30

Analysis of Antibody–Antigen Interactions Using Surface Plasmon Resonance

Thomas C. VanCott

1. Introduction

Accurate analyses of antibody specificities and affinities are critical to understanding their role in antibody-mediated neutralization of HIV-1 in vitro and their potential activity against HIV-1 in vivo. Multiple immunologic tools currently exist for measuring antibody–antigen interactions to include enzyme immunoassays (EIA), Western blotting, radioimmunoassays, and others. However, these techniques all require some form of protein labeling or secondary antibodies for detection of specific binding interactions. Disadvantages of protein labeling involve potential alterations in protein tertiary structure, while the use of secondary detection antibodies prohibits the measurement of binding interactions in real-time. Recently, instruments utilizing the physical principal of surface plasmon resonance (SPR) (BIAcore™, Pharmacia Biosensor, Piscataway, NJ) *(1–4)* have been developed which allow real-time measurements of protein binding interactions without the use of internal labels or secondary antibodies. Proteins of interest are covalently coupled to a flexible biosensor matrix, and kinetic rate constants can be directly extracted from analyses of association and dissociation binding curves. The relative concentration of immobilized protein can be determined allowing, for example, the comparison of binding efficiency of one antibody against a number of different proteins.

Binding of monoclonal antibodies (MAbs) and polyclonal sera (mouse, rabbit, guinea pig, monkey, human) to both whole HIV-1 proteins (gp120, gp160, p24) and peptides can be measured using SPR *(5–7)*. SPR has been used to characterize the binding kinetics of a HIV-1 gp41-specific neutralizing MAb *(8)* and to demonstrate that the dissociation, but not the association, rate con-

From *Methods in Molecular Medicine, Vol 17 HIV Protocols*
Edited by N L Michael and J H Kim © Humana Press Inc , Totowa, NJ

stant describing the interaction between HIV-1 gp160 V3-specific MAbs and monomeric gp120 correlates with the ability of the MAb to neutralize the homologous HIV-1 isolate in vitro *(9)*. The ability to control and quantitate biosensor matrix protein concentrations has allowed direct comparisons of antibody reactivity against several antigens. In this way, HIV-1 infected individuals living in Thailand have been serotyped as clade B or E by studying relative binding of their sera to gp160 from clade B or E *(10)*. Furthermore, studies involving antibody and soluble CD4 binding to recombinant forms of gp120 have indicated minimal disruption of tertiary monomeric gp120 structure upon immobilization allowing for more accurate characterization of antibody binding dependence on overall proper protein structure *(11)*. This has allowed more extensive description of the qualitative nature of the binding properties of antibody elicited using various candidate HIV-1 vaccines *(12)*

Proteins of interest are first covalently linked (immobilized) to the dextran biosensor matrix. Immobilization conditions for each antigen are optimized by conducting a preconcentration step. Once the protein is immobilized, single or multiple antibody interactions can be studied. Bound antibody is dissociated from the matrix (regeneration) allowing the same protein matrix to be used for repeated binding interactions.

2. Materials

2.1. Equipment

1. BIAcore 1000, 2000, or BIAlite™ (Pharmacia Biosensor)
2. BIAcore 1000, 2000, or BIAlite software (Pharmacia Biosensor)

2.2. Reagents and Buffers (see Note 1)

1. HEPES-buffered saline (HBS) running buffer (10 m*M* *N*-[2-hydroxyethyl]piperazine-*N'*-[2-ethanesulfonic acid] [HEPES], 150 m*M* NaCl, 3.4 m*M* ethylene diamine tetra-acetic acid (EDTA), 0.05% BIAcore surfactant P20, pH 7 4) (*see* **Note 2**)
2. Sodium formate immobilization buffer. 10 m*M* sodium formate, pH to 3.5 or pH 3 0 using 1 *M* HCl
3. Sodium acetate immobilization buffer (NaAc) 10 m*M* sodium acetate, pH to 4 0, 4.5, or 5.0 using 1 *M* HCl
4. 2-[*N*-Morpholino]ethanesulfonic acid (*MES*) immobilization buffer: 10 m*M* adjust pH to 6.0 using 1 *M* HCl.
5. 400 m*M* *N*-ethyl-*N'*-(3-diethylaminopropyl) carbodiimide hydrochloride (EDC) (*see* **Note 3**)
6. 100 m*M* *N*-hydroxysuccinimide (NHS) (*see* **Note 3**).
7. 1 *M* Ethanolamine (*see* **Note 3**).
8. Borate buffer. 0 1 *M* borate buffer, adjust pH to 8 5 with 1 *M* NaOH
9. 2-(2-pyridinyldithio)ethaneamine hydrochloride (PDEA) activation solution

(80 m*M*). 4.5 mg of PDEA in 250 µL 0.1 *M* borate buffer pH 8.5 (*see* **Note 4**).
10. 2-Mercaptoethanol (2-ME) 50 m*M* 2-ME in 50 m*M* NaAc, pH 4.5.
11. Regeneration buffers to include HCl, H_3PO_4, formic acid, NaOH, and $MgCl_2$

3. Methods

3.1. Immobilization of Ligand

3.1.1. Preconcentration

1. To select optimal ligand immobilization buffer, prepare ligand at an approximate concentration of 10–50 µg/mL at various pH using some or all of the following buffers: sodium formate pH 3.5, NaAc, pH 4.0, NaAc pH 5.0, and MES, pH 6.0 (*see* **Note 5**)
2. Inject 20–30 µL of ligand at each desired pH across an unactivated matrix surface at a flow rate of 5 µL/min (4–6 min injection). Measure binding 30 s after injection and just prior to completion of ligand injection (*see* **Note 6**). Between each ligand injection, inject 5 µL 10 m*M* HCl to wash matrix of any nonspecifically bound ligand (*see* **Note 7**).
3. Select buffer which yields maximum diffusion signal (from **step 2**) indicating the highest diffusion and concentration of ligand within the biosensor matrix (*see* **Note 8**).
4. Using selected buffer from **step 3**, inject serial dilutions of ligand (20–30 µL at 5 µL/min) across unactivated matrix to determine minimum concentration of antigen with desired diffusion properties (*see* **Notes 9** and **10**).

3.1.2. Immobilization (Amine) (see **Note 11**)

1. Activate biosensor dextran matrix by injecting 35 µL (5 µL/min flow rate) of a 1 1 mixture of EDC and NHS across the matrix (*see* **Note 12**).
2. Inject 30 µL of ligand prepared in selected immobilization buffer across EDC/NHS activated matrix.
3. Deactivate matrix by injecting 30 µL of ethanolamine (*see* **Note 13**).
4. Wash matrix with dilute acid (1 m*M* HCl) or base (1 m*M* NaOH) to remove noncovalently bound ligand from the matrix (*see* **Note 14**).

3.1.3. Immobilization (Thiol) (see **Note 11**)

1. Activate biosensor dextran matrix by injecting 10–35 µL (5 µL/min flow rate) of a 1:1 mixture of EDC and NHS across the matrix (*see* **Note 12**).
2. Inject 30 µL of PDEA across matrix placing an active disulfide group in the matrix for coupling to a free thiol group on the ligand.
3. Deactivate matrix by injecting 30 µL of ethanolamine (*see* **Note 13**)
4. Inject 30 µL of ligand prepared in selected immobilization buffer across PDEA matrix to covalently couple ligand to matrix via the ligand free thiol group.
5. Deactivate matrix by injecting 30 µL of 50 m*M* 2-ME (*see* **Note 15**).
6. Wash matrix with dilute acid (1 m*M* HCl) or base (1 m*M* NaOH) to remove noncovalently bound ligand from the matrix (*see* **Note 14**)

3.2 Measurement of Antibody:Antigen Binding Interactions

3.2.1. Antibody:Antigen Interactions

1 Immobilize ligand of interest as described in **Subheading 3.1.** (*see* **Note 16**)
2 Dilute MAbs (0.1–100 μg /mL) or polyclonal sera (1 100) in HBS running buffer (*see* **Note 17**)
3. Set flow rate (rate at which HBS running buffer flows through the biosensor matrix) at 5 μL/min (*see* **Note 18**)
4 Set injection volume at 30 μL (6-min injection).
5 Inject MAb or polyclonal sera across the matrix with immobilized ligand and across a control matrix (*see* **Note 19**)
6 Regenerate matrix using dilute acid (1 m*M* HCl) or base (1 m*M* NaOH) to remove nonspecifically bound antibody from the matrix (*see* **Note 20**).
7. Repeat **steps 2** and **3** for all MAbs and polyclonal sera (*see* **Notes 21** and **22**)

3.2.2. Multiple Sequential Antibody:Antigen Interactions

1. Immobilize ligand of interest as described in **Subheading 3.1.** (*see* **Note 16**)
2 Dilute MAbs (0 1–100 μg/mL) or polyclonal sera (1.100) in HBS running buffer (*see* **Note 17**)
3 Set flow rate (rate at which HBS running buffer flows through the biosensor matrix) at 5 μL/min (*see* **Note 18**).
4 Set injection volume at 30 μL (6-min injection)
5 Inject MAb or polyclonal sera across the matrix with immobilized ligand and across a control matrix (*see* **Note 19**)
6 Inject second MAb or polyclonal sera across the matrix with immobilized ligand This procedure can be repeated for multiple sequential binding interactions (*see* **Note 23**)
7 Regenerate matrix using dilute acid (1 m*M* HCl) or base (1 m*M* NaOH) to remove nonspecifically bound Ab from the matrix (*see* **Note 20**)
8 Repeat **steps 2** and **3** for all MAbs and polyclonal sera (*see* **Notes 21** and **22**)

3.2.3. Antibody Competition Studies

1 Immobilize MAb of interest as described in **Subheading 3.1.** (*see* **Note 24**)
2. Capture antigen by injecting 30 μL (flow rate 5 μL/min) of gp120 or gp160 at a concentration between 10–50 μg /mL (*see* **Note 25**)
3. Inject 30 μL (flow rate 5 μL/min) of MAb #2 at 5–10 μg/mL (*see* **Note 26**)
4 Regenerate matrix using dilute acid (2 5–10 m*M* HCl) to remove nonspecifically bound MAb from the matrix (*see* **Note 27**).
5. Repeat **steps 2–4** for other MAb combinations (*see* **Note 28**).

3.2.4. Protein Characterization Studies (see **Note 29**)

1 Activate biosensor dextran matrix by injecting 35 μL (5 μL/min flow rate) of a 1 1 mixture of EDC and NHS across the matrix (see Note 12).
2 Inject 30 μL of sCD4 at 20 μg /mL in MES, pH 6.0 at a flow rate of 5 μL/min (*see* **Note 30**)

3 Deactivate matrix by injecting 30 µL of ethanolamine (*see* **Note 13**).
4. Wash matrix with 4.5 *M* $MgCl_2$ to remove noncovalently bound ligand from the matrix (*see* **Note 14**).
5. Capture gp120/gp160 preparation by injecting 30 µL of gp120/gp160 (10 µg/mL) at a flow rate of 2 µL/min across the sCD4 protein matrix (*see* **Note 31**)
6. Inject 30 µL (flow rate 5 µL/min) of MAb (5–10 µg /mL) or polyclonal sera (1.100) (*see* **Note 32**).
7 Regenerate matrix using 4.5 *M* $MgCl_2$
8 Repeat **steps 5–7** to study other MAbs or polyclonal sera.

4. Notes

1. All buffers must be filtered and degassed prior to use. Filter all buffers (0.2 µm) and degas under vacuum (while stirring) for 15–30 min
2. To reduce nonspecific binding of some polyclonal sera to the dextran matrix, an alternate HBS running buffer may be prepared (10 m*M* HEPES, 300 m*M* NaCl, 5.0 m*M* EDTA, 0.05% BIAcore surfactant P20, pH 7 4)
3. EDC, NHS and ethanolamine are stored at –20°C and should be used within 1–2 h of thawing
4 Use PDEA buffer within 1 h of preparation
5 The BIAcore biosensor flow cell is composed of a negatively charged dextran matrix. In order to target and concentrate ligands of interest into this environment, it is necessary to adjust buffer pH to give antigen a positive charge allowing diffusion via electrostatic interactions. Ligands with high isoelectric points (pI > 8 0) will diffuse into the matrix efficiently using MES pH 6.0, while those with lower isoelectric points will require other mentioned buffers In general, the pH of the immobilization buffer needs to be 1–2 pH units lower than the ligand pI.
6. It is important to record sensorgram baseline approx 30 s after injection. Immediately after ligand injection (<30 s), an alteration in baseline results attributable to refractive index differences between the running HBS buffer and the ligand buffer. After 30 s, this baseline equilibrates and any alterations in signal are directly attributable to diffusion of ligand into the matrix The signal should be measured just prior to completion of ligand injection and the difference between this signal and the baseline signal measures the amount of ligand diffusion.
7. It is important for signal to return to baseline after each ligand injection. Since the matrix is not activated no ligand should remain within the matrix after conclusion of the injection step. However, depending on physical properties of the ligand, it is common to have some nonspecifically bound ligand remaining within the matrix which can be removed using 10 m*M* HCl.
8. If no diffusion is observed with ligand prepared in each of the immobilization buffers, it may be necessary to increase the concentration of ligand. Repeat **step 1** using higher ligand concentrations.
9. The amount of ligand immobilized depends on both the diffusion of ligand into the dextran matrix (**Subheading 3.1.**) as well as the presence of active groups on the ligand (described in **Subheading 3.1.2.**). Information on diffusion is

obtained from this preconcentration step, however, information on efficiency of covalent coupling may not be determined until the actual immobilization procedure described in **Subheading 3.1.2.** is conducted

10. Optimal concentration of ligand immobilized within the matrix is dependent upon molecular weight and assay application. For example, for routine screening in which sensitivity is an important consideration, higher concentrations of ligand are desirable, whereas for kinetic applications, lower ligand concentrations are desirable to minimize ligate rebinding to ligand after dissociation. In general, for routine screening, HIV-1 gp120 or gp160 immobilization amounts of 5000–10,000 resonance units (RU) are optimally reactive while for smaller peptides (V3) of 20–30 amino acids, 400–1500 RU are optimally reactive. It is desirable to have preconcentration diffusion values greater than desired immobilization amount owing to the expected less than 100% efficiency in covalent coupling
11 Multiple chemistries have been used to covalently couple ligand to the biosensor matrix and are described in detail elsewhere *(13–21)*. The two most common coupling chemistries described here involve coupling via amine or thiol groups on the ligand to chemically activated groups within the dextran matrix HIV-1 gp120, gp160, p24, and several synthetic peptides corresponding to gp160 have successfully been immobilized using amine-coupling chemistries. Several v3 peptides with terminal cysteine amino acids have successfully been immobilized using thiol chemistries
12. The EDC/NHS injection forms chemically activated NHS-esters within the dextran matrix for covalent coupling to ligand Altering the volume of EDC/NHS injection alters the time the matrix is in contact with the chemicals and therefore the degree of matrix activation Fine tuning the amount of ligand immobilized is most efficiently and successfully accomplished by altering injection volumes of EDC/NHS between 5 and 35 µL
13. Injection of 1 *M* ethanolamine with high ionic strength serves two purposes It weakens electrostatic interactions of noncovalently bound ligands, and it deactivates any unreacted activated NHS-ester groups remaining in the dextran matrix
14 After injection of ligand and matrix deactivation, some noncovalently bound ligand may remain within the matrix and can be removed using 1 m*M* HCl This procedure is termed matrix regeneration, requires some optimization work and will be discussed in more detail below (*see* **Note 20**).
15 Injection of 50 m*M* 2-ME deactivates any unreacted PDEA activated groups remaining in the dextran matrix. Other methods for deactivation have been described elsewhere *(13)*
16 As discussed in **Note 10**, optimal concentration of immobilized ligand is dependent upon the ligand itself and assay application. For routine screening of MAbs or polyclonal sera, the following concentrations yield high sensitivity: gp120/gp160 (5000–10000 RU), p24 (3000–5000 RU), recombinant CD4 receptor (3000–4000 RU), and synthetic peptides 15–30 amino acids in length (400–1500 RU)
17 Selection of polyclonal sera dilution and concentration of MAb for screening purposes will depend on the nature of the antibody·antigen binding interaction

and will require some optimization. Serum dilutions of 1:50 or 1:100 in HBS running buffer appear to provide good signal-to-noise for polyclonal sera from mice, rabbits, guinea pigs, rhesus macaques, and humans. Lower serum dilutions result in increased nonspecific binding of serum components to the dextran matrix Levels of nonspecific binding of MAbs or polyclonal sera should be evaluated initially using a blank matrix or matrix with nonspecific protein immobilized Nonspecific binding signals in the range of 0–20 RU are acceptable background levels. MAbs with affinity constants in the range of 0.1–100 n*M* can be initially successfully screened at concentrations ranging from 0.1–10 μg /mL.

18 Flow rate can be adjusted between 1–100 μL/min. Slower flow rates allow longer time for interaction between Ab and immobilized antigen leading to both increased specific and nonspecific binding. For lower affinity or avidity protein interactions, decreasing flow rate is recommended to increase sensitivity.

19. Sera and MAbs should be tested initially against a blank or control protein matrix to determine extent of nonspecific binding to the dextran matrix. These values should be subtracted from binding values against ligand of interest. Binding of particular Ab is measured as the difference in RU between a time point taken just prior to Ab injection and a time point taken 30 s after completion of Ab injection

20 Optimal regeneration buffers for some HIV-1 antigens include: recombinant gp120 and gp160 (60 m*M* H_3PO_4), oligomeric gp160 (10% formic acid), synthetic v3 peptides (10% formic acid), immunoglobulins (10–50 m*M* HCl), recombinant sCD4 (4.5 *M* $MgCl_2$) and p24 (60 m*M* H_3PO_4) Two factors are important when selecting a regeneration buffer: complete dissociation of bound Ab from antigen and retention of antigen reactivity. These are evaluated by regenerating initially with a weak acid (5 m*M* HCl) and monitoring both efficiency of matrix regeneration and retention of immobilized ligand reactivity

21. Computer-driven software allows construction of programs to inject large series of antibodies against up to four separate matrices (each potentially with different antigens of interest). Abs are injected followed by regeneration and washing of the flow cell. Data is summarized in Microsoft Excel (Microsoft, Redmond, WA) spreadsheet format and individual sensorgrams recording each antibody·antigen interaction are recorded for review.

22. Programs can be written to study Ab binding against multiple ligand matrices simultaneously (BIAcore 2000) or sequentially (BIAcore 1000) This allows for relative binding efficiencies against multiple antigens to be determined. For example, binding of polyclonal sera to native and denatured forms of gp120 have been evaluated by immobilizing native and denatured forms of gp120 in adjacent flow cells and injecting antibody across both. Native/denatured gp120 binding ratios are calculated using the following equation: [(gp120 binding in RU)/(denatured gp120 binding in RU)] * [(amt of denatured gp120 immobilized in RU)/(amt native gp120 immobilized in RU)]. The second part of the equation is a correction for potential concentration differences in the two immobilized proteins.

23. Multiple sequential binding interactions with a single immobilized antigen can be studied For example, MAbs to the HIV-1 envelope V3 region, sCD4 and a

gp41 MAb can be injected sequentially across a gp160 matrix and three binding interactions can be evaluated. This assay will yield information about epitope mapping, and those MAbs with similar specificities will compete with each other. This assay, however, is not optimal for competition studies since it is difficult to completely saturate the immobilized antigen with competing antibody. The capture assay described below is recommended for competition studies

24 Monoclonal antibody #1 can be immobilized directly to the dextran matrix as described above Alternatively, an anti-Ig Ab can be immobilized and used to capture several different MAbs of interest If anti-Ig Ab is immobilized then a blocking step is required after MAb #1 is captured to block remaining unbound anti-Ig. This is accomplished by an injection of a high concentration of nonspecific Ig (concentration of approx 1 mg/mL).

25. The concentration of antigen (gp120/gp160) to be captured will depend upon the affinity of immobilized MAb #1 for the antigen. In most cases with relatively high affinity MAbs, concentrations of 10–20 μg /mL of gp120/gp160 is sufficient. The amount of antigen captured should be high enough to yield sufficient sensitivity to detect binding by MAb #2 (approx 500–1000 RU) If antigen is in short supply, the flow rate can be reduced to 1 or 2 μL/min instead of increasing the concentration of captured antigen.

26. It is essential to include a negative control MAb in order to measure natural dissociation of captured gp120/gp160 from immobilized MAb #1 to serve as a baseline when calculated the amount of MAb #2 bound

27. Regeneration of MAb matrices can be accomplished using 2.5–50 m*M* HCl This will have to be optimized for each individual immobilized MAb.

28 If MAbs #1 and #2 bind to the same epitope then no binding above background will be obtained for MAb #2 since 100% of antigen is bound by MAb #1.

29 This assay has been designed to study and assess reactivity of various HIV-1 envelope glycoproteins to epitope specific MAbs, HIV-1 sera, and sCD4. Preparations of gp120 or gp160 are captured by sCD4 and then bound by various gp160-specific MAbs or HIV-1 sera Amount of bound Ab to captured gp120 or gp160 is then calculated.

30. Amount of sCD4 immobilized using these conditions may vary between 3000–5000 RU. Higher concentrations of immobilized sCD4 should be avoided since high local sCD4 concentrations may interfere with penetration of multimeric forms of gp160 into the matrix and thereby decrease capture efficiency

31 The amount of gp120/gp160 captured can be compared to a control gp120/gp160 preparation and assess the relative concentration of sCD4 binding competent gp160 proteins within any given preparation.

32. This assay will determine relative gp160 epitope accessibility/exposure on various gp160 preparations For example, other surface exposed epitopes on gp160 can be assessed (V3, V1/V2, gp41) to check antigenic structure outside of the sCD4 binding site

References

1. Altschuh, D , Dubs, M. C , Weiss, E , Zeder, L. G., and Van Regenmortel, M (1992) Determination of kinetic constants for the interaction between a mono-

clonal antibody and peptides using surface plasmon resonance. *Biochemistry* **31,** 6298–6304.

2. Fagerstam, L G., Frostell-Karlsson, A., Karlsson, R., Persson, B., and Ronnberg, I (1992) Biospecific interaction analysis using surface plasmon resonance detection applied to kinetic, binding site and concentration analysis. *J Chromatog* **597,** 397–410
3. Karlsson, R , Michaelsson, A., and Mattsson, L. (1991) Kinetic analysis of monoclonal antibody-antigen interactions with a new biosensor based analytical system *J Immunol Meth* **145,** 229–240
4. Sternberg, E., Perssom, B , Roos, H , and Urbaniczky, C (1991) Quantitative determination of surface concentration of protein with surface plasmon resonance by using radiolabelled proteins *J Colloid Interface Sci* **143,** 513–526.
5. Glaser, R. W and Hausdorf, G (1996) Binding kinetics of an antibody against HIV p24 core protein measured with real-time biomolecular interaction analysis suggest a slow conformational change in antigen p24. *J. Immunol Meth* **189,** 1–14
6 Richalet, S P., Deslandres, A , Plaue, S., You, B , Barre, S F , and Van, R. M. (1994) Cross-reactive potential of rabbit antibodies raised against a cyclic peptide representing a chimeric V3 loop of HIV-1 gp120 studied by biosensor technique and ELISA. *FEMS Immunol Med Microbiol.* **9,** 77–87
7. VanCott, T. C., Loomis, L. D., Redfield, R R., and Birx, D. L. (1992) Real-time biospecific interaction analysis of antibody reactivity to peptides from the envelope glycoprotein, gp160, of HIV-1 *J Immunol Meth* **146,** 163–176.
8 Conley, A. J , Kessler, J , Boots, L J., Tung, J S., Arnold, B A , Keller, P M , Shaw, A R , and Emini, E A (1994) Neutralization of divergent human immunodeficiency virus type 1 variants and primary isolates by IAM-41-2F5, an anti-gp41 human monoclonal antibody *Proc Natl Acad Sci USA* **91,** 3348–3352.
9 VanCott, T C , Bethke, F R , Polonis, V R., Gorny, M K , Zolla-Pazner, S , Redfield, R. R , et al. (1994) The dissociation rate of antibody-gp120 binding interactions is predictive of V3-mediated neutralization of HIV-1. *J Immunol* **153,** 449–459.
10 VanCott, T. C , Bethke, F. R., Artenstein, A. W., McCutchan, F. E., McNeil, J G., Mascola, J. R., Redfield, R R , and Birx, D. L. (1994) Serotyping international HIV-1 isolates by V3 peptides and whole gp160 proteins using BIAcore *Methods, a companion to Methods in Enzymol* **6,** 188–198.
11. VanCott, T. C , Bethke, F. R., Kalyanaraman, V , Burke, D S., Redfield, R. R., and Birx, D. L (1994) Preferential antibody recognition of structurally distinct HIV-1 gp120 molecules *J Acquir Immune Defic Synd* **7,** 1103–1115.
12 VanCott, T C , Bethke, F R , Burke, D. S , Redfield, R R , and Birx, D L. (1995) Lack of induction of antibodies specific for conserved, discontinuous epitopes of HIV-1 envelope glycoprotein by candidate AIDS vaccines. *J Immunol* **155,** 4100–4110.
13. BIAcore System Manual, Pharmacia Biosensor, Piscataway, NJ, USA.
14 Gershon, P. D. and Khilko, S (1995) Stable chelating linkage for reversible immobilization of oligohistidine tagged proteins in the BIAcore surface plasmon resonance detector. *J. Immunol Methods* **183,** 65–76

15. Johnsson, B., Lofas, S., and Lindquist, G. (1991) Immobilization of proteins to a carboxymethyldextran-modified gold surface for biospecific interaction analysis in surface plasmon resonance sensors *Anal Biochem* **198,** 268–277
16. Johnsson, B., Lofas, S., Lindquist, G , Edstrom, A., Muller, H. R., and Hansson, A (1995) Comparison of methods for immobilization to carboxymethyl dextran sensor surfaces by analysis of the specific activity of monoclonal antibodies *J. Mol Recognit* **8,** 125–131
17. Johnsson, U , Fagerstam, L., Ivarsson, B., Johnsson, B., Karlsson, R., Lundh, K , Lofas, S., Persson, B , Roos, H , Ronnberg, I , et al (1991) Real-time biospecific interaction analysis using surface plasmon resonance and a sensor chip technology *BioTechniques* **11,** 620–627.
18. Masson, L., Mazza, A., and Brousseau, R. (1994) Stable immobilization of lipid vesicles for kinetic studies using surface plasmon resonance. *Anal Biochem* **218,** 405–412
19 O'Shannessy, D. J , Brigham, B. M., and Peck, K (1992) Immobilization chemistries suitable for use in the BIAcore surface plasmon resonance detector. *Anal Biochem* **205,** 132–136.
20. Scouten, W H , Luoung, J. H. T., and Brown, R. S (1995) Enzyme or protein immobilization techniques for applications in biosensor design. *Trends Biotechnol* **13,** 178–185.
21 Stein, T. and Gerisch, F. (1996) Oriented binding of a lipid-anchored cell adhesion protein onto a biosensor surface using hydrophobic immobilization and photoactive crosslinking *Anal Biochem.* **237,** 252–259.