Appendix A

Investigations of anti-BrdU Antibody Staining of Py-Im Polyamide Treated Cells

This research was conducted in collaboration with Peter B. Dervan (California Institute of Technology).

A.1 Background

A long-standing problem in the field of DNA-binding Py-Im polyamides is to map the location of bound polyamides throughout the genome. Recently, attempts have been made to pull down biotin-conjugated polyamides that are crosslinked to chromatin via streptavidin beads. This strategy proved to be unsuccessful for mapping polyamide binding sites throughout the genome of cancer cells (1,2); however, it was successfully retooled for use with the Bind-N-Seq platform (3). While analogs of the standard 8-ring hairpin polyamides with chemical handles such as biotin substituted for the C-terminus isophthalic acid are effective, there is always the concern as to whether or not the analog behaves in exactly the same manner as the parent compound. This same problem arises when using FITC-substituted polyamides as a means of inferring whether or not the parent molecule is able to traffic to the nucleus in live cells. In animal studies, the issue of monitoring tissue distribution without the use of a dye-conjugated molecule was solved by using a ^{14}C-labeled analog of the parent molecule; the substitution of a single C atom with a radioactive isotope provided a means for sensitive detection of the polyamide with little chance of altering the behavior of the molecule (4,5). If there were a means of recognizing an 8-ring hairpin polyamide without the use of substituting the isophthalic acid for a chemical handle, it would provide a more accurate means of mapping polyamide binding and perhaps another method for staining polyamides in nuclei. One possible method would be through the use of an antibody that can recognize an 8-ring hairpin Py-Im polyamide.

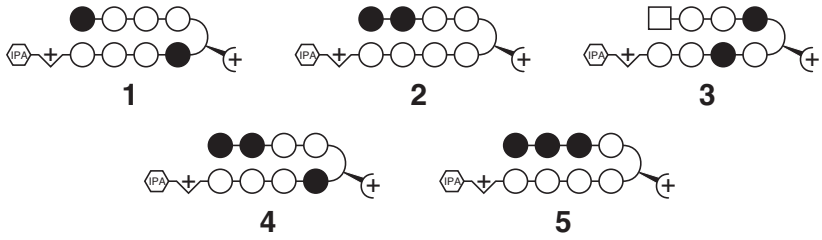

Figure A.1 Ball-and-stick representations of Py-Im polyamides used in this study. Polyamide **1** targets the sequence 5'-WGWWCW-3', polyamide **2** targets the sequence 5'-WGGWWW-3', polyamide **3** targets 5'-WTWCGW-3', polyamide **4** targets 5'-WGGWCW-3', and polyamide **5** targets 5'-WGGGWW-3'.

While attempting to stain for Py-Im polyamide-induced ssDNA foci by detection of incorporated 5-bromo-2'-deoxyuridine (BrdU) via immunocytochemistry (Chapter 2), it was observed that the mouse monoclonal anti-BrdU antibody MoBU-1 resulted in polyamide-dependent intranuclear punctate staining in the absence of BrdU. This result spurred the investigation as to what the antibody was recognizing in polyamide treated DU145 cells, and whether different polyamides cause the same effect (Figure A.1). In this chapter, we report the early studies into MoBU-1 staining and suggest future experiments for this study.

A.2 Results

Mouse monoclonal antibody MoBU-1 stains nuclear foci in Py-Im polyamide treated DU145 cells.

In order to stain BrdU in DU145 cells by immunocytochemistry for detection of ssDNA cells were treated with 50 μM BrdU for 12 h followed by treatment with DMSO vehicle, 10 or 30 μM Py-Im polyamide **1** for 36 h (Figure A.2), or 4 mM HU for 2 h. The cells were then washed three times with ice cold PBS (pH 7.4) and then fixed with fresh 3:1 methanol:acetic acid (MeOH:AcOH) for 20 min at -20ºC. No denaturation step was

used afterward. Next, fixed cells were washed with a solution of .05% Tween 20 in PBS and then blocked using 2% normal goat serum for 30 min in a 37ºC incubator. Immunostaining was carried out overnight at 4ºC with MoBU-1 (Life Technologies) diluted 1:100 in blocking buffer. The next day, cells were washed three times with .05% Tween 20 in PBS before staining with the secondary antibody, Alexa Fluor® 488-conjugated donkey anti-mouse. Finally, cells were mounted using Prolong Gold Antifade

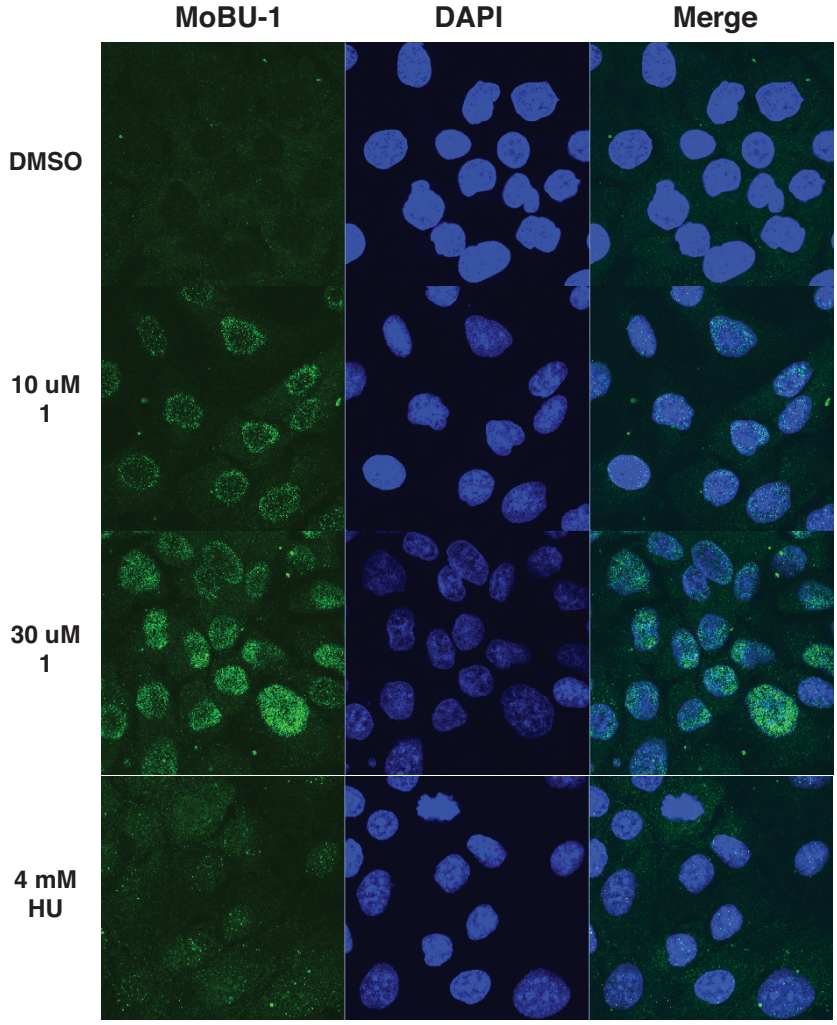

Figure A.2 MoBU-1 immunocytochemistry shows punctate staining in DU145 cells treated with BrdU followed by polyamide **1**. DU145 cells were pre-treated with 50 µM BrdU for 12 h prior to incubation with 10 µM or 30 mM **1** for 36 h. Cells were then fixed with MeOH:AcOH and incubated with MoBU-1 (1:100) and Alexa Fluor® 488 anti-mouse antibody (1:500). Significant punctate staining localized to the nucleus of polyamide treated cells. Only marginal staining was observed in cells treated with 4 mM hydroxyurea (HU) for 2 h.

with DAPI mounting solution for counterstaining of the nuclei. Imaging by confocal fluorescence microscopy (LSM 5 Exciter, 63x oil objective) showed significant nuclear staining in polyamide treated cells, but not in DMSO treated cells. However, cells treated with 4 mM hydroxyurea (HU) showed significantly less staining, which was unexpected given results from other experiments, such as the absence of Chk1 phosphorylation, which suggested that polyamide-induced replication stress was weaker than 4 mM HU-induced stress. Therefore, we tested whether the staining observed in response to polyamide treatment was a false positive.

In order to test whether MoBU-1 may be reacting to an antigen other than incorporated BrdU, the experiment was re-run in the absence of BrdU. Compared to DU145 cells treated with BrdU prior to incubation with **1**, polyamide **1** treated cells lacking BrdU showed equivalent nuclear punctate staining (Figure A.3). Next, we tested whether the fluorophore-conugated secondary antibody might be reacting non-specifically and thus resulting in punctate staining. To test this possibility, polyamide **1** treated cells were stained using only the Alexa Fluor® 488 anti-mouse antibody. Under these conditions, no foci were observed. These results demonstrated that the nuclear staining observed in cells treated with **1** is dependent upon MoBU-1 but not BrdU.

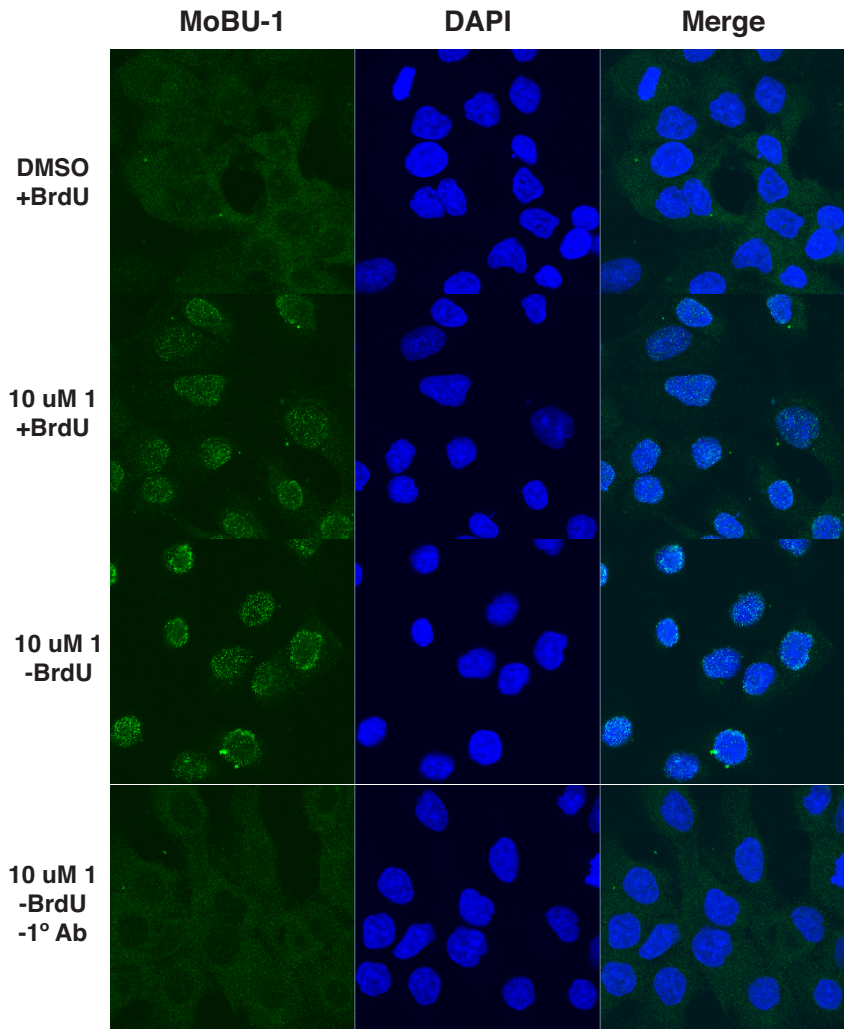

Figure A.3 MoBU-1 stains polyamide **1** treated DU145 cells in the absence of BrdU. DU145 cells were pre-treated with 50 μM BrdU or PBS for 12 h prior to incubation with 10 μM **1** for 36 h. Cells were then fixed with MeOH:AcOH and incubated with MoBU-1 (1:100) or blocking solution and Alexa Fluor® 488 anti-mouse antibody (1:500). Punctate staining is still observed in polyamide treated cells in the absence of **1**, but not when MoBU-1 is also removed.

*MoBU-1 staining of polyamide **1** treated cells is not dependent on MeOH:AcOH fixation.*

The previous results demonstrated that the anti-BrdU antibody MoBU-1 reacts with an unknown antigen in DU145 cells treated with **1** to form punctae. In order to determine whether the antibody is recognizing **1** directly, or whether it can at least be useful as a means of detecting polyamides in cells indirectly, we tested what other dependencies the MoBU-1 reaction might have. Different cell or tissue fixation methods

have been shown alter the structure of chromatin and shape of nuclei (6). Fixation by MeOH:AcOH works by dehydrating cells and does not preserve the 3D structure of chromatin, while fixation by formaldehyde results in protein crosslinking and does preserve the 3D structure of chromatin. Therefore, we next tested if the MoBU-1 staining observed in cells treated with **1** was dependent upon alterations to the chromatin induced by dehydration with MeOH:AcOH.

Polyamide treated cells fixed with MeOH:AcOH were compared to cells fixed with 2% formaldehyde (Figure A.4). For formaldehyde fixation, fresh 2% formaldehyde was prepared by dilution of 16% formaldehyde (10 mL ampule, Ted Pella, Inc.) into PBS. Cells were fixed in formaldehyde for 5 min at room temperature. Next, cells were washed three times with PBS at room temperature and then permeabilized with 0.2% Triton X-100 in PBS. Following permeabilization, cells were blocked with 3% normal goat serum with 0.1% Triton X-100 in PBS for 45 min at room temperature. Immunostaining by MoBU-1 and Alexa Fluor® 488-anti-mouse antibody was carried out as before. Despite the difference in fixation method, robust staining by MoBU-1 in cells treated with 10 μM **1** but no BrdU. However, the staining in formaldehyde fixed cells was uniform throughout the nuclei and did not appear to be punctate as in MeOH:AcOH fixed cells. This difference was also observed in the DAPI stain, which suggests that the difference in staining appearance is likely due to the change in chromatin structure resulting from fixation.

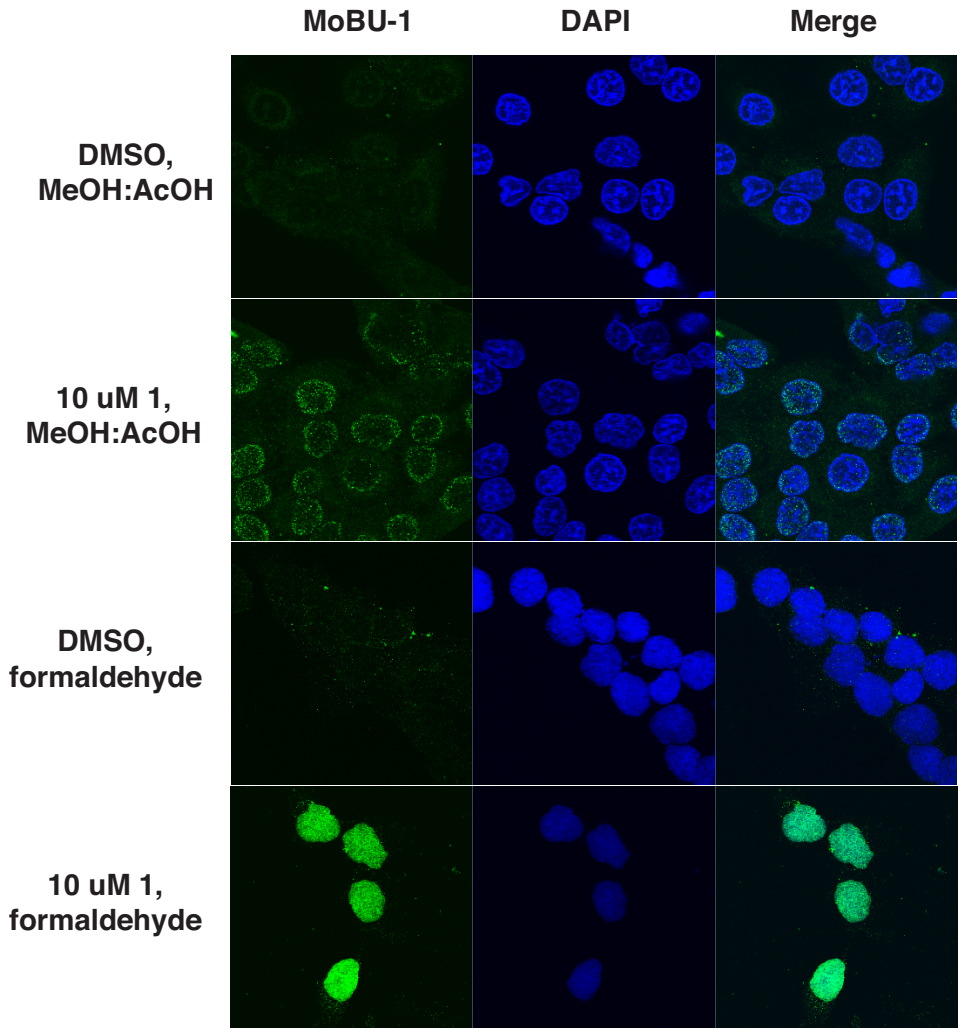

Figure A.4 MoBU-1 stains polyamide **1** treated DU145 cells when fixed with MeOH:AcOH or 2% formaldehyde. DU145 cells were treated with DMSO or 10 μM **1** for 24 h. Cells were then fixed with MeOH:AcOH or 2% formaldehyde and incubated with MoBU-1 (1:100) and Alexa Fluor® 488 anti-mouse antibody (1:500). Significant nuclear staining was observed under both fixation conditions. Staining in formaldehyde fixed cells is more uniform throughout the nucleus.

These results clearly show that MoBU-1 staining is not dependent upon the fixation method. In addition, the appearance of the stain changing with the structure of the chromatin under different fixation conditions suggests that the MoBU-1 reaction occurs at the DNA level. Formaldehyde also has benefits over methanol:acetic acid. If MoBU-1 is reacting directly with polyamide or polyamide:DNA, it is important that the

reaction is not inhibited by formaldehyde used in pulldown studies to crosslink the small molecule to DNA. Formaldehyde fixation is also compatible with immunocytochemistry of nuclear proteins, such as PCNA (Chapter 2) and γ-H2AX. Furthermore methanol fixation will also permeabilize cells (7) and has been shown to alter the subcellular localization of dye-conjugated polyamides (8).

MoBU-1 staining in DU145 cells occurs in response to multiple Py-Im polyamides.

Staining of DU145 cells treated with polyamide **1** by MoBU-1 appeared to be robust, so we next tested how general the phenomenon was among a small library of polyamides targeted to different DNA sequences (Figure A.1). The five polyamides chosen were all 8-ring hairpin motifs with the isophthalic acid conjugated C-terminus and alpha amine substituted turn. These polyamides were chosen because they have been shown to be effective in targeting a variety of gene regulation pathways in previous studies (9-13). All polyamides were dosed at 10 μM for 24 h and fixed with formaldehyde prior to immunostaining. Strong staining by MoBU-1 was observed in response to treatment with **1**, **3**, and **5**, while only marginal staining was observed in response to **2** and **4** (Figure A.5). Interestingly, staining in cells treated with **1** and **3** was localized to the nucleus but staining in cells treated with **5** was exclusively outside the nucleus. A cytoplasmic or membrane counterstain was not used; therefore, specific localization cannot be determined. However, it is plausible the staining occurs only on the cell surface. This result is interesting, because it suggests that if MoBU-1 is reacting directly with polyamides, it is not necessary for the polyamide to be DNA-bound.

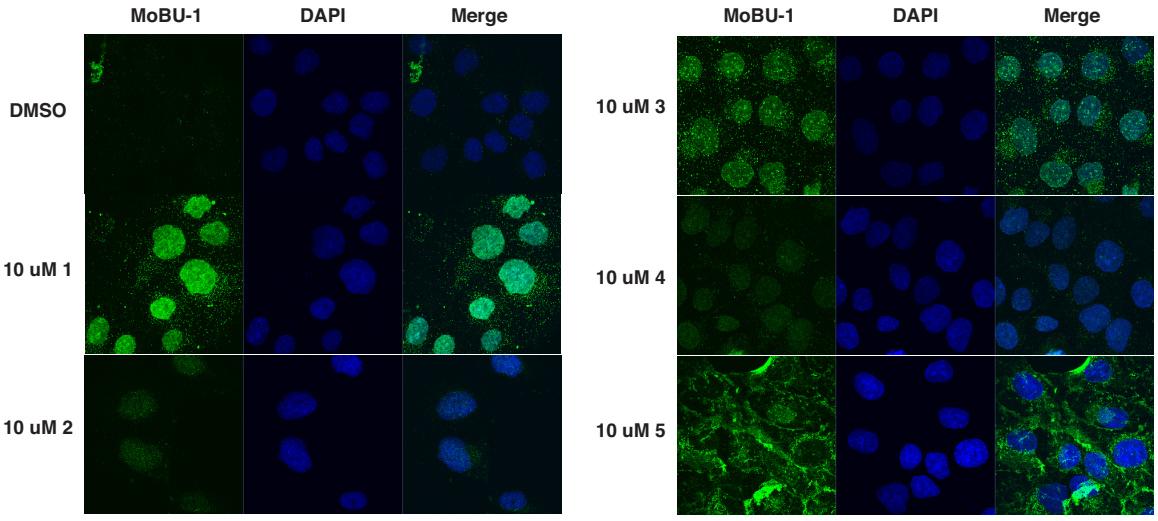

Figure A.5 MoBU-1 staining in response to a small library of polyamides. DU145 cells were treated with DMSO or 10 μM **1**-**5** for 24 h. Cells were then fixed with 2% formaldehyde and incubated with MoBU-1 (1:100) and Alexa Fluor® 488 anti-mouse antibody (1:500). Significant nuclear staining was observed in response to **1**, **3**, and **5**. Staining in cells treated with **5** was observed outside the nucleus.

In order to get a more detailed look at the staining observed in response to **5**, several image slices were acquired by confocal microscopy through the Z-axis and combined to create a "3D" rendered Z-stack (Figure A.6). This Z-stack image clearly shows that MoBU-1 staining in response to **5** is almost exclusively outside the nucleus, while staining observed in response to **1** is predominantly localized to the nucleus.

Next, staining of polyamides **3** and **5** were also tested for dependence upon MoBU-1 and not non-specific binding of the secondary antibody. Just as with polyamide **1**, removal of MoBU-1 from the procedure did not result in immunostaining of cells treated with **3** or **5** (Figure A.7).

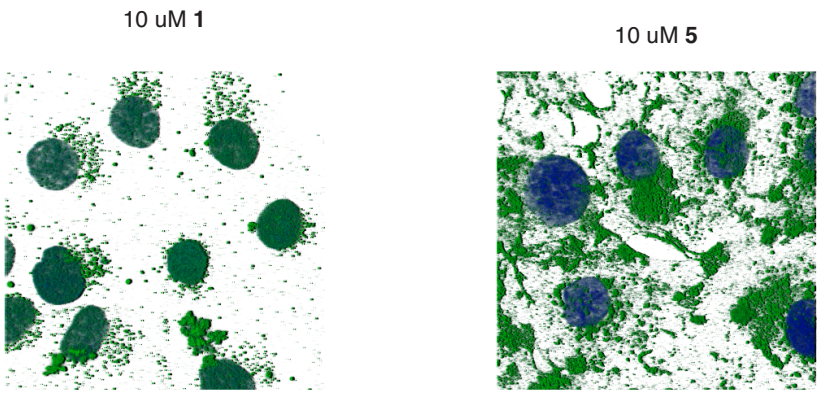

Figure A.6 Z-stack of MoBU-1 stained DU145 cells treated with polyamide **1** or **5**. The 3D rendered image of representative cells treated with **1** or **5** shows differences in localization of the staining.

MoBU-1 staining did not improve when polyamide was dosed after fixation and permeabilization.

If MoBU-1 is reacting directly with polyamide or polyamide:DNA, then the differences in staining observed in response to MoBU-1 might be a result of differences in uptake of polyamides into live cells. Therefore, if polyamides are dosed after fixation and permeabilization of cells, then uptake potential should be roughly equal and all polyamides can be recognized by MoBU-1 It is also possible, however, that MoBU-1 is reacting with an endogenous factor/complex that forms in response to polyamide treatment. If this model is correct, then dosing cells after fixation and permeabilization should prevent this endogenous factor from forming to react with MoBU-1 and no staining would be observed. These hypotheses were tested by fixing untreated cells with formaldehyde and then permeabilizing as before. Next, cells were treated with DMSO or polyamide for 24 h diluted in PBS. Afterward, cells were washed three times with PBS, blocked with goat serum, and then incubated with antibodies. When dosed after fixation,

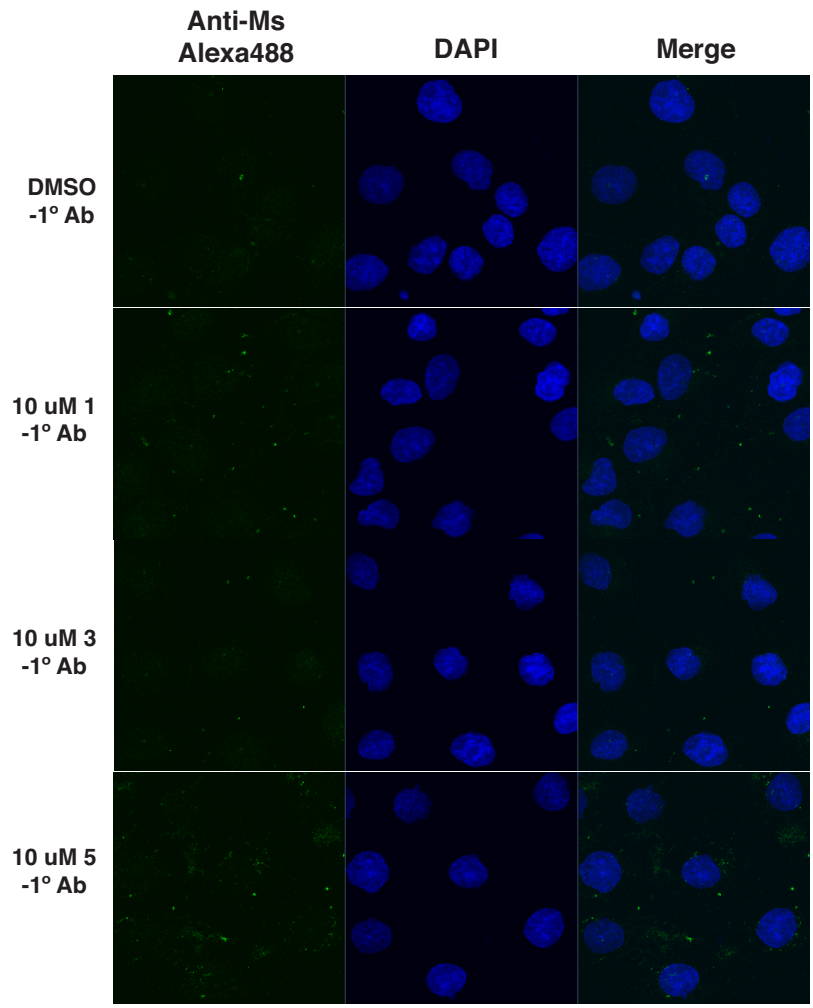

Figure A.7 Staining of polyamide treated cells is dependent upon MoBU-1. DU145 cells treated with 10 μM polyamide followed by fixation and incubation with Alexa Fluor® 488 secondary antibodies showed no staining, proving the dependence on MoBU-1 across all polyamide tested.

cells treated with polyamides **1** and **3** still showed strong nuclear staining by MoBU-1 (Figure A.8). These results demonstrated that the MoBU-1 antigen is not an endogenous factor that forms in response to polyamide treatment. However, polyamide **2** treated cells showed increased staining, but significantly less than observed for **1** and **3**. This is puzzling given uptake should be the same for all polyamides, but may perhaps be due instead to unanticipated differences in binding affinity to chromatin. Interestingly, cells treated with polyamide **5** showed significantly less staining when dosed after fixation.

This result suggests that the MoBU-1 response to polyamide **5** treatment might actually be dependent on a cellular process or protein. One such possibility is if **5** normally interacts with a cell surface protein, which is significantly altered by formaldehyde fixation and Triton X-100 permeabilization, and that this complex is no longer able to form.

At least one other anti-BrdU antibody is capable of staining Py-Im polyamide treated DU145 cells.

The reaction of the mouse monoclonal anti-BrdU antibody MoBU-1 with an unknown antigen in polyamide treated cells showed some generality and potential utility as means of detecting which cells receive polyamides, and possibly even where the polyamide is localized. Before this antibody can be used for these purposes, however, it is critical to understand what the antibody is reacting with and how. One means of inferring what the antibody might react with, is to test whether there is a strong similarity between the intended antigen, BrdU-incporated ssDNA, and the antigen in polyamide treated cells. If other anti-BrdU antibodies demonstrate this same effect, then the antigen in polyamide treated cells must be structurally and electronically similar to BrdU-incorporated ssDNA. If the phenomenon is truly unique to MoBU-1, then the effect might be understood by investigating the difference in the antigen binding site of MoBU-1 from other anti-BrdU antibodies. We know at least one other anti-BrdU antibody is not capable of this phenomenon, as we were able to use the rat monoclonal anti-BrdU antibody ICR1 (Santa Cruz Biotech) to recognize CldU incorporation without observing non-specific effects in polyamide-treated cells (Chapter 2). To test whether a third anti-

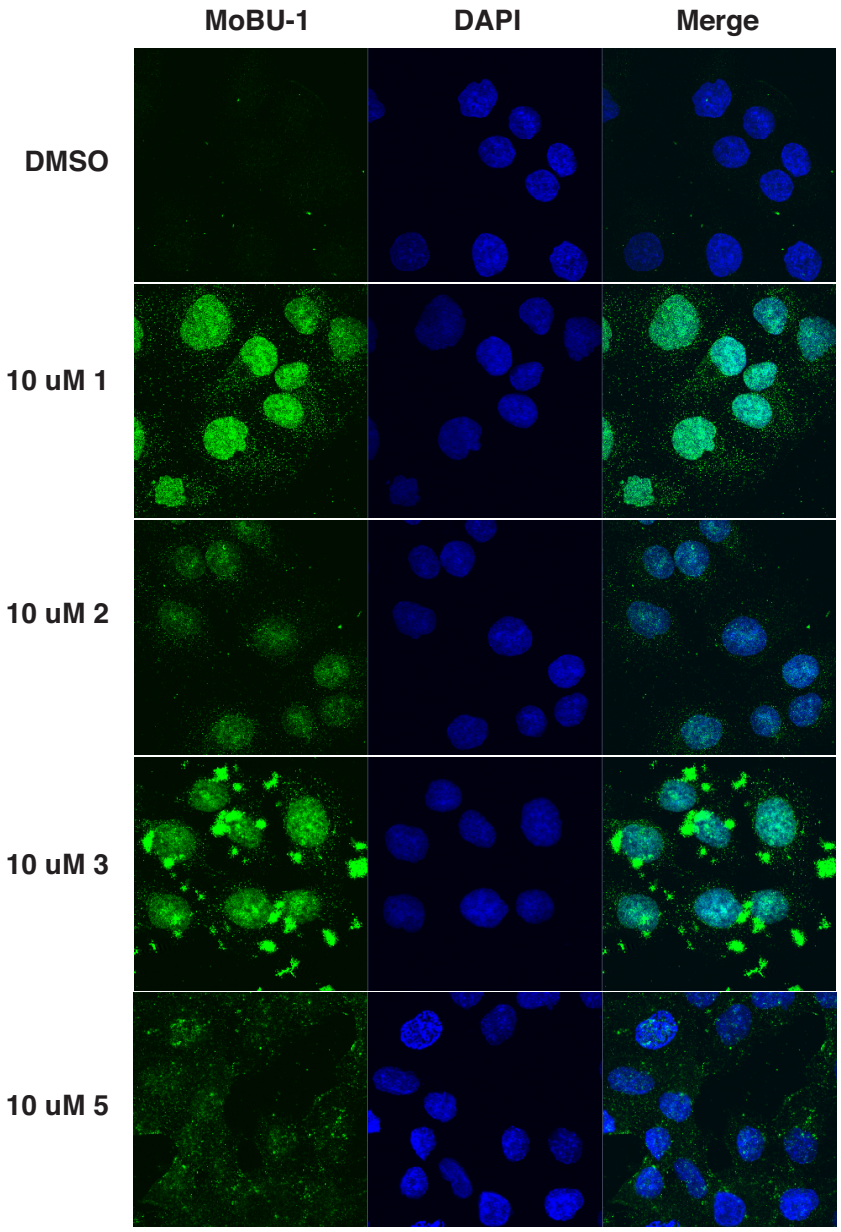

Figure A.8 MoBU-1 staining in DU145 cells treated with Py-Im polyamides after fixation with formaldehyde. Untreated DU145 cells were fixed and permeabilized prior to dosing with 10 μM polyamide for 24 h. Then, cells were blocked with 3% goat serum and incubated with MoBU-1 and Alexa Fluor® 488 secondary antibody. Dosing cells after fixation did not affect staining of cells treated with **1**-**3**. However, staining in response to **5** was significantly reduced.

BrdU antibody might also show the same phenonmenon as MoBU-1, we repeated the staining experiment using ZBU30 (Life Technologies), which is also a mouse monoclonal antibody (Figure A.9). Live DU145 cells treated with 10 μM **1** for 24 h and

then incubated with ZBU30 and Alexa Fluor® 488 anti-mouse secondary antibody showed significant staining of the nucleus, which was comparable to cells stained with MoBU-1 (Figure A.8). Cells treated with DMSO and incubated with ZBU30 showed weak non-specific staining but only outside of the nucleus. Cells treated with **1** and incubated with ICR1 and Alexa Fluor® anti-rat secondary antibody did not result in staining, as expected.

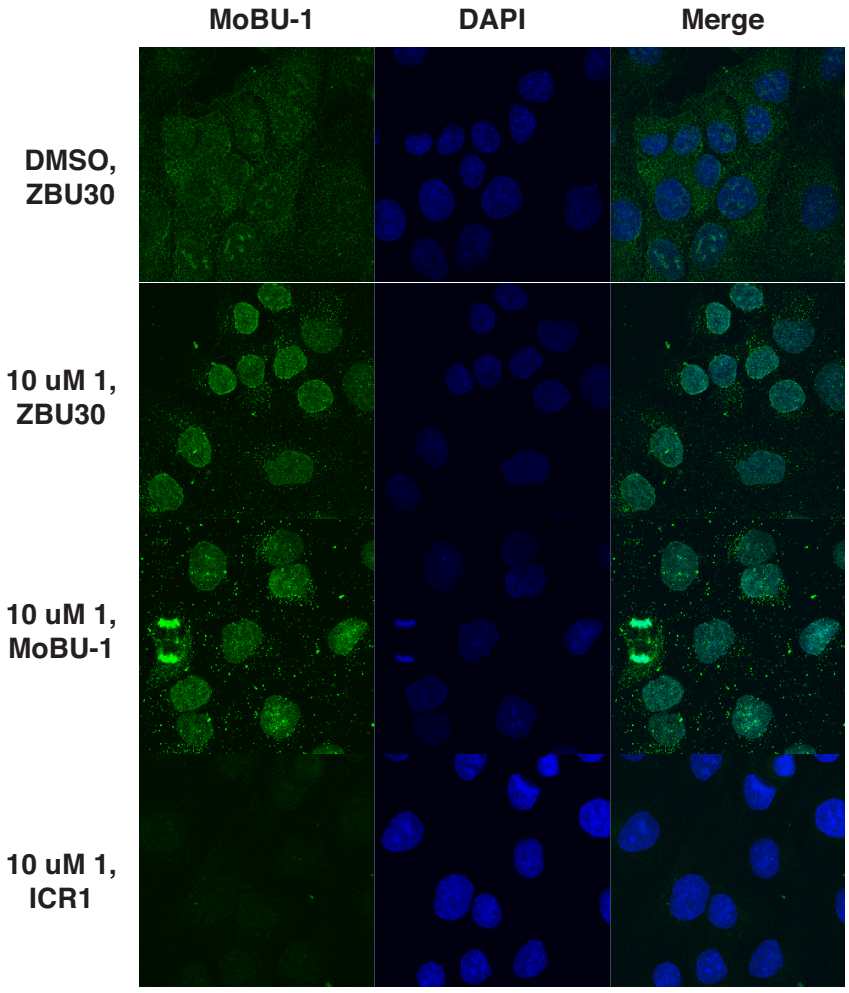

Figure A.9 Anti-BrdU antibody ZBU30 but not ICR1 stains polyamide treated DU145 cells. DU145 cells were treated with DMSO or 10 μM **1** for 24 h. Cells were then fixed with 2% formaldehyde and incubated with either MoBU-1 (1:100), ZBU30 (1:100), or ICR1 (1:10) and Alexa Fluor® 488 anti-mouse antibody (1:500) or anti-rat antibody (1:500). Similar staining was observed using ZBU30 as MoBU-1. ICR1 did result in staining of the cells.

These results demonstrate that there may in fact be a common structural feature between BrdU-incorporated ssDNA and the antigen reacting with MoBU-1 in polyamide treated cells. However, that ICR1 does not show the same effect despite also being capable of recognizing BrdU suggests that it may not be a strong similarity. The cross-reactivities of the three antibodies tested may provide further insight into why this difference exists. MoBU-1 is known to have high specificity for BrdU and not have cross-reactivity with 5-ethynyl-2'-deoxyuridine (EdU) or thymidine. ZBU30 is known to cross-react with 5-iodo-2'-deoxyuridine (IdU) and not thymidine. ICR1, however, is known to cross-react with 5-chloro-2'-dexoyuridine (CldU) and not thymidine. Iodine is a larger halide than bromine while chlorine is smaller. Perhaps the same feature of the antigen binding site that allows for reaction with larger 2' halides in ZBU30 allows it recognize the unknown antigen in polyamide treated cells, while the ability to recognize smaller halides by ICR1 prevents the reaction in polyamide treated cells. It is also interesting to note that the immunogen used to raise MoBU-1 is BrdU conjugated to hemocyanin, an extracellular oxygen carrier protein found in arthropods. The specific immunogens used to raise ZBU30 and ICR1 are unknown.

Attempts to characterize the putative MoBU-1:polyamide:DNA complex by biophysical methods.

Investigations of the MoBU-1 staining phenomenon provided clues into the generality of the effect, but little evidence about its ability to recognize polyamides directly. As a direct means of answering this question, we employed an *in vitro* electrophoretic mobility shift assay (EMSA) to test whether MoBU-1 is capable of

forming a ternary complex with polyamide:DNA (Figure A.10). Polyamide **1** was incubated with 600 fmol Cy5-labeled duplex DNA containing a single match site for 1.5 h at room temperature in 1x TAEMg buffer (40 mM Tris pH 7.5, 1 mM EDTA, 12.5 mM Mg(OAc)2, 20 mM acetic acid). The total reaction volume was 20 µL. The DNA duplex and binding conditions were also used previously to demonstrate ternary complex formation of biotin-analong of **1** (1). Reactions were run on native 6% polyacrylamide gels in 1x TBE at 175 V for 1.5 h. DNA duplex incubated with 300 nM or 1 µM **1** ran higher on the gel than DNA incubated with DMSO, representing the

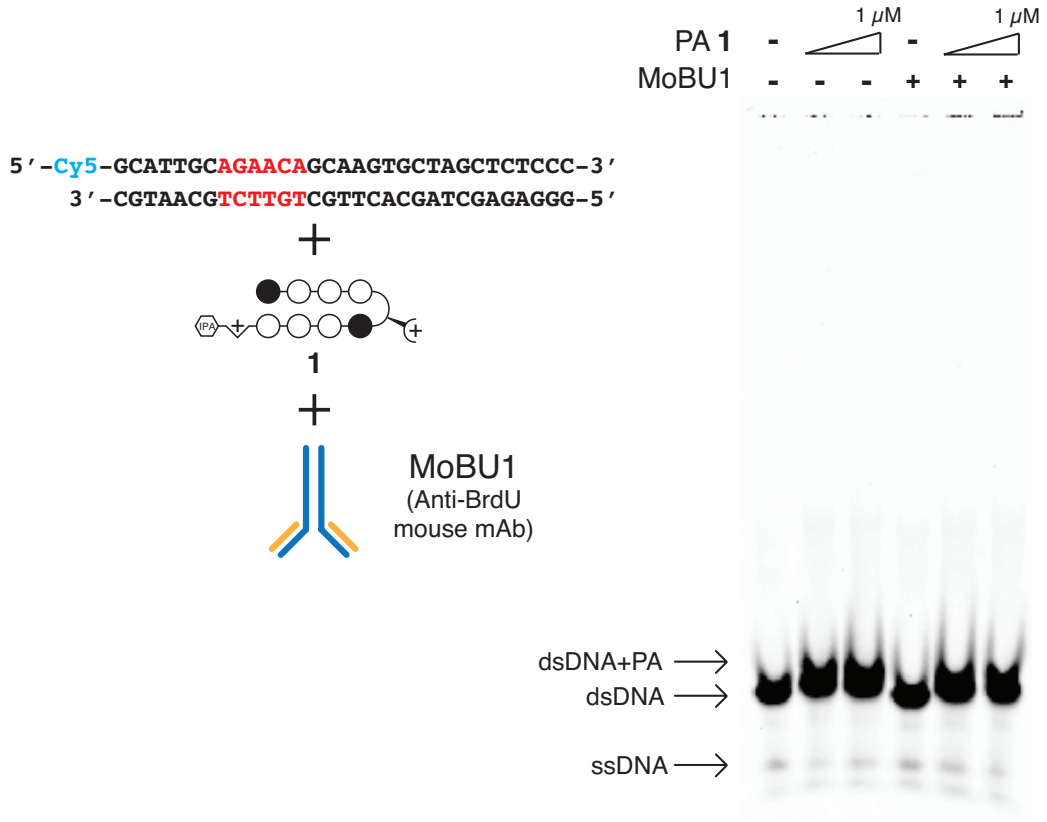

Figure A.10 Electrophoretic mobility shift assay (EMSA) of MoBU-1 and polyamide **1**:DNA. A shift of the Cy5-DNA duplex containing a match site for **1** was observed when incubated with 300 nM and 1 µM **1** in 1x TAEMg. A further shift of this complex representing the ternary complex was not observed when the polyamide:DNA complex was incubated with 133 nM MoBU-1.

higher weight polyamide:DNA complex. When DNA and polyamide were also incubated with 133 nM MoBU-1, no higher shift was observed. These results show that MoBU-1 does not bind to polyamide:DNA directly under these conditions.

We also attempted to probe for MoBU-1 binding to polyamide **1** by surface plasmon resonance (SPR). SPR involves conjugating one of the molecules of interest, or ligand, to the surface of a gold-coated glass slide. The putative binding partner, or analyte, is flowed over the top of the surface-conjugated molecule, and if binding occurs the surface properties of the sensor chip are also altered. These alterations are detected as changes in the angle of incident light needed to induce SPR waves and are proportional to the amount of analyte bound. For this particular study we decided to use the antibody capture method to attach MoBU-1 to the surface of a CM5 Sensor Chip (14). The CM5 chip has an approximately 100 nm thick carboxymethylated dextran layer on top of the gold-coated glass slide, which provides a hydrophilic environment that is favorable to many biomolecular interactions. The negatively charged carboxyl groups also allows for covalent attachment of a variety of biomolecules, such as proteins by amine coupling (14). The antibody capturing method involves covalently coupling an anti-mouse IgG antibody to the surface of the chip by standard amine coupling conditions and then capturing the antibody of interest, MoBU-1, via its interaction with the anti-mouse antibody. For our experiment, we used AffiniPure Sheep anti-Ms IgG (Jackson ImmunoResearch) to conjugate to the surface of the CM5 chip. Conjugation was performed with 1 μM antibody done in 10 mM acetate buffer, pH 5 at a flow rate of 1 μL/min. Capture of MoBU-1 was performed using 100 nM MoBU-1 in 1x HBS-EP+ at

flow rate of 10 μL/min for 120 s. Binding of polyamide **1** to MoBU-1 was investigated as a complex with DNA or alone. The same DNA duplex was used as in the EMSA experiment, except lacking the Cy5 dye. 1 μM polyamide **1**:DNA was used as the analyte and flowed over the chip at 10 μL/min for 30 sec. No response was observed under these conditions relative to a flow cell conjugated with only the anti-mouse antibody. 10 μM **1** alone was also tested as the analyte and no response was observed relative to the control flow cell. ZBU30 was also captured to the chip and tested for binding to both polyamide:DNA and polyamide **1** alone, but not response was observed. Different binding conditions for SPR may be required to observe binding if possible. These results are consistent with the EMSA studies and suggest that the antigen recognized by MoBU-1 in cells might be more complex.

A.3 Conlcusions

The mouse monoclonal anti-BrdU antibodies MoBU-1 and ZBU30 are robustly stain DU145 cells treated with multiple polyamides under both MeOH:AcOH and formaldehyde fixation conditions. Biophysical experiments did not show that MoBU-1 was capable of binding to polyamide **1** bound to DNA, but it is possible that these *in vitro* assays are not accurate models for polyamide bound chromatin in the nuclear environment or polyamide bound to other factors. Perhaps crystallography studies and molecular modeling can provide better insight into direct recognition of polyamides by MoBU-1.

If in the future it can be shown that MoBU-1 is reacting directly with polyamides, it would prove useful for genome-wide mapping studies. The ability of MoBU-1 to pull

down polyamide:DNA can be tested using the Bind-N-Seq methodology, though the *in vitro* studies suggest that this is unlikely to succeed (3). Another area where MoBU-1 can be tested for utility would be in the staining of tissue sections from treated animals. Currently, visualization of polyamides in tissue is done using FITC-analogs. If MoBU-1 is not sufficiently specific for these purposes, current results suggest that it might be possible to have an antibody specifically raised against Py-Im polyamides.

A.4 Acknowledgments

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A.5 References

1. Martinez, T.F. (2011), Candidacy Report, California Institute of Technology.

2. Meier, J.L. (2012), Postdoctoral Report, California Institute of Technology.

3. Meier, J.L., Yu, A.S., Korf, I., Segal, D.J. and Dervan, P.B. (2012) Guiding the design of synthetic DNA-binding molecules with massively parallel sequencing. *J Am Chem Soc*, 134, 17814-17822.

4. Raskatov, J.A., Puckett, J.W. and Dervan, P.B. (2014) A C-14 labeled Py-Im polyamide localizes to a subcutaneous prostate cancer tumor. *Bioorg Med Chem*, 22, 4371-4375.

5. Raskatov, J.A., Szablowski, J.O. and Dervan, P.B. (2014) Tumor xenograft uptake of a pyrrole-imidazole (Py-Im) polyamide varies as a function of cell line grafted. *J Med Chem*, 57, 8471-8476.

6. Kozubek, S., Lukasova, E., Amrichova, J., Kozubek, M., Liskova, A. and Slotova, J. (2000) Influence of cell fixation on chromatin topography. *Anal Biochem*, 282, 29-38.

7. Jamur, M.C. and Oliver, C. (2010) Permeabilization of cell membranes. *Methods Mol Biol*, 588, 63-66.

8. Belitsky, J.M., Leslie, S.J., Arora, P.S., Beerman, T.A. and Dervan, P.B. (2002) Cellular uptake of N-methylpyrrole/N-methylimidazole polyamide-dye conjugates. *Bioorg Med Chem*, 10, 3313-3318.

9. Yang, F., Nickols, N.G., Li, B.C., Marinov, G.K., Said, J.W. and Dervan, P.B. (2013) Antitumor activity of a pyrrole-imidazole polyamide. *Proc Natl Acad Sci U S A*, 110, 1863-1868.

10. Nickols, N.G., Szablowski, J.O., Hargrove, A.E., Li, B.C., Raskatov, J.A. and Dervan, P.B. (2013) Activity of a Py-Im polyamide targeted to the estrogen response element. *Mol Cancer Ther*, 12, 675-684.

11. Raskatov, J.A., Meier, J.L., Puckett, J.W., Yang, F., Ramakrishnan, P. and Dervan, P.B. (2012) Modulation of NF-kappaB-dependent gene transcription using programmable DNA minor groove binders. *Proc Natl Acad Sci U S A*, 109, 1023-1028.

12. Nickols, N.G., Jacobs, C.S., Farkas, M.E. and Dervan, P.B. (2007) Modulating hypoxia-inducible transcription by disrupting the HIF-1-DNA interface. *ACS Chem Biol*, 2, 561-571.

13. Kang, J.S. and Dervan, P.B. (2015), *Unpublished*.

14. (2008) Online Manual: Biacore Sensor Surface Handbook BR-1005-71 Edition AB. *GE Lifesciences*.