Diversity, selection and specificity of immune receptors

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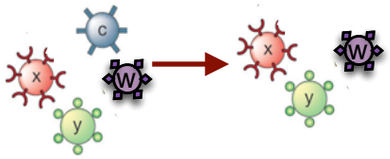
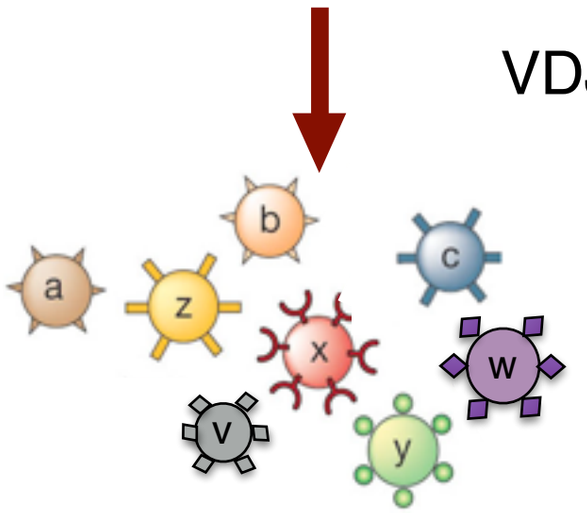
Cold Spring Harbor
Justin Kinney

Repertoire evolution



RECEPTOR GENERATION

VDJ recombination



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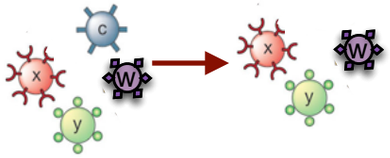

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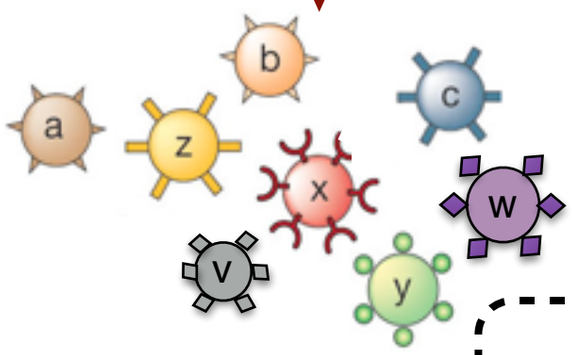
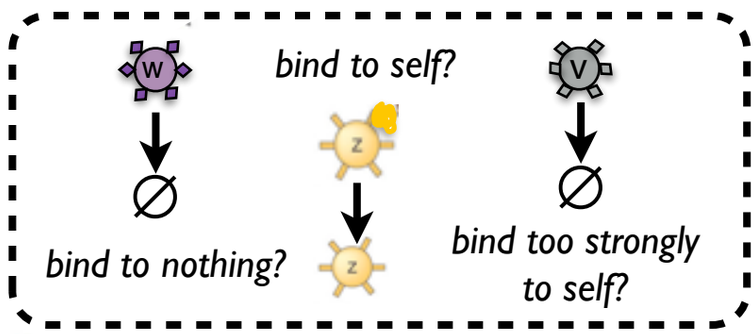
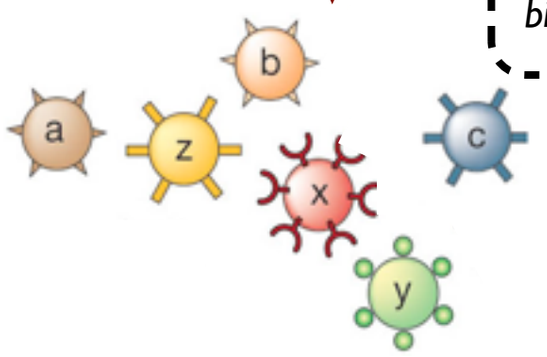


THYMIC SELECTION





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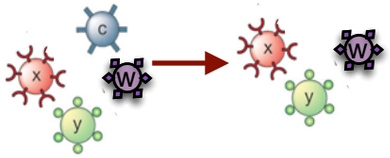

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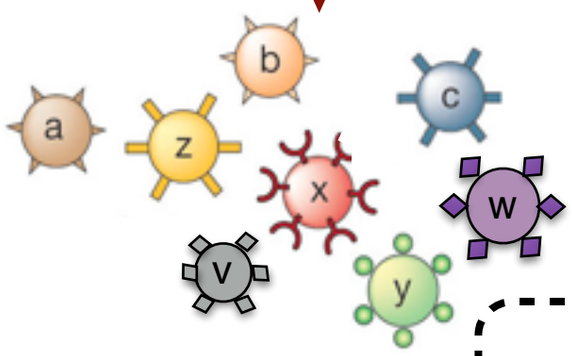

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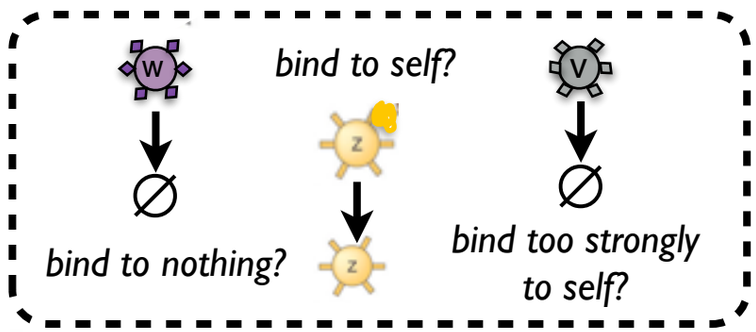
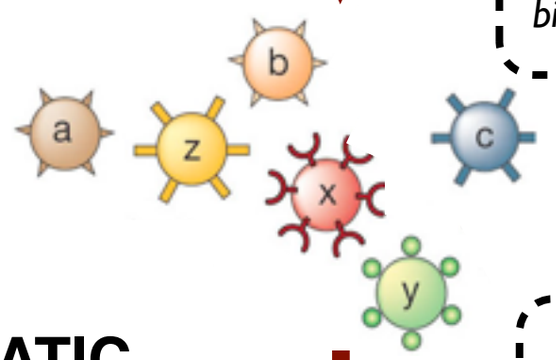
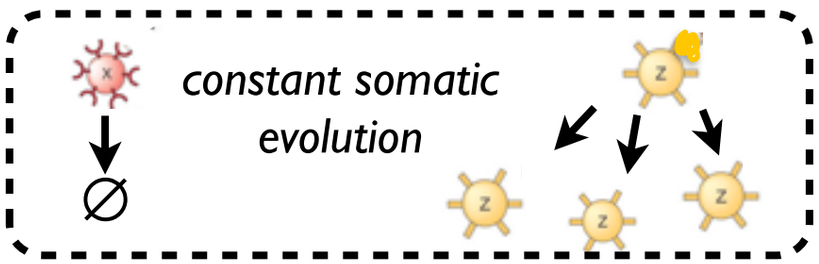
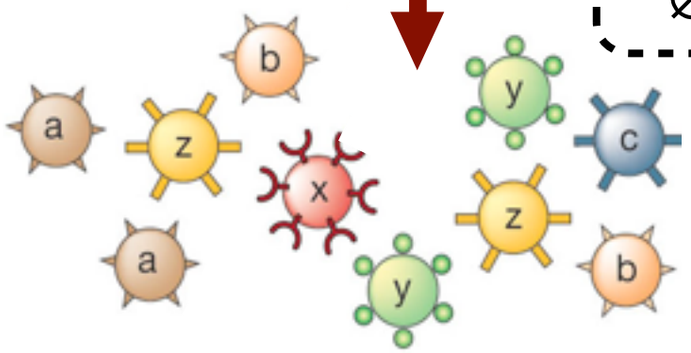


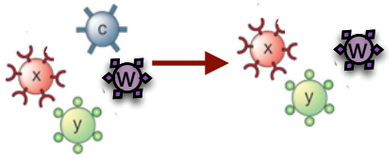


SOMATIC SELECTION





Repertoire evolution



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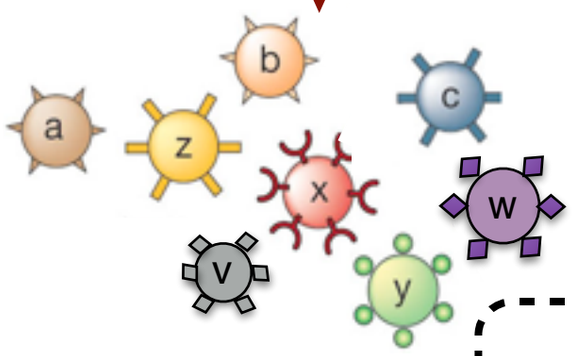

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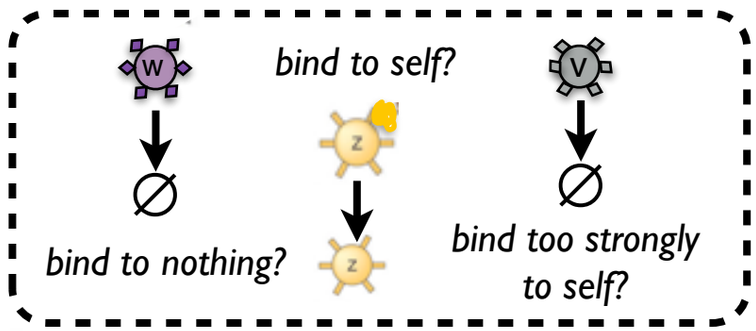

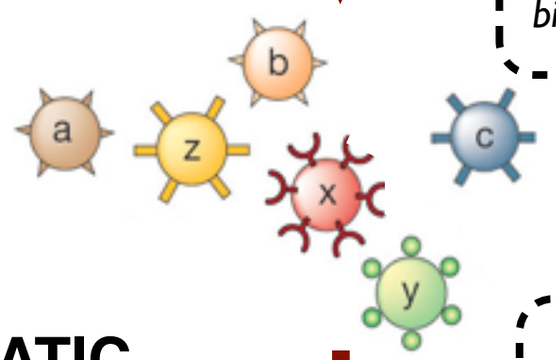

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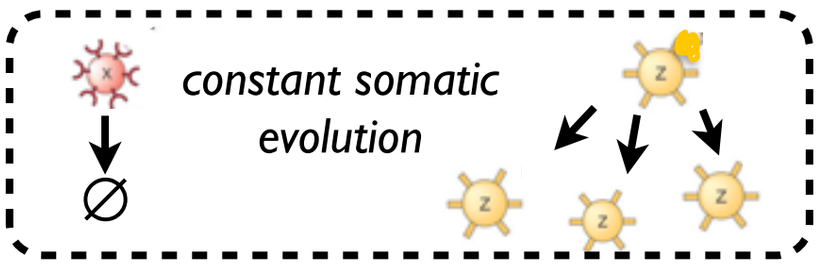
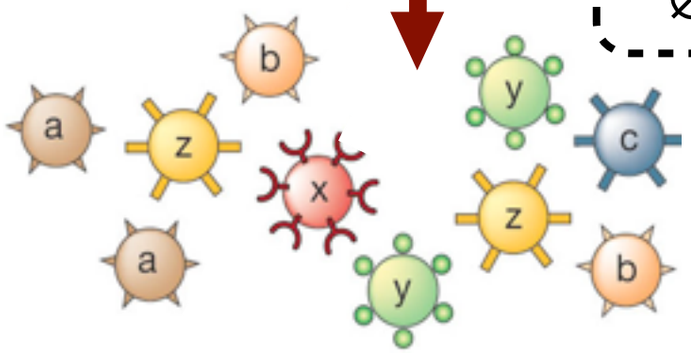
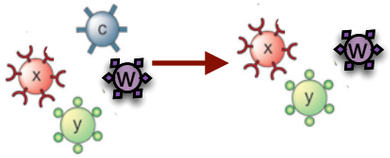


+ Somatic Hypermutations



Repertoire evolution



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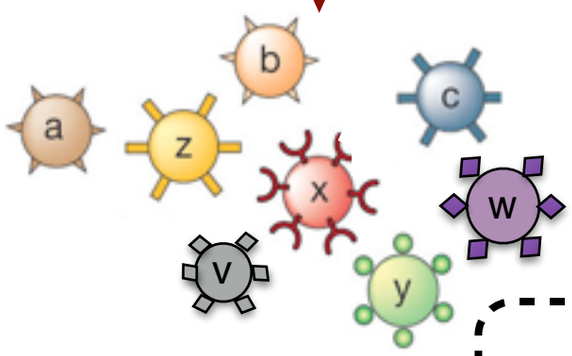

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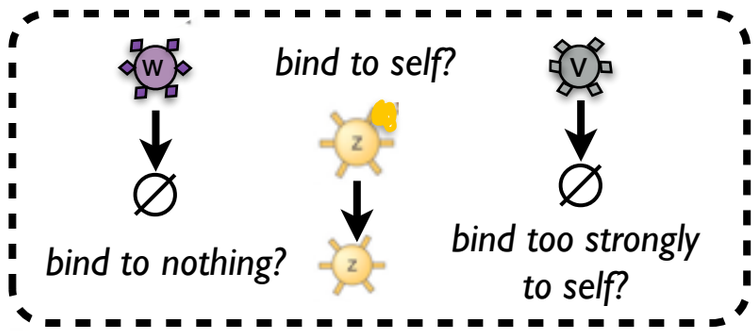

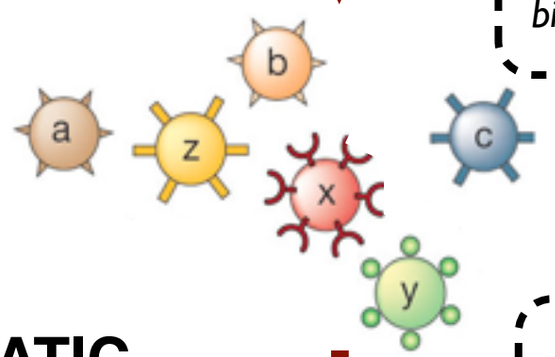

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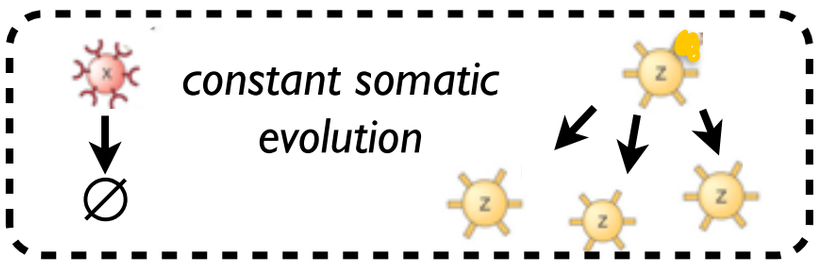

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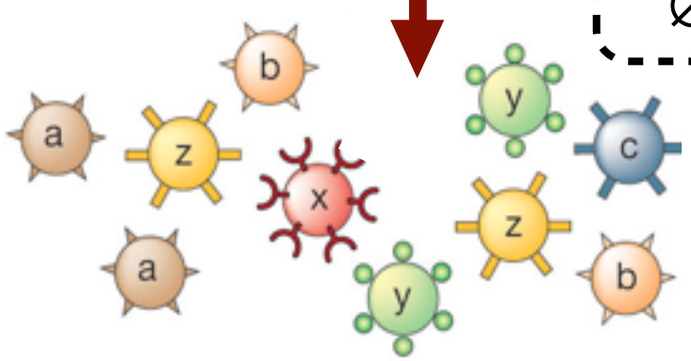
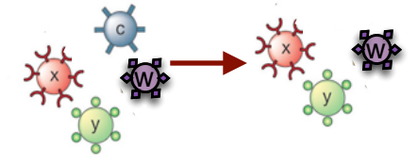


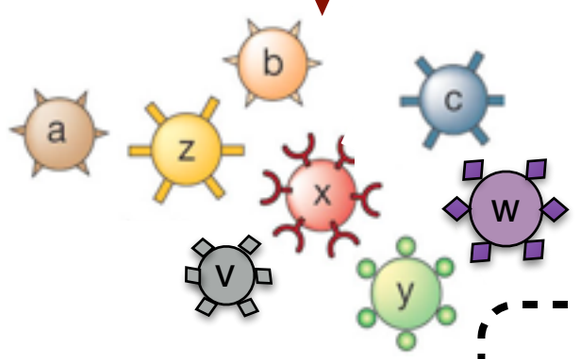
SPECIFICITY

Repertoire evolution



RECEPTOR GENERATION

VDJ recombination



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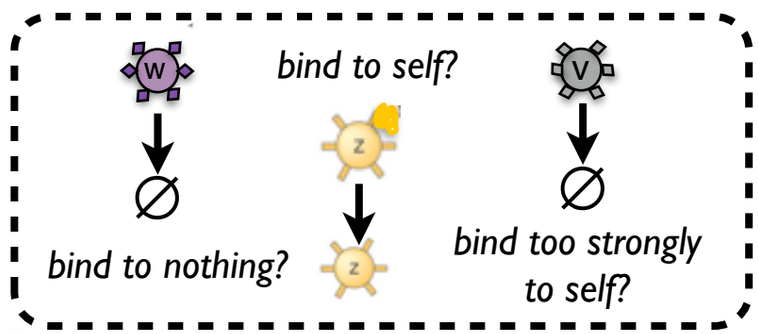

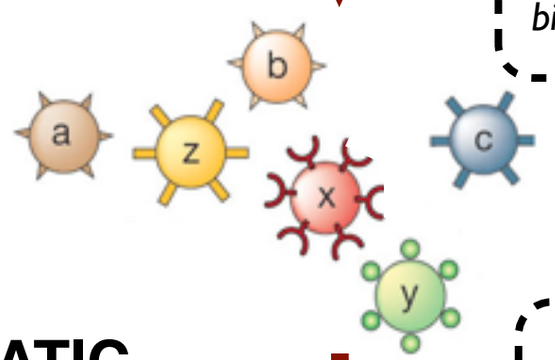

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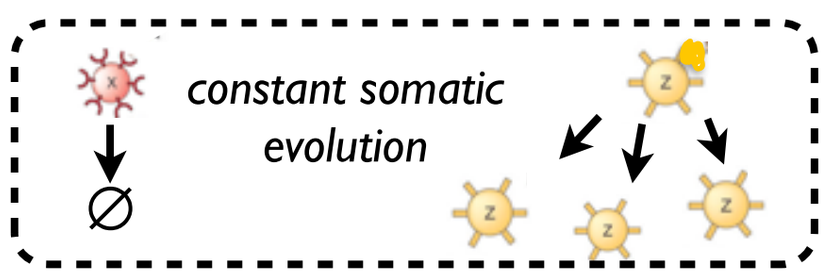

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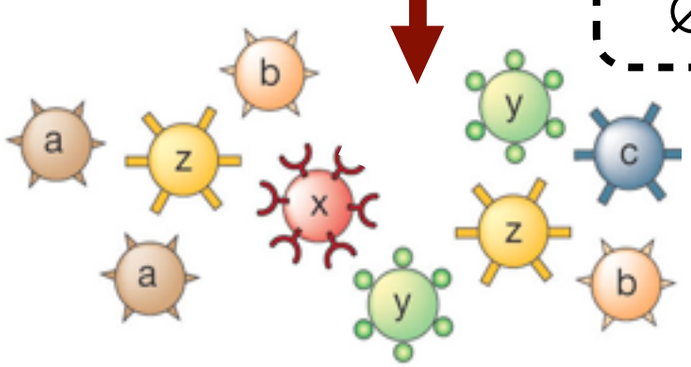
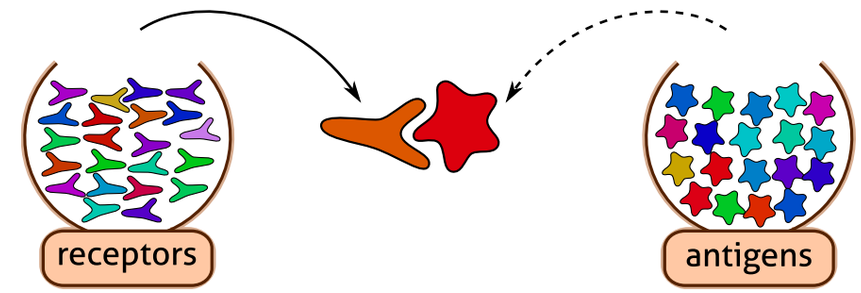


SPECIFICITY

Optimal repertoires



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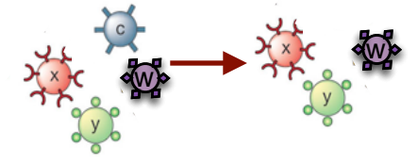
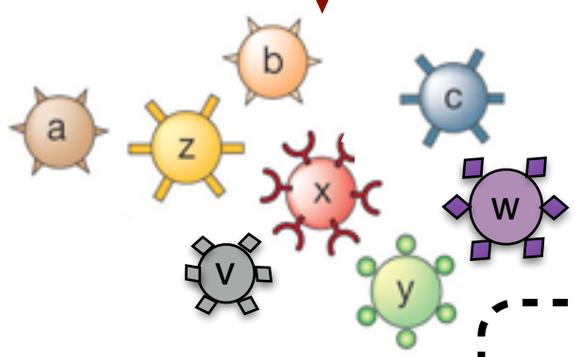
Repertoire evolution

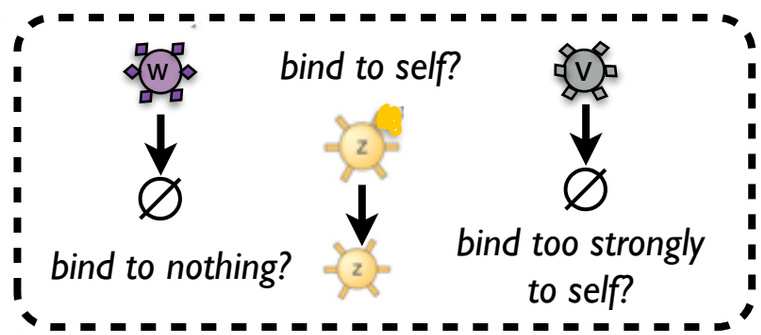


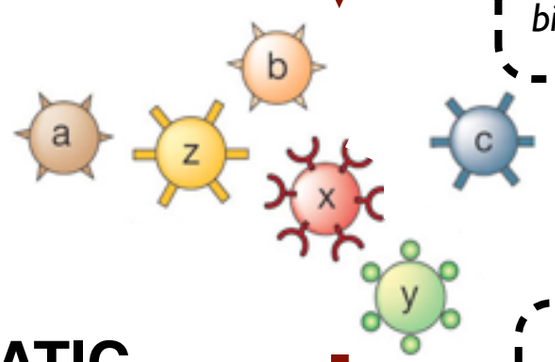
RECEPTOR GENERATION

VDJ recombination



THYMIC SELECTION





SOMATIC SELECTION

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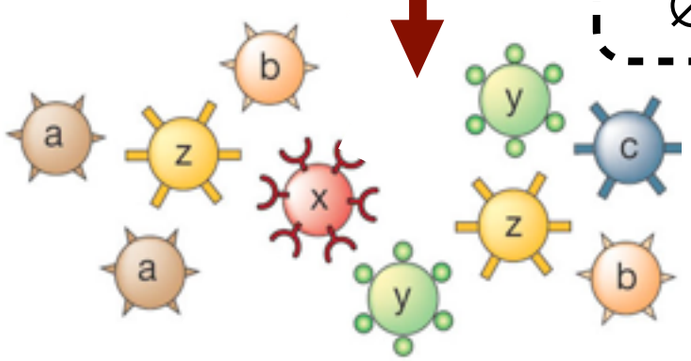
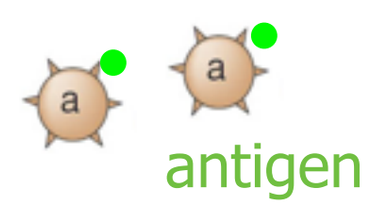
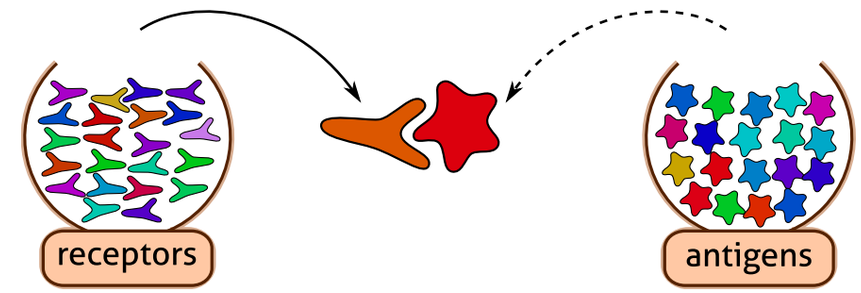


SPECIFICITY



Optimal repertoires



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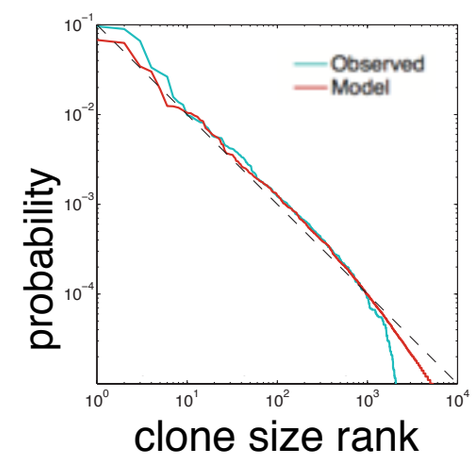
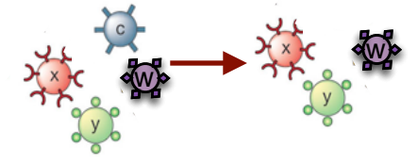
Clone-size distribution



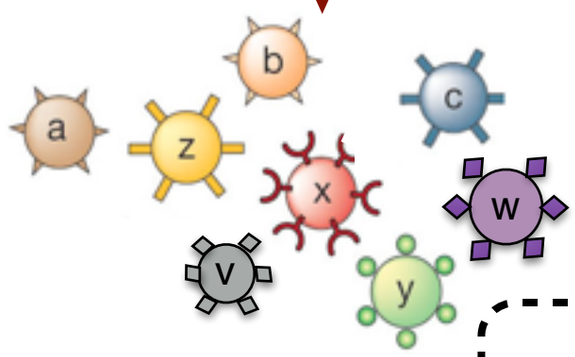
Desponds Mora Walczak PNAS 2016

Repertoire evolution

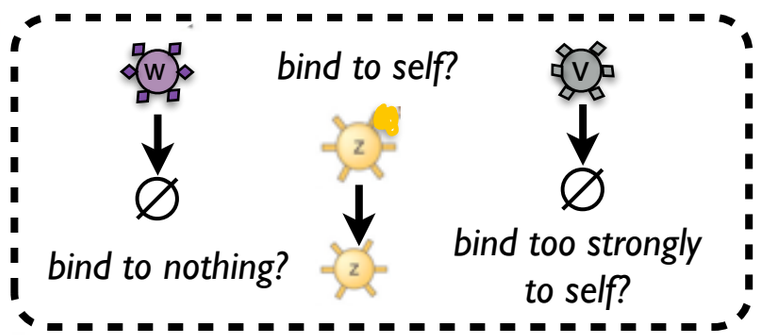


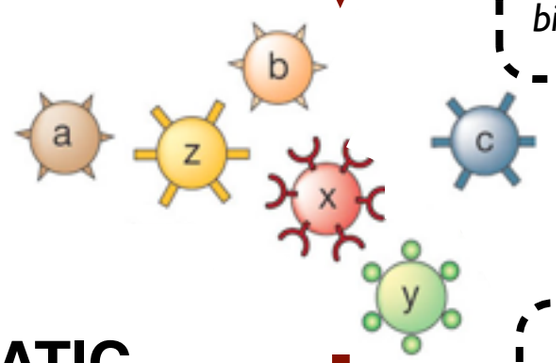
RECEPTOR GENERATION

VDJ recombination



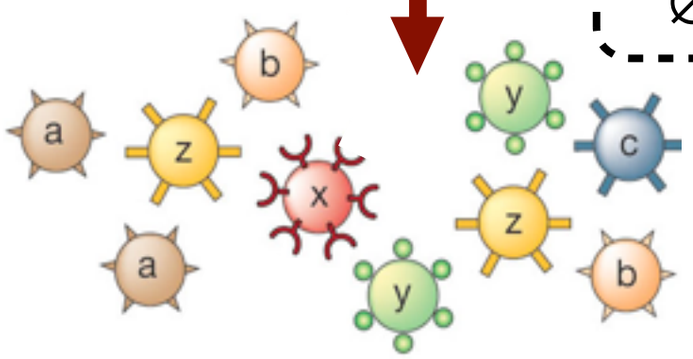
THYMIC SELECTION





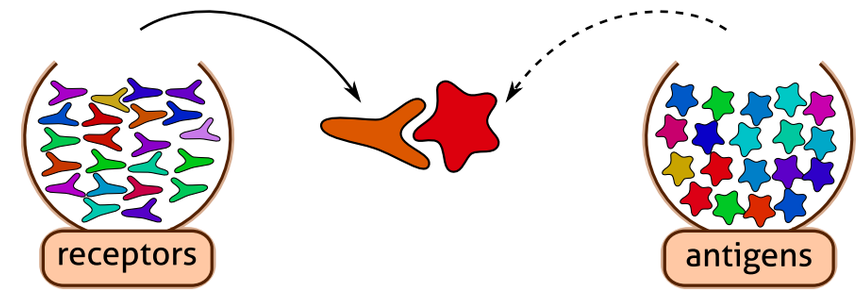
SOMATIC SELECTION

+ Somatic Hypermutations



SPECIFICITY

Optimal repertoires



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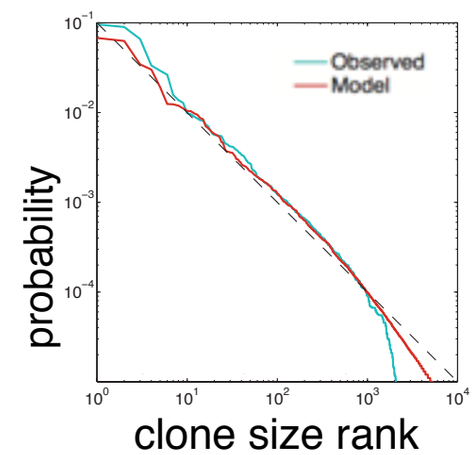
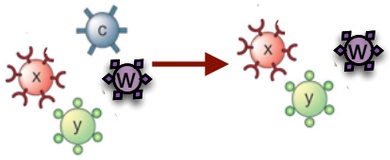


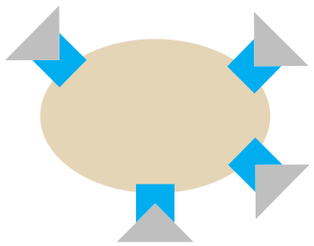
Desponds Mora Walczak PNAS 2016

Sequence-affinity landscape



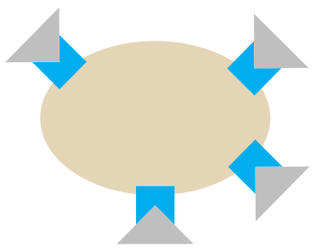
antibody / TCR sequence ⟷ binding affinity

Tite-seq



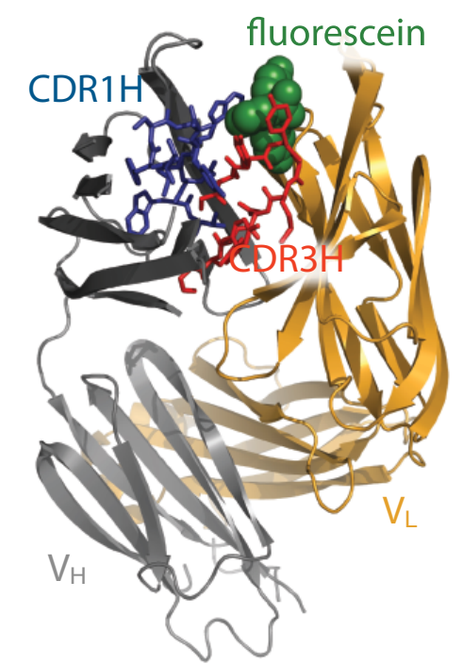
yeast display + FACSort + sequence

Tite-seq

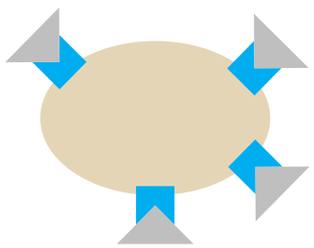


yeast display + FACSort + sequence

- proof of principle: antigen = fluorescein
  antibody = mutagenized scFv



Tite-seq



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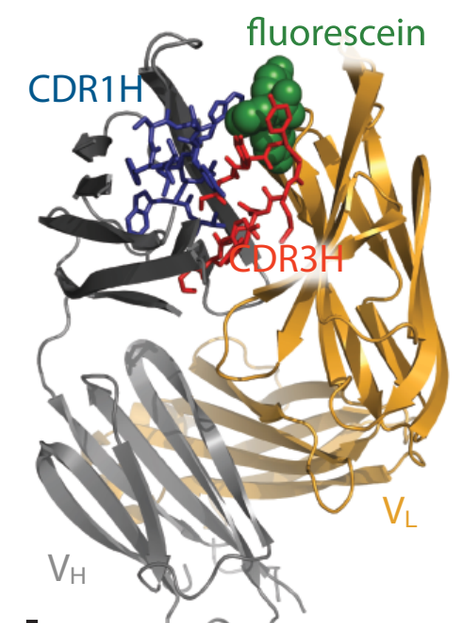
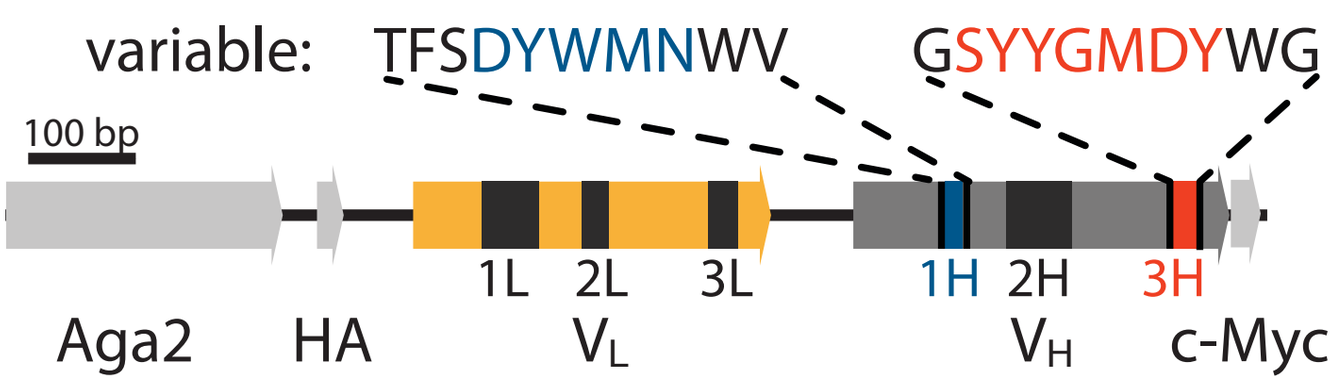
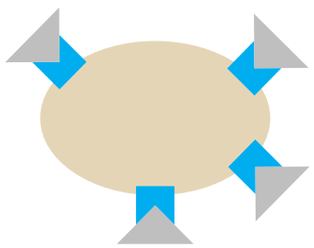


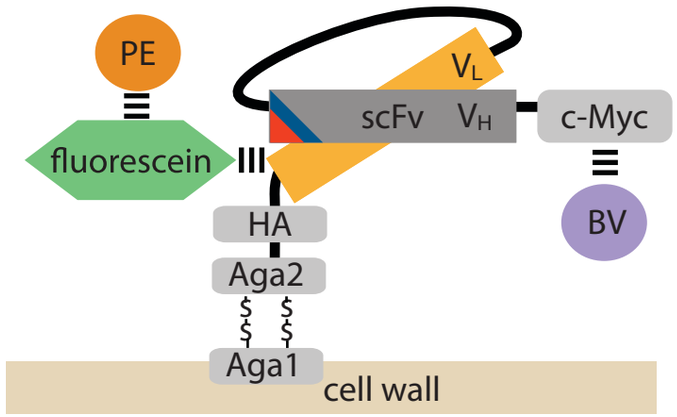
- exhaustive single mutations in CDR1H and CDR3H



variation	1H	3H
1 codon	600	600
2 codon	1100	1100
3 codon	150	150

Tite-seq





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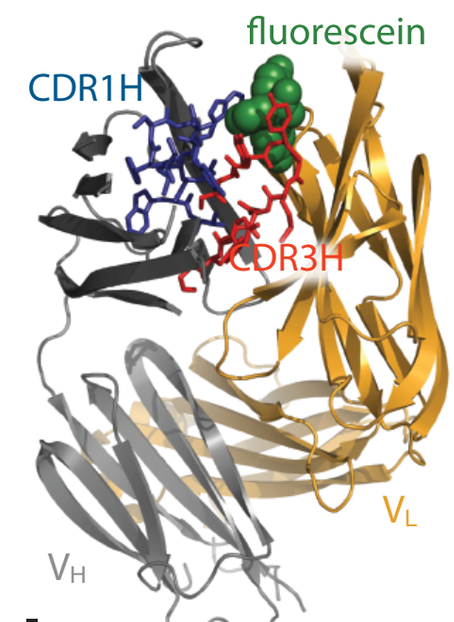

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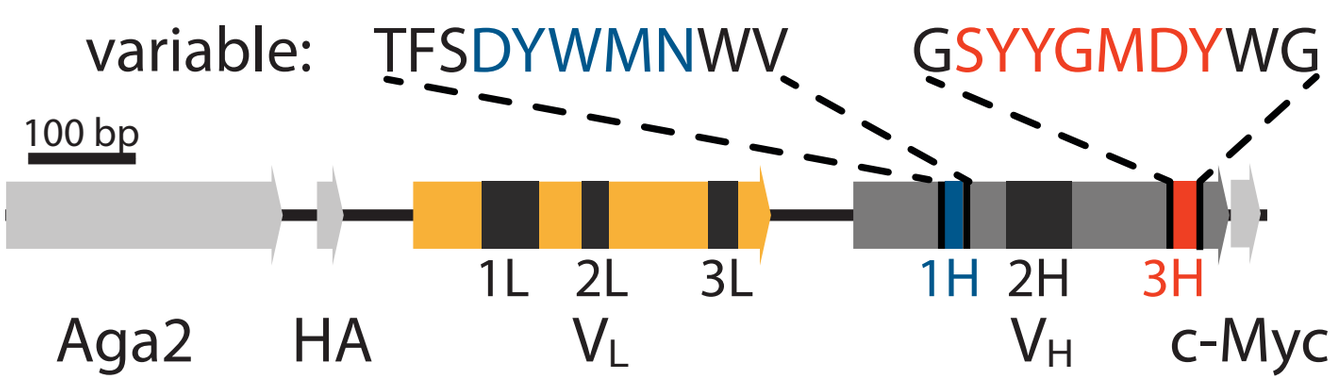

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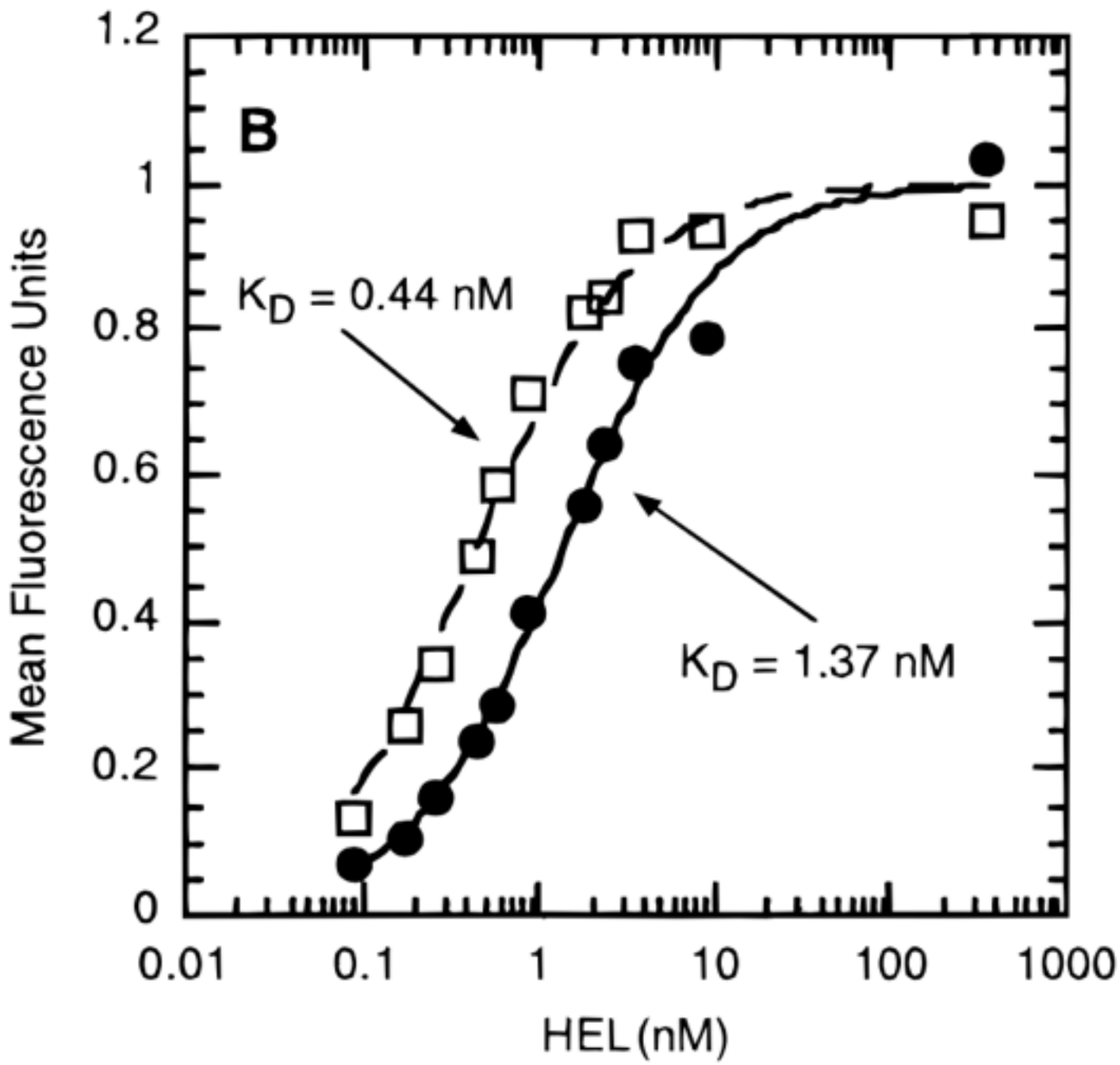
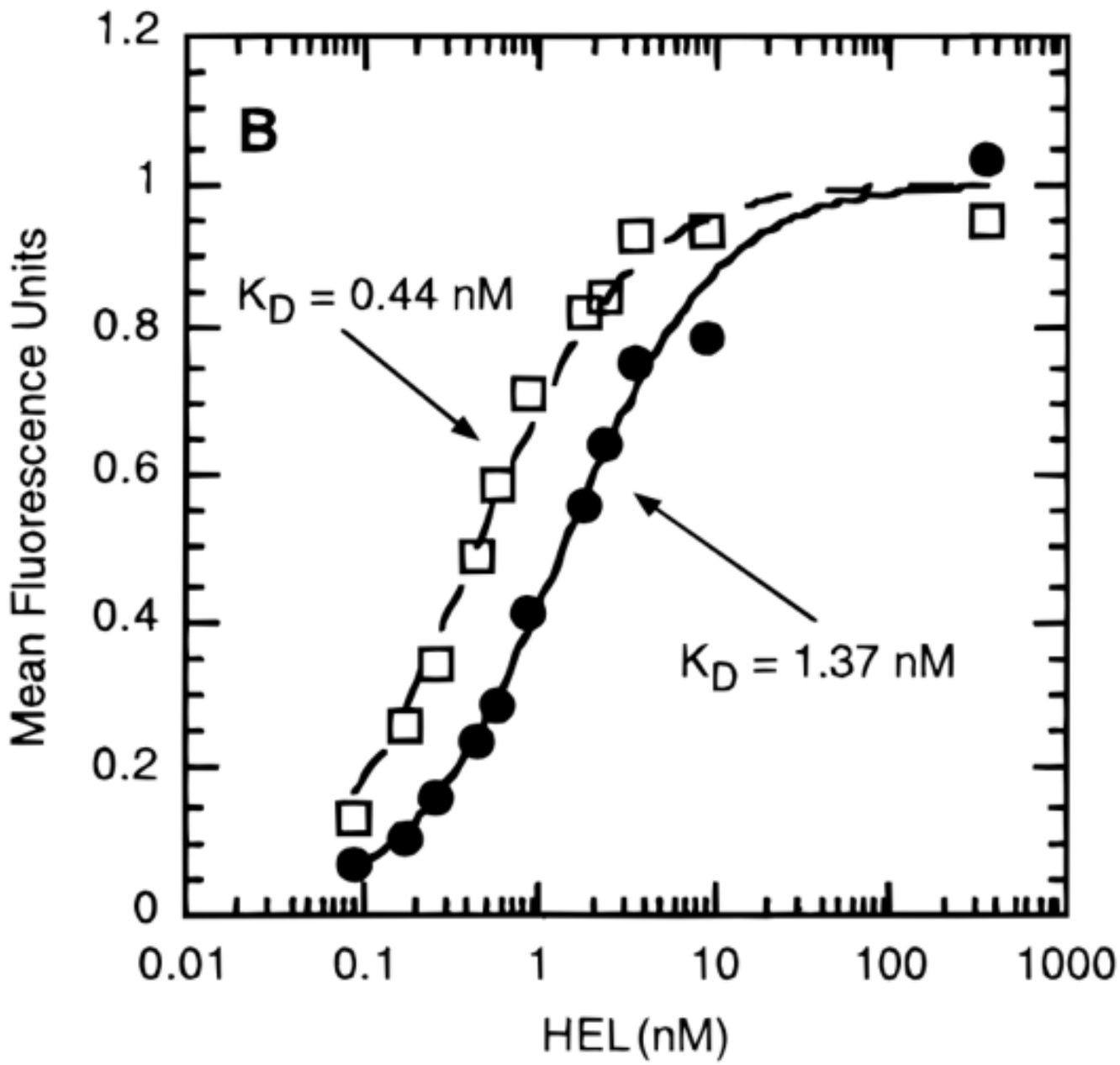
3 codon	150	150

Titration by flow cytometry

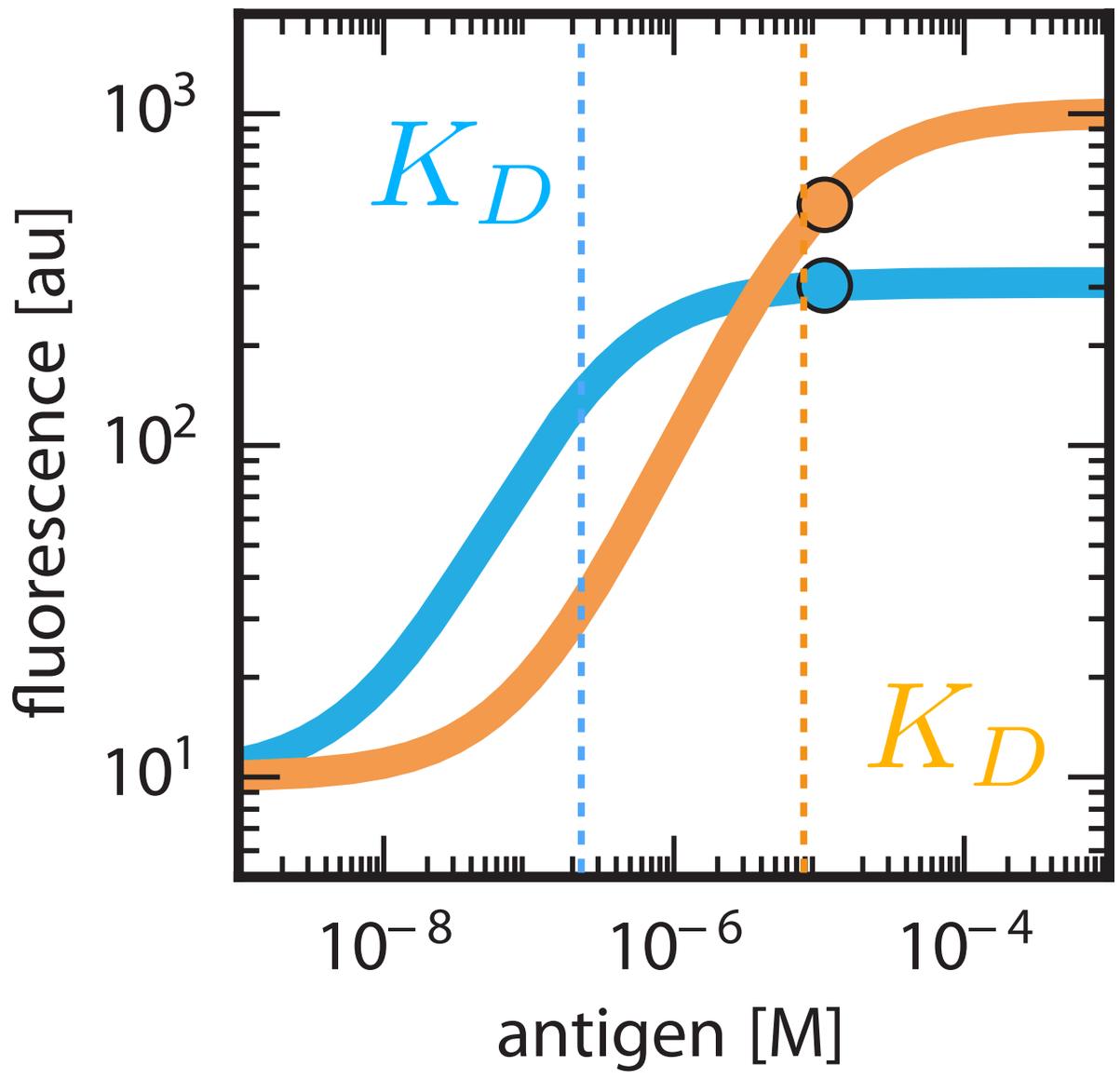


Titration by flow cytometry



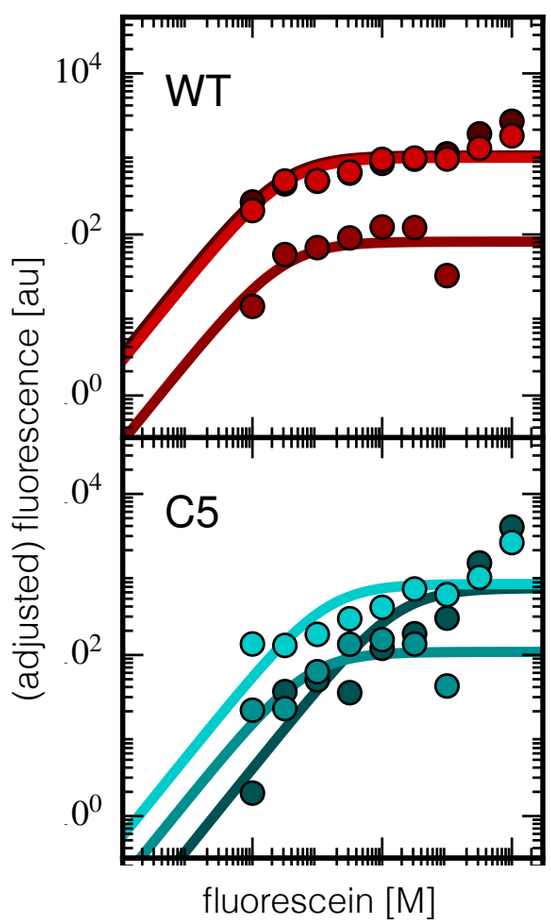
VanAntwerp and Wittrup Biotechnol. Prog. 2000

Titration by flow cytometry

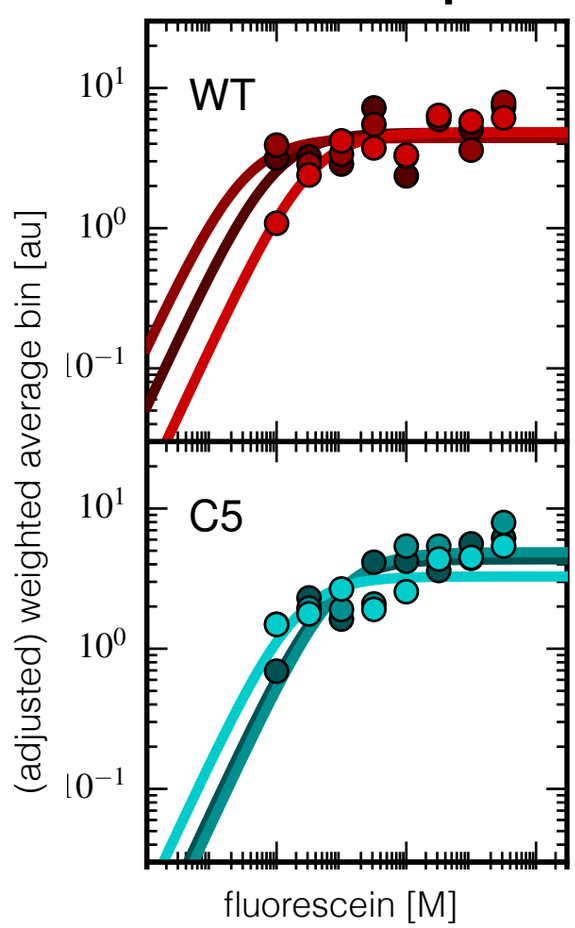


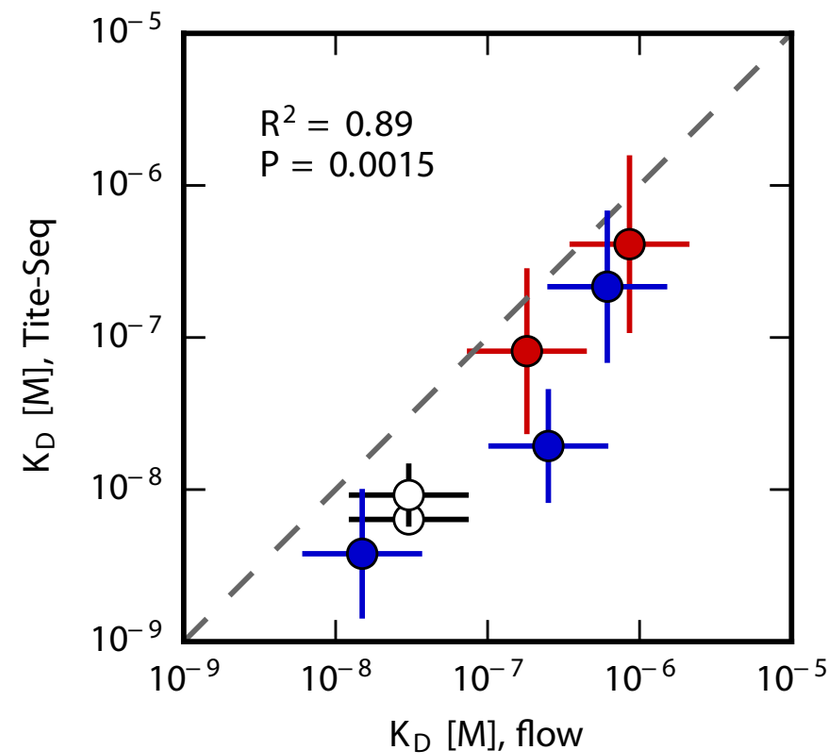
Comparison to direct fluorescence

fluorescence



tite-seq





wide range of affinities

Comparison to direct fluorescence

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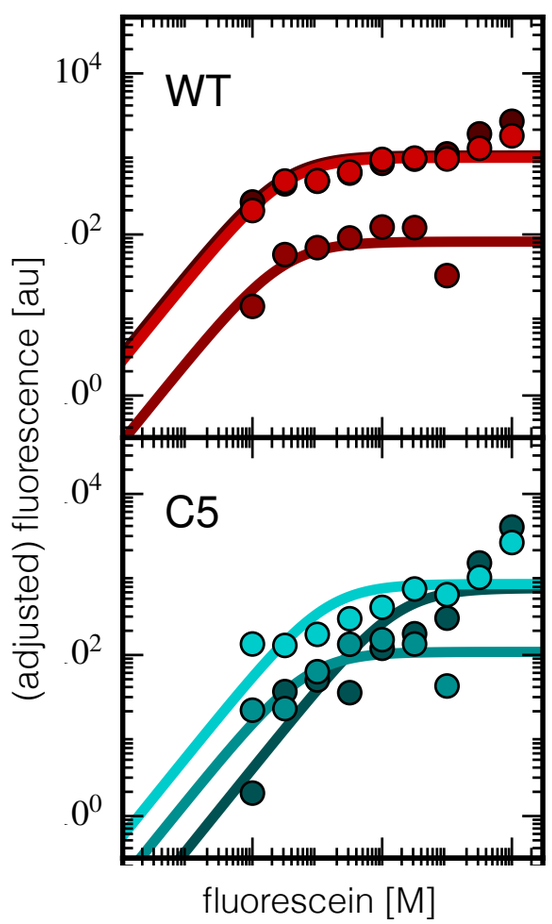
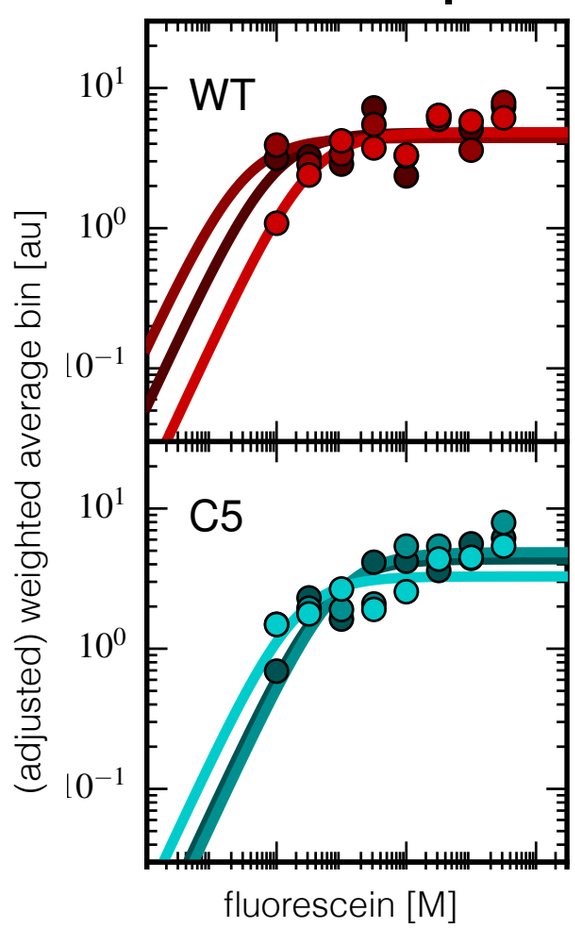
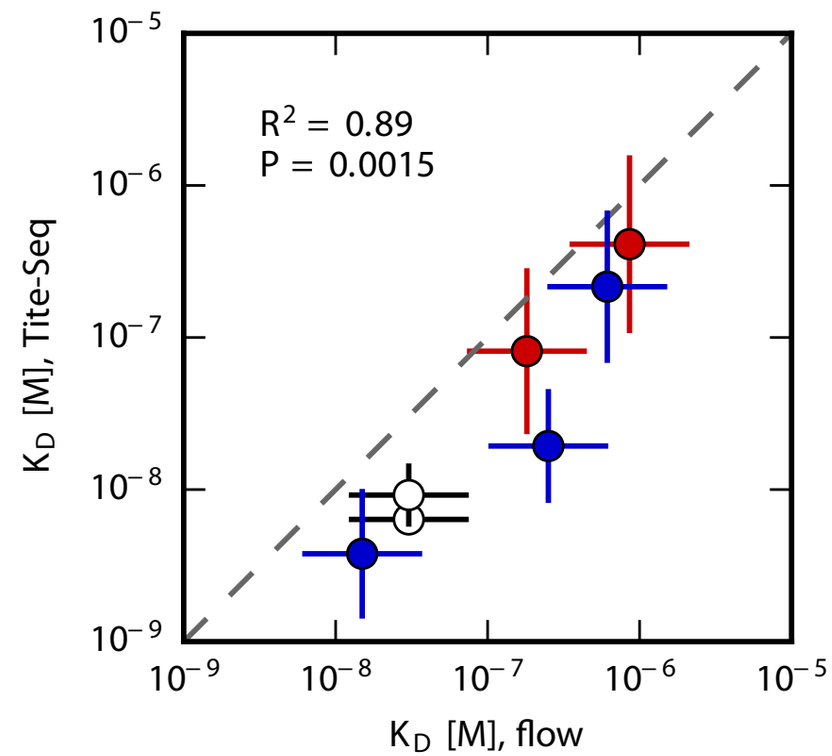


tite-seq

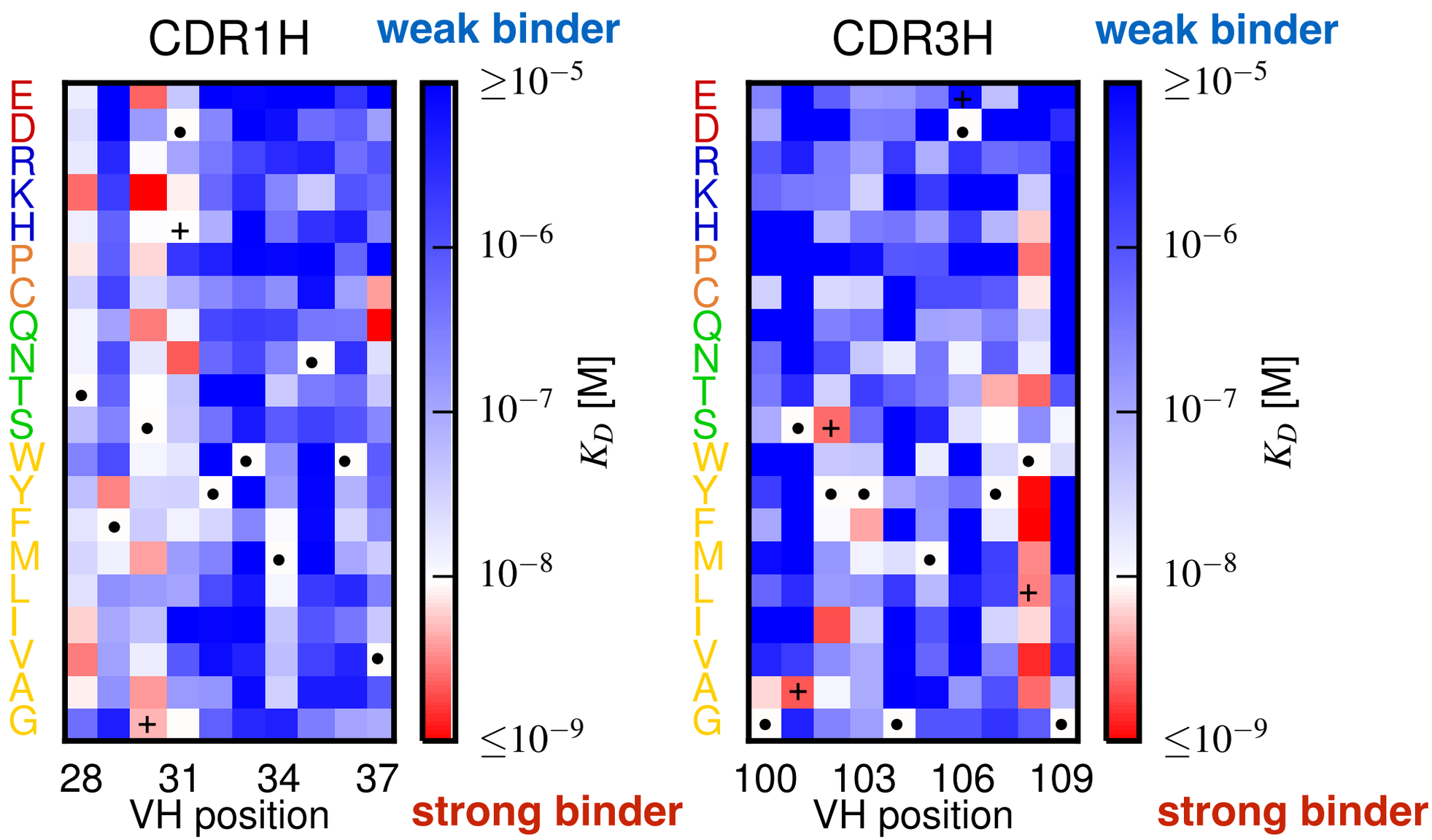




wide range of affinities

Adams Kinney Mora Walczak arXiv 2016

Mutation binding landscape



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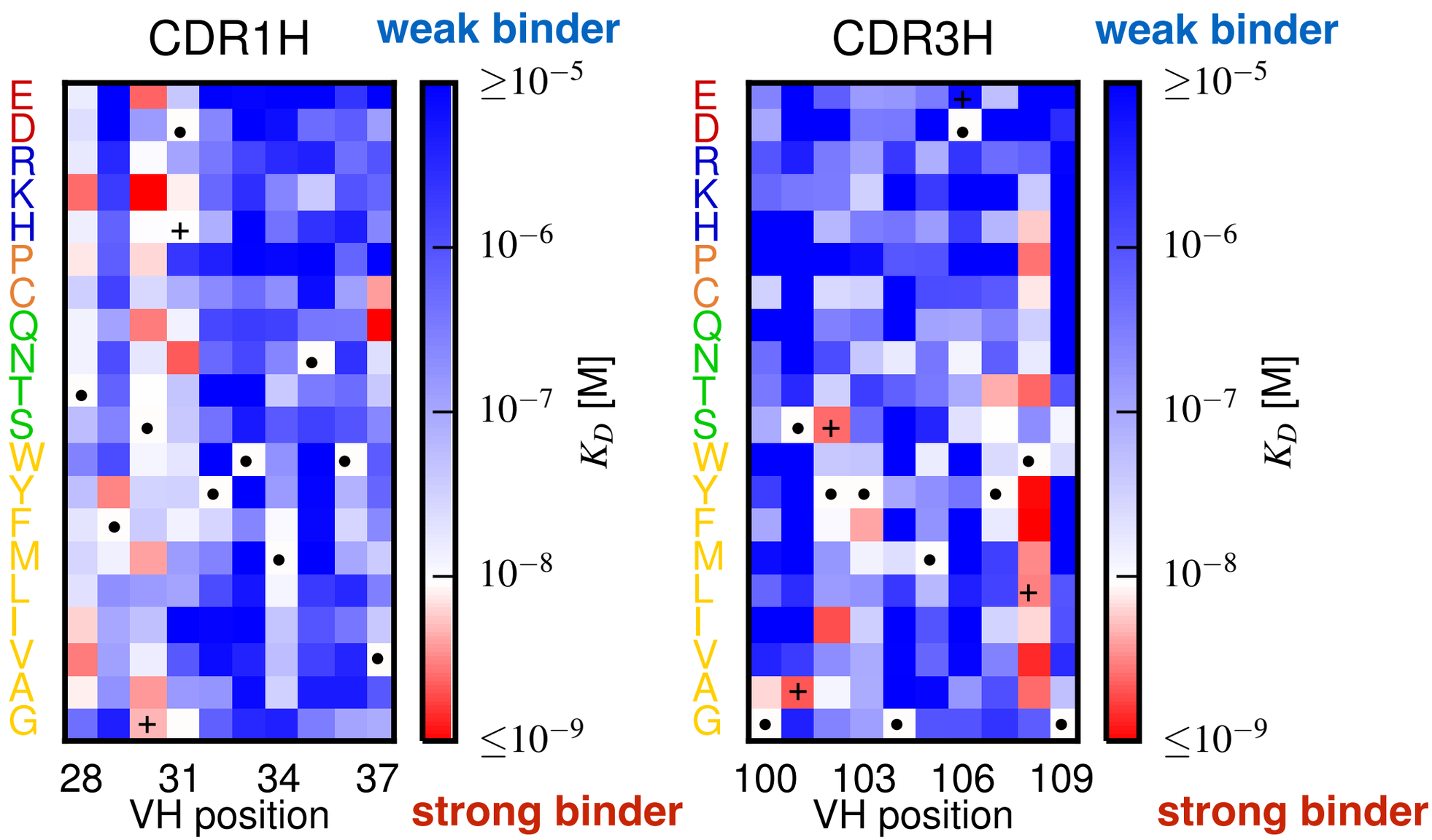
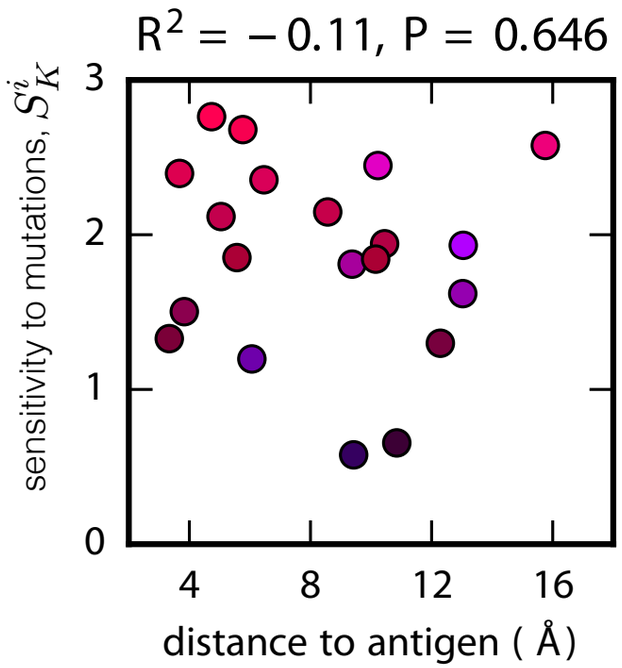
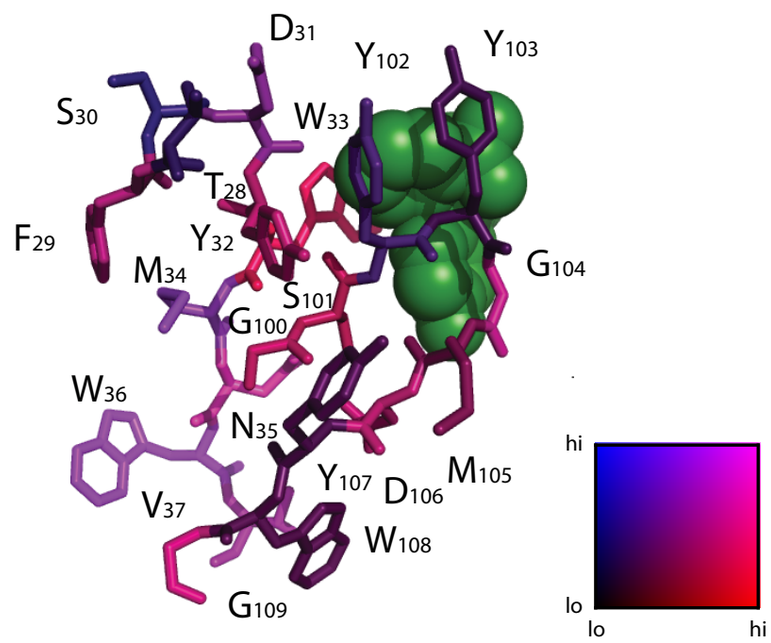


Adams Kinney Mora Walczak arXiv 2016

Effects of mutations

the effect of mutations on affinity: $S_K^i = \sqrt{\left\langle \left(\log_{10} K_D^{ia} - \log_{10} K_D^{\mathrm{WT}}\right)^2 \right\rangle_a}$





- is independent of distance of mutation to antigen

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S_K^i

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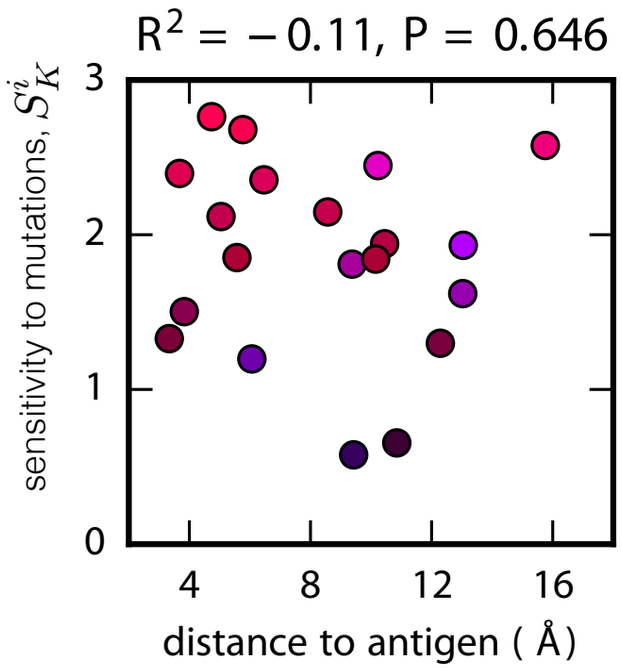

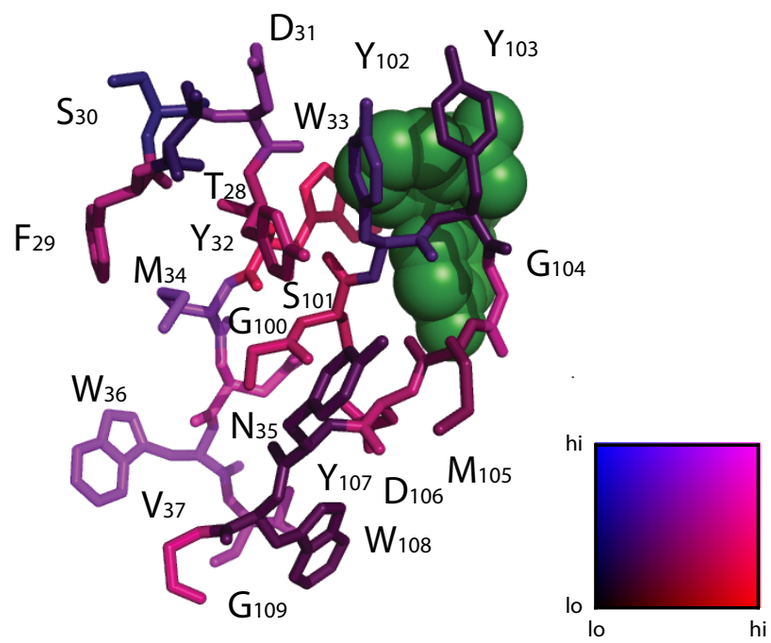

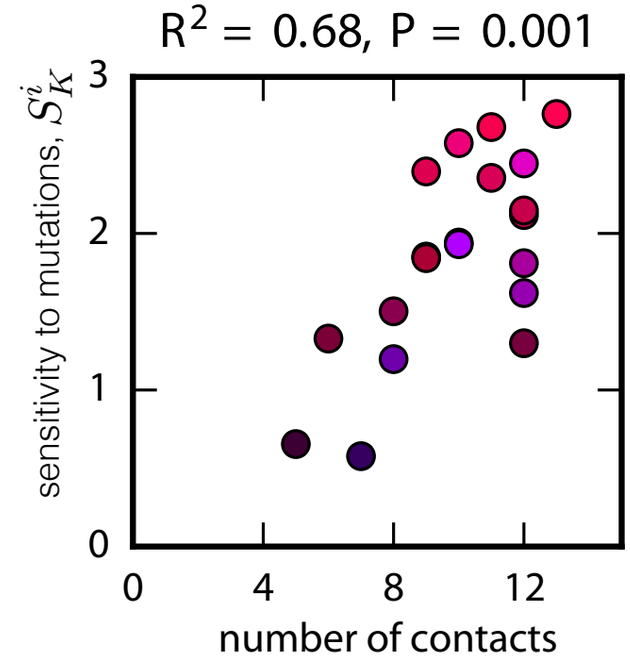

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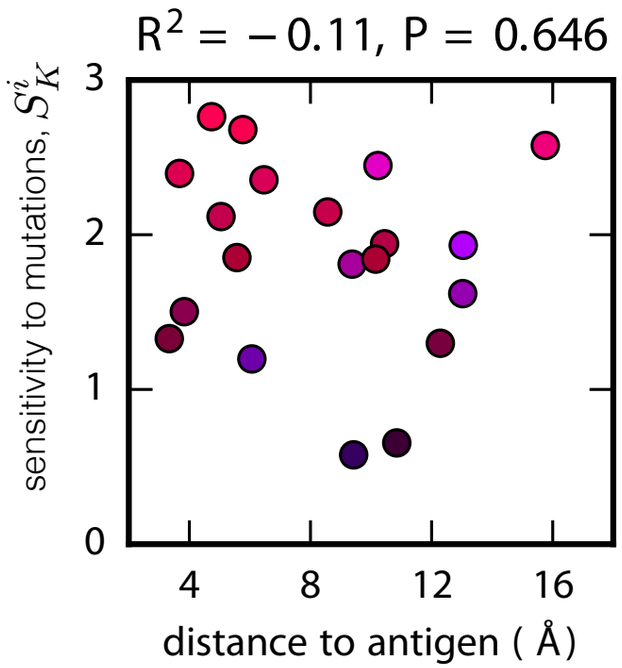

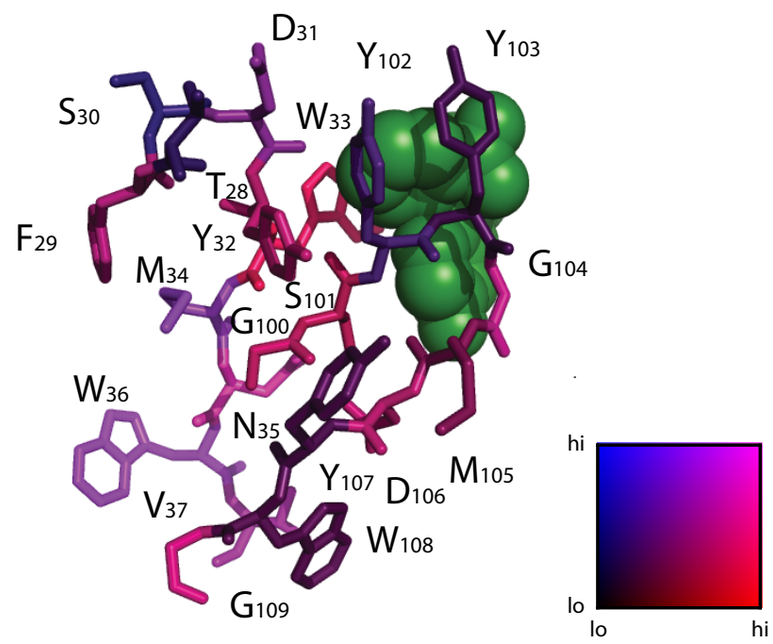

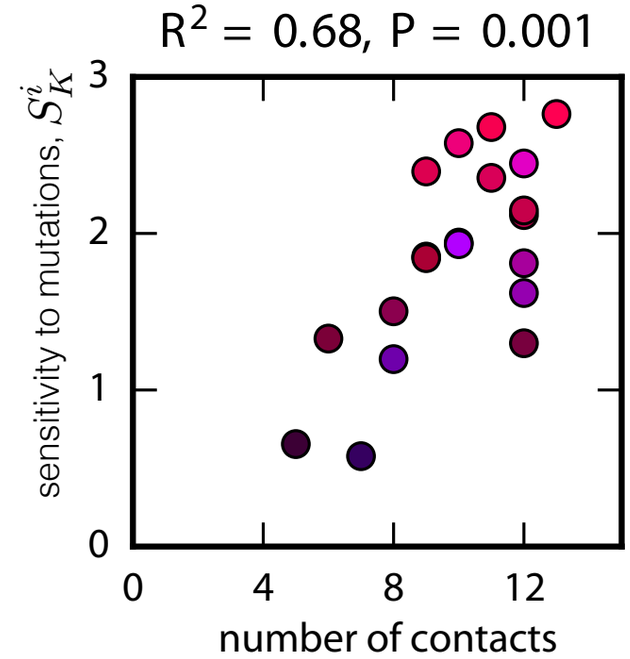

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  → effect of interactions between receptor residues

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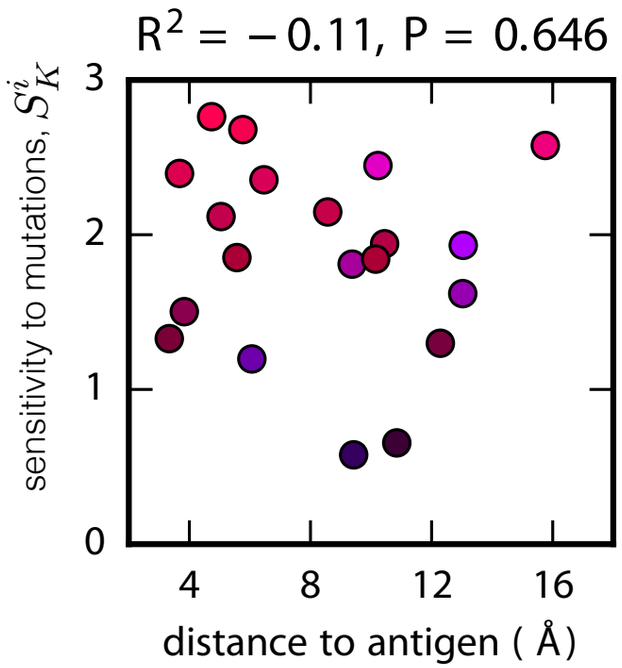
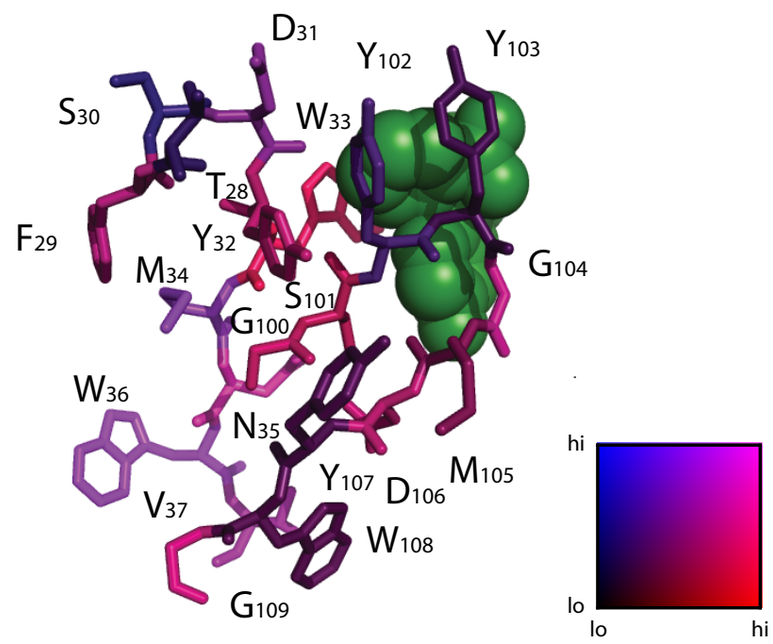
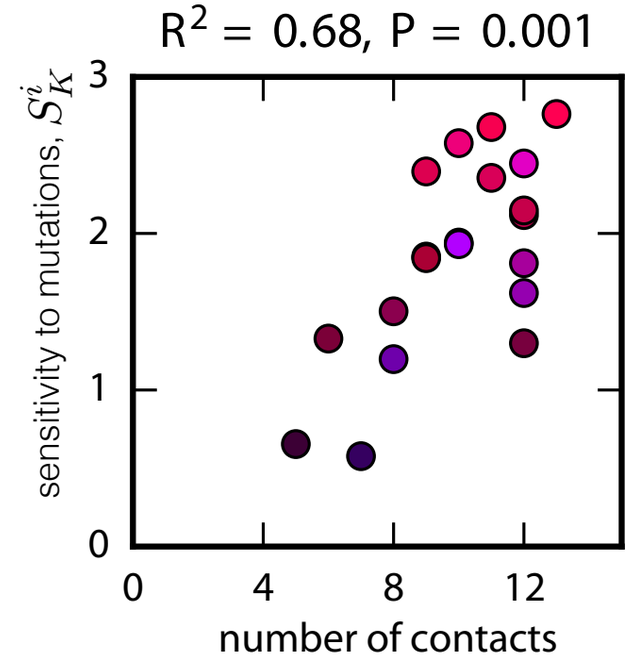






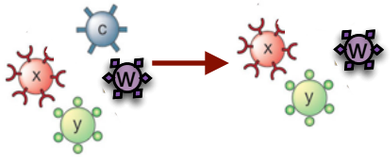
- is independent of distance of mutation to antigen
- depends on the number of contacts a residue makes in the receptor

- non-local effects
  → effect of interactions between receptor residues
- CDR3 mutations have greater effect on affinity
  →more likely to be mutated in functional receptors

S_E^i

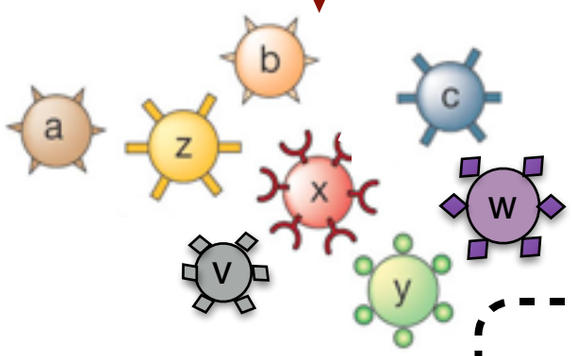
S_K^i

Repertoire evolution

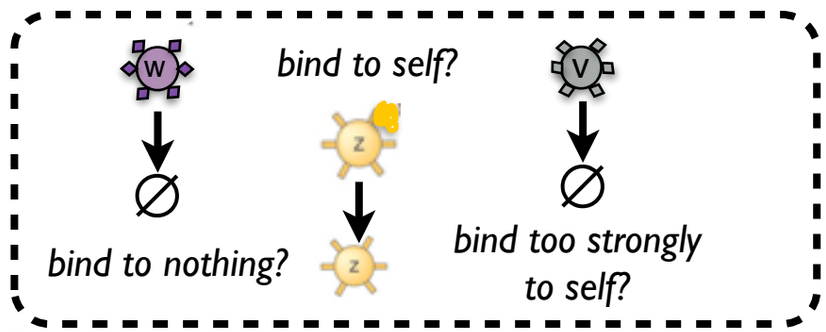


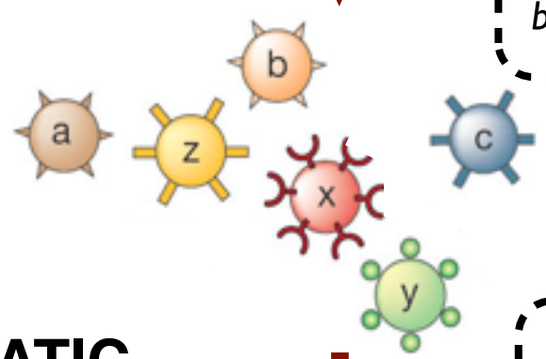
RECEPTOR GENERATION

VDJ recombination

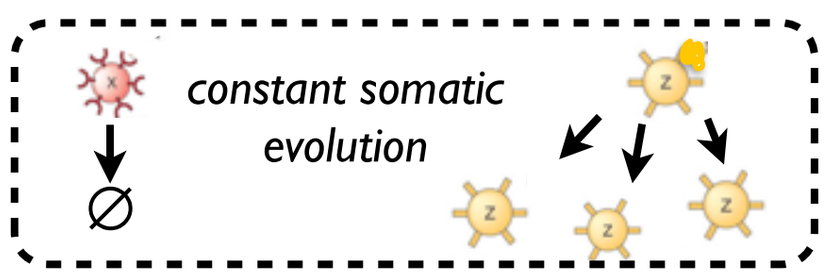


THYMIC SELECTION

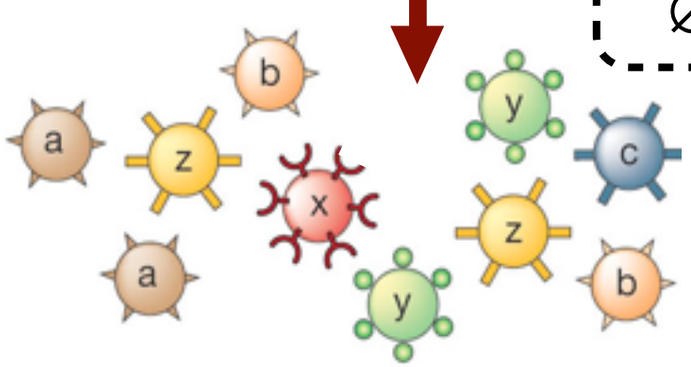




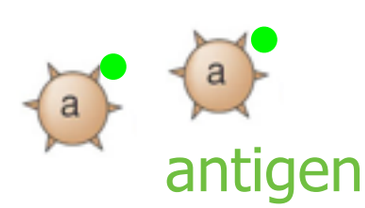
SOMATIC SELECTION



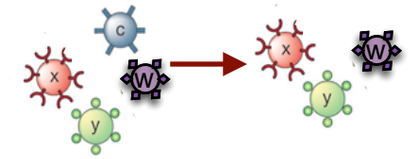
+ Somatic Hypermutations

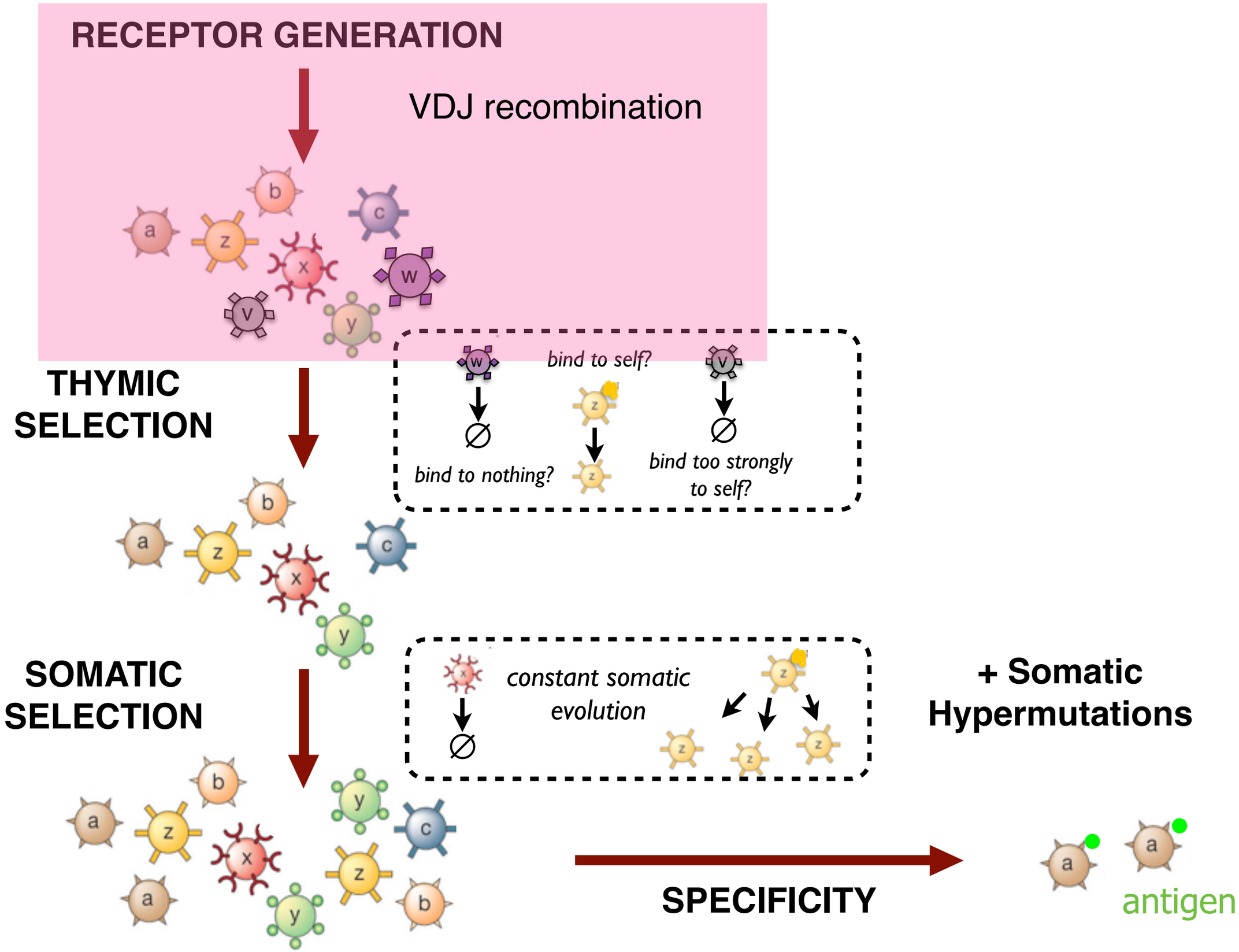


SPECIFICITY

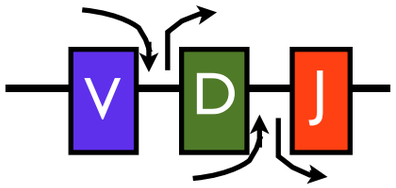


Repertoire evolution





Sequence data



- human T-cell beta chain receptor sequences
- 9 people

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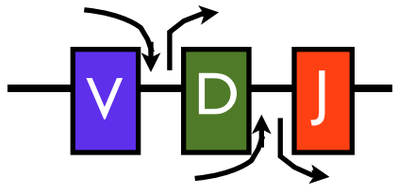
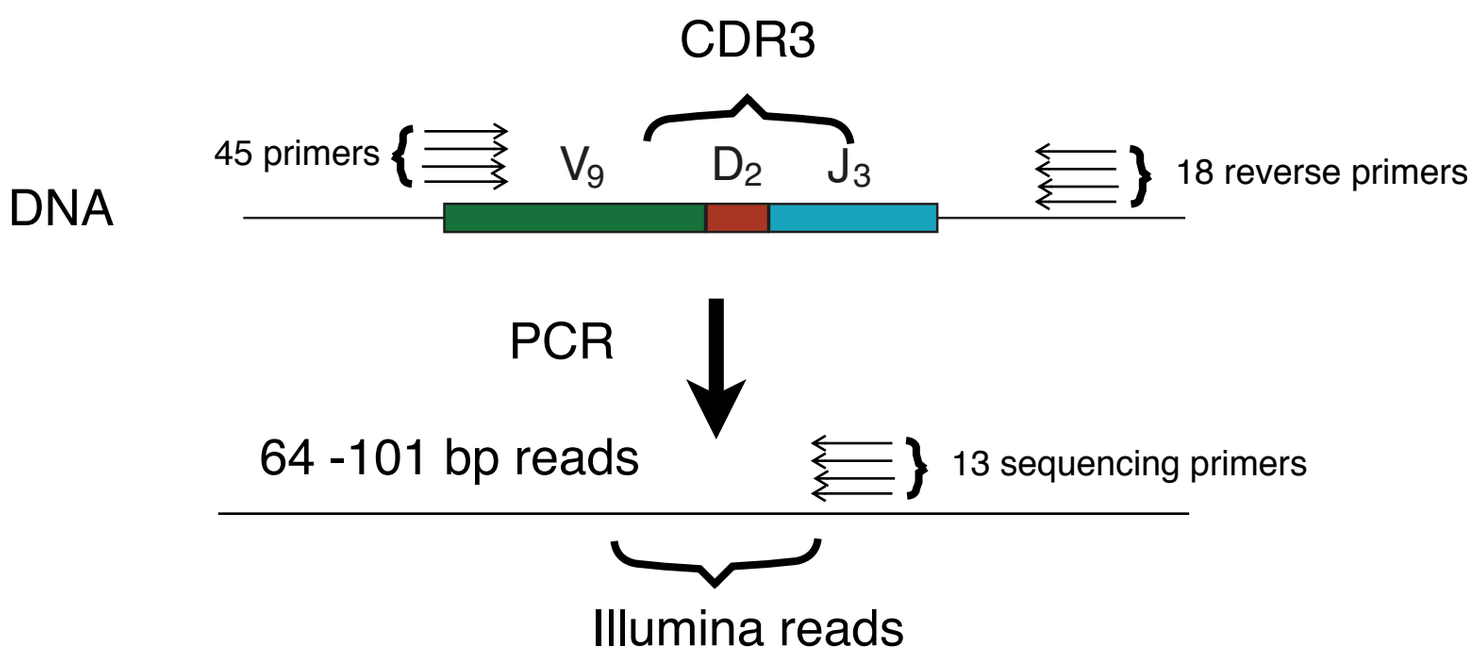


- human T-cell beta chain receptor sequences
- 9 people
- CD4+ naive cells
- out of frame reads (~14%) = 35,000 unique reads ⟶ *generation*
- in frame reads (~235,000 unique reads) ⟶ *selection*

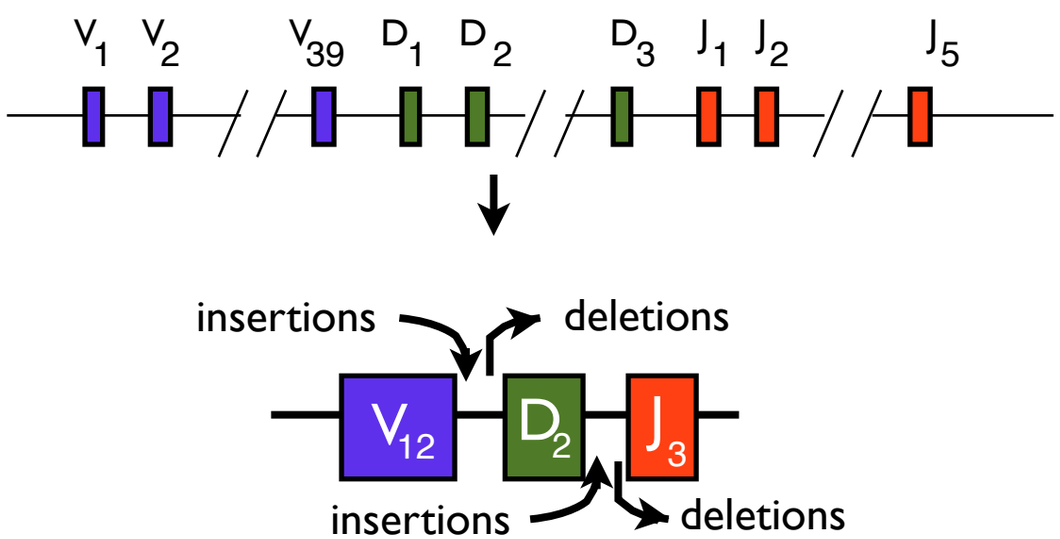


Robins et al, Blood (2009)

data from Robins lab 2009-2012

learning VDJ recombination

sequence generation: VDJ recombination

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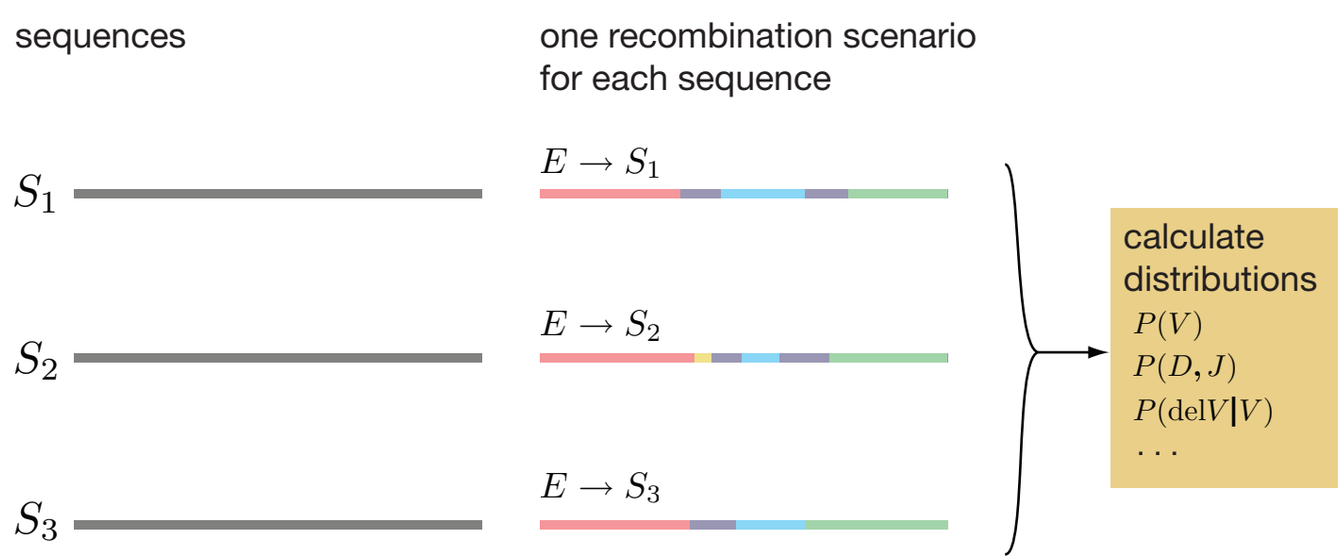
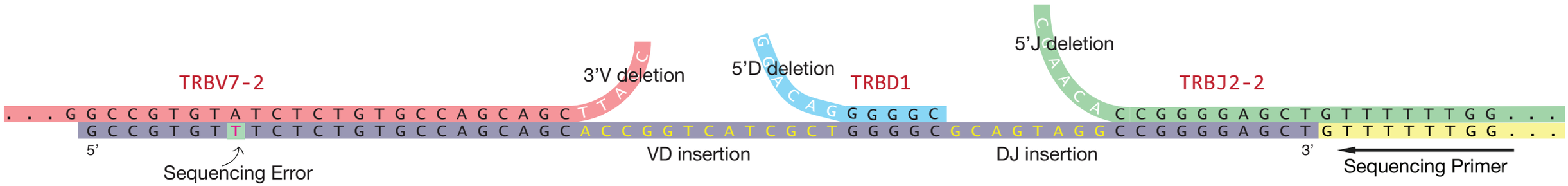
TCCCTCCGGTTTGAAGGGTCTGCTGGCACAGAAGTACACAGATGTTTGGGAGGG
CCGGTGTTCGCACAGCCCTGGGGACCCTGGCGCAAACCCCGCTTCCCTCGAGGA
```

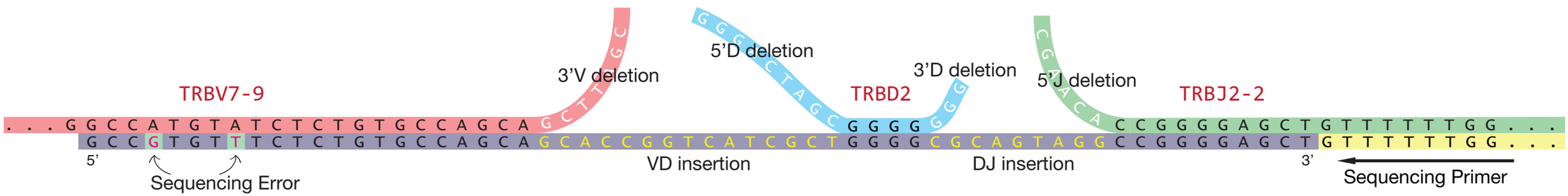
Probability distribution?

- too many possible sequences to sample
- basic approach



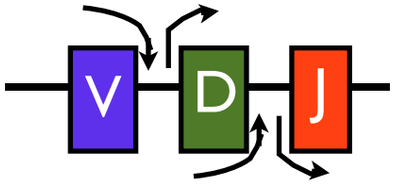
The problem





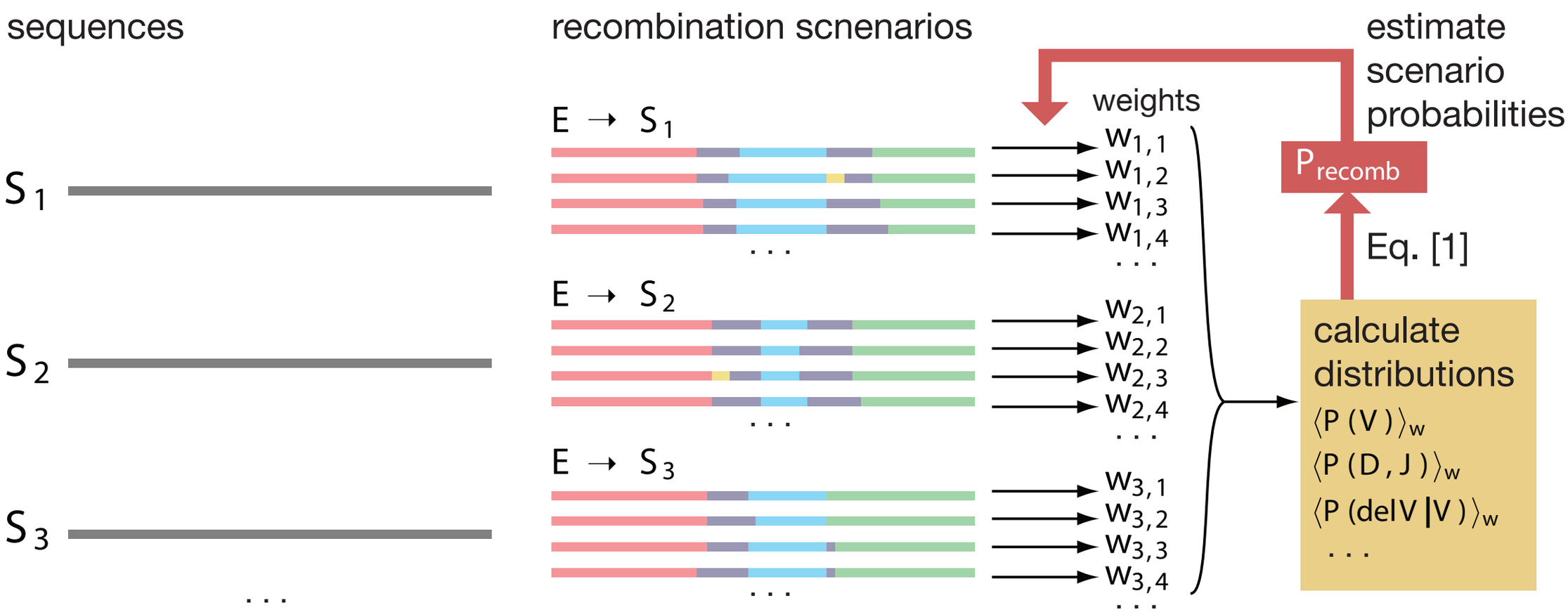
- impossible to reliably assign events (insertions, deletion, ...)
- sequencing errors

Expectation maximization



- probabilistic model for assignment of:
  - genomic VDJ assignment
  - cut position/deletions
  - insertions

$\vec{\sigma}$ - receptor DNA sequence

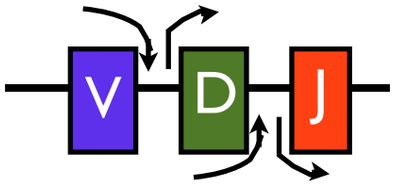


$$\mathrm{P}^{\mathrm{recomb}}(\text{scenario}) = P(V)P(D,J)P(\text{deletions}V|V)P(\text{insertions}DJ)\ldots \qquad [1]$$

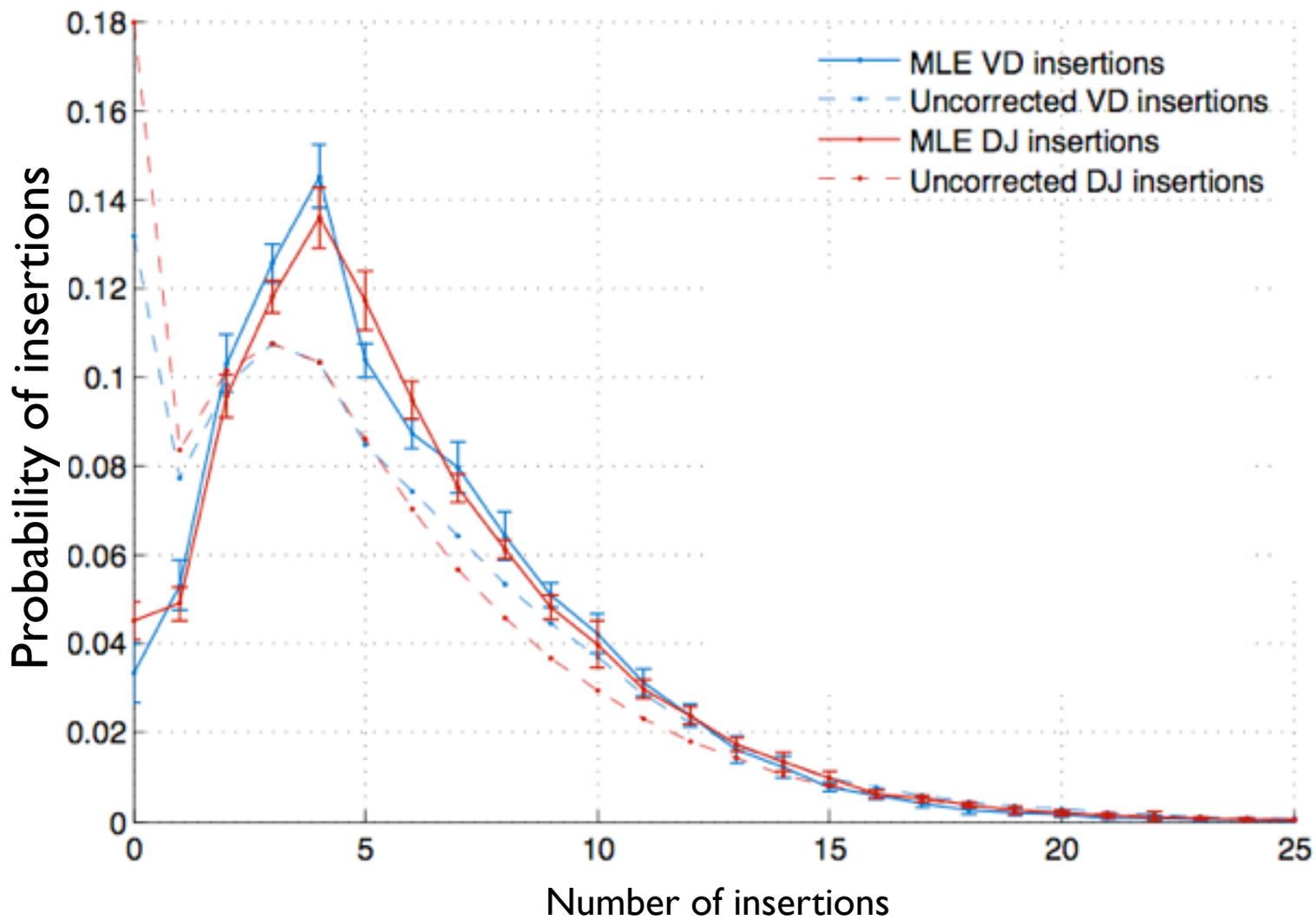
$$P_{gen}(\vec{\sigma}) = \sum_{\substack{\text{scenarios:}\\ V,D,J,\ldots\to\vec{\sigma}}} P^{\mathrm{recomb}}(\text{scenario})$$

Murugan Mora Walczak Callan PNAS (2012)

Universal insertion profiles

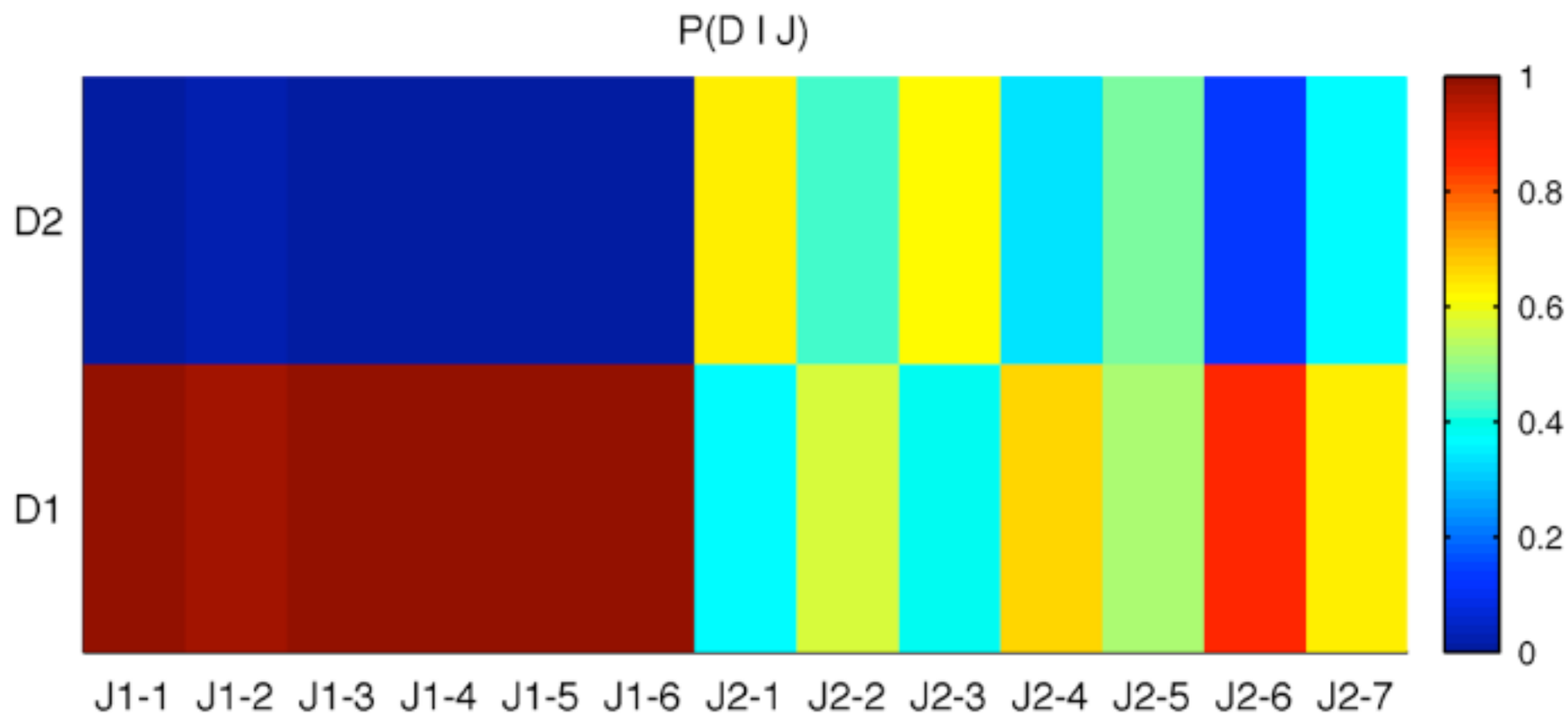


- VD and DJ insertion profiles are identical



Probabilistic is necessary: D and J gene choice

- The choice of J strongly constrains the choice of D



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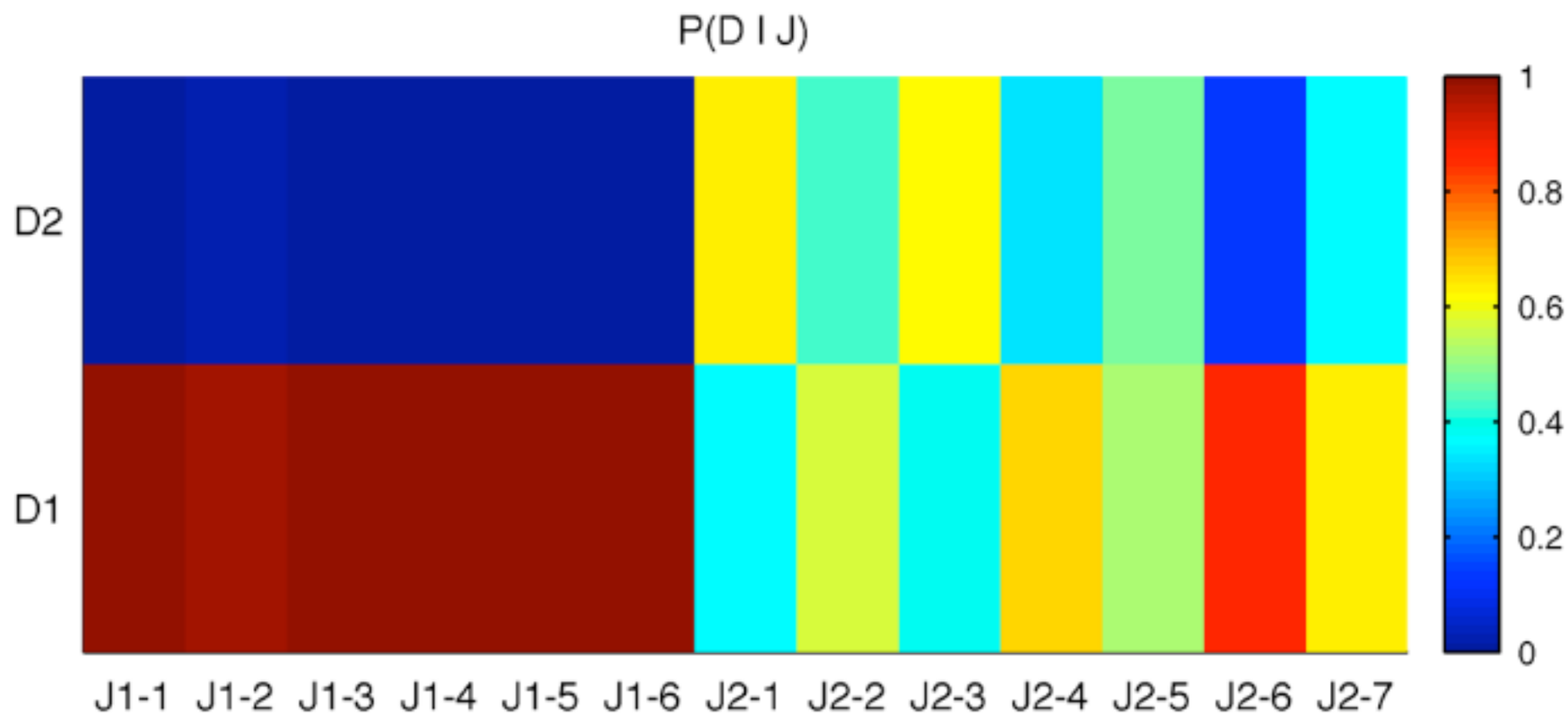

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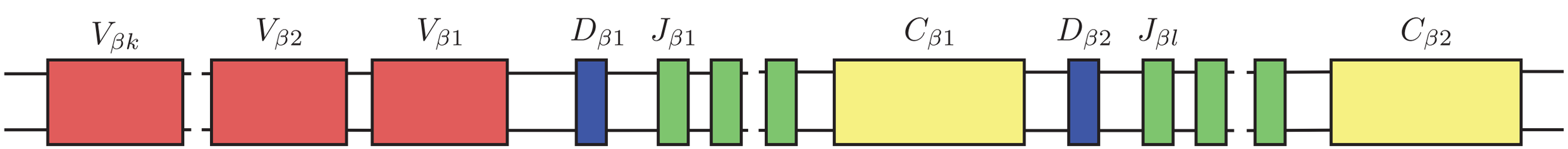
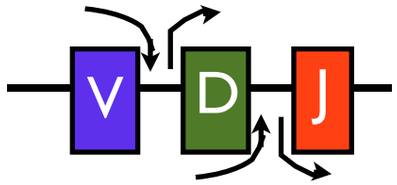


Entropy



- potential sequence diversity of VDJ recombination

all possible sequences - impossible!

$$S_{\text{gen}} = -\sum_{\sigma} P_{\text{gen}}(\vec{\sigma}) \log P_{\text{gen}}(\vec{\sigma})$$

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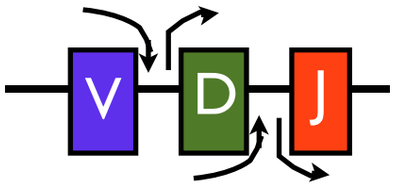

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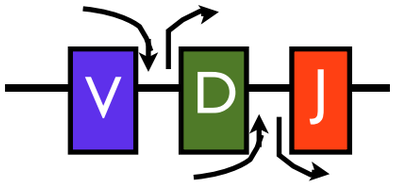
recombination scenario entropy

conditional entropy of recombination events given sequence

Entropy



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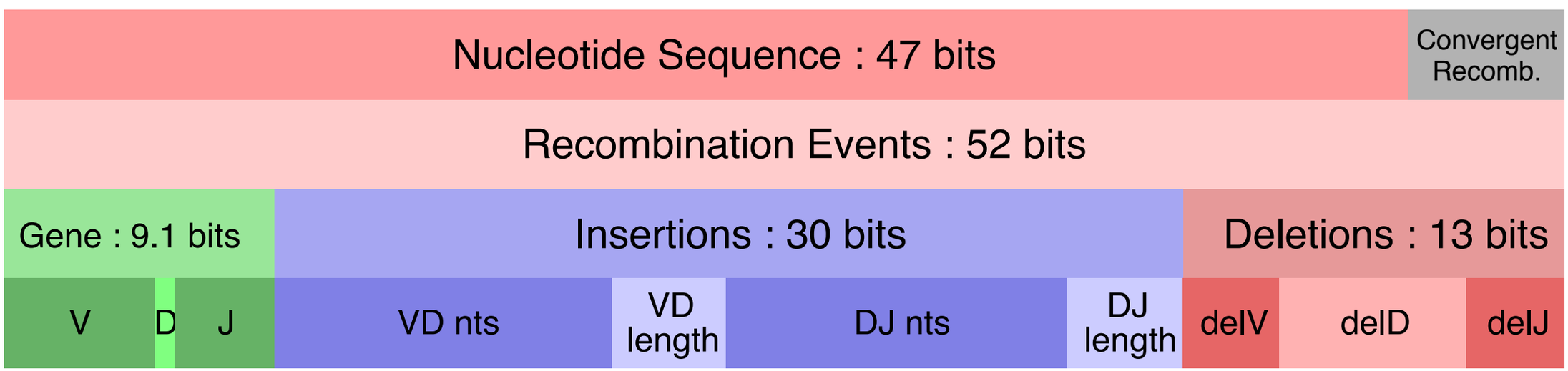
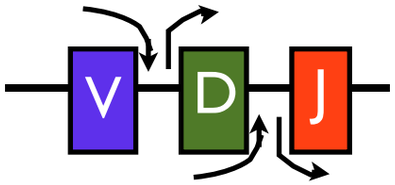


Entropy



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recombination scenario entropy

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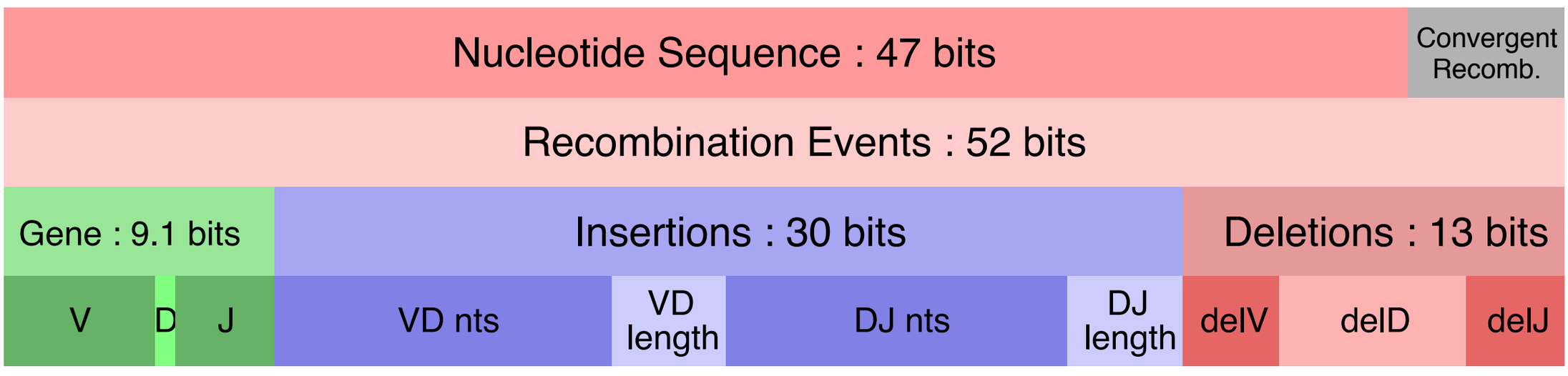
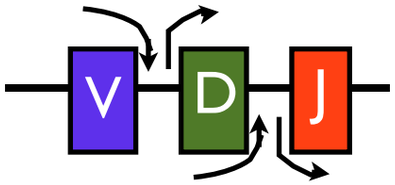


- 47 bits $\Longrightarrow$ repertoire size 10^{14} sequences > 10^{8}*+ unique seqs in individual
  $3\ 10^{11}$ total T-cells in individual

*Robins et al, Blood (2009)

Entropy



- potential sequence diversity of VDJ recombination

all possible sequences - impossible!

$$S_{\text{gen}} = -\sum_{\sigma} P_{\text{gen}}(\vec{\sigma}) \log P_{\text{gen}}(\vec{\sigma})$$

- estimate from

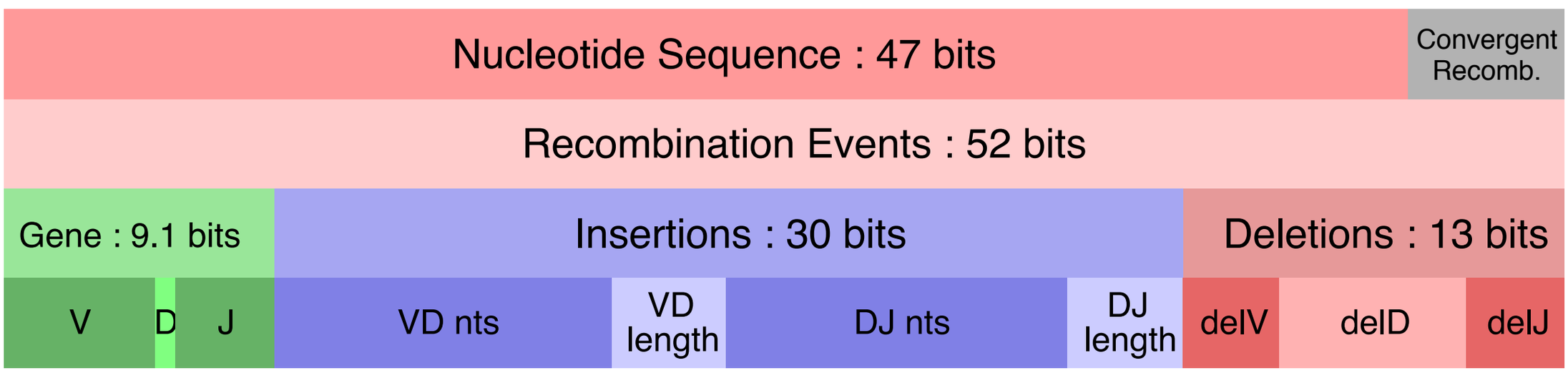
52 bits

5 bits → typical sequence can be generated in 32 different ways

$$S_{\text{gen}} = S_{\text{recomb}} - \langle S(\text{scenario}|\sigma)\rangle_{\sigma}$$

recombination scenario entropy

conditional entropy of recombination events given sequence



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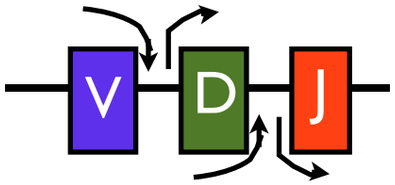
Entropy



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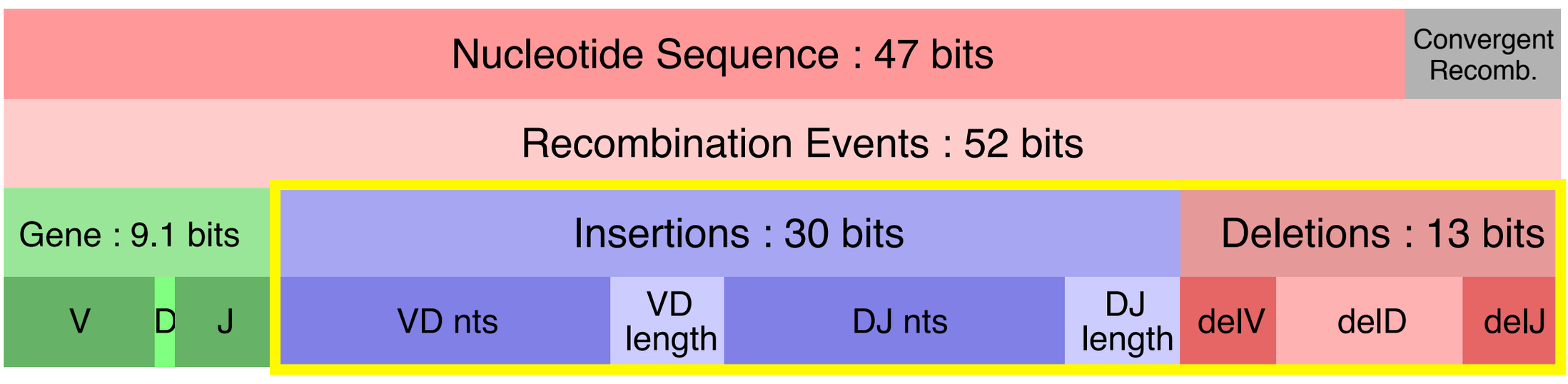
recombination scenario entropy

conditional entropy of recombination events given sequence

- diversity dominated by junctional diversity



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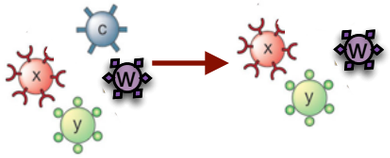
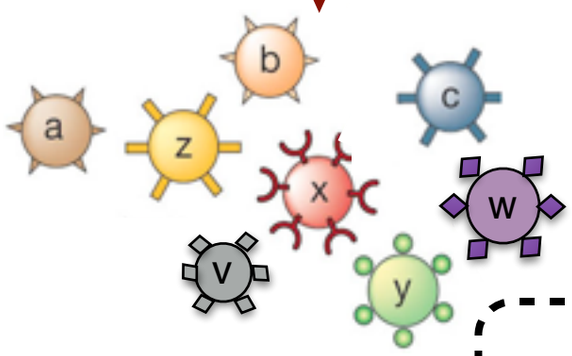
Repertoire evolution

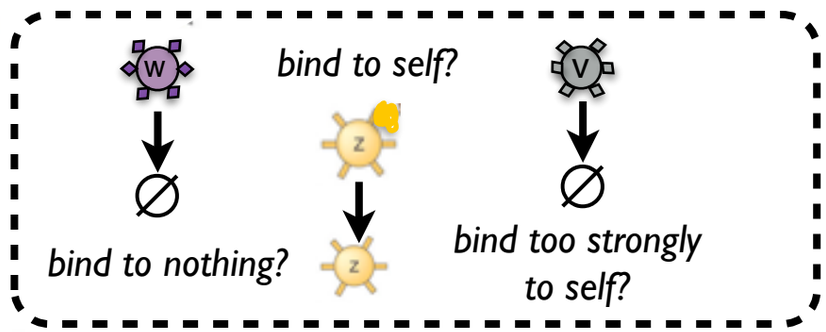


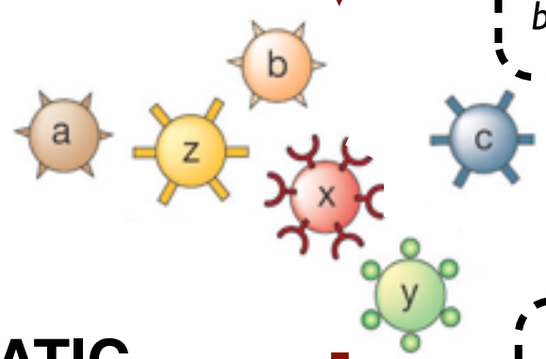
RECEPTOR GENERATION

VDJ recombination

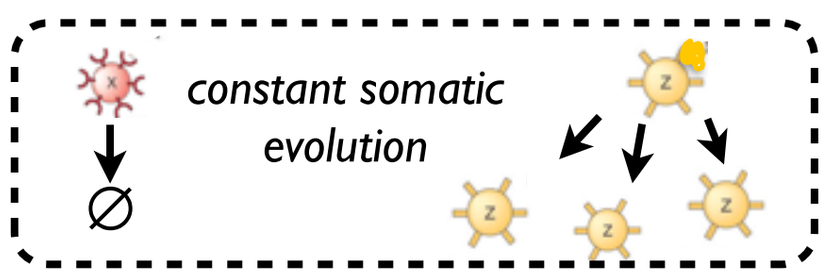


THYMIC SELECTION

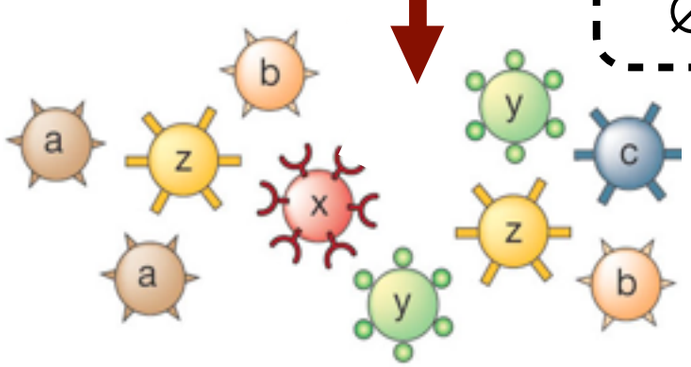




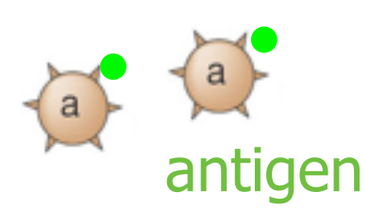
SOMATIC SELECTION



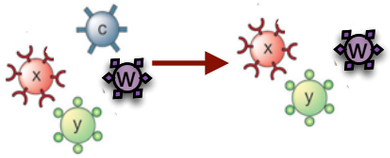
+ Somatic Hypermutations



SPECIFICITY



Repertoire evolution



RECEPTOR GENERATION

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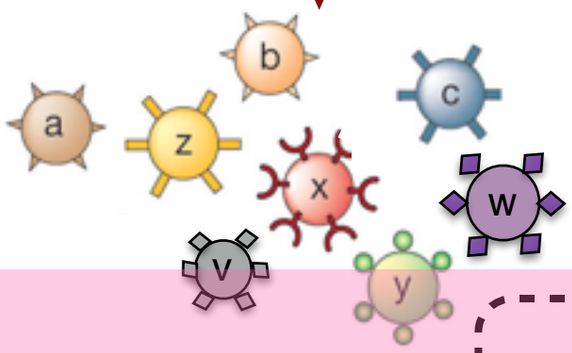
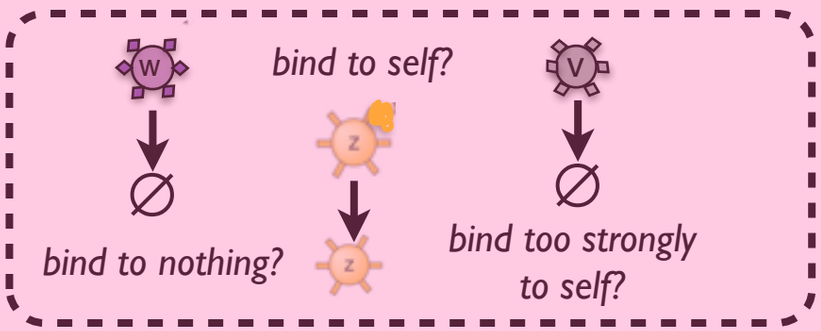
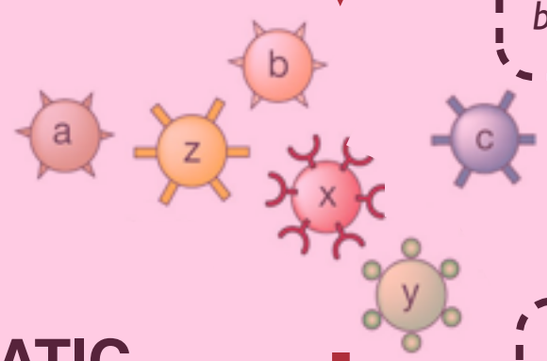
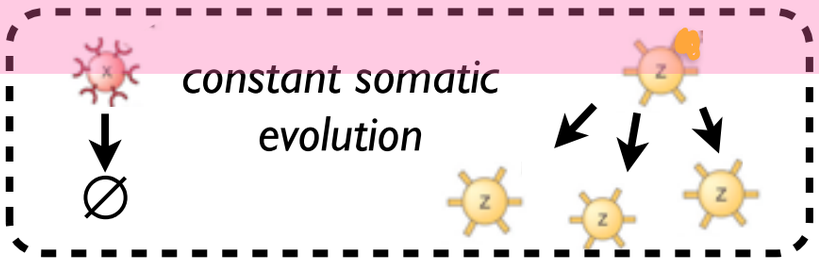


THYMIC SELECTION





SOMATIC SELECTION



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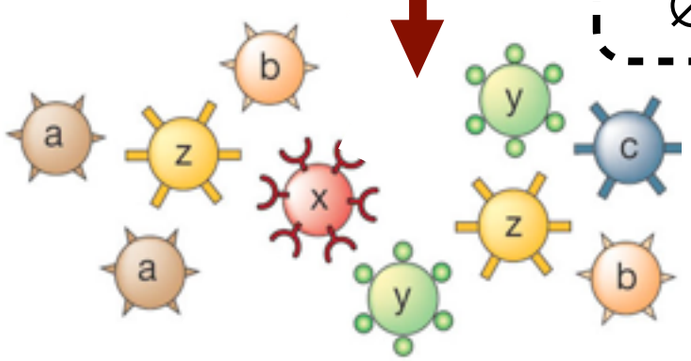
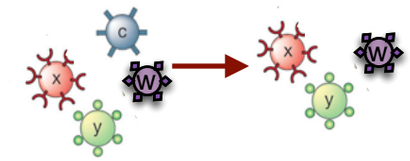
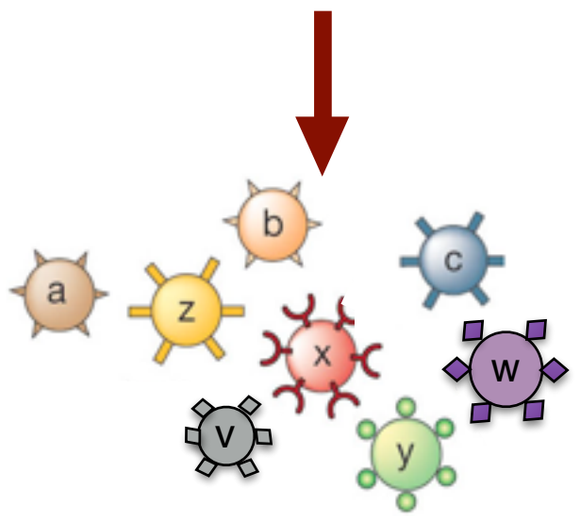


SPECIFICITY

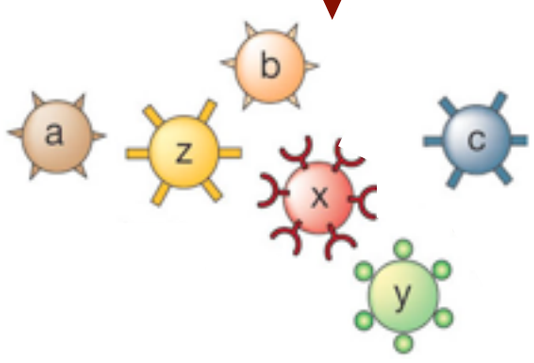
Selection

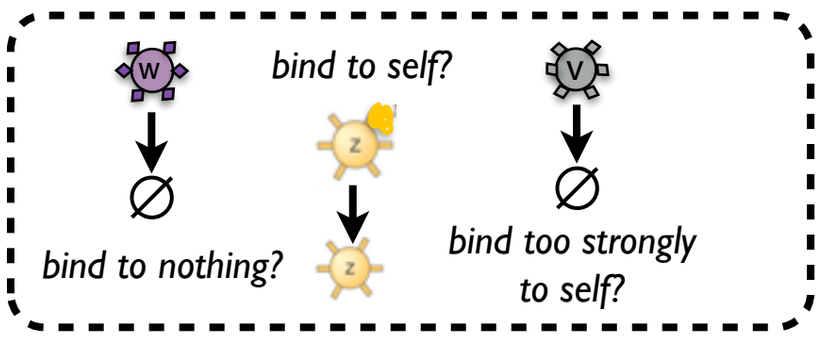


RECEPTOR GENERATION



THYMIC SELECTION

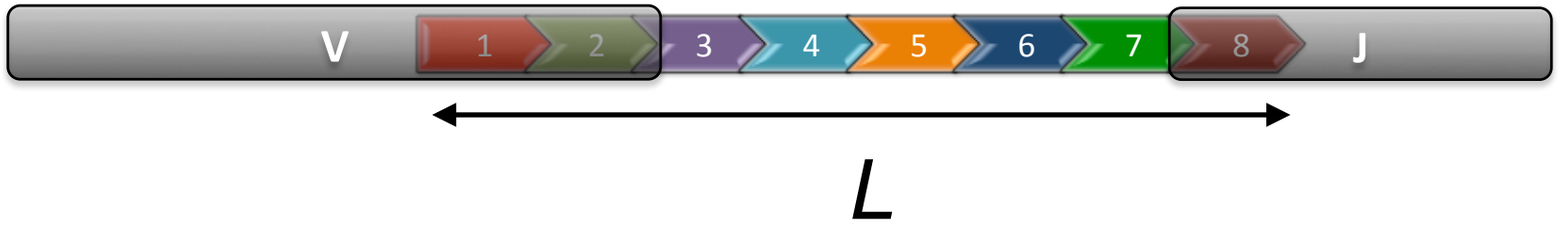




- quantify using selection factors

$$Q(\{\sigma\}) = \frac{P_{\text{post-sel}}(\{\sigma\})}{P_{\text{gen}}(\{\sigma\})}$$

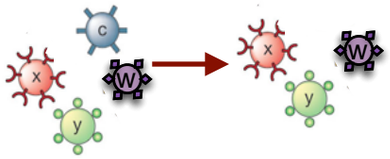
- a model for the observed probabilities

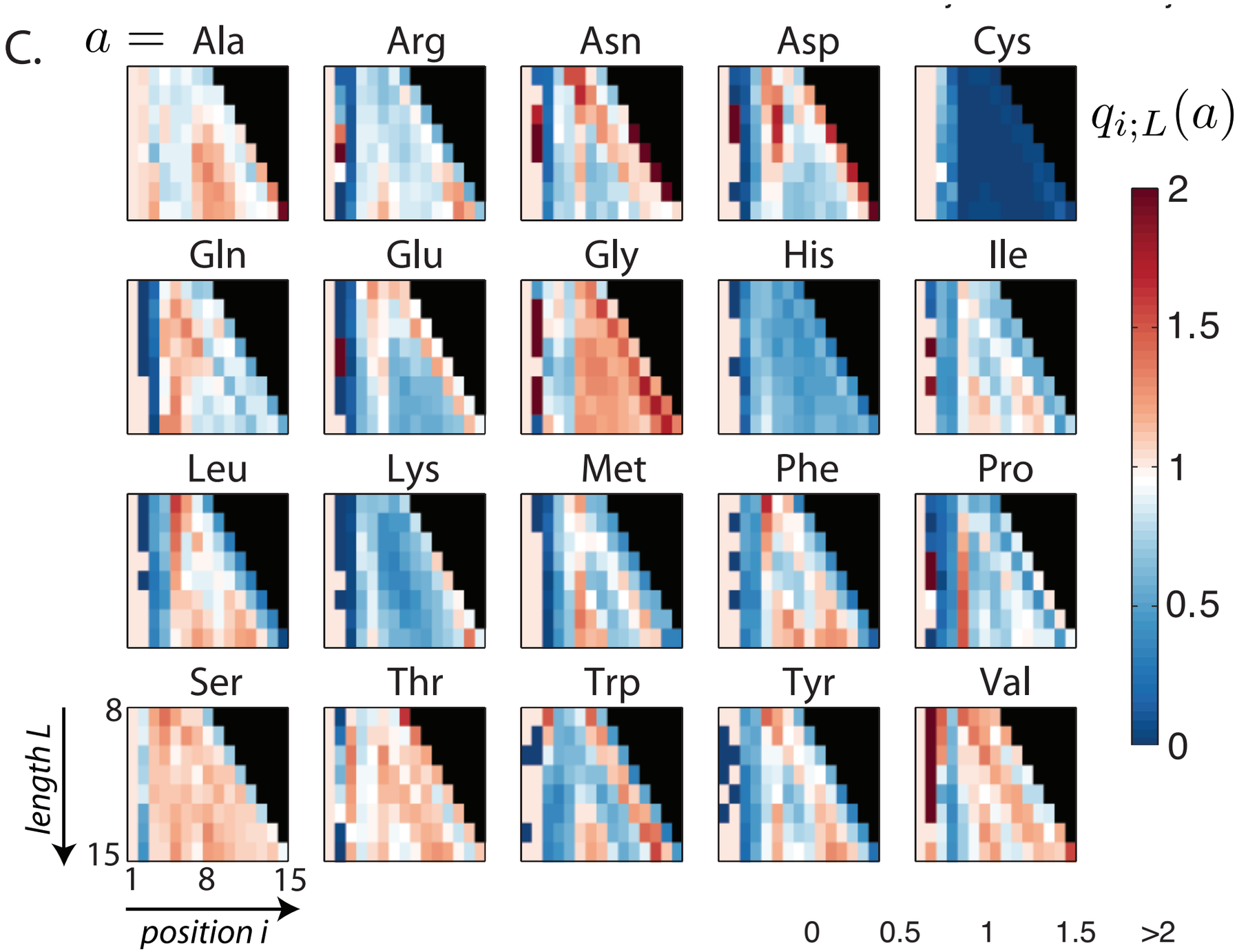


$$Q(\vec{\sigma}, V, J) = \frac{1}{Z}\, q_L\, q_{V,J|L} \prod_{i=1}^{L} q_{i;L}(a_i)$$

Elhanati Callan Mora Walczak PNAS (2014)

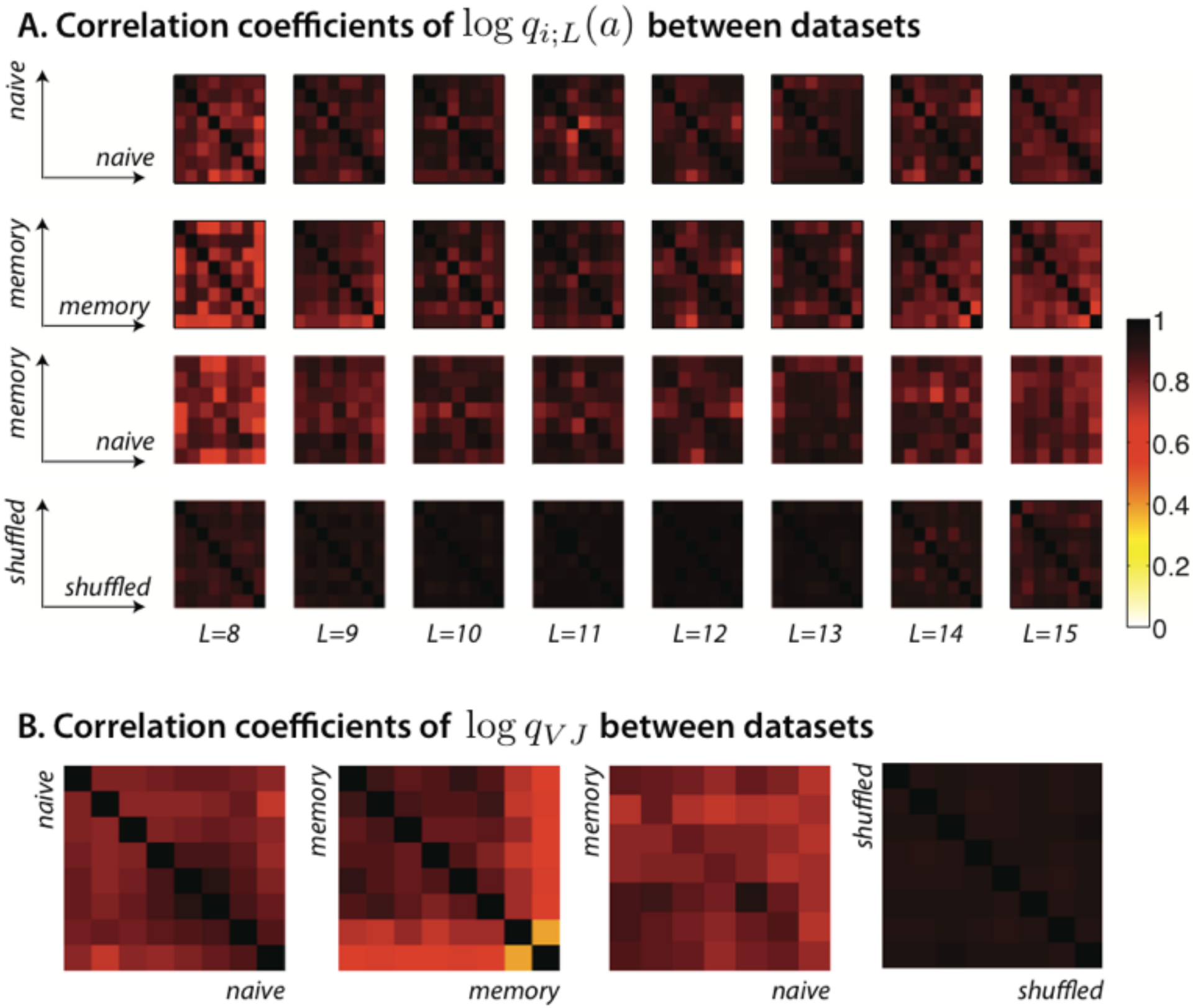
Selection



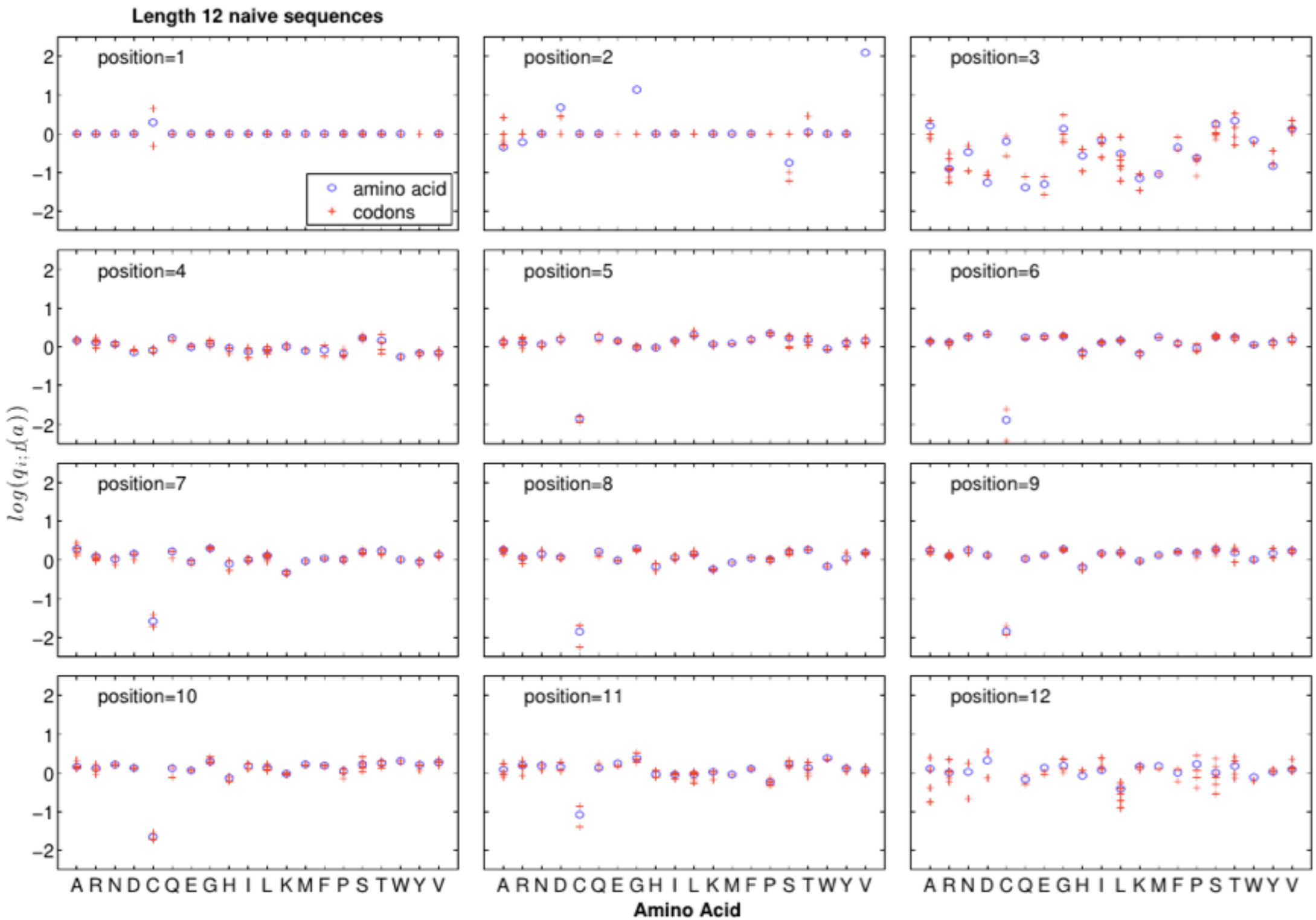


Elhanati Callan Mora Walczak PNAS (2014)

Correlations between individuals

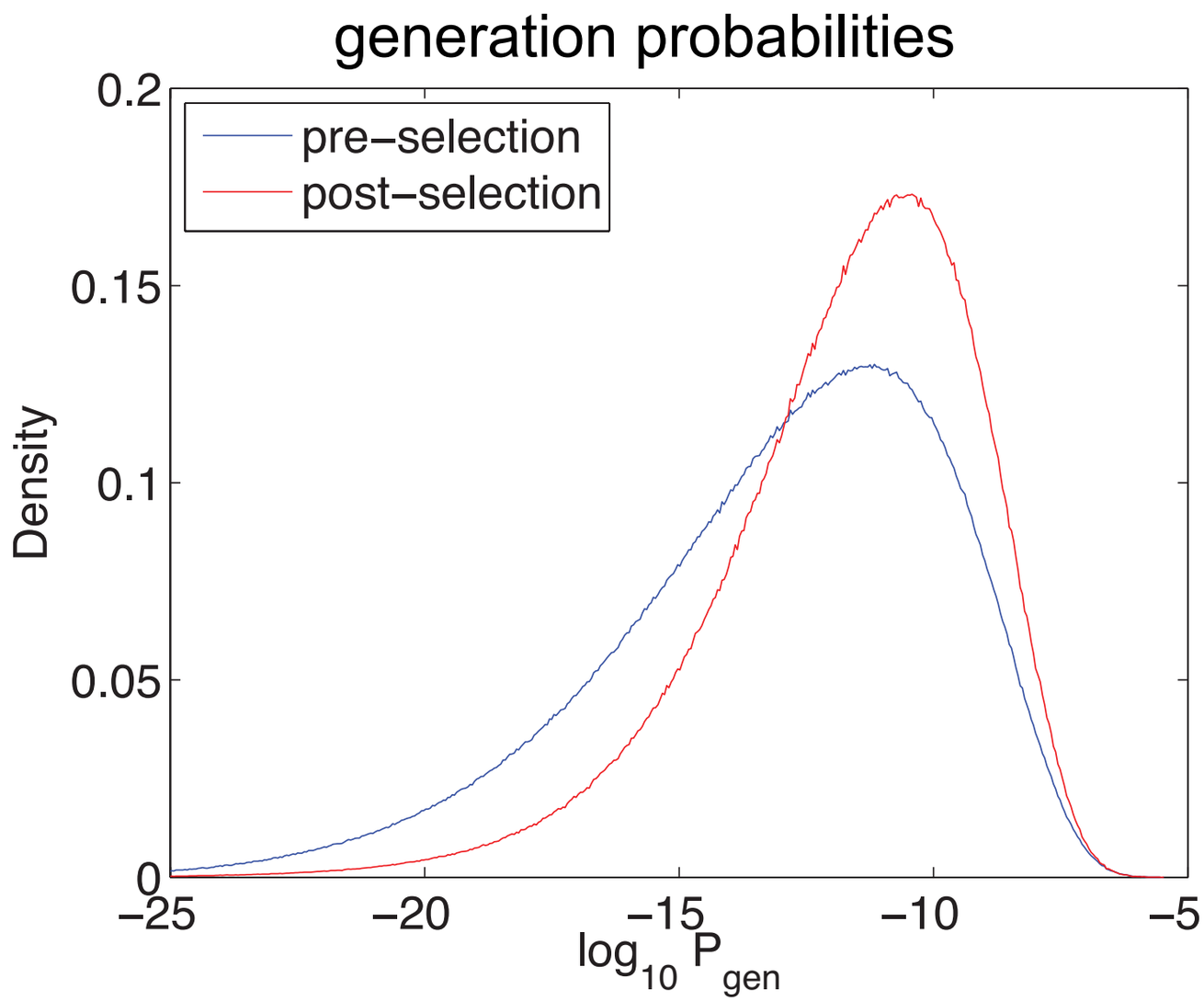


Selection only depends on aa, not codon



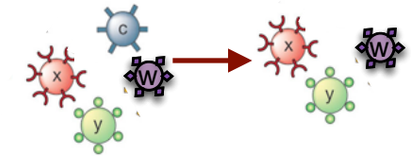
Natural selection anticipates somatic selection

- sequences more likely to be generated → more likely to be selected

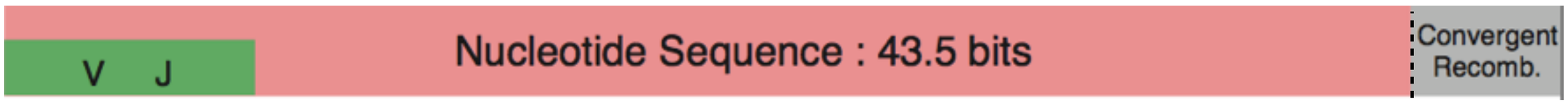


- true for all individuals independently

Entropy, again

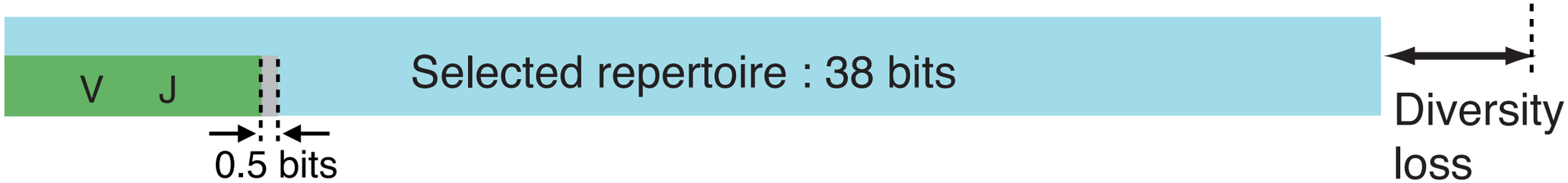


- entropy of generated repertoire



$\Longrightarrow$ repertoire size 10^{13} sequences

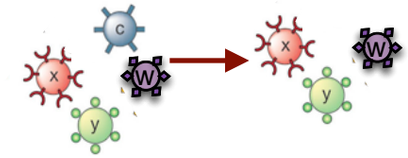
- entropy of post-thymic selection repertoire



$\Longrightarrow$ repertoire size 10^{11} sequences

$\longrightarrow$ thymic selection gives 50-fold reduction in diversity

Entropy, again



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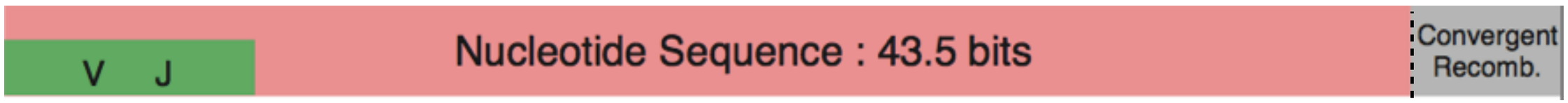

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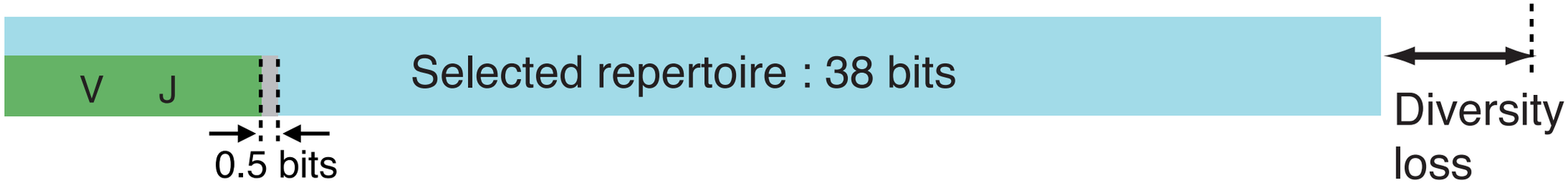


$\Longrightarrow$ repertoire size 10^{11} sequences

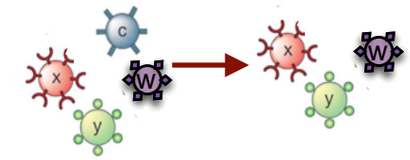
$\longrightarrow$ thymic selection gives 50-fold reduction in diversity

- thymic selection keeps only 2% of diversity

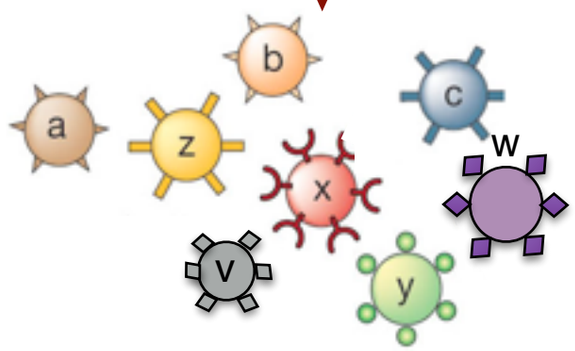
$\longrightarrow$ thymic selection gets rid of rare clones

selection favours clones that are likely to be generated

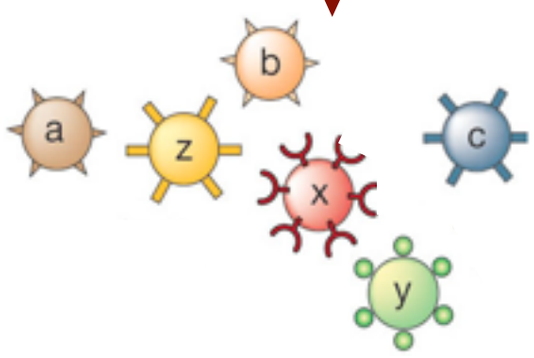
Receptor sharing

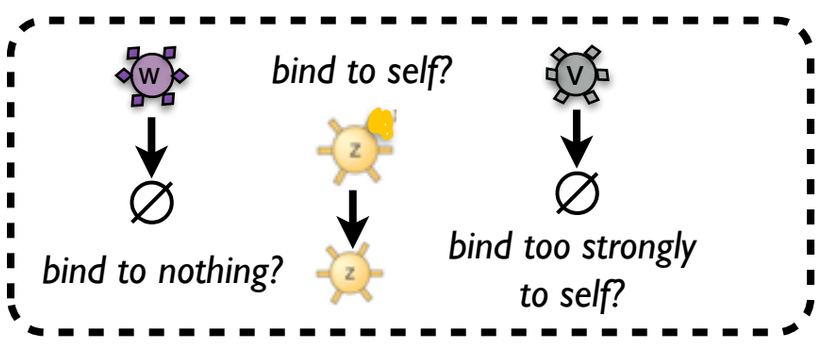


RECEPTOR GENERATION

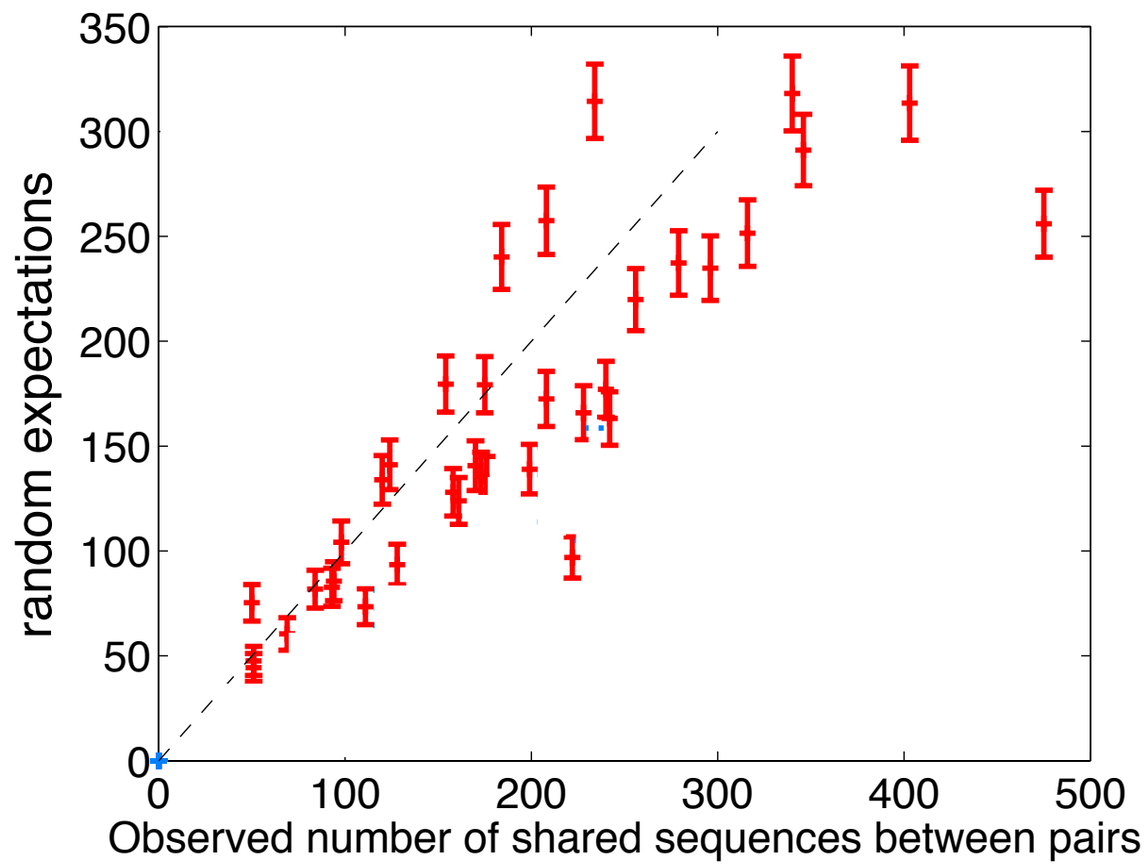


THYMIC SELECTION



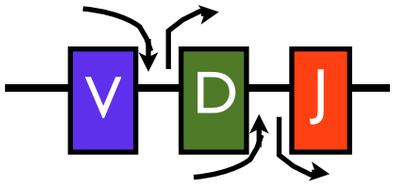


how many shared receptors between 2 people?

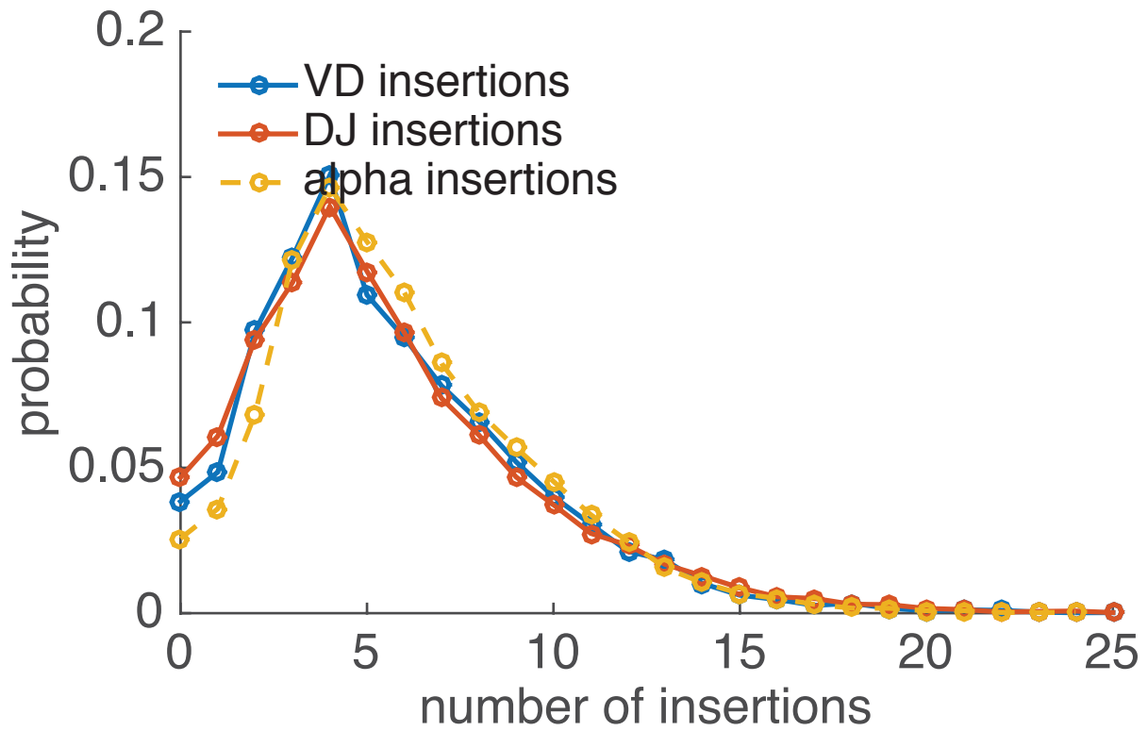


close to random expectations

Other datasets: alpha chains

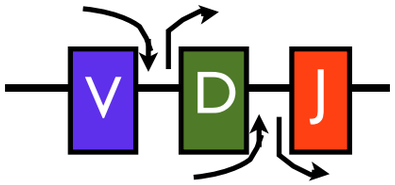


- we can do the same for the alpha chain
- similar insertion profile as beta chain



Elhanati Marcou Mora Walczak arXiv 2015

Other datasets: alpha chains



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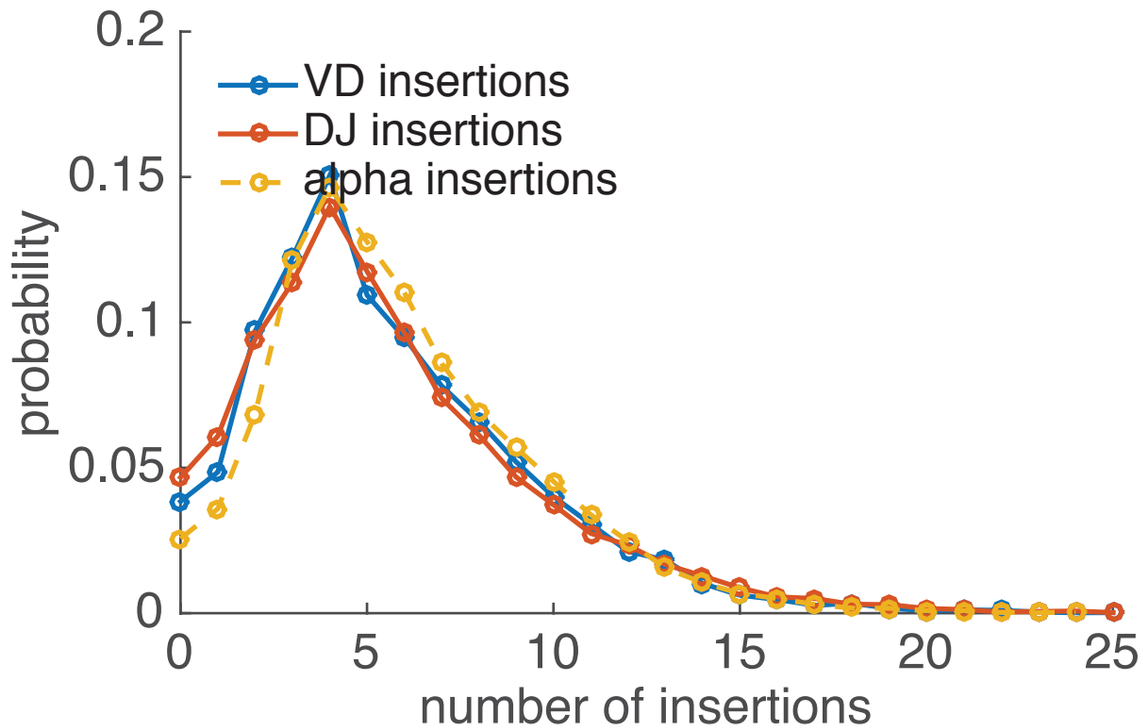
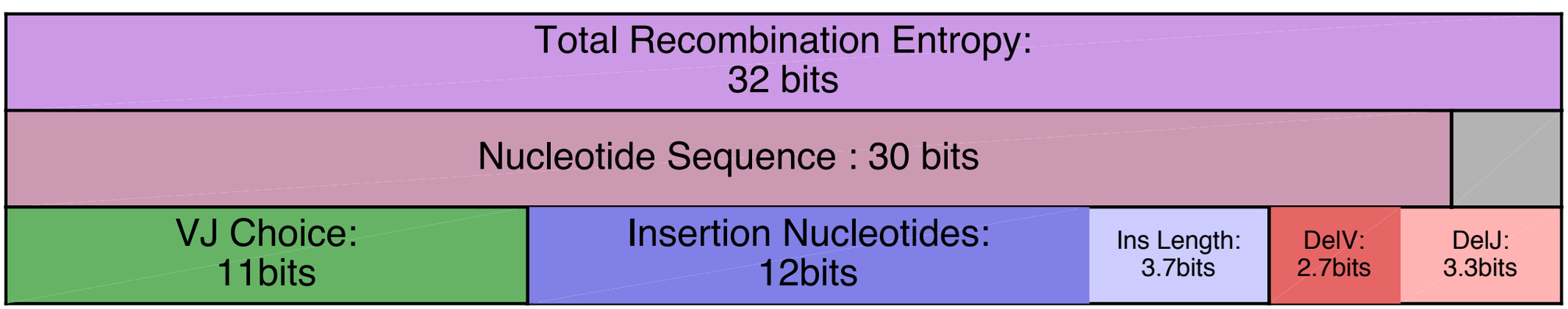
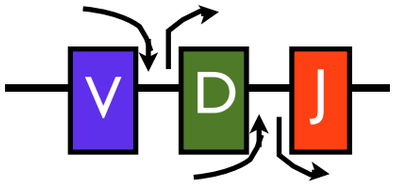


- entropy: 30 (alpha) + 47 (beta) = 77 bits ~ $N = 10^{23}$

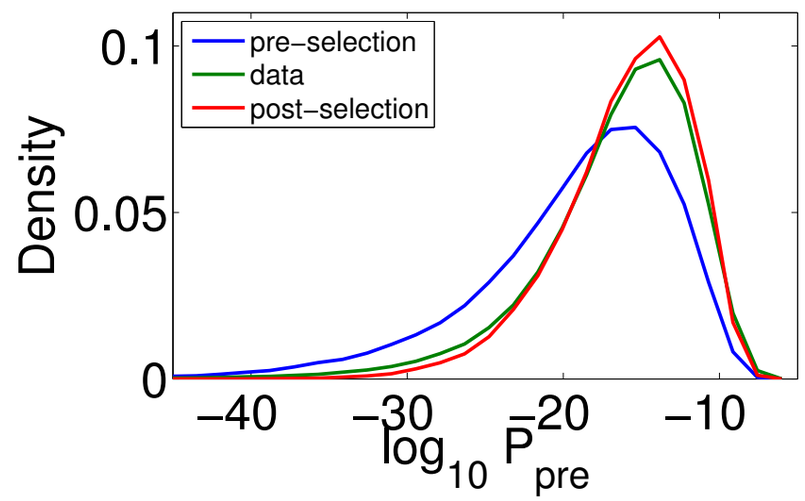


Elhanati Marcou Mora Walczak arXiv 2015

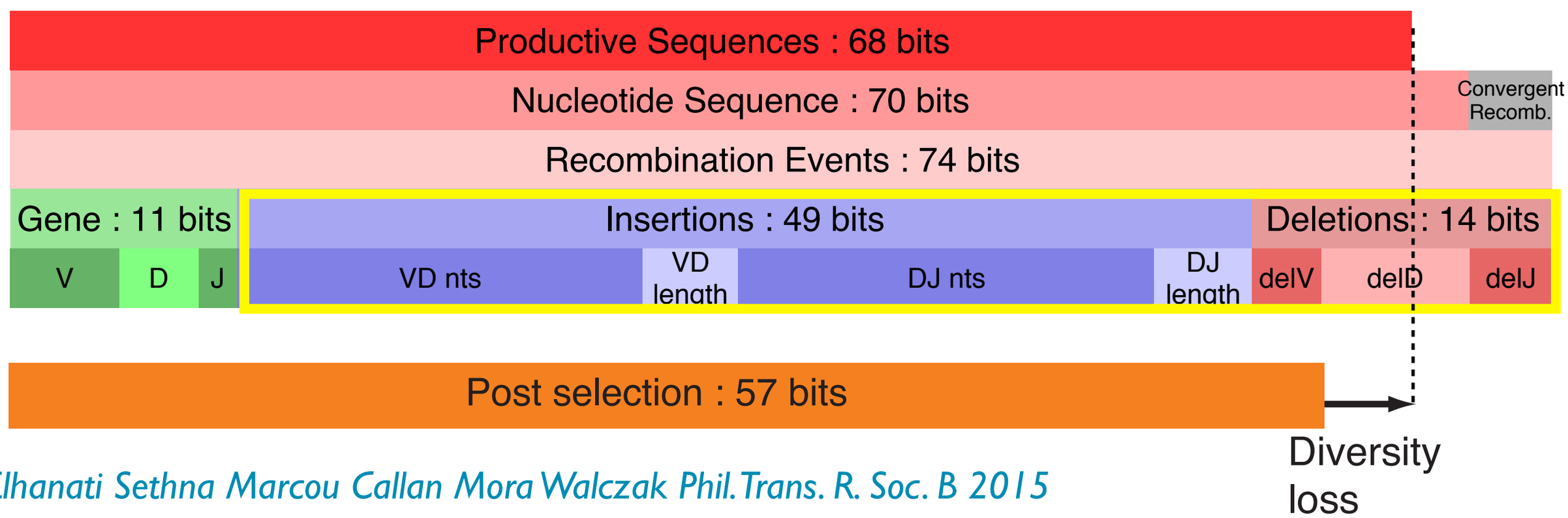
Other datasets: BCR



- we can do the same for B cell receptors: heavy chain
- analyse out-of-frame sequences from naive and memory B cells

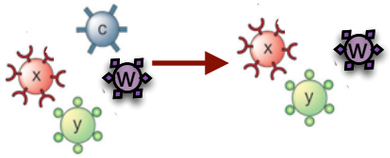


- entropy



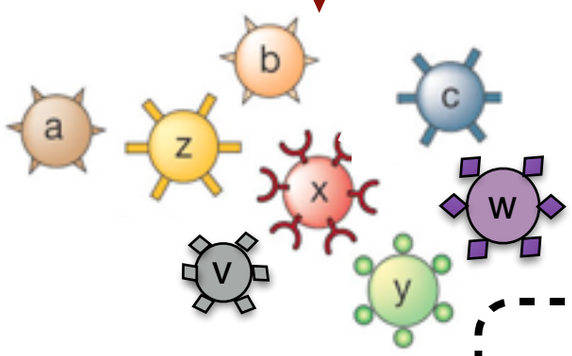
Elhanati Sethna Marcou Callan Mora Walczak Phil. Trans. R. Soc. B 2015

Repertoire evolution

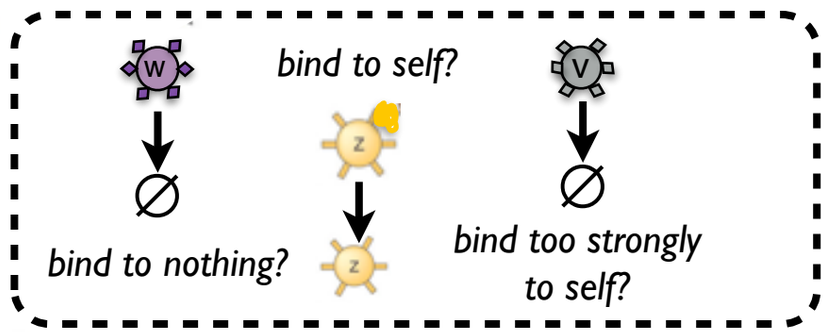


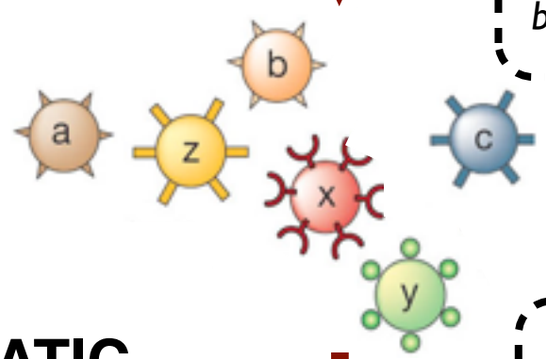
RECEPTOR GENERATION

VDJ recombination

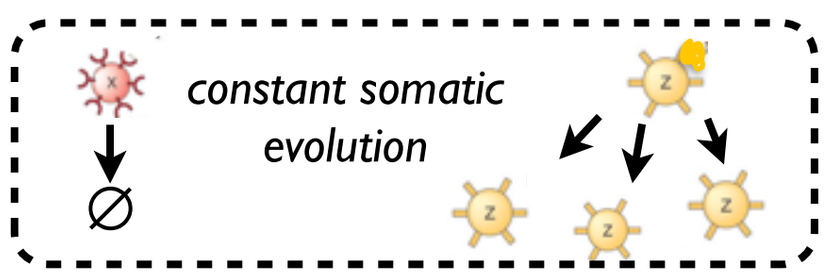


THYMIC SELECTION

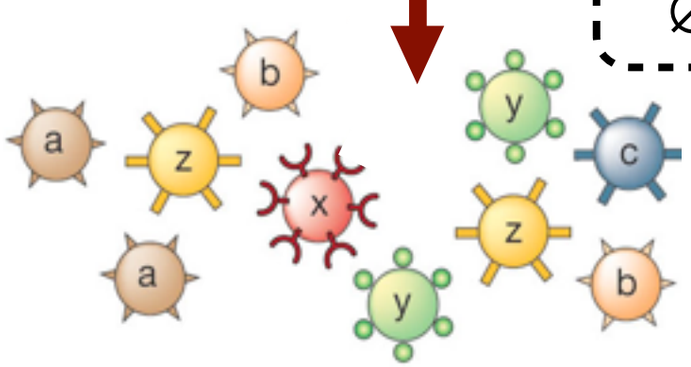




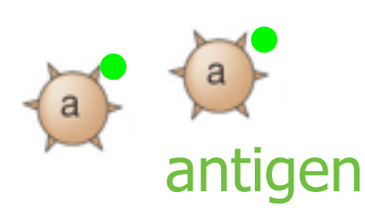
SOMATIC SELECTION



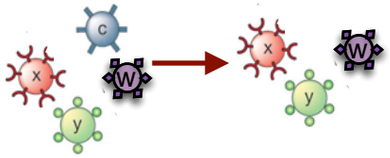
+ Somatic Hypermutations



SPECIFICITY

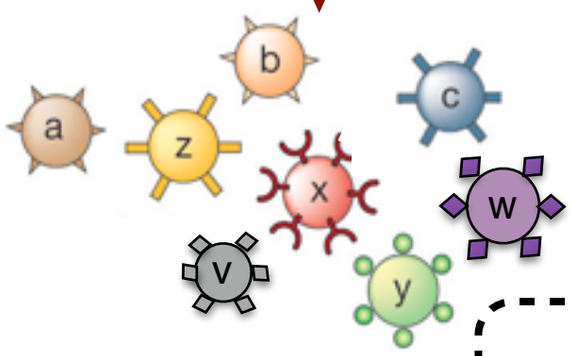


Repertoire evolution

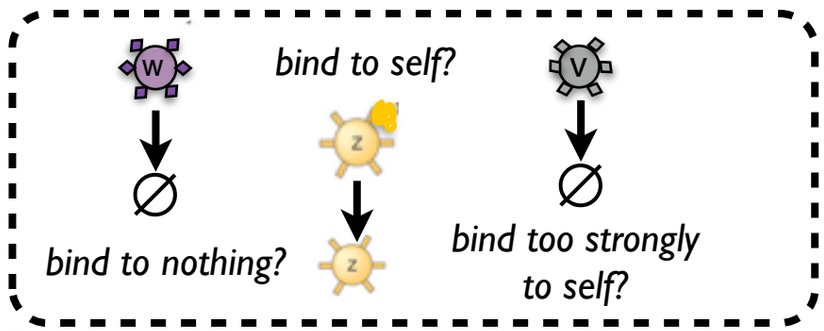


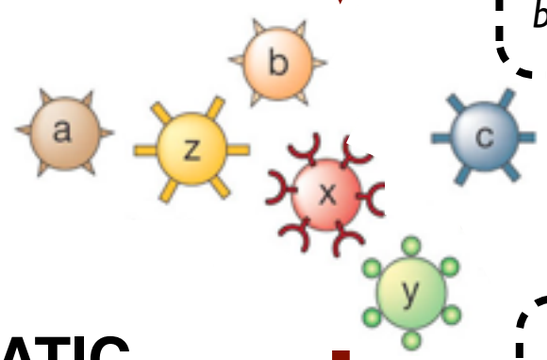
RECEPTOR GENERATION

VDJ recombination

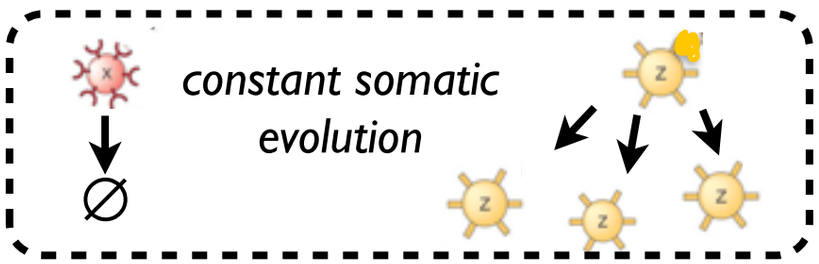


THYMIC SELECTION

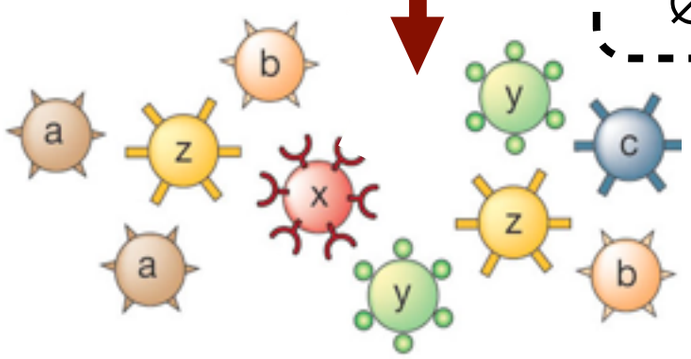




SOMATIC SELECTION

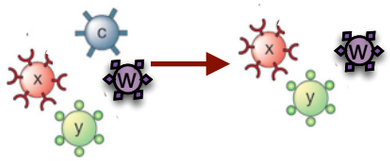


+ Somatic Hypermutations



SPECIFICITY

Somatic hypermutations

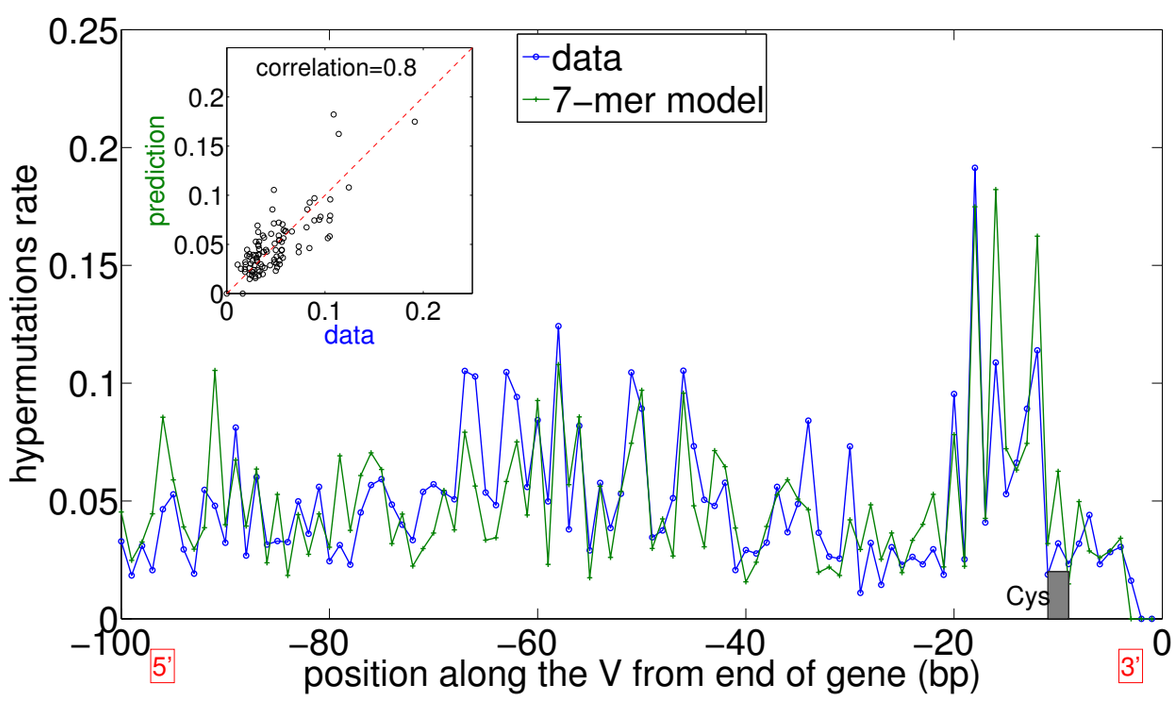


- use out-of-frame sequences from memory B cells
- position-weight matrix model hypermutation hotspots

7-mer

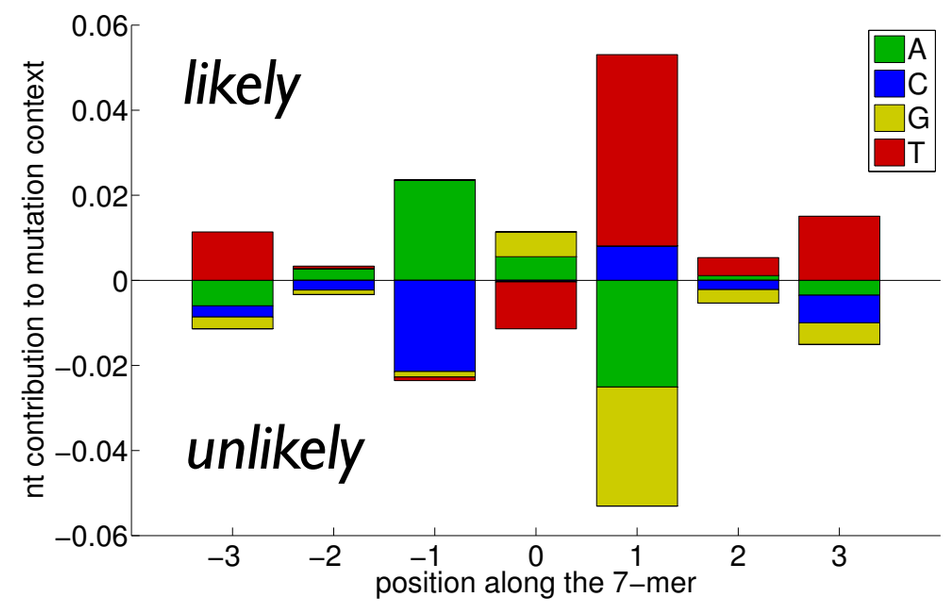
$$p_{\mathrm{SHM}}(\sigma) \propto p_{\mathrm{bg}}(\sigma) \exp[\sum_{i=-3,3} e_i(\sigma_i)]$$

background probability
of that 7-mer



- nt that are likely to hypermutate

hypermutation hotspot 7-mer signature



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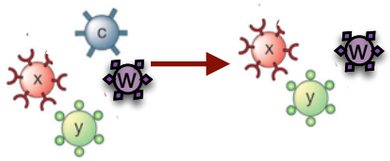
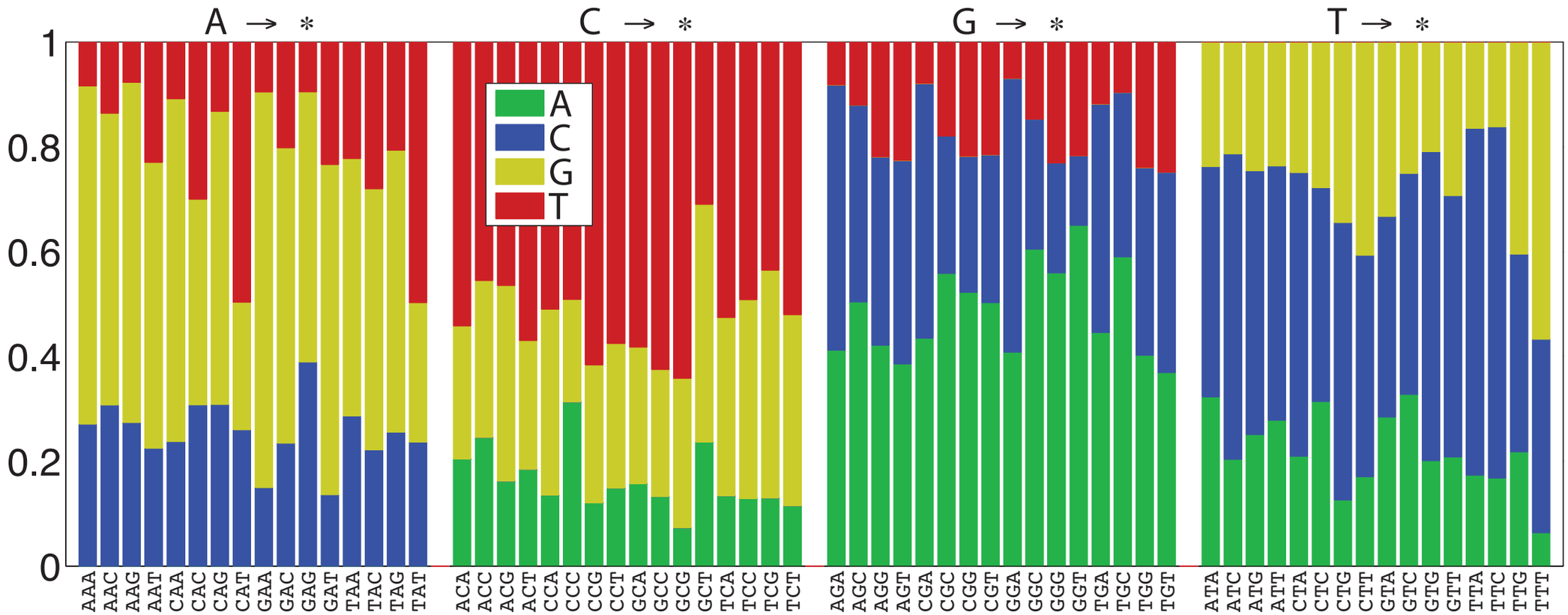
Somatic hypermutations

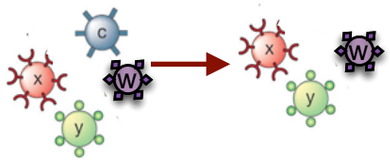


- nt *to which* a nt mutates

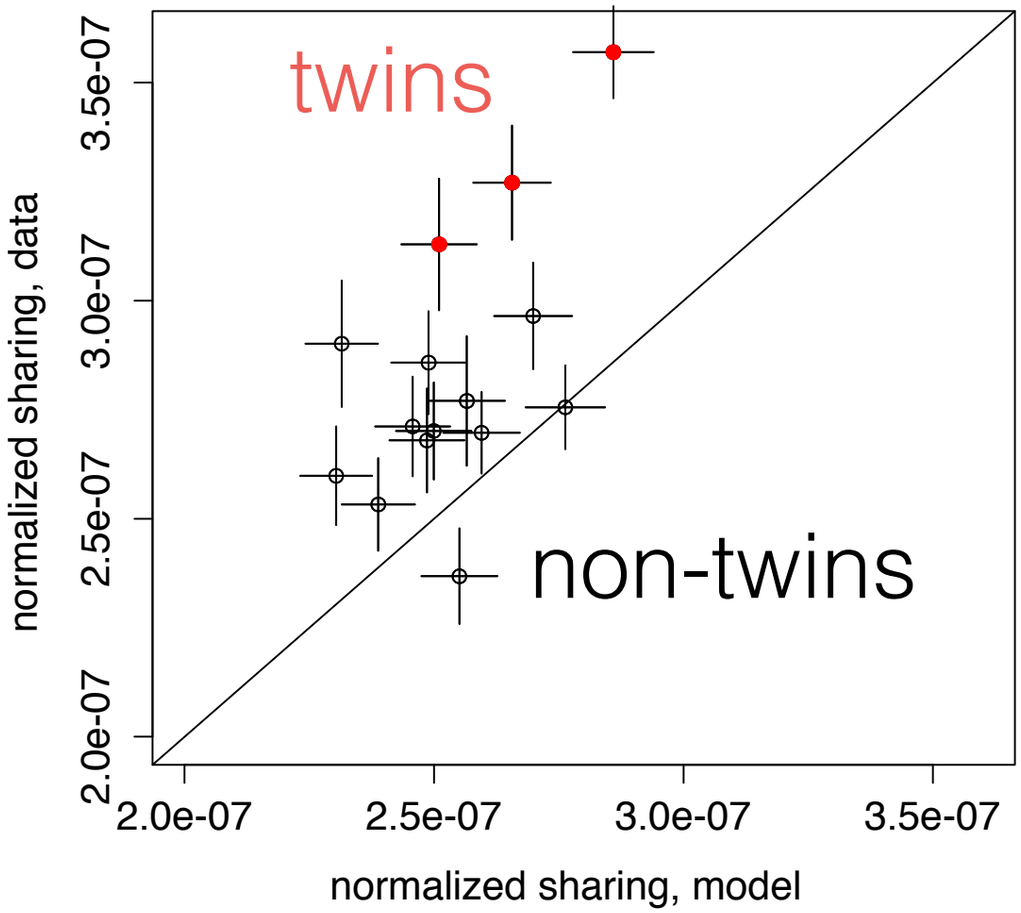


Thank you

Is everyone the same?



at the level of generation



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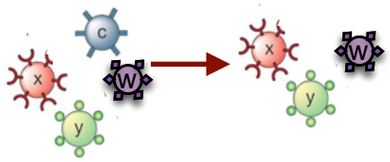
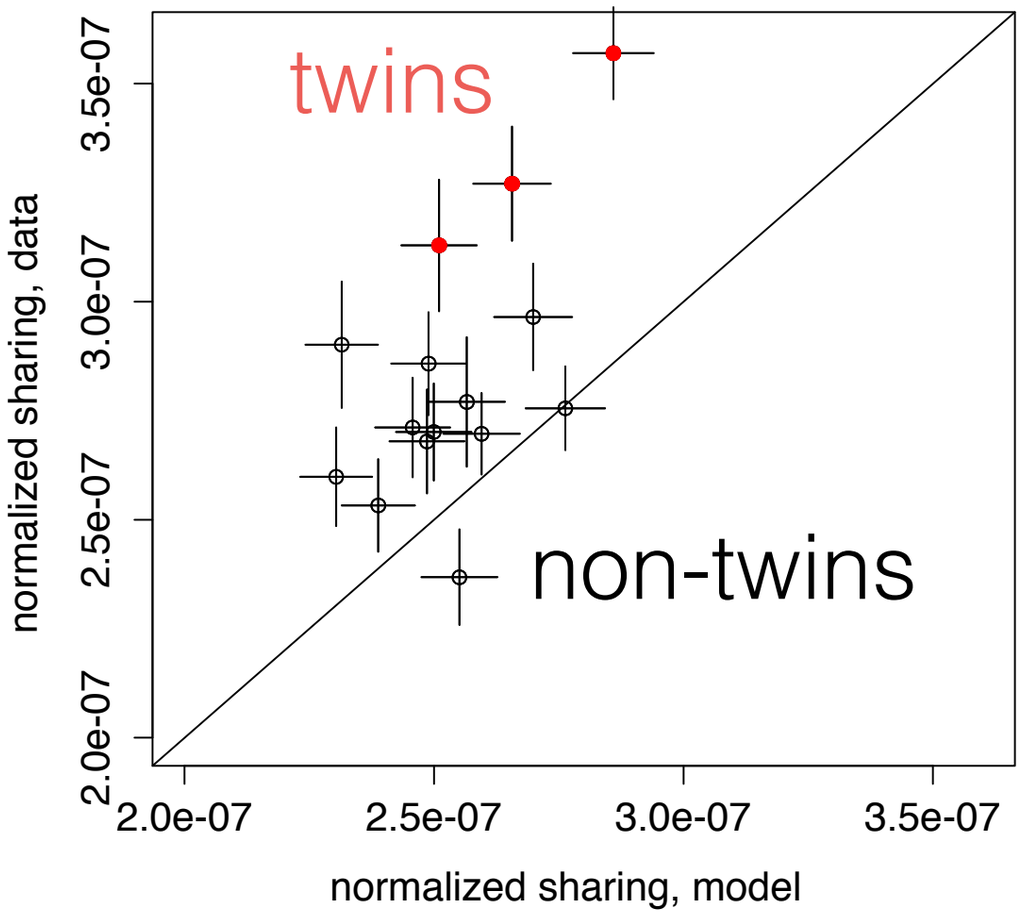
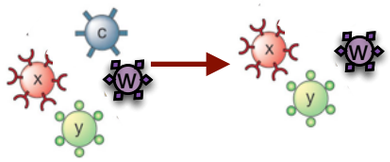


at the level of generation → twins are special



Is everyone the same?



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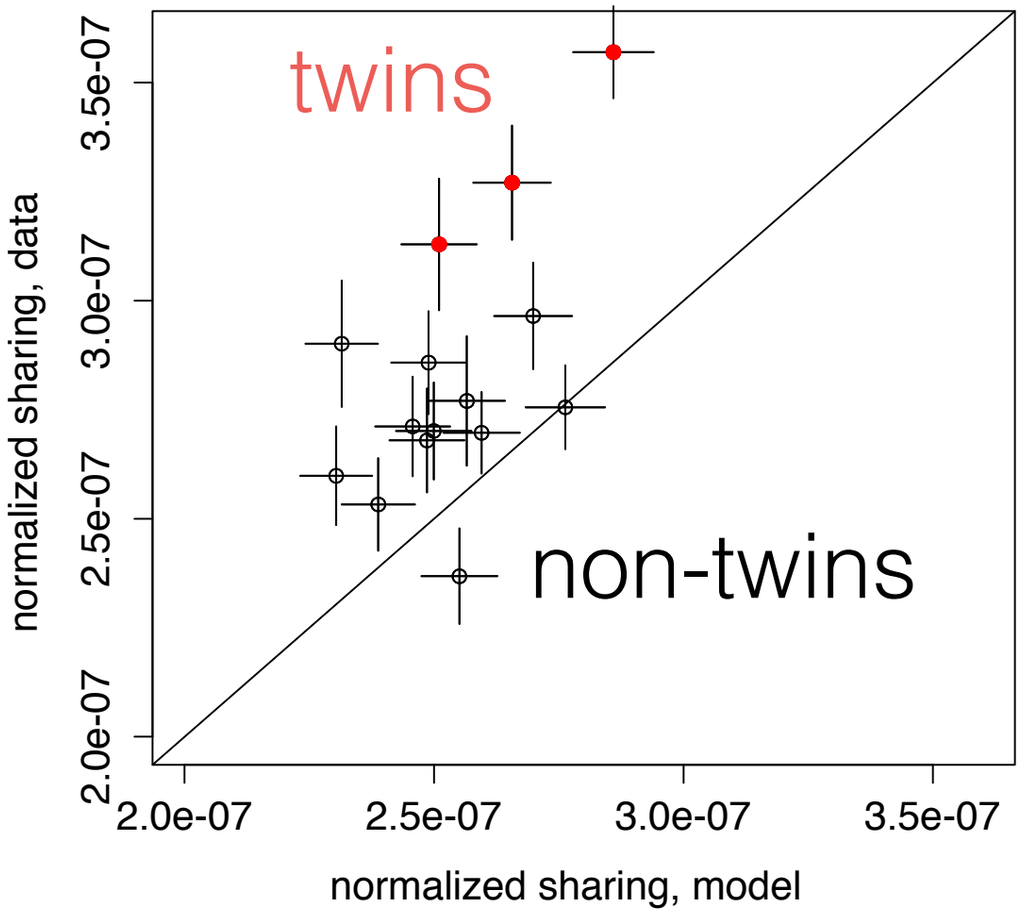
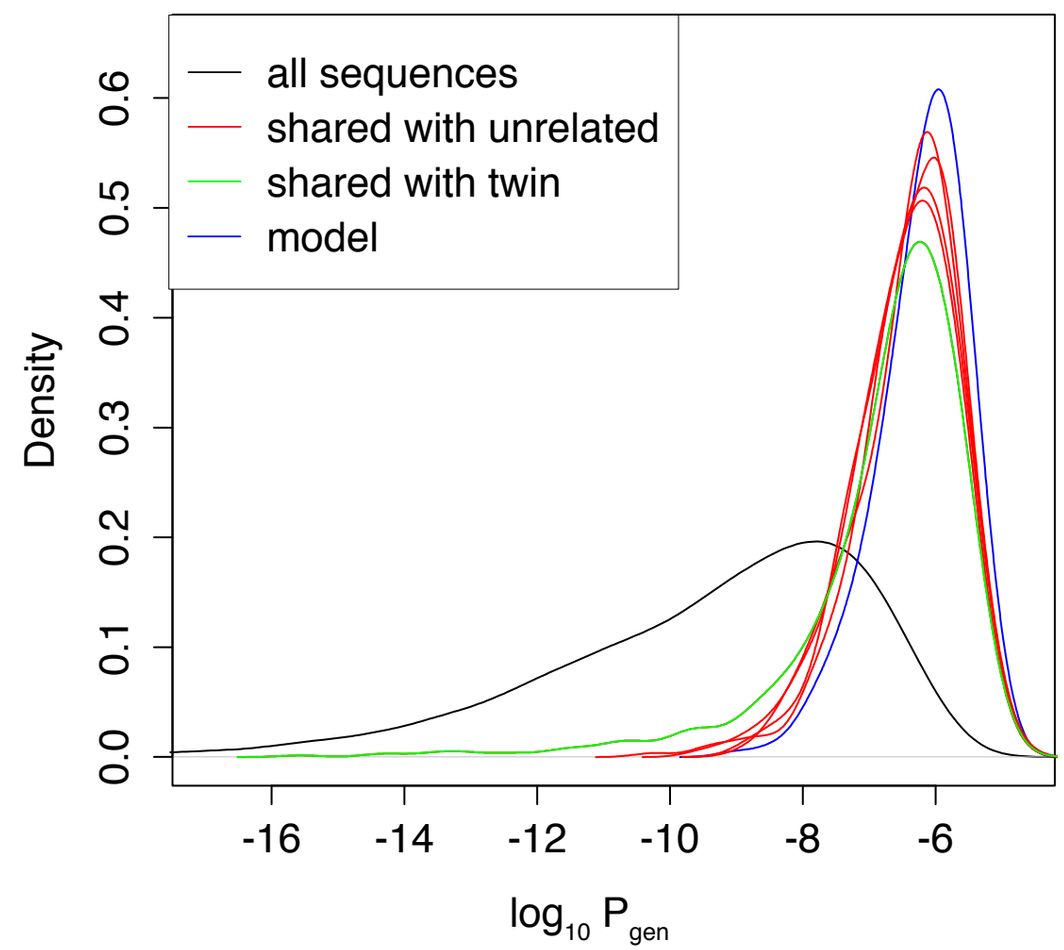
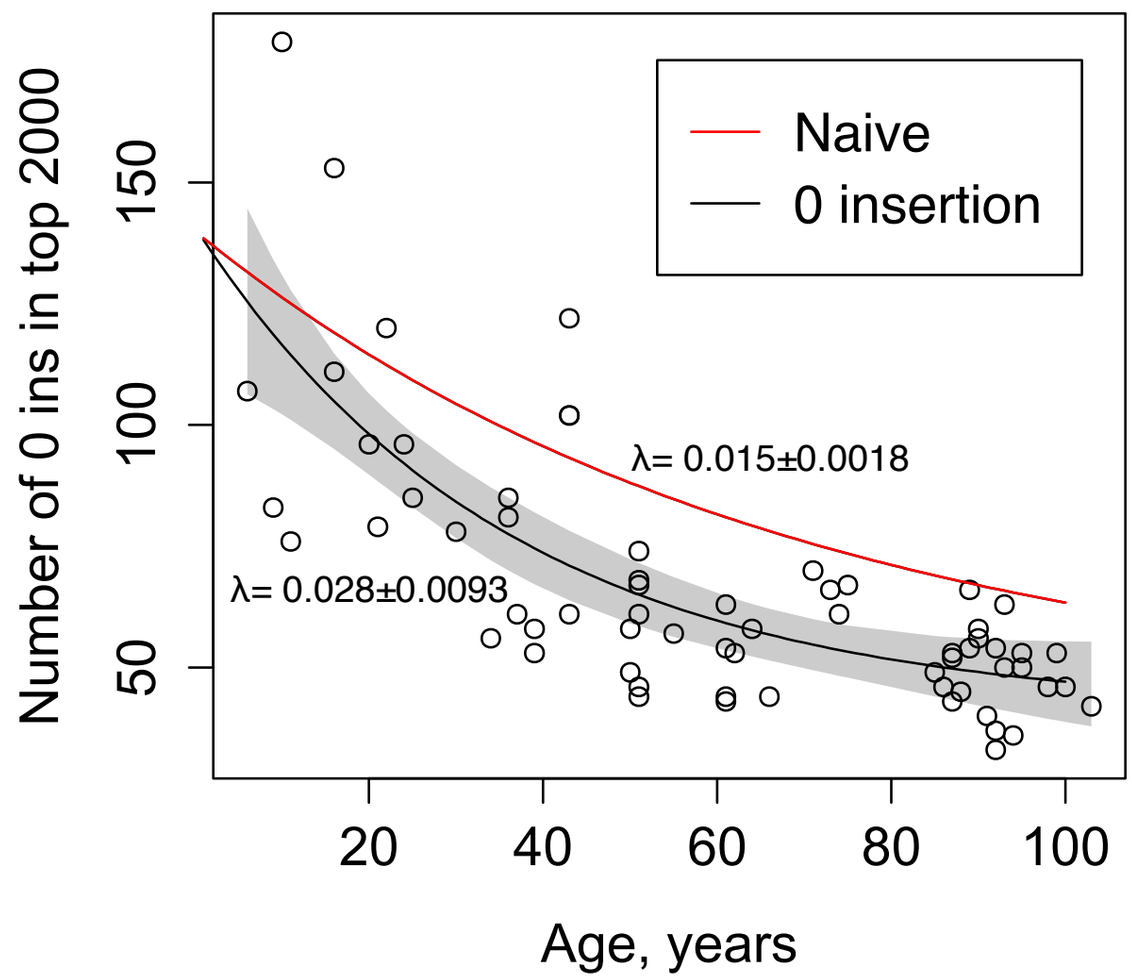


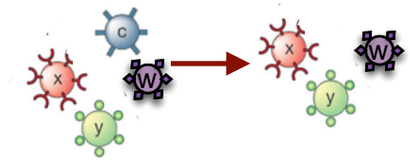


The source: long lived sequences

- last time twins shared blood: before birth
- insertions enzyme less active before birth



The source: long lived sequences



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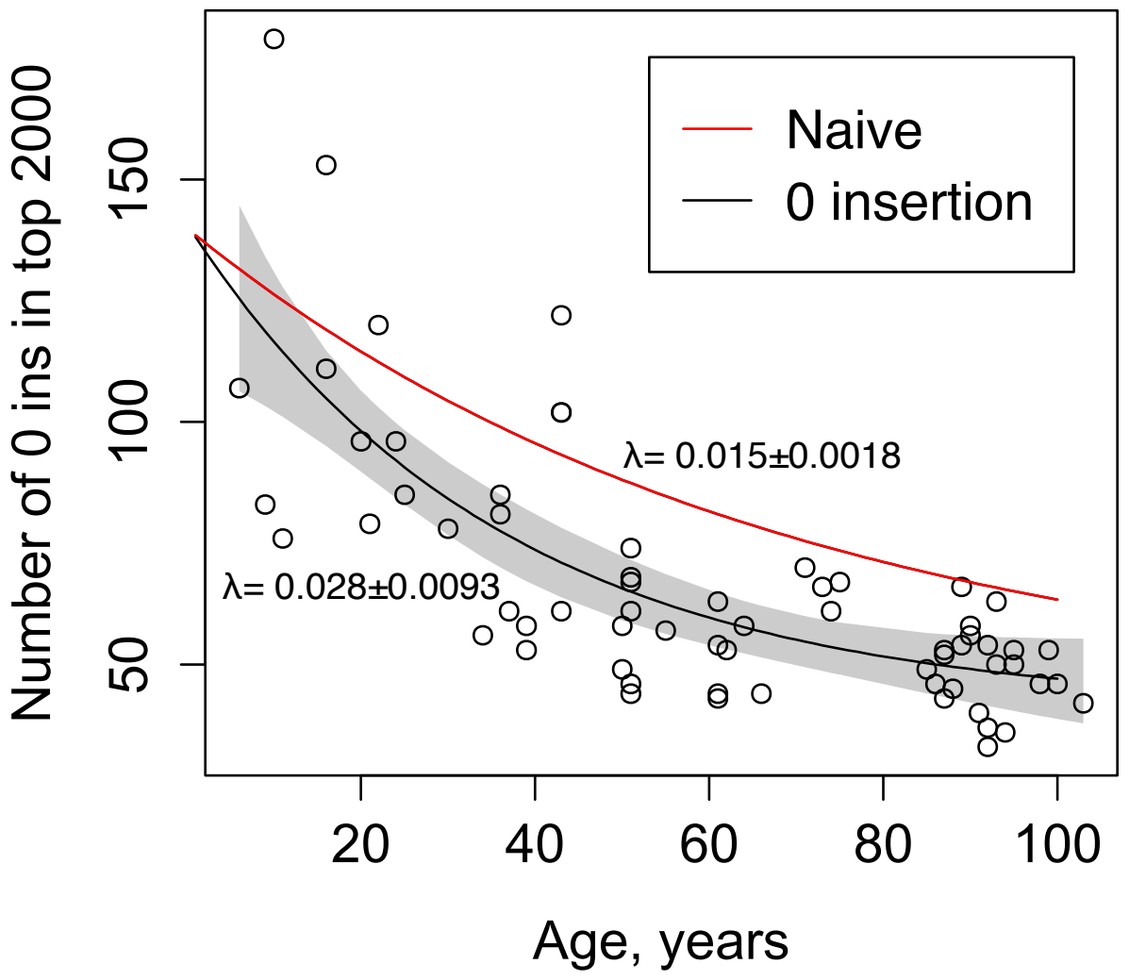
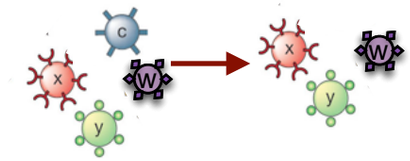


clone lifetime ~ 36 years

The source: long lived sequences



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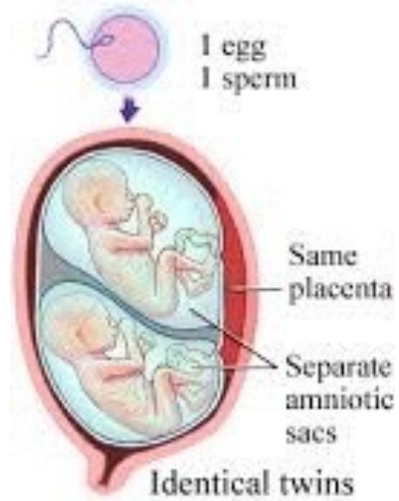

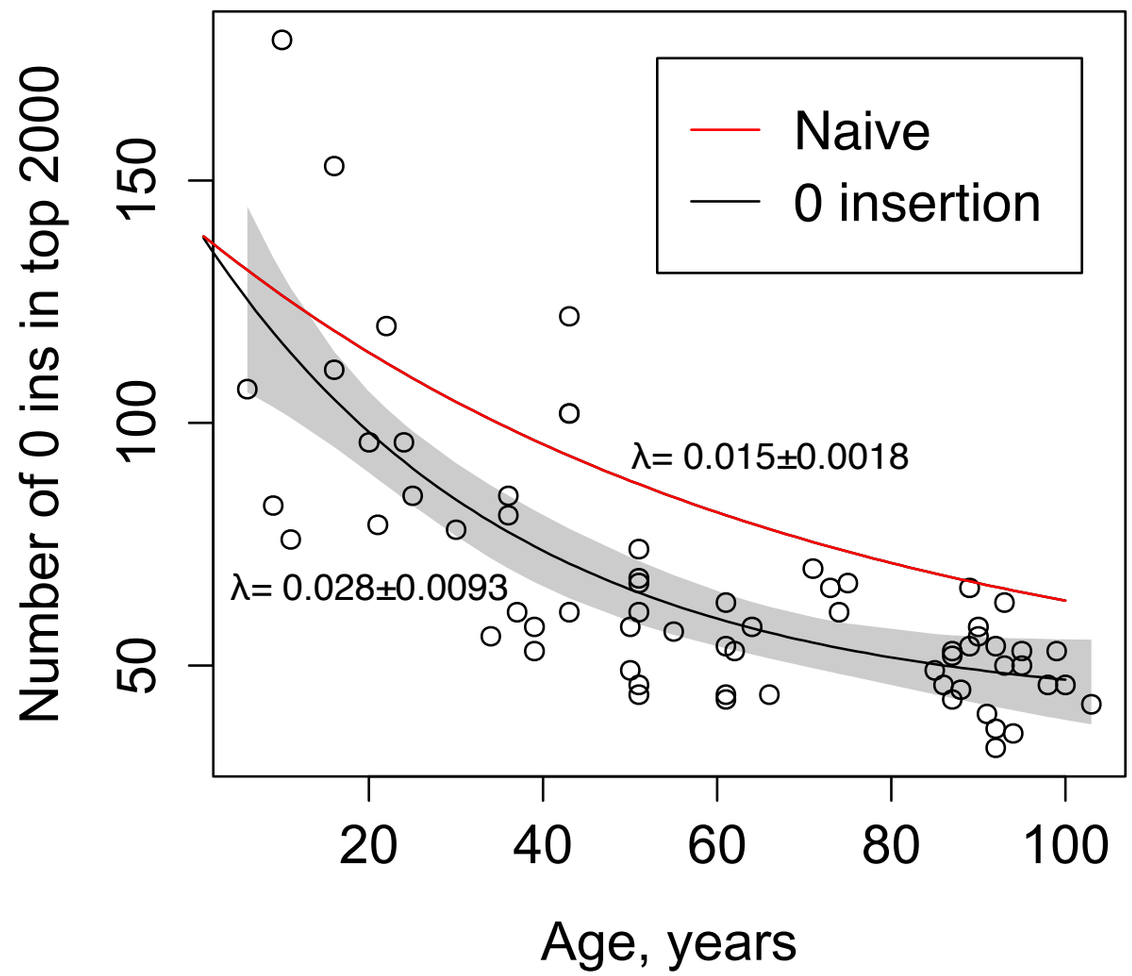

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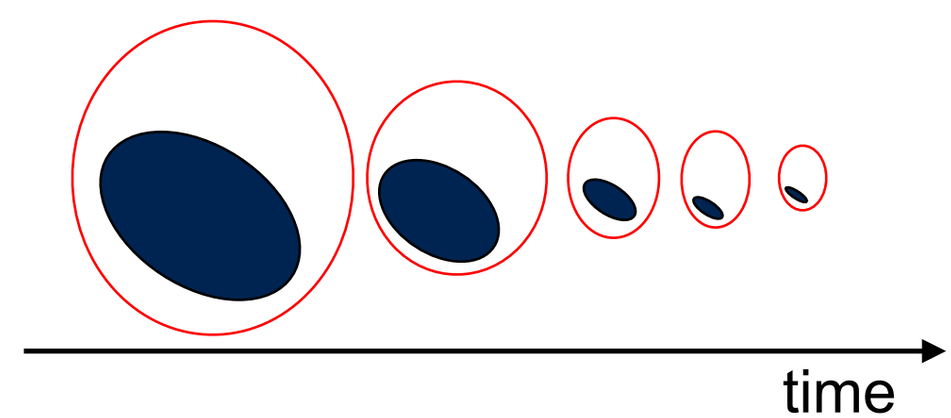

Decay of zero insertion clonotypes:

- zero insertion clonotypes within the naive pool
- size of the total naive pool