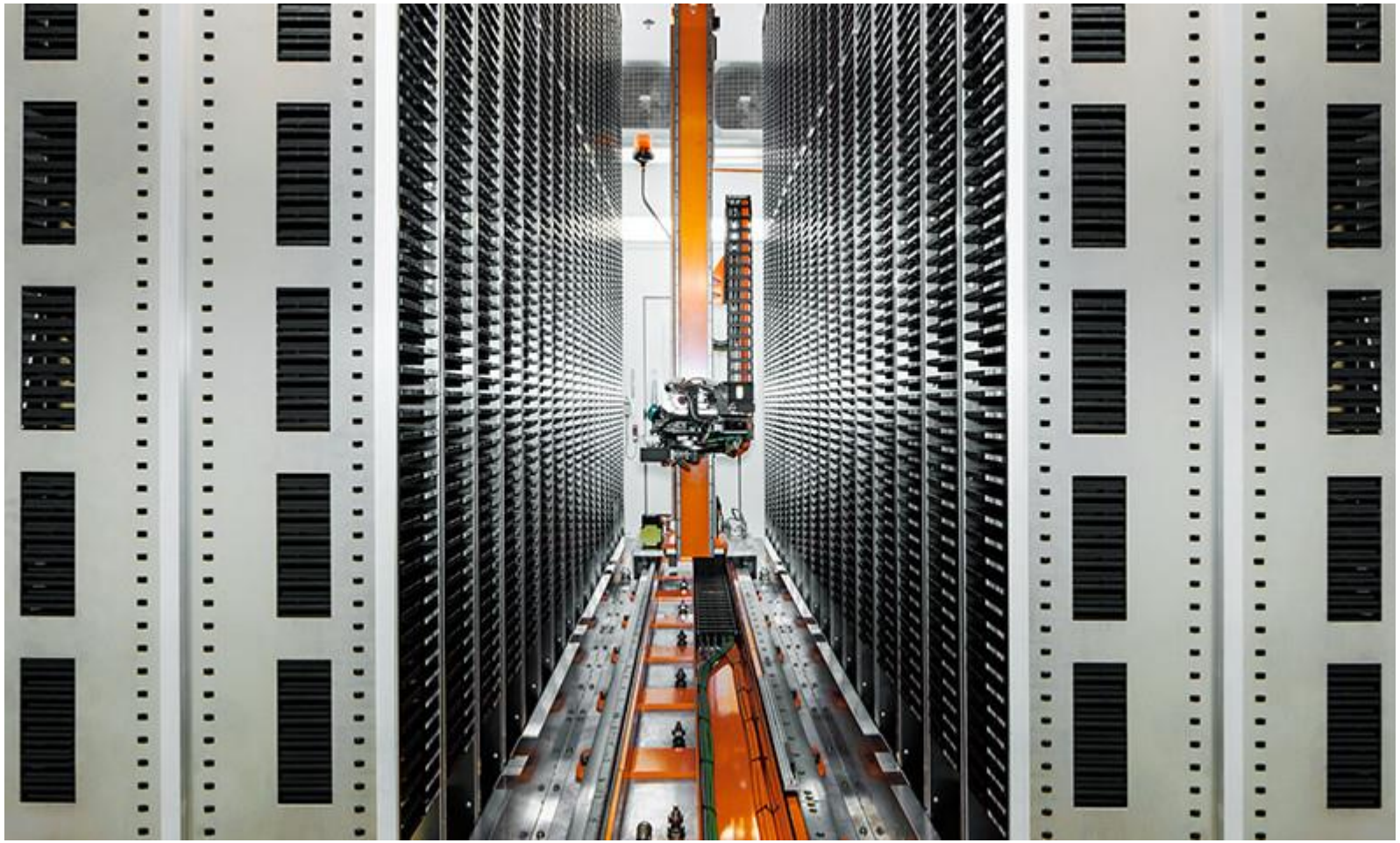
AMIDD Lecture 5: Screening and drug design



The chemical library at Novartis headquarters in Basel currently contains roughly 3 million molecules. We aim to expand that number radically within the next few years.

Jay Bradner, President of NIBR, in an interview in 2017

https://www.novartis.com/news/medical-researchers-using-new-tools-turn-science-fiction-science-fact

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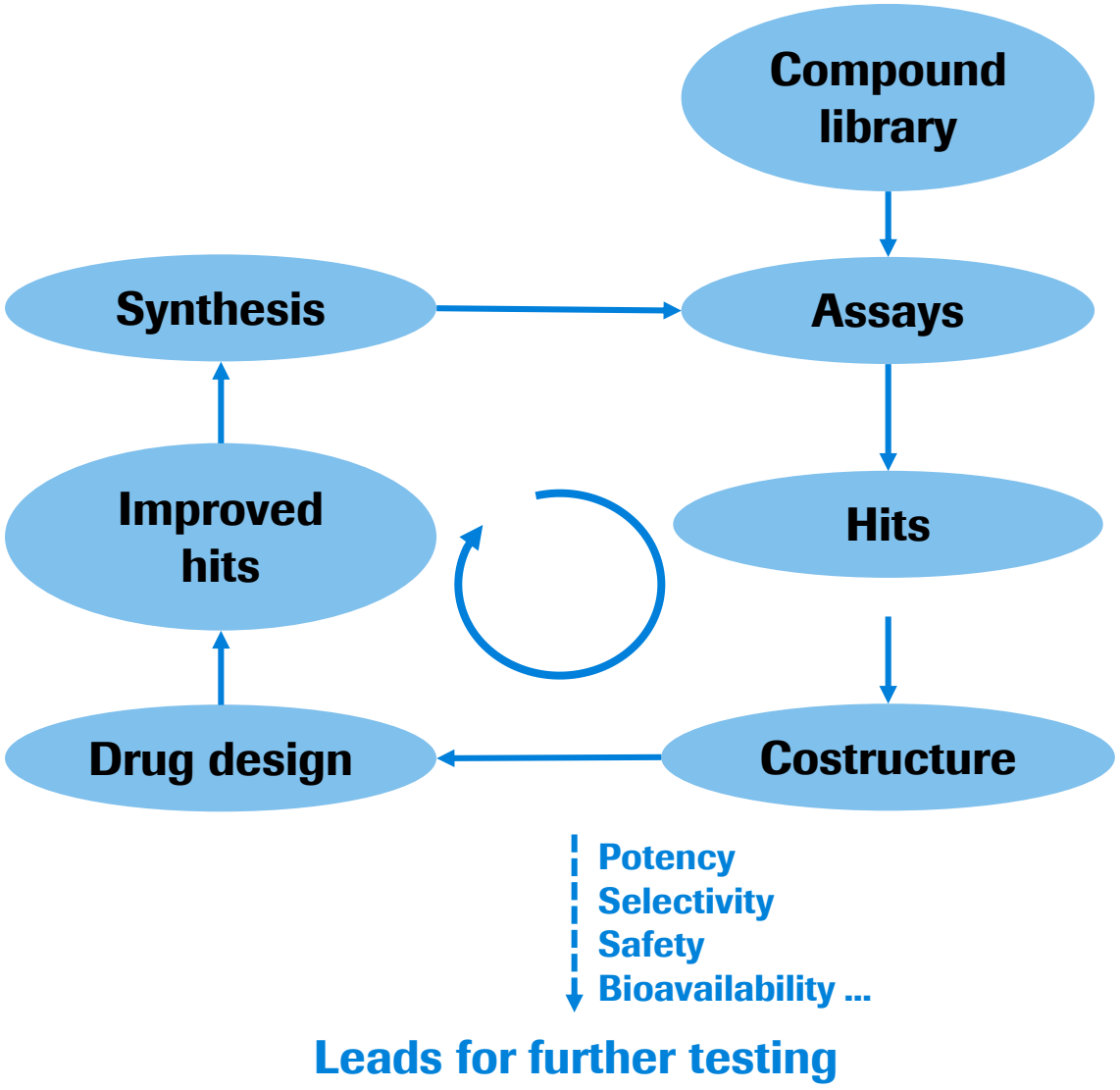
Questions on the PNAS paper by Tsai *et al.*

1. **How many** compounds were screened? What information is available about their **properties**?
2. **How** were the compounds **screened**?
3. What was the **initial chemical structure** that was found to bind to the ATP-binding site?
4. By overlapping structures, the team aimed to optimizing what **two properties of the compounds**?
5. What types of compounds were tested in the **subsequent screenig**?
6. What properties does the PLX4720 compound have that make it **particular attractive** as a drug?

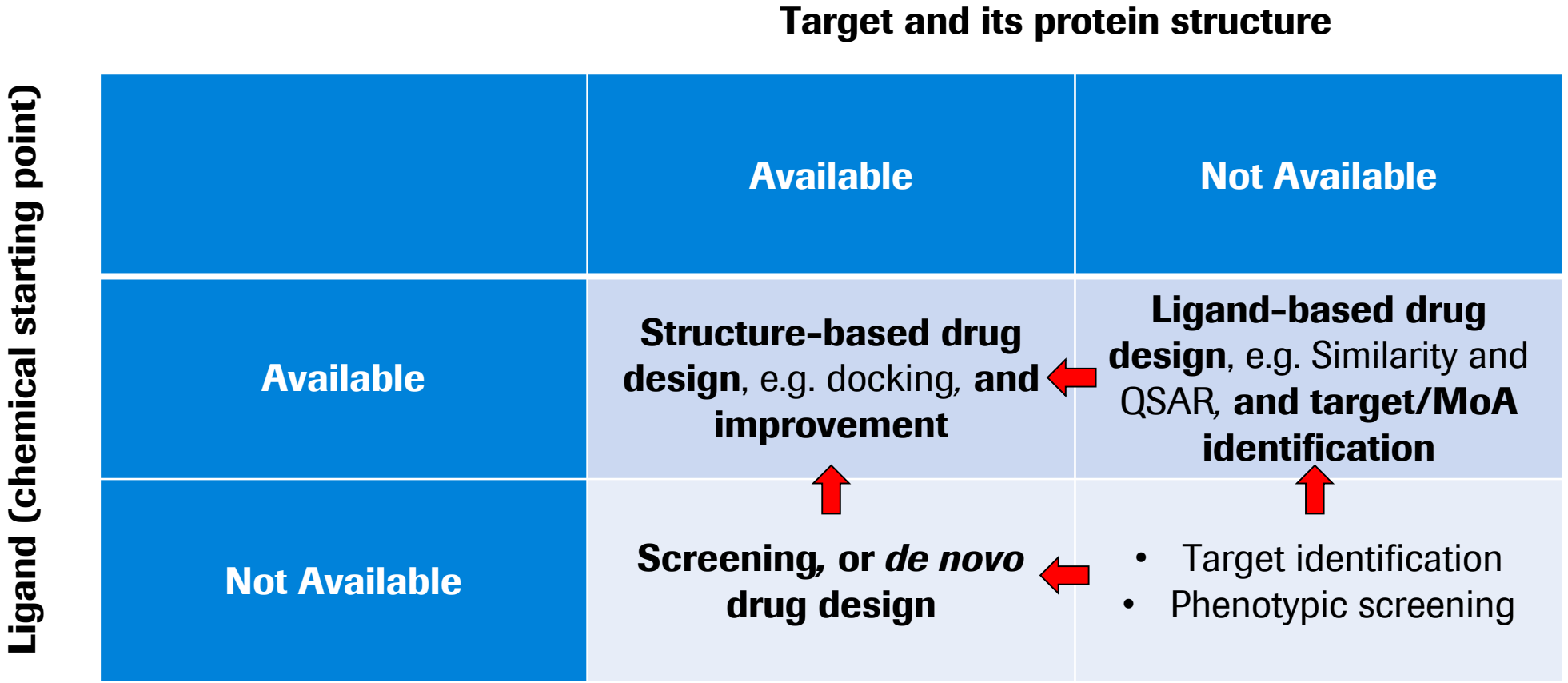
The simplified screening cascade

1. Compound library construction
2. Testing compounds with assays
3. Hit identification
4. Get co-structure of protein (targets or non-targets) and hits
5. Modify drug structure (*drug design)*
6. Analog synthesis and testing (back to step 2)
7. Multidimensional Optimization (MDO): potency, selectivity, safety, bioavailability
8. Further *in vitro*, *ex vivo*, and *in vivo* testing, and preclinical development



A schematic presentation of structure-based drug discovery

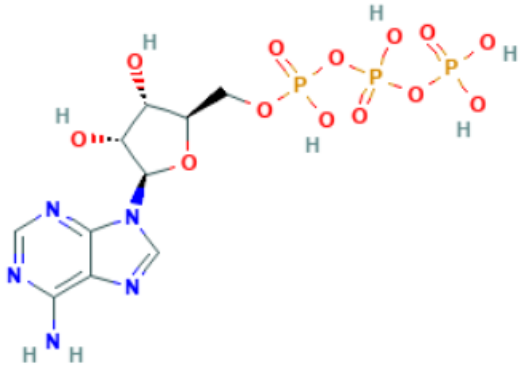
Principles of screening and drug design – an interactive process



QSAR= quantitative structure activity relationship; MoA= mechanism of action, or mode of action

Lipinski's Rule of Five of small-molecule drugs

- No more than **5 hydrogen-bond donors,** *e.g.* the total number of nitrogen–hydrogen and oxygen–hydrogen bonds
- No more than **10 hydrogen-bond acceptors**, *e.g.* all nitrogen or oxygen atoms
- A **molecular mass** less than **500 Daltons**, approximately 500 g/mol.
  - As a reference: ATP has a molecular mass of ~507.
- An **octanol-water partition coefficient (log P)** that does not exceed **5.** (10-based)



ATP

Why PLX4032 (vemurafenib) was finally chosen as the drug?

LETTER

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Clinical efficacy of a RAF inhibitor needs broad target blockade in *BRAF*-mutant melanoma

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B-RAF is the most frequently mutated protein kinase in human cancers[1]. The finding that oncogenic mutations in *BRAF* are common in melanoma[2], followed by the demonstration that these tumours are dependent on the RAF/MEK/ERK pathway[3], offered hope that inhibition of B-RAF kinase activity could benefit melanoma patients. Herein, we describe the structure-guided discovery of PLX4032 (RG7204), a potent inhibitor of oncogenic B-RAF kinase activity. Preclinical experiments demonstrated that PLX4032 selectively blocked the RAF/MEK/ERK pathway in *BRAF* mutant cells and caused regression of *BRAF* mutant xenografts[4]. Toxicology studies confirmed a wide safety margin consistent with the high degree of selectivity, enabling Phase 1 clinical trials using a crystalline formulation of PLX4032 (ref. 5). In a subset of melanoma patients, pathway inhibition was monitored in paired biopsy specimens collected before treatment initiation and following two weeks of treatment. This analysis revealed substantial inhibition of ERK phosphorylation, yet clinical evaluation did not show tumour regressions. At higher drug exposures afforded by a new amorphous drug formulation[4,5], greater than 80% inhibition of ERK phosphorylation in the tumours of patients correlated with clinical response. Indeed, the Phase 1 clinical data revealed a remarkably high 81% response rate in metastatic melanoma patients treated at an oral dose of 960 mg twice daily[5]. These data demonstrate that *BRAF*-mutant melanomas are highly dependent on B-RAF kinase activity.

PLX4032 belongs to a family of mutant B-RAF kinase inhibitors discovered using a scaffold-based drug design approach[6]. The crystallography-guided approach allowed optimization of a compound with modest preference for the mutated form of B-RAF (B-RAF(V600E)) in comparison to wild-type B-RAF. Supplementary Table 1 summarizes the differential ability for PLX4032 to inhibit the activity of over 200 kinases. PLX4032 displays similar potency for B-RAF(V600E) (31 nM) and c-RAF-1 (48 nM) and selectivity against many other kinases, including wild-type B-RAF (100 nM). Whereas the vast majority of kinases are minimally affected, several were found that were also inhibited at <100 nM concentrations in biochemical assays; to date, inhibition of these non-RAF kinases such as ACK1 (also known as TNK2), KHS1 (also known as MAP4K5) and SRMS has not been tested in cellular assays. As previously demonstrated for the related B-RAF inhibitor PLX4720 (ref. 6), the biochemical selectivity of PLX4032 translates to cellular selectivity: potent inhibition of ERK phosphorylation and cell proliferation occurs exclusively in *BRAF*-mutant cell lines[4].

PLX4032 was co-crystallized with a protein construct that contained the kinase domain of B-RAF(V600E). PLX4032 (Fig. 1a) binds in the active site of one of the protomers in the non-crystallographic-symmetry related dimer (Fig. 1). As previously described for the related RAF inhibitor PLX4720 (PDB ID: 3C4C)[6], the PLX4032-bound protomer adopts the DFG-in conformation to enable the formation of a unique hydrogen bond between the backbone NH of Asp 594 and the sulfonamide nitrogen of PLX4032 (Fig. 1b). In addition, PLX4032-binding causes an outward shift in the regulatory αC helix, which may explain why the effect of PLX4720 and PLX4032 on RAF dimerization is in stark contrast to other RAF inhibitors such as AZD-628 and GDC-0879 (Fig. 1c)[7]. The apo-protomer displays the DFG-in conformation with the activation loop locked away from the ATP-binding site by a salt-bridge between Glu 600 and Lys 507 (Fig. 1d).

In *BRAF(V600E)*-mutant xenograft studies, PLX4032 demonstrated dose-dependent inhibition of tumour growth, with higher exposures resulting in tumour regression (Fig. 2a and ref. 4). Efficacy could be demonstrated in cell lines and xenografts bearing either homozygous or heterozygous *BRAF* mutations. By contrast, no effect was observed on melanoma xenograft growth if both *BRAF* alleles were wild-type[4,6]. Due to their consistent pharmacokinetics in rodents, PLX4032 and PLX4720 were prioritized over a panel of related compounds that all had similar activities [illegible]. For further drug development, PLX4032 was chosen (over PLX4720) because its pharmacokinetic properties scaled more favourably in beagle dogs and cynomolgus monkeys.

In order to estimate PLX4032 exposures (as defined by AUC_{0-24}, the area under the plasma concentration time curve over the dosing period of 24 h) that correlated with tumour response, conventionally formulated daily oral doses of PLX4032 were administered in the *BRAF(V600E)*-bearing colorectal cancer COLO205 xenograft model. In this model, tumour growth inhibition was modest at 6 mg kg^{-1} (AUC_{0-24} ~ 50 μM h), tumour stabilization was seen at 20 mg kg^{-1} once daily (QD) (AUC_{0-24} ~ 200 μM h), and significant tumour regressions were observed at 20 mg kg^{-1} twice daily (BID) (AUC_{0-24} ~ 300 μM h). *BRAF(V600E)*-bearing melanoma xenograft models, including NCI-LOX and COLO829 are also sensitive to PLX4032 (ref. 4).

Rats and beagle dogs were dosed for 28 days with increasing doses up to 1,000 mg kg^{-1} day^{-1}, and no toxicity was detected at any dose level. Likewise, no adverse effects were detected in a standard battery of safety pharmacology studies. Subsequent toxicology studies of longer duration, 26 weeks in rats and 13 weeks in dogs, further confirmed the tolerability of the compound. This safety profile was achieved in spite of very high compound exposures, reaching 2,600 μM h in rats and 820 μM h in dogs. The rat exposures exceeded those that were effective in patients (see below). Importantly, no histological changes were observed in the skin in any animal at any dose or duration of treatment, contrasting to results observed with other RAF inhibitors[7].

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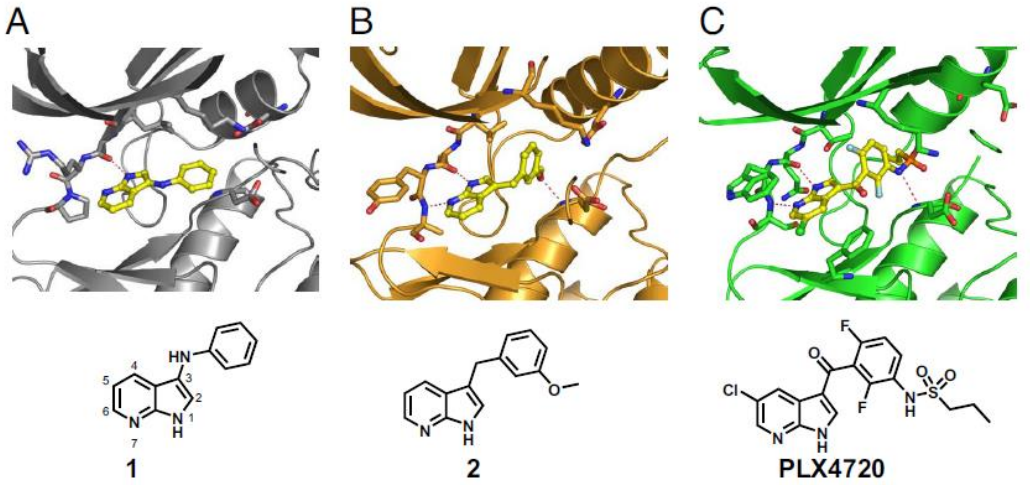

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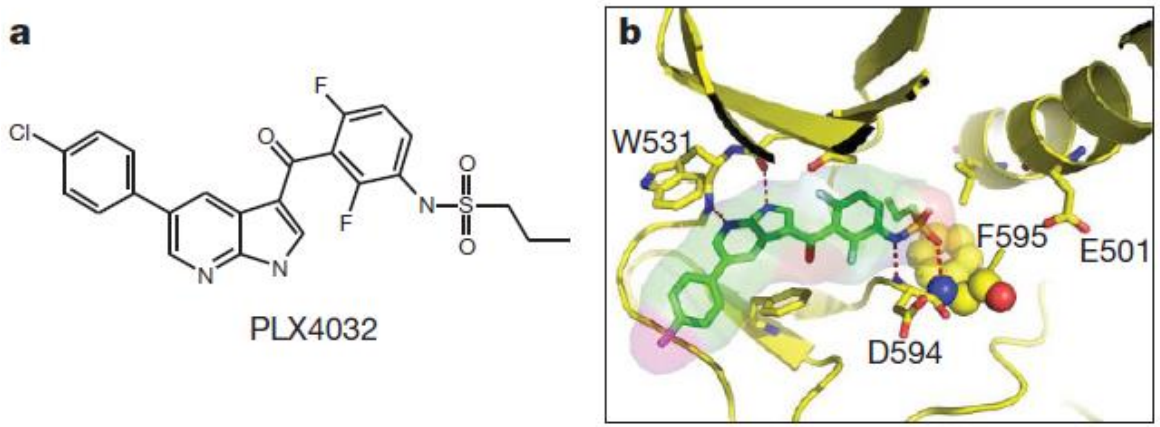

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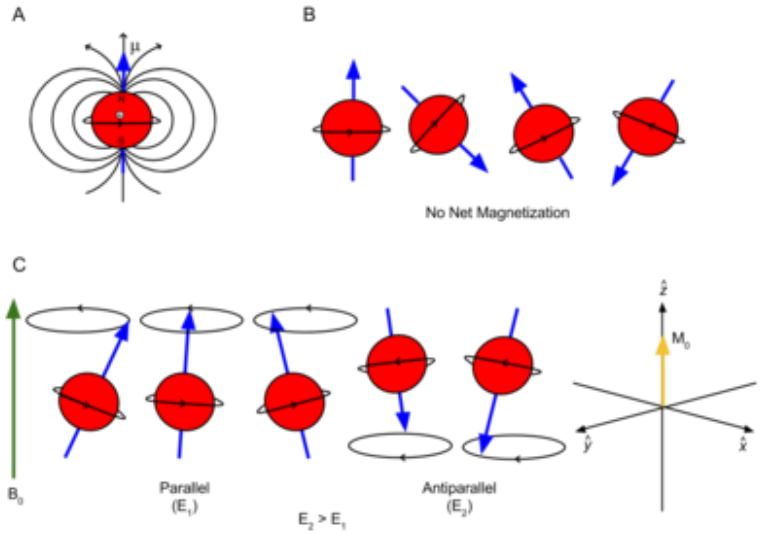
Selected mathematical concepts

- **Affinity**
  - The (bio)physical view
  - The (bio)chemical view
- **The Michaelis–Menton model and enzymatic kinetics**
- Mathematical techniques for structure determination: X-ray, NMR, and CryoEM (post-reading)
- Example of structure-based drug design: molecular docking
- Example of ligand-based drug design: similarity and quantitative structure-activity relationship (QSAR)

Mathematics behind approaches to determine molecular structure

- **Mathematical and physical foundations**
  - Mathematical techniques used in biophysics
  - Background on imaging physics (http://xrayphysics.com)
- **X-ray diffraction by electrons**
  - An AMS Feature Column by Tony Phillips
  - Stanford open course *Fourier transform and its applications*
- **Nuclear Magnetic Resonance (NMR)**
  - A beautiful video tutorial about the principles of magnetic resonance imaging (MRI), which is a variant of NMR
- **Cryo electronic microscopy (CryoEM)**
  - A three-minute introduction to CryoEM
  - Nobel Prize Talk by Joachim Frank
  - Talk on Mathematics of CryoEM, by Prof Amit Singer, with a manuscript available at arXiv: https://arxiv.org/abs/1803.06714

Swiss Light Source, the synchrotron at the Paul Scherrer Institute (PSI), copyright of PSI



Adapted from Bushberg JT, *The Essential Physics of Medical Imaging: Lippincott Williams & Wilkins; 2002*

Downloaded from http://199.116.233.101/index.php/Physics_of_MRI

Summary and Q&A

The Great Wave off Kanagawa『神奈川沖浪裏』, by Katsushika Hokusai, downloaded from wikimedia