SUPPORTING INFORMATION

Mechanism for recognition of polyubiquitin chains: Balancing affinity through interplay between multivalent binding and dynamics

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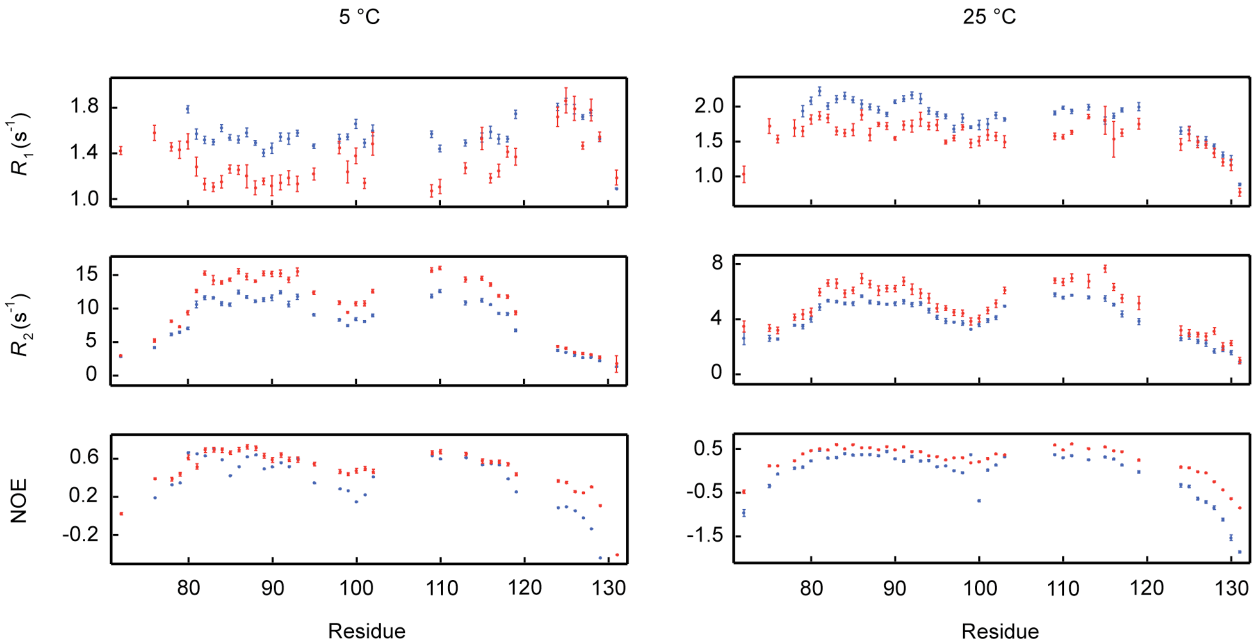


Figure S1. ^{15}N-R_1, -R_2 and {^{1}H}-^{15}N NOE values for RAP80-tUIM at 600 MHz (blue) and 800 MHz (red).

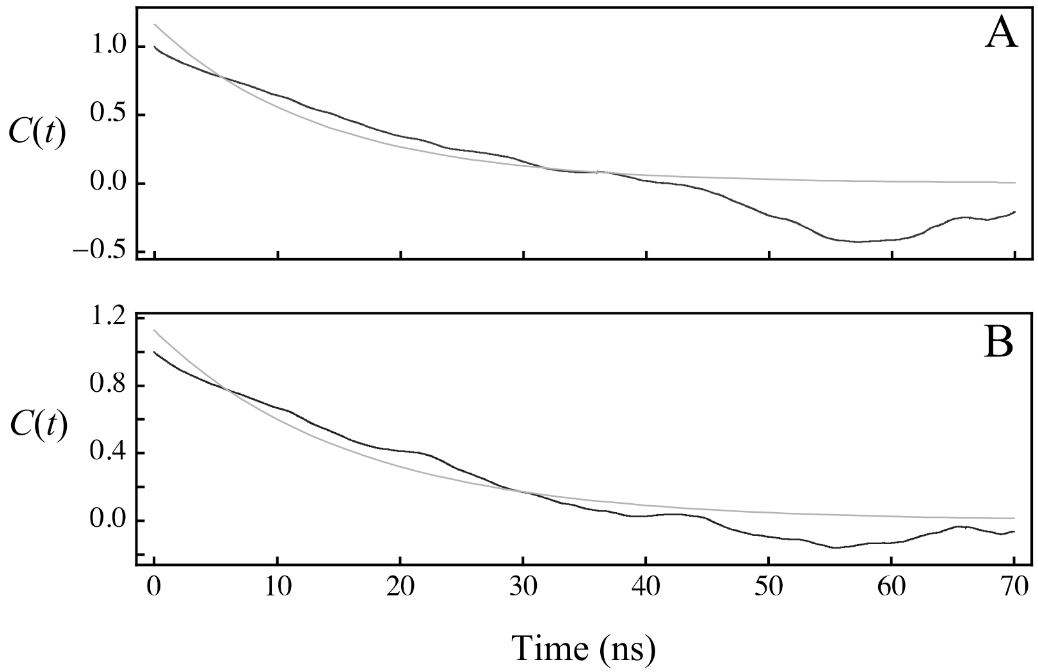
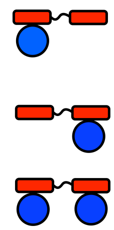


Figure S2. Time correlation functions for vectors from the first two interUb subunits perpendicular (A) and parallel (B) to the long axis of a tandem Ub_3 chain. The correlation functions calculated from the MD trajectory are shown in blue, fits to a two parameter monoexponential decay are shown in red.

Bound states and equilibria for the RAP80-tUIM-monoUb interaction:



$$[RAP80]_{total} = [RAP80] + [RAP80 \bullet Ub]_1 + [RAP80 \bullet Ub]_2 + [RAP80 \bullet 2Ub]$$

$$[Ub]_{total} = [Ub] + [RAP80 \bullet Ub]_1 + [RAP80 \bullet Ub]_2 + 2[RAP80 \bullet 2Ub]$$

$$[RAP80 \bullet Ub]_1 = \frac{[RAP80][Ub]}{K_{D,mono}}$$

$$[RAP80 \bullet Ub]_2 = \frac{[RAP80][Ub]}{K_{D,mono}}$$

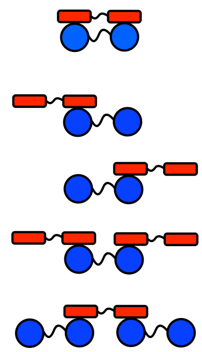
$$[RAP80 \bullet 2Ub] = \frac{[RAP80 \bullet Ub]_1[Ub]}{2K_{D,mono}} + \frac{[RAP80 \bullet Ub]_2[Ub]}{2K_{D,mono}}$$

Expressions for the fraction of [U-^{15}N]-RAP80-tUIM bound to Ub, for the N-terminal and C-terminal UIM.

$$f_{bound}^{[U-^{15}N]-RAP80-tUIM}{}_{N-UIM} = \frac{\left(1[RAP80 \bullet Ub]_1 + 1[RAP80 \bullet 2Ub]\right)}{[RAP80]_{total}}$$

$$f_{bound}^{[U-^{15}N]-RAP80-tUIM}{}_{C-UIM} = \frac{\left(1[RAP80 \bullet Ub]_2 + 1[RAP80 \bullet 2Ub]\right)}{[RAP80]_{total}}$$

Bound states and equilibria for the RAP80-tUIM–Ub$_2$ interaction:



$$[RAP80]_{total} = \begin{pmatrix} [RAP80] + [RAP80 \bullet Ub_2]_1 + [RAP80 \bullet Ub_2]_2 + [RAP80 \bullet Ub_2]_3 \\ +2[2RAP80 \bullet Ub_2]_4 + [RAP80 \bullet 2Ub_2]_5 \end{pmatrix}$$

$$[Ub_2]_{total} = \begin{pmatrix} [Ub_2] + [RAP80 \bullet Ub_2]_1 + [RAP80 \bullet Ub_2]_2 + [RAP80 \bullet Ub_2]_3 \\ +[2RAP80 \bullet Ub_2]_4 + 2[RAP80 \bullet 2Ub_2]_5 \end{pmatrix}$$

$$[RAP80 \bullet Ub_2]_1 = \frac{[RAP80][Ub_2]}{K_{D,mv}}$$

$$[RAP80 \bullet Ub_2]_2 = \frac{[RAP80][Ub_2]}{K_{D,mono}}$$

$$[RAP80 \bullet Ub_2]_3 = \frac{[RAP80][Ub_2]}{K_{D,mono}}$$

$$[2RAP80 \bullet Ub_2]_4 = \frac{[RAP80 \bullet Ub_2]_2[RAP80]}{2K_{D,mono}} + \frac{[RAP80 \bullet Ub_2]_3[RAP80]}{2K_{D,mono}}$$

$$[RAP80 \bullet 2Ub_2]_5 = \frac{[RAP80 \bullet Ub_2]_2[Ub_2]}{2K_{D,mono}} + \frac{[RAP80 \bullet Ub_2]_3[Ub_2]}{2K_{D,mono}}$$

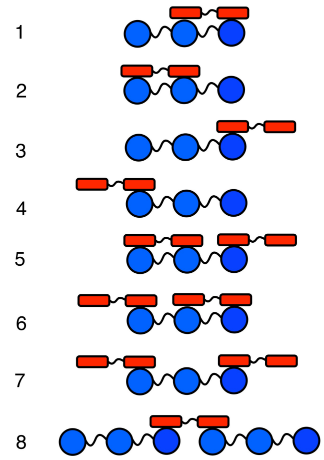
Expression for the fraction of [U-^{15}N]-RAP80-tUIM bound to Ub_2:

$$f_{bound}^{[U-^{15}N]-RAP80-tUIM} = \frac{\begin{pmatrix} 1[RAP80\bullet Ub_2]_1 + 0[RAP80\bullet Ub_2]_2 + 1[RAP80\bullet Ub_2]_3 \\ +1[2RAP80\bullet Ub_2]_4 + 1[RAP80\bullet 2Ub_2]_5 \end{pmatrix}}{[RAP80]_{total}}$$

Expression for the fraction of [U-^{15}N]-Ub_2 bound to RAP80-tUIM:

$$f_{bound}^{[U-^{15}N]-Ub_2} = \frac{\begin{pmatrix} 1[RAP80\bullet Ub_2]_1 + 1[RAP80\bullet Ub_2]_2 + 0[RAP80\bullet Ub_2]_3 \\ +1[2RAP80\bullet Ub_2]_4 + 1[RAP80\bullet 2Ub_2]_5 \end{pmatrix} + \begin{pmatrix} 1[RAP80\bullet Ub_2]_1 + 1[RAP80\bullet Ub_2]_2 + 0[RAP80\bullet Ub_2]_3 \\ +1[2RAP80\bullet Ub_2]_4 + 1[RAP80\bullet 2Ub_2]_5 \end{pmatrix}}{2[Ub_2]_{total}}$$

Bound states and equilibria for the RAP80-tUIM–Ub_3 interaction:



$$[RAP80]_{total} = \begin{pmatrix} [RAP80] + [RAP80 \bullet Ub_3]_1 + [RAP80 \bullet Ub_3]_2 + [RAP80 \bullet Ub_3]_3 + [RAP80 \bullet Ub_3]_4 \\ +2[2RAP80 \bullet Ub_3]_5 + 2[2RAP80 \bullet Ub_3]_6 + 2[2RAP80 \bullet Ub_3]_7 + [RAP80 \bullet 2Ub_3]_8 \end{pmatrix}$$

$$[Ub_3]_{total} = \begin{pmatrix} [Ub_3] + [RAP80 \bullet Ub_3]_1 + [RAP80 \bullet Ub_3]_2 + [RAP80 \bullet Ub_3]_3 + [RAP80 \bullet Ub_3]_4 \\ +[2RAP80 \bullet Ub_3]_5 + [2RAP80 \bullet Ub_3]_6 + [2RAP80 \bullet Ub_3]_7 + 2[RAP80 \bullet 2Ub_3]_8 \end{pmatrix}$$

$$[RAP80 \bullet Ub_3]_1 = \frac{[RAP80][Ub_3]}{K_{D,mv}}$$

$$[RAP80 \bullet Ub_3]_2 = \frac{[RAP80][Ub_3]}{K_{D,mv}}$$

$$[RAP80 \bullet Ub_3]_3 = \frac{[RAP80][Ub_3]}{K_{D,mono}}$$

$$[RAP80 \bullet Ub_3]_4 = \frac{[RAP80][Ub_3]}{K_{D,mono}}$$

$$[2RAP80 \bullet Ub_3]_5 = \frac{[RAP80 \bullet Ub_3]_2[RAP80]}{K_{D,mv} + K_{D,mono}} + \frac{[RAP80 \bullet Ub_3]_3[RAP80]}{K_{D,mv} + K_{D,mono}}$$

$$[2RAP80 \bullet Ub_3]_6 = \frac{[RAP80 \bullet Ub_3]_1[RAP80]}{K_{D,mv} + K_{D,mono}} + \frac{[RAP80 \bullet Ub_3]_4[RAP80]}{K_{D,mv} + K_{D,mono}}$$

$$[2RAP80 \bullet Ub_3]_7 = \frac{[RAP80 \bullet Ub_3]_3[RAP80]}{2K_{D,mono}} + \frac{[RAP80 \bullet Ub_3]_4[RAP80]}{2K_{D,mono}}$$

$$[RAP80 \bullet 2Ub_3]_8 = \frac{[RAP80 \bullet Ub_3]_3[Ub_3]}{2K_{D,mono}} + \frac{[RAP80 \bullet Ub_3]_4[Ub_3]}{2K_{D,mono}}$$

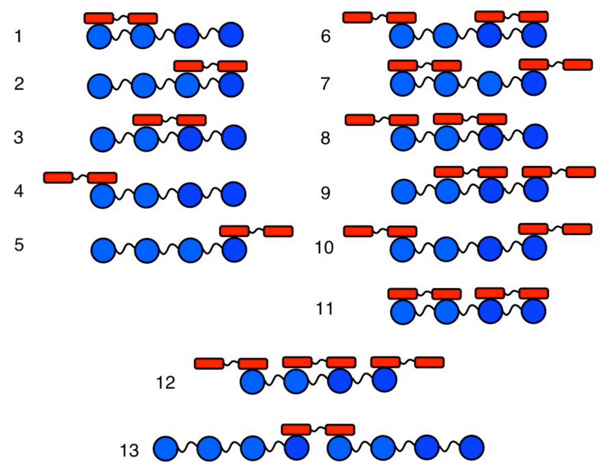
Expression for the fraction of [U-^{15}N]-RAP80-tUIM bound to Ub_3:

$$f_{bound}^{[U-^{15}N]-RAP80-tUIM} = \frac{\begin{pmatrix} 1[RAP80 \bullet Ub_3]_1 + 1[RAP80 \bullet Ub_3]_2 + 1[RAP80 \bullet Ub_3]_3 + 0[RAP80 \bullet Ub_3]_4 \\ +2[2RAP80 \bullet Ub_3]_5 + 1[2RAP80 \bullet Ub_3]_6 + 1[2RAP80 \bullet Ub_3]_7 + 1[2RAP80 \bullet Ub_3]_8 \end{pmatrix}}{[RAP80]_{total}}$$

Expression for the fraction of [U-^{15}N]-Ub_3 bound to RAP80-tUIM:

$$f_{bound}^{[U-^{15}N]-Ub_3} = \frac{\begin{matrix} \begin{pmatrix} 0[RAP80 \bullet Ub_3]_1 + 1[RAP80 \bullet Ub_3]_2 + 0[RAP80 \bullet Ub_3]_3 + 1[RAP80 \bullet Ub_3]_4 \\ +1[2RAP80 \bullet Ub_3]_5 + 1[2RAP80 \bullet Ub_3]_6 + 1[2RAP80 \bullet Ub_3]_7 + 1[2RAP80 \bullet Ub_3]_8 \end{pmatrix} \\ + \\ \begin{pmatrix} 1[RAP80 \bullet Ub_3]_1 + 1[RAP80 \bullet Ub_3]_2 + 0[RAP80 \bullet Ub_3]_3 + 0[RAP80 \bullet Ub_3]_4 \\ +1[2RAP80 \bullet Ub_3]_5 + 1[2RAP80 \bullet Ub_3]_6 + 0[2RAP80 \bullet Ub_3]_7 + 0[2RAP80 \bullet Ub_3]_8 \end{pmatrix} \\ + \\ \begin{pmatrix} 1[RAP80 \bullet Ub_3]_1 + 0[RAP80 \bullet Ub_3]_2 + 1[RAP80 \bullet Ub_3]_3 + 0[RAP80 \bullet Ub_3]_4 \\ +1[2RAP80 \bullet Ub_3]_5 + 1[2RAP80 \bullet Ub_3]_6 + 1[2RAP80 \bullet Ub_3]_7 + 1[2RAP80 \bullet Ub_3]_8 \end{pmatrix} \end{matrix}}{3[Ub_3]_{total}}$$

Bound states and equilibria for the RAP80-tUIM–Ub$_4$ interaction:



$$[RAP80]_{total} = \begin{pmatrix} [RAP80]+[RAP80\bullet Ub_4]_1+[RAP80\bullet Ub_4]_2+[RAP80\bullet Ub_4]_3+[RAP80\bullet Ub_4]_4 \\ +[RAP80\bullet Ub_4]_5+2[2RAP80\bullet Ub_4]_6+2[2RAP80\bullet Ub_4]_7+2[2RAP80\bullet Ub_4]_8 \\ +2[2RAP80\bullet Ub_4]_9+2[2RAP80\bullet Ub_4]_{10}+2[2RAP80\bullet Ub_4]_{11} \\ +3[3RAP80\bullet Ub_4]_{12}+[RAP80\bullet 2Ub_4]_{13} \end{pmatrix}$$

$$[Ub_4]_{total} = \begin{pmatrix} [Ub_4]+[RAP80\bullet Ub_4]_1+[RAP80\bullet Ub_4]_2+[RAP80\bullet Ub_4]_3+[RAP80\bullet Ub_4]_4 \\ +[RAP80\bullet Ub_4]_5+[2RAP80\bullet Ub_4]_6+[2RAP80\bullet Ub_4]_7+[2RAP80\bullet Ub_4]_8 \\ +[2RAP80\bullet Ub_4]_9+[2RAP80\bullet Ub_4]_{10}+[2RAP80\bullet Ub_4]_{11} \\ +[3RAP80\bullet Ub_4]_{12}+2[RAP80\bullet 2Ub_4]_{13} \end{pmatrix}$$

$$[RAP80\bullet Ub_4]_1 = \frac{[RAP80][Ub_4]}{K_{D,mv}}$$

$$[RAP80\bullet Ub_4]_2 = \frac{[RAP80][Ub_4]}{K_{D,mv}}$$

$$[RAP80\bullet Ub_4]_3 = \frac{[RAP80][Ub_4]}{K_{D,mv}}$$

$$[RAP80\bullet Ub_4]_4 = \frac{[RAP80][Ub_4]}{K_{D,mono}}$$

$$[RAP80\bullet Ub_4]_5 = \frac{[RAP80][Ub_4]}{K_{D,mono}}$$

$$[2RAP80\bullet Ub_4]_6 = \frac{[RAP80\bullet Ub_4]_2[RAP80]}{K_{D,mv}+K_{D,mono}} + \frac{[RAP80\bullet Ub_4]_4[RAP80]}{K_{D,mv}+K_{D,mono}}$$

$$[2RAP80 \bullet Ub_4]_7 = \frac{[RAP80 \bullet Ub_4]_1[RAP80]}{K_{D,mv} + K_{D,mono}} + \frac{[RAP80 \bullet Ub_4]_5[RAP80]}{K_{D,mv} + K_{D,mono}}$$

$$[2RAP80 \bullet Ub_4]_8 = \frac{[RAP80 \bullet Ub_4]_3[RAP80]}{K_{D,mv} + K_{D,mono}} + \frac{[RAP80 \bullet Ub_4]_4[RAP80]}{K_{D,mv} + K_{D,mono}}$$

$$[2RAP80 \bullet Ub_4]_9 = \frac{[RAP80 \bullet Ub_4]_3[RAP80]}{K_{D,mv} + K_{D,mono}} + \frac{[RAP80 \bullet Ub_4]_5[RAP80]}{K_{D,mv} + K_{D,mono}}$$

$$[2RAP80 \bullet Ub_4]_{10} = \frac{[RAP80 \bullet Ub_4]_4[RAP80]}{2K_{D,mono}} + \frac{[RAP80 \bullet Ub_4]_5[RAP80]}{2K_{D,mono}}$$

$$[2RAP80 \bullet Ub_4]_{11} = \frac{[RAP80 \bullet Ub_4]_1[RAP80]}{2K_{D,mv}} + \frac{[RAP80 \bullet Ub_4]_2[RAP80]}{2K_{D,mv}}$$

$$[3RAP80 \bullet Ub_4]_{12} = \frac{[RAP80 \bullet Ub_4]_3[RAP80]}{K_{D,mv} + 2K_{D,mono}} + \frac{[RAP80 \bullet Ub_4]_4[RAP80]}{K_{D,mv} + 2K_{D,mono}} + \frac{[RAP80 \bullet Ub_4]_5[RAP80]}{K_{D,mv} + 2K_{D,mono}}$$

$$[RAP80 \bullet 2Ub_4]_{13} = \frac{[RAP80 \bullet Ub_4]_4[Ub_4]}{2K_{D,mono}} + \frac{[RAP80 \bullet Ub_4]_5[Ub_4]}{2K_{D,mono}}$$

Expression for the fraction of [U-^{15}N]-RAP80-tUIM bound to Ub_4:

$$f_{bound}^{[U-^{15}N]-RAP80-tUIM} = \frac{\left(\begin{array}{c} 1[RAP80 \bullet Ub_4]_1 + 1[RAP80 \bullet Ub_4]_2 + 1[RAP80 \bullet Ub_4]_3 + 0[RAP80 \bullet Ub_4]_4 \\ +1[RAP80 \bullet Ub_4]_5 + 1[2RAP80 \bullet Ub_4]_6 + 2[2RAP80 \bullet Ub_4]_7 + 1[2RAP80 \bullet Ub_4]_8 \\ +2[2RAP80 \bullet Ub_4]_9 + 1[2RAP80 \bullet Ub_4]_{10} + 2[2RAP80 \bullet Ub_4]_{11} \\ +2[3RAP80 \bullet Ub_4]_{12} + 1[RAP80 \bullet 2Ub_4]_{13} \end{array} \right)}{[RAP80]_{total}}$$

Expression for the fraction of [U-^{15}N]-Ub_4 bound to RAP80-tUIM:

$$f_{bound}^{[U-^{15}N]-Ub_4} = \frac{\begin{pmatrix} 1[RAP80\bullet Ub_4]_1 + 0[RAP80\bullet Ub_4]_2 + 0[RAP80\bullet Ub_4]_3 + 1[RAP80\bullet Ub_4]_4 \\ +0[RAP80\bullet Ub_4]_5 + 1[2RAP80\bullet Ub_4]_6 + 1[2RAP80\bullet Ub_4]_7 + 1[2RAP80\bullet Ub_4]_8 \\ +0[2RAP80\bullet Ub_4]_9 + 1[2RAP80\bullet Ub_4]_{10} + 1[2RAP80\bullet Ub_4]_{11} \\ +1[3RAP80\bullet Ub_4]_{12} + 1[RAP80\bullet 2Ub_4]_{13} \end{pmatrix} + \begin{pmatrix} 1[RAP80\bullet Ub_4]_1 + 0[RAP80\bullet Ub_4]_2 + 1[RAP80\bullet Ub_4]_3 + 0[RAP80\bullet Ub_4]_4 \\ +0[RAP80\bullet Ub_4]_5 + 0[2RAP80\bullet Ub_4]_6 + 1[2RAP80\bullet Ub_4]_7 + 1[2RAP80\bullet Ub_4]_8 \\ +1[2RAP80\bullet Ub_4]_9 + 0[2RAP80\bullet Ub_4]_{10} + 1[2RAP80\bullet Ub_4]_{11} \\ +1[3RAP80\bullet Ub_4]_{12} + 0[RAP80\bullet 2Ub_4]_{13} \end{pmatrix} + \begin{pmatrix} 0[RAP80\bullet Ub_4]_1 + 1[RAP80\bullet Ub_4]_2 + 1[RAP80\bullet Ub_4]_3 + 0[RAP80\bullet Ub_4]_4 \\ +0[RAP80\bullet Ub_4]_5 + 1[2RAP80\bullet Ub_4]_6 + 0[2RAP80\bullet Ub_4]_7 + 1[2RAP80\bullet Ub_4]_8 \\ +1[2RAP80\bullet Ub_4]_9 + 0[2RAP80\bullet Ub_4]_{10} + 1[2RAP80\bullet Ub_4]_{11} \\ +1[3RAP80\bullet Ub_4]_{12} + 0[RAP80\bullet 2Ub_4]_{13} \end{pmatrix} + \begin{pmatrix} 0[RAP80\bullet Ub_4]_1 + 1[RAP80\bullet Ub_4]_2 + 0[RAP80\bullet Ub_4]_3 + 0[RAP80\bullet Ub_4]_4 \\ +1[RAP80\bullet Ub_4]_5 + 1[2RAP80\bullet Ub_4]_6 + 1[2RAP80\bullet Ub_4]_7 + 0[2RAP80\bullet Ub_4]_8 \\ +1[2RAP80\bullet Ub_4]_9 + 1[2RAP80\bullet Ub_4]_{10} + 1[2RAP80\bullet Ub_4]_{11} \\ +1[3RAP80\bullet Ub_4]_{12} + 1[RAP80\bullet 2Ub_4]_{13} \end{pmatrix}}{4[Ub_4]_{total}}$$

Weights for the contribution of a bound state to the total fraction bound:

The chemical shift changes of [U-^{15}N]-RAP80-tUIM upon titration with unlabeled polyUb chains are described by the equations given above for fraction bound (a function of $K_{D,mv}$). These expressions reflect binding to either the N-terminal or C-terminal UIM. However, not all bound states will contribute to chemical shift changes for a given UIM, and therefore must be weighted accordingly. For example, chemical shift changes for a residue within the N-terminal UIM will not reflect states where the C-terminal UIM is bound but the N-terminal UIM is not. The equations describing fraction [U-^{15}N]-RAP80-tUIM bound, are used to fit binding to either the N-terminal or C-terminal UIM, given the nearly identical $K_{D,mono}$ values for each UIM.

Analysis of the corollary titrations, for which [U-^{15}N]-polyUb chains are titrated with unlabeled RAP80-tUIM are complicated with respect to fraction bound due to the degeneracy of Ub chemical shifts for a given Ub moiety within a polyUb chain. In this case, binding of an individual UIM from

RAP80 to an individual Ub moiety of the chain will result in a chemical shift change. As in the case of [*U*-^{15}N]-RAP80-tUIM, there are a number of states in which some Ub moieties are bound, whereas others are not. To fit polyUb chemical shift changes for these titrations in order to extract $K_{D,mv}$ values, weights are given to each individual bound state. To reach the fully bound chemical shift, all Ub moieties within a chain must be bound to a UIM from RAP80. With respect to degeneracy in chemical shifts for [*U*-^{15}N]-polyUb spectra, one might, in principle, expect slightly different chemical shift changes for individual Ub moeities during titration with RAP80-tUIM. However, in practice, all resonances shift in a similar fashion, likely due to the fact that differences in chemical shifts between free and bound states are small in most cases (< 2 ppm for ^{15}N); we estimate that for a given resonance, the maximum difference in chemical shift difference between individual Ub moieties within a chain during titration is ~0.2 ppm (^{15}N), or ~10% of the maximum chemical shift change upon binding. Given that ^{15}N linewidths for polyUb resonances are ~0.3 ppm, this precludes resolution of differentially shifting resonances from individual Ub moeities. To account for this degeneracy, we averaged the theoretical expression (corresponding to the fraction bound) for differentially shifting resonances for the individual Ub moieties within a polyUb chain to analyze the titration data.

Protein Sequences:

RAP80-tUIM:

GPLGSRKIAQMTEEEQFALALKMSEQEAREVNSQEEEEEELLRKAIAESLNSCRPSDASATRS

Ub:

GPLGSMQIFVKTLTGKTITLEVESSDTIDNVKSKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNI
QKESTLHLVLRLRGG

tandem Ub_2:

GPLGSMQIFVKTLTGKTITLEVESSDTIDNVKSKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNI
QKESTLHLVLRLRGGSMQIFVKTLTGKTITLEVESSDTIDNVKSKIQDKEGIPPDQQRLIFAGKQL
EDGRTLSDYNIQKESTLHLVLRLRGG

tandem Ub_3:

GPLGSMQIFVKTLTGKTITLEVESSDTIDNVKSKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNI
QKESTLHLVLRLRGGSMQIFVKTLTGKTITLEVESSDTIDNVKSKIQDKEGIPPDQQRLIFAGKQL
EDGRTLSDYNIQKESTLHLVLRLRGGSMQIFVKTLTGKTITLEVESSDTIDNVKSKIQDKEGIPPD
QQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG

tandem Ub_4:

GPLGSMQIFVKTLTGKTITLEVESSDTIDNVKSKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNI
QKESTLHLVLRLRGGSMQIFVKTLTGKTITLEVESSDTIDNVKSKIQDKEGIPPDQQRLIFAGKQL
EDGRTLSDYNIQKESTLHLVLRLRGGSMQIFVKTLTGKTITLEVESSDTIDNVKSKIQDKEGIPPD
QQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGGSMQIFVKTLTGKTITLEVESSDTIDNVK
SKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG

Complete Reference 21:

Case, D. A.; Cheatham, III, T.E.; Simmerling, C.L.; Wang, J., Duke, R.E.; Luo, R.; Crowley, M.; Walker, R.C.; Zhang, W.; Merz, K.M.; Wang, B.; Hayik, S.; Roitberg, A.; Seabra, G.; Kolossváry, I.; Wong, K.F.; Paesani, F.; Vanicek, J.; Wu, X.; Brozell, S.R.; Steinbrecher, T.; Gohlke, H.; Yang, L.; Tan, C.; Mongan, J.; Hornak, V.; Cui, G.; Mathews, D.H.; Seetin, M.G.; Sagui, C.; Babin, V.; Kollman, P.A.; AMBER 10, University of California, San Fransico: 2008.