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Binding of Rab29 to the LRRK2 Armadillo domain by Microscale Thermophoresis

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Suzanne Pfeffer

Microscale thermophoresis (MST) is a powerful tool to measure the affinities of interactions between proteins. We present here our method for determining the binding of Rab29 GTPase to the LRRK2 (Leucine rich repeat kinase 2) N-terminal Armadillo domain. Work from several labs has shown that Rab29 can recruit LRRK2 to the Golgi, where it normally resides, or to other compartments, when artificially relocalized to another cellular compartment. MST has enabled us to define the precise binding site for Rab GTPases on the LRRK2 Armadillo domain.

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ASAPCRN

protocol ,

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50221

Eppendorf LoBind tubes 0.5mL Catalog No. 0030108434

Eppendorf LoRetention tips 0.1-10uL Catalog No. 022493018

pET21b HisSumo Rab29 full length wild type (tag cleaved off)

Buffer exchange isolated Proteins

1 Buffer: 50mM Hepes pH 8, 100mM NaCl, 5mM $MgCl_2$, 20µM GTP, 0.2mM TCEP, 5% glycerol

1. Use either a Nanotemper “A-Column” provided in labeling kit or (our preference) 0.5 ml Zeba Spin column 7MWCO. Twist off bottom of column and loosen cap.
2. Prespin column: Centrifuge at 4°C 1500xG for 1 min to remove storage solution.
3. Apply 300µL of buffer to center of resin bed and centrifuge at 1500xG for 1min. Do this 4X.
4. Place column in fresh collection tube. Apply 100µL of your sample to resin and spin at 1500xG for 2min.
5. Protein is in collected flowthrough.

2

2.1 0.

3 'font-size:12.0pt;line-height:107%;font-family:Arial;mso-fareast-font-family:

4 Arial'>

5 font-family:Arial'>Labeling is done with 2nd Generation NHS RED

6 label from Nanotemper

6.1 1.

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7 'font-size:12.0pt;line-height:107%;font-family:Arial;mso-fareast-font-family:

8 Arial'>

9 font-family:Arial'>Dye is resuspended in 25µl DMSO as per protocol from Nanotemper

10 to make it 600µM final stock concentration

10.1 2.

11 'font-size:12.0pt;line-height:107%;font-family:Arial;mso-fareast-font-family:

12 Arial'>

13 font-family:Arial'>Set up 100µL labeling reaction using a 3:1 ratio of dye to

14 protein; Dye is used at final concentration of 30µM; buffer exchanged Armadillo

15 is used at a final concentration of 10µM (note: reducing reagent will interfere

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16 with labeling. Low concentration TCEP is acceptable)

16.1 3.

17 'font-size:12.0pt;line-height:107%;font-family:Arial;mso-fareast-font-family:

18 Arial'>

19 font-family:Arial'>Add above buffer to bring volume to 100µL final volume, mix

20 by flicking tube

20.1 4.

21 'font-size:12.0pt;line-height:107%;font-family:Arial;mso-fareast-font-family:

22 Arial'>

23 font-family:Arial'>Incubate for 30min in dark at room temperature

23.1 5.

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'font-size:12.0pt;line-height:107%;font-family:Arial;mso-fareast-font-family:

Arial'>

font-family:Arial'>Desalt excess dye using column B provided in kit or another

Zeba Spin column as before

27.1 6.

'font-size:12.0pt;line-height:107%;font-family:Arial;mso-fareast-font-family:

Arial'>

font-family:Arial'>Spin labeled sample hard 14000xg for 10min at 4°C to remove

any aggregates

31.1 7.

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32 'font-size:12.0pt;line-height:107%;font-family:Arial;mso-fareast-font-family:

33 Arial'>

34 font-family:Arial'>Take absorbance 280nm of labeled protein and use extinction

35 coefficient and the Nanotemper Degree of Labeling (DOL) calculator to determine

36 concentration and DOL https://nanotempertech.com/dol-calculator/. DOL should be

37 between 0.5-1

38 Normal

39 0

40 false

41 false

42 false

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43 EN-US

44 JA

45 X-NONE

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47 table.MsoNormalTable

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49 mso-tstyle-rowband-size:0;

50 mso-tstyle-colband-size:0;

51 mso-style-noshow:yes;

52 mso-style-priority:99;
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font-family:Calibri;
mso-ascii-font-family:Calibri;
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https://dx.doi.org/10.17504/protocols.io.ewov14mn7vr2/v1

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65 mso-hansi-font-family:Calibri;

66 mso-hansi-theme-font:minor-latin;}

67 Labeling

68 of LRRK2 Armadillo
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