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BIOGRAPHICAL SKETCH

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NAME: Walther Mothes

eRA COMMONS USER NAME (credential, e.g., agency login): WMOTHES

POSITION TITLE: Associate Professor

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)

| INSTITUTION AND LOCATION | DEGREE(if applicable) | Completion DateMM/YYYY | FIELD OF STUDY |
| --- | --- | --- | --- |
| Humboldt University, Berlin, Germany | Diploma | 1993 | Chemistry |
| Humboldt University, Berlin, Germany | Ph.D. | 1998 | Cell Biology |
| Harvard Medical School, Boston, MA | Postdoc | 2001 | Virology |
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**A. Personal Statement**

Our laboratory is interested in various aspects of viral spread and pathogenesis of HIV-1 and other retroviruses. Retroviruses can efficiently spread from cell to cell through contact zones, called virological and infectious synapses. Our laboratory has contributed to the understanding of this process by directly visualizing how cell-cell contracts between infected and uninfected cells form, virus assembly is directed towards cell-cell contact sites and viruses are actively transferred to infect neighboring cells. A major current interest of our laboratory is to monitor viral spread and aspects of retroviral pathogenesis directly in living animals using multi-photon laser scanning microscopy. We are also applying single molecule imaging to understand how conformational events in the HIV-1 envelope protein lead to fusion between viral and cellular membranes. A detailed understanding of these processes will permit the rational design of vaccines and antiviral therapies that prevent virus spreading and the infection of new cells.

1. Sherer NM, Lehmann NM, Jimenez-Soto LF, Horensavitz C, Pypert M, and Mothes W. (2007).

Retroviruses can establish filopodial bridges for efficient cell-to-cell transmission. Nat Cell Biol., 9(3):310-

 315. PMC2628976

2. Jin J, Sherer NM, Heidecker G, Derse D, and Mothes W. (2009). Assembly of the murine leukemia virus

 is directed towards sites of cell-cell contact. PLoS Biology, 7(7):e1000163. PMC2709449

3. SewaldX, LadinskyMS, UchilPD, BeloorJ, PiR, HerrmannC, MotamediN, MurookaTT, BrehmMA,

 GreinerDL, ShultzLD, MempelTR, Bjorkman PJ, Kumar P, Mothes W. (2015). Retroviruses use CD169-

mediated trans-infection of permissive lymphocytes to establish infection. Science, Oct 30;350(6260):563-7.4.

4. Munro JB, Gorman J, Ma X, Zhou Z, Arthos J, Burton DR, Koff WC, Courter JR, Smith III AB, Kwong PD,

 Blanchard SC & Mothes W. (2014). Conformational dynamics of single HIV-1 envelope trimers on the

 surface of native virions. Science 346(6210):759-63.

**B. Positions and Honors**

January 1995-May 1997, Boehringer Ingelheim Fonds Fellowship, Harvard Medical School

August 1998-July 2001, Postdoctoral Fellow of the Jane Coffin Childs Memorial Fund for Medical Research,

Harvard Medical School and Brigham & Women’s Hospital

October 2001-2007, Assistant Professor of Microbial Pathogenesis, Yale University School of Medicine

March 2007-2011, Associate Professor of Microbial Pathogenesis, Yale University School of Medicine

July 2011-present, Associate Professor with tenure, Yale University School of Medicine

Awards: 2003 Searle Scholar; 2002 Hellman Family Fellowship; Keynote Speaker, West coast Retrovirus Meeting, Palm Springs 2012; David Derse Memorial Lecture and Award 2014, NCI.

**C. Contributions to Science**

**1. Molecular mechanisms of biomolecular machines. I received interdisciplinary training in chemistry (Diploma 1993), cell biology (Ph.D. 1998) and retrovirology (Postdoc 2001). My thesis was devoted to the understanding of the molecular mechanisms of protein secretion and membrane protein integration at the endoplasmic reticulum (ER). My work demonstrated that Sec61 forms a channel in the ER membrane for secretory proteins and opens laterally to release hydrophobic membrane anchors into the lipid bilayer. During my postdoc I applied cell biological methods to reveal that the avian leukosis virus enters cells by receptor priming and low pH triggering. In my own laboratory, I applied imaging technologies to directly visualize individual steps of the retroviral life cycle in living cells, and more recently in living animals. The single molecule imaging of the HIV envelope glycoprotein recently established in my laboratory has allowed me to combine my personal fascination with imaging with my scientific roots in the understanding of the molecular mechanism of biomolecular machines.**

a. **Mothes W**, Prehn S, and Rapoport TA. (1994). Systematic probing of the environment of a translocating

 secretory protein during translocation through the ER membrane. **EMBO J**. 13, 3973-3982.

b. **Mothes W**, Heinrich SU, Graf R, Nilsson I, von Heijne G, Brunner J, and Rapoport TA. (1997).

 Molecular mechanism of membrane protein integration into the endoplasmic reticulum. **Cell** 89, 523-533.

c. Heinrich SU, **Mothes W**, Brunner J, and Rapoport TA. (2000). The Sec61p complex mediates the

 integration of a membrane protein by allowing lipid partitioning of the transmembrane domain. **Cell**, 102,

 233-244.

d. **Mothes W**, Boerger AL, Narayan S, Cunningham JM, and Young JAT. (2000). Retroviral entry

 mediated by receptor priming and low pH triggering of an envelope glycoprotein. **Cell**, 103, 679-689.

**2. Retrovirus cell-to-cell transmission.** Retroviruses can efficiently spread from cell to cell through contact zones, called virological and infectious synapses. Our laboratory has contributed to the understanding of this process using the murine leukemia virus (MLV) as a model retrovirus. Generating fluorescently labeled MLV viruses we directly visualized how cell-cell contracts between infected and uninfected cells form, virus assembly is directed towards cell-cell contact sites and viruses are actively transferred to infect neighboring cells. Our time-lapse videos in living cells directly documented how viruses walk from one cell to another. It also revealed a novel process, by which infected cells, expressing viral envelope glycoproteins (Env), capture membranes from neighboring cells expressing receptor to establish filopodial bridges. Viruses then move along the outer surface of the filopodial bridge toward target cells. As such, our work demonstrated that “virus surfing” along filopodia, discovered in our laboratory earlier, is the underlying mechanism for directional spreading of viral infections. Applying 4D imaging to quantify sequential assembly, release, and transmission of individual MLV particles in living cells, we then documented that *de novo* assembly is highly polarized towards zones of cell-cell contact. Our work demonstrated that the infected cell can specifically “sense” the uninfected neighboring cell due to Env and receptor interactions and redirect virus assembly towards the site of cell-cell contact. Our work described a novel role for Env in establishing cell-cell adhesion and polarization of assembly prior to becoming a fusion protein to allow virus entry into cells.

a. Lehmann MJ, Sherer NM, Marks CB, Pypaert M., and **Mothes W**. (2005). Actin- and myosin-driven lateral

movement of viruses along filopodia precedes their entry into cells. **J. Cell Biol.** 170(2), 317-325.

PMC2171413

b. Sherer NM, Lehmann MJ, Jimenez-Soto LF, Horensavitz C, Pypert M, and **Mothes W**. (2007).

Retroviruses can establish filopodial bridges for efficient cell-to-cell transmission. **Nat Cell Biol**., 9(3):310-

 315. PMC2628976

c. Jin J, Sherer NM, Heidecker G, Derse D, and **Mothes W**. (2009). Assembly of the murine leukemia virus

 is directed towards sites of cell-cell contact. **PLoS Biology**, 7(7):e1000163. PMC2709449

d. Li F, Jin J, Herrmann C, and **Mothes W**. Basic residues in the matrix domain and Gag multimerization

target murine leukemia virus Gag to the virological synapse. (2013) **J. Virol.** 87(12):7113-26.

PMC3676119

**3. Visualizing retroviral spread and pathogenesis in living animals. To what extent studies on retroviral spread in tissue culture cells are relevant for the spread of viruses in living organisms has remained unknown. To address this important question, we transferred our fluorescently labeled MLV capsid and Env proteins into the backbone of full-length clones and visualized the behavior of infected primary lymphocytes in the popliteal lymph node of living mice. We first performed a visual screen to identify cell types that were able to form virological synapses within lymph nodes. We identified MLV-infected B cells to be capable of forming long-lived contacts with uninfected B and T cells.** The polarization of Gag-GFP to one side of the cell was entirely dependent on the expression of the viral Env protein. Thus, these in vivo structures exhibited the very same Env-dependent polarization of viral capsid previously described for in vitro virological synapses and thus represent the first documentation of virological synapses in vivo*.* We subsequently visualized the early events when MLV enters an animal through either the lymph or blood. We found that MLV is first captured by subcapsular sinus or metallophillic macrophages. Retrovirus capture was mediated by an interaction between gangliosides on virus particles and Siglec 1/CD169 expressed on macrophages. MLV-laden macrophages then formed long-lived synaptic contacts to trans-infect a specific subset of B cells known as B-1a cells. Infected B-1a cells subsequently migrate to the inter-follicular space to spread the infection to susceptible lymphocytes through virological synapses. Efficient infection of mice required CD169, suggesting that retroviruses utilize a combination of fluid-based movement followed by CD169-dependent trans-infection of permissive lymphocytes to spread in vivo. Our work illustrates the potential of in vivo studies. First, we demonstrate that concepts, developed entirely in vitro to explain efficient retroviral spread, such as trans-infection and virological synapses, exist in vivo. Second, CD169/Siglec-1 and not DC-SIGN is the lectin that recognizes and captures retroviruses for trans-infection. Third, the cell types involved in the specific tissue context are all different from in vitro expectations. Macrophages and not dendritic cells capture retroviruses, and MLV initially infects B-1 and not conventional B-2 cells. This work illustrates the importance of in vivo studies and we are currently expanding our in vivo imaging to study HIV spread and pathogenesis in humanized mice.

1. Sewald X, Gonzalez DG, Haberman AM, and **Mothes, W**. (2012). In vivo imaging of virological synapses. **Nat Commun**. 3:1320. doi: 10.1038/ncomms2338. PMC3784984
2. SewaldX, LadinskyMS, UchilPD, BeloorJ, PiR, HerrmannC, MotamediN, MurookaTT, BrehmMA,

 GreinerDL, ShultzLD, MempelTR, Bjorkman PJ, Kumar P, **Mothes W**. (2015). Retroviruses use CD169-

mediated trans-infection of permissive lymphocytes to establish infection. **Science**, online Oct 2, doi/10.1126/science.aab2749

**4. Single molecule imaging of HIV entry.** Recent progress in the structural understanding of the HIV-1 Env trimer have provided static images of the pre-fusion conformation and post-fusion conformation of HIV Env. However, direct visualization of the conformational transitions, characterization of structural intermediates that lead to membrane fusion have been lacking. To provide insights into the dynamics of the native trimer, we have established single molecule technologies to measure the conformational changes of individual Env molecule in the context of a native trimer on the surface of intact virions. Single molecule imaging is inherently mechanistic as it describes the behavior of hundreds or thousands of single molecules free from assemble averaging, can identify non-accumulating intermediates and thus describes with great detail the molecular mechanism by which biomolecular machines function. The introduction of a pair of donor and acceptor fluorophores into the gp120 subunit allowed the direct observation of conformational changes through time-dependent changes in the efficiency of fluorescence resonance energy transfer (FRET). Our studies revealed that the unliganded HIV-1 Env is intrinsically dynamic, transitioning between three distinct prefusion conformations, whose relative occupancies were remodeled by receptor CD4 and antibody binding. Thus, gp120 exhibited physical properties that typify dynamic enzymes, whose functions are regulated through the modulation of intrinsically accessible conformations. Our analysis also directly reveals molecular and temporal events in gp120 that underlie the two-step activation of HIV-1 Env by CD4 and coreceptor through a necessary structural intermediate. Current work concentrates on the next important goal, to directly visualize the conformational events within gp41 that lead to mixing between viral and cellular membranes.

a. Munro JB, Gorman J, Ma X, Zhou Z, Arthos J, Burton DR, Koff WC, Courter JR, Smith III AB, Kwong PD,

 Blanchard SC & **Mothes W**. (2014). Conformational dynamics of single HIV-1 envelope trimers on the surface

 of native virions. **Science** 346(6210):759-63. PMC4304640

b. Pancera M, Zhou T, Druz A, Georgiev IS, Soto S, Gorman J, Huang J, Acharya P, Chuang Y, Ofek G,

 Stewart-Jones G, Stuckey J, Bailer RT, Joyce MG, Louder MK, Tumba N, Yang Y, Zhang B, Cohen MS,

 Haynes BF, Mascola JR, Morris L, Munro JB, Blanchard SC, **Mothes W**, Connors M, and Kwong PD.

(2014). Structure and immune recognition of trimeric prefusion HIV-1 Env. **Nature** 514(7523):455-61. PMC4348022

c. Munro JB, **Mothes W**. (2015). Structure and dynamics of the native HIV-1 Env trimer. **J. Virol**.

89(11):5752-5755. PMC in progress.

**d.** Patent: PCT/US13/42249 “Reagents and methods for identifying anti-HIV compounds”.

**List of Published Work in MyBibliography (copy/paste link into browser):**

<http://www.ncbi.nlm.nih.gov/sites/myncbi/1XQBcp58uuGAZ/bibliography/40352268/public/?sort=date&direction=ascending>

**D. Research Support**

ACTIVE - independent projects:

**R01 CA098727** (Mothes) 4/01/2013-01/31/2018

NIH/NCI $163,565 DC

Retrovirus cell-to-cell transmission

This application proposes to study retroviral cell-to-cell transmission in primary cells. Intravital microscopy will allow the monitoring of murine leukemia virus generated virological synapses within the lymph node of mice. This will allow us to study the biogenesis of virological synapses and determine the role of integrins in the establishment and stability of virological synapses.

**1 R01 GM116654-01** (Mothes)07/01/2015-06/30/2020

NIH/NIGMS $185,500 DC

Single molecule imaging of HIV-1 entry

This application proposes to develop single molecule methods that allow the visualization of conformational changes within the gp41 fusion machine of HIV-1 Env. The long-term goal is to directly visualize the conformational changes that lead to fusion between viral and cellular membranes.

ACTIVE - collaborative projects:

**P01-GM56550** (Chaiken; PI) 09/30/2013-8/31/2018

NIH/NIGMS $132,969 DC

Structure-Based Antagonism of HIV-1 Envelope Function in Cell Entry.Project 6 (Mothes, Blanchard)

The objective of this scientifically integrated Program Project is to understand at the molecular level how the HIV-1 viral spike protein can be antagonized by small molecule-based entry inhibitors (CD4 antagonists), as novel approach to AIDS chemotherapy.

**10027835-13-M** of **P50GM082545** (Sundquist (PI)) 08/01/2014-7/31/2016

CHEETAH Collaborative Development Program, NIH/NIGMS $64,865 DC

Towards the in vivo visualization of HIV-1 pathogenesis

This pilot grant proposes to develop the tools and surgical methods to visualize various steps of HIV-1 pathogenesis directly in vivo using humanized mouse models.

COMPLETED:

**R21 AI100696** (Mothes (PI), Blanchard) 09/01/2012-01/31/2015

NIH/NIAID

Single molecule imaging of HIV Env

This application proposed the development of single-molecule Fluorescence Resonance Energy Transfer (smFRET) imaging to monitor the conformational landscape of individual trimeric HIV Env molecules on the surface of native HIV viruses. smFRET technology helped to elucidate the mechanism of immune evasion and antibody neutralization, and aided the rational design of vaccines directed against HIV Env.

**R03 AI106444** (Mothes)4/01/2013-12/31/2014

NIH/NIAID

Efficacy of antiretroviral inhibitors in HIV cell-to-cell transmission

This application proposed to test the effectiveness of antiretroviral inhibitors against HIV cell-to-cell transmission. Such a re-evaluation was warranted in the light of reports that had questioned the effectiveness of antiretroviral therapies under conditions of cell-to-cell spread.

**R21AI096999** (Mothes (PI), Rhoades) 03/01/2012-02/28/2014

NIH/NIAID

Monitoring single conformational events during HIV assembly

This application proposed to establish fluorescence correlation spectroscopy (FCS) and single-molecule fluorescence resonance energy transfer (smFRET) imaging to monitor the conformational changes of the capsid and the genome during HIV assembly.

**R01 AI084096** (Multi-PI: Mothes, Agaisse, Kumar) 09/29/2009-11/30/2013

NIH/NIAID

Targeting HIV cell-to-cell transmission

The goal of this grant was to identify targets for novel antiviral therapies that target the ability of HIV to efficiently spread from cell to cell. It led to the isolation of one host gene that is essential for HIV transmission.

PENDING:

ROI (Mothes) 07/01/2016-06/30/2021

NCI/NIH

Monitoring establishment and spread of retroviral infections in living animals

The goal of this grant is to study the early events that lead to the establishment and spread of retroviruses in living mice. It will determine why B-1a cells are highly permissive to murine leukemia virus infections and how the tropism broadens to B-2 cells at later time points. Understanding these events will inform novel antiviral therapies that prevent the establishment of viral infections.

Overlap: None. There is no overlap between any existing or pending grants.