Azure Sapphire™ FL Biomolecular Imager

UNLIMITED POSSIBILITIES, UNCOMPROMISING PERFORMANCE

Applications & Publications
## TABLE OF CONTENTS

### Chemiluminescence imaging
- Western blots.................................................................3
- Protein arrays...............................................................7
- Plant leaf bioluminescence........................................8

### Fluorescence imaging
- Western blots...................................................................9
- In-gel imaging..................................................................10
- Lateral flow assay development....................................11
- 2D DIGE..........................................................................12
- Gel-shift assay..............................................................13
- Organ imaging...............................................................14
- Tumor imaging...............................................................15
- Modified microplates....................................................16
- Tissue imaging...............................................................17
- DNA gels.......................................................................18
- Immunohistochemistry..................................................20

### NIR imaging
- Western blots.................................................................21
- In-cell Western.............................................................23

### Phosphor imaging
- Mobility shift assays..................................................24
- RNA gels.......................................................................25
- Southern blots..............................................................26
- Tissues...........................................................................27
- Thin layer chromatography........................................28

### White light imaging
- Lateral flow immunoassay development....................29
- Clonogenic assay........................................................30

### Table of dyes
- ..................................................................................31

### Sapphire Publications Overview
- .................................................................32
Shikonin Reduces Growth of Docetaxel-Resistant Prostate Cancer Cells Mainly through Necroptosis

Sascha D. Markowitsch, Kira M. Juetter, Patricia Schupp, Kristine Hauschulte, Olesya Vakhrusheva, Kimberly Sue Slade, Anita Thomas, Igor Tsaur, Jindrich Cinatl, Jr., Martin Michaelis, Thomas Efferth, Axel Haferkamp and Eva Juengel

...To explore the expression and activity of cell cycle and cell death regulating proteins, Western blot analysis was performed...The membranes were incubated with ECL detection reagent (AC2204, Azure Biosystems, Munich, Germany) to visualize proteins with a Sapphire Imager (Azure Biosystems, Munich, Germany)...Cell death regulating proteins were normalized to total protein that was quantified by staining total protein from all membranes with Coomassie brilliant blue and measuring with a Sapphire Imager...

Cancers. 2021;13(4):882

Figure 4. Protein expression profile of cell cycle regulating proteins: Representative Western blot images of cell cycle regulating proteins in parental (par) and DX-resistant (res) PC3 (left panel) and DU145 (right panel) cells after 48 h exposure to SHI [0.5 µM].
NonO Is a Novel Co-factor of PRDM1 and Regulates Inflammatory Response in Monocyte Derived-Dendritic Cells

Kyungwoo Lee, Su Hwa Jang, Hong Tian and Sun Jung Kim

Proteins bound by antibody were visualized by ECL (Thermo Scientific, #34580 or Advansta, K-12045) and sapphire biomolecular imager (Azure Biosystems).

Figure 1(A). Nuclear fraction was immunoprecipitated with anti-PRDM1 antibodies and immunoblotting was performed with anti-PRDM1, Flag-NonO, Flag-TP53BP1, or V5-hnRNPM antibody. A representative image from two independent experiments is shown.
WESTERN BLOTS

Effects of prolactin on ventricular myocyte shortening and calcium transport in the streptozotocin-induced diabetic rat

Frank C. Howarth, Gunnar Norstedt, Oleksiy I. Boldyrev, Muhammad A. Qureshi, Ozaz Mohamed, Khatija Parekh, Balaji Venkataraman, Sandeep Subramanya, Anatoliy Shmygol, Lina T. Al Kury

...The blots were developed using the Super Signal West Pico Plus chemiluminescent substrate (34577, Thermo Scientific, Rockford, IL, USA). The blot images were acquired using a Sapphire Biomolecular Imager (Azure Biosystems, Dublin, California, USA) using chemiluminescent detection of HRP, coupled with color image acquisition of the protein ladder...

Heliyon. 2020;6(4):e03797

Figure 1. Expression of PRL receptor (PRLR) protein in ventricle tissue from STZ-induced diabetic and control hearts. Typical Western blots showing expression of PRLR protein in three control (C1 – C3) and 3 diabetic (D1-D3), GAPDH loading control and PRLR protein in tissue from 3 rat mammary glands (MG1-MG3) from 3 female rats, 5 days following delivery.
A dominant negative mitofusin causes mitochondrial perinuclear clusters because of aberrant tethering

Stephanie R Sloat, Suzanne Hoppins

Membranes were developed in Radiance Plus Chemiluminescent HRP Substrate (Azure Biosystems) for 5 min and imaged on a Sapphire Biomolecular Imager (Azure Biosystems). Band intensities were quantified using AzureSpot analysis software (Azure Biosystems). Western blot images for shRNA were acquired on Sapphire, and quantification was performed with AzureSpot. Knockdown quantification was normalized using whole-protein stain (Azure Biosystems).

Figure 7. Mfn1S329P is defective for GTP-dependent oligomerization. (A) Representative blue native–PAGE of mitochondria isolated from Flp-In TReX cells expressing Mfn1WT-FLAG or Mfn1S329P-FLAG after incubation with 0.2 μg/ml TET for 4 h. Mitochondria were untreated or incubated in the presence of GMP-PNP (PNP), followed by solubilization and separation by blue native–PAGE and immunoblotted with α-FLAG. Arrow indicates ~200-kD species, closed arrowhead indicates ~320-kD species, and open arrowhead indicates ~450-kD species. Molecular-weight markers are indicated in kD on left. (A, B) Quantification of native mitofusin species indicated in A. Error bars represent mean ± SEM from n = 3 separate experiments.

Supplemental Figure 6B. Representative Western blot of α-DHC and α-tubulin in whole-protein extract from cells treated with either control shRNA (shLacZ) or shRNA against DHC (shDHC). Molecular-weight markers are indicated in kD on left. Percent knockdown represents mean knockdown of DHC n = 3 quantified by band intensities in Western blot normalized to whole-protein stain.
In vitro Evaluation of ASCs and HUVECs Co-cultures in 3D Biodegradable Hydrogels on Neurite Outgrowth and Vascular Organization

Luís A. Rocha, Eduardo D. Gomes, João L. Afonso, Sara Granja, Fatima Baltazar, Nuno A. Silva, Molly S. Shoichet, Rui A. Sousa, David A. Learmonth and Antonio J. Salgado

...The evaluation of the angiogenic and neurotrophic profile of the previously obtained secretomes was performed using the Human Neuro Discovery Array C1 and Human Angiogenesis Array C1 (RayBiotech, United States)...Finally, the chemiluminescence image of each membrane was obtained using a Sapphire Biomolecular Imager (Azure Biosystems, United States). The intensity of each dot was quantified using the AzureSpot software (Azure Biosystems, United States)...

Front Cell Dev Biol. 2020;8:489

Figure 5. Analysis of the secretomes of ASCs, HUVECs, and their co-culture after 7 days of culture in GG-GRGDS allowed to understand the relative expression of a panel of neuroregulatory and angiotrophic molecules. (A) The secretome of ASCs+HUVECs showed an upregulation on different neurotrophic factors (BDNF, β-NGF, IGF-1, and S-100 B) showing a positive effect of the interaction of both cells on the secretion of these molecules.
Agrobacteria were collected and resuspended in infiltration buffer (10 mM MgCl₂, 10 mM MES, 150 μM acetosyringone) at a final concentration of OD600 = 0.8. Pairwise combinations of suspensions were infiltrated into young tobacco leaves, which were then allowed to grow for 3 d in light. A total 12–16 leaves were collected from five to ten plants, the abaxial side of leaves was sprayed with 1 mM luciferin (Biosynth, L-8220) and kept in the dark at 4 °C for 30 min. The bioluminescence images were captured using Azure Sapphire Biomolecule Imager (Azure Biosystems) and converted to heatmaps using the 16-colour look-up table from ImageJ v.1.53a...
**WESTERN BLOTS**

Engineered unnatural ubiquitin for optimal detection of deubiquitinating enzymes

Wioletta Rut, Mikolaj Zmudzinski, Scott J. Snipas, Miklos Bekes, Tony T. Huang and Marcin Drag

...Biotinylated Ub-based probes were detected with a fluorescent streptavidin Alexa Fluor 647 conjugate (1 : 10 000) in TBS-T with 1% BSA, and UCH-L3 was detected with a mouse anti-human monoclonal IgG1 antibody (1 : 1000) and fluorescent goat anti-mouse (1 : 10 000) using an Azure Biosystems Sapphire Biomolecular Imager and Azure Spot Analysis Software.

Chem Sci. 2020;11(23):6058-6069

Figure 5(D). Detection of UCH-L3 in A-431 cell lysates using Ub-based probes (using a streptavidin Alexa Fluor 647 conjugate) and a UCH-L3 antibody.
Parallel imaging of coagulation pathway proteases activated protein C, thrombin, and factor Xa in human plasma

Sylwia Modrzycka, Sonia Kołt, Stéphanie G. I. Polderdijk, Ty E. Adams, Stanisław Potoczek, James A. Huntington, Paulina Kasperkiewicz and Marcin Drag

...For the simultaneous coagulation factor labeling, human plasma was incubated with 5 μM of each fluorescently labeled probe...and then 5 μL of each sample was run onto a 10% MES (w/v) 15-well gel...The gel was then directly scanned at 520 nm for Cy3, 658 nm for Cy5, and 784 nm for Cy7 detection using an Azure Biosystems Sapphire Biomolecular Imager and Azure Spot Analysis Software.

Chem Sci. 2022;13(23):6813-6029

Figure 6. Coagulation factor (APC, thrombin, fXa) labeling in human plasma. (A) Probe concentration optimization assay. Human plasma was incubated with each fluorescent ABP separately at various probe concentrations ranging from 1 to 20 μM for 60 min at 37 °C. The samples were then subjected to SDS–PAGE analysis, transferred to a membrane, immunostained with the appropriate antibody, and imaged using an Azure Biosystems Sapphire Biomolecular Imager as follows: for APC at 658 nm (for Cy5 detection) and 488 nm (for antibody detection), for thrombin at 784 nm (for Cy7 detection) and 658 nm (for antibody detection), for fXa at 520 nm (for Cy3 detection) and 658 nm (for antibody detection). The results are representative of at least 3 replicates. (B) Graphical scheme of the methodology used for simultaneous coagulation factor detection in human plasma. (C) Simultaneous coagulation factor labeling in human plasma. Human plasma was incubated with 5 μM of each fluorescently labeled ABP and subjected to SDS–PAGE analysis. Direct in-gel analysis was performed with lasers of 520 nm for Cy3, 658 nm for Cy5, and 784 nm for Cy7 using an Azure Biosystems Sapphire Biomolecular Imager. The results are representative of at least 3 replicates.
**LATERAL FLOW ASSAY DEVELOPMENT**

Method for the elucidation of LAMP products captured on lateral flow strips in a point of care test for HPV 16

Lena Landaverde, Winnie Wong, Gabriela Hernandez, Andy Fan & Catherine Klapperich

To identify 5′-FAM-specific LAMP products, unstained agarose and acrylamide gels were imaged under 488-nm laser excitation using the Sapphire Biomolecular Imager...  

Anal Bioanal Chem. 2020;412(24):6199-8209

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**Figure 4.** FAM band comparisons of pvull-digested and undigested lateral flow strip elutions. Three different representations of the LAMP amplicon products are shown. Gel a and b are LAMP amplicons digested with pvull (independent of LFS). Gels c, d, f, and g are eluted products from the LFS itself. Furthermore, gels c and d contain uncleaved LFS-LAMP products (pvull−), whereas gels f and g are cleaved LFS-LAMP products (pvull+). The FAM bands 94–114 (band C) bp and 71–80 bp (b and D) are highlighted as the HPV 16 FAM amplicons. Gel b also shows the previously sequenced bands of 146 (band A) and 92 (band B). The LFS strips that DNA was eluted from are shown in LFS images e and h of Fig. 4. The gels in panels a and b are pvull-digested LAMP products; the lateral flow strips results are equivalent to the LFS shown in panel h.
Proteomic Analysis of Endometrial Cancer Tissues from Patients with Type 2 Diabetes Mellitus

Muhammad Mujammami, Mohamed Rafiullah, Assim A. Alfadda, Khalid Akkour, Ibrahim O. Alanazi, Afshan Masood, Mohthash Musambil, Hani Alhalal, Maria Arafah, Anas M. Abdel Rahman and Hicham Benabdelkamel

"...The gels were scanned with Sapphire Biomolecular Imager (Azure Biosystems...) and digitalized via the image analysis software Sapphire Capture system (Azure Biosystems..."

Life.12(4):491

Figure 1. The representative fluorescent protein of a two-dimensional difference in gel electrophoresis (2D-DIGE) containing tissue samples from EC Diabetic samples labeled with Cy3 (A), EC Non-Diabetic samples labeled with Cy5 (B), pooled internal control labeled with Cy2 (C), and merged image (D).
GEL SHIFT ASSAY
(example of DNA-Protein Binding Assay)

Structure of a RecT/Redβ family recombinase in complex with a duplex intermediate of DNA annealing

Brian J. Caldwell, Andrew S. Norris, Caroline F. Karbowski, Alyssa M. Wiegand, Vicki H. Wysocki & Charles E. Bell

“A gel shift DNA binding assay used two complementary 50-mer oligonucleotides labeled at the 5′-end with either Cy3 or Cy5...For visualization 17.5 μl of each complex was mixed with 7.5 μl Orange G dye...Gels were imaged using a Sapphire Biomolecular Imager (Azure Biosystems) with Sapphire Capture Software (version 1.12.0921.0). Scanning parameters for Fig. 8 were pixel size 100 μm, scan speed high, 2.38mm focus, intensity 2 for Cy5, intensity 4 for Cy3, black lighting 50, white 37186, gamma 1.37. Scanning parameters for Supplementary Fig. 1a, b were intensity 1 for Cy5, intensity 2 for Cy3, black lighting 50, white 15362, gamma 0.88.”

Nat Commun. 2022;13(1):7855

Figure 8 (a). Mutational analysis. Each panel shows a gel-shift assay with 3.6 μM of LiRecT mixed with different combinations of Cy3- and Cy5-labeled 50mer oligonucleotides (25 μM nucleotides). Lanes C3, C5: each oligo without protein. Lanes 3, 5: LiRecT mixed with each individual oligonucleotide (Cy3-50mer or Cy5-50mer) to form a ssDNA complex. Lanes 35: LiRecT incubated with Cy3-50mer at 37° for 15 min, followed by addition of Cy5-50mer and incubation for an additional 15 min to form the duplex intermediate (yellow band).
Neutrophils incite and macrophages avert electrical storm after myocardial infarction


Oxidative stress was imaged after intravenous injection of CellROX Deep Red Reagent (C10422, Thermo Fisher Scientific, 20 μl diluted in 100 μl of PBS). Hearts were sliced in 1-mm sections for immediate imaging using a Sapphire Biomolecular Imager (Azure Biosystems)... Nat Cardiovasc Res. 2022;1(7):649–664

Abbreviations: FRI, fluorescence reflectance imaging; MI, myocardial infarction; ROS, reactive oxygen species; TBR, target-to-background ratio

Figure 3a. Experimental outline. FRI of ROS in hearts 5 hours after MI using the CellROX imaging agent. b, Fluorescence images from cardiac short axis slices after injection of CellROX. c, Quantification of TBR from FRI. Data are from isotype antibody-injected controls (n = 6 mice) and neutrophil-depleted mice (n = 7). Each dot represents a cardiac slice.

Figure 6m. Representative images of TMRE imaging.

Abbreviations: FRI, fluorescence reflectance imaging; MI, myocardial infarction; ROS, reactive oxygen species; TBR, target-to-background ratio
TUMOR IMAGING

Visible Fluorescence

Eliminating mesothelioma by AAV-vectored, PD1-based vaccination in the tumor microenvironment

Zhiwu Tan, Mei Sum Chiu, Chi Wing Yan, Kwan Man, Zhiwei Chen

“...resected REN tumor fluorescence was imaged with a Sapphire Biomolecular Imager (Azure Biosystems) after surgical resection...”

Mol Ther Oncolytics. 2021;20:373–386

Figure 5. Localized injection of rAAV-hsPD1-TWIST1 inhibits human mesothelioma in NSG-huPBL mice. (E) REN tumors in NSG mice (n = 4). 2 x 10^6 REN cells were injected s.c. into NSG mice 3 weeks before i.t. administration of 5 x 10^{11} g.c. rAAV. Left: tumor growth was measured by bioluminescence imaging. Right: representative tumor fluorescence images at the endpoint. Mock, PBS treatment.
Fluorescence Imaging: Modified Microplates

**ZnO Nanowire-Based Early Detection of SARS-CoV-2 Antibody Responses in Asymptomatic Patients with COVID-19**

Jung Kim, Sung Kyun Lee, Jong-Hwan Lee, Hye-Yeon Kim, Nam Hoon Kim, Chang Hoon Lee, Chang-Seop Lee, and Hong Gi Kim

And...NP antigen in coating buffer A (CB07100, Invitrogen, USA) was prepared at 1, 3, or 5 μg mL−1 and added to the ZnO-NW MP. Next, the anti-NP polyclonal antibody (32, 160, or 800 ng mL−1) in assay buffer (DS98200, Invitrogen, USA) was applied to the plate for 1 h, followed by the Alexa Fluor 488-conjugated secondary antibody (A-11034; 1 mg mL−1) for a further hour. The plate was washed between these steps. The fluorescence signal was measured in a microplate reader and an image taken under a laser scanning imager (Sapphire Biomolecular Imager, Azure biosystems, USA)..."
TISSUE IMAGING
White Light and Autofluorescence

Rescue of autoimmune hepatitis by soluble MHC class II molecules in an altered concanavalin A-induced experimental model

Katerina Bakela, Maria Georgia Dimitraki, Evangelia Skoufa, Irene Athanassakis

...Liver tissues were isolated 6 months after the ConA and the ConA + sMHCII treatment of mice and fixed using PFA 4%, under rotation, at 4°C for 24 hours. Azure Biosystems Sapphire™ Biomolecular Imager (Azure Biosystems, Dublin, CA 94568 USA) was used in order to scan the liver tissues in 10-μm resolution. This instrument combines NIR fluorescence (both long and short), RGB fluorescence, chemiluminescent, and phosphor imaging capabilities, while using four solid state lasers as excitation sources (450, 520, 660, and 780 nm). The application of the four-channel fluorescence mode at 10-μm resolution, could detect gross anatomy and morphology of liver tissues, mainly based on tissue autofluorescence...


Figure 6. Livers were isolated, fixed and scanned using a white light beam at 10-μm resolution (Ca) or four-channel fluorescence at 10-μm resolution (Cb).
Supplemental Figure 1. Integration Activity of PFV Intasome Preparations. (b) An ethidium bromide-stained agarose gel showing the integration activity of all the intasomes used in this study. Integration initially results in a linear CI product. Secondary integration events result in a smear of smaller products that extend from the linear CI to the vDNA. Competing intasome inactivation and/or aggregation deplete the active pool and cause the incomplete conversion of the SC DNA to CI products. (c) Quantification of integration activity. Error bars indicate standard deviation from two independent experiments. HS products that migrate slower than the linear DNA were undetectable with ethidium bromide. The Cy3-PFV-ddA did not produce any detectable activity. Secondary integration events that resulted in smearing below the linear CI products, excluding the SC band, were quantified and added to the linear CI product for this analysis. Source data are provided in the Source Data File.
DNA GELS
Visible Fluorescence

DNA strand breaks and gap target retroviral intasome binding and integration

Gayan Senavirathne, James London, Anne Gardner, Richard Fishel, and Kristine E Yoder

...Sequencing ladders were generated using Thermo Sequenase Dye Primer Manual Cycle Sequencing Kit according to the manufacturer’s directions67 with pDrive-601NPS40 as the template and Cy5, Alexa488 or Cy3 labeled oligos complementary to the 5’ end of 601NPS as primers...Products were resolved on 0.8 mm 8% Acrylamide:Bis 19:1/7 M Urea PAGE gels in 1X TBE at 40 W for various times. Gels were scanned on a Sapphire Biomolecular Imager and quantified using AzureSpot software (Azure Biosystems). Alignment of the products with the sequencing ladder was used to determine the integration sites. The integration efficiency was calculated as the fractional intensity of a band relative to the lane. Reactions without INTs were used as controls.⑥⑦


Fig. 4 | Analysis of PFV strand transfer activity...b. Representative denaturing PAGE gels from bulk integration studies. The lengths of ssDNAs derived from a Sanger sequencing ladder are shown. The blue and red bands show DNA fragments containing AF488 and Cy5, respectively. The target DNA substrates used for each lane are shown above (Supplementary Table 1)...d Gel analysis ... of PFV intasomes labeled with Cy3 on the vDNA transferred strand [Cy3-PFV (TS)]. Integration into different 1nt Gap (5’-OH) substrates containing a forward Cy5 label (F-Cy5), reverse Cy5 label (R-Cy5) or unlabeled as illustrated below. The blue, green and red color gel bands correspond to DNA fragments containing AF488, Cy3, and/or Cy5. The brown color bands contain both Cy3 and Cy5. Green arrows indicate location of autointegration (Al) products. The black dots indicate products resulting from strand transfer and red arrowhead the 42 nt alcoholysis product (see: b,c).”
TISSUE IMAGING

Immunohistochemistry

The ISL LIM-homeobox 2 transcription factor is negatively regulated by circadian adrenergic signaling to repress the expression of Aanat in pinealocytes of the rat pineal gland

Mattern KMJ, Blancas–Velázquez AS, Ngo MT, et al.

Immunohistochemistry Cryostat sections of 12-μm thickness were mounted on slides and blocked in 5% normal donkey serum (Abcam). Immunohistochemistry was performed as previously described...Sections were incubated in primary antibody at 4°C overnight and in secondary antibody at room temperature for...1 h if used for whole section imaging...Images of whole sections were taken on an Azure Sapphire Biomolecular Imager (Azure Biosystems). Fluorescence signal intensity after background subraction [sic] was quantified with the Azure Spot software... Western blot for image acquisition, an Azure Sapphire Biomolecular Imager was used. The density of bands was quantified with the Azure Spot software.  

J Pineal Res. 2023;e12905

Figure 3. Diurnal dynamics of Isl2 expression in the pineal gland of the adult rat... (C) Two representative western blot lanes of samples containing protein extracted from pineal glands were harvested at ZT10 and ZT22 to visualize the difference of ISL2 abundancy in daytime versus nighttime samples. Rat ISL2 protein (NP_065204) has a predicted molecular weight of 39.7 kDa. Housekeeping proteins ACTB and GAPDH were used as loading controls... (D) Two representative immunohistochemical stainings in brain sections from brains removed at ZT10 and ZT22 to visualize differences in ISL2 abundancy. Scale bars = 1 mm. (E) Quantification of ISL2 levels in the individual lanes of the western blot shown in (C).

For laser scanning, ISL2 was detected using a sheep anti-hlslet-2 primary antibody and an Alexa Fluor™ 790 donkey anti-sheep secondary antibody. For Western blotting, ISL2 was detected with a sheep-anti-human Islet-2 antibody and a donkey-anti-sheep Alexa Fluor™ 680 secondary antibody. ACTB was detected with a rabbit-anti-beta actin antibody and a donkey-anti-rabbit Alexa Fluor™ 790 secondary antibody. GAPDH was detected with a monoclonal DyLight™ 800 antibody.
**WESTERN BLOTS**

Accumulation and persistence of ivacaftor in airway epithelia with prolonged treatment

Tara N. Guhr Lee, Deborah M. Cholon, Nancy L. Quinney, Martina Gentzsch, Charles R. Esther Jr

...Blots were probed with mouse monoclonal anti-CFTR antibodies and then with IRDye 680–goat anti-mouse immunoglobulin G (Molecular Probes). Anti-actin (Cell Signaling) was used as a loading control. Protein bands were visualized using a Sapphire Biomolecular Imager (Azure Biosystems)... 

**J Cyst Fibros. 2020;19(5):746-751**

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*Fig. 3 (A).* Mature CFTR protein (band C, *) and immature CFTR protein (band B, •) visualized by Western blot analysis of HBE cultures derived from 3 CF (F508del/F508del) patients. Numbers at the top of the lanes represent days since treatment start. Actin is shown as a loading control.
WESTERN BLOTS

Differential influence of Streptococcus mitis on host response to metals in reconstructed human skin and oral mucosa

Lin Shang, Dongmei Deng, Sanne Roffel, and Susan Gibbs

...membranes were washed three times in PBST and further incubated with infrared dye-conjugated secondary antibodies against mouse (1:7500 for TLR3, 4, or 5) or against rabbit (1:7500 for TLR1, 2, 6, or tubulin). After washing, the blots were visualized using Sapphire Biomolecular Imager (Azure biosystems, Dublin, California)...

Contact Dermatitis. 2020;83(5):347–360

Figure 6. Toll-like receptor (TLR) protein expression in reconstructed human skin (RHS) and gingiva (RHG). TLR1 and 4 proteins are shown together with reference tubulin expression. TLR2, 3, 5, and 6 were under the detectable level (data now shown). Data are representative of three independent experiments.
Alboserpin, the Main Salivary Anticoagulant from the Disease Vector Aedes albopictus, Displays Anti–FXa-PAR Signaling In Vitro and In Vivo


...Finally, the plates were scanned at 700 and 800 nm, and the intensity of the labeled proteins was measured using the Azure sapphire biomolecular imager (Azure Biosystems).

Immunohorizons.2022;6(6):373–383

Figure 1. Alboserpin inhibits FXa inflammatory effect in vitro. (E) ERK1/2 protein expression was analyzed using In-Cell Western blots (ICW). (F) Intensity ratio (p-ERK/ERK).
MOBILITY SHIFT ASSAYS

High Dose IFN-β Activates GAF to Enhance Expression of ISGF3 Target Genes in MLE12 Epithelial Cells

Kensei Kishimoto, Catera L. Wilder, Justin Buchanan, Minh Nguyen, Chidera Okeke, Alexander Hoffmann and Quen J. Cheng

...Nuclear extracts...were incubated with P32-labeled oligonucleotide probes...The reaction mixtures were run on a 5% acrylamide (30:0.8) gel with 5% glycerol and TGE buffer (24.8 mM Tris, 190 mM glycine, 1 mM EDTA) at 200V for 1 hour and 45 mins. The gels were dried and imaged on a Sapphire Biomolecular imager in phosphor mode (Azure Biosystems, Dublin, CA).

Front Immunol. 2021;12:651254

Figure 1. IFN-β Induced STAT1 binds to GAS and ISRE motifs. (A) EMSA of GAS and ISRE binding in MLE12 cells treated with IFN-β (100 U/ml). NFY shown as loading control. Data are representative of >5 independent experiments.
RNA GELS

Engineered Viral RNA Decay Intermediates to Assess XRN1-mediated decay

Joseph Russo, Cary T. Mundell, Phillida A. Charley, Carol Wilusz, and Jeffrey Wilusz

...The samples were then resolved via denaturing PAGE, dried, exposed to a phosphor screen, and viewed via an Azure Sapphire Biomolecular Imager providing sufficient sensitivity to observe all required bands with one exposure.  

Host cell-dependent late entry step as determinant of hepatitis B virus infection

Xupeng Hong, Yuka Imamura Kawasawa, Stephan Menne, Jianming Hu

...Viral DNAs were resolved on 1.2% agarose gel and detected by 32P-labeled HBV or WHV DNA probes... DNA signals from Southern blot analysis were detected by Sapphire Biomolecular Imager (Azure Biosystems) and quantified using the Image Lab system 6.0.1 (Bio-Rad).


Figure 4. WCH-17 cells were rendered susceptible to HBV infection after huNTCP expression. WCH-17 (A, B) or WC3 (C, D) parental and huNTCP-expressing cells were plated on regular culture dishes (i.e., with no collagen coating) and infected with ca. 400 genome equivalent of HBV per cell. Three days post infection, the PF DNA (i.e., Hirt DNA) from mock- or HBV-infected cells was extracted by Hirt extraction and treated with Exo I/III followed by Southern blot analysis. Hirt DNA from HBV-infected HepG2-huNTCP cells, loaded at 4-fold less than the Hirt DNA from woodchuck cells, served as the positive control for cccDNA detection.
Preclinical evaluation of $[^{18}\text{F}]$FDG-PET as a biomarker of lymphoid tissue disease and inflammation in Zika virus infection

Carla Bianca Luena Victorio, Joanne Ong, Jing Yang Tham, Marie Jennifer Reolo, Wisna Novera, Rasha Msallam, Satoru Watanabe, Shirin Kalimuddin, Jenny G. Low, Subhash G. Vasudevan, Ann-Marie Chacko

Infected mice were injected i.v. with 10 MBq $[^{18}\text{F}]$FDG and tissues were harvested following a 60-min tracer uptake. Freshly isolated wholemount lymphoid tissues were immediately exposed to multi-purpose phosphoscreen (BAS-IP MS) for 30 min. Fresh tissues with high tracer uptake were exposed to super-resolution (BAS-IP SR) phosphoscreen for 5 min (GE Healthcare Life Sciences, USA). $[^{18}\text{F}]$FDG standards at 2/3 serial dilution from 600 to 0 kBq were mounted together with mouse tissue for calibration of digital autoradiography (DAR) images. Screens were then scanned using the Sapphire Biomolecular Imager (Azure Biosystems, USA) at 100-μm resolution...


Figure 3. Ex vivo assessments of tissue $[^{18}\text{F}]$FDG uptake in acute ZIKV and DENV disease. a, b Representative wholemount tissue ex vivo digital autoradiography (DAR) images of a lymphoid tissues, testes, and liver and b brain, heart, and digestive tract from pre-infection (n = 5), late ZIKV (n = 6), and late DENV (n = 8) mice.

Abbreviations: DENV, dengue virus; ZIKV, Zika virus.
...The lipid extracts were resolved by thin-layer chromatography (TLC) using silica gel G60 TLC plates and chloroform/methanol (90:10 v/v) as the mobile phase. Reference non-radioactive standards were resolved concurrently and developed separately from the blot containing radioactive lipids with phosphomolybdic acid stain. Following this, blot with reference standard was matched with TLC blot to mark resolution of lipids. After development radiolabeled lipids were imaged using Azure Sapphire Biomolecular Imager (Azure Biosystems Inc, Dublin, CA)..."
LATERAL FLOW IMMUNOASSAY DEVELOPMENT

A novel rapid detection for SARS-CoV-2 spike 1 antigens using human angiotensin converting enzyme 2 (ACE2)

Jong-Hwan Lee, Minsuk Choi, Yujin Jung, Sung Kyun Lee, Chang-Seop Lee, Jung Kim, Jongwoo Kim, Nam Hoon Kim, Bum-Tae Kim, Hong Gi Kim

...the intensity of the test and control lines were converted to peak histograms using a Sapphire Biomolecular Imager."

Biosens Bioelectron. 2021;171:112715

Figure 4. Identification of the sandwich pair for detection of SARS-CoV-2 spike antigen. a) Schematic diagram of LFIA using ACE2 as the capture probe and sandwich analysis results obtained from paired antibodies (CR3022, F26G19, and S1mA). SARS-CoV-2 S1 antigen (50 ng) was used as a positive control, and buffer containing no S1 antigen was used as a negative control. After 20 min, the strips were captured by a smartphone, and their peak intensities were analyzed. b) Schematic diagram of LFIA, using antibodies as the capture probe, and their sandwich analysis results. c) Peak intensities of capture probe (PC)–detection probe (PD) pairs. A total of 12 pairs of positive controls (50 ng S1 antigen) were tested, and their intensities were analyzed. Peak intensity was calculated by subtracting the background intensity of the strip from the average intensities of the dots.

Figure 5a. Sensitivity and specificity of the ACE2-based LFA. a) Results of ACE2-based LFA for the detection sensitivity of SARS-CoV-2 S1 antigen. Serially diluted antigen concentrates (concentration range: 500 ng/mL to 5 ng/mL) were tested by ACE2-based LFA. After 20 min, the LFA strips were photographed with a smartphone. Moreover, the intensity of the test and control lines was converted to a peak histogram by an image analyzer.

Figure 6b. Laboratory confirmation of ACE2-based LFIA using clinical samples. b) Results of ACE2-based LFA for the detection sensitivity of cultured SARS-CoV-2. Serially diluted virus concentrates (concentration range: $1.07 \times 10^6$ copies/mL to $5.35 \times 10^6$ copies/mL) were tested. After 20 min, the LFIA strip was taken with a smartphone and scanned with an image analyzer. The line intensities of the test and control lines were converted to peak histograms. Also, the intensity of the test lines was measured by a portable line analyzer (IL: line intensity of test line). Furthermore, human coronavirus (OC43) was tested as a negative control.
CLONOGENIC ASSAY

A novel rapid detection for SARS-CoV-2 spike 1 antigens using human angiotensin converting enzyme 2 (ACE2)

Jong-Hwan Lee, Minsuk Choi, Yujin Jung, Sung Kyun Lee, Chang-Seop Lee, Jung Kim, Jongwoo Kim, Nam Hoon Kim, Bum-Tae Kim, Hong Gi Kim

...The clonogenic recovery potential gives insight into the capability of the cells to form a new tumor (metastasis). Therefore, 500 cells/well were seeded on a 6-well-plate and treated for 10 days with ART. Untreated cells served as controls. RCC cells were subsequently fixed with 85% MeOH/15% AcOH and stained with Coomassie (0.5 g Coomassie Blue G250 (Sigma-Aldrich, Darmstadt, Germany), 75 mL AcOH, 200 mL MeOH, 725 mL distilled water). Amount and size of cell clone colonies were measured with a biomolecular imager (Sapphire, Azure Biosystems, Biozym, Hess. Oldendorf, Germany)...
# TABLE OF DYES

## Visible Fluorescence

<table>
<thead>
<tr>
<th>Dye</th>
<th>Application</th>
<th>Journal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell Tracker Green</td>
<td>Assessment of PI3K/mTOR/AKT Pathway Elements to Serve as Biomarkers and Therapeutic Targets in Penile Cancer</td>
<td>Cancers</td>
</tr>
<tr>
<td>Alexa Fluor® 488</td>
<td>ZnO Nanowire-Based Early Detection of SARS-CoV-2 Antibody Responses in Asymptomatic Patients with COVID-19</td>
<td>Advanced Materials Interfaces</td>
</tr>
<tr>
<td>Alexa Fluor® 546</td>
<td>DNA strand breaks and gap target retroviral intasome binding and integration</td>
<td>Nat Commun</td>
</tr>
<tr>
<td>Alexa Fluor® 647</td>
<td>Comparative Study of Bacterial SPOR Domains Identifies Functionally Important Differences in Glycan Binding Affinity</td>
<td>Journal of Bacteriology</td>
</tr>
<tr>
<td>Bodipy™ FL</td>
<td>SARS-CoV-2 Mpro inhibitors and activity-based probes for patient-sample imaging</td>
<td>Nature Chemical Biology</td>
</tr>
<tr>
<td>Bodipy™ PC</td>
<td>Phospholipase D1 Acts as a Negative Regulator of High Mg²⁺-Induced Leaf Senescence in Arabidopsis</td>
<td>Front Plant Sci.</td>
</tr>
<tr>
<td>Cy5</td>
<td>Parallel imaging of coagulation pathway proteases activated protein C, thrombin, and factor Xa in human plasma</td>
<td>Chemical Science</td>
</tr>
<tr>
<td>Cy3</td>
<td>DNA strand breaks and gap target retroviral intasome binding and integration</td>
<td>Nat Commun</td>
</tr>
<tr>
<td>Cy2</td>
<td>Plasma proteomics reveals an improved cardio-metabolic profile in patients with type 2 diabetes post-liraglutide treatment</td>
<td>Sage Journals</td>
</tr>
<tr>
<td>Ethidium bromide</td>
<td>DNA strand breaks and gap target retroviral intasome binding and integration</td>
<td>Nat Commun</td>
</tr>
<tr>
<td>FAM</td>
<td>A noncanonical RNA-binding domain of the fragile X protein, FMRP, elicits translational repression independent of mRNA G-quadruplexes</td>
<td>Journal of Biological Chemistry</td>
</tr>
<tr>
<td>Flamingo</td>
<td>Defensin-driven viral evolution</td>
<td>Plos Pathogens</td>
</tr>
<tr>
<td>GelRed®</td>
<td>A slow-exchange conformational switch regulates off-target cleavage by high-fidelity Cas9</td>
<td>bioRxiv.org</td>
</tr>
<tr>
<td>GFP</td>
<td>Membrane stretching activates calcium permeability of a putative channel Pkd2 during fission yeast cytokinesis</td>
<td>Molecular Biology of the Cell</td>
</tr>
</tbody>
</table>
**Visible Fluorescence**

<table>
<thead>
<tr>
<th>Dye</th>
<th>Description</th>
<th>Journal</th>
</tr>
</thead>
<tbody>
<tr>
<td>mCherry</td>
<td>Analyzing (Re)Capping of mRNA Using Transcript Specific 5 End Sequencing</td>
<td>Bio-protocol</td>
</tr>
<tr>
<td>SYBR™ Green</td>
<td>Method for the elucidation of LAMP products captured on lateral flow strips in a point of care test for HPV 16</td>
<td>Analytical and Bioanalytical Chemistry</td>
</tr>
<tr>
<td>SYBR™ Gold</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TMRE</td>
<td>Neutrophils incite and macrophages avert electrical storm after myocardial infarction</td>
<td></td>
</tr>
</tbody>
</table>

**NIR Fluorescence**

<table>
<thead>
<tr>
<th>Dye</th>
<th>Description</th>
<th>Journal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azure Spectra 700</td>
<td>Alboserpin, the Main Salivary Anticoagulant from the Disease Vector Aedes albopictus, Displays Anti–FXa-PAR Signaling In Vitro and In Vivo</td>
<td>ImmunoHorizons</td>
</tr>
<tr>
<td>Azure Spectra 800</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cy5.5</td>
<td>A phage mechanism for selective nicking of dUMP-containing DNA</td>
<td>PNAS</td>
</tr>
<tr>
<td>Cy7</td>
<td>A broadly neutralizing antibody protects Syrian hamsters against SARS-CoV-2 Omicron challenge</td>
<td>Chemical Science</td>
</tr>
<tr>
<td>DyLight™ 650</td>
<td>A broadly neutralizing antibody protects Syrian hamsters against SARS-CoV-2 Omicron challenge</td>
<td>Nature Communications</td>
</tr>
<tr>
<td>IRDye 680</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**SAPPHIRE PUBLICATIONS OVERVIEW**

**492 PUBLICATIONS**

As of July 14, 2023

- **300** Western blot
- **14** Northern blot
- **13** Southern blot
- **5** In-cell Western
- **42** Phosphor imaging
- **3** Total protein normalization

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