# Chapter 6

# A Role for Immunology in Nanotechnology

**Bernard F. Erlanger**

**Department of Microbiology, Columbia University, New York, NY 10032**



Until 1985, only two allotropic forms of elemental carbon were known: graphite and diamond. In 1985, a third form was discovered constructed of 60 carbon atoms (i.e. $C_{60}$) and named Buckminsterfullerene because of its geodesic character (*1*). The preparation of fullerenes in workable quantities led first to applications in chemical and engineering processes and subsequently to the suggested use of fullerenes for biological and medical applications (*2-4*) and as templates for the design of experimental pharmaceutical agents, including those with anti-viral (*5-9*), antioxidant (*8-12*), chemotactic (*13*), and neuroprotective (*14*) activities.

We demonstrated that the mouse immune repertoire is diverse enough to recognize and produce antibodies specific for C60-fullerenes (*15*). We succeeded in isolating several monoclonal anti-$C_{60}$ antibodies. The monoclonal antibody under study (1-10F-A8) is an IgG1, kappa and was prepared by standard procedures which included immunization of mice with a fullerene carboxylic acid derivative covalently linked to a protein (bovine thyroglobulin) by competitive inhibition in an ELISA format, in which a fullerene-rabbit serum albumin (RSA) conjugate was used as the target. An apparent binding constant of 22 nM was determined. The sequences of the light and heavy chains were determined, and the three-dimensional structure of the Fab fragment was solved and refined by x-ray crystallographic techniques to a resolution of 2.25 A. Finally, we identified and modeled the probable binding site for C 60 fullerene and described the interatomic interactions that stabilize the antibody-fullerene complex.

## Identification and Modeling of $C_{60}$ Binding to the Fab' Fragment of Monoclonal Antibody 1-10F-A8 by X-ray Crystallization

Identification of the $C_{60}$ binding site in the anti-fullerene antibody was accomplished by X-ray crystallization using accessible surface area calculation and docking/energy minimization procedures. Accessible surface area calculation with a 1.7 A radius probe identified a spherical-shaped cavity in the

 

In Nanotechnology and the Environment; Karn, Barbara, et al.;
ACS Symposium Series; American Chemical Society: Washington, DC, 2004.

interface of the two sequence-variable peptide chains, VH and VL, that make up the binding site of an antibody molecule. The two chains contain within them amino acids that actually contact the antigen, defined as the Complementary Determining Regions (CDRs). There are also the "framework" regions that define the combining site but do not make contact with the fullerene. The V L CDR amino acid residues are Tyr-36, Gln-89, Phe-96 and the V L "framework" residue Phe-98. Included also are the side chains of VH CDR residue Asn-35 and VH framework residues Val-37, Trp-47, and Trp-106. In addition the main chain atoms of the V H residues Ala-97, Thr-98 and Ser-99 (in CDR H3) also define the cavity. The cavity contains two solvent water molecules that form a solvent bridge between the side chains of V L Gln-89 and V H Asn-35.

Accessibility to the fullerene binding site is via a 4.7 A wide channel fashioned by the CDR L3 and the short CDR H3 hypervariable loop. The cavity identified by the V L-V H interface is approximately 7 A in diameter, too small for the $C_{60}$ fullerene of 10 A diameter, incorporating the van der Waals radii of the carbon atoms. Nonetheless, manual docking of a model $C_{60}$ into the cavity and using an energy minimization protocol results in relaxation of the surrounding Fab side chains and 11 degree rotation of the VL and VH domains. The binding process therefore, includes an induced fit mechanism. The antibody surface area buried by the V L and V H interface is relatively small, 1,100 $A^2$, consistent with other anti-hapten antibodies that undergo large relative V L -V H displacements upon hapten binding.

In addition to confirming that the fullerene binding site was the spherical cavity identified by accessible surface area calculation, the modeling and energy minimization of the Fab-$C_{60}$ complex suggests several pi-bond stacking interactions between the fullerene and the antibody. Aromatic side chains of VHTrp-47, VL Tyr-91, and VL Phe-96 as well as side chains for VH Asn-35 and VL Gln-89 lie parallel in the $C_{60}$ molecule. In addition, the potential for a weak hydrogen bond from the VL Tyr-36 hydroxyl to the fullerene (3.15 A) was also noted. Stacking interactions to $C_{60}$ have been previously described in the crystal structure of $C_{60}$ solvated by benzene. The solvent benzene was noted to lie over the electron-rich $C_{60}$ pentagon-pentagon bonds, clearly establishing the nature of the stacking interaction. Pi-system stacking interactions are well established in x-ray crystal structures of antibody-antigen complexes. For example, in the antibody-antigen complex of the anti-lysozyme antibody D44, the stacking interaction involved an aromatic side chain, tryptophan, from the antibody and an arginine from the antigen. It is not surprising, therefore, that the anti-fullerene antibody uses similar stacking interactions for the binding of fullerene.

It should be noted that the stacking and hydrogen bond interactions to the fullerene would be weak interactions.

## Binding of the Anti-Fullerene Antibody to Single Wall Carbon Nanotubes

Single wall carbon nanotubes (SWNTs) are a remarkable new class of nanometer diameter metallic and semiconducting wires that carry current as pi electrons propagating on their graphitic surface. They are physically robust, exhibit great tensile strength, do not oxidize or have surface states under ambient conditions, and show high conductivity. They are easily grown in lengths of tens of microns and can be precisely positioned and manipulated when attached to AFM tips. Their remarkable electrical properties suggest their potential for use in future electronic components.

The immunochemical binding interreaction between SWNTs and the C60 antibody was first demonstrated by a competitive ELISA in which a colloidal suspension of carbon nanotubes was shown to competitively inhibit the binding of anti-fullerene antibody to a fullerene conjugate of rabbit serum albumin. Competition was seen at very high dilutions of the SWNT colloidal suspension. A quantitative measure of binding coefficient will require detailed study as a function of available SWNT surface area, structural type, and extent of SWNT aggregation into ropes.

Binding was confirmed directly by atomic force microscopy. SWNT ropes on mica were initially imaged, the surface was then exposed to antibody solution, and finally, the same SWNT was imaged in air. This sequence distinguishes any preexisting surface particles from bound antibodies. Three drops of aqueous SWNT suspension (0.064 mg/mL in Triton 100X surfactant, from Tubes@Rice) were spun into freshly cleaved mica surface. The sample was imaged by tapping mode AFM in air. Many anti-fullerene antibody molecules could be seen adsorbed to the surface of nanotubes (*16*).

Thus the monoclonal anti-C 60-specific antibody could be visualized binding to carbon nanotubes, presumably because the latter have a curved, hydrophobic pi-electron-rich surface, analogous to C60, itself. The hydrophobic binding site of the antibody is sufficiently flexible to recognize both. Thus our findings bridged 2 disparate disciplines, electrical nanotechnology and monoclonal immunology. We were encouraged, therefore, to explore the possibility of utilizing this combination in biological systems, in particular with the idea that antibody–coated SWNTs can be used advantageously as probes of cell or membrane function. A SWNT rope has a diameter of about 10nm, far smaller than present metal or glass capillary intracellular probes. They should be capable of insertion into and withdrawal from specific regions of cells, with minimal disturbance of cell or membrane function.

We first showed that the monoclonal anti-fullerene antibody could be covalently "decorated" with a fluorescent $Ca^{++}$ probe (Molecular Probes, Eugene OR) without disturbing its specificity for fullerenes and nanotubes. Our

future plans envision insertion of the SWNT- antibody-calcium probe into living cells. It should be possible for the probe molecule(s) to be optically excited or electrically addressed via the conducting SWNT wire. Unlike most semiconductors and metals, SWNTs do not form insulating surface oxides at room temperature, i.e. there should be electrical contact with the antibody. Indeed, recent experiments by others have demonstrated that nanotube electrical properties change with reversible adsorption of molecular species.



## Acknowledgment

(Funding was from CSixty , Inc., Toronto Canada and the National Institutes of Health).

## References

1. Kroto, H.W. et al. *Nature* **1985**, *318*, 162-163 .
2. Jensen, A.W. et al. *Bioorg. Med. Chem.* **1996**, *4*, 767-779.
3. Da Ros, T.& Krato, M. *Chem. Commun.* **1999**, 663-669.
4. Wilson, S.R. *The Fullerene Handbook* Wiley, N.Y. in press
5. Sijbesma, R. et al. *J. Amer. Chem Soc.***1993**, *115*, 6510-6512.
6. Schinazi, R.F. et al. *Antimicrob. Agents Chemother.* **1993**, *37*, 1707-1710.
7. Friedman, S.H. et al. *J. Med. Chem.* **1998**, *41*, 2424-2429.
8. Chang, L.Y. et al. *Chem. Commun.* **1995**, 1283-1284.
9. Lu, L.H. et al. *Br. J. Pharmacol.* **1998**, *123*,1097-1102.
10. Straface, E. et al. *FEBS Lett.* **1999**, *454*, 335-340.
11. Lin, A.M. et al. *J. Neuro. Chem.* **1999**, *72*:,1634-1640.
12. Chueh, S.C. et al. *Transplant Proc.* **1999**, *31*, 4558-4562.
13. Tonolo, C. et al. *J. Med Chem.* **1994**, *37*, 4558-4562.
14. Dugan. L.L. et al. *Proc. Natl Acad. Sci. USA* **1997**, *94*, 9434-9439.
15. Chen, B.-X. et al. *Proc. Natl Acad. Sci. USA* **1998**, *95*, 10809-10813.
16. Erlanger, B.F. et al. *Nano Letters* **2001**, *1*, 465-467.