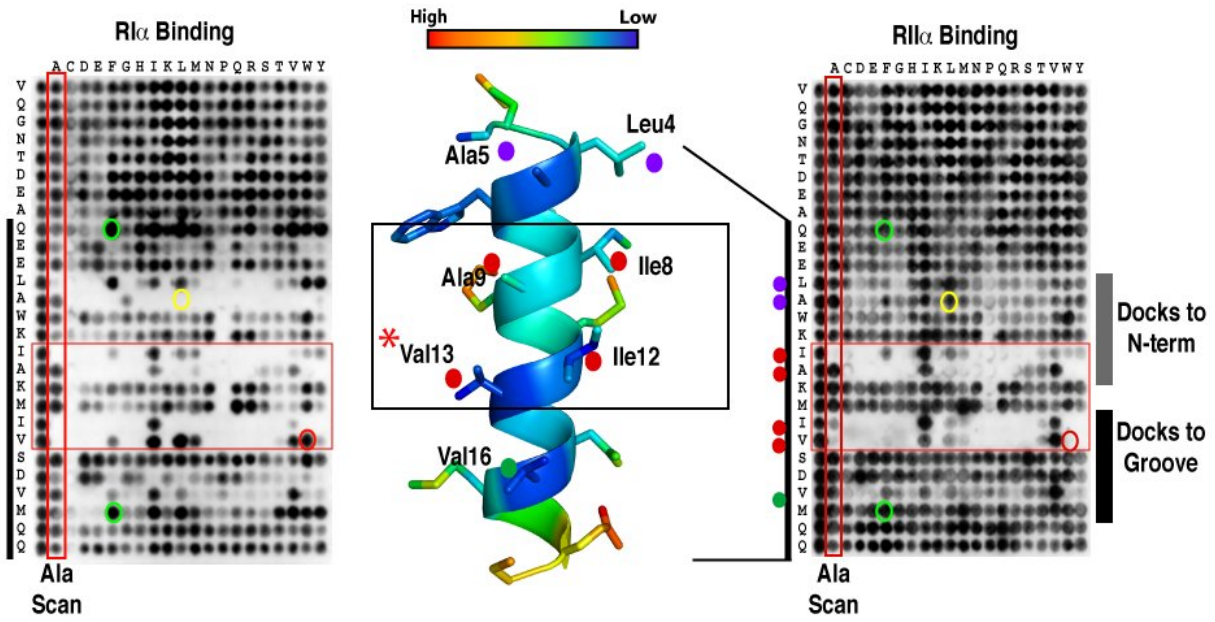
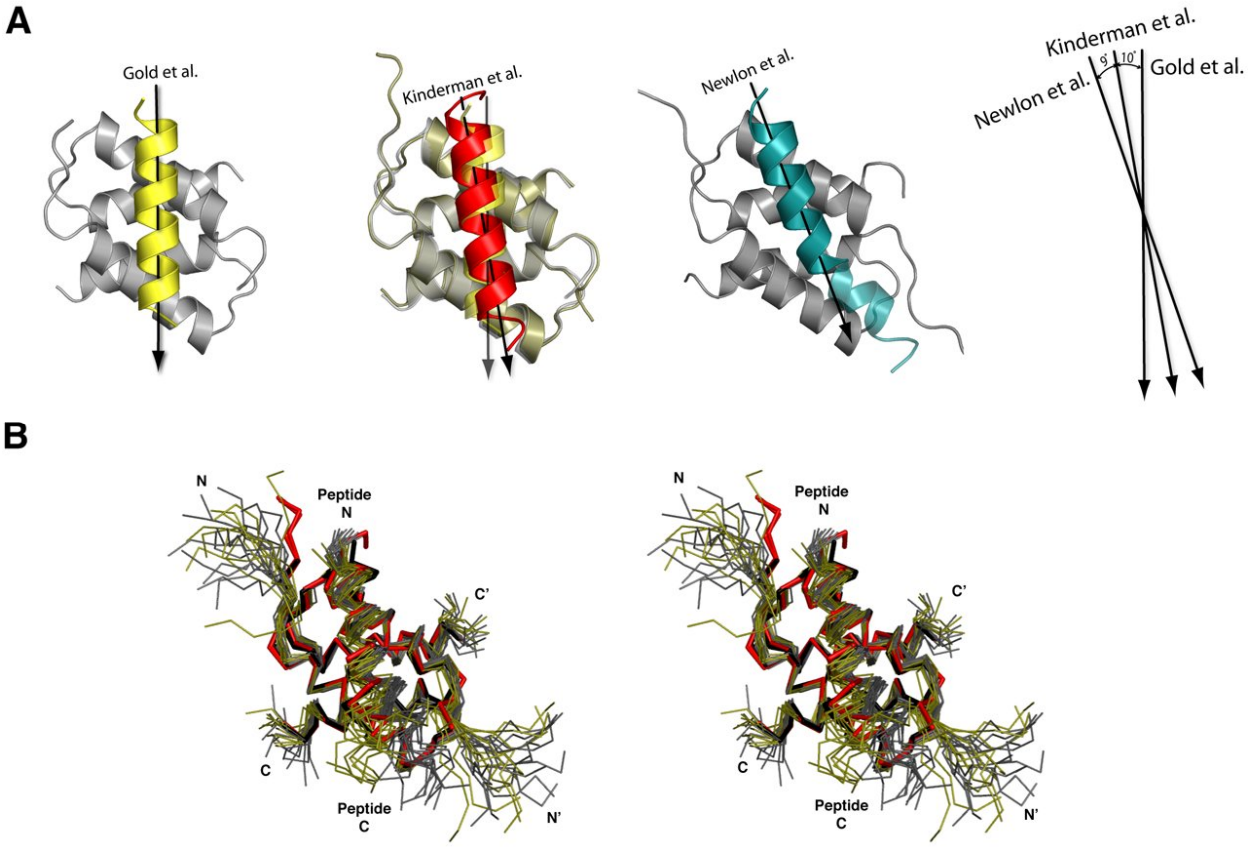


Supplementary Figure 1 – Crystal structure expands on peptide array data and provides new insight into specificity.

Each position of the D-AKAP2 AKB (residues 623-649) was mutated previously (Burns-Hamuro et al., 2003) to every possible residue and tested for binding (binding is indicated by a black spot seen on the array). The side chains highlighted with a red dot (Ile8^{P}, Ala9^{P}, Ile12^{P}, and Val13^{P} from our peptide) are the most intolerant of substitutions. These residues interact with the hydrophobic pocket of the RIIα D/D domain and represent only two turns on the helix. Leu4^{P} and Ala5^{P}, in purple, are crucial to binding, but can accept hydrophobic substitutions, presumably because of the induced fit of the D/D. Val16^{P}, in green, demonstrates less stringent rules for substitutions, but valine is still preferred to other residues. The RIα shows much more stringent requirements for binding to D-AKAP2.



Supplementary Figure 2 – Structural alignment of RIIα D/D domain structure complexes

(A) Three structures of RIIα D/D domain complexes, RIIα D/D domain with a D-AKAP2 peptide (Kinderman et al.), the crystal structure of RIIα D/D domain with AKAP-IS (Gold et al.), and a NMR structure of RIIα D/D domain with Ht31 (Newlon et al.), were aligned and compared. In the center is the structural alignment of the two crystal structures (RIIα D/D domain with a D-AKAP2 peptide in tan/red and RIIα D/D domain with AKAP-IS in silver/yellow) and individual complexes of RIIα D/D:AKAP-IS and RIIα D/D:Ht31 (in gray/teal) are shown on the left and right, respectively. The N-terminus of the D-AKAP2 peptide tilts approximately 10° relative to AKAP-IS and binds the N-terminus of an ordered protomer asymmetrically. The C-terminal region of Ht31 was highly variable in all 13 ensembles of the

RIIα D/D:Ht31 complex (rendered as a transparent helix) and was not considered for the comparison. The N-terminal region of the bound Ht31 (residues 1-15) tilts even more toward one of the protomers of D/D domain.

(B) The structures of the RIIα D/D domain are aligned including the crystal structure complexes with D-AKAP2 (red) and AKAP-IS (black) and the NMR ensembles of the RIIα D/D domain bound to Ht31(grey) and AKAP79 (olive). They are represented here in stereo. The two crystal structures align quite well, while the NMR structure indicate some variability at both termini of the D/D domain and the C-termini of both AKAP peptides.