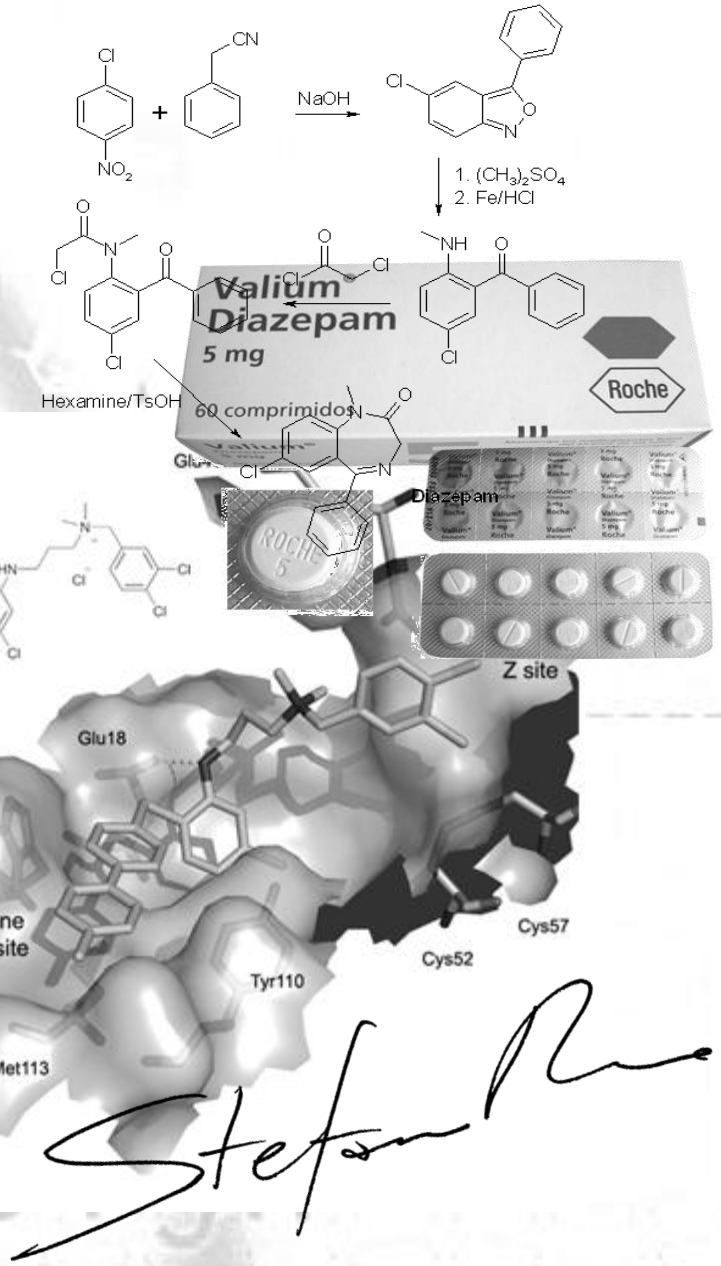
Chimica Farmaceutica



but we will never forget:

PHARMACODYNAMIC (PD) PROFILE

the study of the biochemical and physiological effects of drugs and the mechanisms of their actions, including the correlation of their actions and effects with their chemical structure.

PHARMACOKINETIC (PK)/TOX PROFILE

The study of the process by which a drug is absorbed (A), distributed (D), metabolized (M), and eliminated (E) by the body.

FORMULATION PROFILE

Do you remember costs?

Experiment Typical Cost per Compound (€)

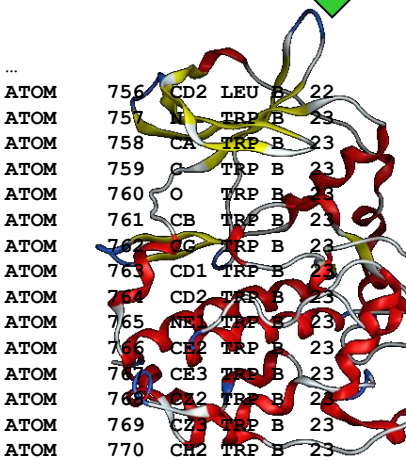
Computer modeling	7
Biochemical assay	270
Cell culture assay	2.700
Rat acute toxicity	8.100
Protein crystal structure	68.000
Animal efficacy trial	200.000
Rat 2-year chronic oral toxicity	550.000
Human clinical trial	3.500.000

Our closer friends…

NMR
Spectroscopy

3D

X-Ray
Crystallography



```
...
ATOM    756  CD2 LEU B  22      31.236 -29.774  -8.020
ATOM    757  N   TRP B  23      27.477 -25.871  -8.251
ATOM    758  CA  TRP B  23      27.021 -24.553  -7.803
ATOM    759  C   TRP B  23      25.532 -24.284  -8.011
ATOM    760  O   TRP B  23      24.919 -23.498  -7.297
ATOM    761  CB  TRP B  23      27.892 -23.448  -8.451
ATOM    762  CG  TRP B  23      27.585 -22.055  -8.009
ATOM    763  CD1 TRP B  23      26.730 -21.178  -8.613
ATOM    764  CD2 TRP B  23      28.090 -21.382  -6.842
ATOM    765  NE1 TRP B  23      26.659 -20.002  -7.892
ATOM    766  CE2 TRP B  23      27.478 -20.101  -6.799
ATOM    767  CE3 TRP B  23      28.990 -21.731  -5.830
ATOM    768  CZ2 TRP B  23      27.738 -19.177  -5.784
ATOM    769  CZ3 TRP B  23      29.251 -20.809  -4.825
ATOM    770  CH2 TRP B  23      28.623 -19.548  -4.812
ATOM    771  N   LYS B  24      24.939 -24.930  -8.991
...
```

Comparative/Homology
Modeling

Cryo-
Electron Microscopy
(Cryo-EM)

We are coordinates hunters!

... and this is our favorite hunting place!

www.rcsb.org

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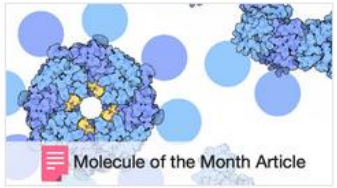
A Structural View of Biology

This resource is powered by the Protein Data Bank archive-information about the 3D shapes of proteins, nucleic acids, and complex assemblies that helps students and researchers understand all aspects of biomedicine and agriculture, from protein synthesis to health and disease.

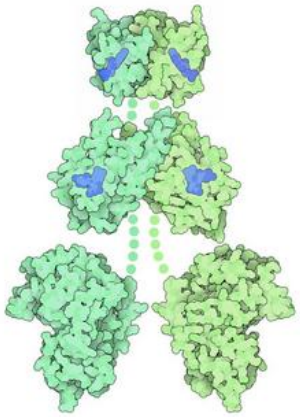
As a member of the wwPDB, the RCSB PDB curates and annotates PDB data.

The RCSB PDB builds upon the data by creating tools and resources for research and education in molecular biology, structural biology, computational biology, and beyond.

Structure and Health Focus: Ebola Virus Proteins



March Molecule of the Month



Phototropin

PDB… in numbers:

RCSB PDB | Deposit | Search | Visualize | Analyze | Download | Learn | More | MyPDB

Other Statistics

PDB Data Distribution by Experimental Method and Molecular Type

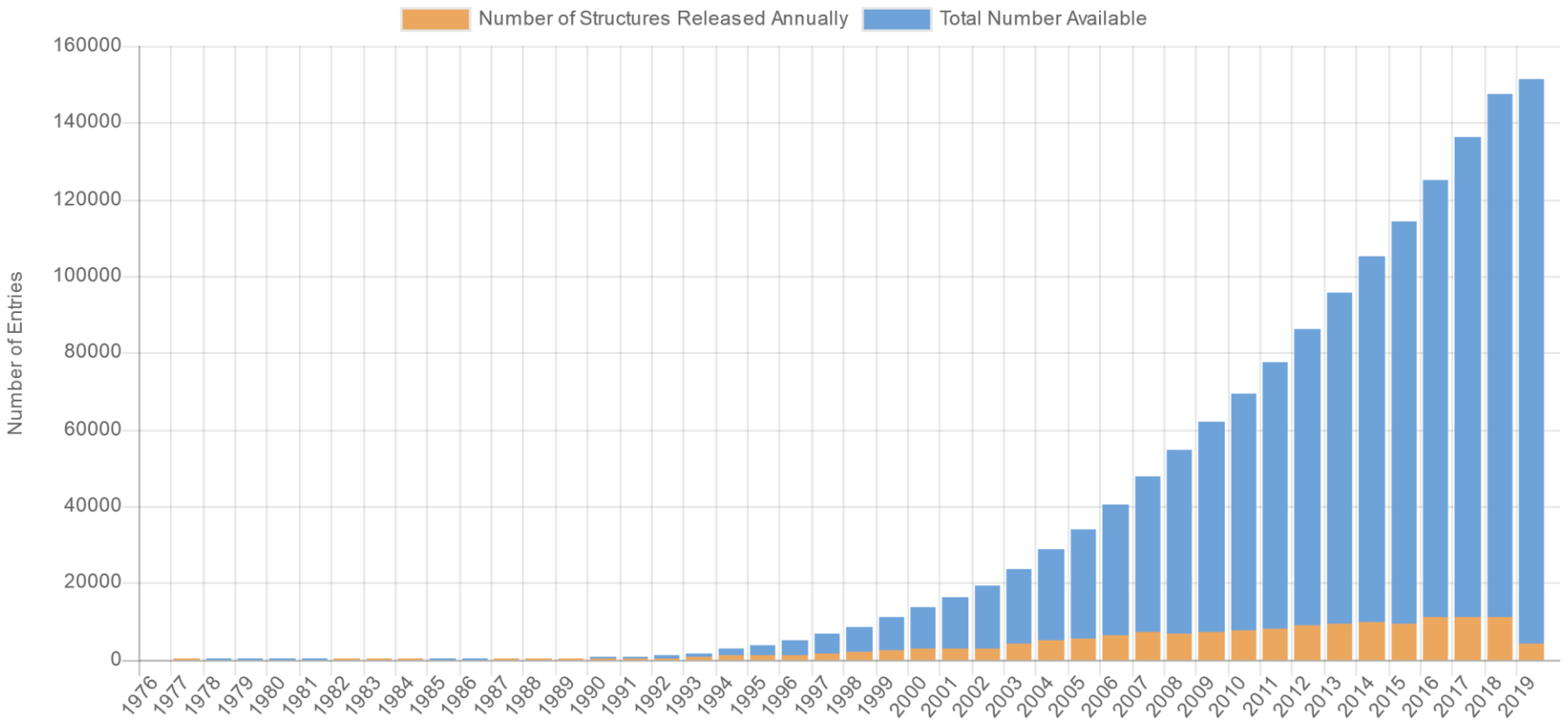
Copy | CSV

Experimental Method	Proteins	Nucleic Acids	Protein/NA Complex	Other	Total
X-Ray	126880	2012	6547	8	135447
NMR	11062	1279	259	8	12608
Electron Microscopy	2277	31	800	0	3108
Other	256	4	6	13	279
Multi Method	129	5	2	1	137
Total	140604	3331	7614	30	151579

125334 structures in the PDB have a structure factor file.
9949 structures in the PDB have an NMR restraint file.
3701 structures in the PDB have a chemical shifts file.
3167 structures in the PDB have a 3DEM map file.

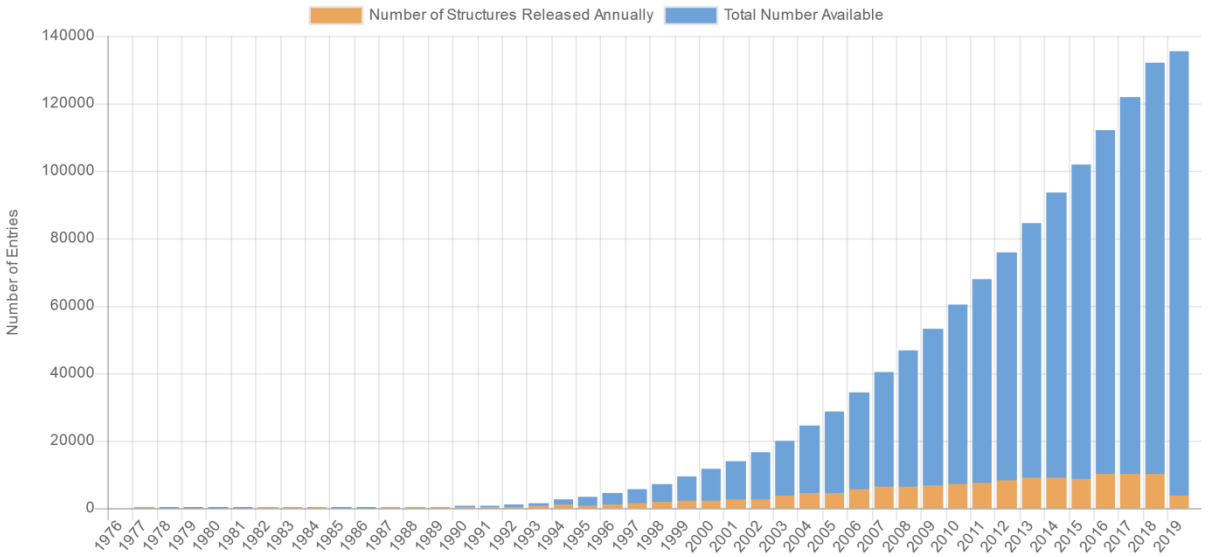
PDB… in numbers:

RCSB PDB Deposit Search Visualize Analyze Download Learn More MyPDB

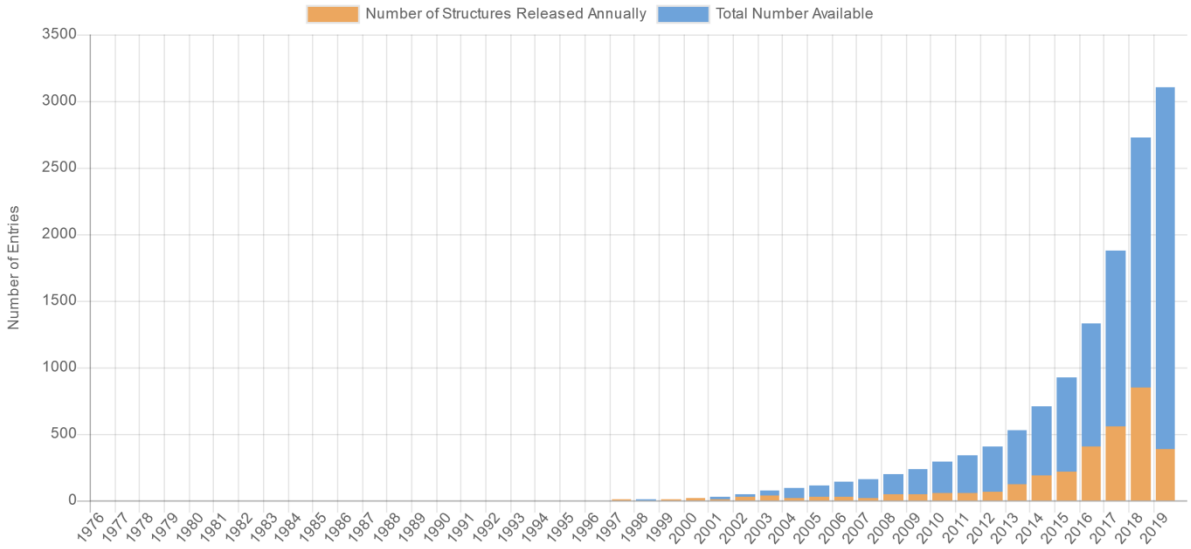


PDB… in numbers:

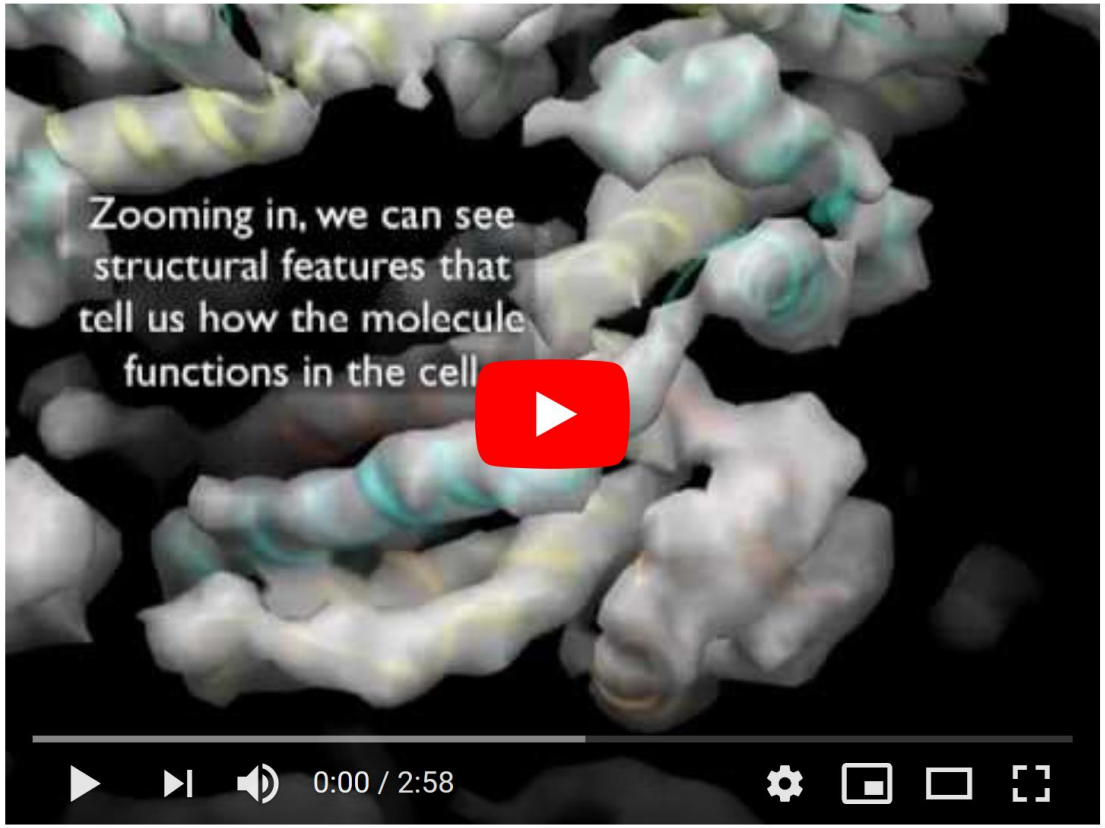
X-Ray Crystallography



Cryo-Electron Microscopy (Cryo-EM)



Cryo-EM: the future of structural biochemistry is today!



A 3 minute introduction to CryoEM

credits: https://www.youtube.com/watch?v=BJKkC0W-6Qk

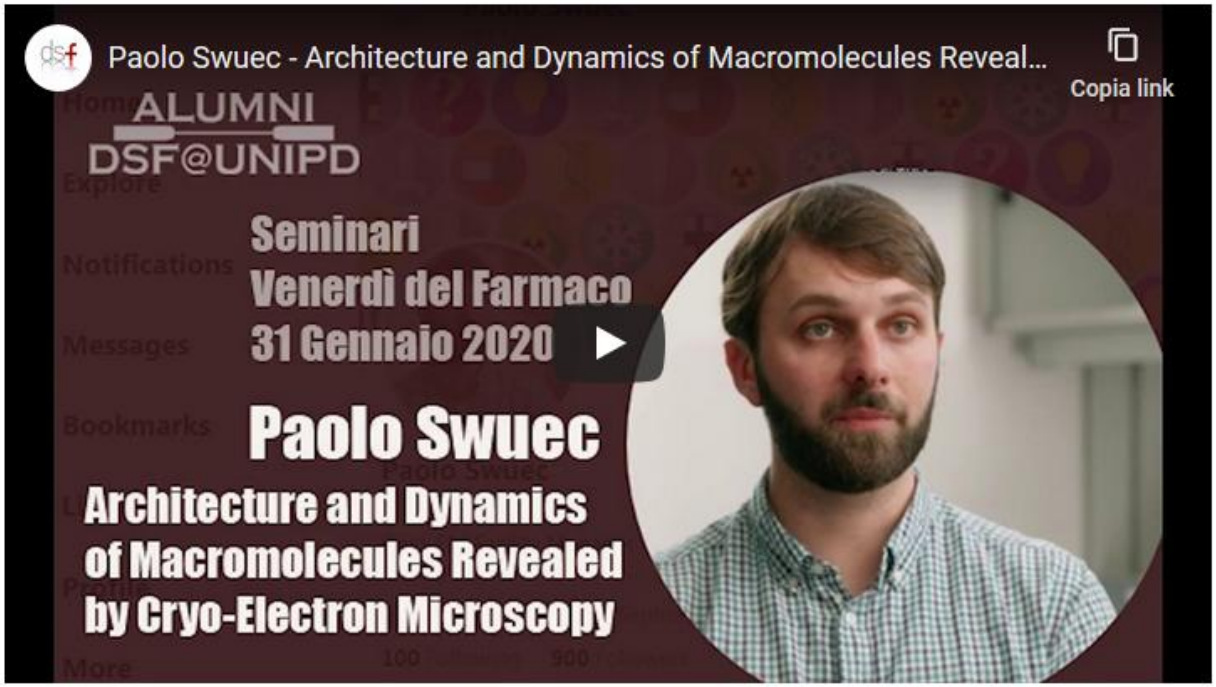
Cryo-EM: the future of structural biochemistry is today!

Venerdì 31 Gennaio 2020 ore 10.00

Aula 2 – Dipartimento di Scienze del Farmaco

Paolo Swuec - Dept. of BioSciences, University of Milan (Italy), "Romeo ed Erica Invernizzi" Paediatric Research Centre, Milan (Italy)

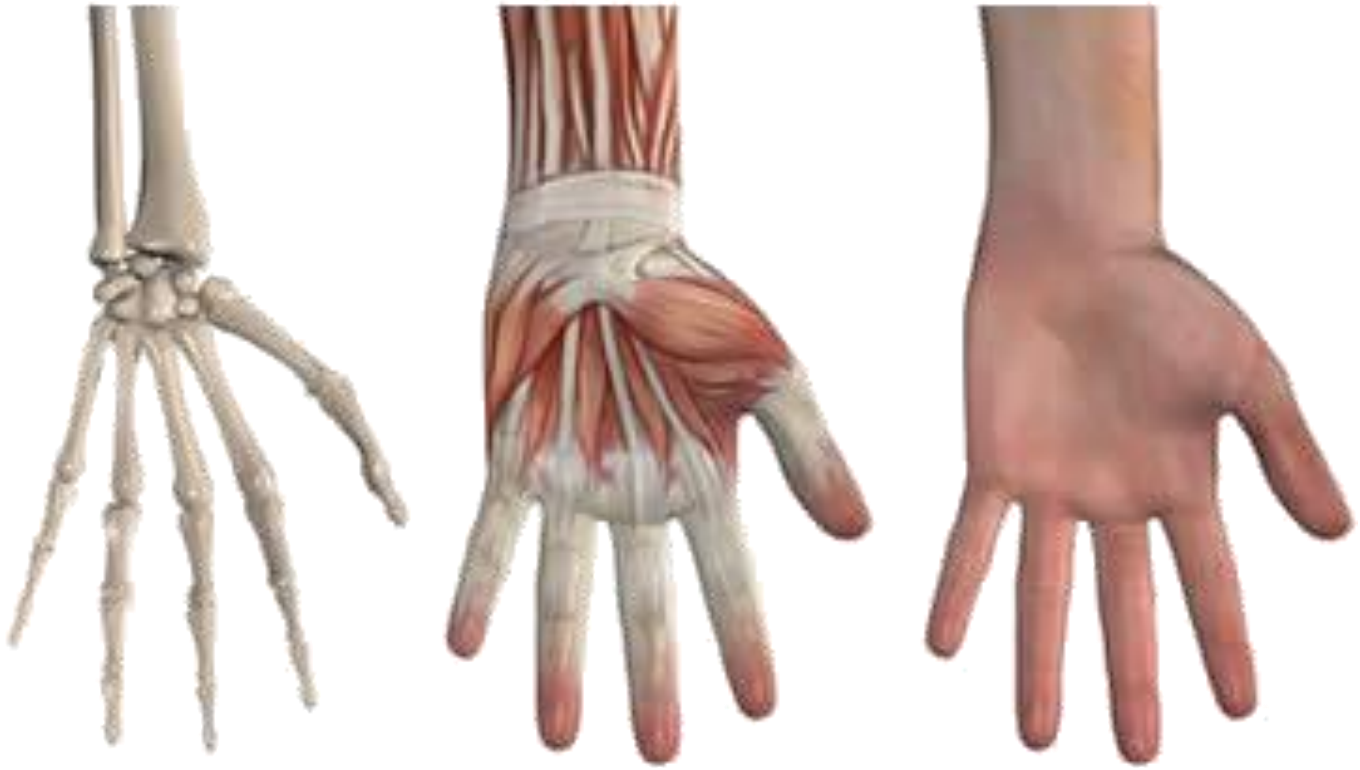
Architecture and Dynamics of Macromolecules Revealed by Cryo-Electron Microscopy



https://youtu.be/BMoRBzW-vvI

Can you understand why pharma companies are very interested to structural biology/biochemistry?

From 3D coordinates to molecular surface



Can we represent 2QC6 (PDB) data?

Protein Section

		Atom type				x	y	z		B-factor	Atom nature
ATOM	1	N	SER	A	7	18.809	-11.548	-9.537	1.00	30.22	N
ATOM	2	CA	SER	A	7	17.758	-10.860	-8.731	1.00	29.23	C
ATOM	3	C	SER	A	7	17.891	-9.329	-8.850	1.00	29.40	C
ATOM	4	O	SER	A	7	18.997	-8.794	-8.756	1.00	29.43	O
ATOM	5	CB	SER	A	7	17.853	-11.306	-7.257	1.00	29.91	C
ATOM	6	OG	SER	A	7	16.761	-10.832	-6.474	1.00	28.29	O
ATOM	7	N	LYS	A	8	16.765	-8.642	-9.075	1.00	29.05	N
ATOM	8	CA	LYS	A	8	16.661	-7.201	-8.846	1.00	28.10	C
ATOM	9	C	LYS	A	8	15.546	-6.888	-7.842	1.00	26.83	C
ATOM	10	O	LYS	A	8	14.668	-7.713	-7.590	1.00	26.71	O
ATOM	11	CB	LYS	A	8	16.479	-6.409	-10.156	1.00	28.45	C
ATOM	12	CG	LYS	A	8	15.230	-6.746	-10.960	1.00	31.04	C
ATOM	13	CD	LYS	A	8	15.113	-5.946	-12.280	1.00	29.28	C
ATOM	14	CE	LYS	A	8	13.733	-6.182	-12.904	1.00	32.90	C
ATOM	15	NZ	LYS	A	8	13.412	-5.378	-14.148	1.00	34.62	N
ATOM	16	N	ALA	A	9	15.607	-5.689	-7.270	1.00	25.59	N
ATOM	17	CA	ALA	A	9	14.514	-5.118	-6.483	1.00	24.63	C
ATOM	18	C	ALA	A	9	13.282	-4.941	-7.363	1.00	23.75	C
ATOM	19	O	ALA	A	9	13.376	-4.516	-8.526	1.00	23.15	O
ATOM	20	CB	ALA	A	9	14.934	-3.766	-5.885	1.00	24.47	C
ATOM	21	N	ARG	A	10	12.130	-5.234	-6.781	1.00	22.67	N
ATOM	22	CA	ARG	A	10	10.856	-5.139	-7.459	1.00	22.08	C
ATOM	23	C	ARG	A	10	10.371	-3.694	-7.515	1.00	21.33	C
ATOM	24	O	ARG	A	10	9.507	-3.357	-8.324	1.00	20.81	O
ATOM	25	CB	ARG	A	10	9.833	-6.045	-6.754	1.00	21.79	C
ATOM	26	CG	ARG	A	10	9.946	-7.504	-7.139	1.00	21.89	C
ATOM	27	CD	ARG	A	10	8.988	-8.414	-6.372	1.00	22.38	C
ATOM	28	NE	ARG	A	10	7.573	-8.170	-6.669	1.00	23.88	N
ATOM	29	CZ	ARG	A	10	6.588	-8.289	-5.780	1.00	24.40	C
ATOM	30	NH1	ARG	A	10	6.863	-8.648	-4.532	1.00	23.52	N
ATOM	31	NH2	ARG	A	10	5.322	-8.034	-6.136	1.00	24.29	N
ATOM	32	N	VAL	A	11	10.931	-2.853	-6.635	1.00	21.41	N
ATOM	33	CA	VAL	A	11	10.640	-1.408	-6.579	1.00	21.07	C
ATOM	34	C	VAL	A	11	11.914	-0.562	-6.456	1.00	21.26	C
ATOM	35	O	VAL	A	11	12.946	-1.050	-5.997	1.00	22.06	O

I would like to be very honest with you!

z

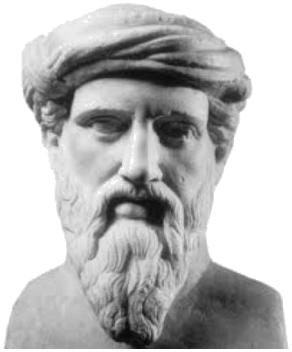
C (x, y, z)

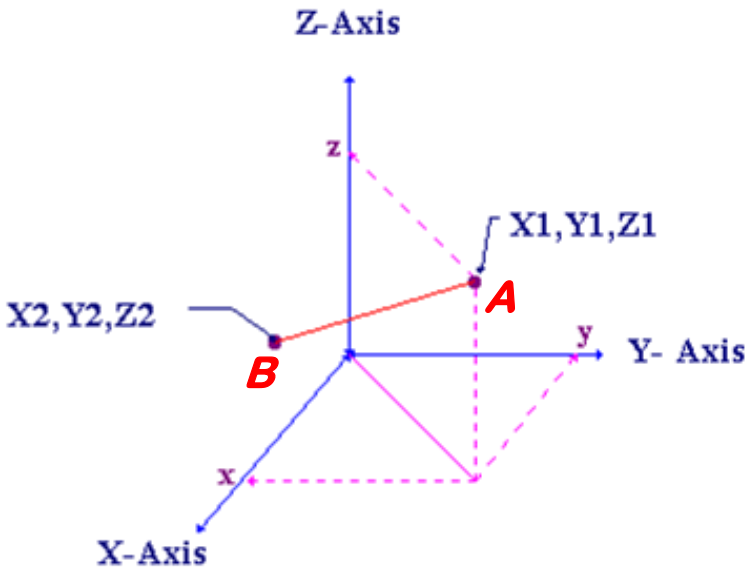
x

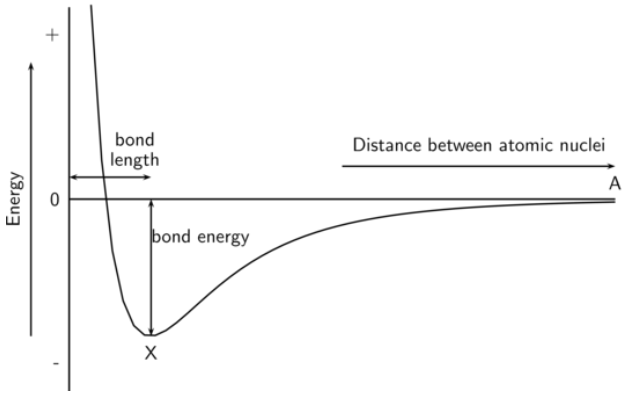
y

= C
= N
= O
= S

What can we easily measure in a 3D Cartesian space?







$$\overline{AB} = \sqrt{(X1-X2)^2 + (Y1-Y2)^2 + (Z1-Z2)^2}$$

Bond	Bond Length (Å)	Bond	Bond Length (Å)
C—C	1.54	N—N	1.47
C═C	1.34	N═N	1.24
C≡C	1.20	N≡N	1.10
C—N	1.43	N—O	1.36
C═N	1.38	N═O	1.22
C≡N	1.16		
		O—O	1.48
C—O	1.43	O═O	1.21
C═O	1.23		
C≡O	1.13		

C

1.54 Å

z

C (x, y, z)

C

x

y

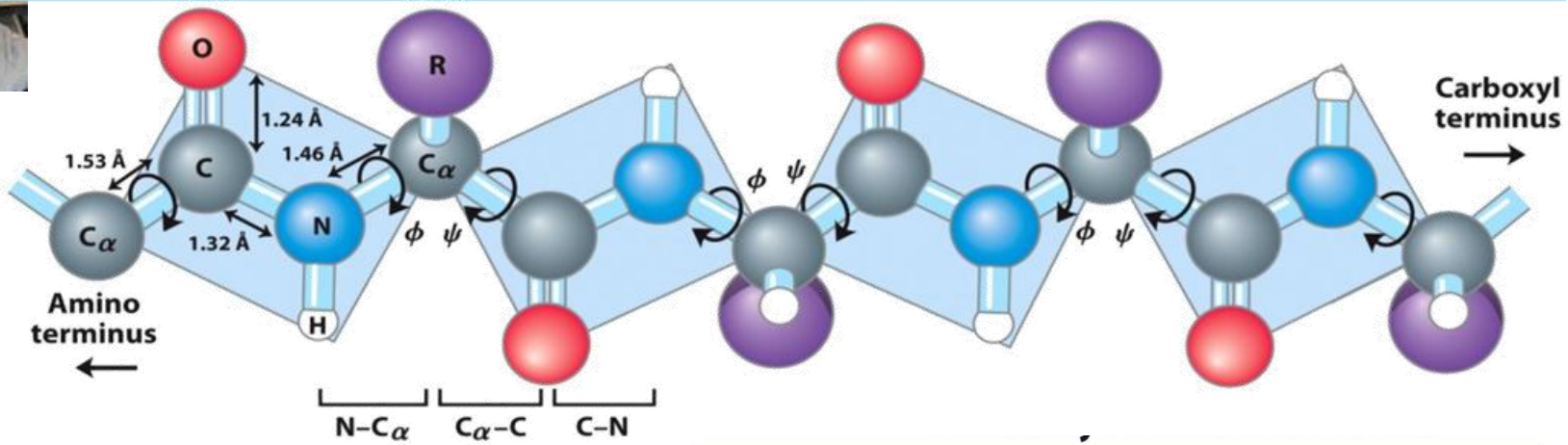
= C

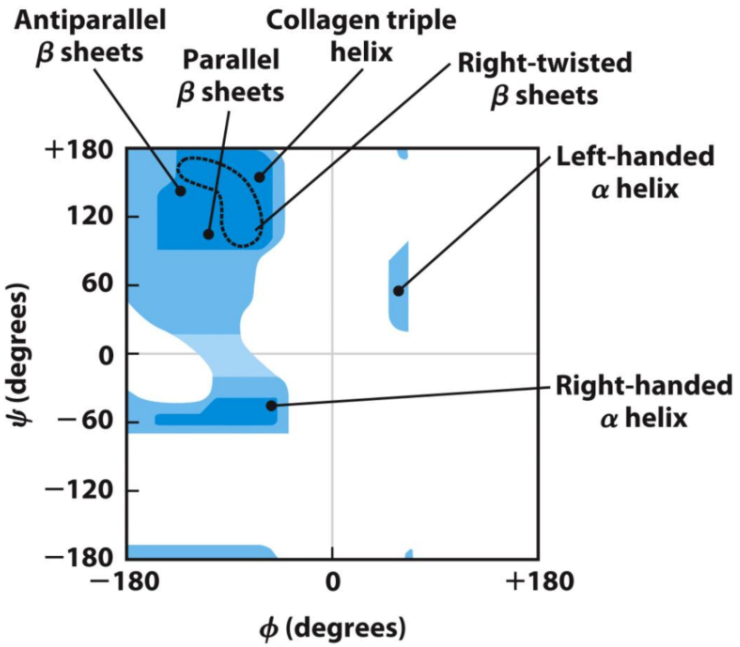
= N

= O

= S

There are some interesting geometrical regularity inside our polymer:

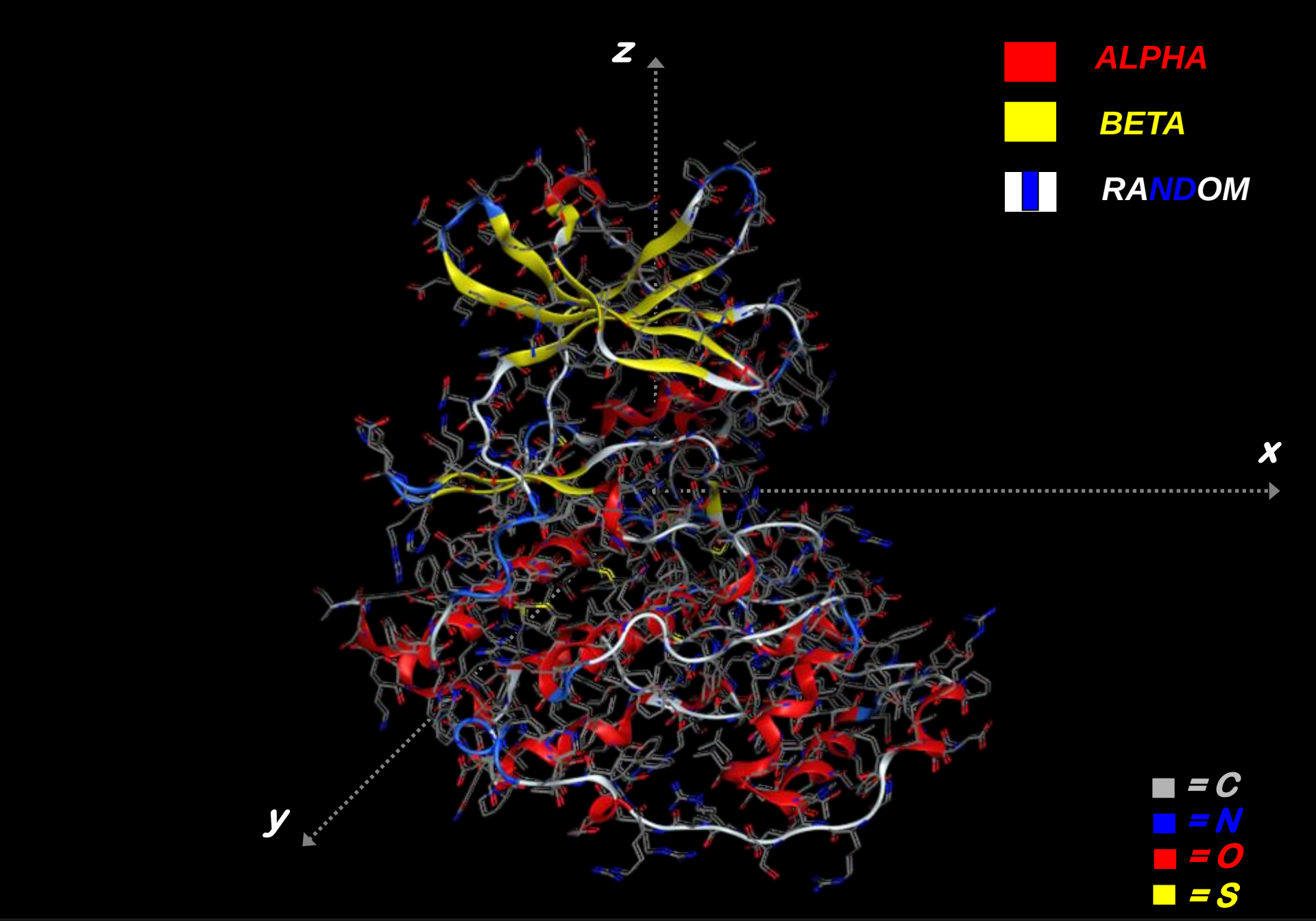




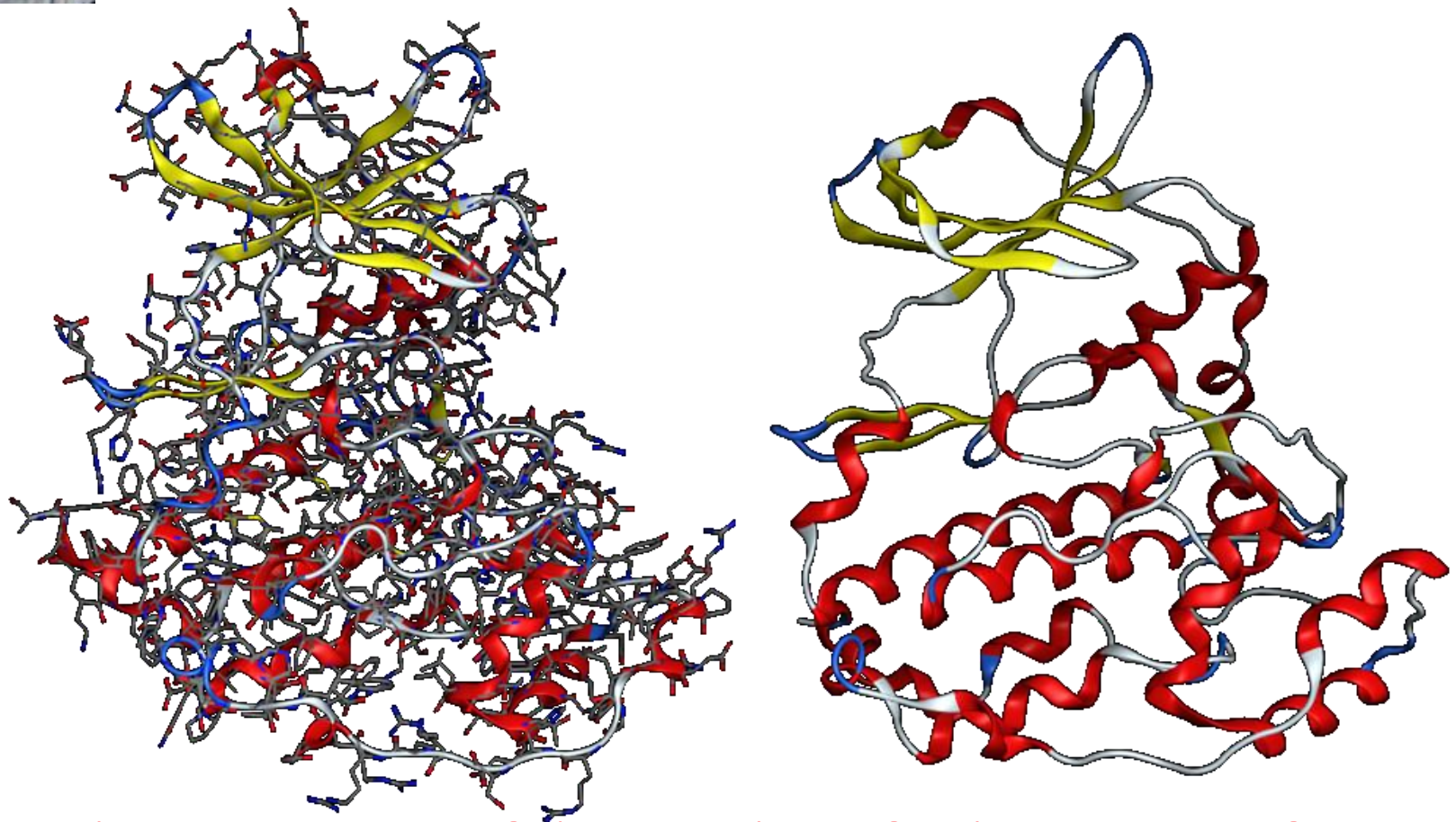
Structure	ϕ	ψ
α Helix	−57°	−47°
β Conformation		
Antiparallel	−139°	+135°
Parallel	−119°	+113°
Collagen triple helix	−51°	+153°
β Turn type I		
i + 1*	−60°	−30°
i + 2*	−90°	0°
β Turn type II		
i + 1	−60°	+120°
i + 2	+80°	0°

Note: In real proteins, the dihedral angles often vary somewhat from these idealized values.

*The i+1 and i+2 angles are those for the second and third amino acid residues in the β turn, respectively.



This is just a useful way to represent geometrical regularity of 3D coordinates and not the real shape of our protein!!!



… but very useful to classified protein from a structural point of view!!!

Atomic radius… could be one?

The atomic radius of a chemical element is a measure of the size of its atoms, usually the mean or typical distance from the nucleus to the boundary of the surrounding cloud of electrons.

Since the boundary is not a well-defined physical enti[illegible]re are various non-equivalent definitions of atomic radius. Three widel[illegible]d definitions of atomic radius are **van der Waals radius, ionic radiu[illegible]nd covalent radius.**

***Johannes Diderik van der Waals** (23 November 1837 – 8 March 1923) was a Dutch theoretical physicist and thermodynamicist famous for his work on an equation of state for gases and liquids.*

His name is primarily associated with the van der Waals equation of state that describes the behavior of gases and their condensation to the liquid phase. His name is also associated with van der Waals forces (forces between stable molecules), with van der Waals molecules (small molecular clusters bound by van der Waals forces), and with van der Waals radii (sizes of molecules).

He became the first physics professor of the University of Amsterdam when it opened in 1877 and won the 1910 Nobel Prize in physics.

WIKIPEDIA
The Free Encyclopedia

***van der Waals radius**: in principle, half the minimum distance between the nuclei of two atoms of the element that are not bound to the same molecule.*

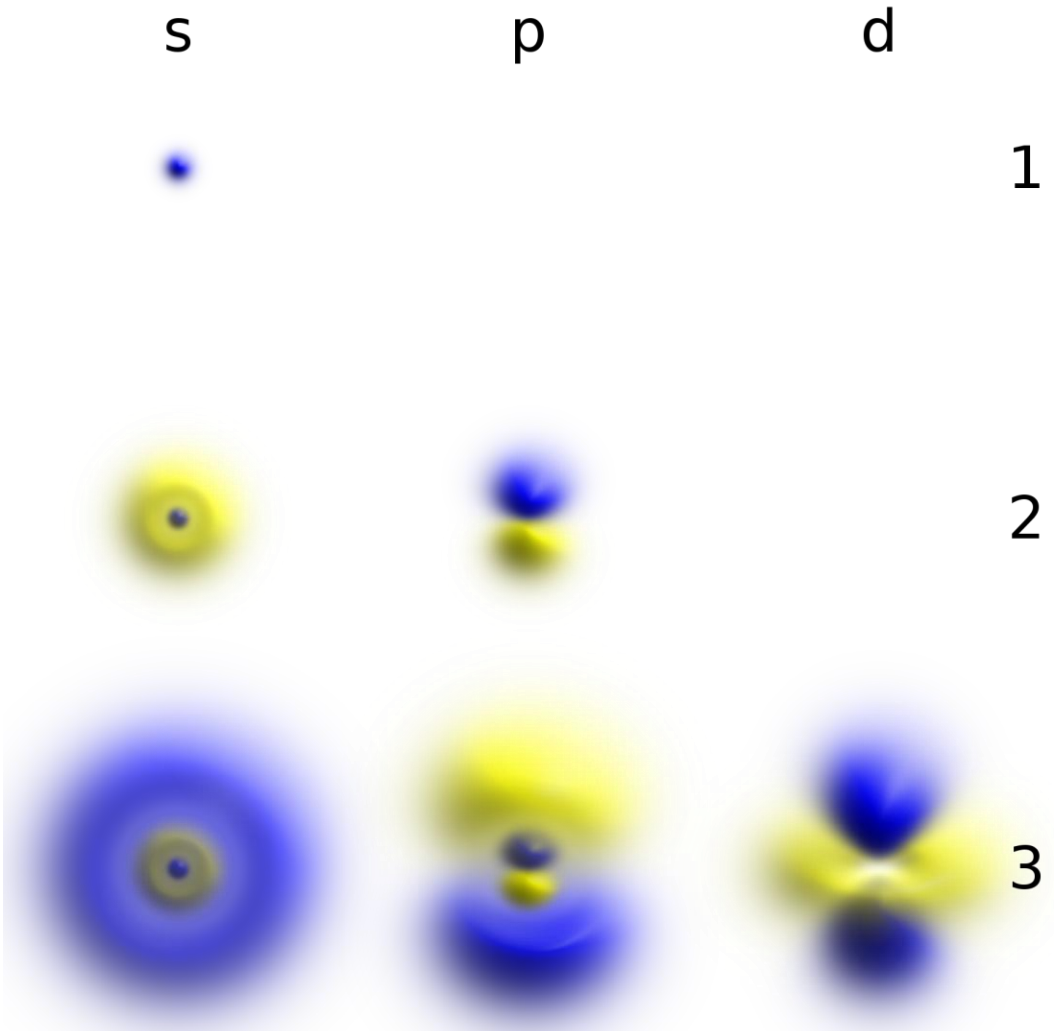
A quick refresh…

Element	radius (Å)
Hydrogen	1.20
Carbon	1.70
Nitrogen	1.55
Oxygen	1.52
Fluorine	1.47
Phosphorus	1.80
Sulfur	1.80
Chlorine	1.75
Copper	1.4

$$V_{vdW} = \frac{4}{3}\pi \; r_{vdW}^{3}$$

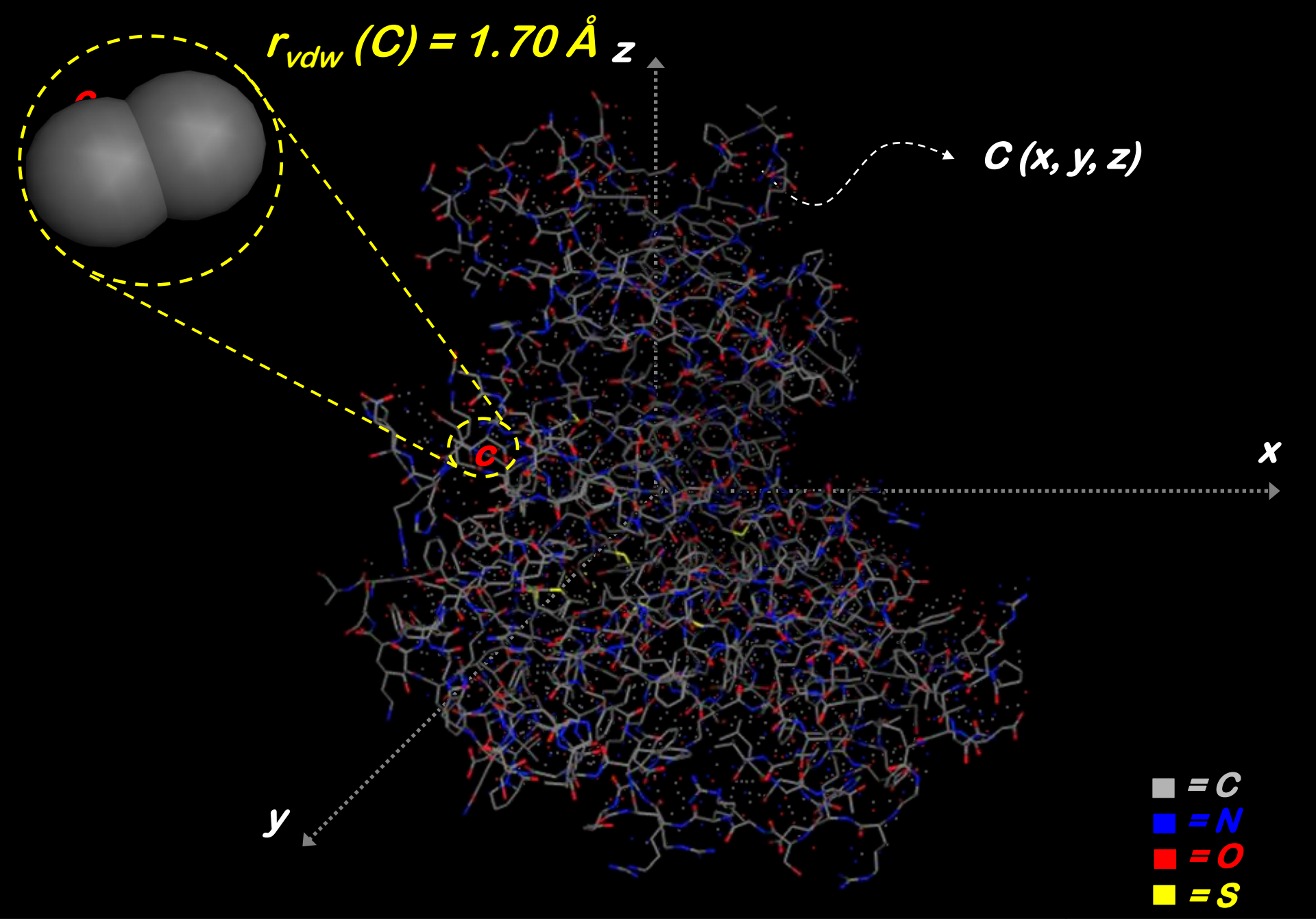
$$r_{vdW} = \sqrt[3]{V_{vdW} \; \frac{3}{4\pi}}$$

Why an atom has a spherical shape?



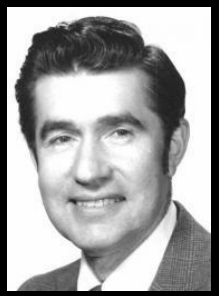
The images are 3D renderings of the spatial density distribution of $|\psi|^2$ with the color depicting the phase of ψ. The spatial distribution is smooth and vanishes for large radii. The cloud is a more realistic representation of an orbital than the more common solid-body approximations.

Credits: https://en.wikipedia.org/wiki/Electron#/media/File:Atomic-orbital-clouds_spd_m0.png



CPK model:

Robert Corey Linus Pauling



Walter Koltun

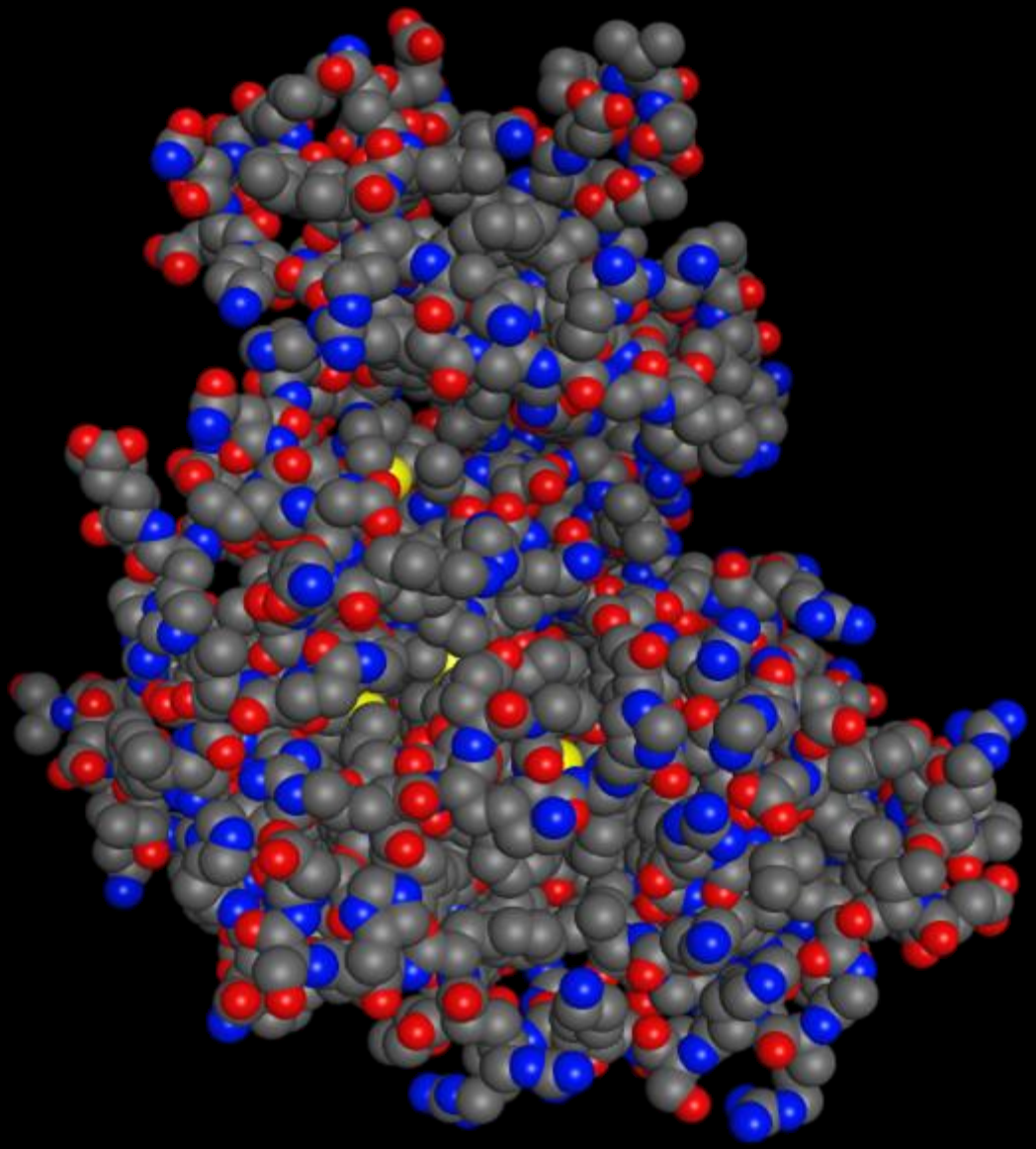
z

x

y

= C
= N
= O
= S

Before proceeding further… what is it missing?



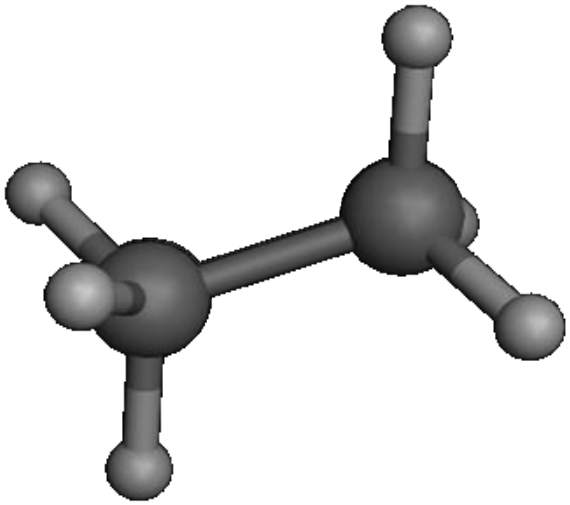
■ = C
■ = N
■ = O
■ = S

Yes, hydrogen atoms!

REMEMBER: X-ray crystallography and Cryo_EM cannot resolve hydrogen atoms in most protein crystals, so in most PDB files, hydrogen atoms are absent. On the contrary, hydrogen atoms are present in NMR-derived structures.

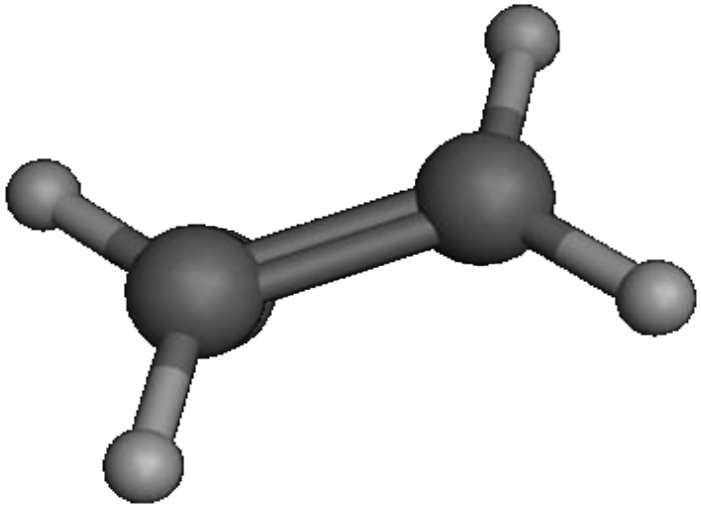
a. Several tools can be used to hydrogen atoms… respecting the valence properties of all heavy atoms and the geometrical characteristics (distances and angles) of all X-H bonds.

Yes, hydrogen atoms!



C_{sp3} angle 109 °

C_{sp3} – H length 1.09 Å

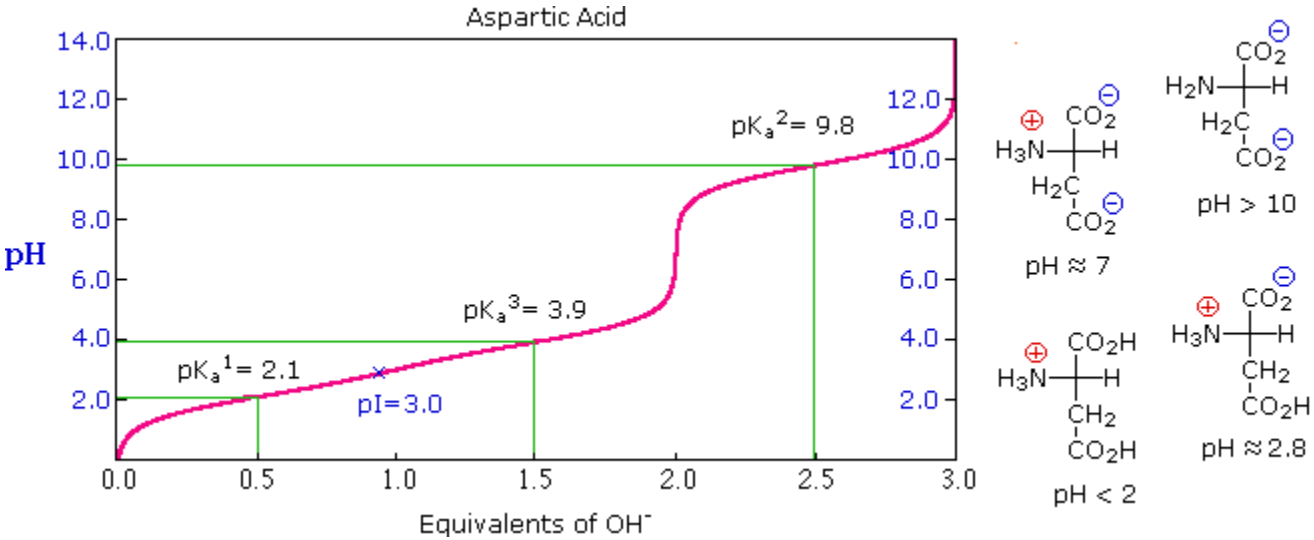


C_{sp2} angle 120 °

C_{sp3} – H length 1.08 Å

but we have hydrogens… and hydrogens!

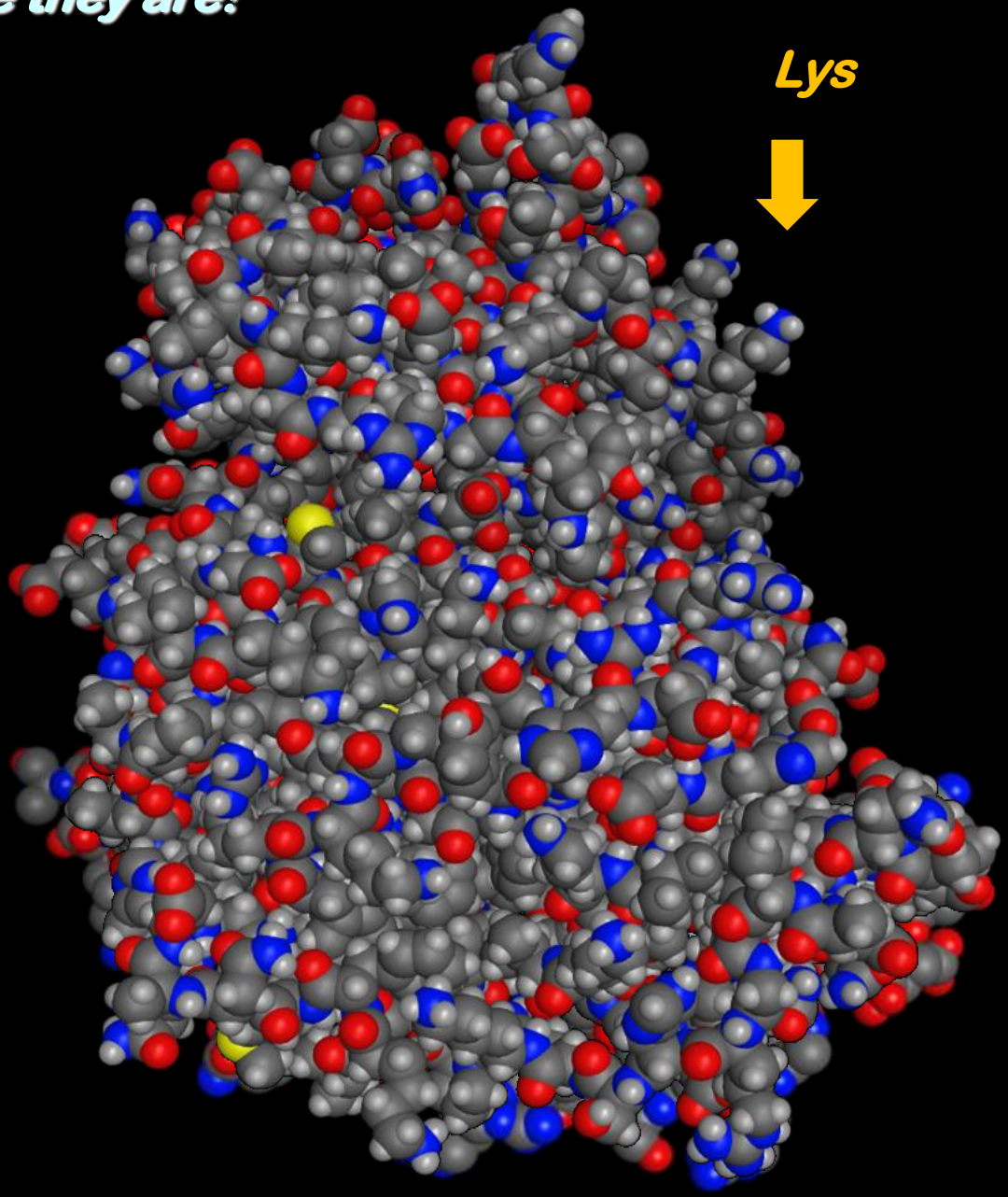
b. Moreover, hydrogen atoms associated with ionizable groups are 'titrated' according to their value of pKa and pH value of medium solvent:



... here they are!

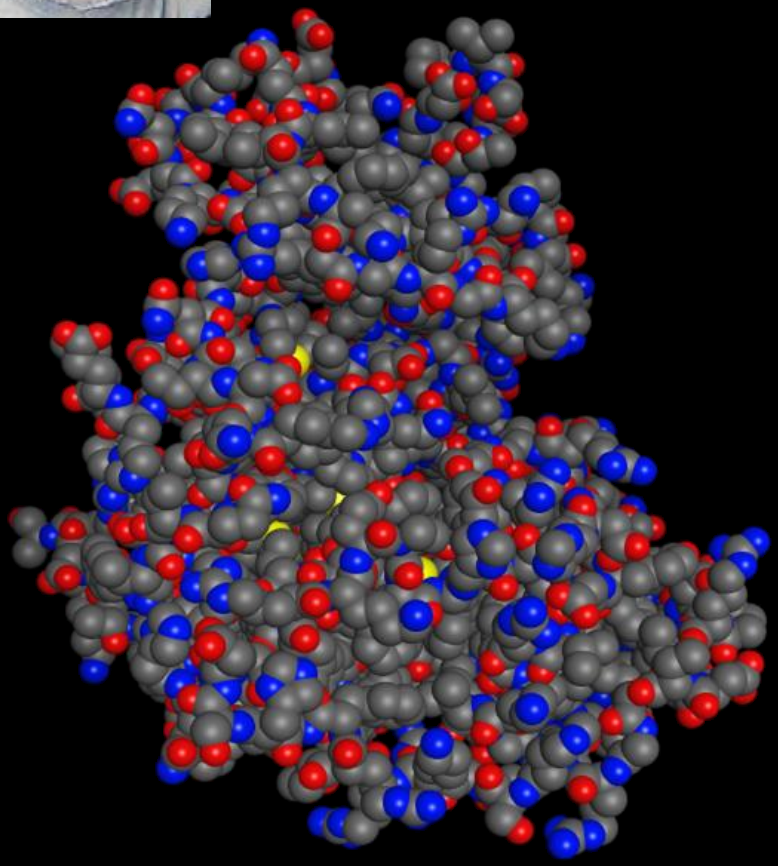
Lys

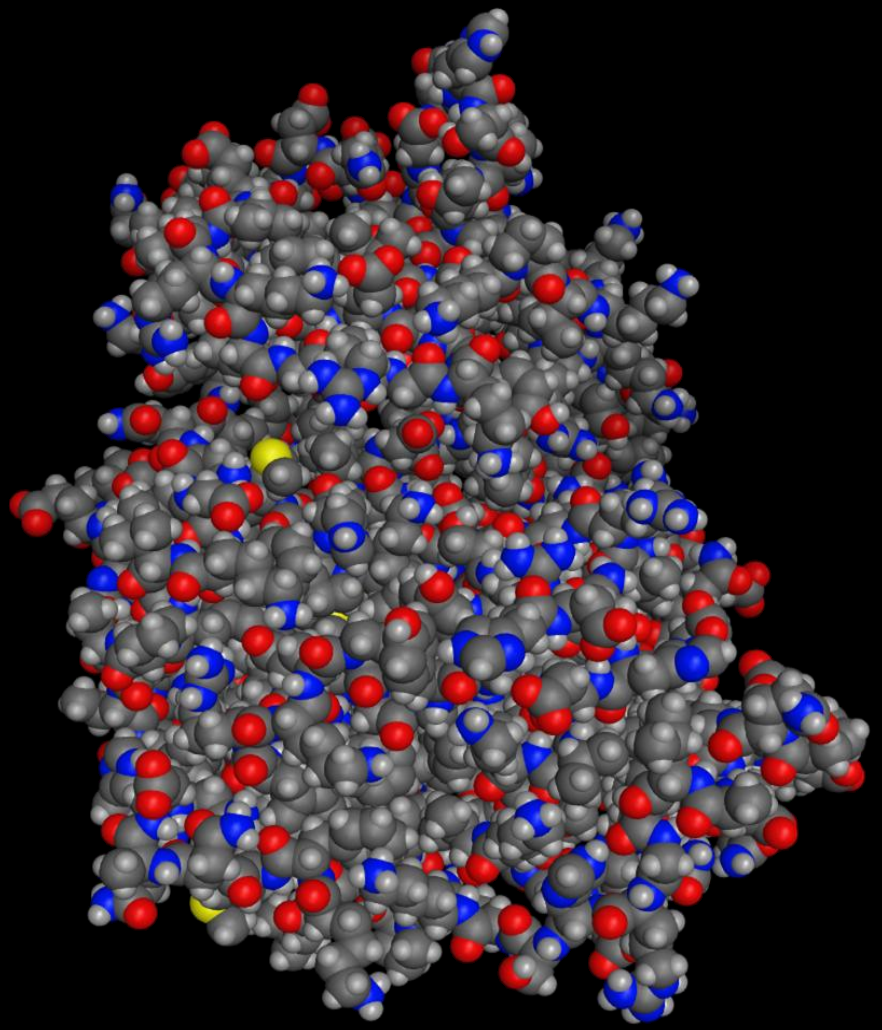
Glu



= C
= N
= O
= S
= H

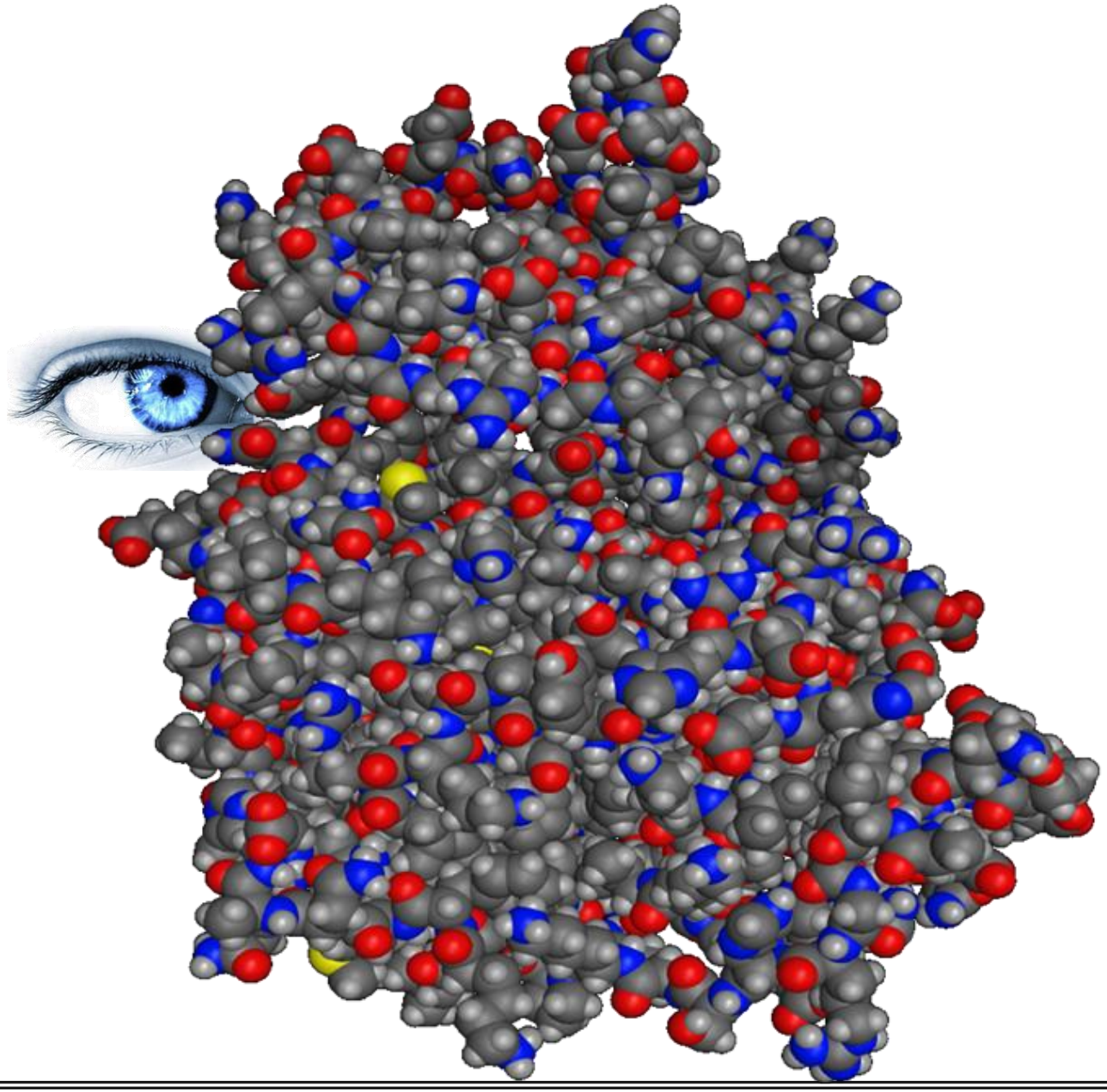
... remember the difference





in this model we have modified the original experimental data!!!

But how they "look" each other?

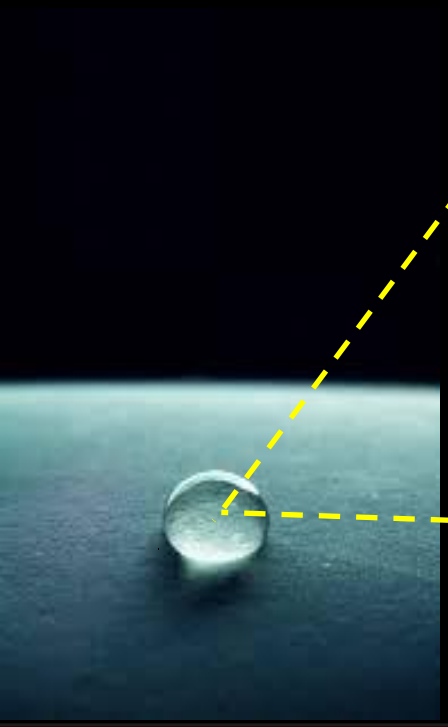


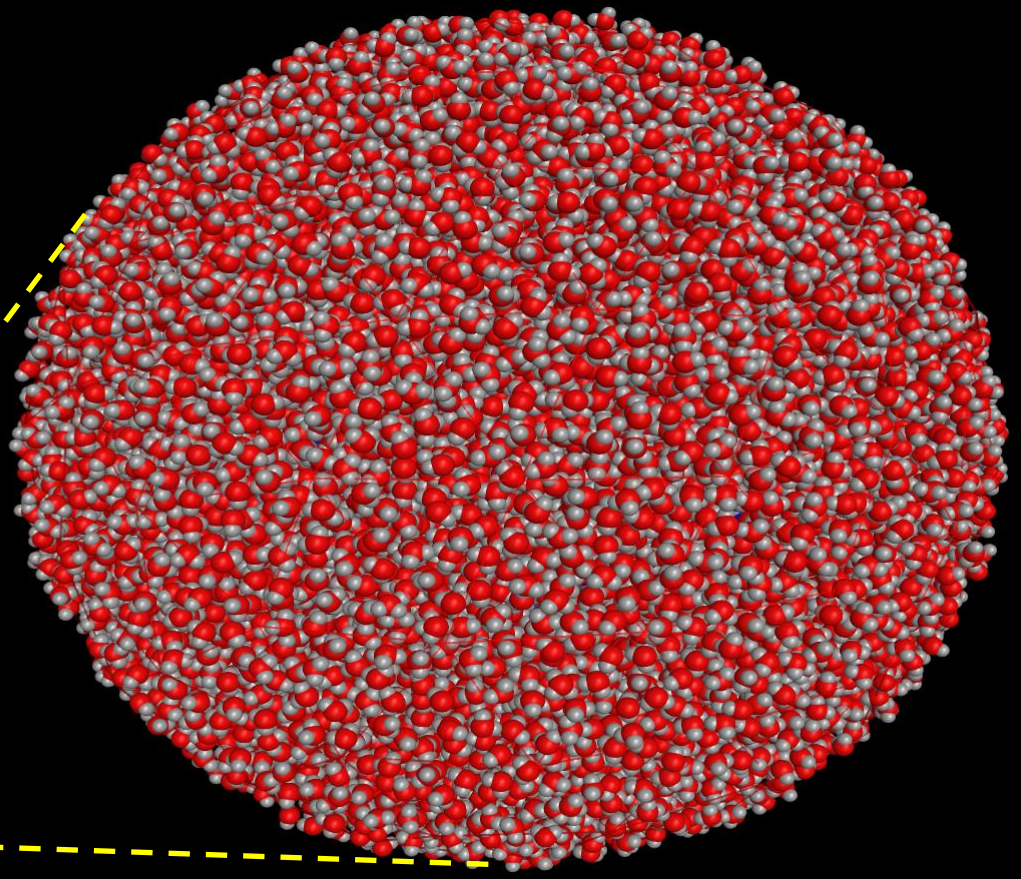
Back when I was a young PhD student.

"It is generally accepted that receptor and substrate molecules recognize each other at the molecular surfaces. The binding strength of a receptor-drug complex depends on the shape of the substrate surface and the distribution of certain properties on this surface. Any method attempting to model biological activity should take into account this information and try to correlate it to biological activity…"

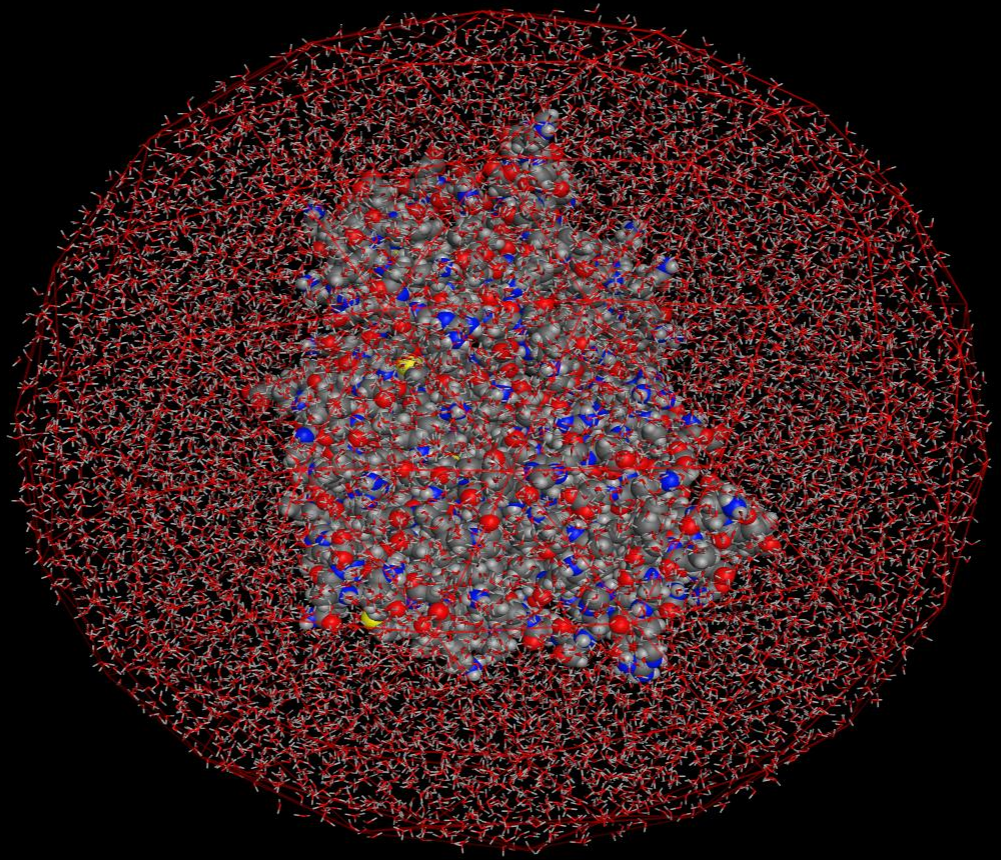
*by Johann Gasteiger et al J.A.C.S. 1995, **117**, 7769-7775*

The magic role of the solvent…

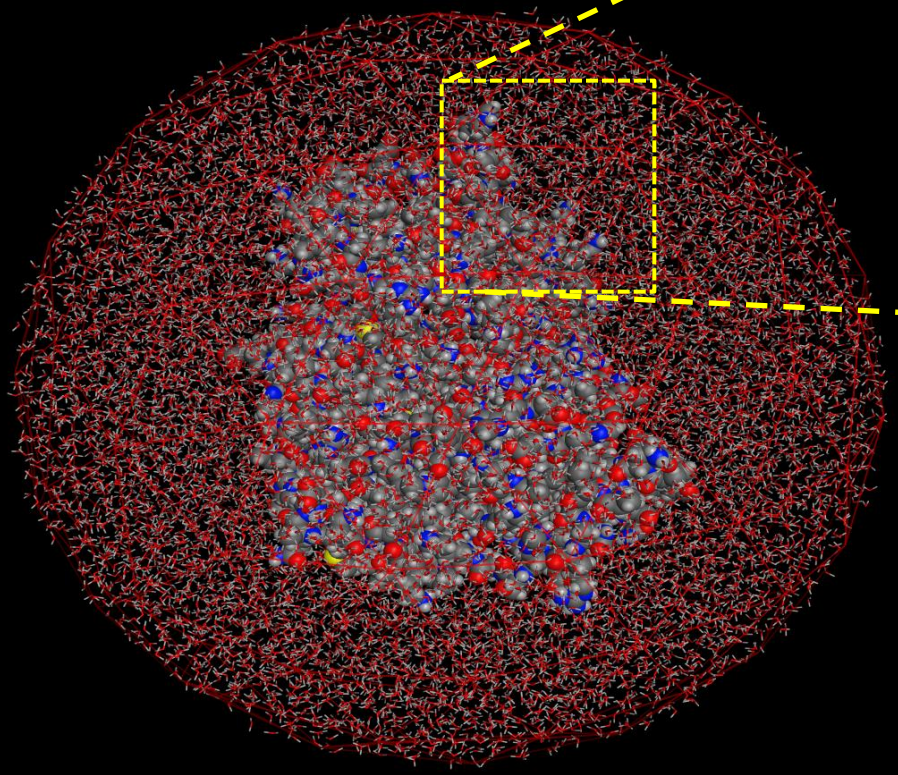


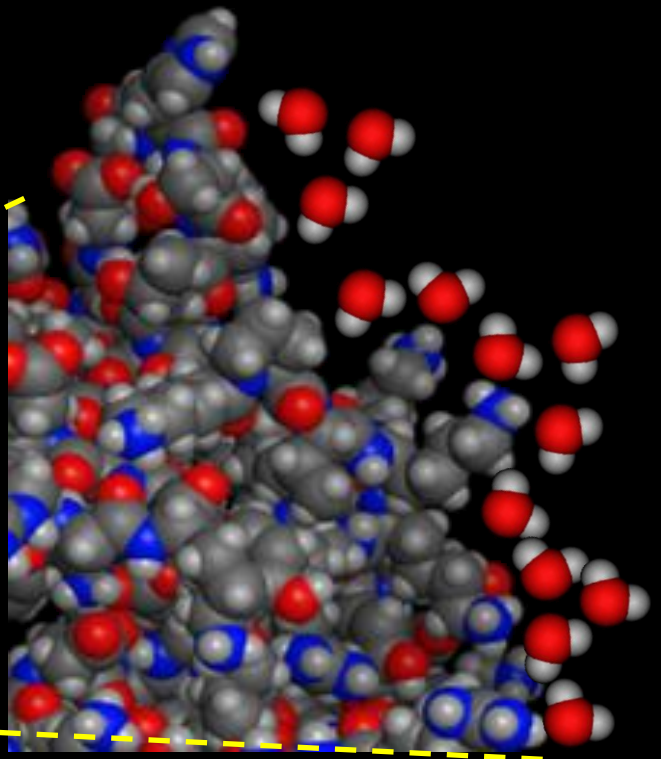


The magic role of the solvent…

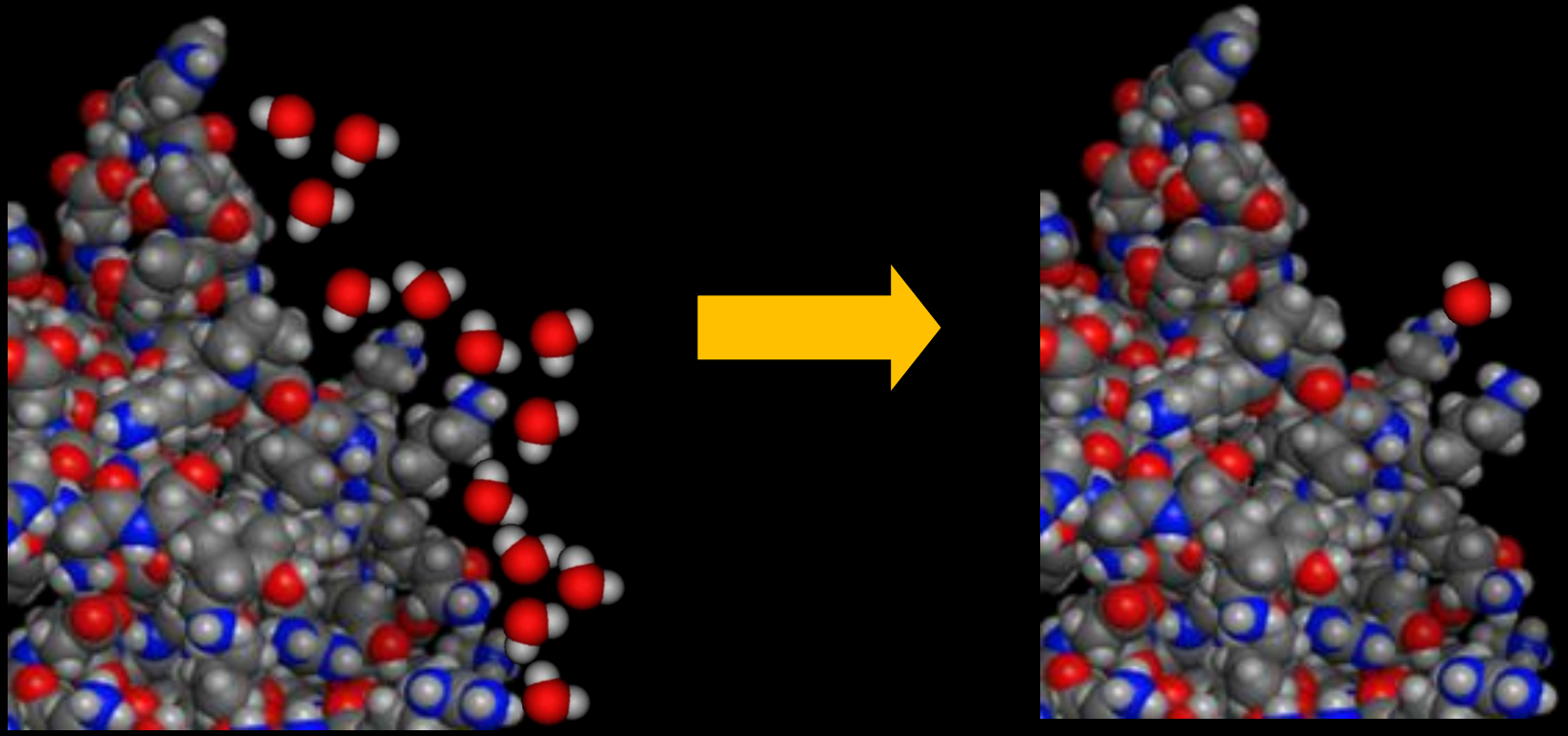


Do you remember the hydration shell concept?

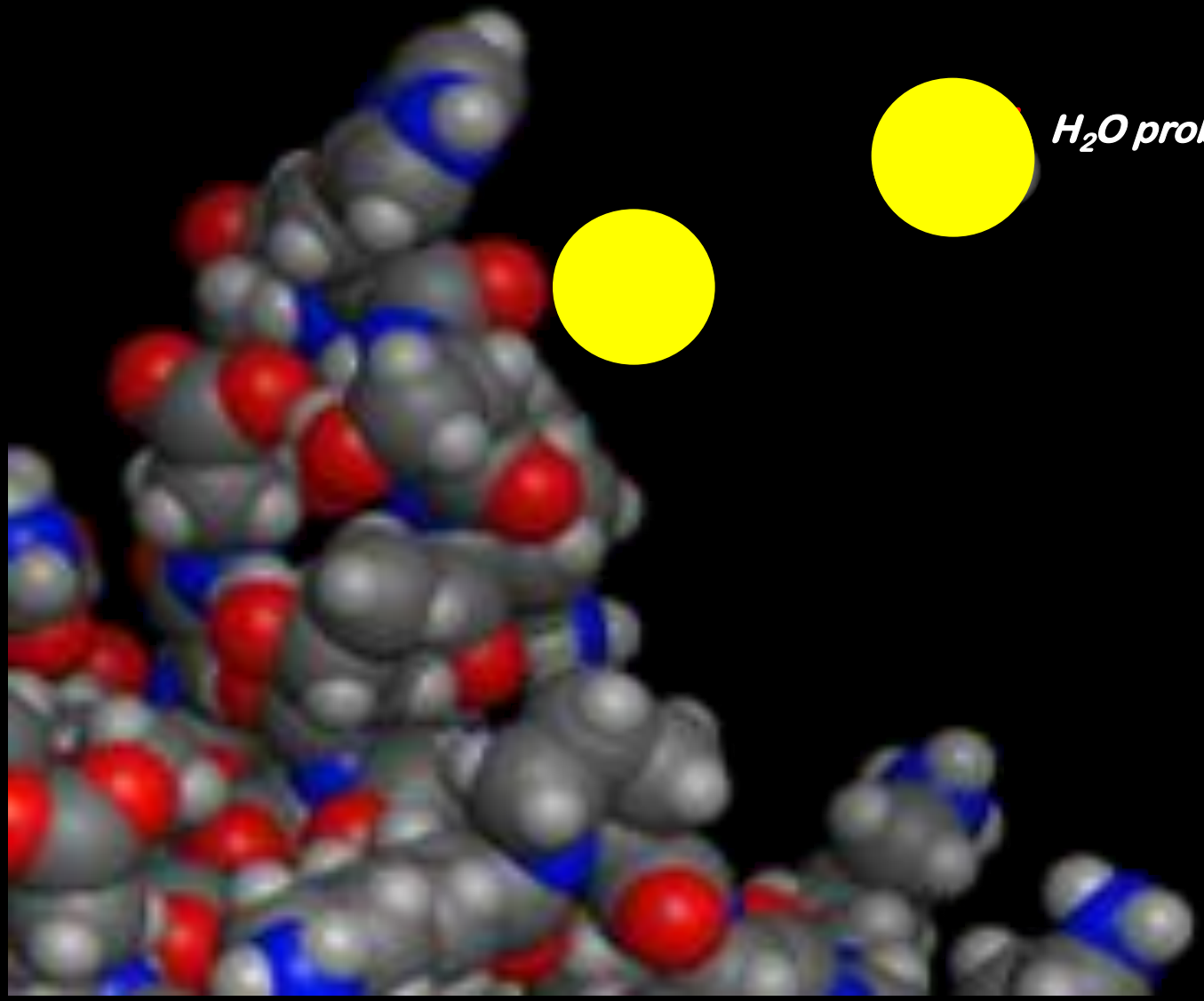




Here is a nice experiment: how we can simulate the first shell of hydration using only ONE water molecule?



From CPK models to molecular surfaces:

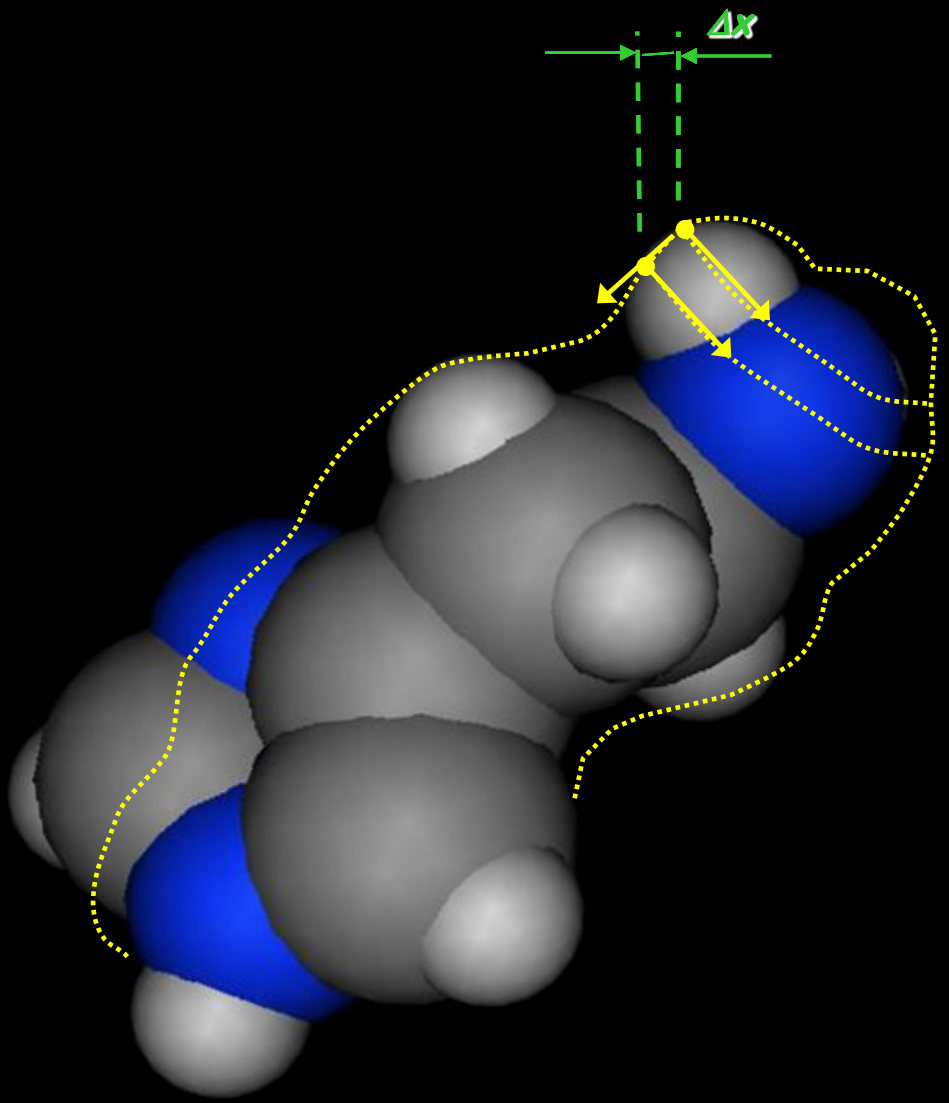


How can we SEE what has TOUCHED the probe?

but, to transform this virtual experiment is an useful experiments we need TWO smart ideas:

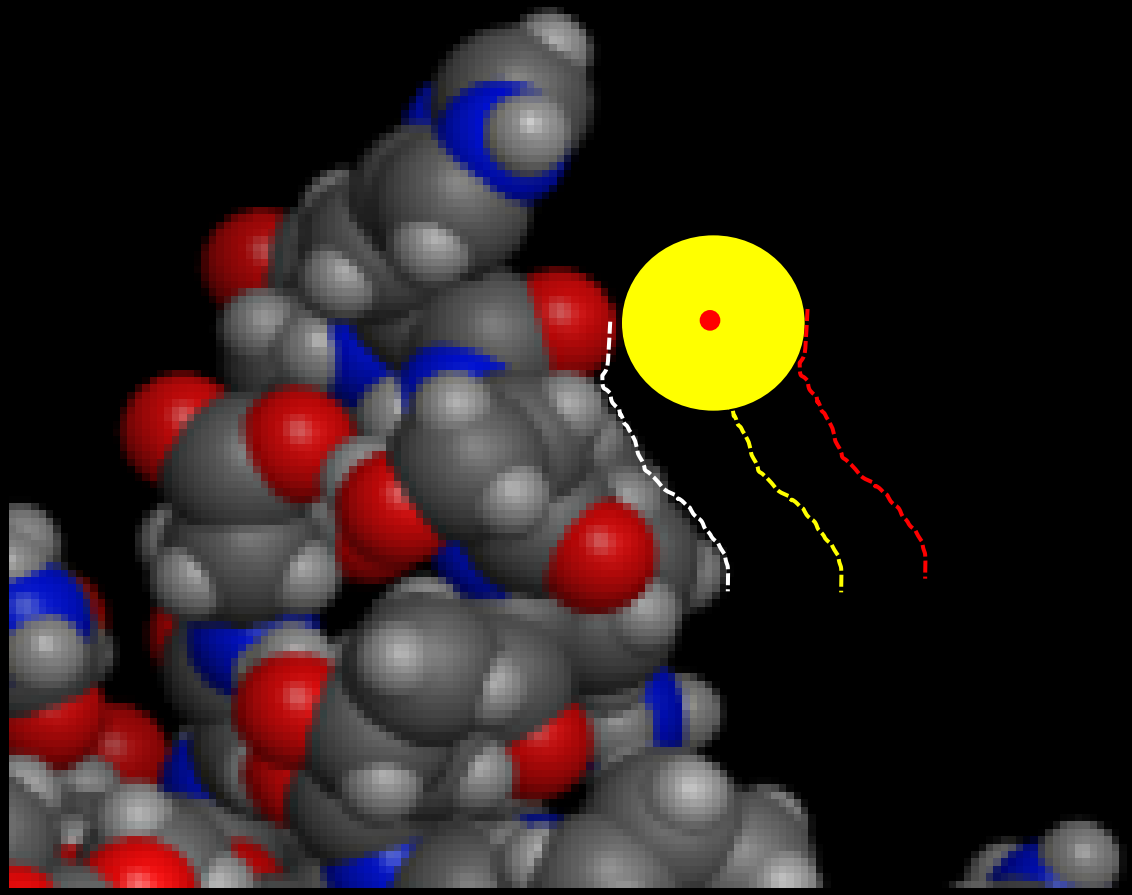
1. Remember Pollicino's fairytale;
2. and…

A bit of algorithm:



Molecular surface representations: Rolling Sphere method

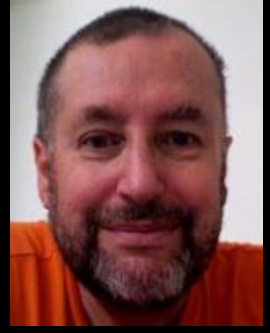
Michael Connolly



The van der Waals Surface (vdWS) is the exterior boundary of the union of van der Waals spheres.

The Solvent Excluded Surface (SES, also known as Connolly surface) is the results of the SAS erosion by the same probe.

The Solvent Accessible Surface (SAS) is the result of the vdW Surface dilatation by a structuring element, or a probe, representing a solvent molecule, typically water.



Michael Connolly

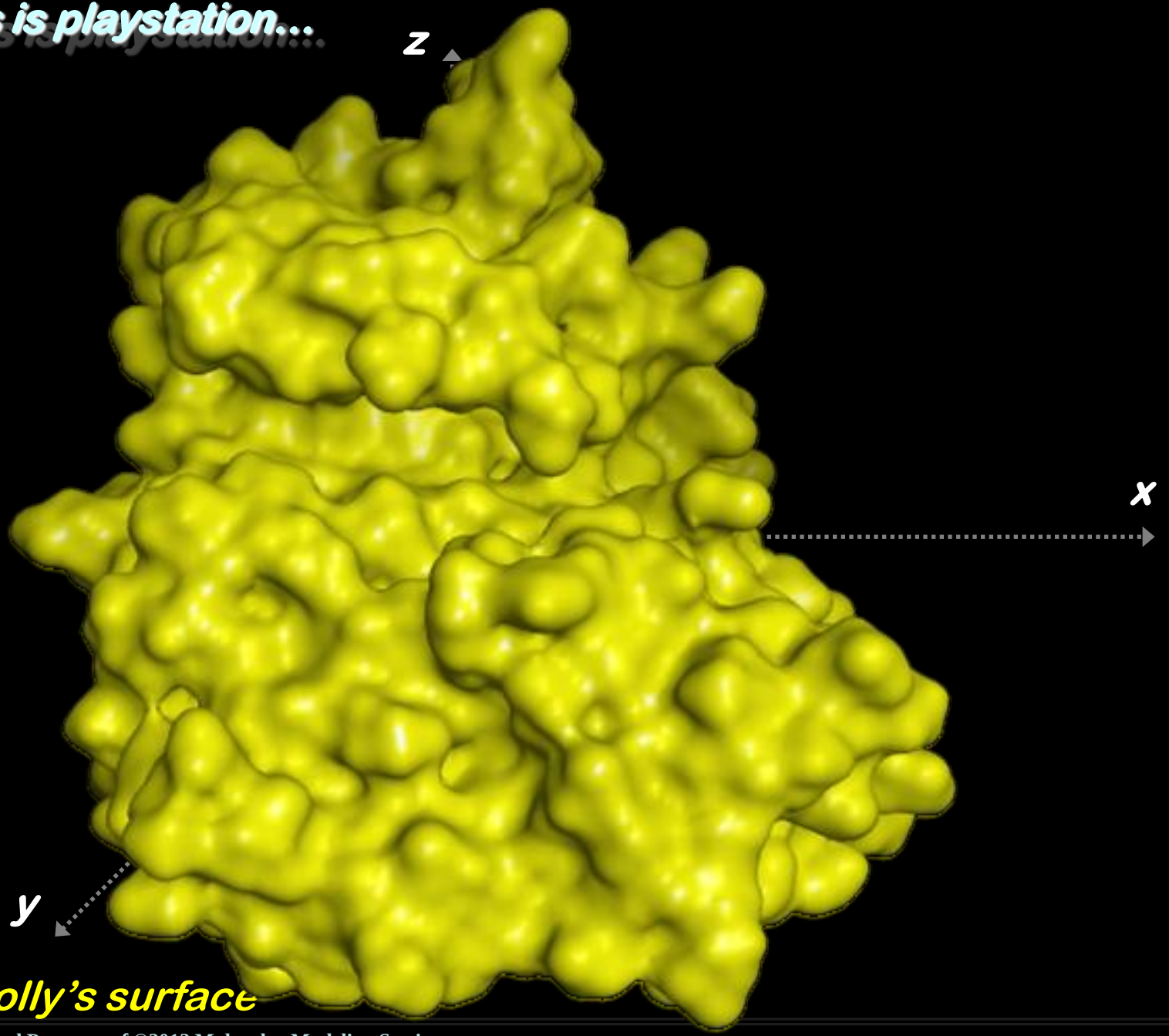
z

x

y

Connolly's surface

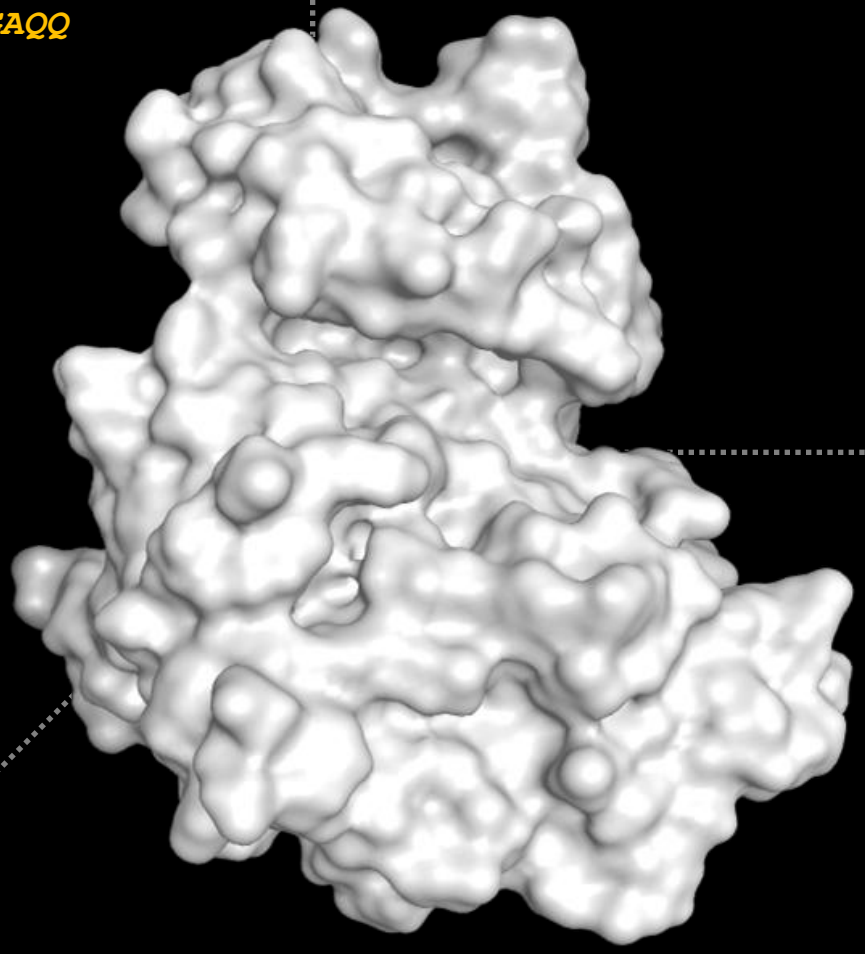
This is playstation...



Connolly's surface

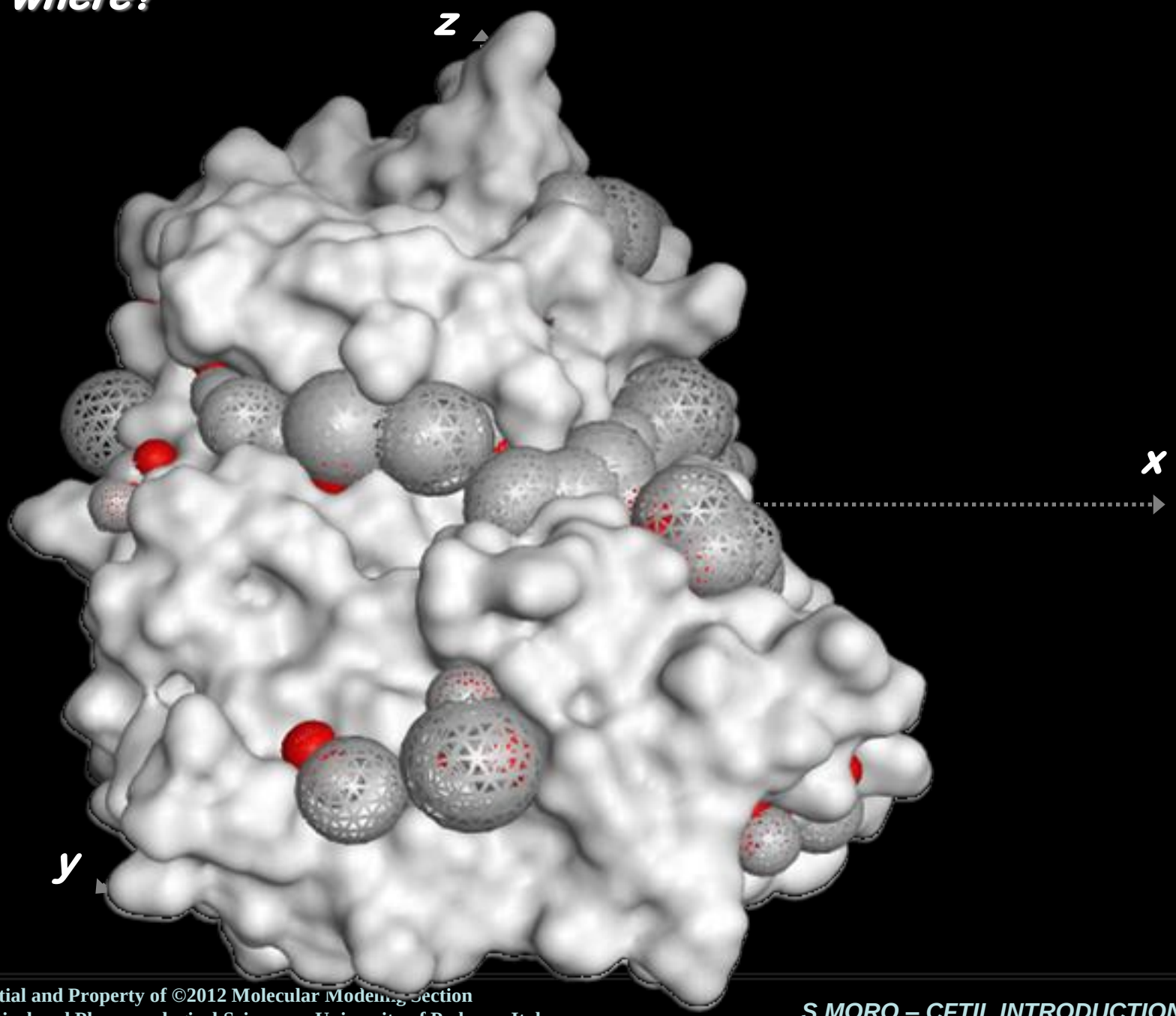
MSGPVPSRARVYTDVNTHRPREYWDYESHVVEWGNQDDYQLVRKLGRGKYSEVFEAINIT
NNEKVVVKILKPVKKKKIKREIKILENLRGGPNIITLADIVKDPVSRTPALVFEHVNNTD
FKQLYQTLTDYDIRFYMYEILKALDYCHSMGIMHRDVKPHNVMIDHEHRKLRLIDWGLAE
FYHPGQEYNVRVASRYFKGPELLVDYQMYDYSLDMWSLGCMLASMIFRKEPFFHGHDNYD
QLVRIAKVLGTEDLYDYIDKYNIELDPRFNDILGRHSRKRWERFVHSENQHLVSPEALDF
LDKLLRYDHQSRLTAREAMEHPYFYTVVKDQARMGSSSMPGGSTPVSSANMMSGISSVPT
PSPLGPLAGSPVIAAANPLGMPVPAAAGAQQ

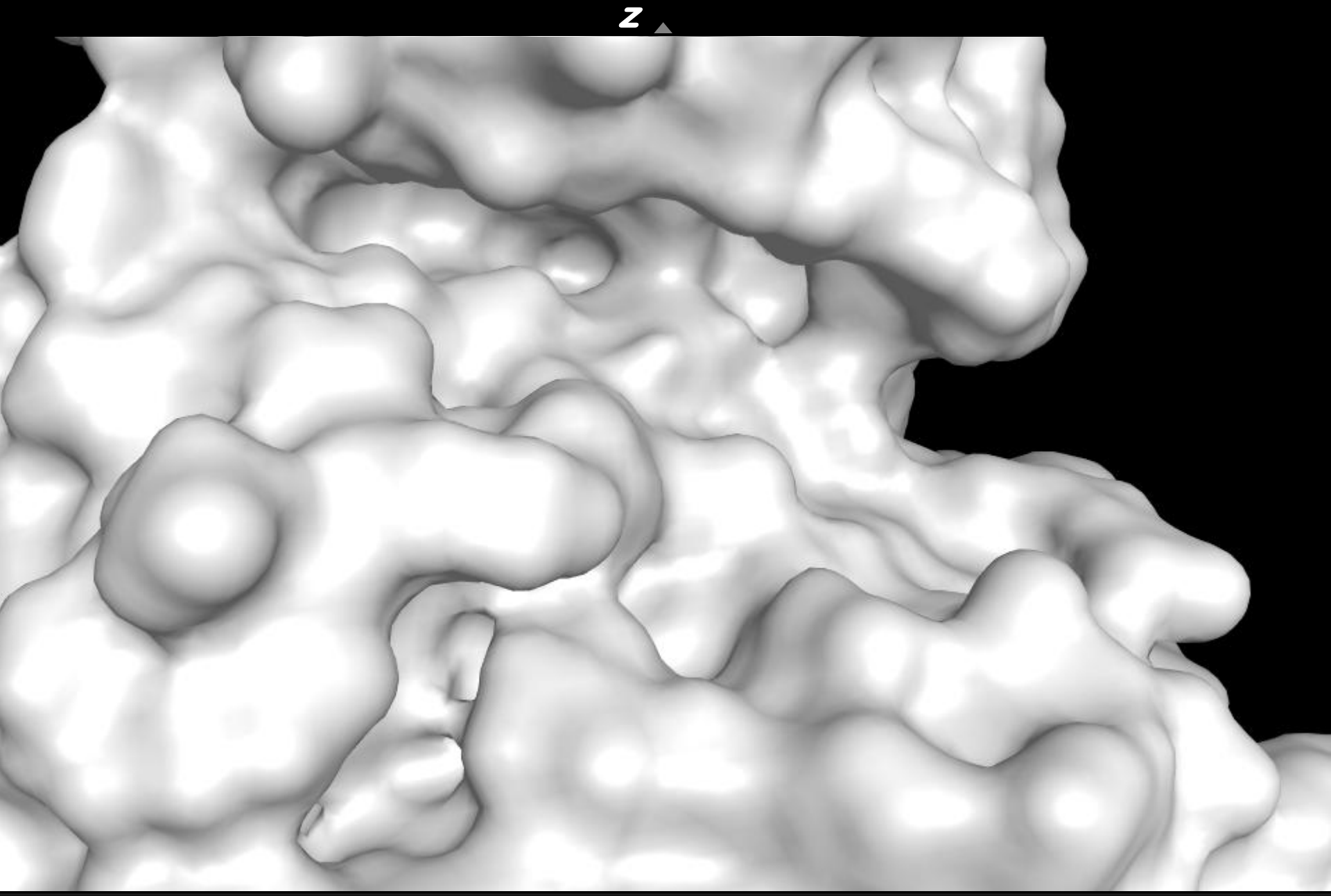
1. where?
2. how?
3. how long?

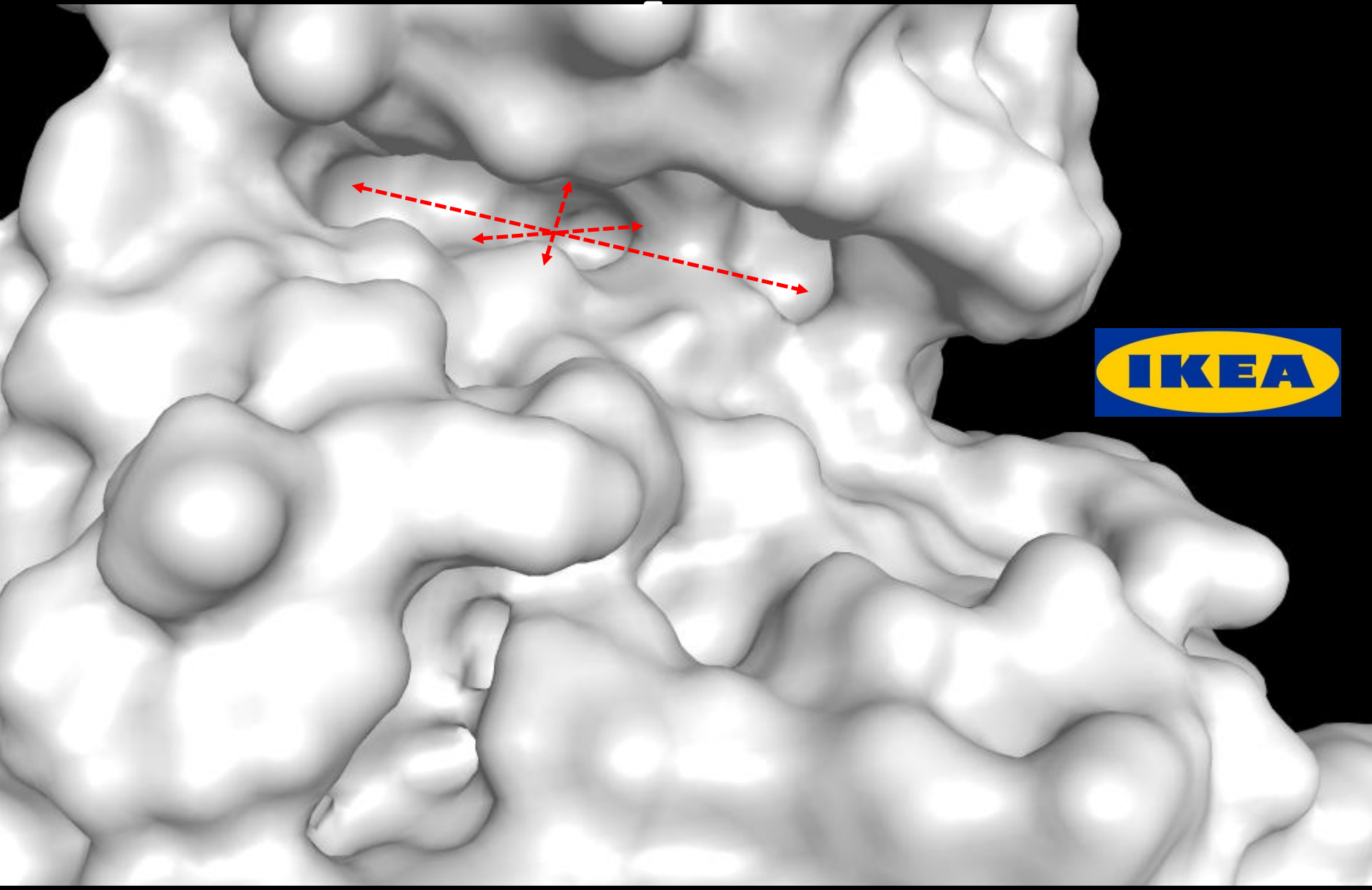


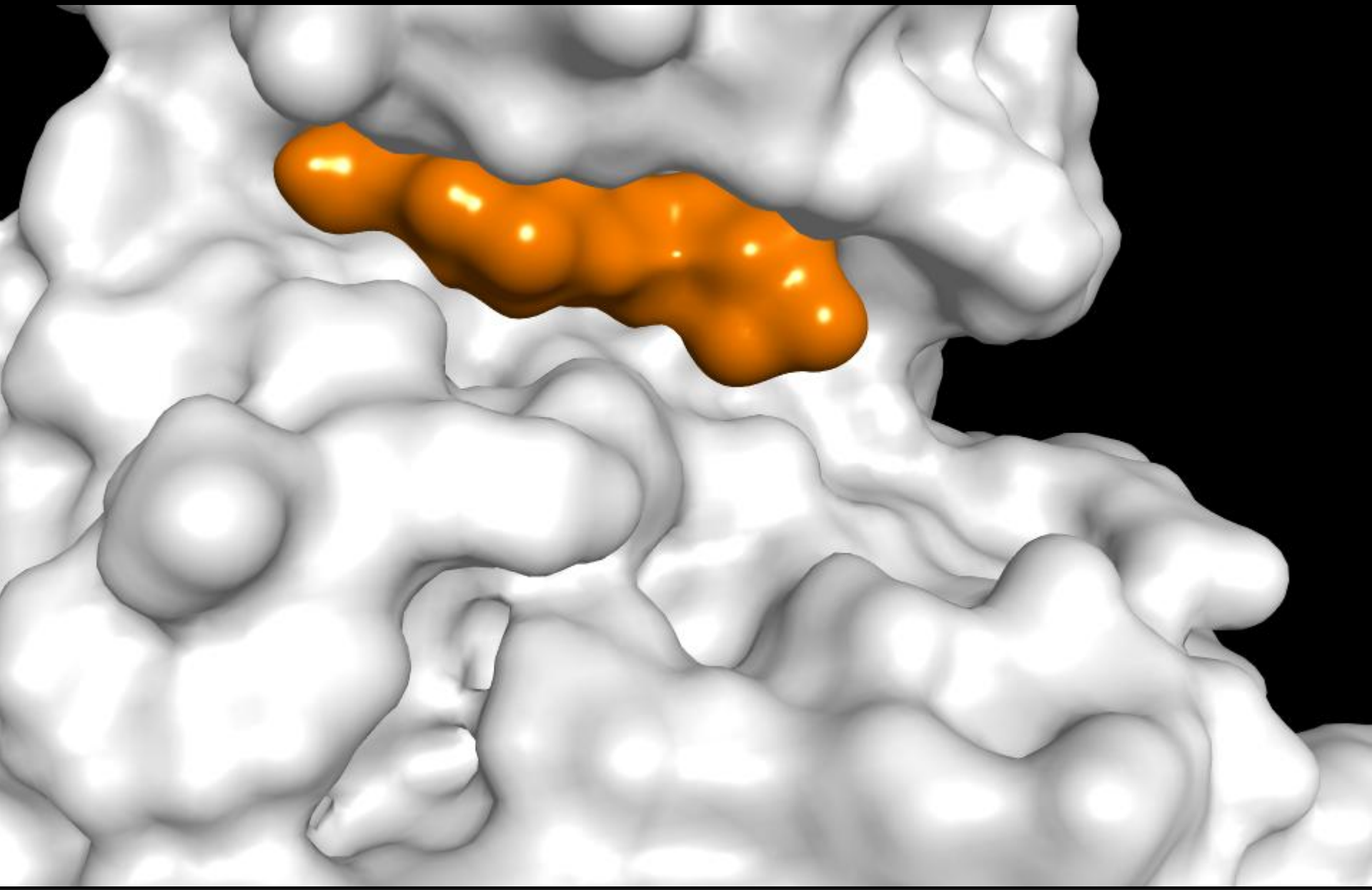
From sequence to topology… from topology to recognition

... where?

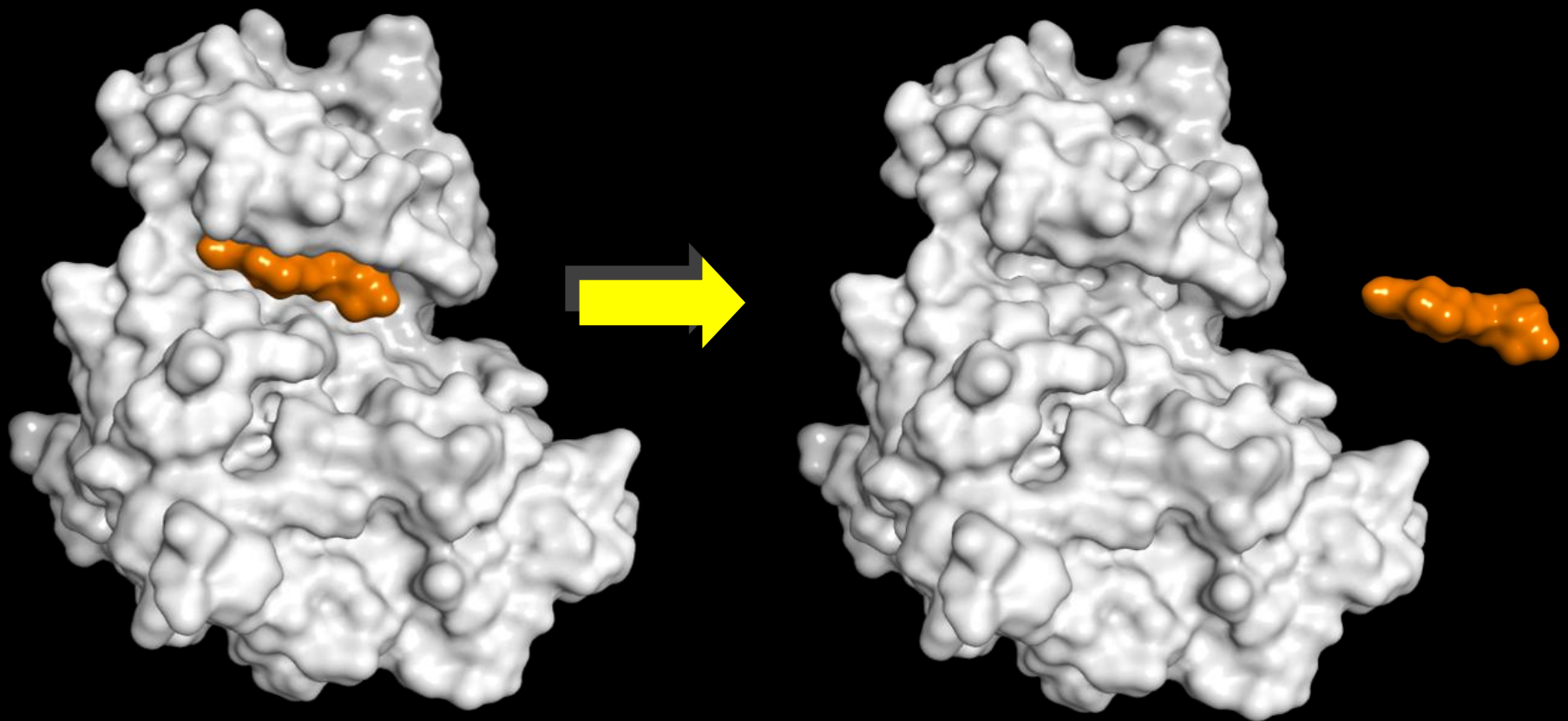




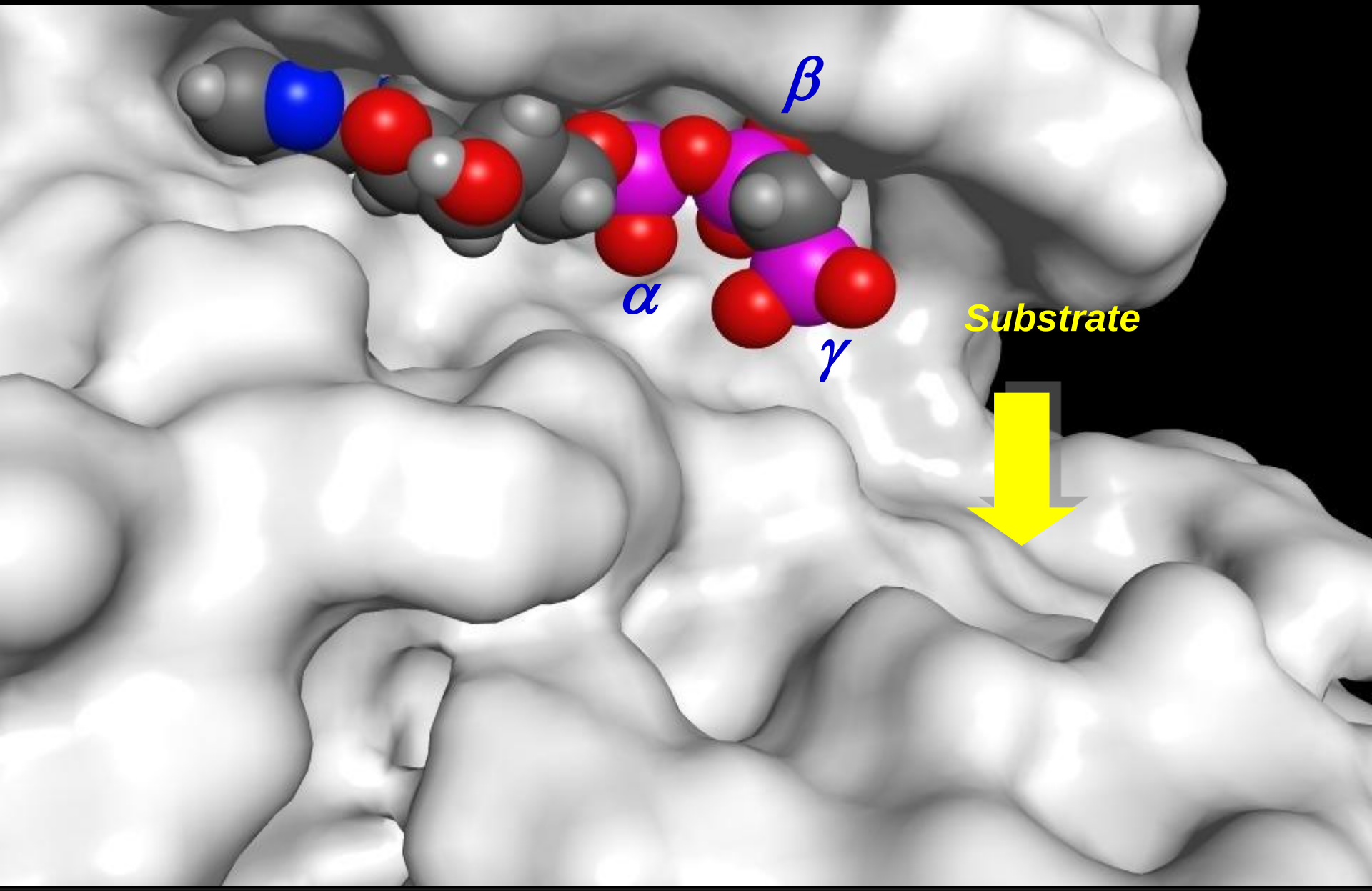

IKEA

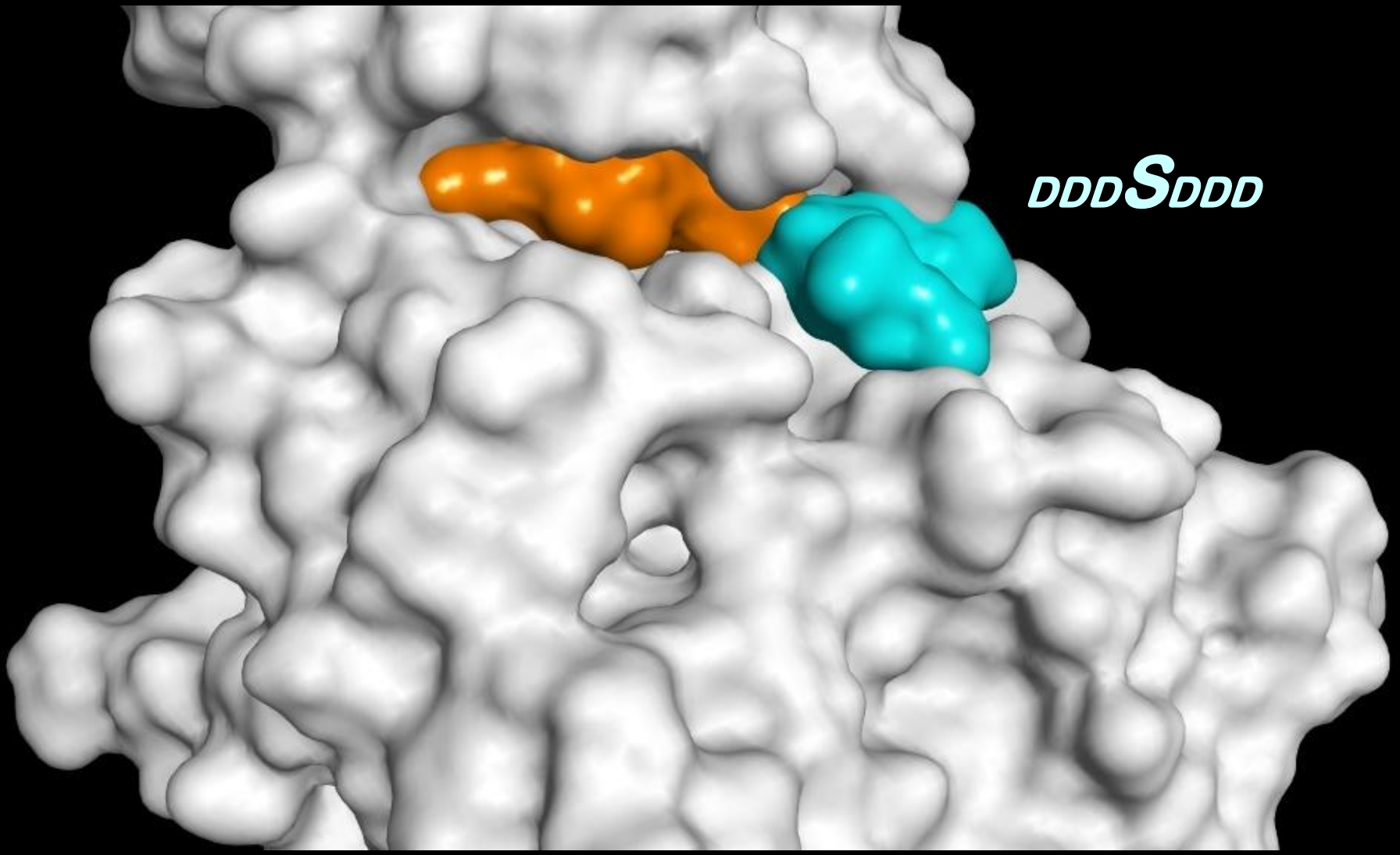


You have now a wonderful tool to estimate the topological complementarity between a cavity and its ligand:

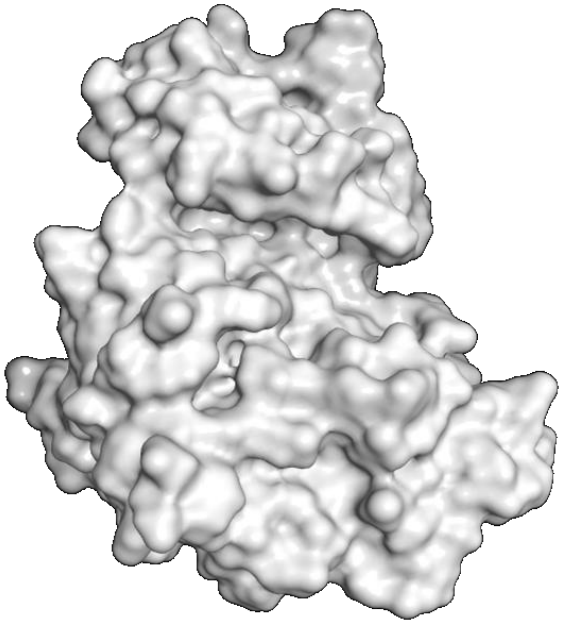


$$Complementarity \propto Vol_{cavity} - Vol_{ligand}$$

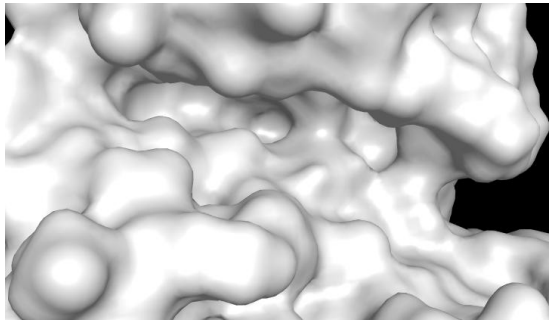




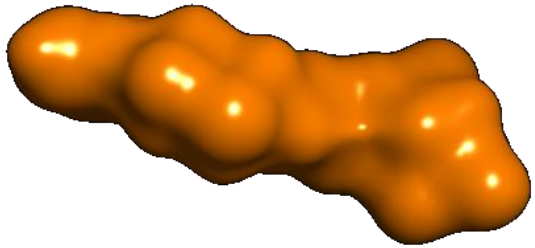
Are we able to measure these volumes?



Volume of the protein ($Å^3$)



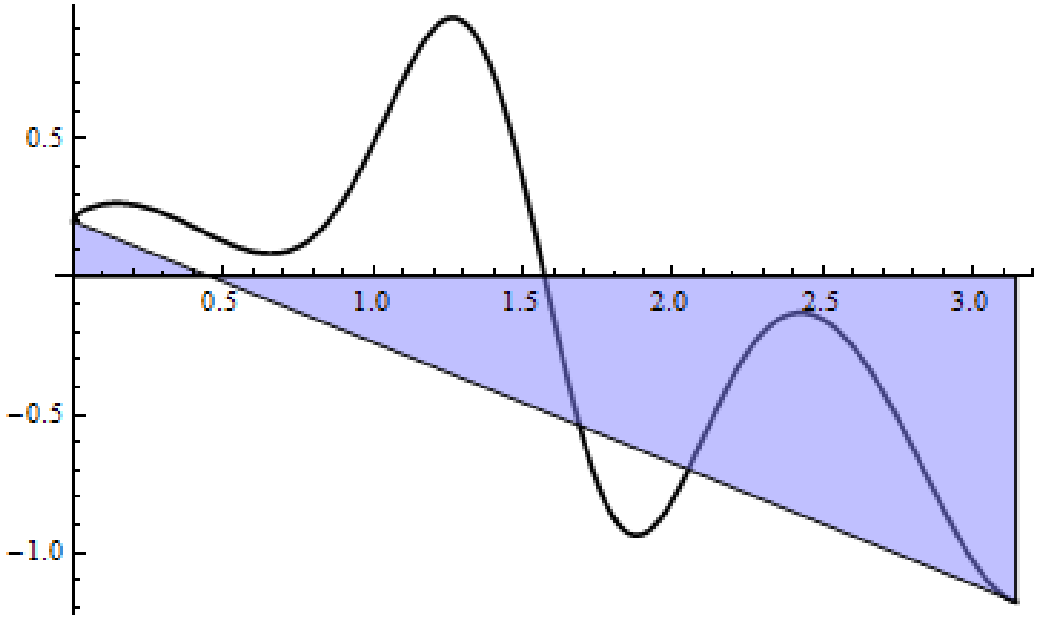
Volume of the cavity ($Å^3$)



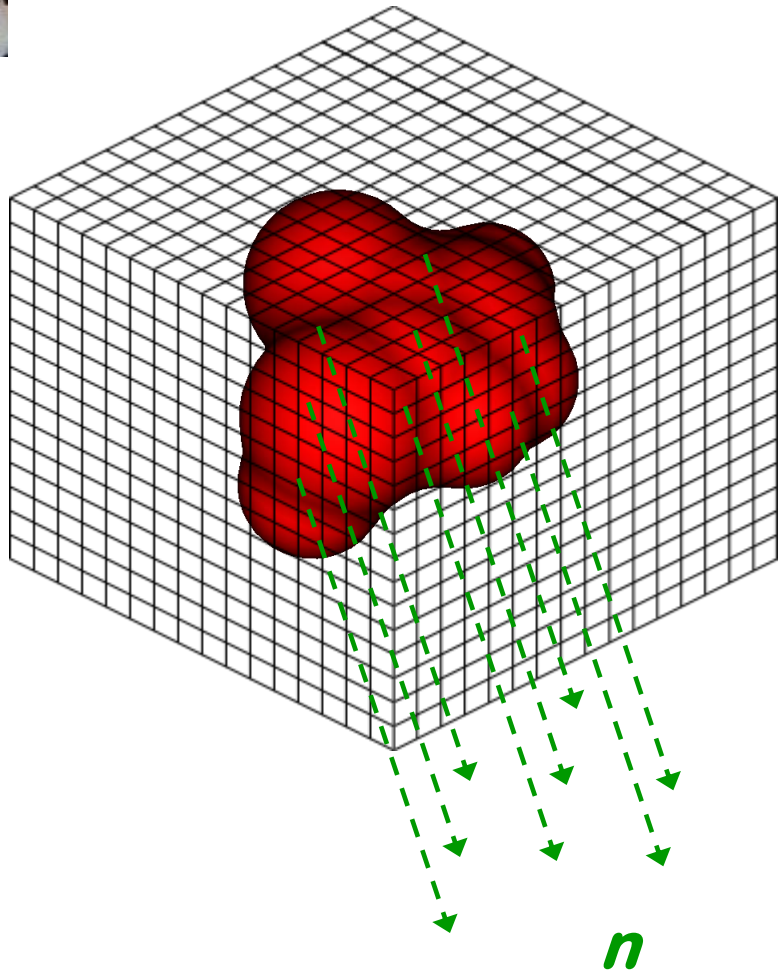
Volume of the ligand ($Å^3$)

do you remember the trapezoidal rule ?

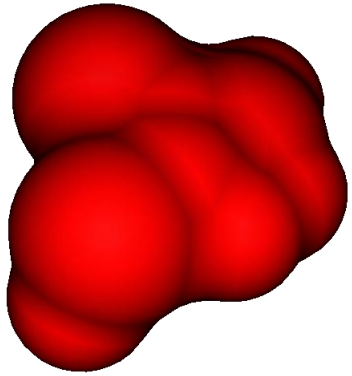
$$\int_a^b f(x)dx \approx (b-a)\frac{f(a)+f(b)}{2}$$



a 3D version of ourtrapezoidal rule

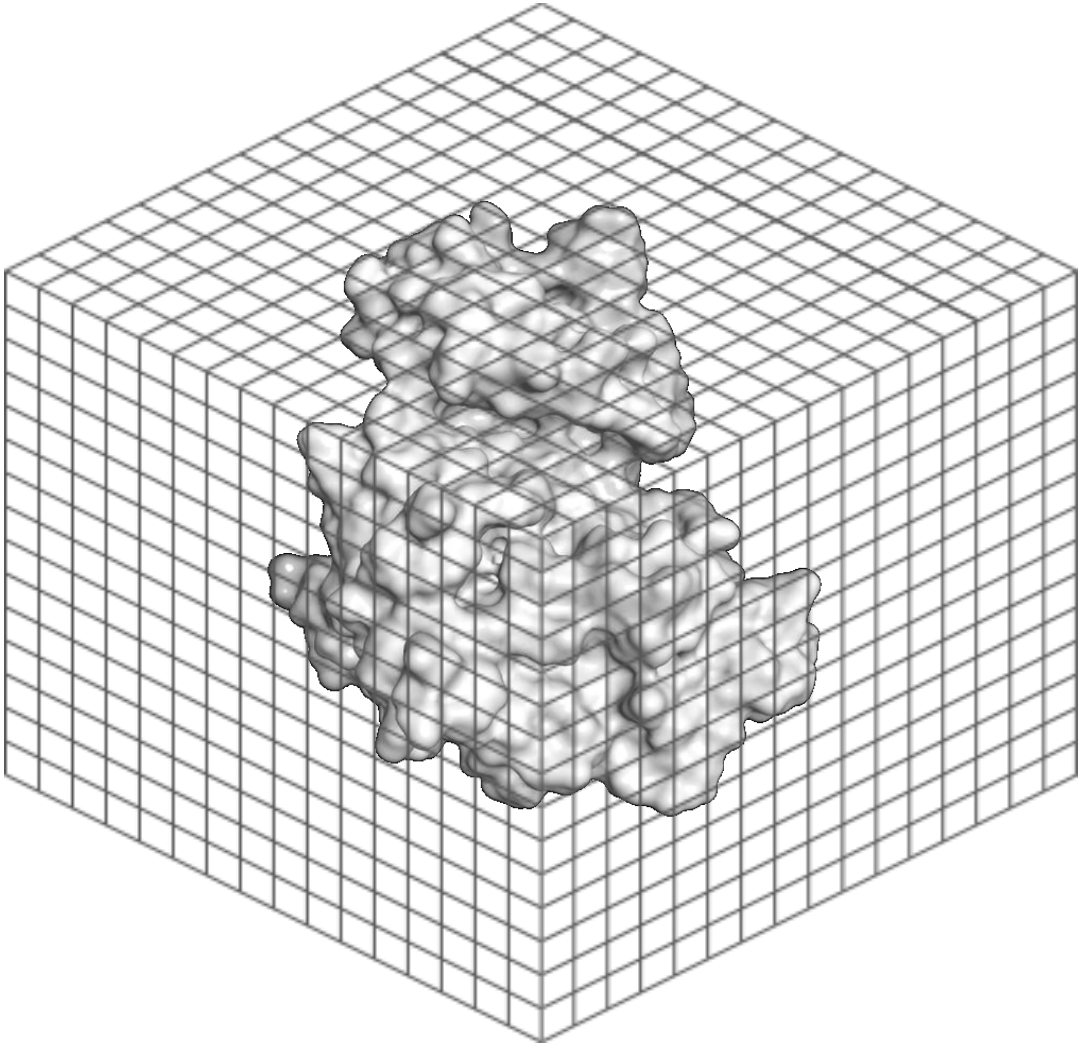


$= V_i$



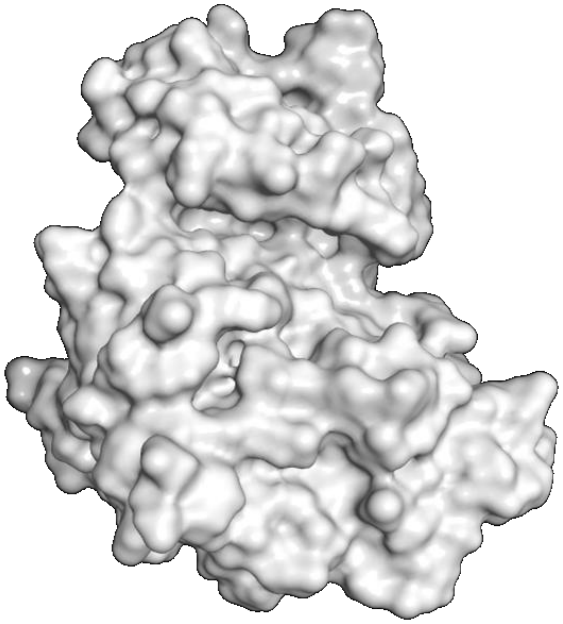
$V_{mol} \cong n\ V_i$

a 3D version of ourtrapezoidal rule



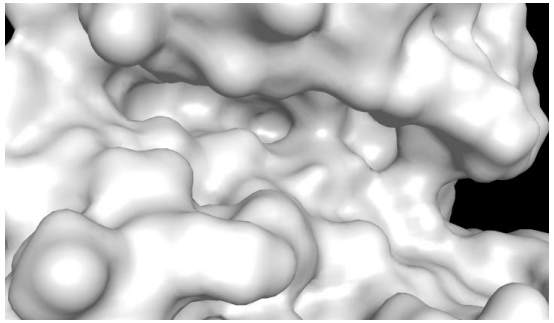
Volume of the protein = 43450 Å^3

Are we able to measure these volumes?



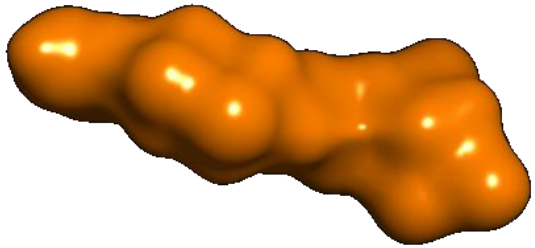
Volume of the protein ($Å^3$)

43450



Volume of the cavity ($Å^3$)

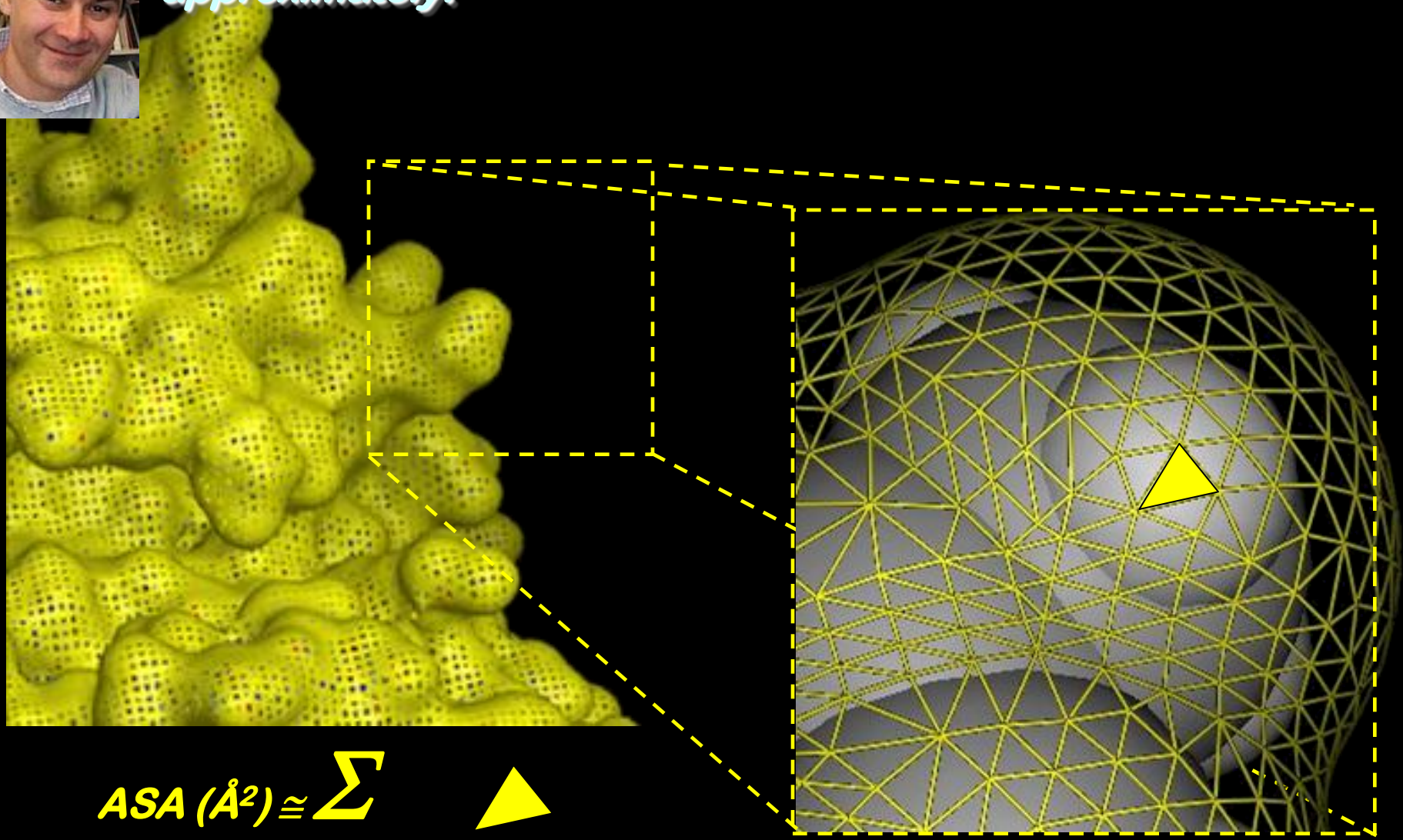
265



Volume of the ligand ($Å^3$)

193

Now also surface extension is very easy to calculate... approximately!



$$ASA\ (Å^2) \cong \sum \blacktriangle$$

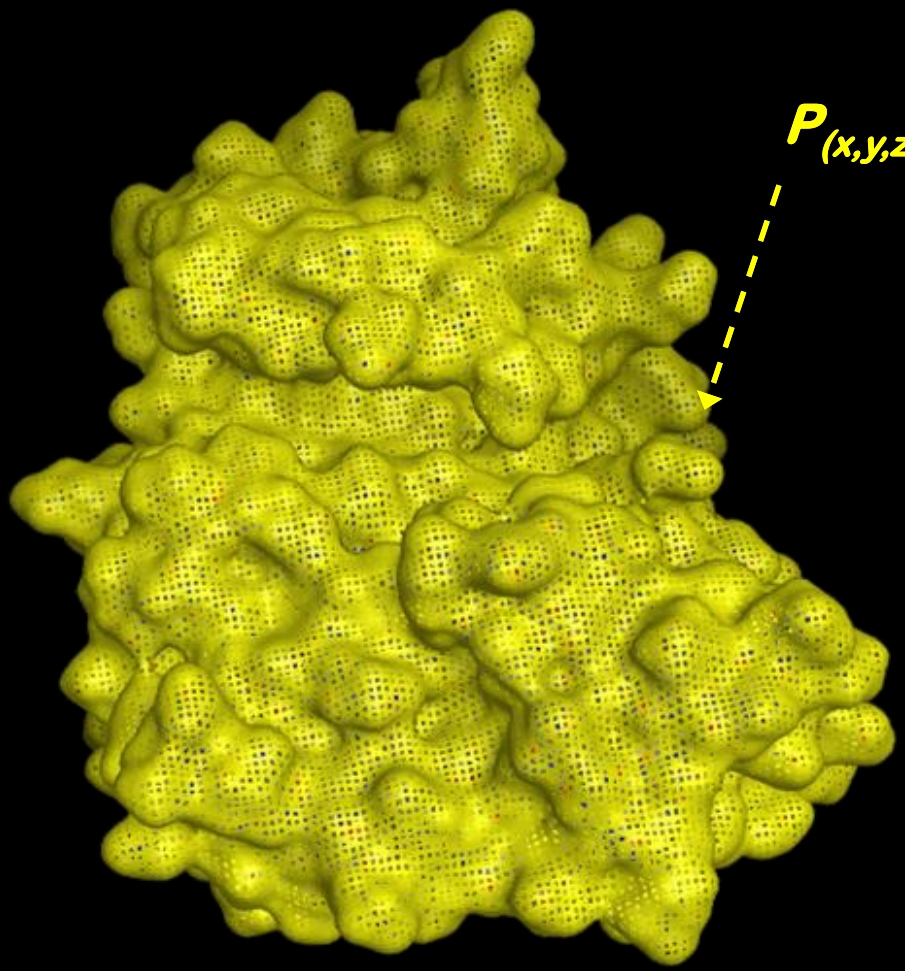
Accessible Surface Area = 15410 Å²

Remember?

*"It is generally accepted that receptor and substrate molecules recognize each other at their molecular surfaces. Therefore, the binding strength of a receptor-drug complex depends on the shape of the substrate surface and on the **distribution of certain properties on this surface**. Any method attempting to model biological activity should take into account this information and try to correlate it to biological activity…"*

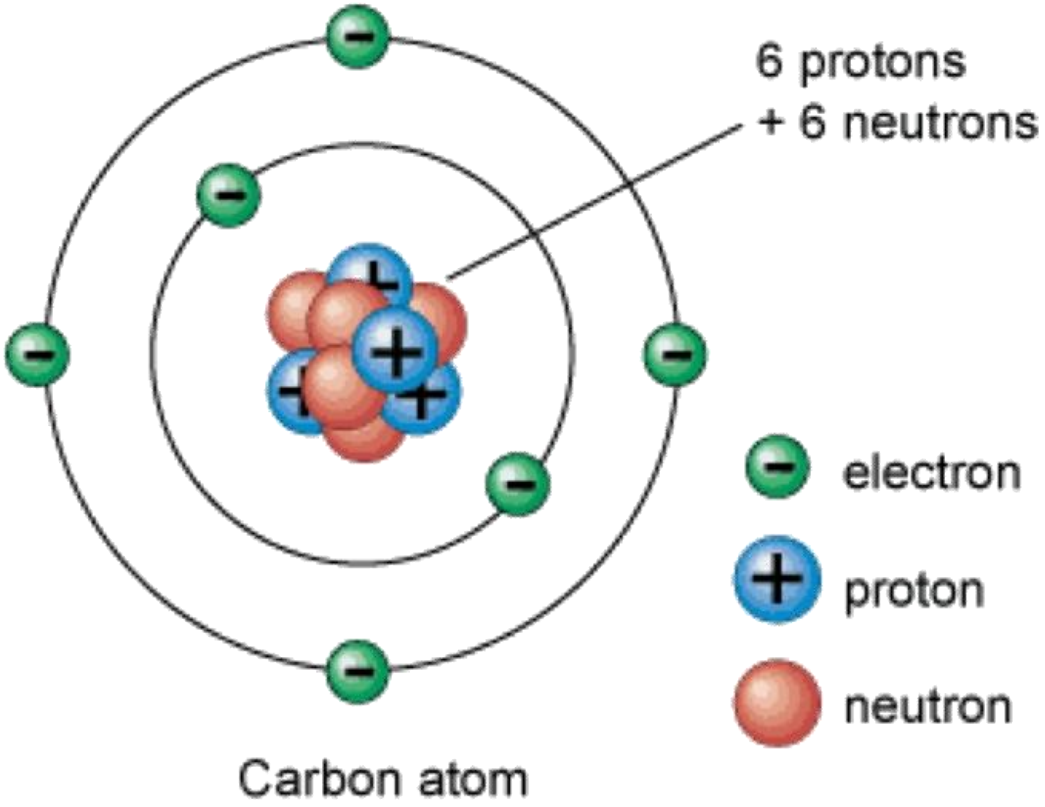
*by Johann Gasteiger et al J.A.C.S. 1995, **117**, 7769-7775*

Molecular surfaces as a locus for the projection of molecular properties:

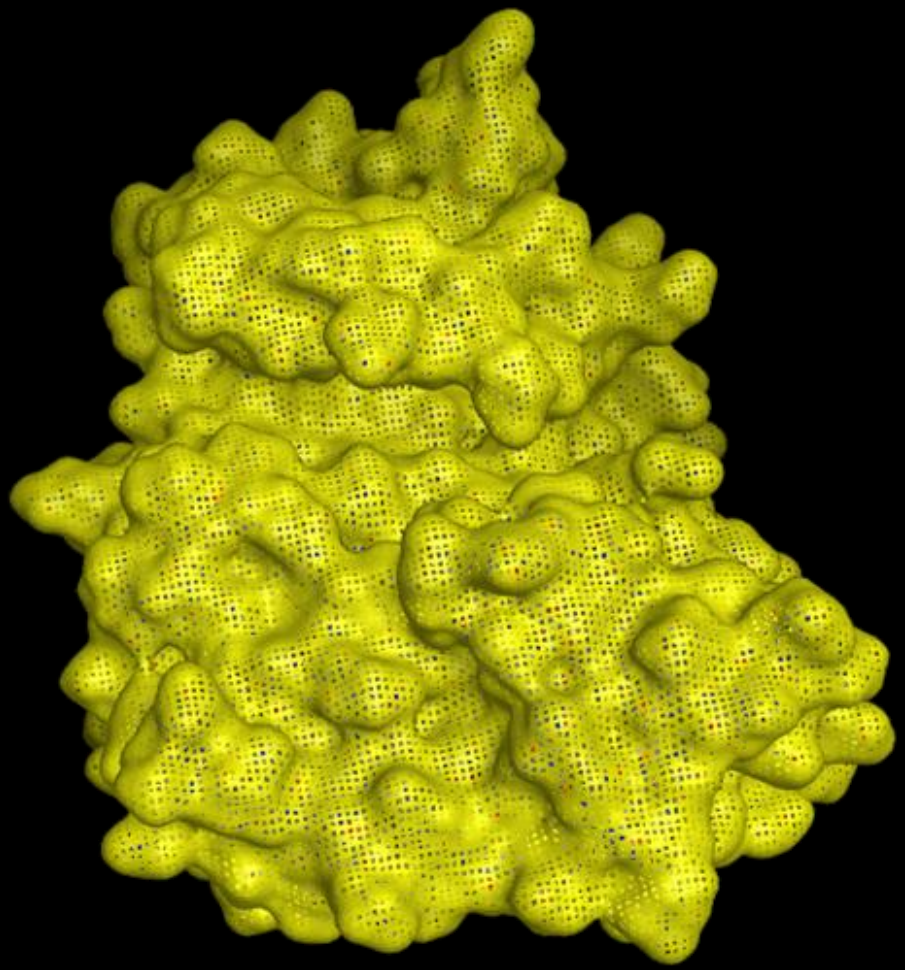


As chemists, what is the first property that you would like to see projected ?

We cannot forget that under the real surface we surely find… charges!



Considering that molecules are an ensemble of electric charges... probably the distribution of their electrostatic potentials?



How we can virtualize the projection of the electrostatic potential o molecular surface?

We can surely start with him!

***Charles Augustin de Coulomb** (Angoulême, 14 giugno 1736 – Parigi, 23 agosto 1806).*

$$F_{el} = k_0 \frac{q_1 q_2}{r^2}$$

$$\text{Coulomb's costant} : k_0 = 9 \times 10^9 \frac{Nm^2}{C^2}$$

$$k_0 = \frac{1}{4\pi\varepsilon_0}$$

where ε_0 is the electric permittivity of free space

$$\varepsilon_0 = 8{,}9 \times 10^{-12} \frac{C^2}{Nm^2}$$

I try to recite it:

"The magnitude of the electrostatic force of interaction between two ***point charges*** *is directly proportional to the scalar multiplication of the magnitudes of charges and inversely proportional to the square of the distance between them."*

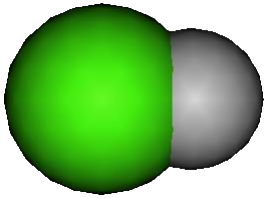
How we can deal with the point charges concept when, in the real world, we usually deal with charge distributions?

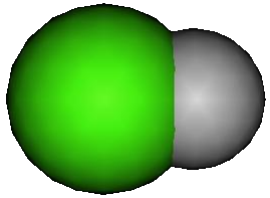
Probably, using a very interesting physical trick:

$\Delta\chi = 1.9$

$\mu = 1.82$ D

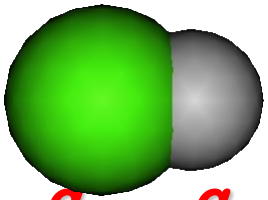
F - H





$\chi_F = 4.0$ $\chi_H = 2.1$

$-\delta$ $+\delta$



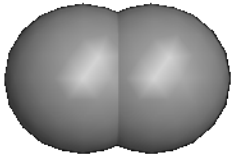
q, partial atomic charge q_F q_H

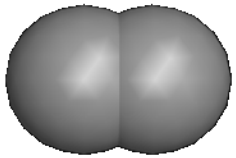
μ **f** $(q_F; q_H)$

Probably, using a very interesting physical trick:

$\Delta\chi = 0$ $\mu = 0\ D$

H - H

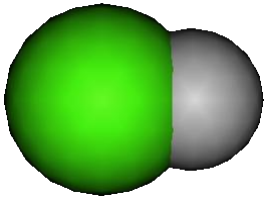


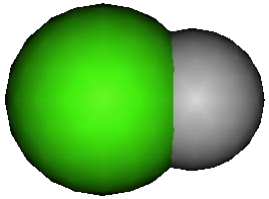


$\chi_H = 2.1$ $\chi_H = 2.1$

$\Delta\chi = 1.9$ $\mu = 1.82\ D$

F - H





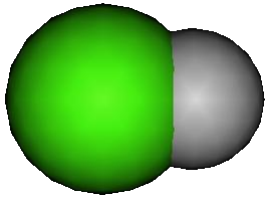
$\chi_F = 4.0$ $\chi_H = 2.1$

$-\delta$ $+\delta$

Probably, using a very interesting physical trick:

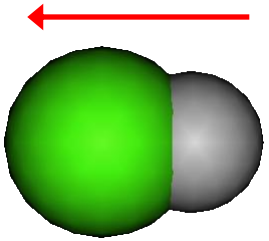
F - H

$\Delta\chi = 1.9$



$\chi_F = 4.0$ $\chi_H = 2.1$

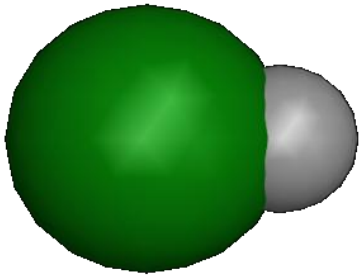
$\mu = 1.82$ D



$-\delta$ $+\delta$

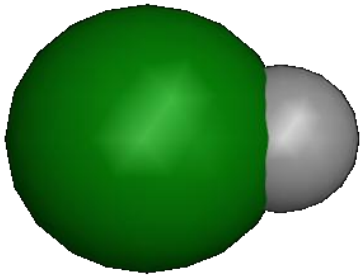
Cl - H

$\Delta\chi = 1.1$



$\chi_{Cl} = 3.2$ $\chi_H = 2.1$

$\mu = 1.08$ D



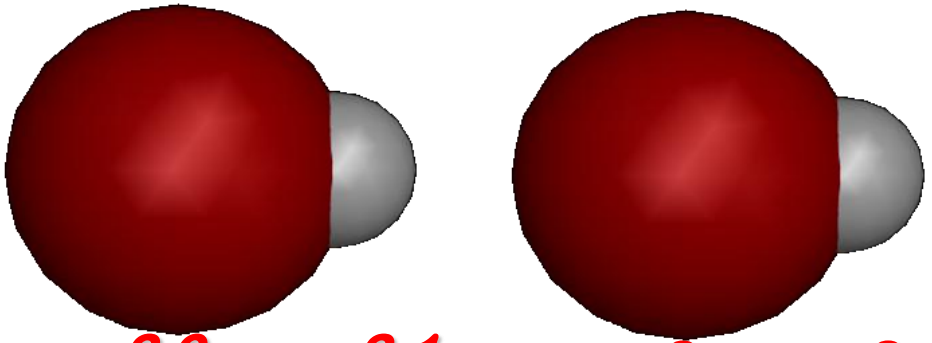
$-\delta$ $+\delta$

Probably, using a very interesting physical trick:

Br - H

$\Delta\chi = 0.8$

$\mu = 0.82$ D



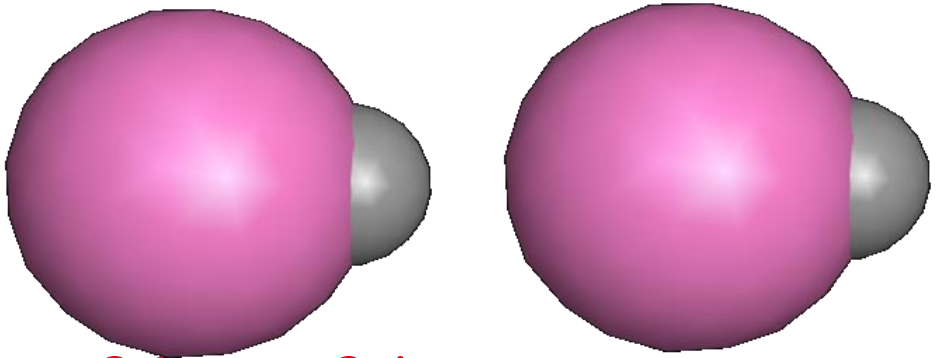
$\chi_{Br} = 2.9$ $\chi_{H} = 2.1$

$-\delta$ $+\delta$

I - H

$\Delta\chi = 0.5$

$\mu = 0.44$ D



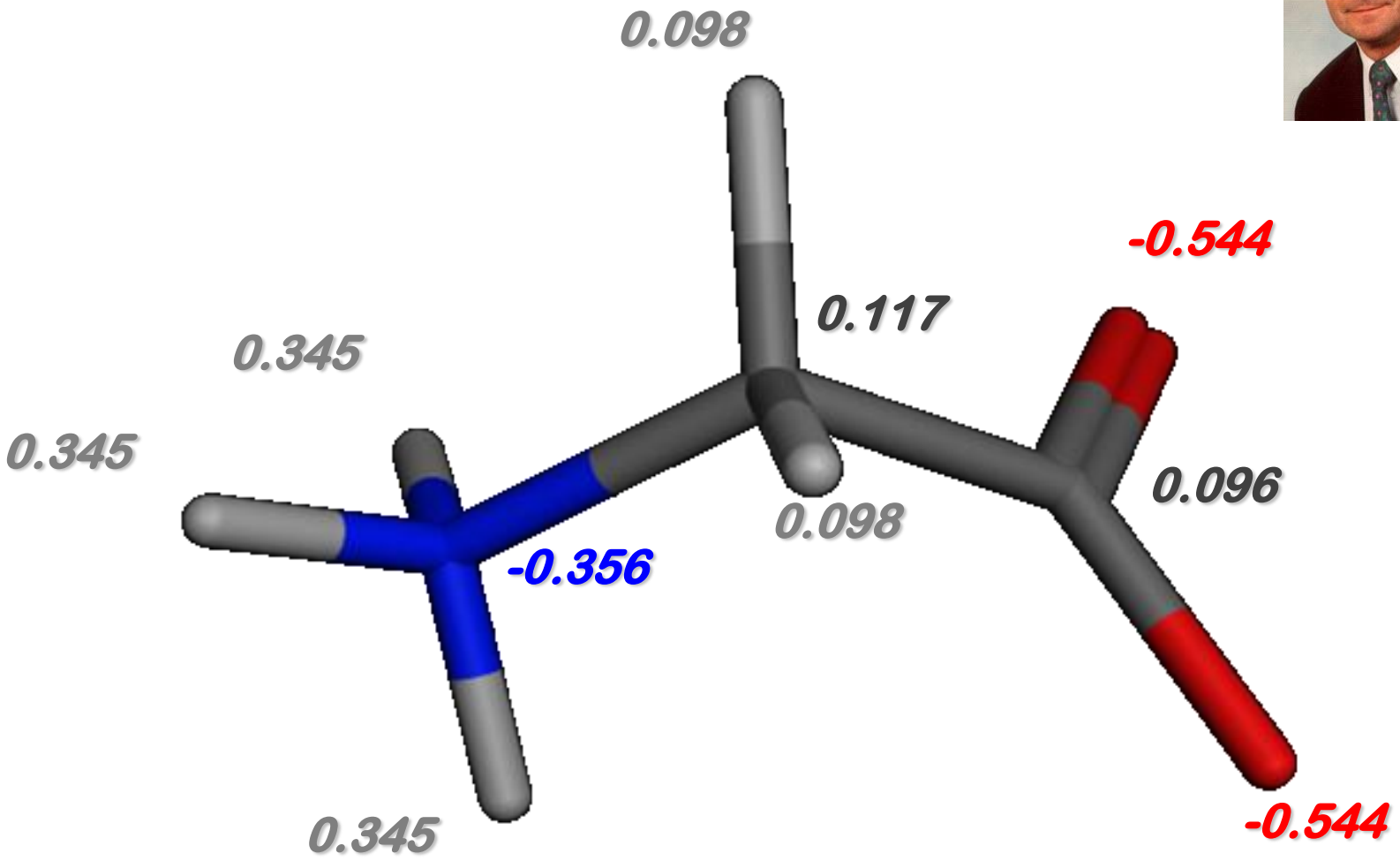
$\chi_{I} = 2.6$ $\chi_{H} = 2.1$

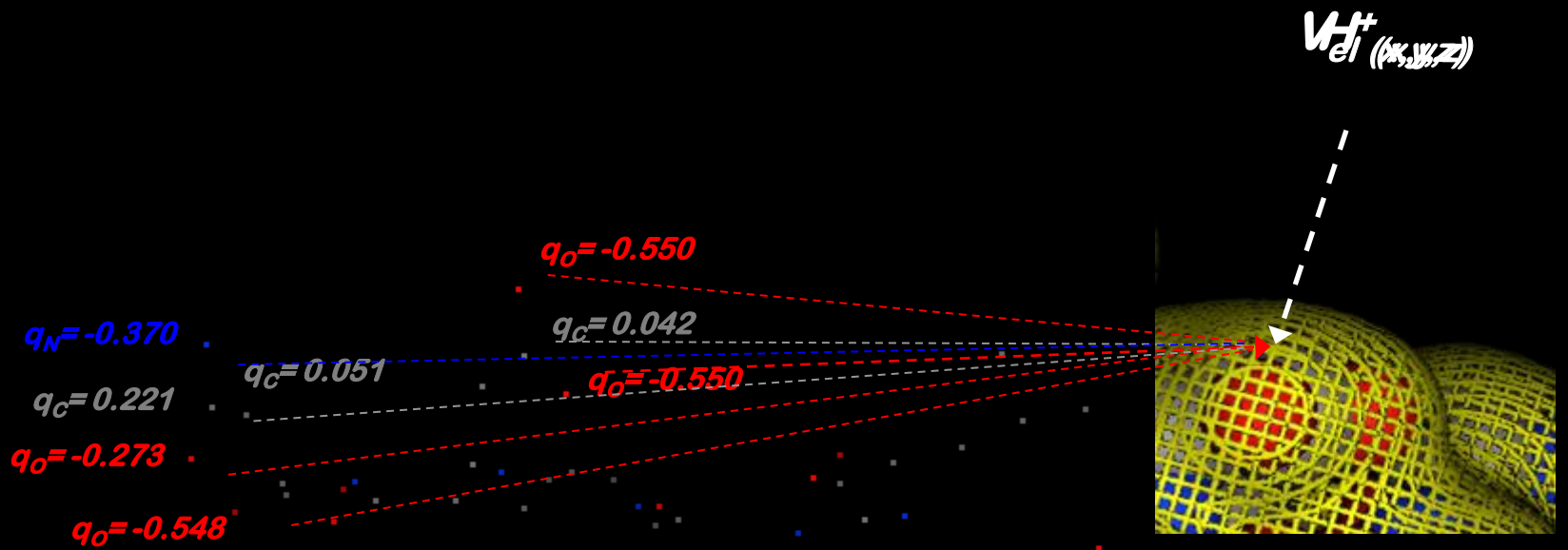
$-\delta$ $+\delta$

Probably, using a very interesting physical trick:

	$\Delta\chi$	μ (D)	q_X	q_H
F - H	1.9	1.82	-0.267	0.267
Cl - H	1.1	1.08	-0.145	0.145
Br - H	0.8	0.82	-0.113	0.113
I - H	0.5	0.44	-0.107	0.107
H - H	0	0	0	0

and now it is very simple to extend:





$$F_{el} = k_0 \sum_{1}^{n} \frac{q_n}{r^2}$$

$$V_{el} = k_0 \sum_{1}^{n} \frac{q_n}{r}$$

... very charming!

DDDSDDD

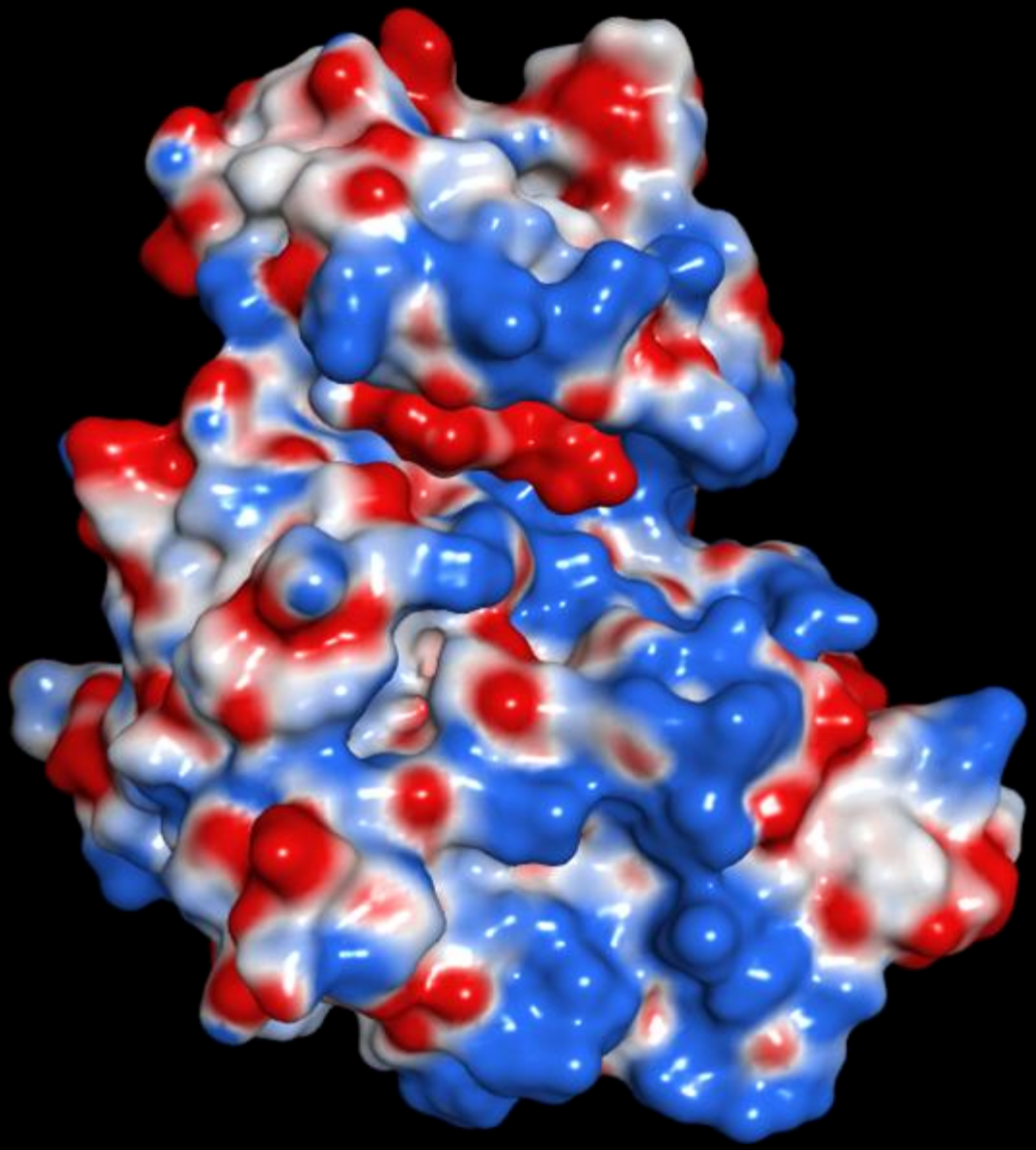
$V_{el} < 0$

$V_{el} = 0$

$V_{el} > 0$

we can reflect for a moment about

$V_{el} = 0$



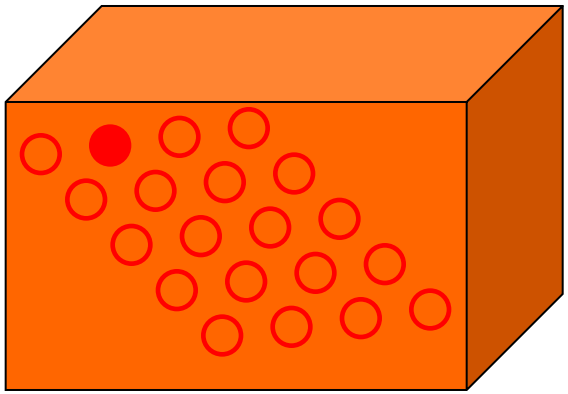
$V_{el} < 0$

$V_{el} = 0$

$V_{el} > 0$

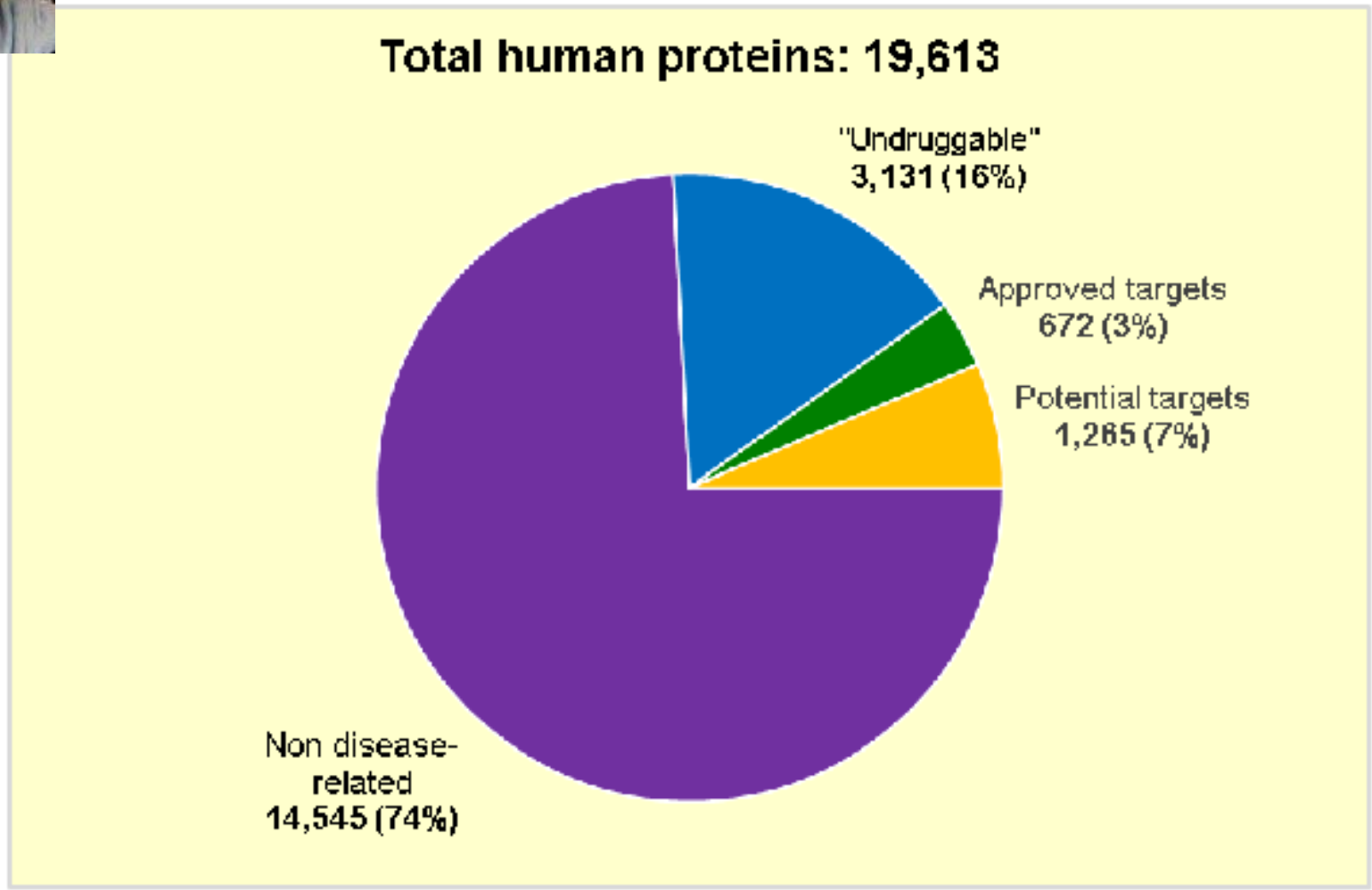
Be patient, I take off the jacket of pharmaceutical and chemical, undeservedly, I wear the jacket of pharmacologist:

● **Biological or "On" Target**



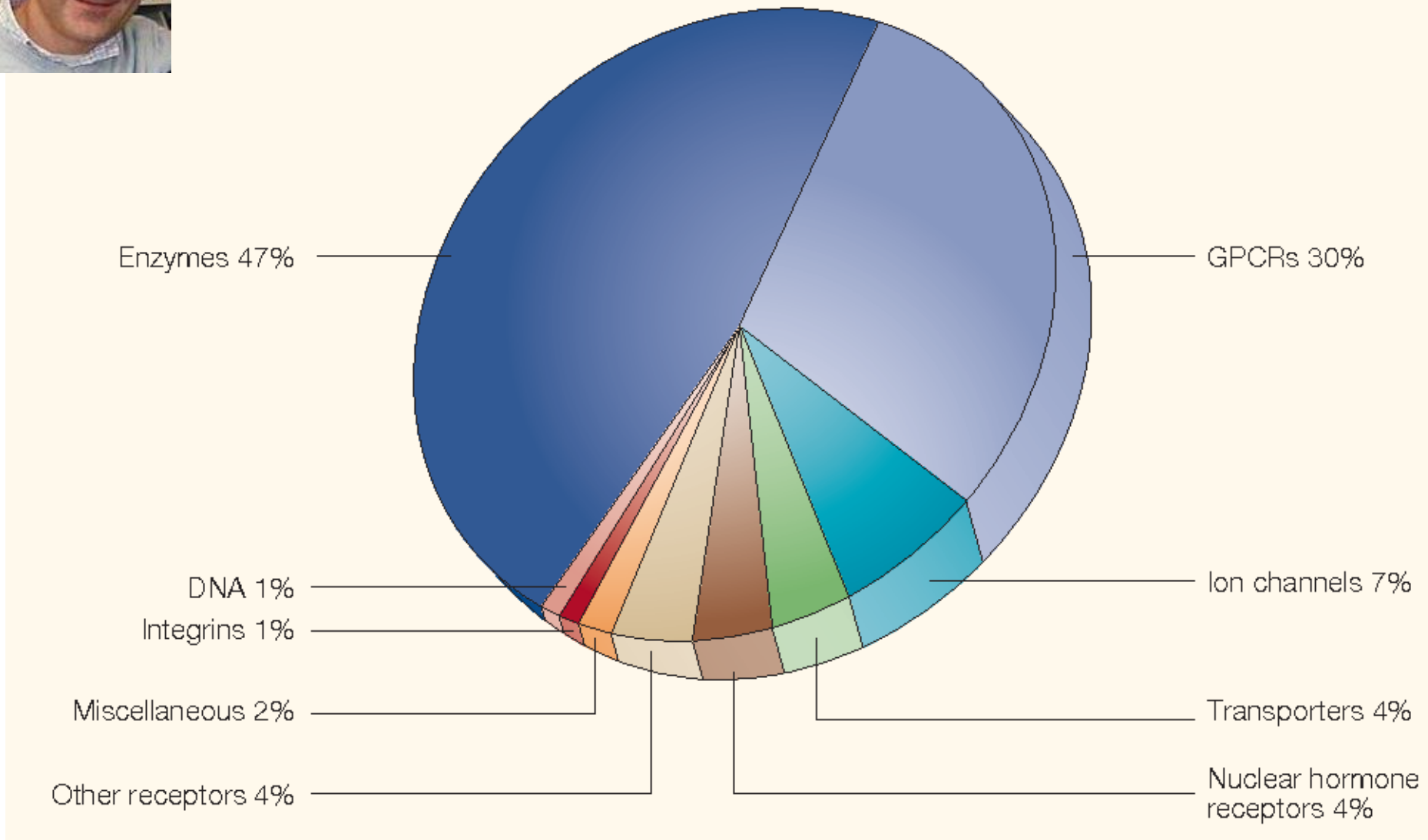
Biological Space

Really very interesting: the Human Protein Atlas…



credits: https://www.proteinatlas.org/

Druggable targets…



credits: https://www.semanticscholar.org/paper/The-druggable-genome-Hopkins-Groom/648a2ef46c4f7b335741111c445ee26adf2f0644/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"CHIMICA FARMACEUTICA E TOSSICOLOGICA II"
DIRECTOR
Stefano Moro
CAMERA
Chimica e Tecnologia Farmaceutiche
DATE
SCENE
TAKE