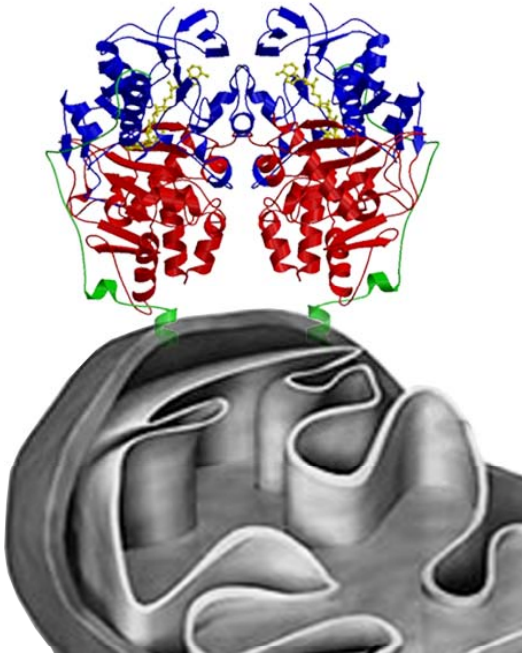
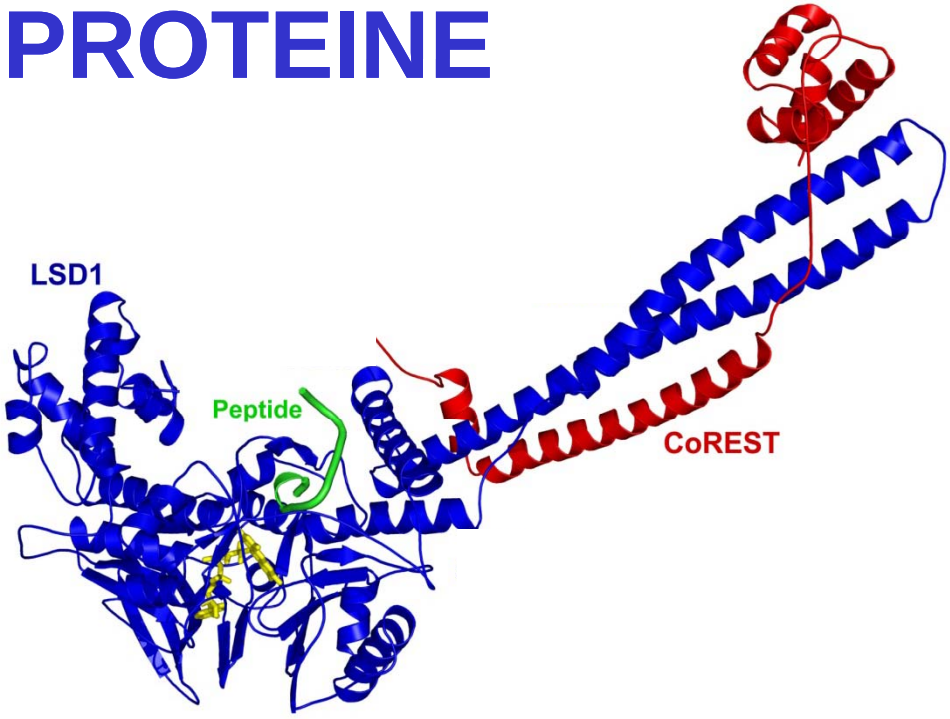
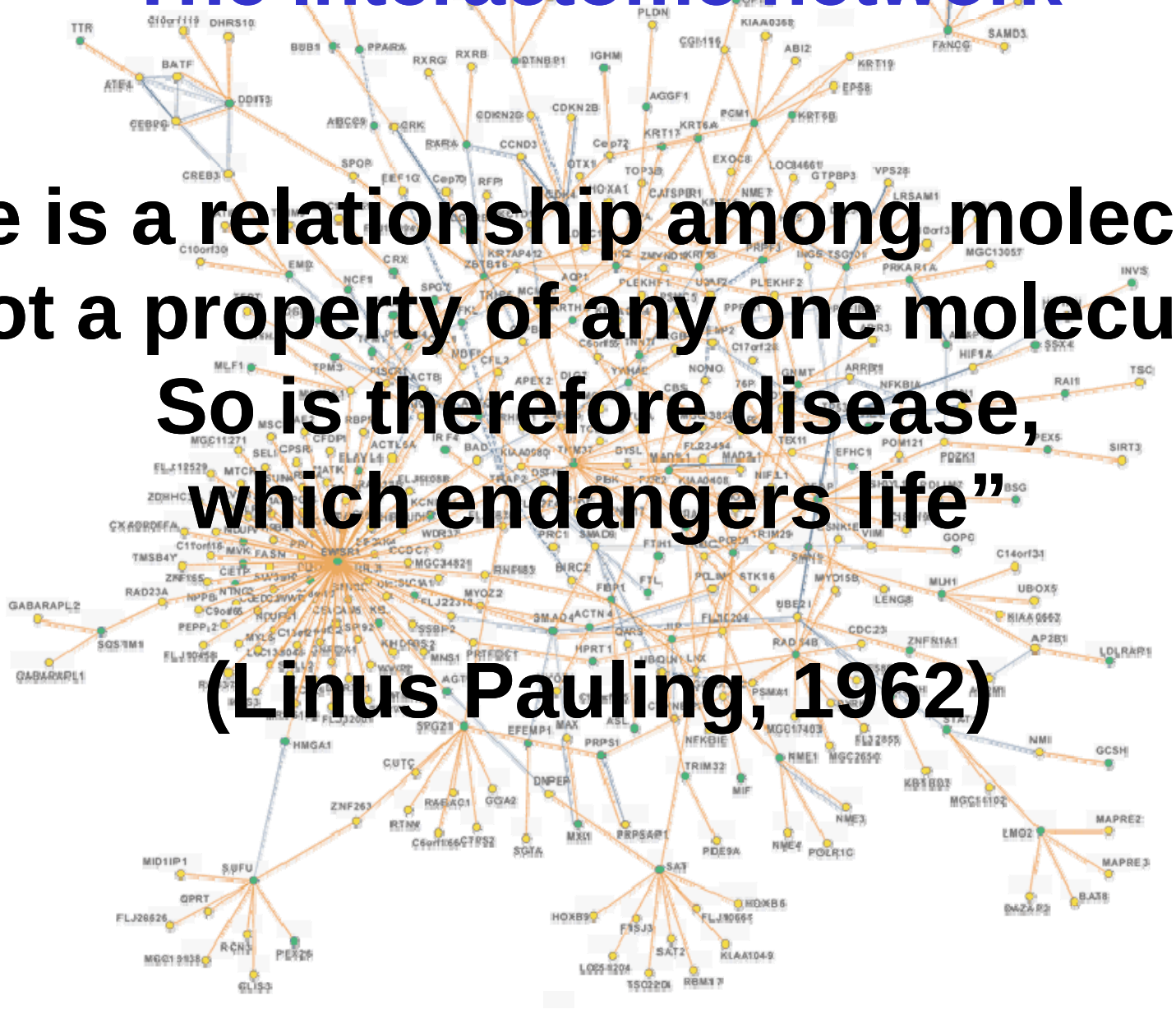
BIOLOGIA STRUTTURALE DEI MECCANISMI DI INTERAZIONE NELLE PROTEINE





Claudia Binda
Dipartimento di Genetica e Microbiologia - Università di Pavia
Dottorato di Ricerca di Biochimica – 1 aprile 2009

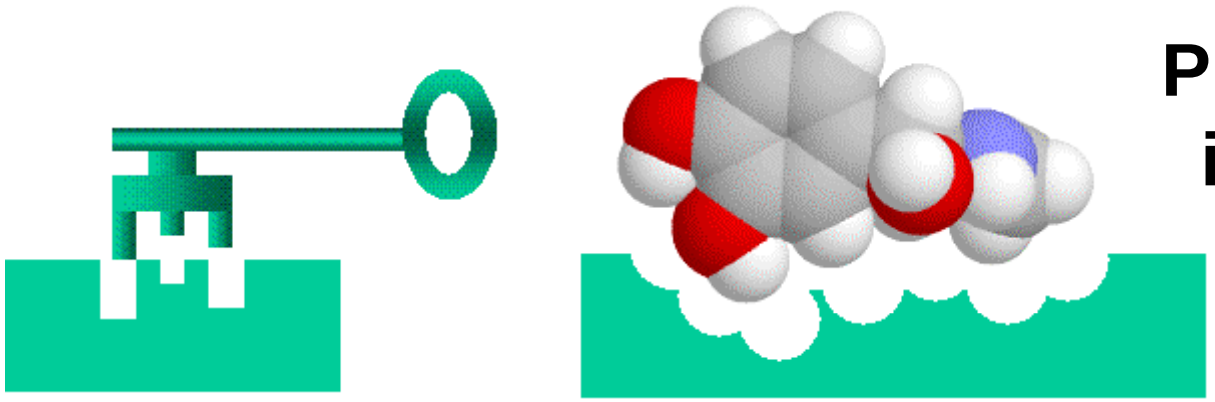
The Interactome network



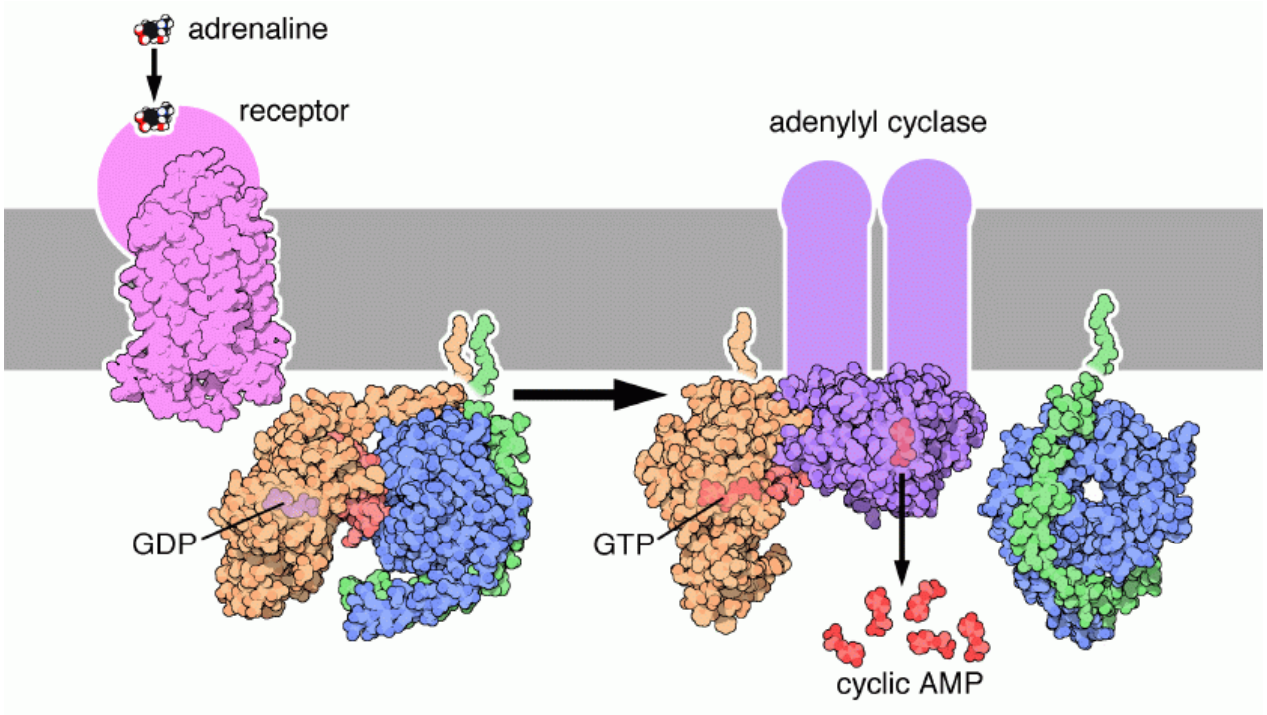
“Life is a relationship among molecules, not a property of any one molecule. So is therefore disease, which endangers life”

(Linus Pauling, 1962)

Structure to function relationship

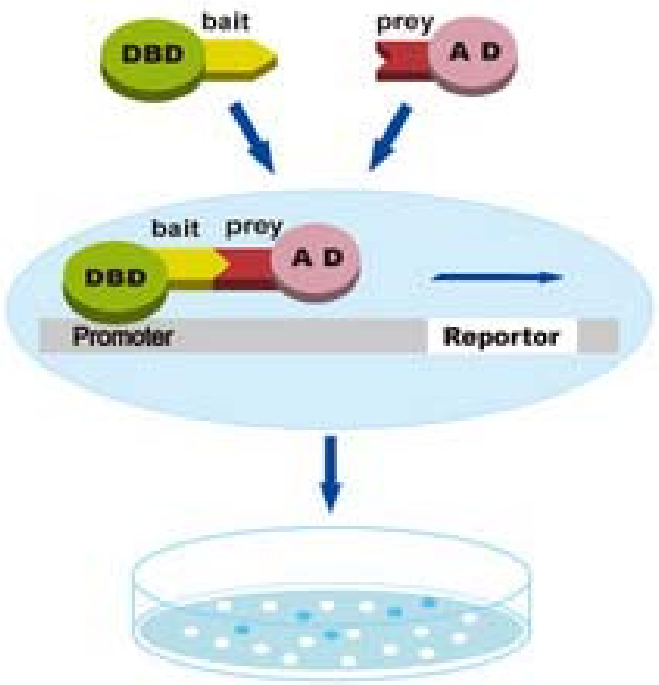


Protein-ligand interactions



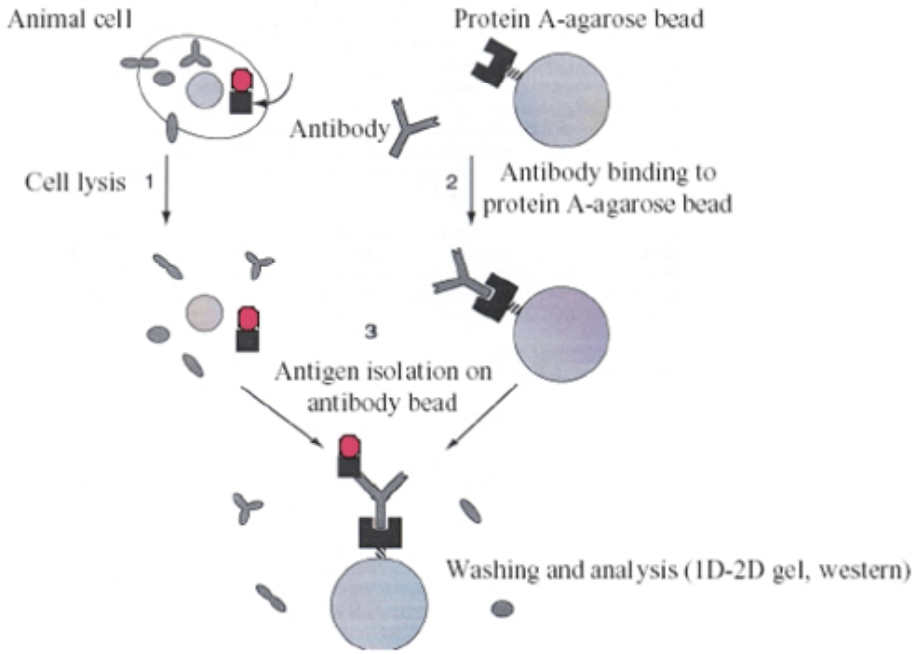
Protein-protein interactions

Yeast two-hybrid system (Y2H)



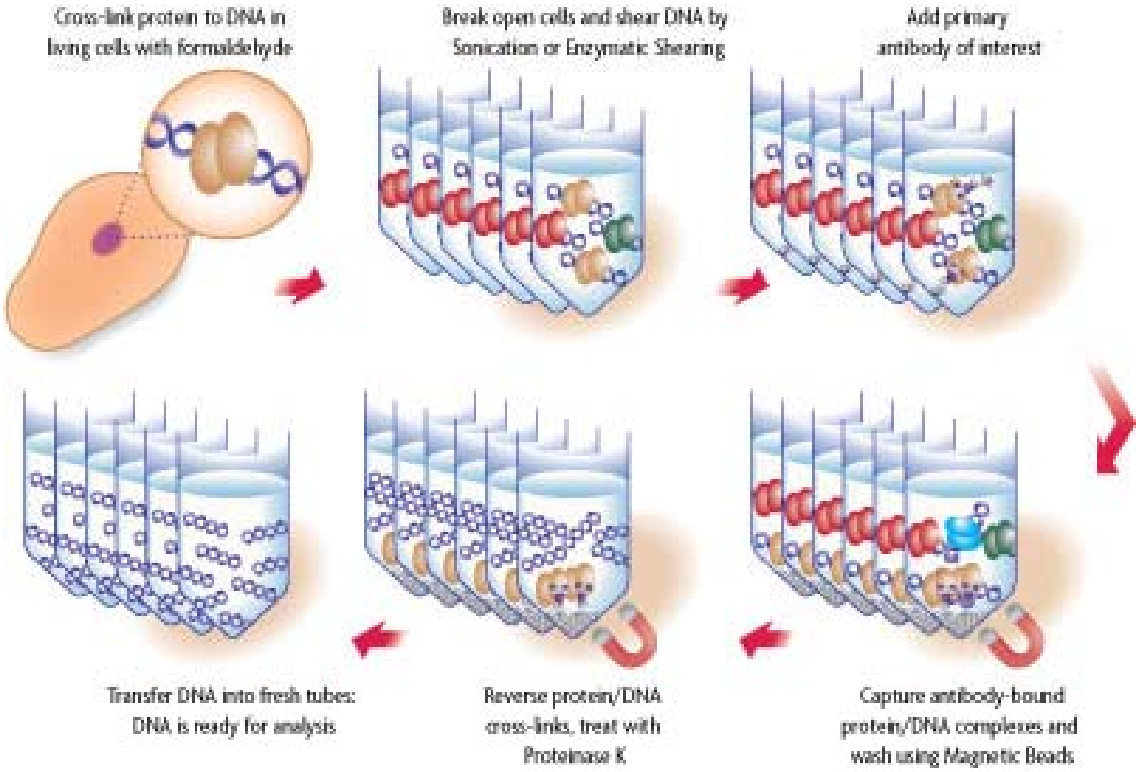
If two proteins (the “bait” and the “prey”) interact, the DNA-binding domain and the activation domain of a transcription factor are connected and the transcription of a reporter gene occurs.

Co-immunoprecipitation (CoIP)



An antibody specific for the protein of interest is incubated with a cell extract and pelleted: associated proteins are analyzed.

Chromatin-immunoprecipitation (ChIP)

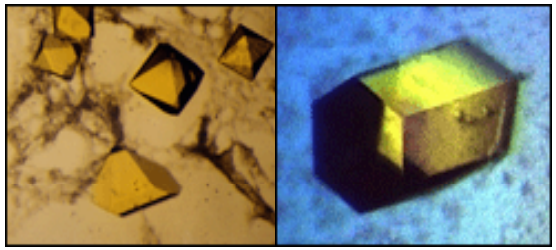


To identify which DNA sequences are bound by the protein of interest

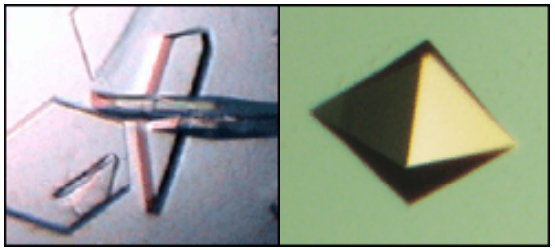
ChIP-on-CHIP: applied on microarray for genome-wide analyses

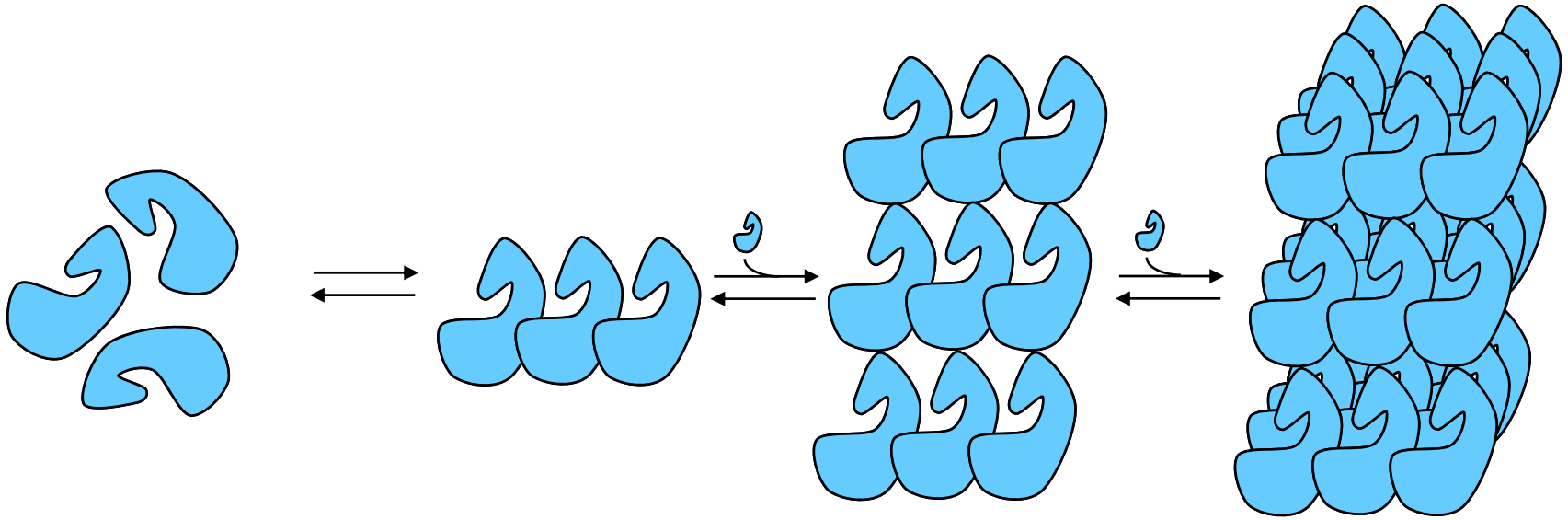
Structural Biology

- cryo-EM
- NMR
- X-ray crystallography



Protein crystals





supersaturated protein solution

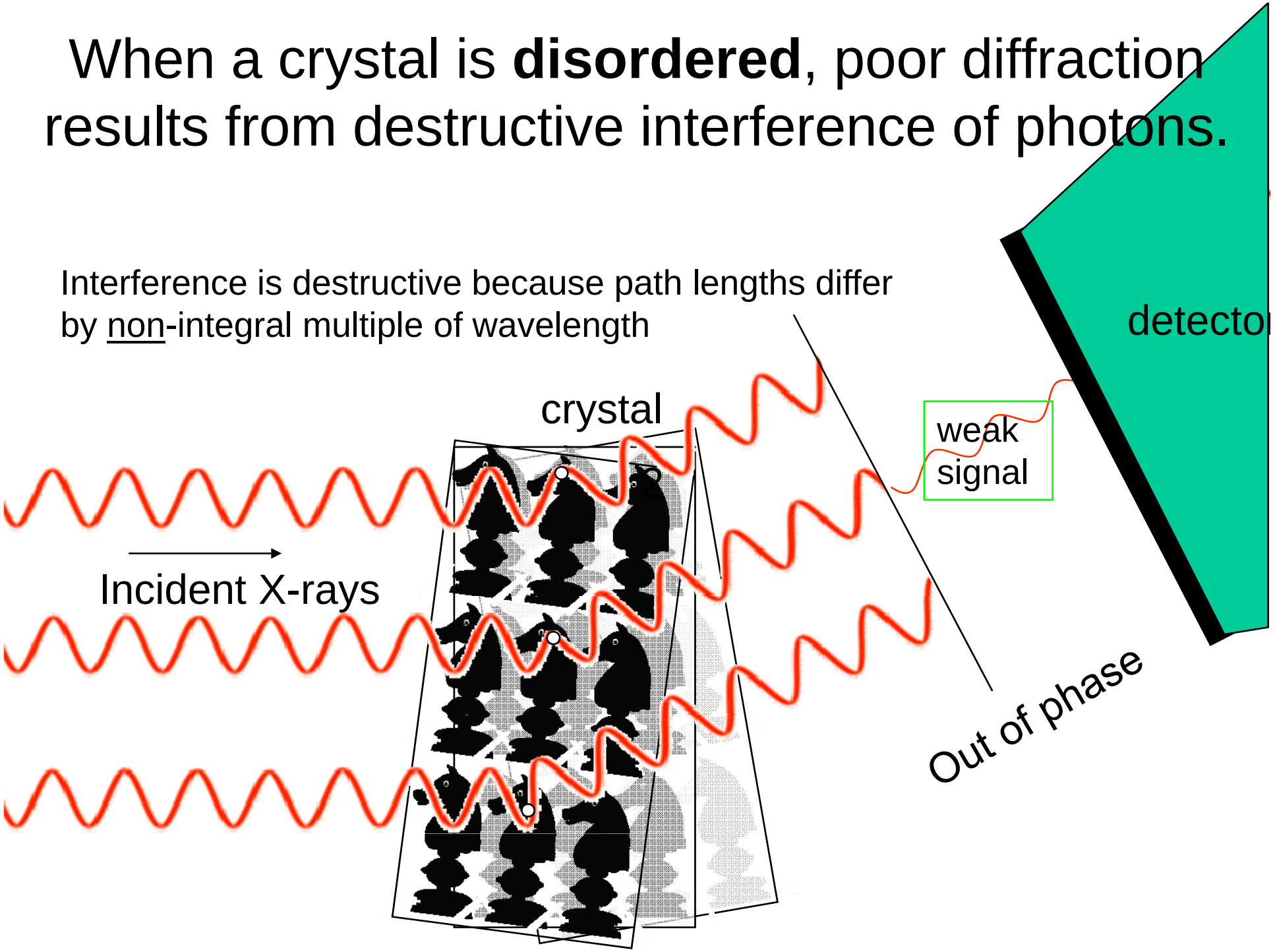
One dimensional order

Two dimensional order

Three dimensional order

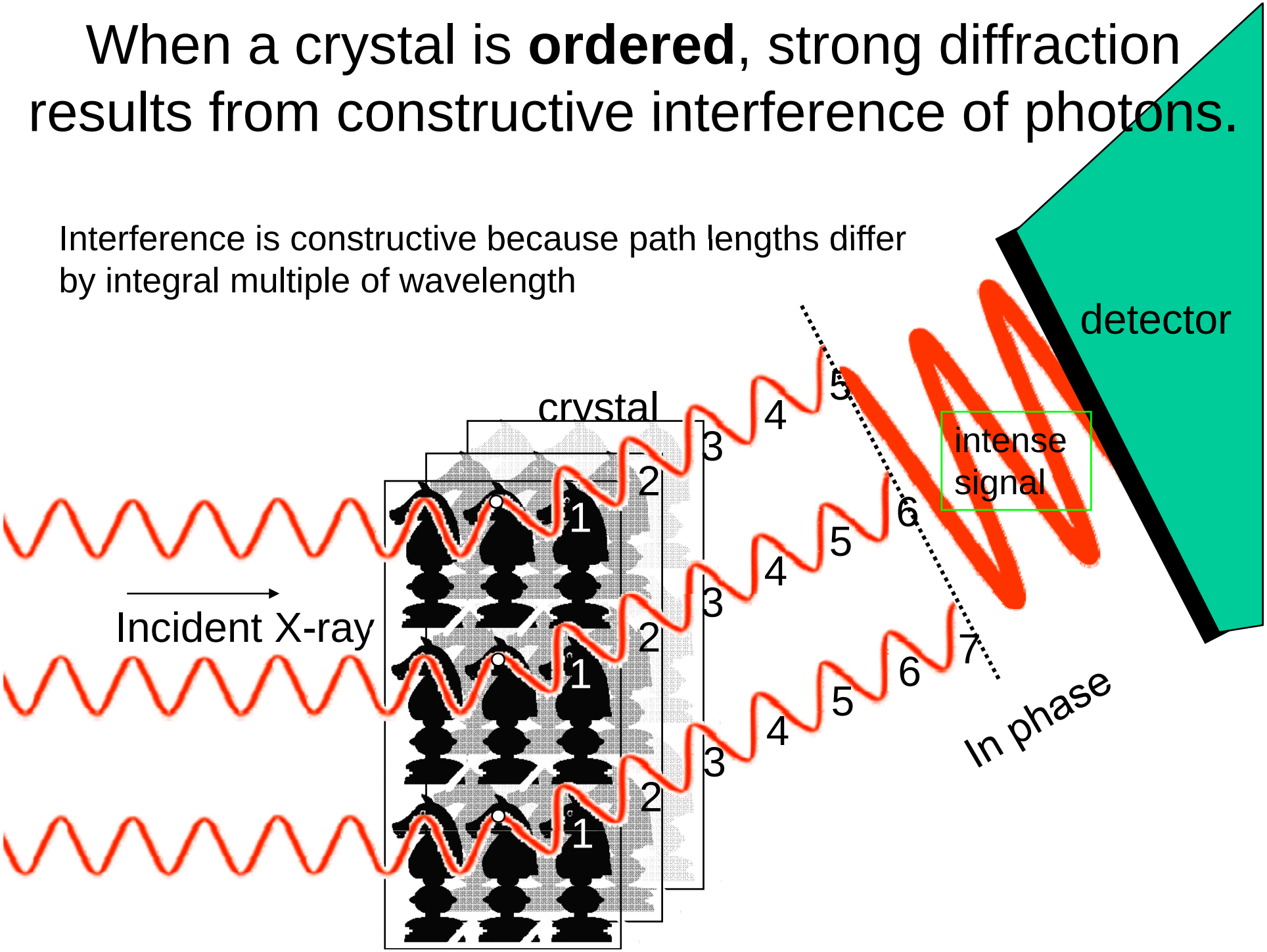
DISORDERED → ORDERED

When a crystal is **disordered**, poor diffraction results from destructive interference of photons.

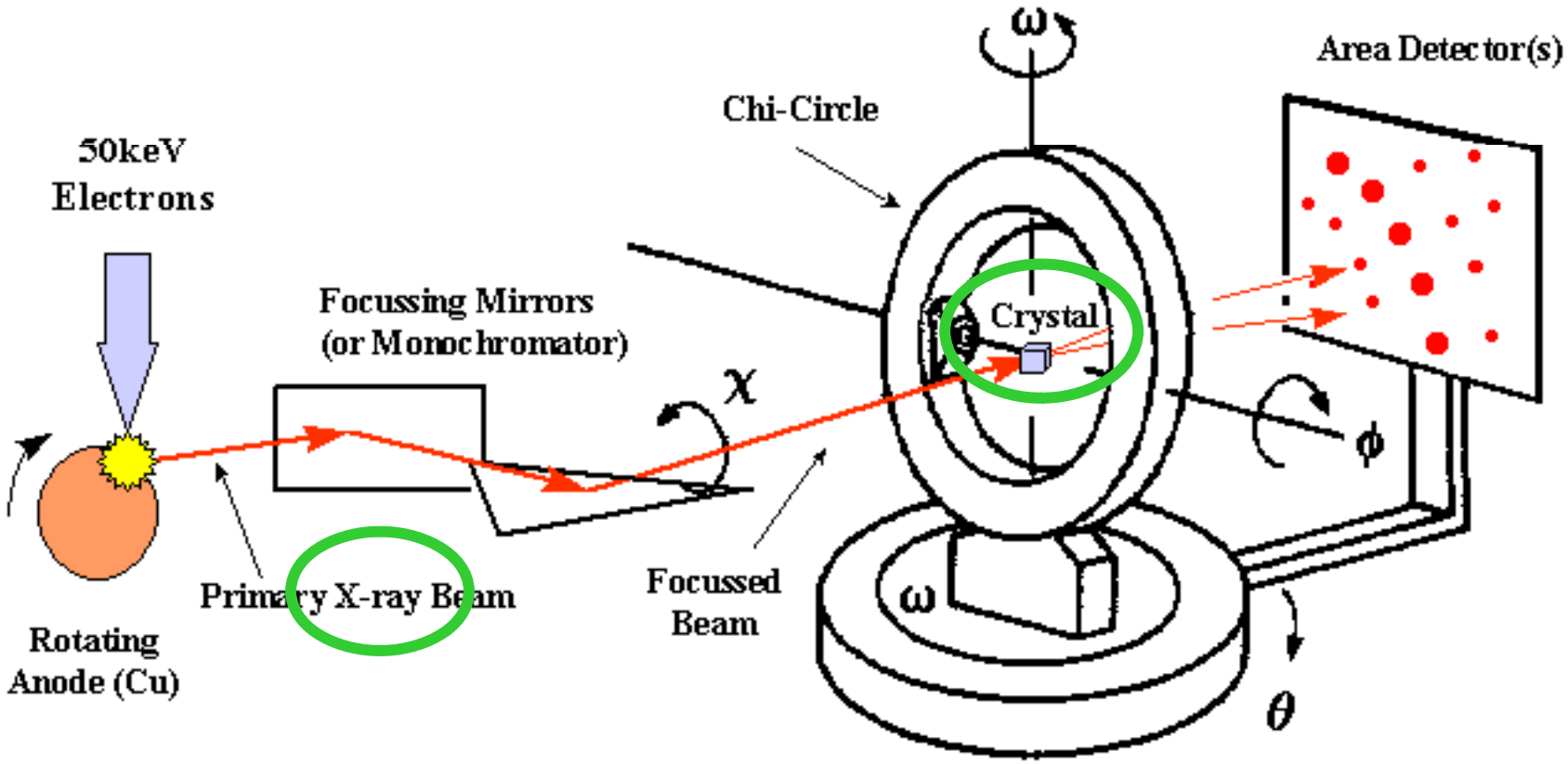


When a crystal is **ordered**, strong diffraction results from constructive interference of photons.

Interference is constructive because path lengths differ by integral multiple of wavelength

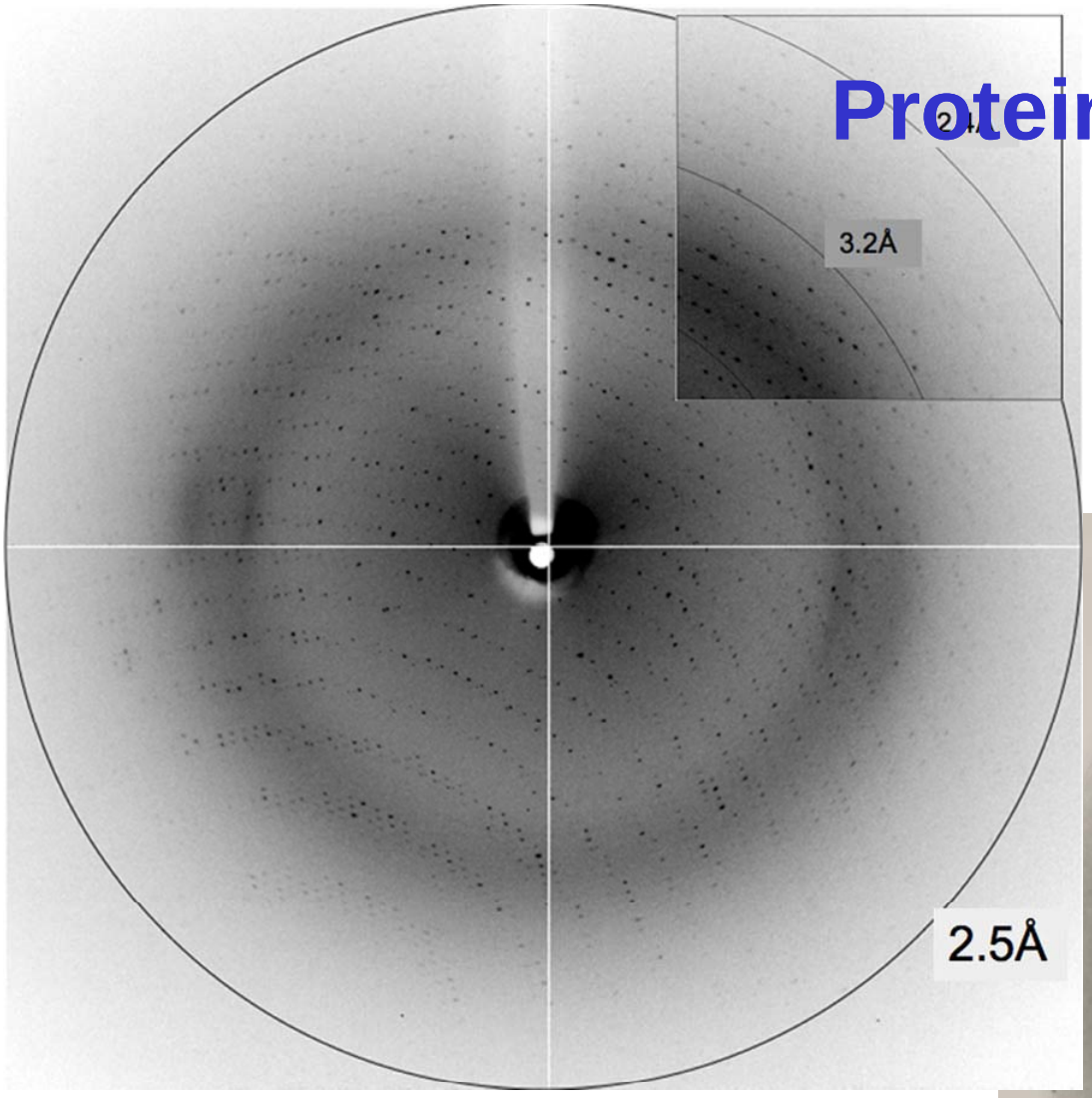


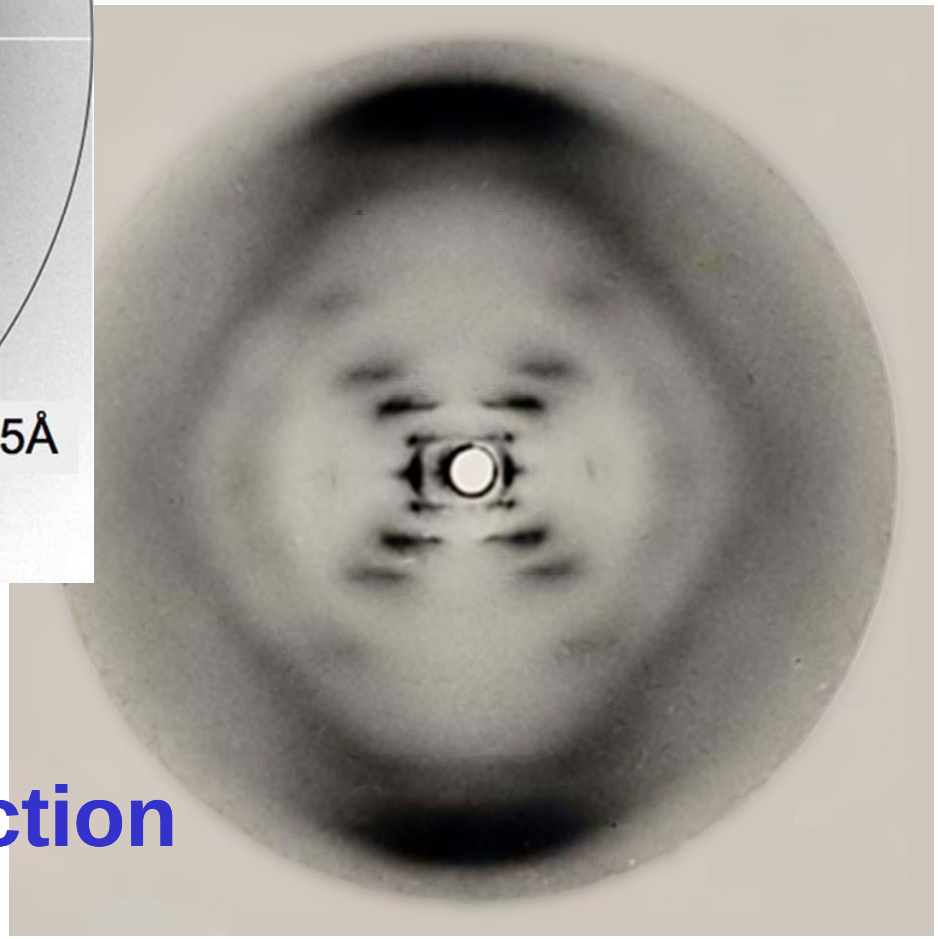
X-ray data collection



X-rays have the proper wavelength (1 Å = 10^{-8} cm) to be scattered by the electron cloud of the atoms in the crystal

Protein crystal diffraction



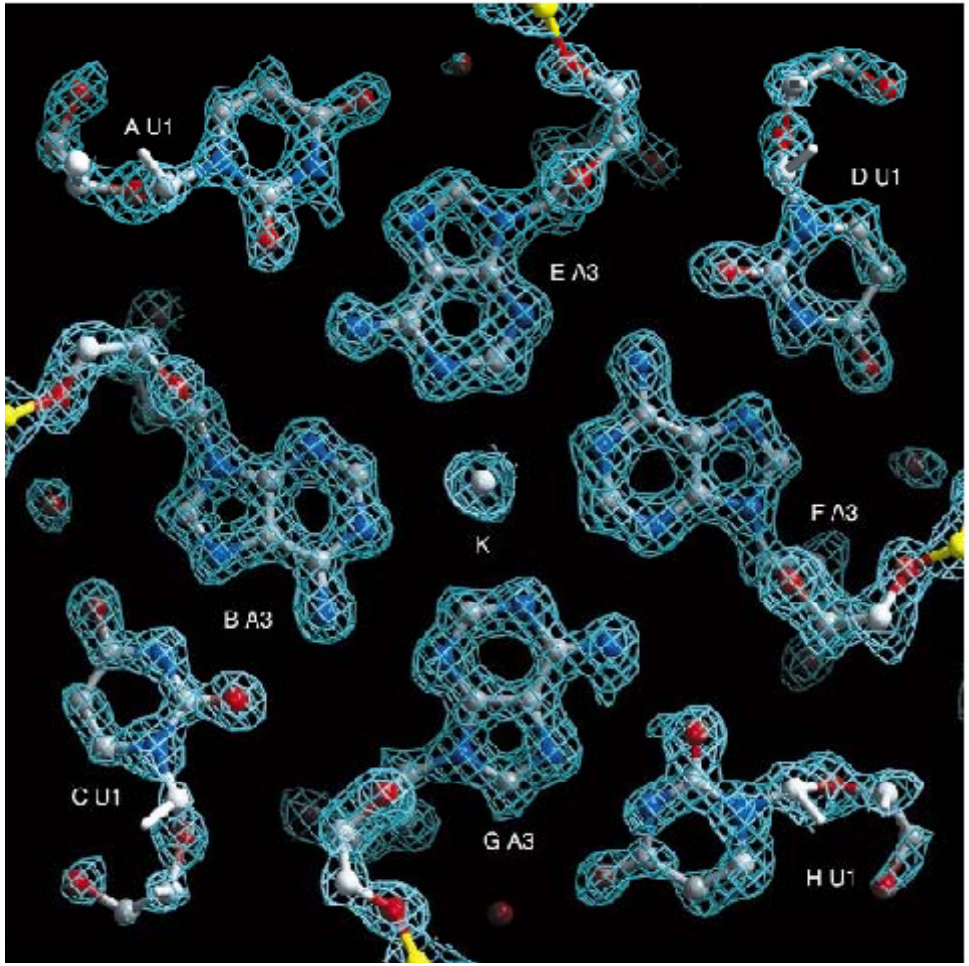


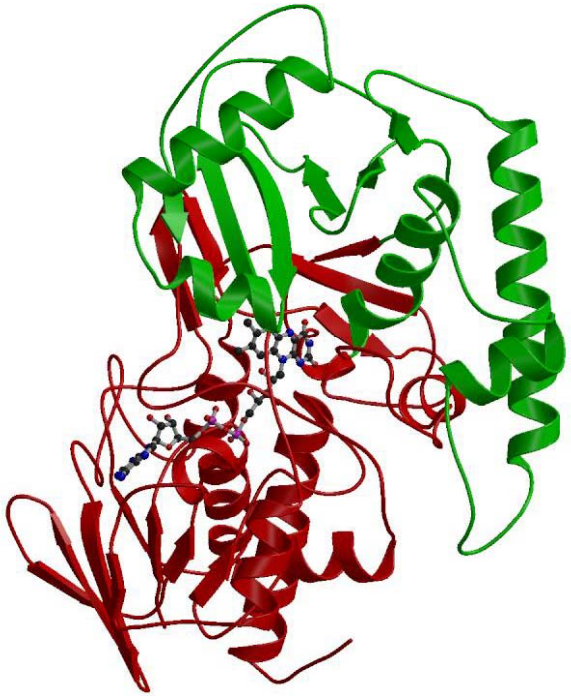
DNA crystal diffraction

Protein structure determination by interpretation of electron density maps

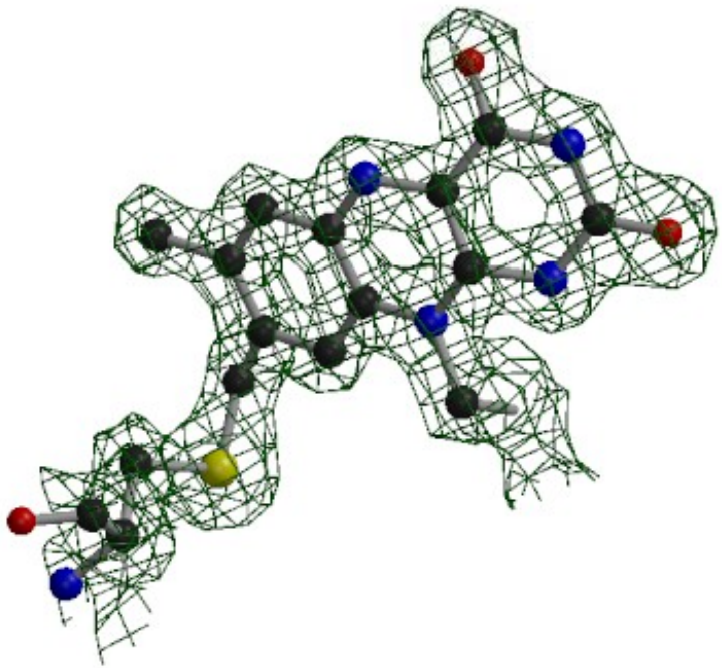
Fourier transform

$$\rho(xyz) = V^{-1} \Sigma\Sigma\Sigma |F_{hkl}| \exp[-2\pi(hx+ky+lz-\alpha_{hkl})]$$

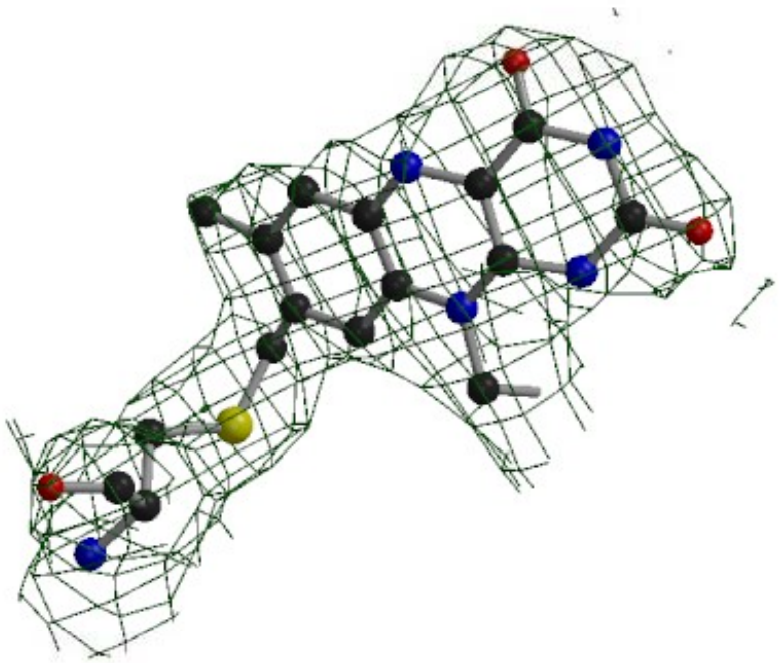




Importance of resolution

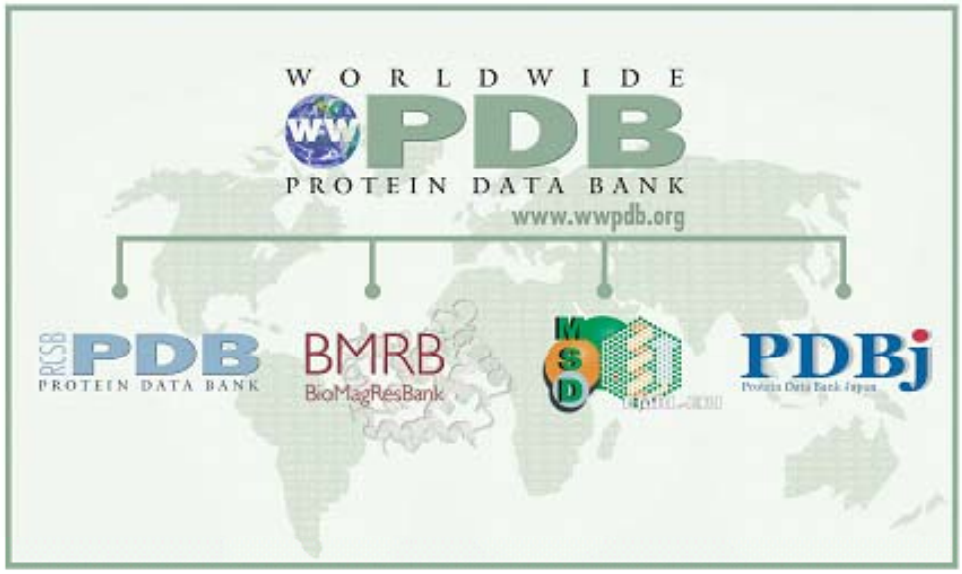


1.7 Å



2.8 Å

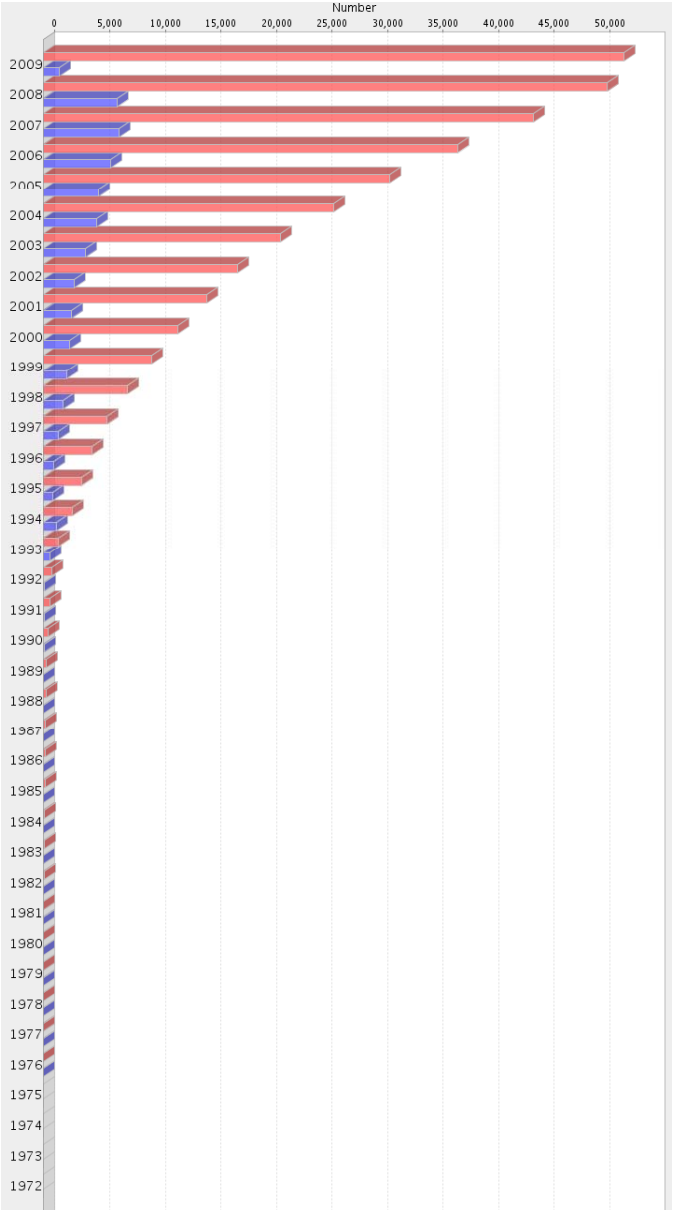
The Protein Data Bank (PDB)
www.rcsb.org



Up to March 2009:

48516	X-ray
7762	NMR
230	cryo-EM

Yearly growth of deposited protein structures



total

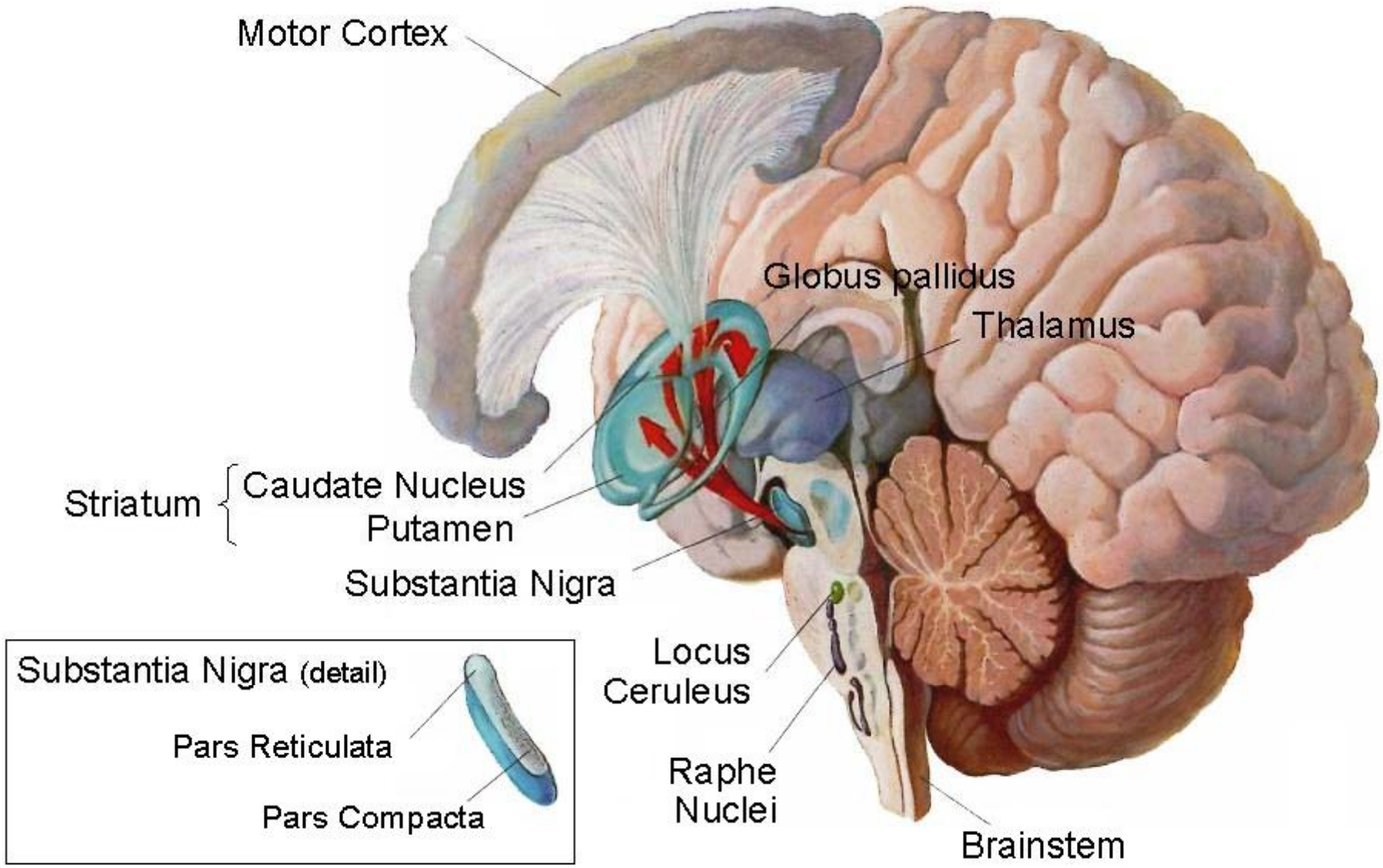
per year

In-house examples

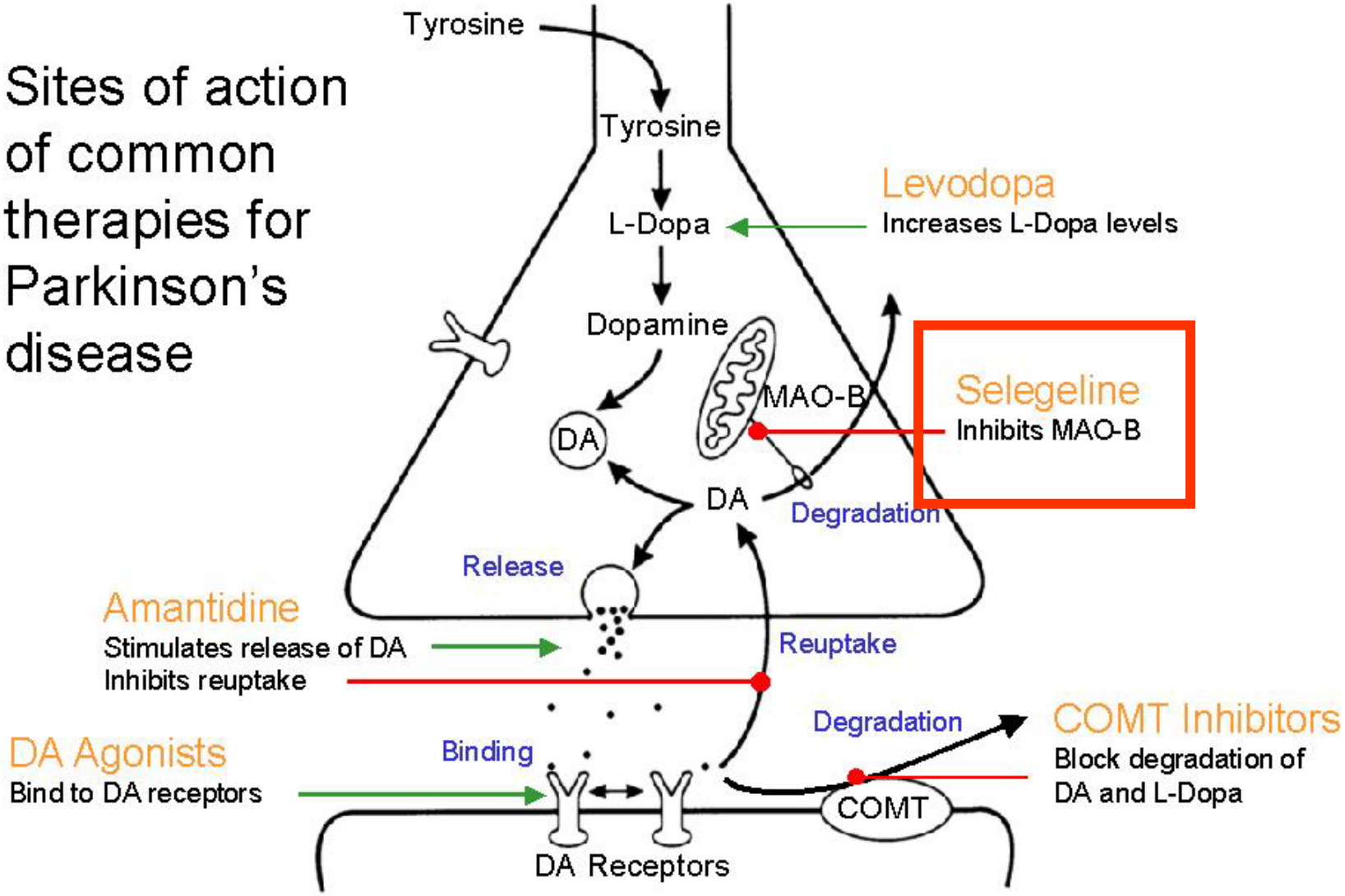
1. Human Monoamine oxidase

2. Human Histone Demethylase

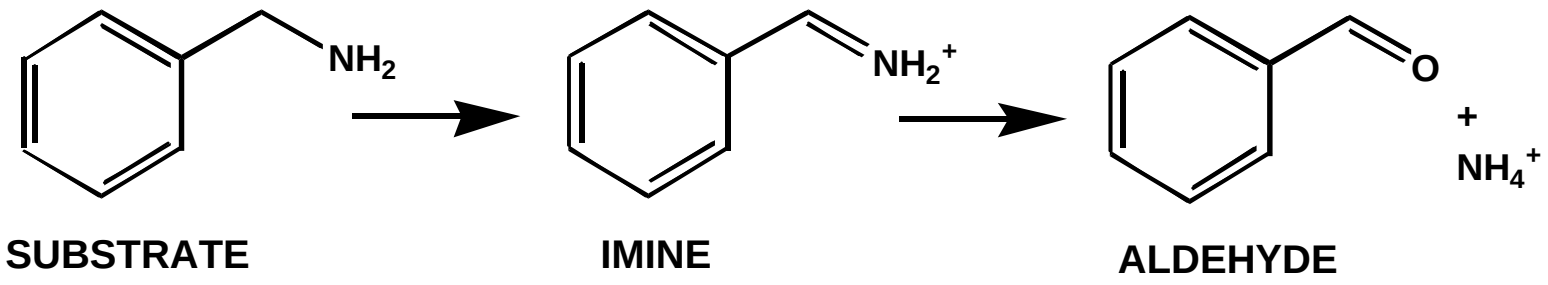
In-house story 1: Monoamine Oxidase



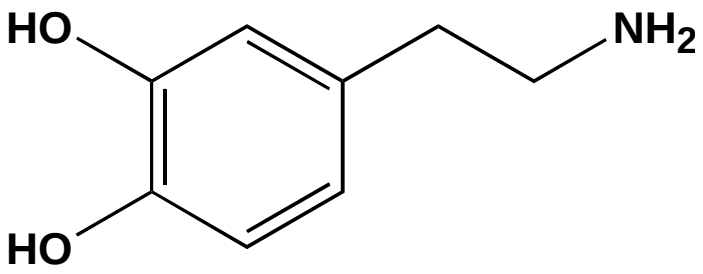
MAO:
a drug target in neurological disorders



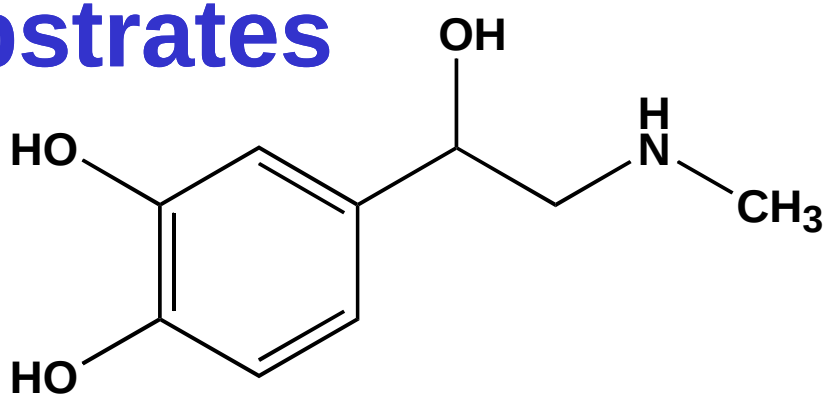
The reaction catalyzed by MAO



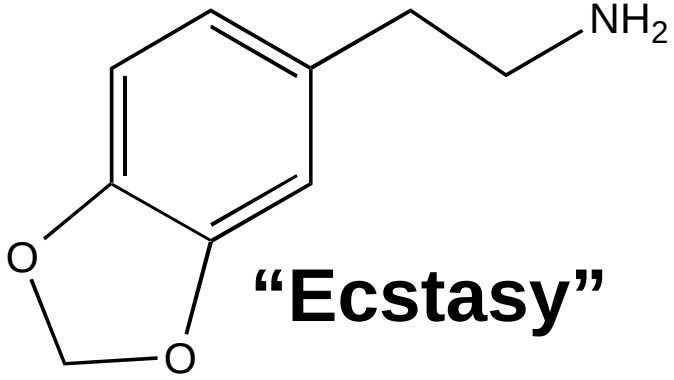
MAO substrates



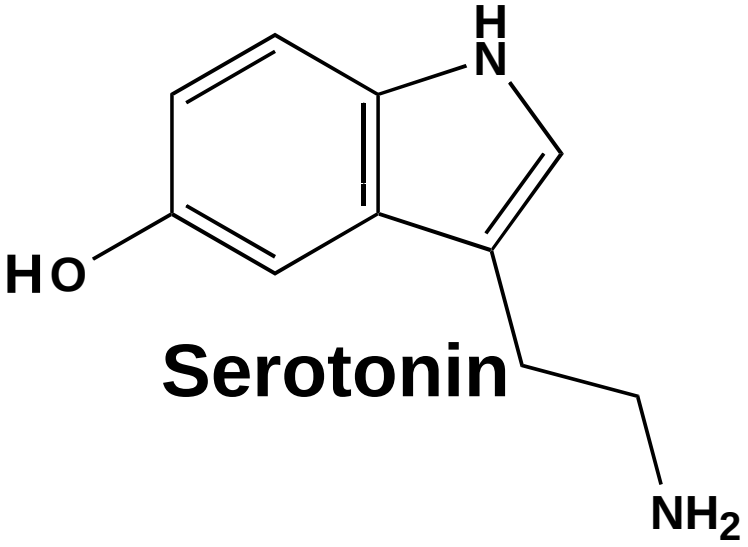
Dopamine



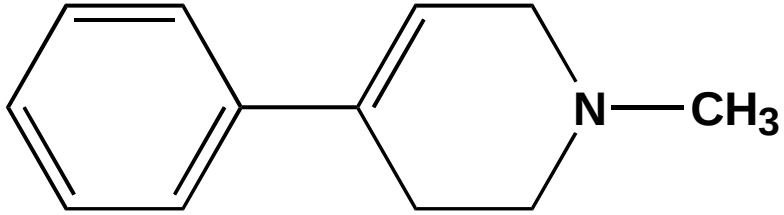
Norepinephrine (noradrenaline)



“Ecstasy”



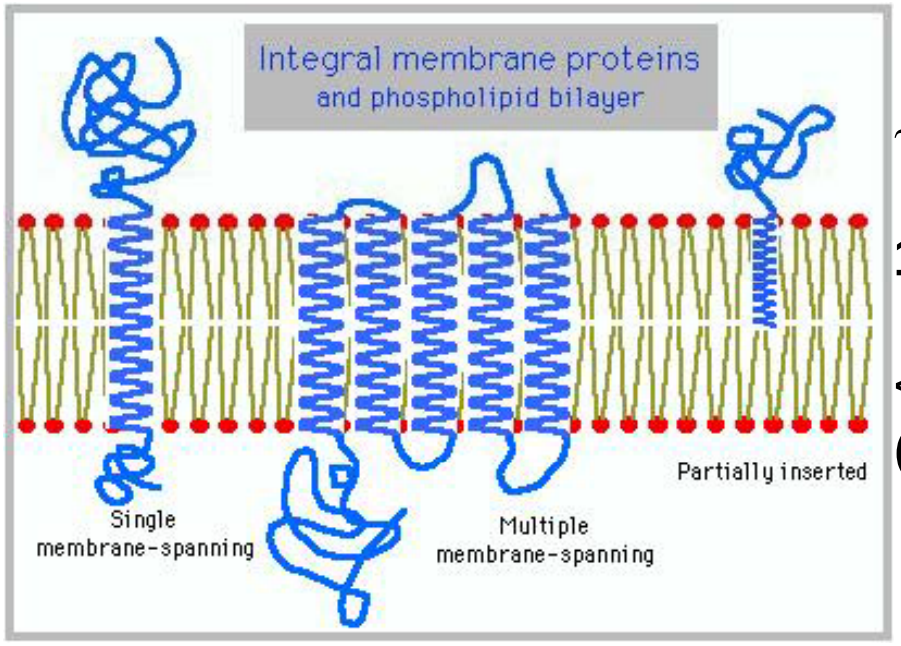
Serotonin



MPTP (heroin impurity)

Human MAO A and MAO B

- Two isoforms with 71% sequence identity
- Partly overlapping substrate specificity
- Anchored to the <u>outer mitochondrial membrane through C-terminal extension</u>



~**30%** of all proteins are membrane-bound

1985: first structure of a membrane protein

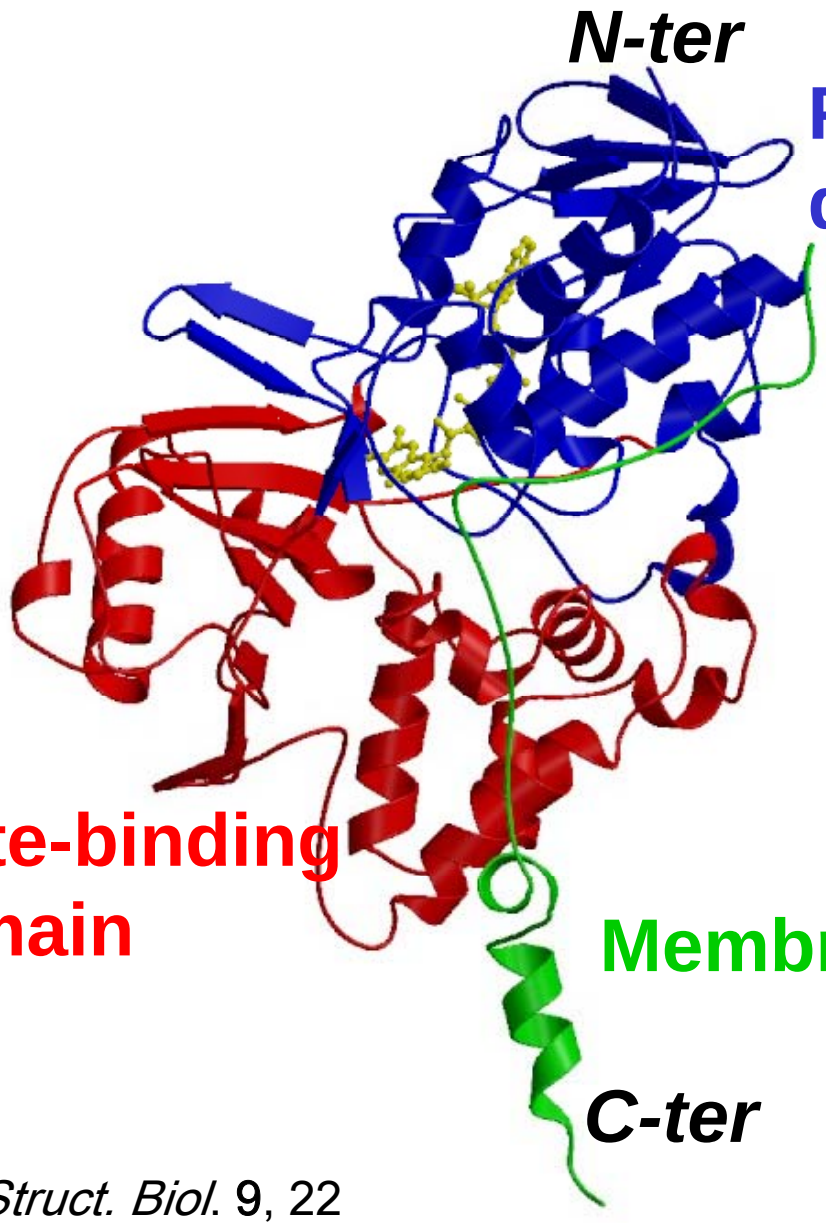
< **150** unique known structures in the PDB
(445 deposited out of the total 56508)

Crystallization of Human MAO B

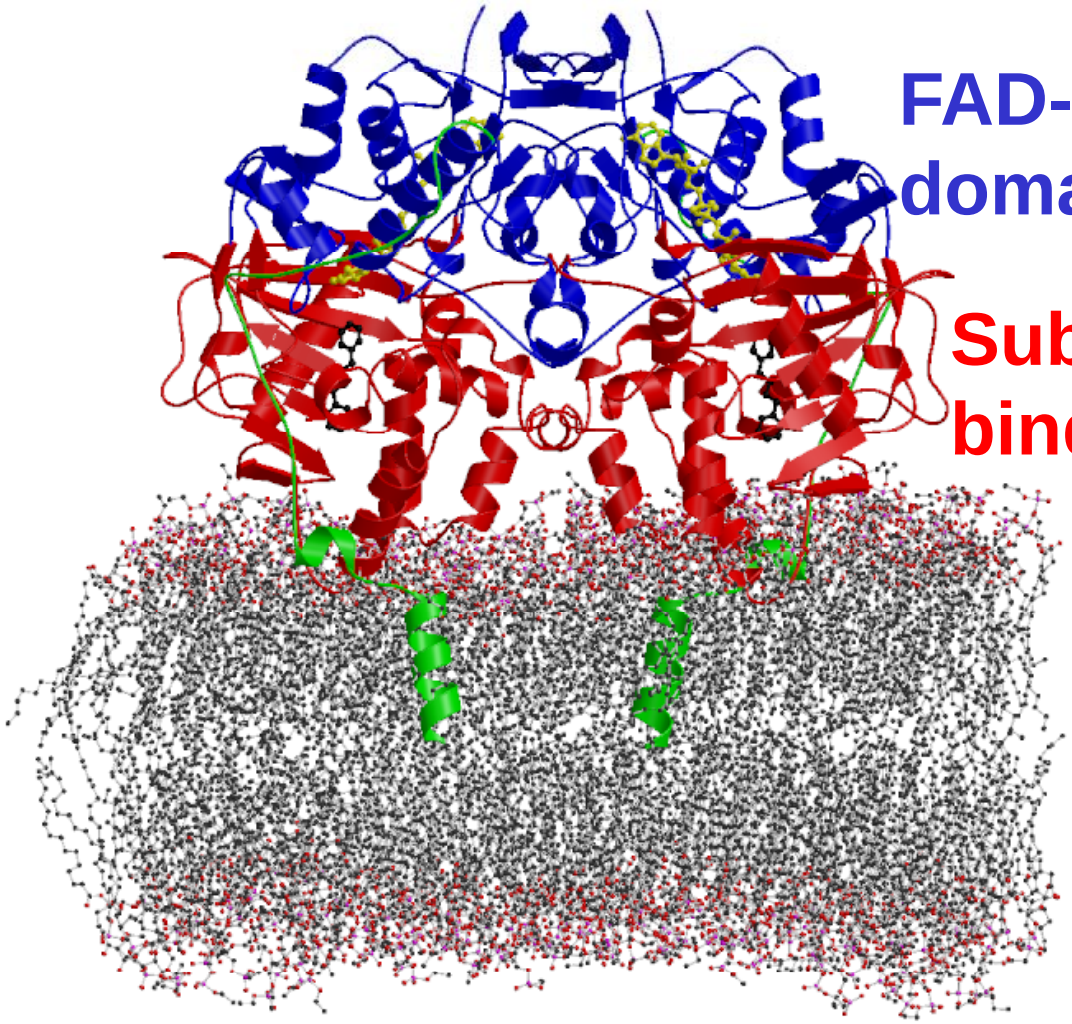
- Expressed in *Pichia pastoris*

- Two crystal forms using different detergents:
  1) Lauryldimethylamine oxide, triclinic
  2) Zwittergent 3-12, orthorombic

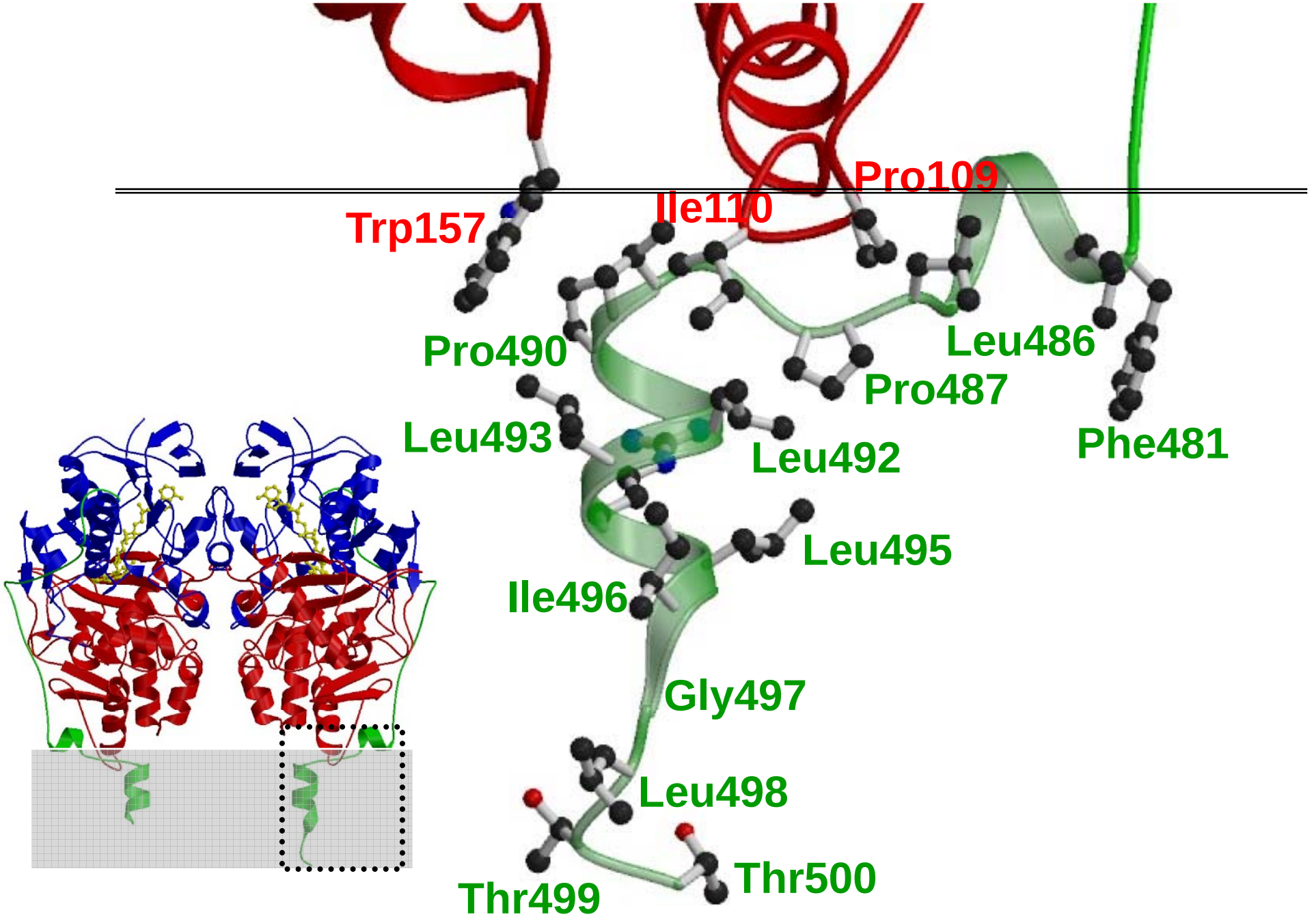
Crystal Structure of Monoamine Oxidase B

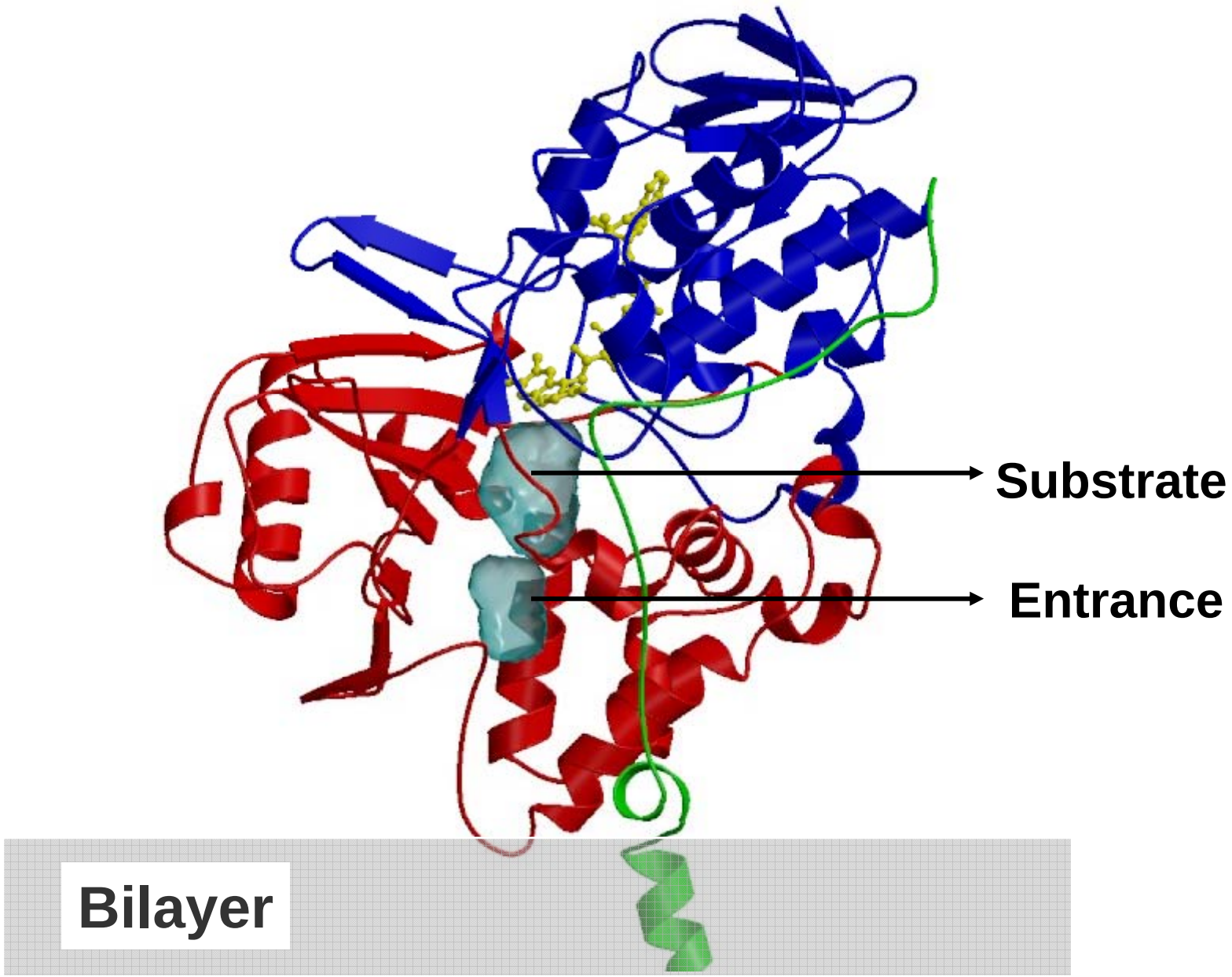


Binda et al. (2002) *Nat. Struct. Biol.* 9, 22



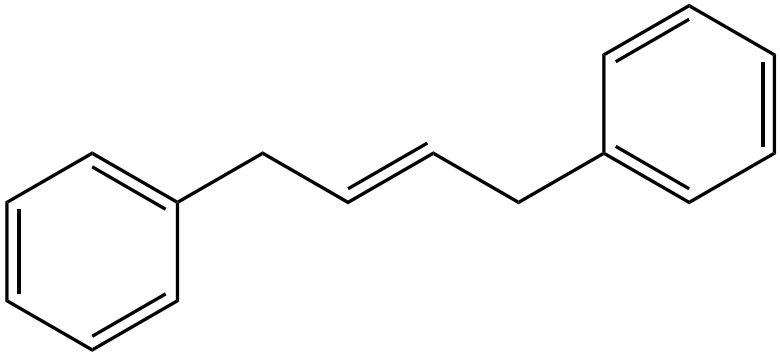
The MAO B dimer


Pro109
Ile110
Trp157
Pro490
Leu486
Pro487
Leu493
Leu492
Phe481
Leu495
Ile496
Gly497
Leu498
Thr500
Thr499

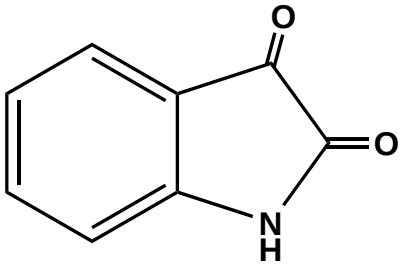


The active site cavities

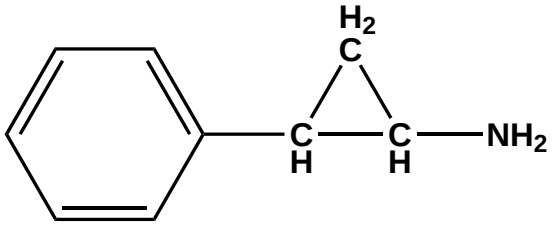
Inhibitor Binding to MAO B



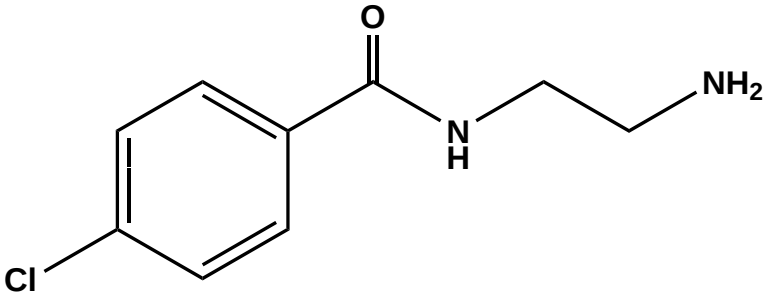
1,4-diphenylbutene



indole-2,3-dione (isatin)

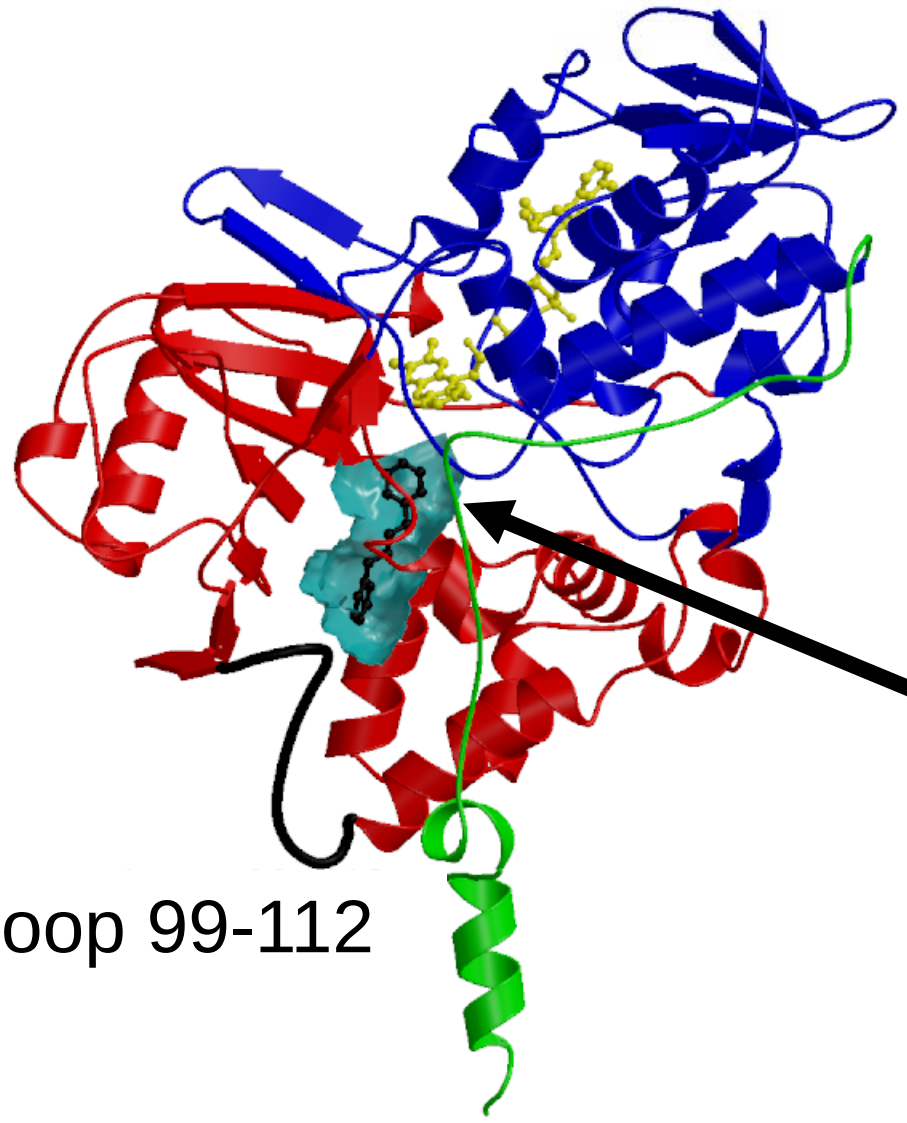


2-trans-phenylcyclopropylamine
(tranylcypromine)



N-(2-aminoethyl)-*p*-chlorobenzamide

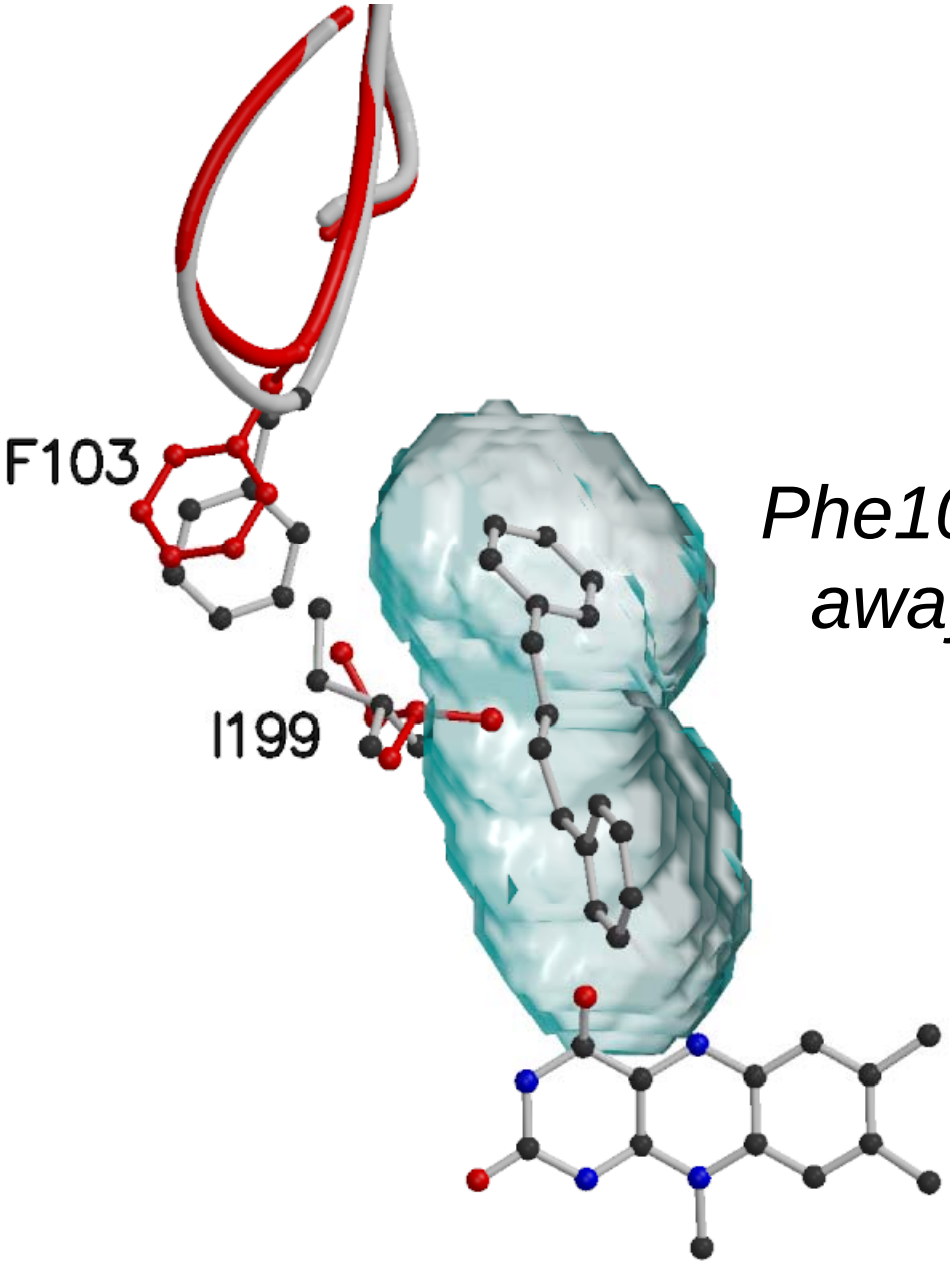
1,4-Diphenylbutene Complex



Loop 99-112

Entrance and Substrate Cavities Merge into a Single Cavity

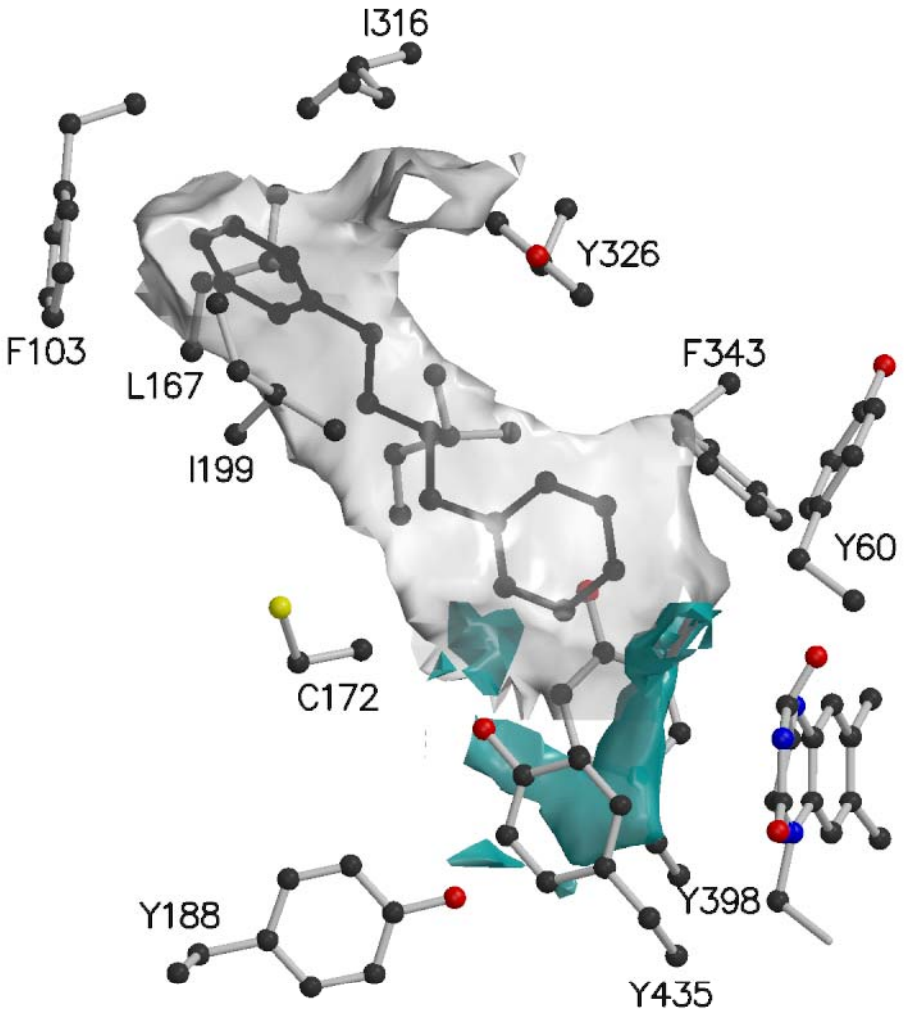
Binda et al. (2003) *PNAS* **100**, 9750



Induced Fit:

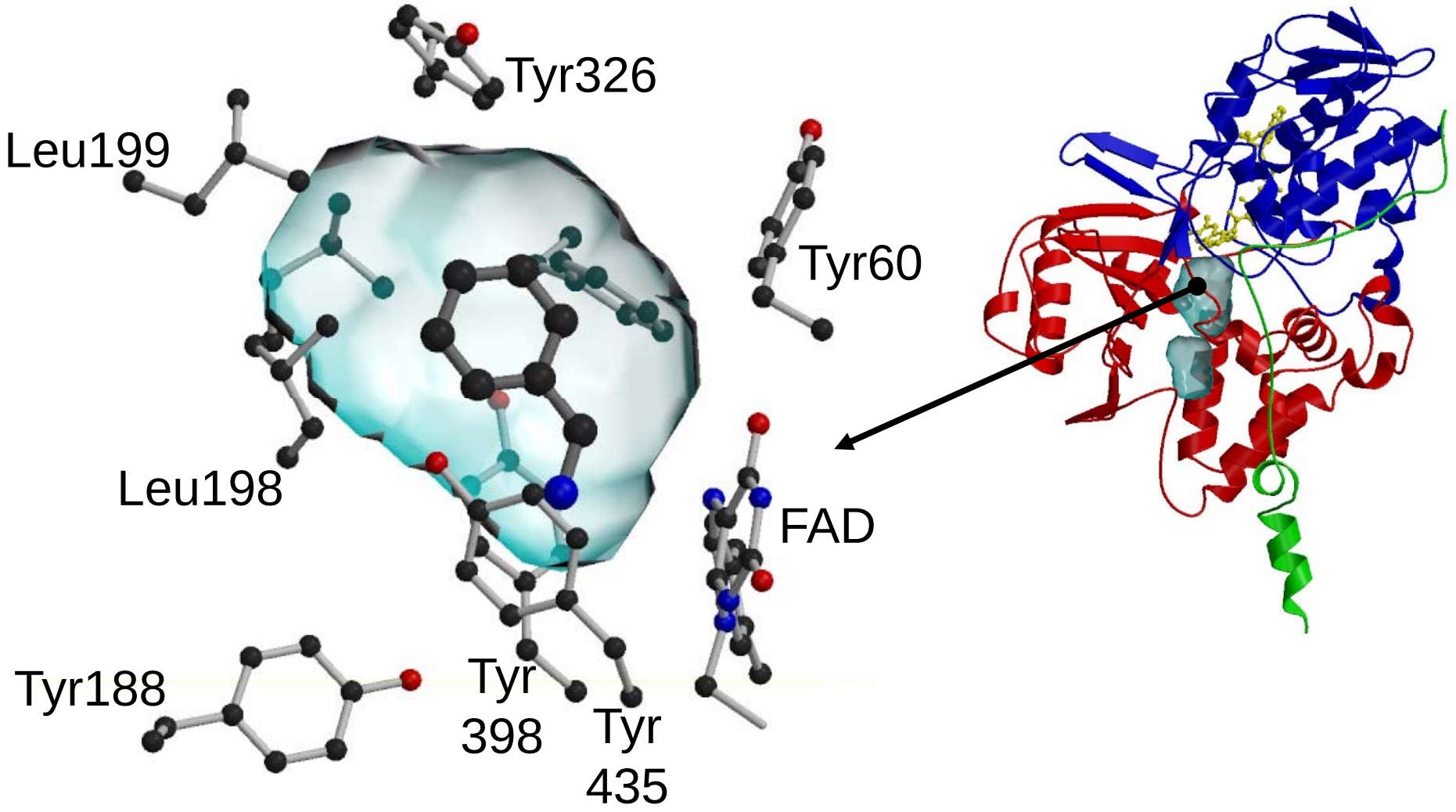
Phe103 and Ile199 move away to allow inhibitor binding

Exploring the Cavity with GRID

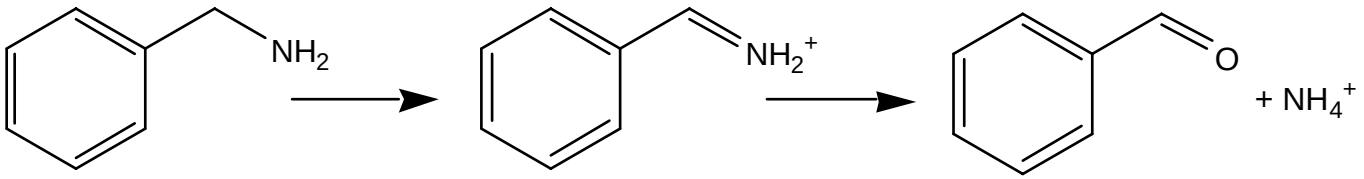


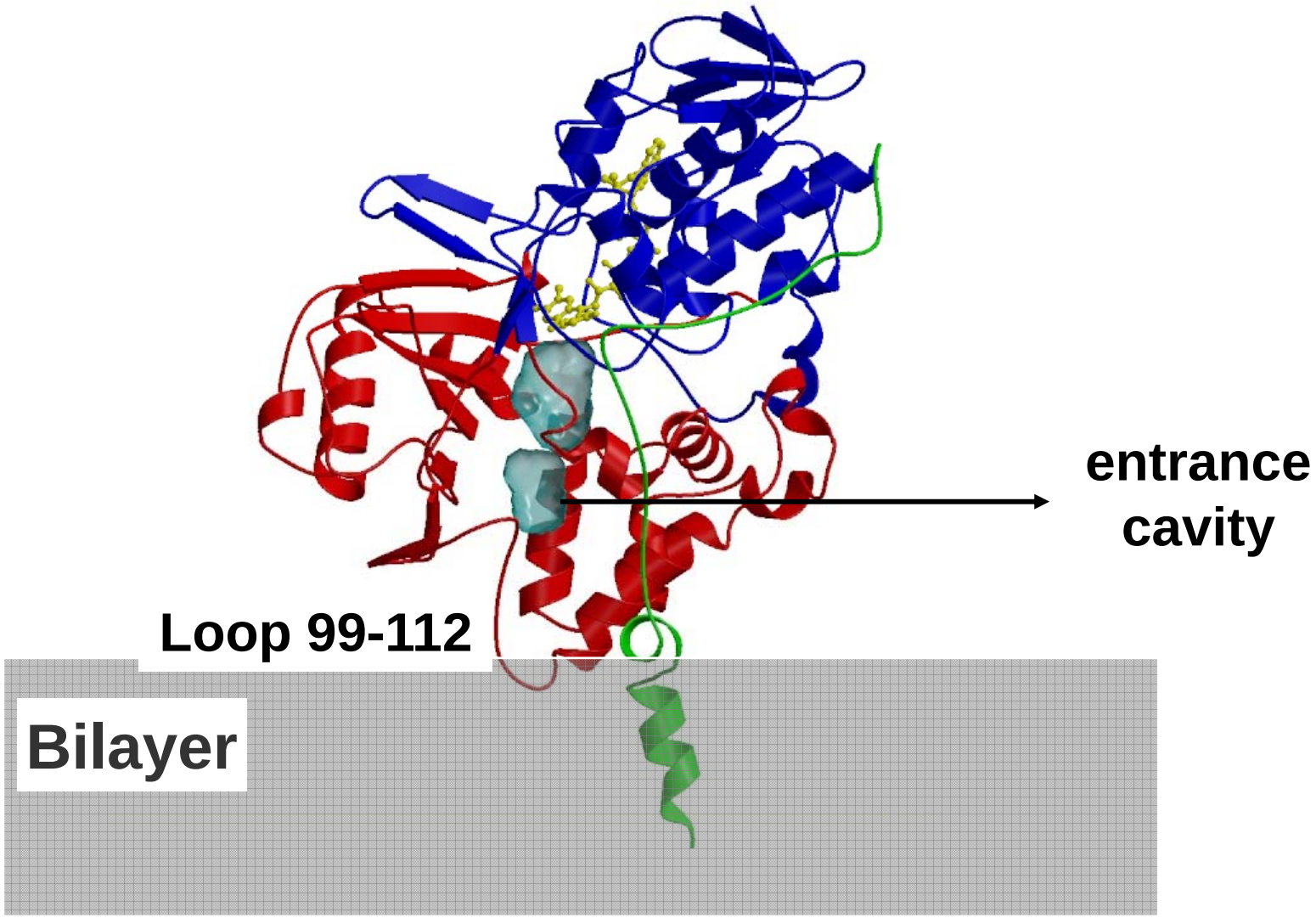
Probes:

- *sp2 carbon*
- *NH_2 group*



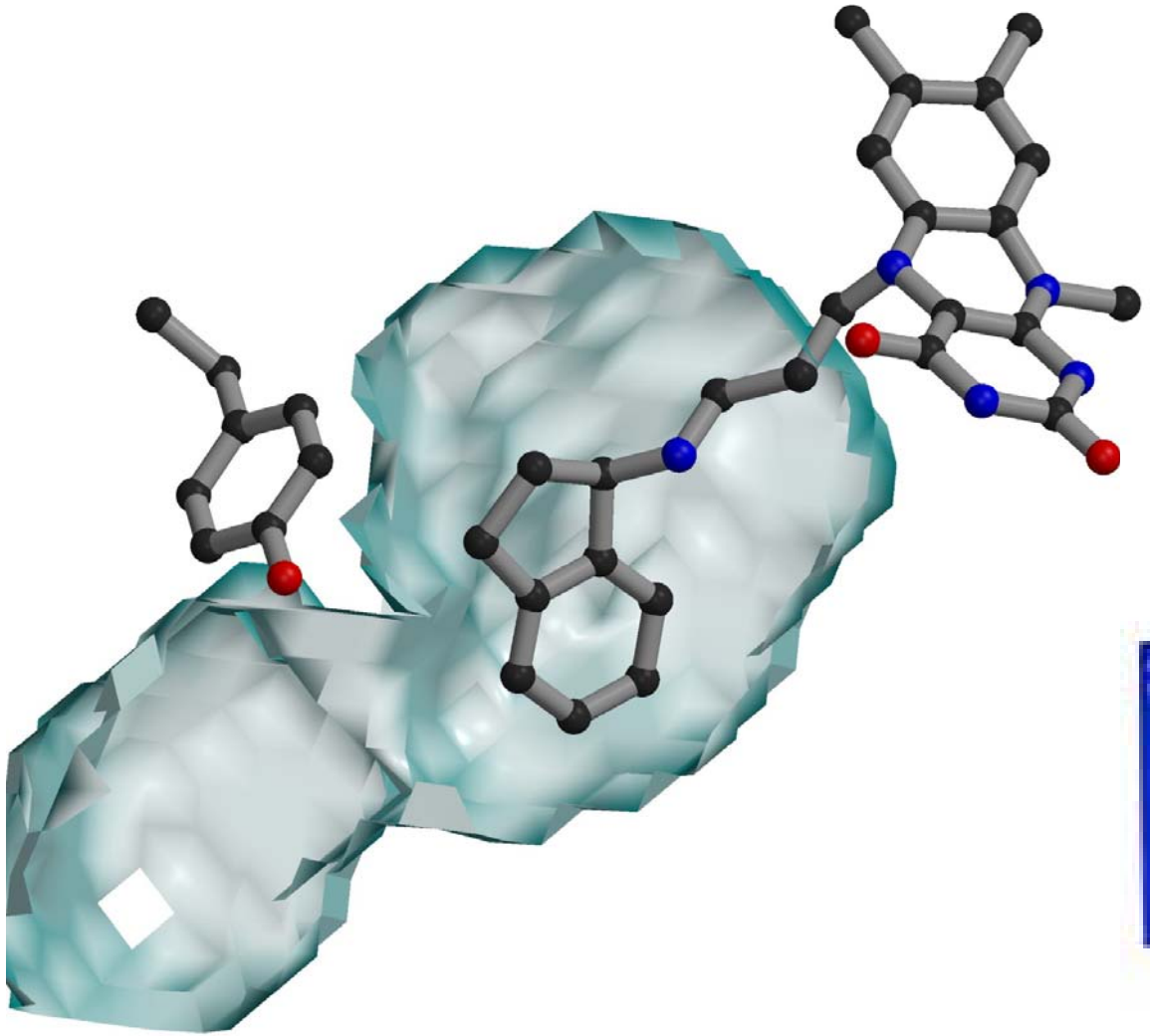
A model for substrate (benzylamine) binding





A passageway for the substrate and a “druggable” cavity

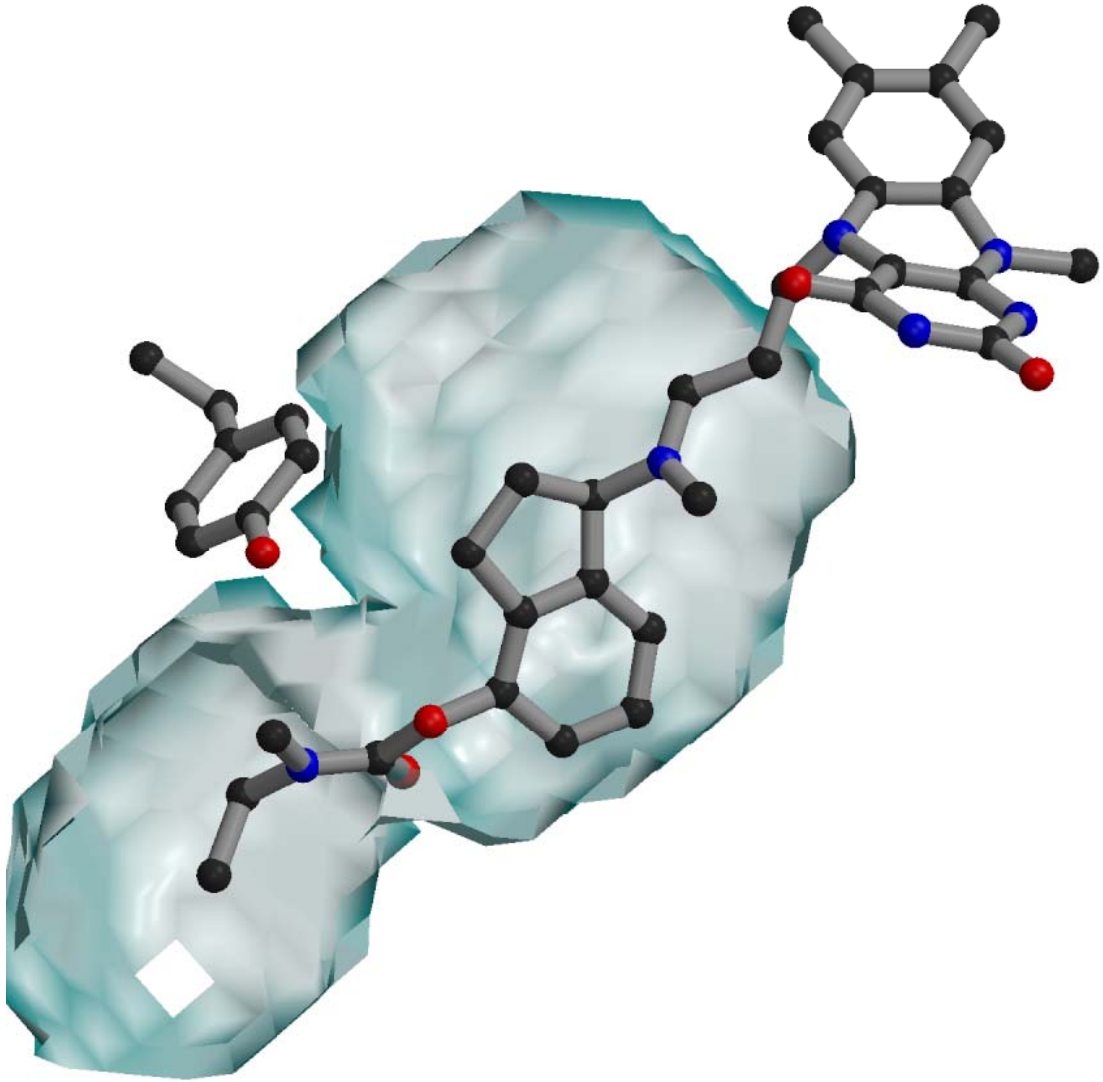
Design of selective MAO B inhibitors: RASAGILINE



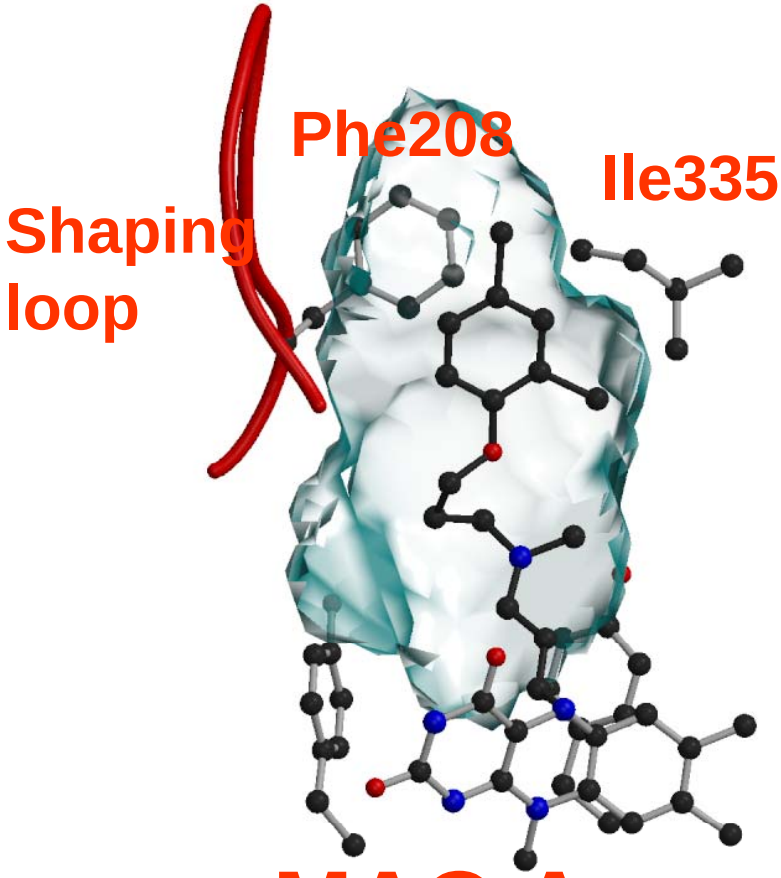
anti-Parkinson drug
on the market since 2005
(Teva Pharmaceuticals, Israel)

Binda et al. (2004) J. Med. Chem. **47**, 1760

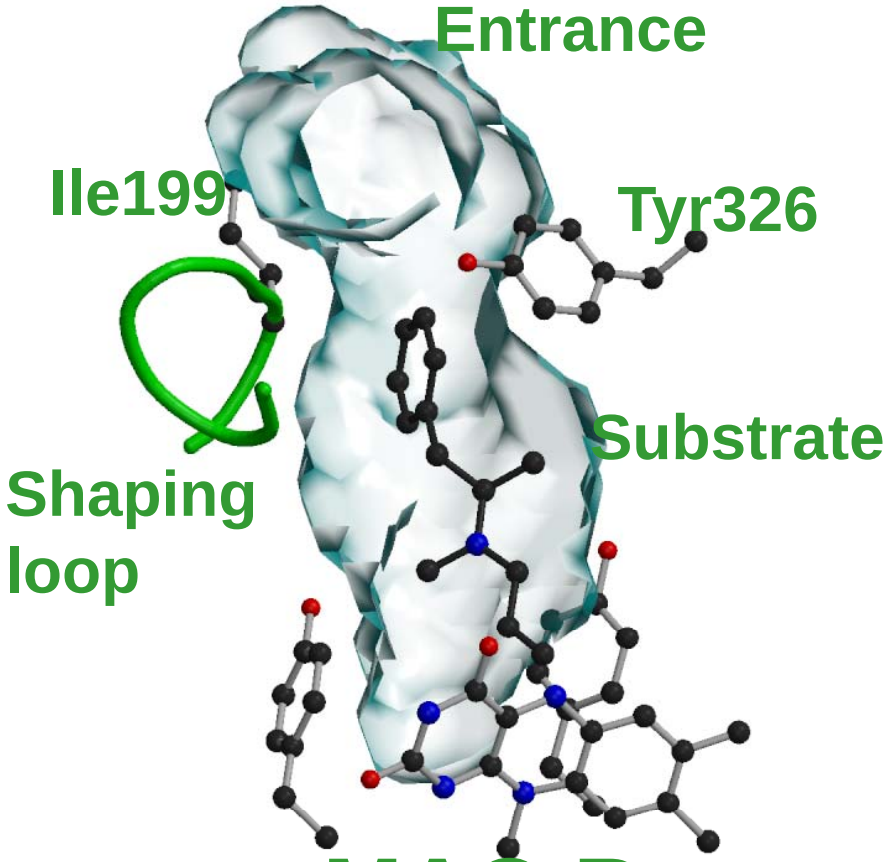
Structure-based optimization



MAO A vs MAO B: Active Site Mutations



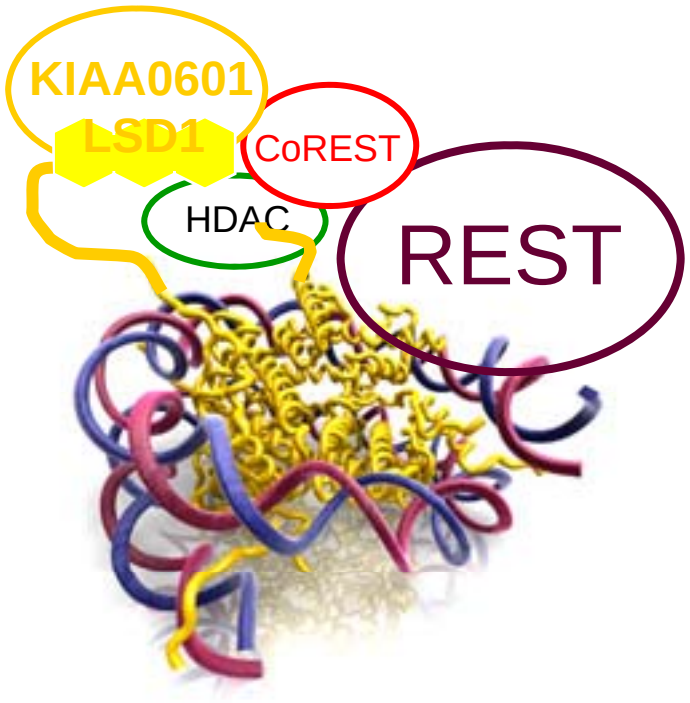
MAO A: single cavity



MAO B: dipartite cavity

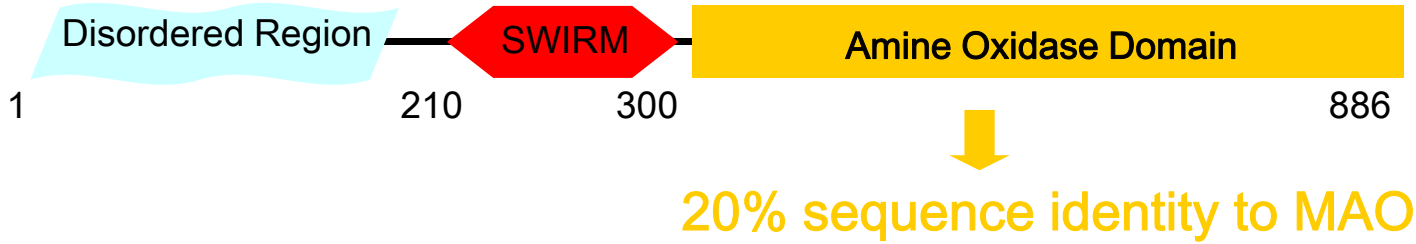
De Colibus et al. (2005) *PNAS* **102**, 12684

In-house story 2: Histone Demethylase



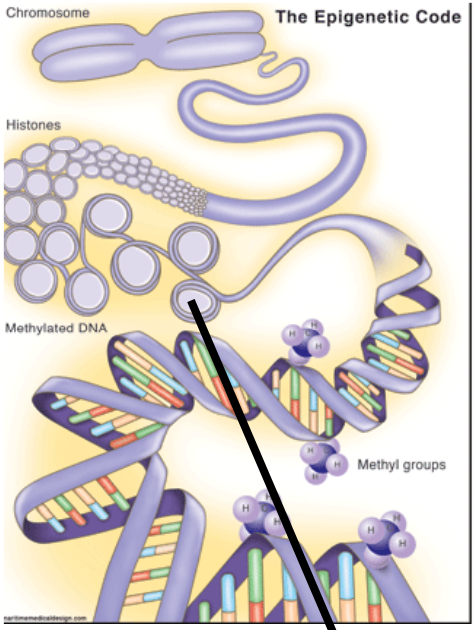
Nuclear protein complex:

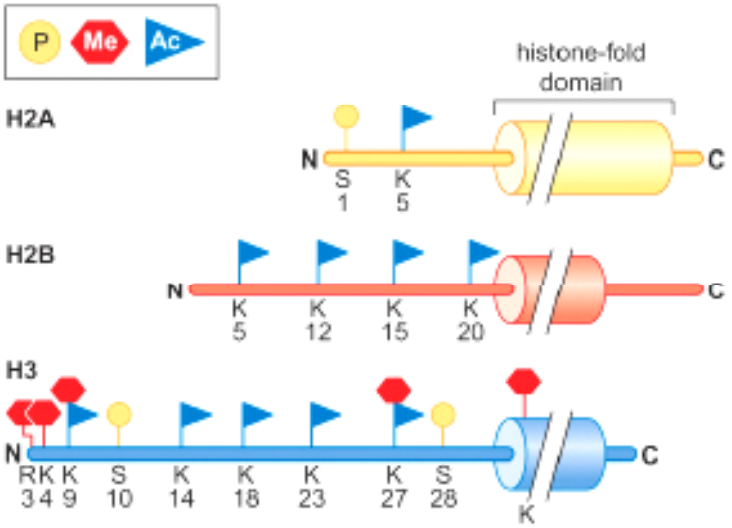
- chromatin remodelling
- regulation of neuronal genes

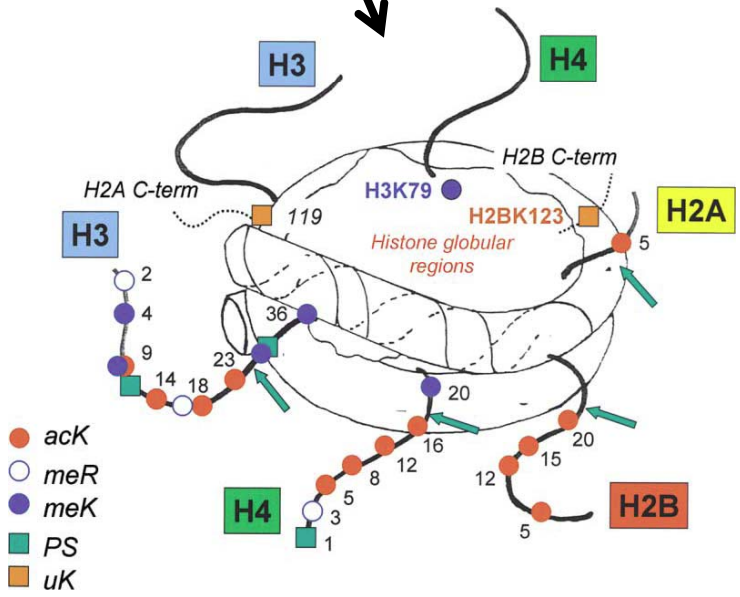


20% sequence identity to MAO

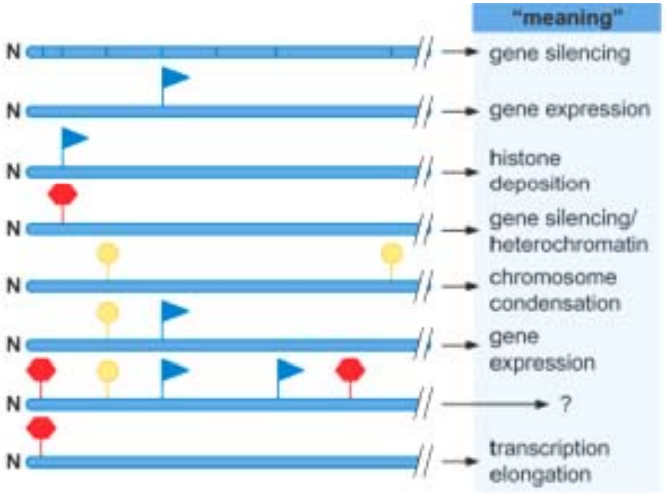
LSD1 is a histone demethylase



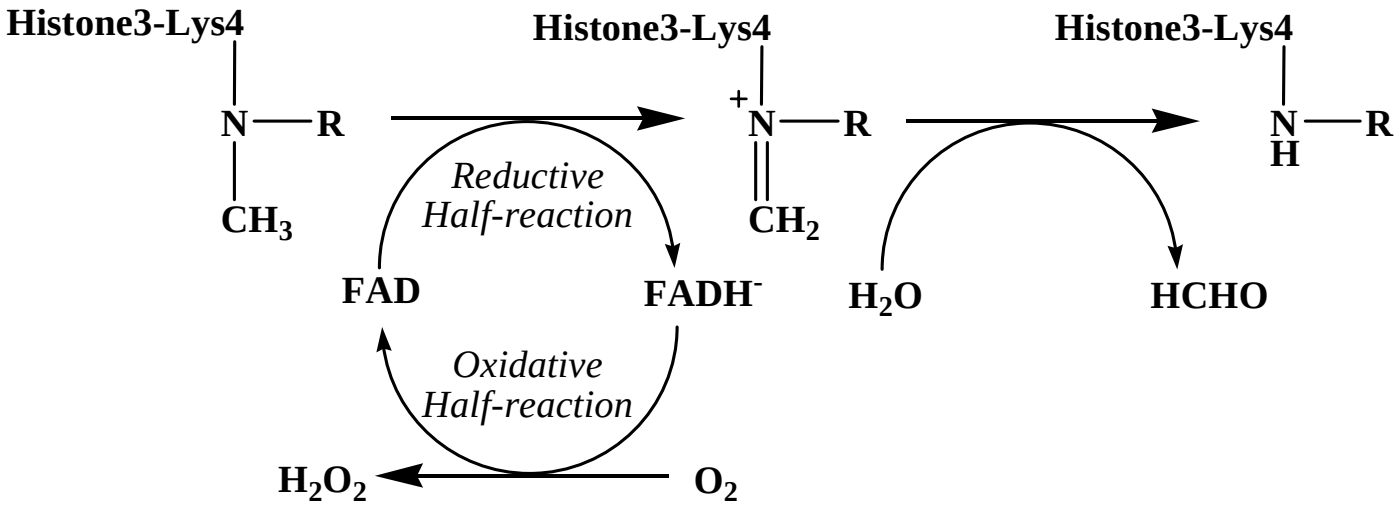




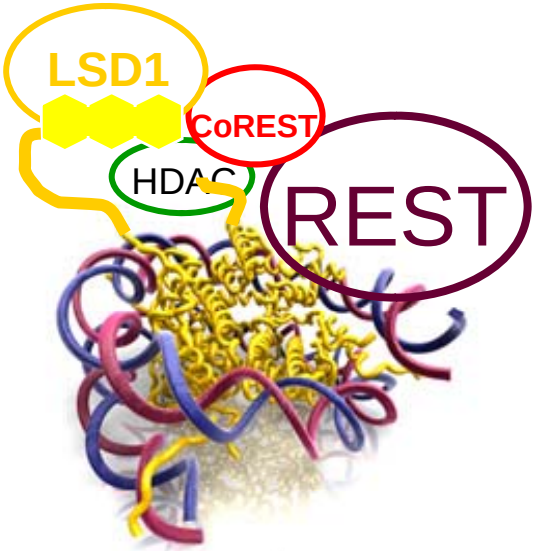
The histone code



LSD1 removes an epigenetic mark in a reaction chemically similar to that of MAO



Shi et al. (2004) Cell **119**, 941
Forneris et al. (2005) FEBS Lett. **579**, 2203

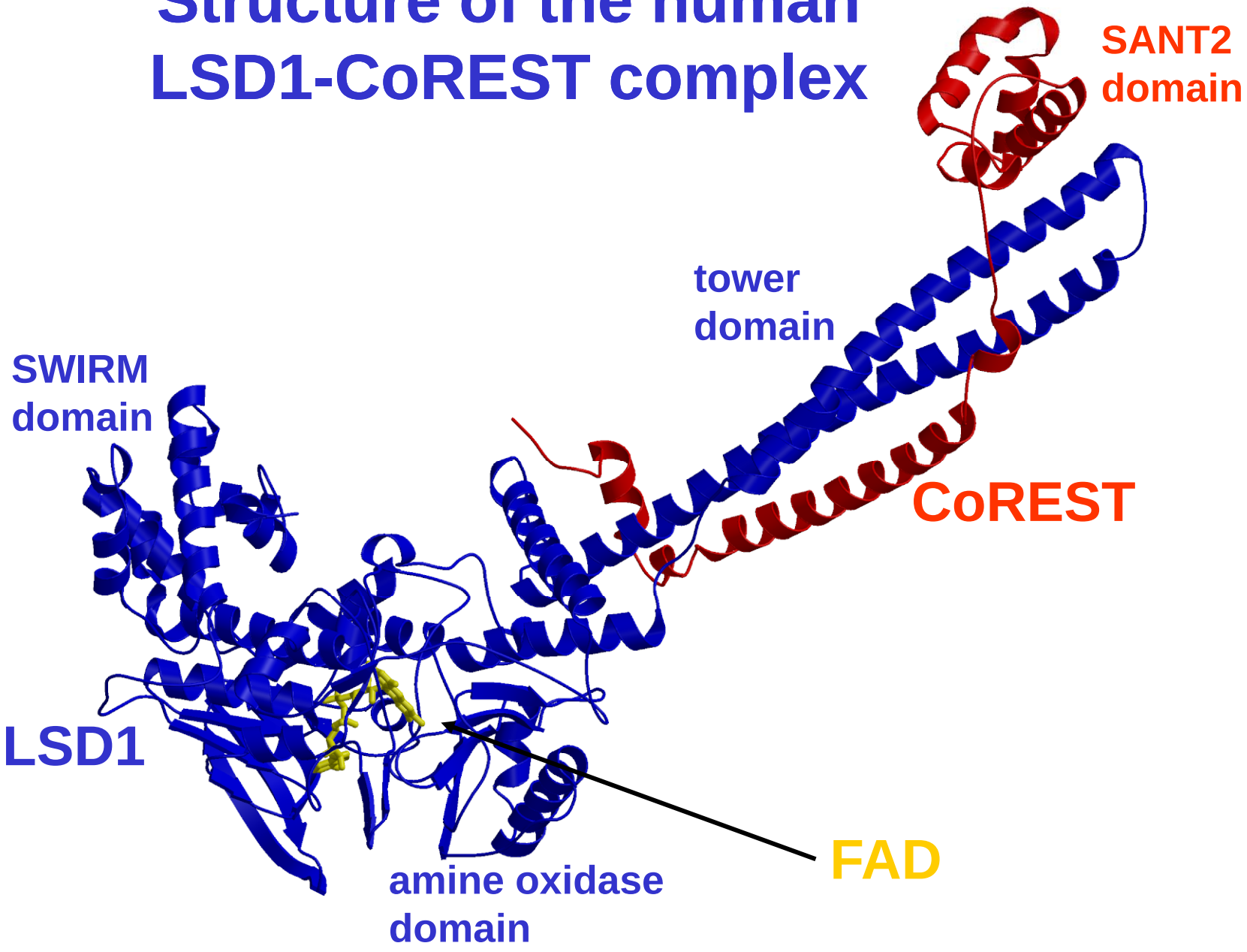


LSD1 and CoREST

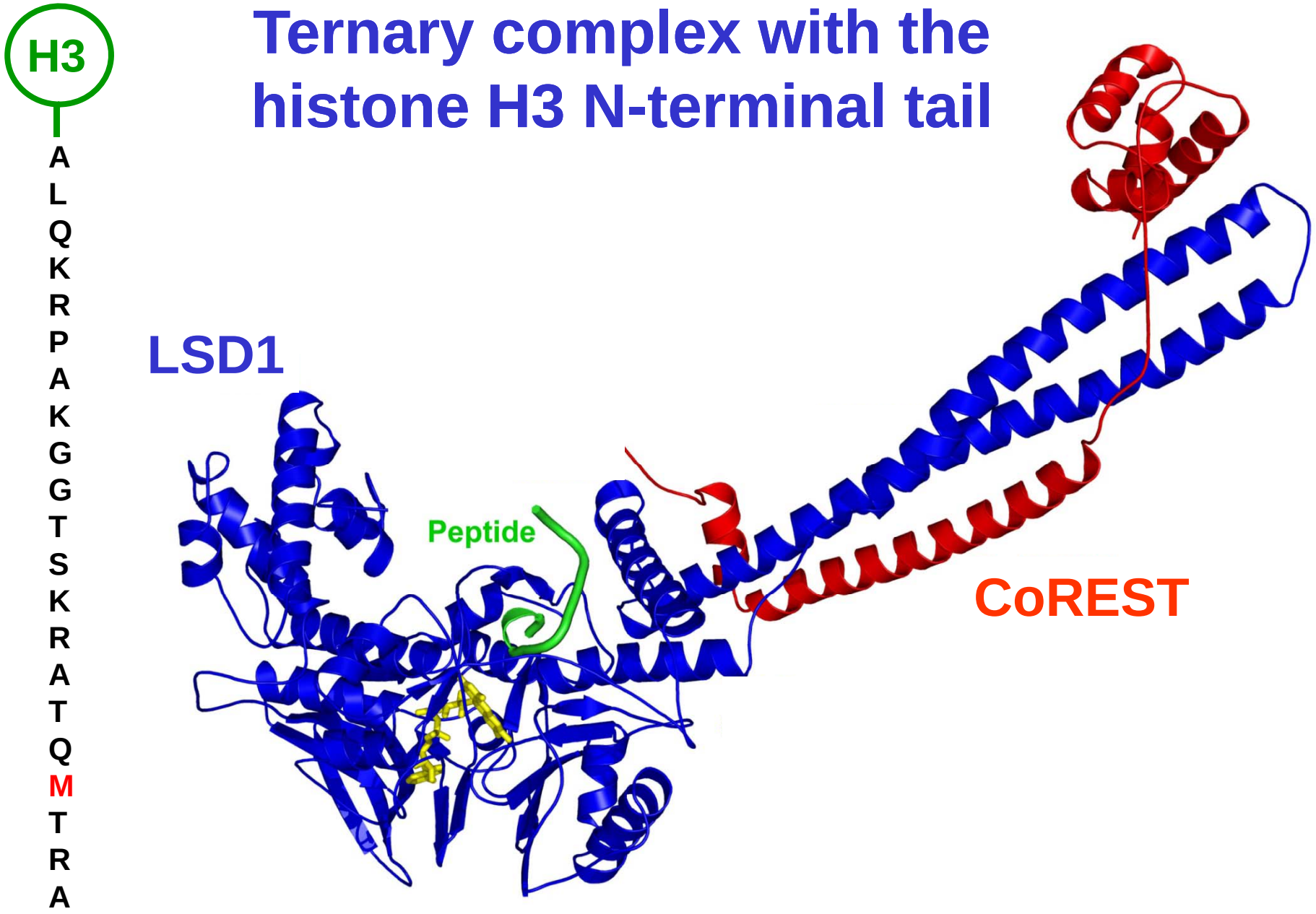
Yeast two-hybrid assay

LSD1	HIS	βGal	CoREST
	+		ELM SANTI SANTII; 1 – 482
	+		293 – 482
	–		1 – 293

Structure of the human LSD1-CoREST complex

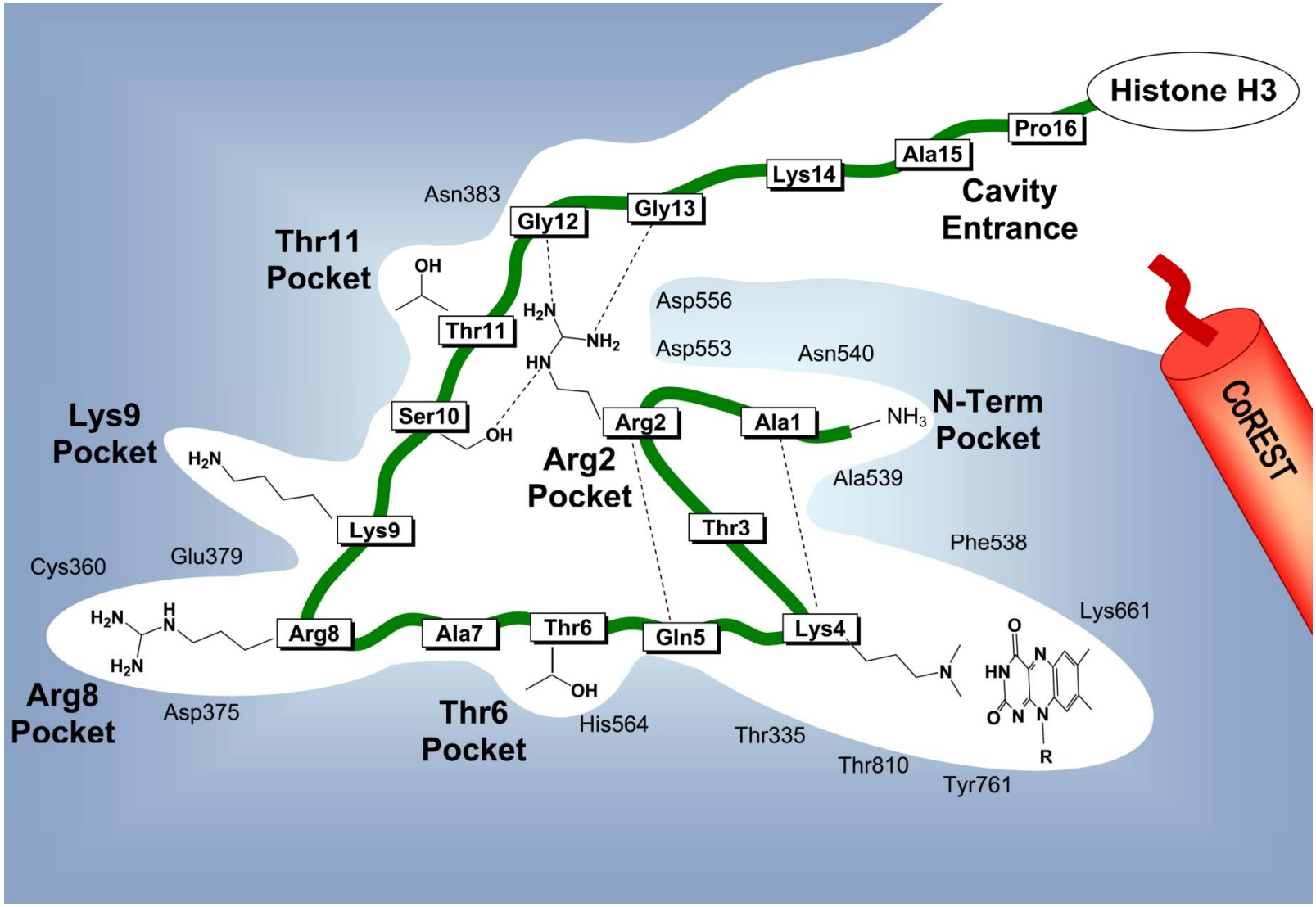


Ternary complex with the histone H3 N-terminal tail



Forneris *et al.* (2007) *J. Biol. Chem.* **282**, 20070

A network of inter- and intra-molecular interactions



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