Ionic Strength Dependence of F-actin and Glycolytic Enzyme Associations: A Brownian Dynamics Simulations Approach Supplemental Information

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Building the Homology Model of Zebrafish LDH

The Homology module of the Insight®II molecular modeling package (Accelrys, San Diego, CA) was used to build the quaternary structure of zebrafish (*Brachydanio rerio*) LDH based on its primary amino acid sequence and known crystal structures of carp (*Cyprinus carpio*) accession code (1V6A) and dogfish (*Squalus sacanthia*) accession code (1LDM). These crystal structures were obtained from the RSCB Protein Data Bank. The primary sequence of the muscle form of zebrafish (*Brachydanio rerio*) LDH with primary accession number (PAN), Q9PVK5) was downloaded from the UniProt protein database. A sequence alignment between this sequence and other available LDH sequence was made (Table S1).

Table S1. Primary Structure Similarity of Some LDH Molecules.

LDH seq	Zebra (1)[a]	Zebra (2)[b]	Carp[c]	Human[d]	Pig[e]	Rabbit[f]	Plasmodium[g]
Zebra (1)	100 %	73.3 %	95 %	67.8 %	77 %	76 %	33 %
Zebra (2)	73.3 %	100 %	73 %	62.3 %	71 %	71 %	32 %
Carp	95 %	73 %	100 %	67 %	75 %	75 %	33 %
Human	67.8 %	62.3 %	67 %	100 %	73 %	72 %	35 %
Pig	77 %	70.9 %	75 %	73 %	100 %	95 %	33 %
Rabbit	76 %	71 %	75 %	72 %	95 %	100 %	33 %
Plasmodium	33 %	32	33 %	35 %	33 %	33 %	100 %

[a]Zebrafish (1) (whole body tissue).
Zebrafish (2) (cytoplasmic).
[c]Carp (Muscle tissue).
[d]Human (Muscle tissue).
[e]Pig (Muscle tissue).
[f]Rabbit (Muscle tissue).
[g]Plasmodium (cytoplasmic).

The results of the sequence similarity are shown on the Table S1. Most of the other LDH sequences are about 73 % similar to the zebrafish LDH sequence on average. Carp, however, shows very high similarity (95 %) with zebrafish LDH. As a result, only the carp reference protein was utilized, to build the tertiary structure of zebra fish LDH (Figure S1). This was done by copying coordinates from the, the carp protein to the zebrafish sequence, using the homology module of Insight®II (Figure S1).

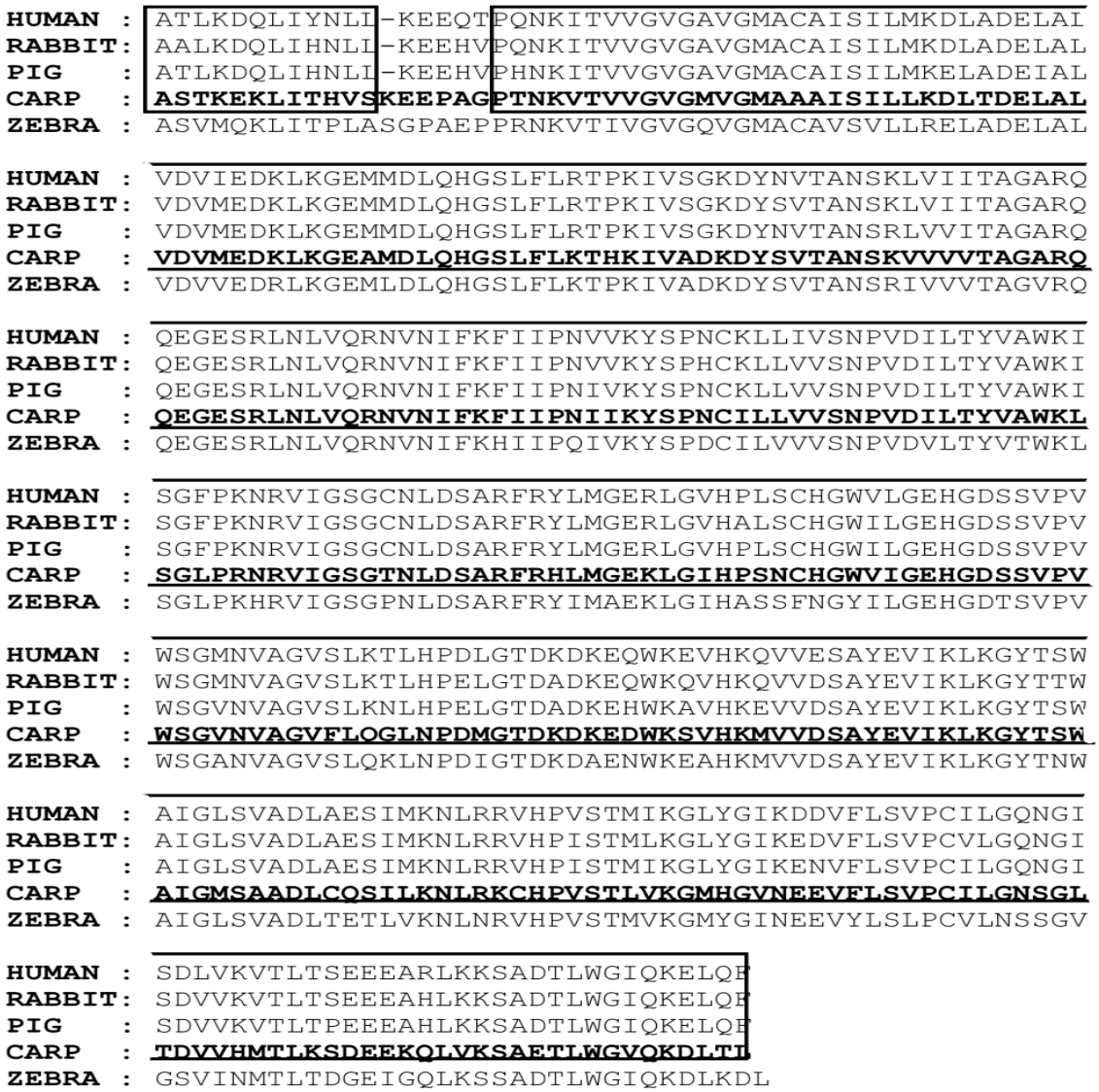

Figure S1. Multiple Sequence Alignment Between Four Different LDH Reference Protein Sequences and Zebrafish LDH. The coordinates of zebrafish LDH were copied from the carp LDH reference protein, whose sequence in bold. The boxes indicate regions of high sequence similarity.

The zebrafish LDH monomer was then energy minimized using the Discover module of Insight®II and the AMBER force field. A strategy of steepest descent, followed by conjugate gradient minimization for 10,000 iterations was performed. The dielectric constant was set at 78.3 for water at a temperature of 298 K. The resulting low energy monomer was then superimposed on each of the subunits of the dogfish LDH homotetramer (1LDM) to form the quaternary structure of zebrafish LDH. Both molecules have a primary amino acid sequence identity of about 71 %. The tetramer was again minimized to relieve atomic overlaps using the same protocol in the Discover_3 module because of the larger number of atoms in the tetramer.

A similar approach was used to build a quaternary structure for zebrafish GAPDH (Q5XJ10) using rabbit skeletal muscle GAPDH (1JOX) as reference protein. Both molecules share a primary amino acid sequence similarity of 86 % (Figure S2). The zebrafish model built is predominant in the embryo. This model was used because of its high sequence similarity to the rabbit enzyme.

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Rabbit: VKVGVNGFGRIGRLVTRAAFNSGKVDVVAINDPFIDLHYMVYMFQYDSTH
Fish  : VKVGINGFGRIGRLVTRAAFLTKKVEIVAINDPFIDLDYMVYMFQYDSTH

Rabbit: GKFHGTVKAENGKLVINGKAITIFQERDPANIKWGDAGAEYVVESTGVFT
Fish  : GKYKGEVKAEGGKLVIDGHAITVYSERDPANIKWGDAGATYVVESTGVFT

Rabbit: TMEKAGAHLKGGAKRVIISAPSADAPMFVMGVNHEKYDNSLKIVSNASCT
Fish  : TIEKASAHIKGGAKRVIISAPSADAPMFVMGVNHEKYDNSLTVVSNASCT

Rabbit: TNCLAPLAKVIHDHFGIVEGLMTTVHAITATQKTVDGPSGKLWRDGRGAA
Fish  : TNCLAPLAKVINDNFVIVEGLMSTVHAITATQKTVDGPSGKLWRDGRGAS

Rabbit: QNIIPASTGAAKAVGKVIPELNGKLTGMAFRVPTPNVSVVDLTCRLEKAA
Fish  : QNIIPASTGAAKAVGKVIPELNGKLTGMAFRVPTPNVSVVDLTVRLEKPA

Rabbit: KYDDIKKVVKQASEGPLKGILGYTEDQVVSCDFNSDTHSSTFDAGAGIAL
Fish  : KYDEIKKVVKAAADGPMKGILGYTEHQVVSTDFNGDCRSSIFDAGAGIAL

Rabbit: NDHFVKLISWYDNEFGYSNRVVDLMVHMASKE
Fish  : NDHFVKLVTWYDNEFGYSNRVCDLMAHMASKE
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Figure S2. Alignment Between Rabbit GAPDH and Zebrafish GAPDH. The unbold letters represent regions in which both sequences have different amino acids.

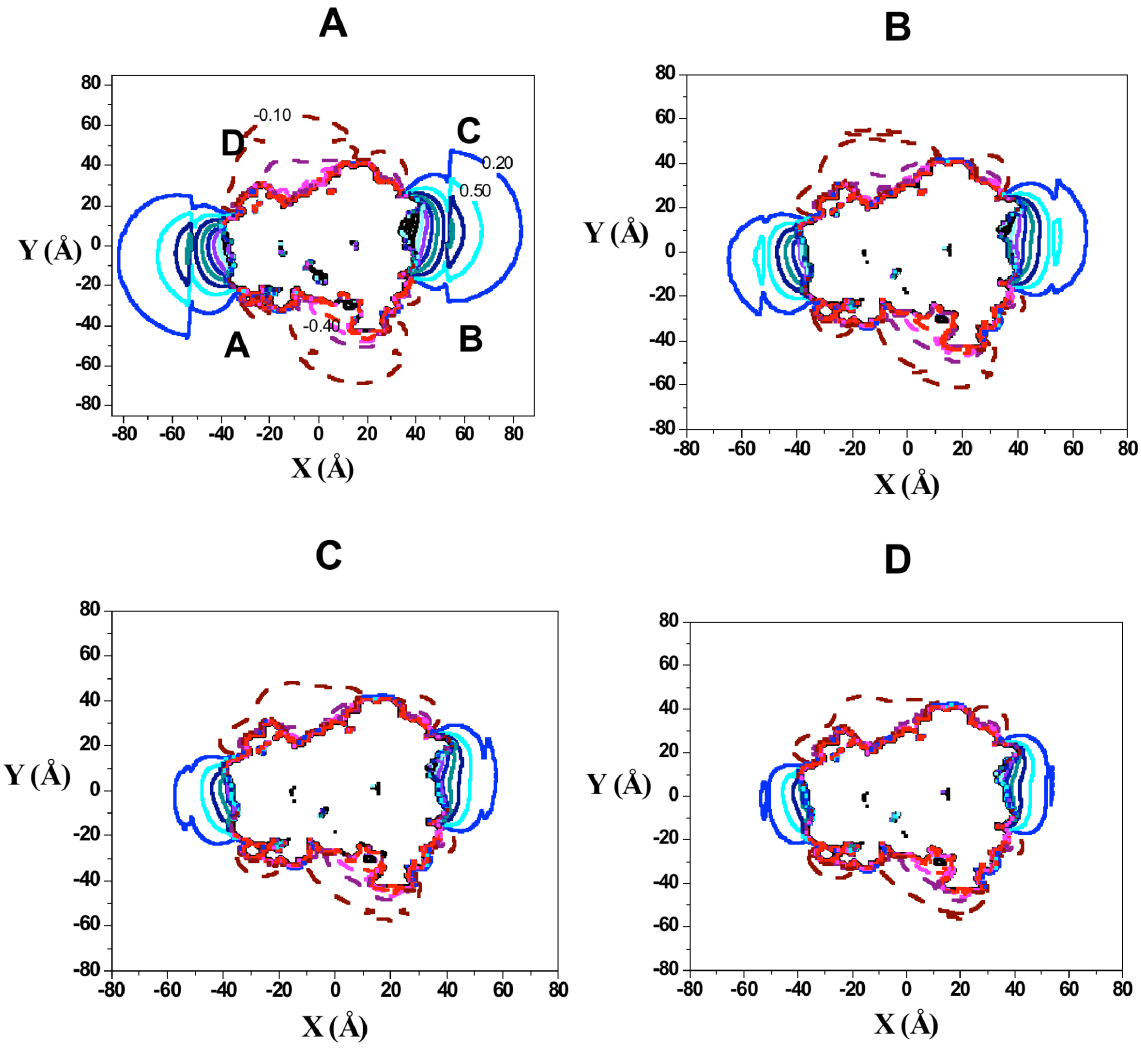

Fig. S3. Electrostatic Potential Contours about Rabbit Aldolase. These are two-dimensional representations of the extension of the electrostatic field around the enzyme. Solid lines represent positive electrostatic potential and dashed lines negative electrostatic potential. The different colors represent different electrostatic potential intensities and also serve for clear differentiation between adjacent contours. (A) I = 0.01 M. (B) I = 0.05 M. (C) I = 0.1 M. (D) I = 0.15 M. In all cases, the contour levels are 0.3 kcal/mol apart. Red (–1 kcal/mol), Magenta (–0.71 kcal/mol), Purple (–0.43 kcal/mol), Dark red (–0.14 kcal/mol), Blue (0.2 kcal/mol), Cyan (0.5 kcal/mol), Navy blue (0.8 kcal/mol), Dark cyan (1.1 kcal/mol), Royal blue (1.4 kcal/mol), Violet (1.7 kcal/mol), Black (2-3 kcal/mol).

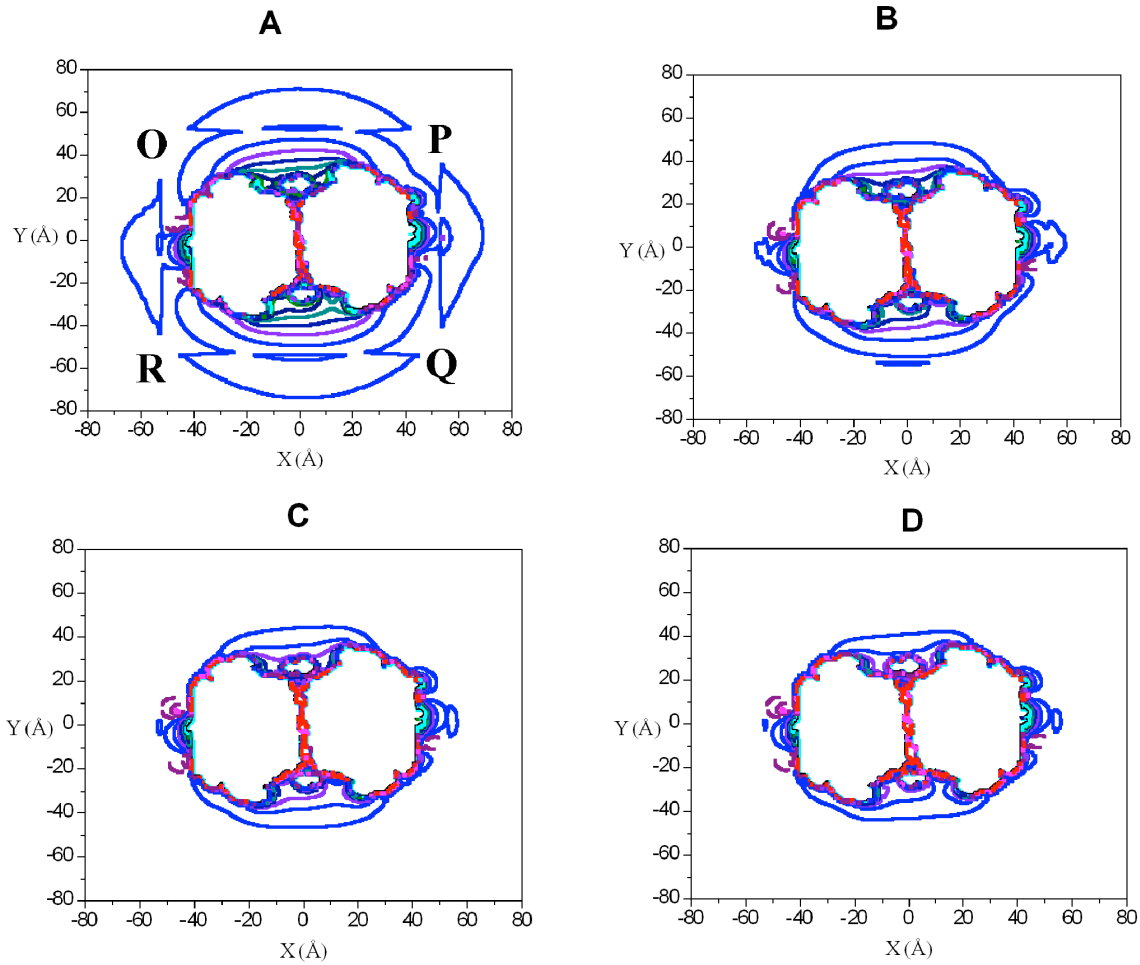

Fig. S4. Electrostatic Potential Contours about Rabbit GAPDH. The color contours and ionic strengths are the same as in Fig. S3. The active sites are found in the grooves on the left and right sides of the enzyme; they show less positive potential extension. At 0.01 M ionic strength, the EP on the GAPDH patches (corners of the tetramer) extend over two subunits as a result of an additive effect caused by the collection of positively charged residues located at the edges of the molecule. This diminishes with increasing ionic strength.

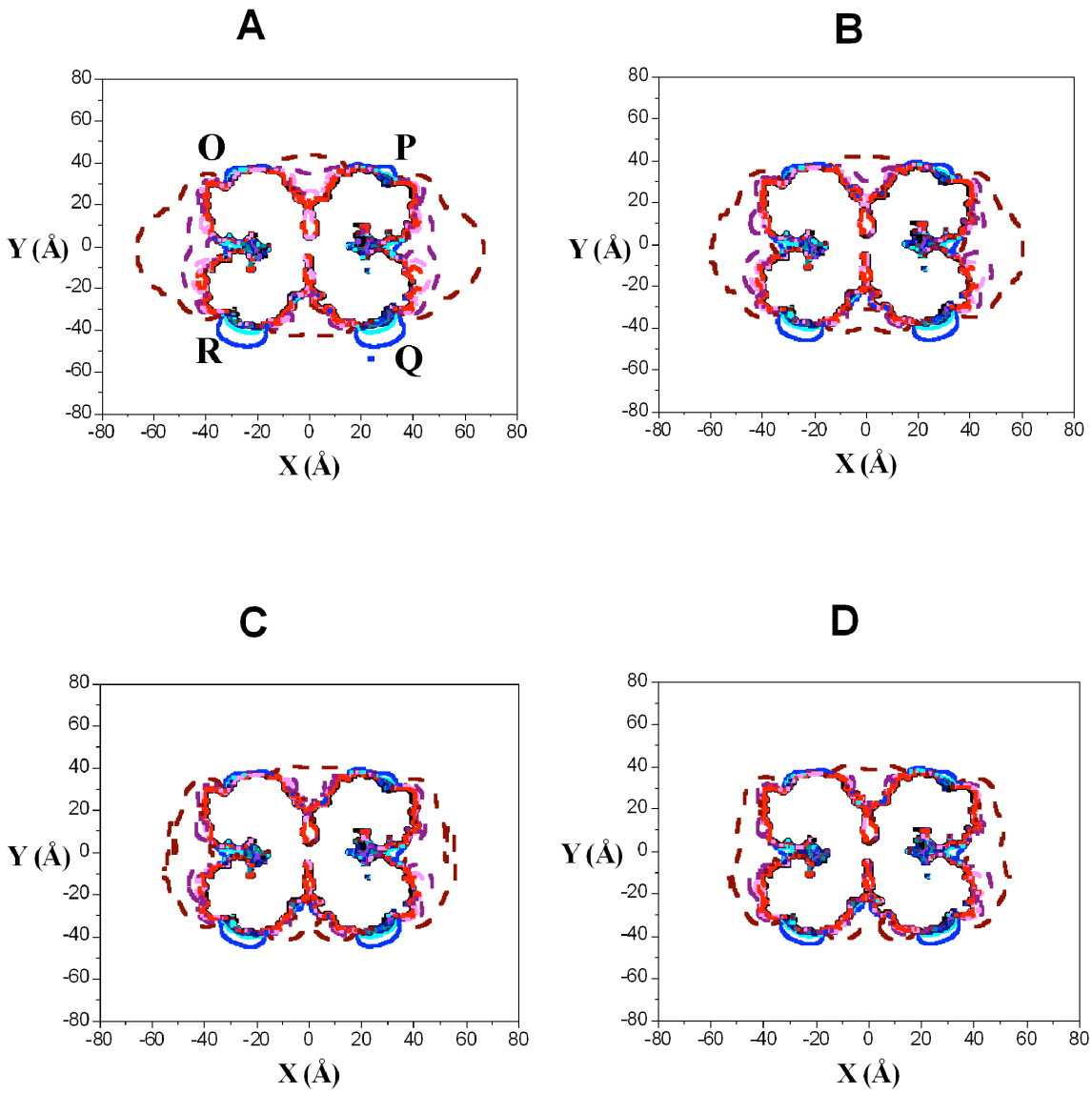

Fig. S5. Electrostatic Potential Contours about Human GAPDH. The color contours and ionic strengths are the same as in Fig. S3.

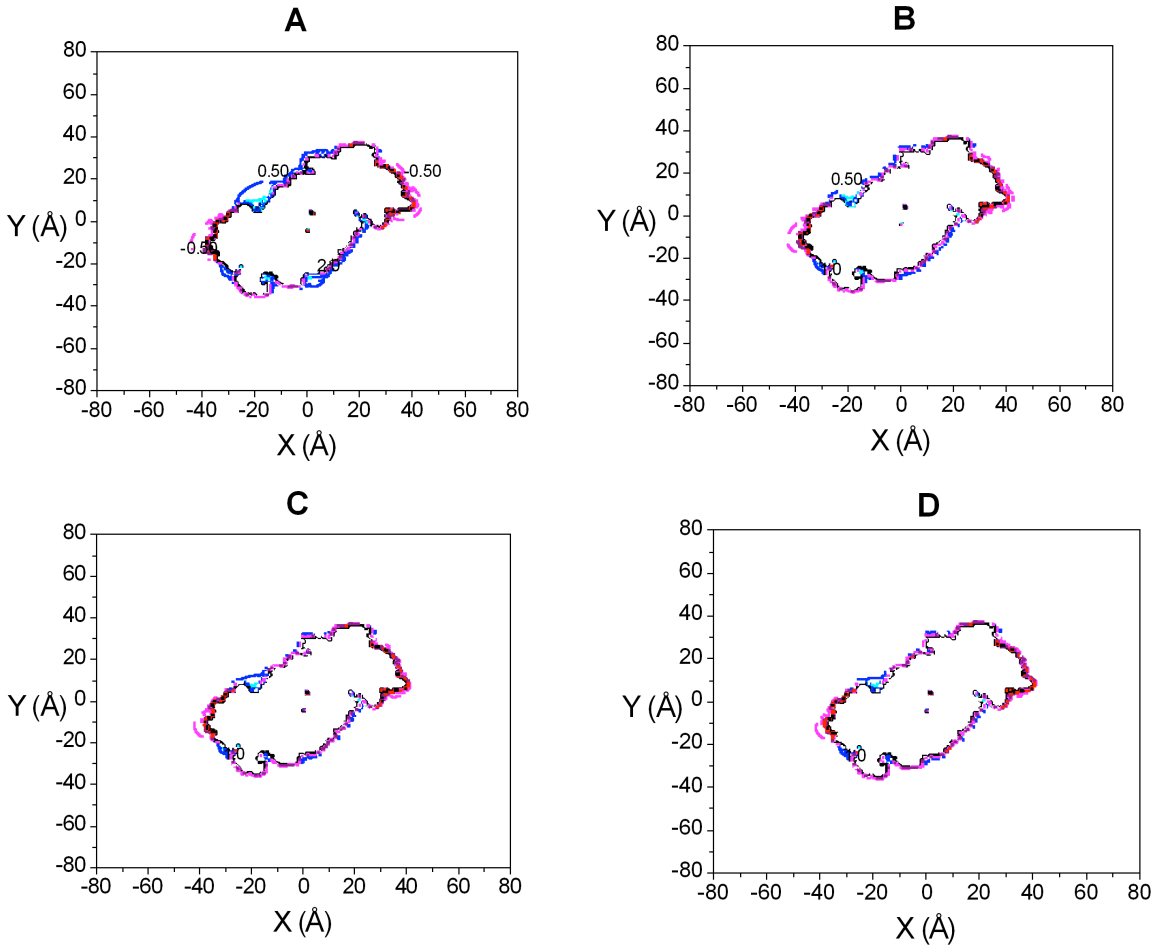

Fig. S6. Electrostatic Potential Contours about Rabbit TPI. The color contours and ionic strengths are the same as Fig. S3.

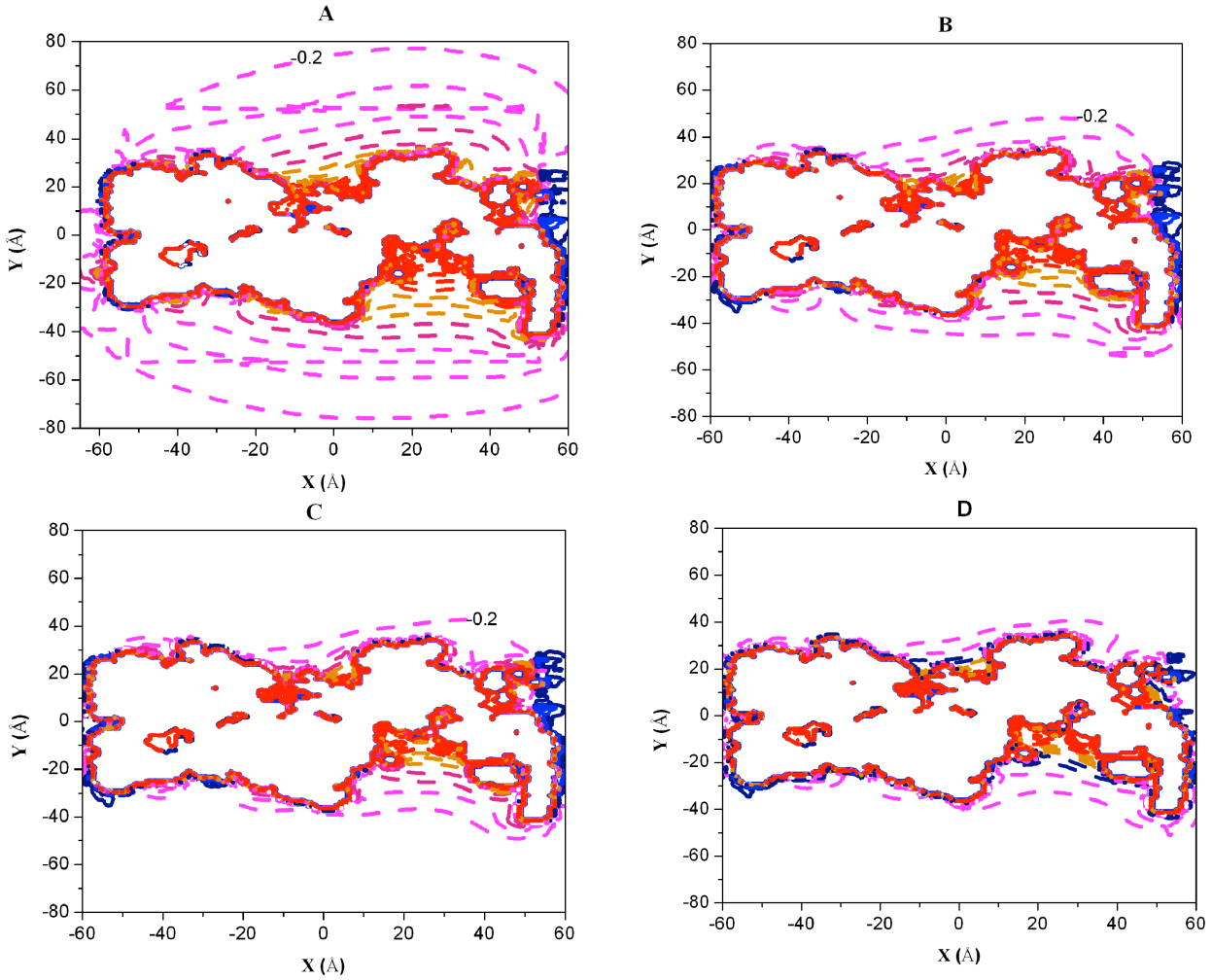

Fig. S7. Electrostatic Potential Contours about Rabbit F-actin. The color contours and ionic strengths are the same as in Fig. 1. This is a representative profile for the highly conserved F-actin molecule used for the simulations. At low ionic strengths there is an extension of negative potential further from the surface, as a result of the aggregation of negatively charged subdomain 1 residues. Zebrafish contours qualitatively very similar to rabbit (rabbit and human being identical).

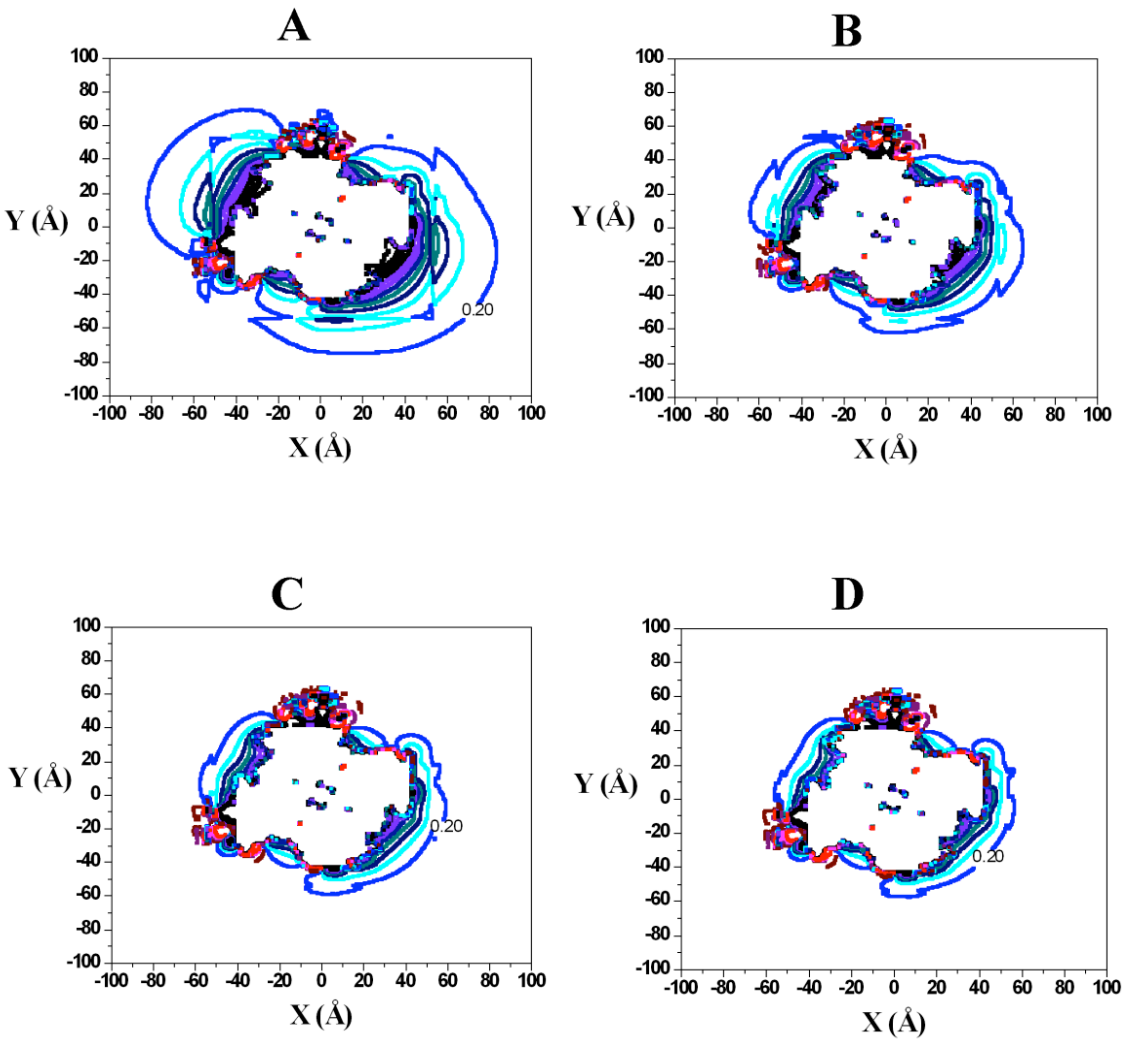

Figure S8. Electrostatic Potential Contours About Human Aldolase. These are two-dimensional representations of the extension of the electrostatic field about the enzyme. Solid lines represent positive electrostatic potential and dashed lines negative electrostatic potential. The different colors represent different electrostatic potential intensities and also serve for clear differentiation between adjacent contours. (A) I = 0.01 M. (B) I = 0.05 M. (C) I = 0.1 M. (D) I = 0.15 M. In all cases, the contour levels are 0.3 kcal/mol apart.

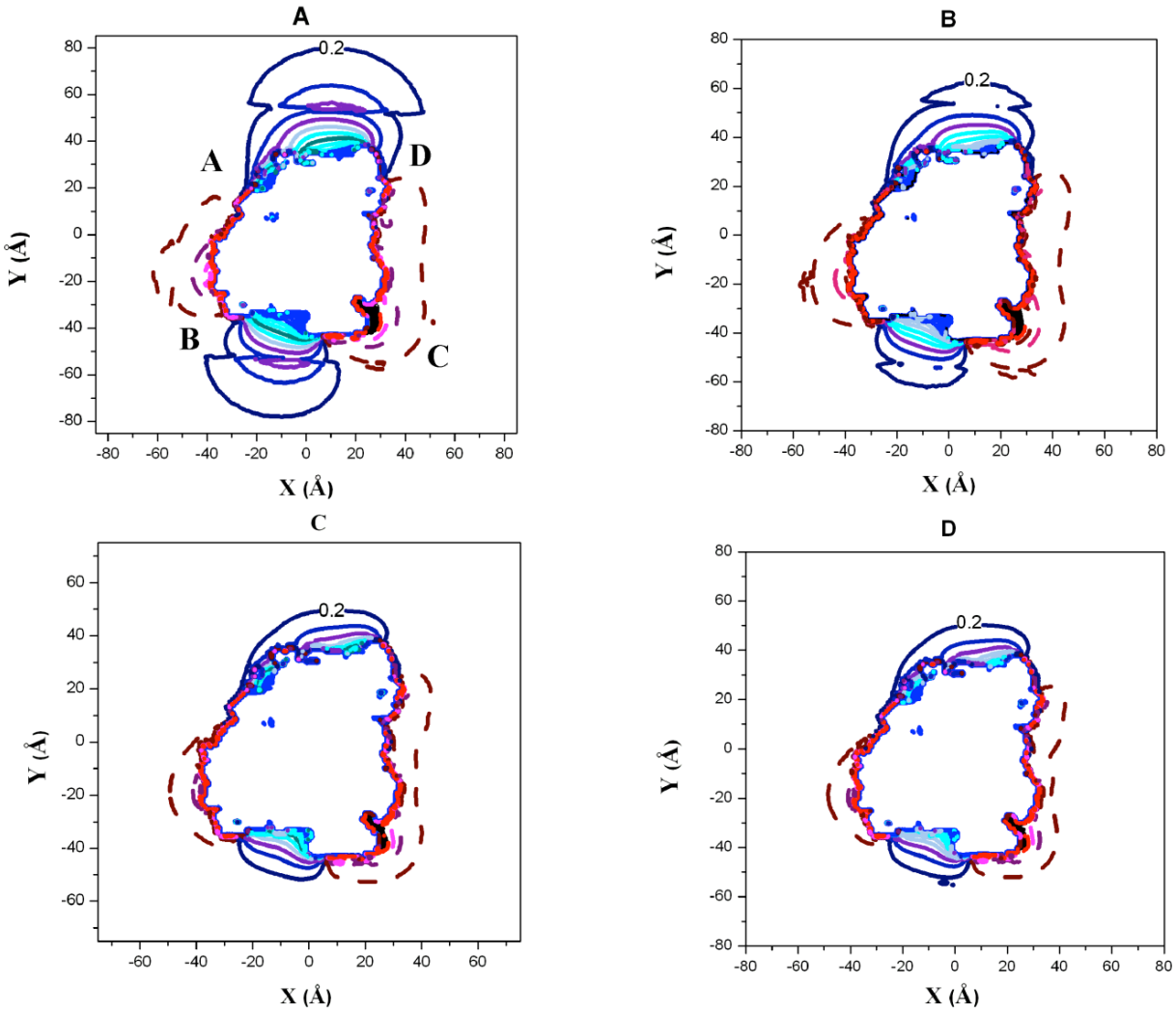

Figure S9. Electrostatic Potential Contours About Zebrafish Aldolase. These are two-dimensional representations of the extension of the electrostatic field about the enzyme. Solid lines represent positive electrostatic potential and dashed lines negative electrostatic potential. The different colors represent different electrostatic potential intensities and also serve for clear differentiation between adjacent contours. (A) I = 0.01 M. (B) I = 0.05 M. (C) I = 0.1 M. (D) I = 0.15 M. In all cases, the contour levels are 0.3 kcal/mol apart.

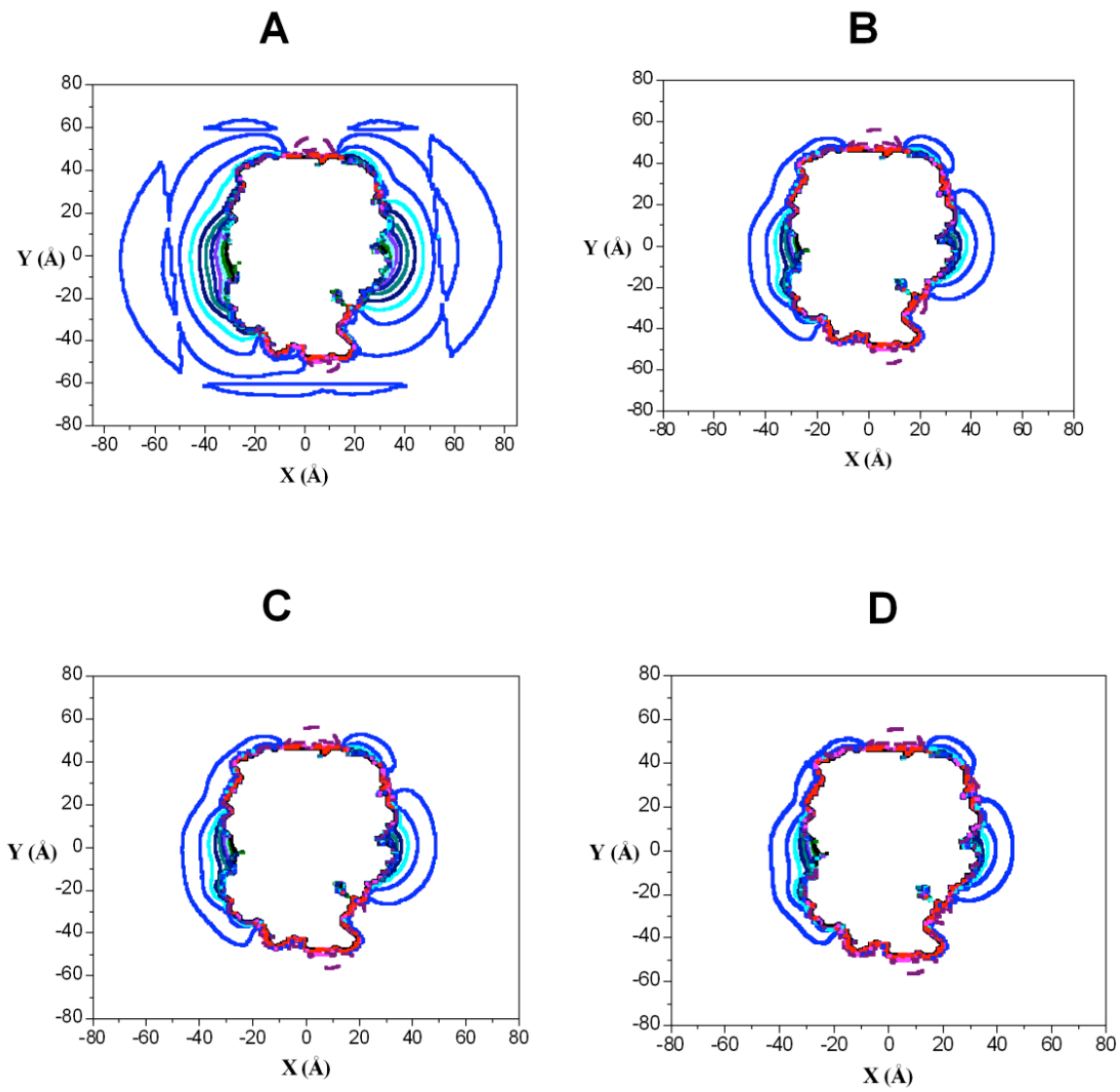

Figure S10. Electrostatic Potential Contours About Human LDH. These are two-dimensional representations of the extension of the electrostatic field about the enzyme. Solid lines represent positive electrostatic potential and dashed lines negative electrostatic potential. The different colors represent different electrostatic potential intensities and also serve for clear differentiation between adjacent contours. (A) I = 0.01 M. (B) I = 0.05 M. (C) I = 0.1 M. (D) I = 0.15 M. In all cases, the contour levels are 0.3 kcal/mol apart.

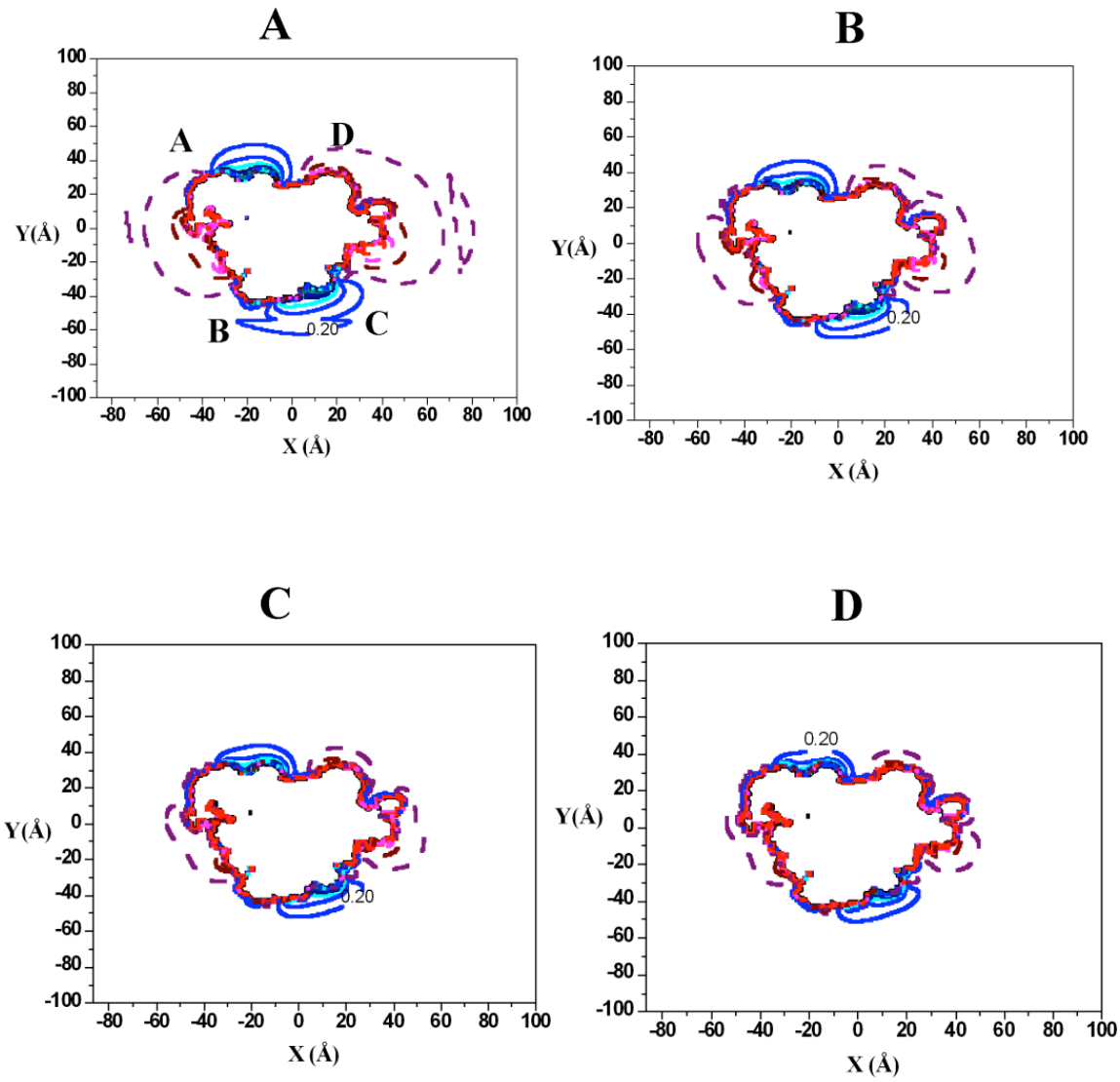

Figure S11. Electrostatic Potential Contours About Zebrafish LDH. These are two-dimensional representations of the extension of the electrostatic field about the enzyme. Solid lines represent positive electrostatic potential and dashed lines negative electrostatic potential. The different colors represent different electrostatic potential intensities and also serve for clear differentiation between adjacent contours. (A) I = 0.01 M. (B) I = 0.05 M. (C) I = 0.1 M. (D) I = 0.15 M. In all cases, the contour levels are 0.3 kcal/mol apart.

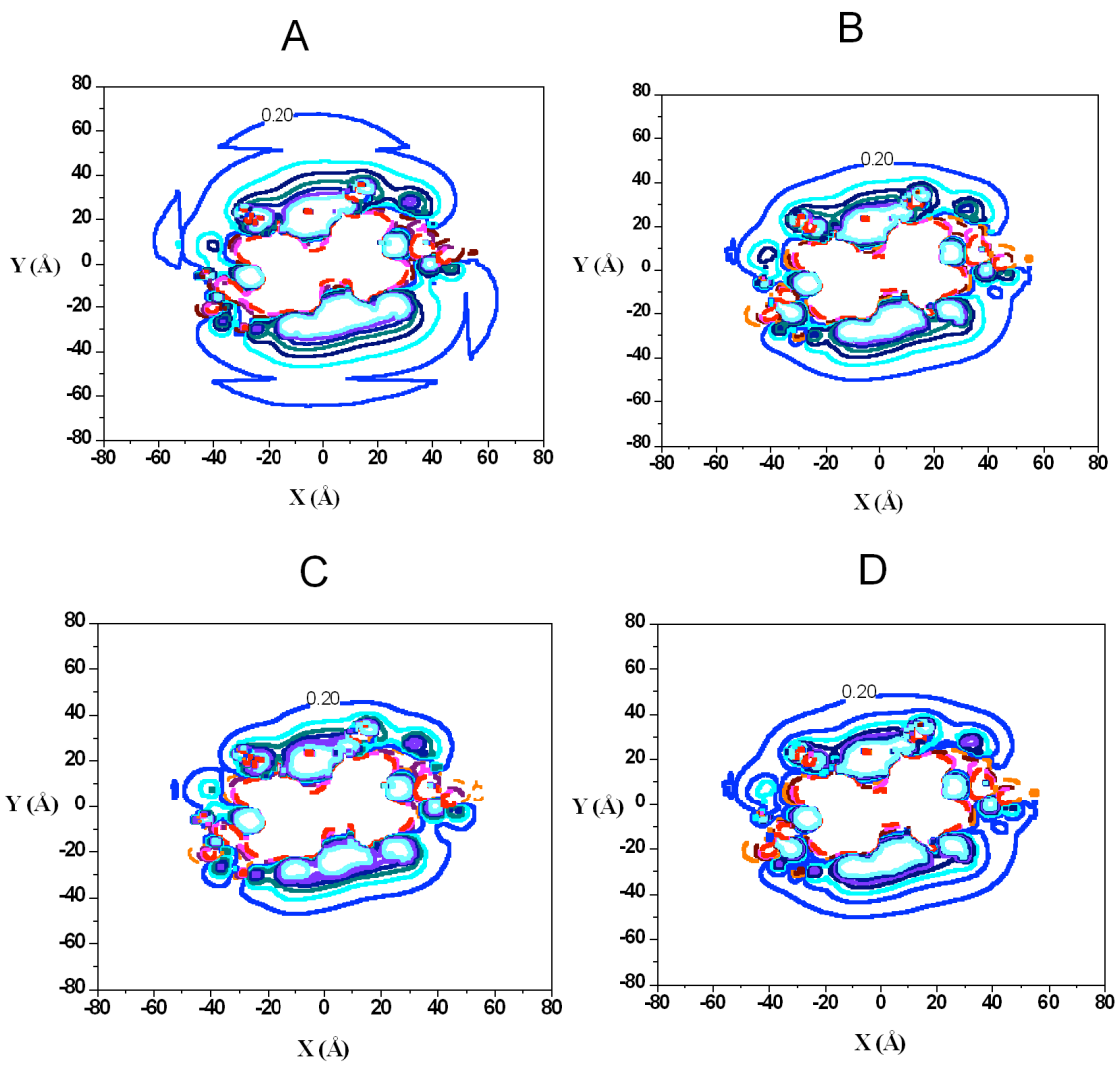

Figure S12. Electrostatic Potential Contours About Zebrafish GAPDH. These are two-dimensional representations of the extension of the electrostatic field about the enzyme. Solid lines represent positive electrostatic potential and dashed lines negative electrostatic potential. The different colors represent different electrostatic potential intensities and also serve for clear differentiation between adjacent contours. (A) I = 0.01 M. (B) I = 0.05 M. (C) I = 0.1 M. (D) I = 0.15 M. In all cases, the contour levels are 0.3 kcal/mol apart.

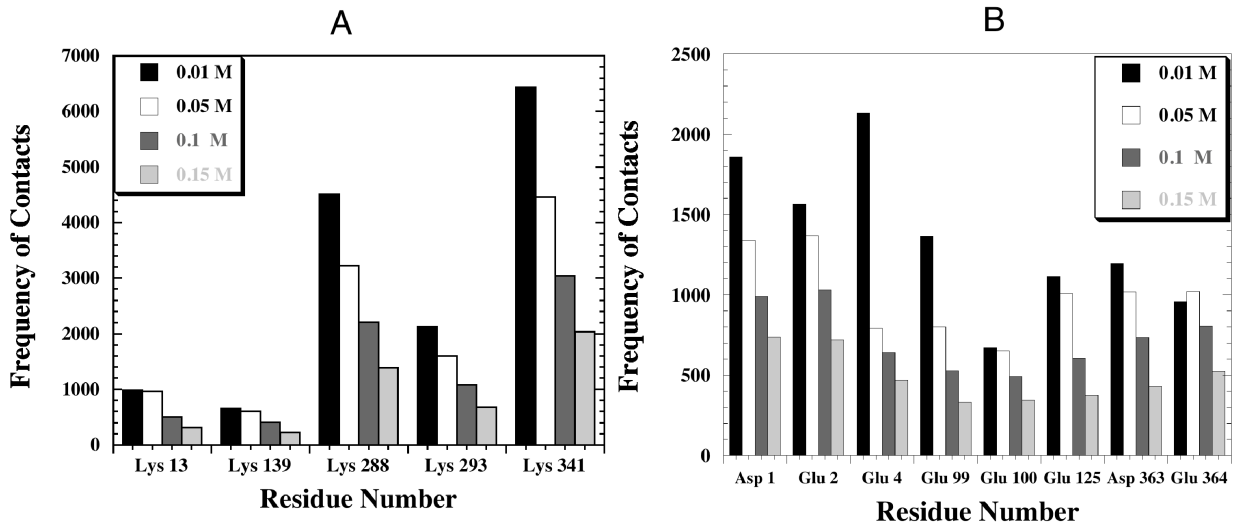

Figure S13. Residues Responsible for the Interaction Between Rabbit Aldolase and F-actin. The bars show the frequency of these residues as a function of ionic strength. (A) Aldolase residues and (B) F-actin residues. Residues involved are similar for zebrafish and human.

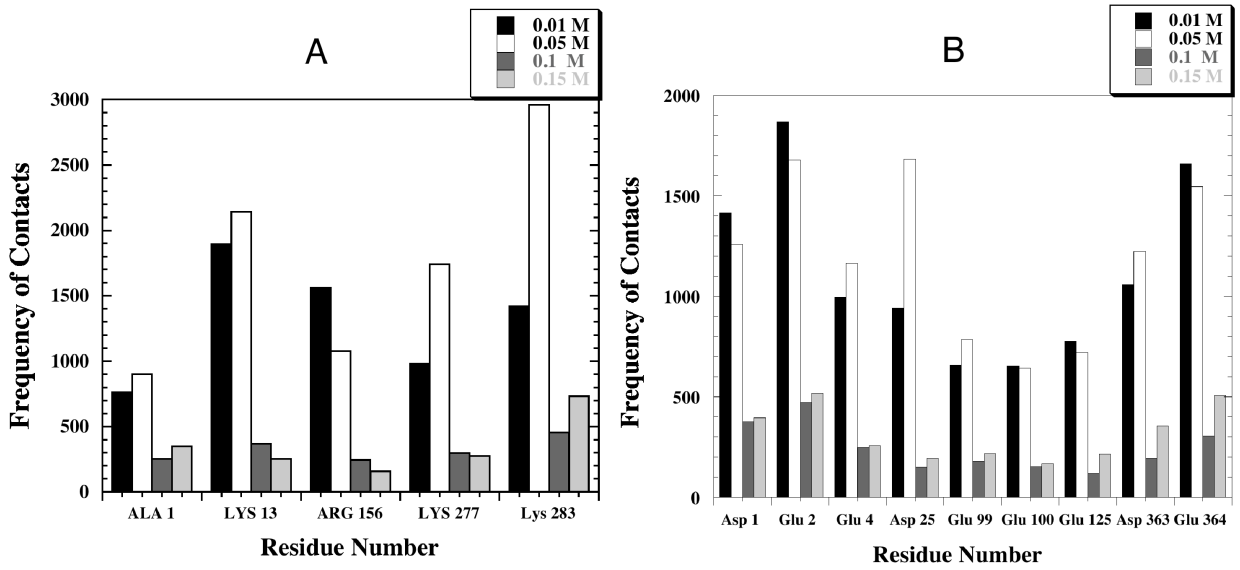

Figure S14. Residues Responsible for the Interaction Between Rabbit LDH and F-actin. The bars show the frequency of these residues as a function of ionic strength. (A) LDH residues and (B) F-actin residues. Residues involved are similar for zebrafish and human.

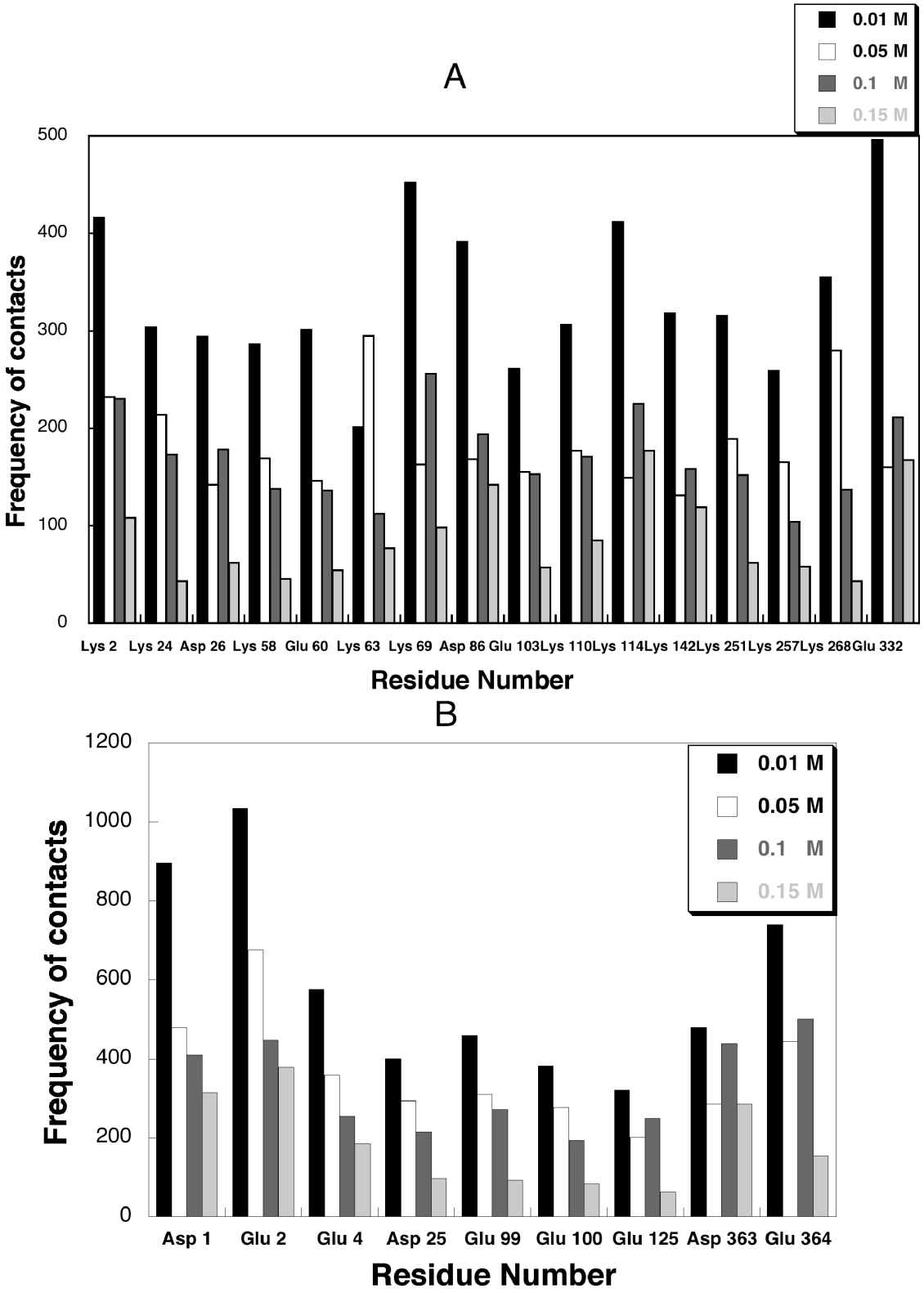

Figure S15. Residues Responsible for the Interaction Between Rabbit GAPDH and F-actin. The bars show the frequency of these residues as a function of ionic strength. (A) GAPDH residues and (B) F-actin residues.

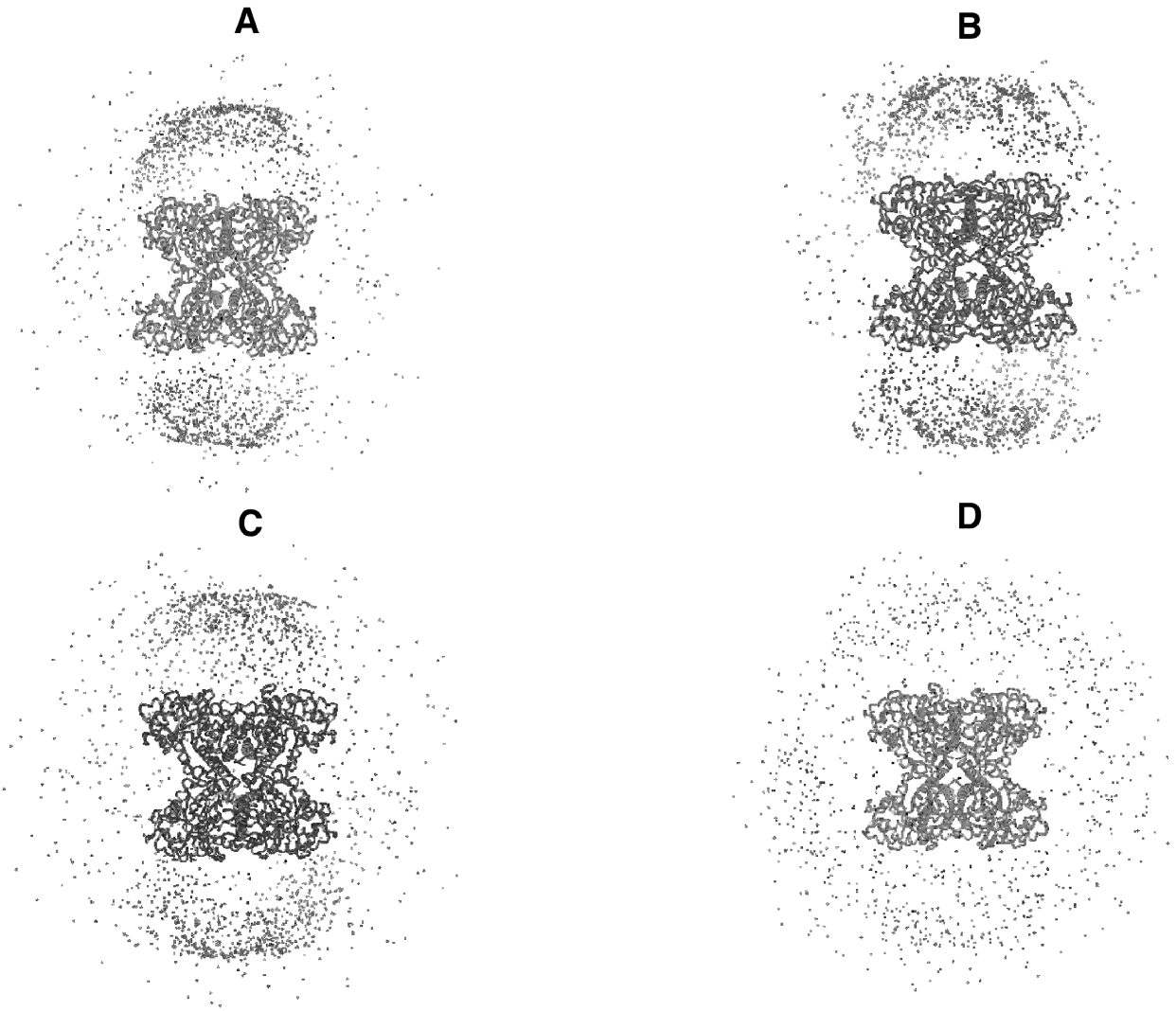

Fig. S16. Center of Mass Profile for the Interaction Between Rabbit Aldolase and F-actin as a Function of Ionic Strength. The small spheres represent center of mass atoms of F-actin in encounter snapshots with aldolase, which is represented by Cα ribbons. Similar profiles are found for human and zebrafish. **(A)** Ionic strength of 0.01 M. **(B)** Ionic strength of 0.05 M. **(C)** Ionic strength of 0.10 M. **(D)** Ionic strength of 0.15 M. Note that shallow grooves on the top and bottom of aldolase interact the most often with F-actin at ionic strengths of 0.1 M and lower, but this specificity is lost when the ionic strength increases to 0.15 M.

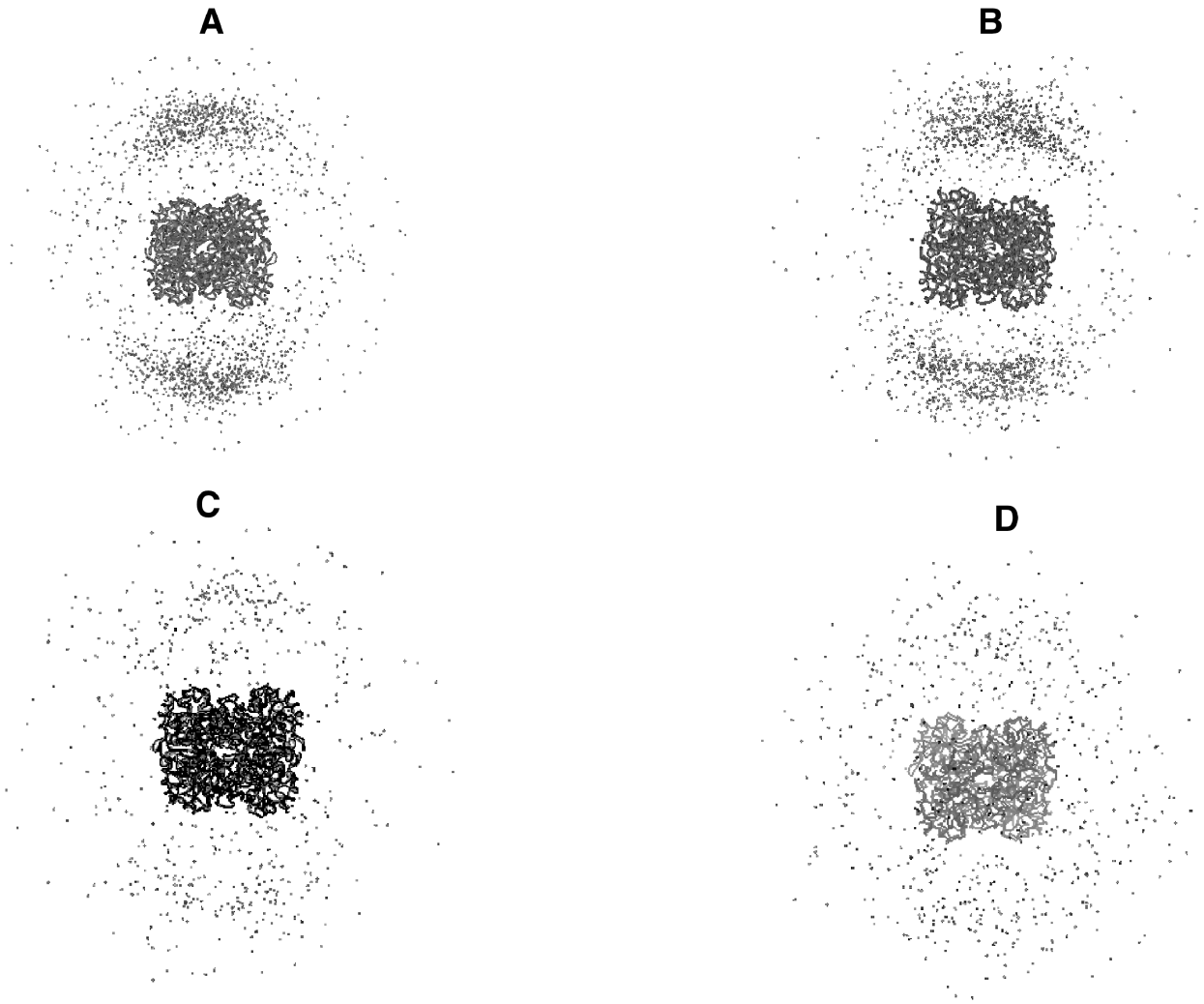

Fig. S17. Center of Mass Profile for the Interaction Between Rabbit Muscle LDH and F-actin as a Function of Ionic Strength. The small spheres represent center of mass atoms of F-actin in encounter snapshots with lactate dehydrogenase, which is represented by Cα ribbons. Similar profiles are found for human and zebrafish.

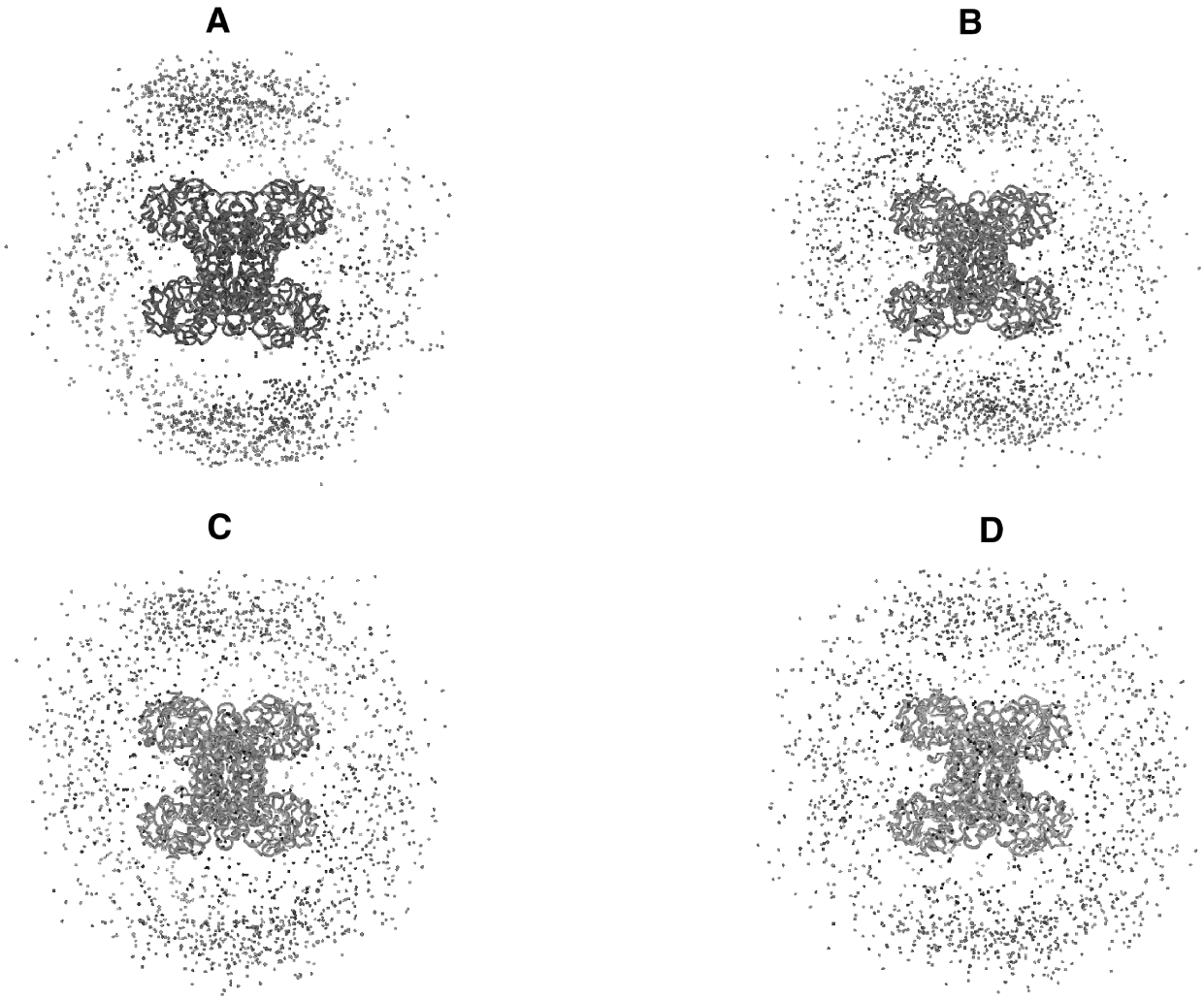

Fig. S18. Center of Mass Profile for the Interaction Between Rabbit GAPDH and F-actin as a Function of Ionic Strength. The small spheres represent center of mass atoms of F-actin in encounter snapshots with GAPDH, which is represented by Cα ribbons. Interactions are nonspecific at all ionic strengths for all the species. **(A)** Ionic strength of 0.01 M. **(B)** Ionic strength of 0.05 M. **(C)** Ionic strength of 0.10 M. **(D)** Ionic strength of 0.15 M. Note that shallow grooves on the top and bottom of GAPDH interact the most often with F-actin at ionic strengths of 0.05 M and lower, but this specificity is lost when the ionic strength increases to 0.10 M.

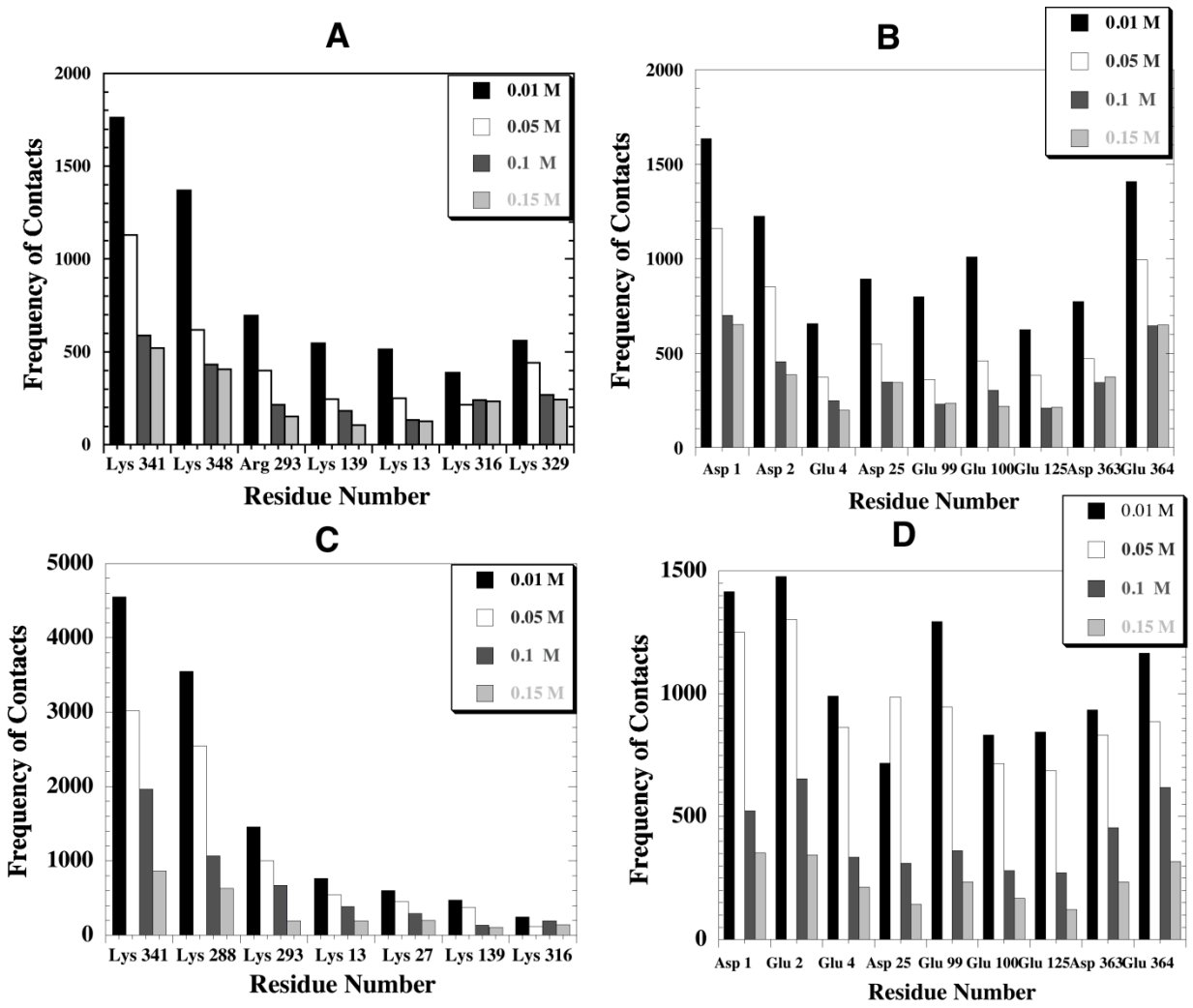

Figure S19. Residues Responsible for the Interaction Between Aldolase and F-actin. The bars show the frequency of these residues as a function of ionic strength. (A) Zebrafish Aldolase, (B) Zebrafish F-actin, (C) Human Aldolase, and (D) Human F-actin.

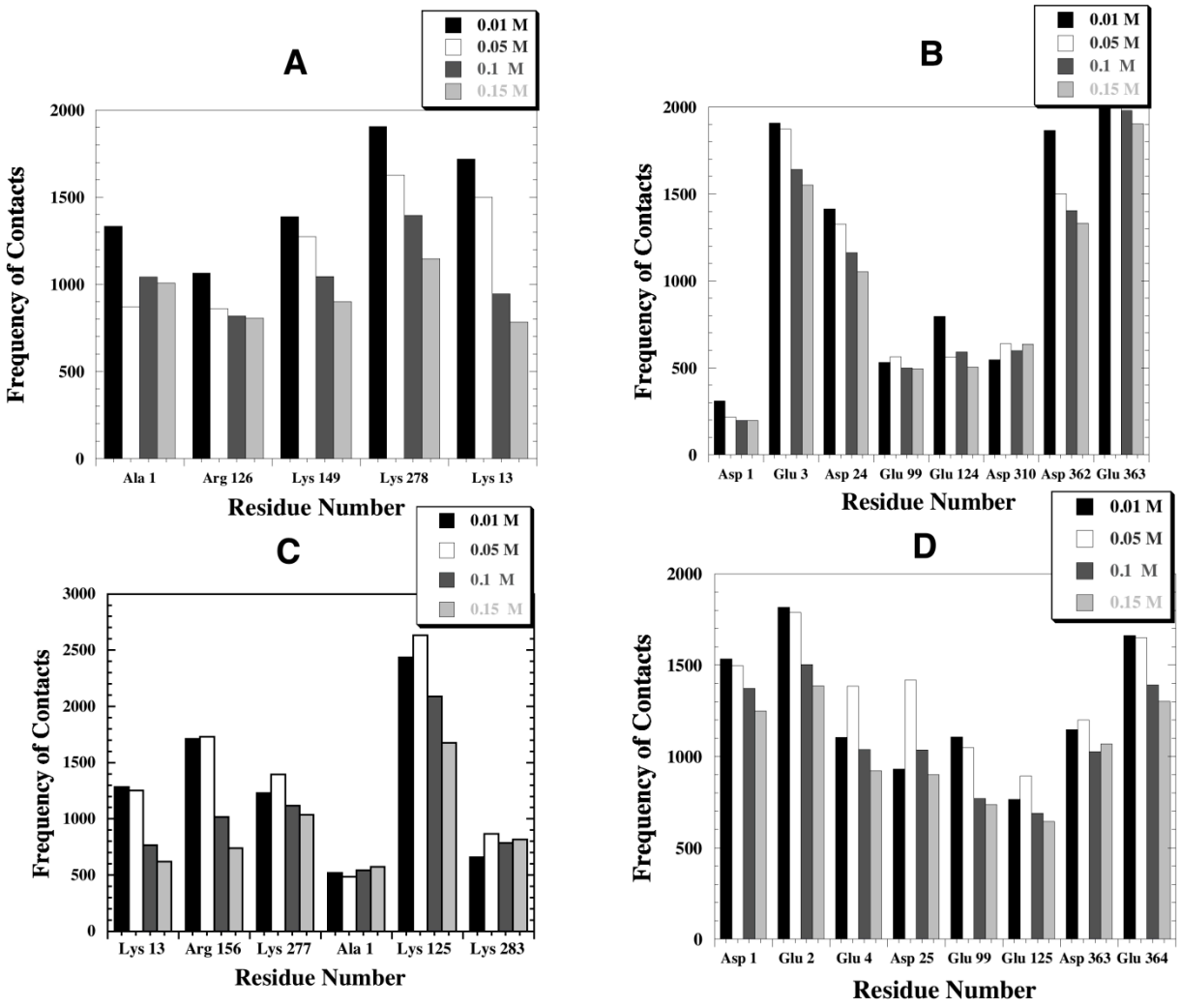

Figure S20. Residues Responsible for the Interaction Between LDH and F-actin. The bars show the frequency of these residues as a function of ionic strength. (A) Zebrafish LDH, (B) Zebrafish F-actin, (C) Human LDH, and (D) Human F-actin residues.

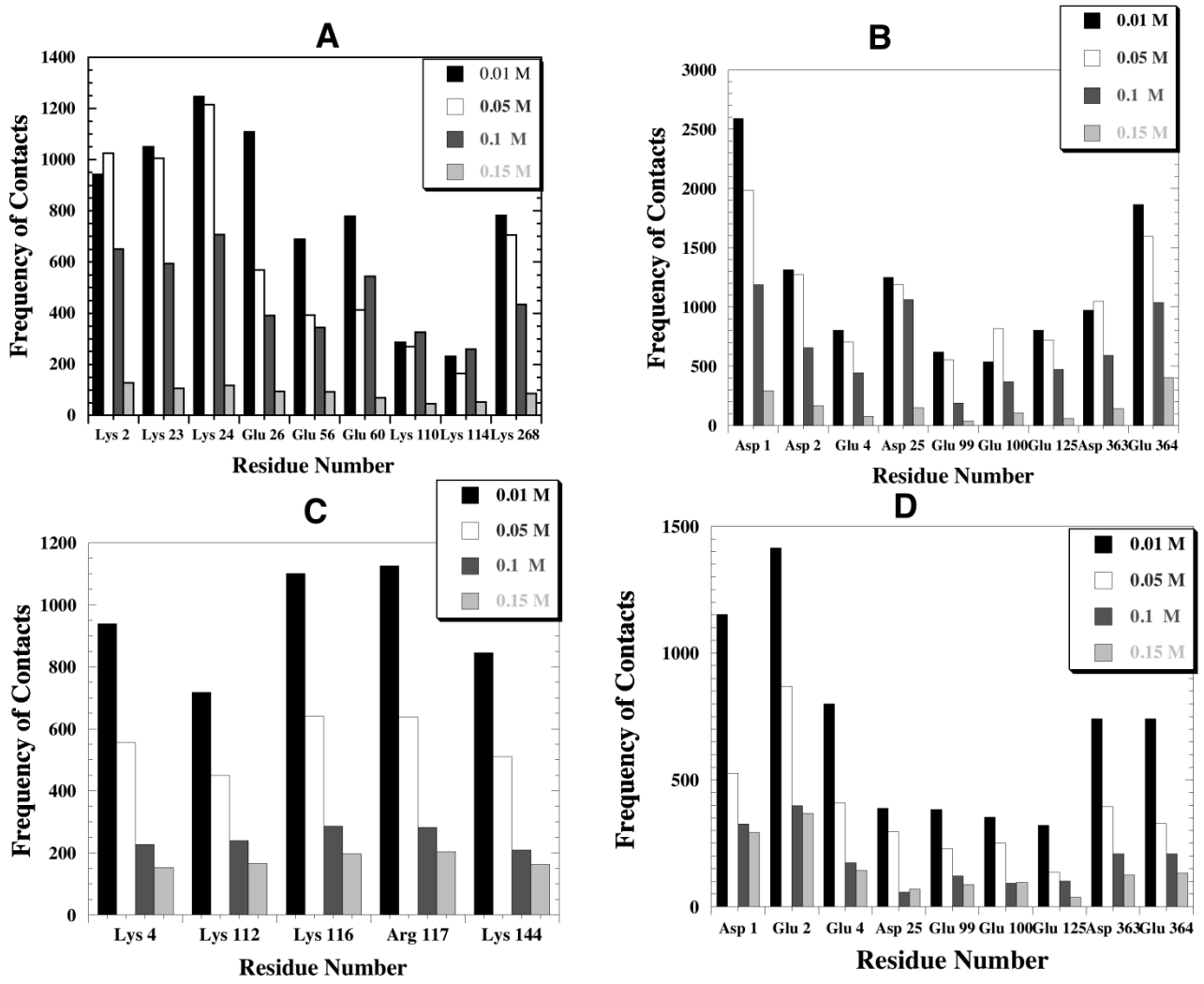

Figure S21. Residues Responsible for the Interaction Between GAPDH and F-actin. The bars show the frequency of these residues as a function of ionic strength. (A) Zebrafish GAPDH, (B) Zebrafish F-actin, (C) Human GAPDH, and (D) Human F-actin.