Structural Errors in Denis et al. Blood 2012

The E2A structure in KIX complex were resolved and compared with MLL - KIX complex in a structural overlay, Denis CM, Chitayat S, Plevin MJ, Wang F, Thompson P, Liu S, Spencer HL, **Ikura M**, LeBrun DP, **Smith SP** et al. 2012.

Previously, we reported nine amino acid Transactivation domains, 9aaTAD, in E2A and MLL activators, Piskacek et al. 2007. Because their 9aaTAD domains did not matched with position in KIX complex, Denis et al. report disproof our 9aaTAD concept.

Recently, we reported structural revised overlay for E2A and MLL in KIX complex, Piskacek et al. 2015. Our data completely differed from report Denis et al. (Figure 1-3, this report). We challenged trueness of the author's structural data and identified number of fundamental errors in their structural data and TADs aliments (E2A, MLL and p53). Differently to the authors, we found that E2A and MLL 9aaTAD domains are in an identical positions (see proper structural overlay in Piskacek et al. 2015).

The structural overlay in Figure 5b and 5c in Denis et al. was obvious error, where MLL and E2A positions ware in wrong place. Their helixes were aligned together regardless of their distal positions about one helix turn. That might be done intentionally or resulted from odd algorithm. We tested Pymol overlay algorithm for generation of the structural overlay for E2A and MLL in KIX complexes (Figure 4-9, this report). Indeed, the Pymol program produced the same error, which authors reported as a result in Figure 5b. Nevertheless, errors like this must be identified as wrong by first inspection by structural biologist (by authors and by reviewers of Blood Journal) and not published. In opposite, such errors are hardly recognizable for the readers, which really on unshakable truth reported by structural biologists, which really on Blood Journal. Such a blindly accepted reports might be and in this case are harmful thereafter.

Furthermore, shorter 3-turn MLL helix was stretched to the length of 4-turn helix of E2A (Figure 5b, Denis et al. and Figure 4 and 8, this report). Even very distal positions were aligned in both structural overlay, e.g. residua K15 to S2847 or L20 to F2852 (Figure 5b, Denis et al.) and in sequence alignment (Figure 5a, Denis et al.). The proline loop responsible for positional shift of L2845 was ignored in sequence alignment (Figure 2 and 5, this report).

The report Denis et al. Blood 2012 mystified readers with their odd structural data, reported disproof of the 9aaTAD concept and harmed so to the research of us and others. Therefore, we demanded from BLOOD Journal correction or full retraction of this report.

Figure 1

Alignment based on odd Structural overlay (done likely by PyMOL) in Figure 5b in Denis et al. Blood 2012
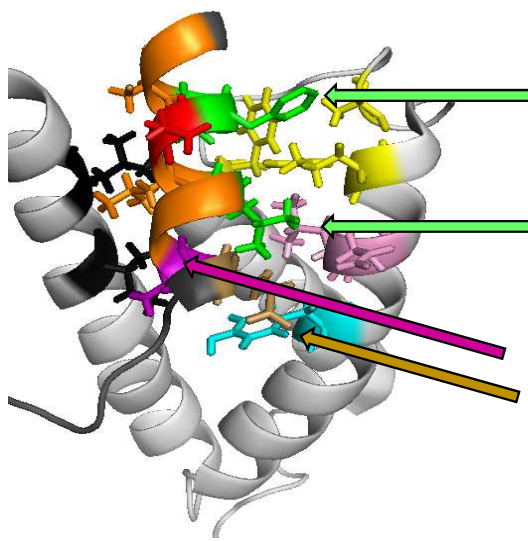

Denis et al. 2012, Figure 5b
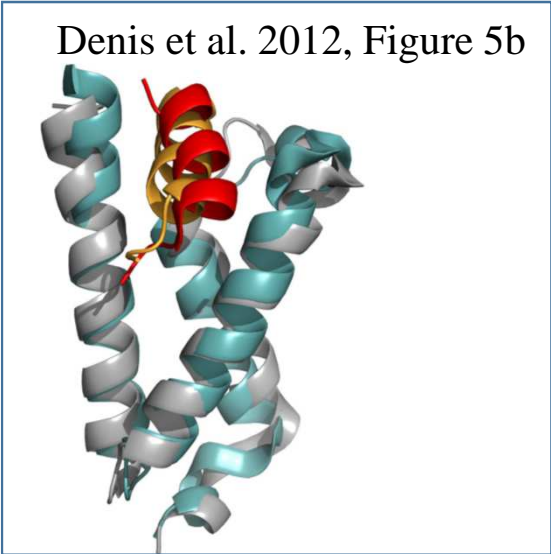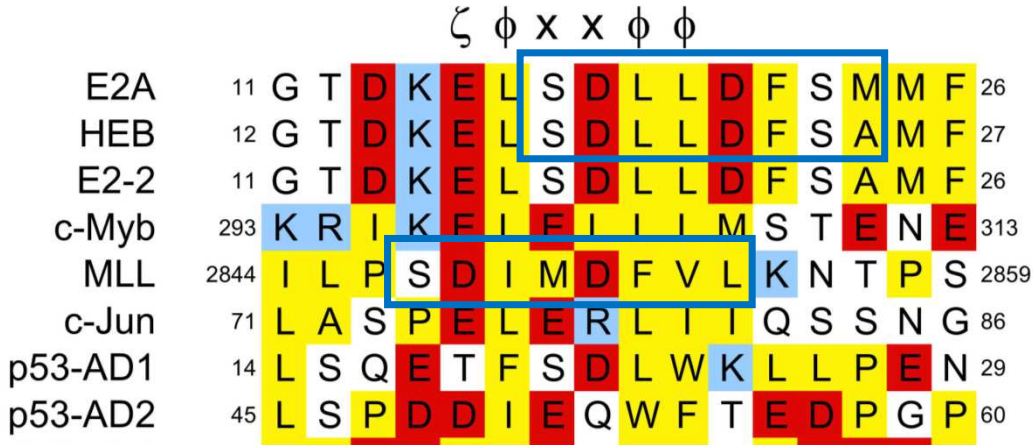
Alignment based on true Structural overlay read from structural data for HEB and MLL (Piskacek et al. Mol Biosyst. 2015)
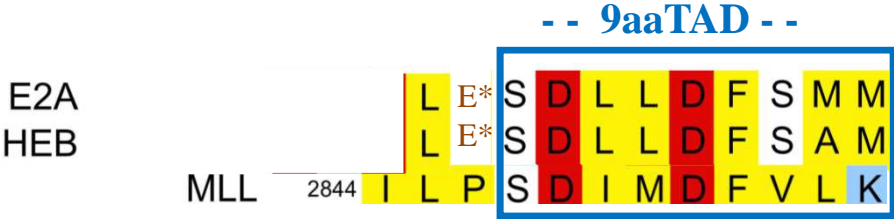
E* are of HEB and P of MLL are in the same place in the KIX complex as the rest of aligned amino acids
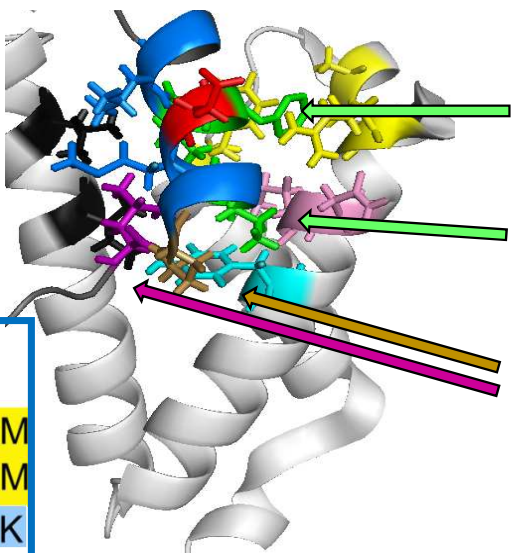
Figure 2

HEB 9aaTAD in MLL binding site of KIX domain

Ala25
Ser24
Phe23   4 turn
Asp22   helix
Leu21
Leu20
Glu19
Ser18
Leu17
Glu16

MLL 9aaTAD in MLL binding site of KIX domain

Leu2854
Val2853
Phe2852   3 turn
Asp2851   helix
Met2850
Ile2849
Asp2848
Ser2847

Pro2846   Proline
Leu2845   loop

Read out from original structural models:

Piskacek et al. 2015

- - 9aaTAD - -

E2A   L E* S D L L D F S M M
HEB   L E* S D L L D F S A M
MLL 2844 I L P S D I M D F V L K
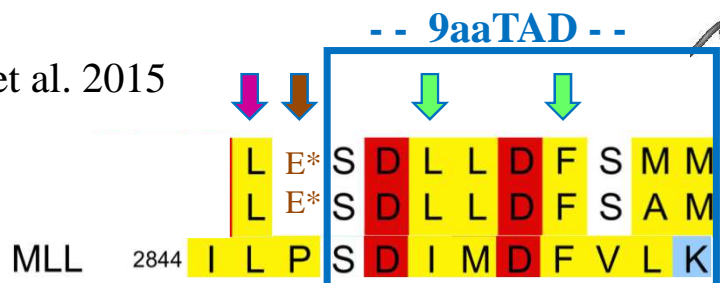
Figure 3

Amphipathic TAD

ξ φx x φφ

9aaTAD domain   Leu –   9aaTAD

			Tyr 631	Ile 660	Tyr 631	Leu 628	Leu 664	Phe 612		
Proximal KIX residues in the complex to TAD's residue											
KIX helix			α2	α3	α2	α2	α3	α2		

TADs aligned according their coordinates in the KIX-TAD complexes:

MLL	2842	GN		IL*	P*	SD IM D FV LK	NTPS	2859
HEB (E2A)	12	GTDK	E*	L*		SD LL D FS AM	FSPP	30
FOXO3	245	GNQTLQ	D	L		LT SD S LS HS	DGG	264

Sequence aligned of the 9aaTAD family:

p53-TADII	40	MDDLM		L	SP	DD IE Q WF TE	DPG	59
p53-TADI	10	VEPP		L	SQ	ET FS D LW KL	LPE	28
Gcn4	85	PIP	E	L	D	DA VV E SF FS	TPT	38
Rtg3	19	MNQTK	V	L		QE TL D FS LV	TPT	38
Gal4	854	GMFNTT	T	M		DD VY N YL FD	DEDTPP	876
Pdr1	1048	VNNG	D	L		ED LY S IL WS	DVYP	1068
Oaf1	1028	GYFG	G	L		DL FD Y DF LF	GNDFA	1047
KBP 2.20	1					SW AV Y EL LF	GS	11
p53-TADII-ECapLL	1			L	TE	EE LI E LW FT		12

* P2846 of MLL merge to E16 of HEB/E2A

Figure 4

Overlay HEB/E2A and MLL in KIX complex

Denis et al. 2012, Figure 5

Pymol reproduction of Figure 5b (Denis et al. 2015)
structural overlay generating incorrect superposition of HEB/E2A
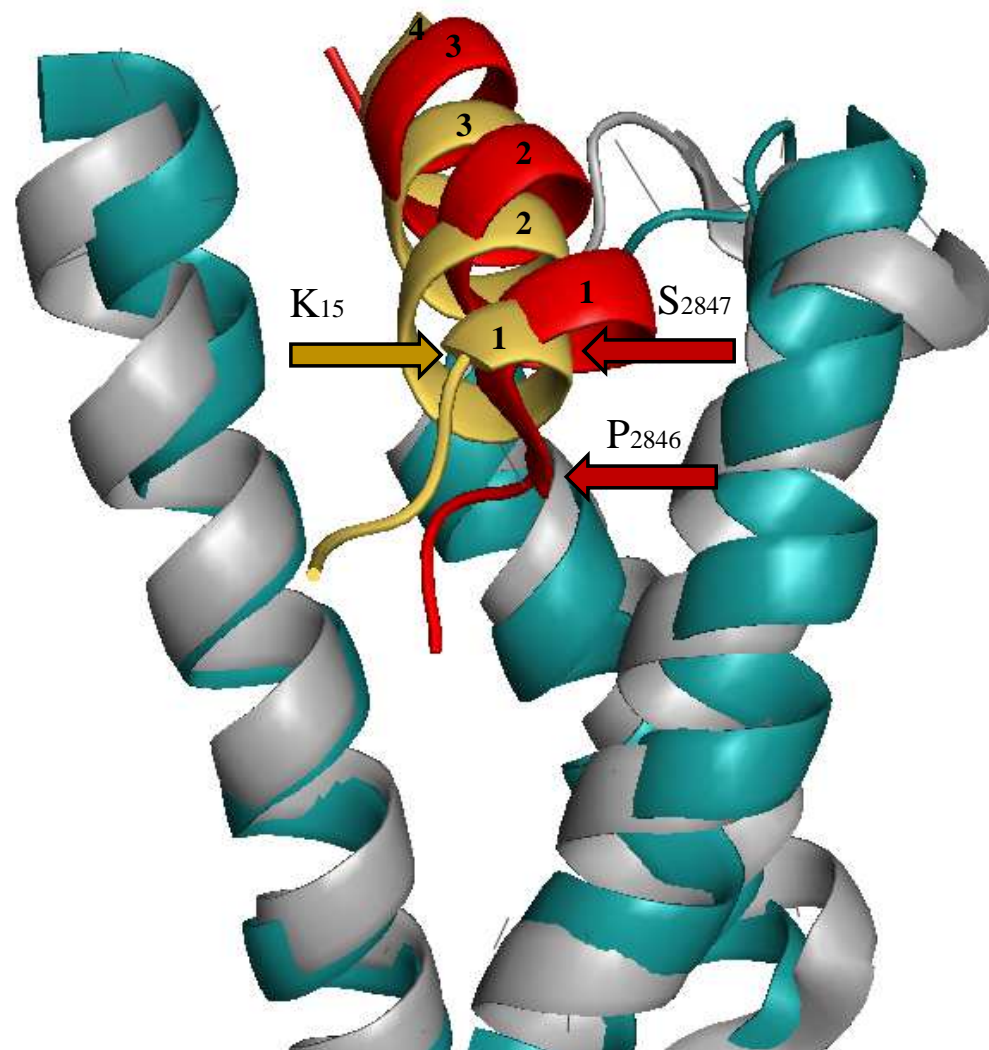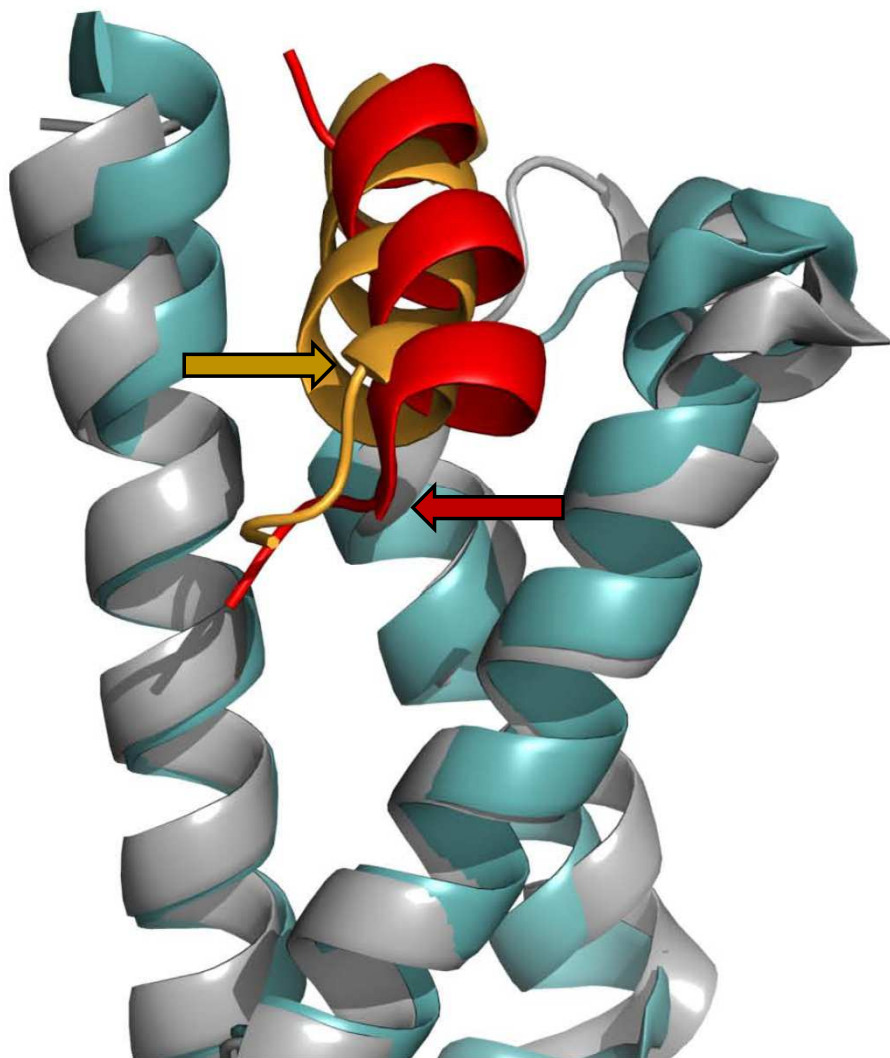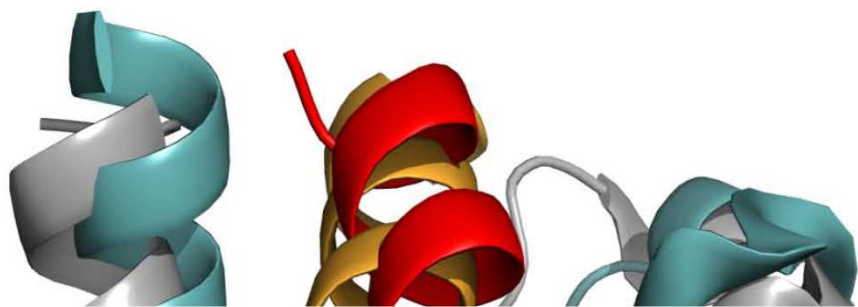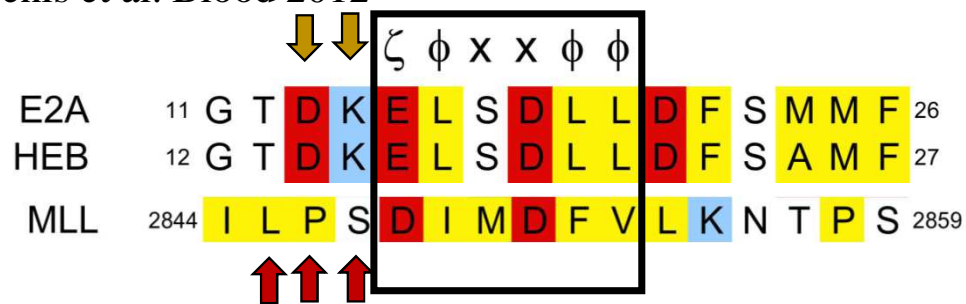and MLL helixes, 3-turn MLL helix stretched in to 4-turn helix

Figure 5

Overlay HEB/E2A and MLL in KIX complex

Pymol reproduction of overlay generating incorrect superposition of E2A and MLL helixes, P-L loop ignored

Denis et al. 2012, Figure 5

Read out from overlay (gap D_K caused by P-L turn is missing):

Denis et al. Blood 2012

			ζ	φ	x	x	φ	φ					
E2A	11 G T	D K	E	L	S	D	L	L	D	F	S	M M F	26
HEB	12 G T	D K	E	L	S	D	L	L	D	F	S	A M F	27
MLL	2844 I L P S	D	I	M	D	F	V	L	K	N	T	P S	2859

Read out from original structural models:

Piskacek et al. 2015

- - 9aaTAD - -

	L	E*	S	D	L	L	D	F	S	M M	F	26
E2A	L	E*	S	D	L	L	D	F	S	M M	F	26
HEB	L	E*	S	D	L	L	D	F	S	A M	F	27
MLL	2844 I L P	S	D	I	M	D	F	V	L	K N	T P S	2859

K₁₅ S₂₈₄₇ D₁₄ P₂₈₄₆ L₂₈₄₅
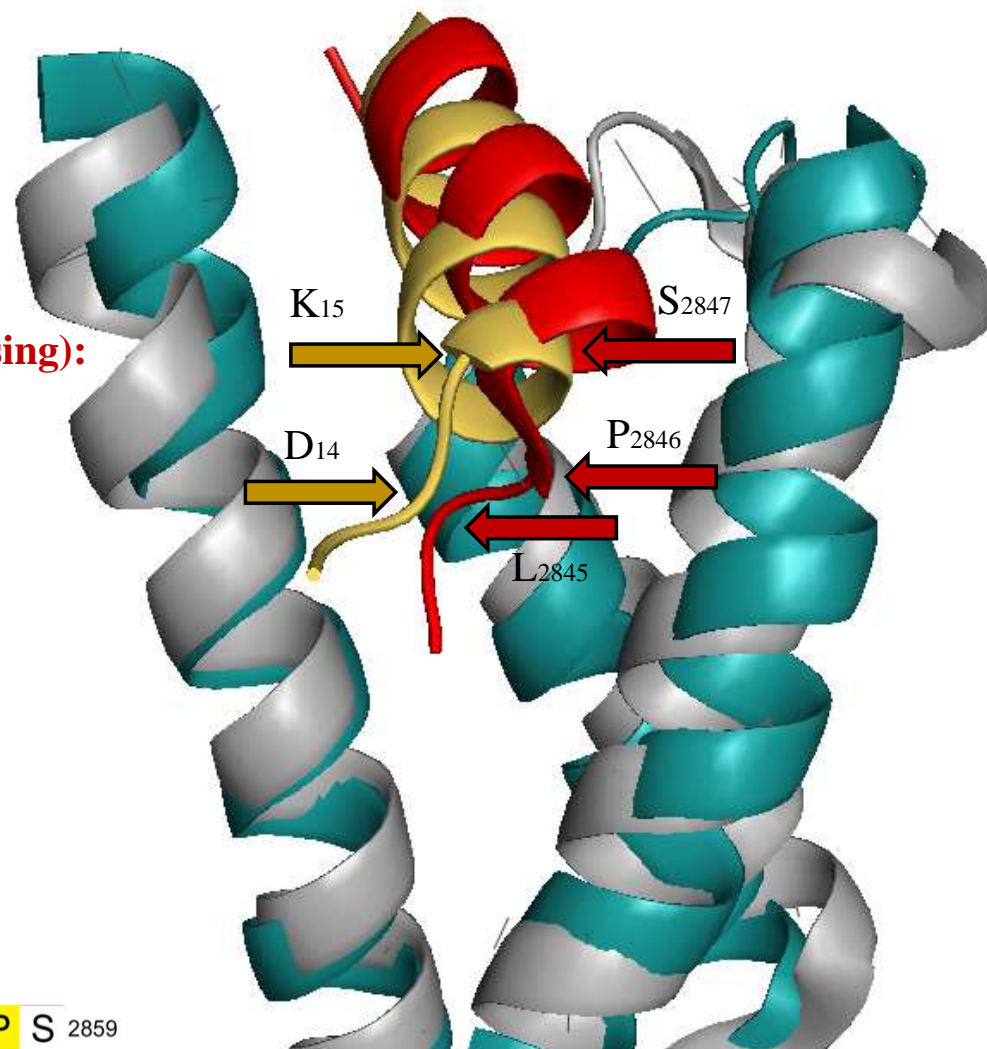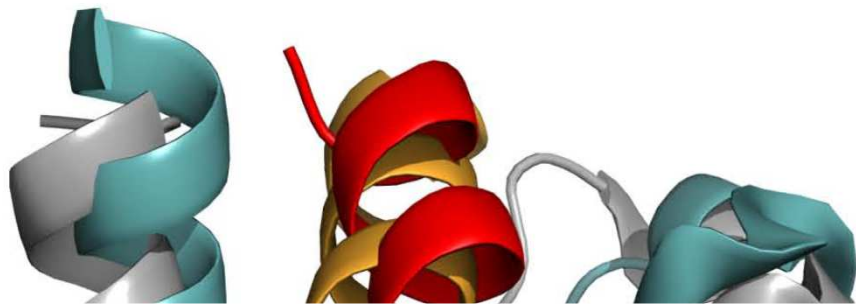
Figure 6

Overlay **HEB/E2A** and **MLL** in **KIX complex**

Denis et al. 2012, Figure 5
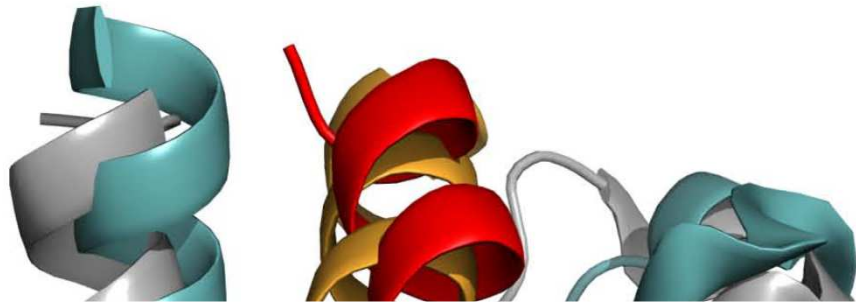
Pymol: K_{15} to S_{2847} versus S_{18} to S_{2847}
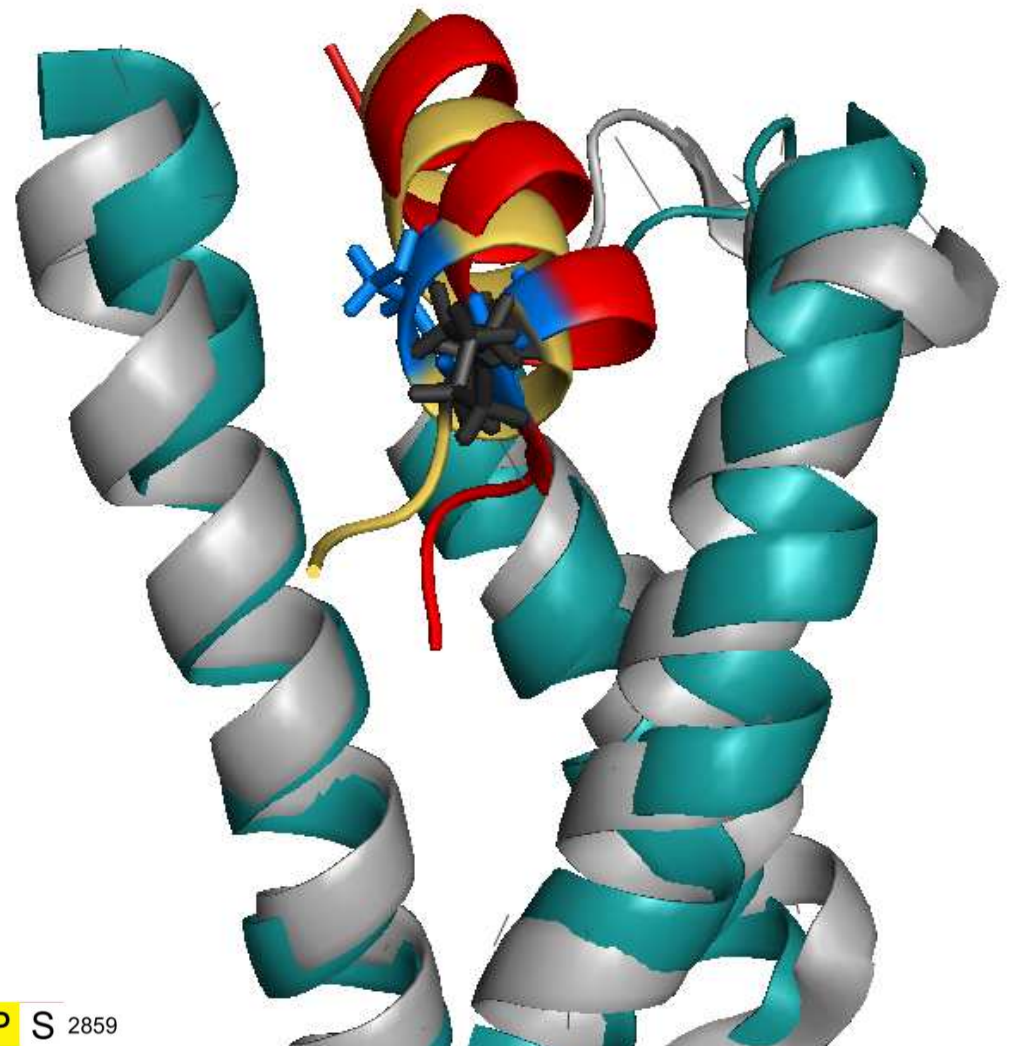
Read out from overlay:

Denis et al. Blood 2012

		ζ	φ	x	x	φ	φ			
E2A	11 G T **D** **K**	**E**	L	S	**D**	L	L	**D**	F S **M M F**
HEB	12 G T **D** **K**	**E**	L	S	**D**	L	L	**D**	F S **A M F**
MLL	2844 I L P S	**D**	I	M	**D**	F	V	L	**K** N T P S

Read out from original structural models:

Piskacek et al. 2015

- - **9aaTAD** - -

E2A	L E*	S	**D**	L	L	**D**	F	S	**M**	**M**	**F**	26
HEB	L E*	S	**D**	L	L	**D**	F	S	**A**	**M**	**F**	27
MLL	2844 I L P	S	**D**	I	M	**D**	F	V	L	**K**	N T P S 2859

Figure 7

Overlay HEB/E2A and MLL in KIX complex

Denis et al. 2012, Figure 5

Pymol: D_{19} to D_{2851} versus D_{19} to D_{2848}

Read out from overlay:

Denis et al. Blood 2012

		ζ	φ	x	x	φ	φ			

E2A 11 G T D K E L S D L L D F S M M F 26
HEB 12 G T D K E L S D L L D F S A M F 27

MLL 2844 I L P S D I M D F V L K N T P S 2859

Read out from original structural models:

Piskacek et al. 2015

|- 9aaTAD - -

E2A L E* S D L L D F S M M F 26
HEB L E* S D L L D F S A M F 27

MLL 2844 I L P S D I M D F V L K N T P S 2859
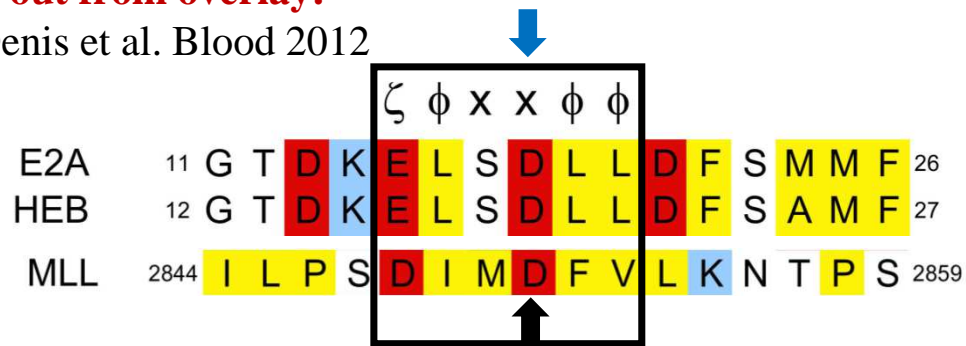
Figure 8

Overlay HEB/E2A and MLL in KIX complex
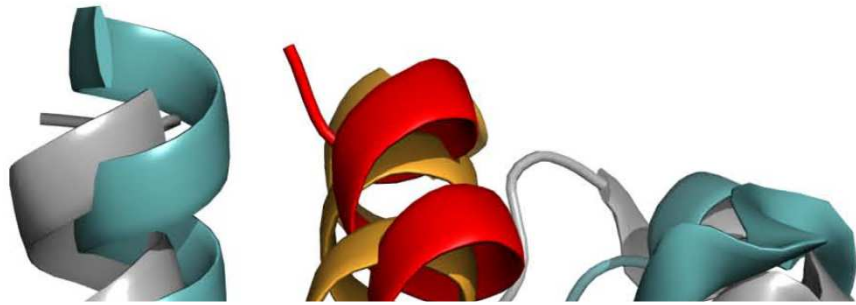
Denis et al. 2012, Figure 5

Pymol: L_{20} to F_{2852} versus F_{23} to F_{2852}

Read out from overlay:

Denis et al. Blood 2012

$\zeta \; \phi \; x \; x \; \phi \; \phi$
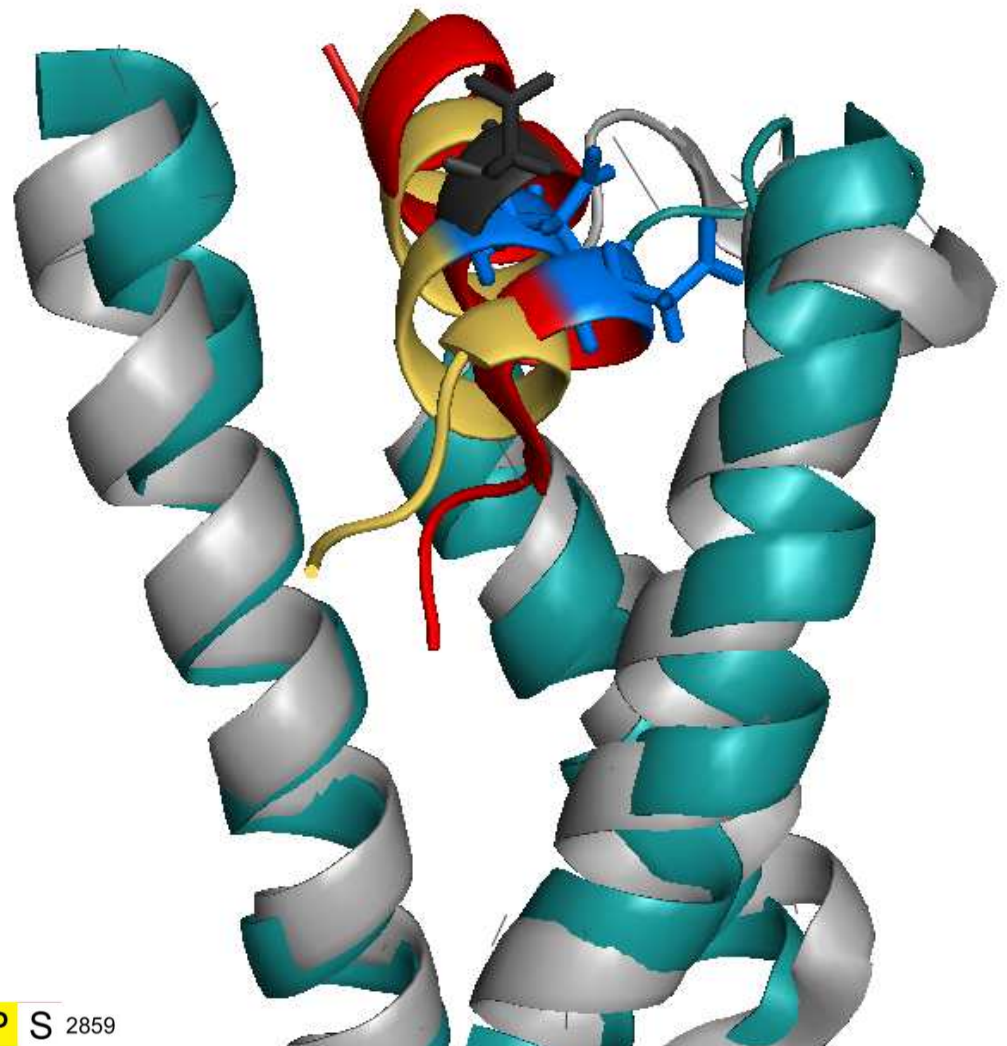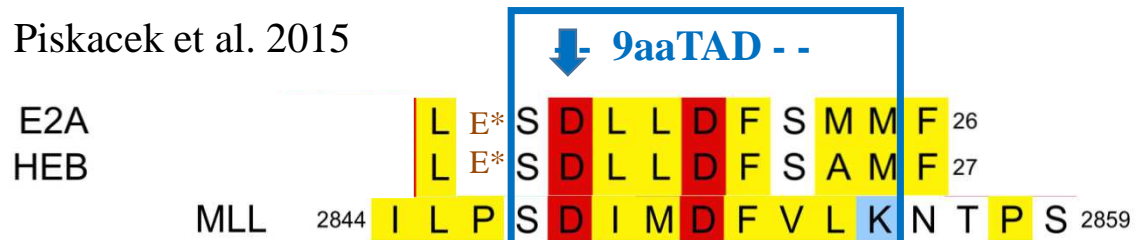
E2A	11 G	T	D	K	E	L	S	D	L	L	D	F	S	M	M	F 26
HEB	12 G	T	D	K	E	L	S	D	L	L	D	F	S	A	M	F 27
MLL	2844 I	L	P	S	D	I	M	D	F	V	L	K	N	T	P	S 2859

Read out from original structural models:

Piskacek et al. 2015

- - 9aaTAD - -

E2A		L	E*	S	D	L	L	D	F	S	M	M	F 26
HEB		L	E*	S	D	L	L	D	F	S	A	M	F 27
MLL	2844 I	L	P	S	D	I	M	D	F	V	L	K	N	T	P S 2859

Pymol reproduction of overlay generating incorrect superposition of E2A and MLL helixes, 3 turn MLL helix stretched in to 4 turn helix.
From original structural model:
E2A turn 4 align to MLL turn 3
E2A turn 3 align to MLL turn 2
E2A turn 2 align to MLL turn 1
E2A turn 1 align to P-L loop outside of the helix

Figure 9

Overlay HEB/E2A and MLL in KIX complex
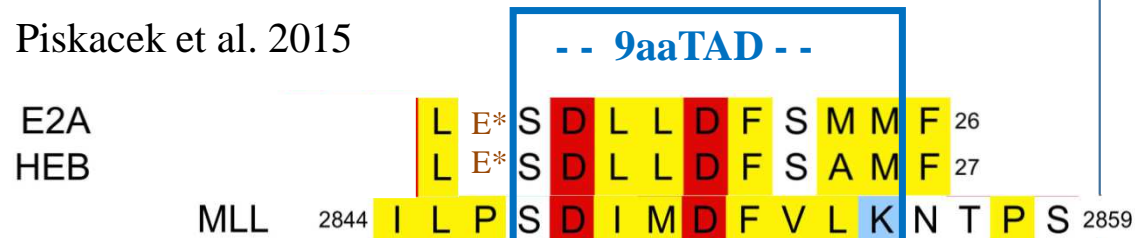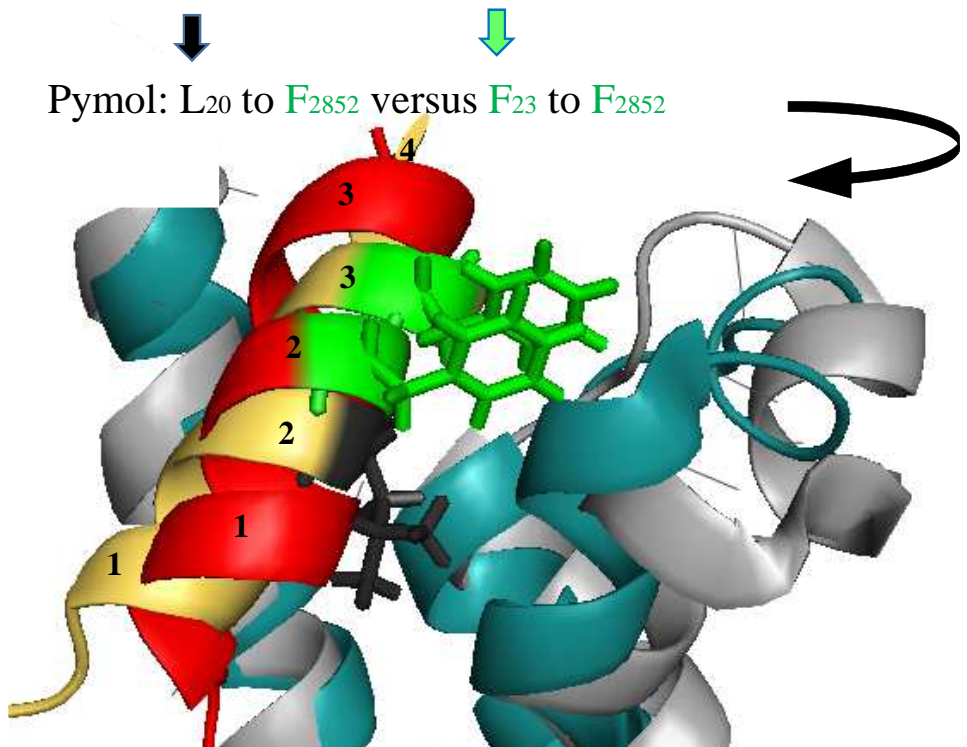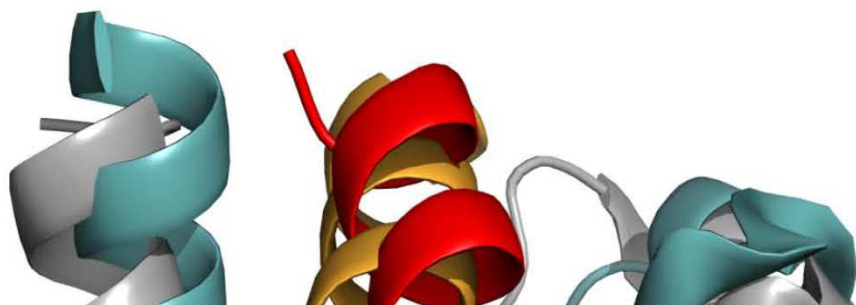
Denis et al. 2012, Figure 5

Pymol: L_{20} to F_{2852} versus F_{23} to F_{2852}

Read out from overlay:

Denis et al. Blood 2012

$$\zeta \quad \phi \quad x \quad x \quad \phi \quad \phi$$

E2A	11	G	T	D	K	E	L	S	D	L	L	D	F	S	M M F 26
HEB	12	G	T	D	K	E	L	S	D	L	L	D	F	S	A M F 27
MLL	2844	I L P S	D	I	M	D	F	V	L	K	N	T	P	S 2859

Read out from original structural models:

Piskacek et al. 2015

-- 9aaTAD --

E2A		L	E*	S	D	L	L	D	F	S	M M F 26
HEB		L	E*	S	D	L	L	D	F	S	A M F 27
MLL	2844	I L P	S	D	I	M	D	F	V	L K	N T P S 2859

Pymol reproduction of overlay generating incorrect superposition of E2A and MLL helixes, 3-turn MLL helix stretched in to 4-turn helix. From original structural model:

E2A turn 4 align to MLL turn 3
E2A turn 3 align to MLL turn 2
E2A turn 2 align to MLL turn 1
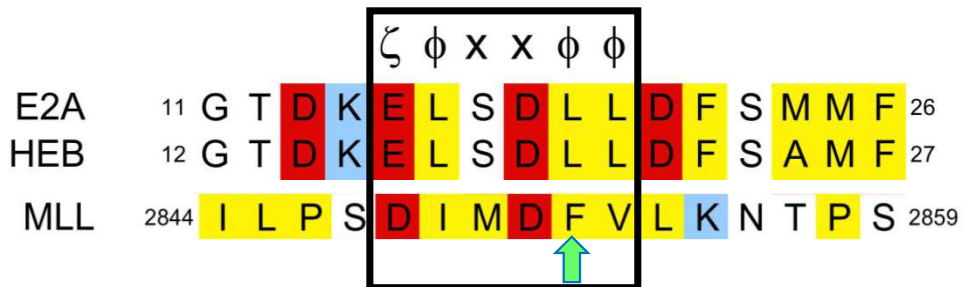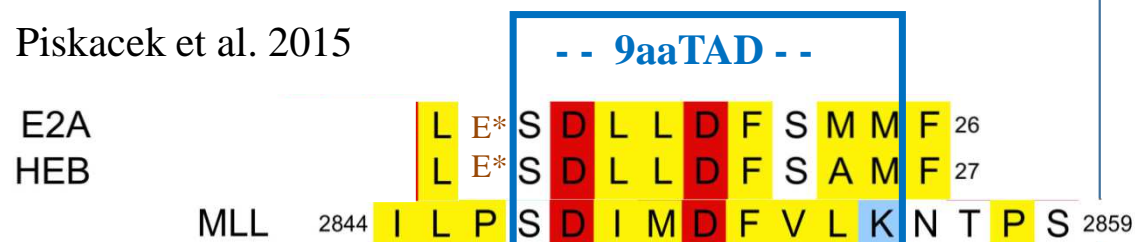E2A turn 1 align to P-L loop outside of the helix