

AD-A219 803

DOCUMENTATION PAGE

Form Approved  
OMB No. 0704-0188

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| 1a SECURITY CLASSIFICATION AUTHORITY<br>NA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |  |                                            | 1b RESTRICTIVE MARKINGS<br>NA                                                                |                                                  |                          |
| 2b DECLASSIFICATION/DOWNGRADING SCHEDULE<br>NA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |  |                                            | 3 DISTRIBUTION/AVAILABILITY OF REPORT<br>Distribution Unlimited                              |                                                  |                          |
| 4 PERFORMING ORGANIZATION REPORT NUMBER(S)<br>NA                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |  |                                            | 5 MONITORING ORGANIZATION REPORT NUMBER(S)<br>NA                                             |                                                  |                          |
| 6a NAME OF PERFORMING ORGANIZATION<br>Stanford University                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  | 6b OFFICE SYMBOL<br>(If applicable)<br>NA  | 7a NAME OF MONITORING ORGANIZATION<br>Office of Naval Research                               |                                                  |                          |
| 6c ADDRESS (City, State, and ZIP Code)<br>Sponsored Projects Office<br>Jordan Quad/Birch/125 Panama St.<br>Stanford, CA 94305                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |  |                                            | 7b ADDRESS (City, State, and ZIP Code)<br>800 N. Quincy St.<br>Arlington, VA 22217-5000      |                                                  |                          |
| 8a NAME OF FUNDING/SPONSORING ORGANIZATION<br>Office of Naval Research                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |  | 8b OFFICE SYMBOL<br>(If applicable)<br>ONR | 9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER<br>N000-14-86-K-0388                          |                                                  |                          |
| 8c ADDRESS (City, State, and ZIP Code)<br>800 N. Quincy St.<br>Arlington, VA 22217-5000                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |  |                                            | 10 SOURCE OF FUNDING NUMBERS<br>PROGRAM ELEMENT NO PROJECT NO TASK NO WORK UNIT ACCESSION NO |                                                  |                          |
| 11 TITLE (Include Security Classification)<br>(U) DETERMINATION OF THE STRUCTURAL BASIS OF ANTIBODY DIVERSITY USING NMR                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |  |                                            |                                                                                              |                                                  |                          |
| 12 PERSONAL AUTHOR(S)<br>Harden M. McConnell                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |  |                                            |                                                                                              |                                                  |                          |
| 13a TYPE OF REPORT<br>FINAL                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |  | 13b TIME COVERED<br>FROM 6/86 to 6/89      |                                                                                              | 14 DATE OF REPORT (Year, Month, Day)<br>90/03/15 |                          |
| 15 PAGE COUNT                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |  |                                            |                                                                                              |                                                  |                          |
| 16 SUPPLEMENTARY NOTATION                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |  |                                            |                                                                                              |                                                  |                          |
| 17 COSATI CODES<br>FIELD GROUP SUB-GROUP<br>06 03                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |  |                                            | 18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)             |                                                  |                          |
| 19 ABSTRACT (Continue on reverse if necessary and identify by block number)<br><br>A group of twelve hybridomas have been prepared that secrete monoclonal antibodies against a paramagnetic nitroxide spin label hapten. The cDNA sequences of both chains of all twelve IgG antibodies have been determined. NMR difference spectra together with specific amino acid deuteration permit the observation of almost high resolution NMR spectra of amino acid residue protons in the combining site regions. Composition, structural and kinetic information concerning these combining sites has been obtained. Single site mutations are in progress to confirm signal assignment and for structure-function studies. There appears to be an excellent correspondence between the NMR results and x-ray crystallographic data on the Fab fragment of antibody AN02. <i>... DNA, cDNA, ...</i> |  |                                            |                                                                                              |                                                  |                          |
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| 22a NAME OF RESPONSIBLE INDIVIDUAL<br>Michael Marron                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |  |                                            | 22b TELEPHONE (Include Area Code)<br>202/696-4760                                            |                                                  | 22c OFFICE SYMBOL<br>ONR |

DD Form 1473, JUN 86

Previous editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE

S/N 0102-LF-014-6603

90 03 90 039

FINAL TECHNICAL REPORT  
OFFICE OF NAVAL RESEARCH CONTRACT  
N00014-86-K-0388

For the period June 15, 1986 - June 14, 1989

Nuclear magnetic resonance spectroscopy was used to study the combining site region of an anti-dinitrophenyl spin label antibody. By continuing to employ strategies developed in previous work, precise distances from the unpaired electron of the bound hapten to tyrosine residues, the contribution of tryptophan residues from the light and heavy chains to the hapten binding pocket, and the change in mobility of a light chain tyrosine residue were determined for the antibody AN02. The amino acid sequence of AN02 was inferred from cDNA clones. A model of the variable domains was built by comparing the AN02 sequence segment by segment to the sequences of FABs with crystallographically determined structure. Each segment of AN02 was given the structure of the most homologous crystal segment. This gross structure was then subjected to energy minimization to remove unfavorable steric contacts.

The NMR data was interpreted in the context of the model structure to give meaningful information on the relative location of hapten and protein. A light chain tryptophan, 91 L, was seen to be likely in a stacking interaction with the hapten dinitrophenyl ring and the heavy chain tryptophan 96 H could also be described as making contact with the hapten. The light chain tyrosine 34 L would most likely be affected by the presence of the hapten. Several site-specific mutants have been made in the cDNAs for the heavy and light chain. Systems have been developed to express these mutant proteins so as to confirm these assignments, assign the remaining tyrosine resonances to which distances have been calculated, and study effects on hapten binding. The model of the combining site region as well as the relative location of the hapten can be refined with the sequence specific tyrosine assignments.

Eleven other anti-DNP spin label antibodies have been prepared and characterized in terms of their cDNA sequences and binding constants. The

sequences are relatively heterogenous, although subsets of the twelve (including AN02) antibodies show high sequence homology. The kinetic reaction rates for four of the relatively homologous antibodies, AN01, AN02, AN03, and AN07, have been investigated. The on-rate constants for these antibodies range over nearly three orders of magnitude with the diffusion limited reaction for AN02 at the high end. Models have been constructed as per AN02 for these eleven new sequences and implicate certain residue differences among the antibodies to account for the observed heterogeneity in binding constants and reaction kinetics.

The germ line gene sequences for the antibodies AN02 and AN07 were determined. The differences that exist compared to the mature protein are likely to have at most a subtle effect on hapten binding. Production of a mutant protein which reflects the germ line sequence is underway and preliminary study of a protein with the germ line AN02 light chain and mature AN02 heavy chain shows a small decrease, less than a factor of two, in binding to spin label hapten.

The Fab fragment of AN02 was crystallized yielding crystals which diffracted to approximately 2.8Å resolution. The resulting structure is in its final stages of refinement. Comparison of NMR and crystallographic structural data is favorable.

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| A-1                                  |                                           |



PUBLICATIONS ACKNOWLEDGING ONR AWARD  
ONR N00014-86-K-0388  
For the period 1986 - 1989

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