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This Week's Citation Classic

Wetmur J G & Davidson N. Kinetics of renaturation of DNA. *J. Mol. Biol.* **31**:349-70. 1968.
[Gates, Crellin and Church Laboratories of Chemistry, California Institute of Technology, Pasadena, CA]

This paper describes a comprehensive investigation of the various phenomena which may affect the second order kinetics of renaturation of complementary DNA strands. These phenomena include properties of the DNA, such as strand length and sequence complexity, and properties of the solvent, such as temperature, ionic strength, and viscosity. [The *SCI*® indicates that this paper has been cited in over 855 publications since 1968.]

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"The work described in this paper was carried out in the laboratory of Norman Davidson when I was a graduate student at the California Institute of Technology. Norman's research had changed from 'pure' physical chemistry to biophysical chemistry of nucleic acids. Yet each of his students took quantum and statistical mechanics and built some sort of machine shortly after his or her arrival in the laboratory. We thought of DNA renaturation as another, albeit important, chemical reaction to which physical principles could be applied in order to elucidate the reaction mechanism. I set out to investigate any variable which might affect DNA renaturation rates. Incidentally, even the T-jump apparatus which I had built turned out to be useful for studying kinetics of some of the faster renaturation reactions.

"We confirmed that DNA renaturation was nearly a second order reaction. The basic mathematical description of such a reaction, as well as the reciprocal plot for determining the rate constant, has been known for about a century.[1] We used these equations to describe the nucleation reaction between complementary sequences in two DNA molecules as well as to describe the total reaction. The equation relating the nucleation rate constant. k_N', and the observed rate constant, k_2, was found to be

$$k_2 = k_N' L^{0.5}/N$$

where L and N are the DNA length and sequence complexity respectively.

"An increase in sequence complexity translates into a decrease in the concentration of any particular nucleation site Britten and Kohne[2] also explored this variable over a wide range of complexities and invented the convenient Cot plot for simultaneous display of the reactions k_N' depends on the temperature, ionic strength, and solvent (microscopic) viscosity but not on the DNA itself. The only remaining variable of significance is the length of the single strands. The square root dependence came as a surprise. If the molecules zipper up to their ends after each nucleation, why $L^{0.5}$ instead of L? After many discussions, I was persuaded by Norman that an excluded volume effect might limit the availability of nucleation sites. This hypothesis fits well into the framework of all that is known concerning the rate of reassociation of nucleic acids.[3] Further studies with molecules of various shapes and sizes[4] have agreed with the excluded volume hypothesis. A practical offshoot of the studies of microscopic viscosity, which affects k_2, and macroscopic viscosity, which does not, is a method for accelerating DNA renaturation.[5]

"I think that this paper is highly cited because it describes the practical and theoretical details of an Important process, in one place. The paper was the result of three years of work. We avoided the temptation to publish the results piecemeal, a luxury too seldom afforded these days. The work itself was to a great extent a product of the environment in Norman's laboratory in the mid-1960s. I could not have done the theoretical work without the physical chemistry training. I could not have done the experimental work without the willing cooperation and assistance of both my friends in the laboratory and Norman's colleagues throughout Caltech."

1. **van 't Hoff J H.** *Studies in chemical dynamics.* London: Williams & Norgate. 1896. p. 12.
2. **Britten R J & Kohne D E.** Repeated sequence in DNA. *Science* **161**:529-40, 1968.
3. **Weimur J G.** Hybridization and renaturation kinetics of nucleic acids. *Annu. Rev. Biophys. Bineng.* **5**:337-61, 1976.
4. **Kinberg-Calhoun J & Wetmur J G.** Circular, but not circularly permuted, DNA reacts slower than linear DNA with complementary linear DNA. *Biochemistry—USA* **20**:2645-50, 1981.
5. **Wieder R & Wetmur J G.** One hundred-fold acceleration of DNA renaturation in solution. *Biopolymers* **20**:1537-47, 1981.