Appendix B

Complete Hit Sequencing Results

	x_1	x_2	x_3	x_4	x_5
hit1	r	r	y	h	r
hit2	m/v	r	w	k	r
hit3	k	r	w	y	y
hit4	w	k	k	k	w
hit5	h	f	f	f	r
hit6	s	r	--	r	r
hit7	r	r	w	h	y
hit8	r	k	w	w	w
hit9	r	w	s	f	r
hit10	r	r	g	w	r
hit11	g	f	r	r	w
hit12	r	t	r	r	w
hit13	m	r	w	k	r
hit14	y	r	k	r	w
hit15	a	--	--	--	--
hit16	r	r	i	r	w
hit17	--	--	k/l	w	--
hit18	r	w	--	--	r
hit19	k/l	r	--	w	r
hit20	w	r	f	r	y
hit21	d/p	y	y	r	r
hit22	r	y	w	k	k
hit23	k/l	r	r	r	w
hit24	y	r	r	k	w
hit25	r	k/l	f	y	r
hit26	r	w	w	k	r

	x_1	x_2	x_3	x_4	x_5
hit27	w	r	--	y	r
hit28	h	r	w	r	r
hit29	w	y	r	k	r
hit30	l	r	f	r	r
hit31	w	k	r	k	k
hit32	r	r	r	w	s/m
hit33	r	r	k	f	w
hit34	r	r	w	r	y
hit35	w	r	h	y	k
hit36	r	r	y	f	r
hit37	w	r	k	w	r
hit38	w	y	--	r	r
hit39	y	r	r	r	h
hit40	y	r	r	r	w
hit41	p	f	y	w	r
hit42	k	y	w	r	k
hit43	r	y	w	h	k
hit44	r	w	h	w	n
hit45	r	h	f	h	h/f
hit46	r	r	--	h	r
hit47	r	y	r	r	r
hit48	y	f	h	h/w	w
hit49	r	r	r	w	y
hit50	w	r	r	r	r/--
hit51	r	w	k	f	h

Table B.1. First-generation anchor ligand screen **An1** results.

	x_1	x_2	x_3	x_4	x_5	x_6
hit1	y	r	w	f	k	f
hit2	h/r	h/r	f	l	l/r	r
hit3	f	r	f	y	y	r
hit4	h/r	f	f	k	l	--
hit5	k	l	f	l	k	l
hit6	l	f	l	w	l	k
hit7	f	f	f	r	y	--
hit8	h/r	f	f	f	r	--
hit9	r	w	w	l	k	f
hit10	h/r	f	f	r	y	y
hit11	l	k	l	f	l	k
hit12	f	r	r	w	w	k
hit13	h/r	y	f	f	k	l
hit14	l	k	f	f	f	k
hit15	h/r	f	f	r	r	--

(**A)**

	x_1	x_2	x_3	x_4	x_5	x_6
hit1	h	l	y	f	l	r
hit2	l	k	l	w	f	k

(**B)**

Table B.2. Second-generation anchor ligand screen **(A) An2a** and **(B) An2b** results. The two anchor ligand candidates (hlyflr and lklwfk) are highlighted in yellow.

	Az_n	x_2	x_3	x_4	x_5	x_6	Az_n
hit1	**Az4**	k	i	w	i	G	
hit2	**Az8**	r	l	w	v	G	**Az4**
hit3	**Az8**	r	r	r	k	r	**Az8**
hit4	**Az4**	l	l	v	i	k	**Az4**
hit5	**Az4**	m	i	l	i	k	
hit6	**Az8**	i	i	i	m	r	**Az4**
hit7	**Az8**	i	i	i	w	r	**Az8**
hit8	**Az4**	n	v	i	i	f	
hit9	**Az4**	i	f	l	v	k	**Az8**
hit10	**Az4**	k	i	w	i	G	**Az8**
hit11	**Az4**	r	r	k	f	r	**Az8**
hit12	**Az4**	r	v	w	l	r	**Az8**
hit13	**Az8**	k	y	r	r	r	**Az4**
hit14	**Az8**	r	r	k	v	w	**Az4**
hit15	**Az4**	i	f	l	v	k	**Az8**
hit16		k	r	k	r	f	**Az4**
hit17	**Az8**	k	i	w	i	k	
hit18	**Az8**	y	r	k	f	k	
hit19	**Az4**	i	f	f	r	v	**Az8**
hit20		a	r	k	k	y	**Az4**
hit 21		r	k	r	t	i	**Az4**
hit 22	**Az8**	k	m	v	f	k	**Az4**
hit23	**Az4**	l	i	m	k	i	**Az4**

Table B.3. In situ biligand screen **Bi1** results. Potential 2° ligand candidates are highlighted in orange.

hit1	f	k	l	w	i	k
hit2	v	w	l	w	G	G
hit3	f	w	f	w	G	G
hit4	k	w	f	w	G	G
hit5	f	k	l	w	l	k
hit6	k	w	f	w	G	G
hit7	w	w	i	w	G	G
hit8	k	G	w	l	w	G
hit9	k	l	w	i	w	G
hit10	l	w	i	w	G	l
hit11	f	k	G	f	l	i
hit12	f	w	i	w	G	k
hit13	l	w	l	w	G	i
hit14	i	i	v	l	w	k
hit15	l	i	i	f	v	
hit16	v	k	f	i	l	l
hit17	l	G	f	f	w	i
hit18	k	k	l	k	k	l
hit19	f	k	l	w	i	k
hit20	w	i	w	G	G	f
hit 21	f	f	l	l	v	k
hit 22	k	f	k	f	w	k
hit23	l	i	k	l	f	v
hit24	l	w	f	w	G	v
hit25	f	w	f	w	G	i
hit26	G	w	f	w	G	v
hit27	G	w	i	w	G	k

(A)

	x_1	x_2	x_3	x_4	x_5	x_6
hit1	k	w	i	w	G	w
hit2	k	w	i	w	G	v
hit3	k	w	l	w	G	l
hit4	k	w	i	w	G	l
hit5	k	w	i	w	G	w
hit6	k	w	l	w	G	l
hit7	G	w	i	w	G	i
hit8	k	i	f	k	i	f

(**B)**

Table B.4. On-bead biligand screen **(A) Bi2a** and **(B) Bi2b** results. Potential 2° ligand candidates are highlighted in yellow/green. Consensus motif w-x_3-w-G (where x_3 = hydrophobic amino acid) is highlighted in red font.

	Az_n	x_2	x_3	x_4	x_5	x_6	Az_n
hit1	**Az4**	n	i	i	i	v	
hit2	**Az4**	i	i	l	l	k	**Az4**
hit3	**Az4**	n	i	i	v	l	
hit4	**Az4**	n	m	i	f	l	**Az4**
hit5	**Az4**	n	v	l	v	l	
hit6	**Az4**	n	l	i	l	f	**Az4**
hit7	**Az4**	n	l	i	l	f	**Az4**
hit8	**Az8**	r	l	w	i	r	**Az4**
hit9	**Az4**	n	l	i	v	f	**Az4**
hit10	**Az4**	r	m	w	v	k	**Az8**
hit11	**Az4**	i	i	l	l	k	**Az8**
hit12	**Az4**	i	l	v	v	r	**Az4**
hit13	**Az4**	n	l	l	f	l	**Az4**
hit14	**Az4**	n	i	i	v	y	
hit15		m	k	r	k	k	**Az8**
hit16	**Az4**	i	l	i	r	w	**Az4**
hit17	**Az8**	i	i	v	f	r	**Az8**
hit18	**Az8**	y	f	t	r	r	
hit19	**Az4**	n	m	i	i	v	**Az4**
hit20	**Az8**	i	l	i	a	k	**Az4**
hit21	**Az4**	i	l	l	r	w	
hit22	**Az8**	i	v	v	f	r	**Az4**
hit23	**Az4**	l	l	l	v	k	**Az4**
hit24	**Az4**	k	v	w	i	k	**Az4**
hit25	**Az4**	i	m	v	l	r	**Az4**

Table B.5. First-generation in situ triligand screen **Tri1** results. Potential 3° ligand candidates are highlighted in orange.

	x_2	x_3	x_4	x_5	x_6	x_7
hit1	r	l	w	l	r	f
hit2	r	l	w	l	r	l
hit3	r	f	f	f	r	f
hit4	r	l	f	l	r	f
hit5	l	f	f	w	f	r
hit6	l	w	f	f	f	r
hit7	l	f	l	w	f	r
hit8	l	w	l	f	f	r
hit9	l	f	f	w	l	r
hit10	r	r	r	l	w	r
hit11	r	l	w	l	r	f
hit12	w	r	r	r	r	w
hit13	r	f	r	f	r	w
hit14	f	w	f	f	w	r

Table B.6. First-generation on-bead triligand screen **Tri2** results. Recall that the focused Library E was used for this screen. Potential 3° ligand candidates are highlighted in orange.

	x_2	x_3	x_4	x_5	x_6	x_7
hit1	n	l	i	v	f	r
hit2	n	l	i	v	l	r
hit3	n	i	i	l	l	r
hit4	i	l	f	l	f	r
hit5	n	l	i	v	l	r
hit6	n	i	i	l	w	r
hit7	n	l	i	v	f	r
hit8	n	l	i	v	f	r

(A)

	x_2	x_3	x_4	x_5	x_6	x_7
hit1	n	l	i	v	f	r
hit2	n	l	i	v	f	r
hit3	n	i	i	v	f	r
hit4	n	i	i	v	f	r
hit5	n	i	i	l	l	r
hit6	n	l	i	v	l	r
hit7	n	l	i	v	f	r

(**B)**

Table B.7. Results of second-generation triligand screens: **(A) Tri3** (in situ) and **(B) Tri4** (on-bead). The final 3° ligand sequence is highlighted in orange.

	x_1	x_2	x_3	x_4	x_5
hit1	w	f	r	r	r
hit2	s	w	v	w	G
hit3	p	v	y	f	w
hit4	d	d	y	w	G
hit5	i	w	a	y	w
hit6	d	n	w	G	f
hit7	a	w	w	a	t
hit8	r	f	r	r	f
hit9	d	w	w	h	t
hit10	r	f	r	w	r
hit11	d	e	w	p	h
hit12	a	w	w	l	w
hit13	a	w	w	a	y
hit14	d	k	k	i	y
hit15	d	w	s	i	e
hit16	s	w	w	f	y
hit17	d	w	l	r	y
hit18	s	w	a	f	y
hit19	d	l	f	l	w
hit20	d	w	a	t	w
hit21	f	k	y	r	s
hit22	d	q	r	w	r
hit23	i	w	s	t	h
hit24	l	i	v	m	w

Table B.8. Azide-free in situ triligand screen **TriX** results (control). Note the poor hit homology, and the lack of resemblance with nlivfr.