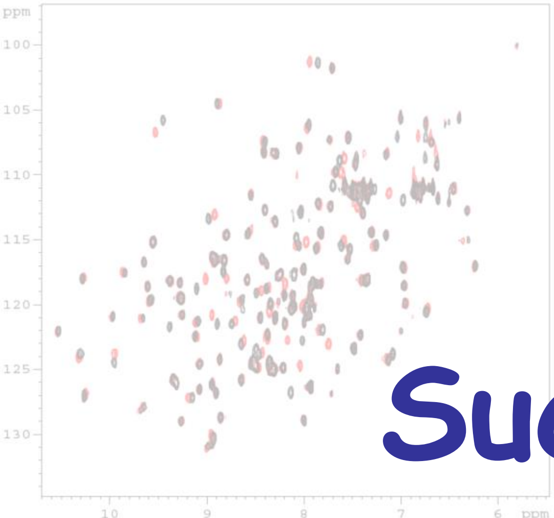
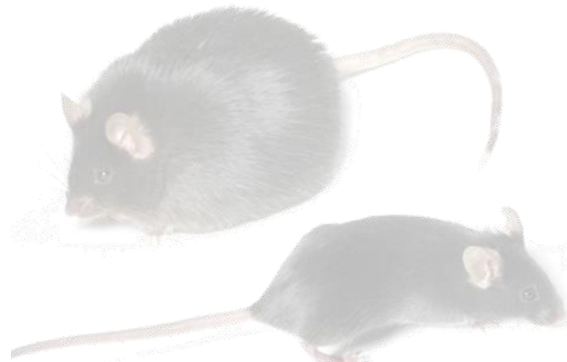
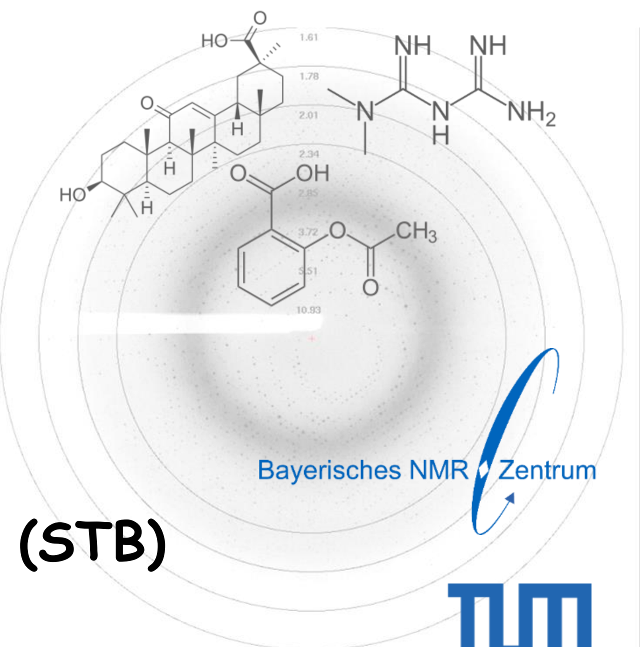




Success stories of structure-based drug discovery



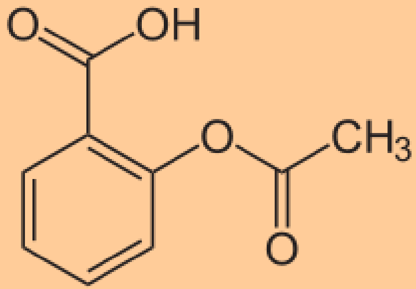
Ana Messias

Institute of Structural Biology (STB)

HelmholtzZentrum münchen
German Research Center for Environmental Health

Technische Universität München

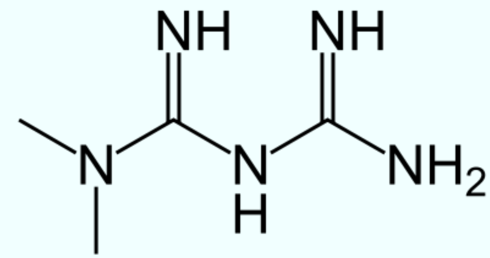
11.10.17



acetylsalicylic acid

(ASPIRIN – Bayer 1853, **1899)**

- antipyretic
- analgesic
- anticoagulant
- pro-drug
- commercially, the most successful drug ever

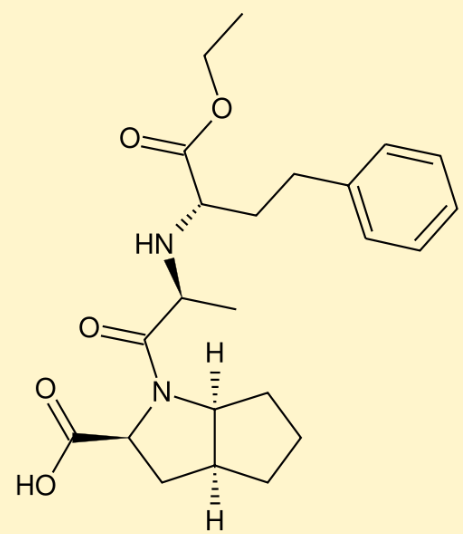


metformin

(**Glucophage** – Rona 1922, **1958**)

- antidiabetic

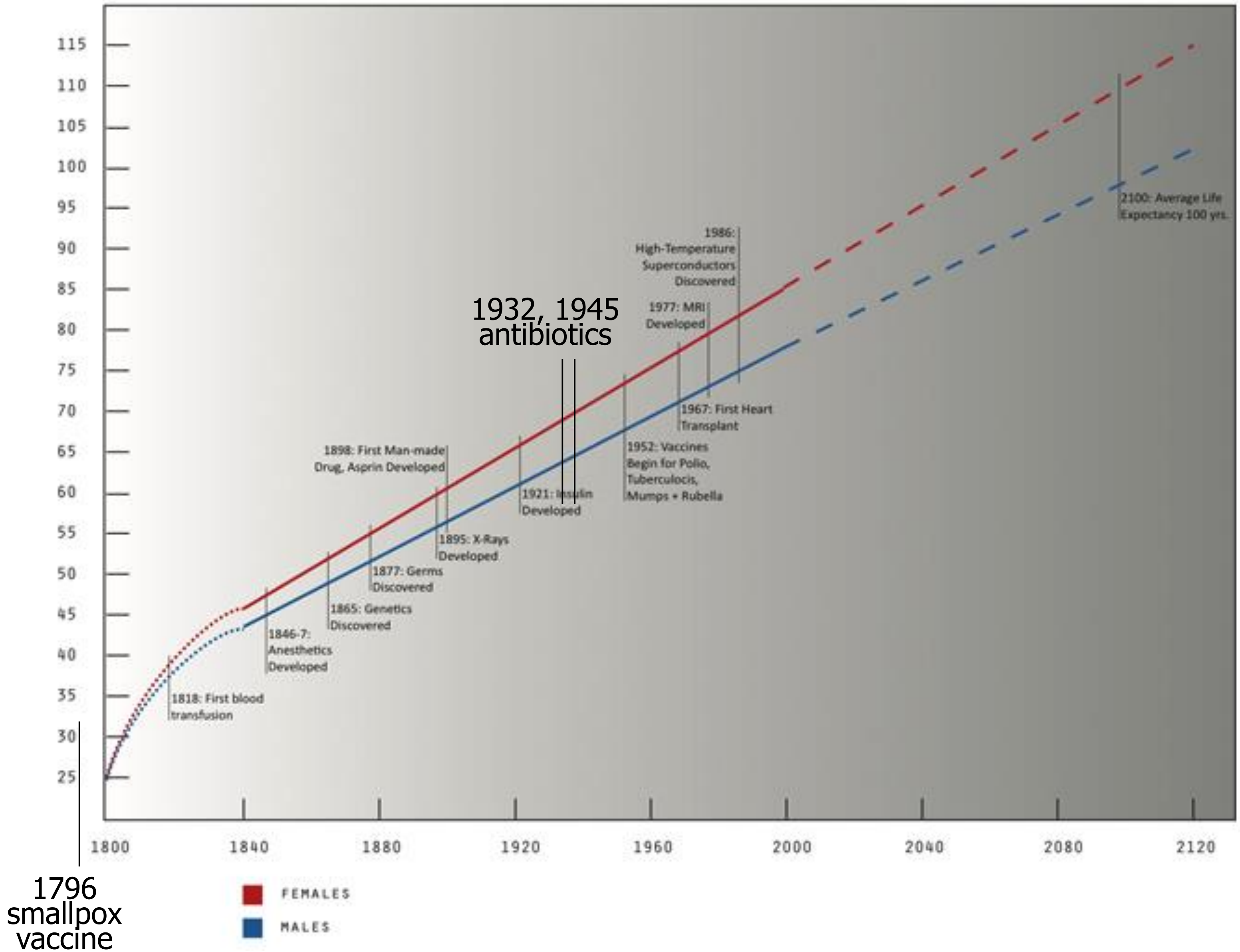
- antihypertensive
- inhibits Angiotensin Converting Enzyme (ACE)
- pro-drug
- designed from viper snake venom



Ramipril

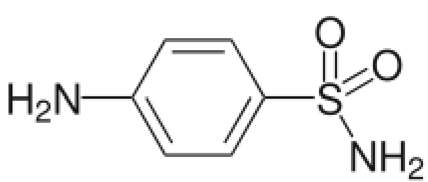
(Altace – Aventis 1991, **1991)**

Discovery and life expectancy



History of the Food & Drug Administration (FDA)

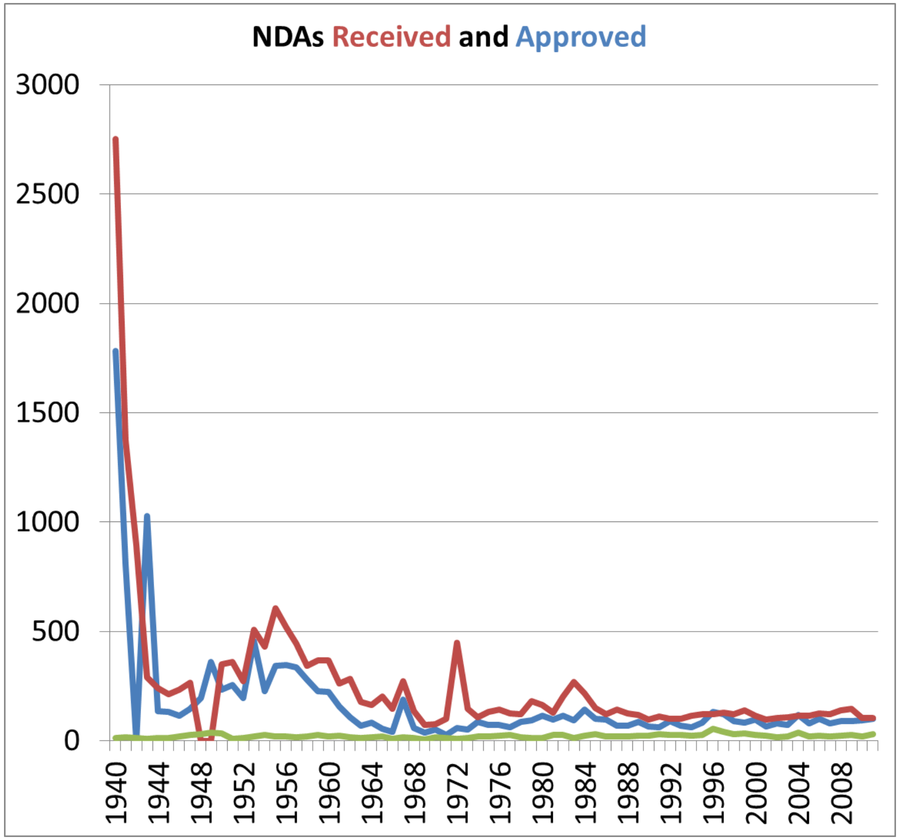
- **1906 Food and Drugs Act** prohibited adulteration or misbranding of pharmaceuticals. Premarket approval of drugs not required - commercialization of hazardous or useless drugs were not prevented.
- **1937** sulfanilamide formulation with untested solvent killed more than 100 people.
- **1938 Food, Drug, and Cosmetic Act** - evidence of **drug safety** required.
- **1962** – required evidence of **effectiveness** through adequate **clinical trials**.

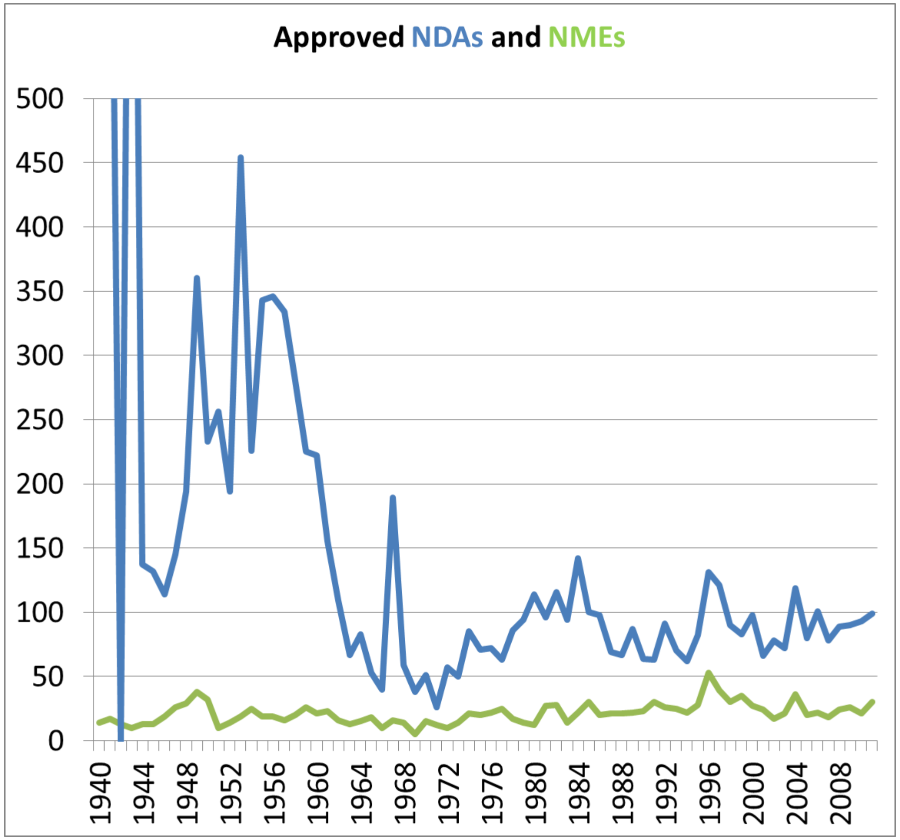


Sulfanilamide
Antibacterial agent used widely during WW2

Chemist Lee Geismer looking over an NDA in the 1960s

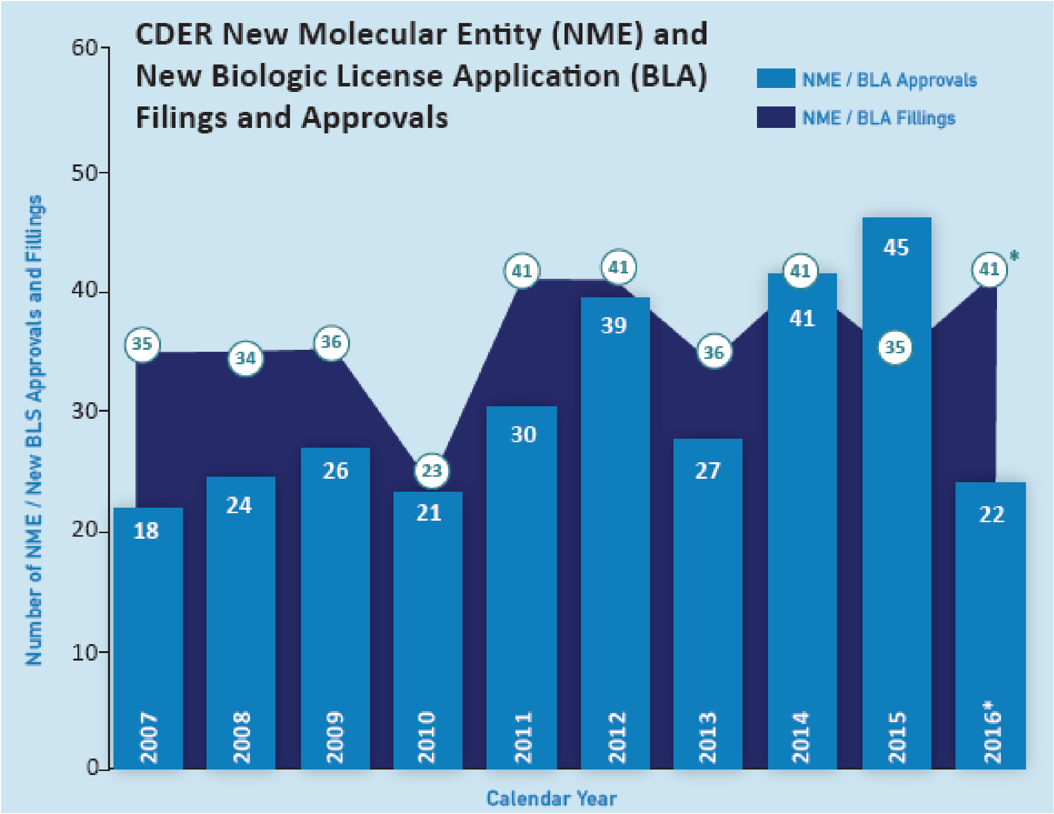
Summary of FDA New Drug Applications (NDAs)





- Average submitted NDAs (1938 - 2011) 254.3/year
- Average approved NDAs (1938 - 2011) 168.9/year
- Average NMEs (1938 - 2011) 21.2/year

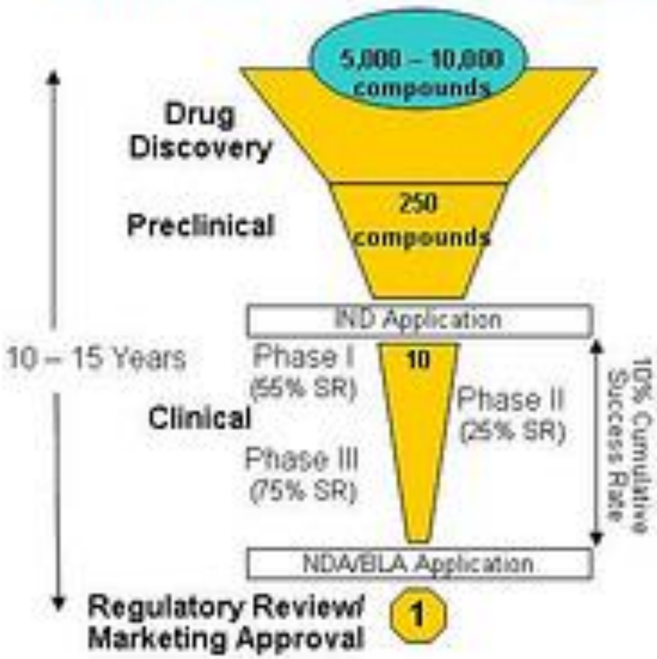
Number of approved drugs by the US FDA



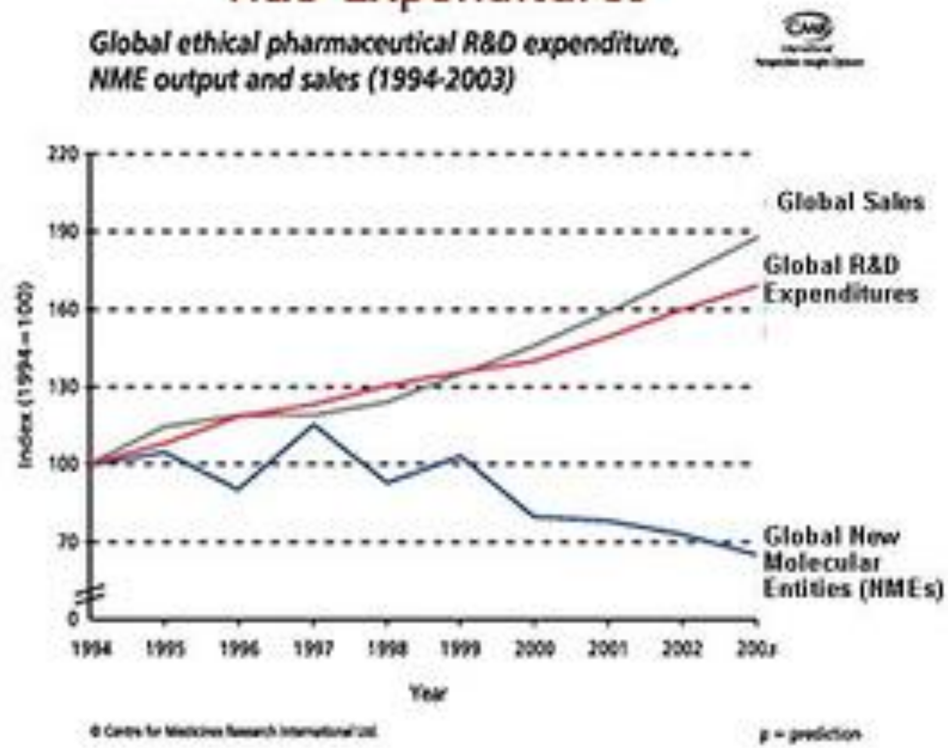
- Between 2007-2015 average 30 approved NMEs/year.
- NME – New Molecular Entity
- BLA – Biologics License Application

Big Pharma :Dramatic Decline in R&D Productivity

Attrition Remains Very High

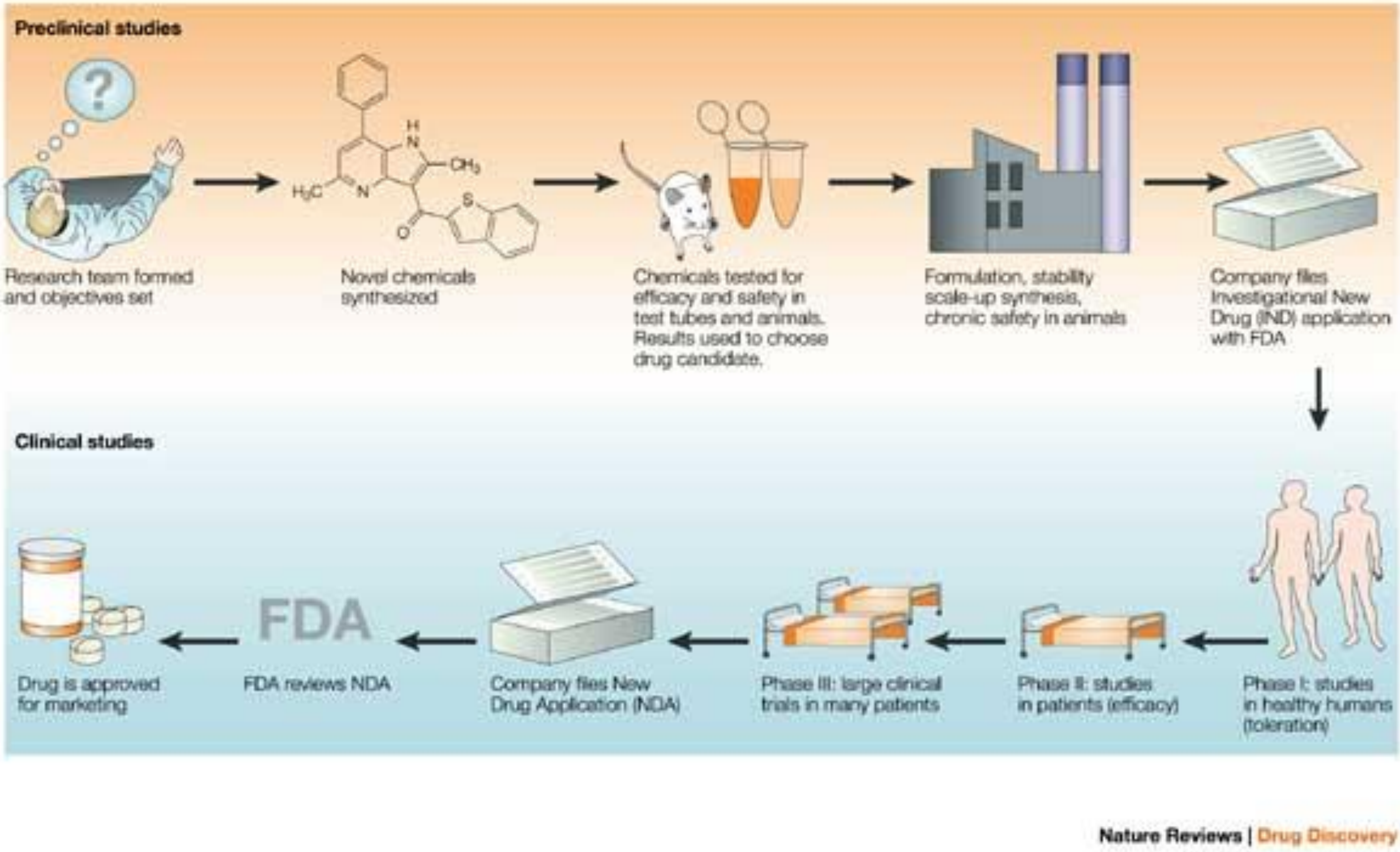


Output Not Keeping Up With R&D Expenditures



Source: PhRMA, CMR, Genentech, Booz Allen Hamilton: *The Global Innovation 1000*, 2006

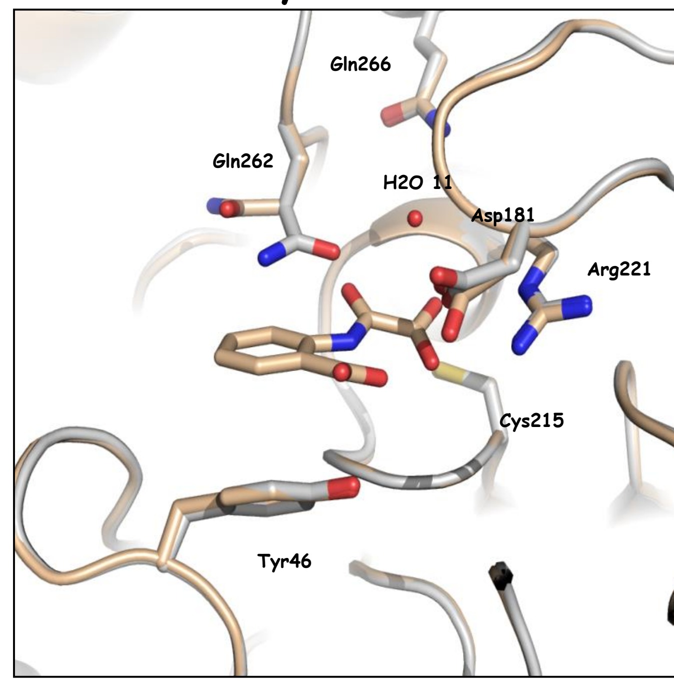
Stages of drug discovery



Structure-based drug design (SBDD)

- Develop new drug candidates for a disease
- Protein target relevant for the disease
- Relies on knowledge of the protein 3D structure
- Find compounds that block (or enhance) protein activity by binding to:
  - catalytic site
  - allosteric site (better for selectivity)
- Structural information of protein-ligand interaction is used to develop new compounds with increased potency and selectivity

PTP1B co-crystallised with OBA



Examples of drugs developed using SBDD

- Dorzolamide (Merck, 1995) – first SBDD approved drug (anti-glaucoma agent; carbonic anhydrase inhibitor)

- Imatinib (Novartis, 2001) – first anti-cancer drug substantially different from previous anti-cancer drugs (inhibitor of the tyrosine kinase *bcr-abl*)

- Vemurafenib (Roche, 2011) – first FBDD approved drug (late stage melanoma; inhibitor of B-Raf (V600E)) - only 6 years from fragment to approval!

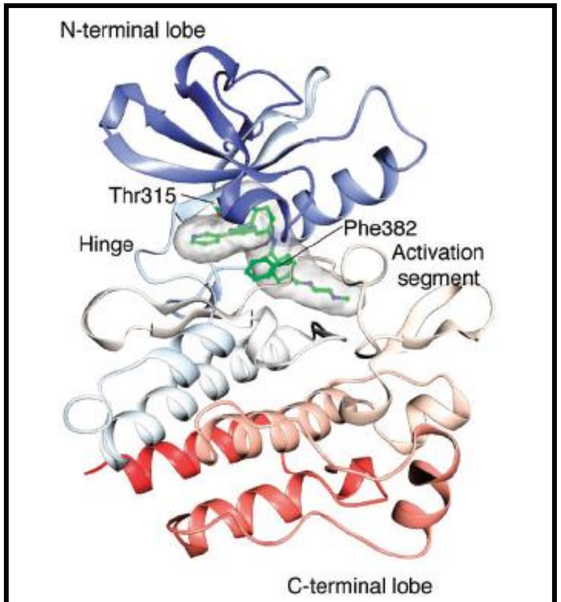
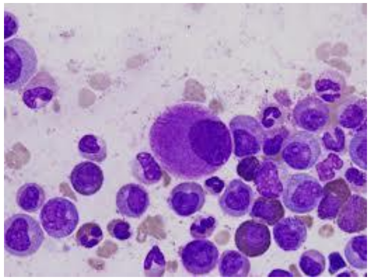
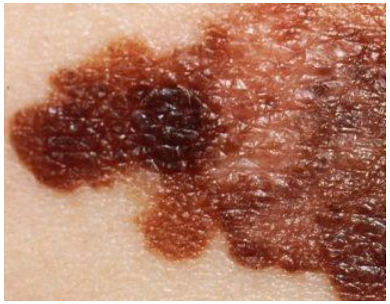


Fig. 1. The structure of the catalytic domain of cAbl in complex with Gleevec (51). The





Structure-based drug discovery

Expertise:

NMR Spectroscopy

- Protein construct optimization
- ligand screening and hit validation
- 3D structure determination

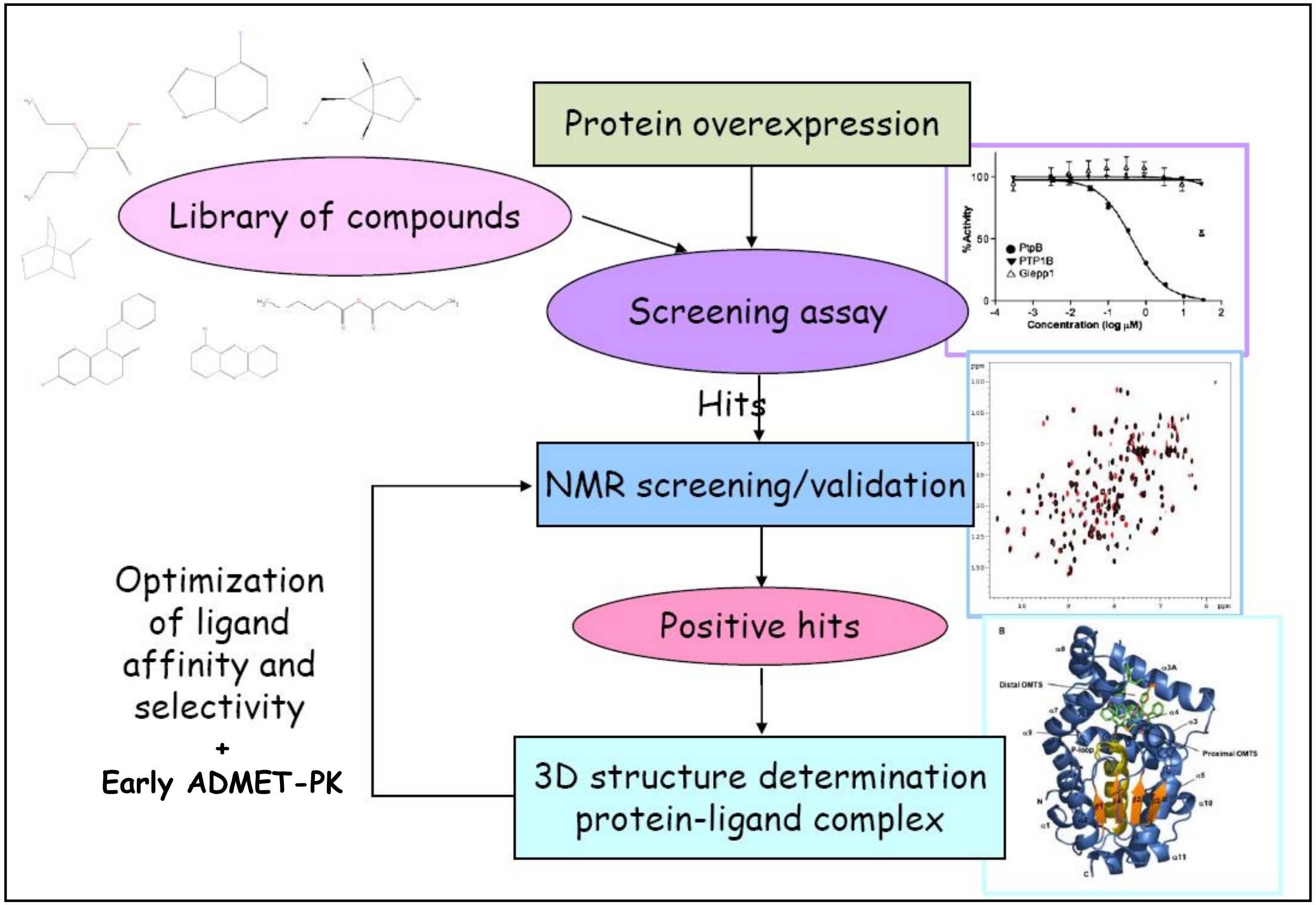
Crystallography

- 3D structure determination

Chemistry

Other techniques

- Biophysical techniques
- Computer modelling and docking of ligands
- SAR (activity assays, binding affinities, competition binding)

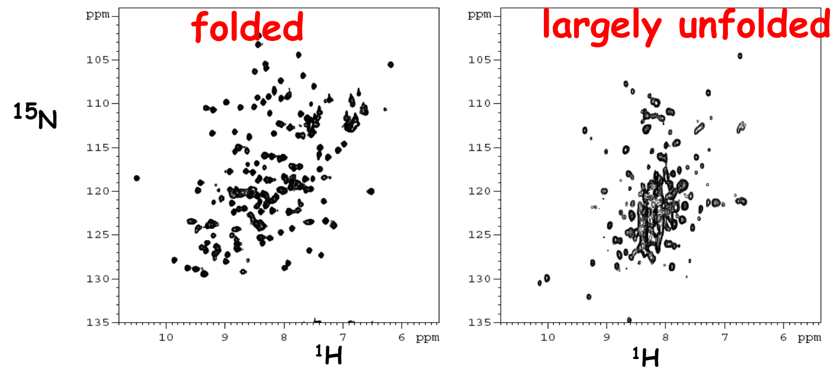


The essentials for a SBDD project

- **Protein:**
  - Easily overexpressed to high amounts
  - Stable (ideally can be frozen or lyophilised)
  - Folded
  - Crystallised into robust (compound soaking) and high-symmetry crystals (reduced acquisition time)
- **Chemical library:**
  - High-purity (> 95%)
  - High amounts (up to 50 mg)
  - Highly soluble in DMSO and water
  - Without reactive or unstable molecules
- **Infrastructure and technology:**
  - Wet-lab with biophysical equipment
  - High-field NMR spectrometers
  - Crystallography facility
  - X-ray generator and access to synchrotron
- **Chemistry support**

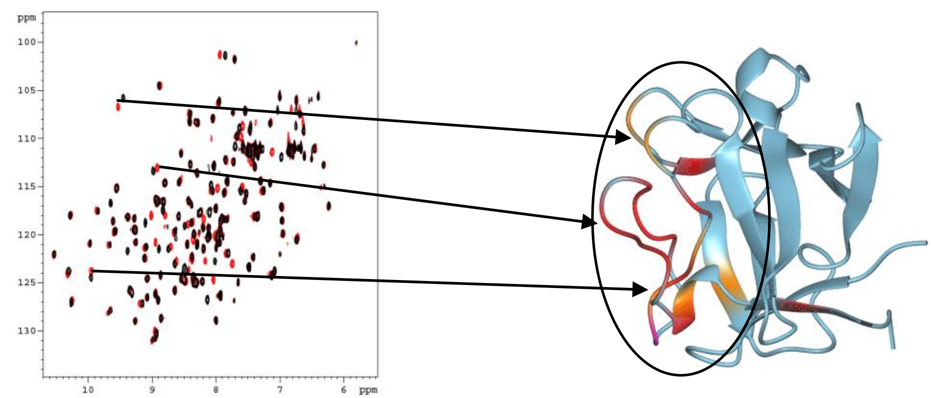
NMR in drug discovery

- Construct optimization of the target protein

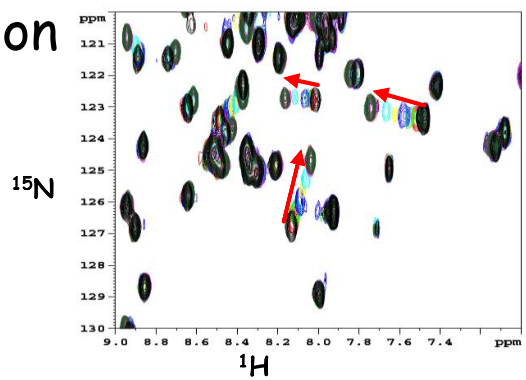


- NMR screening and hit validation

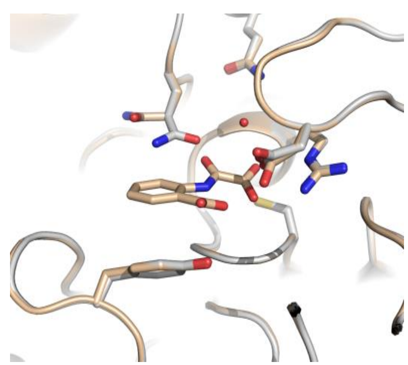
- Map the ligand-binding site



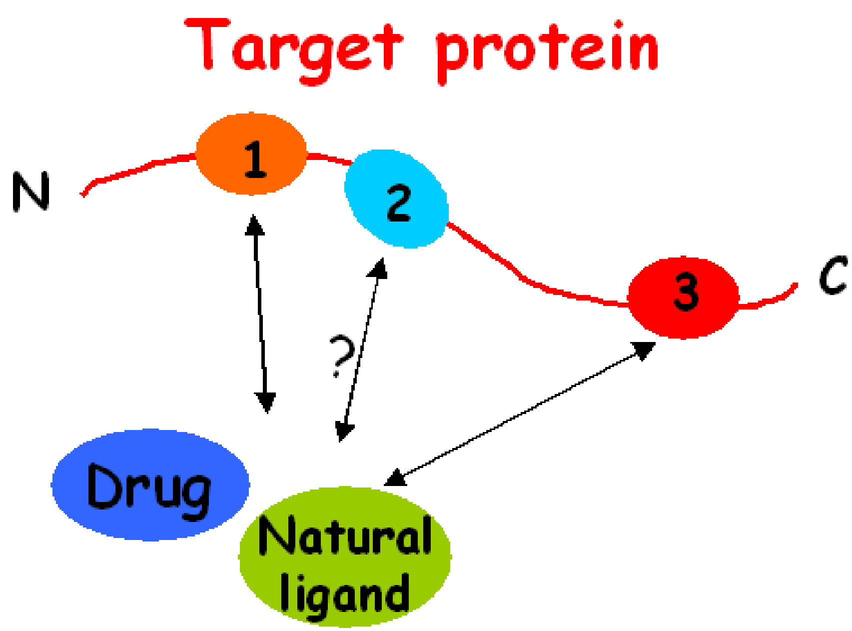
- Characterize the protein-ligand interaction



- Protein-ligand structure determination

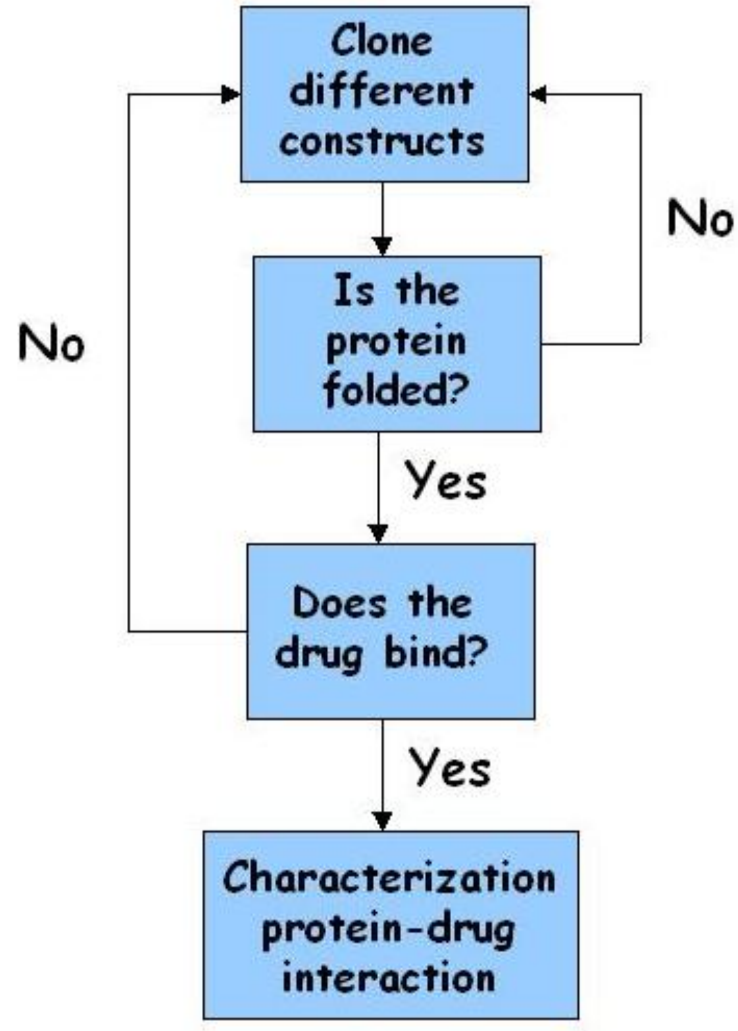


Cloning and expression of the target protein

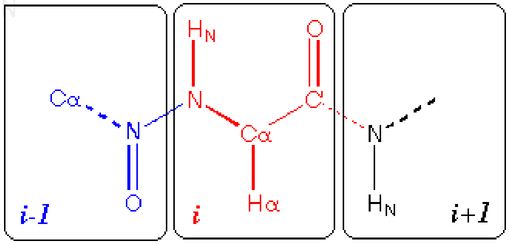


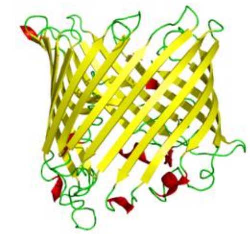
Use of diverse labelling schemes:

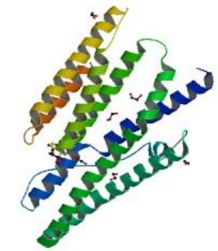
- Uniform ^{15}N labelling
- Uniform ^{15}N, ^{13}C labelling
- ^{2}H,^{15}N,^{13}C labelling



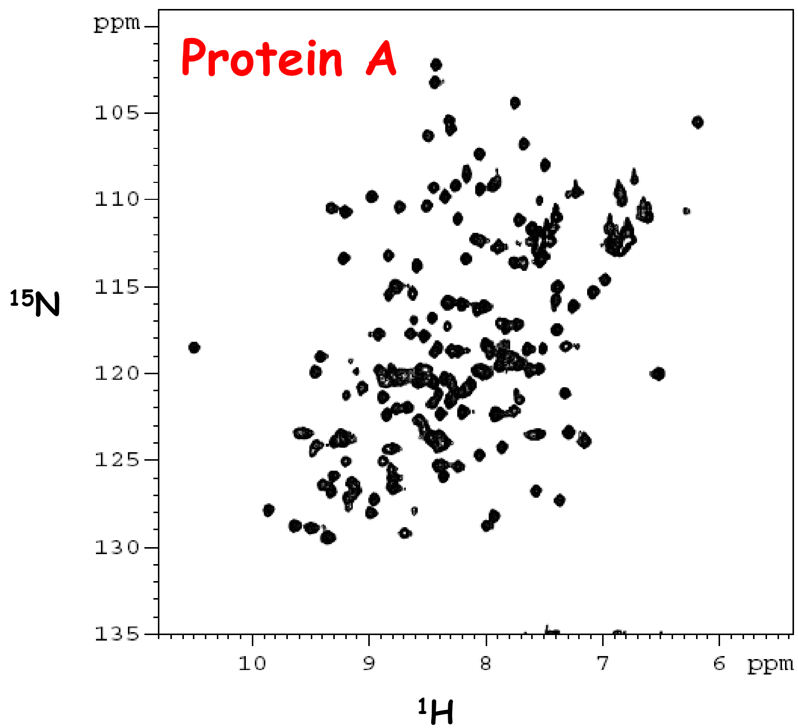
Is the protein folded?

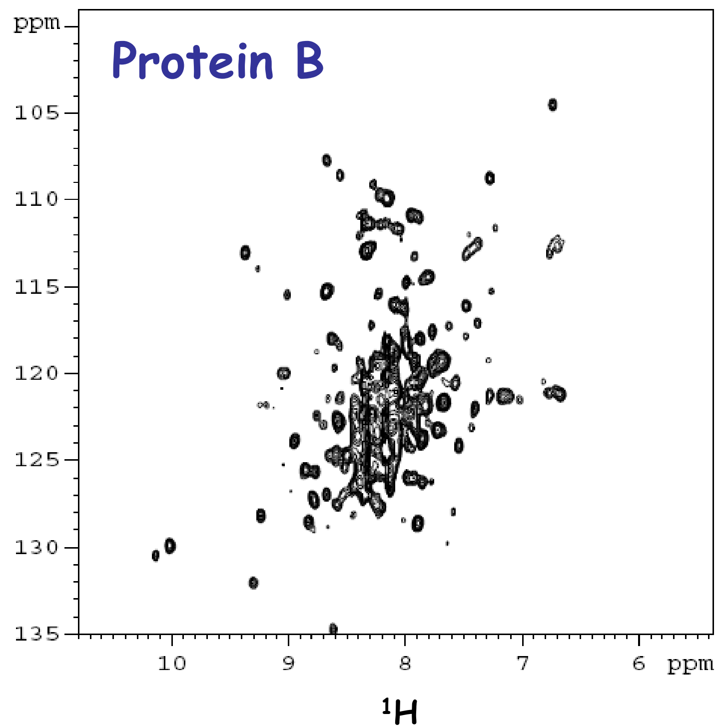






~ 200 amino acid residues





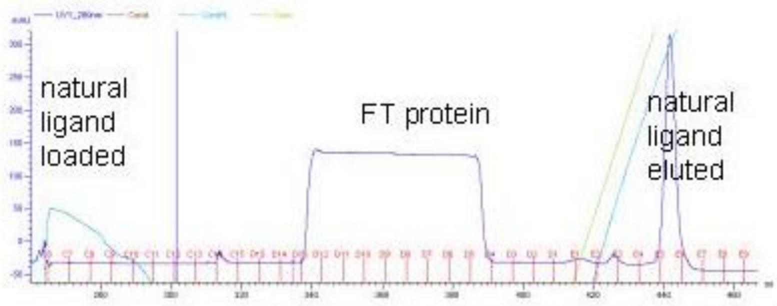
Nicely folded

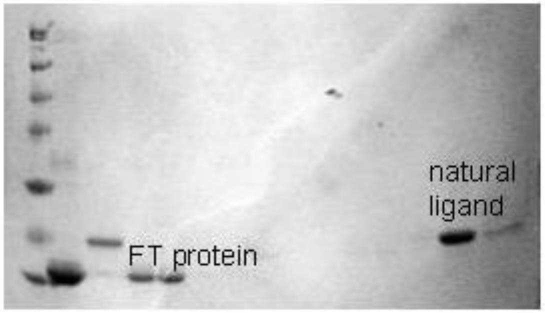
Largely unfolded

⇒ Improve construct/NMR conditions

Does the protein bind to its natural ligands?

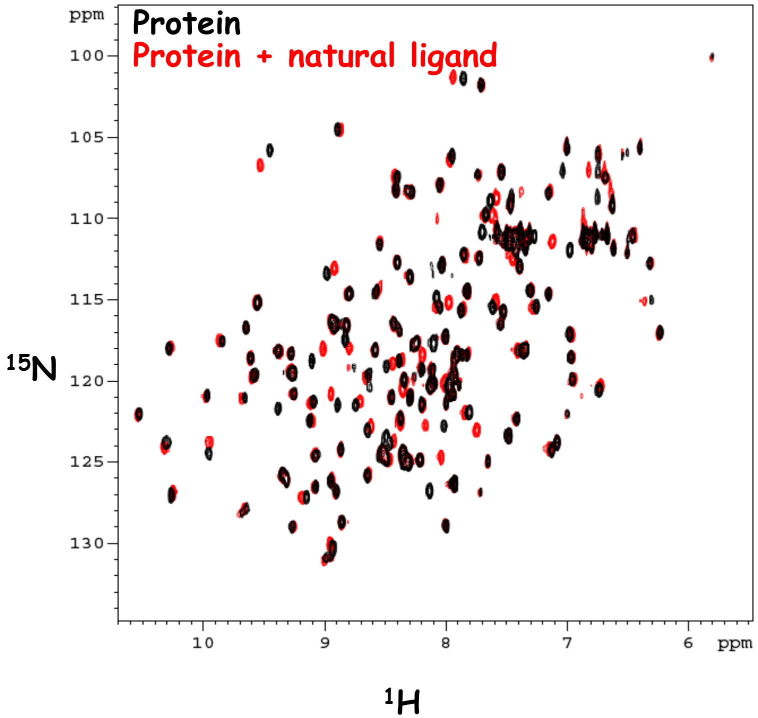
Affinity chromatography





The domain does not bind to its natural ligand! ⇒ construct problem?

2D NMR

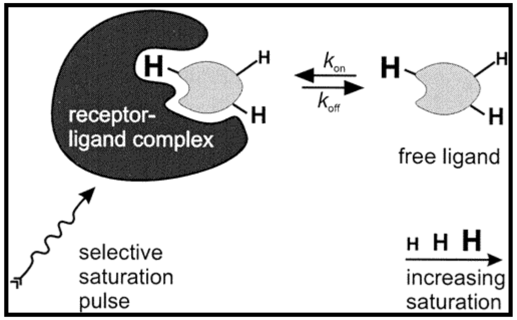


The domain binds to its natural ligand ⇒ the construct is valid!

NMR screening and validation

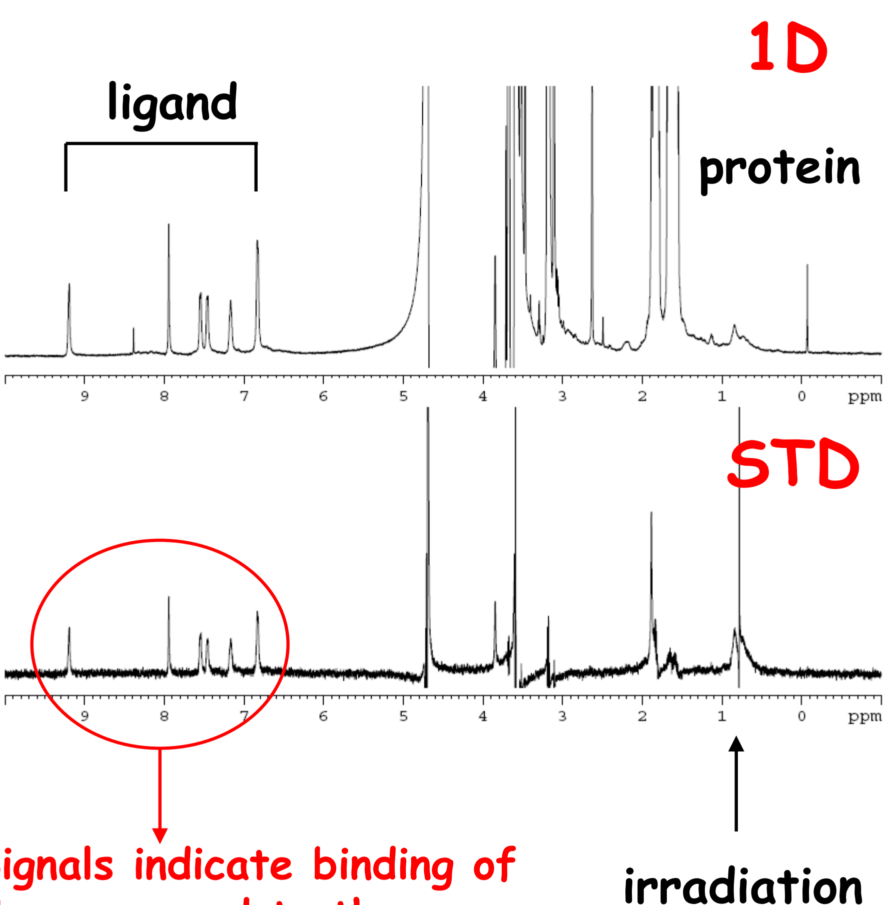
- NMR detects ligand binding mM $\rightarrow$ nM
- Specific binding can be distinguished from unspecific binding
- False positive identification
- Different pH, salt, buffer or redox conditions can be chosen

1D screening



Saturation Transfer Difference (STD) experiments

- Fast
- Unlabelled protein
- Low protein concentrations (~20 μM)
- Compound soluble in buffer (maximum DMSO levels 20%)
- Binding epitope can be inferred



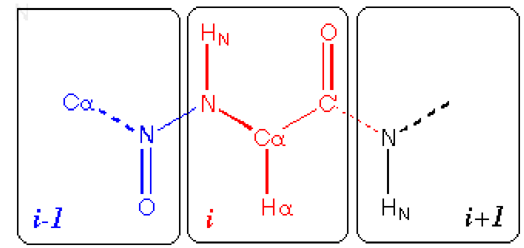
Signals indicate binding of the compound to the protein

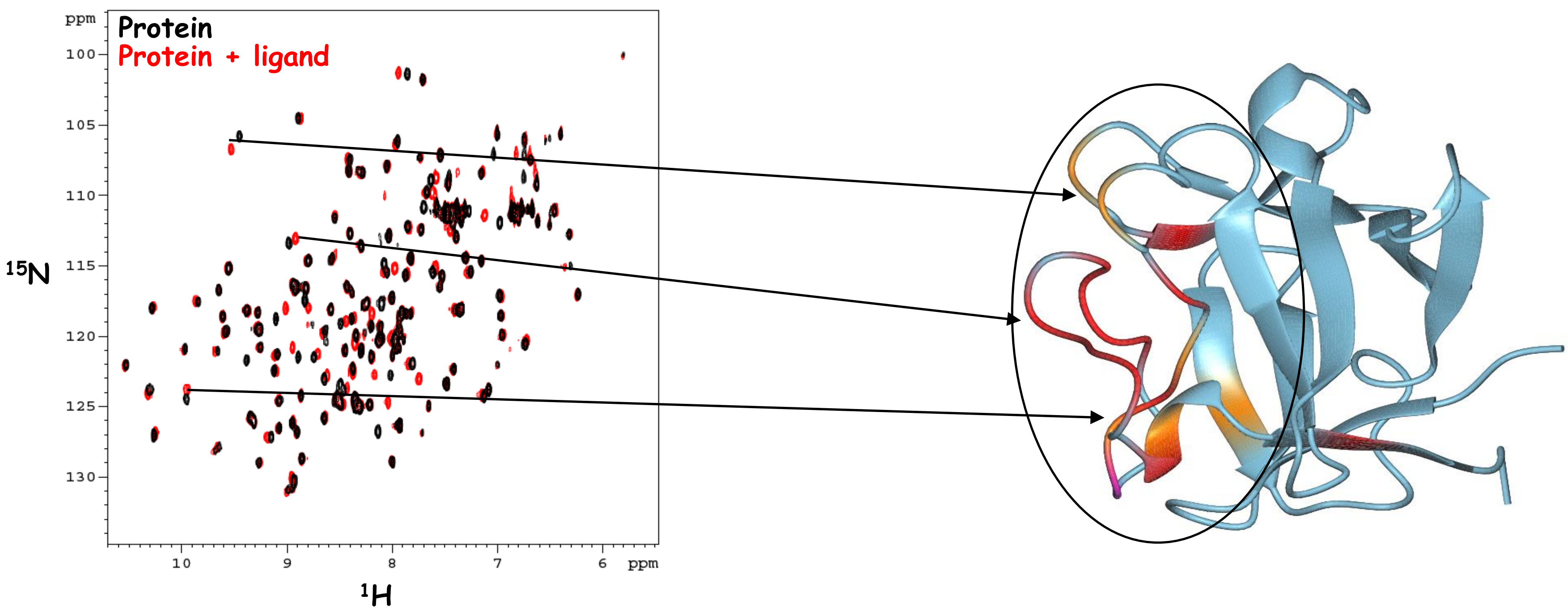
Problems:

- STD signals but non-specific interaction
- No STD signals but specific binding

2D screening

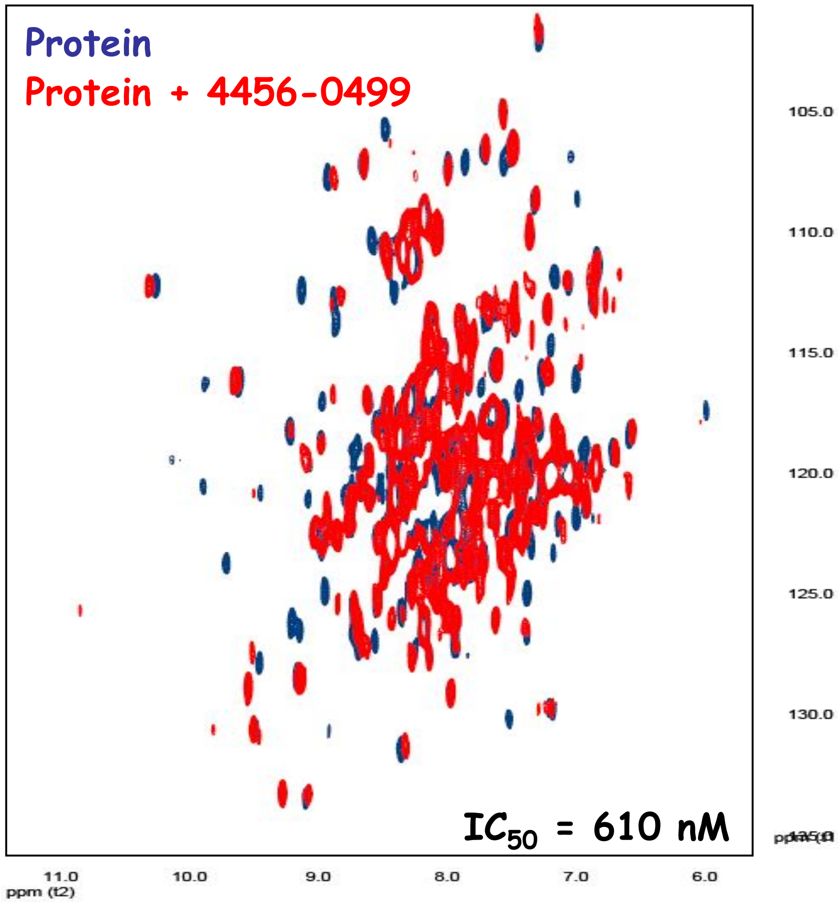
- Identifies specific binding epitopes
- Requirements:
  - ^{15}N-labelled protein
  - Assignment and 3D structure of the protein

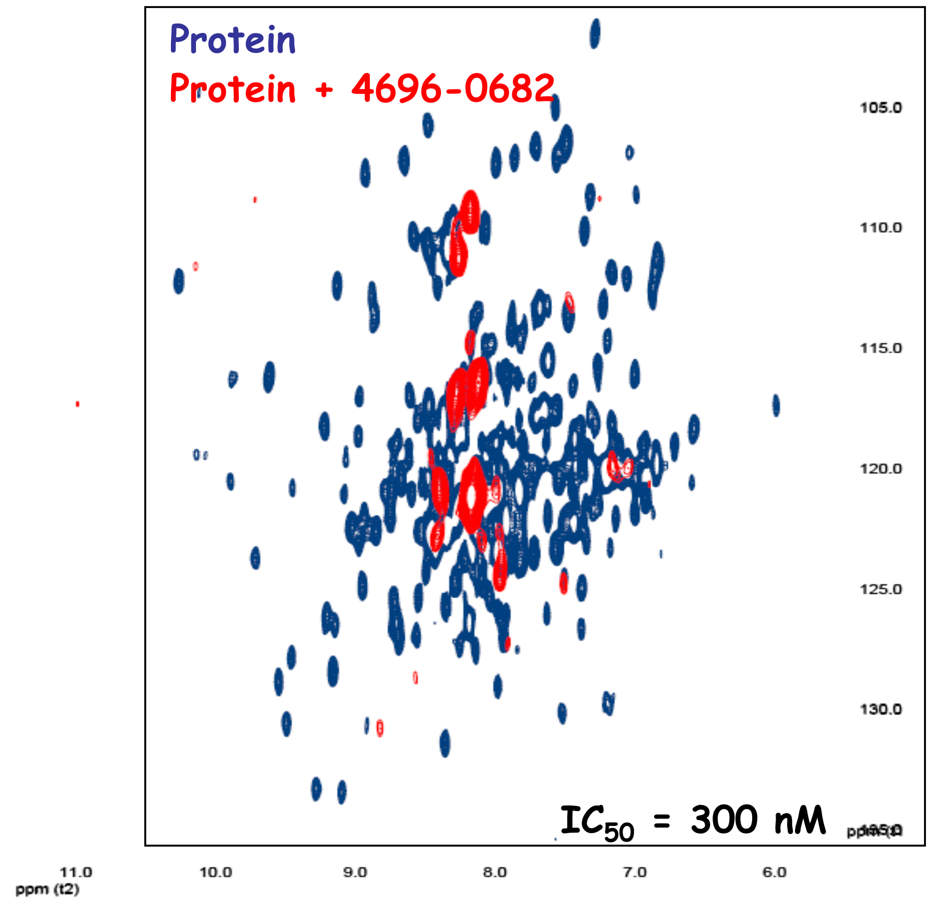




Chemical shift perturbations mapping

Hit validation



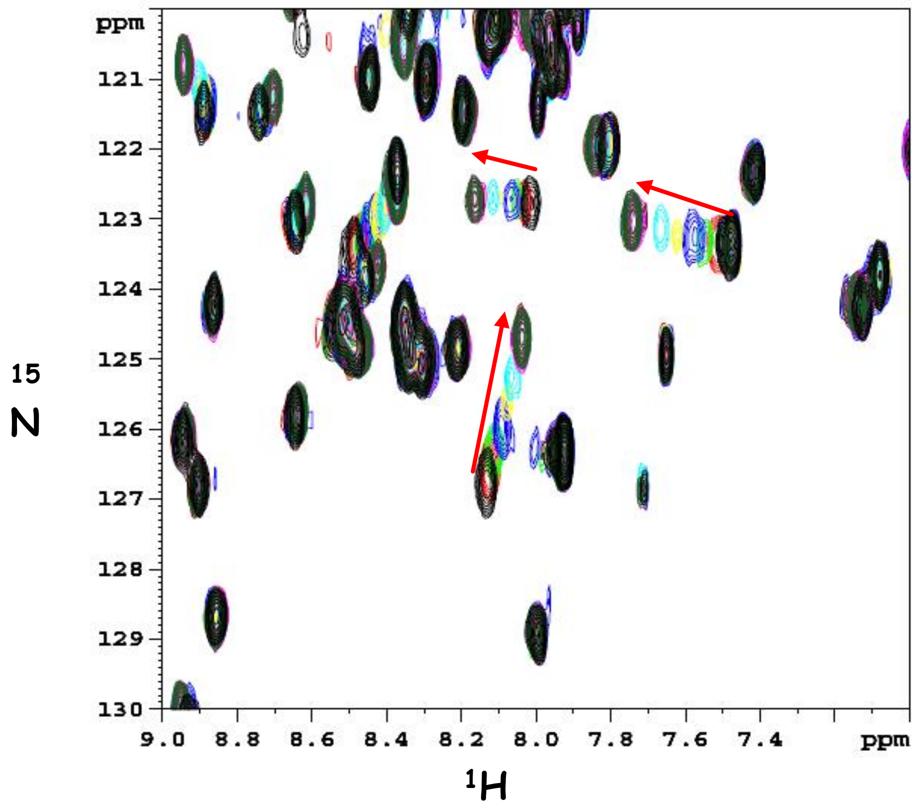


Significant shifts
Positive hit

Protein precipitation
False positive hit

Characterizing protein-ligand interactions

Determining the protein-ligand affinity (K_D)



Ligand titration by NMR

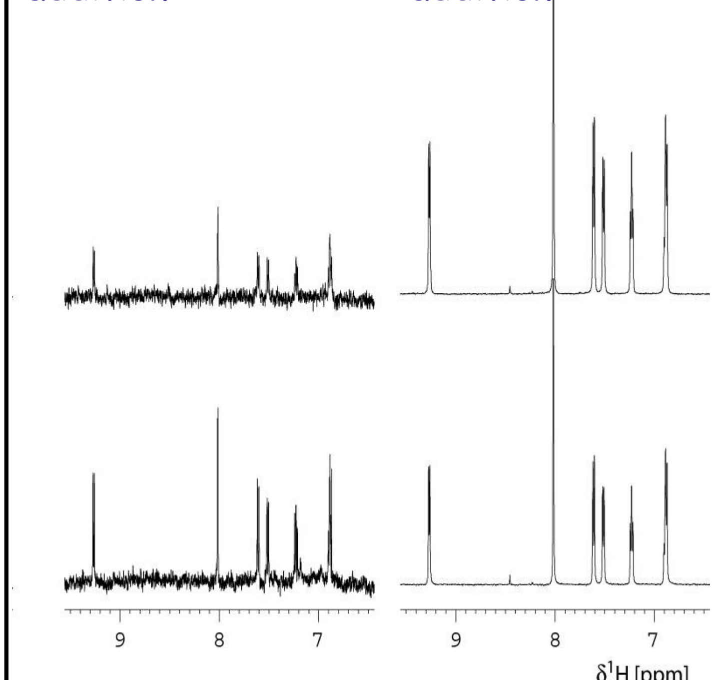
Limitations:

- Simple systems
- Fast exchange
- mM → µM binding
- Higher affinities – other techniques *e.g.* ITC

Determining how the ligand exerts its action

STD effect weakens upon natural ligand addition

Free ligand increases upon natural ligand addition



Addition of natural ligand

Compound with target protein

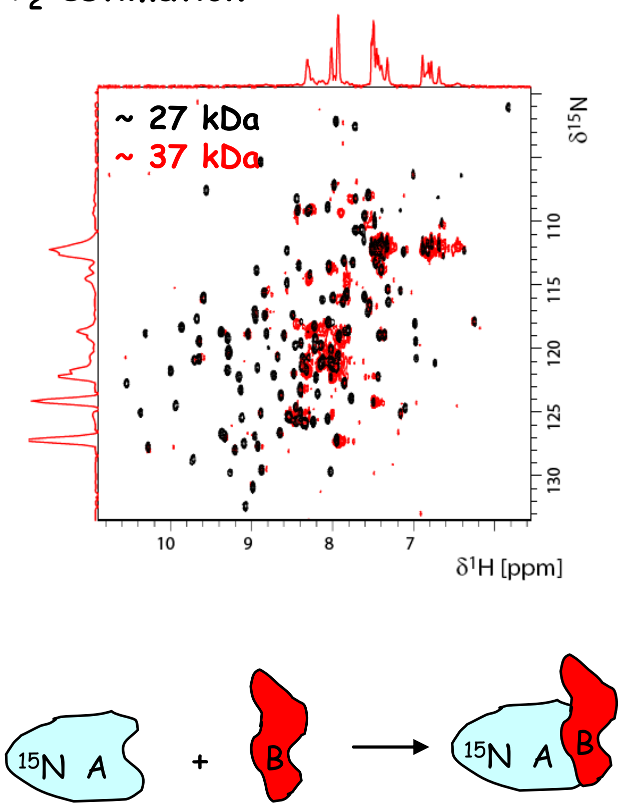
STD

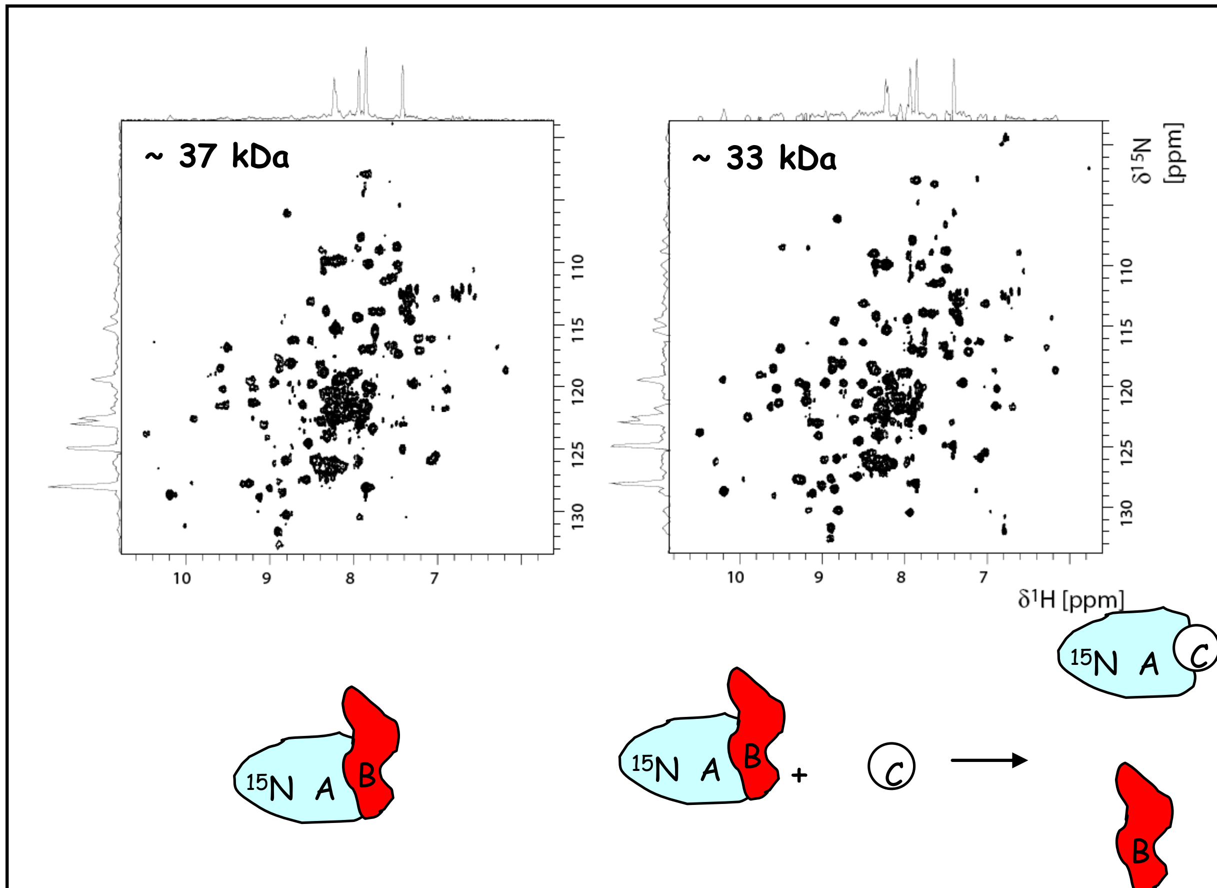
T2-filtered 1D

Competition with a natural ligand

Determining how the ligand exerts its action

T_2 estimation

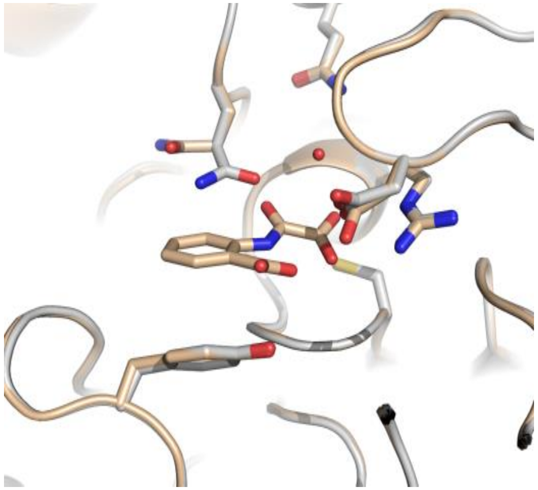




Inhibition of the interaction with biological partners

Crystallography in drug discovery

- 3D structure determination of protein-ligand complexes
- High-resolution – fine details of ligand-protein interactions can be determined and used to improve affinity or selectivity of the compound
- Fast (once you get crystals!)



THE NMEs OF 2011

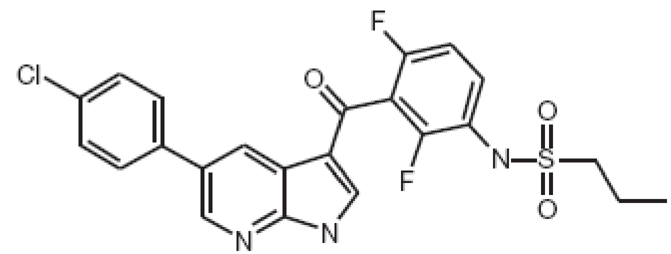
Drug Name	Active Ingredient	Date	What it's used for
Eylea	abliferecept	11/18	To treat wet (neovascular) age-related macular degeneration (AMD), a leading cause of vision loss and blindness in Americans ages 60 and older.
Erwinaze	asparaginase Erwinia chrysanthemi	11/18	For patients with acute lymphoblastic leukemia (ALL), who have developed an allergy (hypersensitivity) to E. coli derived asparaginase and pegapargase chemotherapy drugs used to treat ALL.
Jakafi	ruxolitinib	11/16	To treat patients with the bone marrow disease myelofibrosis.
Onfi	clobazam	10/24	For use as an adjunctive (add-on) treatment for seizures associated with Lennox-Gastaut syndrome in adults and children 2 years of age and older.
Ferriprox	deferiprone	10/14	Iron overload from blood transfusions in patients with thalassemia (genetic disorder causing anemia), who had an inadequate response to chelation therapy.
Xalkori	crizotinib	08/26	Certain patients with late-stage (locally advanced or metastatic), non-small cell lung cancers who express the abnormal anaplastic lymphoma kinase gene.
Firazyr	icatibant	08/25	For the treatment of acute attacks of a rare condition called hereditary angioedema (HAE) in people ages 18 years and older.
Adcetris	brentuximab vedotin	08/19	Hodgkin lymphoma and ALCL (systemic anaplastic large cell lymphoma).
Zelboraf	vemurafenib	08/17	To treat patients with late-stage (metastatic) or unresectable (cannot be removed by surgery) melanoma, the most dangerous type of skin cancer.
Brilinta	ticagrelor	07/20	To reduce cardiovascular death and heart attack in patients with acute coronary syndromes (ACS).
Xarelto	rivaroxaban	07/01	To reduce the risk of blood clots, deep vein thrombosis (DVT), and pulmonary embolism (PE) following knee or hip replacement surgery.
Arcapta Neohaler	indacaterol inhalation powder	07/01	For the long term, once-daily maintenance bronchodilator treatment of airflow obstruction in people with chronic obstructive pulmonary disease (COPD) including chronic bronchitis and/or emphysema.
Nulojix	belatacept	06/15	To prevent acute rejection in adult patients who have had a kidney transplant.
Potiga	ezogabine	06/10	An add-on medication to treat seizures associated with epilepsy in adults.

CY 2011 New Molecular Entities (NMEs)

Drug Name	Active Ingredient	Date	What it's used for
Dificid	fidaxomicin	05/27	For the treatment of *Clostridium difficile*-associated diarrhea (CDAD).
Incivek	telaprevir	05/23	To treat certain adults with chronic hepatitis C infection.
Edurant	rilpivirine	05/20	Treatment of HIV-1 infection in adults who have never taken HIV therapy.
Victrelis	boceprevir	05/13	To treat certain adults with chronic hepatitis C.
Tradjenta	linagliptin	05/02	Addition to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus.
Zytiga	abiraterone acetate	04/28	In combination with prednisone to treat patients with late-stage (metastatic) castration-resistant prostate cancer who have received docetaxel (chemotherapy).
Caprelsa	vandetanib	04/06	To treat adult patients with late-stage (metastatic) medullary thyroid cancer, ineligible for surgery who have disease that is growing or causing symptoms.
Horizant	gabapentin enacarbil	04/06	A once-daily treatment for moderate-to-severe restless legs syndrome (RLS).
Yervoy	ipilimumab	03/25	Late-stage (metastatic) melanoma, the most dangerous type of skin cancer.
Gadavist	gadobutrol	03/14	Magnetic resonance imaging (MRI) of the central nervous system.
Benlysta	belimumab	03/10	To treat patients with active, autoantibody-positive lupus (systemic lupus erythematosus) who are receiving standard therapy, including corticosteroids, antimalarials, immunosuppressives, and nonsteroidal anti-inflammatory drugs.
Daliresp	roflumilast	02/28	To decrease the frequency of flare-ups (exacerbations) or worsening of symptoms from severe chronic obstructive pulmonary disease (COPD).
Edarbi	azilsartan medoxomil	02/25	To treat high blood pressure (hypertension) in adults.
Viibryd	vilazodone HCl	01/21	To treat major depressive disorder in adults.
Natroba	spinosad	01/18	For the treatment of head lice infestation in patients ages 4 years and older.
Datscan	ioflupane i-123	01/14	An imaging drug used to assist in the evaluation of adult patients with suspected Parkinsonian syndromes (PS).

First fragment-based drug approved 17/08/2011

The story of vemurafenib (ZELBORAF)



PLX4032

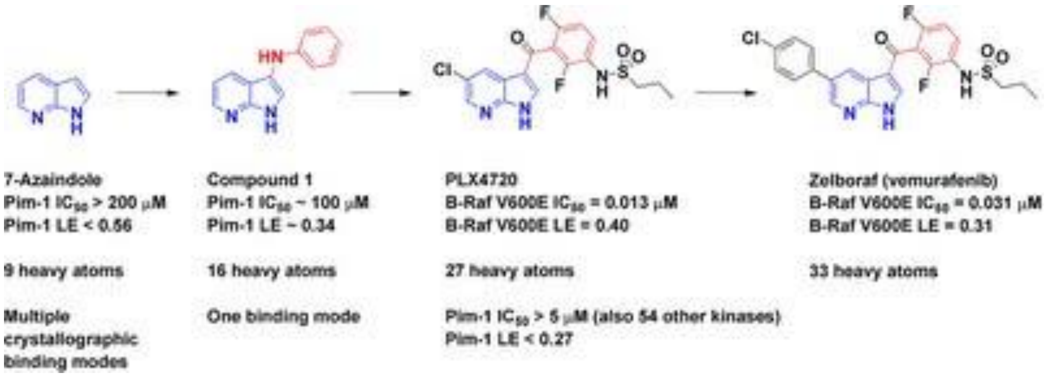
Vemurafenib=PLX4032

- Drug discovered at Plexxikon in partnership with Roche; Plexxikon acquired by Daiichi Sankyo
- 6 years from fragment to approval!

- Treatment of late stage melanoma
- Targets B-Raf (V600E), a Ser-Thr protein kinase
- 50% melanomas carry this mutation
- B-Raf most frequently mutated kinase in human cancers
- Increases survival by approximately 5 months longer
- $9400 /month

Compound evolution

- Initial screen of a 20000 compound library against the ATP-binding site of 3 kinases (Pim-1, CSK, p38)



Zelboraf (PLX4032) has better pharmacokinetic properties in dogs and monkeys than PLX4720

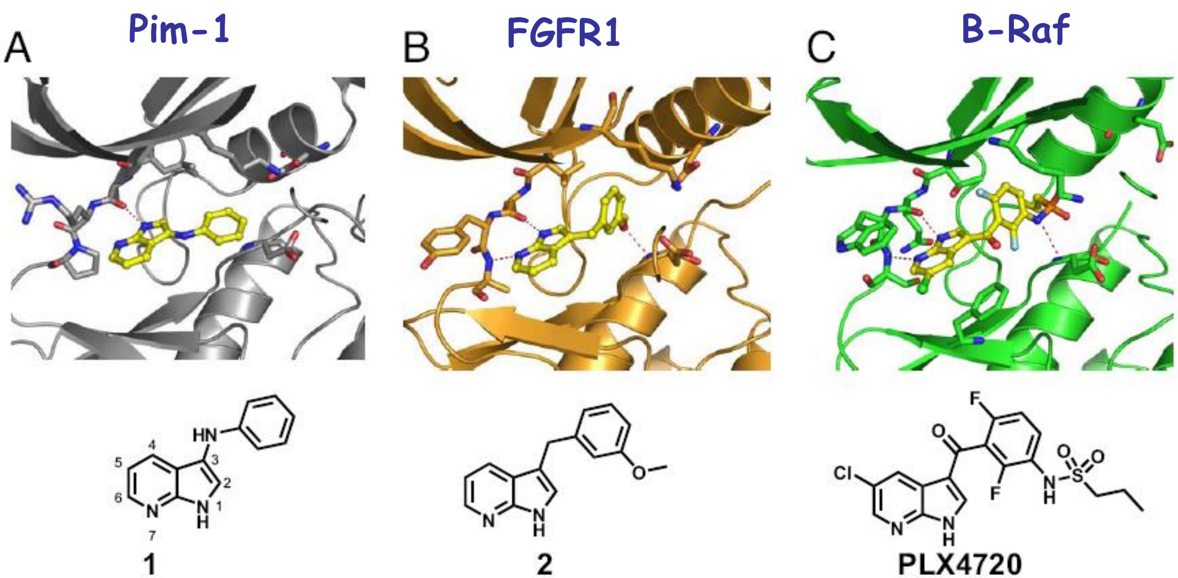


Fig. 1. Structures of individual compounds leading to the discovery of PLX4720 are shown. (*A*) The chemical structure of 3-aminophenyl-7-azaindole (compound 1) is shown beneath its costructure with Pim-1 kinase. (*B*) The chemical structure of 3-(3-methoxybenzyl)-7-azaindole (compound 2) is shown beneath its costructure with the kinase domain of FGFR1. (*C*) The chemical structure of PLX4720 is shown beneath its costructure with B-Raf kinase.

Discovery of a selective inhibitor of oncogenic B-Raf kinase with potent antimelanoma activity.
Tsai J. et al. Proc Natl Acad Sci U S A. 2008 Feb 26;105(8):3041-6

PLX4720 binds preferentially to active B-Raf

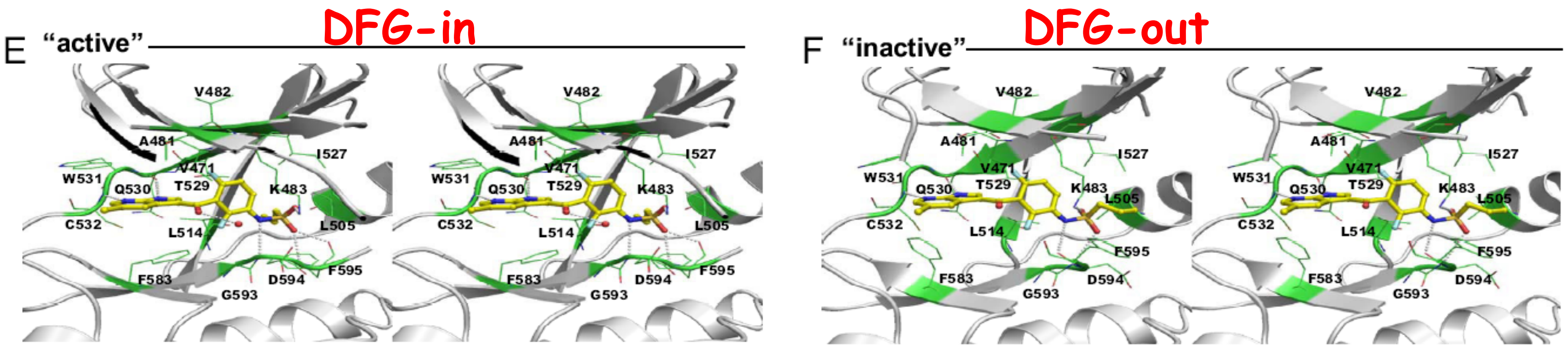
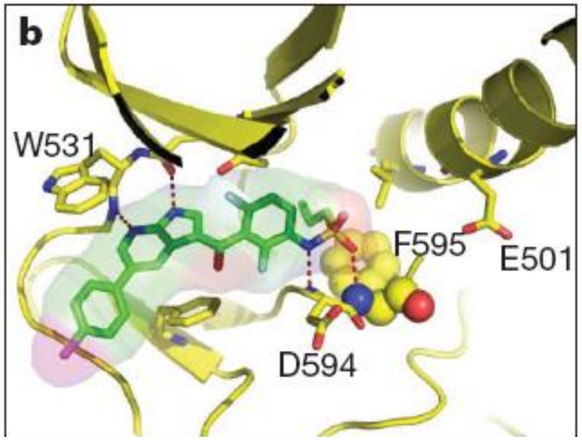


Fig. 2. Depiction of the three-dimensional structure of PLX4720 bound to B-Raf. (*A*) The structure of B-Raf^{V600E} bound to PLX4720 (yellow) is overlayed with an ATP model based on structures of ATP analogs in complex with other tyrosine kinases (orange). This view indicates that the PLX4720 scaffold overlaps with the adenine-binding site, but the tail of PLX4720 binds to a different pocket from the ATP ribose-triphosphate tail. The positions of the hinge, activation loop (A-loop), and phosphate-binding loop (P-loop) are also shown. (*B*) A surface representation shows PLX4720 binding to the B-Raf-selective pocket in the active conformation. (*C*) A surface representation shows PLX4720 binding to the kinase general pocket in the inactive conformation. (*D*) A close-up view shows the overlay PLX4720 bound to both active (green) and inactive (purple) conformations of the V600 protein, and PLX3203 (yellow) bound to V600E protein in the active kinase conformation. (*E*) A stereoview shows the specific interactions of PLX4720 to the active kinase conformation. In this conformation, the phenylalanine of the DFG loop is pointing in toward the compound-binding site. (*F*) A stereoview shows the specific interactions of PLX4720 to the inactive kinase conformation. In this conformation, the phenylalanine of the DFG loop is pointing away from the compound-binding site, and binding of PLX4720 is disfavored, leading to partial occupancy of this site even at the 1 mM compound concentration used in cocrystallography.

Discovery of a selective inhibitor of oncogenic B-Raf kinase with potent antimelanoma activity.
Tsai J, *et al.* Proc Natl Acad Sci U S A. 2008 Feb 26;105(8):3041-6

B-Raf(V600E) in complex with PLX4032



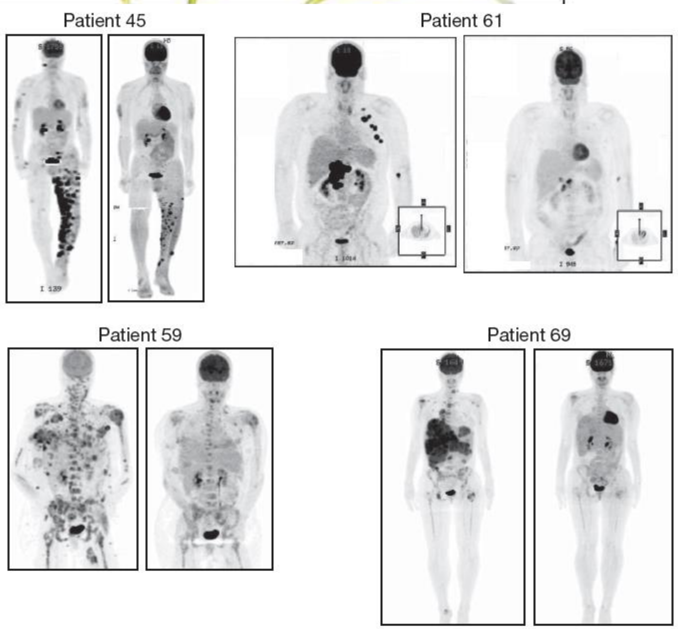


Figure 4 | Representative PET scans for patients taken pre-dose and following 2 weeks of dosing with PLX4032. Each of these image pairs demonstrates significant reduction in FDG uptake following PLX4032 treatment. Note that tumour regressions were later documented for each of these patients: best responses were 70% for patient 45, 70% for patient 59, 68% for patient 61 and 37% for patient 69.

Clinical efficacy of a RAF inhibitor needs broad target blockade in BRAF-mutant melanoma. Bollag G, *et al.* Nature. 2010, 467:596-9.

Supplementary Table 1. Biochemical IC_{50} determinations of the kinase inhibitory activity of PLX4032 versus a panel of kinases

Assay	IC_{50} nM*
B-RAF-V600E	31
C-RAF	48
B-RAF	100
SRMS	18
ACK1	19
MAP4K5 (KHS1)	51
FGR	63
LCK	183
BRK	213
NEK11	317
BLK	547
LYNB	599
YES1	604
WNK3	877
MNK2	1717
FRK (PTK5)	1884
CSK	2339
SRC	2389

*A list of over 200 kinases minimally affected by PLX4032 is included below.
Note that all RAF enzymes and SRMS were assayed at an ATP concentration of 100 μM, while all other kinases in the table above were assayed at an ATP concentration of 10 μM.

Problems: PLX4032 has low brain-blood barrier permeability

Kinases with <20% Inhibition at 1 μM:
ABL1, ABL2, ADRBK1, AMPK_A2, ARK5, Aurora_A-C, BMX, CDC42_BPA, CAMK2A, CDK5_p35, CSF1R, DYRK1B, EPHA5, EPHA8, EPHB4, FES, FLT3, FYN, GSK3beta, JAK1, KDR, KIT, MAP4K2, MAPK3, MARK2, MARK4, MATK, MET, MINK1, NEK1, NEK2, PAK3, PAK6, PDGFRbeta, PHKG1, PKBalpha, PKC_beta_I, PKC_beta_II, PKC_delta, PKC_gamma, PKC_zeta, SRC, STK4, STK24
Kinases with <10% Inhibition at 1 μM:
ACVR1B_(ALK4), ADRBK2_(GRK3), ALK, AMPK_A1/B1/G1, ASK1, AXL, BRSK1_(SAD1), BrSK2, BTK, CAMK1, CAMK1D, CAMK2B, CAMK2D, CaMKIdelta, CaMKIIbeta, CaMKIIdelta, CaMKIIgamma,,CDC42_BPB, CDK1/CyclinB, CDK2/CyclinA, CDK2/cyclinE, CDK3/cyclinE, CDK5_p25, CDK6/cyclinD3, CDK7/CyclinH/MNAT1,CDK9/CyclinT1, CHEK1, CHEK2, CK1delta, CK1gamma1, CK1gamma2, CK1gamma3, CK2alpha2, CLK1, CLK2, CLK3, CSNK1A1, CSNK1D, CSNK1E, CSNK1G1, CSNK1G2, CSNK1G3, CSNK2A1, CSNK2A2, DAPK1, DAPK2, DAPK3_(ZIPK), DCAMKL2_(DCK2), DDR2, DMPK, DRAK1, DYRK1A, DYRK2, DYRK3, DYRK4, EEF2K, EGFR, EPHA1, EPHA2, EPHA3 EPHA4, EPHA7, EPHB1, EPHB2, EPHB3, ERBB2, ERBB4, FER, FGFR1, FGFR2, FGFR3, FGFR4, FLT1, FLT4, FRAP1, GCK, GRK4, GRK5, GRK6, GRK7, GSK3A, HCK, HIPK, HIPK2, HIPK3, HIPK4, IGF1R, IGF-1R, IKBKB, IKBKE, IKKalpha, IKKbeta, INSR, INSRR, IRAK1, IRAK4, ITK, JAK2, JAK2_JH1_JH2, JAK3, JNK1alpha1, JNK2alpha2, LCK, LIMK1, LKB1, LOK, LTK, MAP2K1, MAP2K2, MAP2K6, MAP3K8, MAP3K9, MAP4K4, MAPK1, MAPK10, MAPK11, MAPK12, MAPK13, MAPK14, MAPK2, MAPK8, MAPK9, MAPKAPK2, MAPKAPK3, MAPKAPK5, MARK1, MARK3, MELK, MERTK, MKK7beta, MLCK, MRCKalpha, MRCKbeta, MST1R, MST4, mTOR/FKBP12, MUSK, NEK3, NEK4, NEK6, NEK7, NEK9, NLK, NTRK1, NTRK2, NTRK3, PAK2, PAK4, PAK7_(KIAA1264), PAR-1Balpha, PASK, PDGFRalpha, PDK1, PHKG2, PIK3CA/PIK3R1, PIK3CG, PIM1, PIM2, PIM-3, PKBbeta, PKBgamma, PKCalpha, PKCepsilon, PKCeta, PKCiota, PKCmu, PKCtheta, PKG1alpha, PKG1beta, PKN1, PLK2, PLK3, PRK2, PRKACA, PRKCA, PRKCE, PRKCH, PRKCI, PRKCN, PRKCQ, PRKD1, PRKD2, PRKG1, PRKG2, PRKX, PTK2, PTK2B, RET, RIPK2, ROCK1, ROCK2, ROS1, RPS6KA1, RPS6KA2, RPS6KA3, RPS6KA4, RPS6KA5, RPS6KA6, RPS6KB1, SGK, SGK2, SGKL, SIK, SNF1LK2, SNK, SRPK1, SRPK2, STK3, STK22B, STK22D, STK23, STK25, STK33, SYK, TAK1, TAO3, TAOK2, TBK1, TEC, TEK, TLK2, TXK, TYK2, TYRO3, ULK2, ULK3, VRK2, WNK2, WNK3, ZAP70

THE NOVEL DRUGS OF 2016

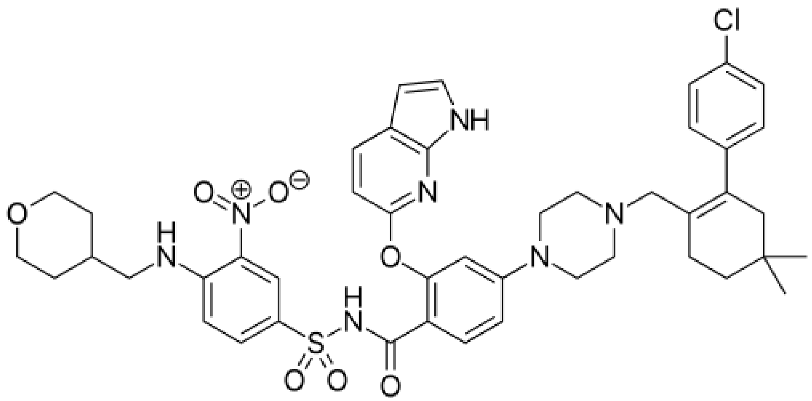
CDER's Novel Drug Approvals of 2016 (Listed in order of approval date).

Drug Name	Active Ingredient	Approval Date	What it is used for
Zepatier	elbasvir; grazoprevir	01/28/2016	To treat patients with chronic hepatitis C virus (HCV) genotypes 1 and 4 infections in adult patients.
Briviact	brivaracetam	02/18/2016	To treat partial onset seizures in patients age 16 years and older with epilepsy.
Anthim	obiltoxaximab	03/18/2016	To treat inhalational anthrax in combination with appropriate antibacterial drugs.
Taltz	ixekizumab	03/22/2016	To treat adults with moderate-to-severe plaque psoriasis.
Cinqair	reslizumab	03/23/2016	To treat severe asthma
Defitelio	defibrotide sodium	03/30/2016	To treat adults and children who develop hepatic veno-occlusive disease with additional kidney or lung abnormalities after they receive a stem cell transplant from blood or bone marrow called hematopoietic stem cell transplantation
Venclexta	venetoclax	04/11/2016	For chronic lymphocytic leukemia in patients with a specific chromosomal abnormality
Nuplazid	pimavanserin	04/29/2016	To treat hallucinations and delusions associated with psychosis experienced by some people with Parkinson's disease
Tecentriq	atezolizumab	05/18/2016	To treat urothelial carcinoma, the most common type of bladder cancer
Axumin	fluciclovine F-18	05/27/2016	A new diagnostic imaging agent to detect recurrent prostate cancer
Ocaliva	obeticholic acid	05/27/2016	To treat rare, chronic liver disease known as primary biliary cirrhosis
Zinbryta	daclizumab	05/27/2016	To treat multiple sclerosis
Netspot	gallium Ga 68 dotatate	06/01/2016	A diagnostic imaging agent to detect rare neuroendocrine tumors

Drug Name	Active Ingredient	Approval Date	What it is used for
Epclusa	sofosbuvir; velpatasvir	06/28/2016	To treat all six major forms of hepatitis C virus
Xiidra	lifitegrast	07/11/2016	To treat the signs and symptoms of dry eye disease
Adlyxin	lixisenatide	07/27/2016	To improve glycemic control (blood sugar levels)
Exondys 51	eteplirsen	09/19/2016	To treat patients with Duchenne muscular dystrophy
Lartruvo	olaratumab	10/19/2016	To treat adults with certain types of soft tissue sarcoma
Zinplava	bezlotoxumab	10/21/2016	To reduce the recurrence of Clostridium difficile infection in patients aged 18 years or older
Eucrisa	crisaborole	12/14/2016	To treat mild to moderate eczema (atopic dermatitis) in patients two years of age and older
Rubraca	rucaparib	12/19/2016	To treat women with a certain type of ovarian cancer
Spinraza	nusinersen	12/23/2016	To treat children and adults with spinal muscular atrophy (SMA)

Second fragment-based drug approved 11/04/2016

The story of venetoclax (VENCLEXTA)



venetoclax=ABT-199

- Drug discovered at AbbVie and Genentech; Initial work done at Abbott
- Two decades from initial 3D structure to approval!

- Second generation drug for the treatment of chronic lymphocytic leukemia (CLL)
- Targets Bcl-2, a protein regulator of apoptosis
- Orphan drug for the thousands of patients with relapsed CLL who have 17p deletion
- In the registration trial, 80% of patients showed a partial or complete remission

Compound evolution until ABT-263

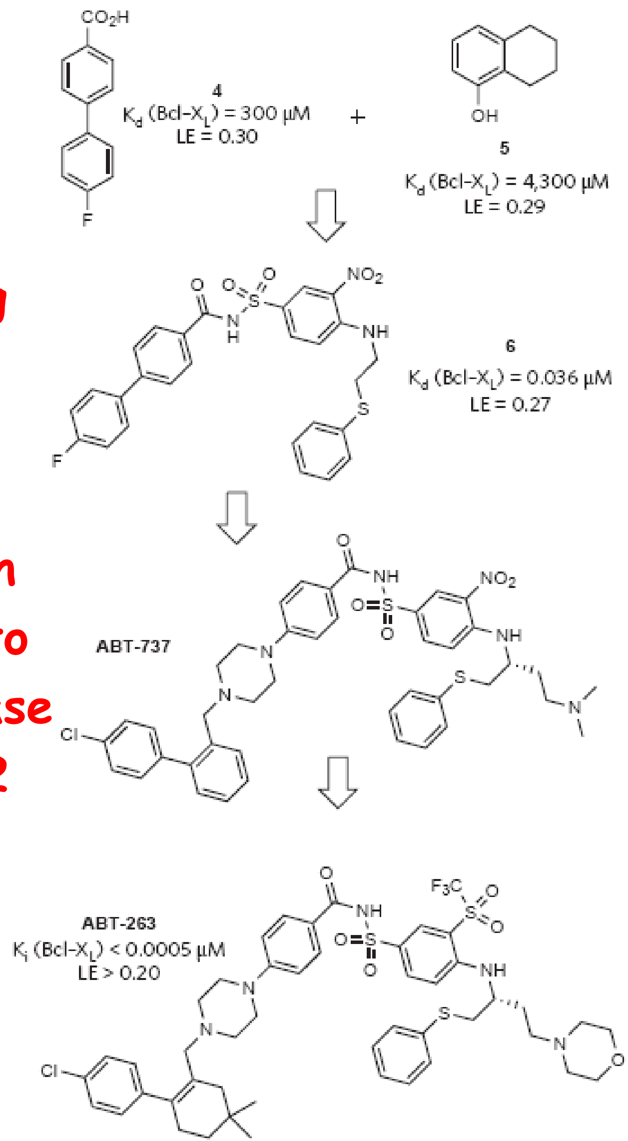
Fragments

Fragment-linking
+ optimization

Lead optimization
remove binding to
albumin and increase
affinity to Bcl-2

Improvement oral
pharmacokinetics

a



b

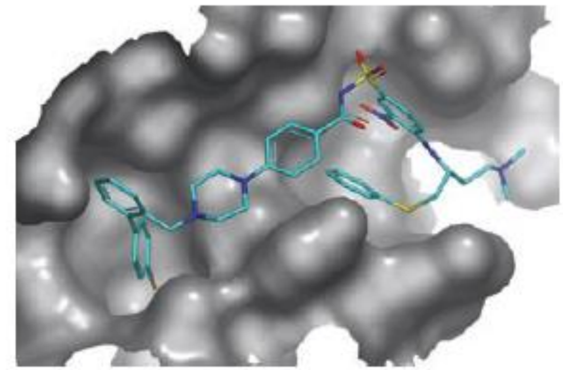
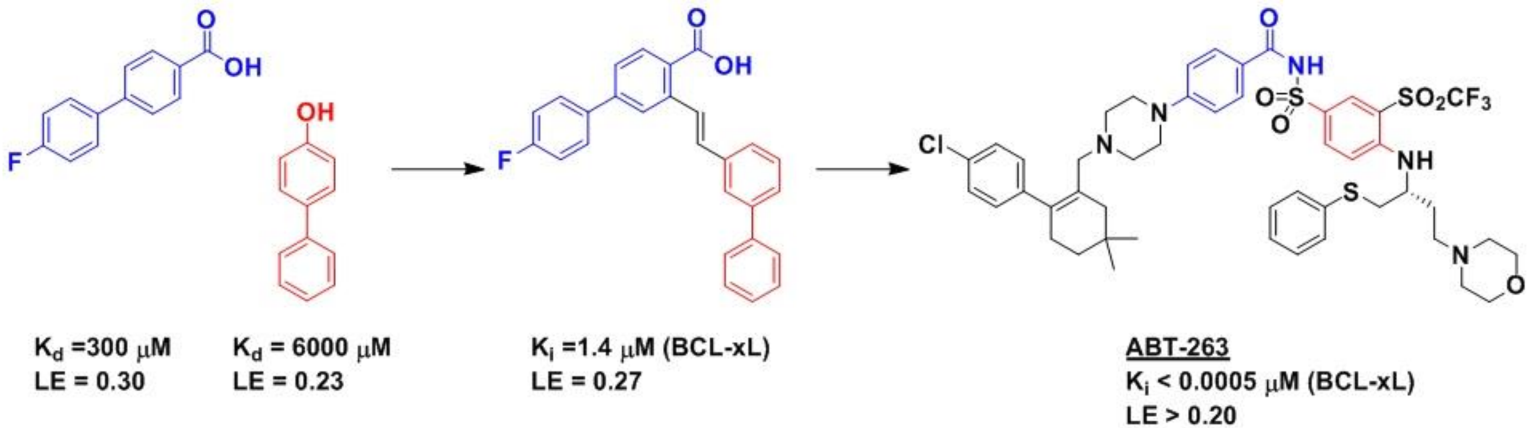
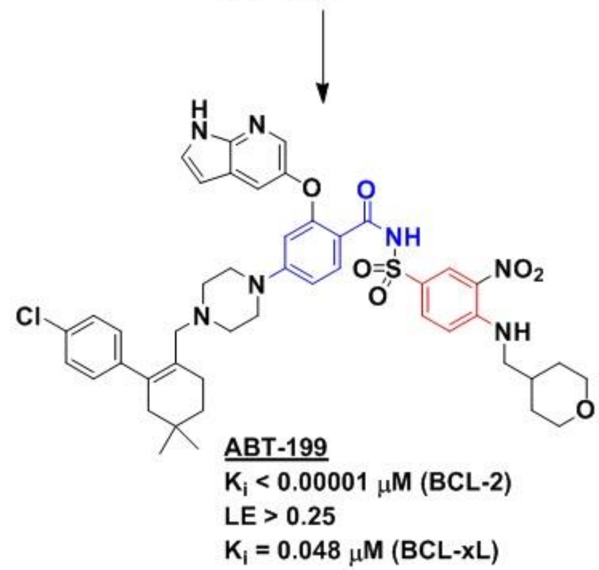


Figure 2 | The discovery of ABT-263, an inhibitor of protein-protein interactions involving Bcl-2 family proteins. a, A 10,000-member fragment library was screened using 2D-NMR leading to the identification of fragment hits **4** and **5**. Subsequent structure determination by NMR spectroscopy showed the compounds bound in proximal pockets and was used in linking the fragments; further elaboration[46,47] led to compound **6**. An early candidate, **ABT-737**, was identified following substantial lead optimization aimed at removing binding to human serum albumin and increasing binding to other Bcl family members[48,49]. The final candidate, **ABT-263**, was discovered following additional iterations of medicinal chemistry focused on improved oral pharmacokinetics[50]. **b**, The experimental binding mode for **ABT-737** on the relatively flat surface of Bcl-X_L.

Murray & Rees (2009) The rise of fragment-based drug discovery. Nature Chem. 1: 187-192

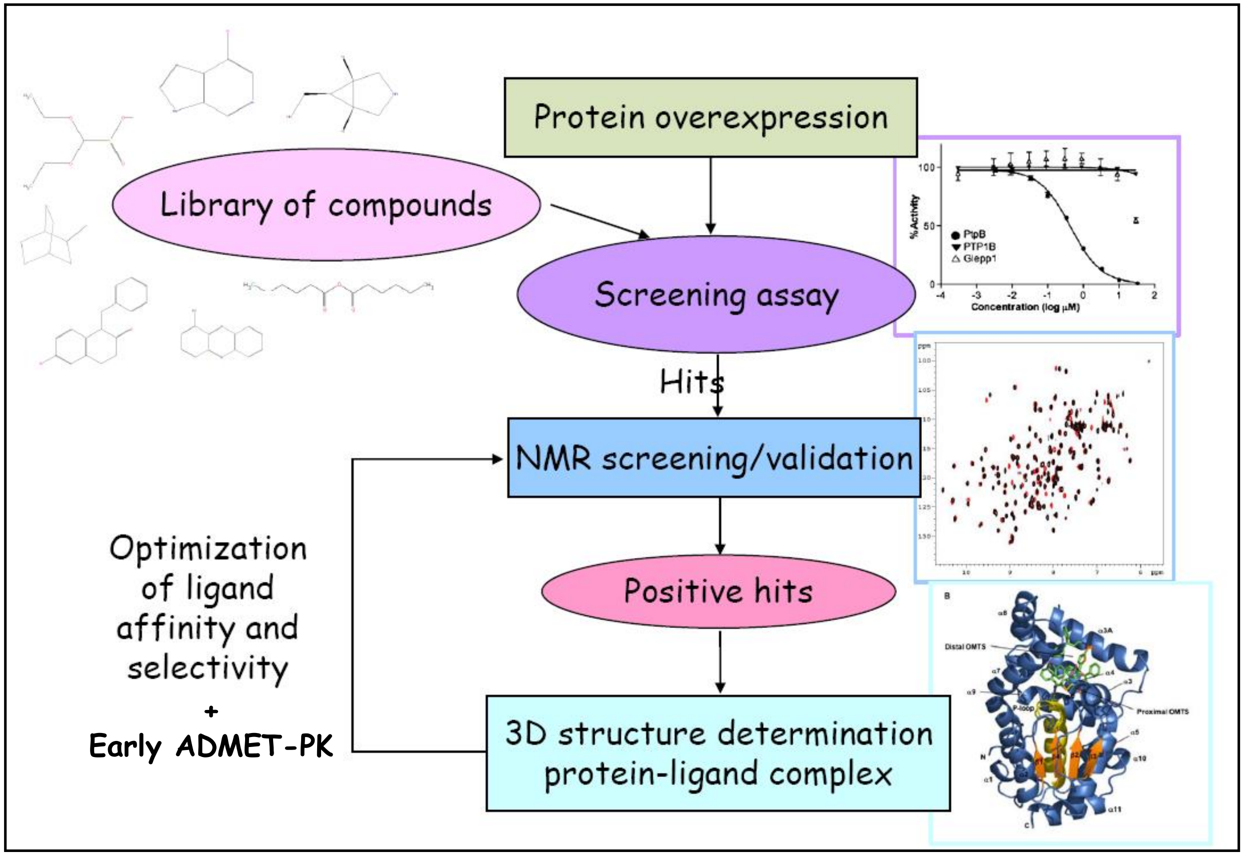
Compound evolution until drug





- Power of SBDD and FBDD to tackle difficult targets
- Violation of the Lipinsky rule of 5
- Contains a nitro group, a moiety red-flagged due to its potential for forming toxic metabolites

Summary



- Structure-based drug discovery is a powerful method for delivering new drugs
- Strategy for screening, hit validation and optimization – lead compound
- Expertise at STB- HMGU (NMR spectroscopy, X-ray crystallography, SBDD, Chemoinformatics)

Lipinski's rules

Lipinski's rule states that, in general, an orally active drug has no more than one violation of the following criteria:

- No more than 5 hydrogen bond donors (the total number of nitrogen–hydrogen and oxygen–hydrogen bonds)
- No more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms)
- A molecular mass less than 500 daltons
- An octanol-water partition coefficient log *P* not greater than 5

Ligand efficiency

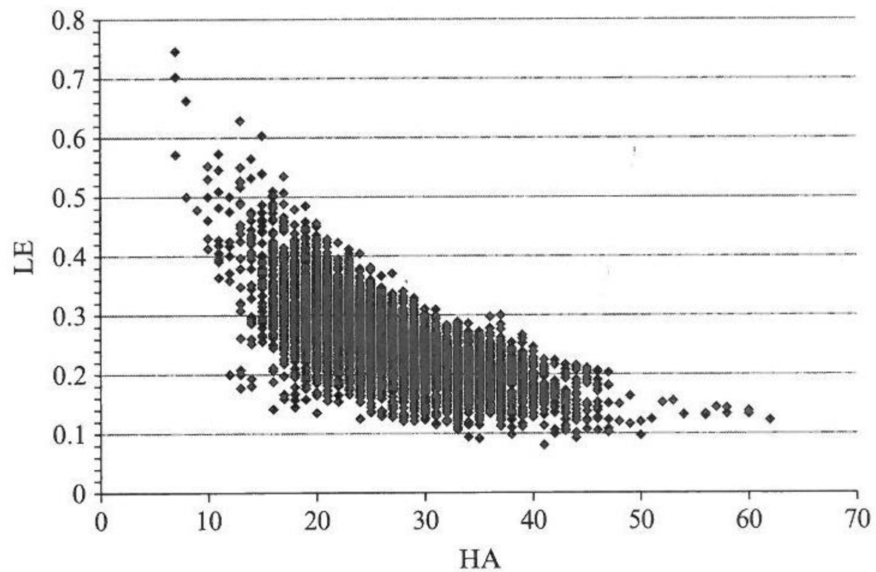


Figure 1.2 Ligand efficiency (LE) shows a precipitous decline between 10 and 25 nonhydrogen atoms (HA). We have extracted the affinity data used in this plot from the BindingDB database developed at the University of Maryland Biotechnology Institute (Liu *et al.*, 2007).

Tounge B. A., Parker M. H. (2011) Designing a Diverse High-Quality Library for Crystallography-Based FBDD Screening. Method Enzymol. 493: 3-20

Important arbiter of progress Ligand Efficiency (LE) - free energy of binding per heavy atom

$LE=\Delta G/HA$

where $\Delta G=-RT\ln K_d$, $-RT\ln K_i$, $-RT\ln IC_{50}$