History of biological structure: sources of radiation

Sealed tube	first proteins/enzymes at medium resolution (25kD)
Rotating anode	hemoglobin (100kD)
Big wheel	tropomyosin
Synchrotron:	
1st generation	viruses
2nd generation	large complexes
3rd generation	larger complexes
4th generation	organelles?
Free electron laser	holography

Biological & Health Sciences

large complexes/systems
membrane-bound proteins
small crystals
ultra-high resolution
time-resolved; detailed dynamics
small-angle scattering
EXAFS
holography

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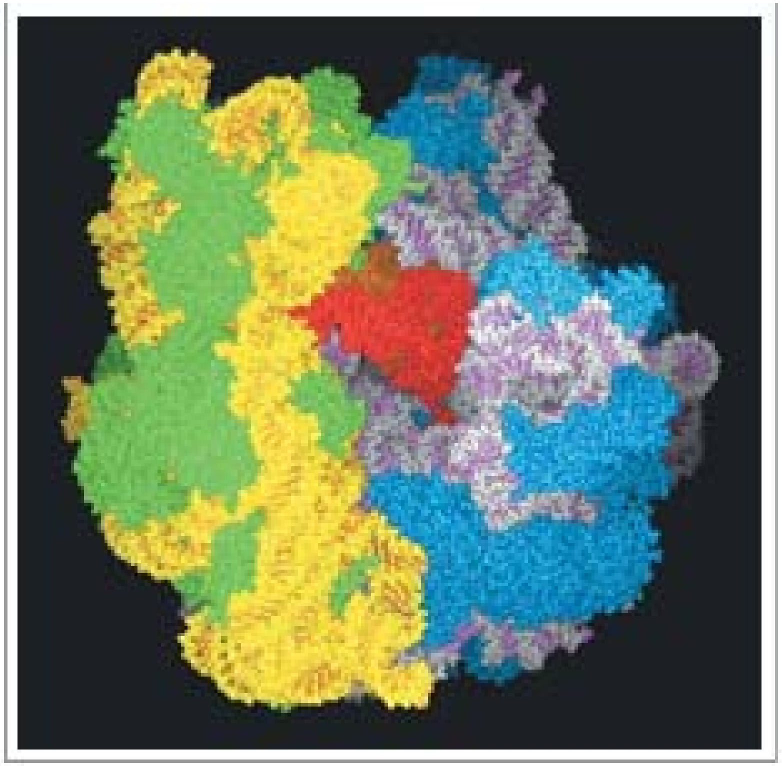
Large Complexes



Ribosome:

Steitz, Moore;

Noller

Ca-ATPase: Toyoshima

cytochrome b oxidase: Tsukihara

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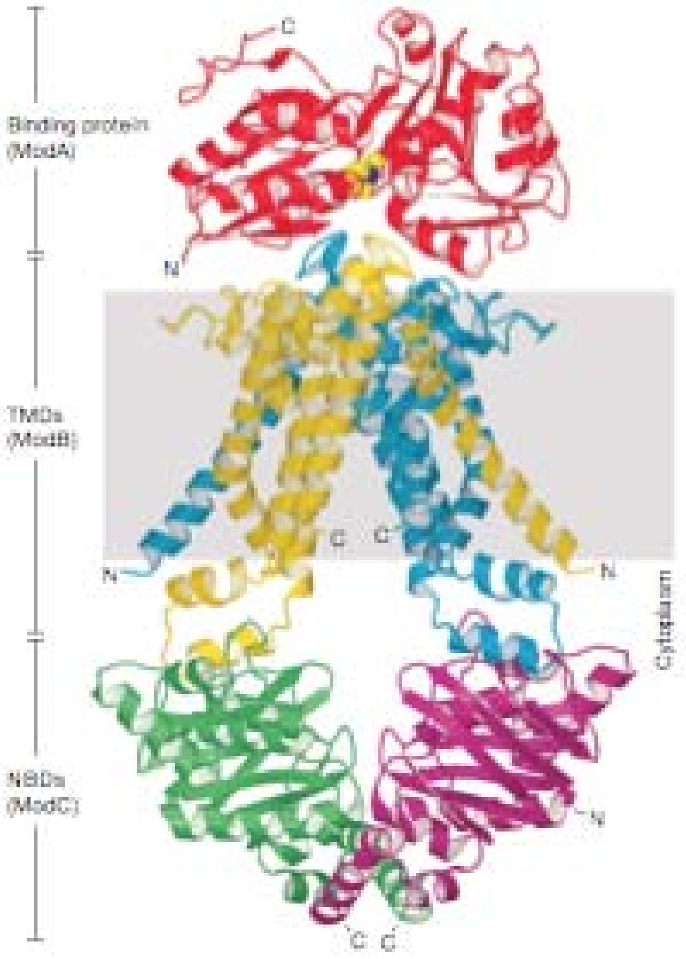
Membrane-bound proteins



Locher: ABC transporter-binding protein complex

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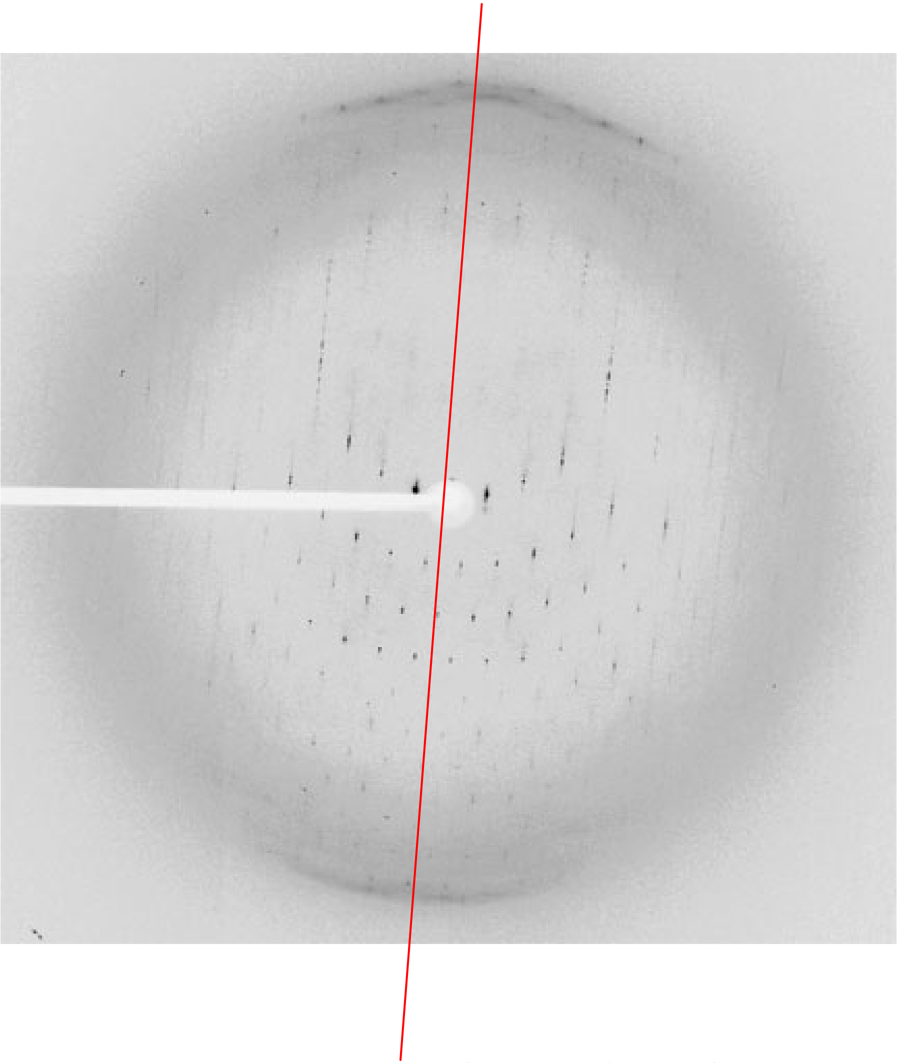
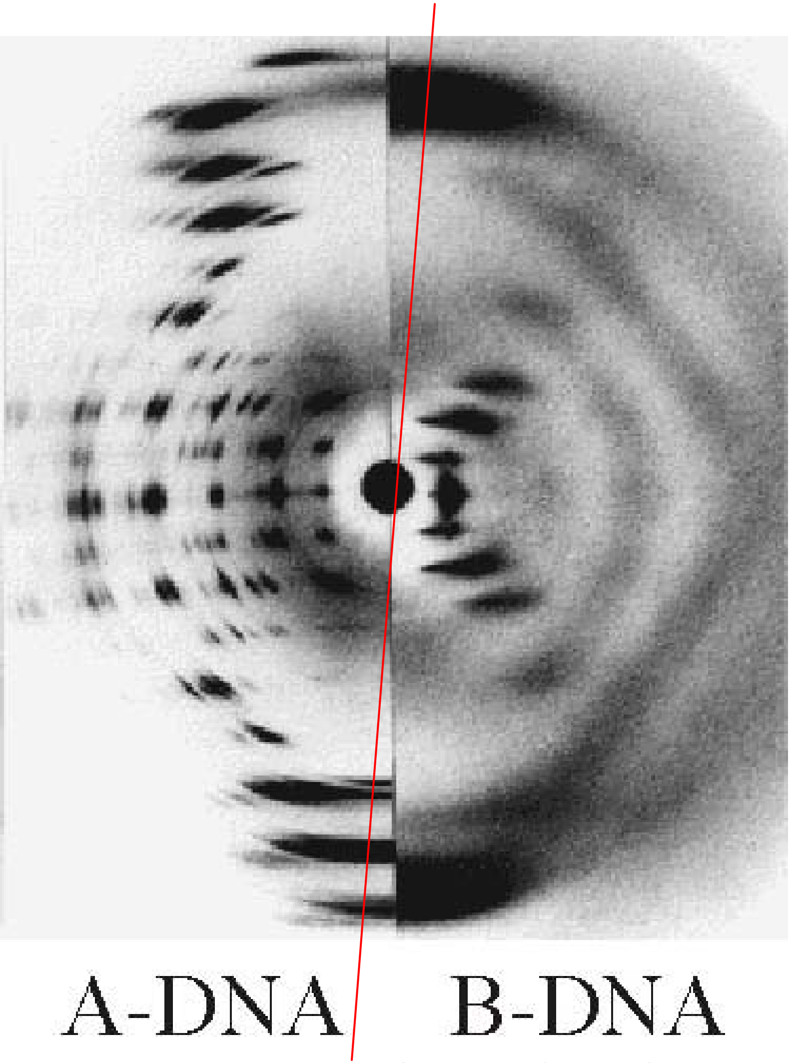
X-ray diffraction from Tbet/DNA co-crystals from condition II

DNA is oriented approximately along the z-axis



DNA axis (in plane of screen)

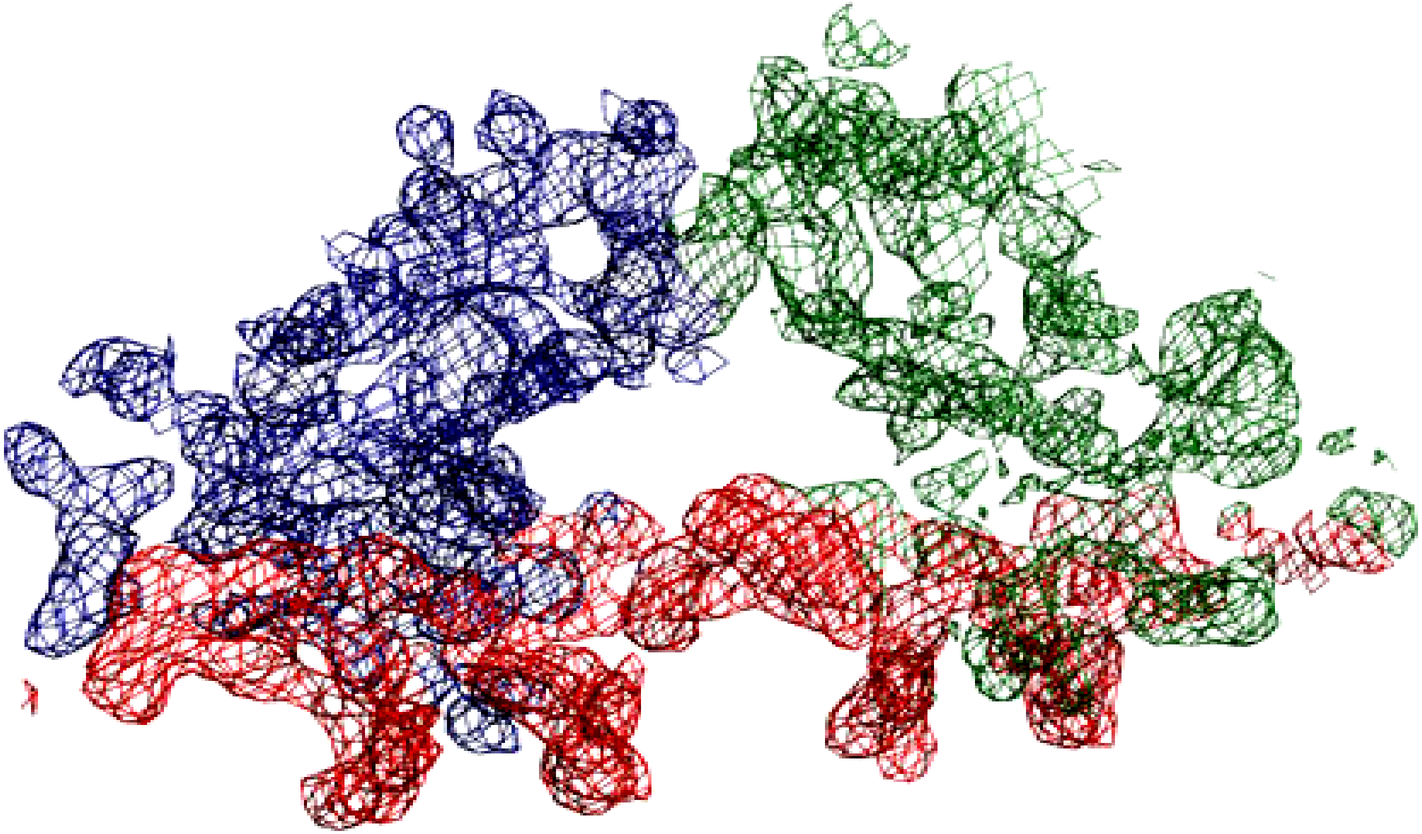
Rosalind Franklin’s original DNA fiber diffraction patterns



DNA axis (in plane of screen)

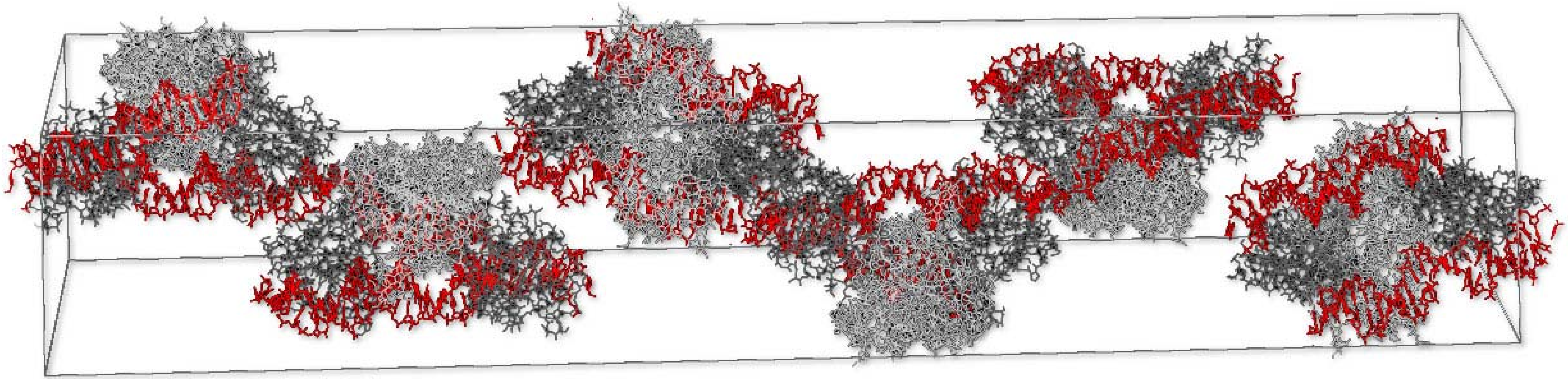
The structure is solved

The DNA and most of the protein backbone fits this electron density relatively well



electron density map (2*Fo* - *Fc*)

DNA orientation is indeed along the *z* (long) axis



Space group: $P6_122$

Unit cell: 70.219 Å *x* 70.219 Å *x* 439.702 Å

(90.000 ° *x* 90.000° *x* 120.000°)

Resolution: ~ 3.2 Å

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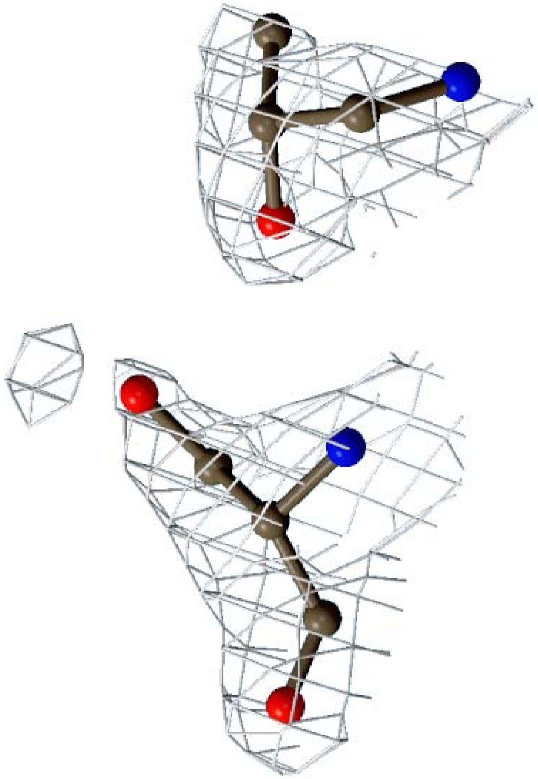
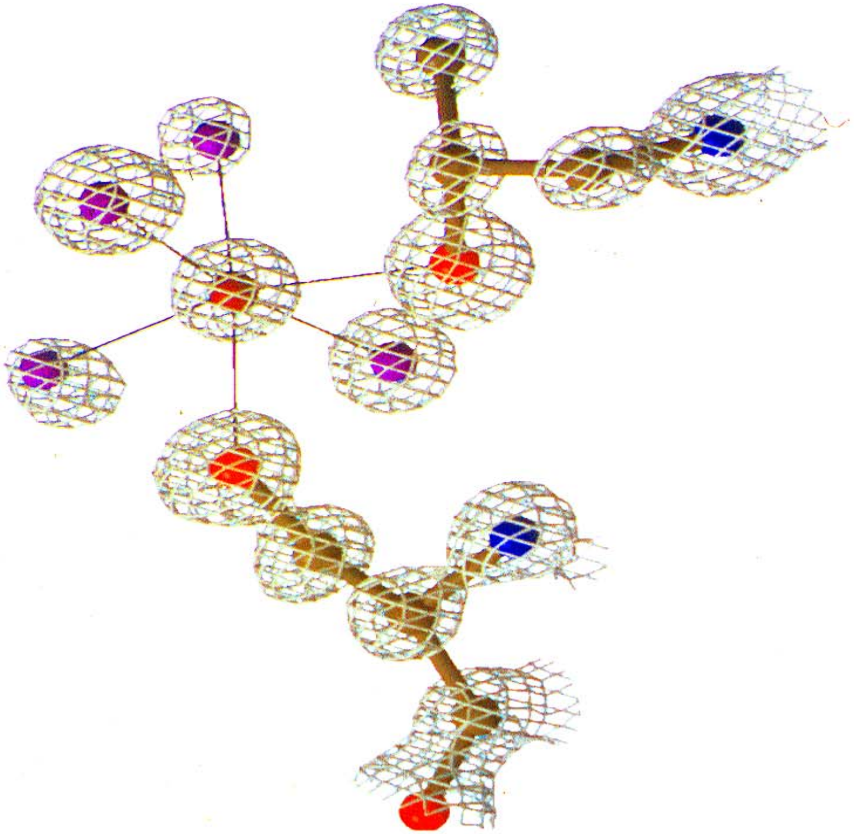
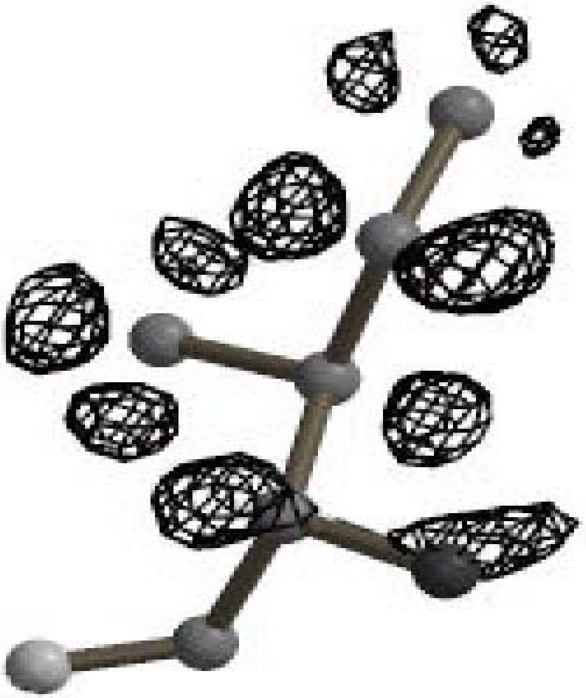
A SURFACE WATER H-BONDED TO A THR AND SER AT 2 Å RESOLUTION



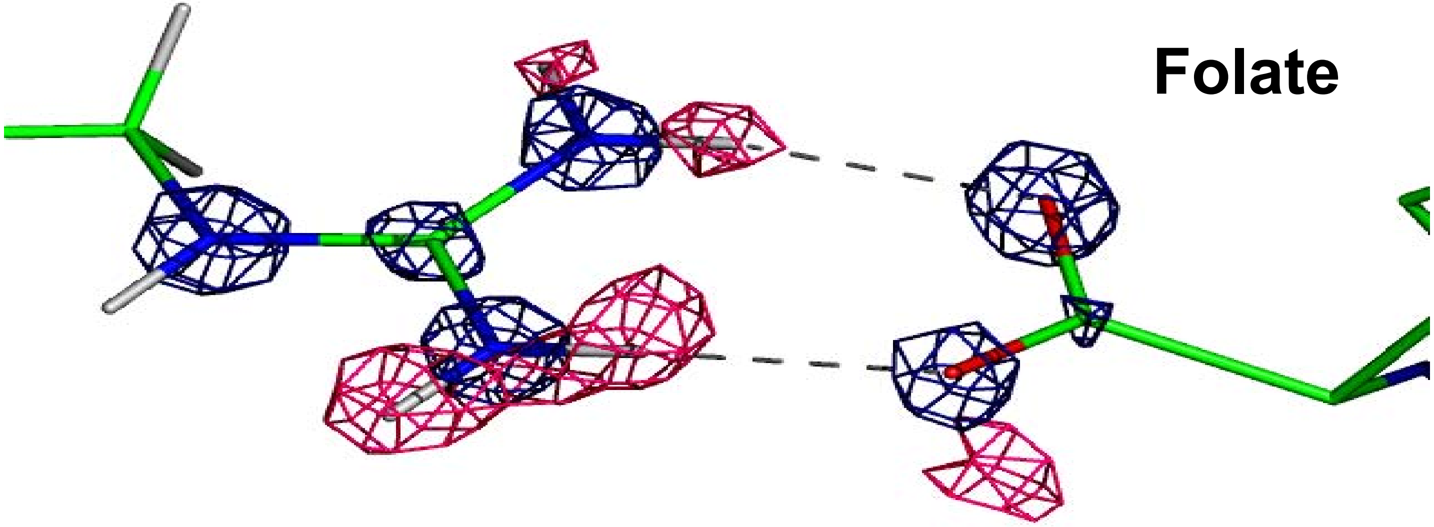
THE “WATER” IS A SODIUM ION (0.95A)



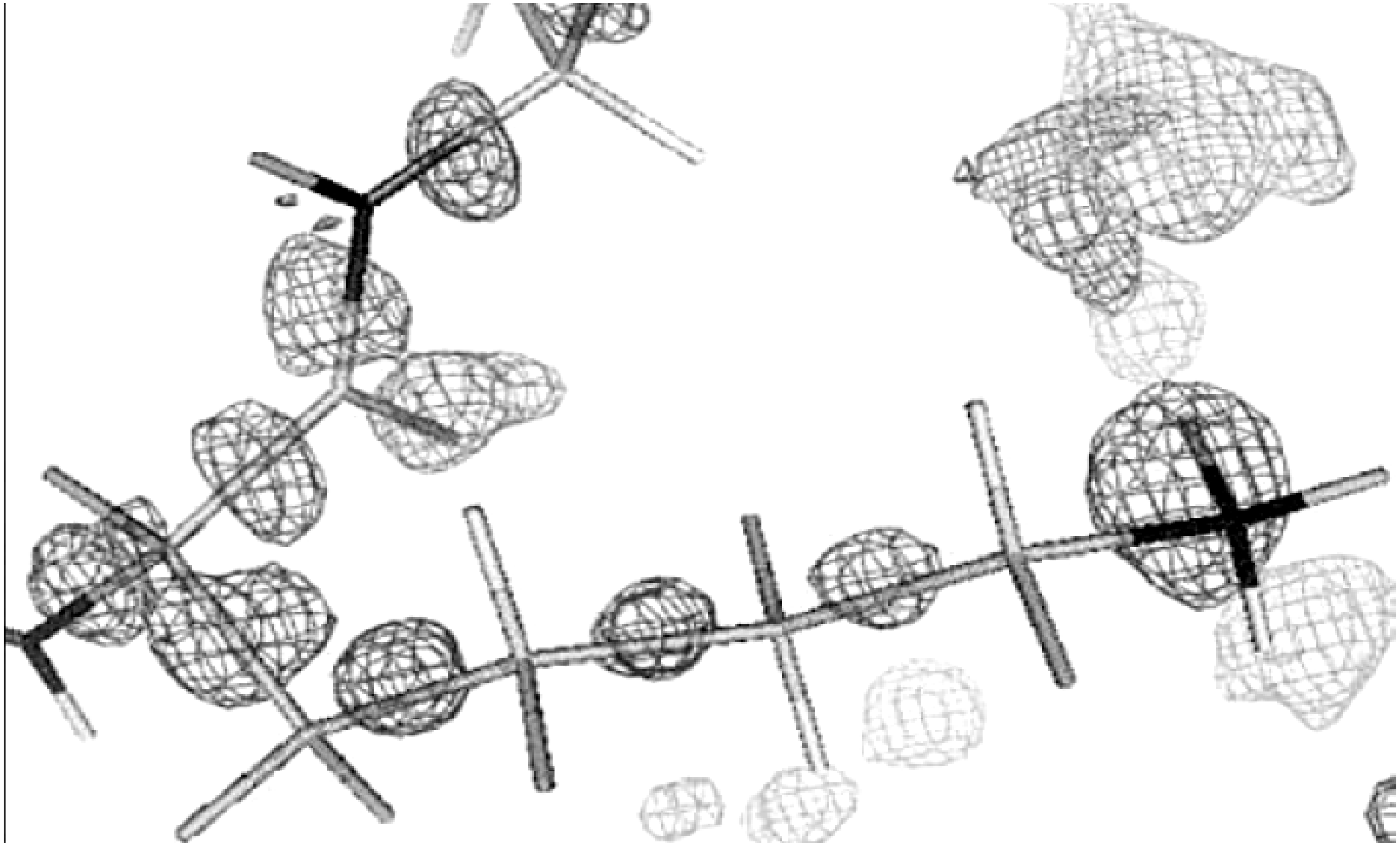
Hydrogens on Ile 26



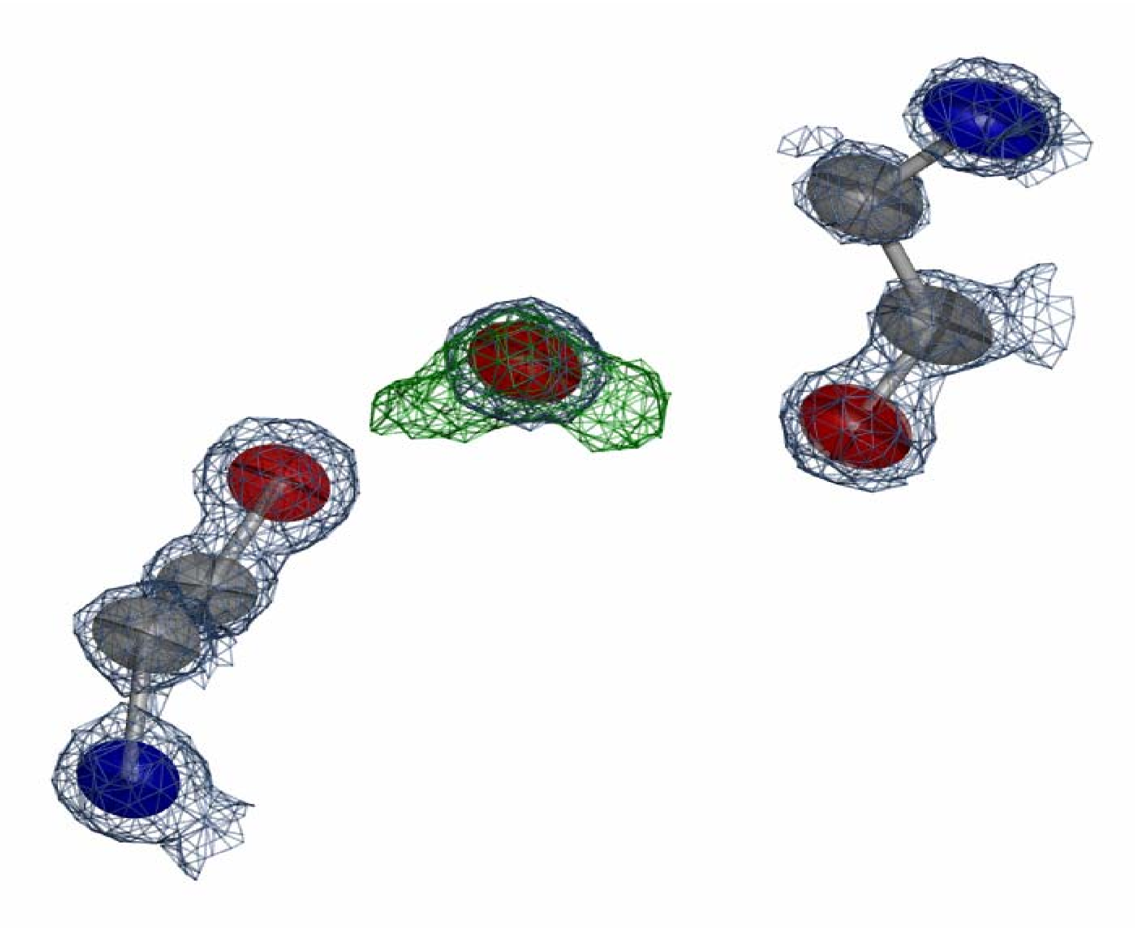
Hydrogen Atom Electron Density in DHFR at 0.95 Å Resolution



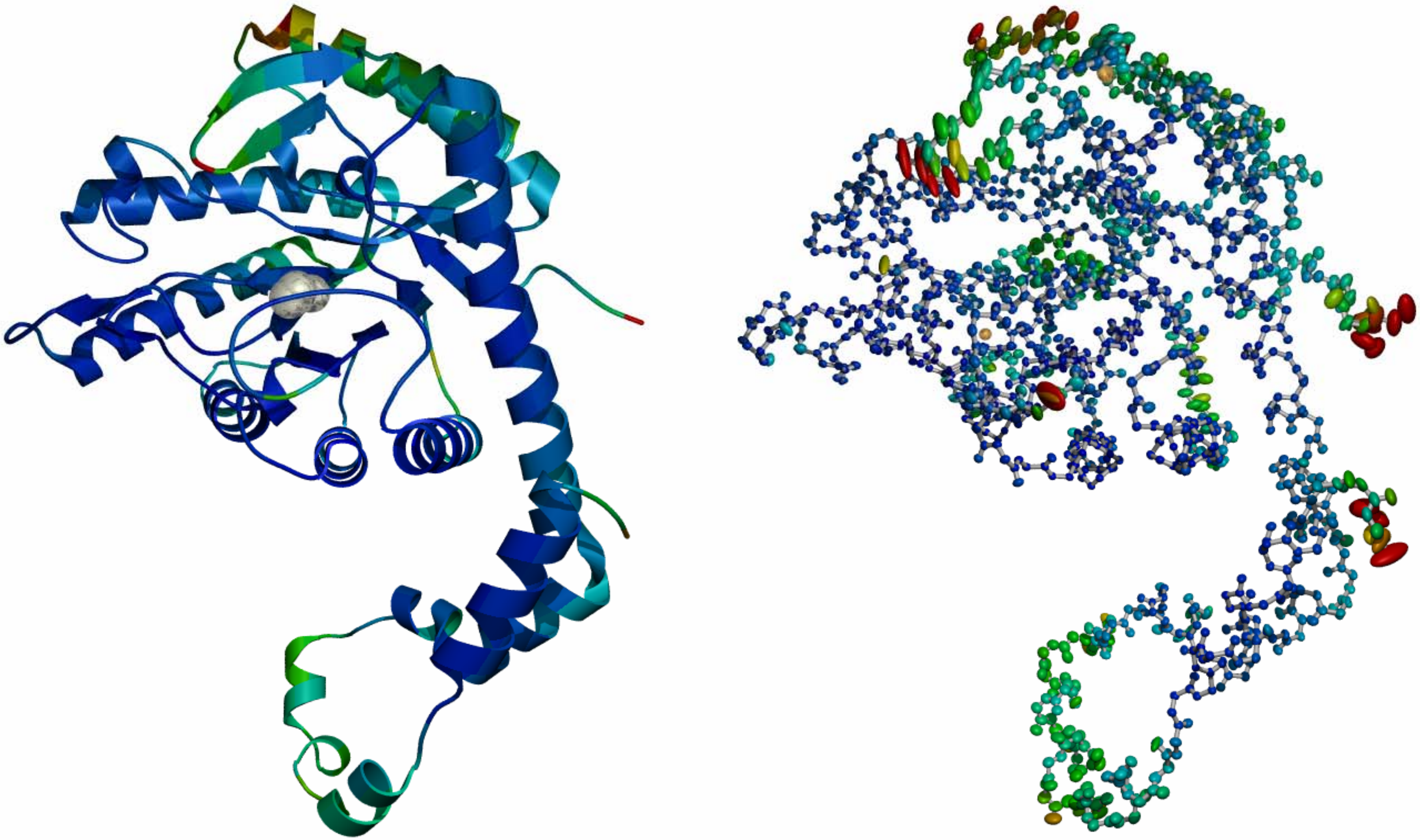
Bonding Electron Density?



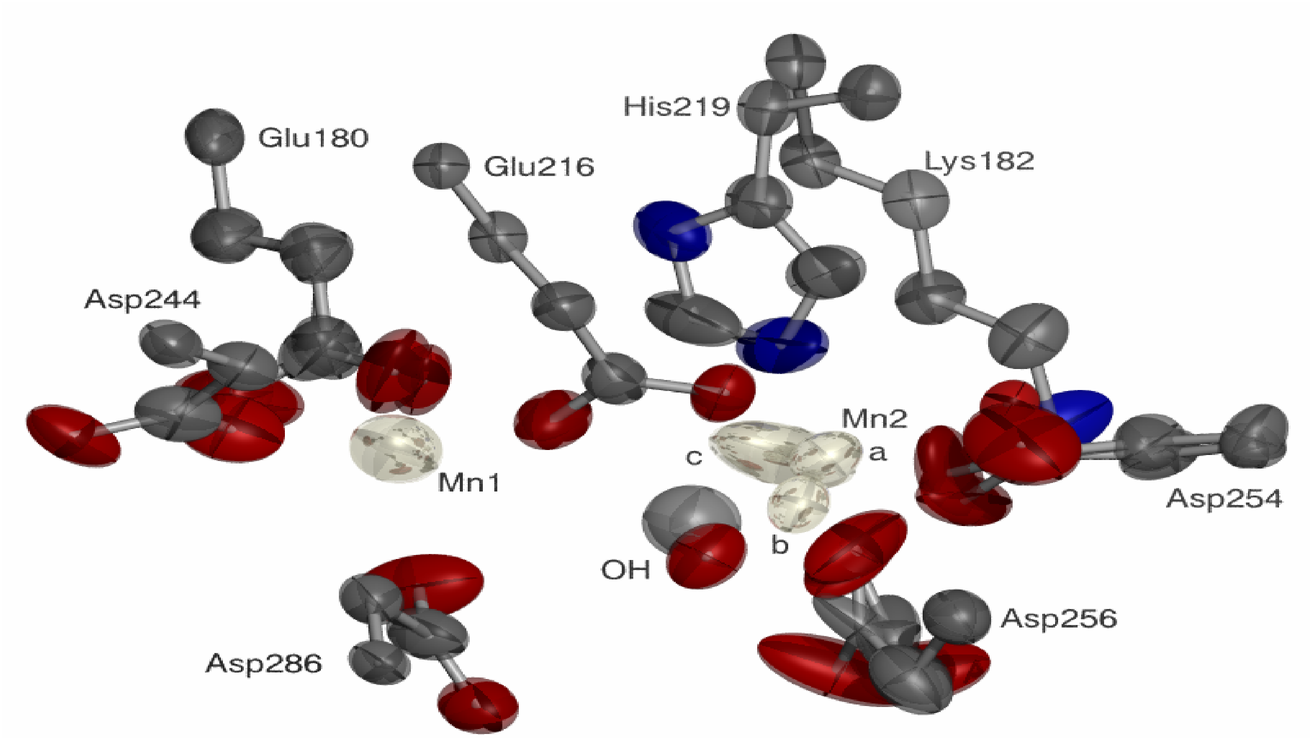
Protonation States



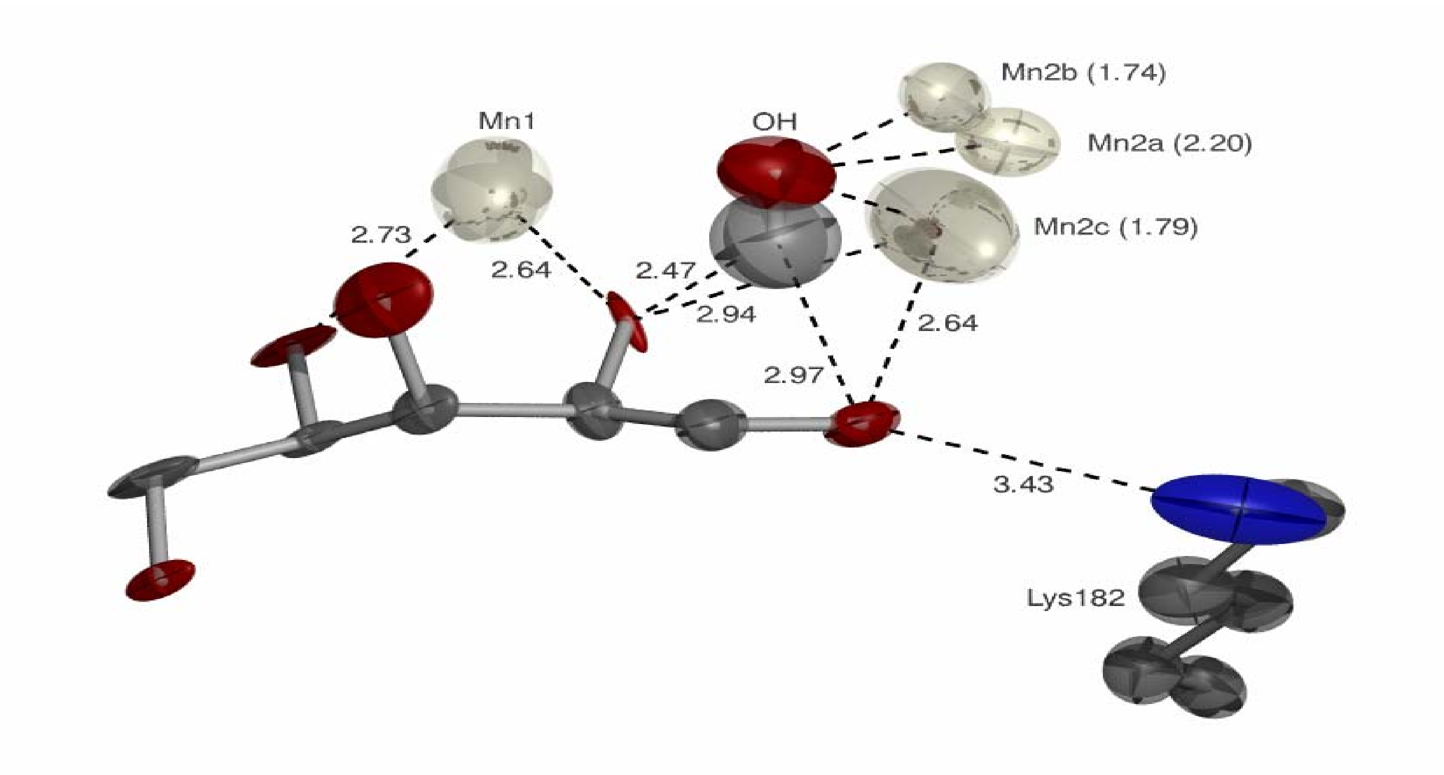
Overall Structure



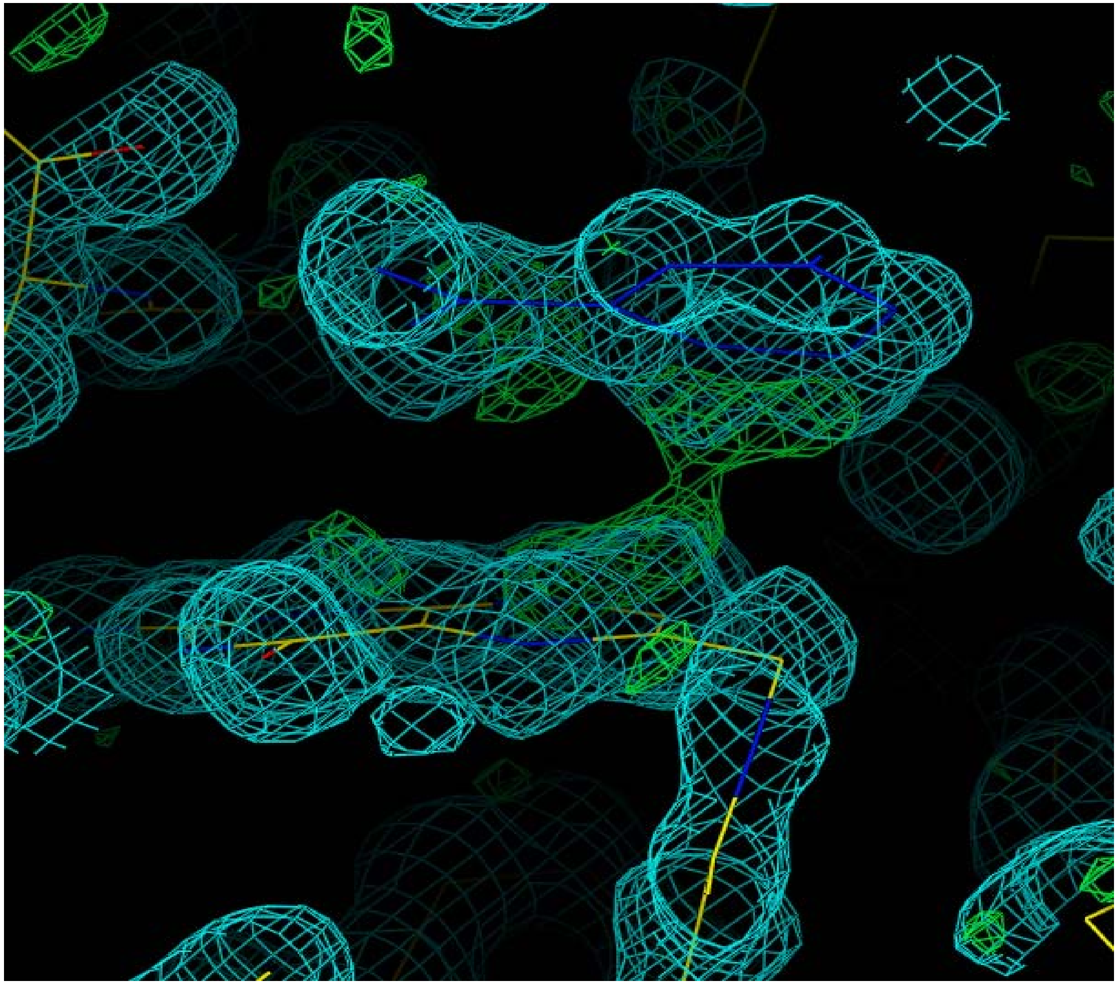
Active Site: Thermal Elipsoids



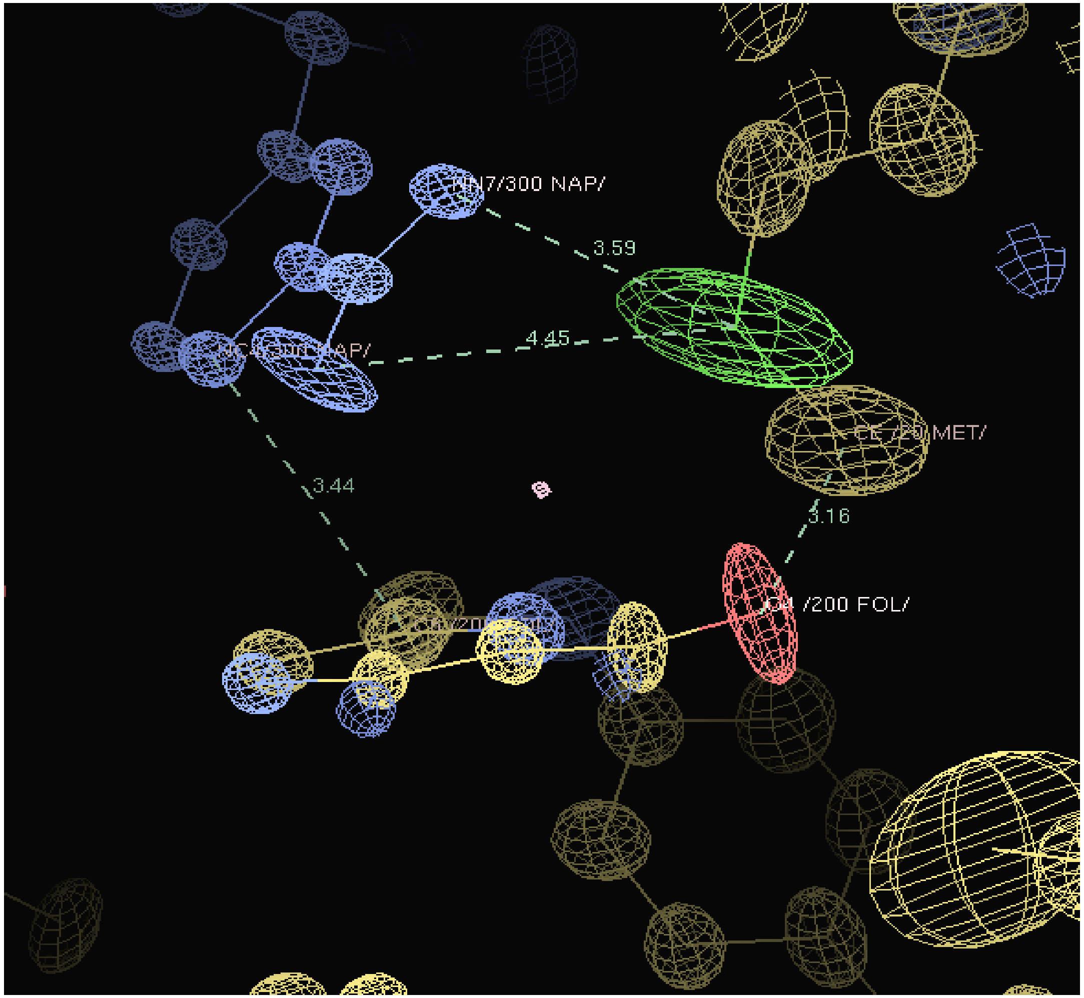
Mechanistic Implications



B. Subtilis DHFR structure determined at 1.1 Å resolution, including difference density



E. coli DHFR 4°C



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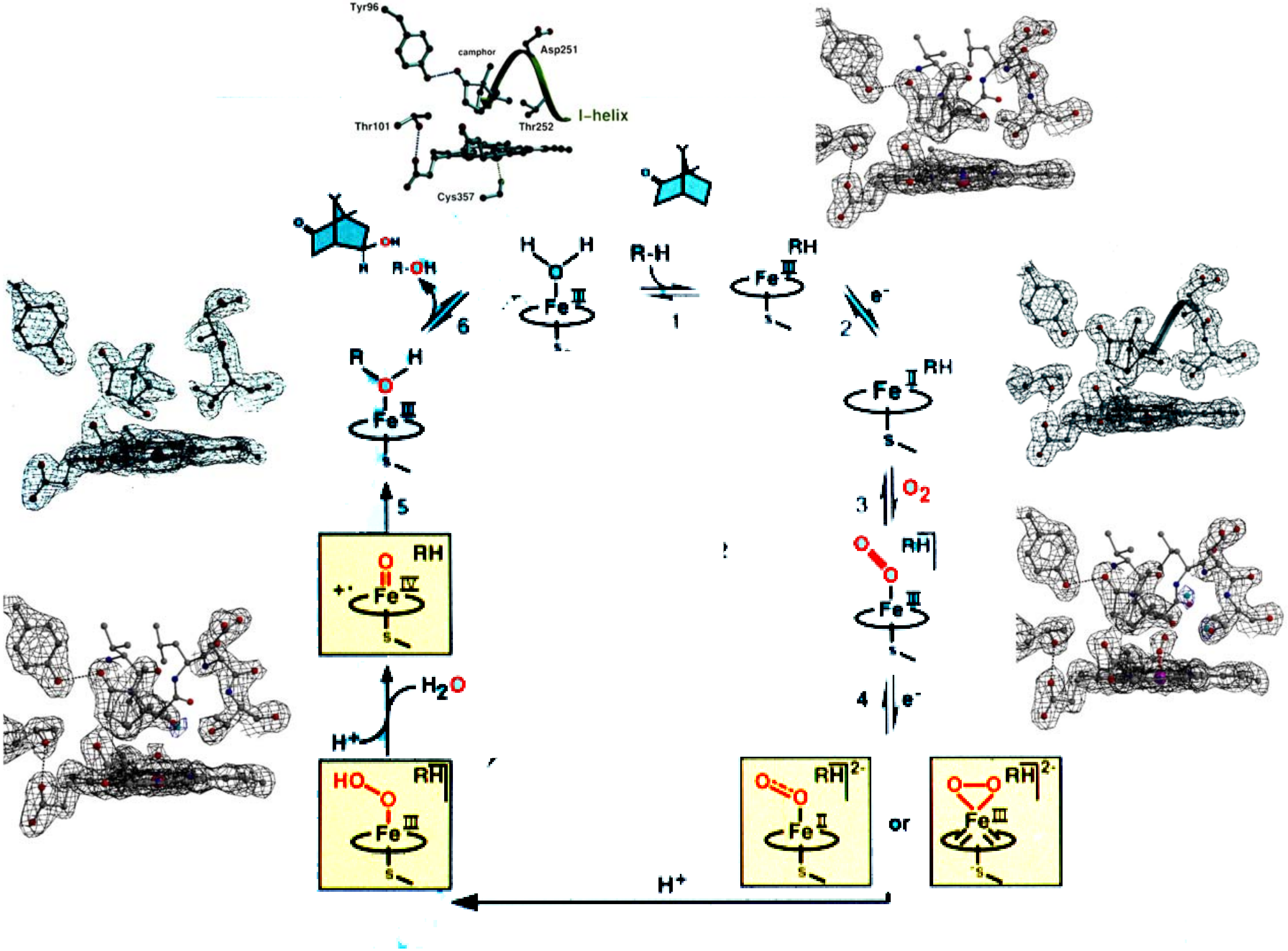
P450: The Movie



Other important capabilities

Small-angle scattering

EXAFS

holography

value

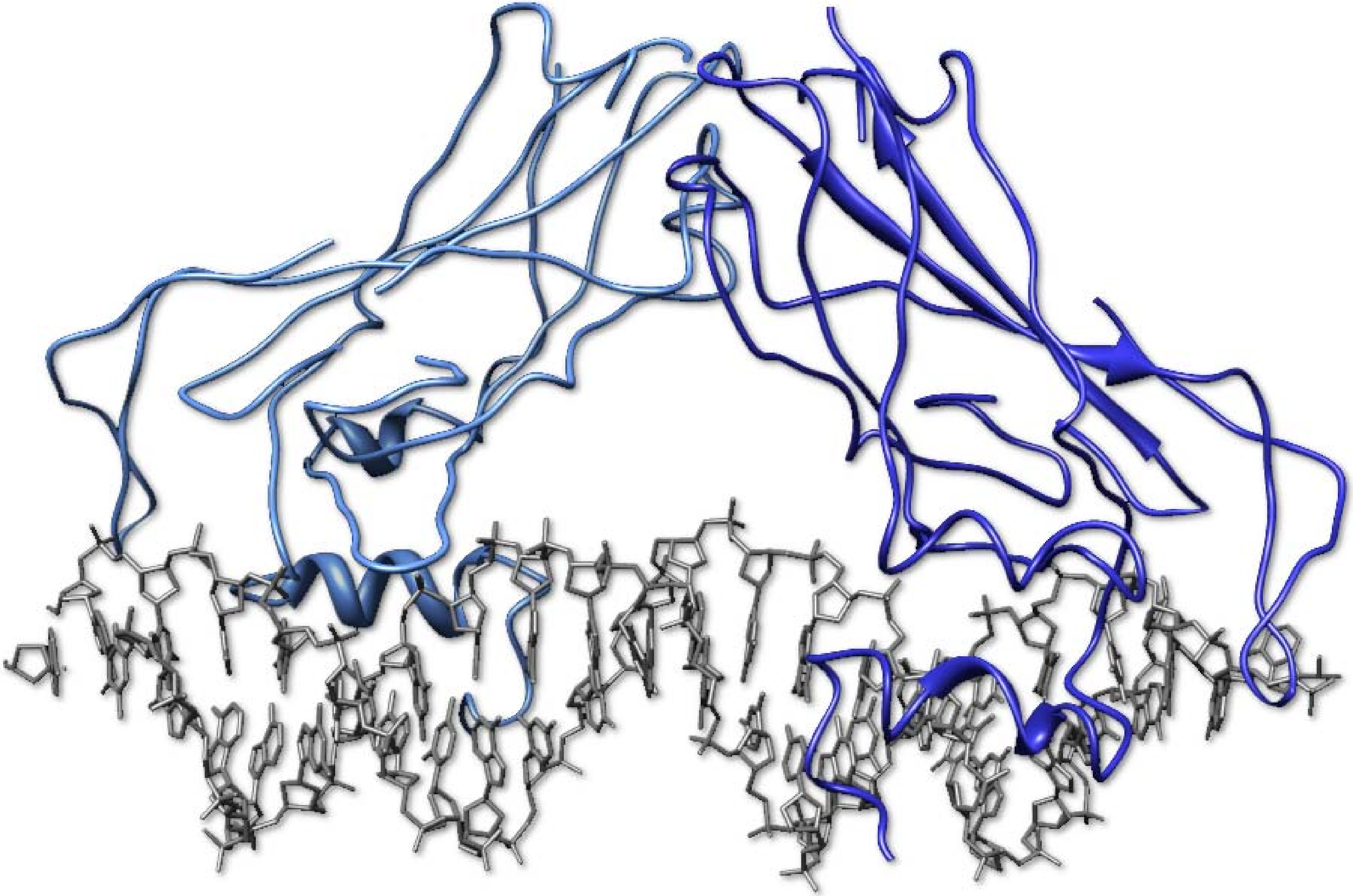
Function

Drug design

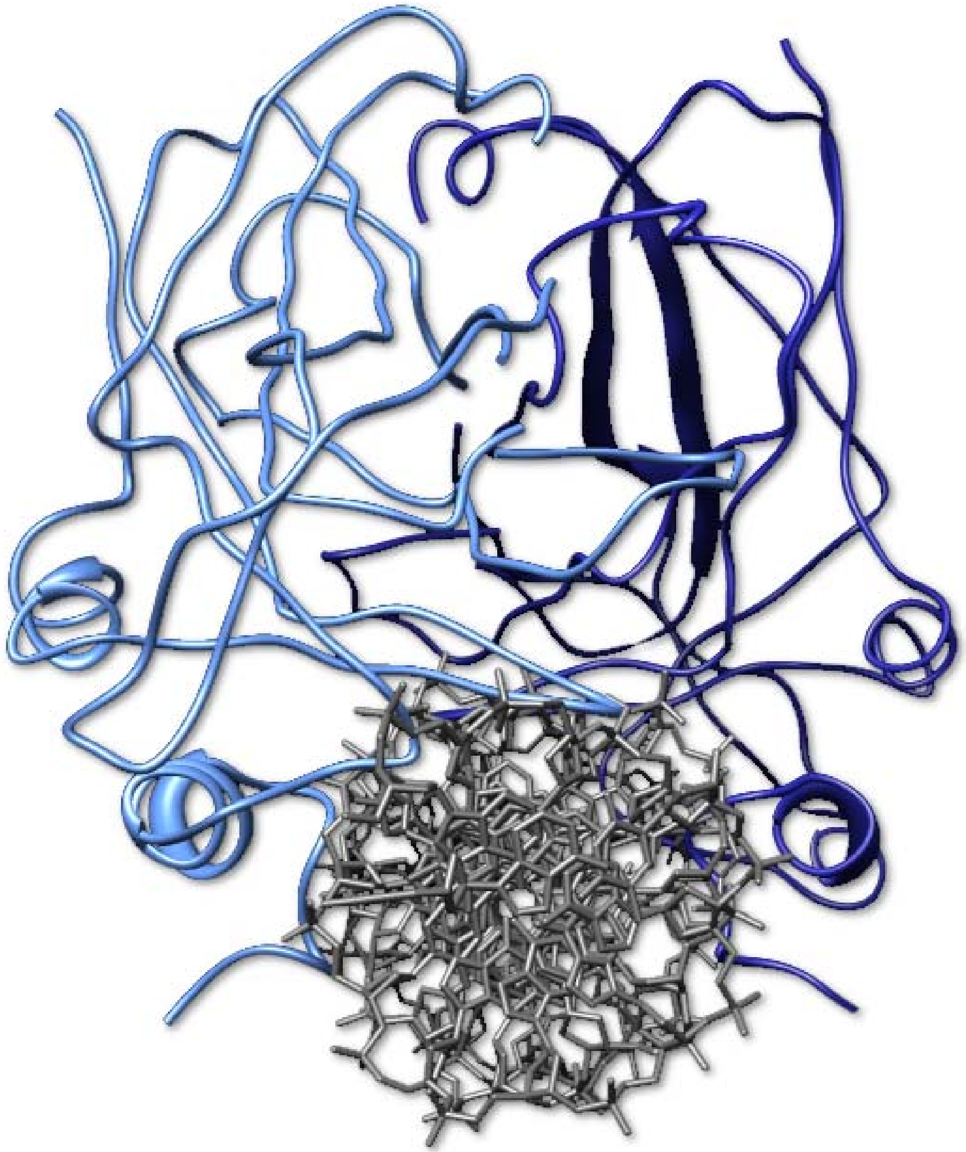
Design of catalysts

Green chemistry

T-box Domain of Human T-bet Bound to Consensus Site

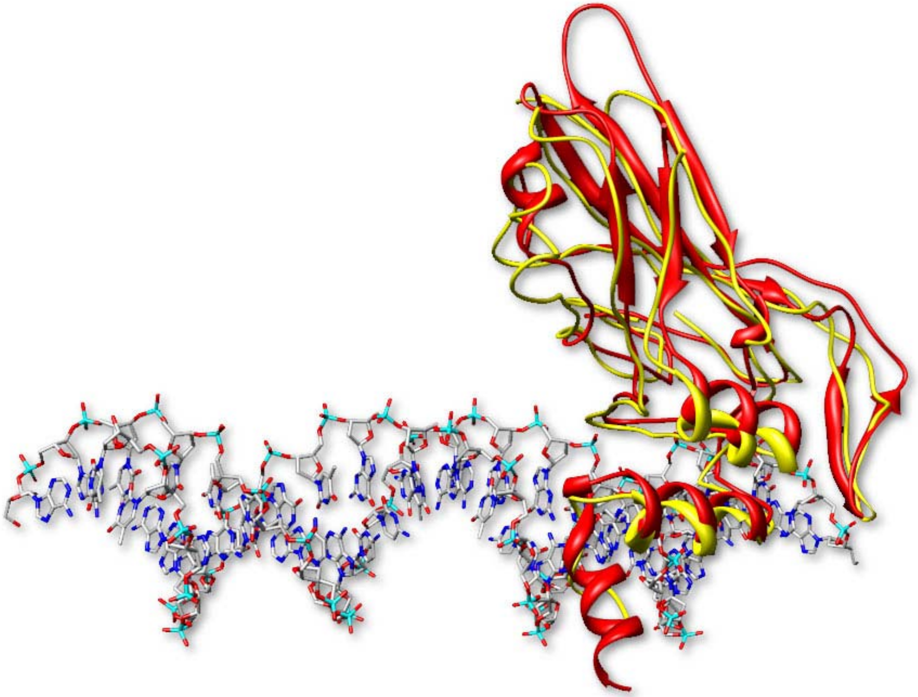


T-box Domain of Human T-bet Bound to Consensus Site



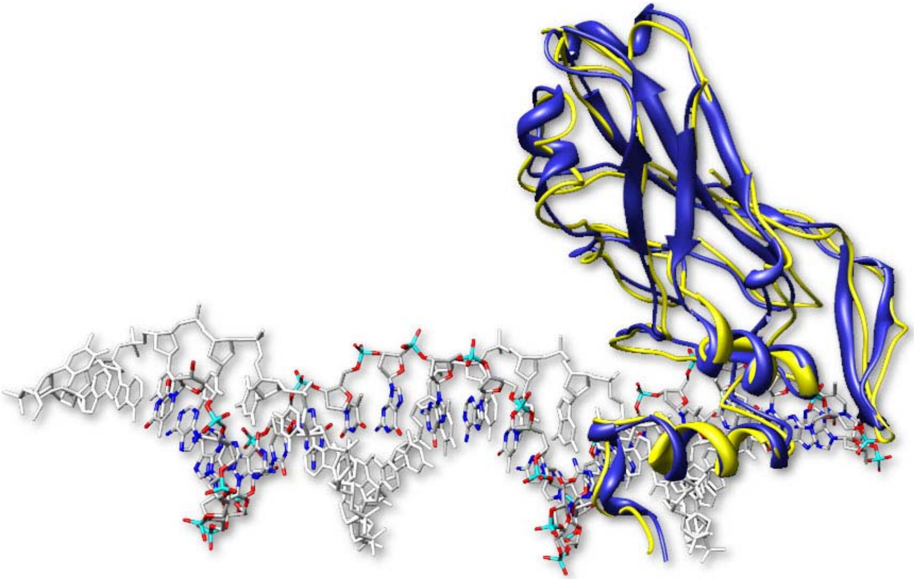
Structural alignment of Tbet with other T-box binding domains

T-bet seems more similar to 1H6F



Xenopus (1XBR) structure: red

T-bet structure: yellow

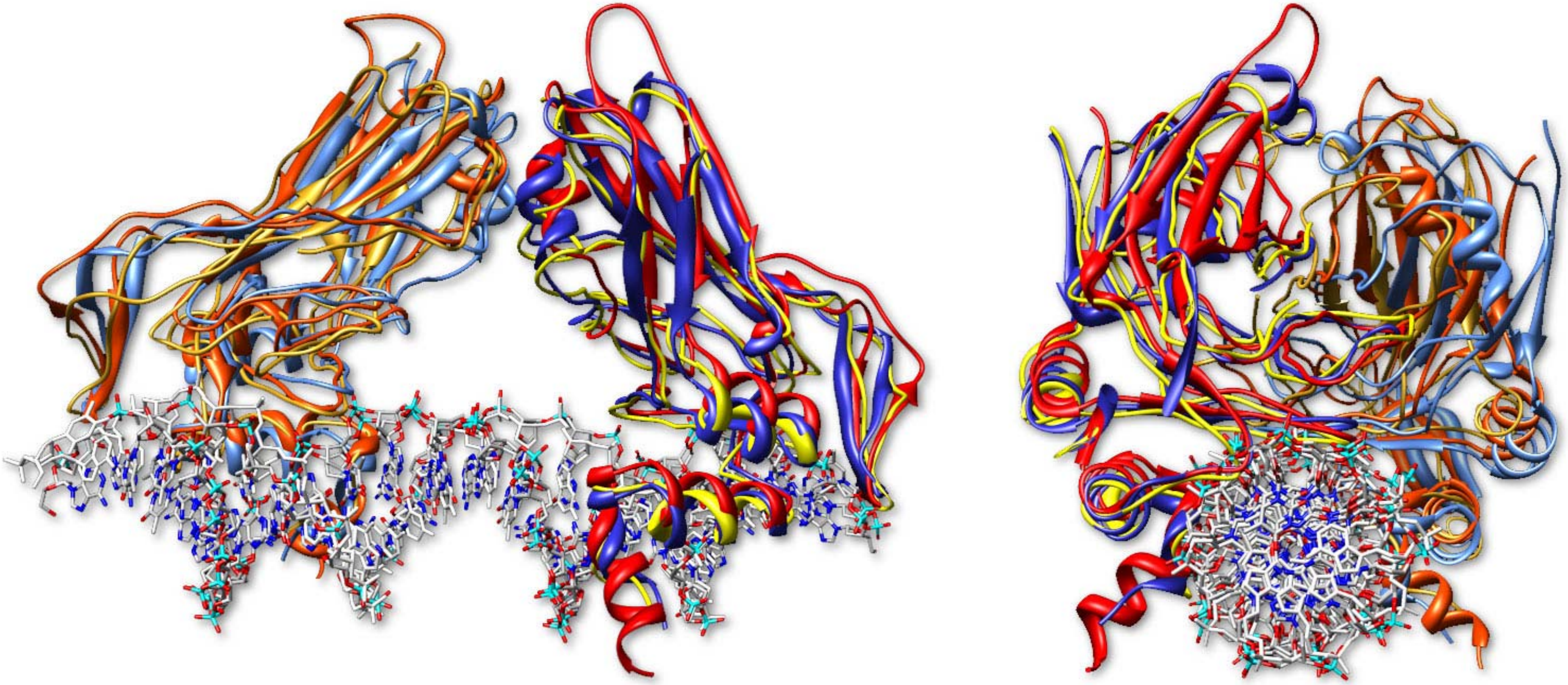


Human (1H6F) structure: blue

T-bet structure: yellow

The dimer interface is of special interest in understanding Tbet function

DNA-binding mode is similar in the three structures, but the interfaces differ considerably

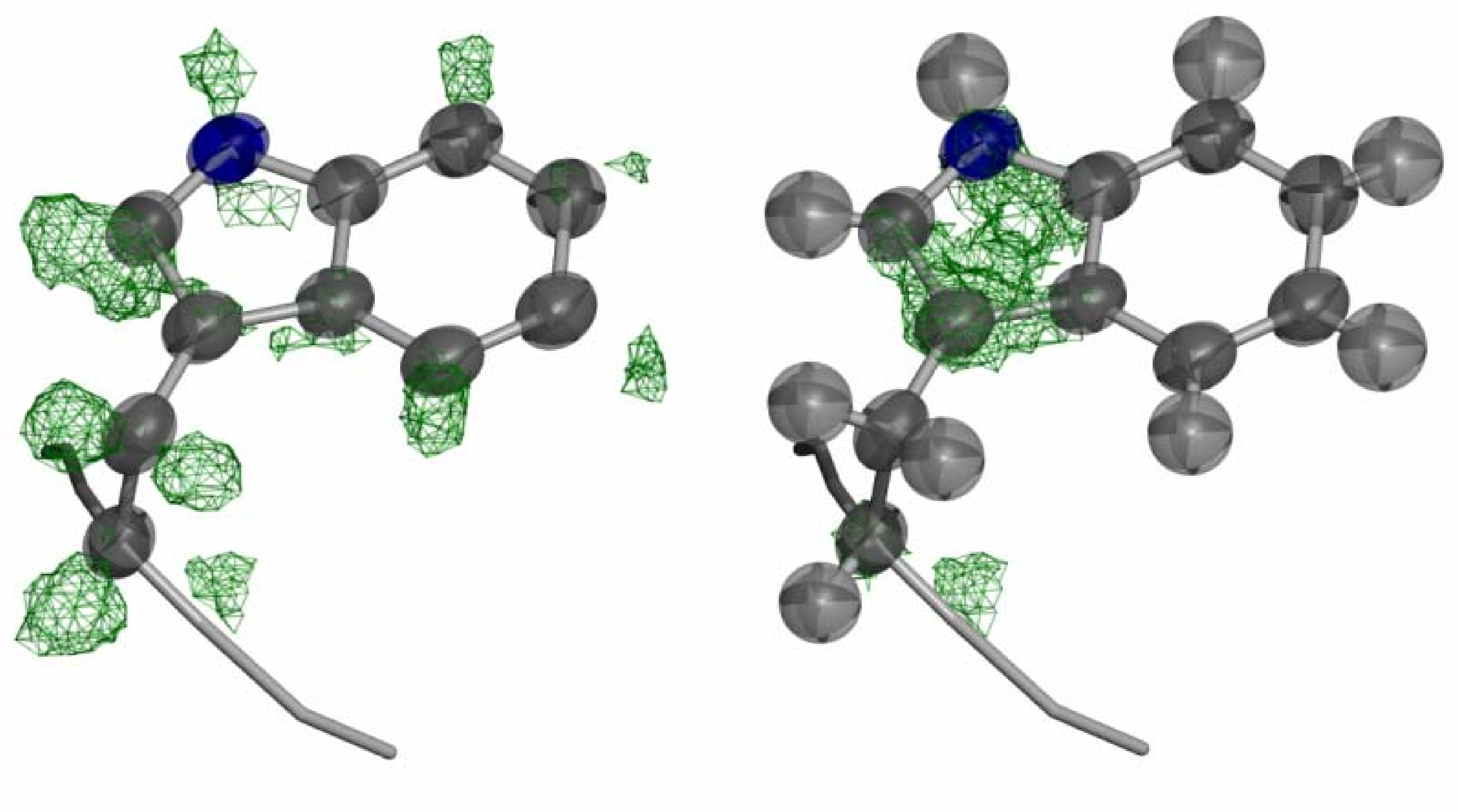


Xenopus (1XBR) structure: red/orange

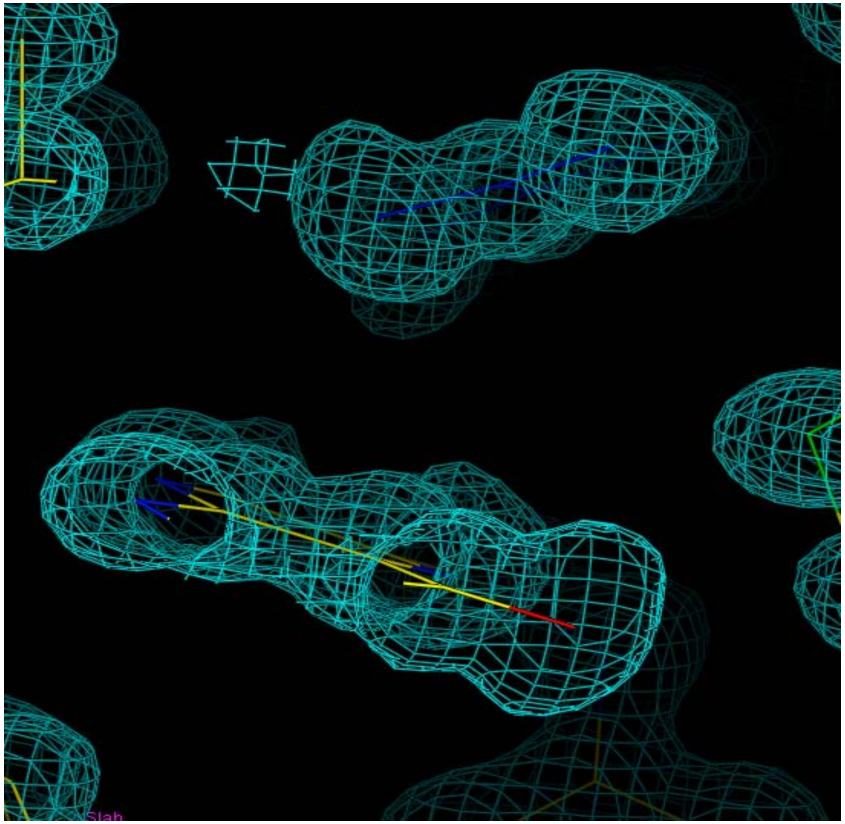
Human (1H6F) structure: light blue/dark blue

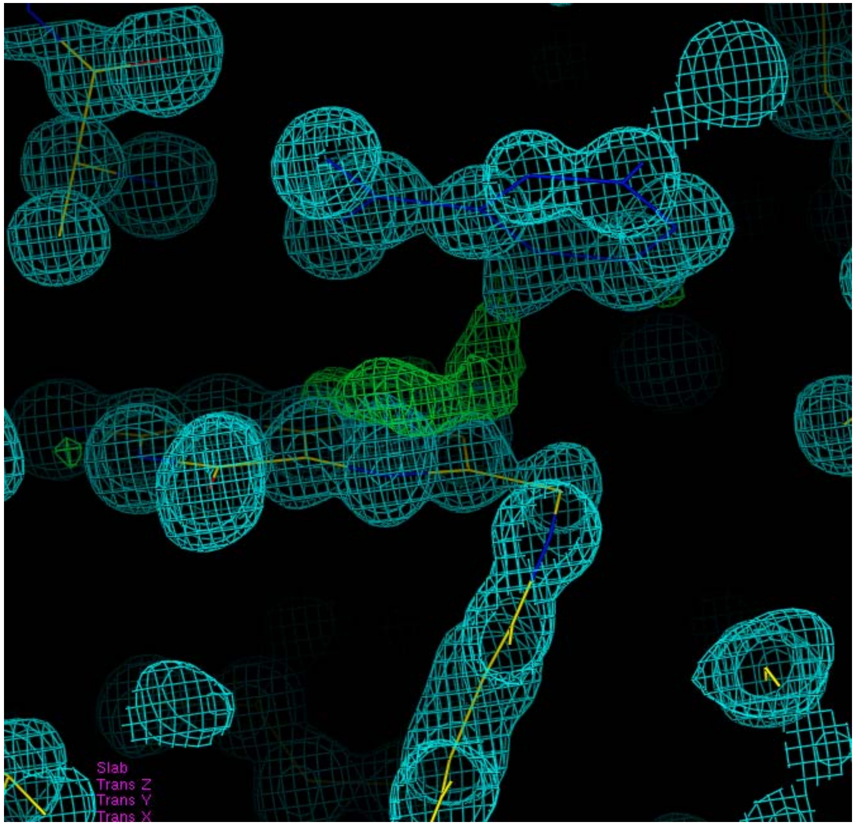
T-bet structure: yellow/gold

Protonation States

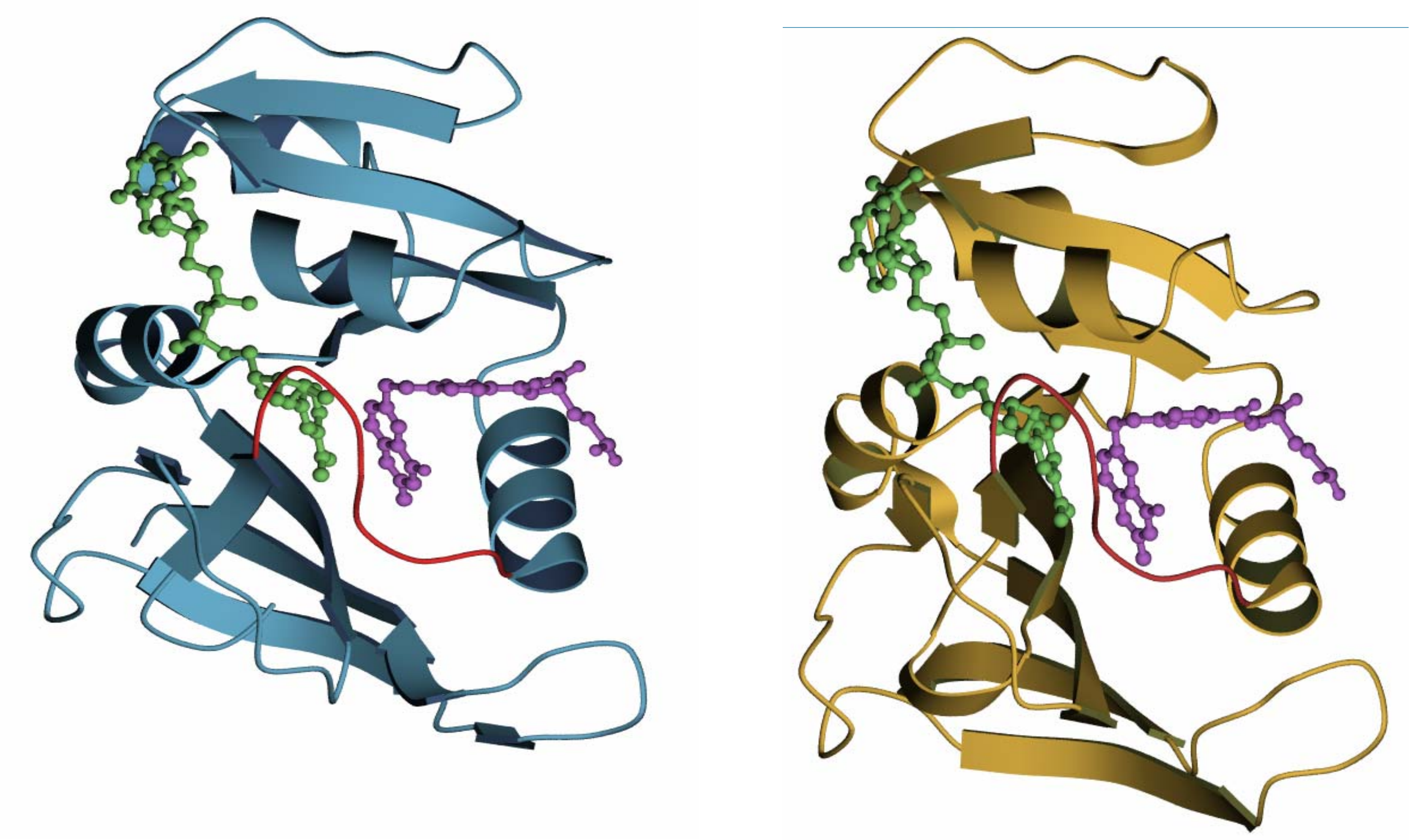


0.95 Å resolution electron density around *E. coli* DHFR catalytic center, including difference density

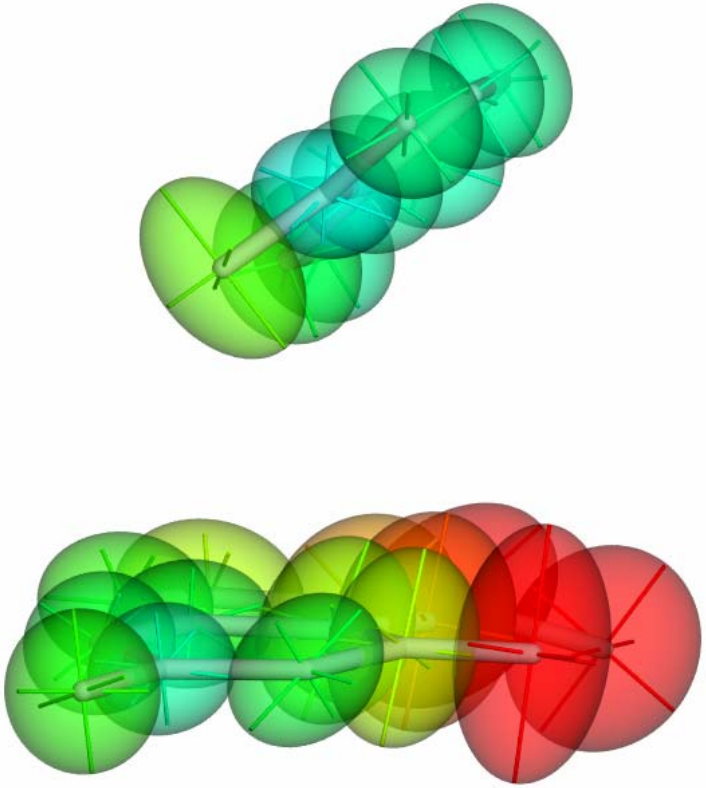


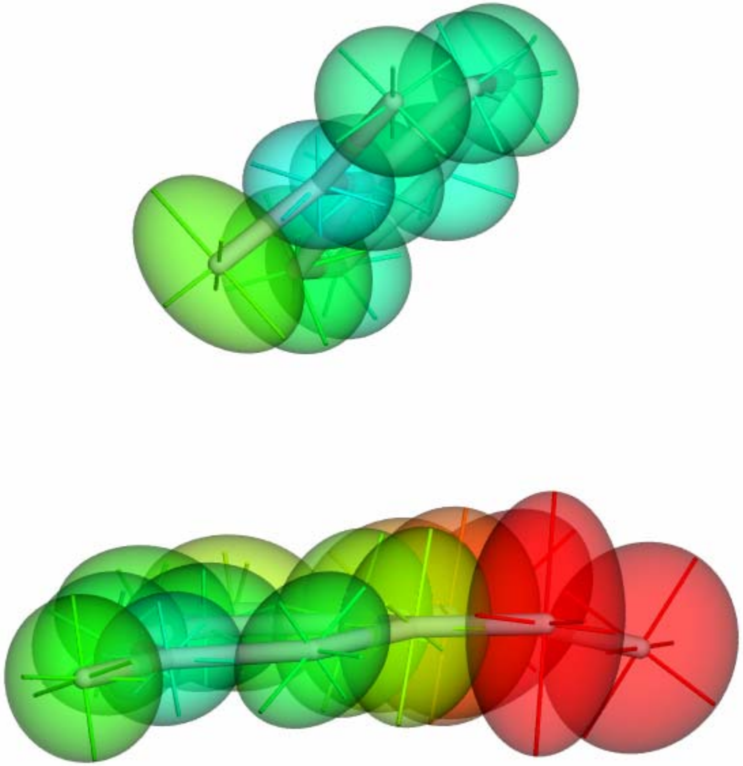


DHFR: *E. coli* vs *B. subtilis*

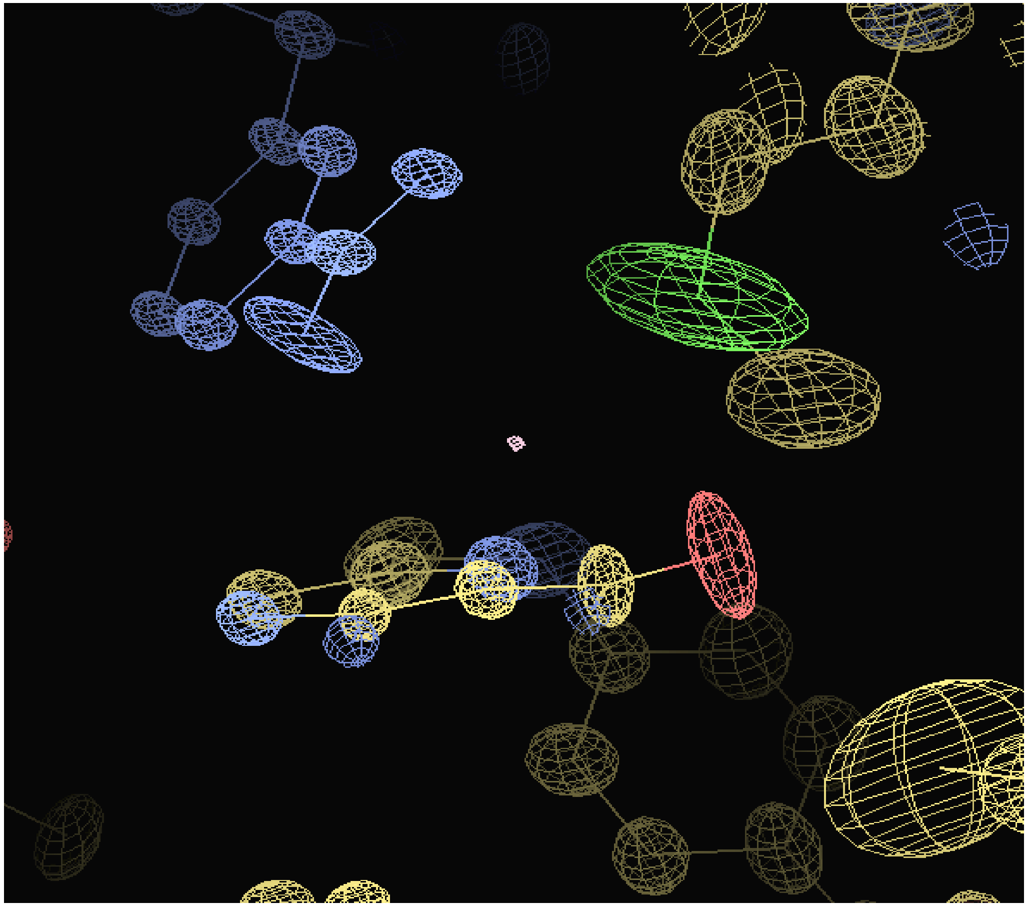


E. coli DHFR **_B. subtilis_**
DHFR





E. coli DHFR 4oC



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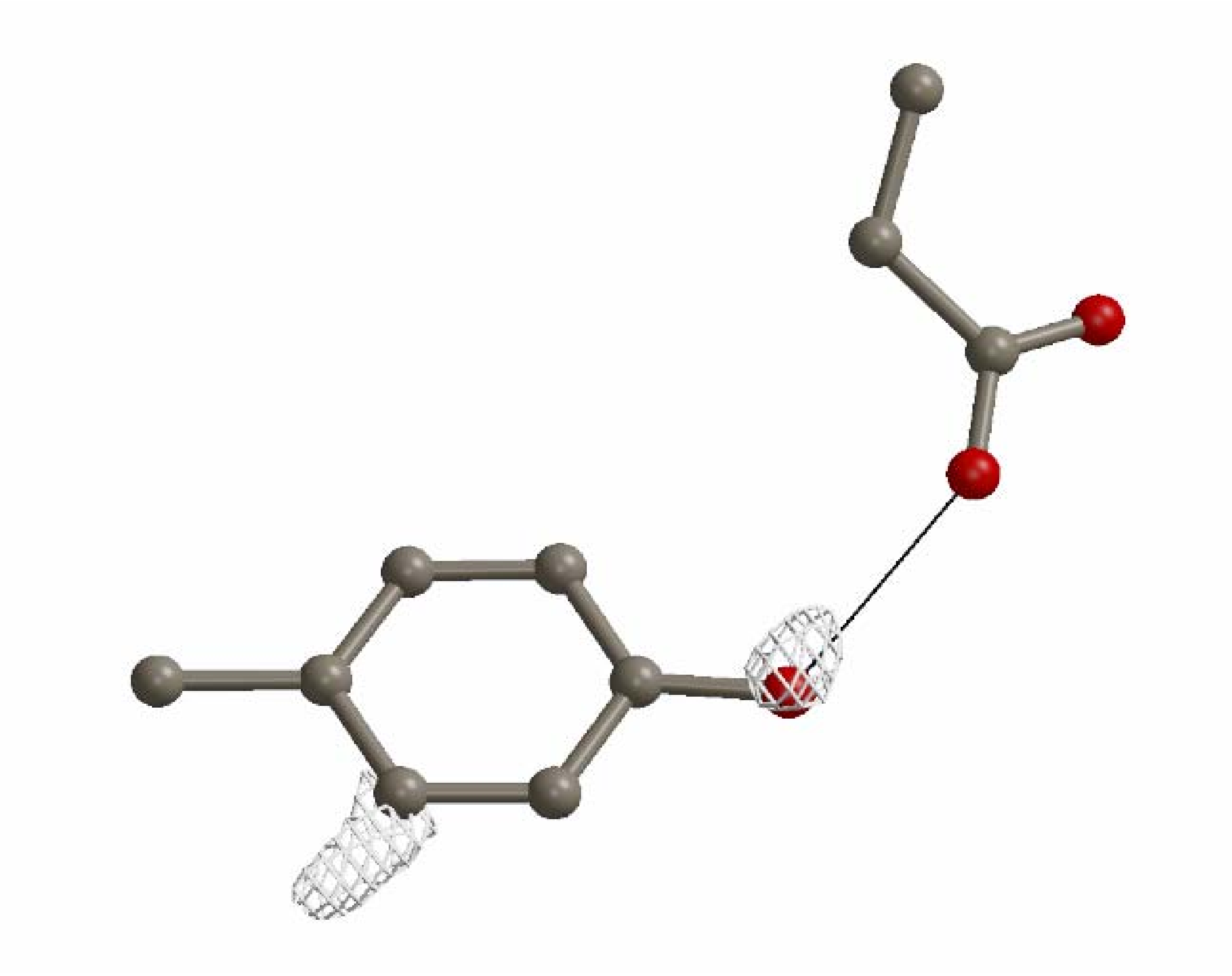
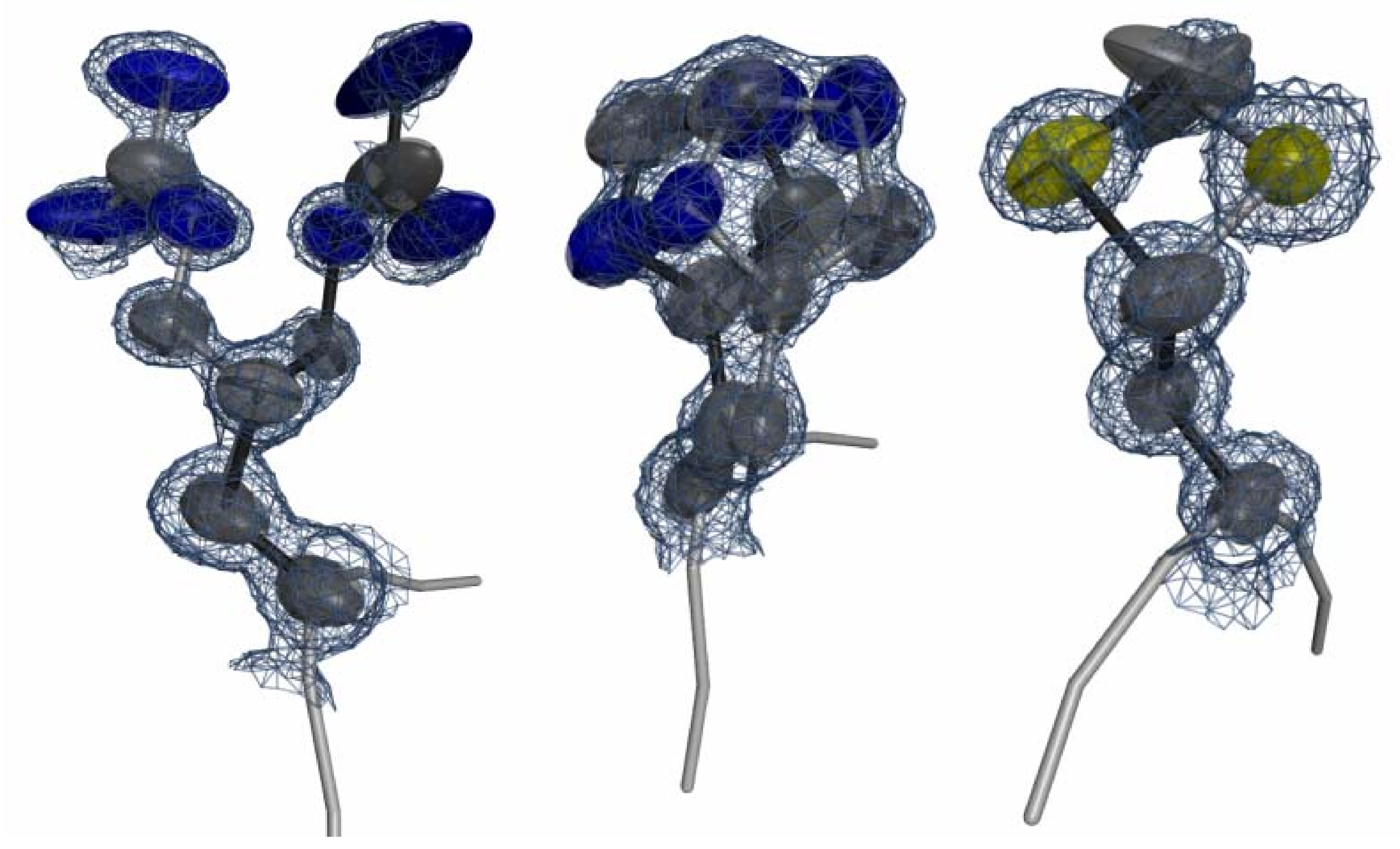
DIFFERENCE ELECTRON DENSITY SHOWS PHENOLIC HYDROGEN



Alternate Conformers



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