein stock (~228 μM).

$A = \frac{0.71549}{\frac{50,070\ M^{-1}}{cm}(cm)} \cdot \frac{4}{1} \times 10^3 \times 10^3 = 57.2\ \mu M$

$57.2\ \mu M \cdot \frac{x}{2.1mL} = 40\ \mu M$ $x = 1.467mL$ of 57.2 stock

$2.1mL - 1.467mL = 0.633mL$ buffer

Measured A of ~40 μM solution:

A = 0.48059

$$c = \frac{0.48059}{50{,}570\ M^{-1}cm^{-1}(cm)} \cdot \frac{4}{1} \cdot 10^3 \cdot 10^3 = 38.4\ \mu M \text{ protein}$$

[CBS] mM ≈ 1.783

MW = 201.2 g/mol

vol ≈ 10 mL

$$0.010L \cdot 1.783 \times 10^{-3}\frac{mol}{L} \cdot \frac{201.2g}{mol} = 0.0036g$$

Actually measured 0.0034g CBS

$$\frac{3.4mg}{X} = \frac{3.6mg}{10} \qquad X = 9.478\ mL\ PBS\ buffer$$

Note CBS was static → stuck to weighing paper so had to rinse off w/ buffer.

CBS, $\varepsilon_{272nm} \approx$ 1307 $M^{-1}cm^{-1}$ (±126)

$\frac{1}{4}$ dilution of ~1.783mM stock

A = 0.63718 @ 272nm → although 274nm looks λ_{max}

$$\frac{0.63718}{1307(1)} \cdot \frac{4}{1} \cdot 10^3 = 1.950\ mM$$ actual CBS concentration

Run 1:

[CA II] ~~mM~~ μM = 38.4

[CBS] mM = 1.783

injects = 10

Vol injects = 10 μL

name = 042711a, saved in DATA/Chodera

run file saved as 10μL - 40μM

*No control injection instead moved plunger down position once x10μL

Run 2:

$A = \frac{0.48678}{50{,}090\ M^{-1}cm^{-1}(cm)} \cdot \frac{4}{1} \times 10^3 \times 10^3 = 38.9\ \mu M$

[CA II] = 38.9 μM

[CBS] = 1.783 mM

injects = 15

Vol injects = 6.7 μL

name = 042711b

Run 3: Heat of dilution

for run # 2, same params

name = 042711c

* Stopped @ pt # 5 b/c of baseline shift after pt # 2

Made 1:2 dilution w/ all remaining protein from dialysis

57.2 μM · $\frac{0.900\ mL}{x}$ = 40 μM + $\frac{60\ \mu L}{x}$ · 52 μM = 40

$x = 1.287$
− 900 protein stock
0.387 buffer

$x = 85.5$
− 60
25.5 μL buffer

Run #4
Repeat heat of dilution w/ Run #2 params.
Name d = 042711d

Run #5
[CAII] x 40 μM
[CBS] = 1.783 mM
# injects = 20
Vol inject = 5 μL
name = 042711e

Ran out of protein added 80 μL, took Abs after w/ leftover
$c = \frac{0.39238}{50,070\ (1cm)} \cdot \frac{4}{1}$
$\times 10^3 \times 10^3$
[CAII] = 31.4 μM

There was a bubble in the cell, happened during loading and dilution of sample. Data from this run isn't any good. Need to repeat.

5/5/2011

Dilution for UV-Vis spec. CA II

$38.4\,\mu M \cdot \frac{1}{4} = 9.60\,\mu M$ for 1cm pL

For nano-drop pL = 0.1 cm (10mm)

should probably do x10 for nano-drop

$C = 95.98\,\mu M$

$A = \varepsilon bc = 50{,}070\,M^{-1}cm^{-1}(0.1\,cm)(9.80\times10^{-5}\,M)$

$A = 0.48059$ ← Target Abs btw 0.1 and 1

[CA II] ~~pp~~ stock = 228 μM

Will do 1:2 dilution for dialysis

228 ÷ 2 = 114 μM after dialysis

Sample for nano-drop: (tomorrow)

$114\,\mu M \cdot \frac{1}{x} = 95.98\,\mu M \quad x = 1.188$

⇒ or $114\,\mu M \cdot \frac{x}{10\,\mu L} = 95.98\,\mu M$

$x = 8.419\,\mu L$ of 114 μM stock

$1.581\,\mu L$ buff.

5/12/11 Heat of Dilution control Experiments

1st 80 μM CAII

From 99.89 μM stock

$$99.89\ \mu M \cdot \frac{1.70\ mL}{X} = 80\ \mu M$$

$X = 2.12266\ mL = 2.123\ mL$

$- 1.70\ mL$ protein stock

$0.423\ mL$ buffer

2nd 40 μM CAII

Used 80 μM from previous run (recovered)

First calc. absorbance w/ nanodrop:

~80 μM → 75.9 μM

(1:10 dilution of ~80 μM → 5 μM?)

After run 051211a → ~75 μM CAII diluted to ~70 μM.

To make 40 μM stock

$70\ \mu M \times \frac{X}{2.1\ mL\ (exact)} = 40\ \mu M$

$X = 1.70\ mL$ recovered 80 μM CAII

$2.1 - 1.7 = 0.4\ mL$ buffer.

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* Had to degass protein sample 2x — was very bubbly
Run name 051211b
Same params as previous

- 3rd Run CAII (10 μM) 20 x 5 μL injections, 446 μM CBS — Follow-up to experiments on 5/6/11

Used ~80 μM CAII recovered stock again

→ $70\,\mu M \cdot \frac{x}{2.1\,mL} = 10\,\mu M$

$x = 0.3$ mL stock protein
1.8 mL PBS

Degassed again
Saved as 051211c

5/19/11

Finish 20 x 5 µL injections

1st 20 x 5 µL CBS into PBS buffer
300s delay
240s spacing
Used 446 µM CBS from 5/12/11
Saved as 051911a

2nd 20 x 5 µL CBS into ~10 µM CA II
Same as above
refilled syringe w/ ~150 µL remaining CBS degassed in small cuvette by simply moving syringe position back to "closed" → purged twice, 10 µL down injection
saved as 051911b

3rd 20 x 5 µL buffer into ~10 µM CA II
Same as above
→ ran out of protein stock, had to add more buffer to cell (see following page)
Saved as 051911c

To do list:

- Protein stock from dialysis O/N
  - store
  - check concentration w/ nano-drop
  - filter + store buffer (dialysis #3 now)

- Check ~10 μM CAII stocks by nano-drop
- Make another 10 μM stock from 80 μM CAII recovered (same as previous) for buffer into protein run

$$\sim 70\,\mu M\ CAII \cdot \frac{x}{2.1\,mL} = 10\,\mu M$$

$x = 0.3\,mL$ CAII stock

$1.8\,mL$ PBS buffer

→ or just add to previous ~10 μM CAII stock

$$\sim 70\,\mu M \cdot \frac{0.175\,mL}{x} = 10\,\mu M$$

$x = 1.225\,mL$ total

$- 0.175\,mL$

$1.050\,mL$ buffer added to ~2mL of 10 μM CAII stock from 5/12/11

Protein for run #3 051911c.

Had ~1.5mL of 8.07μM stock (abs from nano-drop) prior to addition of extra buffer.

To load needed ~2.1mL → 2.1 - 1.5 = 0.6mL buffer added

$8.07\,\mu M \cdot \frac{1.5\,mL}{2.1\,mL} = 5.76\,\mu M$

That's at the lower end; however buffer was added to "top off" the cell after protein solution was loaded, so actual concentration is somewhere btwn the two.

* Can back calculate after run is finished b/c no CBS present

Abs was 0.337 for sample taken after the titration had finished →

Started w/ 1.43mL of [CAII] at unknown concentration. Diluted by 100μL of buffer during the course of titration for a final volume of 1.53 mL

$[CAII]_f = 6.73\,\mu M$

$$6.73\,\mu M \times \frac{1.53\,mL}{1.43\,mL} = 7.20\,\mu M \Leftarrow [CAII]_I$$

Starting [CAII] was 7.20μM prior to titration w/ buffer.