

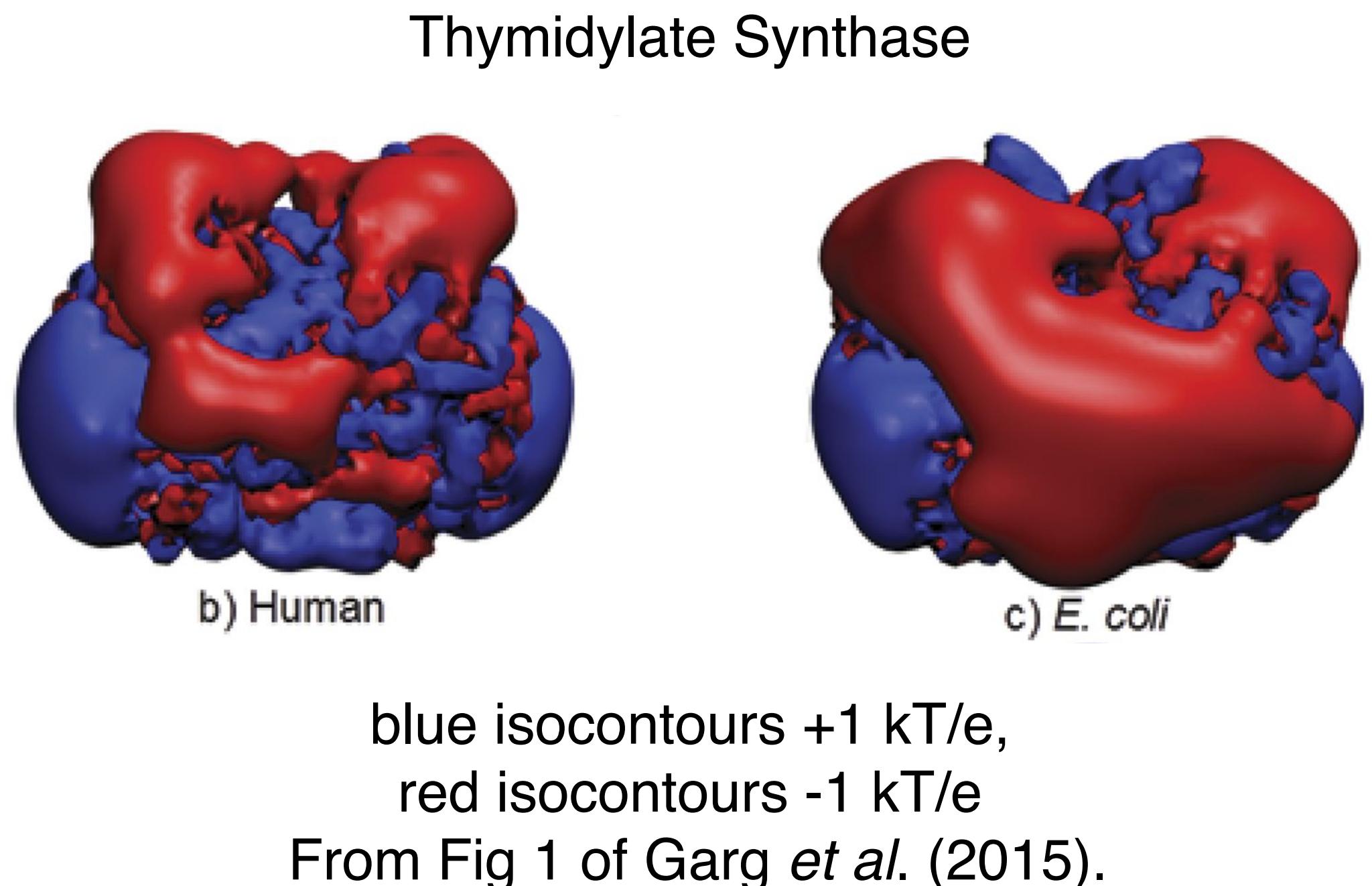
1.2.2 Electrostatics Calculations

- This mini-lecture and tour will be electrostatics calculations
- At the end of this mini-lecture and tour, you should be able to
 - explain, in a general sense, the
 - importance of electrostatics in biological macromolecules
 - origin of the Poisson-Boltzmann equation
 - submit an electrostatics calculation to the APBS server

Electrostatics in Biological Macromolecules

Importance of electrostatics

- Electrostatics important in (at least)
 - binding for
 - steering, facilitating approach of species
 - complexation, as complementarity means lower potential energy
 - enzyme catalysis, as electric potential stabilizes transition state
- Thus, electrostatic potential usually conserved near functional sites



Modeling Electrostatics

- In biological macromolecules, the electrostatic potential is usually calculated based on the Poisson-Boltzmann equation
 - The Poisson equation $\nabla \cdot \epsilon(r) \nabla \phi(r) + 4\pi\rho(r) = 0$ describes the potential $\phi(r)$ due to a given charge density $\rho(r)$ and dielectric $\epsilon(r)$. Atoms in the biomolecule are assumed to have a fixed charge.
 - The Poisson-Boltzmann equation assumes that (infinitely small) ions surround a biomolecule in accordance with the Boltzmann distribution
 - The PB equation is a partial differential equation that is solved numerically
 - The equation is often linearized to be more numerically stable
 - Chun Liu in the Applied Math department at IIT has worked on versions
 - that are time-dependent
 - account for the finite size of ions

The Poisson-Boltzmann equation is also used to model solvation

- It is an *implicit solvent* model that does not account for specific water positions
- Solvent is assumed to modify the dielectric constant

- Coulomb's law is $F = \frac{1}{4\pi\epsilon} \frac{Q_1 Q_2}{r^2}$.

- The permittivity ϵ is related to the permittivity of free space ϵ_0 by the dielectric constant κ , $\epsilon = \kappa\epsilon_0$.
 - ~80 for water
 - ~1 for the protein interior
- The electrostatic component of the solvation energy is modeled as the difference between the energy in water and in vacuum

Review Questions

- Discuss some of the ways in which electrostatics is important for the function of biological macromolecules
- An equation often used to model the electrostatics of biological macromolecules is the Poisson-Boltzmann equation. Why does the equation have this name?
- How can the Poisson-Boltzmann equation be used to calculate the electrostatic component of the solvation free energy?

A tour of some electrostatics calculations

- I will describe how to reproduce key results from [this paper](#):

www.nature.com/scientificreports

SCIENTIFIC REPORTS



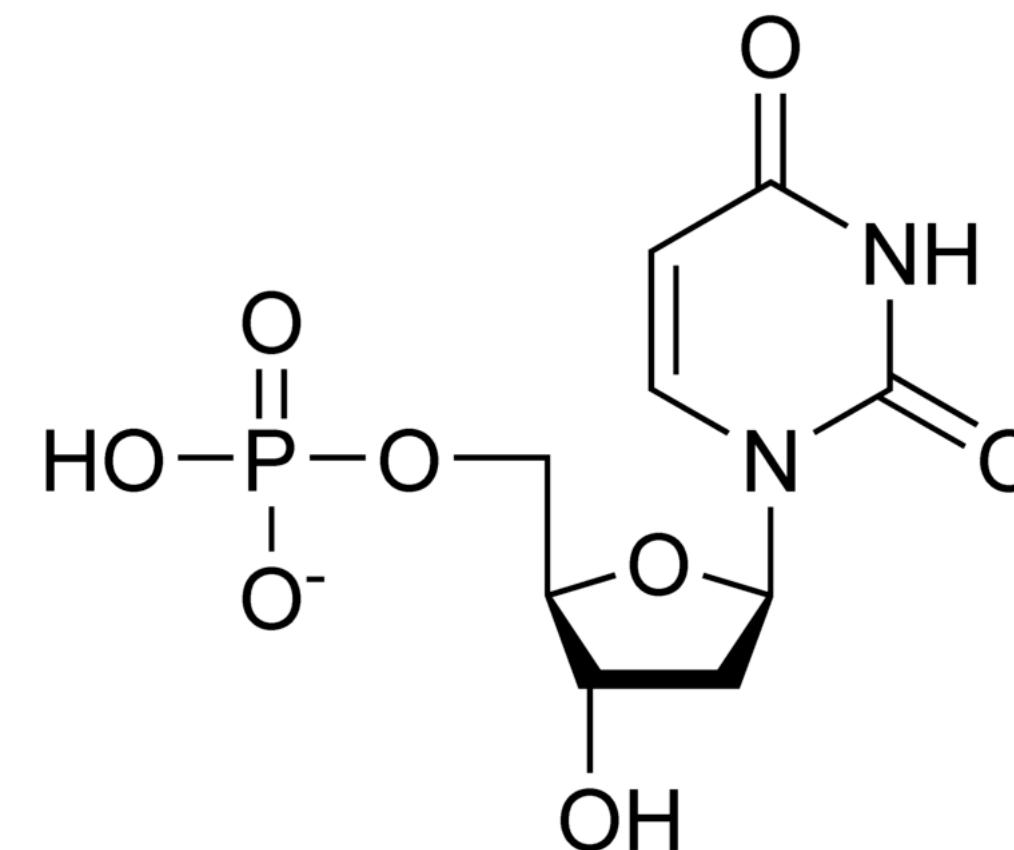
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Conservation and Role of Electrostatics in Thymidylate Synthase

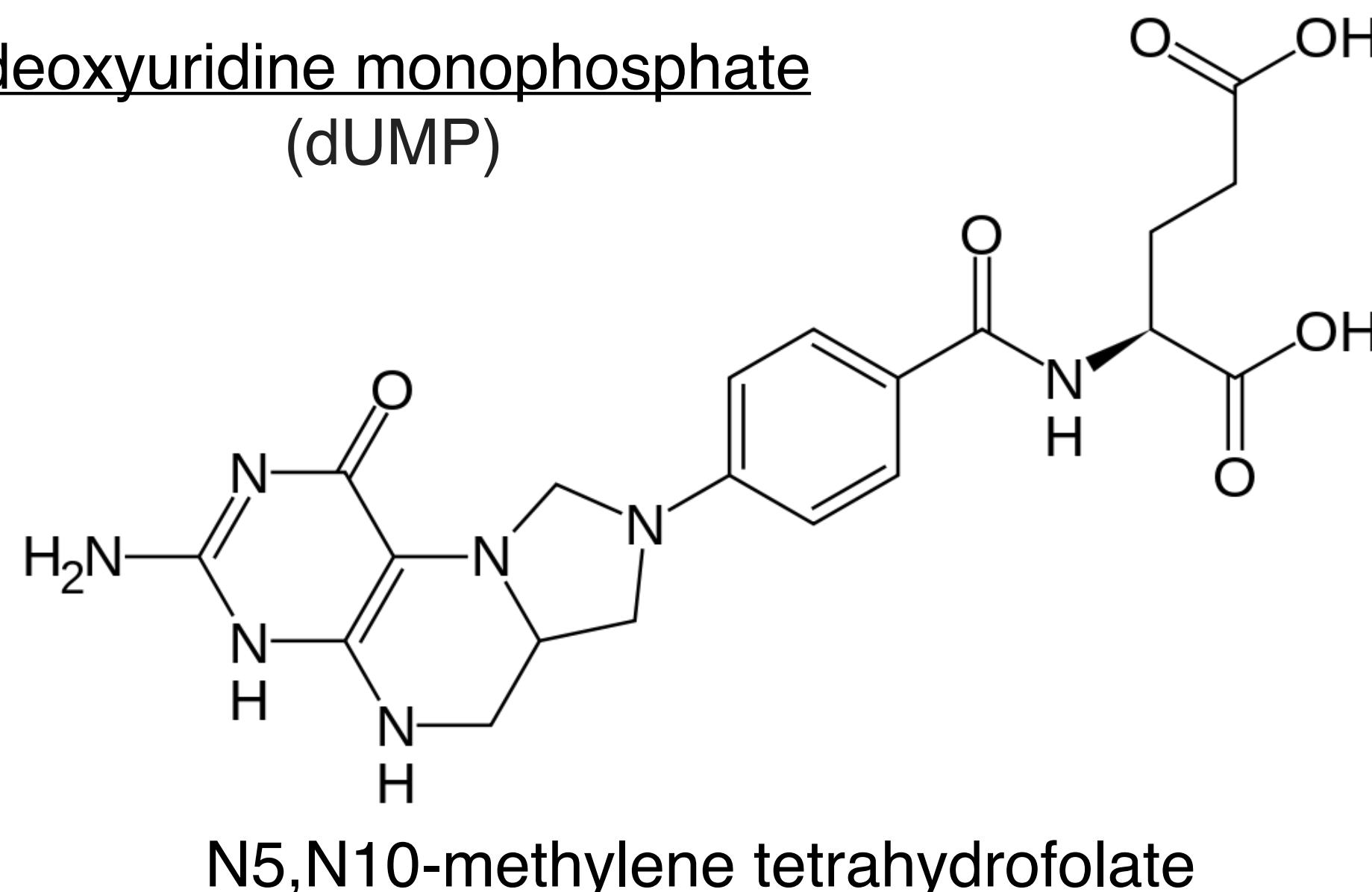
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Published: 27 November 2015

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& Rebecca C. Wade^{1,6,7}

Thymidylate Synthase Catalyzes

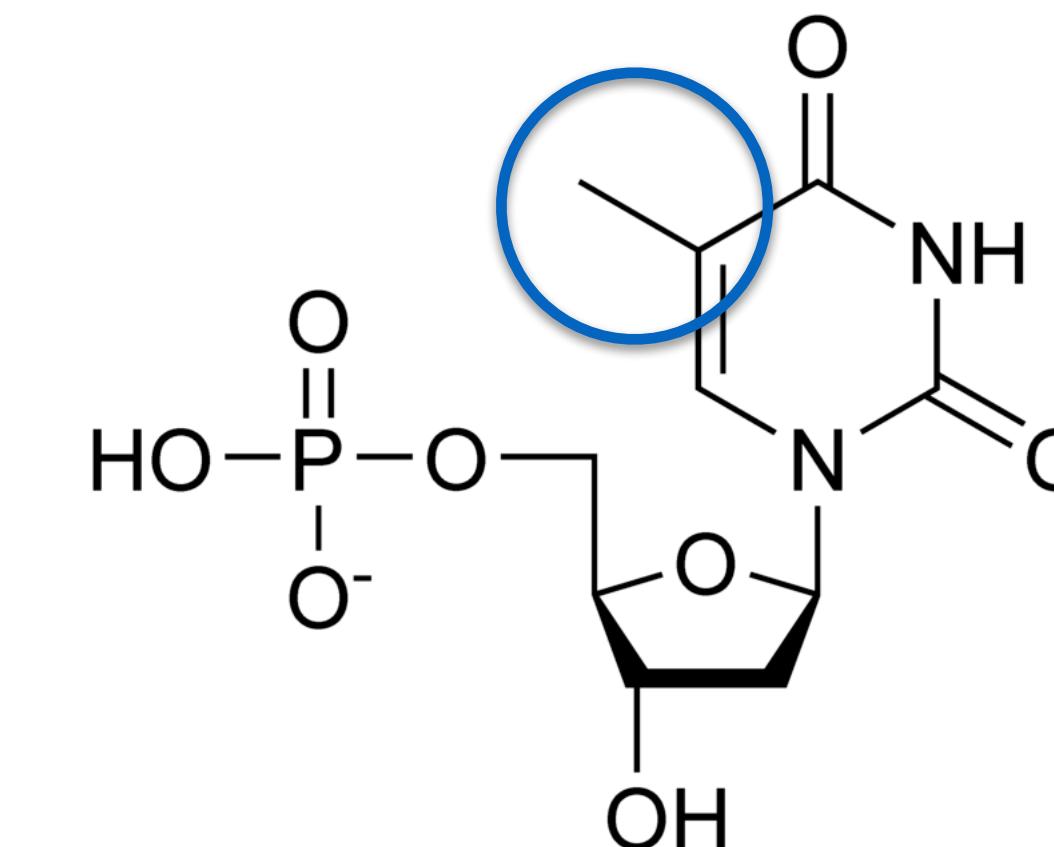


deoxyuridine monophosphate
(dUMP)

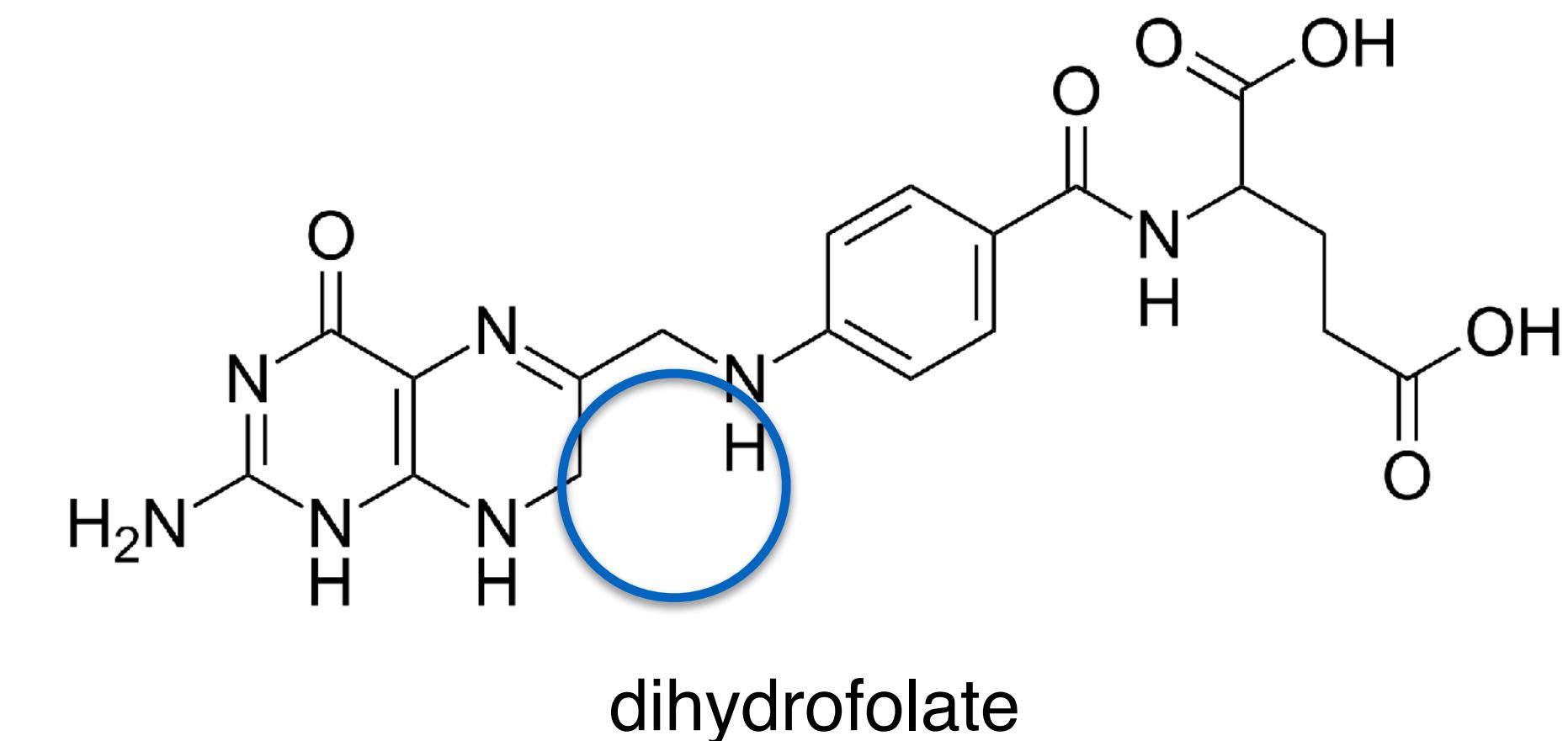


N5,N10-methylene tetrahydrofolate

to



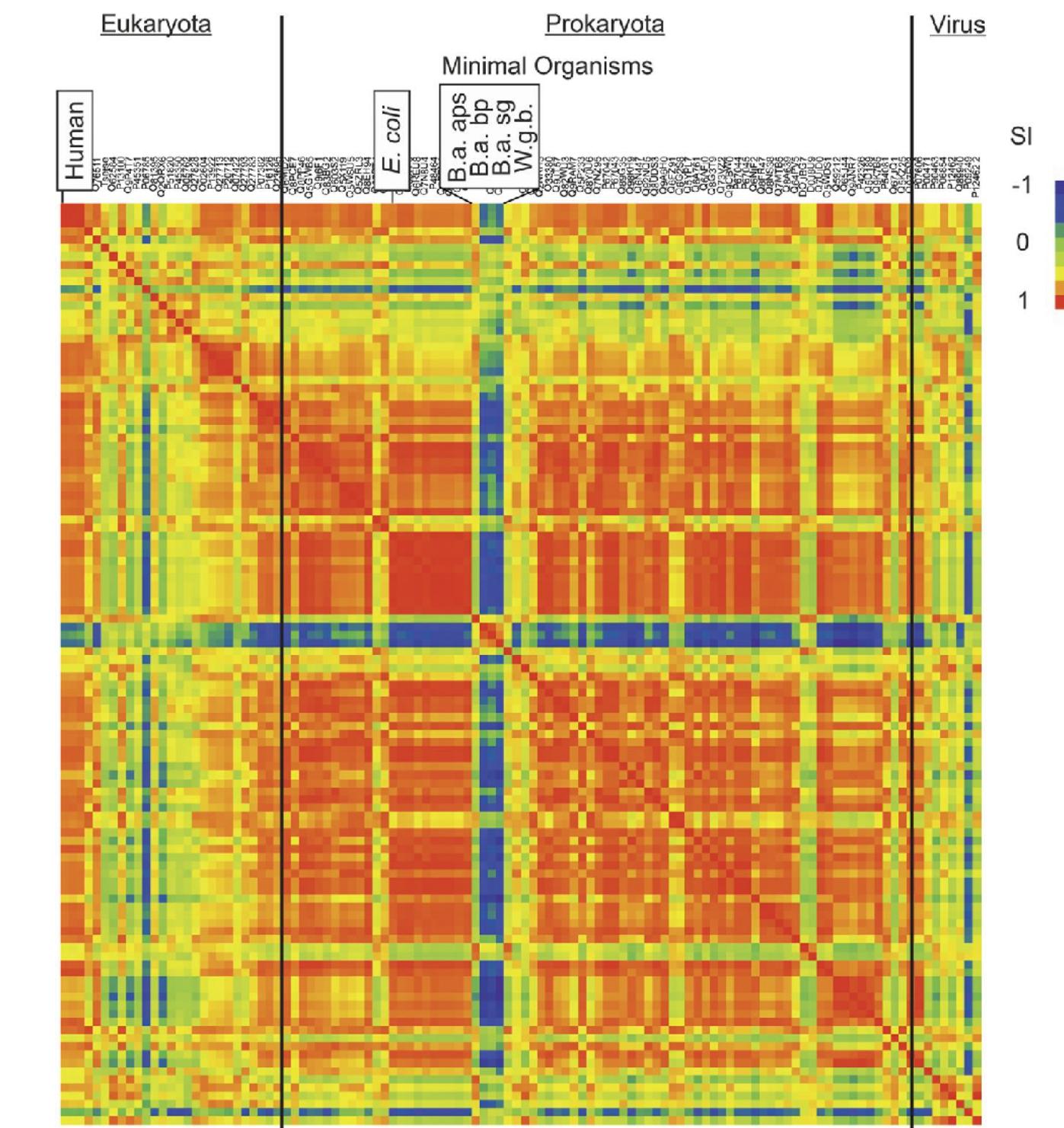
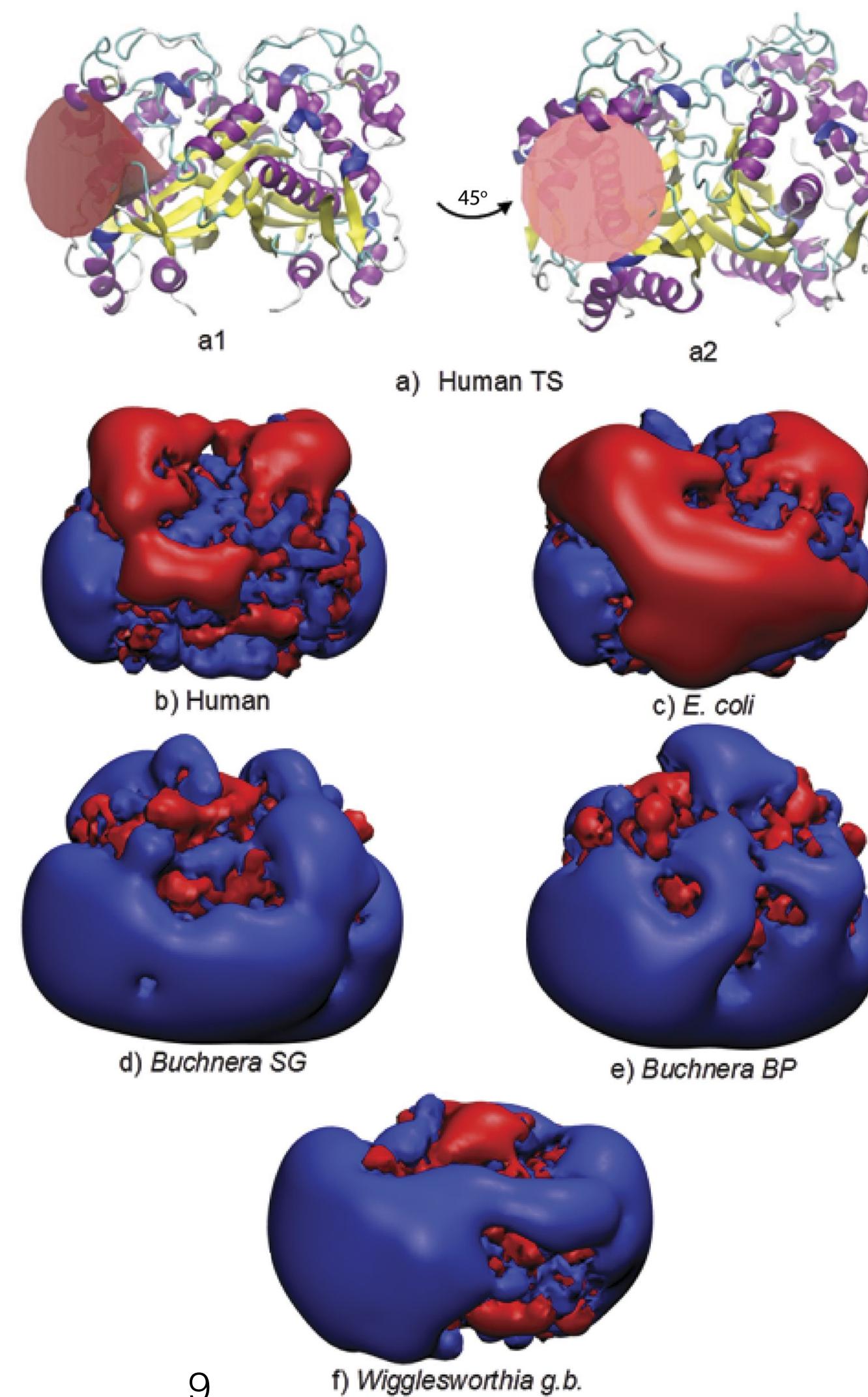
deoxythymidine monophosphate
(dTDP)



dihydrofolate

Summary of “Conservation and Role of Electrostatics in Thymidylate Synthase”

- Built 110 homology models
- Calculated and compared electrostatic potential of the enzyme across species
- Found minimal organisms, including *Wigglesworthia glossinidias brevipalpis* (W.g.b.), to have divergent potential
- Rationalized W.g.b. TS to be functional and unsuccessfully tried to express and purify it
- Mutated *E. coli* TS to be more like W.g.b. TS and found the enzyme to be less active

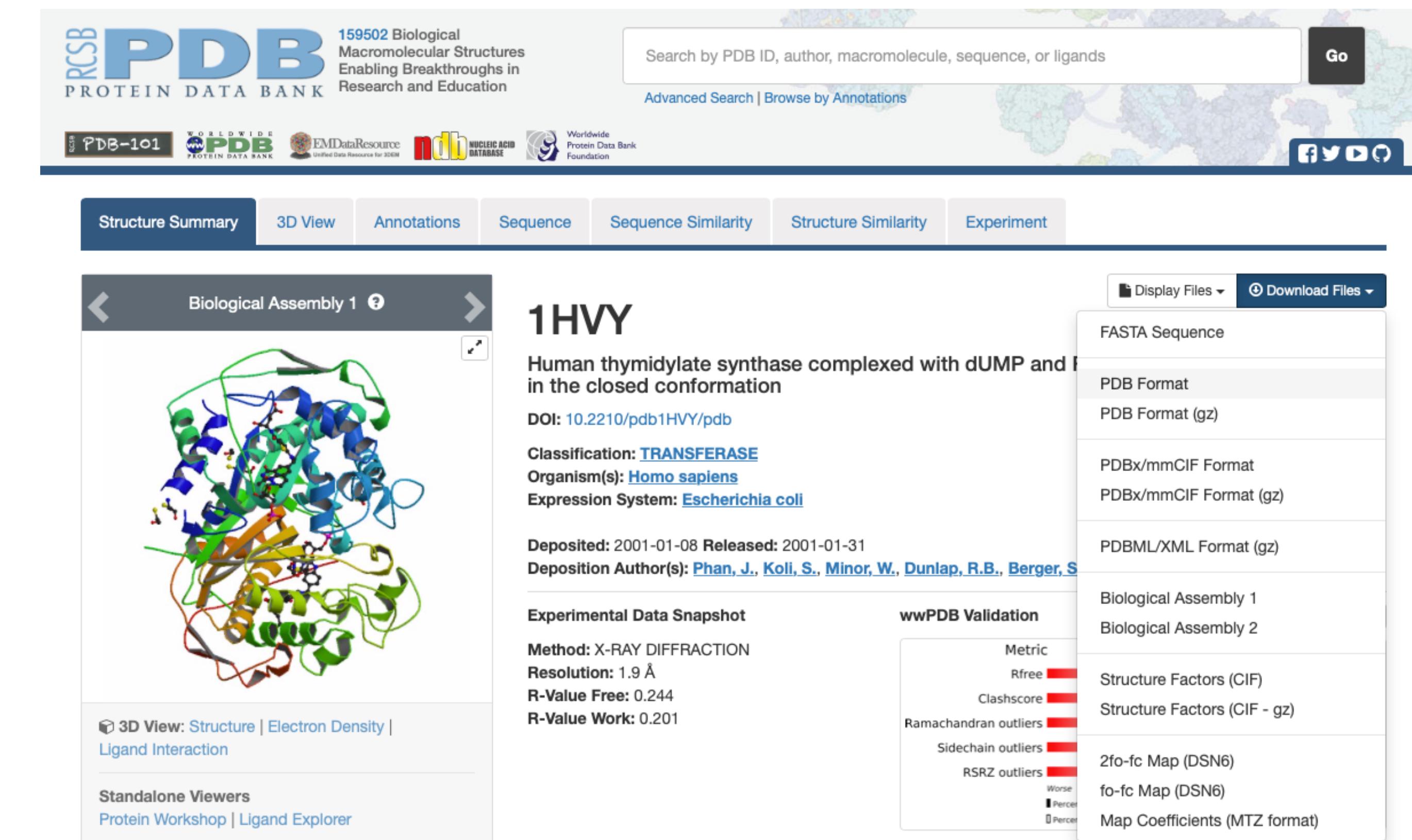


Left: Figure 1 of Garg *et al.* (2015)
Electrostatic potentials and definition
of region for comparative analysis.
Above: Figure 2 of Garg *et al.*
(2015). Heat map of pairwise
similarity index

How I reproduced key results from “Conservation and Role of Electrostatics in Thymidylate Synthase”

- To reproduce the key results, I needed computational models of thymidylate synthase from *homo sapiens*, *E. coli*, and W.g.b.
 - For the former two, there are many structures of TS in the PDB. Garg et. al. used PDB ID 1HVY for *homo sapiens* and 2G8O for *E. coli*
 - For the latter, I used a model from the I-TASSER web server
- Next, I aligned the models with the MultiSeq module in VMD
- I then calculated the electrostatic potential with PDB2PQR and APBS
- Finally, I visualized the results in VMD
- In interest of time, I will not ask you to do every step. Instead, I will guide you through what I did and ask you to download results from previous calculations.

- Getting structures from the PDB (<https://www.rcsb.org/>) is pretty straightforward.
- For a particular crystal structure, you can just click on “Download Files” and select “PDB format”
- We will use the PDB files for 1HVY and 6NNR, which has superceded 2G8O.
- The procedure for homology modeling of W.g.b. was described in the last module.



Structure preparation of thymidylate synthase

- None of the structures were ready for electrostatic potential calculation
- The human structure, 1HVY
 - has 4 chains, but we are only interested in 2
 - I used MultiSeq to write separate files for each chain, merged the files for chains A and B, and then modified “END” after chain A into “TER”
- The E. coli structure, 6NNR
 - is not aligned with the human structure
 - I used MultiSeq to write separate files for each chain, used MultiSeq STAMP structural alignment to superpose E. coli chains A and B on top of the respective human chains, wrote the aligned files, and merged and modified them as with 1HVY
- The W.g.b. homology model
 - is not aligned with the human structure
 - I used MultiSeq STAMP structural alignment to superpose W.g.b. chains A and B on top of the respective human chains, wrote the aligned files, and merged and modified them as with 1HVY

The APBS server

- To reproduce the key results of this paper, I ran electrostatic potential calculations with PDB2PQR and APBS (<http://www.poissonboltzmann.org>) on the PDB2PQR server (http://nbcr-222.ucsd.edu/pdb2pqr_2.1.1/). There were three steps:
 - Uploading the PDB file and selecting parameters. I used the defaults.
 - Running PDB2PQR by pressing “Submit”. This performs some basic structural preparation, e.g. adding missing atoms and optimizing hydrogen positions.
 - Running APBS by following “Click here to run APBS with your results”. This actually solves the PB equation.
- I ran these for TS models from three species. None of these calculations took a very long time.

PDB2PQR Server
Currently using PDB2PQR Version 2.1.1
Return to the [PDB2PQR homepage](#).

This server enables a user to convert PDB files into PQR files. PQR files are PDB files where the occupancy and B-factor columns have been replaced by per-atom charge and radius. pKa calculations are performed by PROPKA.

For more information on PDB2PQR please see the:

- [Home Page](#)
- [Register \(and help support PDB2PQR & APBS\)](#)
- [User Guide](#)
- [Examples](#)
- [Release Notes](#)

If you use the PDB2PQR service in a publication, please cite:

Dolinsky TJ, Nielsen JE, McCammon JA, Baker NA. PDB2PQR: an automated pipeline for the setup, execution, and analysis of Poisson-Boltzmann electrostatics calculations. *Nucleic Acids Research* 32 W665-W667 (2004). [[Link](#)]

Note: In order to distribute server load, the PDB2PQR server currently is limited to a maximum size of 10000 atoms per protein. If you are interested in using PDB2PQR for larger proteins, you are encouraged to download a command line version of PDB2PQR from the [PDB2PQR download page](#). For additional limitations, please see the [PDB2PQR user guide](#).

Note: This server uses automatic refreshing to update the status of your PDB2PQR submission. Do not use the back button on your browser while the server is running.

Please enter either:

a PDB ID:
 upload a PDB file:

Status: complete ✓
Run time: 0:00:34
Current time: Mon Jan 20 13:52:26 2020

Here are the results:

- Input files
 - [TS_human.pdb](#)
- Output files
 - [TS_human.propka](#)
 - [TS_human.pqr](#)
 - [TS_human.in](#)
- Runtime and debugging information
 - [Program output \(stdout\)](#)
 - [Program errors and warnings \(stderr\)](#)

[Click here to run APBS with your results.](#)

Status: complete ✓
Run time: 0:01:36
Current time: Mon Jan 20 13:57:47 2020

Here are the results:

- Input files
 - [15795571126.pqr](#)
 - [apbsinput.in](#)
- Output files
 - [15795571126-pot-PEO.dx.gz](#)
 - [15795571126.cube.gz](#)
- Runtime and debugging information
 - [Program output \(stdout\)](#)
 - [Program errors and warnings \(stderr\)](#)

Visualize your results online:

- [3Dmol](#)
- [Jmol](#)

References

- Garg, D.; Skouloubris, S.; Briffotaux, J.; Myllykallio, H.; Wade, R. C. Conservation and Role of Electrostatics in Thymidylate Synthase. *Sci Rep* 2015, 5 (1), 17356. <https://doi.org/10.1038/srep17356>, adapted under the CC BY 4.0 license.