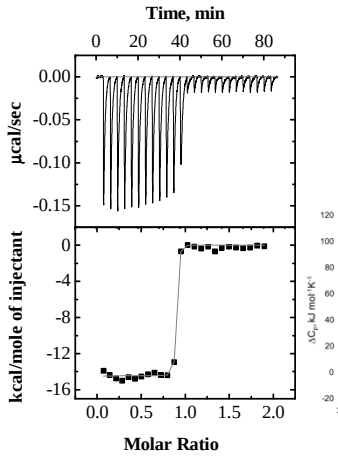
Biothermodynamics & Drug Design Dept.
Vilnius University
Institute of Biotechnology

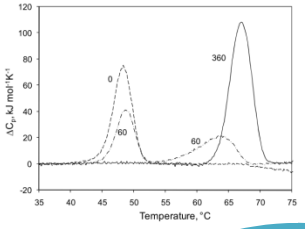
Synthetic compound binding to anticancer drug target proteins: thermodynamics and structure of interaction

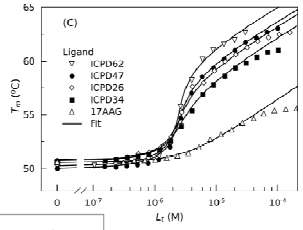
Asta Zubrienė

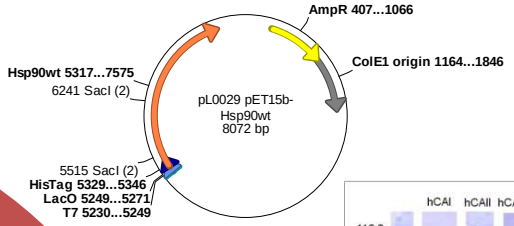
Department of Biothermodynamics and Drug Design
Vilnius University Institute of Biotechnology

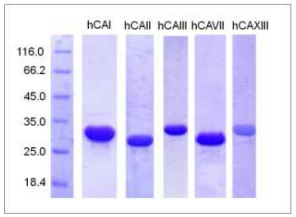
The department profile

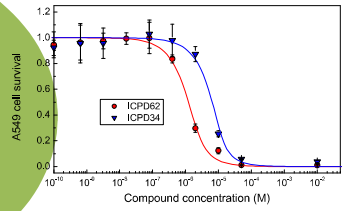


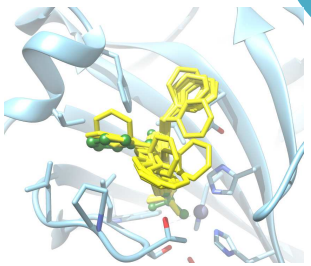


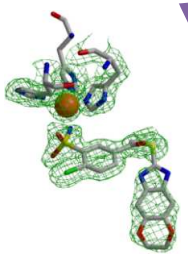


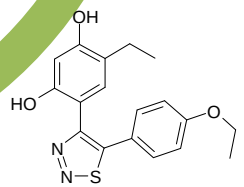


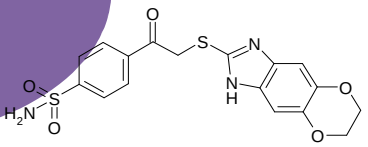


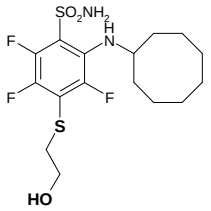












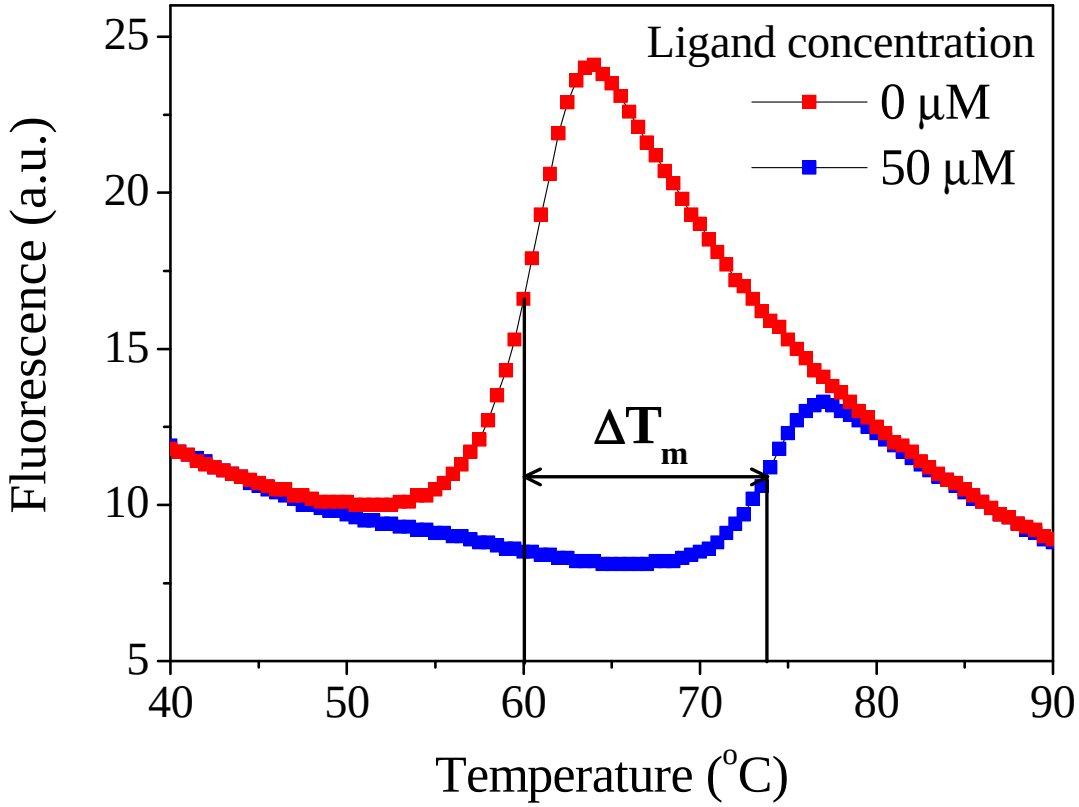
Department designs inhibitors of anticancer drug targets:
carbonic anhydrases, Hsp90 chaperone, histone deacetylases (HDACs, sirtuins)

Outline

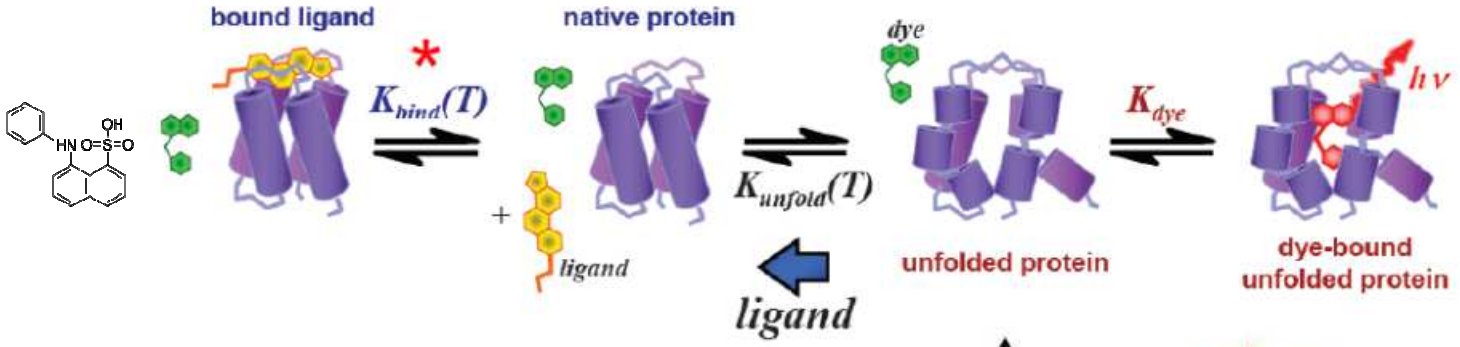
- Protein - ligand interactions: biophysical methods
- Anticancer targets: carbonic anhydrase (CA) and Hsp90
- Discovered inhibitors, selectivity, biological data
- Observed/intrinsic binding parameters
- Structure - binding thermodynamics correlations

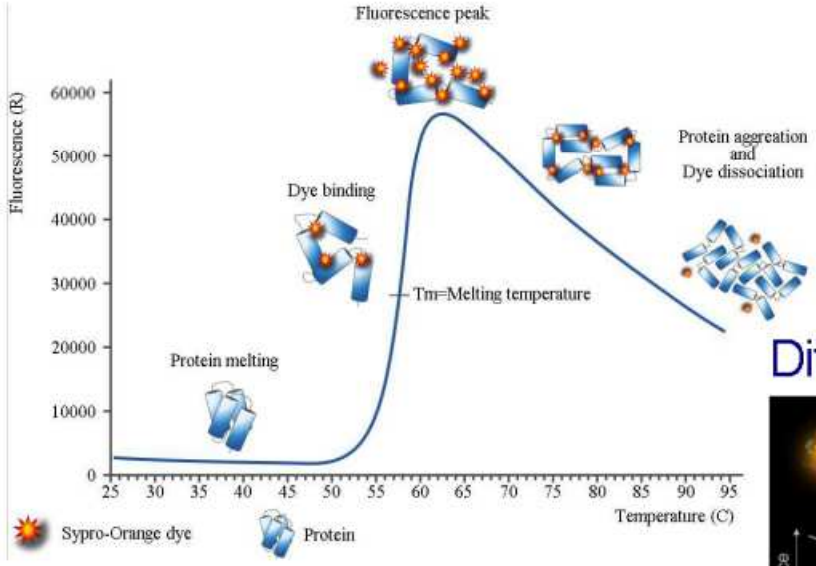
Protein - compound binding assays

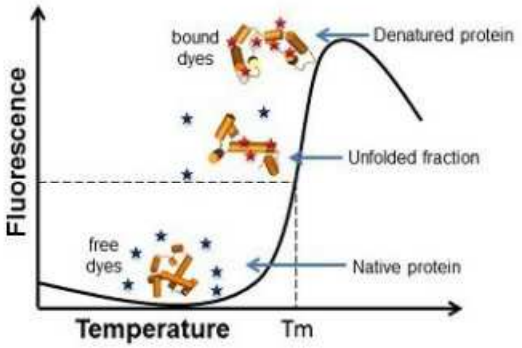
Fluorescent thermal shift assay (ThermoFluor, FTSA)



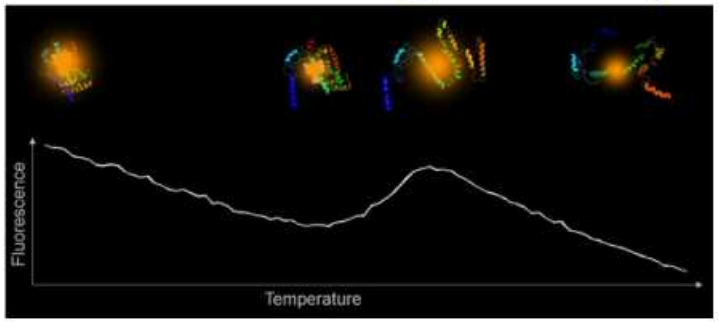
FTSA principle in the literature





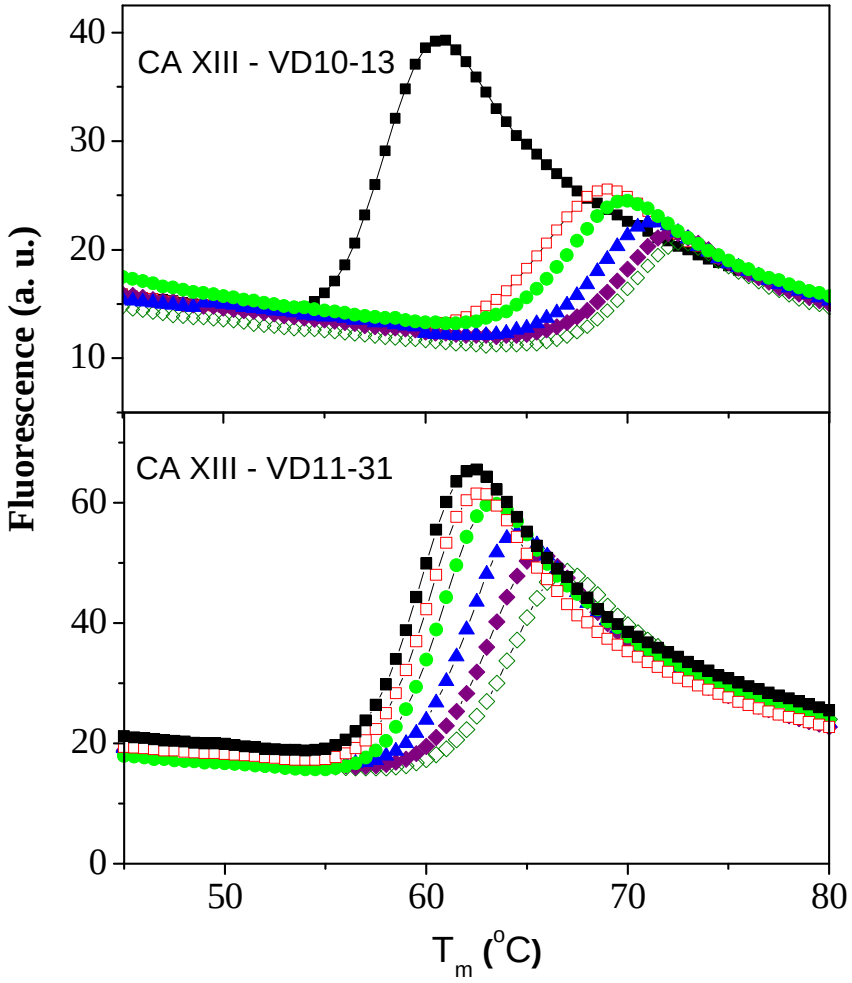


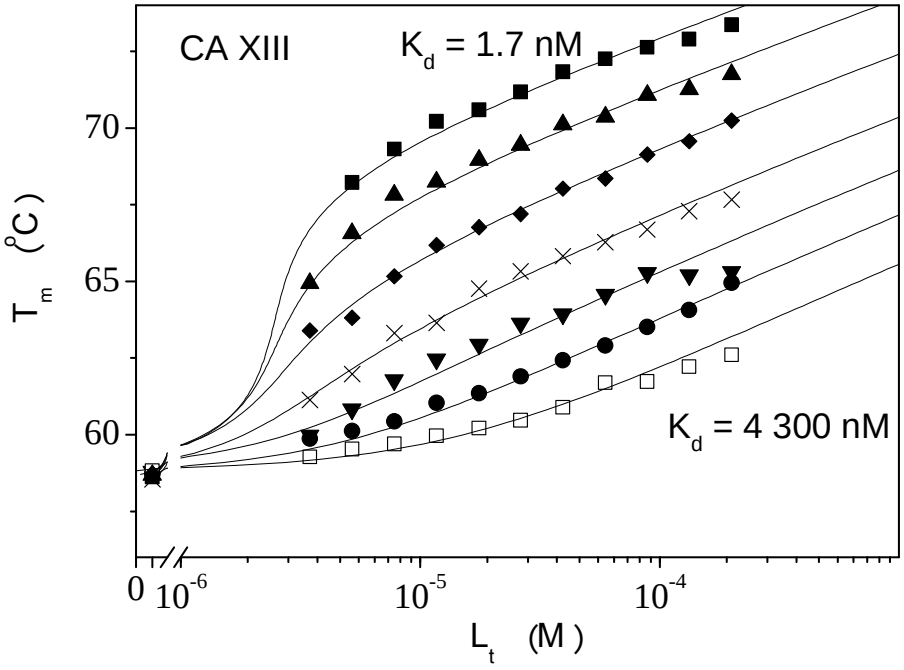
Differential Scanning Fluorimetry



We perform FTSA in rotating RT-PCR Rotorgene apparatus

Strong vs Weak Ligand Dosing Curves

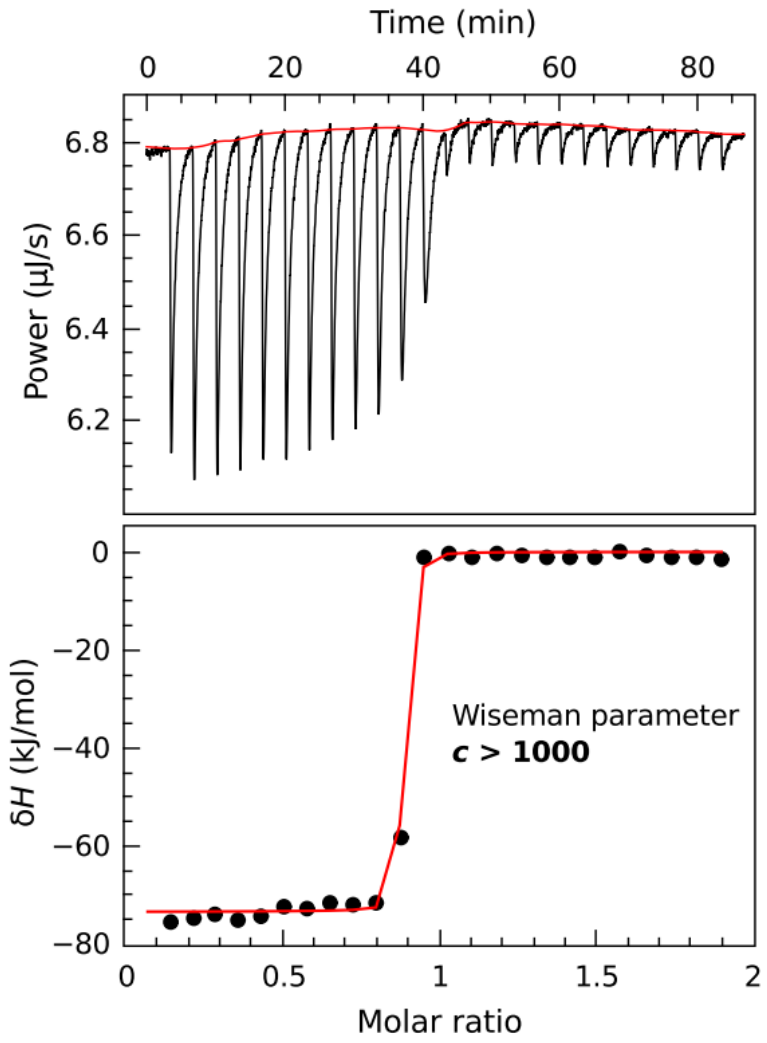




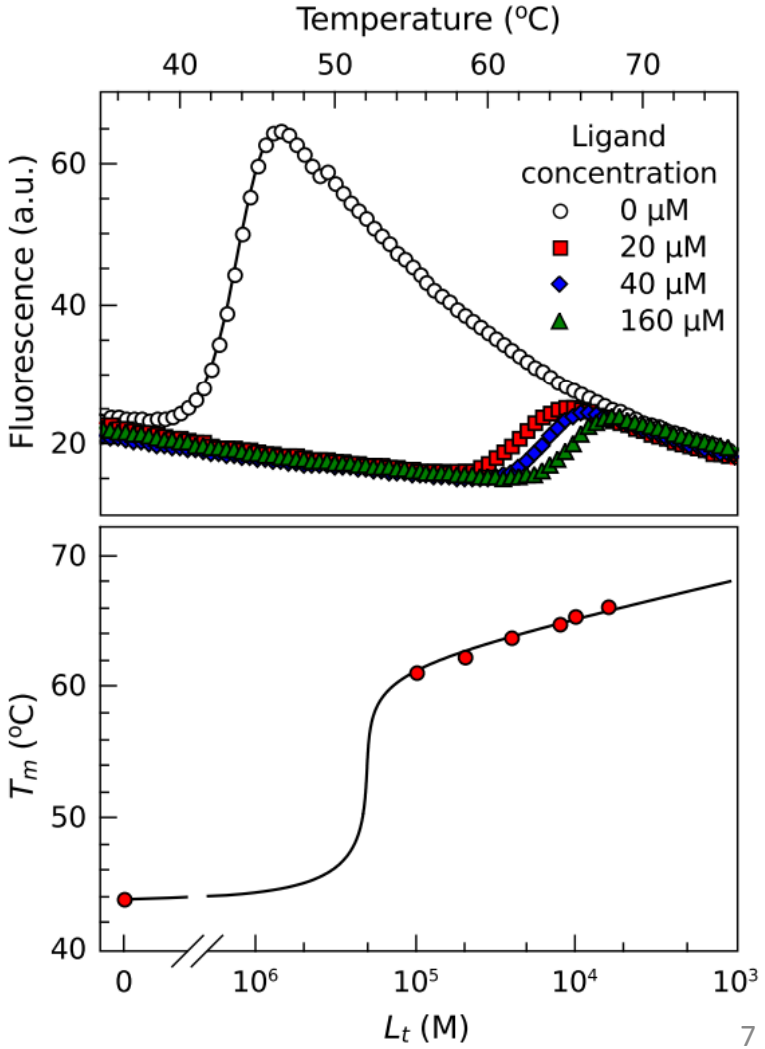
Wide range of affinities by FTSA

Advantage of FTSA over ITC: no limit in affinity

ITC experiment:

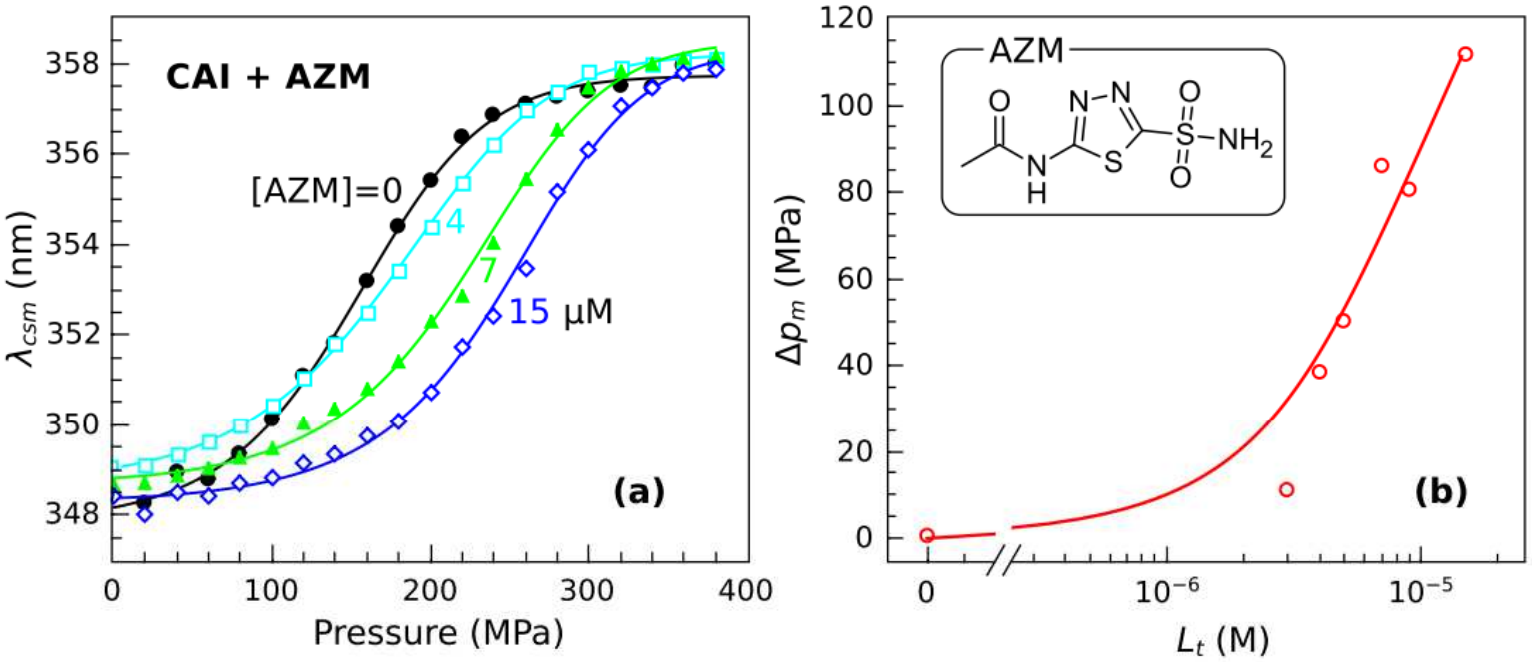


FTSA experiment:



Fluorescent Pressure Shift Assay (FPSA)

The shift in P_m and dosing curves



$$\boxed{K_b} = \exp\left(-\frac{\Delta G_b}{RT}\right) = \left(-\frac{\Delta G_{0_b} + \Delta V_{0_b}(p_m - p_0) + \frac{\Delta \beta_b}{2}(p_m - p_0)^2}{RT}\right)$$

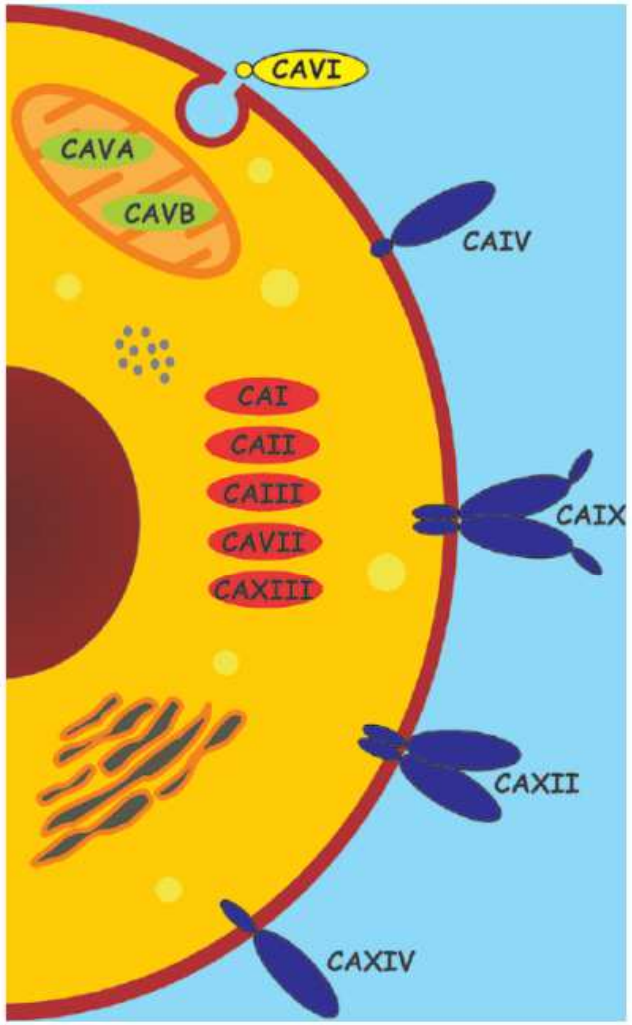
ISS PC1 photon counting spectrofluorimeter equipped with a high-pressure cell connected to a hydrostatic pump

Human carbonic anhydrases (CA) and diseases

Human carbonic anhydrases (CA)

- Humans have 12 active CA isoforms
- The isoforms are involved in numerous physiological and pathological processes: cancer, glaucoma, obesity, epilepsy…
- CA catalyzes the reaction:

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$$



Alterio et al., *Chem. Rev.* 112 (2012), 4421

Human CA IX antibody PET scan

- CA IX is specifically expressed in clear cell variant of renal cell carcinoma (ccRCC)
- Metastases correlate with CA IX expression
  - CAIX is not expressed in the majority of normal tissues, only in gastrointestinal tract.

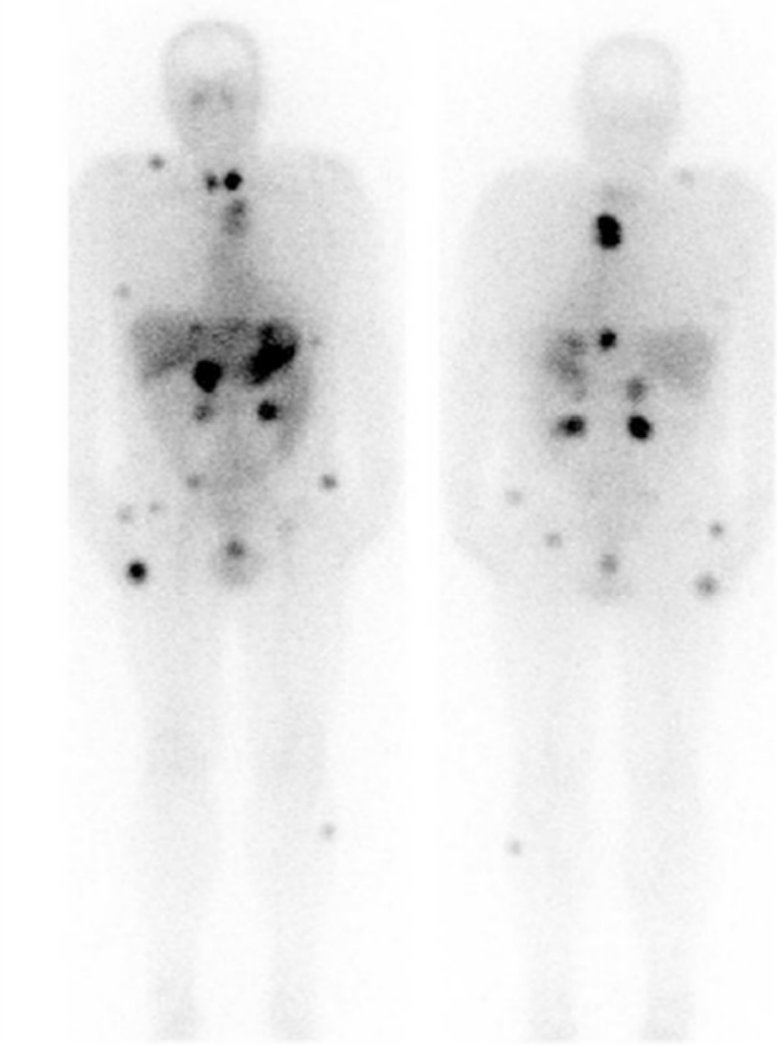
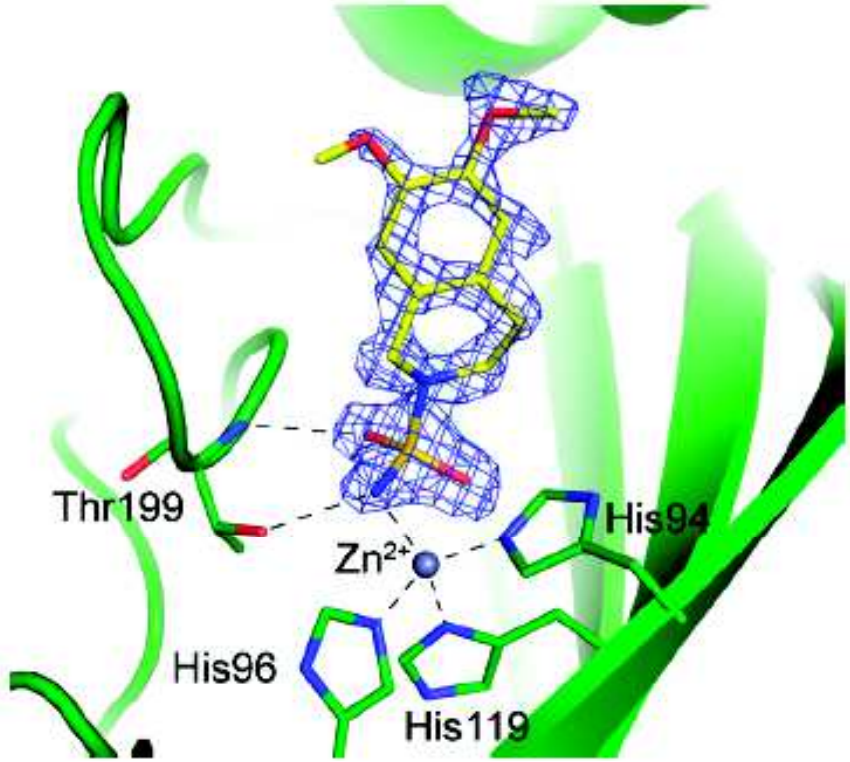


Fig. 10.3 Typical image of patient with mRCC injected with mAbG250, 5 days post-injection. Please note the clear imaging of metastatic lesions and absence of positive images of CAIX-positive organs

Oosterwijk et al, 2013

Structure of CA inhibitors

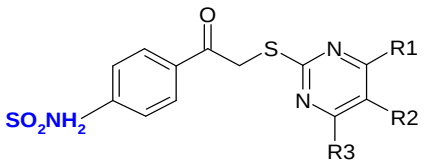


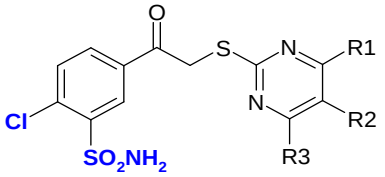
- Majority of CA inhibitors are sulfonamides, they bind to the Zn atom in the active site.
- Development of potent, highly selective inhibitors against CA IX remains an unmet need in anticancer therapeutics.

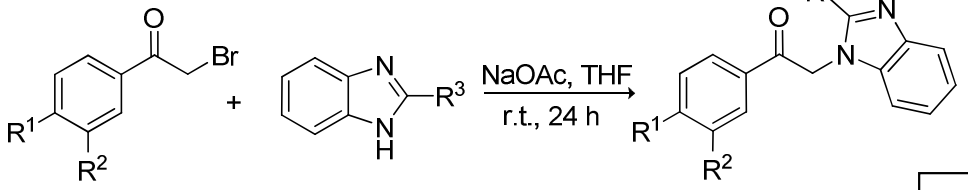
Ligands synthesized in laboratory

~ 400 benzensulfonamide derivatives of different classes during 10 years

Edita Capkauskaite



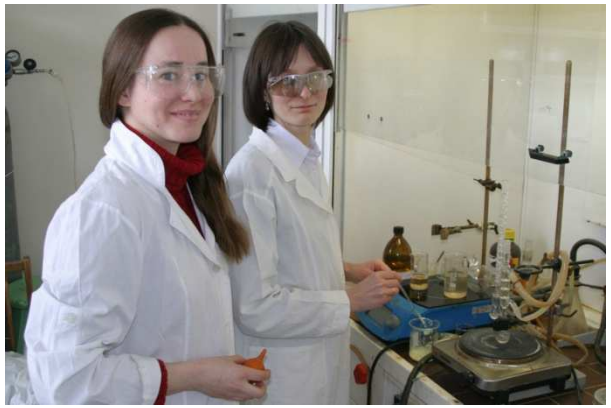




1-3 **a-j** **1-3(a-j)**

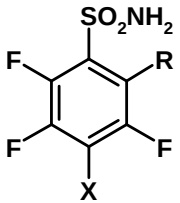
1: R^1=SO_2NH_2, R^2=H
2: R^1=SO_2NH_2, R^2=Cl
3: R^1=H, R^2=SO_2NH_2

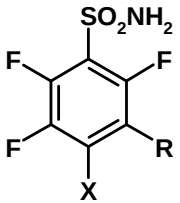
a: R^3=H
b: R^3=Me
c: R^3=CH_2OH
d: R^3=Bn
e: R^3=Et
f: R^3=Pr
g: R^3=Bu
h: R^3=*i*-Pr
i: R^3=*i*-Bu
j: R^3=SMe

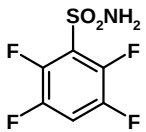


Virginija Dudutiene

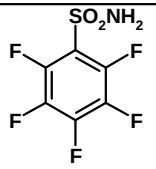
Fluorinated benzenesulfonamides







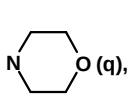
1



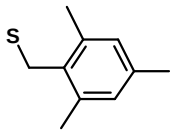
2

3 (a-w)

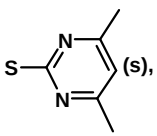
R= $NHNH_2$(a), NHN=CHPh(b), SCH_2CH_2OH (c), $SO_2CH_2CH_2OH$(d), $SCH_2CH_2CH_3$(e), SCH_2COOH(f), SCH_2CH_2COOH(g), $NH(CH_2)_5COOH$ (h), SPh(i), SO_2Ph(j), OPh(k), SCH_2Ph(l), $NHCH_2Ph$(m), $NHCH_2CH_2PhpOH$(n), SCH_2CH_2Ph(o), $SO_2CH_2CH_2Ph$(p),



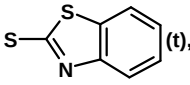
(q),



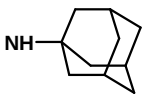
(r),



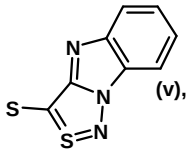
(s),



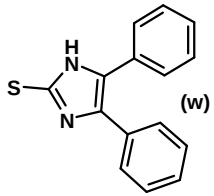
(t),



(u),



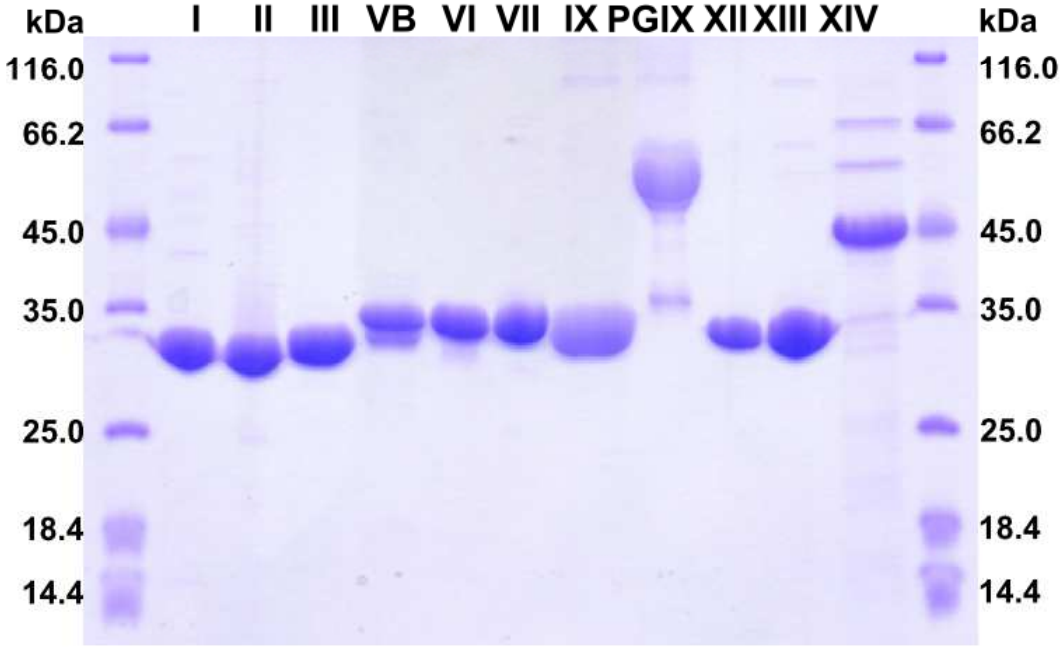
(v),



(w)

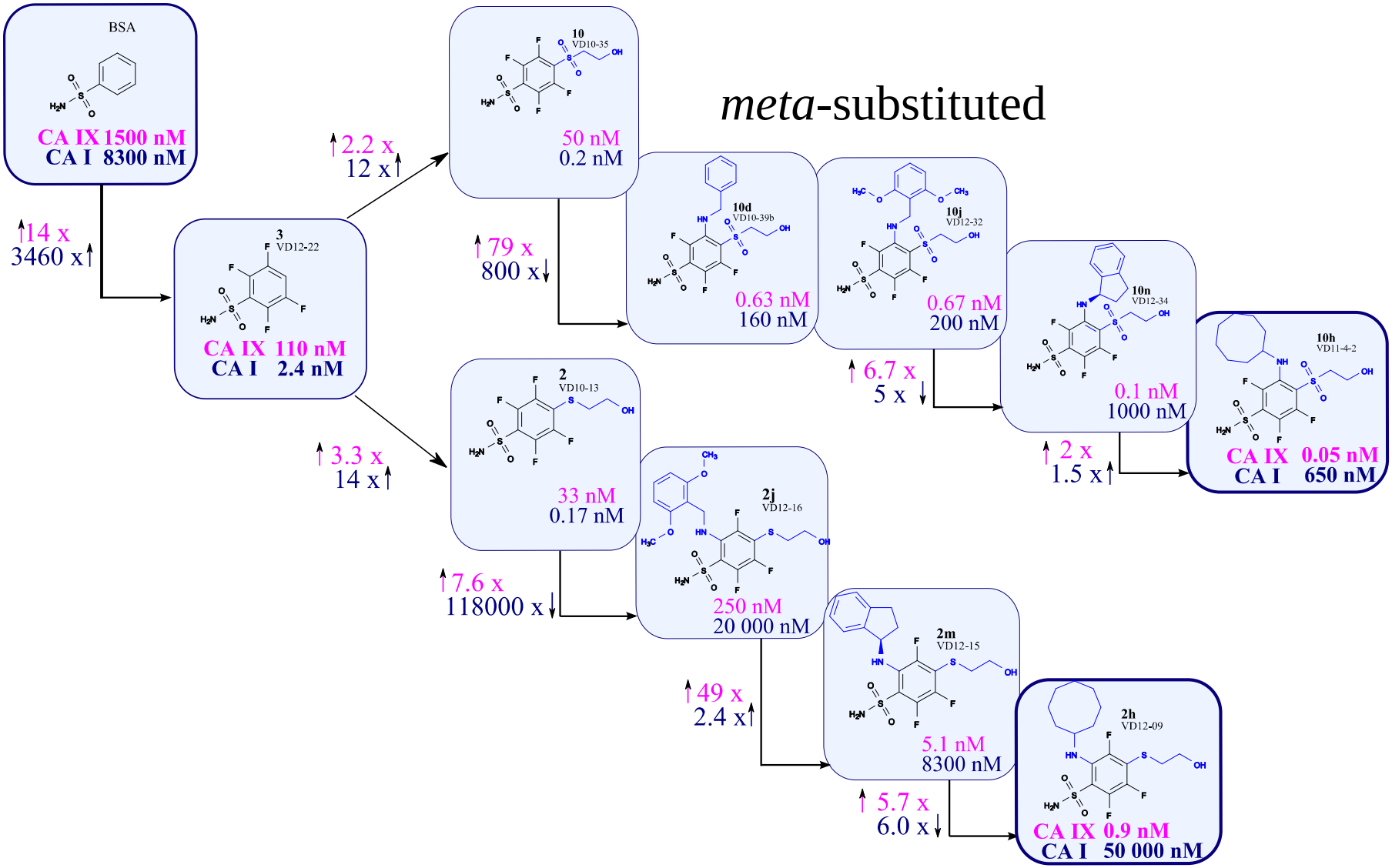
All CAs were produced in the laboratory

- CA I, II, III, IV, VA, VB, VI, VII, XII, XIII and XIV were expressed in Escherichia coli
- CA IV, VI, IX and XII are available from mammalian cell (HEK 293) culture
- Proteins were purified by chromatography, using IMAC, ion-exchange, CA-specific affinity methods

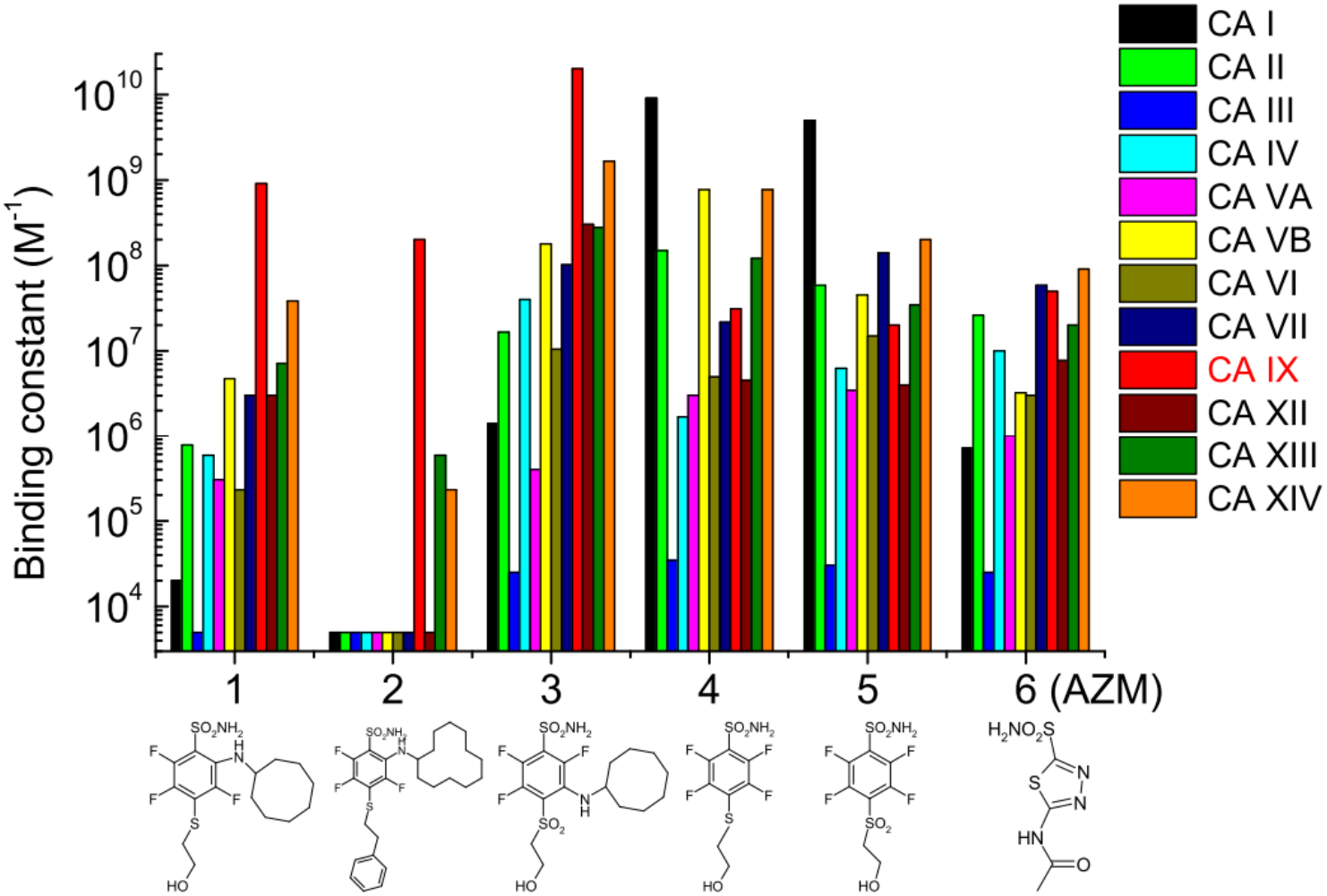


Discovery of CA isoform selective inhibitors

CA IX inhibitor optimization μM→pM

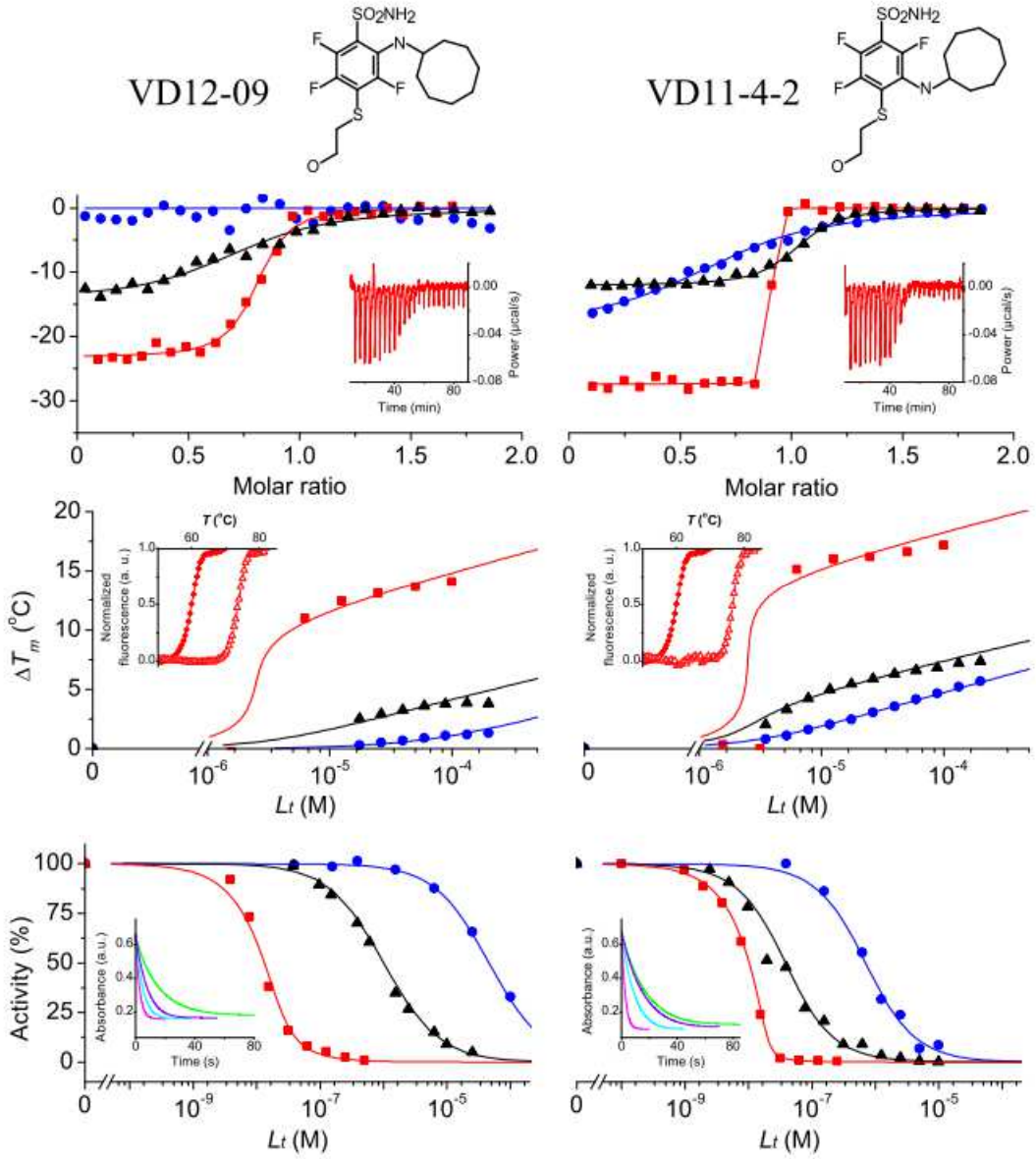


CA IX selective inhibitors

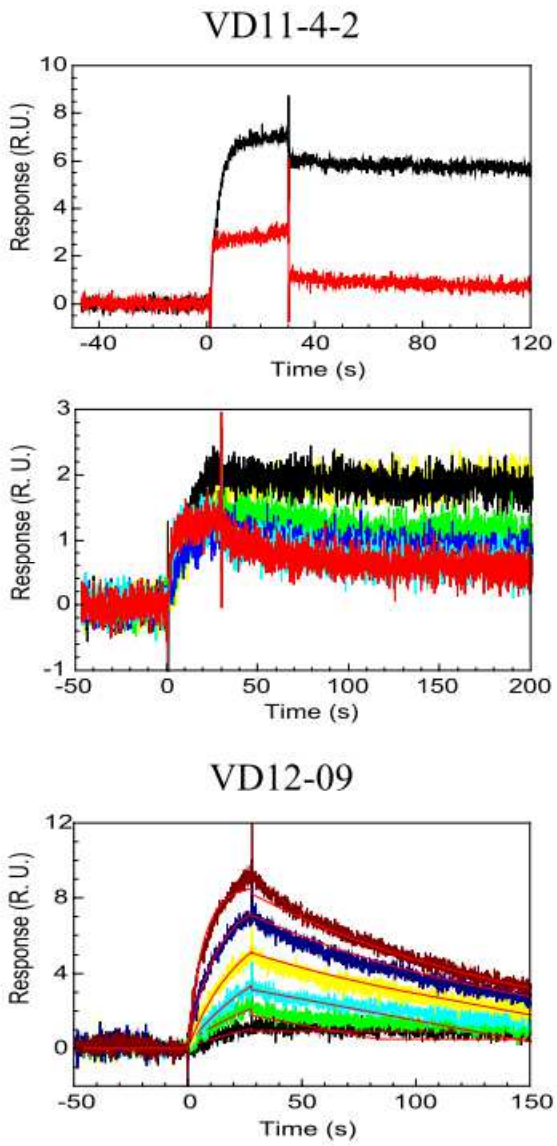


Dudutiene et al., J. Med. Chem. 57 (2014) 2435–46

Selective CA IX inhibitors



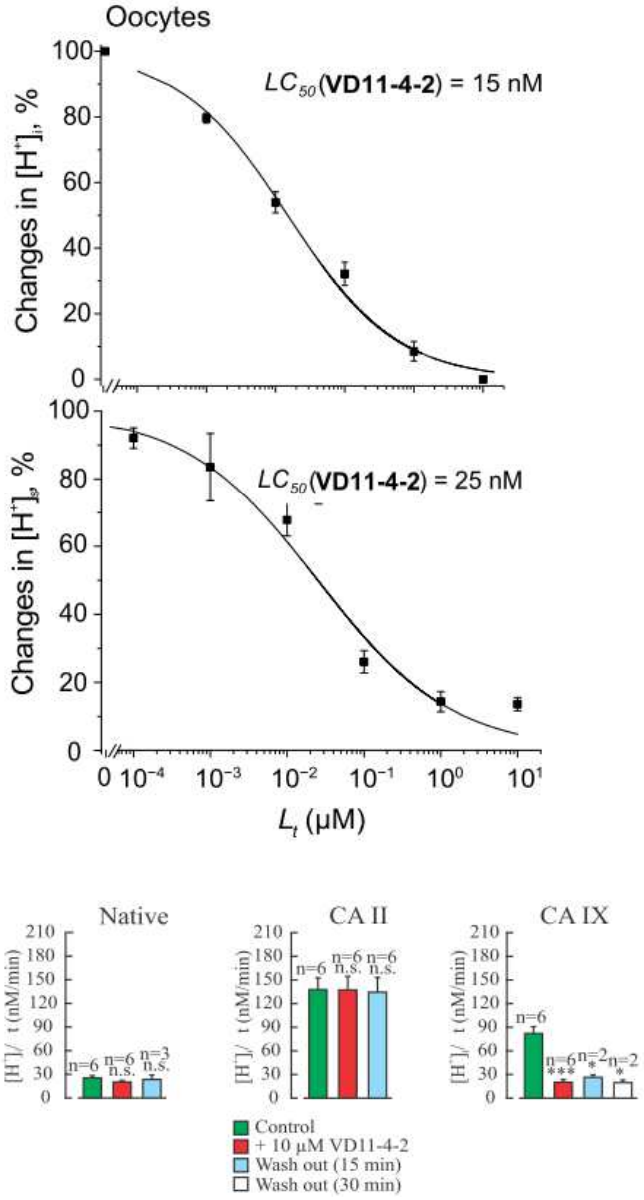
Dudutiene et al., J. Med. Chem. 57 (2014)

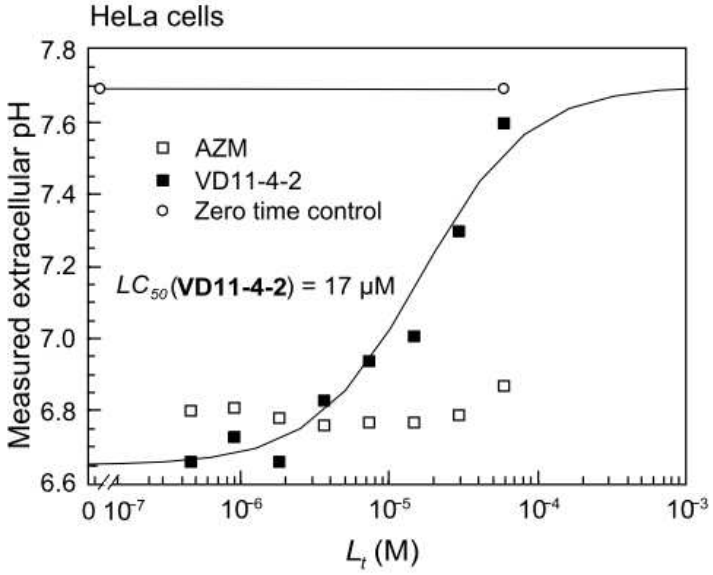


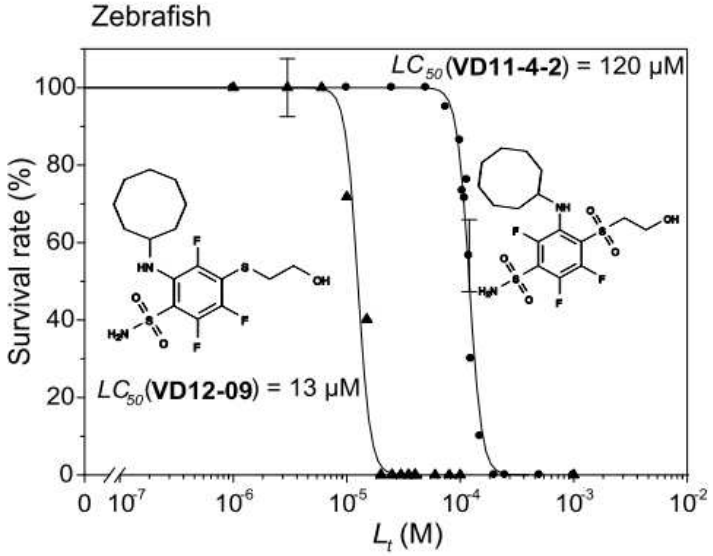
Talibov et al., J. Med. Chem. 59(5) (2016)

Biological assays

Selective CA IX inhibitors in Xenopus, Zebrafish and HeLa cell culture

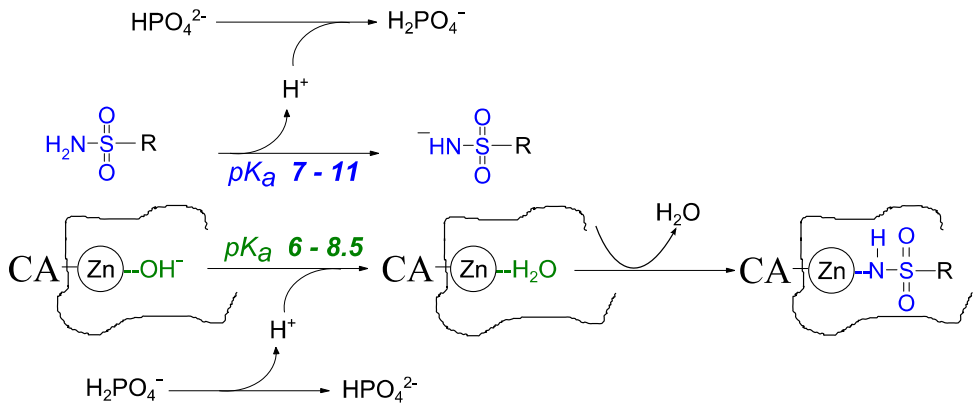






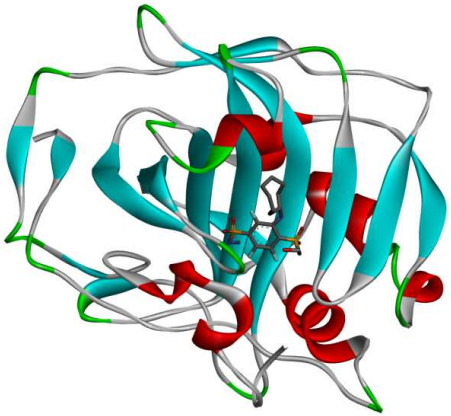
Linked protonation reactions – distinguishing between observed and intrinsic thermodynamic parameters

Observed *vs* intrinsic



observed		**binding-linked events**		**intrinsic**
thermodynamic parameters $K_{b\_obs}, \Delta_b H_{obs}, \Delta_b S_{obs}$	—	structural rearrangements protonation	=	thermodynamic parameters $K_b, \Delta_b H, \Delta_b S$

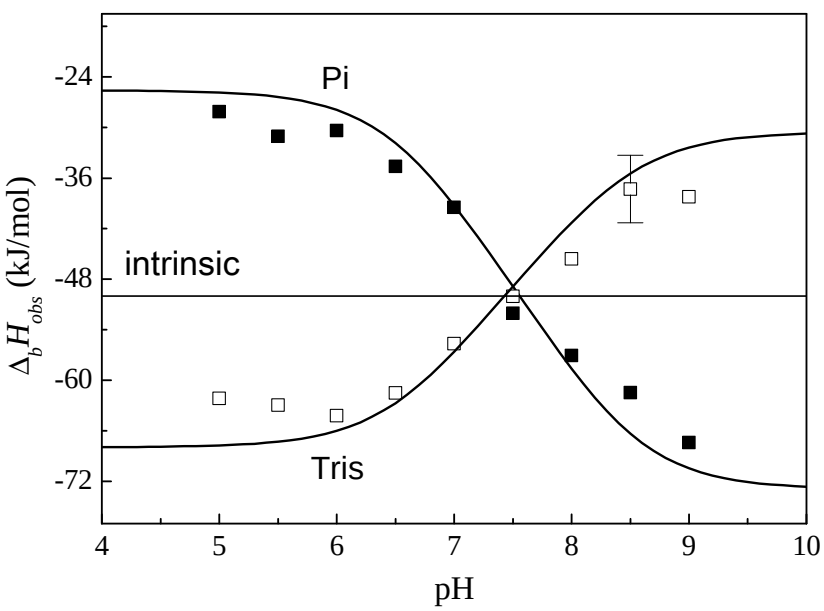
structural data

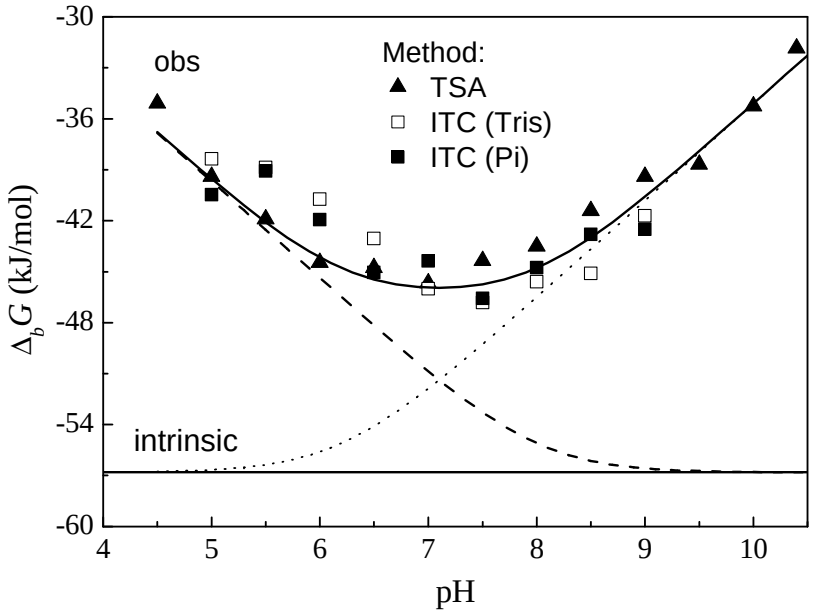


Only intrinsic thermodynamic parameters can be correlated with protein-ligand crystal structures

Observed ΔH and ΔG as a function of pH and buffer

CA XII-EZA ITC





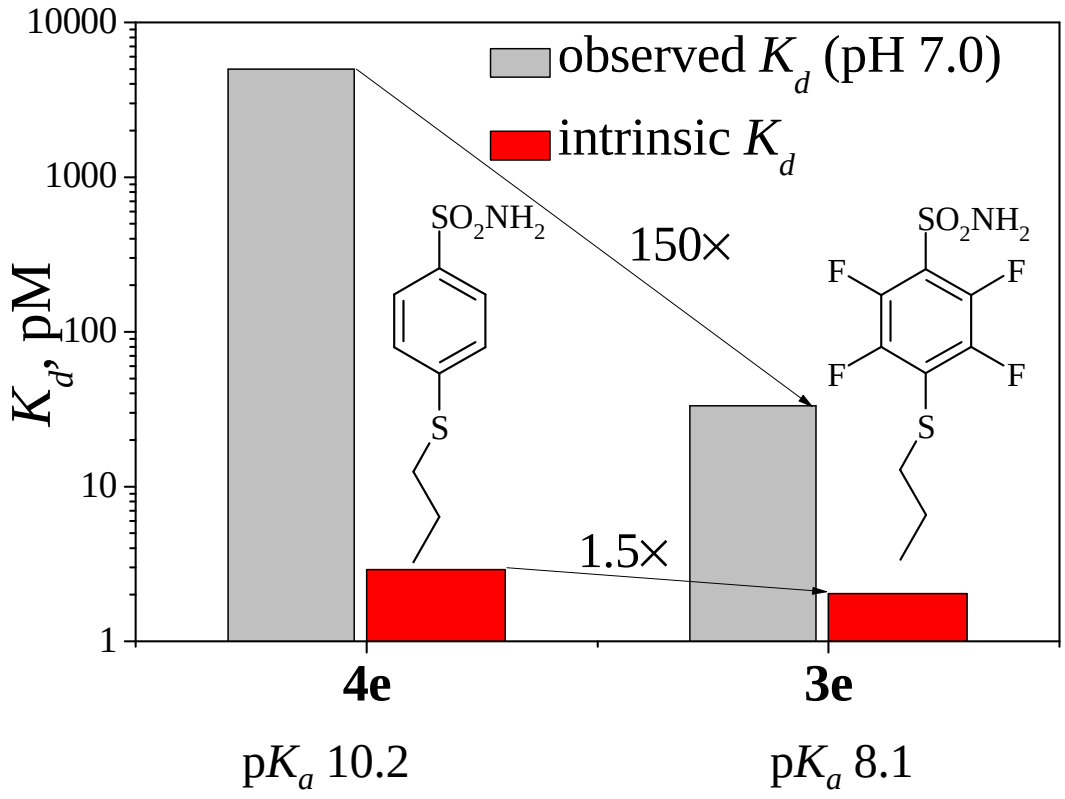
$$K_b = \frac{K_{b\_obs}}{(f_{RSO_2NH^-}\, f_{CAZn\,H_2O})}$$

$$\Delta_b H = \Delta_b H_{obs} - n_{sulf}\Delta_{b\_proton\_sulf} H - n_{CA}\Delta_{b\_proton\_CA} H + n_{buf}\Delta_{b\_proton\_buf} H$$

Observed thermodynamics describe the whole binding event

Observed vs intrinsic parameters

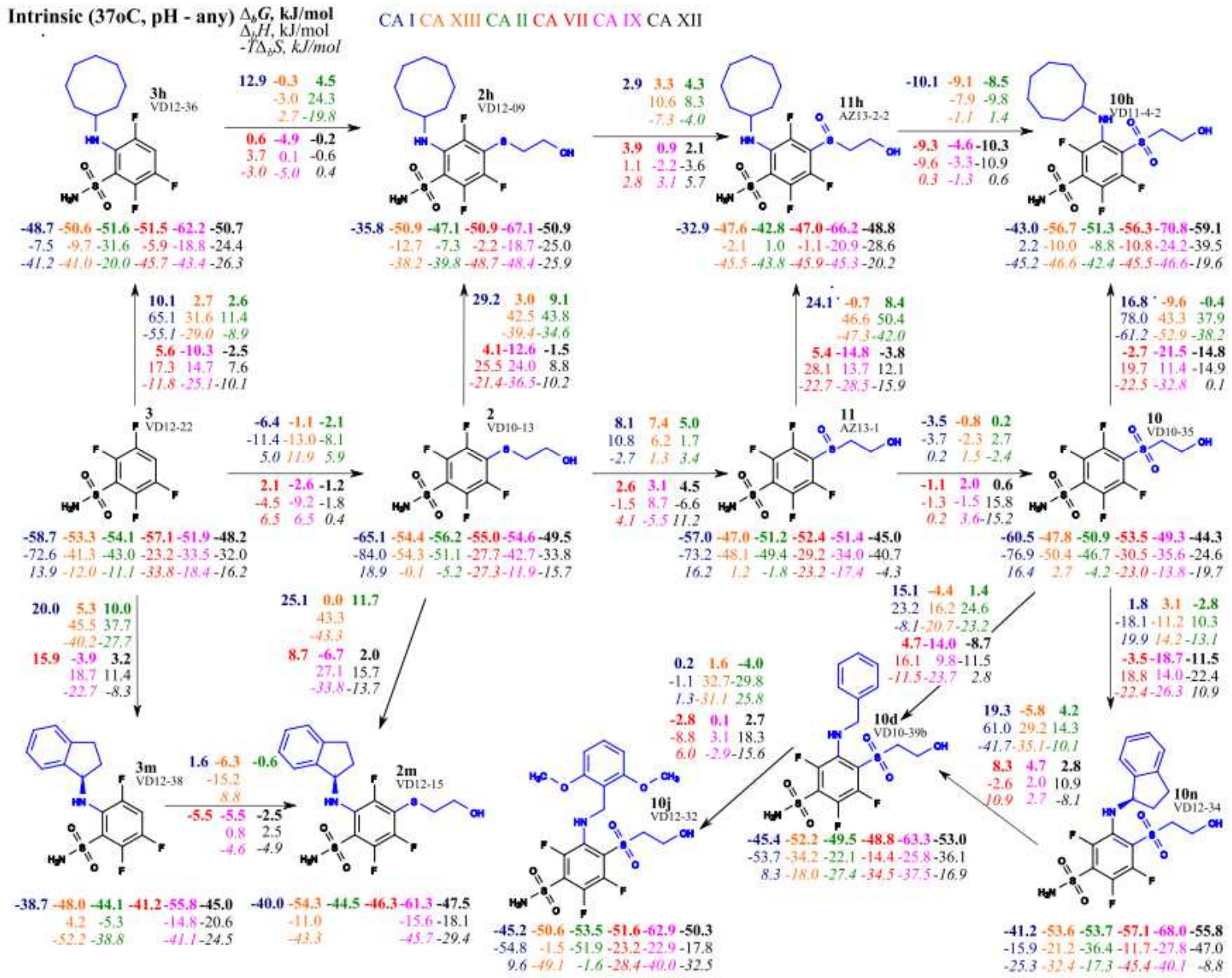
CA I



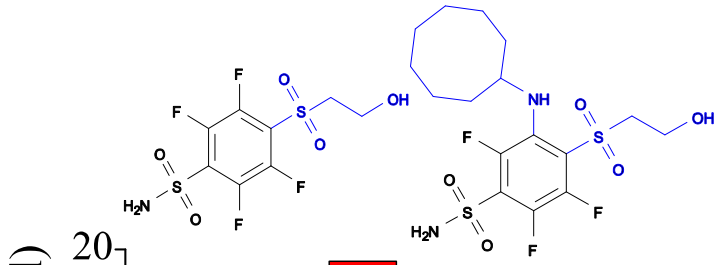
Fluorine does not affect the (intrinsic) affinity

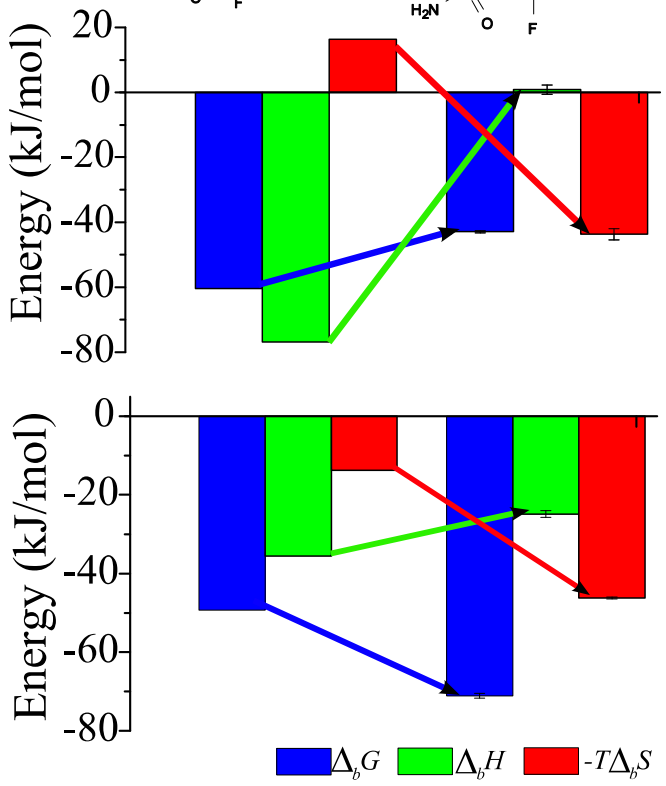
ITC experiments that are performed without examination of protonation effects to the binding process can lead to the improper interpretation of the results

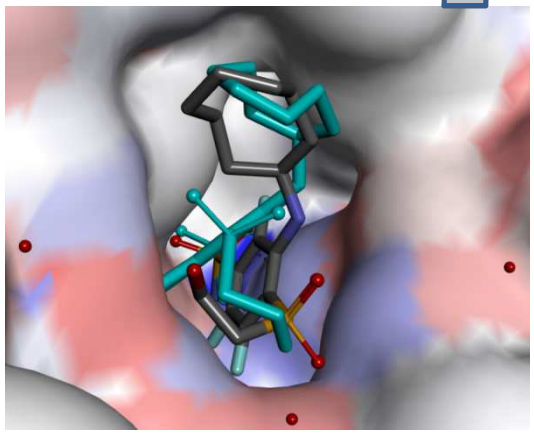
Compound structure – thermodynamics correlation map

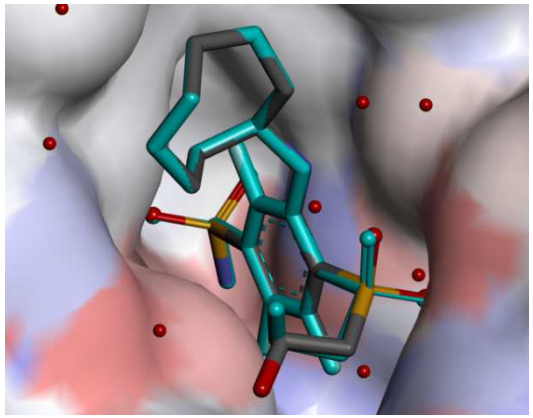


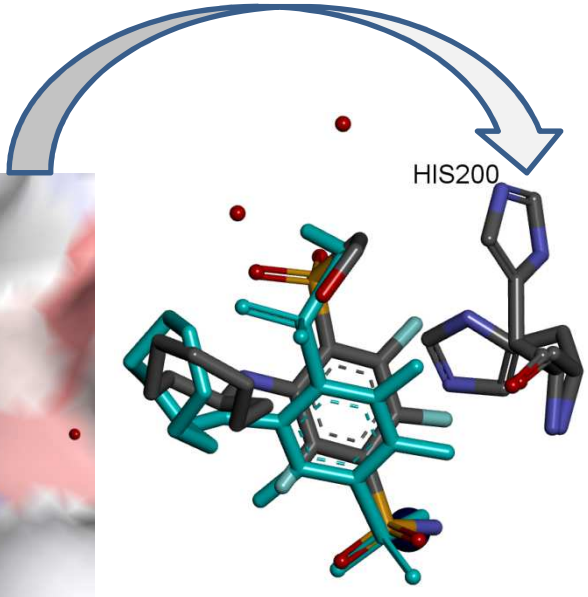
X-ray structure – thermodynamics correlation





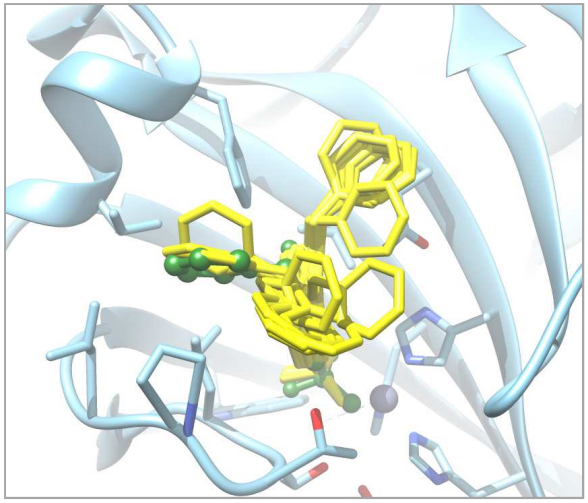


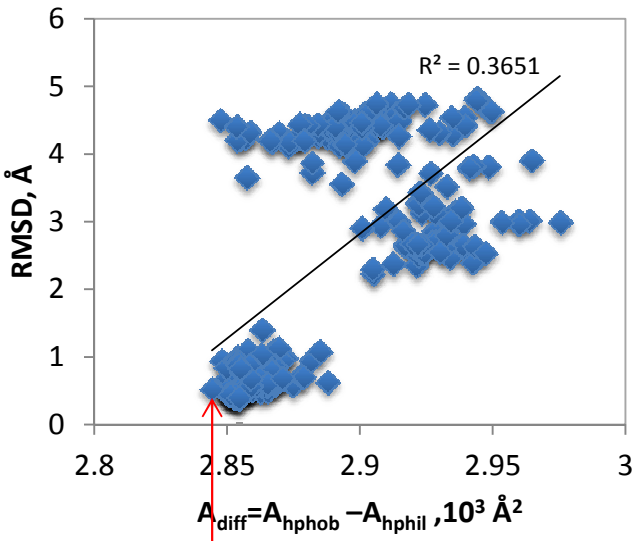




More-in-depth understanding of binding reaction

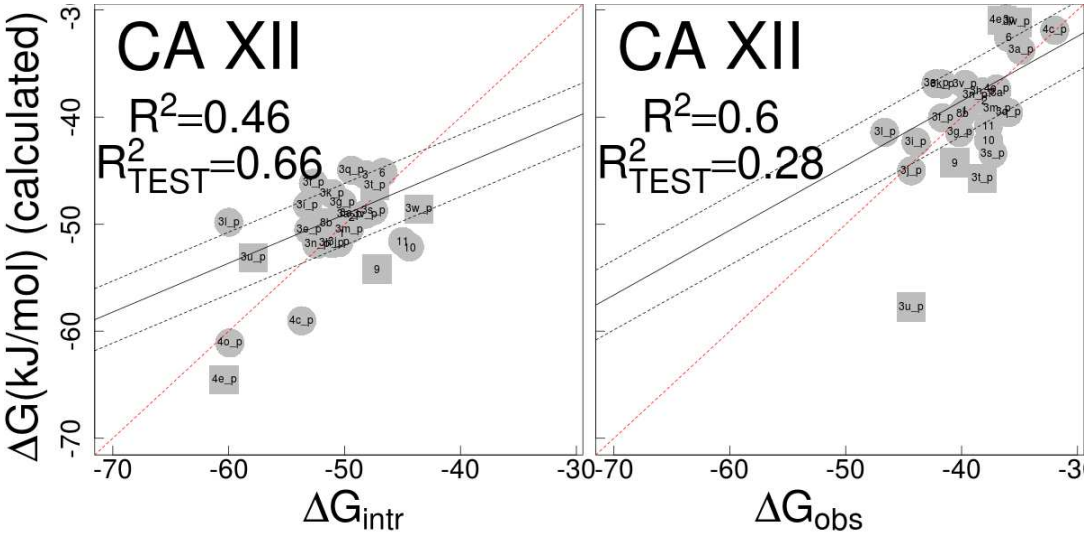
Molecular modeling and QSAR





The lowest A_{diff} gets the right conformation!

Docking often has difficulty putting the "tail" in the right position

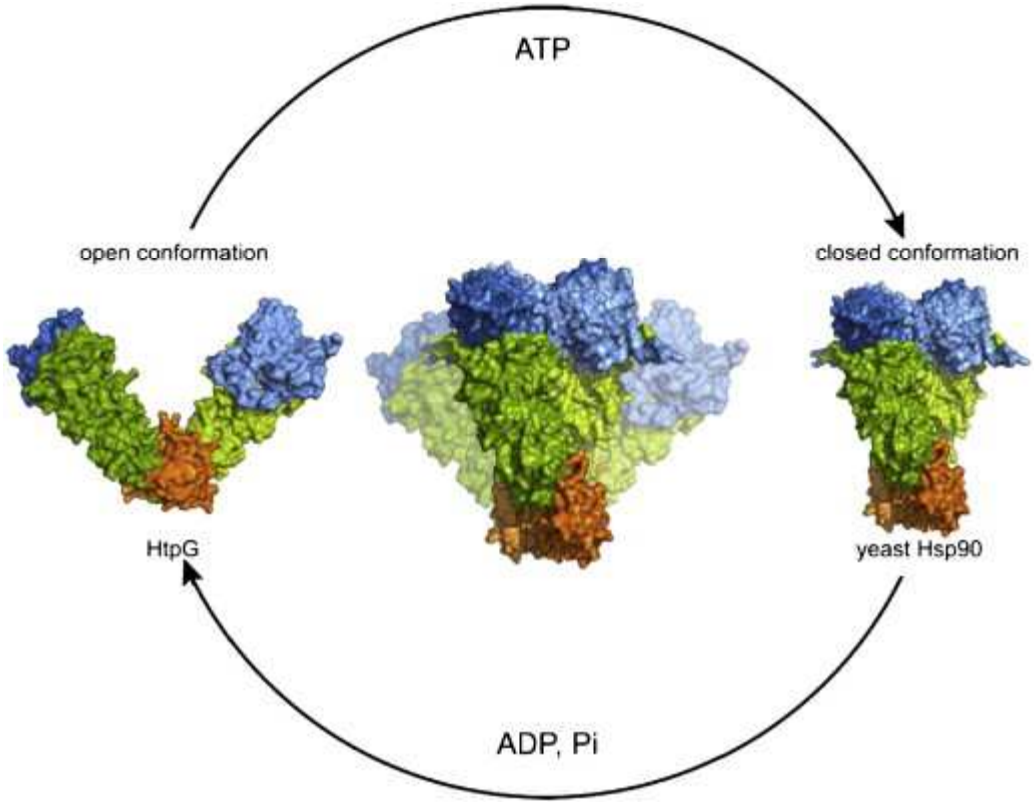


Better correlation between calculated/intrinsic than calculated/observed binding energies

Hsp90 as an anticancer target

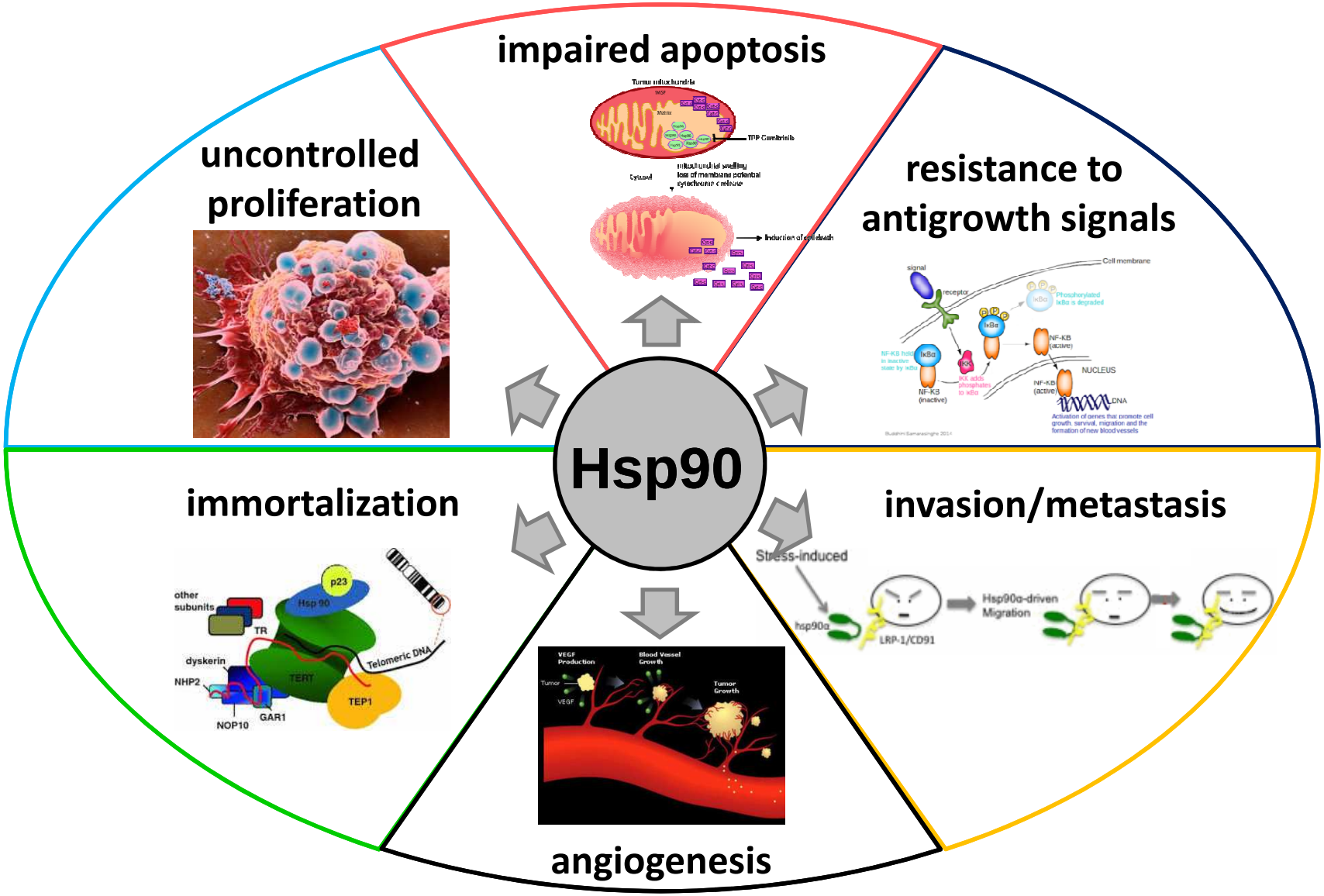
Hsp90

- molecular chaperone
- specialised clientele (kinases, TF, …)
- required for folding and/or function
- ATP-dependent
- cytosolic isoforms:
  - inducible Hsp90α
  - constitutive Hsp90β



Li, Soroka, Buchner, 2012, *Biochimica et Biophysica Acta*

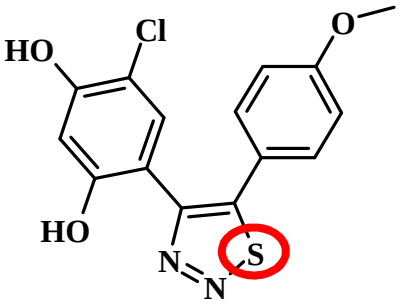
Hsp90 – at the hub of cancer



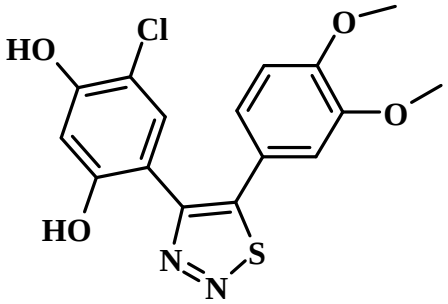
Promising target for anticancer drug research

Hsp90 inhibitors of ICPD series

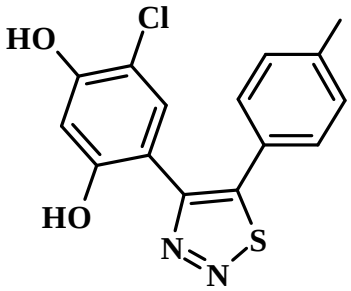
ICPD 26



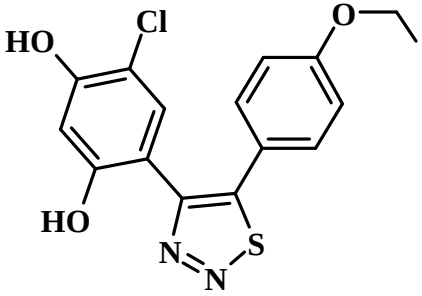
ICPD 34



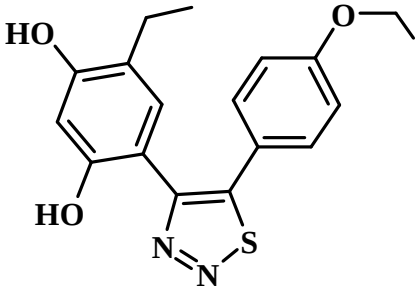
ICPD 39

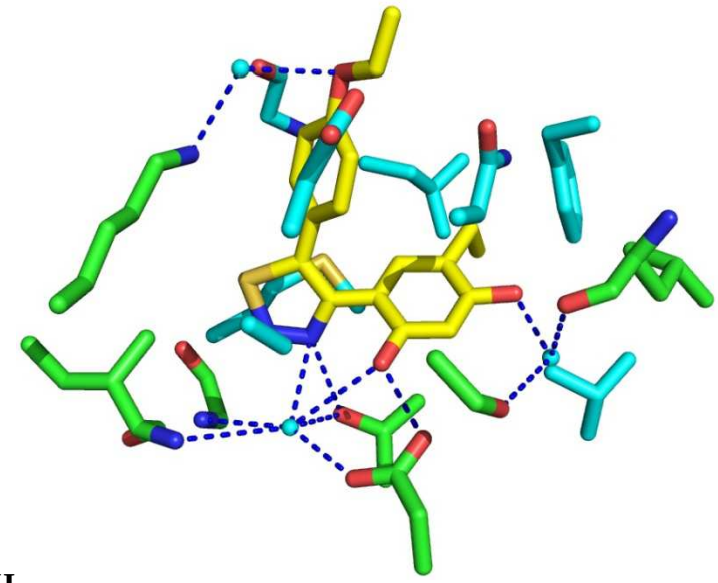


ICPD 47

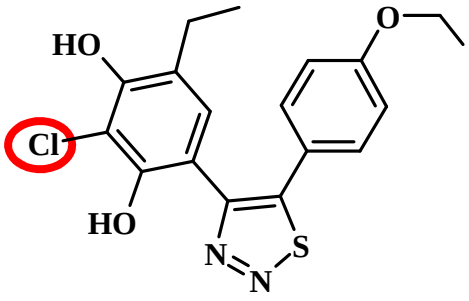


ICPD 62

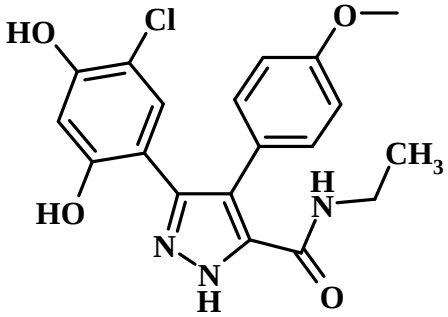




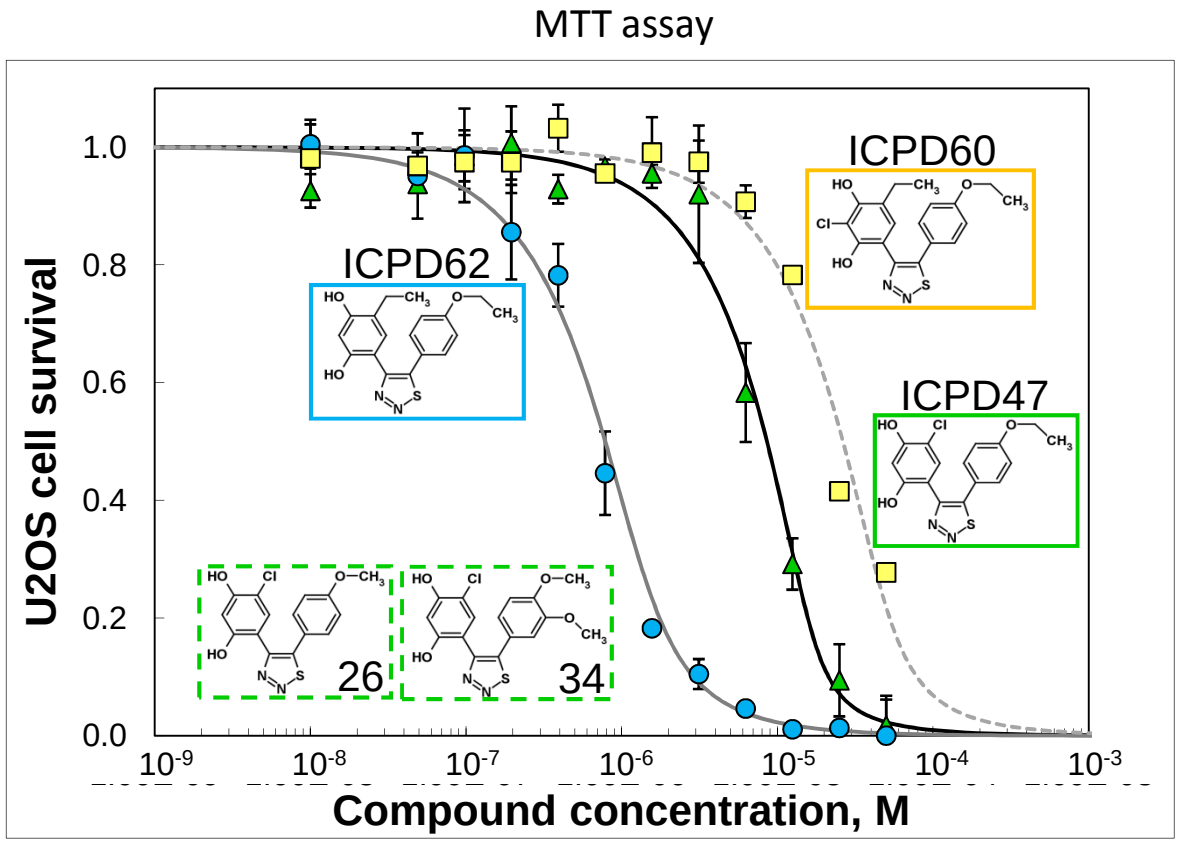
ICPD 60

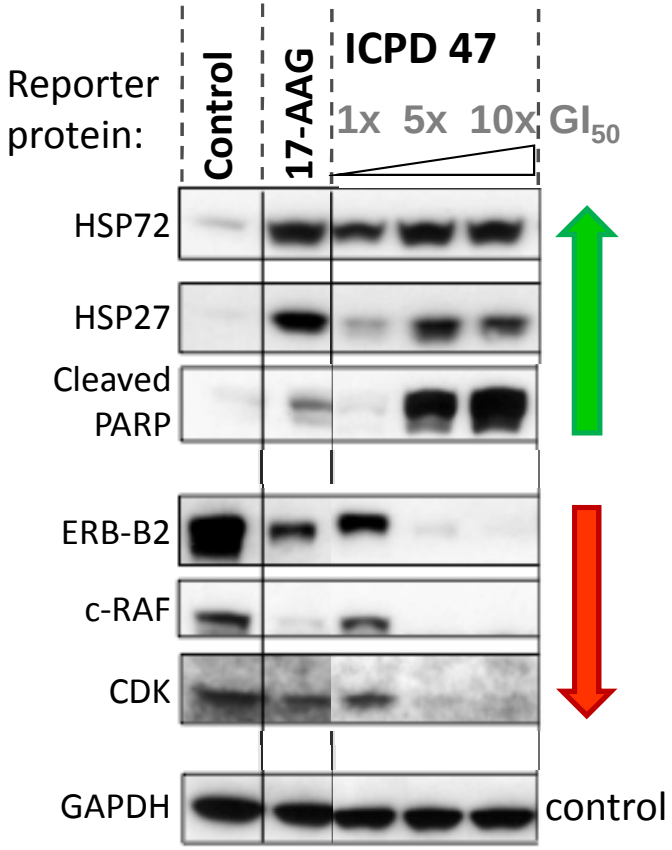


ICPD 72 / VER-49009



ICPD inhibits Hsp90 in cells





dr. S.Y. Sharp *(Institute of Cancer Research, United Kingdom)*

Conclusions

- It is important to distinguish observed from intrinsic binding parameters
- All binding and inhibition assays provide only the observed thermodynamic parameters
- Only intrinsic parameters may be correlated with structure
- A series of Hsp90 and CA IX inhibitors were designed using biothermodynamic and structural methods with the potential to be developed as anticancer drugs

Acknowledgment

Thank you for your attention!