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Advanced Applications in Solving Smoluchowski Equation

Finite element Method Application

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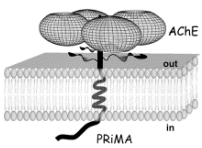
Outline

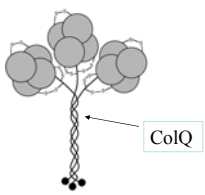
- To apply the SSSE to study mAChE tetramers. conformations; visualization
- To introduce the reaction-determined reactive boundary conditions.
- Preliminary studies on the time-dependent SMOL solver and applications.
  - ➢ Analytical tests
  - ➢ mAChE monomer

The Quaternary Association of AChE

• In mammalian CNS, AChE exists as an amphiphilic tetramer anchored to the membrane by a hydrophobic non-catalytic subunit (PRiMA)

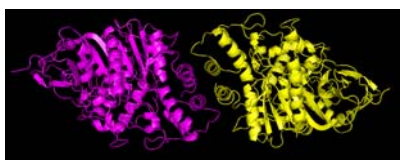


• In NMJ, AChE is an asymmetric form containing 1-3 tetramer associated with the basal lamina by a collagen-like structural subunit ColQ

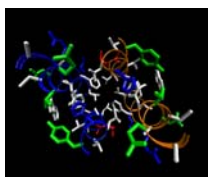


Dimer Interface

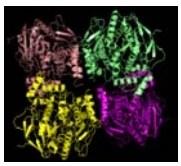
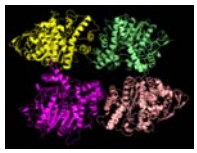
PDB:
1JO6
1JO7



• Four-helix boundle (371-383 and 525-541 in mouse sequense)
• Amphiphilic interface
• Weak affinity, only forms dimer at high concentration in solution



Tetramer: Dimer of Dimers

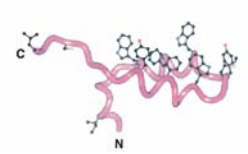




- PDB: 1MAA
- Residues 549-583 deleted
- Only forms monomer or dimer in solution
- Dimer-dimer interaction similar to Fas2-AChE (omega loop = loop II), resulting in two occluded gorges

PDB: 1C2O	1C2B(1EAA)
Trypsin released form of AChE from E. *electrocus*	
Crystal grown at pH8 4°C	pH6 20°C
Compact, square nonplanar	Loose, pseudo-square planar;
four-helix boundle parallel	anti-parallel
two gorges partially blocked	all gorges open

- additional density for C-terminal t peptide observed, but not resolved.

Heteromeric Association with PRAD



Giles, K. (1997) Protein Engineering, 6, 677-685

- t peptide sequence (40 or 41 res) highly conserved
- PRAD = Proline Rich Attachment Domain
- PRAD is required to form tetramer in solution

Bon,S. Coussen, F., Massoulie, J. (1997) JBC, 272, 3016-3021

Bon, S. et al. (2004) Eur. J. Biochem, 271, 33-47

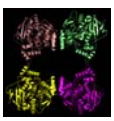
ColQ_ Mouse

```
LPGLDQKKRG SHKACCLLMP PPPPLFPPPF FRGSRSPLLS PDMKNLLELE ASPSPCIQGS
LGSPGPPGQG PPGLPGKTGP KGEKGDLGRP GRKGRPGPPG VPGMPGPVGW PGPEGPRGEK
GDLGMMGLPG SRGPMGSKGF PGSRGEKGSR GERGDLGPKG EKGFPGFPGM LGQKGEMGPK
GESGLAGHRG PTGRPGKRGK QGQKGDSGIM GPHGKPGPSG QPGRQGPPGA PGPPSA
```

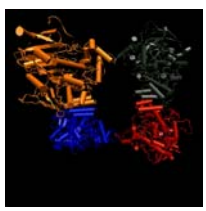
PRMA_ Mouse

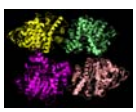
```
MLLRDLVPRH GCCWPSLLLH CALHPLWGLV QVTHAEPQKS CSKVTDSCQH ICQCRPPPPL
PPPPPPPPPP RLLSAPAPNS TSCPAEDSWW SGLVIIVAVV CASLVFLTVL VIICYKAIKR
KPLRKDENGT SVAEYPMSSS QSHKGVDVNA AVV
```

A flexible Tetramer?



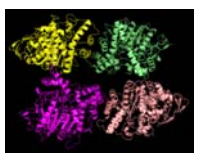
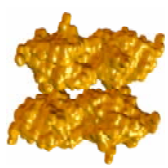
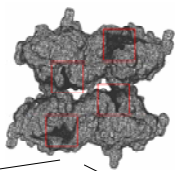
1C2B
Flat square
Quasi-planar



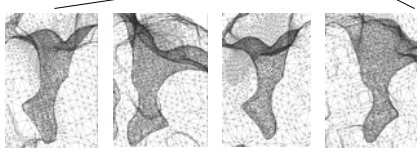


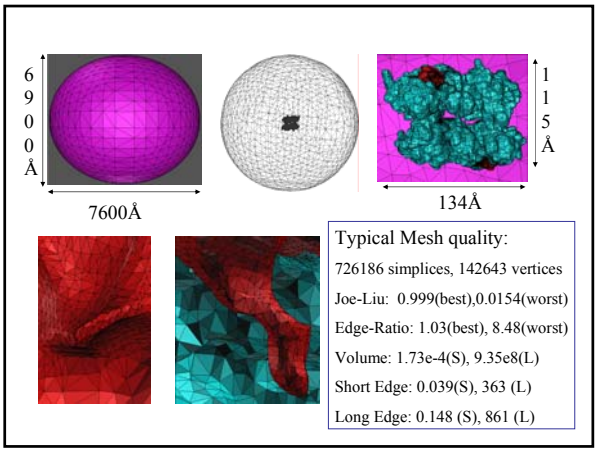
1C2O
Compact

Tetrahedral Mesh for mAChE Tetramer

Reactive surface is assigned according to previous studies on monomeric mAChE





Typical Mesh quality:

726186 simplices, 142643 vertices

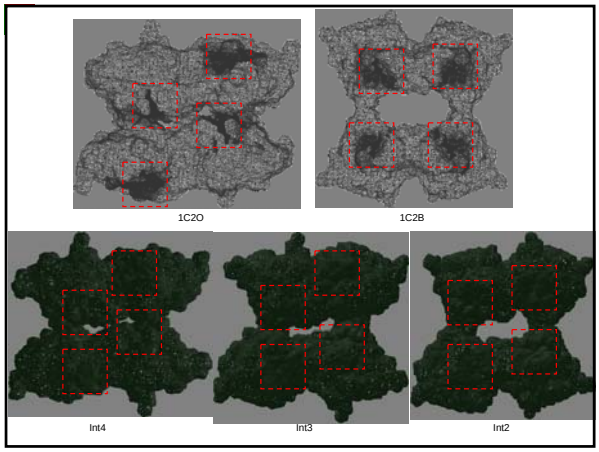
Joe-Liu: 0.999(best),0.0154(worst)

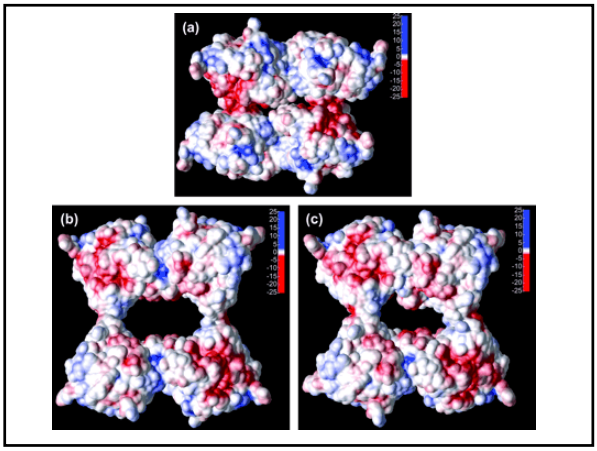
Edge-Ratio: 1.03(best), 8.48(worst)

Volume: 1.73e-4(S), 9.35e8(L)

Short Edge: 0.039(S), 363 (L)

Long Edge: 0.148 (S), 861 (L)





PMF Calculation

- APBS 0.4.0 (http://agave.wustl.edu/)
- Grid hierarchy (0-10)
- Apolar forces and dielectric boundary omitted
- No coupling b/w PMF and diffusing particle (Poisson-Nernst-Planck Eqn)
- No correlation b/w diffusing species

Grid#	dx(Å)	dy(Å)	dz(Å)	*x	*y	*z
0	0.44	0.38	0.38	-	-	-
1	0.89	0.78	0.80	2.0	2.0	2.0
2	1.33	1.16	1.20	1.5	1.5	1.5
3	2.00	1.73	1.80	1.5	1.5	1.5
4	3.00	2.60	2.71	1.5	1.5	1.5
5	4.49	3.91	4.07	1.5	1.5	1.5
6	6.73	5.87	6.11	1.5	1.5	1.5
7	10.11	8.80	9.16	1.5	1.5	1.5
8	15.16	13.20	13.73	1.5	1.5	1.5
9	22.73	19.80	20.60	1.5	1.5	1.5
10	34.09	29.71	30.89	1.5	1.5	1.5

SSSE Solver Details

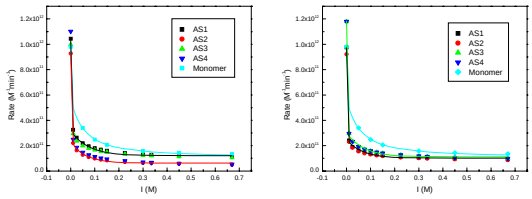
•Based on Mike Holst's Fetk (http://www.fetk.org)

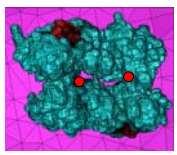
• Diffusing particle (based on TFK+): q = +1e, R=2.0 Å, D = 78000 Å^2/μs

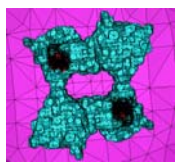
• Ionic strengths: 0.00, 0.01, 0.05, 0.10, 0.20, 0.300, 0.450, and 0.670 M.

• Reactive surface is assigned with zero Dirichlet boundary condition (perfectly absorbing)

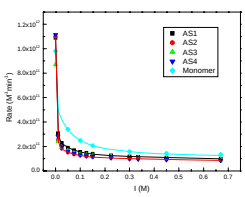
• p_{bulk} = 1.0 M

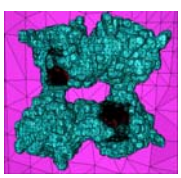






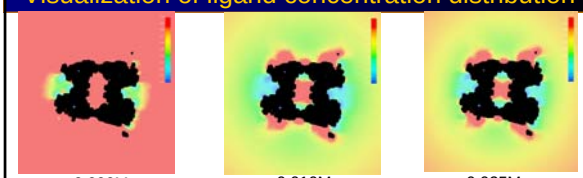
1C2O (compact) 1C2B (loose)



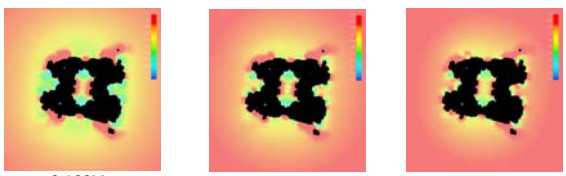


Intermediate b/w 1C2O and 1C2B

Visualization of ligand concentration distribution

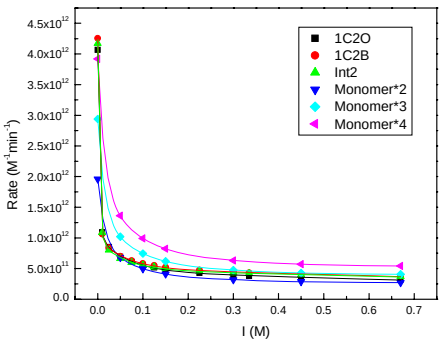


0.000M 0.010M 0.025M

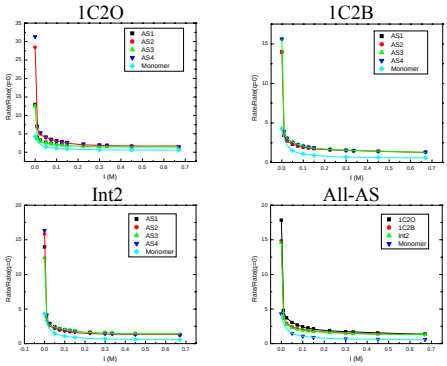


0.100M 0.300M 0.670M

All Active Sites



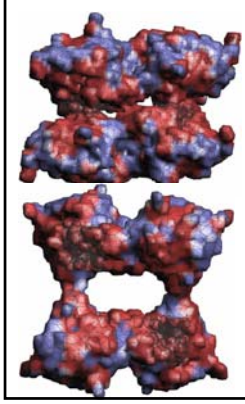
Electrostatic Guidance



What Does it Tell US

- Diffusion rate for the tetramer is between 2 and 3 times that of the monomer at physiological ionic strength (0.1 ~ 0.3 M). Therefore, no enhancing of the catalytic activity. while the tetramer is not perfectly efficient -- there is some competition among the active sites, so that the ratio is less than four -- the electrostatic steering does contribute to the efficiency.
- Tetrameric association probably facilitates the anchoring of AChE to the basal lamina, and it also increases the packing density of AChE.
- Conformational flexibility may be from t peptide-PRAD association, not a result of forming tetramer.
- Finite Element method is a powerful method that can be extended in the synapse simulation.

AChE Reaction Mechchanism



$$S + E \underset{k_s^-}{\overset{k_s^+}{\rightleftharpoons}} ES \xrightarrow{k_{cat}} E + P$$

$$S + SE \underset{k_s^-}{\overset{k_s^+}{\rightleftharpoons}} SES \xrightarrow{bk_{cat}} SE + P$$

θ_1 = [ES]
θ_2 = [SE]
θ_3 = [SES]

$$\frac{d\theta_1}{dt} = k_s^+[ACh](1 - \sum_{i=1}^{3}\theta_i) + k_{ss}^-\theta_3 - (k_s^- + k_{ss}^+[ACh] + k_{cat})\theta_1$$

$$\frac{d\theta_2}{dt} = k_{ss}^+[ACh](1 - \sum_{i=1}^{3}\theta_i) + k_s^-\theta_3 - (k_{ss}^+[ACh] + k_{ss}^-)\theta_2$$

$$\frac{d\theta_3}{dt} = k_{ss}^+[ACh]\theta_1 + k_s^+[ACh]\theta_2 - (k_s^- + k_{ss}^- + bk_{cat})\theta_3$$

Reaction defined Boundary Conditions

$$\hat{n}\cdot D\nabla p_{ACh}(r,t|r_0,t_0)=\begin{cases}-k'_{act,AChE}[k_s^+(1-\sum_{i=1}^3\theta_i)p_{ACh}-\frac{k_s^-\theta_1}{[ACh]_0}], & \text{on } \partial\Omega_{AChE}^{act}\\ 0, & \text{otherwise}\end{cases}$$

Find $p_{ACh}\in V\subset H^1$ such that

$$<F(p_{ACh}),v>=0\ \ \forall v\in V,$$

where

$$<F(p_{ACh},v)>=\int_\Omega(D\nabla p_{ACh}\cdot\nabla v+\frac{\partial p_{ACh}}{\partial t}v)dx$$
$$-\int_{\Omega_{AChE}^{act}}(-k'_{act,AChE}[k_s^+(1-\sum_{i=1}^3\theta_i)p_{ACh}-\frac{k_s^-\theta_1}{[ACh]_0}])dS$$

From SSSE to TDSE

$$\frac{\partial p(\vec{r},t\,|\,\vec{r}_0,t_0)}{\partial t}=-\nabla\cdot De^{-\beta U(\vec{r})}\nabla e^{\beta U(\vec{r})}p(\vec{r},t\,|\,\vec{r}_0,t_0)$$

SSSE:

$$\int_\Omega\nabla v(\vec{r})\cdot\vec{J}p(\vec{r})d\vec{r}^3-\int_{\Gamma_a}v(s)\alpha(s)p(s)ds-\int_{\Gamma_b}v(s)\vec{J}p(s)\cdot\vec{n}(s)ds=0$$

TDSE:

$$\int_\Omega(\nabla v(\vec{r})\cdot\vec{J}p(\vec{r})+\frac{dp}{dt})d\vec{r}^3-\int_{\Gamma_a}v(s)\alpha(s)p(s)ds-\int_{\Gamma_b}v(s)\vec{J}p(s)\cdot\vec{n}(s)ds=0$$

$$Au+\frac{M}{dt}(u-u^0)+Fu=0$$

Three schemes to do timestep marching

$$u_{n+1}=e^{-dt*M^{-1}*A}u_n\cong\frac{M-(1-\theta)*dt*A}{M+\theta*dt*A}u_n$$

1. Forward (explicit) Euler scheme

   |1- dt*M^{-1}*A| <= 1, θ = 0

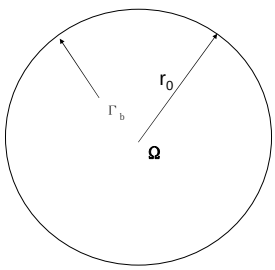
2. Backward (implicit) Euler scheme (unconditional stable)

   |1+ dt*M^{-1}*A| >= 1, θ = 1

3. Crank-Nicholson (implicit) scheme (unconditional stable)

   |2 + dt*M^{-1}*A| >= |2 - dt*M^{-1}*A|, θ = 0.5

Analytical test

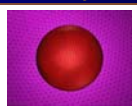


When the potential inside the sphere and the radius of the inner sphere are zero, the analytical solution can be easily written as below:

$$\begin{cases}\frac{\partial p(r;t)}{\partial t}-D\Delta_3 p(r;t)=0\\ p(r;0)=0\\ p(r_0;t)=p_{bulk}\end{cases}$$

$$p(r;t)=p_{bulk}+\frac{2p_{bulk}r_0}{\pi r}\sum_{n=1}^{\infty}\frac{(-1)^n}{n}\sin\frac{n\pi r}{r_0}\exp\left\{-D\left(\frac{n\pi}{r_0}\right)^2t\right\}$$

Analytical test

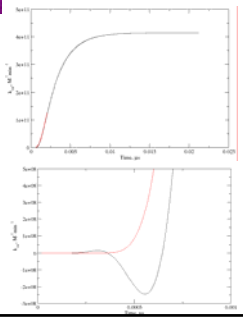


- Vertex number: 109,478
- Simplex number: 629,760

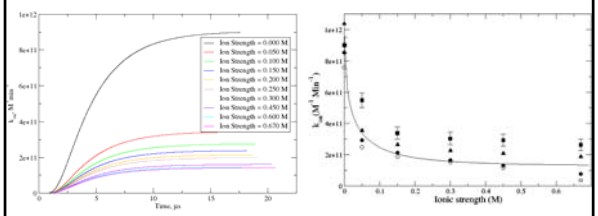
- Vertex number: 857,610
- Simplex number: 5,038,080

- Inner radius: 10 A
- Outer radius: 50 A

- Time step: 5 ps



TDSE on the mAChE monomer



✓At zero ion-strength, the whole system need nearly 20 μ s to reach steady state, and the higher ion strength, the shorter time need to reach steady state.

✓The steady state deriving from the TDSE is very close to the corresponding SSSE results.

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