SUPPLEMENTAL DATA

Molecular determinants of the N-terminal acetyltransferase Naa60 anchoring to the Golgi membrane

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Table S1. All mutations performed on Naa60, purpose of the mutation and consequence observed by microscopy and carbonate wash assay when applied. Abbreviations: <1/2, less than half of the proteins extracted; 1/2, half of the proteins extracted; >1/2, more than half of the proteins extracted; ϕ, hydrophobic amino acid; SF, by subcellular fractionation. Numbers in [] are internal plasmid numbers.

		Localization by microscopy		Org fraction extractable by high pH?
Mutation	**Concept tested**	**Cyto**	**Org**	
Naa60-(1-242)-eGFP [134]	Ctrl, full-length		x	some (<1/2)
C-truncations (Fig 3B/E)				
Naa60-(1-182)-eGFP [439]	Lacking last 61 aa	x		
Naa60-(1-216)-eGFP [207]	Lacking last 26 aa	x	x	
Naa60-(1-225)-eGFP [440]	Lacking last 17 aa	some	x	yes (1/2)
Naa60-(1-230)-eGFP [449]	Lacking last 12 aa	some	x	
Naa60-(1-236)-eGFP [450]	Lacking last 6 aa	?	x	
N-truncations (eGFP targeting assay) (Fig 3C/E)				
eGFP-Naa60-(182-242) [131]	eGFP targeting using the last 61 aa of Naa60		x	
eGFP-Naa60-(192-242) [349]	eGFP targeting using the last 51 aa of Naa60		x	
eGFP-Naa60-(202-242) [363]	eGFP targeting using the last 41 aa of Naa60	x	x	
eGFP-Naa60-(217-242) [132]	eGFP targeting using the last 26 aa of Naa60	x		
Deletions Pred-α1 and extended (Fig 3D/E)				
Delta 191-200 [364]	Most of Pred-α1	x	x	yes (>1/2)
Delta 191-209 [409]	Pred-α1 extended	x		
Delta 203-210 [457]	Between Pred-α-Helices		x (more ER)	
Deletions Pred-α2 and extended (Fig 3D/E)				
Delta 213-222 [365]	Most of Pred-α2	x	x	yes (~ completely)
Delta 213-230 [458]	Pred-α2 extended	x	x	
Delta 213-236 [459]	Pred-α2 further extended	x	x	
Delta 211-216 [421]	1st half of Pred-α2	x	x	
Mutations in Pred-α1 (Fig 4 and S1)				
I190A/L191A/Y193A/I194A [370]	ϕ in Pred-α1 → A	x	x	yes (>1/2)
I190E/L191E/Y193E/I194E [389]	ϕ in Pred-α1 → E	x	x	yes (>1/2)
L197A/L201A/L204A/I209A [428]	ϕ in Pred-α1→ A	x	some	yes (~ completely)
L197E/L201E/L204E/I209E [429]	ϕ in Pred-α1 → E	x		
L197/L201/L204A [435]	**ϕ in Pred-α1 → A**	**x**	**x**	**yes (>1/2)**
L197/L201/L204E [436]	204/209E effective. Skew it Nt dir. E mut	x	some	
L201/L204/I209A [433]	ϕ in Pred-α-Helix1→ A	x	x	
L201/L204/I209E [434]	204/209E effective. Extend it Nt dir. E mut	x		
I190/L191A [451]	ϕ in Pred-α-Helix1 → A		x	
Y193/I194A [454]	ϕ in Pred-α1 → A		x	
Y194/L197A [455]	ϕ in Pred-α1 → A		x	
L197A/L201A [456]	ϕ in Pred-α1 → A		x	
L201/L204A [462]	ϕ in Pred-α1 → A		x	
L204/I209A [430]	ϕ in between Pred-α1 and 2 → A		x	
L204E/I209E [398]	ϕ in between Pred-α1 and 2 → E	x	x	yes(>1/2)
L204E [431]	Effective when comb w 209E. Alone?		x	
I209E [432]	Effective when comb w 204E. Alone?	x	x	
I190E/L191E/Y193E/I194E + L204E/I209E [416]	Mut ϕ in extended Pred-α1 to E	x		

D192A/H196A [366]	Electrostatic interactions?		x	some (like WT)
D192A [468]	Alone?		x	
H185/H196A [464]	Electrostatic interactions?		x	
Mutations in Pred-α2 (Fig 5 and S2)				
I209/V213/Y214A [424]	Mut ϕ in Pred-α2+209 to A	some	x	
I209/V213/Y214E [425]	Mut ϕ in Pred-α2+209 to E	x	x	
V213/Y214A [422]	Mut ϕ in Pred-α2 to A	?	x	
V213/Y214E [423]	Mut ϕ in Pred-α2 to E	some	x	
V213E [443]	Mut ϕ in Pred-α2 to E	some	x	
L220/221E [396]	Mut ϕ in Pred-α2 to E	?	x	
L220/L221/F224/L225A [460]	Mut ϕ in Pred-α2 to A	some	x	yes (1/2)
F224/L225/W227/I230A [426]	Mut ϕ in Pred-α2 to A	some	x	
F224/L225/W227/I230E [427]	Mut ϕ in Pred-α2 to E	some	x	yes (1/2)
F224A/L225A [441]	Mut ϕ in Pred-α2 to A		x	
F224E/L225E [442]	Mut ϕ in Pred-α2 to E	?	x	
H211A/R212A/R215A/H218A [367]	Electrostatic interactions?	SF	x	some (like WT)
H211A/R212A [465]	Electrostatic interactions?		x	
R212A/R215A [466]	Electrostatic interactions?		x	
R215A/H218A [467]	Electrostatic interactions?		x	
Combined mutations in Pred-α1 + Pred-α2 (Fig 5 and S2)		**Cyto**	**Org**	
Delta 191-200 + H211A/R212A/R215A/H218A [392]	Delete most of Pred-α1 + Mut charge in Pred-α2	x	x	
Delta 213-222 + D192A/H196A [394]	Delete most of Pred-α2 + Mut charge in Pred-α1	x	x	
I190E/L191E/Y193E/I194E + H211A/R212A/R215A/H218A [411]	Mut ϕ in Pred-α1 to E + Mut charge in Pred-α2	x	x	
I190E/L191E/Y193E/I194E + V213E [414]	Mut ϕ in Pred-α1 to E + Mut ϕ in Pred-α2 to E	x	some	
I190E/L191E/Y193E/I194E + L220/221E [415]	Mut ϕ in Pred-α1 to E + Mut ϕ in Pred-α2 to E	x		
I190E/L191E/Y193E/I194E + C222S [412]	Mut ϕ in Pred-α1 to E + Mut put palmitoylation site	x	x	
I190E/L191E/Y193E/I194E + F224E/L225E [413]	Mut ϕ in Pred-α1 to E + Mut ϕ in/near Pred-α2 to E	x		
D192A/H196A + H211A/R212A/R215A/H218A [419]	Mut charge in Pred-α1 and Pred-α2 to A	?	x	
L197/L201/L204A + H211A/R212A/R215A/H218A [445]	Mut ϕ in Pred-α1 to A + Mut charge in Pred-α2	x	x	
L197/L201 + I209/V213/Y214A [463]	Mut ϕ in Pred-α1 to A + Mut ϕ in Pred-α2 to A	x	weak	
L197/201/204A + F224/L225/W227/I230A [448]	Mut ϕ in Pred-α1 to A + Mut ϕ in Pred-α2 to A	x		
H211A/R212A/R215A/H218A + K233A/R240A [446]	Mut charge in Pred-α1 + mut charge in C-term		x	
L204E/I209E + L220/221E [418]	Mut ϕ in Pred-α2 + 204/209 to E	x		
L204E + H211A/R212A/R215A/H218A [417]	Mut 204E + Mut charge in Pred-α2	x	x	

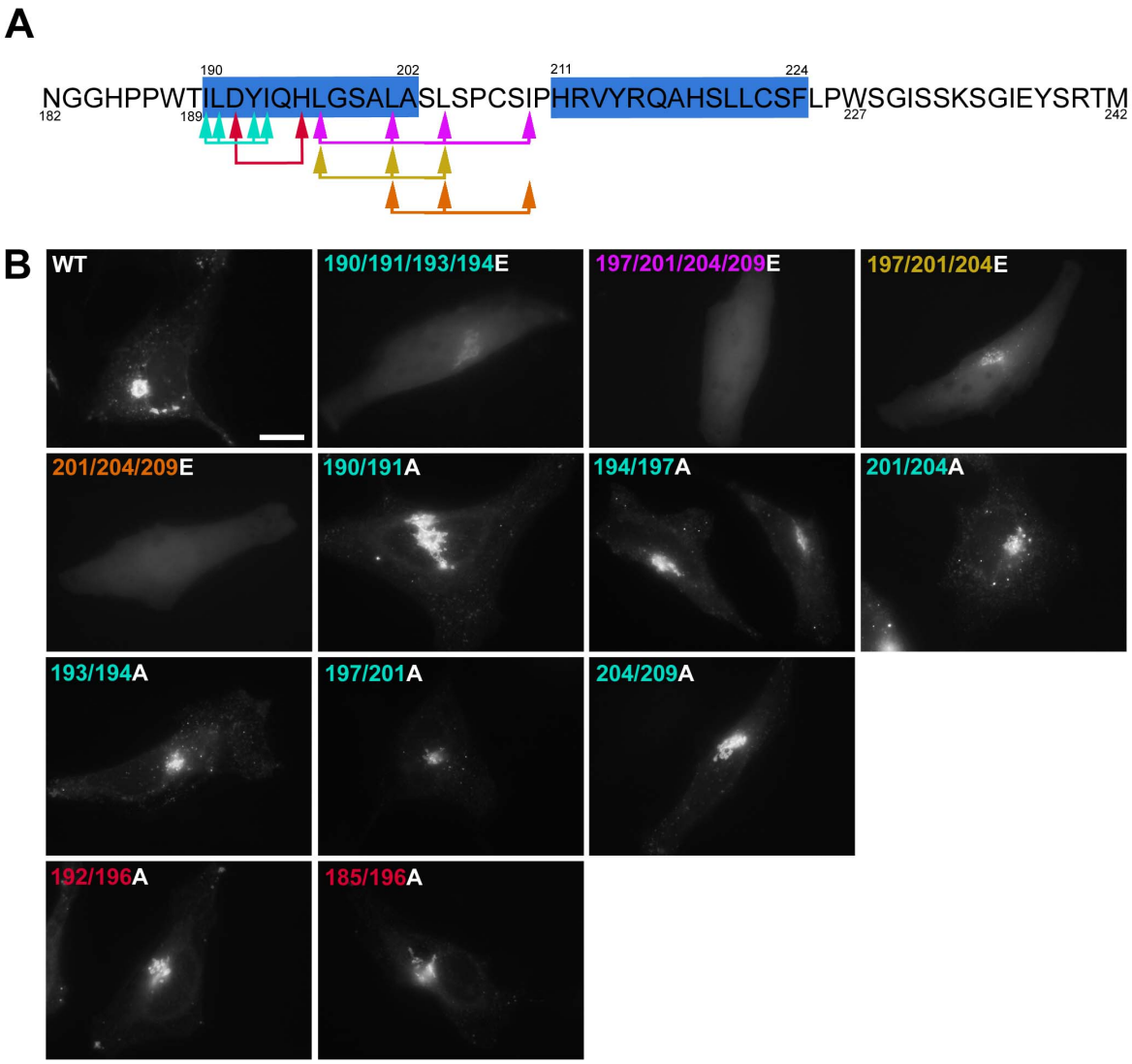
SUPPLEMENTAL FIGURE LEGENDS

Supplemental figure 1. Mutated constructs of Naa60-eGFP demonstrated hydrophobic amino acids within Pred-α1 important for membrane association *in cellulo*. HeLa cells were transfected with the indicated Naa60-eGFP constructs (A) and imaged live (B) or subjected to subcellular fractionation followed by high pH/sodium carbonate mediated protein extraction from membranes (C). **A**. The sequence of the Naa60 C-terminal tail as in Figure 1. Arrows indicate the positions of mutations shown in B and C, and are color coded accordingly. **B**. Subcellular localization of Naa60-eGFP mutated constructs in the area of Pred-α1 as indicated with corresponding colors in A. Scale bar is 10 μm and is representative for all microscopic images. **C**. Cells were transfected with the indicated constructs and subjected to high pH/sodium carbonate mediated protein extraction from membranes following subcellular fractionation and immunoblotting. The peripheral membrane protein GM130 and transmembrane RCAS1 were used as controls for extractable and un-extractable modes of membrane binding. Supplements data in Figure 4B-C.

Supplemental figure 2. Mutated constructs of Naa60-eGFP demonstrated amino acids within Pred-α2 important for membrane association *in cellulo*. HeLa cells were transfected with the indicated Naa60-eGFP constructs (A) and imaged live (B) or subjected to subcellular fractionation followed by high pH/sodium carbonate mediated protein extraction from membranes (C). **A**. The sequence of the Naa60 C-terminal tail as in Figure 1. Arrows indicate the positions of mutations shown in B and C, and are color coded accordingly. **B**. Subcellular localization of Naa60-eGFP mutated constructs in the area of Pred-α1 as indicated with corresponding colors in A. **C**. Cells were transfected with the indicated constructs and subjected to high pH/sodium carbonate mediated protein extraction from membranes following subcellular fractionation and immunoblotting. The peripheral membrane protein GM130 and transmembrane RCAS1 were used as controls for extractable and un-extractable modes of membrane binding. Supplements data in Figure 4D-E.

Supplemental figure 3. Anti-Naa60$_{192-242}$ antibody validation for detection of Naa60$_{189-242}$ peptide. The specificity of the anti-Naa60$_{192-242}$ (Abcam 103800) antibody used to detect the Naa60$_{189-242}$ peptide in pull down experiments was determined by a dot blot. Ratios from 0.1 to 10 pmol Naa60$_{189-242}$ peptide were spotted on a nitrocellulose membrane, in addition to 10 pmol of the negative control peptide SESS (SESSSKS RWGRPVGRRRRPVRVYP). The dot blots were incubated with either anti-Naa60$_{192-242}$ or custom-made anti-Naa10 antibody before detection. Supplements data in Figure 7A.

Supplemental figure 4. Far UV-CD experiments supporting Naa60$_{189-242}$ folding most optimal in the presence of PI4P over all other PI. PI4P liposomes induce the most favorable Naa60$_{189-242}$ helical spectra, when compared to all other PI-liposomes. Far UV-CD spectra of 20 μM Naa60$_{189-242}$ in the presence of 360 μM liposomes containing 92 mol % PC and 8 mol % of either PI3P, PI4P, PI5P, PI(3,4)P2, PI(3,5)P2, PI(4,5)P2 or PIP3. n = 2 for all experiments. Related to data in Figure 7D.


A
190
202
211
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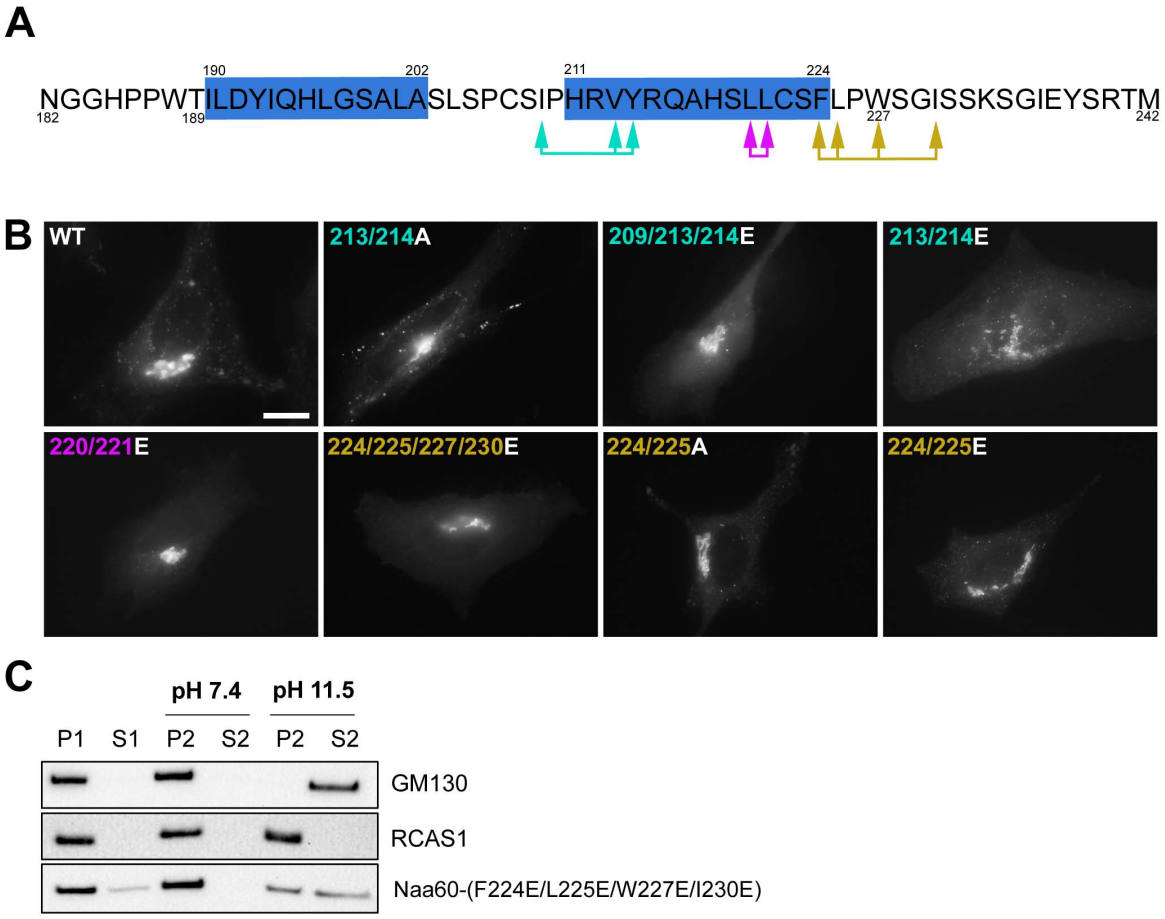
182
189
227
242
B
WT
190/191/193/194E
197/201/204/209E
197/201/204E
201/204/209E
190/191A
194/197A
201/204A
193/194A
197/201A
204/209A
192/196A
185/196A
C
pH 7.4
pH 11.5
P1
S1
P2
S2
P2
S2
GM130
RCAS1
Naa60-(I190E/L191E/Y193E/I194E)
Naa60-(D192A/H196A)


A
190
202
211
224
NGGHPPWTILDYIQHLGSALASLSPCSIPHRVYRQAHSLLCSFLPWSGISSKSGIEYSRTM
182
189
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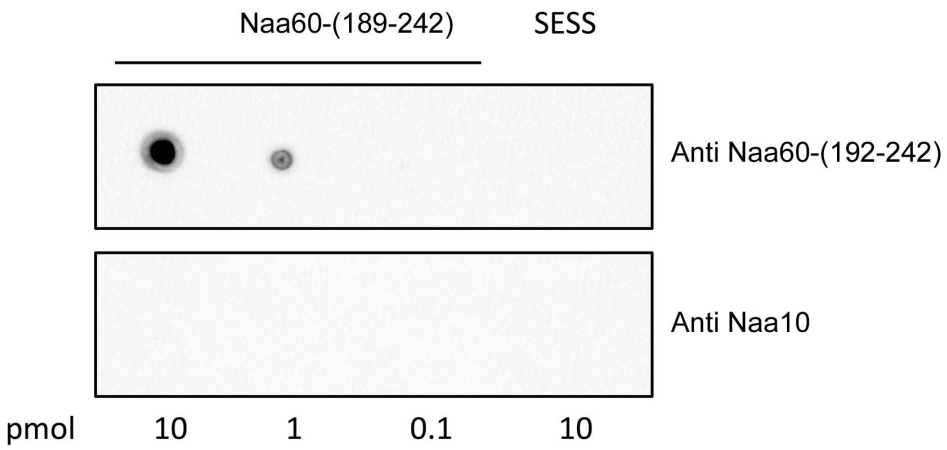
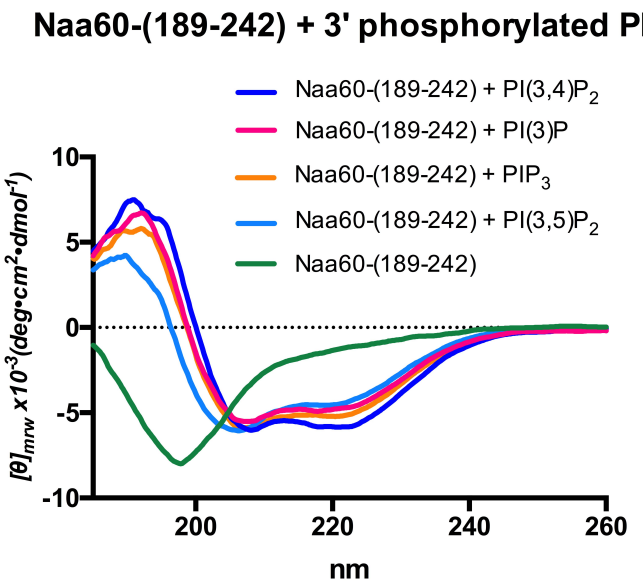
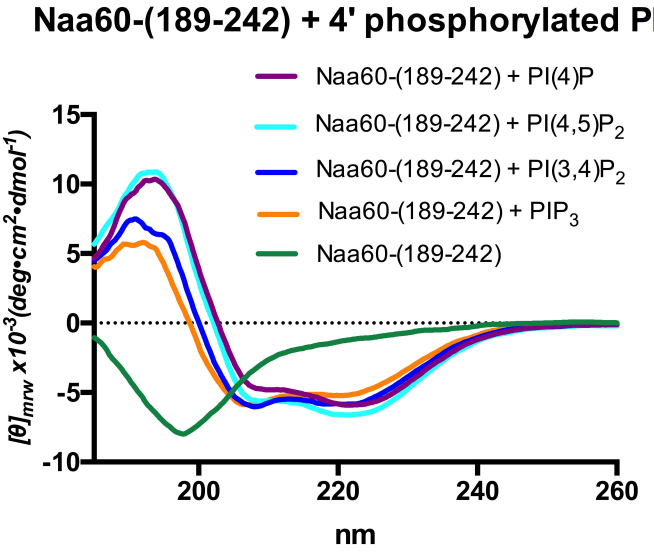
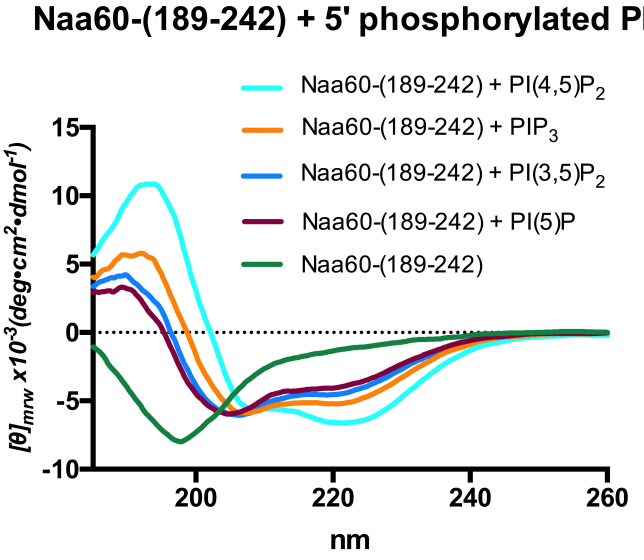
242
B
WT
213/214A
209/213/214E
213/214E
220/221E
224/225/227/230E
224/225A
224/225E
C
pH 7.4
pH 11.5
P1
S1
P2
S2
P2
S2
GM130
RCAS1
Naa60-(F224E/L225E/W227E/I230E)


Naa60-(189-242)
SESS
Anti Naa60-(192-242)
Anti Naa10
pmol
10
1
0.1
10


Naa60-(189-242) + 3' phosphorylated PIs
Naa60-(189-242) + PI(3,4)P2
Naa60-(189-242) + PI(3)P
Naa60-(189-242) + PIP3
Naa60-(189-242) + PI(3,5)P2
Naa60-(189-242)
[θ]mrw x10-3(deg•cm2•dmol-1)
10
5
0
-5
-10
200
220
240
260
nm


Naa60-(189-242) + 4' phosphorylated PIs
Naa60-(189-242) + PI(4)P
Naa60-(189-242) + PI(4,5)P2
Naa60-(189-242) + PI(3,4)P2
Naa60-(189-242) + PIP3
Naa60-(189-242)
[θ]mrw x10-3(deg•cm2•dmol-1)
15
10
5
0
-5
-10
200
220
240
260
nm


Naa60-(189-242) + 5' phosphorylated PIs
Naa60-(189-242) + PI(4,5)P2
Naa60-(189-242) + PIP3
Naa60-(189-242) + PI(3,5)P2
Naa60-(189-242) + PI(5)P
Naa60-(189-242)
[θ]mrw x10-3(deg•cm2•dmol-1)
15
10
5
0
-5
-10
200
220
240
260
nm