MICHIGAN STATE UNIVERSITY

HONGGAO YAN, PH. D.
DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY
313A BIOCHEMISTRY BUILDING

EAST LANSING • MICHIGAN • 48824-1319 • USA
PHONE: 517-353-8786
FAX: 517-353-9334
E-MAIL: YANH@MSU.EDU

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Biocomplexity Faculty Search Committee
c/o Prof. Rob de Ruyter van Steveninck
Biocomplexity Institute, Indiana University
Swain Hall West 117, Bloomington IN47405-7105

Dear Prof. van Steveninck:

I am writing in support of Dr. Lincong Wang's application for a faculty position in your department.

Lincong joined my laboratory in 1993 and graduated with a Ph.D. degree in biochemistry in 1998. His project was the structure-function studies of cellular retinoic acid binding protein II (CRABPII). It's a multidisciplinary project involving molecular genetic, biochemical and biophysical methods. The major part of his project was the determination by multidimensional NMR spectroscopy of the solution structures of the wild-type cellular retinoic acid binding protein II and the site-directed mutant R111M with arginine-111 replaced by methionine. Lincong showed that the solution structure of apo-CRABPII is similar to the crystal structure of holo-CRABPII except the ligand entrance, which is sufficiently enlarged in the apoprotein to be readily accessible to retinoic acid. The enlargement of the ligand entrance of apo-CRABPII relative to that of holo-CRABPII is due mainly to a concerted conformational change in three structural elements, namely the second helix, the βC-βD loop and the βE-βF loop. Furthermore, the ligand-binding pocket of apo-CRABPII showed evidence of dynamic disorder: among the 21 residues that constitute this pocket, 16 residues had weak or no detectable cross-peaks in the two-dimensional ^{1}H-^{15}N heteronuclear single quantum coherence (HSQC) spectrum recorded under conditions of minimal water saturation or dephasing. Apo-CRABPII is largely monomeric in solution, with no evidence for the dimeric structure shown in the crystal structure of apo-CRABPI which was suggested to be a prerequisite for ligand entry. Thus the widening of the ligand entrance required for entry of retinoic acid appears to be a property of monomeric apo-CRABPII.

The solution structure of apo-R111M is similar to that of wild-type apo-CRABPII. However, there are significant conformational differences between the two proteins, localized mainly to three segments (Leu19-Ala36, Glu73-Cys81 and Leu99-Pro105) clustered around the ligand entrance more than 17 Å away from the point mutation. In apo-R111M, all the three segments move towards the center of the ligand entrance so that the opening of the ligand-binding pocket in apo-R111M is much smaller than that in wild-type apo-CRABPII. Furthermore, the ligand-binding pocket of apo-R111M, especially the ligand entrance, is much

less flexible than that of apo-CRABPII. Surprisingly, apo-R111M is more similar to holo-CRABPII than to apo-CRABPII in both structure and dynamical properties. The conformational and dynamical changes caused by the mutation are similar to those induced by binding of retinoic acid, although the magnitudes of the changes caused by the mutation are smaller than those induced by binding of retinoic acid. The results suggest that Arg111 plays a critical role in determining the structure and dynamical properties of CRABPII.

Lincong was initially trained and worked as an engineer. His love for science brought him back to study biology. His interest in combining physical sciences with biology brought him to my laboratory. Lincong is very bright and has very solid training in physical sciences as evidenced by his theoretical work in Dr. Zuiderweg's laboratory. I was most impressed with his love and enthusiasm for science and independent thinking. At the time of his graduation, he could have gone to the best NMR laboratories for solution structure determination of proteins but he chose to study protein dynamics as a postdoctoral fellow. He understood there were more jobs in structure determination than in dynamics studies but he chose to study dynamics because he felt that dynamics plays important roles in protein function and he could make contributions to the field with his solid backgrounds in mathematics and physics.

I strongly support Dr. Lincong Wang's application for the faculty position in your department. Please let me know if you need any further information.

Sincerely yours,

Honggao Yan
Associate Professor of Biochemistry