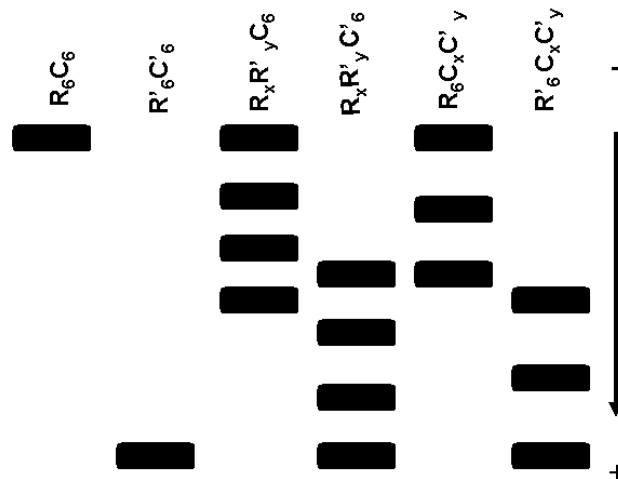


## Aspartate Transcarbamylase Quaternary Structure

Among the many structurally interesting proteins, aspartate transcarbamylase (ATCase) attracted special attention. It catalyzes the first committed step in pyrimidine biosynthesis and a number of metabolites alter its enzymatic activity in physiologically meaningful ways.

*What is the reaction catalyzed by ATCase?*

As it occurs in an *E. coli* cell, native ATCase is a dodecamer ( $R_6C_6$ ) composed of six regulatory (R) subunits and six catalytic (C) subunits. Under mild conditions it is possible to partially dissociate the complex, separate the two classes of subunits, and then recombine the subunits to reform active enzyme. In addition, the native enzyme can be reacted with succinic anhydride that succinylates the epsilon-amino groups of lysine residues and creates a modified active enzyme ( $R'_6C'_6$ ) with a substantial negative charge. By dissociating, isolating, and recombining modified and unmodified subunits, Schachman and coworkers generated various hybrid enzymes and analyzed them by gel electrophoresis under non-denaturing conditions as diagramed below. The more negatively charged dodecamers traveled further toward the positive electrode. The dark spots in the figure below represent the position of various hybrid ATCase oligomers in the gel. Equal amounts of native and modified R subunits when mixed with either native or modified C subunits results in four electrophoretic bands (lanes for  $R_xR'_yC_6$  and  $R_xR'_yC'_6$ , respectively) while the reciprocal combinations yield three bands (lanes for  $R_6C_xC'_y$  and  $R'_6C_xC'_y$ ). The values for x and y sum to 6.



*Identify the spots corresponding to  $R'_6C'_6$  and  $R_6C_6$ .*

*What does the even spacing imply about the net charge on the ATCase hybrid oligomers in each lane?*

*Predict the subunit composition (x and y values) for each spot shown above that is consistent with the number and locations of the various spots in each lane.*

The X-ray structure of *E. coli* ATCase is now known. Constraints of symmetry restrict the possible arrangements of  $R_6C_6$  aggregates. Each subunit is isomorphous with the other subunits of its type. In other words, they are indistinguishable from each other because they fit into the complex in structurally identical ways.

*Identify the symmetry properties of ATCase.*

*Comparing your electrophoretic model with the X-ray model, which subunit interactions break and do not break under the mildly dissociating conditions?*

*Assuming that the reassociation of modified and unmodified subunits occurs randomly, for each lane above, what are the relative amounts of ATCase protein in each of the electrophoretic spots for  $R_xR'_y C_6$  and  $R_6C_xC'_y$  above? Assume a binomial distribution.*

*Draw a diagram that illustrates the dissociation-reassociation and modification procedures involved in this experiment.*

Based on Yang YR and Schachman HK (1987) "Hybridization as a technique for studying interchain interactions in the catalytic trimers of aspartate transcarbamoylase. Anal Biochem 163(1):188-95

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