Acta Physiologica Hungarica, Volume 89 (4), pp. 415–425 (2002)

New trends in studying structure and function of biological membranes

S. **Damjanovich**[1,2], G. **Vámosi**[1], Andrea **Bodnár**[1], L. **Bene**[2]

[1] Cell Biophysics Research Group of the Hungarian Academy of Sciences and
[2] Department of Biophysics and Cell Biology, Medical and Health Science Center,
University of Debrecen, Hungary

Received: April 4, 2002
Accepted: April 31, 2002

Thirty years ago Singer and Nicolson constructed the "fluid mosaic model" of the membrane, which described the structural and functional characteristics of the plasma membrane of non-polarized cells like circulating blood lymphocytes as a fluid lipid phase accommodating proteins with a relatively free mobility. It is a rare phenomenon in biology that such a model could survive 30 years and even today it has a high degree of validity. However, in the light of new data it demands some modifications. In this minireview we present a new concept, which revives the SN model, by shifting the emphasis from fluidity to mosaicism, i.e. to lipid microdomains and rafts. A concise summary of data and key methods is given, proving the existence of non-random co-distribution patterns of different receptor kinds in the microdomain system of the plasma membrane. Furthermore, we present evidence that proteins are not only accommodated by the lipid phase, but they are integral structural elements of it. Novel suggestions to the SN model help to develop a modernized version of the old paradigm in the light of new data.

Keywords: membrane domains, receptor patterns, energy transfer, scanning microscopy, single molecule detection

At the 30th anniversary of the conception of the SN model we intend to give a brief description of the basic statements of the model and the new problems and insights arisen due to accumulating new data gained by fascinating new technologies in the past three decades.

Correspondence should be addressed to
Sándor Damjanovich, MD, PhD, DSc, MHASc
Department of Biophysics and Cell Biology
Medical and Health Science Center
University of Debrecen
H–4012 Debrecen, P.O. Box 39, Hungary
Phone/fax: +36-52-412-623
E-mail: dami@jaguar.dote.hu

Frye and Edidin demonstrated in their classic experiment that after fusion of mouse and human lymphocytes previously labeled with green and red fluorescent dyes on their MHC receptors, respectively, the two differently colored (mouse and human) MHC molecules were intermixed in the plasma membrane of the heterokaryon (23). This result was the firm basis for the invention of the Fluid Mosaic Membrane Model of Singer and Nicolson (43). The basic observation was that cell surface receptors are mobile on the one hand, but on the other hand they may form a mosaic-like structure. These observations and conclusions have a limited validity and apply mostly to the quasi-symmetric circulating blood cells. Cells built into tissues (e.g. cells bordering a lumen) are frequently polarized, having a basal and an apical part. The basal and the apical lipoprotein structures can be distinctly different, containing different proteins or lipids. The sorting of the vesicles transporting the newly synthesized lipoprotein structures to the basal or apical parts of the membranes generally starts as early as the formation of those vesicles. Circulating blood cells, and eminently among them lymphocytes, are assumed to be symmetric. However, their plasma membranes have a microdomain structure.

Microdomains in biological membranes

A microdomain is a segregated lipid and protein formation, which has a certain lifetime in the plasma membrane. Within this lifetime it is practically isolated from other parts of the membrane. Therefore we can define them as locally differentiated patches of the membrane (17). The general conception, mosaicism, of the SN model included the possibility of the existence of subcompartments of membranes, but did not study them in detail. The reason for this was at least partially technical. There were no methods available with a conveniently high resolving power to study these microdomains in living cell membranes. Herewith, we briefly describe the major methods, which are suitable to assess the membrane structure at the currently achievable finest resolution. Fluorescence resonance energy transfer (FRET) and its variations, electron microscopy in combination with immunogold-techniques, scanning force microscopic techniques, confocal laser scanning microscopy (CLSM), fluorescence correlation spectroscopy (FCS), single particle tracking (SPT), and the newest microdot nanotechnology are among those, which resulted in great steps ahead to understand membrane structures in vivo, mentioning just the most basic methods. Even from these selected ones we will discuss mostly those in detail, which have been applied by us so far.

Fluorescence resonance energy transfer, FRET, was introduced by Förster, when he analyzed dipole–dipole interactions between fluorescent molecules (22). The fundamental finding was that an excited donor molecule in the vicinity of a

spectroscopically suitable acceptor molecule with proper dipole orientation was able to transfer its excitation energy to the acceptor, without emitting photons (37). Because the efficiency of this process is proportional to the inverse sixth power of the donor-acceptor distance, the phenomenon can be applied for the determination or comparison of inter- and intramolecular distances. In the last half of the 20th century this method has become one of the most frequently applied spectroscopic methods in biochemistry, biophysics and cell biology that could be applied under physiological conditions, and was also extended into a method of imaging based on the resolution of different fluorescence lifetimes (12). The resolving power of FRET is dependent on the applied donor-acceptor pair, but mostly is confined to distances between 2–10 nm.

Flow cytometric energy transfer (FCET), which reports on proximity relationships at the molecular level in the plasma membranes of large cell populations within a short time interval, was introduced by Chan et al. (6) and, in its more advanced form, by Trón et al. (48, 49). This method obviates problems due to biological variations of the receptor densities on individual cells because data are taken on a cell-by-cell basis over a large cell population within minutes. Thus, these data give a distribution curve of FRET values over the cell population. Photobleaching energy transfer (pbFRET) measurements were introduced in the late 80s and were later extended to image analysis (2, 8, 10, 25–27, 34, 45, 46). The basis of the original method of Jovin and Arndt-Jovin (32) is that the bleaching time of the donor molecules is significantly extended by the presence of the acceptor, because the excited singlet state is depleted due to FRET, and there is less time for photodestruction to occur.

FRET in general measures distances between donor acceptor pairs. Cell surface molecules can be labeled with donor and acceptor dyes representing a supposedly homogenous population of those that are donor labeled and those that are acceptor labeled. Fab fragments of monoclonal antibodies are the best targeting devices. The first results of two-dimensional receptor mapping were published by Bene et al. (4) and Mátyus et al. (38) on the relative distribution of MHC class I and II molecules, IL-2 and transferrin receptors in the membranes of lymphoid cells. After normalization of the energy transfer values to the donor acceptor ratios, average distances determined between different receptors were displayed in two-dimensional grids. Higher normalized energy transfer efficiency indicates smaller distances between donor acceptor pairs. Average proximities can be scaled by known intermolecular distances, e.g. between the light and heavy chain epitopes of MHC class I molecules (28). These distances can be transformed into an epitope-proximity map using a successive triangulation method of map-makers (11).

Thus FCET measurements allow us to determine distances between A and B receptor populations averaged for each cell and determined on a cell-by-cell basis. If we have more than two kinds of receptors or other cell surface molecules, distances can be

determined between A, B, C, D,..., etc. molecules, always measuring FRET efficiency between two entities at a time. Assuming that all molecules investigated this way are within the resolution power of FRET, i.e. the intermolecular distances are between 2 and 10 nm, a two-dimensional map of the receptor co-distribution can be constructed.

A combined application of modern lasers, optical traps, advanced microscopy, fluorescence spectroscopy and imaging technologies has helped to make visualization of single molecules possible. Such methodology "expands the horizon in molecular diagnostics by making it possible to monitor concentrations down to (less than) 10^{-15} M without any need for amplification" (19). Inventive application of these methods to study proximity and mobility of membrane bound molecules may throw light on their dynamic characteristics and interactions at the single molecule level, i.e. the highest possible resolution.

As described above we can define membrane microdomains as a subset of lipids and proteins held together temporarily by their physico-chemical affinity to one another. Single particle tracking experiments may lead us to another operational definition of microdomains. Tomishige et al. (47) studied the interaction of a transmembrane protein having a long cytoplasmic tail (band 3) with the membrane-associated part of the cytoskeleton, the so-called membrane skeleton. The motion of individual proteins labeled with gold beads was followed at a sub-millisecond time resolution. The protein exhibited free Brownian diffusion inside apparent membrane domains of 40–700 nm in diameter for a few seconds then performed "hop diffusion", i.e. it crossed the boundary of the domain. After cleaving the intracellular tail of the protein by enzymatic digestion, the "hop rate" (the mobility between domains) increased significantly. These experiments have shown that the "macroscopic" diffusion of the molecule, which can also be studied by FRAP, is much slower than free short-distance diffusion within a domain (respective diffusion coefficients were D~0.003–0.03 and D~5–10 $\mu m^2/s$). We can now also understand why the diffusion coefficients of proteins measured in reconstituted artificial lipid membranes are orders of magnitude higher than in the plasma membrane of live cells. The generalization of these and similar results is the "membrane skeleton fence model", which assumes that the restricted diffusion of proteins is at least partially due to the physical obstacles set forth by the membrane skeleton (36).

Fluorescence correlation spectroscopy (FCS, introduced by Elson and Magde (20); Ehrenberg and Rigler (18)), offered the first possibility to detect single molecular species in solution. The method is based on fluorescence signal fluctuations. The laser light beam can be focused to volumes as small as 10^{-16} liter. Since a 10^{-9} M solution accommodates 10^{15} particles per liter, a single molecule has a "territorial space" equivalent to 1 femtoliter. In such a "light cavity" having a volume of 0.1–1 fl fluorescence events can be followed by FCS (19). It makes the detection of single

molecules possible, because the short residence time of the diffusing molecule in the small volume element allows detection of single quantum bursts of fluorescent light, and limits the number of the target molecules simultaneously present. Imaging fluorescence correlation spectroscopy visualized molecular clusters by measuring the pixel-to-pixel fluorescence fluctuations of tetramethylrhodamine labeled anti-trinitrophenyl IgE antibodies specifically bound to planar artificial lipid membranes (29). These methods have a very great potential for future biophysical and cell physiological experimentation in general, and in membrane biology in particular (12, 19).

Laser technology and multiple optical traps were used by Finer et al. (21) to determine discrete step-wise movements of about 11 nm of a single myosin molecule during its interaction with a single actin filament. While this was done under low load conditions, isometric conditions revealed 3–4 pN single force transients. A recent approach has further refined the former measurements using a single myosin head instead of the double-headed heavy meromyosin (39). Turnover of individual ATP molecules by single myosin molecules was also measured by imaging single fluorescent molecules (24).

Applying total internal reflection fluorescence microscopy, direct observation of a single kinesin molecule moving along microtubules was made possible (50). Flat mirrors can reflect light beams without a loss of energy. However, if the reflecting plate is very thin and an excitable molecule with suitable fluorescence properties floats into the electro-magnetic field generated by the reflected light beam, it can absorb a photon from this field and emit fluorescence. This single flickering from a single molecule is detectable and we can determine the diffusion of a single molecular species to a surface.

Scanning force microscopy was developed from atomic force microscopy (AFM) invented by Binnig, Quate and Gerber in 1986 (5). Binnig's original idea was to map non-conducting surfaces with a similar efficiency and resolution power to that of the scanning tunneling microscope, which can only be applied to electrically conducting material. The application of a very sharp needle with a tip in atomic dimensions (1–10 nm at the tip) is used to scan material surfaces very closely. The motion pattern of the needle above any surface is modulated by the interactions between the surface molecules and the tip. The quick development of physical and chemical etching of silicon nitride and silicon needles produced very sharp tips, which replaced the original diamond powder "needles". A method invented earlier in scanning tunneling microscopy is applied to measure the motion of the cantilever. The motion is monitored now by a laser beam, which is reflected onto a split photodiode from the cantilever holding the sensor tip. Scanning can be achieved by moving the sample holder or the tip itself by piezo-electric crystals. When the tip is moving and scans the material surface, the instrument is called a stand-alone-type atomic force microscope. Scanning, as in practically all other cases, is based upon the very well controlled and fast changes of the

longitudinal dimensions of piezo-electric rods. These rods, holding the sample stage or a tip, can change their length even in a very fast oscillation mode with a precision of less than 1 Å, depending on the voltage generating this phenomenon and the original "resting" length of the rod. Although the method itself has the capacity for atomic scale resolution, in biology 10–100 nm resolution is achieved in most cases (44). The resolution of rough cell surface structures is handicapped by the strong corrugation of most cell membranes, thereby compromising the resolved structure. The slogan, "the flatter the better", has a strong validity concerning the resolution of a sample surface. This is particularly relevant for lymphocytes and the investigation of important immunological structures. In this case AFM can reveal supramolecular structures only over limited (preferentially flat) cell membrane areas. These areas can be investigated under near physiological conditions. The extension to other parts of the membrane and further verification of such structures can be made by electron microscopy, significantly supporting the existence of such structures over the whole cell surface (16, 31, 52). A combination of atomic force, electron microscopy and flow cytometric as well as photobleaching energy transfer made possible the discovery and description of the clustering of MHC class I molecules at the 2–10 nm and also the μm level (16, 31, 40, 41, 52). This finding reports on the clustering of MHC class I molecules at two different hierarchical levels. Cells were immobilized on slides by photo-cross-linking (35, 51). MHC class I molecules were then double labeled, first with monoclonal antibodies against the heavy or light chains of them. Second antibodies against the first mAbs then targeted colloidal gold beads, 15 and/or 30 nm in diameter, to these molecules. The gold beads visualized in a scanning force microscope under buffered physiological conditions, as well as in dried state indicated a non-random distribution of the MHC class I molecules. This pattern, which was also found in electron microscopic pictures of the same specimens, showed a second, larger-scale topological organization of the MHC class I molecules, in addition to the first one observed by flow cytometric and photobleaching energy transfer over distances of 2–10 nm.

Such defined molecular distributions on the surfaces of cells of the immune system may reflect an underlying specialization of the membrane lipid domains and fulfill important functional roles in cell–cell contacts and signal transduction (13, 14, 16, 30). Small-sized colloidal gold beads, on the order of 1 nm, may be the key labels for individual receptor molecules. However, currently this bead size can be recognized only after silver enhancement procedure, which obscures resolution (42).

Alternatively, the application of scanning near-field optical microscopy (SNOM) for cell surface target localization solves the problem of targeting by using fluorescent labels. SNOM measurements are taken in the visible range of light, however, the current resolution of it is much lower (10–30 nm) than that of the atomic force microscope. SNOM uses an optical fiber to scan the surface of a cell or a molecular object at a very

close distance. A picture of the surface is generated by the photons emitted from the illuminated surface. If we properly label the cell surface by fluorescent dyes, scanning has the capacity to reflect the topography of the fluorescence signals evoked from the labeled molecular elements of the surface (40, 41).

The ascending phase of the application of microdots or quantum dots started relatively recently (7). Microdots (or quantum dots) are semiconductor pieces on the nm scale (built frequently from CdSe) which may offer hitherto non-existing possibilities to study spectroscopic characteristics at a scale, which fills the gap between FRET based measurements and the larger, several 10 nm or even μm scales. These nm-size particles may emit fluorescence of various colors depending on their size. Therefore multicolor analysis is possible by applying single wavelength excitation light, if specific cell surface elements are labeled by these microdots with different diameters. The phenomenon is based on the modulation of the energy band structure of the semiconductor crystals due to spatial confinement.

The application of these technical inventions reflects a new trend in physiology at the level of single molecules; the above list is only a selection of the available technologies.

Information content of lymphocyte membranes

What can we expect from these sophisticated possibilities or, changing the focus of the question, how can we read information from the cell surface mosaicism, receptor patterns, lipid rafts and microdomains? Recently, it was published that cell surface of colo-rectal carcinoma cells, which showed a dimeric distribution pattern for the MHC class I molecules in the plasma membrane, changed the nature of this pattern upon interferon-gamma (INF-γ) treatment. INF-γ increased the density of MHC molecules in the plasma membrane, yet the extent of dimerization was decreased, despite the fact that the size of the cells had not changed. On the other hand, the INF-γ induced ICAM-1 molecules showed close proximity to MHC molecules, which is actually necessary for antigen presentation (1).

Based on this and also on earlier investigations we suggest the following model: The immediate past of a lymphocyte can be marked by the number, density and proximity relations of different specific receptors, the density of ion channels and their overall activation state, which is reflected by the receptor distribution patterns at the cell surface. The receptors at the cell surface of lymphocytes are not static: neither their density nor the distribution patterns are independent of the state of the activation and sequence of receptor expressions in the plasma membrane. The maintenance of cell surface receptor patterns is highly, but not exclusively dependent on lipid

microdomains. Experiments with fused cells showed that protein moieties in the domains have a structural role together with the lipid part that accommodates them. Let us discuss this in a little broader sense. One can ask, what kind of practical aspects can be deduced from these findings for physiology and pathophysiology of the cells in question. We may try to look for diagnostic, prognostic and therapeutic aspects of plasma membrane receptor structures. Specific tumor receptors are the simplest reporters on the deviation of the cell metabolism from the normal. The next step is the appearance of so-called activation antigens.

As it was shown in the above discussion, cell surface receptors are not hetero- or homo-associated with each other freely and randomly. The microdomain structure of the plasma membrane provides the force to maintain molecular vicinity between apparently unrelated or only functionally related molecules. On the other hand we have to emphasize the fact that in several cases the protein structure itself is not only accommodated by the lipid structures, but the protein structures like the membrane spanning alpha-helical segments participate in the maintenance of the microdomain, or even more they participate as an integral part of it. Recently fusion experiments were carried out (41), which intended to repeat the classic Frye–Edidin experiment. The evaluation of the data was carried out with modern techniques, providing a resolution power well beyond that of the classic experiment, which used simple fluorescence microscopic detection, the only sensible way thirty years ago. Cell fusion of human lymphocytes induced by polyethylene-glycol was used to perturb existing small- and large-scale distribution patterns of cell surface proteins and test the kinetics of intermixing between them. The time course of intermixing was followed by pbFRET and SNOM techniques. While we found that the majority of homo- and heteroclusters of molecular species studied by us so far (with a few exceptions like transferrin receptors) intermixed with each other at large scales, mixing at the molecular level, i.e. re-forming of molecular associations was not universal. Intermixing did not take place in the case of MHC II homoclusters; however, it was observed for homoclusters of MHC class I and for heteroclusters of class I and class II MHC molecules. These processes did not depend on the endocytosis-mediated recycling of proteins. These data underlined the integral role of protein and lipid interactions in the maintenance of membrane microdomains. MHC class II has two membrane spanning domains and the lipid rafts accommodating the proteins are rather small-sized. On the other hand, MHC class I and MHC class II could form heteroclusters as the larger lipid rafts with MHC class I could "swallow" the more rigid MHC class II containing rafts in their entirety.

Taken together, the alterations of the SN model have been made necessary by the accumulating data, which could not be included in the frame of the original model. By shifting the emphasis from unrestricted protein mobility to mosaicism and restricted

protein mobility, furthermore accepting the microdomain forming force of lipid–protein interactions inside, above or below the membrane, a new profile can be given to the frame of the old paradigm.

Summarizing the above discussion, it is obvious that microdomains as well as non-random co-distribution patterns of proteins can gain a new functionally and structurally very important meaning.

Acknowledgements

This work was supported by research grants OTKA T30411 (S.D.), ETT 117/2001 (Gy.V.) and ETT 138/2001 (L.B.).

REFERENCES

1. Bacsó Z, Bene L, Damjanovich L, Damjanovich S: INF-gamma rearranges membrane topography of MHC-I and ICAM-1 in colon carcinoma cells. Biochem. Biophys. Res. Commun. 290, 635–640 (2002)
2. Bastiaens PI, Majoul IV, Verveer PJ, Soling HD, Jovin TM: Imaging the intracellular trafficking and state of the AB5 quaternary structure of cholera toxin. EMBO J. 15, 4246–4253 (1996)
3. Bastiaens PI, Wouters FS, Jovin TM: Imaging the molecular state in cells by fluorescence resonance energy transfer. Sequential photobleaching of Förster donor-acceptor pairs. Proc. 2nd Hamamatsu Intern. Symp. Biomolec. Mech. Photonics: Cell-cell communication. (1995)
4. Bene L, Balázs M, Matkó J, Most J, Dierich MP, Szöllősi J, Damjanovich S: Lateral organization of the ICAM-1 molecule at the surface of human lymphoblasts: a possible model for its co-distribution with the IL-2 receptor, class I and class II HLA molecules. Eur. J. Immunol. 24, 2115–2123 (1994)
5. Binnig G, Quate CF, Gerber C: Atomic force microscope. Phys. Rev. Lett. 56, 930–933 (1986)
6. Chan SS, Arndt-Jovin DJ, Jovin TM: Proximity of lectin receptors on the cell surface measured by fluorescence energy transfer in a flow system. J. Histochem. Cytochem. 27, 56–64 (1979)
7. Chan WCW, Maxwell DJ, Gao XH, Bailey RE, Han MY, Nie SM: Luminescent quantum dots for multiplexed biological detection and imaging. Curr. Opin. Biotechn. 13, 40–46 (2002)
8. Clegg RM: Fluorescence resonance energy transfer. Curr. Opin. Biotechn. 6, 103–110 (1995)
9. Clegg RM (1996): Fluorescence resonance energy transfer (FRET). In: Fluorescence Imaging Spectroscopy and Microscopy, eds Wang XF, Herman B. John Wiley & Sons, Inc., New York, pp. 179–252.
10. Clegg RM, Feddersen BA, Gratton E, Jovin TM: Time resolved imaging fluorescence microscopy. SPIE 1604, 448–460 (1992)
11. Damjanovich S, Bene L, Matkó J, Mátyus L, Krasznai Z, Szabó G, Pieri C, Gáspár R, Szöllősi J: Two-dimensional receptor patterns in the plasma membrane of cells. A critical evaluation of their identification, origin and information content. Biophys. Chem. 82, 99–108 (1999)
12. Damjanovich S, Gáspár R, Jr., Pieri C: Dynamic receptor superstructures at the plasma membrane. Q. Rev. Biophys. 30, 67–106 (1997)
13. Damjanovich S, Somogyi B, Trón L: Macromolecular dynamics and information transfer. Adv. Physiol. Sci. 30, 9–15 (1981)
14. Damjanovich S, Szöllősi J, Trón L: Transmembrane signalling in T cells. Immunol Today 13, 12–15 (1992)

15. Damjanovich S, Szöllősi J, Trón L, Edidin M (1994): Mobility and Proximity in Biological Membranes. CRC Press, Boca Raton, Ann Arbor, London, Tokyo.
16. Damjanovich S, Vereb G, Schaper A, Jenei A, Matkó J, Starink JP, Fox GQ, Arndt-Jovin DJ, Jovin TM: Structural hierarchy in the clustering of HLA class I molecules in the plasma membrane of human lymphoblastoid cells. Proc. Natl. Acad. Sci. USA 92, 1122–1126 (1995)
17. Edidin M: Shrinking patches and slippery rafts: scales of domains in the plasma membrane [Review]. Trends Cell Biol. 11, 492–496 (2001)
18. Ehrenberg M, Rigler R: Rotational Brownian motion and fluorescence intensity fluctuations. J. Chem. Phys. 4, 390–401 (1974)
19. Eigen M, Rigler R: Sorting single molecules: application to diagnostics and evolutionary biotechnology. Proc. Natl. Acad. Sci. USA 91, 574–577 (1994)
20. Elson EL, Magde D: Fluorescence correlation spectroscopy. Conceptual basis and theory. Biopolymers 13, 1–27 (1974)
21. Finer JT, Simmons RM, Spudich JA: Single myosin molecule mechanics: piconewton forces and nanometre steps. Nature 368, 113–139 (1994)
22. Förster T: Zwischenmolekulare Energiewanderung und Fluoreszenz. Ann. Phys. 2, 55–75 (1948)
23. Frye LD, Edidin M: The rapid intermixing of cell surface antigens after formation of mouse-human heterokaryons. J. Cell Sci. 7, 319–335 (1970)
24. Funatsu T, Harada Y, Tokunaga M, Saito K, Yanagida T: Imaging of Single Fluorescent Molecules and Individual ATP Turnovers by Single Myosin Molecules in Aqueous Solution. Nature 374, 555–559 (1995)
25. Gadella TWJ, Clegg RM, Jovin TM: Fluorescence lifetime imaging microscopy: pixel-by-pixel analysis of phase-modulation data. Bioimaging 2, 139–159 (1994)
26. Gadella TWJ, Jovin TM, Clegg RM: Fluorescence lifetime imaging microscopy (FLIM): spatial resolution of microstructures on the nanosecond time scale. Biophys. Chem. 48, 221–239 (1993)
27. Gadella TWJ Jr, Jovin TM: Oligomerization of epidermal growth factor receptors on A431 cells studied by time-resolved fluorescence imaging microscopy: A stereochemical model for tyrosine kinase receptor activation. J. Cell Biol. 129, 1543–1558 (1995)
28. Gáspár R Jr, Bagossi P, Bene L, Matkó J, Szöllősi J, Tőzsér J, Fésűs L, Waldmann TA, Damjanovich S: Clustering of class I HLA oligomers with CD8 and TCR: three-dimensional models based on fluorescence resonance energy transfer and crystallographic data. J. Immunol. 166, 5078–5086 (2001)
29. Huang Z, Thompson NL: Imaging fluorescence correlation spectroscopy: nonuniform IgE distributions on planar membranes. Biophys. J. 70, 2001–2007 (1996)
30. Jacobson K, Sheets ED, Simson R: Revisiting the fluid mosaic model of membranes. Science 268, 1441–1442 (1995)
31. Jenei A, Varga S, Bene L, Mátyus L, Bodnár A, Bacsó Z, Pieri C, Gáspár R, Jr., Farkas T, Damjanovich S: HLA class I and II antigens are partially co-clustered in the plasma membrane of human lymphoblastoid cells. Proc. Natl. Acad. Sci. USA 94, 7269–7274 (1997)
32. Jovin TM, Arndt-Jovin D (1989): FRET Microscopy: Digital imaging of fluorescence resonance energy transfer. Application in cell biology. In: Cell Structure and Function by Microspectrofluorometry, eds Kohen E, Hirschberg JG, Academic Press, New York.
33. Jovin TM, Arndt-Jovin D, Marriott G, Clegg RM, Robert NM, Schormann T (1990): Distance, wavelength & time: the versatile 3rd dimension in light emission microscopy. In: Optical microscopy for biology, Wiley-Liss, Inc., New York, pp. 575–602.
34. Jovin TM, Arndt-Jovin DJ: Luminescence digital imaging microscopy. Ann. Rev. Biophys. Chem. 18, 271–308 (1989)
35. Karrasch S, Dolder M, Schabert F, Ramsden J, Engel A: Covalent binding of biological samples to solid supports for scanning probe microscopy in buffer solution. Biophys. J. 65, 2437–2446 (1993)
36. Kusumi A, Sako Y: Cell surface organization by the membrane skeleton. Curr. Opin. Cell Biol. 8, 566–574 (1996)

37. Matkó J, Szöllősi J, Trón L, Damjanovich S: Luminescence spectroscopic approaches in studying cell surface dynamics. Q. Rev. Biophys. 21, 479–544 (1988)
38. Mátyus L, Bene L, Heyligen H, Raus J, Damjanovich S: Distinct association of transferrin receptor with HLA class I molecules on HUT-102B and JY cells. Immunol. Lett. 44, 203–208 (1995)
39. Molloy JE, Burns JE, Kendrick-Jones J, Tregear RT, White DC: Movement and force produced by a single myosin head. Nature 378, 209–212 (1995)
40. Nagy P, Jenei A, Kirsch AK, Szöllősi J, Damjanovich S, Jovin TM: Activation-dependent clustering of the erbB2 receptor tyrosine kinase detected by scanning near-field optical microscopy. J. Cell. Sci. 112, 1733–1741 (1999)
41. Nagy P, Mátyus L, Jenei A, Panyi G, Varga S, Matkó J, Szöllősi J, Gáspár R, Jovin TM, Damjanovich S: Cell fusion experiments reveal distinctly different association characteristics of cell-surface receptors. J. Cell Sci. 114, 4063–4071 (2001)
42. Neagu C, Van der Werf KO, Putman CA, Kraan YM, De Grooth BG, Van Hulst NF, Greve J: Analysis of immunolabeled cells by atomic force microscopy, optical microscopy, and flow cytometry. J. Struct. Biol. 112, 32–40 (1994)
43. Singer SJ, Nicolson GL: The fluid mosaic model of the structure of cell membranes. Science 175, 720–731 (1972)
44. Spudich A, Braunstein D: Large secretory structures at the cell surface imaged with scanning force microscopy. Proc. Natl. Acad. Sci. USA 92, 6976–6980 (1995)
45. Szabó G Jr, Pine PS, Weaver JL, Kasari M, Aszalós A: Epitope mapping by photobleaching fluorescence energy transfer measurements using a laser scanning microscopc system. Biophys. J. 61, 661–670 (1992)
46. Szabó G Jr., Pine PS, Weaver JL, Rao PE, Aszalós A: CD4 changes conformation upon ligand binding. J. Immunol. 149, 3596–3604 (1992)
47. Tomishige M, Sako Y, Kusumi A: Regulation mechanism of the lateral diffusion of band 3 in erythrocyte membranes by the membrane skeleton. J. Cell Biol. 142, 989–1000 (1998)
48. Trón L, Szöllősi J, Damjanovich S, Helliwell SH, Arndt-Jovin DJ, Jovin TM: Flow cytometric measurements of fluorescence resonance energy transfer on cell surfaces. Quantitative evaluation of the transfer efficiency on a cell by cell basis. Biophys. J. 45, 939–946 (1984)
49. Trón L, Szöllősi J, Damjanovich S: Proximity measurements of cell surface proteins by fluorescence energy transfer. Immunol. Lett. 16, 1–9 (1987)
50. Vale RD, Funatsu T, Pierce DW, Romberg L, Harada Y, Yanagida T: Direct observation of single kinesin molecules moving along microtubules. Nature 380, 451–453 (1996)
51. Vereb G, Damjanovich S, Jovin TM: Immobilization of Molecules, Membranes and Cells for Modern Optical and Non-Optical Microscopy by Photo-Cross-Linking. J. Photochem. Photobiol. B: Biol. 27, 275–277 (1995)
52. Vereb G, Matkó J, Vámosi G, Ibrahim SM, Magyar E, Varga S, Szöllősi J, Jenei A, Gáspár R Jr, Waldmann TA, Damjanovich S: Cholesterol-dependent clustering of IL-2R-alpha and its colocalization with HLA and CD48 on T lymphoma cells suggest their functional association with lipid rafts. Proc. Natl. Acad. Sci. USA 97, 6013–6018 (2000)