Labeling L11-S87C with the Cy5 maleimide dye

M-labeling buffer (50 ml):

25 mM Tris 7.0, Add 1.25 mL 1 M stock.
100 mM NaCl, Add 1.25 mL of 4 M stock.
2 mM EDTA, Add 0.5 mL of 200 mM stock.
Bring to volume with deionized water. Store at 4ºC overnight.

- Equilibrate the NAP5 column with 12 mls of labeling buffer.
- Load 0.5 ml (~ 3.4 mg/ml) of L11-87C protein and exchange into M labeling buffer.
- Perform the BioRad protein assay to estimate the concentration of the exchanged protein.

- Make 100mM TCEP: Weigh 29.2 mg of TCEP into 1 ml of water. Adjust pH to 7.0 with 5N KOH
- Make 1 mM TCEP: Add 10 ul of 100mM TCEP to 990 ul of labeling buffer.

Cy5 Dye preparation: Each pack has 250 ug of powder. Add 342 ul of DMF to the pack (vial).
The FWT of the dye is 817. The concentration of Cy5 dye is 894 uM.

MESNA (500mM): Weigh 82 mg of MESNA into 1 mL of M labeling buffer.
MESNA (33mM): Add 66 uL of 500mM MESNA to 934 uL of M.labeling buffer.

Labeling Reaction Solution:	Qty	Final
L11 Protein (from stock uM)	uL	50 uM
TCEP (1mM)	50 uL	50 uM
Cy5 maleimide(894 uM)	uL	75 uM
MESNA (33mM)	91 uL	3 mM
Buffer	uL	
Total volume	1000 uL	

Add the TCEP to protein and incubate for 45 min at RT.
Add the Cy5 dye and incubate @RT for ½, 1, 2, 4 hours. Quench the reactions with MESNA.
Run the quenched aliquots (250uL each) on Source S column. Run a linear gradient from 100% A to 100% B. The labeled protein is eluted around 22 min (1ml/min). Fractions were collected (0.5ml/min).

Purification on Source S FPLC column:

Buffers for FPLC:

Buffer A:

20mM Hepes pH 7.0	1M	20 ml
0.5mM EDTA	0.5M	1 ml
6M Urea (M.Wt is 60.06)	Powder	360.36 g
6mM BME	14.3	0.419 ml
10mM NaCl	4M	2.5 ml
10mM $MgCl_2$	100mM	100 ml

Buffer B:

20mM Hepes pH 7.0	1M	20 ml
0.5mM EDTA	0.5M	1 ml
6M Urea (M.Wt is 60.06)	Powder	360.36 g
6mM BME	14.3	0.419 ml
400mM NaCl	Salt	23.96 g
10mM $MgCl_2$	100mM	100 ml

Labeling L11-S87C with the Cy5 maleimide dye

Wintermaeyer buffer: 1L

	Stock	Qty
50mM Tris pH 7.6	1M	50 ml
30 mM NH4Cl	4M	7.5 ml
70mM KCl	3M	23.33 mL
7mM $MgCl_2$	100mM	70 ml
1mM DTT	1mM	1 mL

Pool the fractions and dialyze against the W. Buffer twice using dialysis cassettes (10 K MWCO) and concentrate on spin filter. Measure the O.D @ 230 nm and @ 650nm and do the BioRad assay on the samples.

Protein concentration was determined by absorbance at 230nm for CTD as described by Xing and Draper (1996), for the NTD as described by Bausch et al.(2005) and for the others using extinction coefficient at 280 nm.

For CTD the Exct.Coeft @ 230nm is $24.8 \times 10^{3}\ M^{-1}\ cm^{-1}$.

For NTD the Exct.Coeft @ 230nm is $45 \times 10^{3}\ M^{-1}\ cm^{-1}$.

Xing, Y and Draper, DE. Cooperative interactions of RNA and thiostrepton antibiotic with two domains of ribosomal protein L11. *Biochemistry* **35**:1581-8. 1996.

Bausch, SL, Poliakova, E and Draper, DE. Interactions of the N-terminal domain of ribosomal protein L11 with tiostrepton and rRNA. *J. Biol. Chem.* **280**:29956-63. 2005.