

Supplementary Information

An Efficient Strategy to Enhance Binding Affinity and Specificity of a Known Isozyme Inhibitor

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Figure S1. Font histogram from screening against (a) *hCAII* and (b) *hCAI* with **Lib 1**

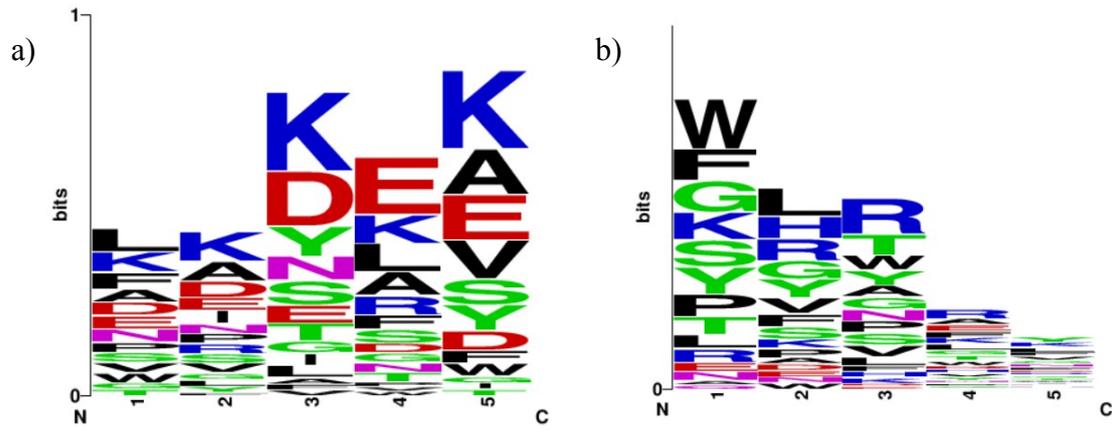


Figure S2. Font histogram from screening against (a) *hCAII* and (b) *hCAI* with **Lib 2**

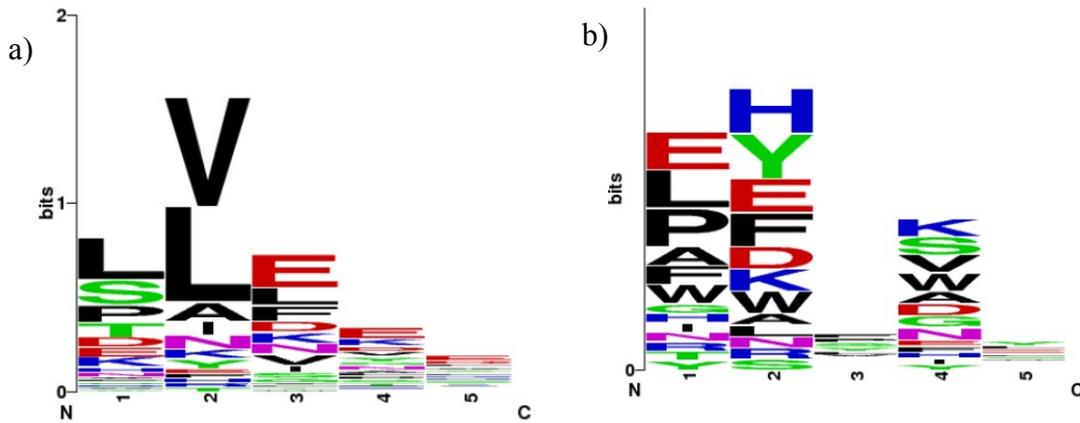


Table S1. Proposed focused library

N-terminal	l	s	d	t		
	l	v	a			
	d	e	f	l	v	
	d	e	k	v	y	
C-terminal	d	e	v	l	w	y

Figure S3. (a) COPAS sorting image from screening of the focused library against *hCAII* [X-axis : TOF; Y-axis : fluorescence intensity (a.u.)]; Excitation : 640 nm solid laser; Detection conditions: Gain = 4, PMT = 750. (b) Font histogram from positive sequences

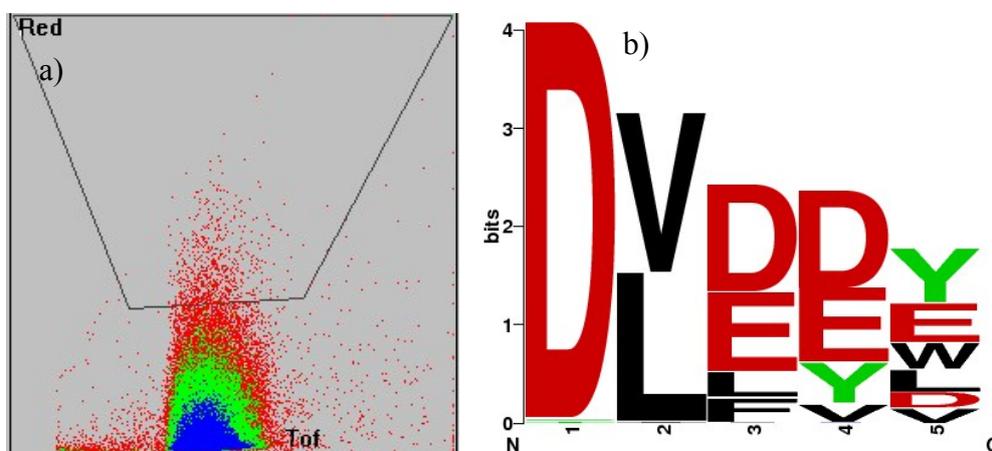


Figure S4. (a) COPAS sorting image from screening of the focused library against *hCAI* [X-axis : TOF; Y-axis : fluorescence intensity (a.u.)]; Excitation : 640 nm solid laser; Detection conditions: Gain = 4, PMT = 750. (b) Font histogram from positive sequences

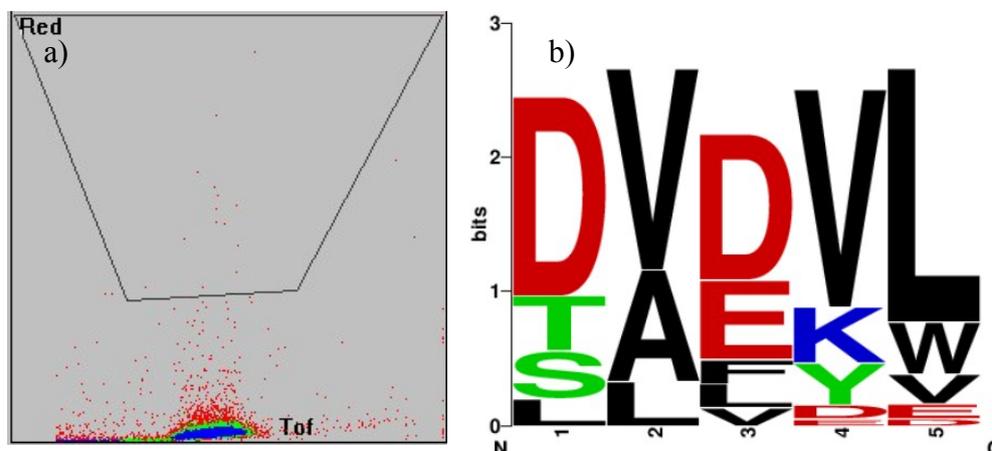


Figure S5. Screening results of **Lib 3** consisting of 18 L-amino acids excluding methionine and cysteine against *hCAII* (a) COPAS sorting image (X-axis : TOF; Y-axis : fluorescence intensity a.u.; Excitation : 640 nm solid laser; Detection conditions: Gain = 4, PMT = 750. (b) Font histogram from sequencing results, PMT = 750, Gains = 4.

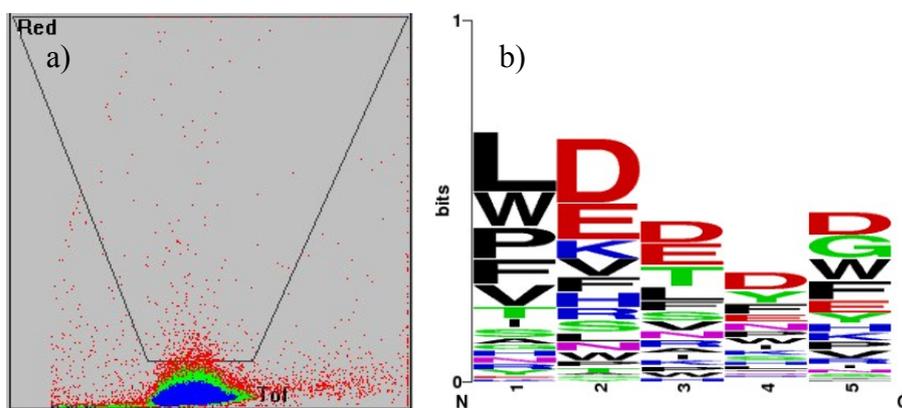
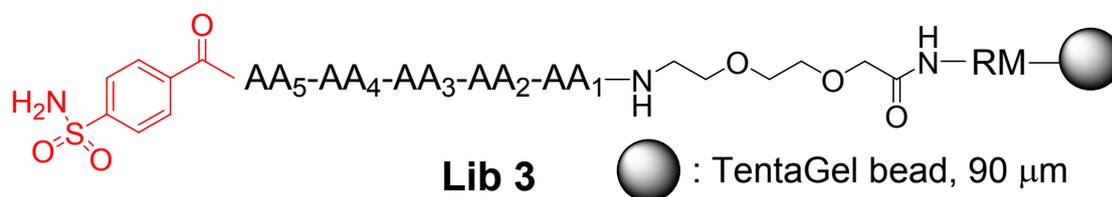


Figure S6. Screening results of a focused library consisting of L-amino acids against *hCAII* (a) COPAS sorting image (X-axis : TOF; Y-axis : fluorescence intensity a.u.; Excitation : 640 nm solid laser; Detection conditions: Gain = 4, PMT = 750) (b) Font histogram from sequencing results, PMT = 750, Gains = 4.

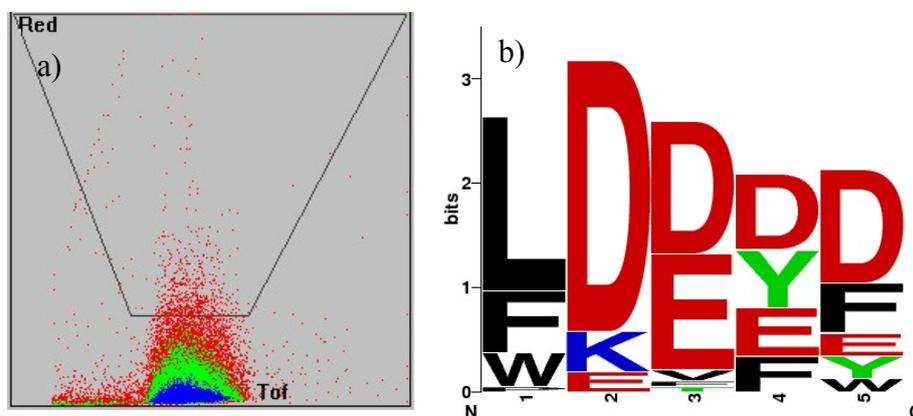
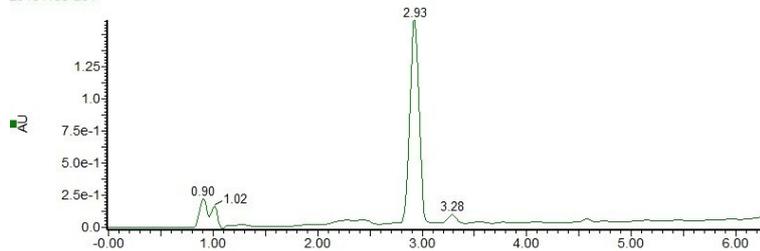


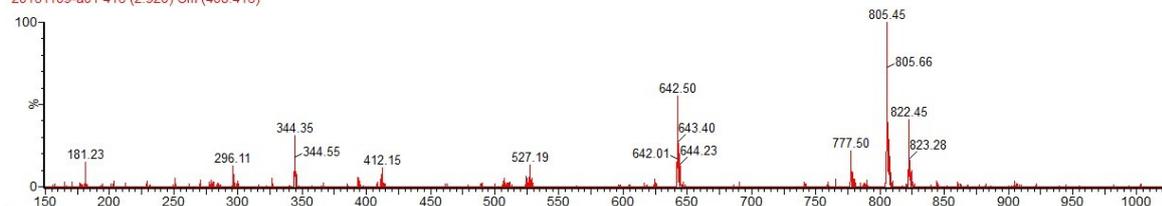
Figure S7. Characterization of peptides studied

01) 4-CBS-dlddy

CA-01) SFA-dlddy_821.82
20151109-a01

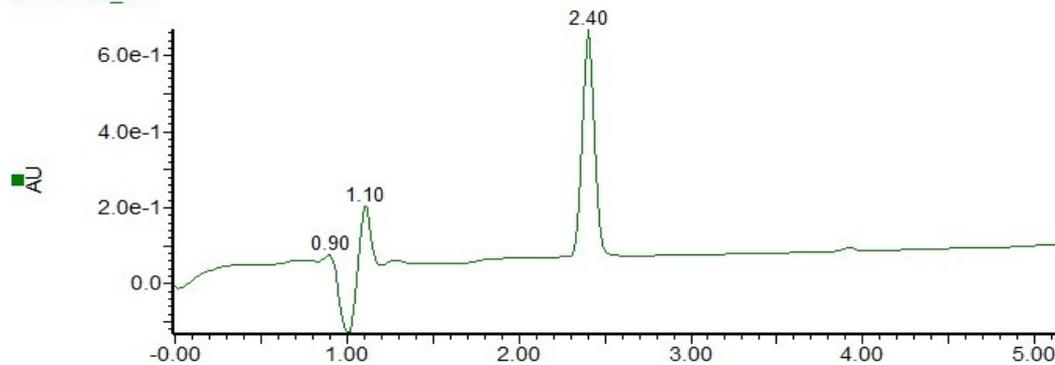


CA-01) SFA-dlddy_821.82
20151109-a01 416 (2.920) Cm (408:416)

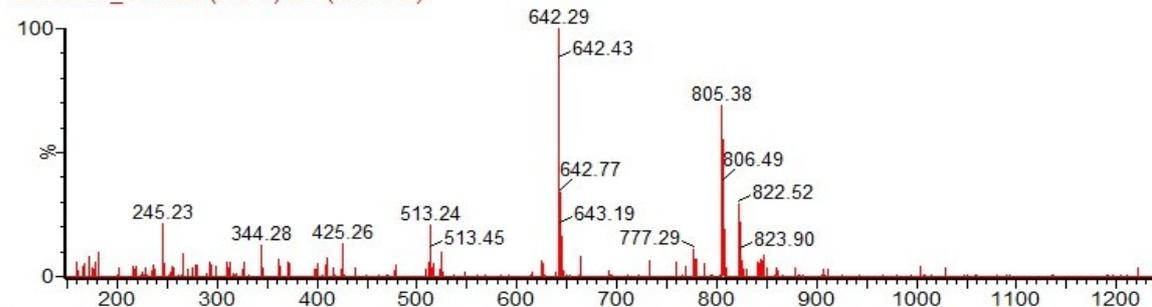


02) 4-CBS-dvdey

CA-02) SFA-dvdey_821.82_1
20151123_a11

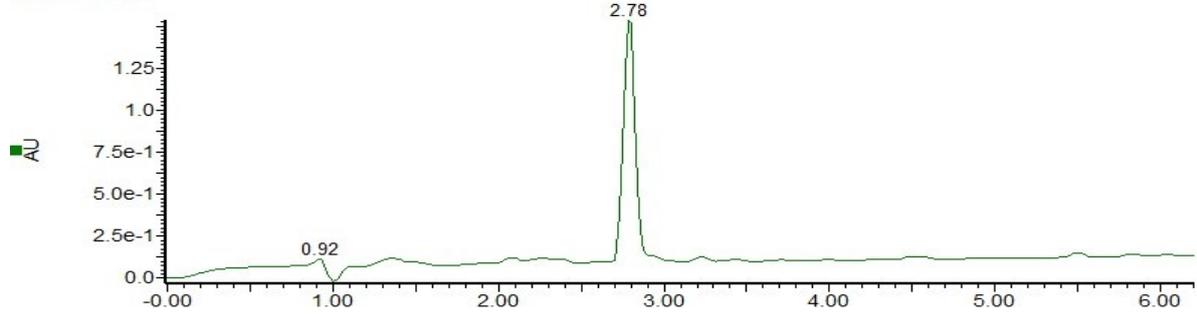


CA-02) SFA-dvdey_821.82_1
20151123_a11 338 (2.373) Cm (334:342)

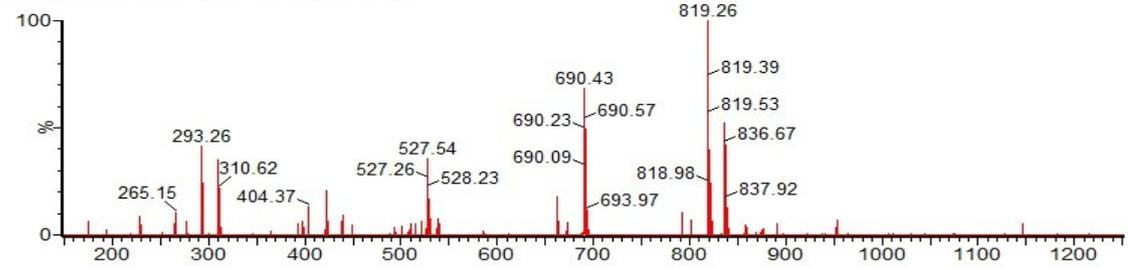


03) 4-CBS-dveye

CA-03) SFA-dveye_835.85
20151109-a03

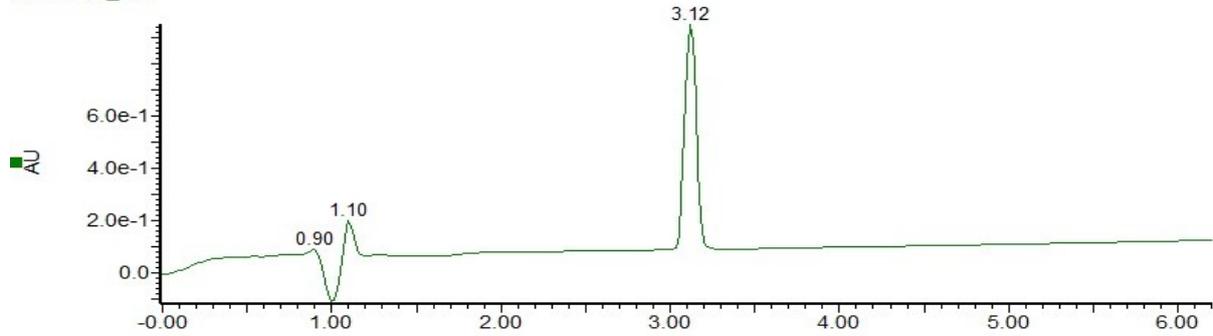


CA-03) SFA-dveye_835.85
20151109-a03 394 (2.766) Cm (392:394)

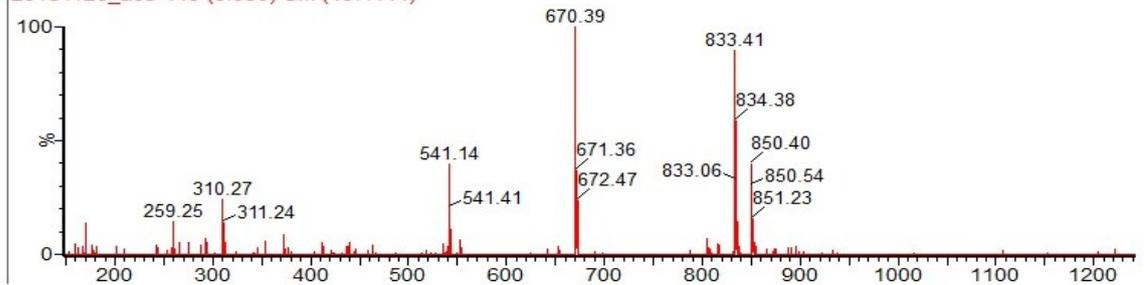


04) 4-CBS-dleey

CA-04) SFA-dleey_849.88
20151123_a09



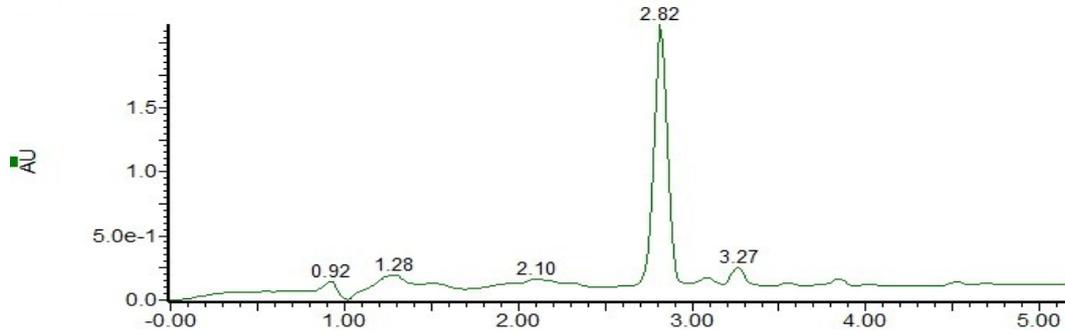
CA-04) SFA-dleey_849.88
20151123_a09 440 (3.090) Cm (437:444)



05) 4-CBS-dvddy

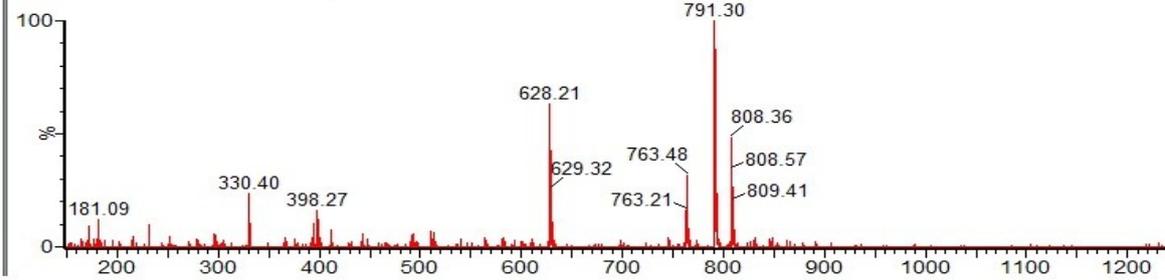
CA-05) SFA-dvddy_807.80

20151109-a05



CA-05) SFA-dvddy_807.80

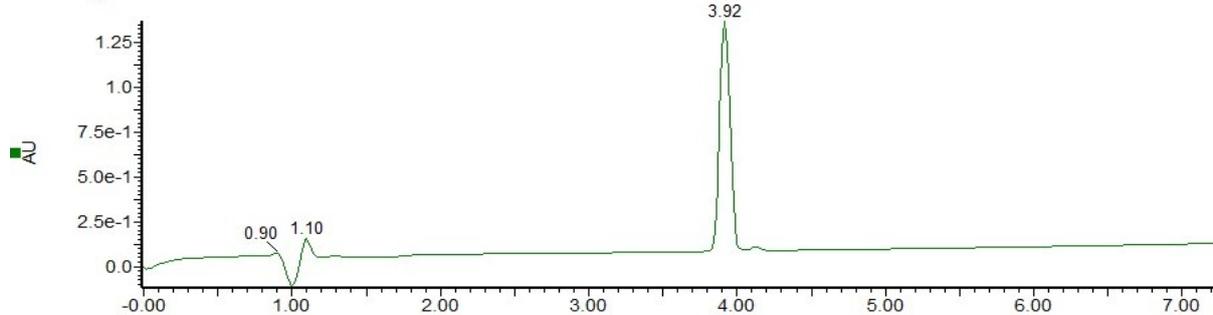
20151109-a05 398 (2.794) Cm (392:405)



06) 4-CBS-dliddw

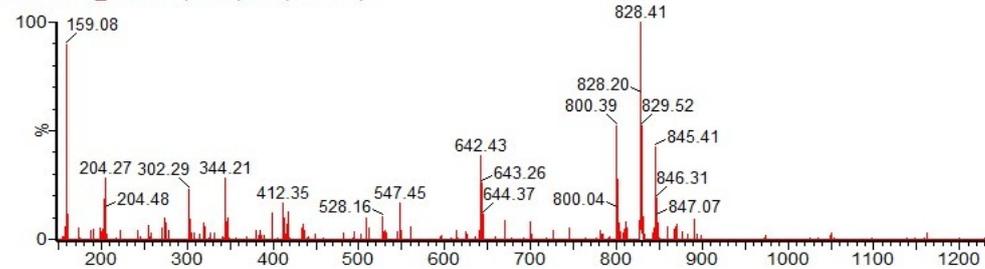
CA-06) SFA-dliddw_844.86

20151123_a08



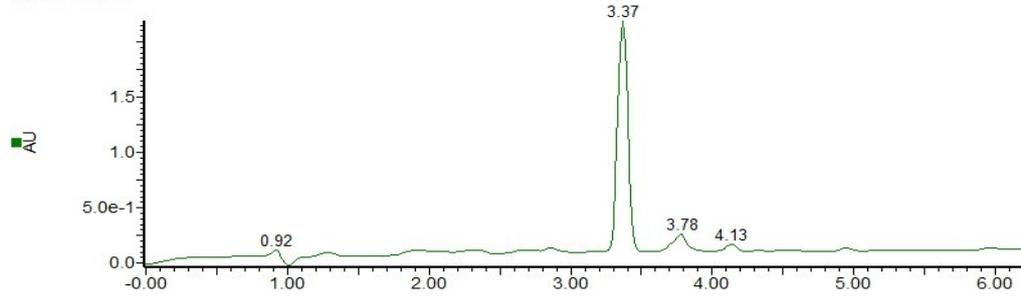
CA-06) SFA-dliddw_844.86

20151123_a08 553 (3.883) Cm (551:557)

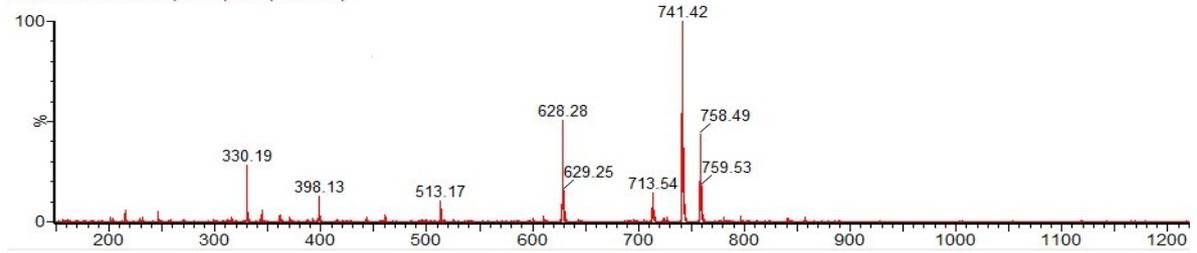


07) 4-CBS-dvddl

CA-07) SFA-dvddl_757.78
20151109-a07

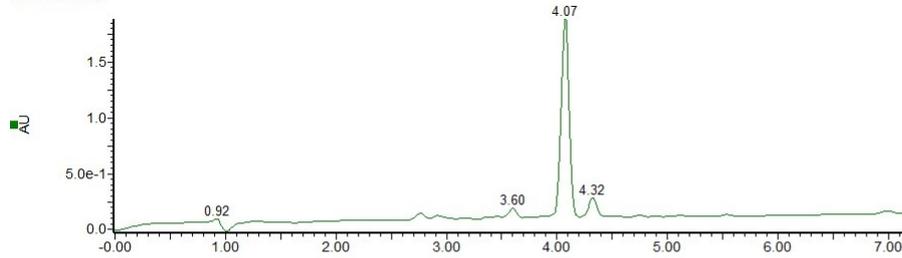


CA-07) SFA-dvddl_757.78
20151109-a07 475 (3.335) Cm (472:486)

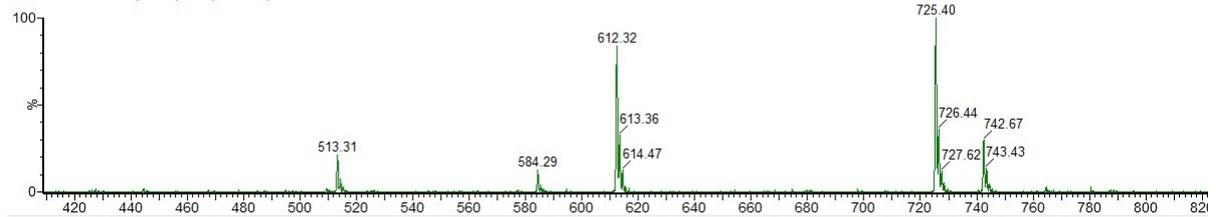


08) 4-CBS-dvdvl

CA-08) SFA-dvdvl_741.82
20151109-a08

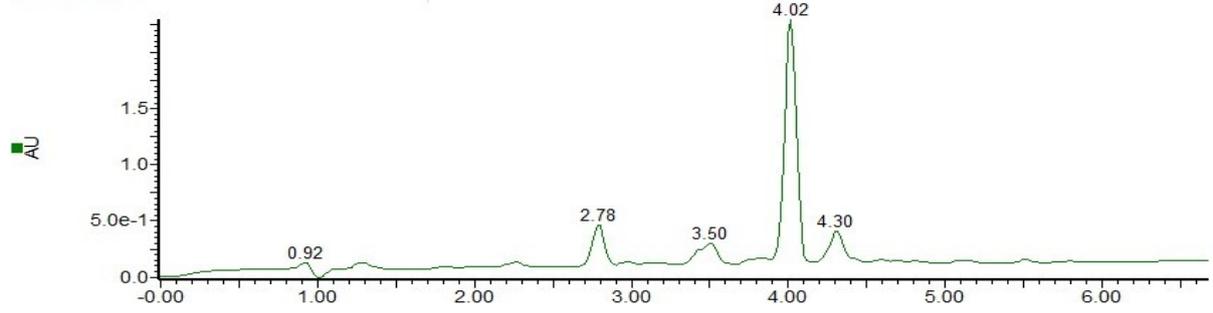


CA-08) SFA-dvdvl_741.82
20151109-a08 574 (4.030) Cm (574:587)

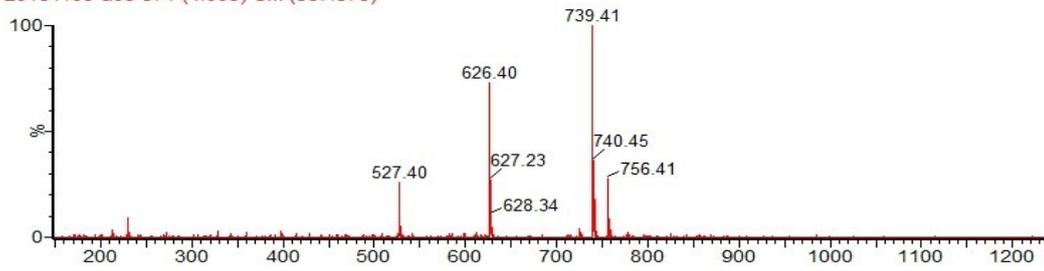


09) 4-CBS-dvevl

CA-09) SFA-dvevl_755.85
20151109-a09

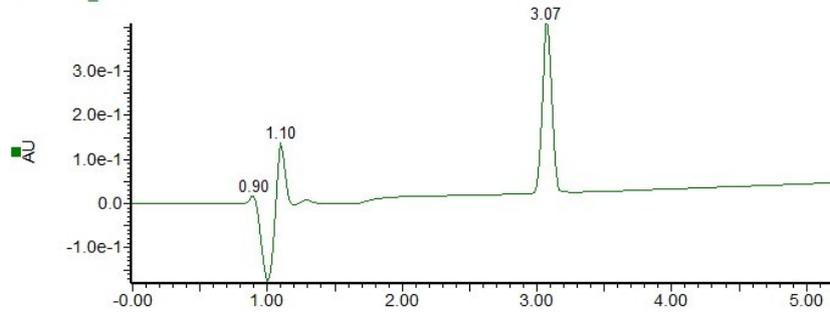


CA-09) SFA-dvevl_755.85
20151109-a09 571 (4.009) Cm (567:575)

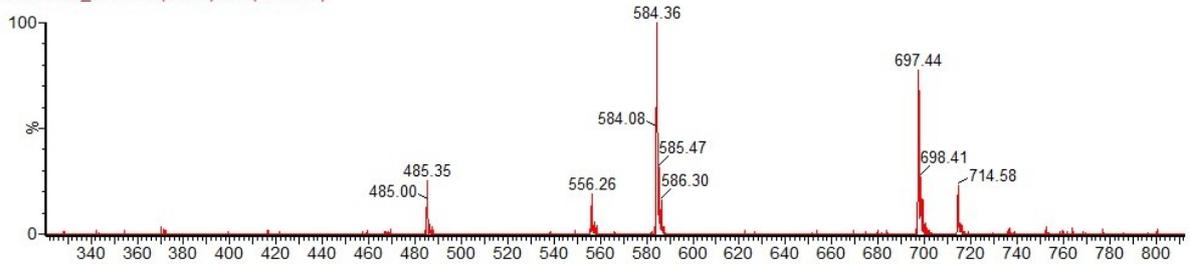


10) 4-CBS-dadvl

CA-10) SFA-dadvl_713.77
20151123_a10

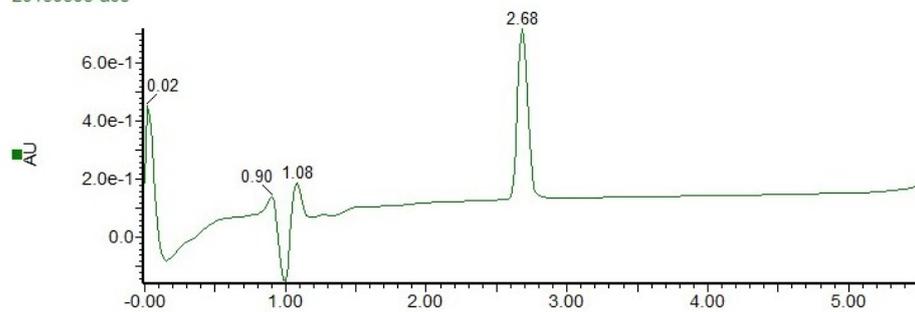


CA-10) SFA-dadvl_713.77
20151123_a10 433 (3.040) Cm (431:437)

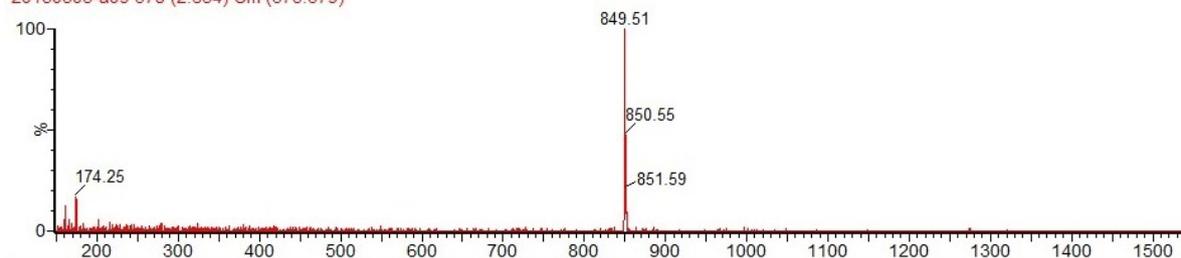


11) 4-CBS-LKEYD

2) SFA-LKEYD_848.96
20150505-a09

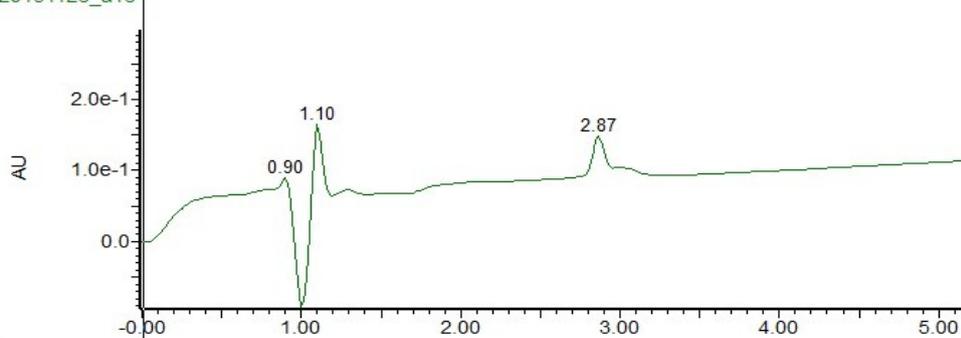


2) SFA-LKEYD_848.96
20150505-a09 378 (2.654) Cm (375:379)

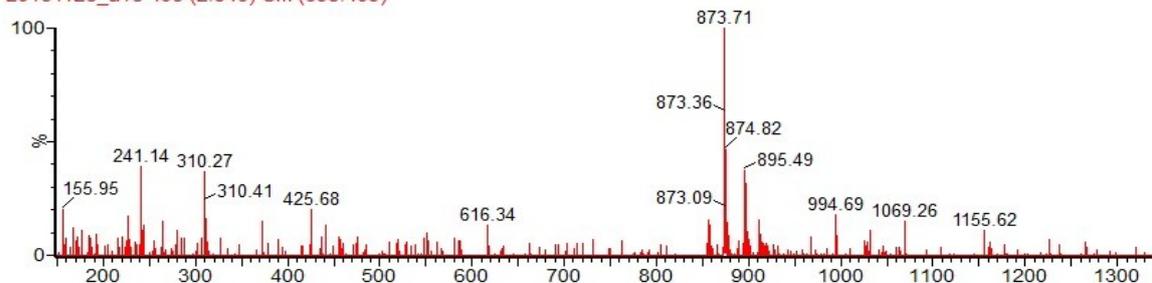


12) 4-CBS-P2-b

CA-A) SFA-p2-b_873.07
20151126_a16



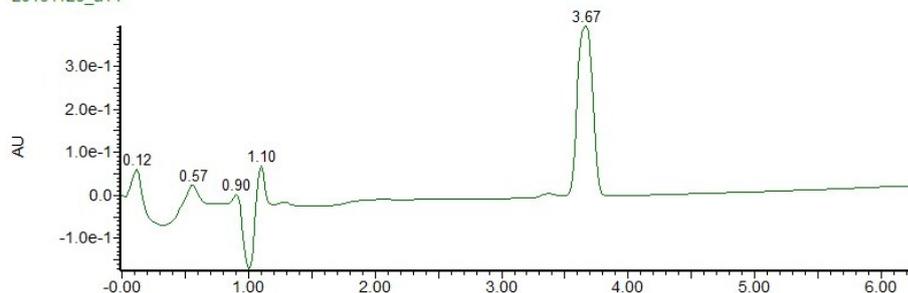
CA-A) SFA-p2-b_873.07
20151126_a16 405 (2.843) Cm (398:409)



13) 4-CBS-dlddy-P2-b

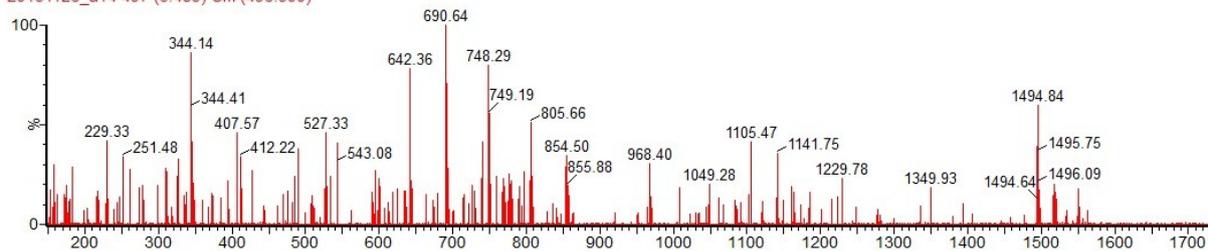
CA-B) SFA-dlddy-p2-b_1494.67

20151126_a14



CA-B) SFA-dlddy-p2-b_1494.67

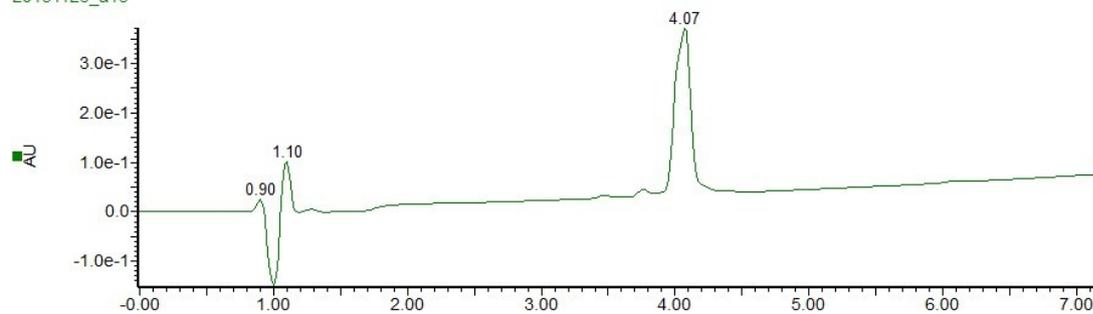
20151126_a14 497 (3.489) Cm (496:500)



14) 4-CBS-dliddw-P2-b

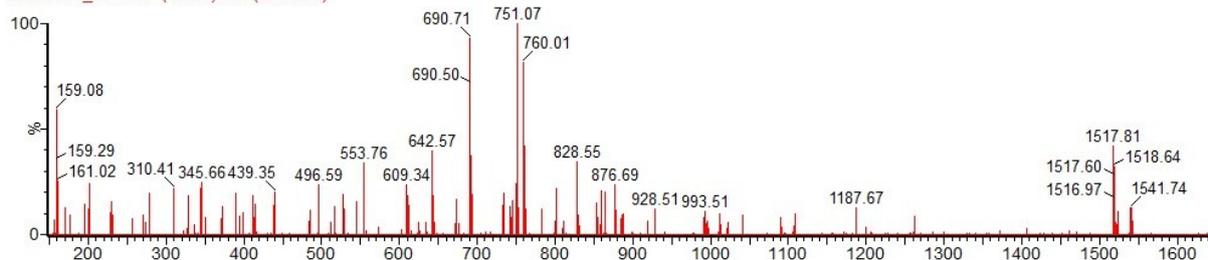
CA-C) SFA-dliddw-p2-b_1517.71

20151123_a13



CA-C) SFA-dliddw-p2-b_1517.71

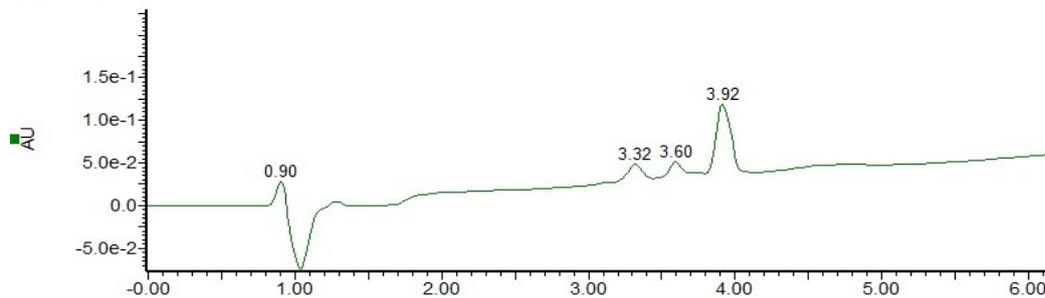
20151123_a13 577 (4.051) Cm (577:578)



15) 4-CBS-dvdvl-P2-b

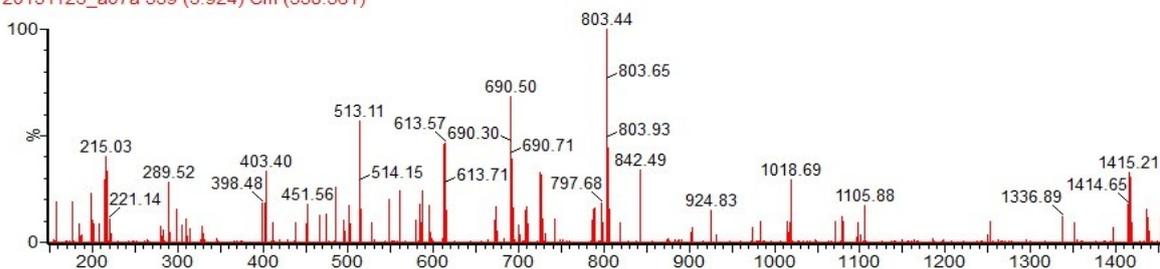
CA-D) SFA-dvdvl-p2-b_1414.67

20151123_a07a



CA-D) SFA-dvdvl-p2-b_1414.67

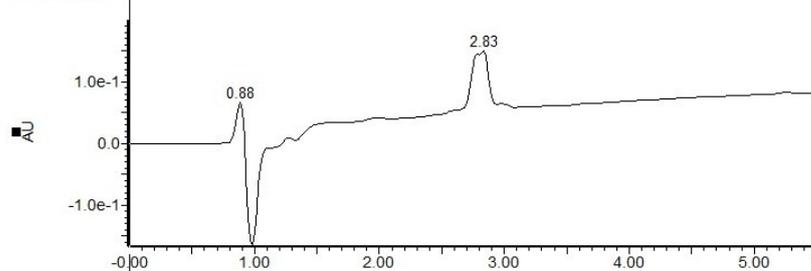
20151123_a07a 559 (3.924) Cm (558.561)



16) 4-CBS-LKEYD-P2-b

B2) SFA-LKEYD-p2-b_1521.78

20150505-a11



B2) SFA-LKEYD-p2-b_1521.78

20150505-a10 418 (2.935) Cm (415.422)

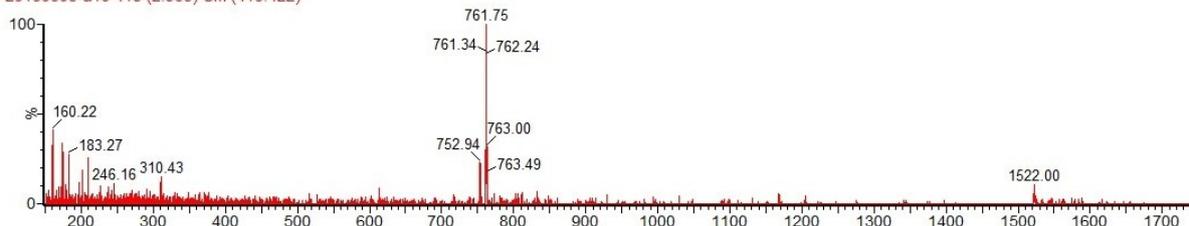
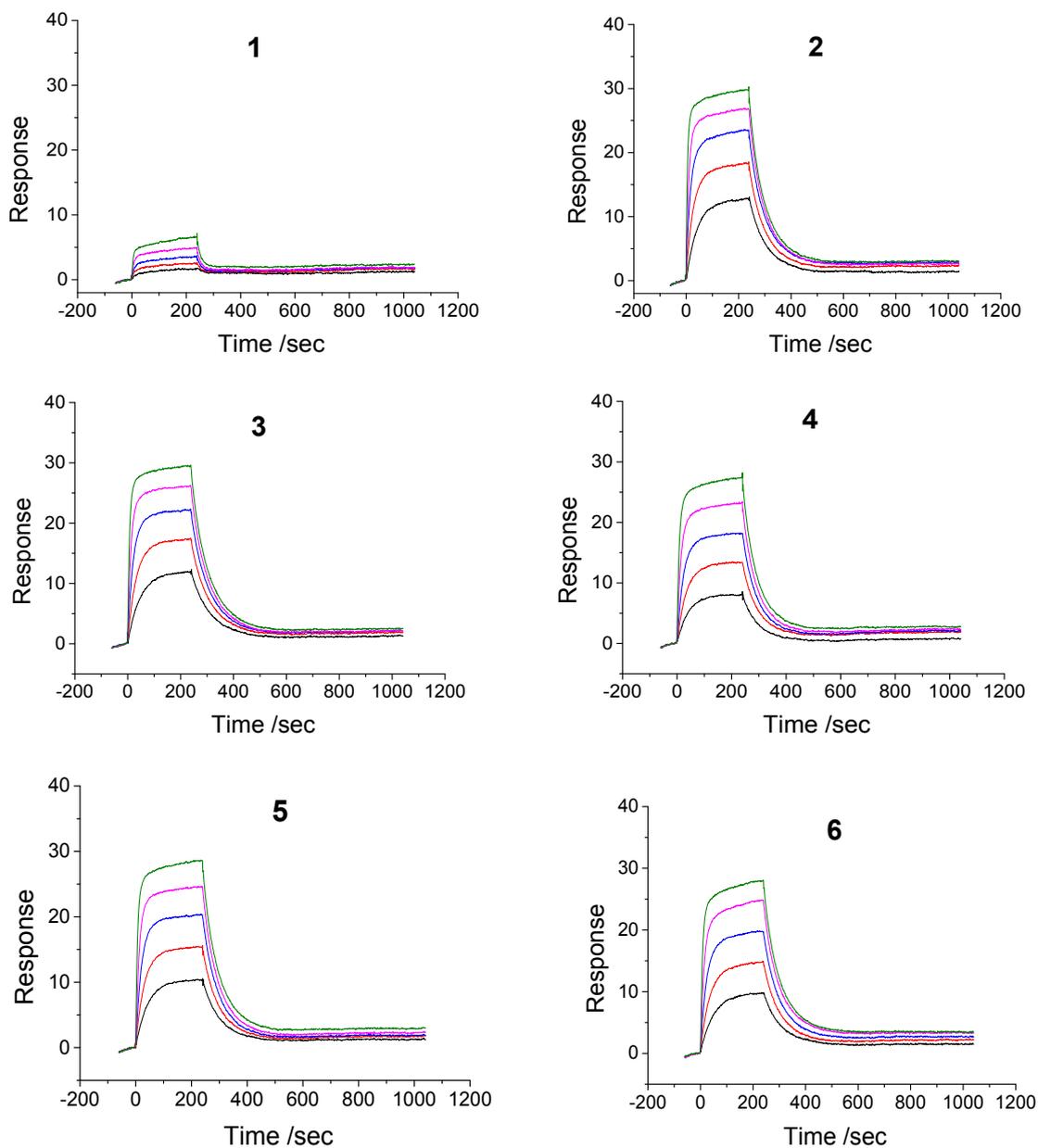
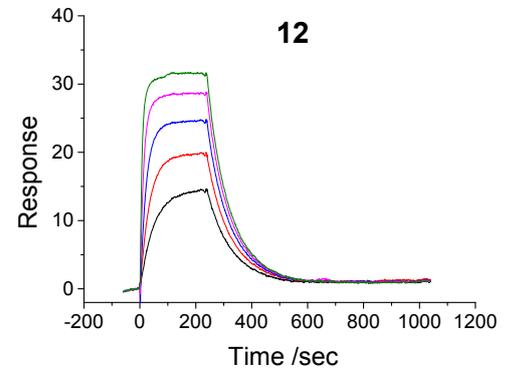
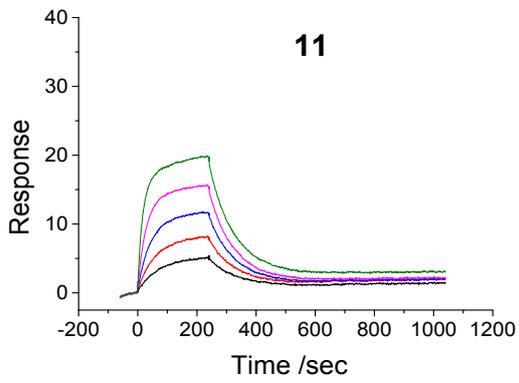
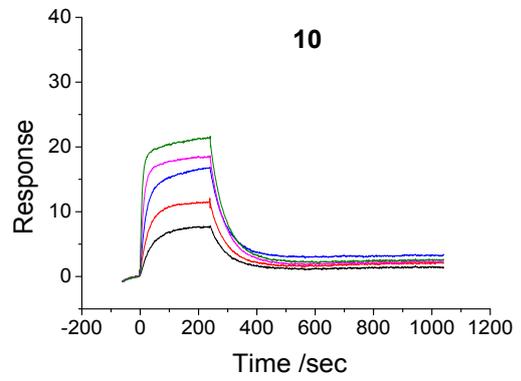
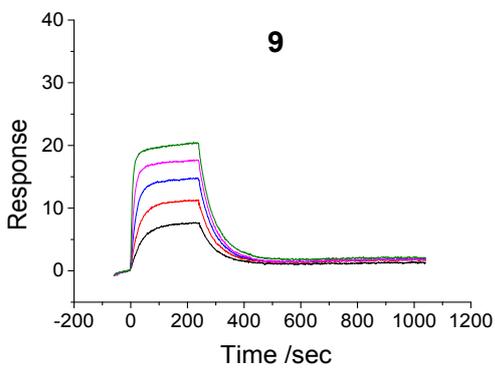
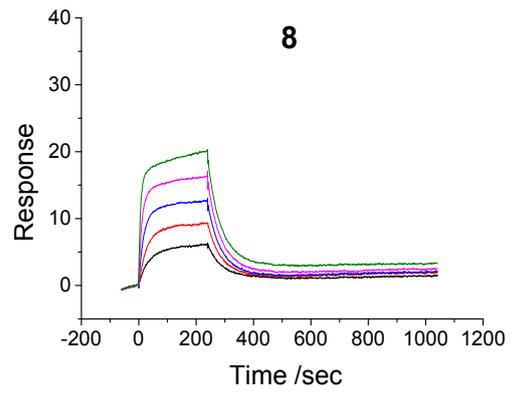
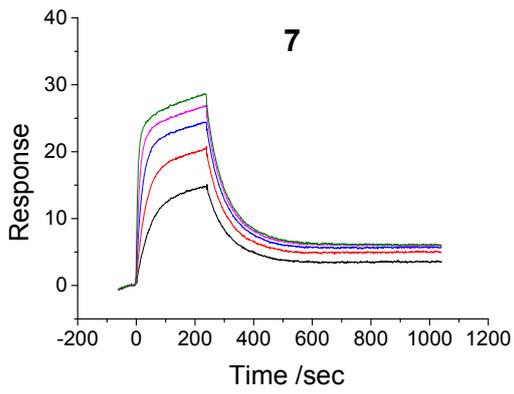


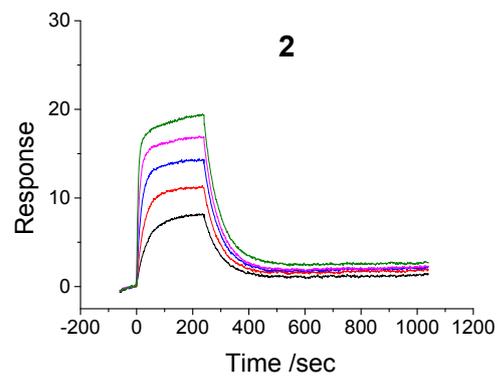
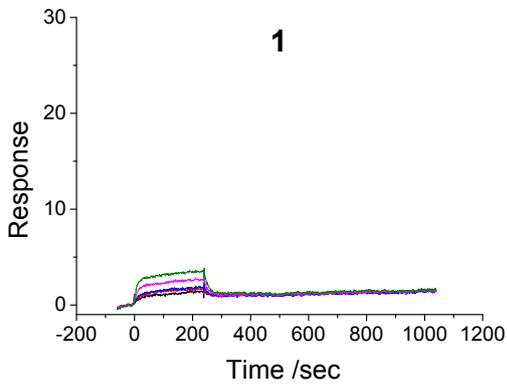
Figure S8. Representative SPR sensorgrams for the binding of **1-12** to (a) *hCAII* (b) *hCAI* and (c) *hCAIX* immobilized on the CM5 sensor chip surface by injecting a concentration series of the analyte (500, 250, 125, 62.5, and 31 nM from top to bottom).

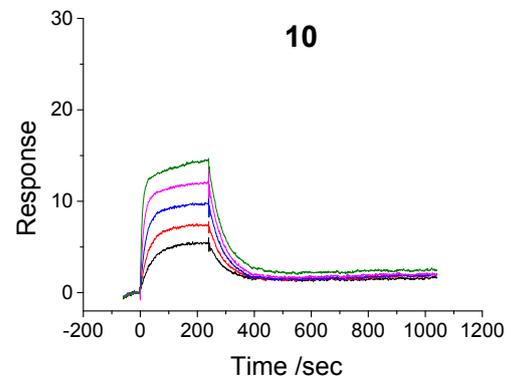
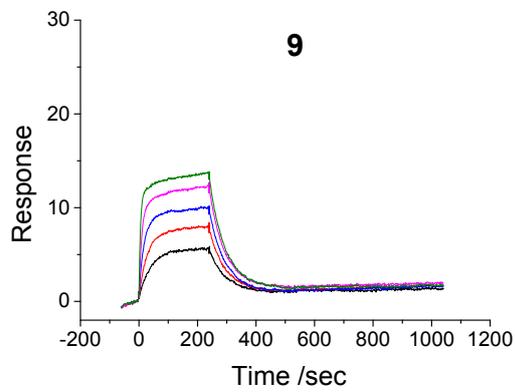
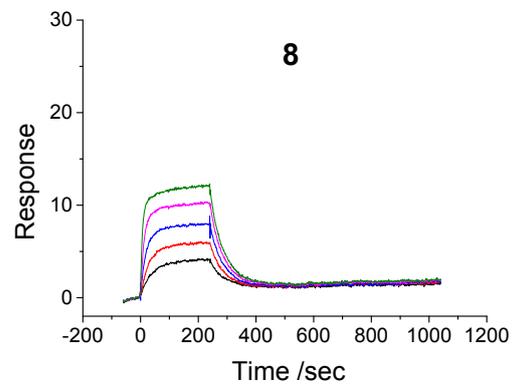
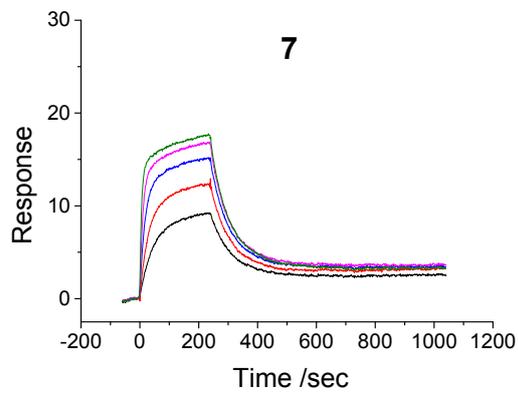
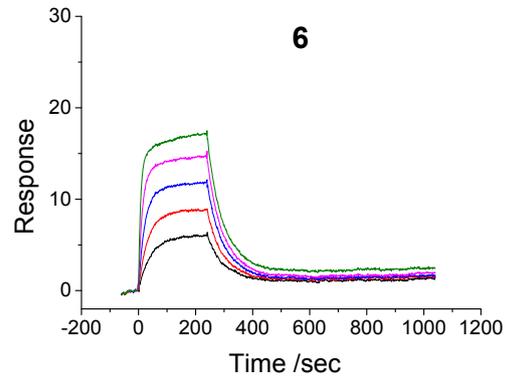
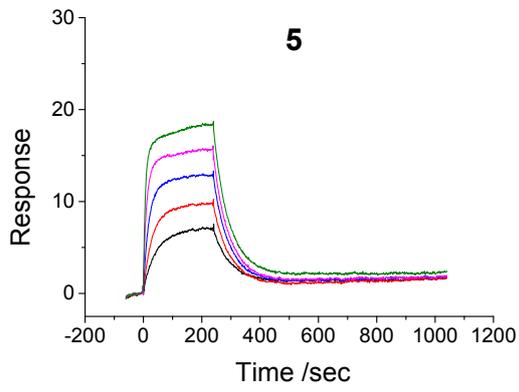
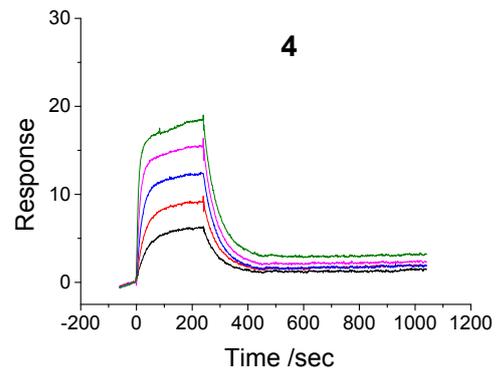
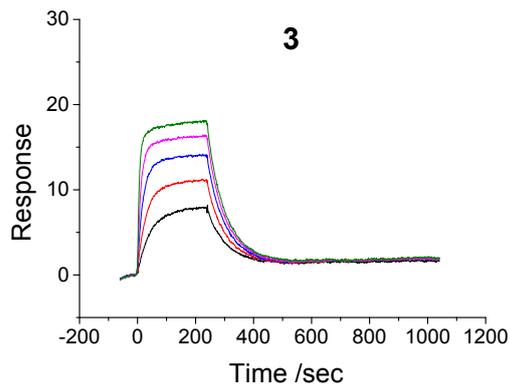
(a) *hCAII*

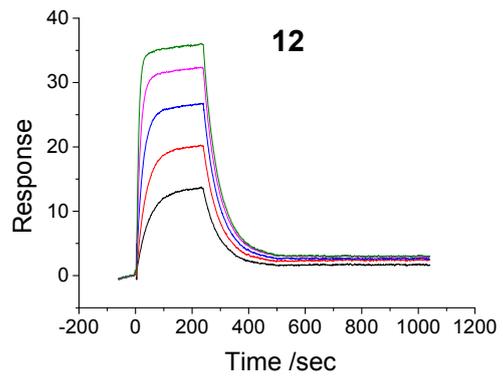
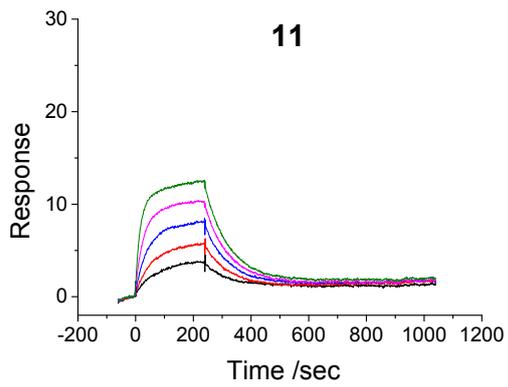




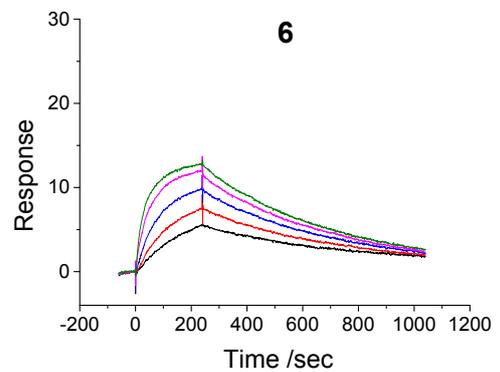
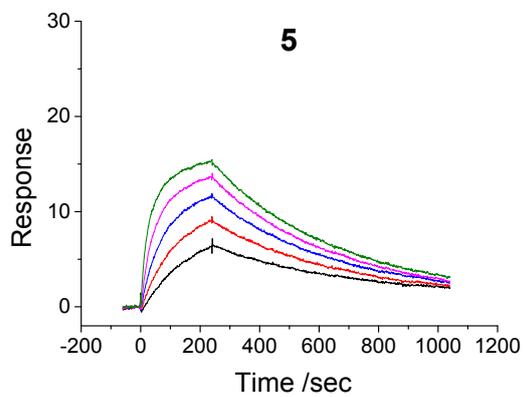
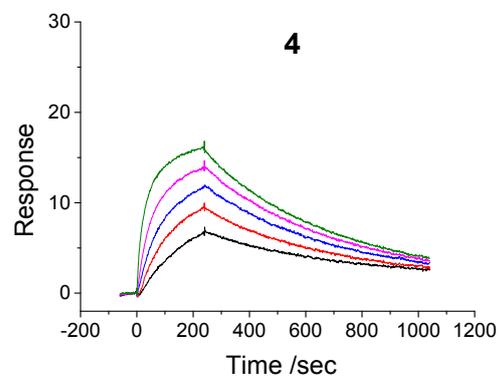
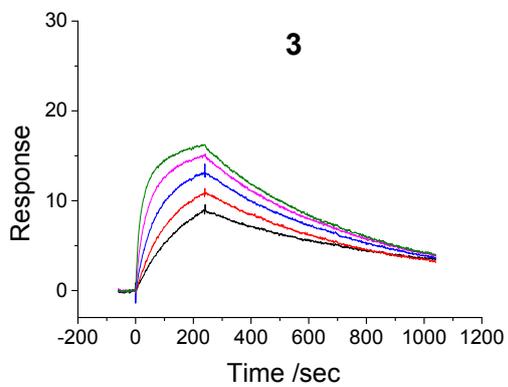
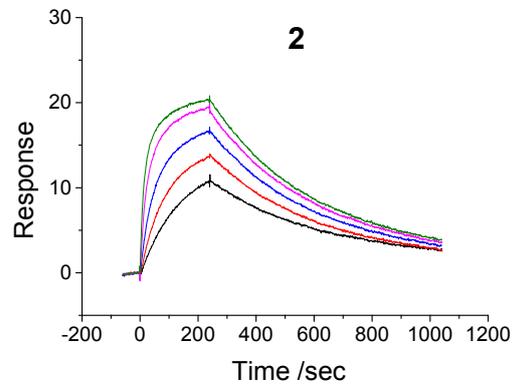
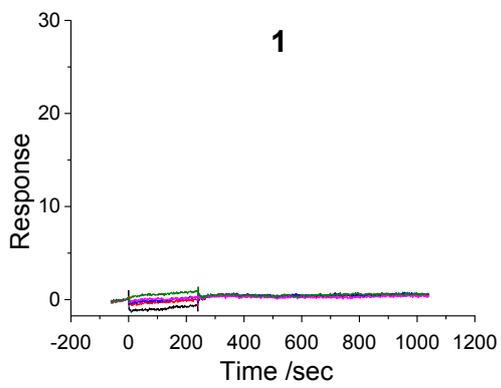
(a) *hCAI*







(b) *hCAIX*



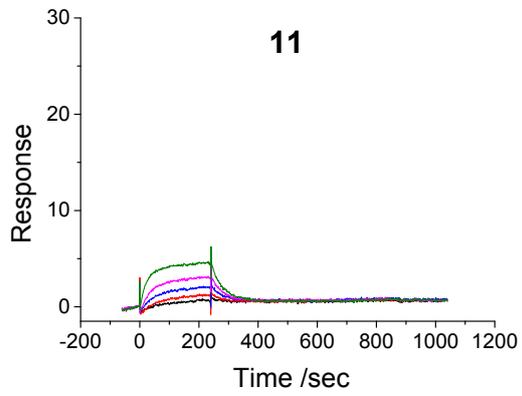
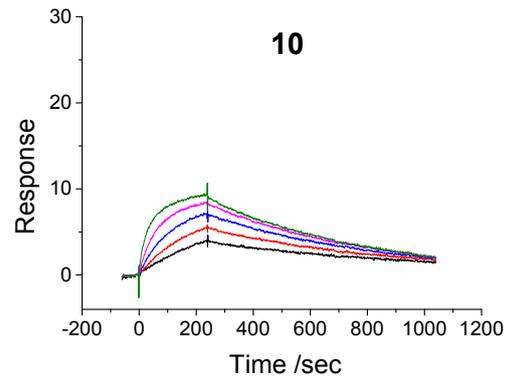
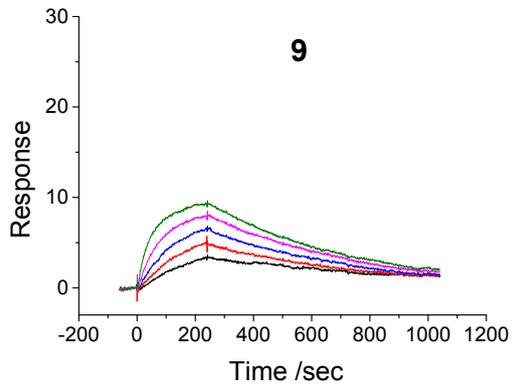
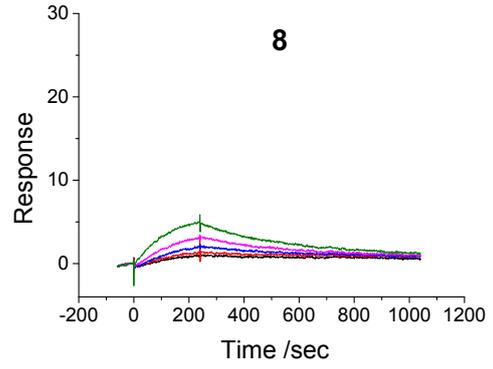
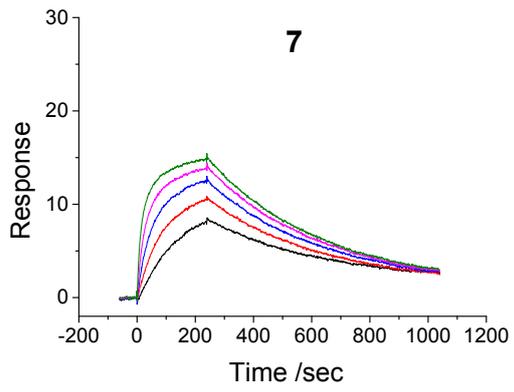


Figure S9 Plot of esterase activity of (a) *hCAI*, (b) *hCAII* and (c) *hCAIX* with selected candidates, **1**, **2**, **7**, **9** and **12**.

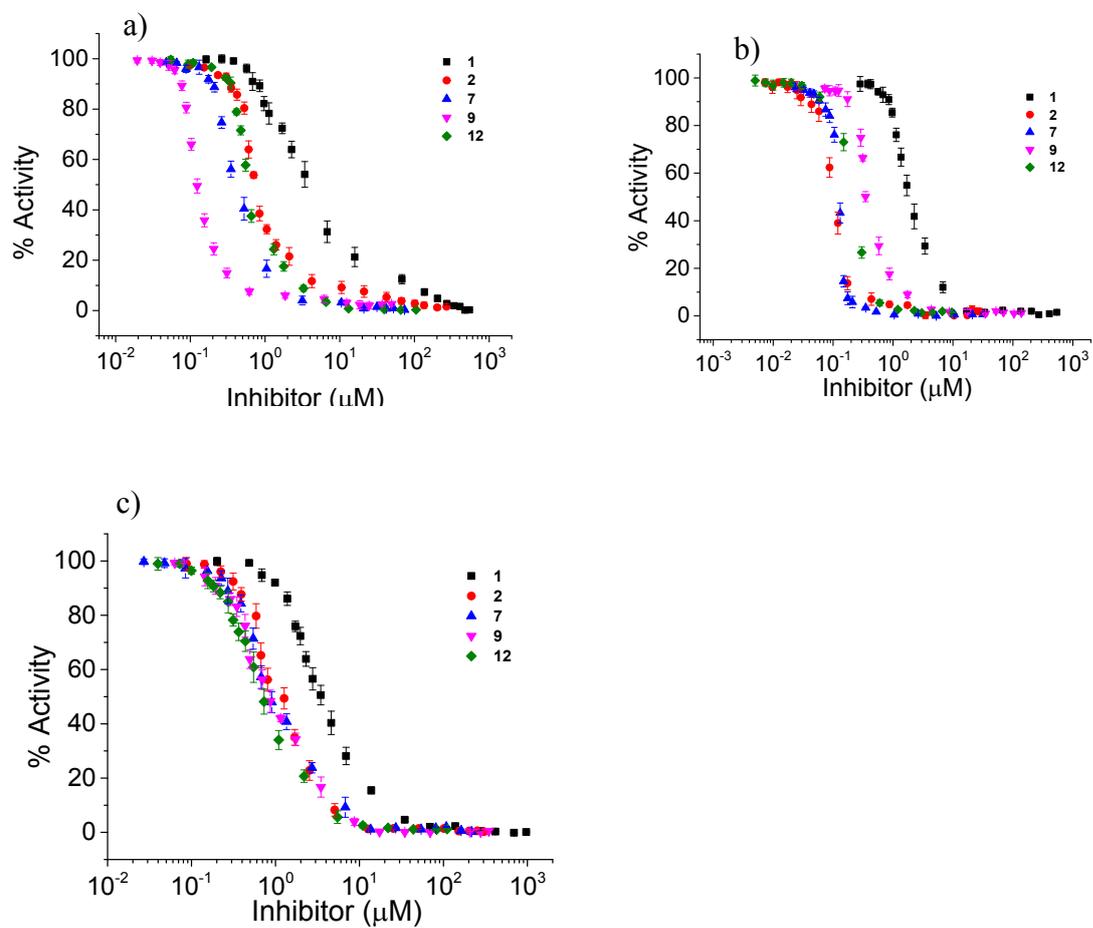


Table S2. Positive peptides from the screening of **Lib 1** against *hCAII*.

liGpw	vdnly	kldea	Indee	kaswa
ppdaw	vifls	lklkf	knGna	dykev
lknfd	psdvd	adtat	pkkka	esers
dtfk	avylk	fGtsG	kknsa	edktk
trvev	laklk	liysv	spsls	fydfe
nikes	apeak	wskek	akktk	weaGk
vdkae	tknek	dayGv	keddf	fvkek
Gakrk	frira	dvdre	nkkfk	
lGyne	Gaske	neddi	skkly	
eklkk	dfkkv	nddev	fesky	
kaeay	eryea	wltek	sniece	

Table S3. Positive peptides from the screening of **Lib 1** against *hCAI*.

rytav	wpdwy	rlted	wafay	rwsG
ksney	pvtep	ffvkk	phwhf	krhek
khkGd	ynvpf	fdywk	frnrn	lhvar
ferfl	wrGrt	wykgv	yynvs	wvrlh
phGne	weldy	qlnyt	whktn	lawlw
Gkrwf	ylyqp	tfrsl	hwl	yswla
kGskl	trrvr	lkalG	wprsl	yllaq
wdttt	Gftrp	Gvhke	tylrl	nlGwh
pyyvd	sGfen	ssrda	Grpnk	GGrdl
ylaGp	wGpfn	fhytk	ekrry	sGrrw
ayeay	svyvt	nfGGr	phsyG	
flvls	snpry	kyprs		
GrwtG	wlwhf	esaff		
aykr	kwsfk	tlrpv		
fpayv	srtfi	nfGGr		

Table S4. Positive peptides from the screening of **Lib 2** against *hCAII*.

llikf	dvpyk	llsvd	lknGe	lleew
lveyp	plisy	llear	lleyh	dleew
rvdGs	ptktd	yvkes	nvvvq	pvsky
pleer	lvese	nanaG	tvekl	hhkvq
syGnk	lktse	andyv	evnfk	lvsla
hllpe	plveG	tldiv	Gnfve	evfle
tlfdy	lvefv	ffeef	lvfkk	svlkd
tvfyd	dvwet	kidre	pllef	lvfvv
sllre	vlvdd	tliny	saetr	seldh
sildv	svneG	laeps	knnkl	eiedn
srvnl	slvel	tvkGe	lveqw	kvfkd
llanf	evlas	kvdGr	lllph	sleli
pyGel	dakty			

Table S5. Positive peptides from the screening of **Lib 2** against *hCAI*.

lhpGk	Gedwy	ryywt	enifq	lknsh
lhgdd	eyyvl	pspkv	pfsey	pwvad
whswv	aeeGy	tavkf	affvp	yhfny
eerdG	pdkaf	eflke	iyssG	fyhil
flpyw	nwyss	wdfhe	lkavl	hrlnn
lGklw	ekwew			

Table S6. Positive peptides from the screening of the 5-mer focused library against *hCAII*

N-terminal	l	s	d	t		
	l	v	a			
	d	e	f	l	v	
	d	e	k	v	y	
C-terminal	d	e	v	l	w	y

dvdey	dvdey	dvfee	dvdve	dldyy
dvedy	dveyy	dlldv	dvddl	dldel
dvdee	dlddy	dvedv	dledv	dledl
dledw	dldel	dvddy	dvedl	dledl
dvddy	dleey	dvldy	dldvd	dveee
dalee	dlddw	dlfed	dvdey	dvldy
dvdee	dvedy	dldyl	dvdye	dldew
dledd	dvfey	dvde	dveyd	dveyv
dldye	dldye	dlddl	dveyd	dlfdy
dveye	dvdye	dvfed	Dveyy	dleey
dlddy	dldvv	dveel	dvddl	dlddy
dllee	dldey	dlfvv	dvdew	dlldd
dledw	dldyd	dvfee	dleey	dvfw
dvlye	dvfel	tlfkl	dveye	dvdv
dveve	dveyy	dvddy	dvddv	dldye
dldew	dldde	dlldd	dlddl	dlldy
dldvl	dvdvd	dlddw	dvdye	dleee
dveee	dvvye	dveee	dvfdd	dvedy
dvddy	dlldv	dlddy	dldey	dledw
dleew	dvfee	dvedv	dvddw	dvdew
dvddl	dvdey	dvddv	dlled	dlldy
dlddy	dveye	dlddw	dvdey	dledy
sllyy	dvddl	dldey	dveew	dvedy
dveew	dledw	dleey	dvdye	dvedw
dveye	dlddl	dvfde	dlddw	dlddl
dveyy	dlldy	dledy	dlddy	dleey
dveye	dleee	dvfyv	dllde	dvddw
dveew	dvdey	dldyd	dldew	

Table S7. Positive peptides from the screening of the 5-mer focused library against *hCAI*

tadyv	tvdvl	dvdivl	llevw	dafvn
dvdivl	dvdivl	dvdivl	dvfdl	dvevl
dvdivl	tvevl	tvevl	ladkl	dadvl
daevl	savke	dvdivl	dvlvl	dvdivl
dvdivl	tadyv	svvkw	dvdel	dadvv
dvvyw	dvdivl	tadvl	dvshk	daevl
sllkw	dadvl	dvevl	svdvd	sldyw
dvevl	saeye	ddl	sadyw	tvevl
daevl	dvevl	lllkl	dllkv	dvdivl
dlfkl	tvedl	dadvl	lvfkw	dvdivl

Table S8. Positive peptides from the screening of **Lib 3** consisting of L-amino acids against *hCAII*

KDPNN	LPHNW	PNRHE	LDEYK	PDPWG
AHSPH	IVRFK	IEVYD	LDDFG	WSDEG
LLFAR	TDLYW	PKPW ^N	WHERR	PEVNY
FWLIW	FEIDE	FDNYG	LDEWP	WWKKG
NFDDK	WFRPF	LDRYW	LLNYD	IESGY
LELEW	SKHDF	PETTW	PKDEF	RSAHG
PEFDY	AHPKK	VEWDG	VEEFY	WDYQF
DWEVL	VDFNP	LNSFV	VDDRW	WVTQH
FEVED	HWPDI	FYEPF	WDENF	NTEPE
LKCLKH	EFLLD	LVSYV	LNTYF	WGDHD
PTKDY	VRTYW	WHAFS	FRTLE	VEFED
WDDNS	YHTAD	TEWFD	SVLIF	TFAIV
PDDL _G	RERN	VDTWL	LDDFP	FDEIG
PDTDF	GRNWD	TKFDV	GPWDF	KVIVE
LDISN	IWES	LDVDW	SKVKA	YSDDI
LDESG	RFSNW	HANPR	LLESD	LSKDP
FDLFD	LDEEG	WRDSE		

Table S9. Positive peptides from the screening of the 5-mer focused library consisting of L-amino acids against *hCAII*

N term	F	L	W	P	
2	D	E	K	V	
3	D	E	F	V	T
4	D	E	F	N	Y
C term	D	E	F	W	Y

LDTDD	LDEDF	LDDDY	FDDFD	WDDDE
PKVED	LDDEF	LDDYD	FDDFD	LDEYY
LDEDD	LDEDF	LDDYD	LKEEF	WDEED
LEDDD	LDDEF	LDEYD	LKEYD	WKDFD
LDDED	LDEDF	LDEYD	LKEYD	FDDDW
LDVEF	LDDFE	FDEED	FDEDF	WKEDE
LDDDF	LDDYD	LDDYE	FDDYD	FDDEW
LDFDD	LDDYD	LDEYD	FDEEF	WEEEE
LDDFD	LDDDY	FDDFD	FDEDY	WEDYD
FDEYW				