NEXT UP:

- **Overview of structural bioinformatics**
  - Major motivations, goals and challenges
- **Fundamentals of protein structure**
  - Composition, form, forces and dynamics
- **Representing and interpreting protein structure**
  - Modeling energy as a function of structure
- **Example application areas**
  - Predicting functional dynamics & **drug discovery**

THE TRADITIONAL EMPIRICAL PATH TO DRUG DISCOVERY

Compound library
(commercial, in-house, synthetic, natural)
↘
High throughput screening
(HTS)
↘
Hit confirmation
↘
Lead compounds
(e.g., μM K_d)
↘
Lead optimization
(Medicinal chemistry)
↓
Potent drug candidates
(nM K_d)
←
Animal and clinical evaluation

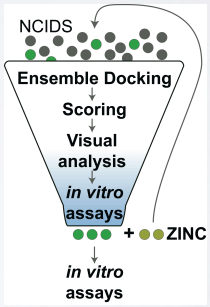
COMPUTER-AIDED LIGAND DESIGN

Aims to reduce number of compounds synthesized and assayed

Lower costs

Reduce chemical waste

Facilitate faster progress



Two main approaches:

(1). **Receptor/Target-Based**

(2). **Ligand/Drug-Based**

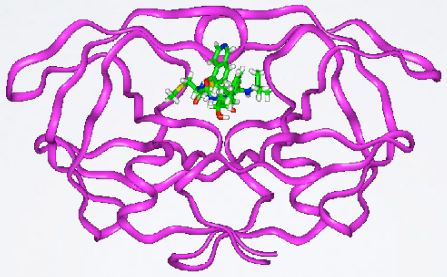
Two main approaches:

(1). **Receptor/Target-Based**

(2). **Ligand/Drug-Based**

SCENARIO I: RECEPTOR-BASED DRUG DISCOVERY

Structure of Targeted Protein Known: Structure-Based Drug Discovery

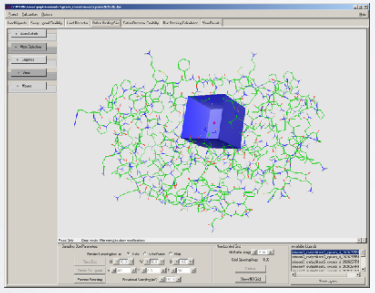


HIV Protease/KNI-272 complex

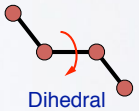
PROTEIN-LIGAND DOCKING

Structure-Based Ligand Design

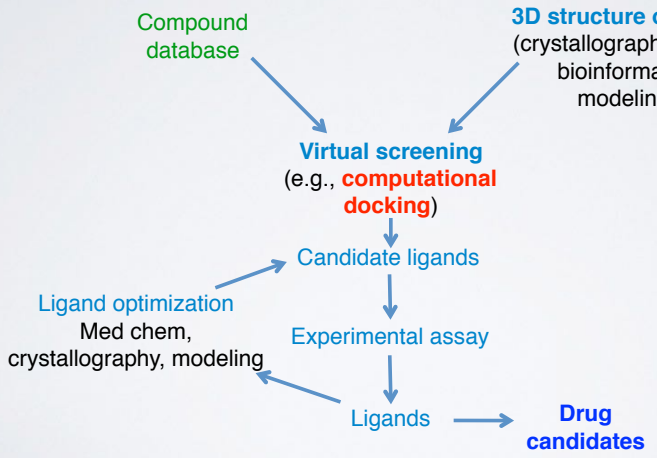
Docking software
Search for structure of lowest energy



Potential function
Energy as function of structure



STRUCTURE-BASED VIRTUAL SCREENING



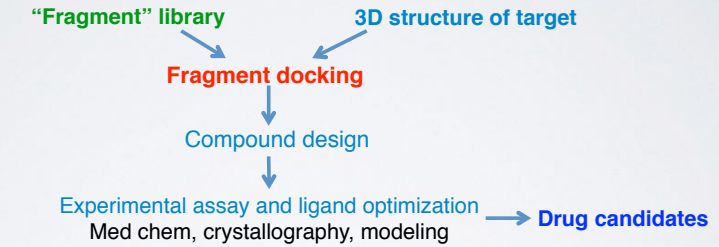
COMPOUND LIBRARIES

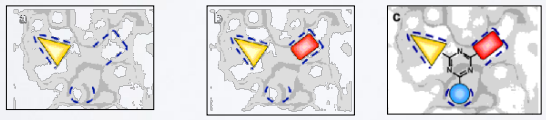
Commercial (in-house pharma)

Government (NIH)

Academia

FRAGMENTAL STRUCTURE-BASED SCREENING

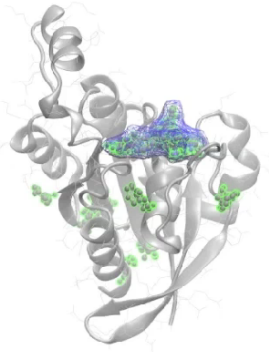


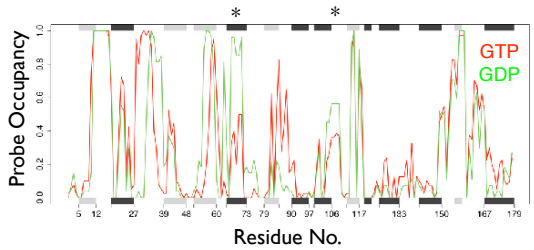


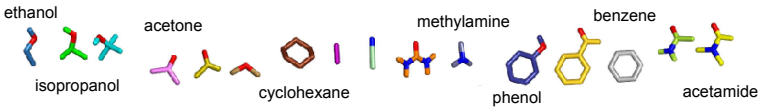
http://www.beilstein-institut.de/bozen2002/proceedings/Jhoti/jhoti.html

Multiple non active-site pockets identified

Small organic probe fragment affinities map multiple potential binding sites across the structural ensemble.



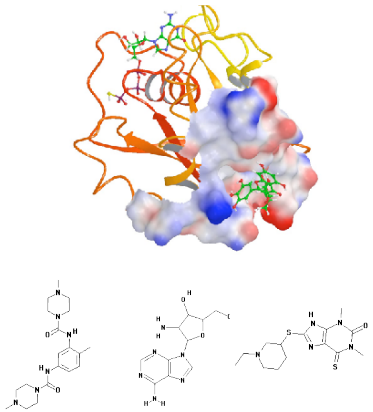




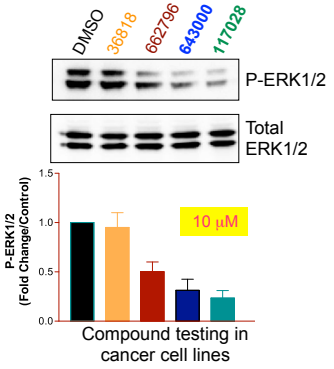
Ensemble docking & candidate inhibitor testing

Top hits from ensemble docking against distal pockets were tested for inhibitory effects on basal ERK activity in glioblastoma cell lines.

Ensemble computational docking

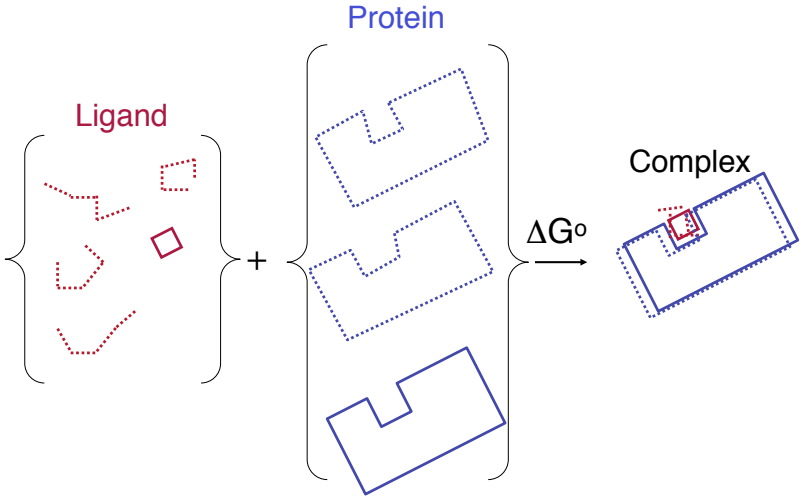


Compound effect on U251 cell line



PLoS One (2011, 2012)

Proteins and Ligand are Flexible



COMMON SIMPLIFICATIONS USED IN PHYSICS-BASED DOCKING

Quantum effects approximated classically

Protein often held rigid

Configurational entropy neglected

Influence of water treated crudely

Two main approaches:

(1). **Receptor/Target-Based**

(2). **Ligand/Drug-Based**

Do it Yourself!

Hand-on time!

https://bioboot.github.io/bggn213_f17/lectures/#12

You can use the classroom computers or your own laptops. If you are using your laptops then you will need to install **VMD** and **MGLTools**

Two main approaches:

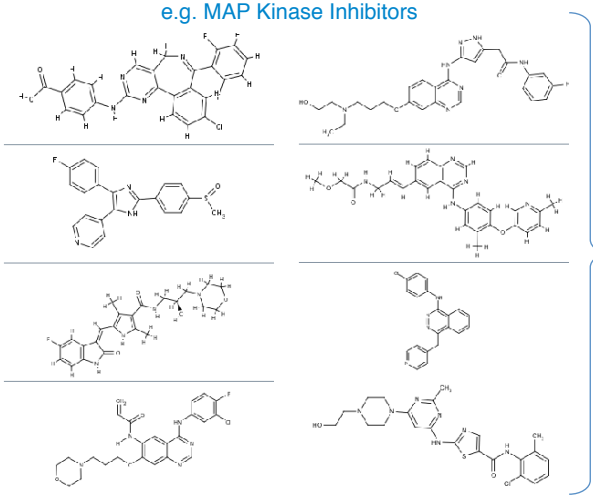
(1). **Receptor/Target-Based**

(2). **Ligand/Drug-Based**

Scenario 2

Structure of Targeted Protein Unknown:
Ligand-Based Drug Discovery

e.g. MAP Kinase Inhibitors



Using knowledge of existing inhibitors to discover more

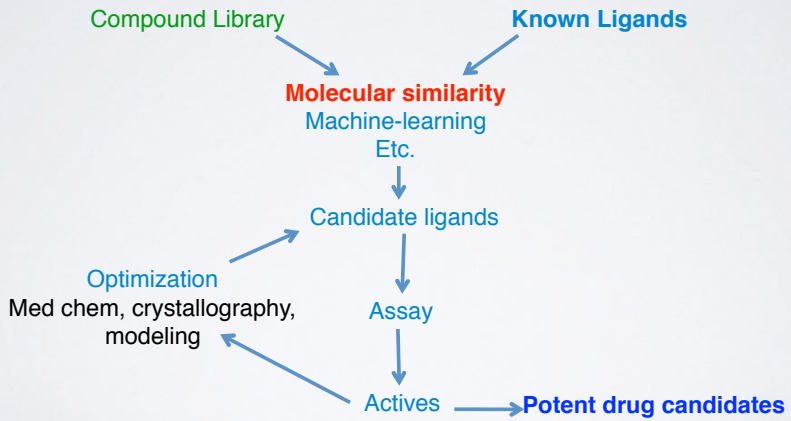
Why Look for Another Ligand if You Already Have Some?

Experimental screening generated some ligands, but they don't bind tightly enough

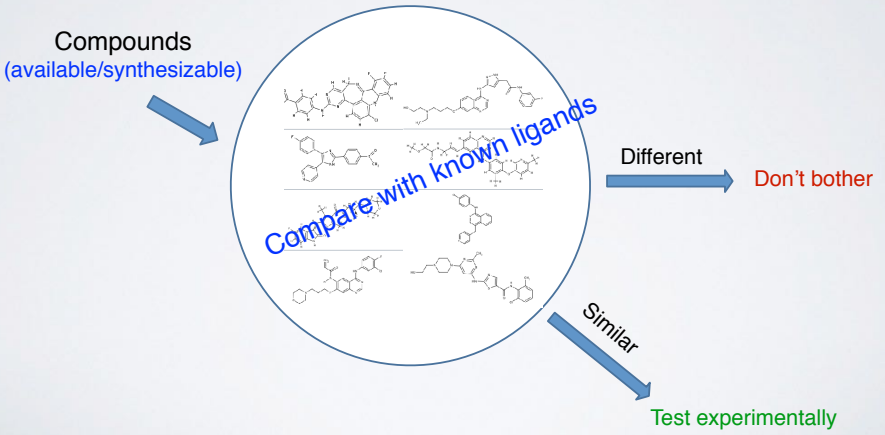
A company wants to work around another company's chemical patents

An high-affinity ligand is toxic, is not well-absorbed, difficult to synthesize etc.

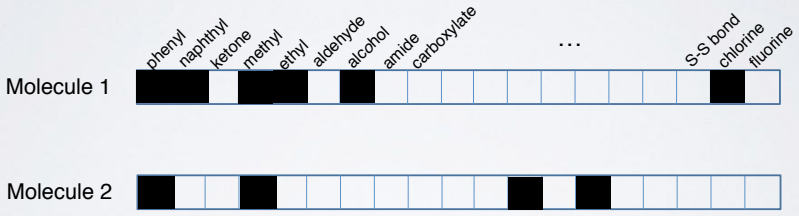
LIGAND-BASED VIRTUAL SCREENING



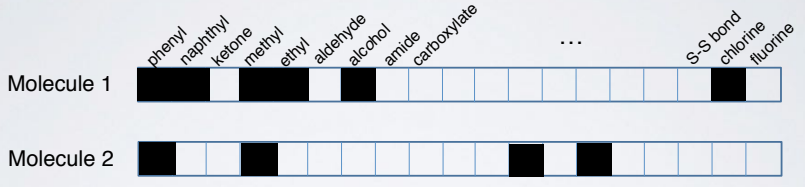
CHEMICAL SIMILARITY LIGAND-BASED DRUG-DISCOVERY



CHEMICAL FINGERPRINTS
BINARY STRUCTURE KEYS

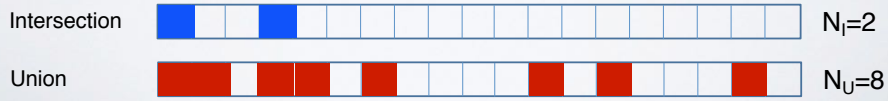


CHEMICAL SIMILARITY FROM FINGERPRINTS



Tanimoto Similarity (or Jaccard Index), T

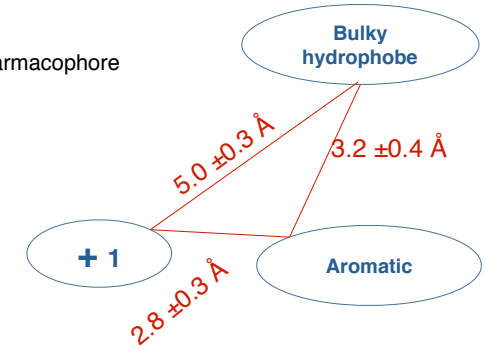
$$T \equiv \frac{N_I}{N_U} = 0.25$$



Pharmacophore Models

Φάρμακο (drug) + Φορά (carry)

A 3-point pharmacophore

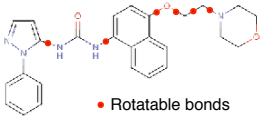


Molecular Descriptors

More abstract than chemical fingerprints

Physical descriptors

- molecular weight
- charge
- dipole moment
- number of H-bond donors/acceptors
- number of rotatable bonds
- hydrophobicity (log P and clogP)



Topological

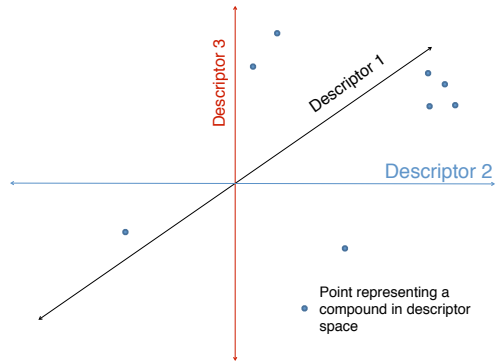
- branching index
- measures of linearity vs interconnectedness

Etc. etc.

A High-Dimensional "Chemical Space"

Each compound is at a point in an n-dimensional space

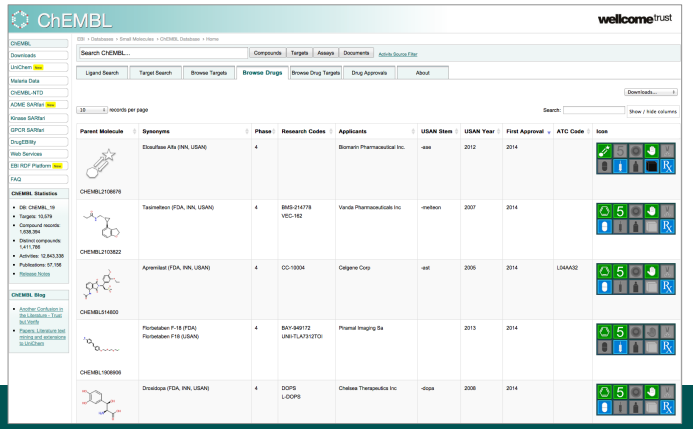
Compounds with similar properties are near each other



Apply **multivariate statistics** and **machine learning** for descriptor-selection. (e.g. partial least squares, support vector machines, random forest, deep learning etc.)

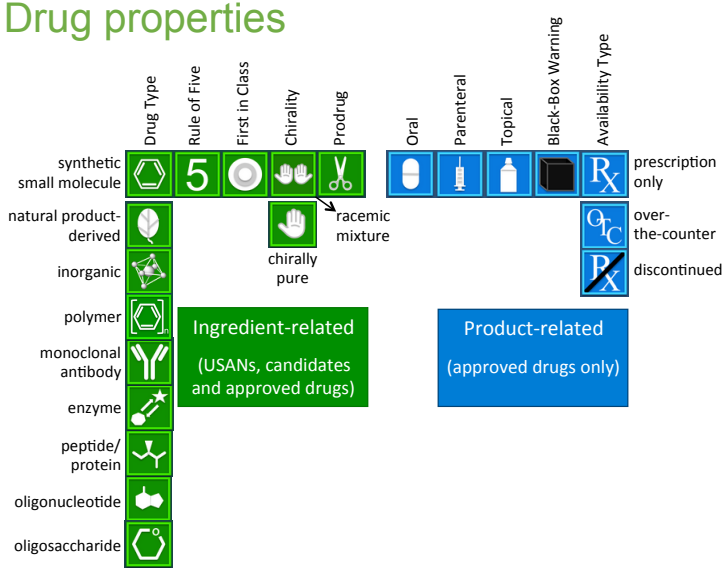
Approved drugs and clinical candidates

- Catalogue approved drugs and clinical candidates from FDA Orange Book, and USAN applications
- Small molecules and biotherapeutics



EMBL-EBI

Drug properties



EMBL-EBI

LIPINSKI'S RULE OF FIVE

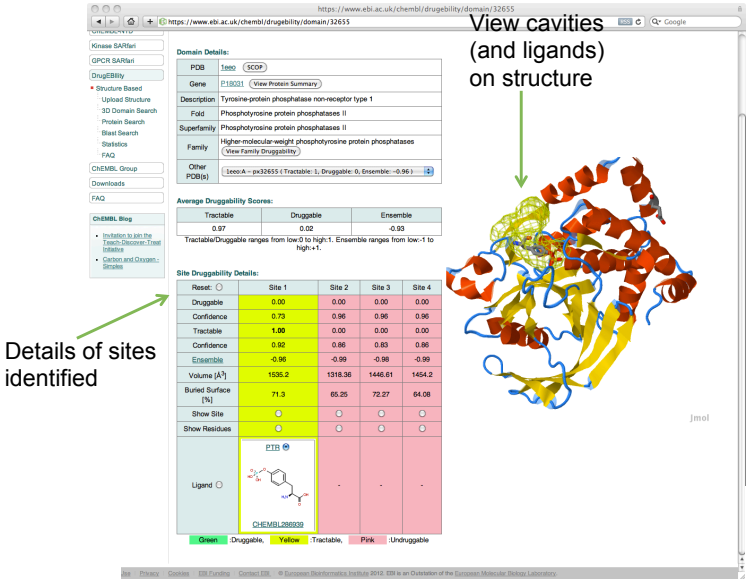
Lipinski's rule of five states that, in general, an orally active drug has no more than one violation of the following criteria:

- Not more than 5 hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms)
- Not more than 10 hydrogen bond acceptors (nitrogen or oxygen atoms)
- A molecular mass less than 500 daltons
- An octanol-water partition coefficient log P not greater than 5

Rules for drug discovery success

- Set of approved drugs or medicinal chemistry compounds and their targets can be used to derive rules for drug discovery success (or failure):
  - What features make a successful drug target?
  - What features make a protein druggable by small molecules?
  - What features of a compound contribute to good oral bioavailability?
  - What chemical groups may be associated with toxicity?

Druggability prediction



Examples

nature Vol 460 | 16 July 2009 | doi:10.1038/nature08160

ARTICLES

The genome of the blood fluke *Schistosoma mansoni*

Matthew Berriman[1], Brian J. Haas[3]†, Philip T. LoVerde[4], R. Alan Wilson[5], Gary P. Dillon[5], Gustavo C. Cerqueira[6,7,8], Susan T. Mashiyama[9,10], Bissan Al-Lazikani[11], Luiza F. Andrade[12], Peter D. Ashton[4], Martin A. Aslett[1], Daniella C. Bartholomeu[3]†, Gaelle Blandin[3], Conor R. Caffrey[9], Avril Coghlan[13], Richard Coulson[2], Tim A. Day[14], Art Delcher[7], Ricardo DeMarco[5,15,16], Appolinaire Djikeng[3], Tina Eyre[1], John A. Gamble[1], Elodie Ghedin[3]†, Yong Gu[1], Christiane Hertz-Fowler[1], Hirohisha Hirai[17], Yuriko Hirai[17], Robin Houston[1], Alasdair Ivens[1]†, David A. Johnston[18]†, Daniela Lacerda[3]†, Camila D. Macedo[6,8], Paul McVeigh[14], Zemin Ning[1], Guilherme Oliveira[12], John P. Overington[2], Julian Parkhill[1], Mihaela Pertea[7], Raymond J. Pierce[19], Anna V. Protasio[1], Michael A. Quail[1], Marie-Adèle Rajandream[1], Jane Rogers[1]†, Mohammed Sajid[9]†, Steven L. Salzberg[7,8], Mario Stanke[20], Adrian R. Tivey[1], Owen White[3]†, David L. Williams[21]†, Jennifer Wortman[3]†, Wenjie Wu[4]†, Mostafa Zamanian[14], Adhemar Zerlotini[11], Claire M. Fraser-Liggett[3]†, Barclay G. Barrell[1] & Najib M. El-Sayed[3,6,7,8]

Schistosoma mansoni is responsible for the neglected tropical disease schistosomiasis that affects 210 million people in 76 countries. Here we present analysis of the 363 megabase nuclear genome of the blood fluke. It encodes at least 11,809 genes, with an unusual intron size distribution, and new families of micro-exon genes that undergo frequent alternative splicing. As the first sequenced flatworm, and a representative of the Lophotrochozoa, it offers insights into early events in the evolution of the animals, including the development of a body pattern with bilateral symmetry, and the development of tissues into organs. Our analysis has been informed by the need to find new drug targets. The deficits in lipid metabolism that make schistosomes dependent on the host are revealed, and the identification of membrane receptors, ion channels and more than 300 proteases provide new insights into the biology of the life cycle and new targets. Bioinformatics approaches have identified metabolic chokepoints, and a chemogenomic screen has pinpointed schistosome proteins for which existing drugs may be active. The information generated provides an invaluable resource for the research community to develop much needed new control tools for the treatment and eradication of this important and neglected disease.

Schistosomiasis is a neglected tropical disease that ranks with malaria and tuberculosis as a major source of morbidity affecting approximately 210 million people in 76 countries, despite strenuous control efforts[1]. It is caused by blood flukes of the genus *Schistosoma* (phylum Platyhelminthes), which exhibit dioecy and have complex life cycles comprising several morphologically distinct phenotypes in definitive human and intermediate snail hosts. *Schistosoma mansoni*, one of the three major human species, occurs across much of sub-Saharan Africa, parts of the Middle East, Brazil, Venezuela and some West Indian islands. The mature flukes dwell in the human portal vasculature, depositing eggs in the intestinal wall that either pass to the gut lumen and are voided in the faeces, or travel to the liver where they trigger immune-mediated granuloma formation and peri-portal fibrosis[2]. Approximately 280,000 deaths per annum are attributable to schistosomiasis in sub-Saharan Africa alone[3]. However, the disease is better known for its chronicity and debilitating morbidity[4]. A single drug, praziquantel, is almost exclusively used to treat the infection but this does not prevent reinfection, and with the large-scale control programmes in place, there is concern about the development of drug resistance. Indeed, resistance can be selected for in the laboratory and there are reports of increased drug tolerance in the field[5].

NATURE CHEMISTRY | ARTICLE

Quantifying the chemical beauty of drugs

G. Richard Bickerton, Gaia V. Paolini, Jérémy Besnard, Sorel Muresan & Andrew L. Hopkins

Affiliations | Contributions | Corresponding author

Nature Chemistry **4**, 90–98 (2012) | doi:10.1038/nchem.1243
Received 01 September 2011 | Accepted 02 December 2011 | Published online 24 January 2012

Citation | Reprints | Rights & permissions | Article metrics

Abstract

Abstract · References · Author information · Supplementary information

Drug-likeness is a key consideration when selecting compounds during the early stages of drug discovery. However, evaluation of drug-likeness in absolute terms does not reflect adequately the whole spectrum of compound quality. More worryingly, widely used rules may inadvertently foster undesirable molecular property inflation as they permit the encroachment of rule-compliant compounds towards their boundaries. We propose a measure of drug-likeness based on the concept of desirability called the quantitative estimate of drug-likeness (QED). The empirical rationale of QED reflects the underlying distribution of molecular properties. QED is intuitive, transparent, straightforward to implement in many practical settings and allows compounds to be ranked by their relative merit. We extended the utility of QED by applying it to the problem of molecular target druggability assessment by prioritizing a large set of published bioactive compounds. The measure may also capture the abstract notion of aesthetics in medicinal chemistry.

Subject terms: Pharmacology · Theoretical chemistry

At a glance

Figures | Compounds

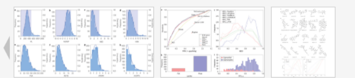


Figure 1 Figure 2 Figure 3

First | 1-3 of 4 | Last

Target prediction models

- Active compounds from ChEMBL can be used to train target prediction models
- Variety of methods used
  - Multi-Category Naïve Bayesian Classifier (e.g., ChEMBL)
  - Chemical similarity between ligand sets (e.g., SEA)
  - 3D similarity between ligands (e.g., SwissTargetPrediction)
  - Protein and ligand descriptors (e.g., Proteochemometric models)
- Open source tools available for many methods
  - E.g., Scikit-learn with RDKit

  Examples at: https://github.com/chembl/mychembl/blob/master/ipython_notebooks

Examples

PLOS | ONE

RESEARCH ARTICLE

Mycobacterial Dihydrofolate Reductase Inhibitors Identified Using Chemogenomic Methods and *In Vitro* Validation

Grace Mugumbate[1], Katherine A. Abrahams[2], Jonathan A. G. Cox[2], George Papadatos[1], Gerard van Westen[1], Joël Lelièvre[3], Szymon T. Calus[2], Nicholas J. Loman[2], Lluis Ballell[3], David Barros[3], John P. Overington[1]*, Gurdyal S. Besra[2]*

1 European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI), Wellcome Trust Genome Campus, Hinxton, Cambridge, United Kingdom, 2 Institute of Microbiology and Infection (IMI), School of Biosciences, University of Birmingham, Edgbaston, Birmingham, United Kingdom, 3 Diseases of the Developing World, GlaxoSmithKline, Severo Ochoa 2, 28760 Tres Cantos, Madrid, Spain

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OPEN ACCESS

Citation: Mugumbate G, Abrahams KA, Cox JAG, Papadatos G, van Westen G, Lelièvre J, et al. (2015) Mycobacterial Dihydrofolate Reductase Inhibitors Identified Using Chemogenomic Methods and *In Vitro* Validation. PLoS ONE 10(3): e0121492. doi:10.1371/journal.pone.0121492

Academic Editor: Anil Kumar Tyagi, University of Delhi, INDIA

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: GM and GvW are grateful to EMBL and Marie Curie Actions for funding this work. GSB acknowledges support in the form of a Personal Research Chair from Mr. James Bardrick, a Royal Society Wolfson Research Merit Award, The Wellcome Trust (681569/Z/06/Z), and the Medical Research Council (MR/K012118/1). The research also received funding from the European Union's 7th framework programme (FP7-2007–2013) under grant agreement ORCHID no. 261378. The funders had no

Abstract

The lack of success in target-based screening approaches to the discovery of antibacterial agents has led to reemergence of phenotypic screening as a successful approach of identifying bioactive, antibacterial compounds. A challenge though with this route is then to identify the molecular target(s) and mechanism of action of the hits. This target identification, or deorphanization step, is often essential in further optimization and validation studies. Direct experimental identification of the molecular target of a screening hit is often complex, precisely because the properties and specificity of the hit are not yet optimized against that target, and so many false positives are often obtained. An alternative is to use computational, predictive, approaches to hypothesize a mechanism of action, which can then be validated in a more directed and efficient manner. Specifically here we present experimental validation of an *in silico* prediction from a large-scale screen performed against *Mycobacterium tuberculosis* (*Mtb*), the causative agent of tuberculosis. The **two** potent anti-tubercular compounds studied in this case, belonging to the tetrahydro-1,3,5-triazin-2-amine (THT) family, were predicted and confirmed to be an inhibitor of dihydrofolate reductase (DHFR), a known essential *Mtb* gene, and already clinically validated as a drug target. Given the large number of similar screening data sets shared amongst the community, this *in vitro* validation of these target predictions gives weight to computational approaches to establish the mechanism of action (MoA) of novel screening hit.

Introduction

The human pathogen, *Mycobacterium tuberculosis* (*Mtb*) is the causative agent of tuberculosis (TB), an infectious disease that is widespread, infecting around one third of the world's population [1]. The discovery of streptomycin in 1943, and the subsequent discovery and

PLOS ONE | DOI:10.1371/journal.pone.0121492 March 23, 2015 1 / 17

ARTICLE doi:10.1038/nature11159

Large-scale prediction and testing of drug activity on side-effect targets

Eugen Lounkine[1]*, Michael J. Keiser[2,3]*, Steven Whitebread[1], Dmitri Mikhailov[1], Jacques Hamon[4], Jeremy L. Jenkins[1], Paul Lavan[4], Eckhard Weber[4], Allison K. Doak[3], Serge Côté[4], Brian K. Shoichet[3] & Laszlo Urban[1]

Discovering the unintended 'off-targets' that predict adverse drug reactions is daunting by empirical methods alone. Drugs can act on several protein targets, some of which can be unrelated by conventional molecular metrics, and hundreds of proteins have been implicated in side effects. Here we use a computational strategy to predict the activity of 656 marketed drugs on 73 unintended 'side-effect' targets. Approximately half of the predictions were confirmed, either from proprietary databases unknown to the method or by new experimental assays. Affinities for these new off-targets ranged from 1 nM to 30 μM. To explore relevance, we developed an association metric to prioritize those new off-targets that explained side effects better than any known target of a given drug, creating a drug–target–adverse drug reaction network. Among these new associations was the prediction that the abdominal pain side effect of the synthetic oestrogen chlorotrianisene was mediated through its newly discovered inhibition of the enzyme cyclooxygenase-1. The clinical relevance of this inhibition was borne out in whole human blood platelet aggregation assays. This approach may have wide application to de-risking toxicological liabilities in drug discovery.

21 JUNE 2012 | VOL 486 | NATURE | 361

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CAUTIONARY NOTES

- **"Everything should be made as simple as it can be but not simpler"**

  A model is **never perfect**. A model that is not quantitatively accurate in every respect does not preclude one from establishing results relevant to our understanding of biomolecules as long as the biophysics of the model are properly understood and explored.

- **Calibration of the parameters is an ongoing and imperfect process**

  Questions and hypotheses should always be designed such that they do not depend crucially on the precise numbers used for the various parameters.

- **A computational model is rarely universally right or wrong**

  A model may be accurate in some regards, inaccurate in others. These subtleties can only be uncovered by comparing to all available experimental data.

SUMMARY

- Structural bioinformatics is computer aided structural biology
- Described major motivations, goals and challenges of structural bioinformatics
- Reviewed the fundamentals of protein structure
- Introduced both physics and knowledge based modeling approaches for describing the structure, energetics and dynamics of proteins computationally
- Introduced both structure and ligand based bioinformatics approaches for drug discovery and design

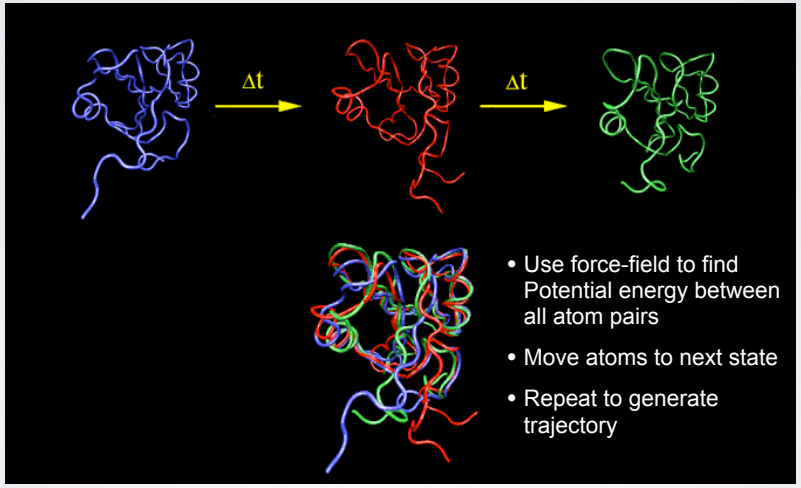
NEXT UP:

- **Overview of structural bioinformatics**
  - Major motivations, goals and challenges
- **Fundamentals of protein structure**
  - Composition, form, forces and dynamics
- **Representing and interpreting protein structure**
  - Modeling energy as a function of structure
- **Example application areas**
  - Predicting **functional dynamics** & drug discovery

PREDICTING FUNCTIONAL DYNAMICS

- **Proteins are intrinsically flexible molecules with internal motions that are often intimately coupled to their biochemical function**
  - E.g. ligand and substrate binding, conformational activation, allosteric regulation, etc.
- **Thus knowledge of dynamics can provide a deeper understanding of the mapping of structure to function**
  - **Molecular dynamics** (MD) and **normal mode analysis** (NMA) are two major methods for predicting and characterizing molecular motions and their properties

MOLECULAR DYNAMICS SIMULATION



McCammon, Gelin & Karplus, *Nature* (1977)
[See: https://www.youtube.com/watch?v=ui1ZysMFcKk]

- Divide **time** into discrete (~1fs) **time steps** (**Δt**)
  (for integrating equations of motion, see below)

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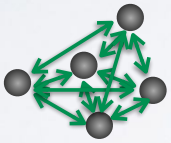
- At each time step calculate pair-wise atomic **forces** (***F(t)***)
  (by evaluating **force-field** gradient)



Nucleic motion described classically

$$m_i \frac{d^2}{dt^2}\vec{R}_i = -\vec{\nabla}_i E(\vec{R})$$

Empirical force field

$$E(\vec{R}) = \sum_{\text{bonded}} E_i(\vec{R}) + \sum_{\text{non-bonded}} E_i(\vec{R})$$

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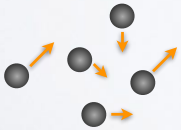
- Use the forces to calculate **velocities** and move atoms to new **positions**
  (by integrating numerically via the "leapfrog" scheme)



$$\begin{aligned} v(t+\frac{\Delta t}{2}) &= v(t-\frac{\Delta t}{2}) + \frac{F(t)}{m}\Delta t \\ r(t+\Delta t) &= r(t) + v(t+\frac{\Delta t}{2})\Delta t \end{aligned}$$

BASIC ANATOMY OF A MD SIMULATION

- Divide **time** into discrete (~1fs) **time steps** (**Δt**)
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- At each time step calculate pair-wise atomic **forces** (***F(t)***)
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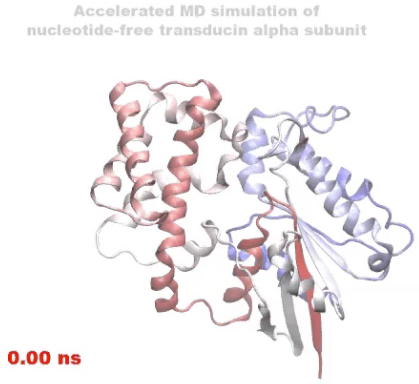
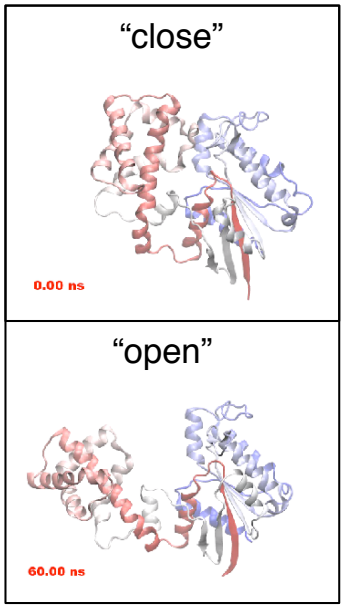
- **REPEAT, (iterate many, many times… 1ms = 10^{12} time steps)**

$$\begin{aligned} v(t+\frac{\Delta t}{2}) &= v(t-\frac{\Delta t}{2}) + \frac{F(t)}{m}\Delta t \\ r(t+\Delta t) &= r(t) + v(t+\frac{\Delta t}{2})\Delta t \end{aligned}$$

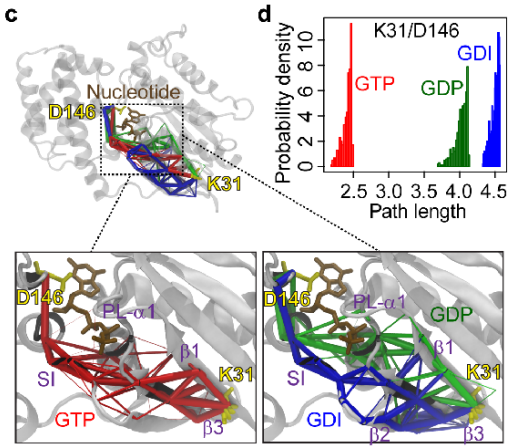
MD Prediction of Functional Motions





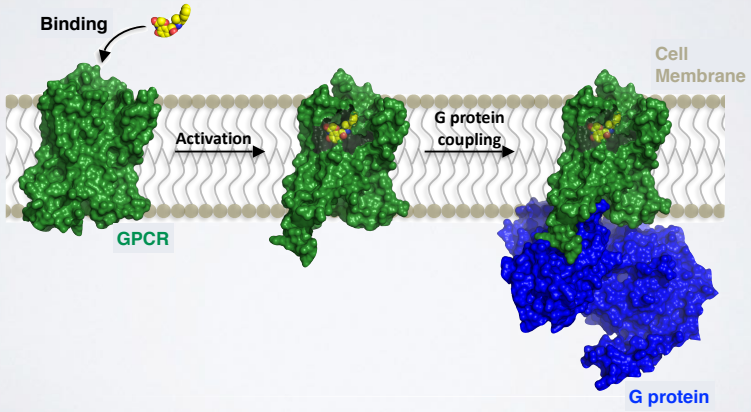
Yao and Grant, Biophys J. (2013)

Simulations Identify Key Residues Mediating Dynamic Activation

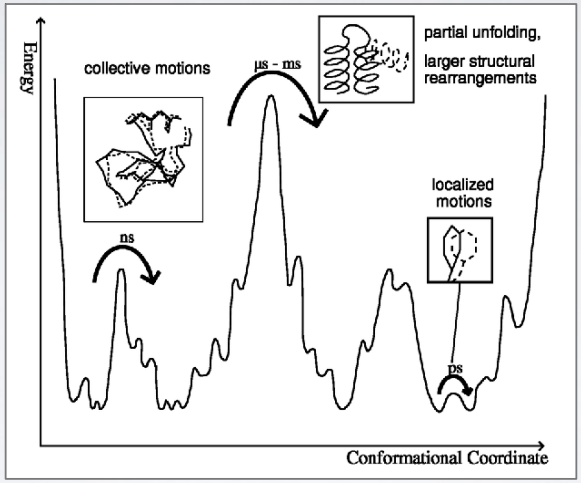


Yao … Grant, Journal of Biological Chemistry (2016)

EXAMPLE APPLICATION OF MOLECULAR SIMULATIONS TO GPCRS



PROTEINS JUMP BETWEEN MANY, HIERARCHICALLY ORDERED "CONFORMATIONAL SUBSTATES"



H. Frauenfelder et al., *Science* **229** (1985) 337

MOLECULAR DYNAMICS IS VERY

Improve this slide

Example: F_1-ATPase in water (183,674 atoms) for 1 nanosecond:
=> 10^6 integration steps
=> $8.4 * 10^{11}$ floating point operations/step
[n(n-1)/2 interactions]

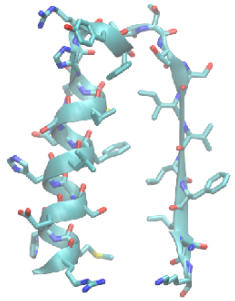
Total: $8.4 * 10^{17}$ flop
(on a 100 Gflop/s cpu: **ca 25 years!**)

… but performance has been improved by use of:

multiple time stepping	ca. 2.5 years
fast multipole methods	ca. 1 year
parallel computers	ca. 5 days
modern GPUs	**ca. 1 day**
(Anton supercomputer	**ca. minutes)**

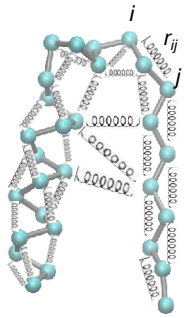
COARSE GRAINING: **NORMAL MODE ANALYSIS** (NMA)

- MD is still time-consuming for large systems
- Elastic network model NMA (ENM-NMA) is an example of a lower resolution approach that finishes in seconds even for large systems.



Atomistic

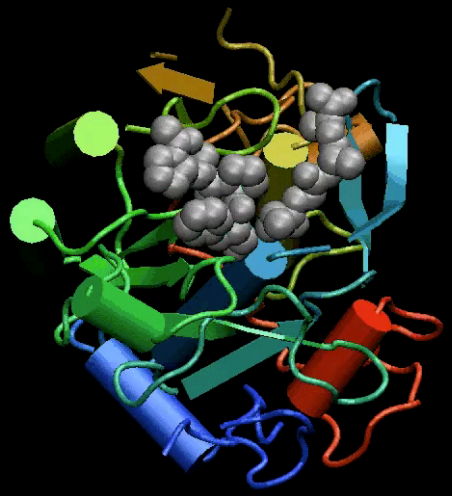
C. G.



Coarse Grained

- 1 bead / 1 amino acid
- Connected by springs

NMA models the protein as a network of elastic strings



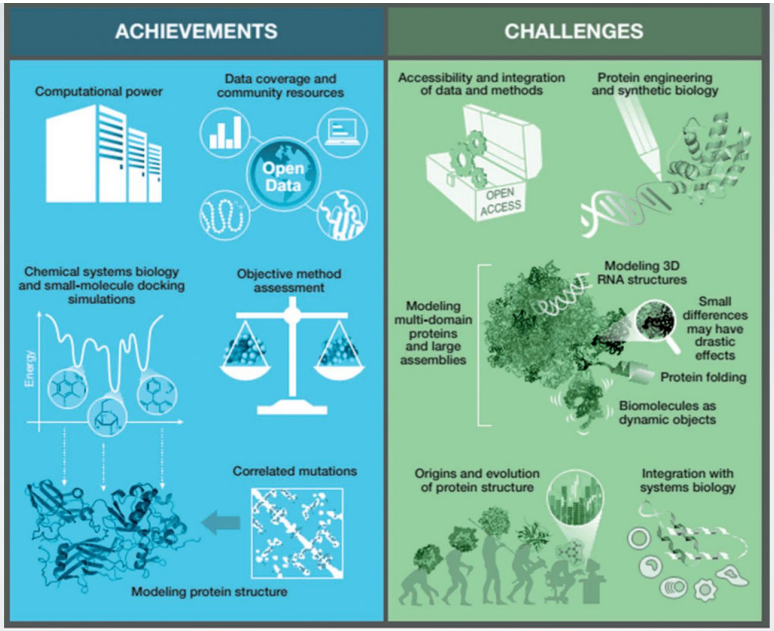
Proteinase K

Do it Yourself!

Hand-on time!

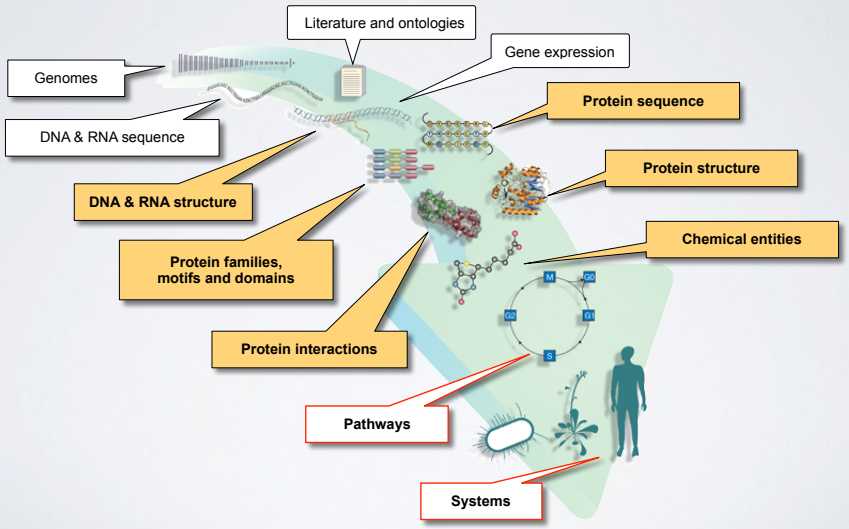
https://bioboot.github.io/bggn213_f17/lectures/#12

Focus on **section 4** exploring **PCA** and **NMA apps**



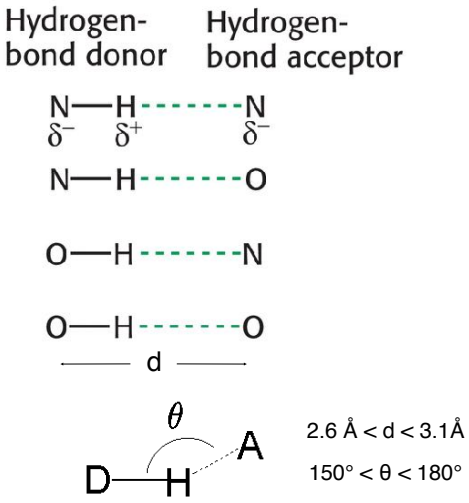
Ilan Samish et al. Bioinformatics 2015;31:146-150

INFORMING SYSTEMS BIOLOGY?



Key forces affecting structure:

- H-bonding
- Van der Waals
- Electrostatics
- Hydrophobicity



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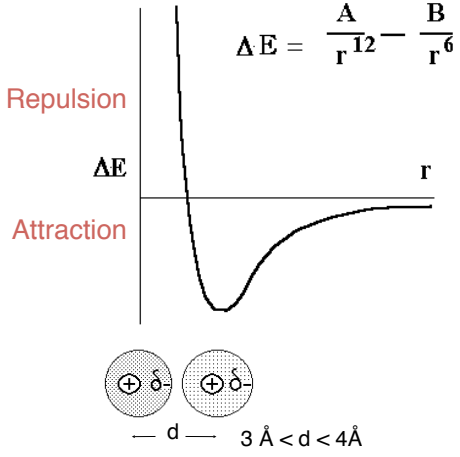

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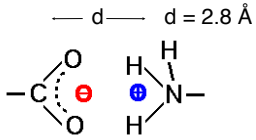
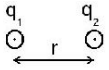


carboxyl group and amino group

(some time called IONIC BONDs or SALT BRIDGEs)



Coulomb's law

$$E = \frac{K q_1 q_2}{D r}$$

E = Energy
k = constant
D = Dielectric constant (vacuum = 1; H_2O = 80)
q_1 & q_2 = electronic charges (Coulombs)
r = distance (Å)

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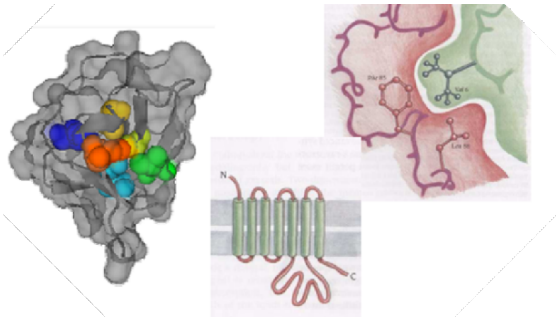

The force that causes hydrophobic molecules or nonpolar portions of molecules to aggregate together rather than to dissolve in water is called Hydrophobicity (*Greek, "water fearing"*). This is not a separate bonding force; rather, it is the result of the energy required to insert a nonpolar molecule into water.

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