Table 1. Data Collection and Refinement Statistics

	Rsc4(36–340)	Rsc4(36–340) Histone (6–18, K14ac) Peptide Soak	Rsc4(1–321) Histone Chimera, Acetylated	Rsc4(1–340) Acetylated
Data Collection				
Space group	$R3_2$	$R3_2$	$C222_1$	$C2_1$
Cell dimensions (Å)	a = b = 95.9, c = 233.5	a = b = 95.0, c = 233.1	a = 86.1, b = 91.0, c = 263.2	a = 123.3, b = 83.2, c = 127.1, β = 109.3°
Resolution (Å)	20–1.80	50–1.75	50–2.20	50–2.35
Resolution Outer Shell (Å)	1.86–1.80	1.81–1.75	2.28–2.20	2.43–2.35
No. reflections	514,412	335,001	548,136	906,673
Unique reflections	37,132	39,000	49,197	50,527
R_{sym} (%)	5.5 (36.9)	6.8 (59.7)	7.8 (49.4)	7.6 (50.3)
$I/\sigma(I)$	25.5 (1.6)	33.7 (2.2)	32.0 (2.8)	26.9 (3.2)
Completeness (%)	96.2 (67.4)	94.6 (72.4)	95.3 (83.9)	99.5 (99.7)
Refinement				
R_{work}/R_{free} (%)	17.6/21.8	18.4/21.9	20.1/24.8	18.0/22.3
Number of Atoms				
Protein	2744	2669	5342	7790
Solvent	351	253	369	506
Average Isotropic B factor(Å)^2	30.6	34.2	51.4	40.7
Ramachandran Plot, Nonglycine Residue in				
Most favorable region (%)	92.1	91.7	91.4	91.7
Allowed region (%)	7.9	8.3	8.2	8.2
Rmsd				
Bond lengths (Å)	0.016	0.016	0.013	0.016
Bond angles (°)	1.241	1.406	1.416	1.211

outside of the primary binding pocket. Nevertheless, this structure supports the genetic and biochemical studies by showing that H3K14ac can bind Rsc4 BD2.

Rsc4 K25ac Binds BD1

In an effort to overcome the problem of low-affinity binding and to visualize a bound H3 peptide better, we took a chimera approach in which a very high local concentration of H3 peptide might be achieved by expressing the H3 tail as a fusion with the Rsc4 N terminus. The fusion included H3 residues 6–18, followed by eight residues containing a thrombin cleavage site, followed by Rsc4 residues 1–321 (Figure 1A). This construct contained the entire N-terminal sequence of Rsc4, whereas our initial crystal structures lacked the first 35 residues. The purified chimeric protein was acetylated at H3K14 using purified recombinant Gcn5, with progress of the reaction monitored by migration of the H3 peptide (following treatment and release with thrombin) using acid-urea gel electrophoresis. Acetylation was also monitored by western analysis, which revealed high levels of H3K14ac and very low levels of H3K9ac (data not shown). To more definitively identify the acetylated lysines, we subjected tryptic fragments to mass spectrometric analysis. This revealed nearly complete acetylation at H3K14 and, surprisingly, nearly complete acetylation at Rsc4 K25. As described below, the modification of K25 was unexpected but highly fortuitous. The acetylated chimeric H3-Rsc4 protein was crystallized and the structure determined. Counter to the design goal, H3 residues were not ordered, and the BD2 pocket appeared to be empty. This was disappointing, but given the lack of information about likely binding orientation, the failure was not surprising. Remarkably, however, Rsc4 K25ac was present in the BD1 pocket, and the surrounding residues were well ordered, starting at residue 19 (Figure 3). An essentially identical arrangement was revealed in the subsequently determined crystal structure of Rsc4(1–340) bearing K25ac. This derivative bore its natural N terminus and lacked the H3 peptide, and quantitative acetylation at K25 (by Gcn5) was verified by mass spectrometry (data not shown). This binding could be recapitulated in *trans*, as a peptide containing Rsc4 residues 18–34 acetylated at K25 interacted with the TBD protein in vitro, though with low affinity (Figure S4).