J Lipid Atheroscler. 2023 Jan;12(1):58-72
https://doi.org/10.12997/jla.2023.12.1.58
pISSN 2287-2892·eISSN 2288-2561

JLA Journal of Lipid and Atherosclerosis

Review

Check for updates

Single-Molecule Imaging of Membrane Proteins on Vascular Endothelial Cells

Jiseong Park, Siwoo Jin, Juhee Jang, Daeha Seo

Department of Physics and Chemistry, Daegu Gyeongbuk Institute of Science & Technology (DGIST), Daegu, Korea

OPEN ACCESS

Received: Aug 19, 2022
Revised: Sep 19, 2022
Accepted: Sep 22, 2022
Published online: Dec 1, 2022

Correspondence to
Daeha Seo
Department of Physics and Chemistry, Daegu Gyeongbuk Institute of Science & Technology (DGIST), 333 Techno jungang-daero, Hyeonpung-eup, Dalseong-gun, Daegu 42988, Korea.
Email: livewire@dgist.ac.kr

ORCID iDs
Jiseong Park
https://orcid.org/0000-0002-4598-6534
Siwoo Jin
https://orcid.org/0000-0002-3044-8055
Juhee Jang
https://orcid.org/0000-0002-1031-1609
Daeha Seo
https://orcid.org/0000-0002-0454-1168

Funding
This work was supported by the National Research Foundation of South Korea (NRF) grants funded by the Korean government (MSIT; Ministry of Science and ICT, NRF-2018R1A5A1025511, NRF-2020R1A2C4002533).

ABSTRACT

Transporting substances such as gases, nutrients, waste, and cells is the primary function of blood vessels. Vascular cells use membrane proteins to perform crucial endothelial functions, including molecular transport, immune cell infiltration, and angiogenesis. A thorough understanding of these membrane receptors from a clinical perspective is warranted to gain insights into the pathogenesis of vascular diseases and to develop effective methods for drug delivery through the vascular endothelium. This review summarizes state-of-the-art single-molecule imaging techniques, such as super-resolution microscopy, single-molecule tracking, and protein–protein interaction analysis, for observing and studying membrane proteins. Furthermore, recent single-molecule studies of membrane proteins such as cadherins, integrins, caveolins, transferrin receptors, vesicle-associated protein-1, and vascular endothelial growth factor receptor are discussed.

Keywords: Vascular endothelium; Membrane protein; Transcytosis; Single molecule imaging; Optical imaging

INTRODUCTION

Blood vessels transport various molecules, including nutrients, hormones, and drugs, to organs and tissues in the body via the circulating blood. During transport, membrane proteins on the vascular endothelial cells regulate the molecular exchange between blood and tissues (**Fig. 1**). The vascular endothelium mediates 2 types of transport known as the transcellular and paracellular pathways. Molecules are transported through appropriate pathways according to their size and solubility, via processes involving receptors expressed on endothelial cells. Transcellular transport includes passive diffusion and membrane protein-associated transport. Small lipophilic molecules and gaseous molecules can pass through cells via passive transport,[1,2] whereas macromolecules are transported via endocytic vesicles or trans-endothelial channels that are regulated by specific membrane proteins such as caveolin, transferrin (Tf) receptor, and plasmalemmal vesicle associated protein-1 (PV-1).[3-5] Paracellular transport, on the contrary, involves molecular trafficking through intercellular junctions and is regulated by proteins such as cadherin and claudin.[6]

JLA Journal of Lipid and Atherosclerosis

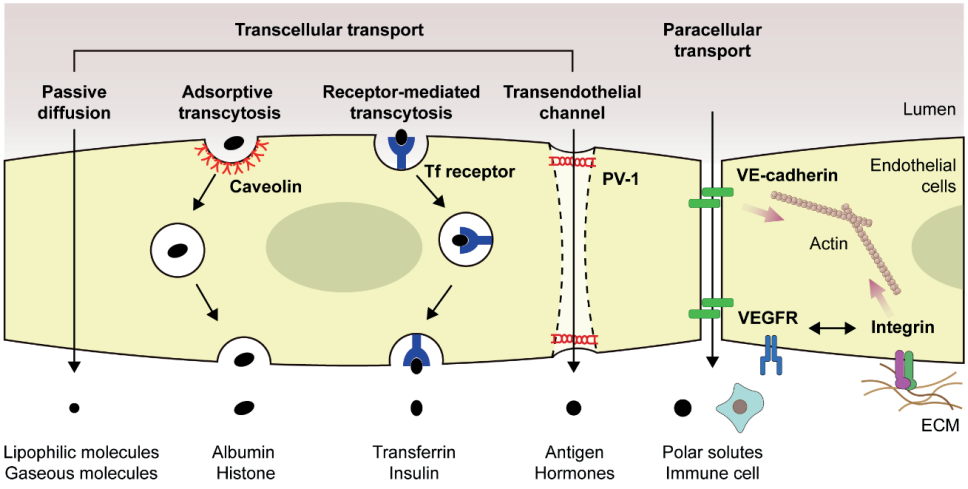

Fig. 1. Transendothelial transport regulated by membrane proteins. Membrane proteins participate in molecular transport through the vascular endothelium, where the transport pathway is determined by the size and polarity of the substance. Molecular transport relies on 2 pathways: paracellular transport, involving VE-cadherin and integrin, and transcellular transport, which is divided into passive diffusion and membrane-associated transport that involves caveolin (adsorptive transcytosis), Tf receptor (receptor-mediated transcytosis), and PV-1 (transendothelial channel).
VE, vascular endothelial; Tf, transferrin; PV-1, plasmalemmal vesicle associated protein-1; VEGFR, vascular endothelial growth factor receptor; ECM, extracellular matrix.

Conflict of Interest

The authors have no conflicts of interest to declare.

Author Contributions

Conceptualization: Park J, Seo D; Supervision: Seo D; Visualization: Park J; Writing - original draft: Park J, Jin S, Jang J; Writing - review & editing: Park J, Jin S, Jang J, Seo D.

The vascular endothelium forms a physical barrier, which restricts the exchange of substances between the blood and tissue and facilitates selective exchange of substances. Therefore, the perturbation of membrane proteins and lipids causes endothelial dysfunction. This leads to various vascular diseases such as atherosclerosis and vascular hyper-permeability. The pathogenesis of atherosclerosis primarily involves the inflammatory process,[7,8] during which the expression of membrane molecules that mediate the adhesion between immune cells and the vascular endothelium, such as vascular cell adhesion protein 1 and E-selectin, is upregulated. Abnormal permeability is a major abnormal property of tumor vasculature, which reduces transcapillary blood flow, increases interstitial pressure, and facilitates tumor cell extravasation.[9] Cancer cells release various chemotactic molecules to induce angiogenesis for nutrient supply and metastasis, whereas vascular endothelial cells receive the signal through membrane receptors and activate angiogenic signaling. The expression and signaling activity of membrane proteins, such as Notch, integrin, and vascular endothelial growth factor receptor (VEGFR), are perturbed during this process.

The endothelial barrier maintains homeostasis due to tight molecular transport regulation but limits drug delivery across the vascular endothelium. In particular, the enhanced permeability and retention effects can be applied for cancer drug transport. The tumor vasculature is more leaky than normal blood vessels, therefore, adjusting the drug size (40–70 kDa) can increase tumor drug delivery. Recently, targeting the transcytosis receptor has become an attractive and efficient strategy for overcoming the endothelial barrier. Tf–Tf receptor (TfR) systems are major components of the receptor-mediated transcytosis (RMT). Tf-conjugation enhances drug delivery using lipid nanocarriers.[10]

As mentioned, membrane proteins in the vascular endothelium play a vital role in the regulation of molecular transport, angiogenesis, and vascular diseases. They diffuse through the plasma membrane and interact with proteins or molecules to trigger cell signaling and to perform their cellular functions. Conventional approaches, e.g., mutation and regulation of gene expression, have primarily been used to understand molecular mechanisms and the

https://doi.org/10.12997/jla.2023.12.1.58

functions of these proteins by quantitatively evaluating gene expression. Methods such as real-time polymerase chain reaction (RT-PCR), microarray, and next-generation sequencing (NGS) have provided a wealth of molecular biological information. However, these ensemble averaging methods are limited in providing insight into spatiotemporal information of the mechanism of protein complex formation, including subcellular localization, single protein diffusion, and temporal dynamics. In this regard, single-molecule imaging and biophysical analyses serve as appealing tools for understanding membrane proteins. Furthermore, as detailed in this review, recent advances in optical microscopy have made it possible to observe single-molecule-level localization and millisecond (ms)-level temporal information in living cells.

OPTICAL MICROSCOPIC TECHNIQUES FOR BIOIMAGING AT SINGLE-MOLECULE LEVEL

1. Labeling of membrane proteins for optical microscopy

Spatiotemporal information on the behavior of a protein is essential for understanding its function and molecular mechanism. Proteins require optical labels, such as fluorophores, for sequential imaging and optical microscopy and spectroscopy utilizing these probes have contributed to the understanding of molecular behaviors of proteins in living cells or under physiological conditions (**Figs. 2** and **3**).

Chemical modification of fluorescent antibodies[17] and production of genetically engineered fluorescent proteins[18,19] have been commonly used as labeling methods since their development. Although antibody labeling has a high affinity for the target protein, it is typically used to alter protein function, for example, as an inhibitor that blocks the binding of proteins. As a result, it is used for imaging fixed cells, but not for studying protein dynamics. Fluorescent proteins have high bio-stability because they are tagged through genetic fusion and therefore do not disrupt cell homeostasis. Various fluorescent proteins of different colors have been designed since the demonstration of green fluorescent proteins (GFPs) as fluorescent tags.[20-22]

Recent research in chemistry and materials science has led to the development of synthetic optical probes such as organic dyes and nanoparticles with the genetically modified fluorescent tag (**Table 1**). Organic dyes are commonly used optical probes because of their smaller size (up to 1 kDa) and better optical properties than those of fluorescent proteins. Recently, nanoparticles have attracted attention for single-molecule imaging due to their higher signal-to-noise ratios and longer lifetime than other optical probes.[26] It is important to note that the preparation methods and surface stoichiometry should be considered when using nanoparticles to prevent perturbation of the target protein though binding to multiple protein molecules due to the large size and surface area of the nanoparticles.

Membrane proteins in vascular endothelial cells need to be specifically labeled with an optical probe to directly observe and analyze single-molecule dynamics. Fluorescent proteins can be directly fused with target proteins via genetic modifications. Although they lack genetic encodability, organic dyes and nanoparticles can be chemically linked to proteins. Antibody-antigen conjugation, peptide tags, and DNA hybridization are commonly used labeling methods for synthetic probes.[17,27,28]

https://doi.org/10.12997/jla.2023.12.1.58

JLA Journal of Lipid and Atherosclerosis

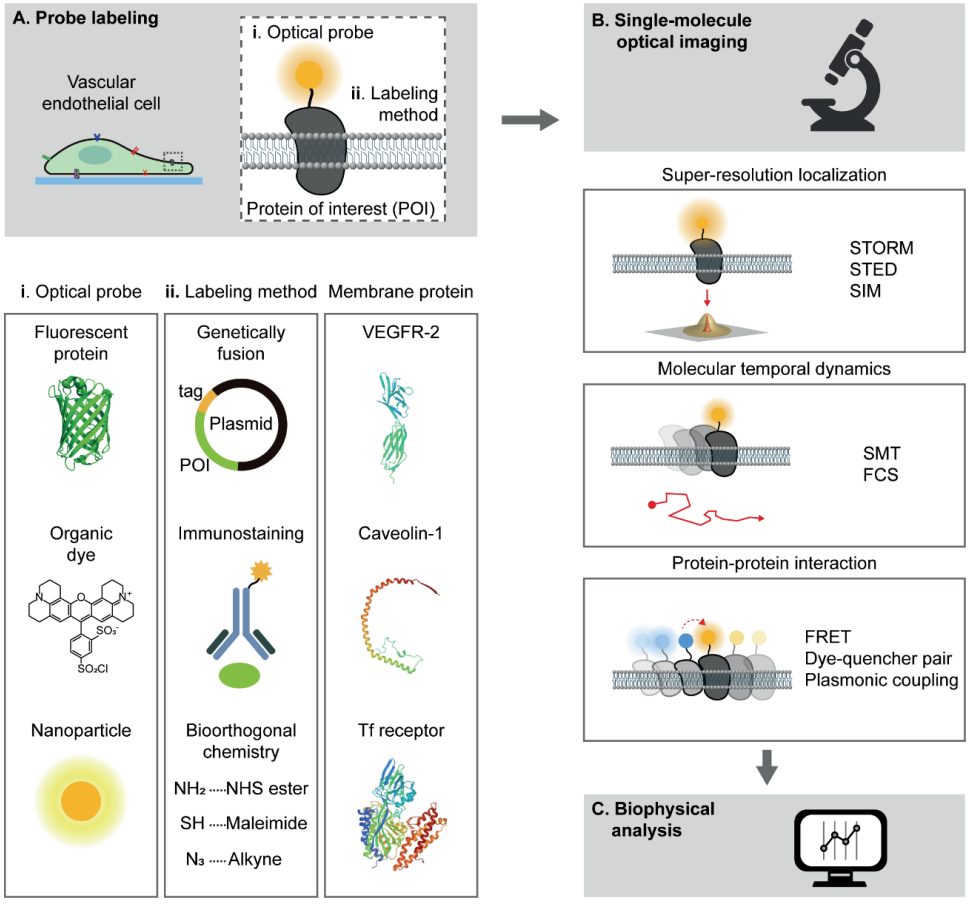

Fig. 2. Single-molecule imaging of membrane proteins in vascular endothelial cells. (A) Optical labeling of membrane proteins. Optical probe (e.g., fluorescent protein, organic dye, and nanoparticle) can be conjugated with membrane proteins (e.g., VEGFR-2: PDB 3V2A, caveolin: PDB 7SC0, and Tf receptor: PDB 6OKD) via proper labeling methods (e.g., genetic fusion, immunostaining, and biorthogonal chemistry). (B) Single-molecule optical imaging. Imaging techniques, such as super-resolution microscopy, single-molecule tracking, and imaging of nm-level protein-protein interactions, can be used to gain high spatiotemporal information on biomolecules. (C) Biophysical analysis. Various algorithms for data processing transform imaging data into biophysical parameters.
VEGFR, vascular endothelial growth factor receptor; POI, protein of interest; Tf, transferrin; STORM, stochastic optical reconstruction microscopy; STED, stimulated emission depletion; SIM, structured illumination microscopy; SMT, single-molecule tracking; FCS, fluorescence correlation spectroscopy; FRET, Fluorescence resonance energy transfer.

These labeling techniques can be combined based on the experimental design. For example, different fluorescent molecules can be used to observe the motion or interaction between molecules, whereas nanoparticles can be used in prolonged observation studies.

2. Super-resolution microscopy for precise measuring of the position of the protein

The diffraction limit (usually up to 200 nm in a conventional microscope) of optical microscopy, discovered by Ernst Abbe in 1873,[29] makes it difficult to resolve neighboring molecules. However, protein clusters on the plasma membrane generally have a sub-diffraction limit. This limitation should be overcome to reveal the molecular composition and relative orientation within the nanometer-sized structure. Since the development of the first working fluorescence microscope in 1911,[30] epifluorescence microscopy[31] and total internal reflection fluorescence (TIRF) microscopy have been designed and developed to enhance the fluorescence signal and to observe only the basal plane (membrane on the glass).[32] Recently, super-resolution microscopy (SRM) has been used to overcome this diffraction barrier.

https://doi.org/10.12997/jla.2023.12.1.58

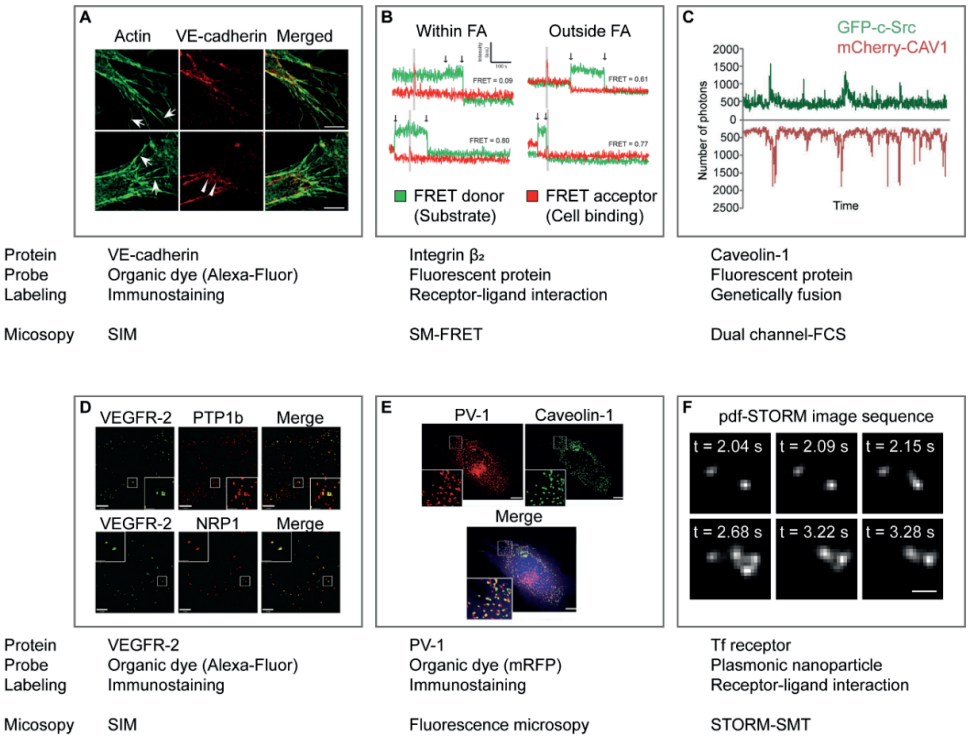

Fig. 3. Representative single-molecule experiments of transmembrane protein involved in endothelial function. (A) SIM images showing a polarized distribution of both VE-cadherin and actin in tip and stalk cells. (B) FRET signal trace within/outside of FA in human foreskin fibroblasts. (C) Single-molecule coincidence analysis of caveolin-1 and c-Src through dual-channel FCS. (D) SIM image revealing the colocalization of VEGFR-2, PTP1, and NRP1 under VEGF stimulation. (E) Fluorescence image showing that the overexpression of PV-1 is colocalized with caveolin-1 to form caveolar diaphragms. (F) STORM image sequences deciphering endosomal dynamics containing Tf-conjugated nanoparticles during intracellular transport. Figures are adapted from the following sources: (A) Cao et al.,[11] CC-BY-4.0; (B) Chang et al.[12] with permission from American Chemical Society; (C) Jung et al.[13] with permission from Rockefeller University Press; (D) Lanahan et al.,[14] CC-BY-3.0; (E) Stan et al.[15] with permission from American Society for Cell Biology; (F) Jin et al.[16] with permission from American Chemical Society.
SIM, structured illumination microscopy; VE, vascular endothelial; FRET, Fluorescence resonance energy transfer; FA, focal adhesion; FCS, fluorescence correlation spectroscopy; VEGFR, vascular endothelial growth factor receptor; PTP1, protein tyrosine phosphatase 1; NRP1, neuropilin 1; VEGF, vascular endothelial growth factor; PV-1, plasmalemmal vesicle associated protein-1; STORM, stochastic optical reconstruction microscopy; Tf, transferrin.

Table 1. Representative optical probes for single-molecule imaging in living cells

Classification	Optical probe	Excitation maximum (nm)	Emission maximum (nm)	Reference
Fluorescent protein	GFP	395 (475)	508	Chalfie et al.[19]
	BFP	381	445	Heim and Tsien[20]
	mOrange	548	562	Shaner et al.[22]
	DsRed	558	583	
	mRFP1	584	607	
	mCherry	587	610	
Organic dye	Fluorescein (FITC)	494	518	Dempsey et al.[23] (Commercially available)
	Atto 520	516	538	
	Cy3	550	570	
	TAMRA	546	575	
	Cy5	649	670	
	Alexa Fluor 647	650	665	
Nanoparticle	Qdot® 525	445	525	Kim and Kim[24] (Commercially available)
	Qdot® 705	445	705	
	Gold nanoparticle (Diameter: 40 nm)	528 (Absorbance)	535 (Scattering)	Jain et al.[25] (Commercially available)

Hell et al. proposed a stimulated emission depletion (STED) microscopy with 35 nm spatial resolution,[33] using a donut-shaped depletion laser that deactivates the fluorescence process in the peripheral regions of the point spread function (PSF). Betzig[34] proposed the basic concept of single-molecule localization microscopy (SMLM), a reconstruction method that

https://doi.org/10.12997/jla.2023.12.1.58

distinguishes individual features within PSF, and demonstrates photoactivated localization microscopy (PALM) with up to 10 nm resolution.[35] Rust et al.[36] reported stochastic optical reconstruction microscopy (STORM) with up to 50 nm resolution. Subsequently, Gustafsson[37] introduced structured illumination microscopy (SIM) with 100 nm resolution, which has advantages in sample preparation and imaging speed. Most microscopes mentioned are marketed and are user-friendly using software and image processing algorithms that are continually in development.

3. Analyzing diffusive dynamics of membrane proteins

Some of the most significant regulatory factors of signal transduction in cell biology in modern systems biology are spatiotemporal and quantitative characteristics, as well as the temporal dependence of biomolecules. Therefore, not only synchronic investigation with high spatial resolution but also accurate temporal resolution is required to understand the regulatory mechanism of protein activation. For this, several imaging techniques, such as fluorescence recovery after photobleaching (FRAP), fluorescence correlation spectroscopy (FCS), and single-molecule tracking (SMT), have been developed to extract dynamic information at the molecular level. In the 1970s, the development of FRAP enabled the detection of protein motility and measurement of the diffusion rate in live cells.[38,39] Although FRAP is still one of the most widely used techniques for measuring molecular diffusion, it provides an average for the number of molecules. FCS was first introduced to analyze diffusive fluctuation of DNA molecules in 1972 and was applied to measure lateral diffusion on lipid membranes during the same decade.[40,41] FCS is a more delicate technique for measuring the temporal fluctuation of emission signals using individual molecules passing through the focal spot. SMT is a direct observation technique of individual molecular motions, thereby facilitating the extraction of dynamic information that cannot be obtained from ensemble data. Automatic tracking algorithms based on video microscopy was first demonstrated to analyze molecular motion in live cells in 1987.[42] In addition to measuring the diffusion rate, additional biophysical analysis, such as analyzing the diffusive state using the hidden Markov model, can extract information such as dimerization, clustering, and conformational changes of proteins.[43-45]

Recent temporal dynamics studies on membrane molecules have been combined with SRM to achieve higher accuracy. Combination of FCS with STED microscopy revealed the existence of nanosized membrane structures by detecting the hindered diffusing states of individual molecules in sub-diffraction limits of detection (30 nm).[46] This technique enabled the measurement of the diffusivity of the viral membrane glycoprotein Env using sub-diffraction-sized virus particles.[47] SMT combined with PALM allowed mapping of molecular motion of the membrane proteins Gag and VSVG with high labeling density.[48]

4. Protein-protein interactions (PPIs)

PPIs, including homo-/hetero-dimerization, ligand–receptor interactions, and protein complex organization, are important for the function of all membrane proteins. Immunoprecipitation is a traditional method which is still widely used to quantify the PPI ensemble average value. In reality, PPI at the single-molecule level can occasionally be transient and time-dependent, therefore, it has recently been measured using various microscopic techniques.

Fluorescence resonance energy transfer (FRET) refers to the non-radiative energy transfer from excited donor molecule to ground state acceptor molecule. Since FRET efficiency is distance-dependent at several nanometers, it can also be used as a spectroscopic ruler to

https://doi.org/10.12997/jla.2023.12.1.58

decipher molecular structures.[49] This method facilitates the monitoring of real-time protein conformation changes in response to various external stimuli. For example, a molecular tension sensor made of an elastic linker between FRET modules can measure the tension placed on the two mechanical sensing proteins.[12,50] Plasmon coupling is a phenomenon that occurs between noble metal nanoparticles and is characterized by the synergistic enhancement of multi-surface plasmon resonance signals. It typically manifests as a distance-dependent phenomenon at zero to tens of nanometers. In particular, long-term tracking of gold nanoparticles using dark-field microscopy allows for the observation of the interaction of membrane proteins, for example, homogeneous association of endothelial growth factor receptor, due to the superior brightness and stability of nanoparticles compared to other fluorescent probes.[45]

IMAGING OF MEMBRANE PROTEINS ON VASCULAR ENDOTHELIAL CELLS AT SINGLE-MOLECULE LEVEL

1. VE-cadherin, a protein that controls cell-to-cell junction for paracellular transport

Vascular permeability controls not only the molecular exchange, but also the infiltration of immune cells, which are regulated by cell adhesion molecules such as cadherins. The extracellular cadherin (EC) domain, which typically comprises 5 EC repeats, forms cell–cell adhesion through calcium-dependent homophilic interactions. The cytoplasmic domain connects to the actin cytoskeleton and regulates cadherin function via its direct association with catenin. Among the cadherin subtypes, vascular endothelial (VE)-cadherin is located at the adherent junction of endothelial cells and regulates vascular permeability.[51] In vivo experiments using anti-VE-cadherin antibodies revealed the crucial role of VE-cadherin in endothelial function.[6,52]

Cadherin forms adherent junctions and mediates outside-in signaling through lateral (cis) dimerization, intermembrane (trans) adhesive interactions, mechanical loading, and actin polymerization. Single-molecule analysis revealed reciprocal interactions between each process and reaction kinetics. Single-molecule FRET experiments showed that cis dimerization and trans interactions are mutually cooperative.[53] This result is also consistent with crosslinking assay results, that showed cis dimerization is a fundamental for cell–cell adhesion.[54] Magnetoplasmonic nanoparticles (MPNs) have been used to localize, visualize, and mechanically activate VE-cadherin at the single-molecule level.[55] This showed that not only local recruitment but also mechanical tension (up to 9 pN) of VE-cadherin are key mechanisms leading to cytoskeleton formation. Single-molecule atomic force microscopy (AFM) has been used to characterize the trans interaction unbinding force (35–55 pN) and binding kinetics (K_D=10^{-3} to 10^{-5} mol/L) of VE-cadherin.[56]

2. Integrin, a protein involved in cell migration and focal adhesion

Integrin is a heterodimeric receptor that mediates cellular adhesion to cells and the extracellular matrix (ECM). These molecules play a critical role in physical connections, as well as in vascular functions such as inflammation, thrombosis, and angiogenesis. Integrin recruits leukocytes to the inflammatory sites through adhesion to the vascular endothelium and transendothelial migration.[57] Integrin, together with the platelet glycoprotein Ibα, plays a significant role in platelet adhesion and aggregation, and is a major molecule involved in thrombosis.[58] Integrin deficient mouse models have shown that integrin plays a key role in angiogenesis and vascularization.[59]

https://doi.org/10.12997/jla.2023.12.1.58

Integrin is linked to the cytoskeleton via adapter proteins, such as talin, kindlin, and vinculin, to form focal adhesions (FAs) and mediate outside-in signaling. Several single-molecule studies have been conducted to study the association of integrins with adapter proteins, which have revealed that integrin exhibits three diffusion states (diffusive, confined, and immobile), and their immobilization has a positive correlation with integrin activation.[60] Most integrins exhibit an immobile state, which refers to the activated states, in FA (69%±2%) and a diffusive state outside of FA. Talin also exhibits an immobile state in FA, but unlike integrin, it has almost no diffusive state outside FA. Quantitative single-molecule colocalization analysis has enabled direct imaging of the spatial coordinates of integrin, talin, and kindlin in a highly crowded FA structure.[61] STORM has revealed that integrin has cis binding to intercellular adhesion molecule (ICAM), which are adhesion molecules expressed on endothelial cells and immune cells.[62] Single-molecule force measurements using a molecular tension sensor that connects both FRET modules with elastic domains showed that the tension received by vinculin in stable FA was up to 2.5 pN and that of integrin heterodimers was 1–7 pN.[12,50]

3. Caveolin, a protein related to endocytosis, transcytosis, and signal transduction

Caveolae, which are 50–100 nm membrane invaginations, play a key role in the regulation of the transcytosis of macromolecules through the vascular endothelium. Caveolin is a membrane protein that mainly contributes to caveola formation and shows high expression levels in endothelial cells.[63] The caveolin subtype, such as caveolin-1 and caveolin-2, form homo-/hetero-oligomers for caveola generation and are located in lipid rafts associated with cholesterol. Caveolin-1 deficient mouse models have shown loss of caveola structure and dysfunction of the vascular function.[64]

Caveola formation is initiated by caveolin oligomerization, followed by cavin recruitment. The invaginated structures are stabilized via actin filament association. SMLM allows the observation of cluster shape (donut-like) and measurement of the size of these nanometer-scale structures (up to 100 nm).[65] This SRM enables visualization of the localization of 2 different nm-spaced constituent molecules: dynamin-2 and caveolin-1. Temporal information regarding the caveolar structure can be obtained via sequential analysis of SMLM and density-based spatial clustering. It revealed the cluster density (0.29±0.12 μm^{-2} min^{-1}), cluster lifetime (τ_1=1.2±0.1 s), and incorporation rate (2.54±0.01 s^{-1}) of the caveolar structure.[66] The coincidence detection technique using 2-color single-molecule spectroscopy can screen molecular interactions through the observation of the co-diffusion between caveolin and other molecules, such as endothelial nitric oxide synthase (eNOS), Fyn, and Src.[13]

4. Tf receptor, a carrier for Fe ions

RMT is a vesicular transport pathway for shuttling a certain large molecule (e.g., insulin, lipoprotein, and Tf) across vascular endothelial cells. TfR, a transmembrane glycoprotein composed of two 90 kDa subunits, mediates the delivery of iron ions from the blood to the brain. The mechanism of iron transport is suggested to involve the specific binding of Tf to TfR, internalization of the TfR-Tf complex, and exocytosis of Tf-bound iron and free iron.[4] It is a unique property that has a high expression level in the brain vascular endothelial cells compared to that in other tissues. Thus, TfR is an efficient target molecule for delivering therapeutic cargo to the brain.[67]

The TfR-Tf complex is internalized from the clathrin-coated pits on the plasma membrane into the endosome. Super-resolution imaging is used to distinguish the shape of the Tf

https://doi.org/10.12997/jla.2023.12.1.58

cluster enclosed by a clathrin coat.[68] The Tf-conjugated gold nanorods were applied to probe endosomal dynamics with a high spatial resolution, thereby allowing the elucidation of efficient searching behavior during intracellular transport.[16] The amount of Tf delivered through specific binding can be quantitatively measured using the FRET-based imaging technique.[69] The anti-TfR antibody was used and visualized using fluorescence microscopy to induce TfR-mediated transcytosis. The accumulation of antibodies is increased in the brain with the decrease in the binding affinity of antibodies, whereas high-affinity antibodies are not delivered due to lower dissociation rates from TfR.[67] Similarly, gold nanoparticles are transported across the vascular endothelial cell depending on the ratio of bound Tf.[70] Consequently, the material transport by the RMT pathway is required to have a proper binding affinity with TfR.

5. PV-1, a protein that forms diaphragm in caveolae, transendothelial channel, and fenestrae

Endothelial subcellular structures, such as the stomatal diaphragm of caveolae, transendothelial channels, and diaphragm of fenestrae, regulate vascular permeability. Structures commonly share a protein named plasmalemma PV-1. PV-1 is a type II membrane glycoprotein with a long extracellular domain (C-terminal) and a short intracellular tail (N-terminal). Additionally, PV-1 forms a homodimer *in situ*, which is shown to localize near the diaphragm.[5] PV-1 homodimers are suggested to form a diaphragm structure (up to 50 nm) with a thin film (up to 5 nm) composed of radial fibrils beginning from the rim (via the N-terminal) and interweaving in a central knob (via the C-terminal).[71] Furthermore, deletion of the PV-1 encoding gene results in the absence of a diaphragm, thus, indicating that PV-1 is key in investigating the permeability in endothelial cells.[72]

Optical fluorescence microscopy was used to elucidate the localization of PV-1 in live cells. In endothelial cells, PV-1 is evenly distributed over the cell surface and colocalizes with related proteins (e.g., caveolin-1, vimentin). The position of PV-1 can be redistributed toward the peripheral area under tumor necrosis factor-α activation in human umbilical vein endothelial cells.[5] Additionally, upregulation and downregulation of PV-1, as observed using the immunofluorescence intensity, affects the cell morphology and newly formed diaphragm. Electron microscopy revealed that the kinetics and magnitude of diaphragm formation changes depending on the endothelial cell type due to the amount of PV-1 on the cell surface. [15,73]

6. VEGFR, one of the best-known factors for controlling permeability

VEGF and VEGFR are key factors in permeability and angiogenesis. VEGFR, composed of seven immunoglobulin-like domains, a single transmembrane domain, and a cytoplasmic tyrosine kinase domain, has three main subtypes: VEGFR-1 (Flt-1), VEGFR-2 (KDR/Flk-1), and VEGFR-3 (Flt-4). Vascular permeability is increased in angiogenesis by the change of adherent and tight junctions, which results in the influx of water and large proteins across the endothelial cell.[74] This process mediates by stimulation of tyrosine phosphorylation of VEGFR-2 and subsequent downstream signaling of the PI-3 kinase-PKB, NOS, and MAP-kinase pathways.[75] Moreover, the deletion of VEGFR-2 in mice results in a lack of vascular development and death in early embryos. Thus, VEGFR is essential to control the formation of new vessels and permeability.[76]

VEGFR dimerization induces phosphorylation of tyrosine kinase residues. Single-molecule tracking is used to observe the movement of VEGFR under VEGF stimulation, thereby changing the mobility from mobile (monomeric state) to restricted motion (non-monomeric

https://doi.org/10.12997/jla.2023.12.1.58

state).[77] The FRET imaging technique was applied to visualize the homo- (VEGFR-1) and hetero-dimerization (VEGFR-1 and VEGFR-2) in live cells.[78] Additionally, AFM was used to observe the distribution of VEGFR using adhesion force mapping, which revealed a slightly higher density of receptors in the native cell periphery.[79] Furthermore, SIM was used to distinguish the colocalization of VEGFR with related proteins (e.g., neuropilin 1 [NRP1], protein tyrosine phosphatase 1B [PTP1B]) for signal transduction.[14] The reduction of vascular permeability was observed in mice upon the elimination of the signaling regulatory protein.[80] Consequently, the permeability of endothelial cells was controlled through the alteration of the adherent junction under the activation of VEGFR signaling.

7. Effects of physical properties of lipid membrane on the membrane molecule function

Membrane proteins are introduced during diffusion on the plasma membrane and 2D lipid bilayer and constantly interact with various membrane components. The molecular composition of the membrane affects biophysical properties, including fluidity and phase separation, which in turn affect the molecular motion of the membrane constituents. Membrane fluidity can be controlled by the lipid composition and is mainly measured using molecular diffusion (rotational diffusion; molecular rotors, translational diffusion; FRAP, FCS, and SMT). Budin et al.[81] showed how membrane viscosity affects the diffusion of ubiquinone, which functions in the electron transport chain in the plasma membranes and cellular respiration of *Escherichia coli*. Membrane heterogeneity, referred to as the lipid raft hypothesis, also affects protein activation via spatial segregation of interacting species. Huang et al.[82] showed that phase separation of LAT, a membrane scaffold protein, plays a crucial role in downstream Ras signal activation. Lipid phase separation is also determined by membrane composition and has been confirmed using detergent resistance assays or NMR.[83,84] Recent advances in SRM and molecular diffusion measurement techniques have enabled the characterization of phase-separated structures at high spatio-temporal resolution.

CONCLUSION

Membrane proteins found in vascular cells are essential for angiogenesis, the selective transport of different substances, and immune cell infiltration. They function by forming dynamic protein complexes through PPIs while diffusing across the cell membrane. The focus of present biological and medical research, however, is on the relationship between quantitative expression and phenotype of membrane proteins as measured by the ensemble average. Nevertheless, recent developments in laser, microscopy, spectroscopy,[85] and computing technologies that can process imaging data may enhance our comprehension of the molecular and biological mechanisms of membrane proteins. Imaging technology provides not only a high level of spatial resolution but also temporal information about living cells, which can be used as a biomarker for disturbed cell functions. In this regard, knowledge of the proteins involved in substance transport in blood vessels will contribute to new avenues for drug discovery and aid in the development of drug delivery systems. High-resolution imaging technologies will also likely be used for drug evaluation and screening.

This review provides a general overview of high-resolution microscopy techniques and suggests that these techniques can aid in the understanding the activation mechanisms of proteins, particularly those of pathologically significant proteins.

https://doi.org/10.12997/jla.2023.12.1.58

REFERENCES

1. Hidalgo IJ, Hillgren KM, Grass GM, Borchardt RT. Characterization of the unstirred water layer in Caco-2 cell monolayers using a novel diffusion apparatus. Pharm Res 1991;8:222-227.
PUBMED | CROSSREF
2. Shin S, Choe J, Park Y, Jeong D, Song H, You Y, et al. Artificial control of cell signaling using a photocleavable cobalt(III)-nitrosyl complex. Angew Chem Int Ed Engl 2019;58:10126-10131.
PUBMED | CROSSREF
3. Schnitzer JE, Oh P, Pinney E, Allard J. Filipin-sensitive caveolae-mediated transport in endothelium: reduced transcytosis, scavenger endocytosis, and capillary permeability of select macromolecules. J Cell Biol 1994;127:1217-1232.
PUBMED | CROSSREF
4. Johnsen KB, Burkhart A, Melander F, Kempen PJ, Vejlebo JB, Siupka P, et al. Targeting transferrin receptors at the blood-brain barrier improves the uptake of immunoliposomes and subsequent cargo transport into the brain parenchyma. Sci Rep 2017;7:10396.
PUBMED | CROSSREF
5. Keuschnigg J, Henttinen T, Auvinen K, Karikoski M, Salmi M, Jalkanen S. The prototype endothelial marker PAL-E is a leukocyte trafficking molecule. Blood 2009;114:478-484.
PUBMED | CROSSREF
6. Corada M, Mariotti M, Thurston G, Smith K, Kunkel R, Brockhaus M, et al. Vascular endothelial-cadherin is an important determinant of microvascular integrity *in vivo*. Proc Natl Acad Sci U S A 1999;96:9815-9820.
PUBMED | CROSSREF
7. Peng HB, Libby P, Liao JK. Induction and stabilization of I κ B α by nitric oxide mediates inhibition of NF-κ B. J Biol Chem 1995;270:14214-14219.
PUBMED | CROSSREF
8. Yellin MJ, Brett J, Baum D, Matsushima A, Szabolcs M, Stern D, et al. Functional interactions of T cells with endothelial cells: the role of CD40L-CD40-mediated signals. J Exp Med 1995;182:1857-1864.
PUBMED | CROSSREF
9. Le Guelte A, Dwyer J, Gavard J. Jumping the barrier: VE-cadherin, VEGF and other angiogenic modifiers in cancer. Biol Cell 2011;103:593-605.
PUBMED | CROSSREF
10. Gupta Y, Jain A, Jain SK. Transferrin-conjugated solid lipid nanoparticles for enhanced delivery of quinine dihydrochloride to the brain. J Pharm Pharmacol 2007;59:935-940.
PUBMED | CROSSREF
11. Cao J, Ehling M, März S, Seebach J, Tarbashevich K, Sixta T, et al. Polarized actin and VE-cadherin dynamics regulate junctional remodelling and cell migration during sprouting angiogenesis. Nat Commun 2017;8:2210.
PUBMED | CROSSREF
12. Chang AC, Mekhdjian AH, Morimatsu M, Denisin AK, Pruitt BL, Dunn AR. Single molecule force measurements in living cells reveal a minimally tensioned integrin state. ACS Nano 2016;10:10745-10752.
PUBMED | CROSSREF
13. Jung W, Sierecki E, Bastiani M, O'Carroll A, Alexandrov K, Rae J, et al. Cell-free formation and interactome analysis of caveolae. J Cell Biol 2018;217:2141-2165.
PUBMED | CROSSREF
14. Lanahan A, Zhang X, Fantin A, Zhuang Z, Rivera-Molina F, Speichinger K, et al. The neuropilin 1 cytoplasmic domain is required for VEGF-A-dependent arteriogenesis. Dev Cell 2013;25:156-168.
PUBMED | CROSSREF
15. Stan RV, Tkachenko E, Niesman IR. PV1 is a key structural component for the formation of the stomatal and fenestral diaphragms. Mol Biol Cell 2004;15:3615-3630.
PUBMED | CROSSREF
16. Jin S, Park J, Lee WJ, Ahn Y, Park Y, Park M, et al. Two GPSes in a ball: deciphering the endosomal tug-of-war using plasmonic dark-field STORM. JACS Au 2022;2:1596-1603.
PUBMED | CROSSREF
17. Coons AH, Creech HJ, Jones RN, Berliner E. The demonstration of pneumococcal antigen in tissues by the use of fluorescent antibody. J Immunol 1942;45:159-170.
18. Prasher DC, Eckenrode VK, Ward WW, Prendergast FG, Cormier MJ. Primary structure of the *Aequorea victoria* green-fluorescent protein. Gene 1992;111:229-233.
PUBMED | CROSSREF

19. Chalfie M, Tu Y, Euskirchen G, Ward WW, Prasher DC. Green fluorescent protein as a marker for gene expression. Science 1994;263:802-805.
PUBMED | CROSSREF
20. Heim R, Tsien RY. Engineering green fluorescent protein for improved brightness, longer wavelengths and fluorescence resonance energy transfer. Curr Biol 1996;6:178-182.
PUBMED | CROSSREF
21. Matz MV, Fradkov AF, Labas YA, Savitsky AP, Zaraisky AG, Markelov ML, et al. Fluorescent proteins from nonbioluminescent Anthozoa species. Nat Biotechnol 1999;17:969-973.
PUBMED | CROSSREF
22. Shaner NC, Campbell RE, Steinbach PA, Giepmans BN, Palmer AE, Tsien RY. Improved monomeric red, orange and yellow fluorescent proteins derived from *Discosoma* sp. red fluorescent protein. Nat Biotechnol 2004;22:1567-1572.
PUBMED | CROSSREF
23. Dempsey GT, Vaughan JC, Chen KH, Bates M, Zhuang X. Evaluation of fluorophores for optimal performance in localization-based super-resolution imaging. Nat Methods 2011;8:1027-1036.
PUBMED | CROSSREF
24. Kim GB, Kim YP. Analysis of protease activity using quantum dots and resonance energy transfer. Theranostics 2012;2:127-138.
PUBMED | CROSSREF
25. Jain PK, Lee KS, El-Sayed IH, El-Sayed MA. Calculated absorption and scattering properties of gold nanoparticles of different size, shape, and composition: applications in biological imaging and biomedicine. J Phys Chem B 2006;110:7238-7248.
PUBMED | CROSSREF
26. Alivisatos P. The use of nanocrystals in biological detection. Nat Biotechnol 2004;22:47-52.
PUBMED | CROSSREF
27. Farlow J, Seo D, Broaders KE, Taylor MJ, Gartner ZJ, Jun YW. Formation of targeted monovalent quantum dots by steric exclusion. Nat Methods 2013;10:1203-1205.
PUBMED | CROSSREF
28. Kim JW, Seo D, Lee JU, Southard KM, Lim Y, Kim D, et al. Single-cell mechanogenetics using monovalent magnetoplasmonic nanoparticles. Nat Protoc 2017;12:1871-1889.
PUBMED | CROSSREF
29. Abbe E. Beiträge zur theorie des mikroskops und der mikroskopischen wahrnehmung. Arch Mikrosk Anat 1873;9:413-468.
CROSSREF
30. Heimstädt O. Das fluoreszenzmikroskop. Z Wiss Mikrosk 1911;28:330-337.
31. Ellinger P, Hirt A. Mikroskopische beobachtungen an lebenden organen mit demonstrationen (intravitalmikroskopie). Naunyn Schmiedebergs Arch Pharmacol 1929;147:63.
CROSSREF
32. Ambrose EJ. A surface contact microscope for the study of cell movements. Nature 1956;178:1194.
PUBMED | CROSSREF
33. Hell SW, Wichmann J. Breaking the diffraction resolution limit by stimulated emission: stimulated-emission-depletion fluorescence microscopy. Opt Lett 1994;19:780-782.
PUBMED | CROSSREF
34. Betzig E. Proposed method for molecular optical imaging. Opt Lett 1995;20:237-239.
PUBMED | CROSSREF
35. Betzig E, Patterson GH, Sougrat R, Lindwasser OW, Olenych S, Bonifacino JS, et al. Imaging intracellular fluorescent proteins at nanometer resolution. Science 2006;313:1642-1645.
PUBMED | CROSSREF
36. Rust MJ, Bates M, Zhuang X. Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM). Nat Methods 2006;3:793-795.
PUBMED | CROSSREF
37. Gustafsson MG. Surpassing the lateral resolution limit by a factor of two using structured illumination microscopy. J Microsc 2000;198:82-87.
PUBMED | CROSSREF
38. Liebman PA, Entine G. Lateral diffusion of visual pigment in photorecptor disk membranes. Science 1974;185:457-459.
PUBMED | CROSSREF

39. Axelrod D, Ravdin P, Koppel DE, Schlessinger J, Webb WW, Elson EL, et al. Lateral motion of fluorescently labeled acetylcholine receptors in membranes of developing muscle fibers. Proc Natl Acad Sci U S A 1976;73:4594-4598.
PUBMED | CROSSREF

40. Magde D, Elson E, Webb WW. Thermodynamic fluctuations in a reacting system-measurement by fluorescence correlation spectroscopy. Phys Rev Lett 1972;29:705-708.
CROSSREF

41. Fahey PF, Koppel DE, Barak LS, Wolf DE, Elson EL, Webb WW. Lateral diffusion in planar lipid bilayers. Science 1977;195:305-306.
PUBMED | CROSSREF

42. Geerts H, De Brabander M, Nuydens R, Geuens S, Moeremans M, De Mey J, et al. Nanovid tracking: a new automatic method for the study of mobility in living cells based on colloidal gold and video microscopy. Biophys J 1987;52:775-782.
PUBMED | CROSSREF

43. Chung I, Akita R, Vandlen R, Toomre D, Schlessinger J, Mellman I. Spatial control of EGF receptor activation by reversible dimerization on living cells. Nature 2010;464:783-787.
PUBMED | CROSSREF

44. Sungkaworn T, Jobin ML, Burnecki K, Weron A, Lohse MJ, Calebiro D. Single-molecule imaging reveals receptor-G protein interactions at cell surface hot spots. Nature 2017;550:543-547.
PUBMED | CROSSREF

45. Park Y, Shin S, Jin H, Park J, Hong Y, Choi J, et al. Single-molecule rotation for EGFR conformational dynamics in live cells. J Am Chem Soc 2018;140:15161-15165.
PUBMED | CROSSREF

46. Eggeling C, Ringemann C, Medda R, Schwarzmann G, Sandhoff K, Polyakova S, et al. Direct observation of the nanoscale dynamics of membrane lipids in a living cell. Nature 2009;457:1159-1162.
PUBMED | CROSSREF

47. Chojnacki J, Waithe D, Carravilla P, Huarte N, Galiani S, Enderlein J, et al. Envelope glycoprotein mobility on HIV-1 particles depends on the virus maturation state. Nat Commun 2017;8:545.
PUBMED | CROSSREF

48. Manley S, Gillette JM, Patterson GH, Shroff H, Hess HF, Betzig E, et al. High-density mapping of single-molecule trajectories with photoactivated localization microscopy. Nat Methods 2008;5:155-157.
PUBMED | CROSSREF

49. Stryer L, Haugland RP. Energy transfer: a spectroscopic ruler. Proc Natl Acad Sci U S A 1967;58:719-726.
PUBMED | CROSSREF

50. Grashoff C, Hoffman BD, Brenner MD, Zhou R, Parsons M, Yang MT, et al. Measuring mechanical tension across vinculin reveals regulation of focal adhesion dynamics. Nature 2010;466:263-266.
PUBMED | CROSSREF

51. Lampugnani MG, Resnati M, Raiteri M, Pigott R, Pisacane A, Houen G, et al. A novel endothelial-specific membrane protein is a marker of cell-cell contacts. J Cell Biol 1992;118:1511-1522.
PUBMED | CROSSREF

52. Gotsch U, Borges E, Bosse R, Böggemeyer E, Simon M, Mossmann H, et al. VE-cadherin antibody accelerates neutrophil recruitment *in vivo*. J Cell Sci 1997;110:583-588.
PUBMED | CROSSREF

53. Thompson CJ, Vu VH, Leckband DE, Schwartz DK. Cadherin cis and trans interactions are mutually cooperative. Proc Natl Acad Sci U S A 2021;118:e2019845118.
PUBMED | CROSSREF

54. Takeda H, Shimoyama Y, Nagafuchi A, Hirohashi S. E-cadherin functions as a cis-dimer at the cell-cell adhesive interface *in vivo*. Nat Struct Biol 1999;6:310-312.
PUBMED | CROSSREF

55. Seo D, Southard KM, Kim JW, Lee HJ, Farlow J, Lee JU, et al. A mechanogenetic toolkit for interrogating cell signaling in space and time. Cell 2016;165:1507-1518.
PUBMED | CROSSREF

56. Baumgartner W, Hinterdorfer P, Ness W, Raab A, Vestweber D, Schindler H, et al. Cadherin interaction probed by atomic force microscopy. Proc Natl Acad Sci U S A 2000;97:4005-4010.
PUBMED | CROSSREF

57. von Andrian UH, Chambers JD, McEvoy LM, Bargatze RF, Arfors KE, Butcher EC. Two-step model of leukocyte-endothelial cell interaction in inflammation: distinct roles for LECAM-1 and the leukocyte β 2 integrins *in vivo*. Proc Natl Acad Sci U S A 1991;88:7538-7542.
PUBMED | CROSSREF

58. Monti M, Iommelli F, De Rosa V, Carriero MV, Miceli R, Camerlingo R, et al. Integrin-dependent cell adhesion to neutrophil extracellular traps through engagement of fibronectin in neutrophil-like cells. PLoS One 2017;12:e0171362.
PUBMED | CROSSREF

59. Madamanchi A, Capozzi M, Geng L, Li Z, Friedman RD, Dickeson SK, et al. Mitigation of oxygen-induced retinopathy in α2β1 integrin-deficient mice. Invest Ophthalmol Vis Sci 2014;55:4338-4347.
PUBMED | CROSSREF

60. Rossier O, Octeau V, Sibarita JB, Leduc C, Tessier B, Nair D, et al. Integrins β1 and β3 exhibit distinct dynamic nanoscale organizations inside focal adhesions. Nat Cell Biol 2012;14:1057-1067.
PUBMED | CROSSREF

61. Fischer LS, Klingner C, Schlichthaerle T, Strauss MT, Böttcher R, Fässler R, et al. Quantitative single-protein imaging reveals molecular complex formation of integrin, talin, and kindlin during cell adhesion. Nat Commun 2021;12:919.
PUBMED | CROSSREF

62. Fan Z, Kiosses WB, Sun H, Orecchioni M, Ghosheh Y, Zajonc DM, et al. High-affinity bent β_2-integrin molecules in arresting neutrophils face each other through binding to ICAMs in *cis*. Cell Reports 2019;26:119-130.e5.
PUBMED | CROSSREF

63. Lisanti MP, Scherer PE, Vidugiriene J, Tang Z, Hermanowski-Vosatka A, Tu YH, et al. Characterization of caveolin-rich membrane domains isolated from an endothelial-rich source: implications for human disease. J Cell Biol 1994;126:111-126.
PUBMED | CROSSREF

64. Drab M, Verkade P, Elger M, Kasper M, Lohn M, Lauterbach B, et al. Loss of caveolae, vascular dysfunction, and pulmonary defects in caveolin-1 gene-disrupted mice. Science 2001;293:2449-2452.
PUBMED | CROSSREF

65. Platonova E, Winterflood CM, Junemann A, Albrecht D, Faix J, Ewers H. Single-molecule microscopy of molecules tagged with GFP or RFP derivatives in mammalian cells using nanobody binders. Methods 2015;88:89-97.
PUBMED | CROSSREF

66. Ma R, Štefl M, Nienhaus GU. Single molecule localization-based analysis of clathrin-coated pit and caveolar dynamics. Nanoscale Horiz 2022;7:385-395.
PUBMED | CROSSREF

67. Yu YJ, Zhang Y, Kenrick M, Hoyte K, Luk W, Lu Y, et al. Boosting brain uptake of a therapeutic antibody by reducing its affinity for a transcytosis target. Sci Transl Med 2011;3:84ra44.
PUBMED | CROSSREF

68. Jones SA, Shim SH, He J, Zhuang X. Fast, three-dimensional super-resolution imaging of live cells. Nat Methods 2011;8:499-508.
PUBMED | CROSSREF

69. Abe K, Zhao L, Periasamy A, Intes X, Barroso M. Non-invasive *in vivo* imaging of near infrared-labeled transferrin in breast cancer cells and tumors using fluorescence lifetime FRET. PLoS One 2013;8:e80269.
PUBMED | CROSSREF

70. Wiley DT, Webster P, Gale A, Davis ME. Transcytosis and brain uptake of transferrin-containing nanoparticles by tuning avidity to transferrin receptor. Proc Natl Acad Sci U S A 2013;110:8662-8667.
PUBMED | CROSSREF

71. Stan RV. Endothelial stomatal and fenestral diaphragms in normal vessels and angiogenesis. J Cell Mol Med 2007;11:621-643.
PUBMED | CROSSREF

72. Stan RV, Tse D, Deharvengt SJ, Smits NC, Xu Y, Luciano MR, et al. The diaphragms of fenestrated endothelia: gatekeepers of vascular permeability and blood composition. Dev Cell 2012;23:1203-1218.
PUBMED | CROSSREF

73. Auvinen K, Lokka E, Mokkala E, Jäppinen N, Tyystjärvi S, Saine H, et al. Fenestral diaphragms and PLVAP associations in liver sinusoidal endothelial cells are developmentally regulated. Sci Rep 2019;9:15698.
PUBMED | CROSSREF

74. Simons M, Gordon E, Claesson-Welsh L. Mechanisms and regulation of endothelial VEGF receptor signalling. Nat Rev Mol Cell Biol 2016;17:611-625.
PUBMED | CROSSREF

75. Lal BK, Varma S, Pappas PJ, Hobson RW 2nd, Durán WN. VEGF increases permeability of the endothelial cell monolayer by activation of PKB/akt, endothelial nitric-oxide synthase, and MAP kinase pathways. Microvasc Res 2001;62:252-262.
PUBMED | CROSSREF

76. Shalaby F, Rossant J, Yamaguchi TP, Gertsenstein M, Wu XF, Breitman ML, et al. Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. Nature 1995;376:62-66.
PUBMED | CROSSREF

77. da Rocha-Azevedo B, Lee S, Dasgupta A, Vega AR, de Oliveira LR, Kim T, et al. Heterogeneity in VEGF receptor-2 mobility and organization on the endothelial cell surface leads to diverse models of activation by VEGF. Cell Reports 2020;32:108187.
PUBMED | CROSSREF

78. King C, Hristova K. Direct measurements of VEGF-VEGFR2 binding affinities reveal the coupling between ligand binding and receptor dimerization. J Biol Chem 2019;294:9064-9075.
PUBMED | CROSSREF

79. Yang Z, Jiang S, Li F, Qiu Y, Gu J, Pettigrew RI, et al. Single-molecule force measurement guides the design of multivalent ligands with picomolar affinity. Angew Chem Int Ed Engl 2019;58:5272-5276.
PUBMED | CROSSREF

80. Lakshmikanthan S, Sobczak M, Li Calzi S, Shaw L, Grant MB, Chrzanowska-Wodnicka M. Rap1B promotes VEGF-induced endothelial permeability and is required for dynamic regulation of the endothelial barrier. J Cell Sci 2018;131:jcs207605.
PUBMED | CROSSREF

81. Budin I, de Rond T, Chen Y, Chan LJ, Petzold CJ, Keasling JD. Viscous control of cellular respiration by membrane lipid composition. Science 2018;362:1186-1189.
PUBMED | CROSSREF

82. Huang WY, Alvarez S, Kondo Y, Lee YK, Chung JK, Lam HY, et al. A molecular assembly phase transition and kinetic proofreading modulate Ras activation by SOS. Science 2019;363:1098-1103.
PUBMED | CROSSREF

83. Yu J, Fischman DA, Steck TL. Selective solubilization of proteins and phospholipids from red blood cell membranes by nonionic detergents. J Supramol Struct 1973;1:233-248.
PUBMED | CROSSREF

84. Schmidt ML, Ziani L, Boudreau M, Davis JH. Phase equilibria in DOPC/DPPC: conversion from gel to subgel in two component mixtures. J Chem Phys 2009;131:175103.
PUBMED | CROSSREF

85. Jeong D, Kim D. Super-resolution fluorescence microscopy-based single-molecule spectroscopy. Bull Korean Chem Soc 2021;43:316-327.
CROSSREF