Towards elucidating the stability, dynamics and architecture of the nucleosome remodeling and deacetylase complex by using quantitative interaction proteomics

Susan L. Kloet1*, H. Irem Baymaz1*, Matthew Makowski1*, Vincent Groenewold2, Pascal W. T. C. Jansen1, Madeleine Berendsen1, Hassin Niazi1, Geert J. Kops2,3 and Michiel Vermeulen1,2,3

1 Department of Molecular Biology, Faculty of Science, Radboud Institute for Molecular Life Sciences, Radboud University Nijmegen, the Netherlands
2 Department of Molecular Cancer Research, UMC Utrecht, the Netherlands
3 Cancer GenomiCs Netherlands, the Netherlands

Keywords
chromatin; mass spectrometry; NuRD; protein–protein interactions; quantitative proteomics

Correspondence
M. Vermeulen, Department of Molecular Biology, M850/3.79 RIMLS, P.O. Box 9101 6500 HB Nijmegen, The Netherlands
Tel: +31 024 3616848
E-mail: m.vermeulen@ncmls.ru.nl

*These authors contributed equally to this work.

(Received 15 July 2014, revised 7 August 2014, accepted 12 August 2014)
doi:10.1111/febs.12972

The nucleosome remodeling and deacetylase (NuRD) complex is an evolutionarily conserved chromatin-associated protein complex. Although the subunit composition of the mammalian complex is fairly well characterized, less is known about the stability and dynamics of these interactions. Furthermore, detailed information regarding protein–protein interaction surfaces within the complex is still largely lacking. Here, we show that the NuRD complex interacts with a number of substoichiometric zinc finger-containing proteins. Some of these interactions are salt-sensitive (ZNF512B and SALL4), whereas others (ZMYND8) are not. The stoichiometry of the core subunits is not affected by high salt concentrations, indicating that the core complex is stabilized by hydrophobic interactions. Interestingly, the RBBP4 and RBBP7 proteins are sensitive to high nonionic detergent concentrations during affinity purification. In a subunit exchange assay with stable isotope labeling by amino acids in cell culture (SILAC)-treated nuclear extracts, RBBP4 and RBBP7 were identified as dynamic core subunits of the NuRD complex, consistent with their proposed role as histone chaperones. Finally, using cross-linking MS, we have uncovered novel features of NuRD molecular architecture that complement our affinity purification-MS/MS data. Altogether, these findings extend our understanding of MBD3–NuRD structure and stability.

Structured digital abstract
- MBD3 physically interacts with ZNF512B, HDAC1, ZMYND8, GATAD2B, SALL4, GATAD2A, ZNF592, MTA3, ZNF687, CDK2AP1, CHD3, ZNF532, HDAC2, MTA2, CHD4, MTA1, KPNA2, CHD5, RBBP4 and RBBP7 by pull down (View interaction)
- CDK2AP1 physically interacts with MBD3, MTA3, HDAC2, GATAD2A, CHD4, CDK2AP1, MTA2, HDAC1, MTA1, CHD3, GATAD2B, MBD2, RBBP4 and RBBP7 by pull down (View interaction)
- MBD3 physically interacts with MTA2, MTA3, RBBP4, RBBP7, HDAC2, HDAC1, CHD4, CHD3 and MTA1 by cross-linking study (View interaction)

Abbreviations
AP, affinity purification; FDR, false discovery rate; GFP, green fluorescent protein; HDAC, histone deacetylase; iBAQ, intensity-based absolute quantification; NuRD, nucleosome remodeling and deacetylase; PDB, Protein Data Bank; SAS, solvent-accessible surface; SILAC, stable isotope labeling by amino acids in cell culture; TAP, tandem affinity purification; TEV, tobacco etch virus.
Dynamics and architecture of the NuRD complex

S. L. Kloet et al.

Introduction

The nucleosome remodeling and deacetylase (NuRD) complex is a conserved chromatin-associated protein complex that was first purified and characterized from Xenopus laevis and human cells in the late 1990s [1–3]. As the name implies, the NuRD complex has both histone deacetylase (HDAC) and ATP-dependent chromatin remodeling activity. In the human complex, HDAC activity is catalyzed by HDAC1 and HDAC2. The large CHD3 and CHD4 subunits have ATPase activity, which is used to move the position of nucleosomes on DNA. In addition to these enzymatic activities, the NuRD complex contains a number of other core subunits: MTA1–MTA3, GATAD2A, GATAD2B, MBD2, MBD3, RBBP4, RBBP7, and CDK2AP1 [4,5]. Some of these proteins, such as MBD2 and MBD3, are mutually exclusive within the NuRD complex. For GATAD2A, GATAD2B, RBBP4, RBBP7, and MTA1–MTA3, it is currently not known whether these proteins exclusively form heterodimers/trimers or form mutually exclusive homodimers/trimers. In addition to the well-described core subunits, a large number of proteins have been reported to interact with the NuRD complex. Examples include SALL4, FOG1, and ZMYND8 [6–8]. These proteins may recruit the NuRD complex to its target sites in the genome.

Owing to the presence of HDACs, which are generally associated with gene silencing, the NuRD complex has long been thought of as a transcriptional corepressor complex. Indeed, in luciferase assays, NuRD subunits repress the transcription of reporter constructs [4]. Furthermore, the methyl-CpG-binding protein MBD2 links MBD2-containing NuRD to methylated CpG islands, and induces gene silencing [9]. However, recent genome-wide profiling studies have revealed that NuRD complexes, such as MBD3–NuRD, are also found at active enhancers and promoters, indicating that NuRD-mediated regulation of transcription is more diverse than previously thought [10,11]. Also, functional experiments have revealed an important role for the NuRD complex in regulating cell fate decisions [12,13]. These different biological functions of the NuRD complex may be driven by subtle changes in subunit composition.

Recently, a number of MS-based methods were developed that can be used not only to confidently identify protein–protein interactions, but also to obtain information about the stoichiometry, dynamics and the interaction surfaces between subunits [14–16]. Here, we applied these different methods to gain further insights into the structure and dynamics of the MBD3–NuRD complex in HeLa cells. We applied label-free quantitative MS to investigate the stoichiometry of the MBD3–NuRD complex, using different buffer stringencies during affinity purification (AP). A stable isotope labeling by amino acids in cell culture (SILAC)-based subunit exchange assay was used to show that RBBP4 and RBBP7 are dynamic core subunits within the NuRD complex. Finally, cross-linking MS was used to identify interprotein contacts within the MBD3–NuRD complex. Altogether, these experiments increase our understanding of the stability, dynamics and architecture of the NuRD complex.

Results

RBBP4 and RBBP7 interactions with the NuRD complex are salt-stable but detergent-sensitive

To facilitate purification of the MBD3–NuRD complex, we created a stable cell line with doxycycline-inducible green fluorescent protein (GFP)-tagged MBD3. Cells were incubated for 16 h in the presence or absence of doxycycline. Expression of GFP–MBD3 occurred only after addition of doxycycline to the medium (Fig. 1A). Nuclear extracts from this cell line were used for GFP AP combined with label-free LC-MS/MS analysis. Briefly, triplicate pulldowns were performed with GFP-Trap_A beads and GFP–MBD3-containing nuclear extracts (+Dox). Simultaneously, triplicate pulldowns were performed with GFP beads on control nuclear extracts (–Dox). Proteins that bind specifically to GFP–MBD3 will be enriched in the GFP pulldowns and appear on the right side of the

![Fig. 1. Label-free proteomics reveal that RBBP4 and RBBP7 interactions with NuRD are NP-40-sensitive. (A) An anti-MBD3 western blot of GFP–MBD3 HeLa cells after 16 h of incubation with doxycycline (+Dox) or without doxycycline (–Dox). Nonspecific bands are marked with an asterisk. (B–D) Volcano plots from label-free GFP pulldowns of GFP–MBD3 HeLa cell nuclear extracts with varying salt and NP-40 concentrations. Statistically enriched proteins in the GFP–MBD3 pulldown are identified by a permutation-based FDR-corrected t-test. The label-free quantification (LFQ) intensity of the GFP pulldown relative to the control [fold change (FC), x-axis] is plotted against the –log10-transformed P-value of the t-test (y-axis). The proteins in the upper right corner represent the bait and its interactors. (E) Stoichiometry of NuRD core subunits. The iBAQ value of each protein group is divided by the iBAQ value of the GFP–MBD3 bait determined with the same data as in (E), but with a differently scaled axis.](image-url)
To determine the stability of the NuRD complex, several NP-40 and NaCl concentrations were used during the APs (Fig. 1B–D). In each of these pulldowns, known NuRD core subunits were identified. In addition, several previously described NuRD interactors were detected, including ZMYND8, ZNF592, and SALL4 [8,14,17]. Interestingly, we did not pull down LSD1 in any of our GFP–MBD3 purifications. LSD1 has previously been reported as a putative subunit of the NuRD complex [18], but our data on the MBD3–NuRD complex do not agree with this observation. Next, the intensity-based absolute quantification (iBAQ) values were compared between all NuRD subunits and interactors. The iBAQ algorithm normalizes the total MS intensity for each protein according to the number of theoretically observable peptides [19]. This allows estimation of the relative abundance of large and small proteins detected in APs. As the NuRD complex contains many paralogs that overlap at the peptide level, iBAQ values were summed for all paralogs (Table S1).

Comparison of the iBAQ values relative to the GFP–MBD3 bait revealed that most core subunits of NuRD remained tightly bound to each other and to MBD3 despite the presence of high salt (1 M NaCl) and NP-40 (0.5%) concentrations in the wash steps (Fig. 1E). Surprisingly, the core RBBP4 and RBBP7 subunits were very sensitive to NP-40. Increasing the NP-40 concentration from 0.15% to 0.5% led to decreases in the amounts of RBBP4 and RBBP7 associated with the complex (stoichiometry of 5.5 reduced to 4).

In addition, the association of several zinc finger domain-containing proteins with NuRD was very sensitive to high salt concentrations. The association of ZNF512B decreased nearly eight-fold when the salt concentration in the wash was increased from 300 mM to 1 M NaCl (Fig. 1F). A reduction in SALL4 binding was also observed with increased salt concentration. In contrast, other substoichiometric interactors, such as ZMYND8 and KPNA2, remained tightly associated with the core complex under increased salt and NP-40 concentrations. Together, these results suggest that the core NuRD complex is very stable when challenged with high salt concentrations, indicating that interactions within the NuRD complex are at least partially driven by hydrophobic interactions. The partial dissociation of RBBP4 and RBBP7 observed at high NP-40 concentrations may suggest that these proteins are less tightly associated with the rest of the core complex. Finally, although other detected interactors had a low iBAQ value relative to the core complex (1–2% relative to the core subunits), some of these interactions were very stable at high salt and NP-40 concentrations, suggesting a functional role for these proteins in relation to the NuRD complex.

RBBP4 and RBBP7 partially dissociate from the NuRD complex in a subunit exchange assay

To further study the potential dynamics of NuRD subunit interactions, a SILAC-based subunit exchange assay was used (Fig. 2A) [15]. HeLa cells expressing GFP–CDK2AP1 were labeled in culture with heavy amino acids (forward) or light amino acids (reverse). Similarly, wild-type HeLa cells were labeled with light or heavy amino acids. GFP pulldowns were performed immediately after mixing of the nuclear extracts (T0) or after overnight incubation (TON). Proteins that are more dynamically associated with NuRD will dissociate from the complex during the overnight incubation step and may be replaced by proteins from the other, differentially labeled extract. This eventually results in a decrease in detected SILAC ratios, whereby dynamic core subunits and/or interactors will move towards the background cloud in the scatter plot.

At T0, all NuRD core subunits were significantly enriched according to boxplot statistics (Fig. 2B). However, after overnight incubation, RBBP4 and RBBP7 clearly separated from the other NuRD subunits, and migrated towards the background cloud (Fig. 2C). To more directly compare the two scatter plots, the difference in forward and reverse ratios between the plots was visualized in a graph (Fig. 2D). A protein with no change in ratios between experiments would have a value of 0. This graph clearly shows that RBBP4 and RBBP7 are the most dynamic NuRD core subunits. These observations are in agreement with recent structural studies suggesting that MTA and histone H4 compete for RBBP binding [20]. Furthermore, RBBP4 and RBBP7 are present in many protein complexes other than the NuRD complex (Sin3 complex, PRC2), which also could explain their observed dynamic behavior [21,22].

Cross-linking MS reveals novel architectural features of the human NuRD complex

To further characterize the structural interactions between NuRD complex core subunits, we conducted cross-linking MS to identify interprotein interaction surfaces (Fig. 3A). The NuRD complex was isolated from HeLa nuclear extracts by the use of tandem AP with a GFP–His-MBD3 bait, and subsequently cross-linked with BS3 for LC-MS/MS analysis. Cross-linking
was monitored by silver staining to ensure complete cross-linking of all NuRD subunits (Fig. 3B). High-confidence cross-linked peptides [false discovery rate (FDR) of < 0.05] were then identified with PLINK by using default settings and searching against a database of NuRD core components (Fig. 3C,D) [23]. In total, 336 spectra matching with high confidence to 16 non-ambiguous interprotein cross-links, 87 nonambiguous
A) Collect cells

Hela cells

Extract nuclear proteins

GFP bead purification

MBD3

Goat anti-His

Ni²⁺-NTA beads

On bead cross-linking

Ni²⁺-NTA beads

Enzymatic digestion

LC-MS/MS analysis

Identification and validation of cross-linked peptides

P K W Y F R V L K P F W N

B) GFP bead purification

MTA2/2/3

HDAC1/2

RBBP4/7

MBD3

C) Estimated FDR curve

Number of spectra

FDR (%)

D) MRPSKTLF

180°

E) PK W Y F R V L K P F W N

F) Dynamics and architecture of the NuRD complex

S. L. Kloet et al.

intraprotein cross-links and 46 ambiguous cross-links encompassing a variety of homomers or heteromers or intraprotein cross-links were identified (Table S2).

Structural validation was confounded by a lack of overlap between the few existing NuRD crystal structures and our identified cross-links. For example, although five HDAC1 and 11 HDAC2 intraprotein cross-links and four ambiguous HDAC1/HDAC2 cross-links were identified, all of them were located in the undetermined region of the known HDAC1 crystal structure [Protein Data Bank (PDB) 4BKKX]. However, seven cross-links mapping to the CHD4 chromodomain-ATPase region (residues 500–1298), which has a yeast CHD1 ortholog with known structure (PDB 3MWY), were identified. To our knowledge, this represents the highest number of mappable cross-links from our dataset to any single structure. Structural validation of our CHD4 chromodomain-ATPase cross-links was performed with a homology modeling approach. The human CHD4 sequence was aligned to the yeast CHD1 template with HHpred, and homology models were constructed with MODELLER (Human CHD4 Uniprot: Q14839) [24–27]. In 10 overlapping homology models, well-ordered regions from the crystal structure yielded consistently low divergence models, whereas short alignment gaps or structurally undetermined loops were clearly disordered (Fig. 3E). All homology models were highly similar, except for very small, flexible regions, so one model was chosen as representative, and cross-links were validated by calculating the solvent-accessible surface (SAS) distance with xWALK [28]. All seven cross-links showed an SAS distance of < 30 Å, as expected from the maximum length of the BS3 cross-linker. Three cross-links mapped to a well-ordered region, and four mapped to a disordered region that probably possesses a highly flexible structural conformation. Therefore, all seven CHD4 chromodomain-ATPase cross-links seem to be structurally plausible, and we conclude that our cross-linking data are of good quality.

Few NuRD subcomplexes have resolved structures, most notably the MBD2–GATAD2A coiled-coil subcomplex, the RBBP4–MTA1 subcomplex, and the HDAC1–MTA1 subcomplex [20,29,30]. None of these studies described comprehensive protein structures for both binding partners, so the complete interaction interfaces remain unknown. Therefore, our interprotein cross-linking data reveal novel features of the NuRD complex architecture and extend our understanding of NuRD structure–function relationships (Fig. 3F). For example, robust contacts were observed between MTA1–MTA3 and RBBP4 and RBBP7 subunits. Our data do not show whether these RBBP4 and RBBP7 cross-link sites indicate binding of a single MTA paralog or a multimer, nor do they enable us to distinguish between potentially dynamic or paralog-specific interactions at each site. However, mapping cross-linked residues onto the known RBBP4 structure shows that Lys25 and Lys307 fall on either side of the known MTA1 interaction interface (PDB 4PC0 [20]) (Fig. 4A). As RBBP4 Lys25 maps relatively close to the known MTA1-binding pocket, whereas Lys307 sits across a large, adjacent hydrophobic region, these cross-links could outline the opposite ends of the MTA1–MTA3 binding platform (Fig. 4B). Intriguingly, this would explain our observation that MTA1–MTA3 binding to RBBP4 and RBBP7 is stabilized by hydrophobic interactions under high-salt conditions. Similarly, although the MBD3 structure is not known, we identified a short MBD3 region (residues 92–124) that contained five cross-links with GATAD2A/GATAD2B, two of which represented unique cross-links for GATAD2A, one that was unique for GATAD2B, and two that were ambiguous for the GATAD2A/GA-
TAD2B peptide (Fig. 4C). The MBD2 and MBD3 sequences were aligned in EBI’s CLUSTAL OMEGA in order to map any MBD3–GATAD2A cross-links with the solved MBD2–GATAD2A coiled-coil structure (PDB 2L2L [30,31]). Only two cross-linked lysines could be mapped to a representative conformer GATAD2A structure (Lys163 and Lys178), and no cross-links could be mapped to the MBD2 structure (Fig. 4D). Furthermore, one GATAD2A cross-link site, Lys178, was located at the tail end of the conformer, which showed a highly flexible conformation and thus offers little structural information. It is possible that we observed low cross-link coverage within the MBD2–GATAD2A coiled-coil domain because, as the authors of that study noted, this interaction hides at least 1337 Å² of the solvent-accessible area, potentially hindering BS3 accessibility. Thus, our cross-links may outline an interaction interface rather than reveal a binding footprint. However, in agreement with previous work, our data show a single binding interface between MBD3 and GATAD2A/GATAD2B paralogs. Overall, these cross-linking MS data greatly improve
our understanding of the NuRD complex subunit architecture, and will help to direct future structural studies.

Discussion

Here, we have used a variety of MS-based methods to increase our understanding of the dynamics, stability and architecture of the MBD3–NuRD complex. GFP-based APs combined with a label-free method to estimate protein complex stoichiometry revealed that the RBBP4 and RBBP7 core subunits of the NuRD complex are sensitive to high detergent concentrations. Increasing the NP-40 concentration from 0.1% to 0.5% in the wash buffer resulted in a reduced amount of RBBP4 and RBBP7 copurified with MBD3. This finding illustrates an important limitation of AP-MS experiments, namely that some of the interactions that occur in vivo may be lost during AP. Conversely, interactions can be formed in the extract that may not occur in vivo. To overcome these limitations, cross-linking prior to cell lysis can be used as an alternative approach. However, this approach is technically very challenging, owing to experimental and computational issues.

Except for MBD3 and HDAC1/HDAC2, the stoichiometry values that we obtained in our GFP–MBD3 purifications are generally in good agreement with our previously published NuRD stoichiometry results [14,32]. Previously, we made use of a BAC GFP–MBD3 HeLa cell line that expresses MBD3 with a GFP tag at near-endogenous levels. In the current study, we used a doxycycline-inducible GFP–MBD3 cell line. This cell line may express MBD3 at slightly higher levels, which may result in different MBD3 and HDAC1/HDAC2 stoichiometry values. It is important to note that the final stoichiometry values that we obtained represent an average value based on a large number of potentially heterogeneous complexes. On the basis of our data, we cannot be sure whether paralogous proteins such as MTA1–MTA3 or GATAD2A/GATAD2B are present within the same complex, or whether these proteins are, in fact, mutually exclusive, as we have previously shown for MBD2 and MBD3 [33]. It is highly likely that a large number of distinct NuRD complexes exist. However, our cross-linking data cannot distinguish unambiguously between homomeric and heteromeric interactions for HDAC1/HDAC2, MTA1–MTA3 and GATAD2A/GATAD2B. Interestingly, we were able to detect unambiguous interprotein cross-links for CHD3 and CHD4, indicating that these proteins may form a heterodimer. Nevertheless, NuRD complex heterogeneity most likely creates a significant challenge for high-resolution determination of the NuRD molecular architecture.

In addition to the core NuRD subunits, a number of substoichiometric interactors were identified in the GFP–MBD3 purifications. Interestingly, these interactors showed differential sensitivity to salt and detergent. ZMYND8 and ZNF532/592/687 copurified with GFP–MBD3 even when challenged with 1 mM NaCl and 0.5% NP-40. The stability of these interactions indicates that they may be functionally relevant. Interestingly, these proteins have previously been reported to interact with each other as a central hub in a large transcriptional network [34]. Given the presence of a large number of putative DNA-binding zinc fingers in the ZMYND8/ZNF module, we hypothesize that this module may recruit the NuRD complex to a subset of its target genes. The same holds true for other substoichiometric interactors. Further work is needed to determine the functional significance of the ZMYND8/ZNF–NuRD interaction.

Experimental procedures

Cloning

A tandem AP (TAP) tag was cloned into pcDNA5 FRT/TO. This tag consists of enhanced GFP followed by two tobacco etch virus (TEV) protease cleavage sites and a His6 tag. The TEV sites and His tag were added to enhanced GFP via PCR. MBD3 was amplified with BamHI and XhoI, and cloned into the multiple cloning site of pcDNA5 FRT/TO.

Cell culture and SILAC

HeLa FRT cells were transfected with pcDNA5 FRT/TO TAP–MBD3 by the use of Lipofectamine LTX Plus (Invitrogen, Glasgow, Scotland). Cells underwent selection for 10 days with hygromycin, and single colonies were picked, expanded, and screened for expression of TAP–MBD3. TAP–MBD3 expression was induced by treatment with 1 μg·mL⁻¹ doxycycline for 16 h. SILAC treatment of HeLa cells was performed as described previously [35].

Nuclear extracts

Nuclear extracts were prepared essentially according to Dignam et al. [36]. Cells were harvested with trypsin, washed twice with PBS, and centrifuged at 400 g for 5 min at 4 °C. Cells were allowed to swell for 10 min at 4 °C in five volumes of Buffer A (10 mM Hepes/KOH, pH 7.9, 1.5 mM MgCl₂, 10 mM KCl), and then pelleted at 400 g for 5 min at 4 °C. Cells were resuspended in two volumes of Buffer A plus pro-
Dynamics and architecture of the NuRD complex

S. L. Kloet et al.

tease inhibitors and 0.15% NP-40, and transferred to a Dou-

once homogenizer. After 30–40 strokes with a Type B pestle,
the lysates were spun at 3200 g for 15 min at 4 °C. The
nuclear pellet was washed once with PBS, and spun at 3200 g
for 5 min at 4 °C. The nuclear pellet was resuspended in
Buffer C (420 mM NaCl, 20 mM Hepes/KOH, pH 7.9, 20%
$v/v$ glycerol, 2 mM MgCl$_2$, 0.2 mM EDTA) with 0.1%
NP-40, protease inhibitors, and 0.5 mM dithiothreitol. The
suspension was incubated with rotation for 1 h at 4 °C, and
then spun at 18 000 g for 15 min at 4 °C. The supernatant
was saved, aliquoted, and stored at −80 °C until further use.

**Label-free pulldowns and cross-linking MS**

Label-free GFP pulldowns, LC-MS/MS and data analysis
were performed as described previously [14]. For cross-link-
ing MS, 8 mL of HeLa nuclear extract (5 mg·mL$^{-1}$ plus
50 μm·L$^{-1}$ ethidium bromide) containing doxycycline-
induced TAP–MBD3 was incubated with 400 μL of GFP-
trap beads (Chromotek) for 2 h at 4 °C, and incubating for 15
min at 4 °C. The supernatant was collected, and GST
–

TEV protease was

incubated with 10 μL of TEV cleavage buffer containing 100 units of GST–TEV protease (US Biological) and incubated overnight at 4 °C in a rotation wheel. The supernatant was incubated with 10 μL of GST beads and incubating for 15 min at 4 °C in a rotation wheel. The eluate was then incubated with 20 μL of Ni$^{2+}$–nitritolriacetic
tic acid beads (Qiagen) for 45 min at 4 °C in a rotation wheel. Beads were washed once with 1 mL of TEV cleavage buffer and three times with 1 mL of cross-linking buffer (25 mM Hepes/KOH, pH 7.9, 300 mM KCl, 5% glycerol, 0.05% Tween-20). Beads were then resuspended in 50 μL of cross-linking buffer containing 250 μM BS3 cross-
linker (Sigma), and incubated for 30 min at 37 °C in a thermoshaker (1100 r.p.m.). The cross-linking reaction was quenched by adding 7 μL of 0.5 M ammonium bicarbonate and incubating for 5 min at room temperature. Finally, beads were washed twice with 0.5 mL of PBS, after which bound cross-linked proteins were digested overnight with on-bead LysC/trypsin digestion. Tryptic peptides were de-
salted and purified with stagetips prior to LC-MS/MS analysis.

**Analysis of cross-linking MS data**

Thermo RAW MS files were converted to mgf format with the mconvert tool from proteowizard, and analyzed with plink, with default settings for HCD with BS3 as a cross-
linking agent [23,37]. Cross-linked peptides matched to spectra with an FDR of < 0.05 were considered to be high

confidence, and were used for further analysis. Spectra were

visually analyzed and annotated with plabel [38]. Template
structures for homology modeling were searched with
hhpred, and homology models were built with the model-

ler interface in ucsf chimera [24,26,39]. The yeast CHD1
chromodomain and ATPase residues 38–800 (PDB 3MWY)
were used as a template for residues 500–1298 from human

CHD4 (Uniprot Q14839) for homology modeling [27].
Calculations of rmsd values were performed in ucsf chi-
mera. SAS distances were calculated for a representative
CHD4 model with xwalk. xinet was used to visualize
high-confidence intraprotein and interprotein cross-links in
network form (Rappsilber Laboratory, University of Edin-
burgh, Scotland; crosslinkviewer.org). All protein structure
visualizations were produced with ucsf chimera.

**Acknowledgements**

This work was supported by the EU FP7 framework

program 4CellFate. We thank I. Poser and A.

Hyman for providing the BAC GFP–CDK2AP1 cell

line. We thank N. Vermeulen-Hubner and H. Stunnen-
berg for providing access to the Q Exactive mass

spectrometer.

**Author contributions**

SLK, HIB, VG, PWTCJ, MB, HN, and MV per-
formed experiments. SLK, HIB, MM, and VG ana-
alyzed data. GJK contributed reagents. SLK, HIB, MM, and
MV wrote the paper with input from all authors.

**References**

decaylase and nucleosome remodeling activities. Cell 95, 279–289.


Supporting information
Additional supporting information may be found in the online version of this article at the publisher’s web site:

Table S1. iBAQ values used to calculate NuRD subunit stoichiometry.

Table S2. Overview of all assigned cross-links with FDR < 0.05 as calculated in PLINK.