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NMR-based screening of combinatorial libraries to target protein-protein interactions with reversible or covalent agents

North Jersey ACS NMR Topical Group
2020 Virtual NMR Symposium
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1. Background: NMR-guided drug discovery targeting protein-protein interactions.

2. Application of the HTS by NMR against a protein-protein interaction involving EphA4-LBD and the ephrin ligands

3. Introduction of an anchoring moiety in the library (focused HTS by NMR) and applications to various targets including metalloproteases, and the BIR3 domains of IAP proteins

4. Design of Lys/Tyr covalent agents using sulfonyl-fluorides or fluoro-sulfates

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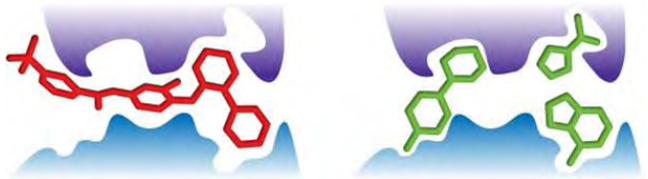
Protein-protein interactions (PPIs) represent a large class of potentially viable therapeutic targets that are deemed "*undruggable*"

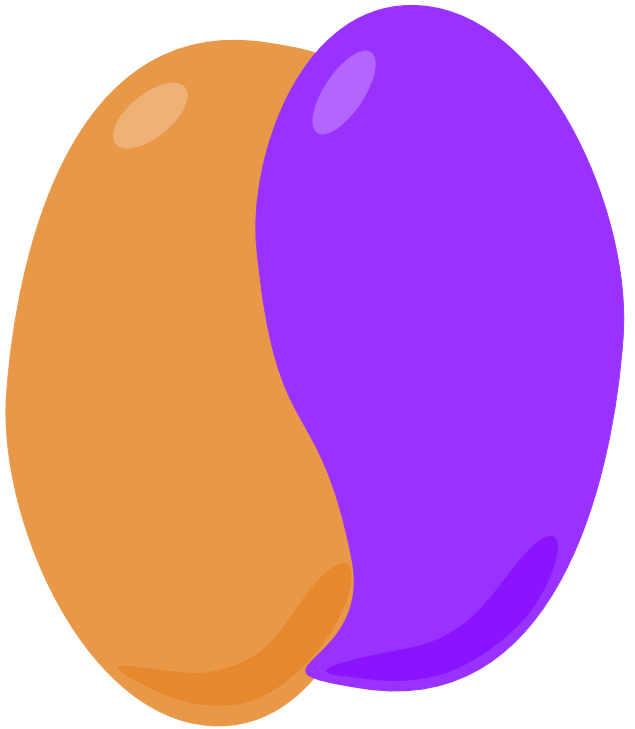
- Targeting PPIs has been notoriously difficult using conventional biochemical HTS approaches (identification of artefacts or PAINS compounds)
- Contact surface area is typically too large for a "small-molecule" (MW 500 -1000)
- Binding pockets tend to be flat with induced fit grooves
- Lack natural small molecule binders onto which design mimetics
- Some success using fragment- and structure based approaches (i.e. SAR by NMR)



- Short peptides or peptide-mimetics targeting PPIs (HTS by NMR) as chemical probes or even therapeutics

Enzyme + Inhibitor
(or substrate or cofactor)

Protein-Protein interaction



SAR by NMR discovery of the first FDA approved PPIs antagonist Venetoclax (ABT199, Bcl-2 antagonist)

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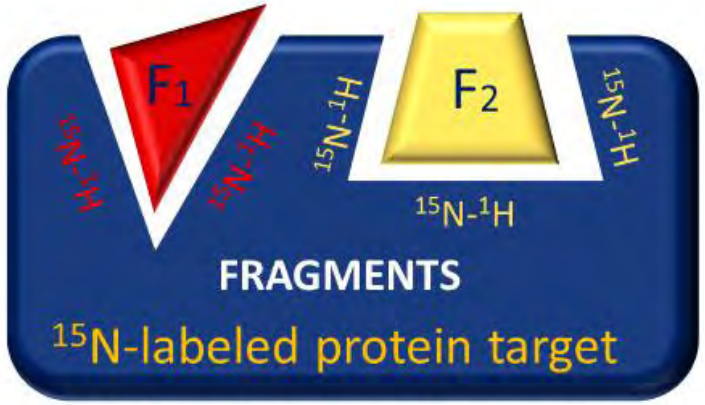
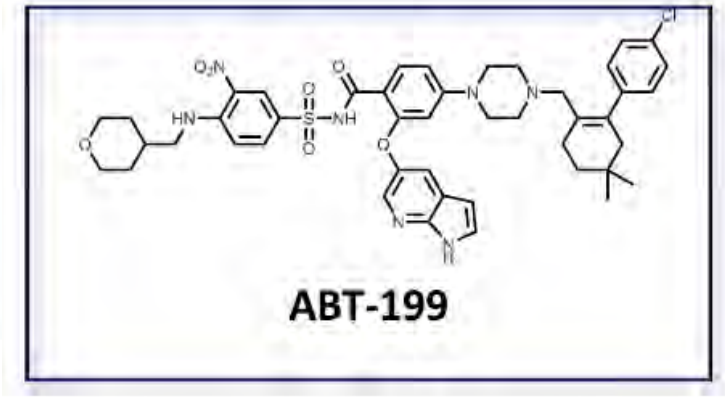
The design of potent (low nM) and selective PPIs antagonists is not a trivial task

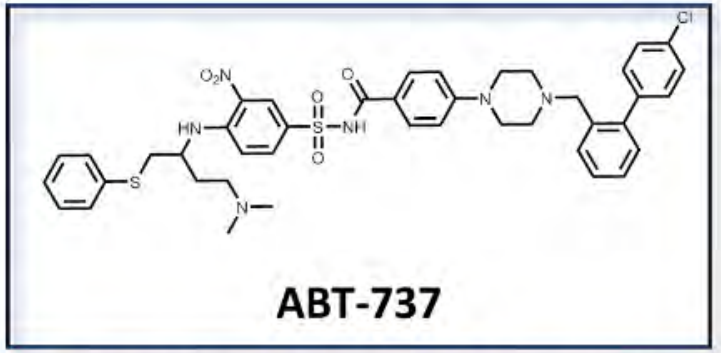


NMR-protein based screening and second site screening

Synthesis of bi-dentate compounds



3D structure of ternary complex with two optimized fragments



Medicinal chemistry and structure based optimizations

Shuker SB, Hajduk PJ, Meadows RP, Fesik SW. **Science. (1996)** 274(5292):1531-4. Discovering high-affinity ligands for proteins: SAR by NMR.

Oltersdorf et al. **Nature (2005)** 435, 677-681. An inhibitor of Bcl-2 family proteins induces regression of solid tumours

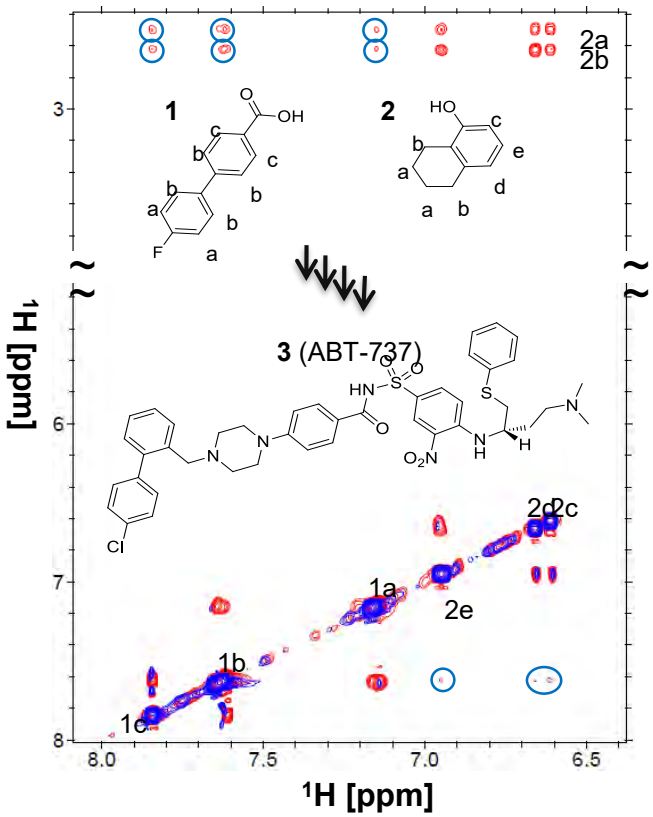
Souers et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. **Nature Medicine (2013)** 19, 202–208.

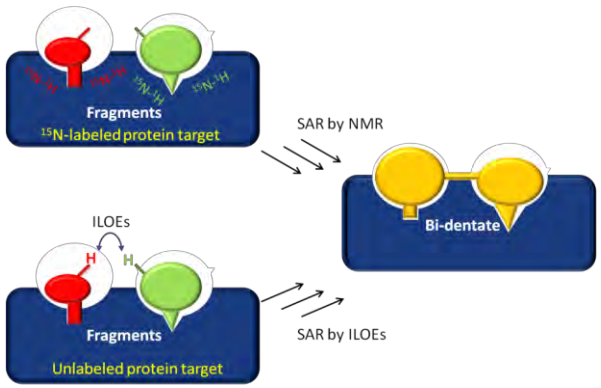
20+ years!

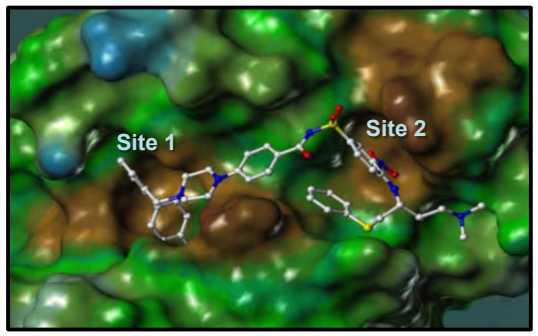
FDA approves Venetoclax (2016) https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm495253.htm

Spin diffusion measurements

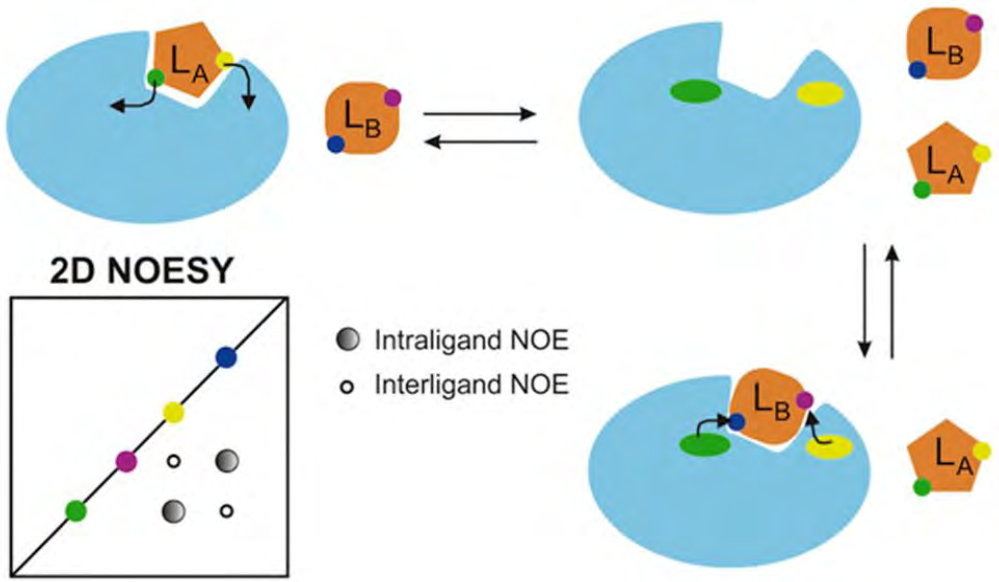
*SAR by ILOEs







**INPHARMA



*Rega et al. Pellecchia J Med Chem. 2011 Sep 8; 54(17): 6000–6013.

Carlomagno, T. *et al.* Identification of new hit scaffolds by INPHARMA-guided virtual screening. *Med. Chem. Commun.* **6, 1501–1507 (2015).

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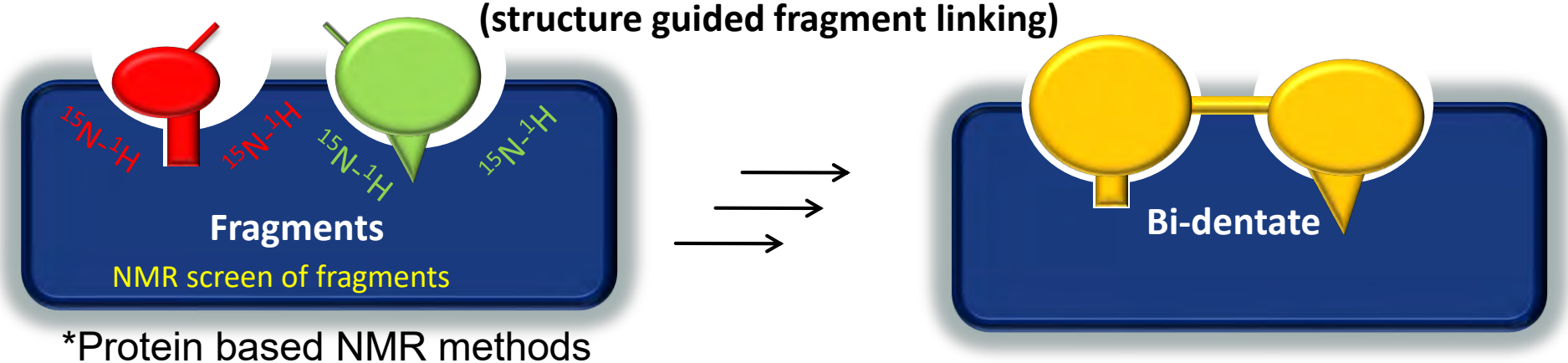
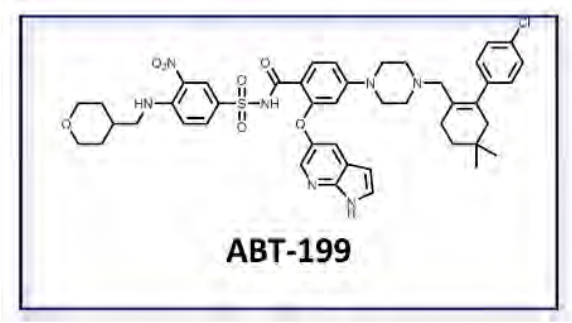
Fragment based drug discovery (FBDD)

SAR by NMR
(structure guided fragment linking)



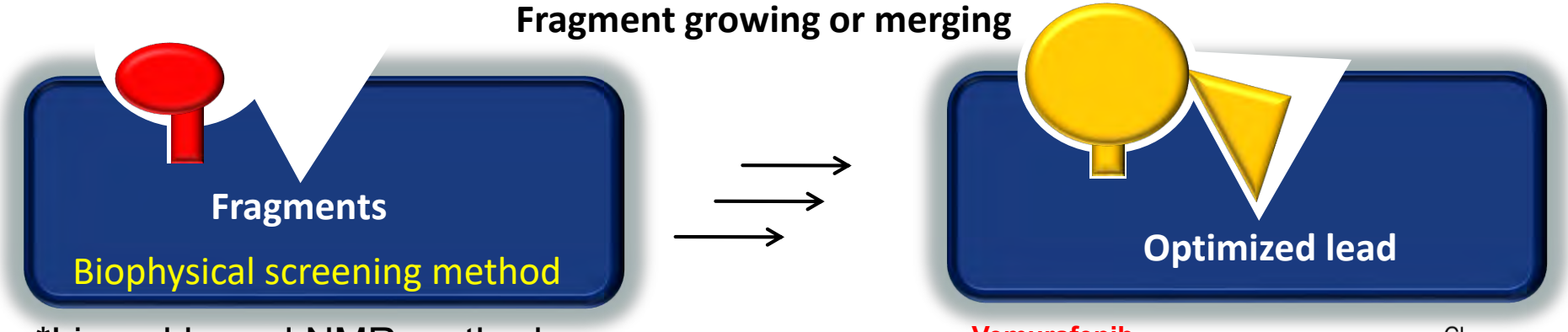
*Protein based NMR methods



Venetoclax

First rationally designed PPI inhibitor

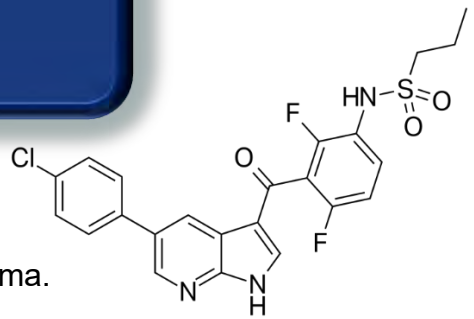
Structure guided
Fragment growing or merging



*Ligand based NMR methods such as STD, and other biophysical methods (SPR, thermal shifts, etc.)

Vemurafenib.

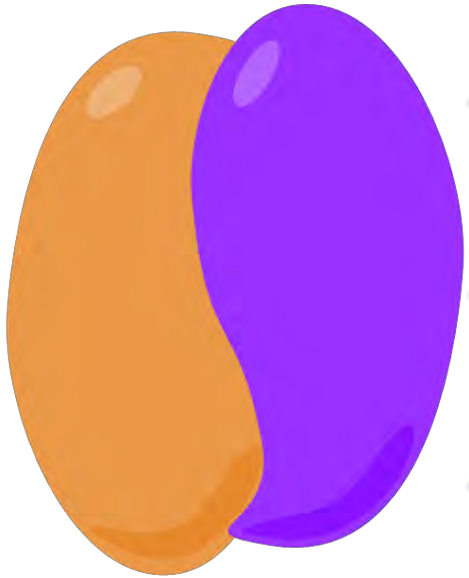
First FBDD designed kinase inhibitor .
Targets V600E- RAF protein in melanoma.



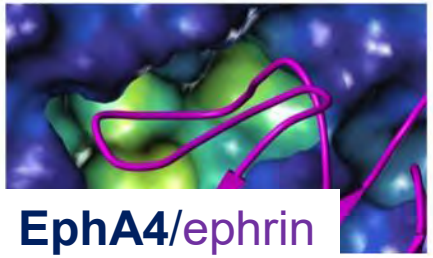
***Perspectives on NMR in drug discovery: a technique comes of age Nature Rev Drug Disc. 2008 Sep;7(9):738-45**

Maurizio Pellecchia[1], Ivano Bertini, David Cowburn, Claudio Dalvit, Ernest Giralt, Wolfgang Jahnke, Thomas L James, Steve W Homans, Horst Kessler, Claudio Luchinat, Bernd Meyer, Hartmut Oschkinat, Jeff Peng, Harald Schwalbe, Gregg Siegal

When targeting PPIs, short peptide mimetics can be effective pharmacological agents and even therapeutics

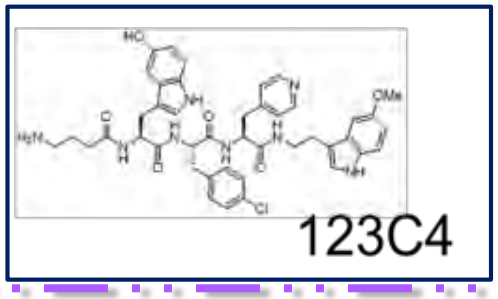


(a) loop



EphA4/ephrin

Inhibitors



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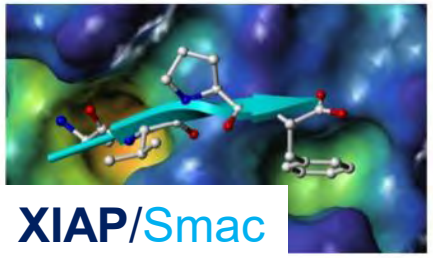
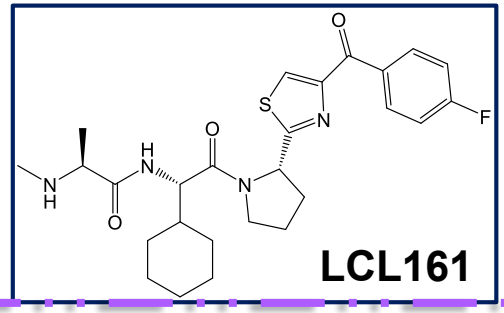
Wu et al., Pellecchia, *Cell Chem. Biol* 2016
Development stage (150E8)

(b) β-strand

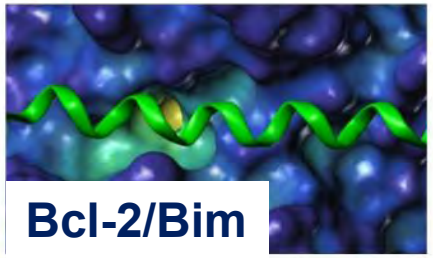


XIAP/Smac

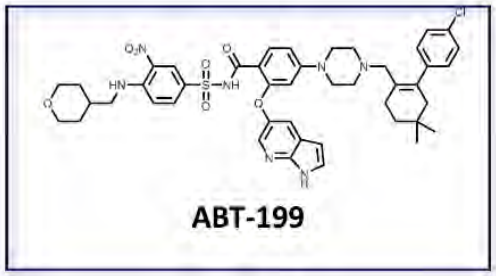


Genentech
Chen, K. F. et al., *Biochem Pharm.* 2012
Phase II

(c) α-helix



Bcl-2/Bim



Abbott
Souers et al., *Nature Medicine* 2013
FDA approved 2016

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HTS by NMR as a way to design/discover potent and selective peptide mimetics: basic principles

For a library of all possible tri-peptides or tetra-peptides composed by let's say 50 natural or non natural aminoacids:

- Synthesize a library of **50 x 50 x 50 = 125,000 molecules**
- Test them in assays that are sensitive enough to detect weak binders against PPIs (NMR)
- For tetra-peptides and using let's say 50 amino-acids **50 x 50 x 50 x 50 => 6 Million**

However we can reduce the complexity by synthesizing pools using the position scanning method and by testing the mixtures by protein NMR:

P1	P2	P3
A X X	X A X	X X A
G X X	X G X	X X G
.	.	.
.	.	.
.	.	.
W X X	X W X	X X W

50 + 50 + 50 = 150 mixtures

A X X = A A A, AAG, AGA, …, AWW

50 x 50 = 2500

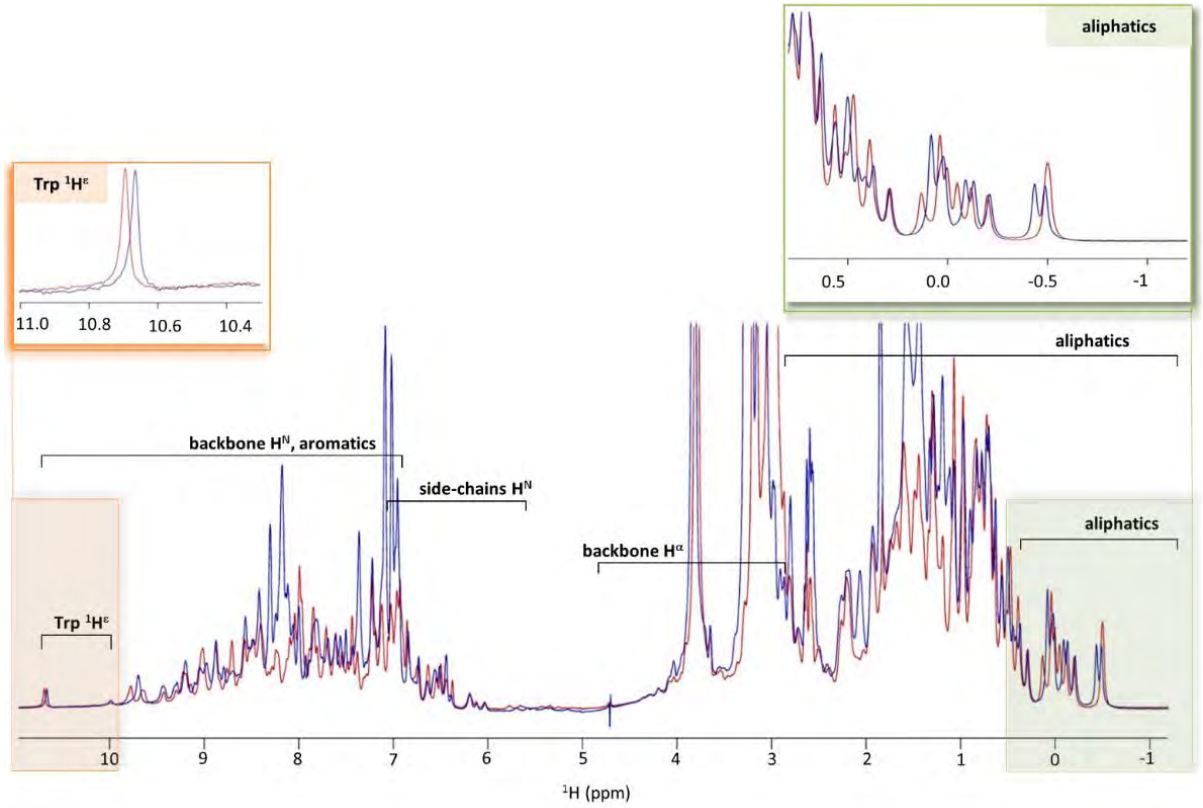
If **AGW** is a positive then we expect that the mixtures **A**XX, X**G**X, and XX**W** will be positive by NMR

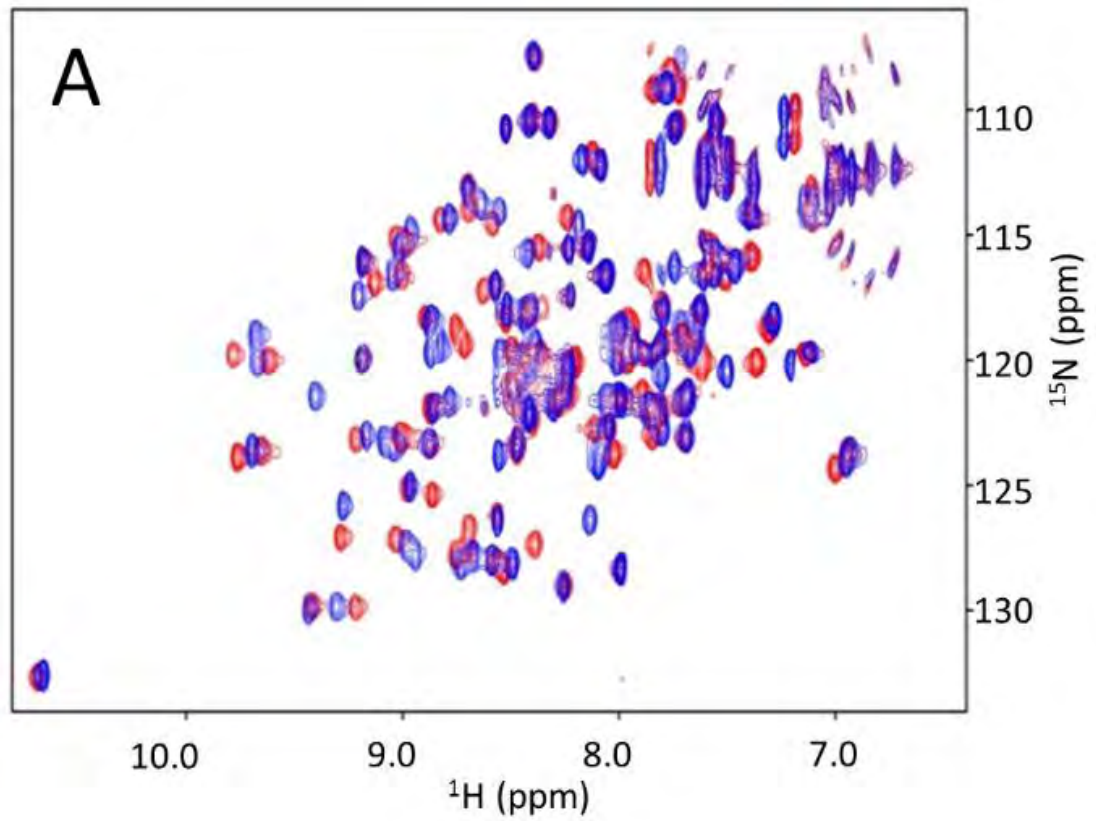
Pinilla, C., et al. *2001 Cancer Res.* **61**: 5153; Wu et al., Pellecchia Chem. Biol. 2013

Protein concentrations 5-20 μM
Mixtures 1-2 mM (individual agents ~ 1-2 μM)

Ligand binding by solution NMR spectroscopy

Overlay of NMR spectra measured in **absence** and **presence** of test ligands





Barile and Pellecchia, *Chem. Rev.* 2014, 114, 9, 4749-4763

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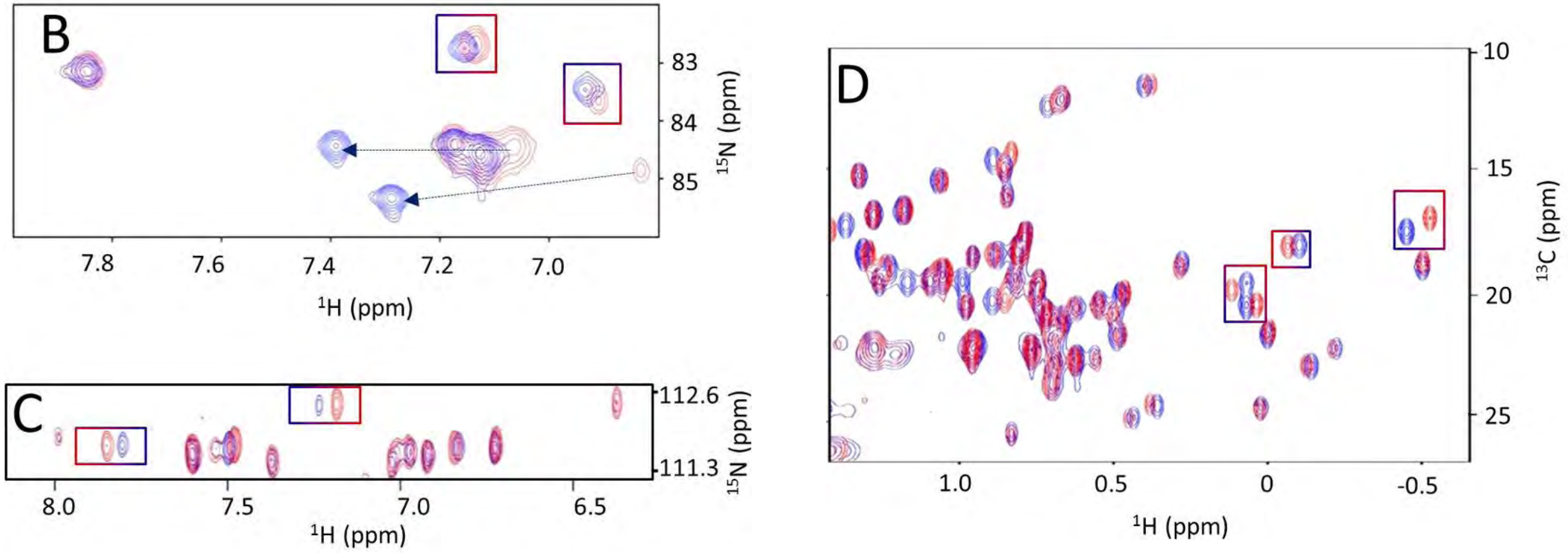
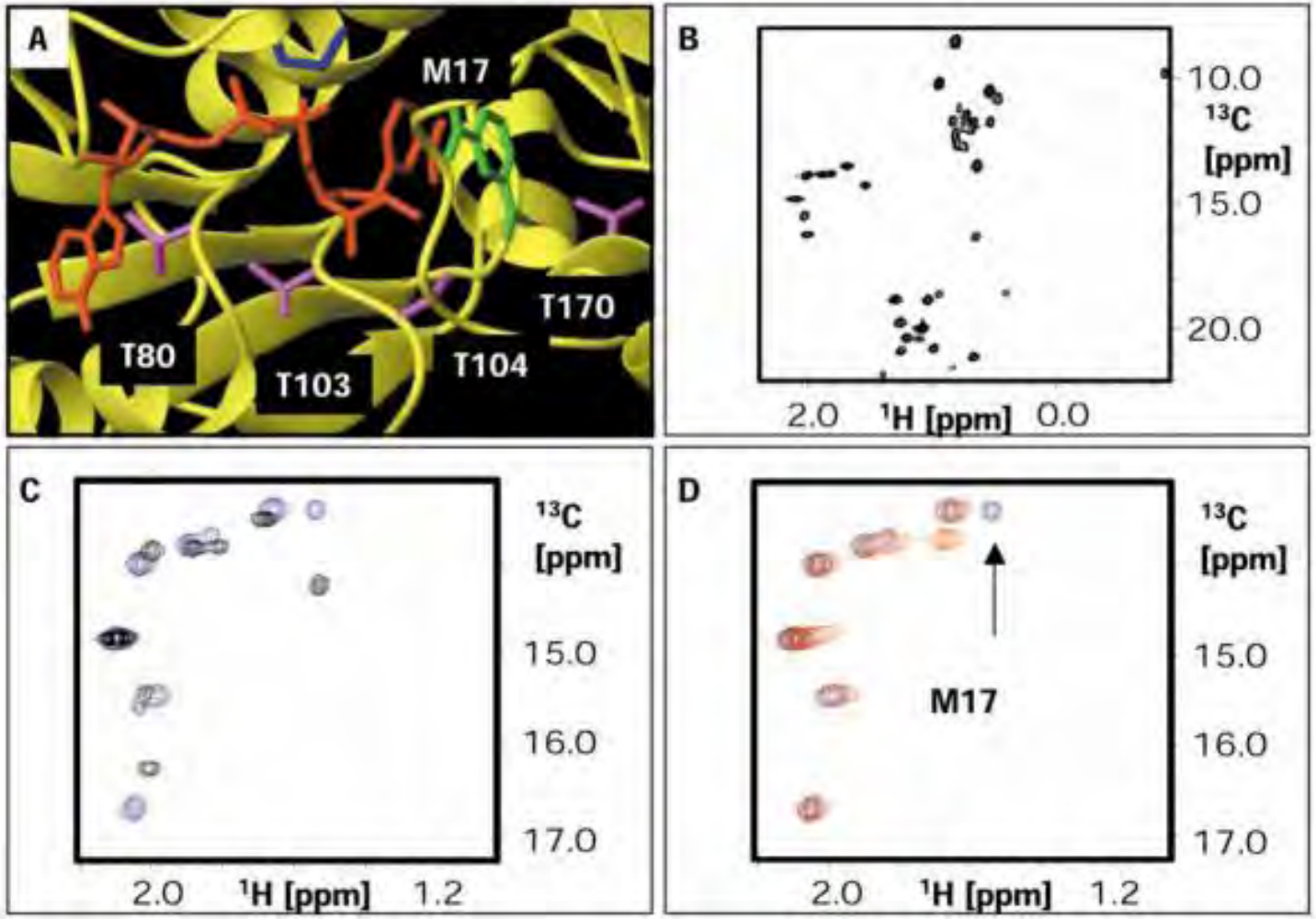


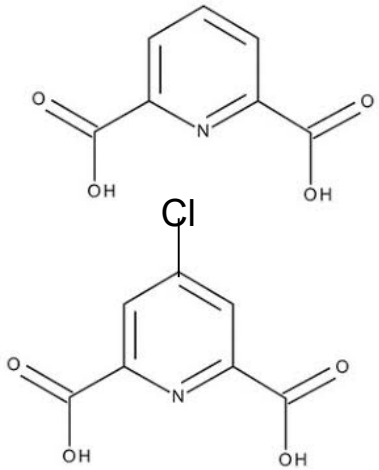
Barile and Pellecchia, Chem. Rev. 2014, 114, 9, 4749-4763

Ligand binding by solution NMR spectroscopy for large proteins



Dihydrodipicolinate reductase (DHPR), a homo-tetramer of **120 kDa**, which is involved in the biosynthesis of lysine and bacterial cell wall components.

Assignment of active site ^{13}Cε/^{1}Hε Met via mutagenesis or differential chemical shift



2D [^{13}C,^{1}H] HMQC spectra recorded with a 150 µl of a 50 µM sample of **U-^{2}H, ^{13}Cε/^{1}Hε Met, ^{13}C/^{1}H Thr, ^{13}Cδ/^{1}Hδ Ile labeled DHPR ([MIT]-DHPR)**. (C) and (D): Met ^{13}Cε/^{1}Hε sub-spectra. (C) Black, unbound [MIT]-DHPR; blue, [MIT]-DHPR bound to PDC. (D) Red, [MIT]-DHPR bound to 4-Cl PDC; blue, [MIT]-DHPR bound to PDC. Pellecchia *et al*. *J Biol NMR* 22: 165–173, 2002.

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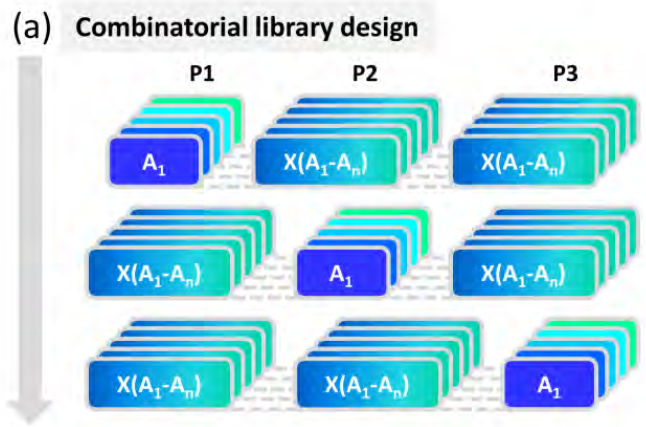
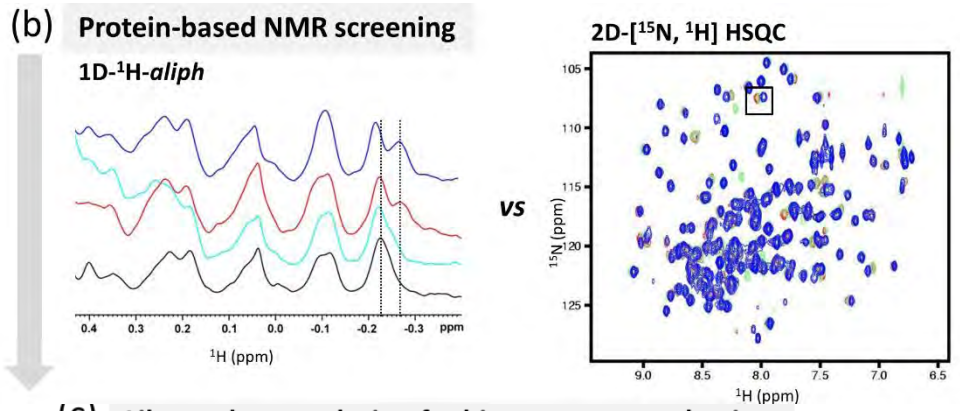
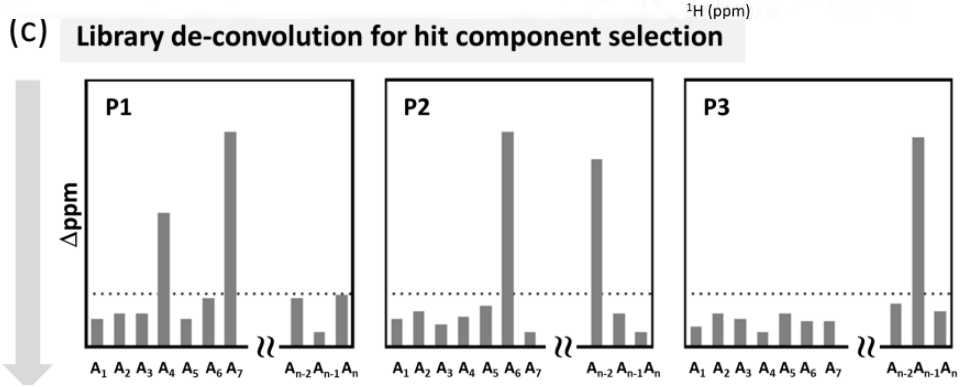
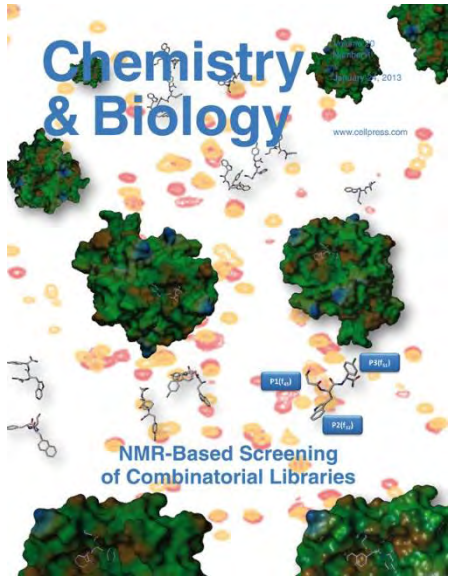
HTS by NMR as a way to design/discover potent and selective peptide mimetics: basic principles





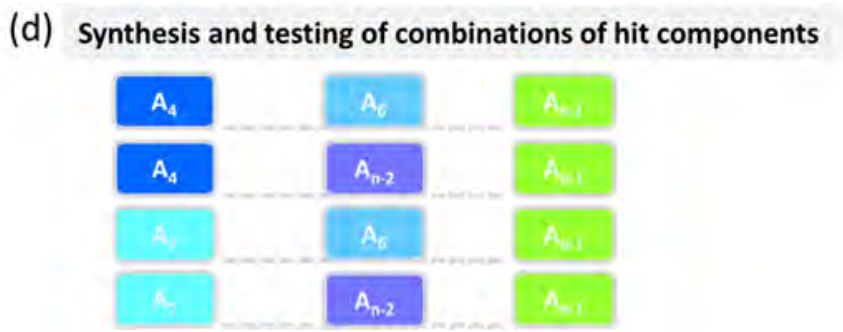




Wu et al. Pellecchia Chem & Biol 2013

General POS libraries of

- **3-mers or 4-mers using natural and non-natural aa.**
- **Biophysical detection/selection**
- **Optimization strategies**



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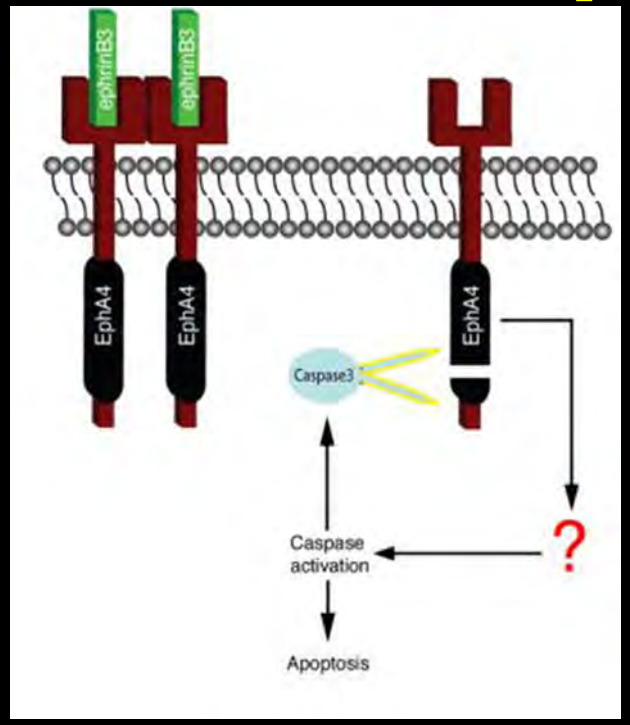
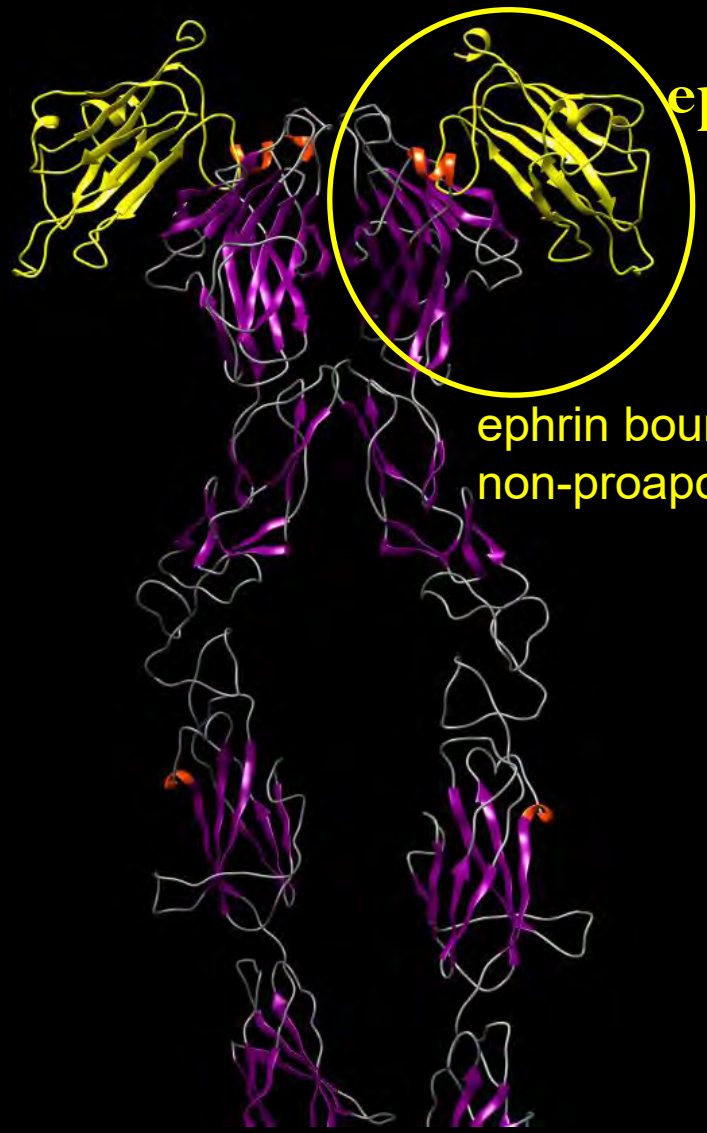
EphA4/ephrin interactions modulate MN cell death and viability: ephrin mimetics (EphA4 agonists) may revert EphA4 induced MN cell death



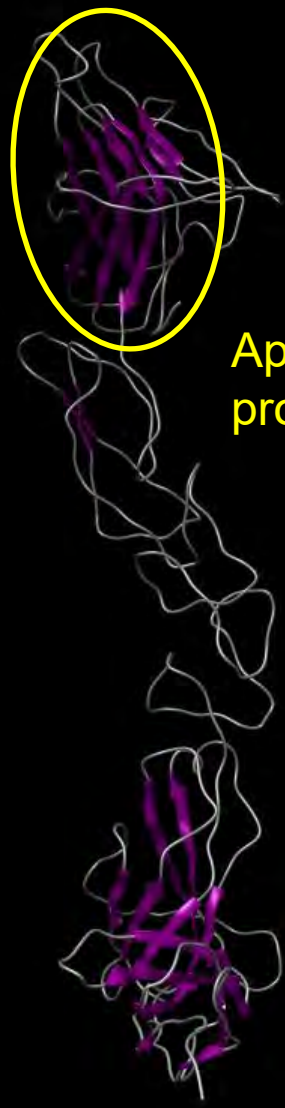
Journal of Biomolecular Screening, (2012) 17, 785-795

ephrin

ephrin



ephrin bound EphA4
non-proapototic



Ligand binding domain

Apo EphA4
proapototic

Xua, et al., Nikolov. PNAS (2013) 110, 14634–14639

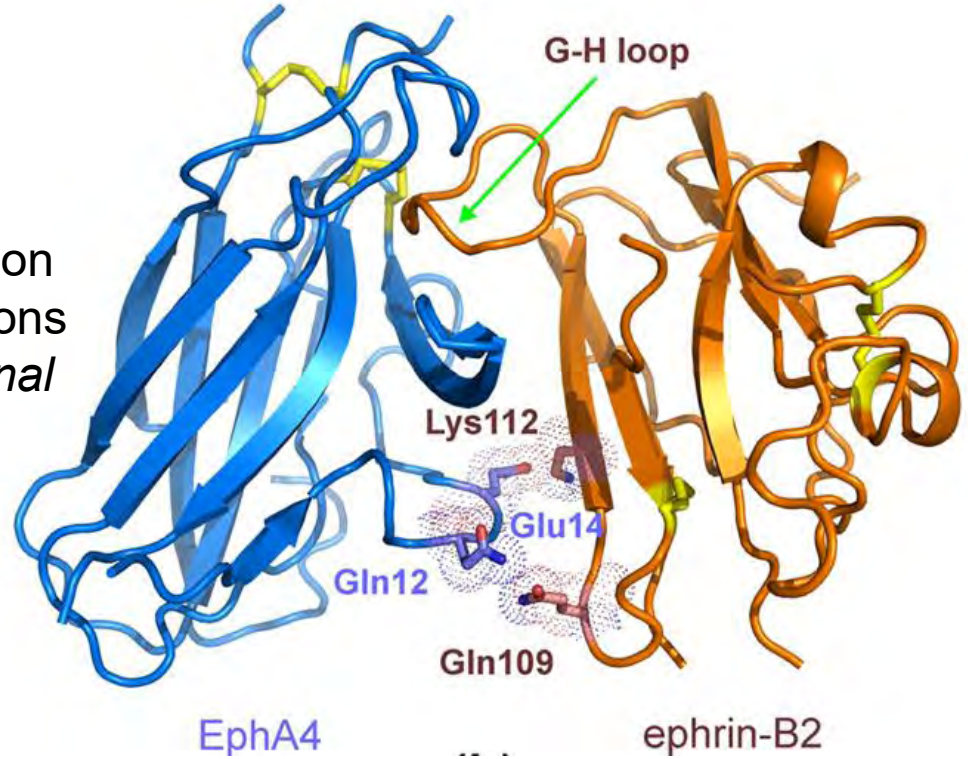
Targeting the LBD of EphA4 with antagonists of protein-protein interactions (PPIs)

EphA4 subtype is involved in preventing nerve regeneration, and is associated with spinal cord injury and ALS.

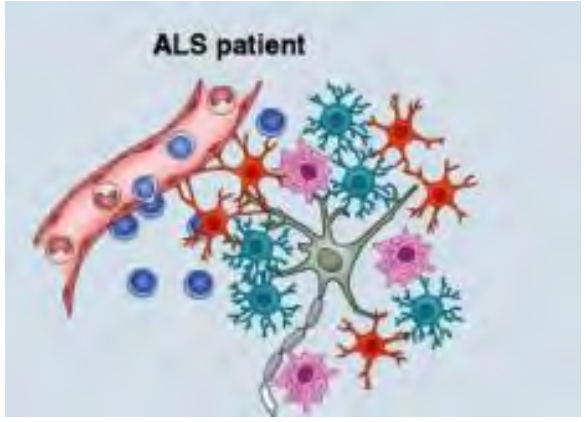
However:

Targeting the EphA4 receptor with small molecules is challenging because its ephrin binding site is large

EphA4 on
Motor neurons
Forward signal



ephrinB2 on
Reactive astrocytes
Reverse signal



Qin, H. et al. J. Biol. Chem. 2010;285:644-654

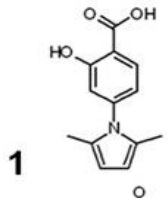
Introducing…the PAINS (Pan Assay INterference compoundS)

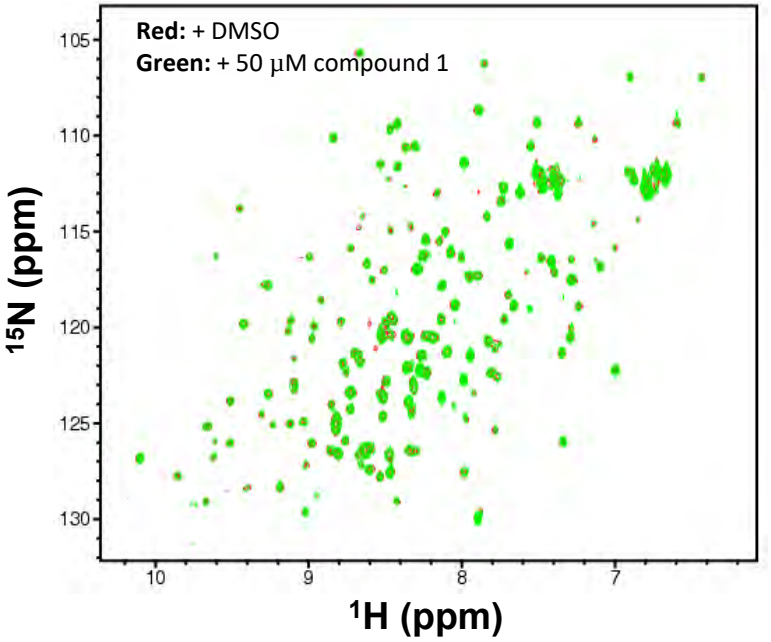
Phage display identified a weak 12-mer linear peptide (KYL or KYLPYWPVLSSL)[1].
Subsequent HTS campaigns (300,000 compounds) using biochemical assays resulted in in 1 hit molecule (compound 1)[1]

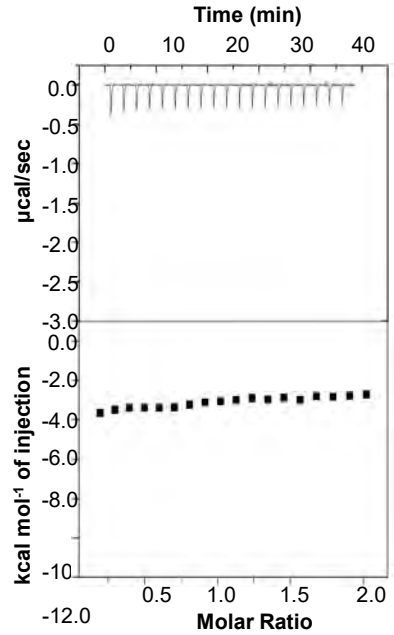
However:

- **When we tested compound 1 by NMR or by ITC**

we could not detect any appreciable binding to the EphA4 !

Structure	MW	% Inhib. EphA4 KYL	IC_{50} (μM) EphA4 KYL	IC_{50} (μM) EphA4 ephrin-A5
 1	231	99 99	3±0.5	13±1.3





[1]Murai KK, et al. (2003)
Mol Cell Neurosci 24(4):1000–1011

[2]Noberini R et al. J. Biol. Chem.
2008;283:29461-29472

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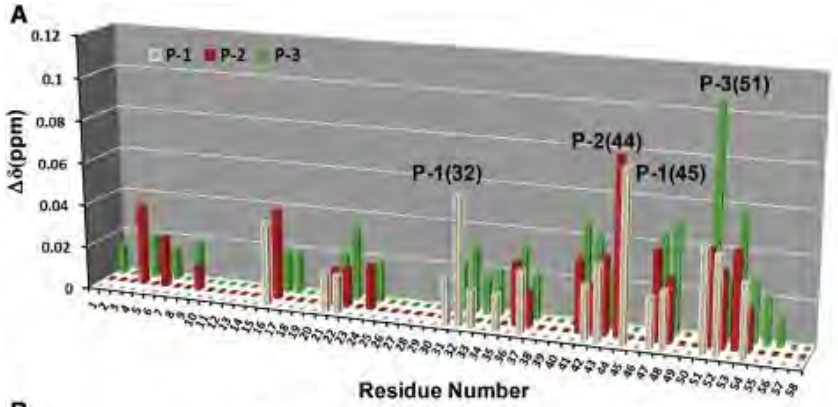
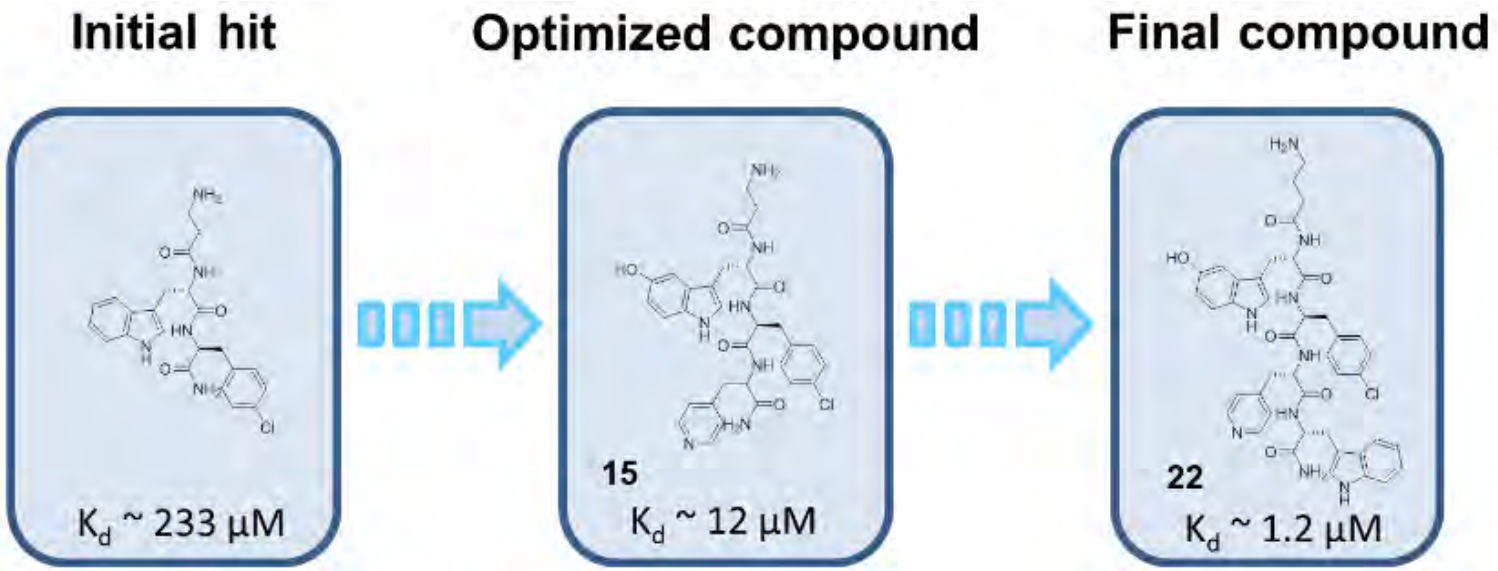
HTS by NMR targeting EphA4-LBD

Tested by NMR a combinatorial library of 48 x 48 x 48 tripeptoids

(144 mixtures covering >110,000 peptoids)





Wu et al. Pellecchia, Chemistry & Biology, 2013

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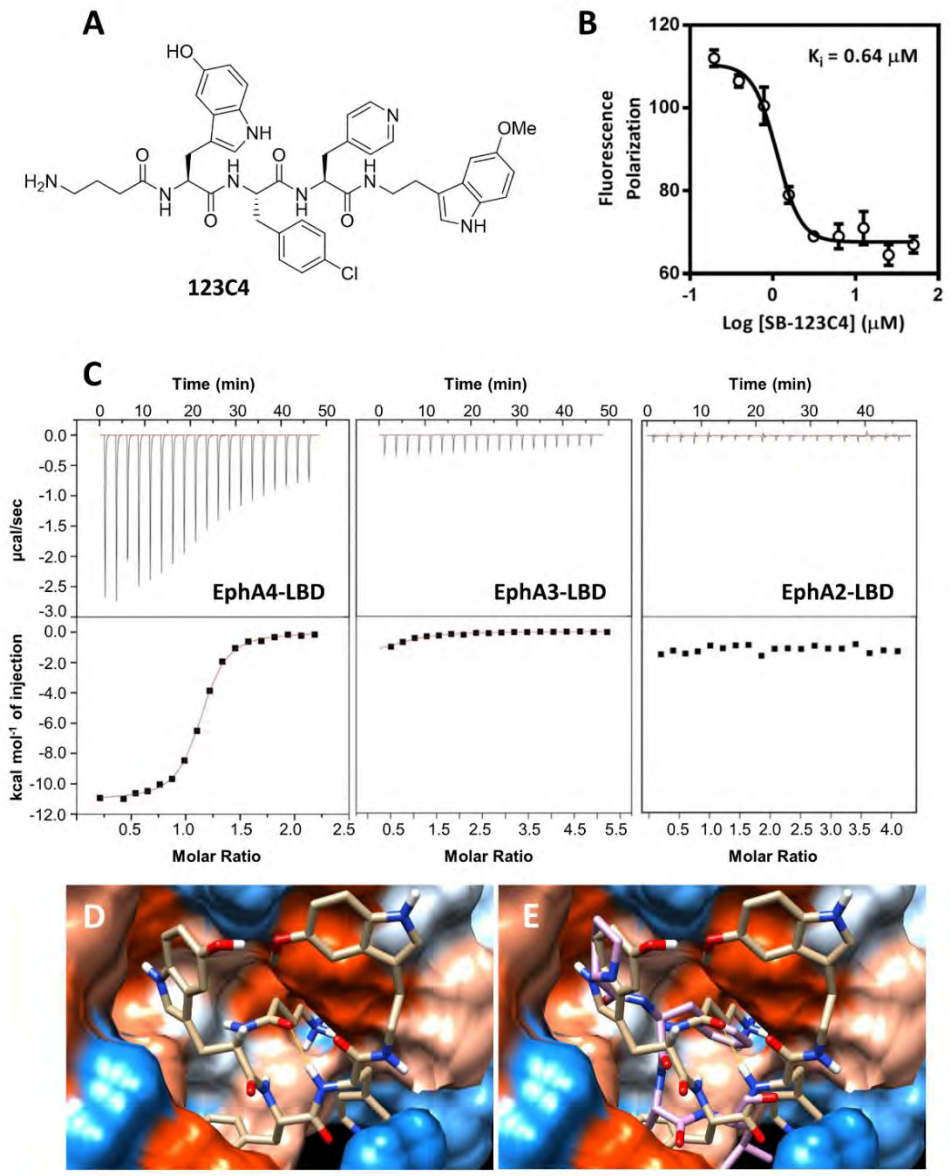
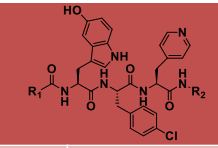
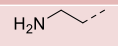
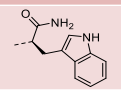
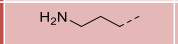
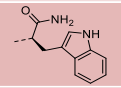
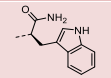
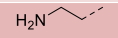
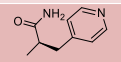
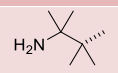
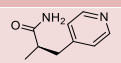
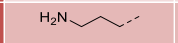
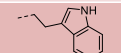
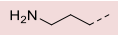
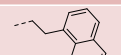
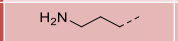
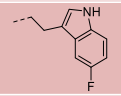
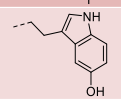
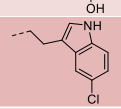
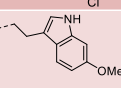
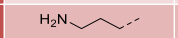
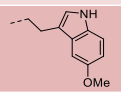
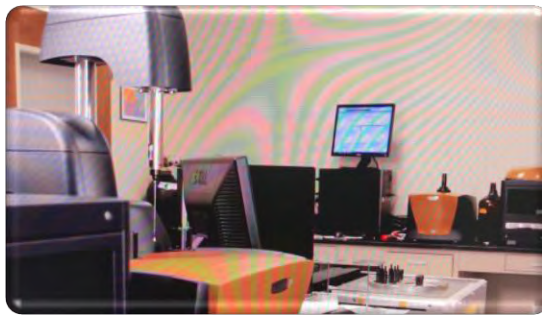
HTS by NMR targeting EphA4-LBD





ID	R_1	R_2	K_i (μM) by FPA	K_d (μM) by ITC
compound 22			2.14	1.2
120H10			0.96	ND
123B1			3.04	ND
120G1			13.96	ND
120G2			>100	ND
123C2			1.59	1.1
123C12			7.67	ND
123C3			2.22	ND
123C5			2.57	2.2
123C6			3.14	ND
123C7			1.59	0.54
123C4			0.64	0.42

Potent and Selective EphA4 Agonists for the Treatment of ALS.
Wu *et al.*, Pellecchia Cell Chem Biol 2017



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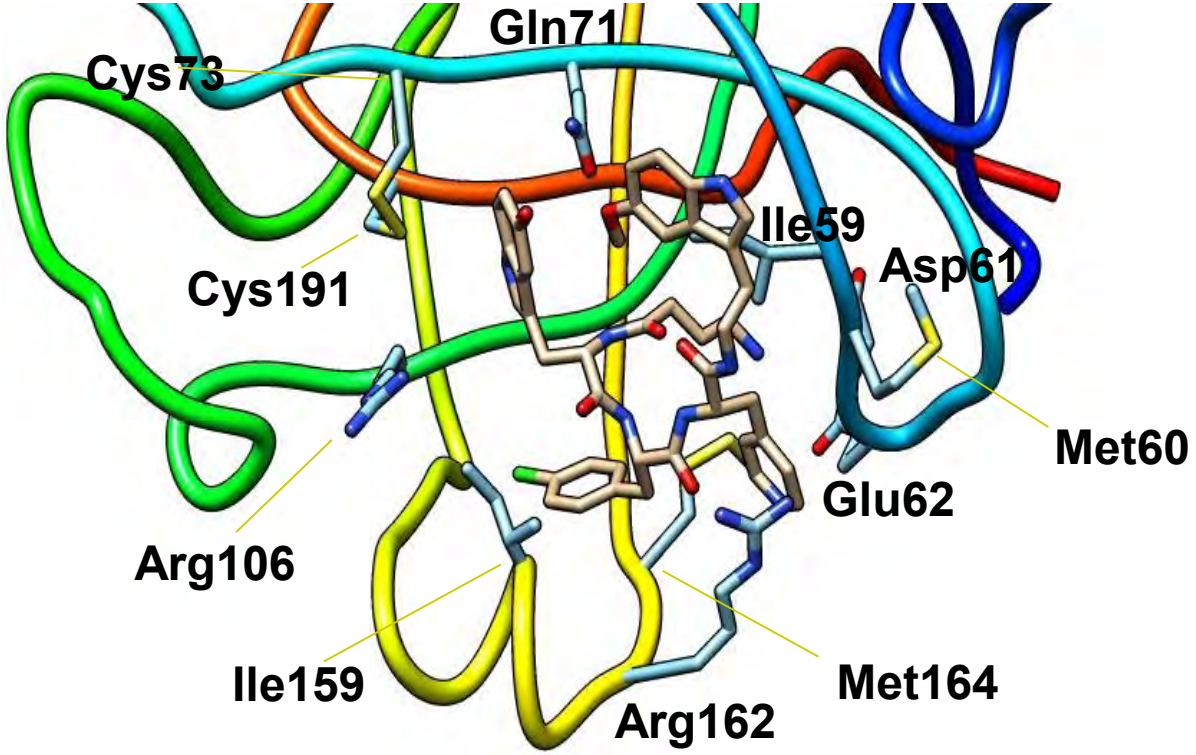
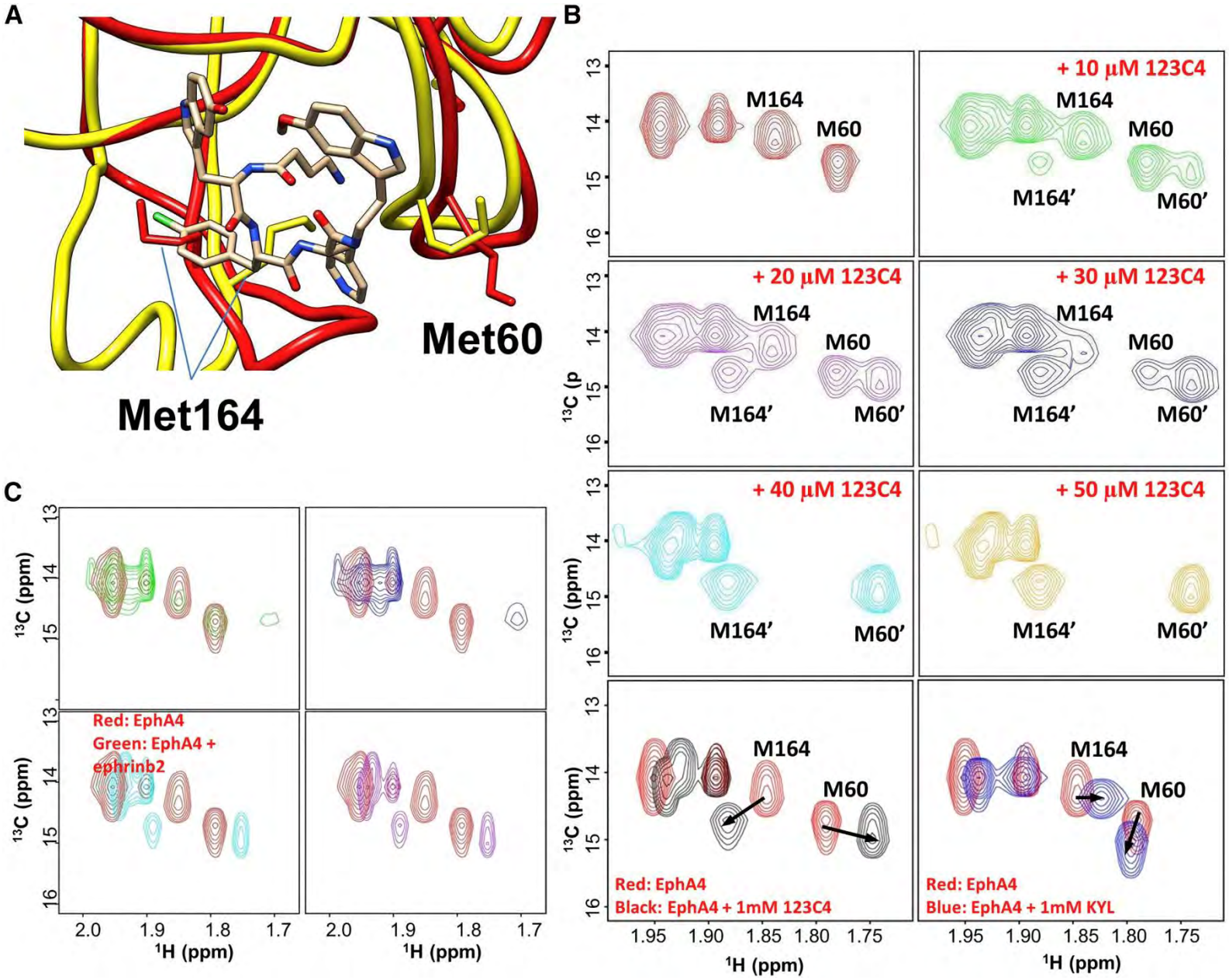
Molecular basis for the selectivity of 123C4



Protein	K_d (μM)	ΔH (kcal/mol)	TΔS (kcal/mol)
WT-EphA4 LBD	0.42 ± 0.02	-11.06 ± 0.06	-2.42
WT-EphA3 LBD	4.55 ± 1.09	-1.54 ± 0.30	5.75
WT-EphA2 LBD	N.B.	-	-
I59G-EphA4 LBD	3.66 ± 0.71	-5.69 ± 0.74	1.68
I59A-EphA4 LBD	9.09 ± 1.49	-3.43 ± 0.41	3.43
I159A-EphA4 LBD	0.29 ± 0.02	-8.53 ± 0.06	0.34
M60A-EphA4 LBD	0.35 ± 0.03	-9.98 ± 0.21	-1.24
M164A-EphA4 LBD	0.062 ± 0.01	-11.14 ± 0.13	-1.38

Targeting the LBD of EphA4



Cell Chemical Biology DOI: (10.1016/j.chembiol.2017.01.006)

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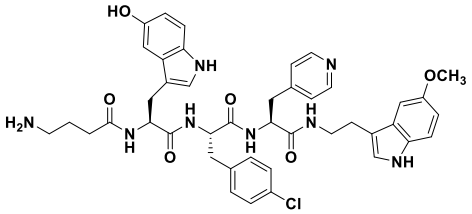
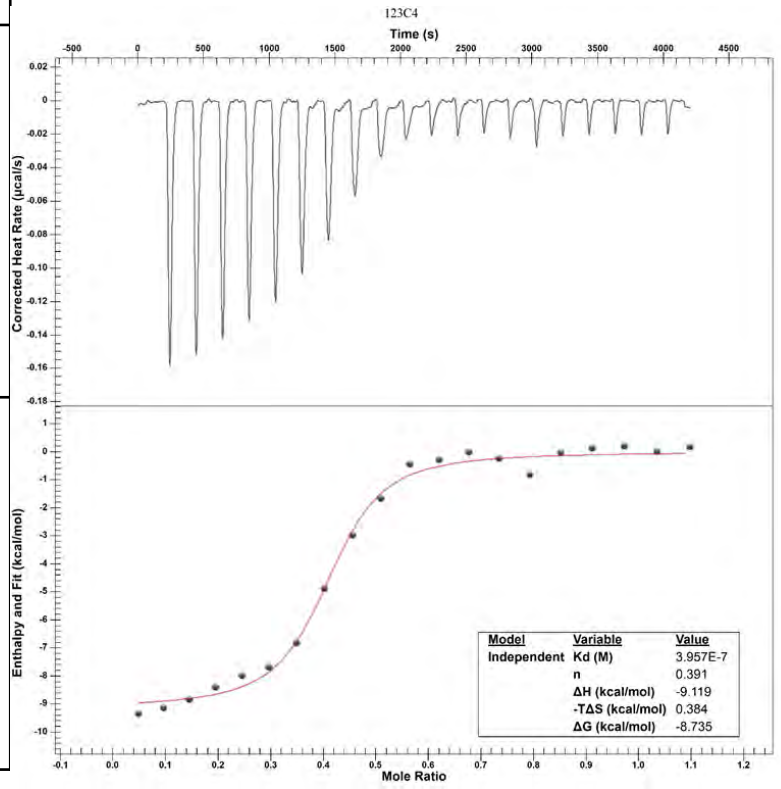
HTS by NMR versus phage display

ID	Structure
123C4	MW ~ 800 Kd ~ 400 nM 
146C8 (APY-d3)	MW ~ 1300 Kd ~ 67 nM **βA-PYCVYR-βA-SWSC-CONH$_2$**

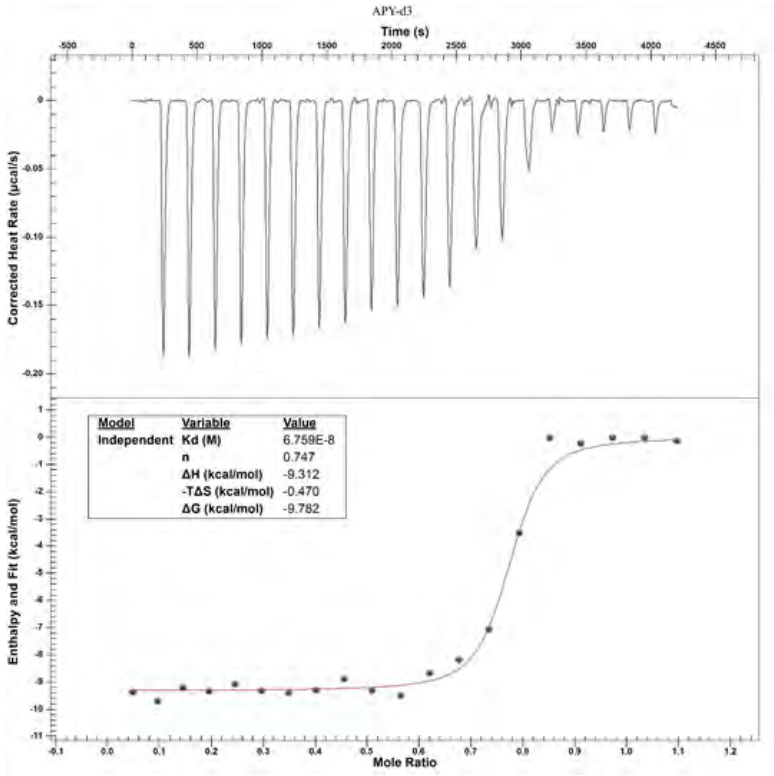
HTS by NMR derived*

123C4



Phage display derived and optimized**

APY-d3



*Wu et al., Pellecchia, Cell Chem. Biol 2017;
Baggio et al., Pellecchia,2020 in preparation

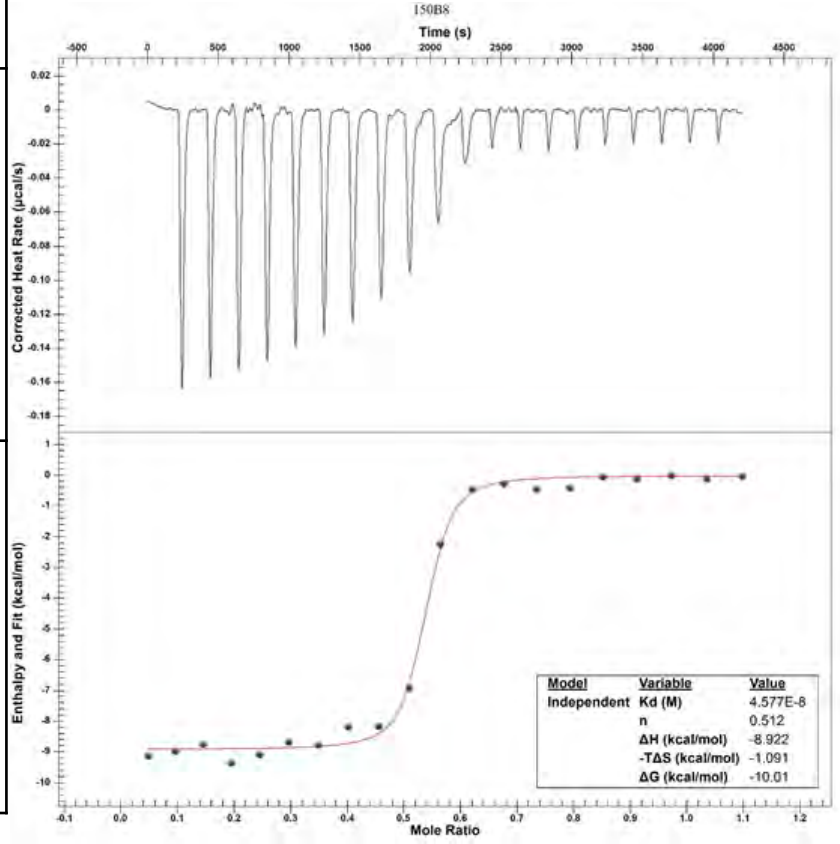
** Lamberto et al. Pasquale ACS Chem Bio, 2014;
**Lechtenberg at al. Pasquale ACS Med Chem Lett 2016

HTS by NMR versus phage display

ID	Structure
150B8	MW ~800 Kd ~ 45 nM
146C8 (APY-d3)	MW ~ 1300 Kd ~ 67 nM βA-PYCVYR-βA-SWSC-CONH$_2$

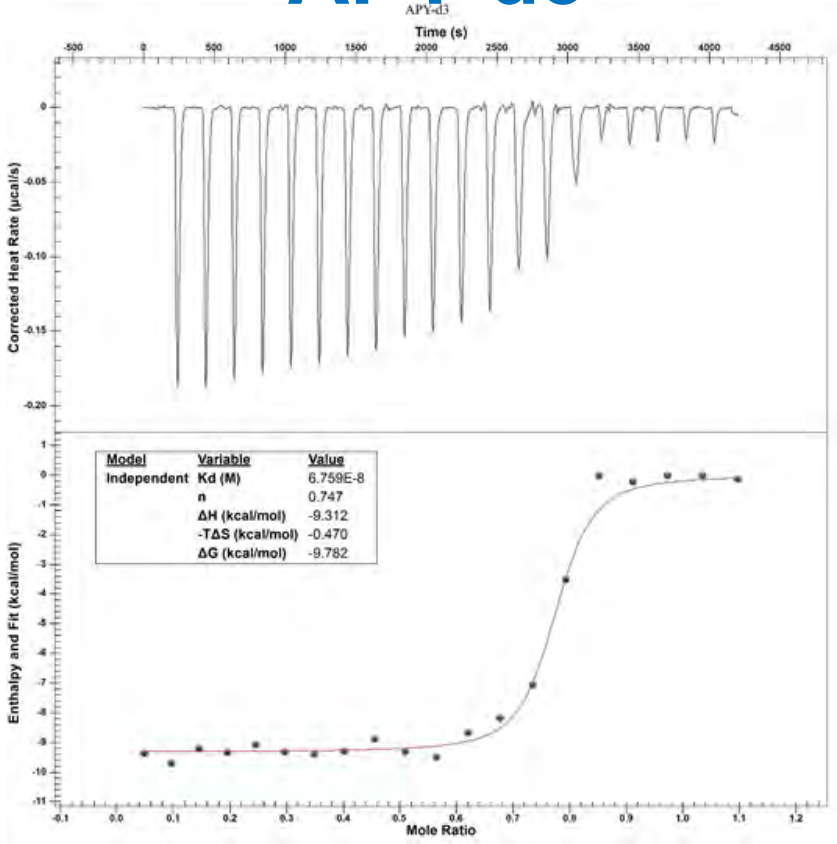
HTS by NMR derived and optimized*

150B8



Phage display derived and optimized**

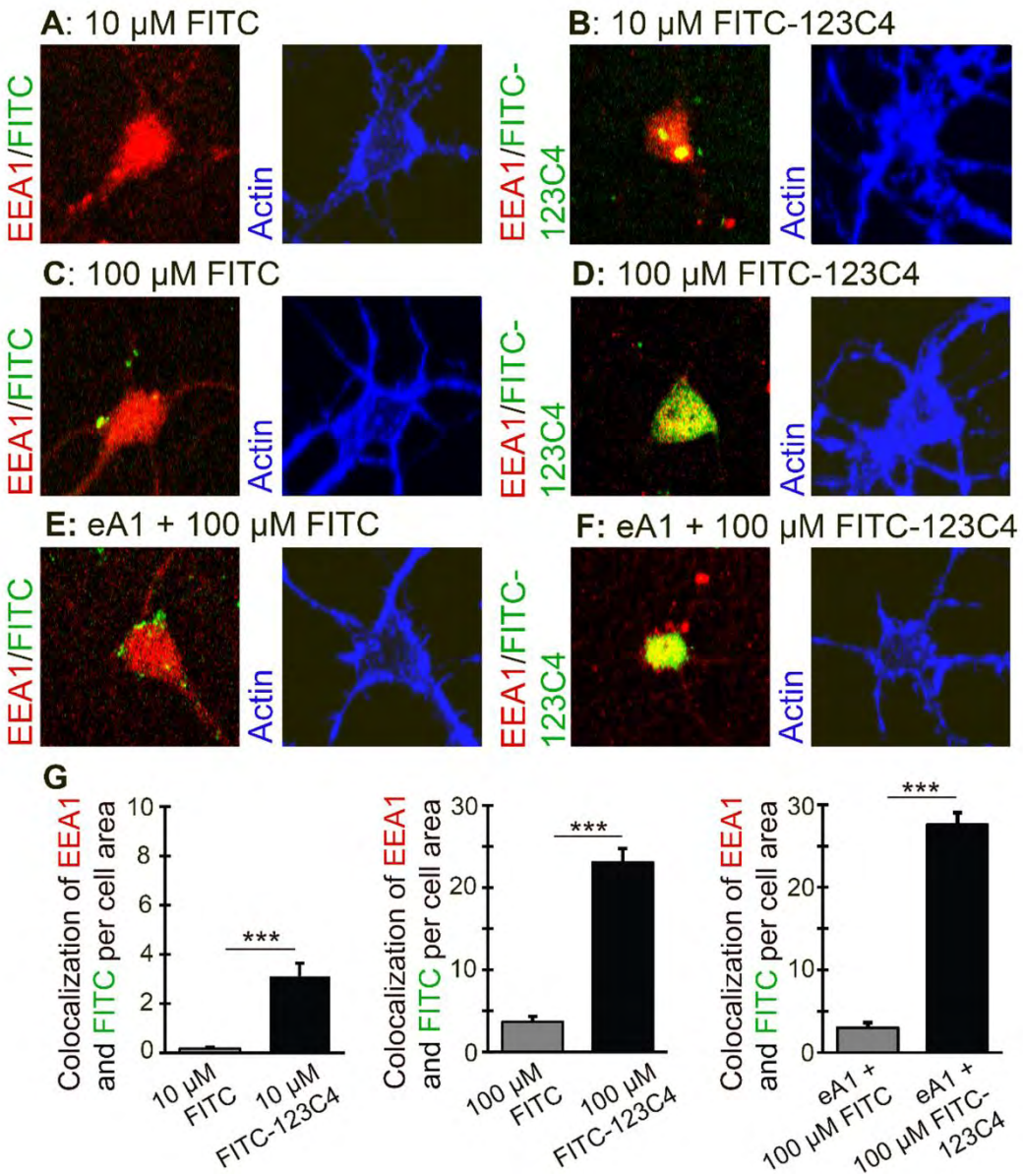
APY-d3

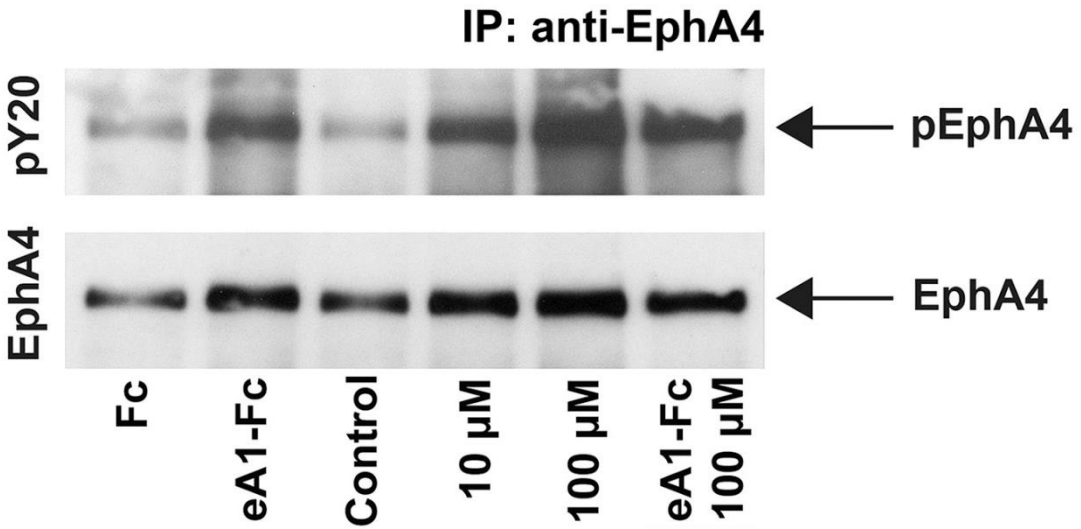


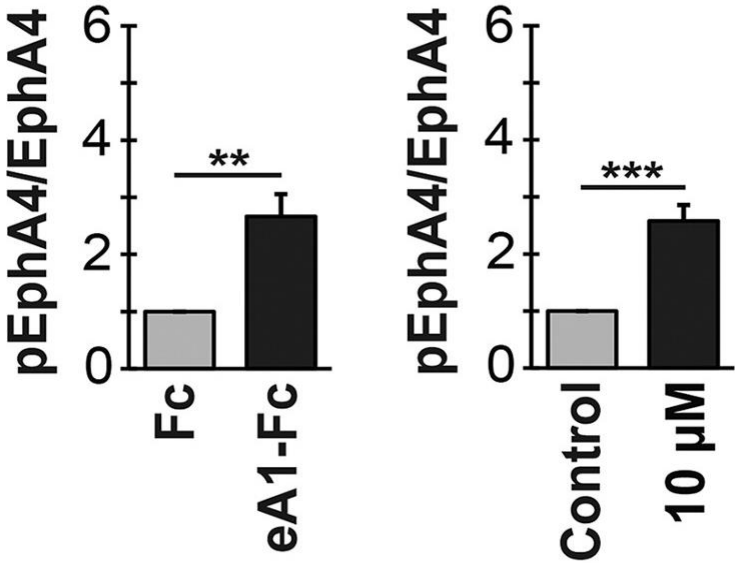
Wu et al., Pellecchia, Cell Chem. Biol 2017;
*Baggio et al., Pellecchia,2020 in preparation

** Lamberto et al. Pasquale ACS Chem Bio, 2014;
Lechtenberg at al. Pasquale ACS Med Chem Lett 2016

123C4 is an effective EphA4 agonist







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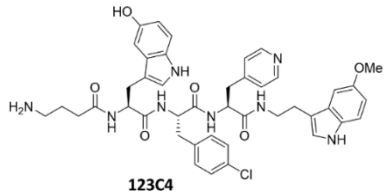
123C4 is effective in animal models of ALS

Potent and Selective EphA4 Agonists for the Treatment of ALS. Wu *et al.,* Pellecchia
Cell Chem Biol 2017

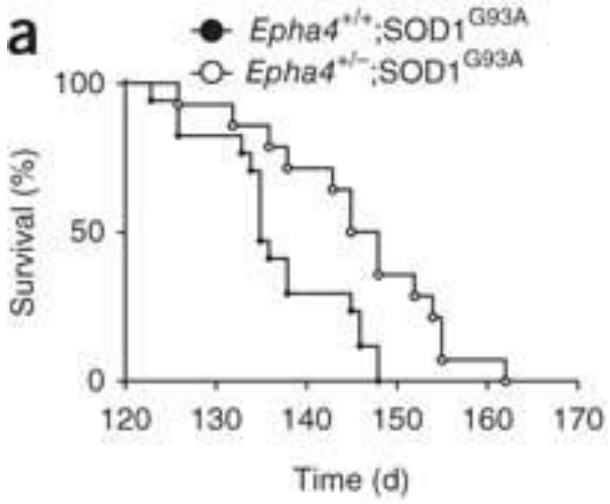
EphA4 is a disease modifier of amyotrophic lateral sclerosis in animal and in humans.

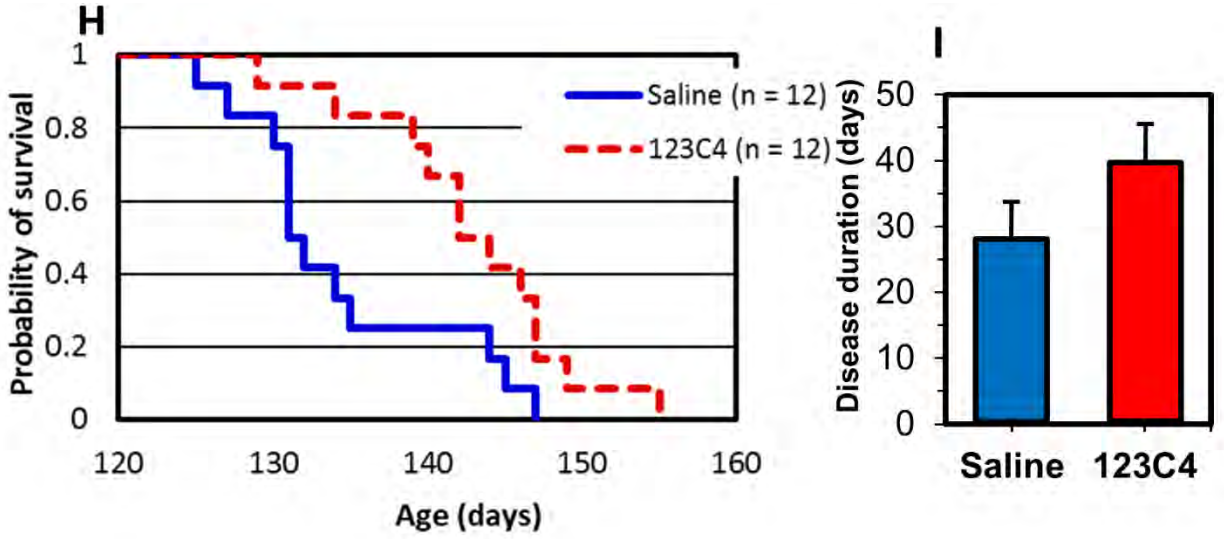
Hoecke et al., Nature Med. 2012 (9):1418-22.

123C4 crosses the BBB (%F Brain /IV ~64% after 30 min).



Compound 123C4 in saline was injected daily (i.p. 30 mg/kg) in SOD1(G93A) mice.





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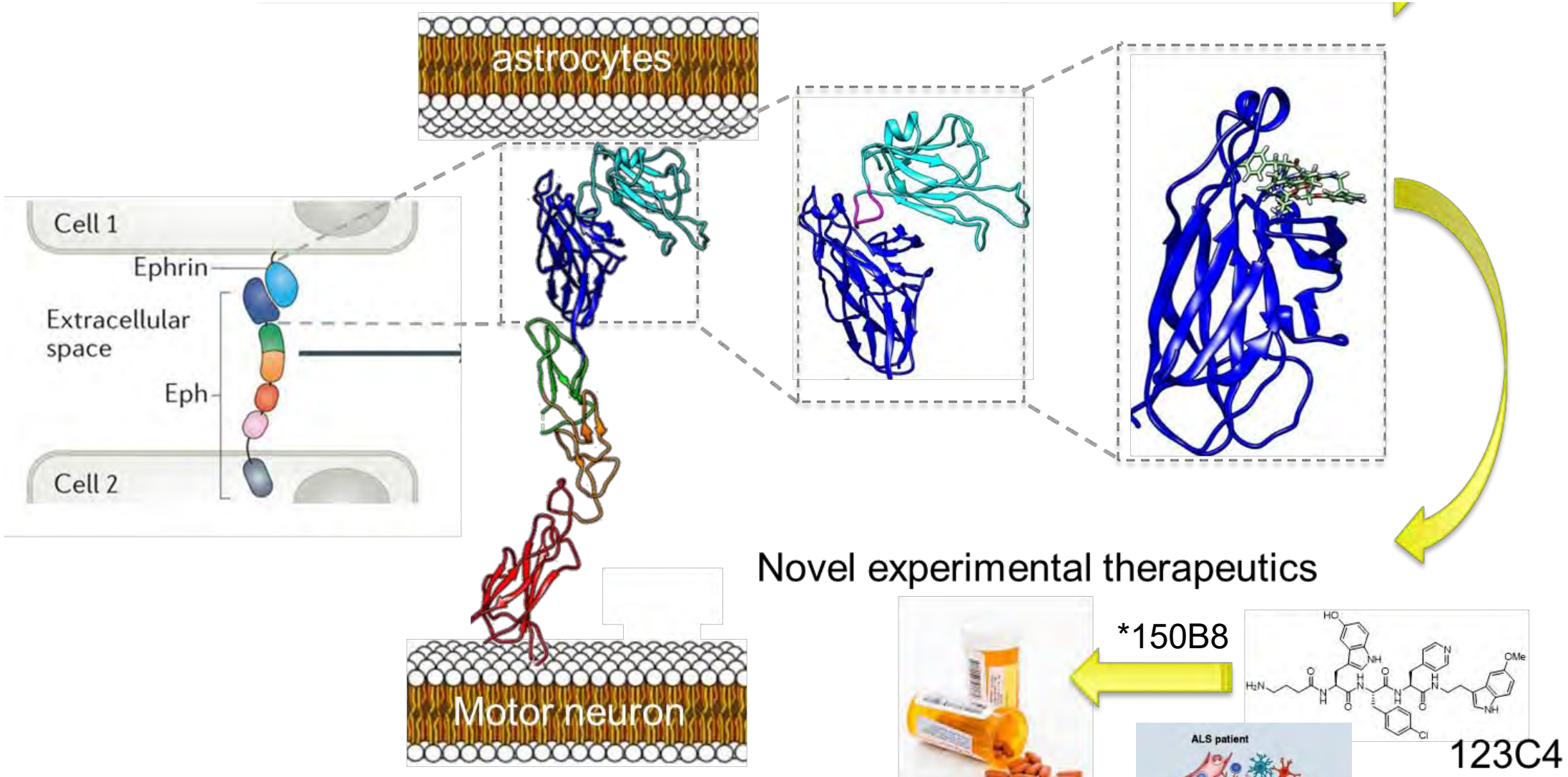
HTS by NMR derived potent EphA4 agonistic agents for the treatment of ALS



Wu et al., Ethell and Pellecchia, Cell Chem Biol 2017

*Baggio et al., Pellecchia, 2020 in preparation

1. Background: NMR-guided drug discovery targeting protein-protein interactions.

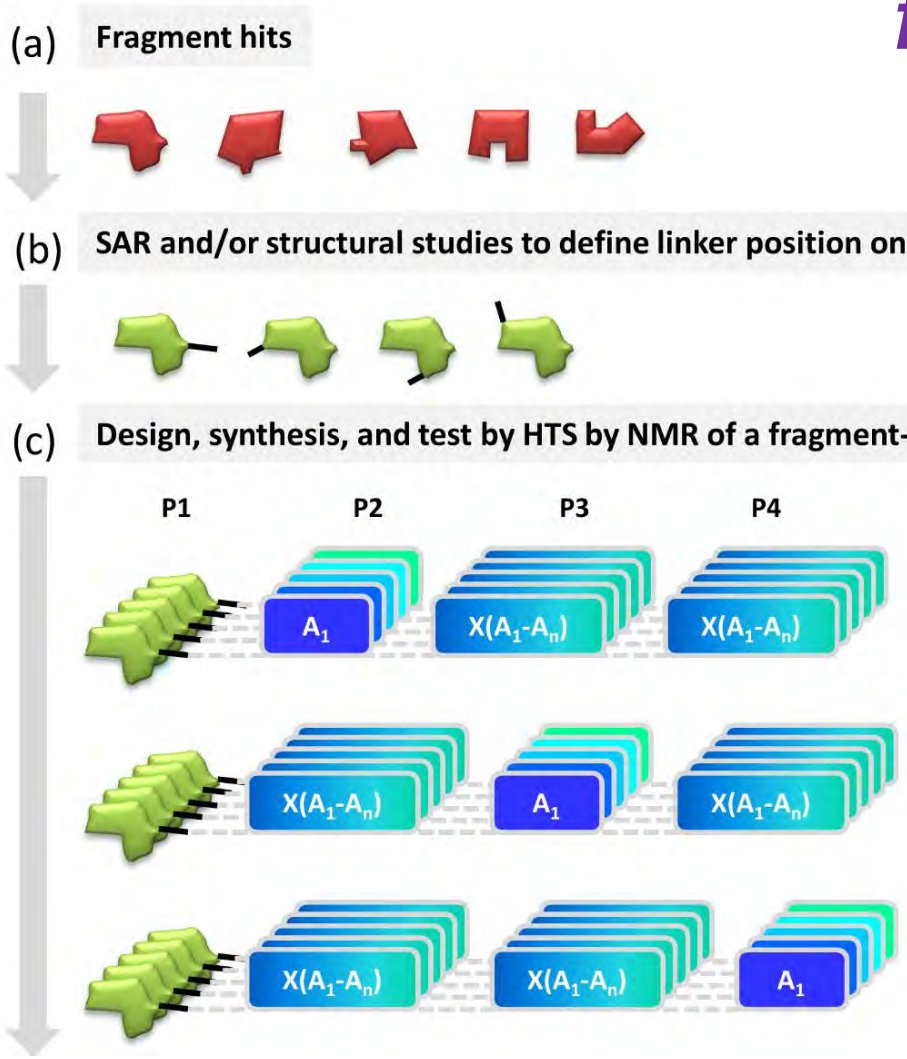
2. Application of the HTS by NMR against a protein-protein interaction involving EphA4-LBD and the ephrin ligands

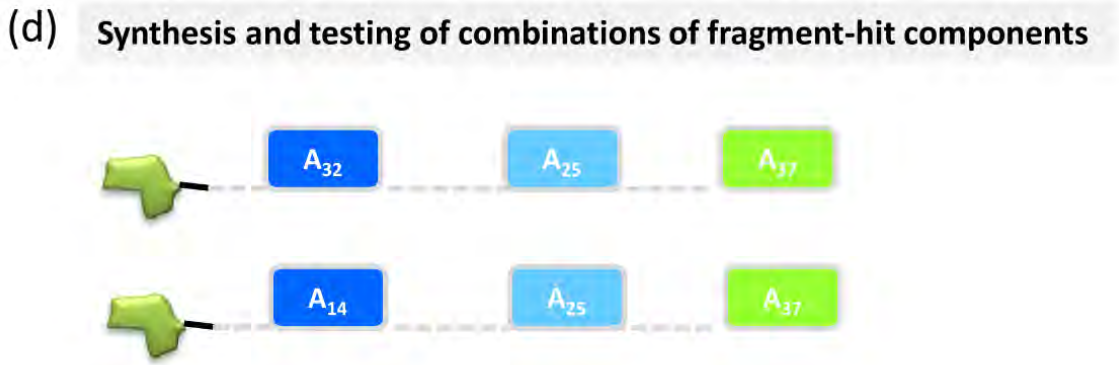
3. Introduction of an anchoring moiety in the library (focused HTS by NMR or *f*HTS by NMR) and applications to various targets including metalloproteases, and the BIR3 domains of IAP proteins

4. Design of Lys/Tyr covalent agents using sulfonyl-fluorides or fluoro-sulfates

Focused POS using an anchoring moiety: *f*HTS by NMR

*f*HTS by NMR





(e) Biophysical studies, SAR, cell based assays

Curr Top Med Chem. 2015; 15(20): 2032–2042.
High-throughput screening by Nuclear Magnetic Resonance (HTS by NMR) for the identification of PPIs antagonists
Bainan Wu, Elisa Barile, Surya K. De, Jun Wei, Angela Purves, and Maurizio Pellecchia*

Baggio et al. Pellecchia *ACS Med. Chem. Lett.*, **2018**, *9* (2), pp 137–142

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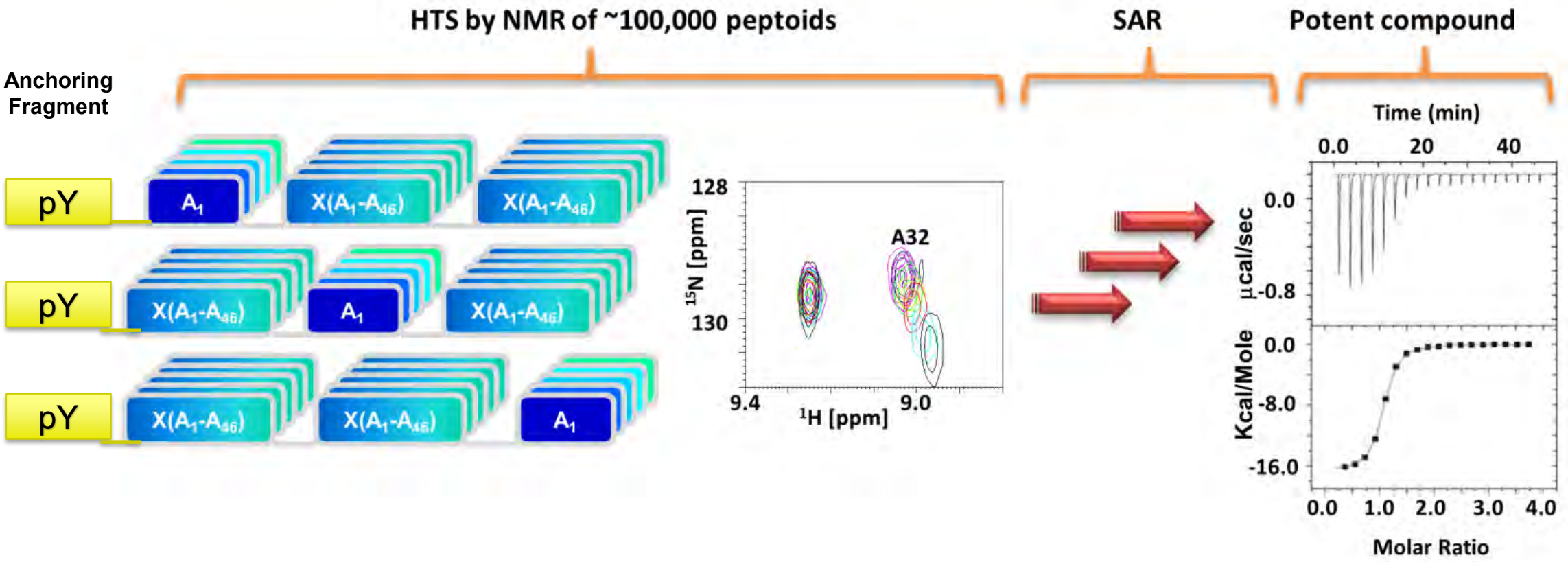
*f*HTS by NMR using pY as anchoring moiety for phospho-Tyr binding proteins led to rapid optimization of ligands



Bottini, Wu, Barile, Leone, Pellecchia
HTS by NMR Guided Identification of Novel Agents Targeting the Protein Docking Domain of YopH
ChemMedChem. 2016 Apr 19;11(8):919-27

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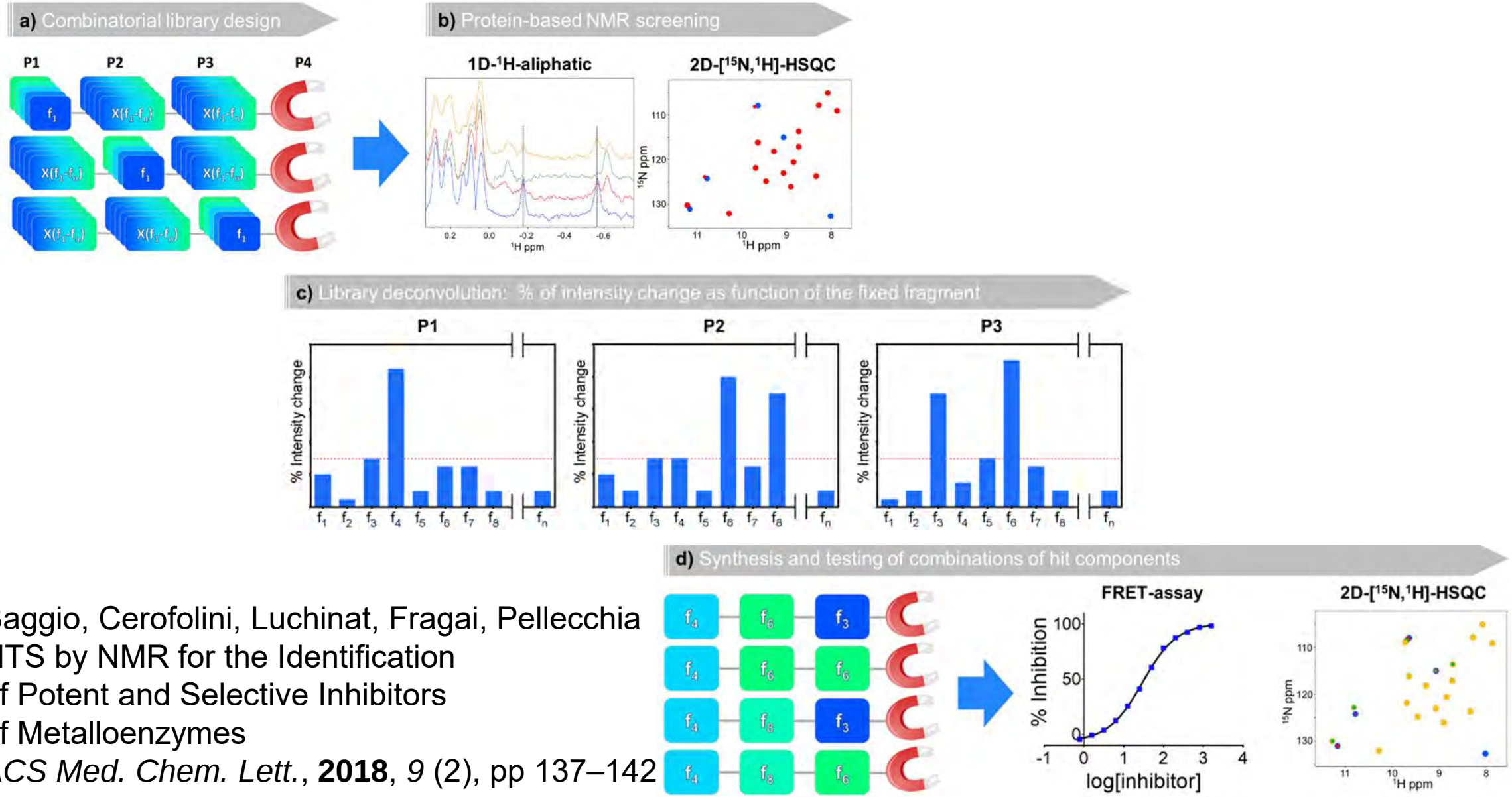
*f*HTS by NMR using -CONHOH as anchoring moiety for metallo-proteins



Baggio, Cerofolini, Luchinat, Fragai, Pellecchia
HTS by NMR for the Identification
of Potent and Selective Inhibitors
of Metalloenzymes
ACS Med. Chem. Lett., **2018**, *9* (2), pp 137–142

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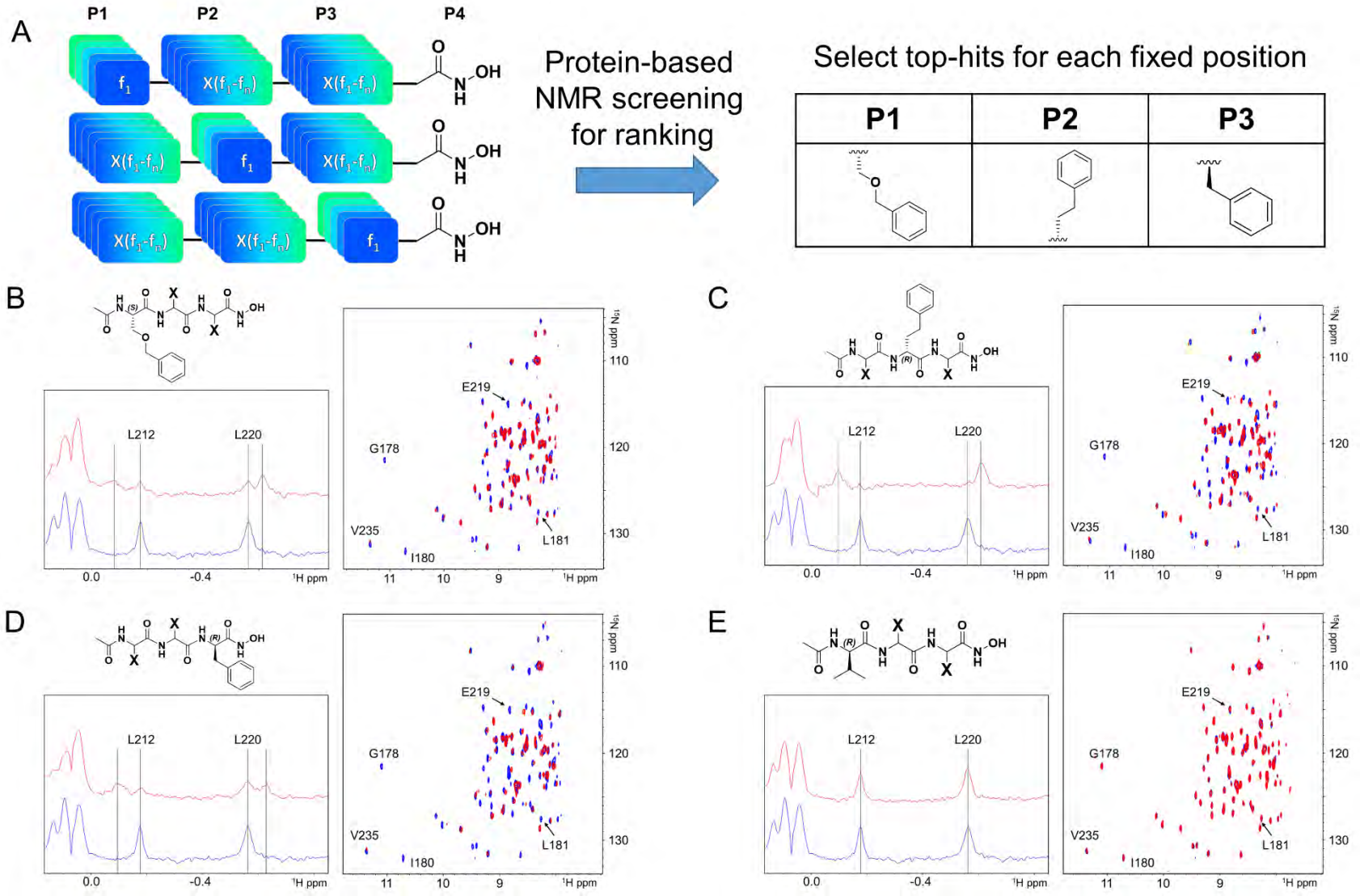
*f*HTS by NMR using -CONHOH as anchoring moiety for metallo-proteins



Baggio, Cerofolini, Luchinat, Fragai, Pellecchia, *ACS Med. Chem. Lett.*, **2018**, *9* (2), pp 137–142

Baggio et al. Fragai, Nordgren, Pellecchia, *J. Med. Chem* **2020** in press

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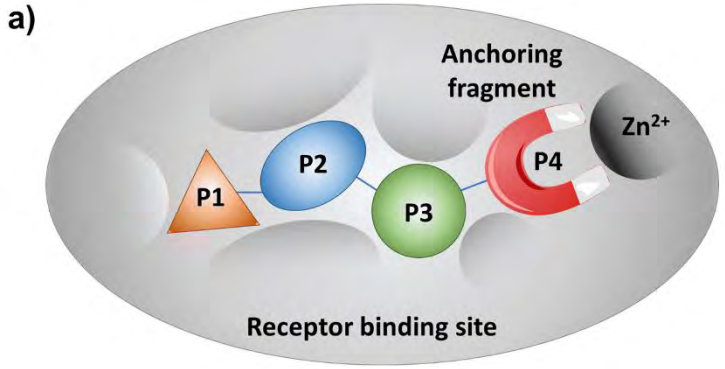
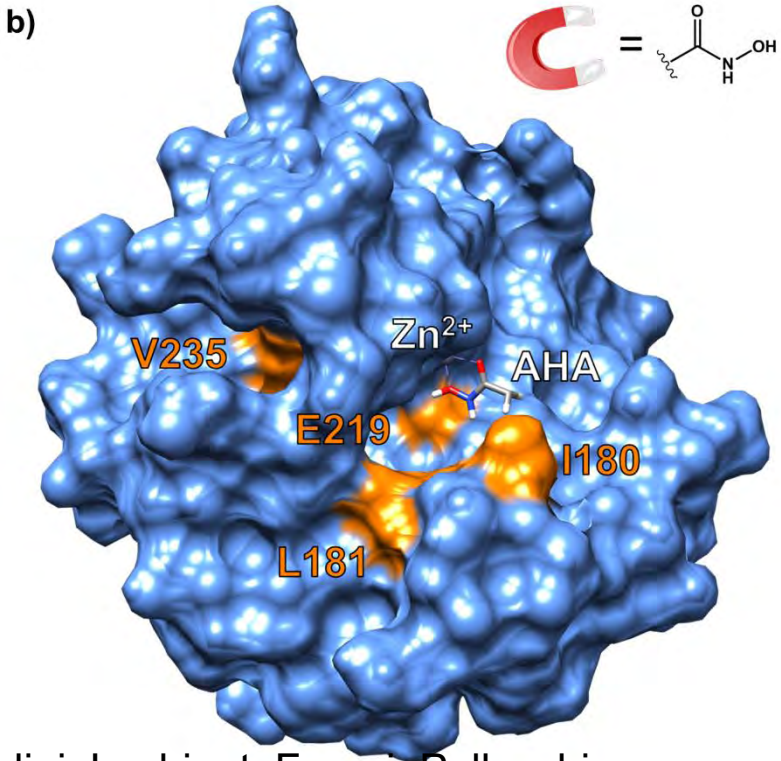
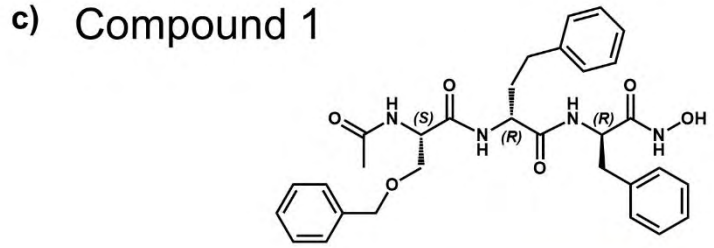
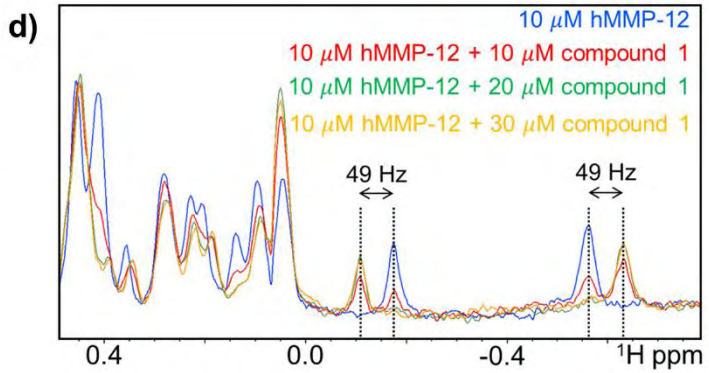
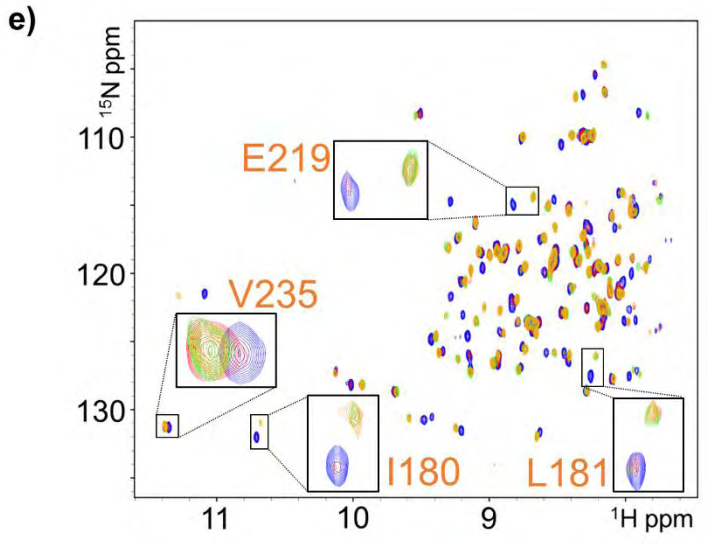
*f*HTS by NMR using -CONHOH as anchoring moiety for metallo-proteins











Baggio, Cerofolini, Luchinat, Fragai, Pellecchia,
ACS Med. Chem. Lett., 2018, 9 (2), pp 137–142

*f*HTS by NMR using -CONHOH as anchoring moiety for metallo-proteins

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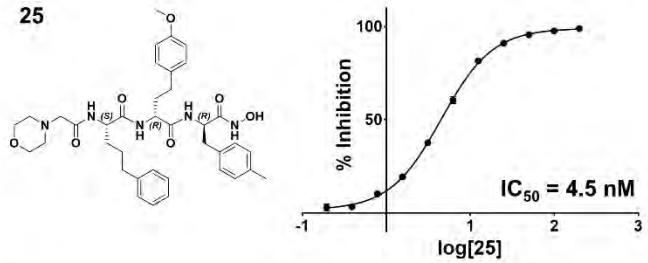
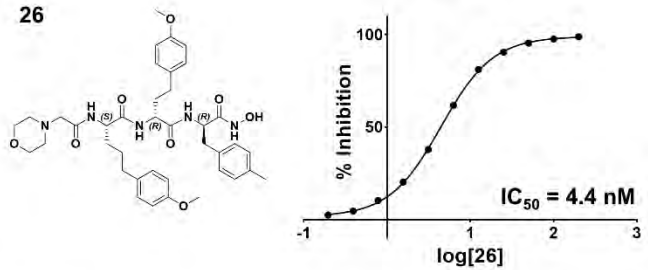
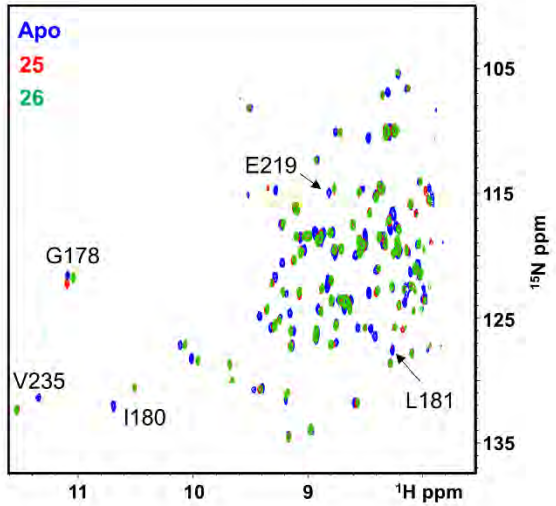
A



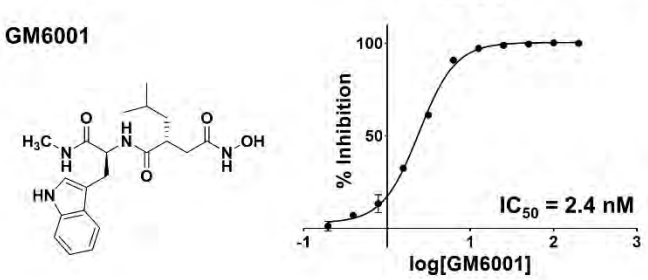
B



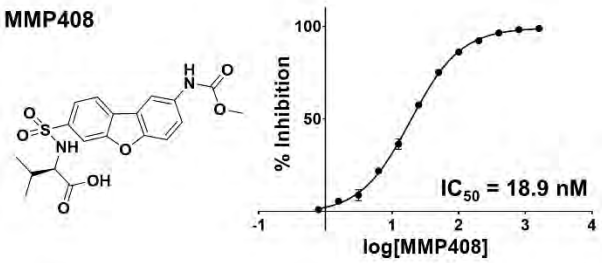
C



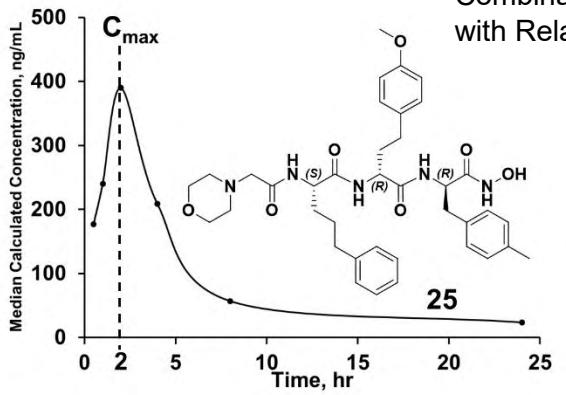
D

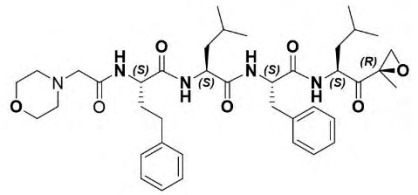


E

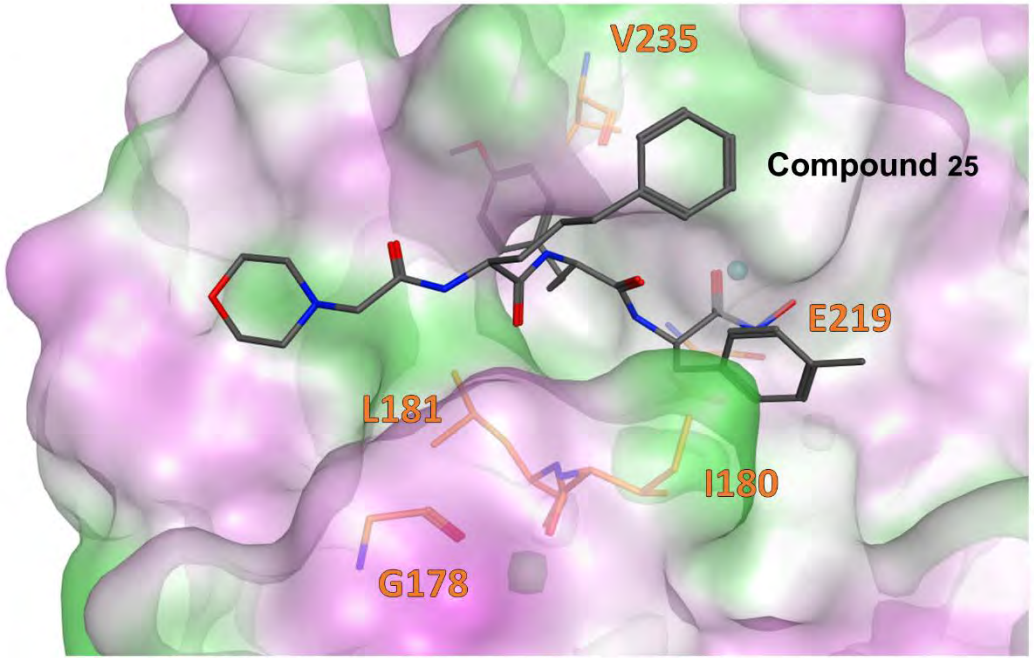


FDA Approves New Kyprolis (Carfilzomib) Combination Therapy for the Treatment of Patients with Relapsed or Refractory Multiple Myeloma





Carfilzomib



Baggio et al. Fragai, Nordgren, Pellecchia, *J. Med. Chem* **2020** in press

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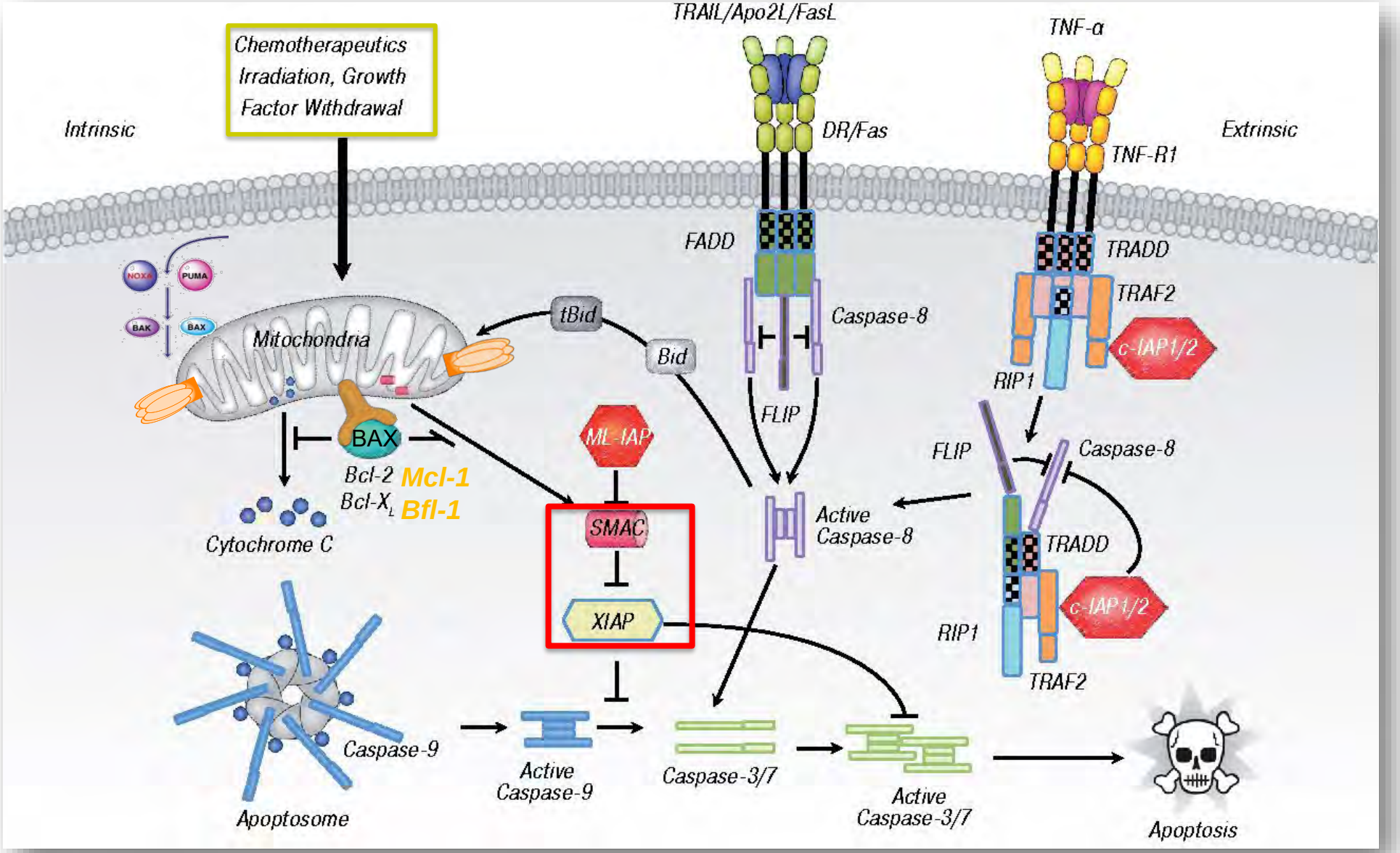
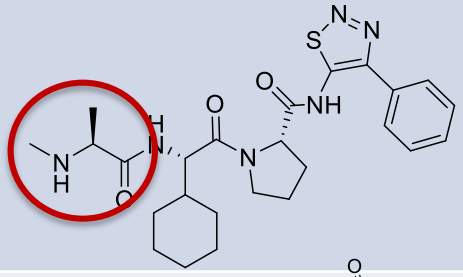
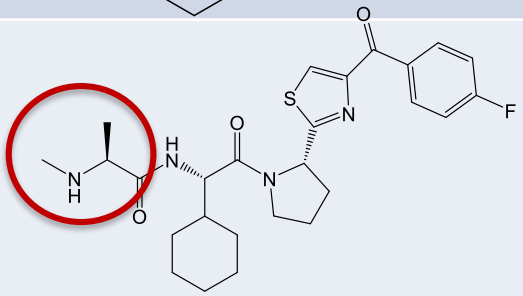
Inhibition of apoptosis and cancer resistance

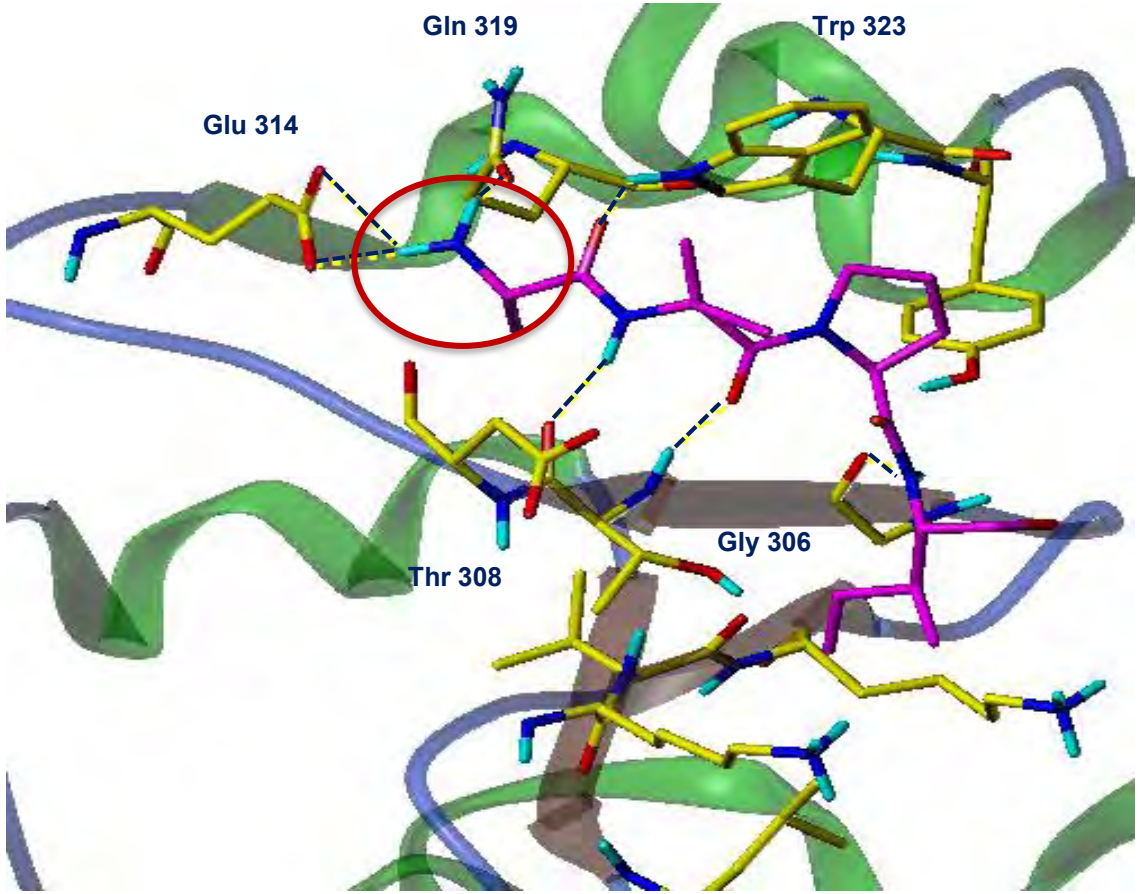


Adapted from: Biology, Medicine Experimental oncology. The inhibitor of\apoptosis (IAP) proteins are critical regulators of signaling pathways and targets for anti-cancer therapy by De Almagro and Vučić

*f*HTS by NMR using Ala as anchoring moiety for XIAP targeting agents

AVPI (or AVPF) mimetics as potent Smac mimetics targeting IAPs

A ϕ P ϕ motifs	
GDC-0152 (Genentech)	
LCL161 (Novartis)	



Wu G. et al. Structural basis of IAP recognition by Smac/DIABLO. *Nature* **2000** , 408, 1008-1012.
Liu et al. Structural basis for binding of Smac/DIABLO to the XIAP BIR3 domain. *Nature* **2000** , 408, 1004-1008.
Sun et al. NMR Structure and Mutagenesis of the Third Bir Domain of the Inhibitor of Apoptosis Protein XIAP, *JBC*, **2000**, 275, pp. 33777–33781.

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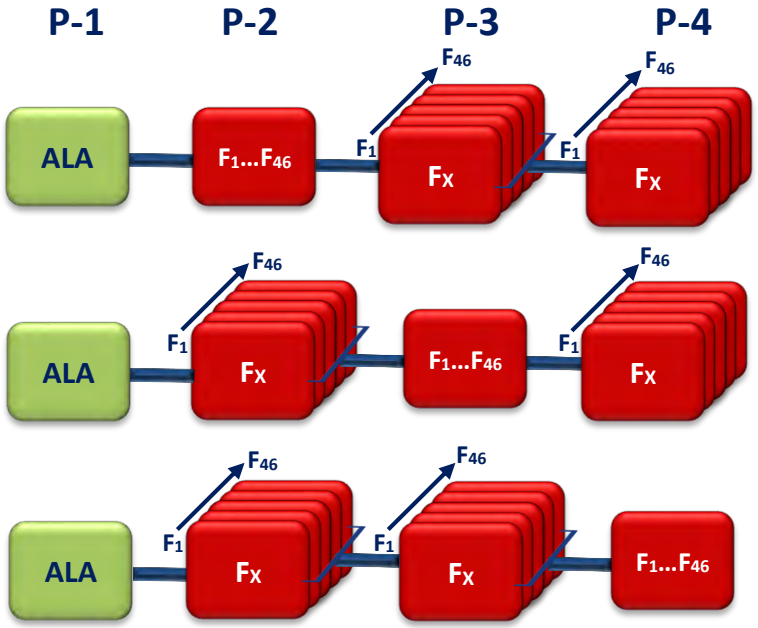
Focused HTS by NMR using an anchoring moiety

Synthesis of positional scanned library of peptide mimetics

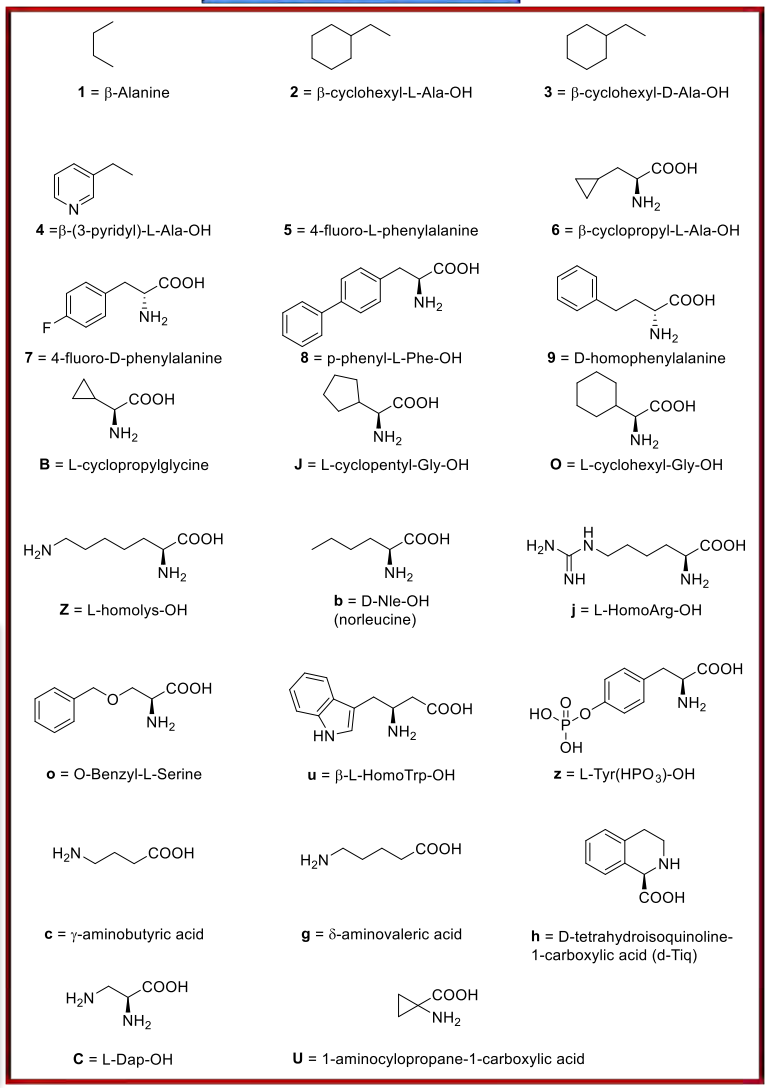


H-AXXX-NH_2 LIBRARY:

A tri-peptoid library of 46 natural and non-natural amino acids (23 L/D aa + 23 modified aa) has been synthesized.

- **46x3 =138 mixtures,**
- **each containing 1x46x46= 2116 compounds**
- **46x46x46 = 97.336 compounds have been tested**
- **Average MW: 450 Da.**

modified aa



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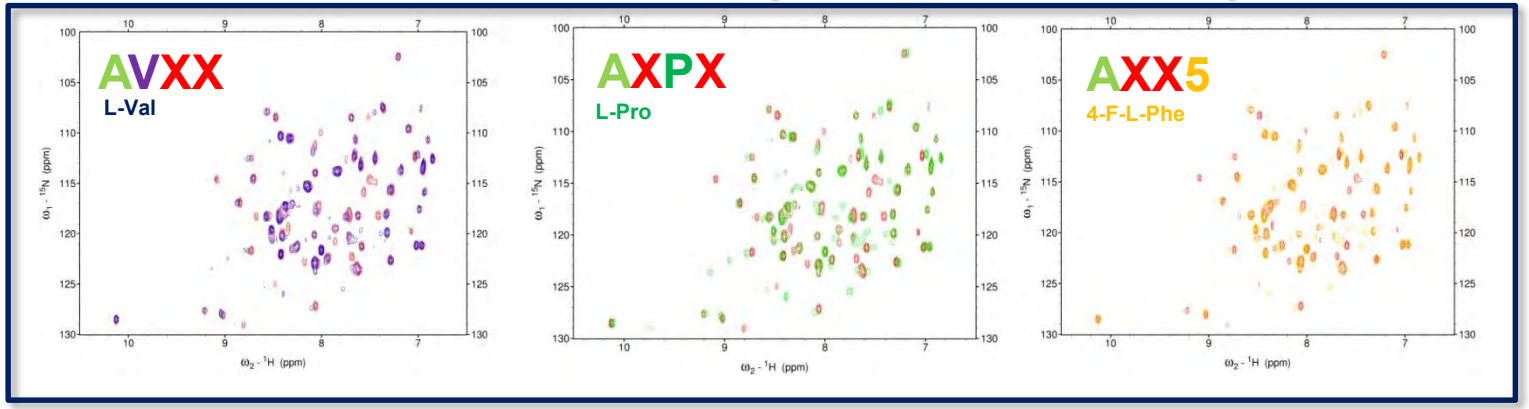
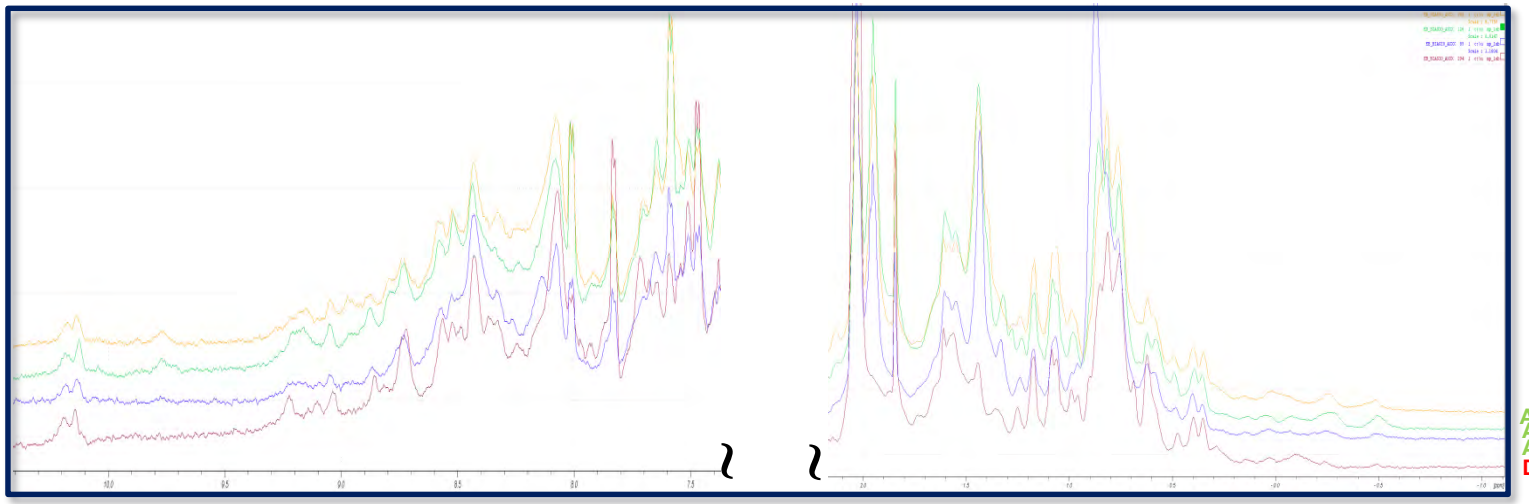
*f*HTS by NMR using Ala as anchoring moiety reveals the known binding motif, AVPI/F, for the Bir3 domain of XIAP

2D [^{1}H,^{15}N]



The known consensus is identified

1D ^{1}H *aliph*



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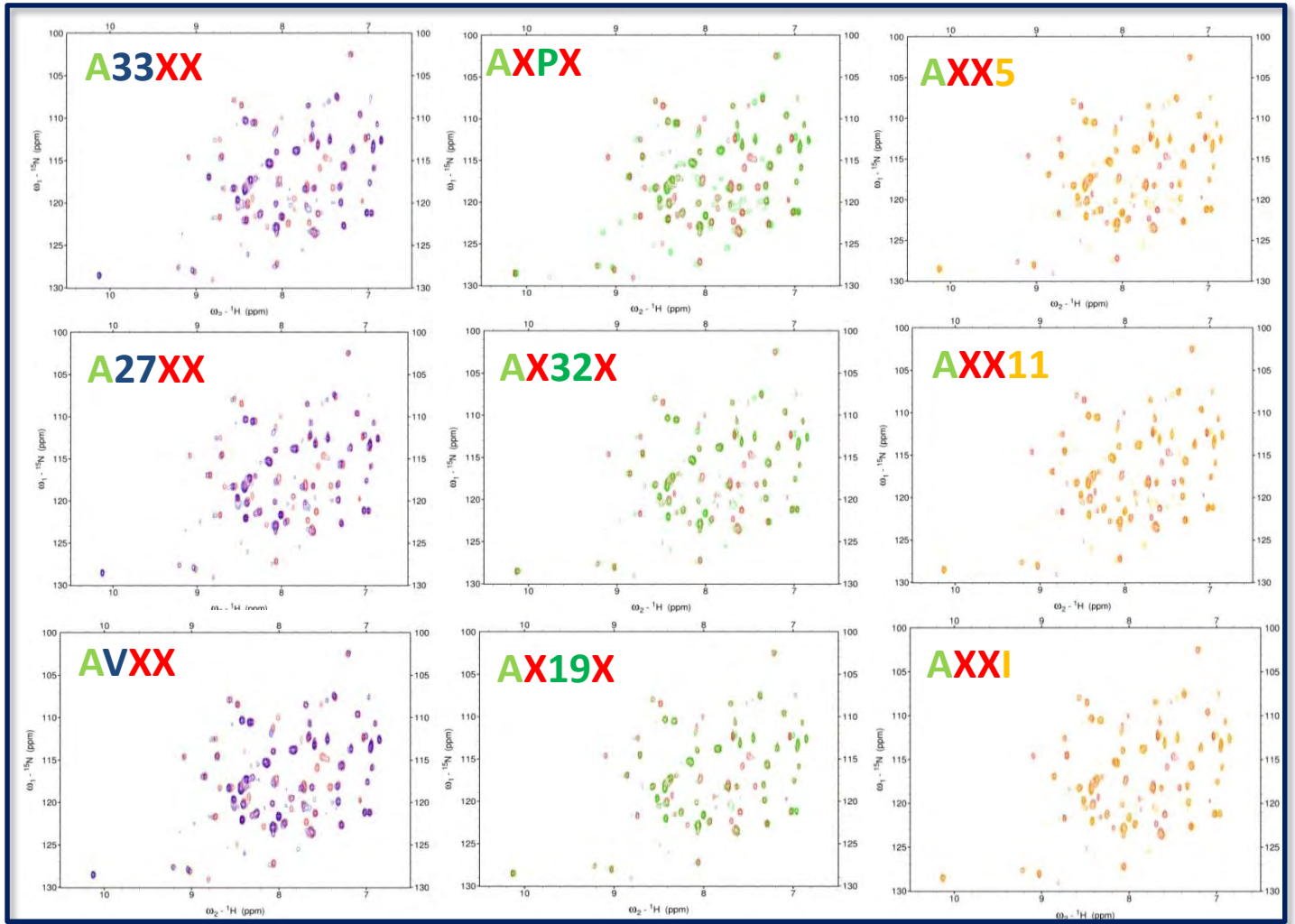
*f*HTS by NMR using Ala as anchoring moiety reveals novel binding motifs for the Bir3 domain of XIAP

The fHTS by NMR also identifies new binding elements



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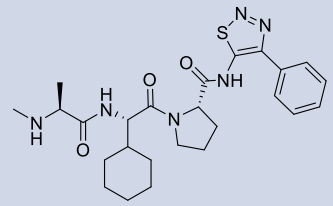
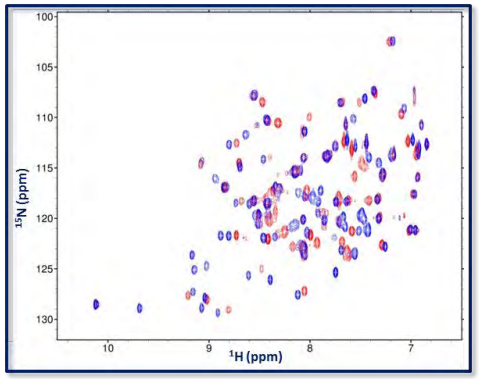
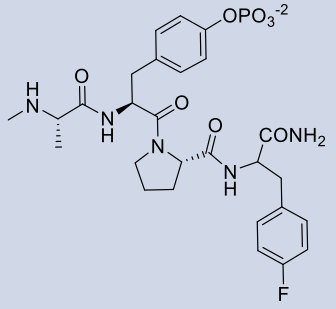
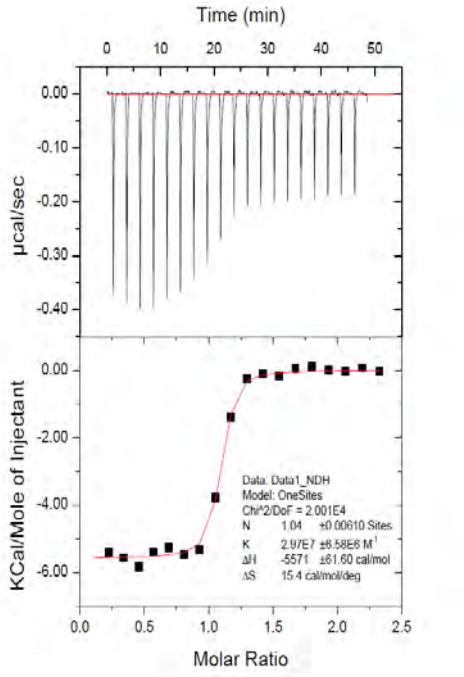
*f*HTS by NMR using Ala as anchoring moiety reveals novel binding motifs for the Bir3 domain of XIAP

Synthesis of few agents lead to initial compound that seems as potent but more selective than GDC0152 towards XIAP

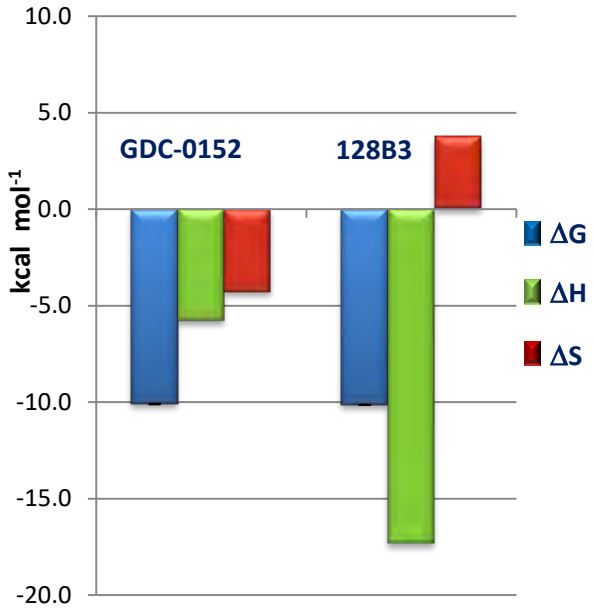
GDC-0152	

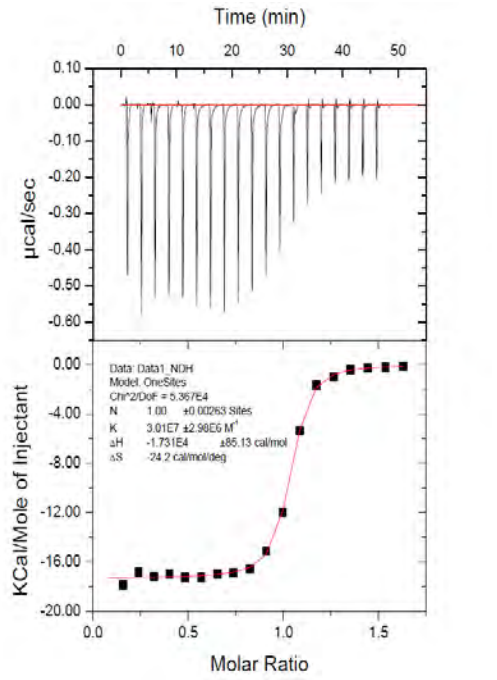


New agent	



Kd= 33.7 nM





Kd= 33.2 nM

Flygare J.A. et al. Discovery of a Potent Small-Molecule Antagonist of Inhibitor of Apoptosis (IAP) Proteins and Clinical Candidate for the Treatment of Cancer (GDC-0152). *Journal of Medicinal Chemistry* **2012** , 55, 4101-4113.

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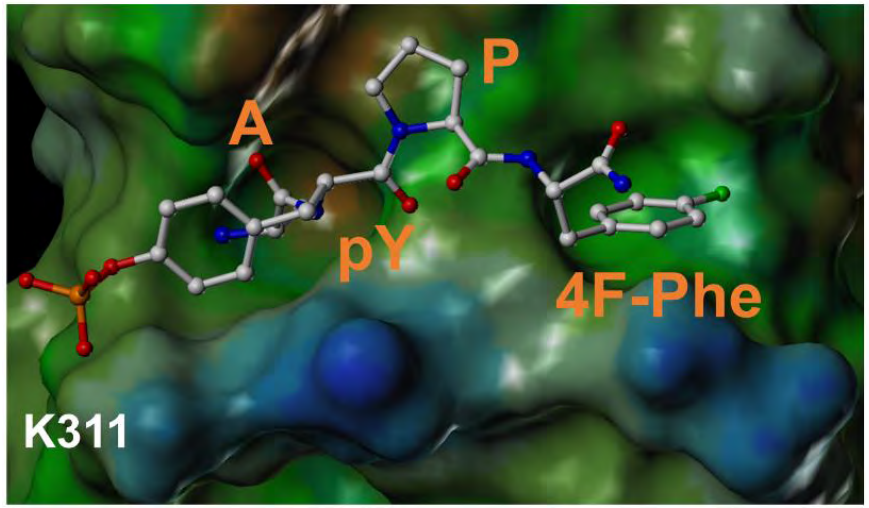
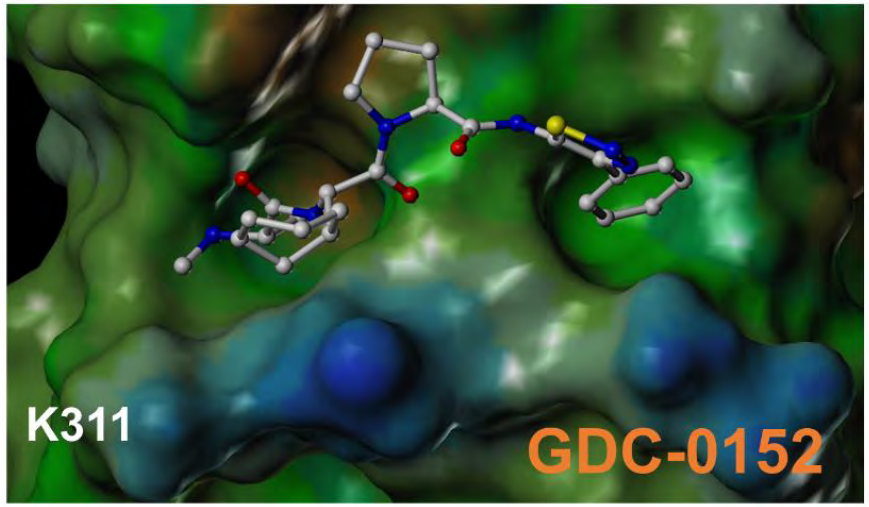
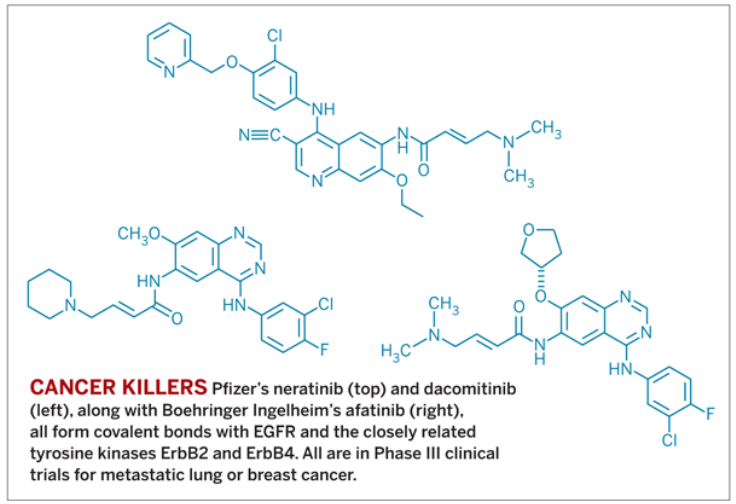
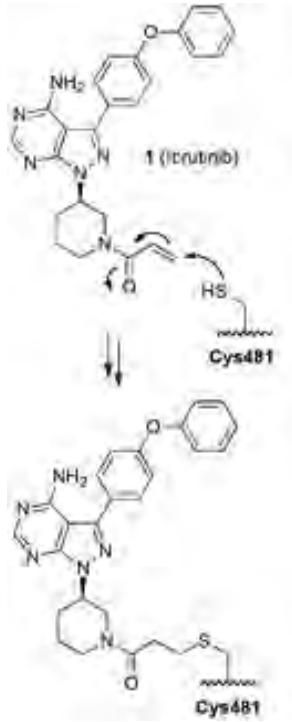
HTS by NMR using Ala as anchoring moiety reveals novel binding motifs for the Bir3 domain of XIAP









- **Can we likewise identify suitable electrophiles for the design of Lys covalent PPIs targeting ligands ?**

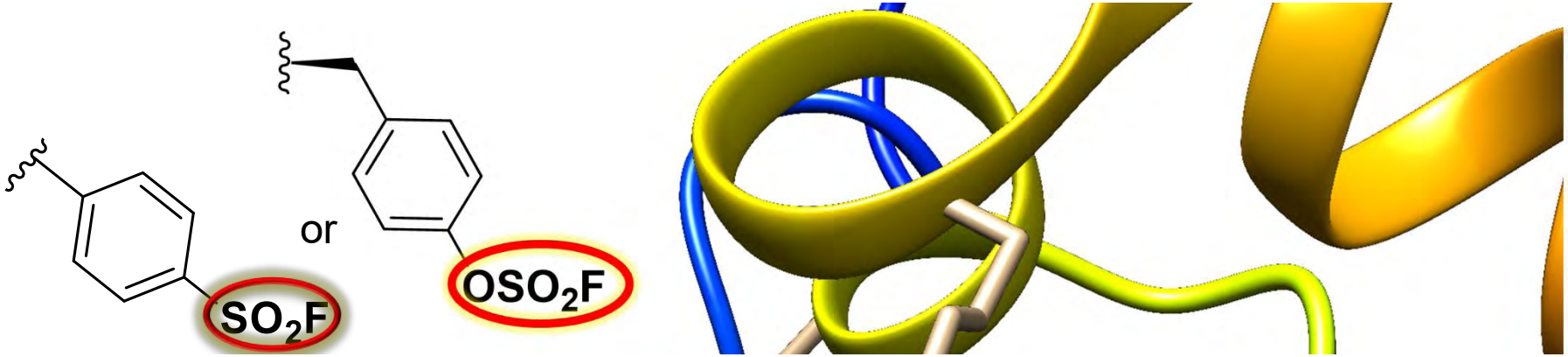
1. Background: NMR-guided drug discovery targeting protein-protein interactions.

2. Application of the HTS by NMR against a protein-protein interaction involving EphA4-LBD and the ephrin ligands

3. Introduction of an anchoring moiety in the library (focused HTS by NMR or *f*HTS by NMR) and applications to various targets including metalloproteases, and the BIR3 domains of IAP proteins

4. Design of Lys/Tyr covalent agents using sulfonyl-fluorides or fluoro-sulfates

Sulfonyl fluorides and fluoro-sulfates as useful warheads to target Lys, Tyr, and His



- Can sulfonyl-fluorides or fluoro-sulfates be used for covalent PPIs targeting ligands ?
  Can the agents react efficiently and selectively with any of these amino acids in PPIs

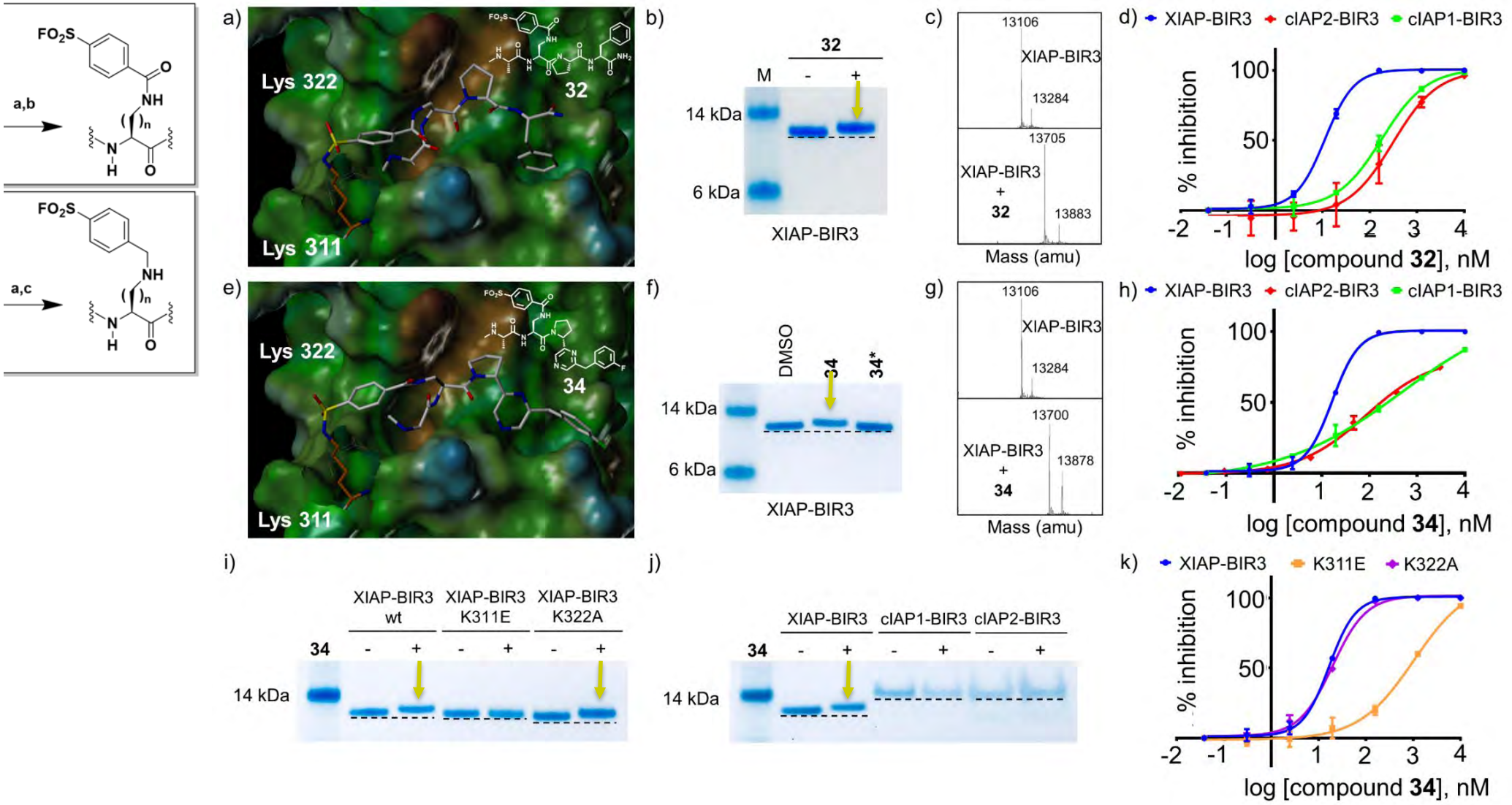
- Are the resulting agents suitable as chemical probes for Lys, Tyr, Ser, Thr, or His?
  Stable in buffer and media, cell permeable, engage the target in cell

- Are the resulting agents suitable as possible therapeutics
  In vivo stability and bioavailability

Sulfur(VI) Fluoride Exchange (SuFEx): Another Good Reaction for Click Chemistry, Dong et al., Sharpless (2014) Ang. Chem.; Morteson, et al., Sharpless and Kelly J. Am. Chem. Soc. 2018, 140, 1, 200-210
Chem et al., Sharpless and Kelly J. Am. Chem. Soc. 2016, 138, 23, 7353-7364

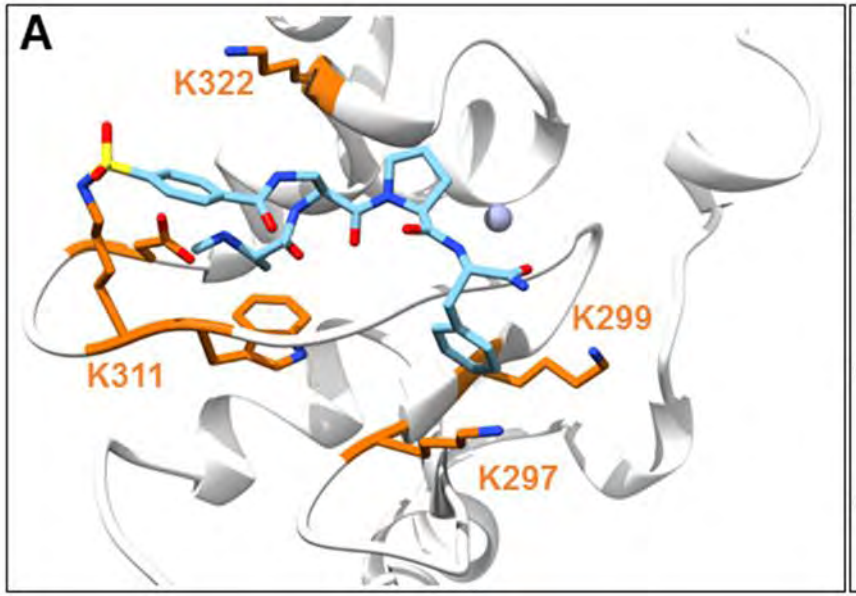
XIAP BIR3 Lys-covalent inhibitors

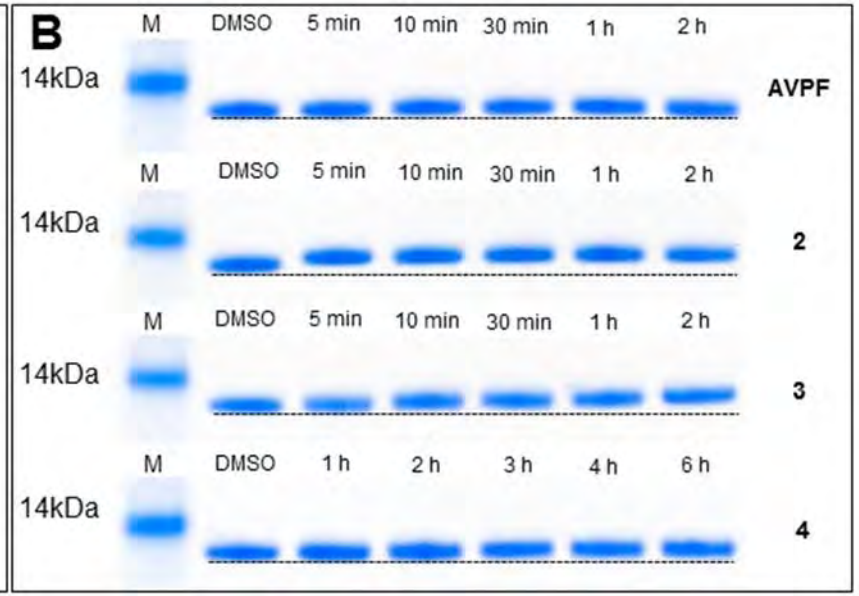
Design of Potent pan-IAP and Lys-Covalent XIAP Selective Inhibitors Using a Thermodynamics Driven Approach. Baggio et al. and Pellecchia *J. Med. Chem.*, **2018**, 61 (14), pp 6350–6363

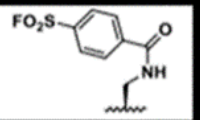
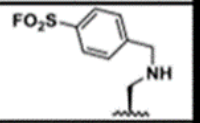
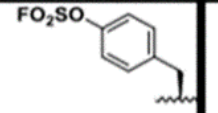


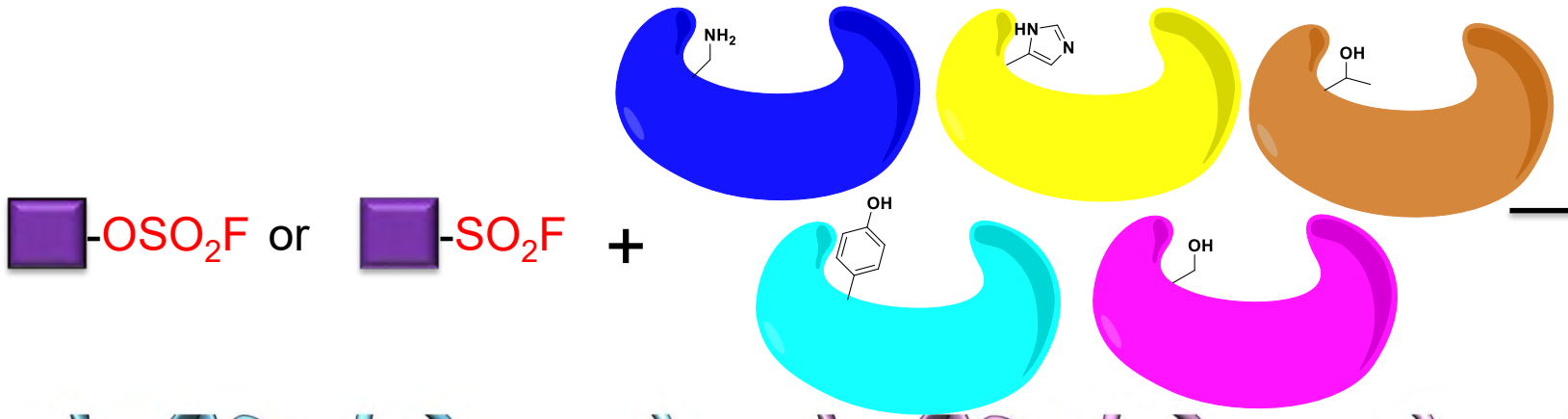
Sulfonyl fluorides and fluoro-sulfates as useful warheads to target Lys, Tyr, and His

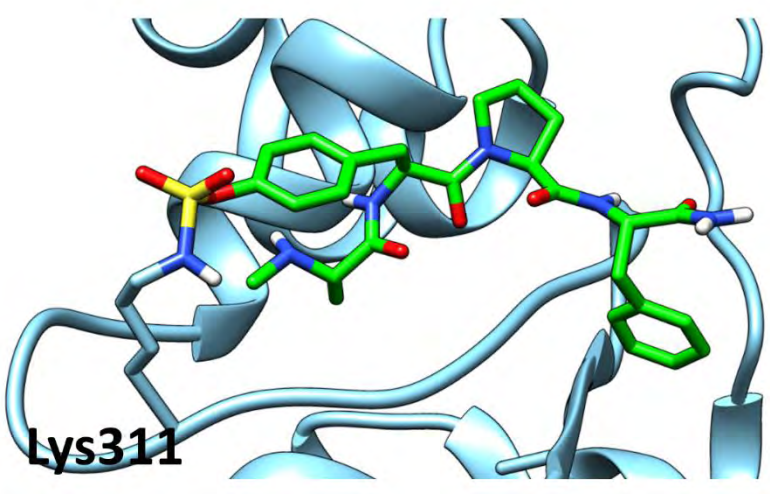
Sulfonyl fluorides agents rapidly react with Lys 311

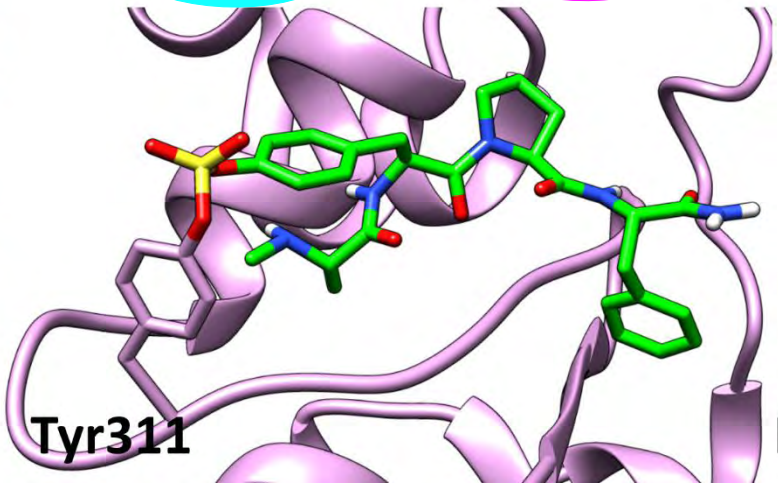


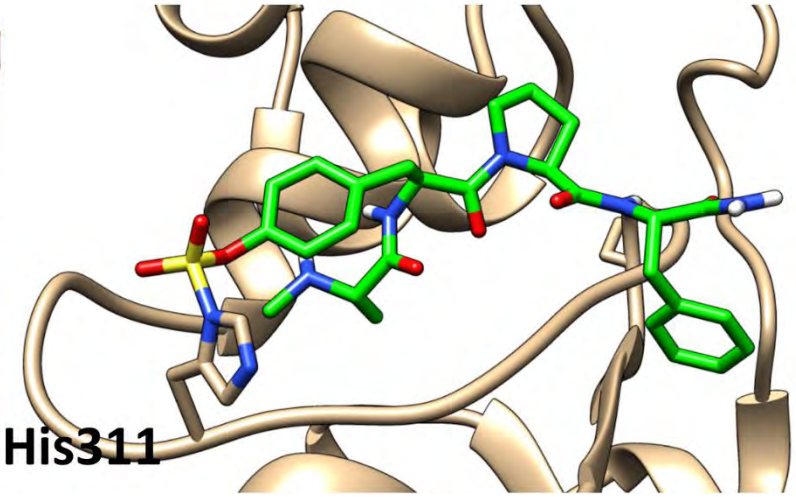


Agent		BIR3wt	
ID	X	IC_{50}[a] [nM]	ΔT_m [°C][b]
2		11 ± 1 13 ± 1	31 37
3		17 ± 0.1 8 ± 0.1	35.5 35
4		71 ± 7 24 ± 1	8.5 33.0









Wt-BIR3 Lys311Tyr BIR3 Lys311His BIR3

Covalent Inhibitors of Protein-Protein Interactions Targeting Lysine, Tyrosine, or Histidine Residues.
Gambini L, Baggio C, Udompholkul P, Jossart J, Salem AF, Perry JJP, Pellecchia M.
J Med Chem. **2019** 62(11):5616-5627

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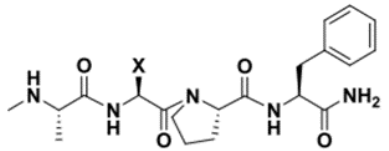
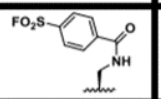
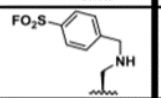
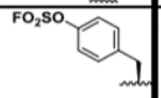
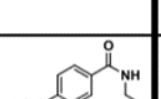
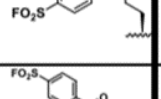
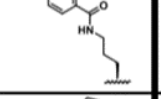
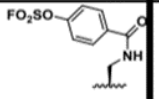
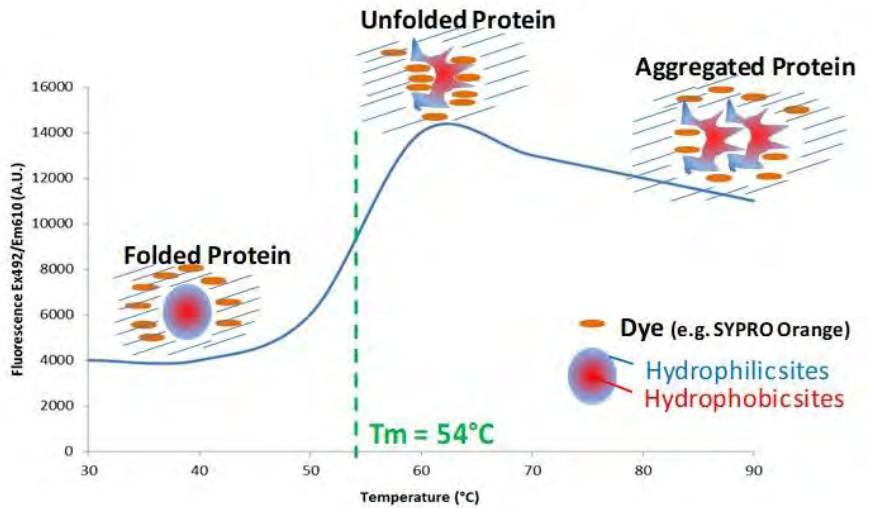
Sulfonyl fluorides and fluoro-sulfates as useful warheads to target Lys, Tyr, and His



Agent		BIR3wt		Lys311Ala		Lys311Tyr		Lys311His		Lys311Thr		Lys311Ser	
ID	X	IC_{50}[a] [nM]	ΔT_m [°C][b]	IC_{50} [nM][a]	ΔT_m [°C][b]	IC_{50} [nM][a]	ΔT_m [°C][b]	IC_{50} [nM][a]	ΔT_m [°C][b]	IC_{50} [nM][a]	ΔT_m [°C] b	IC_{50} [nM][a]	ΔT_m [°C][b]
2		11 ± 1 13 ± 1	31 37	86 ± 2 59 ± 6	1.5 8.5	12 ± 2 10 ± 0.8	37.5 34.5	26 ± 0.1 21 ± 1	35.5 28.5	201 ± 1 72 ± 2	4.0 6.5	160 ± 8 80 ± 9	5 11
3		17 ± 0.1 8 ± 0.1	35.5 35	61 ± 4 27 ± 0.1	5 7.5[d]	6.4 ±0.1 3.5 ±0.1	36 33	62 ± 14 16 ± 1	31 28.5	161 ± 9 45 ± 2	5 6[d]	145 ± 7 39 ± 1	5 7.5[d]
4		71 ± 7 24 ± 1	8.5 33.0	65 ± 1 55 ± 0.2	11 13.5	37 ± 5 21 ± 2	8.5 35.5	113± 22 63 ± 4	11 12.5	140±20 120±10	9.5 11.5	177 ± 4 126 ± 2	10.5 14.5
5		190± 20 105± 10	11.5 16	86 ± 9 95 ± 4	10.5 11.5	55± 16 71 ± 8	10.5 11.5	137 ± 4 110 ± 3	8.5 8.5	231±20 220±10	8.5 9.5	241 ± 1 230± 10	6.5 13.5
6		140 ± 1 100 ± 5	11.5 15.0	89 ± 0.2 108 ± 6	10.5 11.5	63 ± 10 85 ± 4	10.5 11.5	140± 24 130± 10	11.5 12	240±12 220±20	7.5 9.5	250±1 276±1 4	8 13.5
7		590± 65 100 ± 7	4.5 31[c]	218 ±8 270± 25	8 8.5	113 ± 2 48 ± 6	5.5 32	270±70 260±20	36.5[e] 38[e]	620±40 630±20	5.5 6.5	713± 3 646± 32	8.5 10.5
8		2000± 20 90 ± 1	11.5 28.5[c]	70 ± 2 70 ± 7	11 12.5	80 ± 2 86 ± 13	10.5 29.5[c]	126± 14 98 ± 1	11 29.5[c]	180± 40 165± 3	8.5 10	250± 1 237± 13	10.5 10.5

Two values are for **2 hr or 6 hr incubation time**, respectively

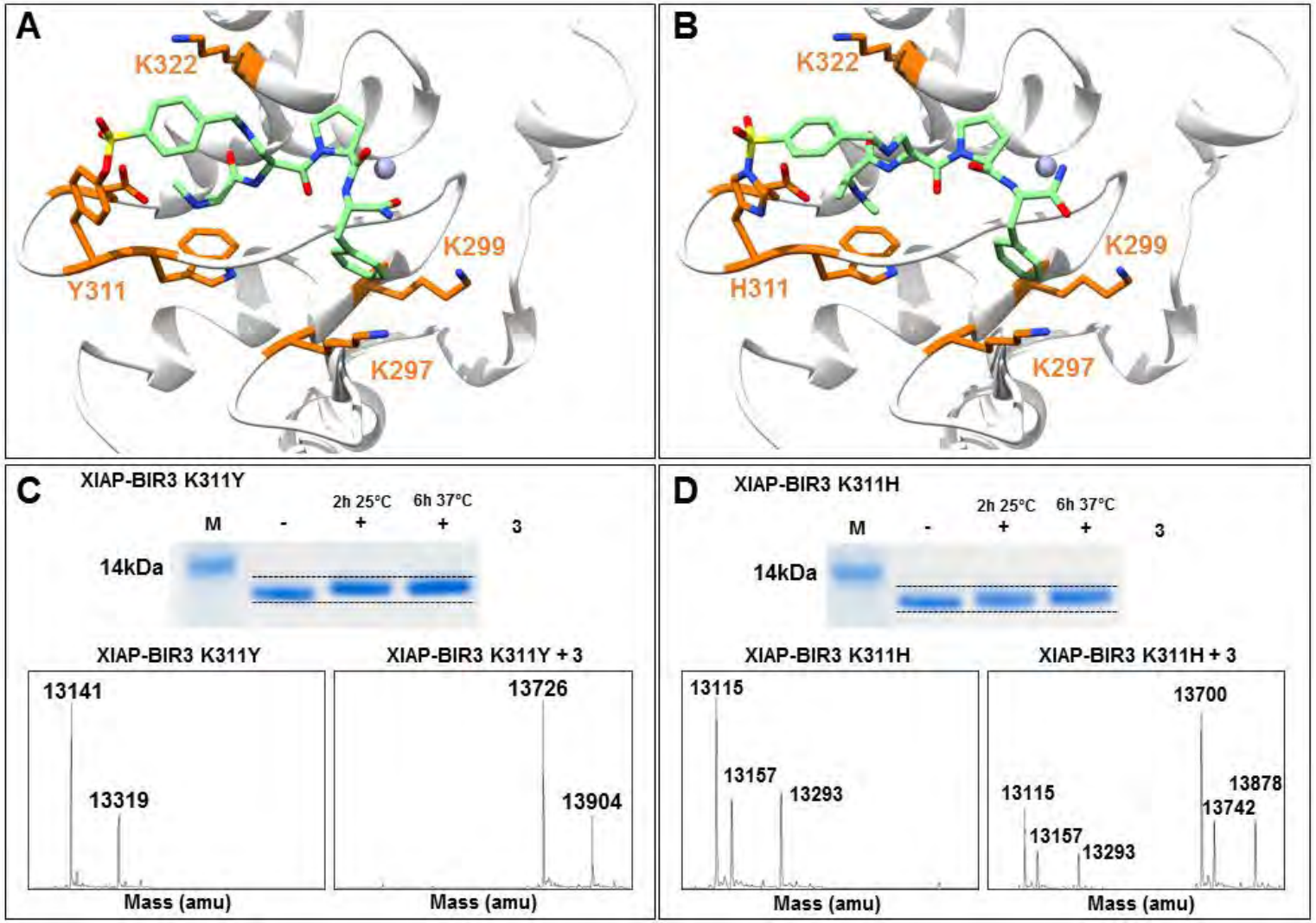
Thermal shift (ΔTm) assay



*Gambini et al., Pellecchia, J Med Chem **2019,** 62(11):5616-5627

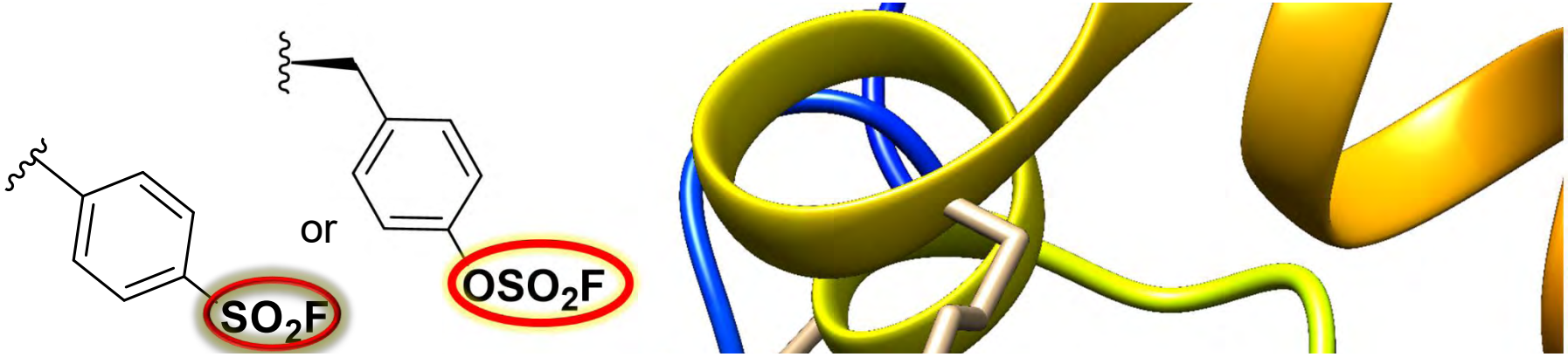
Sulfonyl fluorides and fluoro-sulfates as useful warheads to target Lys, Tyr, and His

Sulfonyl fluorides and fluorosulfates with Lys311Tyr and Lys311His mutants



*Gambini et al., Pellecchia, J Med Chem **2019,** 62(11):5616-5627

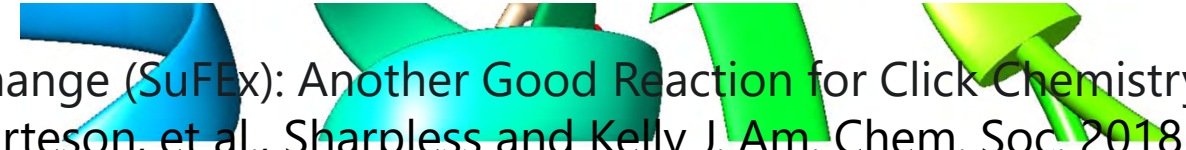
Sulfonyl fluorides and fluoro-sulfates as useful warheads to target Lys, Tyr, and His



- Can sulfonyl-fluorides or fluoro-sulfates be used for covalent PPIs targeting ligands ?
  Can the agents react efficiently and selectively with any of these amino acids in PPIs

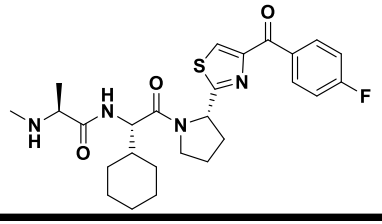
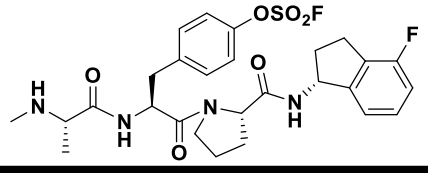
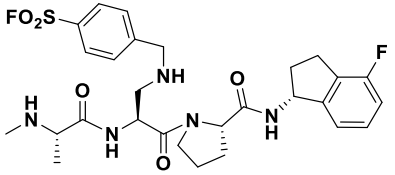
- Are the resulting agents suitable as chemical probes for Lys, Tyr, Ser, Thr, or His?
  Stable in buffer and media, cell permeable, engage the target in cell

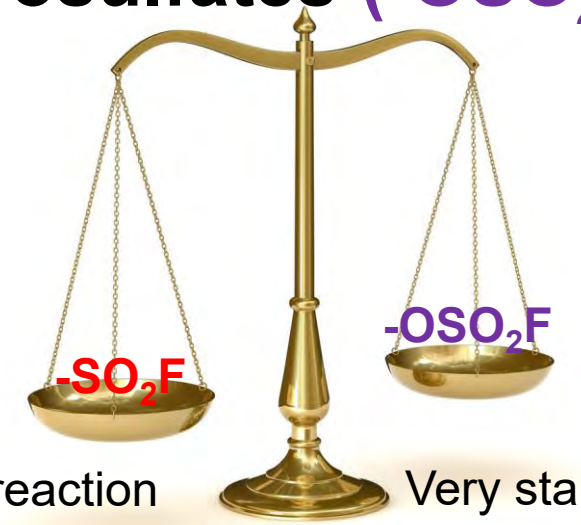
- Are the resulting agents suitable as possible therapeutics
  In vivo stability and bioavailability



Sulfur(VI) Fluoride Exchange (SuFEx): Another Good Reaction for Click Chemistry, Dong et al., Sharpless (2014) Ang. Chem.; Morteson, et al., Sharpless and Kelly J. Am. Chem. Soc. 2018, 140, 1, 200-210
Chem et al., Sharpless and Kelly J. Am. Chem. Soc. 2016, 138, 23, 7353-7364

Sulfonyl fluorides (-SO_2F) versus aryl-fluorosulfates (-OSO_2F)

ID	Structure	IC_{50} XIAP-BIR3 [nM][a]	Chemical stability	Plasma Stability[c]	ΔT_m [°C][d]
LCL161		48 ± 5 53 ± 3	> 5 h	> 2h	14 18.5
10		63 ± 6 28 ± 6	> 5 h	> 2h	**9.5** **33.5**
11		24 ± 1 3.5 ± 0.1	~ 2.5 h	**~15 min**	35 34.5



Rapid reaction with target

=>

Rapid reaction with water and plasma

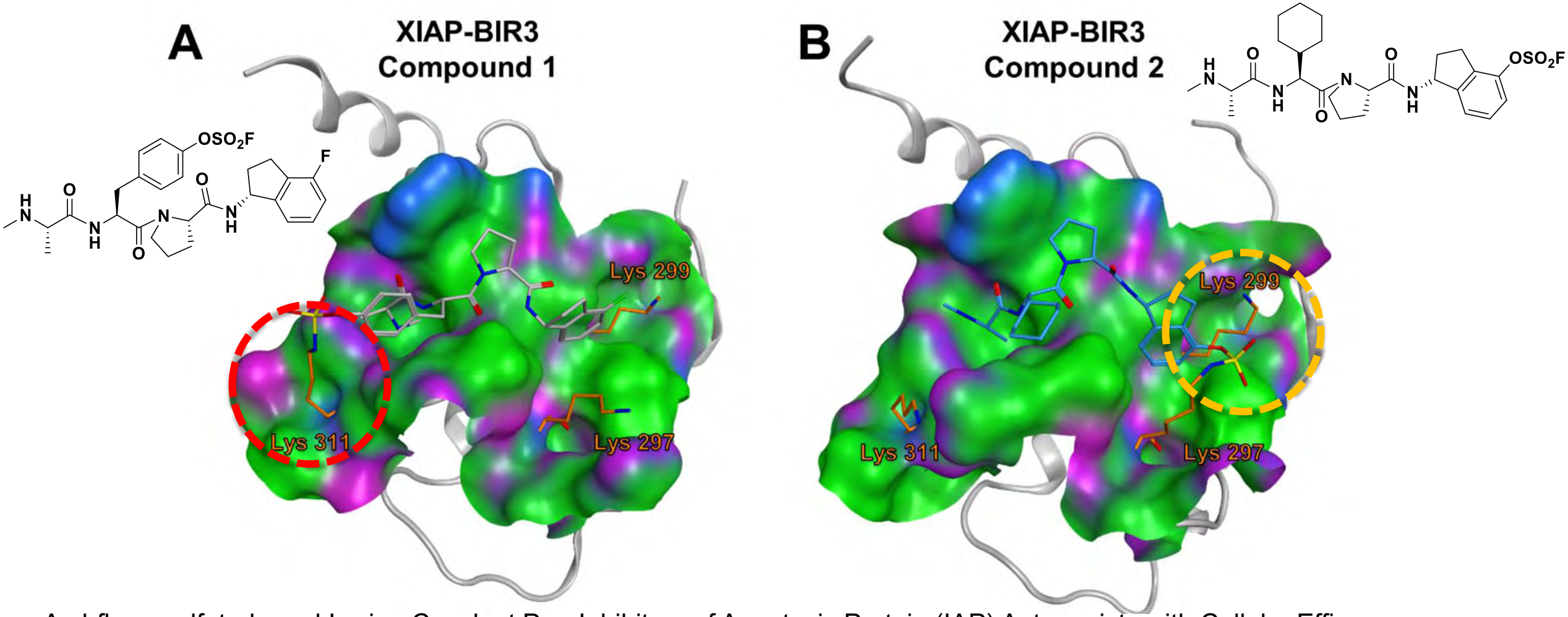
Very stable in water and plasma

=>

Slower reaction with target

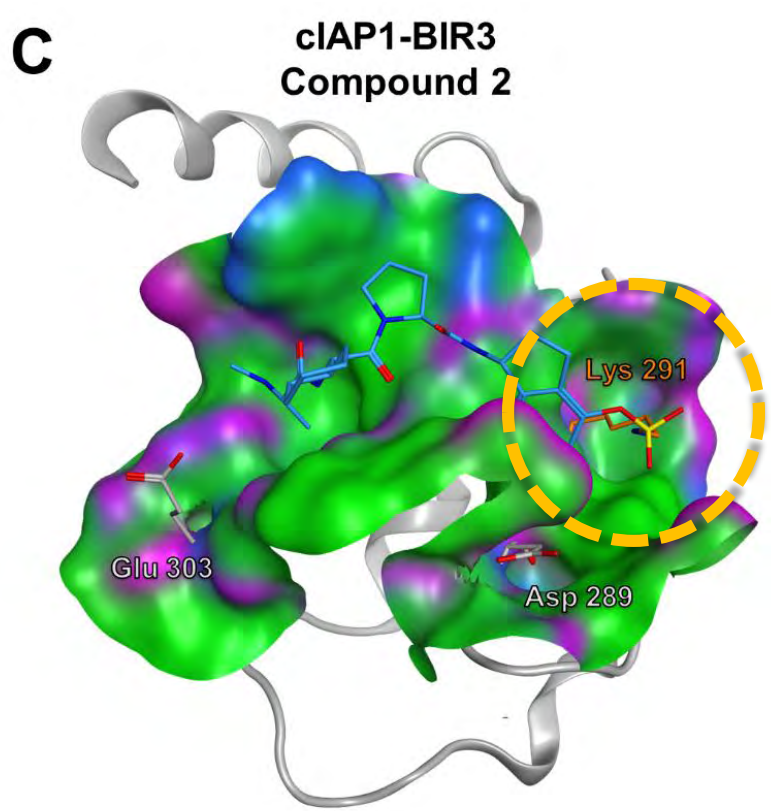
Lys311 distal –OSO_2F

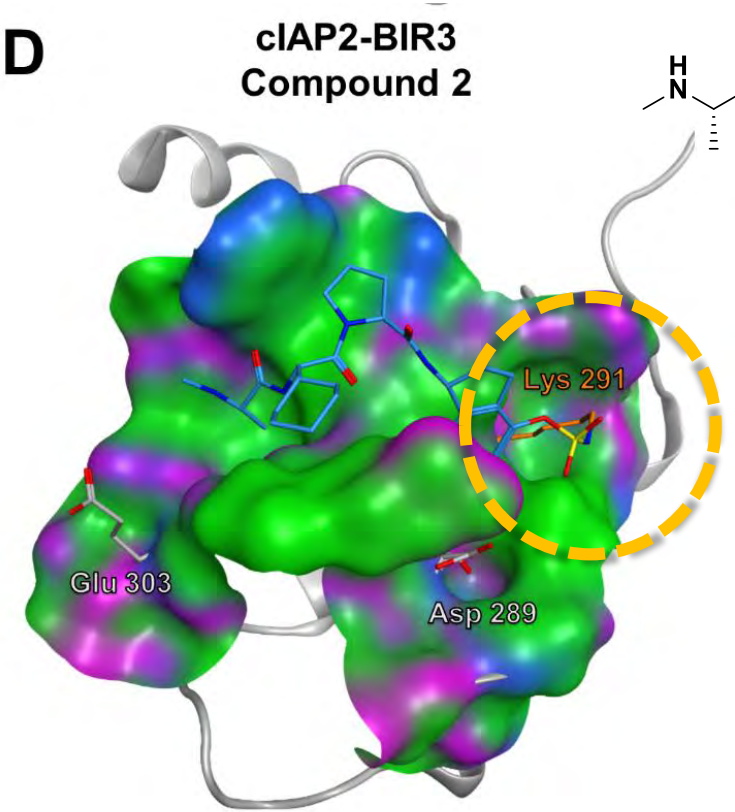
Lys297 proximal –OSO_2F

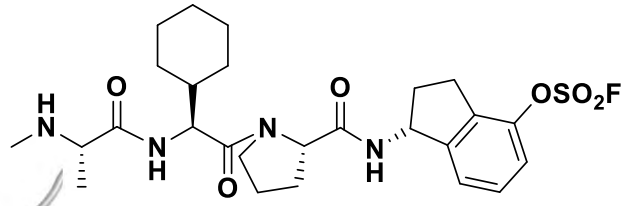


Aryl-fluorosulfate-based Lysine Covalent Pan-Inhibitors of Apoptosis Protein (IAP) Antagonists with Cellular Efficacy
Baggio, C., Udompholkul P., Gambini L., Salem AF., Jossart J., Perry JJP, and Pellecchia. M
J Med Chem **2019** 62(20):9188-9200 – **F1000 selected**

Lys291 proximal –OSO_2F







Compound 2

Aryl-fluorosulfate-based Lysine Covalent Pan-Inhibitors of Apoptosis Protein (IAP) Antagonists with Cellular Efficacy
Baggio, C., Udompholkul P., Gambini L., Salem AF., Jossart J., Perry JJP, and Pellecchia. M
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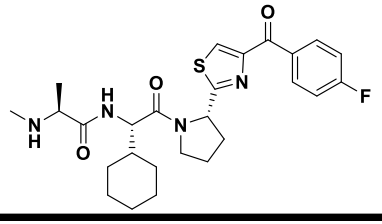
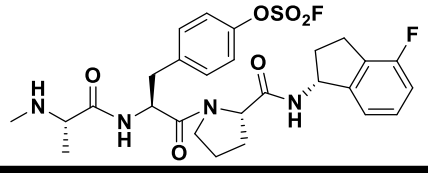
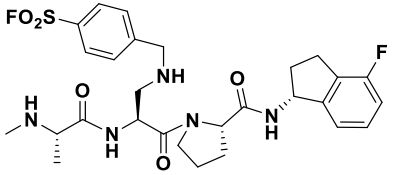
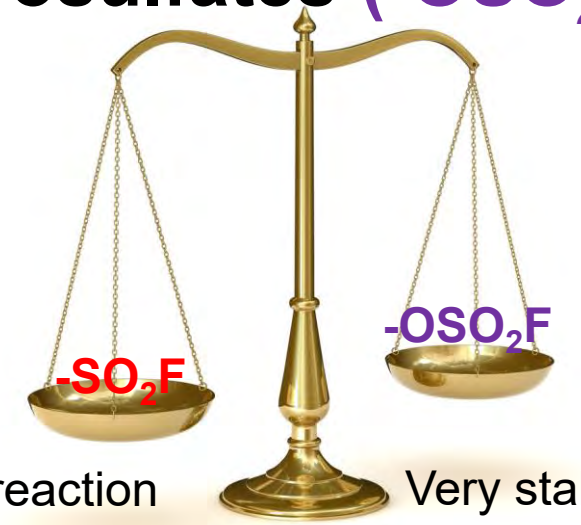
Sulfonyl fluorides (-SO_2F) versus aryl-fluorosulfates (-OSO_2F)

ID	Structure	IC_{50} XIAP-BIR3 [nM][a]	Chemical stability	Plasma Stability[c]	ΔT_m [°C][d]
LCL161		48 ± 5 53 ± 3	> 5 h	> 2h	14 18.5
10		63 ± 6 28 ± 6	> 5 h	> 2h	**9.5** **33.5**
11		24 ± 1 3.5 ± 0.1	~ 2.5 h	**~15 min**	35 34.5



Rapid reaction with target

=>

Rapid reaction with water and plasma

Very stable in water and plasma

=>

Slower reaction with target

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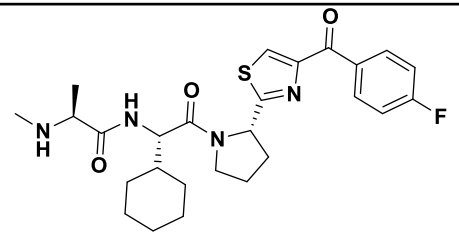
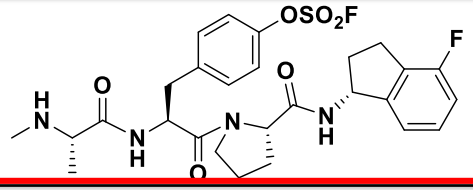
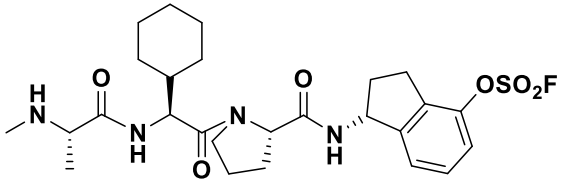
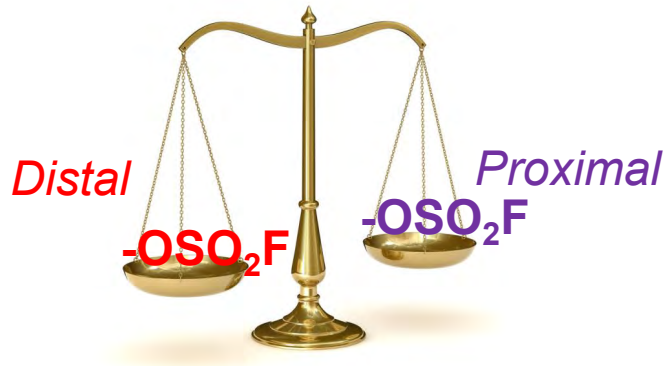
Aryl-fluorosulfate-based Lysine Covalent Pan-Inhibitors of Apoptosis Protein (IAP) Antagonists with Cellular Efficacy

ID	STRUCTURE	XIAP-BIR3		cIAP1-BIR3	cIAP2-BIR3
		IC_{50} [nM][a]	ΔT_m [°C][b]	IC_{50} (nM)	IC_{50} (nM)
LCL161		50 ± 5 40 ± 14	14.0 18.5	18.6 ± 0.1 21 ± 3	11 ± 4 19 ± 2
1		63 ± 6 28 ± 6	9.5 33.5	24 ± 1 20 ± 5	40 ± 3 47 ± 23
2		38 ± 1 12 ± 2	37.5 38.0	25 ± 1 14 ± 2	28 ± 1 9 ± 3



Plasma stable

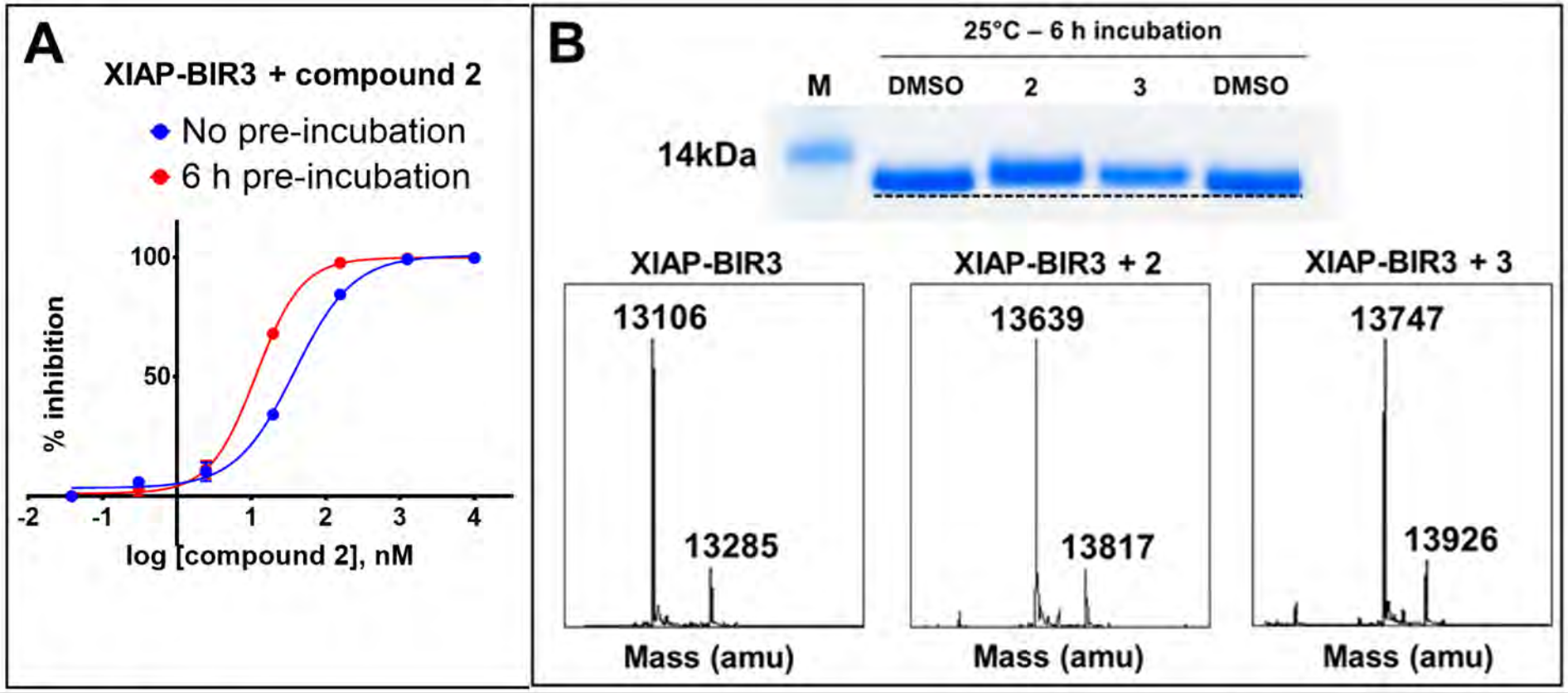
Plasma stable

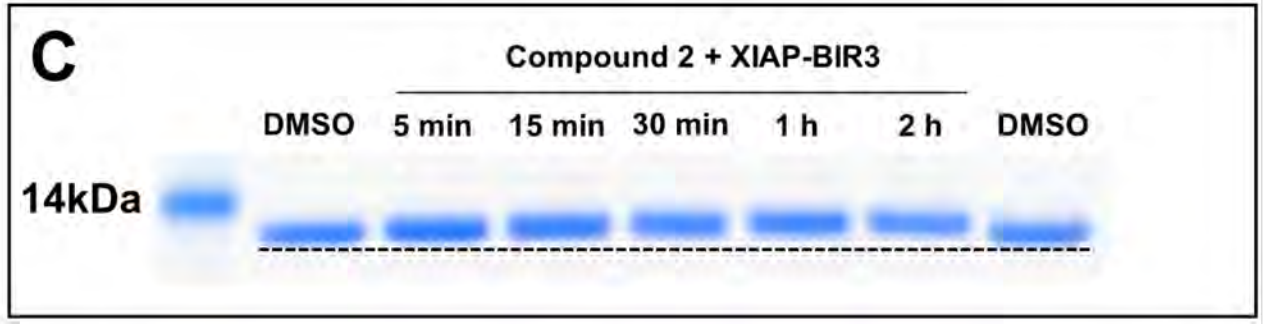
Slow reaction with target

Fast reaction with target

Aryl-fluorosulfate-based Lysine Covalent Pan-Inhibitors of Apoptosis Protein (IAP) Antagonists with Cellular Efficacy
Baggio, C., Udompholkul P., Gambini L., Salem AF., Jossart J., Perry JJP, and Pellecchia. M
J Med Chem **2019** 62(20):9188-9200 – **F1000 selected**

Aryl-fluorosulfate-based Lysine Covalent Pan-Inhibitors of Apoptosis Protein (IAP) Antagonists with Cellular Efficacy





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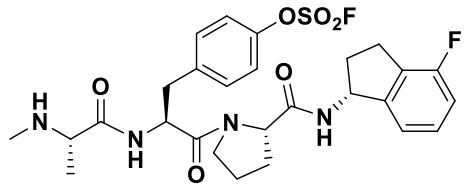
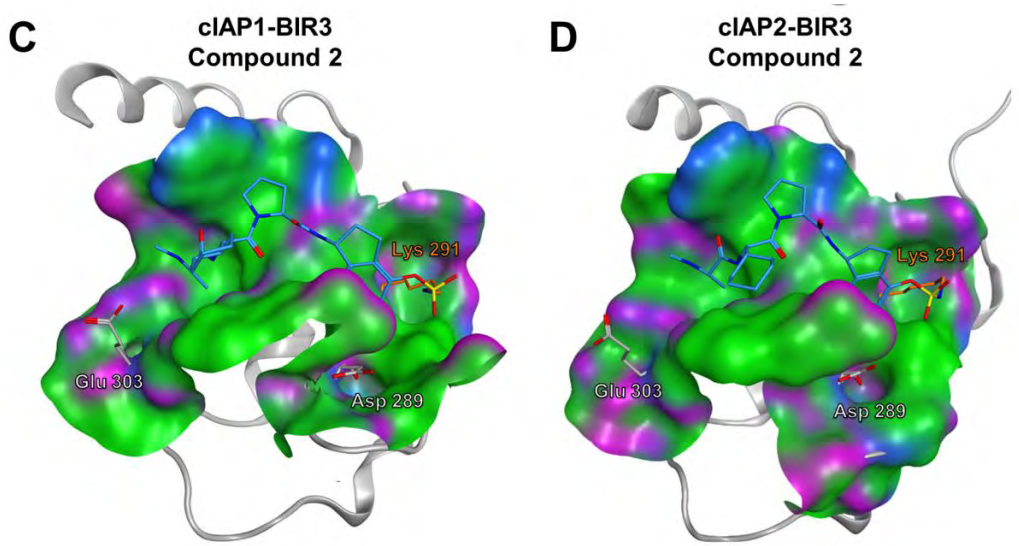
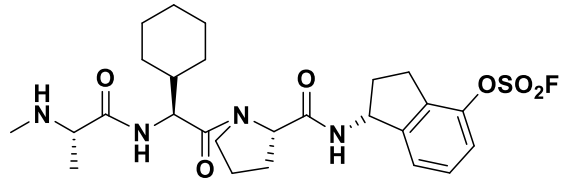
Aryl-fluorosulfate-based Lysine Covalent Pan-Inhibitors of Apoptosis Protein (IAP) Antagonists with Cellular Efficacy

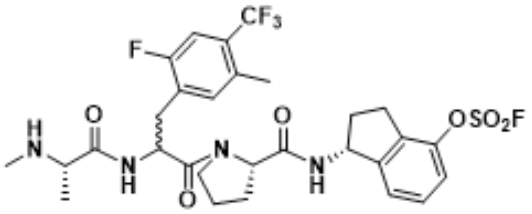


Compound 1

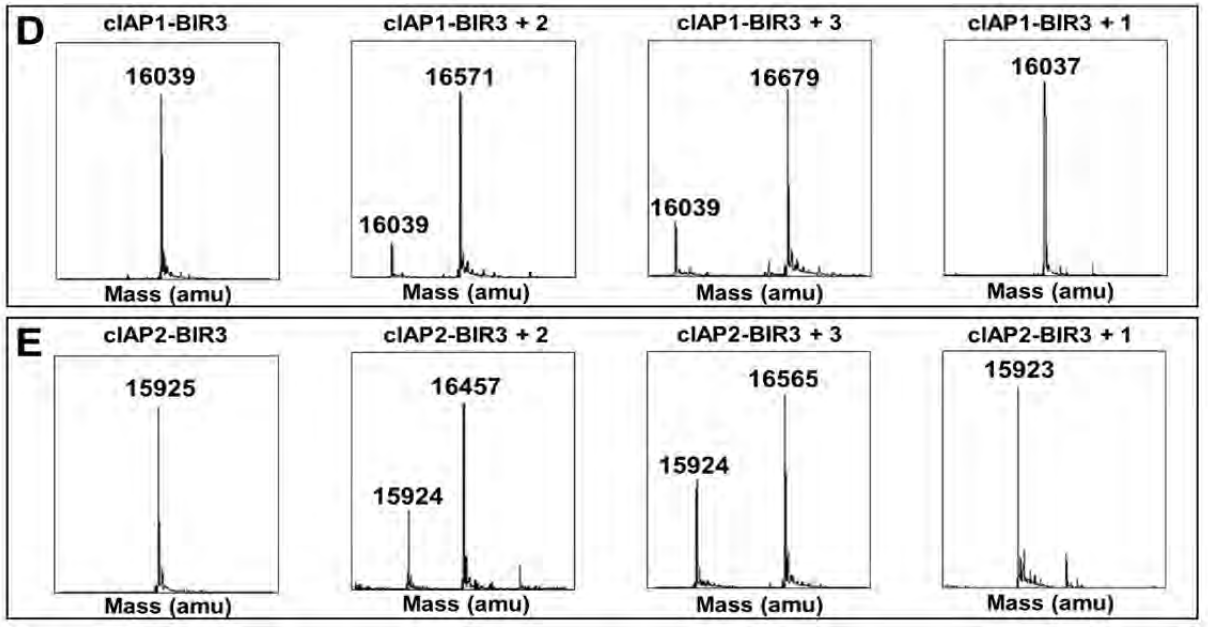




Compound 2

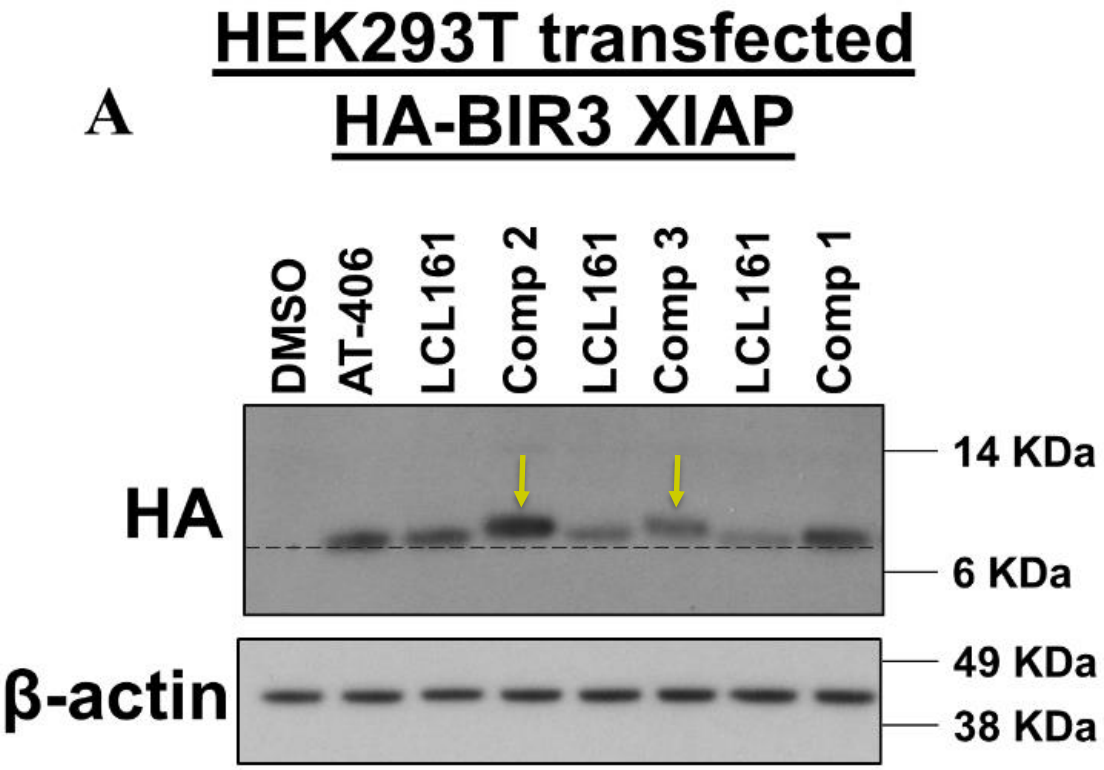


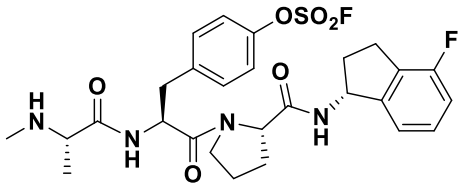
Compound 3



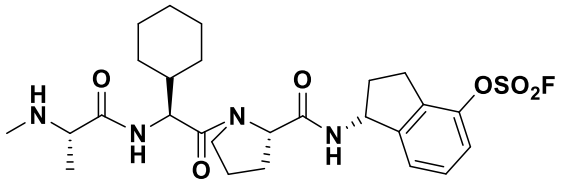
Aryl-fluorosulfate-based Lysine Covalent Pan-Inhibitors of Apoptosis Protein (IAP) Antagonists with Cellular Efficacy

Cell permeability assay

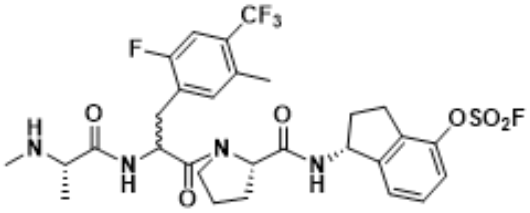




Compound 1



Compound 2



Compound 3

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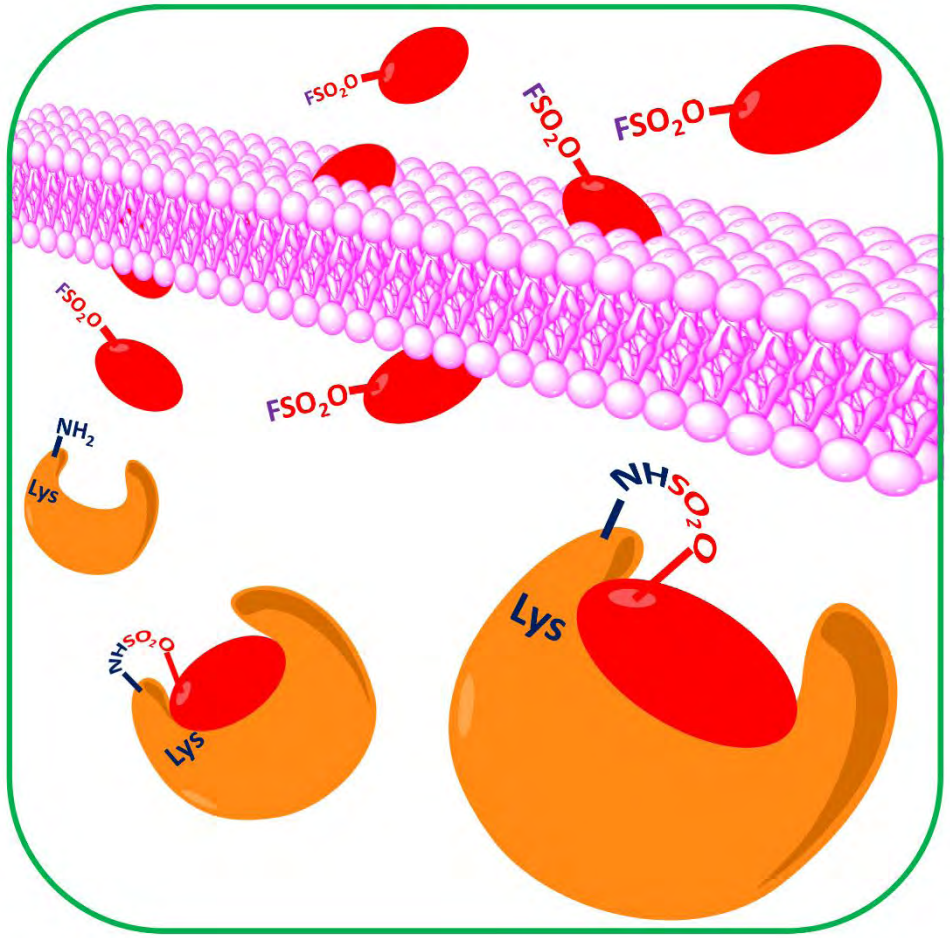

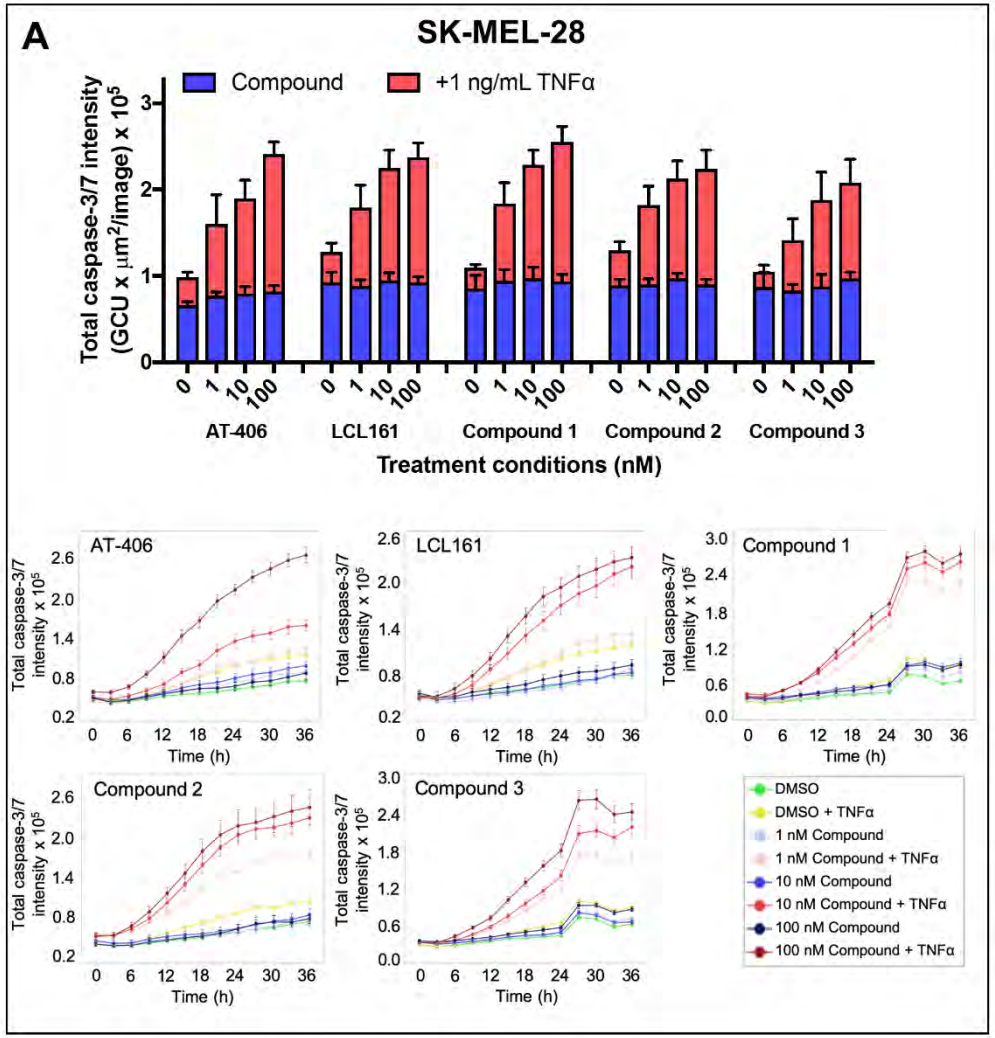

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Baggio, C., Udompholkul P., Gambini L., Salem AF., Jossart J., Perry JJP, and Pellecchia. M
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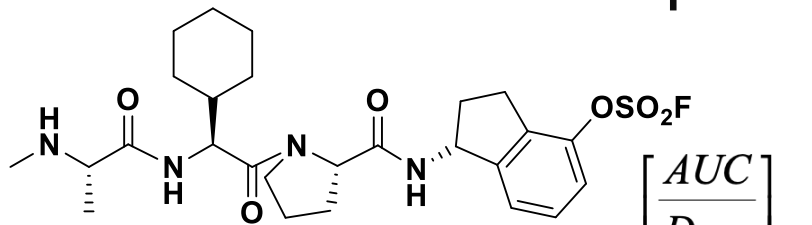
In vivo PK studies (mice)

Aryl-fluorosulfate-based pan-IAP antagonist is bioavailable

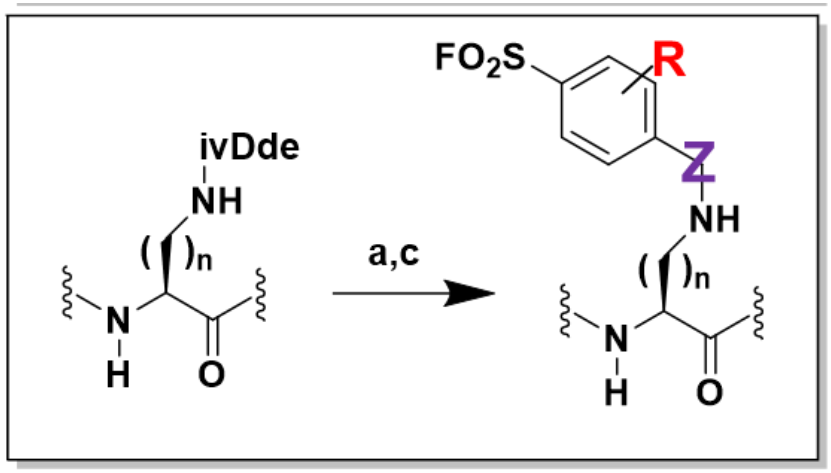


$$F = \frac{\left[\frac{AUC}{Dose}\right]_{ev}}{\left[\frac{AUC}{Dose}\right]_{iv}}$$

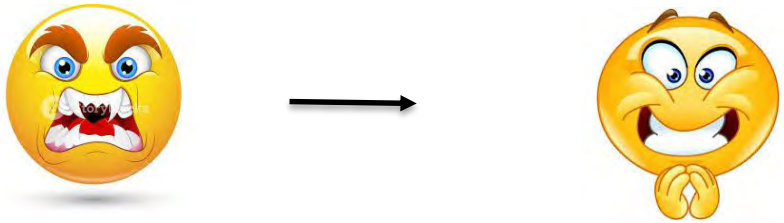
F_{PO} = 36%

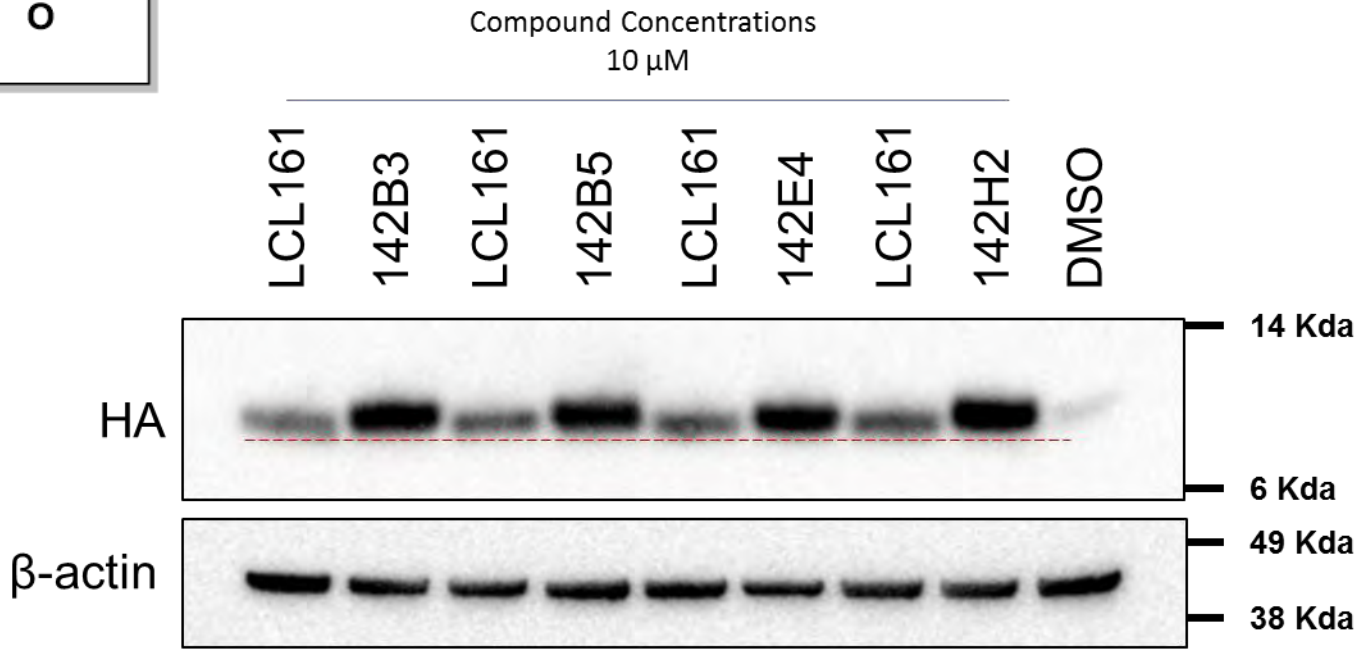
F_{IP} = 38%

PK data from the UCSD In vivo pharmacology core facility (ms in preparation)



Taming the sulfonyl fluorides





Stability and Cell Permeability of Sulfonyl Fluorides in the Design of Lys-Covalent Antagonists of Protein-Protein Interactions. Gambini L, Udompholkul P, Salem AF, **Baggio C**, Pellecchia M. *ChemMedChem*. **2020** Aug, in press

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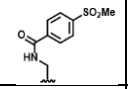
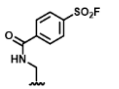
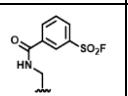
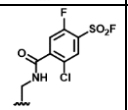
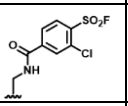
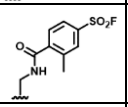
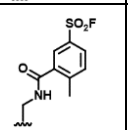
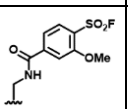
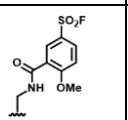
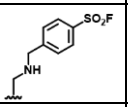
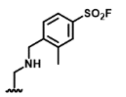
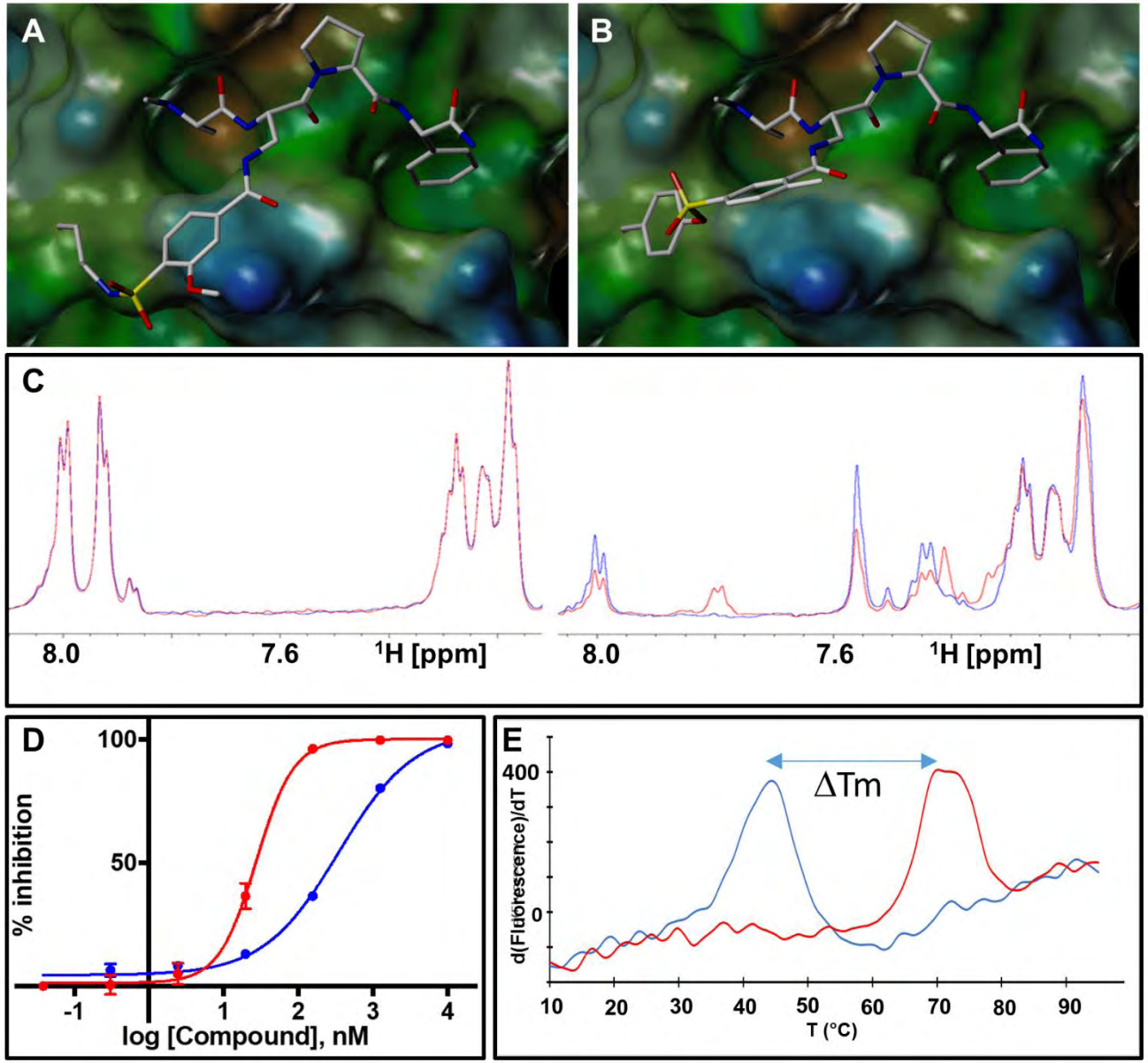
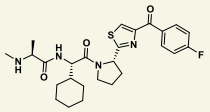
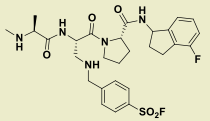
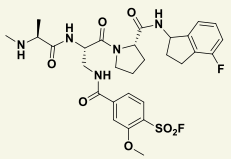
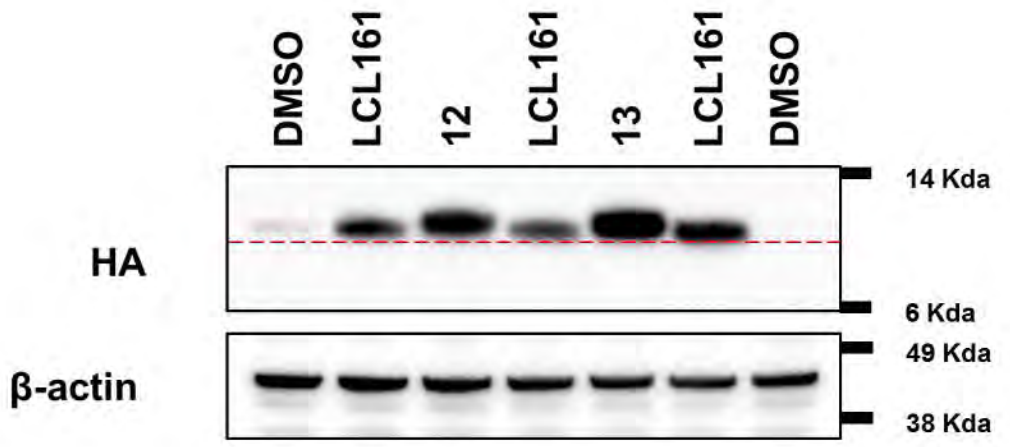
Taming the sulfonyl fluorides

ID	X group	aqueous stability [a]	DELFIA IC_{50} [nM][b]	ΔT_m [°C] [c]	
				wt-BIR3 (Lys311)	*mut*-BIR3 (Lys311Tyr)
1		>99% >99%	348 ± 8	4.7 ± 0.1 5.4 ± 0.1	3.0 ± 0.2 2.7 ± 0.5
2		54% 0%	15 ± 2	30.1 ± 0.3 29.9 ± 0.4	25.8 ± 0.2 24.7 ± 0.1
3		57% 0%	47± 3	5.1 ± 0.1 5.2 ± 0.3	24.8 ± 0.1 23.4 ± 0.2
4		39% 0%	173 ± 6	6.7 ± 0.2 6.1 ± 0.1	26.6 ± 0.2 25.2 ± 0.2
5		58% 0%	12 ± 1	28.5 ± 0.4 28.4 ± 0.4	25.9 ± 0.1 24.7 ± 0.1
6		80% 15%	21 ± 3	30.4 ± 0.1 30.0 ± 0.2	26.4 ± 0.1 25.1 ± 0.1
7		94% 73%	122 ± 1	25.8 ± 0.4[d] 25.5 ± 0.2[d]	26.3 ± 0.2 25.0 ± 0.1
8		96% 46%	28 ± 3	26.8 ± 0.7 27.4 ± 0.3	26.0 ± 0.2 24.6 ± 0.1
9		90% 71%	261 ± 16	5.3 ± 0.4 26.0 ± 0.4[c]	24.6 ± 0.1 23.5 ± 0.2
10		92% 47%	47 ± 2	25.8 ± 0.2 26.0 ± 0.1	25.5 ± 0.2 24.3 ± 0.1
11		86% 39%	268 ± 5	5.5 ± 0.2 25.6 ± 0.5	25.2 ± 0.1 24.1 ± 0.1



Taming the sulfonyl fluorides

ID	Structure	Thermal shift data ΔTm [°C] (30min/2hr incubation) XIAP-BIR3	DELFIA IC_{50} values [nM]		
			XIAP-BIR3	cIAP1-BIR3	cIAP2-BIR3
LCL161		9.8 ± 0.3 10.8 ± 0.1	48 ± 5	16 ± 2	10 ± 2
12		26.7 ± 0.2 26.5 ± 0.2	24.0 ± 0.7	22 ± 4	58.5 ± 0.1
13		27.0 ± 0.2 27.2 ± 0.1	15.6 ± 0.8	141 ± 15	492 ± 26



Stability and Cell Permeability of Sulfonyl Fluorides in the Design of Lys-Covalent Antagonists of Protein-Protein Interactions. Gambini L, Udompholkul P, Salem AF, Baggio C, Pellecchia M. *ChemMedChem*. **2020** Aug, in press

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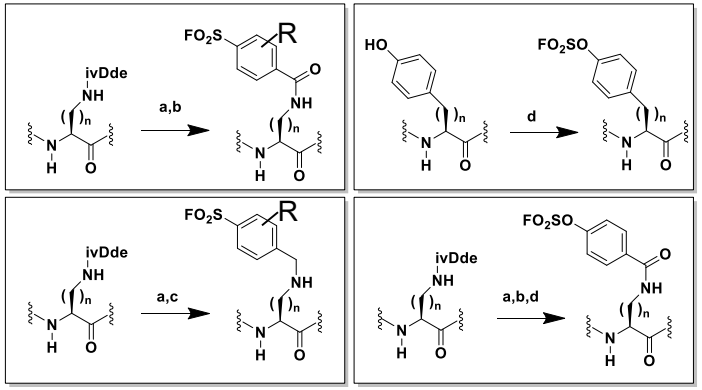
Design of potent covalent inhibitors of PPIs

HTS by NMR (general library)
fHTS by NMR (target specific library)

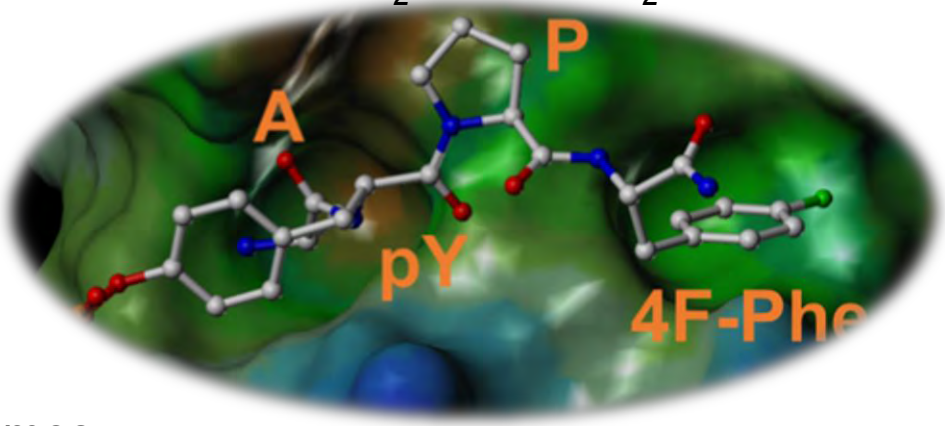
Identify initial peptide mimetic (Kd << 10 μM)

Systematic introduction of –SO_2F or –OSO_2F (library)



Design of covalent PPIs antagonists

Structural studies and design of –SO_2F or –OSO_2F derivatives



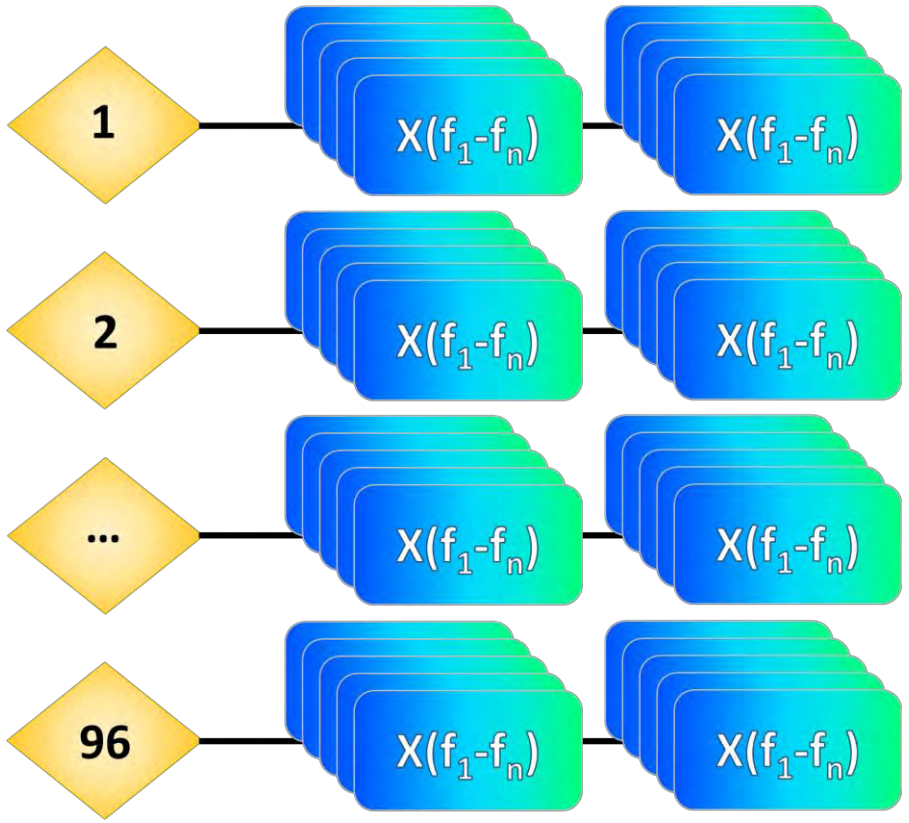
Target-based evaluations

- IC_{50} at different incubation times
- Measurements of Thermal Shifts
- Mass spec analysis
- SDS Gel Electrophoresis
- Single point mutations and verifications

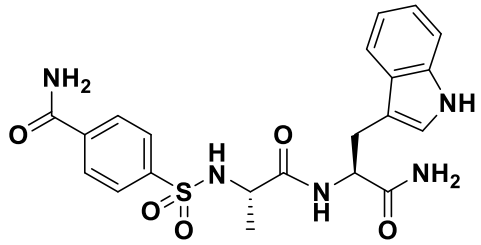
Summary

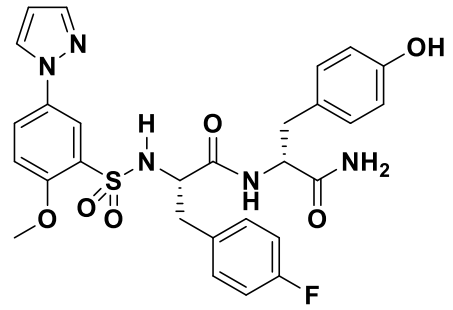
- NMR based screening of POS combinatorial libraries (HTS by NMR) can be powerful in the identification and optimization of potent and selective peptide mimetics and lead agents.
- Expanding the method to focused POS combinatorial libraries using an anchoring moiety (*f*HTS by NMR) allows rapid identification of low micromolar to nanomolar hits.
- Introduction of proper aryl-sulfonyl-fluorides or aryl-fluoro-sulfates can lead to potent, selective, cell permeable (pharmacologically viable) covalent agents targeting Lys and Tyr residues (expanding the target space for covalent drugs).
- *cov*HTS by NMR with fluorosulfates ? pY as a probe for Lys?

HTS by NMR – new sulfonamide based library



Examples of elements of the library:





MW **400 +/- 100**
cLogP **1 +/- 3**
HB donors and acceptors < 10

Possible combinations: 96 x 36 x 36 = ~ 124,000 agents

To be tested: **96 MIXTURES** (36 x 36 = 1,296 agents)

Baggio, Alboreggia, *et al.* Pellecchia, under investigation

UCR | School of Medicine

Thank you!

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