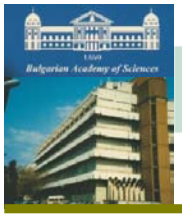
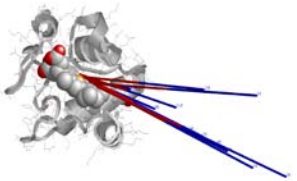
Fig. S1-1



PHEMTO

Protein pH-dependent Electric Moments TOols

Institute of Organic Chemistry, Bulgarian Academy of Sciences, Biophysical Chemistry of Proteins Group



If you have need for electrostatic calculations on your protein - use Internet address:

http://phemto.orgchm.bas.bg/

The only what you have to have are the atomic coordinates of your protein in PDB format. Than wide variety of pH-dependent properties you can predict :

PHEMTO functionality

- **μ**(pH) - electric or dipole moments – visualized vectors with preselected length
- μ(pH) - electric or dipole moments – visualized scalars in wide pH region (2-12)
- Mut(pH) – predefined set of electrostatic mutants (D,E,H,C,Y,K,R → any)
- Difference analysis – subtract any basic pH-function from their derivatives
- F (pH) - electric forces (field gradients) – scalars and vectors */in development/*
- Any function of molecular domain/subunit within all others parts */in development/*

PHEPS functionality **(automatic link)**

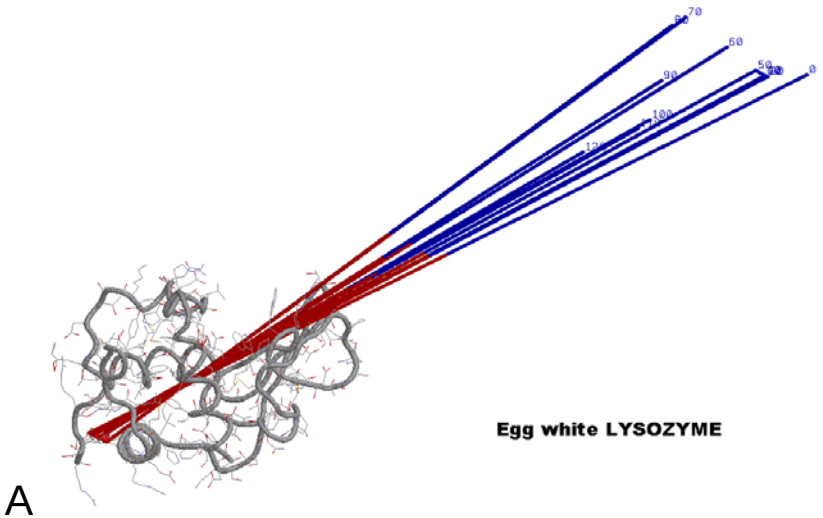
• **Z(pH)** – proton bonding (potentiometric titration) curve; • **s_i(pH)** – degree of ionization of each *i*-th ionisable site; • **ΔG_{el}(pH), ΔG_{tot}(pH)** – electrostatic and total free energy (stability), • **$E_{el,i}$(pH)** – Coulomb energy of each ionic group with whole charge multipole, • **Φ_j** – Electrostatic potential at you selected pH on every j-th atom in your protein; • **φ_i(pH)** – Electrostatic potential at each *i*-th point within or close to molecule; • **$\langle SA \rangle_l$** - Averaged relative Static Atomic accessibility to the solvent; • **$\langle SR \rangle_j$** - Averaged relative Static Residue accessibility to the solvent;

and many others

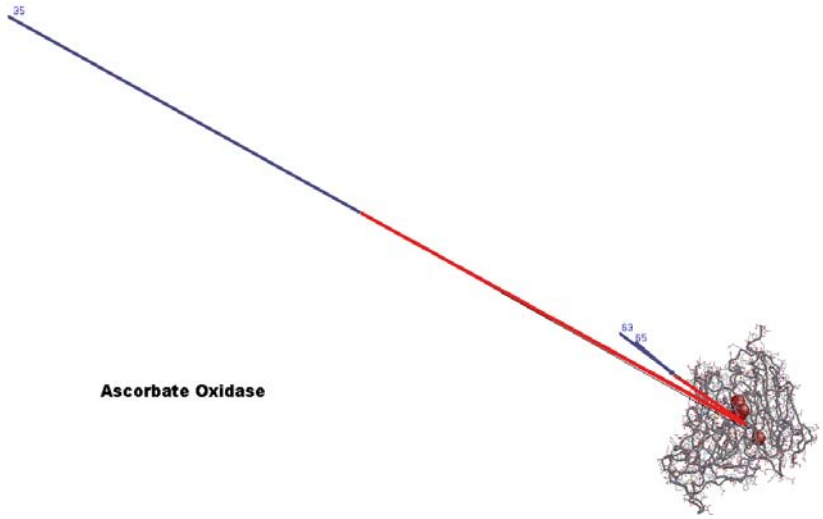
See the NEXT →

SUPPLEMENT – 1

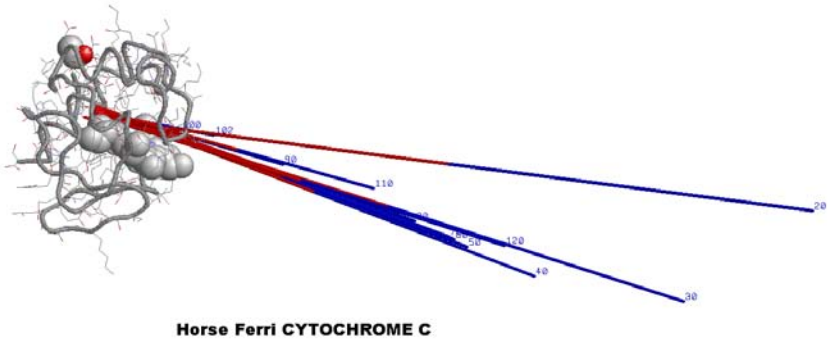
Fig. S1-2



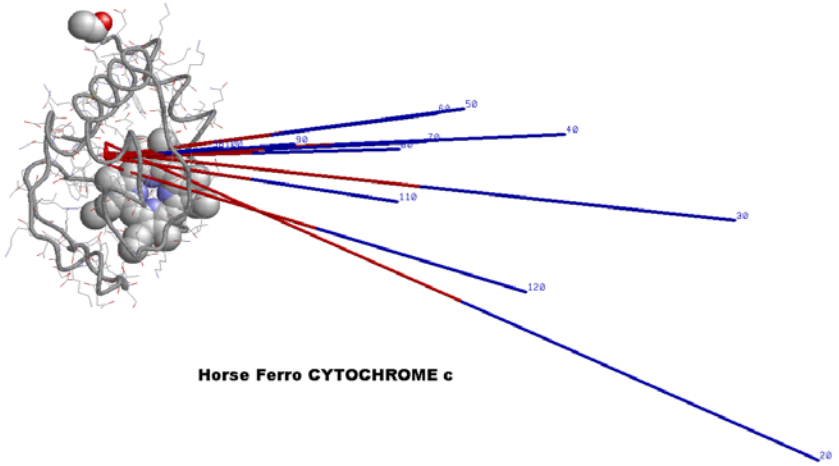
A



B

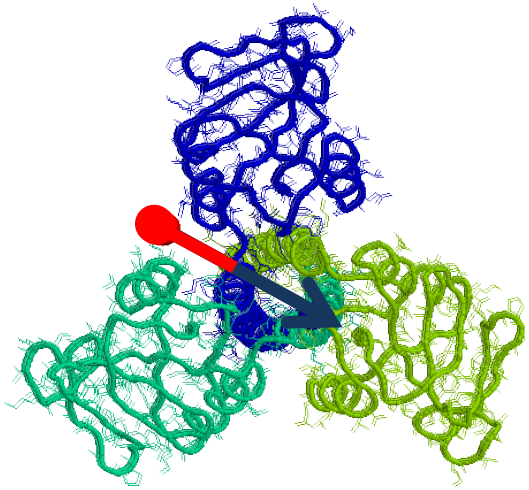


C

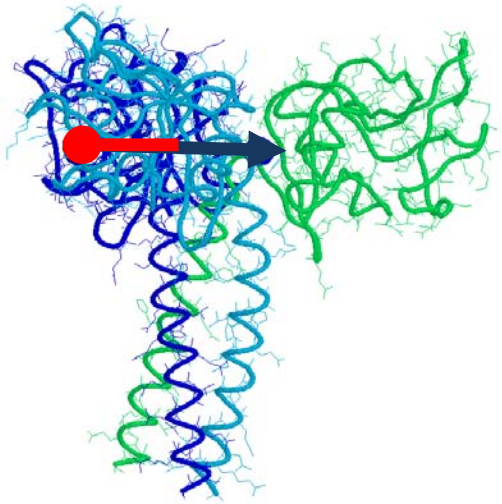


D

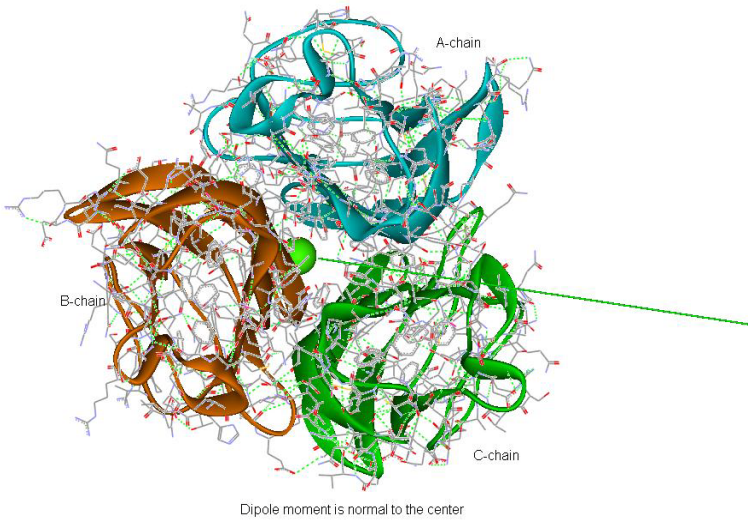
Fig. S1-3



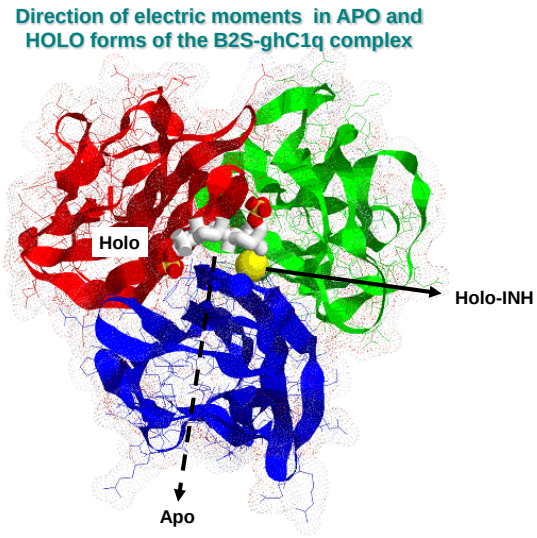
A



B


A-chain
B-chain
C-chain
Dipole moment is normal to the center

C


Direction of electric moments in APO and HOLO forms of the B2S-ghC1q complex
Holo
Holo-INH
Apo

D

Fig. S1-4

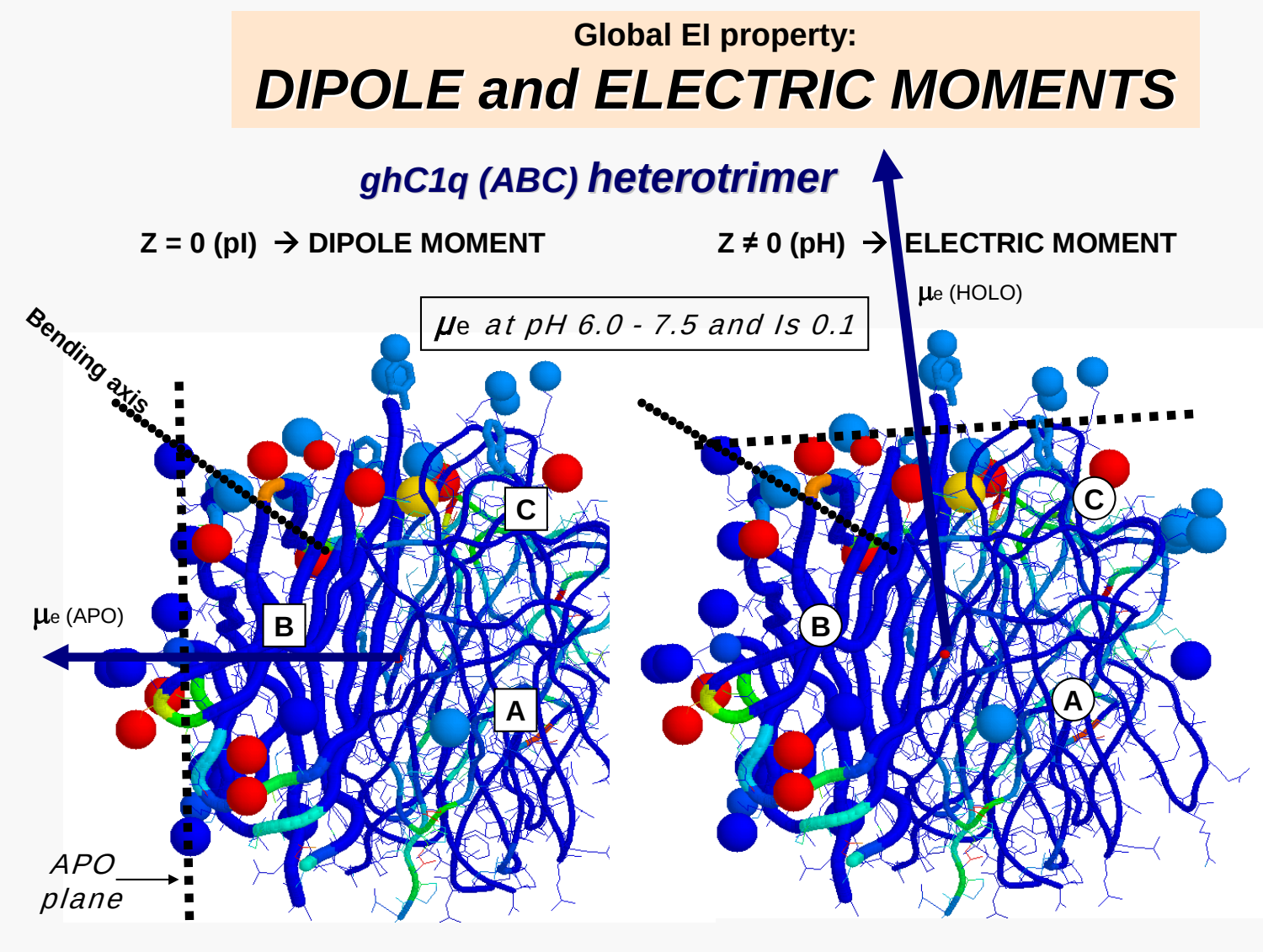

Global EI property:
DIPOLE and ELECTRIC MOMENTS
ghC1q (ABC) heterotrimer
Z = 0 (pI) → DIPOLE MOMENT
Z ≠ 0 (pH) → ELECTRIC MOMENT
μe (HOLO)
μe at pH 6.0 - 7.5 and Is 0.1
Bending axis
μe (APO)
C
B
A
APO plane
C
B
A

Fig. S1-5

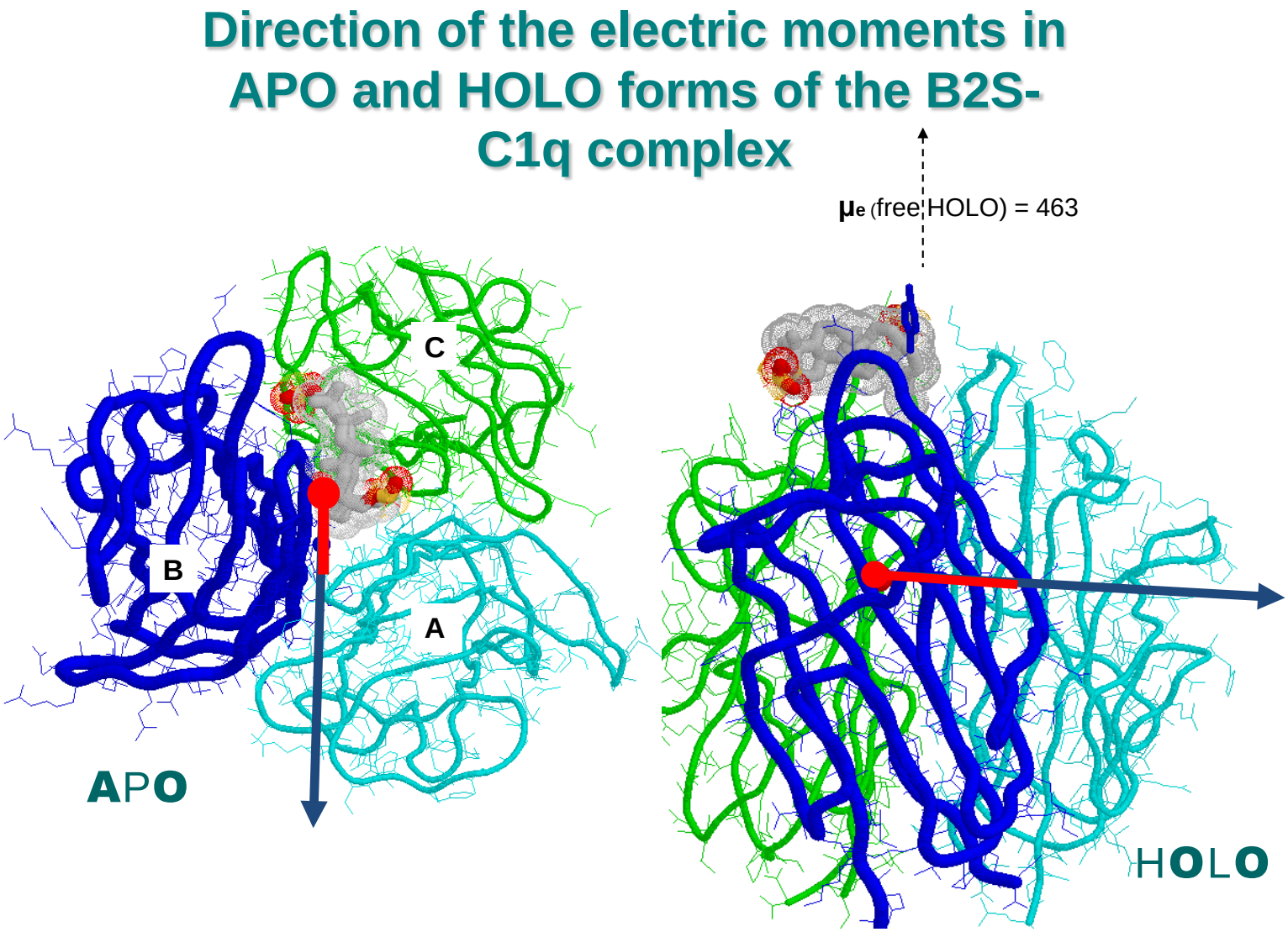
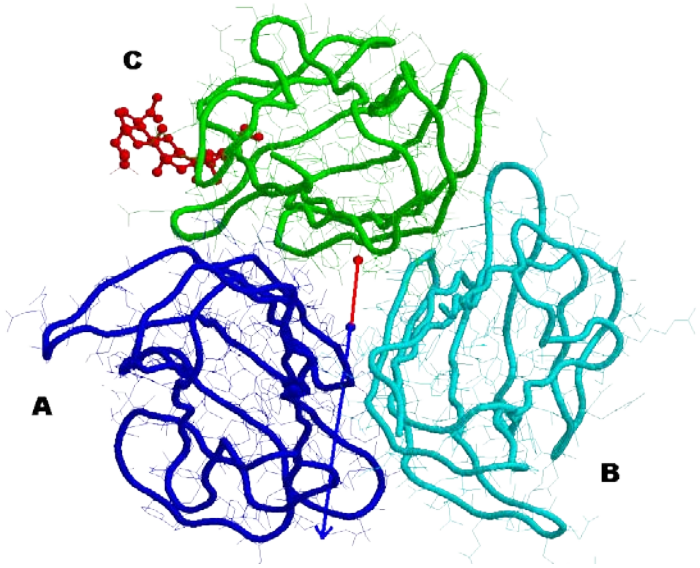
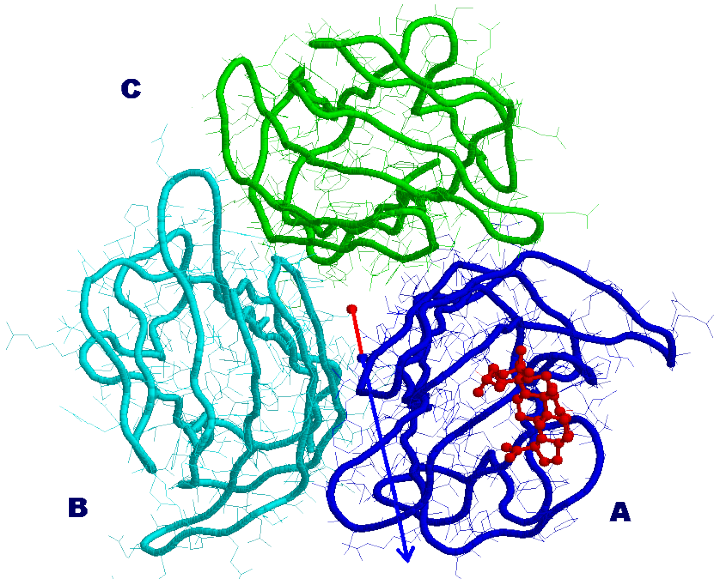

Direction of the electric moments in APO and HOLO forms of the B2S-C1q complex
μe (free HOLO) = 463
C
B
A
APO
HOLO

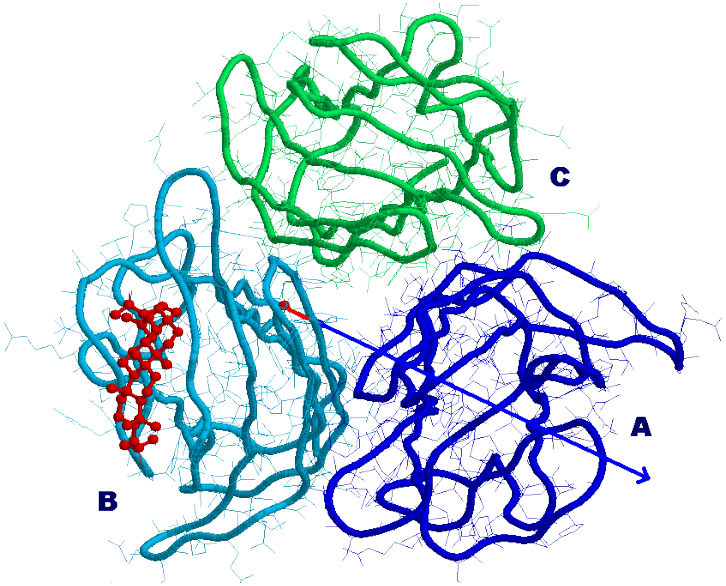
Fig. S1-6



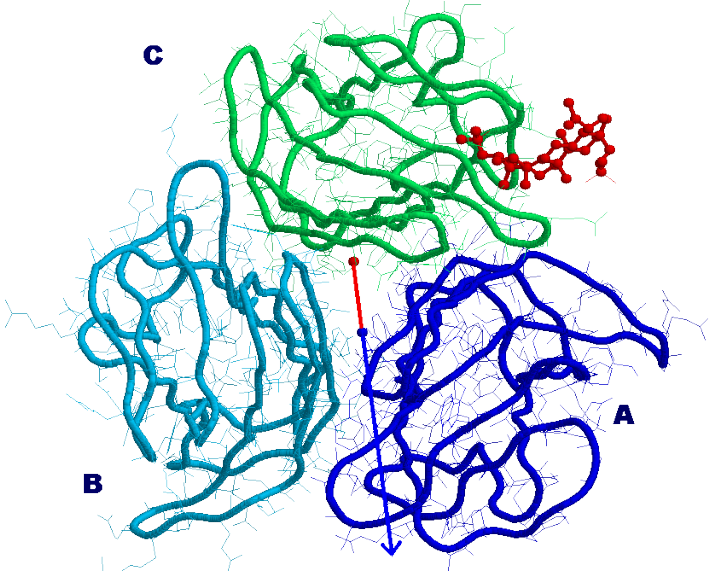
A



B

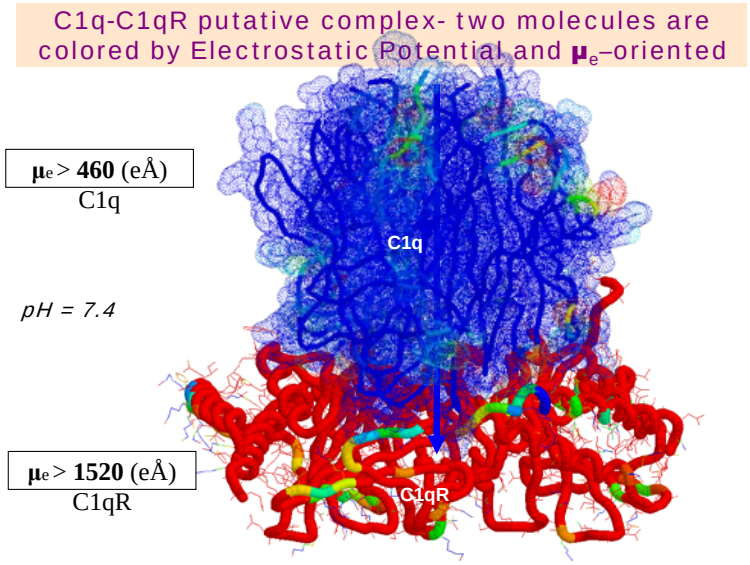


C

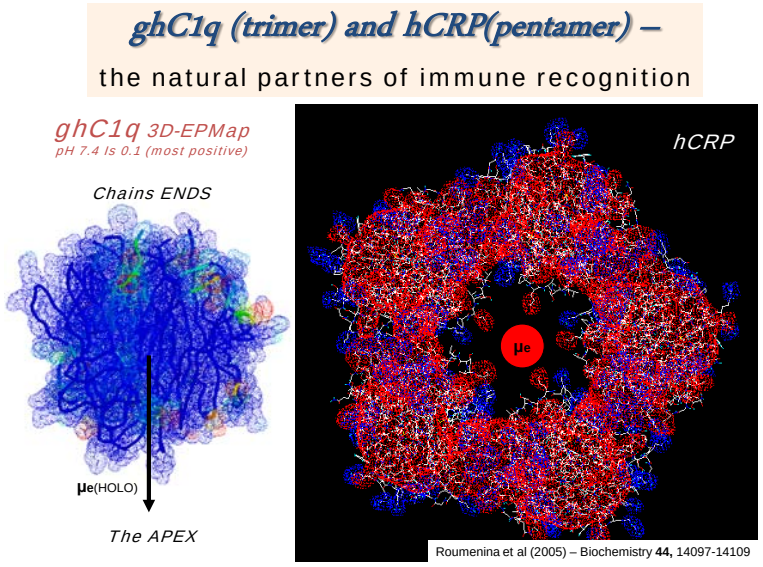


D

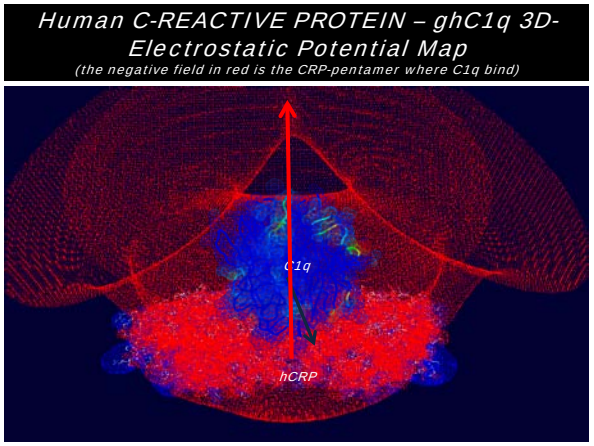
Fig. S1-7


C1q-C1qR putative complex- two molecules are colored by Electrostatic Potential and μe–oriented
μe > 460 (eÅ)
C1q
pH = 7.4
μe > 1520 (eÅ)
C1qR
C1q
C1qR

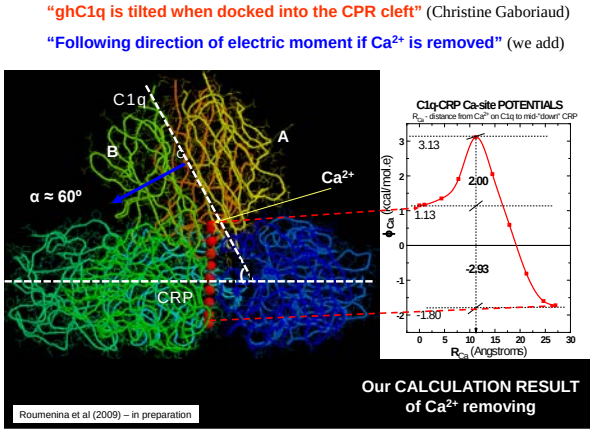
A


ghC1q (trimer) and hCRP(pentamer) –
the natural partners of immune recognition
ghC1q 3D-EPMap
pH 7.4 Is 0.1 (most positive)
Chains ENDS
μe(HOLO)
The APEX
hCRP
μe
Roumenina et al (2005) – Biochemistry 44, 14097-14109

B


Human C-REACTIVE PROTEIN – ghC1q 3D-Electrostatic Potential Map
(the negative field in red is the CRP-pentamer where C1q bind)
C1q
hCRP

C


"ghC1q is tilted when docked into the CPR cleft" (Christine Gaboriaud)
"Following direction of electric moment if Ca2+ is removed" (we add)
C1q
A
B
C
Ca2+
α ≈ 60°
CRP
C1q-CRP Ca-site POTENTIALS
3.13
2.00
1.13
-2.93
-1.80
Our CALCULATION RESULT of Ca2+ removing
Roumenina et al (2009) – in preparation

D

Fig. S1-8

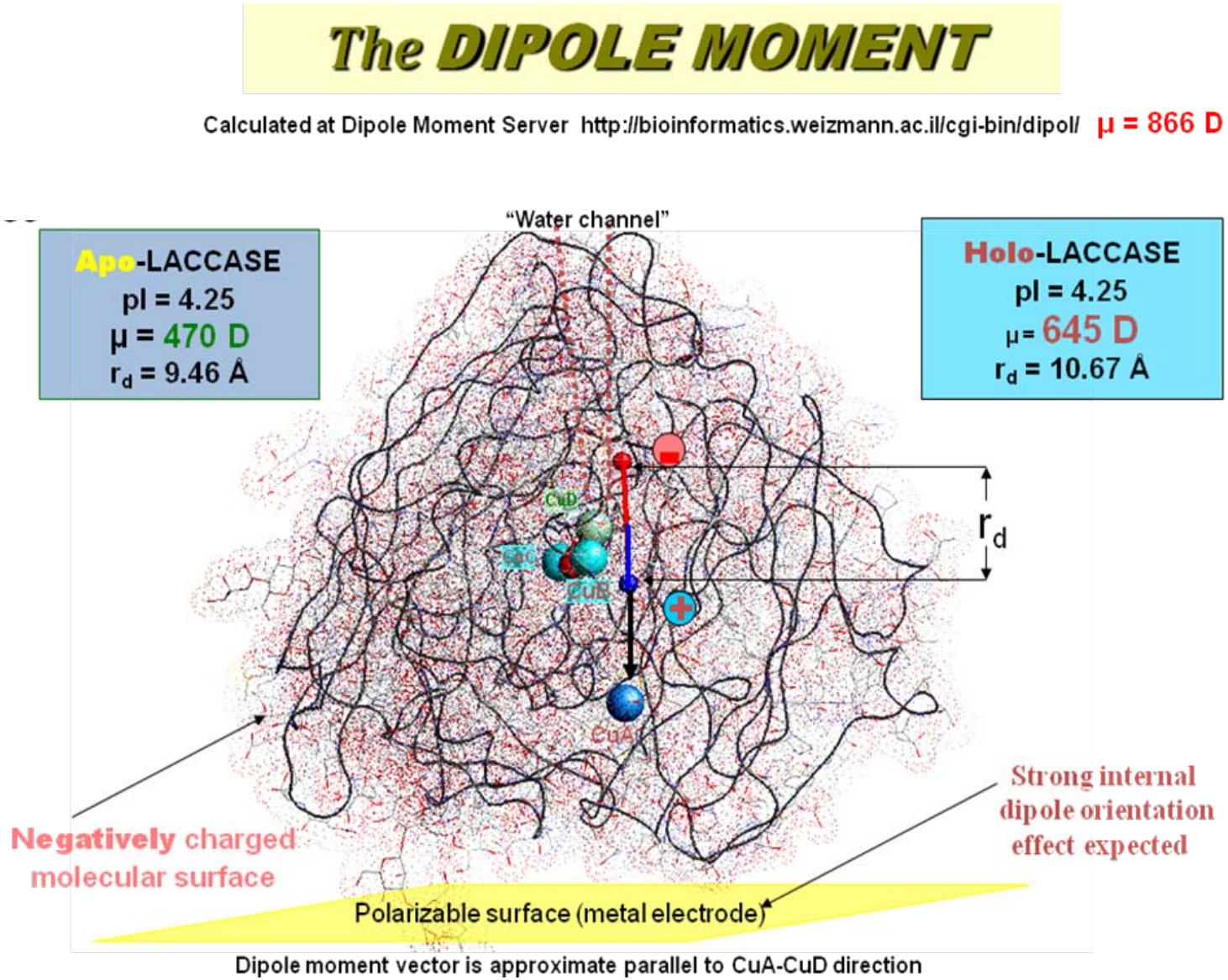
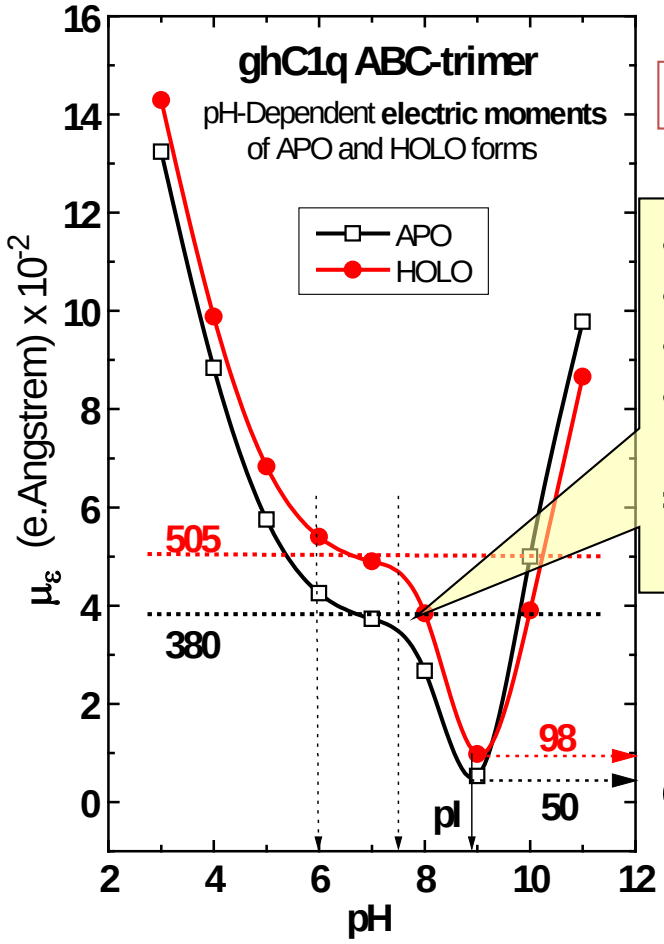

The DIPOLE MOMENT
Calculated at Dipole Moment Server http://bioinformatics.weizmann.ac.il/cgi-bin/dipol/ μ = 866 D
"Water channel"
Apo-LACCASE
pI = 4.25
μ = 470 D
r_d = 9.46 Å
Holo-LACCASE
pI = 4.25
μ = 645 D
r_d = 10.67 Å
CuD
CuC
CuB
CuA
r_d
Negatively charged molecular surface
Strong internal dipole orientation effect expected
Polarizable surface (metal electrode)
Dipole moment vector is approximate parallel to CuA-CuD direction

Fig. S1-9

ghC1q ELECTRIC MOMENTS

and the recognition process



$\Delta\mu_e$(HOLO–APO) ≈ 120 eÅ (pH 7.5 – 6.0)

- ***Blood serum pH = 7.4***
- ***At inflammation pH drop with 1.0-1.5 pH***
- ***μ_e are relatively constant at pH 6-7.5***
- ***In this pH region $\Delta\mu_{e(HOLO–APO)}$ is also relative constant***

==========

FUNCTIONAL MEANING?

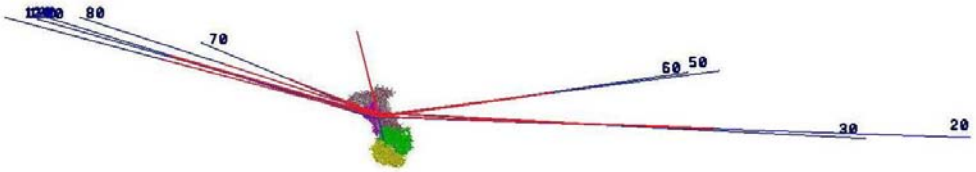
For recognition are important not only absolute values and directions of electric moments but as well as the ratio

μ_e (HOLO) / μ_e (APO) = 1.3 ± 0.1 !

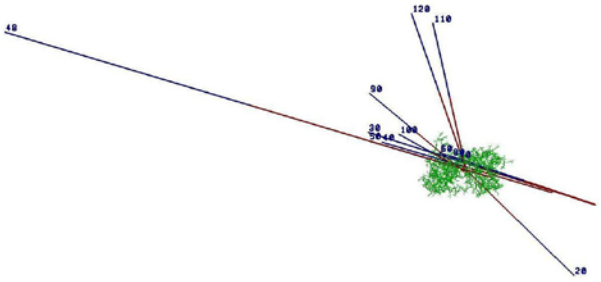
A

m(ph).org

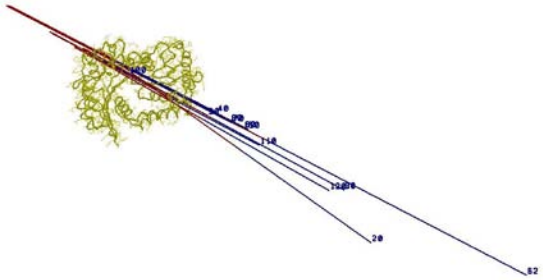
Cytochrome Reductase (CytBC1)
B Vectors



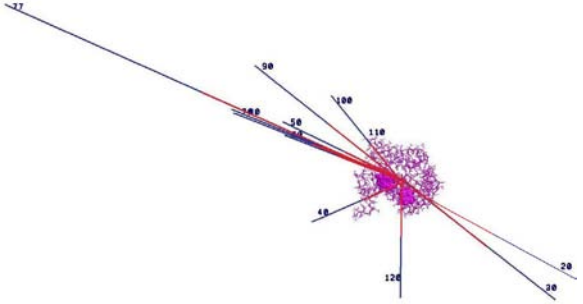
ABCDEFGHIJGK-chains



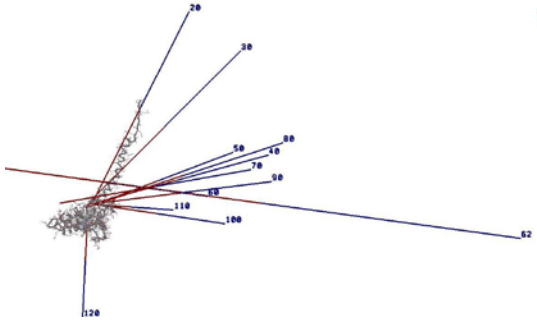
A-chain



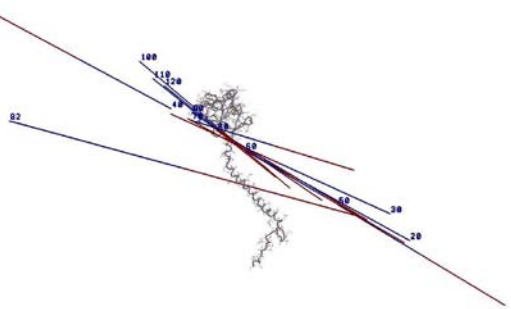
B-chain



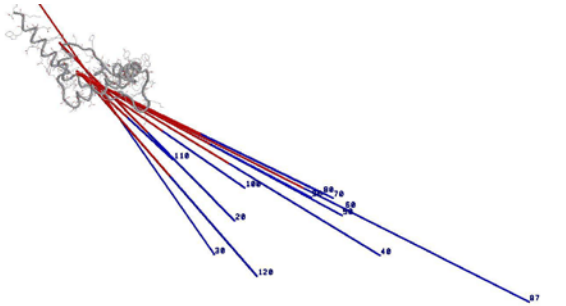
C-chain



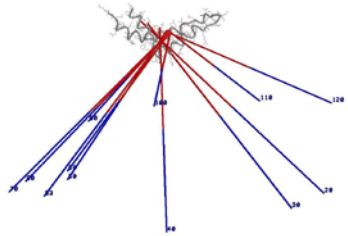
D-chain



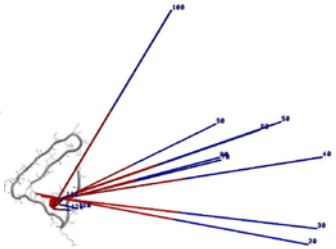
E-chain



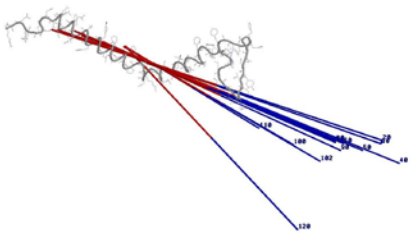
F-chain



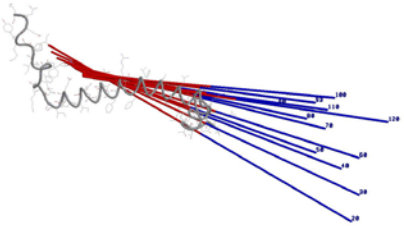
H-chain



I-chain



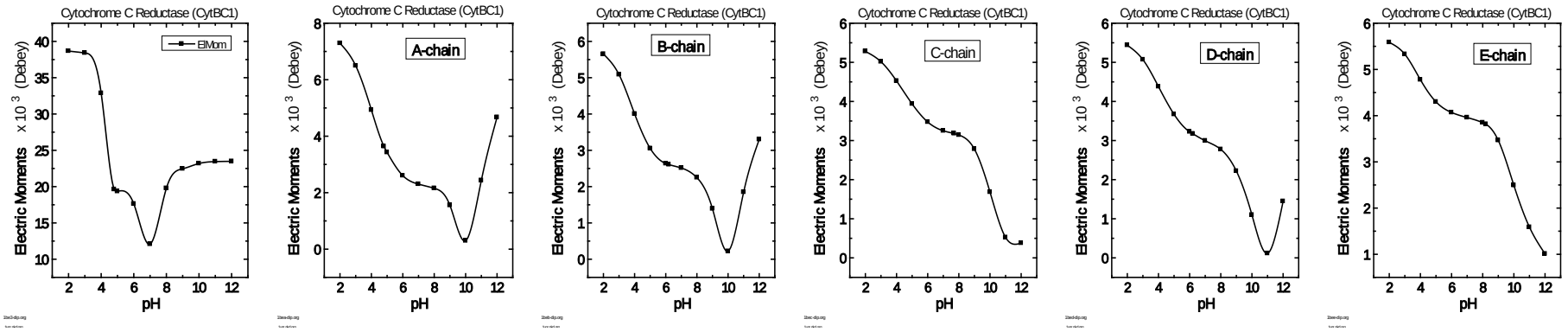
J-chain



G-chain

C Scalars

pH DEPENDENT ELECTRIC MOMENTS (scalars) of CYTOCHROME REDUCTASE



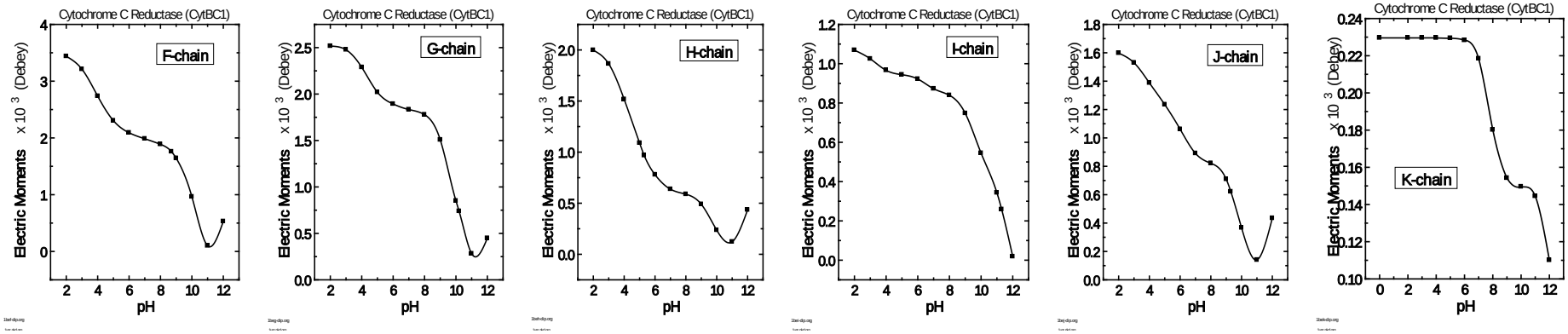


Fig. S1-9

Global EI property:
DIPOLE and ELECTRIC MOMENTS

TEM1 ß-lactamase reaction cycle intermediates (RI)

	CYCLE	Electric moments (μ_e) at pH			Dipole moments (μ_d) at pI (in Debye) (e.Å)		
No	RI	4.0 (e.Å)	6.5 (e.Å)	9.0 (e.Å)	pI/ΔG_{el} (kcal/mol)	Distance (Å)	Dipole moment (e.Å)
1.	FE	531	254	388	5.25 / -17.46	3.35	49
2.	MC	413	379	556	5.25 / -19.5	2.76	105
3.	T1	486	344	514	5.25 / -17.9	3.13	59
4.	AE	614	184	342	5.75 / -19.8	3.44	45
5.	T2	614	185	357	5.75 / -19.8	3.52	46
6.	PE	514	298	457	5.25 / -22.7	3.00	30

Fig. S1-10 A

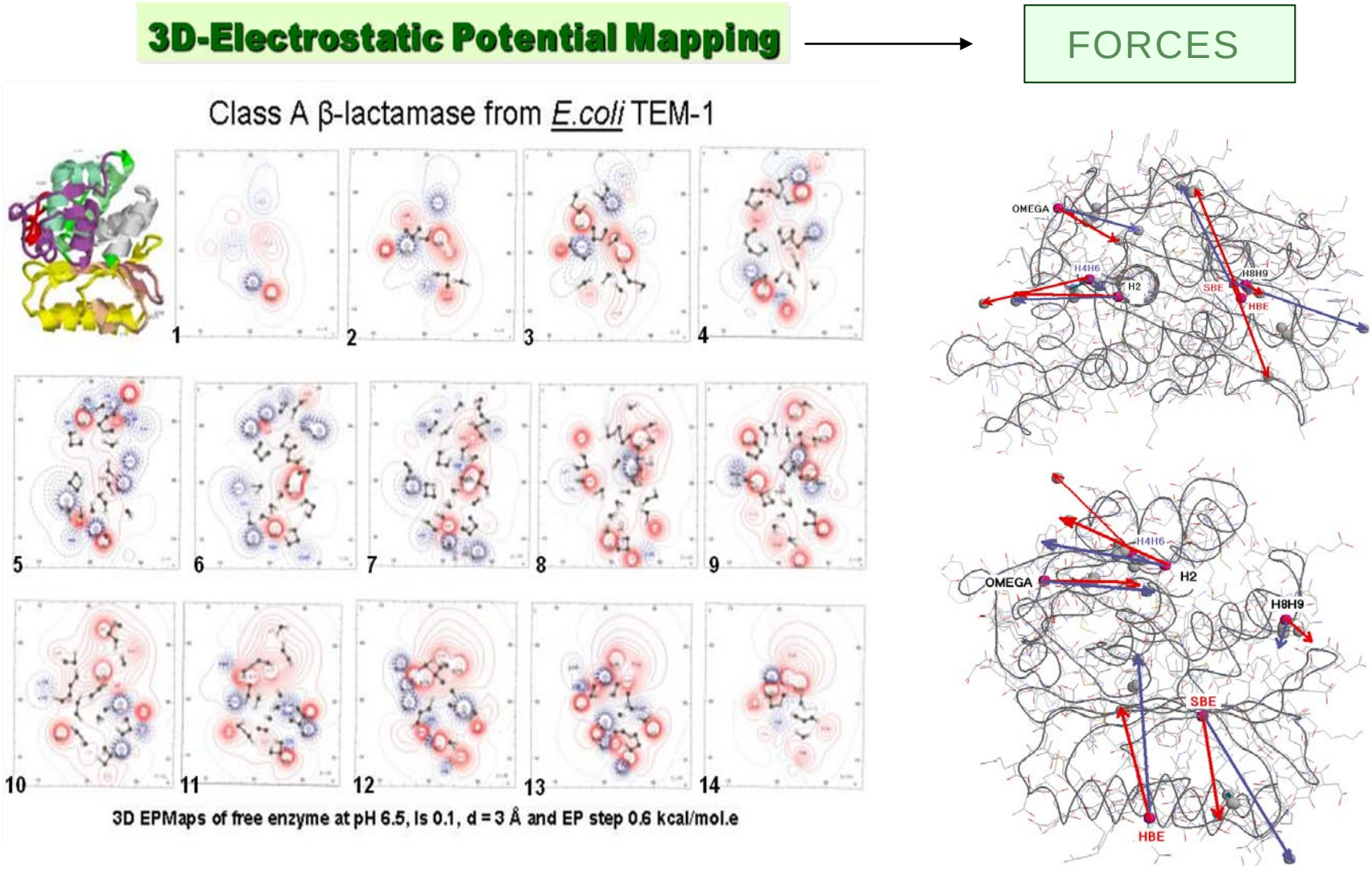

3D-Electrostatic Potential Mapping
FORCES
Class A β-lactamase from E.coli TEM-1
1
2
3
4
5
6
7
8
9
10
11
12
13
14
3D EPMaps of free enzyme at pH 6.5, Is 0.1, d = 3 Å and EP step 0.6 kcal/mol.e
OMEGA
H4H6
H2
H8H9
SBE
HBE

Fig. S1-10 B

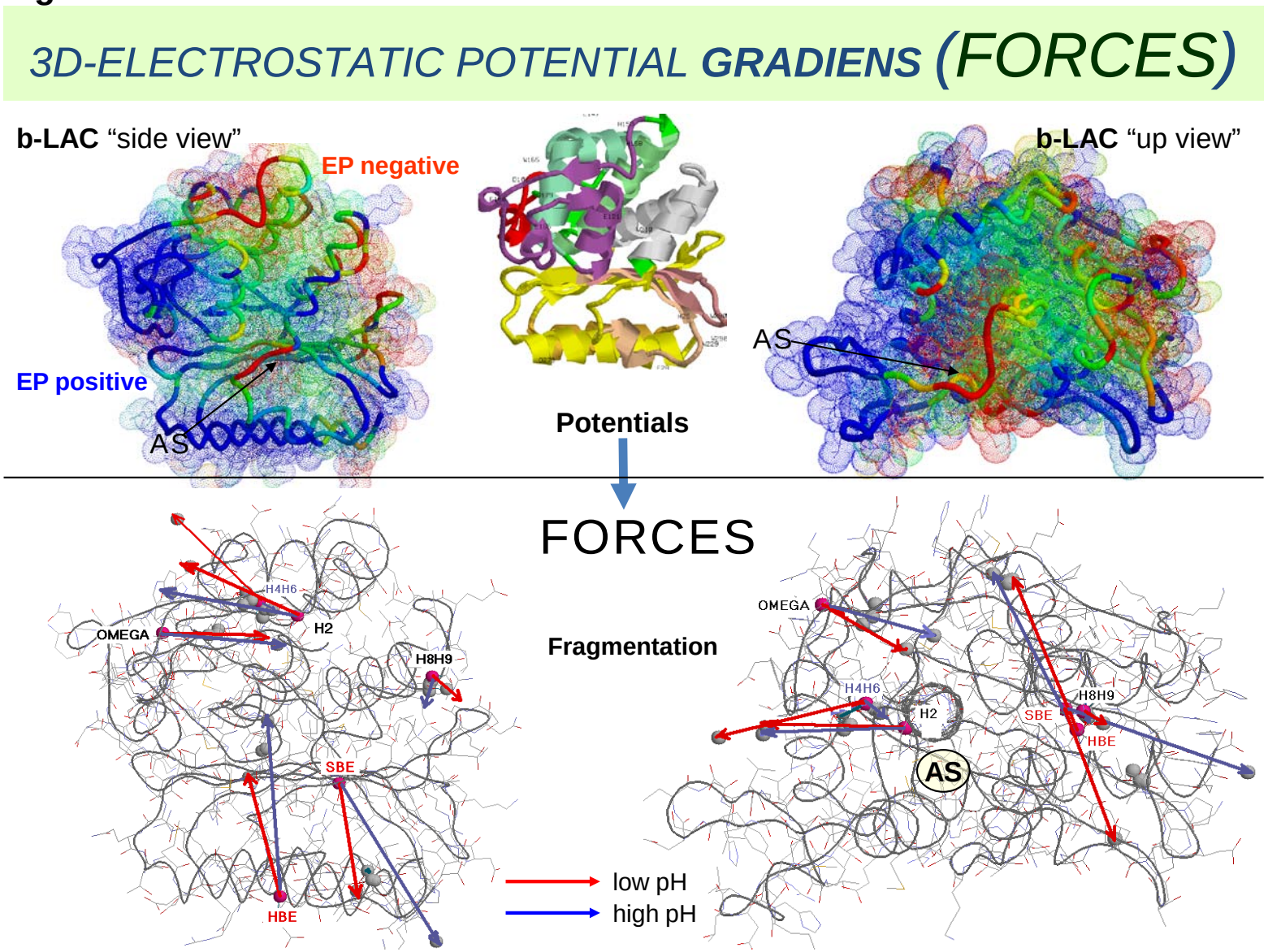
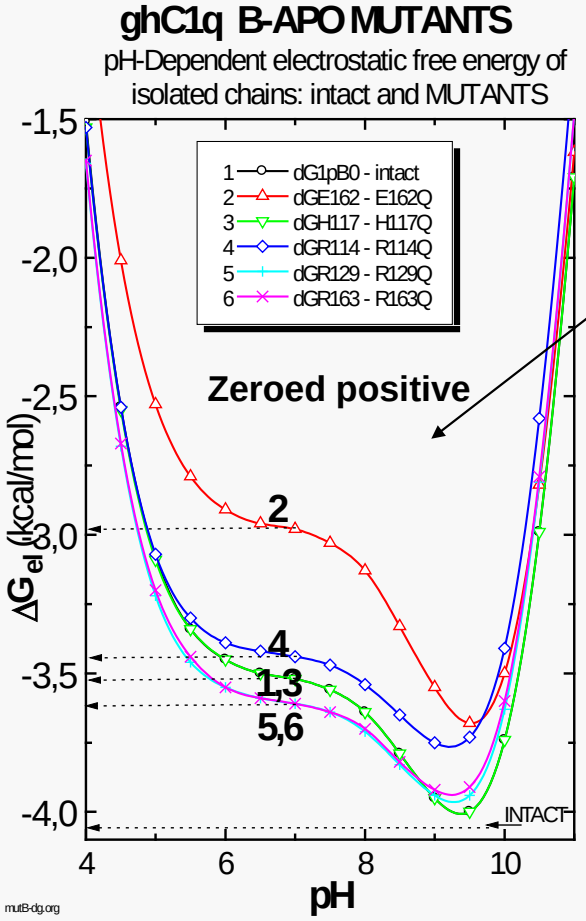

3D-ELECTROSTATIC POTENTIAL GRADIENS (FORCES)
b-LAC "side view"
EP negative
EP positive
AS
Potentials
b-LAC "up view"
AS
FORCES
OMEGA
H4H6
H2
H8H9
SBE
HBE
Fragmentation
OMEGA
H4H6
H2
H8H9
SBE
HBE
AS
low pH
high pH

Fig. S1-11 A

"Computer Mutagenesis"
Electrostatic Interaction Analysis



Manual plots like that

Difference plots by:

ΔS_i
ΔZ_i
$\Delta pK_{a,i}$
ΔE_{el}
$\delta\Delta G_{el,i}$
$\Delta\varphi_{el.i}$

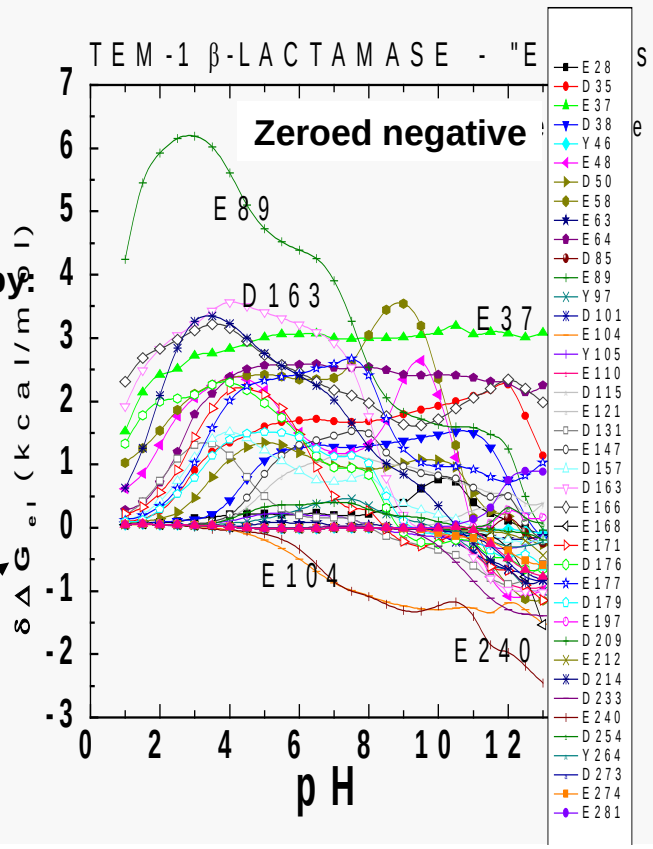
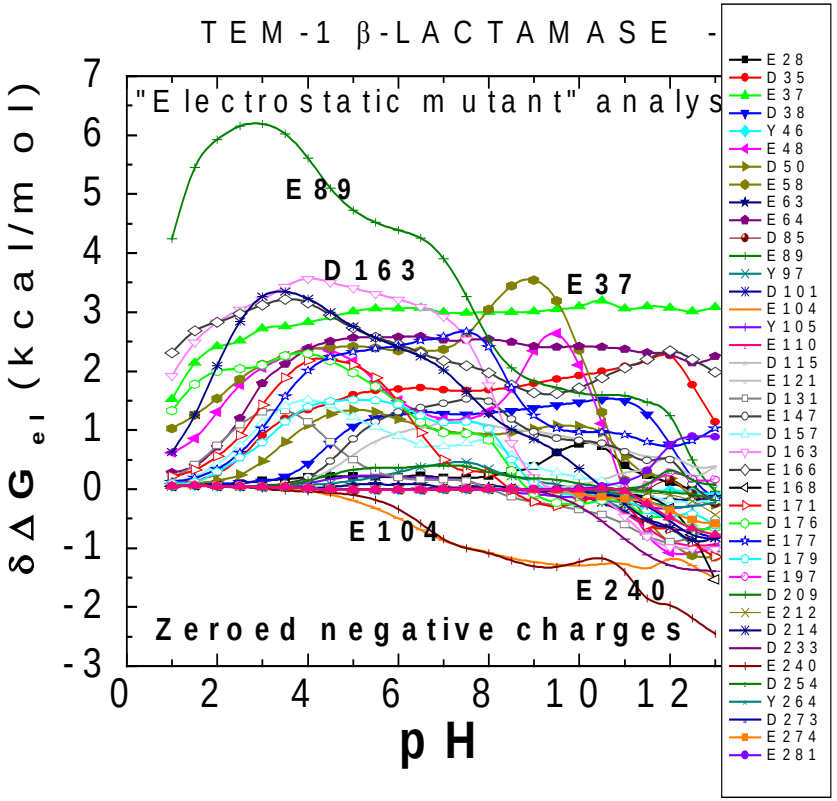
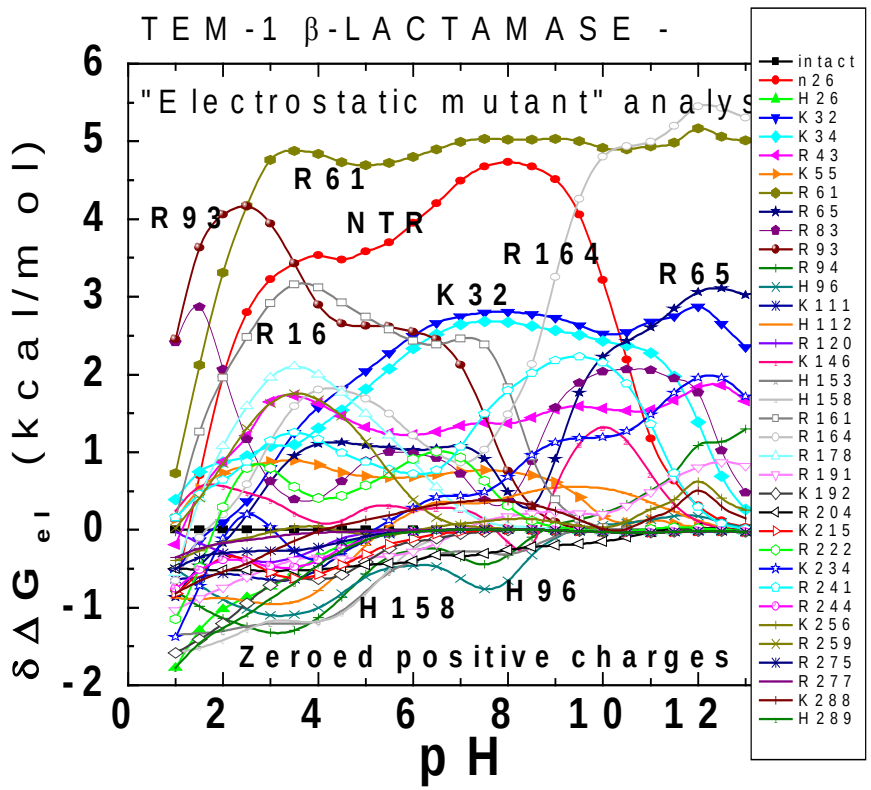


Fig. S1-11



B



C

FINE