All-atom Molecular Dynamics Simulations of EGFR with Prediction of Inhibitors Fold Resistance

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AMS 535

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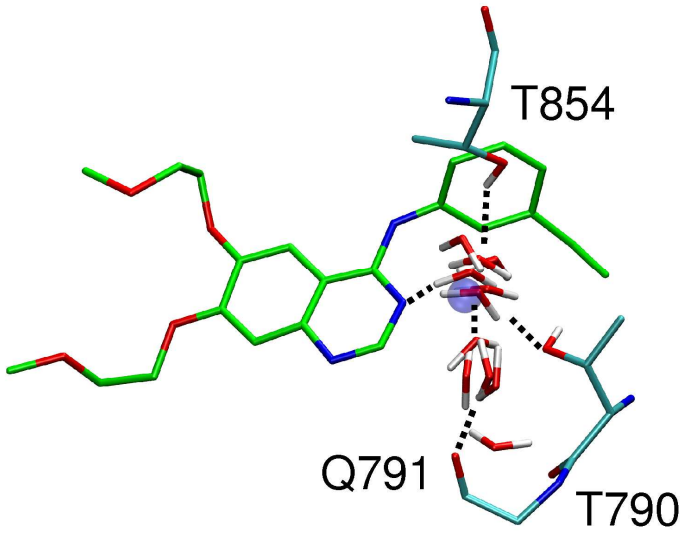
Overview

- Background on EGFR
  - Motivations
  - Literature binding values
- Molecular Dynamics Simulations
- Post-processing Methods
  - Background on MM-GBSA
  - Molecular Footprint
- Results
- Conclusions



Balius, T. E.; Rizzo, R. C., Quantitative prediction of fold resistance for inhibitors of EGFR. *Biochemistry* **2009,** 48, (35), 8435-48.

EGFR Background

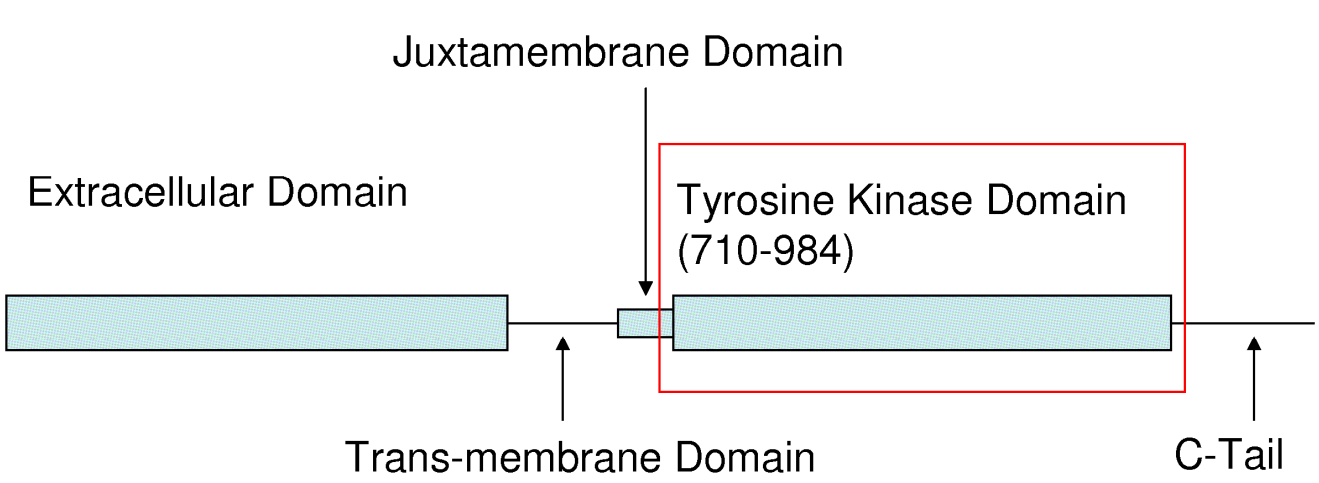
Cancer Background

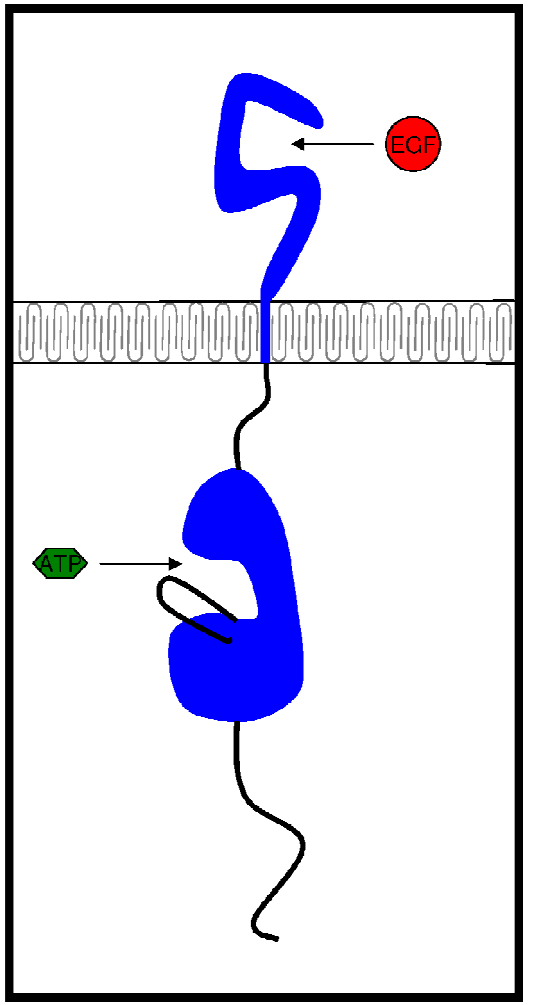
- leading cause of death in US under the age of 85
- 2nd highest cause of death in US
- lung & bronchial cancer is leading cause of death of cancer
- non-small cell lung cancer (NSCLC) largest subset of lung cancer
- EGFR is a target for NSCLC
- member of the ErbB family: EGFR, ErbB2, ErbB3, & ErbB4

Jemal, A., et al. (2008) Cancer statistics, 2008, CA Cancer J Clin 58, 71-96.

Hynes, N. E., and Lane, H. A. (2005) ERBB receptors and cancer: the complexity of targeted inhibitors, Nat Rev Cancer 5, 341-354.

EGFR: A Chemotherapeutic Target

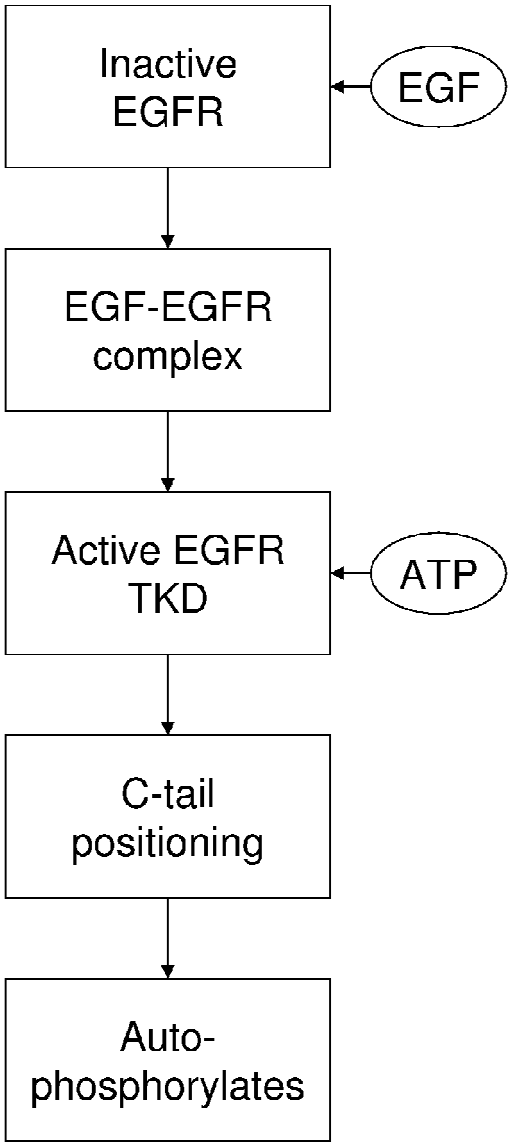


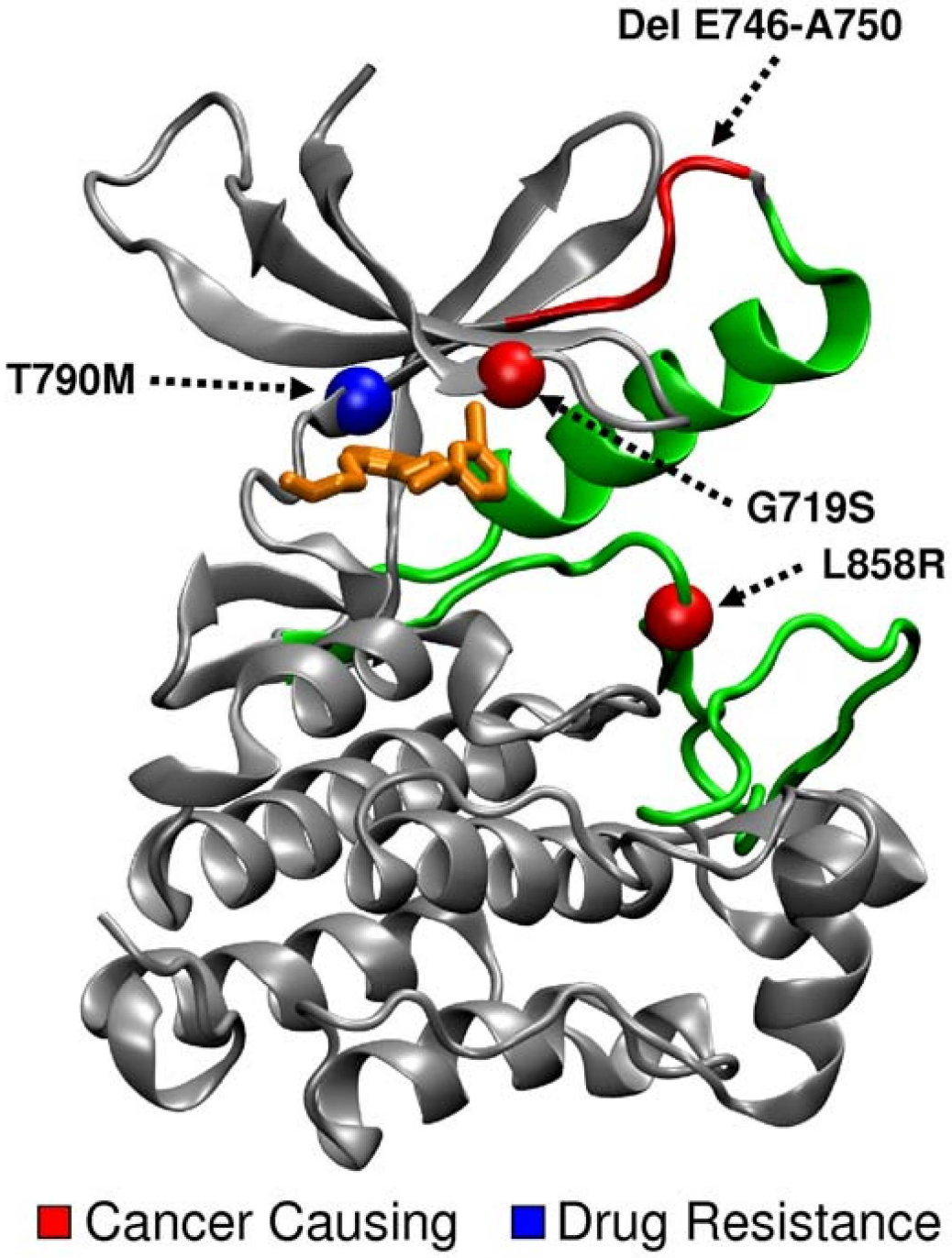


- two classes of anticancer agents
  - monoclonal antibodies (extracellular)
  - small molecule inhibitors (intracellular)

EGFR Pathway

- ligand-free EGFR is a monomer
- EGFR is inactive as a monomer
- EGF binds, the EGFR homo- or hetero dimerizes
- EGFR is active as a dimer
- ATP binds Tyrosine Kinase Domain (TKD)
  - EGFR auto-phosphorylates C-tail
- leads to signal transduction
  - pathways involved in cellular proliferation





Key EGFR Mutations

- Cancer Causing
  - L858R

    increase binding to erlotinib & gefitinib
  - Del E746-A750

    increase binding to erlotinib & gefitinib
  - G719S

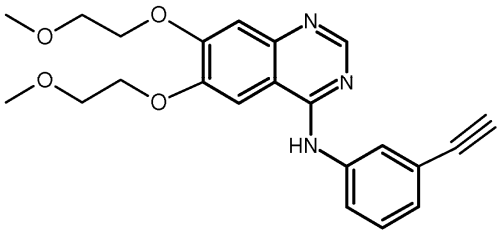
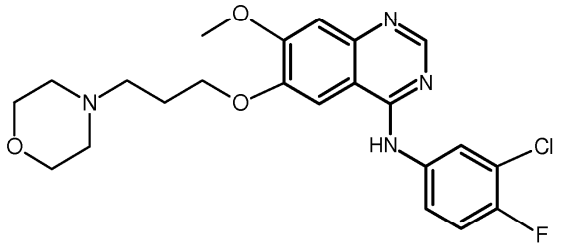
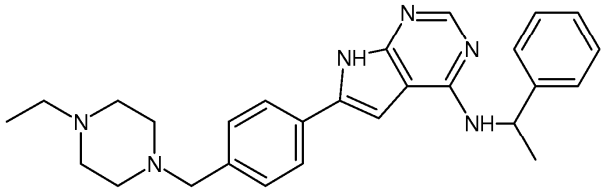
    decrease binding to gefitinib

- Drug Resistance
  - T790M

    decrease binding to all

Ligands

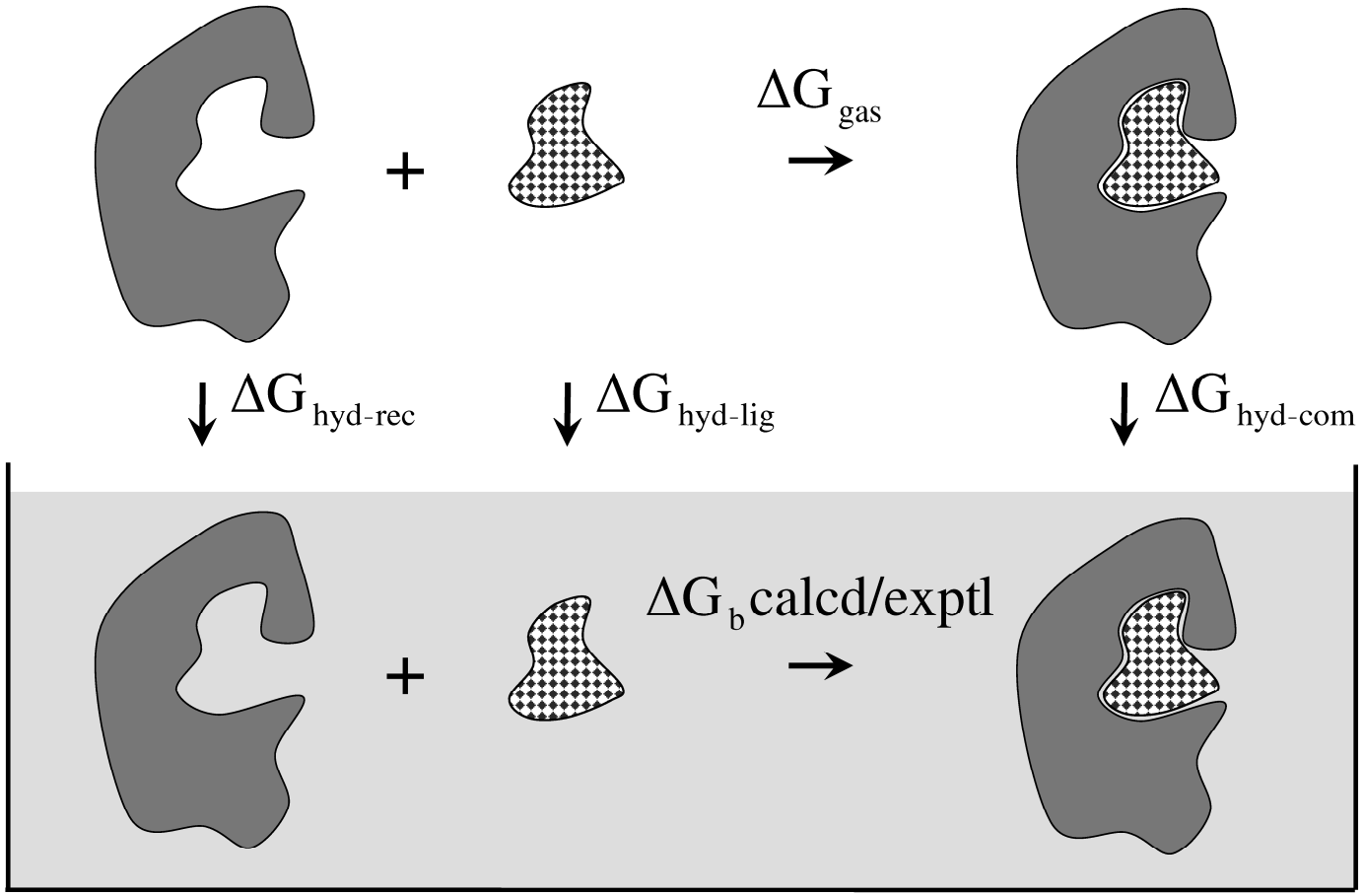
Table 1. Experimental Fold Resistance (FR) values for ATP-competitive inhibitors with EGFR.

Inhibitor	Structure	Experimental Fold Resistance[a]		
		L858R / WT	**L858R&T790M / L858R**	**G719S / WT**
erlotinib		6.25 / 17.5 nM[b] 0.36 FR −0.61 $\Delta\Delta G_{FR}$	>10000 / 12.5 nM[c] >800 FR >3.96 $\Delta\Delta G_{FR}$	–
gefitinib		2.4 / 35.3 nM[d] 0.068 FR −1.59 $\Delta\Delta G_{FR}$	10.9 / 2.4 nM[d] 4.54 FR 0.90 $\Delta\Delta G_{FR}$	123.6 / 53.5 nM[e] 2.31 FR 0.50 $\Delta\Delta G_{FR}$
AEE788		1.1 / 5.3 nM[d] 0.21 FR −0.92 $\Delta\Delta G_{FR}$	18.6 / 1.1 nM[d] 16.9 FR 1.68 $\Delta\Delta G_{FR}$	11.3 / 10.9 nM[e] 1.04 FR 0.02 $\Delta\Delta G_{FR}$

[a]Fold Resistance (FR) = ratio of experimental activities. $\Delta\Delta G_{FR}$ exptl ≈ RTln(FR) at 298.15 K in kcal/mol. [b]Ki values (nM) from Carey, K. D., et al. , Cancer Res 66, 8163-8171. (2006). [c] IC_{50} values (nM) from Ji, H., et al., Proc Natl Acad Sci U S A 103, 7817-7822. (2006). [d] Kd values (nM) from Yun, C. H., et al., Proc Natl Acad Sci U S A 105, 2070-2075. (2008). [e]Kd values (nM) from Yun, C. H., et al., Cancer Cell 11, 217-227. (2007).

Using Molecular Dynamics generated ensembles

Thermodynamic Cycle

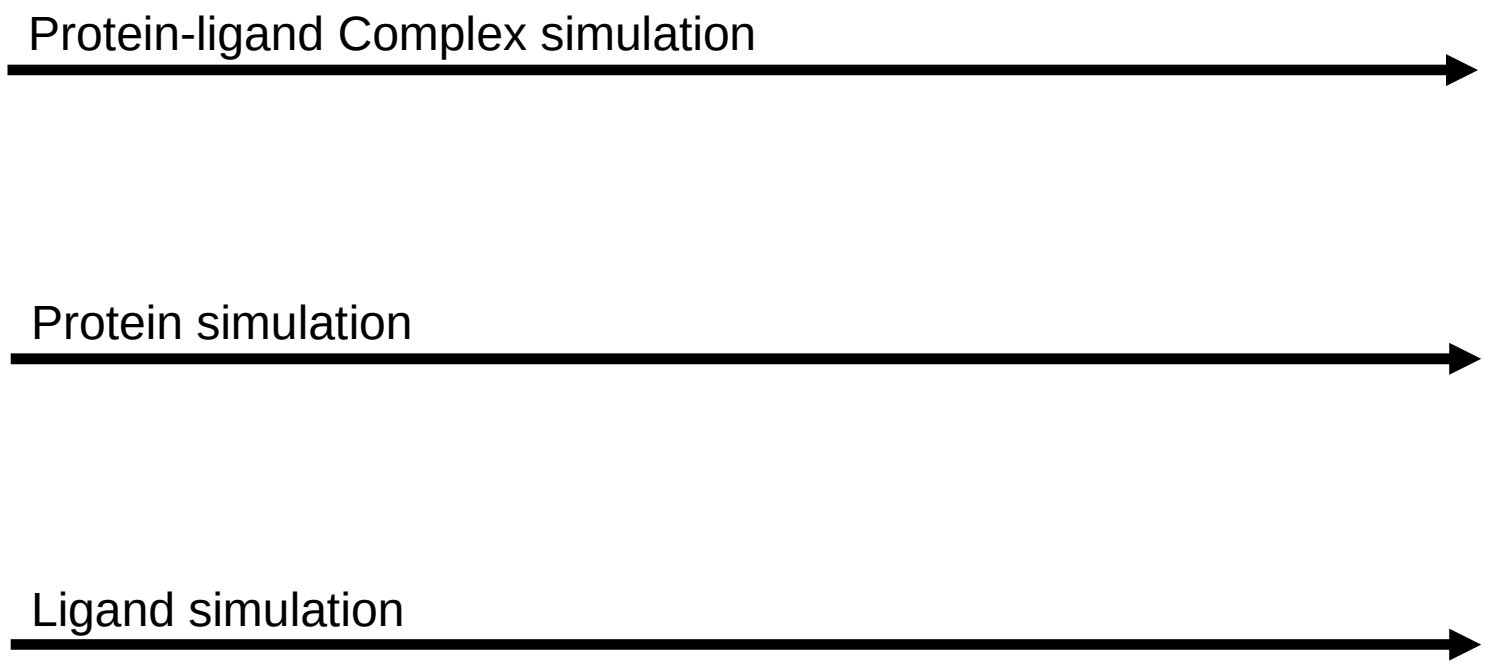


$$\Delta G_b \text{ exptl} \approx \Delta G_b \text{ calcd} = \Delta G_{gas} + \Delta G_{hyd\text{-}com} - (\Delta G_{hyd\text{-}rec} + \Delta G_{hyd\text{-}lig})$$

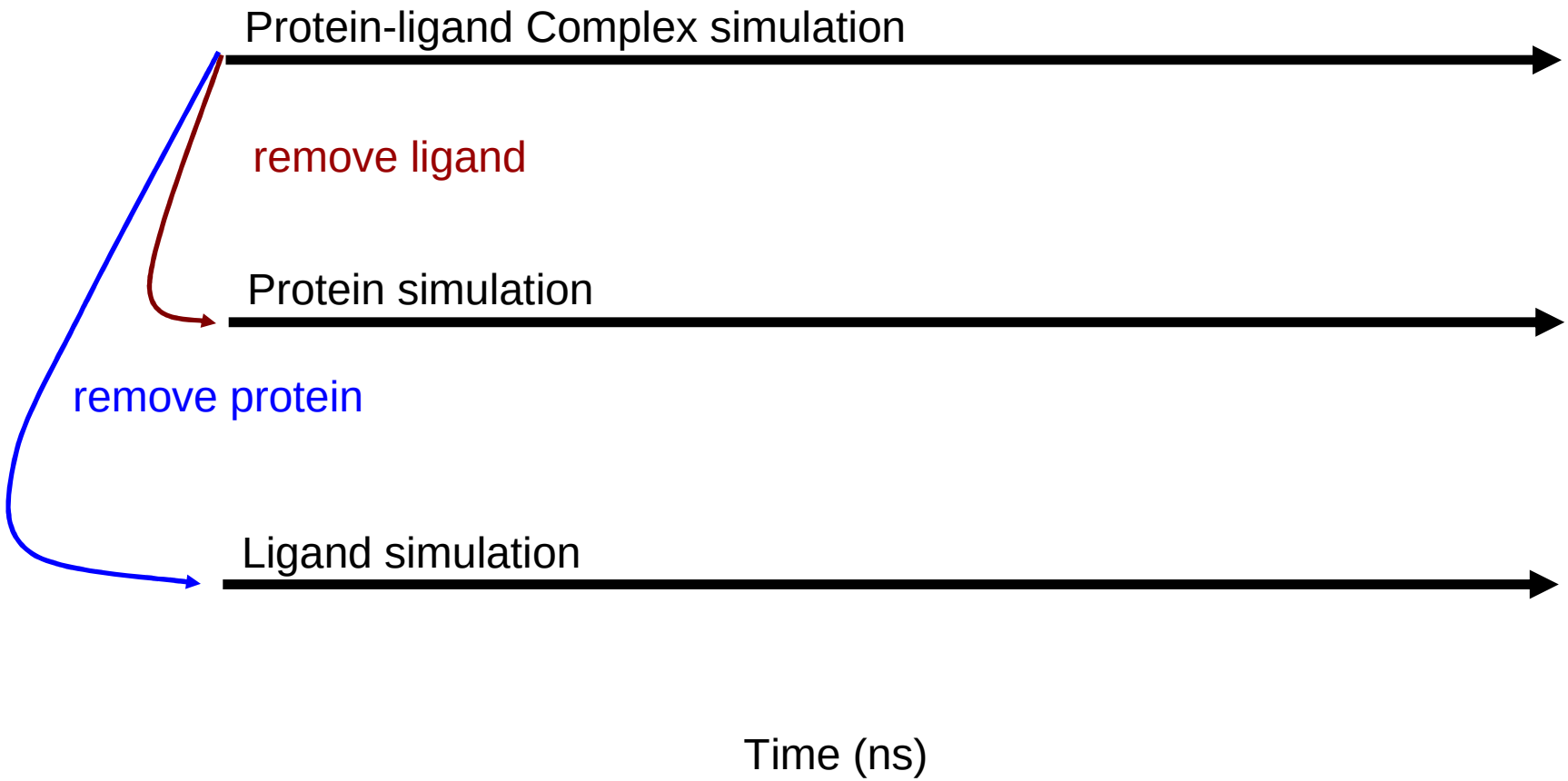
$$\Delta G_{gas} = \Delta E_{vdw} + \Delta E_{coul}$$

$$\Delta G_{hyd\text{-}species} = \Delta G_{polar} + \Delta G_{nonpolar}$$

Run 3 independent Simulations



Run 1 Simulations



Simulation Methods

- force field: FF99SB (protein); GAFF (ligand) augmented with CHELPG charges (6-31G* basis set);TIP3P (water)

- 9 step equilibration (minimization + MD) with decreasing restraints (amber 8 package)

- final production run for 5 ns @ 298.15 K
  - constant T & P with periodic boundary conditions

- post processing
  - root mean-squared deviations (RMSD)
  - ΔG_{bind} estimation via MM-GBSA method
  - energy component decomposition
  - per-residue H-bond and energetic footprints

MM-GBSA Background

MM-GBSA Equations

$$G = E_{MM} + \Delta G_{hyd} - TS$$

such that

$$E_{MM} = E_{bond} + E_{angle} + E_{tors} + E_{vdw} + E_{es}$$

TS is calculated using quasi harmonic analysis normal mode analysis

$$\Delta G_{hyd} = \Delta G_{polar} + \Delta G_{nonpolar}$$

where the polar and the nonpolar terms are defined in the following way

ΔG_{polar} - is defined by solving the PB set of differential equations

or by using the GB equation.

$$\Delta G_{nonpolar} = \alpha \cdot SA + \beta$$

$$\Delta G = G_{complex} - G_{protein} - G_{ligand}$$

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Generalized Born Equations

$$G_{GB} = -166\left(1-\frac{1}{\varepsilon}\right)\sum_{i=1}^{n}\sum_{\substack{j=1\\ j\neq i}}^{n}\frac{q_i q_j}{f_{GB}\left(r_{i,j},\alpha_{i,j}\right)} \qquad \alpha_{i,j} = \sqrt{\alpha_i \alpha_j}$$

$$f_{GB}(r,\alpha) = \sqrt{r^2 + \alpha^2 \exp\left(-D(r,\alpha)\right)} \qquad D(r,\alpha) = \frac{r^2}{4\alpha^2}$$

- The trick to GB is calculating the Born Radii
- Born Radii are dependent on conformation

Still, W. C.; et al., J. Am. Chem. Soc 1990, 112, 6127-6129

Hawkins Born Radii Model

$$\hat{\alpha}_i = \frac{1}{\frac{1}{\rho_i} - \frac{1}{2}\sum_{j=1}^{n}\left[\left(\frac{1}{L_{i,j}} - \frac{1}{U_{i,j}}\right) + \frac{r_{i,j}}{4}\left(\frac{1}{L_{i,j}^{\,2}} - \frac{1}{U_{i,j}^{\,2}}\right) + \cdots \left(\frac{1}{2r_{i,j}}\ln\frac{L_{i,j}}{U_{i,j}}\right) + \left(\frac{1}{L_{i,j}} - \frac{1}{U_{i,j}}\right)\right]}$$

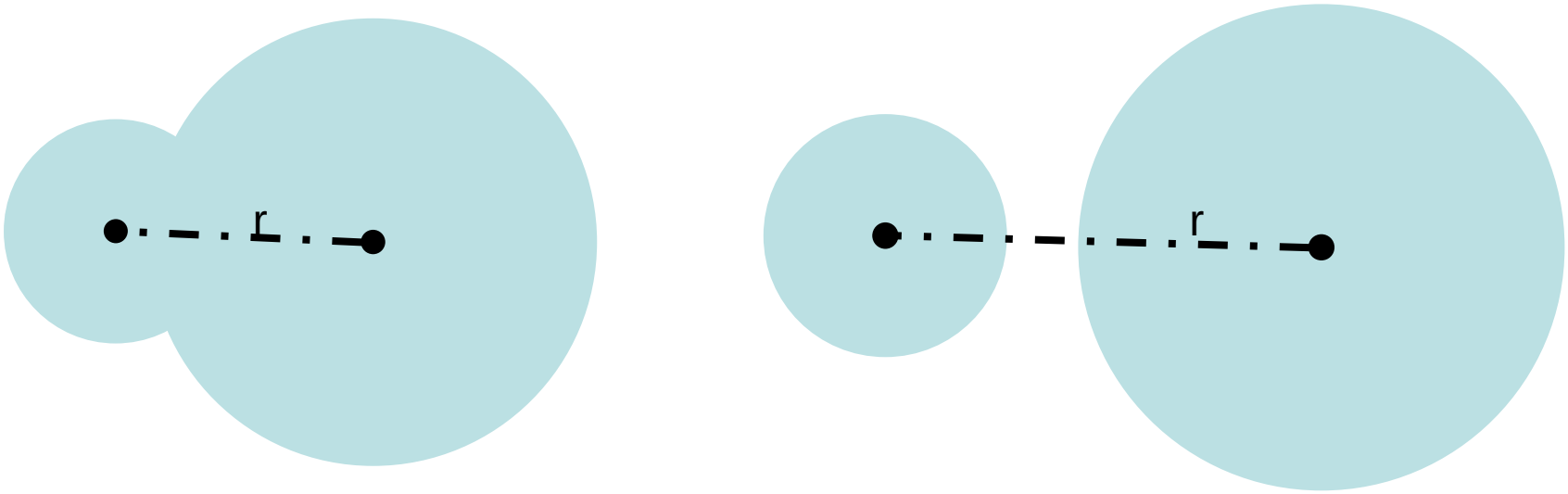
$$\alpha_i = \max\left(0, \hat{\alpha}_i\right)$$

$$L_{i,j} = \begin{cases} 1 & \text{if } r_{i,j} + s_j\rho_j \le \rho_i \\ \rho_i & \text{if } r_{i,j} - s_j\rho_j \le \rho_i < r_{i,j} + s_j\rho_j \\ r_{i,j}\text{-}s_j\rho_j & \text{if } \rho_i \le r_{i,j}\text{-}s_j\rho_j \end{cases}$$

$$U_{i,j} = \begin{cases} 1 & \text{if } r_{i,j} + s_j\rho_j \le \rho_i \\ r_{i,j}\text{-}s_j\rho_j & \text{if } \rho_i < r_{i,j} + s_j\rho_j \end{cases}$$

Hawkins, G. D. et al. J. Phys. Chem., Vol. 100, No. 51, 1996 19824-19839

Generalized Born as a Function of atom position



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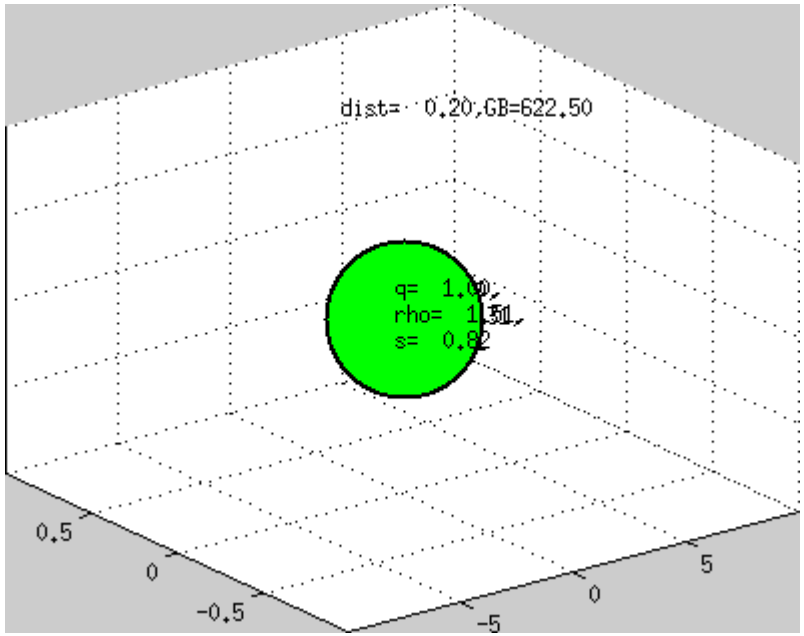
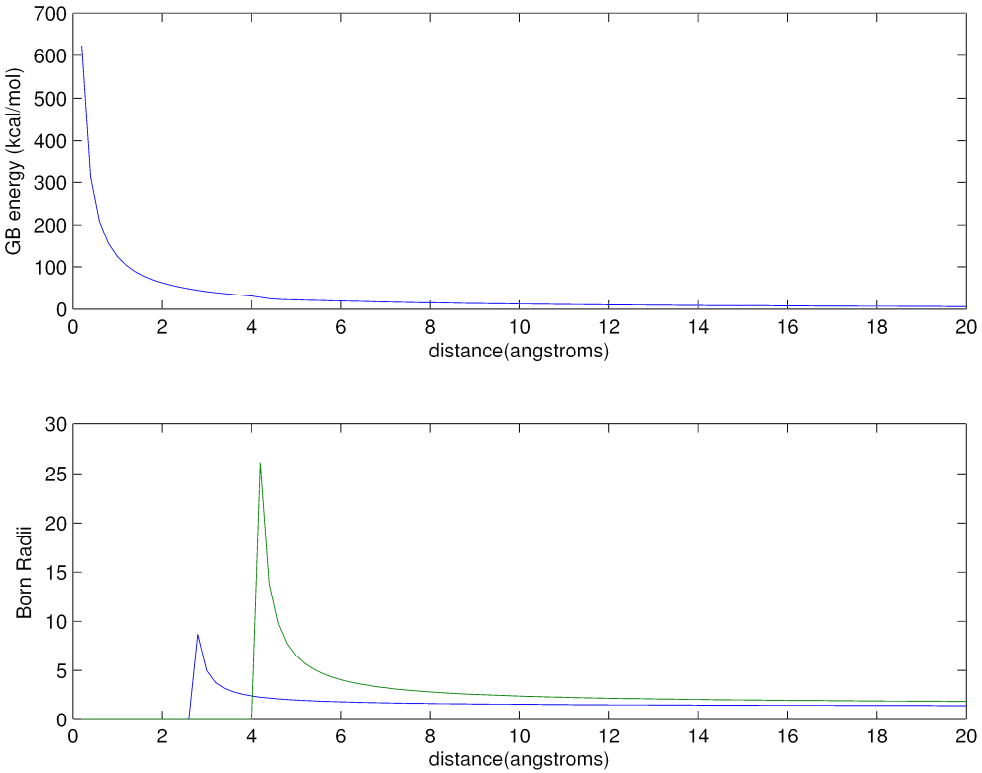


2 atoms movie



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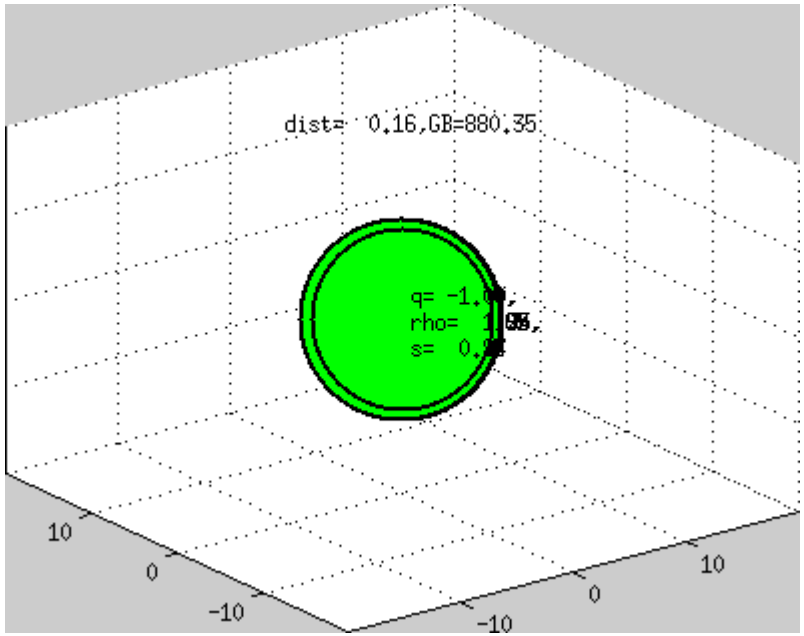
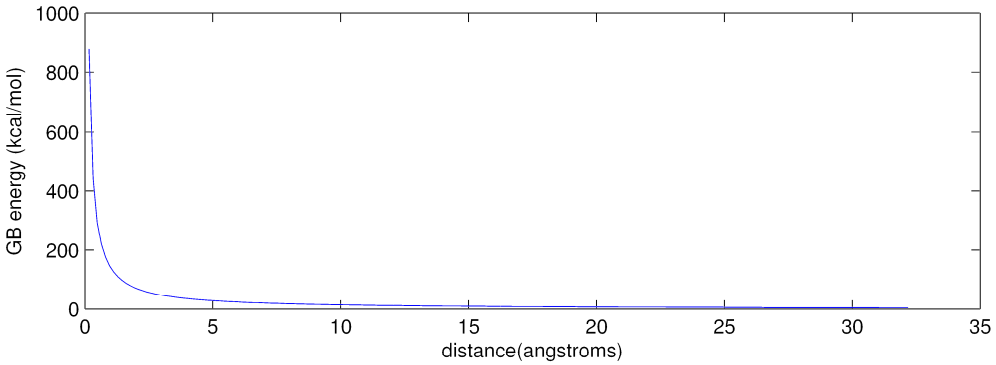
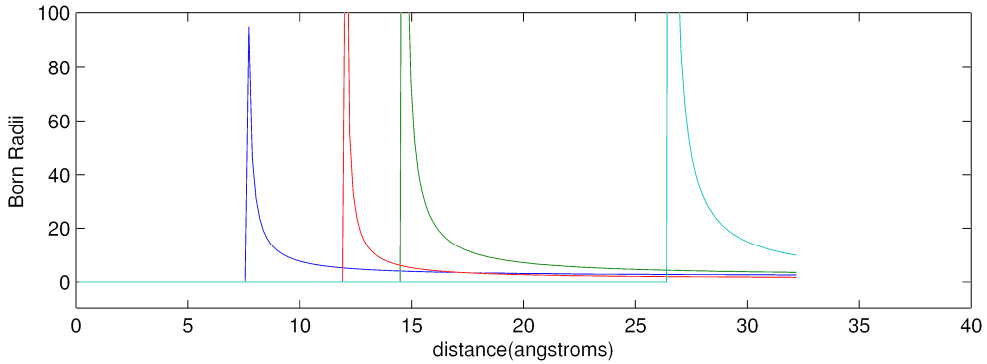


4 atoms movie



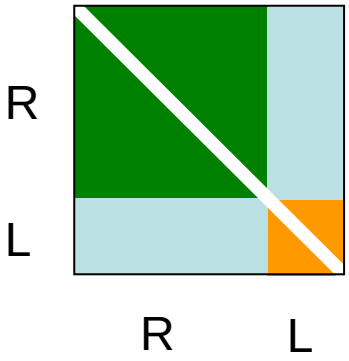


Molecular Footprints -- Per-residue Decomposition of Interactions

Footprint Introduction

$$E_{\text{vdw}}\text{comp} = \frac{1}{2}\sum_{a\in\text{comp}}\sum_{\substack{b\in\text{comp}\\ \text{s.t. } b\neq a}} E_{\text{vdw}}(a,b)$$

pair wise interaction



$$= \frac{1}{2}\sum_{a\in\text{rec}}\sum_{b\in\text{rec}} E_{\text{vdw}}(a,b) + \frac{1}{2}\sum_{a\in\text{lig}}\sum_{b\in\text{lig}} E_{\text{vdw}}(a,b) + \sum_{a\in\text{lig}}\sum_{b\in\text{rec}} E_{\text{vdw}}(a,b)$$

$$= E_{\text{vdw}}\text{rec} + E_{\text{vdw}}\text{lig} + \sum_{a\in\text{lig}}\sum_{b\in\text{rec}} E_{\text{vdw}}(a,b)$$

intermolecular component

$$\Delta E_{\text{vdw}} = E_{\text{vdw}}\text{comp} - (E_{\text{vdw}}\text{rec} + E_{\text{vdw}}\text{lig})$$

vdw component of binding energy

$$= \sum_{a\in\text{lig}}\sum_{b\in\text{rec}} E_{\text{vdw}}(a,b)$$

This same analysis can be done for other through space interactions

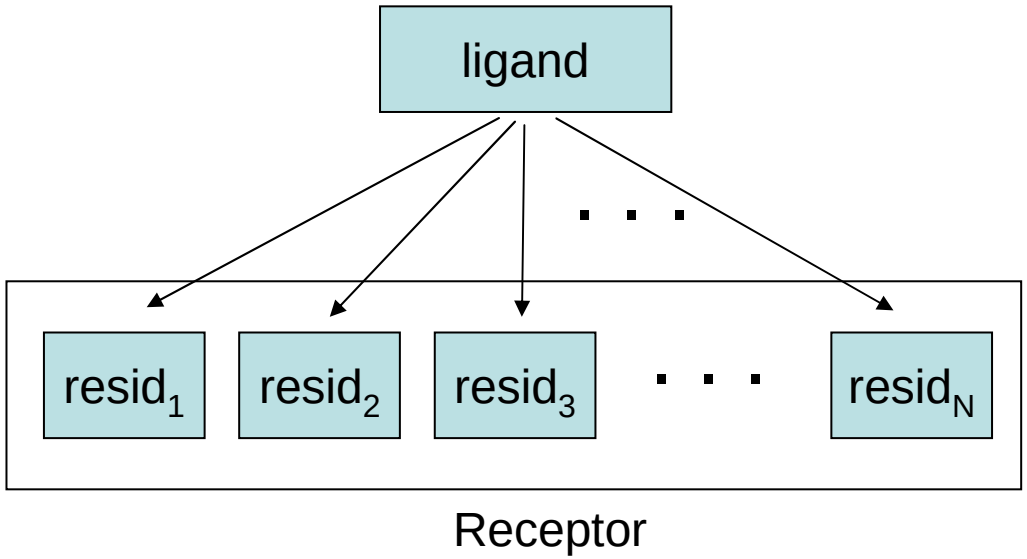
Coulombic, GB, SASA, . . .

Footprint Introduction

$$\Delta E_{\text{vdw}} = \sum_{a \in \text{lig}} \sum_{b \in \text{rec}} E_{\text{vdw}}(a,b)$$

$$= \sum_{a \in \text{lig}} \left[\sum_{i=1}^{N} \left(\sum_{b \in \text{resid}(i)} E_{\text{vdw}}(a,b) \right) \right]$$

$$= \sum_{i=1}^{N} \left[\sum_{a \in \text{lig}} \left(\sum_{b \in \text{resid}(i)} E_{\text{vdw}}(a,b) \right) \right]$$



$$\Delta E_{vdw} = \text{sum}\left(\vec{E}_{\text{vdw,fp}}\right)$$

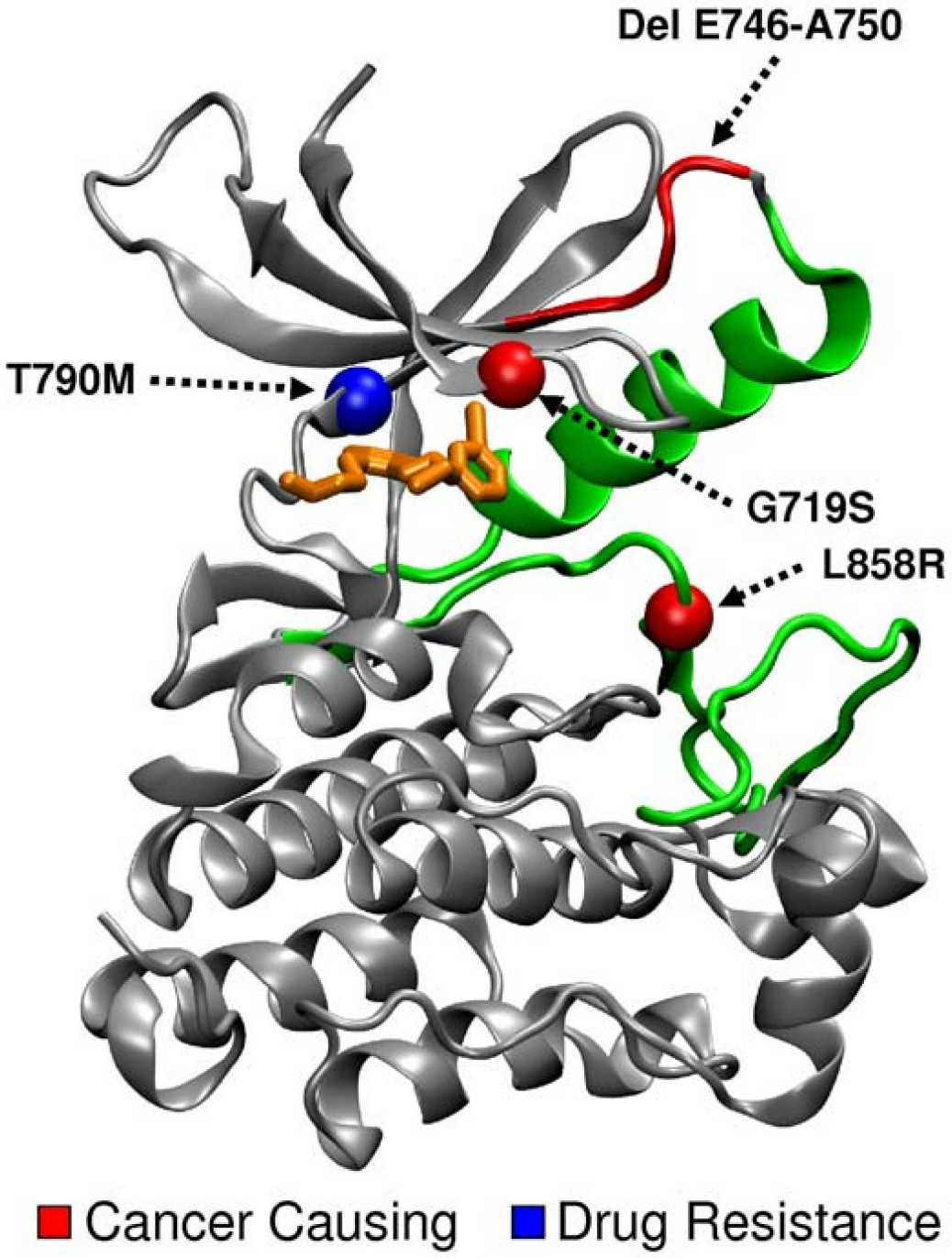
$$\vec{E}_{\text{vdw, fp}} = \left[\sum_{a \in \text{lig}} \left(\sum_{b \in \text{resid}(i)} E_{vdw}(a,b) \right) \right]$$

footprint vector each element corresponds to a residue

This same analysis can be done for other through space interactions

Coulombic, GB, SASA, . . .

Results



Key EGFR Mutations

- Cancer Causing
  - L858R

    increase binding to erlotinib & gefitinib
  - Del E746-A750

    increase binding to erlotinib & gefitinib
  - G719S

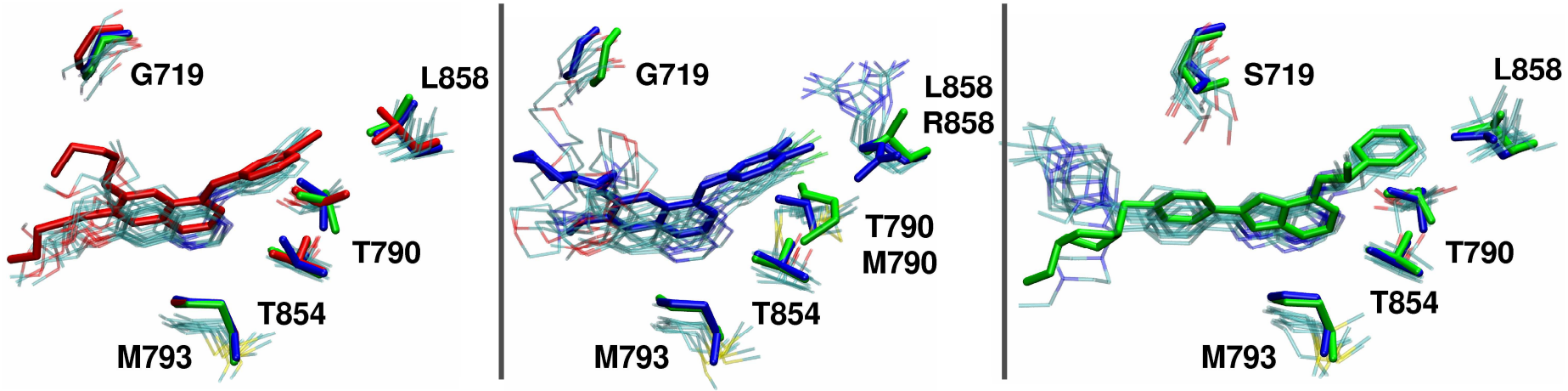
    decrease binding to gefitinib

- Drug Resistance
  - T790M

    decrease binding to all

Simulations vs. Crystallographic Structures

(a) erlotinib **(b) gefitinib** **(c) AEE788**



red=1M17 (erlotinib, WT)

blue=2ITY (gefitinib,WT)

green=2J6M (AEE788, WT)

blue=2ITZ (gefitinib,L858R)

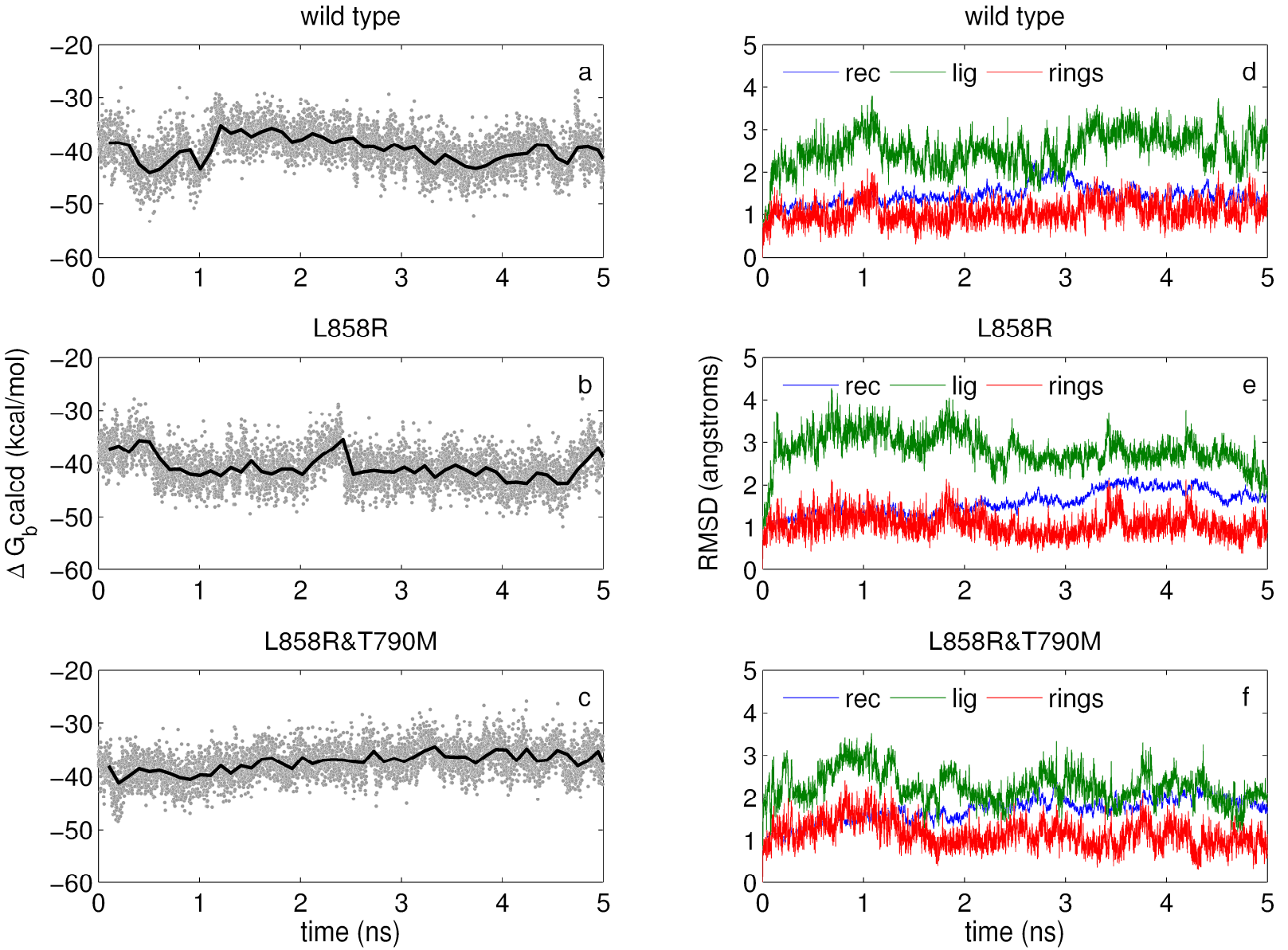
green=2JIU (AEE788,T790M)

blue=2ITO (gefitinib, G719S)

green=2ITP (AEE788, G719S)

Overlaid MD snapshots (thin lines N=10) with available crystal structure complexes of EGFR (bold lines)

Simulation and System Stability: Erlotinib



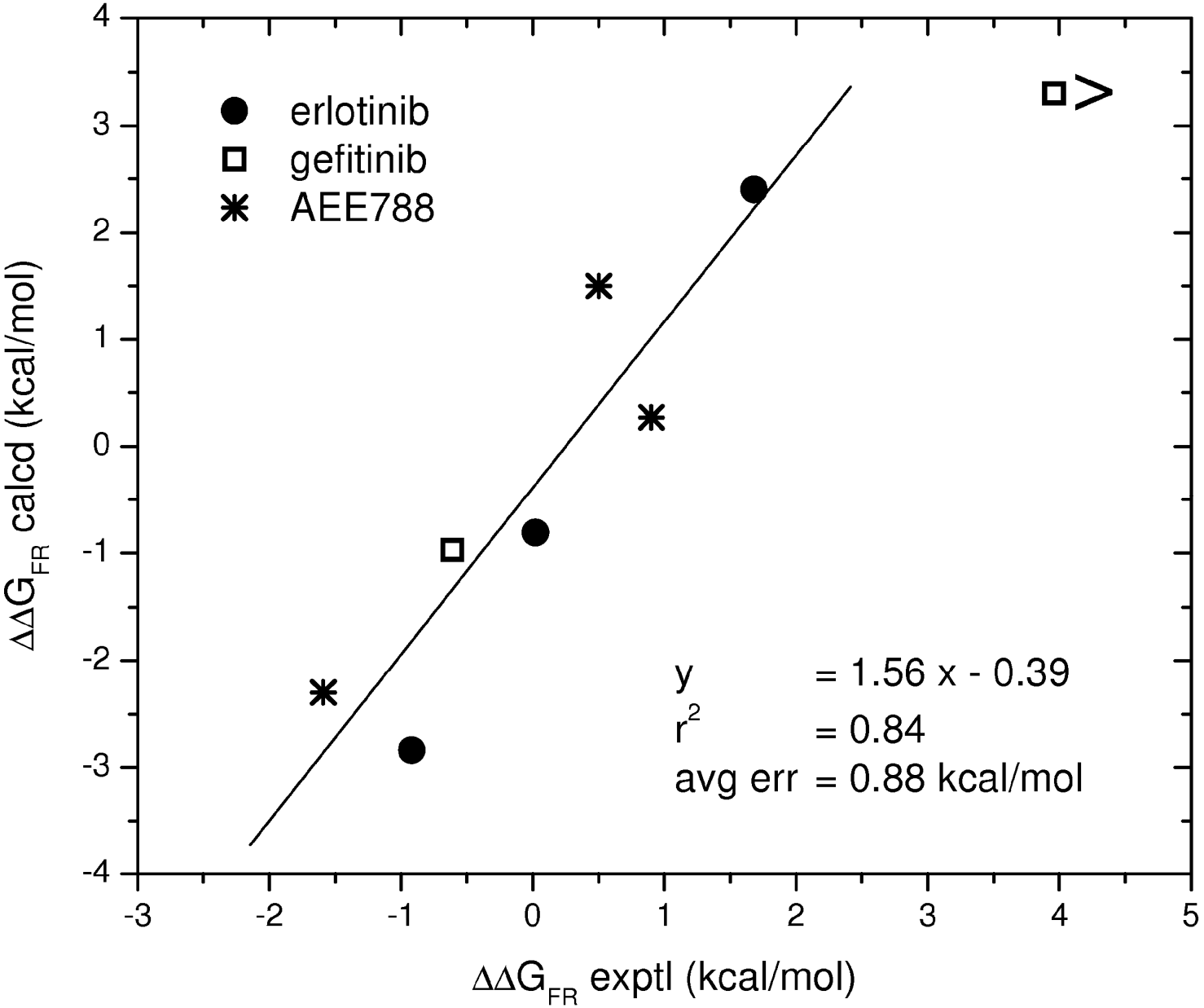
Relative Free Energies and Components

Table 2. Experimental versus calculated Fold Resistance (FR) energies ($\Delta\Delta G_{FR}$) and energy components for ligands with EGFR.

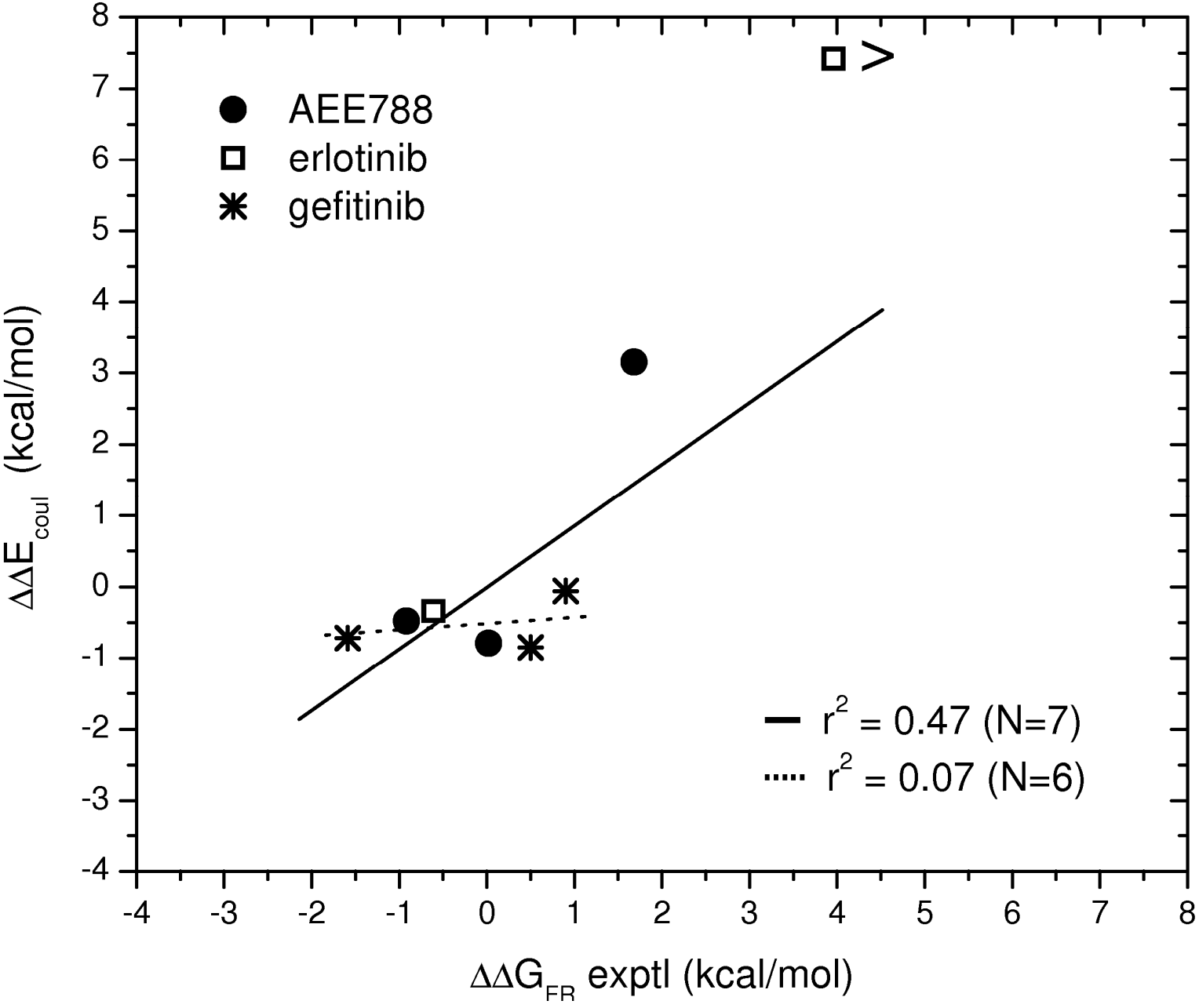
inhibitor	$\Delta\Delta E_{vdw}$ A	$\Delta\Delta E_{coul}$ B	$\Delta\Delta G_{polar}$ C	$\Delta\Delta G_{nonpolar}$ D	$\Delta\Delta G_{FR}$ calcd E =(A+B+C+D)	$\Delta\Delta G_{FR}$ exptl F
			L858R – WT			
erlotinib	–0.86 ± 0.06	–0.34 ± 0.13	0.21 ± 0.11	0.06 ± 0.003	–0.97 ± 0.07	–0.61
gefitinib	–0.99 ± 0.06	–0.72 ± 0.07	–0.58 ± 0.06	–0.01 ± 0.004	–2.30 ± 0.07	–1.59
AEE788	–2.41 ± 0.06	–0.48 ± 0.07	0.36 ± 0.06	–0.30 ± 0.005	–2.84 ± 0.06	–0.92
			L858R&T790M – L858R			
erlotinib	2.30 ± 0.06	7.42 ± 0.11	–6.56 ± 0.10	0.09 ± 0.003	3.30 ± 0.06	>3.96
gefitinib	–0.10 ± 0.05	–0.06 ± 0.07	0.49 ± 0.06	–0.06 ± 0.004	0.27 ± 0.06	0.90
AEE788	3.39 ± 0.07	3.15 ± 0.09	–4.33 ± 0.07	0.20 ± 0.004	2.40 ± 0.08	1.68
			G719S – WT			
erlotinib	–2.08 ± 0.06	–0.05 ± 0.12	–0.24 ± 0.11	0.04 ± 0.003	–2.38 ± 0.07	not reported
gefitinib	0.74 ± 0.07	–0.85 ± 0.07	1.59 ± 0.07	0.04 ± 0.004	1.50 ± 0.08	0.50
AEE788	–0.65 ± 0.06	–0.78 ± 0.06	0.55 ± 0.05	0.08 ± 0.005	–0.81 ± 0.07	0.02
r^2 =	0.70	0.47	0.19	0.30	0.84	7 data points[c]

[a]$\Delta\Delta G_{FR}$ calcd derived from the difference of two independent simulations (eg L858R – WT) computed using eqs 1-3. [b]$\Delta\Delta G_{FR}$ exptl values from Table 1. Correlations coefficients (r^2 values) obtained from fitting the change in each energy component to $\Delta\Delta G_{FR}$ exptl. All energies in kcal/mol ± standard errors of the mean from 5000 MD snapshots. [c]Data point for erlotinib with double mutant (>3.96) excluded from r^2 calculations given ambiguity in the experimental $\Delta\Delta G_{FR}$ measurement.

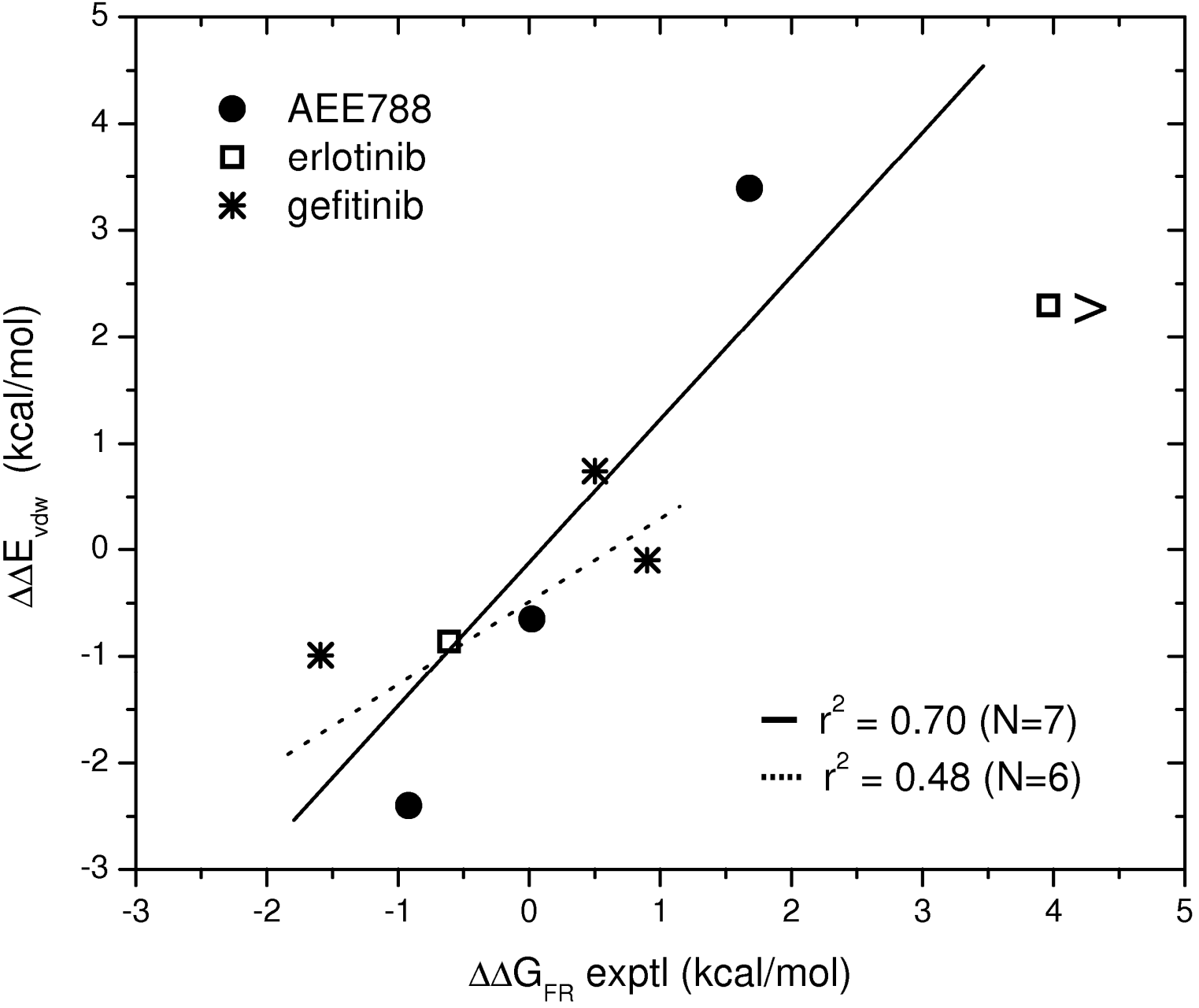
Correlation With Experimental Fold Resistance



Component Correlations



Component Correlations



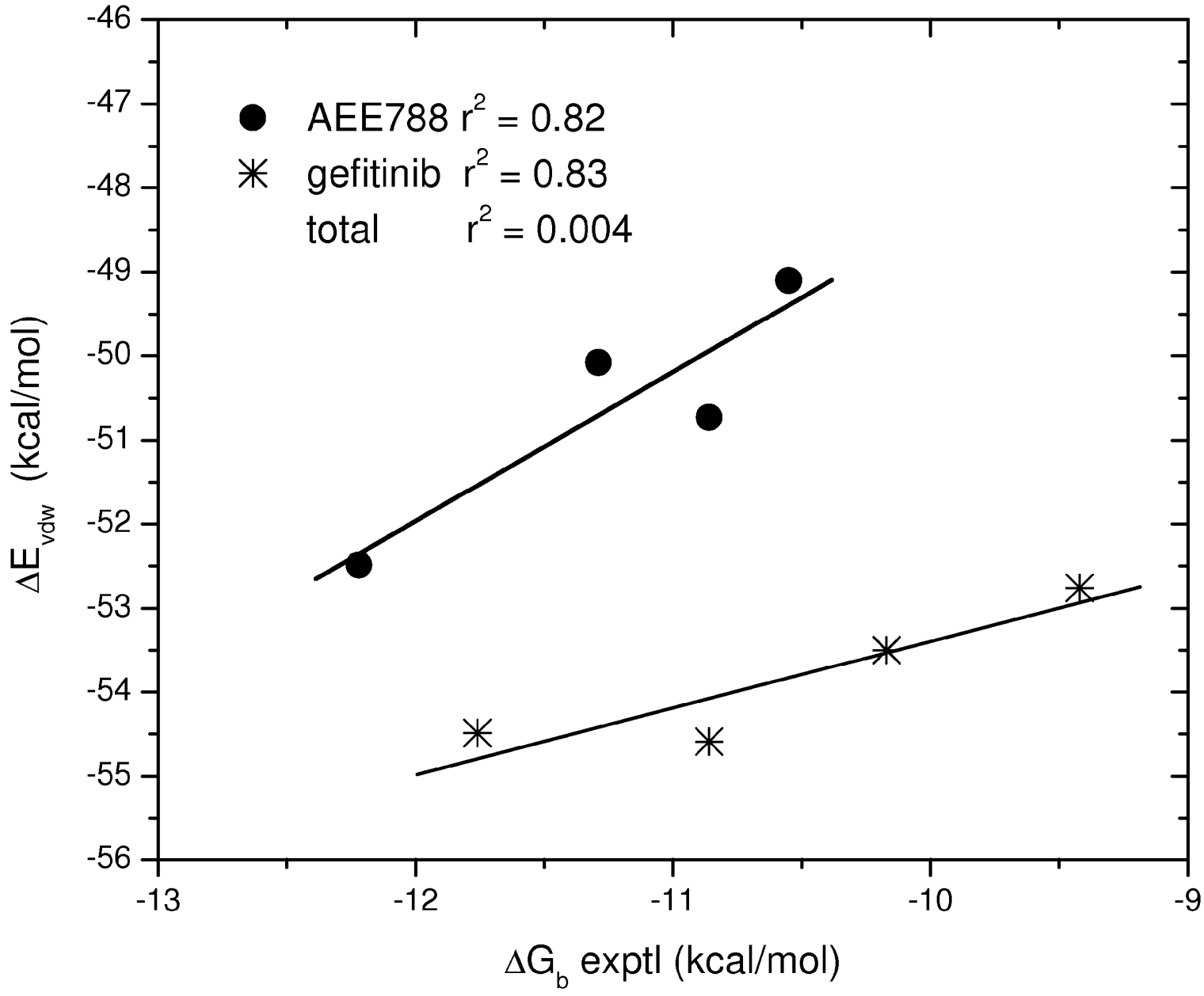
Absolute Free Energies and Components

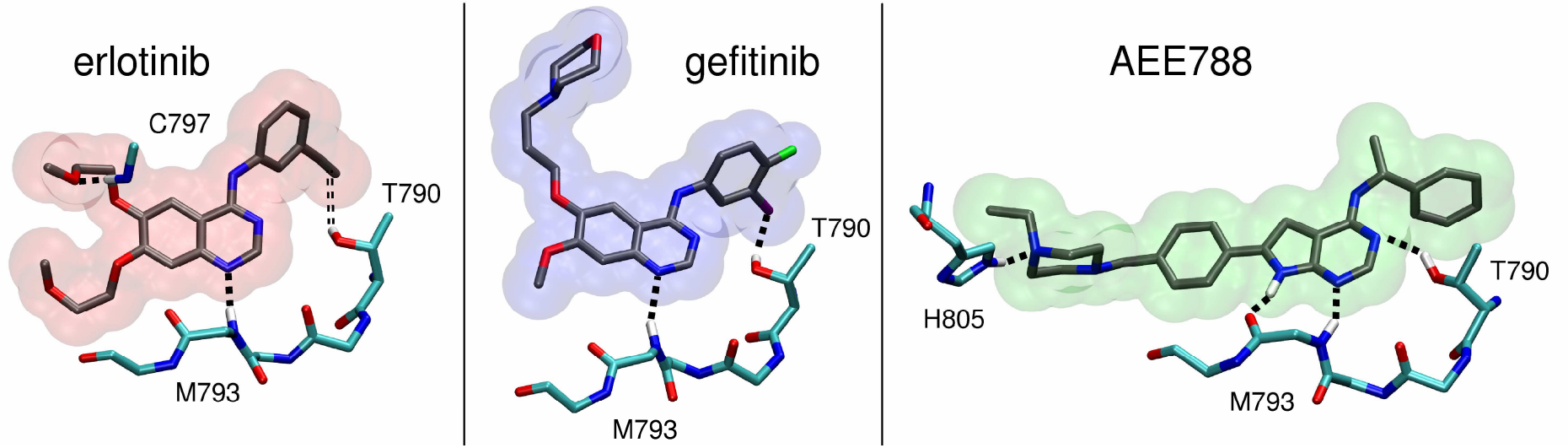
Table 3. Absolute free energies and component decomposition for inhibitors with EGFR.

system	ΔE_{vdw} A	ΔE_{coul} B	ΔG_{polar} C	$\Delta G_{nonpolar}$ D	ΔG_b calcd E=A+B+C+D	ΔG_b exptl[a] F	Hbond G
			erlotinib				
wildtype	−49.01 ± 0.04	−24.71 ± 0.09	39.73 ± 0.08	−6.05 ± 0.002	−39.69 ± 0.05	−10.58[b]	1.82
L858R	−49.86 ± 0.04	−25.04 ± 0.09	39.94 ± 0.07	−5.99 ± 0.002	−40.66 ± 0.05	−11.19[b]	2.17
L858R&T790M	−47.57 ± 0.05	−17.62 ± 0.07	33.38 ± 0.06	−5.89 ± 0.002	−37.36 ± 0.04	> −6.82[c]	0.99
G719S	−51.09 ± 0.04	−24.76 ± 0.08	39.49 ± 0.07	−6.01 ± 0.003	−42.07 ± 0.05	not reported	1.95
			gefitinib				
wildtype	−53.50 ± 0.05	−14.02 ± 0.05	28.80 ± 0.04	−6.30 ± 0.003	−45.01 ± 0.06	−10.17[d]	1.16
L858R	−54.49 ± 0.04	−14.74 ± 0.04	28.22 ± 0.04	−6.31 ± 0.003	−47.32 ± 0.05	−11.76[d]	1.24
L858R&T790M	−54.59 ± 0.04	−14.80 ± 0.05	28.71 ± 0.05	−6.37 ± 0.003	−47.05 ± 0.05	−10.86[d]	1.05
G719S	−52.76 ± 0.04	−14.87 ± 0.06	30.39 ± 0.05	−6.26 ± 0.002	−43.51 ± 0.05	−9.42[e]	1.08
			AEE788				
wildtype	−50.08 ± 0.05	−21.77 ± 0.04	31.97 ± 0.03	−5.93 ± 0.004	−45.81 ± 0.05	−11.29[d]	2.02
L858R	−52.49 ± 0.04	−22.26 ± 0.06	32.33 ± 0.05	−6.24 ± 0.003	−48.65 ± 0.04	−12.22[d]	2.19
L858R&T790M	−49.10 ± 0.06	−19.11 ± 0.07	28.00 ± 0.05	−6.03 ± 0.003	−46.25 ± 0.07	−10.55[d]	2.48
G719S	−50.73 ± 0.04	−22.56 ± 0.04	32.52 ± 0.03	−5.85 ± 0.003	−46.62 ± 0.04	−10.86[e]	1.99

[a]ΔG_b exptl ≈ RTln(activities) at 298.15 K in kcal/mol. [b]Ki values (nM) from Carey et al. [c]IC_{50} values (nM) from Ji et al. [d]Kd values (nM) from Yun et al (2008). [e]Kd values (nM) from Yun et al (2007).

Component Correlations

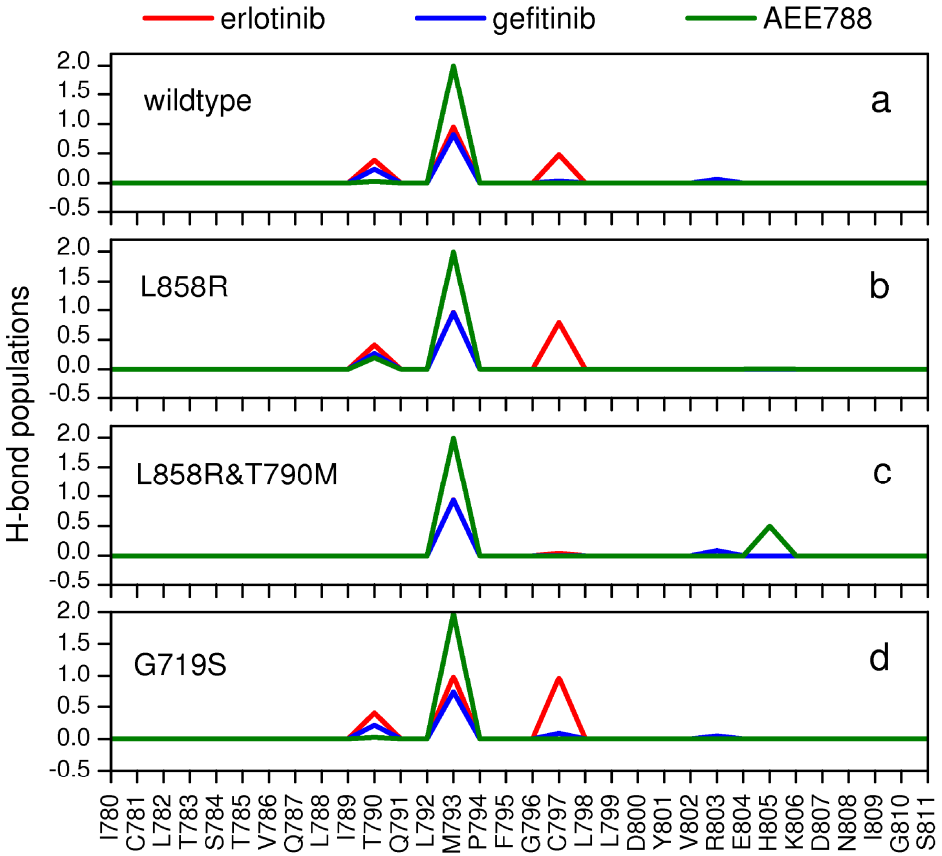




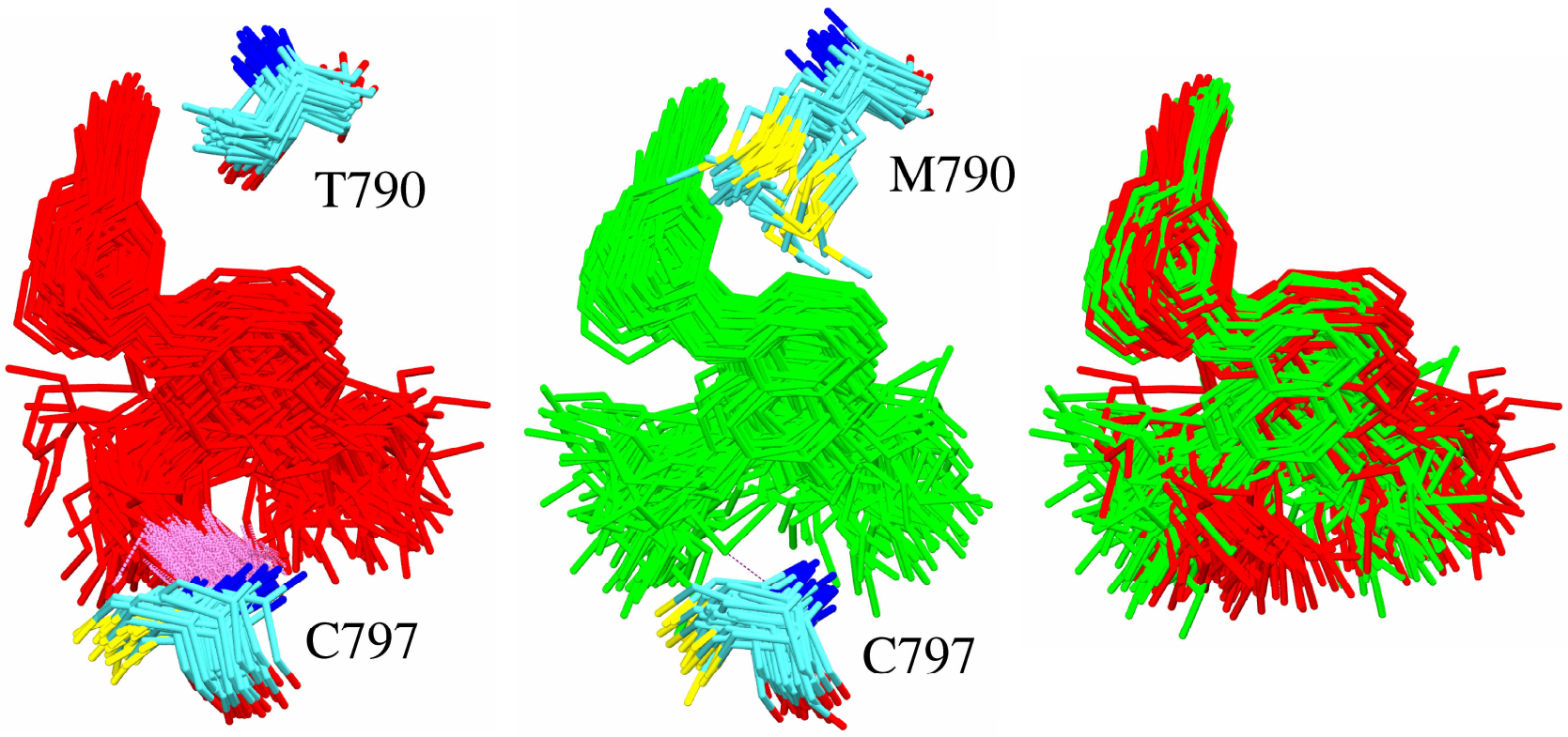
Key Inhibitor H-bonding

- M793 at backbone
- interactions with T790
  - erlotinib Pi-type h-bond
  - gefitinib Cl-type h-bond
  - AEE788 scaffold

- erlotinib at C797
- AEE788 at 805



Erlotinib Binding Comparison



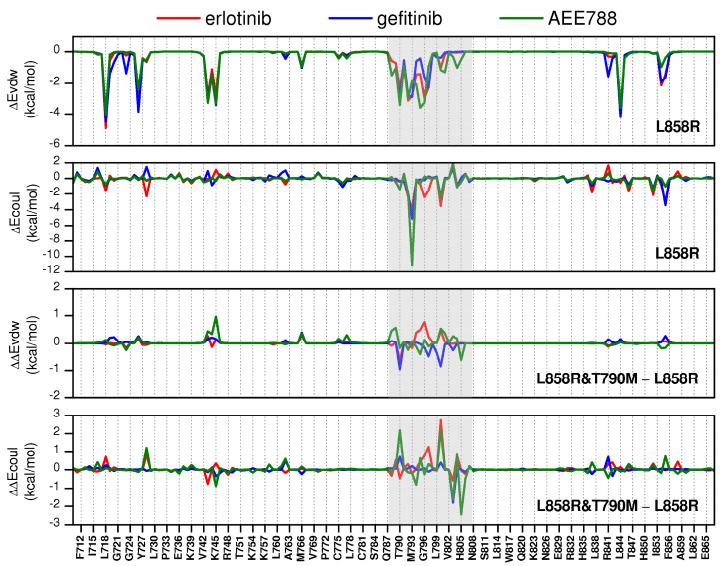
L858R simulations

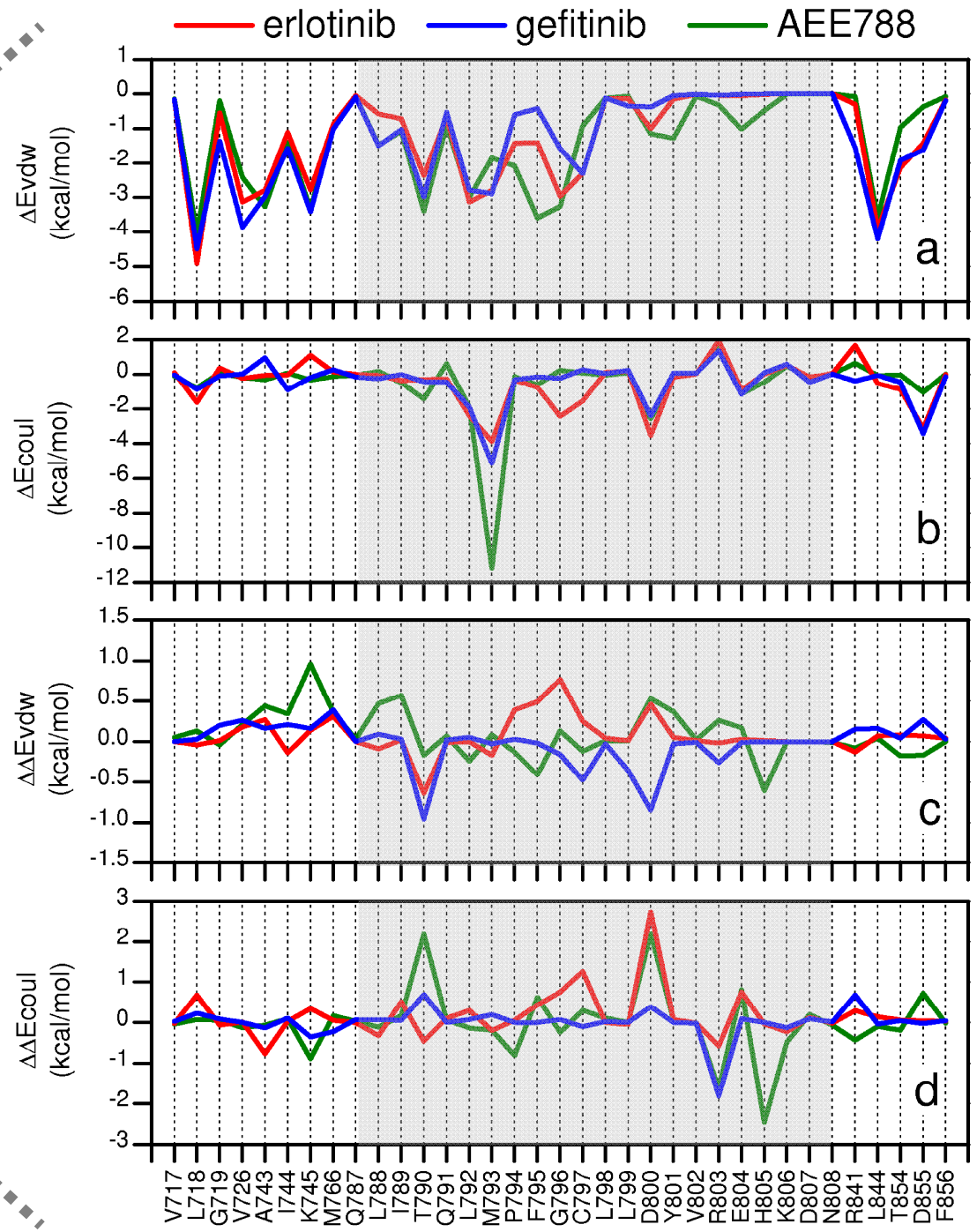
L858R&T790M simulations

overlay

The T790M mutation does not lead to a steric clash with erlotinib however there is change in H-bonding at position C797

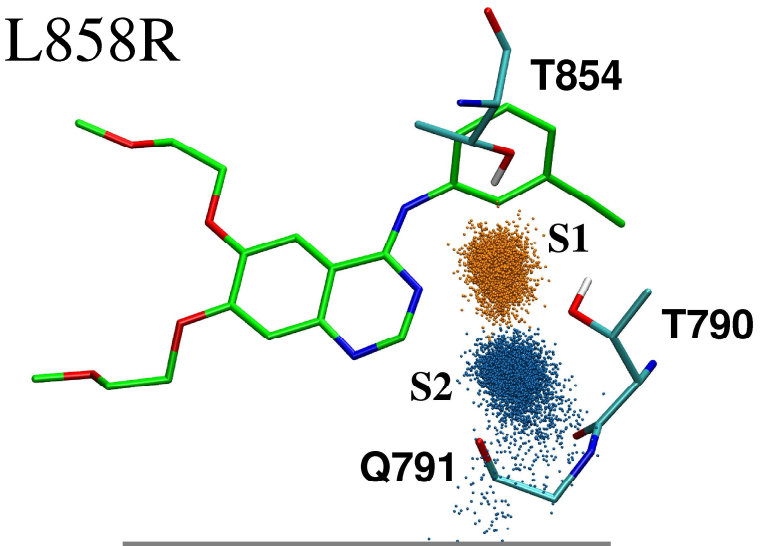
Footprints: Energy

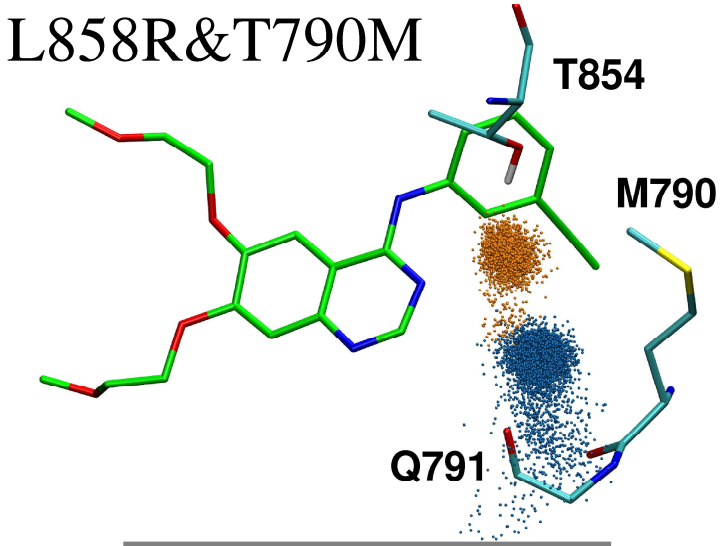




Water Mediated Interactions

a



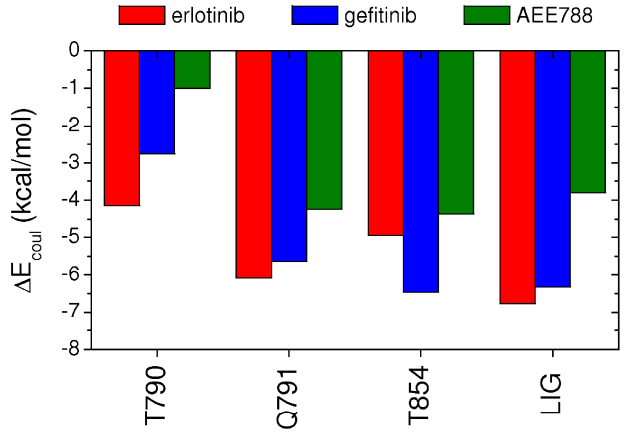


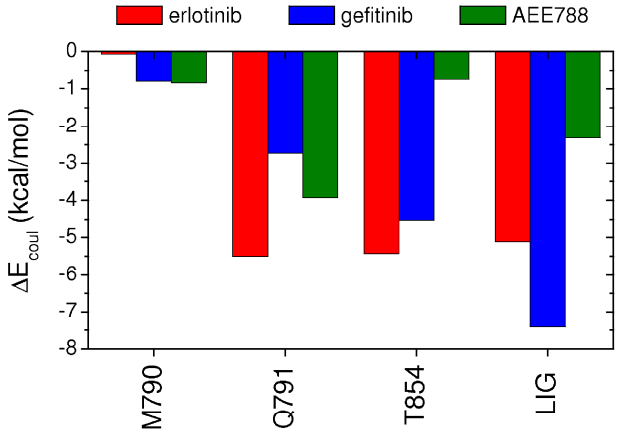
b

	S1 count	S2 count
erlotinib	4628	4168
gefitinib	4544	4385
AEE788	2489	5076

	S1 count	S2 count
erlotinib	3279	4111
gefitinib	3126	2536
AEE788	172	3563

c





Conclusions

- Good agreement ($\Delta\Delta$Gb calcd vs. exptl correlation, $r^2 = 0.84$, N=7)
- VDW is the most correlated term
- Coul. is important for orienting the ligand in the pocket
- FP regions with similar and dissimilar energies suggest good convergence/reproducibility
- Increased VDW interactions at M790 suggest this is not a steric clash mechanism
- Coulombic energies mirror H-bond trends (AEE788 shows largest interactions at M793)
- Flatter difference FP profiles for gefitinib shows agreement with exptl FR trend
- Water-mediated interaction is meaningful
- Mutants effect on affinity for ATP is not the sole reason for modulated affinity for the three inhibitors
- Differences of direct and water-mediated interactions contribute to changes in energies

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Questions??