GEORGETOWN UNIVERSITY
CHEMISTRY
ANALYTICAL ★ INORGANIC ★ PHYSICAL ★ ORGANIC ★ BIOPHYSICAL
THEORETICAL ★ BIOCHEMISTRY ★ MATERIALS

Biophysics of Life in Extreme Environments

Toshiko Ichiye

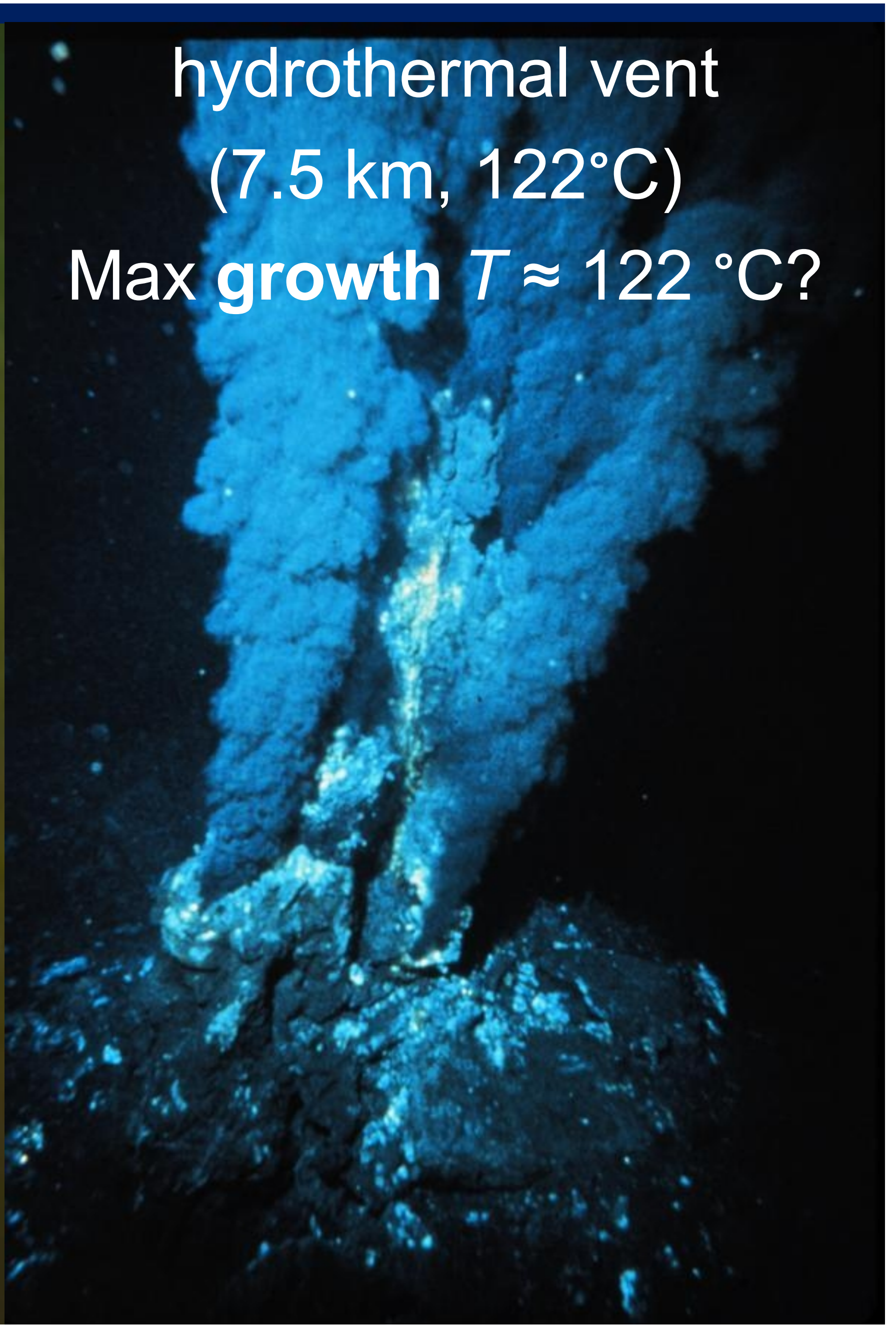
MASPG
October 16, 2019

Life Found at Amazing Extremes
“Extremophiles”

Mariana Trench (11 km, 2°C)

Max growth $P \approx 1.1$ kbar?

atm ≈ bar = 0.1 MPa; 10 m/bar depth/pressure

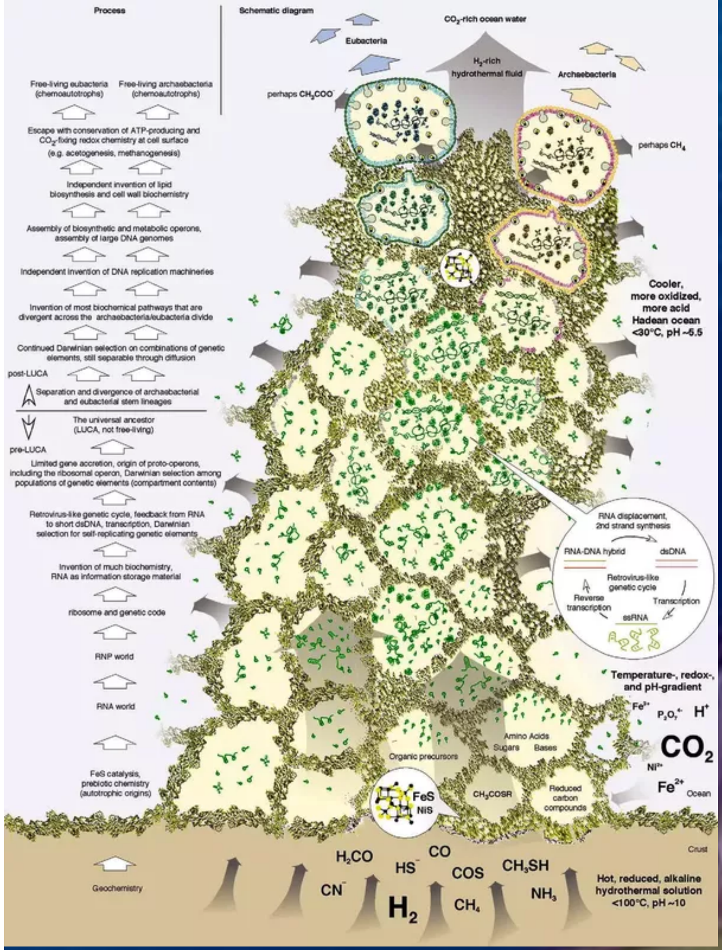


hydrothermal vent

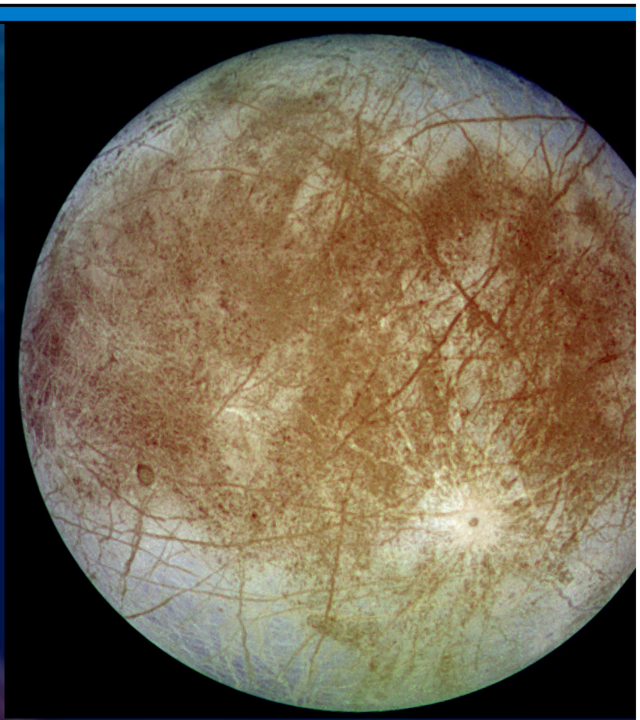
(7.5 km, 122°C)

Max **growth** $T \approx 122$ °C?

Why Study Life Under Extremes?



Origin of life?



Extraterrestrial life?

Climate change?

Why Study "Death" Under Extremes?

Pasteurization (<100 °C)

Max **survival** $T < 100$ °C?

Pascalization (6-8 kbar)

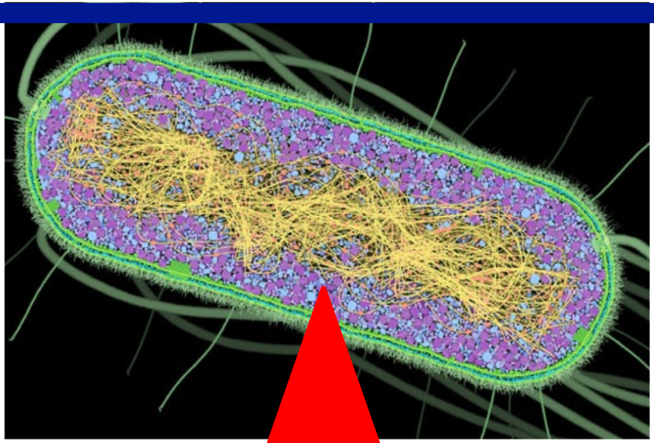
Max **survival** $P < 8$ kbar?

For Organisms to Live at Extremes, Their Macromolecules Must Work at Extreme

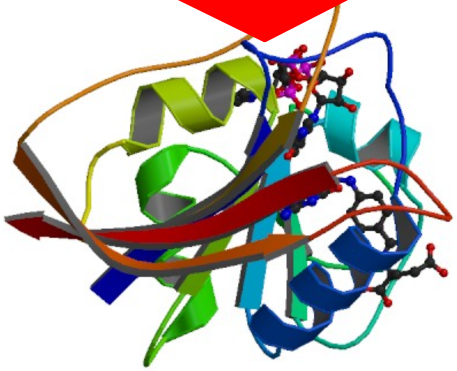
Biologists

to

Chemists &
Physicists



Escherichia coli (Goodsell)



Dihydrofolate reductase (PDB)

Computational
methods can
provide link

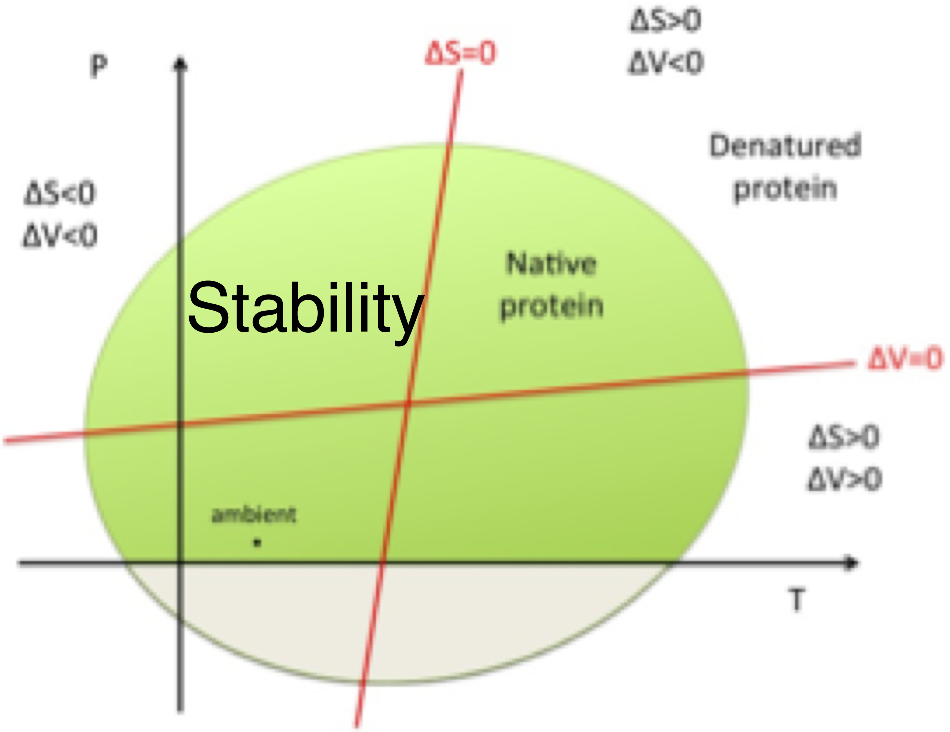
What are maximum *PTX* that proteins can function at?

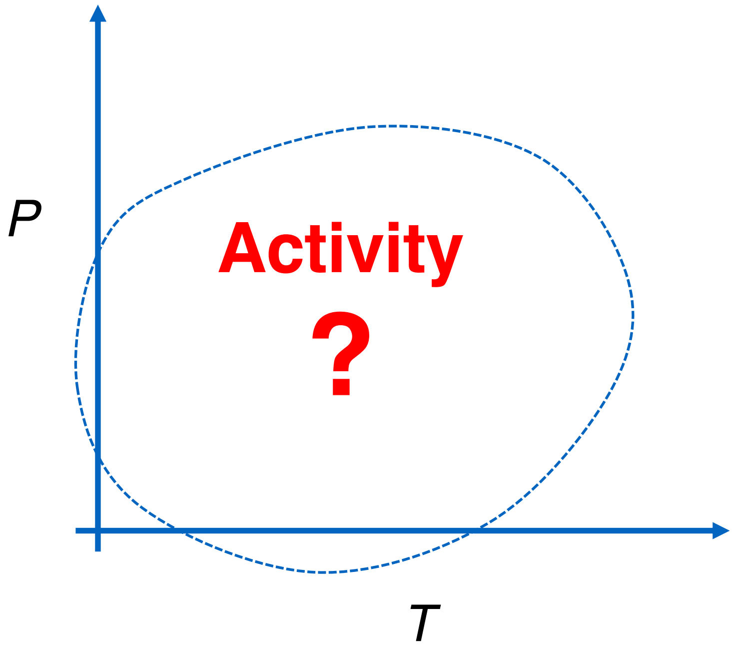
Focus on material properties

(Huang, Tran, Rodgers, Bartlett, & Ichiye, Condensed Matter Physics 2016)

What Makes Enzymes Work:
Activity: Stability + Flexibility

Ichiye, Phys. Biol. (2016) 13, 063001; Sem. Cell Dev. Biol. (2018) 84, 138



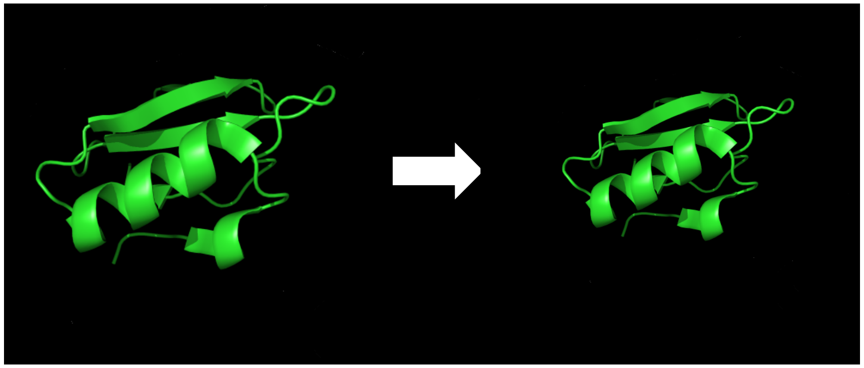


- Activity needs stability *and flexibility*
- Both are functions of *P* & *T*
- Our focus on *material* properties of protein

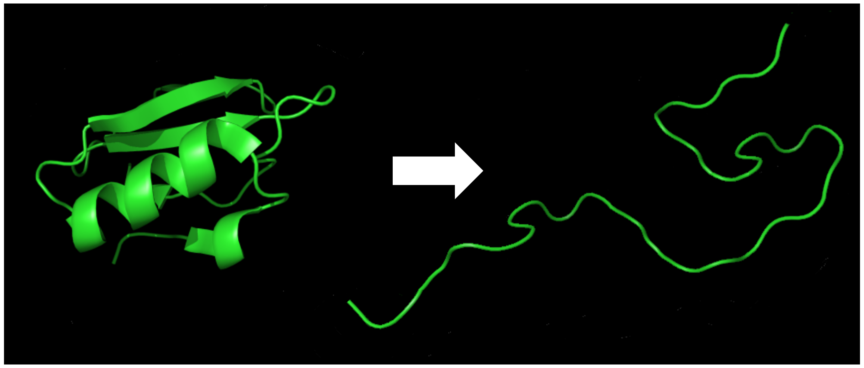
Pressure Effects on Proteins

Gross & Jaenicke, Eur. J. Biochem,. 1994, 221, 617

- Compresses proteins
- ➢ (Decreases flexibility???)



- Unfolds proteins (> 2 kbar)
- ➢ (Really increases flexibility???)



- Simplistically, seem like opposing effects?

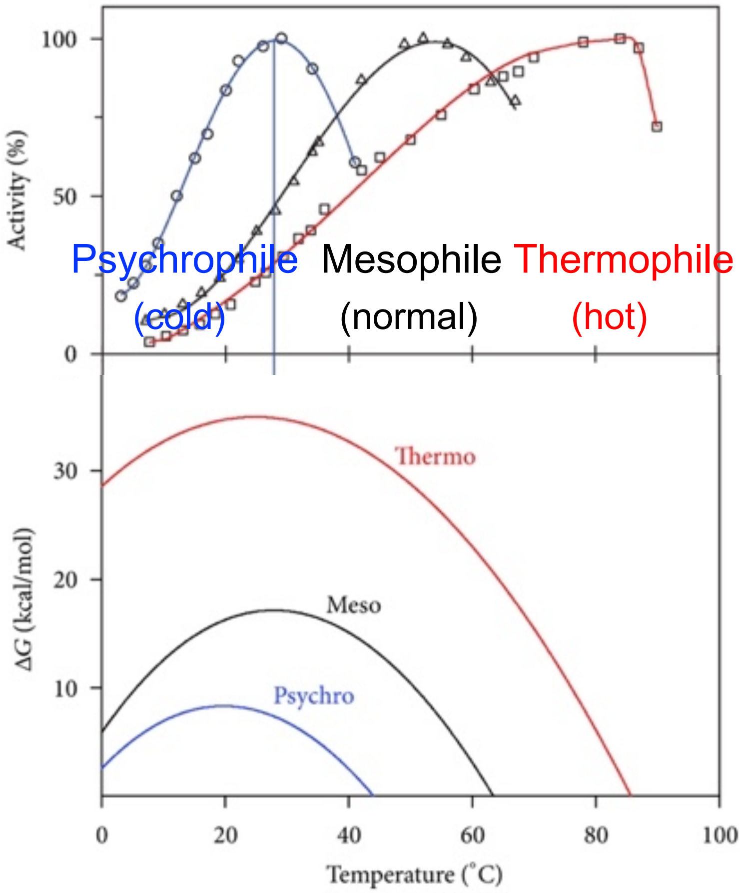
Question I:
How do Enzymes from Extremophiles Adapt to *P*-*T*?

Growth *Temperature* T_G

Jaenicke, EJB (1991) 202,715;
Somero, Ann Rev Physiol (1995) 75,43)

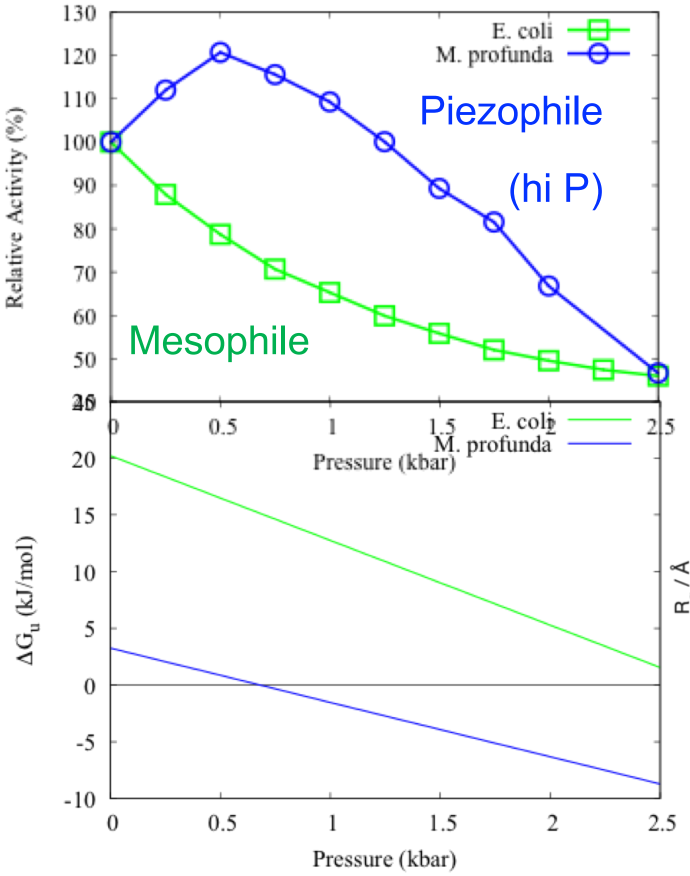
Growth *Pressure* P_G

Huang et al., Cond. Mat. Phys (2016)
Ichiye Phys. Biol. (2016) 13, 063001



Psychrophile (cold) Mesophile (normal) Thermophile (hot)

β-amylase, Feller, Scientifica (2013) 512840

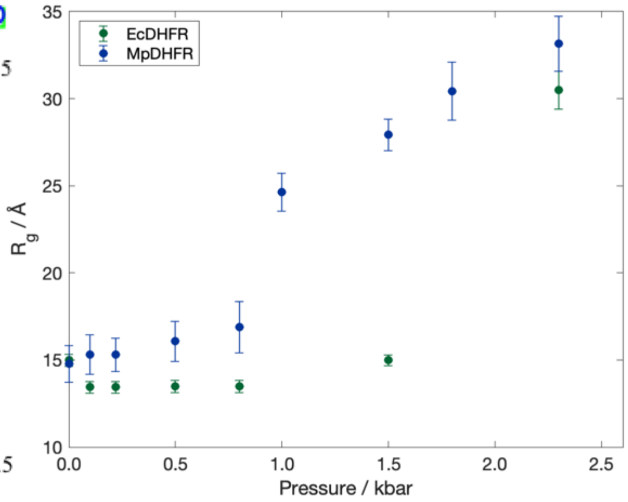


Piezophile (hi P)

Mesophile

DHFR, Ohmae et al., BBA (2012) 1824, 511

SANS confirms low *P* unfolding of MpDHFR; Penhallurick, Marujo-Teixeira & T. Ichiye, unpublished



Similar Activity (Stability & Flexibility) at "Corresponding States" T_G P_G

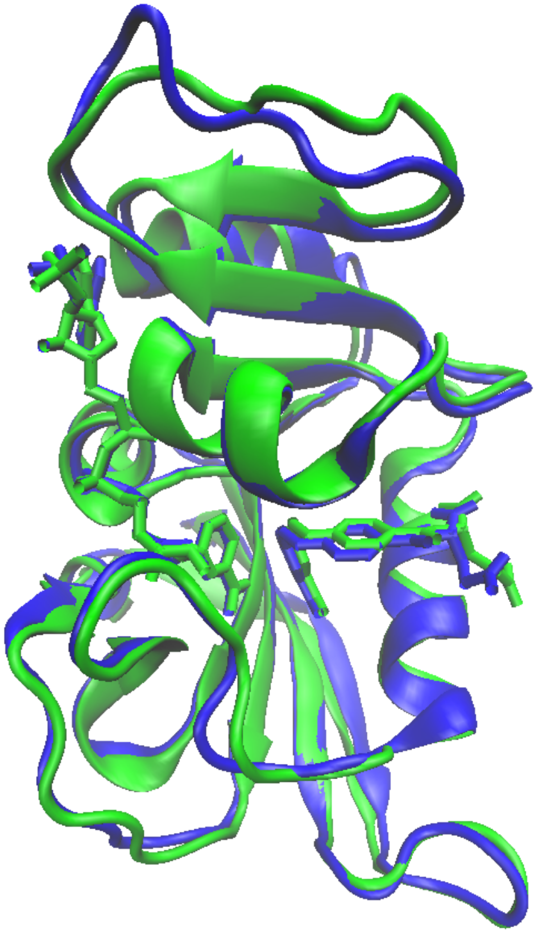
Molecular Dynamics Computer Simulations of Enzymes from Extremophiles at Extremes of *P-T*

Dihydrofolate Reductase (DHFR)

E. coli (Ec) (PDB: 1RX2) Mesophile: GTP=310 K,1 bar

Moritella profunda (Mp) (PDB: 2ZZA) Psychropiezophile: GTP=279 K, 220 bar

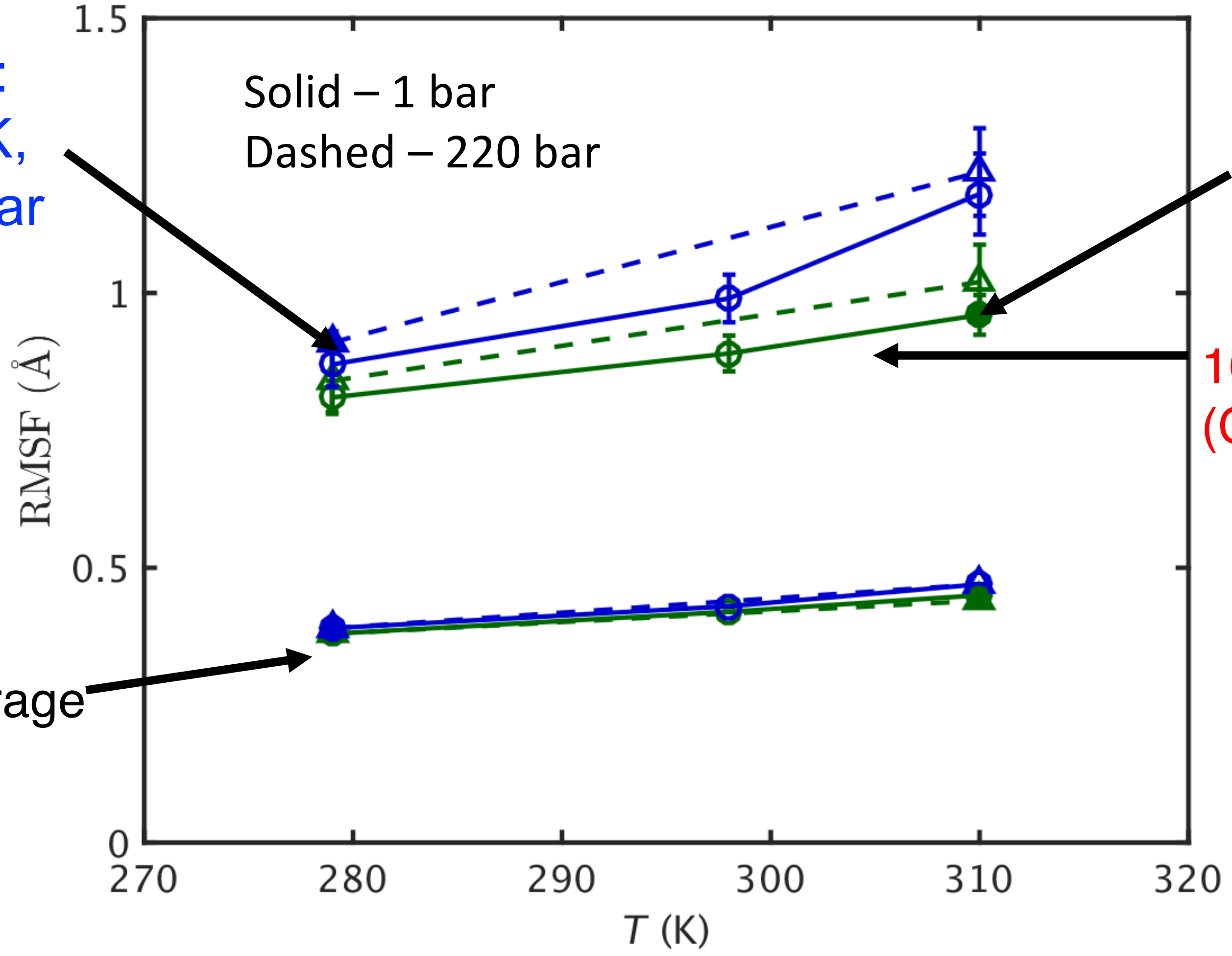
GTP = growth temperature and pressure



No obvious differences in xtal structure

Average RMS Atomic Fluctuations: A Measure of Flexibility

Huang, Rodgers, & Ichiye, JCC (2017) 48, 1174



MpDHFR:
T_G = 279 K,
P_G = 220 bar

EcDHFR:
T_G = 310 K
P_G = 1 bar

10 ns average
(Collective)

10 ps average
(Atomic)

Collective motions show differences:

- larger for piezophile at given *T* (more flexible)
- larger *at higher P* (come back to this!)

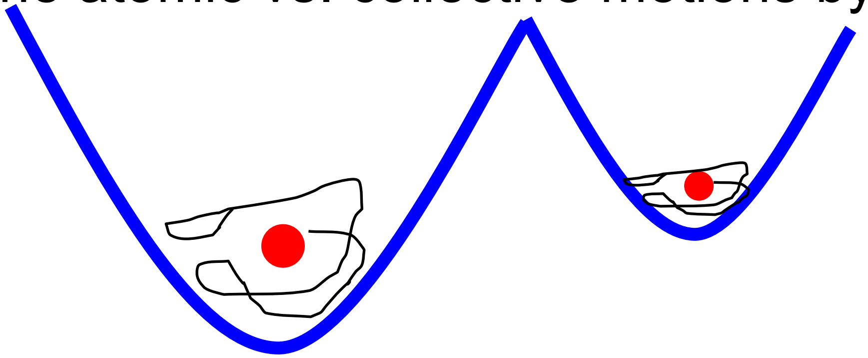
➢ same at GTP of each: corresponding state flexibility?

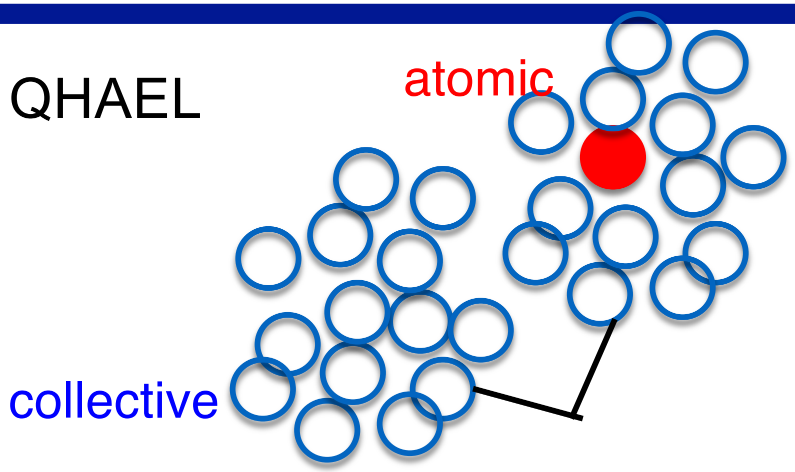
How Do You Measure Material Properties of a Protein?

Quasi-harmonic Approximation of Energy Landscape (QHAEL)

Rodgers, Hemley, & Ichiye, JCP (2017) 147, 125103

- Determine atomic vs. collective motions by QHAEL





- Quasi-harmonic frequencies vary only with *V*, so as material expands or contracts with *P* or *T*, frequencies change due to change in available space.

- Our analysis shows force constant *k* $T >> T_g$ varies with *P* & *T* as

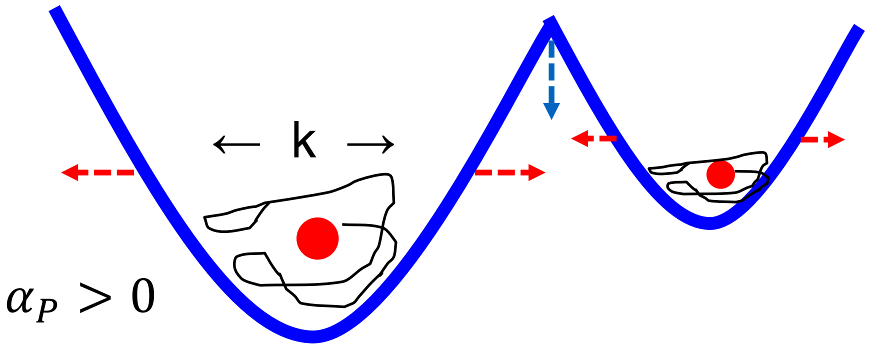
$$k(P,T) = k_0 \exp\left(-\frac{2\alpha_P \Delta T}{3}\right)\left(1 + \kappa_{T,0}\Delta P\right)^{2/3}$$

thermal expansivity α_P is constant; compressibility $\kappa_T = \kappa_{T,0}\left(\frac{V}{V_0}\right)$

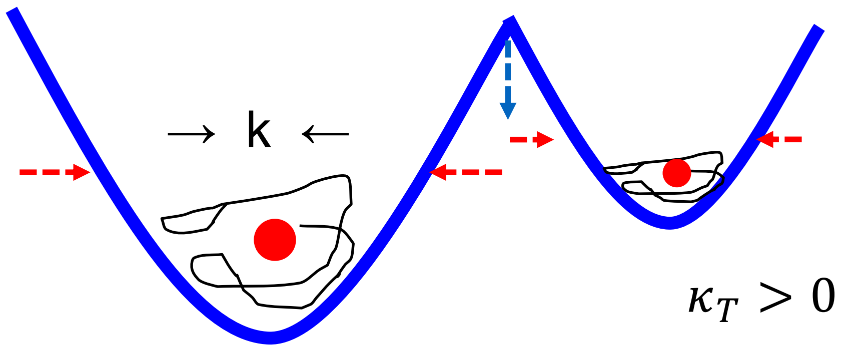
- Above T_g, $\alpha_P > 0$ atomic + collective. Below T_g, $\alpha_P = 0$ only atomic

QHAEL Analysis of *P*-*T* Effects on DHFR

(Huang, Rodgers, Hemley, & Ichiye, JCPB 2018, 21, 5527)



- *T increases width of local potential well (atomic motions)*
- *T increases transitions above T_g (collective motions)*



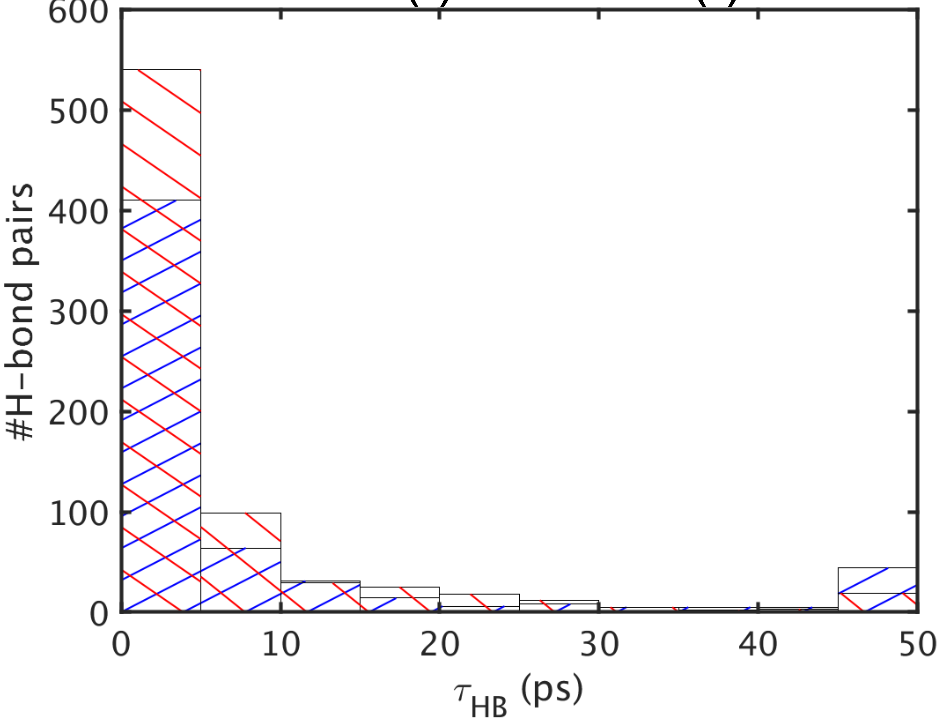
- *P decreases width of* local potential well (atomic motions)
- *P increases transitions above* T_g (collective motions)
- *Consistent with previous results on nsec+plus RMSF*

What leads to lower transitions at high *P*?

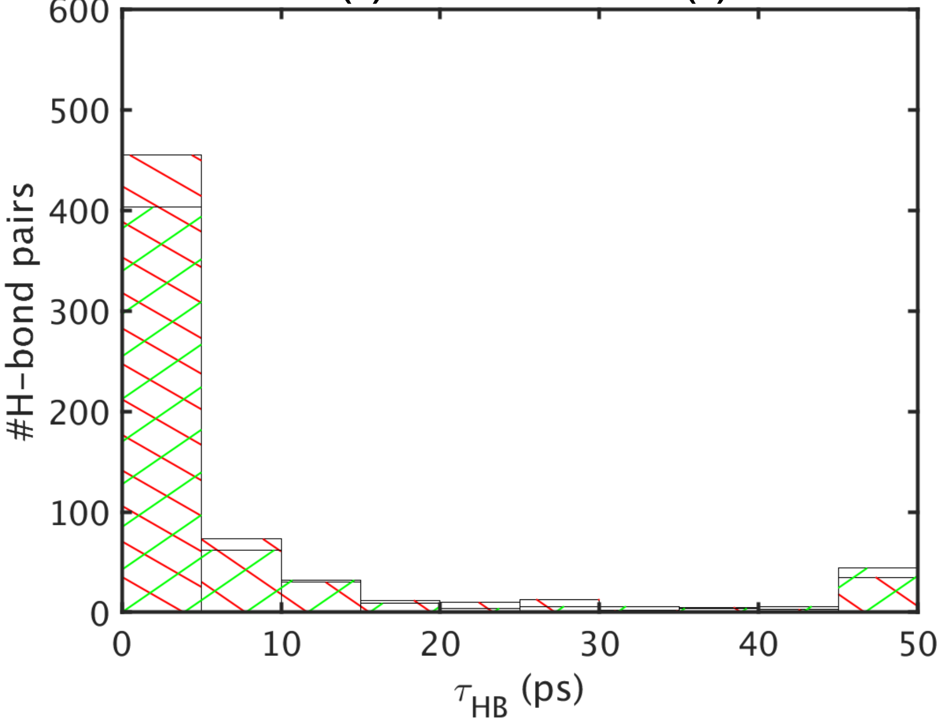
Physical Origin of *T*-*P* Effects on Collective Motions

(Huang, Rodgers, Hemley, & Ichiye, Internatl J Molec Sci (2019) 20, 1452)

EcDHFR at 1 atm:
279 K (/) & 310 K (\)



MpDHFR at 279 K:
1 (/) & 220 atm (\)

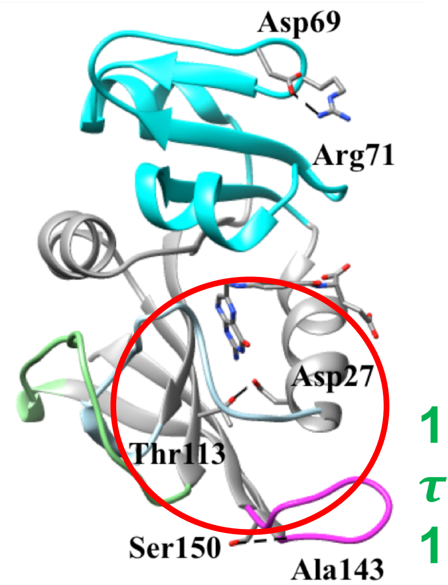


- Collective motions increase at higher *T* by surmounting barriers to break H-bonds
- Collective motions increase at higher *P* because most H-bonds weakened, which lowers barriers

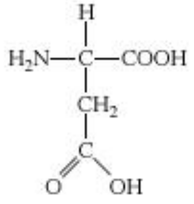
Hydrogen Bonds!

Pressure Adaptation ?

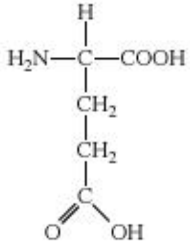
(Huang, Rodgers, Hemley, & Ichiye, High Pressure Research (2019) 39, 225)



EcDHFR

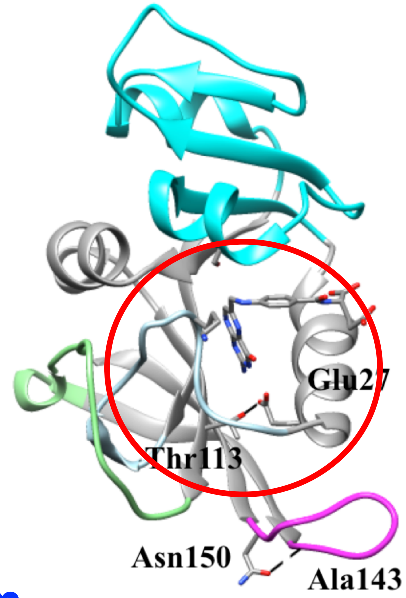


aspartic acid
(Asp, D; Asx or B)



glutamic acid
(Glu, E; Glx or Z)

MpDHFR

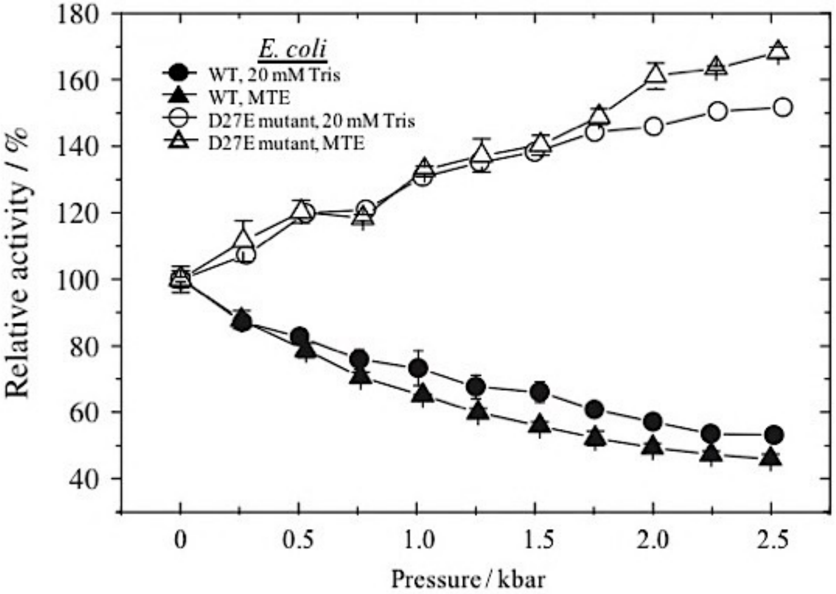


113-27 H-bond
τ_{HB} = ~25 ns,
100% occupied
at 279 K, 220 atm

113-27 H-bond
τ_{HB} = 48 ps,
77% occupied
at 279 K, 220 atm

Exp: D27E EcDHFR is piezotolerant

Ohmae et al., *Biochim Biophys Acta* **2013,** *1834*, 2782

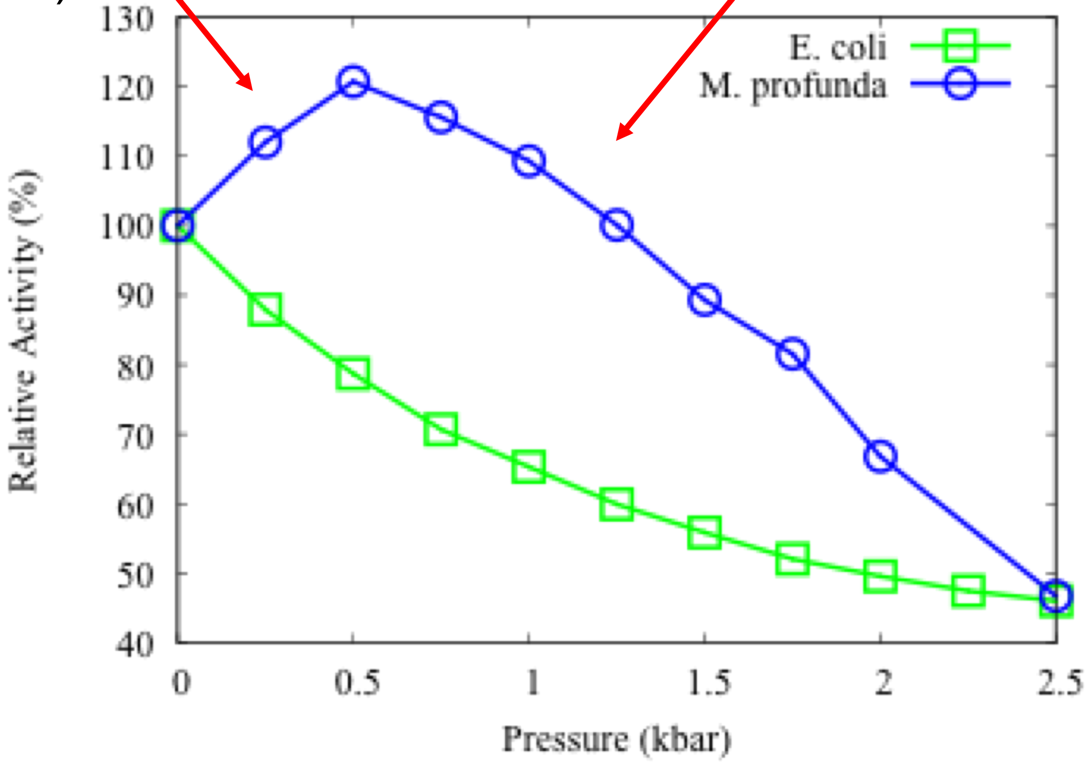


The biology:
1 carbon makes difference in pressure sensitivity!

Adaptations for P-T Effects on Hydrogen Bonds

Rise due to weak 113-27 H-bond
(less correlated)

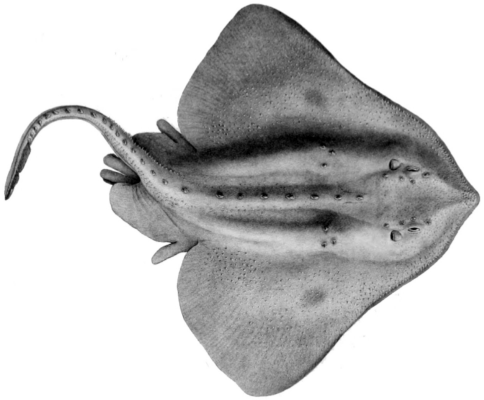
Fall due to overall weaker H-bonds
(less stable), maybe for cold

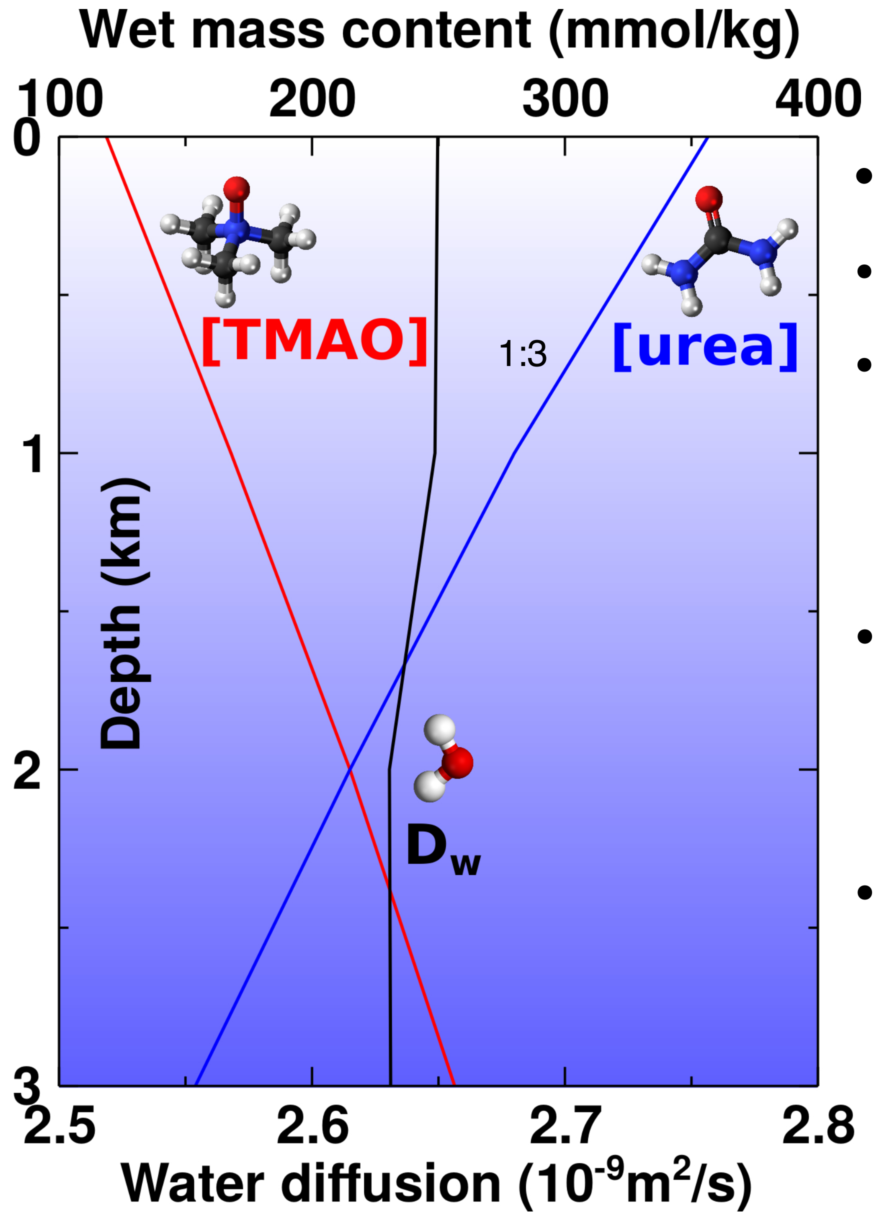


- Hydrogen bonds!

Question II
Can “Piezolytes” Adapt Cellular Environment?

Sharks, rays, etc.
Yancey’s group





(300 bar)

- Urea is denaturant
- TMAO is stabilizer
- 2 urea : 1 TMAO cancel
- How does TMAO counteract urea at 1 atm?
- How does TMAO act as piezolyte at higher *P*?

Molecular Dynamics Computer Simulations:

Water Diffusion & Hydrogen Bonds at 0.5 M vs *P*

Teng & Ichiye, to be submitted

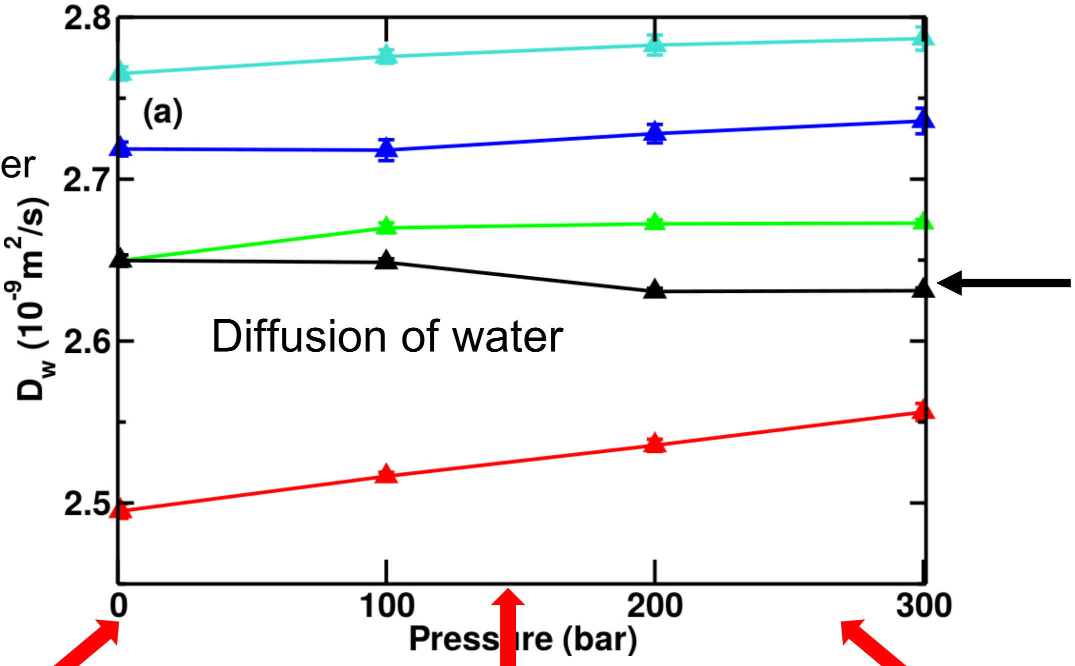
Pure water

Urea-water

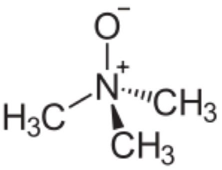
3:1 urea-TMAO-water

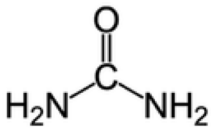
Shark ratio urea-TMAO-water

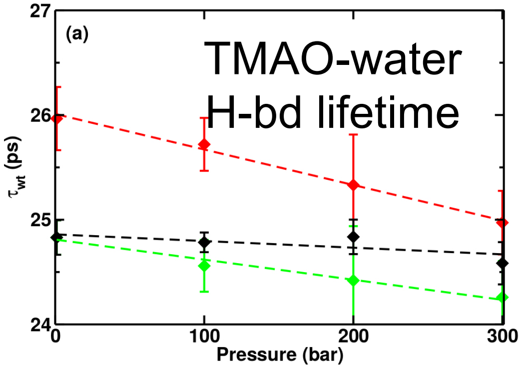
TMAO-water



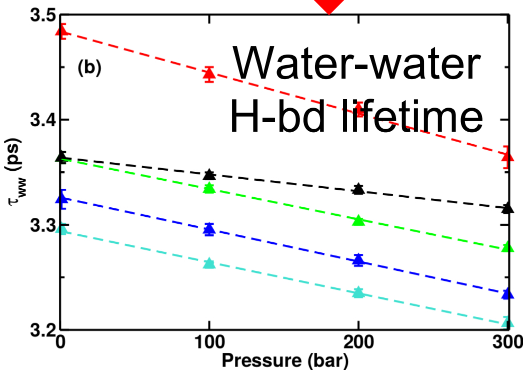
homeostasis



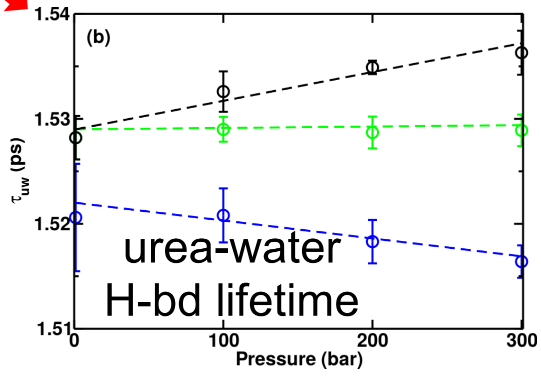




TMAO-water H-bd lifetime



Water-water H-bd lifetime



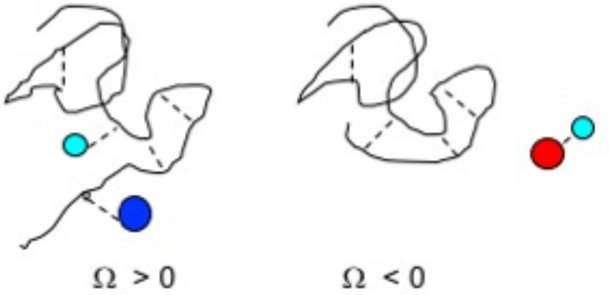
urea-water H-bd lifetime

- Shark ratio water diffusion independent of *P*
- TMAO slows down water due to strong H-bond

Dynamic Theory of Osmolyte Effects

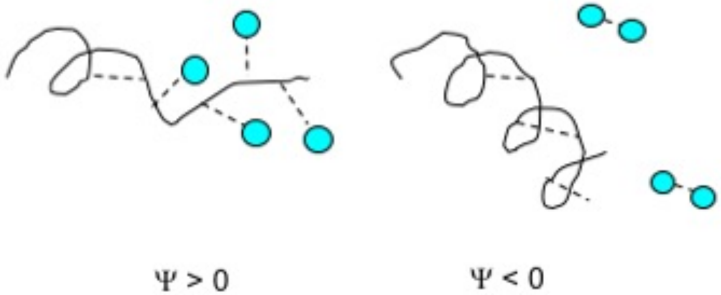
- Hydrogen bonding propensity of cosolute to protein

$$\Omega = \frac{d}{dt}[\text{free Hbd of solute in soln}] - \frac{d}{dt}[\text{free Hbd of water molecule in } H_2O]$$



- Hydrogen bonding propensity of water (to *unfolding* protein)

$$\Psi = \frac{d}{dt}[\text{free Hbd of water in soln}] - \frac{d}{dt}[\text{free Hbd of water molecule in } H_2O]$$



Denaturing/stabilizing effects determined by inverse H-bd lifetimes

TMAO Effects on Urea Denaturation at High *P*

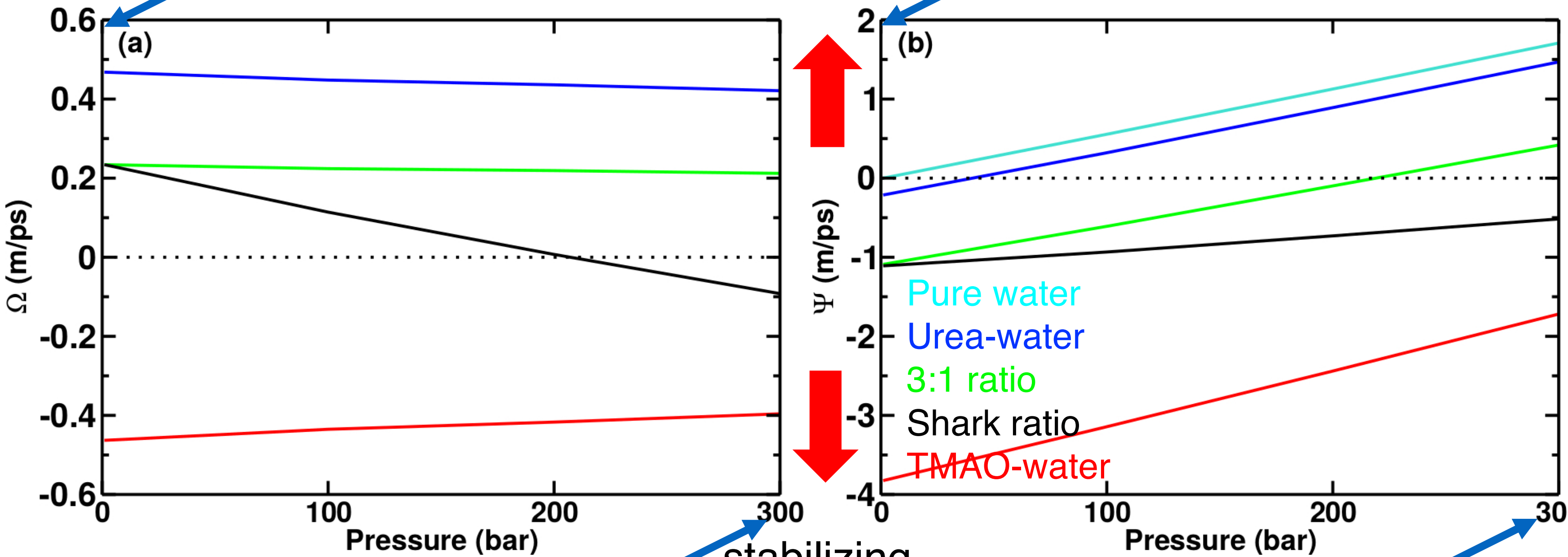
H-bond Propensity of Osmolyte

H-bond Propensity of Water

TMAO replaces urea

TMAO reduces hydration

denaturing



stabilizing

more TMAO because *P* breaks intraprotein H-bonds

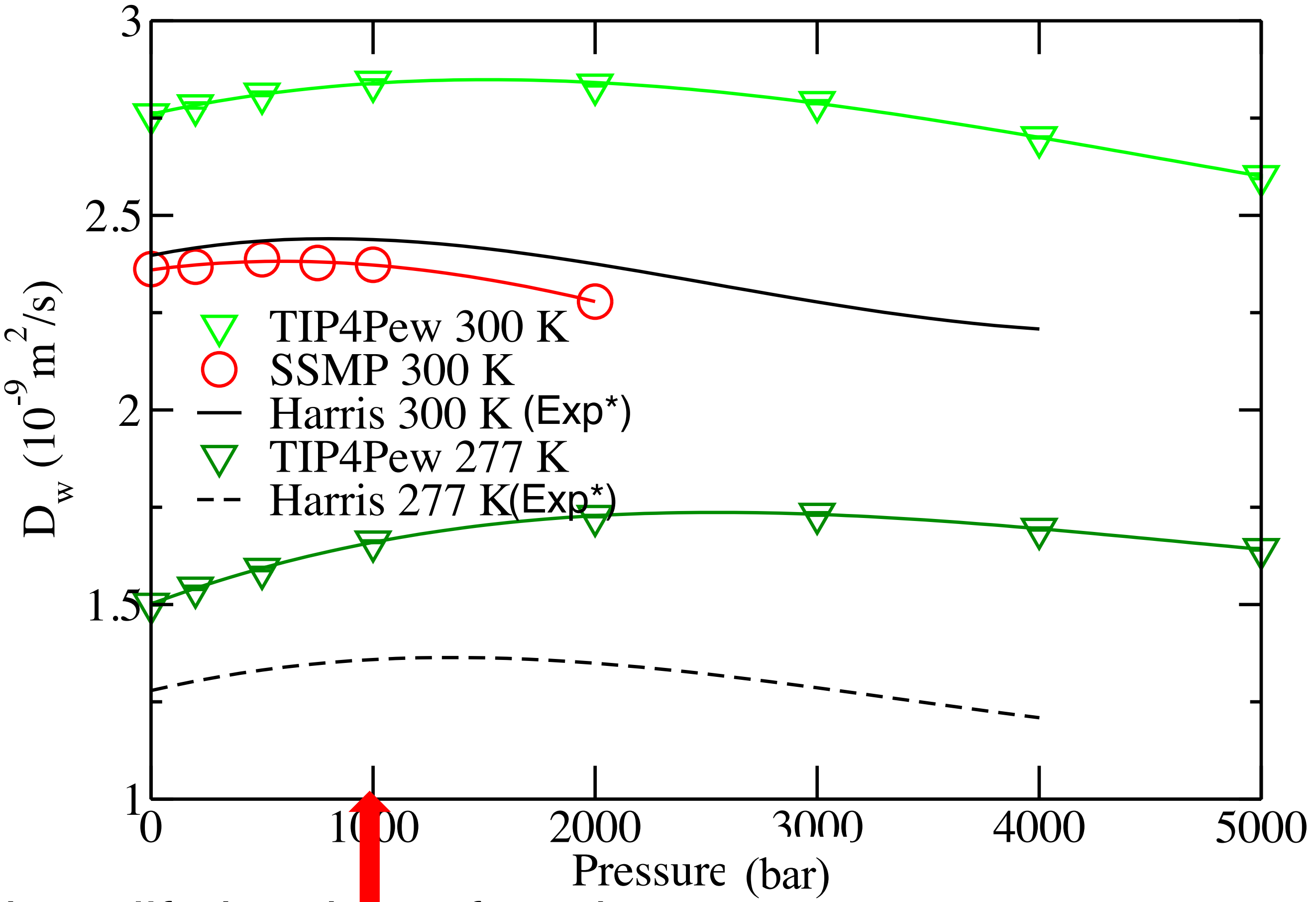
more TMAO because *P* breaks water-water H-bonds

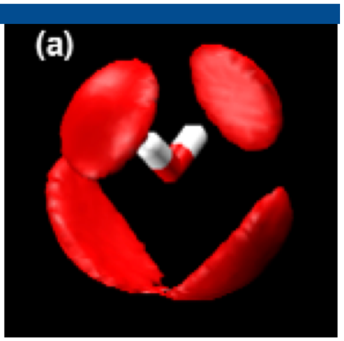
TMAO compensates for *P* effects on protein and on water

The Anomalous Diffusion of Water under Pressure

Teng, Liu, Ichiye, to be submitted

IP4P-Ew





SSMP

Max *P* where life has been found

Life so far where coordination number of water < 5

*Harris & Woolf, JCS Faraday I, 1980, 76, 377

Conclusions

Question I: Adaptations in extremophile enzymes

- *Material* properties of enzyme are important
- Unlike *T*, high *P* has opposing effects
  - compresses local potential energy well, ↓ local motion
  - usually weakens H-bonds, ↑ collective motion
  - increases *correlation, may strengthen H-bonds*, allostery?

Question II: How piezolytes work

- Model based on H-bond lifetimes explains effects of osmolytes on proteins
- Sharks? Counteracting effects of TMAO - decreases H-bond capacity of water

Acknowledgments

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PSC: ANTON
Georgetown: Advanced Research Computing