## **Supporting Information for:**

Nuclear Magnetic Resonance Structural Mapping Reveals Promiscuous Interactions between Clathrin-Box Motif Sequences and the N-Terminal Domain of the Clathrin Heavy Chain

Yue Zhuo<sup>1\*</sup>, Kristin E. Cano<sup>1\*</sup>, Liping Wang<sup>1</sup>, Udayar Ilangovan<sup>1</sup>, Andrew P. Hinck<sup>1</sup>, Rui Sousa<sup>1</sup>, and Eileen M. Lafer<sup>1</sup>

<sup>1</sup>Department of Biochemistry and Center for Biomedical Neuroscience, University of Texas Health Science Center at San Antonio, San Antonio, TX 78229

\*these two authors contributed equally to the study

^Corresponding Author

Address Correspondence to:

Dr. Eileen M. Lafer

Department of Biochemistry

University of Texas Health Science Center at San Antonio

7703 Floyd Curl Drive

San Antonio, TC 78229

+1-210-567-3764 (phone)

+1-210-567-6595 (fax)

Lafer@uthscsa.edu

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	A	AD0	Α.	D0	A DO :-		
(a) _	Approximate	AP2 peptide expt.	A	P2 peptide expt.		eptide expt.	
	[Peptide]/[TD]	[TD] uM		[Peptide] uM	Exact	Peptide]/[TD]	
	-						
			le:				
	0	765.00		0.00		0.00	
	1	400.00	,	400.00		1.00	
	2	371.13		739.18		1.99	
	3	345.54		1039.92		3.01	
	4	322.82		1306.85		4.05	
	5	303.70		1531.53		5.04	
	6	286.71		1731.16		6.04	
	7.5	264.20		1995.61		7.55	
	9	245.14		2219.64		9.05	
		(					
(b)	Approximate	AP180 peptide 1 expt.	AP1		AP180 p	eptide 1 expt.	
` '	[Peptide]/[TD]	[TD] uM		[Peptide] uM	Exact [	Peptide]/[TD]	
	0	500.00		0.00		0.00	
	1	469.26		494.37		1.05	
	2	443.27		912.21		2.06	
	3	420.09		1284.90		3.06	
	4	399.43		1617.12		4.05	
	5	381.04		1912.91		5.02	
	6	363.49		2195.08		6.04	
	7.5	340.04		2572.17		7.56	
	9	320.17	2	2891.72		9.03	
		323.17		20011112		0.00	
(c)	Approximate	AP180 peptide 2 expt.	AP1	180 peptide 2 expt.	AP180 r	peptide 2 expt.	
(c)	Approximate [Peptide]/[TD]	AP180 peptide 2 expt.	AP1				
(c)	Approximate [Peptide]/[TD]	AP180 peptide 2 expt. [TD] uM	AP1	180 peptide 2 expt. [Peptide] uM		peptide 2 expt. Peptide]/[TD]	
(c)			AP1				
(c)	[Peptide]/[TD]	[TD] uM	AP1	[Peptide] uM		Peptide]/[TD]	
(c)	[Peptide]/[TD]	[TD] uM	AP1	[Peptide] uM		Peptide]/[TD] 0.00	
(c)	[Peptide]/[TD]  0 1	500.00 453.07	AP1	0.00 459.87		0.00 1.01	
(c)	[Peptide]/[TD]  0 1 2	500.00 453.07 414.58	AP1	0.00 459.87 837.14		0.00 1.01 2.02	
(c)	[Peptide]/[TD]  0 1 2 3	500.00 453.07 414.58 383.11	AP1	0.00 459.87 837.14 1145.54		0.00 1.01 2.02 2.99	
(c)	[Peptide]/[TD]  0 1 2 3 4	500.00 453.07 414.58 383.11 355.20	AP1	0.00 459.87 837.14 1145.54 1419.04		0.00 1.01 2.02 2.99 4.00	
(c)	[Peptide]/[TD]  0 1 2 3 4 5	500.00 453.07 414.58 383.11 355.20 331.52	AP1	0.00 459.87 837.14 1145.54 1419.04 1651.11		0.00 1.01 2.02 2.99 4.00 4.98	
(c)	[Peptide]/[TD]  0 1 2 3 4 5 6	500.00 453.07 414.58 383.11 355.20 331.52 310.45	AP1	0.00 459.87 837.14 1145.54 1419.04 1651.11 1857.56		0.00 1.01 2.02 2.99 4.00 4.98 5.98	
(c)	[Peptide]/[TD]  0 1 2 3 4 5 6 7.5	500.00 453.07 414.58 383.11 355.20 331.52 310.45 283.15	AP1	0.00 459.87 837.14 1145.54 1419.04 1651.11 1857.56 2125.13		0.00 1.01 2.02 2.99 4.00 4.98 5.98 7.51	
(c)	[Peptide]/[TD]  0 1 2 3 4 5 6	500.00 453.07 414.58 383.11 355.20 331.52 310.45	AP1	0.00 459.87 837.14 1145.54 1419.04 1651.11 1857.56		0.00 1.01 2.02 2.99 4.00 4.98 5.98	
	[Peptide]/[TD]  0 1 2 3 4 5 6 7.5	500.00 453.07 414.58 383.11 355.20 331.52 310.45 283.15 260.80		0.00 459.87 837.14 1145.54 1419.04 1651.11 1857.56 2125.13 2344.20	Exact [i	0.00 1.01 2.02 2.99 4.00 4.98 5.98 7.51 8.99	eptide expt.
(c)	[Peptide]/[TD]  0 1 2 3 4 5 6 7.5 9	500.00 453.07 414.58 383.11 355.20 331.52 310.45 283.15 260.80		0.00 459.87 837.14 1145.54 1419.04 1651.11 1857.56 2125.13 2344.20	Exact [i	0.00 1.01 2.02 2.99 4.00 4.98 5.98 7.51 8.99	
	[Peptide]/[TD]  0 1 2 3 4 5 6 7.5	500.00 453.07 414.58 383.11 355.20 331.52 310.45 283.15 260.80		0.00 459.87 837.14 1145.54 1419.04 1651.11 1857.56 2125.13 2344.20	Exact [i	0.00 1.01 2.02 2.99 4.00 4.98 5.98 7.51 8.99	
	[Peptide]/[TD]  0 1 2 3 4 5 6 7.5 9	500.00 453.07 414.58 383.11 355.20 331.52 310.45 283.15 260.80		0.00 459.87 837.14 1145.54 1419.04 1651.11 1857.56 2125.13 2344.20	Exact [i	0.00 1.01 2.02 2.99 4.00 4.98 5.98 7.51 8.99	
	[Peptide]/[TD]  0 1 2 3 4 5 6 7.5 9  Approximate [Peptide]/[TD]	500.00 453.07 414.58 383.11 355.20 331.52 310.45 283.15 260.80 AP2 mutant peptide examples of the period of the pe	xpt.	0.00 459.87 837.14 1145.54 1419.04 1651.11 1857.56 2125.13 2344.20	Exact [i	0.00 1.01 2.02 2.99 4.00 4.98 5.98 7.51 8.99	tide]/[TD]
	[Peptide]/[TD]  0 1 2 3 4 5 6 7.5 9  Approximate [Peptide]/[TD]	500.00 453.07 414.58 383.11 355.20 331.52 310.45 283.15 260.80 AP2 mutant peptide example of the control of the	xpt.	0.00 459.87 837.14 1145.54 1419.04 1651.11 1857.56 2125.13 2344.20	Exact [i	0.00 1.01 2.02 2.99 4.00 4.98 5.98 7.51 8.99	0.00
	[Peptide]/[TD]  0 1 2 3 4 5 6 7.5 9  Approximate [Peptide]/[TD]	500.00 453.07 414.58 383.11 355.20 331.52 310.45 283.15 260.80  AP2 mutant peptide example of the second of the se	).00 3.58	0.00 459.87 837.14 1145.54 1419.04 1651.11 1857.56 2125.13 2344.20	Exact [I	0.00 1.01 2.02 2.99 4.00 4.98 5.98 7.51 8.99	0.00 1.02
	[Peptide]/[TD]  0 1 2 3 4 5 6 7.5 9  Approximate [Peptide]/[TD]  0 1 2	500.00 453.07 414.58 383.11 355.20 331.52 310.45 283.15 260.80  AP2 mutant peptide example of the control of th	0.00 3.58 2.67	0.00 459.87 837.14 1145.54 1419.04 1651.11 1857.56 2125.13 2344.20  AP2 mutant peptic [Peptide] ul	Exact [I	0.00 1.01 2.02 2.99 4.00 4.98 5.98 7.51 8.99	0.00 1.02 2.01
	[Peptide]/[TD]  0 1 2 3 4 5 6 7.5 9  Approximate [Peptide]/[TD]  0 1 2 3	500.00 453.07 414.58 383.11 355.20 331.52 310.45 283.15 260.80  AP2 mutant peptide example of the control of th	0.00 3.58 2.67 5.18	0.00 459.87 837.14 1145.54 1419.04 1651.11 1857.56 2125.13 2344.20  AP2 mutant peptic [Peptide] ul	0.00 470.60 869.89	0.00 1.01 2.02 2.99 4.00 4.98 5.98 7.51 8.99	0.00 1.02 2.01 3.02
	[Peptide]/[TD]  0 1 2 3 4 5 6 7.5 9  Approximate [Peptide]/[TD]  0 1 2 3 4	500.00 453.07 414.58 383.11 355.20 331.52 310.45 283.15 260.80  AP2 mutant peptide example (TD) uM	D.000 3.58 2.67 5.18 D.70	0.00 459.87 837.14 1145.54 1419.04 1651.11 1857.56 2125.13 2344.20  AP2 mutant peptic [Peptide] ul	0.00 470.60 869.89 1225.11	0.00 1.01 2.02 2.99 4.00 4.98 5.98 7.51 8.99	0.00 1.02 2.01 3.02 4.05
	[Peptide]/[TD]  0 1 2 3 4 5 6 7.5 9  Approximate [Peptide]/[TD]  0 1 2 3 4 5	500.00 453.07 414.58 383.11 355.20 331.52 310.45 283.15 260.80  AP2 mutant peptide example (TD) uM	).00 3.58 5.18 ).70 3.91	0.00 459.87 837.14 1145.54 1419.04 1651.11 1857.56 2125.13 2344.20  AP2 mutant peptic [Peptide] ul	0.00 470.60 869.89 1225.11 1541.32 1822.86	0.00 1.01 2.02 2.99 4.00 4.98 5.98 7.51 8.99	0.00 1.02 2.01 3.02 4.05 5.08
	[Peptide]/[TD]  0 1 2 3 4 5 6 7.5 9  Approximate [Peptide]/[TD]  0 1 2 3 4 5 6	500.00 453.07 414.58 383.11 355.20 331.52 310.45 283.15 260.80  AP2 mutant peptide example of the second of the se	xpt. 3.00 3.58 2.67 5.18 3.70 3.91 3.66	0.00 459.87 837.14 1145.54 1419.04 1651.11 1857.56 2125.13 2344.20  AP2 mutant peptic [Peptide] ul	0.00 470.60 869.89 1225.11 1541.32 1822.86 2058.64	0.00 1.01 2.02 2.99 4.00 4.98 5.98 7.51 8.99	0.00 1.02 2.01 3.02 4.05 5.08 6.04
	[Peptide]/[TD]  0 1 2 3 4 5 6 7.5 9  Approximate [Peptide]/[TD]  0 1 2 3 4 5	500.00 453.07 414.58 383.11 355.20 331.52 310.45 283.15 260.80  AP2 mutant peptide example of the second of the se	).00 3.58 5.18 ).70 3.91	0.00 459.87 837.14 1145.54 1419.04 1651.11 1857.56 2125.13 2344.20  AP2 mutant peptic [Peptide] ul	0.00 470.60 869.89 1225.11 1541.32 1822.86	0.00 1.01 2.02 2.99 4.00 4.98 5.98 7.51 8.99	0.00 1.02 2.01 3.02 4.05 5.08

Figure S1. Peptide and TD concentrations used for each step of the four NMR titrations performed in the study. AP2 peptide (panel a), AP180 peptide 1 (panel b), AP180 peptide 2 (panel c), and AP2 mutant peptide (panel d).

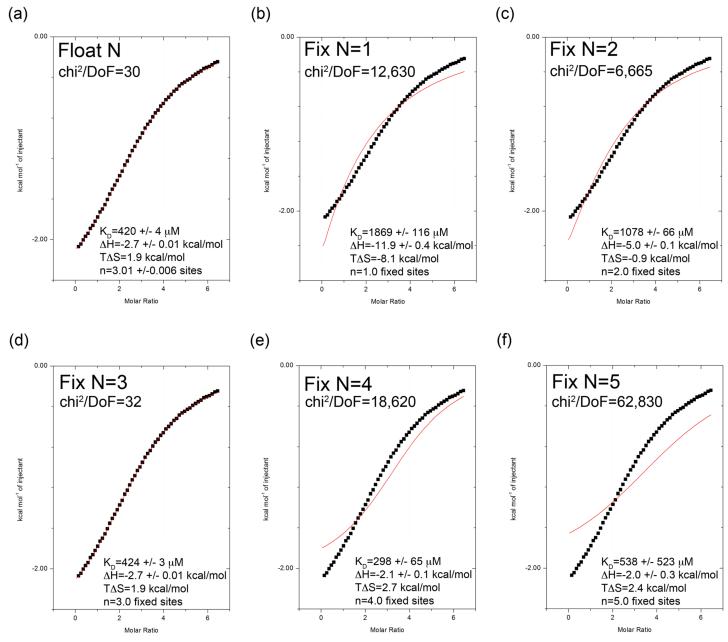


Figure S2. Analysis of ITC data for the titration of AP2 peptide into clathrin TD. The thermogram displayed in Fig. 1a was integrated and fit with Microcal modified Origin 7 software to an N independent binding sites model with either N floated (panel a), or fixed to 1 (panel b), 2 (panel c), 3 (panel d), 4 (panel e) or 5 (panel f).