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ORIGINAL PAPER

# Temperature dependence of fluctuations in HIV1-protease

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**Abstract** Revealing the structure and the intrinsic dynamics of a protein is paramount to understand its function and other properties of evolutionary importance. Several schemes to obtain such insight in silico were developed over the decades. A computationally efficient protocol approximates the molecular dynamics around its native state by a harmonic potential. In this paper, we introduce a new methodology to combine the various harmonic approaches to understand the folding/unfolding dynamics and the dynamics around the native structure of the protein in a temperature dependent way. We apply this new protocol to the HIV1-protease and discuss the results in the light of events in the adaptive evolution towards drug resistance, which is a major problem in HIV infection.

**Keywords** HIV1-protease · Elastic network models · Self-consistent pair-contact probability method · Statistical mechanics · Molecular dynamics

## Introduction

The function of proteins and enzymes in particular is largely determined by the structure and the dynamics of the molecule. To investigate these characteristics computational protocols were proposed, such as molecular dynamics. Typical molecular dynamics protocols (Karplus and McCammon 2002; Dodson et al. 2008) suffer, however, from the problem of high computational requirements and sampling issues.

A partial resolution was suggested by models that compute fluctuations around the native state of a biomolecule; namely traditional normal mode analysis (NMA) (Brooks and Karplus 1983; Brooks et al. 1995; Kitao and Go 1999; Roux and Karplus 1988) and elastic network models (Tirion 1996; Bahar et al. 1997; Atilgan et al. 2001; Doruker et al. 2000), which were recently reviewed (Soheilifard et al. 2008).

Functional studies in molecular biophysics press for computations of fluctuations of biomolecular complexes around their native states. One can deduce several features such as entropy changes upon binding (Lazaridis 2002), probable binding sites for drugs (Micheletti et al. 2002) or overall stability and function of complexes (Keskin et al. 2002) from the detailed analysis of those fluctuations.

The area of applicability of such elastic network models to ligand binding (Housaindokht et al. 2008; Haliloglu et al. 2008) was suggested, as well as extensions to larger systems (Tama et al. 2000; Doruker et al. 2002; Lu and Ma 2008; Sen et al. 2006), protein–protein interactions (Erman 2006; Dobbins et al. 2008), sidechain orientations (Micheletti et al. 2004), investigations on the susceptibility to external forces (Eyal and Bahar 2008), even proteome annotation (Demirel and Keskin 2005), and specific molecular systems such as tRNAs (Bahar and Jernigan 1998), tryptophan synthase (Bahar and Jernigan 1999), cowpea chlorotic mottle virus (Tama and Brooks 2002) or tubulin (Keskin et al. 2002).

We have extended these models by sequence specific parameterization (Hamacher and McCammon 2006) and for macromolecular self-assembly processes (Hamacher et al. 2006b). We, however, also discussed the limitations

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of these models previously (Hamacher et al. 2006a). The field of elastic network models and NMA is therefore an active and promising area of research.

At the same time viral enzymes and other proteins of these infectious pathogens are under scrunity for several reasons; e.g. to understand evolutionary dynamics and adaptation or for more practical purposes such as drug design. Kurt et al. (2003) used an elastic network model to investigate the effect of a drug bound to the HIV1-protease. Now, drug resistance due to mutation of the protease has however become a major problem (Little et al. 2002). Recently, we were able to annotate the selective advantage of (adaptive) evolution in HIV1-protease by elastic network models (Hamacher 2008).

Up to now, however, these models do not allow for the investigation of the influence of temperature on the (un)folding transitions and the fluctuations in the system. There exists a trivial thermodynamics only, as the free energy consists just of equipartition energy due to a Gaussian Boltzmann weight in the harmonic approximation of the potential.

In this paper, we set to close the methodological gap and extent these useful models to the realm of temperature susceptibility.

## Methods

### Temperature dependent fluctuation model

We start out with the Self-Consistent Pair Contact Probability Method by Micheletti et al. (2001). The naming convention (SCPCP) is due to other studies that facilitated the SCPCP e.g. to investigate binding phenomena (Canino et al. 2002). The SCPCP is at first inspection another elastic network model, but with a term resembling breaking of native contacts:

$$H = \frac{TK}{2}\sum_{i=1}^{N-1}\Xi_{i,i+1}\tilde{X}^2_{i,i+1} + \sum_{i,j=1}^{N}\frac{\Delta_{ij}}{2}\left[R^2 - X^2_{i,j}\right] \cdot \Theta\left(R^2 - \tilde{X}^2_{i,j}\right) \tag{1}$$

with

$$\tilde{X}^2_{i,j} := \left(\mathbf{r}_{i,j} - \mathbf{r}^0_{i,j}\right)^2 = \left(\Delta\mathbf{r}_i - \Delta\mathbf{r}_j\right)^2 \tag{2}$$

$\Xi_{i,i+1}$ is 1 if $i$ and $i+1$ are covalent bonds and 0 otherwise. The contact matrix $\Delta_{ij}$ is 1 if the euclidean distance between residues $i$ and $j$ is smaller than some threshold $R_c$. This distance is computed as the eucledian distance of the $C_\alpha$-atoms of the amino acids. $N$ is the number of amino acids and the summation in Eq. 1 runs over all amino acids in the protein. The displacements $\Delta\mathbf{r}_i$ and $\Delta bf\ r_j$ are taken relatively to an input structure, e.g. obtained in experiment, whose coordinates are given by the distance vectors $\mathbf{r}^0_{i,j}$.

The main difference to simple elastic network models is the ability of the SCPCP to "break" bonds by means of the Heaviside-step-function $\Theta\left(R^2 - \tilde{X}^2_{i,j}\right)$: an unbounded separation is not possible, instead the bond between two residues breaks after the strain in the contact becomes to large.

Still no analytic treatment of the model in Eq. 1 is feasible and one faces the same problem as in molecular dynamics simulations: the need to construct a trajectory. If we define the contact probability $p_{ij} := \langle\Theta(R^2 - \tilde{X}^2_{i,j})\rangle_H$ as the thermodynamical average of the contact defining function with respect to the Hamiltonian we can solve for $p_{ij}$ in a mean-field fashion by a recursion. The recursion mapping reads for the $(n+1)$-iteration

$$p^{(n+1)}_{ij} = \Gamma\left(\frac{3}{2}; \frac{R^2}{2G^{(n)}_{ij}}\right) \tag{3}$$

where $G^{(n)}_{ij} = M^{(n)}_{ii} + M^{(n)}_{jj} - M^{(n)}_{ij} - M^{(n)}_{ji}$ and

$$M^{-1\,(n)}_{ij} := \begin{cases} K\left(\Xi_{i,i+1} + \Xi_{i,i-1}\right) + 2\sum_l \Delta_{il}p^{(n)}_{il}/T & i = j \\ -2p^{(n)}_{ij}\Delta_{ij}/T - K\left(\delta_{i,j+1} + \delta_{i,j-1}\right) & i \neq j \end{cases} \tag{4}$$

We define an accuracy measure $\epsilon := \max_{i,j}\left|p^{(n+1)}_{ij} - p^{(n)}_{ij}\right|$ to stop iterating the mean-field-equation.

After convergence we can compute several ensemble-properties, like the fraction of native contacts in the thermodynamic equilibrium $Q := \frac{1}{N_c}\sum_{i,j=1}^{N} p^{(\infty)}_{ij}$, where $N_c = \sum_{i,j=1}^{N}\Delta_{ij}$ is the number of contacts in the pdb-structure. $Q$ is therefore a thermodynamic quantity. We note here we see an advantage of the SCPCP over other, ensemble-based methods like molecular dynamis or Monte-Carlo: the SCPCP allows the immediate calculation of thermodynamic expectation values just from the set of converged $p^{(\infty)}_{ij}$.

Closer inspection of Eq. 1 reveals that the SCPCP does indeed show subtle temperature effects beyond typical Gaussian models. SCPCP in fact captures the rich thermodynamics of breaking bonds and provides for a finite-size-version of phase transitions—in this case protein unfolding.

After convergence to the set of contact probabilities $p^{(\infty)}_{ij}$ we can take up two positions: (1) in fact the SCPCP determines interaction weights that are modulated and softened by temperature, that reveal themselves in the $p^{(\infty)}_{ij}$ and can then be used as *effective* interactions in an elastic network model, or (2) in the recurrence relation of the SCPCP we immediately can obtain eigenmodes of SCPCP-models by a spectral decomposition of the Hessian given by the $p_{ij}$. We note that due to symmetries we need to perform singular-value-decomposition (Press et al. 1995)

instead of optimized eigenvector-algorithms (Golub and Loan 1996).

Here, we take the first of the two equivalent points of view. After replacing the Heaviside-function in Eq. 1 by the converged $p_{ij}^{(\infty)}$ we obtain an effective elastic network model—in fact a Gaussian network model (GNM) (Erman 2006; Keskin et al. 2000) with softened bond strengths.

We therefore perform the following computations: we first converge the SCPCP for a given temperature $T$. We then construct (again) a Hesse matrix with the reweighted bond strengths and perform a spectral decomposition, thus obtaining eigenmodes and eigenvalues/-frequencies. This is achieved within the GNM (Bahar et al. 1997) with the—by temperature softened—potential

$$V_{\mathrm{GNM}} = \frac{1}{2}\left[\sum_{i,j}^{N}\left(\Xi_{i,i+1}\cdot TK + \Delta_{ij}\cdot\left(1-\Xi_{i,i+1}\right)\cdot p_{ij}^{(\infty)}\right)\times\left(\Delta R_j - \Delta R_i\right)^2\right]$$

for the displacements $\Delta R_j$ and $\Delta R_i$.

From here on we have all information available: eigenvectors and eigenfrequencies, from which we can compute e.g. temperature factors discussed elsewhere (Hamacher and McCammon 2006). From here on we solve the recurrence relation (3) numerically to an accuracy of $\varepsilon = 10^{-12}$.

### Protein structure preparation

The three-dimensional protein structure of HIV1-protease (Reiling et al. 2002) with pdb-code 1KZK was used; we neglected the atoms of the heteromolecule within the pdb-file. We note that the contact matrix $\Delta_{ij}$ obtained from this structure with the chosen $R_c = 6.75$ Å is not symmetric under exchange of the two protein chains in the homo-dimer. We refrained from artificially symmetrizing this structure towards a perfect $C_2$ symmetry. We set $R = R_c$ in Eq. 1.

### Parameter derivation

We scanned for an optimal value of $K$ in Eq. 1, so as to co-locate the peak in the specific heat as well as the transition at $Q = 0.5$ at the same temperature. This guarantees that the picture of a 'phase transition' is obeyed by this system. To this end, we found $K = 0.05$ to be a good choice. See also (Micheletti et al. 2001) for details. We note in passing that the peak of the specific heat $C$ is broad—this indicates that the SCPCP neglects some cooperativity: this comes as no surprise, as the interaction is only local in Eq. 1 and does not contain long-range interactions as e.g. Coulomb-potentials.

More challenging parameter optimizations—longing for agreement with other experimentally accessible quantities can be done with efficient optimization techniques (Hamacher 2005; Hamacher 2006; Hamacher and Wenzel 1999). Figure 1 shows that this $K$ values indeed realizes the desired behavior.

## Results

With the choice of parameters described above, we investigated now the behavior of eigenmodes under varying temperatures and thus softened contact in the thermodynamical ensemble.

As a first application of the new protocol we investigated shifts in the energy spectrum. These can already be deduced from the free energy as $F \sim \log(\det M) = \log\left(\prod_{k=1}^{N}\lambda_k\right) = \sum_{k=1}^{N}\log(\lambda_k)$. But the composition of the spectrum—that is the change of the individual eigenvalues—proves to be a valuable source of additional information; shown in Fig. 2. The data show the softening of the contact at the sharp transition of the spectrum to a flat function close to the folding temperature $T_f$.

Small frequencies stand for localized motions. The unfolding event on the other hand is a global, delocalized structural change so that all modes participate, thus softening the spectrum almost everywhere.

Now we can proceed to investigate how stable particular eigenmodes are under temperature increase. In Fig. 3 we show the effect of temperature on the preservation or modification of modes. To quantify this we compute the overlap or angle between the $i$th eigenvector $\mathbf{v}_{0.001}^{(i)}$ at $T = 0.001$ and the $i$th eigenvector $\mathbf{v}_T^{(i)}$ as $\cos(\alpha_{\mathrm{ref};T}) = \mathbf{v}_{0.001}^{(i)}\cdot\mathbf{v}_T^{(i)}$ for normalized eigenvectors.

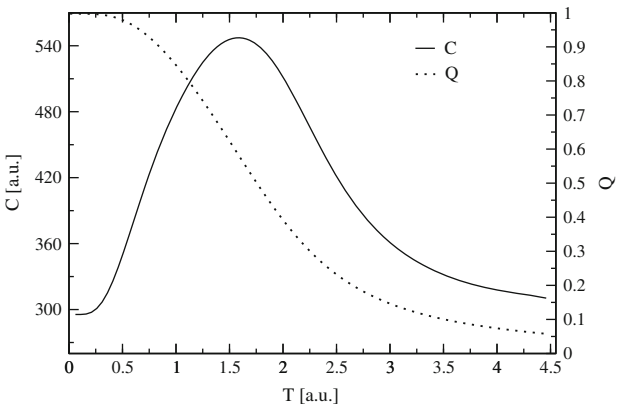


**Fig. 1** The specific heat $C$ and the fraction of native contacts $Q$ in HIV1-Protease as a function of temperature $T$. A phase transition towards a disordered state is clearly visible at the "folding temperature" $T_f = 1.76$ where $Q = 0.5$ and $C$ shows a peak

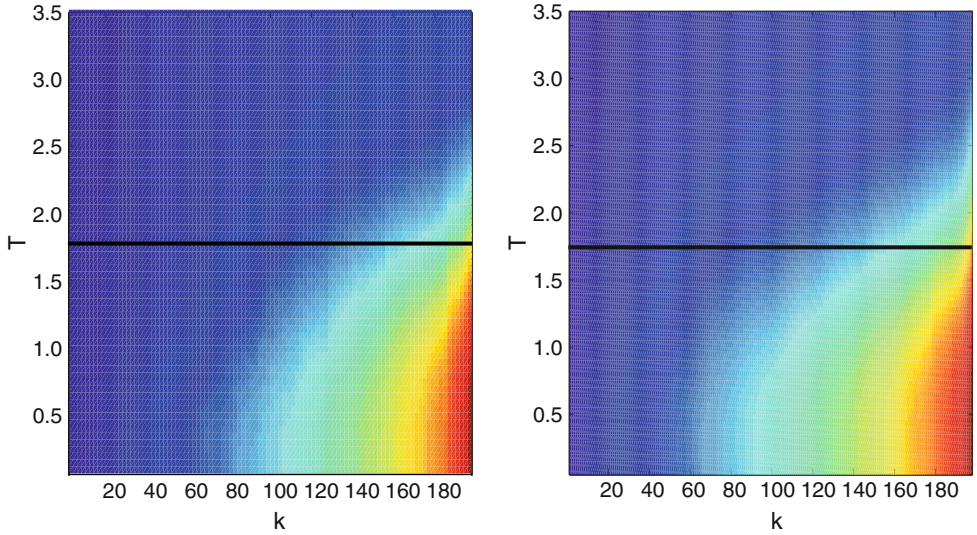


**Fig. 2** Color coded eigenvalues $\lambda_k$ of mode $k$ at temperature $T$. The energy spectrum $\omega_k = \sqrt{\lambda_k}$ becomes smoother for higher temperatures while at low temperatures pronounced modes with high frequencies prevail. The *left* graph displays the results for the monomer, the *right* one for the dimer. The *black line* indicates the transition temperature $T_f = 1.76$ of the dimer. *Blue* low values, *red* high values of $\lambda_k$ [arbitrary units]

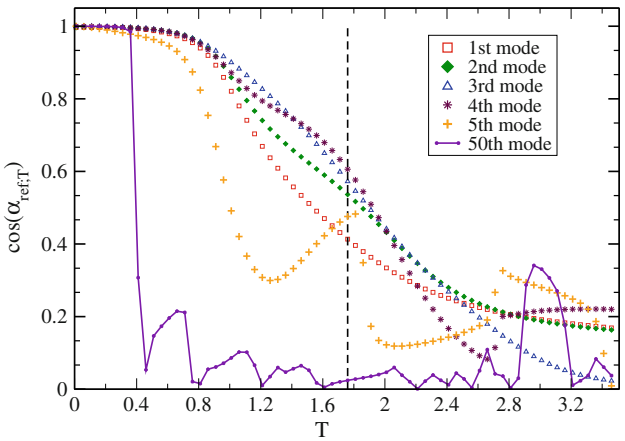


**Fig. 3** The overlap measured by $\cos(\alpha_{\mathrm{ref};T})$ between various low energy modes as a function of $T$ with respect to their counterparts at $T = 0.001$. The *broken line* indicates $T_f$. Clearly the slowest modes are still present before reaching $T_f$. The faster mode 5 is subject to a more pronounced change. While the smooth change of the modes 1–4 indicates a gradual redirection, the kinks in the data for mode 5 and the kink for mode 4 at $T = 2.71$ show that some other modes have taken over in the frequency/energy ordering, replacing the eigendirection at smaller temperatures. For comparison we plot also a higher mode which is destroyed 'frequently' for temperature increase

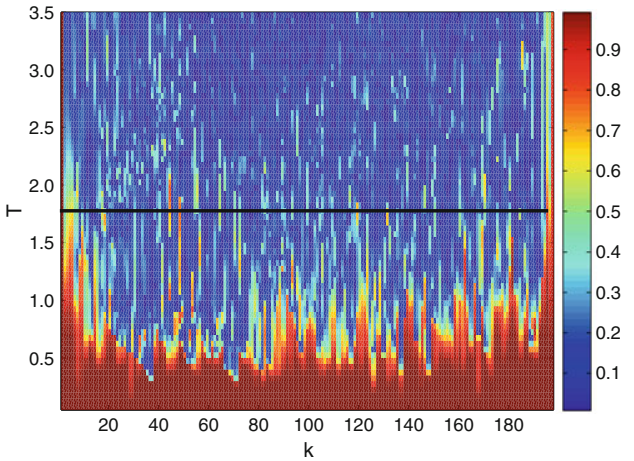


**Fig. 4** The overlap measured by $\cos(\alpha_{\mathrm{ref};T})$ between all the residues as a function of $T$ with respect to their counterparts at $T = 0.001$. The line indicates $T_f$. The first $\sim 6$ modes are more stable against temperature change than the next ones

We hereby prove the assertion that the small-frequency modes are responsible for stabilization of the molecule.

We expand the results of Fig. 3 by looking at all modes in Fig. 4. We here see that certain modes "desolve" more rapidly upon temperature increase than others.

But what are the modes being so stable? In Fig. 5 we show the contribution of these modes to the overall flexibility (as given by the computed temperature factors). Clearly we see that the basis and a "strip" of residues in the core of the dimer—including, however, also the residues in the "flaps".

The first modes are not affected on a larger scale by an increasing temperature. We conclude from Figs. 3 and 5 that the opening and curling the flaps (upper part of the protein) is a low-energy motion. Therefore these modes are substantially realized. Second we conclude from our results that this characteristic movement stays important throughout the whole temperature range scanned. The structural properties of HIV1-protease thus lead to a functional property that is 'conserved' for a broad temperature range. HIV1-protease evolved to a structure that is capable to function over a broad temperature range.

For a quantitative impression on the cooperativity of the various modes we computed the squared entries of the respective eigenvectors. The larger this value, the more an amino acid participates in this mode and thus the more cooperative its dynamics. To quantify the cooperativity we

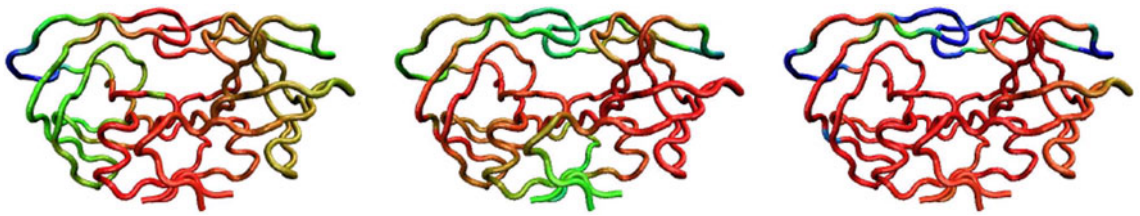

**Fig. 5** The three lowest energy modes at $T_f$ (from *left* to *right*). *Red* indicates small, *blue* and *green* large fluctuations

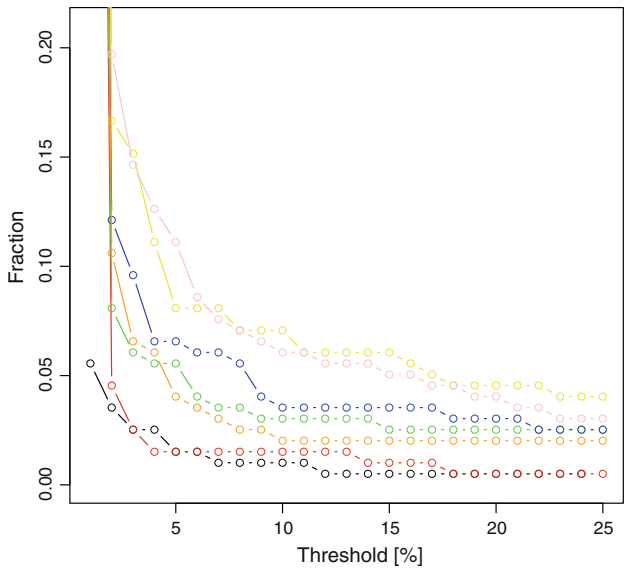


**Fig. 6** For $T_f$ we show the cooperativity of the low-frequency modes. On the *x*-axis we plot a threshold *t* for the contribution of amino acids to the respective modes. The *y*-axis gives the fraction of amino acids that participate with more than *t* in the modes. First mode: *black*; second mode: *red*; third mode: *green*; fourth mode: *blue*; fifth mode: *yellow*; sixth mode: *orange*; seventh mode: *pink*

scan each mode with a threshold *t*. The results are shown in Fig. 6.

## Discussion and summary

We extended an analytic procedure for the self-consistent determination of fluctuations, binding strengths and other thermodynamical quantities by combining it with a throughly investigated model for the computation of eigenmovements. Our results show the unfolding mechanism as indicated by a smoothing of the frequencies of the fluctuations as well as by temperature induced disordering of eigenvectors of the underlying dynamics.

The importance of obtaining insight into the thermostability of viral proteins is two-fold: one the one hand, we can learn about general stability of such drug targets and therefore improve rational drug design efforts; on the other hand, we will be able to understand better the evolution of viruses in general. If key-proteins involved in viral infection are strongly conserved, but also contribute to thermostability then this stability seems to be a selective pressure on the virus. We can therefore (partially) annotate viral evolution by thermostability issues.

In application to an important enzyme of viral infection progression (HIV1-protease) we were able to illustrate the advantages of the new methodology and point to an evolved stabilizing core of the protein that partially includes the flaps, which were identified in sophisticated molecular dynamics studies (Perryman et al. 2004) to be involved in drug resistance mechanisms.

Improvements to leverage the emerging Gaussian probability distributions in the SCPCP for molecular design purposes (Hamacher 2007) are currently underway.

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