Functional modes of proteins are among the most robust ones

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It is shown that a small subset of modes which are likely to be involved in protein functional motions of large amplitude can be determined by retaining the most robust normal modes obtained using different protein models. This result should prove helpful in the context of several applications proposed recently, like for solving difficult molecular replacement problems or for fitting atomic structures into low-resolution electron density maps. Moreover, it may also pave the way for the development of methods allowing to predict such motions accurately.

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In the case of two-domain proteins, it is well known that a few low-frequency normal modes can provide a fair description of their large amplitude motion upon ligand binding. More recently, it has been shown that this is also true for proteins with more complex architectures, as long as their functional motion is a collective one, i.e. if it concerns large parts of the structure. For instance, a single low-frequency mode of the T form of hemoglobin is enough to describe accurately its conformational change upon oxygen binding.

This result has been successfully applied for exploiting fiber diffraction data, solving difficult molecular replacement problems, or fitting atomic structures into low-resolution electron density maps. The principle of these applications is to perturb a known structure along its low-frequency modes so as to get a deformed structure that is consistent with low-resolution biophysical data, which are obtained after the protein has undergone some large amplitude conformational change. It was also shown that when variations of a few key distances are known, through spectroscopic measurements for instance, it is possible, using linear response theory, to identify which modes are the most involved in the conformational change. However, if such experimental data are missing, it is difficult to guess which low-frequency modes are the functional ones. Hereafter, we show that they are among the most robust ones, i.e. among the most conserved modes when different descriptions of a given protein are considered. The robustness of the functional modes was recognized when it was shown that they can be obtained with simple protein descriptions, like Elastic Network (EN) models. Herein, this property is used so as to identify them.

First, standard normal modes were calculated for a set of five proteins of different sizes and architectures after preliminary energy-minimization. The CHARMM program was used, with the EEF1.1 implicit solvent model and the corresponding electrostatic and non-bonded options, as done in recent studies performed at this level of detail. Then, for each energy-minimized structure, low-frequency normal modes were calculated with the all-atom EN model proposed by M. Tirion, where the standard, many-parameters, empirical energy function $E_p$ used in programs like CHARMM is replaced by:

$$E_p = \sum_{d_{ij} < R_c} C(d_{ij} - d_{ij}^0)^2$$

where $d_{ij}$ is the distance between atoms $i$ and $j$, $d_{ij}^0$ being their distance in the studied structure. The strength of the potential $C$ is a constant assumed to be the same for all interacting pairs. It is required only in order to define energy (and frequency) units. As done in previous studies, the cut-off parameter, is set to 5 Å.

In order to compare both sets of normal modes, $n_i^{eff}$, the effective number of EN modes involved in the description of standard mode $i$, is calculated as follows:

$$n_i^{eff} = \exp\left(-\sum_{j} \alpha I_{ij}^2 \ln(I_{ij}^2)\right)$$

where $n$ is the number of EN modes taken into account ($n=100$ herein), $I_{ij}$ being the scalar product between standard mode $i$ and EN mode $j$. The normalization factor $\alpha$ is such that: $\sum \alpha I_{ij}^2 = 1$. Thus, $n_i^{eff}$ gives the effective number of non-zero (normalized) $I_{ij}^2$. It ranges from 1 to $n$. As shown in Fig. 4 for each protein considered, several of its standard normal modes can be described accurately with less than 5-6 EN modes. Moreover, all these robust modes have low rankings, namely, below #15.

Next, two other EN models were considered. In both cases, as often done, only Cα atoms are kept. In the first model, as proposed by M. Tirion [see Eq. (1)], pairs of interacting neighbors are determined according to a distance cutoff criterion, namely, $R_c = 12$ Å. With such a criterion, for Adenylate Kinase, $n_c$, the average number of interacting neighbors per Cα atom, is 25 ± 7, ranging from 10 to 42, as a function of the degree of burial of the amino-acid in the protein interior. Note that $R_c$ cannot be set to a value lower than 8-10 Å, a limit which depends upon the structure considered. Otherwise, the number of zero-frequency modes

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becomes larger than six, as a consequence of the splitting of the elastic network into several independant ones.

The second model was designed so as to keep \( n_c \) as constant as possible from one amino-acid to the other. To do so, we use the following algorithm. First, all pairs of \( \text{C}_\alpha \) atoms are sorted, according to their distance. Then, starting from the pair separated by the largest distance, they are removed one after the other, unless one atom of the pair has already \( n_c \) neighbors. With this algorithm, setting \( n_c = 10 \), the average distance between pairs of interacting neighbors is 6.2 ± 1.8Å, ranging from 3.0 to 10.8Å. Note that in the case of Adenylate Kinase \( n_c \) can be set to a value as low as 7 without splitting the network into independant ones.

As done above, normal modes obtained with both EN models were compared, seeking for robust ones, using a set of twenty-two proteins considered in previous studies performed with the distance-cutoff criterion[7, 8, 11]. Like in the case of all-atom models, modes are considered to be robust whenever \( n_{\text{eff}} \leq 6 \).

Statistics of the number of robust modes found for all studied proteins are shown in Fig. 2 (zero-frequency modes are not taken into account). In most cases, the number of robust modes is four or less. In only three cases, it is larger than seven. Interestingly, the DNA polymerase of bacteriophage RB69 (pdb code 1ih7), which is the protein of our dataset with the largest number of robust modes (eleven), has a quite complex architecture, with three well-known structural domains. It is also among the largest cases considered herein (897 amino-acids).

In four cases, no robust mode is found. Interestingly, the known conformational change of these proteins, namely, Tyrosine Phosphatase, Triose Phosphate Isomerase, Che Y, and HIV-1 protease (pdb codes are 1yts, 3tim, 3chy, 1hhp, respectively), is a small amplitude one, with a \( \text{C}_\alpha \) root-mean-square displacement (r.m.s.d.) of 1.5Å at most.

Then, it was checked that robust modes yield accurate descriptions of protein functional motions. To do so, \( Q_d \), the quality of the motion description is calculated as follows[5, 8]:

\[
Q_d = 100 \sum_{i=1}^{n} \frac{I_{id}^2}{I_{id}^2}
\]

where \( n \) is the number of modes taken into account in the description and \( I_{id} \) is the scalar product between mode \( i \) and the direction of the conformational change observed by crystallographers. Note that \( Q_d = 100\% \) when all modes are included in the description, since they form a complete basis set[27].

In Fig. 3 the conformational change of lactoferrin is shown. It can be described accurately (\( Q_d \) over 85%) as a linear combination of the seven lowest-frequency modes of the "open" form (see Fig. 4). Interestingly, all seven
modes are found to be robust. In Fig. 4 $Q_d$ is given as a function of the amplitude of the functional motion of each protein considered when $n = 100$ normal modes or only the robust ones are taken into account in the description. For most proteins with small amplitude motions, i.e. of less than 2-3Å of r.m.s.d., robust modes fail to capture any information about the nature of the known conformational change, while in several cases some information is indeed present in the normal modes. For instance, as mentioned above, for HIV-1 protease, no robust mode is found, although a single EN mode is enough for describing 50% of its conformational change upon ligand binding. If two other EN modes are added to the description, $Q_d$ can reach a value of 77% (with $n=100$, $Q_d=89%$).

On the other hand, when considering proteins with large amplitude motions, the description of the conformational change with robust modes is almost as accurate ($Q_d$ over 75%) as when $n = 100$ normal modes are taken into account. The only counter example is Adenylate Kinase, whose r.m.s.d. upon ligand binding is 5.3Å (the corresponding pdb codes of the open and closed crystallographic structures are 4ake and 1ake). As a matter of fact, when standard normal modes of Adenylate Kinase are compared to all-atom EN ones, only a single robust mode is found (see Fig. 4), and it is not involved in the conformational change ($Q_d=4%$). However, using $C_\alpha$-EN models, six robust modes are found and they allow for an almost perfect description of the conformational change ($Q_d=91%$).

Of course, when using all atom models, more robust modes can be obtained by raising the robustness criterion. In the case of Adenylate Kinase, if a given mode is said robust whenever $n_i^{eff} \leq 10$, then five robust modes are found. However, it is still not enough ($Q_d=73%$) for describing its conformational change as well as with robust modes obtained using $C_\alpha$-EN models. Raising the robustness criterion so as to obtain six robust modes does not change significantly the quality of the description ($Q_d=77%$). As a matter of fact, robust modes obtained using all-atom models always yield poorer description of protein functional motions than simpler models, in which only $C_\alpha$ atoms are kept (open circles are below open squares in Fig. 4). This is mainly due to the fact that standard normal mode analysis requires a preliminary energy-minimization, during which the structure is significantly distorted, while normal mode analysis of EN models does not, as illustrated by the case of DNA polymerase $\beta$. For this protein, when the $C_\alpha$-EN model is built using the crystal structure (pdb code 1bpx), seven robust modes are found, which are able to describe accurately ($Q_d=84%$) the conformational change upon nucleotide binding (pdb code 1bpy). However, when it is built using the energy-minimized structure, only three robust modes are found, which are not able to describe the conformational change ($Q_d=21%$) much better than the three ones obtained using all-atom models ($Q_d=16%$). In that case, the distortion during the energy-minimization process is unusually large (r.m.s.d.=2.5Å), probably as a consequence of the removal of the large ligand, namely, a sixteen base pair DNA (1bpx is the structure of a binary complex while 1bpy is the structure of a ternary complex), prior to the calculation. Even though the amplitude of the distortion is almost as large as the amplitude of the functional motion itself (r.m.s.d.=2.8Å), the above result is not straightforward, since the distortion does not occur along the direction of the conformational change. Indeed, with respect to the energy-minimized structure, the amplitude of the functional motion remains

Figure 3: The conformational change of Lactoferrin upon ligand binding. Left: apo (or "open") state (pdb code 1cb6). Right: holo (or "closed") state (1lfg). In the latter case, the iron ligands are not shown. Drawn with Molscript.

Figure 4: Quality of the description of the closure motion of Lactoferrin upon ligand binding, as a function of the number of low-frequency normal modes (black points) considered. Boxes: contribution of each robust mode to the description.
Figure 5: Quality of the description of protein functional motions with 100 low-frequency modes (filled symbols) or with only the robust ones (open symbols), as a function of the amplitude of the motion. Five proteins were studied at the all-atom level (circles) and the other ones at the amino-acid level (squares).

large (r.m.s.d. = 2.4 Å).

In the present study, modes obtained with standard all-atom, many parameters, protein models were compared to those obtained with elastic network models, as proposed by M. Tirion [18]. For most protein cases, several robust modes are found, confirming results obtained previously [7, 18, 19, 20], namely, that the lowest-frequency modes are little sensitive to details in the protein description. Since such EN models rely on a distance-cutoff criterion for defining atomic interactions, this can be explained in two different ways. First, these modes may capture informations about the protein mass distribution in space. Second, they may capture informations about the rigidity of the protein in the vicinity of each amino-acid residue. Indeed, with a distance-cutoff criterion, amino-acids in the protein interior are more rigid (more neighbors) than those on the surface (less neighbors). So, we designed a novel Cα-EN model whose main raison d’être was to decide between these two possibilities. In this model, each Cα atom has a given number of interacting neighbors and rigidity is fairly constant from one point of a protein to another. When modes obtained with this model are compared to those obtained with a Cα-EN model based on the distance-cutoff criterion, the same robust modes are found. This means that they are also not sensitive to the distribution of rigidity in the protein.

Moreover, we have shown that these robust modes are likely to be involved in protein functional motions, at least when the functional motion is a large amplitude one (r.m.s.d. ≥ 2-3 Å). This result should prove helpful in the context of applications like those mentioned in the Introduction, since they all concern large amplitude conformational changes [11, 12, 13, 14, 15].

This result could also pave the way for the development of methods allowing to predict such motions accurately, i.e. to predict their amplitude, since exploring a subspace of small dimensionality (three or four in most cases considered) should be enough for finding conformations close to functional ones. Interestingly, seeking for robust modes could also indicate whether a given protein can exhibit large amplitude functional motions or not. Indeed, the functional motions of the four proteins with no robust mode are small amplitude ones.

[1] J. A. McCammon, B. R. Gelin, M. Karplus, and P. Wolynes, Nature 262, 325 (1976).
[2] W. Harrison, Biopolymers 23, 2943 (1984).
[3] B. R. Brooks and M. Karplus, Proc. Natl. Acad. Sci. USA 82, 4995 (1985).
[4] O. Marques and Y.-H. Sanejouand, Proteins 23, 557 (1995).
[5] D. Perahia and L. Mouawad, Comput. Chem. 19, 241 (1995).
[6] J. Ma, Structure 13, 373 (2005).
[7] F. Tama and Y.-H. Sanejouand, Protein Engineering 14, 1 (2001).
[8] M. Delarue and Y.-H. Sanejouand, J. Mol. Biol. 320, 1011 (2002).
[9] W. G. Krebs, V. Alexandrov, C. A. Wilson, N. Echols, H. Yu, and M. Gerstein, Proteins 48, 682 (2002).
[10] M. Tirion, D. ben Avraham, M. Lorenz, and K. Holmes, Biophys. J. 68, 5 (1995).
[11] K. Suhre and Y.-H. Sanejouand, Act. Cryst. D 60, 796 (2004).
[12] K. Suhre and Y.-H. Sanejouand, Nucl. Ac. Res. 32, W610 (2004).
[13] M. Delarue and P. Dumas, Proc. Natl. Acad. Sci. USA 101, 6957 (2004).
[14] F. Tama, O. Miyashita, and C. L. Brooks III, J. Mol. Biol. 337, 985 (2004).
[15] K. Hinsen, N. Reuter, J. Navaza, D. L. Stokes, and J. J. Lacapere, Biophys. J. 88, 818 (2005).
[16] M. Ikeguchi, J. Ueno, M. Sato, and A. Kidera, Phys. Rev. letters 94, 078102 (2005).
[17] W. Zheng and B. R. Brooks, Biophys. J. 88, 3109 (2005).
[18] M. Tirion, Phys. Rev. Lett. 77, 1905 (1996).
[19] I. Bahar, A. R. Atilgan, and B. Erman, Folding & Design 2, 173 (1997).
[20] K. Hinsen, Proteins 33, 417 (1998).
[21] B. R. Brooks, R. E. Bruccoleri, B. D. Olafson, D. J. States, S. Swaminathan, and M. Karplus, J. Comp. Chem. 4, 187 (1983).
[22] T. Lazaridis, Proteins 52, 176 (2003).
[23] G. Li and Q. Cui, Biophys. J. 86, 743 (2004).
[24] R. Bruschweiler, J. Chem. Phys. 102, 3396 (1995).
[25] A. Atilgan, S. Durell, R. Jernigan, M. Demirel, O. Keskin, and I. Bahar, Biophys. J. 80, 505 (2001).
[26] H. Goldstein, Classical Mechanics (Addison-Wesley, 1950), reading, ma ed.
[27] P. Kraulis, J. Appl. Cryst. 24, 946 (1991).