Molecular characterisation of weak Lu^a and Lu^b expression associated with altered *LU*01* and *LU*02* alleles

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Donor samples

- 10 suspected **Lu(b-)** donor samples identified by serological screening at NHSBT South London using diluted monoclonal anti-Lu^b
- referred to IBGRL for phenotype confirmation
- At IBGRL, donor cells tested with anti-Lu^a and two examples of undiluted anti-Lu^b

One sample = $Lu(a+^{wk}b+^{wk})$

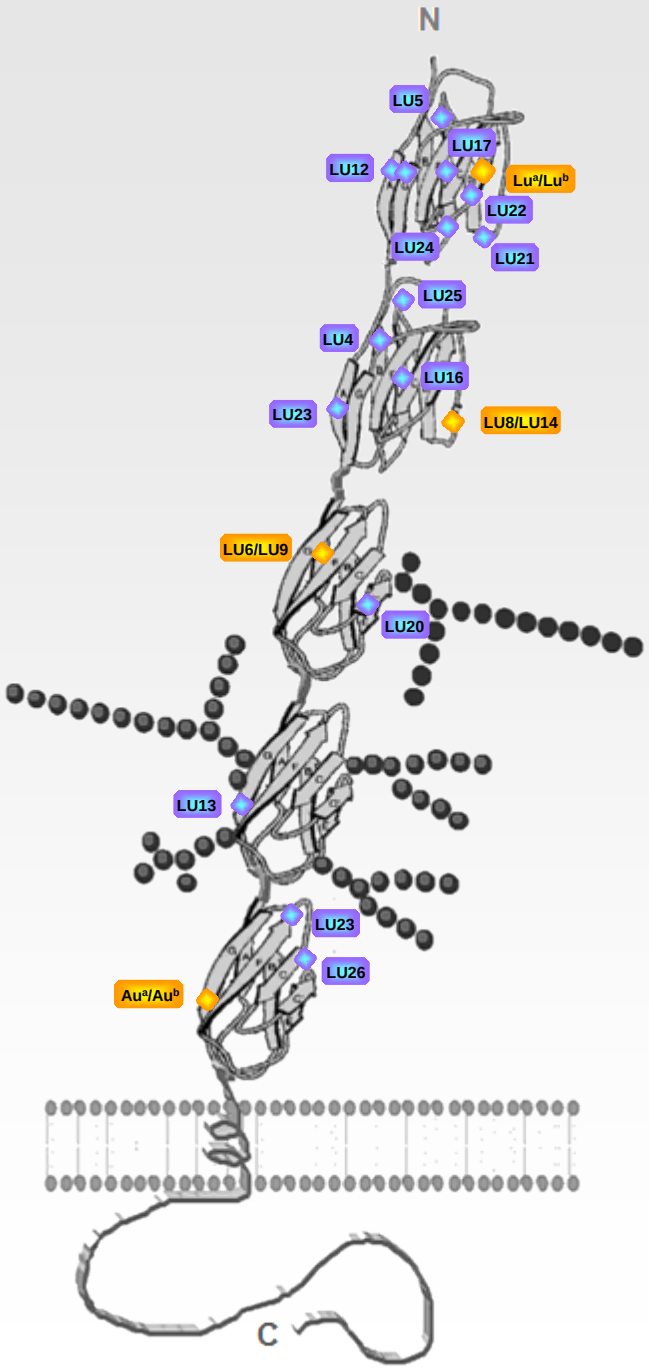
Nine samples = $Lu(a+b+^{wk})$

UK National Rare Donor Panel

- Currently donors with rare blood types identified through serological screening at NHSBT South London and smaller regional programs and routine extended phenotyping
- Aim to identify donors whose cells lack high incidence antigens
- First established in the UK in 1952 to meet the demand for rare blood types
- NRDP includes ~ 2000 active donors

Co^a, Ge, I, k, Kp^b, Lu^b, Wr^b, Vel, Lan, Yt^a

Lutheran system



- Cell adhesion molecule, laminin receptor
- 24 antigens (LU1-LU26) carried on two glycoprotein isoforms - Lutheran and BCAM
- LU10 and LU15 designated obsolete

- 4 pairs are antithetic antigens: Lu^a/Lu^b (LU1/LU2), LU6/LU9, LU8/LU14, Au^a/Au^b (LU18/LU19)
- Remaining 16 antigens are of high frequency

Lutheran null phenotypes

Lu(a-b-) dominant type; In(Lu)

Heterozygous for a dominant suppressor gene KLF1

In(Lu) suppresses all Lutheran antigens but also a number of red cell antigens in other systems [AnWj, P_1, i, CD44 (Indian system), CR1, MER2]

Numerous molecular backgrounds
Rare

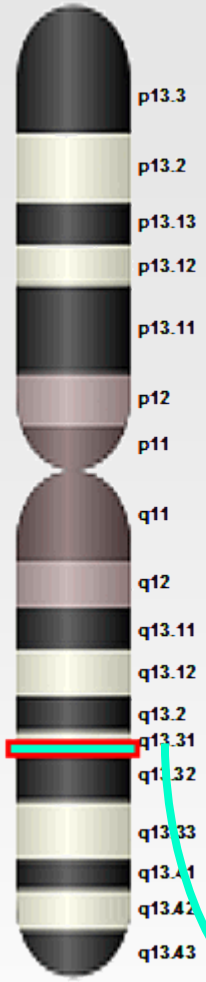
Lu(a-b-) recessive type

Homozygous for a recessive allele at the LU locus

Cells lack all Lutheran antigens

Five different molecular backgrounds
Very rare

BCAM (*LU*) gene

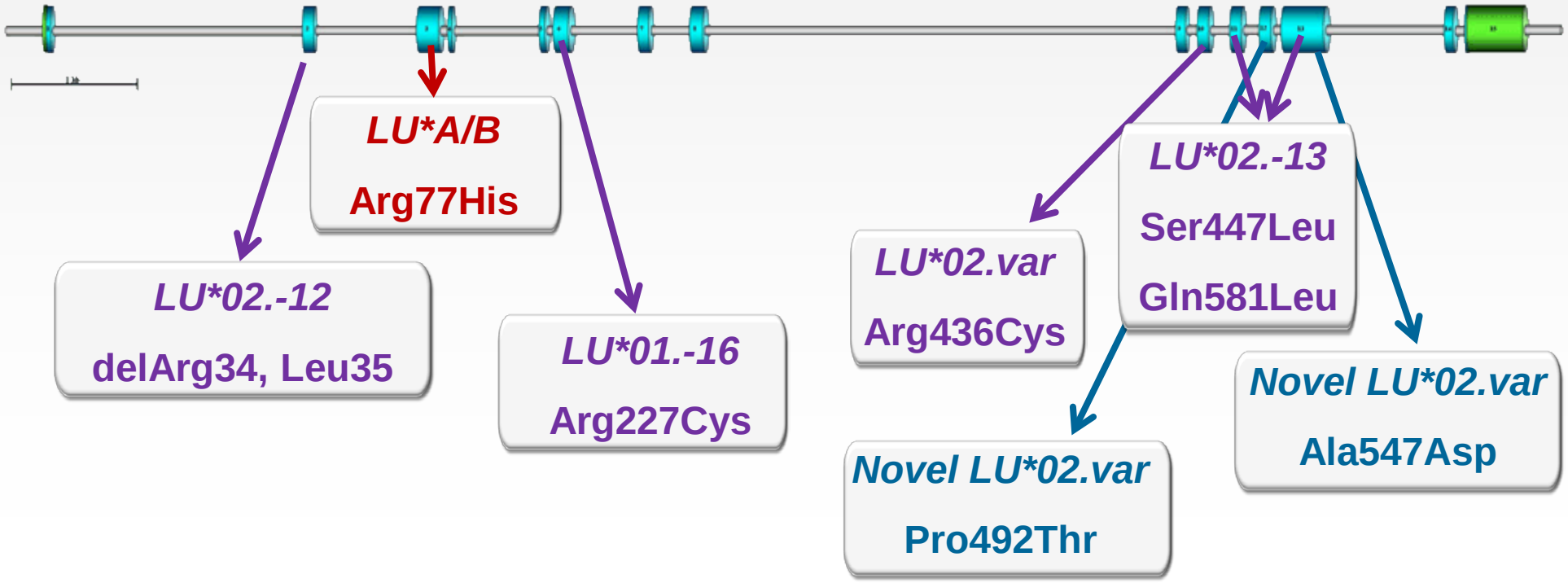


- Lu-glycoprotein is encoded by a single gene *BCAM* (*LU*)
- *LU* locus on chromosome 19 q13.3, 2.5 kb in size, organised in 15 exons
- At IBGRL Sanger sequencing of the all exons of *LU* since 2001

LU sequencing of the 10 donors

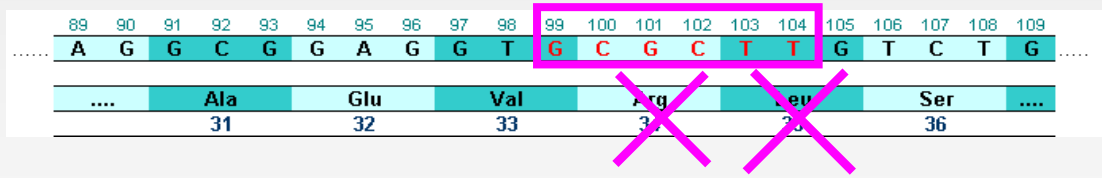
All 10 heterozygous for *LU*A/B* (*LU*01/02*)

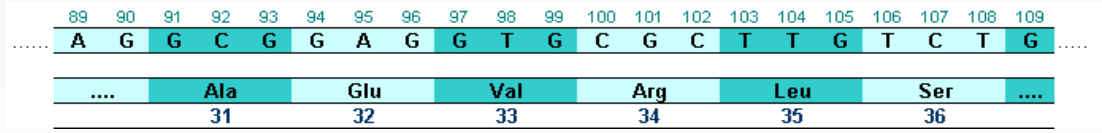
All 10 heterozygous for at least one missense or indel mutation in *LU* (associated either with a lack of HFA or novel amino acid change)



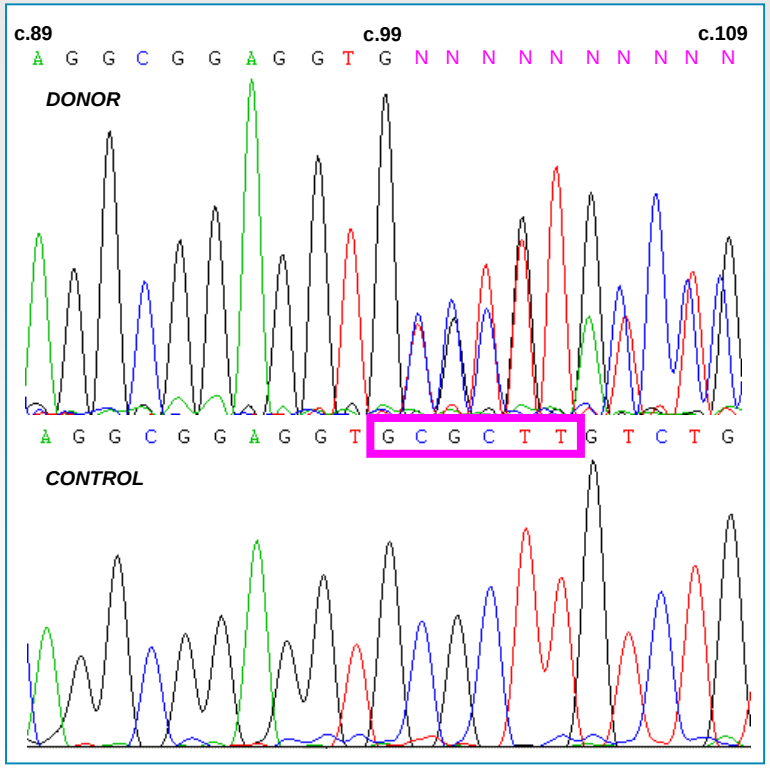
*LU*02.-12* (*LU*B* with c.99_104del)

- 4 donors heterozygous for *LU*02.-12*





***LU* exon 2**



c.99_104delGCGCTT

delArg34, Leu35

*LU*02.-12* (*LU*B* with c.99_104del)

- 4 donors heterozygous for *LU*02.-12*

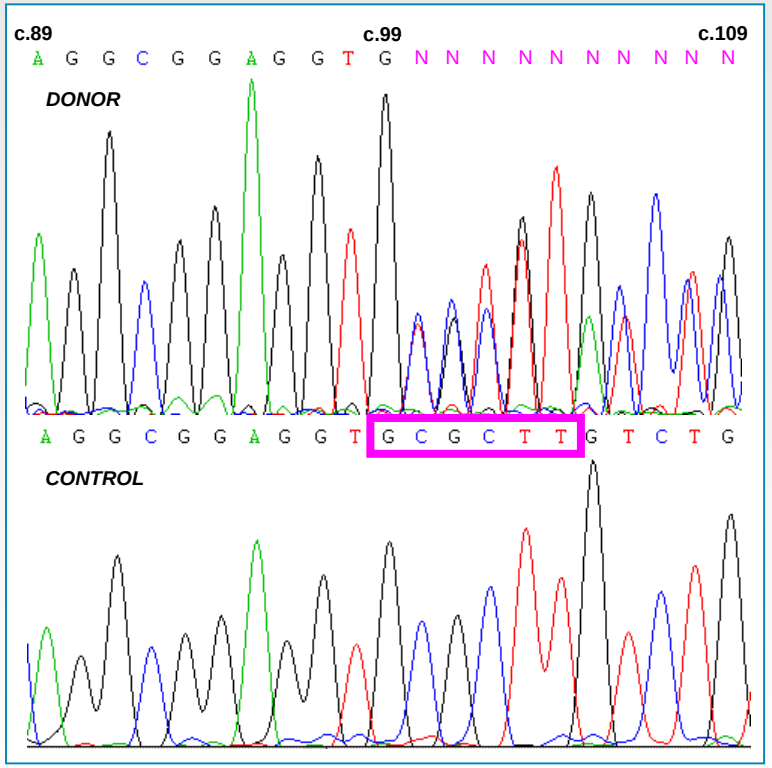
89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108
..... A	G	G	C	G	G	A	G	G	T	G	T	C	T	G	.....				

....	Ala	Glu	Val	Ser	....
	31	32	33	34	35

89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107	108	109
..... A	G	G	C	G	G	A	G	G	T	G	C	G	C	T	T	G	T	C	T	G .....

....	Ala	Glu	Val	Arg	Leu	Ser	....
	31	32	33	34	35	36	

***LU* exon 2**

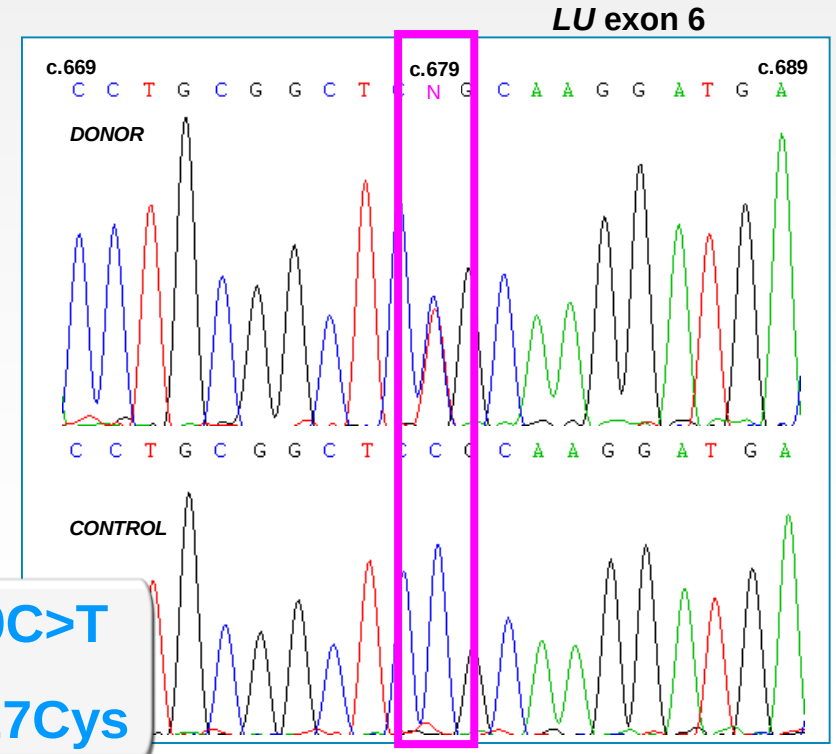


c.99_104delGCGCTT

delArg34, Leu35

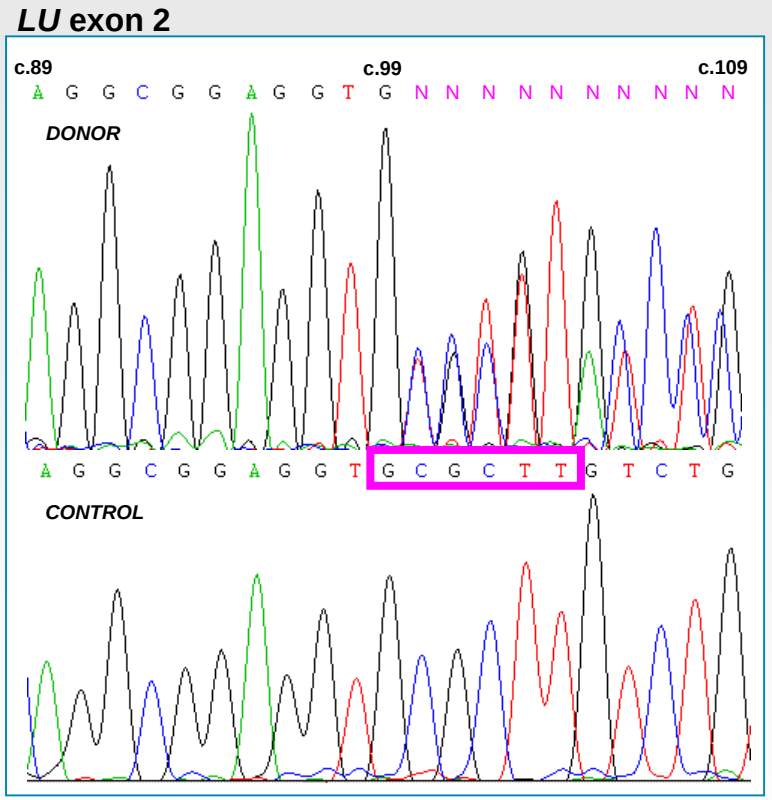
*LU*02.-12* and *LU*01.-16*

- 1 donor compound heterozygous for *LU*02.-12 (LU*B* allele) and *LU*01.-16* (*LU*A* allele)



c.679C>T

Arg227Cys



c.99_104delGCGCTT

delArg34, Leu35

*LU*02.-13 with LU*B(1306T)*

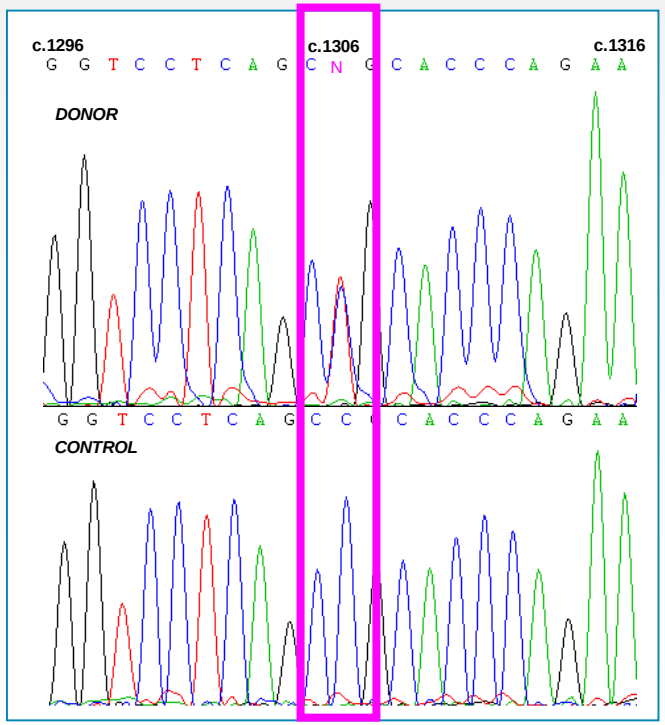
- 2 donors heterozygous for *LU*02.-13*

and *LU*B(1306T)* in exon 10

exon 11: c.1340C>T, Ser447Leu

exon 13: c.1742A>T, Gln581Leu

exon 13: silent 1671C>T



c.1306C>T

Arg436Cys

*LU*02.-13 with KLF1*BGM06*

- 1 donor was serologically Lu(a+^{wk} b+^{wk}):

exon 11: c.1340C>T, Ser447Leu

exon 13: c.1742A>T, Gln581Leu

exon 13: silent 1671C>T

- heterozygous for *LU*02.-13*
- and heterozygous for *KLF1* duplication associated with *KLF1*BGM06* genotype and In(Lu) phenotype

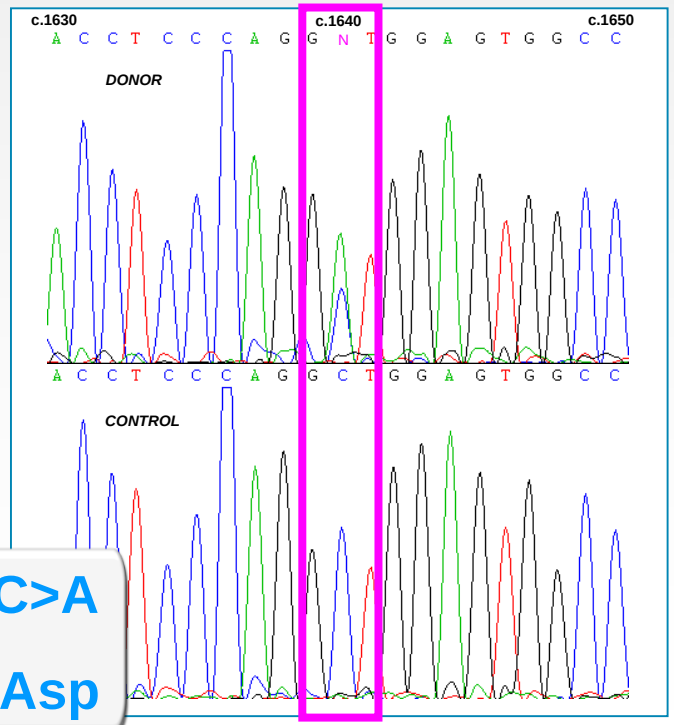
c.954dupG

Arg319Glu fs Ter

*LU*02* with novel mutations

- 2 donors were heterozygous for novel missense mutations in *LU*

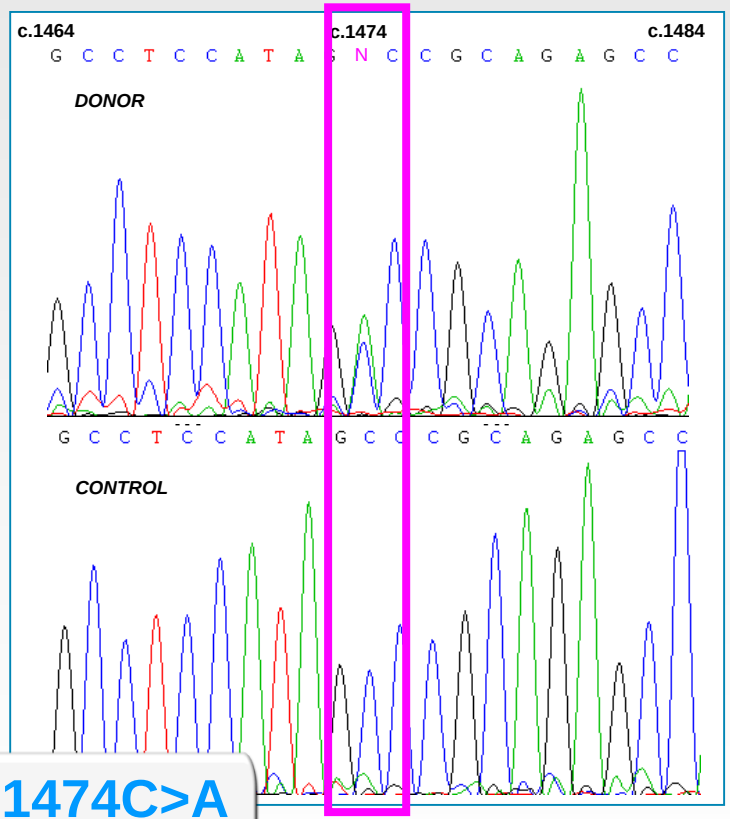
DONOR 2 *LU* exon 13



c.1640C>A

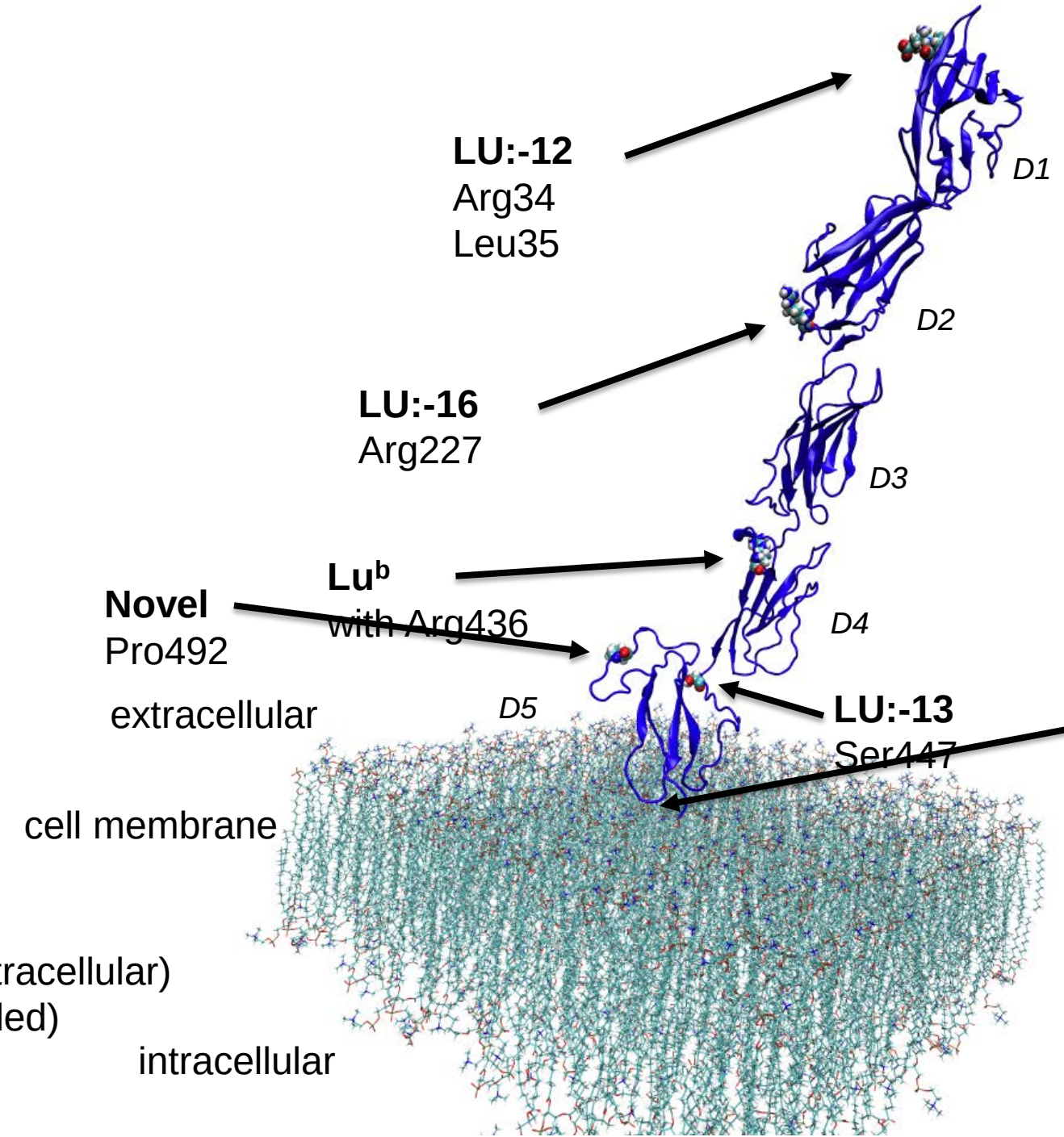
Ala547Asp

DONOR 1 *LU* exon 12



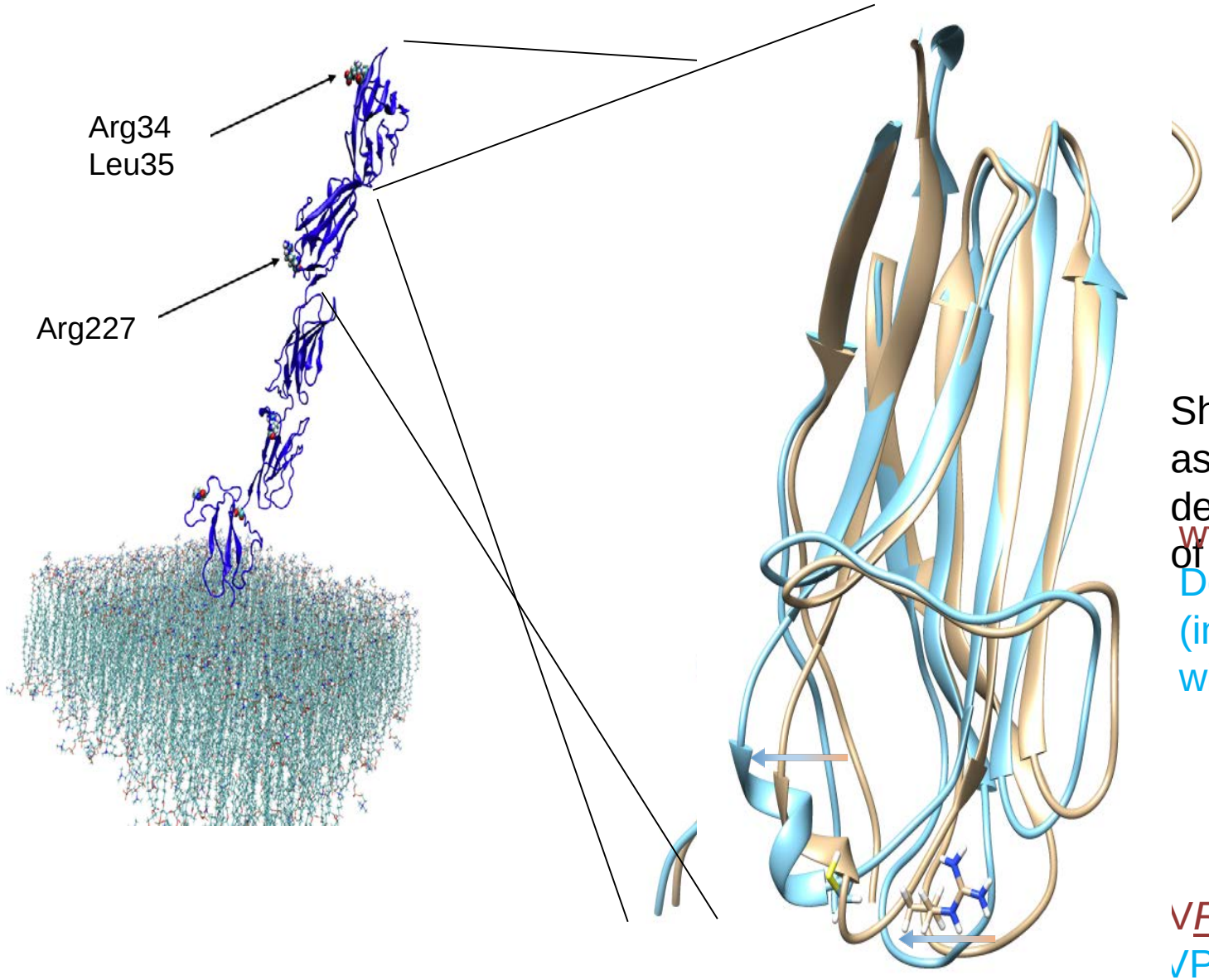
c.1474C>A

Pro492Thr



Novel
Ala547 (adjacent to transmembrane helix)
(not modelled)

LU:-12 and LU:-12 with Arg227Cys:



Shifts in both loops as a result of deletion (in direction of arrows)

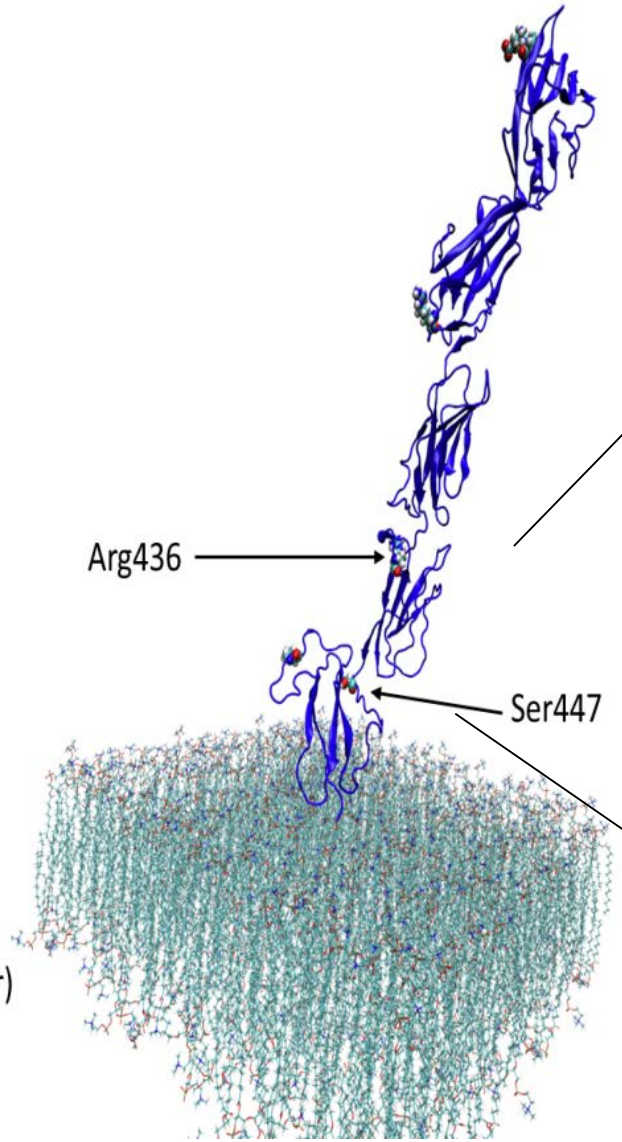
wt Arg227

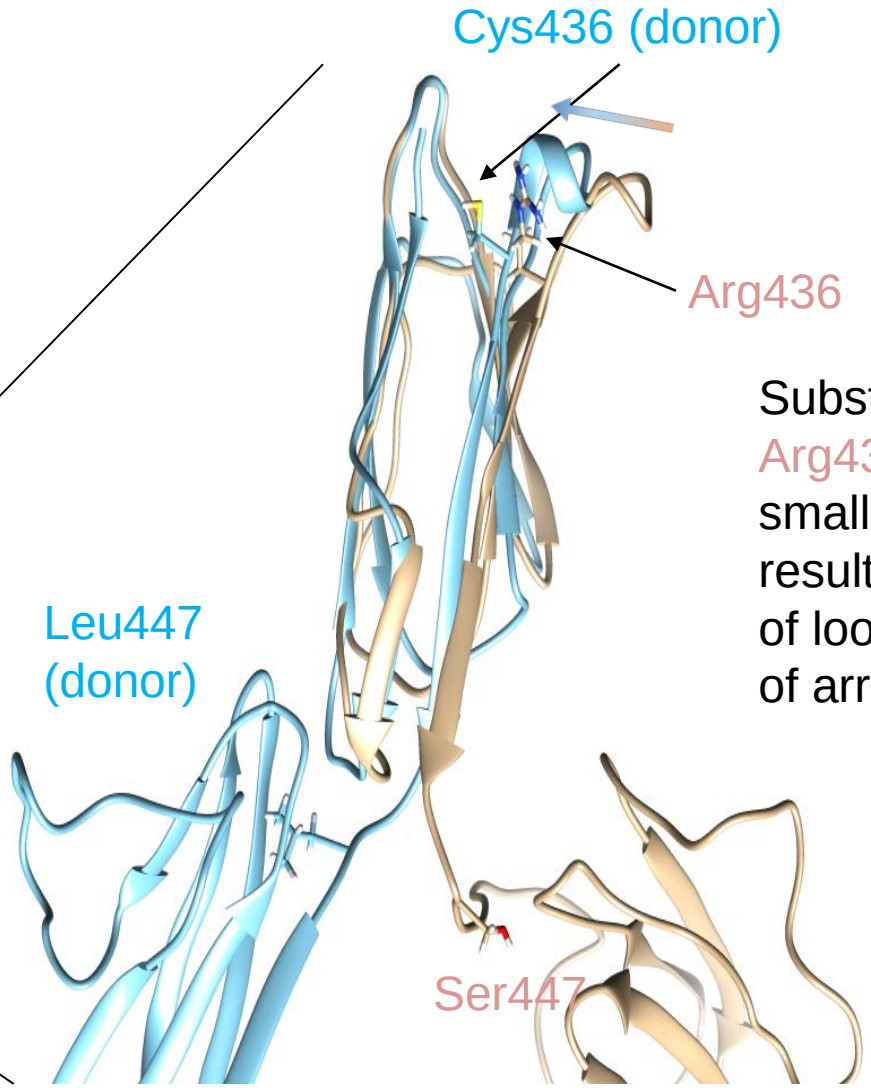
Donor Arg227Cys (in combination with c.99_104Δ)

V*RL*SVPPL…

VPPL…

LU:-13 with Arg436Cys:

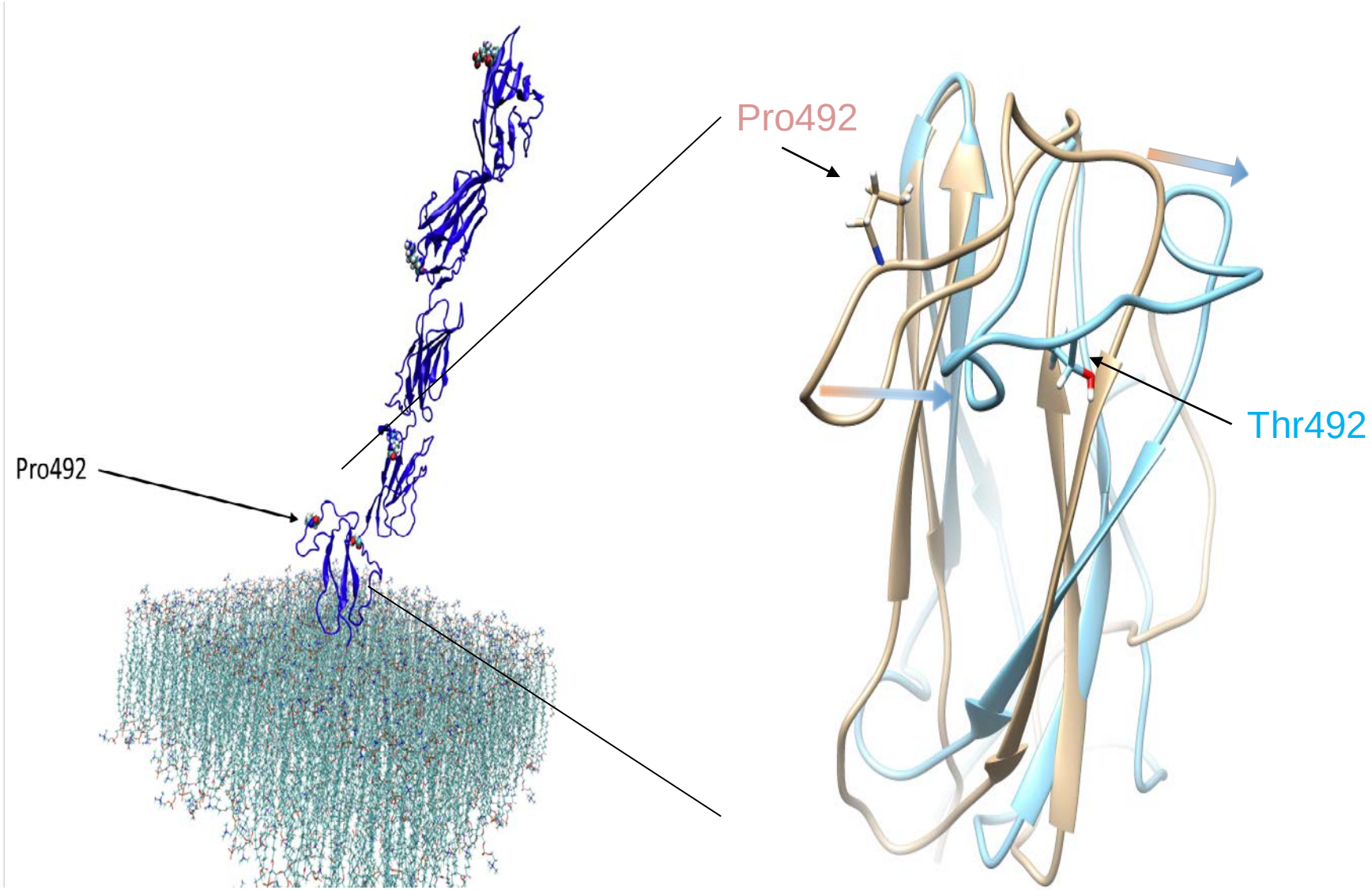




Substitution of Arg436 with smaller Cys436 results in shrinking of loop (in direction of arrow)

Considerable flexibility in hinge *D4-D5* results in strongly different conformations (Ser447Leu)

Novel allele encoding Pro492Thr:



Substitution of Pro492 for Thr492 (donor) predicts strong conformational change in the loop containing Pro492 (shift in direction of arrows)

Summary

Serological typing

9 donor samples were found to be Lu(a+b+^{wk}) and one Lu(a+^{wk}b+^{wk})

Molecular study – six different molecular backgrounds

Samples heterozygous for:

4 were *LU*02.-12* (delArg34, Leu35)

1 was *LU*02.-12* (delArg34, Leu35) with *LU*01.-16* (Arg227Cys)

2 were *LU*02.-13* (Ser447Leu, Gln581Leu) with *LU*02(1306T)*

1 was *LU*02.-13* (Ser447Leu, Gln581Leu) with *KLF1*BGM06*; In(Lu)

2 were novel variant alleles (Pro492Thr and Ala547Asp)

Thank you for your attention

University of BRISTOL

NHS Blood and Transplant