SUPPLEMENTARY MATERIAL AND METHODS

Peptide Synthesis and Purification

Peptide N1 was purified by RP-HPLC with a gradient of 9–27% acetonitrile in H_2O + 0.1% TFA on a Vydac C18 column to obtain 17 mg pure peptide. Peptide identity was confirmed by MALDI-TOF MS (*m/z* expected 4128.3, observed 4127.8). Peptide C1 was purified by RP-HPLC on a Grace Vydac C18 column with a 27-36% gradient of acetonitrile / 0.1% TFA to generate 21 mg pure peptide. Peptide identity was confirmed by MALDI-TOF MS (*m/z* expected 4554.5, observed 4555.7).

Synthesis of the 56 amino acid peptide M1 was completed on mercaptopropionamide-MBHA resin. Synthesis was closely monitored by ninhydrin testing. After coupling of 37 residues (H3-K56ac residues 54-90), 100 mg of peptide-resin was cleaved to follow the progress of the synthesis. While the presence of the expected peptide was detected by RP-HPLC and MALDI-TOF MS, typical RP-HPLC protocols utilizing a gradient of acetonitrile / H_2O + 0.1% TFA could not achieve single-peak resolution (data not shown). After significant protocol optimization, a gradient of isopropanol / 0.1% TFA at 40°C over a Grace Vydac C4 column was determined to provide optimal product separation.

While the primary species in the crude cleavage mixture for full-length peptide M1 was the desired product, analysis and purification were complicated by the appearance of an additional species at +16 Da (Met species: *m/z* expected 5098.7, observed 5099.2; Met(O) species: *m/z* expected 5115.7, observed 5115.4). Oxidation of methionine residues [Met(O)] is a commonly observed side product of HF cleavage and may also evolve following prolonged storage of resin prior to cleavage. Met and Met(O) containing M1 species could not be resolved under RP-HPLC purification conditions, but established protocols exist for the subsequent conversion of Met(O) to Met in thioester peptides[1]. However, we determined that these

conditions result in partial conversion of N-terminal Thz to Cys, and subsequent cyclization of peptide M1 via intramolecular ligation (Data not shown) and are thus unsuitable to immediate application to the M1 peptide. These species were therefore pooled and carried through the remainder of the ligation steps, and Met(O) reduction was carried out only on final synthetic H3(K56ac) products. Crude M1 peptide was purified by RP-HPLC on a Vydac C4 column with a gradient of 24-38% isopropanol / 0.1% TFA at 45°C. Fractions containing both the Met and Met(O) species were pooled and lyophilized; peptide identity was confirmed by MALDI-TOF MS (for Met species, *m/z* expected 6782.6, observed 6782.8. For Met(O) species, *m/z* expected 6798.6, observed 6798.8). Despite the inclusion of the Met(O) containing species in the product pool, final peptide M1 yield was 2 mg by weight from 80 mg of crude for a final purification yield of 2.5%.

Following synthesis and HF cleavage, peptide N2 was purified by RP-HPLC with a gradient of 11–24% acetonitrile / 0.1% TFA on a Vydac C18 column. Peptide identity was confirmed by MALDI-TOF MS (*m/z* expected 4958.8, observed m/z 4955.4). A total of 70 mg of pure N2 peptide was obtained. Synthesis of the 44 residue middle segment M2 was carried out using double-coupling of each amino acid to improve crude yields. Peptide M2 was purified using an optimized gradient of 33-47% acetonitrile / 0.1% TFA at room temperature (RT) with a Supelco Widebore C18 column. Peptide identity was confirmed by MALDI-TOF MS (*m/z* expected 5526.0, observed 5526.5) with only minimal Met(O) observed. Purification yields were ~5% pure product from the initial crude cleavage mixture (3.7 mg pure by weight from 71 mg crude). Peptide C2 was initially purified by RP-HPLC on a Vydac C18 column with a 27-41% gradient of acetonitrile / 0.1% TFA. Peptide identity was verified by MALDI-TOF MS (*m/z* expected 5082.7, observed 5083.1). A purification yield of ~5% was typically observed under these conditions. Subsequent purifications were carried out over a Supelco Widebore C18 column, which increased purification yield significantly to generate 19.6 mg pure C2 peptide.

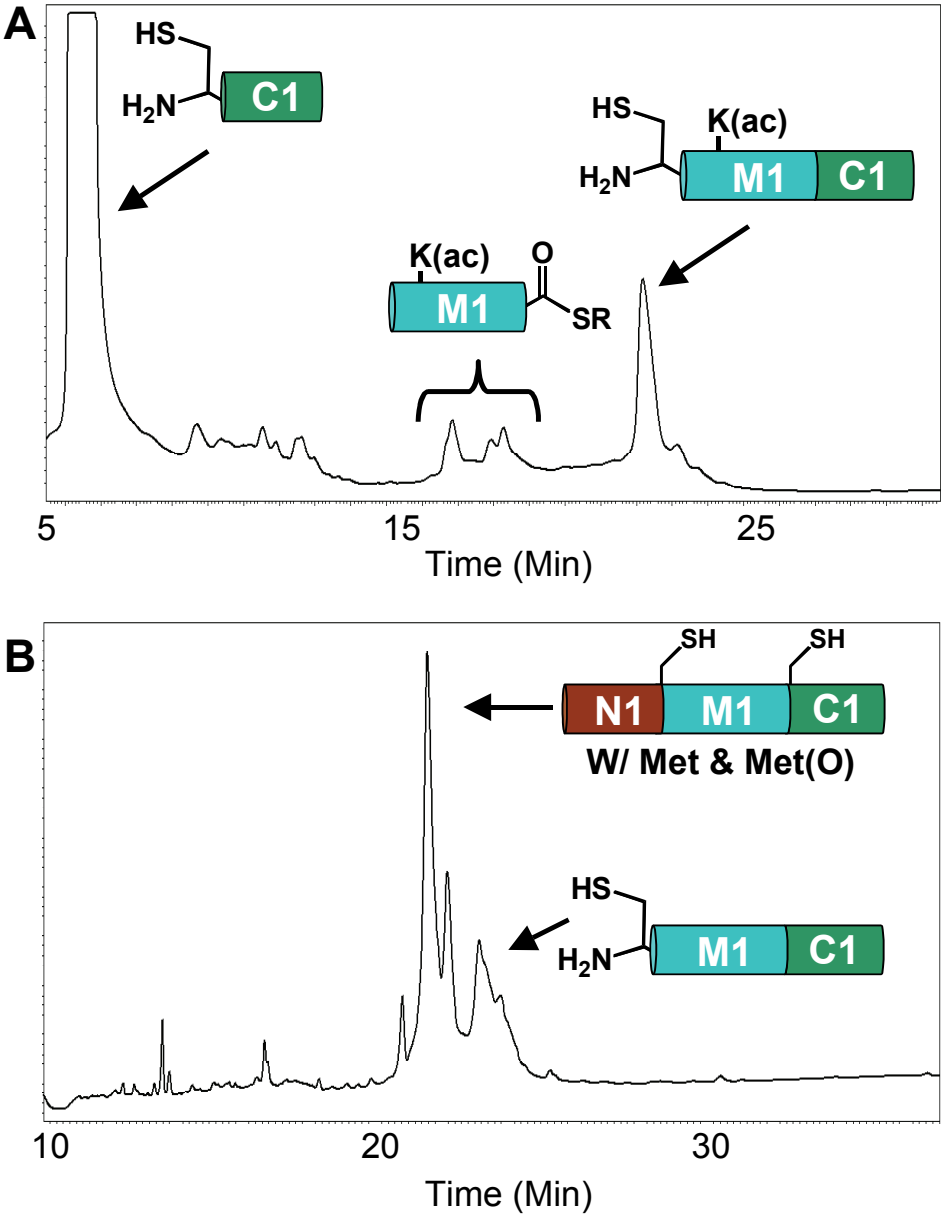
Electrophoretic Mobility Shift Assay of LexA Binding

In order to verify that the reduction in FRET upon addition of LexA to nucleosomes containing the 601-LexA-end construct was due to LexA binding to the nucleosome, we conducted Electrophoretic Mobility Shift Assays (EMSA) of LexA bound to the nucleosome. Nucleosomes were incubated with 0-3 μM LexA in 0.5xTE for 2 minutes at 20°C before resolving on a 5% native polyacrylamide gel (PAGE) in 0.3xTBE at 300V. As previously reported[3], we did not observe a detectable gel shift until a LexA concentration much greater than the $S_{0.5\text{-nuc}}$ for binding of 601-LexA-end observed by FRET (data not shown). Therefore we employed glutaraldehyde fixation to trap specifically bound LexA. To demonstrate that glutaraldehyde does not affect the specific binding of LexA to naked DNA we performed EMSA on 601-LexA-end DNA. DNA at 0.1 nM was incubated with 0-1 μM LexA in 0.5xTE or 5 mM HEPES pH 8.0 for 2 minutes at 20°C. Glutaraldehyde was added to 0.05% final concentration to the reactions containing HEPES, incubated for 2 minutes at 20°C before quenching with a final concentration of 50 mM Tris pH 8.0, and resolved by EMSA (Fig S2 A,B). The fraction of free DNA as a function of LexA concnetration was quantified for n=3 experiments (Fig. S2 C) and fit to a cooperative binding curve to determine the concentration of half saturation ($S_{0.5\text{-DNA}}$) and cooperativity parameter (p) without glutaraldehyde fixation ($S_{0.5\text{-DNA-glut}}$ = 0.32 ± 0.04, p = 2.1 ± 0.2, R^2 = 0.9995) and with glutaraldehyde fixation ($S_{0.5\text{-DNA+glut}}$ = 0.36 ± 0.04, p = 1.4 ± 0.2, R^2 = 0.9984). $S_{0.5\text{-DNA}}$ is unaffected by glutaraldehyde fixation and the only apparent effects glutaraldehyde has on LexA binding is that it decreases the cooperativity parameter by ~25% and alters the mobility of non-specific DNA-LexA complex at higher [LexA].

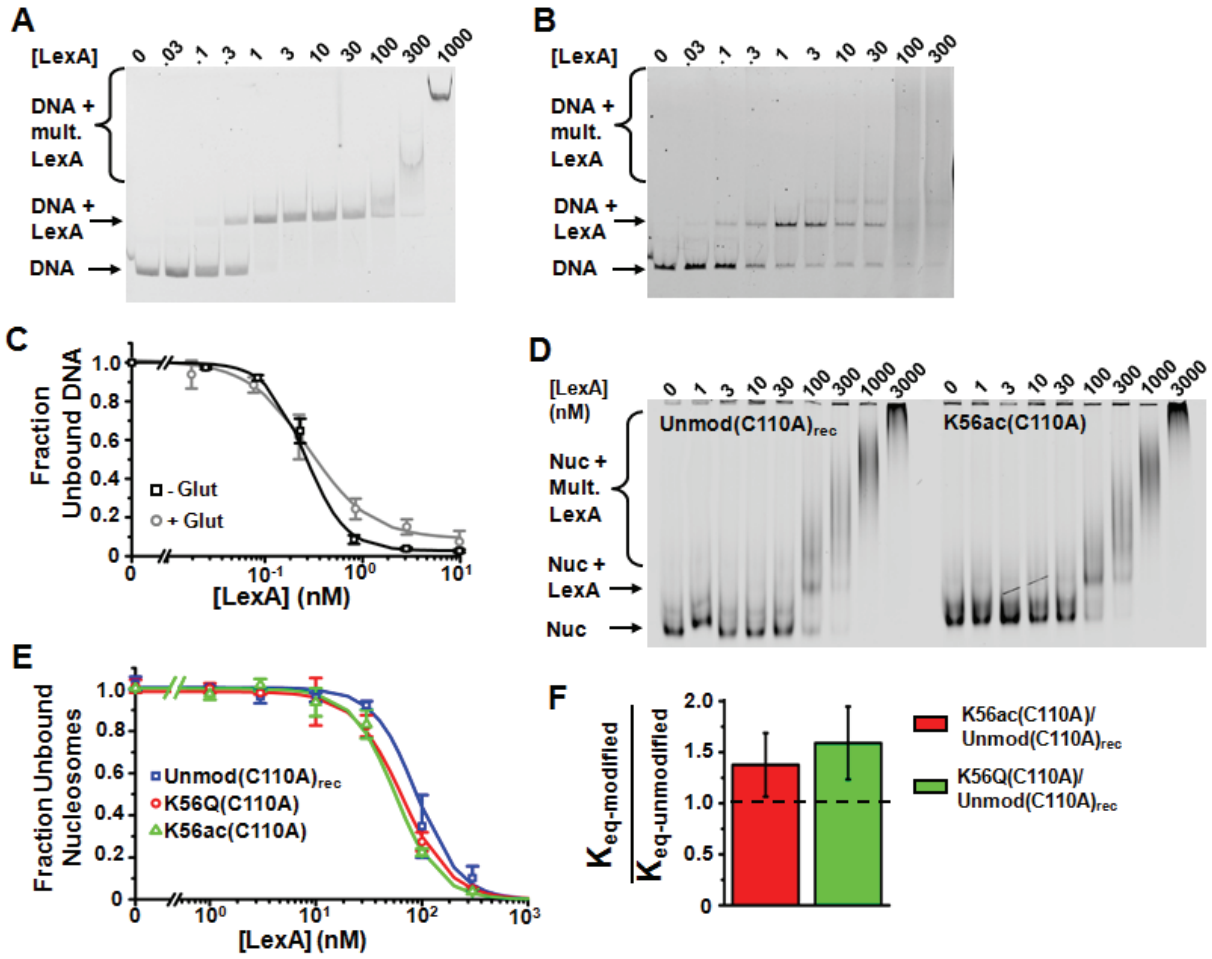
For binding to 601-LexA-end nucleosomes, nucleosomes at 5 nM in 5 mM HEPES pH 8.0 were incubated with LexA, fixed with glutaraldehyde and resolved by 5% native PAGE as described above (Fig S2 D). A well-defined hyper-shifted band, absent without glutaraldehyde fixation, was observed from 30-300 nM LexA. As this is the range of LexA concnetrations in

which fluorescence measurements show a decrease in FRET, we attribute this band to specific LexA-nucleosome complex. The fraction of free nucleosomes as a function of LexA concnetration was quantified for n=3 experiments (Fig. S2E) and fit to a cooperative binding curve for nucleosomes containing H3(C110A) ($S_{0.5\text{-Nuc}}$ = 88 ± 13, R^2 = 0.9995), H3(K56Q,C110A) (64 ± 4, R^2 = 0.9984), or H3(K56ac,C110A) (56 ± 3, R^2 = 0.9984). We determined the site exposure equilibrium constant, K_{eq}, from the half saturation value of LexA binding to its target sequence within the nucleosome and to naked DNA, since $S_{0.5\text{-}nuc} = S_{0.5\text{-}DNA} / K_{eq}$, in the limit that the K_{eq} is much less than 1 (See Materials and Methods for details). From this equation, we determined the equilibrium constant for site exposure by EMSA for nucleosomes containing H3(C110A) (0.0036 ± 0.0007), H3(K56Q,C110A) (0.0050 ± 0.0007) and H3(K56ac,C110A) (0.0058 ± 0.0007), which is similar to those measure by FRET. The change in the site exposure equilibrium of H3(K56Q) and H3(K56ac) relative to unmodified nucleosomes is equal to the probability that LexA can bind to its site that extends 27 base pairs into the nucleosome by 1.4 ± 0.3 and 1.6 ± 0.3 times, respectively, which within the uncertainty of the measurements agree with the FRET measurements (Fig. S2 F).

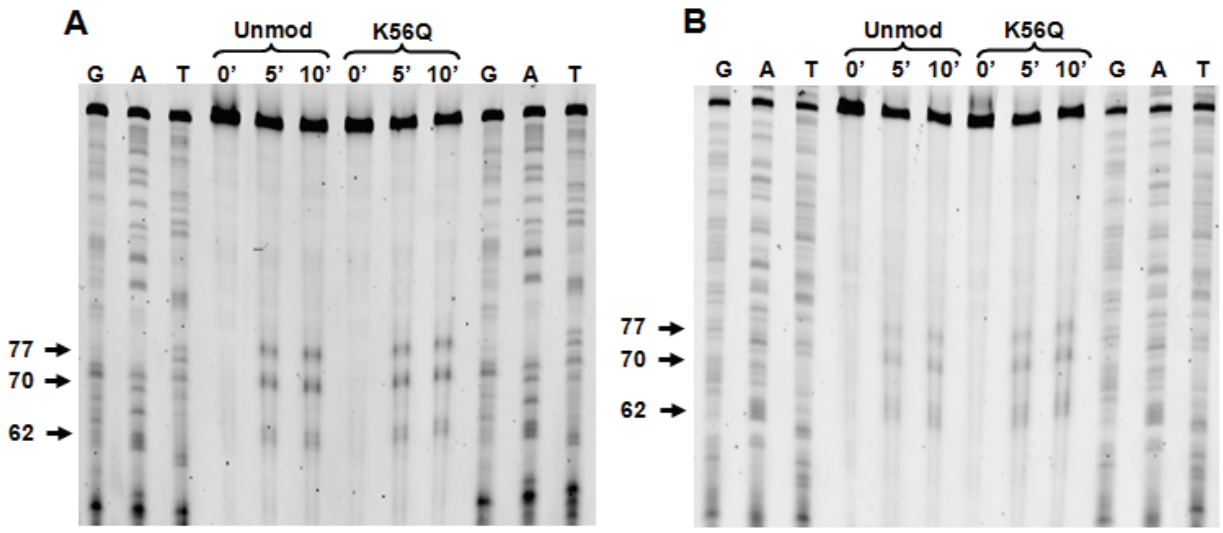
SUPPLEMENTARY FIGURES



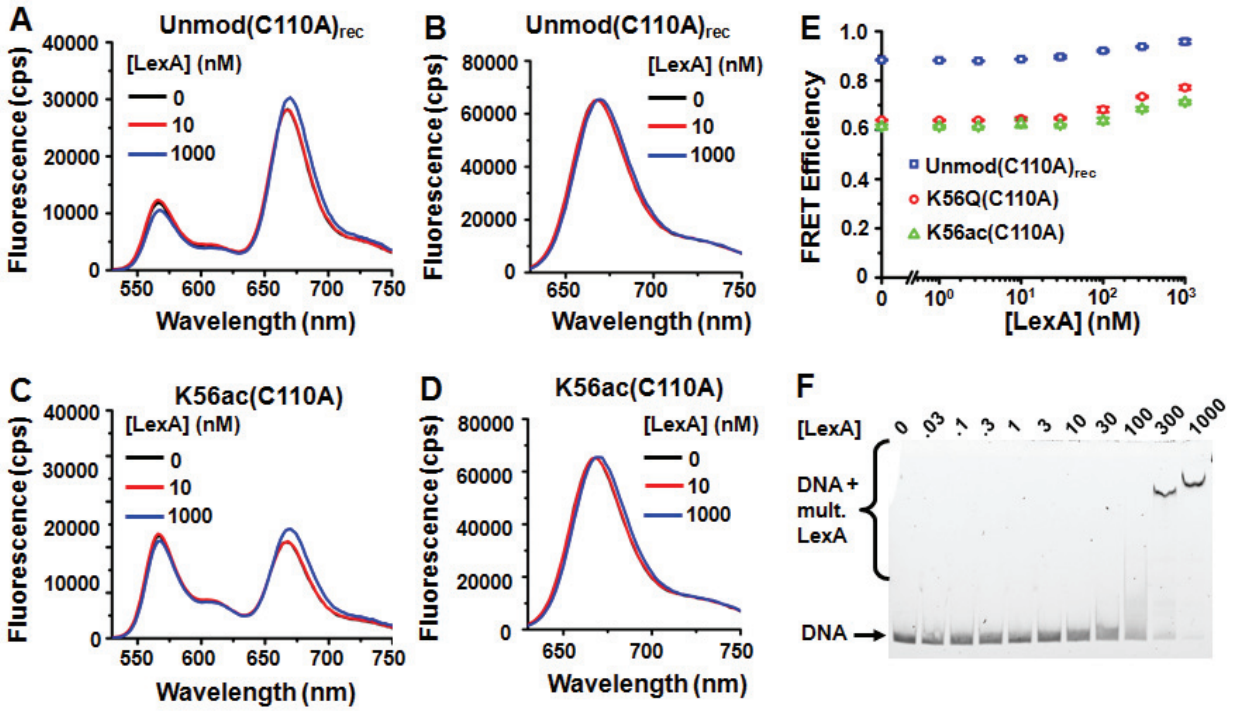
Supplementary Fig. 1. Purification of sequential native chemical ligation products to generate H3(R40C,K56ac,S96C,C110A). (**A**) RP-HPLC chromatogram of reaction mixture of the first ligation step to generate M1C1, after ring-opening to reveal the N-terminal Cys. RP-HPLC was run with a gradient of 22.5-50% isopropanol / 0.1% TFA on a C4 column at 45°C. (**B**) RP-HPLC chromatogram of the reaction mixture for the second ligation step to generate H3(R40C,K56ac,S96C,C110A) with a step gradient of acetonitrile / 0.1% TFA on a C18 column as follows: 11-16% over 5 min, 16-43% over 5 min and 43-66% over 30 min. Note that peptide N1 eluted in the solvent front and is not shown in this trace.



Supplementary Fig. 2. Electrophoretic Mobility Shift Assays (EMSA) of LexA binding to naked DNA and nucleosomes containing the 601-LexA-end DNA construct. (**A**) EMSA of LexA binding to naked 601-LexA-end DNA without glutaraldehyde fixation. DNA was incubated with the indicated amount of LexA in 0.5xTE and resolved by 5% native PAGE. (**B**) EMSA of LexA binding to naked 601-LexA-end DNA with glutaraldehyde fixation. DNA was incubated with the indicated amount of LexA in 5 mM HEPES pH 8.0, fixed with 0.05% glutaraldehyde, and resolved by 5% native PAGE. (**C**) Average fraction of unbound DNA as a function of [LexA] with (gray) and without (black) glutaraldehyde fixation for the experiments in **A** and **B** and two additional experiments each. The error bars are the standard deviation of the three mesaurements. (**D**) EMSA of LexA binding to 601-LexA-end nucleosomes containing H3(C110A) and H3(K56ac,C110A). Nucleosomes were incubated with the indicated amount of LexA in 5 mM HEPES pH 8.0, fixed with 0.05% glutaraldehyde, and resolved by 5% native PAGE. (**E**) Average fraction of unbond nucleosomes as a function of [LexA] for nucleosomes containing H3(C110A) (blue), H3(K56Q,C110A) (red) and H3(K56ac,C110A) (green) for the experiment in **D** and two additional experiments. The error bars are the standard deviation of the three measurements. The data was fit to a cooperative binding cure which determines $S_{0.5\text{-}nuc}$, the LexA concentration at which 50% of the nucleosomes are bound by LexA. (**F**) Equilibrium constants of H3(K56Q,C110A) relative to H3(C110A) (1.4 ± 0.3), and H3(K56ac,C110A) relative to H3(C110A) (1.6 ± 0.3),



Supplementary Fig. 3. LexA binding within the nucleosome does not reposition the nucleosome. . (**A**) and (**B**) Denaturing polyacrylimide gel electorphoresis of the nucleosomal DNA cleaved by FeBABE for 0, 5 and 10 minutes in the presence of 1000 nM LexA. Within each gel, lanes 1-3 and 10-12 contain sequencing tracks terminated with ddGTP,ddATP and ddTTP respectively, lanes 4-6 contain nucleosomes with H3(C110A) and lanes 7-9 contain nucleosomes with H3(K56Q,C110A). (**A**) and (**B**) are images of denaturing gels with the top and bottom DNA strands, respectively, are visualized by cy3 fluorescence. The cleavage patterns remain unchanged compared to FeBABE cleavage with out LexA (See Figure 7).



Supplementary Fig. 4. LexA binding to nucloeosomes without a LexA binding site. (**A**) and (**B**) Fluorescence emission traces for H3(C110A) nucleosomes containing the 601-end DNA construct in the presence of LexA at 0 nM (black), 10 nM (red), 1000nM (blue), excited at 510nm and 610nm, respectively. (**C**) and (**D**) Fluorescence emission traces for H3(K56ac,C110A) nucleosomes containing the 601-end DNA construct in the presence of LexA at 0 nM (black), 10 nM (red), 1000nM (blue), excited at 510nm and 610nm, respectively. (**E**) Energy transfer efficiency, as determined by the $(ratio)_A$ method[2], versus LexA concentration for nucleosomes containing H3(C110A) (blue), H3(K56Q,C110A) (red), and H3(K56ac,C110A) (green). The error bars are the standard deviation of the separate measurements. (**F**) EMSA of naked 601-end DNA incubated with the indicated amount of LexA in 0.5xTE and resolved by 5% native PAGE.

SUPPLEMENTARY REFERENCES

1. **Hackenberger, C. P. (2006). The reduction of oxidized methionine residues in peptide thioesters with NH4I-Me2S. *Org Biomol Chem* 4, 2291-5.**

2. **Clegg, R. M. (1992). Fluorescence resonance energy transfer and nucleic acids. *Methods Enzymol* 211, 353-88.**

3. Li, G. & Widom, J. (2004). Nucleosomes facilitate their own invasion. *Nat Struct Mol Biol* 11, 763-9.