Defining binding Efficiency and Specificity of auxins for $SCF^{TIR1/AFB}$-Aux/IAA co-receptor complex formation

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Supplementary Data

Figure S1.

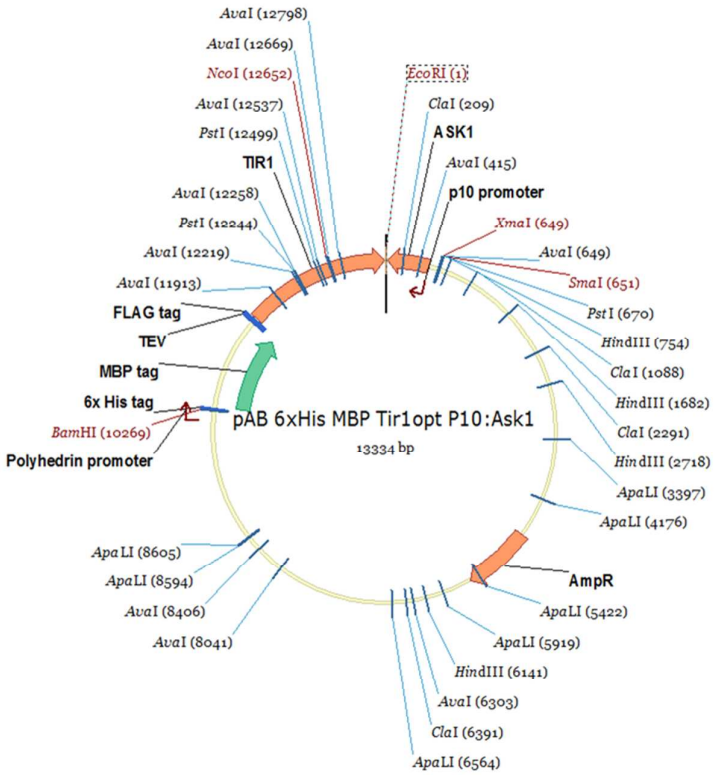

Figure S1a: Construct for baculovirus co-expression[5] of ASK1 and TIR1 (as His-MBP-TEV-FLAG-TIR1). The sequence of the TIR1 fusion protein is:

MSG**HHHHHH**GDQLVEE*MAEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKF*
PQVAATGDGPDIIFWAHDRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEAL
SLIYNKDLLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDI
KDVGVDNAGAKAGLTFLVDLIKNKHMNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSKVN
YGVTVLPTFKGQPSKPFVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSY
EEELAKDPRIAATMENAQKGEIMPNIPQMSAFWYAVRTAVINAASGRQTVDEALKDAQTNSSSN
*NNNNNNNNNLGIE*GRG**ENLYFQA**AIA**DYKDDDDK**GLNMQKRIALSFPEEVLEHVFSFIQLDK
DRNSVSLVCKSWYEIERWCRRKVFIGNCYAVSPATVIRRFPKVRSVELKGKPHFADFNLVPDG
WGGYVYPWIEAMSSSYTWLEEIRLKRMVVTDDCLELIAKSFKNFKVLVLSSCEGFSTDGLAAIA
ATCRNLKELDLRESDVDDVSGHWLSHFPDTYTSLVSLNISCLASEVSFSALERLVTRCPNLKSL
KLNRAVPLEKLATLLQRAPQLEELGTGGYTAEVRPDVYSGLSVALSGCKELRCLSGFWDAVPA
YLPAVYSVCSRLTTLNLSYATVQSYDLVKLLCQCPKLQRLWVLDYIEDAGLEVLASTCKDLREL
RVFPSEPFVMEPNVALTEQGLVSVSMGCPKLESVLYFCRQMTNAALITIARNRPNMTRFRLCII
EPKAPDYLTLEPLDIGFGAIVEHCKDLRRLSLSGLLTDKVFEYIGTYAKKMEMLSVAFAGDSDL
GMHHVLSGCDSLRKLEIRDCPFGDKALLANASKLETMRSLWMSSCSVSFGACKLLGQKMPKL
NVEVIDERGAPDSRPESCPVERVFIYRTVAGPRFDMPGFVWNMDQDSTMRFSRQIITTNGL

The short epitope tags are shown in **<u>BOLD</u>**, The TEV protease site is in **BOLD** (no underscore), MBP is in *ITALICS* and the first residue of TIR1 is underscored for ease of reference.

Figure S2.

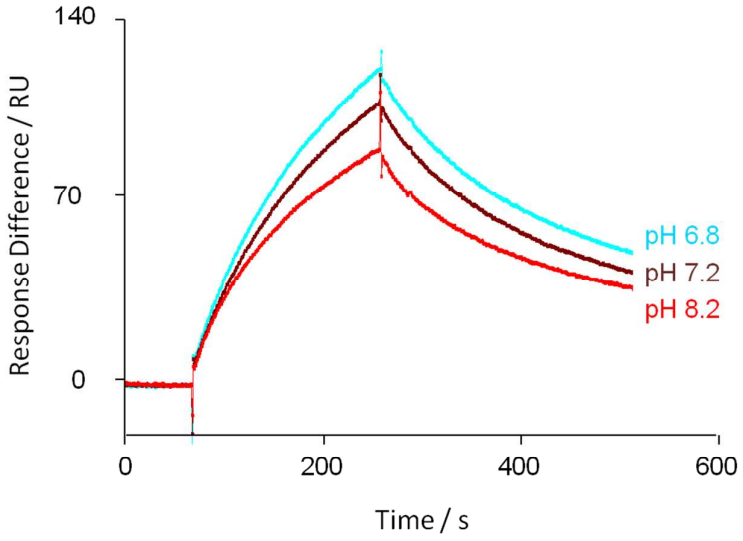

Figure S2. The assembly of the TIR1 co-receptor complex shows marginal dependence on pH between pH 6.8 – pH 8.2.

Table S1

Summary of compounds, binding data, bioactivity scores and previous classifications. Compounds are arranged by family as in Figure 4. Binding is given initially as the magnitude of the association normalised to the binding measured for IAA. This is converted to a score for comparison to the scores assigned in other classification schemes: up to 10% = 0; 10-30% = 1; 30-60% = 2; over 60% = 2. Boxes are colour-coded for ease of comparison, with the most highly active (3) red, 2 orange and 1 yellow. * see Walsh et al., 2006.

	Compound	CAS number	TIR1	AFB5	TIR1 binding score	AFB5 binding score	Ferro et al	Katekar	Ferro class	Tomic class
			Binding as % IAA	Binding as % IAA			bioactive score	bioactive score	molecular dynamics	molecular dynamics
1	indole-3-acetic acid	87-51-4	100	100	3	3	3	3	4	1
2	1-naphthylacetic acid	86-87-3	76	59	3	3	3	3	3	1
3	2-naphthylacetic acid	581-96-4	25	0	1	0	1	1	7	2
4	2-naphthoxyacetic acid	120-23-0	0	12	0	1	2	2	11	
5	indole-3-butyric acid	133-32-4	15	5	1	0	3		5	4
6	2,4-dichlorophenoxypropanoic acid (Dichloroprop, Chirality unknown)	120-36-5	56	30	2	2				1
7	2,4,5-trichlorophenoxypropionic acid (Chirality unknown)	93-72-1	70	35	3	2				
8	p-hydroxycinnamic acid ((E)-3-phenylprop-2-enoic acid)		0	2	0	0				
9	tryptophan	73-22-3	0	0	0	0	0			
10	tryptophol	526-55-6	7	2	0	0				
11	tryptamine	61-54-1	7	6	0	0				
12	indole-3-lactic acid	832-97-3	0	0	0	0	1		1	
13	indole-3-propionic acid	830-96-6	54	27	2	1	1		3	2
14	indole-3-glycolic acid		0	0	0	0	1		2	
15	indole-3-pyruvic acid	392-12-1	54	27	2	1	0		1	
16	indole-3-carbinol	700-06-1	0	0	0	0				
17	indoxylacetic acid		0	0	0	0				
18	indole-3-carboxylic acid	771-50-6	0	0	0	0	2		10	
19	indole-3-acetaldehyde	2591-98-2	0	0	0	0				
20	benazolin	3813-05-6	0	0	0	0				
21	benzoic acid	65-85-0	8	0	0	0	0	0	1	3
22	4-hydroxybenzoic acid	99-96-7	3	0	0	0				

23	2,4-dichlorobenzoic acid	50-84-0	6	0	0	0	0	0	4	
24	2,5-dichlorobenzoic acid	50-79-3	2	0	0	0	1	1	5	
25	2,5-dihydroxybenzoic acid	490-79-9	1	0	0	0				
26	2-chloro-5-bromomethoxybenzoic acid		7	0	0	0				
27	amiben (3-amino-2,5-dichlorobenzoic acid)	133-90-4	0	0	0	0	1	2	7	
28	2-amino-3,5-diiodobenzoic acid	609-86-9	3	0	0	0				
29	3,5-diiodo-2-hydroxybenzoic acid	133-91-5	0		0	0				
30	3,5-diiodo-2-hydroxybenzoic acid	133-91-5	0	8	0	0				
31	2,3,6-trichlorobenzoic acid	50-31-7	17	9	1	0	3		9	
32	Gallic acid (3,4,5-trihydroxybenzoic acid)	149-91-7	2	0	0	0				
33	2,3,5-triiodobenzoic acid	88-82-4	0	2	0	0		3		4
34	phenyl acetic acid	103-82-2	55	5	2	0	1	1	7	
35	4-hydroxyphenylacetic acid	156-38-7	38	3	2	0				
36	4-hydroxyphenylacetic acid	156-38-7	35	1	2	0				
37	4-chlorophenylacetic acid	1878-66-6	5	8	0	0				
38	phenoxy acetic acid	122-59-8	15	2	1	0	0	0	11	3
39	2-formylphenoxyacetic acid	6280-80-4	6	6	0	0				
40	4-chlorophenoxyacetic acid	122-88-3	35	1	2	0	2	2	4	
41	2,4-D	94-75-7	78	45	3	2	3	3	4	1
42	2,5-D	582-54-7	44	1.6	2	0	2	2	3	1
43	2,6-D	575-90-6	13	0	1	0	1	0	7	
44	2,4,5-trichlorophenoxyacetic acid	93-76-5	42	4	2	0	3	3	7	1
45	2,4,6-trichlorophenoxyacetic acid	575-89-3	4	1	0	0	0	0	3	3, 4
46	picloram	1918-02-1	45	103	2	3				
47	Fluoxypyr	2840-00-8	80	143	3	3				
48	trichlopyr	55335-06-3	60	90	3	3				
49	DAS534*		79	191	3	3				
50	DAS805*		0	5	0	0				
51	clopyralid	1702-17-6	0	0	0	0				
52	nicotinic acid	59-67-6	0	0	0	0				
55	quinclorac	84087-01-4	0	81	0	3				
56	1-naphthylacetic-L-aspartic acid		0	1	0	0				
57	indole-3-acetic-L-aspartic acid		0	0	0	0				
58	benzoyl aspartic acid		0		0					
59	o-benzoyl-L-malic acid	22138-51-8	1		0					
60	indole-3-acetic acid ethyl ester	778-82-5	15	18	1	1				

61	**quinic acid (tetra-hydroxy-cyclohexane carboxylic acid**	**77-95-2**	**0**	**0**	**0**	**0**				
62	**L-phenylalanine**	**63-91-2**	**0**	**0**	**0**	**0**				
63	**abscisic acid**	**14375-45-2**	**0**	**1**	**0**	**0**				

Table S2

Table of auxins and related compounds tested and represented in Figure 5, but sorted (column 1, N) according to binding activity as shown for Figure 6. CAS is the Chemical Registry number for each compound. The columns labelled TIR1 and AFB5 give the amplitude of the association at report point 1 as a percentage of the response for IAA (100%). The following columns are: **$Ave_{(Tir1\text{-}Afb5)}$**, the average of TIR1 and AFB5 binding activities as well as Factors 1 and 2 (Figure 6a); (Effic.), binding Efficiency level (Figure 6); (Spec.)the predicted specificity from the quantum chemical structures; (Class), classification based on electronic structure differences (unspec = class not specified).

N	Compound	Labels	CAS	TIR1	AFB5	$Ave_{(Tir1\text{-}Afb5)}$	Effic.	Spec.	Class
1	Tryptophan	1	73-22-3	0	0	0	1	2	Unspec
2	Indole-3-lactic ac.	l	832-97-3	0	0	0	1	2	Unspec
3	Indole-3-glycolic ac.	n	3050-37-1	0	0	0	1	2	Unspec
4	Indole-3-carbinol	p	700-06-1	0	0	0	1	2	Unspec
5	Indoxylacetic ac.	q		0	0	0	1	2	Unspec
6	Indole-3-carboxylic ac.	r	771-50-6	0	0	0	1	2	Unspec
7	Indole-3-acetaldehyde	s	2591-98-2	0	0	0	1	2	Unspec
8	Benazolin	t	3813-05-6	0	0	0	1	2	Unspec
9	Amiben	A	133-90-4	0	0	0	1	2	Unspec
10	3,5-diI-2-OH-benzoic ac.	C	133-91-5	0	0	0	1	2	Unspec
11	Clopyralid	V	1702-17-6	0	0	0	1	2	Unspec
12	Nicotinic ac.	W	59-67-6	0	0	0	1	2	Unspec
13	IAA-L-aspartic ac.	Z		0	0	0	1	2	Unspec
14	Benzoyl aspartic ac.	2		0	0	0	1	2	Unspec
15	Quinic acid	5	77-95-2	0	0	0	1	2	Unspec
16	L- phenylalanine	6	63-91-2	0	0	0	1	2	Unspec
17	2,5-dihydroxybenzoic ac.	y	490-79-9	1	0	0,5	2	2	Unspec
18	NAA aspartate	Y		0	1	0,5	2	2	Unspec
19	o-benzoyl-L-malic ac.	3	22138-51-8	1	0	0,5	2	2	Unspec
20	ABA	7	14375-45-2	0	1	0,5	2	2	Unspec
21	p-hydroxycinnamic ac.	h	4120-62-1	0	2	1	2	2	Unspec
22	2,5-diCl-benzoic ac.	x	50-79-3	2	0	1	2	2	Unspec
23	Gallic acid	E	149-91-7	2	0	1	2	2	Unspec
24	2,3,5-I-benzoic ac.	F	88-82-4	0	2	1	2	2	Unspec
25	4-OH-benzoic ac.	v	99-96-7	3	0	1,5	2	2	Unspec
26	2-NH2-3,5-di-I-benzoic ac.	B	609-86-9	3	0	1,5	2	2	Unspec
27	2,4,6-T	Q	575-89-3	4	1	2,5	2	2	Unspec
28	2,4-diCl-benzoic ac.	w	50-84-0	6	0	3	3	2	Unspec
29	2-Cl-5-Br-methoxybenzoic ac.	z		7	0	3,5	3	2	Unspec
30	Benzoic ac.	u	65-85-0	8	0	4	3	2	Unspec
31	Tryptophol	j	526-55-6	7	2	4,5	3	2	Unspec
32	2-naphthoxyacetic ac.	d	120-23-0	0	12	6	3	3	AFB5
33	2-formylphenoxyacetic ac.	K	6280-80-4	6	6	6	3	2	Unspec
34	Tryptamine	k	61-54-1	7	6	6,5	3	2	Unspec
35	4-Cl-phenylacetic ac.	I	1878-66-6	5	8	6,5	3	2	Unspec
36	2,6-D	O	575-90-6	13	0	6,5	3	2	Unspec
37	Phenoxy acetic ac.	J	122-59-8	15	2	8,5	3	2	Unspec
38	IBA	e	133-32-4	15	5	10	3	2	Unspec
39	2-NAA	c	581-96-4	25	0	12,5	3	1	TIR1
40	2,3,6-Cl-benzoic ac.	D	50-31-7	17	9	13	3	2	Unspec
41	IAA-ethyl ester	4	778-82-5	15	18	16,5	4	2	Unspec
42	4-Cl-phenoxyacetic ac.	L	122-88-3	35	1	18	4	1	TIR1
43	4-OH-phenylacetic ac.	H	156-38-7	38	3	20,5	4	1	TIR1
44	2,5-D	N	582-54-7	44	1,6	22,8	4	1	TIR1
45	2,4,5-T	P	93-76-5	42	4	23	4	1	TIR1

46	Phenyl acetic ac.	G	103-82-2	55	5	30	5	1	TIR1
47	Indole-3-propionic ac.	m	830-96-6	54	27	40,5	5	1	TIR1
48	Indole-3-pyruvic ac.	o	392-12-1	54	27	40,5	5	1	TIR1
49	Quinclorac	X	84087-01-4	0	81	40,5	5	3	AFB5
50	Dichlorprop	f	120-36-5	56	30	43	5	1	TIR1
51	2,4,5-TP	g	93-72-1	70	35	52,5	5	1	TIR1
52	2,4-D	M	94-75-7	78	45	61,5	5	1	TIR1
53	NAA	b	86-87-3	76	59	67,5	5	1	TIR1
54	Picloram	R	1918-02-1	45	103	74	5	3	AFB5
55	Trichlopyr	T	55335-06-3	60	90	75	5	2	Unspec
56	IAA	a	87-51-4	100	100	100	5	1	TIR1
57	Fluoxypyr	S	69377-81-7	80	143	111,5	5	3	AFB5
58	DAS534	U		79	191	135	5	3	AFB5

Table S3: Coefficients of linear discriminants for binding efficiency and specificity.

	Efficiency				Specificity	
	LD1	LD2	LD3	LD4	LD1	LD2
V1	-0,0001	-0,0003	0,0000	0,0000	-0,0008	0,0000
V2	0,0035	0,0002	0,0007	-0,0004	0,0048	0,0010
V3	0,0037	-0,0009	0,0006	-0,0001	-0,0059	0,0019
V4	-0,0058	0,0018	-0,0007	0,0003	-0,0019	-0,0017
V5	-0,0100	-0,0017	-0,0040	0,0001	-0,0291	-0,0038
V6	0,0099	-0,0001	-0,0006	-0,0006	-0,0117	0,0119
V7	-0,0079	0,0010	-0,0039	0,0004	-0,0216	0,0035
V8	-0,0178	0,0048	0,0010	0,0000	-0,0083	-0,0094
V9	0,0132	-0,0020	-0,0004	-0,0019	-0,0118	0,0185
V10	0,0154	0,0149	0,0036	-0,0007	0,0342	0,0017
V11	0,0420	-0,0118	-0,0031	0,0032	-0,0028	0,0298
V12	0,0872	0,0387	0,0040	0,0025	0,0348	0,1199
V13	-0,0332	-0,0332	-0,0280	0,0032	-0,0286	0,1021
V14	0,0777	0,0201	-0,0598	-0,0018	-0,0012	-0,0530
V15	0,0792	0,0806	-0,0309	-0,0046	-0,1296	0,0359
V16	0,0614	-0,0841	0,0021	0,0080	0,0486	-0,0783
V17	-0,1622	0,0526	-0,0263	-0,0122	-0,1625	-0,0970
V18	-0,0706	0,0502	-0,0016	-0,0246	-0,0293	-0,0606
V19	-0,0284	0,0852	0,0253	0,0273	0,2246	0,0618
V20	0,2768	0,1488	-0,0250	-0,0011	-0,1142	0,4034
V21	-0,1159	-0,1178	0,0022	0,0126	-0,2391	-0,0362
V22	0,5165	0,0701	0,0077	0,0319	-0,0803	0,1692
V23	0,2218	0,2642	-0,0032	0,0020	0,4020	0,0064
V24	-0,4674	-0,0027	0,2484	0,0227	-0,3074	-0,2332
V25	0,1009	-0,0021	-0,0232	0,0675	-0,8526	0,1701
V26	-0,6789	-0,1309	-0,1220	-0,1778	-0,2079	-0,0363
V27	-0,6246	0,1944	0,0774	0,0198	0,0651	0,0987
V28	-0,1249	0,1548	-0,0453	0,0192	-1,1494	0,2555
V29	0,0287	-0,2051	0,0634	0,0245	-1,2714	0,2283
V30	0,1455	-0,2532	-0,2201	0,0173	0,0523	0,0541
V31	0,0669	-0,2370	0,3371	-0,0868	0,2894	-0,1502
V32	0,1947	0,3327	-0,1696	-0,0453	-1,2572	-0,0819
V33	0,0533	-0,6659	0,0558	-0,1047	0,3387	-0,9031
V34	1,2424	-0,3514	0,3572	0,0546	5,0707	0,3418
V35	0,7379	0,7599	0,1739	0,1084	1,0469	-0,0376
V36	-1,6349	0,9755	-0,0409	-0,0389	0,0281	-1,0148
V37	-1,2160	-0,4155	0,1674	-0,0588	-3,7130	-0,0563
V38	0,1683	-1,3711	0,9712	0,0624	1,2376	-0,8936
V39	-0,0534	-0,8389	-1,4114	0,0142	-2,4853	-1,2819
V40	0,5278	2,6549	-0,7737	-0,0461	0,7391	-0,6191
V41	-2,3350	-3,3178	0,5732	0,1695	-1,0719	-2,5540
V42	-1,9693	0,4135	-0,0991	-0,4127	0,4658	-1,8107
V43	1,8941	0,5215	-0,4729	0,1246	-0,3029	-1,6816
V44	0,7419	-0,2081	0,1938	-0,0962	-0,1692	-1,0608
V45	-6,2229	2,1608	-0,0917	0,1961	-1,0633	-1,3860
V46	1,7544	0,2472	-2,2746	-0,5282	-6,4901	-1,7251
V47	-6,9869	-1,5953	-1,2653	0,7409	1,0124	-8,4634
V48	1,0149	1,2677	-0,1394	0,5484	4,9508	-1,9105
V49	-5,8126	1,2335	6,9949	0,2773	5,3395	-1,1611
V50	-18,7608	5,6521	-0,6418	-0,1812	-4,2312	-7,5737
V51	17,7378	1,6562	-6,6741	0,5226	4,0870	7,1268
V52	7,0208	-9,1066	-2,2938	0,3224	-6,0149	-10,3939
V53	-4,5536	9,4754	3,7428	0,2410	2,2736	8,1695
V54	-7,5793	-1,4238	-5,7529	1,1402	3,8513	-1,3792
Prop. Trace	0,76	0,17	0,07	0	0,79	0,21