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A COLLABORATIVE WHITE PAPER FROM ASTRAZENECA, CHALMERS UNIVERSITY OF TECHNOLOGY, THE UNIVERSITY OF GOTHENBURG, AND CAMECA



Introducing DrugSIMS: a new approach unveils drug targets inside the "black box" of the cell

Even the most elegantly designed drug is useless when it can't make its way to its destination. The pharmaceutical industry requires effective tactics to address the problem of developing drugs to reach intracellular drug targets. Methods applied in the past have produced inadequate results — so that in significant ways, until now the cell has represented a "black box," with key interactions occurring inside that remain hidden from precise analysis.

This issue may now be the most important problem facing drug discovery researchers. A key focus: the failure of plasma concentration measurements to accurately determine drug exposure inside the cell.

A novel approach — DrugSIMS™ — applies advanced imaging instrumentation within a specialized workflow to create a proven new solution. In addition, the DrugSIMS methodology itself is the product of a new collaboration, which embeds a research incubator group inside a large pharmaceutical manufacturer. (See "The collaboration" below.)

The problems

Plasma concentration

This key issue facing drug discovery efforts is outlined in a paper[1] by C. T. Dollery of GlaxoSmithKline. A drug molecule may pass through the cell wall and successfully reach the cytoplasm. However, inside the cell, basic molecules may be retained by organelles such as lysosomes, accumulating there in large concentrations and failing to complete their therapeutic missions. So the traditional metric of exposure — *plasma concentration* — often inaccurately represents the actual exposure of drug components to potential targets inside the cell. Thus a concept fundamental to drug discovery's decision-making process, whereby the exposure of the drug is compared to response, is in many cases fatally flawed.

Researchers must undertake a fundamental reexamination to ensure clear understanding of pharmacokinetics at the subcellular level, with a specific focus on drug molecules that become sequestered in organelles.

Fluorescent microscopy

Some researchers attempt to assess intracellular activity using *fluorescent microscopy*: labeling drug molecules with fluorescent probes to inspect their activity within a cell.

Unfortunately, none of the microscopes used for this inspection possess the ultra-fine, nanometer-scale sensitivity necessary to observe quantitative results at the low molecule counts characteristic of therapeutic concentrations. Instead, researchers must increase concentrations well beyond therapeutic levels. In addition, fluorescent dyes themselves may skew results for efficacy, uptake, and potency.

Also, since the fluorescent method produces only a relative measurement, it offers limited usefulness in a database. All other database measurements are likely to be absolute — presenting a specific number. So a true comparison of differing values to assess intracellular targeting would require redoing all other experiments that used absolute results to yield relative results instead: not a practical strategy.

Fractionation

Other researchers assess intracellular activity using *fractionation*: centrifuging samples to break cells down, in an attempt to label cell components and measure their associated drug concentrations.

This method does provide sufficient sensitivity, but it presents cell materials out of intracellular context. This requires numerous assumptions, perhaps without adequate evidence. And the lack of context degrades the usefulness of any conclusions.

Comments in a seminal paper[1] by C. T. Dollery of GlaxoSmithKline:

"To predict the pharmacologic effect accurately, there must be data concerning the concentration at the target, which is difficult to measure."

"The distribution of drugs into and within individual cells is arguably one of the most neglected areas of pharmacology, pharmacokinetics, and therapeutics."

"In drug discovery, molecules that can engage novel targets such as oligonucleotides, antibodies to cancer cell epitopes carrying a target load, and stapled peptides often have difficulty accessing their intracellular targets."

The collaboration

A novel academic/industry collaboration has produced a proven new solution that overcomes the problems above. The group involves three partners: Chalmers University of Technology in Gothenburg, Sweden; the University of Gothenburg; and the global pharmaceutical firm AstraZeneca, via the BioVentureHub incubator facility on its Gothenburg campus. The two universities jointly manage a Chemical Imaging Infrastructure (CII) at the hub, utilizing tools such as SEM, TEM, and SIMS technology (see below).

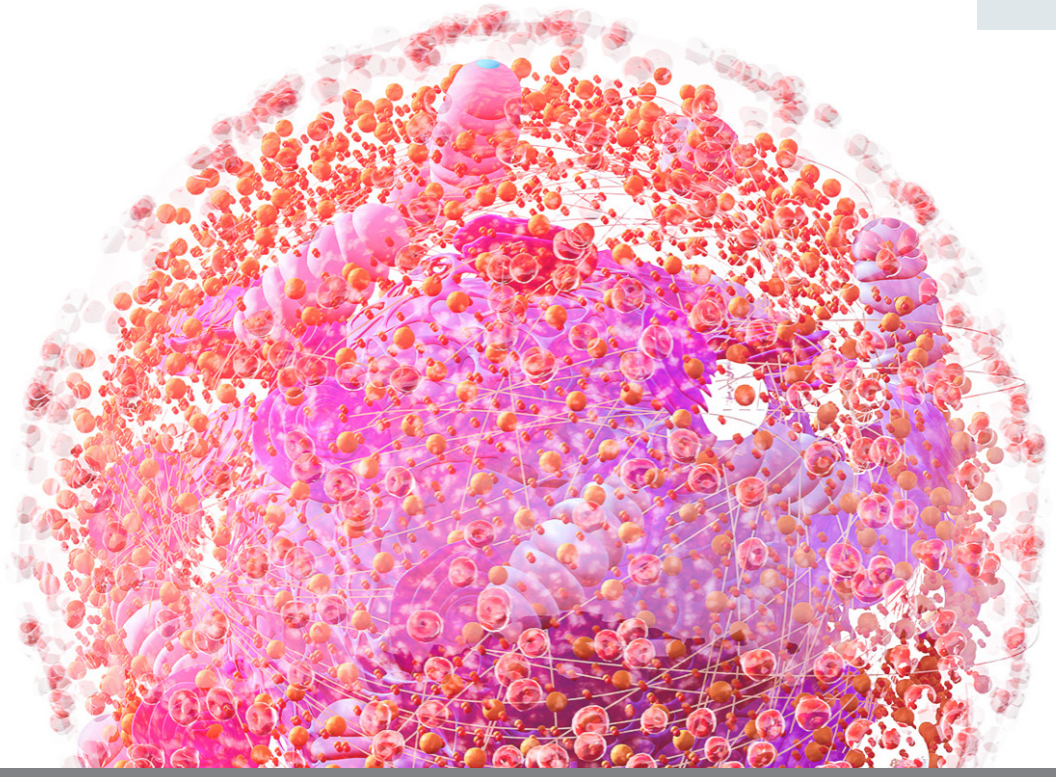
Normally, such collaborations target specific research topics or well-defined problems. However, too many traditional studies are governed by contractual project plans and perceived outcomes. So since its founding in 2015, BioVentureHub has taken a somewhat different approach — innovatively sharing knowledge, models, and tools in a more pragmatic manner to catalyze collaborative innovation between industry and academia. Focusing on investigating areas of common interest, AstraZeneca drug discovery research and advanced imaging capabilities are merged to advance basic pharmacokinetics knowledge.

Innovation is almost always a collaborative endeavor. The BioVentureHub model maximizes shared problem definition and resolution, enabling researchers to be guided and inspired to an uncommon extent by scientific curiosity. Disruptive innovation is also difficult to plan. By freeing great minds to access — without many of the usual constraints — the infrastructure provided by a large pharmaceutical company such as AstraZeneca, the collaboration can devise fresh frameworks for progressing pharmaceutical science in new directions.



AstraZeneca BioVentureHub

This open innovation ecosystem is located at the heart of AstraZeneca's strategic R&D center in Gothenburg, Sweden. Today, the hub provides 34 emerging biotech firms and pharmaceutical companies, plus academic groups, a unique opportunity to co-locate and interact — with AstraZeneca experts, and with each other. BioVentureHub's goals: to advance life sciences, health care, and patient wellbeing.



The solution: DrugSIMS

Paramount aim of the collaboration has been to develop a portfolio of methods to measure drug concentration inside sample cells with various labelling strategies. Together, the group has successfully validated a unique new method to quantify these subcellular drug concentrations.[2]

Designated DrugSIMS™, this novel methodology enables accurate measurement of drug uptake and endosomal escape, yielding a thorough understanding of protein and lipid trafficking through absolute quantification of new drug modalities.

The DrugSIMS method utilizes advanced imaging equipment technologies, sometimes including *scanning electron microscope (SEM)* and/or *transmission electron microscope (TEM)* instruments.

But its key differentiator is the use of the hub's NanoSIMS 50L ion microprobe, from the leading microanalytical/metrology instrumentation firm CAMECA. This unique tool optimizes *secondary ion mass spectrometry (SIMS)* performance, imaging and measuring elemental and isotopic distribution with great sensitivity and ultra-high spatial resolution — commensurable to organelle sizes. So it enables imaging of drugs and nanoparticles at an organelle level.

Where fluorescent microscopy can produce only a relative value for the pharmacokinetic properties of a given drug molecule, based on a *qualitative* image, DrugSIMS analysis yields an exact value from *quantitative* imaging, thanks to the 50 nanometer (nm) resolution possible with NanoSIMS.

Thus researchers get an absolute measurement of exposure at a given target, which can be usefully compared to benchmarks and other values in a well-organized database. When stored, this data can also provide continuity from experiment to experiment. In addition, DrugSIMS uses molecules at therapeutic levels. Plus it won't affect efficacy, uptake, or potency — a game changer in pharmacokinetics analysis.

Finally, as opposed to the lack of context found in fractionation methodology, DrugSIMS provides reliable measurements within their full intracellular context.



We often rationalize our results by speculating how our therapeutics behave inside the cells we target. The big idea behind DrugSIMS is to allow researchers to probe much deeper and actually see where their drugs end up and at what concentration.

— Michael E. Kurczy
Associate Principal Scientist
AstraZeneca

A DrugSIMS workflow: subcellular drug exposure measurement[3]

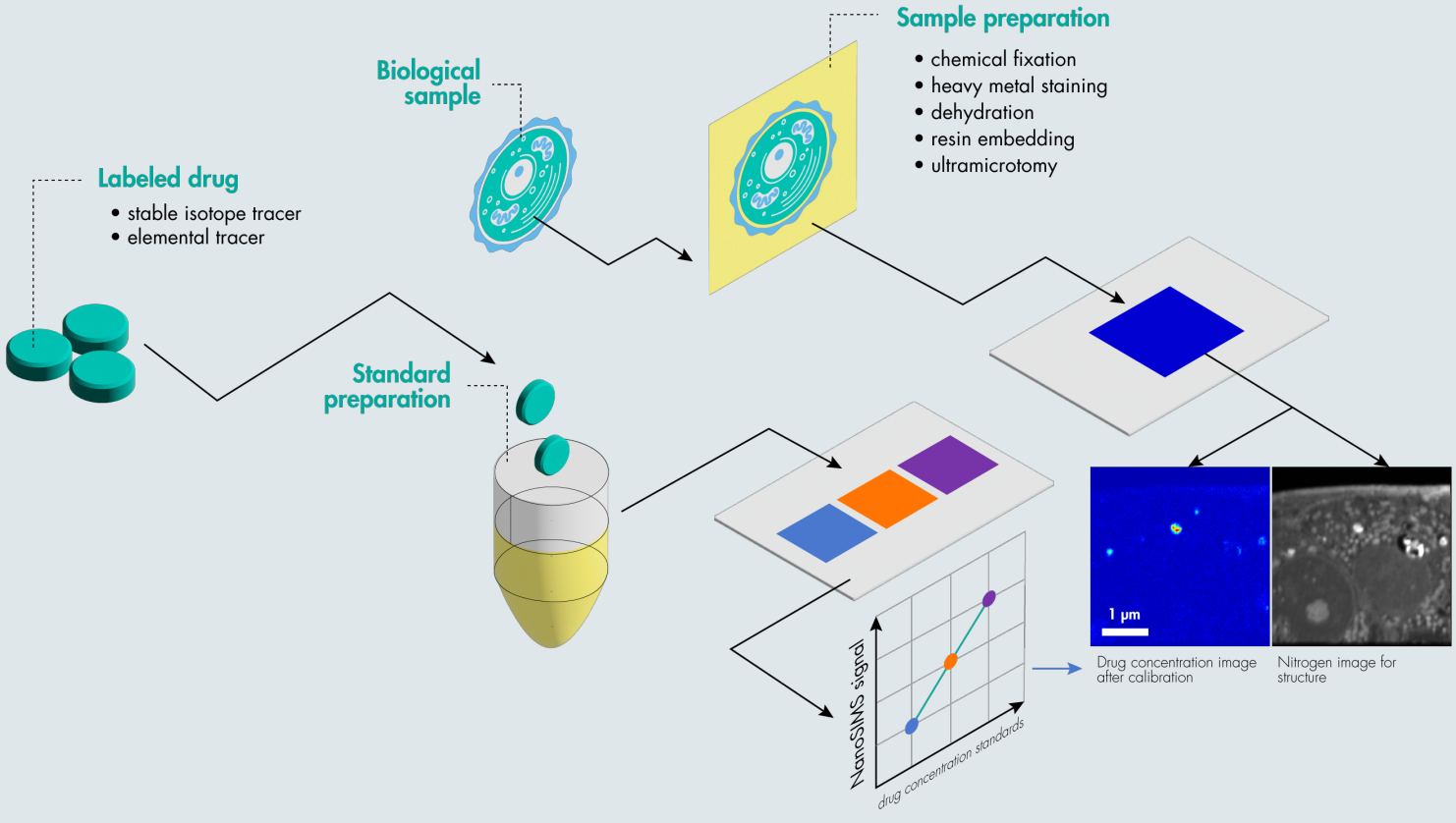
In a typical DrugSIMS workflow, a team's chemist and biologist start by selecting their labeling strategies: either isotopic — for example, carbon-13 (^{13}C), nitrogen-15 (^{15}N), or sulfur-34 (^{34}S) — or elemental — for example, fluorine (F), bromine (Br), or iodine (I). Their choice is guided by the precision needed for concentration measurement; by constraints on how labelling may affect the potency of the given drug; and finally by commercial availability of the starting material. The incubation of cell or tissue with the drug remains standard.

Sample preparation (which is generally allotted 2 days in a typical DrugSIMS workflow plan) is largely inspired by electron microscopy (EM) procedures. The sample undergoes fixing and/or heavy metal staining, and is then embedded in epoxy resin. The resin preserves the sample's subcellular structure, as epoxy replaces the water content that originally accounted for 60% to 70% of cell/tissue volume. Moreover, since the carbon content of the cell/tissue matches the carbon content of standard Agar100 resin, the cured resin is considered a blank sample with a homogeneous matrix.

Ultramicrotomed slices are sectioned from the cured resin and deposited on a substrate. SEM and TEM imaging (2 days) may optionally be used to target regions of interest, which are then subjected to NanoSIMS analysis (2 weeks). Lastly, NanoSIMS data mining (2 weeks) finalizes the workflow.

Results: the researchers are able to derive reliably accurate drug concentration measurements in organelles and other sample cell components. These metrics may be used with confidence for informed decision-making in the drug discovery process.

Typical DrugSIMS Workflow

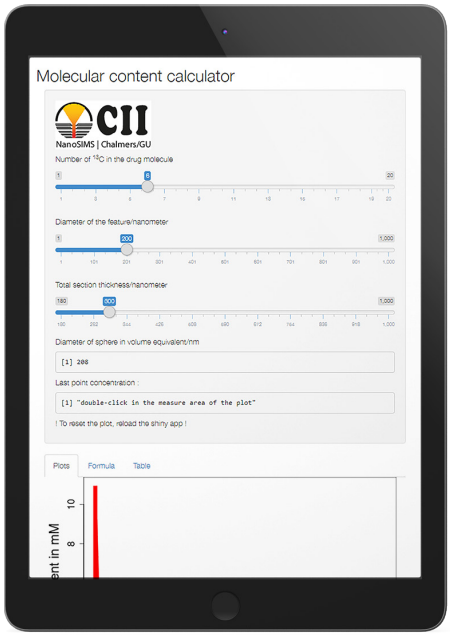


The DrugSIMS Web App: streamlining the workflow

To ensure the method's ease of use, the group has created a DrugSIMS web application[4] that incorporates limits of quantification, guiding the biologist or chemist in choosing the most appropriate labelling element for their drug. They plug their chosen parameters into the app, which thereby calculates the sensitivity necessary; the researchers can then judge if this is sufficient for their proposed experiment.

Four standards should be accessible, either as physical specimens or as standardized formulations. Work will continue to develop intuitive, ergonomic software solutions to complement existing data-processing tools such as ImageJ and CAMECA WinImage. These will be seamlessly integrated into the DrugSIMS workflow.

The goal: an easily understood method realized in software that can be readily applied to resolve important questions in the pharmaceutical industry.



Addendum: contract research

The Chemical Imaging Infrastructure shared by Chalmers University of Technology and the University of Gothenburg currently offers determination of subcellular drug concentration as a contract research service. Where academic researchers, biotech startups, and smaller pharmaceutical companies can't devote the time, effort, and resources to developing these capabilities themselves, this service may provide a welcome — and economically feasible — alternative.

Service and support are available for a wide array of projects. The group has standards available for many atomic labeling strategies, and can advise on appropriate strategies for a given compound. Laboratories worldwide may simply treat samples with standardized TEM preparation, then ship them to the hub. Provided that labeled compounds and appropriate standards are available, a complete workflow — from experiment to data report delivery — typically can be completed in 4 weeks or less.

Learn More About DrugSIMS



The Chemical Imaging Infrastrure research team in front of the NanoSIMS tool hosted on the AstraZeneca Gothenburg campus. From left to right, Emma Kay and Michael Kurczy (AstraZeneca), Elias Rajbari (University of Gothenburg), Per Malmberg (Chalmers University of Technology), and Cécile Becquart (AstraZeneca/University of Gothenburg).

Conclusion

- New pressures to reach targeted intracellular drug concentrations have forced the pharmaceutical industry to seriously consider pharmacokinetics occurring inside the cell: until now comparable to evaluating the contents of a "black box."
- Integration into existing pharmaceutical frameworks requires a common language. Without absolute concentration metrics, mass spectrometry imaging may get lost in translation.
- The most elegantly designed drug concept will never become a product if it cannot reach its target.
- Current methodologies such as plasma concentration measurement, fluorescent microscopy, and fractionation suffer from serious shortcomings.
- A new approach to academic/industry collaboration — BioVentureHub — has created a novel solution to address these problems.
- The DrugSIMS solution implements advanced NanoSIMS imaging technology within a specialized workflow to present absolute measurements of therapeutic-level concentrations for intracellular drug targeting. This DrugSIMS methodology should revolutionize the drug discovery process.

Advanced DrugSIMS methodologies have been collaboratively developed by AstraZeneca BioVentureHub, Chalmers University of Technology, the University of Gothenburg, and CAMECA.

BIO VENTURE HUB

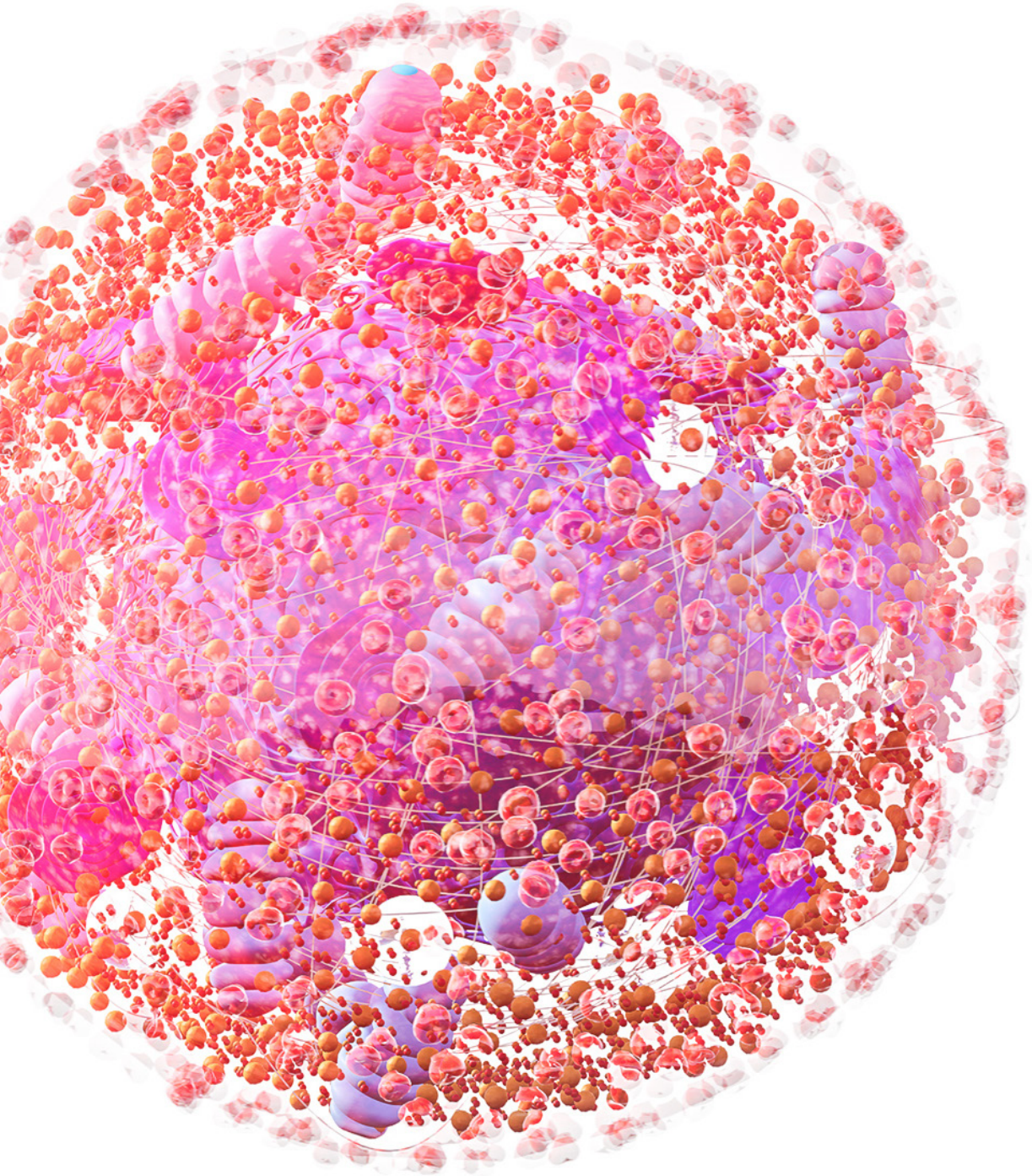
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2. A. Thomen et al., "Subcellular Mass Spectrometry Imaging and Absolute Quantitative Analysis across Organelles," *ACS Nano* (2020)
3. C. Becquart et al., "Intracellular Absolute Quantification of Oligonucleotide Therapeutics by NanoSIMS," *Analytical Chemistry* (2022)
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