Specificity and methylation sensitivity analysis of HoxB13

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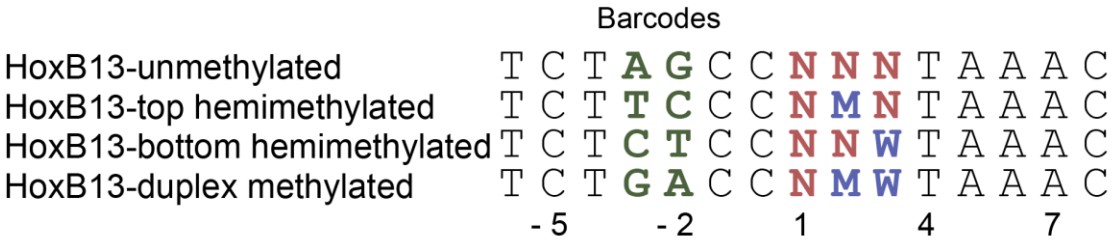
Table of Contents

Introduction

HoxB13, is a homeobox protein recently found to process methylation sensitivity and two distinct motifs, i.e., TCGTAAA and CAATAAA. To quantify this, I designed following randozmied dsDNA libraries covering the unmethylated, top hemimethylated, bottom hemimethylated, duplex methylated binding sites at positions 2 and 3 respectively by chemical synthesis, thus we can assay the strand-specific methyl-C contribution to the binding affinity of each site.



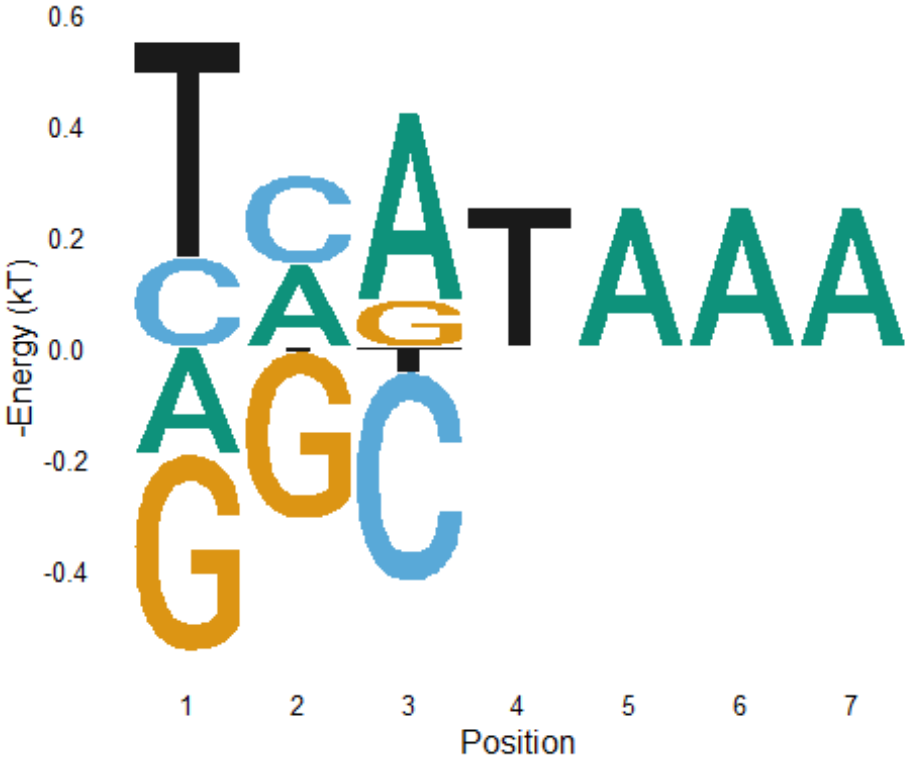
Importing and preoprocessing data

```
load("../data/HoxB13.RData")

(HoxB13 <- HoxB13 %>%
  dplyr::mutate("Bound/Unbound" = Bound/Unbound,
                Energy = -log(Bound/Unbound)))

## # A tibble: 100 x 6
##    Sequence Property               Bound Unbound `Bound/Unbound` Ene
rgy
```

```
##    <chr>    <chr>                  <dbl>   <dbl>           <dbl>  <d
bl>
##  1 CCATAAA  top Hemimethylated    119049    3949           30.1  -3.
41
##  2 TCGTAAA  duplex Methylated     246730   16406           15.0  -2.
71
##  3 TCATAAA  top Hemimethylated    113243    8850           12.8  -2.
55
##  4 TCGTAAA  top Hemimethylated    157847   12818           12.3  -2.
51
##  5 TCGTAAA  unmethylated           66312   12149            5.46 -1.
70
##  6 ACATAAA  top Hemimethylated     42781    8102            5.28 -1.
66
##  7 TCGTAAA  bottom Hemimethylated  40646    8448            4.81 -1.
57
##  8 TCATAAA  unmethylated           15940    9614            1.66 -0.
506
##  9 CCGTAAA  duplex Methylated      23539   15208            1.55 -0.
437
## 10 CAATAAA  unmethylated           41067   27166            1.51 -0.
413
## # ... with 90 more rows
```

Building specificity model

```
HoxB13 %>%
  dplyr::filter(Property == "unmethylated") %>%
  TFCookbook::buildEnergyModel() %>%
  TFCookbook::getEnergyMatrix() %>%
  TFCookbook::addAnchorMatrix(anchor = "TAAA", position = 4, height = 0
.25) %>%
  TFCookbook::plotEnergyLogo() +
  scale_y_continuous(breaks = seq(-0.8, 0.8, 0.2))
```



Building methylation sensitivity model

It is easy to build methylation effect model by comparison of each methylated ste with its unmethylated counterpart

```
HoxB13.unmethylated <- subset(HoxB13, Property == "unmethylated")
HoxB13.methylated <- subset(HoxB13, Property %in% c("top Hemimethylated
", "bottom Hemimethylated", "duplex Methylated"))

(HoxB13.paired <- left_join(HoxB13.methylated, HoxB13.unmethylated, by
= "Sequence") %>%
  dplyr::mutate(Energy = Energy.x - Energy.y) %>%
  dplyr::select(Sequence,
                Property = Property.x,
                Energy))
```

```
## # A tibble: 36 x 3
##    Sequence Property              Energy
##    <chr>    <chr>                  <dbl>
##  1 CCATAAA  top Hemimethylated    -3.08
##  2 TCGTAAA  duplex Methylated     -1.01
##  3 TCATAAA  top Hemimethylated    -2.04
##  4 TCGTAAA  top Hemimethylated    -0.814
##  5 ACATAAA  top Hemimethylated    -2.86
##  6 TCGTAAA  bottom Hemimethylated  0.126
##  7 CCGTAAA  duplex Methylated     -1.25
##  8 CCGTAAA  top Hemimethylated    -0.977
```

```
##  9 TAGTAAA  bottom Hemimethylated -0.632
## 10 ACGTAAA  top Hemimethylated    -0.902
## # ... with 26 more rows
```

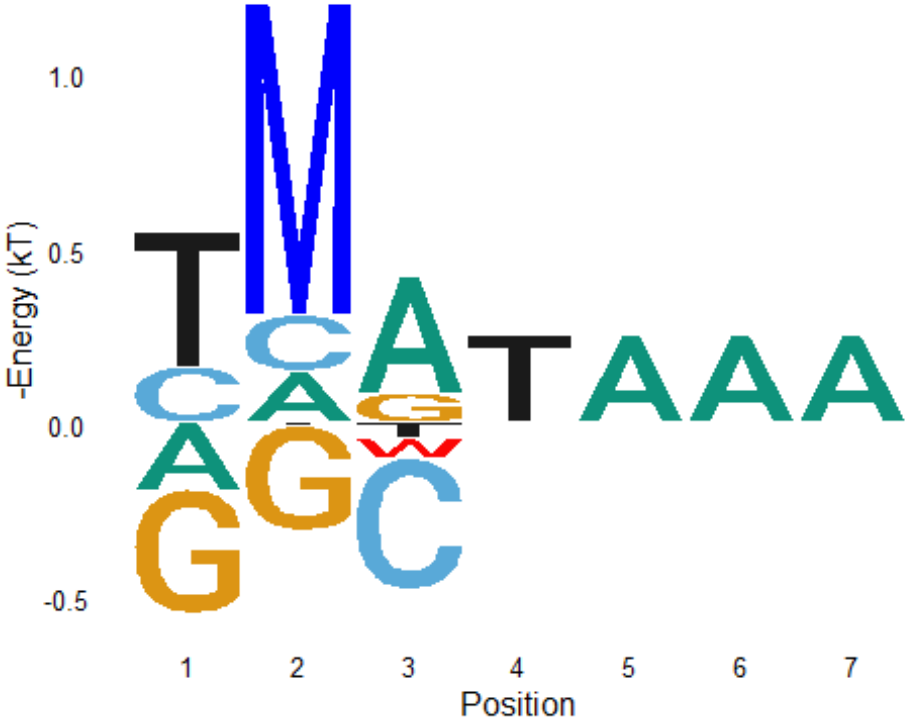
Overall, we can create a compositive model to include both specificity and methylation effect

```
HoxB13.MethylModel <- HoxB13.paired %>%
  dplyr::mutate(Sequence = dplyr::case_when(
                                    Property == "top Hemimethylated" ~ st
ringi::stri_sub_replace(Sequence, from=2,to=2, replacement = "M"),
                                    Property == "bottom Hemimethylated" ~
 stringi::stri_sub_replace(Sequence, from=3,to=3, replacement = "W"),
                                    Property == "duplex Methylated" ~ str
ingi::stri_sub_replace(Sequence, from=2,to=3, replacement = "MW")
                                    )
                ) %>%
  TFCookbook::buildMethylationModel()
```

```
## Warning: Expected 14 pieces. Additional pieces discarded in 36 rows
[1, 2, 3, 4,
## 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, ...].
```

```
HoxB13 %>%
  dplyr::filter(Property == "unmethylated") %>%
  TFCookbook::buildEnergyModel() %>%
  TFCookbook::getEnergyMatrix() %>%
  TFCookbook::addAnchorMatrix(anchor = "TAAA", position = 4, height = 0
.25) %>%
  TFCookbook::addMethylMatrix(MethylModel = HoxB13.MethylModel, encodin
g = "(3+2)L+1") %>%
  TFCookbook::plotEnergyLogo()
```

```
## Warning: Expected 21 pieces. Additional pieces discarded in 64 rows
[1, 2, 3, 4,
## 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, ...].
```

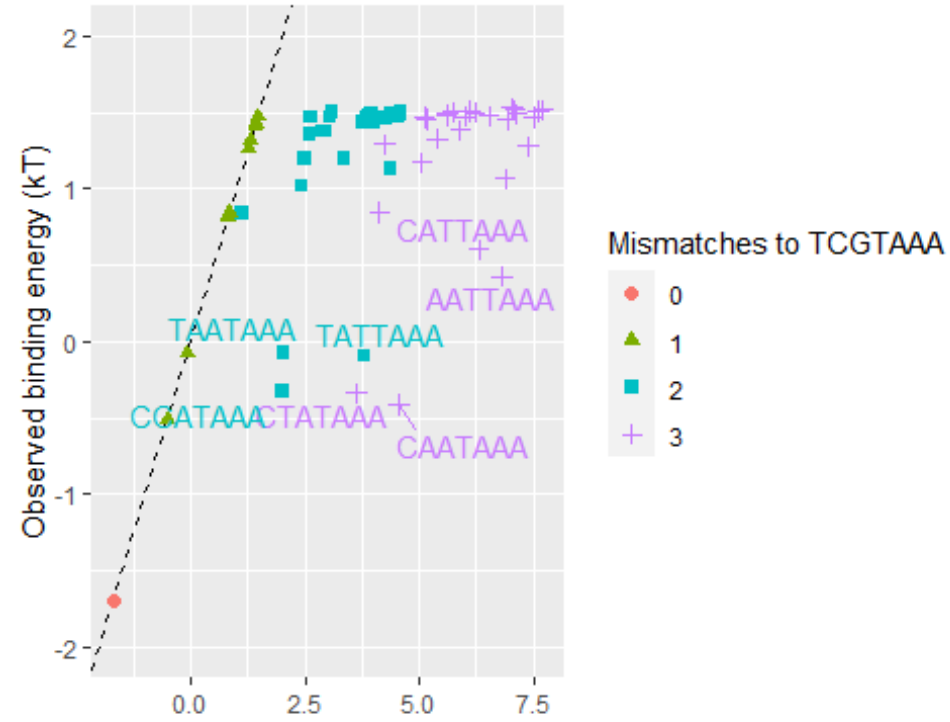


The existence of secondary motif and the necessity to use composite "Min" model to predict binding energy

Since among all unmethylated sequences, TCGTAAA has the lowest binding energy and hightest affinity, we can build a specificity model based on all those single variants to TCGTAAA as below. Using this model to predict the binding energy for all other sites and compare them with experimentally observed values, it is clear that small fraction of sequences, e.g., CAATAAA and CTATAAA, deviate significantly from the diagnoal lines, meaning that they are not properly represented in the energy model made by TCGTAAA and its single variants. The most intuitive explanation is that there exists another structural conformation for the recognition of DNA sequence CAATAAA and its related variants. We can build another model from CAAATAAA and its single variants, called HoxB13.CAA.Model as below. However, this "CAA" model still wouldn't explain all the observed values, particularly for sequence like TCGTAAA. Alternatively, if for each sequence we take the minimal value predicted by "TCG" and "CAA" models as the composite predicted value and compare it with the observed number, they match very well, so it is fair to say the TCG and CAA models are both required for proper understanding the specificity behavior of HoxB13 to its unmethylated sites.

```
HoxB13.TCG.Model <- HoxB13 %>%
  dplyr::filter(Property == "unmethylated") %>%
  TFCookbook::selectVariants(reference = "TCGTAAA", maxMismatches = 1)
%>%
  TFCookbook::buildEnergyModel()
```

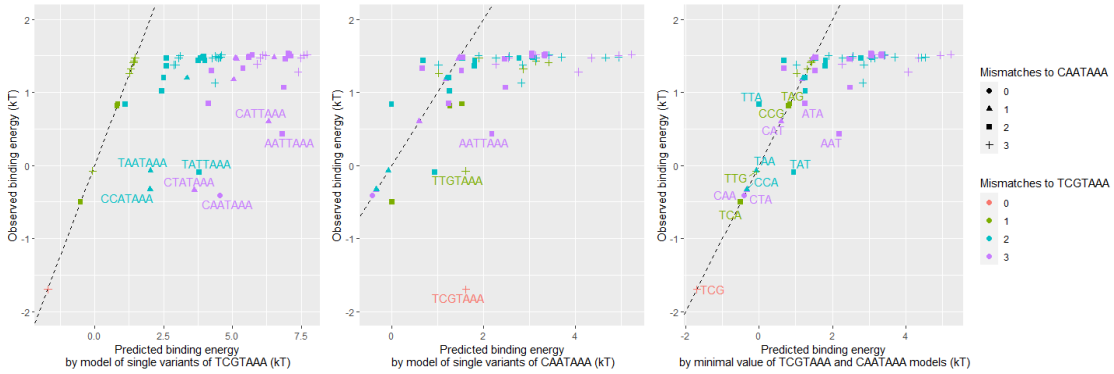
```
HoxB13 %>%
  dplyr::filter(Property == "unmethylated") %>%
  mutate(Predicted.Energy = TFCookbook::predictEnergy(Sequence, HoxB13.
TCG.Model),
         Mismatch.TCG = as.factor(TFCookbook::countMismatch(Sequence, "
TCGTAAA"))) %>%
  ggplot(aes(x = Predicted.Energy, y = Energy, label = Sequence, color
= Mismatch.TCG, shape = Mismatch.TCG)) +
  geom_abline(slop = 1, linetype = "dashed") +
  geom_point(size = 2) +
  geom_text_repel(data = function(x) filter(x, ((Predicted.Energy - Ene
rgy) > 2) & (Energy < 0.8)), show.legend = FALSE) +
  xlab("Predicted binding energy by model of single variants of TCGTAAA
 (kT)") +
  ylab("Observed binding energy (kT)") + ylim(-2, 2) +
  labs(color = "Mismatches to TCGTAAA", shape = "Mismatches to TCGTAAA"
)
```



binding energy by model of single variants of TCGTAAA (kT)

```
HoxB13.CAA.Model <- HoxB13 %>%
  dplyr::filter(Property == "unmethylated") %>%
  TFCookbook::selectVariants(reference = "CAATAAA", maxMismatches = 1)
%>%
  TFCookbook::buildEnergyModel()

HoxB13 %<>%
  dplyr::filter(Property == "unmethylated") %>%
```

```r
  dplyr::mutate(Mismatch.TCG = as.factor(TFCookbook::countMismatch(Sequ
ence, "TCGTAAA")),
                Mismatch.CAA = as.factor(TFCookbook::countMismatch(Sequ
ence, "CAATAAA")),
                Predicted.Energy.TCG = TFCookbook::predictEnergy(Sequen
ce, HoxB13.TCG.Model),
                Predicted.Energy.CAA = TFCookbook::predictEnergy(Sequen
ce, HoxB13.CAA.Model)) %>%
  dplyr::mutate(Predicted.Energy.min = pmin(Predicted.Energy.TCG, Predi
cted.Energy.CAA))

HoxB13 %>%
  ggplot(aes(x=Predicted.Energy.TCG, y=Energy, label=Sequence, color=Mi
smatch.TCG, shape = Mismatch.CAA)) +
  geom_abline(slop = 1, linetype = "dashed") +
  geom_point(size = 2, show.legend = FALSE) +
  geom_text_repel(data = function(x) filter(x, ((Predicted.Energy.TCG -
 Energy) > 2) & (Energy < 0.8)), show.legend = FALSE) +
  xlab("Predicted binding energy\n by model of single variants of TCGTA
AA (kT)") +
  ylab("Observed binding energy (kT)") + ylim(-2, 2) -> plot.TCG

HoxB13 %>%
  ggplot(aes(x=Predicted.Energy.CAA, y=Energy, label=Sequence, color=Mi
smatch.TCG, shape = Mismatch.CAA)) +
  geom_abline(slop = 1, linetype = "dashed") +
  geom_point(size = 2, show.legend = FALSE) +
  geom_text_repel(data = function(x) filter(x, ((Predicted.Energy.CAA -
 Energy) > 1.5) & (Energy < 0.8)), show.legend = FALSE) +
  xlab("Predicted binding energy\n by model of single variants of CAATA
AA (kT)") +
  ylab("Observed binding energy (kT)") + ylim(-2, 2) -> plot.CAA

HoxB13 %>%
  ggplot(aes(x = Predicted.Energy.min, y = Energy, label = substr(Seque
nce, 1,3), color = Mismatch.TCG, shape = Mismatch.CAA)) +
  geom_abline(slop = 1, linetype = "dashed") +
  geom_point(size = 2) +
  geom_text_repel(data=function(x) filter(x, (abs(Predicted.Energy.min
- Energy) > -0.1) & (Energy < 1)), show.legend = FALSE) +
  xlab("Predicted binding energy\n by minimal value of TCGTAAA and CAAT
AAA models (kT)") +
  ylab("Observed binding energy (kT)") + ylim(-2, 2) +
  labs(color = "Mismatches to TCGTAAA", shape = "Mismatches to CAATAAA"
) -> plot.min

cowplot::plot_grid(plot.TCG, plot.CAA, plot.min, align = "h", ncol = 3,
 rel_widths = c(1,1,1.45))
```


Observed binding energy (kT)
Predicted binding energy
by model of single variants of TCGTAAA (kT)
CATTAAA
AATTAAA
TAATAAA
TATTAAA
CTATAAA
CCATAAA
CAATAAA
Predicted binding energy
by model of single variants of CAATAAA (kT)
AATTAAA
TTGTAAA
TCGTAAA
Predicted binding energy
by minimal value of TCGTAAA and CAATAAA models (kT)
TTA
TAG
CCG
ATA
CAT
AAT
TAA
TAT
TTG
CCA
CAA
CTA
TCA
TCG
Mismatches to CAATAAA
0
1
2
3
Mismatches to TCGTAAA
0
1
2
3