avoid jitter. Also, the physical processes under investigation must completely reset before each pump-probe cycle repeats in order to maintain a consistent causality between the reconstructed event sequences. For the time frames for molecular imaging reported by Wolter *et al.*, meeting these two conditions in conventional pump-probe studies is difficult because time jitter can easily set in at the 10-fs time scale. Also, image formation can be obscured because photoinduced molecular dissociation can proceed on multiple fragmentation pathways. These different outcomes normally can only be sampled statistically because tracking individual events is prohibited by the uncertainty principle.

In fact, the reported ~1-fs resolution is 10 times shorter than the optical cycles (~10 fs) of the mid-infrared pulses used to initiate the events. The ability to go beyond the traditional pulse-envelope–limited resolution and even sort out complex reactions is made possible by using "tagging" to isolate information. Taking their lead from coincidence schemes frequently used in the nuclear scattering experiments, Wolter *et al.* acquired the signals of both departing electrons and positive ions in their detection scheme to isolate specific excitation channels. Furthermore, given the precise timing of the optical field that also encodes a periodic time-energy (t-E) correlation on the departing electrons by way of high-field tunnel ionization, events can be delicately sorted out by analyzing the energy of the electrons arriving at the detectors.

From the knowledge of these correlations, post-collection analysis of events distributed over a 1-eV energy window (δE) yields a mere 0.2-fs shutter speed (δt), according to classical trajectory calculations of the tunnel-ionization process. This extremely high temporal resolution may at first glance appear to violate the uncertainty principle, that $\delta E \delta t > h$ (Planck's constant). In this case, it would impose an ~4-fs uncertainty in timing of the specific electron arriving at the detector if such a measurement were conducted.

However, the power of this resolution by correlation is not in sidestepping the uncertainty principle but in relying on the knowledge of the phase-space structure of the outgoing electrons. Although the overall phase space (t-E) of single electrons still extends over h, the exact correlation in t and E developed during the extraction process allows the identification of t by measuring E, to the precision of ~0.6 fs in this case. The penalty is taken in the sensitivity. The counting conducted in the specific energy-time window of the restricted electron phase space is scarce and relies on large statistical samplings, which fortunately is not prohibitive because of the nearly jitter-free nature of this imaging approach.

In spite of this exquisite demonstration of time resolution in molecular imaging, the technology in its current form is still limited in the dynamical range set by the optical cycle (~10 fs) and has a modest structure resolution. In terms of applications, these pros and cons seem to be orthogonal to the conventional ultrafast electron and x-ray diffraction technologies, which are more flexible in deployment but nonetheless face challenges in reaching the sub-10-fs time scale because of timing jitter.

However, the idea of improving resolution by tagging the events through correlation as demonstrated here is more general. In fact, the next generation of ultrafast electron-imaging technology development aims at circumventing the limitations posed by space-charge–driven expansion through actively controlling the beam dynamics (*6*). In one central aspect, the objective is to delicately shape the phase-space structure developed through the space-charge effect by designing new actively controlled optical components to reconstruct the electron pulse's phase space for optimizing performance (*7*). In another aspect, high-brightness beams created by controlling the photoemission can lead to highly compact electron pulses that can retrieve structural information in single shots, extending the pump-probe scheme to irreversible scenarios (*8*).

These strategies of dynamical phase-space control also provide means to encode information that can be used to improve the time resolution. For example, the phase-energy correlation developed through active fields used for pulse compression (*9*) can be exploited to resequence the events scrambled by timing jitter based on post-selection of pulse energy determined through diffraction itself. These various new developments, although operating in very different settings, share the key aspects of constraining and manipulating the phase space of the imaging electrons to transcend current resolution limits, presenting a bright future for electron-based ultrafast imaging technologies. ■

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ANTIBODIES

Hitting Ebola, to the power of two

Bispecific antibodies are engineered to thwart ebolavirus entry into cells

By **Aran F. Labrijn**[1] *and* **Paul W. H. I. Parren**[1,2]

Passive immunotherapy with therapeutic antibodies is one of the most promising treatments for Ebola virus infection. Despite disappointing initial results using single monoclonal antibodies (mAbs) (*1*), successful post-exposure protection in nonhuman primate models of Ebola virus infection was eventually achieved using "designer polyclonals" that mixed individual mAbs (*2*). The efficacy of an optimized three-mAb cocktail (ZMapp) in protecting nonhuman primates (*3*) was the basis of its compassionate use and clinical evaluation during the 2014 to 2015 West African Ebola virus outbreaks. The limited protective breadth of available antibodies against specific ebolavirus species, however, raises concerns with respect to a general preparedness for filovirus outbreaks (*4*). On page 350 of this issue, Wec *et al.* (*5*) describe exploiting the powerful nature of bispecific antibodies (bsAbs)—the ability to recognize two different proteins or epitopes with a single antibody—to block entry of multiple ebolavirus species into cells. This may lead to the development of antiviral immunotherapy with cross-filovirus activity.

"...bsAb technology may... provide exciting therapeutic opportunities..."

Ebola virus, like other filoviruses, is unusual in that it bears a glycoprotein that exposes its receptor binding site only after entry and processing in an intracellular vesicle (endosome). Productive infection requires the virus to escape from the endosome by

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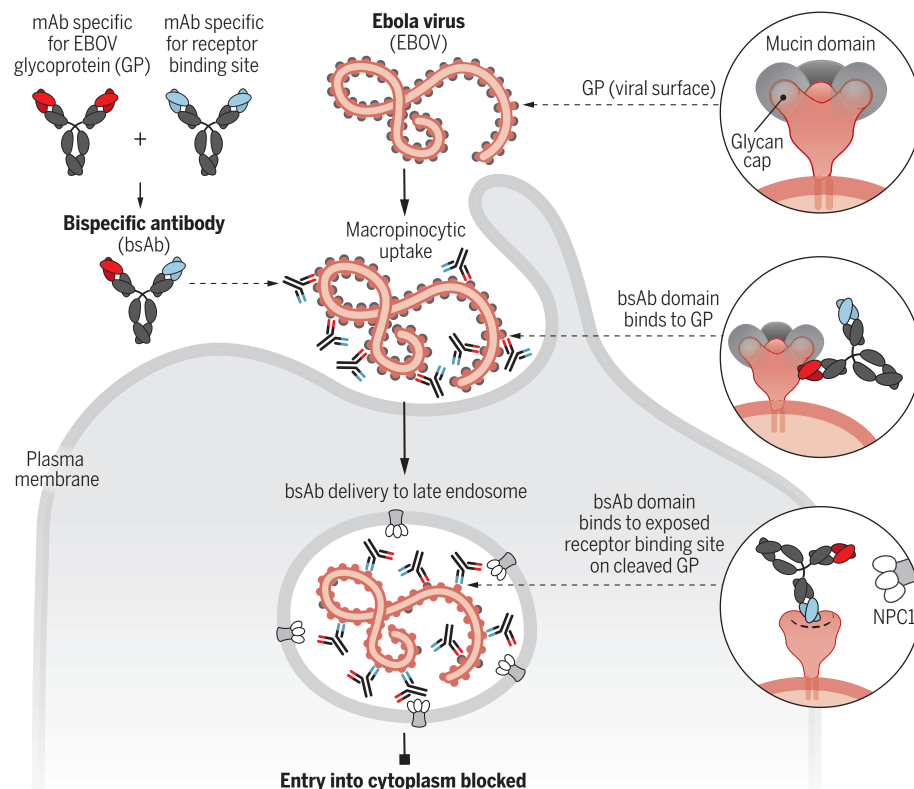
interacting with its receptor, an endosomal transporter protein (*6, 7*). Both the conserved nature of the viral glycoprotein's binding site for the host cell receptor and its critical role in filovirus infection would make it an attractive target for protective antibodies, were it not for the inaccessibility of the binding site to antibodies. Therefore, Wec *et al.* generated bsAbs that bear both the binding domain of a mAb specific for a conserved and exposed epitope present on all known ebolavirus glycoproteins, and the binding domain of an antibody that interrupts interaction between the cleaved viral glycoprotein and the endosomal transporter protein Niemann-Pick C1 (NPC1). Hence, the bsAb binds to the virus extracellularly and holds on while it enters the endosome. Once in the endosome, the second domain of the bsAb binds to the glycoprotein epitope that is revealed upon cleavage, and blocks viral entry into the host cell (see the figure). This "Trojan horse" approach could thus be used to outsmart the formidable fortifications of this lethal virus and create a single agent with an unprecedented effectiveness. Wec *et al.* show that the bsAb neutralized all known ebolaviruses in vitro and conferred postexposure protection against lethal challenge with multiple viruses in mouse models. The power of the bsAb approach is highlighted by the fact that simple mixtures of the parent antibodies were ineffective.

Wec *et al.* provide a dramatic example of the improvement that can be achieved by physically connecting an appropriate combination of antibody binding specificities. Indeed, therapeutic applications that are unique to bsAbs involve those in which the combination of two antibody specificities generates a new functionality not present in the parent antibodies. Because of the dependence on the two binding domains for activity, such antibodies have been termed "obligatory bsAbs" (*8*), a few examples of which now exist. In oncology, obligatory bsAbs are being used to redirect effector cells for the killing of tumor cells. Most of these bsAbs, including the marketed therapeutic bsAb blinatumomab, combine an agonistic antibody binding domain that recognizes a T cell co-receptor (CD3), with a binding domain that recognizes a tumor-associated antigen; thus, the T cells and tumor cell are brought together. In addition, the redirection of natural killer lymphocytes (*9*) to target tumor cells is also being pursued. Emicizumab, a bsAb that binds the blood clotting factors X and IXa, has been designed to replace the activity of factor VIII in patients with hemophilia A (*10*) and is currently in phase 3 clinical trials. In another application, bsAbs that can selectively activate or inhibit pairs of targets on a single cell are being developed. Examples include a bsAb directed against fibroblast growth factor receptor 1 (FGFR1) and βKlotho (KLB) to treat diabetes (thus, activating FGFR1 only in the presence of KLB) (*11*) and a bsAb that targets the epidermal growth factor receptor (EGFR) and the hepatocyte growth factor receptor (cMET) for cancer treatment (blocking both receptors to prevent treatment escape) (*12*). Also, the "hijacking" of transcytosis pathways (which transport macromolecules, in vesicles, through a cell barrier) would give bsAbs access to immunoprivileged sites such as the human brain (*13*), and represents another attractive application of obligatory bsAbs.

The bispecific's powers

Combining binding specificities into one antibody can lead to functionalities not present in the individual antibodies. An example of a bispecific antibody that blocks Ebola virus infection is shown.



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The design of new functionalities in a bsAb is not straightforward and may often combine knowledge-based and empirical approaches. As shown by Wec *et al.*, insights into the molecular mechanisms of filovirus infection, in combination with the availability of mAbs against the relevant epitopes, were critical in selecting the binding domains of the bsAb. As the biological activity of both arms of the bsAb occurs sequentially and does not require simultaneous binding, the relative epitope topology is likely to play a subordinate role in such applications and can thus be exploited by bsAb formats of different design (*8*). By contrast, for applications where simultaneous binding is a prerequisite, such as for selective agonists like emicizumab, the selection of binding arms of a bsAb is much more dependent on empirical approaches in which identifying just the right combination of binding specificities may require the screening of thousands of binding pairs. In addition, architecture of the selected bsAb format and positioning of the binding arms may impact the antibody's activity.

After decades of optimization, bsAb technology may finally start to deliver on its promise in which obligatory bsAbs with new functionalities provide exciting therapeutic opportunities, the design of which may only be limited by our imagination. ■

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