TCREMP supplementary

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Properties of TCREMP distances

First, lets show that TCREMP distances for CDR3β region behave as expected. Given sequence similarities s_{ij} one can obtain a metric $d_{ij} = s_{ii} + s_{jj} - 2s_{ij}$ which can be also computed on-the-fly by transforming substitution scoring (e.g. BLOSUM matrix with gaps for linear gaps) appropriately.

We'll first analyze CDR3β distances for $n = 3000$ prototypes mapped to themselves and answer two questions:

- **Q1**: What is the distribution of alignment scores d_{ij} and pairwise Euclidean distances D_{ij} in embedding space
- **Q2**: The properties of Euclidean distances are well-known, but are the alignment scores additive (so PCA can be applied)?
- **Q3**: How does pairwise distances in embedding space agree with actual alignment scores

Load data and compute alignment metric

```
data.1 <- read_tsv("p1000_p1000.txt.gz") |>
  rename(from = id) |>
  mutate(from = as.character(from)) |>
  melt() |>
  filter(grepl("cdr3", variable)) |>
  mutate(to = str_split_fixed(variable, "_", 2)[,1]) |>
  select(-variable)
```

```
## Rows: 994 Columns: 3001
## -- Column specification --------------------------------------------------------
## Delimiter: "\t"
## dbl (3001): id, 0_v_score, 0_j_score, 0_cdr3_score, 1_v_score, 1_j_score, 1_...
##
## i Use `spec()` to retrieve the full column specification for this data.
## i Specify the column types or set `show_col_types = FALSE` to quiet this message.
## Using from as id variables
```

```
ids <- intersect(data.1$from, data.1$to)

data.1 <- data.1 |>
  filter(from %in% ids, to %in% ids)

data.1 <- data.1 |>
  rename(Sij = value) |>
  group_by(from) |>
  mutate(Sii = Sij[from == to]) |>
  group_by(to) |>
  mutate(Sjj = Sij[from == to]) |>
  ungroup() |>
  mutate(Dij = Sii + Sjj - 2 * Sij)

glimpse(data.1)
```

```
## Rows: 988,036
## Columns: 6
## $ from <chr> "0", "1", "2", "3", "4", "5", "6", "7", "8", "9", "10", "11", "12~
## $ Sij  <dbl> 730, -250, 150, -80, 10, 0, -250, -140, -230, -140, 10, 10, -240,~
## $ to   <chr> "0", "0", "0", "0", "0", "0", "0", "0", "0", "0", "0", "0", "0", ~
## $ Sii  <dbl> 730, 400, 550, 410, 610, 500, 270, 250, 350, 280, 670, 580, 340, ~
## $ Sjj  <dbl> 730, 730, 730, 730, 730, 730, 730, 730, 730, 730, 730, 730, 730, ~
## $ Dij  <dbl> 0, 1630, 980, 1300, 1320, 1230, 1500, 1260, 1540, 1290, 1380, 129~
```

Compute embedding metric, append values

```
data.1m <- data.1 |>
  dcast(from ~ to, value.var = "Dij")

rownames(data.1m) <- data.1m$from
data.1m$from <- NULL
data.1m <- as.matrix(data.1m)
data.1d <- dist(data.1m) |>
  as.matrix() |>
  melt()
colnames(data.1d) <- c("from", "to", "DDij")
data.1d$from <- as.character(data.1d$from)
data.1d$to <- as.character(data.1d$to)
data.1 <- left_join(data.1, data.1d)
```

```
## Joining with `by = join_by(from, to)`
```

```
glimpse(data.1)
```

```
## Rows: 988,036
## Columns: 7
## $ from <chr> "0", "1", "2", "3", "4", "5", "6", "7", "8", "9", "10", "11", "12~
## $ Sij  <dbl> 730, -250, 150, -80, 10, 0, -250, -140, -230, -140, 10, 10, -240,~
## $ to   <chr> "0", "0", "0", "0", "0", "0", "0", "0", "0", "0", "0", "0", "0", ~
## $ Sii  <dbl> 730, 400, 550, 410, 610, 500, 270, 250, 350, 280, 670, 580, 340, ~
## $ Sjj  <dbl> 730, 730, 730, 730, 730, 730, 730, 730, 730, 730, 730, 730, 730, ~
## $ Dij  <dbl> 0, 1630, 980, 1300, 1320, 1230, 1500, 1260, 1540, 1290, 1380, 129~
## $ DDij <dbl> 0.000, 9756.895, 8095.134, 12501.840, 6352.008, 9569.922, 11944.4~
```

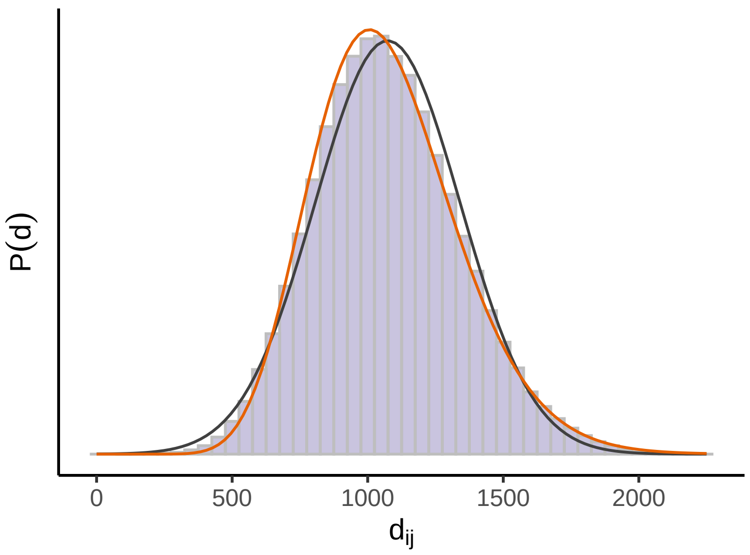
We provide two fits for d_{ij}, first is $\mathcal{N}(\mu, \sigma)$ and the second one that recaptures the right-skewness is $\Gamma(\alpha = \mu^2/\sigma^2, \lambda = \mu/\sigma^2)$, the Gamma distribution. This is in line with Pang, H., Tang, J., Chen, SS. et al. Statistical distributions of optimal global alignment scores of random protein sequences. BMC Bioinformatics 6, 257 (2005). A thorough theoretical proof for this is left for the reader. We will just note that if, for first half of

CDR3β sequence $d^l_{ij} \sim \Gamma(\cdot)$ then $d_{ij} = d^l_{ij} + d^r_{ij} \sim \Gamma(\cdot)$ due to the nature of Gamma distribution. Also note that number of matches between two random strings of amino acids can be modeled as a Poisson process which leads to Gamma distribution. > N.B. We use Gamma distribution instead of Erlang distribution as sequence alignment scores may be non-integer

```r
data.1diag <- data.1 |>
  filter(as.integer(from) < as.integer(to))

mu <- mean(data.1diag$Dij)
sigma <- sd(data.1diag$Dij)
alpha <- mu * mu / sigma / sigma
lambda <- alpha / mu

data.1diag |>
  ggplot(aes(x = Dij)) +
  geom_histogram(aes(y = after_stat(density)),
                 binwidth = 50,
                 color = "grey",
                 fill = "#b2abd2",
                 alpha = 0.7) +
  stat_function(fun = dnorm,
                args = list(mean = mu, sd = sigma),
                color = "grey25") +
  stat_function(fun = dgamma,
                args = list(shape = alpha, rate = lambda),
                color = "#e66101") +
  xlab(TeX("$d_{ij}$")) + ylab(TeX("$P(d)$")) +
  theme_classic() +
  theme(axis.text.y = element_blank(),
        axis.ticks.y = element_blank())
```



The distribution of Euclidean distances D_{ij} looks similar to Planck radiation law distribution for wavelengths: $D_{ij} \sim P(A, B)$, $P(x|A, B) = \frac{A}{x^\nu} \frac{1}{e^{B/x}-1}$. Assume that the support is $x \in [1, \infty)$ so we don't have to deal with the infinity at $x = 0$, we can shift the distances accordingly. Using method of moments for parameter estimation would require calculating integrals like $I_\nu = \int_1^\infty \frac{A/x^\nu dx}{e^{B/x}-1}$ which can lead to expressions such as $I_1 = \frac{A}{B}\left(\mathrm{Li}_2(e^{-B}) + B\ln(1 - e^{-B})\right)$ and $I_2 = -A\ln(1 - e^{-B})$ that are quite complex, so we would rather choose parameters A and B by hand for $\nu = 5$.

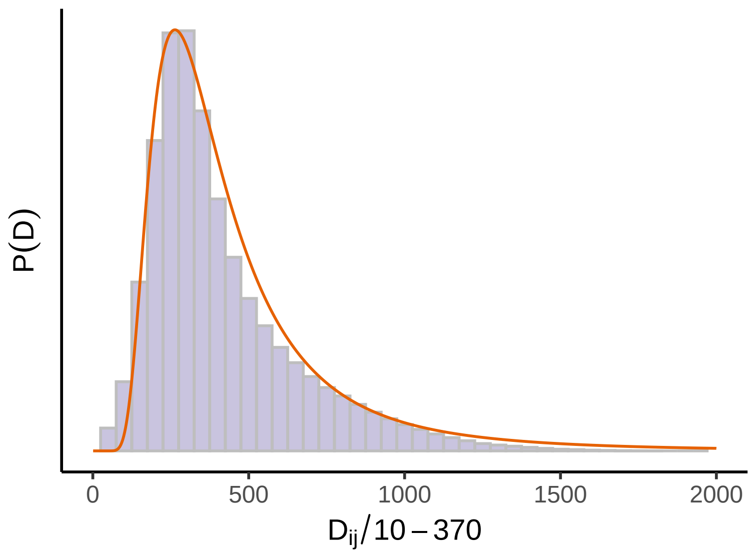
```r
# choose coef by hand
hh <- 6.62607015e-34
cc <- 299792458
TT <- 11000
kB <- 1.380649e-23
A <- 2.0 * hh * cc * cc
B <- hh * cc / kB / TT
scale <- 0.0000033 / 8E+11

data.1diag |>
  ggplot(aes(x = DDij / 10 - 370)) + # choose coef by hand
  geom_histogram(aes(y = after_stat(density)),
                 binwidth = 50,
                 color = "grey",
                 fill = "#b2abd2",
                 alpha = 0.7) +
  geom_line(data = tibble(x = 1:2500,
                          y = scale * (A / (x * 1e-9) ^ 5.0) /
                            (exp(B / (x * 1e-9)) - 1)), # choose coef by hand
                          aes(x, y),
                color = "#e66101") +
  scale_x_continuous(TeX("$D_{ij} / 10 - 370$"),
                     limits = c(0, 2000)) + ylab(TeX("$P(D)$")) +
  theme_classic() +
  theme(axis.text.y = element_blank(),
        axis.ticks.y = element_blank())
```

```
## Warning: Removed 754 rows containing non-finite outside the scale range
## (`stat_bin()`).

## Warning: Removed 2 rows containing missing values or values outside the scale range
## (`geom_bar()`).

## Warning: Removed 500 rows containing missing values or values outside the scale range
## (`geom_line()`).
```



Q1 : $d_{ij} \sim \Gamma, D_{ij} \sim$ Plank □

Check additivity and triangle rule, circle through triplicates

```
data.1tri <- expand_grid(a = 0:999, b = 0:999, c = 0:999) |>
  filter(a < b, b < c) |>
  mutate(a = as.character(a),
         b = as.character(b),
         c = as.character(c)) |>
  left_join(data.1 |>
              select(a = from, b = to, Dab = Dij, DDab = DDij) |>
              filter(a < b)) |>
  left_join(data.1 |>
              select(a = from, c = to, Dac = Dij, DDac = DDij) |>
              filter(a < c)) |>
  left_join(data.1 |>
              select(b = from, c = to, Dbc = Dij, DDbc = DDij) |>
              filter(b < c))
```

```
## Joining with `by = join_by(a, b)`
## Joining with `by = join_by(a, c)`
## Joining with `by = join_by(b, c)`
```

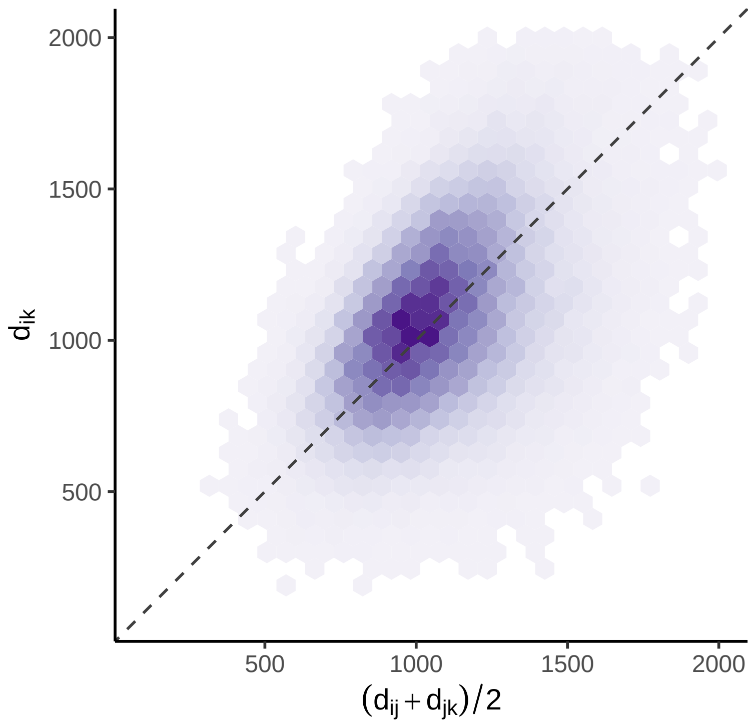
Plot them

```
set.seed(42)
data.1tri |>
  sample_n(100000) |>
  ggplot(aes(x = (Dab + Dbc) / 2, y = Dac)) +
  geom_hex(bins = 30) +
  scale_fill_distiller(palette = "Purples", direction = 1, guide = F) +
  geom_abline(slope = 1, intercept = 0,
              linetype = "dashed", color = "grey25") +
  scale_x_continuous(TeX("$(d_{ij} + d_{jk})/2$"),
                     limits = c(100, 2000)) +
  scale_y_continuous(TeX("$d_{ik}$"),
                      limits = c(100, 2000)) +
  theme_classic() +
  theme(aspect.ratio = 1)
```

```
## Warning: Removed 19262 rows containing non-finite outside the scale range
## (`stat_binhex()`).

## Warning: The `guide` argument in `scale_*()` cannot be `FALSE`. This was deprecated in
## ggplot2 3.3.4.
## i Please use "none" instead.
## This warning is displayed once every 8 hours.
## Call `lifecycle::last_lifecycle_warnings()` to see where this warning was
## generated.

## Warning: Removed 1 row containing missing values or values outside the scale range
## (`geom_hex()`).
```

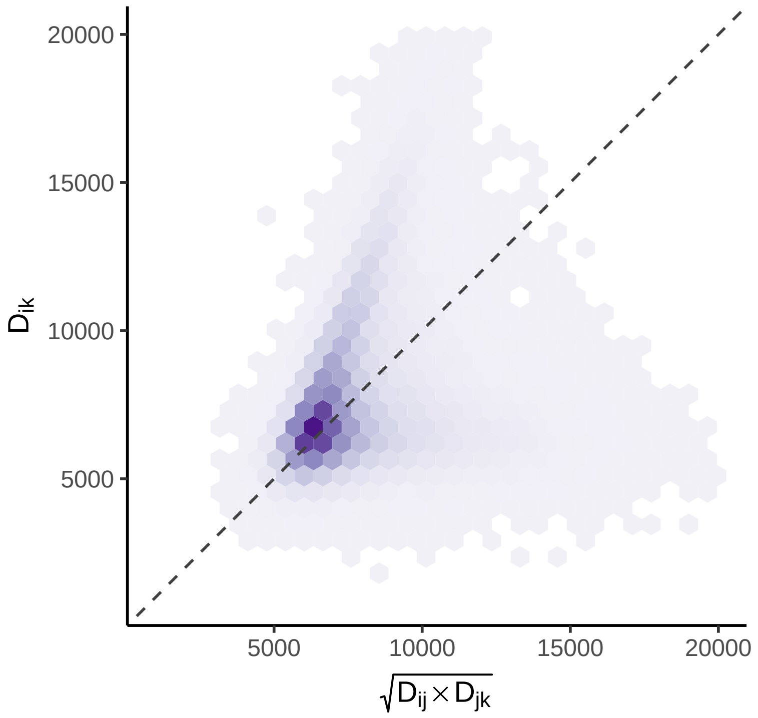


```
with(data.1tri,
     cor.test(Dab + Dbc, Dac))
```

```
##
##  Pearson's product-moment correlation
##
## data:  Dab + Dbc and Dac
## t = 5873.1, df = 134308859, p-value < 2.2e-16
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  0.4519066 0.4521757
## sample estimates:
##       cor
## 0.4520411
```

```
data.1tri |>
  sample_n(100000) |>
  ggplot(aes(x = sqrt(DDab * DDbc), y = DDac)) +
  geom_hex(bins = 30) +
  scale_fill_distiller(palette = "Purples", direction = 1, guide = F) +
  geom_abline(slope = 1, intercept = 0,
              linetype = "dashed", color = "grey25") +
  scale_x_continuous(TeX("$\\sqrt{D_{ij} \\times D_{jk}}$"),
                     limits = c(1000, 20000)) +
  scale_y_continuous(TeX("$D_{ik}$"), limits = c(1000, 20000)) +
  theme_classic() +
  theme(aspect.ratio = 1)
```

```
## Warning: Removed 19068 rows containing non-finite outside the scale range
## (`stat_binhex()`).
```



```
with(data.1tri,
     cor.test(sqrt(DDab * DDbc), DDac))
```

```
##
##  Pearson's product-moment correlation
##
## data:  sqrt(DDab * DDbc) and DDac
## t = 2798.4, df = 134308859, p-value < 2.2e-16
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  0.2345626 0.2348822
## sample estimates:
##       cor
## 0.2347224
```

Q2 : $d_{i,j} \sim d_{i,\cdot} + d_{\cdot,j}, \log D_{i,j} \sim \log D_{i,\cdot} + \log D_{\cdot,j}$ □

Compare alignment and embedding distances

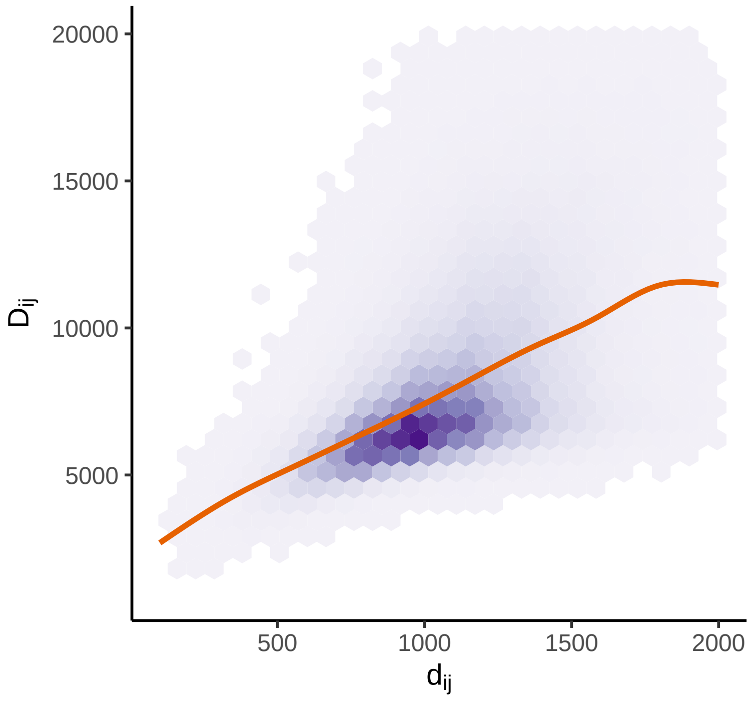
```
data.1diag |>
  ggplot(aes(x = Dij, y = DDij)) +
  geom_hex(bins = 30) +
  scale_fill_distiller(palette = "Purples", direction = 1, guide = F) +
  geom_smooth(fill = NA, color = "#e66101") +
  scale_x_continuous(TeX("$d_{ij}$"), limits = c(100, 2000)) +
  scale_y_continuous(TeX("$D_{ij}$"), limits = c(1000, 20000)) +
  theme_classic() +
  theme(aspect.ratio = 1)
```

```
## Warning: Removed 360 rows containing non-finite outside the scale range
## (`stat_binhex()`).

## `geom_smooth()` using method = 'gam' and formula = 'y ~ s(x, bs = "cs")'

## Warning: Removed 360 rows containing non-finite outside the scale range
## (`stat_smooth()`).

## Warning: Removed 13 rows containing missing values or values outside the scale range
## (`geom_hex()`).
```



```r
with(data.1diag,
     cor.test(Dij, DDij))
```

```
##
##  Pearson's product-moment correlation
##
## data:  Dij and DDij
## t = 469.74, df = 493519, p-value < 2.2e-16
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  0.5539145 0.5577704
## sample estimates:
##       cor
## 0.5558455
```

Q3 : $D_{i,j} \sim d_{i,j}$ $\square$

Check difference between generated and real prototypes

Load similarity scores and compute Euclidean distances for prototypes obtained from VDJ rearrangement model ("Murugan") or real-world repertoires ("Britanova"). Note that here we use CDR3β scores and omit Vβ, Jβ as they are quite predictable/discrete and are subject to batch effect/bias.

```r
Sys.setenv(VROOM_CONNECTION_SIZE = 2^25)
read_proto <- function(prefix = "v_p", sz = 3000) {
  tmp <- read_tsv(paste0(prefix, sz, ".txt.gz")) |>
    select(cloneId, matches("b_\\d+_cdr3$")) |>
    melt(id.vars = "cloneId") |>
    dcast(cloneId ~ variable, mean)
  rownames(tmp) <- as.character(tmp$cloneId)
  tmp$cloneId <- NULL
  tmp |>
    as.matrix() |>
    dist() |>
    as.matrix() |>
    melt() |>
    rename(from = Var1, to = Var2, Dp = value)
}
v_p3000_d <- read_proto("v_p", 3000)
```

```
## Rows: 954 Columns: 18009
## -- Column specification --------------------------------------------------------
## Delimiter: "\t"
## chr     (7): a_cdr3aa, a_v, a_j, b_cdr3aa, b_v, b_j, antigen.epitope
## dbl (18002): tcremp_id, cloneId, a_0_v, a_0_j, a_0_cdr3, a_1_v, a_1_j, a_1_c...
##
## i Use `spec()` to retrieve the full column specification for this data.
## i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

```r
v_b3000_d <- read_proto("v_b", 3000) |> rename(Db = Dp)
```

```
## Rows: 954 Columns: 18009
## -- Column specification --------------------------------------------------------
## Delimiter: "\t"
## chr     (7): a_cdr3aa, a_v, a_j, b_cdr3aa, b_v, b_j, antigen.epitope
## dbl (18002): tcremp_id, cloneId, a_0_v, a_0_j, a_0_cdr3, a_1_v, a_1_j, a_1_c...
##
## i Use `spec()` to retrieve the full column specification for this data.
## i Specify the column types or set `show_col_types = FALSE` to quiet this message.
```

```r
v_bp3000_d <- merge(v_p3000_d, v_b3000_d)
```

Plot and correlate

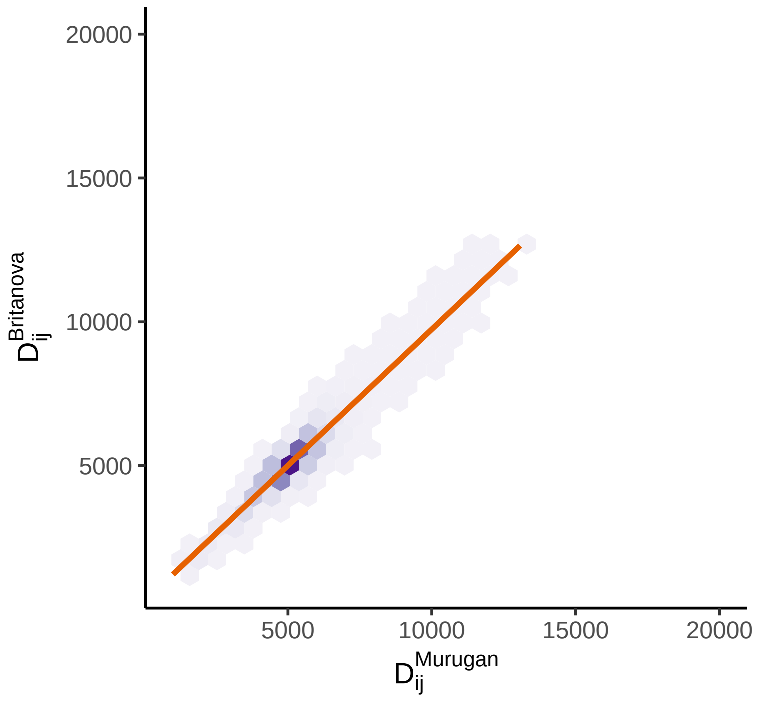
```r
v_bp3000_d |>
  ggplot(aes(x = Dp, y = Db)) +
  geom_hex(bins = 30) +
  scale_fill_distiller(palette = "Purples", direction = 1, guide = F) +
  geom_smooth(method = "lm", fill = NA, color = "#e66101") +
  scale_x_continuous(TeX("$D_{ij}^{Murugan}$"), limits = c(1000, 20000)) +
  scale_y_continuous(TeX("$D_{ij}^{Britanova}$"), limits = c(1000, 20000)) +
  theme_classic() +
  theme(aspect.ratio = 1)
```

```
## Warning: Removed 7917 rows containing non-finite outside the scale range
## (`stat_binhex()`).
```

```
## `geom_smooth()` using formula = 'y ~ x'
```

```
## Warning: Removed 7917 rows containing non-finite outside the scale range
## (`stat_smooth()`).
```

```
## Warning: Removed 1 row containing missing values or values outside the scale range
## (`geom_hex()`).
```



```r
with(v_bp3000_d,
     cor.test(Dp, Db))
```

```
##
##  Pearson's product-moment correlation
##
## data:  Dp and Db
## t = 2484.8, df = 560999, p-value < 2.2e-16
## alternative hypothesis: true correlation is not equal to 0
## 95 percent confidence interval:
##  0.9572298 0.9576657
## sample estimates:
##       cor
## 0.9574483
```

$\mathbf{Q4}: D_{ij}^{Model} \sim D_{ij}^{RepSeq}$ □

Check effect fom the number of prototypes

Here we will again operate with CDR3β, look at the behavior of embeddings with $n_{proto} \in (100, 1000, 2000, 3000)$ prototypes.

```r
v_px <- bind_rows(
  read_proto("v_p", 100) |> mutate(n_prot = 100),
  read_proto("v_p", 1000) |> mutate(n_prot = 1000),
  read_proto("v_p", 2000) |> mutate(n_prot = 2000),
  read_proto("v_p", 3000) |> mutate(n_prot = 3000)
) |>
  left_join(read_tsv("v_p100.txt.gz") |>
              select(from = cloneId, ag = antigen.epitope) |>
              unique())
```

```
## Rows: 954 Columns: 609
## -- Column specification --------------------------------------------------------
## Delimiter: "\t"
## chr   (7): a_cdr3aa, a_v, a_j, b_cdr3aa, b_v, b_j, antigen.epitope
## dbl (602): tcremp_id, cloneId, a_0_v, a_0_j, a_0_cdr3, a_1_v, a_1_j, a_1_cdr...
##
## i Use `spec()` to retrieve the full column specification for this data.
## i Specify the column types or set `show_col_types = FALSE` to quiet this message.
## Rows: 954 Columns: 6009
## -- Column specification --------------------------------------------------------
## Delimiter: "\t"
## chr    (7): a_cdr3aa, a_v, a_j, b_cdr3aa, b_v, b_j, antigen.epitope
## dbl (6002): tcremp_id, cloneId, a_0_v, a_0_j, a_0_cdr3, a_1_v, a_1_j, a_1_cd...
##
## i Use `spec()` to retrieve the full column specification for this data.
## i Specify the column types or set `show_col_types = FALSE` to quiet this message.
## Rows: 954 Columns: 12009
## -- Column specification --------------------------------------------------------
## Delimiter: "\t"
## chr     (7): a_cdr3aa, a_v, a_j, b_cdr3aa, b_v, b_j, antigen.epitope
## dbl (12002): tcremp_id, cloneId, a_0_v, a_0_j, a_0_cdr3, a_1_v, a_1_j, a_1_c...
##
## i Use `spec()` to retrieve the full column specification for this data.
## i Specify the column types or set `show_col_types = FALSE` to quiet this message.
## Rows: 954 Columns: 18009
## -- Column specification --------------------------------------------------------
## Delimiter: "\t"
## chr     (7): a_cdr3aa, a_v, a_j, b_cdr3aa, b_v, b_j, antigen.epitope
## dbl (18002): tcremp_id, cloneId, a_0_v, a_0_j, a_0_cdr3, a_1_v, a_1_j, a_1_c...
##
## i Use `spec()` to retrieve the full column specification for this data.
## i Specify the column types or set `show_col_types = FALSE` to quiet this message.
## Rows: 954 Columns: 609
## -- Column specification --------------------------------------------------------
## Delimiter: "\t"
## chr   (7): a_cdr3aa, a_v, a_j, b_cdr3aa, b_v, b_j, antigen.epitope
## dbl (602): tcremp_id, cloneId, a_0_v, a_0_j, a_0_cdr3, a_1_v, a_1_j, a_1_cdr...
##
## i Use `spec()` to retrieve the full column specification for this data.
## i Specify the column types or set `show_col_types = FALSE` to quiet this message.
## Joining with `by = join_by(from)`
```

Do UMAP

```
set.seed(42)

do_umap <- function(data) {
  config <- umap.defaults
  config$n_neighbors <- config$n_neighbors * 3
  config$min_dist <- config$min_dist * 3
```

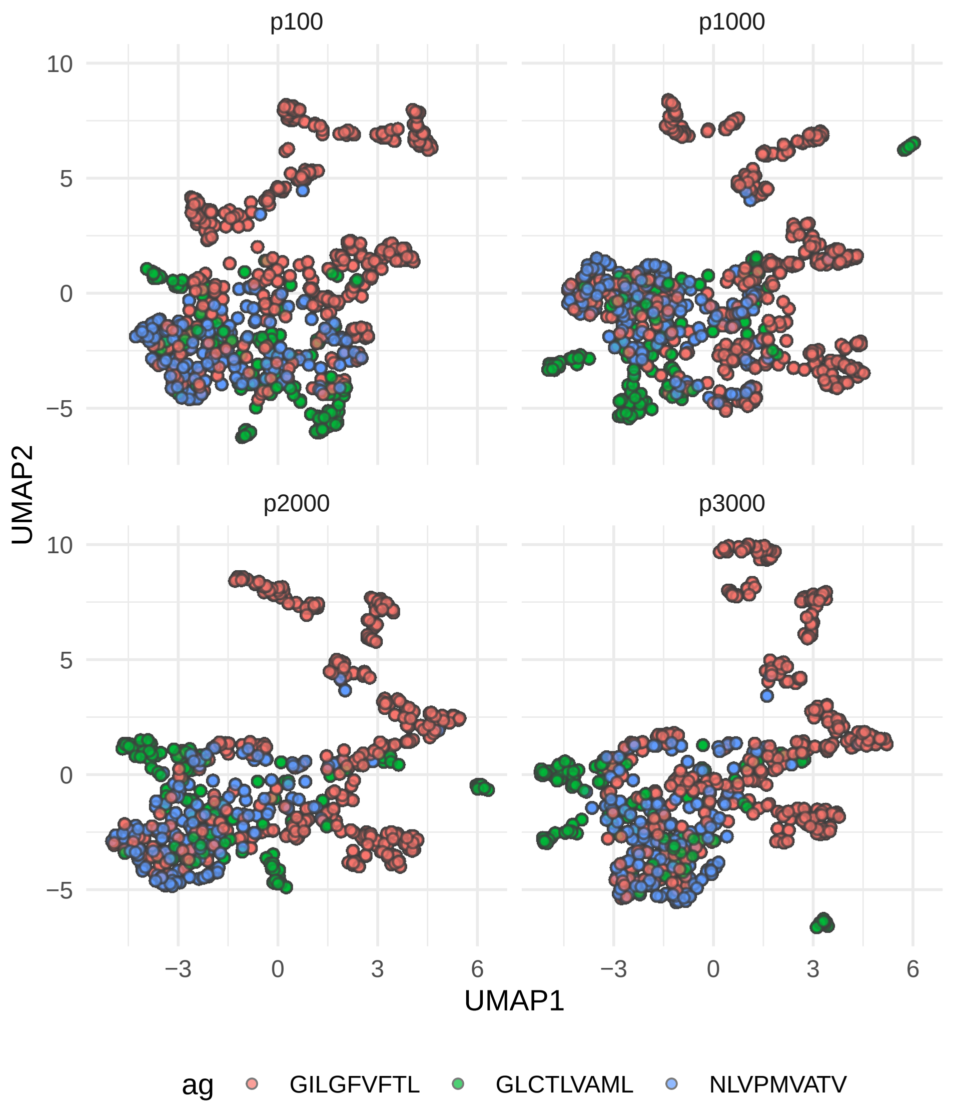
```r
  emb <- data |>
    dcast(from ~ to, value.var = "Dp") |>
    umap(config = config)
  res <- emb$layout |>
    as.tibble()
  colnames(res) <- c("UMAP1", "UMAP2")
  cbind(data |> select(from, ag), res)
}

v_px_umap <- v_px |>
  group_by(n_prot) |>
  group_modify(~ do_umap(.x))
```

```
## Warning: `as.tibble()` was deprecated in tibble 2.0.0.
## i Please use `as_tibble()` instead.
## i The signature and semantics have changed, see `?as_tibble`.
## This warning is displayed once every 8 hours.
## Call `lifecycle::last_lifecycle_warnings()` to see where this warning was
## generated.
```

```
## Warning: The `x` argument of `as_tibble.matrix()` must have unique column names if
## `.name_repair` is omitted as of tibble 2.0.0.
## i Using compatibility `.name_repair`.
## i The deprecated feature was likely used in the tibble package.
##   Please report the issue at <https://github.com/tidyverse/tibble/issues>.
## This warning is displayed once every 8 hours.
## Call `lifecycle::last_lifecycle_warnings()` to see where this warning was
## generated.
```

```r
v_px_umap |>
  mutate(n_prot = paste0("p", n_prot)) |>
  group_by(n_prot) |>
  mutate(sgn_GIL_1 = sign(sum(UMAP1 * startsWith(ag, "GIL"))),
         sgn_GIL_2 = sign(sum(UMAP2 * startsWith(ag, "GIL")))) |>
  ungroup() |>
  ggplot(aes(x = UMAP1 * sgn_GIL_1, y = UMAP2 * sgn_GIL_2, fill = ag)) +
  geom_point_rast(shape = 21,
                  color = "grey25",
                  alpha = 0.7) +
  facet_wrap(~n_prot) +
  scale_color_brewer(palette = "PuOr") +
  xlab("UMAP1") + ylab("UMAP2") +
  theme_minimal() +
  theme(aspect.ratio = 1,
        legend.position = "bottom")
```



Q5 : UMAP stabilizes for $n_{proto} \geq 1000$ ☐

```
print("              ")
```

```
## [1] "              "
```