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Conformational Dynamics of Wild-Type Neuroserpin in Relation to Human Protein Misfolding Diseases

ABSTRACT

Wild-type human neuroserpin, a member of the serine protease inhibitor superfamily, is expressed in neurons of the central and peripheral nervous system, as well as in the adult brain. Polymerization of certain mutants of neuroserpin is associated with dementia caused by familial encephalopathy, but the protein can also inhibit the toxicity of amyloid-β-peptides in Alzheimer's disease by binding to them[1]. We have performed hydrogen/deuterium exchange mass spectrometry in order to monitor the structural stability and flexibility of different regions of the neuroserpin structure. We found that a critical region thought to be involved in polymerization is less stable and more labile in neuroserpin than in other serpins such as alpha-1 antitrypsin and antithrombin. This may explain why wild-type neuroserpin is more susceptible to polymerization than other serpins. Molecular dynamic simulations of the wild-type neuroserpin and a disease associated mutant were performed in order to probe the molecular motions at atomic level detail. Principle component analysis was then used to interpret the molecular dynamic simulations. Correlation diagrams show that the mutant neuroserpin simulations may have more correlated movements than the wild-type neuroserpin. Furthermore, the mutant showed distortions near the top of the central beta-sheet, a region believed to be a critical site for polymer formation. The distortion could explain why the mutant protein is more likely to polymerize than the wild type protein.

INTRODUCTION

Misfolding of a protein is the cause of many human diseases. In the case of wild-type human neuroserpin, misfolding can cause dementia. Affected individuals show signs of cognitive difficulties including reduced attention and concentration, response regulation difficulties, and impaired visuospatial skills[2]. Mutation of certain amino acids in this protein also leads to diseases such as myoclonus, epilepsy, and chorea as well. Therefore, studying the folding mechanism and the global interactions of wild-type human neuroserpin will be particularly useful in understanding these diseases and then leading to possible treatment.

Crystal Zhou

Crystal Zhou is a senior at Case Western Reserve University studying Biochemistry, with minors in Biology, Chemistry, and Sociology. She has been an active member of the Asian American Alliance and is the President this year. She also serves as the Vice President of Intellectual Development (Scholarship Chair) of her sorority, Alpha Chi Omega, and gives back to the community by participating in various service and philanthropy events with her sorority sisters. Some of her future goals are to continue her research and design drugs that may one day cure cancer.

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Hydrogen/deuterium exchange measured by mass spectrometry helped us determine if parts of the protein were flexible or stable. Flexibility of the protein was determined by monitoring which peptides exchanged first, and stability of the protein was determined by monitoring which peptides exchanged the least or did not exchange at all. The information from the exchange also allowed us to determine which parts of the protein are exposed to the environment and which parts are folded inside, as well as which regions of the protein are less stable and susceptible to polymerization.

Molecular dynamic simulations showed how wild-type human neuroserpin and a mutant form act at the atomic level. From the simulations, we were able to gather information about the movement of the protein due to its interactions with the environment. Principle component analysis was useful because it allowed us to figure out which regions of the protein experienced correlated movements, the root mean square distance between residues, and the energy levels of the protein by using the first, second, and third principle components. Energy landscape diagrams helped determine which parts of the protein are more stable and change little over time. Our results provide insight into the structural stability and reasons why the mutant form of neuroserpin is more prone to polymerization than the wild-type.

MATERIALS AND METHODS

Purification:

A seed culture of BL21 cells was grown in LB media overnight. The cells were then allowed to grow in 2L of 2XYT media for 3 hours. Cells were then induced to express wild-type neuroserpin by adding IPTG to the media and incubated at 20° C for 17 hours. The cells were then harvested and allowed to incubate in lysozyme and PMSF on ice-water for half an hour. The cells were disrupted by sonication, washed, and incubated in the cold room for 45 minutes with Ni-NTA beads. The Ni-NTA agarose was then washed with Buffer A (50mM sodium phosphate, 10mM imidazole, 500mM sodium chloride, pH 7.8), packed into a column, connected to an FPLC system, and washed again with Buffer A followed by 20mM sodium phosphate buffer, pH 7.8. The protein was eluted with 250mM imidazole, 20mM sodium dihydrogen phosphate, pH 7.8. The eluate was diluted fourfold with Buffer C (20mM Tris-hydrochloride, 20mM sodium chloride, pH 7.4), washed with Buffer C in a Hitrap Q HP and eluted. The final eluate was diluted twofold with Buffer B. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed to verify that a protein was present in the sample. The purified sample of neuroserpin was separated into 2 μL aliquots and kept in the -80 °C storage.

Hydrogen/Deuterium Exchange-Mass Spectrometry:

Seven aliquots were used for this experiment. Each aliquot was diluted 25-fold with D2O Buffer (6.4M Guanidine Hydrochloride, 10mM sodium phosphate, 50mM sodium chloride, pD 7.8) and pulse labeled for either 10, 50, 100, 500, 1000, 2000, or 3000 seconds. After the time point was reached, the reaction was quenched by diluting the sample twofold with 100mM sodium dihydrogen phosphate, pH 2.4. We used pepsin to digest the protein, which was prepared by diluting pepsin from porcine gastric mucosa with 0.05% trifluoroacetic acid in water in a 1:1 ratio. The pepsin was allowed to dissolve for an hour. 6 μL of pepsin was then added to the protein and allowed to digest for 5 minutes. The sample was then injected into the mass spectrometer and run over a short gradient of 10-45% 0.05% trifluoroacetic acid in acetonitrile for 15 minutes.

Molecular Dynamic Simulations:

The wild-type neuroserpin structure was taken from the PDB (3FGQ). VMD was used to build the original protein file. The protein molecule was then relaxed to find a local minimal energy state, then solvated and ionized to an ionic strength of 50mM of sodium chloride to match the conditions we used experimentally. The protein was then heated to a temperature of 37° C. All simulations and steps were done using the NAMD engine on Case Western Reserve University's high performance computing cluster. The simulations were completed 5 nanoseconds at a time until we reached 50 nanoseconds.

Data Analysis:

Xcalibur was used to view and find peaks in the spectrums generated by the mass spectrometer. Selected peaks were

transferred to MagTran in order to obtain the centroid mass. PyMOL and Chimera were used to create figures of the crystal structure of neuroserpin, and Microsoft Excel was used to map the percentage of deuterium exchanged over time. The percentage of deuterium exchanged was calculated according to the following equation:

$$\%D = \frac{Dt - Do}{Dm - Do} \times 100$$

Where Dt is the mass of a specific peptide after 10, 50, 100, 500, 1000, 2000, or 3000 seconds, Do is the mass of the original peptide before hydrogen/deuterium exchange, and Dm is the mass of the peptide after it has experienced the maximum amount of hydrogen/deuterium exchange.
Carma was used to generate correlation and energy diagrams of the different principle components obtained from the molecular dynamic simulations.

RESULTS AND DISCUSSION

Hydrogen/Deuterium Exchange Measured by Mass Spectrometry:

The amide hydrogens of peptides that are exposed to the solvent exchange faster than those that are hidden by the secondary stucture of the protein (Table 1). Hydrogen exchange indicates that helices D, F, G and beta sheet A are highly flexible in neuroserpin (Figure 1). Beta sheets A and B are both more labile in neuroserpin than in other serpins such as antitrypsin and antithrombin. Large movements in both these regions are thought to be required during the formation of pathological polymers. The high flexibility seen in neuroserpin may explain why even wild type neuroserpin is more polymerization prone than other serpins. Figure 1 also shows that the reactive center loop (marked in magenta) of neuroserpin exchanges faster in some places and slower in others. The rate of exchange also increases as time progresses, and the protein is allowed to incubate in D2O buffer (Figure 2). Figure 1 also leads us to conclude that different regions of the protein have very different flexibilities due to the highly variant rates of exchange. While some peptides are still exchanging after 1000 seconds, others have finished exchanging after just 10 seconds. The central beta sheets of the wild-type protein each exchange at different rates, which is consistent with its unstable nature. On the other hand, α1-antrypsin is not as wobbly as neuroserpin and experiences a more stable structure, as determined by hydrogen/deuterium exchange and molecular dynamic simulations similar to the ones performed on neuroserpin. Figure 3 also shows that wild-type human neuroserpin is very flexible, since most regions of the protein have large ranges of fluctuation as indicated by the vectors on the protein.
This means that neuroserpin likes to exist as least two stable structures and alpha-1 antrypsin likes to exist in only one stable structure. With this information, we can conclude that neuroserpin is less stable and more flexible than other serpins such as alpha-1 antitrypsin or antithrombin.

Table 1. Select peptides of wild-type human neuroserpin and their hydrogen/deuterium exchange rates at different time points. Peptides in red indicate regions more exposed to solvent.

Peptide	MH	Mass	Deuterated	10s	% exc	50s	% exc	100s	% exc	500s	% exc	1000s	% exc	2000s	% exc	3000s	% exc
A.IADLSVNM.Y	1	862.43	666.5	863.41	0.241	664.4	0.484	664.76	0.572	865.74	0.613	665.79	0.823	866.72	1.054	866.35	0.963
A.VANYINKWVENNTNNL.V	2	953.47	956.66	954.03	0.176	954.34	0.273	956.39	0.915	956.86	1.063	956.83	1.053	955.66	0.687	955.77	0.721
D.ENILFSPLSIA.L	2	602.33	605.22	603.76	0.495	604.12	0.62	603.95	0.561	604.65	0.803	604.71	0.824	605.13	0.969	605.22	1
E.IDLKDVLKA.L	2	507.6	510.77	507.9	0.034	508.03	0.077	510.6	0.943	510.36	0.862	510.49	0.906	510.7	0.976	510.71	0.98
E.IFIKDANL.T	1	933.54	938.04	936.55	0.669	937.33	0.842	937.05	0.78	938.02	0.996	938. 5	1.002	938.12	1.018	938.36	1.071
E.QEIDLKDVL.K	1	1072.6	1075.6	1073.7	0.374	1073.7	0.377	1073.7	0.364	1073.6	0.401	1073.9	0.424	1073.9	0.45	1074.1	0.467
E.VQIPMM.Y	1	718.36	720.91	719.09	0.286	719.08	0.282	719.18	0.322	719.1	0.29	719.2	0.329	719.3	0.369	719.33	0.38
F.HVNEEF.L	1	774.34	780.27	776.18	0.31	776.52	0.368			77 .88	0.428	776.62	0.384	776.78	0.411	776.74	0.405
F.IKDANLIGLSDNKEIF.L	2	889.46	894.67	892.98	0.676	893.73	0.82	893.9	0.852	894.13	0.896	894.21	0.912	894.59	0.985	894.49	0.965
F.LEVNEEGSE.A	2	503.21	505.39	503.67	0.211	504.01	0.367	504.66	0.665	503.93	0.33	503.83	0.284	504.36	0.528	504.27	0.486
F.LKEFSNMVT.A	1	1066.5	1074.05	1073.7	0.936	1073.7	0.94	1073.8	0.951	1073.6	0.953	1073.9	0.966	1073.9	0.96	1074.1	1.005
F.LSKAIHKSF.L	1	1030.6	1033.25	1032.6	0.743	1032.7	0.808	1031.8	0.464	1032.2	0.619	1033	0.891	1032.4	0.66	1032.9	0.853
F.MGRVMHPETMNTSGHD.F	2	900.38	904.43	903.05	0.659	903.04	0.657			903.13	0.679	903.1	0.672	903.17	0.669	903.42	0.751
F.TVEQEIDLKDVL.K	2	701.37	704.85	703.88	0.721	704.36	0.859	704.23	0.822	704.81	0.989	704.83	0.994	704.81	0.989	704.81	0.989
F.YYGEFSDGSNEAGGIY.Q	1	1728.7	1737.1	1734.1	0.64	1734.4	0.681	1740.9	1.455	1734.7	0.719	1735.1	0.757	1735.8	0.846	1735.5	0.807
L.EVNEEGSEAAAVSGMIA.I	2	832.37	836.7	834.81	0.564	834.84	0.57			834.64	0.524	835.23	0.661	835.4	0.7	835.6	0.746
L.FSPLSIAL.A	2	424.24	427.31	425.14	0.293	425.04	0.261	425.11	0.283	425.22	0.319	425.17	0.303	425.26	0.332	425.47	0.401
L.FVQNGF.H	1	711.34	713.3	712.4	0.541	712.64	0.663			713.07	0.883	713. 2	1.01	713.22	0.959	713.44	1.071
L.SVNMYNRL.R	1	996.49	1002.2	998.39	0.333	997.56	0.187			998.02	0.266	996.67	0.362	999.05	0.448	999.55	0.536
L.VKDLVSPRD.F	1	1028.6	1035.32	1032.6	0.593	1031	0.363	1031.6	0.449	1032.2	0.535	1033	0.649	1032.8	0.625	1032.9	0.636
M.MLVLSRQEVPLATL.E	2	785.45	789.95	786.33	0.196	787.04	0.353	788.7	0.722	788.31	0.636	786.52	0.682	788.62	0.749	788.77	0.738
M.VTAKESQY.V	2	463.23	467.22	464.73	0.376	465.14	0.479	465.29	0.516	465.52	0.574	465.77	0.637	466.14	0.729	466.14	0.729
M.YNRLRATGEDENIL.F	2	832.41	638.78	834.62	0.346	634.84	0.383	834.74	0.367	834.66	0.354	835.22	0.443	835.4	0.471	835.59	0.501
N.LVKDLVSPRDFDAAFY.L	2	905.46	912.33	908.12	0.387	908.29	0.412	906.3	0.413	909.46	0.437	908.61	0.459	909.24	0.55	907.91	0.357
Q.VLEIPYEGDEIS.M	1	1363.7	1369.32	1365.6	0.334	1365.8	0.376			1366.5	0.495	1366.7	0.541	1367.6	0.687	1367.3	0.645
S.FLKEFSNM.V	2	508.24	510.77	510.4	0.854	510.4	0.854	510.56	0.917	610.54	0.909	510.49	0.669	510.7	0.972	610.72	0.98
S.FTKDDESEVQIPMM.Y	2	835.37	838.76	837.42	0.605	837.35	0.584	836.89	0.448	837.45	0.614	837.49	0.625	837.68	0.681	837.79	0.714

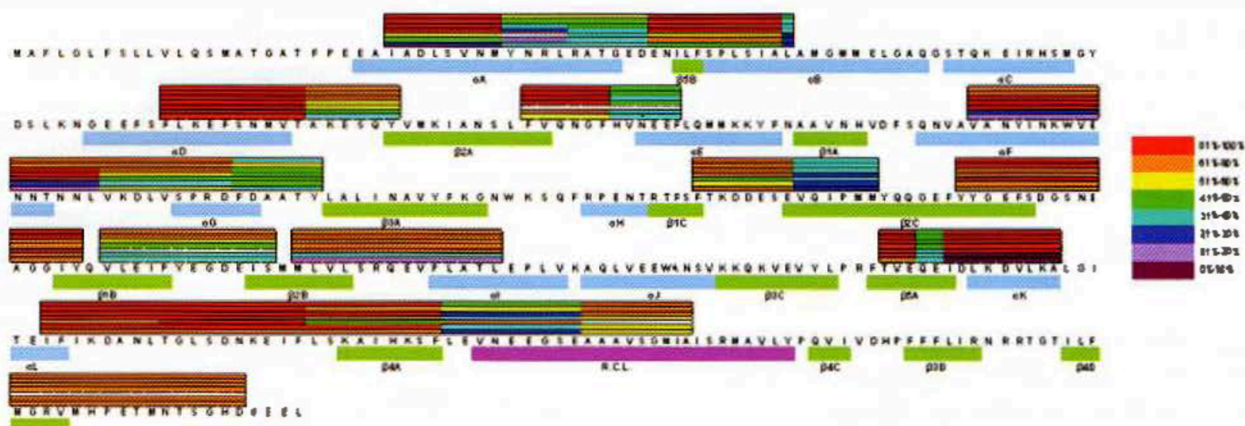

Figure 1. Rate of hydrogen/deuterium exchange of specific peptides at different time points (10, 50, 100, 500, 1000, 2000, and 3000 seconds) with 10 seconds being closest to the sequence and 3000 seconds being farthest away. Alpha helix regions are marked in blue, beta-pleated sheet regions are marked in green, and the reactive center loop is marked in magenta.

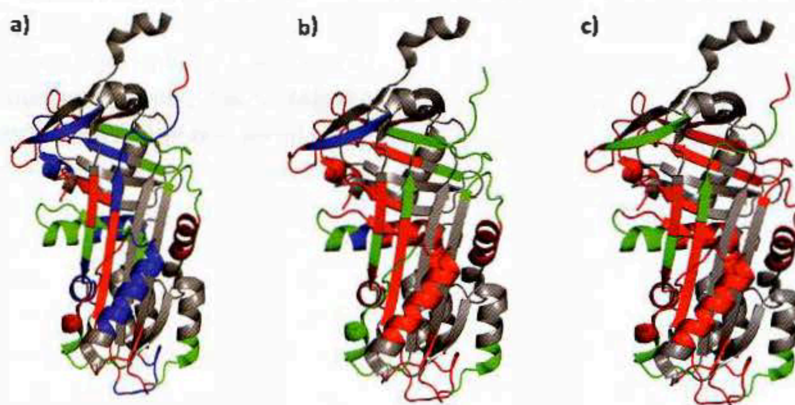

Figure 2. Rate of hydrogen/deuterium exchange of certain peptides at different time points where a) is after 10 seconds of exchange, b) is after 500 seconds of exchange, and c) is after 3000 seconds of exchange. Blue symbolizes 0%-30% exchange, green symbolizes 31%-60% exchange, and red symbolizes 61%-100% exchange.

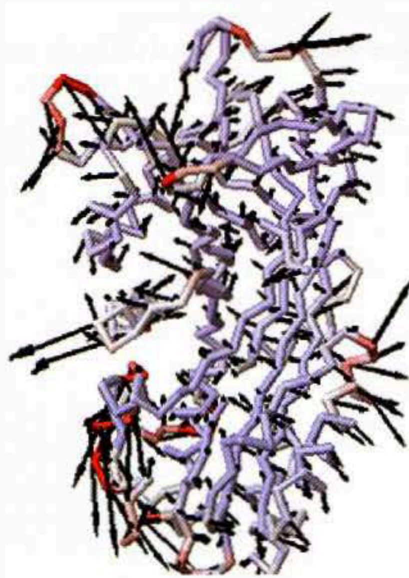

Figure 3. Vector diagram of wild-type human neuroserpin. Longer vectors equate to greater fluctuations and the red regions are more mobile than the blue regions.

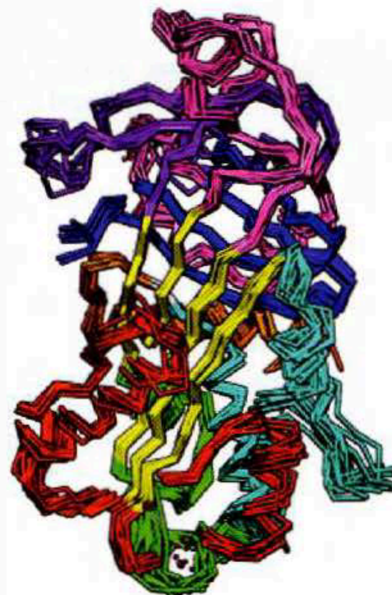

Figure 4. Fluctuations of the different regions of wild-type human neuroserpin, where more compact and solid regions equate to regions of less flexibility and regions that occupy a larger range of space indicate peptides that display more mobility.

Molecular Dynamic Simulations:

The protein on a global level is very mobile and unstable, as shown by Figures 3 and 4. The more flexible regions allow for easier exchange between deuterium and the amide hydrogens. Molecular dynamics simulations of both the wild-type and a His338-->Arg mutant structure were generated using VMD (Figure 6). The correlation diagrams (Figure 5) show that most of the alpha helical structures move in the same directions (shown in red) whereas other regions of the protein, such as beta sheets, either move in opposite directions (shown in blue) or independently of one another (shown in yellow). As we can see, most of the red that goes down the diagonal line from top left to bottom right corresponds to the movement of the alpha helices. When we compare the wild-type (Figure 5 left) to the mutant (Figure 5 right), we can see that the His338-->Arg mutation alters the global dynamics of neuroserpin, leading to more correlated and anti-correlated motions (Figure 5). Locally, the mutants show distorting motions at the top of beta sheet A (Figure 6 right) and increased mobility in helix F (Figure 6 left). Disruption of both sheet A and helix F has been linked to polymer formation in other serpins. The perturbed structure and dynamics in these regions in the pathological His338Arg mutant of neuroserpin may facilitate polymer formation. The free energy landscape diagram of the principle component planes shows that wild-type human neuroserpin has at least two well-populated energy wells (Figure 7 left), whereas alpha-1 antitrypsin only has one well-populated energy well (Figure 7 right).

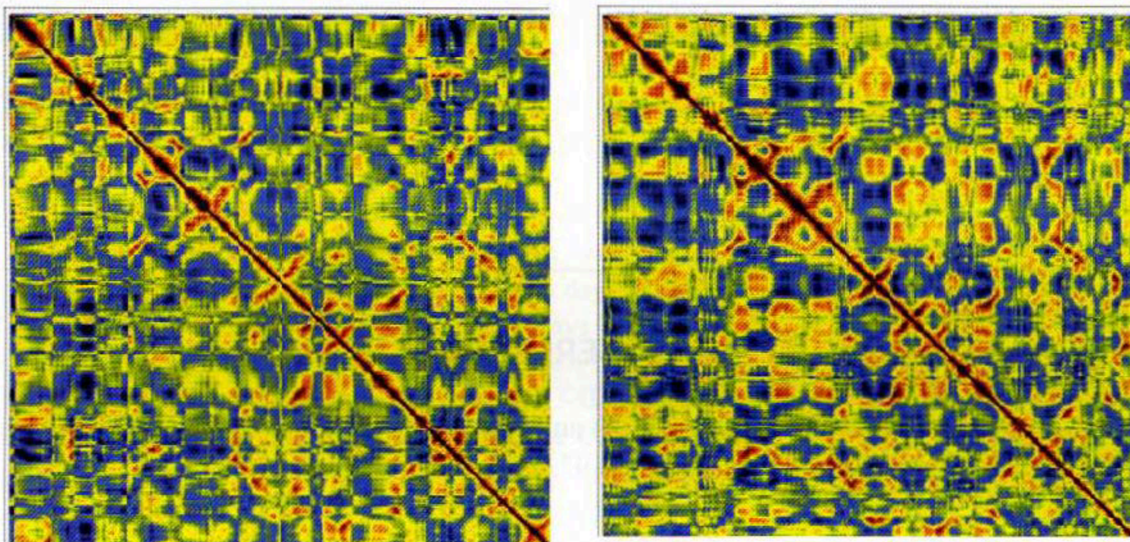

Figure 5. Variance-covariance matrices of wild-type human neuroserpin (left) and neuroserpin mutant (right). Red regions indicate residues that move in a correlated manner, blue regions indicate residues that move in an anti-correlated manner, and yellow regions indicate residues that move independently.

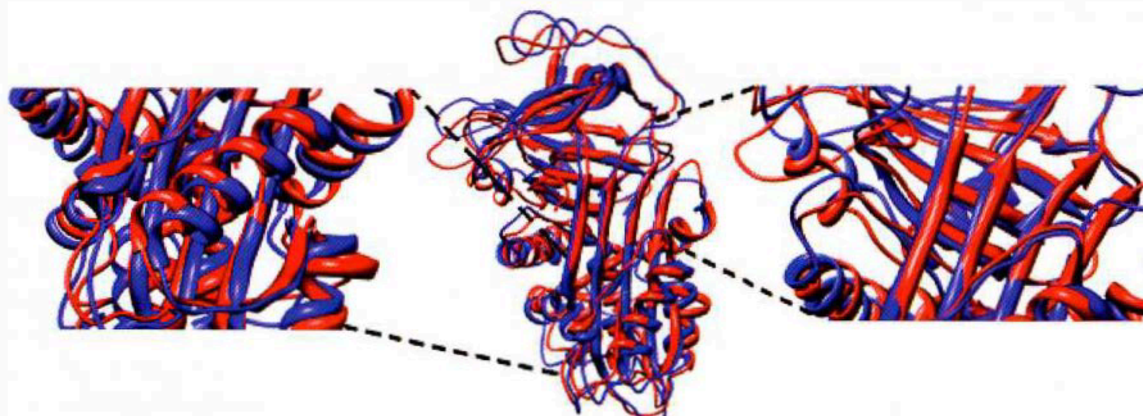

Figure 6. Molecular dynamic simulations of wild-type neuroserpin, in red, superimposed upon its mutant structure, in blue. Distinguishing factors between the two structures are emphasized by focusing on the f-helix (left) and the central beta-sheet (right).

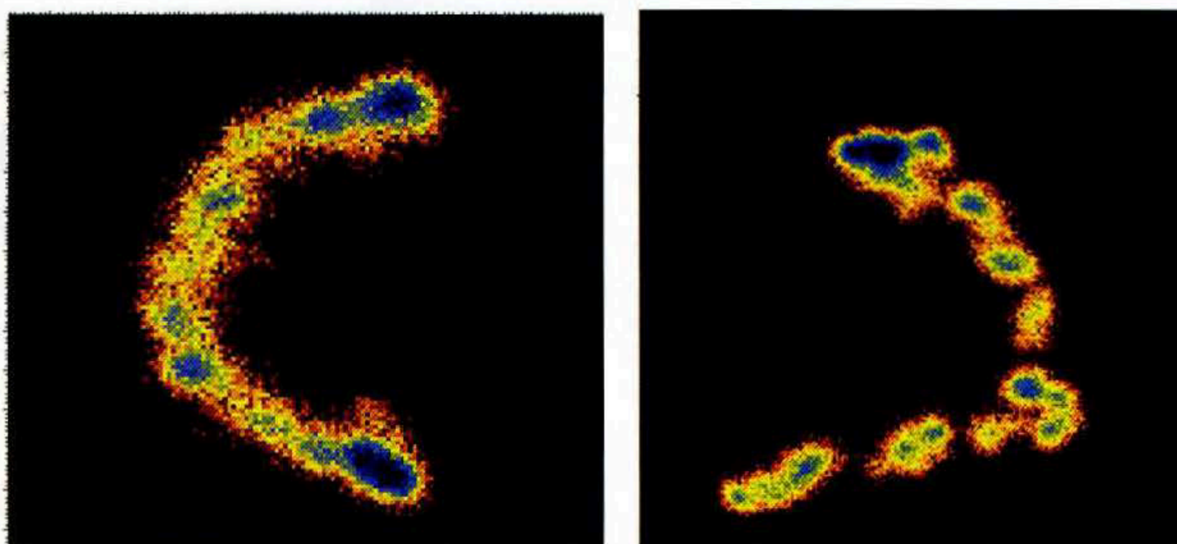

Figure 7. Free energy landscape diagram on the principle component planes defined by the eigenvectors corresponding to the first and second highest eigen values. The one on the left is of wild-type human neuroserpin and the one on the right is of α1-antitrypsin.

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