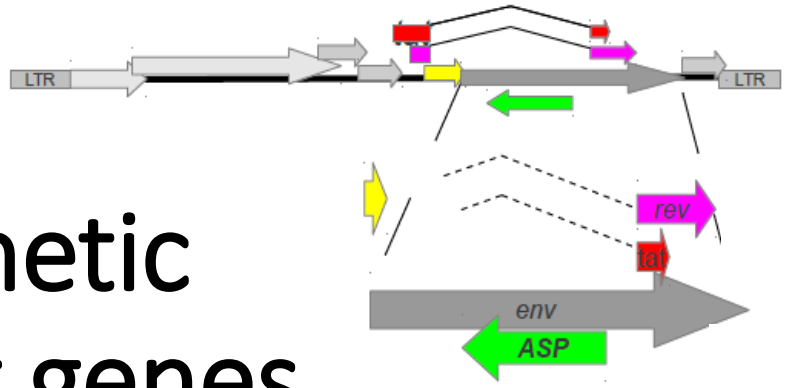

Deciphering the genetic code of overlapping genes

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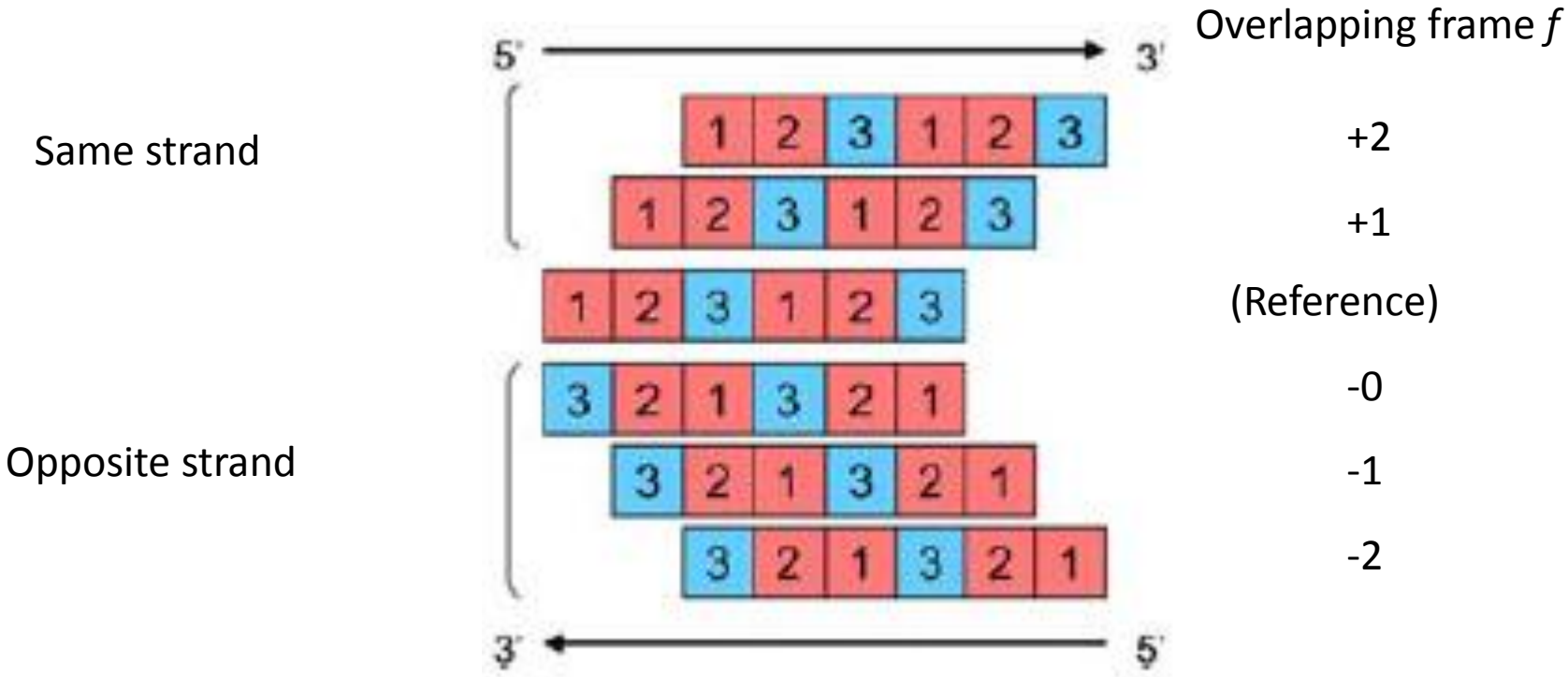
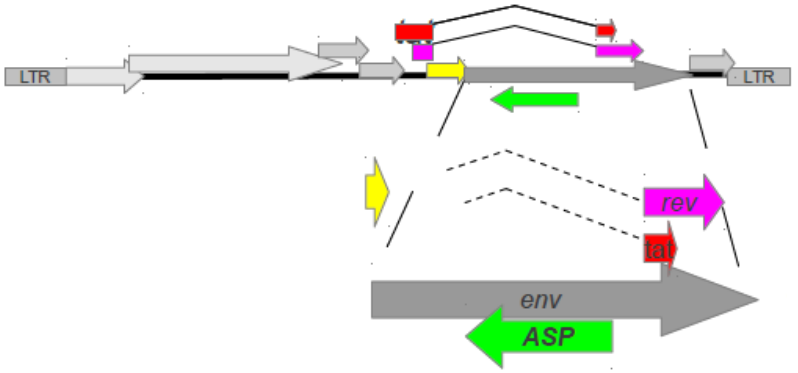
Overlapping genes



- 1977: a single DNA sequence may code for several overlapping genes
- Genetic code: DNA bases {a,c,g,t} amino ➔ 1 amino acid
- One sequence
  - different reading frame
  - same or opposite strain

5 frames

Overlapping genes



- First (70s)
  - non-viral species
  - multiple functions : regulation, translational coupling, genome imprinting…
- Recently
  - Number of overlapping genes could be greater than expected
  - Especially in the virus world

    HIV : 3 overlapping genes (env, tat, rev)

    HTLV-1 (Human T-cell leukemia virus): HBZ gene
  - Favored therapeutic targets

    ➔ ***Highly conserved*** DNA sequences are subject to strong evolutionary constraints

    ➔ ***Prevent*** the ***rapid adaptation*** of viruses

    and fast appearance of ***resistance mutations***.

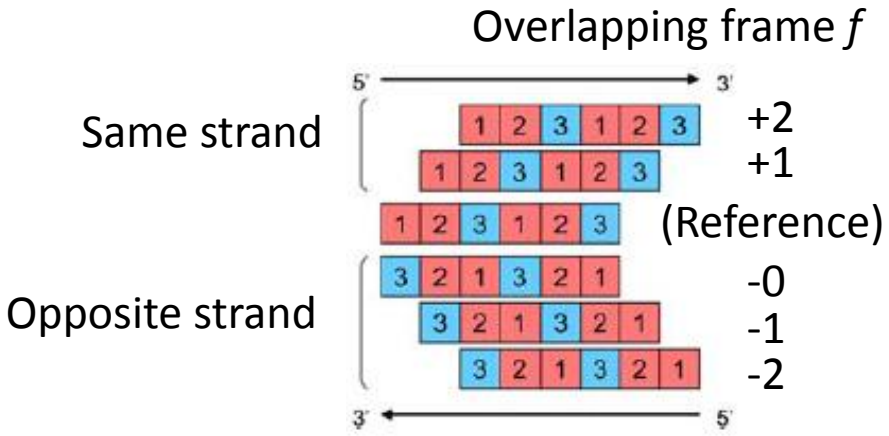
Degrees of freedom ?

- Mathematical results in the 1980s
  - Sander and Schultz (1979)
  - Siegel and Fitch ((1980)
  - Smith and Waterman (1980) : conditional information
    - ➔ Frame dependent
    - ➔ Frame *f* = -2 : « very rare in nature »
- But: HBZ (HTLV), ASP (HIV) overlap in *f* = -2
- Still questions…

➔amino-acid (n-peptide) composition
➔detecting selection pressure
➔searching for overlapping genes

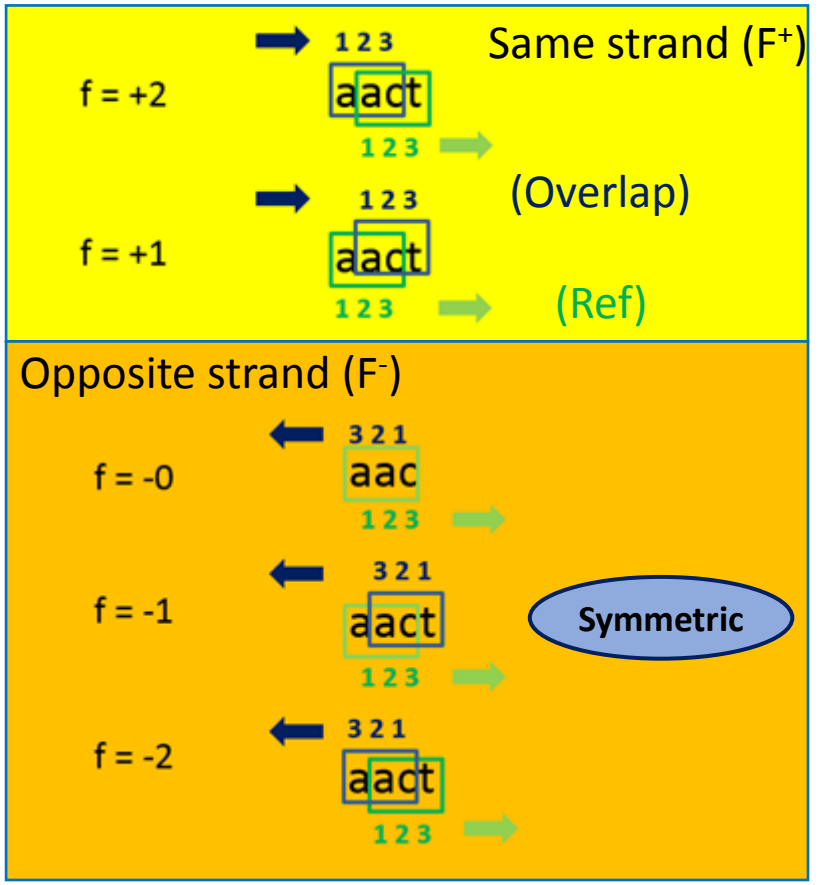
- Looking for explicit constraints:
  1) For 2 overlapping “proteins” ***(symmetric view)***
  2) When 1 protein is known ***(asymmetric view)***

Opposite frames F⁻

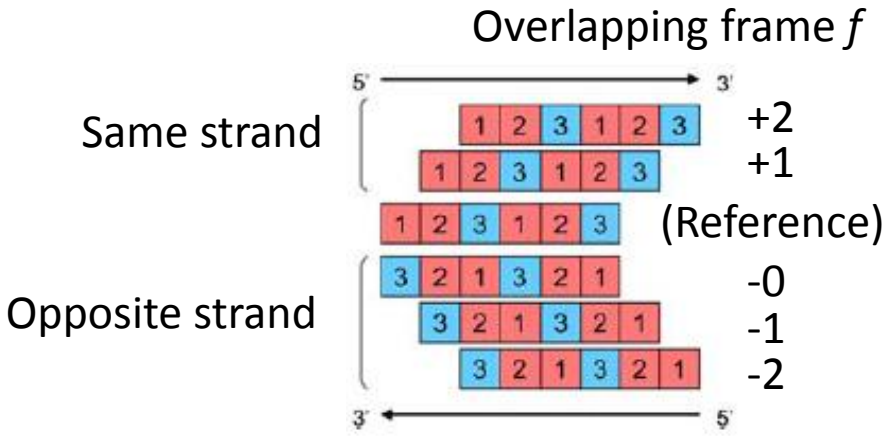


- Let R_f be the relation such that $xR_f y$ whenever sequence y overlap the reference sequence x with frame *f*

$$f \in F^- = \{-2,-1,-0\}: \quad xR_f y \Leftrightarrow yR_f x$$



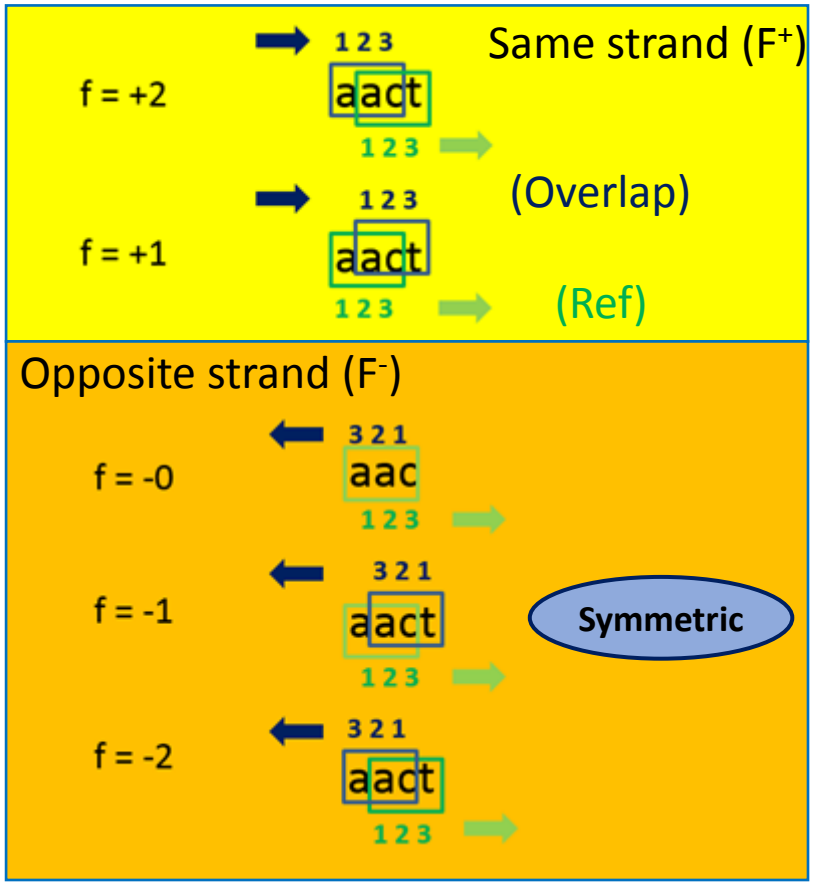
Same sense frames F+



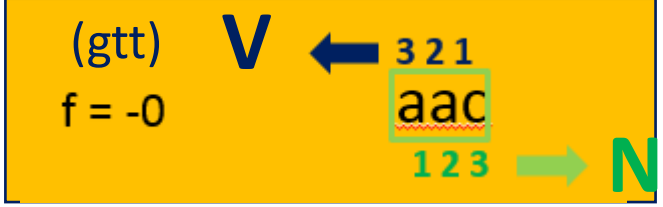
- Let R_f be the relation such that $xR_f y$ whenever sequence y overlap the reference sequence x with frame *f*

$$f \in F^- = \{-2,-1,-0\}: \quad xR_f y \Leftrightarrow yR_f x$$

$$F^+ = \{+1,+2\}: \quad xR_{+1} y \Leftrightarrow yR_{+2} x$$



Amino acid constraints



- **Frame *f* = -0**

(opposite strand, without shift)

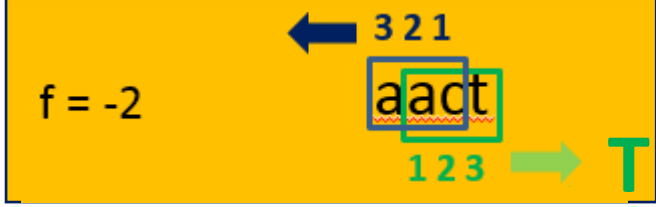
➔**5 constraints**

(Ref) **F + L = q + k + e** (Overlap)
Q + K + E = f + l
I + M + V = y + h + n + d
Y + H + N + D = i + m + v
+ the trivial constraint (S + P + T + A + C + W + R + G)

- From the genetic code (without stop in the 2 reading frames)
  1) Reference frame 'aac' (**N**) ➔ 'gtt' (**v**) in overlapping frame
  2) 'aat' (**N**) ➔ 'att' (**i**)
  3) 'gtc', 'gta', 'gtg' (**v**) ➔ 'gac' (**D**), 'tac' (**Y**), 'cac' (**H**)
  4) 'atc', 'ata' (**i**) ➔ 'gat' (**D**), 'tat' (**Y**)
  5) … ➔ …
- But for the other overlapping frames ?

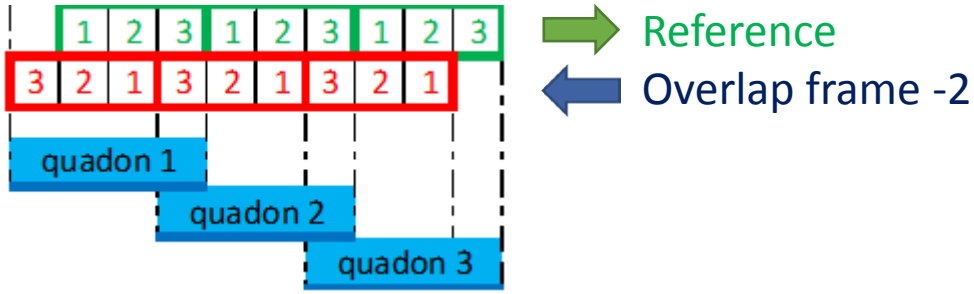
Partial codon overlap ➔ Dependency

Linear Algebraic approach



- ***Quadons***

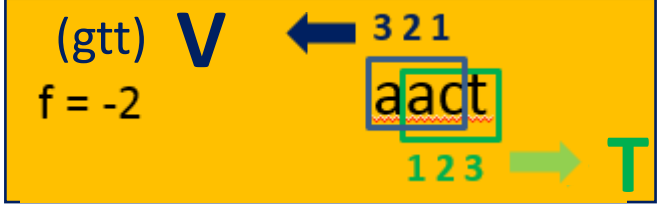
In all frames $f \neq -0$, 4 DNA bases describe 2 overlapping codons (amino acids in both reading frame)



- Vector Q of size (4^4- #stops) gives the number of occurrences of *quadons* or 4-letter words in the sequence ***(except Stops)***
- Vector N of size 40 gives the number of occurrences of 20 amino acids (without a stop) in ***both*** frames (reference and overlap),

$$N = \left([A]_1, [C]_1, [D]_1, \ldots [Y]_1, [A]_2, [C]_2, [D]_2, \ldots, [Y]_2 \right).$$

Linear Algebraic approach



$$\begin{pmatrix} A_1 \\ \dots \\ T_1 \\ \dots \\ Y_1 \\ A_2 \\ \dots \\ V_2 \\ \dots \\ Y_2 \end{pmatrix} = \begin{pmatrix} \cdots 0 \cdot 0 \cdots\cdots\cdots\cdots \\ 0 \cdot 0 \\ \cdots 1 \cdot 0 \cdots\cdots\cdots\cdots \\ 0 \cdot 0 \\ 0 \cdot 1 \\ 0 \cdot 0 \\ 0 \cdot 0 \\ \cdots 1 \cdot 0 \cdots\cdots\cdots\cdots \\ 0 \cdot 0 \\ \cdots 0 \cdot 1 \cdots\cdots\cdots\cdots \end{pmatrix} \begin{pmatrix} aaaa \\ aaac \\ \dots \\ aact \\ \dots \\ atat \\ \dots \\ \dots \\ \dots \\ tttt \end{pmatrix}$$

$$N_{[40]} = M_{f\left[40\times\left(4^4-24\right)\right]} Q_{\left[4^4-24\right]}$$

# of AA
(Ref & overlap)

# of quadons
(- 24 Stop)

$Rank(M_f)$ ➔ Remaining degrees of freedom

- M_f has not full rank: $\sum_{i=1}^{20} L_i = \sum_{i=1}^{20} L_{20+i}$ **(➔ Trivial constraint)**

- This may be the only constraint

Frame f = -1 : only one (trivial) linear constraint
between reference/overlapping protein amino acid composition

- For all other frame shifts, additional constraints do exist

➔ Equality constraints correspond to the set of linear combinations of the lines of matrix M_f

➔ Number of equality constraints = $2 \times 20 - Rank(M_f)$

Constraints list for all frames

Frame *f* = -2 ➔ 10 constraints

(Ref) (Overlap)

A = a
Y = y
G = p
P = g
T = v
V = t
H+Q = c + w
C + W = h + q
I + M = i + m

+the trivial constraint (F + L + S + N + K + D + E + R)

Symmetric

Extension to di-peptides

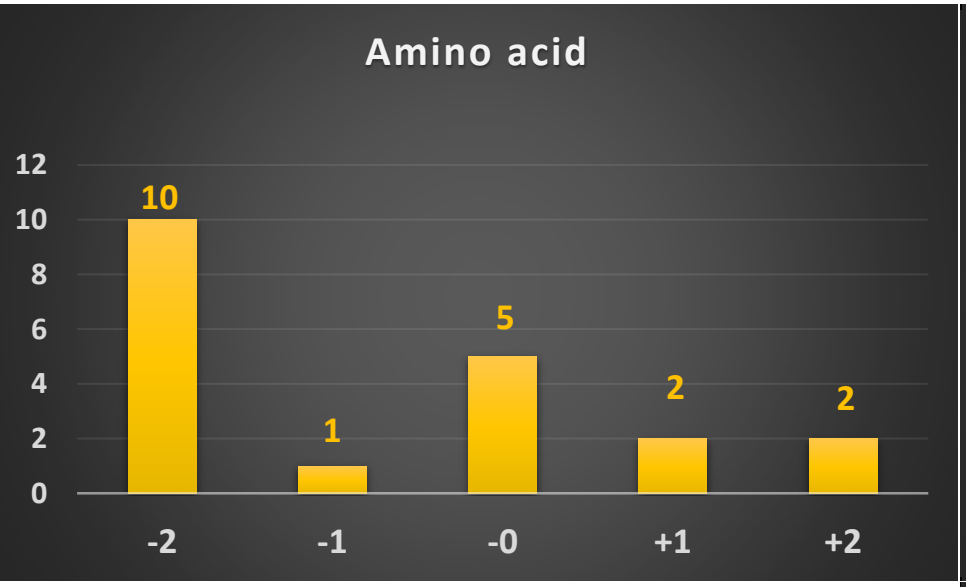
Frame *f* = -2 ➔ 125 di-pep constraints

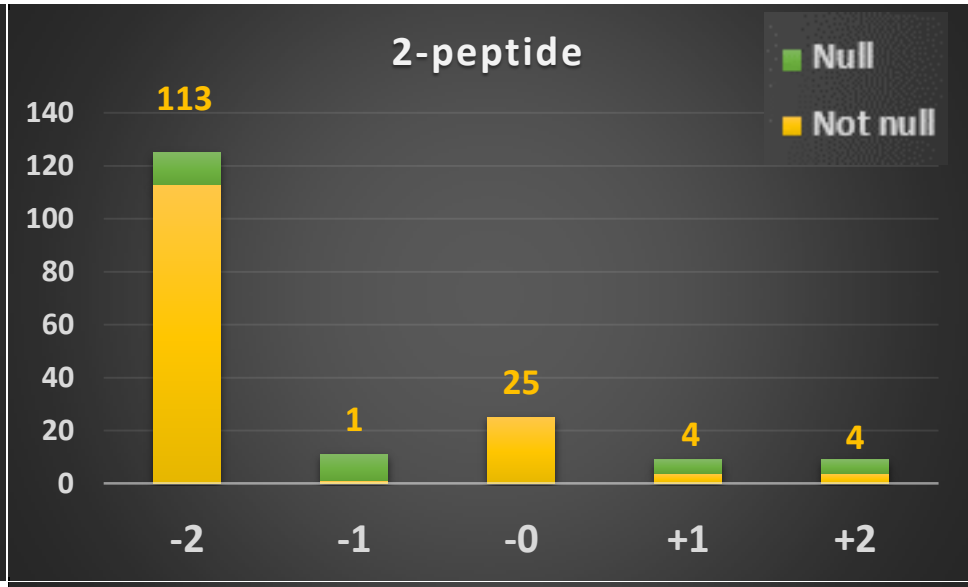
2x 6 null constraints (2 frames)
FY = 0, YY = 0, HY = 0, NY = 0, DY = 0, CY = 0

(➔ STOP in overlapping: YY {'tat', 'tac'}*2 overlap in frame *f*=-2 with 'taa' or 'tag')

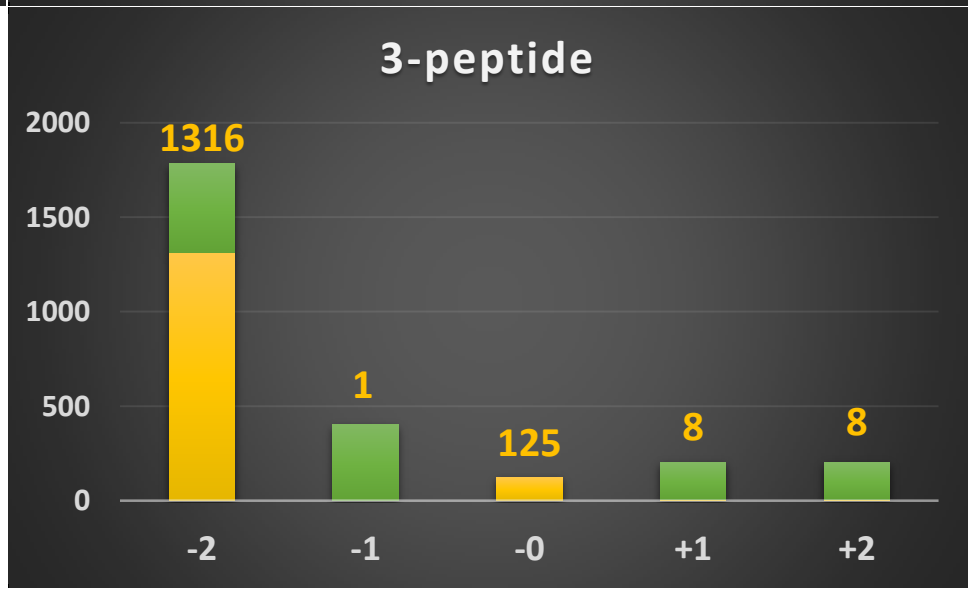
Number of equality constraints

Null constraint = **STOP** in at least 1 frame





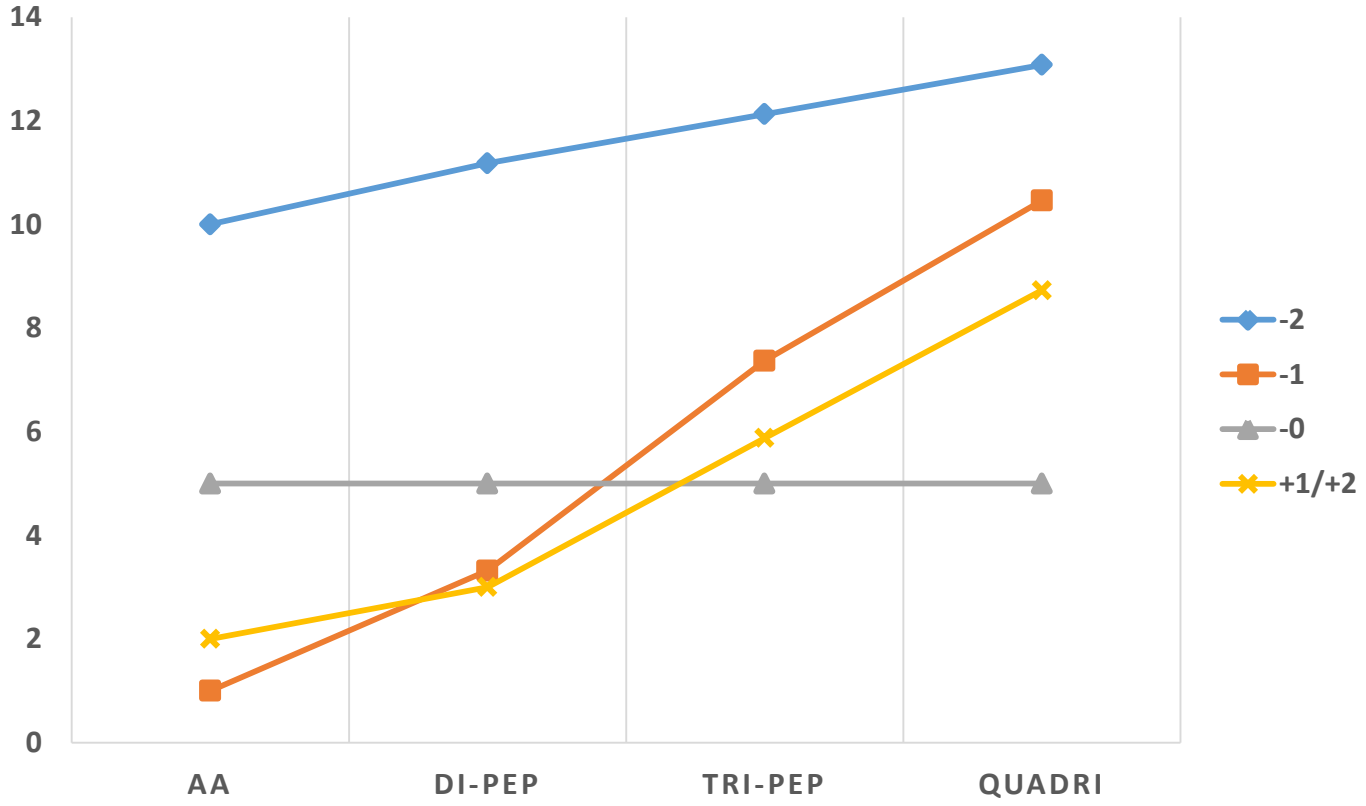
- Tri-peptides
- Higher order
  (Graph traversal algorithm)



Normalized number of equality constraints

Normalized number of constraints = $\sqrt[n]{C_n}$

where C_n is the # of constraints for peptides of length n



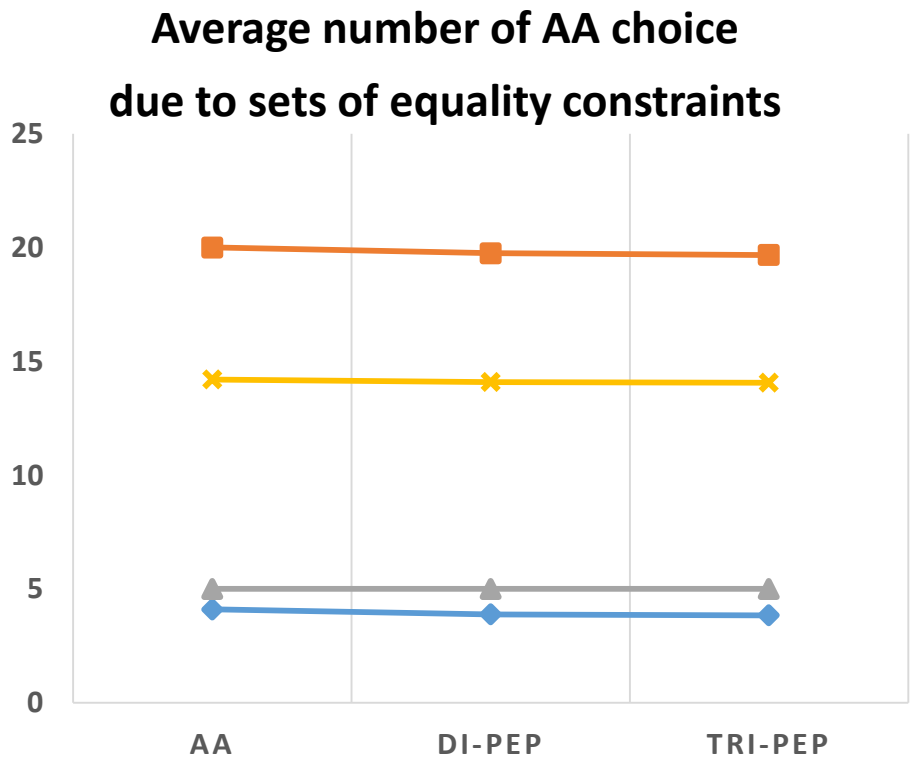
Average number of amino acid choice

$$S_n^f = \left(\frac{1}{\#\text{Pep}} \sum_{c=1}^{\#constraints} \left| Pep_{1,c}^f \right| . \left| Pep_{2,c}^f \right| \right)^{\frac{1}{n}}$$

- Example

  (2-peptide constraints, f = -2)

$$S_{n=2}^{f=-2} = \sqrt{\frac{1+1+1+0+0+2\times 2+2\times 2+6\times 4+\ldots}{20^2}}$$



AA = AA

AG = PA

PA = AG

YY = 0

0 = YY

AH + AQ = CA + WA

CA + WA = AH + AQ

YF + YL + YS + YN + YK + YR = LY + KY + EY + RY

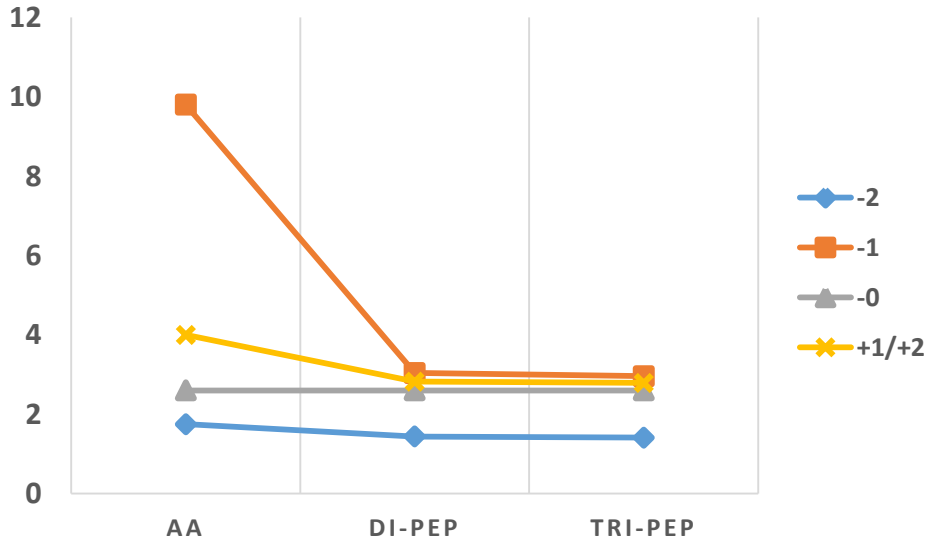
…

When one protein is given…

$$S_n^f = \left(\frac{1}{20^n} \sum_{i=1}^{20^n} \left| Pep_i^f \right| \right)^{\frac{1}{n-1}}$$

➔ Local n-peptide constraints

Average # of AA choice due to local constraints



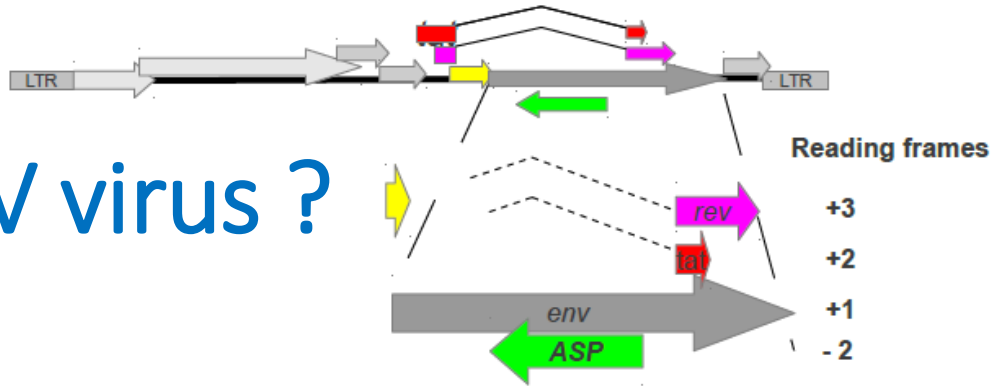
Smith & Waterman (1980)

TABLE 2

The Average Conditional Information per Codon Obtained from Eqs. (8) and (9) When the Encoding of Each Amino Acid Defines a Codon Class.

Reading frame m	$I_m(C\|C)$	$I_m(C\|C\times C)$
Ref	4.218	—
+2	2.144	1.709
+1	2.144	1.729
-0	1.532	—
-1	3.424	1.832
-2	0.821	0.644

a 10th protein in HIV virus ?



- env : gene coding for the virus capside
- ASP may be a protein coded by a gene overlapping env with *f* =-2
- ASP

➔ 189 amino acids : 103 fixed + 86 flexibles

➔ Average Hydrophobicity (Kyte Doolittle)

Fixed AA: **0,9**

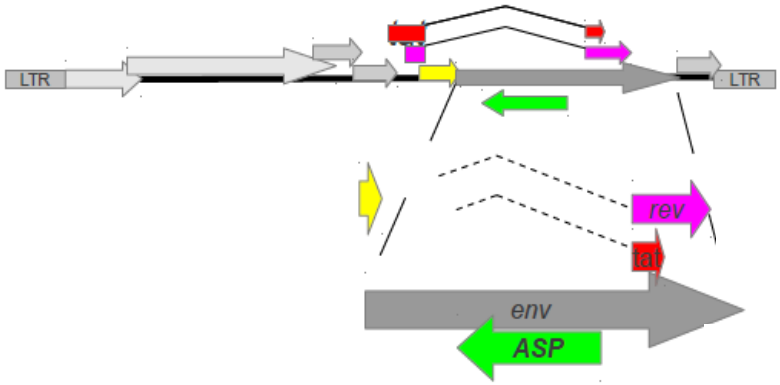
Flexible AA:

- Observed: **0,9**
- Expected: **0,2** (**σ^2= 0,06**)

Highly Hydrophobic

Frame *f* = -2
env aa composition
HIVb aa frequency

Conclusion



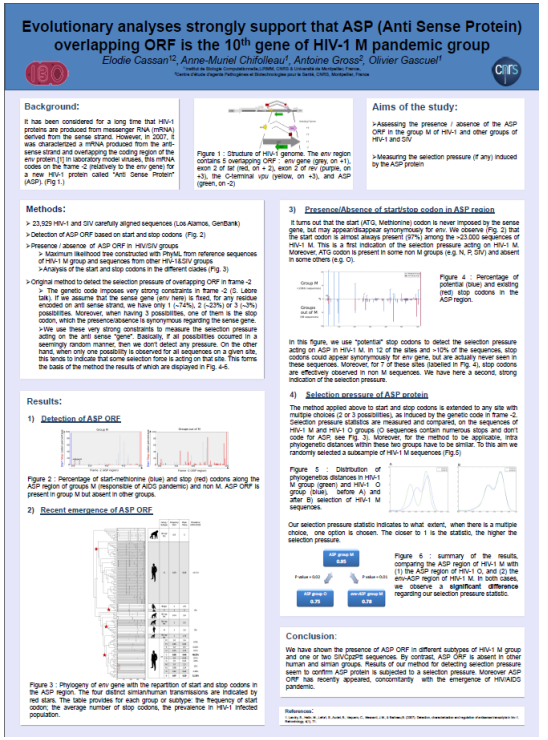
- 2 points of view
  - 2 proteins: peptide equality constraints
  - 1 known protein

- Tools for studying pression selection ?

➔ Poster

Evolutionary analyses strongly support that ASP (Anti Sense Protein) overlapping ORF is the 10th gene of HIV-1 M pandemic group

Elodie Cassan , Anne-Muriel Chifolleau, Antoine Gross, Olivier Gascuel.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Elodie Cassan[1,2], Anne-Muriel Chifolleau[1], Antoine Gross[2], Olivier Gascuel[1]

Background:

Aims of the study:

Methods:

Results:

Conclusion: