The two *RH* genes and their *Rhesus boxes*

FF Wagner

Priv.-Doz. Dr. Franz F Wagner
Abt. Spenderlabor
Institut Springe
31830 Springe

RH: The most complex blood group

- Immunohematology
  - 48 different antigens
  - Antigen D split in >30 different epitopes
- Genetics
  - >100 known haplotypes
  - Similar phenotypes of different alleles
- Proteomics
  - Membrane expression depends on RhAG
  - Direct binding to Ankyrin

RH Blood Group

- Rh protein structure
  - Structural model
  - Differences of RhD and RhCE
- Genetics
  - Structure of an *RH* gene
  - Structure of *RH* locus and the *RHD* deletion
- Prediction of the phenotype
  - Molecular basis of antigen D
  - Molecular basis of other main antigens
  - Testing strategies, pitfalls and limitations

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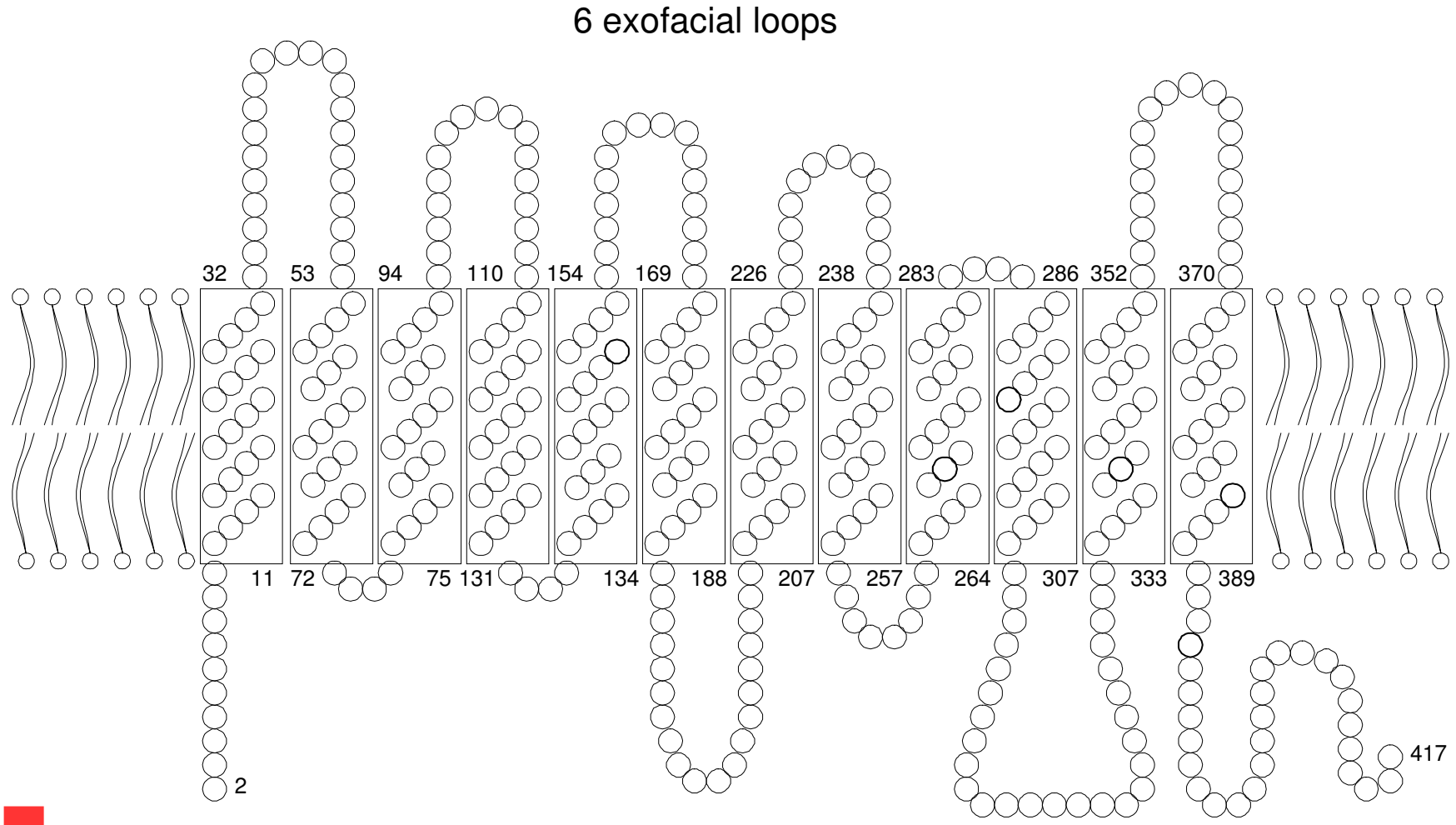
Rh protein in the membrane

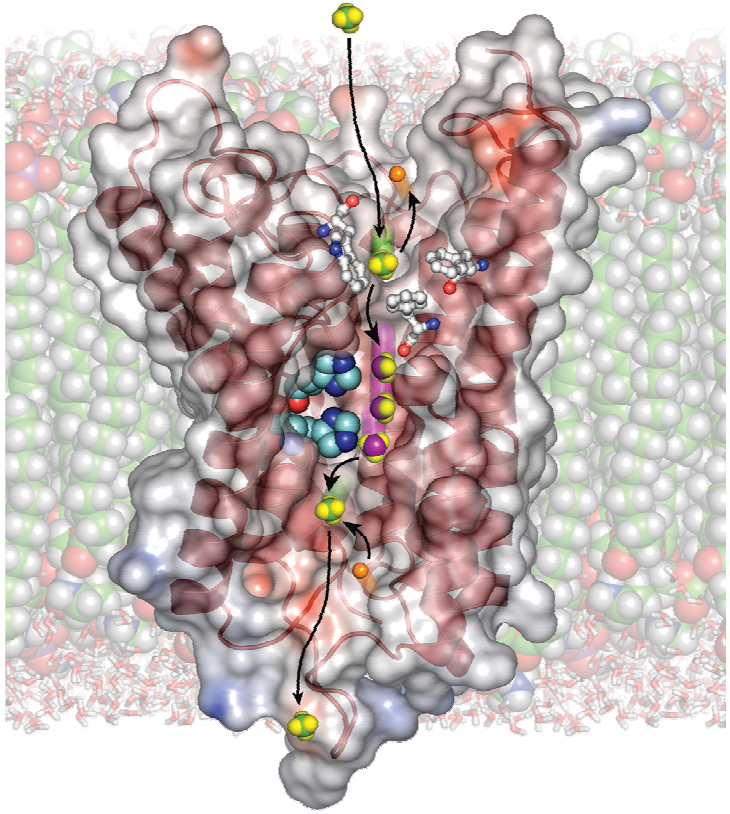
- Complex with RhAG
- Direct interaction with ankyrin

Nicolas JBC 2003

Schematic structure of Rh protein



Added complexity: AmtB structure resolved



Khademi et al. Science 2004; 305: 1587

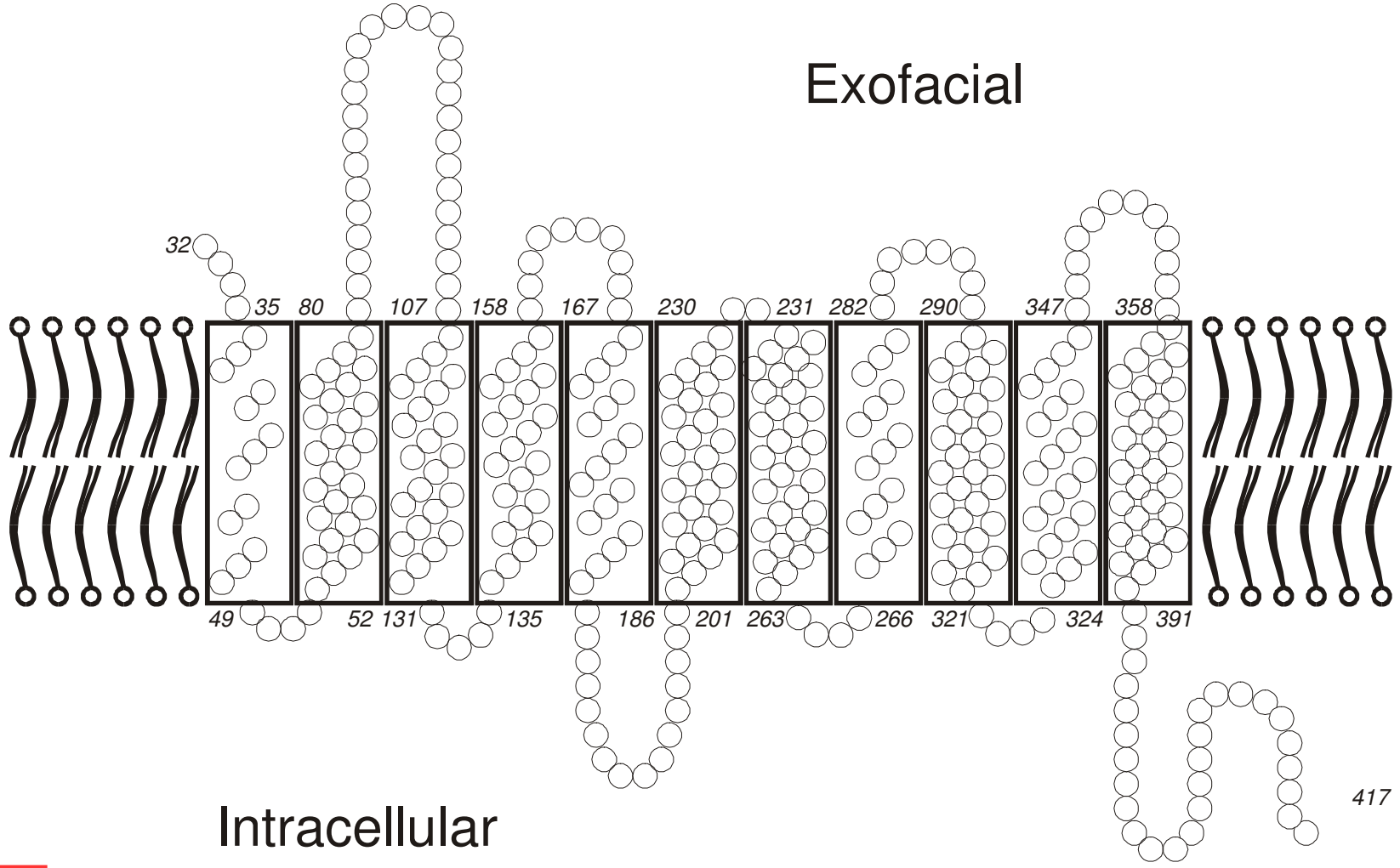
- AmtB:
  Ammonia transporter
  of E. coli
- Homology to the Rh
  proteins
- 3D structure resolved:
  - 3 protein complex
  - 11 helices

AmtB

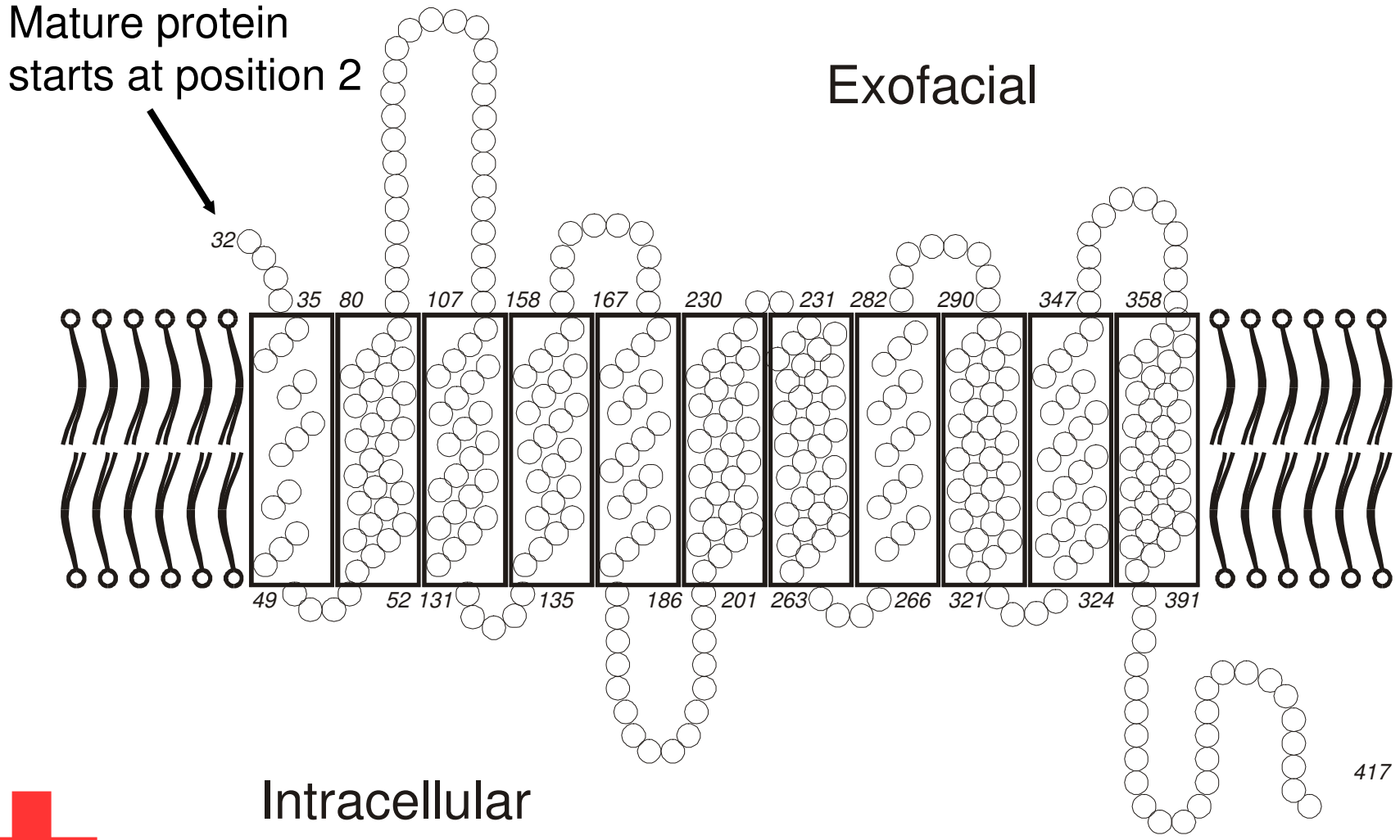
- extracellular aminoterminal end
- helix 1 near the axis

Khademi et al. Science 2004; 305: 1587

RhD structure modeled on AmtB



RhD structure modeled on AmtB



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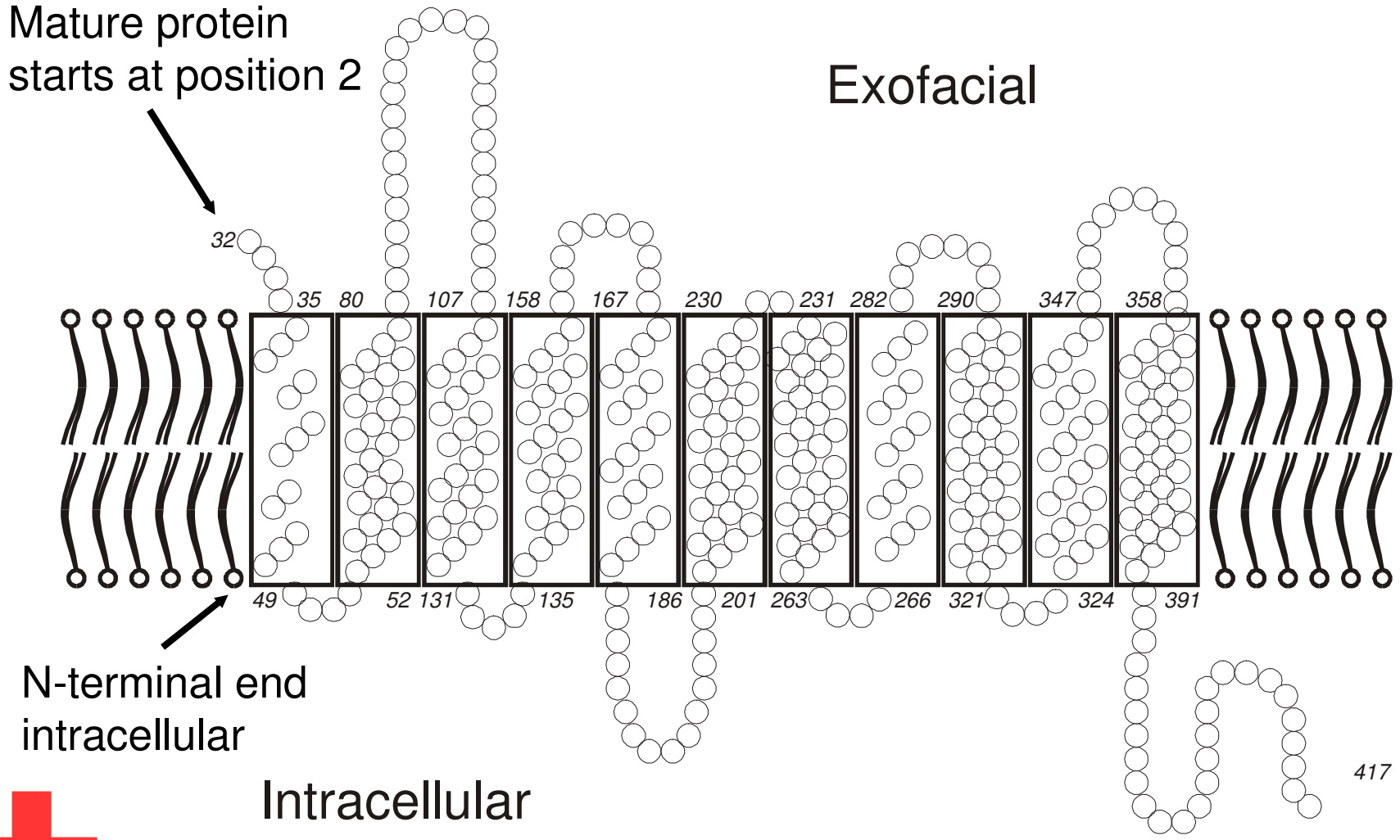
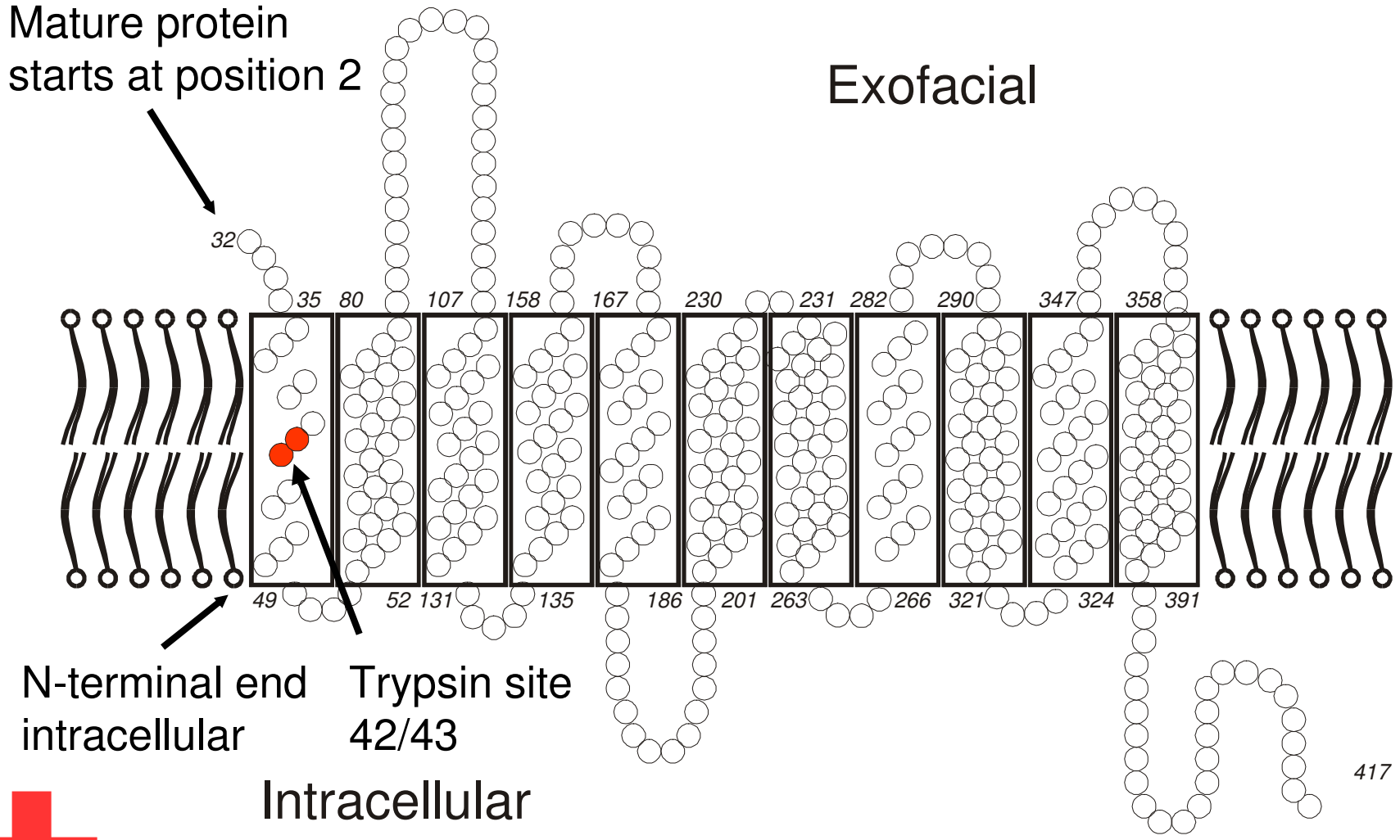


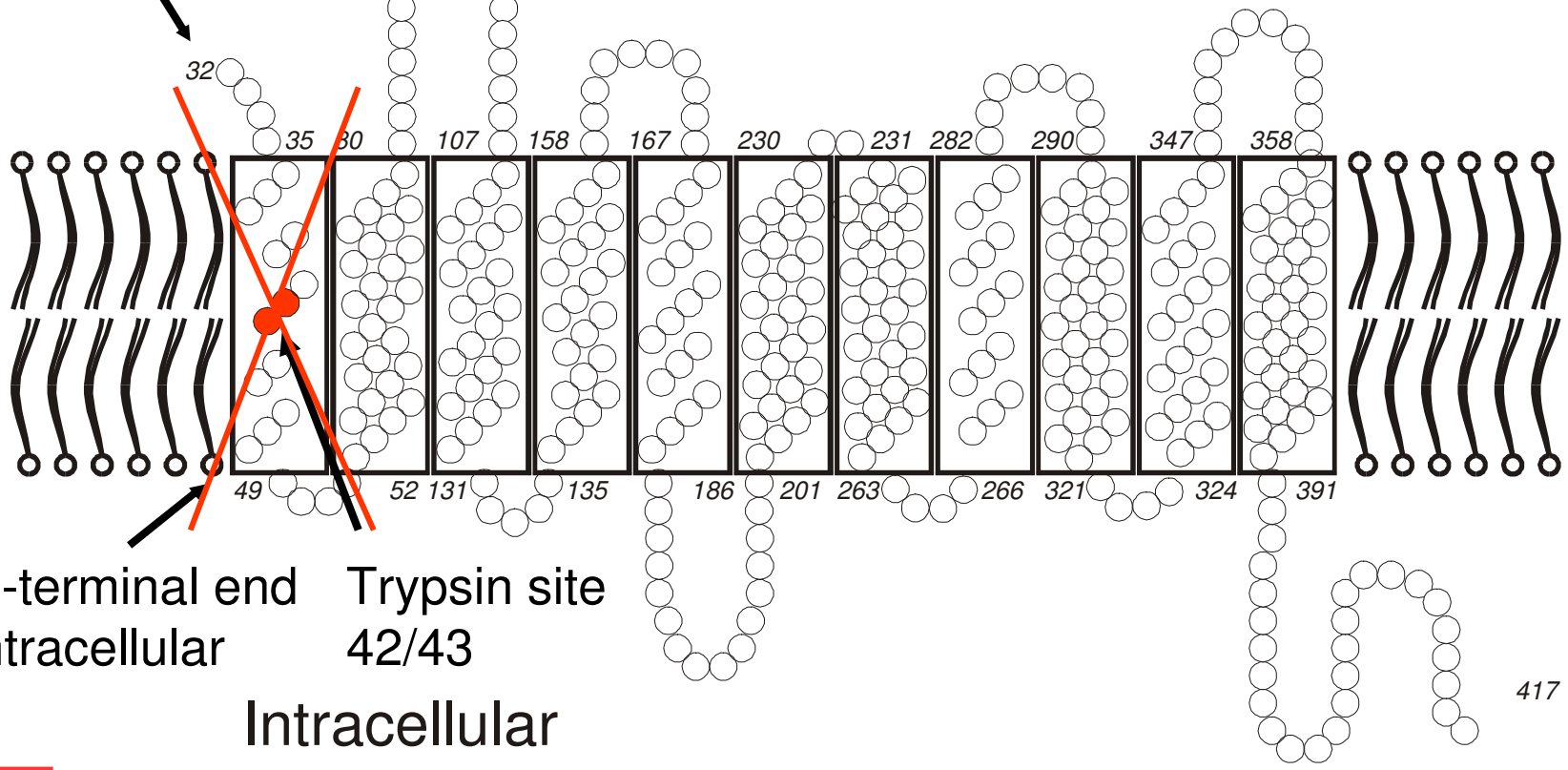
RhD structure modeled on AmtB



RhD structure modeled on AmtB

Mature protein
starts at position 2

The AmtB model seems to fail
at the aminoterminal end.

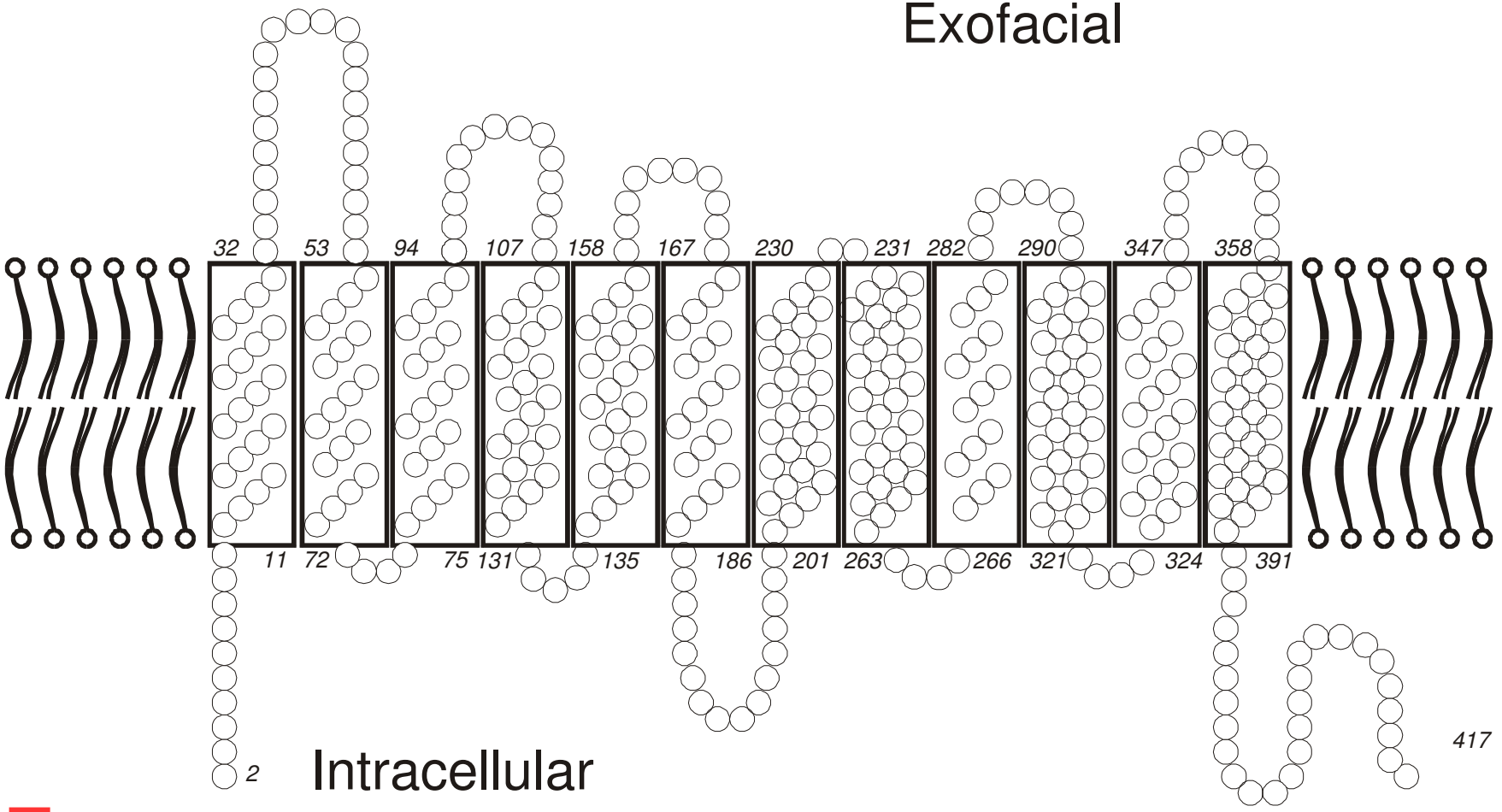


N-terminal end
intracellular

Trypsin site
42/43

Intracellular

RhD structure partially modeled on AmtB



RhD structure and AmtB

- Amino acids lining Ammonia channel
  - 103 Phe
  - 107 Phe
  - 148 Trp
  - 219 Ser
  - 168 His
  - 318 His

RhD structure and AmtB

- Amino acids lining Ammonia channel
  - 103 Phe
  - 107 Phe conserved through RhAG, RhBG, RhCG
  - 148 Trp
  - 219 Ser
  - 168 His conserved through RhAG, RhBG, RhCG
  - 318 His conserved through RhAG, RhBG, RhCG

RhD structure and AmtB

- Amino acids lining Ammonia channel
  - 103 Phe not conserved in RhCE and RhD
  - 107 Phe not conserved in RhCE and RhD
  - 148 Trp not conserved in RhCE and RhD
  - 219 Ser not conserved in RhCE and RhD
  - 168 His not conserved in RhCE and RhD
  - 318 His not conserved in RhCE and RhD

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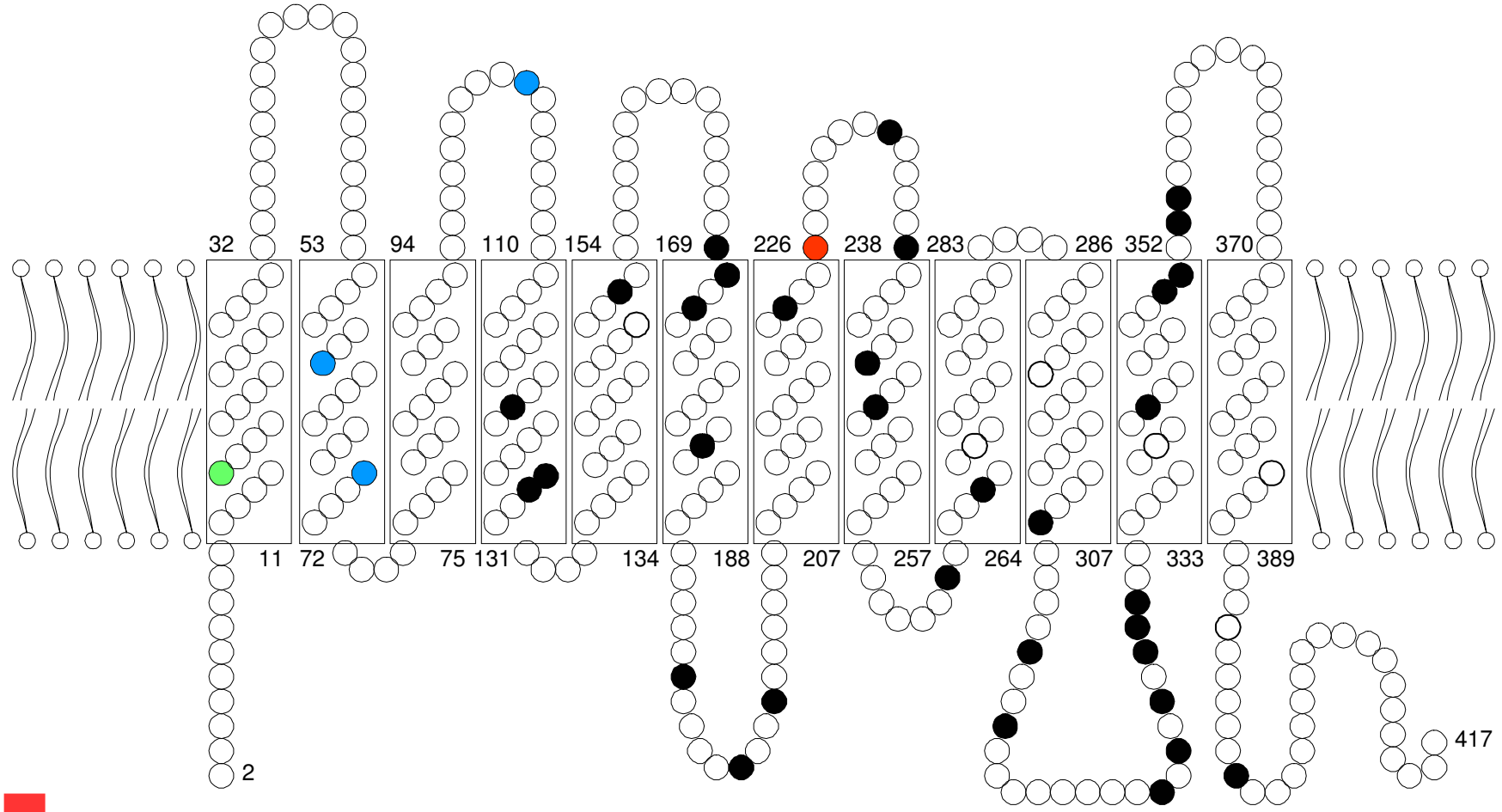
RhD vs RhCE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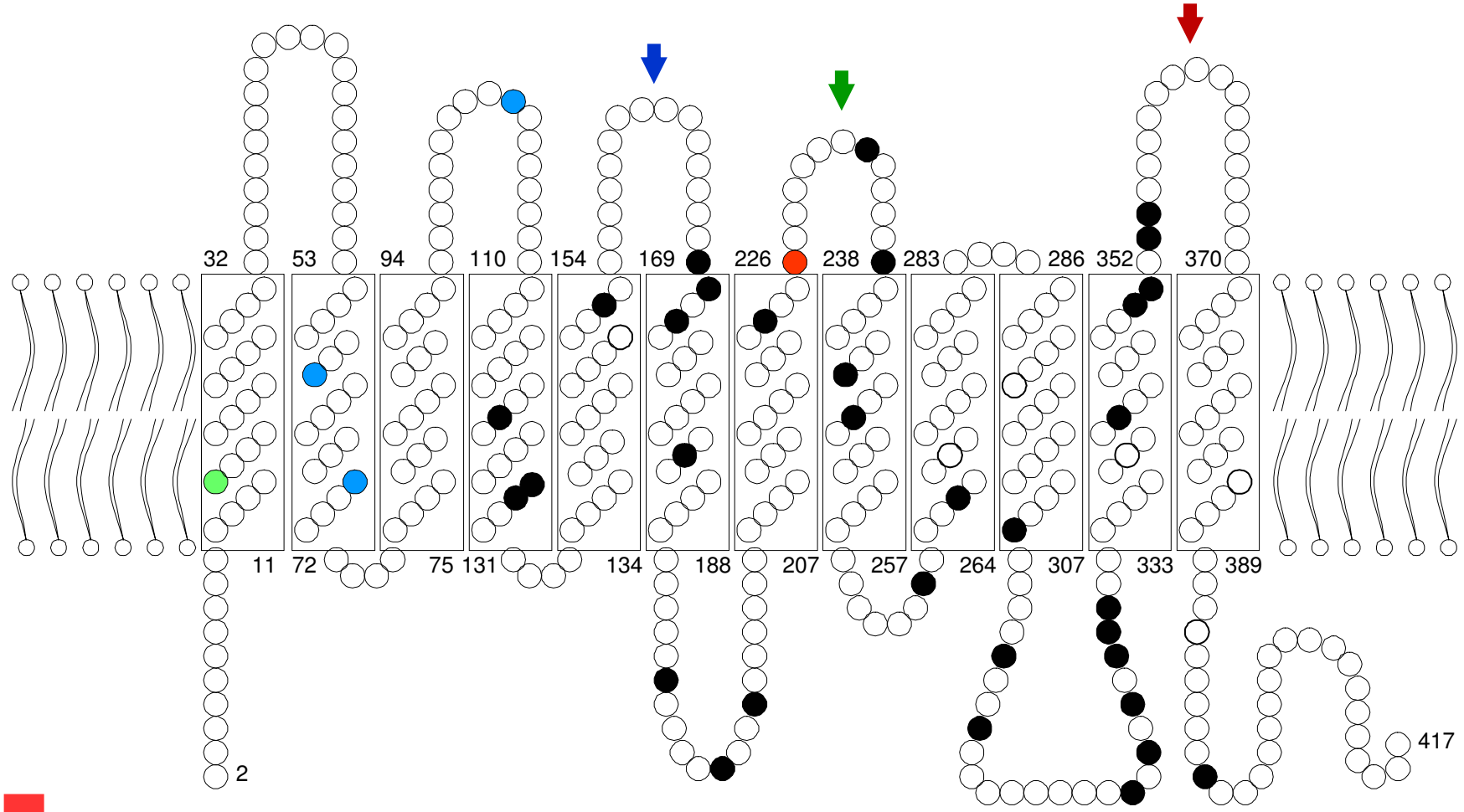
417

RhD vs RhCE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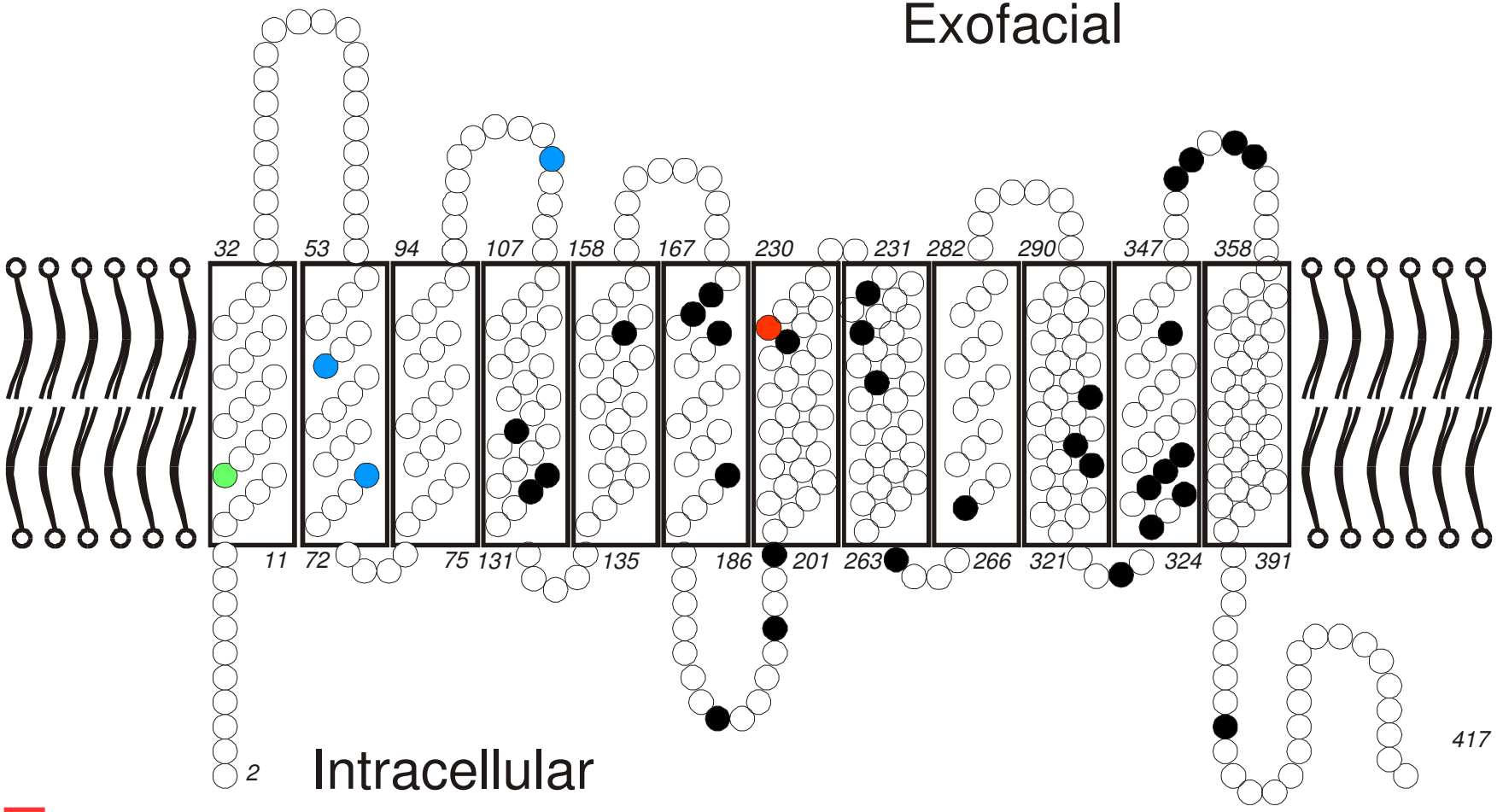
RhD vs RhCE (new model)



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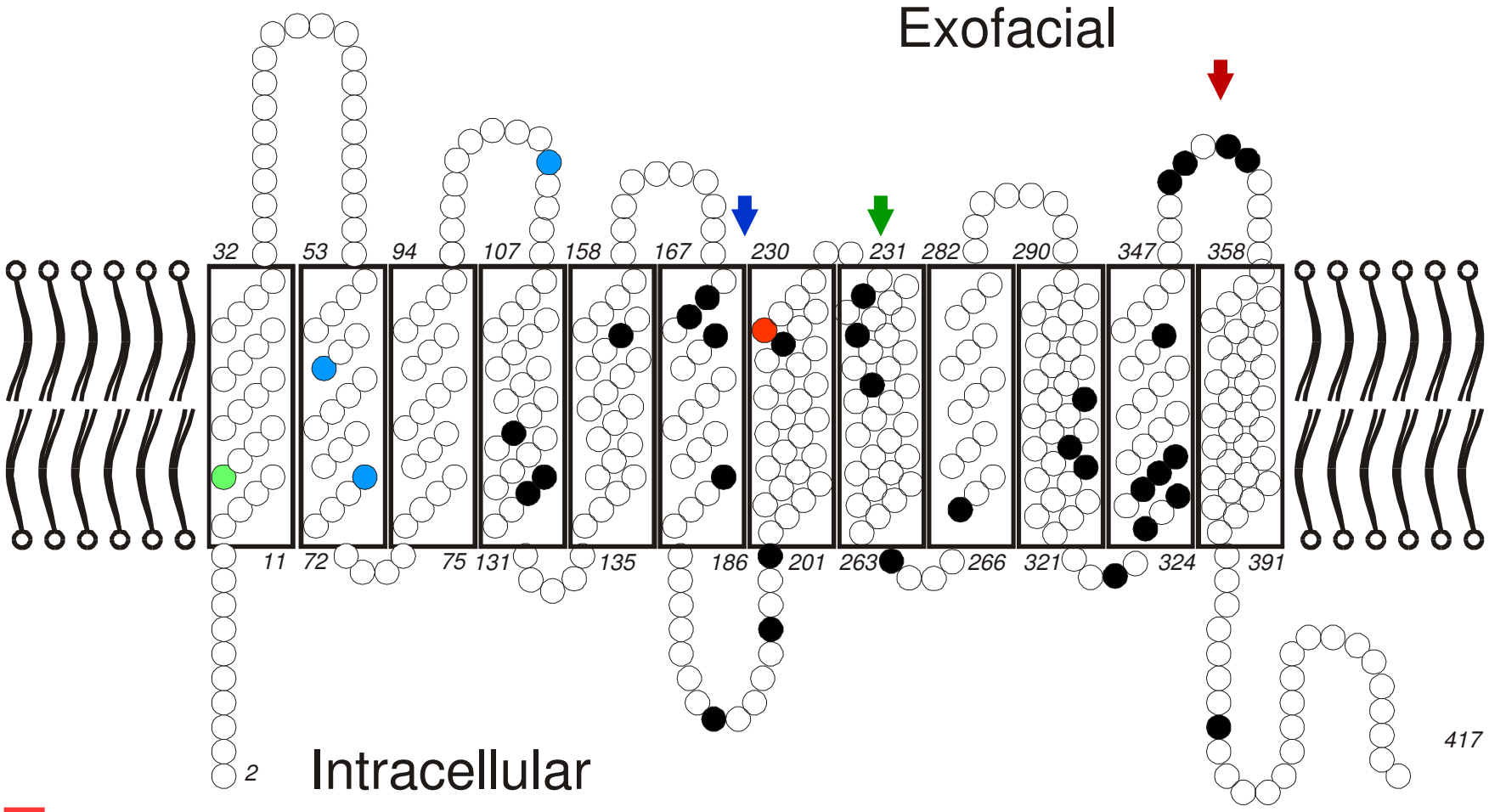

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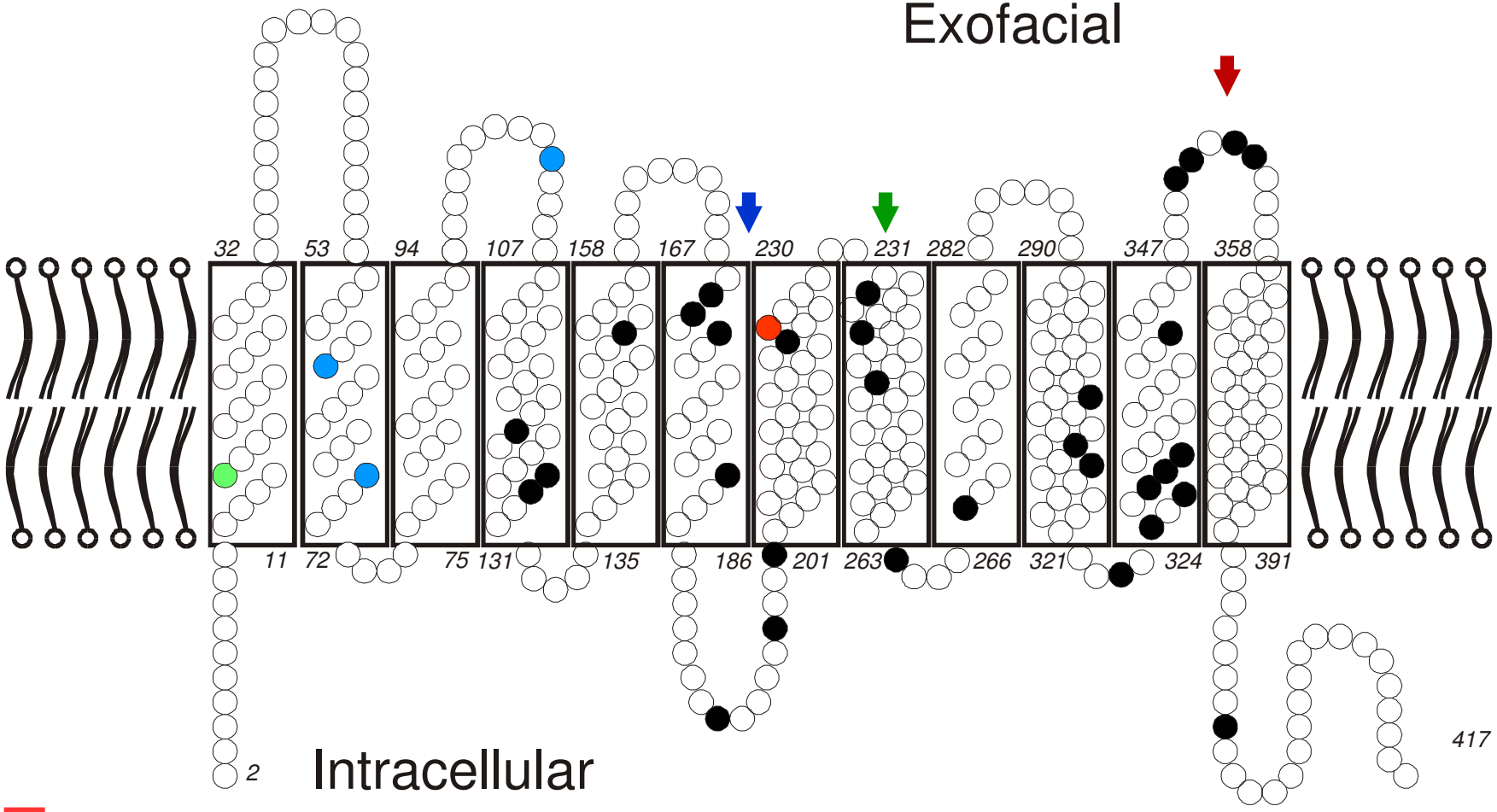

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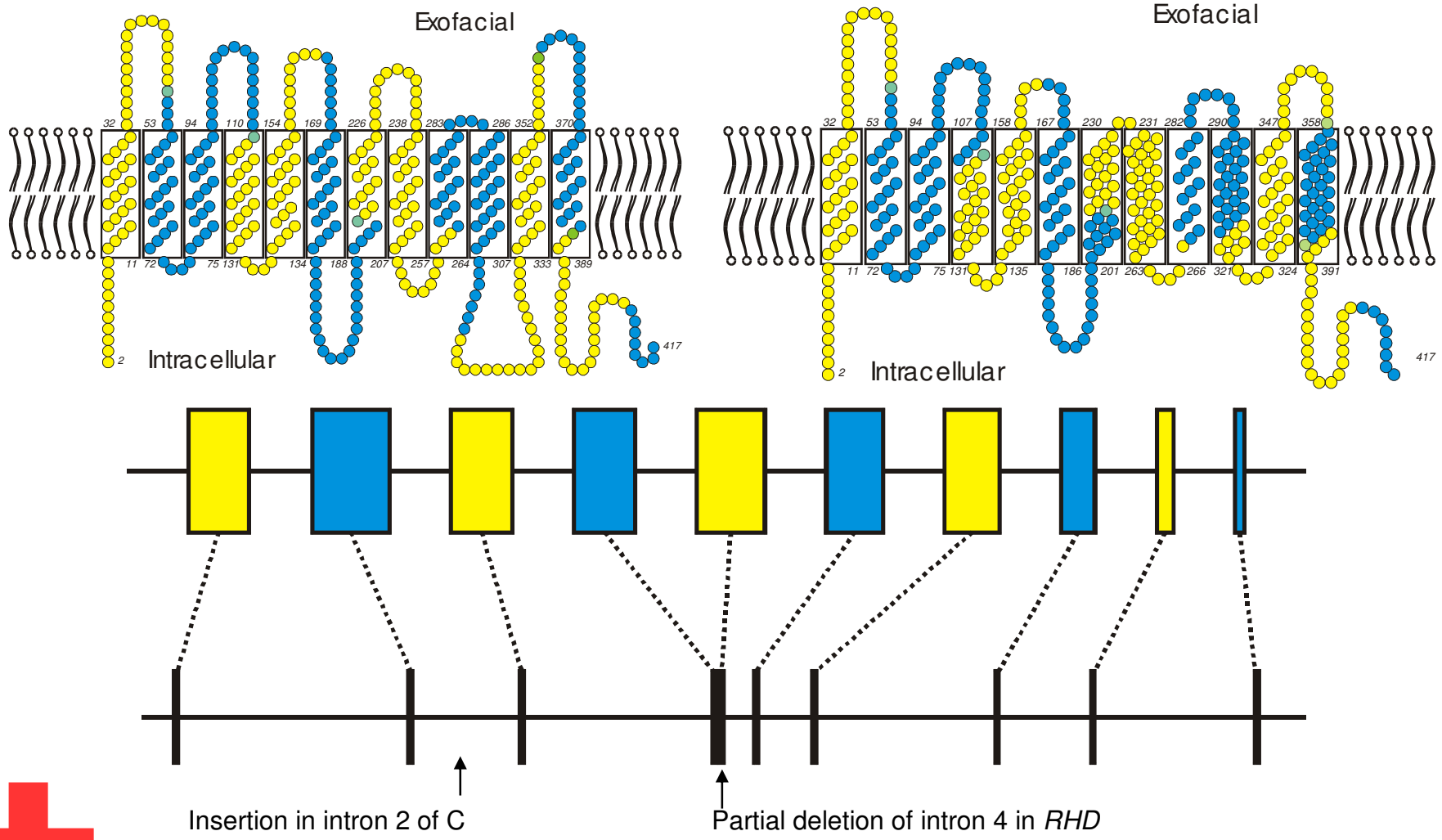
Genomic structure



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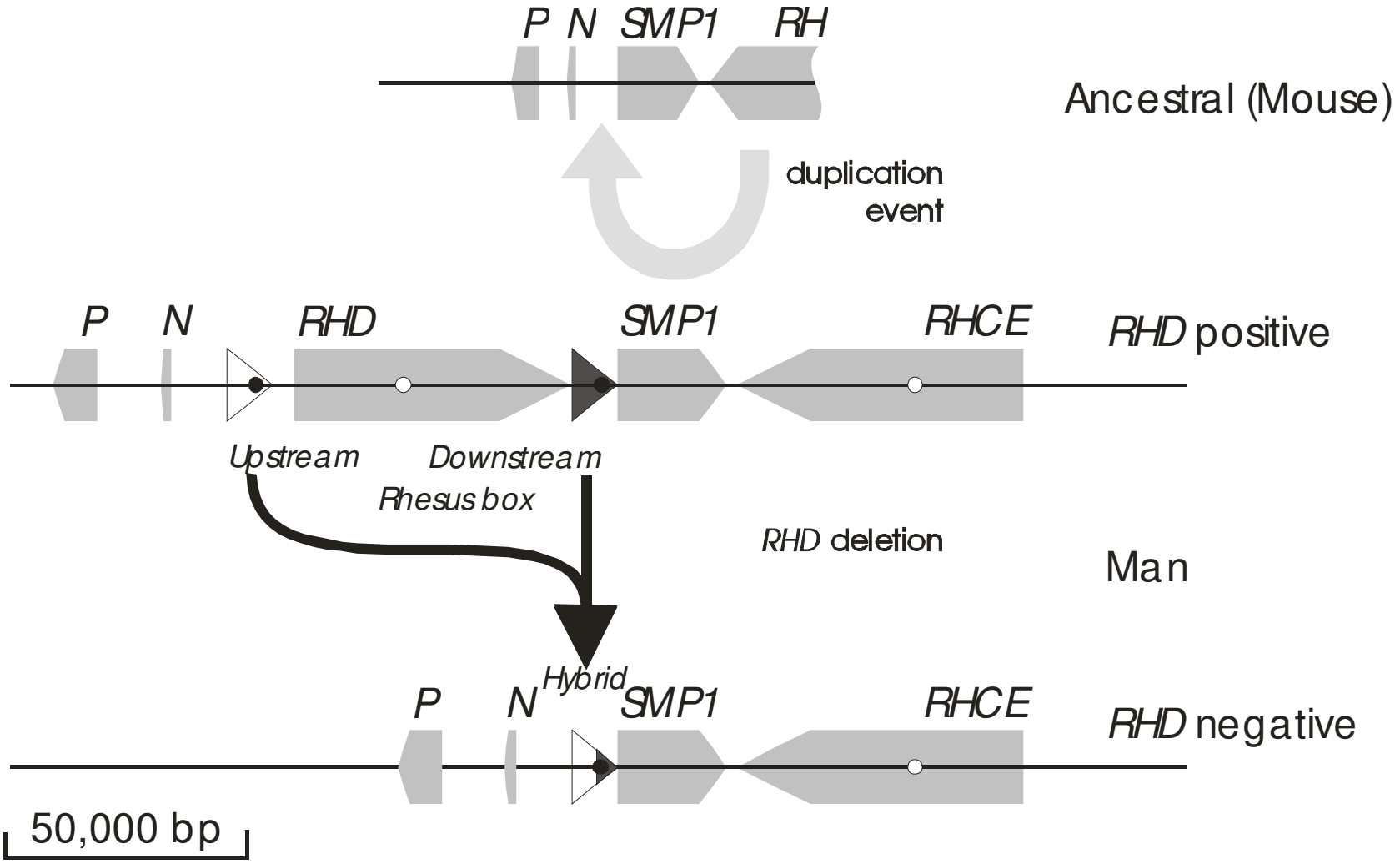
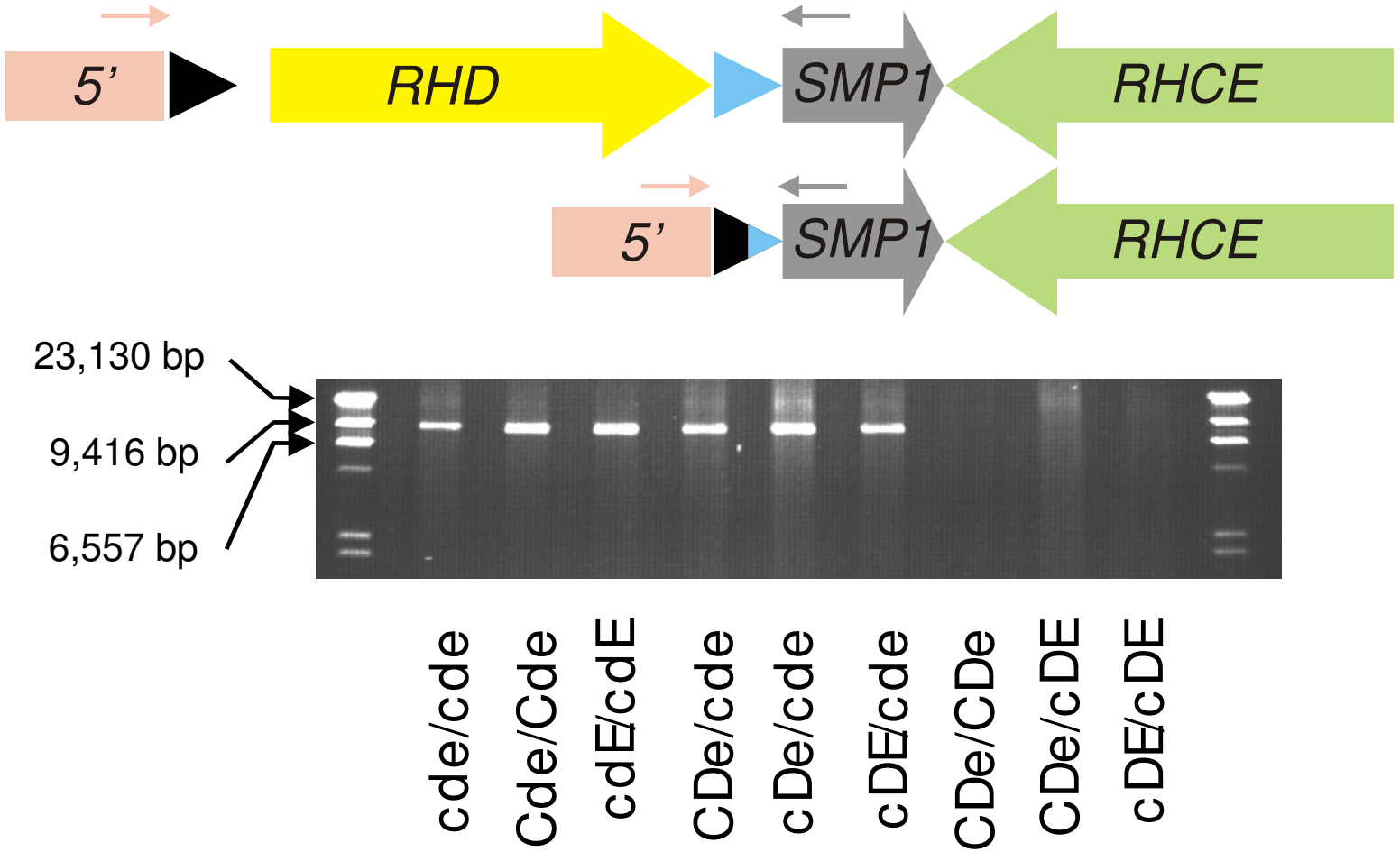
RH locus


P N SMP1 RH
Ancestral (Mouse)
duplication event
P N RHD SMP1 RHCE
RHD positive
Upstream
Downstream
Rhesus box
RHD deletion
Man
Hybrid
P N SMP1 RHCE
RHD negative
50,000 bp

Detection of *RHD* deletion by PCR



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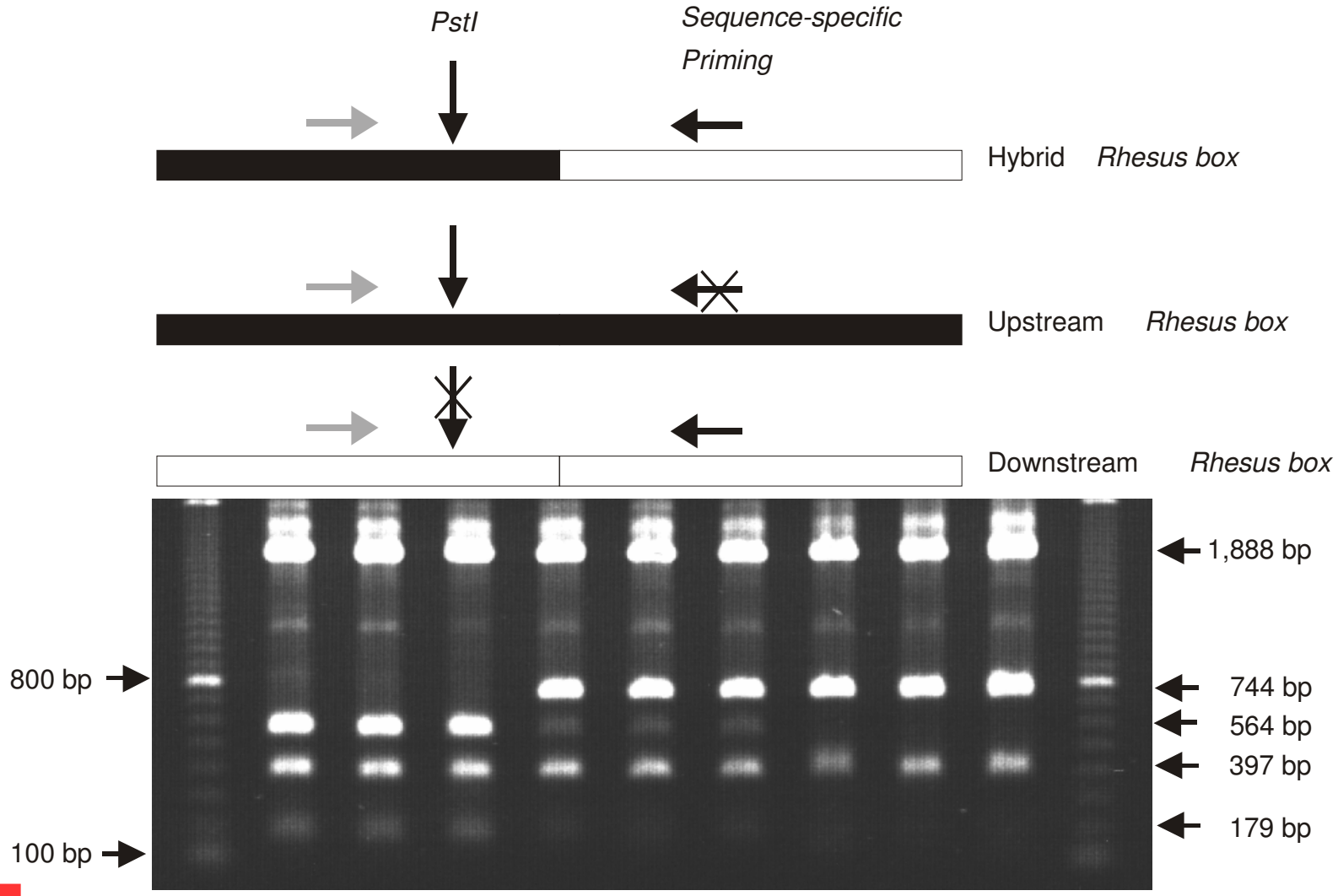

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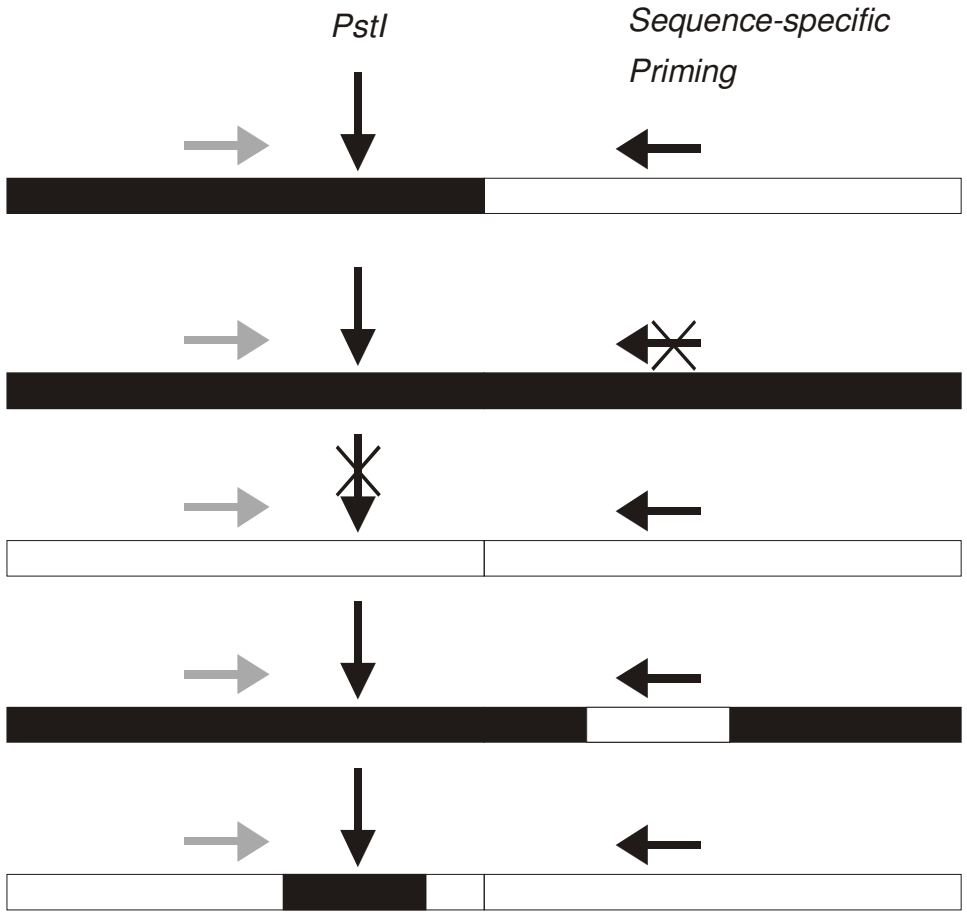


Hybrid *Rhesus box*

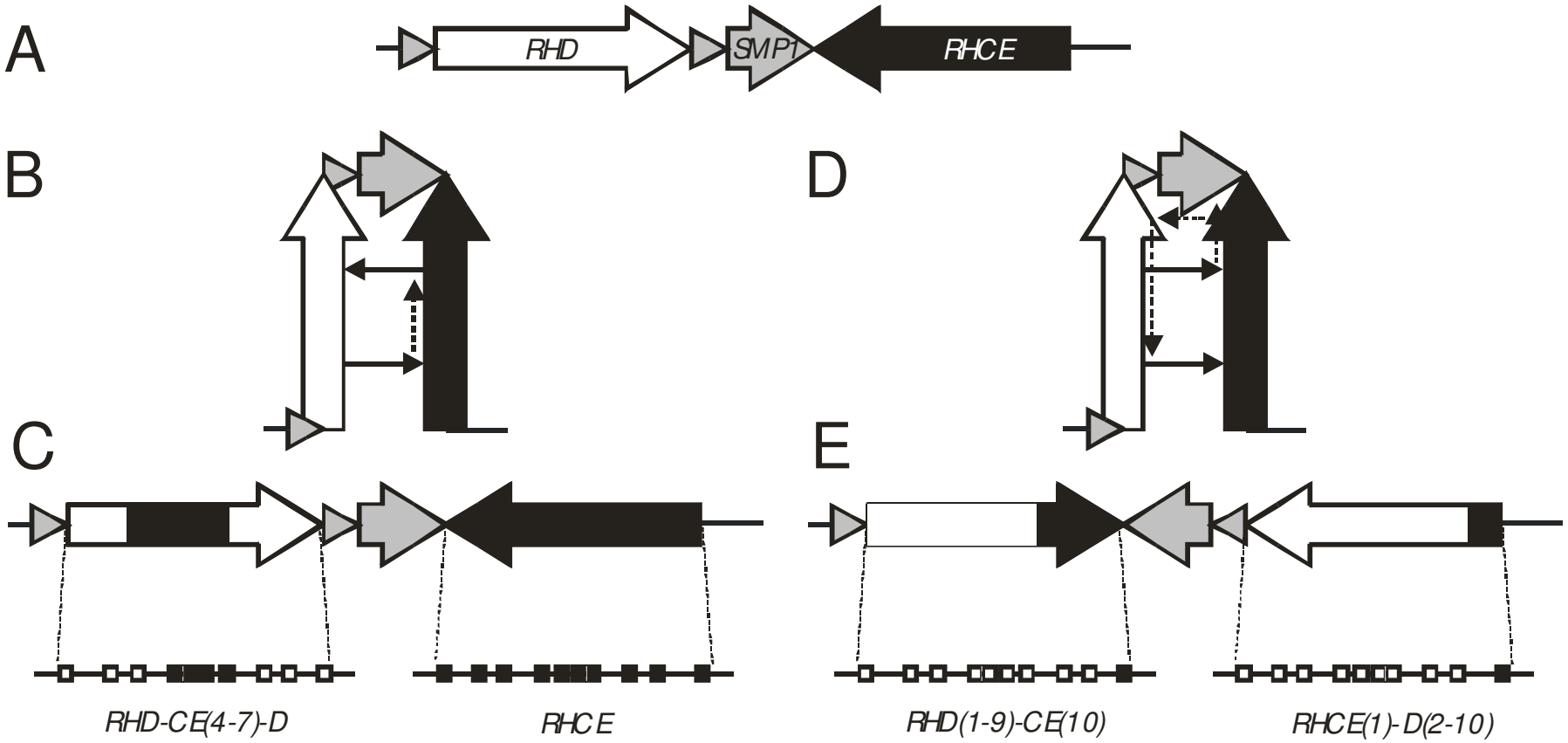
Upstream *Rhesus box*

Downstream *Rhesus box*

Upstream *Rhesus box*
(*RHD* psi, DAU-1, -3)

Downstream *Rhesus box*
(Weak D type 4.1)

RH locus



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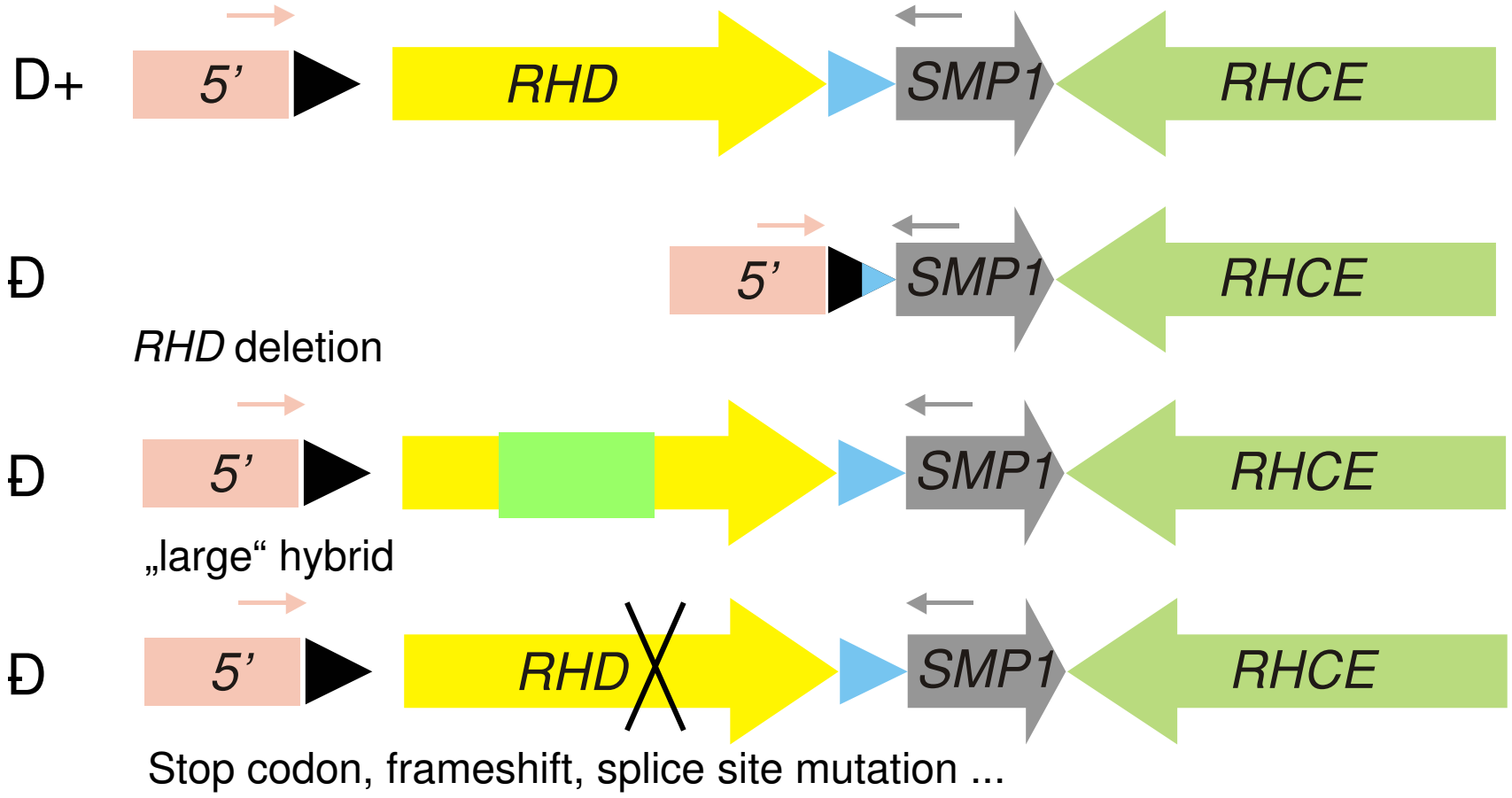
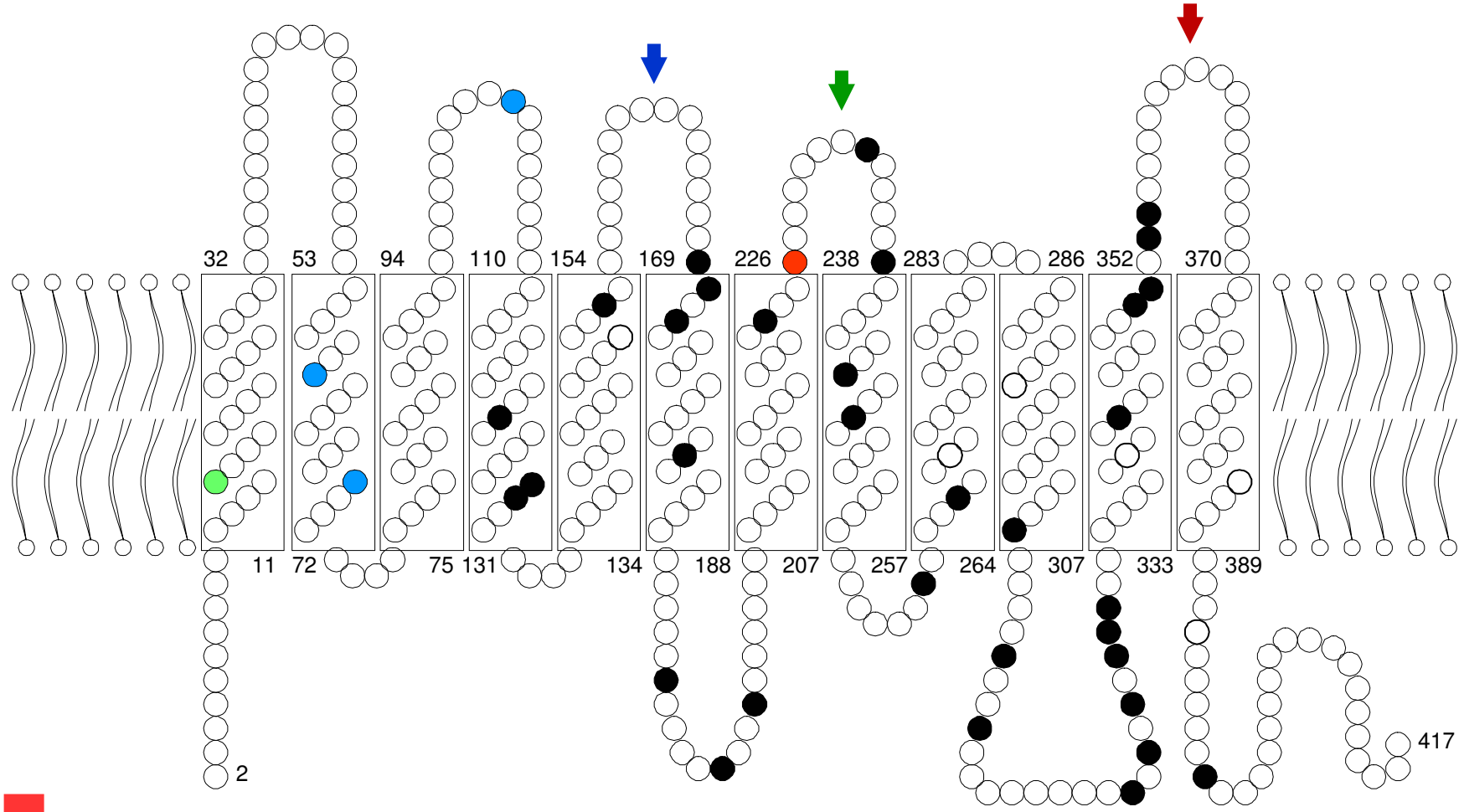
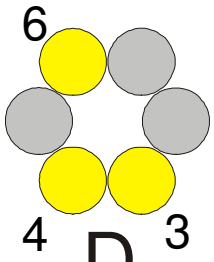
Three types of D neg

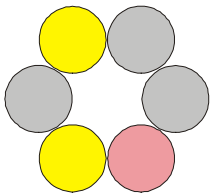


Molecular basis of antigen D

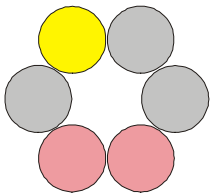


RhD loops and D antigen





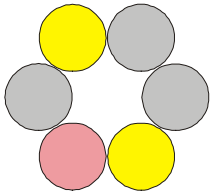
FPTT



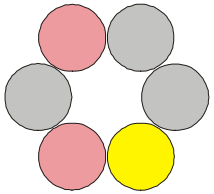
BARC

DFR

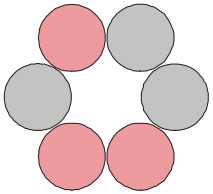
D cat VI



D^{W}



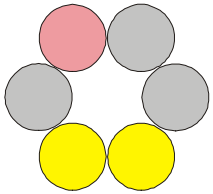
Rh32



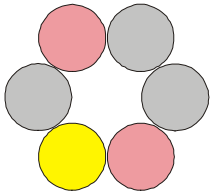
D cat V

DBT

CE



Evans

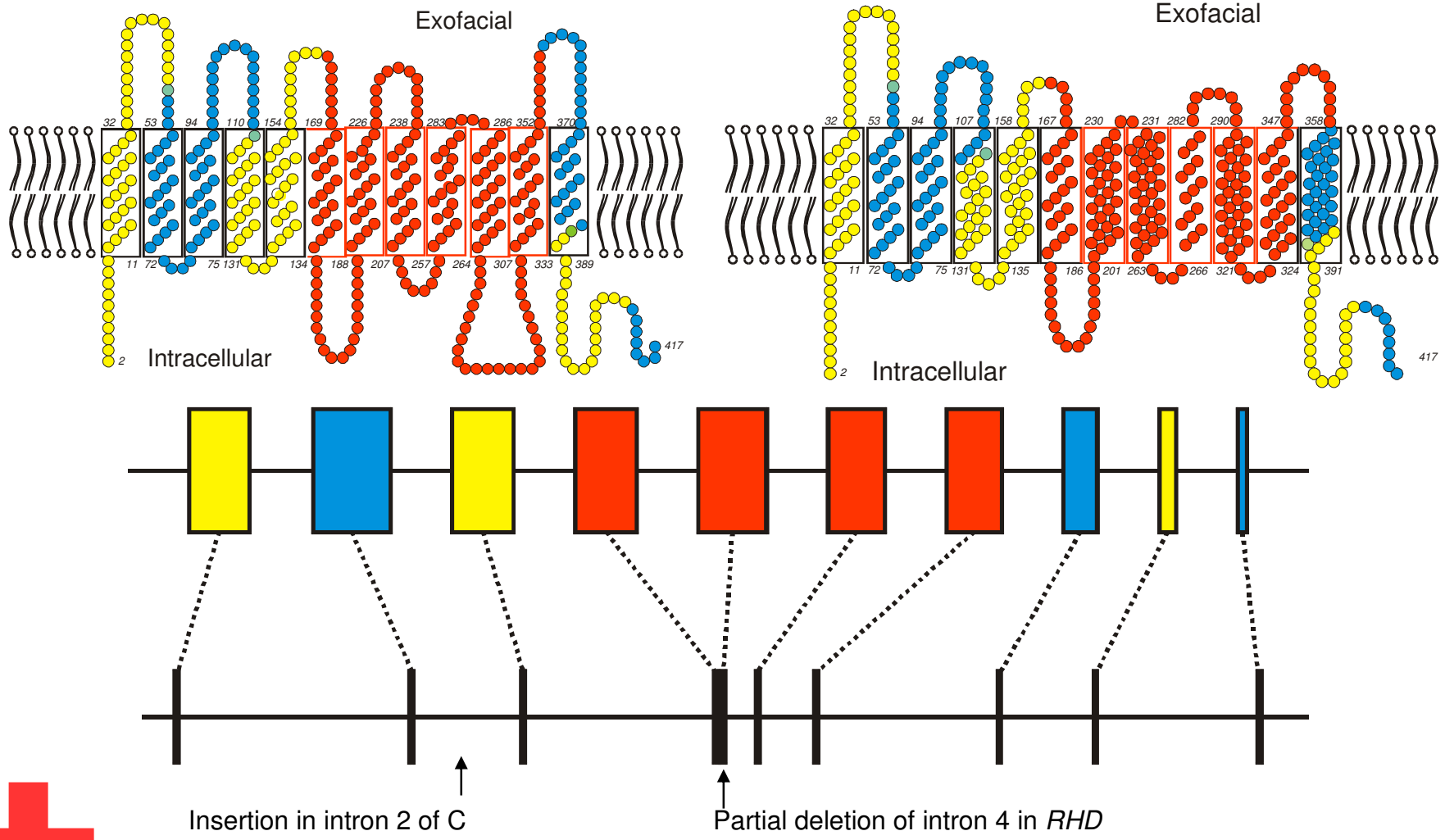


Rh33

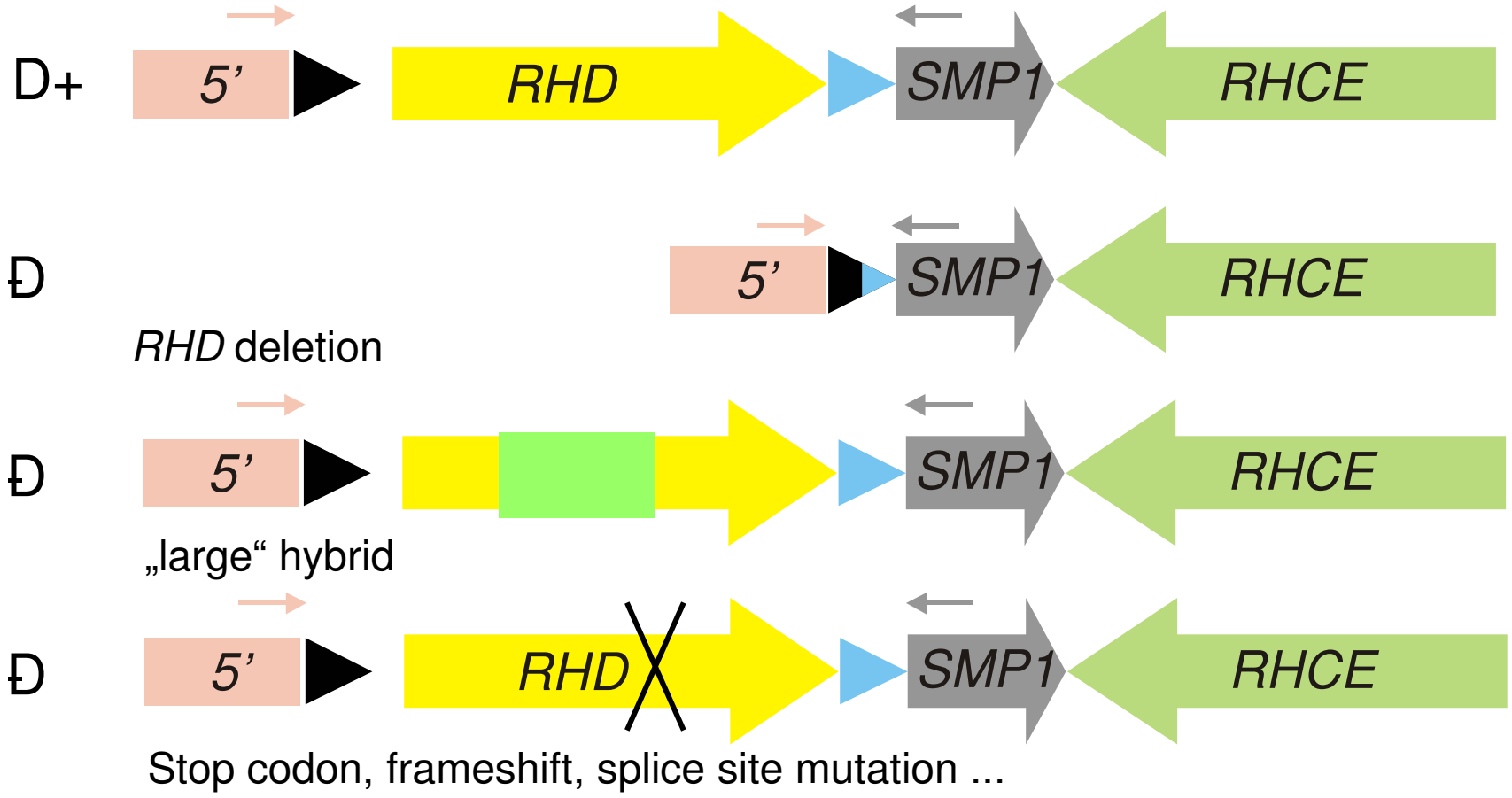
D cat IV

DHAR

D negative *RHD-CE-D* hybrid alleles



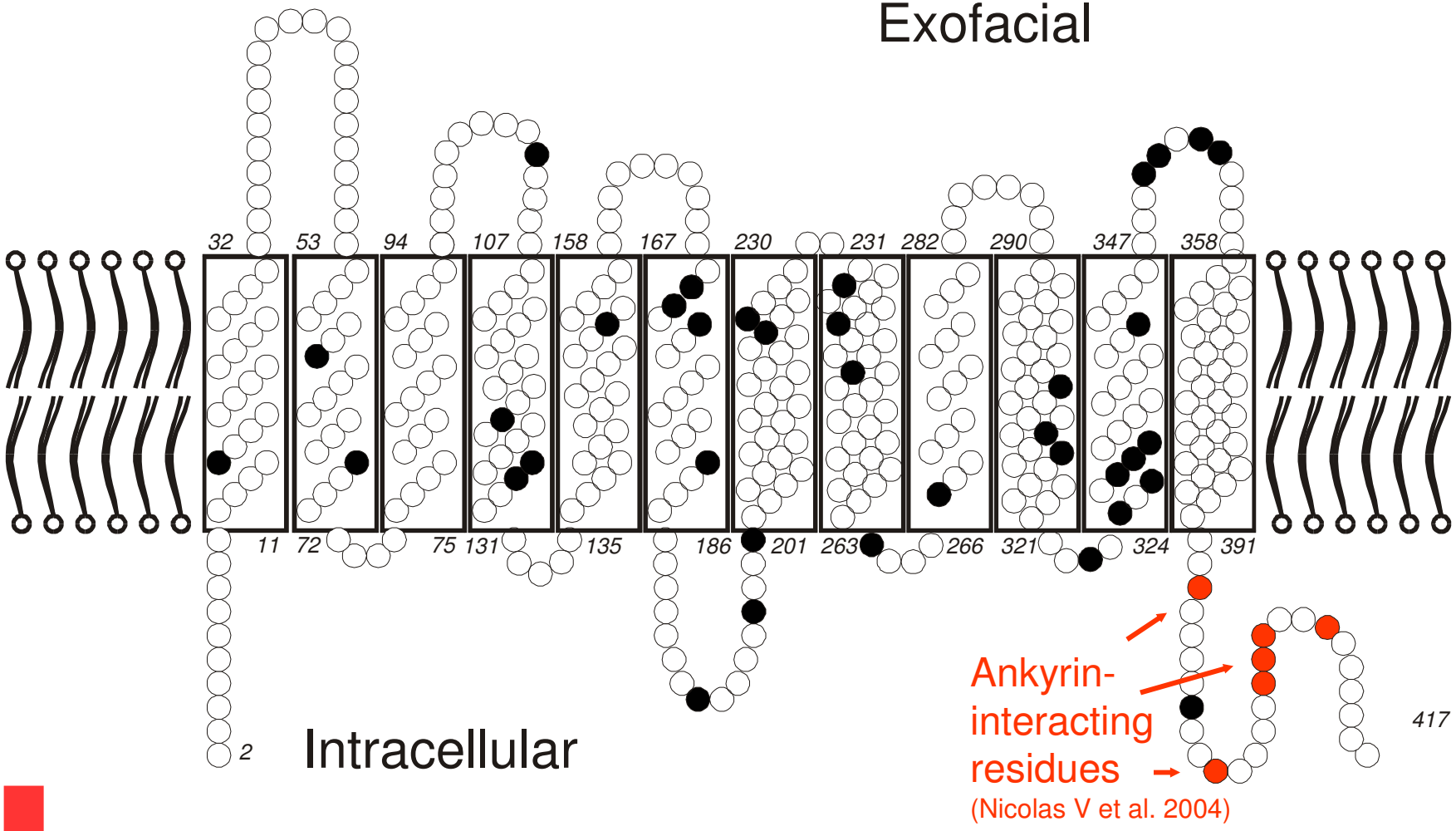
Three types of D neg



Other D negative *RHD* alleles

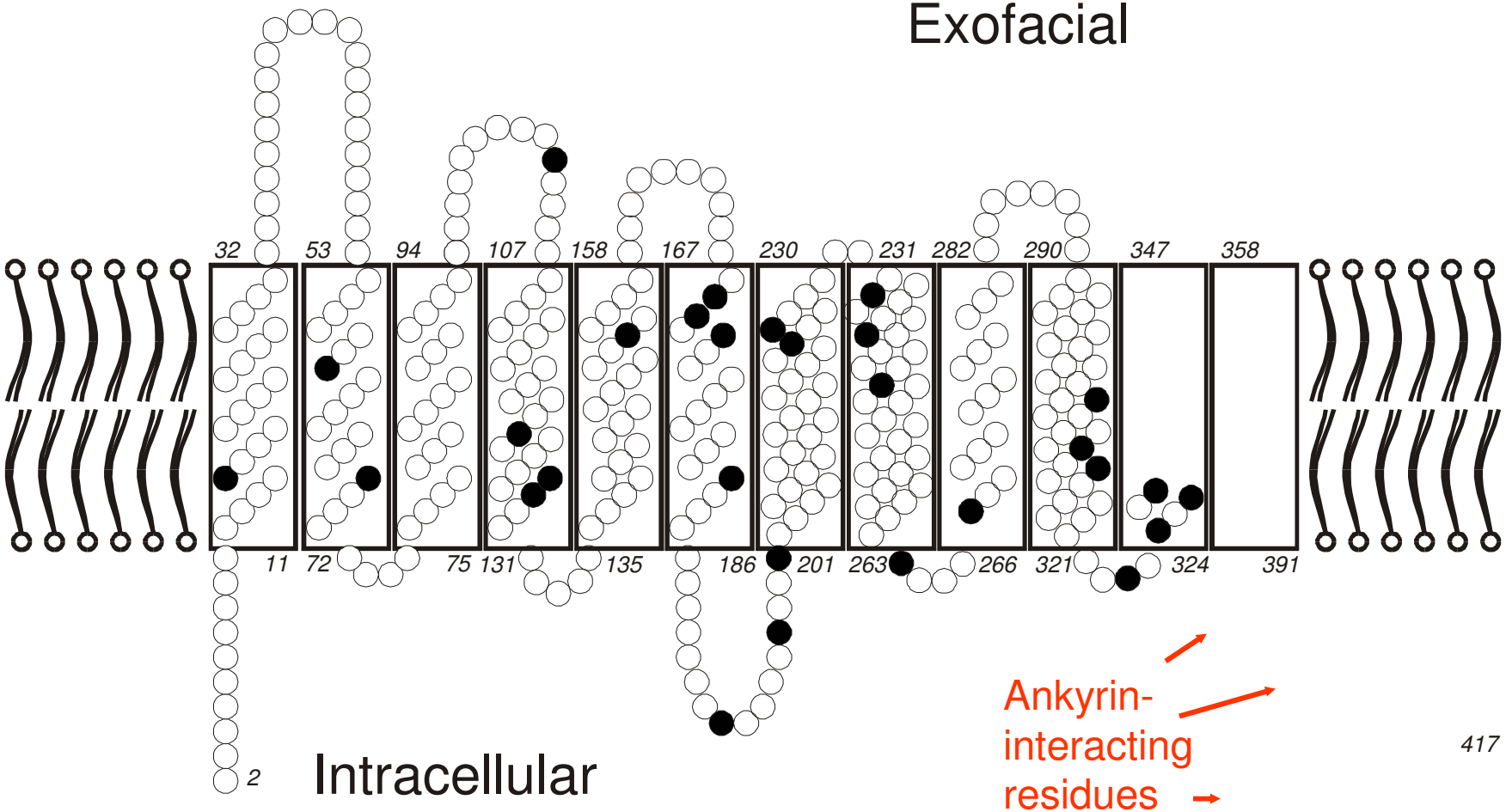
- Nonsense mutations
  - *RHD*(W16X), *RHD*(W90X), *RHD*(Y330X)
- „Small“ deletions/insertions causing frameshift
  - *RHD*(488del4), *RHD*(del711),*RHD*(1253instT)
- Splice site mutations (may express some D)
  - *RHD*(IVS3+1G>A), *RHD*(G212V), *RHD*(1227G>A)
- Complex changes
  - *RHDΨ*
  - Unusual hybrid pattern, „normal“ *RHD*

Importance of C-terminal end



(Nicolas V et al. 2004)

Shortened proteins don‘t fit the membrane



D variants

- Partial D
  - Qualitative change of antigen D
  - Anti-D immunization possible
  - Loss of epitopes defined by monoclonal antibodies
  - Antigen density normal, decreased or enhanced
  - Molecular causes:
    - *RHD-CE-D* hybrid alleles
    - Single amino acid substitution in or near the extracellular loops
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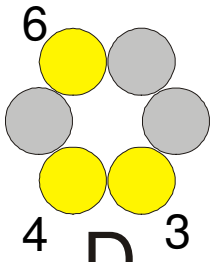
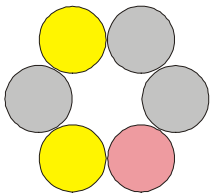
RhD loops and D antigen





FPTT

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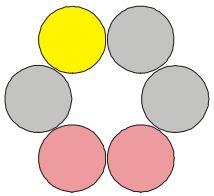
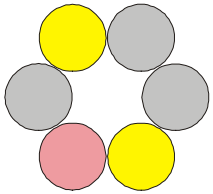


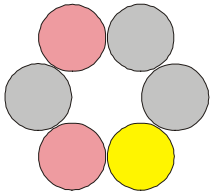
BARC

D cat VI



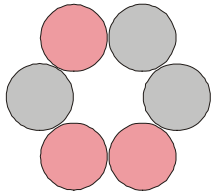
D^w

D cat V

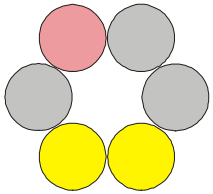


Rh32

DBT

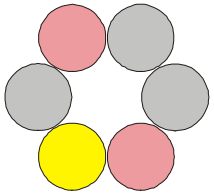


CE



Evans

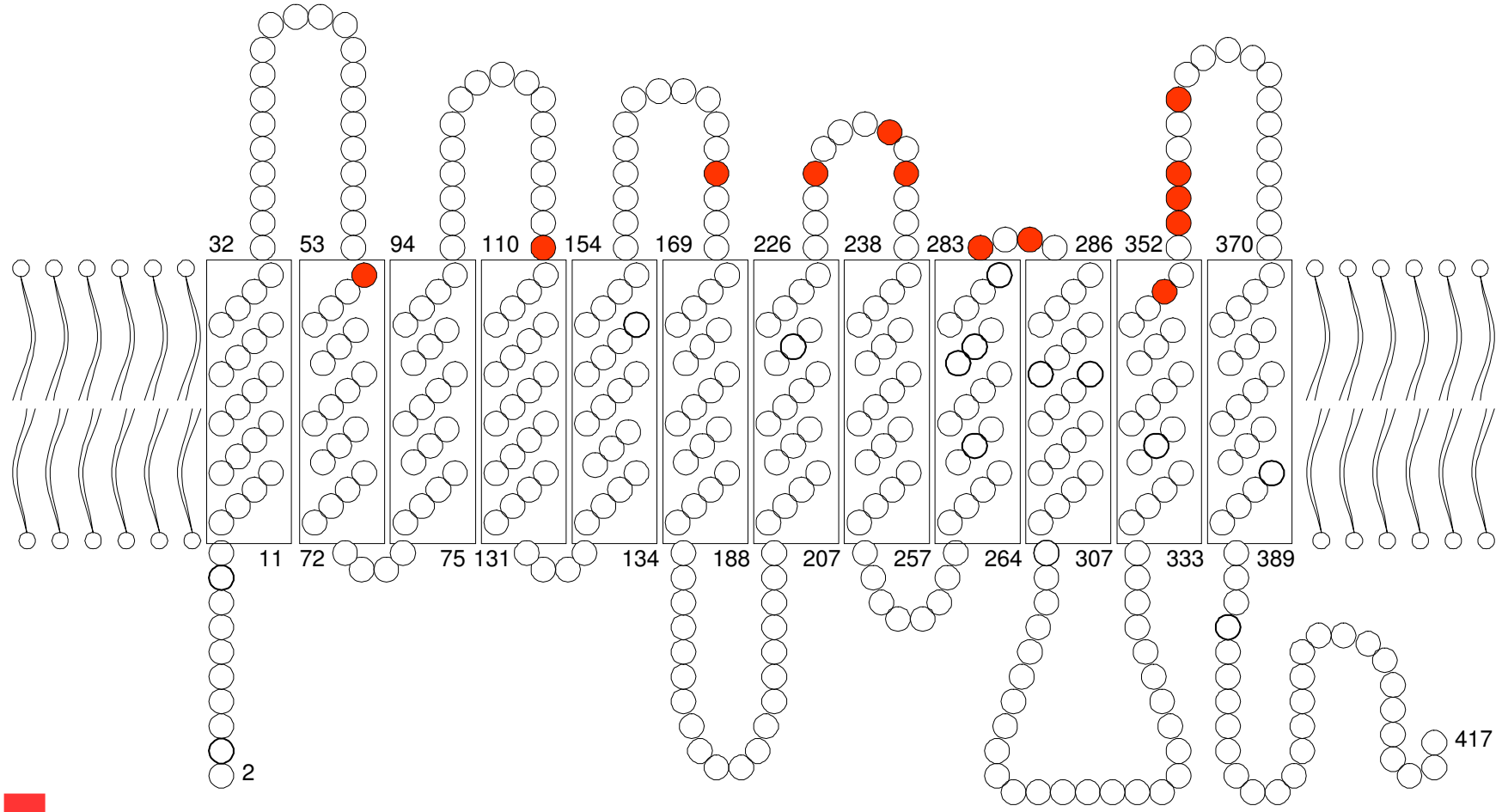
D cat IV



Rh33

DHAR

Single amino acid substitutions in partial D



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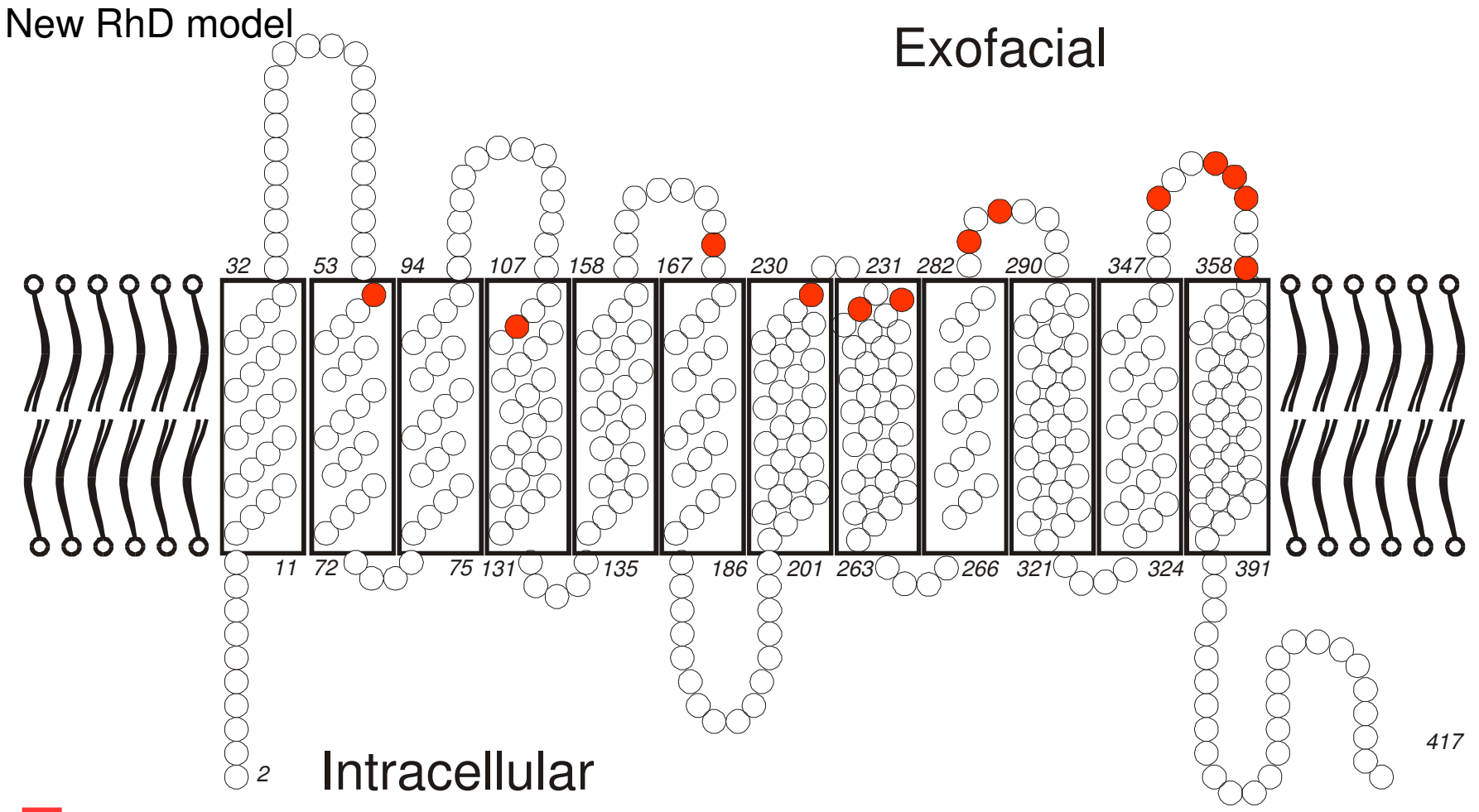

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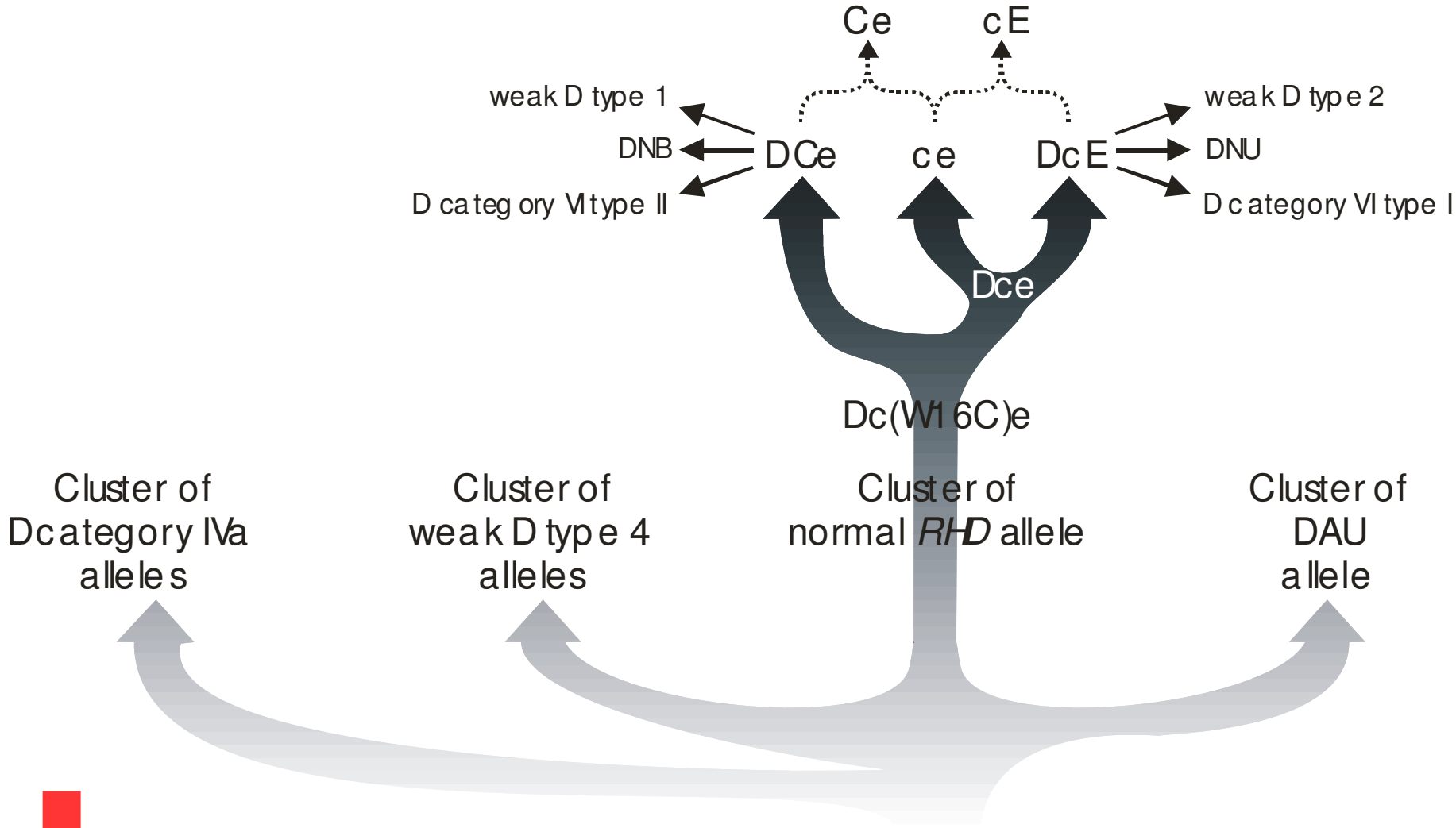
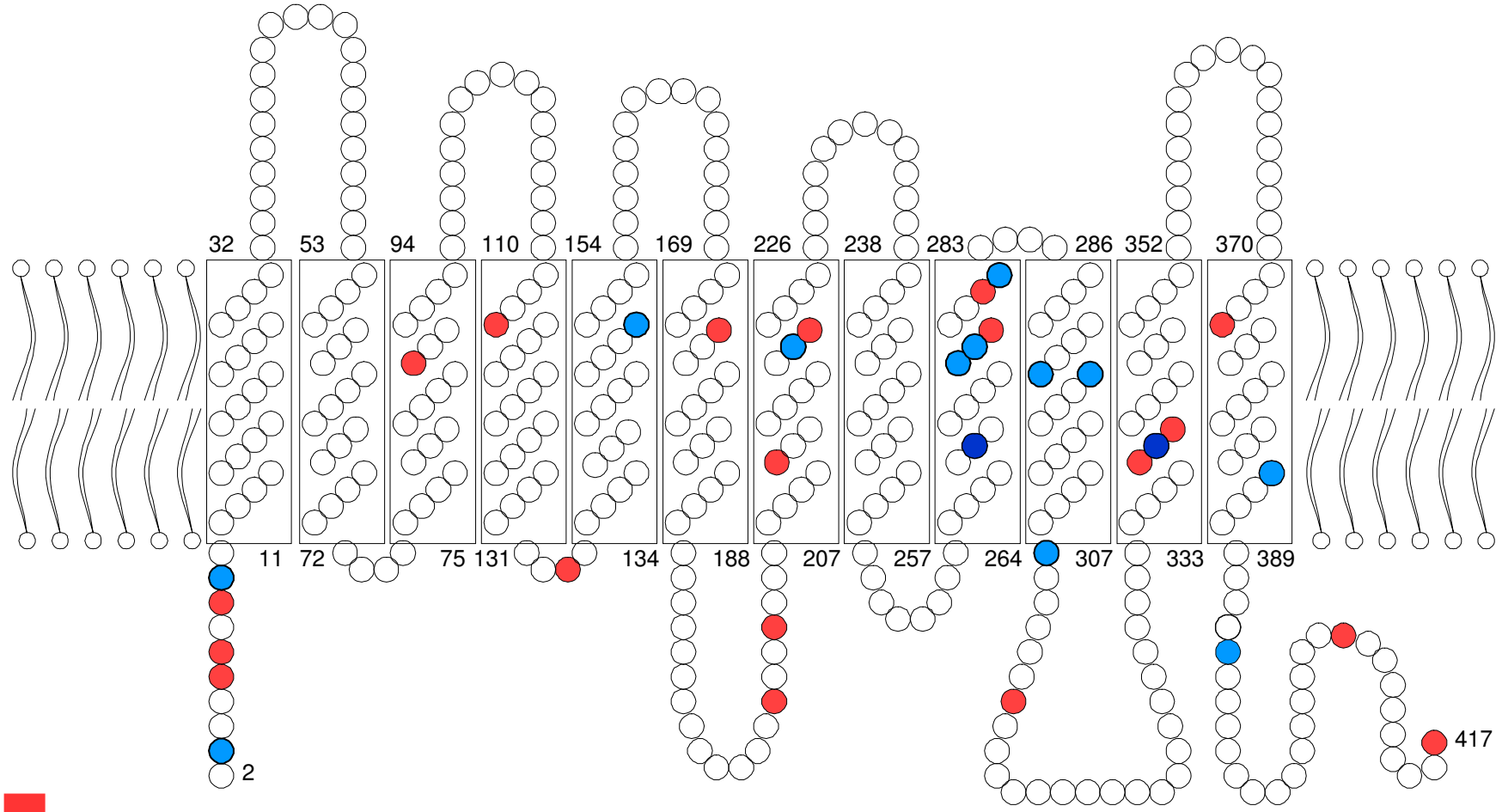
RH phylogeny



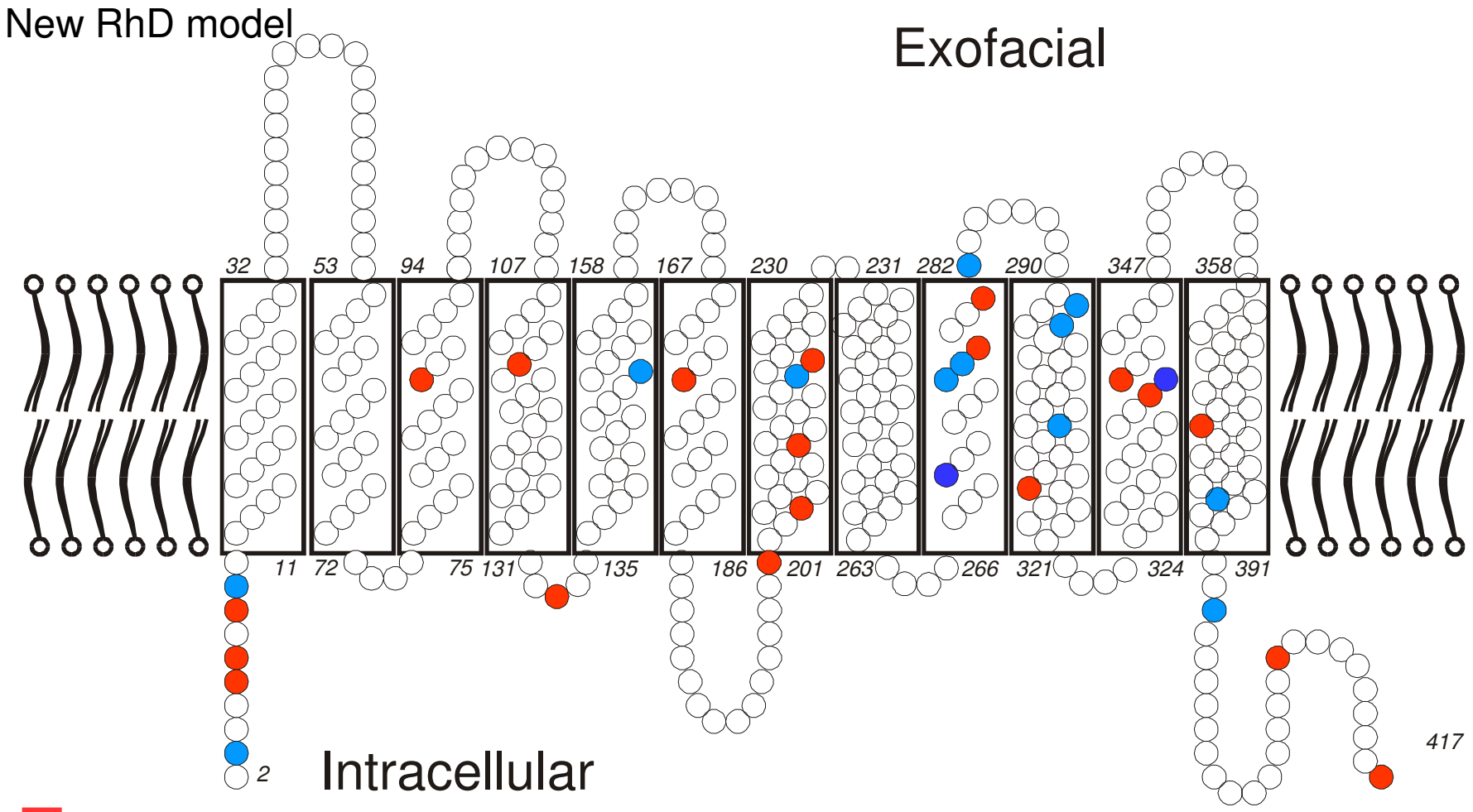
D variants

- Weak D and D_{el}
  - Mainly quantitative reduction of antigen D
  - Usually no Anti-D immunization observed
  - Molecular causes:
    - Single amino acid substitution in transmembraneous and intracellular parts of the Rh protein
    - Multiple dispersed amino acid substitutions
    - Splice site mutations (D_{el})

Single amino acid substitutions in weak D



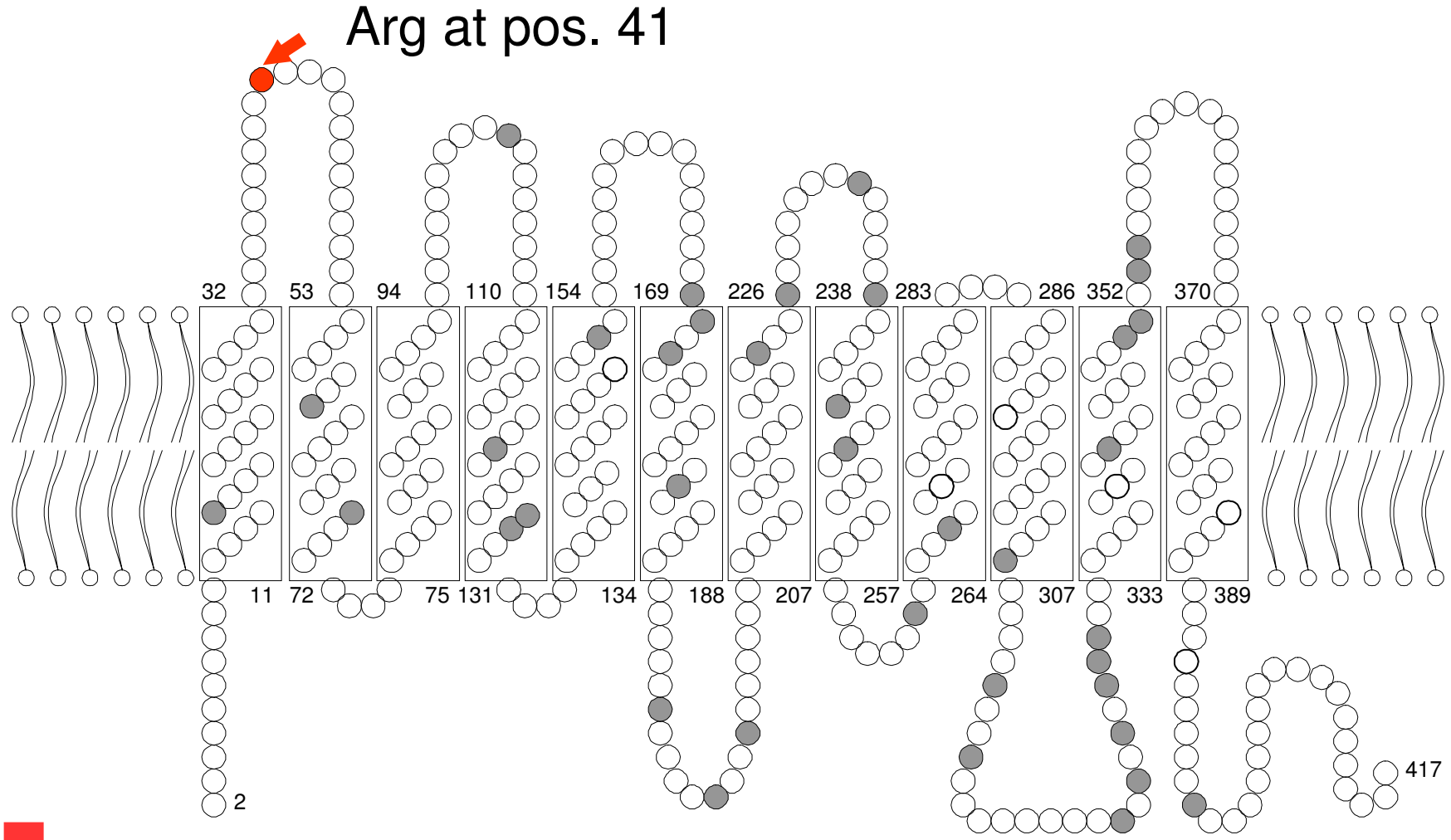
Single amino acid substitutions in weak D



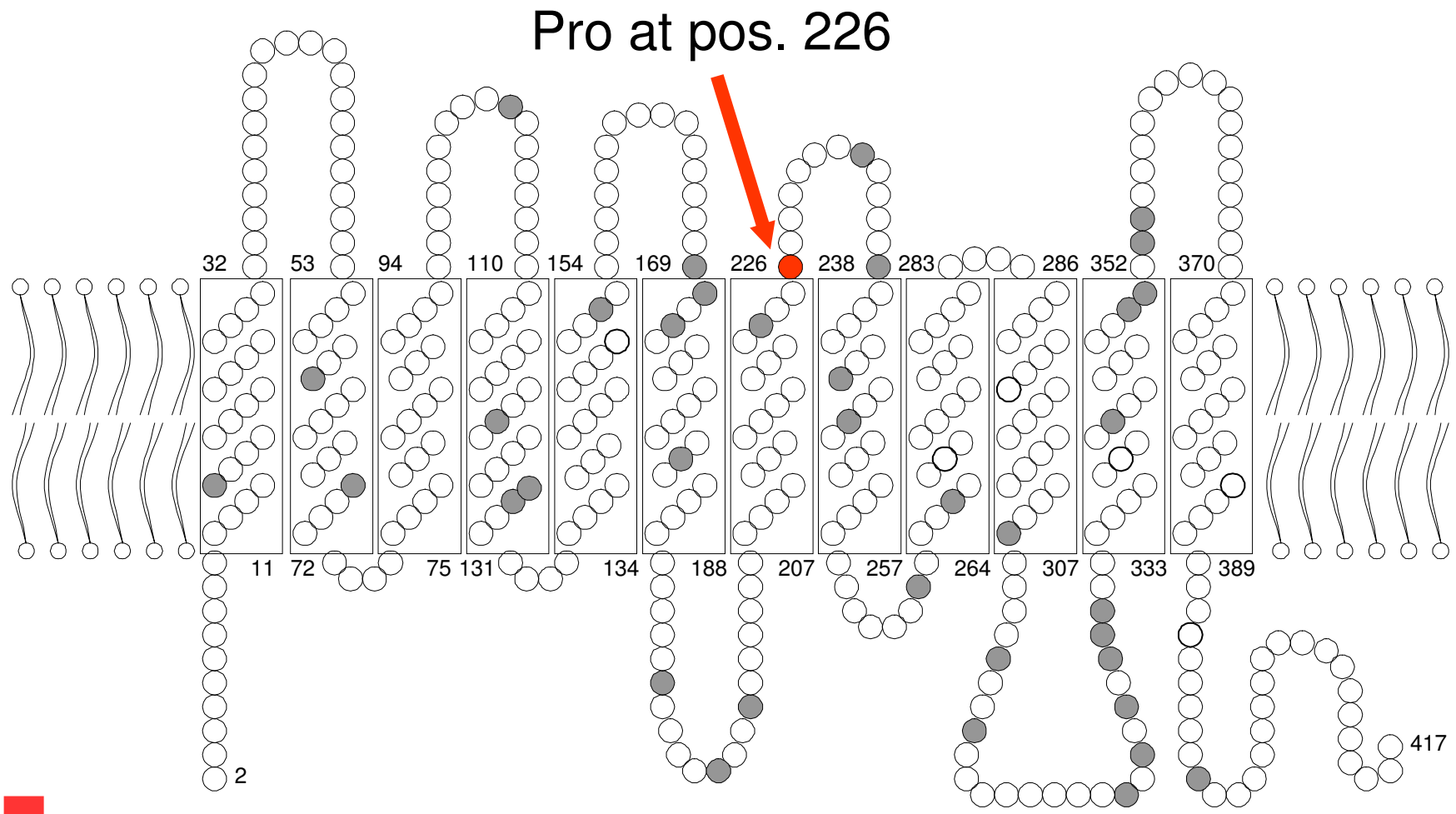
RH Blood Group

- Rh protein structure
  - Structural model
  - Differences of RhD and RhCE
- Genetics
  - Structure of an *RH* gene
  - Structure of *RH* locus and the *RHD* deletion
- Prediction of the phenotype
  - Molecular basis of antigen D
  - Molecular basis of other main antigens
  - Testing strategies, pitfalls and limitations

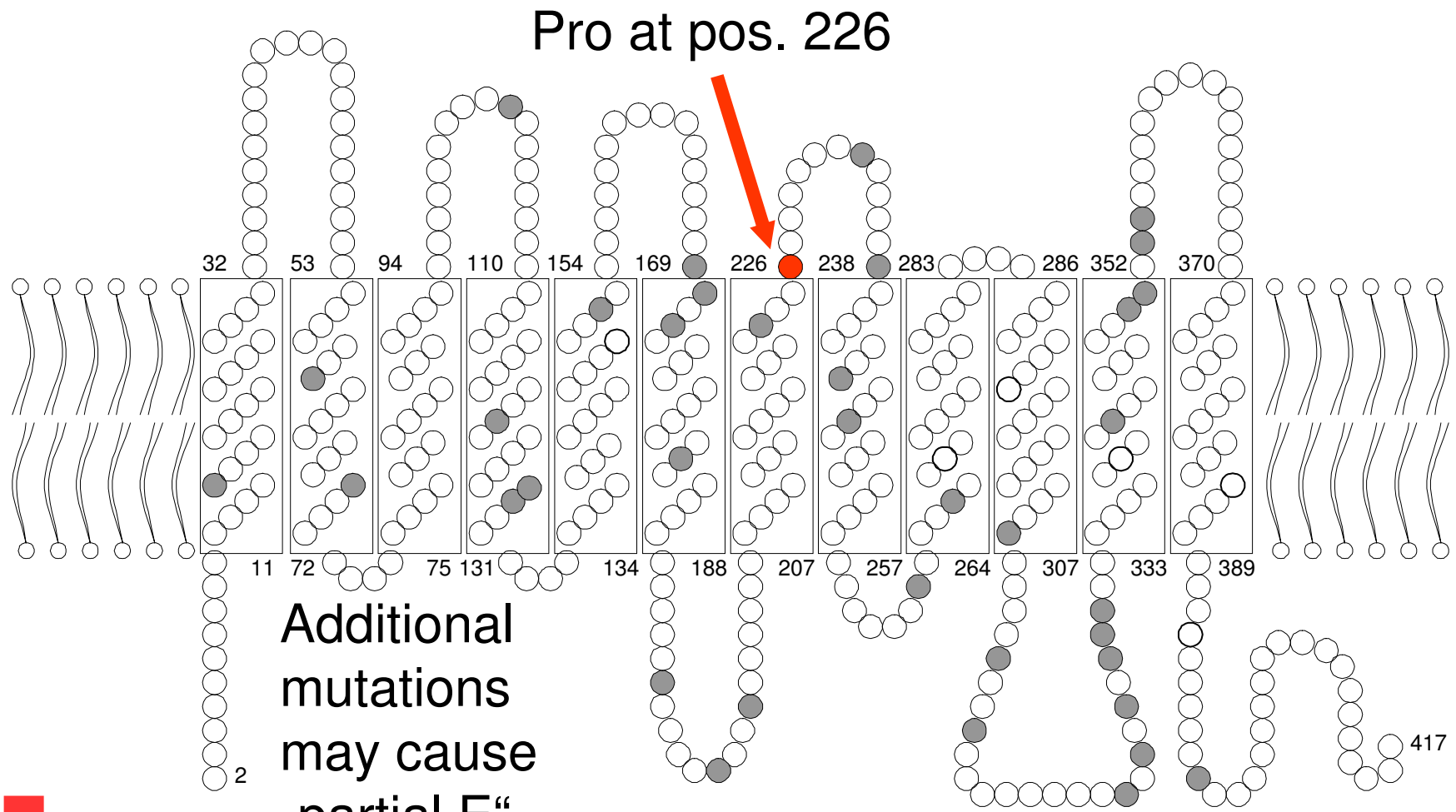
Molecular basis of antigen C^w



Molecular basis of antigen E

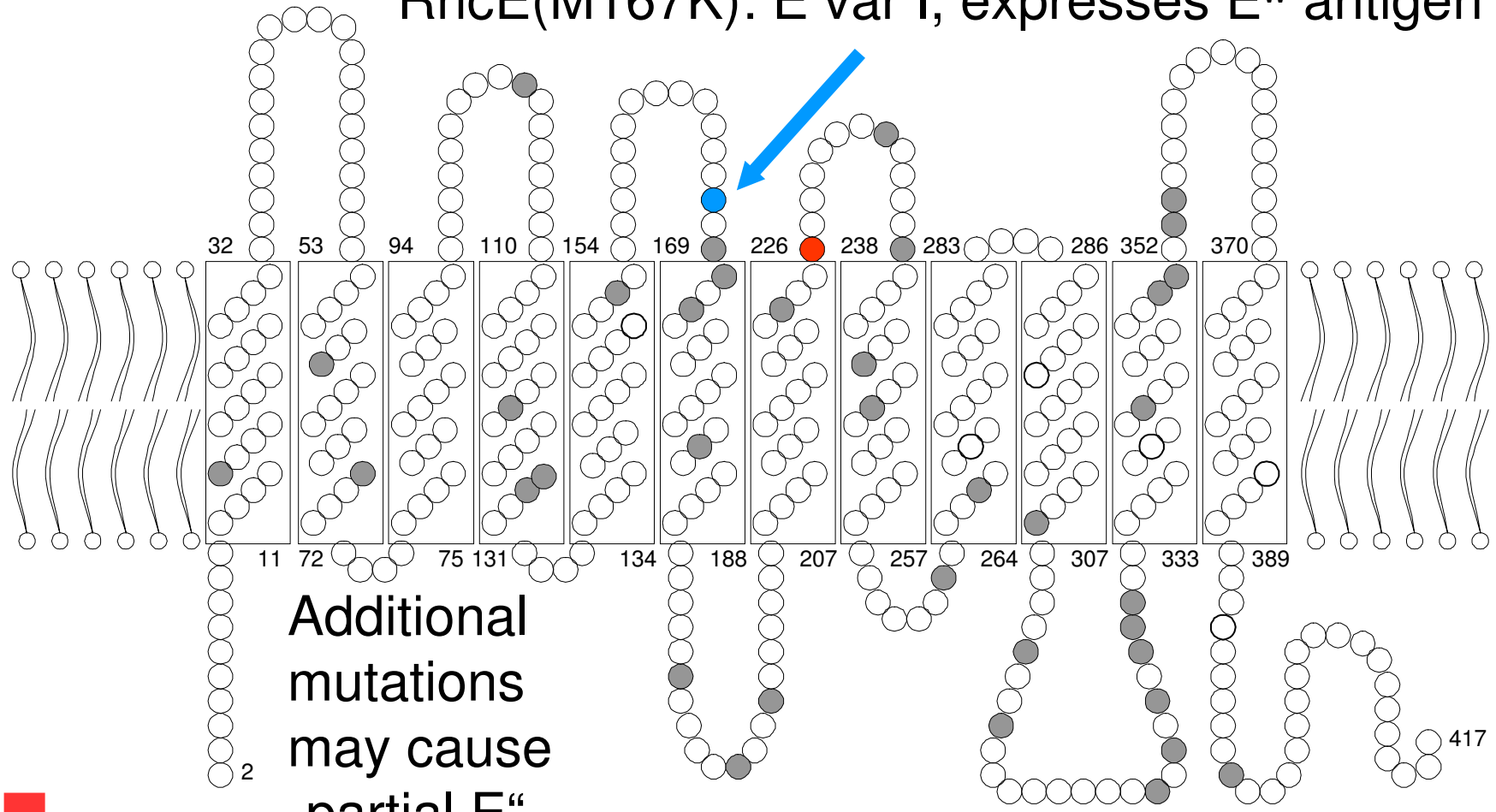


Molecular basis of antigen E



Molecular basis of antigen E

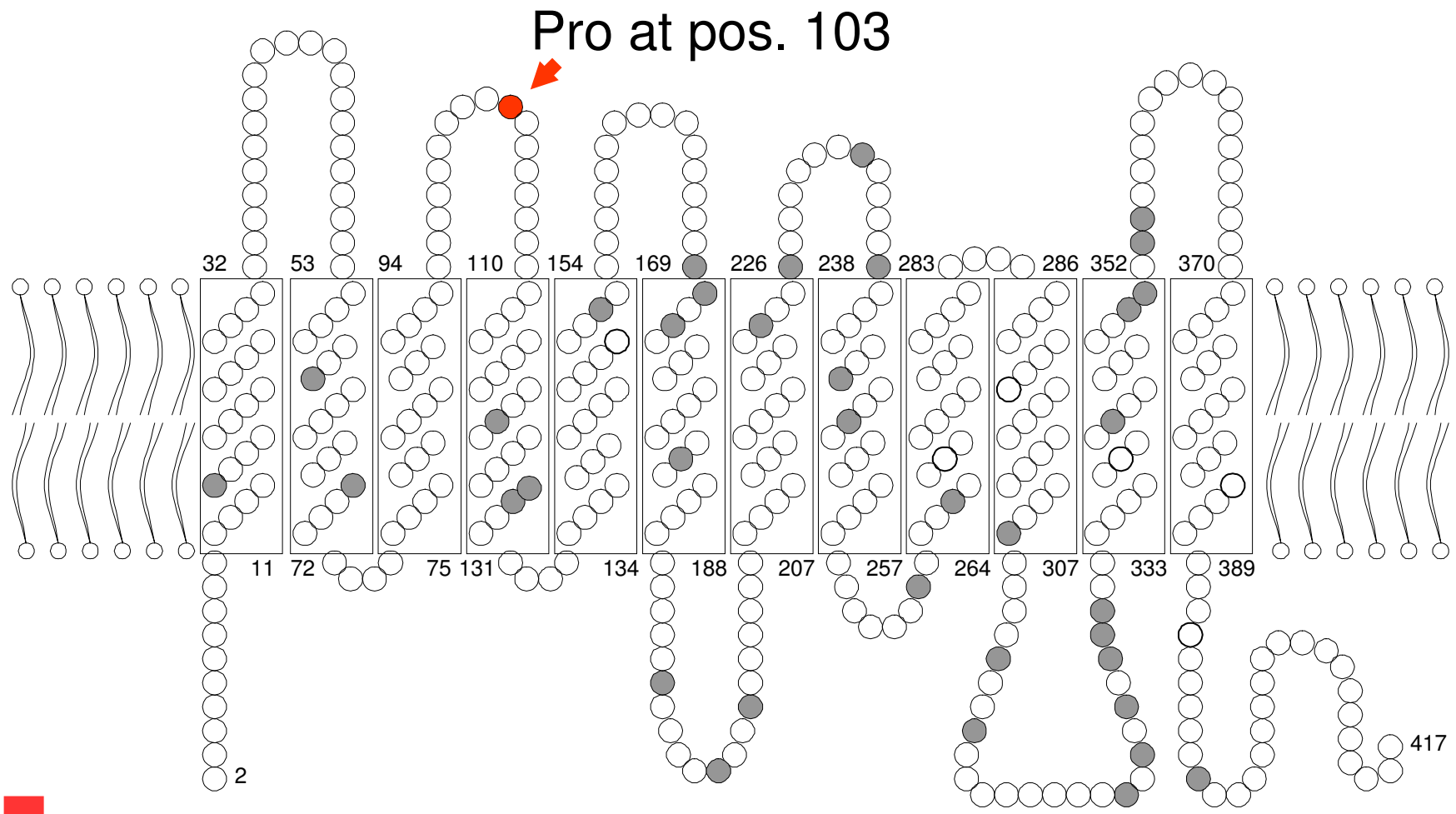
RhcE(M167K): E var I, expresses E^{w} antigen



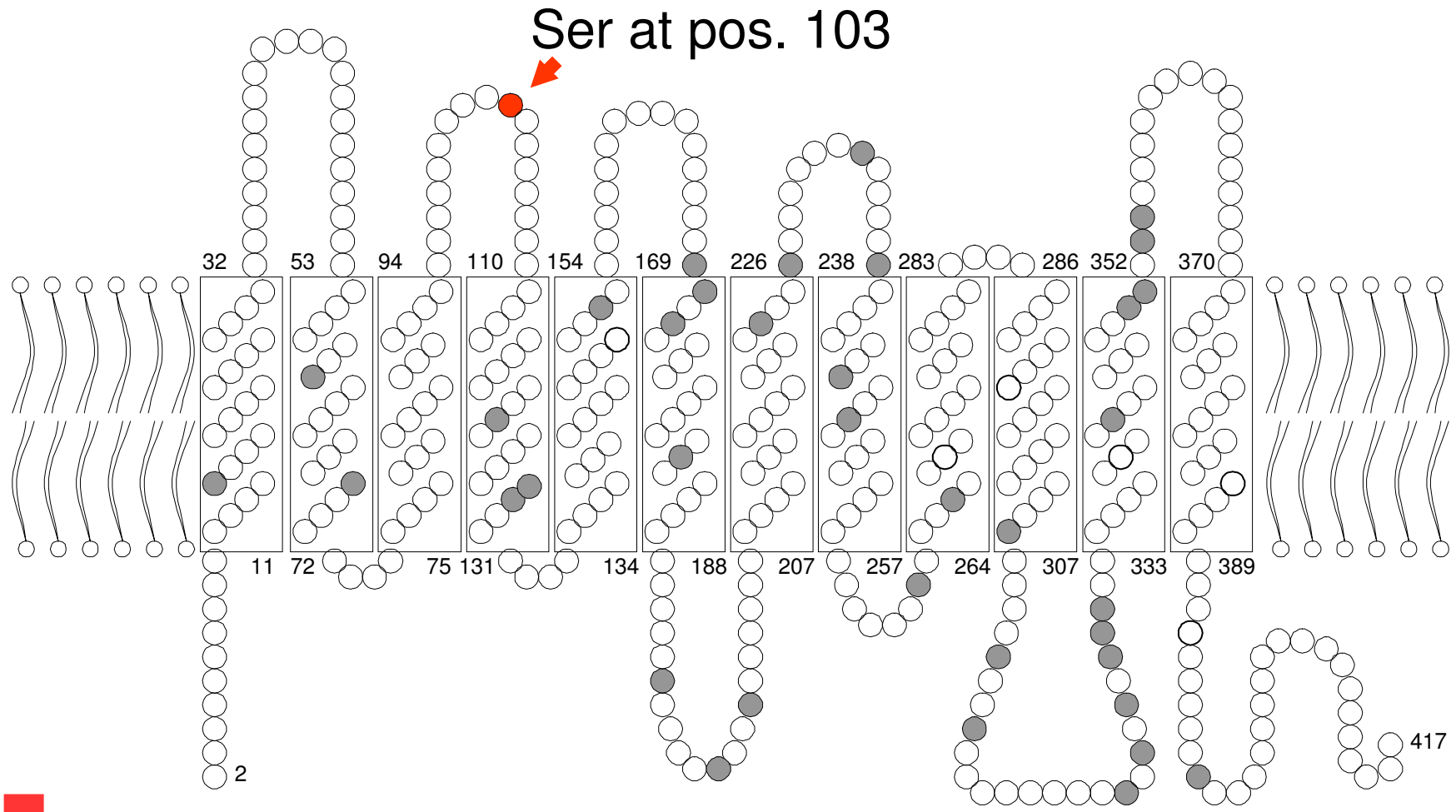
Additional mutations may cause „partial E“

Strobel et al. 2004

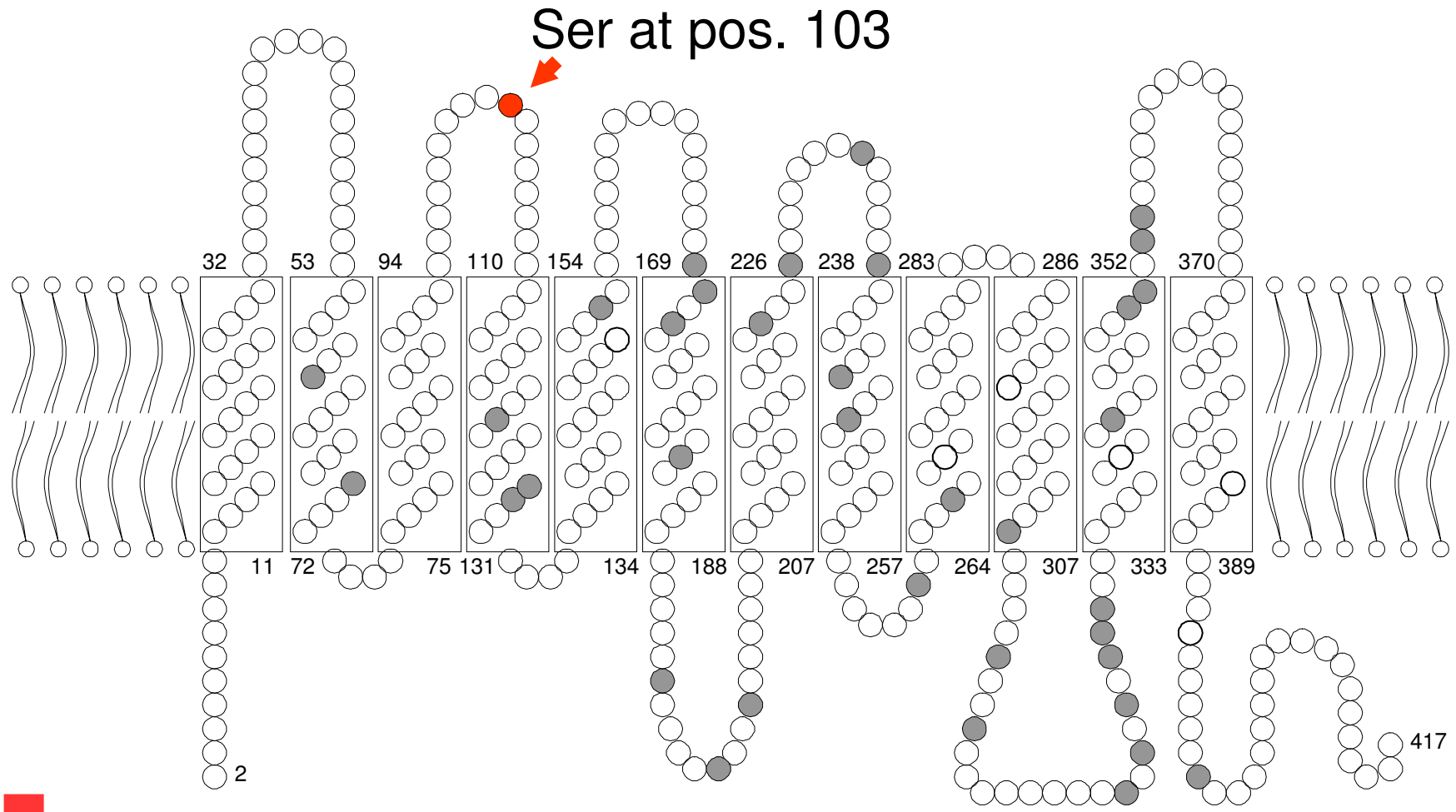
Molecular basis of antigen c



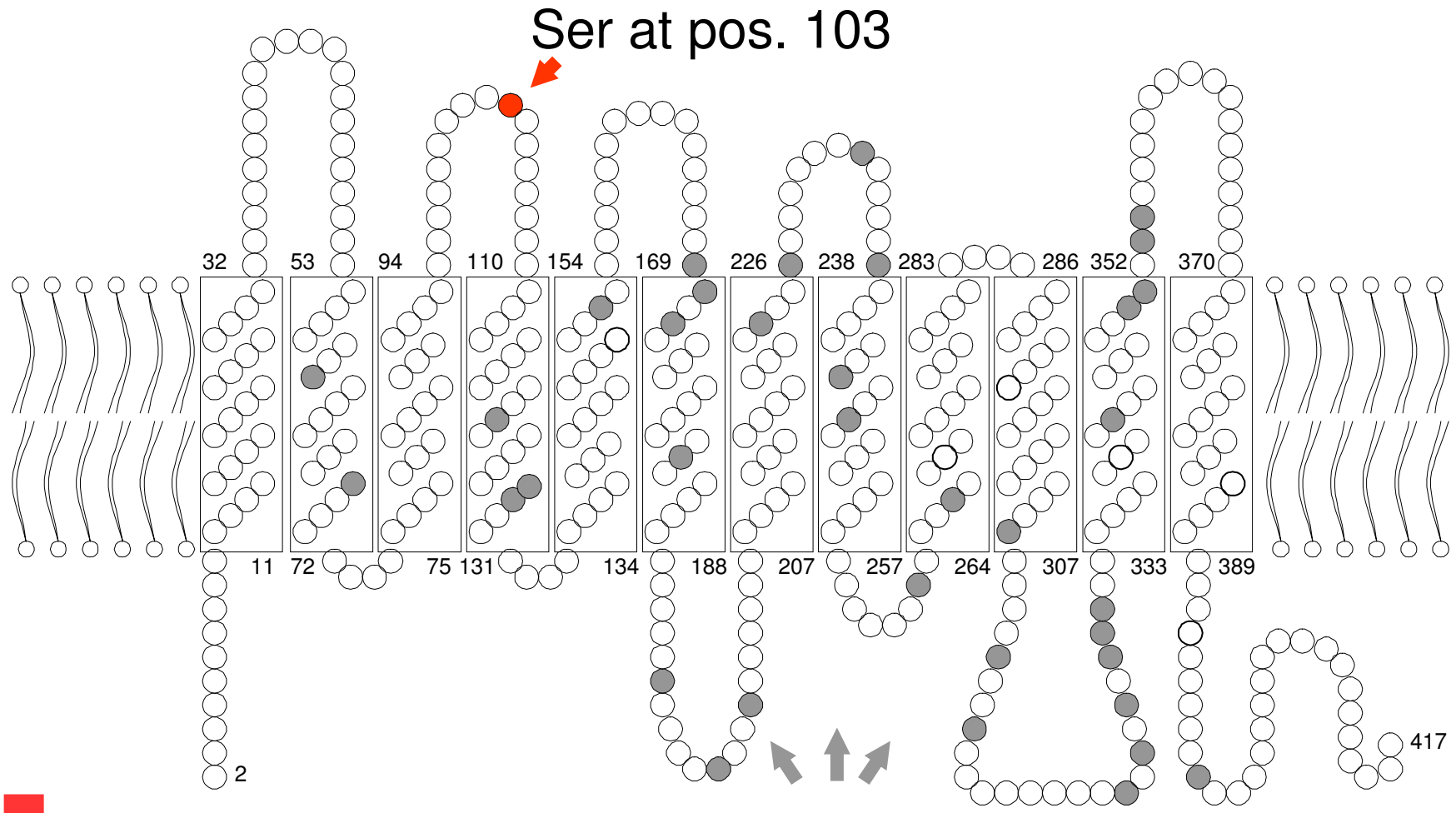
Molecular basis of antigen G



Molecular basis of antigen C

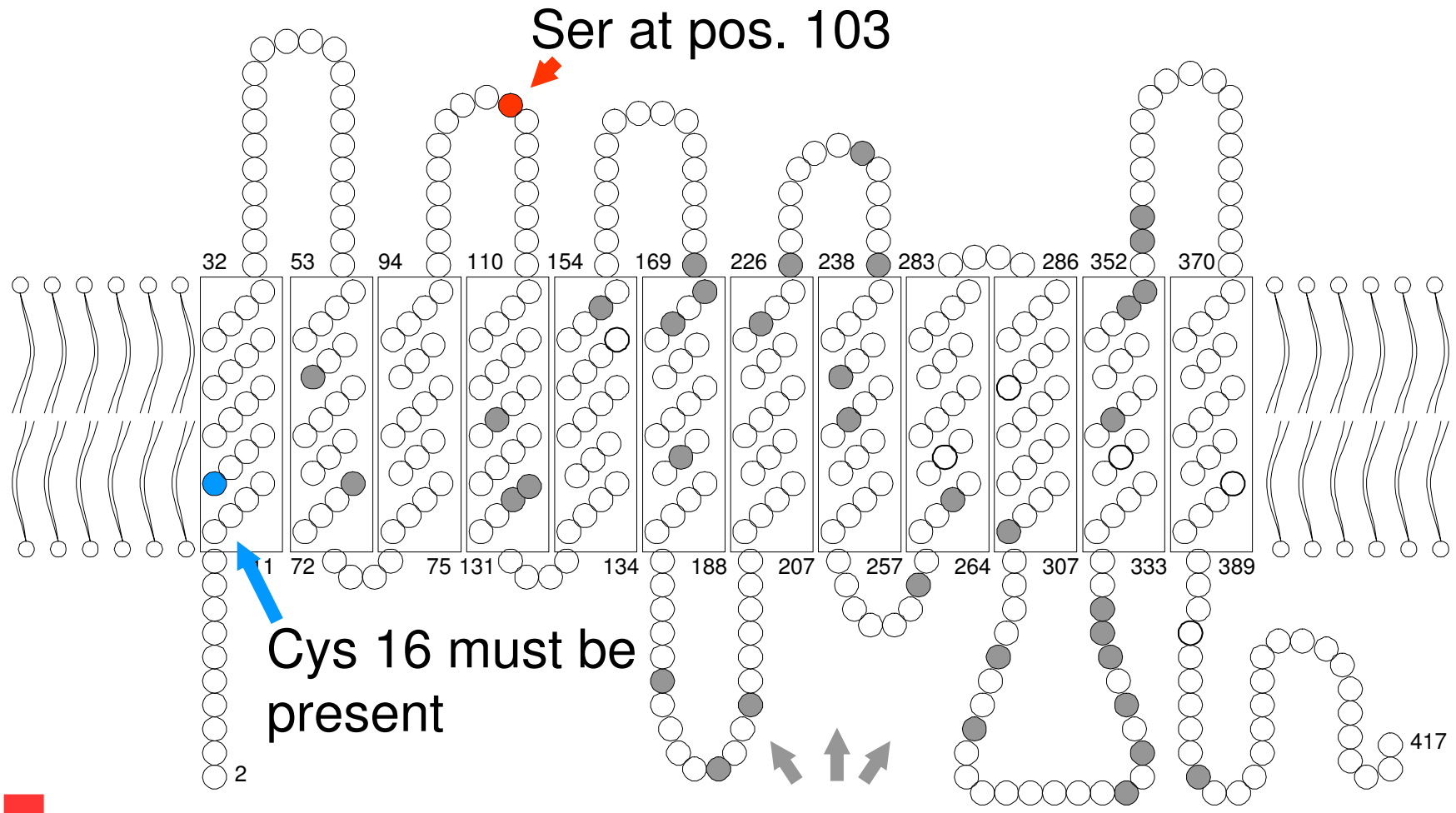


Molecular basis of antigen C

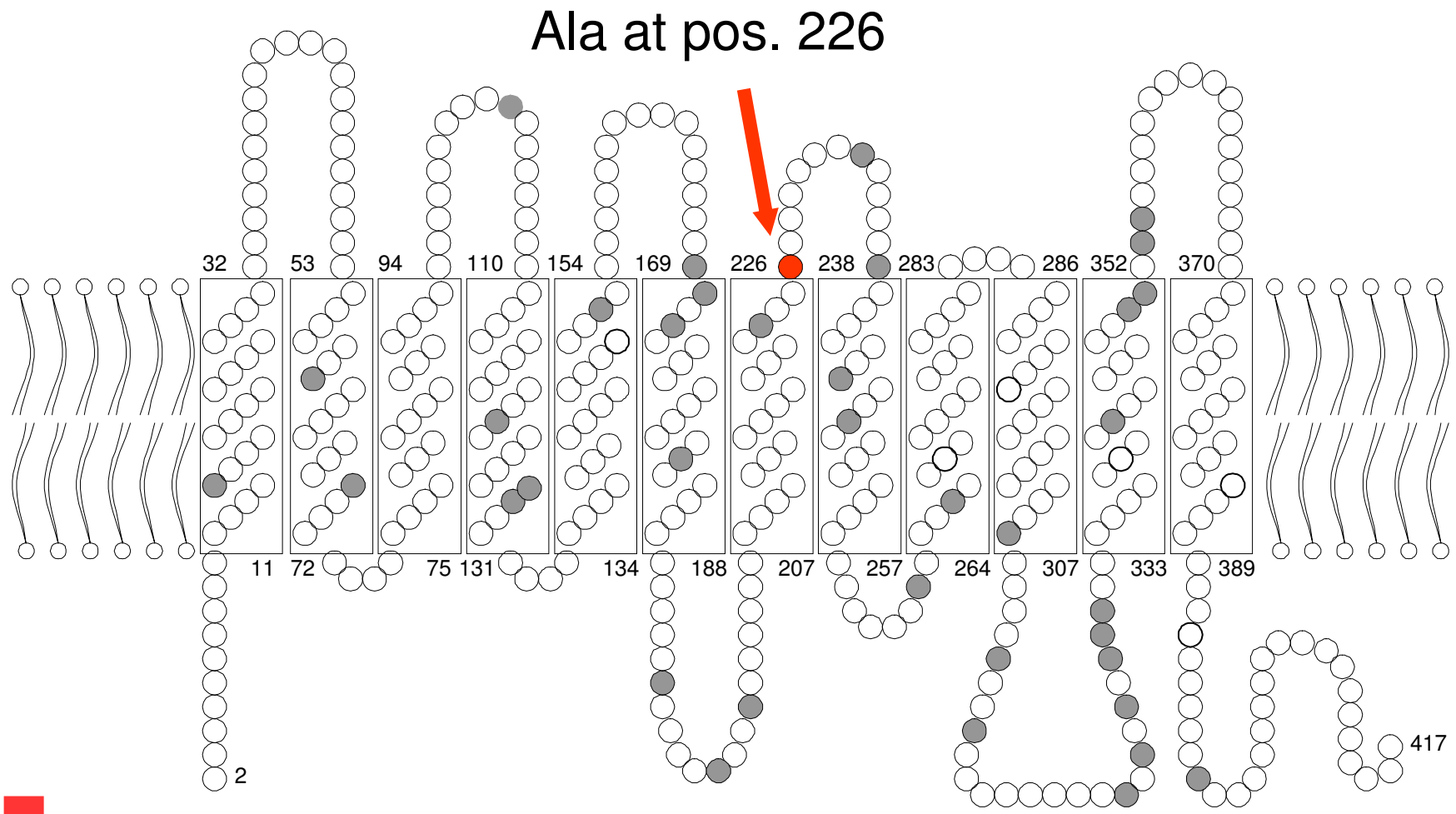


Overall structure must be RhCE

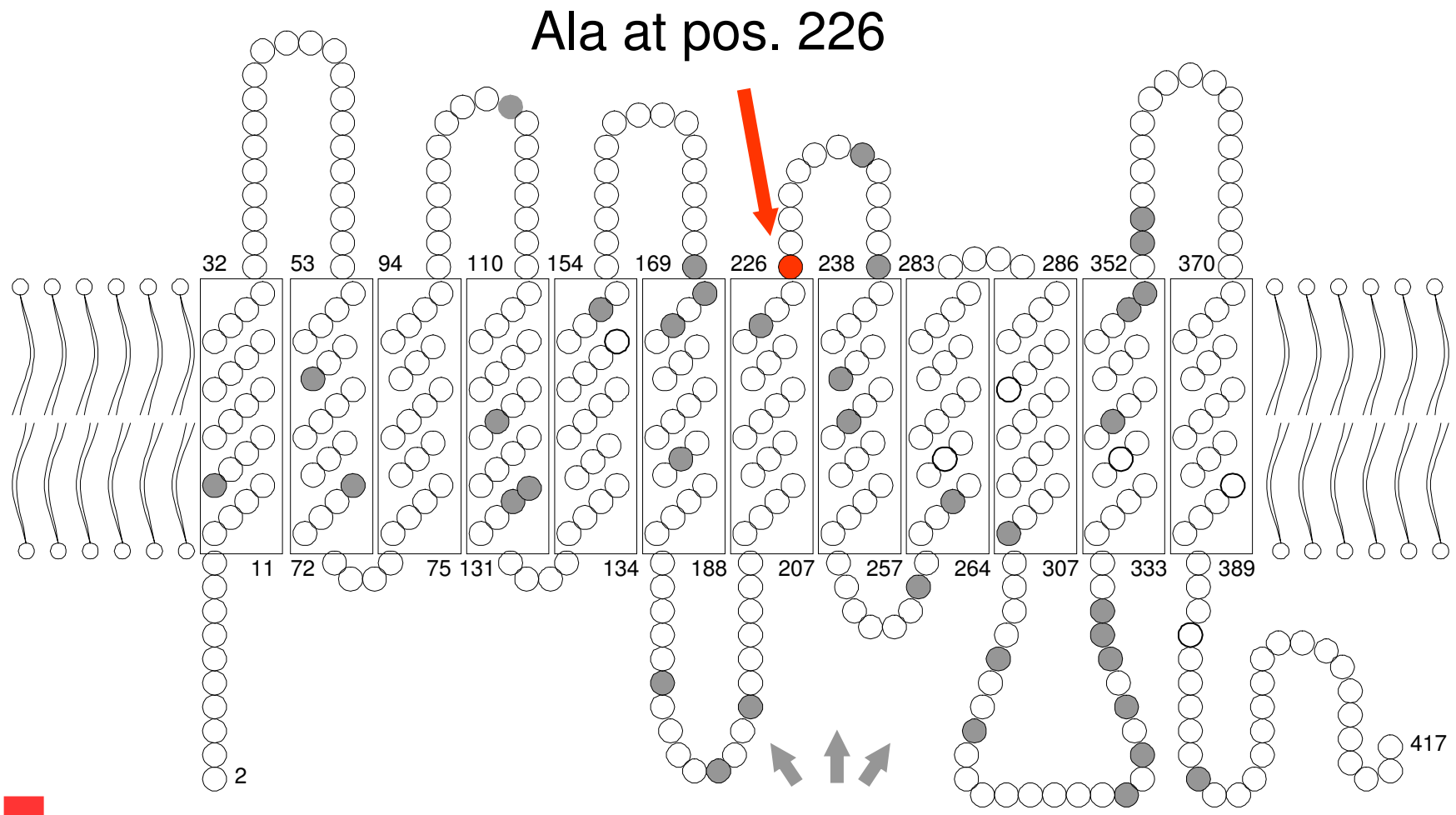
Molecular basis of antigen C



Molecular basis of antigen e

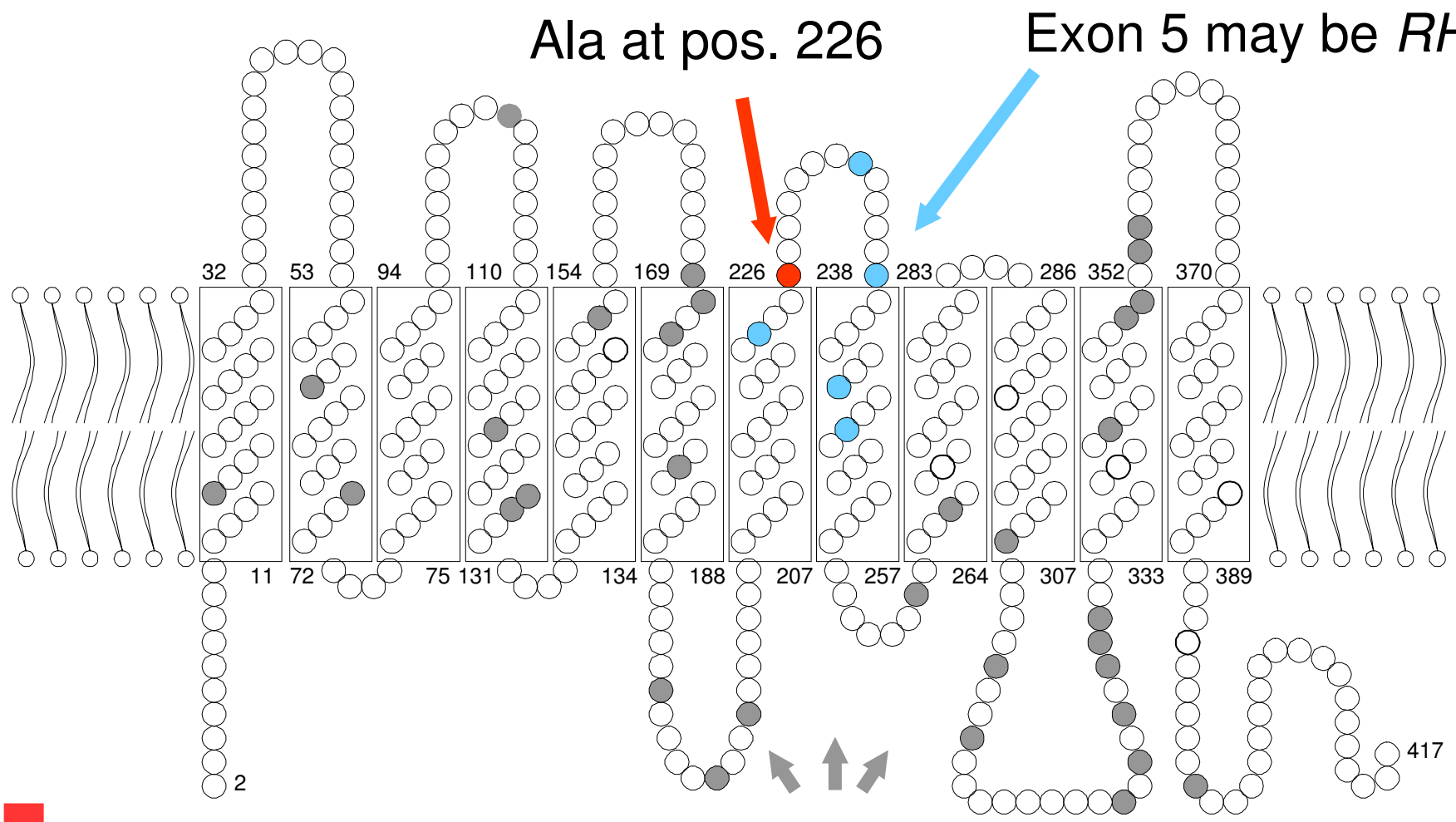


Molecular basis of antigen e



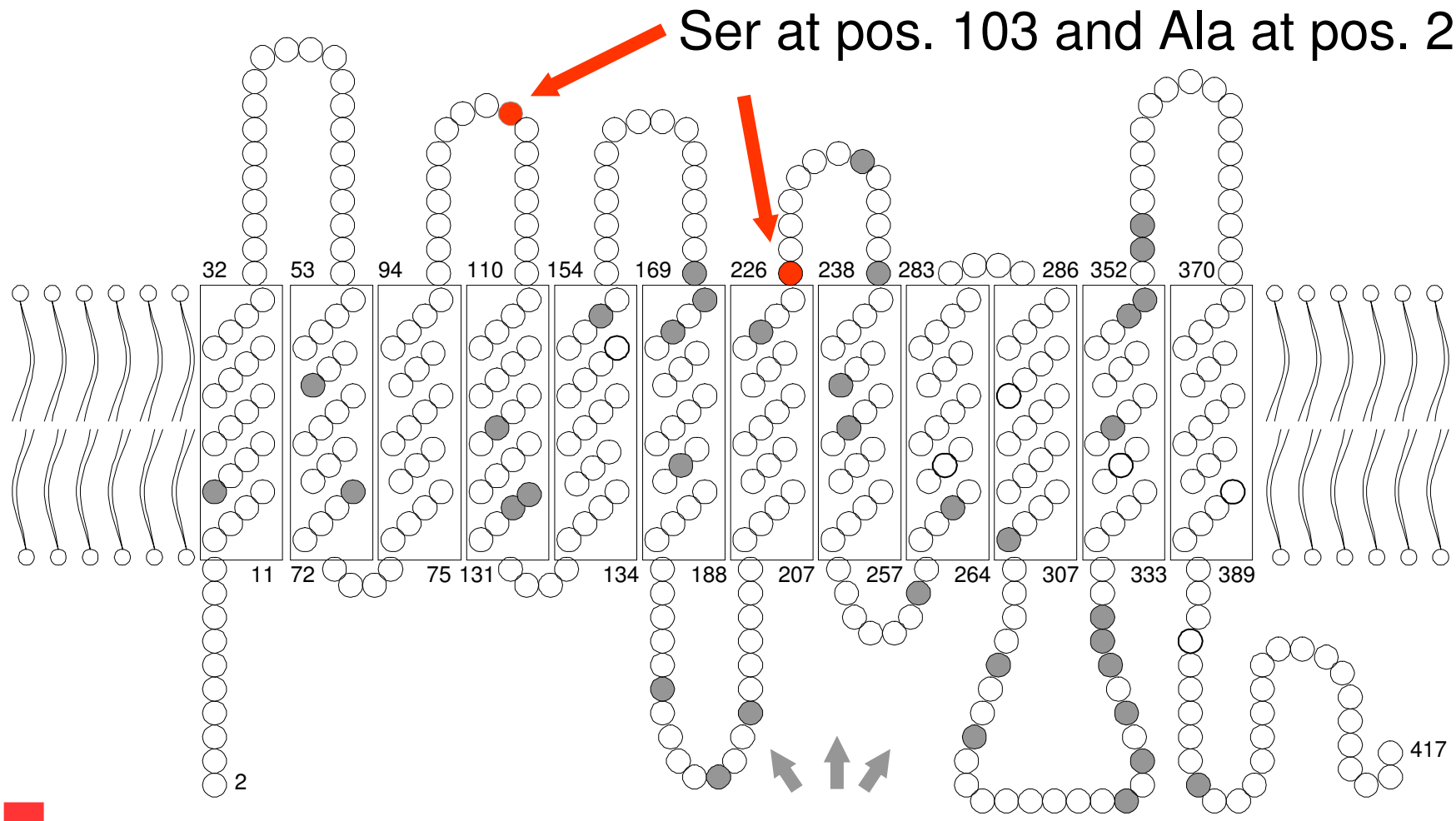
Overall structure must be RhCE

Molecular basis of antigen e



Overall structure must be RhCE

Molecular basis of antigen Ce (Rh7)



Overall structure must be RhCE

Further complexities

- „African“ *RHCE*-variants
- -D-
- Rh_{null}
  - Amorph type:
    defect of *RH*
  - Regulator type:
    defect of *RHAG*

RH Blood Group

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  - Molecular basis of other main antigens
  - Testing strategies, pitfalls and limitations

Purpose of molecular testing

- Antigen prediction in the absence of suitable RBC
  - Prenatal diagnoses
  - Recently transfused patients
  - Positive DAT and similar serologic problems
- Detection of weak antigens
  - Donor screening
  - Confirmation of serologic results
- Characterization of antigenic variants
  - Discrimination weak D / partial D alleles
  - Characterization of variants of other antigens

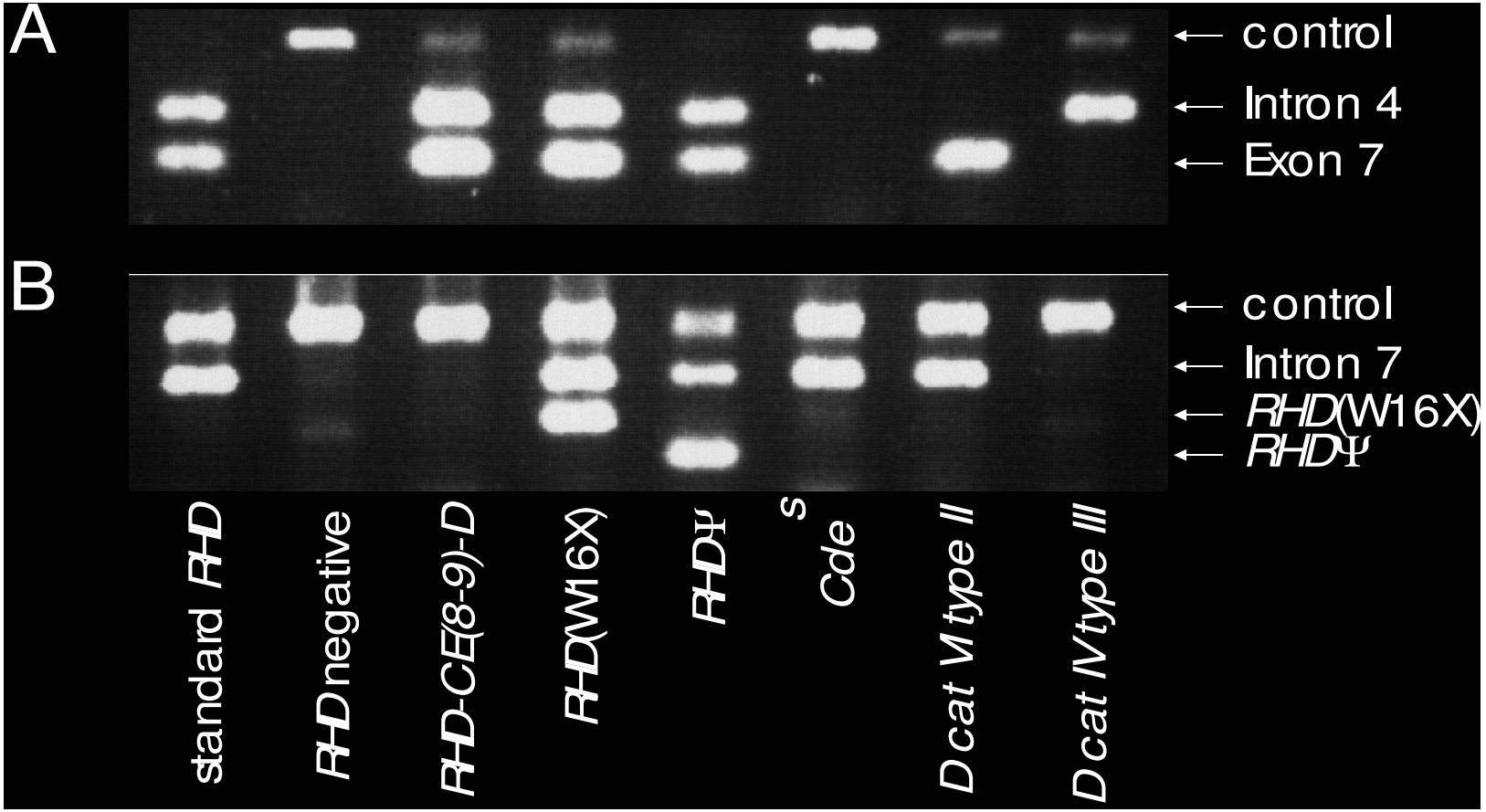
Testing strategies

- Direct check for antigenic polymorphisms
  - Antigen C^w prediction
- Indirect check for antigenic polymorphisms
  - Antigen C prediction based on intron 2
  - Antigen D prediction based on intron 4
  - Antigen D prediction based on non-exofacial positions
- Exclusion of additional polymorphisms

Strategies for the prediction of D antigen

- *RHD* PCR should focus on loops 3 to 6
  - Corresponds to *RHD* exon 4 to 7
    - Absence of *RHD* in these loops => D negative
    - Presence of *RHD* in all loops => likely D positive
    - Presence of *RHD* in some loops => likely partial D
  - Many partial D and hybrid-type D negative will be typed correctly
- „Other“ changes must be detected individually
  - *RHD*psi, frequent stop/splice/frameshift mutations

A solution to antigen D prediction



Exclusion of additional polymorphisms

- Which alleles must be detected?
  - Alleles found as single case report?
  - Alleles detected in a single region?
  - Alleles detected by a single group?
  - Alleles with very low frequency?

  - ✓ D negative alleles
  - ? D_{el} alleles
  - ? Partial D alleles
  - ? Weak D alleles
  - ? *RHCE*-alleles with unexpected D-expression (e.g. ceRT)

Strategies for the prediction of antigen C

- Cys at position 16
  - Shared by most c-alleles of cDe (Africans!)
  - Absence predicts C negative phenotype
- *RHD* exon 2
  - Also present in *RHD* (e.g. cDE)
  - Absence predicts C negative phenotype
- C-specific insertion in intron 2
  - Best predicting polymorphism in Europeans
  - Fails in *Ccde*^{s}

Strategies for the prediction of antigen e

- Usual approach: Ala 226 in *RHCE* exon 5
  - Fails in *RHCE-D(5)-CE*
  - No information for *RHD-CE(5)-D*
  - Minimal *RHCE*-context unknown

Prediction of antigen Ce (Rh7)

- Simultaneous presence of Ser 103 and Ala 226 in *RHCE*
  - Ser 103 coded by exon 2
  - Ala 226 coded by exon 5
- Two problems:
  - Distance on DNA: ~ 17,000 bp
  - Simultaneous testing of 3 determinants:
    - Ser 103, Ala 226, RhCE

Additional pitfalls in Rh antigen prediction

- Regulator-type Rh_{Null}
  - Normal Rh genes, no Rh antigens
- Sporadic mutations
  - How many samples and regions must be tested?
- Unexplained non-expression or Rh proteins
  - D negative type with anti-D despite „normal“ *RHD*

More facts --- more questions